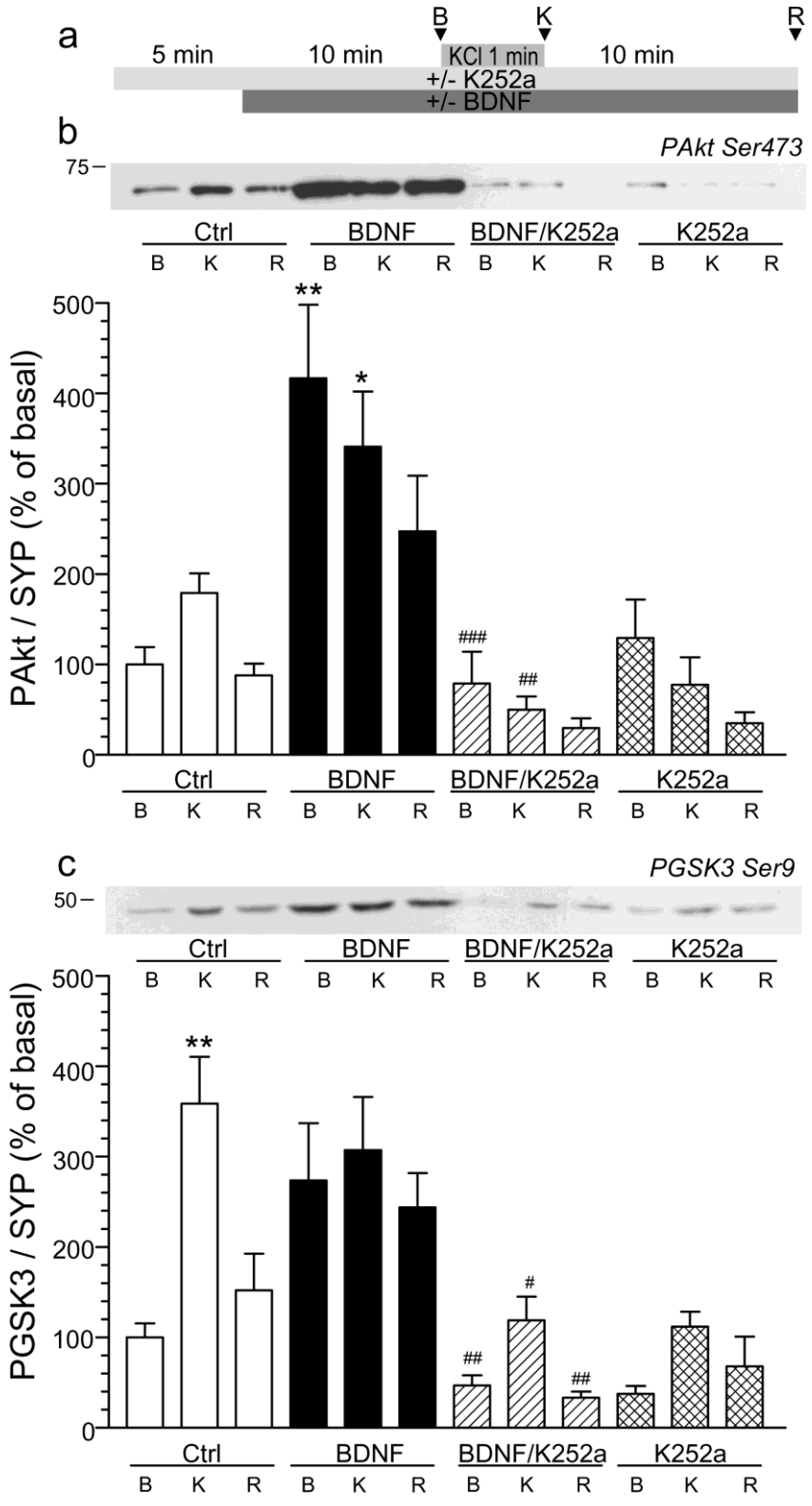
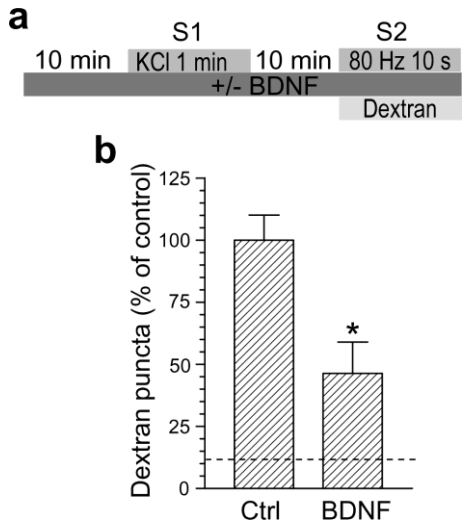
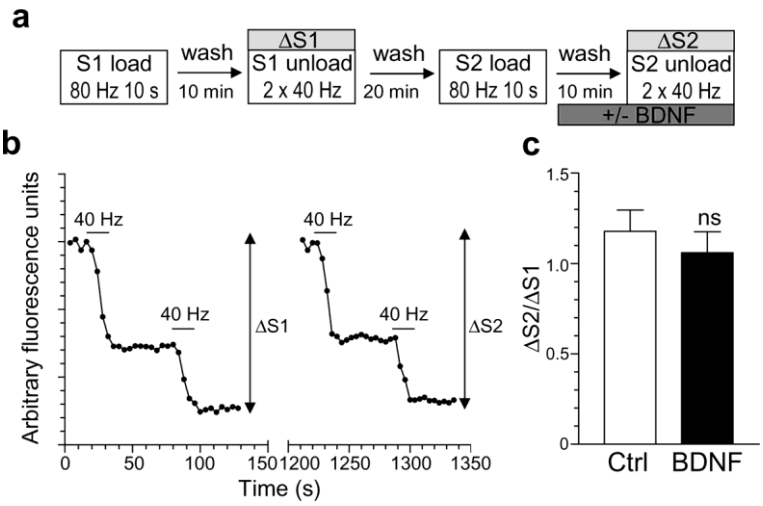

