

A dependency map enhanced with next-generation 3D cancer models

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Despite advances in precision oncology, effective personalized treatments are still lacking for most patients with cancer¹. The Cancer Dependency Map (DepMap) accelerates this field by systematically identifying cancer vulnerabilities in diverse preclinical models. Data from over 1,300 cell lines have led to the discovery of new therapeutic strategies across multiple tumour types². However, mapping cancer vulnerabilities using traditional cell lines has limitations, including insufficient cancer subtype representation and the impact of culture conditions on perturbation responses. Here we perform 147 genome-scale CRISPR screens and multi-omic characterizations of next-generation (NextGen) cancer models (organoids and spheroids) across 10 cancer types. This strategy enables the expansion of DepMap to cover new genomic and molecular subtypes and to identify new biomarker-associated vulnerabilities. These new models also preserve transcriptional programs that are silenced in traditional cell lines and facilitate the discovery of specific gene dependencies associated with these programs. Comparisons of traditional and NextGen cancer models enable further identification of distinct effects of growth format and culture medium on gene essentiality. The integrated dataset combines data from both model types to offer a valuable, expansive resource for exploring cancer vulnerabilities and is accessible via the DepMap portal.

Advances in understanding cancer genomes have led to mutation-tailored therapies that have improved outcomes in cancers such as chronic myeloid leukaemia and melanoma³. However, despite this progress, most patients still lack targeted treatments customized to the molecular makeup of their tumours. DepMap has accelerated personalized cancer medicine by identifying therapeutic targets through genomic profiling and functional screening of cancer models^{2,4}. Having profiled over 1,300 models, DepMap has uncovered multiple targets that have advanced to clinical trials, but current limitations—including underrepresentation of many cancer subtypes and a reliance on monolayer cultures with serum-containing media—constrain its scope⁵.

Three-dimensional (3D) patient-derived models such as organoids and spheroids⁶, termed NextGen models, have emerged as a

complement to conventional cell lines. Their specialized culture conditions support the propagation of diverse, underrepresented cancer subtypes and may better recapitulate key features of the tumour microenvironment that are lost in traditional two-dimensional (2D) cultures^{7,8}.

Here we profile NextGen models via genome-scale CRISPR screens, whole-genome sequencing (WGS) and RNA sequencing (RNA-seq), and compare and integrate these data with traditional cell line data. The NextGen models expand tumour subtype coverage in DepMap and preserve tumour-relevant gene expression programs that are silenced in traditional cell lines, which enables the discovery of new biomarker-linked dependencies. A comparison of 2D adherent and 3D organoid models further identifies dependencies shaped by culture environment, a result that highlights the advantages of the integrated traditional and NextGen dataset.

Overview of models and data generation

We collected 314 NextGen models comprising 237 carcinoma organoids and 77 central nervous system (CNS) tumour spheroids^{8–11} (Fig. 1a,b and Extended Data Fig. 1a,b) and generated WGS and RNA-seq profiles from 291 and 309 models, respectively. WGS confirmed expected recurrent genomic alterations, including the following changes: *TP53* and *APC* mutations and chromosome 18q loss in colorectal models; *TERT* promoter mutations in CNS models; and *TP53* and *KRAS* mutations and chromosome 9p loss in pancreas models (Fig. 1c and Extended Data Fig. 1a,b). NextGen models also captured mutations not represented in traditional models, such as *NRAS* in colorectal cancer (4.4% prevalence)¹² and *ESR1* in breast cancer (7% prevalence)¹², and expanded prostate cancer representation with previously underrepresented alterations (*SPOP*, *RBI1*, *APC* and *CTNNB1*; Fig. 1c,d and Extended Data Fig. 1a,b).

We assessed the fidelity of NextGen models in recapitulating tumour transcriptional cell states using Celligner (Fig. 1e), which jointly embeds gene expression data from tumour samples (acquired from TCGA¹³, TARGET and Treehouse (referred to as TCGA-plus) and from MET500¹⁴; $n = 13,104$) and cancer models¹⁵. Analyses of 300 NextGen models, 532 traditional cell lines and 266 patient-derived xenograft (PDX) models¹⁶ enabled us to infer the lineage for each model on the basis of 25 nearest neighbour tumour samples.

Celligner-based lineage inference matched annotated lineage for 69% of NextGen models, outperforming PDX models (60%) and traditional cell lines (35%) (Fig. 1f and Extended Data Fig. 2a,b). The lower accuracy of traditional cell lines was partly due to their enrichment in an ‘undifferentiated cluster’ associated with epithelial–mesenchymal transition (EMT) and loss of lineage-specific gene expression¹⁵. In detail, >25% of the traditional models fell into this cluster compared with <2% of NextGen, PDX and tumour samples (Extended Data Fig. 2c).

NextGen models substantially outperformed traditional cell lines for CNS tumours (93% versus 11%) and oesophagus–stomach tumours (83% versus 24%; Fig. 1f and Extended Data Fig. 2a,b,d). This result is consistent with previous reports of marked transcriptional divergence between traditional CNS models and corresponding tumours^{7,17}. Prostate cancer was an exception, for which traditional cell lines (4 out of 7 correct) outperformed NextGen models (0 out of 12; Fig. 1f and Extended Data Fig. 2a,b). This result probably reflects the derivation of prostate organoids from castration-resistant or neuroendocrine tumours and their known transcriptional heterogeneity^{9,18}. Pancreas lineage prediction was challenging owing to its proximity to oesophagus–stomach samples in Celligner (Fig. 1e) and the scarcity of pancreas tumour samples. All Celligner data, including 414 additional Human Cell Model Initiative (HCMI) models^{19,20}, are accessible through the Celligner portal (<https://depmap.org/portal/celligner/>) (Extended Data Fig. 2e).

We performed genome-scale CRISPR screens on 147 NextGen models using the Humagne enAsCas12a single-guide RNA (sgRNA) library²¹ (Extended Data Fig. 3). Screen quality was comparable between NextGen models and traditional cell lines screened using spCas9 ($n = 1,351$) or enAsCas12a ($n = 36$) sgRNA libraries. Moreover, both showed clear separation of non-essential and essential sgRNA score distributions and similar receiver operating characteristic area under the curve (ROC-AUC) and null-normalized mean difference (NNMD) scores (Fig. 1g and Extended Data Fig. 4a,b).

CNS models were screened as spheroids or on matrix-coated plates (termed ‘coat’ hereafter) and organoids were screened in Matrigel domes or in coat conditions, all in serum-free media (Supplementary Table 1). Gene expression and essentiality profiles were nearly identical across CNS growth formats (Pearson’s $r = 0.996$ and 0.990 , respectively), and organoid gene dependencies also strongly agreed between the two conditions ($r = 0.984$; Extended Data Fig. 4c–h). These results suggest that growth format does not significantly affect CRISPR screen outcomes under serum-free conditions. This finding is in contrast to

a previous study that used serum-containing media²²—a discrepancy that may be attributable to differences in medium composition and the comparison strategy used. All models were profiled for WGS, RNA-seq and CRISPR under identical conditions.

To integrate data across models and libraries, we used Chronos²³, which corrects for copy number (CN) effects, screen quality and library-specific batch effects (Fig. 1h,i). Because all NextGen models used the Humagne library, library-specific batch effects were confounded with organoid model effects. We therefore pre-trained Chronos on all traditional and NextGen CNS models before applying the pretrained parameters to organoids. The integrated dataset—encompassing WGS, RNA-seq and CRISPR data and growth-format annotations—is available through the DepMap portal (<https://depmap.org/portal>)²⁴.

Biomarker-associated dependencies

To identify therapeutic targets in the NextGen CRISPR dataset, we classified genes into five dependency profile classes: non-dependency, weakly selective, strongly selective, high variance and pan-dependency²⁵ (Extended Data Fig. 5a). Focusing on the three strong dependency classes, we analysed three representative dependency–biomarker associations—expression addictions, paralogue dependencies and dependencies associated with oncogene gain-of-function (GOF) or tumour suppressor gene (TSG) loss-of-function (LOF)—to identify gene dependencies that were predictable by cellular genetic or molecular features.

Expression addiction, in which gene essentiality correlates with increased levels of its own expression (common for oncogenes and lineage-defining transcription factors^{23,25–27}), was identified for 51 strong dependencies ($r < -0.3$, $q < 0.005$). These included 14 oncogenes ($P = 6.21 \times 10^{-10}$, one-sided Fisher’s exact test) and 21 transcription factors ($P = 3.09 \times 10^{-14}$; Fig. 2a,b and Supplementary Table 2). Among these were *HNRNP1* and *PROX1*, both implicated in brain tumours^{28,29} (Fig. 2c and Extended Data Fig. 5b). The dependency–expression correlation was stronger for both genes in NextGen models, and both displayed increased expression in brain tumours relative to normal tissue (Extended Data Fig. 5c). *HNRNP1* also exhibited elevated expression in biliary tract cancers, as did *PROX1* in colorectal cancers, which may represent tumour-specific vulnerabilities.

Notably, expression addictions for certain lineage-specific oncogenes were found only in traditional cell lines. *ESR1* addiction, which is central to oestrogen-receptor-positive breast cancer, was absent in NextGen models ($r = 0.033$ for NextGen versus $r = -0.637$ for traditional; Fig. 2c). This result is consistent with insufficient *ESR1* expression in breast organoids (Fig. 2c and Extended Data Fig. 5d) and previous reports of its progressive loss in organoid culture^{30,31}. Similarly, *ERBB2* addiction was absent in organoids, with only 3 out of 146 NextGen models expressing *ERBB2* at levels to cause its dependency ($\log_2[\text{transcripts per million (TPM)} + 1] > 7.5$; Extended Data Fig. 5b,d). These findings indicate that there are differences between NextGen and traditional models in their dependencies on oncogenes.

Paralogue dependencies arise when the loss of a gene increases reliance on its paralogue^{26,32,33}. We evaluated all paralogue pairs with >25% identity and identified 106 strong dependencies linked to reduced paralogue expression ($r > 0.3$, $q < 0.005$; Fig. 2d and Supplementary Table 2). Several were stronger in NextGen models, including *ENO1* dependency linked to low *ENO2* expression, than in traditional models ($r = 0.455$ versus $r = 0.233$, respectively; Fig. 2e). *ENO2*, a neuron-specific enolase³⁴, showed lineage-appropriate expression only in NextGen models (Fig. 2e). Its reduced expression in colorectal and prostate cancers relative to normal tissue (Extended Data Fig. 5c) suggests that *ENO1* inhibition could selectively target these tumours. Previous work has also linked *ENO1* CN deletion to *ENO2* dependency in glioblastoma (GBM)³⁵, which indicates that there

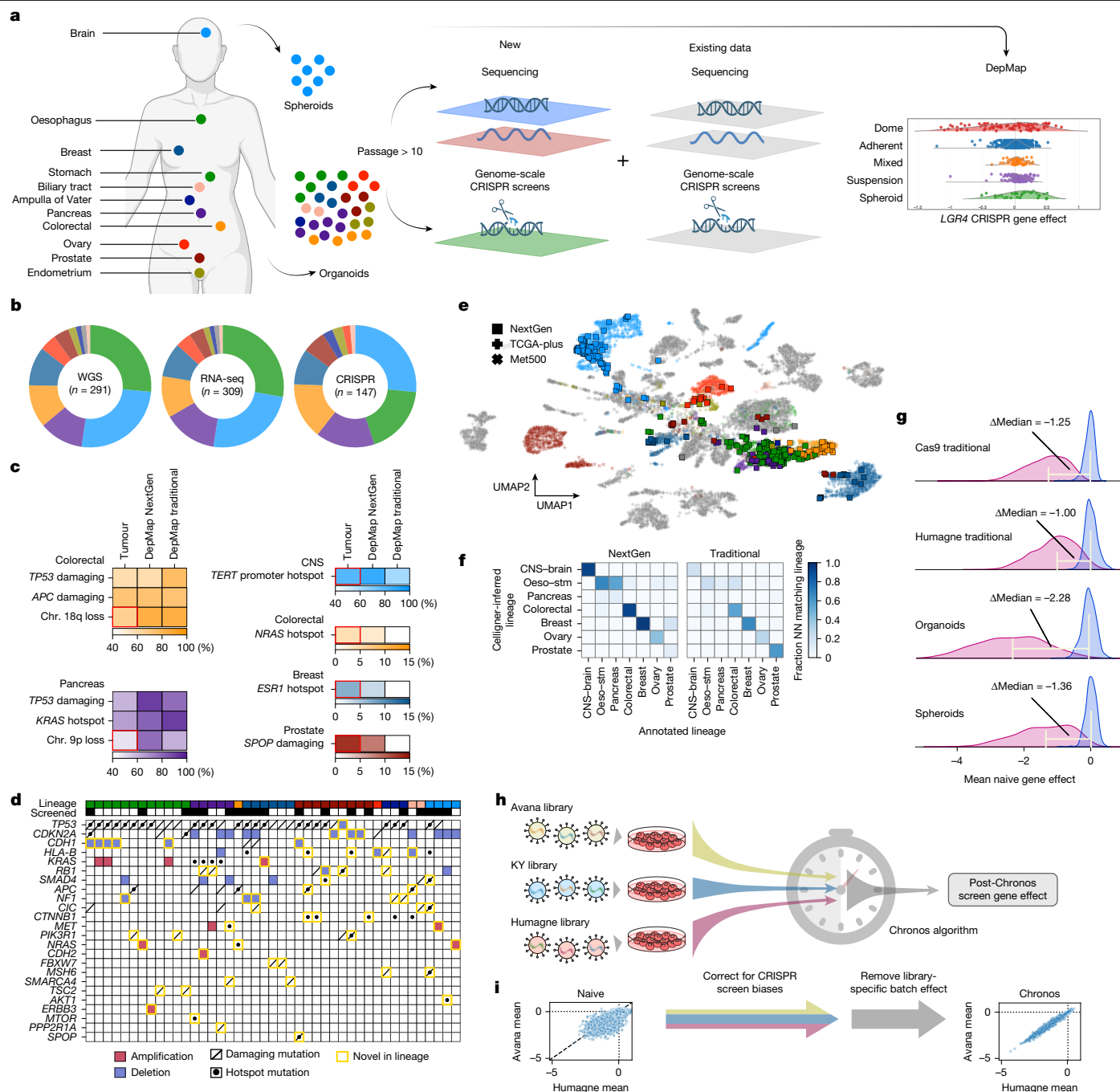


Fig. 1 | Multiomics and functional profiling of NextGen cancer models.

a, Illustration of NextGen model profiling in DepMap. The combination of multiomic characterization and functional genomic profiling at DepMap is extended to NextGen models. The colour code for individual cancer lineages shown here is used in **b–e**. **b**, Lineage composition of the NextGen models profiled for each of the assay types. **c**, Comparison of alteration frequencies per lineage for selected genes across TCGA tumours, NextGen models and traditional cell lines. The intensity of the colour represents the proportion of tumours or models with the indicated genomic alteration. Red frames indicate that values are sourced from publications describing a similar tumour dataset^{12,58–60} but not from the same TCGA dataset¹³ used in other parts of this study. **d**, Oncoprint of NextGen models with mutations and CN alterations of

driver genes that were not represented in the traditional DepMap models of their respective lineages. **e**, Celligner embedding of gene expression for tumour samples and NextGen cancer models. **f**, Lineage prediction based on nearest neighbour (NN) tumour samples in Celligner. Oeso–stm, oesophagus–stomach. For each of the NextGen and traditional models, the lineages of the 25 nearest neighbour tumour samples (y axis) were plotted for each of the annotated lineages of the models (x axis). **g**, Distributions of mean dependency log fold changes (naive gene effect) of nonessential (blue) and common essential (maroon) genes. **h, i**, Illustration of data integration steps across screens using Chronos (**h**) with a plot of Avana and Humagne data shown for pre-Chronos and post-Chronos processing (**i**). Schematic in **a** created in BioRender; Vazquez, F. <https://biorender.com/fd5u98k> (2026).

is mutual paralogue dependency. Conversely, some relationships were weaker in NextGen models. These included *ELMO2* dependency tied to low *ELMO3* expression ($r = -0.110$ (NextGen) versus $r = 0.545$ (traditional); Extended Data Fig. 5e), which suggests that there are

different requirements for *ELMO2*-mediated cytoskeletal rearrangement across model types.

To find dependencies related to oncogene GOF and TSG LOF, we analysed 6 oncogenes and 13 TSGs with sufficient NextGen

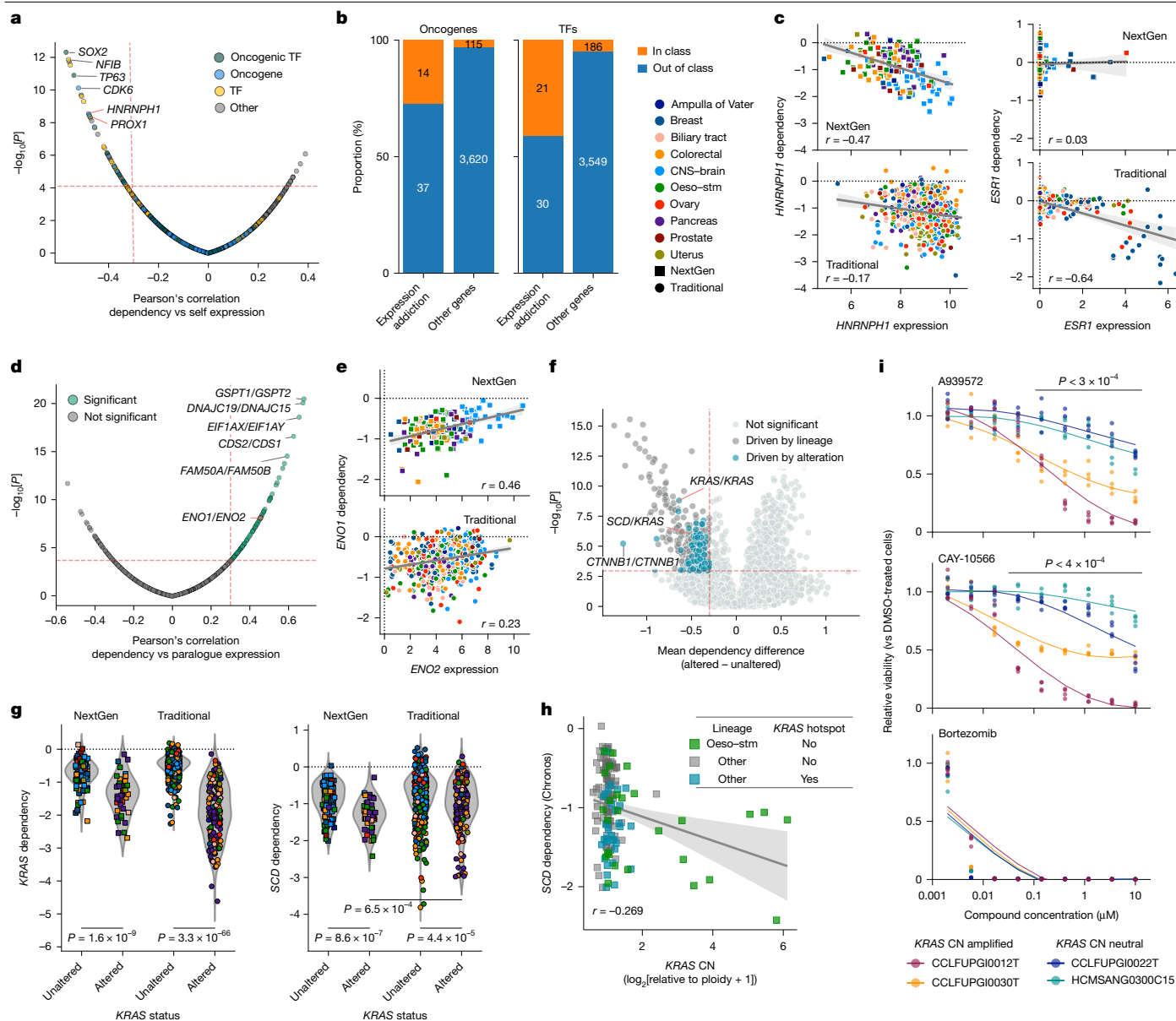


Fig. 2 | Biomarker-associated vulnerabilities identified in NextGen models.

a–c, Expression addition. Dependency–expression correlation on the same genes was scored (**a**; significance thresholds, $q = 0.005$; two-tailed Pearson's correlation, $r = -0.3$; $n = 146$ (model), 3,786 (gene)). Red label lines indicate that further analysis was carried out. Oncogenes and transcription factor (TF)-encoding genes are enriched in expression additions (**b**). *HNRNP1* and *ESR1* show strong expression addition in NextGen and traditional models, respectively (**c**). In **c**, **e** and **g**, model lineages are indicated using the colour code in **c**. In **c**, **e** and **h**, regression lines with 95% confidence intervals are displayed. **d**, **e**, Paralogue dependencies were assessed by correlating gene dependency with paralogue expression (**d**; significance thresholds, $q = 0.005$; two-tailed Pearson's correlation, $r = 0.3$; $n = 146$ (model), 3,787 (paralogue pair)). Several points are labelled as dependency gene/paralogue gene. *ENO1* dependency in *ENO2*-low models is more common in NextGen models (**e**). **f**, **g**, Dependencies associated with oncogene GOF or TSG LOF. In **f**, blue points

are associations not driven by lineage bias (dark grey, associations driven by lineage bias; light grey, not significant, $q \geq 0.05$ (two-tailed Mann–Whitney *U*-test) or Δ mean dependency ≥ -0.3 ; $n = 147$). Several points are labelled as dependency gene/(altered) cancer gene. Representative model distributions are plotted (**g**) with P values calculated using two-tailed Mann–Whitney *U*-tests. $n = 105$ and 42 for altered and unaltered, respectively (NextGen); and $n = 428$ and 169 for altered and unaltered, respectively (traditional). **h**, **i**, *KRAS* CN amplification correlates with enhanced *SCD* dependency ($P = 9.89 \times 10^{-4}$, two-tailed Pearson's correlation; $n = 147$) (**h**). *KRAS* CN-amplified oesophagus–stomach organoids show enhanced sensitivity to *SCD* inhibitors (**i**). $P = 6.2 \times 10^{-6}$, $P = 1.7 \times 10^{-5}$, $P = 1.2 \times 10^{-4}$, $P = 2.2 \times 10^{-4}$, $P = 9.5 \times 10^{-6}$ for 0.14, 0.41, 1.2, 3.4 and 10 μM , respectively (A939572); $P = 4.0 \times 10^{-6}$, $P = 4.4 \times 10^{-6}$, $P = 2.0 \times 10^{-5}$, $P = 6.3 \times 10^{-5}$, $P = 3.4 \times 10^{-4}$ for 0.14, 0.41, 1.2, 3.4 and 10 μM , respectively (CAY-10566). *KRAS* amplified versus neutral; two-tailed two-way analysis of variance (ANOVA) ($n = 4$).

model representation ($n \geq 5$, with at least one hotspot mutation model) and compared dependency scores between altered and unaltered models. After removing associations driven by lineage bias, we identified 83 genetic alteration–dependency pairs with significant dependency enrichment (Δ mean dependency < -0.3 , $q < 0.05$) in altered models, including the expected *KRAS* dependency

enrichment in *KRAS* GOF models (Fig. 2f,g and Supplementary Table 2).

This analysis also revealed an association between *KRAS* GOF and dependency on stearoyl-CoA desaturase (*SCD*), a key enzyme in de novo lipogenesis (Fig. 2f,g). This link has been reported in the context of radiosensitizing lung tumours with *KRAS* mutations³⁶. In our data,

this association was most robust in oesophagus–stomach organoids with *KRAS* CN amplification (Fig. 2h). Pharmacological validation with SCD inhibitors confirmed that *KRAS*-amplified oesophageal adenocarcinoma organoids were significantly more sensitive to both the compounds tested (Fig. 2i), which suggests that SCD inhibitors could target these cancers via a mechanism orthogonal to *KRAS* inhibitors. The SCD dependency–*KRAS* GOF link was stronger in NextGen than traditional models (Δ mean dependency = -0.442 versus -0.241 , respectively; Fig. 2g), which suggests that the lipid-rich, serum-containing conditions of traditional cell culture mask this dependency by reducing reliance on de novo lipogenesis.

Collectively, these three classes of biomarker-associated dependencies highlight the utility of NextGen models to uncover therapeutically relevant vulnerabilities missed by traditional cell lines.

Dependencies tied to GBM gene expression

We next explored transcriptional differences between NextGen and traditional models, with the aim of gaining insight into distinct dependency profiles. To that end, we characterized their differentially activated biological programs using 41 gene expression meta-programs (MPs), as identified in a previous study³⁷. These MPs represent recurrent gene expression modules that account for intratumour heterogeneity (Fig. 3a) and largely reflect environmentally driven gene expression changes. We therefore reasoned that the distinct culture conditions of NextGen and traditional models would lead to differential MP activation. To evaluate this hypothesis, we quantified MP activity by comparing NextGen ($n = 76$) and traditional ($n = 97$) CNS models and organoids ($n = 229$) with lineage-matched traditional cell lines ($n = 451$), and each organoid lineage against its traditional counterpart. Substantial variations in MP activity were observed between NextGen and traditional CNS models and between organoids and traditional cell lines (Fig. 3a). Notably, NextGen CNS models exhibited significantly increased expression of five MPs associated with neural lineages (Δ expression score > 0.79 , $q < 2 \times 10^{-21}$, two-tailed Mann–Whitney U -test), which prompted additional investigations.

Focusing on GBM, Celligner revealed that most NextGen GBM models (90.0%) clustered with the majority of GBM tumours (93.1%), whereas most traditional GBM cell lines (87.3%) grouped separately with only 4.2% of tumours (Fig. 3b and Extended Data Fig. 6a). Differential gene expression analyses indicated that the former cluster expressed glial or classical markers (*SOX2* and *OLIG1*)³⁸, whereas the latter expressed mesenchymal markers (*VEGFC* and *IGFBP6*)^{39,40} (Fig. 3c and Extended Data Fig. 6b), which led us to label them ‘glial’ and ‘mesenchymal’, respectively. All five neural-lineage-specific MPs were significantly increased in glial models relative to mesenchymal models ($q < 2 \times 10^{-35}$), reaching levels comparable to GBM tumours (Extended Data Fig. 6c,d). A companion study¹⁹ confirmed that traditional culture conditions induced the expression of mesenchymal markers and suppressed glial markers in a NextGen GBM model. This result demonstrates that culture conditions drive cell-state changes.

Next, we explored vulnerabilities tied to glial gene expression. We identified an enrichment of *CDK6* dependency in glial compared with mesenchymal models (Δ dependency = -0.44 , $P = 0.013$) (Fig. 3d,e). Although this dependency ranked second in effect size, it fell short of the multiple-testing significance threshold ($q = 0.28$), which may be due to the small sample size of glial GBMs ($n = 36$) analysed. Comparisons with other NextGen models confirmed that this enrichment was driven by the glial state rather than serum-free media (Fig. 3e and Extended Data Fig. 6e). Encouraged by the clinical impact of CDK4 and CDK6 (CDK4/6) inhibitors on treatment outcomes, we pursued *CDK6* dependency and its associated biomarker.

Although no recurrent GBM mutations correlated closely with *CDK6* dependency (Extended Data Fig. 6f), significant associations were found with CNs of genes, including those on chromosome 9p21.3

(Fig. 3f). Of these genes, *CDKN2A*—which encodes p16INK4A and is the most frequently deleted gene in GBM⁴¹—exhibited the third strongest correlation with *CDK6* dependency across adult GBM models ($r = 0.520$, $q = 4.12 \times 10^{-5}$) and the strongest in glial GBM models specifically ($r = 0.640$, $q = 0.0340$; Extended Data Fig. 6g). Given the established role of *CDKN2A* in CDK6-mediated cell cycle control, we postulated that *CDKN2A* CN loss is a biomarker for *CDK6* dependency in glial GBM.

Validation with individual sgRNAs confirmed that *CDK6* knockout caused greater viability loss than *CDK4* knockout in two glial GBM models with *CDKN2A* loss (Extended Data Fig. 6h,i). This result is consistent with the reported exclusive *CDK6* dependency in GBM stem cells with *CDKN2A* and *CDKN2B* loss⁴². Glial models with *CDKN2A* loss were also significantly more sensitive to *CDK6* depletion than mesenchymal models with the same deletion (Fig. 3g). This finding indicates that glial gene expression and *CDKN2A* loss independently contribute to *CDK6* dependency.

To pharmacologically validate this association, we profiled seven glial GBM models—five with *CDKN2A* CN loss and two CN neutral—against three CDK4/6 inhibitors (abemaciclib, palbociclib and ribociclib) using the dye drop method⁴³. *CDKN2A* CN-loss models were more sensitive (mean growth rate AOC (GR_{AOC}) = 0.35, 0.25 and 0.15, respectively) to all three inhibitors than CN-neutral models (GR_{AOC} = 0.06, -0.03 and -0.07 , respectively), with significant differences for abemaciclib ($P = 7.1 \times 10^{-4}$) and palbociclib ($P = 0.021$) (Fig. 3h and Extended Data Fig. 6j). CN-loss models also displayed reductions in the S phase fraction at doses that did not affect CN-neutral models (Fig. 3i and Extended Data Fig. 6k), which—together with a previous report on CRISPR-mediated *CDKN2A* depletion⁴⁴—provides support for a causal role of *CDKN2A* loss in enhancing CDK4/6 inhibitor sensitivity.

In summary, *CDKN2A* loss increases *CDK6* dependency and CDK4/6 inhibitor sensitivity in glial GBM. Given that 92% of glial and classical GBM tumours exhibit homozygous 9p21.3 deletion⁴⁵, these findings indicate that CDK6 inhibition is a relevant therapeutic strategy. These results also underscore the value of NextGen CNS models in uncovering clinically relevant vulnerabilities because they preserve tumour transcriptional states.

Mucinous differentiation program in organoids

We proceeded to examine genetic vulnerabilities linked to programs that are preserved in organoids. Out of 41 gene expression MPs, 5 exhibited significant differential expression between the organoid and lineage-matched traditional models ($|\Delta$ expression score| > 0.5 , $q < 1 \times 10^{-30}$; Figs. 3a and 4a). In detail, stress, secreted II, pancreatic ductal adenocarcinoma (PDAC)-classical, cilia and glutathione were increased, whereas cell cycle G1/S was reduced in organoids. The PDAC-classical program displayed the largest difference ($|\Delta$ expression score| = 2.30, $q = 3.86 \times 10^{-45}$), which spurred further investigation.

Although first described in the classical subtype of PDAC⁴⁶, this program is recognized across cancer types with aberrant mucin production³⁷. GeneTEA overrepresentation analysis⁴⁷ showed strong enrichment for terms related to epithelial differentiation, mucous membrane and gastrointestinal tissues (Extended Data Fig. 7a), thereby linking this program to the common developmental path of mucosal gastrointestinal epithelium⁴⁸. We therefore termed it the PDAC-classical and mucinous differentiation (MUC) program (PDAC-classical/MUC).

This program was highly expressed (mean score > 1.9) across gastrointestinal tumours (pancreas, oesophagus–stomach and colorectal) but low (< 0.5) in non-gastrointestinal tumours (breast, ovary and prostate) (Extended Data Fig. 7b). This gastrointestinal-specific pattern was preserved in 3D organoids (mean score > 3.0 in gastrointestinal; < 0.6 in non-gastrointestinal), but not in traditional cell lines, which showed low-to-moderate (< 1.6) expression across all lineages (Fig. 4b,c and Extended Data Fig. 7c). This result extends previous findings that organoids maintain the classical gene expression profile in PDACs⁸.

