Dynamin I phosphorylation by GSK3 controls activity-dependent bulk endocytosis of synaptic vesicles

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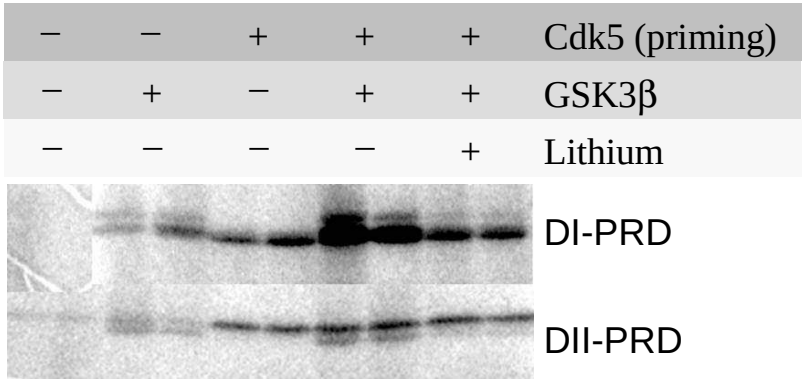
a



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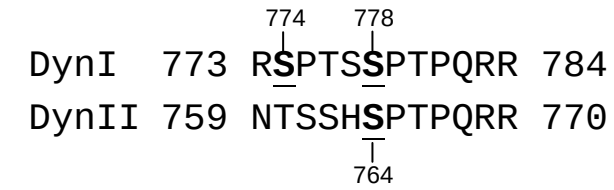
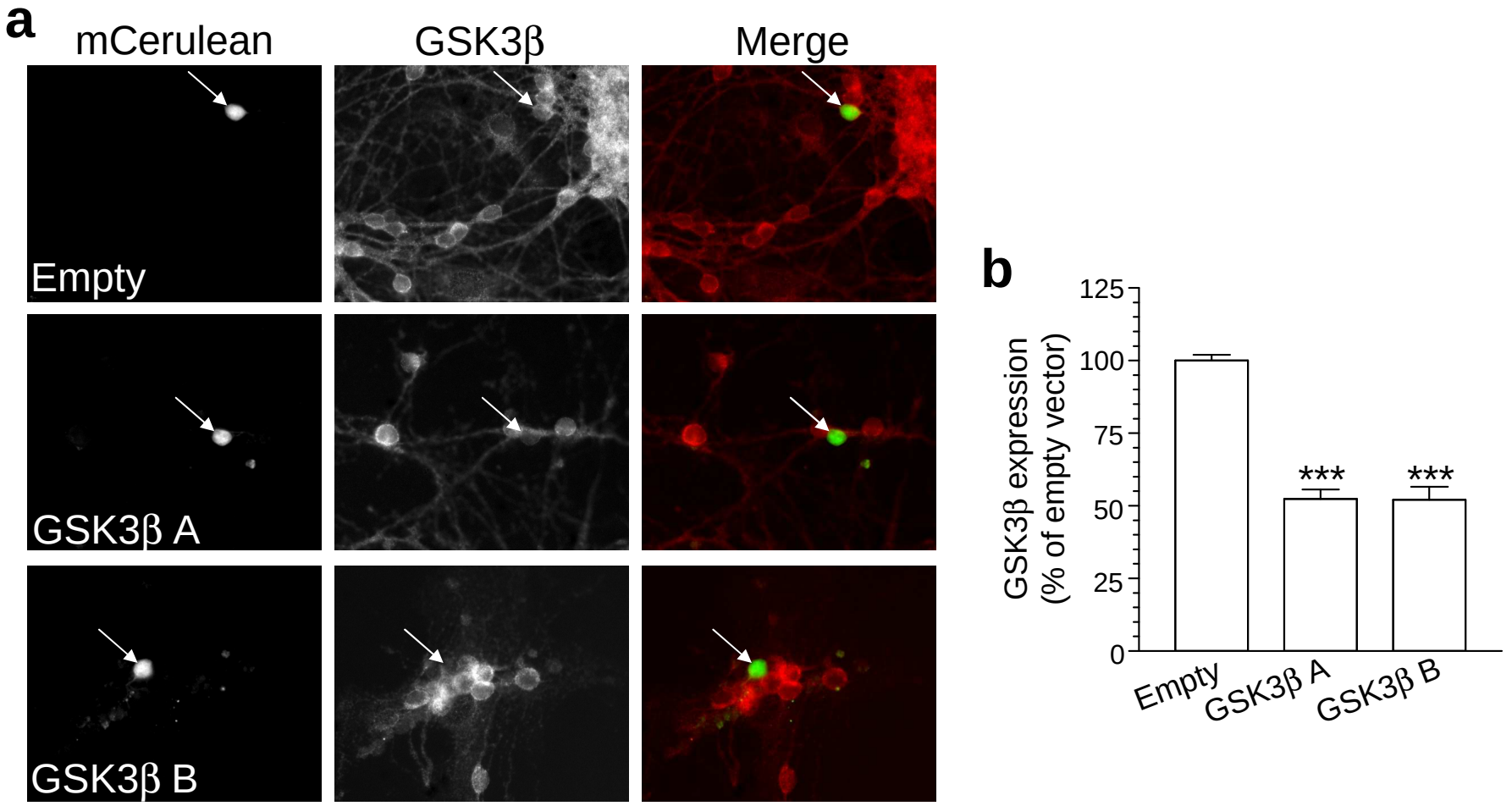
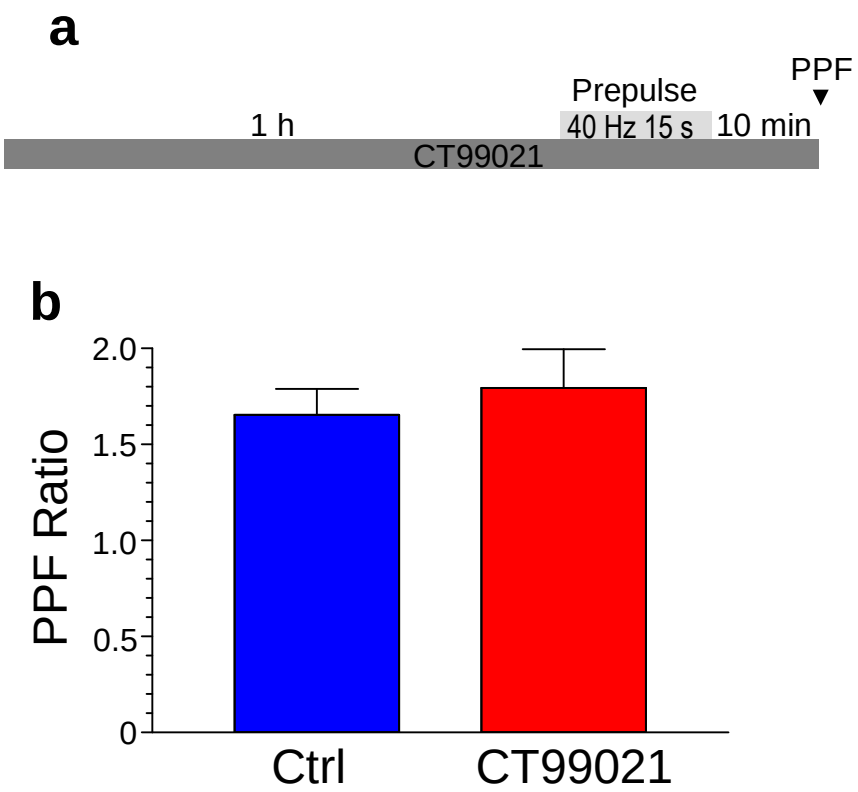


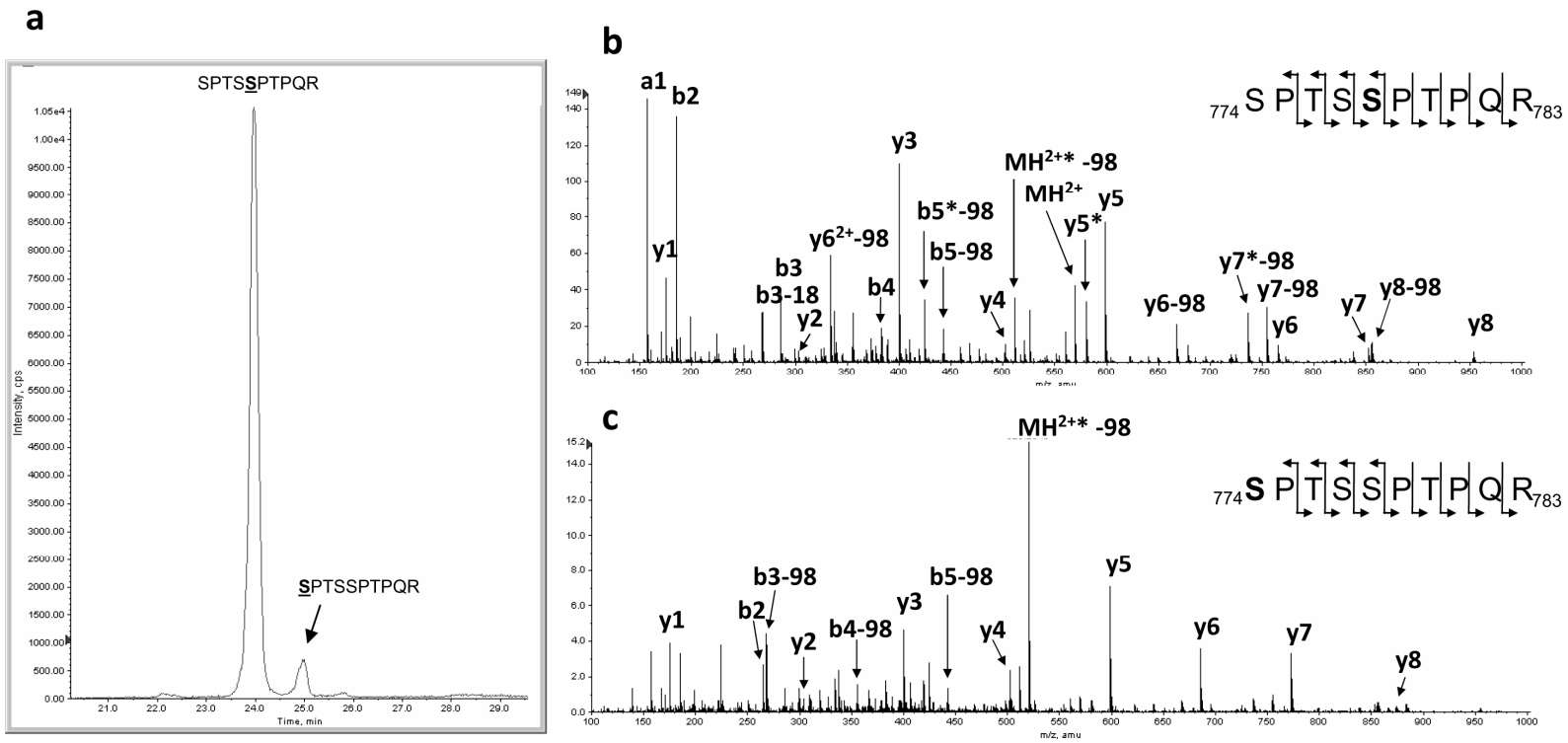
Supplementary Figure 1 - *Dynamin II is not phosphorylated by GSK3β.* **a**) DynI-PRD and DynII-PRD were treated in an identical manner to Figure 1**b** and visualized by autoradiography (upper panel). No GSK3β -dependent phosphorylation of DynII-PRD was observed. **b)** Partial amino acid sequence alignment of dynamin I and II is shown (lower panel). Note that while Ser-778 is conserved in the amino acid sequence of dynamin II (Ser-764), Ser-774 is not. Results are shown in duplicate.



