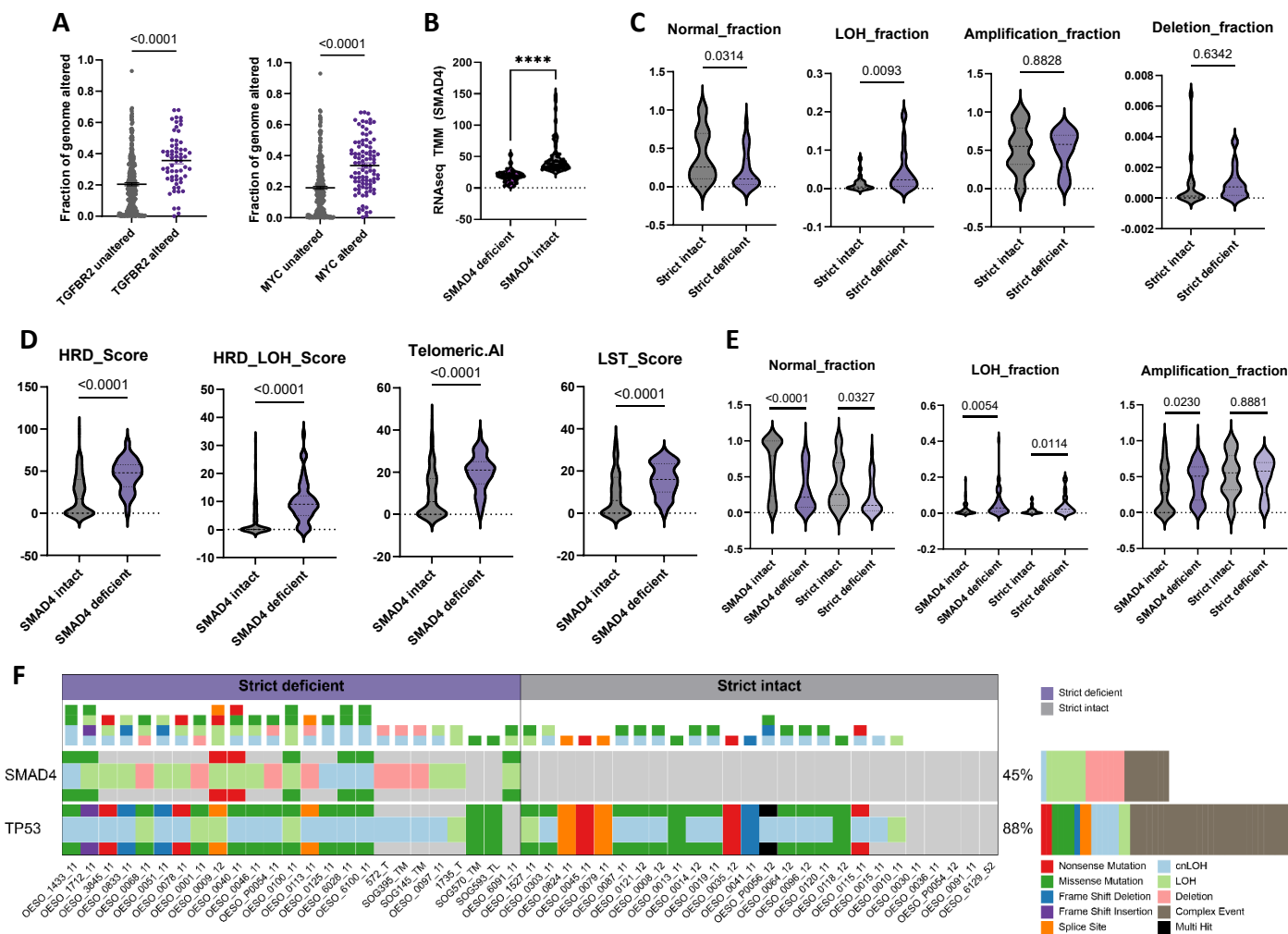


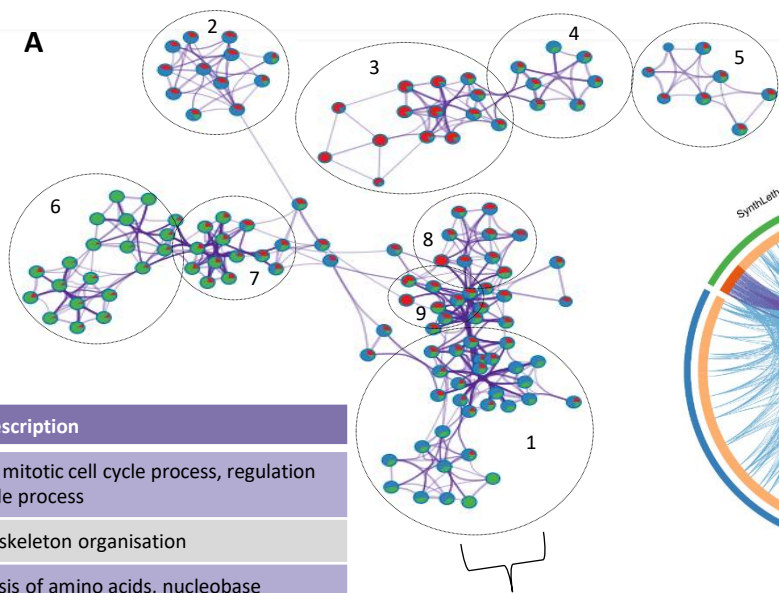
Figure S1



Supplementary Figure S1 SMAD4 loss is associated with chromosomal instability. (A) Dot plots showing fraction of the genome altered in TCGA and MSK EAC patient samples as a function of alteration of *TGFBR2* and *MYC* genes. Error bars represent SEM. (B) Violin plots indicating median and quartiles of trimmed mean of M values (TMM) for *SMAD4* mRNA expression in *SMAD4* deficient or intact samples. (C) Violin plots indicating median and quartiles of chromosomal aberrations in UQ EAC patient samples, stratified by *SMAD4* status with "strict" criteria. (D) Violin plots indicating median and quartiles of HRD score factors in UQ EAC patient samples. (E) Violin plots depicting