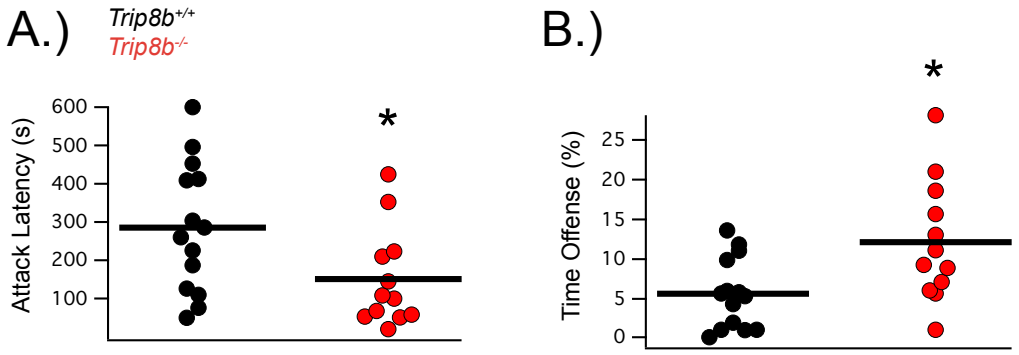
SUPPLEMENTARY MATERIALS

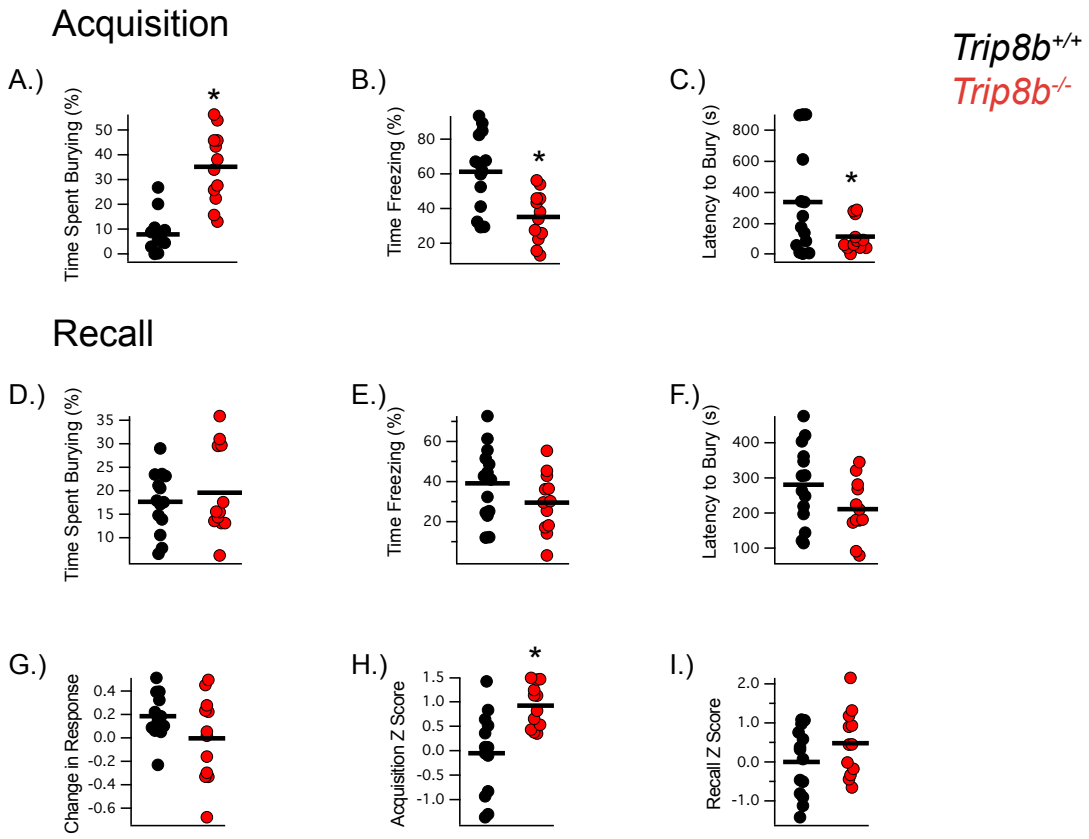
HCN channels in the hippocampus regulate active coping behavior

Daniel W. Fisher*, Ye Han*, Kyle A. Lyman*, Robert J. Heuermann, Linda A. Bean, Natividad Ybarra, Kendall M. Foote, Hongxin Dong, Daniel A Nicholson, Dane M. Chetkovich

