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Supplemental information

ADAR1 Zα domain P195A mutation activates the MDA5-dependent RNA-sensing signaling pathway in brain without decreasing overall RNA editing

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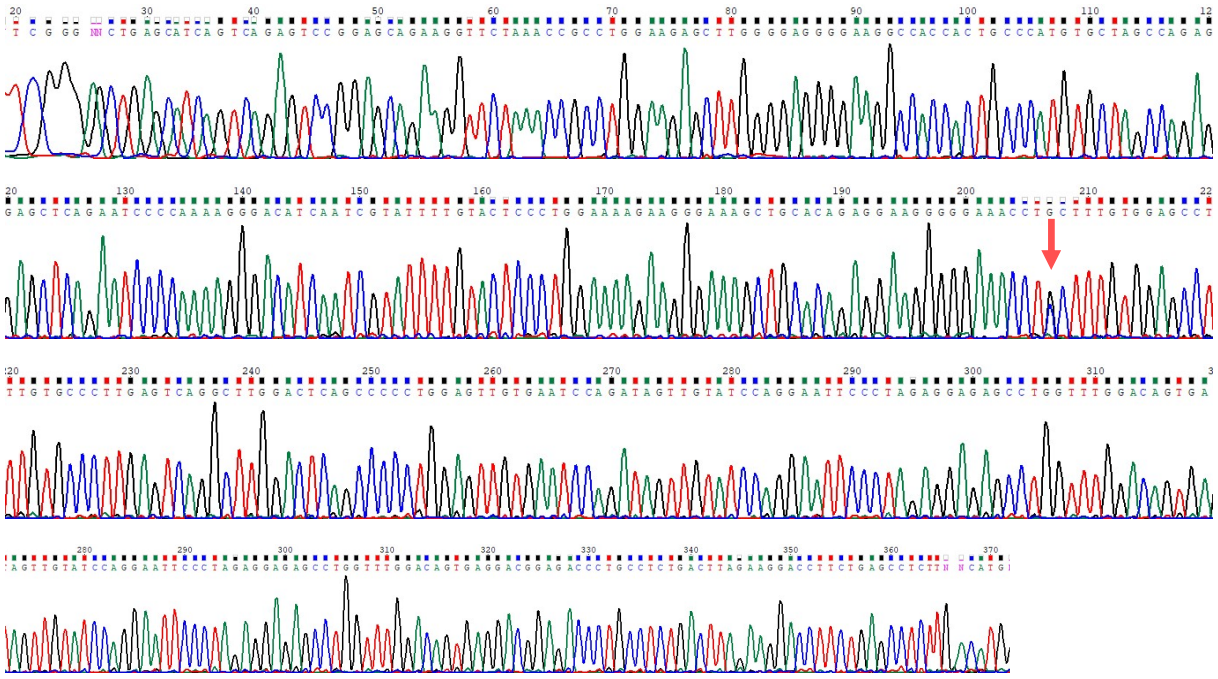
Suppl. Figure 1. Sanger sequencing of *Adar* alleles



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Ref.    1    TCGGGAGCTGAGCATCAGTCAGAGTCCGGAGCAGAAGGTTCTAAACCGCCTGGAAGAGCT  60
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Mut.    1    TCGGGAGCTGAGCATCAGTCAGAGTCCGGAGCAGAAGGTTCTAAACCGCCTGGAAGAGCT  60

Ref.    61   TGGGGAGGGGAAGGCCACCACTGCCCATGTGCTAGCCAGAGAGCTCAGAATCCCCAAAAG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mut.    61   TGGGGAGGGGAAGGCCACCACTGCCCATGTGCTAGCCAGAGAGCTCAGAATCCCCAAAAG  120

Ref.    121  GGACATCAATCGTATTTTGTACTCCCTGGAAAAGAAGGGAAAGCTGCACAGAGGAAGGGG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mut.    121  GGACATCAATCGTATTTTGTACTCCCTGGAAAAGAAGGGAAAGCTGCACAGAGGAAGGGG  180

Ref.    181  GAAACCTCCTTTGTGGAGCCTTGTGCCCTTGAGTCAGGCTTGGACTCAGCCCCCTGGAGT  240
             ||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Mut.    181  GAAACCTGCTTTGTGGAGCCTTGTGCCCTTGAGTCAGGCTTGGACTCAGCCCCCTGGAGT  240

