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## Supplemental information

# Malignant A-to-I RNA editing by ADAR1 drives T cell acute lymphoblastic leukemia relapse via attenuating dsRNA sensing

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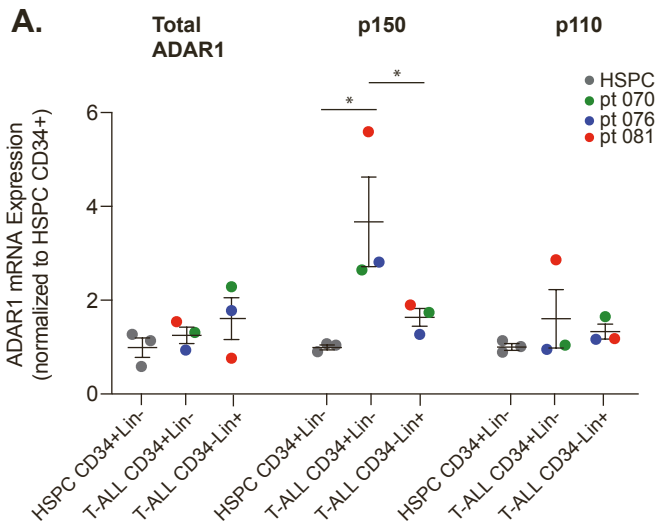
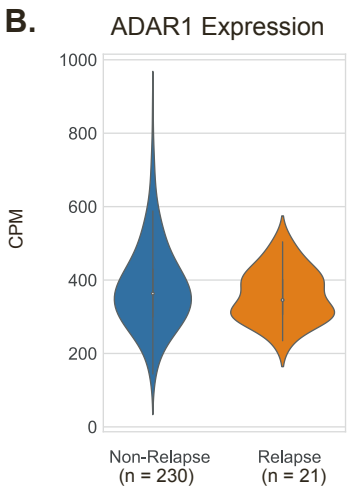
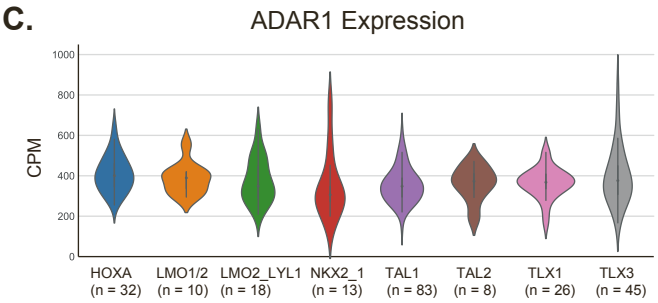
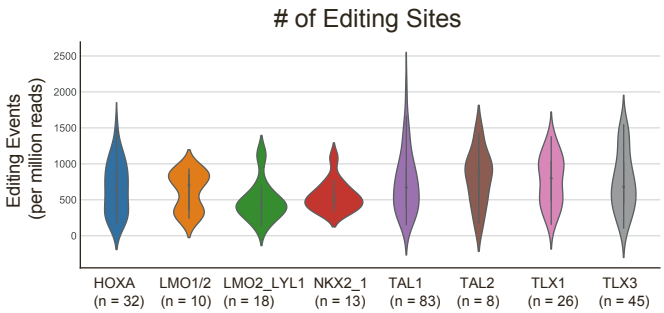
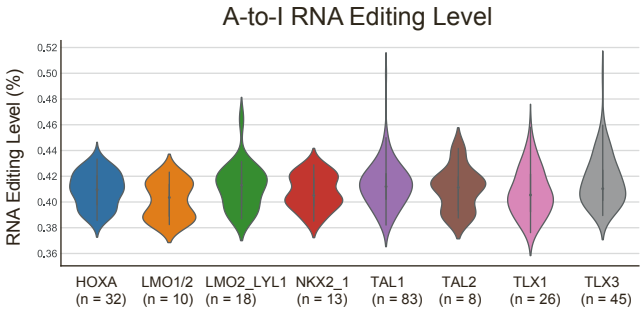
## Supplemental Figure 1