a
B
K
R
5 min
10 min
KCl 1 min
10 min
+/- K252a
+/- BDNF
b
PAkt Ser473
75
Ctrl
BDNF
BDNF/K252a
K252a
B K R B K R B K R B K R
PAkt / SYP (% of basal)
500
400
300
200
100
0
**
*
###
##
c
PGSK3 Ser9
50
PGSK3 / SYP (% of basal)
**
#
##
##

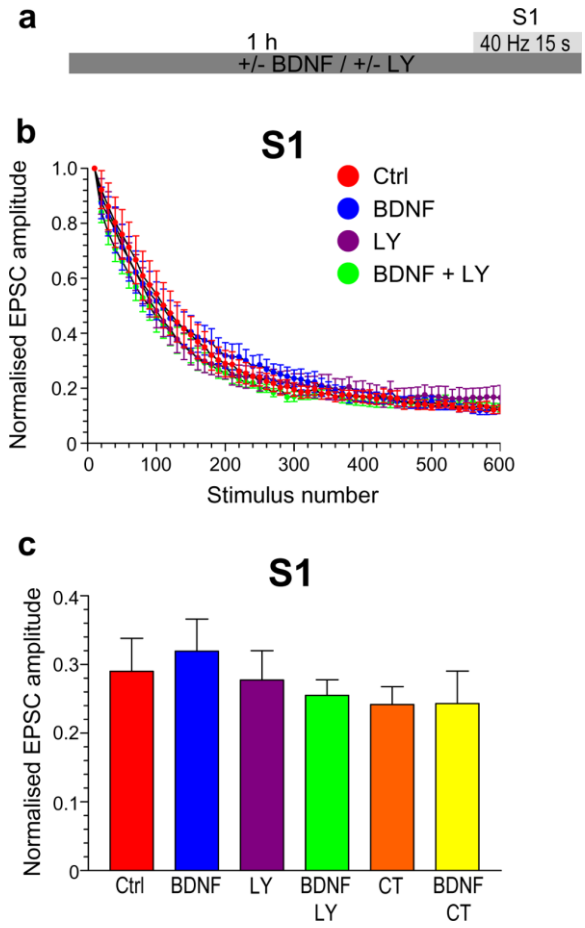
Supplementary Figure S1 – BDNF inhibits GSK3 via a TrkB cascade. **a)** CGNs were placed in incubation medium for 10 minutes before stimulation with 50 mM KCl (1 min). Following stimulation CGNs were repolarized for 10 minutes. Samples were prepared from cultures before stimulation (basal, B), directly after KCl stimulation (K) or after 10 minutes repolarization (R) as indicated by arrowheads. BDNF (100 ng / ml) or K252a (200 nM) were present throughout the experiment where indicated. Lysates were separated by SDS-PAGE and probed for either **b**) phospho-Ser-473 on Akt (*PAkt Ser473*) or **c**) phospho-Ser-9 on GSK3 (*PGSK3 Ser9*) on immunoblots. Quantitative analysis is shown in the graphs in **b,c**. These graphs display the extent of phosphorylation of either Ser-473 on Akt (**b**) or Ser-9 on GSK3 (**c**). All values were normalised to the amount of synaptophysin (SYP) as a loading control, and expressed as a percentage of control Basal ± SEM (PAkt n = 6, PGSK3 n = 8 for all conditions). One-way ANOVA in ** = $p < 0.01$, * = $p < 0.05$ compared to control basal; ### = $p < 0.001$, ## = $p < 0.01$, # = $p < 0.05$ compared to same condition with BDNF.



