Expanded View Figures

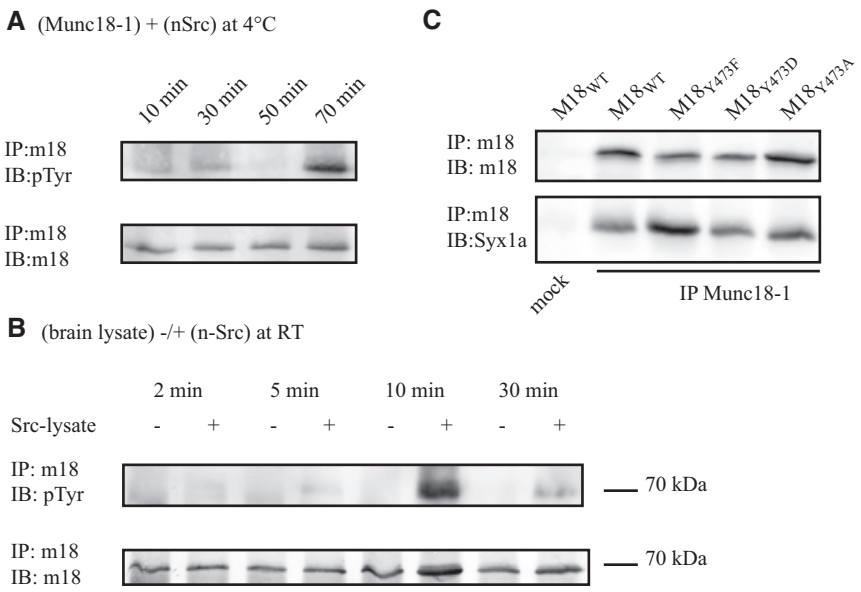

Figure EV1. *In vitro* kinase assays and Syntaxin binding of Munc18-1 variants.

A *In vitro* kinase assay in which lysates from HEK cells expressing n-Src or Munc18-1 were combined and incubated in the presence of PTP inhibitor vanadate at 4°C. Samples were denatured after different incubation times and Munc18-1 was immunoprecipitated and immunoblotted against phosphotyrosine.

B Same as (A) but using neuronal Munc18-1 from brain lysate and incubation at room temperature.

C Y473 mutants all bind Syntaxin1. Munc18-1 variants were co-expressed with Syntaxin1 in HEK293T cells. Munc18-1 and Munc18-1-bound proteins were immunoprecipitated from cell lysate and immunoblotted for detection.

Source data are available online for this figure.