1. SUPPLEMENTARY FIGURES and FIGURE LEGENDS

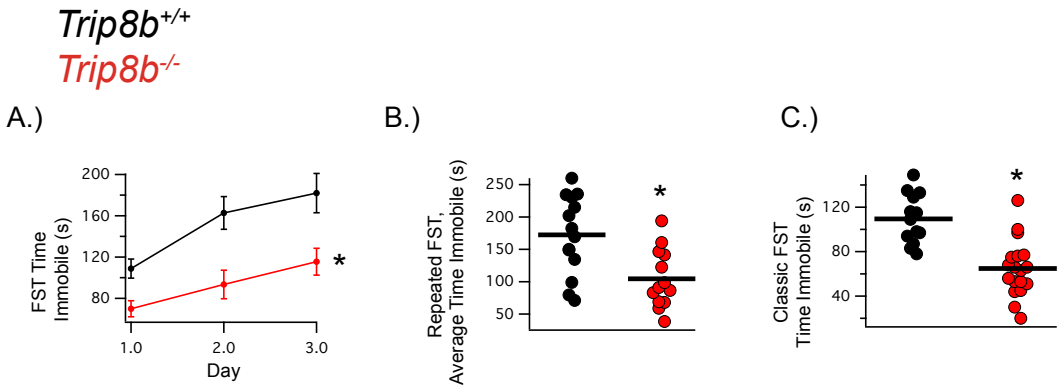


Supplementary Figure 1: *Trip8b*$^{-/-}$ mice show increased active coping in the Resident Intruder Test (RIT). (Related to Figure 1)
A) *Trip8b*$^{-/-}$ mice showed decreased attack latencies (*Trip8b*$^{+/+}$:285±45.24s, *Trip8b*$^{-/-}$: 150.91±37.01s, t_{24} = 2.248, $p < 0.05$) and **B)** increased time displaying offensive aggressive behavior (*Trip8b*$^{+/+}$:5.64±1.18%, *Trip8b*$^{-/-}$: 12.13±2.19%, t_{24} = 2.710, $n_{subjects}$= 14, 12, $p < 0.05$) compared to *Trip8b*$^{+/+}$.

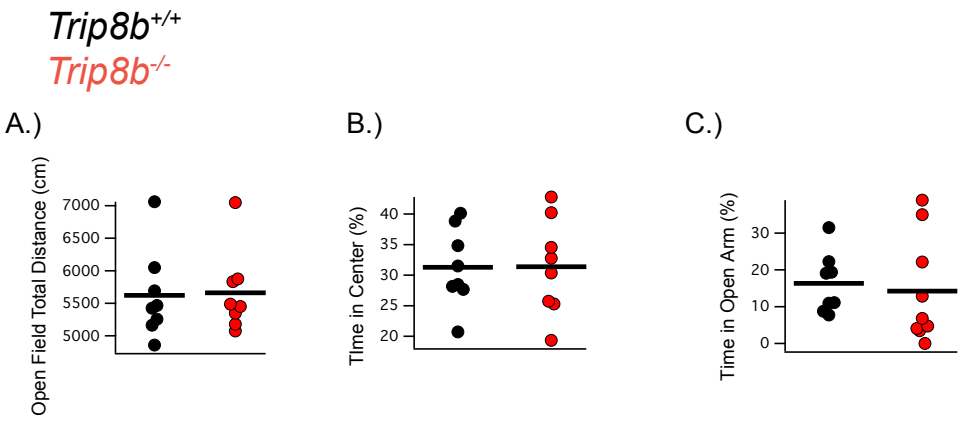


Supplementary Figure 2: *Trip8b*$^{-/-}$ mice show increased active and decreased passive coping in the Shock Probe Burying Task (SPBT). (Related to Figure 1) **A)** During the SPBT Acquisition Trial, the *Trip8b*$^{-/-}$ mice showed increased time spent burying (*Trip8b*$^{+/+}$:7.85±2.19%, *Trip8b*$^{-/-}$: 14.31±2.04%, $t_{24} = 2.155$, $p < 0.05$, $n_{subjects} = 14, 12$), **B)** decreased time freezing (*Trip8b*$^{+/+}$:61.17±5.86%, *Trip8b*$^{-/-}$: 35.12±4.15%, $t_{24} = 3.509$, $p < 0.01$), **C)** and a decreased burying latency (*Trip8b*$^{+/+}$:339±92.2s, *Trip8b*$^{-/-}$: 117.75±29.03s, $n_{subjects} = 14, 12$ $t_{24} = 2.139$; $p < 0.05$) compared to *Trip8b*$^{+/+}$ mice. **D)** On the SPBT Recall Trial, *Trip8b*$^{-/-}$ mice did not show statistical differences in burying (*Trip8b*$^{+/+}$:17.63±1.72s, *Trip8b*$^{-/-}$: 19.58±2.69s, $t_{24} = 0.6257$, $p = 0.54$), **E)** freezing (*Trip8b*$^{+/+}$:39.09±4.89%, *Trip8b*$^{-/-}$: 29.48±4.28%,$t_{24} = 1.454$, $p = 0.16$), or **F)** burying latency (*Trip8b*$^{+/+}$:280.57±30.5s, *Trip8b*$^{-/-}$: 211.00±23.76s, $t_{24} = 1.754$, $p = 0.09$). **G)** To determine if mice had a difference in remembering the shock probe context, the Change In Response was measured by taking the total time spent freezing and burying in the Recall Trial, subtracting the total time freezing and burying in the Acquisition Trial, and dividing by the total freezing and burying during the Acquisition Trial. *Trip8b*$^{+/+}$ and *Trip8b*$^{-/-}$ mice had similar Changes in Response from the Acquisition trial (*Trip8b*$^{+/+}$:0.18±0.05, *Trip8b*$^{-/-}$: -0.00±0.1, $t_{23} = 1.661$; $p > 0.05$; $n_{subjects} = 13, 12$), suggesting that a difference memory was unlikely to influence behavior. **H)** Endpoints for the SPBT were z-score normalized based on the wildtype population statistics and averaged to give a final z-score for the Acquisition and Recall trials. *Trip8b*$^{-/-}$ mice

showed increased active coping during the SPBT Acquisition Trial (*Trip8b*$^{+/+}$:-0.05±0.23, *Trip8b*$^{-/-}$: 0.92±0.12,t_{23} = 3.251, p < 0.01, $n_{subjects}$= 12, 13) **I)** but no statistically significant increase in active coping behavior in the Recall Trial (*Trip8b*$^{+/+}$:0±0.23, *Trip8b*$^{-/-}$: 0.47±0.24,t_{24} = 1.426, p = 0.17, $n_{subjects}$ = 14, 12)).