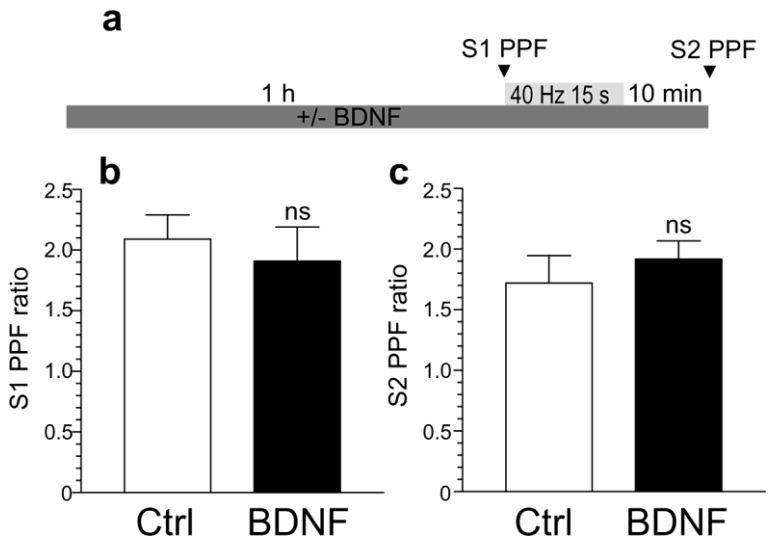
Supplementary Figure S2 – BDNF inhibits ADBE in primary cultures of hippocampal neurons. **a**) Primary hippocampal cultures were placed in imaging medium for 10 min before a priming stimulus of 50 mM KCl (1 min). Cultures were repolarized for 10 min before stimulation with 800 action potentials (80 Hz) in the presence of 50 :M tetramethylrhodamine dextran. BDNF (100 ng / ml) was present throughout the experiment where indicated. Dextran was perfused away immediately after stimulation. **b**) The bar graph displays the number of dextran puncta expressed as a percentage of control ± SEM (Control n = 5; BDNF n = 4, student's t test p = 0.012). Dotted line indicates background uptake in absence of stimulation.



