

## Supporting Information:

### **Proteomic analysis of brain region and sex-specific synaptic protein expression in the adult mouse brain**

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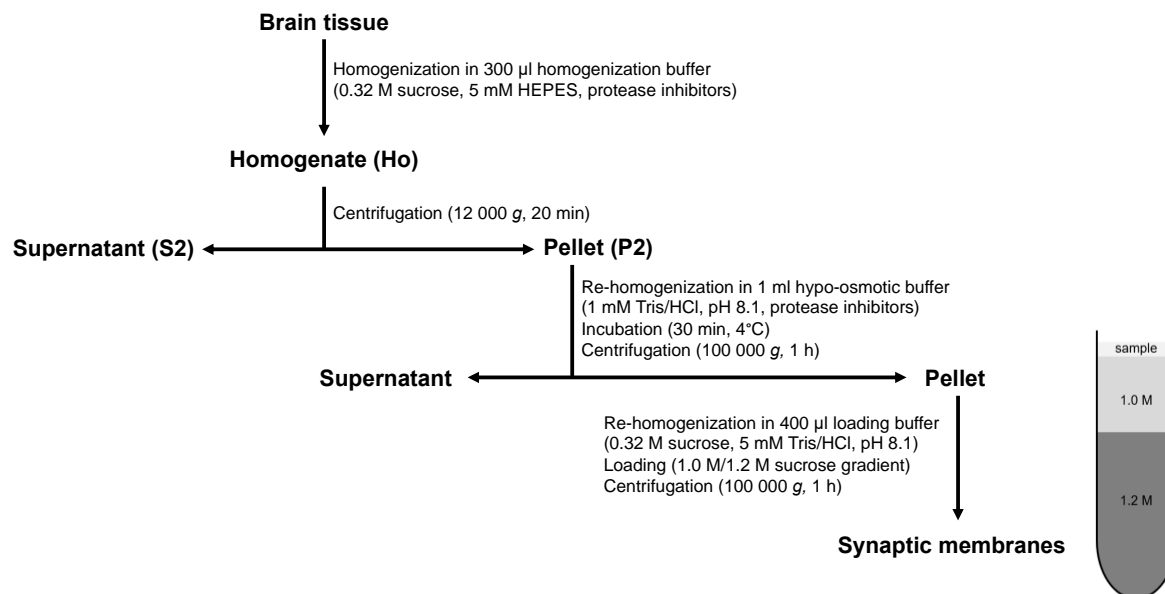
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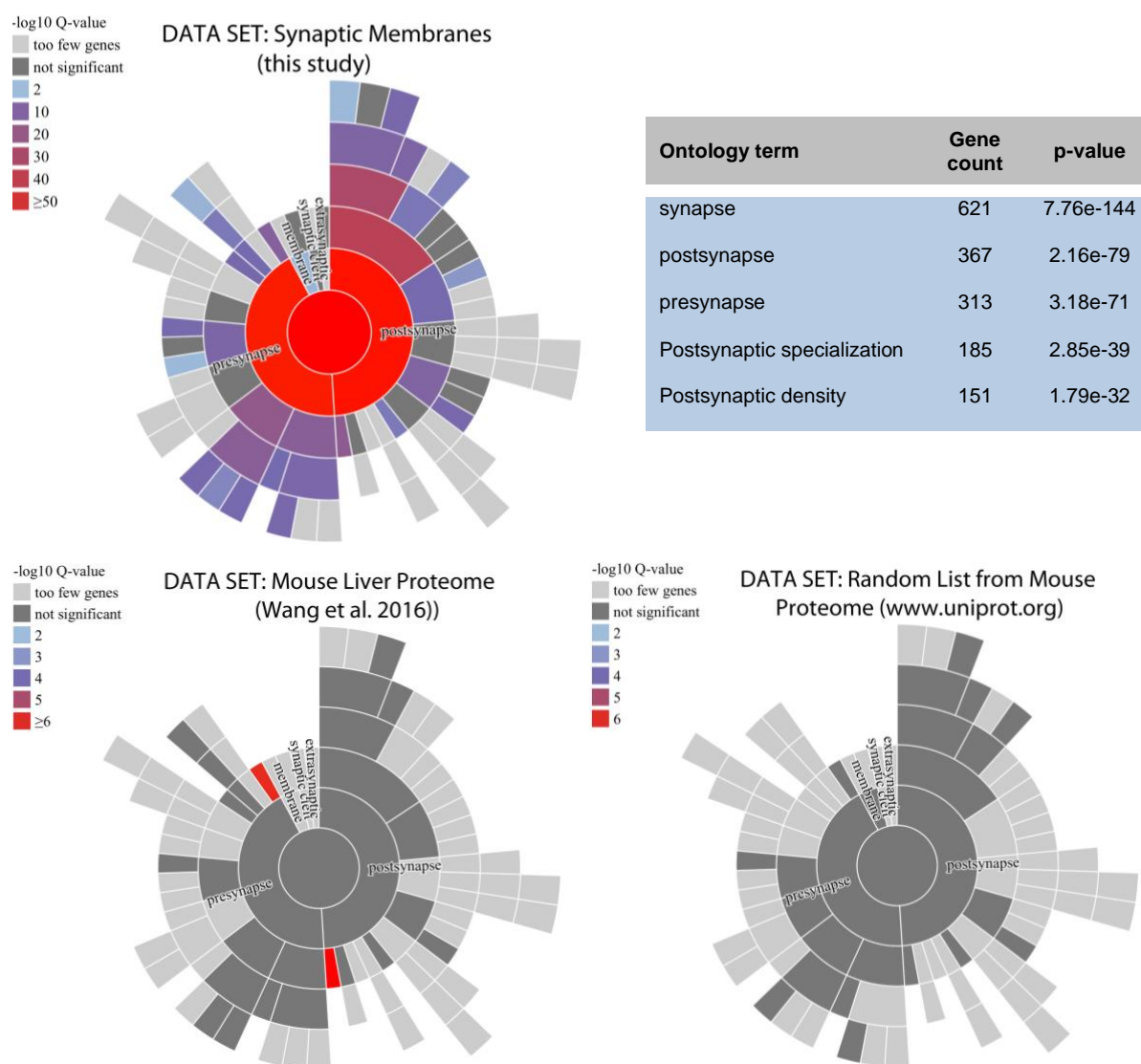
**Supplementary Figure S1. Isolation of synaptic membranes.** For preparation of protein samples enriched for synaptic membrane structures, tissue was first homogenized in 300 µL homogenization buffer (0.32 M sucrose, 5 mM HEPES and Complete™ protease inhibitor cocktail). Afterwards, samples were centrifuged at 12,000 x g for 20 min. Resulting supernatants were discarded and pellets reconstituted in 1 mL of 1 mM Tris/HCl (pH 8.1) containing protease inhibitors and incubated for 30 min at 4°C. After incubation, samples were centrifuged at 100,000 x g for 1 h. Resulting supernatants were discarded and pellets reconstituted in 400 µL of 0.32 M sucrose with 5 mM Tris/HCl (pH 8.1). Afterwards, samples were loaded on a 1.0 M/1.2 M sucrose step gradient and centrifuged at 100,000 x g for 1.5 h. Synaptic membranes were collected at the 1.0 M/1.2 M sucrose interface. For proteome analysis, samples were resuspended in PBS and pelleted to reduce sucrose levels.

