SUPPLEMENTARY ITEMS

Table S1: Antibodies used for the PRISM-RNAi screen.

Target	Supplier and Cat. No.	Host, clonality and isotype	Imaged with	Conjugation method
MAP2	Novus Biologicals NB300-213	Chicken polyclonal	Secondary IF	--
PSD95	Cell Signaling Technology 3450	Rabbit monoclonal	Secondary PRISM	--
Gephyrin	Synaptic Systems 147208	Rat IgG1	Secondary PRISM	--
GluR2	Synaptic Systems 182 105	Guinea Pig polyclonal	Secondary PRISM	--
NR2A	Neuromab 75-288	Mouse IgG2a	Secondary PRISM	--
Shank3	Synaptic Systems 162311	Mouse IgG1	Secondary PRISM	--
F-actin	BACHEM H7643	Phalloidin	Direct PRISM – p2	SMCC
vGAT	Synaptic Systems 131011	Mouse IgG3	Direct PRISM – p3	SMCC
Bassoon	Enzo Life Sciences ADI-VAM-PS003	Mouse IgG2a	Direct PRISM – p8	SMCC
Synapsin1	Synaptic Systems 106011	Mouse IgG1	Direct PRISM – p9	SMCC
Homer1	Synaptic Systems 160011	Mouse IgG1	Direct PRISM – p10	SiteClick
vGlut1	Synaptic Systems N1602	Camelid sdAb	Direct IF	
anti-Rabbit	Invitrogen A16126	Goat polyclonal	PRISM – p1	SMCC
anti-Mouse IgG1	Abcam ab98689	Goat polyclonal	PRISM – p4	SMCC
anti-Guinea Pig	Invitrogen A18777	Goat polyclonal	PRISM – p6	SMCC
anti-Rat	Invitrogen A18873	Goat polyclonal	PRISM – p7	SMCC
anti-Mouse IgG2	Novus Biologicals nb7513	Goat polyclonal	PRISM – p12	SMCC

Table S2: Docking and Imaging strand sequences.

Sequence Name	Docking Strand sequence (5' to 3')	ssLNA imaging probe sequence (5' to 3')	Fluorophore on LNA imaging probe
p1	TTATACATCTA	**T**AGA**TG**TATAA	Atto 565
p2	TTATCTACATA	TATG**TAG**ATAA	Atto 565
p3	TTTCTTCATTA	TAA**TGA**AGAAA	Atto 655
p4	TTATGAATCTA	T**A**GA**TT**CATAA	Atto 655
p6	TTAATTGAGTA	**TAC**TCAATTAA	Atto 655
p7	TTAATTAGGAT	**AT**CC**T**AATTAA	Atto 655
p8	TTATAATGGAT	**AT**C**C**ATTATAA	Atto 565
p9	TTTAATAAGGT	**A**C**CT**TATTAAA	Atto 565
p10	TTATAGAGAAG	**CT**T**C**TCTATAA	Atto 565
p12	TTATAGTGATT	**A**AT**CA**CTATAA	Atto 655

Table S3: Accell siRNA mixtures and TaqMan RTqPCR primers.

Gene	**Accell siRNA Cat. No.**	**TaqMan RTqPCR Assay ID**
Nontargeting	D-001910-10-20	
Dyrk1a	E-090285-00	Rn00562940_m1
Trio	E-087912-00	Rn01524285_m1
Cul1	E-094875-00	Rn01501284_m1
Setd5	E-095571-00	Rn01425934_m1
Sp4	E-090979-00	Rn00562717_m1
Chd8	E-098578-00	Rn00576005_m1
Herc1	E-101129-00	Rn01421830_m1
Shank3	E-080173-00	Rn00572344_m1
Scn2a	E-097072-00	Rn00680558_m1
Gria3	E-088270-00	Rn00583547_m1
Cacna1g	E-089308-00	Rn00709287_m1
Fmr1	E-091941-00	Rn00709627_m1
Syngap1	E-080169-00	Rn00710435_m1
Setd1a	E-051358-00	Mm00626141_m1
Grin2a	E-091573-00	Rn00561341_m1
Pten	E-080104-00	Rn00477208_m1
Xpo7	E-087157-00	Rn01493200_m1
Actb		Rn00667869_m1

