Supplemental Materials

Molecular Biology of the Cell

Toft-Bertelsen et al.

Supplementary figure legends

Figure S1 – Overexpression of syntaxin-1A does not increase SNAP-25 immunoavailability.

A. Confocal mid sections of WT adrenal chromaffin cell, WT cell expressing syntaxin-1A, and WT cell expressing EGFP stained against SNAP-25. Scale bar: 3 µm.

B. Quantification of the SNAP-25 immunolabeling identified no significant increase in SNAP-25 immunofluorescence upon syntaxin-1a overexpression (one-way ANOVA: not significant). N=3, n=29-32.

Figure S2 - Overexpression of Doc2B in chromaffin cells causes a marked reduction in calcium influx.

A. Time course of the intracellular calcium concentration, measured by microfluorimetry in chromaffin cells from Doc2B WT or Doc2B KO mice and Doc2B KO cells overexpressing Doc2b, using a repetitive stimulation protocol consisting of 6 brief (10 ms) and 4 long (100 ms) membrane depolarizations from -70 mV to +20 mV.

B. Steady-state calcium current amplitudes evoked by a brief (10 ms) membrane depolarization in the same experimental groups as in panel A from -70 mV to +20 mV. ***, $p < 0.0001$, (one-way ANOVA with Tukey's post hoc test). Doc2B WT, n = 24; Doc2B KO, n = 26; Doc2B KO + Doc2B o.e., n = 21.

Figure S3 - Overexpression of ubMunc13-2 induces a massive increase in catecholamine secretion from chromaffin cells upon intracellular calcium uncaging.

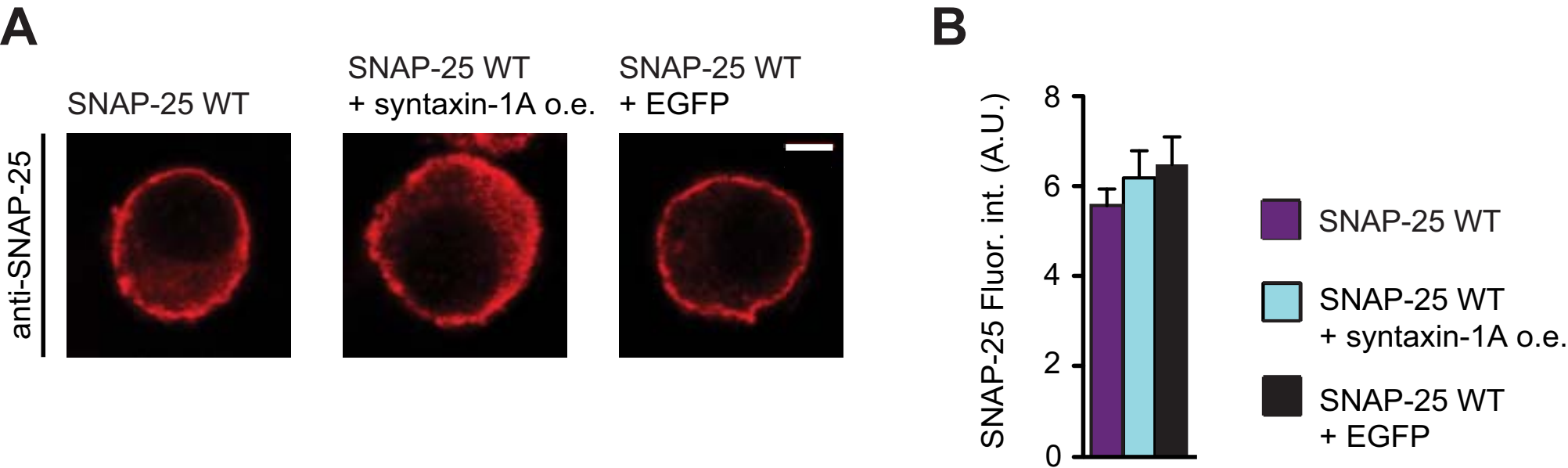
A. Average (± SEM) $[Ca^{2+}]_i$ attained after uncaging, measured by microfluorimetry.

