Supplemental Material for

A comparative analysis of ADAR mutant mice reveals site-specific regulation of RNA editing

Pedro Henrique Costa Cruz[1], Yuki Kato[1], Taisuke Nakahama[1], Toshiharu Shibuya[1],

Yukio Kawahara[1*]

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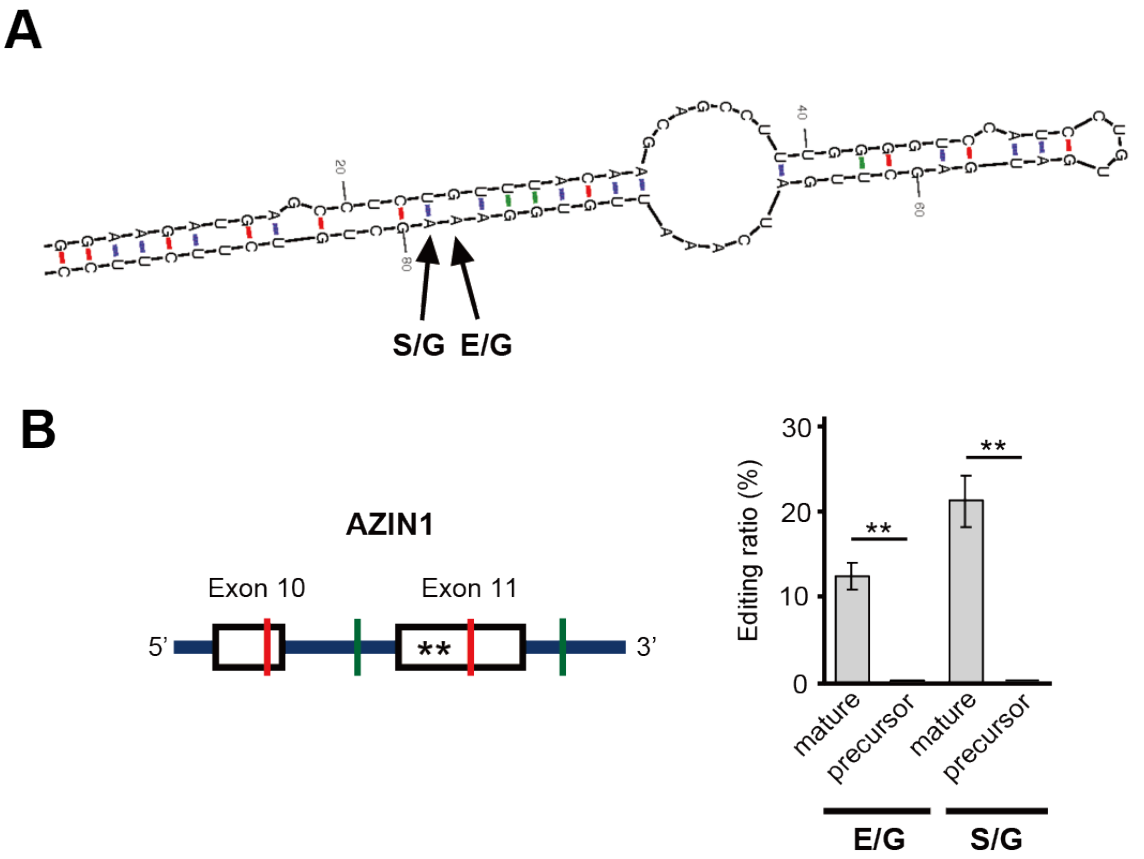
[1]Department of RNA Biology and Neuroscience, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan

