

Supplemental information

Targeting addiction to HMGB2-driven transcriptional programs in pancreatic cancer

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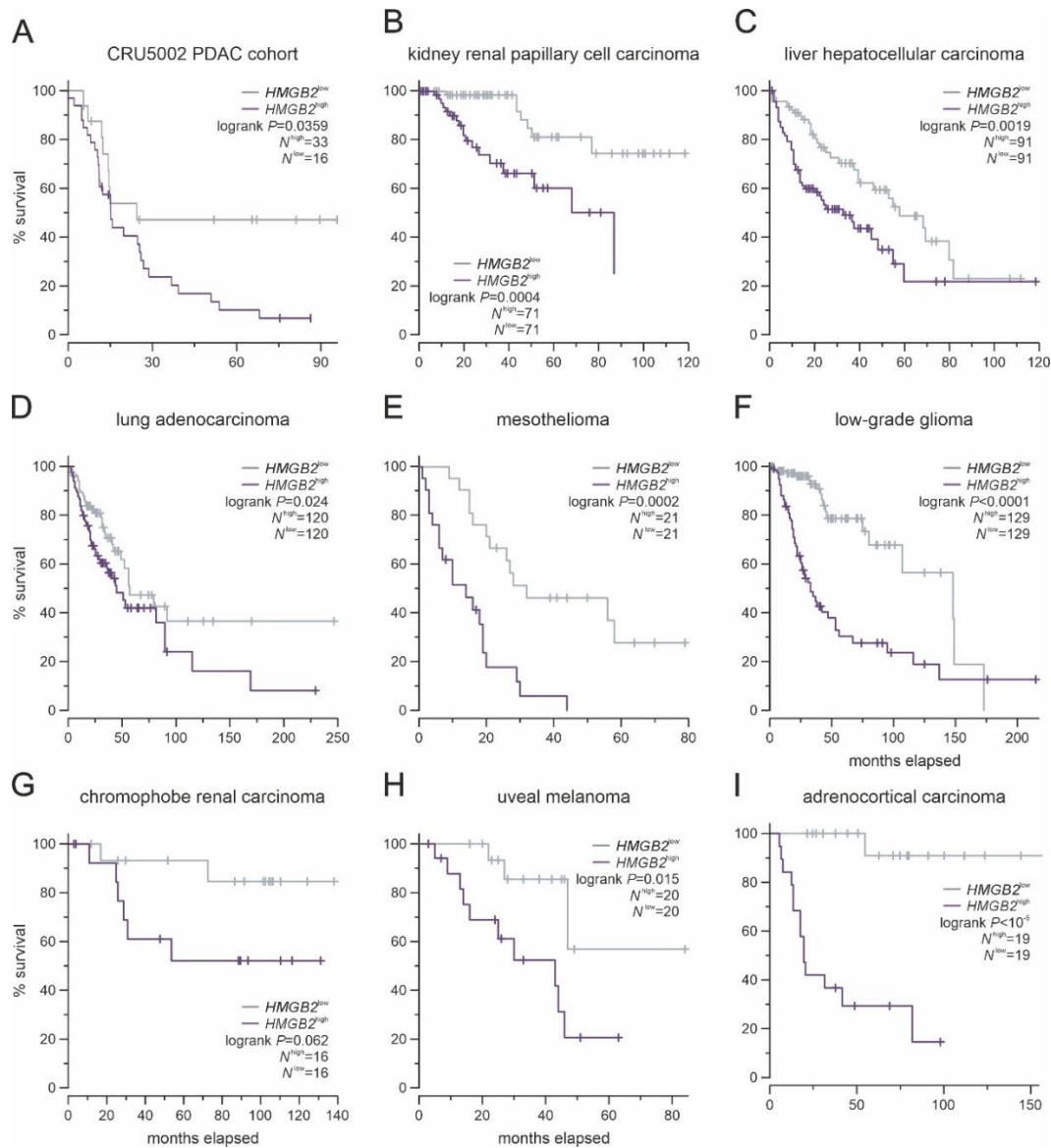


Figure S1. HMGB2 levels are prognostic in multiple cancer types, Related to Figure 1.

(A) Kaplan-Meier plot of overall CRU5002 PDAC patient survival in the top (purple, $HMGB2^{\text{high}}$) or bottom $HMGB2$ expression quantile (grey, $HMGB2^{\text{low}}$) with the associated log-rank P -value for the number of patients (N) indicated.

(B) As in panel A, but for kidney renal papillary cell carcinoma TCGA patients (data from GEPIA2²²).

(C) As in panel B, but for liver hepatocellular carcinoma patients.

(D) As in panel B, but for lung adenocarcinoma patients.

(E) As in panel B, but for mesothelioma patients.

(F) As in panel B, but for liver low-grade glioma patients.

