

Expanded View Figures

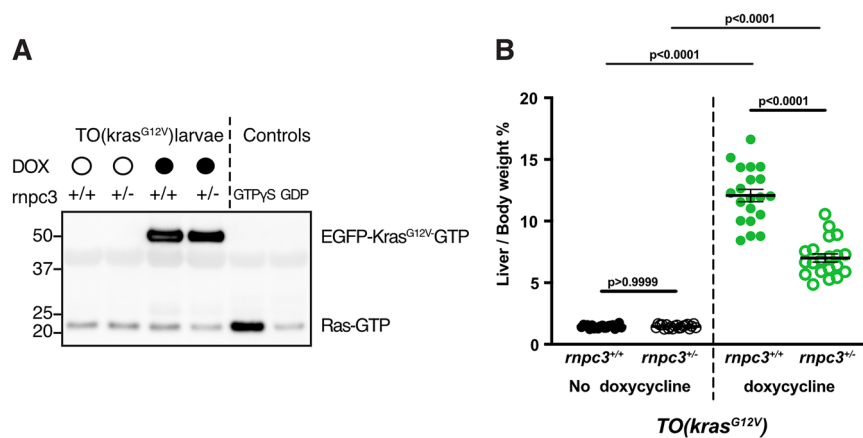


Figure EV1. Heterozygous loss of *rnpc3* reduces tumour burden in a *kras*^{G12V}-driven zebrafish model of hepatocellular carcinoma (HCC).

(A) Western blot of active Ras-GTP protein following active Ras pull-down from TO(*kras*^{G12V}) larvae of the indicated *rnpc3* genotype. (B) *rnpc3*^{+/+} and *rnpc3*^{+/-} liver/body mass ratio (%) of adult male TO(*kras*^{G12V}) (3.5–4 months of age) with and without dox treatment. *n* = 20, 2 pooled independent experiments. Mean ± SEM, significance was assessed by one-way ANOVA with Tukey's multiple comparison test.

