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Supplementary Information for:
Decoupling expression and editing preferences of ADAR1 p150 and p110 isoforms

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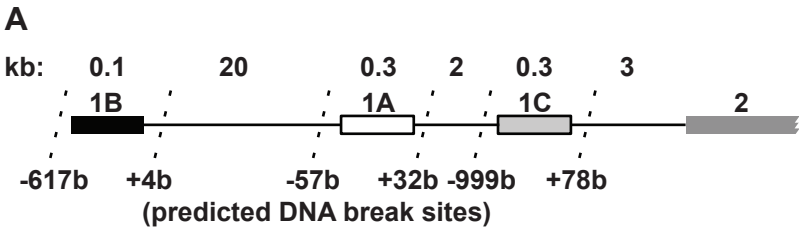

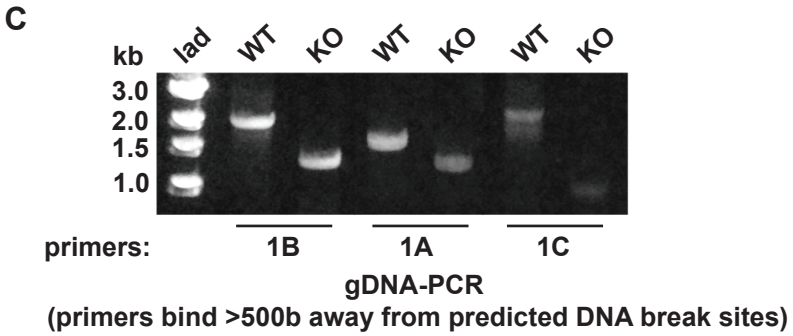

predicted gDNA-PCR products (kb)

	1B	1A	1C
WT	2.1	1.8	2.4
KO	1.3	1.4	1.1

Figure S1. Generation and validation of ADAR1 exon-specific knockout clones

(A) Part of the human ADAR1 gene locus is shown with predicted Cas9-mediated break sites around exons 1B, 1A, and 1C shown using dotted arrows. The approximate lengths of the exons and introns are indicated above in kilobases. Guide RNA sequences are listed in SI Appendix, Table S1.

(B) The schematic shows the iterative process of CRISPR-Cas9 gene engineering followed by single-cell cloning used to generate the final Δ1B/Δ1C/Δ1A (ADAR1 knockout) cell line.

(C) The gel shows PCR products generated from genomic DNA extracted from WT and Δ1B/Δ1C/Δ1A (KO) cell lines. The bands for the ADAR1 KO sample reveal deletion of exons 1B, 1A, and 1C. Primer sequences are listed in SI Appendix, Table S2.

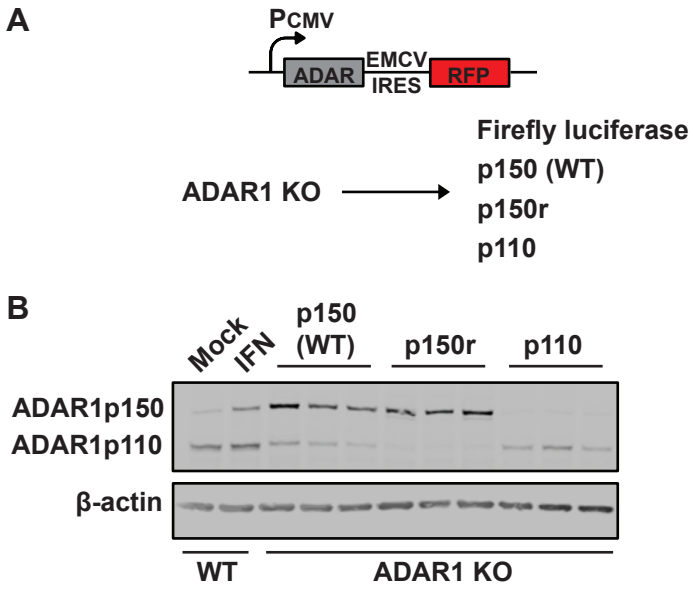

Figure S2. Generation of clones for editing analysis

(A) The schematic shows the bicistronic expression plasmid used for stable expression of firefly luciferase, WT p150, p150r, and p110 coding sequences in ADAR1 KO cells. The plasmids were first packaged into lentivirus, which was then used to deliver the sequences into the genome of ADAR1 KO cells.

(B) The immunoblot shows a comparison of ADAR1 p150 and p110 expression levels between endogenous (exon 1B, 1A, and 1C promoters) and exogenous promoters (in cells stably integrated with the plasmids in panel A).