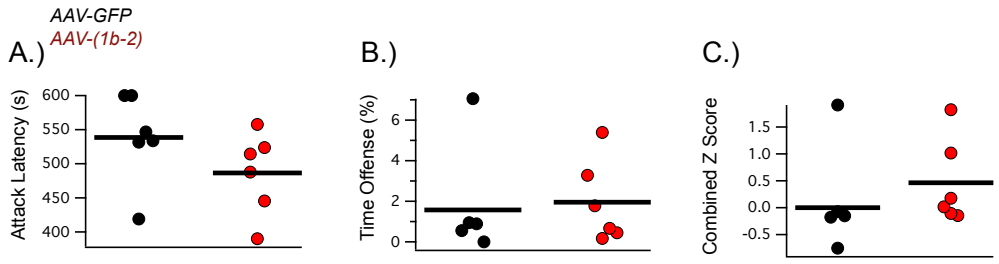


Supplementary Figure 3: *Trip8b*$^{-/-}$ mice show decreased passive coping in the repeated Forced Swim Test (rFST). (Related to Figure 1)
A) *Trip8b*$^{+/+}$ and *Trip8b*$^{-/-}$ mice were both able to learn to increase their passive coping over the three trials (Repeated Measures Two-Way ANOVA; effect of trial $F(2,50) = 27.30$, $p < 0.0001$; effect of genotype $F(1,25) = 11.58$, $p < 0.01$; and no interaction, $p > 0.05$)). Sidhak's Test confirmed immobility differences between the first and third trial for *Trip8b*$^{+/+}$ ($p < 0.0001$) and *Trip8b*$^{-/-}$ mice ($p < 0.001$). **B)** *Trip8b*$^{-/-}$ mice showed decreased average immobility time across the three rFST trials ($t_{25} = 3.256$, $p < 0.01$, $n_{subjects} = 14, 13$). **C)** *Trip8b*$^{-/-}$ mice also showed decreased immobility ($t_{24} = 4.413$, $p < 0.001$, $n_{subjects} = 13, 13$) during the last 4 minutes of the first rFST trial, representing the common antidepressant screening test used in previous reports.