(G) As in panel B, but for chromophobe renal cell carcinoma patients.

(H) As in panel B, but for uveal melanoma patients.

(I) As in panel B, but for adrenocortical carcinoma patients.

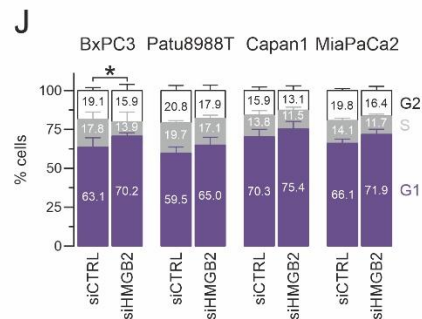
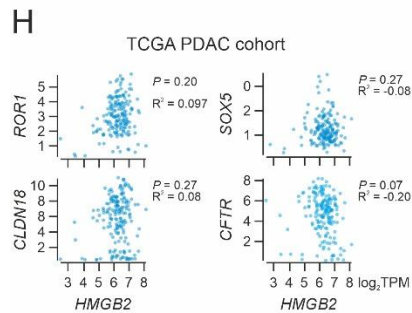
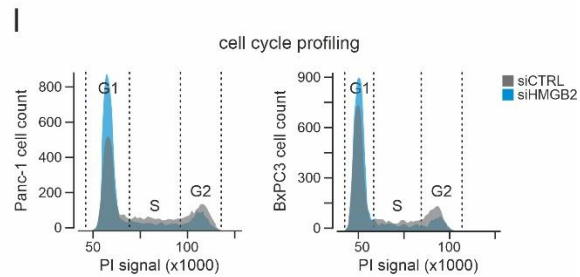
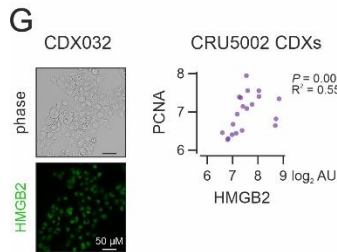
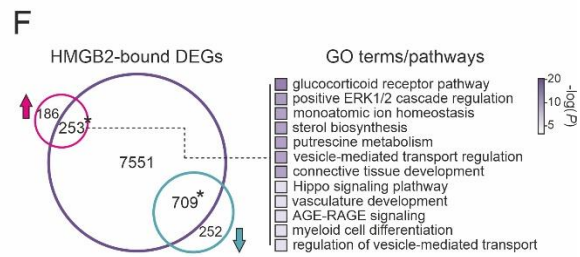
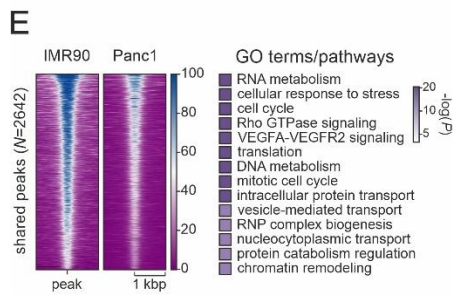
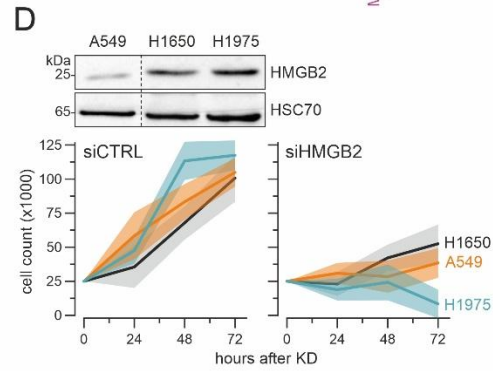
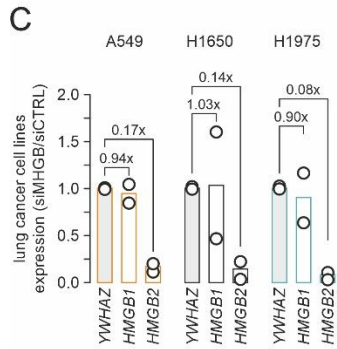
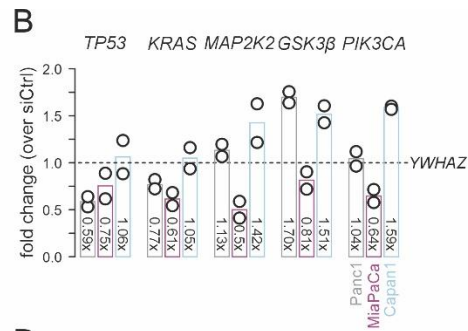
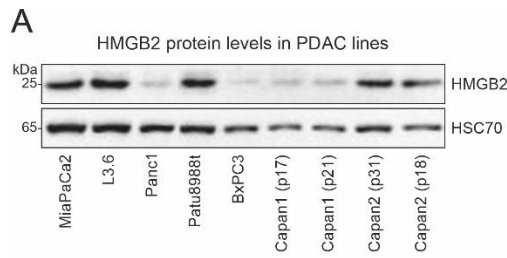


Figure S2. HMGB2 availability affects cell cycle progression, Related to Figure 2.