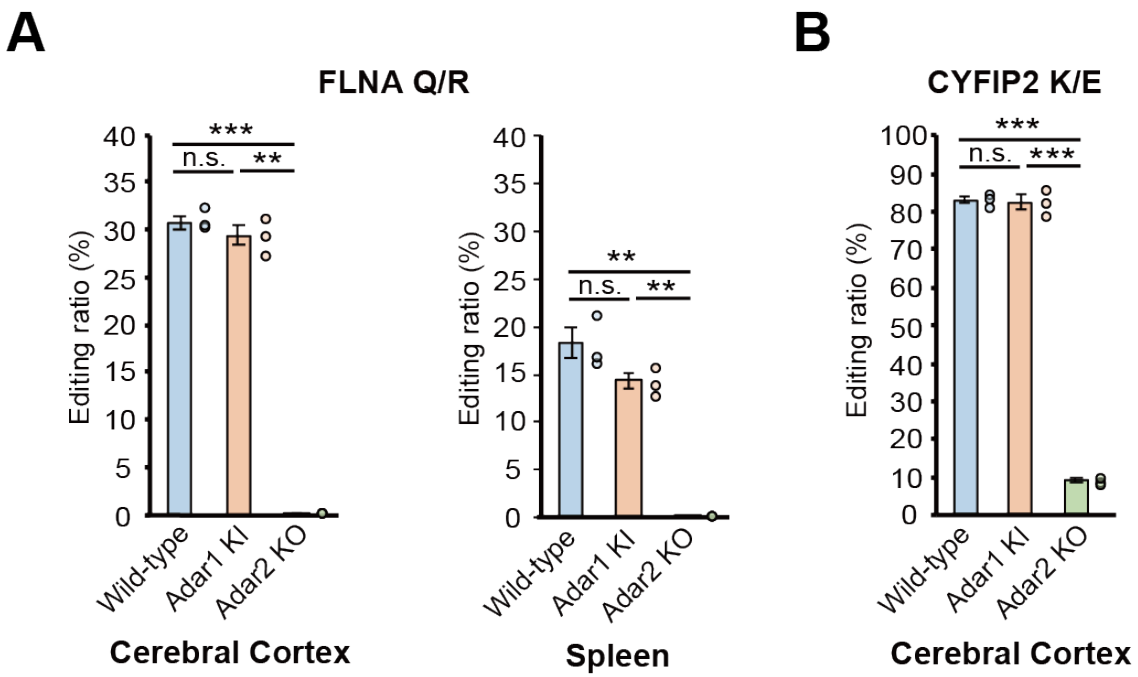
*Correspondence: ykawahara@rna.med.osaka-u.ac.jp

Other supplemental material for this article includes the following:
Supplemental Tables (Excel file)
Supplemental Charts (Excel file)