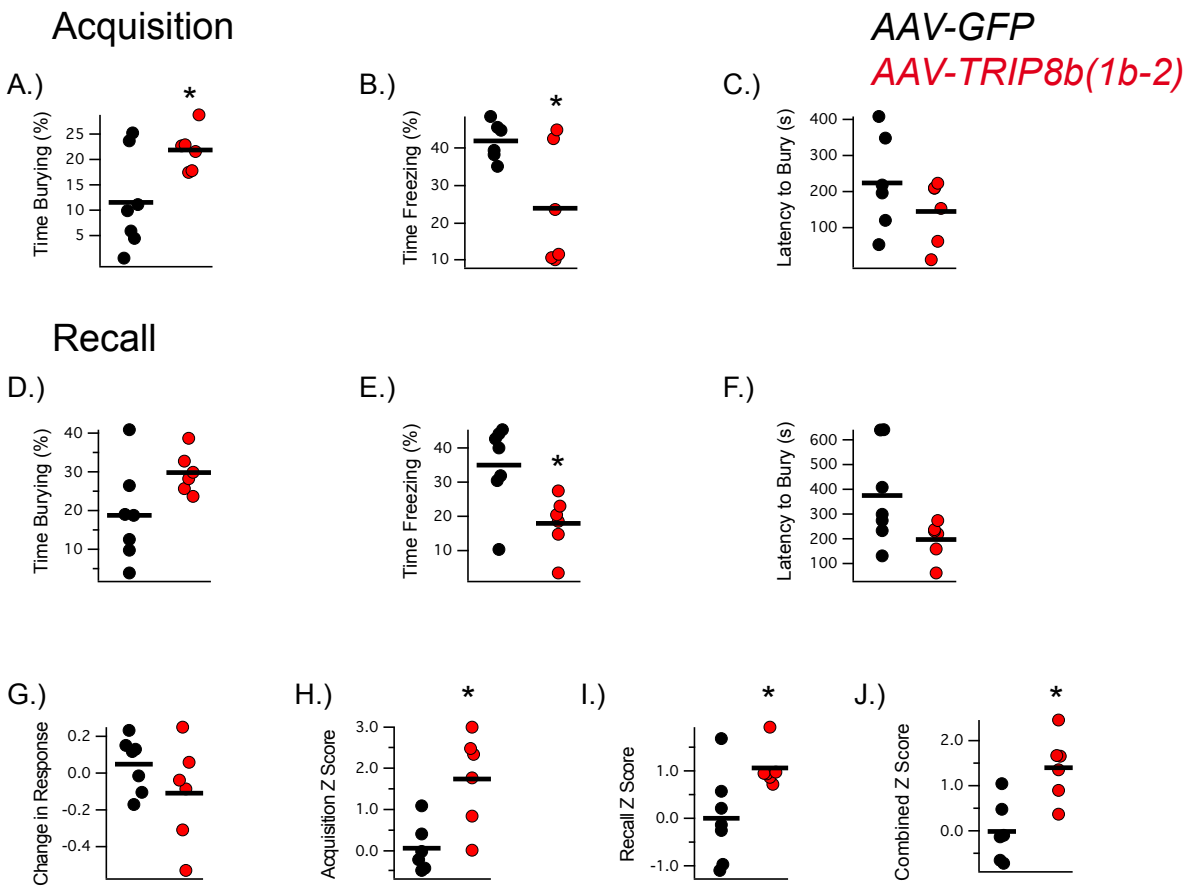


Supplementary Figure 4: *Trip8b*$^{-/-}$ mice perform similarly to *Trip8b*$^{+/+}$ mice on tasks measuring memory, locomotion, or anxiety-like behavior. (Related to Figure 1)

A) On the Open Field Test (OFT), *Trip8b*$^{+/+}$ and *Trip8b*$^{-/-}$ mice traveled similar distances in the arena (*Trip8b*$^{+/+}$:5621.29 ±240.91cm, *Trip8b*$^{-/-}$: 5661.81±221.57cm, t_{14} = 0.1238; p > 0.05). **B)** *Trip8b*$^{+/+}$ and *Trip8b*$^{-/-}$ mice also spent similar amounts of time in the center of the OFT arena (*Trip8b*$^{+/+}$:31.29±2.27%, *Trip8b*$^{-/-}$: 31.38±2.78%, t_{14} = 0.02502, p > 0.05, n = 8, 8) **C)** *Trip8b*$^{+/+}$ and *Trip8b*$^{-/-}$ mice spent similar amounts of time in the open arm of the Zero Maze (*Trip8b*$^{+/+}$:16.33±2.89%, *Trip8b*$^{-/-}$: 15.41±5.29%, t_{15} = 0.3636, p > 0.05, $n_{subjects}$= 8, 9).

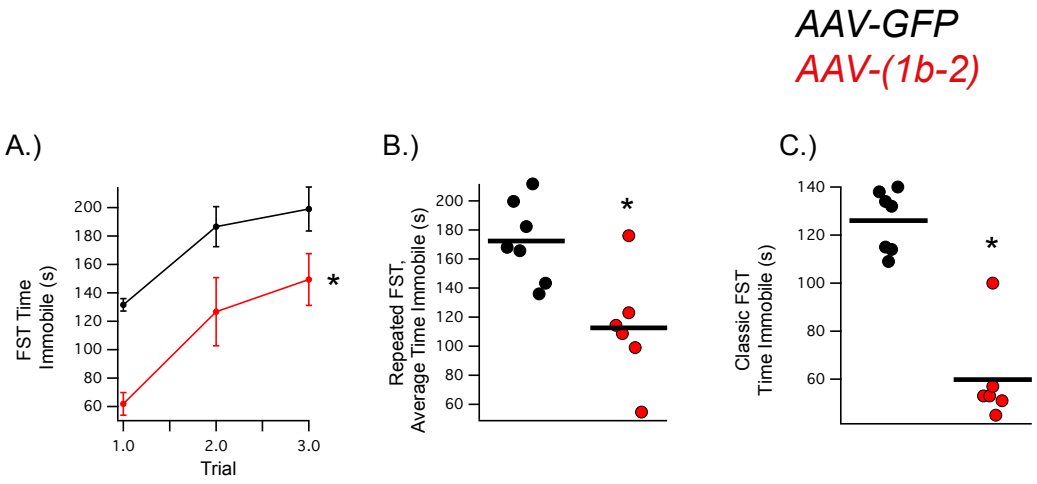


Supplementary Figure 5: AAV-(1b-2) mice did not show statistical differences from AAV-GFP mice on the Resident Intruder Test (RIT). (Related to Figure 5)
A) AAV-(1b-2) mice did not show a statistically significant difference in attack latency (AAV-GFP: 538.5±27.06s, AAV-(1b-2): 486.44±24.65s, $t_{10} = 1.422$, $p > 0.05$) or **B)** time displaying offensive aggression (AAV-GFP: 1.57±1.10%, AAV-(1b-2): 1.95±0.83% $t_{10} = 0.2739$, $p > 0.05$) compared to AAV-GFP mice.