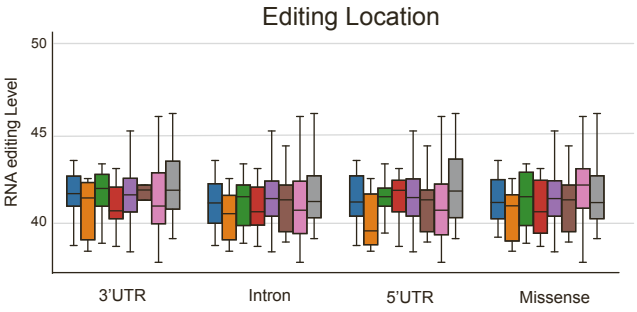


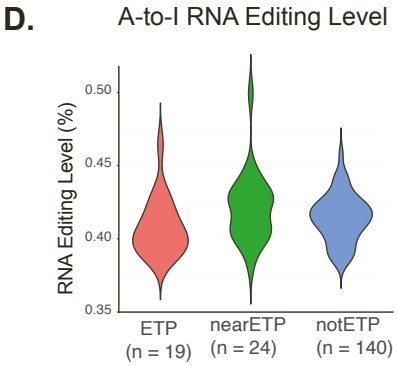



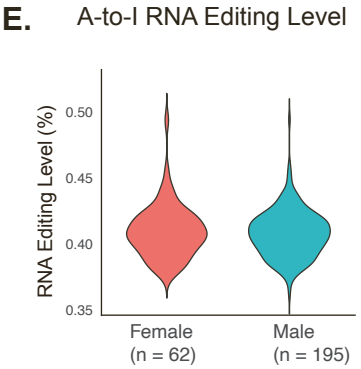



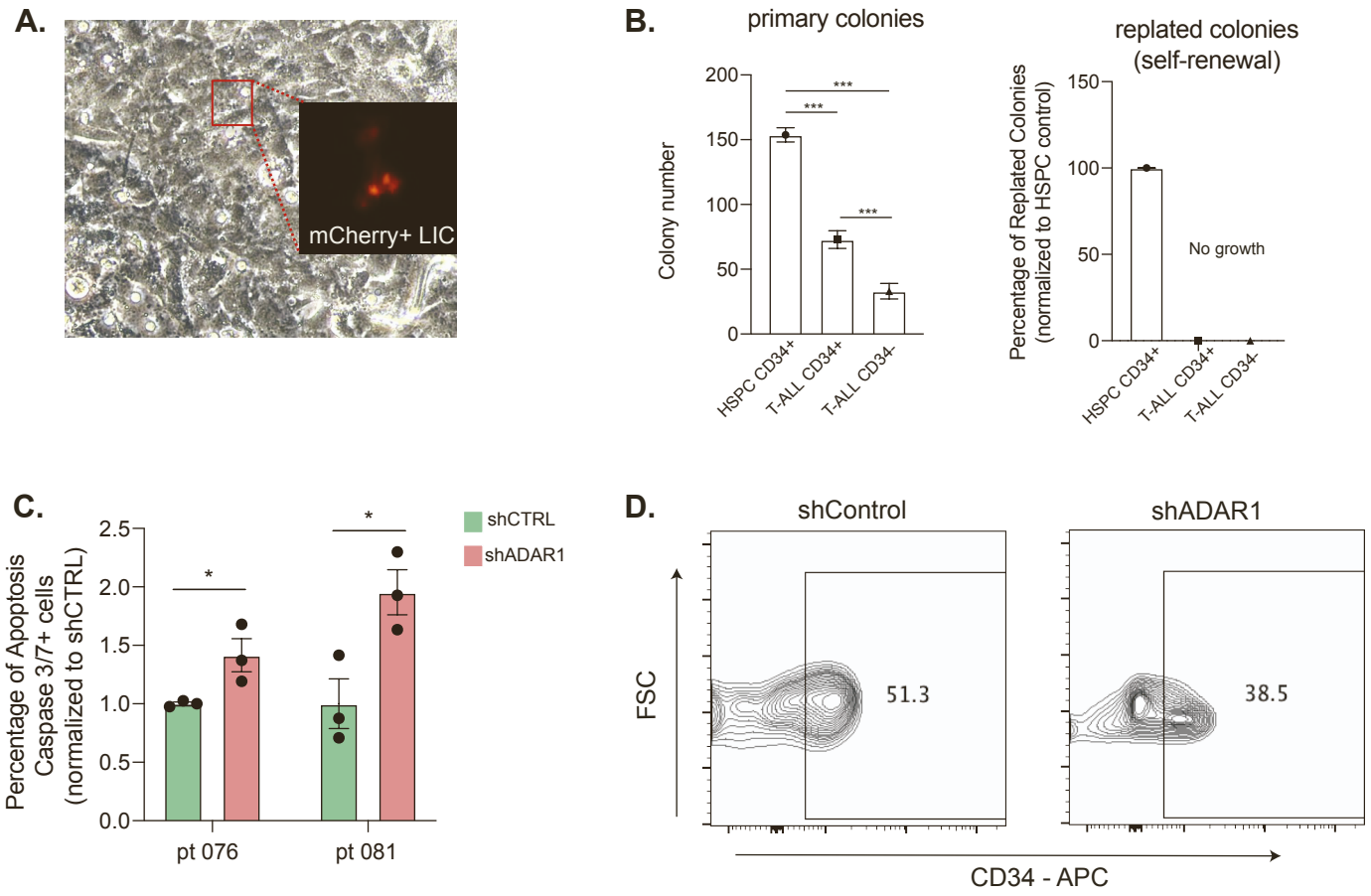



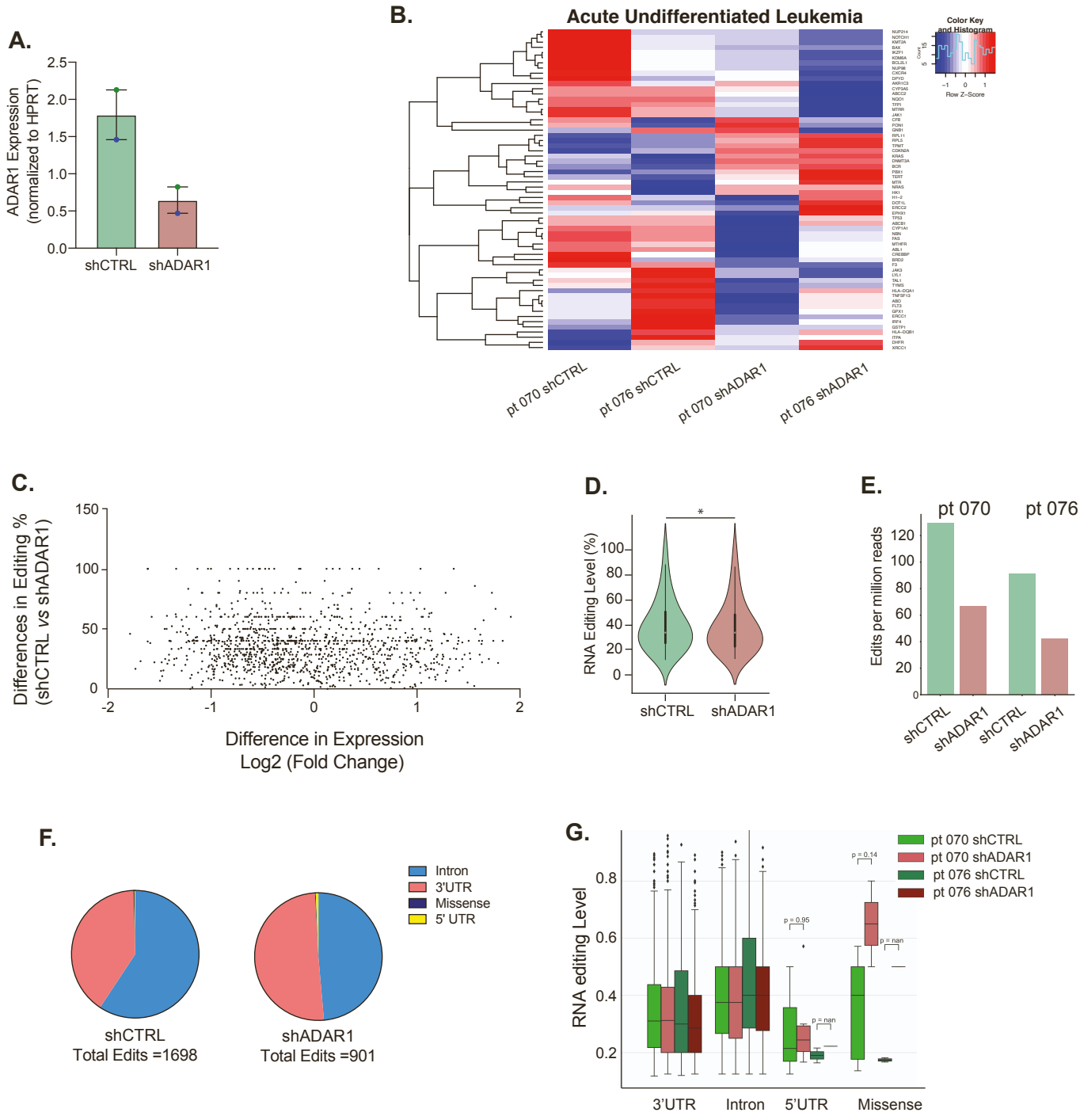

**Supplemental Figure. 1, related to Figure 1. ADAR1 expression and the A-to-I Editing landscape in T-ALL**. **A**.Expression levels of total ADAR1, ADAR1 p150 and ADAR1 p110 were quantified by RT-qPCR in cord blood derived CD34+Lin- HSPC (n = 3 cord blood) and CD34+Lin- or CD34-Lin+ T-ALL LIC (n = 3 patients, pt 070, pt 076 and pt 081). Error bars represents mean with SEM. * p< 0.05, unpaired student t-test. **B**. Expression of ADAR1 in non-relapsed and relapsed T-ALL cohort. **C**. Violin plots depicting ADAR1 expression, RNA editing level, number of editing events, and editing location were quantified based on patient molecular characteristics of NCI T-ALL TARGET dataset. **D-E**. RNA editing level based on ETP status (D) or gender (E). No significance by t-test (**B** and **E**) or one-way ANOVA (**C-D**) .

