SUPPLEMENTARY INFORMATION

Supplementary Figure 1. Synaptic localization of TAP tagged PSD-95 in primary neurons. DIV14 neurons from wt and PSD-95$^{TAP/TAP}$ mice were simultaneously stained with PSD-95 and Synaptophysin antibodies (top panels), PSD-95, MAP2B and GluR1 antibodies (middle panels) and NR1, MAP2B and FLAG antibodies (bottom panels). Arrows show the puncta labeling with each protein. Bar=10 μm**.**

Supplementary Figure 2. Short-term plasticity is normal in PSD95$^{TAP/TAP}$ mice. (**A**) Sample traces of paired-pulse recordings of fEPSPs in wt and PSD95$^{TAP/TAP}$ mice. (**B**) Average paired-pulse facilitation values are not different (P = 0.182, Student's t-test) between wt (15 slices from 4 animals) and PSD95$^{TAP/TAP}$ mice (13 slices from 4 animals).

Supplementary Figure 3. (**A**) TAP tagged PSD-95 was affinity purified with FLAG antibody from forebrain extracts from PSD-95$^{TAP/TAP}$ mice, then cleaved with TEV protease (TEV) and monitored using immunoblotting with a FLAG antibody. The eluted (El) and column retained PSD-95 (BB) is shown. TEV protease was added to the reaction as indicated (TEV) or control without TEV (non TEV). Input, total lysate; El, Elution after TEV reaction; BB, beads boiled with Laemmli sample buffer after TEV cleavage. Only a small amount of TAP tagged PSD-95 was not cleaved (TEV, BB lane). (**B**) TAP tagged PSD-95 complex was affinity purified by FLAG antibody, cleaved by TEV (Single-step) and recovered and eluted from a Ni^{2+} column (Tandem). The same amount of eluted material from each purification was loaded. One fifth of the protein loaded in both purifications lanes was loaded in the Input lane. The antibodies used for blotting are indicated. Input: Total lysate; Single: Single-step purification; Tandem: Tandem step purification. (**C**) Validation of known PSD-95 interactors. Immunoprecipitation from forebrain extracts with indicated antibodies (labeled above panels) and immunoblotting with antibodies directed against PSD-95. c-: mouse total IgG was used for immunoprecipitation control, IP: antibodies used for immunoprecipitation.

Supplementary Figure 4. Proteins from a single step and four independent tandem purifications (Supplementary Table 1) were classified into 10 functional categories. The graph represents the percentage of proteins in each category relative to the total number of proteins in each purification (single step and tandem purifications).

Supplementary Figure 5. The ratio of protein emPAI values for proteins in the tandem purification/single step purification calculated in the Supplementary Table 4 were used to plot the bar charts. 20 known primary interactors of PSD-95 are indicated across the distribution of emPAI ratios, 19 (95%) of which are proteins with a tandem/single step purification ratio of > 0.5.

Supplementary Figure 6. Comparison of PSD-95 (40 proteins) and MASC (90proteins) networks. 17 common proteins are colored in the MASC network (right) (Pocklington et al., 2006). The cluster 1 in the MASC network centered 10 of the 16 proteins (p<10e-3).

Supplementary Table 1. Proteins identified in the single step and tandem purifications by LC-MS/MS. Approved gene symbols, MGI IDs, UniProt accession numbers, Gene accession numbers in the Genes to Cognition database (G2Cdb) (http://www.genes2cognition.org) and number of approved peptides for each protein identified by LC-MS/MS analysis in the single step and 4 tandem replicates are shown. See Material and Methods for peptide approval criteria. Genes that are also in the MASC/NRC, AMPA receptor (AMPA), metabotrobic glutamate receptor 5 (mGluR5), PSD and PSP lists (Collins et al., 2006) are shown. Common proteins with the PSD-95 immunoprecipitation reported by Dosemeci et al. are also shown (Dosemeci et al., 2007). Using Panther categories (http://www.pantherdb.org/), the molecular function and biological process of each protein is indicated. Numbers of peptides from proteins identified in the PSD-95$^{TAP/TAP}$ mice are separated by the symbol | from number of peptides that are also in the wild type purification. Genes marked with an asterisk represent genes whose peptides are common to other genes.
Cpne5: Cpne4, Cpne8. Eif1a1*: Eif1a2. Tuba1a*: Hist1h2bj*: Hist1h2bm Hist1h2be Hist1h2bn Hist1h2bg Hist1h2bp Hist1h2bh Hist1h2bf Hist1h2bb Hist3h2bb Hist1h2bc Hist1h2bl Hist2h2bb. Tuba1b, Tuba4c, Tuba1b. Tubb2b*:Tubb5, Tubb2a, Tubb2c, Tubb4. Uba52*: Ubc, Ubb. Vamp2*: Vamp3.*