chromosomal aberrations in UQ EAC patient samples with locus 18q21.2 removed. (F) Oncoplot showing distribution of genomic alterations in *SMAD4* and *TP53* in UQ EAC patient samples in "strict" criteria. (A-E) Unpaired Student's t-test.

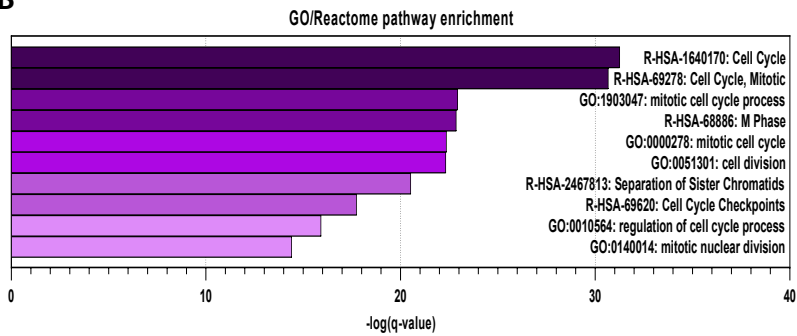
Figure S2

A

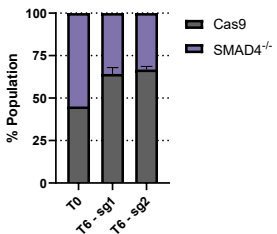
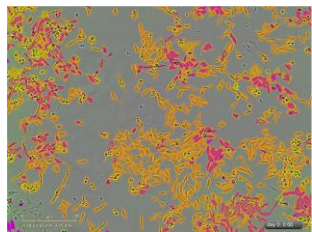


ID	Cluster description
1	Cell cycle, mitotic cell cycle process, regulation of cell cycle process
2	Actin cytoskeleton organisation
3	Biosynthesis of amino acids, nucleobase containing small molecule metabolic process
4	Heterocycle biosynthetic process
5	Protein localization to organelle
6	Metabolism of RNA, mRNA metabolic process
7	Translation
8	Amyotrophic lateral sclerosis
9	HIV infection

