SUPPLEMENT FIGURE LEGENDS

Supplementary Figure 1. Extent of rapid endocytosis is coupled to Dynamin-1 GTPase activity. Chromaffin cells expressing VMAT2-pHluorin were exposed to low (5.5) pH for 10 sec after an initial 15 sec stimulation with 56 mM K^+ in the presence of bafilomycin. Low pH-insensitive events (endocytic events) are expressed as % of total fusion events. Endocytosis is similar in cells expressing Dyn1WT and Control cells (i.e., no significant difference). Expression of Dyn1(T141A) with enhanced GTPase resulted in significantly more endocytosis. Conversely, expression of Dyn1(T65A) with reduced GTPase activity reduced endocytosis. Dyn1(T65A) and Dyn1(T141A) are significantly different from WT ($^{+++}P<0.001$; binomial probability). Data are from 7 cells for Control, 7 cells for Dyn1WT, 10 cells for Dyn1(T141A), and 9 cells for Dyn1(T65A).

Supplementary Figure 2. Polarized-TIRFM responses to granule fusion in a Control cell (without transfected dynamin). (**A**) With granule fusion and release of NPY (left panel), an **increase** in p (middle panel) and **decrease** in s (right panel) is observed. The dotted line indicates the frame before granule fusion (time 0). (**B**) Images corresponding to graphs shown in **A**. White arrows indicate areas where the **increase** in p intensity or **decrease** in s intensity is observed. (**C, D**) Based upon these data, *P/S* and *P+2S* were calculated as described in the **Methods**. Scale bar, 1 μm.

Supplementary Figure 3. Dynamin-1 GTPase activity has little effect on amperometric spikes. Quantal size of amperometric spikes (**A**) and spike rise time (**C**, cutoff of 5 ms) was not different between groups. (**B**) Spike half-width between Control and Dyn1WT cells was significantly different (9.875 ± 0.5118 Control; 8.298 ± 0.5716, Dyn1WT; $P<0.05$ Student's

unpaired *t* test). Otherwise, no significant differences were observed between Control and Dyn1WT, or between the dynamin mutants and Dyn1WT.

Supplementary Figure 4. Expression level of Dynamin-1 in transfected cells compared to endogenous Dynamin. (**A**) To assess dynamin membrane expression, mean pixel intensity of a thin section (width ~500 nm) of the cell periphery was calculated. The mean pixel intensity of the cytoplasm (**B**) was then subtracted from the intensity in the thin section to obtain membrane dynamin expression. (**B**) To assess dynamin expression in the cytoplasm, mean pixel intensity of the cell interior was calculated (excluding the thin section membrane section). Each point in (**A**) and (**B**) represents the intensity for an individual cell.

Supplementary Figure 5. Dimming of SNAP25 fluorescence is due to exclusion of the membrane protein from the area by the fused granule. Chromaffin cells were co-transfected with NPY-Cherry and Cer-SNAP25(1-100). A decrease in cerulean fluorescence was observed upon granule fusion. Cer-SNAP25(1-100) is the inactive SN1 SNARE motif of SNAP25 and is membrane-bound; the loss of intensity reflects exclusion of the probe from the area by the fused granule. Intensity values of Cer-SNAP25(1-100) from individual fusion events were normalized to the average of 20 pre-fusion frames, aligned to the pre-fusion frame (dotted line), and averaged. n=18 events from 3 cells (data presented as mean ± SEM). Scale bar, 1 μm.

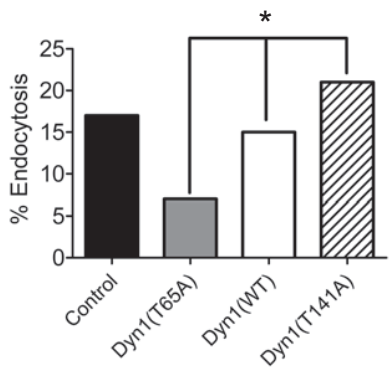
*
% Endocytosis
25
20
15
10
5
0
Control
Dyn1(T65A)
Dyn1(WT)
Dyn1(T141A)