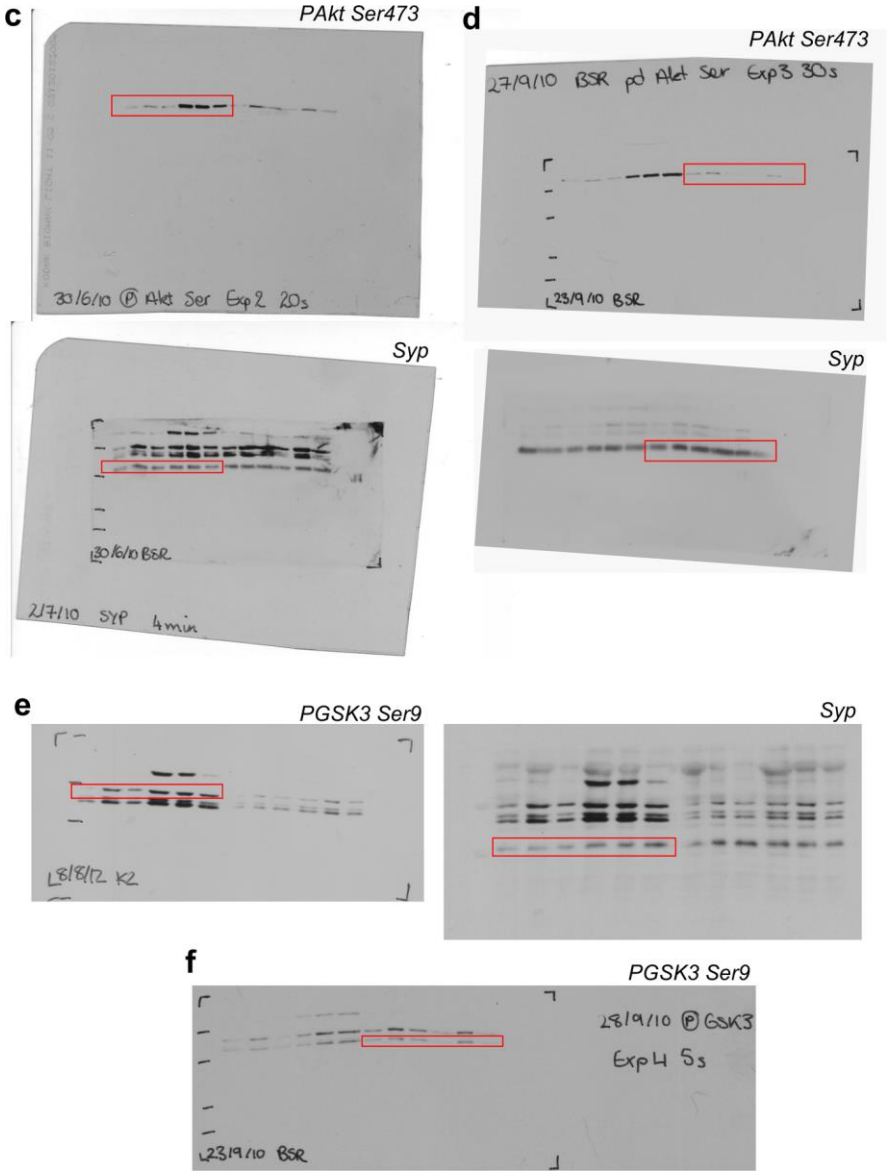
Supplementary Figure S3 – BDNF does not affect the unloading of FM2-10. **a)** CGNs were loaded and unloaded with FM2-10 using the protocol displayed. In both S1 and S2, dyes were loaded with 800 action potentials (80 Hz) followed by immediate washout of dye. After a 10 min rest period, unloading of dye was stimulated by 2 sequential stimuli of 400 action potentials (40 Hz). CGNs were preincubated with BDNF (100 mg / ml) for 10 min before and during S2 unloading as indicated. Control (Ctrl) was in the absence of drug at both S1 and S2. **b)** An example trace displaying the unloading of FM2-10 at both S1 and S2 in the presence and absence of BDNF is shown. Bars indicate the period of stimulation and arrows indicate ΔS1 and ΔS2 respectively. **c**) Mean ΔS2/ΔS1 response ∀ SEM (n = 10 for Ctrl and n = 4 for BDNF, ns, Student=s t test p = 0.57).



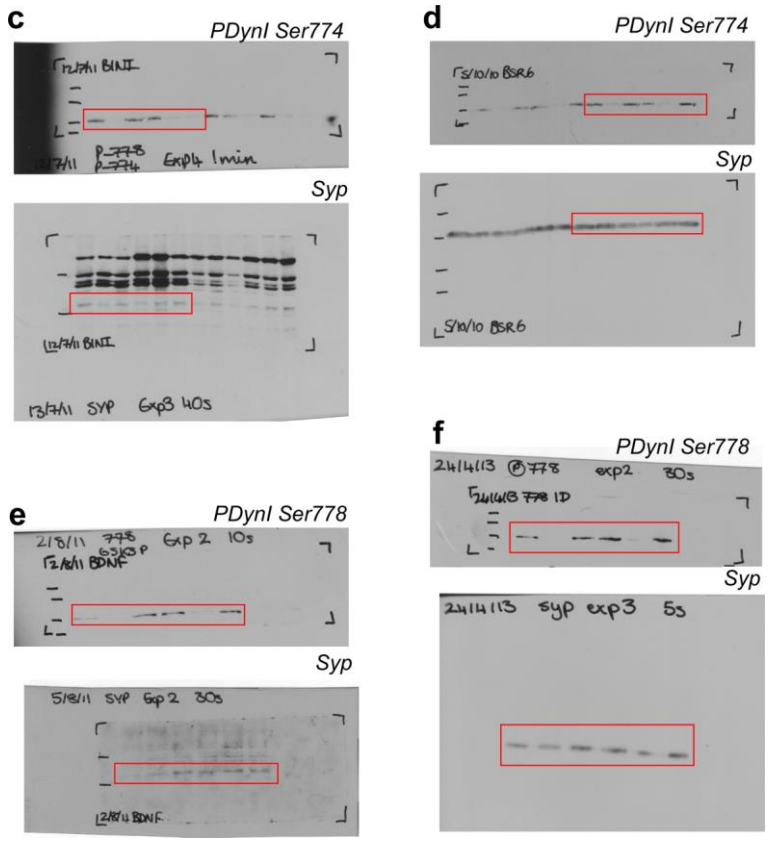
Supplementary Figure S4 – BDNF has no effect on synaptic depression at the parallel fibre – Purkinje cell synapse during an acute action potential train. **a)** Cerebellar slices were incubated with combinations of either BDNF (100 ng / ml), LY294002 (LY 10 :M) or CT99021 (CT, 2 :M) for 1 hour before being transferred to the recording chamber. Slices were then challenged with 600 action potentials (40 Hz, S1). **b)** Summary plots showing the dependence of the EPSC amplitude on the stimulus number at S1. The first EPSC was normalised to 1 to allow direct comparison of the different recording conditions on the rundown of the amplitudes of the EPSCs. **c)** The mean EPSC amplitude after 200 action potentials is displayed for each experimental condition ± SEM (Ctrl n = 11, BDNF n = 9, LY n = 7, BDNF/LY n = 9; CT n = 9, BDNF/CT n = 9, one-way ANOVA all not significant).