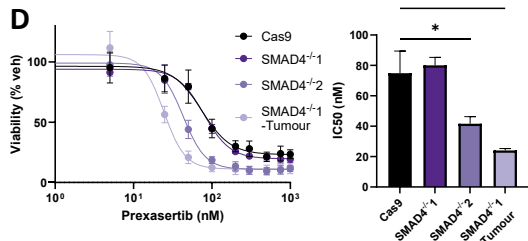
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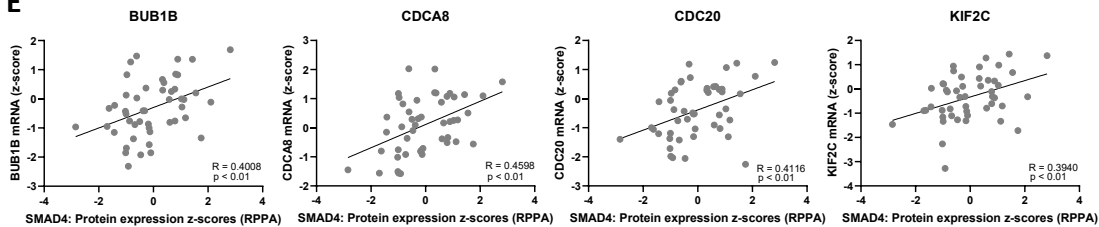
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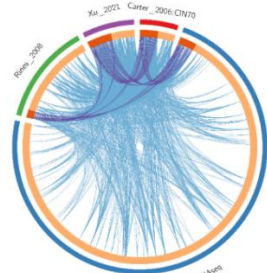
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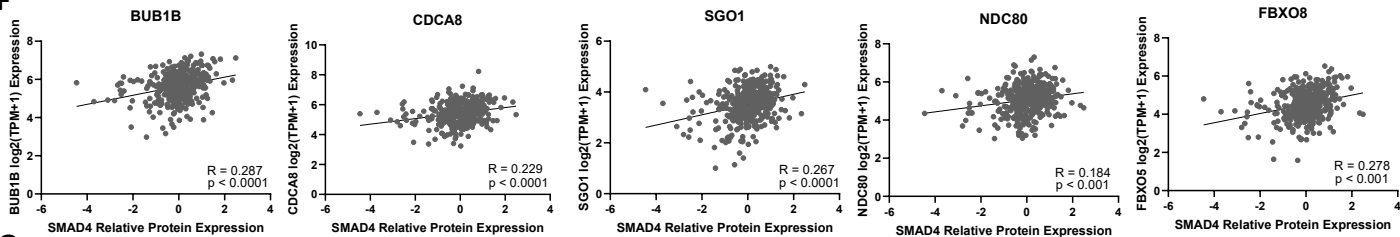
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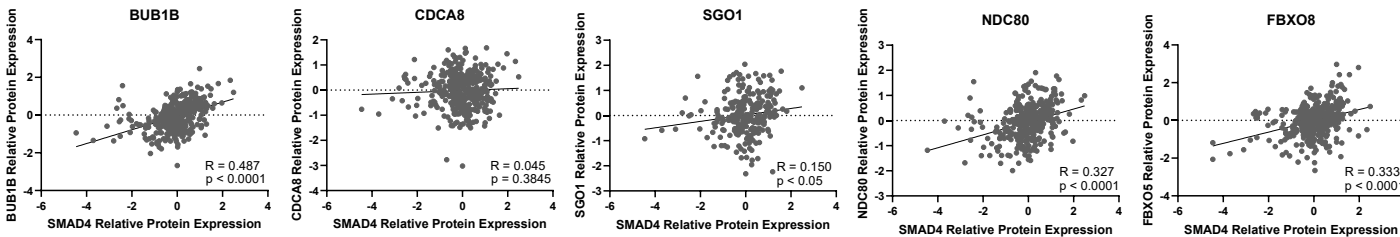
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F