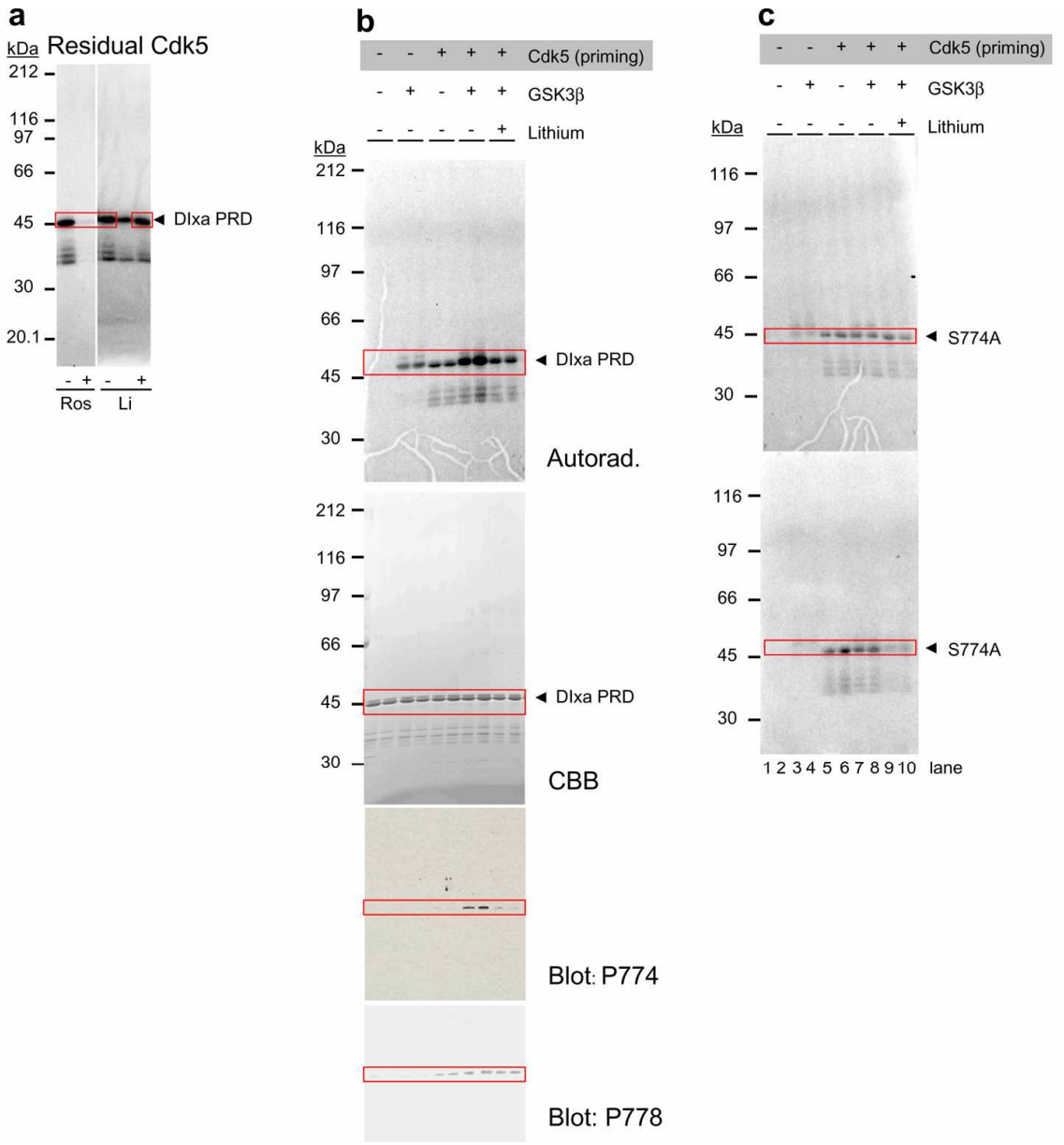
Supplementary Figure 2 - *Knockdown of GSK3β expression in granule neuron cultures* **a)** Granule neuron cultures were transfected with shRNA against GSK3β or empty shRNA vector. After 72 hours GSK3β expression was quantified using immunofluorescence. Left panels show transfected neurones with either empty shRNA vector, GSK3β A or GSK3β B oligos. Middle panels show GSK3β expression. Right panels show a merged image with transfected neuron in green and GSK3β in red. Arrows highlight GSK3β expression levels in transfected neurons. **b)** Quantification of GSK3β knockdown in the cell body of shRNA expressing neurones. (n = 6 Ctrl, n = 4 GSK3β A, n = 3 GSK3β B ± SEM, *** = $p < 0.001$, one-way ANOVA).