This protocol is based on previously published work by various laboratories [1–3] and was modified slightly to adapt to smaller sized samples, i.e. in the range below 100 mg per sample. These modifications concern the following changes:

(1) After homogenization, removal of nuclei and cell debris by low speed centrifugations has been waived assuming that these denser particles will be localized later anyway underneath the 1.2 M sucrose layer together with mitochondria.

(2) In our protocol, we omitted the separation of synaptosomes and subjected the crude membrane fraction (P2) directly to a hypo-osmotic shock using, however, the same conditions as in the protocols cited above. Afterwards, shocked membranes were collected by highspeed centrifugation and separated on a discontinuous sucrose gradient with the resulting "synaptic membrane fraction" at the 1.0 M/1.2 M sucrose interface (similar to the cited protocols) while myelin and "light membranes" remain on top of the gradient and mitochondria, nuclei, debris are found beneath the 1.2 M sucrose layer.

The "synaptic membrane" fraction generated by the present procedure contains synaptic membranes as previously described [1–3] according to their physicochemical properties but may, of course, contain additional material. Therefore, at least an enrichment in synaptic membranes in this fraction can be assumed. In order to proof this assumption, we conducted bioinformatic and immunoblot analyses (see Supplementary Figures 2 and 3).

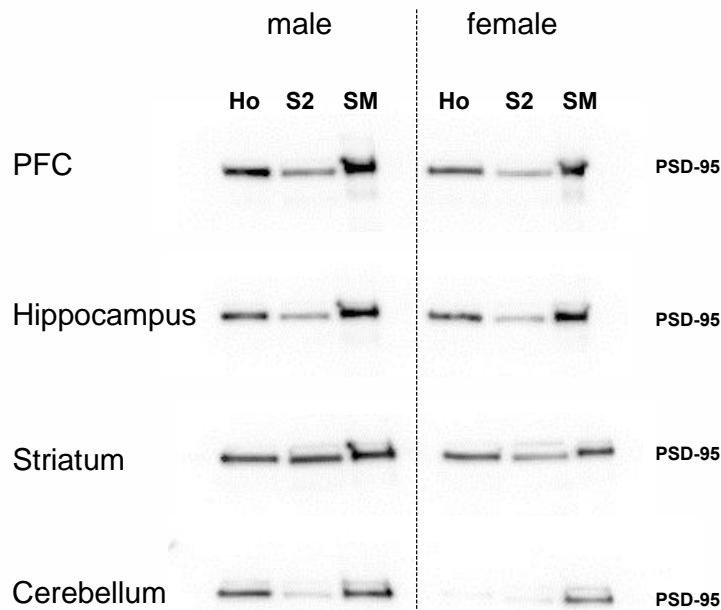


# **Supplementary Figure S2. Comparison of the present dataset with the SynGO protein database.**

To confirm that our preparations are representative for synapse (sub)structures, we compared the full complement of all proteins identified in our study in different conditions to the expert-curated, manually annotated synapse protein database SynGO ([www.syngoportal.org](http://www.syngoportal.org); 1,018 non-redundant protein entries) [4]. To exclude that synapse-associated GO terms are detected in our dataset just by chance, we compared also the overlap of the full mouse liver proteome containing 4,535 independent protein entries [5] and a randomly generated list of 2,000 proteins out of the mouse proteome (generated from [www.uniprot.org](http://www.uniprot.org)). The present figure depicts the overlap for the 3 datasets with the SynGO database as sunburst diagrams.

The dataset generated in the present study shows a high overlap with the SynGO database entries and the major SynGO terms are directly related to the term "synapse" and sub-terms thereof on top. The