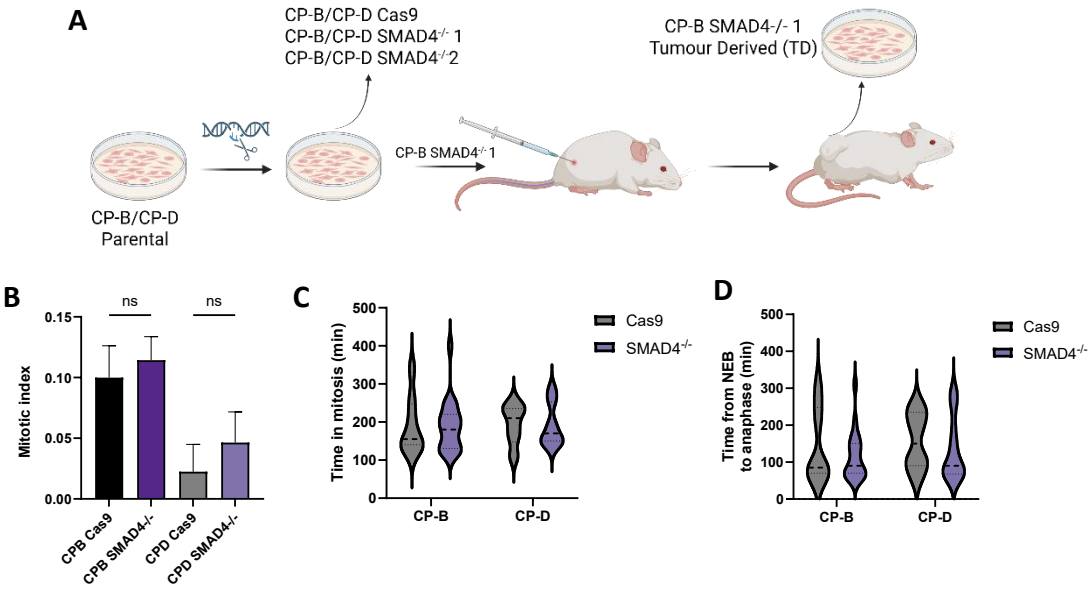


G

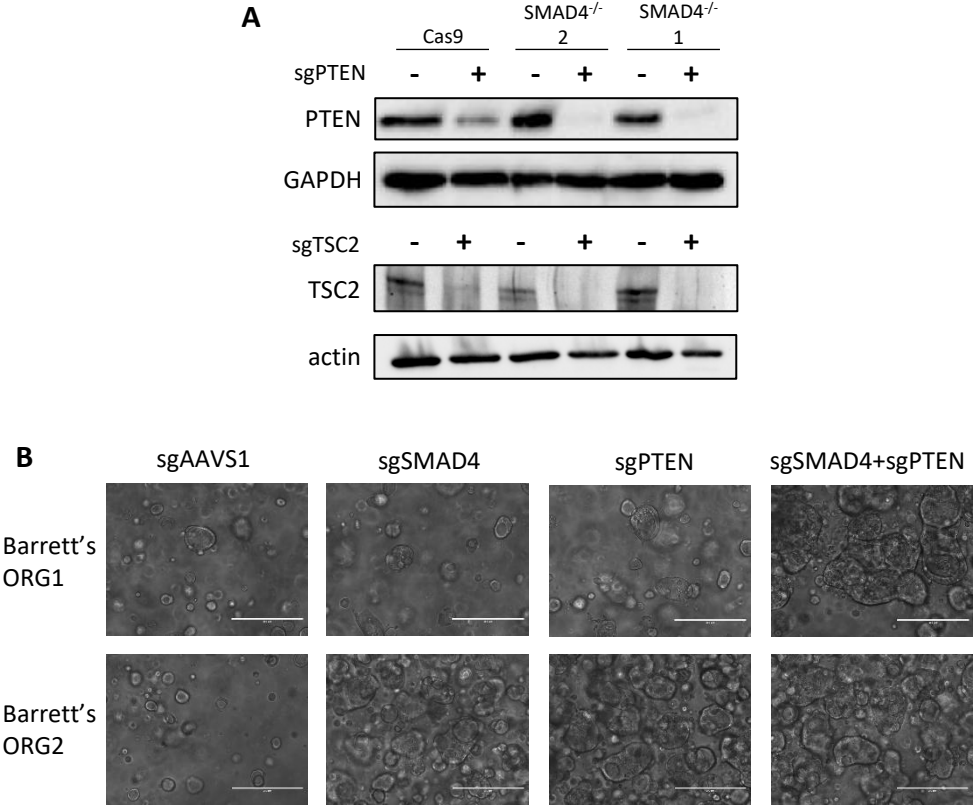


Supplementary Figure S2 SMAD4 loss is associated with a deregulated mitosis signature. (A) (Top) Network of enriched terms illustrated by nodes, colour-coded by dataset. Blue = RNAseq, red = proteomics, green = synthetic lethal CRISPR. Table lists demarked clusters in node network (1-9). (Right) Circos plot depicting overlap between gene lists from RNA-seq (blue arc), proteomics (red arc) and synthetic lethal (green arc) datasets, where the inner circle (orange) represents the total gene set in each dataset, identical genes between datasets are linked by purple curves and genes in shared enriched ontology terms are linked by light blue curves. (B) Top significantly enriched terms in “cell cycle” cluster. (C) Representative phase contrast image showing CP-B cells present at end point of competitive assay, after transduction with sgANAPC11, where Cas9-only only cells are masked in yellow and SMAD4ko (GFP-expressing) are masked in pink, with quantitative histograms for two different sgRNAs (n = 