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Supplementary Materials for

Remodeling of the Homer-Shank interactome mediates homeostatic plasticity

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stke.sciencemag.org/cgi/content/full/14/681/eabd7325/DC1)

Data file S1 (Microsoft Excel format). QMI data.

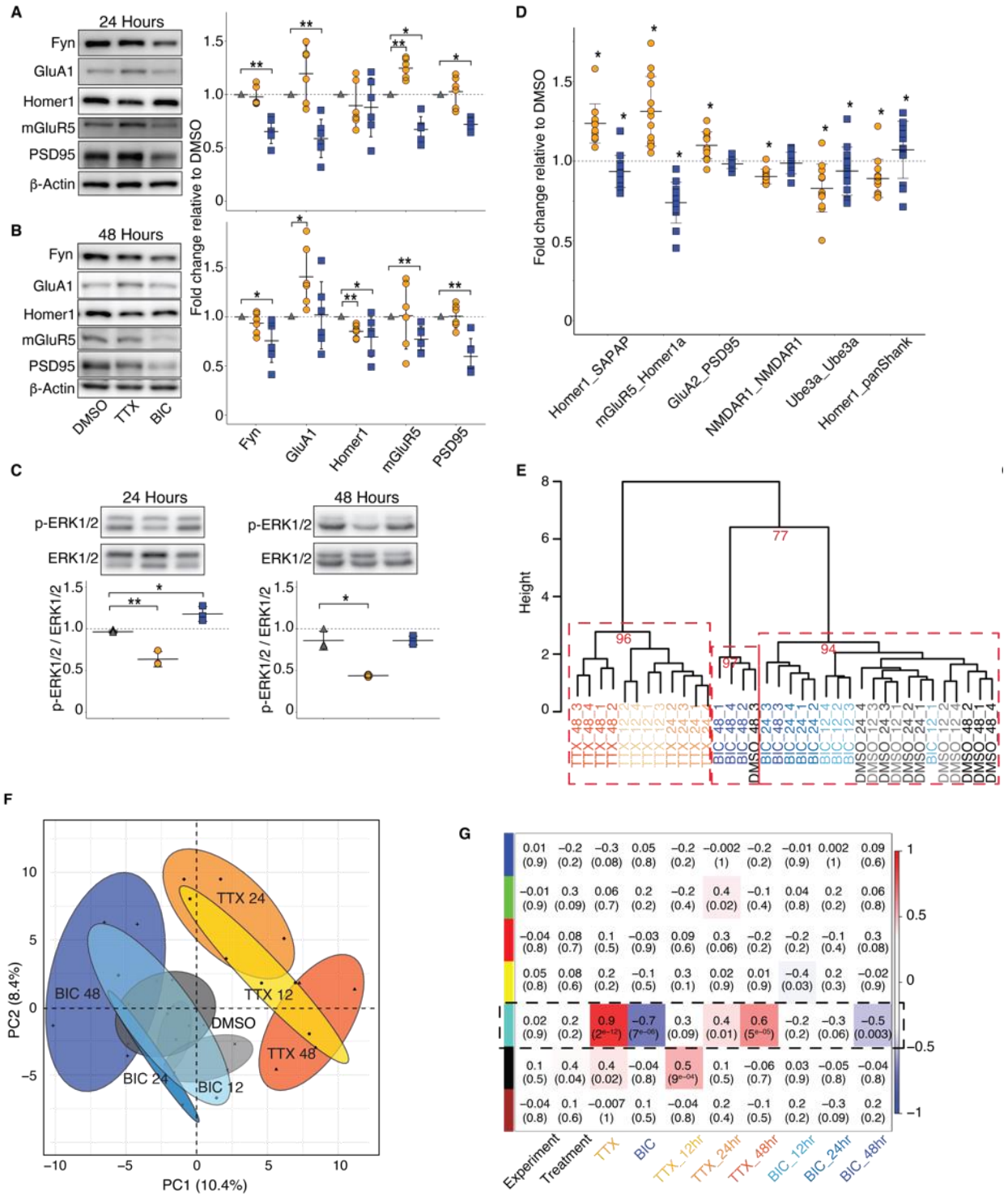

Fig. S1. In vitro scaling; related to Fig. 1. (A and B) Representative Western blots of total levels of select synaptic proteins after 24 (A) or 48 (B) hours of DMSO, TTX, or BIC treatment. Mean protein levels relative to control after treatment are shown in the scatter plot to the right (N=6 biological replicates). **P* < 0.05, ***P* < 0.01 by one-way ANOVA **(C)** Representative Western blots of total levels of ERK1/2 and p-ERK1/2 after 24 or 48 hours of treatment. Plots are mean ratios of phospho-to-total ERK (N=3 biological replicates). **P* < 0.05, ***P* < 0.01 by one-way ANOVA. **(D)** Scatter plots of mean