Figure S1

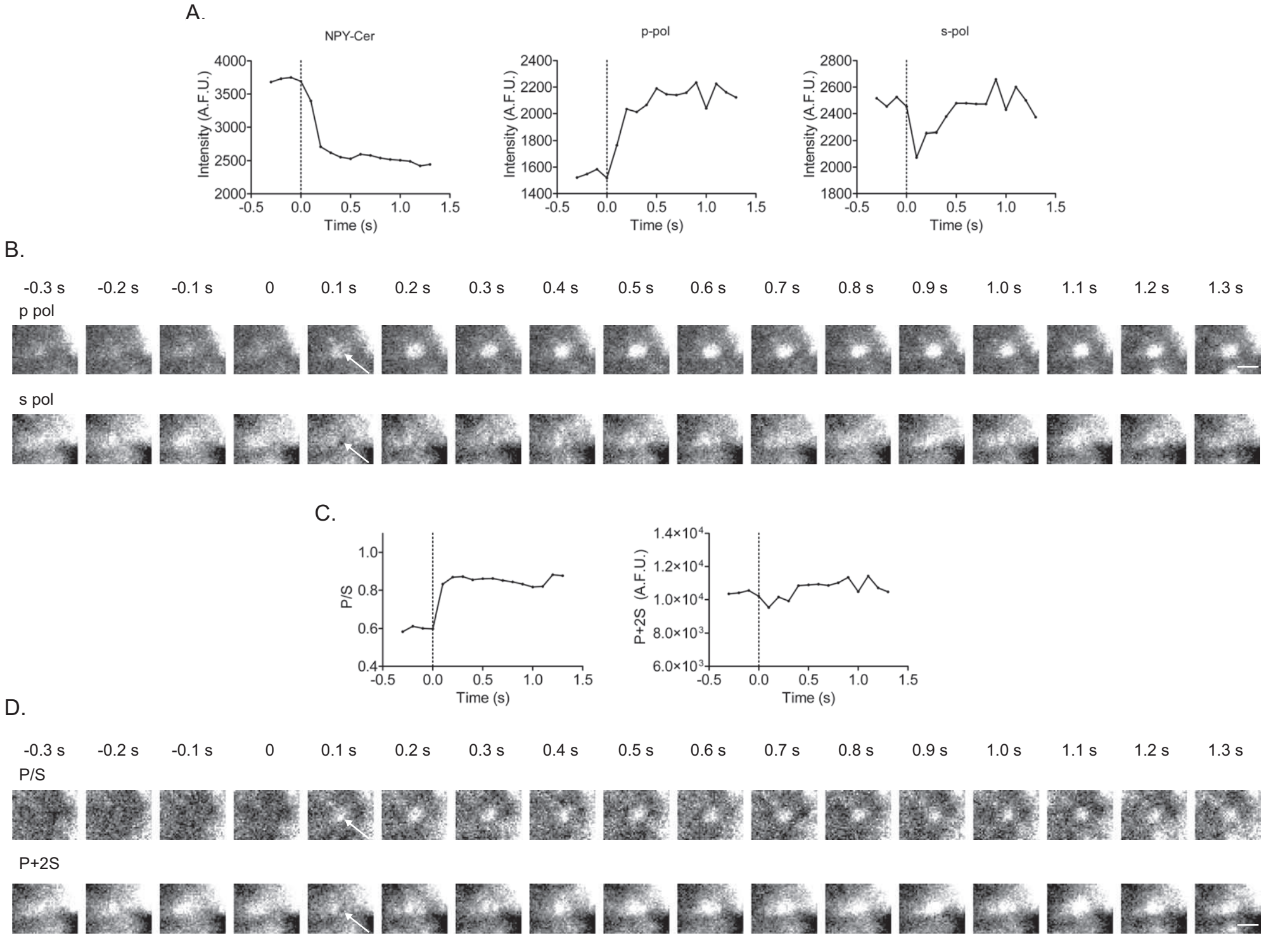
A.
NPY-Cer
p-pol
s-pol
Intensity (A.F.U.)
Time (s)
B.
-0.3 s
-0.2 s
-0.1 s
0
0.1 s
0.2 s
0.3 s
0.4 s
0.5 s
0.6 s
0.7 s
0.8 s
0.9 s
1.0 s
1.1 s
1.2 s
1.3 s
p pol
s pol
C.
P/S
P+2S (A.F.U.)
D.
P/S
P+2S

Figure S2

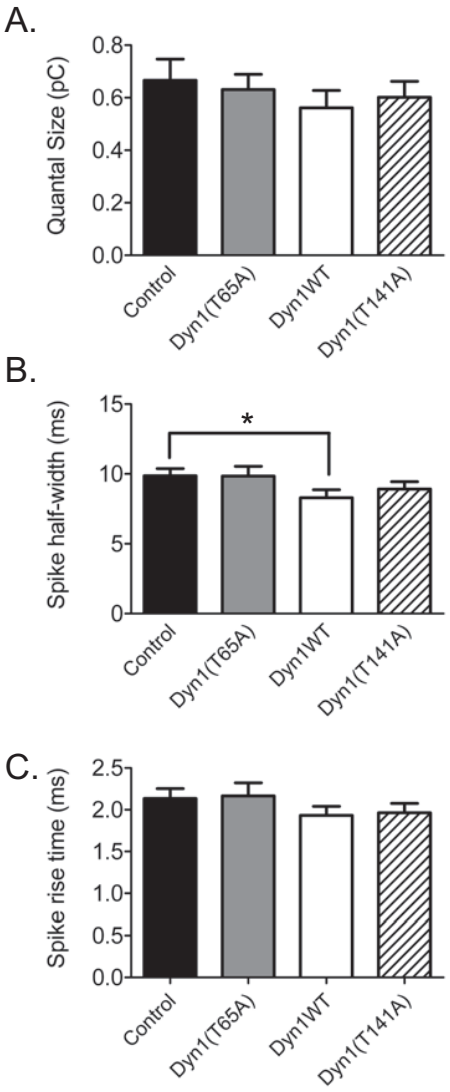
A.
Quantal Size (pC)
0.8
0.6
0.4
0.2
0.0
Control
Dyn1(T65A)
Dyn1WT
Dyn1(T141A)
B.
Spike half-width (ms)
15
10
5
0
*
Control
Dyn1(T65A)
Dyn1WT
Dyn1(T141A)
C.
Spike rise time (ms)
2.5
2.0
1.5
1.0
0.5
0.0
Control
Dyn1(T65A)
Dyn1WT
Dyn1(T141A)