- (A) HMGB2 western blotting across PDAC cell lines; HSC70 provides a loading control.
- (B) Plots of fold-changes in the expression of five PDAC-related genes in Panc-1 (grey) MiaPaCa-2 (purple) or Capan-1 cells (light blue) upon *HMGB2*-knockdown (RT-qPCR data from two independent replicates); changes to *YWHAZ* levels (dotted line) provide a baseline.
- (C) As in panel B, but for *HMGB1* and *HMGB2* levels in three lung cancer cell lines.
- (D) Top: HMGB2 western blotting in three lung cancer lines; HSC70 provides a loading control. Bottom: Changes in cell numbers (mean $\pm$ SEM) for the three lines in the 72 h following transfection with control (siCtrl) or HMGB2-targeting siRNAs (siHMGB2).
- (E) Left: Tornado plots of ChIPmentation signal from IMR90 and Panc-1 cells in the 2 kbp around all shared HMGB2-bound sites. Right: GO terms associated with genes under shared peaks.
- (F) Left: Venn diagram of the overlap of genes assigned to Panc-1 HMGB2 peaks with those differentially up- (magenta) or downregulated upon HMGB2 knockdown (green). Right: GO terms associated with HMGB2-bound and upregulated genes. * $P < 0.001$, hypergeometric test.
- (G) Correlation of HMGB2 and PCNA protein levels (right) as deduced via immunofluorescence experiments (left) in a collection of CRU5002 patient-derived CDX lines. Scale bar: 50 μ m.
- (H) As in panel G, but for *HMGB2* levels relative to the expression of *ROR1*, *CLDN18*, *SOX5* and *CFTR* in TCGA PDAC patients (data retrieved from GEPIA2²²). The associated *P*-values and Spearman's correlation coefficients (R^2) are shown.
- (I) Distribution of PI-stained Panc-1 (top) or BxPC3 cells (bottom) treated with control (siCTRL) or HMGB2-targeting siRNAs (siHMGB2) in the three cell cycle phases as deduced by flow cytometry.
- (J) Bar plots of the percent of cells in different cell cycle phases as deduced by flow cytometry upon *HMGB2*-KD. * $P < 0.001$ and $X^2 > 100$, Chi-square test; $N=2$ independent replicates for BxPC3 and $N=3$ independent replicates for all other lines.