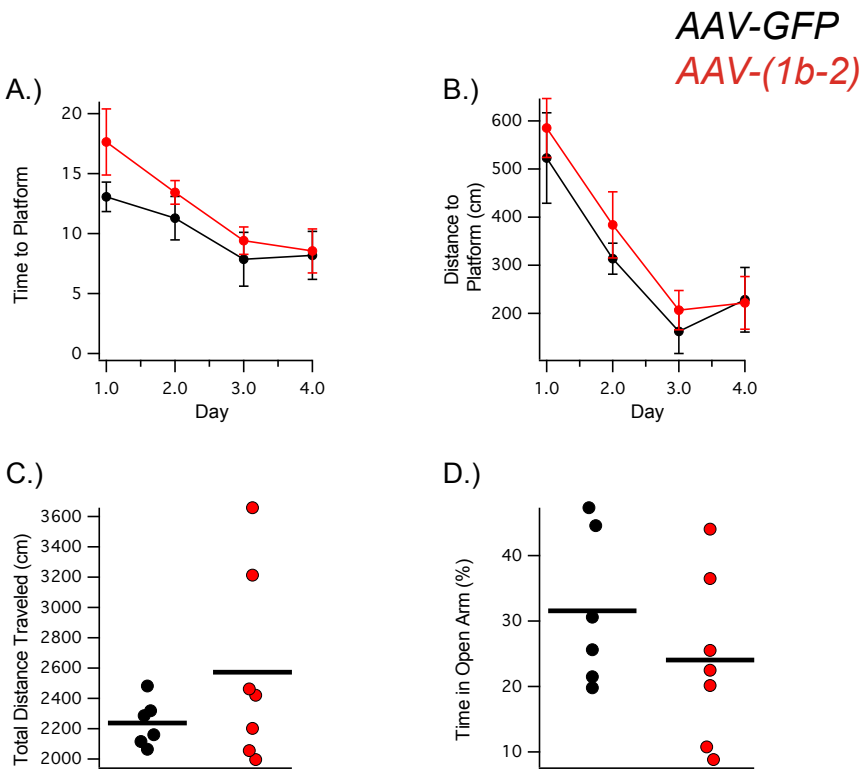


Supplementary Figure 6: AAV-(1b-2) mice show increased active and decreased passive coping in the Shock Probe Burying Task (SPBT). (Related to Figure 5) **A)** Mice were bilaterally injected with AAV-GFP or AAV-(1b-2) and tested for active coping behavior at peak viral expression 4 weeks after surgery. During the SPBT Acquisition Trial, AAV-(1b-2) mice spent more time burying (AAV-GFP: 11.53±3.58%, AAV-(1b-2): 21.85±1.69%, $t_{11} = 2.457$, $p < 0.05$, $n_{subjects} = 7, 6$). **B)** less time freezing (AAV-GFP: 41.85±2.06%, AAV-(1b-2): 23.98±6.53%, $t_{10} = 2.609$, $p < 0.05$, $n_{subjects} = 6, 6$), **C)** and no statistically significant difference in latency to bury (AAV-GFP: 223.83±54.8s, AAV-(1b-2): 144.66±36.2s, $t_{10} = 1.205$, $p > 0.05$) compared to AAV-GFP mice. **D)** On the SPBT Recall Trial, AAV-(1b-2) displayed a trend towards more burying (AAV-GFP: 18.76±4.6%, AAV-(1b-2): 29.81±2.19%, $t_{11} = 2.049$, $p = 0.06$), **E)** a significant decrease in time spent freezing (AAV-GFP: 34.96±4.66%, AAV-(1b-2): 17.94±3.37%, $t_{11} = 2.865$, $p < 0.05$), **F)** and a trend towards a decreased latency to burying (AAV-GFP: 375.14±75.23s, AAV-(1b-2): 197.16±31.02s, $t_{11} = 2.055$, $n_{subjects} = 7,6$, $p = 0.06$) compared to AAV-GFP mice. **G)** The Change in Response from the Acquisition to Recall Trial was similar between the two groups (AAV-GFP: 0.04±0.05, AAV-(1b-2): -0.10±0.11, $t_{11} = 1.315$, $p > 0.05$). **H)** Endpoints for the SPBT were z-score normalized based on the AAV-GFP population statistics and averaged to give a final z-score for the Acquisition and Recall trials. AAV-1b2 mice showed increased active coping during the Acquisition trial (AAV-GFP: 0.06±0.24, AAV-(1b-2): 1.73±0.45, $t_{10} =$

3.238, $p < 0.01$, $n_{subjects}$= 6, 6) and **I)** Recall Trials (AAV-GFP: 0.00±0.35, AAV-(1b-2): 1.06±0.17, $t_{11} = 2.508$, $p < 0.05$, $n_{subjects} = 7, 6$) compared to AAV-GFP mice.



Supplementary Figure 7: AAV-(1b-2) mice show decreased passive coping in the repeated Forced Swim Test (rFST). (Related to Figure 5)
A) AAV-GFP and AAV-(1b-2) mice were both able to learn to increase their passive coping over the three trials (Repeated Measures Two-Way ANOVA; effect of trial $F(2,22) = 41.31$, $p < 0.0001$; effect of genotype $F(1,11) = 10.32$, $p < 0.01$; and no interaction, $p > 0.05$; $n_{subjects} = 7, 6$)). Sidhak's Test confirmed immobility differences between the first and third trial for AAV-GFP ($p < 0.0001$) and AAV-(1b-2) ($p < 0.001$). **B)** AAV-(1b-2) mice showed decreased average immobility time across the three rFST trials (AAV-GFP: 172.38±10.48s, AAV-(1b-2): 112.61±16.00s $t_{11} = 3.212$, $p < 0.01$, $n_{subjects} = 7, 6$). **C)** AAV-(1b-2) mice also showed decreased immobility (AAV-GFP: 126.00±4.86s, AAV-(1b-2): 59.83±8.19s $t_{11} = 2.565$, $p < 0.05$, $n_{subjects} = 7, 6$) during the last 4 minutes of the first rFST trial, representing the common antidepressant screening test used in previous reports.



