Synaptic Signatures and Disease Vulnerabilities of Layer 5 Pyramidal Neurons

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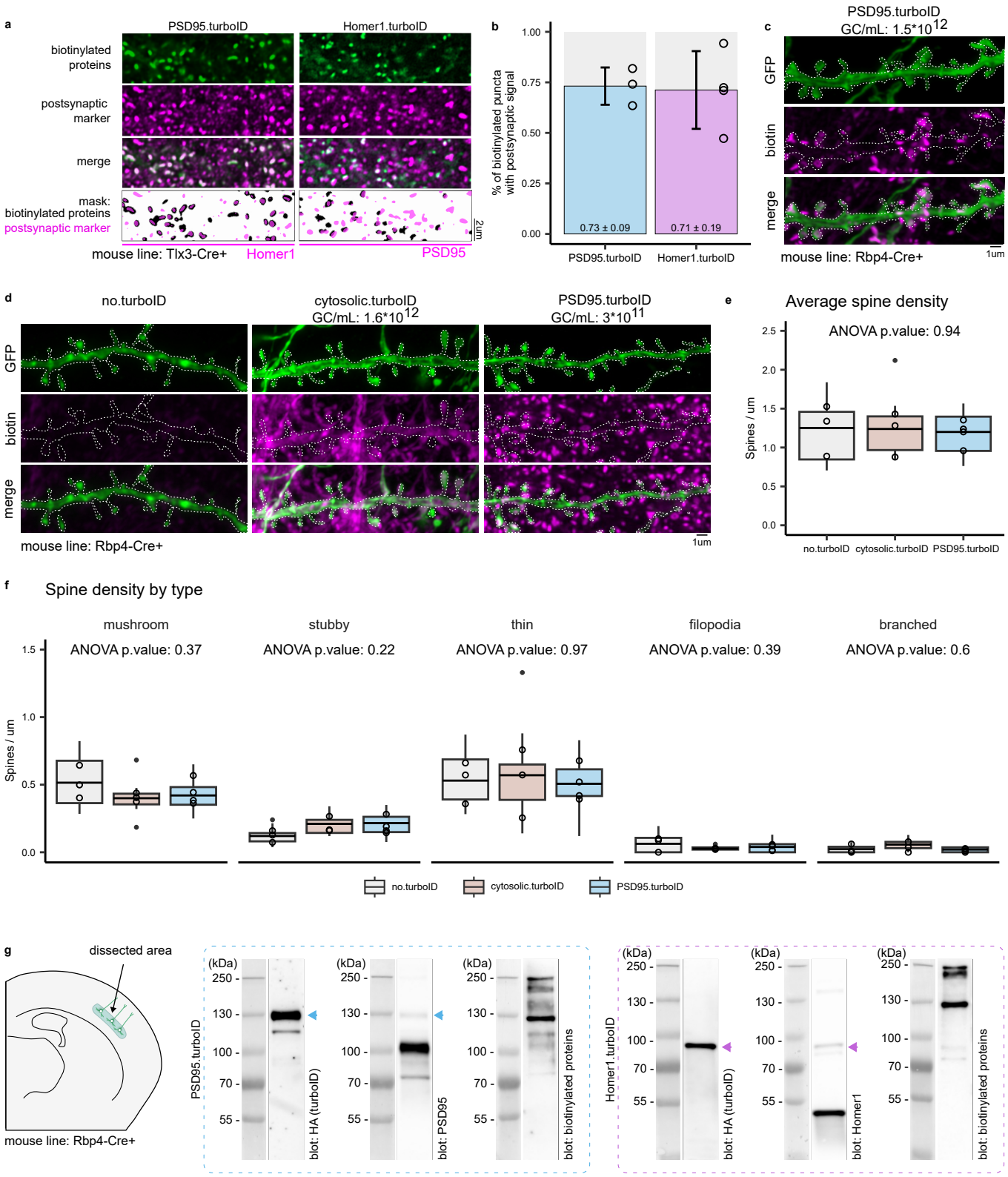
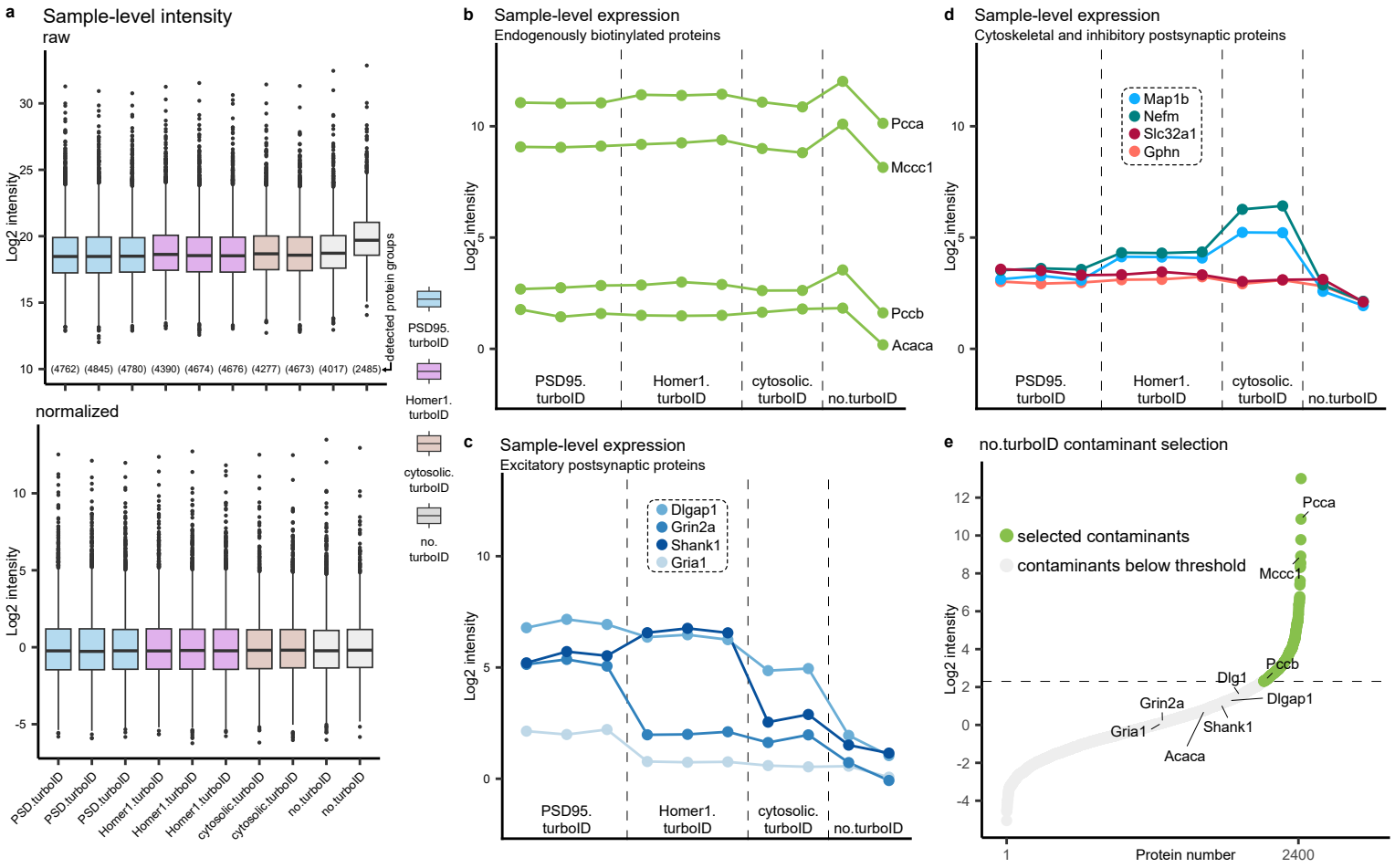
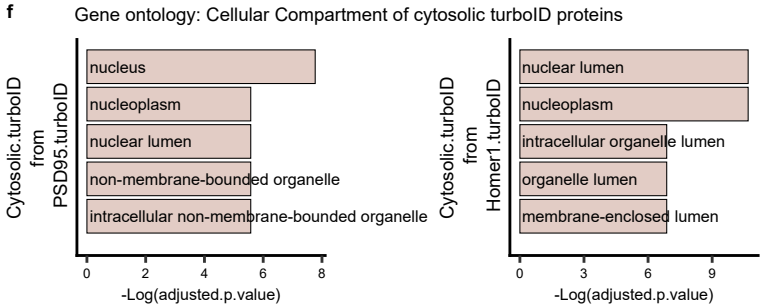
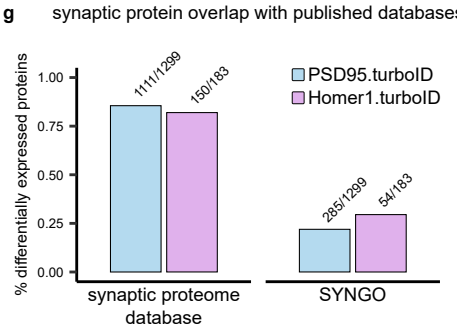