Supplementary Table 2. Proteins identified by LC-MS/MS in three tandem purifications from wild type mice. Gene symbols, UniProt and IPI accession numbers and number of approved peptides for each protein identified by LC-MS/MS analysis in the 4 tandem purifications from PSD-95$^{TAP/TAP}$ mice (T1, T2, T3, T4) and 3 tandem purifications from wt mice (WT1, WT2, WT3) are shown.

Supplementary Table 3. List of known PSD-95 primary interactors extracted from UniHI database and manually curated. The HGNC symbols and number of peptides obtained in the single step and four tandem replicates are shown. The PubMed ID and the method used to see the interaction with PSD-95 are also shown.

Supplementary Table 4. EmPAI values for each protein present in both single step and tandem purifications were normalised to the total protein emPAI in each set and then normalised to the emPAI value of the bait protein (PSD-95). EmPAI ratio tandem purification/single step purification was calculated to plot the chart in Supplementary Figure 5.

Supplementary Table 5. Numbers of common proteins found in the MASC/NRC, AMPA receptor (AMPA), metabotrobic glutamate receptor 5 (mGluR5), PSD and PSP lists (Collins et al., 2006) and PSD-95 immunoprecipitation list (Dosemeci et al., 2007) with all the proteins indentified in this study (301 proteins, Supplementary Table 1) and in the PSD-95 core complexes (118 proteins).

Supplementary Table 6. Protein nodes of the interaction network's MCC ranked by average shortest path (ASP) calculated as the average number of shortest paths between the node and all the other nodes in the network.

Supplementary Table 7. Primers used for the gene targeting vector generation and genotyping screening.

MATERIALS AND METHODS

TAP transgenic Mice.

Mice used for experimental analysis were 2- to 7-month-old males from the second generation of intercrosses between the chimeras and C57BL/6 strain mice. All animal experiments were conducted in a licenced animal facility in accordance to guidelines determined through the UK Animals (Scientific Procedures) Act, 1986 and all procedures were approved through the British Home Office Inspectorate.

Electrophysiology

Hippocampal slices

Hippocampal slices of 3-4 months old litter-matched wt and PSD-95$^{TAP/TAP}$ mice were prepared using procedures described in detail elsewhere. Experimenter was blind to the genotype of mice. Electrophysiological measurements were performed using MEA120-4 multielectrode array-based system (Multi Channel Systems, Reutlingen, FRG) using 5x13 3D MEA chips with electrode height of 35-45 μm and electrode spacing of 140 and 200 μm (Ayanda Biosystems, Lausanne, Switzerland). Four MEA1060-BC set-ups were run in parallel and monopolar stimulation of Schäffer collateral/commissural fibres through array electrodes was performed by STG2008 stimulus generator (Multi Channel Systems, Reutlingen, FRG). For LTP experiments, a single principal recording electrode was picked in proximal part of CA1 *stratum radiatum*. To stimulate control and test pathways, two stimulation electrodes were assigned on the subicular side and on the CA3 side of *stratum radiatum* respectively. The distance from the recording electrode to the test stimulation electrode was 400-510 m and to the control stimulation electrode 280-510 μm. To evoke orthodromic fEPSPs, test and control pathways were activated in succession at a frequency of 0.02

Hz. Baseline stimulation strength was adjusted to evoke a response that corresponded to 40% of the maximal attainable fEPSP at the recording electrode located in proximal *stratum radiatum*. Amplitude of the negative part of fEPSPs was used as a measure of the synaptic strength. To induce LTP, 10 bursts of baseline strength stimuli were administered at 5 Hz to test pathway with 4 pulses given at 100 Hz per burst (total 40 stimuli). LTP plots were scaled to the average of the first five baseline points. Normalisation of LTP values was performed by dividing the fEPSP amplitude in the tetanised pathway by the amplitude of the control fEPSP at corresponding time points. Normalised LTP values averaged across the period of 61-65 min after theta-burst stimulation were used for statistical comparison. Paired stimulation with interpulse interval of 50 ms was used to observe paired-pulse facilitation (PPF) in baseline conditions in the test pathway before LTP induction. PPF was calculated by dividing the negative amplitude of fEPSP obtained in response to the second pulse by the amplitude of fEPSP amplitude evoked by the preceding pulse.

