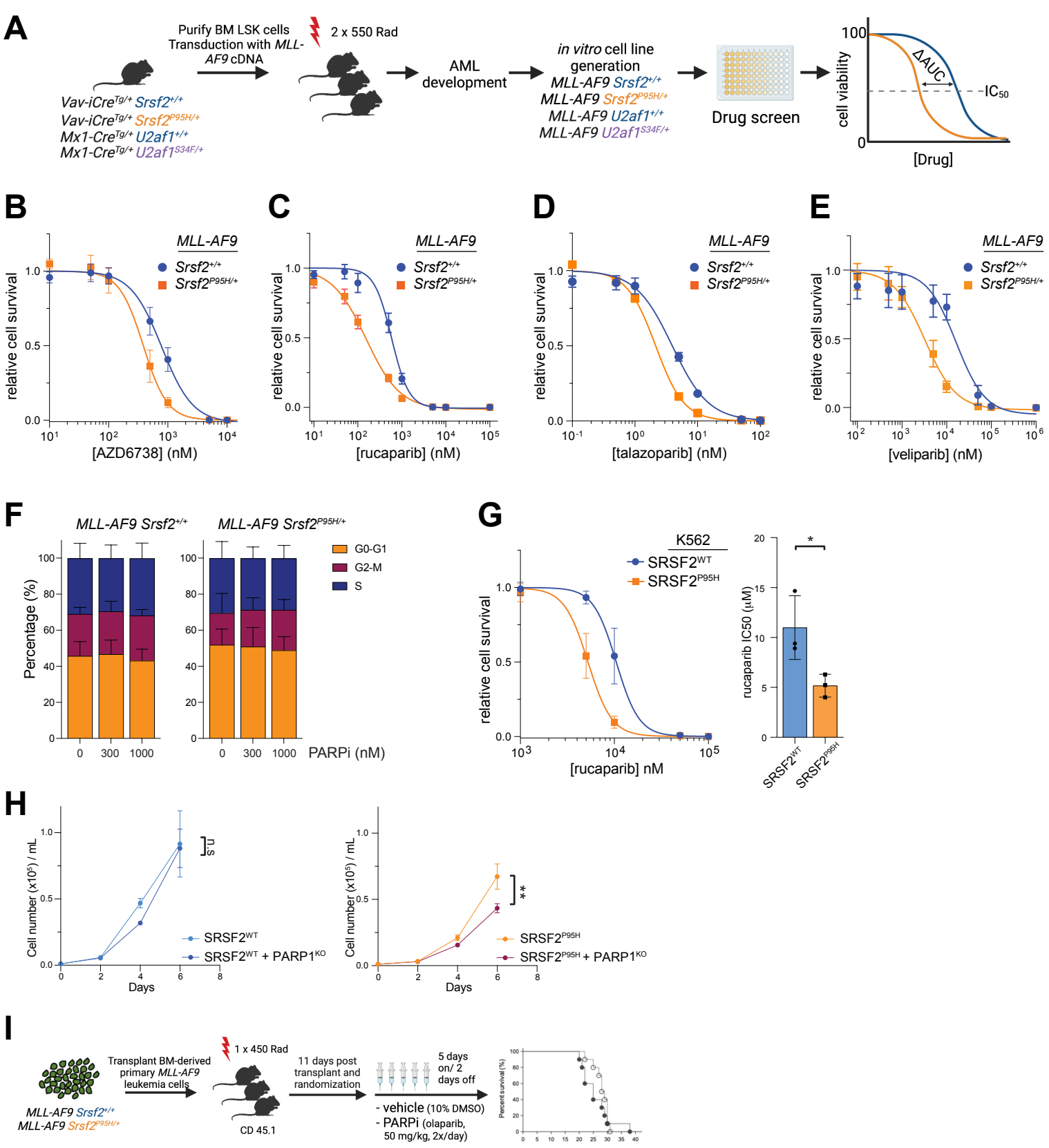
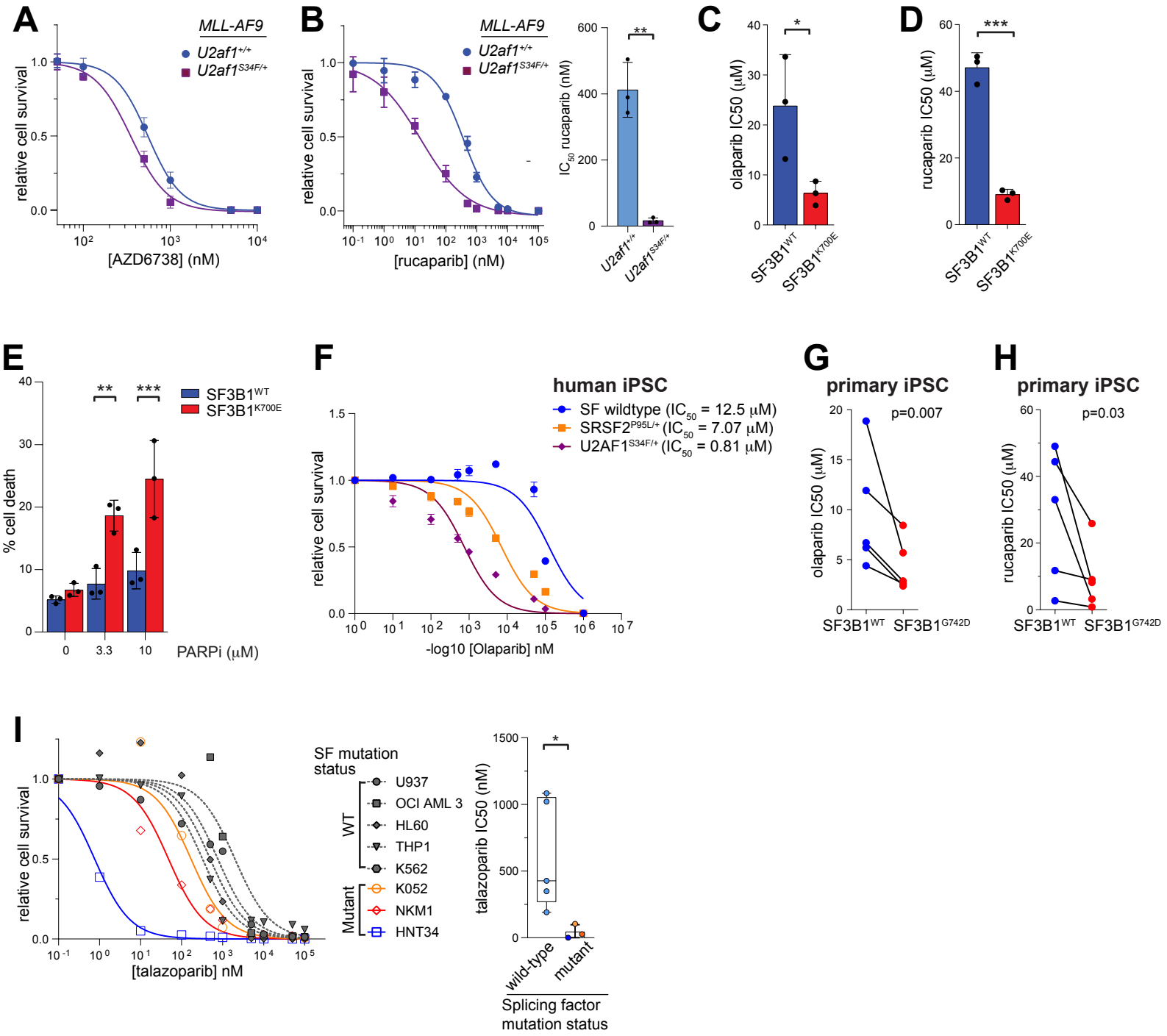