2). (D) Dose response graph depicting cell viability of CP-B cells after 120 h exposure to indicated doses of Prexasertib, normalised to vehicle, and (right) corresponding IC50 values (n = 3). Scatter plots depicting (E) Scatter plots depicting mRNA expression z-score of genes as a function of SMAD4 protein expression z-score in TCGA and MSK EAC patient samples. (E) mRNA expression transcripts per million (TPM) plus 1 and (F) relative protein expression BUB1B, CDCA8, SGO1, NDC80 and FBXO8 as a function of SMAD4 protein expression in pan-cancer cell lines (n = 365). (G) Circos plot depicting overlap between gene lists from RNA-seq (this study) and Rines (2008)(40), Xu (2021)(41) and Carter (2006)(39), where the inner circle represents the gene lists, identical genes are linked by purple curves and genes that occur in shared enriched ontology terms are linked by light blue curves. Error bars represent SEM. Correlation determined by simple linear regression and Pearson’s R.

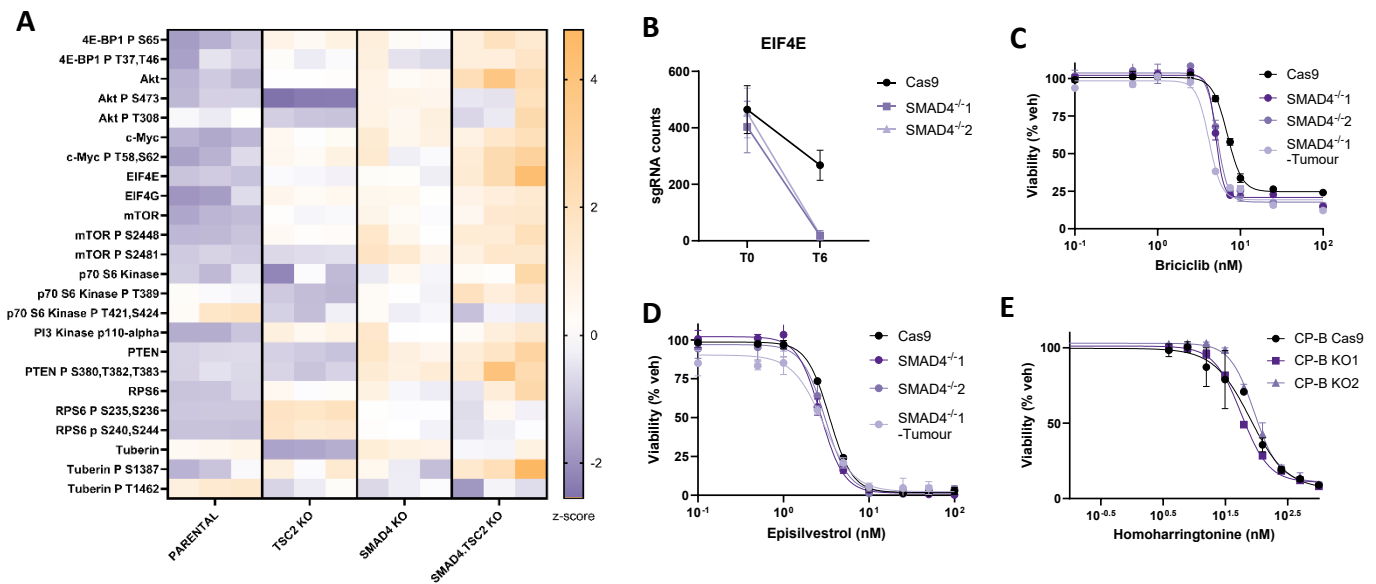
(G) Correlation determined by simple linear regression and Pearson’s R.



