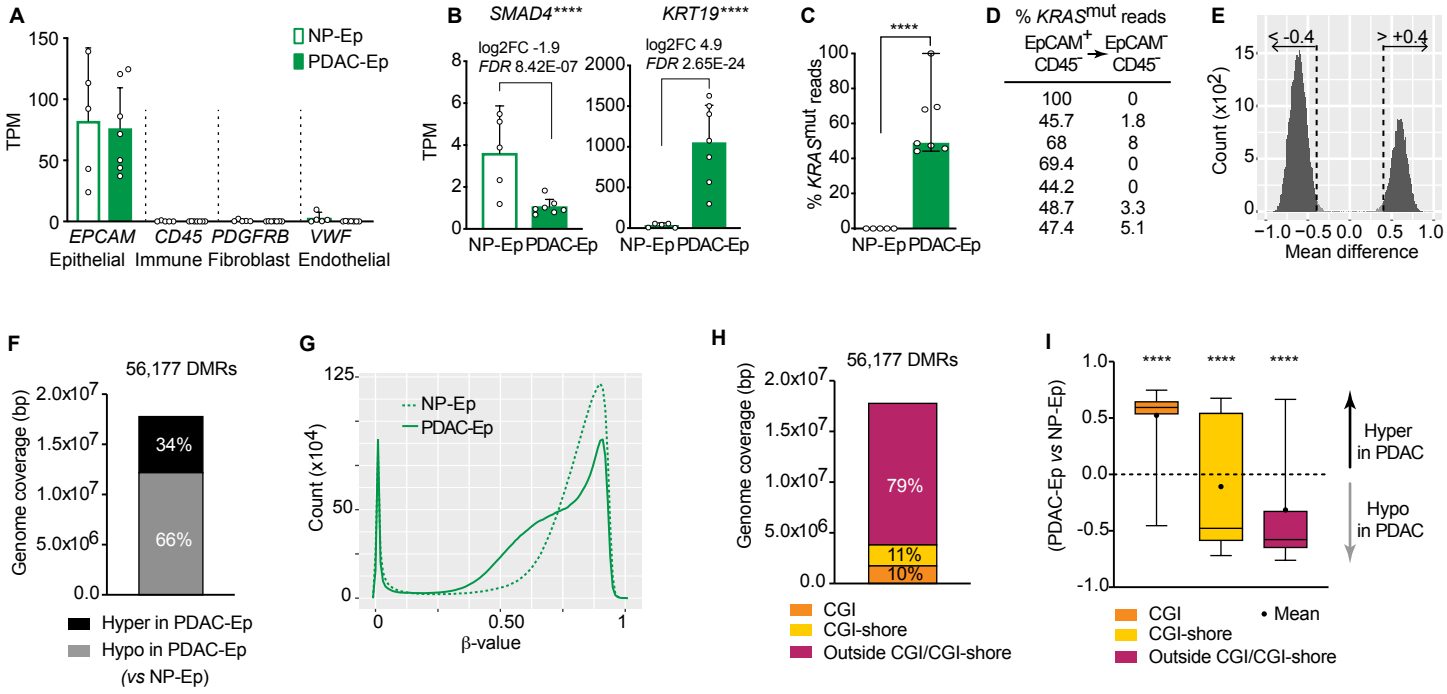
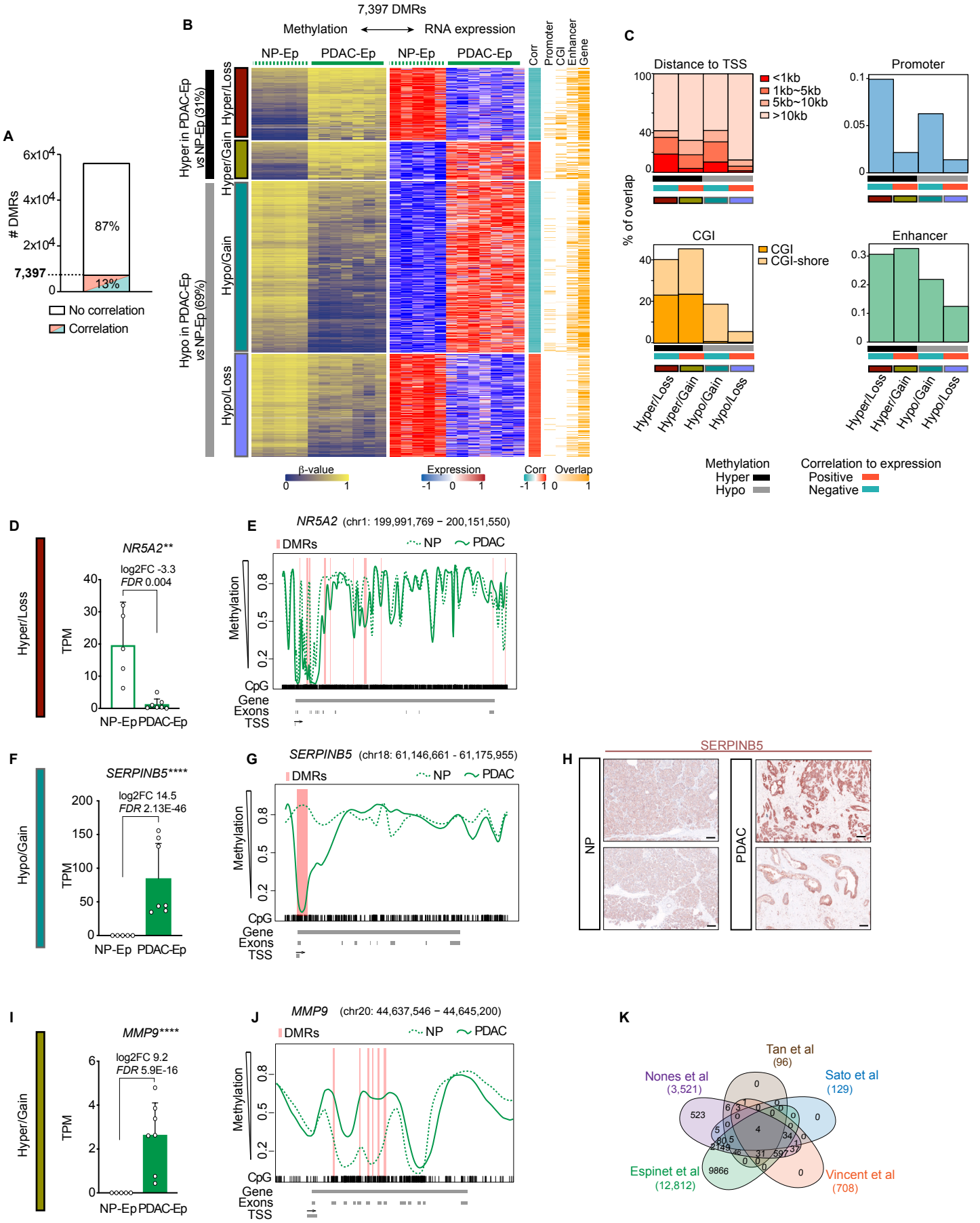
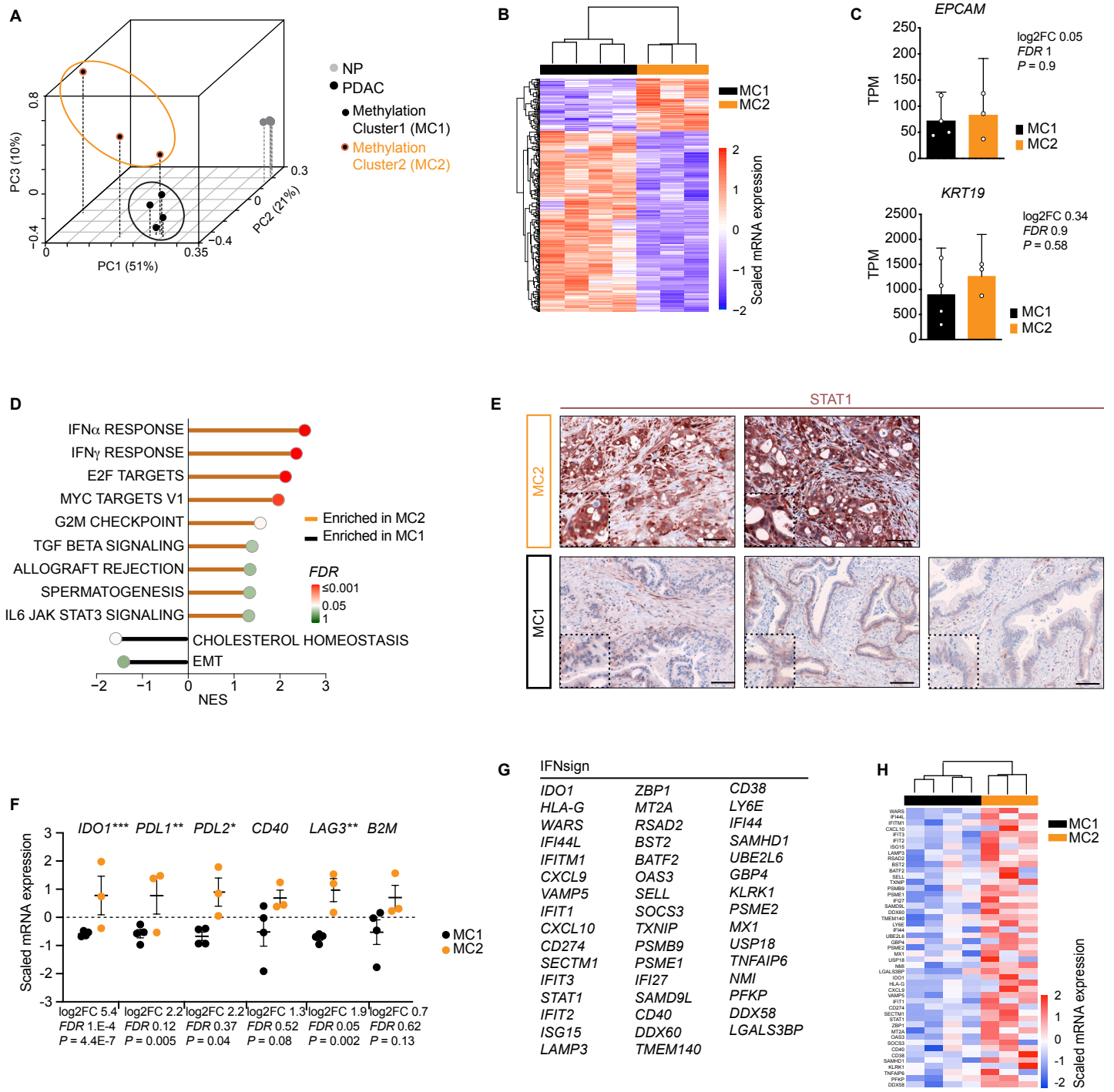