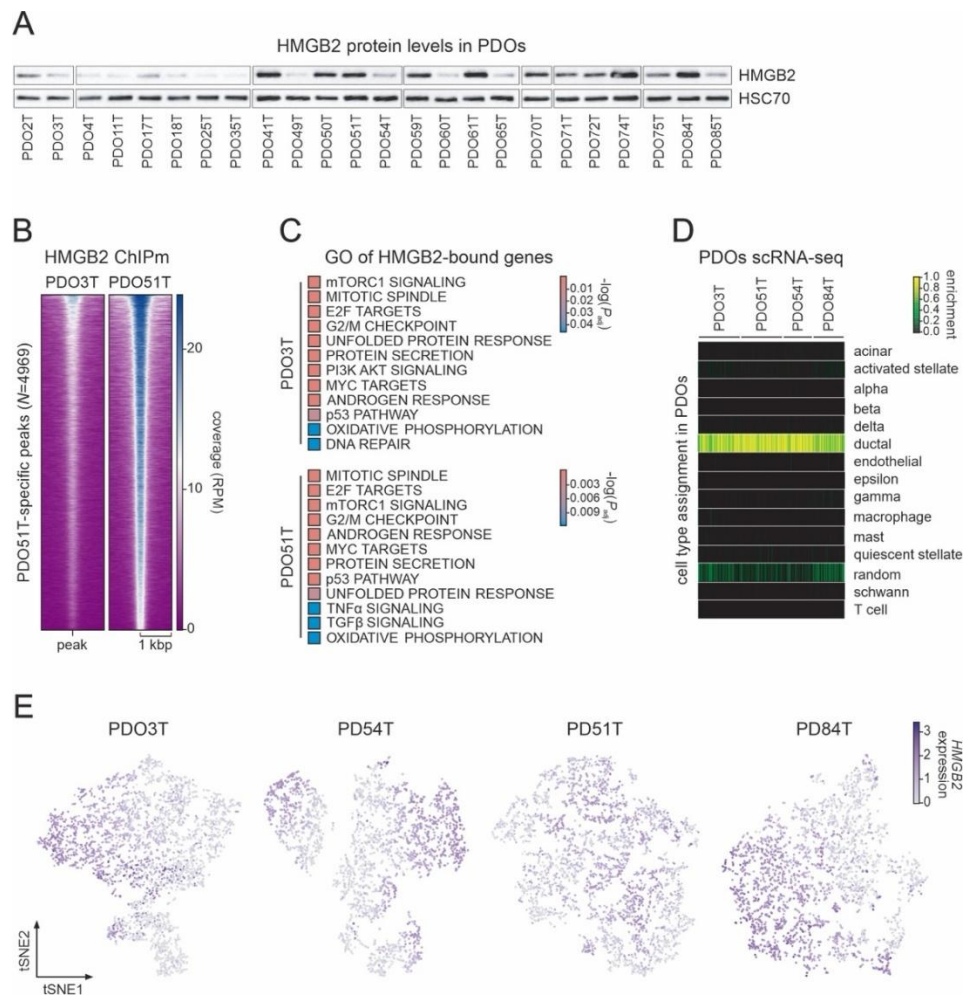


Figure S3. Molecular characteristics of CRU5002 PDOs, Related to Figure 3.

(A) HMGB2 western blotting across patient-derived PDAC organoids from the CRU5002 cohort; HSC70 provides a loading control.

(B) Tornado plots of ChIPmentation signal in the 2 kbp around PDO51T-specific HMGB2 peaks.

(C) GO terms associated with HMGB2-bound genes in PDO3T and -51T.

(D) Unsupervised cell type determination of PDO composition based on scRNA-seq data.

(E) Feature plots of *HMGB2* expression levels in individual cells from each of four PDOs.