fold change of select PiSCES after 48 hours of treatment (N=13 biological replicates). *$P < 0.05$ by ANC. **(E)** Hierarchical clustering of three sets of experiments (N=4 biological replicates per set per condition) covering three time points (12, 24, and 48 hours) shows separation of all 12 TTX samples into one significant cluster, regardless of time, while BIC samples form distinct sub-clusters. AU *P*-values calculated by pvclust are shown in red. **(F)** PCA of all PiSCES measurements (MFI>100) shows separation of TTX and BIC conditions along PC1. **(G)** Module-trait relationship heatmap showing the correlation (top number) and *P*-value calculated by CNA for each module-trait pair.

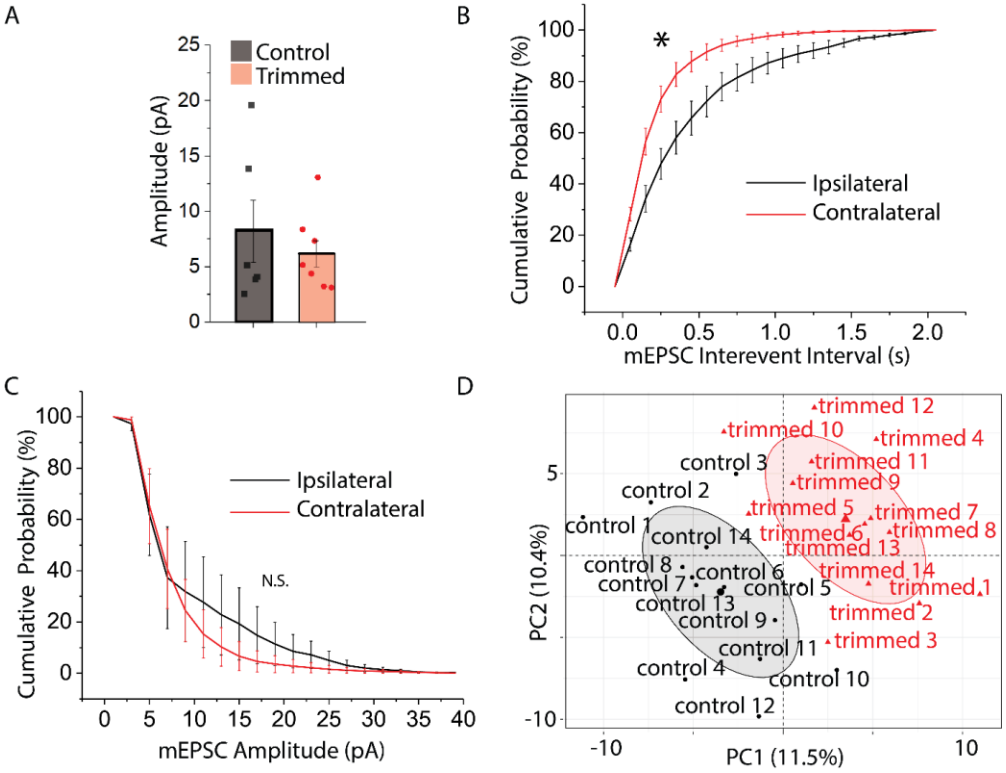

Fig. S2. In vivo experience-dependent plasticity; related to Fig. 2. (A) Mean amplitude of mEPSCs following unilateral whisker trimming from ipsilateral control (black) and contralateral trimmed (red) barrel cortex. **(B)** Cumulative probability of mEPSC inter-event intervals (0-2s, 0.1s bins) following unilateral whisker trimming. (*) *P*-value (hemisphere) < 0.05 by two-way ANOVA. **(C)** Cumulative probability of mEPSC amplitudes (0-30pA, 2pA bins) following unilateral whisker trimming. NS, not significant, *P*-value (hemisphere) = 0.14 by two-way ANOVA. For A-C, Ipsilateral: N=6 cells from 4 animals, Contralateral: N=7 cells from 4 animals. **(D)** PCA of 14 control and 14 trimmed hemispheres demonstrating separation of treatment groups across PC1/2.