All reported values represented mean ± standard error of the mean. Unpaired Student's *t*-test was used to examine differences in LTP and PPF levels between wt and PSD95$^{TAP/TAP}$ mice.

Immunoprecipitation

For each immunoprecipitation two forebrains were homogenized and clarified as described for the Tandem Affinity Purification. 200 μg of protein was incubated with 2 μg of antibody at 4 ºC. Then, 20 μl of slurry Sepharose beads with Protein G (GE Healthcare) was added and incubated at 4 ºC. The pellets were washed three times with the lysis buffer and twice with 50 mM Tris pH 8.0. The beads were heated at 65 ºC for 10 min with LDS buffer (NuPAGE, Invitrogen, CA) containing 100 mM DTT. The antibodies used for immunoprecipitation were: rabbit Ablim1 (Abnova), mouse Arc/Arg3.1 goat SAP102, rabbit SAP97, goat Gda (Santa Cruz), mouse Nsf, goat PSD-93 (Abcam), mouse PSD-95, rabbit NMDAR2A (Affinity Bioreagents), mouse NMDAR1, rabbit MapkI (Zymed), rabbit CamKIIa (Chemicon), mouse IRSp53 (BD Biosciences) and mouse Rac1, mouse NMDAR2B (Upstate).

Immunoblotting

Protein samples were subjected to reducing SDS electrophoresis (NuPAGE, Invitrogen, CA) and transferred to polyvinyldifluoride membrane (Hybond^{TM}-P, GE Healthcare). The membranes were blocked in 5% non-fat milk, 0.01% Tx100 in PBS and the antibodies used were: rabbit Ablim (Abnova), mouse PSD-95 (Affinity Bioreagents), mouse FLAG (Sigma), rabbit Synaptophysin, mouse NR1, mouse CamKII (Chemicon), goat SAP102, goat Chapsyn/PSD-93, rabbit SynGAP (Abcam), mouse NSF, rabbit NR2A, mouse NR2B (Upstate), mouse Arc/Arg3.1, goat Gda (Santa Cruz), rabbit GluR1, mouse GluR2, rabbit MapkI (Zymed), mouse IRSp53, mouse SAP97, mouse Nsf (BD Biosciences). Detection of signals was carried out using peroxidase-linked secondary IgGs

(Jackson) and enhanced chemiluminescence (GE Healthcare).

Immunohistochemistry

Mice were euthanized with tribromoethanol and perfused via the heart with 0.1 M sodium phosphate buffer (pH 7.4) followed by 4 % Paraformaldehyde in 0.1M sodium phosphate buffer (pH 7.4). The brain was removed and placed in fixative for a further 1 h and then equilibrated overnight in 30 % sucrose in PBS. Frozen sagital sections were cut at 16 μm and mounted onto treated slides (VWR International). Immunohistochemistry was performed on an Automated Ventana Discovery machine according to the manufacturer's instructions with the polyclonal rabbit PSD-95 (10 μg/ml) (Zymed) and secondary antibody biotin-conjugated donkey anti-rabbit (Jackson). Standard procedures were used for mounting and coverslipping.

Immunocytochemistry

Hippocampal primary neurons were dissected from E17.5 $PSD95^{TAP/TAP}$ and wt C57Bl/6 mouse embryos and digested in papain. The culture conditions and the immnocytochemistry protocol were performed as described in (Valor et al., 2007) Primary antibodies were diluted as follows: mouse IgG2A PSD-95 1/350 (Affinity Bioreagents, Golden, CO), IgG1 mouse Synaptophysin 1/1000, rabbit GluR1 1/100 and rabbit MAP2B 1/300 (Chemicon, Temecula, CA), IgG1 mouse FLAG 1/700 (Sigma) and chicken MAP2B (Abcam, Cambridge, UK). Secondary antibodies were used as follows: IgG1 specific-Cy2 1/200 and IgG2A specific-Cy3 1/300 (Jackson Immunoresearch Laboratories, West Grove, PA), chicken specific-Cy2 1/200 and rabbit specific-AlexaFluor 633 1/300 (Invitrogen). No staining was detected when primary antibodies were not added in parallel preparations (data not shown). All images were taken on a Zeiss 510 META confocal microscope using a 63x Plan-apochromat objective. Z-stacks were taken at 0.8 μm intervals and maximal projections were made to give the images showed.

