
Figures and figure supplements

MicroRNA-138 controls hippocampal interneuron function and short-term memory in mice

Reetu Daswani et al

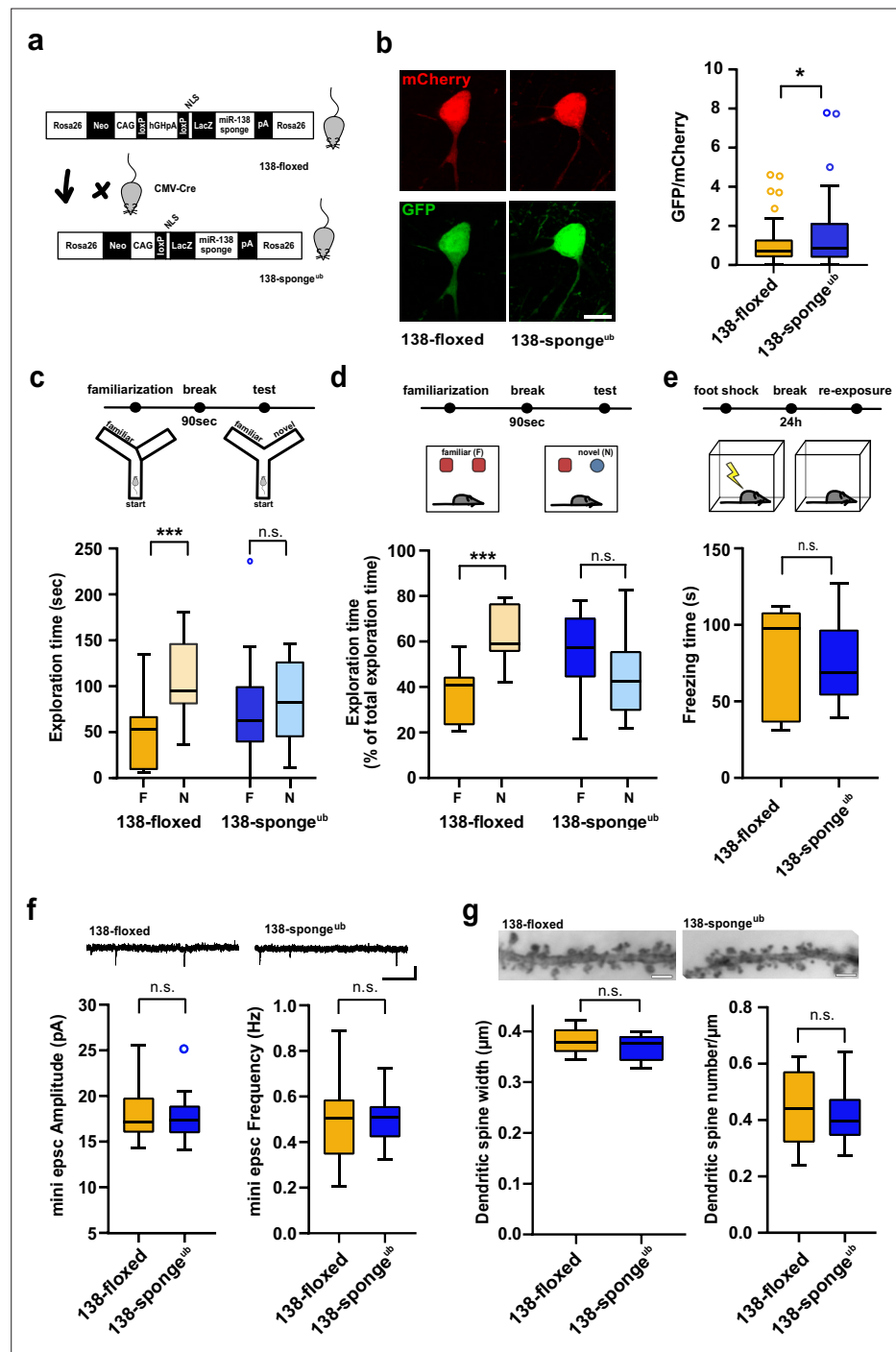


Figure 1. Impaired working memory in ubiquitous 138-sponge mice. **(a)** Schematic overview of the strategy for generating 138-sponge^{ub} mice. **(b)** Left: representative images of mCherry and GFP expression in hippocampal neurons from 138-floxed and 138-sponge^{ub} mice, respectively. Right: bar graphs of GFP/mCherry ratios from CA1 hippocampal neurons infected with a 138-pbds sensor construct; 138-floxed: n=105 cells from two mice, 138-sponge^{ub}: n=127 cells from three mice; p=0.02 (KS-test). **(c)** Upper panel: schematic representation of the Y-maze novelty preference task; lower panel: exploration time spent in familiar (F) and novel (N) arm; 138-floxed: n=12 mice; 138-sponge^{ub}: n=14 mice; ***p=0.005; n.s.=0.711 (Student's two-tailed heteroscedastic t-test). **(d)** Upper panel: schematic representation of the novel object recognition task; lower panel: exploration time presented as percentage of total time spent with either novel or familiar object; 138-floxed: n=12 mice; 138-sponge^{ub}: n=12 mice; ****p<0.00002; n.s. p=0.11 (Student's two-tailed heteroscedastic t-test). **(e)** Upper: schematic representation of the contextual fear conditioning task; lower: time (s) mice spent freezing 24 hr after

Figure 1 continued on next page

Figure 1 continued

the foot shock was administrated; 138-floxed: n=7 mice; 138-sponge^{ub}: n=7 mice; n.s. p=0.97 (Student's two-tailed heteroscedastic t-test). (f) mEPSC recording in CA1 pyramidal neurons. Upper panel: example traces; scale bar: 20 pA, 500 ms. Lower panel left: mEPSC amplitude (138-floxed: range, from 14.3 to 25.6 pA; median, 17.2 pA; interquartile range [IQR], 3.9 pA. 138-sponge^{ub}: range, from 14.1 to 25.2 pA; median, 17.4 pA; IQR, 3.1 pA; n.s. p=0.74 Student's two-tailed heteroscedastic t-test). Lower panel right: mEPSC frequency (138-floxed: range, from 0.2 to 0.9 Hz; median, 0.5 Hz; IQR, 0.2 Hz. 138-sponge^{ub}: range, from 0.3 to 0.7 Hz; median, 0.5 Hz; IQR, 0.1 Hz; n.s. p=0.91 Student's two-tailed heteroscedastic t-test). 138-floxed: n=13 cells/4 mice; 138-sponge^{ub}: n=13 cells/3 mice. (g) Upper panel: representative images of Golgi-stained CA1 pyramidal neuron dendritic segments of the indicated genotypes. Lower panel: quantification of dendritic spine width (left) and density (number/ μm ; right) based on Golgi staining; 138-floxed: n=15 cells/3 mice (1312 spines total); 138-sponge^{ub}: n=18 cells/3 mice (1687 spines total) (n.s., p=0.25 (width); p=0.49 (density); Student's two-tailed heteroscedastic t-test). mEPSC, miniature excitatory postsynaptic current.