## Supplemental Figure 2



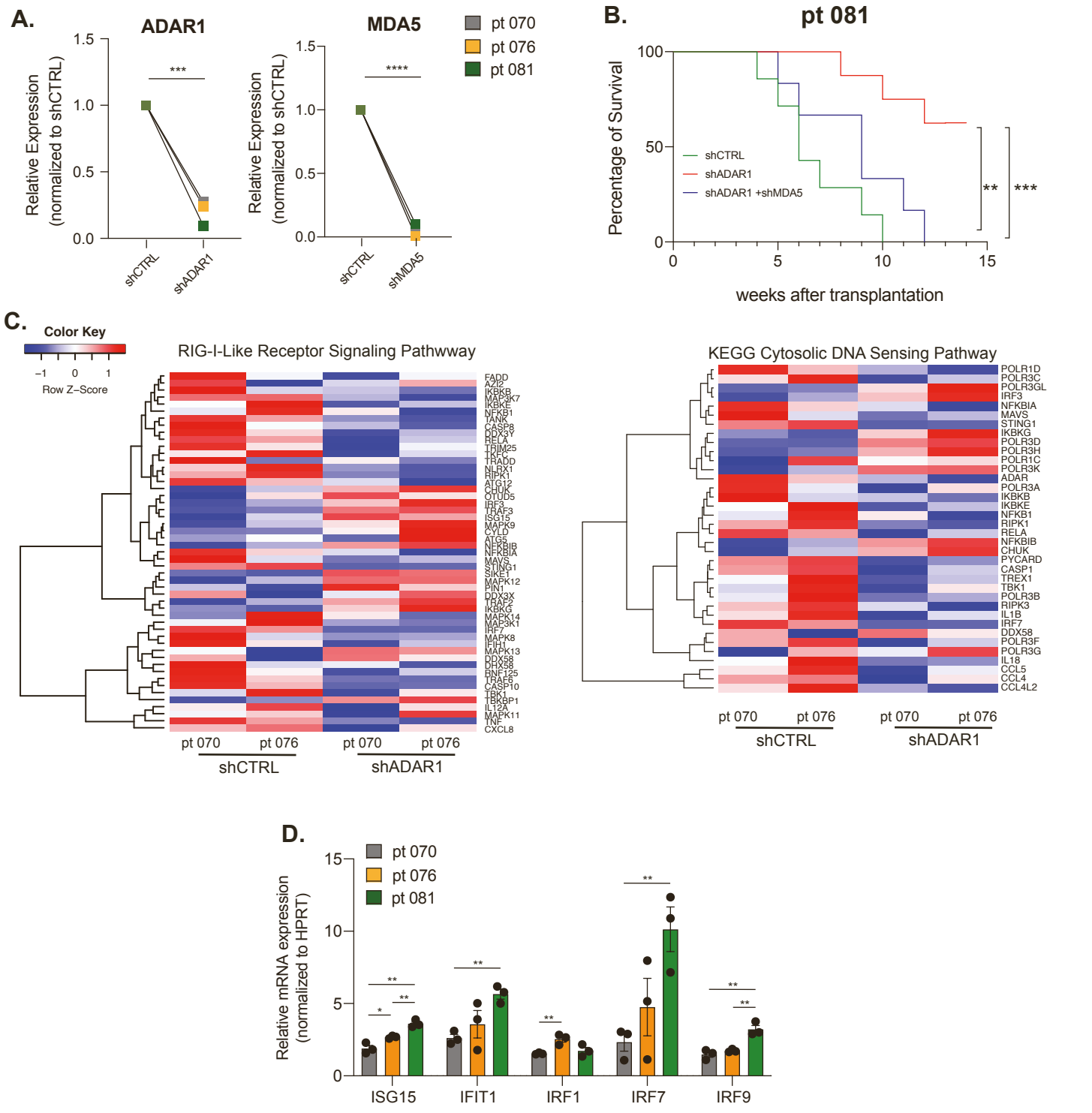

**Supplemental Figure. 2, related to Figure 2. Functional characterization of T-ALL LICs in ATO co-culture and PDX systems. A.** Images of mCherry-labeled T-ALL LICs in the Artificial Organoid System (ATO)**. B**. Bar plot showing number of primary colonies per 10,000 cells and percentage of replated secondary colonies. CD34+Lin- cord blood HSPC (n = 3), CD34+Lin- T-ALL LIC, and CD34-Lin+ T-ALL cells were sorted from PDX mouse bone marrow (n = 3, pt 070, pt 076, and pt 081). **C**. Percentage of apoptosis after ADAR1 knockdown in T-ALL LIC (pt 076 and pt 081, n = 3 experiments). **D**.Representative flow of CD45+Lin-CD34+ LICs cultured in ATOs. Cells were gated on CD45+Lin-population. T-ALL LICs were transduced with control or shRNA lentiviral vectors targeting ADAR1 for 3 days and cultured on MS5-DLL4 stroma. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, unpaired student t-test.