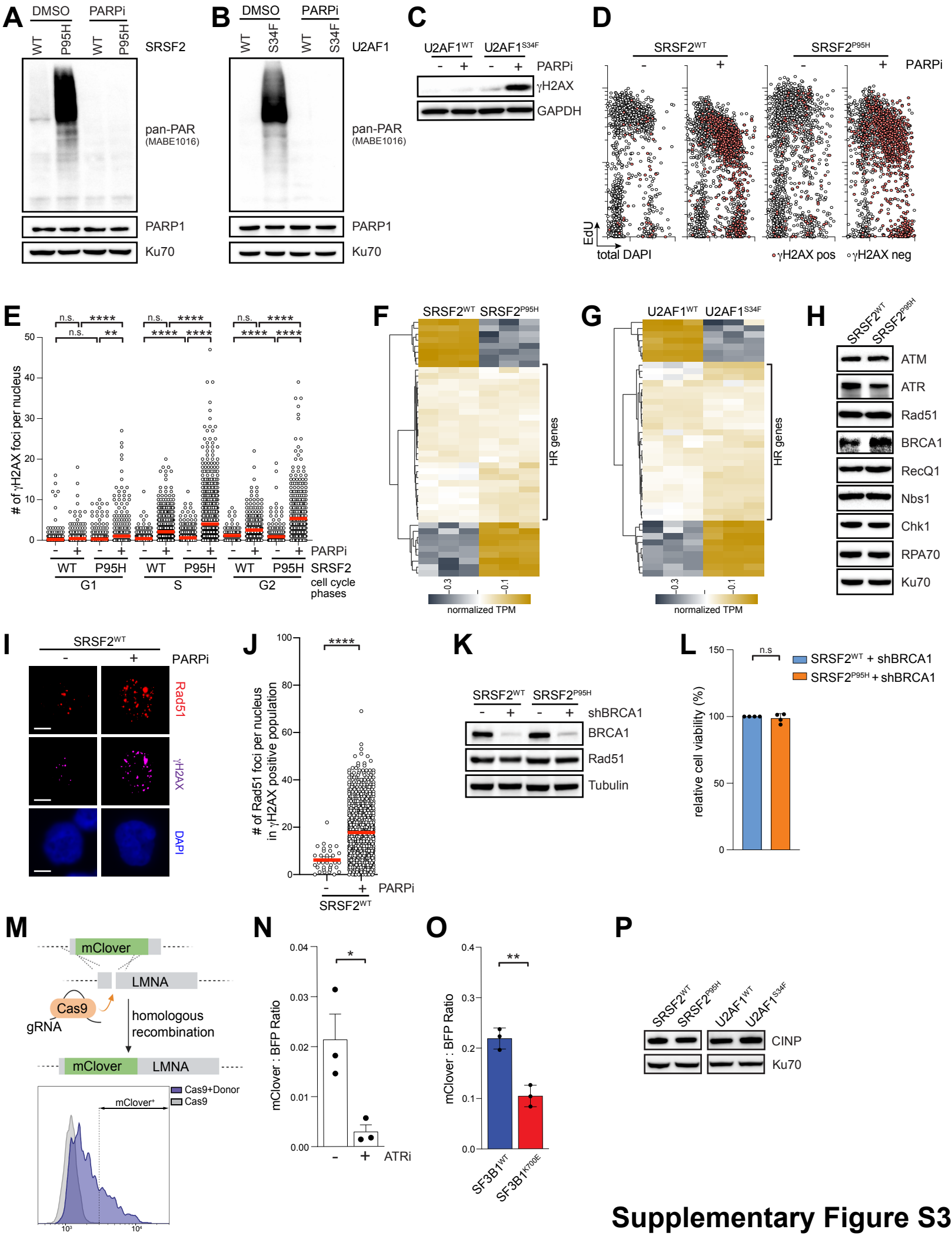
Supplementary Figure S1

Supplementary Figure S1. (A) Experimental schema for the generation of primary *MLL-AF9* murine leukemia on indicated genetic backgrounds for *in vitro* drug screening. **(B-E)** Relative cell viability of murine *MLL-AF9 Srsf2*$^{+/+}$ and *MLL-AF9 Srsf2*$^{P95H/+}$ cells were treated with indicated inhibitors for 72 hours (n=3 independent experiments). Error bars represent standard deviation. **(F)** Cell cycle analysis of murine *MLL-AF9 Srsf2*$^{+/+}$ and *MLL-AF9 Srsf2*$^{P95H/+}$ cells treated with DMSO or olaparib (300 nM and 1 μM) (n=3 independent experiments). Error bars represent standard deviation. **(G)** Relative cell viability, left, and IC_{50} values, right of K562 *SRSF2*^{WT} and *SRSF2*^{P95H} cells in response to rucaparib treated for 7 days (n=3 independent experiments). Statistical analysis was performed using unpaired two-tailed Student's t-test (*, $p<0.05$). **(H)** Viable cell numbers of K562 SRSF2^{WT} and SRSF2^{P95H} cells with or without *PARP1* deletion were determined using trypan blue exclusion method. Error bars represent standard deviation (n=3 independent experiments). Statistical analysis using 2-way ANOVA method was performed (n.s. and ** indicate not significant and $p<0.01$, respectively). **(I)** Experimental schema for the Kaplan-Meier survival curves of mice transplanted with *Srsf2*$^{+/+}$ and *Srsf2*$^{P95H/+}$ leukemia cells followed by treatment with vehicle or PARPi (olaparib).