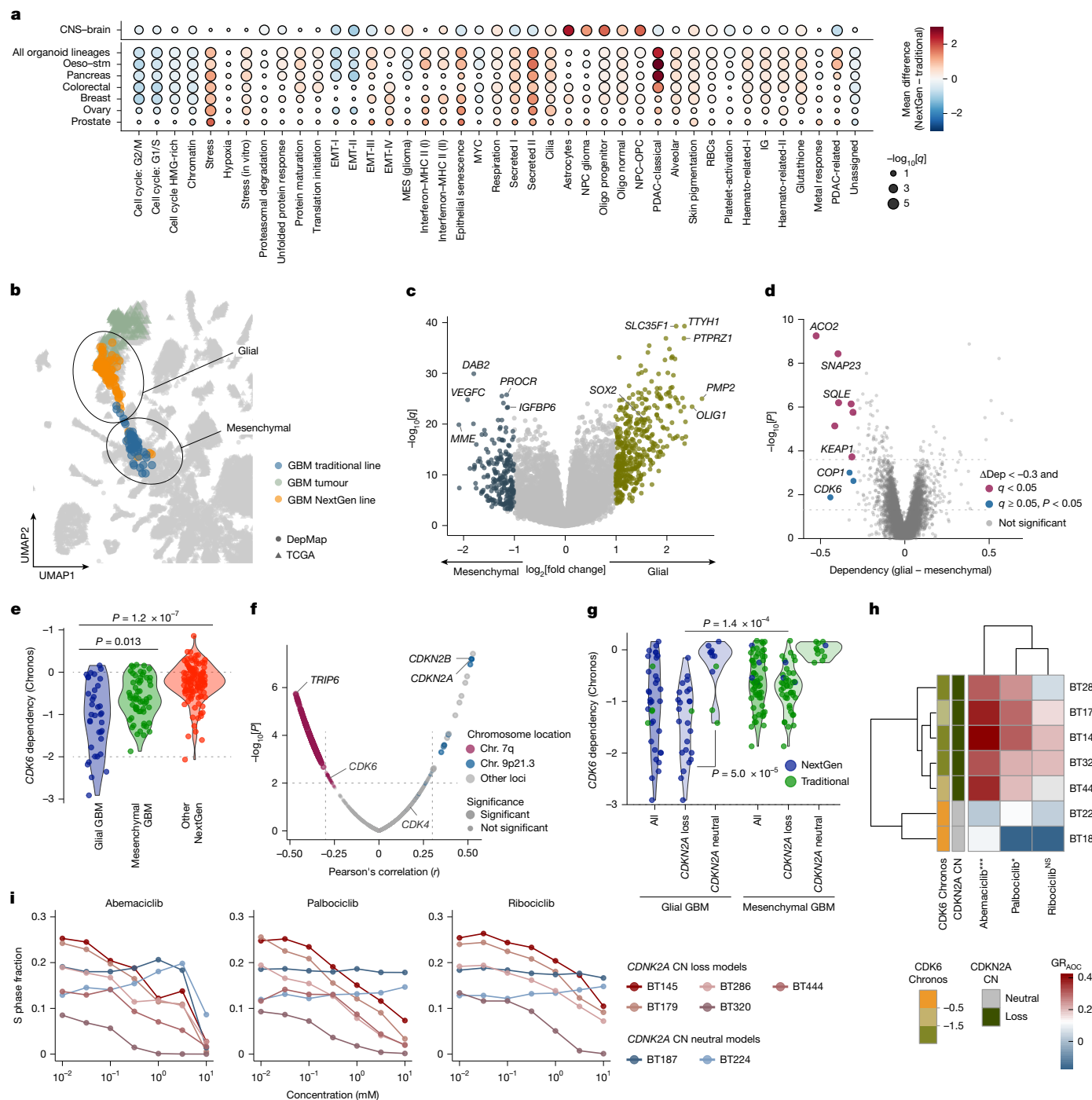


Fig. 3 | Identification of vulnerabilities linked to the glial state of GBM.

a, Mean difference in 41 gene expression MP scores between NextGen and traditional CNS (top) and carcinoma (bottom) models. The colour indicates the score difference. The point size reflects significance (q values; two-tailed Mann–Whitney U -test). $n = 76$ and 97 (CNS–brain); 229 and 451 (all organoid models); 86 and 78 (oesophagus–stomach); 44 and 54 (pancreas); 34 and 86 (colorectal); 28 and 70 (breast); 13 and 66 (ovary); 12 and 7 (prostate) for NextGen and traditional models, respectively. HMG, high-mobility group; MES, mesenchymal; MHC, major histocompatibility complex; NPC, neural progenitor cells; OPCs, oligodendrocyte-progenitor cells; Haemato, haematological; RBCs, red blood cells. **b**, Celligner uniform manifold approximation and projection (UMAP) of GBM tumour and models, with the latter classified into glial and mesenchymal groups. **c**, Differential gene expression between glial ($n = 67$) and mesenchymal ($n = 54$) GBM models (highlighted genes exhibited $|\log_2[\text{fold change}]| > 1$ values, $q < 0.001$, two-tailed Student's t -test). **d**, Differential gene dependencies between glial ($n = 36$)

and mesenchymal ($n = 60$) GBM models. Vulnerabilities enriched in glial GBM models are highlighted in maroon and blue. **e**, $CDK6$ dependency in glial, mesenchymal and non-glial NextGen models ($n = 115$). Here and in **g**, significance was scored using two-tailed Mann–Whitney U -tests. **f**, Correlation of $CDK6$ dependency with gene CNs across GBM models ($n = 95$). Significant associations ($|r| > 0.3$, $q < 0.005$; large points) and genes on chromosome 7p (maroon) and chromosome 9p21.3 (blue) are highlighted. **g**, $CDK6$ dependency in glial and mesenchymal GBM models stratified by $CDKN2A$ CN. For glial models, $n = 36$ (all), 25 ($CDKN2A$ loss) and 11 (neutral); for mesenchymal models, $n = 55$ (all), 44 ($CDKN2A$ loss) and 11 (neutral). **h**, Heatmap of $CDK4/6$ inhibitor GR_{AOC} metrics in seven glial GBM models, with $CDKN2A$ status and $CDK6$ dependency. *** $P = 7.1 \times 10^{-4}$, * $P = 0.021$ and $P = 0.051$ (not significant (NS)) for abemaciclib, palbociclib and ribociclib, respectively ($CDKN2A$ neutral versus loss; two-tailed Student's t -test). **i**, Response curves illustrating S phase cell cycle fractions across indicated concentrations of the $CDK4/6$ inhibitors.

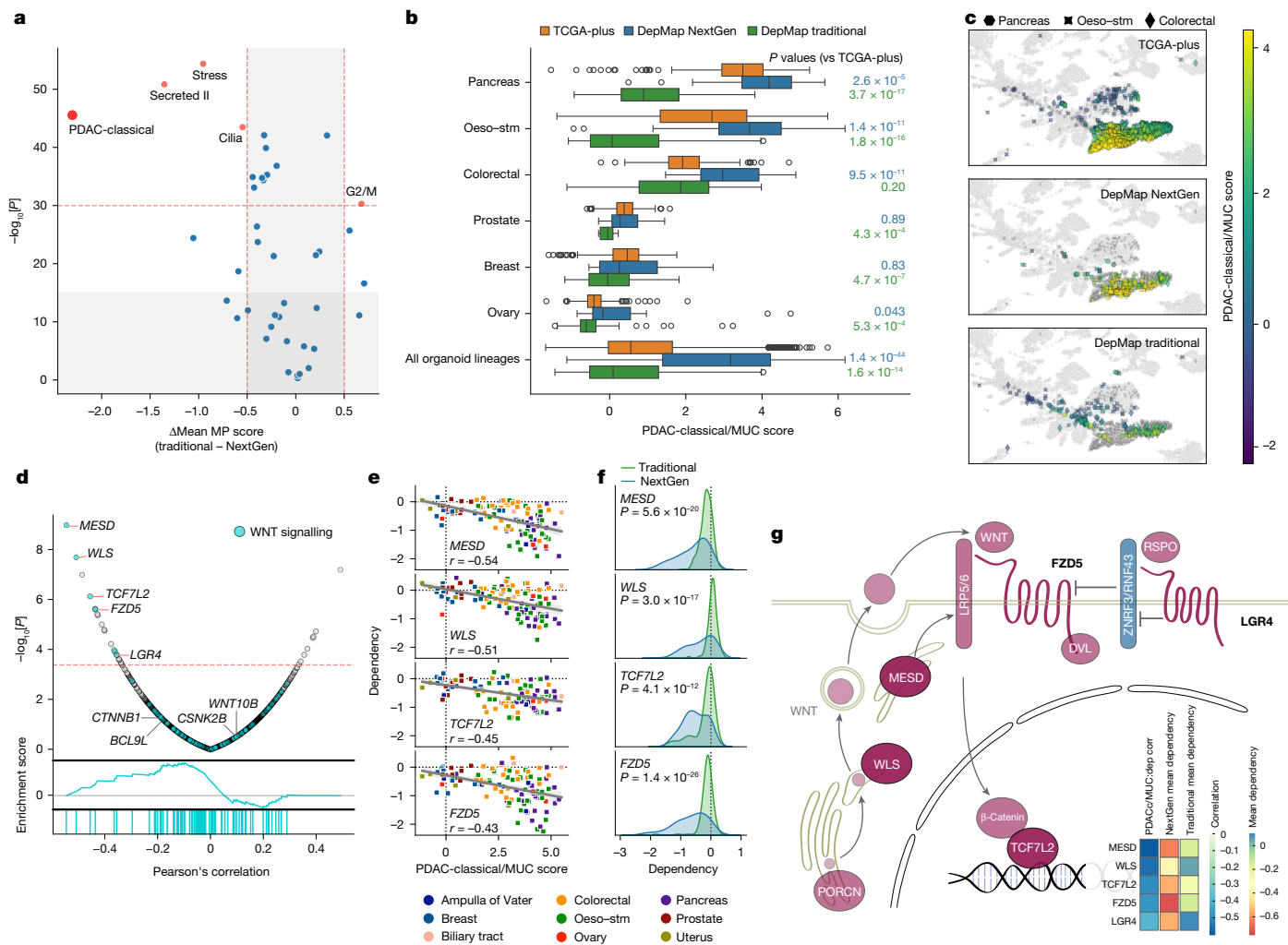


Fig. 4 | PDAC-classical/MUC program and WNT dependency link revealed in organoids. **a**, Differences in MP scores between NextGen organoids and traditional cell lines. Highly significant ($P < 1 \times 10^{-30}$, two-tailed Mann–Whitney U -test; $n = 229$ (NextGen), 451 (traditional)) differences with large effect sizes ($|\text{mean difference}| > 0.5$) are highlighted (red). **b**, PDAC-classical/MUC program expression is high in TCGA-plus tumours and organoids of pancreas, oesophagus–stomach and colorectal lineages. The centre line and box denote median and 25th to 75th percentile data points, respectively, and whiskers extend up to 1.5 times the interquartile range from box edges. $n = 180$, 44 and 54 (pancreas); 602, 86 and 78 (oesophagus–stomach); 384, 34 and 86 (colorectal); 496, 12 and 7 (prostate); 1,099, 28 and 70 (breast); 427, 13 and 66 (ovary); 3,463, 229 and 451 (all organoid models) for TCGA-plus, NextGen and traditional, respectively. P values were scored using two-tailed Mann–Whitney U -tests. **c**, Celligner plots show that PDAC-classical/MUC high expression in pancreas, oesophagus–stomach and colorectal samples cluster centrally in TCGA-plus tumours and organoids, but not in traditional models. **d–f**, Correlation between PDAC-classical/MUC expression and gene dependency across organoids ($n = 3,521$ (gene) and 108 (model)); significant

correlations, $q < 0.05$, two-tailed Pearson’s correlation; **d**, top). Several components of the ‘GOBP WNT signalling pathway’ gene set (cyan points) exhibited enhanced dependency in PDAC-classical/MUC high expression models as revealed by gene set enrichment analysis (GSEA) (**d**, bottom). The dependencies on WNT signalling components that were correlated closely with PDA-classical/MUC scores (*MESD*, *WLS*, *TCF7L2*, *FZD5* and *LGR4*) were investigated further (red label lines; panels **e–g** and Extended Data Fig. 8a,b). Representative dependency versus PDAC-classical/MUC score is plotted (**e**) with regression lines and 95% confidence intervals. Dependency distributions in NextGen versus traditional models are compared (**f**), with P values from two-tailed Mann–Whitney U -tests ($n = 108$ (NextGen) and 485 (traditional)). **g**, WNT pathway components with enriched dependency in high PDAC-classical/MUC expression models (maroon, positive; blue, negative regulators). Bold text (*WLS*, *MESD*, *FZD5*, *LGR4* and *TCF7L2*) indicates factors with increased dependency in high PDAC-classical/MUC models. Correlation and mean dependency scores in NextGen versus traditional models are also indicated (bottom right). PDACc/MUC:dep corr, PDAC-classical/MUC score versus dependency correlation.

Furthermore, the results demonstrated that the PDAC-classical/MUC program is broadly preserved across gastrointestinal organoid lineages but suppressed in traditional cell lines.

Among oesophagus–stomach tumour subtypes, all displayed high PDAC-classical/MUC expression (>2.9 ; Extended Data Fig. 7d) except gastrointestinal stromal tumours and oesophageal squamous cell carcinoma (ESCC). Although ESCCs are overrepresented in traditional oesophagus–stomach models, removing them did not eliminate the significant reduction in program expression in traditional cell lines

($P < 2.1 \times 10^{-13}$, two-tailed Mann–Whitney U -test; Extended Data Fig. 7e). PDAC-classical/MUC scores correlated strongly with previously defined classical subtype scores⁴⁶ ($r = 0.969$) and were predominantly high in pancreas, oesophagus–stomach and colorectal tumours, with high levels (>3) also appearing in some lung and cervix tumours (Extended Data Fig. 7f,g). Scores did not correlate with tumour purity ($r = 0.061$ to -0.193 across lineages; Extended Data Fig. 7h), and cells with high PDAC-classical/MUC expression were significantly enriched in colon tumours compared with normal tissues³⁷. This result supports the

relevance of the PDAC-classical/MUC program to gastrointestinal cancers.

To identify dependencies associated with this program, we correlated PDAC-classical/MUC scores with dependency scores of strong dependency genes (3 strong dependency classes in organoids; $n = 3,521$). The top correlated dependencies were enriched for canonical WNT signalling regulators, including *MESD* (ranked first; $r = -0.544$), *WLS* (second; $r = -0.507$), *TCF7L2* (fifth; $r = -0.454$), *FZD5* (seventh; $r = -0.435$) and *LGR4* (21st; $r = -0.354$) (Fig. 4d,e). This association held when restricting the analysis to organoids grown with R-spondin 1 and WNT3A (Extended Data Fig. 7i), which indicated that it was not driven solely by the presence of WNT activators in culture media. Notably, other WNT regulators (for example, *CTNNB1* and *BCL9L*) did not correlate significantly (Fig. 4d and Extended Data Fig. 7j), which suggests that the association is limited to selective WNT signalling components.

A similar WNT pathway dependency is known in traditional PDAC models with *RNF43* LOF⁴⁹. However, in organoids, these dependencies correlated with the PDAC-classical/MUC transcriptional state irrespective of *RNF43*, *APC*, *CTNNB1* or *AXIN2* mutations (Extended Data Figs. 7k and 8a,b). All 19 organoids strongly dependent on the five listed WNT regulators (average Chronos dependency score < -1) were PDAC-classical/MUC-high (expression score > 2.8), including those with and without WNT-activating genetic alterations (Extended Data Fig. 8b). This result suggests that enhanced WNT dependency is driven by the transcriptional cell state rather than genetic alterations.

These findings extend previous work on the loss of WNT3A and R-spondin dependence during PDAC organoid niche-adaptation⁵⁰ by linking specific WNT signalling dependencies to a conserved mucinous differentiation program active across multiple gastrointestinal tumour types. The expression of this program in a subset of lung and cervix tumours suggests a shared WNT-associated vulnerability in these rare, poor-prognosis cancers^{51,52}. Notably, *MESD*, *WLS*, *TCF7L2* and *FZD5* dependencies were significantly weaker in lineage-matched 2D models ($P < 4 \times 10^{-12}$ for all; Fig. 4f,g and Extended Data Fig. 8c), which provides further support that the PDAC-classical/MUC program, maintained only in organoids, drives these dependencies.

Culture conditions and screen outcomes

To better understand the combined dataset of NextGen and traditional models, we studied differences in gene essentiality between NextGen 3D organoids and traditional 2D models. We compared 3D organoids grown in Matrigel domes with serum-free media (containing EGF and FGF10) to lineage-matched 2D adherent models cultured directly on plastic with serum-containing media (referred to as 3D ($n = 94$) and 2D ($n = 433$) models, respectively). We maintained consistent culture conditions in each cohort to elucidate the effects of these conditions.

Examination of dependencies on frequently mutated oncogenes and TSGs (mutated in $>1\%$ in TCGA⁵³) revealed increased *CDK4* dependency in 2D models (mean 3D dependency score $= -0.26$, mean 2D dependency score $= -0.83$, $q = 9.54 \times 10^{-21}$; Extended Data Fig. 8d,e), which probably reflects greater reliance on this cell cycle driver in these fast-growing models. Similarly, *MDM2* dependency was enriched in 2D models (mean 3D dependency score $= -0.27$, mean 2D dependency score $= -0.54$, $q = 4.23 \times 10^{-12}$; Extended Data Fig. 8d,e), which held for *TP53* wild-type (WT) models (mean 3D dependency score $= -0.54$, mean 2D dependency score $= -1.09$, $P = 8.26 \times 10^{-4}$). This result suggests that 2D culture conditions trigger p53 activation, an effect exposed by reduced MDM2-mediated p53 degradation.

Summarizing pathway-level differences in gene dependencies⁵³ revealed that most 2D models (65.6%) showed positive mean dependency scores for p53 pathway TSGs (*TP53* and *ATM*) compared with only 21.3% of 3D models (Extended Data Fig. 8f,g). This result further suggests that the p53 pathway is more growth-suppressive in 2D than 3D culture conditions. Conversely, knockout of a TSG did not generally

cause a stronger growth benefit in 3D organoids than in 2D models, a result that is in contrast to a previous study analysing serum-containing spheroid cultures²² (Extended Data Fig. 8d,f).

To broadly compare gene essentialities, we calculated the difference in mean dependency scores between 3D and 2D models for every gene ($n = 16,691$; excluding common essential genes), and identified gene sets (Hallmark and KEGG gene sets from MSigDB; $q < 0.05$) that were significantly overrepresented at the extremes of this difference (Fig. 5a,b). Clustering these gene sets by component overlap (>0.3 ; Extended Data Fig. 8h) merged two sets (adhesion and cytoskeleton (adhesion–cytoskeleton), and cell cycle), whereas five others (lipid metabolism, mTORC1, oxidative phosphorylation, UV response and systemic lupus erythematosus) remained separate.

We focused on the adhesion–cytoskeleton and lipid metabolism gene sets owing to their probable connection to the physical and metabolic culture environments, respectively. The 3D versus 2D growth format difference plausibly affects cell–cell and cell–extracellular matrix (ECM) interactions and associated dependencies⁵⁴. By contrast, differential lipid content in media—2D models use lipid-rich RPMI supplemented with fetal bovine serum (FBS), whereas organoid media contain only linoleic and linolenic acids and lack cholesterol—probably influences lipid-metabolism dependencies.

Accordingly, we generated a focused enAsCas12a sgRNA minipool library targeting 250 genes from 9 core functional classes (5 related to adhesion–cytoskeleton, 1 for lipid metabolism and an additional 3 from the 3D versus 2D dependency comparison; Fig. 5c, Extended Data Fig. 9a and Supplementary Table 3). Viability screens using this library were conducted across seven models, testing combinations of growth format (2D plastic or 3D Matrigel dome) and medium type (serum-containing RPMI–FBS or serum-free OPAC) for a total of 18 screens (Extended Data Fig. 9b).

After applying Chronos to the minipool screen data (which correlated well with genome-wide data, $r > 0.59$; Extended Data Fig. 9c), we used linear regression to distinguish growth-format and culture-medium effects. This analysis identified seven genes for which dependencies were altered by growth formats ($q < 0.05$), including three integrin subunits (*ITGB1*, *ITGAV* and *ITGA3*) and two actin regulators (*NCKAP1* and *PTK2*; Fig. 5d and Extended Data Fig. 9d). Eight genes also exhibited dependency changes across different media, three of which regulate lipid metabolism (*SPTLC1*, *SPTLC2* and *SQLE*; Fig. 5e and Extended Data Fig. 9e).

A global analysis of the minipool screen results across the nine functional gene classes did not reveal collective enhancement or impairment of dependencies between different growth formats and media, except for a minor enhancement (Δ dependency $= 0.09$) in the actin regulation class under RPMI–FBS conditions compared with OPAC (Extended Data Fig. 9f,g). To capture opposing effects in gene classes (for example, *ITGB1* dependency increases whereas *ITGAV* decreases in dome culture), we analysed the variance in dependency score differences between conditions (dome versus plastic and OPAC versus RPMI–FBS). Growth format had significantly larger effects on integrin ($q = 7.3 \times 10^{-5}$) and actin regulation genes ($q = 7.2 \times 10^{-14}$), whereas culture medium significantly affected lipid metabolism genes ($q = 1.5 \times 10^{-4}$; Fig. 5f,g). These data demonstrate functional class-specific effects of growth format and medium.

To validate these findings, we knocked out *ITB1*, *ITGAV*, *MSMO1* or *SQLE* in KP3 and HS766T pancreas models under 2D plastic (with RPMI–FBS) and 3D dome (with RPMI–FBS or OPAC) cultures. Cholesterol biosynthesis gene dependencies (*MSMO1* and *SQLE*) varied by medium, whereas integrin subunit dependencies (*ITGAV* and *ITGB1*) were sensitive to growth format (Fig. 5h and Extended Data Fig. 10a). Immunofluorescence analyses confirmed that integrin $\beta 1$ (encoded by *ITGB1*) colocalized with FAK in 3D but not 2D cultures, whereas integrin αV (encoded by *ITGAV*) showed enhanced FAK colocalization in 2D relative to 3D cultures, which was partially due to reduced cell surface

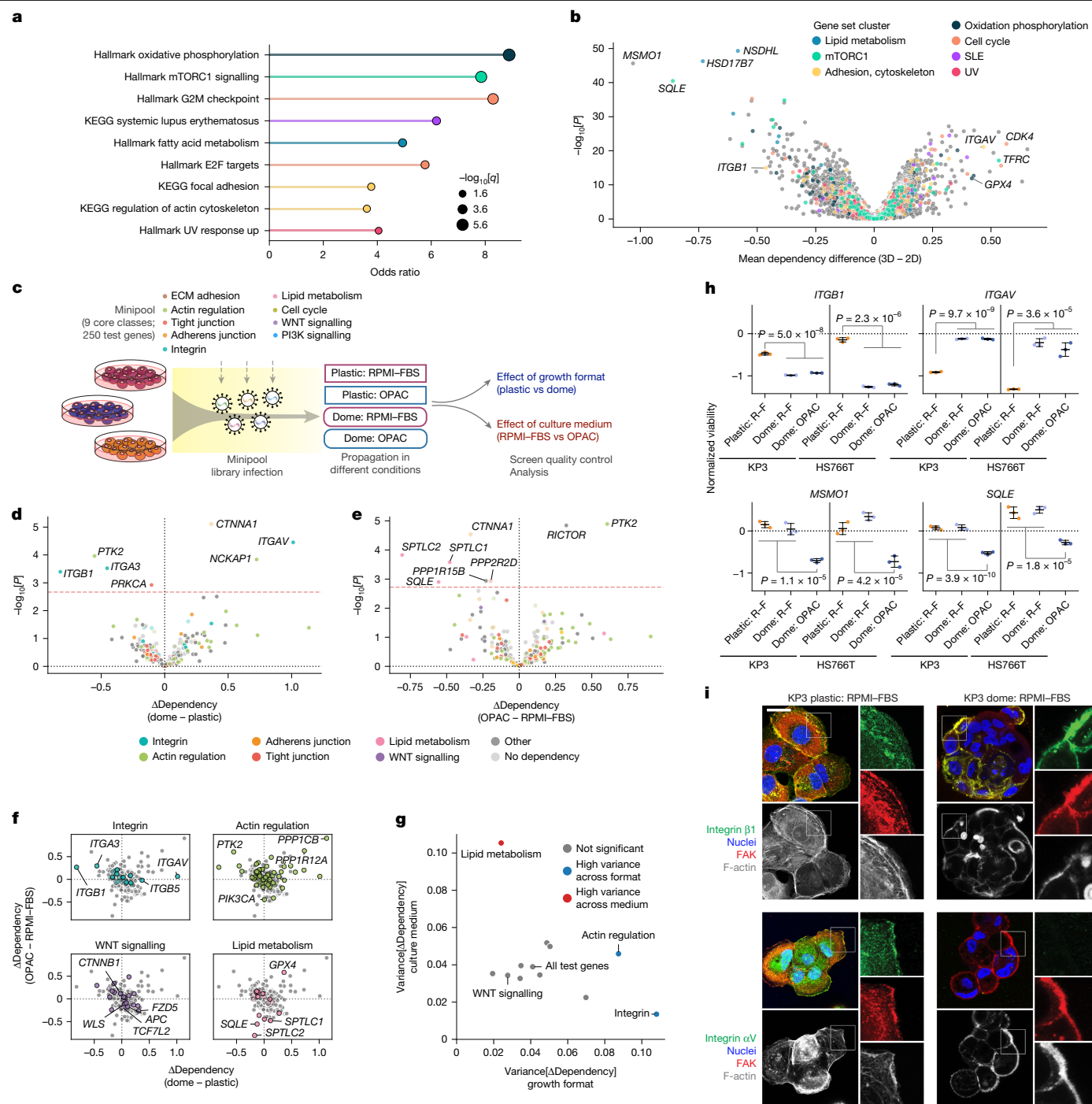


Fig. 5 | Comparing 2D and 3D dependencies to disentangle the effects of culture conditions. a, b, Gene sets enriched for differential dependencies between 3D and 2D models ($n = 94$ (3D) and 433 (2D)). Odds ratios for the 9 significant gene sets ($q < 0.05$) overrepresented in the 200 outlier genes were plotted, clustered and coloured by gene overlap (>0.3 ; **a**). Mean dependency differences and their significance for individual genes (two-tailed Mann–Whitney U -test; gene $n = 3,521$), coloured by the gene set clusters from **a** were also plotted (**b**). **c–e,** Minipool screens were performed to elucidate the differing effects of growth format and culture medium. A minipool screen (250 test genes) was designed (**c**) to separate the effects of growth format (plastic versus dome) and culture medium (RPMI–FBS versus OPAC) on gene essentiality. The screens identified genes for which dependencies are primarily altered by growth format (**d**) or culture medium (**e**), with red dashed lines indicating significance threshold ($q = 0.05$, two-sided linear regression t -test)

and the point colours indicating gene classes in the minipool library (bottom). Screen $n = 11$ (dome), 7 (plastic), 9 (OPAC), 9 (RPMI–FBS). **f, g,** Comparisons across growth formats and culture media. **f,** Different gene classes represented in the minipool library are highlighted. Integrin and actin regulation classes exhibited significant variance across growth formats. **g,** By contrast, lipid metabolism class were affected by culture medium ($q < 0.05$, two-tailed F -test; gene $n = 200$). **h,** Differential effects on integrin and cholesterol homeostasis dependencies assessed by viability after test gene knockout. Values are the mean $\pm$ s.d. ($n = 3$). P values calculated using two-tailed two-way ANOVA. R–F, RPMI–FBS. **i,** Colocalization of integrin chains (green) with FAK (red) in KP3 cells under plastic or dome growth conditions (both in RPMI–FBS). Nuclei (blue) and actin (white) are also shown. Representative images from three independent experiments are displayed. Scale bar, 20 μ m.

expression in 3D (Fig. 5i and Extended Data Fig. 10b–e). These findings align with their differential roles in cell–ECM interactions across different growth formats⁵⁵.

Notably, neither growth format nor medium significantly affected dependencies on the WNT signalling regulators *FZD5* and *WLS* (Fig. 5f), which were essential only in organoids with high PDAC-classical/MUC program levels (Fig. 4f,g). This result probably reflects stable maintenance of the PDAC-classical/MUC program in organoids rather than its rapid induction by the short-term shift in culture conditions. This result is in contrast to integrin, actin and lipid metabolism dependencies, which seem to be more responsive to acute changes in growth conditions. Together, these findings highlight how dependencies on specific cellular processes vary with culture environment and underscore the importance of selecting appropriate model systems for the biological question being pursued.

Discussion

We generated a comprehensive CRISPR viability dataset for NextGen cancer models—carcinoma organoids and CNS spheroids—and integrated them with traditional cell line data. Incorporating these NextGen models substantially expands the representation of tumour subtypes and genomic alterations in DepMap (Fig. 1a–f). We view both traditional cell lines and NextGen models as valuable and complementary resources for advancing our understanding of cancer dependencies.

NextGen models preserve key tumour-relevant transcriptional programs that are lost in traditional cell lines. These include the PDAC-classical/MUC program in gastrointestinal cancer organoids (Fig. 4b,c and Extended Data Fig. 7c) and neural-lineage-specific programs and a glial state in GBM spheroids (Fig. 3b and Extended Data Fig. 6a–d). The maintenance of these programs correlated with selective dependencies, which highlights the utility of the NextGen models in identifying certain transcriptional-state-specific vulnerabilities missed by the traditional models.

Our data also revealed that oncogenic signalling roles differ significantly between model types. WNT signalling is required for organoids with a PDAC-classical/MUC program but is largely dispensable in traditional models (Fig. 4). By contrast, dependencies on *ESR1* and *ERBB2* were exclusive to traditional cell lines (Fig. 2c and Extended Data Fig. 5b,d), which probably stem from reduced expression of these oncogenes in the organoids (Extended Data Fig. 5d) owing to composition differences in the media. Together, these findings demonstrate that multiple oncogenic signalling pathways are highly context-dependent, which reinforces the fact that cellular microenvironments substantially shape requirements for these pathways^{56,57}.

We further demonstrated that growth format (3D Matrigel dome versus 2D monolayer) and medium (serum-free OPAC versus serum-containing RPMI) differentially affect gene essentiality (Fig. 5). Dependencies on integrin and actin genes were sensitive to growth format, whereas lipid metabolism dependencies were influenced by medium, a result that revealed gene class-specific effects. This finding highlights the distinct advantages and disadvantages of NextGen and traditional models and underscores the need to understand the limitations of each model type when studying dependencies.

In summary, the current work enhances DepMap by incorporating NextGen models and integrating their data with traditional cell line data. As more NextGen models are profiled, the expanded DepMap will accelerate the discovery of vulnerabilities across a broader range of cancers, which will enable identification of therapeutic strategies tailored not only to tumour genotypes but also to transcriptional states.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information,

acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-10843-7>.

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Source and culture conditions of NextGen cancer models

The source of each of the NextGen models is provided in Supplementary Table 1. In brief, breast cancer organoid models were derived by the laboratories of A.V.L., S.O. and D.L.S. Most of the NextGen CNS tumour models were derived by the laboratory of K.L.L. Colorectal and oesophagus–stomach cancer organoid models were derived by staff at the Cancer Cell Line Factory at the Broad Institute. Ovarian and uterus cancer organoid models were derived by the laboratory of S.J.H. Pancreas cancer organoid models were derived by the laboratories of A.J.A. and S.R. Prostate cancer organoid models were derived by the laboratory of H.B. and the laboratory of Y. Chen. Additional models derived by the HCMI were obtained via the American Type Culture Collection (ATCC). Models were confirmed for the absence of mycoplasma contamination (Mycoplasma PCR Detection kit; ABM, G238) and authenticated by short tandem repeat (STR) profiling (LabCorp) before expansion. These procedures were repeated monthly during expansion and screening.

