Supplementary materials

A structural determinant required for RNA editing

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Abbreviations: Hsa (*Homo sapiens*); Ptr (*Pan troglodytes*); Mmul (*Macaca mulatta*); Cfa (*Canis familiaris*); Eca (*Equus caballus*); Bta (*Bos taurus*); Rno (*Rattus norvegicus*); Mmu (*Mus musculus*); Mdo (*Monodelphis domestica*); Oan (*Ornithorhynchus anatinus*); Gga (*Gallus gallus*); Ain (*Aspidoscelis inornata*); Cre (*Chinemys reevesii*); Xla (*Xenopus laevis*) ; Dre (*Danio rerio*); Fru (*Fugu rubripes*).

Supplementary Figure 1-3

Supplementary Figure Table S1

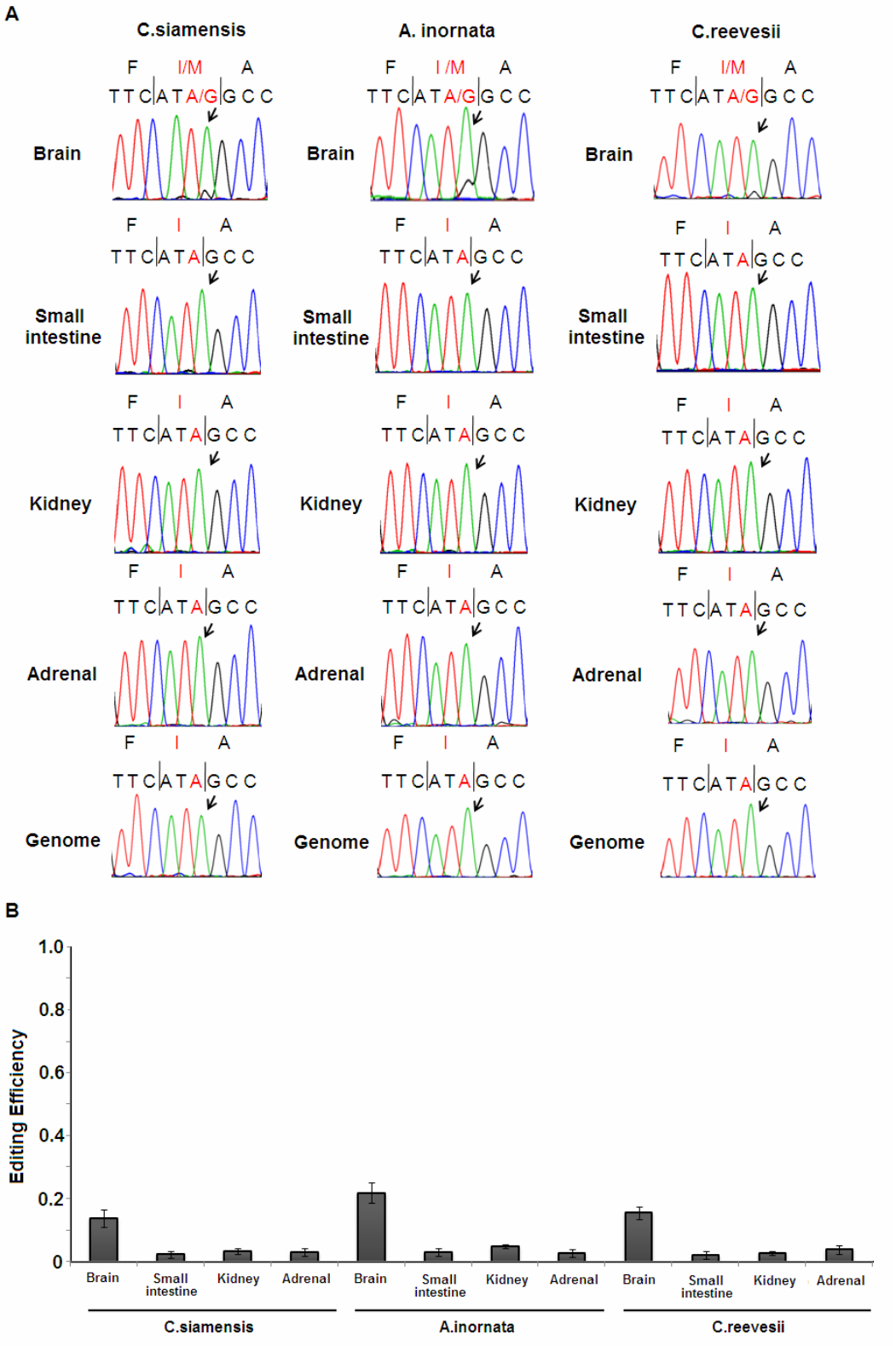

Figure S1 Tissue specificity regulation of Gabra-3 RNA expression and editing (I/M site)