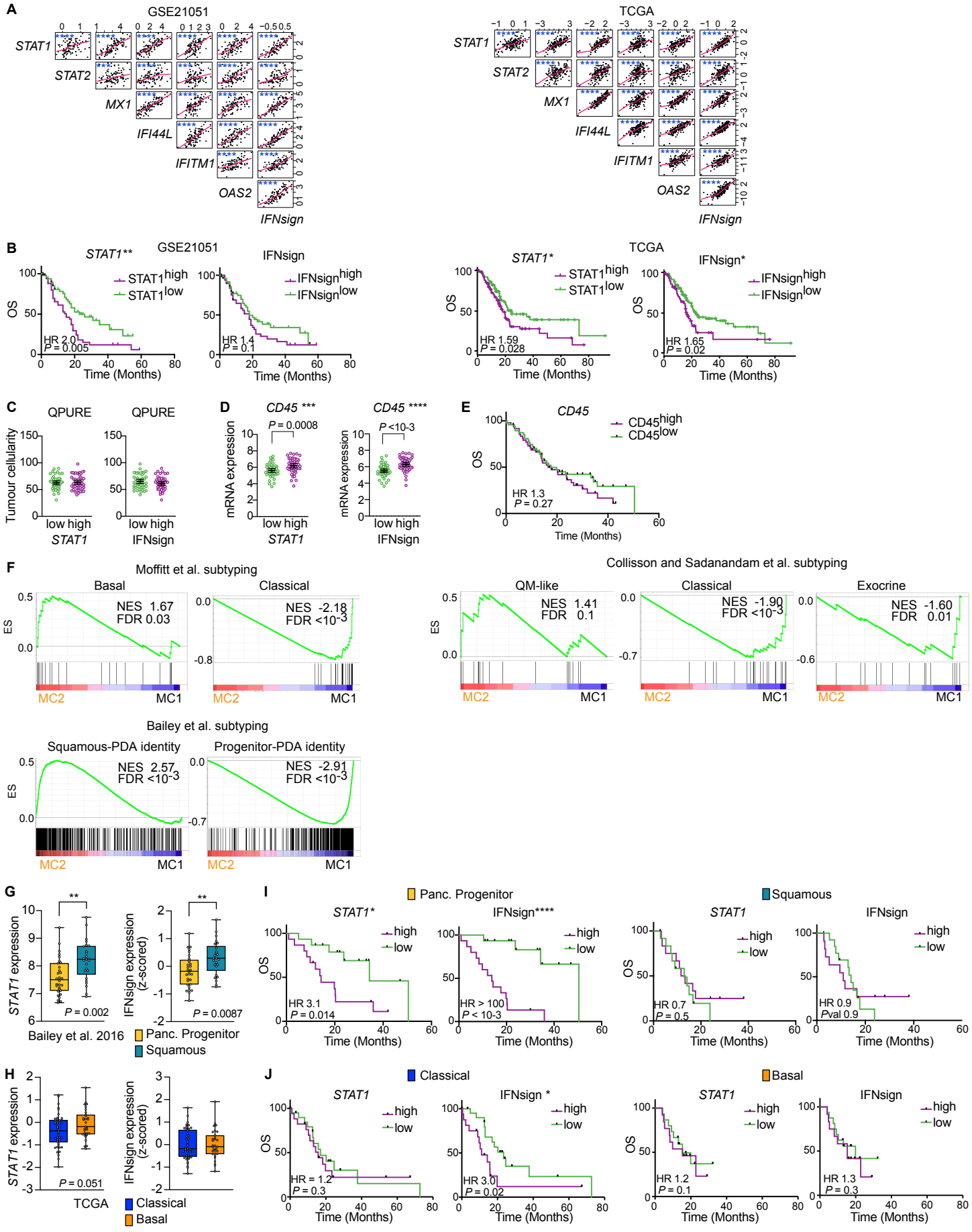
Supplementary Figure S1. Transcriptome and methylome of PDAC-Ep and NP-Ep cells. A,B, Normalized RNA counts (transcripts per million, TPM) of indicated cell-type markers. Two-tailed Wald-test. **C,** Percentage of RNAseq reads within *KRAS* gene containing a coding mutation. Data are median ± 95%CI. Two-tailed *t*-test. **D,** Percentage of RNAseq reads within *KRAS* gene containing a coding mutation from EpCAM^{+}/CD45^{-} and corresponding EpCAM^{-}/CD45^{-} populations data. **E,** Distribution of PDAC-Ep *vs*. NP-Ep DMRs; the cut-off used for downstream analyses (+/-0.4) is indicated. **F,** Extent of genome (in base pairs and relative percentage) covered by differentially methylated regions (DMRs). Direction in the change of methylation is highlighted. "Hyper" and "Hypo" indicate less and more methylation in PDAC-Ep *vs* NP-Ep, respectively. **G,** Distribution of mean β-values of NP and PDAC epithelial samples. **H,** Extent of genome covered by DMRs overlapping to CGIs, CGI-shores (± 2Kb) or other regions. **I,** Differences in methylation levels (as beta values) at different genomic regions. Boxes extend from 25th to 75th percentiles with line representing median. Mean is shown as a dot. Whiskers indicate 5th and 95th percentile. One sample two-tailed *t*-test (theoretical mean = 0). **** *FDR*/*P* < 0.0001.



