Figures and figure supplements

Dynamin phosphorylation controls optimization of endocytosis for brief action potential bursts

Moritz Armbruster, et al.
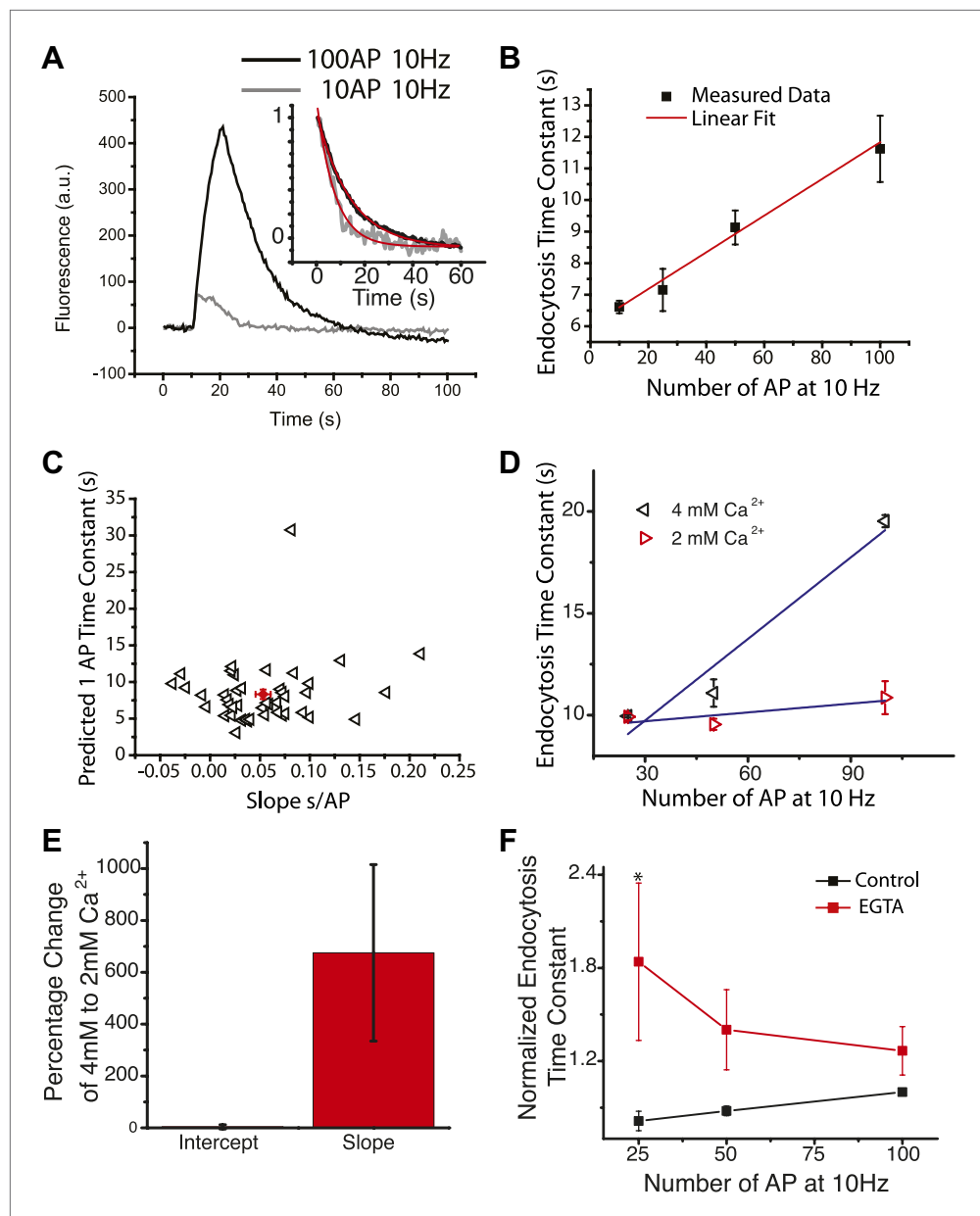
Figure 1. Calcium slows endocytosis at 30°C. (**A**) Endocytosis decays from a 100 AP 10 Hz run (black) and 10 AP 10 Hz runs (2–3 runs averaged) (gray), inset shows endocytosis phase fit with a single exponential decay (red) to measure endocytosis time constant = 13.9 ± 0.10 s, 6.8 ± 0.3 s 47 boutons. adj. R-square of fits 0.95, 0.997 respectively. (**B**) An example cell probed multiple times at 10, 25, 50, 100 AP at 10 Hz at 30°C, fit with a linear dependence with a slope of 0.058 ± 0.004 s, a predicted 1 AP time constant of 6.09 ± 0.11 s. (**C**) Across 44 cells, the slope (s/AP) is plotted against the predicted 1 AP time constant (s). Average slope 0.053 ± 0.008 s, average predicted 1 AP time constant 8.31 ± 0.64 s. There is a CV of 100% in the slope and 52% in the predicted 1 AP time constant. (**D**) Endocytosis for 25, 50 and 100 AP delivered in 2 mM and 4 mM external Ca^{2+} for the same set of boutons from one cell. Each condition was probed 1–4 times and averaged over 46 ROIs. Slope of 2 mM is 0.01 ± 0.01 s/AP, 4 mM is 0.13 ± 0.03 s/AP. The intercept of 2 mM is 