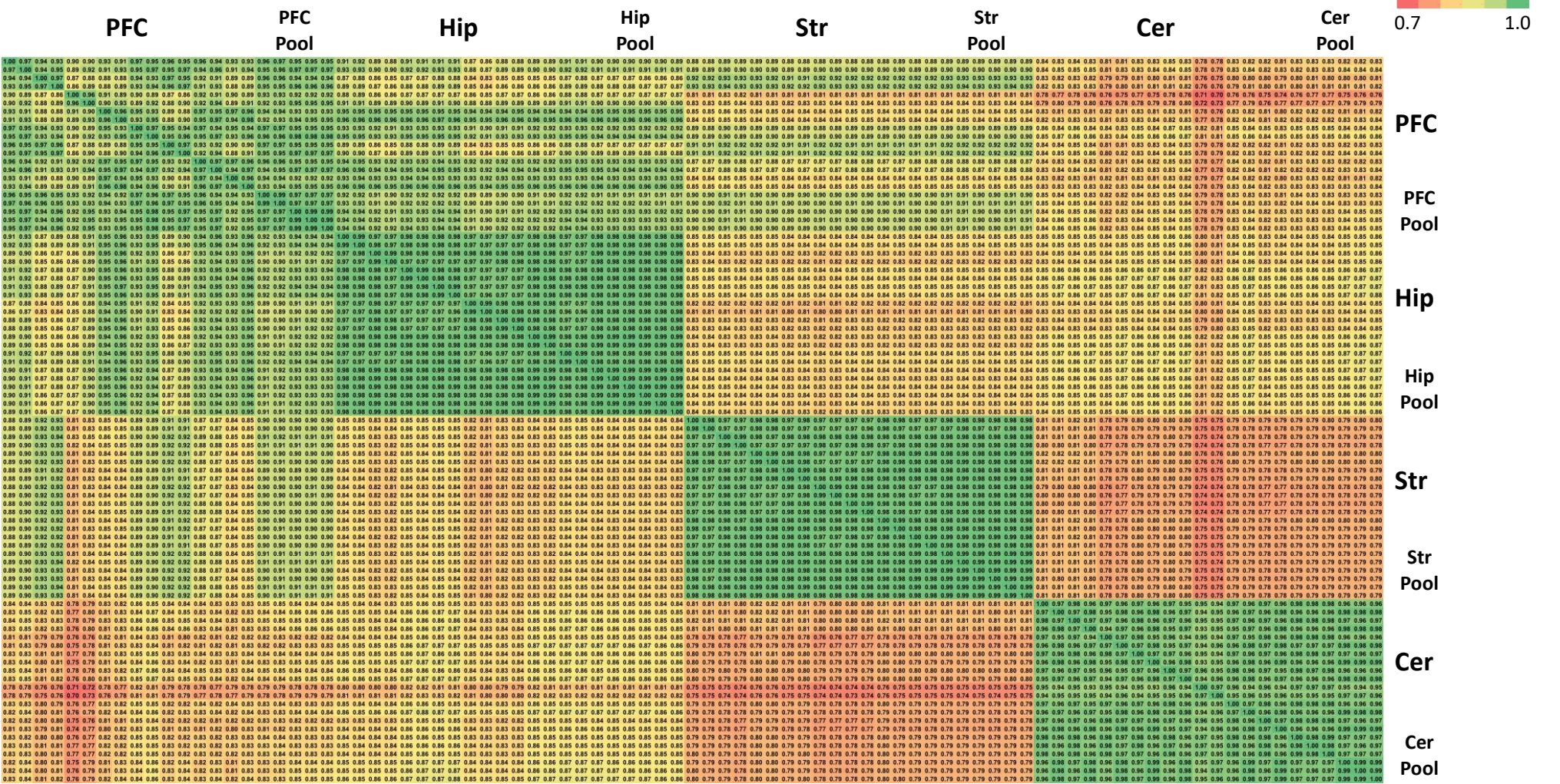
extremely high significance is also reflected in a  $p$ -value below  $10^{-60}$  for the term “synapse”. This is completely different for the mouse liver proteome and even more to the random protein list out of the mouse proteome. Overall, we conclude that our preparation protocol indeed leads to an enrichment of synaptic structures. Based on current knowledge meta-analyses of our protein dataset clearly indicate "synapse" and directly related subterms to be the top GO as well as SynGO terms for cellular compartment and thus localization.



**Supplementary Figure S3. Enrichment of the postsynaptic scaffold protein PSD-95 indicates successful preparation of synaptic proteins.** For each brain region, we performed Western Blot analyses of the following fractions (see also Supplementary Figure 1): homogenate (Ho), soluble fraction (S2), synaptic membrane (SM).