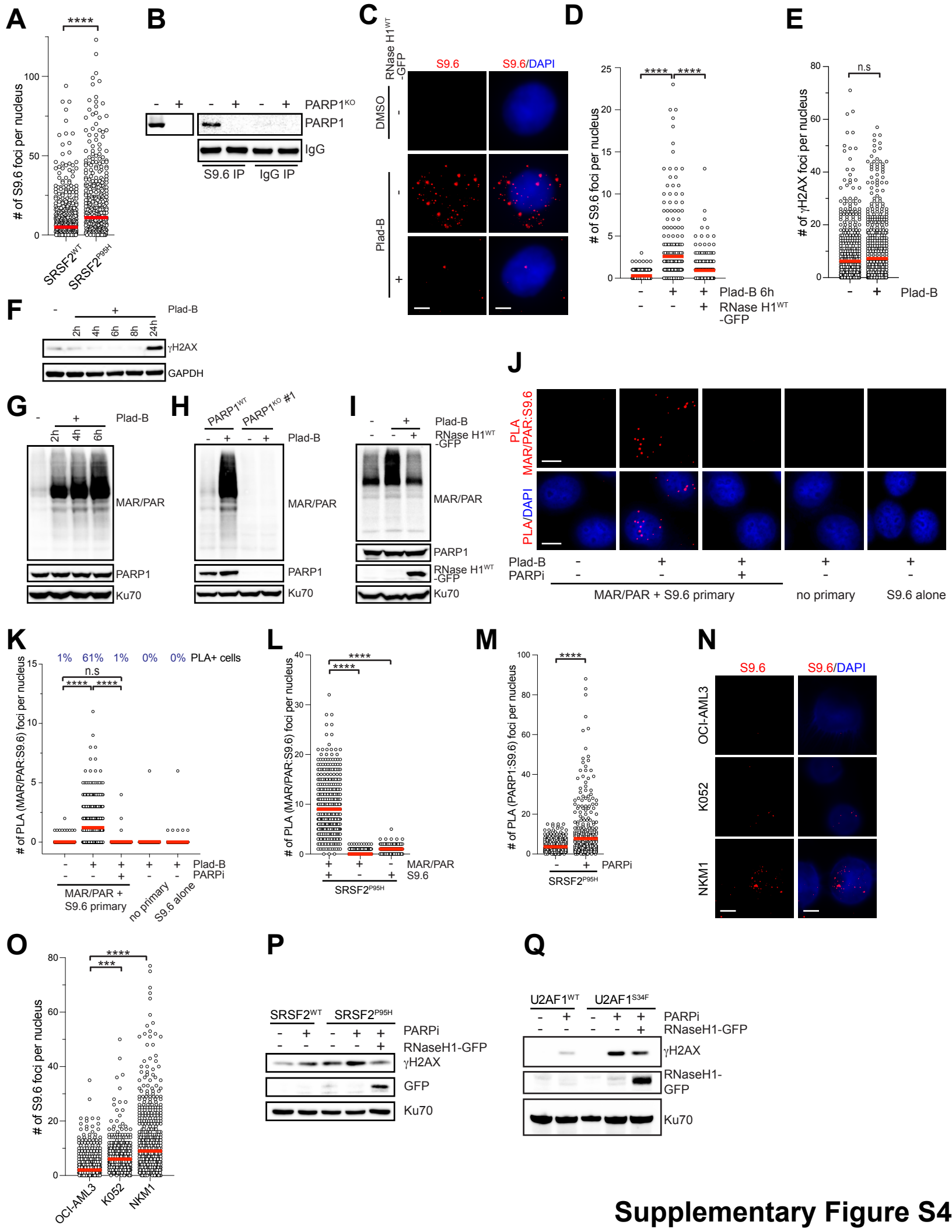
Supplementary Figure S2

Supplementary Figure S2. (A) Relative cell viability of murine *MLL-AF9 U2af1*$^{+/+}$ and *MLL-AF9 U2af1*$^{S34F/+}$ cells in response to AZD6738 72 hours post treatment. **(B)** Relative cell viability and IC_{50} of murine *MLL-AF9 U2af1*$^{+/+}$ and *MLL-AF9 U2af1*$^{S34F/+}$ cells in response to rucaparib 72 hours post treatment. Statistical analysis was performed using unpaired two-tailed Student's t-test (*, $p<0.05$). **(C-D)** IC_{50} of K562 SF3B1^{WT} and SF3B1^{K700E} cells for PARP inhibitors olaparib (C) and rucaparib (D). Statistical analysis was performed using unpaired two-tailed Student's t-test (*, ***, $p<0.05$, $p<0.0001$, respectively). **(E)** K562 SF3B1^{WT} and SF3B1^{K700E} cells were treated with DMSO or olaparib (PARPi; 3.3 and 10 μM) for 24h followed by Annexin V analysis. Error bars represent standard deviation one-way analysis of ANOVA followed by Tukey's post-hoc test was used to adjust for multiple comparison (**, ***, $p<0.01$, $p<0.001$, respectively). **(F).** Relative cell viability of human iPSC-HPCs that are spliceosome wildtype (SF wildtype) or spliceosome mutated *SRSF2*^{P95L} and *U2FA1*^{S34F} respectively were determined 96 hours post olaparib treatment. **(G-H)** MDS patient-derived iPSC-HPCs harboring either *SF3B1*^{WT} or *SF3B1*^{G742D} mutation were treated with olaparib (G) or rucaparib (H). IC_{50} values for each biological replicate were calculated. Statistical analysis was performed using ratio paired Student's t-test (n=5 independent experiments). **(I)** Relative cell viability and IC_{50} of indicated human leukemia cell lines that are either wildtype for splicing factor mutations or carry the splicing factor mutations in response to talazoparib 7 days post treatment. Statistical analysis was performed using unpaired two-tailed Student's t-test (*, $p<0.05$).