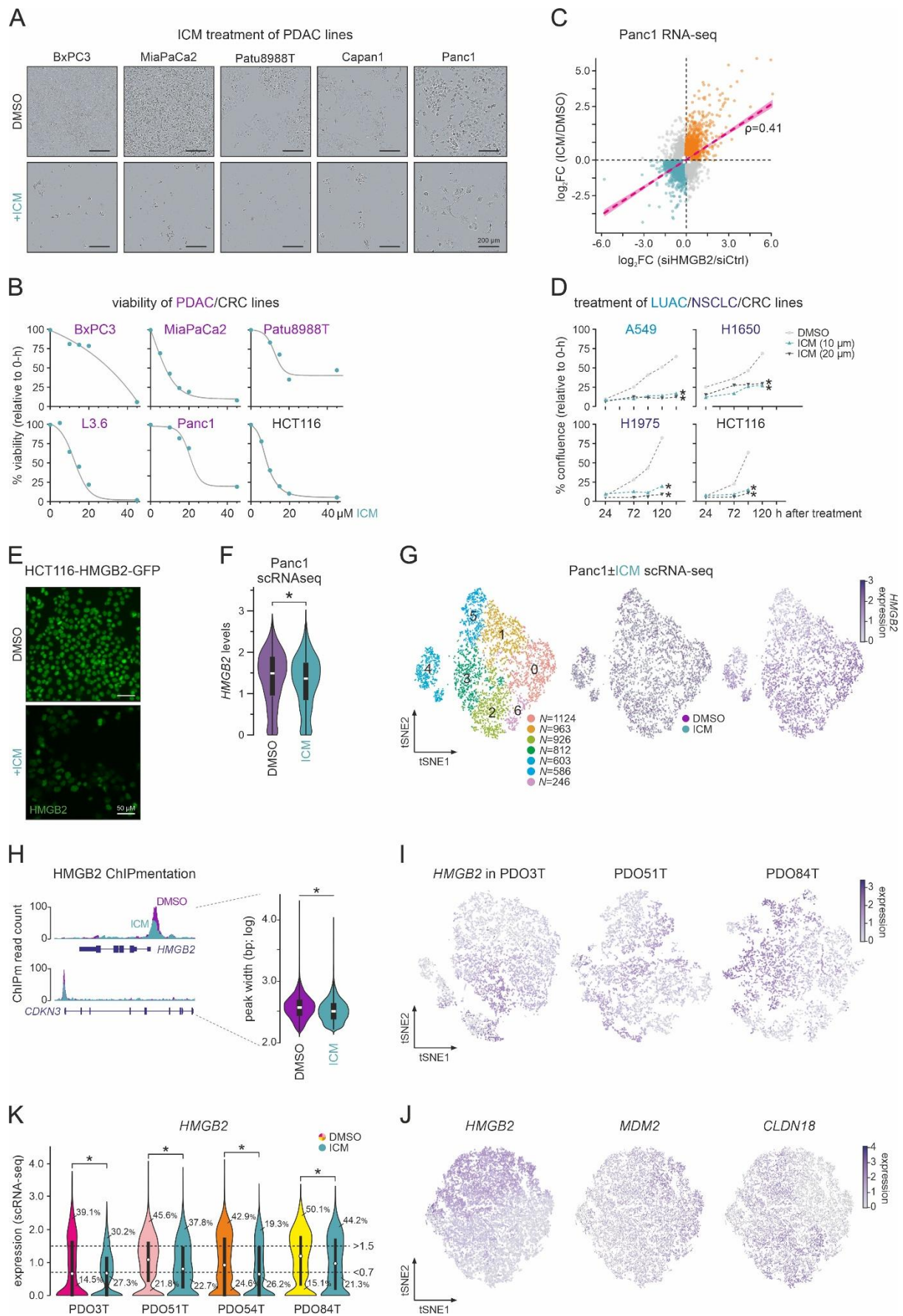


Figure S4. Effects of ICM treatment on cell lines and PDOs, Related to Figure 4.

(A) Representative images of PDAC cell lines treated with DMSO or 10 μ M ICM at $t=112$ h. Scale bars: 200 μ m.

(B) Plots of percent viability of PDAC (purple) and colorectal cell lines (black) upon increasing ICM concentrations.

(C) Correlation of expression changes ($\log_2$ FC) in Panc-1 cells treated with ICM or with *HMGB2*-targeting siRNAs; only genes with $FDR < 0.05$ were considered and the Pearson's correlation coefficient (ρ) is indicated.

(D) Plots of changes in confluence upon treatment of lung adenocarcinoma (light blue), non-small cell lung cancer (dark blue) and colorectal cancer lines (black) with DMSO or ICM. $*P < 0.01$, unpaired two-tailed Student's test at the last time-point.

(E) Images of HCT116 colorectal cancer cells expressing endogenous *HMGB2* fused to mClover (green) and treated with DMSO (left) or 10 μ M ICM for 3 days (right). Scale bars: 50 μ m.

(F) Violin plots comparing *HMGB2* levels in scRNA-seq data from Panc-1 cells treated (green) or not with 20 μ M ICM for 3 days (purple).

(G) tSNE plots of Panc-1 scRNA-seq data showing unsupervised clustering (left), distribution of control (DMSO) and ICM-treated cells (middle), and *HMGB2* expression levels in each cell (right).

(H) Left: Representative genome browser views of *HMGB2* ChIPmentation data in Panc-1 treated (green) or not with ICM (purple). Right: Violin plots of *HMGB2* ChIPmentation peak widths. $*P < 0.01$, Wilcoxon-Mann-Whitney test.

(I) Feature plots of *HMGB2* expression levels in individual cells from each PDO.

(J) As in panel H, but *HMGB2*, *MDM2* and *CLDN18* expression across all PDOs.

(K) Violin plots of *HMGB2* expression in individual cells of each PDO treated or not with ICM. $*P < 0.01$, Wilcoxon-Mann-Whitney test. The percentage of cells with high (>1.5 counts) or low *HMGB2* expression (<0.7 counts) for each PDO are shown.

