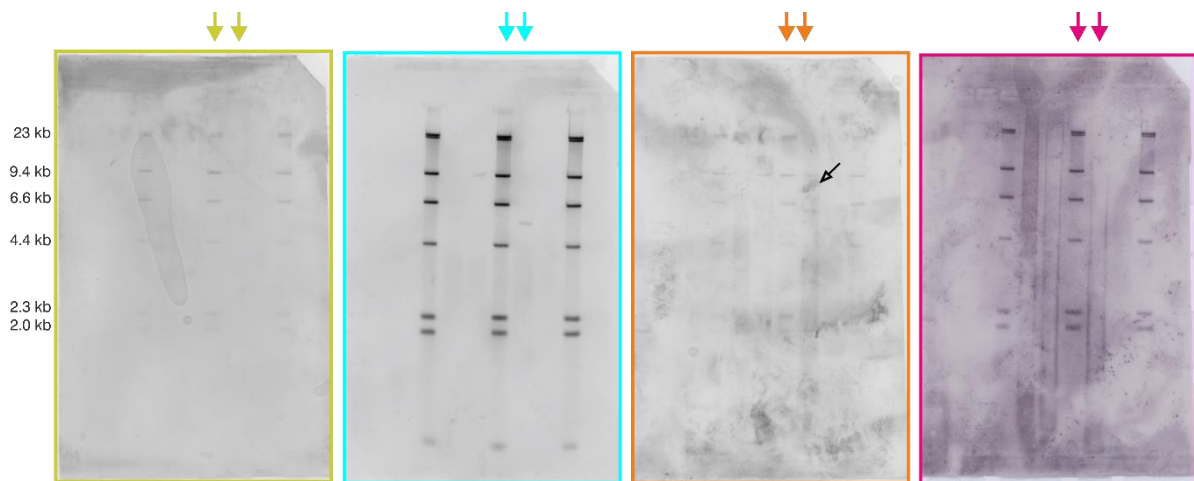
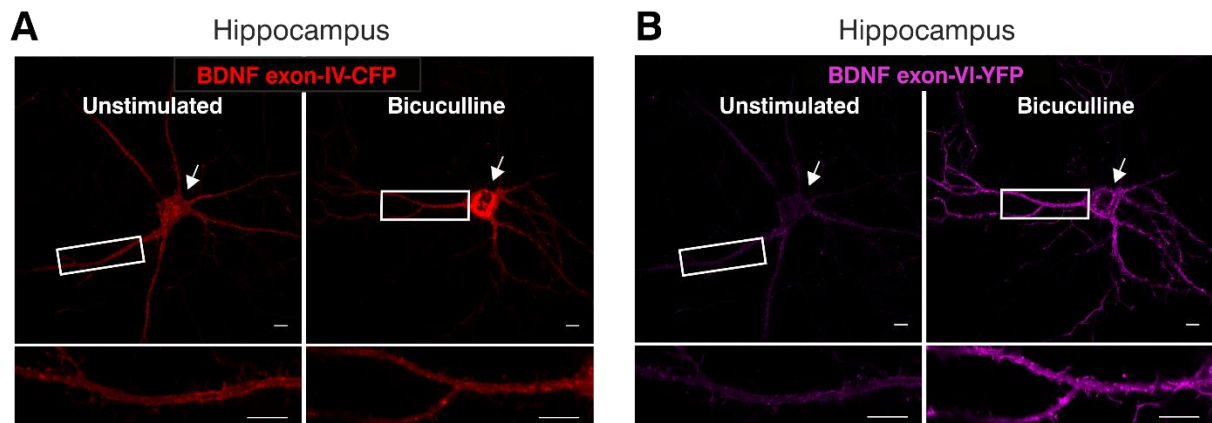