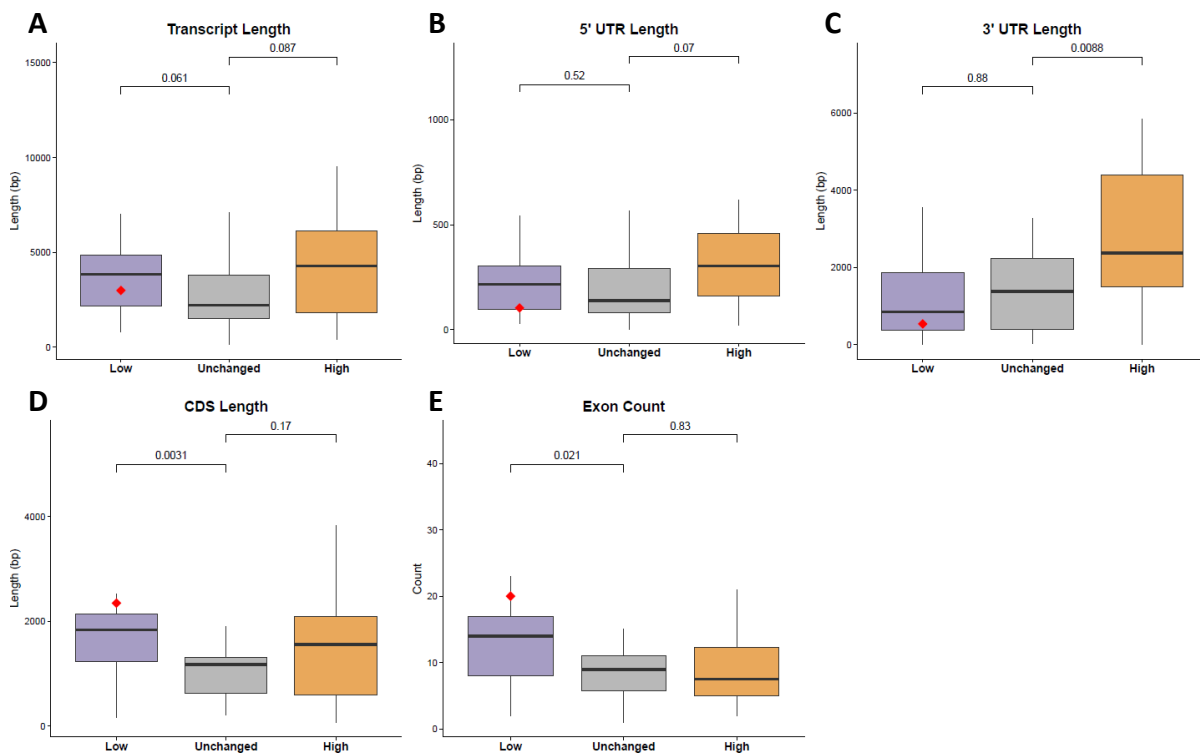
Supplementary Figure S3 SMAD4 loss is associated with a deregulated mitosis signature. (A) Schematic depicting model: CP-B/CP-D Cas9 and SMAD4^{-/-} 1 & 2 lines were derived from respective parental cell lines. CP-B SMAD4^{-/-} tumor-derived cells (TD) were derived from tumours that arose from CP-B SMAD4^{-/-} 1 cells that had been xenografted into NSG mice. (B) Bar chart depicting mitotic index of CP-B and CP-D cells with or without SMAD4. Violin plots showing (C) time in mitosis and (D) time from nuclear envelope breakdown (NEB) to anaphase onset. Error bars represent SEM. (B) Unpaired Student's t-test.



Supplementary Figure S4 SMAD4 loss and mTOR deregulation co-operate to drive tumorigenesis. (A) Immunoblot of PTEN or TSC2 in CP-B cells transduced with sgPTEN or sgTSC2, respectively. GAPDH and actin were used as loading controls. (B) Representative brightfield microscope images of two Barrett's esophagus organoid cultures (Barrett's ORG1 and 2) nucleofected with sgRNAs targeting *AAVS1* (sgAAVS1), *SMAD4* (sgSMAD4), *PTEN* (sgPTEN) and *SMAD4* and *PTEN* (sgSMAD4+sgPTEN). Scale bars represent 200 μ m.



Supplementary Figure S5 SMAD4-deficient cells and tumours upregulate mTOR signaling and cap-dependent translation initiation. (A) Heat map depicting protein and phospho-protein expression in CP-B cells as determined by RPPA, depicted as z-scores. (B) Raw sgRNA counts for EIF4E in CP-B cells at day 0 (T0) and 6 (T6) in genome-wide CRISPR-Cas9 screen. Dose response curves for (C) Briciclib, (D) Episilvestrol and (E) Homoharringtonine in CP-B cells (n=3).



Supplementary Figure S6. Structural features of translationally reprogrammed mRNAs. Box plots showing the distribution of (A) transcript length, (B) 5' UTR length, (C) 3' UTR length, (D) CDS length, and (E) exon count for genes with high (n=24), low (n=25), and unchanged (n=24) translation efficiency. Red diamonds represent CDK11B mRNA. Bars represent median $\pm$ IQR.