a
PSD95.turboID
Homer1.turboID
biotinylated proteins
postsynaptic marker
merge
mask: biotinylated proteins
postsynaptic marker
2um
mouse line: Tlx3-Cre+
Homer1
PSD95
b
% of biotinylated puncta with postsynaptic signal
1.00
0.75
0.50
0.25
0.00
0.73 ± 0.09
0.71 ± 0.19
PSD95.turboID
Homer1.turboID
c
PSD95.turboID
GC/mL: 1.5*10^12
GFP
biotin
merge
mouse line: Rbp4-Cre+
1um
d
no.turboID
cytosolic.turboID
GC/mL: 1.6*10^12
PSD95.turboID
GC/mL: 3*10^11
GFP
biotin
merge
mouse line: Rbp4-Cre+
1um
e
Average spine density
ANOVA p.value: 0.94
Spines / um
2.5
2.0
1.5
1.0
0.5
0.0
no.turboID
cytosolic.turboID
PSD95.turboID
f
Spine density by type
mushroom
ANOVA p.value: 0.37
stubby
ANOVA p.value: 0.22
thin
ANOVA p.value: 0.97
filopodia
ANOVA p.value: 0.39
branched
ANOVA p.value: 0.6
Spines / um
1.5
1.0
0.5
0.0
no.turboID
cytosolic.turboID
PSD95.turboID
g
dissected area
mouse line: Rbp4-Cre+
(kDa)
250
130
100
70
55
PSD95.turboID
blot: HA (turboID)
blot: PSD95
blot: biotinylated proteins
Homer1.turboID
blot: HA (turboID)
blot: Homer1
blot: biotinylated proteins

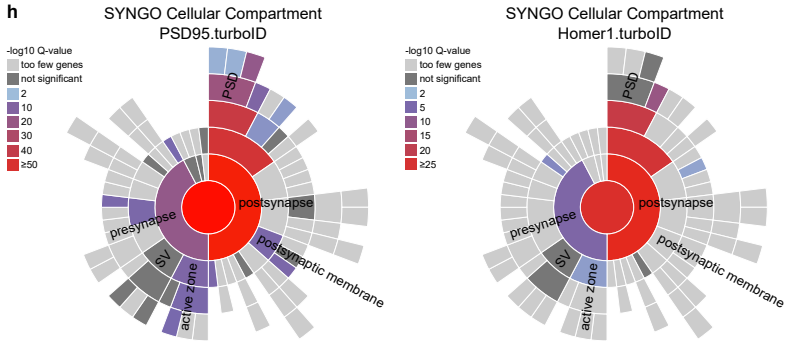
Supplementary Figure 1. TurboID expression has no effect on dendritic spine density or morphology.

a, High resolution images of biotinylated proteins produced by synaptic TurboID constructs, corresponding postsynaptic markers and threshold masks used for quantification in (b). Scale bar is 2 µm. **b,** Fraction of biotinylated objects that contain postsynaptic marker signal for synaptic TurboID constructs. Error bars show standard deviation. Mean and standard deviation are indicated on the graph. N = 3 mice per condition (PSD95.turboID) or 4 mice per condition (Homer1.turboID), 1 single-plane field of view per mouse is quantified as a replicate.Source data are provided as a Source Data file. **c,** Representative image of Supernova-labeled L5 neuron dendrite of Rbp4-Cre mice injected with high titer AAV-PSD95.turboID. Viral titer is indicated as Genome Copies (GC) per mL. Scalebar is 1 µm. **d,** Representative images of Supernova-labeled dendrites of control L5 neurons and L5 neurons expressing different TurboID constructs. Viral titer is indicated as Genome Copies (GC) per mL. Scalebar is 1 µm. **e, f,** Quantification of spine density (e) and quantification of spine density by spine type (f). N = 3 mice per replicate except PSD95.turboID = 4 mice per replicate, average of 2 dendrites per mouse. Boxplots show spine density distribution of individual dendrites, empty dots display the average spine density per replicate. The line across the box describes the mean. The lower and upper hinges correspond to the first and third quartiles. The upper whisker extends from the hinge to the largest value no further than 1.5 * inter-quartile range. The lower whisker extends from the hinge to the smallest value at most 1.5 * inter-quartile range of the hinge. Data beyond the end of the whiskers (outliers) are plotted individually as a small, solid dot. ANOVA statistical analysis is performed on the replicate means. Source data are provided as a Source Data file. **g,** AAV expressing Cre-dependent GFP was co-injected with AAV-PSD95.TurboID or AAV-Homer1.TurboID in the somatosensory cortex of Rbp4-Cre mice. Tissue was dissected as close as possible to the cell bodies to minimize inclusion of non-transduced material. Western blot analysis of the TurboID tag (HA) shows the molecular weight of the PSD95.turboID (blue arrow) and Homer1.turboID (purple arrow) constructs. Molecular weight markers in kDa are indicated on the left. Western blot analysis with an antibody against endogenous PSD95 and Homer1 proteins shows low expression levels of TurboID-fusion proteins compared to endogenous proteins levels. Biotinylated protein analysis confirms successful transduction and TurboID activity. These experiments were independently repeated more than 2 times with similar results.