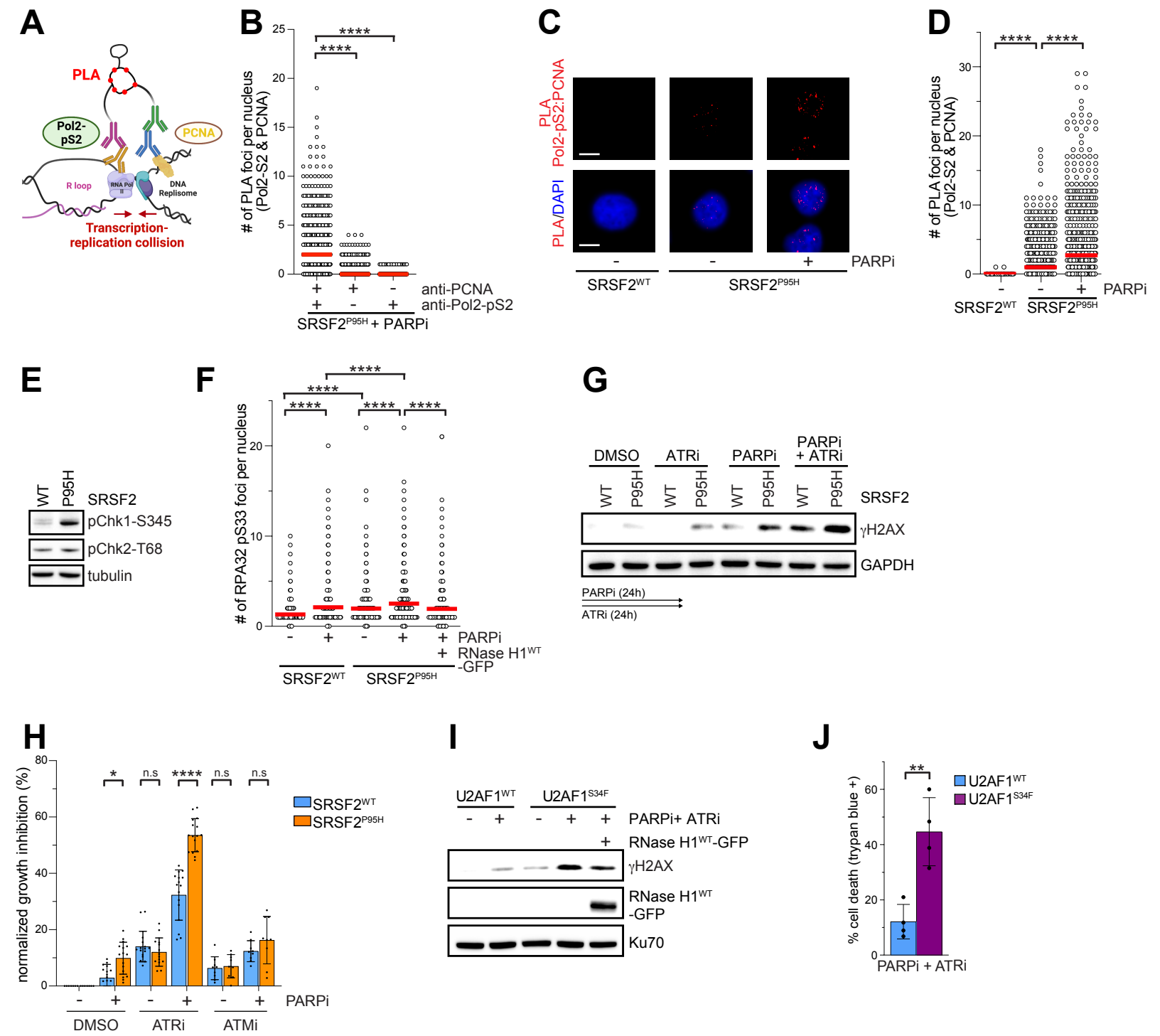
Supplementary Figure S3

Supplementary Figure S3. (A-B) Immunoblot analysis of total pan-PARylation (pan-PAR) and PARP1 levels in K562 $SRSF2^{WT}$ and $SRSF2^{P95H}$ cells (A) and $U2AF1^{WT}$ and $U2AF1^{S34F}$ cells (B) at steady state or following acute olaparib treatment (PARPi, 10 μM) for 1h. **(C)** Assessment of γH2AX levels in K562 $U2AF1^{WT}$ and $U2AF1^{S34F}$ cells following PARPi treatment (olaparib, 10 μM for 24h). **(D)** QBIC analysis of K562 $SRSF2^{WT}$ and $SRSF2^{P95H}$ cells treated with DMSO or olaparib (PARPi, 10 μM for 24 hours). Cells were pulsed for 30 mins at the end of the treatment. The pink dots represent γH2AX positive cells. **(E)** QBIC specific quantification of γH2AX foci numbers per nucleus (n>2000) for each experimental condition in Fig. 2F. Red bars represent the mean of the indicated groups. Statistical analysis was obtained using ordinary one-way ANOVA (****, $p<0.0001$, n.s., non-significant). **(F-G)** Heatmaps of HR gene expression in the KEGG pathway hsa03440 in transcripts per million (TPM) normalized using SciKit-learn L1 normalization, compared against randomly selected genes that are significantly differentially expressed in K562 $SRSF2^{P95H}$ (F) and $U2AF1^{S34F}$ (G) cells compared to their respective isogenic wildtype control. **(H)** Immunoblot analysis of indicated protein levels in K562 $SRSF2^{WT}$ and $SRSF2^{P95H}$ cells. **(I-J)** K562 $SRSF2^{WT}$ cells were treated with olaparib (PARPi,10 μM) for 24 hours. Chromatin-bound Rad51 foci in γH2AX-positive cells were analyzed. Representative images (scale bar = 5 μm) and quantification of Rad51 foci numbers per γH2AX-positive nucleus in (n>30) are shown in I and J, respectively. Red bars represent the mean in each group. Statistical analysis was obtained using unpaired two-tailed Student's t-test (****, $p<0.0001$). **(K)** Immunoblot analysis of indicated protein levels in K562 $SRSF2^{WT}$ and $SRSF2^{P95H}$ cells expressing either shControl or shBRCA1. **(L)** Relative cell viability of K562 $SRSF2^{WT}$ and $SRSF2^{P95H}$ cells expressing shBRCA1 were measured by CellTiter-Glo and normalized to respective shControl cells. **(M)** Top, experimental schema of the CRISPR-Cas9/mClover assay. Bottom, representative flow cytometry results to gate for mClover-positive cells within the BFP-positive population, a marker for positive infectivity. **(N)** K562 cells were treated with DMSO or ATRi (VE-821, 1 μM) for 48 hours for mClover assay. Error bars represent standard deviation (n=3). Statistical analysis was performed using unpaired two-tailed Student's t-test. (*, $p<0.05$) **(O)** Assessment of HR repair efficiency by mClover:BFP ratio in K562 $SF3B1^{WT}$ and $SF3B1^{K700E}$ cells. Error bars represent standard deviation (n=3 independent experiments). Statistical analysis was performed using unpaired two-tailed Student's t-test (**, $p<0.01$). **(P)** Immunoblot analysis of CINP protein levels in indicated K562 cells.