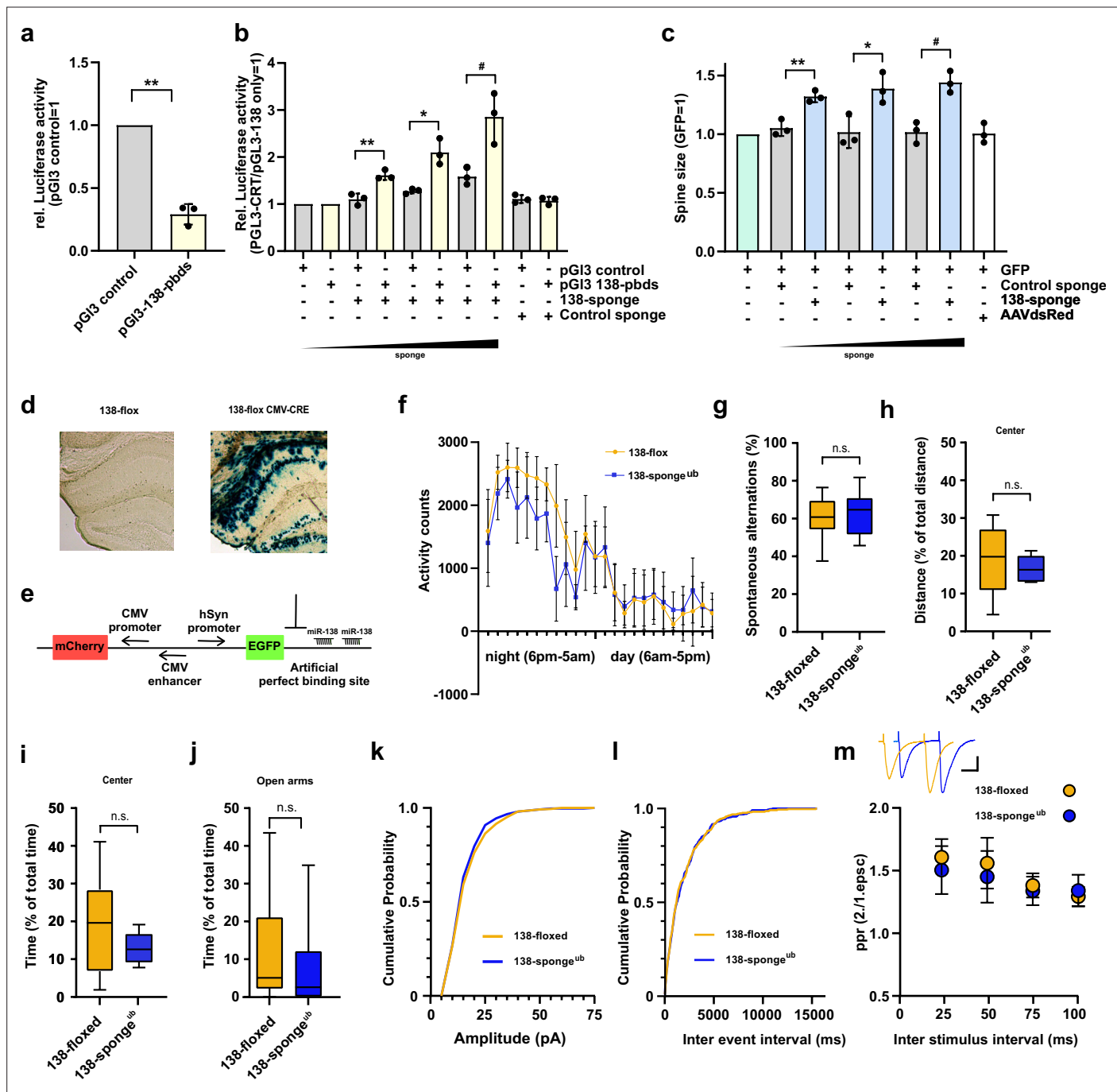


Figure 1—figure supplement 1. Validation, behavioural and electrophysiological characterization of ubiquitous miR-138 sponge mice. **(a)** Relative luciferase activity in hippocampal neurons (DIV 12–17) transfected with pGL3 CTR (control) or pGL3-138 pbds (sensor) constructs. pGL3-CTR=1. n=3 independent transfections. **p=0.0013 (One sample t-test). **(b)** Relative luciferase activity in hippocampal neurons (DIV 12–17) transfected as in **(a)**, in addition with either control (100 ng) or increasing amounts of 138 sponge (25–100 ng). pGL3-CTR/pGL3-138 only=1. n=3 independent transfections, **p=0.005, *p=0.032, #p=0.045 (Student's two-tailed heteroscedastic t-test). **(c)** Quantification of relative dendritic spine volume in rat hippocampal neurons (DIV10–18) transfected with GFP and increasing amounts (25–100 ng) of either control or 138 sponge. GFP only=1. n=3 independent transfections; each value represents at least 150 spines per cell, from six individual neurons per experiment. **p=0.006, *p=0.027, #p=0.004 (Student's two-tailed heteroscedastic t-test). **(d)** Representative enzymatic b-Gal staining of hippocampal slices obtained from 138-floxed mice (P21) injected with either saline (left) or rAAV-CMV-CRE (right). **(e)** Schematic of the sensor principle. **(f)** Activity counts of mice from indicated genotypes monitored over 24 hr in their home cage; 138-floxed: n=7 mice, 138-sponge^{ub}: n=7 mice; data shown as mean \pm s.d. **(g)** Percentage of spontaneous alternations in the Y-Maze. 138-floxed: n=14 mice; 138-sponge^{ub}: n=14 mice; p=0.43 (Student's two-tailed heteroscedastic t-test). **(h, i)** Percentage of total distance travelled **(h)**/time spent **(i)** in the center of an open field arena during 30 min exploration by mice of the indicated genotypes; 138-floxed: n=14 mice;

Figure 1—figure supplement 1 continued on next page

Figure 1—figure supplement 1 continued

138-sponge^{ub}: n=14 mice; (h) p=0.30, (i) p=0.054 (Student's two-tailed heteroscedastic t-test). (j) Percentage of total time spent in the open arms of an elevated plus maze (EPM) during 5 min exploration by mice of the indicated genotypes; 138-floxed: n=14 mice; 138-sponge^{ub}: n=14 mice; p=0.50 (Mann-Whitney test). (k, l) Cumulative distribution mEPSC amplitude (p=0.65; Kolmogorov-Smirnov [KS] test) (k) and frequency (p=0.85; KS test) (l). (m) PPR of stimulated EPSCs in CA1 pyramidal neurons. Upper panel: example traces of PPR (inter stimulus interval of 50 ms) for 138-floxed (orange) and 138-sponge^{ub} (blue); scale bar: 100 pA, 20 ms. Lower panel: PPRs for different interstimulus intervals ranging from 25 ms to 100 ms (138-floxed vs. 138-sponge^{ub} [mean \pm s.d.]: 25 ms: 1.6 \pm 0.1 vs. 1.5 \pm 0.2 [n=13]; 50 ms: 1.6 \pm 0.2 vs. 1.5 \pm 0.2 [n=13]; 75 ms: 1.4 \pm 0.1 vs. 1.3 \pm 0.1 [n=11]; 100 ms: 1.3 \pm 0.1 vs. 1.3 \pm 0.1 [n=11]. n.s. p=0.14, 0.09, 0.17, and 0.13 for 25, 50, 75, and 100 ms inter stimulus intervals, respectively. Mann-Whitney test. PPR, paired-pulse ratio.