i

proteins detected in all conditions: 5036

sample-level normalization
remove contaminants present in no.turboID
keep only proteins identified 3 times in at least one group

proteins detected (filtered): 4468

t-test PSD95.turboID vs cytosolic.turboID
t-test Homer1.turboID vs cytosolic.turboID
FDR < 0.1 s0 > 01

synaptic proteins (combine PSD95.turboID and Homer1.turboID): 1359

t test PSD95.turboID vs Homer1.turboID
FDR < 0.05 s0 > 0.1

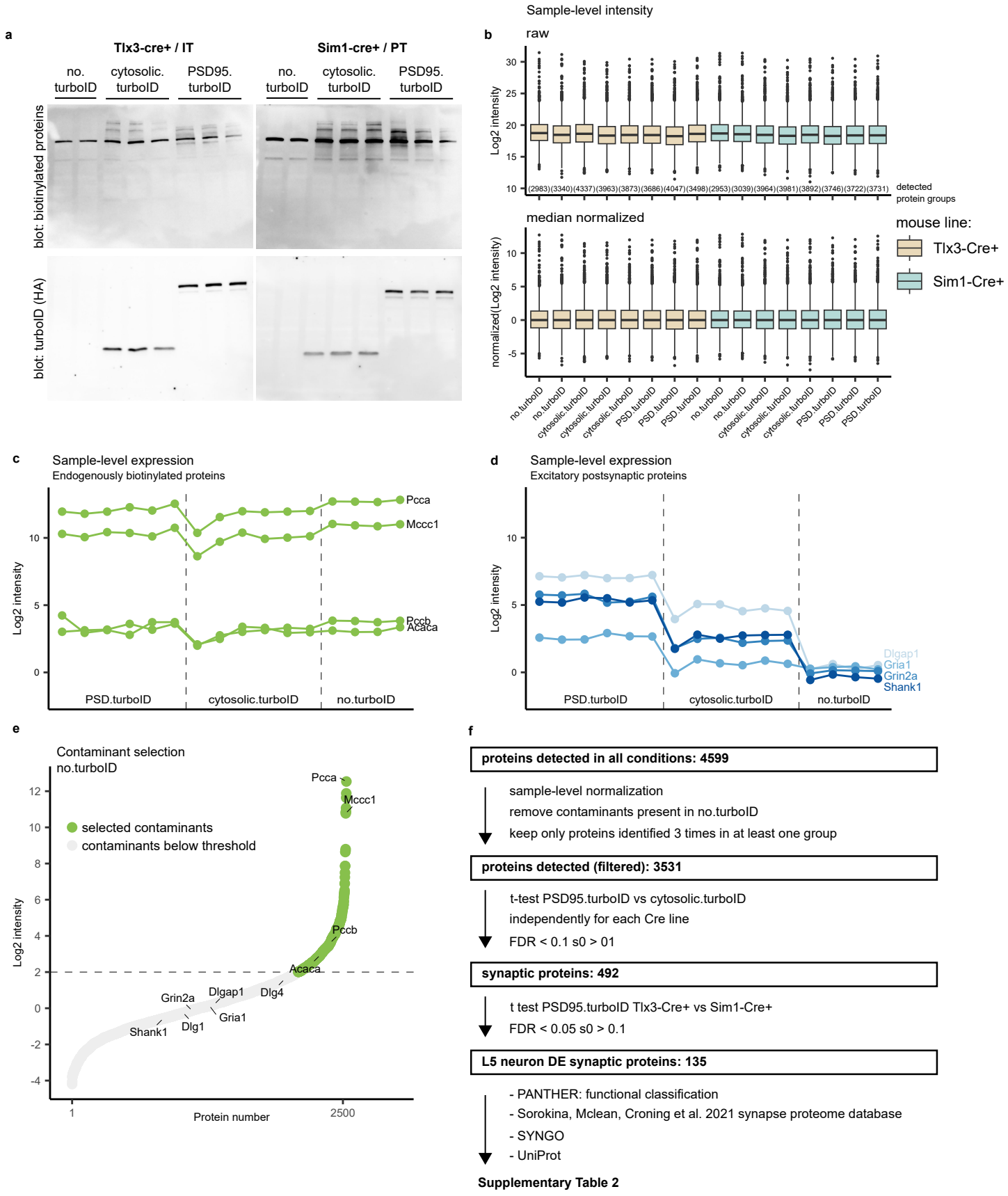
PSD95- and Homer1-specific enriched synaptic proteins: 695