Supplementary Figure 1



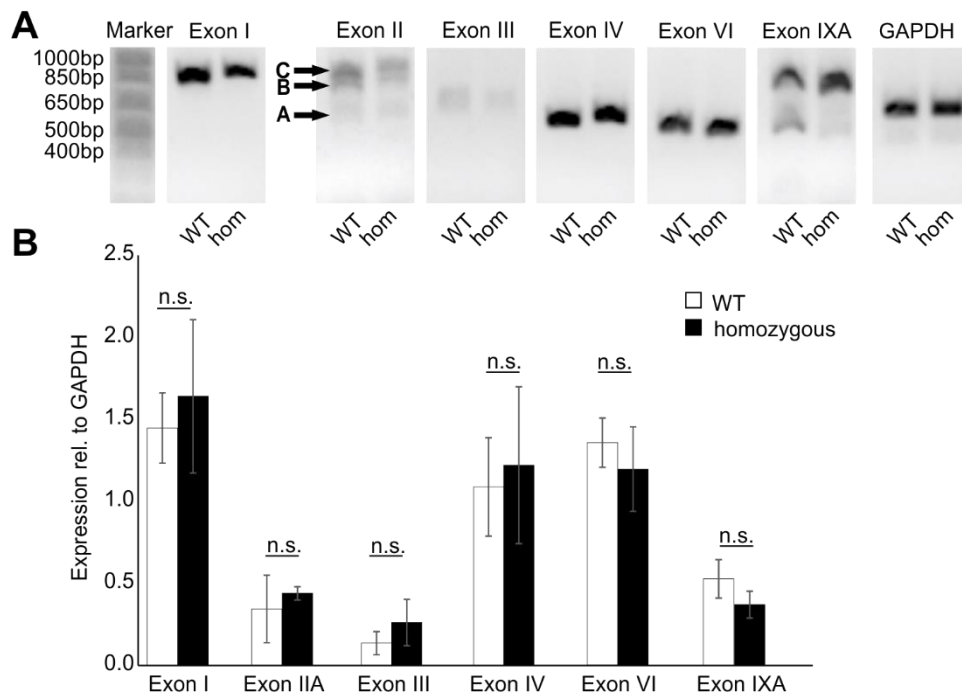
Supplementary Figure 1 Original Southern Blots used for Figure 3 C. Four different probes were used for Southern Blot analyses. Probe 1 (green), specific for the 5'-end: WT band 9.8 kb, transgenic allele 3 kb; probe 2 (blue), covering parts of exon-IV transgene: WT band 9.8 kb, transgenic allele 5.3 kb; probe 3 (orange), covering parts of the exon-VI transgene: WT band 9.8 kb, transgenic allele 6.3 kb; probe 4 (pink), specific for the 3'-end: WT and transgenic allele 12.7 kb. Colored arrows indicate lanes used in Figure 3C. All lanes used for Figure 3C are derived from the same ES cell clone. Southern Blots were inverted and the contrast was increased for all pixels similarly to increase visibility of the band. For probe 3 an unspecific spot was darkened between the 9.8 and 6.3 kb bands, otherwise the bands would not have been clearly visible (black open arrow).