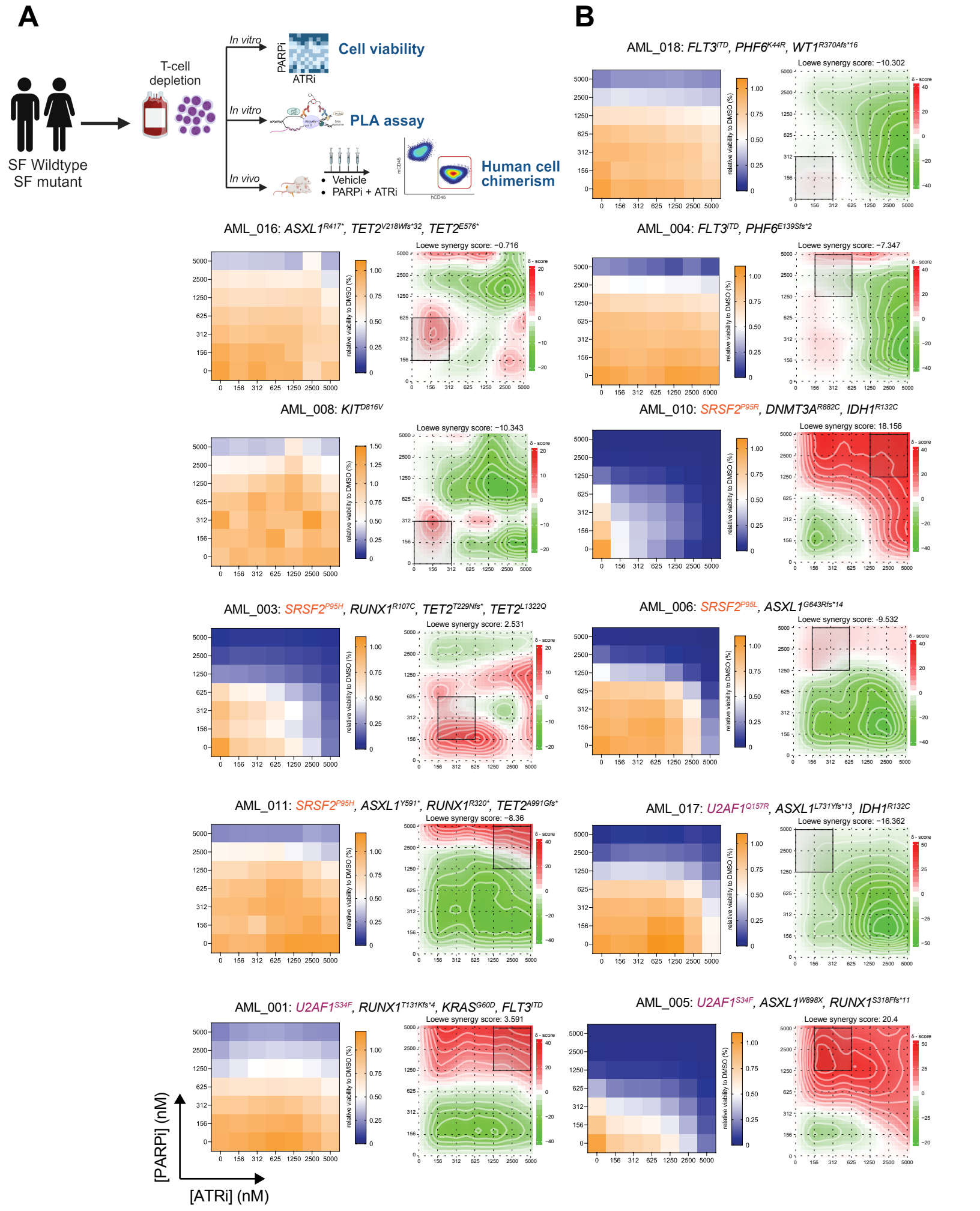
Supplementary Figure S4

Supplementary Figure S4. (A) Quantification of S9.6 foci numbers per nucleus (n>400) for K562 SRSF2^{WT} and SRSF2^{P95H} cells. Red bars represent the median in the indicated groups. Statistical analysis was obtained using unpaired two-tailed Student's t-test (****, p<0.0001). **(B)** Co-immunoprecipitation in K562 cell lysates using S9.6 or mouse IgG antibodies and assessment of PARP1 association by western blot. **(C-D)** HeLa cells that inducibly expressing RNase H1^{WT}-GFP were treated with either DMSO or Plad-B (3 nM) for 6h. RNase H1^{WT} expression was induced by addition of doxycycline (100 ng/mL) for 24h. Representative images (scale bar = 5 μm) and quantification of S9.6 foci numbers per nucleus (n>300) for each condition are shown in C and D, respectively. Red bars represent the mean in the indicated groups. Statistical analysis was obtained using ordinary one-way ANOVA (****, p<0.0001). **(E)** Quantification of γH2AX foci numbers per nucleus (n>600) in HeLa cells treated with DMSO or pladienolide-B (Plad-B, 3 nM) for 6 hours. Red bars represent the mean in each groups. Statistical analysis using unpaired two-tailed Student's t-test was performed (n.s., non-significant). **(F)** HeLa cells were treated with Plad-B (3 nM) for different timepoints and subjected to western blot analysis. **(G)** Immunoblot analysis of total Mono-/poly-ADPribosylation (MAR/PAR) and PARP1 levels in HeLa cells treated with Plad-B (3 nM) at the indicated timepoints. **(H)** Parental or *PARP1*-knockout HeLa cells were treated with Plad-B (3 nM) for 6 hours, followed by immunoblot analysis. **(I)** HeLa cells inducibly expressing RNase H1^{WT}-GFP were treated with either DMSO or Plad-B (3 nM) for 6 hours and subjected to western