Figure S3

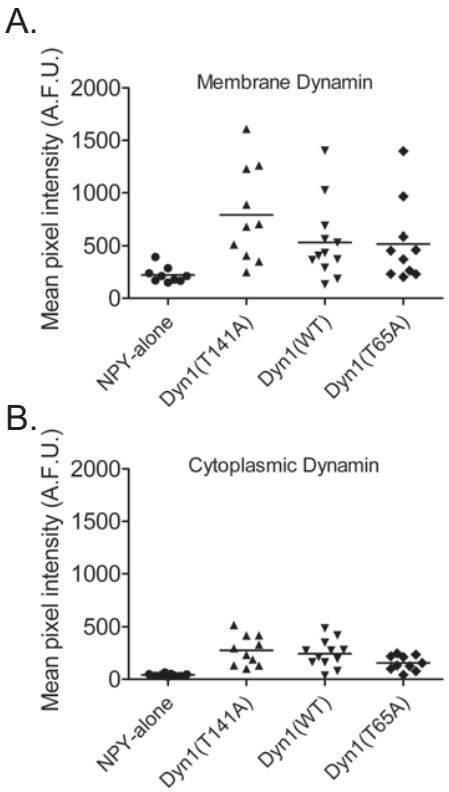
A.
Membrane Dynamin
Mean pixel intensity (A.F.U.)
2000
1500
1000
500
0
NPY-alone
Dyn1(T141A)
Dyn1(WT)
Dyn1(T65A)
B.
Cytoplasmic Dynamin
Mean pixel intensity (A.F.U.)
2000
1500
1000
500
0
NPY-alone
Dyn1(T141A)
Dyn1(WT)
Dyn1(T65A)

Figure S4

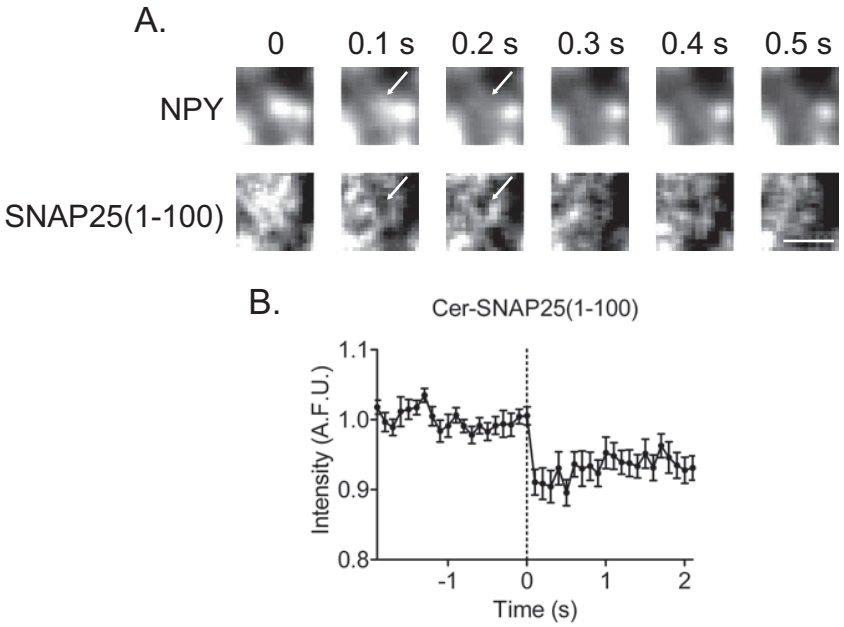
A.
0
0.1 s
0.2 s
0.3 s
0.4 s
0.5 s
NPY
SNAP25(1-100)
B.
Cer-SNAP25(1-100)
Intensity (A.F.U.)
1.1
1.0
0.9
0.8
-1
0
1
2
Time (s)

Figure S5