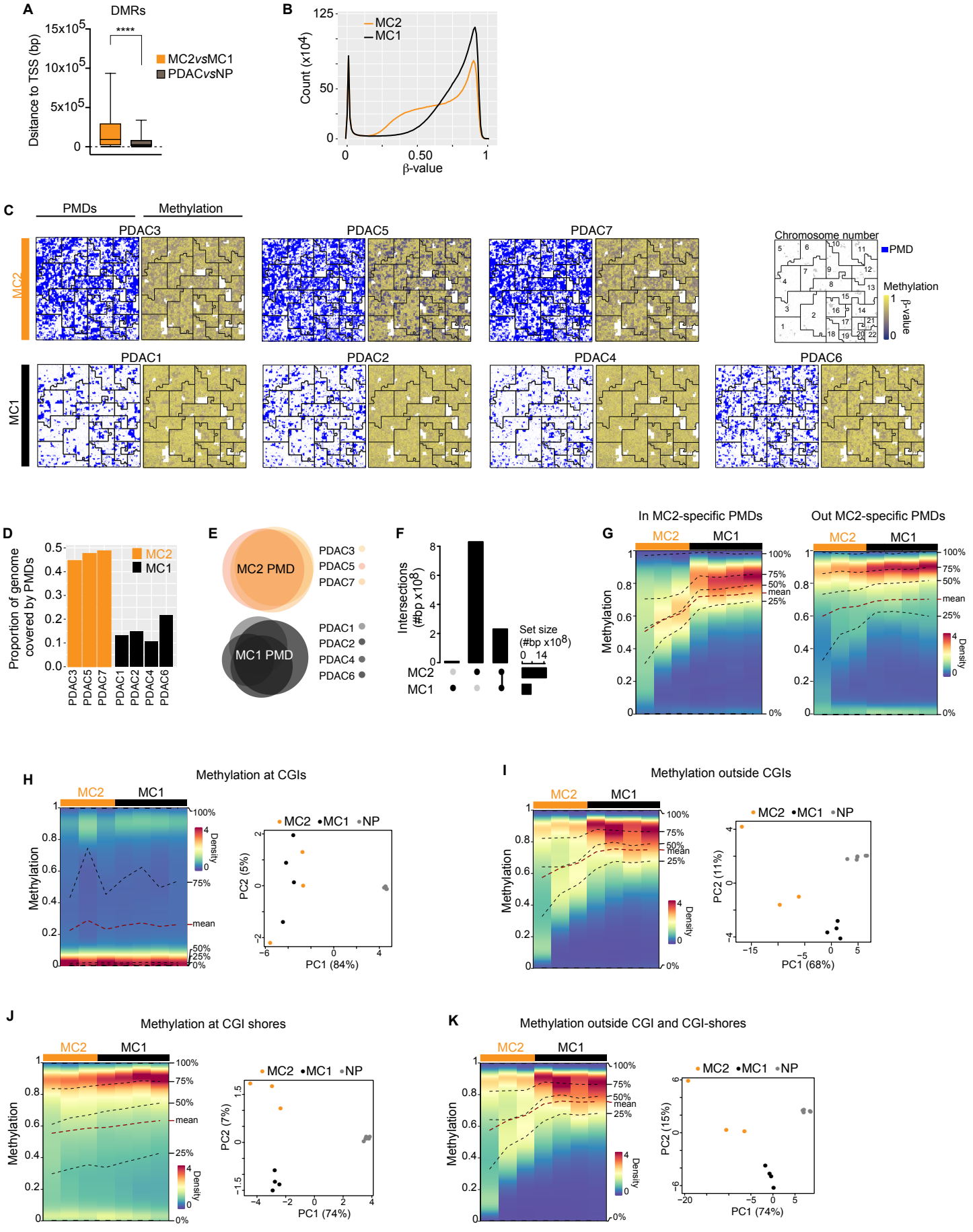
Supplementary Figure S2. Correlation of transcriptome and methylome of PDAC-Ep and NP-Ep cells. A, Number of DMRs correlating or not to changes in gene expression. **B,** Subset of DMRs that correlate with changes in gene expression. Left heatmap shows methylation (beta value) per DMR per sample; right heatmap shows mRNA expression per sample (z-scored) of the closest gene to the DMR. For each DMR correlation to gene expression changes (corr), and overlap to different genomic features are indicated. “Loss” and “Gain” indicate loss and gain of expression in PDAC-Ep *vs* NP-Ep, respectively. “Hyper” and “Hypo” indicate less and more methylation in PDAC-Ep *vs* NP-Ep, respectively. **C,** Distance to transcription start site (TSS) and overlap with different genomic features of DMRs from the groups shown in (B). **D,** mRNA levels of indicated gene (transcript per million, TPM). Mean + 95% CI; two-tailed Wald-test; **** *FDR* < 0.0001. **E,** Methylation profile of indicated gene presented as beta values (average of biological replicates per group; *n* = 7 (PDAC-Ep); 5 (NP-Ep)); DMRs are highlighted in pink. **F,** See D. **G,** See E. **H,** SERPINB5 staining in two NP and two PDAC human tissues. Scale bar = 100µm. **I,** See D. **J,** See E. **K,** Overlap of genes identified in the indicated studies as differentially methylated bewteen PDAC and NP.



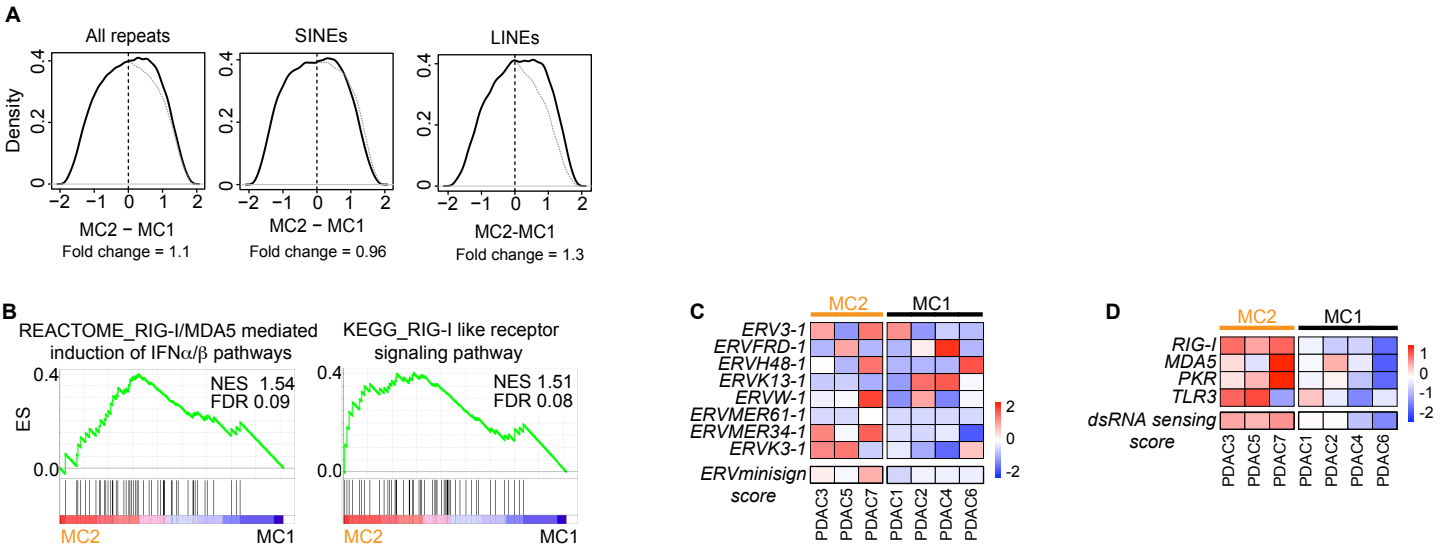
Supplementary Figure S3. MC2 samples are enriched in interferon response signature genes. A, Unsupervised Principal component analysis using the 10k most variable windows between samples (window size 5kb, coverage ≥ 10X per sample). Percentage indicates proportion of variance explained by each component. **B,** Differentially expressed genes between MC1 and MC2 (*FDR* < 0.01). **C,** TPM of indicated genes. Two-tailed Wald-test. **D,** Hallmark gene signatures enriched in MC2 or MC1 by gene set enrichment analysis. *NES*, normalized enrichment score; *FDR*, false discovery rate. **E,** STAT1 staining in MC1 and MC2 samples. Scale bar = 100µm. **F,** mRNA expression (z-scored) of indicated immune checkpoint genes in MC1 and MC2. Lines are mean ± s.e.m.; two-tailed Wald-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **G,** Genes of IFNsign. **H,** Scaled mRNA expression of interferon signature genes (IFNsign).



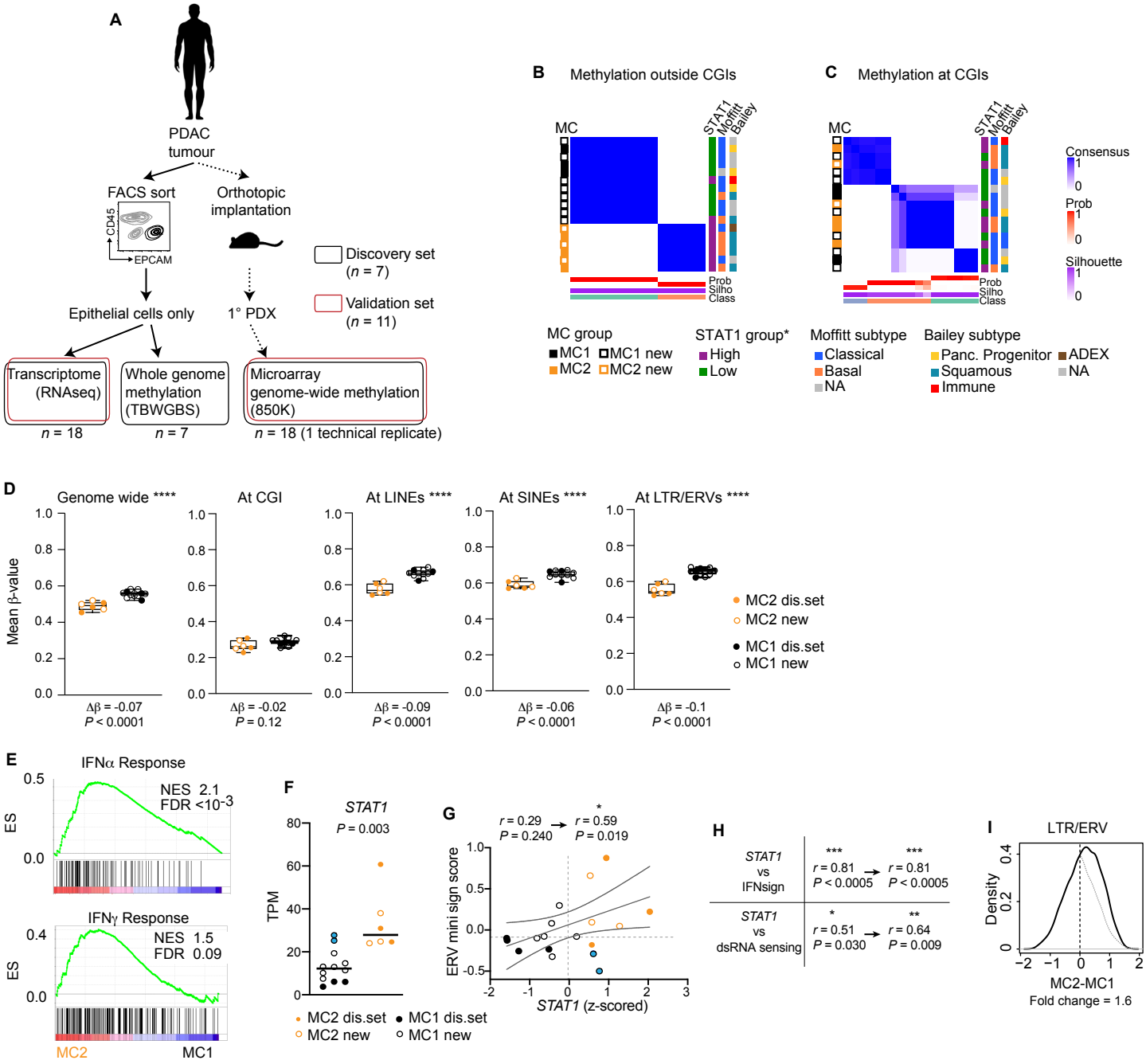
Supplementary Figure S4. IFNsign^{high} status associates with worse prognosis and the squamous/basal subtype. A, Correlative expression of IFN-relatde genes in indicated patient cohorts. Each dot is one patient; Spearman´s rank correlation coefficient. **B,** Kaplan-Meier curves of overall survival (OS) of patients with low or high expression of *STAT1* or IFNsign in indicated cohorts. *HR*, Hazard ratio; logrank-test. **C,** QPURE score in patients from Fig. 1I. **D,** *CD45* mRNA levels in patients from Fig. 1I. Two-tailed Mann-Whitney test. **E,** Kaplan-Meier curve of OS of patients from Fig. 1I according to *CD45* expression. *HR*, Hazard ratio; logrank-test. **F,** Gene set enrichment analyses of PDAC-subtype signatures in MC2 *vs* MC1. *ES*, enrichment score; *NES*, normalized enrichment score; *FDR*, false discovery rate. **G,H,** *STAT1* or IFNsign expression in squamous and pancreatic progenitor-like PDAC patients (Bailey cohort, G) or in classical and basal (TCGA cohort, high tumour content samples only, H). Two-tailed Mann Whitney test. ** $P < 0.01$. **I,J,** Kaplan-Meier curves of OS of patients in (G) and (H) according to *STAT1* or IFNsign expression. *HR*, Hazard ratio; logrank-test. * $P < 0.05$; *** $P < 0.001$.