Ref.    241  TGTGAATCCAGATAGTTGTATCCAGGAATTCCCTAGAGGAGAGCCTGGTTTGGACAGTGA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mut.    241  TGTGAATCCAGATAGTTGTATCCAGGAATTCCCTAGAGGAGAGCCTGGTTTGGACAGTGA  300

Ref.    301  GGACGGAGACCCTGCCTCTGACTTAGAAGGACCTTCTGAGCCTCTTGACATGGCTA  356
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mut.    301  GGACGGAGACCCTGCCTCTGACTTAGAAGGACCTTCTGAGCCTCTTGACATGGCTA  356
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Fig. S1. Sanger sequencing of ADAR1 mutant allele

Sanger sequencing analysis confirmed the genomic sequences of the ADAR1 P195A mutant mice. The top panel shows a histography of the sequencing of a heterozygous mouse. The arrow indicates the C>G mutation site, which shows both the C and G peaks. The bottom panel shows the alignment of the sequences of a homozygous P195A mutant mouse and a WT mouse from the Sanger sequencing analysis. The mismatch showing the C>G replacement is highlighted.

Suppl. Figure 2. RNA editing levels at known editing sites

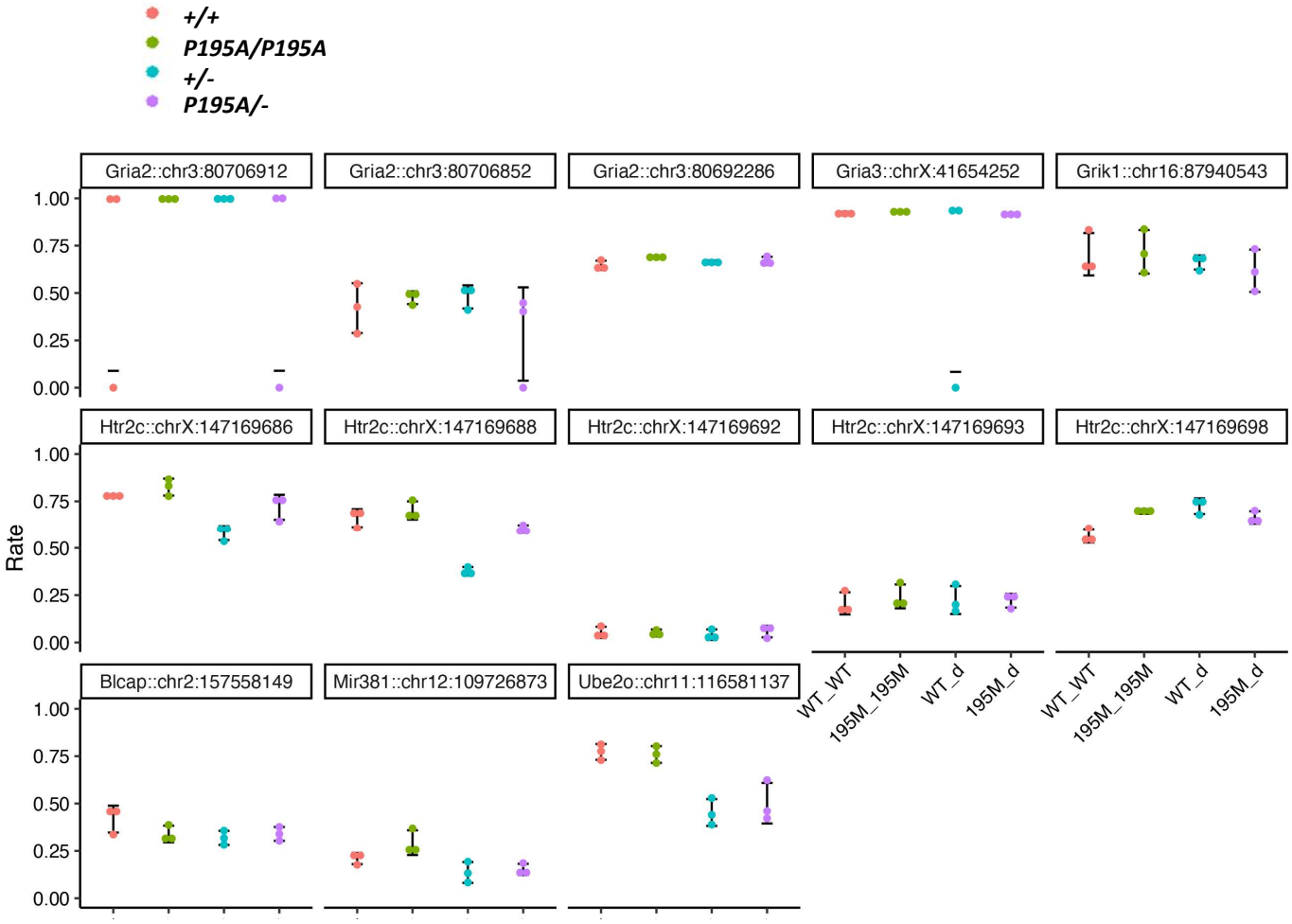

Fig. S2. RNA editing levels at known neuronal RNA substrates from RNA-seq data.
Shown are the RNA editing levels calculated from RNA-seq data at known editing sites in neuronal RNA substrates, including RNA *Gria2* Q/R, *Gria2* +60, *Gria2* R/G, *Gria3* R/G, *Grik1* Q/R, *Htr2c* A, *Htr2c* B, *Htr2c* C, *Htr2c* D, *Htr2c* E, and in *Mir381*and mRNAs of *Blcap* and *Ube2o*. The editing rate = the number of A/G or T/C variant reads/total number of reads at the variant site. The editing rates of three mice of each genotype were shown. No significant differences were observed at all tested sites in $Adar^{+/+}$, $Adar^{+/-}$, $Adar^{P195A/P195A}$; $Ifih1^{-/-}$ and $Adar^{P195A/-}$; $Ifih1^{-/-}$ mice, except at *Htr2c* A, B sites and in *Mir381* and *Ube2o* mRNA where RNA editing rates were lower in $Adar^{+/-}$ and $Adar^{P195A/-}$; $Ifih1^{-/-}$ mice than those in $Adar^{+/+}$ and $Adar^{P195A/P195A}$; $Ifih1^{-/-}$ mice; and at *Htr2c* B sites, editing rates in $Adar^{+/-}$ mice is even lower than in $Adar^{P195A/-}$ mice, which are consistent with those assessed by Sanger sequencing analysis.

Suppl. Fig 3. Variation of editing sites identified by RNA-seq analysis from three mice of each genotype