9.3 ± 0.7 s, 4 mM is 5.7 ± 2.0 s. (**E**) Across 10 cells the percentage change in slope and predicted 1 AP time constant when changing from 2 mM to 4 mM external Ca^{2+} slope and predicted 1 AP time constant. The change in slope is significantly different from 0, (log corrected one sample t-test p<0.004). (**F**) 25, 50, and 100 AP at 10 Hz endocytosis time constant compared before and after 90 s load of 100 μM EGTA-AM reveals an acceleration phase of endocytosis for low stimulus number (25 AP significantly different before and after EGTA treatment, paired sample t-test p<0.03, N = 6 cells).
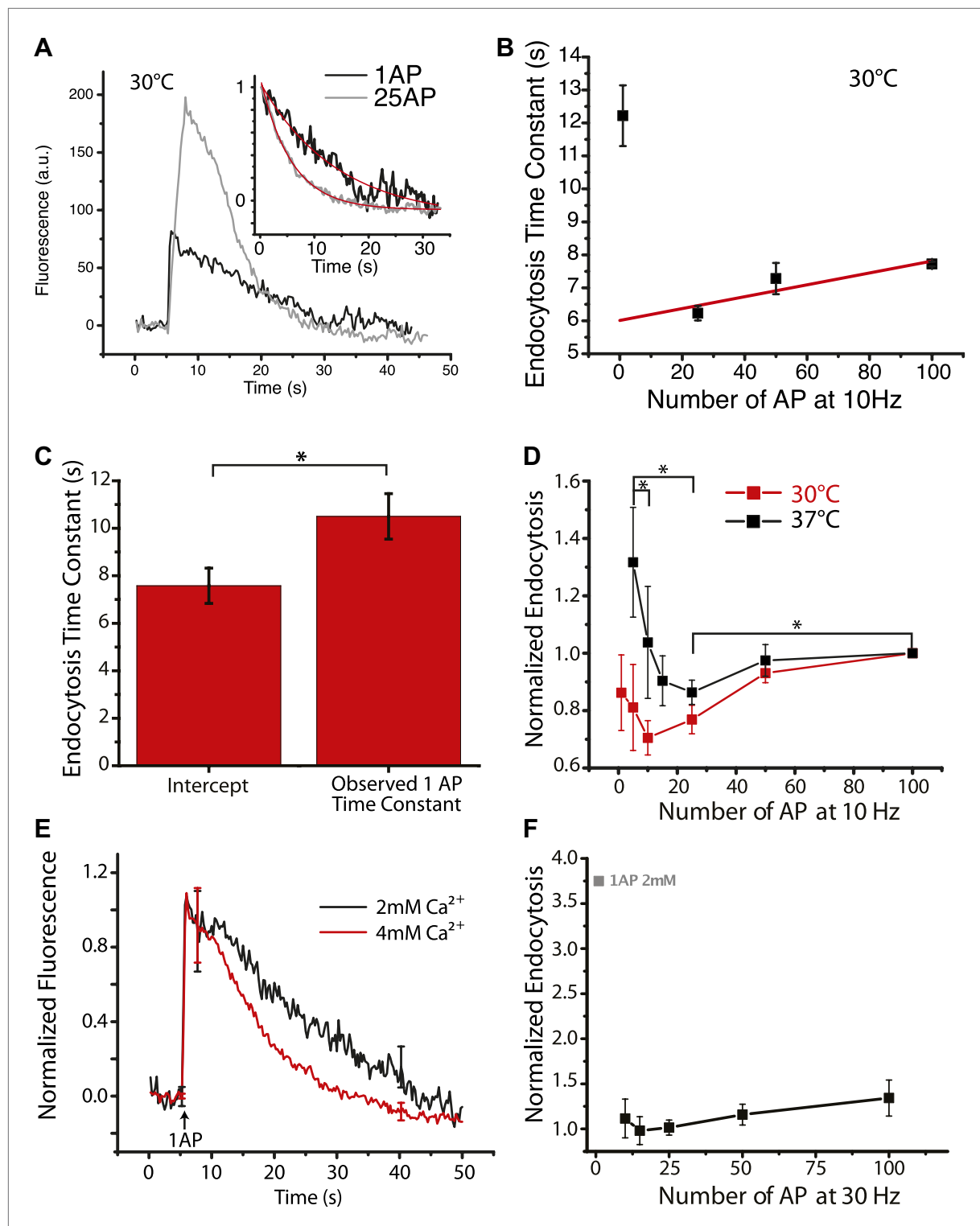DOI: 10.7554/eLife.00845.003

Figure 2. Acceleration of endocytosis for small stimuli. (**A**) Individual example traces of a cell probed with 1 AP (black) and 25 AP at 10 Hz (gray), inset shows endocytosis phase with their fits (red) at 30°C. 1 AP time constant τ = 12.36 ± 1.1 s, 25 AP 10 Hz time constant τ = 8.57 ± 0.67 s, adj. R-square of fits 0.93, 0.99 respectively. (**B**) The same example cell probed at 1, 25, 50, 100 AP at 10 Hz at 30°C, with a linear fit to the 25, 50, 100 AP data. Each point is an average of 2–3 runs based on 76 ROIs. Predicted 1 AP time constant = 6.55 ± 0.54 s. (**C**) Across 10 cells, the predicted 1 AP