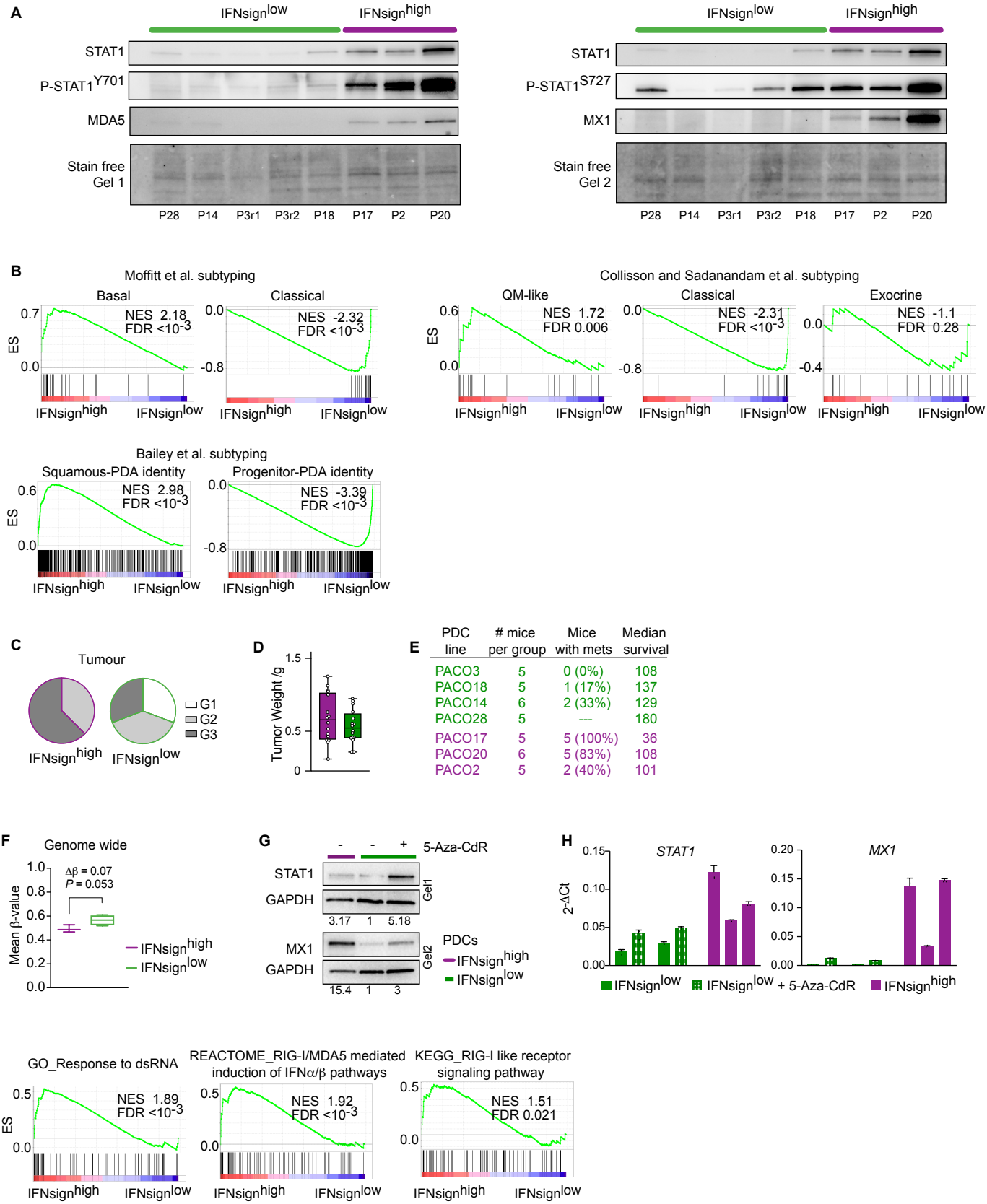
Supplementary Figure S5. Hypomethylation discriminates tumors types and occurs mainly at non-CGIs. A, Box plot illustrating the distance to transcription start site (TSS) of DMRs identified between PDAC-Ep and NP-Ep or between MC2 and MC1. Boxes extend from 25th to 75th percentiles with line representing median. Whiskers indicate minimum and maximum values. Two-tailed Mann-Whitney test. *** $P < 0.001$. **B,** Distribution of mean β-values of MC1 and MC2-PDAC epithelial samples. **C,** Hilbert curve plot showing the methylation values along the genome (right) and the genomic distribution of PMDs (left) per sample. **D,** Proportion of the genome per sample covered by partially methylated domains (PMDs). **E,** Overlap of PMDs in MC2 (top) or MC1 (bottom) samples. **F,** UpSet plot showing number and intersection of partially methylated domains (PMDs) quantified per group. **G,** Methylation density plot at MC2-PMDs (left) or in regions outside of MC2-PMDs (right). Lines indicate quartiles and mean values. **H-K,** Methylation density plot (left) and PCA plots at different genomic regions (right, see methods for details). See also Extended Data Figure 2.