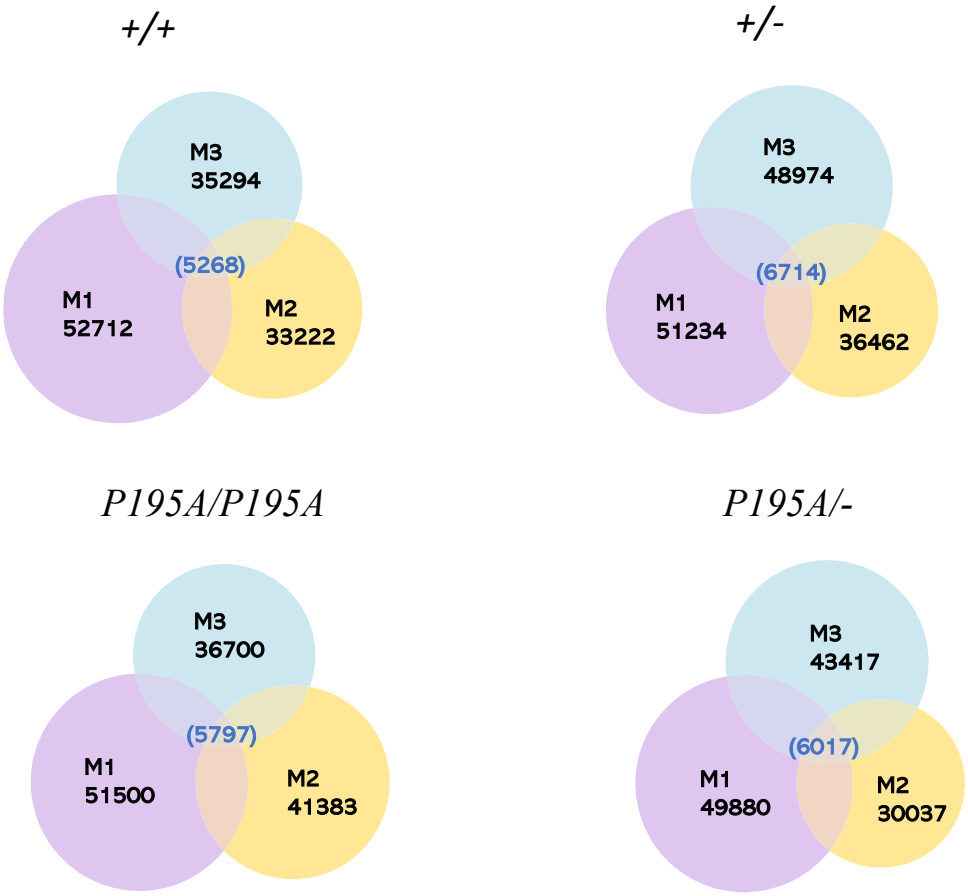

Fig. S3. RNA editing levels at the 36 ADAR1 p150 isoform-specific editing sites in 3'UTRs from RNA-seq data.

The reported 36 ADAR1 p150 isoform-specific editing sites in 3'UTR regions and their editing levels calculated from the RNA-seq data are shown here. There is no significant difference at any of these editing sites in *Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ mice versus *Adar*$^{+/+}$ mice, or in *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice versus *Adar*$^{+/-}$ mice.

Suppl. Fig 4. P195A mutation does not affect editing at P150 isoform specific sites-1