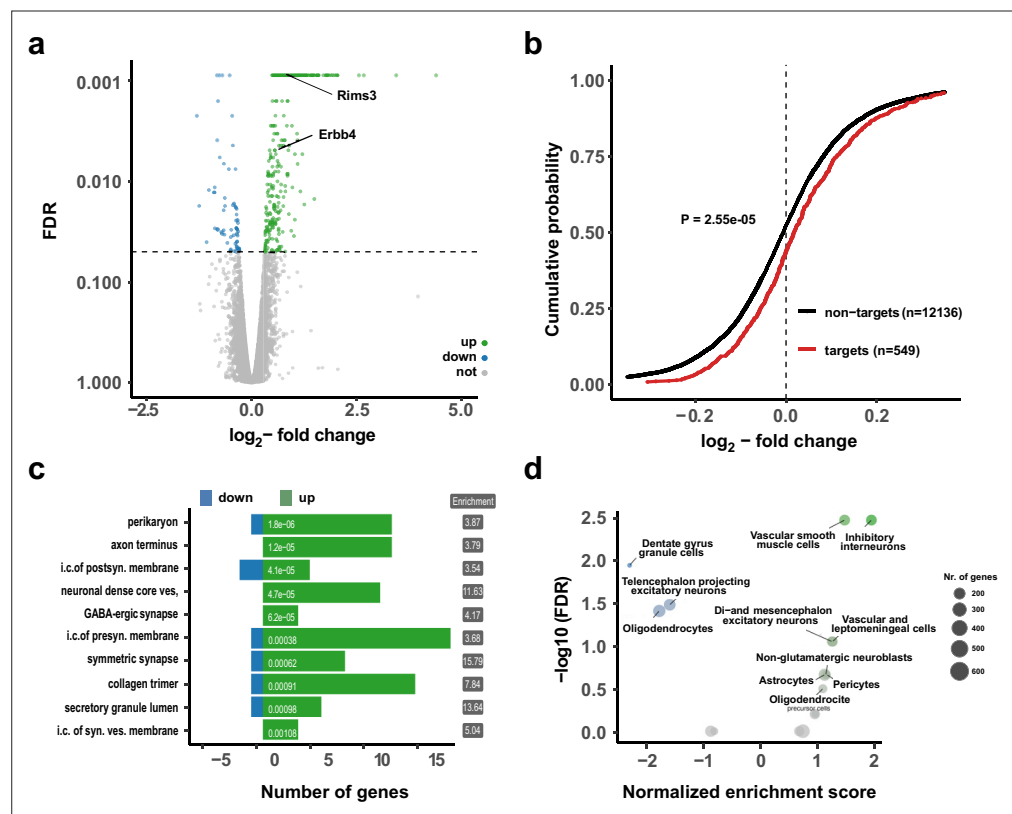


Figure 2. Upregulation of interneuron-enriched synaptic genes in ubiquitous miR-138 sponge mice. **(a)** Volcano plot of differentially expressed genes (DEGs) obtained from polyA-RNAseq of total hippocampal RNA from 138-flox and 138-sponge^{ub} mice. N=3. Genes with FDR < 0.05 are labeled blue (downregulated) or green (upregulated). Rims3 and Erbb4 are indicated. **(b)** Cumulative distribution plots of \log_2 -fold expression changes (138-sponge^{ub}/138-floxed) for genes either containing (targets, red curve) or not containing (non-targets, black curve) predicted miR-138 binding sites. $p=2.55e-05$ (KS-test). **(c)** Gene ontology (GO) term analysis for DEGs. Top ten enriched cellular component (CC) GO terms with less than 200 total genes are shown. **(d)** Enrichment analysis of DEGs in different brain cell types based on published single-cell RNA-seq data (Zeisel et al., 2018). Normalized enrichment score > 0: upregulated in 138-sponge^{ub} mice. KS, Kolmogorov-Smirnov.

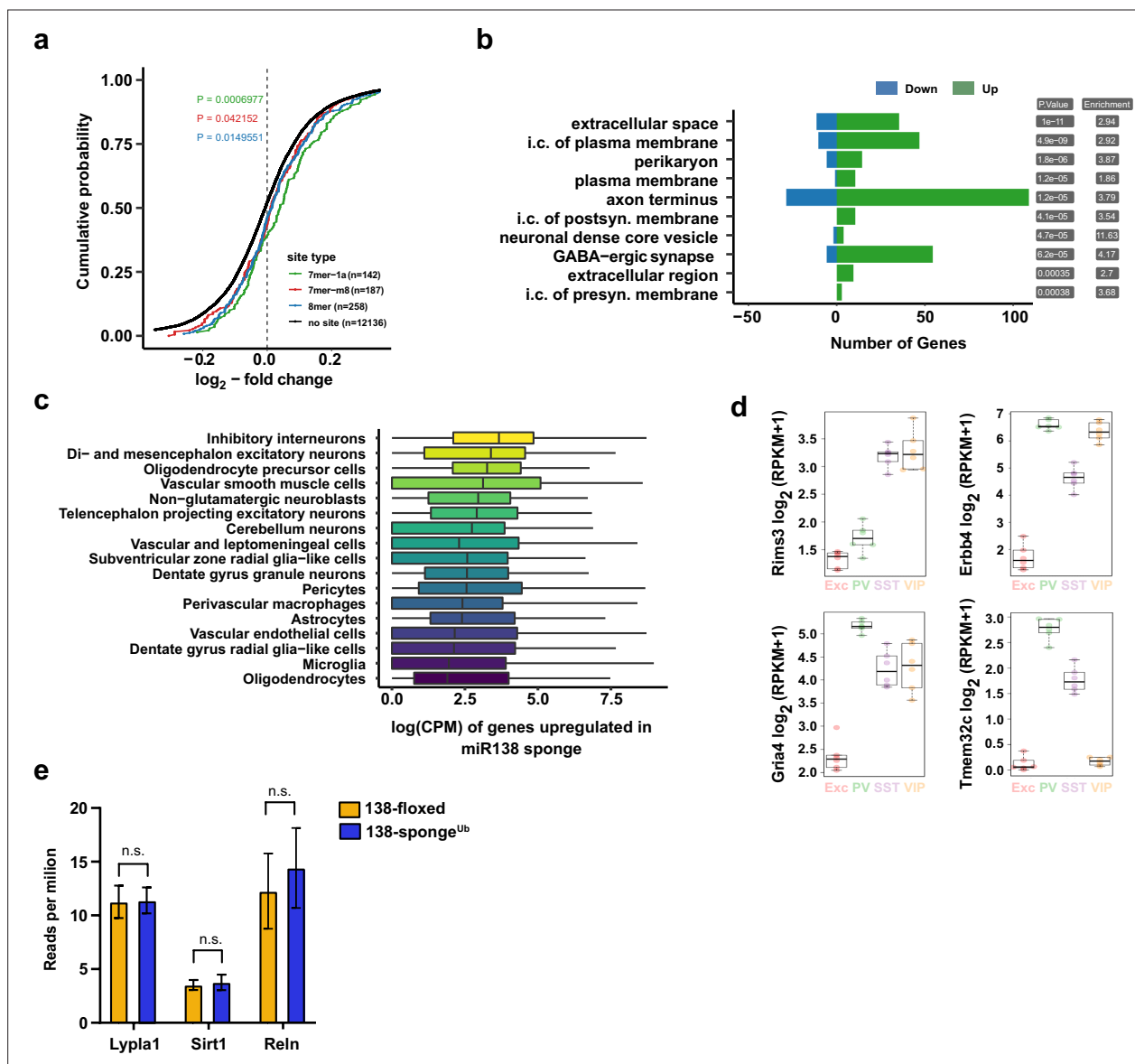


Figure 2—figure supplement 1. Gene expression analysis in ubiquitous miR-138 sponge mice. **(a)** Cumulative distribution plots of \log_2 -fold expression changes (138-sponge^{ub}/138-floxed) for genes either containing 7mer-1a (green), 7mer-m8 (red), 8mer (blue), or no (no site, black curve) predicted miR-138 binding sites. P value is calculated compared to the no site population and indicated in the graph (KS-test). **(b)** Gene ontology (GO) term analysis for DEGs. Top ten enriched cellular component (CC) GO terms are shown. **(c)** Normalized expression levels, in single-cell clusters from **Zeisel et al., 2018**, of the genes upregulated in the miR-138 sponge. The genes are most abundantly expressed in inhibitory neurons. **(d)** Expression plots of selected miR-138 binding site containing transcripts in different neuronal subtypes. EXC: excitatory neurons; PV: parvalbumin+interneurons; SST: somatostatin +interneurons; VIP: Vasoactive intestinal peptide+interneurons based on <http://research-pub.gene.com/NeuronSubtypeTranscriptomes/>. **(e)** Expression of validated miR138-5p targets based on RNA-seq in 138-floxed and 138-sponge^{ub} mice. n=3 mice. Lypla1 n.s. p=0.90; Sirt1 n.s. p=0.66; Reln n.s. p=0.50 (Student's two-tailed heteroscedastic t-test). DEG, differentially expressed gene; KS, Kolmogorov-Smirnov.