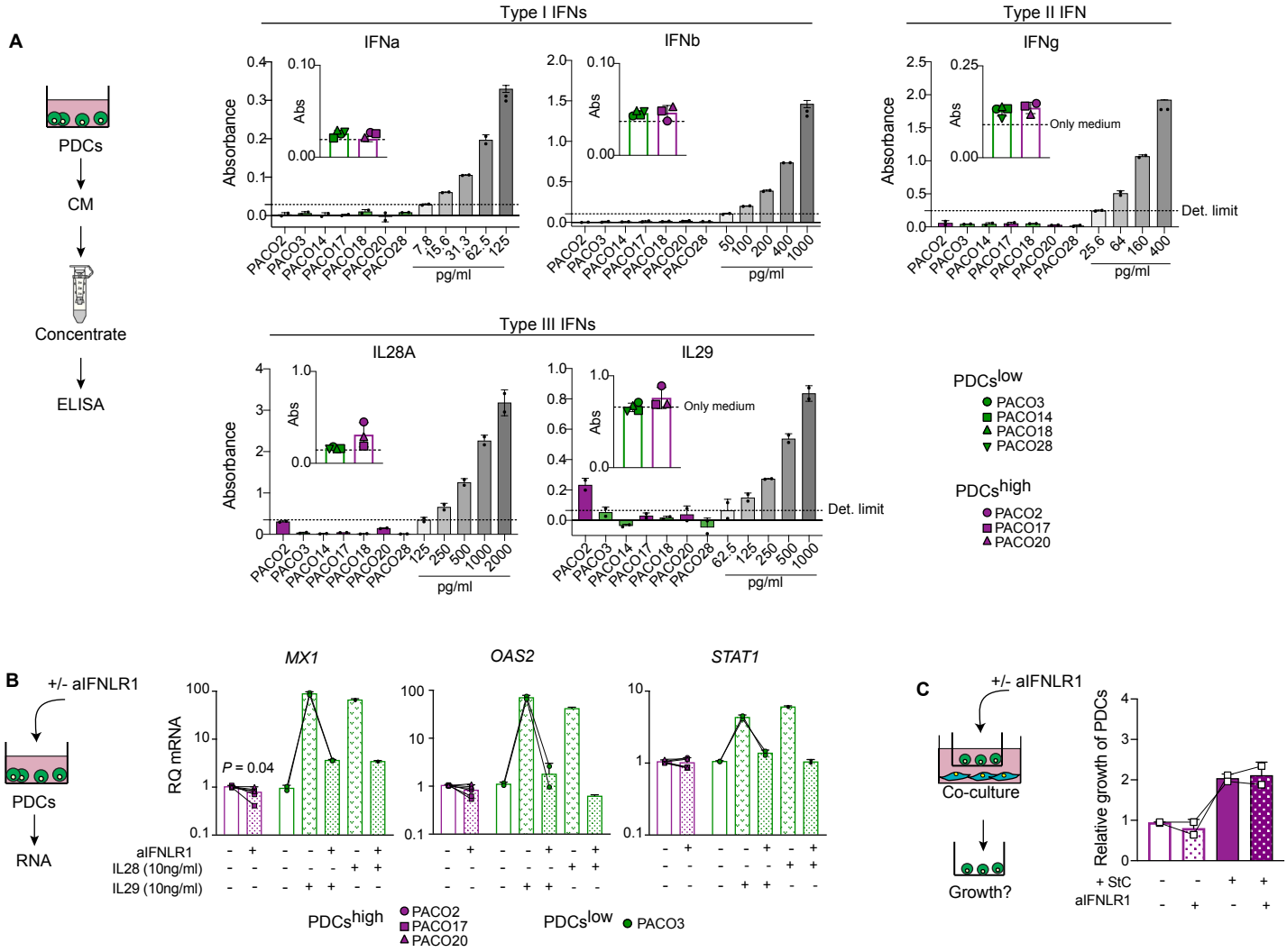
Supplementary Figure S6. Low methylated PDACs engage a dsRNA sensing response. A, Distribution of expression difference of repeatitive elements between MC2 and MC1. Fold change of area under the curve is shown. Gray line mirrors the line at negative values. **B,** Gene set enrichment analysis (GSEA) results of the indicated gene sets in MC2 *vs* MC1 samples. *ES*, enrichment score; *NES*, normalized enrichment score; *FDR*, false discovery rate. **C,D,** Heatmap showing scaled expression of indicated genes and mean expression of the gene signatures in MC2 and MC1 samples.



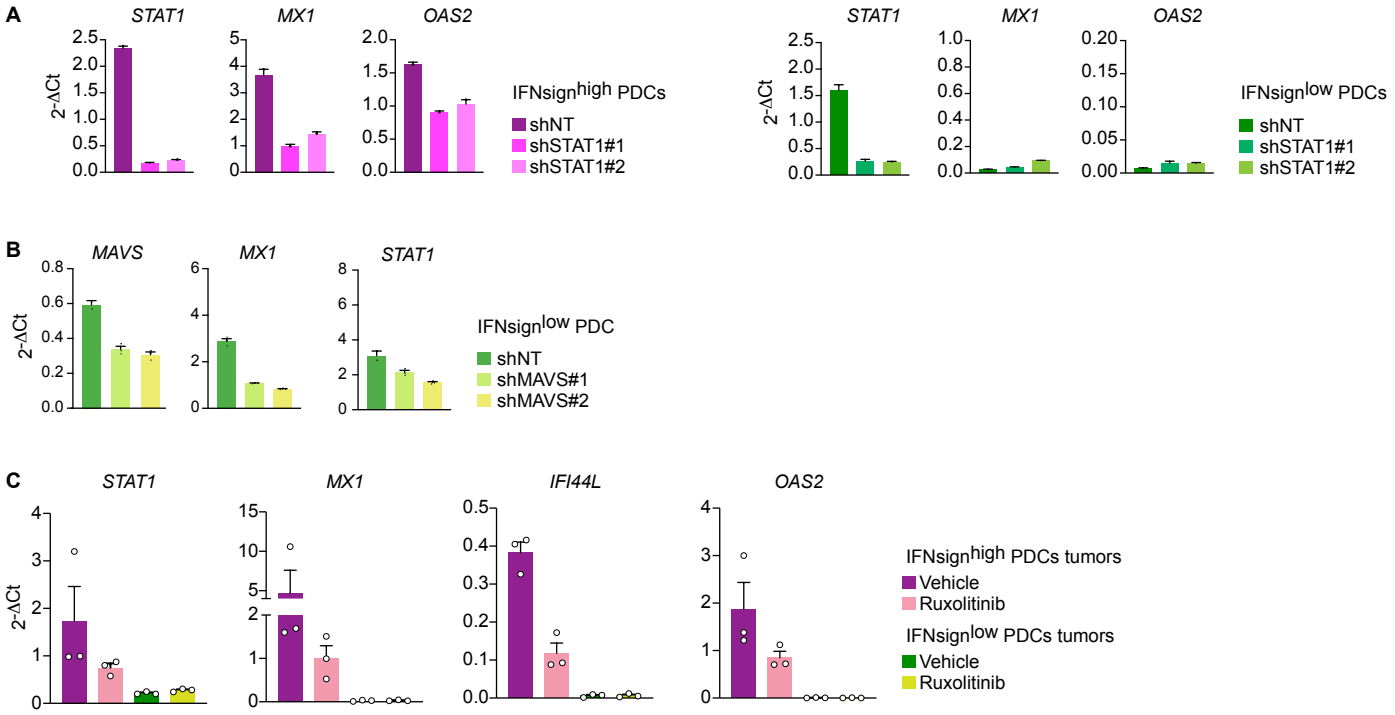
Supplementary Figure S7. Validation of methylation and expression pattern in an independent tumor cohort. A, Scheme of all the tumour samples used in the study. **B,C,** Consensus clustering of PDX samples using methylation of probes outside of (B) or at (C) CGI sites. (See also Extended Data Figure 2). **D,** Mean methylation values of samples at different genomic features. Values are calculated per sample and plotted per group. Boxes extend from 25th to 75th percentiles with line representing median. Whiskers indicate minimum and maximum values. Δβ are MC2-MC1. (B-D): MC1-PDXs: *n* = 12 (11 + one technical replicate. Mean of technical replicates was used for plotting); MC2-PDX: *n* = 6. **E,** Gene set enrichment analysis GSEA results of the epithelial gene expression of MC2 *vs* MC1 samples. *ES*, enrichment score; *NES*, normalized enrichment score; *FDR*, false discovery rate. **F,** *STAT1* epithelial expression (transcripts per million, TPM) of MC1 and MC2 tumors. Mann-Whitney test. **G,** Correlation of expression between *STAT1* and ERVminisign in MC1 and MC2 epithelial cells. Of note, the two samples in MC1 group with STAT1 expression levels comparable to those of MC2 samples (blue circles) showed low levels of ERVs. This could indicate that STAT1 expression is regulated by other means than ERV derepression in these samples. Statistics are Spearman correlation of all data or after exclusion of the two blue samples. **H,** Correlation between STAT1 expression and indicated signatures considering all samples or after exclusion of the two blue samples. **I,** Distribution of expression difference of ERVs/LTRs between MC1 and MC2 (see methods for details). Fold change of area under the curve is shown. Gray line mirrors the line at negative values. E-I: MC1: *n* = 12; MC2: *n* = 6.



