- Supplementary Information -

Temporal quantitative proteomics of mGluR-induced protein translation and phosphorylation in neurons

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Supplementary Dataset 1. Overview of *de novo* synthesized proteins upon DHPG stimulation.

Supplementary Dataset 2. Significantly regulated phosphorylation sites upon DHPG stimulation.

Supplementary Figures

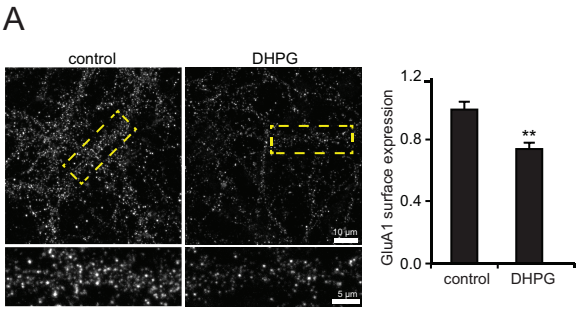
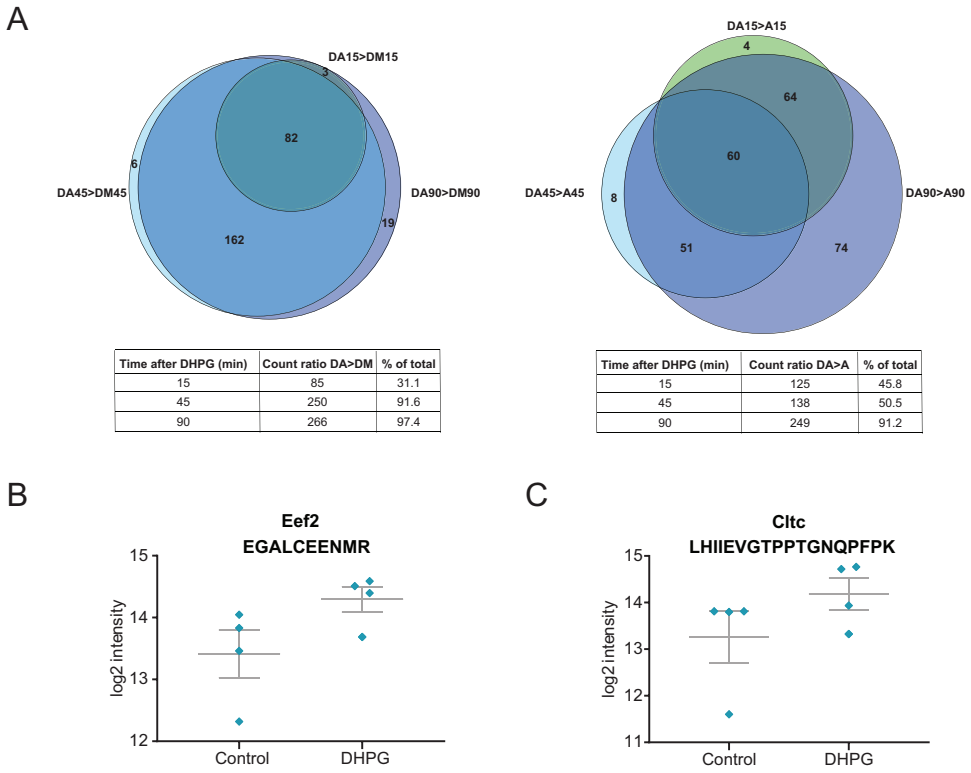


Figure S1. DHPG induces mGluR-mediated AMPAR internalization. DHPG stimulation induces significant GluA1 internalization. Data are represented as mean±SEM. ** p<0.01.



Time after DHPG (min)	Count ratio DA>DM	% of total
15	85	31.1
45	250	91.6
90	266	97.4

Time after DHPG (min)	Count ratio DA>A	% of total
15	125	45.8
45	138	50.5
90	249	91.2

Figure S2. Evaluation of the AHA and TMT dataset. (A) Venn diagrams showing the overlap of proteins identified in the different experimental conditions. As expected, the number of translated proteins increase over time, and the vast majority of proteins identified are stably identified among all studied time points. DA – DHPG and AHA, DM – DHPG and methionine, A – AHA only. Quantification of SRM traces of Eef2 (B) and Cltc (C) 15 minutes after DHPG stimulation versus control.