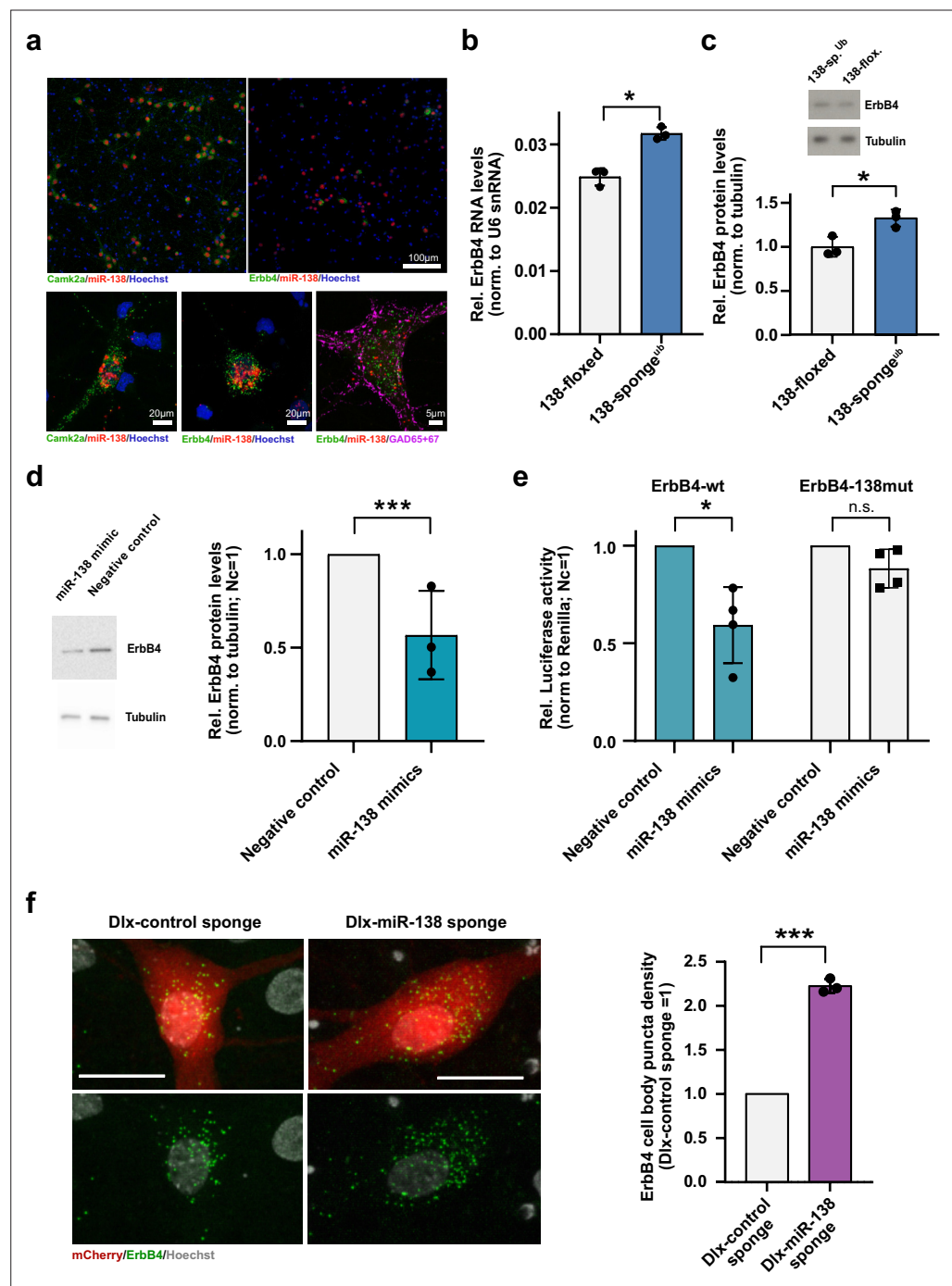


Figure 3. *Erbb4* is a direct miR-138 target in rat hippocampal interneurons. (a) Single-molecule (Sm) FISH analysis of miR-138 (red) together with *Camk2a* or *Erbb4* mRNA to label excitatory or inhibitory neurons, respectively. Hoechst was used to counterstain nuclei. GAD65/67 antibody staining was used to identify GABAergic neurons. Scale bar = 100 μ m (upper); 20 μ m (lower left and center), 5 μ m (lower right). (b) qPCR analysis of *Erbb4* mRNA in total hippocampal RNA obtained from 138-floxed or 138-sponge^{ub} mice. U6 snRNA was used for normalization. n=3 mice; *p=0.003, (Student's two-tailed heteroscedastic t-test). (c, d) Western blot analysis of *Erbb4* protein in hippocampal lysates from 138-floxed or 138-sponge^{ub} mice (c) or lysates from rat hippocampal neurons (DIV12) transfected with miR-138 or control mimic (d). Tubulin was used for normalization. (c) n=3 mice; *p=0.025 (Student's two-tailed heteroscedastic t-test); (d) n=3 independent transfections; ***p=0.0009 (one sample t-test). (e) Relative luciferase activity in rat cortical neurons (DIV9-12) transfected with *Erbb4* 3'UTR constructs with (138mut) or without (wt) a mutation in the miR-138 binding site, together with miR-138 or negative control mimics. Negative control

Figure 3 continued on next page

Figure 3 continued

mimic =1. n=4 independent transfections, *p=0.025, n.s. p=0.09 (Student's two-tailed heteroscedastic t-test). (f) Sm FISH analysis of *ErbB4* (green) in rat hippocampal interneurons infected with Dlx-control-sponge or miR-138-sponge. Left panel: representative neurons, scale bar =20 μ m. Green: *ErbB4* FISH; gray: DAPI (nuclei); red: mCherry (*Dlx5/6* expressing interneurons). Right panel: *ErbB4* FISH quantification. Control sponge =1. N=3 independent infections (10–12 cells per condition), ***p=0.0004 (one-sample t-test).