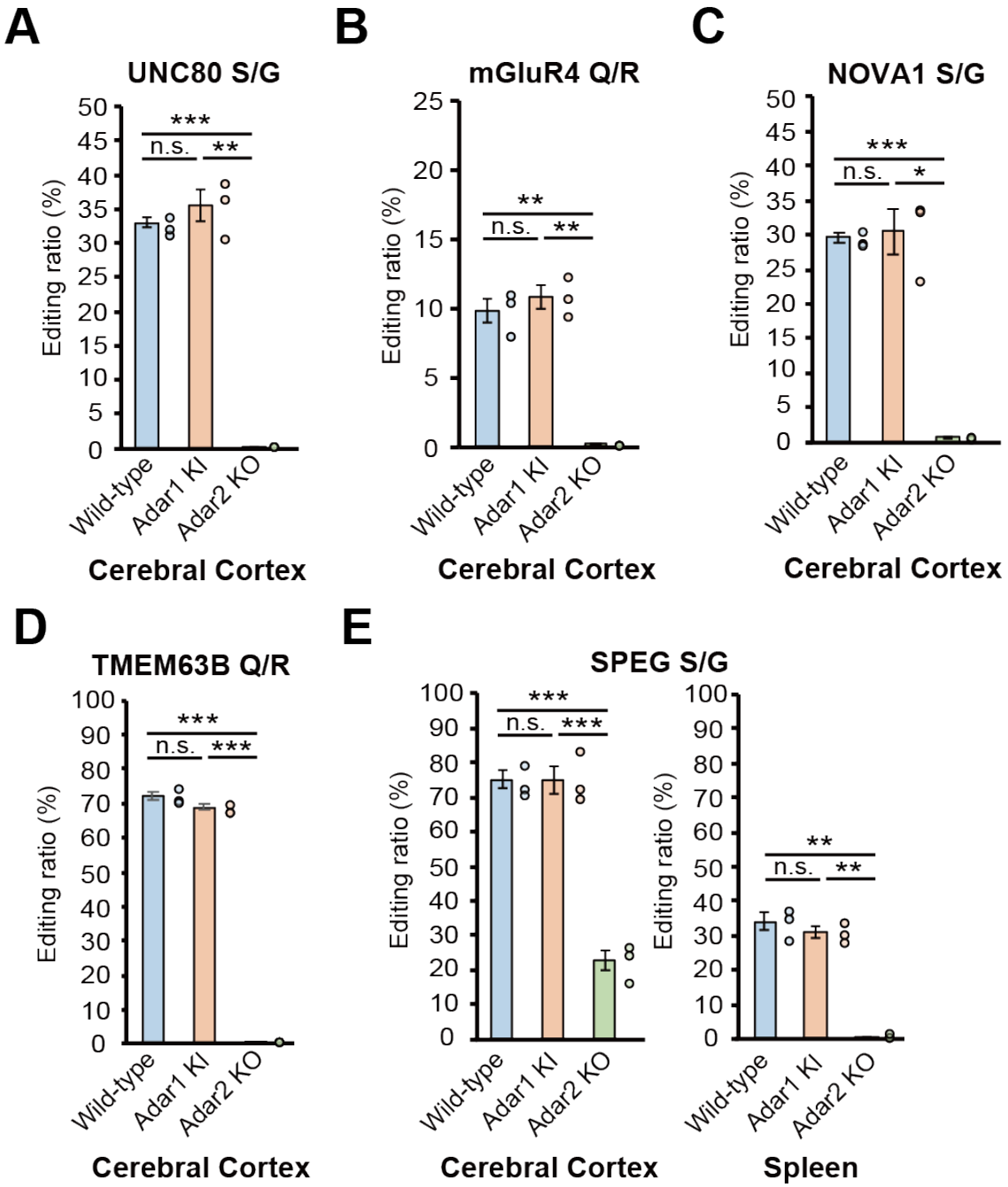
Supplemental Figures



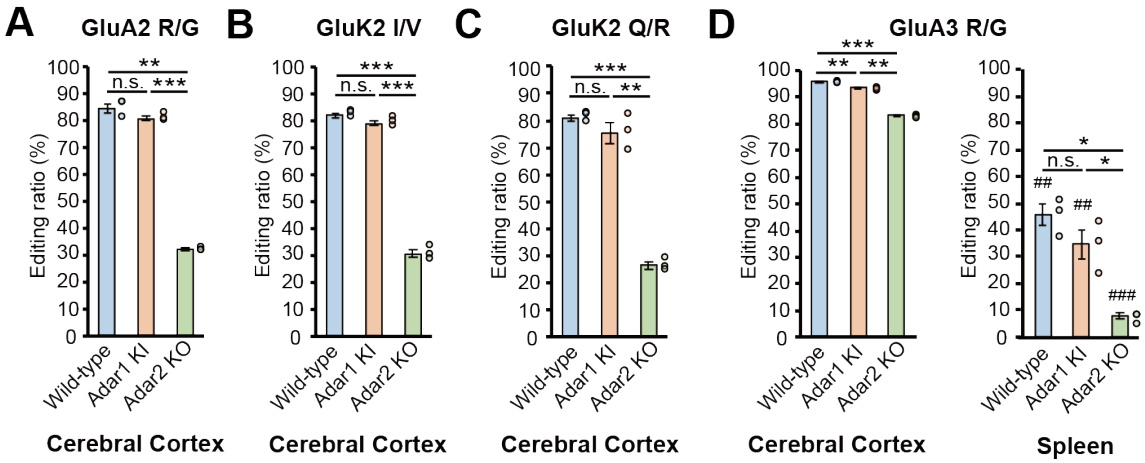
SUPPLEMENTAL FIGURE S1. Estimated dsRNA structure required for editing at AZIN1 E/G and S/G sites. *(A)* The secondary structure within the exon containing AZIN1 E/G and S/G sites was estimated using Mfold. The number indicates the position from the 5' end of this exon. dsRNA, double-stranded RNA; E/G, glutamic acid/glycine; S/G, serine/glycine. *(B)* Editing ratios for AZIN1 E/G and S/G sites in mature messenger RNA (mRNA) and precursor mRNA in the spleen of wild-type mice are shown. The locations of the two editing sites (asterisks) and primers for mature mRNA (in red) and for precursor mRNA (in green) are indicated in the left panel. Editing ratios are displayed as the mean ± SEM (n=3 mice; Student's *t*-test, $^{**}p < 0.01$).