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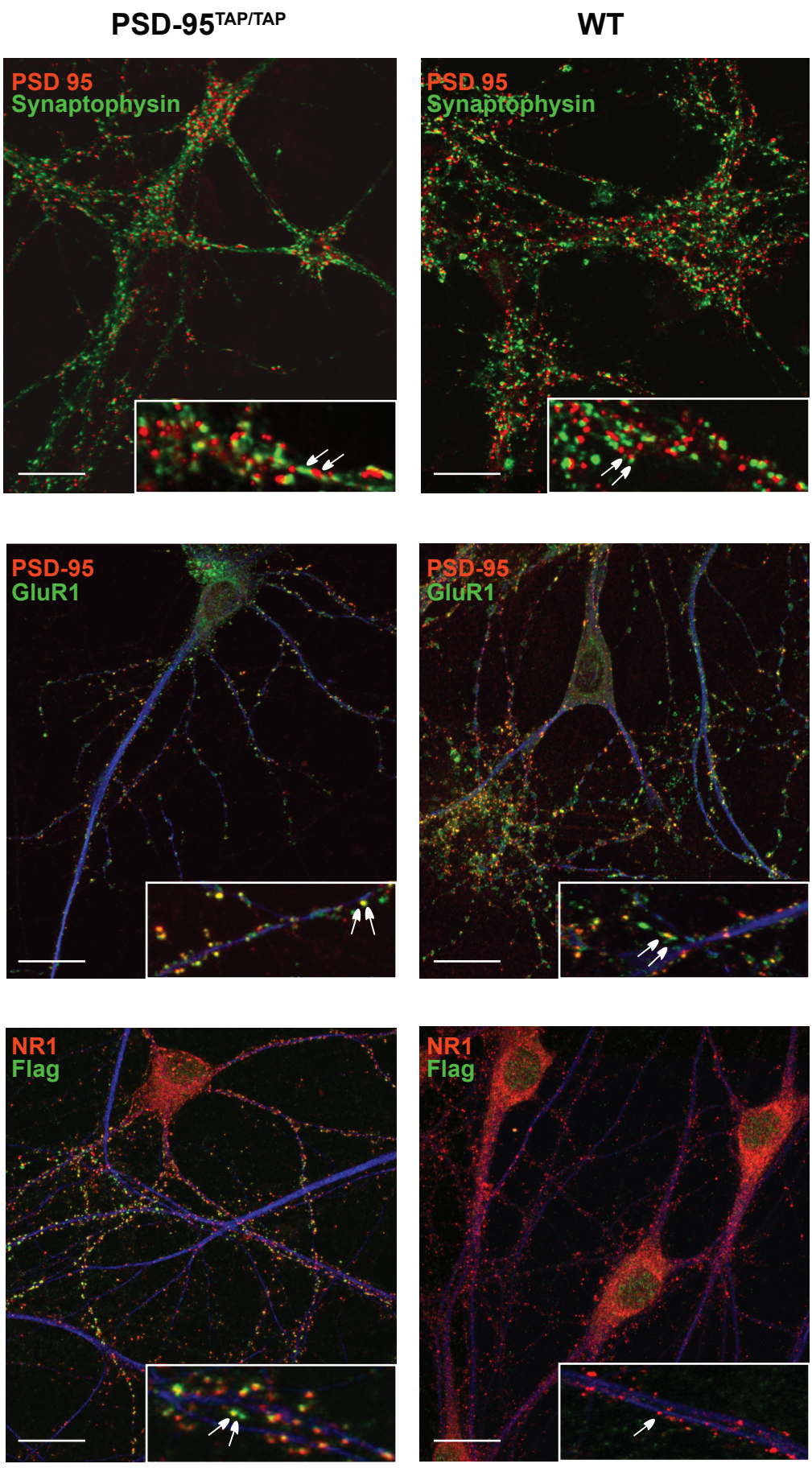
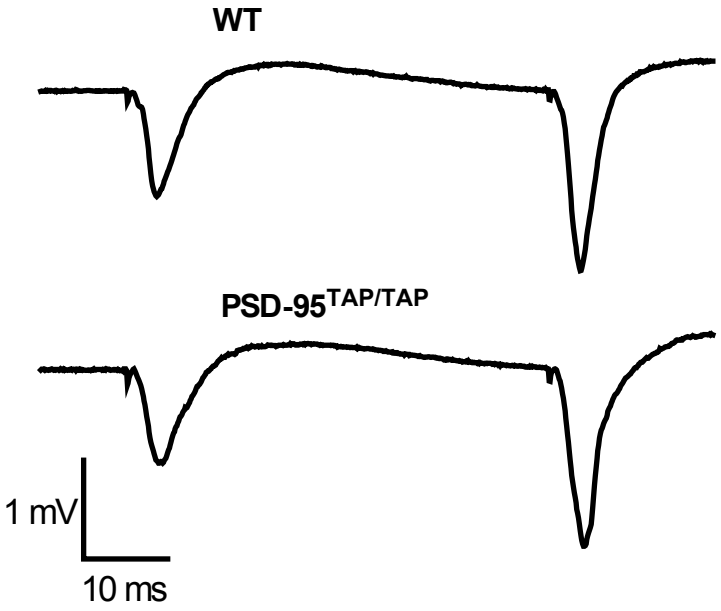
Supplementary Figure 1