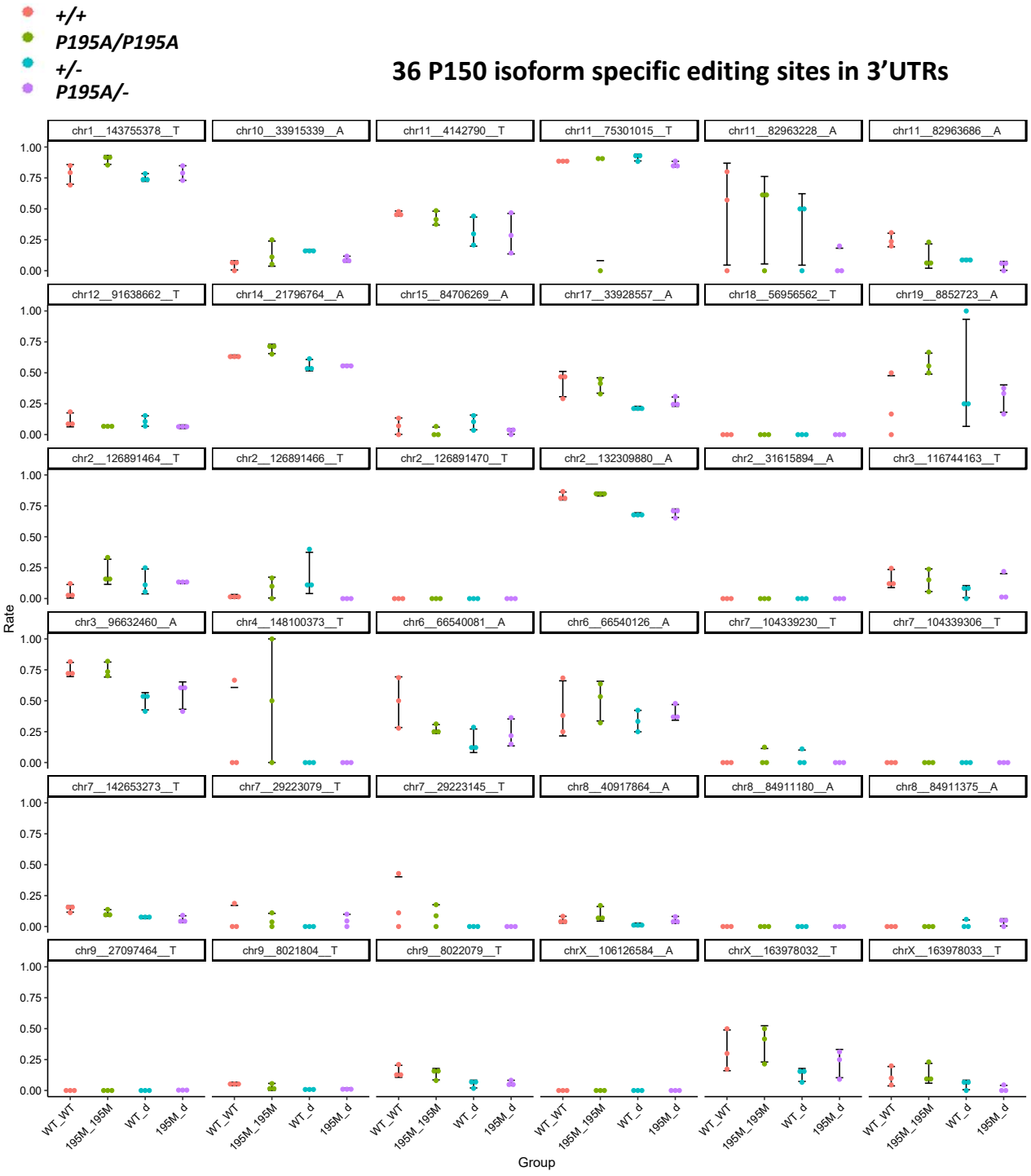

Fig. S4. RNA editing levels at the 17 ADAR1 p150 isoform-specific editing sites in introns from RNA-seq data.

The reported 17 ADAR1 p150 isoform-specific editing sites in introns and their editing levels calculated from the RNA-seq data are shown here. There is no significant difference at any of these editing sites in *Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ mice versus *Adar*$^{+/+}$ mice, or in *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice versus *Adar*$^{+/-}$ mice.

Suppl. Fig 5. P195A mutation does not affect editing at P150 isoform specific sites-2