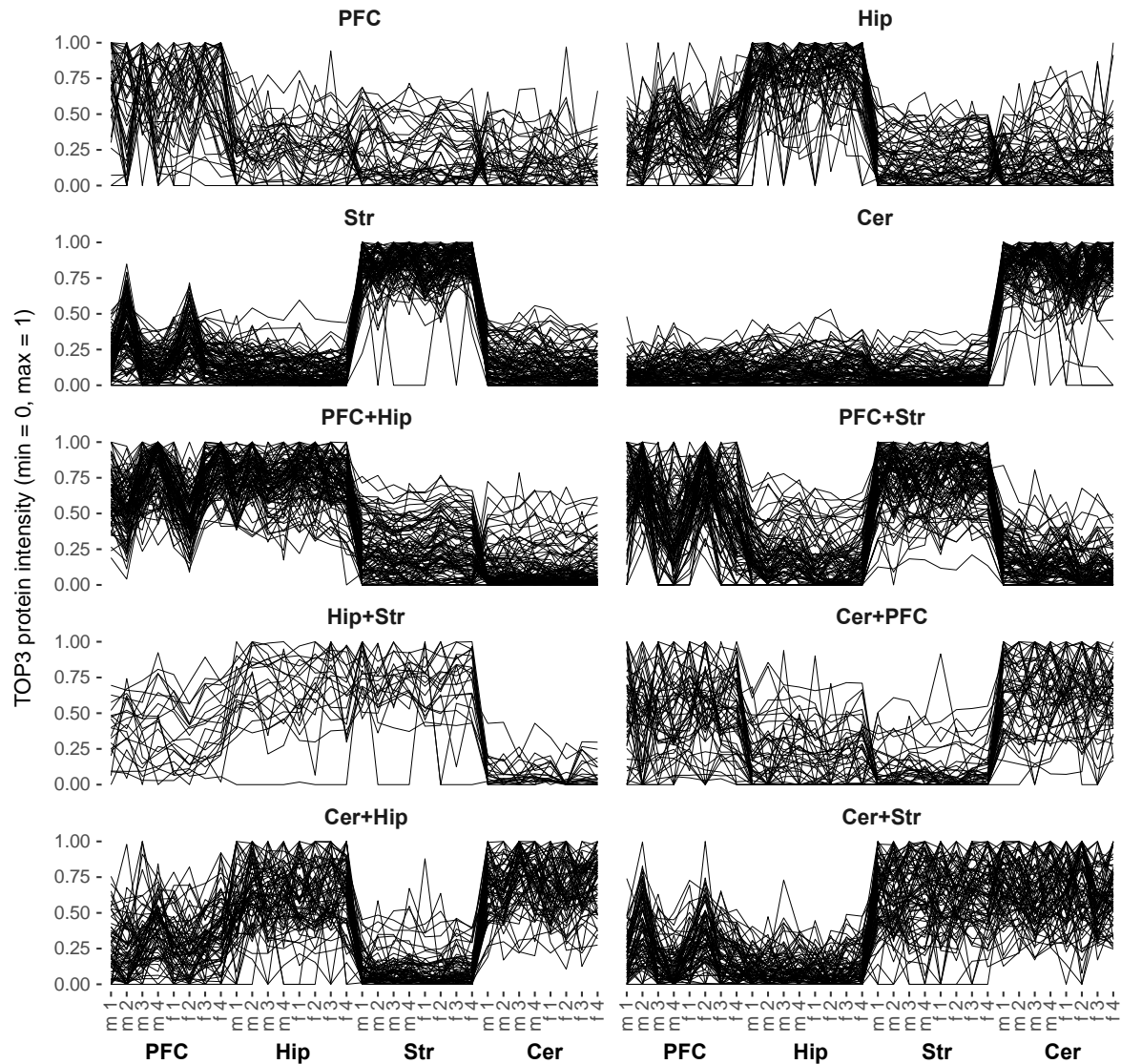
For Western Blot analysis, samples (10 µg of total protein) were mixed with 5x sodium dodecyl sulfate (SDS) loading buffer (10 mL buffer containing 600 µL 1 M Tris base pH 6.8, 1000 µL glycerol, 2000 µL 10%-SDS, 500 µL 0.1%-bromophenol blue, 500 µL β-mercaptoethanol and 5.4 mL Aqua dest.) and heated up to 95 °C for 5 min to denaturate proteins. Protein amounts were estimated using the Molecular probes® Qubit Protein Assay Kit and the Qubit® 2.0 Fluorometer (Thermo Fisher, Waltham, MA, USA) in accordance to the manufacturer's protocols. Samples and Page Ruler prestained protein ladder (SM0671, Thermo Fisher, Waltham, MA, USA) were loaded on an SDS polyacrylamide gel and subjected to electrophoresis. Afterwards, samples were transferred to a 0.45 µm PVDF membrane by semi-dry electroblotting using a TRANS-BLOT® SD Semi Dry Transfer Cell (Bio-Rad, Munich, Germany). The membrane was incubated with an antibody against PSD-95 (1:1000, ab18258, Abcam, Cambridge, UK) over night at 4°C. Protein detection was performed using the BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit) following the manufacturer's instructions (Roche, Basel, Switzerland). As compared to the homogenate/S2 fractions, PSD-95 was found to be enriched in the "synaptic membrane" fraction of each brain region indicating successful preparation of synaptic proteins.





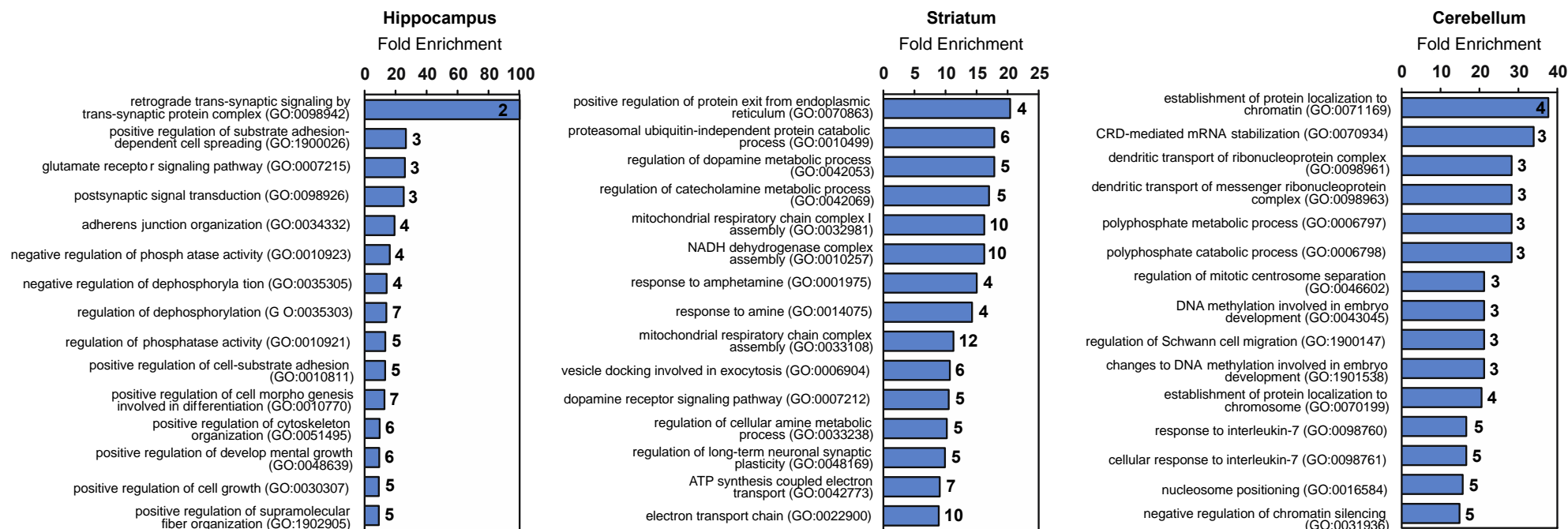
**Supplementary Figure S4. Correlation of protein abundances for all identified synaptic proteins in different brain regions.** In total, we analyzed four biological replicates for each condition (male/female, brain region). Moreover, biological replicates of each brain region independent of gender were pooled and analyzed in five to six technical replicates to monitor LC-MS performance. Calculated Pearson's correlation coefficients between individual LC-MS runs using TOP3-quantification values of all identified proteins indicate high correlation between biological and technical replicates.