## Supplemental Figure 3



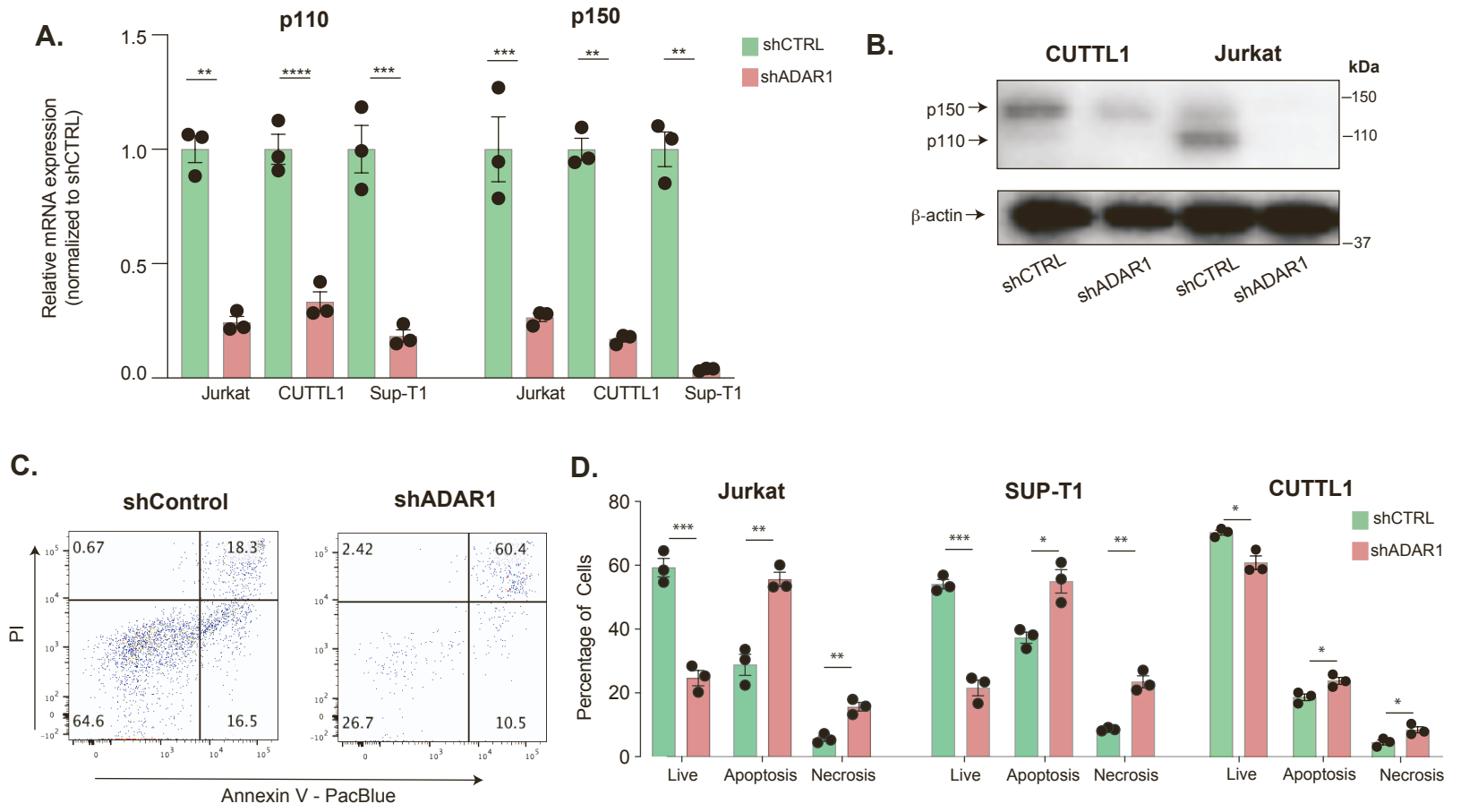

**Supplemental Figure. 3, related to Figure 3. ADAR1 knockdown reduces A-to-I RNA editing and leads to differential gene expression in T-ALL LICs. A.** ADAR1 expression in LICs after transduction with shADAR1 by RT-qPCR (n = 2 patient samples). **B.** Heatmap depicting genes in Acute Undifferentiated Leukemia pathway in T-ALL LICs with ADAR1 knockdown. **C.** Correlation of differential expression and differential RNA editing level per gene in shCTRL cells compared with shADAR1 T-ALL LICs (n = 2 samples). **D.** Overall RNA editing level was quantified by RNA-seq (n = 2 samples). *p<0.05, unpaired student t-test. **E.** Number of edits per million reads in each patient. **F.** Pie chart showing RNA editing locations in scramble control or shADAR1 in T-ALL LICs (n = 2 samples). **G.** Level of RNA editing (A-to-G ratio) in RNA locations.