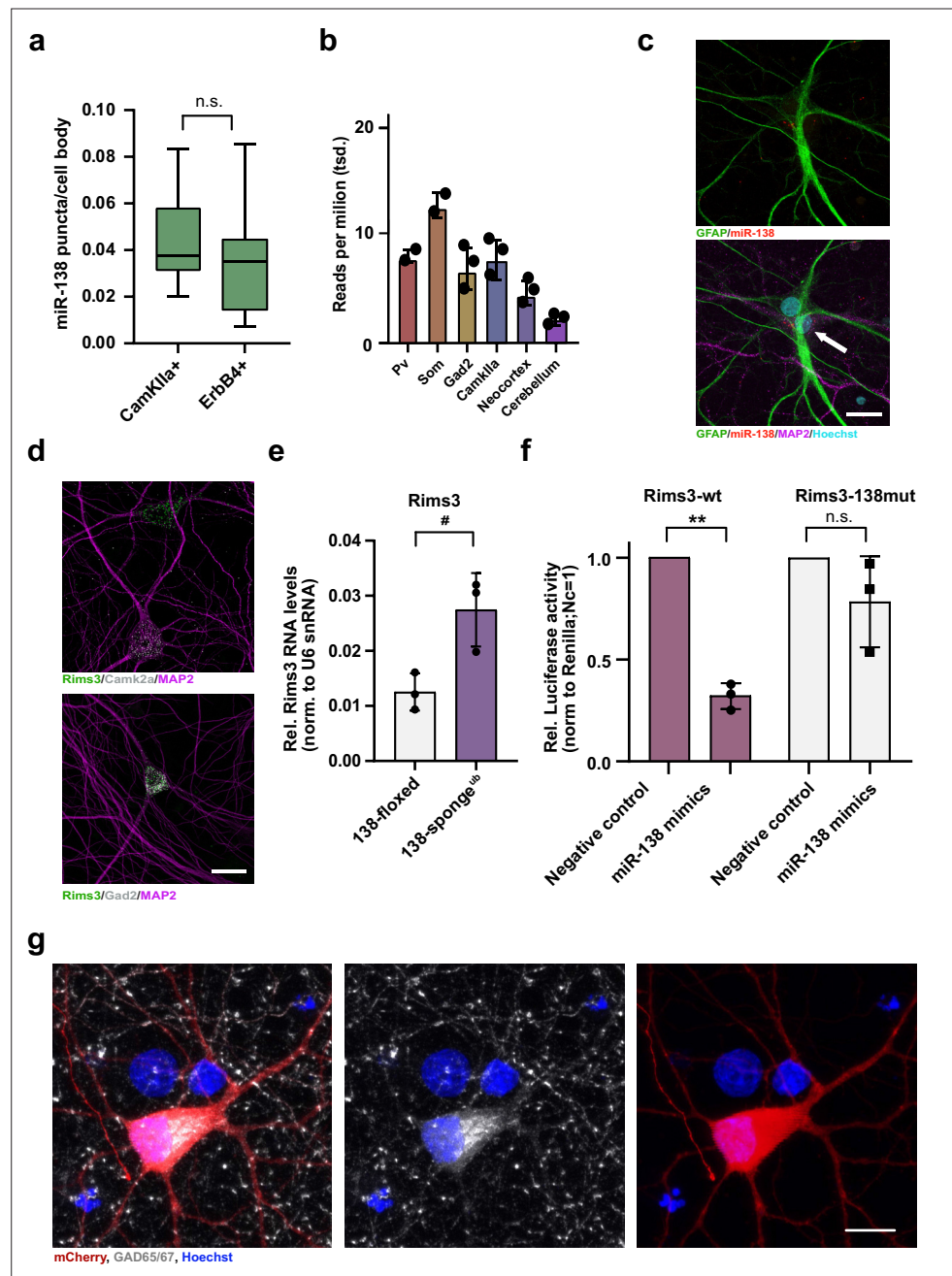


Figure 3—figure supplement 1. Validation of miR-138 target genes in rat hippocampal interneurons. **(a)** Quantification of *miR138-5p* smFISH in Camk2a- and ErbB4-positive neurons. Camk2a-positive n = 10 cells; ErbB4-positive n = 7 cells, p = 0.48 (Student's two-tailed heteroscedastic t-test). **(b)** Quantification of *miR138-5p* levels in mouse brain tissue based on small RNA-seq data from He et al., 2012. **(c)** Single-molecule FISH analysis of miR-138 (red) together with GFAP antibody stain (green) to label glia cells. Hoechst was used to counterstain nuclei. Arrow points to miR-138/MAP2 positive, GFAP-negative neuron adjacent to glial cell. Scale bar = 20 μ m. **(d)** Single-molecule FISH analysis of *Rims3* mRNA (green) together with *Camk2a* (gray; left) or *Gad2* (gray; right) mRNA to label glutamatergic and GABAergic neurons, respectively. Scale bar = 20 μ m. **(e)** qPCR analysis of *Rims3* mRNA in total hippocampal RNA obtained from 138-floxed or 138-sponge^{ub} mice. U6 snRNA was used for normalization. n = 3 mice; *p = 0.04 (Student's two-tailed heteroscedastic t-test). **(f)** Relative luciferase activity in rat cortical neurons (DIV9-12) transfected with *Rims3* 3'UTR constructs with (138mut) or without (wt) a mutation in the miR-138 binding site, together with miR-138 or negative control mimics. Negative control mimic = 1. n = 3 independent transfections, *p = 0.002, n.s. p = 0.23 (Student's two-tailed heteroscedastic t-test). **(g)** Representative picture of primary rat hippocampal neurons infected with Dlx5/6-mCherry-138 sponge (red) and stained for GAD65/67 (gray) and Hoechst (blue). Scale bar = 10 μ m. smFISH, single-molecule fluorescence in situ hybridization.

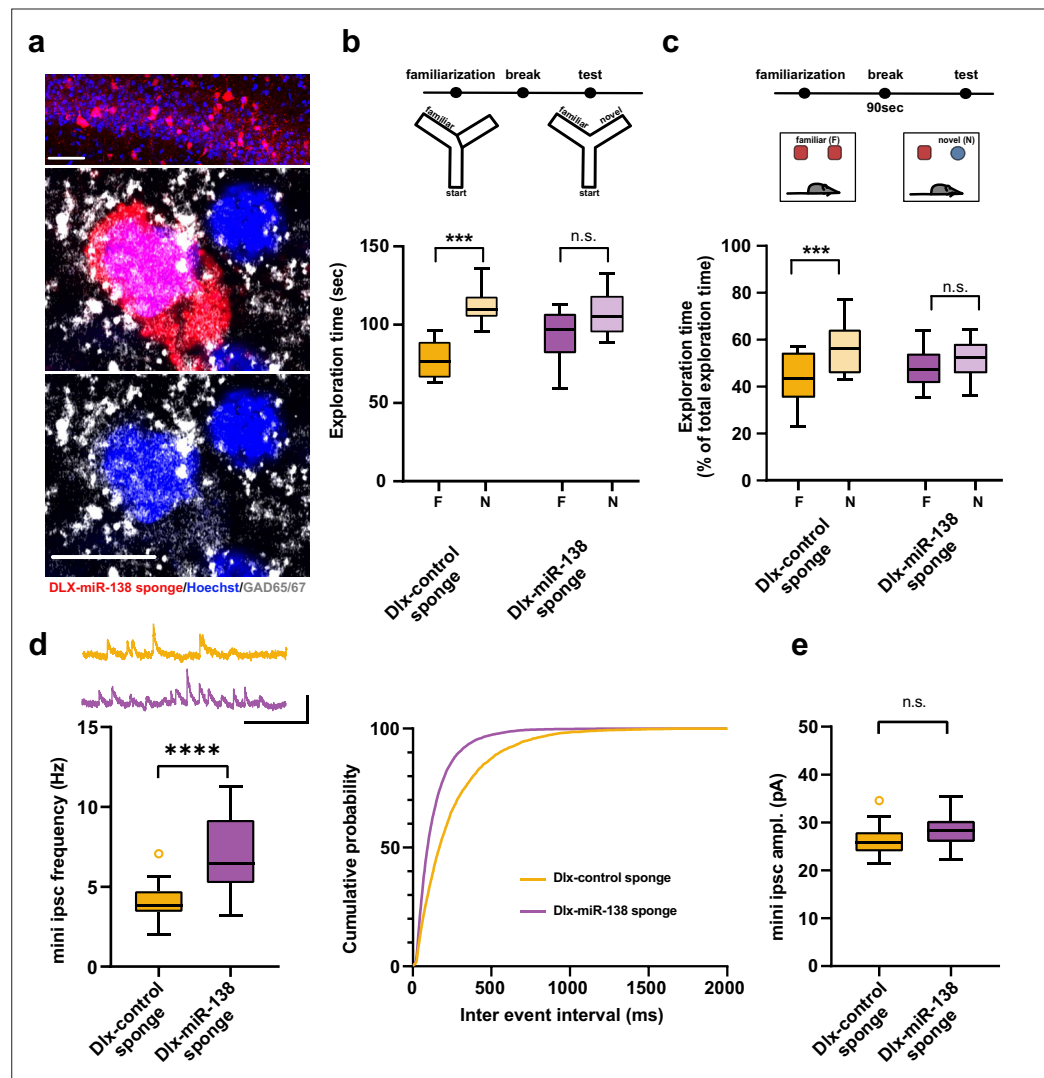


Figure 4. Impaired short-term memory and inhibitory synaptic transmission upon miR-138 inhibition in hippocampal interneurons. **(a)** Representative pictures of hippocampal interneurons in vivo infected with rAAV-Dlx-138-sponge. Upper panel: infected interneurons in hippocampal area CA1. Scale bar = 50 μ m. Middle and lower panels: neurons in hippocampal area CA1 at higher magnification. Left neuron: infected with rAAV-Dlx-138-sponge, expressing GAD65/67; right neuron: not infected, no GAD65/67 expression. Scale bar = 10 μ m. red: mCherry; gray: GAD65/67, blue: Hoechst nuclei. **(b)** Upper panel: schematic representation of the Y-maze novelty preference task; lower panel: exploration time spent in familiar (F) and novel (N) arm; Dlx-control sponge n=8 mice; Dlx-miR-138 sponge n=9 mice; ****p=0.00005; n.s.=0.079 (Student's two-tailed heteroscedastic t-test). **(c)** Upper panel: schematic representation of the novel object recognition task; lower panel: exploration time presented as percentage of total time spent with either novel or familiar object; Dlx-control sponge n=9 mice; Dlx-miR-138 sponge n=10 mice; *p=0.03; n.s.=0.33 (Student's two-tailed heteroscedastic t-test). **(d)** mIPSC frequency in CA1 pyramidal neurons. Upper panel left: example traces, Dlx-control sponge in orange, Dlx-miR-138 sponge in purple, scale bar: 50 pA, 200 ms. Lower panel left: mIPSC frequency (Dlx-control sponge: range, from 2.0 to 7.1 Hz; median, 3.8 Hz; IQR, 1.3 Hz. Dlx-miR-138 sponge: range, from 3.2 to 11.3 Hz; median, 6.4 Hz; IQR, 4.0 Hz; ****p<0.0001, Student's two-tailed heteroscedastic t-test). Right panel: Cumulative distribution mIPSC frequency (p<0.0001; Kolmogorov-Smirnov test). **(e)** mIPSC amplitude in CA1 pyramidal neurons (Dlx-control sponge: range, from 21.5 to 34.6 pA; median, 25.9 pA; IQR, 4.0 pA. Dlx-miR-138 sponge: range, from 22.3 to 35.5 pA; median, 28.3 pA; IQR, 4.4 pA; n.s. p=0.13 Student's two-tailed heteroscedastic t-test). Dlx-control sponge n=19 cells/2 mice; Dlx-miR-138 sponge n=19 cells/2 mice. IQR, interquartile range; mIPSC, miniature inhibitory postsynaptic current.