Supplementary Figure 2

A



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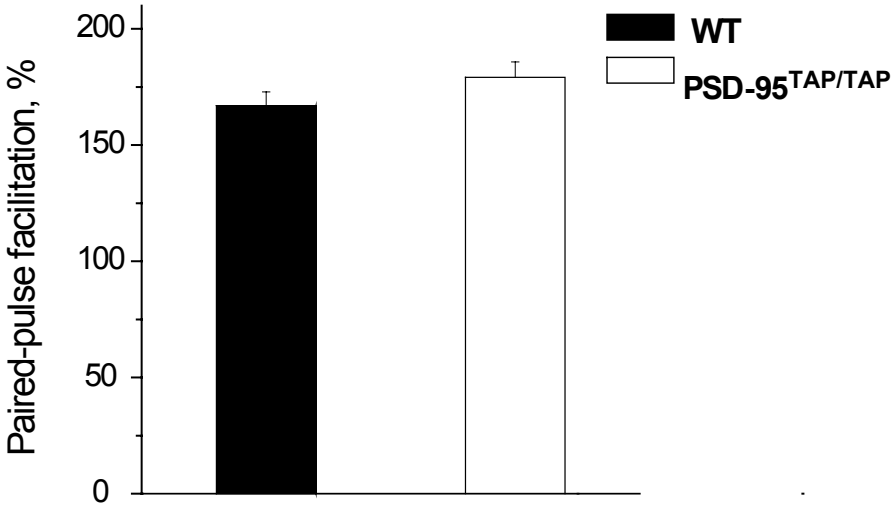
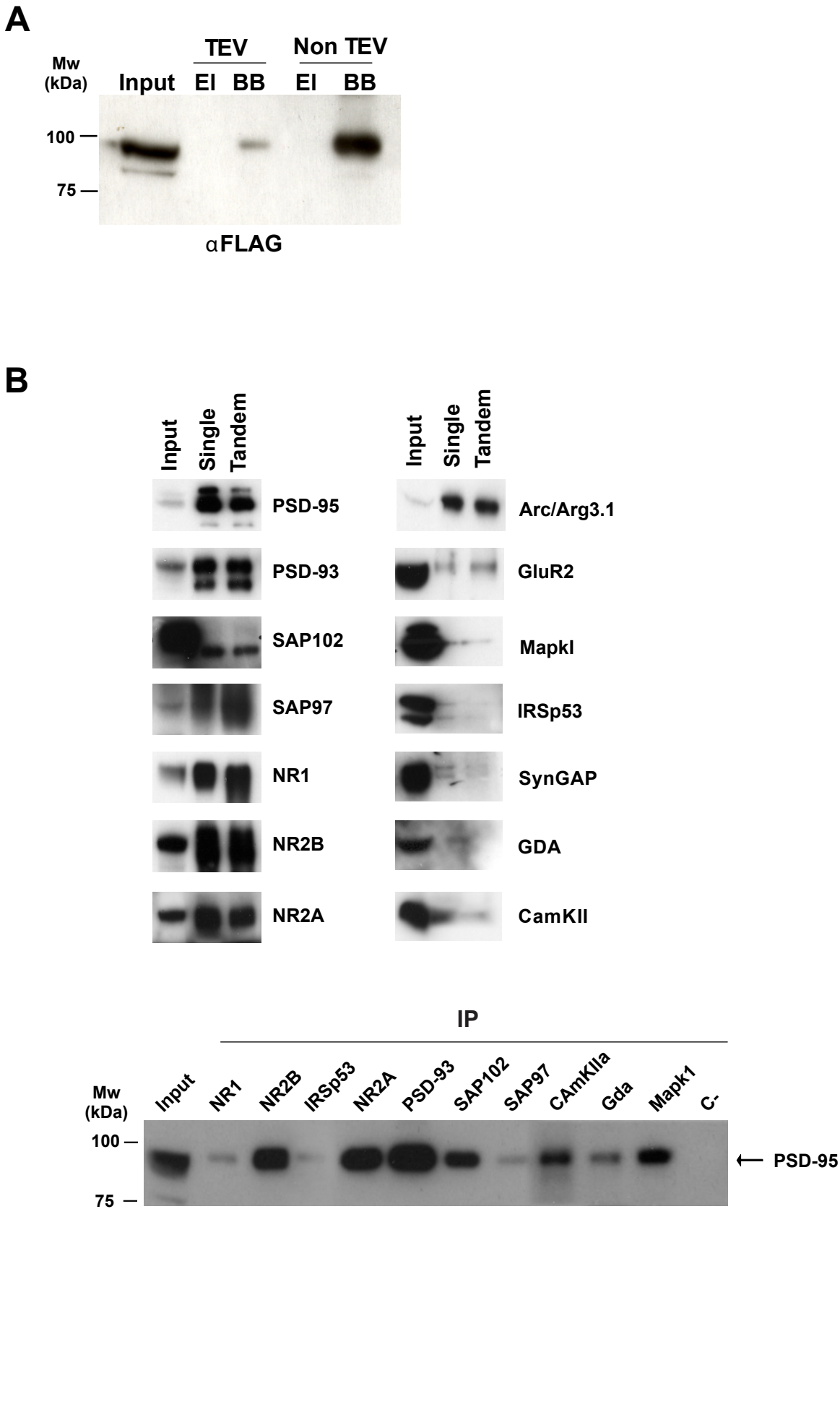