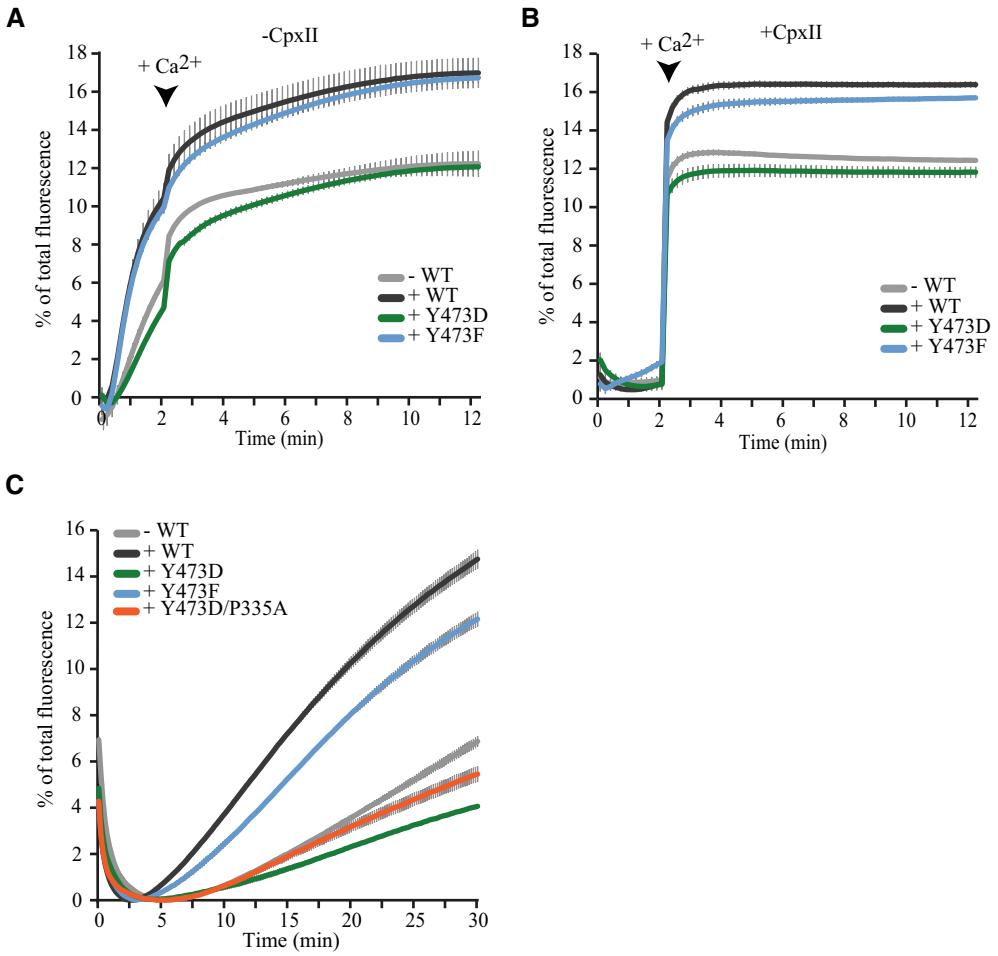

Figure EV2. Effect of Munc18-1 variants on content-mixing assays.

A Content-mixing assay; t-SNARE GUVs. Same as Fig 1F but with a content-mixing readout.
B Content-mixing assay; t-SNARE GUVs. Same as Fig 1G but with a content-mixing readout.
C Content-mixing assay; Syntaxin GUVs. Same as Fig 5D but with a content-mixing readout.

Data information: Content mixing was monitored by the increase of sulforhodamine B fluorescence for 30 min. Data were normalized to the maximum fluorescence after liposome lysis by detergent. The minimum fluorescence was set to 0%. Error bars = s.e.m., $N = 3$.

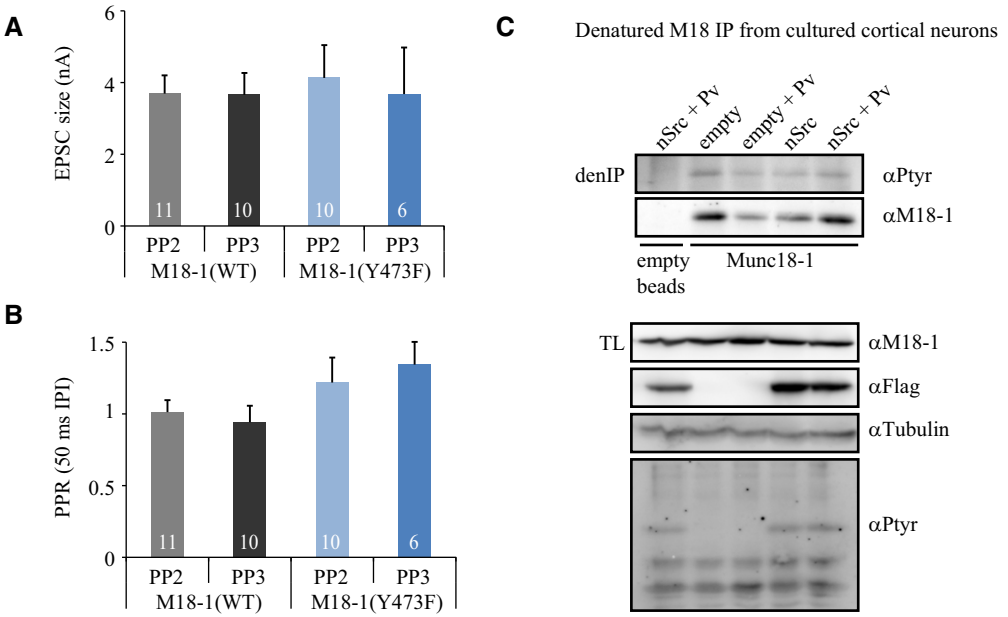

Figure EV3. Src activity in cultured neurons.

A Autaptic excitatory *munc18-1* neurons expressing $M18_{WT}$ or $M18_{Y473F}$ were subjected to 1-h incubation with Src family kinase inhibitor PP2 or its inactive form PP3. EPSC size was unaffected by drug treatment ($M18_{WT}$ + PP2: 3.69 ± 0.51 nA, n = 11; $M18_{WT}$ + PP3: 3.65 ± 0.62 nA, n = 10; $M18_{Y473F}$ + PP2: 4.13 ± 0.92 nA, n = 10; $M18_{Y473F}$ + PP3: 3.68 ± 1.30 nA, n = 6).

B Two pulses were given with an interpulse interval of 50 ms. Paired-pulse ratio (PPR: EPSC#2/EPSC#1) was not affected by drug treatment ($M18_{WT}$ + PP2, n = 11: 1.01 ± 0.08; $M18_{WT}$ + PP3: 0.94 ± 0.12, n = 10; $M18_{Y473F}$ + PP2: 1.22 ± 0.17, n = 10; $M18_{Y473F}$ + PP3: 1.34 ± 0.16, n = 6).

C Neuronal cultures were subjected to protein tyrosine phosphatase inhibitor vanadate and/or overexpression of Flag-tagged n-Src. Munc18-1 tyrosine phosphorylation was determined by denatured Munc18-1 IP and immunoblotting with a phosphotyrosine antibody.

Data information: The error bars represent s.e.m.
Source data are available online for this figure.