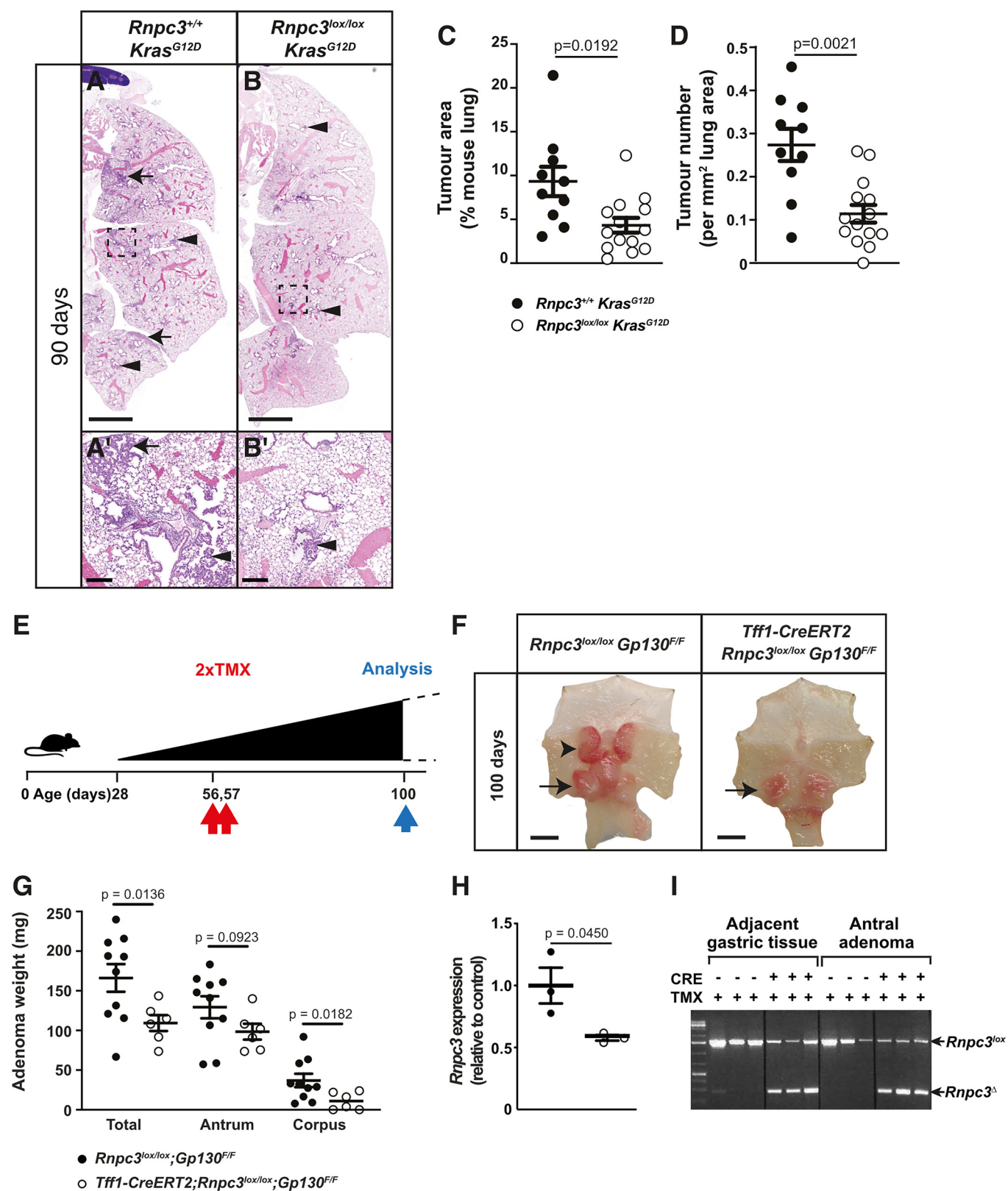


Figure EV2. Recombination of *Rnpc3*^{lox} alleles in mice reduces tumour burden in *Kras*^{G12D}-driven lung adenocarcinoma and a *Gp130*^{F/F} gastric adenoma model.

(A, B) Histological analysis of lung hyperplasia. Areas of AAH (atypical adenomatous hyperplasia; arrows) and bronchial hyperplasia (arrowheads) were observed in the lungs 90 d after adenoviral Cre recombinase administration. Scale bar is 2 mm. (A', B') Higher magnification images of region in dashed box. Scale bar is 200 μ m. (C, D) Quantitation of the frequency and area of the hyperplastic lesions. Data are expressed as mean $\pm$ SEM, $n = 10$ or 14 per genotype. Significance was assessed using an unpaired Student's t test with Welch's correction. (E) Schematic diagram of gastric adenoma formation in *Gp130*^{F/F} mice and treatment with tamoxifen (2 $\times$ TMX) on days 56 and 57 (red arrows) to induce recombination of *Rnpc3*^{lox} alleles. (F) Representative stomachs with adenomas in the corpus (arrowhead) and antrum (arrow). Scale bar is 5 mm. (G) Quantitation of adenoma weight. Data are expressed as mean $\pm$ SEM, $n = 6$ or 10 per genotype. Significance was assessed using an unpaired Student's t test with Welch's correction. (H) RT-qPCR analysis of *Rnpc3* mRNA in single antral adenomas from three independent mice per genotype. Data are expressed as mean $\pm$ SEM. Significance was evaluated with a two-tailed Student's t test. (I) PCR of genomic DNA for *Rnpc3* alleles in normal glandular stomach and adenomas harvested from TMX-treated, *Tff1-CreERT2;Rnpc3*^{lox/lox};*Gp130*^{F/F} mice, $n = 3$.