## Supplemental Figure 4



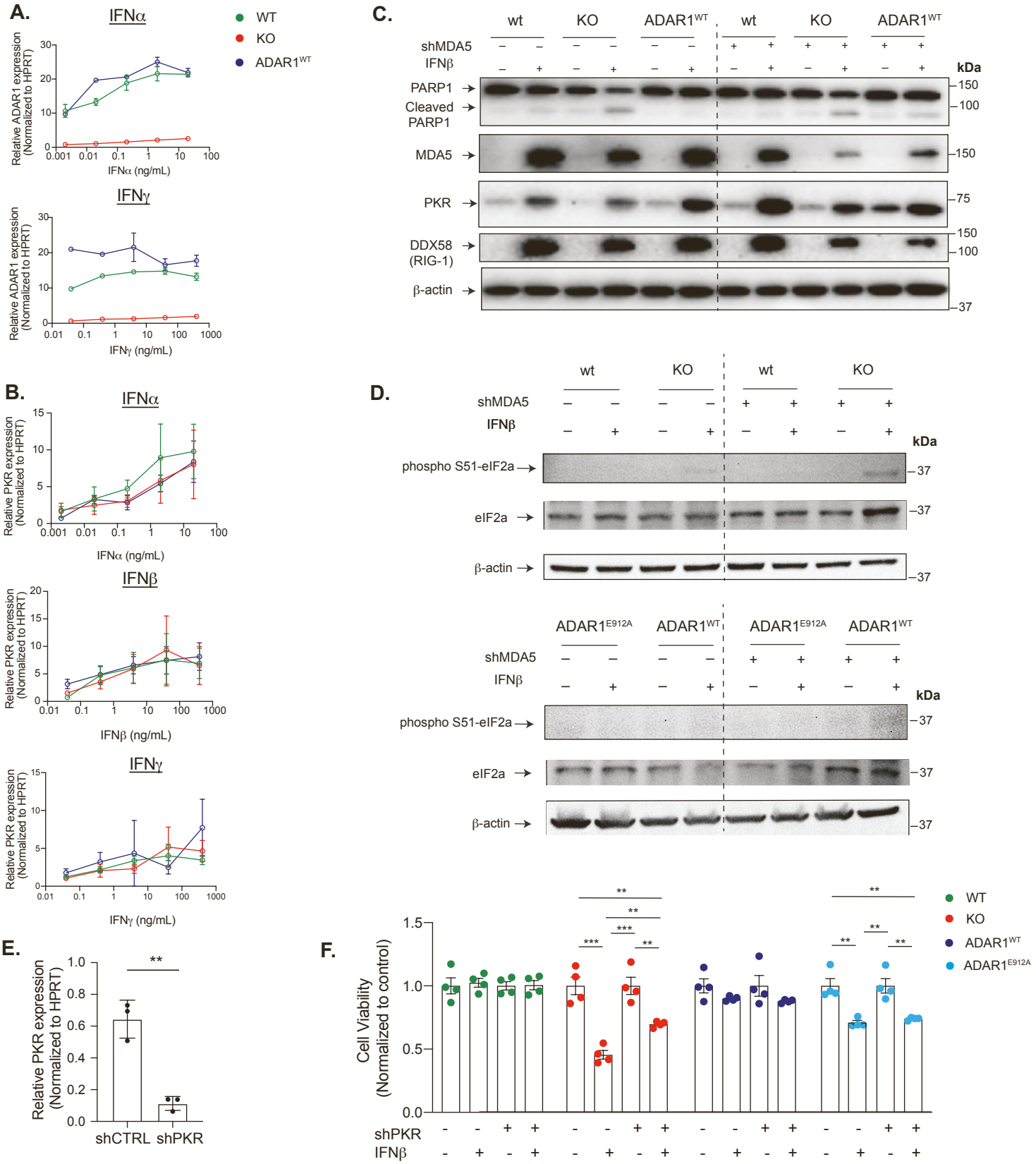

**Supplemental Figure. 4, related to Figure 4. Cell intrinsic differences of dsRNA sensing in T-ALL LICs. A.** Expression of ADAR1 and MDA5 was quantified in double-knockdown in FACS-sorted mCherry+EGFP+ T-ALL LICs (n = 3, pt 070, pt 076, and pt 081). **B.** Survival curve of mice serial transplanted with pt 081 T-ALL LICs isolated from scramble control shRNA (shCTRL), shADAR1, or co-knockdown of shADAR1 and shMDA5 primary xenografts (n = 6-8 mice per group). **p<0.01, ***p<0.001, p value determined by the Mantel–Cox log-rank test. **C.** Heatmap shows the upregulation (red) and downregulation (blue) of genes that were differentially expressed in RIG-I signaling pathway and Cytosolic DNA sensing pathways during comparison of T-ALL LICs (CD34+Lin-) transduced with a scramble shRNA lentivirus or ADAR1 targeting shRNA (n = 2 patient samples, patient 070 and patient 076). **D.** Expression of ISGs and IFN signaling components were quantified by RT-qPCR in T-ALL LICs. n = 3 independent experiments. **A** and **D**. Error bars represent mean with SEM. *p<0.05, **p<0.01, ***p<0.001, unpaired student t-test.