- PANTHER: functional classification
- Sorokina, Mclean, Croning et al. 2021 synapse proteome database
- SYNGO
- UniProt

Supplementary Table 1

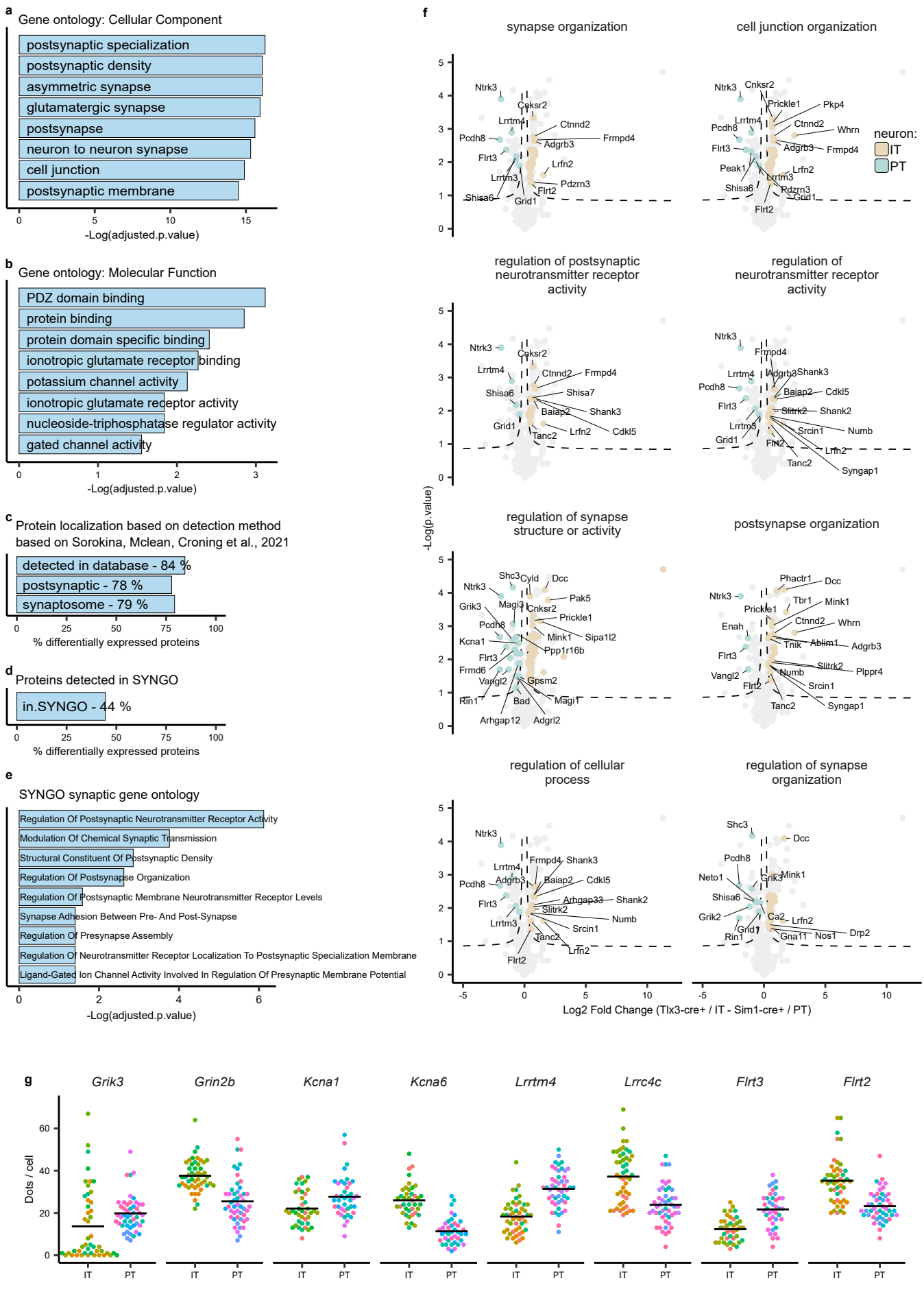
Supplementary Figure 2. Quality control and analysis for PSD95.turboID and Homer1.turboID synaptic proteome workflow.