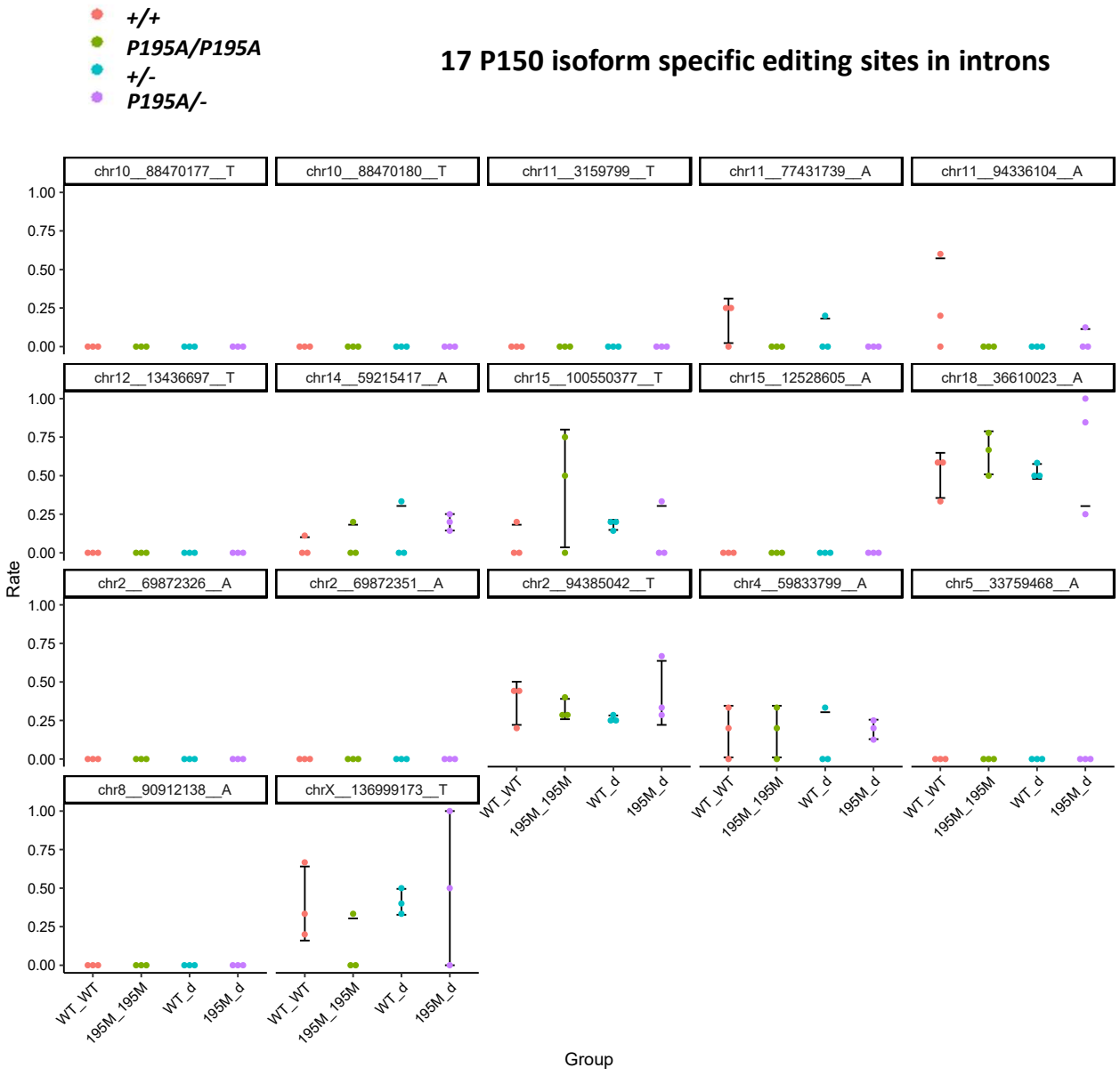

Fig. S5. Variation of editing sites identified by RNA-seq analysis from three mice of each genotype shown by Venn diagram.

RNA editing sites identified by RNA-Seq data analysis from each of three mice carrying wildtype ADAR1 protein (*Adar*$^{+/+}$, *Adar*$^{+/-}$) or P195A mutant ADAR1 protein (*Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ and *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$) were compared. Most editing sites found in a mouse were largely different from any other mouse with the same genotype. Three mice of each genotype