B. The changes in cell membrane capacitance (mean traces for all recorded cells).

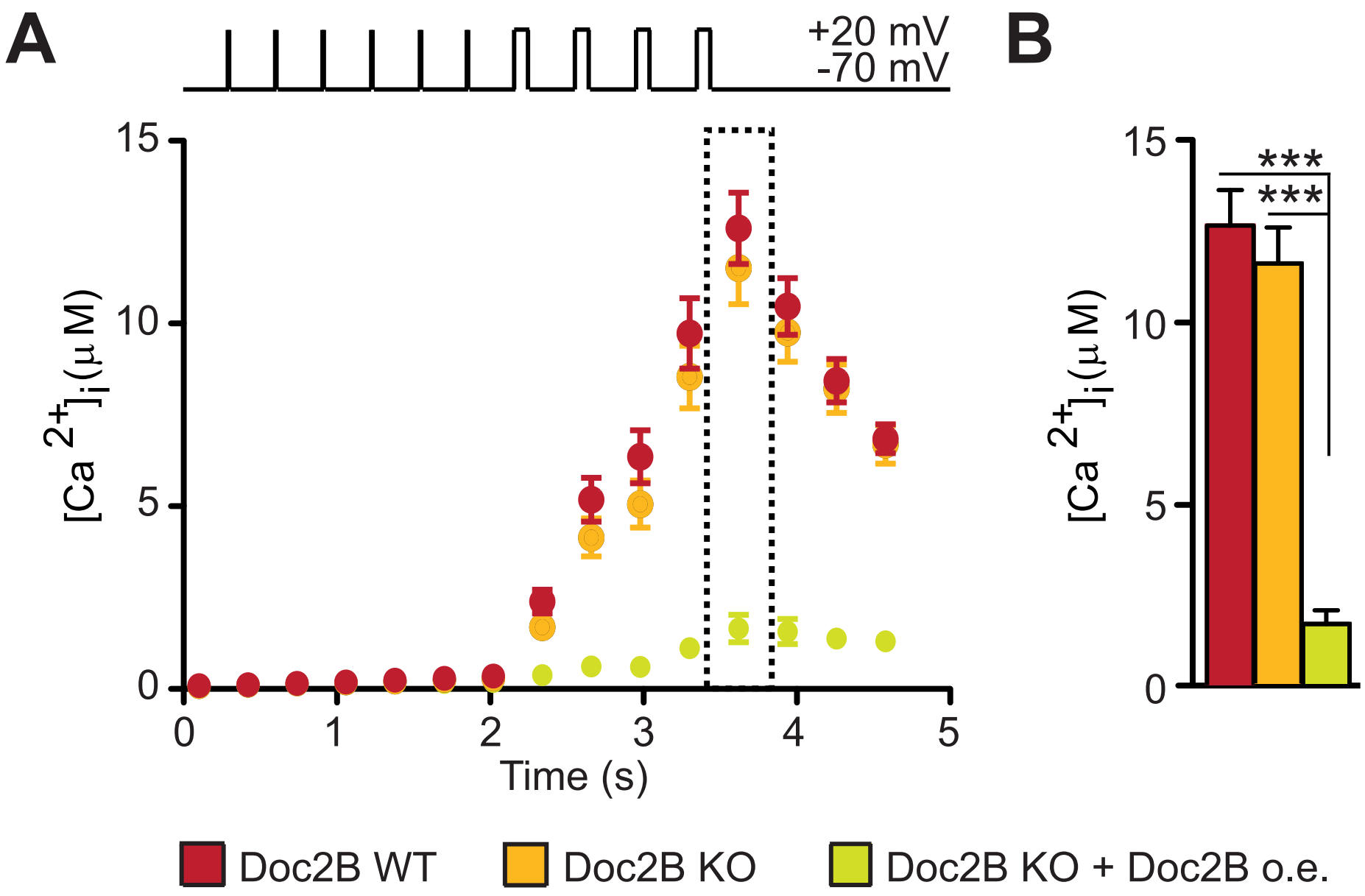
C. Amperometric signal (mean traces); left ordinate axis: amperometric current; right ordinate axis: amperometric charge. Dark lilac traces, WT cells (n = 12); magenta traces, WT cells overexpressing ubMunc13-2 (n = 11).

Figure S4 - Recruitment of syntaxin from plasma membrane clusters by SNAP-25 downregulates Ca^{2+} channels.