Supplementary Figure 2



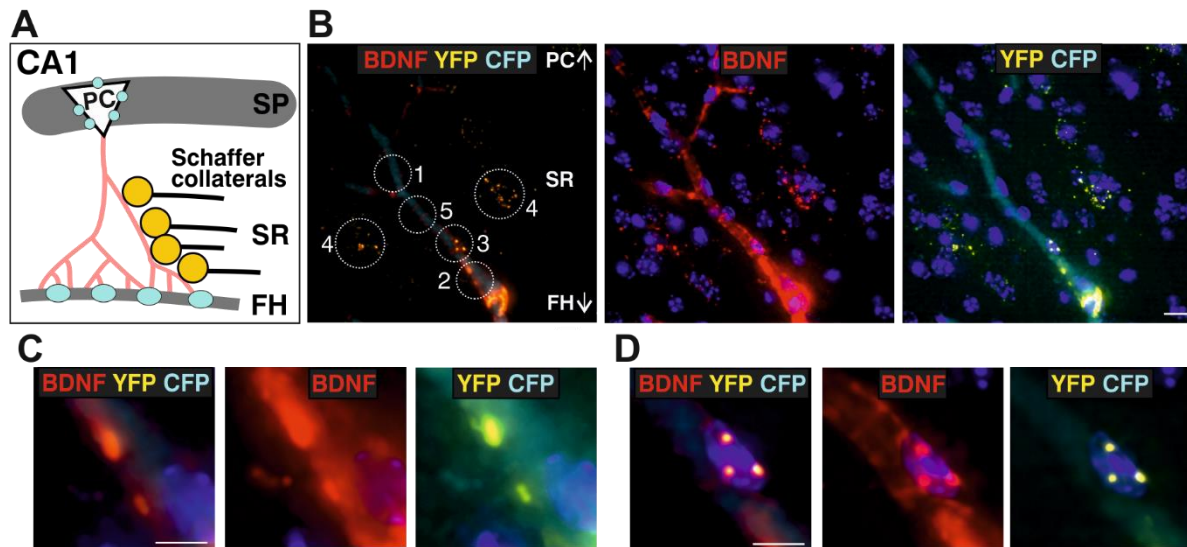
Supplementary Figure 2 Transfected rat primary hippocampal neurons (embryonic day E18) without (left panel) and with bicuculline (right panel) stimulation. Staining against HA (coupled to *Bdnf* exon-IV-CFP) (**A**) and cMyc (coupled to *Bdnf* exon-VI-YFP) (**B**) was performed with specific antibodies against the HA and c-Myc tags, respectively. Lower panels show higher magnification of area in solid frame. (**A**) Increased expression of *Bdnf* exon-IV-CFP detected preferentially in cell bodies after stimulation with bicuculline (right panel, arrow). (**B**) Increased expression of *Bdnf* exon-VI-YFP after treatment with bicuculline predominantly detected in proximal and distal regions of the neurites (right panel, solid frame). Scale bars: 10 μ m.

Supplementary Figure 3



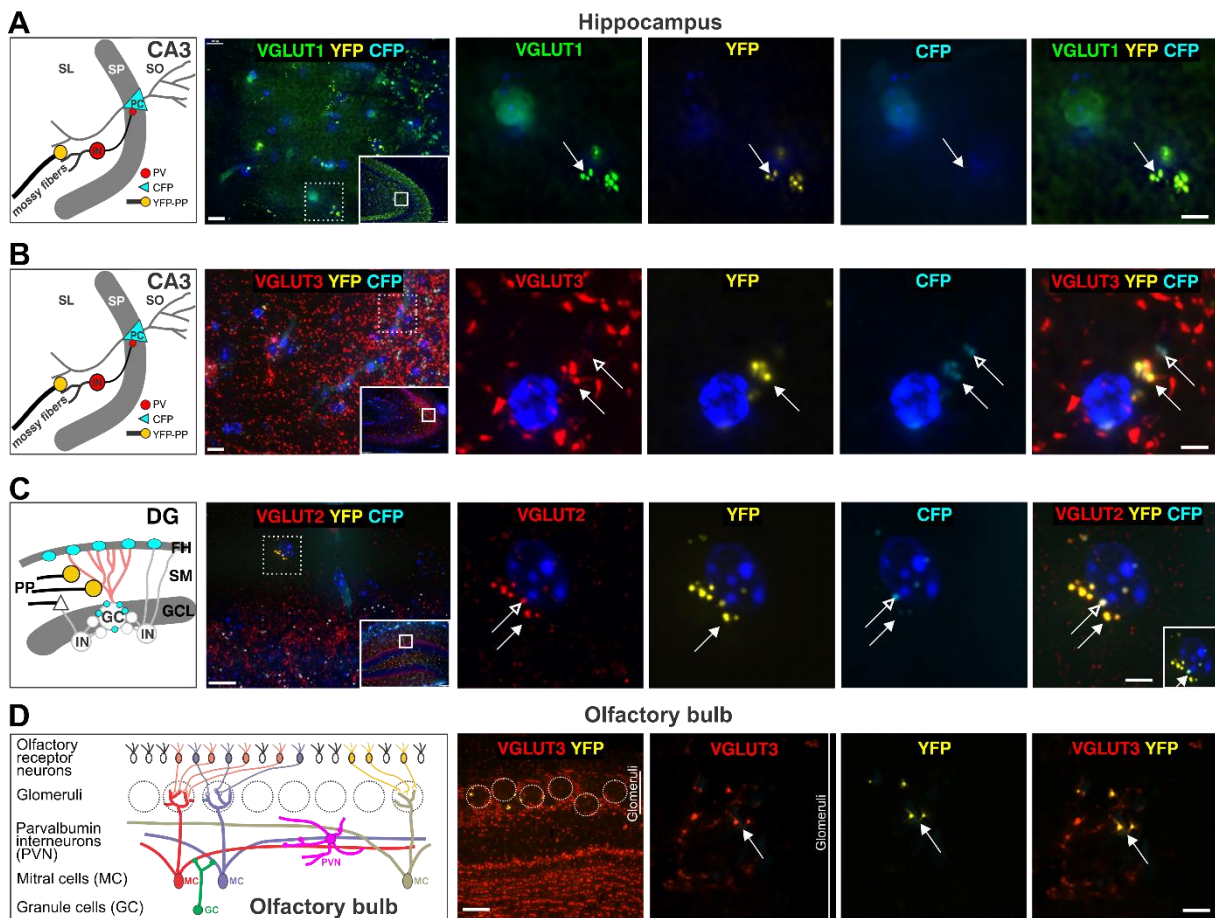
Supplementary Figure 3 Analysis of mRNA expression of the untranscribed *Bdnf* exons I, II, III, IV, VI, IXA by RT-PCR in the hippocampus of BLEV mice for wildtype (WT) and homozygous (hom) animals. **(A)** Representative gel picture for *Bdnf* exon I, II, III, IV, VI and IXA. The previously described different transcript variants of *Bdnf* exon II (A, B, C) (Aid et al., 2007) are indicated by arrows. Due to the very similar size of the transcript variants B and C of exon II a separate quantification was not possible. Housekeeping gene: GAPDH. For original picture see Supplementary Figure 6D. **(B)** Quantitative analyses of the expression of *Bdnf* exon I, IIA, III, IV, VI, and IXA did not reveal any significant differences between WT and homozygous BLEV mice. Data represented as mean \pm SEM (2-sided Student's *t*-test with $\alpha = 0.05$, for details see Supplementary Table 2).