a, Sample-level intensity values for each replicate before and after median normalization. Protein groups detected in each replicate are reported above the x-axis on the top graph. **b,** Intensity levels in each sample for 4 endogenously biotinylated proteins. **c,** Intensity levels in each sample for 4 known PSD proteins. **d,** Intensity levels in each sample for 2 known inhibitory postsynapse proteins and 2 cytoskeletal proteins. **e,** Proteins detected in all 4 no.turboID replicates are ranked by intensity. Endogenously biotinylated proteins and known PSD proteins are labelled. Threshold for selecting contaminants is based on the expression of endogenously biotinylated proteins and known PSD components. **f,** Gene ontology analysis of cytosolic proteins in PSD95.turboID and Homer1.turboID samples (refers to Figure 2f). **g,** Overlap of the enriched proteins with the synaptic proteome database and SYNGO. **h,** SYNGO cellular compartment enrichment analysis plots of synaptic proteins in PSD95.turboID and Homer1.turboID samples showing distribution between pre- and postsynapse. **i,** Proteomic analysis workflow to enrich for synaptic proteins. See Supplementary Data 1 for complete annotation.


a
Tlx3-cre+ / IT
Sim1-cre+ / PT
no. turboID
cytosolic. turboID
PSD95. turboID
blot: biotinylated proteins
blot: turboID (HA)
b
Sample-level intensity
raw
Log2 intensity
(2983)(3340)(4337)(3963)(3873)(3686)(4047)(3498)(2953)(3039)(3964)(3981)(3892)(3746)(3722)(3731)
detected protein groups
median normalized
normalized(Log2 intensity)
mouse line:
Tlx3-Cre+
Sim1-Cre+
c
Sample-level expression
Endogenously biotinylated proteins
Pcca
Mccc1
Pccb
Acaca
PSD.turboID
cytosolic.turboID
no.turboID
d
Sample-level expression
Excitatory postsynaptic proteins
Dlgap1
Gria1
Grin2a
Shank1
e
Contaminant selection
no.turboID
selected contaminants
contaminants below threshold
Dlg4
Dlg1
Protein number
f
proteins detected in all conditions: 4599
sample-level normalization
remove contaminants present in no.turboID
keep only proteins identified 3 times in at least one group
proteins detected (filtered): 3531
t-test PSD95.turboID vs cytosolic.turboID
independently for each Cre line
FDR < 0.1 s0 > 01
synaptic proteins: 492
t test PSD95.turboID Tlx3-Cre+ vs Sim1-Cre+
FDR < 0.05 s0 > 0.1
L5 neuron DE synaptic proteins: 135
- PANTHER: functional classification
- Sorokina, Mclean, Croning et al. 2021 synapse proteome database
- SYNGO
- UniProt
Supplementary Table 2