blot. RNase H1^{WT} expression was induced by addition of doxycycline (100 ng/mL) for 24h. **(J)** Representative images of S9.6:MAR/PAR PLA foci in HeLa cells treated with DMSO or Plad-B (3 nM) alone or combined with olaparib (PARPi, 10 μM) for 6 hours, scale bar = 5 μm. **(K)** Quantification of MAR/PAR:S9.6 PLA foci numbers per nucleus (n>300) for each condition in (J). Red bars represent the median in the indicated groups. Statistical analysis was obtained using ordinary one-way ANOVA (****, p<0.0001, n.s., non-significant). **(L)** Quantification of MAR/PAR:S9.6 PLA foci numbers per nucleus (n>400) in K562 SRSF2^{P95H} with single antibody control. Red bars represent the median in the indicated groups. Statistical analysis was obtained using ordinary one-way ANOVA (****, p<0.0001). **(M)** Quantification of PARP1:S9.6 PLA foci numbers per nucleus (n>400) in K562 SRSF2^{P95H} treated with olaparib (PARPi, 10 μM for 1 hour). **(N)** Representative images of S9.6 foci in indicated cells, scale bar = 5 μm. **(O)** Quantification of S9.6 foci numbers per nucleus (n>450) for each condition in (N). Red bars represent the median in the indicated groups. Statistical analysis was obtained using ordinary one-way ANOVA (***, **** indicate p<0.001, p<0.0001, respectively). **(P)** K562 SRSF2^{WT} and SRSF2^{P95H} cells inducibly expressing nuclear, GFP-tagged RNase H1^{WT} by addition of doxycycline (100 ng/mL) were treated with either DMSO or olaparib (PARPi, 5 μM) for 48 hours. The relative γH2AX levels were assessed by immunoblot. **(Q)** HeLa U2AF1^{WT} and U2AF1^{S34F} cells inducibly expressing nuclear RNase H1^{WT} by addition of doxycycline (400 ng/mL) were treated with either DMSO or olaparib (PARPi, 5 μM) for 72 hours. The relative γH2AX levels were assessed by immunoblot.