Organoid media were prepared using the recommendations provided for each of the models (Supplementary Table 1). The conditioned media were prepared by culturing WNT3A-producing L cells (L Wnt-3A; ATCC, CRL-2647) and R-spondin-1-producing 293T cells (Cultrex R-spondin 1 (RSPO1) cells; Trevigen, 3710-001-K) in DMEM with 10% serum. After reaching 80% confluency, cells were washed once with PBS and incubated with Advanced DMEM–F12 medium (Thermo Fisher, 12634028) containing 100 mM HEPES, 1% penicillin–streptomycin and 1% 100× Glutamax (Ad+++ for another week. The conditioned medium from this culture was then collected, filtered and stored at –80 °C before being used for organoid cultures. All organoids were grown initially as domes composed of 75% growth-factor-reduced, Phenol Red-free Matrigel (50 µl per dome; Corning, 356231) in the recommended medium diluted 1:1 with Ad+++ . The screening of organoids was conducted either in Matrigel dome cultures or in adherent cultures on Matrigel-coated plates (see the section ‘Genome-wide CRISPR screening of organoids’ below).

The NextGen CNS models were grown in tumour stem medium, the composition of which is described in Supplementary Table 1. The conditioned medium was collected routinely during expansion and preserved for subsequent cultures. Cells were seeded in a mixture of 30% conditioned medium and 70% fresh medium. Some of the models were tested for their ability to grow on a Matrigel-coated plate. In brief, cells were plated in 1% Matrigel-coated flasks and cells attached to the coating were carried forward as adherent models. Models that failed to attach to coated plates were grown as spheroids in ultra-low attachment (ULA) vessels (Corning, 3814). Models that were initially derived as adherent cultures on laminin-coated plates were maintained in the same conditions. To coat plates, Matrigel or laminin (Corning, 354232) was diluted in ice-cold PBS at a concentration of 1%. This solution was then added to culture vessels at approximately one-half the recommended volume of medium for a given vessel (for example, 5 ml solution in a T75 flask). Flasks were incubated at 37 °C overnight, and the solution was aspirated immediately before plating the cells. The growth format of each model is listed in Supplementary Table 1.

Preparation of samples for WGS and RNA-seq

To generate pellets from the organoids, the medium was first aspirated and then the Matrigel domes were scraped and transferred to a 20× volume of pre-warmed TrypLE (Thermo Fisher, 12604021) with 10 µM ROCK inhibitor (Peprotech, 129830-38-2). This mixture was incubated at 37 °C for 30 min. Subsequently, the cells were pelleted by centrifugation, resuspended in a mixture of medium and Matrigel and seeded at a density of 50,000 cells per dome. After 1 week of growth, cell pellets were collected without ROCK inhibitor for WGS and RNA-seq.

For the NextGen CNS tumour models, cells were seeded and propagated for 7–14 days, depending on their growth, and dissociated with TrypLE for 5 min at 37 °C. Following dissociation, the cells were counted, and the pellets of 1×10^6 cells were collected and submitted for WGS and RNA-seq.

WGS analysis

WGS for all the DepMap NextGen models was conducted at the Broad Institute Genomics Platform using the PCR-free human WGS procedure. In brief, libraries were constructed and sequenced at 30× coverage on either an Illumina NovaSeq 6000 or NovaSeq X system, with the use of 150 base-pair paired-end reads. The FASTQ files from sequencing were de-multiplexed, aggregated and aligned using the DRAGEN germline pipeline to create BAM files.

Mutation calling from WGS

The mutation calls from the WGS results were generated using Mutect2 (ref. 61), annotated and filtered downstream. The detailed strategies are described in a publicly shared document (https://storage.googleapis.com/shared-portal-files/Tools/25Q3_Mutation_Pipeline_Documentation.pdf). In short, variants were aligned to the GRCh38.p14 human genome assembly (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.40/) and mutation calling was conducted with the following parameters: default format of VCF and HG38; Gatk_docker: broadinstitute/gatk:4.2.6.1; M2_extra_args: –genotype-germline-sites true –genotype-pon-sites true for getting access to germline calls; Filter_funcotations to False PoN: gs://gatk-best-practices/somatic-hg38/1000g_pon.hg38.vcf.gz; Run_funcotator to True; Run_orientation_bias_mixture_model_filter to True. The pipeline was run such that no bait sets were needed.

CN calling from WGS

Relative CN data were generated from the WGS data by running the GATK CN pipeline aligned to hg38 (ref. 62). Absolute CN data from WGS or whole-exome sequencing were generated using PureCN⁶³. Arm-level CN values were calculated using PureCN segment-level CN as previously described⁶⁴. Gene-level amplifications were defined as having a relative CN ($\log_2[\text{relative to ploidy} + 1]$) greater than 3, and deletions were defined as having a relative CN less than 0.25.

RNA-seq

RNA-seq for all the DepMap NextGen models was conducted at the Broad Institute Genomics Platform. In brief, poly-A RNAs were captured with oligo-dT beads, from which libraries (450–550 bp insert size) were prepared using a strand-specific Illumina TruSeq library preparation protocol. These libraries were sequenced to 100 million paired reads on either an Illumina NovaSeq 6000 or NovaSeq X system, with the use of 151 bp paired-end reads. The FASTQ files from sequencing were processed using the DRAGEN RNA-seq pipeline to create BAM files.

Source of traditional cell lines

The traditional cell lines used were obtained from DepMap, and the original source can be found on the DepMap portal (<https://DepMap.org/portal>). These included several commonly misidentified cell lines registered by the International Cell Line Authentication Committee (<https://iclac.org/databases/cross-contaminations/>). We reasoned that even though these cell lines are commonly misidentified, their inclusion in the DepMap dataset would be advantageous for comprehensiveness, provided they were confidently identified and authenticated. Accordingly, we performed STR profiling on the cell lines that are frequently misidentified. After confirming their identities, we decided to profile these lines and include their data in DepMap. Mycoplasma testing and STR profiling were performed after receiving the cell lines and every 3 months of culture period thereafter using a Mycoplasma PCR Detection kit (G238, ABM). Cells were grown in RPMI 1640 (Corning,

10-040-CV) supplemented with 2 mM glutamine, 50 U ml⁻¹ penicillin, 50 U ml⁻¹ streptomycin (Gibco, 10378016) and 10% FBS (MilliporeSigma, F4135) and incubated at 37 °C in 5% CO₂.

Lineage prediction with Celligner

To predict the lineage of cancer models (NextGen and traditional) using Celligner, the 25 nearest neighbour tumours from the TCGA, TARGET and Met500 datasets ($n = 13,104$) were identified for each model in Celligner's aligned gene expression space according to pairwise Euclidean distance. For each lineage of the models, we computed the lineage representation among the neighbouring tumours as a global measure of model–tumour lineage similarity. To obtain per-model lineage predictions, we classified each model by the most frequent lineage found among its nearest neighbour tumours.

Models that fell into the 'undifferentiated cluster' were excluded in a part of the analysis shown in Extended Data Fig. 2a, as this cluster was highly enriched with adherent models from various lineages that exhibited loss of lineage-specific gene expression patterns, which made it difficult to predict lineages of the models in this cluster. The undifferentiated cluster was defined by applying the following criteria to the 79 clusters identified using Celligner: (1) number of DepMap models in the cluster > number of tumour samples in the cluster; and (2) average Hallmark epithelial mesenchymal transition ssGSEA enrichment score among the DepMap models in the cluster > 0.

This procedure identified four clusters with Celligner (clusters 28, 59, 62 and 67), which were collectively designated as the undifferentiated cluster.

Genome-wide CRISPR screening of organoids

After initial expansion and collection of viable stocks and pellets (used for fingerprinting and omics profiling; Extended Data Fig. 3), the organoids were tested for blasticidin sensitivity to identify the minimal concentration of blasticidin that gives less than 1% viability of uninfected cells in 10–14 days. Fourteen organoid models were screened as adherent cultures on Matrigel-coated plates, whereas the rest of the organoids were screened in 3D Matrigel dome cultures. To select the culture conditions for organoid screens, each of the organoid models was subjected to a growth format assay by seeding 50,000 cells into a well of a 24-well non-tissue-culture-treated plate (Corning, 351147) either as a Matrigel dome (75% Matrigel, 50 µl per dome) or on top of a Matrigel coat, referred to as either dome or coat format, respectively. The coated wells were prepared by first adding 1% Matrigel in ice-cold PBS to 50% of the standard filling volume of the well and allowing it to solidify by incubating overnight at 37 °C. Before cell plating, the PBS–Matrigel mixture was aspirated, leaving the Matrigel coat behind, and the cell suspension was added on top of the coat. The growth of cells in the dome and coat formats was compared after 1 week of culture via a viability assay with CellTiter-Glo 3D (3D CTG; Promega, G9683). Models with at least 30% viability on coat relative to dome format were propagated and screened in the coat format. This process enabled us to screen these models in a similar manner to traditional adherent cell lines, but with a thin layer of matrix support, thus decreasing the amount of required Matrigel by approximately tenfold.

Subsequently, all organoids were infected with lentivirus expressing enAsCas12a (Addgene, 136476). For this infection, 1.5×10^6 cells were exposed to the lentivirus in a 24-well ULA plate (Corning, 3473) in the presence of 8 µg ml⁻¹ polybrene and 10 µM ROCK inhibitor. After 18 h, the cells were collected by centrifugation and washed once with PBS to remove the virus, and cells were then plated in Matrigel domes at 5×10^4 cells per dome. At 24 h after seeding the cells in the Matrigel domes, the medium was replaced with selection medium containing blasticidin at 30 µg ml⁻¹. A 3D CTG assay was carried out 10–14 days after infection to ensure efficient elimination of uninfected cells with blasticidin and to measure the infection rate from the enAsCas12a virus. Cells were

maintained at a constant blasticidin (as determined using a blasticidin sensitivity assay) concentration throughout the screening process.

Cas12a activity assessment was conducted as previously described⁶⁵ (a detailed protocol is available as 'Cas9/Cas12a Activity Assay' at the Broad Institute Genetic Perturbation Platform (GPP) web portal: <https://portals.broadinstitute.org/gpp/public/resources/protocols>) using two different types of lentivirus, both of which express GFP, a sgRNA (or sgRNAs) against GFP and the puromycin-resistance gene. One type of these virus expresses two GFP-targeting sgRNA sequences optimized for enAsCas12a (pRDA_221; Addgene, 169142), whereas the other virus type expresses a GFP-targeting sgRNA sequence optimized for spCas9 (pXPR_047; Addgene, 107145), the latter of which served as a negative control. Cells were infected with these two different virus types separately as described above and selected with puromycin for 4 days. GFP expression was measured in the FITC channel by flow cytometry (CytoFLEX LX, Beckman Coulter) 14 days after pRDA_221/pXPR_047 infection with a Zombie Aqua viability stain (BioLegend, 423102) applied to gate out dead cells. Using the non-infection control (NIC) as a negative control, GFP positivity was determined for both the pRDA_221 and pXPR_047 infected cells. Cas activity was calculated using the following formula: (GFP (%) of pXPR_047 – GFP (%) of pRDA_221)/(GFP (%) of pXPR_047).

Cell lines with Cas activity above 70% were selected for screening. However, some lines were screened with activity as low as 44% if they showed sufficiently rapid growth of at least one population doubling per week.

Library titrations were carried out to determine the volume of sgRNA library virus required to achieve an infection efficiency of around 50%. Infections were conducted on 24-well ULA plates, with each well containing 5×10^5 cells in 600 µl medium with 4–8 µg ml⁻¹ polybrene, 10 µM ROCK inhibitor and 50–200 µl library virus. The plate was incubated at 37 °C for 18 h, followed by collection of the cells by centrifugation and a wash with PBS to remove the virus. Cells were subsequently plated in Matrigel domes on a 24-well plate. Puromycin (3 µg ml⁻¹) was added 48 h after virus infection. After 4 days of selection, infection efficiency was determined using a 3D CTG cell viability assay.

For the screens, cells were first infected using the same protocol as for library titration, but only with the virus volume selected to give an infection rate of about 50%. The number of cells used for infection was determined such that sufficient number of cells survived puromycin selection to produce at least 500× representation of the library. Before July 2022, the screens were performed using either the C or D versions of the Humagne library (Addgene, 172650 and 172651) at full representation for each library. By contrast, screens after July 2022 were performed in two technical replicates at half representation using a combined C and D library to enable measurement of replicate correlation. After 18 h of incubation with virus in ULA plates, the virus was washed out, and infected cells were plated at 5×10^4 cells per dome. To test infection efficiency, we seeded four NIC domes and four infected domes in a 24-well plate (one dome per well). After 24 h, the medium was replaced with the selection medium containing puromycin for the infected plates and half of the domes (two NIC domes and two infected domes) for the infection efficiency assay. Puromycin was washed out and blasticidin treatment was resumed on day 6, when a 3D CTG viability measurement for the infection efficiency assay was conducted to estimate actual screen representation. Screens with less than 200× representation were excluded. The medium was replaced on days 13 and 20, and the samples were passaged on day 9 and optionally on day 17 depending on the confluence of the model, which was determined by visual inspection, each time maintaining minimum target representation. All the cells were collected and frozen as pellets on day 23.

gDNA samples were prepared from the screen pellets using a KingFisher Apex System (Thermo Fisher, 5400940). After preparing gDNA samples, the DNA concentration of each sample was measured using a Qubit4 Fluorometer (Thermo Fisher, Q33238), and the

Article

presence of library DNA was confirmed by performing PCR with a set of library-specific primers. gDNA samples were subsequently submitted for library DNA amplification and sequencing to the GPP at the Broad Institute. PCR and sequencing were performed in accordance with the 'sgRNA/shRNA/ORF PCR for Illumina Sequencing' protocol available at the Broad Institute GPP web portal (<https://portals.broadinstitute.org/gpp/public/resources/protocols>). DNA samples were sequenced at a scale matched with the size of the screen: 68 µg gDNA per replicate for screens with 500× representation and 134 µg gDNA per replicate for screens with 1,000× representation.

Genome-wide CRISPR screening of NextGen CNS tumours

The pipeline for genome-wide CRISPR screens of the NextGen CNS tumour models was adapted in part from previous work⁶⁶ and is composed of the same processes to what is described above in the section 'Genome-wide CRISPR screening of organoids' except for the following differences.

CNS models were tested for the growth format and seeding density that resulted in optimal growth rates and for polybrene sensitivity, which was used at a concentration that resulted in >85% survival. For growth testing, cells were plated in both Matrigel-coated and ULA 6-well tissue culture plates. Specifically, cells were seeded in duplicate at densities of 100,000, 200,000 and 300,000 cells per well for both the Matrigel-coated plates and ULA plates. Cells were allowed to grow for 7 days, with a medium change performed at day 3 or 4 as needed. After 7 days, cells from all the conditions were counted and a doubling time was calculated for each condition. The condition that gave the lowest doubling time while maintaining high cell viability was used for the screen. Blasticidin and puromycin were used at a standardized concentration of 50 µg ml⁻¹ and 3 µg ml⁻¹, respectively. For enAsCas12a transduction, 1.5 × 10⁶ cells were exposed to lentivirus expressing enAsCas12a in a 24-well ULA plate in the presence of the optimized concentration of polybrene. At 18 h after infection, cells were washed with PBS to remove the virus and the cells were plated in the optimal growth format at 2× the optimal seeding density. Subsequently, cells were selected with blasticidin. A CellTiter-Glo cell viability assay (CTG; Promega, G7572) was carried out 14 days after infection to ensure efficient elimination of uninfected cells with blasticidin and to measure the infection rate from the enAsCas12a virus.

For library titration, infections were conducted on 24-well ULA plates, with each well containing 1.5 × 10⁶ cells in 600 µl medium, the optimized concentration of polybrene and 50–200 µl library virus. The volume of virus required for an infection efficiency of about 50% was determined as described above in 'Genome-wide CRISPR screening of organoids'. Blasticidin treatment was resumed on day 7 instead of day 6 after infection, and a CTG viability measurement for the infection efficiency assay was performed on the same day to estimate actual screen representation. Medium was replaced on days 10 and 17, and the samples were passaged on days 7 and 14, each time maintaining minimum target representation. All cells were collected and frozen as pellets on day 21, from which gDNA samples were prepared, and the library DNA was amplified by PCR and sequenced.

Genome-wide CRISPR screens of cancer cell lines

The original source for all the cell lines is available at the DepMap portal (<https://DepMap.org/portal>). The CRISPR screens of the traditional cancer cell lines with the spCas9 sgRNA libraries (Avana and KY) were carried out as previously described^{67,68}.

For the CRISPR screens of the traditional cancer cell lines with the enAsCas12a sgRNA library (Humagne-CD), the cell lines were first tested for doubling time and cell size. Blasticidin and puromycin were used at a standard concentration of 40 µg ml⁻¹ and 2 µg ml⁻¹, respectively. To infect adherent cells with lentivirus expressing enAsCas12a (Addgene, 136476), 5 × 10⁶ cells were exposed to the virus in a T175 flask (Corning, 354487) in the presence of 4 µg ml⁻¹ polybrene. For suspension cells,

1.8 × 10⁷ cells were exposed to the virus in a 12-well plate (Corning, 3513) in the presence of 4 µg ml⁻¹ polybrene, which was followed by centrifugation at 930g, 37 °C for 2 h. One day after infection, cells were washed with PBS to remove the virus and cells were then plated at around 50% confluence. Blasticidin was added to the medium 2–3 days after infection. A CTG assay was carried out after 3 days of blasticidin selection to ensure efficient elimination of uninfected cells with blasticidin and to estimate the infection rate from the enAsCas12a virus. Cells were maintained in blasticidin for at least 10 days after transduction and throughout the screening process. After enCas12a transduction and blasticidin selection, enCas12a activity was assessed as described in the section 'Genome-wide CRISPR screening of organoids'.

Library titrations were performed in 12-well tissue-culture-treated plates, with each well containing 1.5 × 10⁶ cells in 2 ml medium, 4 µg ml⁻¹ polybrene and 0, 10, 25, 75, 200 or 500 µl virus. These 12-well plates were spun at 930g, 37 °C for 2 h, after which an additional 2 ml medium was added to each well for a final volume of 4 ml per well. After 24 h of infection, the cells in each well of the 12-well infection plate were split into 2 wells of a 6-well plate with 0 and 2 µg ml⁻¹ puromycin in fresh medium. An optimal seeding density was chosen such that the unselected population reached <70% confluence after selection. After 3 days of selection, infection efficiency for a given virus volume was determined as the ratio of live cells in the puromycin-containing well (2 µg ml⁻¹) to the number of live cells in the puromycin non-containing well. A linear regression of the virus volume to the transduction efficiency was performed to estimate the volume of virus required to transduce 50% of the population.

The remaining procedures of the screen were the same as for the procedures described above in the section 'Genome-wide CRISPR screening of organoids' except for the following modifications. For library infection, after 24 h of incubation with virus in 12-well plates, the virus was washed out with PBS and infected cells were plated with puromycin at an appropriate seeding density and flask size. After 72 h of selection, puromycin was washed out and blasticidin treatment was resumed. Screens with less than 250× representation or at an infection efficiency of higher than 60% were excluded. Cells were passaged every 3–6 days depending on the confluence of the model, as determined by visual inspection, each time maintaining minimum target representation. All cells were collected and frozen as pellets on day 22–23.

Genome-wide CRISPR screening data processing and quality control evaluation

All screens were initially evaluated for sequencing quality and selected to have a mean of at least 185 reads per gene. Naive gene effect scores were calculated by taking the log of the ratio of normalized reads for each gene in screened samples to that of normalized reads for each gene in the corresponding plasmid library. For screens using the two Humagne C and D libraries, the log fold change scores of libraries were averaged together. An ROC-AUC was calculated on the basis of per cent of true positive hits (essential genes with log fold change below -1) for every possible false discovery rate. An NNMD was calculated for each screen by taking the difference between the average log fold changes for essential and non-essential genes and dividing by the standard deviation of the non-essential genes⁶⁹. Screens that had an NNMD greater than -1.25 or a correlation between replicates less than 0.19 were excluded from downstream analyses. Chronos scores were then calculated using the open-source code Chronos (v.2.0.8; <https://github.com/broadinstitute/chronos>) as previously described²³ for each type of guide library (Avana spCas9, KY spCas9 or Humagne CD enAsCas12a) independently. To mitigate batch effects between guide libraries, the mean Chronos score per gene in individual libraries was regularized towards the global mean across all libraries. On the DepMap portal, the file 'ScreenGeneEffect' contains Chronos gene effect estimates for each individual screen, with library effects corrected out whereas organoid-specific effects are preserved. Beginning with the 26Q1

release, a similar strategy will be used to preserve organoid-specific effects in 'CRISPRGeneEffect', which integrates all screens of the same model into a model-indexed table that can be used with portal tools.

Comparison of gene expression profiles between spheroid and adherent cultures of the NextGen CNS models

Nine NextGen CNS models were adapted to grow as adherent cultures on Matrigel-coated plates (coat culture) for 4 weeks or more. RNA samples were collected both before and after adaptation and profiled by RNA-seq using a similar protocol as described above. For these 18 samples (nine models $\times$ two conditions (spheroid and coat)), a correlation matrix was calculated based on the expression of the top 10% highly variable genes ($n = 1,919$). Subsequently, hierarchical clustering of the correlation matrix was conducted using the `hclust` and `heatmap.2` functions in R (Extended Data Fig. 4c). To compare the expression levels of individual genes between the nine samples of spheroid culture and the nine samples of coat culture, the difference in expression levels of all the genes measured in this analysis ($n = 19,193$) were analysed using two-tailed Mann–Whitney *U*-tests (Extended Data Fig. 4f).

Comparison of gene dependency profiles between different growth formats of NextGen CNS models and organoids

For the NextGen CNS tumour models and organoids, the gene dependency profiles were compared between the two different culture conditions used for each of these model groups. Specifically, for the NextGen CNS models, the dependency scores between spheroid culture ($n = 14$) and coat culture ($n = 25$) and for organoids, and the dependency scores between Matrigel dome culture ($n = 55$) and coat culture ($n = 14$) were compared for every gene assessed in the CRISPR screen of these models ($n = 18,159$) using two-tailed Mann–Whitney *U*-tests (Extended Data Fig. 4h). For the organoids in Matrigel dome cultures, only the models from lineages that have corresponding coat-cultured models (ampulla of Vater, colorectal, oesophagus–stomach, ovary, prostate and uterus) were included in this comparison.

Classification of dependency profiles

The classification of dependency profiles (Extended Data Fig. 5a) was conducted primarily based on a published strategy²⁵ using the entire set of the NextGen ($n = 147$) and organoid ($n = 108$) models. First, we identified a negative control set of non-expressed genes per model as having a $\log_2[\text{TPM} + 1]$ expression value of less than 0.2. We then inferred the probability that the score represents a true dependency by using an expectation–maximization step until convergence independently in each screen or model. The dependent distribution was derived from the list of essential genes, which was generated from the intersection of previously published lists of essential genes^{70,71}. The null distribution was determined from non-expressed gene scores for the respective model if the expression data were available, or otherwise from the non-essential gene list inferred from a published study⁷⁰.

To identify pan-dependencies, we selected a gene rank threshold T based on the dependency rank of all the genes in their 90th percentile least depleted model. The distribution of this 90th percentile rank was bimodal, and T was defined as the rank that gives the minimum density between the two peaks. To identify high variance genes, we first selected an expression threshold by analysing the distribution of expression values across all NextGen (or organoid) models, which was bimodal, and selecting the value corresponding to the minimum density between the two modes. Genes with average expression less than this value were designated as lowly expressed. The variance threshold V was determined as the 99th percentile variance over lowly expressed genes. To identify strongly selective genes, we computed the product S of the skew and kurtosis of the distribution of gene dependencies over all NextGen (or organoid) models for each gene.

Using the scores defined above, we classified the genes in the following order:

- 1) Pan-dependency: ranked in the 90th percentile least depleting line above T .
- 2) Strongly selective dependency: $S < -0.86$, has a probability of dependency >0.5 in at least three models, and does not meet criterion (1).
- 3) High variance dependency: the variance in the dependency score is above V and does not meet criteria (1) or (2).
- 4) Weakly selective dependency: has a probability of dependency >0.5 in at least one model and does not meet criteria (1)–(3).
- 5) Non-dependency: probability of dependency <0.5 for all models and does not meet criteria (1)–(4).

Identification of biomarker-associated dependencies

To identify dependencies with expression addiction, we scored the Pearson's correlation coefficient (r) and the significance (P) of correlation between the expression level ($\log_2[\text{TPM} + 1]$) and dependency (Chronos gene effect) of the same gene across all the NextGen models that were profiled by both RNA-seq and CRISPR screening ($n = 146$) (Fig. 2a). This process was conducted for all the genes that have expression data and were classified as strong dependency (strongly selective, high variance or pan-dependency) in the NextGen model CRISPR screen dataset ($n = 3,786$). The false discovery rate (or q value) was calculated using the Benjamin–Hochberg procedure for this and subsequent analyses unless otherwise indicated. The genes that met the criteria for significance ($r < -0.3$ and $q < 0.005$) were identified as dependencies with expression addiction. For each of the strong dependency genes identified from the NextGen model data, the correlation between expression and dependencies was also computed across the profiling results of the traditional cell lines that have lineage-matched NextGen models and gene expression data ($n = 572$).

Paralogue dependencies were identified by calculating the Pearson's correlation coefficient and the significance of correlation between the dependency (Chronos gene effect) on a gene and the expression level ($\log_2[\text{TPM} + 1]$) of its paralogue partner (Fig. 2d). We conducted this process for all the strong dependency genes in the NextGen model CRISPR screen dataset ($n = 3,800$), comparing their dependency with the expression level of the respective paralogue partners listed on Ensembl genome browser 113 (<https://www.ensembl.org/>). Paralogue pairs were extracted via BioMart query for all human paralogues with valid HGNC symbols. Only gene pairs with at least 25% sequence identity between paralogues and with variance in expression >1 over all the NextGen models were considered for analyses. The dependency gene–paralogue pairs that met the significance criteria ($r < -0.3$ and $q < 0.005$) were identified as paralogue dependencies. Here again, we computed correlations for the same dependency–expression paralogue pairs in the traditional model profiling results ($n = 572$).

To find dependencies that are associated with oncogene GOF or TSG LOF, we first identified models with oncogene GOF as either harbouring a hotspot mutation or CN gain ($\log_2[\text{relative to ploidy} + 1] > 3$) of the oncogene, and TSG LOF as either having a damaging mutation or CN loss ($\log_2[\text{relative to ploidy} + 1] < 0.25$) of the TSG for all the oncogenes and TSGs listed in OncoKB¹ (<https://www.oncokb.org/>). We selected 6 oncogenes and 13 TSGs that had at least 1 model of hotspot mutation and 5 or more models of relevant alteration among the NextGen models with CRISPR screen profiles ($n = 147$). For each of these oncogenes and TSGs, we assessed whether any of the strong dependencies were significantly enriched in the NextGen models with the respective cancer gene alteration ('altered') compared with the rest of these models ('unaltered') using two-tailed Mann–Whitney *U*-tests with a threshold of $q < 0.05$ (Fig. 2f).

To test whether the observed association between the cancer gene alteration and dependency was consistently beyond the effects caused by lineage bias of the cancer gene alteration, we also computed the differences in mean dependency between NextGen screens in each lineage and the remaining NextGen screens, only considering lineages

Article

that have at least five models screened. If the dependency difference observed across the two classes of models distinguished by the presence of a cancer gene alteration was outside the range of all dependency differences observed across all lineages, then we deemed the dependency–biomarker relationship to be driven by the cancer gene alteration. Otherwise, the dependency pattern was attributed to lineage effects. The mean dependency scores for the altered and unaltered groups, as well as the significance of these mean dependency scores, were similarly calculated for all these 6 oncogenes and 13 TSGs in the traditional models ($n = 597$).

SCD inhibitor sensitivity assay in oesophageal adenocarcinoma organoids

To examine whether the increased reliance on SCD of oesophageal adenocarcinoma organoids with *KRAS* CN amplification, identified via CRISPR screens, can be reproduced with pharmacological SCD inhibition, we tested two chemical inhibitors of SCD: A939572 and CAY-10566. Specifically, we tested four oesophageal adenocarcinoma models, two with *KRAS* CN amplification (CCLFUPGI0012T and CCLFUPGI0030T) and two with neutral *KRAS* CN (CCLFUPGI0022T and HCMSANGO300C15). On day 0, for each of these four models, 10,000 cells per well were seeded into 120 wells of 96-well ULA plates (Revvity, 6055802) in a slurry culture with 100 μ l of 5% Matrigel–OPAC per well. One of the following compounds was added to the culture at the same time as seeding using a D500e Digital Dispenser (Tecan Life Sciences): A939572, CAY-10566 and bortezomib. For each of these compounds, nine different doses with serial dilutions (2 nM to 10 μ M, 2.9-fold dilution between the neighbouring doses) and DMSO control were tested in quadruplicate. On day 3, 50 μ l fresh medium containing the corresponding concentration of the compounds was added to the culture.

To measure viability of cells following compound treatment, 50 μ l 3D CTG assay reagent was added to the culture and mixed by pipetting on day 7. This was followed by an incubation of the plates at room temperature (RT) for 30 min on a digital microplate shaker (Thermo Scientific, 88882006) at 300 rpm. Subsequently, luminescence emission was measured using a CLARIOstar Plus plate reader (BMG LABTECH).

Scoring the activity of gene expression MPs

We used a published method³⁷ to score the activity of 41 different gene expression MPs in each of the tumour samples and culture models. In brief, MP scores were calculated as the average relative expression of the MP gene set minus the average relative expression of a control gene set; that is,

$$MP_{i,j} = \text{mean}(\text{expression}(G_j, i)) - \text{mean}(\text{expression}(\text{Ctrl}_j, i))$$

in which $MP_{i,j}$ indicates the score of MP_j in sample i , G_j is the gene set for MP_j and Ctrl_j is a control gene set for MP_j . To define the control gene set, all analysed genes were grouped into 25 bins on the basis of their mean expression levels. Subsequently, for every gene in the MP gene set, 100 genes were selected randomly from the same expression bin to constitute the control gene set.

MP expression across different sample types (for example, traditional versus NextGen models, 2D models versus organoids, tumours versus traditional models, tumours versus NextGen models, GBM tumours versus GBM models with specific gene expression subtype, and between different subtypes of oesophagus–stomach tumours) (Figs. 3a and 4a,b and Extended Data Figs. 6d and 7d,e) were compared using two-tailed Mann–Whitney U -tests.

NextGen GBM model dependency analyses

To analyse gene expression and associated dependencies in the NextGen GBM models, the transcriptional landscape of GBM models was visualized in a 2D UMAP using Celligner embedding (Fig. 3b). The cohort

included 55 traditional and 70 NextGen GBM models, alongside 189 tumour samples from the TCGA-plus and Met500 GBM cohort. Two clusters were identified visually on the UMAP plot on the basis of distinct groupings of data points. Differential gene expression analysis was conducted to compare the transcriptional profiles of the clusters by computing $\log_2$ fold changes to quantify expression differences and performing two-tailed Student's t -tests to assess significance for each gene. Significance was determined using adjusted P values (q values), with thresholds applied at $q < 0.001$ and $|\log_2[\text{fold change}]| > 1$ (Fig. 3c).

Differential dependency analysis was performed between the glial GBM models (screen $n = 36$) and either mesenchymal GBM models (screen $n = 60$) (Fig. 3d) or all the other non-glial NextGen models (organoids and non-glial NextGen CNS models; $n = 115$) (Extended Data Fig. 6e). Common essential genes were excluded from the analysis. Significant dependencies (effect size < -0.3 , $q < 0.05$; $P < 0.05$ cutoff was also used for Fig. 3d) were identified and visualized using a volcano plot.