Supplementary Figure 4



Supplementary Figure 4 Triple images of BDNF IR (red), YFP and CFP (A) Schematic overview of the CA1 region containing the stratum pyramidale (SP), the stratum radiatum (SR) and the fissura hippocampalis (FH). (B) Merged image of Figure 5B. Left picture: BDNF-IR in 5 different characteristic regions at the level of the SR co-localized with *Bdnf* exon-VI-YFP and *Bdnf* exon-IV-CFP. Middle picture: BDNF-IR (red). Right picture: Co-localization of *Bdnf* exon-VI-YFP and *Bdnf* exon-IV-CFP. Nuclei were stained with DAPI (blue). Scale bars: 100 μ m. (C) Merged image of Figure 5E. Left picture: BDNF-IR co-localized with *Bdnf* exon-VI-YFP close to a *Bdnf* exon-IV-CFP positive capillary in the FH. Middle picture: BDNF-IR (red). Right picture: Co-localization of *Bdnf* exon-VI-YFP and *Bdnf* exon-IV-CFP. Nuclei were stained with DAPI (blue). Scale bar: 10 μ m (D) Merged image of Figure 5H. Left picture: BDNF-IR co-localized with *Bdnf* exon-VI-YFP-positive puncta on *Bdnf* exon-IV-CFP-positive capillaries. Middle picture: BDNF-IR (red). Right picture: Co-localization of *Bdnf* exon-VI-YFP and *Bdnf* exon-IV-CFP. Nuclei were stained with DAPI (blue). Scale bar: 10 μ m.