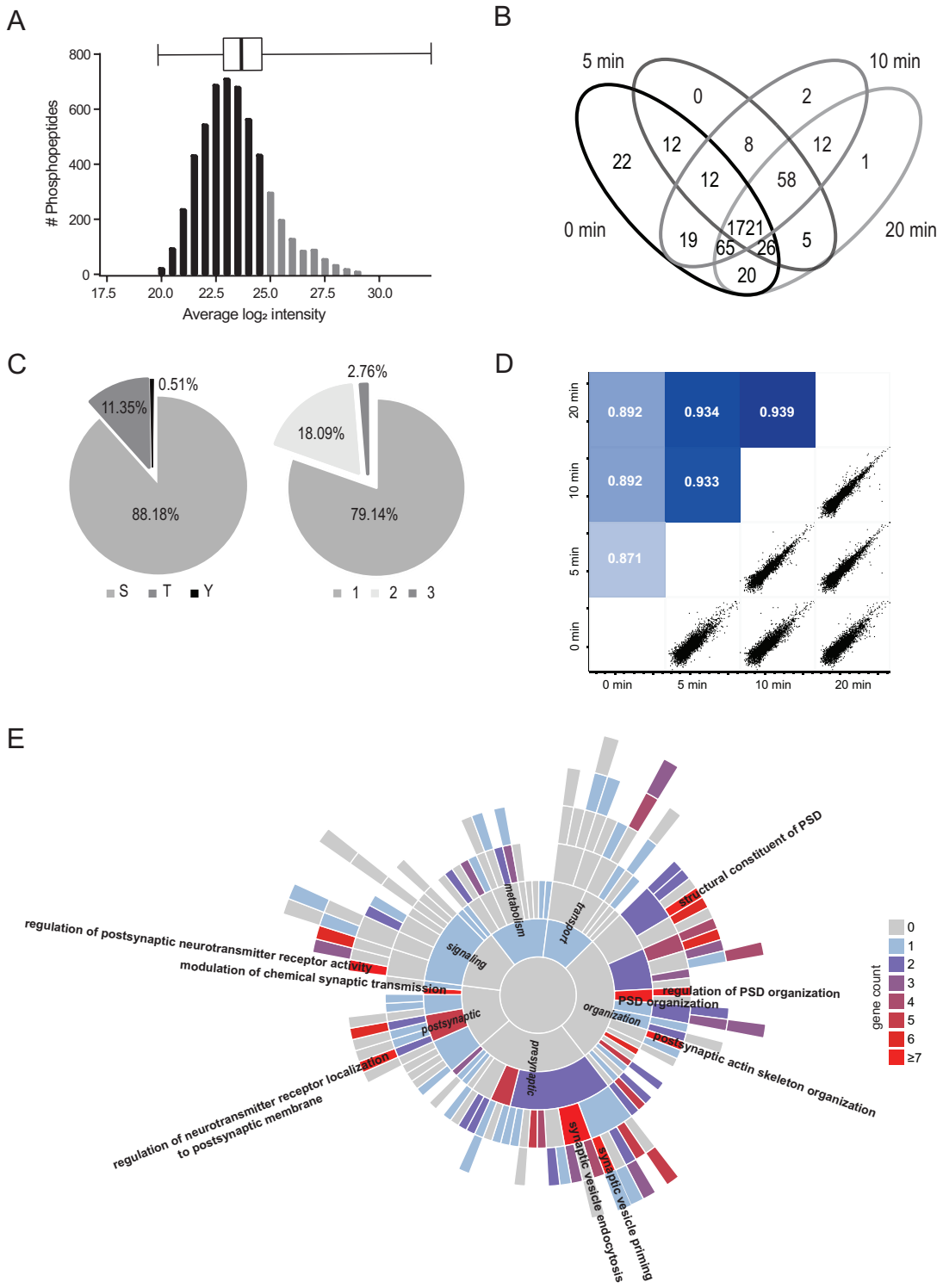


Figure S3. Evaluation of the phosphoproteomics dataset. (A) Distribution of phosphopeptide abundance, displaying normal distribution. Light grey bars indicate the top 25% most abundant phosphopeptides. (B) Venn diagram of the overlap between proteins identified in the 5, 10, and 20 minutes DHPG experiment, as well as the control condition. Biological replicates were combined. (C) Percentages of enriched serine, threonine, and