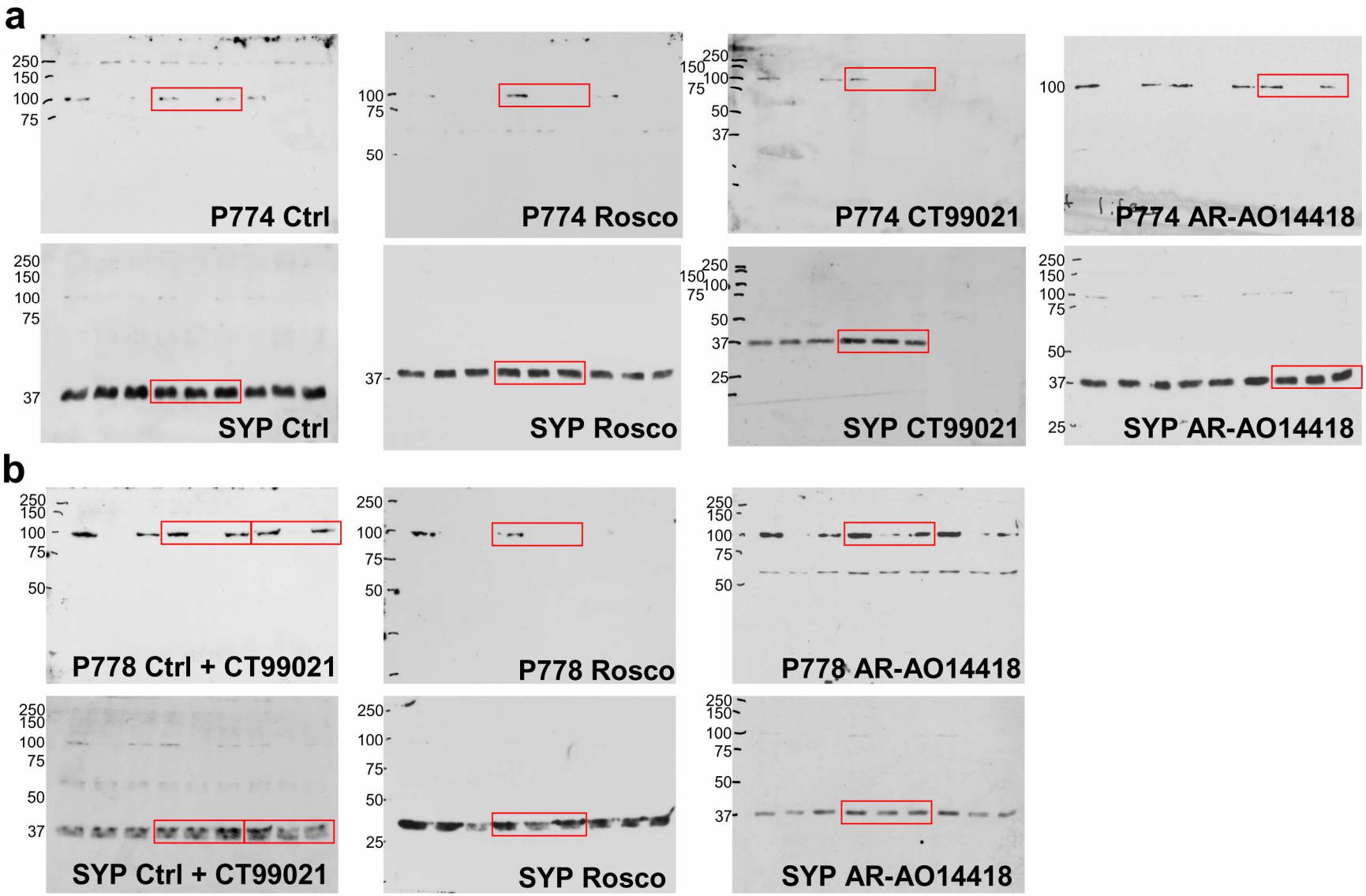
Supplementary Figure 3 - *Inhibition of GSK3 does not affect SV release probability.* **a)** Hippocampal slices were incubated with or without CT99021 (2 mM) for 1 hour before being transferred to the recording chamber. Slices were challenged with a prepulse of 600 action potentials (40 Hz) 10 minutes before recording. Paired pulse facilitation (PPF) was recorded in slices challenged with 2 stimuli separated by 25 milliseconds (indicated by arrowhead). **b)** Bar graph displays the PPF ratio (EPSC amplitude for second stimulus / first stimulus) for slices where CT99021 (2 mM) was only present in the pipette-filling solution (Control, Ctrl) or where CT99021 (2 mM) was also included in the external recording solution (CT99021). N = 7 for Ctrl, n = 11 for CT99021, Student's t test, p = 0.58.