Supplementary Figures

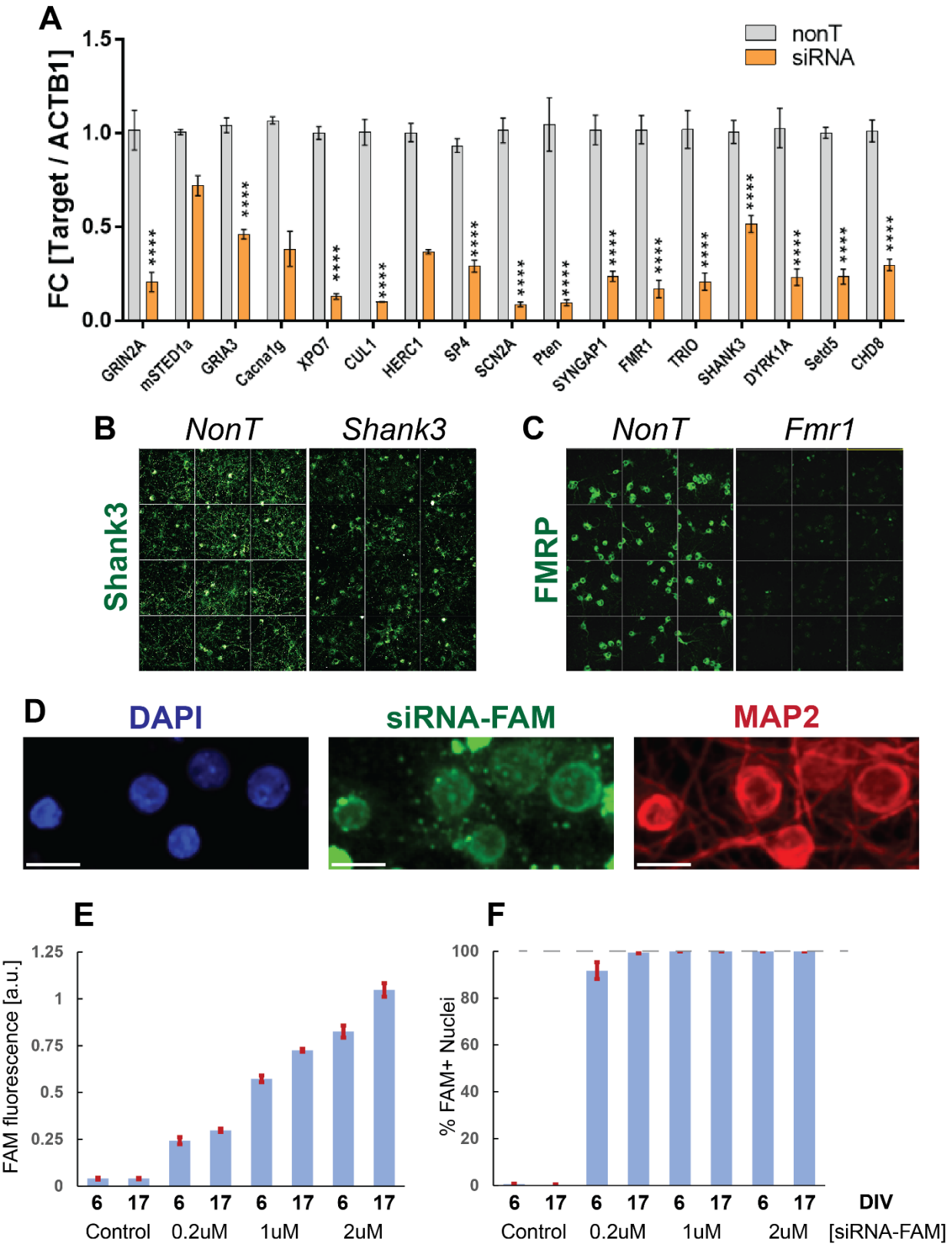

Figure S1: RNAi knockdown validation. Related to Figure 2. A) RTqPCR of transcript abundances of the perturbed genes in rat embryonic hippocampal neurons after siRNA treatment at DIV 5 and harvest at DIV 21. Y-axis is normalized to NonT and to ACTB1 mRNA levels. Error bars indicate SEM across cultures. ****p<0.001. B,C) Immunofluorescence of Shank3 (B) and FMRP (C) in siRNA-treated vs NonT-treated cultures. D) Images of neurons treated with a FAM-labeled nontargeting siRNA. Scale bar is 50um. E) Average fluorescence integral of FAM over DAPI-identified nuclei for the treatment conditions. F) Fraction of nuclei with significantly above background levels of FAM fluorescence. 'Control' indicates cultures treated with a FAM-labeled non-self-transfecting short oligo. Error bars are SEM across 3-4 cultures.