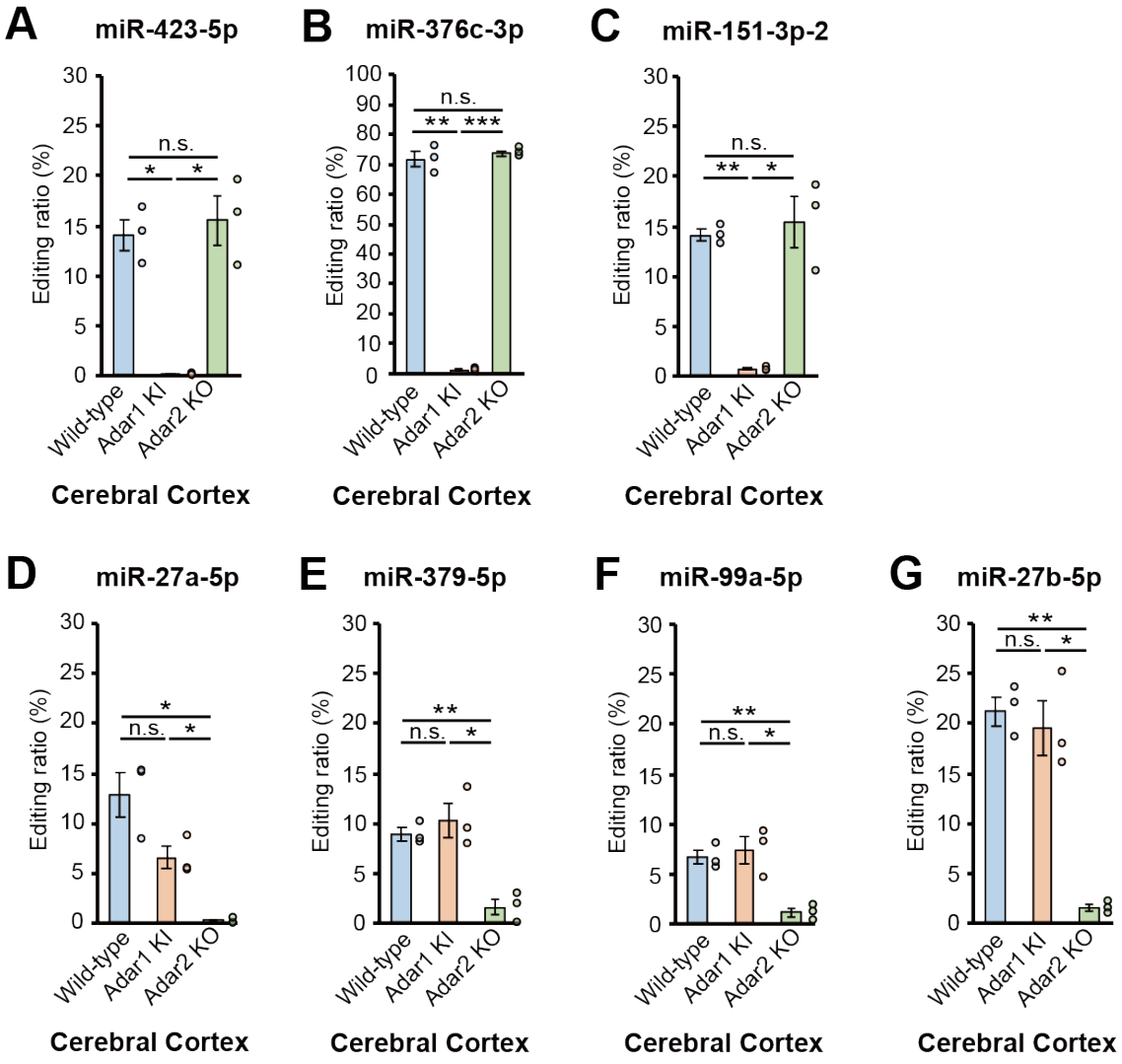
SUPPLEMENTAL FIGURE S2. Retention of RNA editing at known ADAR2 sites in Adar1 KI and Adar2 KO mice. (*A–B*) Editing ratios for FLNA Q/R (*A*) and CYFIP2 K/E (*B*) sites in the indicated tissues isolated from wild-typ, *Adar1*$^{E861A/E861A}$*Ifih*$^{-/-}$ mice (Adar1 KI), and *Adar2*$^{-/-}$*Gria2*$^{R/R}$ (Adar2 KO) mice are shown. Editing ratios are displayed as the mean ± SEM (n=3 mice for each group; Student's *t*-test, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s., not significant). The editing ratio for each mouse is also displayed as a circle on the right side. Q/R, glutamine/arginine; K/E, lysine/glutamic acid.