(A) Bulk-sequence analysis of Gabra3 RT-PCR amplicons in different tissues. The chromatogram of RT-PCR from lizard (Ain), crocodile (Csi), and turtle (Cre) brain showed A-to-I editing as a mixed A/G peak. But in small intestine, kidney, adrenal of them is only A

signal in electropherogram trace. (B) Quantitative analyses of Gabra3 mRNA levels in brain,small intestine, kidney, adrenal of lizard, crocodile and turtle.

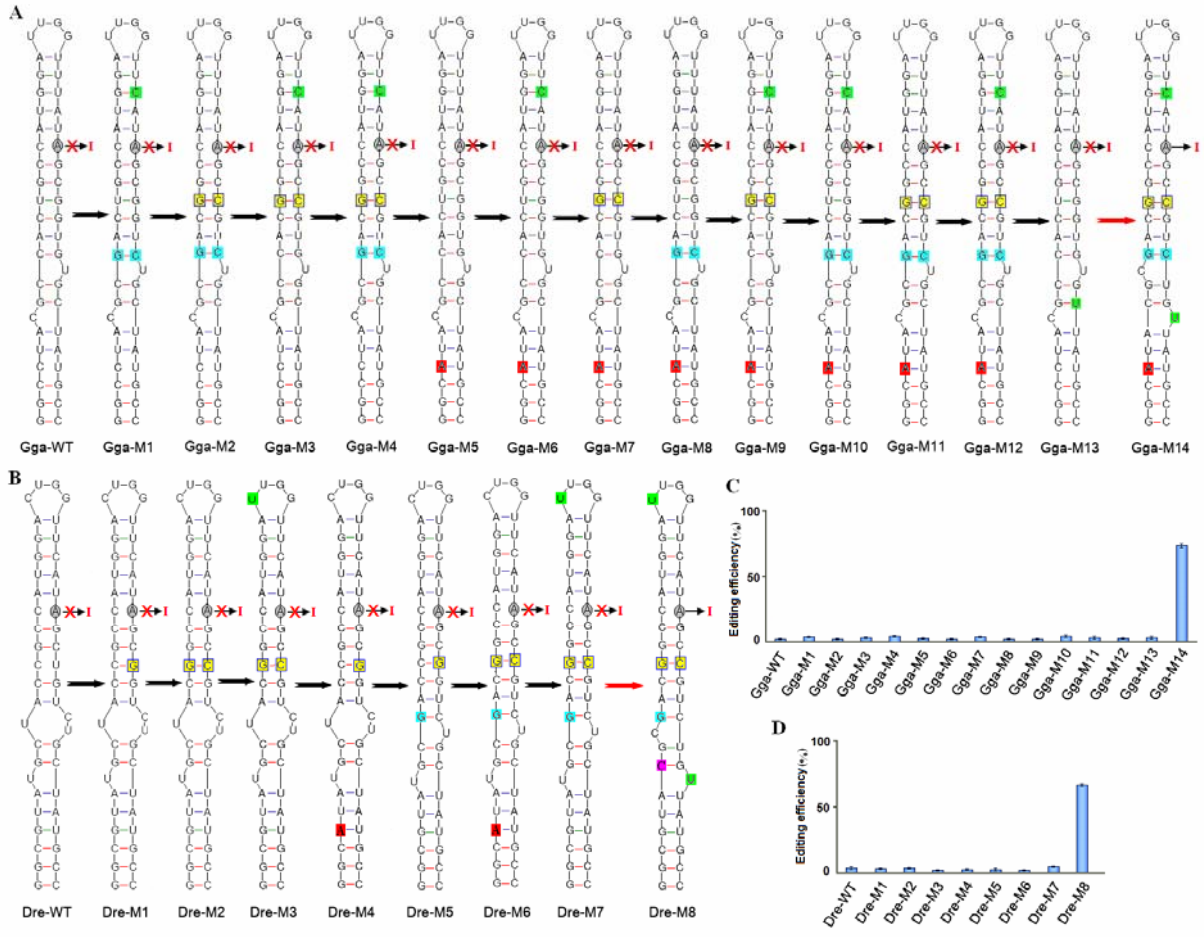

Figure S2. A series of mutants were mimicked a duplex structure to analyzed the effect on RNA editing. (A, B) Schematic diagrams of wild-type (WT) and mutant (M) minigene constructs involved in *in vivo* editing assays, based on chicken Gabra1 (A) and *D. rerio* Gabra5 (B). The edited A is circled, and the mutated nucleotides are shaded in colour. A red cross marks the failure to detect RNA editing (<5%). The conserved stems (Stem 1, 2, 3) are