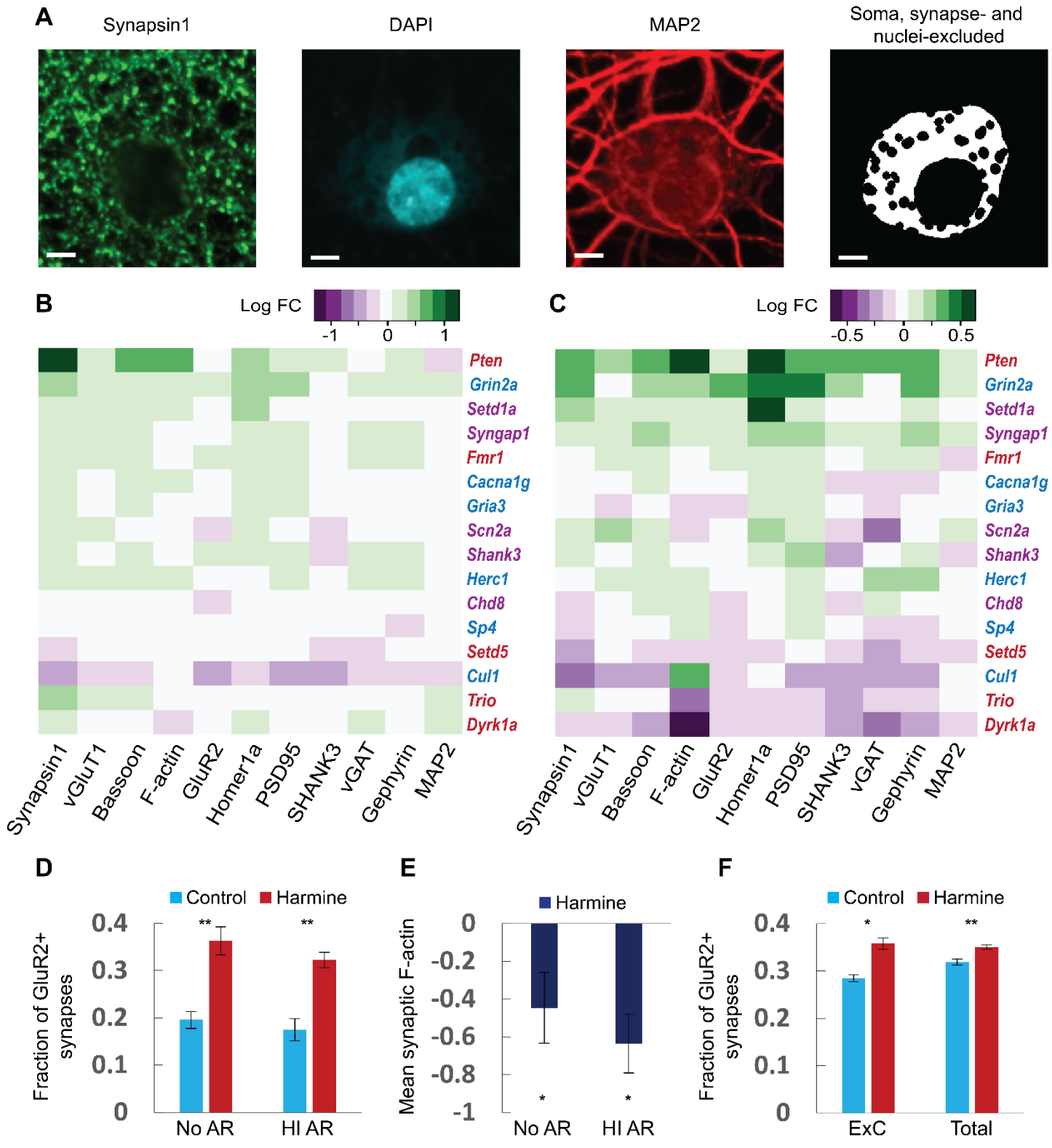

Figure S2: Additional measurements of synaptic protein phenotypes. Related to Figure 2. A) Representative images of Synapsin1, DAPI and MAP2 used to define synapse- and nuclei-excluded soma to quantify somatic protein levels. B) Log-fold-change of average somatic levels of each protein under each gene knockdown, relative to nontargeting control. C) Log-fold changes of average overall levels (mean intensity value over nucleus-excluded image). D-E) Effect of Harmine treatment on fraction of GluR2-positive Synapsin1 puncta (D) and average synaptic F-actin (E) in staining with no antigen retrieval step (No AR) and with heat and acid-induced antigen retrieval (HI AR). F) Effect of Harmine treatment on externalized GluR2 (stained via an N-terminal targeting antibody without permeabilization) and total GluR2 (stained after permeabilization). *p<0.05, **p<0.01, two-sided t-test. D-F data are mean ± s.e.m.