## Supplemental Figure 5



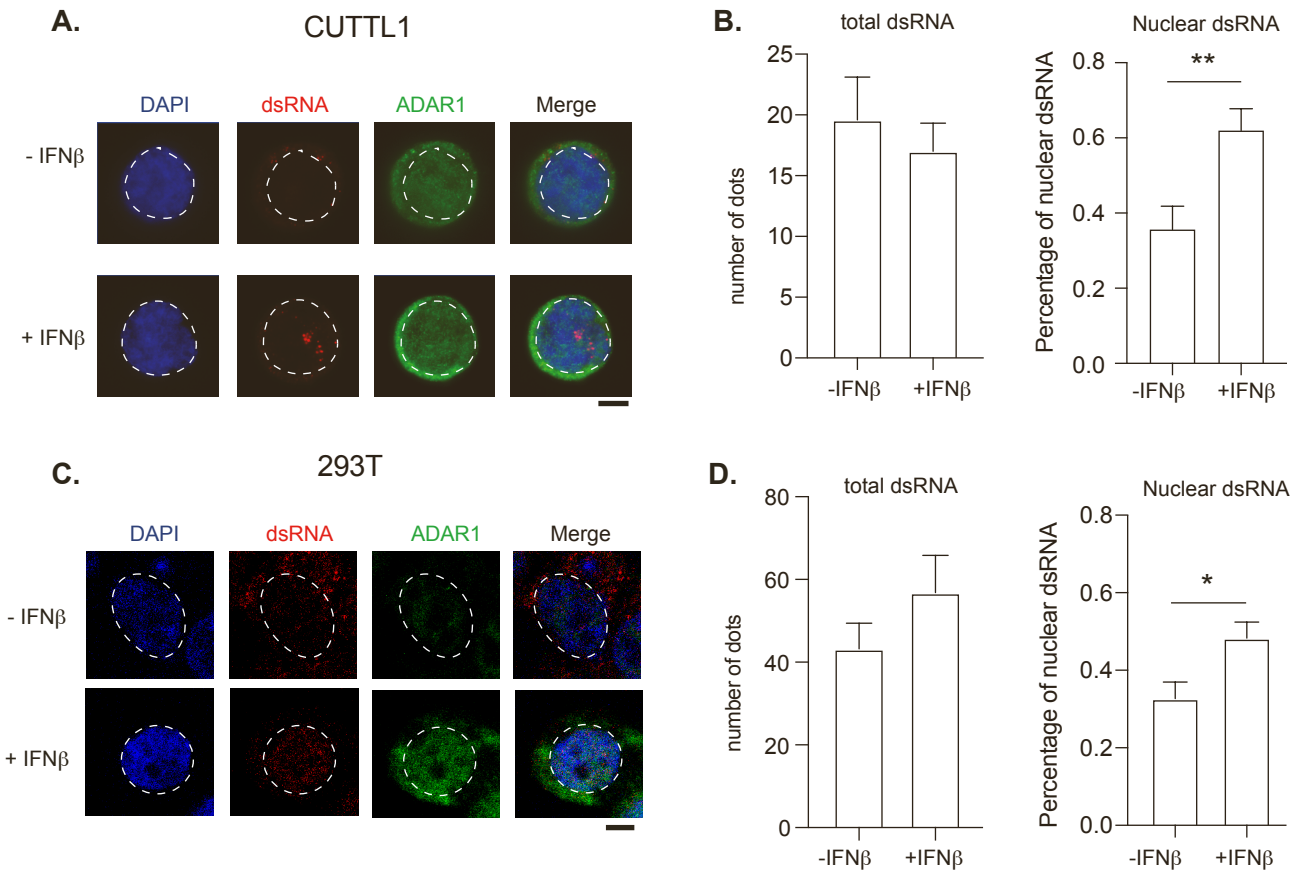

**Supplemental Figure 5, related to Figure 5. ADAR1 knockdown induces apoptosis in T-ALL cell lines.** **A-B.** Expression of ADAR1 was quantified by RT-qPCR (A, 3 independent experiments) or western blot (B) in T-ALL cell lines . **C**. Representitive flow showing increased Annexin V and PI staining in SUP-T1 cells after ADAR1 knockdown. **D**. Quantification of live, apoptosis, and necrosis poplations in control and ADAR1 knock-down conditions (3 independent experiments) . *p<0.05, **p<0.01, ***p<0.001, unpaired student t-test.

## Supplemental Figure 6




**Supplemental Figure 6, related to Figure 6. ADAR1 suppresses ISG activation during interferon stimulation. A-B.** Jurkat cells were stimulated with IFNα, IFNβ, or IFNγ and the gene expression of ADAR1 (A) and PRK (B) was determined (n = 2 independent experiments). **C.** Western blot showing levels of dsRNA sensor (PRK, MDA5, DDX58) and apoptosis markers (PARP1 and cleaved PARP1) in cells stimulated with IFNβ (1 ng/mL). β-actin is used as loading control. **D.** Western blot of total eIF2a and phosphorylated eIF2a in wildtye, ADAR1 KO, ADAR1$^{WT}$, and ADAR1$^{E912A}$ Jurkat cells. Cells were stimulated with IFNβ (1 ng/mL) for 24 hours. **E.** PKR expression after shRNA knockdown (n = 3 experiment). **F.** Effect of PKR knockdown on cell viability after IFNβ (1 ng/mL, 24 hours) stimulation (n = 4 experiments). Error bars represent mean with SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, unpaired student t-test.