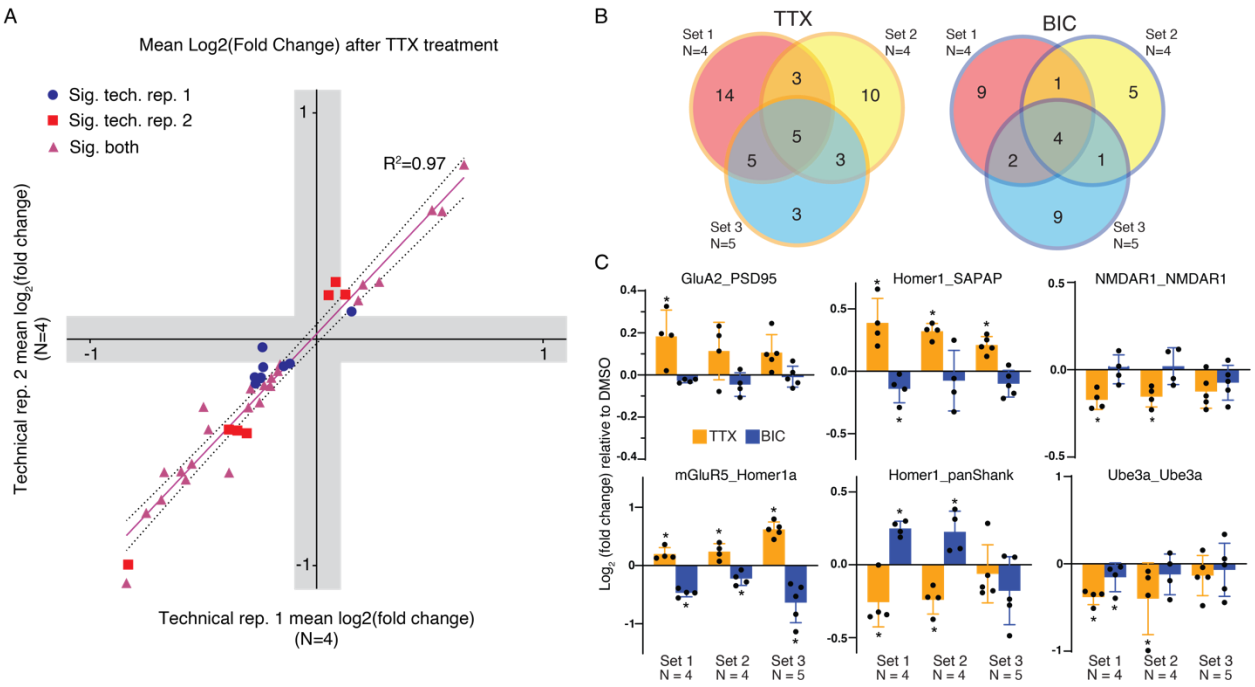

Fig. S3. QMI detects a subset of high-confidence PiSCES changes; related to Fig. 1. (A) Mean $\log_2$(fold change) of all PiSCES that changed significantly in at least one of two technical replicate sets (N=4 biological replicates per set) for set 1 (X-axis) and set 2 (Y-axis). Sig. tech. rep. = significant in technical replicate N, by ANC∩CNA. **(B)** Venn diagrams showing the overlap of significant PiSCES changes (by ANC∩CNA) for each of three independent sets of experiments (N=4 or 5 biological replicates per set). **(D)** Bar graphs showing the mean $\log_2$(fold change) of select PiSCES after 48 hours of TTX (orange) or BIC (blue) treatment for each set. *$P < 0.05$ by ANC∩CNA in the given condition.

Table S1. Proteins targeted by the QMI panel.