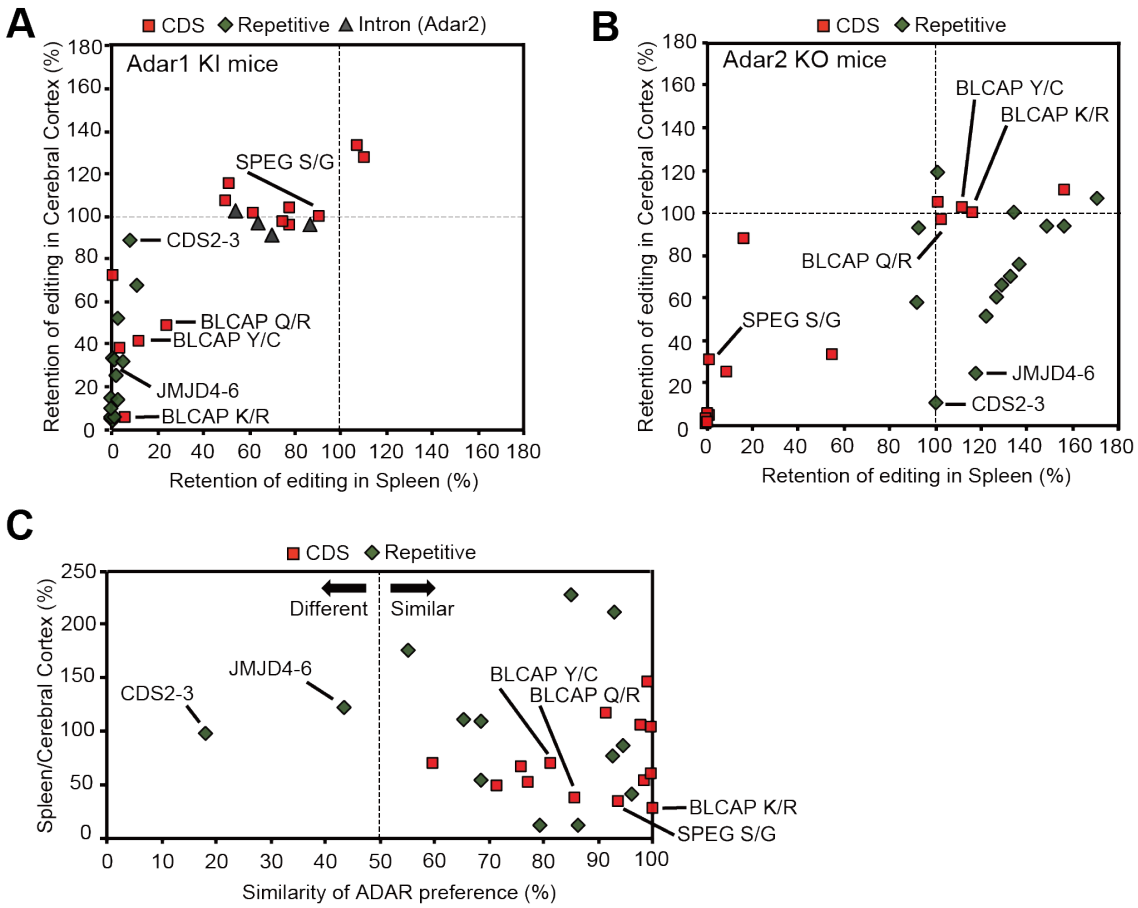
SUPPLEMENTAL FIGURE S3. Novel ADAR2 sites. (*A–E*) Editing ratios for UNC80 S/G (*A*), mGluR4 Q/R (*B*), NOVA1 S/G (*C*), TMEM63B Q/R (*D*) and SPEG S/G (*E*) sites in the indicated tissues isolated from wild-type, *Adar1*$^{E861A/E861A}$*Ifih*$^{-/-}$ mice (Adar1 KI), and *Adar2*$^{-/-}$*Gria2*$^{R/R}$ (Adar2 KO) mice are shown. Editing ratios are displayed as the mean ± SEM (n=3 mice for each group; Student's *t*-test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, n.s., not significant). The editing ratio for each mouse is also displayed as a circle on the right side of each column. S/G, serine/glycine; Q/R, glutamine/arginine.