To identify a CN of a gene that can serve as a biomarker to predict *CDK6* dependency, the Pearson's correlation between *CDK6* dependency and CN of genes (only those in the top 10% high variance of CN in the DepMap GBM models) was calculated and plotted across DepMap GBM models (with CRISPR and CN data; $n = 95$; Fig. 3f). The correlation between *CDK6* dependency and CN of genes was similarly calculated using only the DepMap GBM models that exhibited the glial pattern of gene expression ($n = 36$; Extended Data Fig. 6g). Associations with $|r| > 0.3$ and $q < 0.05$ were considered as significant. We also analysed the difference in *CDK6* dependency between mutant and non-mutant screens for all the hotspot or damaging mutations that have ≥ 5 mutant cases in the DepMap GBM models (5 mutations met this criterion; Extended Data Fig. 6f) again using the data from the entire DepMap GBM model cohort. None of these mutations was significantly associated with *CDK6* dependency (all $q > 0.95$ by a two-tailed Mann–Whitney U -test).

CDK6 dependency validation in patient-derived short-term cultures

We used seven patient-derived (NextGen) GBM models (BT286, BT179, BT145, BT320, BT444, BT224 and BT187) to evaluate their sensitivity to *CDK4/6* inhibition. Patient-derived models were maintained at 37 °C and 5% CO_2 . Cells were plated at 500–2,000 cells per well in 60 μ l medium in 384-well CellCarrier plates (Perkin Elmer, 6057302) and allowed to adhere for a minimum of 24 h. Cells were then treated with drugs in half-log dilution series with a D300 digital drug dispenser (Hewlett Packard). Palbociclib, ribociclib and abemaciclib (MedChem Express, HY-50767, HY-15777, HY-16297A, respectively) were identity and purity verified by LC–MS and prepared as 10 mM stocks in DMSO. Palbociclib was diluted to 5 mM before dispensing owing to solubility limitations. At the time of drug addition, a time = 0 control plate was fixed. Following 96 h or 120 h in drug, treated plates were fixed.

Plates (both time = 0 and treated plates) were stained, fixed, imaged and analysed according to the Dye Drop or Deep Dye Drop protocols⁴³. In brief, for Dye Drop, cells were stained with Hoechst 33342 (1:5,000, Thermo Fisher, 62249) and LIVE/DEAD far red fluorescent dye (LDR, 1:2,000, Thermo Fisher, L10120), prepared in a 10% solution of OptiPrep (Sigma, D1556) in PBS to displace the growth medium, for 30 min at RT. Cells were then fixed with 4% formaldehyde, prepared in 20% OptiPrep (a denser solution to displace the stain solution and media column), for 30 min at RT. Fix, stain and medium were aspirated and 80 μ l PBS was added per well. Plates were then sealed and stored at 4 °C until image acquisition. For Deep Dye Drop, cells were stained with LDR and pulsed with EdU (Lumiprobe, 30540) in a 10% solution of OptiPrep in PBS for 1 h before fixation with 4% formaldehyde, prepared in 20% OptiPrep for 30 min. Wells were aspirated and cells were then permeabilized with 0.5% Triton X-100 (Sigma, T8787) in 10% OptiPrep for 20 min at RT, and EdU was then labelled with cy3-azide (Lumiprobe, C1030) by Click chemistry in 20% OptiPrep for 30 min at RT. Following

aspiration, cells were blocked in Odyssey buffer (LI-COR Biosciences, 927-40150) for 1 h at RT, and then stained with anti-phospho histone H3 Alexa 488 (pH3, 1:2,000, Cell Signaling Technologies, 3465S) and Hoechst 33342 (1:5,000) overnight at 4 °C. Cells were washed once with PBST, twice with PBS and sealed for storage at 4 °C until image acquisition. All wash steps were performed with an EL406 washer equipped with a 96-channel head (Biotek).

Images were acquired on an Operetta (Perkin Elmer) with a $\times 10$ objective. Six fields of view were imaged per well. Nuclear segmentation was performed based on Hoechst intensity with Columbus (v.2.7.0, Perkin Elmer). A ring around the nucleus was drawn and the intensity of each marker was measured in the nuclear and ring masks. The intensity in the ring was subtracted from the nuclear intensity to correct for deviations in local background intensities. Cells were classified as live or dead on the basis of LDR signals, cells positive for EdU or pH3 were classified as S phase or M phase, respectively, and the integrated Hoechst intensity was used to measure DNA content and to assign cells doubly negative for EdU and pH3 to the G1 (DNA content = 2N) or G2 (DNA content = 4N) phases of the cell cycle. Cells negative for EdU with intermediate DNA content were assigned 'S dropout'. All gating was performed with custom Python scripts that are publicly available (<https://github.com/datarail/DrugResponse/wiki>). Live cell counts were used to calculate growth rate inhibition values⁷².

PDAC-classical/MUC program expression analyses

To assess the relationship between PDAC-classical/MUC program expression and the classical score (based on a previously defined 'classical' gene set⁴⁶) of tumour samples, the classical score was determined using the same strategy as the calculation of the MP expression score described above (in the section 'Scoring the activity of gene expression MPs'). The relationship between the purity of tumour samples and the level of PDAC-classical/MUC program expression was analysed by compiling the ABSOLUTE tumour purity score of the TCGA tumour samples of pancreas, oesophagus–stomach and colorectal lineages ($n = 149, 375$ and 448 , respectively) from previous studies^{59,73}.

The expression levels of the PDAC-classical/MUC program in various sample types (tumours, NextGen models and traditional cell lines) were also visualized by the colour of the points on the Celligner plots (Fig. 4c and Extended Data Fig. 7c) to show the similarity of the overall gene expression profiles of the models and tumours displaying high expression levels of this program across different lineages (pancreas, oesophagus–stomach and colorectal).

Dependencies associated with PDAC-classical/MUC program expression

To identify genetic dependencies associated with the level of PDAC-classical/MUC program expression, the Pearson's correlation coefficient and the significance of correlation (two-tailed) were calculated between the level of the PDAC-classical/MUC program and the dependency scores of strong dependency genes (strongly selective + high variance + pan-dependency; $n = 3,521$) identified from the organoid screen data using all the organoid models with gene expression and CRISPR screen data ($n = 108$ (all organoids), $n = 60$ (organoids grown with R-spondin 1 and WNT3A); Fig. 4d and Extended Data Fig. 7i). The enrichment of the MSigDB gene set 'GOBP WNT signalling pathway' in the dependencies associated with PDAC-classical/MUC program expression was assessed by gene set enrichment analysis, which was conducted using the open-source code GSEapy (v.1.1.5; <https://pypi.org/project/gseapy/>) (Fig. 4d).

The PDAC-classical/MUC program-associated dependencies on the selective components of the WNT signalling pathway, namely *TCF7L2*, *WLS*, *FZD5* and *MESD* (plus *LGR4* for some analyses), were investigated further as follows. The dependency on these WNT pathway components were compared between the organoid models ($n = 108$) and the traditional cell lines that have lineage-matched organoid models (screen

$n = 485$) using the density plots and two-tailed Mann–Whitney *U*-tests (Fig. 4f). To test whether dependencies on these WNT pathway components observed in organoids (with high levels of PDAC-classical/MUC program expression) is driven by specific mutations, 14 hotspot mutations and 573 damaging mutations that have 2 or more mutant models in the cohort of CRISPR-screened organoids ($n = 108$) were selected. For each of these 587 mutation types, dependencies on the 5 WNT pathway components listed above were compared between the mutant and non-mutant models (Extended Data Fig. 7k). For mutations and CN alterations that result in the activation of the WNT pathway, namely, *APC* damaging mutation, *CTNNB1* hotspot mutation, *CTNNB1* CN amplification and *RNF43* damaging mutation, dependencies on *TCF7L2*, *WLS*, *FZD5* and *MESD* were also compared between the organoids that have at least one of these alterations ($n = 30$) and those that do not have any of them ($n = 78$) (Extended Data Fig. 8a,b).

Cancer gene dependencies in 3D organoids versus 2D models

To compare dependencies on cancer genes (that is, oncogenes and TSGs) between the 2D models (screen results from traditional adherent models that have lineage-matched 3D organoids; $n = 433$) and organoids ($n = 94$), 90 cancer genes (40 oncogenes and 50 TSGs) with frequent (>1%) mutation rates in the TCGA cohort were selected based on a previous publication⁵³. *CDKN2B* was not included as none of the organoids has a CRISPR screen result for this TSG. The dependencies on each of these cancer genes were compared between the 2D models and 3D organoids. The significance of the difference was assessed using two-tailed Mann–Whitney *U*-tests. For the cancer genes for which mutation (hotspot mutation for the oncogenes and damaging mutation for the TSGs) is annotated in DepMap, the same analysis was conducted after removing the mutant models to see whether the differing dependencies between the 2D models and 3D organoids is attributable to the differing rates of mutation between these two model types (Extended Data Fig. 8d).

To assess whether 2D models and 3D organoids exhibit difference in dependencies on a group of oncogenes or TSGs in the same signalling pathway, each of the 89 cancer genes frequently mutated in TCGA tumours were classified into one of the ten oncogenic signalling pathways based on the function of the protein they encode⁵³. For the groups of oncogenes and the groups of TSGs that belong to the same pathway (13 groups), mean dependency scores for the genes in the same group were calculated for each model. Subsequently, the mean dependency scores of the 2D models and 3D organoids were compared using two-tailed Mann–Whitney *U*-tests (Extended Data Fig. 8f).

Differential dependency analysis between 3D organoids and 2D models

To identify functional classes that are enriched for genes exhibiting differing degrees of dependency between 2D models (screen results from traditional adherent models that are lineage-matched with 3D organoids; $n = 433$) and 3D organoids ($n = 94$), we ranked all genes ($n = 16,691$; excluding common essential genes) according to their mean difference in dependency score between organoid models and lineage-matched adherent models. The genes with the 100 most extreme differences in each direction were combined into one set. Gene sets from the Hallmark and KEGG legacy collections, which contain 101–249 member genes that were ranked in this analysis, were tested for enrichment among this set of 200 genes using a hypergeometric test (or one-sided Fisher's exact test). The gene sets that achieved significance at $q < 0.05$ were compared for similarity using the overlap coefficient:

$$\text{overlap}(A, B) = \frac{|A \cap B|}{\min(A, B)}$$

Gene sets with pairwise overlap similarity >0.3 were identified as belonging to the same functional cluster (Extended Data Fig. 8h).

Article

Clusters were successively combined using their original component gene sets until all clusters contain no gene sets having similarity >0.3 with any gene sets belonging to a different cluster.

Lentiviral production for the minipool screen and its validation

Lentiviral production was conducted using 293FT cells (Thermo Fisher, R70007) in accordance with the 'Lentiviral Production in Flasks' protocol available at the Broad Institute GPP web portal (<https://portals.broadinstitute.org/gpp/public/resources/protocols>). In brief, the lentiviral particles were generated by the co-transfecting the lentiviral plasmid with a packaging plasmid (psPAX2; 12260, Addgene) and VSV-G envelope (pMD2.G; Addgene, 12259) into 293FT cells using PEIpro transfection reagent (Polyplus, 101000033). The medium was replaced 12 h after transfection, and the virus-containing medium was collected after 36–48 h.

Library preparation for minipool screens

For the minipool screens, to evaluate the effects of growth formats and culture media on gene essentiality, we decided to select test genes from nine different functional classes. Most of these gene classes are related to the regulation of adhesion–cytoskeleton, lipid metabolism or other processes highlighted in the systematic comparison of genome-wide CRISPR screen data between 2D models and 3D organoids (Fig. 5a,b). The following gene classes were included: integrin (genes encoding an integrin α or β subunit); ECM adhesion (adhesome, <https://adhesome.org/>); actin regulation (MSigDB gene set and KEGG regulation of actin cytoskeleton); adherens junction (MSigDB gene set and KEGG adherens junction); tight junction (MSigDB gene set and KEGG tight junction); lipid metabolism (a union of two MSigDB gene sets, Hallmark cholesterol homeostasis and Reactome metabolism of lipids); WNT signalling (MSigDB gene set and GOBP WNT signalling pathway); PI3K–mTOR signalling (MSigDB gene set and Hallmark PI3K–mTOR signalling); cell cycle (MSigDB gene set and GOBP cell cycle); and other genes of particular interest (manual curation). To select 250 test genes from these 9 core functional gene classes, we used the dependency profiles of the gene between 2D models and 3D organoids to prioritize genes that exhibited selective enrichment of dependency in either of these model types. Many of these test genes belong to multiple different classes, and the patterns of membership of individual genes across the nine classes and the number of test genes that exhibit the specific pattern are presented in Extended Data Fig. 9a. We then added 650 non-dependency genes and 100 pan-dependency genes, genes that are universally essential across 2D models and 3D organoids, to compile a list of 1,000 genes (Supplementary Table 3). We subsequently designed 4 enAsCas12a sgRNA sequences for each of these 1,000 genes using the CRISPick web tool (<https://portals.broadinstitute.org/gppx/crispick/public>), which were assembled into 2 constructs (2 sgRNA sequences for the same gene per construct; 2 constructs per gene) of the pRDA_052 backbone (Addgene, 136474). The resulting library with 2,000 constructs was used for minipool screening.

Minipool screening

Before screening, models were tested for their growth in an alternative medium (1:1 OPAC organoid medium for 2D models and RPMI with 10% FBS for 3D organoids) and with alternative growth format (3D Matrigel dome growth for 2D models and 2D monolayer growth for 3D organoids) for at least two passages. Only the combination of medium type and growth format that enabled a comparable rate of growth with the original conditions (<2-fold difference in the doubling times) and reasonably rapid growth (doubling time <120 h), as well as the original conditions of growth format–culture medium pair, were used for the screening. Moreover, all the models selected for screening were subjected to titrations of library virus and puromycin to determine the dose of virus to achieve optimal infection rates and the concentration of puromycin to kill uninfected cells within 3 days, respectively.

For the screen, selected models were infected with the amount of library virus required for infecting 30% of the cells in accordance with the result of virus titration on day 0. The infected cells were selected with the concentration of puromycin determined in the puromycin titration between day 1 and day 4. On day 4, cells were seeded into up to 4 conditions (2D monolayer culture on plastic plates (plastic culture) with RPMI + 10% FBS (RPMI–FBS); plastic culture with 1:1 OPAC; 3D Matrigel dome culture (dome culture) with RPMI + 10% FBS; and dome culture with 1:1 OPAC), depending on how many conditions survived the above-mentioned selection criteria for the respective model. For plastic culture, 2 million cells were resuspended with 10 ml of the respective medium and seeded on a Falcon 100 mm TC-treated cell culture dish (Corning, 53003). For dome culture, 2 million cells were resuspended with 0.5 ml of the medium used for the condition and subsequently mixed with 1.5 ml of Matrigel. Approximately 40 domes, each with 50 μ l of Matrigel–cell suspension were placed on a 100 mm TC-treated culture dish, which was followed by the incubation of the plate at 37 °C for 30 min to allow the Matrigel to solidify. Subsequently, 10 ml of the culture medium was added before further incubation. We prepared two technical replicates per each condition.

These cultures were maintained until day 14 with refreshment of the medium every 3 days. If the culture showed signs of confluency (coverage of more than 80% of the bottom surface by the cells for monolayer culture on plastic plates and appearance of a dark spot in the middle of the domes for Matrigel dome culture), we passaged the cells instead of replacing the medium while keeping the number of cells after passaging to 2 million cells per dish. Trypsinization (with 0.25% trypsin and 0.1% EDTA (Corning, 25-053-CI)) was used for the passaging of cells in plastic cultures, whereas cells in dome cultures were passaged in accordance with the strategy described above in the section 'Source and culture conditions of NextGen cancer models'. At day 14, cells were collected by trypsinization and counted. gDNA samples were prepared from these cells and the abundance of individual sgRNA sequences in the genome of these cells were analysed by PCR amplification of the sgRNA sequences, preparation of sequencing libraries from the PCR products and subsequent sequencing of these libraries as described in the section 'Genome-wide CRISPR screening for gDNA isolation and sequencing'.

Minipool screen analysis

Minipool screens were evaluated for sequencing quality by having a total read count of at least 500,000 reads across replicates. Gene scores and NNMDs were computed in the same manner as the genome-wide screens. Replicate correlation was computed as the Pearson's correlation coefficient over log fold changes in all non-control genes. Screens with NNMDs greater than –1.5 and replicate correlations less than 0.2 were excluded from downstream analyses. Screens with a single replicate were retained if their other metrics passed these quality control thresholds. Chronos scores were calculated as previously described²³, with adjusted hyperparameters (smoothing regularization parameter = 0.3, kernel width = 5) for the smaller library size. Subsequently, we modelled the Chronos score $Y_{g,k}$ for each gene g and screen k with the generalized linear regression model:

$$Y_{g,k} = \beta_0 + \beta_d I_d + \beta_s I_s + \beta_{ds} I_d I_s + \sum_{m \in M} \beta_m I_m$$

The first term captures the baseline viability effect of knocking out gene g . The second and third terms capture the growth format effect and medium effect, where I_d and I_s are indicator variables representing the types of growth format (Matrigel dome culture (dome) versus monolayer culture on plastic plates (plastic)) and culture medium (serum-free OPAC medium versus serum-containing RPMI–FBS medium), respectively. The fourth term captures interaction effects between these two conditions, and the final term captures model effects to account for multiple screens in the same cell line. Differences in

effect sizes across growth format and serum status were extracted as β_d and β_s , respectively. Significance values corresponding to the same coefficients were extracted from the P values calculated using two-sided linear regression t -test.

To assess the effects of growth format (or culture medium) on the core functional classes of gene targets included in our minipool library, we calculated the variance in β_d (or β_s) across members of the same class and compared it to the variance in β_d (or β_s) across all non-control genes outside of class. For each gene set, we tested whether the in-class variance was significantly greater than the out-of-class variance using one-tailed F -tests.

Single-gene validation viability assay

The viability effect (10 days after sgRNA transduction) of CRISPR-mediated knockout of *ITGB1*, *ITGAV*, *MSMO1* and *SQLE* (Fig. 5h and Extended Data Fig. 10a) was assessed using CTG cell viability assays. Specifically, KP3 and HS766T pancreatic cancer cells, both of which were engineered to express enAsCas12a, were infected with several different types of sgRNA-expressing lentivirus on day 0, which included CRISPR cutting controls (sgCh2 and sgAAVS1), sgRNAs targeting common essential genes (sgPOLR2D, sgSF3B1 and sgKIF11) and sgRNAs against the experimental genes (sgITGB1, sgITGAV, sgMSMO1 and sgSQLE). The infected cells were selected with $2 \mu\text{g ml}^{-1}$ puromycin between day 1 and day 3. On day 3, cells were trypsinized, counted and reseeded without puromycin. For the 2D monolayer culture on a plastic plate (or plastic culture), cells were seeded at 2,000 cells per well in 100 μl RPMI medium with 10% FBS onto an opaque 96-well plate (Corning, 3903). For the 3D Matrigel dome culture (or dome culture), 10,000 cells were resuspended with 50 μl Matrigel and plated as a dome onto a 12-well plate, which was subsequently incubated (Corning, 356231) at 37 °C for 30 min to allow the Matrigel to solidify. The Matrigel dome was then covered with 1,200 μl per well of either the RPMI medium with 10% FBS or the 1:1 OPAC medium before further incubation. The medium was replaced every 3 days until day 10. Puromycin ($2 \mu\text{g ml}^{-1}$) was added back to the culture at the first medium replacement.

On day 10, the medium of the plastic-cultured cells was replaced with 100 μl per well of fresh RPMI with 10% FBS, and 40 μl per well of CTG assay reagent was subsequently added. Following incubation at RT for 30 min with constant shaking, luminescence emission was measured using a SpectraMax M5 Multi-Mode microplate reader (Molecular Devices, M5). For the dome-cultured cells, the medium was replaced with 350 μl per well of fresh RPMI with 10% FBS and 350 μl per well of 3D CTG assay reagent was subsequently added. Following incubation at RT for 30 min with constant shaking, the dissolution of Matrigel was confirmed by microscopy. Next, 150 μl of the lysate was transferred to an opaque 96-well plate to measure luminescence emission via a microplate reader. The luminescence signal from each of the experimental wells was normalized using a scale in which the average value of the cutting control wells (six wells; triplicate wells for each of sgCh2 and sgAAVS1) was scored as 0 and the average value for the common essential control wells (nine wells; triplicate wells for each of sgPOLR2D, sgSF3B1 and sgKIF11) was scored as -1. The normalized viability score for each of the experimental wells was plotted. The relative viability scores before normalization were also plotted (Extended Data Fig. 10a). The experiments were repeated twice. Each of these experiments was conducted with technical replicates ($n = 3$).

Immunofluorescence and flow cytometry of single-gene validation

For immunostaining of cells grown in either plastic culture or the dome culture conditions, cells were propagated on a Nunc 8-Well Lab-Tek chamber slide (Thermo Fisher, 177402). For plastic culture, the slide was first incubated with 100 $\mu\text{g ml}^{-1}$ collagen I (Corning, 354236) and 20 $\mu\text{g ml}^{-1}$ laminin (SouthernBiotech, 1415-01) for 60 min at 37 °C. Subsequently, 20,000 cells were seeded with 200 μl RPMI

with 10% FBS per well. For dome culture, 30,000 cells were resuspended with 30 μl Matrigel and placed as a dome at the centre of a well of an 8-well chamber slide. After incubating at 37 °C for 30 min to solidify Matrigel, 200 μl RPMI with 10% FBS was added to the well before further incubation. Fixation and staining were performed essentially as previously described⁵⁷. In brief, after 3–5 days of culture, cells were washed twice with PBS before fixation. Plastic-cultured cells were fixed with 2% paraformaldehyde in PBS for 20 min at RT. Dome-cultured cells were fixed with 2% paraformaldehyde and 0.2% glutaraldehyde in PBS for 20 min at RT, washed twice with PBS and incubated with 0.2% sodium borohydride for 30 min at 4 °C for the quenching of glutaraldehyde. For both plastic and dome cultures, this fixation procedure was followed by two washes with PBS and two rounds of incubations with 0.1 M glycine in PBS at RT for 10 min for the quenching of paraformaldehyde. After permeabilizing the cells with 0.5% Triton X-100 in PBS for 5 min on ice, the slides were incubated with IF buffer (0.1% BSA, 0.2% Triton-X, 0.05% Tween-20 and 0.05% NaN_3 in PBS + 10% normal goat serum (Jackson ImmunoResearch, 005-000-121)) for 1 h at RT for blocking. For dome culture, slides were additionally incubated with M.O.M. blocking reagent (Vector Laboratories, MKB-2213-1) for 1 h at RT to reduce endogenous mouse IgG staining of Matrigel. Subsequently, the slides were incubated with primary antibody diluted in IF buffer overnight at RT. After washing with TBS-T (0.05% Tween-20 in TBS) 3 times for 10 min each at RT, slides were incubated with secondary antibody diluted in IF buffer for 1 h at RT. This was followed by washing with PBS-T 4 times for 10 min each at RT and then the slides were incubated with PBS containing 1 $\mu\text{g ml}^{-1}$ 4'-diamidino-2-phenylindole (DAPI; MilliporeSigma, D9452) and Phalloidin-iFluor 647 conjugate (Cayman Chemical, 20555; 1:1,000 dilution) for 30 min at RT for nuclear counterstaining and enhancement of F-actin staining, respectively. For post-fixation, slides were incubated with 1% paraformaldehyde in PBS for 5 min at RT, washed with PBS and incubated with 0.1 M glycine in PBS at RT for 5 min. Finally, cover glasses (no. 1.5) were mounted on the slides with ProLong Gold Antifade mountant (Thermo Fisher, P36930). The following primary and secondary antibodies were used for the staining: anti-FAK (MilliporeSigma, 05-537 (4.47, mouse); 1:200 dilution); anti-FAK (MilliporeSigma, 06-543 (rabbit); 1:200); anti-integrin $\beta 1$ (BD Pharmingen, 556048 (HUTS-21); 1:50); anti-integrin αV (MilliporeSigma, AB1930; 1:500); goat anti-mouse IgG secondary antibody Alexa Fluor 488 (Thermo Fisher, A-11001; 1:500); goat anti-rabbit IgG secondary antibody Alexa Fluor 488 (Thermo Fisher, A-11008; 1:500); goat anti-mouse IgG secondary antibody Alexa Fluor 568 (Thermo Fisher, A-11004; 1:500); and goat anti-rabbit IgG secondary antibody, Alexa Fluor 568 (Thermo Fisher, A-11011; 1:500).

Images were acquired using confocal microscopy, which was performed on a Zeiss LSM 710 laser scanning confocal system equipped with Axio Observer (Carl Zeiss). A $\times 63$ objective lens was used for all imaging (Fig. 5i and Extended Data Fig. 10e). Z-stack images were acquired with a step size of 0.36 μm (for images acquired with $\times 25$, $\times 40$ and $\times 63$ objectives). Images represent maximum intensity projections of five consecutive focal planes. Images were processed using Photoshop (Adobe) for contrast and brightness adjustment, which were applied equally to all the samples from the same experiment.

To quantify colocalization between integrin chains (integrin $\beta 1$ and integrin αV) and FAK in KP3 cells grown in either plastic or dome cultures, images of integrin staining and FAK staining from the same region were loaded onto Fiji (ImageJ v.1.54p). Subsequently, the regions of interest were manually defined by drawing boundaries around single cells and the intensity correlation quotient (Li's ICQ)⁷⁴ of the two stainings in the region of interest was calculated using the Coloc2 plugin (v.3.1.0) for Fiji. Approximately 20 single cells were analysed per sample (Extended Data Fig. 10b).

The cell surface expression levels of integrin $\beta 1$ and integrin αV in cells grown in either plastic or dome conditions were analysed by flow

cytometry. For this, KP3 cells were grown in either plastic or dome conditions in RPMI with 10% FBS for 5 days. For collection, cells were first washed with PBS twice. Plastic-cultured cells were subsequently detached from the plate by incubating with 5 mM EDTA–PBS for 10 min at 37 °C and collected. Dome-cultured cells were collected by scraping and centrifugation. The pellets containing cells and Matrigel were resuspended in 5 mM EDTA–PBS and incubated at RT for 30 min with continuous rotation to dissolve Matrigel. Subsequently, cells were fixed with 2% paraformaldehyde in PBS for 20 min at RT, washed twice with PBS and incubated with 0.1 M glycine in PBS at RT for 10 min twice for the quenching of paraformaldehyde. The cells were then incubated with 10% normal goat serum–PBS for 1 h at RT for blocking. For primary antibody staining, 1×10^6 cells were incubated at RT for 1 h with one of the following antibodies, all diluted in 10% normal goat serum–PBS: anti-integrin $\beta 1$ (Developmental Studies Hybridoma Bank (DSHB), A1B2; 1:40 dilution); anti-integrin αV (DSHB, P3G8; 1:58); rat IgG1k isotype control (Thermo Fisher, 14-4301-82; 1:1,000); or mouse IgG1k isotype control (Thermo Fisher, 14-4714-82; 1:500). This was followed by three washes with PBS for 10 min each at RT with continuous rotation and secondary antibody staining, during which cells were incubated at RT for 1 h with one of the following antibodies, all diluted in 10% normal goat serum–PBS: goat anti-rat IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11006; 1:500); or goat anti-mouse IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11001; 1:500). The cells were then washed with PBS three times for 10 min each at RT with continuous rotation and analysed by flow cytometry, which was conducted on a CytoFLEX S flow cytometer (Beckman Coulter) (Extended Data Fig. 10c,d).

Statistical analysis

Statistical analyses were conducted as follows. For Fig. 2a, Pearson's correlation between dependency and expression of the same gene across the NextGen models (gene $n = 3,786$ (strong dependency), model $n = 146$) was calculated for testing the degree and significance of the expression addition relationship. Genes that met $r < -0.3$ and $q < 0.005$ (two-tailed) were considered significant. The false discovery rate (q values) was determined using the Benjamini–Hochberg procedure here and throughout the rest of the study.

For Fig. 2b, the enrichment of oncogenes and transcription factors in the group of strong dependency genes that were classified as significant expression addition ($n = 51$) relative to the rest of strong dependency genes ($n = 3,735$) was tested by one-sided Fisher's exact test (hypergeometric test).

For Fig. 2d, Pearson's correlation between the (strong) gene dependency and expression of its paralogue gene across the NextGen models (paralogue pair $n = 3,787$, model $n = 146$) was calculated for testing the degree and significance of the paralogue dependency relationship. Paralogue pairs that satisfied $r > 0.3$ and $q < 0.005$ (two-tailed) were considered significant.

For Fig. 2f, the significance of difference in the gene dependency scores between the group of NextGen models that have a specific oncogene or TSG mutation (altered) and the rest of the NextGen models (unaltered) was calculated using two-tailed Mann–Whitney U -test (model $n = 147$). Oncogene hotspot mutations (and/or amplification) and TSG damaging mutations (and/or deletion) with sufficient representation in the NextGen models ($n \geq 5$ and at least one model with a hotspot mutation) were included in the analysis (oncogene $n = 6$; TSG $n = 13$).

For Fig. 2g, the significance of difference in *KRAS* dependency and *SCD* dependency among the following groups of screens was assessed by two-tailed Mann–Whitney U -test: NextGen unaltered (without *KRAS* GOF), $n = 105$; NextGen altered (with *KRAS* GOF), $n = 42$; traditional unaltered, $n = 428$; traditional altered, $n = 169$.

For Fig. 2h, Pearson's correlation between *KRAS* CN and *SCD* dependency in the cohort of NextGen models ($n = 147$) was calculated.

For Fig. 2i, the significance of difference in *SCD* inhibitor (A939572 and CAY-10566) sensitivity between oesophageal adenocarcinoma organoid models with (CCLFUPGI0012T and CCLFUPGI0030T) and without (CCLFUPGI0022T and HCMSANG0300C15) *KRAS* CN amplification was determined by two-tailed two-way ANOVA. Relative cell viability corresponding to the same dose of inhibitor, measured in quadruplicate in each of the models, was compared between the *KRAS* CN-neutral and *KRAS* CN-amplified groups.

For Fig. 3a, the expression scores for each of the previously identified 41 gene expression MPs³⁷ were compared between the following groups using two-tailed Mann–Whitney U -test: NextGen CNS models ($n = 76$) versus traditional CNS models ($n = 97$); all organoids ($n = 229$) versus lineage-matched traditional models ($n = 451$); oesophagus–stomach organoids ($n = 86$) versus oesophagus–stomach traditional models ($n = 78$); pancreas organoids ($n = 44$) versus pancreas traditional models ($n = 54$); colorectal organoids ($n = 34$) versus colorectal traditional models ($n = 86$); breast organoids ($n = 28$) versus breast traditional models ($n = 70$); ovary organoids ($n = 13$) versus ovary traditional models ($n = 66$); and prostate organoids ($n = 12$) versus prostate traditional models ($n = 7$).

For Fig. 3c, expression levels of individual genes were compared between glial ($n = 67$) and mesenchymal ($n = 54$) GBM models using two-tailed Student's t -test. Genes that met $|\log_2[\text{fold change}]| > 1$ and $q < 0.001$ were considered as significantly differentially expressed genes (gene $n = 18,965$).

For Fig. 3d, enrichment of gene dependency in the glial subgroup of GBM models (screen $n = 36$) relative to the mesenchymal subgroup (screen $n = 60$) was analysed by two-tailed Mann–Whitney U -test. All the genes evaluated in the genome-wide CRISPR screens, except for common essential genes, were included in the analysis (gene $n = 16,659$). Gene dependencies that met Δ dependency (glial – mesenchymal) < -0.3 and $P < 0.05$ were considered as significantly enriched in the glial subgroup.

For Fig. 3e, *CDK6* dependency among the following groups of models were compared by two-tailed Mann–Whitney U -tests: glial GBM models (screen $n = 36$); mesenchymal GBM models (screen $n = 60$); and NextGen models other than glial GBM (screen $n = 115$).