gradually mutated to be nearly or even completely identical to mammalian gabra3. However, the resulting structure still failed to restore editing. By contrast, only mutation in combination with substitution in a nonconserved region could restore editing to wild-type level. (C, D) Editing levels of the various transcripts after injection *Xenopus* oocyte nuclear. RNA editing level of mutants was quantified using a restriction enzyme appropriate for each mutated editing site (see Methods).

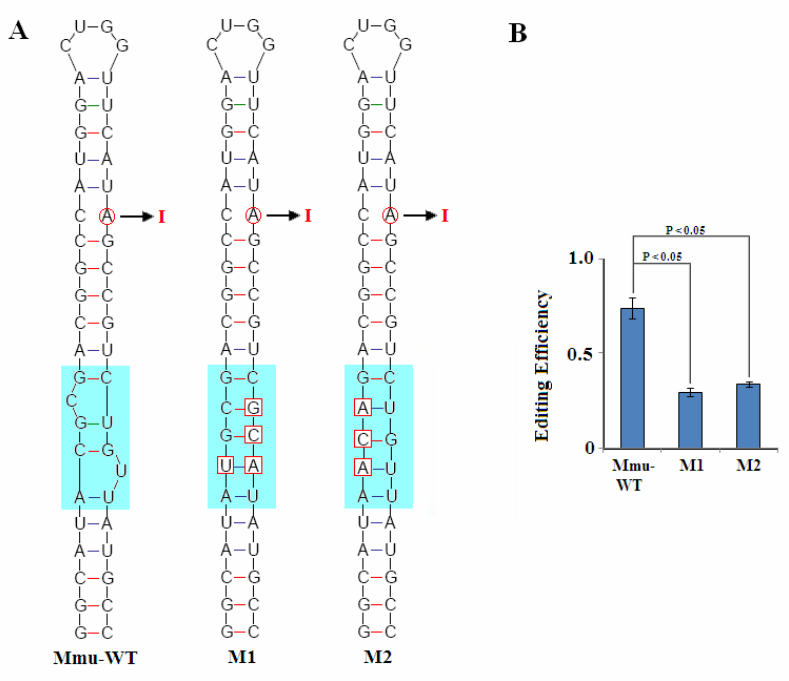

Figure S3. Perfect pairing at variable region by mutation led to marked decrease of editing level.(A) RNA secondary structure of wild-type (W) and mutant (M) minigene constructs involved in *in vivo* editing assays. The edited A is circled, and the mutated nucleotides are shaded in colour. (B) Editing levels of the various transcripts after injection *Xenopus* oocyte nuclear.