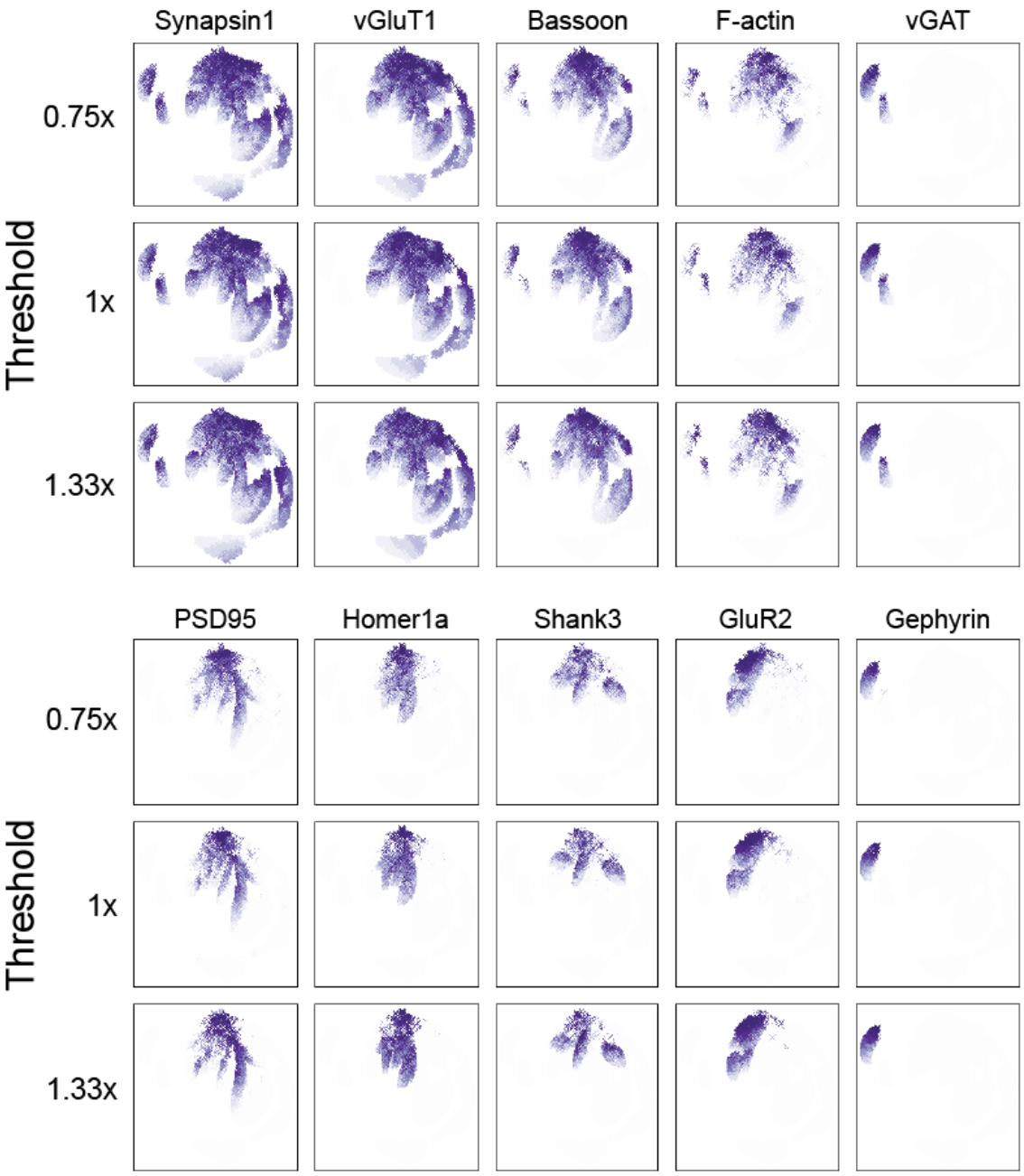

Figure S3: UMAP plot of data sampled from synapses thresholded at three levels, colored by protein level. Related to Figure 3. Synapses for each threshold exhibit clusters defined by presence and absence of protein combinations.

