Supplementary Information Methods

Receptor mediated endocytosis assay
U2OS cells were cultured in DMEM supplemented with 10% fetal bovine serum at 37°C in 5% CO2 in a humidified incubator. Quantitative analysis of the inhibition of Tf endocytosis in U2OS cells was performed on large numbers of serum-starved cells by an automated process as previously described (Quan et al. 2007). U2OS cells were grown in fibronectin-coated (50 µg/ml) 96 well plates and pre-incubated with varying concentrations of drugs for 30 min prior to addition of 4 µg/ml Tf-A594 for 8 min at 37°C in triplicate. Cells were washed with an acid wash solution (0.2 M acetic acid, 0.5 M NaCl, pH 2.8) at 4°C for 10 min, then ice cold PBS for 5 min to remove surface bound Tf. Cells were fixed in 4% PFA (pH 7.5). Nuclei were stained with DAPI. Red (Tf-A594) and blue (DAPI) images were collected using an automated acquisition and analysis system (ImageXpress Micro, Molecular Devices, Sunnyvale, CA). Nine images were collected from each well, averaging 40-50 cells per image. The average integrated intensity of Tf-A594 signal per cell was calculated for each well (MetaXpress, Molecular Devices) and the data expressed as a percentage of vehicle treated control cells. The average number of cells for each data point was ~1,200. IC50 values were calculated using GraphPad Prism 5 and data was expressed as mean ± 95% confidence interval (CI) for 3 wells and ~1,200 cells.

Supplementary Figure Legends

Supplementary Figure 1 - Dynamin I mutants and peptides. (A) Schematic representation of the full length dynamin I mutants employed. All mutations were in the phospho-box of dynamin I and are as follows (DynIdmA-mCer - Ser 774/778 Ala; DynIdmE-mCer - Ser 774/778 Glu; DynIPB2-mCer - Arg 772/773 Glu). All mutants are tagged at their C-termini by the fluorescent protein mCerulean. (B) Schematic representation of dynamin I phospho-box peptides employed. Both peptides mimic the phospho-box of dynamin I (769-784) except DynI769-784AA contains the substitutions Ser 774/778 Ala and DynI769-784EE contains the substitutions Ser 774/778 Glu. Both peptides are tagged at their N-termini with a penetratin sequence (RRMKWKK) to aid delivery into the nerve terminal cytosol.

Supplementary Figure 2 - Dynamin I null phospho-peptides do not block transferrin uptake. The effect of two phospho-box peptides on endocytosis of Tf-A594 in U2OS cells was
determined for 1-300 µM peptide preincubated with the cells for 30 min prior to addition of transferrin. Results are means and standard error for triplicate determinations from two independent experiments.

**Supplementary Figure 3** - Overexpression of full length dynamin I mutants does not affect SV exocytosis. (A) Granule neuron cultures were loaded and unloaded with FM dyes using the protocol displayed. Dyes were loaded with 800 action potentials (80 Hz) and then washed away immediately. Dye unloading was stimulated by a 30 second stimulus of 50 mM KCl. Traces were aligned to the start point of stimulation. The start point and end point of dye unloading were normalized to 1 and 0 respectively. Panels B and D display a time course of dye unloading from a typical experiment that has undergone this analysis (B) Black circles represent untransfected neurons and grey circles represent neurones transfected with DynI<sup>dmA</sup>-mCer. (D) Open circles represent untransfected neurons and grey circles represent neurones transfected with DynI<sup>dmA</sup>-mCer. (C and E) The time taken for the nerve terminals to lose 50% of their content was determined for each experiment (t<sub>1/2</sub>) and these values were averaged across experiments. The average t<sub>1/2</sub> of dye loss for either FM1-43 (C) or FM2-10 (E) is displayed as a percentage of untransfected controls (FM1-43 n = 14 untransfected, n = 3 Dyn<sup>WT</sup>, n = 4 Dyn<sup>dmA</sup>, n = 4 Dyn<sup>dmE</sup>, n = 3 Dyn<sup>PB2</sup>; FM1-43 n = 16 untransfected, n = 3 Dyn<sup>WT</sup>, n = 4 Dyn<sup>dmA</sup>, n = 5 Dyn<sup>dmE</sup>, n = 4 Dyn<sup>PB2</sup>; all ± SEM). One-way ANOVA analysis performed, all values > 0.05.

**Reference List**