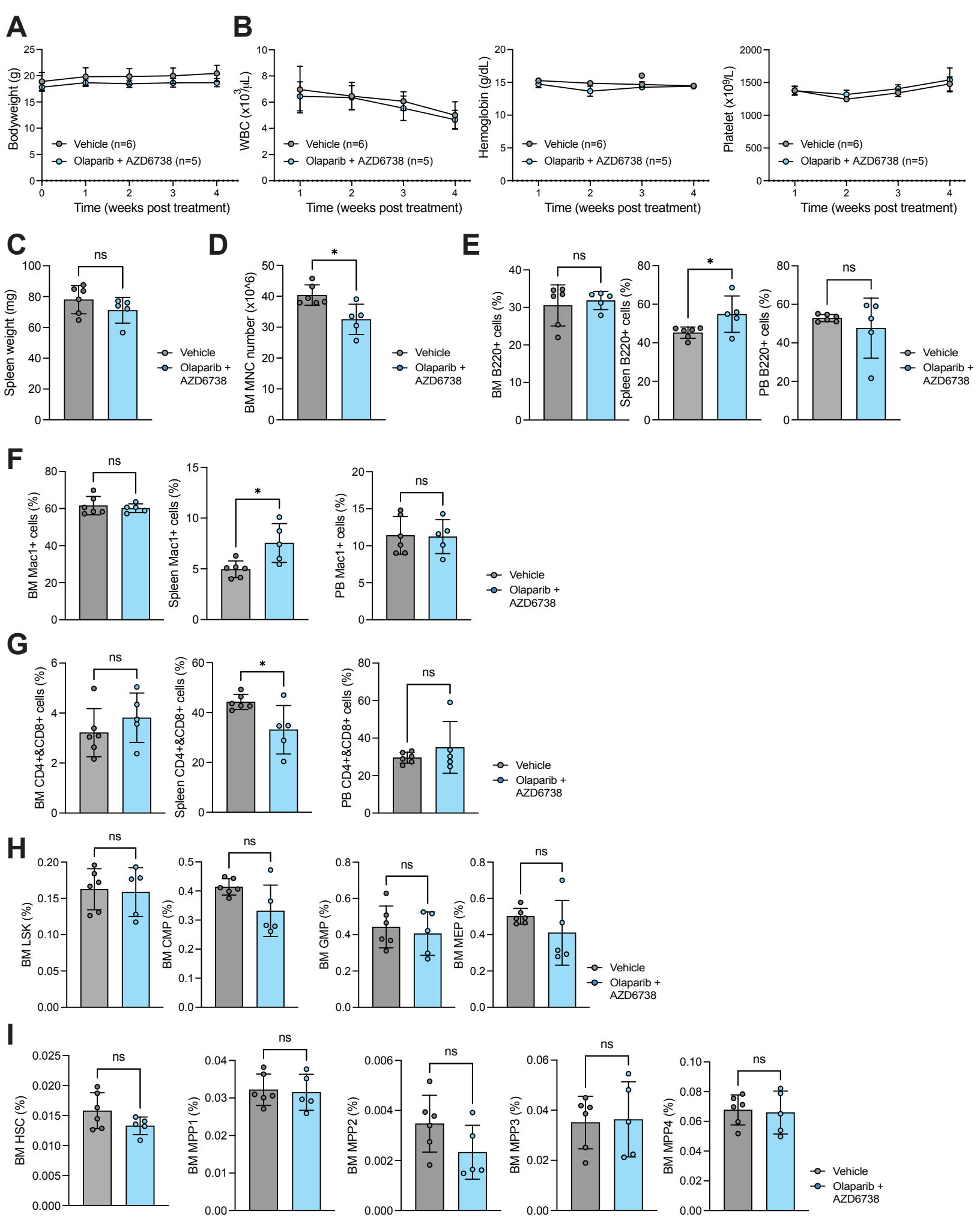
Supplementary Figure S5

Supplementary Figure S5. (A) Illustration of transcription-replication collision PLA using RNA Pol2-pS2 and PCNA antibodies. **(B)** Quantification of Pol2-pS2 and PCNA PLA foci numbers per nucleus (n>400) in K562 SRSF2^{P95H} treated with olaparib (PARPi, 3 μM, 24 hours) with single antibody control. Red bars represent the median in the indicated groups. Statistical analysis was obtained using one-way ANOVA (****, p<0.0001). **(C)** Representative images of Pol2-pS2:PCNA PLA in K562 SRSF2^{WT} and SRSF2^{P95H} treated with olaparib (PARPi, 3 μM, 24h; scale bar = 5 μm). **(D)** Quantification of Pol2-pS2:PCNA PLA foci numbers per nucleus (n>900) for each condition in (C). Red bars represent the mean in the indicated groups. Statistical analysis was obtained using ordinary one-way ANOVA (****, p<0.0001). **(E)** Immunoblot analysis pChk1-S345 and pChk2-T68 levels in K562 SRSF2^{WT} and SRSF2^{P95H} cells. **(F)** Quantification of RPA32 pS33 foci numbers per nucleus (n>2000). K562 SRSF2^{WT} and SRSF2^{P95H} cells inducibly expressed RNase H1^{WT} (100 ng/mL dox) while treated with DMSO or olaparib (PARPi, 3 μM, 24 hours). Red bars represent the mean in the indicated groups. Statistical analysis was obtained using one-way ANOVA (****, p<0.0001). **(G)** K562 SRSF2^{WT} and SRSF2^{P95H} cells were treated with either DMSO, PARPi (olaparib, 3 μM), ATRi (AZD6738, 3 μM), or combined PARPi+ATRi for 24 hours. **(H)** K562 SRSF2^{WT} and SRSF2^{P95H} cells were treated with PARP inhibitor (3 μM) alone or combination with either ATRi (3 μM) and ATMi (3 μM). Percent normalized growth inhibition was measured by CellTiterGlo (n=3 independent experiments). Error bars represent standard deviation. Statistical analysis using 2-way ANOVA was performed (*, ****, p<0.05, p<0.0001, respectively). **(I)** K562 U2AF1^{WT} and U2AF1^{S34F} cells were treated with DMSO, olaparib (PARPi, 3 μM), AZD6738 (ATRi, 1 μM) or combined for 24 hours. Doxycycline (400 ng/mL) was added in U2AF1^{S34F} cells to induce nuclear RNase H1^{WT} expression for the whole drug treatment duration. **(J)** K562 U2AF1^{WT} and U2AF1^{S34F} cells were treated with DMSO or combined PARPi (olaparib 1 μM) and ATRi (AZD67381 μM) for 5 days. Percent cell apoptosis was quantified by trypan blue-positive cells. Error bars represent standard deviation. Statistical analysis using unpaired two-tailed Student's t-test (**, p<0.01).