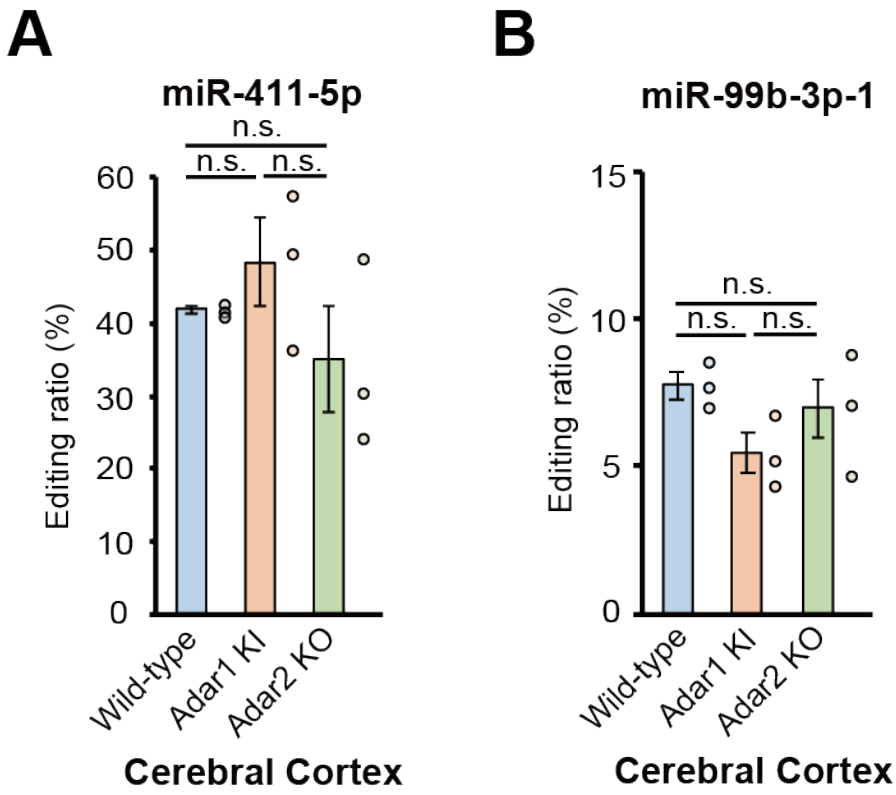
SUPPLEMENTAL FIGURE S4. Compensatory editing of glutamate receptor subunits by ADAR1 in the absence of ADAR2 activiy. (*A–D*) Editing ratios for GluA2 R/G (*A*), GluK2 I/V (*B*), GluK2 Q/R (*C*) and GluA3 R/G (*D*) sites in the indicated tissues isolated from wild-type, *Adar1*$^{E861A/E861A}$*Ifih*$^{-/-}$ mice (Adar1 KI), and *Adar2*$^{-/-}$*Gria2*$^{R/R}$ (Adar2 KO) mice are shown. Editing ratios are displayed as the mean ± SEM (n=3 mice for each group; Student's *t*-test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, n.s., not significant). The editing ratio of each mouse is also displayed as a circle on the right side of each column. Significant differences in editing ratios between the cerebral cortex and spleen in the same mutant mice are indicated by hashes ($^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001). R/G, arginine/glycine; Q/R, glutamine/arginine; I/V, isoleucine/valine.