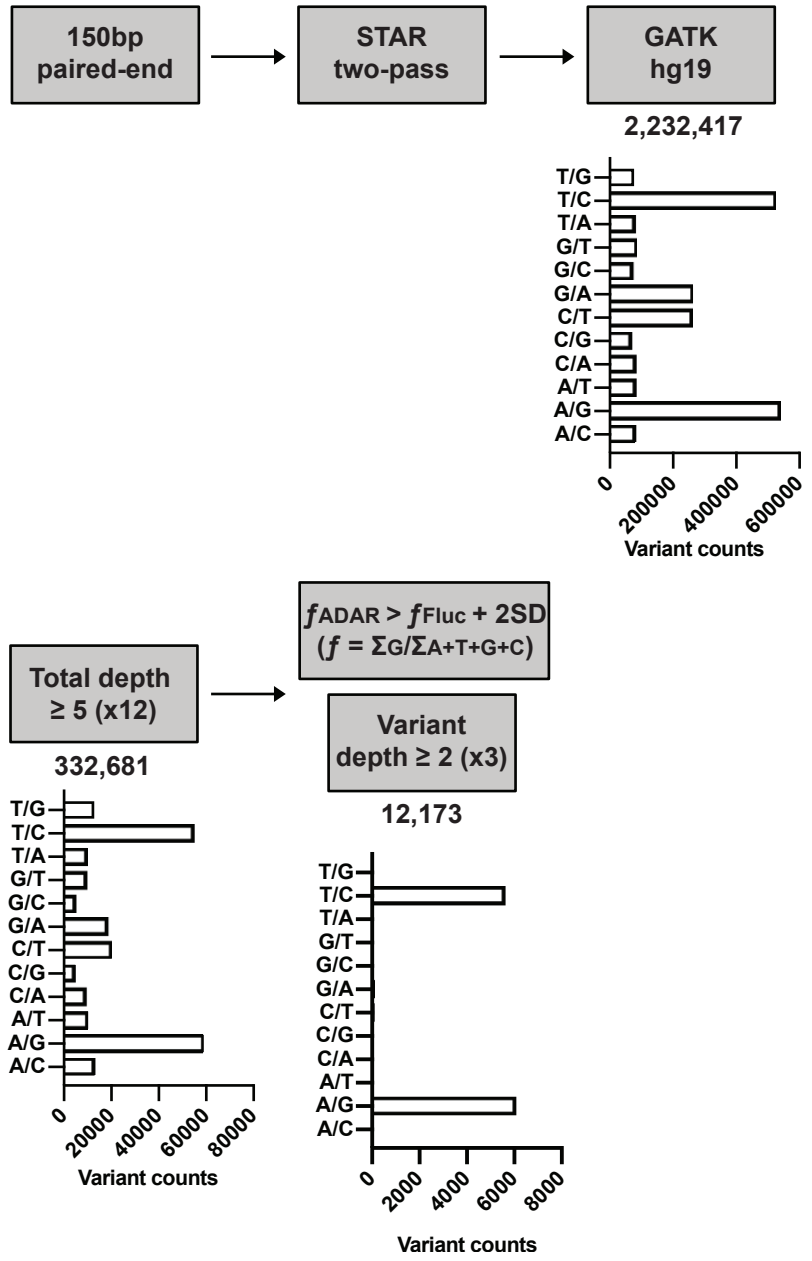

Figure S3. Editing analysis workflow

Following sequencing of total RNA and alignment of reads, variant calling yielded counts as shown in the bar graphs for the twelve possible types of mutations. The counts of total variants at each filtering step are shown above the bar graphs. Further details regarding filtering and identification of putative ADAR1-edit sites are in the methods section.