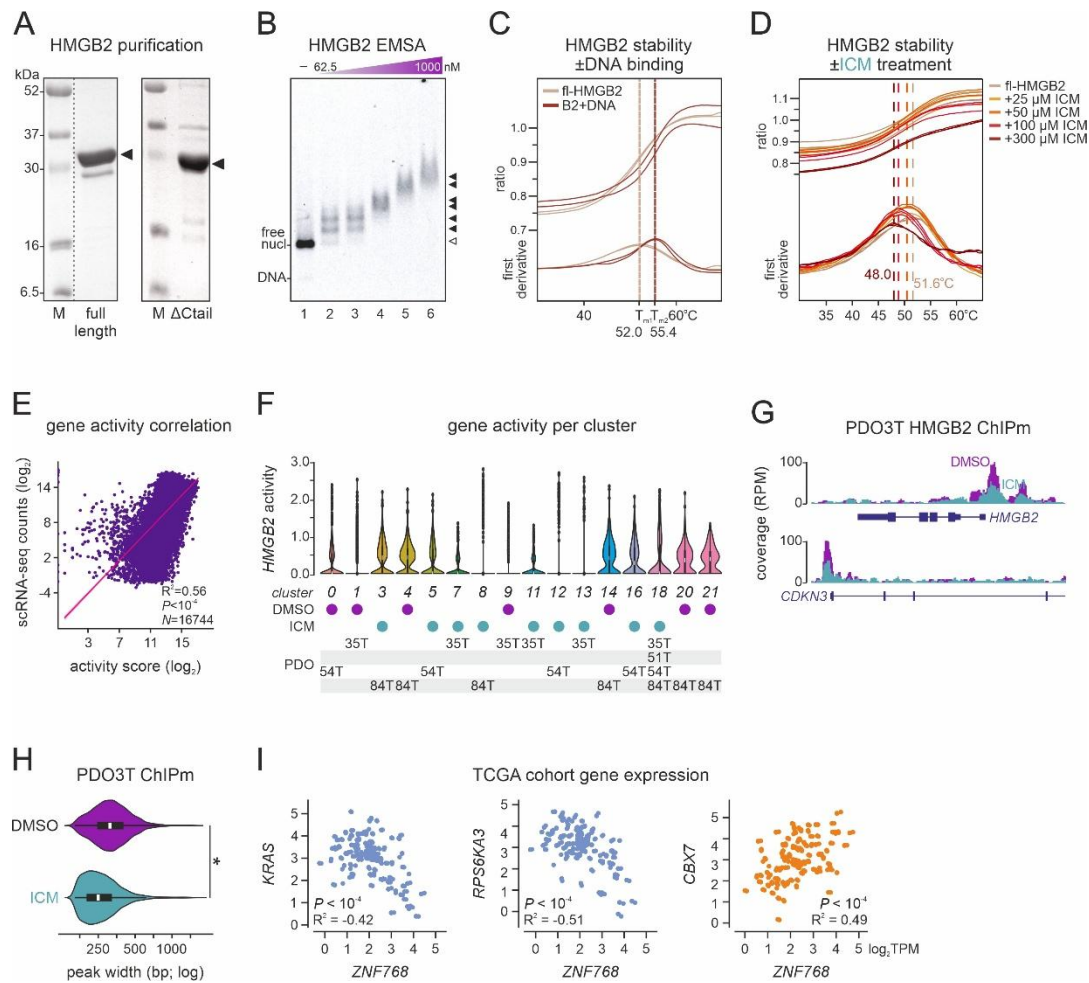


Figure S5. Characterization of HMGB2 association with chromatin, Related to Figure 5.

(A) SDS-PAGE and Coomassie blue staining of purified full length (left) and Δ Ctail HMGB2 (right) with protein marker (M) sizes indicated.

(B) Electromobility shift assay (EMSA) using Cy5-tagged nucleosomes with a DNA linker (600 fm) with increasing concentrations of HMGB2 (lanes 2-6). The migration positions of free DNA, nucleosome (open triangle) and higher-order nucleosome::HMGB2 complexes (closed triangles) are indicated.

(C) Stability of 15 μ M full-length HMGB2 assessed by nanoDSF, where intrinsic tryptophan fluorescence was measured at 330 and 350 nm over a temperature gradient from 20°C to 95°C in the absence or presence of DNA. Deduced T_m values are shown at the bottom of each graph.

(D) As in panel C, but in the presence of increasing concentrations of ICM.

(E) Correlation of gene activity scores (from scATAC-seq) and expression (scRNA-seq counts). The deduced Spearman's correlation coefficients and P -values are shown.

(F) Violin plots showing *HMGB2* activity scores from scATAC-seq data in the different tSNE clusters from control (purple dots) or ICM-treated PDOs (green dots) from Figure 5A.

(G) Genome browser views of HMGB2 ChIPmentation in PDO3T treated (green) or not with ICM (purple).

(H) Violin plots of HMGB2 peak width distribution from the data in panel G. *: $P < 0.01$, Wilcoxon-Mann-Whitney test.

(I) Correlation of *ZNF768* expression with that of *KRAS*, *RPS6KA3*, and *CBX7* in TCGA PDAC patients.

