Phosphatidylserine is critical for vesicle fission during clathrin-mediated endocytosis

(Running title: PS in vesicle fission during CME)

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Supplementary results

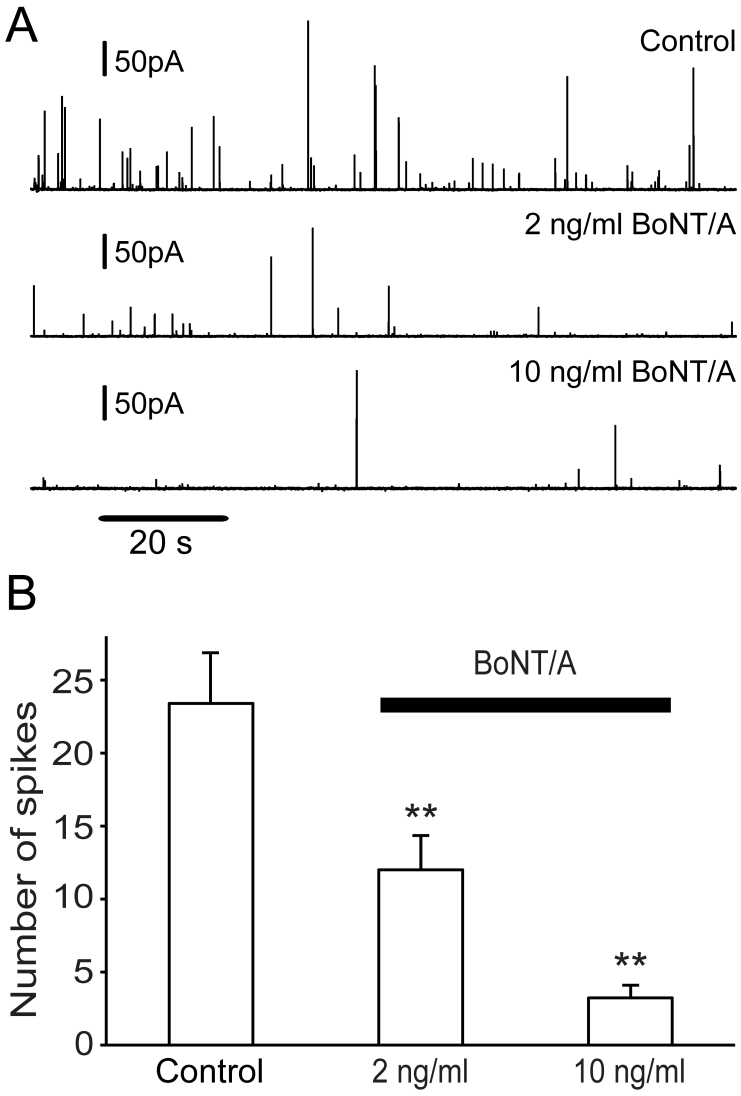

Fig. S1. Treatments with BoNT/A, which cleaves the last 9 amino acids of SNAP-25 c-terminus, inhibited exocytosis in chromaffin cells using carbon fiber amperometry. **A.** Representative amperometric traces in a control cell (*top*), cells with a 45-min incubation of BoNT/A at a concentration of either 2 (*middle*), or 10 ng/ml (*bottom*). **B.** Quantifications show that BoNT/A treatments reduced the number of amperometrical spikes in a concentration dependent manner

(Control: 23.4 ± 3.47, n = 18 cells, 2 ng/ml BoNT/A: 12.0 ± 2.36, n = 18 cells, 10 ng/ml BoNT/A: 3.2 ± 0.81, n = 18 cells; one-way ANOVA followed by Tukey's post hoc test, $F_{(2, 51)}$ = 17.7513, *** $p < 0.0001$, control vs. 2 ng/ml BoNT/A: ** $p = 0.0042$, control vs. 10 ng/ml BoNT/A: ** $p = 0.0010$). Data was collected from 4 independent cultures.