were labeled M1, M2, and M3, and each mouse's number of editing sites was indicated. Only a relatively small number of editing sites (blue color in the overlap area) were shared by all three mice in each group.

Suppl. Fig 6. Differential editing ratio in 418 shared sites: *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice vs *Adar*$^{+/+}$ and *Adar*$^{+/-}$ mice

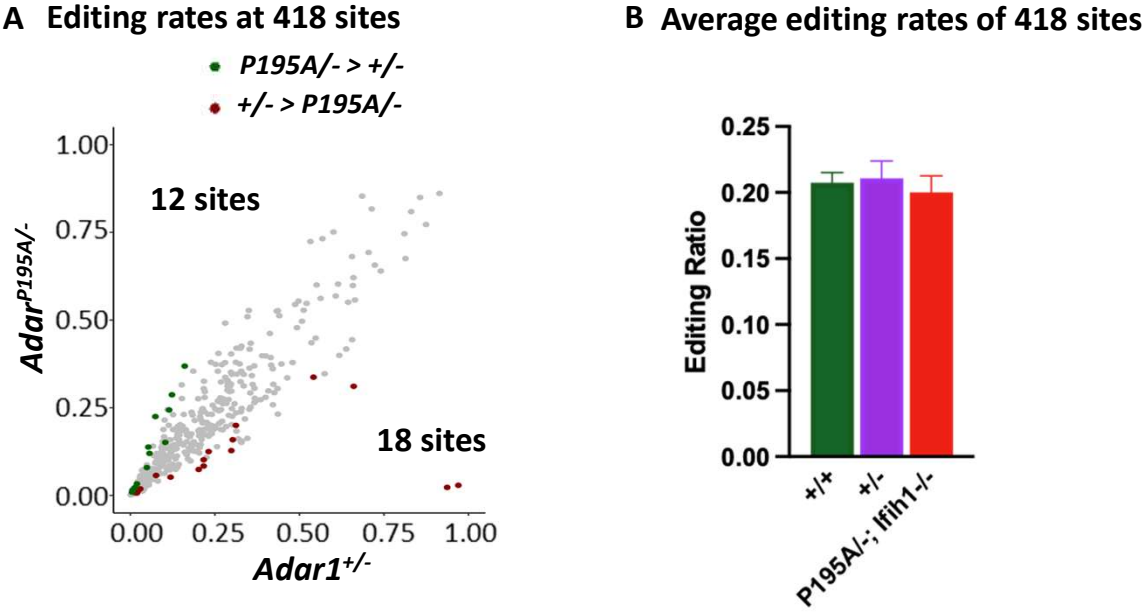

Fig. S6. Differential editing ratio at 413 unique editing sites identified by RNA-seq analysis in *Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ mice shared with wildtype mice.