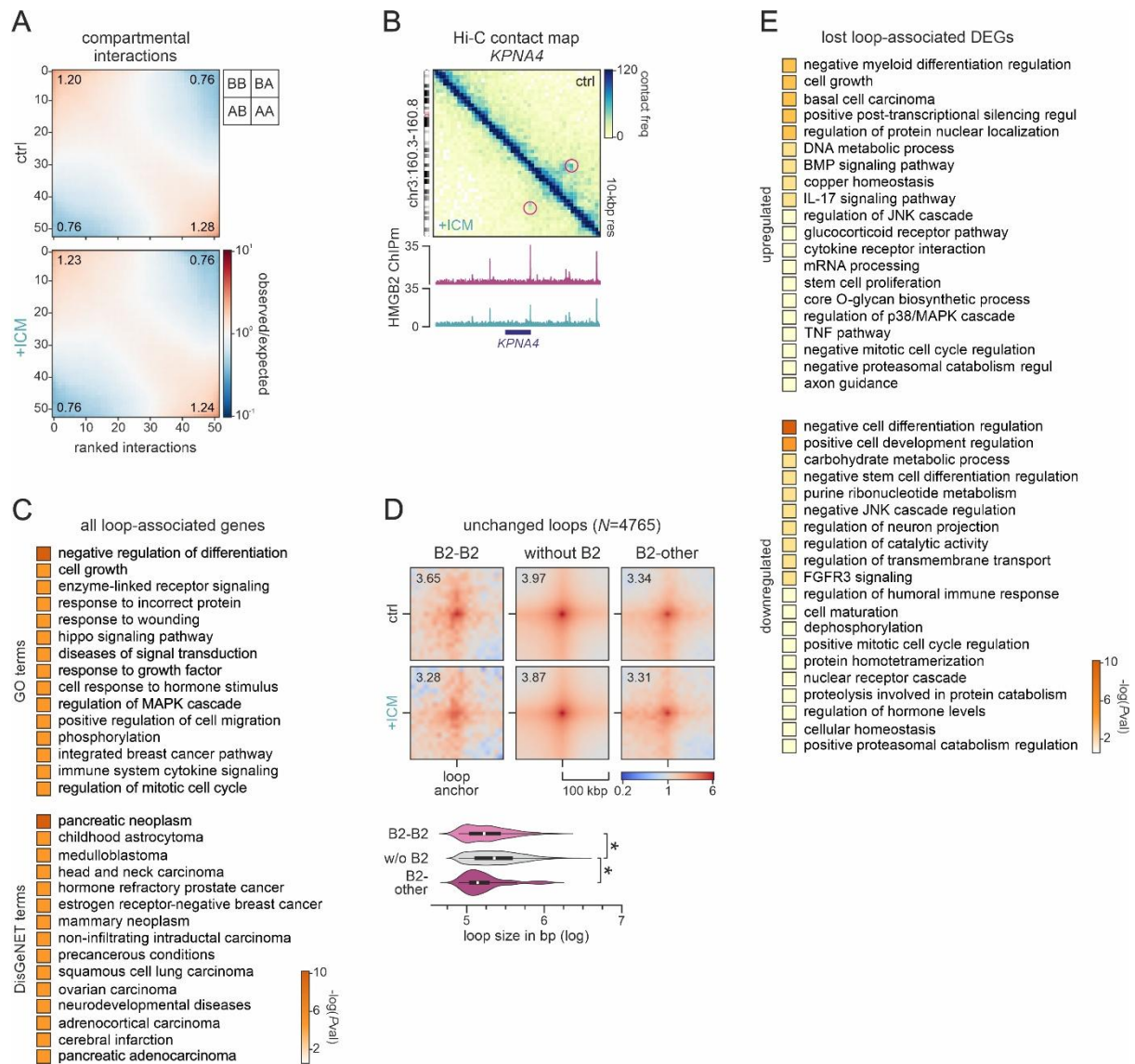


Figure S6. Multi-scale interactions and associations in PDO₃T Hi-C data, Related to Figure 6.

(A) Saddle plots of compartmental interactions calculated at a 100-kbp resolution in Hi-C data from DMSO- (ctrl) or ICM-treated PDO₃T cells (+ICM).

(B) 10-kbp resolution contact map showing loop interactions (circle) involving the *KPNA4* promoter aligned to HMGB2 ChIPmentation data from DMSO- (ctrl) or ICM-treated PDO₃T cells (+ICM).

(C) Top 15 most enriched GO terms (top) and disease associations (bottom) for all active genes overlapping loop anchors in PDO₃T cells.

(D) Top: APA plots of loops that remained unchanged between DMSO- (ctrl) and ICM-treated PDO₃T cells (+ICM) and have both (B2-B2), one (B2-other) or no anchor bound by HMGB2 (without B2). Bottom: Violin plots showing the distribution of loop sizes in each group. * $P < 0.01$, Wilcoxon ranked-sum test.

(E) As in panel D, but for GO terms associated with differentially-expressed genes (DEGs) associated with loop anchors lost upon ICM treatment.