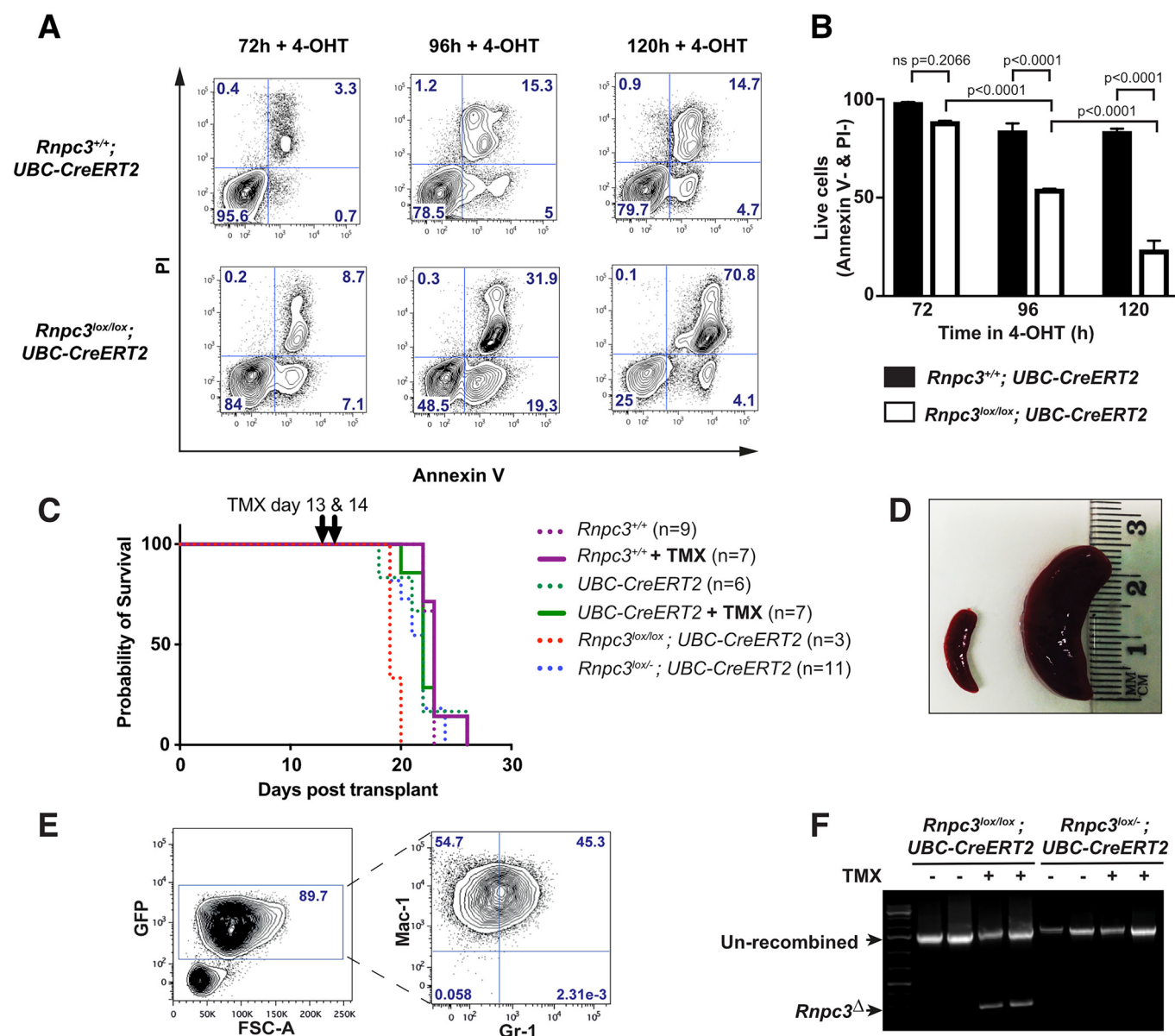


Figure EV3. Recombination of the *Rnpc3* locus in MLL-ENL AML cells in vitro reduces cell viability.