time constant based upon linear fit to 25, 50, 100 AP 10 Hz data compared to observed 1 AP time constant. The difference is significant paired sample *t*-test p<0.01. (**D**) At physiological temperature, (37°C black) probing 5, 10, 15, 25, 50, 100 AP at 10 Hz, normalized for 100 AP 10 Hz tau for each cell, showing the acceleration

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of endocytosis. N = 8 cells. 5 AP (maximum) vs 25 AP (minimum) is significant p<0.01, 25 AP vs 100 AP is significant p<0.03 paired sample *t*-tests, 5 AP vs 10 AP is significant p<0.009 paired sample *t*-tests. At 30°C (red) there is no significant acceleration over a similar range of stimuli 1 AP vs 10 AP (minimum) p>0.05 paired *t*-test. N = 8, 7, 12, 14, 12, 14 cells for 1, 5, 10, 25, 50, and 100 AP 10 Hz respectively. (**E**) Paired comparisons of 1 AP stimulation at 2 mM and 4 mM extracellular Ca^{2+} showing a Ca^{2+} dependent acceleration (N = 6 cells, significant difference in remaining fluorescence at 20 s paired *t*-test p<0.05). (**F**) Acceleration of endocytosis measured for 30 Hz AP bursts normalized to the value obtained at 15 AP, but including the relative value measured for 1 AP (2 mM) in **D**. N = 3, 4, 5, 4, 4 cells for stimuli 100, 50, 25, 15 10 AP at 30 Hz.