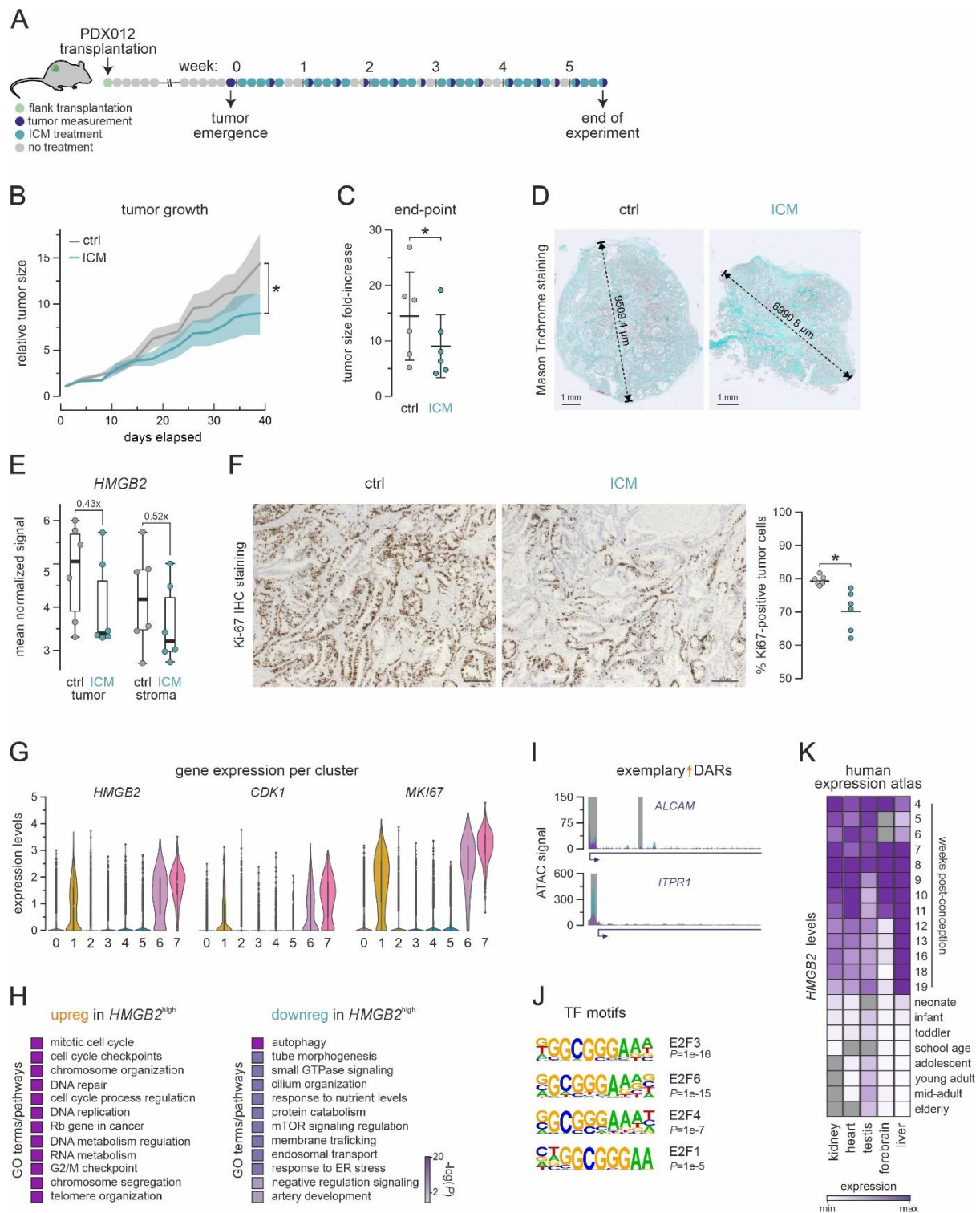


Figure S7. Effects of ICM treatment on PDX tumors *in vivo*, Related to Figure 7.

(A) Treatment strategy of flank-transplanted PDX012 tumors in SCID mice.

(B) Mean (normalized to initial tumor volume $\pm$ SEM) placebo- (grey) and ICM-treated tumor growth (green) over the course of 5.5 weeks. * $P < 0.05$, two-way ANOVA test.

(C) Plot of end-point relative changes in volumes of placebo- (grey) and ICM-treated tumors (green) in each transplanted mouse. * $P < 0.01$, Wilcoxon-Mann-Whitney test.

(D) Representative longitudinal sections from ctrl and ICM-treated PDX tumors following Masson Trichrome staining at the experimental end-point. The longest tumor axis is indicated. Scale bar: 1 mm.

(E) Plots of normalized HMGB2 levels in PDX012-derived tumors (left) and surrounding stromal tissue (right) in placebo- (grey) and ICM-treated mice (green).

(F) Left: Representative immunohistochemistry staining for Ki-67 in control and ICM-treated PDX tumors. Scale bar: 200 μ m. Right: Plot showing quantification of Ki-67 levels in six tumors per group.

* $P < 0.05$, unpaired two-tailed Student's t-test.

(G) Violin plots of *HMGB2*, *CDK1* and *MKI67* expression levels in the different scRNA-seq tSNE clusters from Figure 7G.

(H) GO term analysis of genes up- (left) or downregulated in *HMGB2*^{high} compared to *HMGB2*^{low} cells in orthotopic tumor scRNA-seq data (from Figure 7G).

(I) Representative genome browser views of differentially accessible regions (DARs) in orthotopic tumor scATAC-seq data from Figure 7J.

(J) TF motifs rendered inaccessible upon ICM treatment of orthotopically transplanted Panc-1 cells. Enrichment P -values for each motif are indicated.

(K) Heatmap of *HMGB2* expression levels across different tissues and developmental stages from the human expression atlas (<https://www.ebi.ac.uk/gxa>).