tyrosine phosphosites, and the distribution of singly, doubly and triply phosphorylated peptides. (D) Heatmap of Pearson correlations and correlation plots for the different biological replicates in the DHPG stimulated and control samples, showing high quantitative reproducibility between all measurements. (E) SynGO enrichment analysis of biological process. Processes with highest gene counts are labeled.

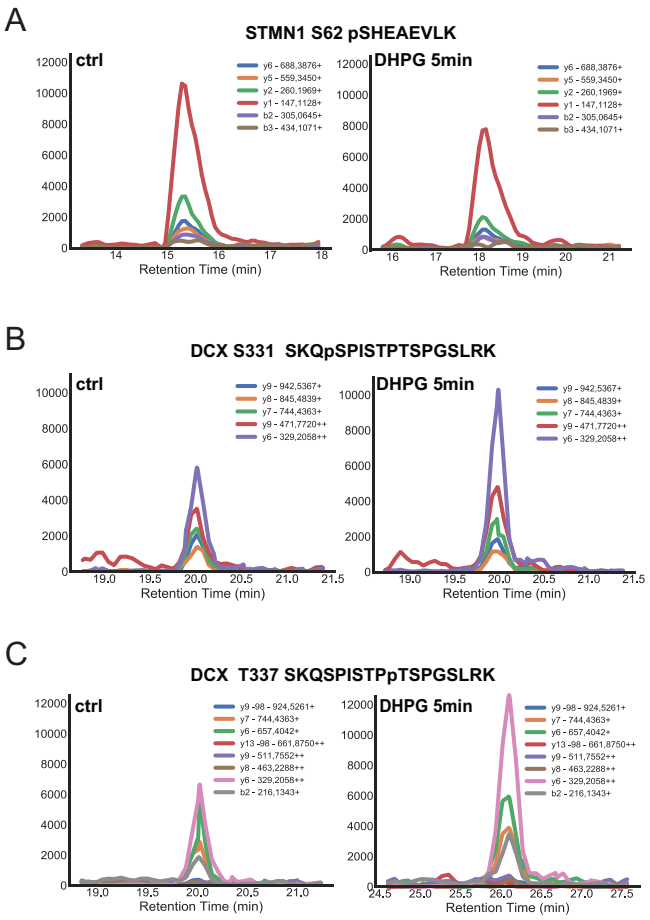


Figure S4. SRM validation of significantly regulated phosphorylation sites.