(A) Representative fluorescence-activated cell sorting (FACS) plots of cell viability using AnnexinV and PI staining. Numbers in each quadrant indicate the percentage of cells in each category. (B) Quantitation of the percentage of viable (AnnexinV⁻, PI⁻) cells over time enumerated by FACS. Percentage viable cells expressed as mean $\pm$ SEM, $n = 3$ for all cohorts. Significant differences were assessed with a one-way ANOVA with Tukey's multiple comparison test. (C) Kaplan-Meier survival plot of mice harbouring tertiary transplants of MLL-ENL AML cells with all control genotypes in the presence (solid line) and absence (dotted line) of TMX treatment 13 and 14 d (black arrows) following transplantation. There is no significant difference in median survival (19–23 d) between all control cohorts (i.e. no *Rnpc3*^{lox} alleles and no TMX treatment), $n = 311$. Significance was assessed with a Mantel-Cox test. (D) Spleen from a WT mouse not transplanted with AML cells next to a spleen harvested from a mouse transplanted with UBC-CreERT2;*Rnpc3*^{lox/lox} AML cells and treated with TMX. (E) Example FACS analysis of UBC-CreERT2;*Rnpc3*^{lox/lox} AML cells. (F) Genomic analysis of the *Rnpc3* locus in tertiary transplanted AML cells.