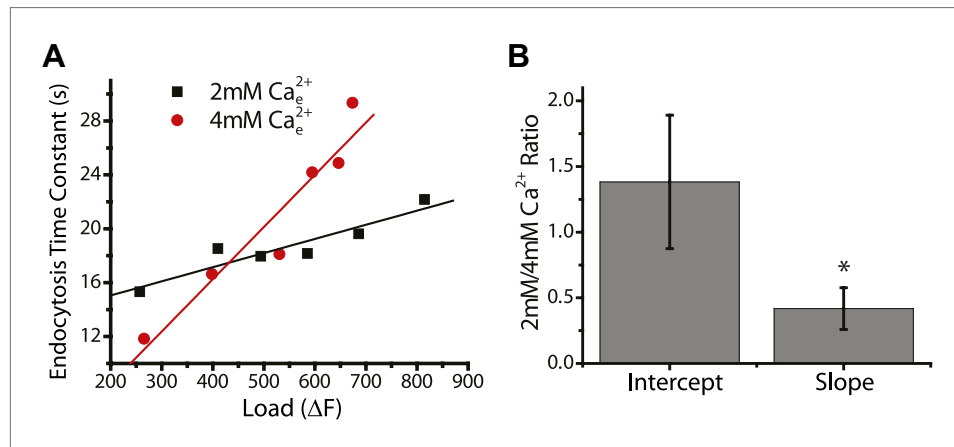DOI: 10.7554/eLife.00845.004

Figure 2—figure supplement 1. Comparing the effects of 2 mM and 4 mM external Ca^{2+} on the slopes of endocytosis corrected for changes in exocytosis. (**A**) Example from a single cell plotting endocytosis time constant for a variety of stimuli at 2 mM and 4 mM compared to the endocytic load (Fluorescence at the end of the stimulus). Linear fits to the slopes at 2 mM and 4 mM Ca^{2+}; Slopes 0.01 ± 0.002 s/ΔF, 0.039 ± 0.006 s/ΔF respectively; Intercepts at 0 ΔF were 13.0 ± 1.2 s, 0.7 ± 3.3 s respectively. (**B**) Statistics on the ratios of the slopes (s/ΔF), 0.42 ± 0.16, and the intercepts (s), 1.4 ± 0.5. The slope is significantly different from 1, one-sample *t*-test p<0.01. This indicates that the changes in slope associated with changes in Ca^{2+} are not due to differences in the endocytic load. N = 10 cells.
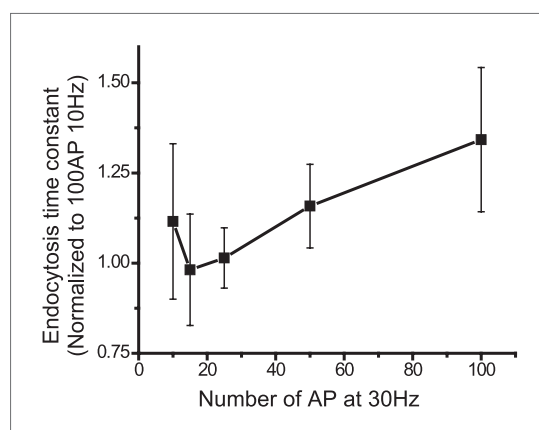
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Figure 2—figure supplement 2. Mapping the acceleration notch curve at 30 Hz, at 100, 50, 25, 15, 10 AP. Each cell is normalized to its 100 AP 10 Hz time constant. The acceleration minimum is difficult to resolve with the increased Ca^{2+} influx; N=3, 4, 5, 4, 4 cells.
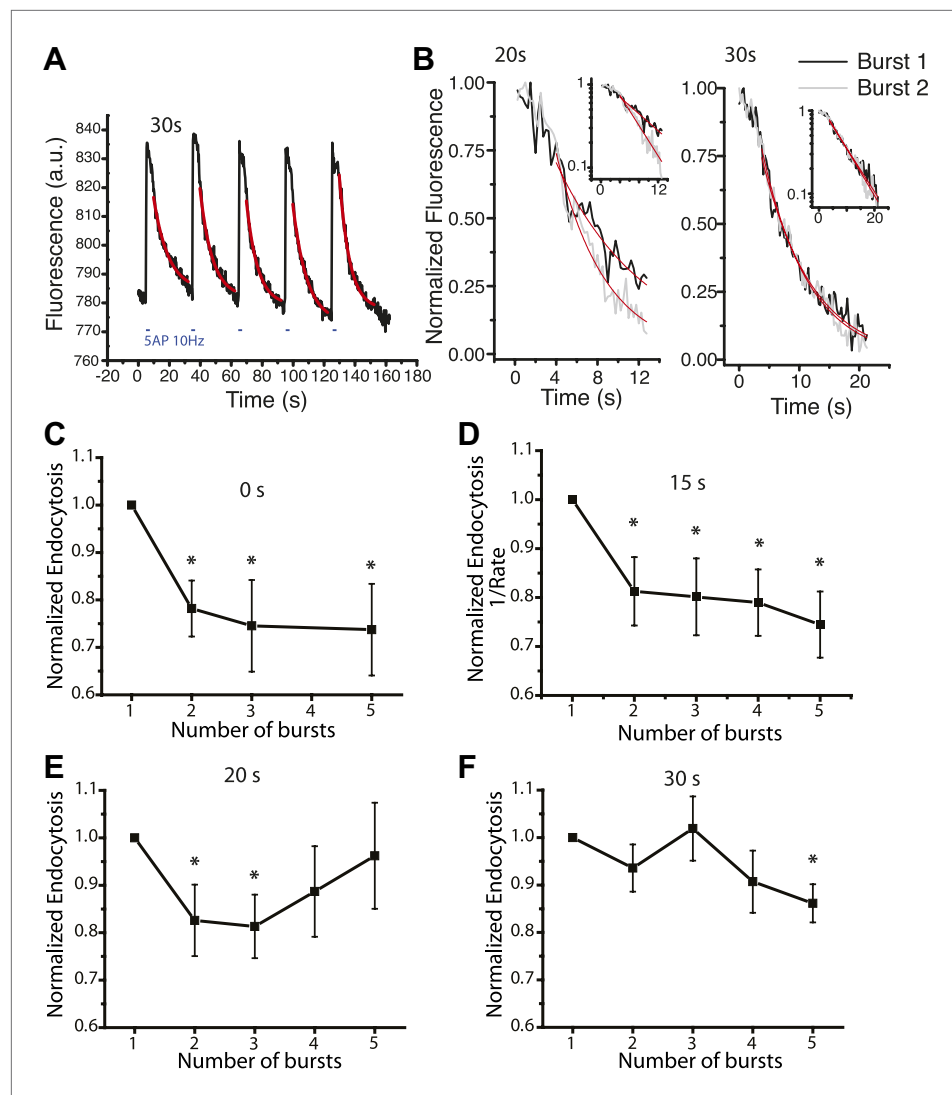