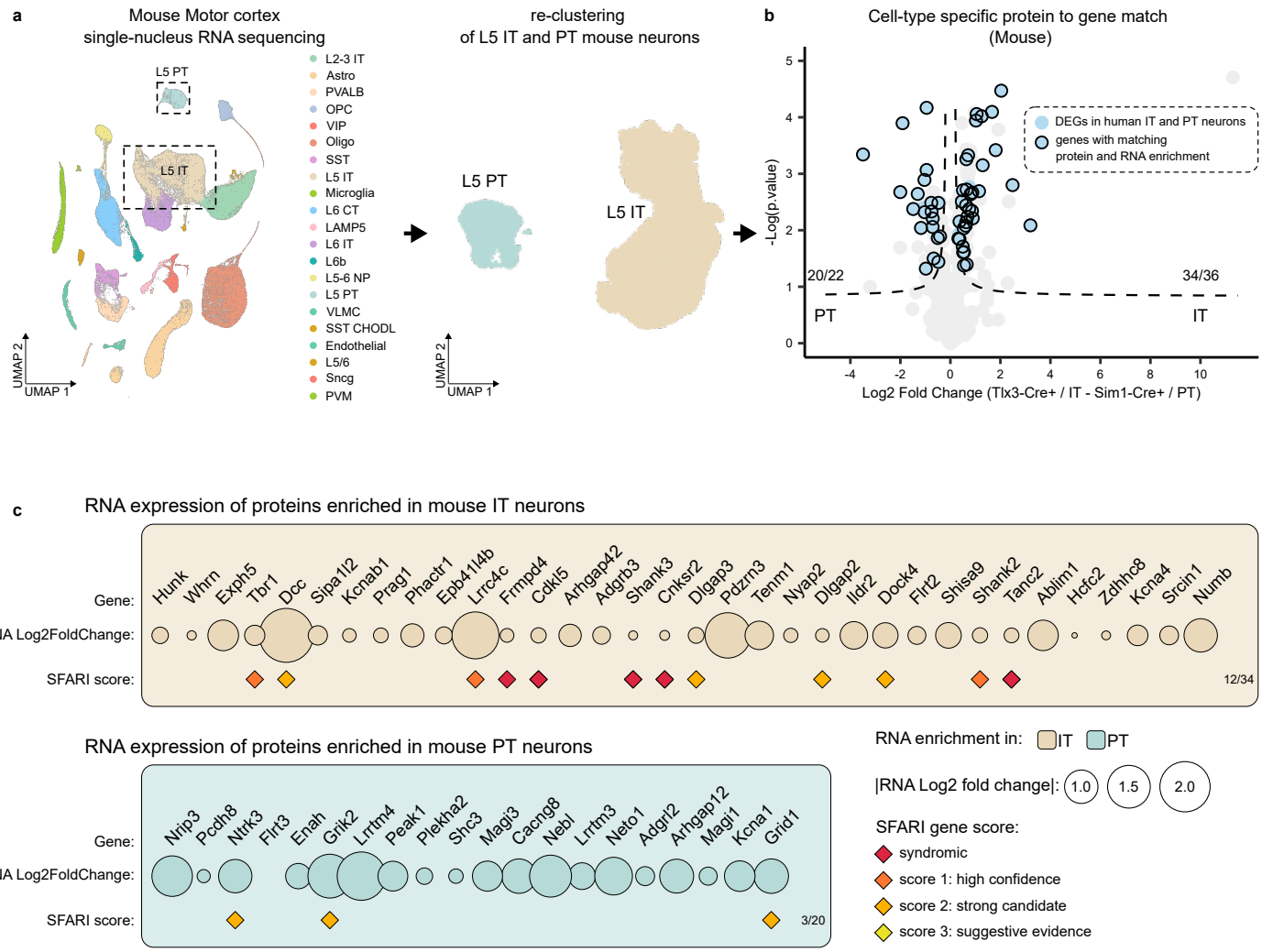
Supplementary Figure 3. Quality control and analysis for cell type-specific TurboID synaptic proteome workflow.

a, Western blots for biotinylated proteins and TurboID (HA) on replicates confirm successful biotinylation in every sample and correct TurboID construct expression. **b,** Sample-level intensity values for each replicate before and after median normalization. Protein groups detected in each replicate are reported on the top graph. **c,** Intensity levels in each sample for 4 endogenously biotinylated proteins. **d,** Intensity levels in each sample for 4 known PSD proteins. **e,** Proteins detected in all 4 no.turboID replicates are ranked by intensity. Endogenously biotinylated proteins and known PSD proteins are labelled. Threshold for selecting contaminants is based on the expression of endogenously biotinylated proteins and known PSD components. **f,** Proteomic analysis workflow to enrich for synaptic proteins and select candidates from differentially expressed proteins. See Supplementary Data 2 for complete annotation.


a
Gene ontology: Cellular Component
postsynaptic specialization
postsynaptic density
asymmetric synapse
glutamatergic synapse
postsynapse
neuron to neuron synapse
cell junction
postsynaptic membrane
-Log(adjusted.p.value)
b
Gene ontology: Molecular Function
PDZ domain binding
protein binding
protein domain specific binding
ionotropic glutamate receptor binding
potassium channel activity
ionotropic glutamate receptor activity
nucleoside-triphosphatase regulator activity
gated channel activity
-Log(adjusted.p.value)
c
Protein localization based on detection method
based on Sorokina, Mclean, Croning et al., 2021
detected in database - 84 %
postsynaptic - 78 %
synaptosome - 79 %
% differentially expressed proteins
d
Proteins detected in SYNGO
in.SYNGO - 44 %
% differentially expressed proteins
e
SYNGO synaptic gene ontology
Regulation Of Postsynaptic Neurotransmitter Receptor Activity
Modulation Of Chemical Synaptic Transmission
Structural Constituent Of Postsynaptic Density
Regulation Of Postsynapse Organization
Regulation Of Postsynaptic Membrane Neurotransmitter Receptor Levels
Synapse Adhesion Between Pre- And Post-Synapse
Regulation Of Presynapse Assembly
Regulation Of Neurotransmitter Receptor Localization To Postsynaptic Specialization Membrane
Ligand-Gated Ion Channel Activity Involved In Regulation Of Presynaptic Membrane Potential
-Log(adjusted.p.value)
f
synapse organization
cell junction organization
regulation of postsynaptic neurotransmitter receptor activity
regulation of neurotransmitter receptor activity
regulation of synapse structure or activity
postsynapse organization
regulation of cellular process
regulation of synapse organization
neuron:
IT
PT
-Log(p.value)
Log2 Fold Change (Tlx3-cre+ / IT - Sim1-cre+ / PT)
g
Grik3
Grin2b
Kcna1
Kcna6
Lrrtm4
Lrrc4c
Flrt3
Flrt2
Dots / cell
IT
PT