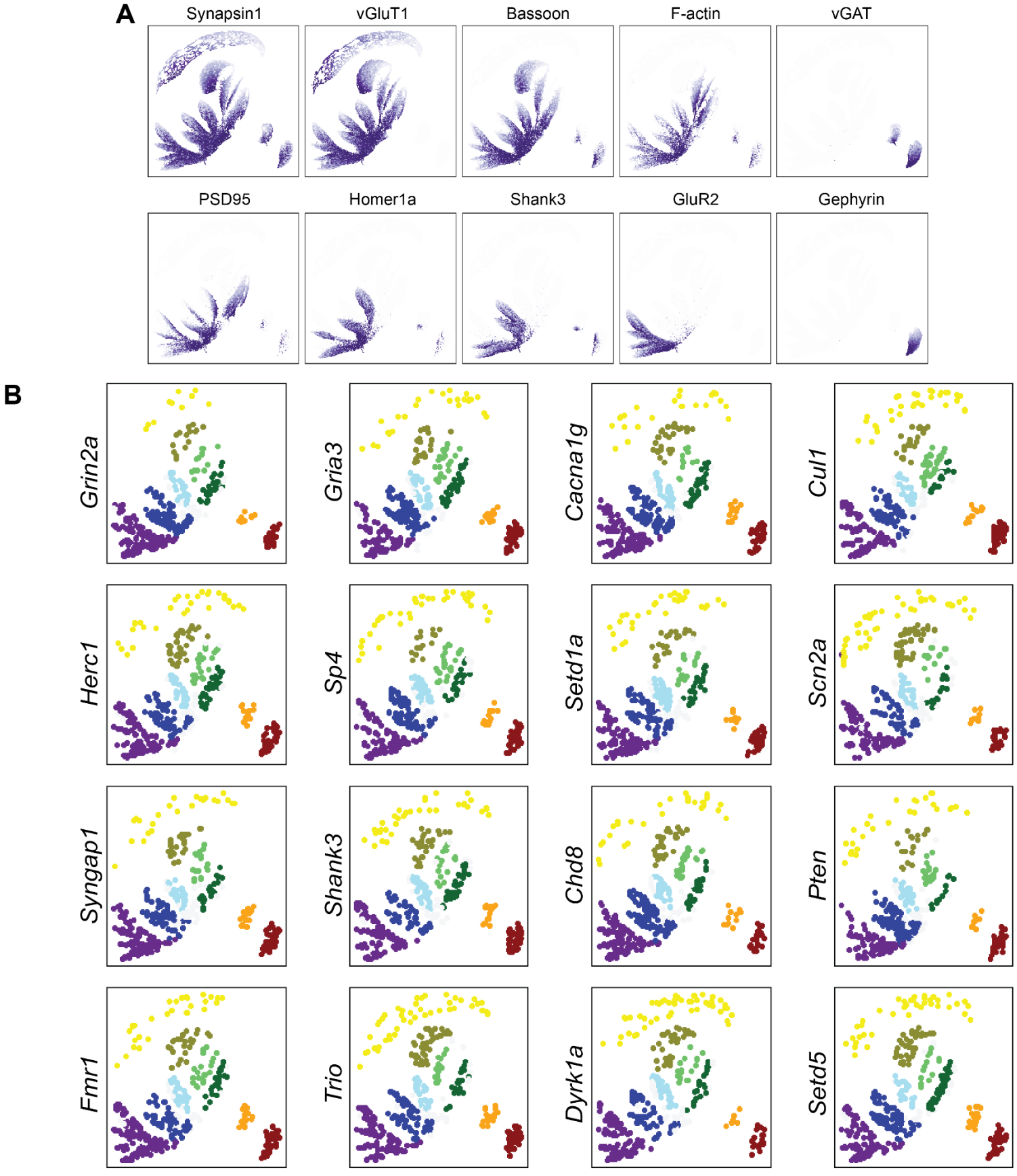

Figure S4: A) UMAP projections colored by levels of individual proteins. B) UMAP projections of synapses from each treatment group. Related to Figure 3.

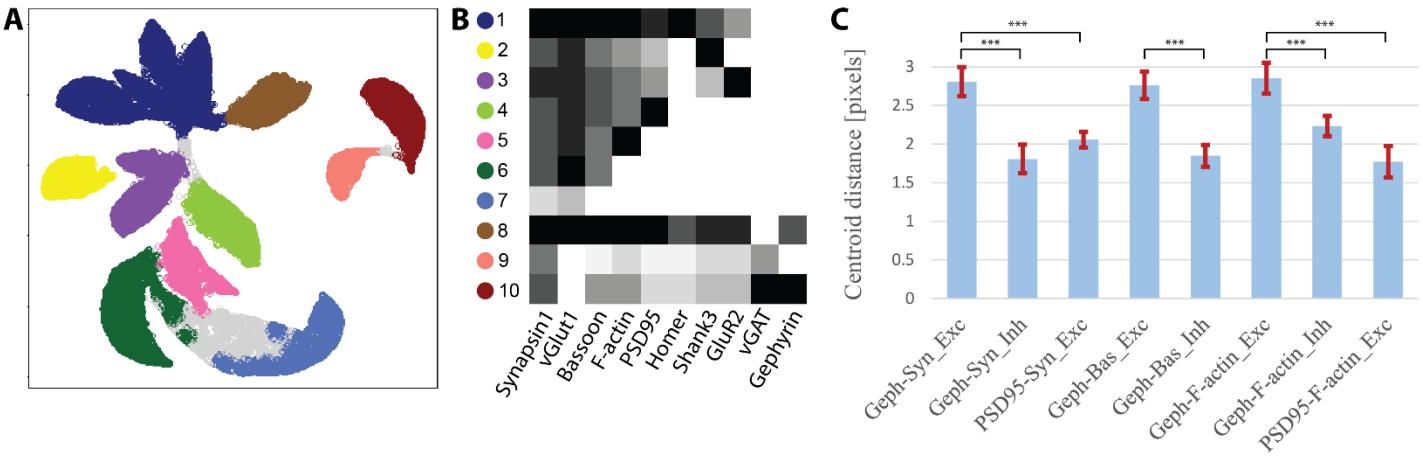

Figure S5: Multiplexed information-based data cleanup. Related to Figure 3 and Star Methods. A,B) UMAP projection and unsupervised density-based clustering (A) of all synapses, and heatmap of average protein levels per cluster (B) similar to figure 3 but with an additional cluster (#8) of synapses positive for Gephyrin and vGluT. C) Average distances (in pixels) between centroids of puncta of different proteins in synaptic subsets. Left to right: Gephyrin-Synapsin in cluster #8, Gephyrin-Synapsin in cluster #10, PSD95-Synapsin across all PSD95+ excitatory synapses, Gephyrin-Bassoon in cluster #8, Gephyrin-Bassoon in cluster #10, Gephyrin-F-actin in cluster #8, Gephyrin-F-actin in cluster #10, and PSD95-F-actin across all PSD95+, F-actin+ excitatory synapses. Error bars are standard deviations across all wells. ***p<0.0001. Bars are mean ± s.e.m.