Supplementary Figure 3



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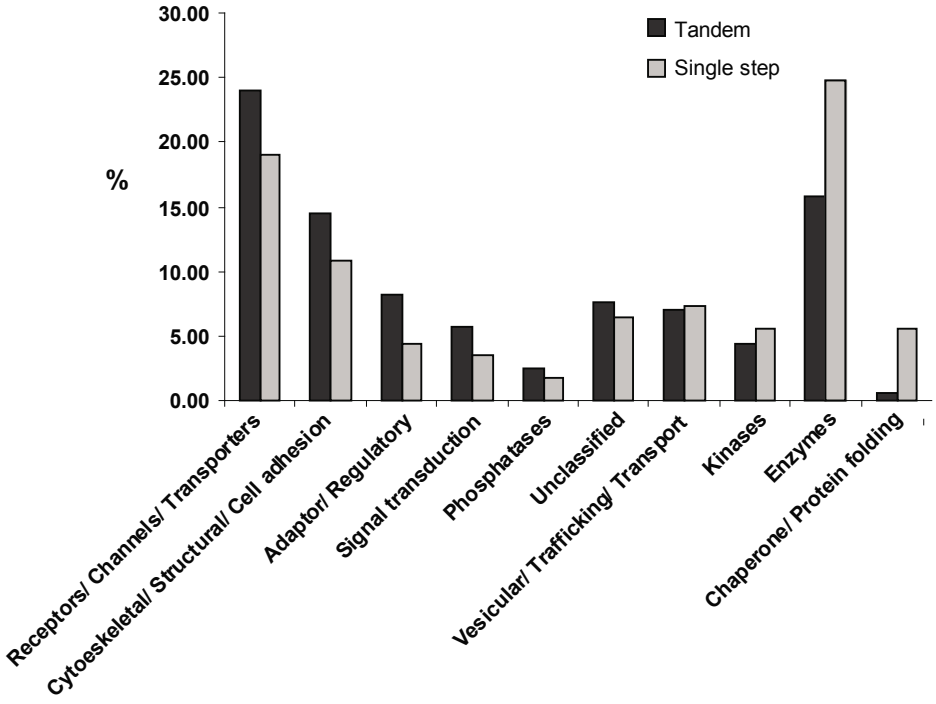