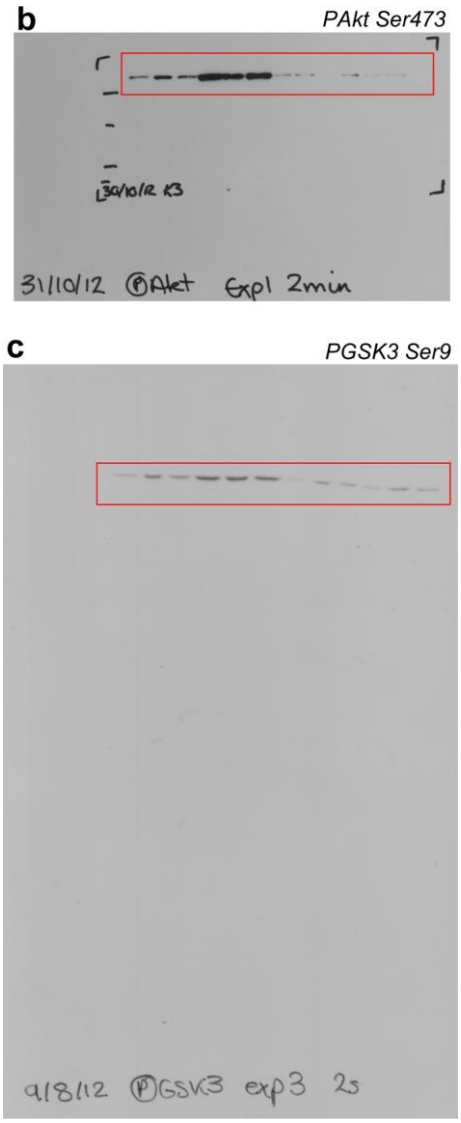
Supplementary Figure S5 – BDNF does not affect SV release probability. **a)** Cerebellar slices were incubated with or without either BDNF (100 ng / ml) for 1 hour before being transferred to the recording chamber. Slices were challenged with a prepulse of 600 action potentials (40 Hz) 10 minutes (S1). After a 10 minute rest period slices were challenged with an identical stimulus (S2). Paired pulse facilitation (PPF) was recorded in slices challenged with 2 stimuli separated by 25 milliseconds (indicated by arrowhead). **b,c)** Bar graph displays the PPF ratio (EPSC amplitude for second stimulus / first stimulus) for slices in the presence and absence of BDNF at either S1 (**b**) or S2 (**c**) ± SEM (n = 11 for Ctrl, n = 9 for BDNF, ns, Student=s t test, p = 0.60 for S1; p = 0.50 for S2).



Supplementary Figure S6 – Whole unaltered Western blots from Figure 1. Labelling is consistent with Figure 1. All membranes for these experiments were cut in half to allow simultaneous probing for dynamin I and Akt/GSK3. The synaptophysin blot in (**d**) is also used in (**f**) since the same membrane was probed for both Akt and GSK3.



Supplementary Figure S7 – Whole unaltered Western blots from Figure 2. Labelling is consistent with Figure 2. All membranes for these experiments were cut in half to allow simultaneous probing for dynamin I and Akt/GSK3.



Supplementary Figure S8 – Whole unaltered Western blots from Supplementary Figure S1. Labelling is consistent with Figure S1.