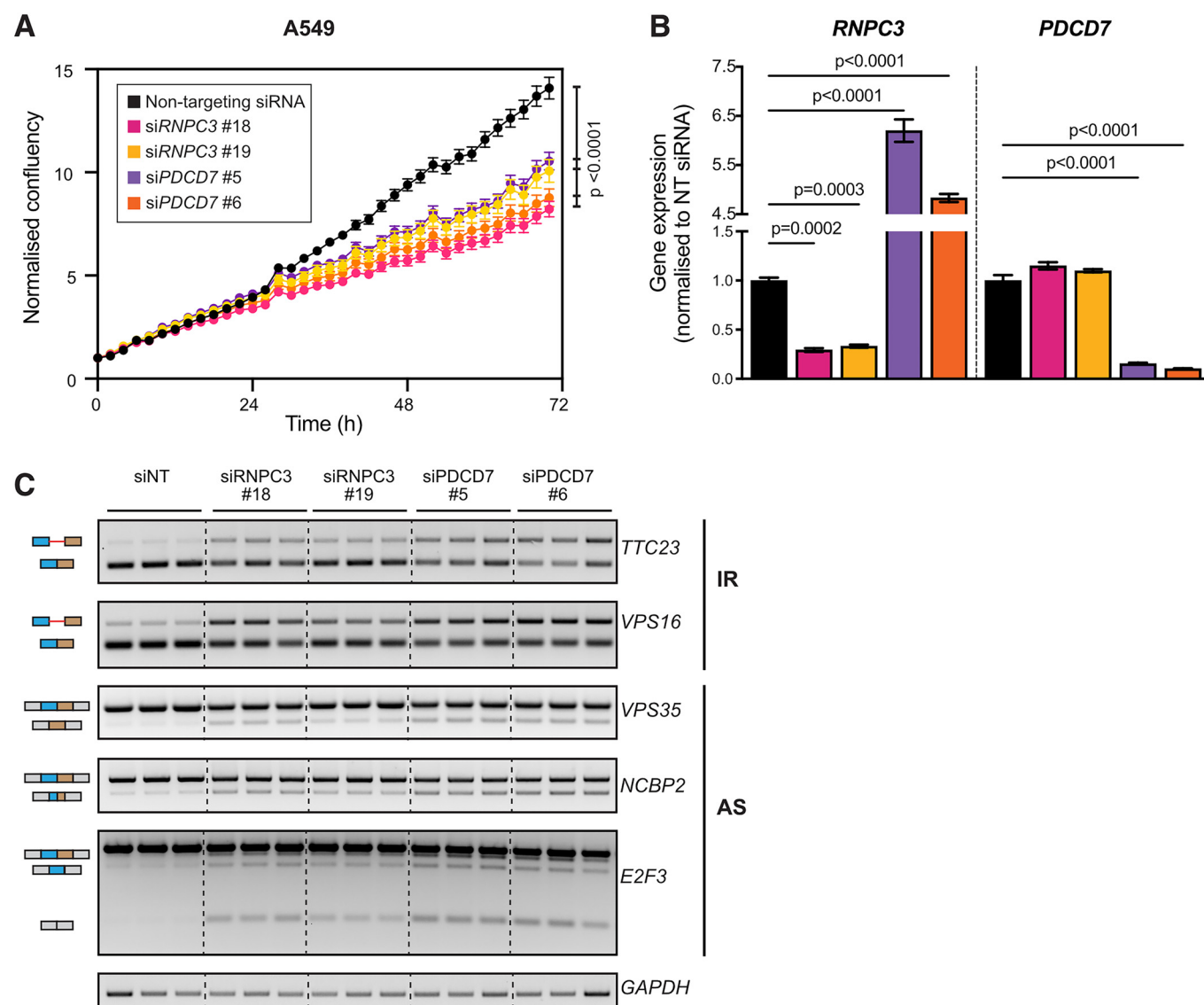


Figure EV4. Analysis of cell proliferation and aberrant splicing of MIGs in A549 cells treated with siRNPC3 and siPDCD7 for 72 h.

(A) Quantification of A549 cell growth over 72 h treatment with non-targeting (NT), two independent *RNPC3* or 2 independent *PDCD7* siRNAs. Data are represented as mean $\pm$ SEM ($n = 3$, 25 images per well, every hour). Significance was assessed by 2way ANOVA with Dunnett's multiple comparisons test. Note: the siNT and siRNPC3 data are the same as shown in Fig. 5B. (B) RT-qPCR analysis of *RNPC3* and *PDCD7* mRNA in A549 cells after 72 h treatment with siRNAs. Data are represented as mean $\pm$ SEM ($n = 3$), significance was tested using a one-way ANOVA with Tukey's multiple comparisons test. (C) RT-PCR analysis of example MIG splicing changes, identified in A549 cells treated with siRNPC3 or siPDCD7 for 72 h. Schematised IR and AS events are represented on the left with the minor intron in red and the flanking upstream and downstream exons in blue and orange, respectively. Exons not separated by a minor intron are grey.