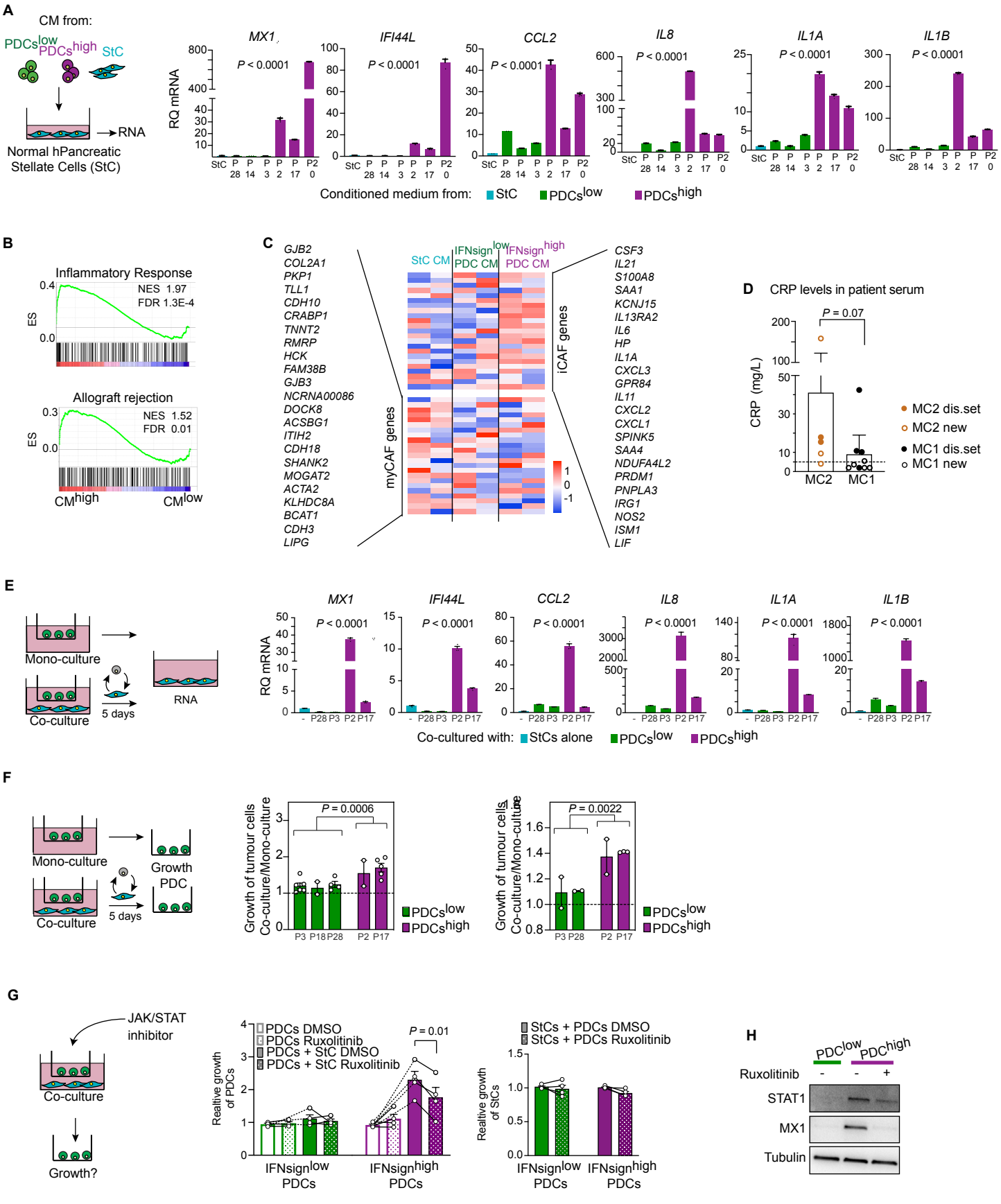
Supplementary Figure S8. IFNsign^{high} patient derived primary cells show lower methylation and engaged response to dsRNA. A, Western blot of indicated proteins in PDAC patient-derived cells (PDCs). **B,** Gene set enrichment analysis (GSEA) results of PDAC-subtypes signatures in IFNsign^{high} *vs* IFNsign^{low} PDCs. *ES*, enrichment score; *NES*, normalized enrichment score; *FDR*, false discovery rate. **C,** Grade of tumors formed by IFNsign^{low} (*n* = 13) or IFNsign^{high} (*n* = 8). **D,** Weight of tumours from Fig. 3C. **E,** Number of mice, incidence of metastasis and median survival per cell line. **F,** Genome wide mean methylation of PDCs. Values are calculated per sample and plotted per group. *n* = 4 (IFNsign^{low}: PACO3, 14, 18, 28), *n* = 3 (IFNsign^{high}: PACO2, 17, 20). Boxes extend from 25th to 75th percentiles with line representing median. Whiskers indicate minimum and maximum values. Two-tailed *t*-test. **G,** Western blot of STAT1 and MX1 in IFNsign^{low} PDC (PACO3) treated with decitabine (5-Aza-CdR). One IFNsign^{high} cell line (PACO2) is plotted for comparison. GAPDH serves as loading control. Numbers indicate quantification of above plot normalized to loading control. **H,** mRNA expression of indicated genes in two IFNsign^{low} PDCs (PACO2 and 14) treated with decitabine (5-Aza-CdR) and three IFNsign^{high} PDCs (PACO2, 17, 20), measured by RT-qPCR and relative to *RPLPO*. Data are mean + s.e.m. **I,** GSEA of signatures of response to dsRNA in IFNsign^{high} *vs* IFNsign^{low} PDCs.



Supplementary Figure S9. IFNs independent activation of an IFNsign^{high} status A, ELISAs of Type I, II and III IFNs in concentrated conditioned medium (CM) of PDCs. Dotted line in bigger plot indicates absorbance values of the detection limit. Dotted line in inner plot indicate absorbance values of medium used to grow PDCs, concentrated and processed in the same manner as the PDC CM. **B,** mRNA expression of indicated genes in PDCs treated with anti-IFNLR1 blocking antibody or IgG1 control (-), measured by RT-qPCR and relative to *HPRT1* and normalized to IgG control. Recombinant IL28 and IL29 were used in PACO3 as control. PDCs^{high}: Bars are mean of three different cell lines; each dot of a cell lines is the mean of two independent experiments. PDCs^{low} IL29: Bars are mean of two independent experiments; each dot is one independent experiment. PDCs^{low} IL28: Dots are technical replicates. Two-tailed paired *t*-test. **C,** Realtive growth of PDCs (PACO17) treated with an anti-IFNLR1 blocking antibody or IgG control (-) and in co-cultured with stellate cells (StCs).



Supplementary Figure S10. dsRNA sensing and JAK/STAT pathway blockade. A, mRNA expression of indicated genes in PDCs from Fig. 3J, measured by RT-qPCR and relative to the expression of *HPRT1*. Data are mean + s.e.m. **B,** mRNA expression of indicated genes in *MAVS* knock-down IFNsign^{low} PDC (PACO3), measured by RT-qPCR and relative to *HPRT1*. Data are mean + s.e.m. **C,** mRNA expression of indicated genes in tumors (*n* = 3) from mice treated with vehicle or Ruxolitinib, measured by RT-qPCR relative to the expression of *GAPDH* and *HPRT1*. Data are mean + s.e.m.