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Figure 3. Persistence time of endocytic acceleration. (**A**) A sequence of 5 bursts of 5 AP 10 Hz with inter-burst interval of 30 s apart from one cell averaged over 4 runs, each decay is fit with an exponential decay. Endocytosis time constants = 9.7 ± 0.8 s, 7.9 ± 0.5 s, 7.2 ± 0.4 s, 9.2 ± 0.5 s, 6.7 ± 0.3 s respectively for pulses 1–5. (**B**) Example endocytic decays from pulses 1 and 2 with 20 s and 30 s spacing illustrating the acceleration of endocytosis for 20 s inter-burst interval spacing: 20 s time constants 8.6 ± 0.5 s and 4.8 ± 0.2 s for first and second burst respectively, adj. R-square of fits 0.88, 0.93 respectively; 30 s time constants 8.3 ± 0.2 s and 7.7 ± 0.2 s first and second burst respectively, adj. R-square of fits 0.96 and 0.97 respectively. Data were averaged over 5–12 runs across 30–50 boutons. (**C**) Inter-burst interval of 0 s, based upon data from *Figure 2D*, normalized to 5 AP 10 Hz shows significant acceleration of endocytosis, $p < 0.02$ for 10 AP, 15 AP, and 25 AP 10 Hz pulses n = 8 cells. (**D**) 15 s spacing with linear fits for the decays, plotting 1/Rate of endocytosis, showing significant acceleration of endocytosis compared to the first burst for all subsequent bursts, $p < 0.03$ for bursts 2–5. n = 11 cells. (**E**) 20 s spacing, fit with exponential time constants, bursts 2 and 3 are significantly accelerated compared to the first burst $p < 0.05$, n = 9 cells. (**F**) 30 s inter-burst interval with exponential fits, only the fifth burst is significantly accelerated compared to the first burst $p < 0.04$, n = 9 cells. All tests one sample *t*-test.