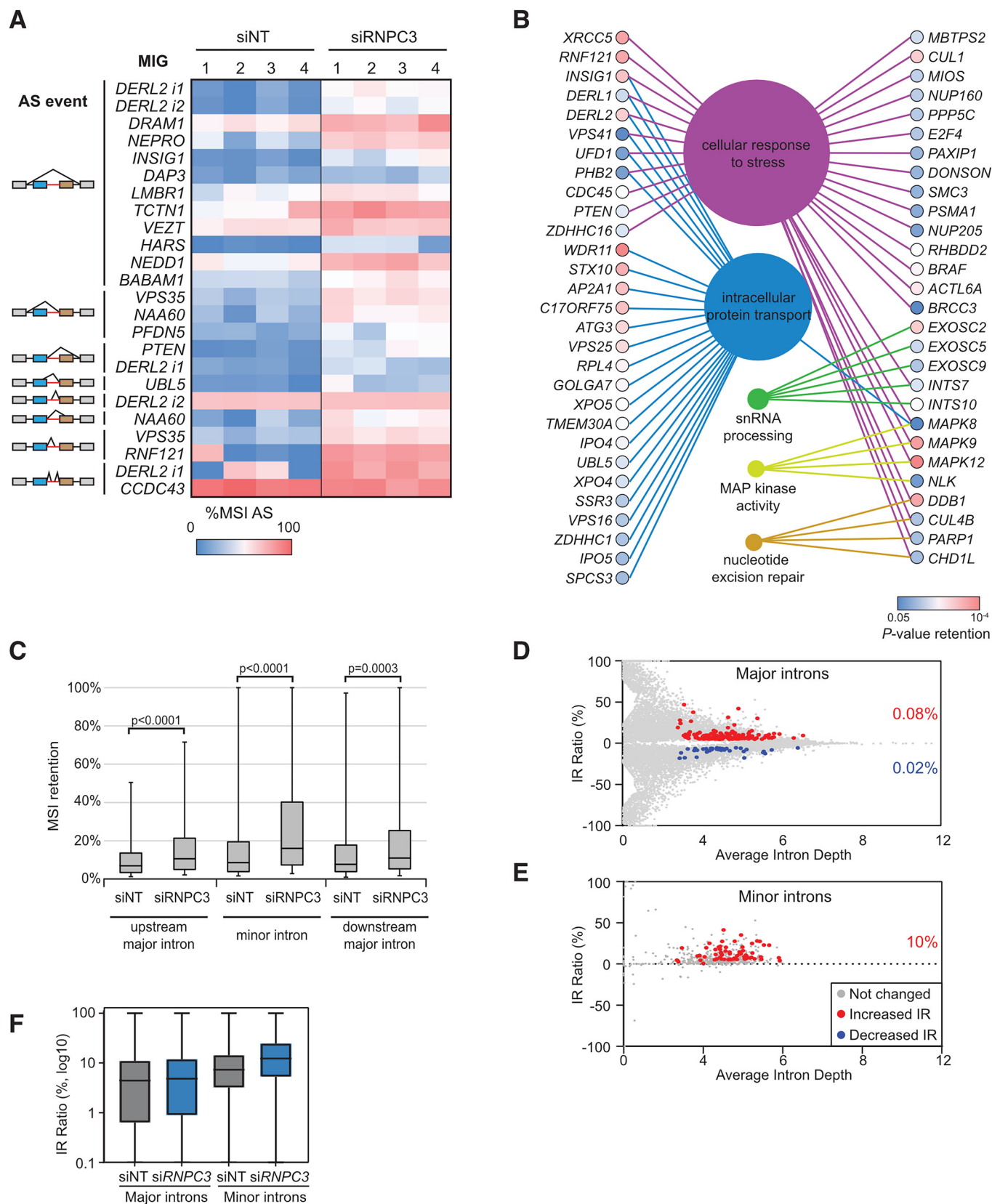
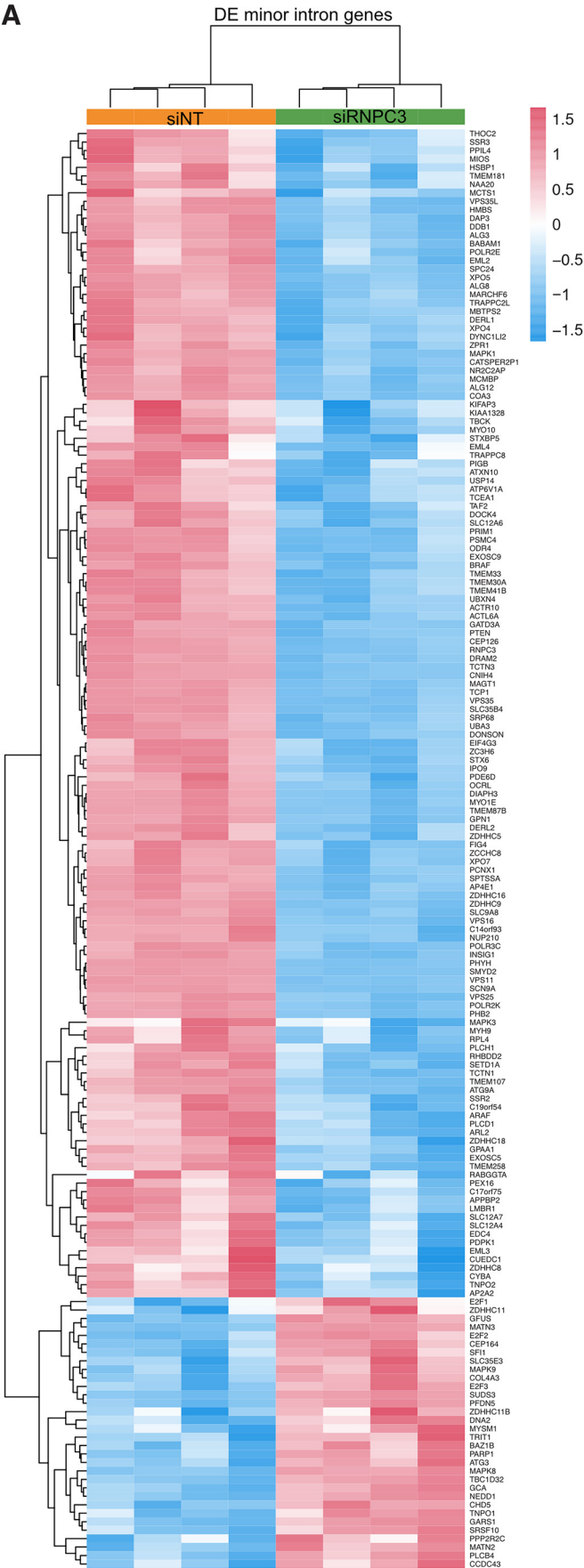