Supplementary Figure 8: AAV-(1b-2) mice perform similarly to AAV-GFP mice on tasks measuring memory, locomotion, or anxiety-like behavior (Related to Figure 5)

A) In the Morris Water Maze (MWM) hidden platform trials, Repeated Measures Two-Way ANOVA revealed an effect by trial for latency to reach the hidden platform but no effect for injection condition nor an interaction between trial x injection condition ($F_{Trial}(3,3) = 9.382$, $p = 0.0001$; $F_{Injection}(1,11) = 1.415$, $p > 0.05$; $F_{Interaction}(3,33) = 0.6612$, $p > 0.05$). **B)** Repeated Measures Two-Way ANOVA revealed an effect of trial for distance traveled to the hidden platform but no effects for injection condition or interaction ($F_{Trial}(3,33) = 16.89$, $p < 0.0001$; $F_{Injection}(1,11) = 0.7347$, $p > 0.05$; $F_{Interaction}(3,33) = 0.1822$, $p > 0.05$). During MWM probe trial, Two-way ANOVA yield a significant effect for Quadrant but not for Injection Condition nor an Interaction ($F_{Quadrant}(3,44) = 51.60$, $p < 0.0001$, $F_{Injection}(1,44) = 8.373 \times 10^{-8}$, $p > 0.05$, $F_{Interaction}(3,44) = 1.272$, $p > 0.05$).**C)** In the Open Field Test, AAV-(1b-2) and AAV-GFP mice traveled similar distances (AAV-GFP: 2237±63.11cm, AAV-(1b-2): 2572±237.16cm, $n_{subjects} = 6,7$, $t_{11}=-1.267$, $p > 0.05$) and spent similar amounts of time in the center of the arena (AAV-GFP: 14.84±3.16%, AAV-(1b-2): 12.74±2.07%, $n_{subjects} = 6,7$, $t_{11}=-0.57$, $p > 0.05$). **D)** AAV-GFP and AAV-(1b-2) mice spent similar amounts of time in the open arms of the Zero Maze (AAV-GFP: 31.55±4.80%, AAV-(1b-2): 24.02±4.83%, $n_{subjects} = 6,7$, $t_{11}=1.09$, $p > 0.05$).

2. SUPPLEMENTARY TABLES

Offensive Aggressive Behavior

	Lateral Threat	Upright	Clinch/Bite	Keep Down	Chase
Trip8b $^{+/+}$	1.1 (0.4)	0.8 (0.3)	1.6 (0.4)	1.7 (0.4)	0.5 (0.2)
Trip8b $^{-/-}$	3.3 (1.0)	0.9 (0.3)	3.7 (0.8)	3.2 (0.8)	1.0 (0.4)

Social Behavior

	Social Explore	AG Sniff	Social Groom	Mount
Trip8b $^{+/+}$	19.6 (1.8)	9.1 (1.2)	7.0 (1.6)	0.2 (0.2)
Trip8b $^{-/-}$	13.4 (1.7)	8.1 (1.8)	7.4 (2.2)	0.2 (0.1)

Exploratory Behavior

	Sniff	Rearing	Cage Exploration
Trip8b $^{+/+}$	15.3 (1.6)	3.8 (0.9)	26.8 (3.2)
Trip8b $^{-/-}$	10.6 (1.3)	4.5 (1.0)	30.2 (3.8)

Defensive/Submissive Behaviors

	On Back	Submissive Freeze	Being Groomed	Defensive Upright	Flee	Defensive Sideways
Trip8b $^{+/+}$	0 (0)	0 (0)	0.9 (0.3)	0 (0)	1.3 (0.5)	0 (0)
Trip8b $^{-/-}$	0 (0)	0 (0)	0.3 (0.2)	0 (0)	0.8 (0.3)	0 (0)

Other Behaviors

	Inactivity/ Rest	Groom	Digging
Trip8b $^{+/+}$	4.8 (1.3)	3.3 (0.4)	2.3 (0.9)
Trip8b $^{-/-}$	3.3 (1.0)	4.8 (1.0)	4.2 (1.7)

Behaviors by group

	Offensive Behavior	Social Behavior	Exploratory Behavior	Defensive/ Submissive Behaviors	Other Behaviors
Trip8b $^{+/+}$	5.6 (1.2)	35.9 (3.9)	45.9 (3.0)	2.2 (0.7)	10.4 (1.0)
Trip8b $^{-/-}$	12.1 (2.2)	29.1 (5.2)	45.3 (4.4)	1.2 (0.5)	12.3 (1.5)

Supplementary Table 1: Descriptive statistics for behaviors observed in the Resident Intruder Test for *Trip8b*$^{+/+}$ and *Trip8b*$^{-/-}$ animals (Related to Figure 1)
Values displayed represent percent of total time with standard error of the mean shown in parentheses.

Shock Probe Burying Test - Acquisition

	Burying	Freezing	Ambulation	Prod Exploring	Grooming	Rearing
Trip8b $^{+/+}$	7.9 (2.4)	63.4 (6.4)	20.4 (4.4)	5.4 (0.6)	1.4 (0.5)	1.7 (0.6)
Trip8b $^{-/-}$	15.3 (2.0)	35.1 (4.2)	35.9 (3.3)	6.9 (0.8)	4.9 (1.8)	1.9 (0.6)

Shock Probe Burying Test - Recall

	Burying	Freezing	Ambulation	Prod Exploring	Grooming	Rearing
Trip8b $^{+/+}$	17.6 (1.8)	39.1 (5.3)	30.1 (2.8)	6.9 (0.7)	2.6 (0.7)	3.7 (1.0)
Trip8b $^{-/-}$	19.6 (2.7)	29.5 (4.3)	33.9 (3.1)	7.1 (0.9)	5.7 (2.3)	4.2 (1.1)

Supplementary Table 2 Descriptive statistics for behaviors observed in the Shock Probe Burying Test for *Trip8b*$^{+/+}$ and *Trip8b*$^{-/-}$ animals (Related to Figure 1)
Values displayed represent percent of total time with standard error of the mean shown in parentheses.