For Fig. 3f, Pearson's correlation between CN of the genes (genes with top 10% highest variance of CN in the GBM models with CRISPR screens; gene $n = 1,914$, screen $n = 95$) and *CDK6* dependency was calculated. CN of genes that met $|r| > -0.3$ and $q < 0.005$ (two-tailed) were considered significantly correlated with *CDK6* dependency.

For Fig. 3g, *CDK6* dependency among the following groups of models were compared by two-tailed Mann–Whitney U -tests: glial GBM models with *CDKN2A* CN loss ($n = 25$); glial GBM models with *CDKN2A* CN neutral (screen $n = 11$); mesenchymal GBM models with *CDKN2A* CN loss (screen $n = 44$); and mesenchymal GBM models with *CDKN2A* CN neutral (screen $n = 11$).

For Fig. 3h, the mean GR_{AOC} values⁷² were compared between *CDKN2A* CN neutral (BT187 and BT224) and *CDKN2A* CN loss (BT145, BT179, BT286, BT320 and BT444) GBM models for each of the three *CDK4/6* inhibitors (abemaciclib, palbociclib and ribociclib) using two-tailed Student's t -tests.

For Fig. 4a, the expression scores for each of the previously identified 41 gene expression MPs³⁷ were compared between all organoids ($n = 229$) and lineage-matched traditional models ($n = 451$) using two-tailed Mann–Whitney U -tests.

For Fig. 4b, the expression scores of the PDAC-classical/MUC program were compared among the following groups of samples using two-tailed Mann–Whitney U -tests: pancreas TCGA-plus tumour ($n = 180$); pancreas NextGen models ($n = 44$); pancreas traditional models ($n = 54$); oesophagus–stomach TCGA-plus tumours ($n = 602$); oesophagus–stomach NextGen models ($n = 86$); oesophagus–stomach traditional models ($n = 78$); colorectal TCGA-plus tumours ($n = 384$); colorectal NextGen models ($n = 34$); colorectal traditional models

($n = 86$); prostate TCGA-plus tumours ($n = 496$); prostate NextGen models ($n = 12$); prostate traditional models ($n = 7$); breast TCGA-plus tumours ($n = 1,099$); breast NextGen models ($n = 28$); breast traditional models ($n = 70$); ovary TCGA-plus tumours ($n = 427$); ovary NextGen models ($n = 13$); ovary traditional models ($n = 66$); all organoid lineages TCGA-plus tumours ($n = 3,463$); all organoid lineages NextGen models ($n = 229$); and all organoid lineages traditional models ($n = 451$).

For Fig. 4d, Pearson's correlation between the gene dependency and expression score of the PDAC-classical/MUC program across the organoid models (gene $n = 3,521$ (strong dependency in organoids), model $n = 108$) was calculated for identifying dependencies associated with the expression of this program. Gene dependencies that satisfied $q < 0.05$ (two-tailed) and $r < -0.3$ were considered as dependencies significantly associated with PDAC-classical/MUC program expression.

For Fig. 4f, dependencies on WNT signalling regulators (*MESD*, *WLS*, *TCF7L2* and *FZD5*) were compared between the NextGen models ($n = 108$) and lineage-matched traditional models (screen $n = 485$) using two-tailed Mann-Whitney *U*-tests.

For Fig. 5a, the top 100 genes in either extreme of the distribution of mean dependency differences (3D organoids ($n = 94$) versus lineage-matched 2D models ($n = 433$)) were combined to form a set of 200 genes with differential dependencies. Gene sets from the Hallmark and KEGG legacy collections were tested for enrichment among this set of differential dependency genes using hypergeometric test (or one-sided Fisher's exact test). The gene sets that achieved significance at $q < 0.05$ were considered as significantly overrepresented in differential dependency genes. These gene sets were tested for the mutual overlap of their constituent genes. Gene sets exhibiting high-degrees of overlap (overlap coefficient > 0.3) were merged with each other (Extended Data Fig. 8h).

For Fig. 5b, the dependency scores for each of the strong dependency genes in organoids ($n = 3,521$) were compared between the 3D organoids ($n = 94$) and lineage-matched 2D traditional models ($n = 433$) using two-tailed Mann-Whitney *U*-tests.

For Fig. 5d,e, in the minipool screen analysis, the effects of growth format (dome – plastic) and culture medium (OPAC – RPMI – FBS) on gene dependency (Chronos score) were determined as coefficients in a linear regression model (see Methods, 'Minipool screen analysis'), which was also used for calculating the *P* values against the null hypothesis that these variables have no effect on gene dependency (two-sided linear regression *t*-test). The number of screens for each condition is as follows: dome format, $n = 11$; plastic format, $n = 7$; OPAC medium, $n = 9$; RPMI – FBS medium, $n = 9$.

For Fig. 5g, the variances of dependency between different growth formats and different culture media were scored for all nine of the core functional classes of minipool target genes. The variances were compared between in-class genes and out-of-class genes (that is, rest of the 250 test genes in the minipool library) for each of the 9 core functional classes using one-tailed *F*-tests. The functional class is considered to be significantly affected by the growth format or culture medium if the corresponding *q* value was less than 0.05. The numbers of genes in each of the 9 core functional classes are as follows: actin regulation, $n = 75$; adherens junction, $n = 30$; cell cycle, $n = 53$; ECM adhesion, $n = 89$; integrin, $n = 22$; lipid metabolism, $n = 20$; PI3K – mTOR signalling, $n = 20$; tight junction, $n = 34$; WNT signalling, $n = 29$.

For Fig. 5h, the effects of growth formats on the viability of KP3 and HS766T cells following integrin (*ITGB1* and *ITGAV*) knockout, as well as the effects of culture media on the viability of these cells following the knockout of cholesterol synthesis mediators (*MSMO1* and *SQLE*), were analysed by two-tailed two-way ANOVA. The viability of the KP3 and HS766T cells following gene knockout was measured as triplicates.

For Extended Data Fig. 2a, the accuracies of Celligner-based model lineage prediction were compared between the 'DepMap: Traditional (all)' group and other sample groups, including 'DepMap: NextGen', 'DepMap: Traditional (out of undifferentiated cluster)', and 'Novartis:

PDX', using two-sided Fisher's exact test. The number of samples in each sample group – lineage category pair is as follows: DepMap: NextGen, $n = 300, 76, 83, 44, 34, 27, 12$ and 13 ; DepMap: Traditional (all), $n = 532, 81, 78, 54, 86, 70, 7$ and 66 ; DepMap: Traditional (out of undifferentiated cluster), $n = 400, 12, 67, 48, 84, 63, 7$ and 48 ; Novartis: PDX, $n = 266, 7, 19, 59, 70, 55, 0$ and 34 for 10 lineages, CNS, oesophagus – stomach, pancreas, colorectal, breast, prostate and ovary, respectively.

For Extended Data Fig. 2c, the proportions of samples in the undifferentiated cluster (see the section 'Lineage prediction with Celligner' in the Methods) were compared between the 'DepMap: Traditional' group and other sample groups, including 'DepMap: NextGen', 'TCGA-plus and Met500: Tumour', and 'Novartis: PDX', using two-sided Fisher's exact tests. The number of samples in each sample group – lineage category pair is as follows (note that certain models have multiple profiles and Celligner coordinates were calculated separately for them): DepMap: NextGen, $n = 375, 101, 126, 49, 34, 28, 13$ and 13 ; DepMap: Traditional, $n = 542, 86, 78, 59, 86, 70, 7$ and 66 ; TCGA-plus and Met500: Tumour, $n = 5185, 1244, 639, 202, 406, 1,254, 651$ and 453 ; Novartis: PDX, $n = 266, 7, 19, 59, 70, 55, 0$ and 34 for 10 lineages, CNS, oesophagus – stomach, pancreas, colorectal, breast, prostate and ovary, respectively.

For Extended Data Fig. 4d, Pearson's correlation of gene expression (among top 10% high variance genes ($n = 1,919$)) between 2 different samples of the gene expression analysis of 9 NextGen CNS samples each propagated in 2 different culture conditions (spheroid and coat) was calculated. The distributions of Pearson's correlation coefficient among the following groups of sample pairs were compared using two-tailed Mann-Whitney *U*-tests: same model under coat and spheroid culture conditions ($n = 9$); different models under different culture conditions ($n = 72$); different models both in coat cultures ($n = 36$); different models both in spheroid cultures ($n = 36$).

For Extended Data Fig. 4e, Pearson's correlation of the mean expression levels of individual genes ($n = 19,193$) between coat ($n = 9$) and spheroid ($n = 9$) cultures of the NextGen CNS models was calculated.

For Extended Data Fig. 4f, the mean expression levels of individual genes in the spheroid ($n = 9$) versus coat ($n = 9$) cultures were compared for all the genes ($n = 19,193$) measured in RNA-seq by two-tailed Mann-Whitney *U*-tests.

For Extended Data Fig. 4g, Pearson's correlation of the mean dependency scores of individual genes ($n = 18,159$) between coat ($n = 25$) and spheroid ($n = 14$) cultures of the NextGen CNS modes as well as coat ($n = 14$) and dome ($n = 55$) cultures of the NextGen organoid models was calculated.

For Extended Data Fig. 4h, the mean dependency scores of individual genes in the spheroid ($n = 14$) versus coat ($n = 25$) cultures of the NextGen CNS modes as well as dome ($n = 55$) versus coat ($n = 14$) cultures of the NextGen organoid models were compared for all the genes assessed by the CRISPR screen ($n = 18,159$) using two-tailed Mann-Whitney *U*-tests.

For Extended Data Fig. 5c, the expression levels of *HNRNP1*, *PROX1* and *ENO2* were analysed between the normal and tumour samples of the combined TCGA, TARGET and GTEx cohort (across 9 tissue types; $n = 3,192$ (normal), $4,143$ (tumour)) downloaded from the Xena browser (<https://xenabrowser.net/>) using two-tailed Mann-Whitney *U*-tests. The numbers of normal and tumour samples in each of the lineages shown in the pilots are as follows: biliary tract, normal $n = 9$; biliary tract, tumour $n = 36$; CNS – brain, normal $n = 1,151$; CNS – brain, tumour $n = 689$; colorectal, normal $n = 355$; colorectal, tumour $n = 383$; prostate, normal $n = 152$; and prostate, tumour $n = 496$.

For Extended Data Fig. 6d, the expression scores of the five neural-lineage-specific MPs (astrocytes, NPC (glioma), NPC and OPC, oligo progenitor and oligo normal) were compared among the three groups of samples, GBM tumours ($n = 180$), glial GBM models ($n = 67$) and mesenchymal GBM models ($n = 54$), using two-tailed Mann-Whitney *U*-tests.

For Extended Data Fig. 6e, enrichment of gene dependency in the glial subgroup of GBM models (screen $n = 36$) relative to the non-glial GBM NextGen models (Other NextGen; $n = 115$) were analysed by two-tailed Mann–Whitney U -tests. All the genes evaluated in the genome-wide CRISPR screens, except for common essential genes, were included in the analysis (gene $n = 16,659$). Gene dependencies that met Δ dependency (glial – other NextGen) < -0.3 and $q < 0.05$ were considered as significantly enriched in the glial subgroup of GBM.

For Extended Data Fig. 6f, the significance of difference in *CDK6* dependency between the GBM models that have a specific gene mutation (altered) and the rest of the GBM models (unaltered) was calculated using two-tailed Mann–Whitney U -tests (screen $n = 95$). Hotspot or damaging mutations with sufficient representation in the GBM models ($n \geq 5$) were included in the analysis.

For Extended Data Fig. 6g, Pearson's correlation between CN of the genes (genes with top 10% highest variance of CN in the glial GBM models with CRISPR screens; gene $n = 1,914$, screen $n = 36$) and *CDK6* dependency across the glial GBM models was calculated. The significance of correlation was scored by two-tailed Pearson's correlation.

For Extended Data Fig. 6h,i, the effects of *CDK6* or *CDK4* knockout on the viability of two glial GBM models with *CDKN2A* CN loss (BT145 and BT1718) were analysed by two-tailed Student's t -test. The viability of these GBM models following gene knockout was measured as triplicates.

For Extended Data Fig. 7a, overrepresentation of individual terms in the description of genes that constitute the PDAC-classical MP (MP30)³⁷ was evaluated by a hypergeometric test (one-sided Fisher's exact test) as a part of the GeneTEA algorithm⁴⁷. The effect size shows the sum of term frequency–inverse document frequency values per term across the constituent genes (gene $n = 50$) of the PDAC-classical meta-program.

For Extended Data Fig. 7d, the expression scores of the PDAC-classical/MUC program were compared between the oesophageal squamous adenocarcinoma subtype and other subtypes of the TCGA oesophagus–stomach tumour samples using two-tailed Mann–Whitney U -tests. The number of tumour samples in each of the subtypes of TCGA oesophagus–stomach tumour subtypes are as follows: all oesophagus–stomach tumours, $n = 602$; diffuse-type stomach adenocarcinoma, $n = 68$; oesophageal adenocarcinoma, $n = 89$; oesophageal squamous cell carcinoma, $n = 92$; gastrointestinal stromal tumour, $n = 6$; intestinal-type stomach adenocarcinoma, $n = 72$; mucinous stomach adenocarcinoma, $n = 20$; papillary stomach adenocarcinoma, $n = 7$; signet ring cell carcinoma of the stomach, $n = 12$; stomach adenocarcinoma, $n = 159$; and tubular stomach adenocarcinoma, $n = 76$.

For Extended Data Fig. 7e, the expression scores of the PDAC-classical/MUC program were compared between oesophagus–stomach samples of TCGA tumours, NextGen models and traditional models after excluding the oesophageal squamous adenocarcinoma samples. The number of remaining samples in each class is as follows: TCGA tumours, $n = 510$; NextGen models, $n = 86$; and traditional models, $n = 52$.

For Extended Data Fig. 7f, Pearson's correlation of previously published classical scores⁴⁶ and previously defined PDAC-classical/MUC scores³⁷ across the cohort of TCGA-plus solid tumour samples ($n = 11,029$) was calculated.

For Extended Data Fig. 7h, Pearson's correlation of ABSOLUTE tumour purity score and the PDAC-classical/MUC score was calculated individually for TCGA-plus tumours of colorectal ($n = 375$), oesophagus–stomach ($n = 448$) and pancreas ($n = 149$) lineages.

For Extended Data Fig. 7i, Pearson's correlation between the gene dependency and expression score of the PDAC-classical/MUC program across the organoid models that were propagated in the presence of R-spondin 1 and WNT3A ligands (gene $n = 3,521$ (strong dependency in organoids), model $n = 60$) was calculated for identifying dependencies associated with the expression of this program.

For Extended Data Fig. 7k, for each of the hotspot and damaging mutations that have ≥ 2 mutated organoids (with CRISPR screen data;

$n = 108$ (model), 587 (mutation)), dependencies on the five regulators of WNT signalling, including *MESD*, *WLS*, *TCF7L2*, *FZD5* and *LGR4*, were compared between the non-mutated and mutated organoids using two-tailed Mann–Whitney U -tests.

For Extended Data Fig. 8a, the dependency scores of the five WNT pathway genes (*MESD*, *WLS*, *TCF7L2*, *FZD5* and *LGR4*) and the PDAC-classical/MUC program expression scores were compared between organoid models without ($n = 78$) versus with ($n = 30$) WNT-activating gene mutations via two-tailed Mann–Whitney U -tests.

For Extended Data Fig. 8b, Pearson's correlation between PDAC-classical/MUC program expression scores and the dependency scores of the five WNT pathway genes (*MESD*, *WLS*, *TCF7L2*, *FZD5* and *LGR4*) as well as the mean dependency scores of these five genes was calculated across the cohort of organoid models ($n = 108$).

For Extended Data Fig. 8d,e, dependency on frequently mutated oncogenes and TSGs (40 oncogenes and 50 TSGs) were compared between the organoid models grown in 3D Matrigel domes in serum-free conditions ($n = 94$) and lineage-matched 2D adherent models cultured directly on plastic plates with serum-containing media (screen $n = 433$) using two-tailed Mann–Whitney U -tests. For *MDM2* dependency, we also tested if the difference in dependency holds true when we limit the analysis to *TP53* WT models (b, right; $n = 22$ (3D), 97 (2D)).

For Extended Data Fig. 8f,g, mean dependency scores on the groups of oncogenes and groups of TSGs that belong to the same pathway were calculated for the 10 oncogenic signalling pathways (oncogene or TSG group $n = 13$). These mean scores were compared between the 3D ($n = 94$) and 2D (screen $n = 433$) models using two-tailed Mann–Whitney U -tests.

For Extended Data Fig. 8h, overlap coefficients were calculated between individual groups of the 9 gene sets with significant overrepresentation of the 200 outlier genes in the distribution of mean dependency differences (Fig. 5a). The numbers of member genes in each of these gene sets are as follows: 200 (Hallmark oxidative phosphorylation); 200 (Hallmark MTORC1 signalling); 200 (Hallmark G2M checkpoint); 139 (KEGG systemic lupus erythematosus); 158 (Hallmark fatty acid metabolism); 200 (Hallmark E2F targets); 199 (KEGG focal adhesion); 213 (KEGG regulation of actin cytoskeleton); and 158 (Hallmark UV response up). Gene sets exhibiting high degrees of overlap (overlap coefficient > 0.3) were merged with each other.

For Extended Data Fig. 9c, Pearson's correlation of the gene dependency scores between the genome-wide screen and minipool screen was calculated across all the genes tested in the minipool screens ($n = 1,000$). Five pairs of genome-wide and minipool screens (KP3 plastic versus RPMI–FBS; HS766T plastic versus RPMI–FBS; UWB1289 plastic versus RPMI–FBS; PANFR0185T2 dome versus OPAC; and HCM-BROD115C16 dome versus OPAC), for which these two types of screens were conducted under the same culture conditions (growth format and culture medium), were evaluated.

For Extended Data Fig. 9d,e, for the seven pairs of minipool screens that were conducted with the same model, same medium but different growth format (dome versus plastic), the Chronos dependency scores of all the test genes in the minipool library ($n = 250$) were compared using two-tailed paired t -tests (d). Similarly, for the five pairs of minipool screens that were conducted with the same model, same format but different culture medium (OPAC versus RPMI–FBS), the Chronos dependency scores of all the test genes in the minipool library were also compared using two-tailed paired t -tests (e). All genes with significant differential dependencies in either of these comparisons as well as *CDK4* and *GPX4* are shown in the plots.

For Extended Data Fig. 9f,g, effects of growth formats (f) and culture media (g) on gene dependencies (calculated by a linear regression model (see the section 'Minipool screen analysis' of the Methods)) were compared between in-class genes and out-of-class genes (that is, the rest of the 250 test genes in the minipool library) for each of the 9 core functional classes of the minipool library. Significance of

the difference was assessed by two-tailed Mann–Whitney *U*-test. The numbers of genes in each of the 9 core functional classes are as follows: actin regulation, *n* = 75; adherens junction, *n* = 30; cell cycle, *n* = 53; ECM adhesion, *n* = 89; integrin, *n* = 22; lipid metabolism, *n* = 20; PI3K–mTOR signalling, *n* = 20; tight junction, *n* = 34; and WNT signalling, *n* = 29.

For Extended Data Fig. 10a, the viabilities of KP3 enAsCas12a and HS766T enAsCas12a cells that were transduced with various sgRNAs were compared against the viability of corresponding cells expressing cutting control sgRNAs (sgCh2 and sgAAVS). The viabilities were measured as triplicates, and significance was calculated by two-tailed two-way ANOVA.

For Extended Data Fig. 10b, the degrees of colocalization between integrin chains (integrin β 1 and integrin α V) and FAK, scored by intensity correlation quotient (Li's ICQ)⁷⁴, were compared among the following sample groups using two-tailed Mann–Whitney *U*-tests: integrin β 1 FAK staining, plastic *n* = 20; integrin β 1 FAK staining, dome *n* = 19; integrin α V FAK staining, plastic *n* = 18; and integrin α V FAK staining, dome *n* = 21.

Figure preparation

The figure panels were prepared primarily using Adobe Illustrator 2025 (v.29.8.3). The plots were generated using custom code written in R (v.4.4.2) or Python (v.3.11.5). Other plots, including Figs. 2i and 3d and Extended Data Figs. 2a,c (bottom), 4e–h, 6a,e,f,h,i, 7a,i, 8c and 10a, were produced using GraphPad Prism (v.10.6.0), whereas the plots shown in Extended Data Fig. 10c,d were produced with FlowJo (v.10.10.0).

Material availability

Plasmids generated in this study and the mini-pool library (Supplementary Table 3) will be deposited into Addgene.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Raw sequencing data of WGS and RNA-seq for most of the NextGen models profiled in this study are available in the dbGaP database (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs003444.v4.p1; dbGaP Study accession: phs003444.v4.p1). Raw profiling data (including data for mutation, CN, gene expression and gene dependency) and processed data required for running the custom code used in this study as well as the metadata of the samples are available for download from the DepMap portal (https://depmap.org/portal/data_page/?tab=allData; file set name: 'NextGen Model Manuscript 2026'). Other analytical results from this study are available in a Figshare collection (<https://doi.org/10.6084/m9.figshare.29472362>)⁷⁵. Some data used in this study were obtained from public repositories. Specifically, in Fig. 1c, tumour mutation data were collected through the cBioPortal (<https://www.cbioportal.org/>) from the following studies in the TCGA PanCancer Atlas: COAD/READ, PAAD, PRAD and BRCA⁷⁶; except for the ones labelled with red rectangles, which were acquired from following publications: ref. 60 for the rate of CNS tumour with *TERT* promoter hotspot mutation; TCGA-COAD/READ⁵⁸ for the rate of colorectal tumours with chromosome 18q loss; TCGA-PAAD⁵⁹ or the pancreatic tumour chromosome 9p loss rate; and MSK-MET¹² for the rates of colorectal tumours with *NRAS* hotspot mutation, breast tumours with *ESR1* hotspot mutation, and prostate tumours with *SPOP* damaging mutation. In Fig. 1c,d and Extended Data Fig. 1a,b, GRCh38.p14 human genome assembly (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.40/) was used as the reference sequence in the mutation calls. In Extended Data Fig. 5c, gene expression data for the combined cohort of TCGA, TARGET and GTEx samples (*n* = 8,167 (normal) and 10,530 (tumour)) were obtained through the UCSC Xena browser (<https://xenabrowser.net/>; study name: TCGA TARGET GTEx).

In Extended Data Fig. 7h, ABSOLUTE tumour purity data of TCGA tumours were compiled from the following publications: ref. 73 and TCGA-PAAD⁵⁹. Source data are provided with this paper.

Code availability

Code to perform all the downstream analyses after initial processing to obtain data for mutation, CN, gene expression and gene dependency can be found at GitHub (<https://github.com/broadinstitute/DepMap-NextGen-Public>).

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Additional information

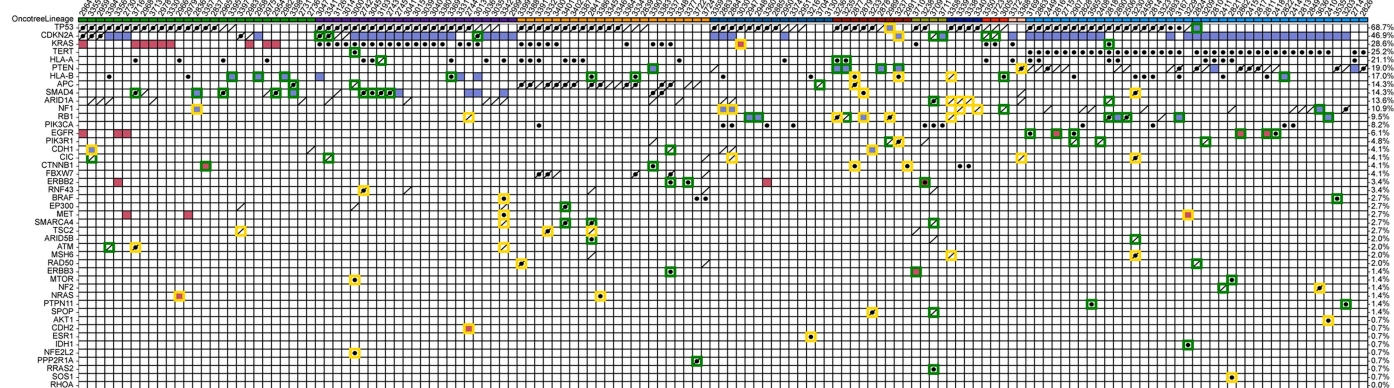
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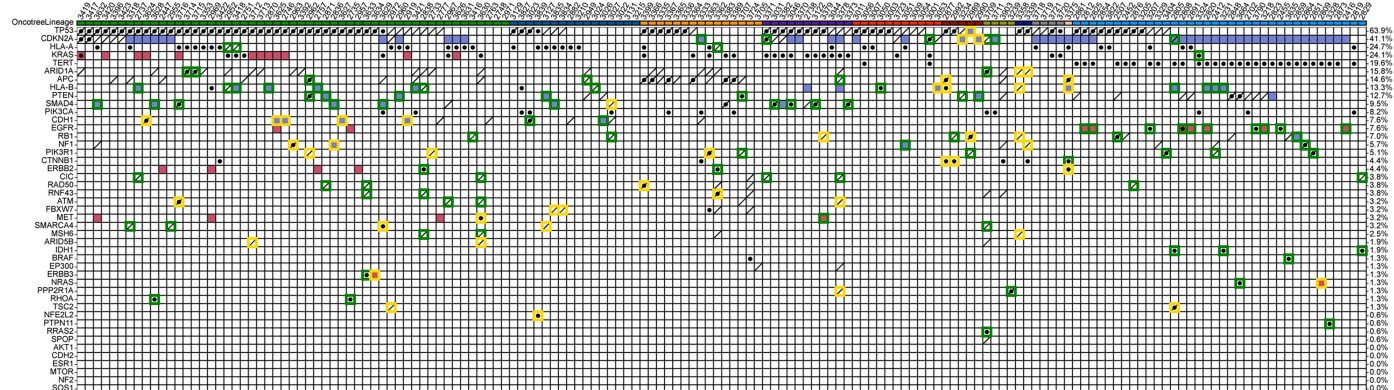
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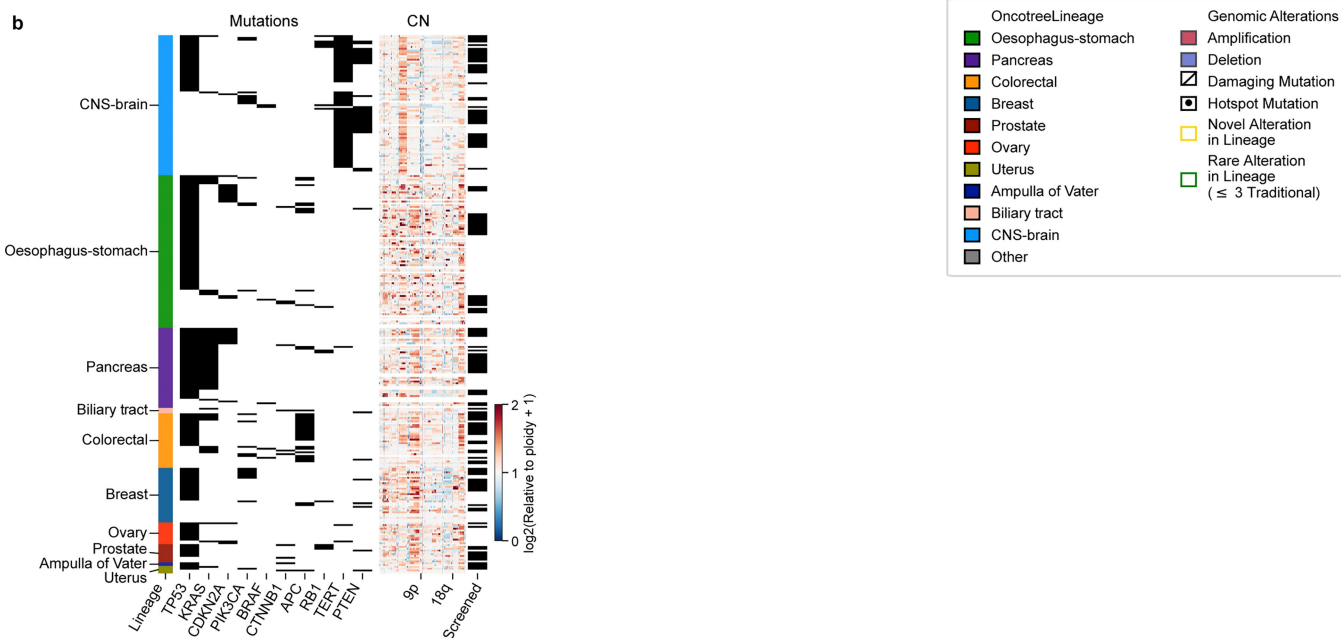
a Models with CRISPR screen data



Models only with omics profiling (WGS with or without RNAseq) data

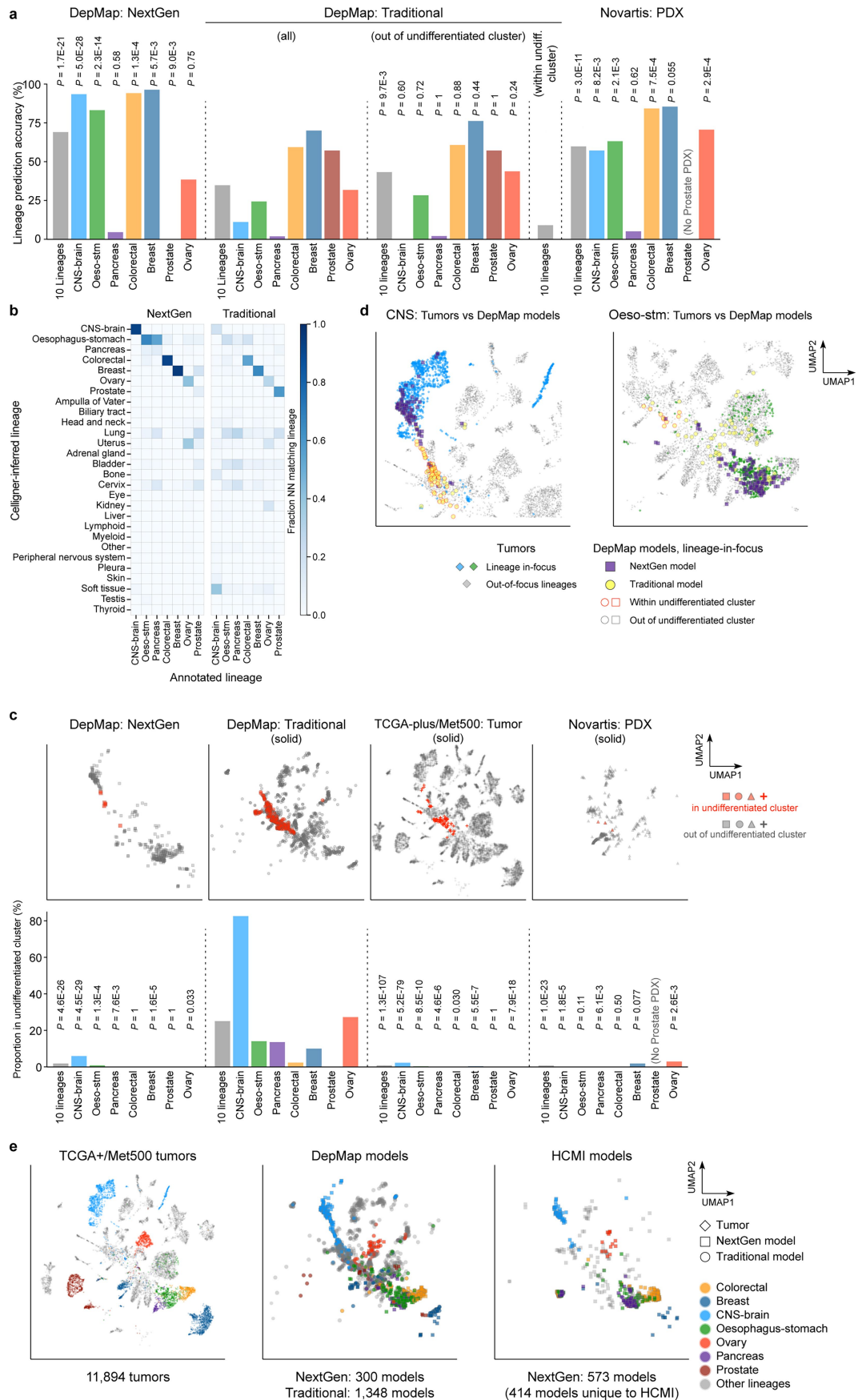


b



Extended Data Fig. 1 | Summary for the WGS and RNAseq data of DepMap NextGen models. **a**, Oncoprint for DepMap NextGen models. The models with ($n = 147$) and without ($n = 144$) the CRISPR screen data are shown on the top and bottom panels, respectively. The mutation and copy number alteration status of oncogenes and tumor suppressor genes (TSGs) that result in oncogene gain-of-function (GOF) (i.e., copy number [CN] gain and hotspot mutation) or TSG loss-of-function (LOF) (i.e., CN loss, hotspot and damaging mutations) are indicated for individual NextGen models. Each column represents a single model and is labeled by the last four digits of the ACH ID (the model ID used for the DepMap models). All the oncogenes and TSGs, for which at least one of

DepMap NextGen models have a hotspot mutation, are listed. The yellow blank squares indicate the type of genetic alterations that are not harbored by any lineage-matched DepMap traditional cell lines, while the green blank squares show the type of changes possessed by ≤ 3 traditional cell lines of the respective lineage. **b**, Summary of the WGS data of NextGen models. Each model is represented by a row. For the mutations, models harboring a hotspot mutation of the oncogenes and either a hotspot or damaging mutation of the TSGs are indicated by the black color. Arm-level copy number variation is indicated by the blue-to-red color gradient, while the models that have CRISPR screen scores are indicated by the black color.