Supplementary Figure 5

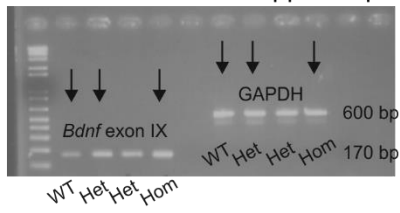


Supplementary Figure 5 Immunostaining of the presynaptic marker proteins VGLUT1, VGLUT2 and VGLUT3. **(A)** Schematic overview of the CA3 region containing the stratum lucidum (SL), stratum pyramidale (SP), the stratum oriens (SO), parvalbumin (PV) positive interneurons (IN) and pyramidal cells (PC). Second panel: VGLUT1 IR in green co-localized with *Bdnf* exon-VI-YFP and *Bdnf* exon-IV-CFP within the CA3 region. Inset: lower magnification of the same area (solid frame). Scale bar: 100 μ m. Right panels: higher magnification depicting single channels of VGLUT1 (green), YFP and CFP demonstrate co-localized VGLUT1 and YFP positive dots (arrows). Scale bar: 10 μ m. Nuclei were stained with DAPI (blue) **(B)** Schematic overview of the CA3 region. Second panel: VGLUT3 IR in red co-localized with *Bdnf* exon-VI-YFP and *Bdnf* exon-IV-CFP. Inset lower magnification of the area (solid frame). Scale bar: 100 μ m. Right panels: higher magnification depicting single channels of VGLUT3 (red), YFP and CFP show co-localization of VGLUT3 and YFP (arrows). Some few dots show a co-localization of VGLUT3 (red) and CFP (open arrows). Scale bar: 10 μ m. Nuclei were stained with DAPI (blue) **(C)** Schematic overview of the dentate gyrus (DG) region containing the fissura hippocampalis (FH), stratum moleculare (SM), the granular cell layer (GCL), granular cells (GC), interneurons (IN) and the perforant path (PP). Second panel: VGLUT2 IR (red) co-localized with *Bdnf* exon-VI-YFP and *Bdnf* exon-IV-CFP. Inset: lower magnification of the same area (solid frame). Scale bar: 100 μ m. Right panels: higher magnification depicting single channels of VGLUT2, YFP and CFP, which demonstrate a co-localization of VGLUT2 (red) and YFP (arrows). Some few dots show a co-localization of VGLUT2 (red), YFP and CFP (open arrows). Inset in the right panel shows Scale bar: 10 μ m. Nuclei were stained with DAPI (blue). **(D)** Olfactory bulb. Left panel: Schematic overview of the olfactory bulb containing the olfactory receptor neurons, the glomeruli, parvalbumin-positive interneurons (PVN), the mitral cells (MC), and the granule cells (GC). Second panel:

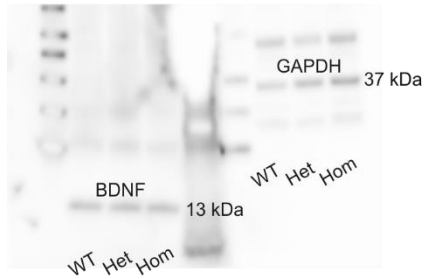
VGLUT3 IR (red) in the olfactory bulb co-localized with *Bdnf* exon-VI-YFP. Scale bar: 100 μm . Right panels: higher magnification of the glomeruli. A clear co-localization of VGLUT3 IR (red) and *Bdnf* exon-VI-YFP was observed within the glomeruli. Scale bar: 10 μm .