**Supplementary Figure S5. Protein correlation profiling reveals distinct expression patterns of synaptic proteins across different brain regions.** The present graph depicts the abundance profiles of the “Top 100” proteins significantly associated to either a single brain region or multiple regions (see Supplementary Table 3). Protein abundances are scaled between zero and their maximum intensity (which has been set to 1) across all biological replicates and brain regions. Group associations for all identified proteins can be found in Supplementary Table 3.



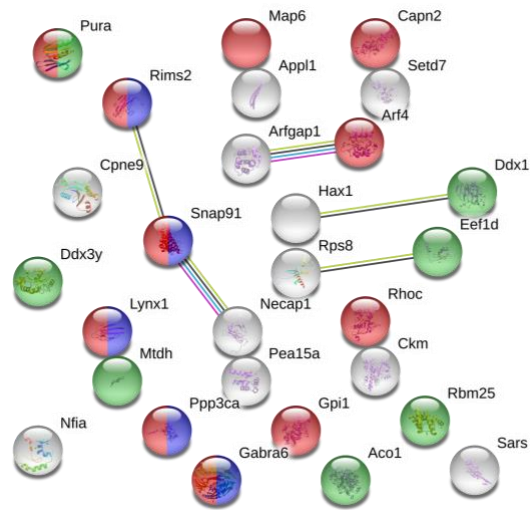


**Supplementary Figure S6. Gene ontology analysis of brain-region specific synaptic proteomes.** Top 15 biological processes (“fold enrichment”) of synaptic

proteins that are significantly associated with a certain brain region (Benjamini-Hochberg correction,  $p < 0.05$ ,  $\text{Log}_2$  fold change compared to other regions  $> 1$ ).

In case of PFC-specific synaptic proteins, no biological process was significantly enriched. In the hippocampus, we mainly detected proteins that are involved in (synaptic) signaling as well as growth regulation. In the striatum, most of the region-specific synaptic proteins are associated with mitochondrial functions. In the cerebellar synapse, we detected a strong enrichment of proteins related to RNA processing.

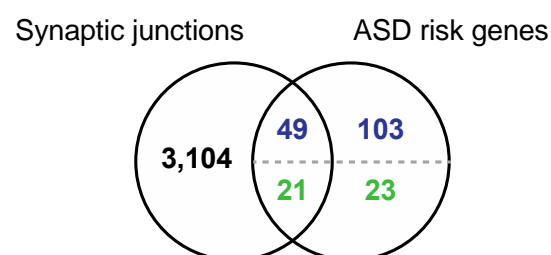




| Cellular Component (GO) |   |                   |                      |
|-------------------------|---|-------------------|----------------------|
| GO-term                 | description                             | count in gene set | false discovery rate |
| GO:0097458              | neuron part                             | 12 of 1732        | 0.00014              |
| GO:0043005              | neuron projection                       | 11 of 1429        | 0.00014              |
| GO:0120025              | plasma membrane bounded cell projection | 12 of 2172        | 0.00049              |
| GO:0043227              | membrane-bounded organelle              | 24 of 9775        | 0.00049              |
| GO:0005829              | cytosol                                 | 14 of 3326        | 0.00068              |
| Biological Process (GO) |   |                   |                      |
| GO-term                 | description                             | count in gene set | false discovery rate |
| GO:0050789              | regulation of biological process        | 25 of 9594        | 0.00065              |
| GO:0050794              | regulation of cellular process          | 24 of 9045        | 0.00077              |
| GO:0009987              | cellular process                        | 26 of 12459       | 0.0076               |
| GO:0010468              | regulation of gene expression           | 14 of 3720        | 0.0108               |
| GO:0007268              | chemical synaptic transmission          | 5 of 321          | 0.0108               |
| Molecular Function (GO) |   |                   |                      |
| GO-term                 | description                             | count in gene set | false discovery rate |
| GO:0005488              | binding                                 | 25 of 10884       | 0.0025               |
| GO:0003723              | RNA binding                             | 7 of 986          | 0.0226               |
| GO:0005515              | protein binding                         | 17 of 6454        | 0.0432               |

**Supplementary Figure S8. Protein interaction network of differentially regulated proteins between male and female mice in the cerebellum.** Network analysis was performed using STRING (version 11.0, <http://string-db.org>). We detected multiple proteins involved in neuron projection and synaptic transmission, such as the scaffold protein RIMS2 or clathrin-associated protein AP180, that are differentially expressed between male and female mice at the cerebellar synapse. Another group of differentially regulated proteins between the two genders is involved in RNA binding.

|  | No. of Genes | Total no. of Genes |
|--|--------------|--------------------|
| SFARI autism gene database                             |              | <b>91</b>          |
| - High confidence (category 1)                         | 25           |                    |
| - Strong candidate (category 2)                        | 66           |                    |
| Rubeis et al. (2014)                                   | 107          | <b>107</b>         |
| Doan et al. (2019)                                     |              | <b>59</b>          |
| - ASD risk genes with LOF mutations                    | 41           |                    |
| - Disease-associated genes with LOF/missense mutations | 18           |                    |
| All studies (w/o duplicates)                           |              | <b>215</b>         |
| Genes/candidates not available for mouse               |              | 19                 |
| Final ASD risk candidates for comparison               |              | <b>196</b>         |
| - Reported exclusively in one study                    | 152          |                    |
| - Reported by multiple studies                         | 44           |                    |