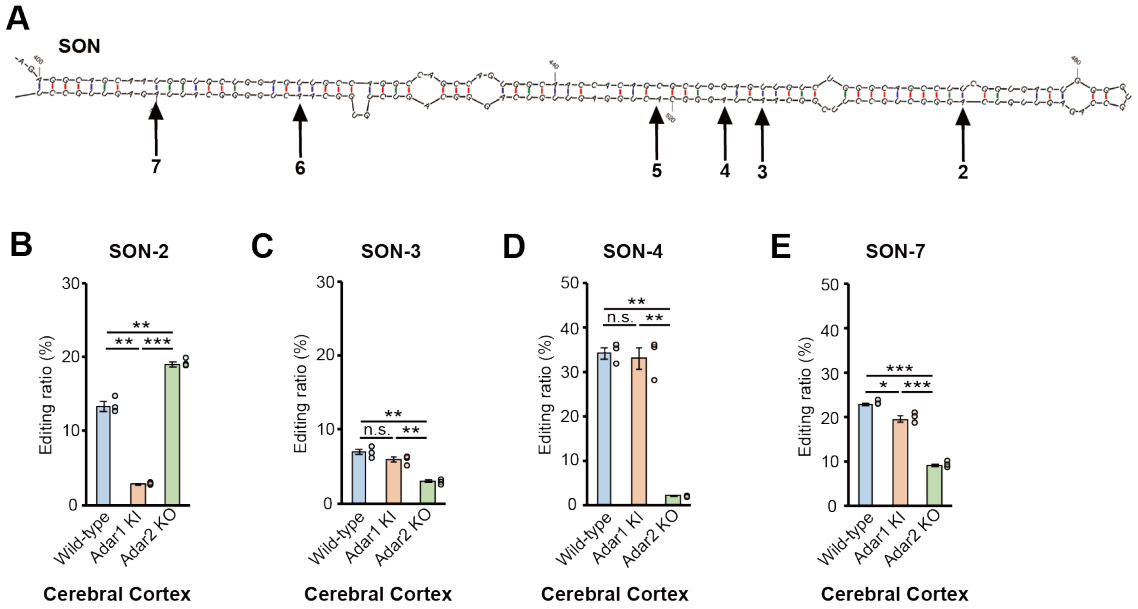
SUPPLEMENTAL FIGURE S5. Retention of RNA editing at sites in miRNAs in Adar1 KI and Adar2 KO mice. (*A*–*G*) Editing ratios for the -4 position of miR-423-5p (*A*), the +6 position of miR-376c-3p (*B*), the +3 position of miR-151-3p (*C*), the +1 position of miR-27a-5p (*D*), the +5 position of miR-379-5p (*E*), the +1 position of miR-99a-5p (*F*), and the -6 position of miR-27a-5p (*G*) in cerebral cortex isolated from wild-type, *Adar1*$^{E861A/E861A}$*Ifih*$^{-/-}$ mice (Adar1 KI), and *Adar2*$^{-/-}$*Gria2*$^{R/R}$ (Adar2 KO) mice are shown. Editing ratios are displayed as the mean ± SEM (n=3 mice for each group; Student's *t*-test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, n.s., not significant). The editing ratio of each mouse is also displayed as a circle on the right side of each column.