Supplementary Figure 4 - *In-vitro phosphorylation of dynamin I PRD with cdk5 for 10 minutes yields predominantly mono-phosphorylated 778.* DynI-PRD was phosphorylated with cdk5 for 10 minutes using the same assay conditions in Figure 1. **a**) Extracted ion chromatogram of the mono-phosphorylated peptide SPTSSPTPQR and the area under the chromatographic peak indicates their abundance. The peptide phosphorylated on S778 elutes before the peptide phosphorylated on S774. **b**) The MS/MS spectrum and identity of the first chromatographic peak matching the peptide phosphorylated on S778 and **c**) The MS/MS spectrum and identity of the second chromatographic peak matching the peptide phosphorylated on S774. Bold "**S"** indicates the phosphorylated Serine, "-98" indicates the neutral loss of H_3PO_4 from the phosphorylated fragment ion and * indicates the neutral loss of H_2O.



Supplementary Figure 5 – *Full length gels, autorads and Western blots for Figure 1*. Experimental protocols were performed as described in figure 1. Original blots are displayed. Red boxes indicate the region selected for Figure 1.



Supplementary Figure 6 – *Full length Western blots for Figure 2*. Experimental protocols were performed as described in figure 2. Original blots are displayed showing either **a**) serine 774 phosphorylation (P-774) or **b**) serine 778 phosphorylation (P-778). Membranes were then reprobed for the presence of synaptophysin (SYP) as a loading control. Red boxes indicate the region selected for Figure 2.