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Supplemental Data

TRIP8b Splice Variants Form a Family of Auxiliary Subunits that Regulate Gating and Trafficking of HCN Channels in the Brain

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Supplementary Figure Legends

Supplementary Figure 1. The TRIP8b gene and open reading frame (ORF).

(A) Schematic representation of the TRIP8b gene on mouse chromosome 3. Exons encoding the open reading frame of the protein are drawn as grey rectangles, and numbered as referred to in the text. Exons 10-16 are clustered within a region of 10kb, and encode the tetratricopeptide repeats. Drawing is not to scale. A detailed description of the structure of the TRIP8b intron/exon boundaries can be found at http://www.ncbi.nlm.nih.gov/projects/CCDS/CcdsBrowse.cgi (CCDS ID 17302). Only a subset of the TRIP8b exons encoding the open reading frame of the protein encode complete codons.

(B) Amino acid sequence corresponding to each of the exons identified above. Alternatively spliced exons are labeled separately (as indicated) whereas the constant region of the TRIP8b protein is labeled continuously (exons 5-16). Blue letters identify the N-terminal domain of TRIP8b, which shows no homology to other proteins and contains the putative trafficking motifs (underlined; see Results). Black and red letters identify the C-terminal domain of TRIP8b, which has 57% sequence identity to the C-

terminal domain of Pex5. The tetratricopeptide repeats (TPR) are underlined and identified in red.

Supplementary Figure 2. TRIP8b(1a-4) and TRIP8b(1a) are the most abundant splice variants in the adult mouse brain.

Western blot analysis was carried out to directly compare the size of the TRIP8b(1a-4) and TRIP8b(1a) splice variants heterologously expressed in *Xenopus* oocytes (as indicated), with the size of the protein species identified by an anti-TRIP8b polyclonal antibody in hippocampal extracts prepared from adult mouse brain (hippocampus). A third faint band is visible in the hippocampal extract, which exhibits a molecular weight slightly larger than TRIP8b(1a-4); this species co-migrates with the heterologously expressed TRIP8b(1b-2) splice variant (data not shown). Note that the same three protein species are detected in extracts from adult mouse neocortex or whole brain (data not shown).

The origin of the two faint bands of lower molecular weight in the *Xenopus* ocytes expressing TRIP8b(1a-4) is unclear (possible products of degradation, cleavage or premature translation stops).

Supplementary Figure 3. The effects of TRIP8b on channel expression are specific to HCN1 channels.

Xenopus oocytes were co-injected with cRNA encoding HCN1, KAT1 or Kv1.2 (as indicated) plus either GFP or GFP-TRIP8b(1a-4), and two-microelectrode voltage-clamp current measurements performed 3 d after injection. For HCN1, Boltzmann curves were obtained and maximal tail current amplitude plotted as in Figure 1. For KAT1, tail current amplitudes following a 3 s step to -100 mV were measured and plotted; bath solution contained (in mM): 96 KCl, 2 NaCl, 10 HEPES, and 2 MgCl2, pH 7.5. For Kv1.2, a

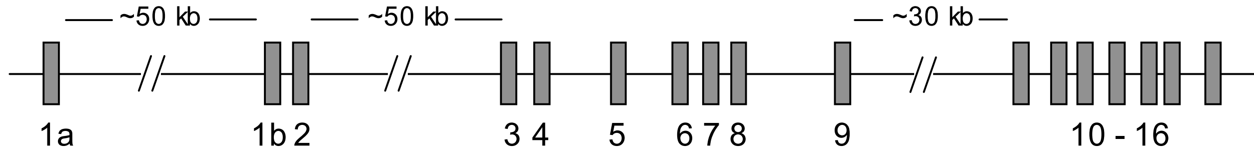
depolarizing voltage in a single step to 0 mV was applied from a holding potential of -80 mV, and steady-state current amplitudes measured and plotted; step duration was 100 ms; bath solution was as for HCN1 recordings (see Experimental Procedures). For recordings of Kv1.2, linear leak current was subtracted. To assess the leak current, membrane voltage was stepped from -110 mV to -90 mV four times. Mean values and s.e.m. (N): HCN1 + GFP, 1.12 ± 0.03 μA (n=140); HCN1 + GFP-TRIP8b(1a-4), 3.63 ± 0.07 μA (n=80) ($P<10^{-14}$; *t*-test); KAT1 + GFP, 5.38 ± 0.33 μA (n=22); KAT1 + GFP-TRIP8b(1a-4), 5.52 ± 0.41 μA (n=22) ($P>0.36$; *t*-test); Kv1.2 + GFP, 47.04 ± 2.06 μA (n=20); Kv1.2 + GFP-TRIP8b(1a-4), 54.43 ± 3.07 μA (n=20) ($P<0.02$; *t*-test). Note that, though statistically significant, the difference in Kv1.2 current amplitude was small (15.7% increase) compared to the 324% increase seen for HCN1.