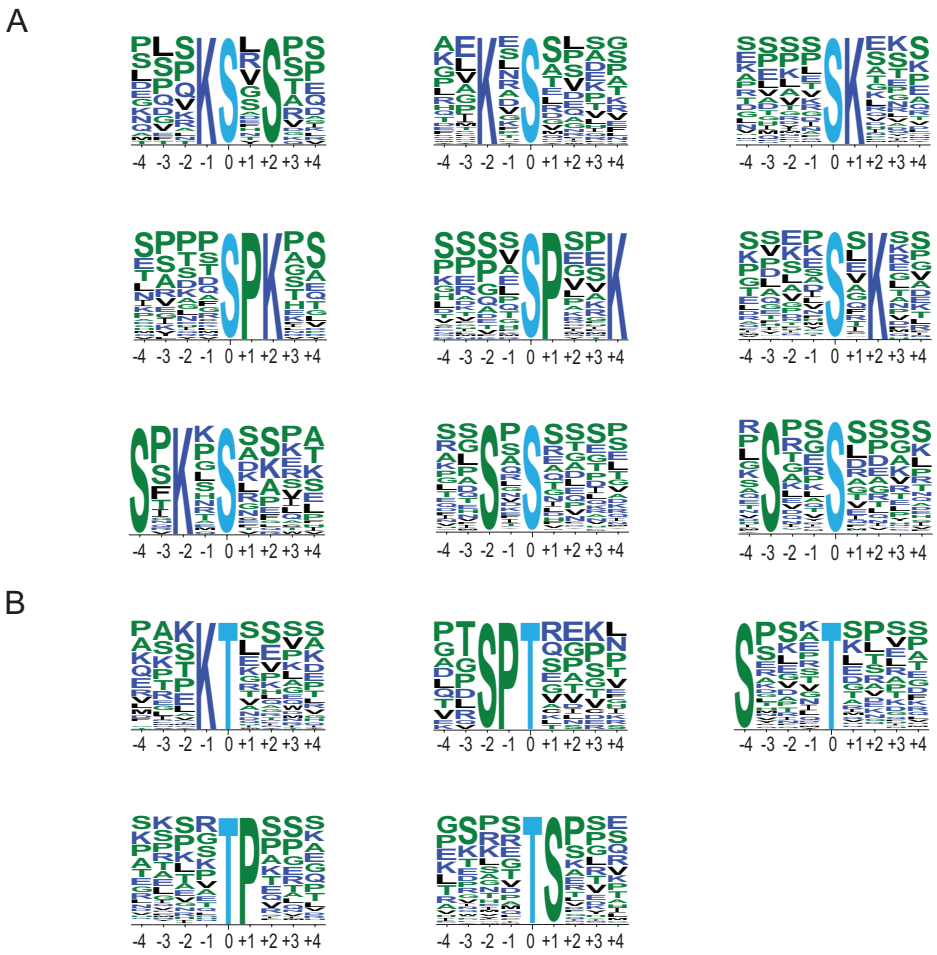


Figure S5. Phosphorylation sequence motifs. (A) Significantly enriched serine-directed phosphorylation motifs as generated with MotifX. (B) Significantly enriched threonine-directed phosphorylation motifs.

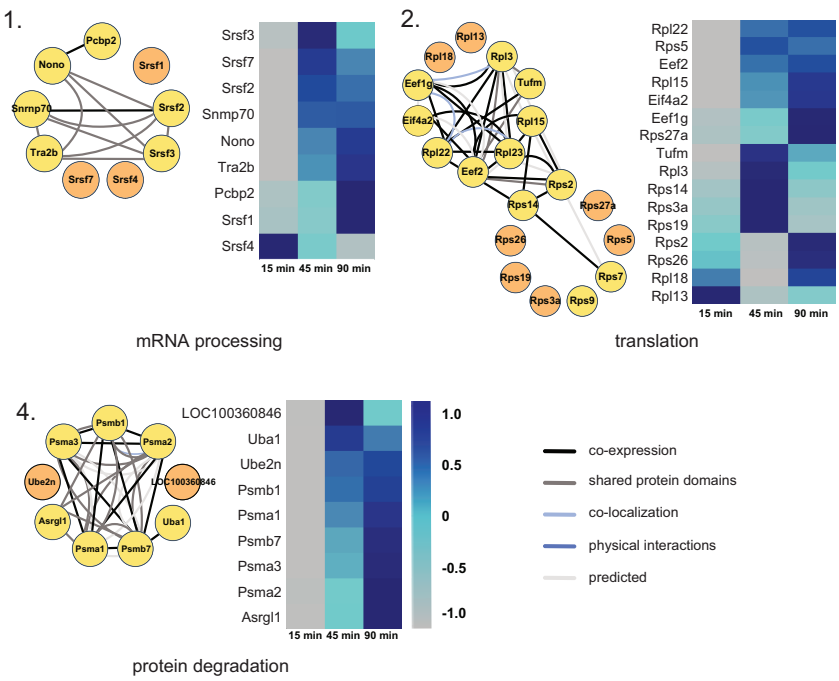


Figure S6. Supplemented interaction-based protein clusters of newly translated proteins. Protein clusters of enriched GO terms are displayed with their known interaction profiles (yellow), supplemented with less studied proteins from the translation dataset with similar function (orange). Heatmaps represent the z-score normalized ratio DHPG AHA / pool over the three measured time points.