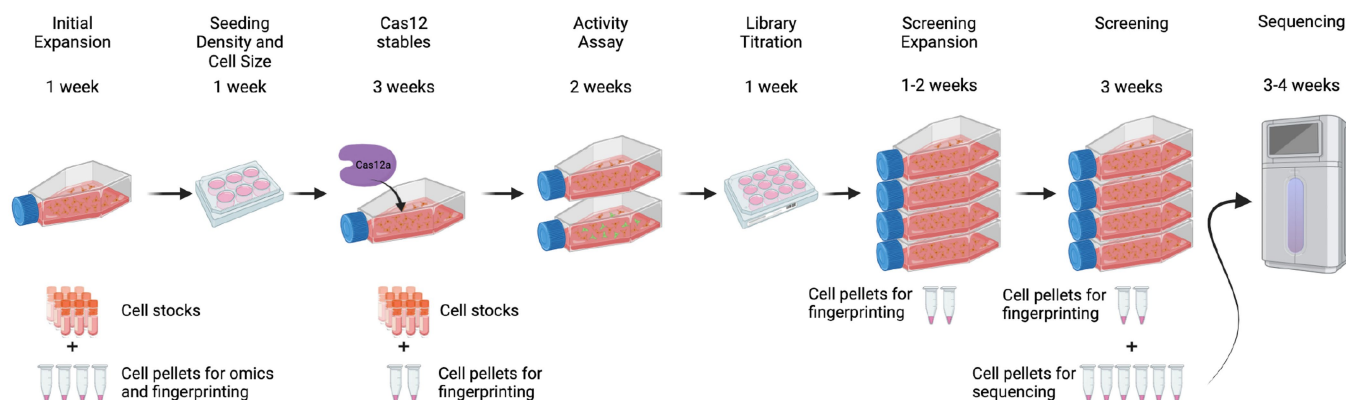
Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Comparison of the lineage fidelity of NextGen vs traditional cancer models. **a**, Elevated Celligner lineage prediction accuracy for the NextGen models and PDXs relative to the traditional cell lines. The lineage of each cancer model was predicted based on the lineages of nearest neighbor tumor samples in the Celligner-aligned space (see **b** and Methods) for each of the three model groups (DepMap NextGen models, DepMap traditional cell lines and Novartis patient-derived xenografts [PDXs]¹⁶). The probability of the agreement between the annotated lineage and the lineage inferred from the Celligner alignment were plotted per individual lineage and for all the 10 NextGen model lineages combined. For the traditional cell lines, the probability of agreement was also calculated only for the models out of the undifferentiated cluster (see **c**) and plotted. DepMap: NextGen, $n = 300, 76, 83, 44, 34, 27, 12$ and 13 ; DepMap: Traditional (all), $n = 532, 81, 78, 54, 86, 70, 7$ and 66 ; DepMap: Traditional (out of undifferentiated cluster), $n = 400, 12, 67, 48, 84, 63, 7$ and 48 ; Novartis: PDX, $n = 266, 7, 19, 59, 70, 55, 0$ and 34 for 10 lineages, CNS, oeso-stm, pancreas, colorectal, breast, prostate and ovary, respectively. Here and in **c**, P -values were scored by two-sided Fisher's exact test (vs DepMap: Traditional [all]). **b**, Lineage prediction based on nearest neighbor tumor samples on Celligner. For each of the NextGen and traditional culture models, the lineages of the 25 nearest neighbor (NN) tumor samples (y-axis) were identified on Celligner. The distribution of these NN tumor lineages was plotted for each of the annotated lineages of the culture models (x-axis). A cropped version of this panel is also presented as Fig. 1f. **c**, The undifferentiated cluster enriched with DepMap traditional cell lines. The clusters of tumors and models on the Celligner embedding were evaluated based on the epithelial-mesenchymal transition (EMT) score of the samples and the abundance of DepMap models over the tumors to identify four clusters (clusters #28, 59, 62 and 67) with high EMT scores and relative enrichment of DepMap models over TCGA+/Met500 tumors (Methods). These four clusters were combined to form

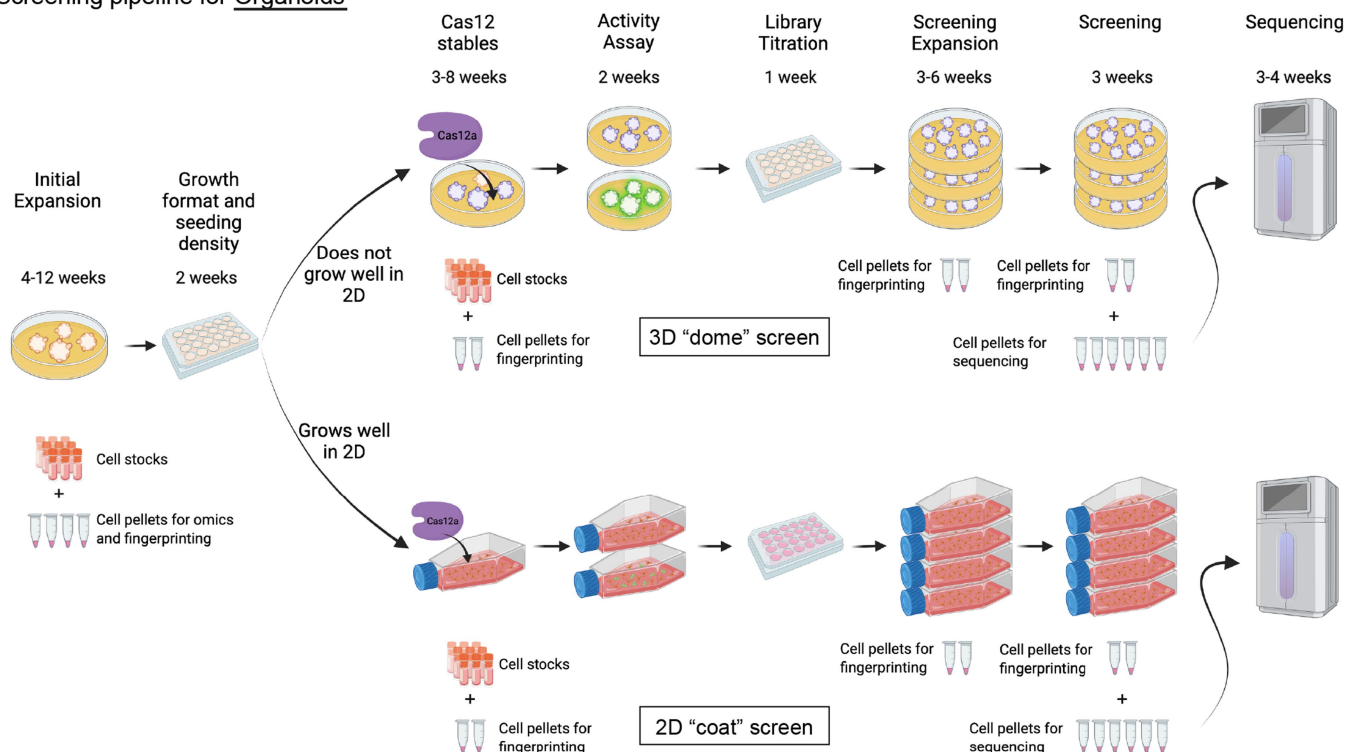
the undifferentiated cluster. TCGA-plus/Met500 solid tumors, DepMap NextGen models and DepMap traditional cell lines (only solid tumor models) and Novartis PDXs were all plotted on the Celligner embedding (top) with samples within the undifferentiated cluster indicated by the red color of the points. The proportions of samples in the undifferentiated cluster per individual lineage with > 5 NextGen models (CNS, oeso-stm, pancreas, breast, colorectal, prostate and ovary), or for all the 10 NextGen model lineages combined, were also plotted (bottom). DepMap: NextGen, $n = 375, 101, 126, 49, 34, 28, 13$ and 13 ; DepMap: Traditional, $n = 542, 86, 78, 59, 86, 70, 7$ and 66 ; TCGA-plus/Met500: Tumor, $n = 5185, 1244, 639, 202, 406, 1254, 651$ and 453 ; Novartis: PDX, $n = 266, 7, 19, 59, 70, 55, 0$ and 34 for 10 lineages, CNS, oeso-stm, pancreas, colorectal, breast, prostate and ovary, respectively. **d**, Close modeling of the tumor transcriptome by the NextGen CNS and oeso-stm models. TCGA-plus/Met500 solid tumors and DepMap models of the CNS (left) and oeso-stm (right) lineage were compared on Celligner. For the tumors, the lineage in focus was labeled with a color (light blue and green for CNS and oeso-stm, respectively). For both lineages, DepMap NextGen models (purple squares) clustered together with the tumors of the respective lineage, while DepMap traditional cell lines (yellow circles) were largely distributed separately from this cluster, indicating superior preservation of tumor lineage-specific gene expression by the NextGen models. Models within the undifferentiated cluster (see **b**) are indicated by the red frame of the points. **e**, Distribution of TCGA-plus/Met500 solid tumors, DepMap models and HCMI models on the Celligner embedding. Samples in lineages with five or more DepMap NextGen models are indicated by the color (see legend). Some of the models were shared by DepMap and HCMI, although they were profiled separately and therefore have different Celligner coordinates. Within the HCMI cohort, 414 models were unique to HCMI while the remaining 161 models were also profiled separately by DepMap and incorporated in the DepMap cohort as well.

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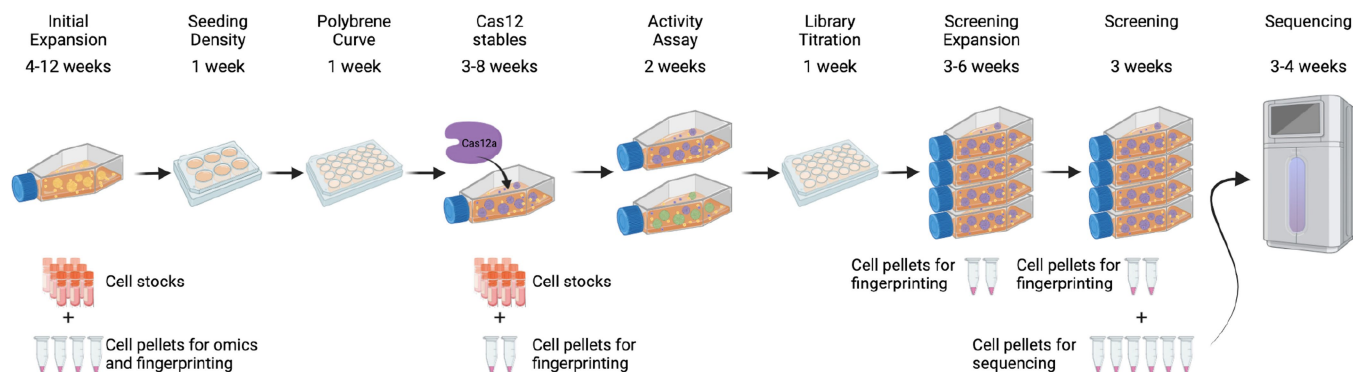
Screening pipeline for Traditional models (w. enAsCas12a library)



Screening pipeline for Organoids



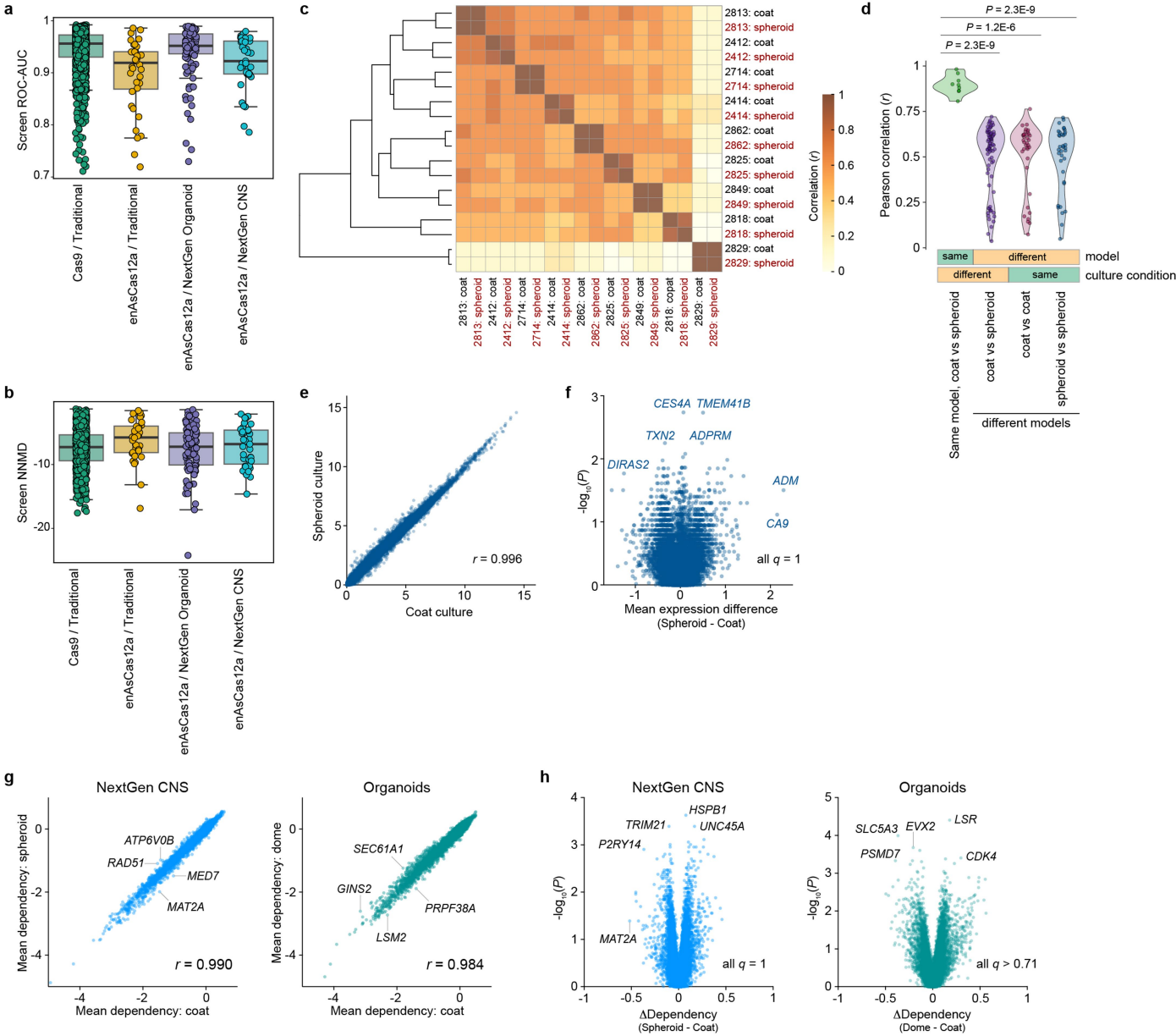
Screening pipeline for NextGen CNS models



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Overview of CRISPR screen workflow for different model types. DepMap has been profiling three different model types (traditional cell lines [top], organoids [middle], and NextGen CNS tumor models [bottom]) with the genome-scale CRISPR loss-of-function screen. The workflows of screening for these three model types are illustrated. For all these model types, the screen process starts with the initial expansion to obtain enough cells for subsequent processes as well as enough frozen vials. This was followed by antibiotics/polybrene titration to determine optimal concentrations of puromycin, blasticidin, polybrene as well as optimal seeding density (the types of titration required are different among different model types). Subsequently, the lentiviral transduction of enAsCas12a, Cas12a activity assay, library titration, and screening with a genome-scale Cas12a sgRNA library (Humagne-CD), were conducted sequentially. The typical durations of the entire screen process (initial expansion to sequencing) range from 16 weeks (traditional cell lines) to 30+ weeks (organoids and NextGen CNS

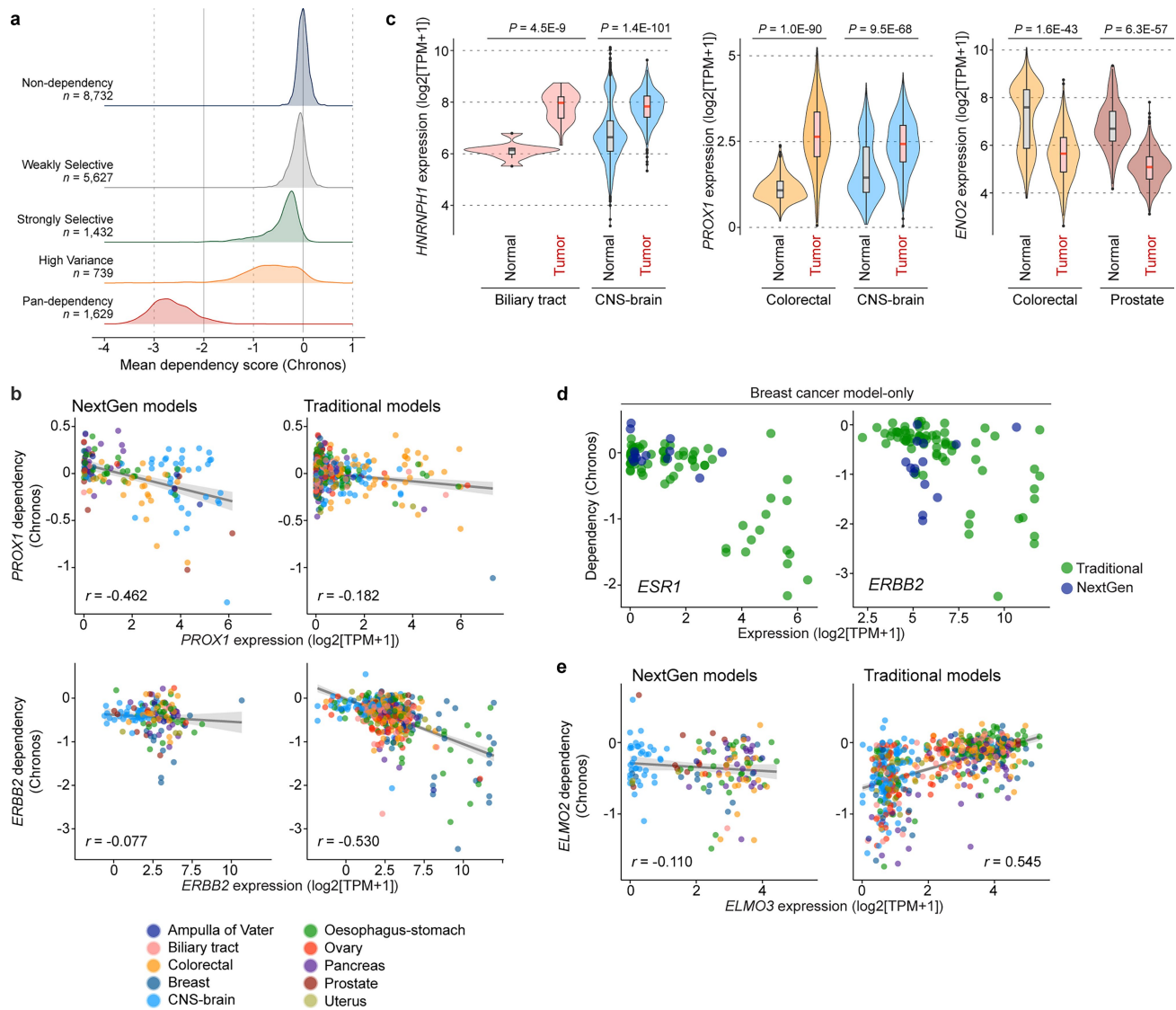
models). For the organoids, all the models were initially derived in the 3D Matrigel dome culture. Some models were converted to adherent culture on laminin- or Matrigel-coated plates prior to screen ("coat" culture), while the others were screened in the original dome culture. Likewise, for the NextGen CNS models, all the models were initially derived as 3D spheroids. Some models were converted to coat culture using Matrigel- or laminin-coated plates, while the others were screened in the original spheroid culture. The transition from 3D spheroid culture to adherent culture on laminin-coated plates minimally affected the transcriptome of the NextGen CNS models (see Extended Data Fig. 5). In addition, the CRISPR screening outcomes between the 3D dome and 2D coat cultures of the organoids, and between 3D spheroid and 2D coat cultures of the NextGen CNS models, were not discernibly different (see Extended Data Fig. 6). Schematics created in BioRender; top, Brenan, L. <https://biorender.com/1w9lcwr> (2026); middle, Brenan, L. <https://biorender.com/r2dcgt5> (2026); bottom, Brenan, L. <https://biorender.com/4ifc5pn> (2026).



Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Comparison of gene expression and CRISPR screen outcomes between 2D and 3D cultures. a, b, CRISPR screen QC metrics. In **a**, ROC-AUC (Receiver Operating Characteristic Area Under the Curve) scores for common essential (positive controls; defined as the intersection of essential genes in Hart et al.⁷⁰ and Blomen et al.⁷¹) and nonessential (negative controls; defined by Hart et al.⁷⁰) controls were calculated using the naïve gene effect scores and plotted for all the DepMap CRISPR screens. In **b**, NNMD (Null-Normalized Median Difference) score between the common essential and nonessential controls was calculated for each screen. The screens evaluated here included Cas9 traditional model screens ($n = 1,351$), enAsCas12a traditional model screens ($n = 36$), enAsCas12a NextGen organoid screens ($n = 108$), and enCas12a NextGen CNS model screens ($n = 39$). For both metrics, scores from the NextGen models were comparable to those from the Cas9 traditional model screens and enAsCas12a traditional model screens. The centre (for consistency with the main text) line and box denote median and 25th to 75th percentile data points, respectively, and whiskers extend up to 1.5 times the interquartile range from box edges. **c, d,** Clustering of samples from NextGen CNS tumor models in 3D spheroid culture and 2D adherent culture on Matrigel-coated plates (“coat” culture) by the correlation of gene expression. Nine NextGen CNS tumor spheroid models were either maintained in the spheroid culture or propagated in the coat culture for > 4 weeks. In **c**, gene expression profiles of these 18 samples (9 models x 2 conditions) were compared and clustered using the correlation of expression across top 10% highly variable genes ($n = 1,919$). These samples were primarily clustered by cell identity, but not by the culture conditions. The distributions of correlation between two different samples were also plotted (**d**), exhibiting the high degree of correlation ($r > 0.8$) between two samples with different conditions (spheroid and adherent cultures) of the same model. *P*-values were calculated with two-tailed Mann-Whitney *U* test. $n = 9$ (same model under coat and spheroid culture conditions), 72 (different models under different culture conditions), 36 (different models both in coat cultures), and 36 (different models both in spheroid cultures). **e, f,** High concordance of gene expression between spheroid and coat cultures

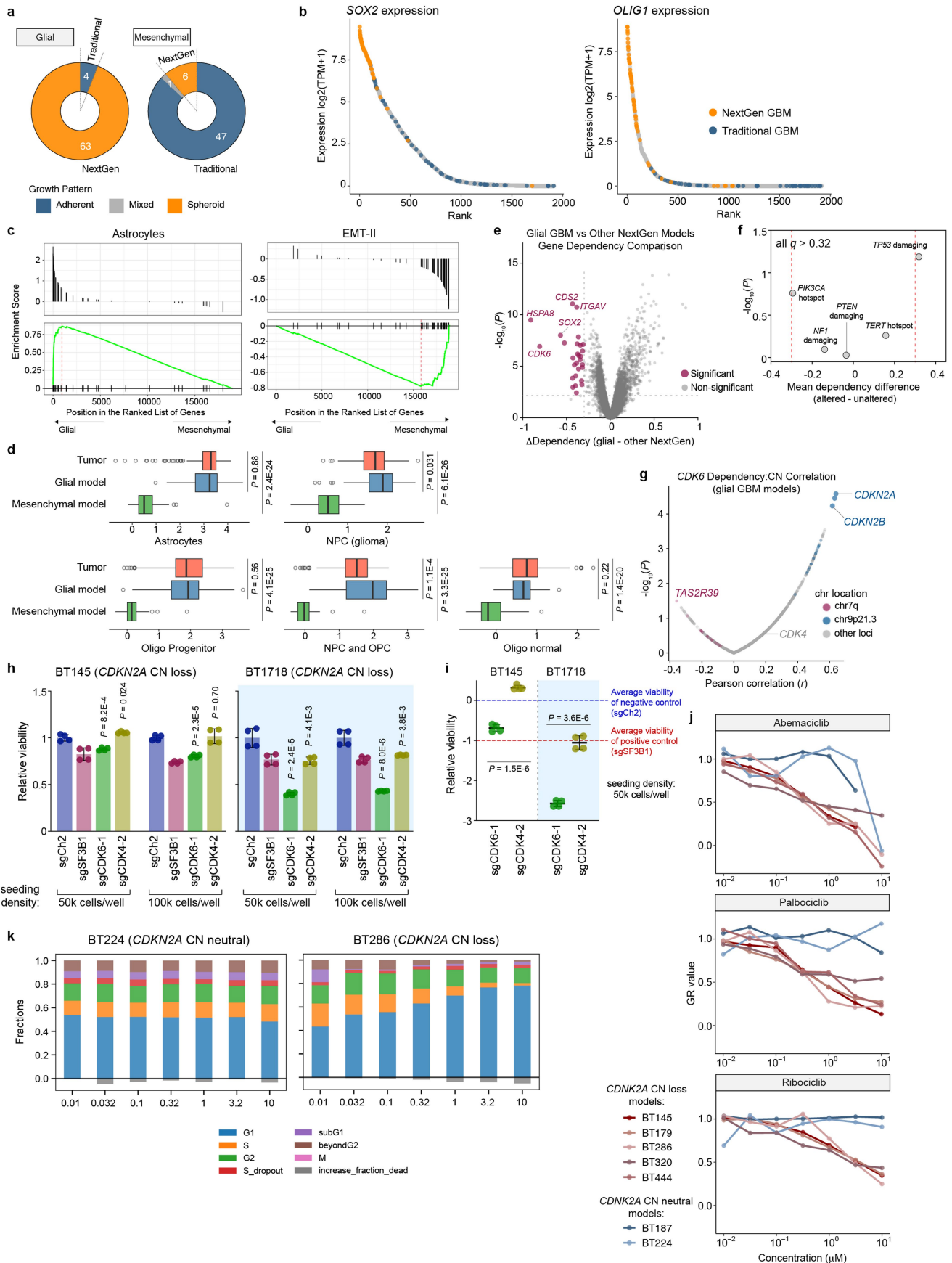
of the NextGen CNS models. The mean expression levels in the nine spheroid culture samples (x-axis) and the nine coat culture samples (y-axis) were plotted for all the genes ($n = 19,193$), which exhibited a very high degree of concordance ($r = 0.996$) between these two culture conditions (**e**). The difference in the mean expression levels (x-axis) and the statistical significance of the difference (by a two-tailed Mann-Whitney *U* test; y-axis) were also plotted (**f**). The false discovery rate (FDR; q) was 1 for all the genes, indicating that none of the genes exhibited statistically significant differences. **g, h** Concordance of Chronos dependency scores between 3D culture and 2D coat culture. The mean dependency scores of each gene in the NextGen CNS models screened under 3D spheroid cultures ($n = 14$; x-axis) and in the NextGen CNS models screened under adherent cultures on laminin- or Matrigel-coated plates (“coat” cultures; $n = 25$; y-axis) were plotted (**g**, left). Similarly, the mean dependency scores of genes in the organoid models screened under 3D Matrigel dome cultures ($n = 55$; x-axis) and in the organoid models screened under coat cultures ($n = 14$; y-axis) were plotted (**g**, right). For the organoids in Matrigel dome cultures, only the models from lineages that have corresponding coat-cultured models (ampulla of vator, colorectal, oeso-stm, ovary, prostate and uterus) were included in this comparison. In both cases, a high degree of concordance was observed between the two different conditions of culture ($r = 0.990$ and 0.984 for CNS models and organoids, respectively). The difference in the mean dependency scores between the spheroid and coat culture of the CNS models (**h**, left) and between the dome and coat cultures of the organoid models (**h**, right) (x-axis) and the significance of the difference (y-axis; by a two-tailed Mann-Whitney *U* test) were also plotted. In both cases, none of the genes exhibited a statistically significant difference of the mean dependency scores between two different culture conditions ($q = 1$ for all the genes [CNS]; $q > 0.71$ for all the genes [organoids]). Note that gene expression comparison between spheroid and coat cultures of the NextGen CNS models (**c-f**) were conducted with matched model groups, while the comparison of CRISPR outcomes (**g, h**) were performed between unmatched, different groups of models.



Extended Data Fig. 5 | Dependencies linked to gene expression identified in NextGen vs traditional models. a, Representative distribution of dependency scores for each dependency category. Each gene evaluated by the CRISPR screen was classified into either of these five dependency classes based on the distribution of its Chronos dependency scores across DepMap NextGen models (or organoid models). We used a previously published strategy for this classification²⁵ (also see Methods). **b,** Correlation of expression and dependency for *PROX1* and *ERBB2* in the NextGen (left) and traditional (right) models. Elevated expression of *PROX1* correlated with enhanced dependency on its own more strongly in the NextGen models ($r = -0.462$) than in the traditional cell lines ($r = -0.182$). In contrast, while the traditional cell lines exhibit very strong association ($r = -0.530$) between the expression and dependency levels of *ERBB2*, this relationship was much weaker ($r = -0.077$) in the NextGen models. Here and in **e**, regression lines with 95% confidence intervals are displayed. **c,** Increased expression of *HNRNP1* and *PROX1* and reduced expression of *ENO2* in tumors relative to lineage-matched normal tissues. The expression levels of *HNRNP1*, *PROX1* and *ENO2* in the tumor tissues and corresponding normal tissues were analyzed per lineage in the combined TCGA/TARGET/GTEX dataset downloaded from the Xena browser (<https://xenabrowser.net/>). $n = 9$ (biliary tract, normal), 36 (biliary tract, tumor), 1,151 (CNS-brain, normal), 689 (CNS-brain, tumor), 355 (colorectal, normal), 383 (colorectal, tumor), 152 (prostate, normal), and 496 (prostate, tumor). Expression values were plotted for the lineages with tumor-specific upregulation (*HNRNP1* and *PROX1*) or downregulation (*ENO2*) of these genes.