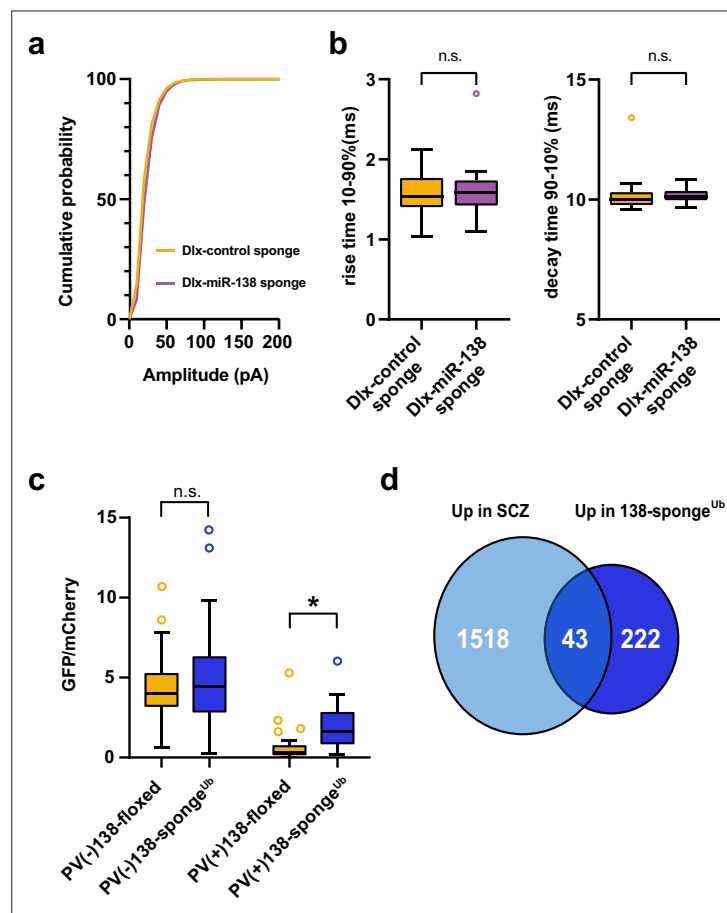


Figure 4—figure supplement 1. Inhibitory synaptic transmission upon miR-138 inhibition in hippocampal interneurons and validation of miR-138 sponge activity in PV-expressing hippocampal interneurons. **(a)** Cumulative distribution of mIPSC amplitude ($p < 0.0001$; Kolmogorov-Smirnov test). **(b)** mIPSC rise (10%–90%) and decay (90%–10%) time. Left panel: mIPSC rise (10%–90%) time (Dlx control sponge: range, from 1.0 to 2.1 ms; median, 1.5 ms; IQR, 0.4 ms. Dlx miR-138 sponge: range, from 1.1 to 2.8 ms; median, 1.6 ms; IQR, 0.3 ms; n.s. $p = 0.76$ Mann-Whitney test). Right panel: mIPSC decay (90%–10%) time (Dlx control sponge: range, from 9.6 to 13.4 ms; median, 10.0 ms; IQR, 0.6 ms. Dlx miR-138 sponge: range, from 9.7 to 10.8 ms; median, 10.1 ms; IQR, 0.4 ms; n.s. $p = 0.3$ Mann-Whitney test). Dlx control sponge $n = 19$ cells/2 mice; Dlx miR-138 sponge $n = 19$ cells/2 mice. **(c)** Bar graphs of GFP/mCherry ratios from PV+ or PV– CA1 hippocampal neurons in 138-floxed or 138-sponge^{Ub} mice infected with a 138-pbds sensor construct; 138-floxed/PV+: $n = 32$ (two mice); 138-floxed/PV–: $n = 40$ (two mice); 138-sponge/PV+: $n = 32$ (three mice); 138-sponge/PV–: $n = 42$ (three mice); n.s. $p = 0.113$; $*p = 3.6 \times 10^{-6}$ (Kolmogorov-Smirnov-Test). **(d)** Venn diagram describing the overlap between transcripts upregulated in SCZ patients (Gandal et al., 2018) and miR-138 sponge mice (Figure 2a). Fold enrichment = 1.43; $p = 0.00859$ (Fisher test). IQR, interquartile range; mIPSC, miniature inhibitory postsynaptic current; SCZ, schizophrenia.

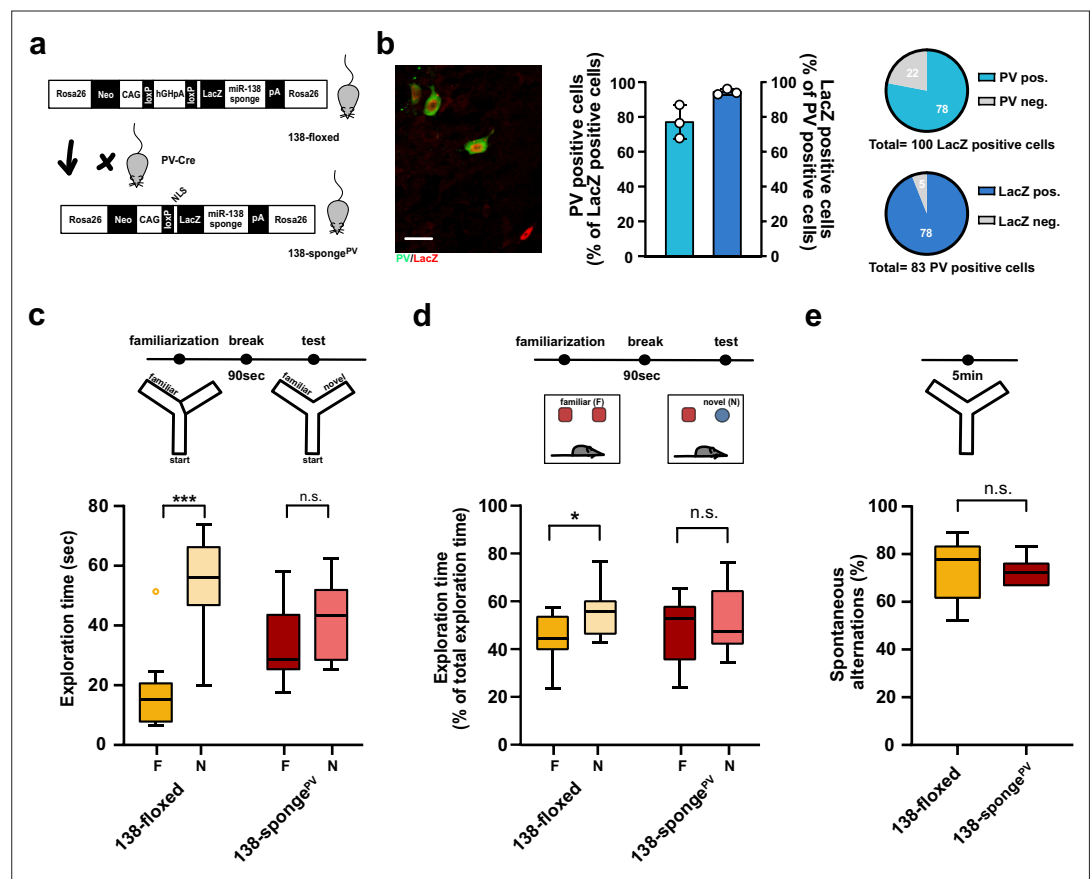


Figure 5. Impaired short-term memory in PV-expressing interneuron specific miR-138 sponge mice. **(a)** Schematic overview of the strategy for generating miR-138 sponge^{PV} mice. **(b)** Beta-gal expression is largely restricted to PV expressing interneuron. Left panel: representative picture from a CA1 region of a 138-sponge^{PV} hippocampal slice co-stained for the lacZ product beta-galactosidase (red) and PV (green). Scale bar = 20 μ m. Right panel: quantification of PV+/lacZ+ cells in 138-sponge^{PV} mice. n=3 mice. **(c)** Behavioral characterization of 138-sponge^{PV} mouse line, upper: schematic representation of the Y-maze novelty preference task; lower: exploration time spent in familiar (F) and novel (N) arm; 138-floxed n=10 mice; 138-sponge^{PV} n=10 mice; ***p=0.0002; n.s. p=0.19 (Mann-Whitney test). **(d)** Upper: schematic representation of the novel object recognition task; Lower: exploration time presented as percentage of total time spent with either novel or familiar object; 138-floxed n=10 mice; miR-138 sponge n=10 mice; *p=0.035, n.s. p=0.57 (Student's two-tailed heteroscedastic t-test). **(e)** Percentage of spontaneous alternations in the Y-Maze. 138-floxed n=10; 138-sponge^{PV} n=10; n.s. p=0.90 (Student's two-tailed heteroscedastic t-test). PV, parvalbumin.