A
CM from:
PDCs low
PDCs high
StC
RNA
Normal hPancreatic Stellate Cells (StC)
MX1
IFI44L
CCL2
IL8
IL1A
IL1B
P < 0.0001
RQ mRNA
StC
P 28
P 14
P 3
P 2
P 17
P2 0
Conditioned medium from: StC PDCs low PDCs high
B
Inflammatory Response
NES 1.97
FDR 1.3E-4
Allograft rejection
NES 1.52
FDR 0.01
ES
CM high
CM low
C
GJB2
COL2A1
PKP1
TLL1
CDH10
CRABP1
TNNT2
RMRP
HCK
FAM38B
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CDH18
SHANK2
MOGAT2
ACTA2
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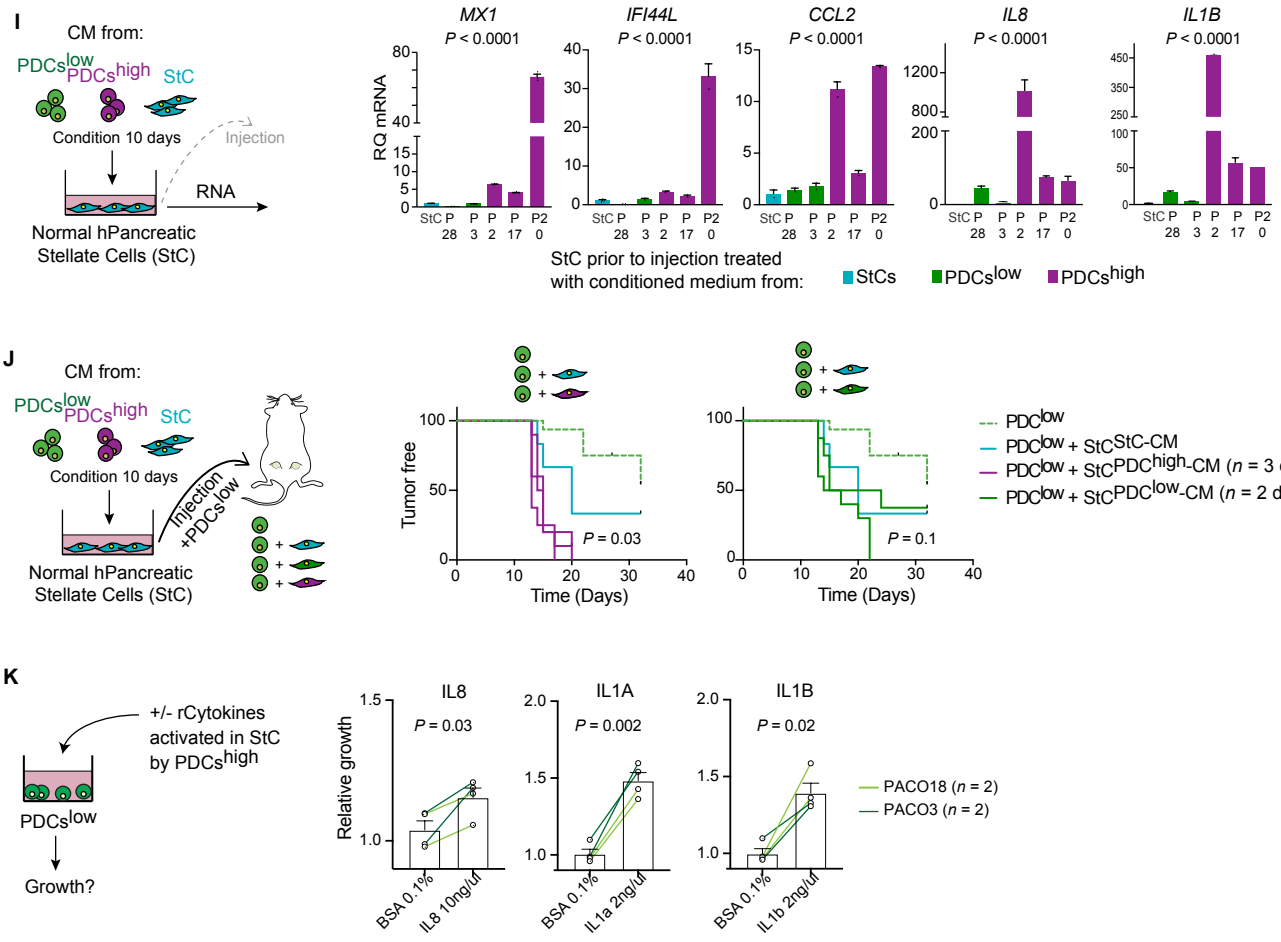
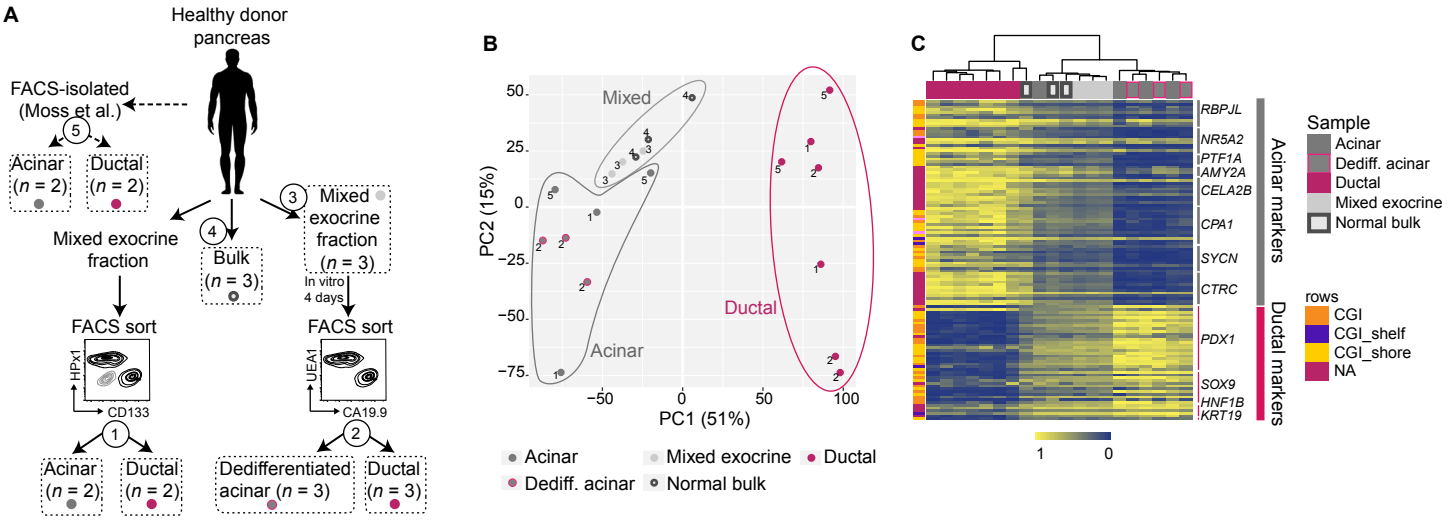
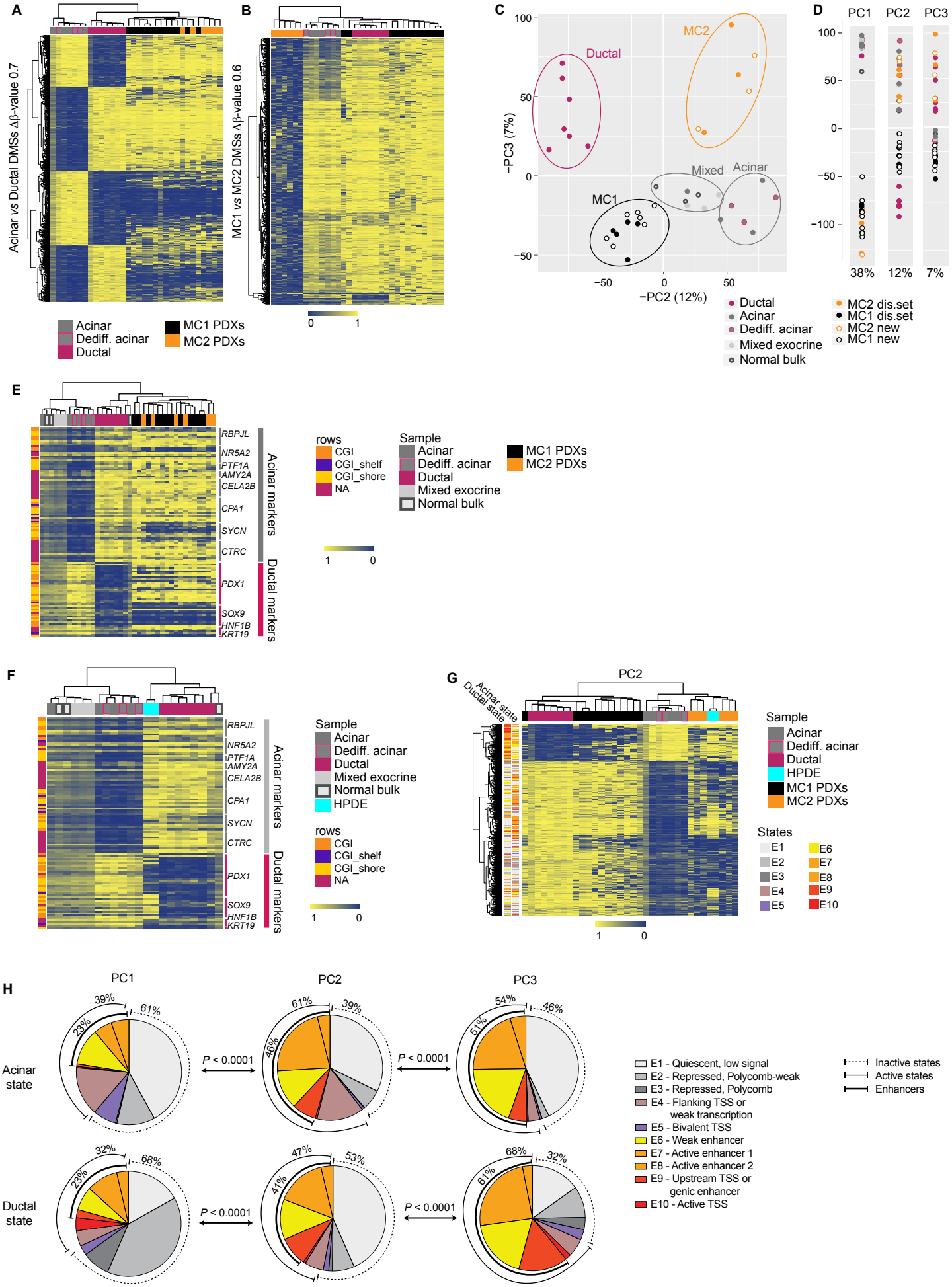
CDH3
LIPG
StC CM
IFNsign low PDC CM
IFNsign high PDC CM
myCAF genes
iCAF genes
CSF3
IL21
S100A8
SAA1
KCNJ15
IL13RA2
IL6
HP
IL1A
CXCL3
GPR84
IL11
CXCL2
CXCL1
SPINK5
SAA4
NDUFA4L2
PRDM1
PNPLA3
IRG1
NOS2
ISM1
LIF
D
CRP levels in patient serum
P = 0.07
CRP (mg/L)
MC2
MC1
MC2 dis.set
MC2 new
MC1 dis.set
MC1 new
E
Mono-culture
Co-culture
5 days
RNA
MX1
IFI44L
CCL2
IL8
IL1A
IL1B
P < 0.0001
RQ mRNA
- P28 P3 P2 P17
Co-cultured with: StCs alone PDCs low PDCs high
F
Mono-culture
Co-culture
5 days
Growth PDC
P = 0.0006
P = 0.0022
Growth of tumour cells Co-culture/Mono-culture
P3 P18 P28
P2 P17
P3 P28
PDCs low
PDCs high
G
JAK/STAT inhibitor
Co-culture
Growth?
PDCs DMSO
PDCs Ruxolitinib
PDCs + StC DMSO
PDCs + StC Ruxolitinib
P = 0.01
Realtive growth of PDCs
IFNsign low PDCs
IFNsign high PDCs
StCs + PDCs DMSO
StCs + PDCs Ruxolitinib
Realtive growth of StCs
H
PDC low
PDC high
Ruxolitinib
STAT1
MX1
Tubulin