Supplementary Figure 4. TRIP8b(1a) co-expression alters the intracellular distribution of HCN1 channels.

Xenopus oocytes were injected with cRNA encoding a GFP-HCN1 fusion protein, with or without cRNA encoding TRIP8b(1b-2) or TRIP8b(1a), as indicated. Live imaging of direct GFP fluorescence was carried out on an inverted laser scanning confocal microscope (Santoro et al., 2004). All images are single focal planes. Notice the redistribution of labeling into puncta when GFP-HCN1 is co-expressed with either TRIP8b(1b-2) or TRIP8b(1a), splice variants that induce down-regulation of HCN current density (see text; Santoro et al, 2004).

Supplementary Figure 1

A



B

Exon 1a
MYQGHMQ

Exon 1b
MSDSEMDGRTHIPSLLNALLSRNRVMQMSYL

Exon 2
KSKEQG**Y**GK**L**SSDEDLEIIVDQKQ

Exon 3
VVGVTLKKKWHCLQKSDLTLAL

Exon 4
GKGSRAADKAVAMVMKEIPREESA**E**EKP**LL**TMTSQ

Exon 5-16
LVNEQQ**E**SRP**LL**SPSIDDFLCETKSEAIAKPVTSNTAVLTTGLDLLDLSEPVSQTQTKA
KKSESSSKSSSLKKKA**D**GSD**LI**SADAEQRAQALRGPETSSLDLDIQTQLEKWDDVKF
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APAQARLTKEHRWGSALLSRNHSLEEEFERAKAAVESDTEFWDKMQAEWEEMARR
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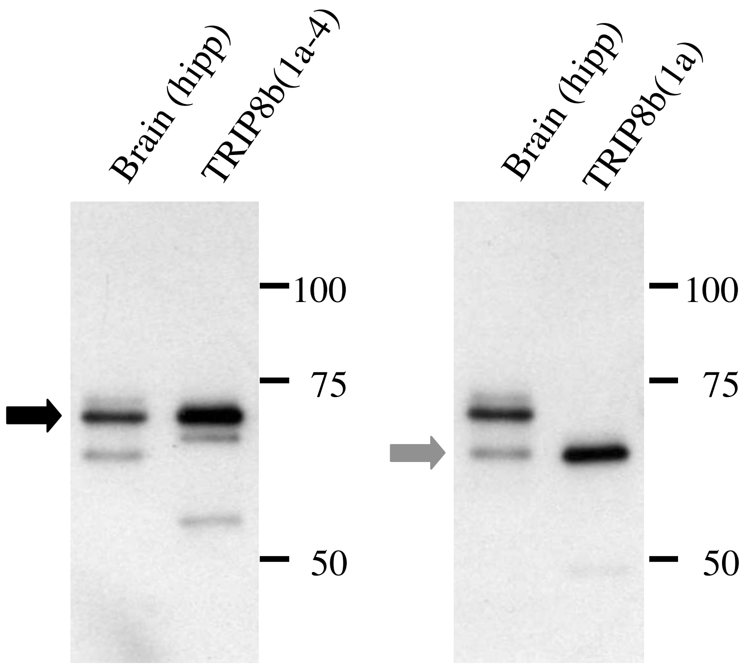
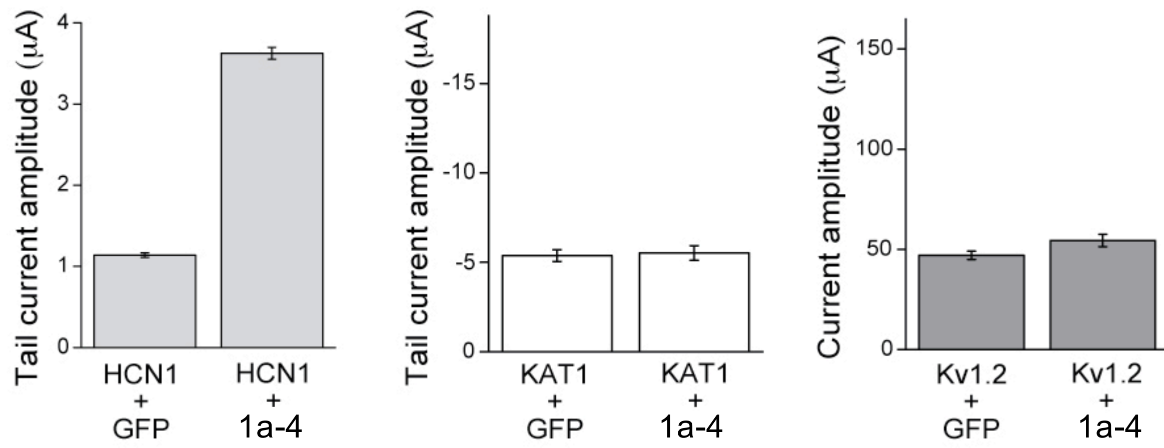
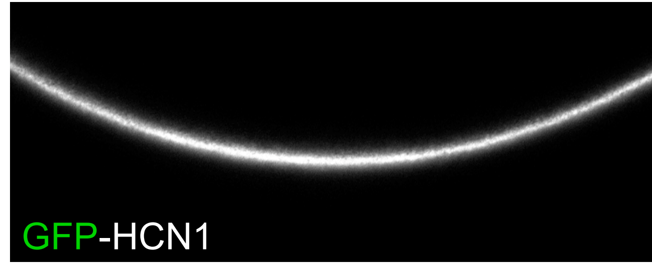
Supplementary Figure 2