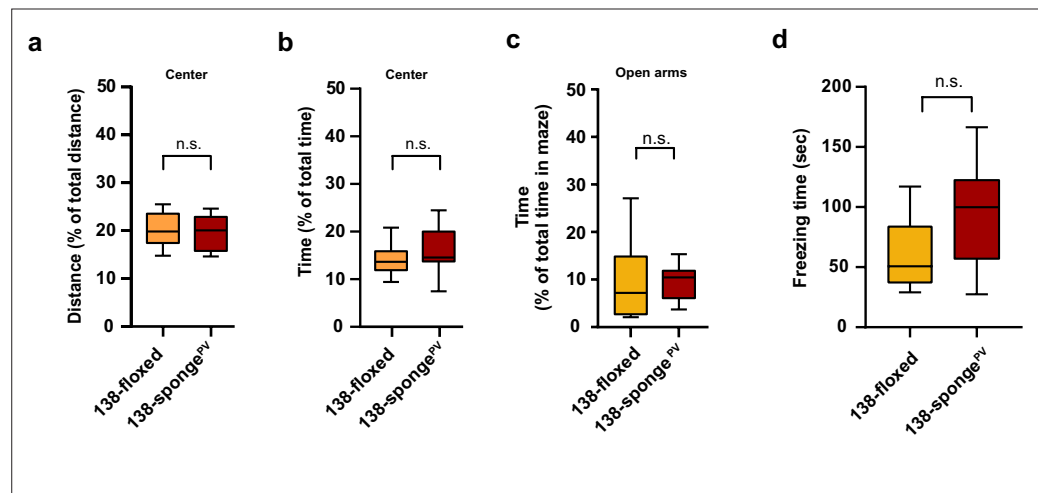


Figure 5—figure supplement 1. Behavioural characterization of PV-expressing interneuron specific miR-138 sponge mice. (a, b) Percentage of total distance travelled (a)/time spent (b) in the center of an open field arena during 30 min exploration by mice of the indicated genotypes; 138-floxed n=10 mice; 138-sponge^{PV} n=10 mice; (a) p=0.74 (b) p=0.28 (Student's two-tailed heteroscedastic t-test). (c) Percentage of total time spent in the open arms of an elevated plus maze (EPM) during 5 min exploration by mice of the indicated genotypes; 138-floxed n=10 mice; 138-sponge^{PV} n=10 mice; p=0.53 (Mann-Whitney test). (d) Time (s) mice spent freezing 24 hr after the foot shock was administrated; 138-floxed n=9 mice; 138-sponge^{PV} n=9 mice; p=0.075 (Student's two-tailed heteroscedastic t-test).

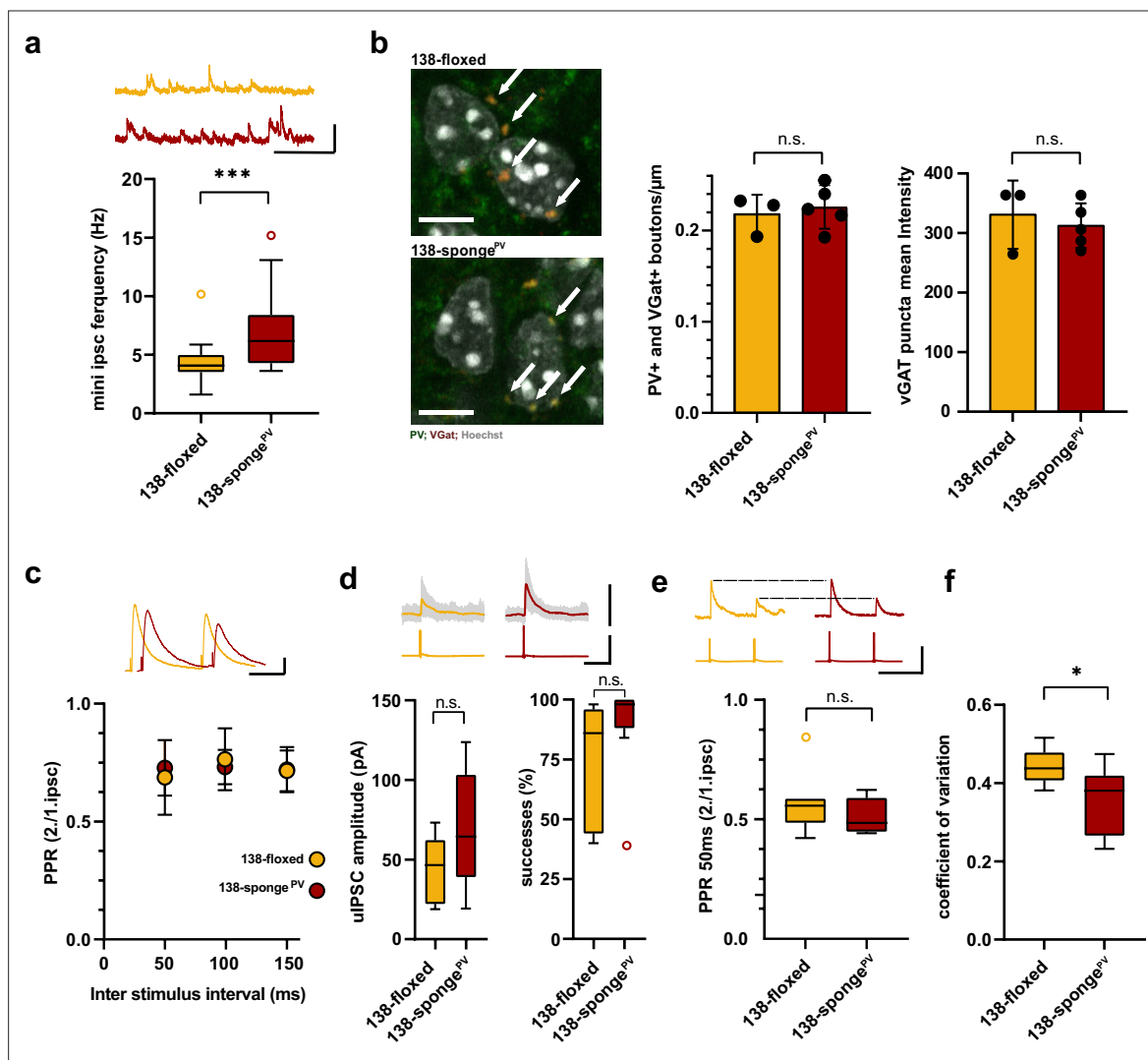


Figure 6. Enhanced inhibitory synaptic transmission onto hippocampal pyramidal neurons in PV-expressing interneuron specific miR-138 sponge mice. (a) mIPSC frequency in CA1 pyramidal neurons. Upper panel: example traces, 138-floxed in orange, 138-sponge^{PV} in red, scale bar: 50 pA, 200 ms. Lower panel: mIPSC frequency (138-floxed: range, from 1.6 to 10.2 Hz; median, 4.1 Hz; IQR, 1.5 Hz. 138-sponge^{PV}: range, from 3.6 to 15.2 Hz; median, 6.2 Hz; IQR, 4.2 Hz; ***p=0.0002, Mann-Whitney test). 138-floxed n=22 cells/5 mice; 138-sponge^{PV} n=23 cells/5 mice. (b) PV+, VGAT+ bouton density. Left panel: representative pictures (arrows point the PV+; VGAT+ boutons). Middle panel: number of boutons per CA1 pyramidal neuron cell perimeter based on Hoechst counterstain. Right panel: VGAT puncta mean intensity; 138-floxed: n=73 cells/3 mice; 138-sponge^{PV}: n=95 cells/5 mice; data represents the average per mouse \pm s.d.; n.s., p=0.65 (bouton density), n.s., p=0.59 (mean intensity) (Student's two-tailed heteroscedastic t-test); (c) Paired pulse ratio (PPR) of stimulated IPSCs in CA1 pyramidal neurons. Upper panel: example traces of PPR (inter stimulus interval of 100 ms) for 138-floxed (orange) and 138-sponge^{PV} (red); scale bar: 100 pA, 50 ms. Lower panel: PPRs for different interstimulus intervals from 50 to 150 ms (138-floxed vs. 138-sponge^{PV} [mean \pm s.d.]: 50 ms, 0.7 ± 0.2 vs. 0.7 ± 0.1 [n=13]; 100 ms: 0.8 ± 0.1 vs. 0.7 ± 0.1 [n=13]; 150 ms: 0.7 ± 0.1 [n=12] vs. 0.7 ± 0.1 . n.s. p=0.45, p=0.69, and p=0.89 for 50, 100, and 150 ms, respectively. Mann-Whitney test). (d) Unitary connections between presynaptic fast-spiking interneurons and postsynaptic CA1 pyramidal cells. Upper panel: example traces, 138-floxed: average of 50 sweeps in orange, 26 single sweeps in gray, 138-sponge^{PV}: average of 50 sweeps in red, 26 single sweeps in gray; scale bar: 100 pA, 100 mV, 25 ms. Lower panel left: uIPSC amplitude (138-floxed: range, from 18.9 to 73.2 pA; median, 46.6 pA; IQR, 40.2 pA. 138-sponge^{PV}: range, from 19.3 to 123.8 pA; median, 64.5 pA; IQR, 64.4 pA; n.s. p=0.11 Student's two-tailed heteroscedastic t-test). Lower panel right: Success rate (138-floxed: range, from 40% to 98%; median, 86%; IQR, 54%. 138-sponge^{PV}: range, from 38% to 100%; median, 98%; IQR, 12%; n.s. p=0.14 Mann-Whitney test). 138-floxed n=7 pairs/3 mice; 138-sponge^{PV} n=9 pairs/5 mice. (e) PPR of unitary connections. Upper panel: example traces, 138-floxed in orange, 138-sponge^{PV} in red, uIPSCs are normalized to the first uIPSC, scale bar: 100 mV, 50 ms. Lower panel: PPR (2nd/1st uIPSC) (138-floxed: range, from 0.42 to 0.85; median, 0.56; IQR, 0.10. 138-sponge^{PV}: range, from 0.44 to 0.62; median, 0.49; IQR, 0.14; n.s. p=0.34 Student's two-tailed heteroscedastic t-test). 138-floxed n=7 pairs/3 mice; 138-sponge^{PV} n=9 pairs/5 mice. (f) Coefficient of variation (138-floxed: range, from 0.38 to 0.52; median, 0.44; IQR, 0.07. 138-sponge^{PV}: range, from 0.23 to 0.47; median, 0.38; IQR, 0.15; *p=0.028, Student's two-tailed heteroscedastic t-test). 138-floxed n=7 pairs/3 mice; 138-sponge^{PV} n=9 pairs/5 mice. IQR, interquartile range; mIPSC, miniature inhibitory postsynaptic current; uIPSC, unitary inhibitory postsynaptic current.