Supplementary Figure 4. Gene ontology analysis of differentially expressed proteins in layer 5 neurons.

a, Gene ontology for Cellular Compartment and **b,** Molecular Function categories using differentially expressed proteins as input. **c,** Analysis of differentially expressed proteins using the synapse proteome database[63]. 85% of differentially expressed proteins were detected in the database; 79% and 82% were identified in postsynaptic fractionation studies and synaptosome fractionation studies, respectively. **d,** 44% of differentially expressed proteins were detected in the SYNGO database[46]. **e,** SYNGO gene ontologies. **f,** Annotation of proteins found in the Biological Process gene ontology terms (refers to Figure 4b). Not all protein names are displayed due to space limitations, see Supplementary Data 2 for complete annotation. **g,** Plots from Figure 4f and 4h showing distribution of the analyzed cells. Each color represents a different mouse, each dot represents a single cell.


a
Mouse Motor cortex
single-nucleus RNA sequencing
L5 PT
L5 IT
L2-3 IT
Astro
PVALB
OPC
VIP
Oligo
SST
L5 IT
Microglia
L6 CT
LAMP5
L6 IT
L6b
L5-6 NP
L5 PT
VLMC
SST CHODL
Endothelial
L5/6
Sncg
PVM
UMAP 2
UMAP 1
re-clustering
of L5 IT and PT mouse neurons
L5 PT
L5 IT
b
Cell-type specific protein to gene match
(Mouse)
DEGs in human IT and PT neurons
genes with matching
protein and RNA enrichment
-Log(p.value)
20/22
34/36
PT
IT
Log2 Fold Change (Tlx3-Cre+ / IT - Sim1-Cre+ / PT)
c
RNA expression of proteins enriched in mouse IT neurons
Gene:
RNA Log2FoldChange:
SFARI score:
Hunk Whrn Exph5 Tbr1 Dcc Slpa1l2 Kcnab1 Prag1 Phactr1 Epb41l4b Lrrc4c Frmpd4 Cdkl5 Arhgap42 Adgrb3 Shank3 Cnksr2 Dlgap3 Pdzrn3 Tenm1 Nyap2 Dlgap2 Ildr2 Dock4 Flrt2 Shisa9 Shank2 Tanc2 Ablim1 Hcfc2 Zdhhc8 Kcna4 Srcin1 Numb
12/34
RNA expression of proteins enriched in mouse PT neurons
Gene:
RNA Log2FoldChange:
SFARI score:
Nrip3 Pcdh8 Ntrk3 Flrt3 Enah Grik2 Lrrtm4 Peak1 Plekha2 Shc3 Magi3 Cacng8 Nebl Lrrtm3 Neto1 Adgrl2 Arhgap12 Magi1 Kcna1 Grid1
3/20
RNA enrichment in: IT PT
|RNA Log2 fold change|: 1.0 1.5 2.0
SFARI gene score:
syndromic
score 1: high confidence
score 2: strong candidate
score 3: suggestive evidence

Supplementary Figure 5. Comparison of differentially expressed synaptic proteins with mouse transcriptomic data.

a, L5 IT and PT neurons were extracted from a published mouse motor cortex snRNA-Seq dataset[61] and re-clustered. **b,** Differentially expressed genes (DEGs) between mouse IT and PT neurons (adjusted p-value < 0.01 and log fold change > 0.25) were plotted on top of mouse synaptic proteins enriched in IT and PT neurons (two-sided, unpaired t-test with permutation-based FDR correction at 5%, s0 > 0.1) to evaluate their overlap. 20/22 DEGs matched PT neuron-enriched synaptic proteins, 34/36 DEGs matched IT neuron-enriched synaptic proteins. **c,** Synaptic proteins from mouse L5 IT and PT neurons that show matching differential gene expression in mouse motor cortex L5 IT and PT neurons are shown. SFARI scores are annotated.