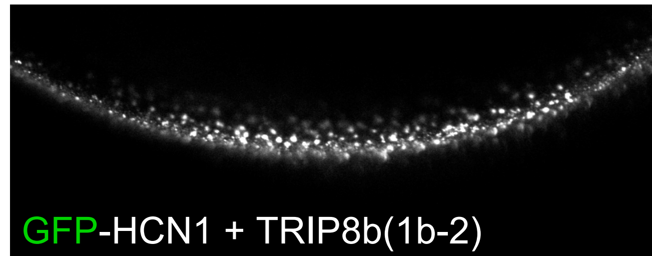


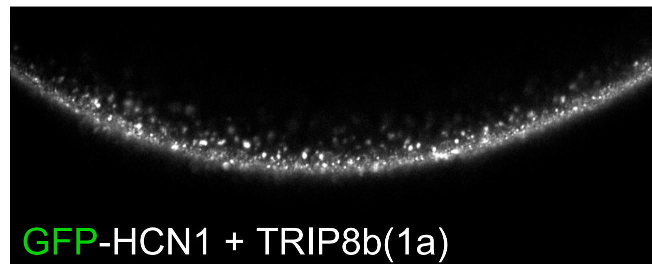
Supplementary Figure 3



Supplementary Figure 4







Supplementary Table 1

NAME	SEQUENCE
1a-for	GAGCAGAATGTACCAGGGACACATG
1b-for	GGAAGGACTCACATTCCATCTCTAC
1a4-for	GAATGTACCAGGGACACATGCAGGG
1a5-for	ATGTACCAGGGACACATGCAGCTGG
3-forA	CAGGTAGTAGGTGTAACCTTGAAG
3-forB	AAAGTGACCTGACCCTGGCTTTG
56-for	TGACATCCAACACAGCCGTGTTGAC
7-for	TCAAGTTTCACGGTGACCGAACAAG
2-rev	TTCCAGGTCCTCGTCACTGCTTAGC
25-rev	TTGTTGCTCATTCACCAGCTGCTTC
34-rev	TTTCCCAAAGCCAGGGTCAGGTCAC
45-rev	CACCAGCTGTGATGTCACAGTAAGG
5-rev	TGGATGTCACTGGCTTTGCAATGGC
6-rev	CTTGAGGGACGAGCTCTTTGATGAG
8-rev	AGCTCTGGCTGAGATCTGTGTTCTG

Supplementary Table 1. Oligonucleotides for real-time PCR. Numbering indicates exons within the TRIP8b coding sequence (see Supplementary Figure 1, and Figure 1A). All DNA sequences are indicated in 5’ -> 3’ direction; for, forward primer; rev, reverse primer.