Supplementary Figure 6

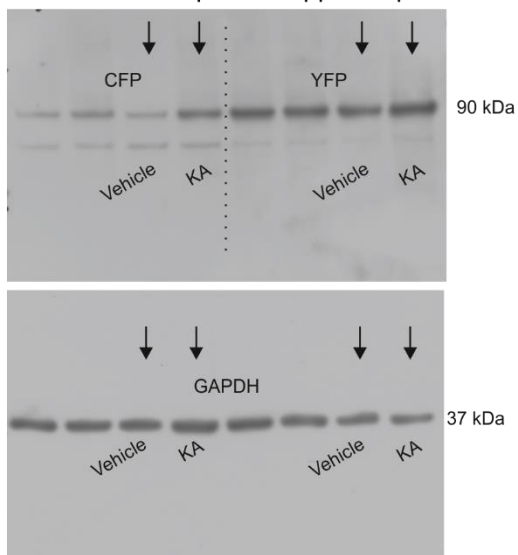
A *Bdnf* exon-IX mRNA hippocampus



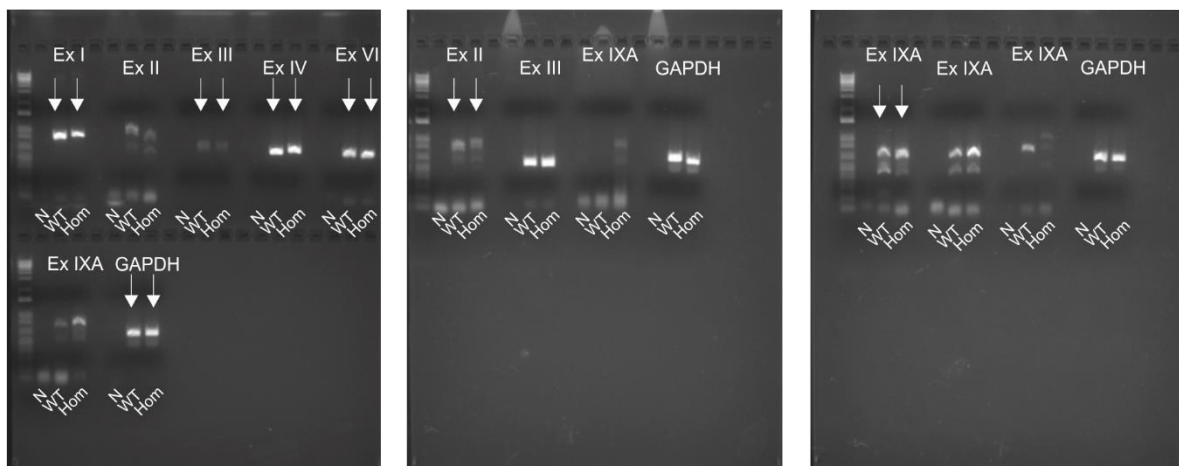
B BDNF protein hippocampus



C CFP and YFP protein hippocampus



D RT-PCR untranslated *Bdnf* exons



Supplementary Figure 6 Original PCR and Western Blots for Figures 4C/D and 7A. **(A)** Picture from the original PCR gel used for Figure 4C. Black arrows indicate bands used for the figure. As two heterozygous animals were used for PCR, one is left out. **(B)** Original Western blot used for Figure 4D. Contrast was increased for better visibility. For quantification the unmodified image was used. **(C)** Original Western blot used for Figure 7A. Black arrows indicate bands used for the figure. As other probes were run on the same blot relevant bands were cut. Contrast was increased for better visibility. For quantification the unmodified image was used. **(D)** Original PCR gel used for Supplementary Figure 2. Arrows indicate the used lanes. For quantification the unmodified images were used.

Supplementary Table 1 Antibody information for immunohistochemistry and Western blot

Primary Antibodies						
Immunohistochemistry	Antibody	Protein name	Product number	Source	Dilution	Protein size
Brain sections	Mouse anti-BDNF	Brain-derived neurotrophic factor	BDNF #9-b	Developmental Studies Hybridoma Bank University of Iowa	1:50	
	Rabbit anti-parvalbumin	Parvalbumin	ab11427	Abcam	1:2,000	
	Mouse anti-desmin	Desmin	ab8976	Abcam	1:100	
	Rabbit anti-Arc	Activity regulated cytoskeletal protein	156003	Synaptic Systems	1:500	
	Rabbit anti-IBA-1	Ionized calcium-binding adaptor molecule 1	016-20001	Wako Chemicals	1:500	
	Rabbit anti-GFAP	Glial fibrillary acidic protein	Z 0334	Dako	1:2,000	
	Rabbit anti-p75NGFR	p75 neurotrophin receptor	AB1554	Millipore	1:100	
	Rabbit anti-b1-GC	b1-subunit of the nitric oxide-sensitive guanylate cyclase		Müllerhausen et al. 2004	1:200	
	Guinea pig anti-vGLUT1	Vesicular glutamate transporter 1	135304	Synaptic System	1:1,500	
	Rabbit anti-vGLUT2	Vesicular glutamate transporter 2	135403	Synaptic System	1:500	
	Rabbit anti-vGLUT1	Vesicular glutamate transporter 3	135203	Synaptic System	1:1,000	
Cell culture	Mouse anti-Myc	Myc-tagged protein	2276	Cell Signaling	1:500	
	Rabbit Anti-HA	HA-tagged protein	AB9110	Abcam	1:500	