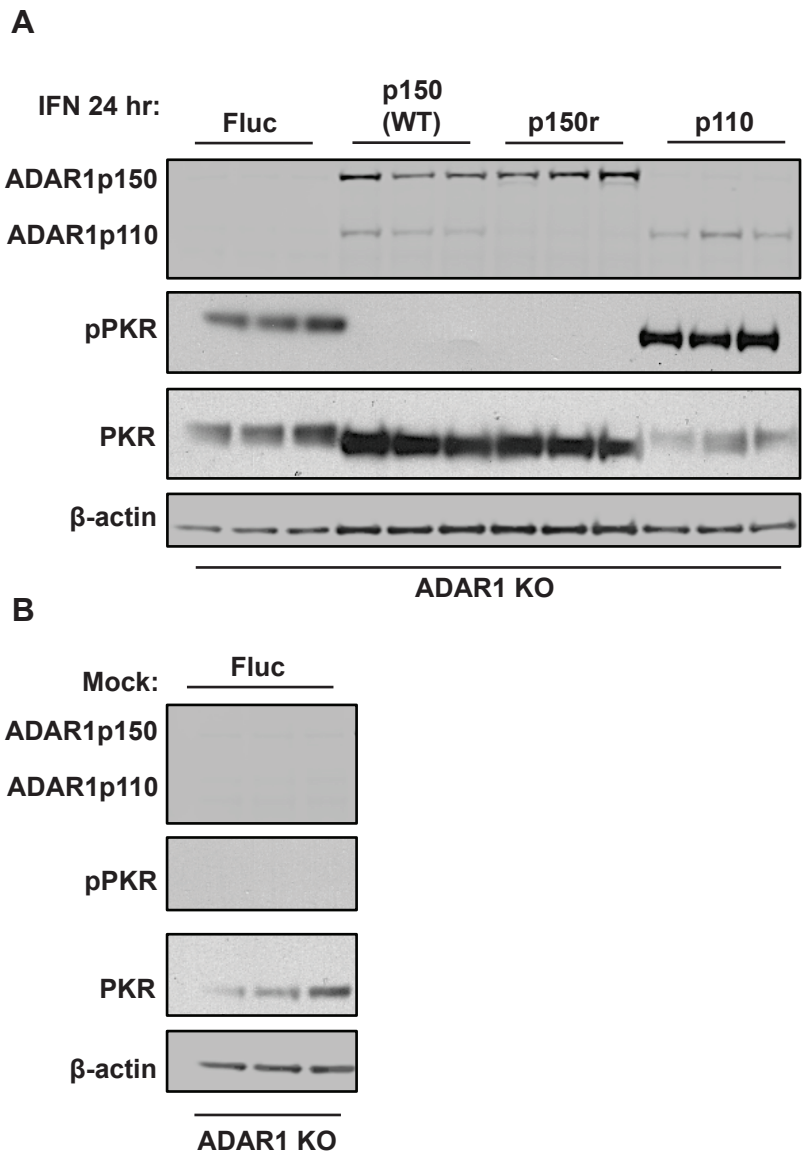

Figure S4. ADAR1 isoform-selective suppression of PKR activation

(A) The immunoblot shows activation (phosphorylation) of PKR following interferon treatment of ADAR1 KO cells that are stably expressing firefly luciferase (Fluc), WT p150, p150r, or p110 coding sequences. Three different clones are shown for each group.

(B) The immunoblot shows that PKR activation specifically occurs when ADAR1 KO cells are treated with interferon.

Table S1. Guide RNA sequences

ADAR1 exon	Target sequence with PAM
1B-upstream	CAAACTCTCATCTAGAGGCC(TGG)
1B-downstream	AGAAGGACAGAGGCTCTTAC(CGG)
1C-upstream	GATACTGCTTAGTAGTGAAG(AGG)
1C-downstream	GCTACTCTTGGCCCAAATC(TGG)
1A-upstream	CGTAGTTCTCATGCAGCGGA(GGG)
1A-downstream	CTTGGACCTTCGCCGCCGTC(TGG)

Table S2. Primer sequences

Primer name	Sequence
ATM-F	CATACAGCAGGCCATAGACC
ATM-R	GGCCCACAGCAACCTTCACCTCCCAG
ADAR1-F	ATGAATCCGCGGCAGGGGTATTC
ADAR1-R	GGTAAGGCCAATATTTTTAGCCAAATTC
M296L-F	GATCCTCTTGAGTTTTTAGACCTCGCCGAGATCAAGGAGAAAATC
M296L-R	GATTTTCTCCTTGATCTCGGCGAGGTCTAAAAACTCAAGAGGATC
KOZAK1-F	TCAGGCTTGGAACCAGCACAG
KOZAK1-R	AGTCGCAGATTTTCTCCTTGATCTTTAGCATAAAAAAAAACTCAAGAGGATCTTCCAAG
KOZAK2-F	CTGCCTTGGAAGATCCTCTTGAGTTTTTTTTTATGCTAAAGATCAAGGAGAAAATCTGCG
KOZAK2-R	TTTGATGTGGGTATATTACAGG
TMEM120B-RT	GGGCTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNAACTCTTTGGGTGAGGCATGG
TMEM120B-PCR1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCAAGACAGAAAGGACTTCCAGCC
PDE12-RT	GGGCTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNCCATTTCTCCAAACTATTCCACAGG
PDE12-PCR1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGATATACACACTTTTAAAAGGATTTATT GCGC
i5-UDP0001 (X1)	AATGATACGGCGACCACCGAGATCTACACTCGTGGAGCGTCGTCGGCAGCGTC
i7-UDP0001 (X1)	CAAGCAGAAGACGGCATACGAGATCGCTCAGTTCGTCTCGTGGGCTCGG
i5-UDP0002 (X2)	AATGATACGGCGACCACCGAGATCTACACCTACAAGATATCGTCGGCAGCGTC
i7-UDP0002 (X2)	CAAGCAGAAGACGGCATACGAGATTATCTGACCTGTCTCGTGGGCTCGG
i5-UDP0003 (X3)	AATGATACGGCGACCACCGAGATCTACACTATAGTAGCTTCGTCGGCAGCGTC
i7-UDP0003 (X3)	CAAGCAGAAGACGGCATACGAGATATATGAGACGGTCTCGTGGGCTCGG
i5-UDP0004 (C1)	AATGATACGGCGACCACCGAGATCTACACTGCCTGGTGGTCGTCGGCAGCGTC
i7-UDP0004 (C1)	CAAGCAGAAGACGGCATACGAGATCTTATGGAATGTCTCGTGGGCTCGG
i5-UDP0005 (C7)	AATGATACGGCGACCACCGAGATCTACACACATTATCCTTCGTCGGCAGCGTC
i7-UDP0005 (C7)	CAAGCAGAAGACGGCATACGAGATTAATCTCGTCGTCTCGTGGGCTCGG
i5-UDP0006 (C10)	AATGATACGGCGACCACCGAGATCTACACGTCCACTTGTTCGTCGGCAGCGTC
i7-UDP0006 (C10)	CAAGCAGAAGACGGCATACGAGATGCGCGATGTTGTCTCGTGGGCTCGG
i5-UDP0007 (F5)	AATGATACGGCGACCACCGAGATCTACACTGGAACAGTATCGTCGGCAGCGTC
i7-UDP0007 (F5)	CAAGCAGAAGACGGCATACGAGATAGAGCACTAGGTCTCGTGGGCTCGG
i5-UDP0008 (F7)	AATGATACGGCGACCACCGAGATCTACACCCTTGTTAATTCGTCGGCAGCGTC
i7-UDP0008 (F7)	CAAGCAGAAGACGGCATACGAGATTGCCTTGATCGTCTCGTGGGCTCGG