## Supplemental Figure 7




**Supplemental Figure 7, related to Figure 6. Increased nuclear dsRNA upon IFNβ stimulation.** **A** and **C**. Immunofluorescent staining to detect the localization of dsRNA in CUTTL1 (A) and 293T (C) cells stimulated with IFNβ (1ng/mL, 24 hrs). Scale bars represent 5μm. **B** and **D**. Quantification of total dsRNA dots and percentage of nuclear dsRNA from CUTTL1 (B) and 293T (D) cells shown in panel C (n = 10 cells/condition). Error bars represent mean with SEM. *p<0.05, **p<0.01, unpaired student t-test.

**Table S1. Cell surface markers used for FACS sorting, related to STAR Methods.**

| Name | Phenotype |
|---|---|
| Lin- | CD8- CD56- CD4- CD3- CD19- CD2- CD14- |
| Stem | hCD45+ Lin- CD34+ CD38- |
| Progenitor | hCD45+ Lin- CD34+ CD38+ |

**Table S2. Patient characteristics, related to STAR Methods.**

| Sample ID | Diagnosis | Collection Timepoint | Sample Type | Age (years) | Karyotype at diagnosis | cytogenetic abnormalities at diagnosis | Experiments |
|---|---|---|---|---|---|---|---|
| 070 | T-Lymphoblastic Lymphoma | Diagnostic | Bone Marrow | 13 | 46,XY[20] | No abnormalities detected | RNA-seq, PDX, ATO |
| 076 | T-Lymphoblastic Lymphoma | Diagnostic | Blood | 17 | 46,XY,add(9)(p13),t(11;14)(p13;q11.2)[11]/46,XY[9] | CDKN1a (9p21) deletion | RNA-seq, PDX, ATO |
| 081 | T-Lymphoblastic Lymphoma | Diagnostic | Blood | 18 month | t(5;14) | TLX3-BCL11B fusion or NKX2-5-BCL11B fusion, CDKN1a (9p21) deletion | PDX, ATO |

**Table S6. Primers for RT-qPCR used in this study, related to STAR Methods**

| RT-qCPR Primers | Sequence (5' to 3') |
|---|---|
| ADAR1 fw (total ADAR1) | TGC TGC TGA ATT CAA GTT GG |
| ADAR1 rev (total ADAR1) | TCG TTC TCC CCA ATC AAG AC |
| ADAR1 fw (ADAR1p150) | AAC GAA AGC GAA ATT GAA CC |
| ADAR1 rev (ADAR1p150) | GGG TGT AGT ATC CGC TGA GG |
| ADAR1 fw (ADAR1p110) | GAC TGA AGG TAG AGA AGG CTA CG |
| ADAR1 rev (ADAR1p110) | TGC ACT TCC TCG GGA CAC |
| PKR fw | TGG AAA GCG AAC AAG GAG TAAG |
| PKR rev | CCA TCC CGT AGG TCT GTG AA |
| MDA5 fw | GCC TAA TTT ACA GCA ACC ATG A |
| MDA5 rev | TCA TCA ATG GAT AAC TCC CAT GT |
| ISG15 fw | GGC TGG GAG CTG ACG GTG AAG |
| ISG15 rev | GCT CCG CCC GCC AGG CTC TGT |
| IFIT1 fw | AGG CAA TTA GCC CAG AAG GT |
| IFIT1 rev | CTC CTC TCT CGG GAA GGT CT |
| IRF1 fw | CAG CCC AAG AAA GGT CCT C |
| IRF1 rev | TTG AAC GGT ACA GAC AGA GCA |
| IRF7 fw | AGC TGT GCT GGC GAG AAG |
| IRF7 rev | CAT GTG TGT GTG CCA GGA A |
| IRF9 fw | AGC CTG GAC AGC AAC TCA G |
| IRF9 rev | GAA ACT GCC CAC TCT CCA C |
| CD34 fw | TCT GGA TCA AAG TAG GCAG GA |
| CD34 rev | GAT CCAG CCTC AGA GGA AGA |
| CD44 fw | ATG AGC ACT AGT GCT ACA GC |
| CD44 rev | AGACGTACCAGCCATTTGTGT |
| LMO2 fw | AGG CAA TTA GCC CAG AAG GT |
| LMO2 rev | CTC CTC TCT CGG GAA GGT CT |
| hHPRT fw | TCA GGG ATT TGA ATC ATG TTT GTG |
| hHPRT rev | CGA TGT CAA TAG GAC TCC AGA TG |