Western blot	Rabbit anti-RCFP	Reef coral fluorescent protein pan antibody	632475	Clontech	1:1,000	30-35 kDa
	Rabbit anti-BDNF	Brain-derived neurotrophic factor	sc-546	Santa Cruz Biotechnology Inc.	1:400	15 kDa
	Mouse anti-GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	ab8245	Abcam	1:10,000	40 kDa
Secondary antibodies						
Immunohistochemistry						
Brain sections						
	Antibody	Protein name	Product number	Source	Dilution	Protein size
	Cy3-conjugated goat anti-mouse antibody		115-1665-062	Jackson ImmunoResearch Laboratories	1:3,000	
	AlexaFluor 488 goat anti-guinea pig		A-11073	Molecular Probes	1:500	
Cell culture	AlexaFluor 568 conjugated goat anti-rabbit		A-11011	Invitrogen	1:500	
	AlexaFluor 647 conjugated goat anti-mouse		A-21235	Invitrogen	1:500	
Western blot	Cy3-conjugated goat anti-rabbit antibody		111-166-003	Jackson ImmunoResearch Laboratories	1:1,500	
	Alexa488 - conjugated anti-mouse antibody		A11001	Molecular Probes, MoBiTec	1:500	
	Alexa488 - conjugate		A11073	Molecular Probes, MoBiTec	1:500	

	d anti-guinea pig antibody					
	ECL anti-mouse IgG HRP linked		NA 931-100µl	GE Healthcare UK Limited	1:2,500	
	ECL anti-rabbit IgG HRP linked		NA 934-100µl	GE Healthcare UK Limited	1:2,500	

Supplementary Table 2 Statistical information

		Statistical test	Test value	Degrees of freedom	p-value	Post-hoc test with p-value		n-number
Fig. 1E		1-way ANOVA			p = 0.0002	post-test 2-sided Student's t-test		done in duplicate for 4 independent experiments
			F (3, 36) = 8.35			CFP	p = 0.008	
						YFP	p = 0.003	
Fig. 4B								WT n = 9 Het n = 11 Hom n = 11 animals
Fig. 4C	PCR (mRNA)	1-way ANOVA	F(2,9) = 0.15		p = 0.86			n = 3 animals / genotype
Fig. 4D	Westen blot (Protein)	1-way ANOVA	F(2,12) = 0.20		p = 0.82			n = 4 animals / genotype
Fig. 4E	click-ABR	1-way ANOVA	F(2, 26) = 1.988		p = 0.16			WT n = 20/10 ears/animals het n = 30/15 ears/ animals hom n = 10/5 ears/animals
	f-ABR	2-way ANOVA	F(18, 2665) = 0.50		p = 0.96			
Fig. 7A	Western blot (CFP)	1-sided Student's t-test	t = 6.25	DF = 4	p = 0.002			n = 3 animals / group
	Western blot (YFP)	1-sided Student's t-test	t = 1.99	DF = 6	p = 0.04			n = 4 animals / group
Fig. 7D	Integrated density (YFP)	1-sided Student's t-test	t = 4.14	DF = 3	p = 0.0128			n = 3 animals / group
Fig. 7F	Integrated density (CFP)	1-sided Student's t-test	t = 2.32	DF = 4	p = 0.0405			n = 2-3 animals / group
Supp. Fig. 2B	PCR (mRNA)	2-sided Student's t-test	Exon I t = 0.7327 Exon IIA t = 0.3913 Exon III t = 1.007 Exon IV t = 0.3009 Exon VI t = 0.5474	DF = 6 DF = 6 DF = 6 DF = 6 DF = 6	p = 0.4914 p = 0.7091 p = 0.3526 p = 0.7737 p = 0.6132 p = 0.3560			n = 3 animals / genotype; 4-5 replicates / animal

			Exon IXA t = 0.9908					
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