i5-UDP0009 (F8)	AATGATACGGCGACCACCGAGATCTACACGTTGATAGTGTCGTCGGCAGCGTC
i7-UDP0009 (F8)	CAAGCAGAAGACGGCATACGAGATCTACTCAGTCGTCTCGTGGGCTCGG
i5-UDP0010 (Z2)	AATGATACGGCGACCACCGAGATCTACACACCAGCGACATCGTCGGCAGCGTC
i7-UDP0010 (Z2)	CAAGCAGAAGACGGCATACGAGATTCGTCTGACTGTCTCGTGGGCTCGG
i5-UDP0011 (Z6)	AATGATACGGCGACCACCGAGATCTACACCATACACTGTTCGTCGGCAGCGTC
i7-UDP0011 (Z6)	CAAGCAGAAGACGGCATACGAGATGAACATACGGGTCTCGTGGGCTCGG
i5-UDP0012 (Z8)	AATGATACGGCGACCACCGAGATCTACACGTGTGGCGCTTCGTCGGCAGCGTC
i7-UDP0012 (Z8)	CAAGCAGAAGACGGCATACGAGATCCTATGACTCGTCTCGTGGGCTCGG
1B-screening-F	GGAAGTTTCCTTCTCTTTTCCCC
1B-screening-R	CTCCGCTAATTGCATACTTGGG
1C-screening-F	GTGTGTCGTCTTGCCAAGCAGC
1C-screening-R	CAGCCCTTGGGGTTTCCTCTCC
1A-screening-F	TTACTAAAGCCTTCAGACCTG
1A-screening-R	CTATAAAGTGGTTAACAAGCTTTC

Dataset S1 (separate file). Putative ADAR1-edit sites

The 12,173 putative ADAR1-edit sites are listed with total read depth (DP), mismatch read depth (AC), mismatch frequency (AF), and annotations for all samples in groups X (firefly luciferase), C (WT p150), F (p150r), and Z (p110).

Dataset S2 (separate file). Putative ADAR1 p150-selective edit sites

The 192 putative p150-selective edit sites are listed with total read depth (DP), mismatch read depth (AC), mismatch frequency (AF), and annotations for all samples in groups X (firefly luciferase), C (WT p150), F (p150r), and Z (p110).

Dataset S3 (separate file). Putative ADAR1 p150+p110-shared edit sites

The 119 putative p150/p110-shared edit sites are listed with total read depth (DP), mismatch read depth (AC), mismatch frequency (AF), and annotations for all samples in groups X (firefly luciferase), C (WT p150), F (p150r), and Z (p110).

Dataset S4 (separate file). Genes with putative ADAR1 p150-selective edit sites

Genes containing putative p150-selective edit sites are listed.

Dataset S5 (separate file). Genes with putative ADAR1 p150+p110-shared edit sites

Genes containing putative p150/p110-shared edit sites are listed.