**Supplementary Figure S9. ASD risk gene products identified in our dataset.** We compared the quantitative datasets of altered proteins between male and female wildtype animals with selected autism-associated target genes. In total, we selected 215 ASD risk genes compiled from three sources for the comparison: i) the SFARI autism gene database (<https://gene.sfari.org>) as well as the studies from ii) Rubeis *et al.* [6] and iii) Doan *et al.* [7]. Regarding the SFARI gene set, we included the high confidence (category 1) and strong ASD candidates (category 2) comprising 25 and 66 genes, respectively. Moreover, we incorporated the set of 107 autosomal ASD risk genes (FDR < 0.3) reported by Rubeis *et al.* [6]. In a recent study, Doan *et al.* [7] screened a large ASD cohort for recessive mutations and describe a set of 41 genes that display biallelic loss-of-function mutations and were specifically knocked out in individuals diagnosed with ASD but not in the control group. Moreover, the authors describe 18 disease-associated genes found either in their loss-of-function dataset and/or in a group of genes that harbor biallelic, damaging missense mutations in ASD cases but not in controls. Both, the LOF genes as well as the disease-associated genes, were included in the comparison with our data. Out of the 215 ASD risk candidates, 19 candidates were excluded from the comparison (i.e. no known mouse homologue available, mouse protein not characterized yet, candidate is a non-coding RNA) (Supplementary Table 4). Comparing our data to the selected ASD genes, we identified 70 gene products of ASD risk candidates across the different brain regions.

Blue Letters: ASD risk candidates reported exclusively in one study; green letters: ASD risk candidate reported in multiple studies

| DDX3X_MOUSE (Q62167) ATP-dependent RNA helicase DDX3X |                              |                     |                     |                     | DDX3Y_MOUSE (Q62095) ATP-dependent RNA helicase DDX3Y |                                      |                             |                              |                              |
|---|------------------------------|---------------------|---------------------|---------------------|---|--------------------------------------|-----------------------------|------------------------------|------------------------------|
| 10  | 20                           | 30                  | 40                  | 50                  | 10  | 20                                   | 30                          | 40                           | 50                           |
| MSHVA <b>V</b> ENAL                                   | GLDQQF <b>A</b> GLD          | LNSSDNQ <b>S</b> GG | STASKGRY <b>I</b> P | PHLRNRE <b>A</b> TK | MSQVA <b>A</b> ESTA                                   | GLDQQF <b>V</b> GLD                  | LKSSDNQ <b>N</b> GG         | GNTESKGRY <b>I</b>           | P <b>P</b> HLRNRE <b>T</b> S |
| 60  | 70                           | 80                  | 90                  | 100                 | 60  | 70                                   | 80                          | 90                           | 100                          |
| G <b>F</b> YDKDSSGW                                   | SSSKDK <b>D</b> AYS          | SFGSRGDSRG          | KSSFFGDRGS          | GSRGRFDD <b>R</b> G | KG <b>V</b> CDKDSSG                                   | WSCSKDK <b>D</b> AY                  | SSFSGSRDSRG                 | KPNYFSDRG <b>S</b>           | GSRGRFDD <b>H</b> G          |
| 110   | 120                          | 130                 | 140                 | 150                 | 110   | 120                                  | 130                         | 140                          | 150                          |
| RGDYDGIGGR  | GDRSGFGKFE                   | RGGNSRW <b>C</b> DK | SDEDDWSKPL          | PPSERLEQEL          | RNDYDGIGGR  | DR <b>T</b> GF <b>G</b> KFER         | SGHSRW <b>S</b> DRS         | DEDDWSKPLP                   | P <b>S</b> ERLEQELF          |
| 160   | 170                          | 180                 | 190                 | 200                 | 160   | 170                                  | 180                         | 190                          | 200                          |
| FSGNGTGINF  | EKYDDIPVEA                   | TGNNCP <b>P</b> HIE | SFSD <b>V</b> EMGEI | IMGNIELTRY          | SGGNTG <b>I</b> NF                                    | KYDDIPVEAT                           | GNNCP <b>P</b> HIE <b>N</b> | FSD <b>I</b> EMGE <b>I</b>   | MGNIELTRY <b>T</b>           |
| 210   | 220                          | 230                 | 240                 | 250                 | 210   | 220                                  | 230                         | 240                          | 250                          |
| TRPTPVQKHA  | IPII <b>K</b> EK <b>R</b> DL | MACAQTGS <b>G</b> K | TAAFL <b>L</b> PILS | QIY <b>A</b> DGPGEA | RPTPVQKHAI  | PII <b>K</b> E <b>K</b> RDL <b>M</b> | ACAQTGS <b>G</b> K <b>T</b> | AAFL <b>L</b> PIL <b>S</b> Q | IY <b>T</b> DGPGEAL          |
| 260   | 270                          | 280                 | 290                 | 300                 | 260   | 270                                  | 280                         | 290                          | 300                          |
| L <b>R</b> AMKENG <b>R</b> Y                          | GRRKQY <b>P</b> ISL          | VLAP <b>T</b> RELAV | QIY <b>E</b> EARKFS | YRSRV <b>R</b> PCVV | KAMKENG <b>R</b> Y                                    | RRKQY <b>P</b> ISL <b>V</b>          | LAP <b>T</b> RELAVQ         | IY <b>E</b> EARKFSY          | RSRV <b>R</b> PCVVY          |
| 310   | 320                          | 330                 | 340                 | 350                 | 310   | 320                                  | 330                         | 340                          | 350                          |
| YGG <b>A</b> EIGQ <b>Q</b> I                          | RDLERG <b>C</b> HLL          | VATPGRLVDM          | MERGKIGLDF          | CKYLVLDEAD          | GG <b>A</b> DT <b>V</b> Q <b>Q</b> IR                 | DLERG <b>C</b> HLLV                  | ATPGRLVDM                   | ERGKIGLDFC                   | KYLVLDEAD <b>R</b>           |
| 360   | 370                          | 380                 | 390                 | 400                 | 360   | 370                                  | 380                         | 390                          | 400                          |
| RMLDMGFEPQ  | IRRI <b>V</b> EQDTM          | PPKGVRHTMM          | FSATFPKEIQ          | MLARDFLDEY          | MLDMGFEPQI  | RRIVEQDTMP                           | PKGVRHTMMF                  | SATFPKEIQM                   | LARDFLDEY <b>I</b>           |
| 410   | 420                          | 430                 | 440                 | 450                 | 410   | 420                                  | 430                         | 440                          | 450                          |
| IFLAVGRVGS  | TSENITQK <b>V</b> V          | WVEE <b>I</b> DKRSF | LLDLLNATGK          | DSLTLVFVET          | FLAVGRVGST  | SENITQK <b>V</b> VW                  | VEE <b>L</b> DKRSFL         | LDLLNATGKD                   | SLTLVFVETK                   |
| 460   | 470                          | 480                 | 490                 | 500                 | 460   | 470                                  | 480                         | 490                          | 500                          |
| KKGADSL <b>E</b> DF                                   | LYHEG <b>Y</b> ACTS          | IHGDRSQ <b>R</b> DR | EEALHQFRSG          | KSPILVATAV          | KGADSL <b>E</b> NFL                                   | FQ <b>E</b> RYACTSI                  | HGDRSQ <b>K</b> DRE         | EALHQFRSGR                   | KPILVATAV <b>A</b>           |
| 510   | 520                          | 530                 | 540                 | 550                 | 510   | 520                                  | 530                         | 540                          | 550                          |
| AARGLDISNV  | KHVINFDLPS                   | DIEEYVHRIG          | RTGRVGNLGL          | ATSFNERN <b>I</b>   | ARGLDISNVK  | HVINFDLPSD                           | IEEYVHRIGR                  | TGRVGNLGLA                   | TSFFNERN <b>L</b> N          |
| 560   | 570                          | 580                 | 590                 | 600                 | 560   | 570                                  | 580                         | 590                          | 600                          |
| NITKDLLDL <b>L</b>                                    | VEAKQEVPSW                   | LENMA <b>F</b> EHY  | KGSSRGRSKS          | SRFSGGFGAR          | ITKDLLDL <b>L</b> IV                                  | EAKQEVPSWL                           | ESMAY <b>E</b> HHYK         | GSSRGRSKSR                   | FSGGFGARDY                   |
| 610   | 620                          | 630                 | 640                 | 650                 | 610   | 620                                  | 630                         | 640                          | 650                          |
| DYRQSSGASS  | SSFSSSRASS                   | SRSGGGG <b>H</b> GG | SRGFGGGGYG          | GFYNSDGYGG          | RQSSGS <b>A</b> NAG                                   | FNSN <b>R</b> ANSSR                  | SSGSS <b>H</b> NRGF         | GGGGYGGFYN                   | NDGYGGNYNS                   |
| 660   |                              |                     |                     |                     |   |                                      |                             |                              |                              |
| NYNSQ <b>G</b> VDWW                                   | GN                           |                     |                     |                     | QA <b>V</b> DWWGN                                     |                                      |                             |                              |                              |