Offensive Aggressive Behavior

	Lateral Threat	Upright	Clinch/Bite	Keep Down	Chase
AAV-GFP	0.2 (0.2)	0.3 (0.2)	0.6 (0.4)	0.2 (0.2)	0.2 (0.2)
AAV-(1b-2)	0.6 (0.4)	0.2 (0.1)	1.0 (0.3)	0.1 (0.1)	0.1 (0.0)

Social Behavior

	Social Explore	AG Sniff	Social Groom	Mount
AAV-GFP	21.1 (1.7)	9.0 (1.4)	14.5 (2.5)	0 (0)
AAV-(1b-2)	20.9 (2.1)	7.8 (2.0)	11.8 (1.2)	0 (0)

Exploratory Behavior

	Sniff	Rearing	Cage Explore
AAV-GFP	10.8 (1.9)	5.8 (1.3)	22.9 (3.9)
AAV-(1b-2)	11.8 (3.5)	7.0 (2.8)	24.2 (2.9)

Defensive/Submissive Behaviors

	On Back	Submissive Freeze	Being Groomed	Defensive Upright	Flee	Defensive Sideways
AAV-GFP	0.1 (0.1)	2.3 (1.2)	0.6 (0.3)	0.4 (0.3)	1.2 (0.5)	0.5 (0.2)
AAV-(1b-2)	0.1 (0.1)	1.5 (0.4)	1.1 (0.6)	0.5 (0.3)	1.3 (0.5)	0.6 (0.3)

Other Behaviors

	Inactivity/ Rest	Groom	Digging
AAV-GFP	2.7 (1.0)	3.0 (1.2)	3.7 (2.3)
AAV-(1b-2)	3.0 (0.9)	4.1 (1.5)	2.3 (0.6)

Behaviors by group

	Offensive Behavior	Social Behavior	Exploratory Behavior	Defensive/ Submissive Behaviors	Other Behaviors
AAV-GFP	1.6 (1.1)	44.6 (3.3)	39.5 (4.6)	5.0 (2.2)	9.3 (2.0)
AAV-(1b-2)	2.0 (1.0)	40.6 (3.3)	42.9 (3.8)	5.1 (1.6)	9.5 (2.1)

Supplementary Table 3: Descriptive statistics for behaviors observed in the Resident Intruder Test for AAV-GFP and AAV-(1b-2) treated animals (Related to Figure 5)
Values displayed represent percent of total time with standard error of the mean shown in parentheses.

Shock Probe Burying Test - Acquisition

	Burying	Freezing	Ambulation	Prod Exploring	Grooming	Rearing
AAV-GFP	13.4 (3.6)	41.9 (2.1)	30.6 (3.3)	7.3 (0.7)	3.2 (1.0)	3.4 (0.6)
AAV-(1b-2)	21.9 (1.7)	24.0 (6.5)	36.7 (4.6)	8.4 (1.5)	1.8 (0.9)	3.4 (1.2)

Shock Probe Burying Test - Recall

	Burying	Freezing	Ambulation	Prod Exploring	Grooming	Rearing
AAV-GFP	18.8 (4.6)	35.0 (4.7)	29.2 (1.6)	6.6 (1.0)	5.5 (1.1)	4.9 (1.1)
AAV-(1b-2)	29.8 (2.2)	17.9 (3.4)	34.4 (3.2)	6.7 (1.0)	4.5 (1.8)	5.4 (1.3)

Supplementary Table 4: Descriptive statistics for behaviors observed in the Shock Probe Burying Test for AAV-GFP and AAV-(1b-2) treated animals (Related to Figure 5)
Values displayed represent percent of total time with standard error of the mean shown in parentheses.

3. SUPPLEMENTARY METHODS

Immunohistochemistry

Mice were anesthetized with isoflurane and perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. Brains were then removed and fixed in 4% PFA overnight at 4^{o}C. After 48-72 hours, 30 µm coronal sections were made on a vibratome (Leica, Buffalo Grove, IL) at room temperature. Antigen retrieval was performed with 10 mM Na-citrate, pH 9.0, for 10 minutes at 80^{o}C, and the tissue was then allowed to cool for 30 minutes back to room temperature. Afterward, the tissue was blocked in PBS with 5% normal goat serum and 0.03% Triton X-100 for 1 hour at room temperature. Primary antibodies were diluted in blocking solution and applied overnight at 4^{o}C with gentle agitation. The next day, sections were washed 3 times in PBS with 0.03% Triton X-100 (PBS-T) prior to a 1 hour incubation at room temperature in secondary antibody, followed by 3 additional washes in PBS-T. 1mM DAPI was included in the final PBS-T wash, and tissue was then mounted on glass slides with PermaFluor (Thermo Fisher Scientific, Fremont, CA). All imaging was performed at the Northwestern University Center for Advanced Microscopy on a Nikon A1R confocal microscope using NIS Elements software (Nikon, Melville, NJ). Primary antibodies used were custom (Han et al. 2017) guinea pig anti-HCN1, guinea pig anti-HCN2, and rabbit anti-TRIP8b, and rabbit anti-GFP (RRID:AB_1587096, Millipore, Temecular, CA). All secondary antibodies were purchased from Invitrogen. For quantification of images, custom written routines in MATLAB (Mathworks, Natick, MA) were used, as in our previous report(Han et al. 2017). Briefly, regions of interest (ROI) were drawn over the stratum oriens (SO) and stratum pyramidale (SP). A larger ROI was also drawn over the region encompassing the stratum radiatum (SR) and stratum lacunosum moleculare (SLM) and then subdivided into ten equally spaced ROIs. The mean intensity of the staining within each ROI was then used for subsequent downstream analyses. Within each slice, the staining intensity of the injected hemisphere was divided by the intensity of the staining in the corresponding ROI from the contralateral (uninjected) hemisphere.