SUPPLEMENTAL FIGURE S6. Comparison of editing retention between the cerebral cortex and spleen. (*A–B*) The retention of editing in *Adar1*$^{E861A/E861A}$*Ifih*$^{-/-}$ mice (Adar1 KI) mice (*A*) and *Adar2*$^{-/-}$*Gria2*$^{R/R}$ (Adar2 KO) mice (*B*) was compared between the cerebral cortex and spleen. (*C*) The degree of similarity in ADAR preference between the cerebral cortex and spleen. Values of ADAR preferences were compared between the cerebral cortex and spleen; small values were defined as "C" and large ones as "D"; the similarity in ADAR preferences was calculated using the following formula: 100–(D–C). In this calculation, 100% indicates the contribution of the same ADAR between the cerebral cortex and spleen to a certain editing site, while 0% indicates the contribution of different ADARs between the cerebral cortex and spleen. The relative value calculated by dividing the editing ratio in the spleen by that in the cerebral cortex isolated from wild-type (WT) mice is shown on the vertical axis as a percentage. The red squares, green diamonds and grey triangles represent editing sites in coding sequences (CDS), repetitive elements (REs), and introns, respectively. See Supplemental Charts to access an interactive version of these charts in which each editing site can be identified.



SUPPLEMENTAL FIGURE S7. Comparable retention of RNA editing in two miRNAs from Adar1 KI and Adar2 KO mice. (*A–B*) Editing ratios at the +5 positon of miR-411-5p (*A*) and -1 position of miR-99b-3p (*B*) in the indicated tissues isolated from wild-type, *Adar1*$^{E861A/E861A}$*Ifih*$^{-/-}$ mice (Adar1 KI), and *Adar2*$^{-/-}$*Gria2*$^{R/R}$ (Adar2 KO) mice are shown. The 5' end of the mature micro (mi)RNA sequence is defined as the +1 position (See Supplemental Table S1). Editing ratios are displayed as the mean ± SEM (n=3 mice for each group; Student's *t*-test, n.s., not significant). The editing ratio of each mouse is also displayed as a circle on the right side of each column.



SUPPLEMENTAL FIGURE S8. Comparion of RNA editing pattern among multiple sites in SON. *(A)* The secondary structure aroung multiple editing sites in SON was estimated using Mfold. The number (2–7) indicates each editing site. (*B–E*) Editing ratio for SON-2 (*B*), SON-3 (*C*), SON-4 (*D*) and GluK2 Q/R (*E*) sites in the indicated tissues isolated from wild-type, *Adar1*$^{E861A/E861A}$*Ifih*$^{-/-}$ mice (Adar1 KI), and *Adar2*$^{-/-}$*Gria2*$^{R/R}$ (Adar2 KO) mice are shown. Editing ratios are displayed as the mean ± SEM (n=3 mice for each group; Student's *t*-test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, n.s., not significant). The editing ratio of each mouse is also displayed as a circle on the right side of each column.