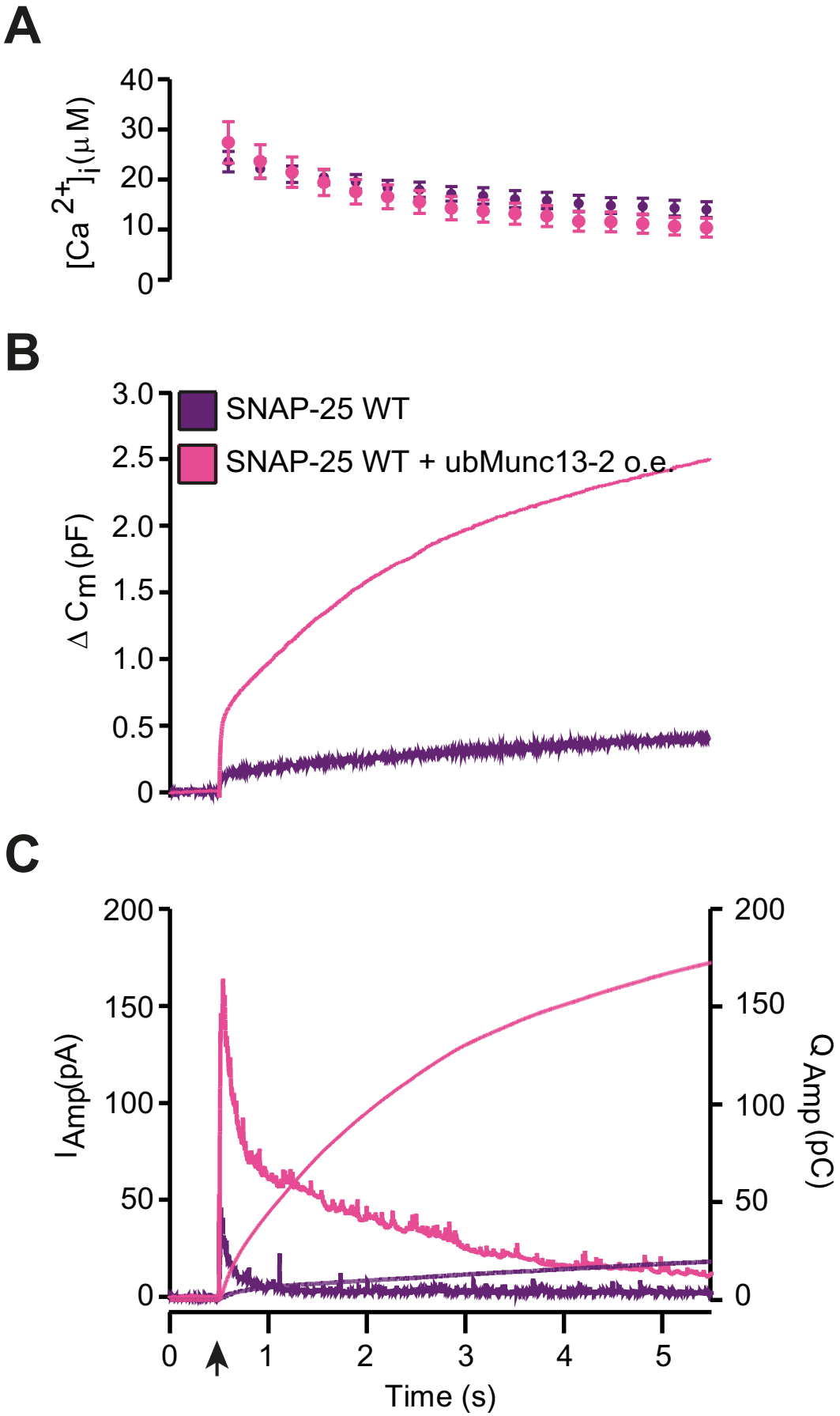
Recruitment of syntaxin-1 from dense clusters by SNAP-25 leads to down regulation of Ca^{2+} channels, possibly because the recruited syntaxin-1 becomes available for direct interaction with Ca^{2+} channels.



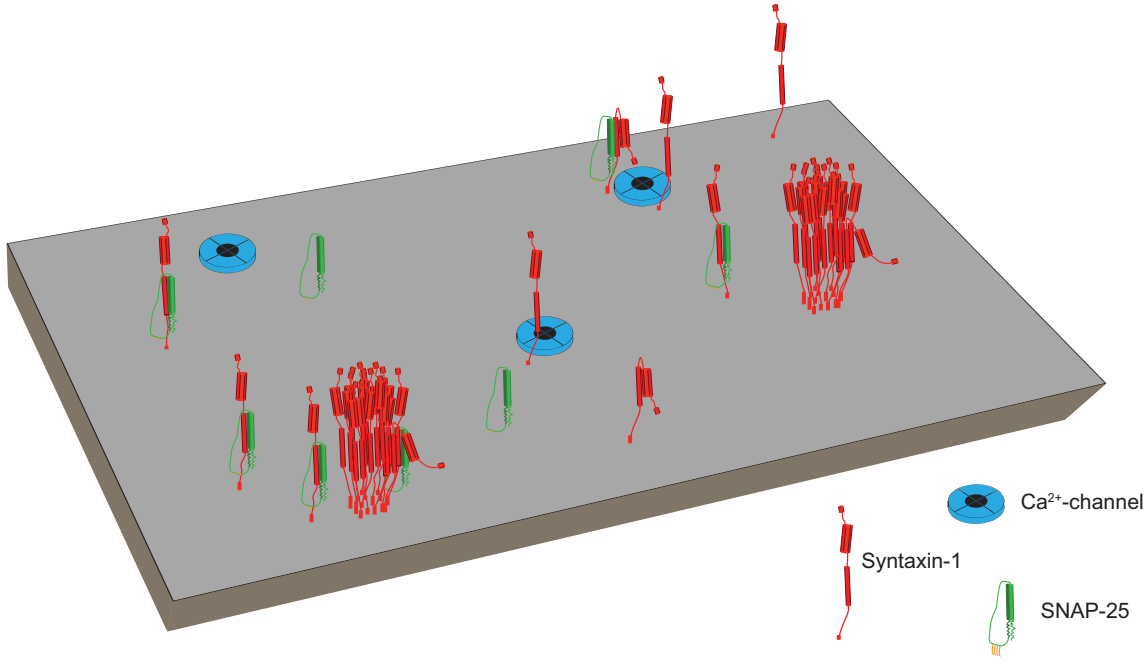
Toft-Bertelsen et al., Figure S1



Toft-Bertelsen et al., Figure S2



Toft-Bertelsen et al., Figure S3



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