Antibodies

Primary antibodies used were custom(Chung *et al.* 2009, Lewis *et al.* 2009, Shin & Chetkovich 2007, Shin *et al.* 2006) guinea pig anti-HCN1, guinea pig anti-HCN2, and rabbit anti-TRIP8b, rabbit anti-MAP2 (RRID:AB_309685, Millipore Temecular, CA), mouse anti-tubulin (RRID:AB_570921, Millipore Temecular, CA), and rabbit anti-GFP (RRID:AB_1587096, Millipore, Temecular, CA). All secondary antibodies were purchased from Invitrogen.

Electrophysiology

Electrophysiology was performed as previously described(Han et al. 2017). Mice were anesthetized with isoflurane, decapitated, and the whole brain was rapidly dissected into ice-cold sucrose solution containing (in mM): 190 sucrose, 10 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 dextrose; pH 7.4 with continuous bubbling with 95% O2/5% CO_2. 300 µm sagittal slices were made using a vibratome (Leica) and immediately transferred to a 35^{o}C holding chamber containing ACSF (125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 25 dextrose; pH 7.4) for a 25 min incubation period. Afterward, the chamber was allowed to equilibrate to room temperature for ≥30 min before recording began. Individual slices were transferred to a custom chamber perfused with oxygenated, room temperature (22±1^{o}C) ACSF at a rate of 1-2 mL/min. Electrodes (4-6 MΩ) were pulled on a Sutter P87 pipette puller and filled with intracellular solution containing: 115 K-gluconate, 20 KCl, 10 HEPES, 10 Na- phosphocreatine, 2 Mg-ATP, 0.3 Na-GTP, 0.2% biocytin. KOH was added to pH 7.3. Whole-cell recordings were made with a PC-ONE amplifier (Dagan), filtered at 3 kHz, and digitized at 20 kHz using an InstruTECH ITC16. A calculated liquid junction potential of 13 mV was compensated prior to approaching each cell. Series resistance was monitored throughout each experiment, and cells were discarded if the series resistance exceeded 30 MΩ. Data acquisition and analysis was performed in IgorPro 6 (WaveMetrics) using custom macros. I_h density at -130 mV was obtained by subtracting the instantaneous current after the capacitive transient from the steady-state current at the end of a 2 s step. Current clamp recordings were performed with a holding current to maintain cells at -70mV.

Western Blotting

Western blotting was performed as previously described(Han et al. 2017). Primary antibodies used were: custom rabbit anti-HCN1, rabbit anti-HCN2, and guinea pig anti-TRIP8b, rabbit anti-MAP2 (Millipore Temecular, CA), and mouse anti-tubulin (Millipore Temecular, CA). Primary antibodies were diluted in blocking solution containing 5% milk and 0.1% Tween-20 in TBS (TBS-T). Band intensities were quantified using ImageStudio (Li-Cor, Lincoln, NE) software and normalized to the anti-tubulin signal for each sample.

4. SUPPLEMENTARY REFERENCES

Chung, W. K., Shin, M., Jaramillo, T. C. et al. (2009) Absence epilepsy in apathetic, a spontaneous mutant mouse lacking the h channel subunit, HCN2. *Neurobiology of Disease,* **33,** 499-508.

Lewis, A. S., Schwartz, E., Savio Chan, C. et al. (2009) Alternatively Spliced Isoforms of TRIP8b Differentially Control H Channel Trafficking and Function. *J. Neurosci.,* **29,** 6250-6265.

Han, Y., Heuermann, R. J., Lyman, K. A., Fisher, D., Ismail, Q. A. and Chetkovich, D. M. (2017) HCN-channel dendritic targeting requires bipartite interaction with TRIP8b and regulates antidepressant-like behavioral effects. *Molecular Psychiatry,* **22,** 458-465.

Peña, C. J., Kronman, H. G., Walker, D. M. et al. (2017) Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science,* **356,** 1185-1188.

Shin, M. and Chetkovich, D. M. (2007) Activity-dependent Regulation of h Channel Distribution in Hippocampal CA1 Pyramidal Neurons. *J. Biol. Chem.,* **282,** 33168-33180.

Shin, M., Simkin, D., Suyeoka, G. M. and Chetkovich, D. M. (2006) Evaluation of HCN2 abnormalities as a cause of juvenile audiogenic seizures in Black Swiss mice. *Brain Research,* **1083,** 14-20.