(A) 413 unique editing sites were identified by RNA-seq analysis in *Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ mice which were shared by *Adar*$^{+/+}$ and *Adar*$^{+/-}$ mice. Comparison of these 413 shared editing sites in *Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ mice versus *Adar*$^{+/+}$ mice found ten sites with significantly higher editing levels in *Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ mice and eleven sites were editing significantly higher editing levels in *Adar*$^{+/+}$ mice.

(B) The average editing levels of these 413 editing sites in *Adar*$^{+/+}$, *Adar*$^{+/-}$, and *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice were not significantly different.

Suppl. Fig 7. Differential editing ratio in 413 shared sites: *Adar*$^{P195A/P195A}$; *Ifih1*$^{-/-}$ mice vs *Adar*$^{+/+}$ and *Adar*$^{+/-}$ mice.

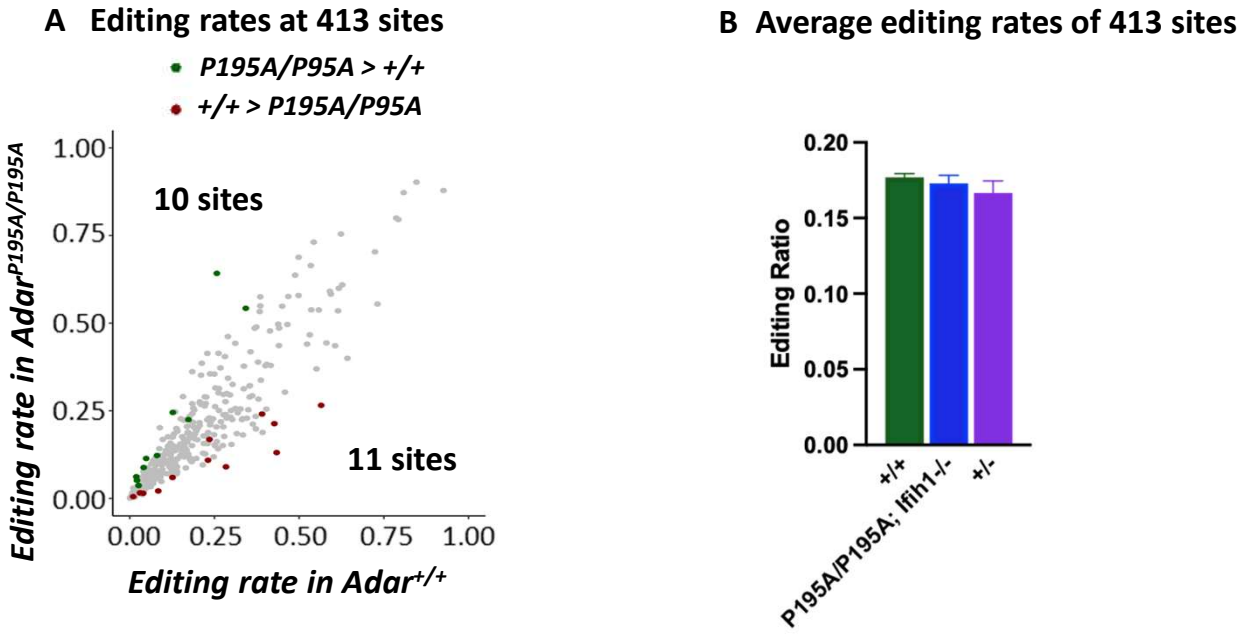

Fig. S7. Differential editing ratio at 418 unique editing sites identified by RNA-seq analysis in *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice shared with wildtype mice.

(A) 418 unique editing sites were identified by RNA-seq analysis in *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice which were shared by *Adar*$^{+/+}$ and *Adar*$^{+/-}$ mice. A comparison of the editing rates of these 418 shared editing sites found 12 sites with significantly higher editing levels in *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice, and 18 sites with significantly higher editing levels in *Adar*$^{+/-}$ mice.

(B) The average editing levels of these 418 editing sites in *Adar*$^{+/+}$, *Adar*$^{+/-}$, and *Adar*$^{P195A/-}$; *Ifih1*$^{-/-}$ mice were not significantly different.