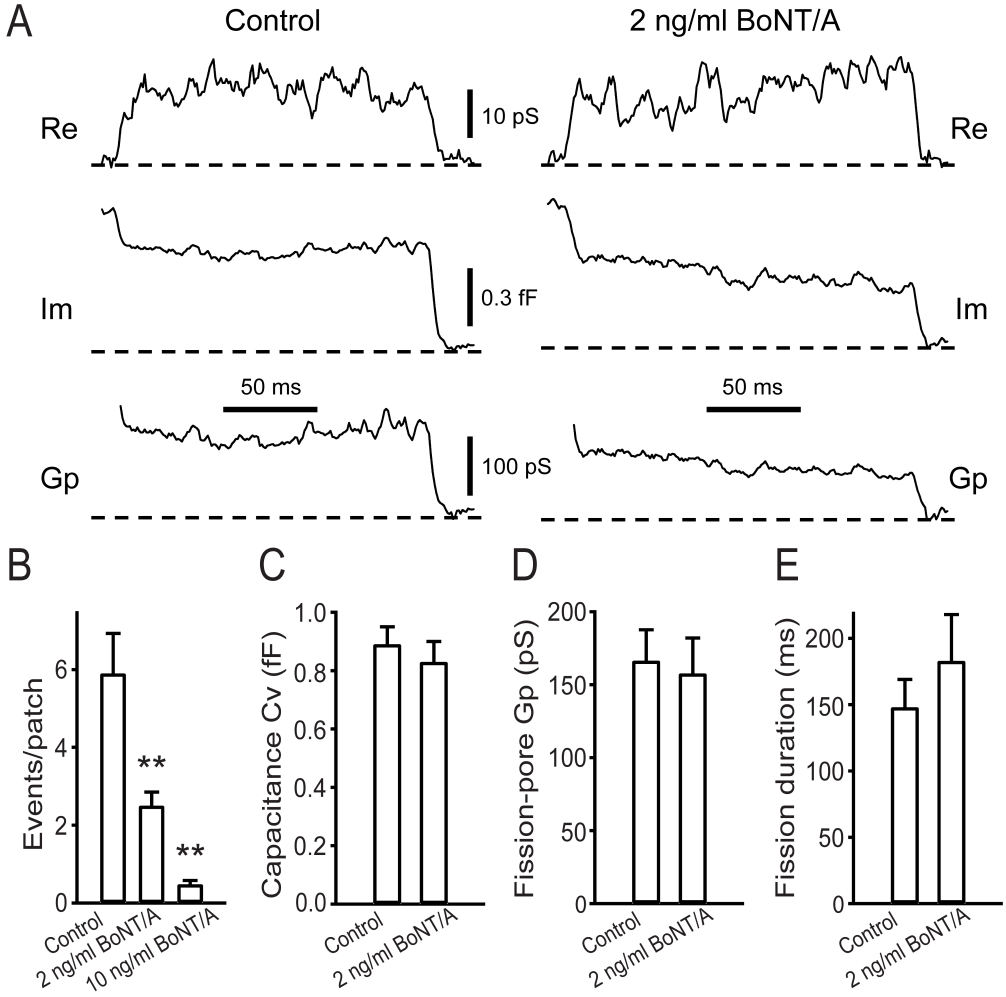

Fig. S2. The treatment of BoNT/A at 2 ng/ml had no obvious effect on the fission-pore duration using cell-attached capacitance recordings. **A.** Representative endocytic events, as membrane

conductance (Re), membrane capacitance (Im) and the fission-pore conductance (Gp) recorded in the cell-attached configuration at 2 mM $[Ca^{2+}]_e$ from control cells (left) or cells treated with BoNT/A (right). **B**. The number of endocytic events within the first 5 min time of cell-attached recordings was significantly reduced by BoNT/A in a concentration dependent manner (Control: 5.90 ± 1.03, n = 86 cells, 2 ng/ml BoNT/A: 2.50 ± 0.35, n = 108 cells, 10 ng/ml BoNT/A: 0.48 ± 0.10, n = 87 cells; one-way ANOVA followed by Tukey's post hoc test, $F_{(2, 278)} = 20.3879$, *** p < 0.0001, control vs. 2 ng/ml BoNT/A: ** p = 0.001056, control vs. 10 ng/ml BoNT/A: ** p = 0.001043). **C-E**. The capacitance Cv of endocytic vesicles (Control: 0.89 ± 0.06, n = 43 events, 2 ng/ml BoNT/A: 0.83 ± 0.07, n = 41 events; unpaired Student's t-test, $t_{(82)} = 0.5542$, p = 0.5809) (C), the fission-pore Gp (Control: 166.4 ± 21.2, n = 43 events, 2 ng/ml BoNT/A: 157.7 ± 24.3, n = 41 events; unpaired Student's t-test, $t_{(82)} = 0.3657$, p = 0.7155) (D), and the fission-pore duration (Control: 148.1 ± 21.2, n = 42 events, 2 ng/ml BoNT/B: 183.0 ± 35.2, n = 41 events; unpaired Student's t-test, $t_{(82)} = 0.8673$, * p = 0.3883) (E) were statistically comparable among these 3 groups. Data was collected from 4 independent cultures.

	No. of events	Amplitude pA	Half-width ms	Quantal size pC	50-90% rise time ms	Foot duration ms
Control	421	46.5 ± 2.53	6.78 ± 0.26	0.42 ± 0.03	0.92 ± 0.07	3.05± 0.26
BoNT/A	249	41.0 ± 2.70	8.58 ± 0.42**	0.41 ± 0.03	1.02 ± 0.09	3.87 ± 0.37*

Table. S1. Effects of BoNT/A at 2 ng/ml on kinetics of single amperometric spikes. While the quantal size, peak amplitude and 50-90% rise time remained unaltered, the half-width ($t_{(668)}$ =

2.8577, p = 0.0044) and foot duration (Control: n = 255; BoNT/A: n = 166; $t_{(419)}$ = 2.0549, p = 0.0405) were significantly increased by 2 ng/ml BoNT/A. Data are presented as mean ± SEM of individual events for each parameter. Data was collected from 4 independent cultures.