QMI Name	Gene Name	Protein Name	Function
Scaffolding proteins			
Homer1	*Homer1*	Homer protein homolog 1	Scaffold
panShank	*Shank1, Shank2, Shank3*	SH3 and multiple ankyrin repeat domains protein	Scaffold
panHomer	*Homer1, Homer2, Homer3*	Homer protein homolog	Scaffold
PSD95	*DLG4*	Disks large homolog 4	MAGUK Scaffold
SAP97	*DLG1*	Disks large homolog 1	MAGUK Scaffold
SAPAP	*DLGAP1*	Disks large-associated protein 1	Scaffold
Shank1	*Shank1*	SH3 and multiple ankyrin repeat domains protein 1	Scaffold
Shank3	*Shank3*	SH3 and multiple ankyrin repeat domains protein 3	Scaffold
Glutamate receptors			
GluA1	*Gria1*	Glutamate receptor AMPA type 1	Receptor
GluA2	*Gria2*	Glutamate receptor AMPA type 2	Receptor
NMDAR1	*Grin1*	Glutamate receptor ionotropic, NMDA 1	Receptor
NMDAR2A	*Grin2a*	Glutamate receptor ionotropic, NMDA 2A	Receptor
NMDAR2B	*Grin2b*	Glutamate receptor ionotropic, NMDA 2B	Receptor
mGluR5	*Grm5*	Metabotropic glutamate receptor 5	Receptor
Signal transducers			
CaMKII	*CaMKII*	Calcium/calmodulin-dependent protein kinase type II alpha chain	Kinase
Fyn	*Fyn*	Tyrosine-protein kinase Fyn	Kinase
Homer1a	*Homer1*	Immediate early gene protein Homer1A	Immediate early gene
NL3	*NLGN3*	Neuroligin-3	Cell adhesion
PI3K	*Pik3ca*	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	Kinase
PIKE	*Agap2*	Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 2	GTPase
SynGAP	*Syngap1*	Ras/Rap GTPase-activating protein SynGAP	GTPase-activating
Ube3a	*Ube3a*	Ubiquitin-protein ligase E3A	Ubiquitin ligase

Table S2. Top-ranked PiSCES from three models of homeostatic plasticity. The listed PiSCES are significantly different by Wilcoxon rank-sum test for the comparison indicated in the table header; *P*-values are shown. N=7 sets (TTX), 7 sets (BIC), and 4 sets (Trim) with 4 biological replicates per set.

TTX vs. BIC	*P*-value	TTX vs. Trim	*P*-value	BIC vs. Trim	*P*-value
CaMKII_PIKE	0.002	Homer1_mGluR5	0.011	Fyn_Fyn	0.011
Homer1_mGluR5	0.002	Homer1_SAPAP	0.011	Homer1_mGluR5	0.011
mGluR5_Homer1	0.002	mGluR5_Fyn	0.011	mGluR5_Homer1a	0.011
Ube3a_panShank	0.002	NL3_PIKE	0.011	SAP97_PSD95	0.011
Ube3a_Shank3	0.002	Ube3a_panShank	0.011	Ube3a_Fyn	0.011
NMDAR1_NMDAR1	0.003	Ube3a_Shank3	0.011	mGluR5_Fyn	0.018
Homer1_SAPAP	0.005	GluA2_GluA1	0.018	mGluR5_panHomer	0.018
CaMKII_Homer1a	0.011	NMDAR1_NMDAR1	0.018	CaMKII_Homer1a	0.030
mGluR5_mGluR5	0.011	Shank1_Homer1a	0.018	Shank1_SAPAP	0.030
Ube3a_Ube3a	0.015	Shank1_NMDAR2B	0.018	SynGAP_NMDAR1	0.030
CaMKII_panShank	0.021	Ube3a_Fyn	0.018	Homer1_Homer1a	0.047
Homer1_NMDAR1	0.021	Ube3a_Ube3a	0.018	Homer1_NMDAR1	0.047
mGluR5_Homer1a	0.021	CaMKII_panShank	0.030	mGluR5_NMDAR2A	0.047
PSD95_PSD95	0.021	Homer1_Homer1a	0.030	NL3_PIKE	0.047
SAP97_PSD95	0.021	PSD95_PSD95	0.030		
NL3_PIKE	0.030	Shank1_Fyn	0.030		
Shank1_SAPAP	0.030	mGluR5_NMDAR2A	0.047		
Homer1_Shank1	0.041	Shank1_SynGAP	0.047		
NMDAR2B_SAPAP	0.041	SynGAP_SynGAP	0.047		

Data file S1. QMI data. This Excel file (in the online supplementary materials) contains median fluorescence intensity (MFI) values for each PiSCES measured in each tissue type for all experiments in the manuscript. The "read me" tab lists date of each experiment, the treatment type [DMSO, bicuculline (BIC) or tetrodotoxin (TTX) for cultured cortical neuron experiments; barrel cortex contralateral to the shaved whiskers (S) or ipsilateral to the shaved whickers (NS) for the whicker trimming experiments; and *Homer* or *Shank3* knockout (KO) or wild-type littermate (WT)]. Individuals are listed in the "Samples" column, where each text string represents the descriptor for an experimental N, separated by commas. Subsequent tabs are arranged by the figure in which the data are presented. MFI values are calculated by taking the median bead distribution for each bead class in each well, then taking the mean of two technical replicates for each N. PiSCES are represented in the IP_Probe column, and identified by the immunoprecipitation antibody target protein, followed by an underscore (_), followed by the probe antibody target protein.