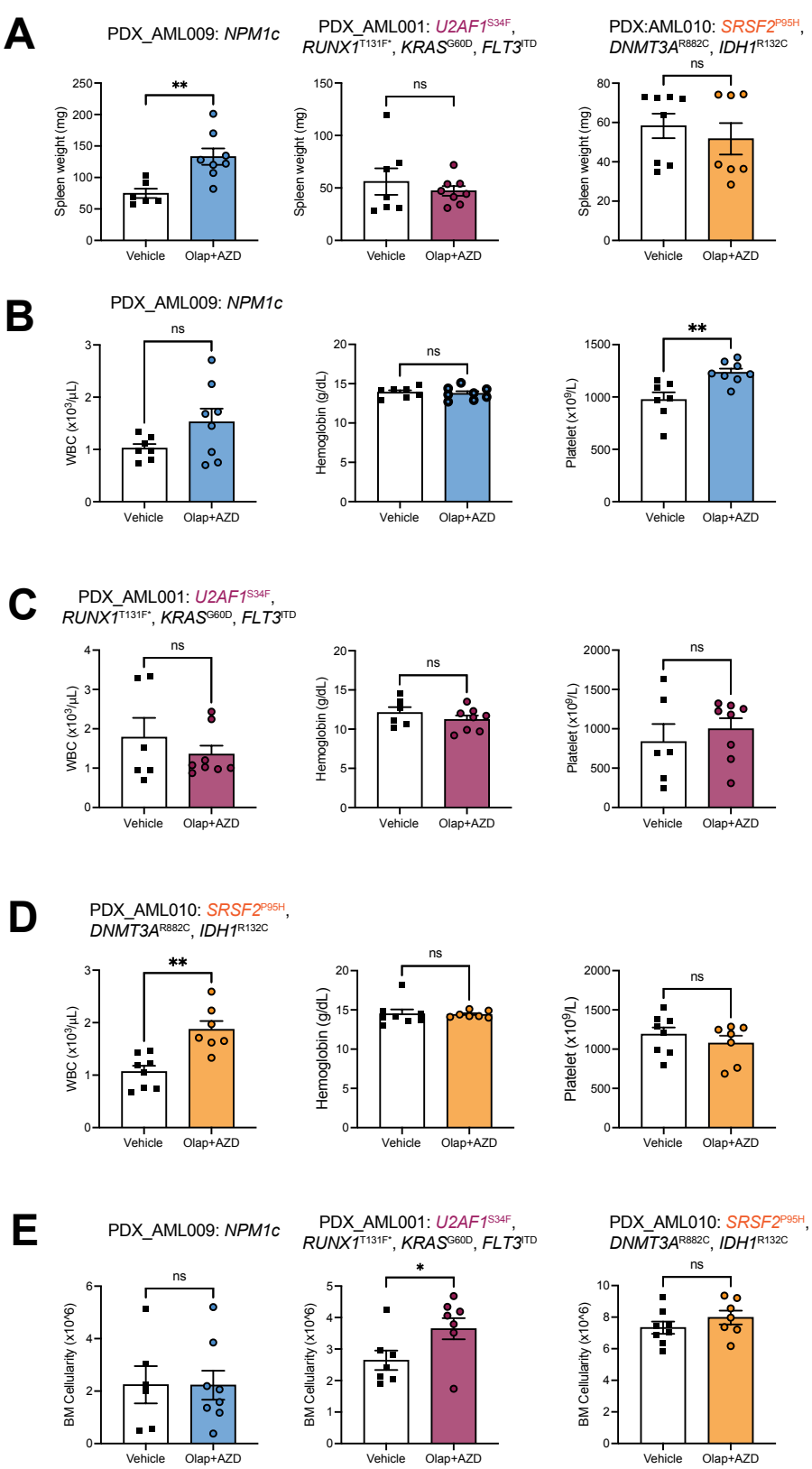
Supplementary Figure S6

Supplementary Figure S6. (A) Experimental schema for primary AML based *ex vivo* assays (PLA and cell viability) and *in vivo* PDX model generation and combination drug treatment. **(B)** Relative viability heatmaps and synergy analysis of human primary AML cells in response to combined treatment of olaparib (PARPi) and AZD6738 (ATRi). Cell viability relative to DMSO-treated cells was assessed by CellTiterGlo 96 hours post treatment (n=4 splicing wildtype, n=4 *SRSF2*-mutant and n=3 *U2AF1*-mutant AML samples). Loewe synergy score was determined for each sample using SynergyFinder 3.0.



Supplementary Figure S7

Supplementary Figure S7. Toxicity test of PARP and ATR combination treatment in C57BL/6 mice. **(A)** Bodyweight measurement of mice over a period of 4 weeks after receiving combined treatment with olaparib and AZD6738 or vehicle. **(B)** White blood cell (WBC), hemoglobin and platelet count from peripheral blood of mice over a period of 4 weeks after receiving combined treatment with olaparib and AZD6738 or vehicle. **(C)** Measurement of spleen weight and **(D)** bone marrow mononuclear cell number (BM MNC) after receiving 4 weeks of combined treatment with olaparib and AZD6738 or vehicle. Percentages of $B220^+$ cells **(E)**, $Mac1^+$ cells **(F)** and $CD4^+$ and $CD8^+$cells **(G)** in the bone marrow, spleen and peripheral blood after receiving 4 weeks of combined treatment with olaparib and AZD6738 or vehicle. **(H)** Percentages of BM LSK, CMP, GMP and MEP cell populations after receiving 4 weeks of combined treatment with olaparib and AZD6738 or vehicle. **(I)** Percentages of BM HSC, MPP1, MPP2, MPP3 and MPP4 cell populations after receiving 4 weeks of combined treatment with olaparib and AZD6738 or vehicle. Each data point represents one mouse and error bars represent standard deviation. Statistical analysis was performed using unpaired two-tailed Student's t-test (n.s. and * indicate not significant and $p<0.05$, respectively).



Supplementary Figure S8

Supplementary Figure S8. (A) The effect of combined olaparib+AZD6738 (Olap+AZD) or vehicle treatment on spleen weight of NSG-SGM3 mice in primary AML PDX models. Data are represented as mean ± s.d. **(B-D)** The effect of combined olaparib+AZD6738 (Olap+AZD) or vehicle treatment on the blood cell counts of NSG-SGM3 mice transplanted with (B) splicing wildtype, (C) *SRSF2*-mutant and (D) *U2AF1*-mutant primary AML patient samples. **(E)** The effect of combined olaparib+AZD6738 (Olap+AZD) or vehicle treatment on bone marrow cellularity of NSG-SGM3 mice in AML PDX models. Each data point represents one mouse and error bars represent standard deviation. Statistical analysis was performed using unpaired two-tailed Student's t-test (*, ** and n.s. indicate $p<0.05$, $p<0.01$ and not significant, respectively).