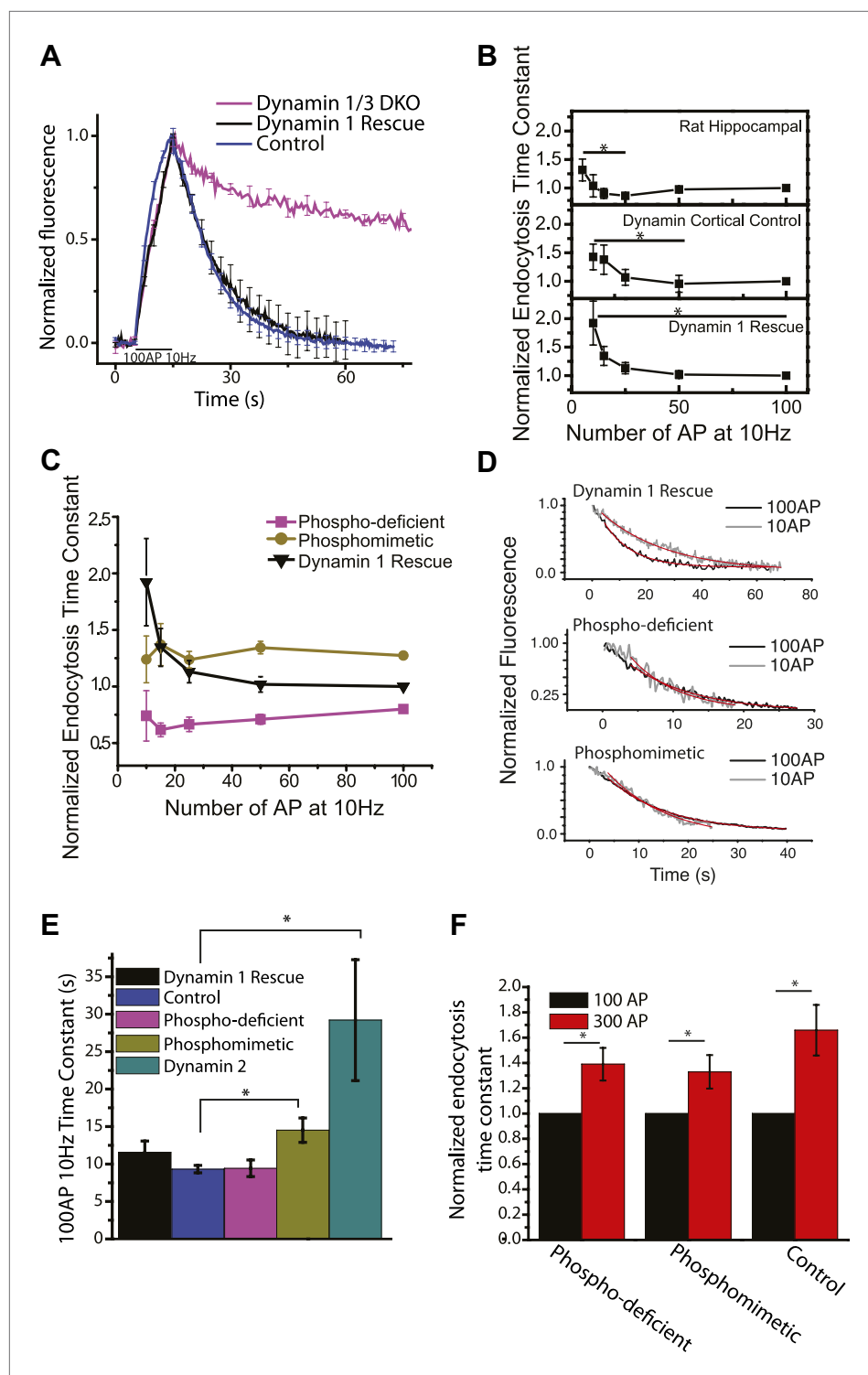
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Figure 4. Dephosphin control of endocytic acceleration. (**A**) 100 AP 10 Hz stimulation of dynamin 1/3 DKO, dynamin 1 rescue expressed in DKO, or the dynamin 1 Het dynamin 3 KO control genotype at 37°C. n = 5, 7, 10 cells respectively. (**B**) Comparison of endocytosis acceleration in rat neurons (top) cortical mouse neurons (middle) and cortical mouse dynamin 1/3 DKO neurons rescued with dynamin 1. For each cell data is normalized to the value obtained for 100 AP 10 Hz, N = 8 rat hippocampus, 7 mouse cortical, 9 dynamin 1 rescue. Mouse cortical 10 AP compared to 50 AP (minimum) is significant p<0.03; dynamin 1 rescue 10 AP compared to 100 AP (minimum) is significant p<0.05 paired sample *t*-tests. (**C**) Endocytosis vs stimulation for dynamin 1/3 DKO rescued with the full

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length dynamin 1 (black, replotted from **B**, bottom), phospho-deficient, S774/8A (pink), or the phosphomimetic S774/8D mutants of dynamin 1 (gold) shows that mutations at these serines block acceleration and lock endocytosis in a fast or slow state. Individual traces are normalized to the 100 AP 10 Hz value for the dynamin 1 rescue. N = 9 cells dynamin 1 rescue, N = 6 cells phospho-deficient, N = 8 cells phosphomimetic. (**D**) Example traces of 10 AP compared to 100 AP for dynamin 1 rescue (adj. R-square of fits 0.97, 0.97 100 AP, 10 AP respectively), phospho-deficient rescue (adj. R-square of fits 0.98, 0.89 100 AP, 10 AP respectively), and phosphomimetic rescue (adj. R-square of fits 0.999, 0.97 100 AP 10 AP respectively) of dynamin 1/3DKO showing the lack of acceleration for the phosphorylation mutants. Single cells based upon 30–50 boutons and 1–3 runs. Time constants of decays: dynamin 1 rescue 25.1 ± 0.4 s and 9.7 ± 0.4 s, phospho-deficient rescue 6.8 ± 1.2 s and 9.4 ± 0.5 , phosphomimetic rescue 12.4 ± 1.1 s and 12.0 ± 0.1 s for 10 AP and 100 AP respectively. (**E**) The 100 AP 10 Hz time constants of the dynamin 1/3 DKO rescued with dynamin 1, phosphorylation mutants and dynamin 2. Phosphomimetic is significantly different from control KS-test $p<0.003$. Dynamin 2 is significantly different from control KS-test $p<0.0004$. N = 7, 10, 9, 12, and 11 cells respectively. (**F**) Paired 100 AP and 300 AP 10 Hz stimulus, endocytosis time constants for the phosphomutants and control. Data normalized to 100 AP 10 Hz paired *t*-test $p<0.03$ for all conditions. 300 AP τ_{endo} phospho-deficient 1.39 ± 0.13 s, phosphomimetic 1.33 ± 0.13 s, control 1.66 ± 0.20 s. N = 9, 15, 9 cells respectively.
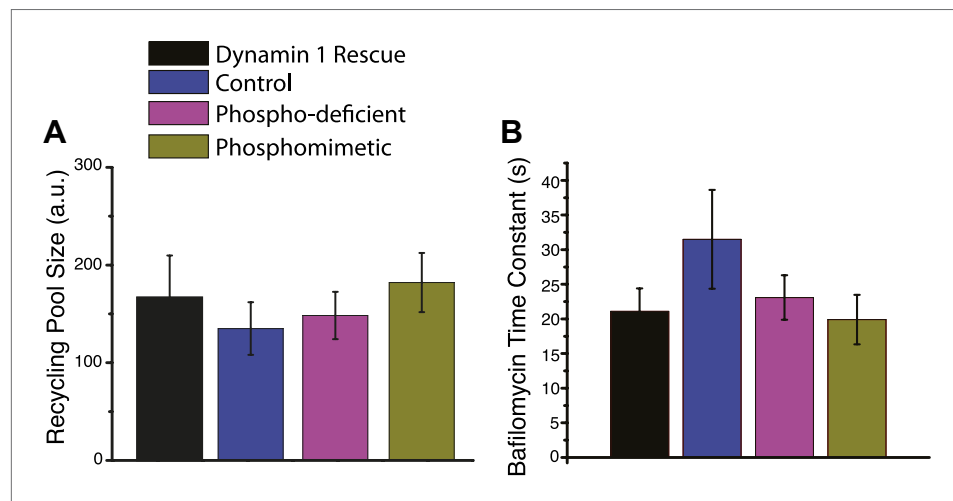DOI: 10.7554/eLife.00845.008

Figure 4—figure supplement 1. Exocytosis and pool size controls for dynamin rescues. Exocytosis and recycling pool size are measure by applying the vesicular proton pump inhibitor bafilomycin and stimulating for 1000 AP at 10 Hz. The rate of fluorescent increase gives a measure of exocytosis and the absolute fluorescence represents the size of the recycling pool. (**A**) No changes are observed in the size of the recycling pool, for the full length dynamin 1 rescue, control, dynamin 1 phospho-deficient mutant, or dynamin 1 phosphomimetic mutant as measured with the bafilomycin assay. (**B**) No significant change is observed in the bafliomycin time constant, a measure of exocytosis for the full length dynamin 1 rescue, control, dynamin 1 phospho-deficient mutant, or dynamin 1 phosphomimetic mutant. N = 9, 5, 5, 7 for dynamin 1, control, phospho-deficient, phosphomimetic.
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