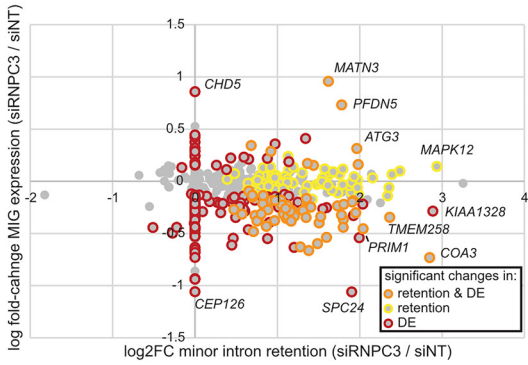
Figure EV5. RNAseq analysis of A549 cells treated with siRNPC3 for 72 h reveals defects in MIG splicing.

(A) Heatmap of RNAseq identified MIG AS events. (B) Selected enriched GO terms of MIGs with significant intron retention. Significance was assessed by Welch's *t* test. (C) Box plot of intron retention at minor introns and their upstream and downstream major introns. Significance was assessed by Mann-Whitney *U* test, *n* = 4 replicate RNAseq samples/genotype. (D, E) Transcriptome-wide IRFinder analysis showing median IR for all introns. (D) Major introns showing significantly upregulated IR (solid red circles) and significantly downregulated IR (solid blue circles). (E) Minor introns showing significantly upregulated IR (solid red circles). For an intron to be called as significantly retained in siRNPC3 samples, we required the following, FDR JQ 0.05, 100% coverage of the intron in siRNPC3 samples, 10% IRratio in siRNPC3 and 5% IRratio over NT samples. Likewise, for an intron to be significantly retained in NT samples, FDR JQ 0.05, 100% coverage across the intron in NT samples, 10% IRratio in NT cells and 5% IRratio over siRNPC3 samples. (F) Box plot of intron retention (% IRratio log10) across 234,057 major and 599 minor introns. Significance was assessed by paired *t* test.

A



B



**Figure EV6. A549 cells treated for 72 h with *RNPC3* siRNA exhibit differential expression of genes and MIGs that are enriched in cancer.**

(A) Heatmap of all identified differentially expressed MIGs, showing logCPM corrected for replicate and scaled for each gene. (B) Log-fold change plot of all MIGs highlighting those that exhibit significant changes in intron retention, differential expression or both when *RNPC3* is knocked down.

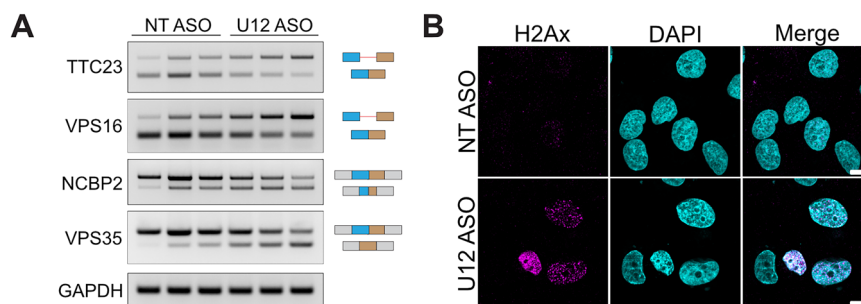


Figure EV7. A549 cells treated for 72 h with U12 ASO exhibit aberrant MIG splicing and markers of DNA damage.

(A) RT-PCR analysis of MIG splicing changes in A549 cells after 72 h treatment with 10 nM ASO. Schematic depictions of the obtained amplicons are shown on the right with the minor intron in red and the upstream and downstream exons coloured blue and orange, respectively. Exons not separated by a minor intron are grey. (B) Representative images of γ H2Ax (Ser139) staining in A549 cells 72 h after 10 nM ASO treatment. Scale bar is 10 μ m.