SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. TRIP8b isoform sequence alignment.

A, Overlay of TRIP8b isoforms identified in rat brain. Numbering corresponds to each individual isoform. Asterisk indicates beginning of IsoC1. Tan colored region is required for TRIP8b binding to HCN1Δ3. Light purple and light green colored regions represent TPR set I and II, respectively.

Supplemental Figure 2. TRIP8b isoforms cause hyperpolarization of activation of HCN1.

A, Representative tail currents elicited upon return to holding potential of HEK293T cells cotransfected with GFP and HCN1 cDNA from whole cell voltage clamp in which cell was held at -40 mV and stepped from -125 to -25 in 5 mV increments. ***B-G,*** representative tail currents elicited from cells cotransfected with HCN1 and IsoA2, IsoA4, IsoA5, IsoB1, IsoB3 and IsoB4 respectively with corresponding Boltzmann plot below. For each plot, data from TRIP8b-containing cotransfection is represented in grey while the data obtained from the GFP cotransfection is represented in black. Plot of V_{50} is shown in Fig. 2 for each transfection condition. Horizontal scale bar in ***G*** applies to all traces.

Supplemental Figure 3. Characterization of custom antibodies.

A, Custom guinea pig (gp) antibodies to total TRIP8b are specific. Cell lysates from untransfected HEK293T cells, cells transfected with IsoA4, and lysate from adult mouse brain were separated by SDS-PAGE and immunoblotted using gp α-TRIP8b (left

panel) or previously characterized rabbit (rab) α-TRIP8b (right panel, (Shin et al., 2008)), demonstrating that gp α-TRIP8b antibody recognizes TRIP8b specifically. ***B,*** Custom gp and rab antibodies to green fluorescent protein (GFP) are specific. Cell lysates from untransfected HEK293T cells, cells transfected with GFP-fused to amino acid residues 167-474 of rat O-linked N-acetylglucosamine transferase [OGT(167-474)-GFP], or GFP alone, and lysate from adult rat brain were separated by SDS-PAGE and immunoblotted using gp α-GFP (left panel) or rab α-GFP (right panel).

Supplemental Figure 4. IsoA4 and IsoB2 are localized in dendrites.

Cultured hippocampal neurons were transfected with IsoA4 (***A-C***) or IsoB2 (***D-F***), fixed at day *in vitro* (DIV) 10-11, and stained using antibody to total TRIP8b (green; ***A, D***) or the dendritic marker MAP2 (blue; ***B, E***). Distribution of TRIP8b isoforms in MAP2-positive processes indicates dendritic expression. Merged images are shown in ***C*** and ***F***. Scale bar, 10 μm.

Supplemental Figure 5. TRIP8b isoform expression varies by brain region

A, Lysates were prepared from adult rat cerebellum (Cb), cortex (Ctx), hippocampus (Hc), and striatum and thalamus (together referred to as subcortex, (Sub)), and were immunoblotted using TRIP8b exon-specific antibodies or antibody to total TRIP8b. α-tubulin was used as a loading control. ***B,*** Quantitation of blots shown in ***A*** demonstrate that TRIP8b isoform and total TRIP8b expression varied regionally within adult rat brain ($n = 3$).

Supplemental Table 1. Sequence of oligonucleotide primers used for PCR amplification of cDNA following reverse transcription.

Exon 1a, 1b, and 1c 5'-untranslated region (UTR) TRIP8b primers were used to detect the variably spliced N-termini of TRIP8b by priming within the UTR 5' to the start of splice isoforms from promoters A, B, and C, respectively. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used to control for cDNA loading. The remaining primers, IsoA1-5 and IsoB1-4 were designed to amplify specific TRIP8b splice isoforms. Although IsoB1 consists of three different mRNA structures, all are predicted to code for the same protein and the IsoB1 PCR primer was designed to amplify all three mRNA species.

Supplemental Table 2. Membrane parameters in control and TRIP8b shRNA-expressing neurons.

Assessment of electrophysiologic membrane parameters of cultured hippocampal neurons infected with FUGWlinker lentivirus expressing shControl (shRNA control), empty FUGWlinker lentivirus (empty vector), and uninfected neurons (all three pooled to derive control group) and FUGWlinker expressing TRIP8b-sh4 (shRNA).