The centre line and box denote median and 25th to 75th percentile data points, respectively, and whiskers extend up to 1.5 times the interquartile range from box edges. P -values were scored by two-tailed Mann-Whitney U test.

d, Reduced expression of *ESR1*/*ERBB2* underlies the absence of expression addition for these oncogenes in the NextGen breast cancer models. The association between the expression and dependency of two breast cancer oncogenes, *ESR1* and *ERBB2*, was plotted for the NextGen (green circles) and traditional (navy circles) breast cancer models. None of the 14 breast cancer organoids exhibited high ($\log_2[\text{TPM} + 1] > 3.4$) expression of *ESR1*, while 11 out of the 51 traditional breast cancer models showed high expression of this oncogene. All the traditional cell lines with strong (Chronos score < -1) *ESR1* dependency displayed high expression of *ESR1*. Similarly, only one out of the 14 NextGen models, and 14 out of the 51 traditional cell lines, exhibited high expression of *ERBB2* ($\log_2[\text{TPM} + 1] > 7.5$) and the majority (10/12) of the traditional cell lines whose screen results exhibit strong *ERBB2* dependencies were found to have high *ERBB2* expression. Hence, the absence of expression addition relationship for *ESR1* and *ERBB2* in the NextGen breast cancer models appears to be attributable, in part, to the reduced expression of these oncogenes in these models as compared to the levels observed in their traditional counterparts. **e,** *ELMO2* paralog dependency in the NextGen (left) vs traditional (right) models. *ELMO2* dependency was correlated strongly ($r = 0.545$) with reduced expression of its paralog *ELMO3* in the traditional cell lines, while this relationship was not observed ($r = -0.110$) in the NextGen models.

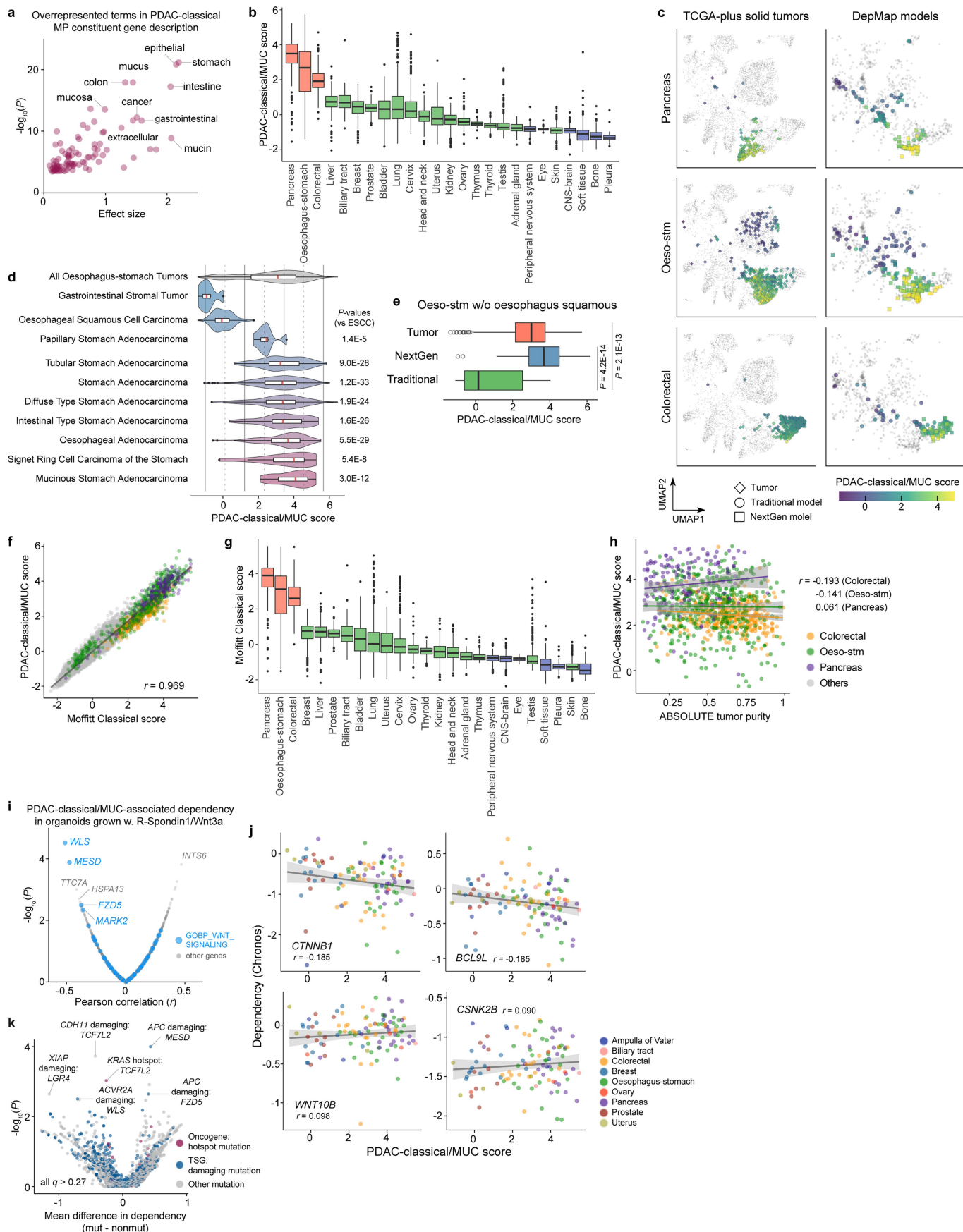


Extended Data Fig. 6 | See next page for caption.

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Extended Data Fig. 6 | Enhanced *CDK6* dependency linked to glial gene expression of NextGen GBM models. **a**, Differential clustering of NextGen and traditional GBM models on the Celligner embedding. Celligner-based comparison of overall gene expression patterns (see Fig. 3b) showed that NextGen GBM models and traditional GBM models are clustered separately. The cluster with the majority of NextGen models was named as “glial”, while the other cluster enriched with most of the traditional cell lines was labeled as “mesenchymal”. The number of models in each of these clusters per model type (NextGen or traditional) and growth pattern (adherent, mixed or spheroid) was plotted. **b**, Expression levels of *SOX2* and *OLIG2*, two markers for the glial subtype GBM, between NextGen and traditional GBM models. The NextGen models exhibited significantly higher expression of both markers than the traditional cell lines ($P < 4 \times 10^{-16}$ by two-tailed Mann-Whitney *U* test). **c**, Differential activation of gene expression meta-programs (MPs) between GBM models in the glial vs mesenchymal clusters. A gene set enrichment analysis (GSEA) was applied to the differential gene expression data between the GBM models of the glial and mesenchymal clusters to compare expression levels of genes defining each of the 41 MPs (see Fig. 3b,d). The five neuronal lineage-specific MPs, including the “Astrocyte” program (left), were selectively activated by models in the glial cluster, while other programs, including the “EMT-II” program (right), were expressed specifically by models in the mesenchymal cluster. **d**, Expression levels of the five neuronal lineage-specific MPs in DepMap GBM models and TCGA-plus GBM tumors. For all these five neuronal MPs, GBM tumors ($n = 180$) and glial GBM models ($n = 67$) exhibited comparable levels of expression, which were far higher than the expression levels observed in the mesenchymal GBM models ($n = 54$). The centre line and box denote median and 25th to 75th percentile data points, respectively, and whiskers extend up to 1.5 times the interquartile range from box edges. Here and in **e**, **f**, *P*-values were scored by two-tailed Mann-Whitney *U* test. **e**, Differential dependency analysis comparing glial GBM models ($n = 36$) and all other non-glial organoids and spheroids from 10 lineages ($n = 115$). Selective dependencies were defined as genes with $q < 0.05$ (horizontal dashed line) and gene effect < -0.3 (vertical dashed line) and highlighted in maroon. **f**, *CDK6* dependency association with mutation. For the mutations

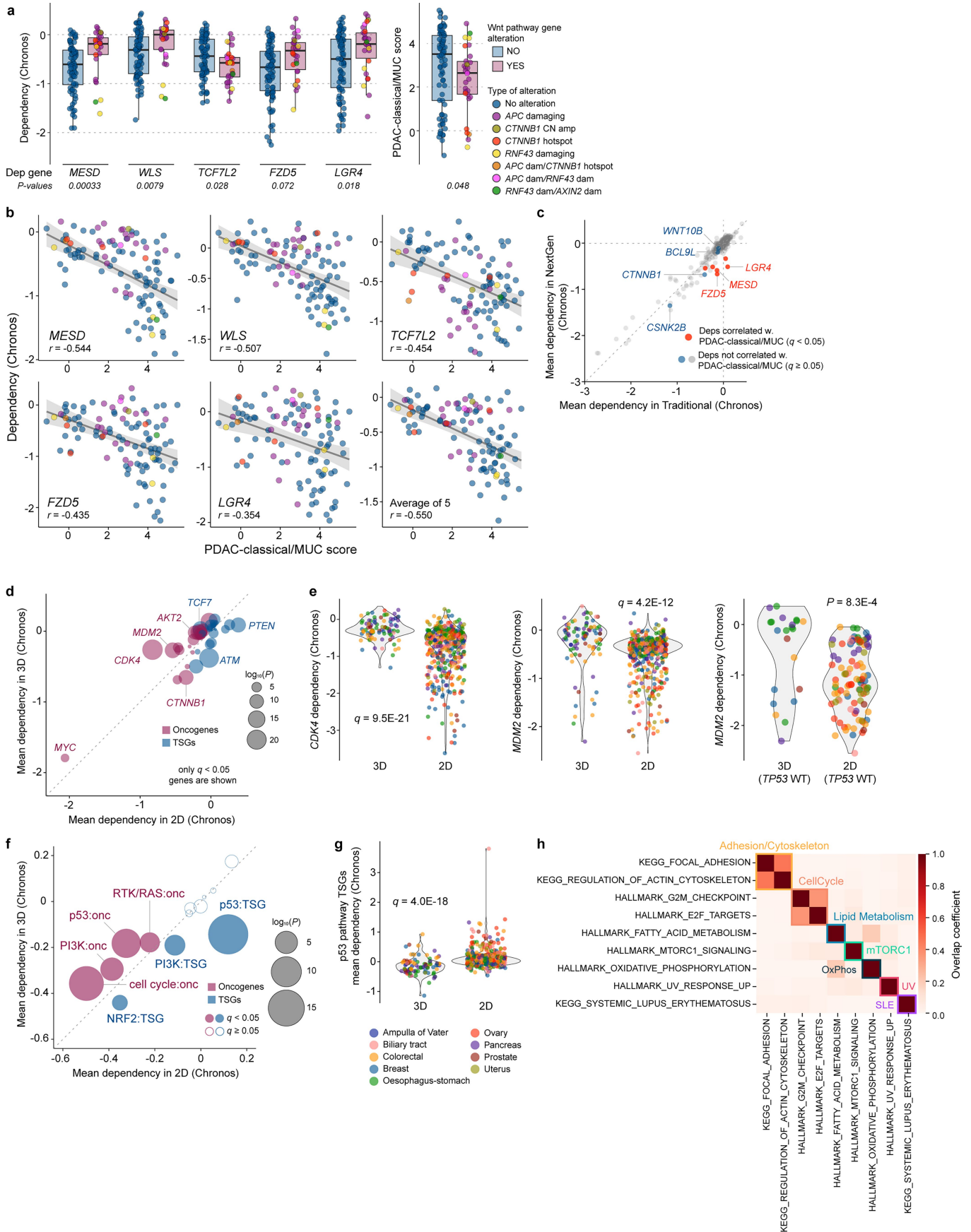
(hotspot or damaging) with ≥ 5 GBM screens of mutant models (five different mutations), *CDK6* dependency was compared between mutant and nonmutant models, and the difference in mean Chronos dependency scores (x-axis) and the significance of difference (y-axis) were plotted (screen $n = 95$). Significant dependency difference was not observed for any of these five mutation types ($q > 0.32$). **g**, Correlation between *CDK6* dependency and gene copy numbers (CNs) across DepMap glial GBM models. Correlation of *CDK6* dependency with log2-transformed CN values (log2[relative to ploidy +1]) of the genes within the top 10% high variance of CN across DepMap glial GBM models (with CRISPR scores; screen $n = 36$) was plotted. Genes on Chr7q and Chr9p21.3 are indicated by maroon and blue dots, respectively. *P*-values were scored by two-tailed Pearson correlation. **h,i**, Viability of *CDKN2A* CN loss glial GBM models after *CDK6/CDK4* knockout. Two NextGen glial GBM models with *CDKN2A* CN loss—BT145 (*CDKN2A* log2[relative to ploidy+1] = 2.54×10^{-6}) and BT1718 (*CDKN2A* log2[relative to ploidy+1] = 1.91×10^{-6}), both of which were also engineered to express Cas12a, were infected with lentivirus expressing the indicated sgRNAs and successfully infected cells were reseeded at the indicated densities into a 24-well plate. These cells were subjected to viability assay 10 days after the infection. The viability relative to the cells receiving a cutting control sgRNA (sgCh2) are plotted (**h**). These scores were also normalized to the average viability of cells transduced with the cutting control sgRNA (set as 0) and the average viability of cells with knockout (KO) of a pan-dependency gene (sgSF3B1; set as -1), and the normalized values for the samples with the low seeding density (50k cells/well) were plotted (**i**). Values = means $\pm$ SD ($n = 4$). *P*-values were scored by two-tailed Student's *t*-test. **j,k**, Inhibition of cell growth by CDK4/6 inhibitor treatment of the NextGen GBM models. Seven NextGen glioblastoma models, among which two models (BT187 and BT224; blue lines) were *CDKN2A* copy number (CN) neutral while the remaining five models (red lines) had loss of *CDKN2A* CN, were treated with various concentrations of CDK4/6 inhibitors (Abemaciclib, Palbociclib, and Ribociclib). In **j**, the relative viabilities of the drug-treated cells against the mock control (growth rate [GR] value) were plotted. Distributions of the drug-treated cells to different phases of cell cycle were also plotted for one *CDKN2A* CN neutral model (BT224) and one *CDKN2A* CN loss model (BT286) (**k**).



Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | PDAC-classical/MUC program activity and associated Wnt dependency. **a**, GeneTEA⁴⁷ identified terms associated with the constituent genes of the PDAC-classical/MUC program. The plot shows the sum of TF-IDF (Term Frequency-Inverse Document Frequency) for all the constituent genes (x-axis) and the significance of overrepresentation (one-sided Fisher's exact test; y-axis), enumerating frequently appearing terms in the gene annotations. **b**, PDAC-classical/MUC program expression was plotted for 11,014 TCGA-plus solid tumor samples by lineage. This program is highly expressed (mean score > 1.9) in the three gastrointestinal (GI) lineages (pancreas, oeso-stm, and colorectal), intermediately expressed (0.7 < mean score < 0.9) in other GI lineages (biliary tract and liver), and low in non-GI tumors (mean score < 0.5). Here and in **g**, these groups are color-coded: red for high-expression GI lineages, green for other epithelial (carcinoma) lineages, and blue for mesenchymal lineages. *n* = 180 (pancreas), 602 (Oesophagus-stomach), 384 (colorectal), 398 (liver), 37 (biliary tract), 1099 (breast), 496 (prostate), 407 (bladder), 1015 (lung), 307 (cervix), 524 (head and neck), 238 (uterus), 1019 (kidney), 427 (ovary), 121 (thymus), 515 (thyroid), 156 (testis), 243 (adrenal gland), 193 (peripheral nervous system), 81 (eye), 476 (skin), 1229 (CNS-brain), 518 (soft tissue), 262 (bone), 87 (pleura) (in **b**, **g**). Also, in **b**, **d**, **e**, **g**, the centre line and box denote median and 25th to 75th percentile data points, respectively, and whiskers extend up to 1.5 times the interquartile range from box edges. **c**, PDAC-classical/MUC program expression in individual lineages shown on the Celligner embedding. The levels of PDAC-classical/MUC program expression are indicated by the color on the Celligner plots for pancreas (left), (middle), and colorectal (right) samples of the TCGA-plus tumors (top) and DepMap models (traditional cell lines and NextGen organoids; bottom). **d**, PDAC-classical/MUC program expression varied across oesophagus-stomach (oeso-stm) tumor subtypes. In TCGA-plus samples, expression was very low (mean < 0) in gastrointestinal stromal tumor and oesophageal squamous cell carcinoma (ESCC), but uniformly high (mean > 2.1) in other oeso-stm cancer subtypes. Notably, the two subtypes with the highest mean expression—signet ring cell carcinoma and mucinous stomach adenocarcinoma—are both known for mucin production. *n* = 602 (all oesophagus-stomach tumors), 6 (gastrointestinal stromal tumors), 92 (oesophageal squamous cell carcinoma), 7 (papillary stomach adenocarcinoma), 76 (tubular stomach adenocarcinoma), 159 (stomach adenocarcinoma), 68 (diffuse type stomach adenocarcinoma), 72 (intestinal type stomach adenocarcinoma), 89 (oesophageal adenocarcinoma), 12 (signet ring cell carcinoma of the stomach), and 20 (mucinous stomach adenocarcinoma). *P*-values were determined by two-tailed Mann-Whitney *U* test (vs. ESCC [**d**], vs. tumor [**e**]). **e**, PDAC-classical/MUC program expression levels in the oeso-stm models and tumors excluding ESCC. ESCCs exhibit low levels of PDAC-classical/MUC program expression and are enriched in the traditional models (35.4%) relative to the organoids (0%) and tumors (15.3%). However, the traditional cell lines showed the lowest PDAC-classical/MUC

program expression even after removing the ESCC samples. *n* = 510 (tumor), 86 (NextGen) and 52 (traditional). **f**, Comparison of the Classical score based on the study by Moffitt et al.⁴⁶ with the PDAC-classical/MUC program score defined by Gavish et al.³⁷ The Classical score and the PDAC-classical/MUC program expression score was calculated for all the TCGA-plus solid tumor samples (*n* = 11,029). These two scores correlated strongly overall (*r* = 0.969). Here and in **h**, **j**, regression lines with 95% confidence intervals are displayed. **g**, Levels of Moffitt et al. Classical score in the TCGA-plus tumor samples per lineage. The Moffitt et al. Classical scores were plotted for the TCGA-plus solid tumor samples as in **b**. The Moffitt et al. Classical score was also high in the three GI lineages (pancreas, oeso-stm and colorectal; mean expression score > 2.6), but low (mean expression score < 0.7) in the other lineages. **h**, Lack of constant association between ABSOLUTE tumor purity score and PDAC-classical/MUC program expression. The ABSOLUTE tumor purity score, compiled from previous studies^{59,73}, were plotted against the PDAC-classical/MUC program levels for the pancreas, oeso-stm and colorectal tumors (*n* = 149, 375 and 448, respectively). The expression scores of this program did not show constant association with tumor purity. **i**, Strong dependencies correlated with PDAC-classical/MUC program expression in the NextGen organoids propagated in conditioned media containing Wnt3a/R-Spondin1 (*n* = 60). The correlation (Pearson *r*; x-axis) between the PDAC-classical/MUC program expression and dependency scores for all the strong dependency genes identified from the organoid screen data (*n* = 3,521) and the significance of correlation ($-\log_{10}[P]$ /y-axis; two-tailed Pearson correlation) were plotted. The dependencies on selective regulators of the Wnt signaling pathway (including *WLS*, *MESD*, and *FZDS*) exhibited strong correlation (*r* < -0.37) with the level of PDAC-classical/MUC program expression even when the analysis was limited to the organoids that were propagated in media containing Wnt3a/R-spondin1. Blue points indicate members of the MSigDB 'GOBP_WNT_SIGNALING_PATHWAY' gene set. **j**, Wnt pathway genes whose dependencies did not correlate significantly with the PDAC-classical/MUC program expression. None of the four Wnt pathway genes shown here correlated significantly with the PDAC-classical/MUC program expression (*r* > -0.2 for all), while each of them had at least one model that is strongly dependent (Dependency score < -1) on the respective gene. **k**, Association between dependencies on Wnt pathway regulators (*MESD*, *WLS*, *TCF7L2*, *FZDS* and *LGR4*) and mutations. For each of the hotspot or damaging mutations that have ≥ 2 mutated organoids (with CRISPR screen data; *n* = 108 [model], 587 [mutation]), the dependencies on these regulators of the Wnt signaling pathway were compared between the non-mutated and mutated organoids. The mean difference of dependency (x-axis) and the significance of the difference ($-\log_{10}[P]$ /y-axis; two-tailed Mann-Whitney *U* test) were plotted, where none of them were significant (*q* > 0.27).

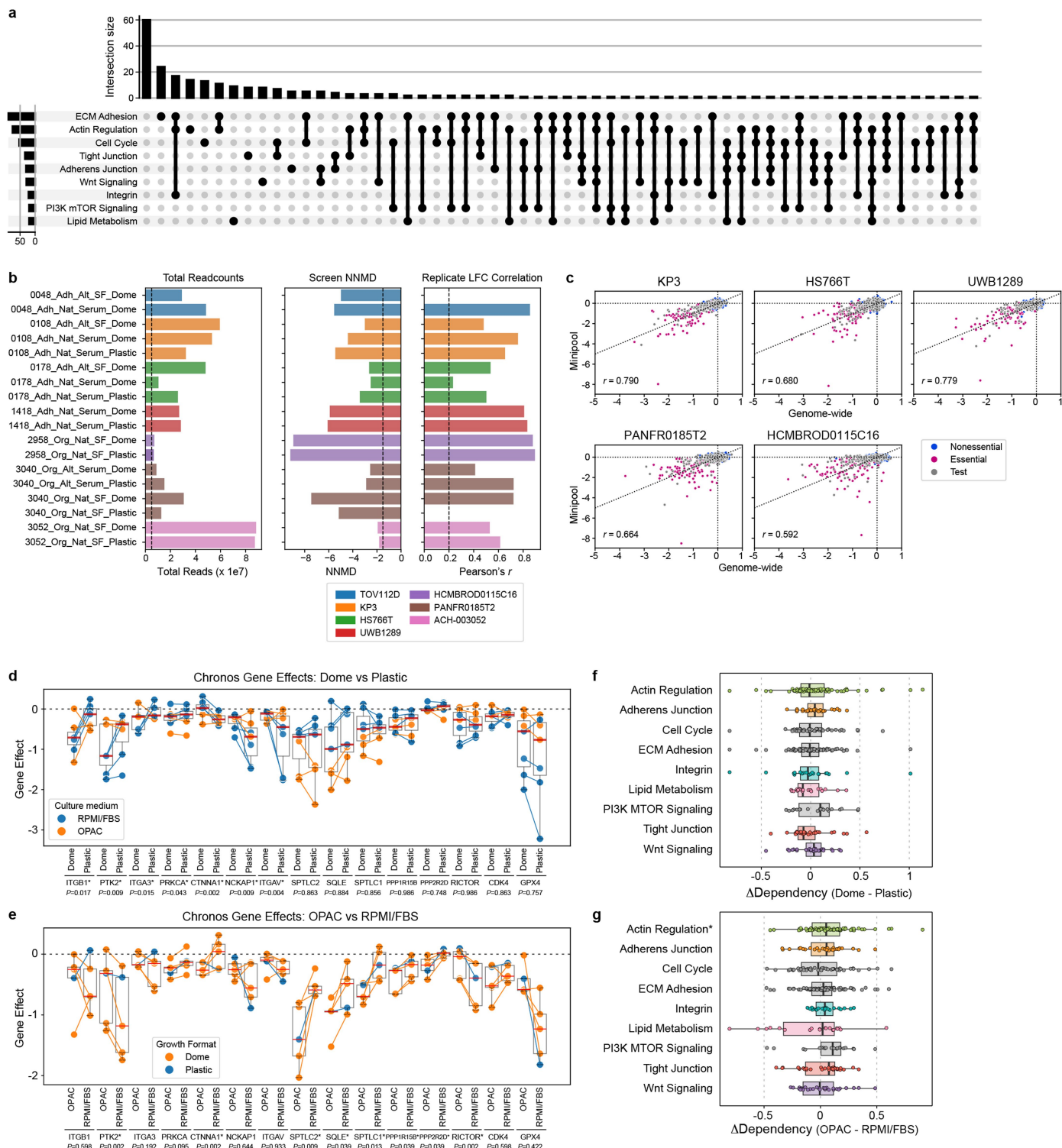


Extended Data Fig. 8 | See next page for caption.

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Extended Data Fig. 8 | Extended analyses of Wnt dependency and other cancer gene dependencies. **a**, Lack of constant association between Wnt-activating genetic alteration and Wnt regulator-dependency. All the CRISPR-screened organoids ($n = 108$) categorized into Wnt pathway gene alteration-positive ($n = 30$) or -negative ($n = 78$) groups based on mutations/CN alterations in *CTNNB1*, *APC*, *AXIN2*, or *RNF43*. The positive group included 21 *APC*, 1 *AXIN2*, 5 *CTNNB1* hotspot, 5 *RNF43* damaging mutations, and 1 *CTNNB1* CN amplification, with some models harboring multiple alterations. Dependencies on the Wnt signaling pathway regulators (*MESD*, *WLS*, *TCF7L2*, *FZD5* and *LGR4*) were plotted for each of these two groups (left). The expression levels of the PDAC-classical/MUC program were also plotted. For the Wnt pathway gene alteration-positive (red box) group, the point colors indicate the type of alteration. Significance of the difference was assessed by two-tailed Mann-Whitney U test. The centre line and box denote median and 25th to 75th percentile data points, respectively, and whiskers extend up to 1.5 times the interquartile range from box edges. Harboring Wnt-activating alterations did not have constant effects on Wnt regulator dependencies: it reduced the dependencies on *MESD*, *WLS*, *FZD5* and *LGR4*, but enhanced *TCF7L2* dependency. **b**, Mutation-independent association between PDAC-classical/MUC program expression and dependencies on Wnt regulators. The correlation between PDAC-classical/MUC program expression and dependency on the five regulators of Wnt signaling (*MESD*, *WLS*, *TCF7L2*, *FZD5* and *LGR4*) as well as their mean dependency scores in the organoid models were plotted, where Wnt-activating genetic alterations were indicated by the color code shown in **a**. All the organoids with strong mean dependency scores on these Wnt regulators (mean Chronos score < -1 ; $n = 21$) exhibited enhanced PDAC-classical/MUC program expression (expression score > 2.8) regardless of Wnt-activating genetic alterations. Regression lines with 95% confidence intervals are also displayed. **c**, Wnt dependency in the traditional cell lines and organoids. The mean dependency in the organoids ($n = 108$; y-axis) and lineage-matched traditional models (screen $n = 485$; x-axis) were plotted for all the members of the MSigDB 'GOBP_WNT_SIGNALING_PATHWAY' gene set. For the seven members that exhibited significant correlation with the PDAC-classical MP expression (*MESD*, *WLS*, *TCF7L2*, *FZD5*, *LGR4*, *MARK2* and *SOX9*; see Fig. 4d), the NextGen organoids exhibited stronger dependency than lineage-matched traditional cell lines (mean[NextGen] $>$ mean[traditional]). However, this held true for less than a half of the remaining members of this gene set (198/438 [45.2%]). **d, e**, Differential dependencies on frequently mutated oncogenes and tumor suppressor genes (TSGs) between 2D models and 3D organoids. Dependency on frequently mutated ($>1\%$ mutation rate in the TCGA cohort⁵³) oncogenes and TSGs (40 oncogenes and 50 TSGs) were compared between the 3D organoids grown in serum-free conditions ($n = 94$) and lineage-matched 2D adherent models cultured directly on plastic plates with serum-containing media ($n = 433$). In **d**, the mean dependency scores in 2D vs 3D models are represented in the x- and y-axes, respectively. Here and in **f**, the color of the points indicates the type of the cancer genes (red: oncogenes, blue: TSGs) and

the point sizes indicate the significance of the difference. Only 22 oncogenes and 20 TSGs that exhibited statistically significant ($q < 0.05$) difference of dependency were shown here, while in **c**, the pathways displaying significant and non-significant ($q \geq 0.05$) differences are distinguished by filled and open circles, respectively. We also compared the dependency on each of these cancer genes after removing the mutant (hotspot mutation for oncogenes; damaging mutations for TSGs) models for the respective gene, but it remained significant for all the 42 genes shown here. In **e**, the distribution of dependency in individual models were plotted for two representative cancer genes (*CDK4* and *MDM2*), where the lineage was indicated by the point color (see **g** for the legend). For *MDM2*, the dependency distribution in *TP53* WT models ($n = 22$ [3D organoids], 97 [2D models]) were also plotted (right). Here and in **g**, the statistical significance was assessed by two-tailed Mann-Whitney U test. **f, g**, A pathway-level summary of the differential dependencies on frequently mutated oncogenes and TSGs. Oncogenes and TSGs analyzed in **a** were classified into the 10 oncogenic signaling pathways in accordance with a previous publication⁵³. For each of these 10 pathways, mean dependency scores on the oncogenes or TSGs that belong to the respective pathway were calculated and these mean scores were compared between 3D (y-axis; $n = 94$) and 2D (x-axis; $n = 433$) models (**f**). The pathway:cancer gene type (oncogene or TSG) pairs with two or more genes were plotted. The mean dependency scores of the individual models were also plotted for the TSGs of the p53 pathway (**g**). **h**, Clustering of the gene sets that are enriched for differential dependencies between 2D models and 3D organoids. In the global comparison of gene dependencies, the nine gene sets with significant overrepresentation ($q < 0.05$ by one-sided Fisher's exact test) of the 200 outlier genes (top 100 genes in either extreme) in the distribution of mean dependency differences (see Fig. 5a) were clustered by the degree of overlap in their component genes. Note that this analysis was conducted not only on the selected cancer genes but on all the genes measured in the screen except for the common essential genes (as opposed to **d-g**). The number of member genes within each of these gene sets is: 200 (HALLMARK_OXIDATIVE_PHOSPHORYLATION), 200 (HALLMARK_MTORC1_SIGNALING), 200 (HALLMARK_G2M_CHECKPOINT), 139 (KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS), 158 (HALLMARK_FATTY_ACID_METABOLISM), 200 (HALLMARK_E2F_TARGETS), 199 (KEGG_FOCAL_ADHESION), 213 (KEGG_REGULATION_OF_ACTIN_CYTOSKELETON), and 158 (HALLMARK_UV_RESPONSE_UP). Two pairs of gene sets exhibited a high-degree of overlap (overlap coefficient > 0.3) with each other and therefore we merged each of them into one gene set: • KEGG_FOCAL_ADHESION and KEGG_REGULATION_OF_ACTIN_CYTOSKELETON (merged and renamed as 'Adhesion/Cytoskeleton') • HALLMARK_G2M_CHECKPOINT and HALLMARK_E2F_TARGETS ('Cell Cycle') The remaining five gene sets, HALLMARK_FATTY_ACID_METABOLISM ('Lipid Metabolism'), HALLMARK_MTORC1_SIGNALING ('mTORC1'), HALLMARK_OXIDATIVE_PHOSPHORYLATION ('OxPhos'), HALLMARK_UV_RESPONSE_UP ('UV'), and KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS ('SLE') were not merged with others. Also see Fig. 5a,b.