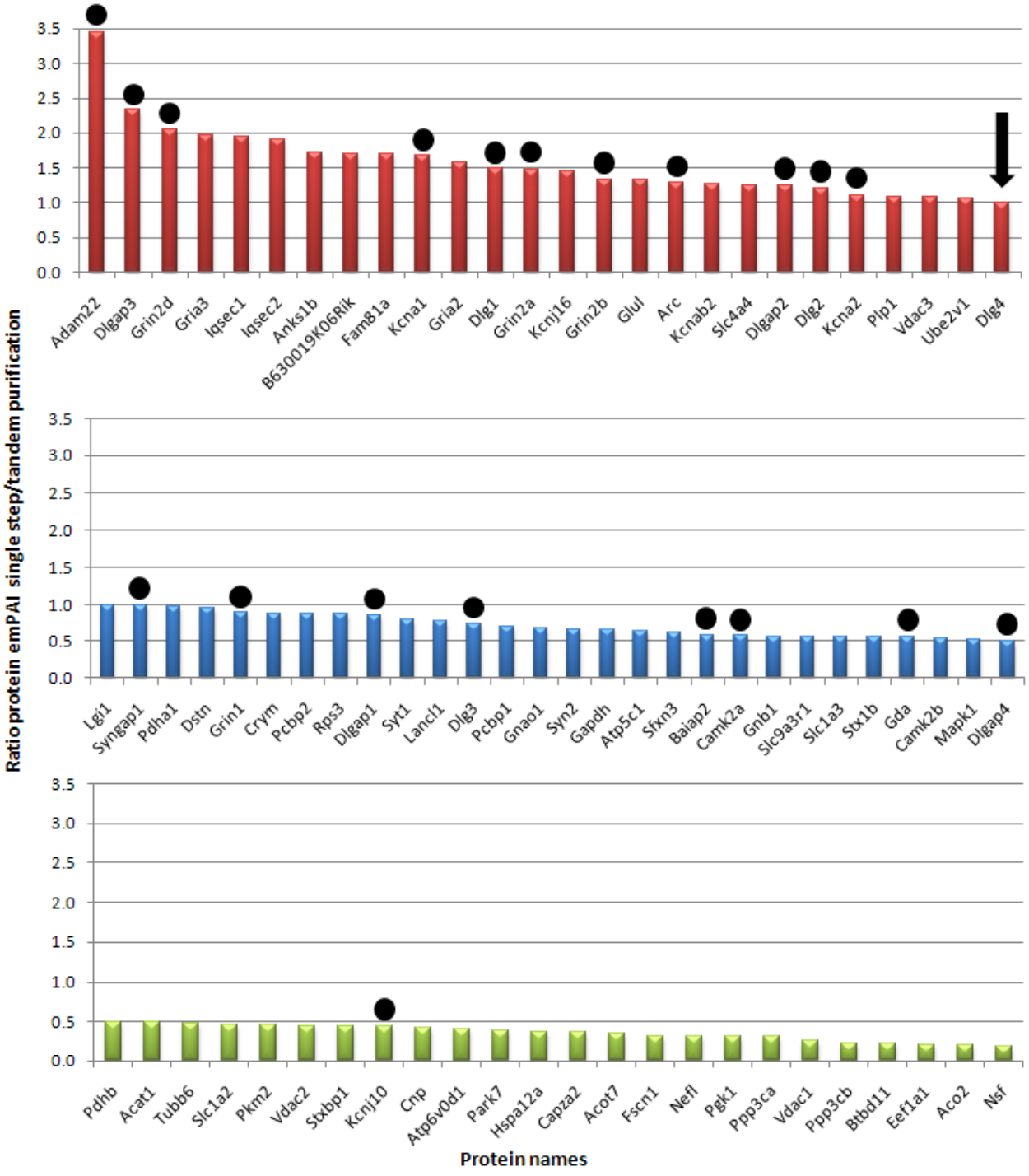


Supplementary Figure 5



Supplementary Figure 6