## Supplementary Figure S10. Sequence coverage of DDX3X and DDX3Y. DDX3X and DDX3Y

exhibit a sequence homology of 90%. Bold red letters indicate amino acids that differ between the two protein sequences. To estimate which peptides will be generated and could be theoretically identified in our bottom-up proteomics experiment, we performed an in-silico digest of both proteins mimicking the experimental settings of our proteomics approach (i.e. trypsin digestion) as well as the filter criteria applied for peptide identification (i.e. zero missed cleavages, minimum length of 7 amino acids). Blue letters mark homologue peptides between DDX3X and DDX3Y. Red letters highlight peptides that are not shared between DDX3X and DDX3Y. Theoretically, around 85% and 88% of the DDX3X and DDX3Y sequences could be covered. In total, we identified nineteen shared as well as five unique peptides for each protein. In case, of DDX3X we additionally detected three peptides that are not shared with DDX3Y but with another protein, DDX3L.

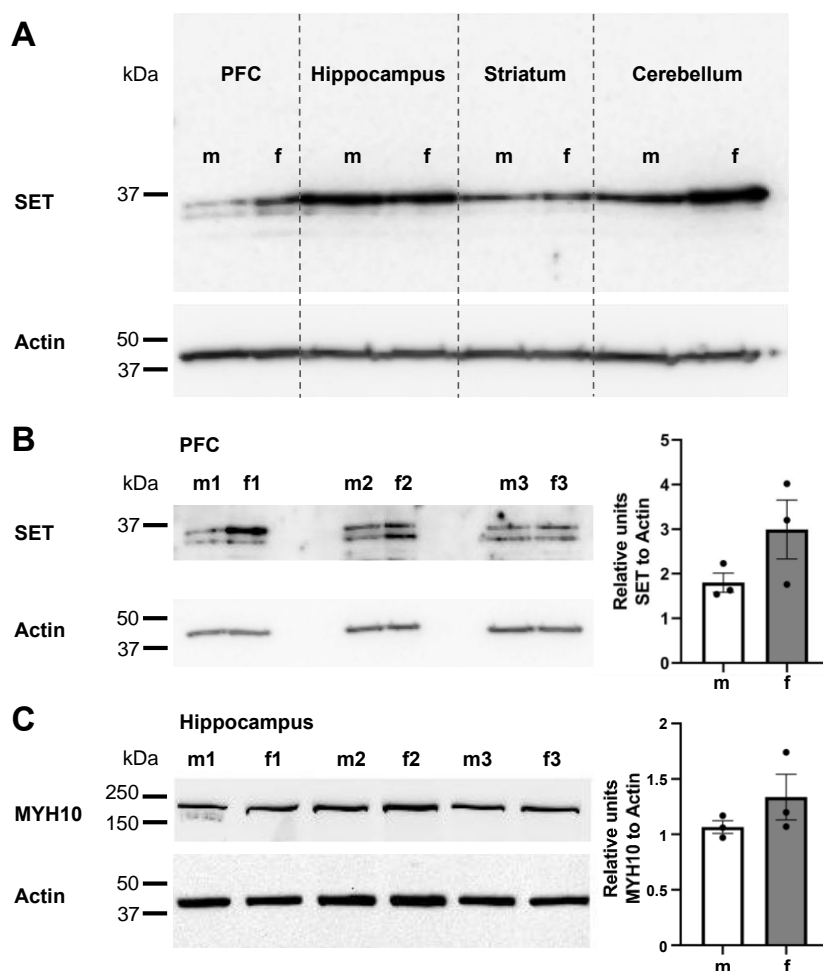
Orange box: identified unique peptides; grey box: identified shared peptides between DDX3X and DDX3Y; purple box: identified peptides in DDX3Y that are not shared with DDX3Y but other proteins (i.e. DDX3L)