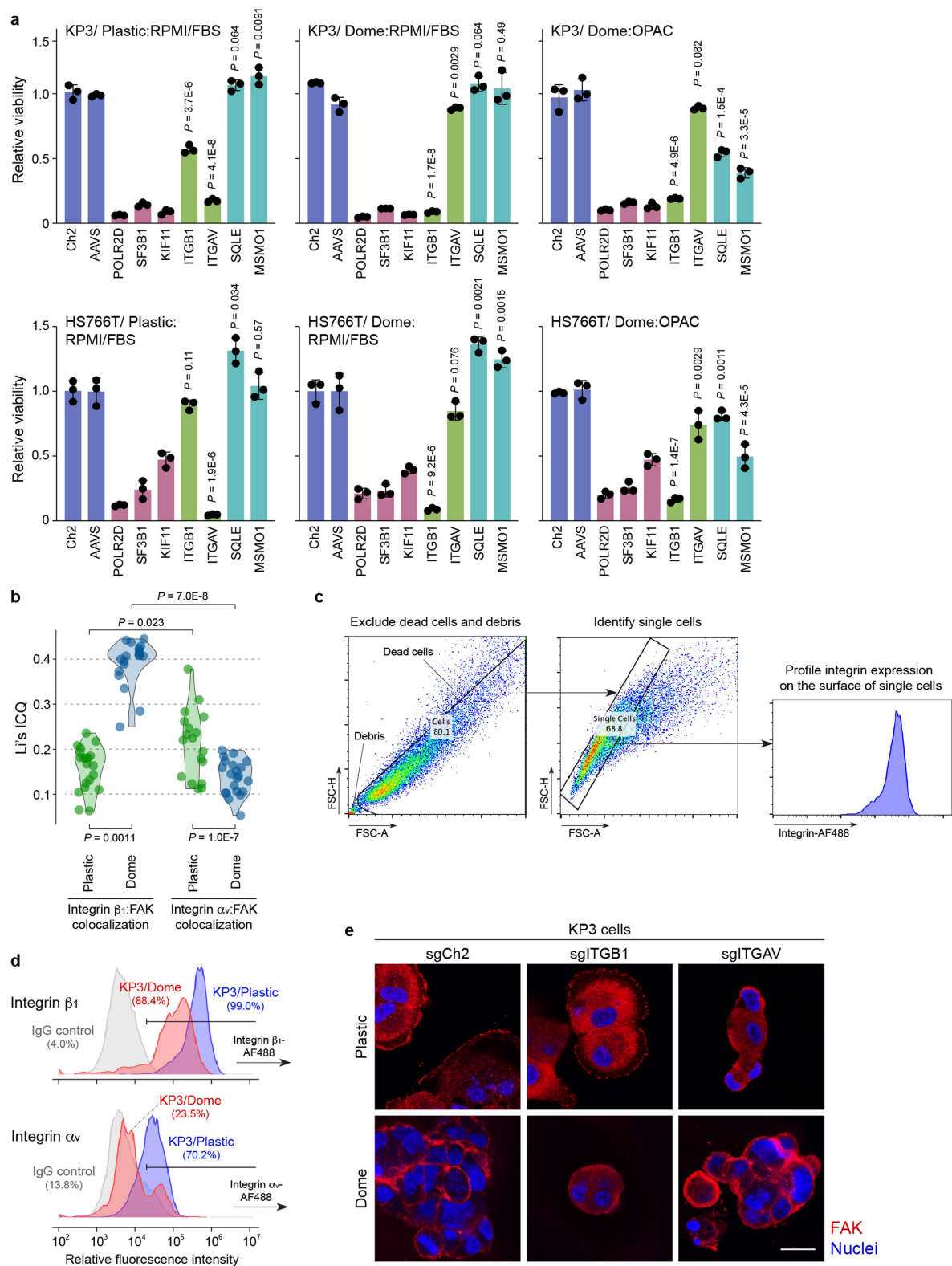


Extended Data Fig. 9 | See next page for caption.

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Extended Data Fig. 9 | CRISPR minipool screens in different growth formats and medium types. **a**, Composition of test genes in the minipool library ($n = 250$). The library for the minipool screen was designed primarily to evaluate the essentiality of genes that exhibit large differences in the systematic comparison of gene dependencies between 2D models and 3D organoids (see Fig. 5a,b). These genes include those involved in the regulation of adhesion/cytoskeleton (further divided into “ECM Adhesion”, “Actin Regulation”, “Tight Junction”, “Adherens Junction” and “Integrin” classes) and the control of cholesterol homeostasis (as a part of the “Lipid Metabolism” class). They were categorized into nine core classes that are listed on the left based on the function of the encoded protein. Some of the test genes belong to multiple classes, while others do not belong to any of these classes but are still included in the library based on specific interests. The membership patterns of individual genes across the nine functional classes, along with the count of test genes exhibiting each specific pattern, are shown (top). The number of test genes that belong to each of the nine classes were also plotted (left). **b**, Quality control (QC) test of individual minipool screens. Seven models were subjected to screening with the minipool library under different combinations of growth formats (adherent culture on plastic dishes [plastic] and Matrigel dome culture [dome]) and culture media (RPMI/FBS and OPAC serum-free media). 18 screens passed the criteria for QC, which were total readcounts $\geq 5 \times 10^6$ and null-normalized mean difference (NNMD) ≤ -1.5 . These QC metrics for individual screens as well as the log fold change (LFC) correlation of replicate samples (where replicate samples were available) are presented. **c**, Correlation between the genome-wide screen results and minipool screen results. The dependency scores from the genome-wide CRISPR screen (x-axis) and minipool screen (y-axis) were plotted for all the genes (nonessential [negative] controls, common essential [positive] controls and test genes) that were targeted by the minipool library for all the five models screened in minipool format under the same conditions as the genome-wide screens. This showed high concordance ($r > 0.59$) between the genome-wide screen result and minipool screen result for all these pairs. **d,e**, Impact of the growth format and culture medium on the Chronos gene effect of representative genes measured by the minipool screen. All genes with significant differential dependency across conditions, in addition to *CDK4* and *GPX4*, are shown. In **d**, the Chronos gene effects under the Matrigel dome culture (dome) and adherent culture on a plastic plate (plastic) were plotted for pairs of matched screens (same model type, same culture

medium; $n = 7$). Results from cultures with the RPMI/FBS medium and OPAC medium are indicated by the blue and orange points, respectively. In **e**, the Chronos gene effects under cultures with the RPMI/FBS medium (serum-containing) and OPAC medium (serum-free) were plotted for pairs of matched screens (same model type, same growth format; $n = 5$). Results from the dome culture and plastic culture are indicated by the blue and orange points, respectively. In both plots, the results from the same pair are indicated by the connecting line and the red horizontal line indicates the median gene effect score of the sample group. The statistical significance of the difference in the gene effects under dome vs plastic cultures as well as under RPMI/FBS vs OPAC media, assessed by a two-tailed paired t -test, are also indicated. Genes whose dependency was significantly ($P < 0.05$) affected by the growth formats (dome vs plastic) or culture media (RPMI/FBS vs OPAC) are indicated by the asterisks. The dependencies on three integrin subunits (*ITGB1*, *ITGAV* and *ITGA3*) and *NCKAP1*, a regulator of cytoskeletal organization, were specifically influenced by the growth format but not by the culture medium. In contrast, the dependencies on three mediators of lipid metabolism (*SPTLC1*, *SPTLC2* and *SQLE*) and *RICTOR*, an oncogene in the PI3K/mTOR pathway, were affected by the culture medium but not by the growth format. The dependency on *PTK2*, a gene encoding FAK—a central mediator of adhesion-dependent signaling, was significantly altered by both growth format and culture medium. Here and in **f,g**, the centre line and box denote median and 25th to 75th percentile data points, respectively, and whiskers extend up to 1.5 times the interquartile range from box edges. **f,g**, The effects of growth formats and culture media on gene dependencies per gene class. Differential dependencies resulting from different growth formats (dome - plastic; **f**) and different culture media (OPAC - RPMI/FBS; **g**), both calculated as coordinates in a linear regression model (see Methods), were plotted for each of the nine classes (see **a**) of minipool test genes. None of these gene classes exhibited significant enrichment or depletion of dependencies (relative to the rest of the test genes) upon changes in growth format or culture medium, except for the effect of culture medium on ‘Actin Regulation’ gene dependencies, which were enhanced with the RPMI/FBS medium as compared to the OPAC medium ($q = 0.0224$ by a two-tailed Mann-Whitney U test; indicated by the asterisk). $n = 75$ (actin regulation), 30 (adherens junction), 53 (cell cycle), 89 (ECM adhesion), 22 (integrin), 20 (lipid metabolism), 20 (PI3K/mTOR signaling), 34 (tight junction) and 29 (WNT signaling).



Extended Data Fig.10 | See next page for caption.

Extended Data Fig. 10 | Growth formats and culture media respectively influence dependencies on integrins and cholesterol biosynthesis mediators. **a**, Specific effects of growth format and culture medium on the dependencies on integrin and regulators of cholesterol homeostasis. The KP3-enAsCas12a and HS766T-enAsCas12a cells were infected with lentivirus expressing cutting control sgRNAs (sgCh2 and sgAAVS), sgRNAs against pan-dependency genes (sgPOLR2D, sgSF3B1 and sgKIF11), or sgRNAs for indicated test genes. Following a puromycin selection to eliminate uninfected cells, these cells were propagated under different growth formats (monolayer culture on plastic dishes [plastic] vs Matrigel dome culture [dome]) and culture media (RPMI/FBS or OPAC medium) for seven days and viability scores relative to the average viability of cells transduced with cutting control sgRNAs were scored and plotted. Values = means $\pm$ SD ($n = 3$). The same experimental results were also presented in Fig. 5h, where the average viability of cells transduced with cutting control sgRNAs was set as 0, the average viability of cells with knockout (KO) of pan-dependency genes was set as -1 and the viability scores of cells that received sgRNAs against the test genes were plotted on this scale. *P*-values were scored by two-tailed two-way ANOVA test (vs samples with cutting control sgRNAs [sgCh2 and sgAAVS]). **b**, Colocalization of FAK with different integrin chains between plastic and dome growth formats. The KP3 cells were grown under plastic or dome conditions with the RPMI/FBS medium for five days and immunostained for either integrin β_1 and FAK or integrin α_v and FAK (see Fig. 5i). Colocalization between integrin subunits and FAK was scored by calculating intensity correlation quotient, or Li's ICQ⁷⁴, on background-subtracted images. Shown are the aggregated results of two independent experiments. *P*-values were scored by two-tailed Mann-Whitney *U* test. $n = 20$ (integrin β_1 /FAK staining, plastic), 19 (integrin β_1 /FAK staining, dome),

18 (integrin α_v /FAK staining, plastic) and 21 (integrin α_v /FAK staining, dome). **c,d**, Flow cytometry analysis of cell-surface integrin β_1/α_v expression under different growth formats. The KP3 cells, grown under plastic or dome conditions with the RPMI/FBS medium for 5 days, were stained for integrin β_1 and integrin α_v without permeabilization and analyzed by flow cytometry to assess the cell-surface expression levels of these integrin chains. The gating strategy for flow cytometry analysis is shown in **c**. Thus, debris and dead cells were first excluded based on forward scatter-area (FSC-A) and side scatter-area (SSC-A) profiles. Subsequently, singlets were identified based on FSC-A and forward scatter-height (FSC-H) profiles. These singlets were then subjected to integrin expression analysis. The cell-surface expression level of integrin α_v , but not that of integrin β_1 , was reduced to the negative control (isotype-matched IgG) level under the dome growth (**d**). **e**, Differing roles of integrin β_1 and integrin α_v in controlling the localization of FAK under plastic vs dome culture conditions. KP3-enAsCas12a cells were infected with lentivirus expressing a cutting control sgRNA (sgCh2), or sgRNAs targeting *ITGB1* or *ITGAV*. These cells were propagated under plastic or dome conditions with the RPMI/FBS medium for 4 days and immunostained for FAK (red). The nuclei of these cells were also visualized by DAPI staining (blue). In the control (sgCh2) cells, FAK accumulated to the cell periphery under both two culture conditions (dot- or oval-like patterns in plastic culture and uniform membrane staining in dome culture). The knockout (KO) of *ITGB1* prevented this accumulation specifically in the dome but not in plastic culture. In contrast, the KO of *ITGAV* prevented FAK localization to cell periphery only in the plastic but not dome culture. Representative images from three independent experiments are displayed. Bar = 20 μ m.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Cancer model profiling data were obtained in-house as described in Methods.
Data analysis	For some of the analyses, we used following open source code: Chronos (ver 2.0.8) and GSEAPy (ver 1.1.5). For other analyses, we used custom code written in R (ver 4.4.2) or Python (ver 3.11.5), which is available on Github (https://github.com/broadinstitute/achilles-pipeline-2.0 [Achilles pipeline for gene effect]; https://github.com/broadinstitute/DepMap-NextGen-Public [downstream analyses]). For the colocalization analysis on micrographs, we used ImageJ (ver 1.54p) with the Coloc 2 (ver 3.1.0) plugin.

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Data

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All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Raw sequencing data of WGS and RNAseq for most of the NextGen models profiled in the present study are available in the dbGaP database (<https://>

www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs003444.v4.p1; dbGaP Study Accession: phs003444.v4.p1). Raw profiling data (including data for mutation, copy number, gene expression, and gene dependency) and processed data required for running the custom codes used in this study as well as the metadata of the samples are available to be downloaded from the DepMap portal (https://depmap.org/portal/data_page/?tab=allData; file set name: "NextGen Model Manuscript 2026"). Other analytical results from this study are available in a Figshare collection (<https://doi.org/10.6084/m9.figshare.29472362>).

Some data used in this study were obtained from public repositories. Specifically, in Fig. 1c, tumor mutation data were collected through the cBioPortal (<https://www.cbioportal.org/>) from the following studies within the TCGA PanCancer Atlas: COAD/READ, PAAD, PRAD, and BRCA; except for the ones labeled with red rectangles, which were acquired from following publications: Olympios et al. (PMID: 33800183) for the rate of CNS tumor with TERT promoter hotspot mutation, TCGA-COAD/READ (PMID: 22810696) for the rate of colorectal tumors with chromosome (Chr) 18q loss, TCGA-PAAD (PMID: 22810144) for the pancreatic tumor Chr9p loss rate, and MSK-MET (PMID: 35120664) for the rates of colorectal tumors with NRAS hotspot mutation, breast tumors with ESR1 hotspot mutation, and prostate tumors with SPOP damaging mutation. In Figs. 1c,d and Extended Data Figs. 1a,b, GRCh38.p14 human genome assembly (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.40/) was used as the reference sequence in the mutation calls. In Extended Data Fig. 5c, gene expression data for the combined cohort of TCGA, TARGET and GTEx samples (n = 8,167 [normal] and 10,530 [tumor]) were obtained through the UCSC Xena browser (<https://xenabrowser.net/>; Study name: TCGA TARGET GTEx). In Extended Data Fig. 7h, ABSOLUTE tumor purity data of TCGA tumors were compiled from following publications: Liu et al. (PMID: 29622466), and TCGA-PAAD.

Research involving human participants, their data, or biological material

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Reporting on sex and gender

Among the 314 NextGen cancer models used in this study, 169 were derived from male patients, 138 were derived from female patients, and 7 were derived from patients with unknown sex. Information on gender and sexual orientation was not collected.

Reporting on race, ethnicity, or other socially relevant groupings

Among the 314 NextGen models used in this study, 78 were derived from Caucasian patients, 5 were derived from Latino or Hispanic patients, 5 were derived from Asian patients, 8 were derived from Black or African American patients, and 218 were derived from patients of unknown race.

Population characteristics

Among the 314 NextGen models used in this study, 268 have information of the age of the patient from whom the model was derived. The average age of the patients was 61.0. Treatment status of the patients at the time of model derivation was also recorded and shown in Supplementary Table 1.

Recruitment

The selection of patients for model derivation was based in part on the specific interest on particular cancer subtype or genotype (e.g., neuroendocrine and castration-resistant prostate cancers, KRAS-wild-type pancreas cancers). To avoid potential bias caused by this, we limited our analysis mostly to the association between genetic dependency and gene expression across cancer subtypes while minimizing focused analysis on specific cancer lineage, subtype and genotype.

Ethics oversight

Protocols for the acquisition of patient tumor samples and establishment of models from these samples were approved by each of the five institutions (Broad Institute, Dana-Farber Cancer Institute, University of Pittsburgh, Memorial Sloan-Kettering Cancer Center and Cold Spring Harbor Laboratory) involved in the derivation of the respective model. Profiling of these models and analyses of the data from them were approved by the Broad Institute.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

The sample size in this study was determined on the basis of model availability. No sample size calculation was performed. A number of highly statistically significant observations on the difference between NextGen and traditional cancer models (e.g., CDK6 dependency in glial glioblastoma (GBM) models, dependency on Wnt signaling mediators between organoid models and 2D adherent models) indicate that the sample size was sufficient to give confidence on these findings.

Data exclusions

For the genome-wide CRISPR screens, we excluded screen data that do not meet either of the following criteria:

- Reads per gene ≥ 185
- NNMD (null-normalized median difference) ≤ -1.25
- Correlation between replicates ≥ 0.19

For the minipool screens, we excluded screen data that do not meet either of the following criteria:

- NNMD ≤ -1.5
- Correlation between replicates ≥ 0.2

Also, data from models that do not have complete dataset for the specific analysis (e.g., gene expression data from models that do not have CRISPR data in the analysis for the association between gene expression and gene dependency) were excluded. In addition, traditional models that were included in the "undifferentiated cluster" on the Celligner gene expression map (n = 132) were excluded from a part of the plot

(labeled as “out of undifferentiated cluster”) in Extended Data Fig. 2a due to the strong enrichment of traditional models in this cluster and associated difficulty in predicting the model lineage based on the similarity to tumor gene expression.

Replication

For the genome-wide CRISPR screens, all the screens after July 2022 were performed in two technical replicates at $\geq 250\times$ representation using a combined “Humagne C” and “Humagne D” library. The majority (16 out of 18 screens) of minipool screens were also conducted with technical duplicates. Screens with the Pearson correlation coefficient between the replicate below 0.19 (for genome-wide screens) or 0.2 (for minipool screens) were excluded from the analysis.

The assessment of sensitivity to SCD inhibitors (A939572, CAY-10566) in a series of four esophageal adenocarcinoma organoids (Fig. 2i) was conducted twice each with four technical replicates and the results were reproducible.

The analyses for viability and cell cycle phase following the treatment of NextGen glioblastoma (GBM) models with CDK4/6 inhibitors (Abemaciclib, Palbociclib, and Ribociclib) were repeated twice and the results were concordant.

The measurement of viability of the KP3 and HS766T cells after the knockout of ITGB1, ITGAV, MSMO1, or SQLE (Fig. 5h, Extended Data Fig. 10a) were conducted twice each with three technical replicates and the results were reproducible. Immunostaining of the KP3 cells with or without the knockout of ITGB1 or ITGAV (Fig. 5i, Extended Data Fig. 10e) was conducted three times and the results were consistent. Extended Data Fig. 10b shows the aggregated result of two experiments.

Randomization

Randomization of experimental samples is not relevant for the present study, since whenever samples were grouped (e.g., CDKN2A CN-neutral and -loss GBM models), group allocation was defined by the feature of the models without arbitrariness.

Blinding

Blinding is not relevant for the present study, since whenever samples were grouped (e.g., CDKN2A CN-neutral and -loss GBM models), group allocation was defined by the feature of the models without arbitrariness.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- | | |
|-------------------------------------|---|
| n/a | Involved in the study |
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

Methods

- | | |
|-------------------------------------|--|
| n/a | Involved in the study |
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

Antibodies

Antibodies used

We used following antibodies in the present study:

- anti-CD29/Integrin beta1 (BD Biosciences, 556048)
- anti-integrin beta 1(Developmental Studies Hybridoma Bank [DSHB], A1IB2)
- anti-Integrin alphaV (MilliporeSigma, AB1930)
- anti-integrin alpha V (DSHB, P3G8)
- anti-FAK (MilliporeSigma, 05-537)
- anti-FAK (MilliporeSigma, 06-543)
- anti-phospho-Histone H3, Alexa Fluor 488 (Cell Signaling, 3465S)
- rat IgG1 kappa Isotype Control (Thermo Fisher, 14-4301-82)
- mouse IgG1 kappa Isotype Control (Thermo Fisher, 14-4714-82)
- goat anti-mouse IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11001)
- goat anti-rabbit IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11008)
- goat anti-rat IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11006)
- goat anti-mouse IgG secondary antibody, Alexa Fluor 568 (Thermo Fisher, A-11004)
- goat anti-rabbit IgG secondary antibody, Alexa Fluor 568 (Thermo Fisher, A-11011)

Validation

Below we summarize the vendor, catalog number, information on antibody validation on the manufacturers' website and use cases of each antibody in the literature:

- anti-CD29/Integrin beta1 (BD Biosciences, 556048): According to the manufacturer, this antibody is routinely tested by flow cytometric analysis. This antibody has been repeatedly used for immunofluorescence in the literature (e.g., Shibue et al. 2012, Cancer Discov [PMID: 22609699]; Petridou and Skourides 2016, Nat Commun [PMID: 26952307]; Benedicto et al. 2017, Nat Commun [OMID: 28524846]).
- anti-integrin beta 1(Developmental Studies Hybridoma Bank [DSHB], A1IB2): According to the manufacturer, this antibody is recommended for ELISA, FACS, FFPE, function blocking, IF, IHC, IP and WB. This antibody has been repeatedly used for FACS in the literature (e.g., Zawistowski et al. 2013, Mol Cell Biol [PMID: 23530058]; Spiess et al. 2018, J Cell Biol [PMID: 29632027]; Grimm et al. 2020 J Cell Biol [PMID: 33119040]).
- anti-Integrin alphaV (MilliporeSigma, AB1930): According to the manufacturer, this antibody has been published & validated for use

in ELISA, IH, IP, radioactive immunoassays & WB. This antibody has been repeatedly used for immunofluorescence in the literature (e.g., Goossens et al. 2009, BMC Dev Biol [PMID: 19126199]; Shibue et al. 2012, Cancer Discov [PMID: 22609699]; King et al. 2016, Sci Adv [PMID: 23876551]).

- anti-integrin alpha V (DSHB, P3G8): According to the manufacturer, this antibody is recommended for ELISA, FACS, FFPE, IHC and IP. This antibody has been repeatedly used for FACS in the literature (e.g., Kobinski et al. 2002, J Biol Chem [PMID: 12070442]; Jia et al. 2013, Anticancer Drugs [PMID: 23275294]; Li et al., 2017, Nat Materials [PMID: 28783156]).

- anti-FAK (MilliporeSigma, 05-537): According to the manufacturer, this antibody is routinely evaluated by immunoblot on 3T3/A31 RIPA cell lysate, PC-12, Hep-G2, SW 620 or Jurkat RIPA cell lysates. This antibody has been repeatedly used for immunofluorescence in the literature (e.g., Shibue and Weinberg 2009, Proc Natl Acad Sci USA [PMID: 19502425]; Petridou and Skourides 2016, Nat Commun [PMID: 26952307]; Imamoto et al. 2020, Life Sci Alliance [PMID: 32041892]).

- anti-FAK (MilliporeSigma, 06-543): According to the manufacturer, 10 ug/mL of this antibody showed positive immunostaining for Fak in Ref 52 cells fixed with 3.7% formaldehyde. This antibody has been repeatedly used for immunofluorescence in the literature (e.g., Lawson et al. 2012, J Cell Biol [PMID: 22270917]; Shibue et al. 2013, Cancer Cell [PMID: 24035453]; Zhang et al. 2022, NPJ Breast Cancer [PMID: 35332167]).

- anti-phospho-Histone H3, Alexa Fluor 488 (Cell Signaling, 34655): According to the manufacturer, this antibody detects endogenous levels of histone H3 only when phosphorylated at Ser10. The antibody does not cross-react with other phosphorylated histones or with acetylated histones. This antibody has been repeatedly used for immunofluorescence in the literature (e.g., Mich and Chen 2011, Development [PMID: 22069185]; Tatrai and Gergely 2022, EMBO J [PMID: 35678476]; Mills et al. 2022, Nat Commun [PMID: 36376301]).

- rat IgG1 kappa Isotype Control (Thermo Fisher, 14-4301-82): According to the manufacturer, this antibody has been commonly used as a negative control in experiments, as it does not bind to many mammalian antigens. This antibody has been repeatedly used for flow cytometric analysis (e.g., Sindhava et al. 2017, J Clin Invest [PMID: 28346226]; Moreira-Teixeira et al. 2020, Nat Commun [PMID: 33149141]; Schick et al. 2023, Elife [PMID: 36753434]).

- mouse IgG1 kappa Isotype Control (Thermo Fisher, 14-4714-82): According to the manufacturer, this antibody is useful as an isotype control. This antibody has been repeatedly used for flow cytometric analysis (e.g., Hota et al. 2022, Nature [PMID: 35082446]; Kim et al. 2022, JCI Insight [PMID: 35674135]; Molino et al. 2023, Aging Cell [PMID: 37254368]).

- goat anti-mouse IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11001): According to the manufacturer, this antibody has been cross-adsorbed against human IgG and human serum. This antibody has been repeatedly used for immunofluorescence and flow cytometric analysis (e.g., Nakagawa et al. 2026, Nat Neurosci [PMID: 42026254]; Yang et al. 2026, J Clin Invest [PMID: 41842971]; Kahlhofer et al. 2025, EMBO J [PMID: 41116060]).

- goat anti-rabbit IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11008): According to the manufacturer, this antibody has been cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. This antibody has been repeatedly used for immunofluorescence and flow cytometric analysis (e.g., Park et al. 2025, Nat Commun [PMID: 41203647]; Wu et al. 2026, EMBO Mol Med [PMID: 41708990]; Zhao et al. 2026, Nat Biotechnol [PMID: 41826551]).

- goat anti-rat IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11006): According to the manufacturer, this antibody has been cross-adsorbed against mouse IgG, mouse serum, and human serum. This antibody has been repeatedly used for immunofluorescence and flow cytometric analysis (e.g., Rahman et al. 2022, Cell Rep [PMID: 36417863]; Endo et al. 2025, Elife [PMID: 40990917]; Verdu Schlie et al. 2025, Genes Dev [PMID: 40210435]).

- goat anti-mouse IgG secondary antibody, Alexa Fluor 568 (Thermo Fisher, A-11004): According to the manufacturer, this antibody has been cross-adsorbed against human IgG and human serum. This antibody has been repeatedly used for immunofluorescence and flow cytometric analysis (e.g., MacDonald et al. 2024, Mol Cell [PMID: 38423013]; Kim et al. 2024, J Cell Biol [PMID: 38477830]; Hoover et al. 2025, Nature [PMID: 40562940]).

- goat anti-rabbit IgG secondary antibody, Alexa Fluor 568 (Thermo Fisher, A-11011): According to the manufacturer, this antibody has been cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. This antibody has been repeatedly used for immunofluorescence and flow cytometric analysis (e.g., Sakai et al. 2024, PNAS [PMID: 39292748]; Airich et al. 2025, EMBO Rep [PMID: 41174261]; Shen et al. 2023, Nat Methods [PMID: 36481965]).

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

The NextGen cancer models (organoids and spheroids) used in this study were either derived by one of the authors' laboratory or obtained through American Type Culture Collection (ATCC; for models derived by Human Cancer Model Initiative [HCMI]). The traditional cell lines used for the WGS/RNAseq and CRISPR screen were obtained from DepMap and the original source can be found on the DepMap portal (<https://DepMap.org/portal>). The cell lines used for the minipool screen experiments were obtained from ATCC or Japanese Collection of Research Bioresource (JCRB): KP3, JCRB; HS766T, ATCC, TOV112D: ATCC, UWB1289, ATCC. The 293FT cells, Wnt3a-producing L cells (L Wnt-3A), and R-Spondin1-producing 293T cells were obtained from Thermo Fisher Scientific, ATCC, and R&D Systems, respectively.

Authentication

All the NextGen and traditional cancer models used in this study were authenticated by short tandem repeat (STR) profiling (conducted by LabCorp) prior to expansion. This procedure was repeated monthly during expansion and screening.

Mycoplasma contamination

All the NextGen and traditional cancer models used in this study were confirmed for the absence of mycoplasma

Mycoplasma contamination

contamination (using Mycoplasma PCR Detection Kit; ABM, G238) prior to expansion. This procedure was repeated monthly during expansion and screening.

Commonly misidentified lines
(See [ICLAC](#) register)

To make the Cancer Dependency Map (DepMap) a comprehensive resource for the cancer model data, we reasoned that including cell lines that are commonly misidentified would still be beneficial as long as they were confidently identified on our end. Accordingly, we conducted STR profiling of commonly misidentified cell lines and for those whose identity was confirmed, we decided to profile them and incorporate the data from them into DepMap. Specifically, following commonly misidentified cell lines were used as a part of the traditional model cohort in the present study. All of them are confirmed for their STR profiles before being used for the analysis.

EJ-1 (ACH-003100), GOS-3 (ACH-000027), HHUA (ACH-002026), KCI-MOH1 (ACH-001098), KP-1N (ACH-001107), KPL-1 (ACH-000028), LN-319 (ACH-000128), LN-443 (ACH-000673), LN-464 (ACH-000676), MEL-HO (ACH-000450), MKN28 (ACH-002161), ONCO-DG-1 (ACH-000013), OVMIU (ACH-002183), PLB-985 (ACH-000034), SF767 (ACH-000490), SH-2 (ACH-002675), SK-MG-1 (ACH-002304), SK-N-MC (ACH-000039), SNG-M (ACH-000974), U-118 MG (ACH-000040)

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

KP3 cells were grown in either plastic or dome conditions in RPMI with 10% FBS for five days. For harvesting, cells were first washed with PBS twice. Plastic-cultured cells were subsequently detached from the plate by incubating with 5 mM EDTA/PBS for 10 min at 37 °C and collected. Dome-cultured cells were collected by scraping and centrifugation. The pellets containing cells and Matrigel were resuspended in 5 mM EDTA/PBS and incubated at RT for 30 min with continuous rotation to dissolve Matrigel. Subsequently, cells were fixed with 2% paraformaldehyde in PBS for 20 min at RT, washed twice with PBS and incubated with 0.1M glycine in PBS at RT for 10 min twice for the quenching of paraformaldehyde. The cells were then incubated with 10 % normal goat serum/PBS for 1 hour at RT for blocking. For primary antibody staining, 1x 10⁶ cells were incubated at RT for 1 hour with either of the following antibodies, all diluted in 10 % normal goat serum/PBS: anti-integrin β 1 (Developmental Studies Hybridoma Bank [DSHB], AIB2; 1:40 dilution), anti-integrin α v (DSHB, P3G8; 1:58), rat IgG1 kappa Isotype Control (Thermo Fisher, 14-4301-82; 1:1000), mouse IgG1 kappa Isotype Control (Thermo Fisher, 14-4714-82; 1:500). This was followed by three washes with PBS for 10 min each at RT with continuous rotation and secondary antibody staining, where cells were incubated at RT for 1 hour with either of the following antibodies, all diluted in 10 % normal goat serum/PBS: goat anti-rat IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11006; 1:500), goat anti-mouse IgG secondary antibody, Alexa Fluor 488 (Thermo Fisher, A-11001; 1:500). The cells were then washed with PBS three times 10 min each at RT with continuous rotation and analyzed by flow cytometry.

Instrument

CytoFLEX S Flow Cytometer (Beckman Coulter)

Software

The CytExpert software (Beckman Coulter) that accompanies the CytoFLEX S instrument was used for the data collection. Subsequent analyses were conducted on FlowJo 10 (ver. 10.10.0) (Becton Dickinson).

Cell population abundance

Approximately 80% of all the acquires samples fell into the "Cell" fraction, which corresponds to the remaining samples after excluding dead cells and debris. Approximately 70% of the "Cell" subpopulation fell into the "Single cell" fraction, which conforms to the singlets.

Gating strategy

The debris and dead cells were first excluded based on forward scatter-area (FSC-A) and side scatter-area (SSC-A) profiles. Subsequently, singlets were identified based on FSC-A and forward scatter-height (FSC-H) profiles. These singlets were then subjected to integrin expression analysis.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.