Table S1 Primers used for the RT-PCR and PCR analysis

Primer	Sequence	Primer	Sequence
***Gabra-α3* cloning and sequencing**			
Csi -5-1	GACCACATTAAGTATCAGCG	Csi -3-1	GTGAAGTAGTTGACGGTGGCA
Ain -5-1	GACTACTCTGAGCATCAGCGC	Ain -3-1	CGTGAAGTAGTTGACCGCTGCA
Cre -5-1	ATGACCACACTGAGCATC	Cre -3-1	CCGCTTGGTGAAGTAGTTGA
Cor -5-1	ATGACCACATTAAGTATC	Cor -3-1	ACAAAGGCGTAGCACACAGC
Universal primer-5-1	GG(A/G/T/C)GTCACCAC(A/G/T/C)GT(T/G)CT(T/G/A/C)AC(C/T)ATGAC	Universal primer-3-1	AC(C/T)TT(C/T)TT(G/A/T/C)CC(A/G)TCCCAAGCCCA(G/A)CT
RNA editing analysis			
Hsa3-wt-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCAT	Hsa3-wt-3-1	AAGGCATAACAGACGGC
Bta3-wt-5-1	TAATACGACTCACTATAGGGGTGGCATATGCGACGGCCATGGAT	Bta3-wt-3-1	AAGGCATAACACACGGC
Gga1-mu-5-1	TAATACGACTCACTATAGGGTGGCATACGCCACTGCCATGGA	Gga1-mu-3-1	AAGGCATAGCACACCGC
Dre5-mu-5-1	TAATACGACTCACTATAGGGTGGCGTATGCTACCGCCATGGA	Dre5-mu-3-1	AAGGCATAGCAGACCGC
Mmu3-wt-5-1	TAATACGACTCACTATAGGGTGGCATACGCGACGGCCAT	M1-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACAGGT
M2-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACTCGT	M3-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACTGCT
M4-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACGGT	M5-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACTTGC
M6-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACTTGGCT	M7-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGTCTGGTT
M8-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACTGGTTTAT	M9-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCATGGACTGGTTGAT
M10-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCTTGGACTGGTTCAA	M11-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCGTGGACTGGTTCAC
M12-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCCTGGACTGGTTCAG	M13-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCAAT
M14-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCTAT	M15-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCGAT
M16-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGGCAT	M16-3-1	AAGGCATAACAGACGGG
M17-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGTCAT	M17-3-1	AAGGCATAACAGACGGT
M18-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGACAT	M18-3-1	AAGGCATAACAGACGGA
M19-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGTCGTGGACTGGTTCAC	M20-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGTCCTGGACTGGTTCAG
M21-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGACCTGGACTGGTTCAG	M22-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGGCCTGGACTGGTTCAG
M23-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACCGCCA	M23-3-1	AAGGCATAACAGACCGC
M26-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACAGCCAT	M26-3-1	AAGGCATAACAGACAGC
M27-5-1	TAATACGACTCACTATAGGGTGGCTTATGCGACGGCCAT	M27-3-1	AAGGCTTAACAGACGGC
M29-5-1	TAATACGACTCACTATAGGGTCGCATATGCGACGGCCAT	Mmu-M1-5-1	TAATACGACTCACTATAGGGTGGCATACGTGACGGCCAT
Mmu-M1-3-1	AAGGCATAACGGACGGC	Mmu-M3-5-1	TAATACGACTCACTATAGGGTGGCATACGCGACGGCCAT
Mmu-M3-3-1	AAGGCATAGCGGACGGC	Mmu-M4-5-1	TAATACGACTCACTATAGGGTGGCATATGCGACGGCCAT
Mmu-M4-3-1	AAGGCATAGCAGACGGC	Mmu-M8-5-1	TAATACGACTCACTATAGGGTGGCATACGCCACGGCCAT
D1-5-1	TAATACGACTCACTATAGGGATGCGACGGCCAT	D1-3-1	AACAGACGGCTATGAACCAGTCCAT

D2-5-1	TAATACGACTCACTATAGGGTACGCGACGGCCAT	D2-3-1	AACAGACGGCTATGAACCAGTCCAT
D3-5-1	TAATACGACTCACTATAGGGCGACGGCCATGGATTGGTTCAT	D3-3-1	ACACACGGCTATGAACCAAT
D4-5-1	TAATACGACTCACTATAGGGCGACGGCCATGGACTGGTTCAT	D4-3-1	AGACGGCTATGAACCAGTCCAT
D5-5-1	TAATACGACTCACTATAGGGACGGCCATGGATTGGTTCAT	D5-3-1	CACGGCTATGAACCAAT
D6-5-1	TAATACGACTCACTATAGGGTCGGCCAT	D6-3-1	ACGGCTATGAACCAATCCAT
D7-5-1	TAATACGACTCACTATAGGGGGCCAT	D7-3-1	CGGCTATGAACCAATCCAT
D8-5-1	TAATACGACTCACTATAGGGCGCCAT	D8-3-1	GGCTATGAACCAATCCAT