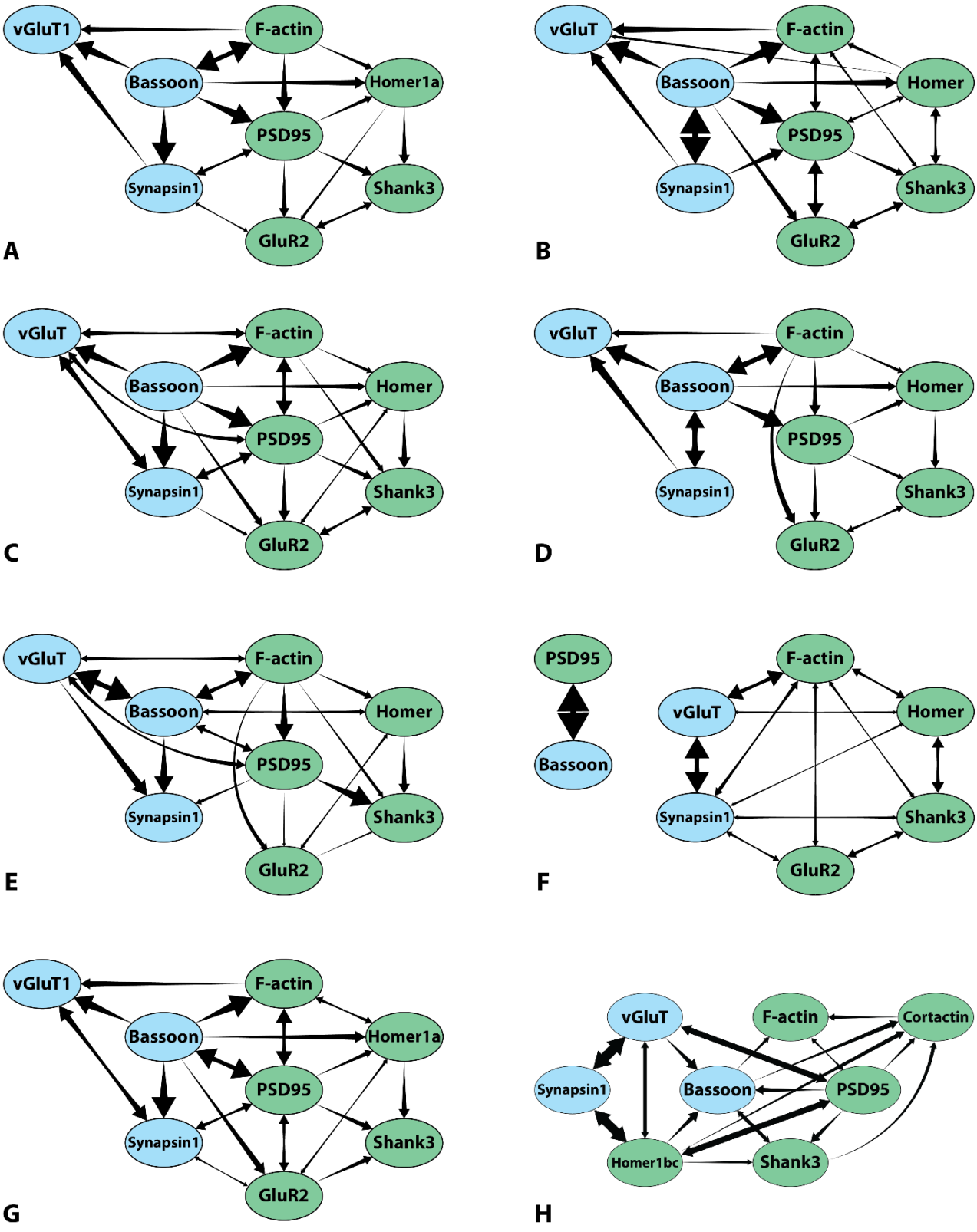

Figure S6: Bayesian networks derived from modified data. Related to Figure 5. A) Original network. B) Network derived only from the subset of synapses which are positive for all components. C) Network derived on data with 25% lower threshold. D) Network derived on data with 33% higher threshold. E) Network derived on data in which synapses were identified (during CellProfiler analysis) not by Synapsin1 puncta but by a combination of F-actin and PSD95 puncta. F) Network derived from the original data, but with data for Bassoon and PSD95 levels randomly permuted together. *G)* Bayesian network derived on data with Shank3 knockdown cultures excluded. *H)* Bayesian network derived on data from a previous study. 6 proteins are the same in both studies – Synapsin1, vGluT1, Bassoon, Shank3, F-actin and PSD95 – but different antibodies were used. Homer1bc was replaced by Homer1a in this study, and Cortactin by GluR2.