| Sequence                 | PFC male | PFC female | Hip male | Hip female | Str male | Str female | Cer male | Cer female | Entries Post homology  |
|--------------------------|----------|------------|----------|------------|----------|------------|----------|------------|--|
| KPILVATAVAAR             | X        |            | X        |            | X        |            | X        |            | DDX3Y_MOUSE  |
| VVWVEELDKR               | X        |            | X        |            | X        |            | X        |            | DDX3Y_MOUSE  |
| GADSLNLFQER              | X        |            | X        |            | X        |            | X        |            | DDX3Y_MOUSE  |
| GVCDKSSGWSCSK            | X        |            | X        |            | X        |            | X        |            | DDX3Y_MOUSE  |
| TAAFLLPILSQIYTDGPGEALK   | X        |            |          |            | X        |            |          |            | DDX3Y_MOUSE  |
| SSFFGDR                  |          |            |          |            |          |            | X        | X          | DDX3X_MOUSE  |
| QSSGASSSSFSRSSR          |          |            |          |            |          |            |          | X          | DDX3X_MOUSE  |
| GFYDKDSSGWSSSK           | X        | X          | X        |            | X        | X          | X        | X          | DDX3X_MOUSE  |
| VRPCVVYGGAEIGQQIR        | X        | X          | X        | X          | X        | X          | X        | X          | DDX3X_MOUSE  |
| TAAFLLPILSQIYADGPGEALR   | X        | X          |          |            | X        | X          | X        | X          | DDX3X_MOUSE  |
| SPILVATAVAAR             | X        | X          |          | X          |          | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE                                       |
| QEVPSWLENMAFEHHYK        |          |            |          |            |          |            | X        |            | DDX3L_MOUSE, DDX3X_MOUSE                                       |
| GADSFLEDFLYHEGYACTSIHGDR | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE                                       |
| GLDISNVK                 | X        | X          | X        | X          |          |            | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| FSGGFGAR                 | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| LVDMMER                  | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| DKDAYSSFGSR              | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| HAIPK                    | X        | X          | X        | X          |          |            | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| IGLDFCK                  | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| YTRPTPVQK                | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| DLLDLLVEAK               | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| VGSTSENITQK              |          |            |          |            |          |            | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| SFLDLLNATGK              | X        | X          |          |            | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| DREEALHQFR               | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| ELAVQIYEER               | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| VGNLGLATSFNER            | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| DFLDEYIFLAVGR            | X        | X          |          |            | X        | X          | X        |            | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| LEQELFSGGNTGINFEK        | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| HVINFDLPDIEEYVHR         | X        | X          | X        | X          | X        | X          | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| DLMACAQTGSGK             | X        | X          | X        | X          | X        |            | X        | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| RDLMACAQTGSGK            |          | X          |          | X          |          |            |          | X          | DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE                          |
| MLDMGFEPQIR              | X        | X          | X        | X          | X        | X          | X        | X          | DDX17_MOUSE, DDX3L_MOUSE, DDX3X_MOUSE, DDX3Y_MOUSE, DDX5_MOUSE |

**Supplementary Figure S11. Overview of identified DDX3X and DDX3Y peptides.** Orange: identified unique peptides; grey: identified shared peptides

between DDX3X, DDX3Y and other proteins; purple: DDX3X peptides that are shared with DDX3L.



**Supplementary Figure S12. Expression levels of SET and MYH10 differ in male and female animals in synaptic membranes of distinct brain regions.** (A) Immunoblot analysis of SET in synaptic membranes enriched from different brain regions (PFC, Hippocampus, Striatum, Cerebellum) reveals slightly higher SET expression levels in female as compared to male animals in the PFC and the cerebellum. Higher SET expression in the PFC of female mice was also observed by LC-MS. (B,C) Comparison of (B) SET and (C) MYH10 levels from female (f) and male (m) mice in synaptic membranes from PFC and Hippocampus, respectively. Samples were analyzed by immunoblotting using antibodies against SET (cat. no. GTX113834, GeneTex, Irvine, CA, USA), MYH10 (cat. no. sc-376942, Santa Cruz Biotechnology, Dallas, TX, USA) and  $\beta$ -Actin (cat. no. A1978, Merck, Darmstadt, Germany). Protein detection was performed using the BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit) following the manufacturer's instructions (Roche, Basel, Switzerland). Signals were quantified using the QuantityOne software from Bio-Rad (version 4.6.9). Intensities of SET and MYH10 were normalized to the Actin signal and are plotted in the right panels in (B) and (C).

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