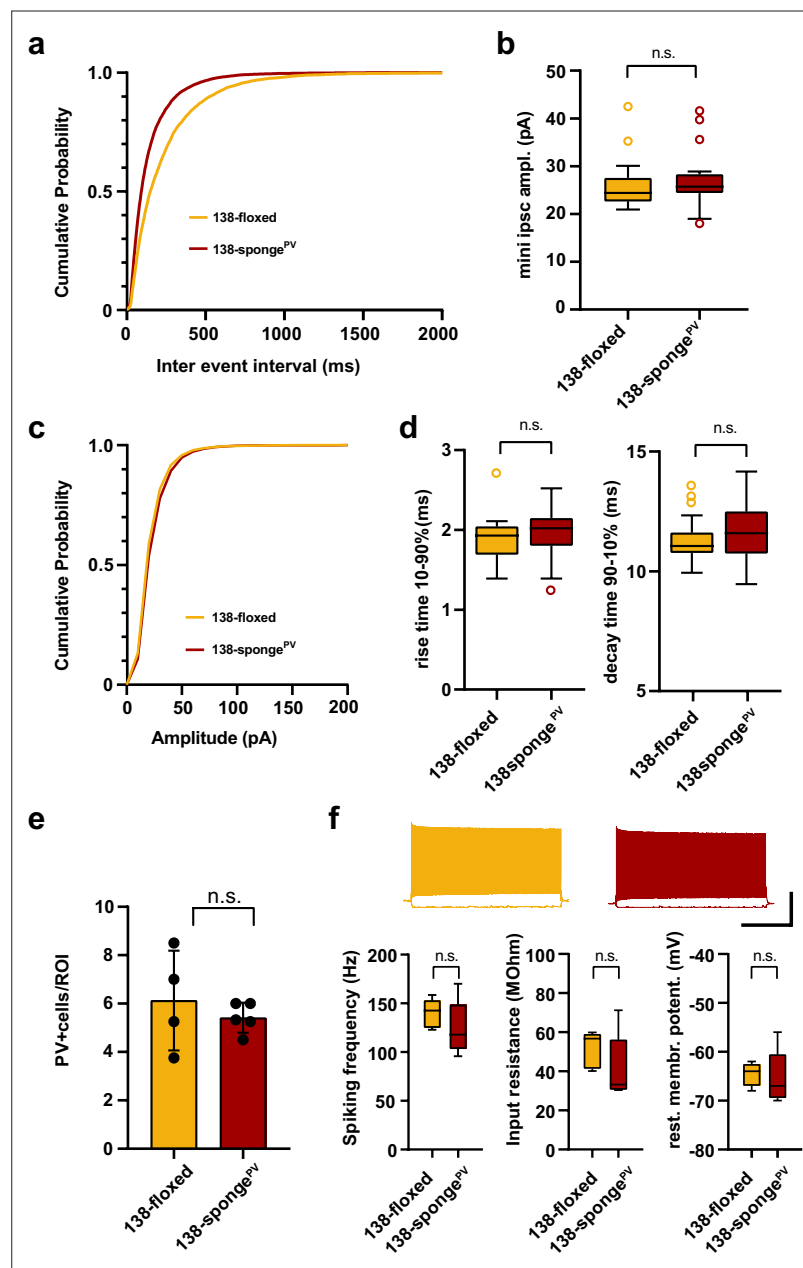


Figure 6—figure supplement 1. Electrophysiological characterization of hippocampal neurons in PV-expressing interneuron specific miR-138 sponge mice. (a–d) mIPSC in CA1 pyramidal neurons. (a) Cumulative distribution of mIPSCs frequency ($p < 0.0001$; Kolmogorov-Smirnov [KS] test). (b) mIPSC amplitude (138-floxed: range, from 20.9 to 42.5 pA; median, 24.4 pA; IQR, 5.0 pA. 138-sponge^{PV}: range, from 18.0 to 41.6 pA; median, 25.7 pA; IQR, 4.0 pA; n.s. $P = 0.22$ Mann-Whitney test). (c) Cumulative distribution of mIPSCs amplitude ($p < 0.0001$ KS test). 138-floxed $n = 22$ cells/5 mice; 138-sponge^{ub} $n = 23$ cells/5 mice. (d) mIPSC rise (10%–90%) and decay (90%–10%) time. Left panel: mIPSC rise (10%–90%) time (138-floxed: range, from 1.4 to 2.7 ms; median, 1.9 ms; IQR, 0.4 ms. 138-sponge^{PV}: range, from 1.3 to 2.5 ms; median, 2.0 ms; IQR, 0.4 ms; n.s. $p = 0.28$ Mann-Whitney test). Right panel: mIPSC decay (90%–10%) time (138-floxed: range, from 9.9 to 13.6 ms; median, 11.1 ms; IQR, 0.9 ms. 138-sponge^{PV}: range, from 9.5 to 14.2 ms; median, 11.6 ms; IQR, 1.8 ms; n.s. $p = 0.20$ Mann-Whitney test). 138-floxed $n = 22$ cells/5 mice; 138-sponge^{ub} $n = 23$ cells/5 mice. (e) Density of PV+ interneurons in CA1 hippocampus of mice with the indicated genotype. Values are expressed relative to a defined region of interest (ROI). 138-floxed: $n = 16$ ROIs/4 mice; 138-sponge^{PV}: $n = 18$ ROIs/5 mice; data represents the average per mouse \pm s.d.; n.s. $p = 0.8049$ (Mann-Whitney test). (f) Properties of fast-spiking interneurons. Upper panel: example traces, 138-floxed in orange, 138-sponge^{PV} in red, scale bar: 50 mV, 500 ms. Lower panel left: spiking frequency (138-floxed: range, from 123

Figure 6—figure supplement 1 continued on next page

Figure 6—figure supplement 1 continued

to 159 Hz; median, 143 Hz; IQR, 29 Hz. 138-sponge^{PV}: range, from 96 to 170 Hz; median, 118 Hz; IQR, 46 Hz; n.s. p=0.31 Student's two-tailed heteroscedastic t-test). Lower panel middle: input resistance (138-floxed: range, from 40 to 60 M Ω ; median, 57 M Ω ; IQR, 18 M Ω . 138-sponge^{PV}: range, from 30 to 71 M Ω ; median, 33 M Ω ; IQR, 26 M Ω ; n.s. p=0.22 Mann-Whitney test). Lower panel right: resting membrane potential (138-floxed: range, from -68 to -62 mV; median, -64 mV; IQR, 4.5 mV. 138-sponge^{PV}: range, from -70 to -56 mV; median, -67 mV; IQR, 9 mV; n.s. p=0.78 Student's two-tailed heteroscedastic t-test). 138-floxed n=5 cells/3 mice; 138-sponge^{ub} n=5 cells/5 mice. IQR, interquartile range; mIPSC, miniature inhibitory postsynaptic current.