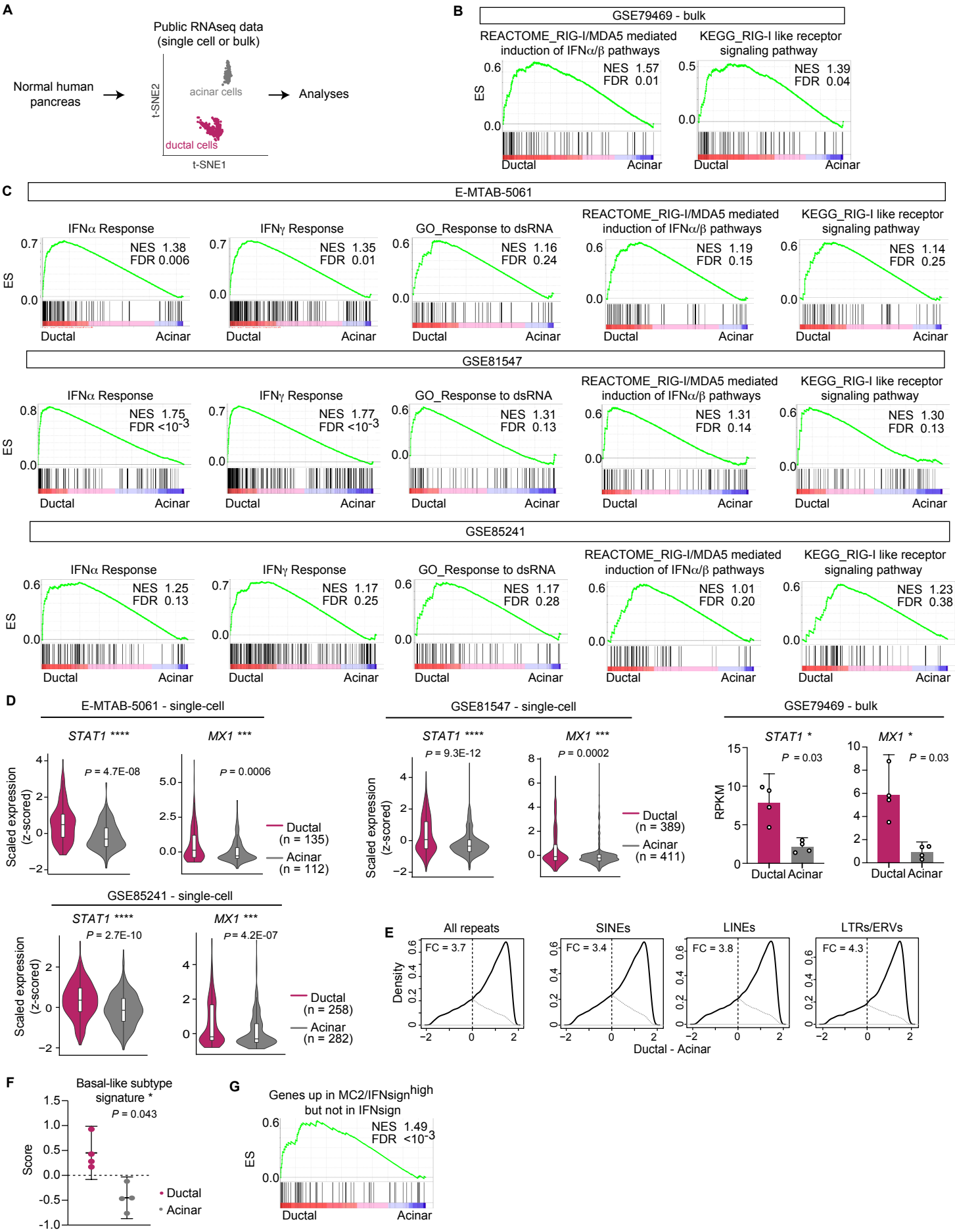
Supplementary Figure S11. IFNsign^{high} tumor cells activate interferon and pro-inflammatory genes in stromal cells. A,I mRNA expression of indicated genes in stellate cells (StC) treated with conditioned medium (CM) from PDCs, measured by RT-qPCR and relative to *HPRT1* and normalized to StC alone. One-way Anova. **B,** Gene set enrichment analysis (GSEA) of stromal subtypes signatures in StC treated with CM from IFNsign^{high} versus IFNsign^{low} cells. *ES*, enrichment score; *NES*, normalized enrichment score; *FDR*, false discovery rate. **C,** Heatmap showing the expression of genes associated to iCAFs or myCAFs in StC treated with different CM. **D,** C-reactive protein levels in serum of MC1 and MC2 patients. Data indicate mean + 95% CI. Two tailed Mann-Whitney test. *n* = 5 MC2, *n* = 9 MC1. **E,** mRNA expression of indicated genes in StC co-cultured with PACOs in 6-wells, measured by RT-qPCR and relative to *HPRT1* and normalized to StC alone. One-way Anova. **F,** Growth of tumour cells co-cultured with StC relative to mono-culture growth. Left: co-culture in 24-well plates and 2,500 StCs. Right: co-culture in 6-well plates and 12,500 StCs. Data are mean ± s.e.m.; each dot is an independent experiment. Two-tailed nested *t*-test. **G,** Realtive growth of PDCs (left; PACO3, PACO17) or StCs (right) after mono-culture or co-culture with StC and treated with the JAK/STAT inhibitor Ruxolitinib. Two-tailed paired *t*-test. **H,** WB of IFNsign^{high} (PACO17) PDCs treated with 100nM Ruxolitinib. One IFNsign^{low} (PACO3) is shown as control. **I,** mRNA expression of indicated genes in StC injected *in vivo*, measured by RT-qPCR and relative to *HPRT1* and normalized to StC alone. One-way Anova. **J,** Appearance of subcutaneous tumors arising from IFNsign^{low} PDCs (PACO3) co-injected with StC reprogrammed by (left) IFNsign^{high} or (right) IFNsign^{low} CM. Appearance of PDCs injected alone (dotted green line) or with control StC (i.e. reprogrammed by conditioned StC CM) (blue line) are shown as control. Log-rank test. **K,** Relative growth of IFNsigh^{low} PDCs treated with indicated recombinant cytokines. *n* = 2 biological and 2 technical replicates. Two-tailed paired *t*-test.



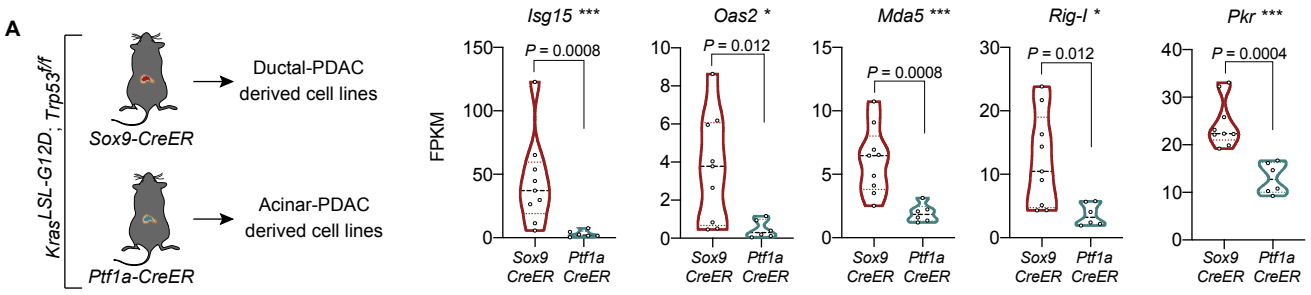
Supplementary Figure S12. Methylation profile of normal control samples. A, Scheme illustrating the source of the normal control samples used in the study. **B,** Unsupervised principal component analysis of normal samples. Numbers refer to panel A. **C,** Methylation status of acinar and ductal samples in control samples.



Supplementary Figure S13. Acinar and ductal methylation footprint in PDAC tumours. A, Top acinar *vs* ductal differentially methylated sites (DMSs). **B,** Top MC1 *vs* MC2 DMSs. **C,** PCA of the cohort showing PC2 and PC3. **D,** Distribution of samples according to PC1, PC2 and PC3. **E,** Methylation of acinar and ductal markers in control and PDX samples. **F,** Methylation of acinar and ductal markers in control normal samples and HPDE cells. **G,** Clustering of samples using the top 1,000 CGs driving PC2. **H,** Annotation of the top 1,000 CGs associated to PC1, PC2 and PC3 to acinar and ductal states. Chi-square test summarizing E2+E3 and E6-E9.



Supplementary Figure S14. Ductal cells in normal pancreas express IFN related genes and repetitive elements. A, Illustration of data analysed in panels B-C. Adapted from Segerstolpe, Palasantza et al. **B-C,** GSEA in ductal *vs* acinar samples of indicated datasets. *ES*, enrichment score; *NES*, normalized enrichment score; *FDR*, false discovery rate. **D,** RNA expression of indicated IFN-related genes in ductal and acinar cells from different studies. E-MTAB-5061, GSE81547, GSE85241: Violin plots show scaled expression (z-scored); Two-tailed Mann-Whitney test. GSE79469: Reads per Kilobase Million (RPKM). Each dot is one biological replicate (n = 4 per group); bars indicate mean + 95% CI. **E,** Distribution of expression difference of repeat-derived transcripts between ductal and acinar cells (GSE79469). Fold change of area under the curve is shown. Gray line mirrors line at negative values. **F,** Expression of the basal-like PDAC subtype gene signature in acinar and ductal cells. GSE79469: n = 4 ductal, 4 acinar; Lines are mean ± 95% CI; paired two-tailed t-test. **G,** GSEA in ductal *vs* acinar samples of genes upregulated in MC2 *vs* MC1 excluding IFNsign genes.



Supplementary Figure S15. Ductal-derived mouse PDAC tumors express interferon-related genes. A, Normalized expression of indicated genes (FPKM - fragments per kilobase of transcript per million mapped reads) in mouse cell lines from ductal [*n* = 9 cell lines from 9 tumors (8 mice)] or acinar [*n* = 6 cell lines from 6 tumors (4 mice)] derived PDAC mouse tumors. Black dotted line indicates median. Coloured dotted lines indicate upper and lower quartiles. Mann-Whitney test.