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Supplemental Information

Two Pools of Vesicles Associated with Synaptic Ribbons Are Molecularly Prepared for Release

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SUPPLEMENTARY MATERIAL

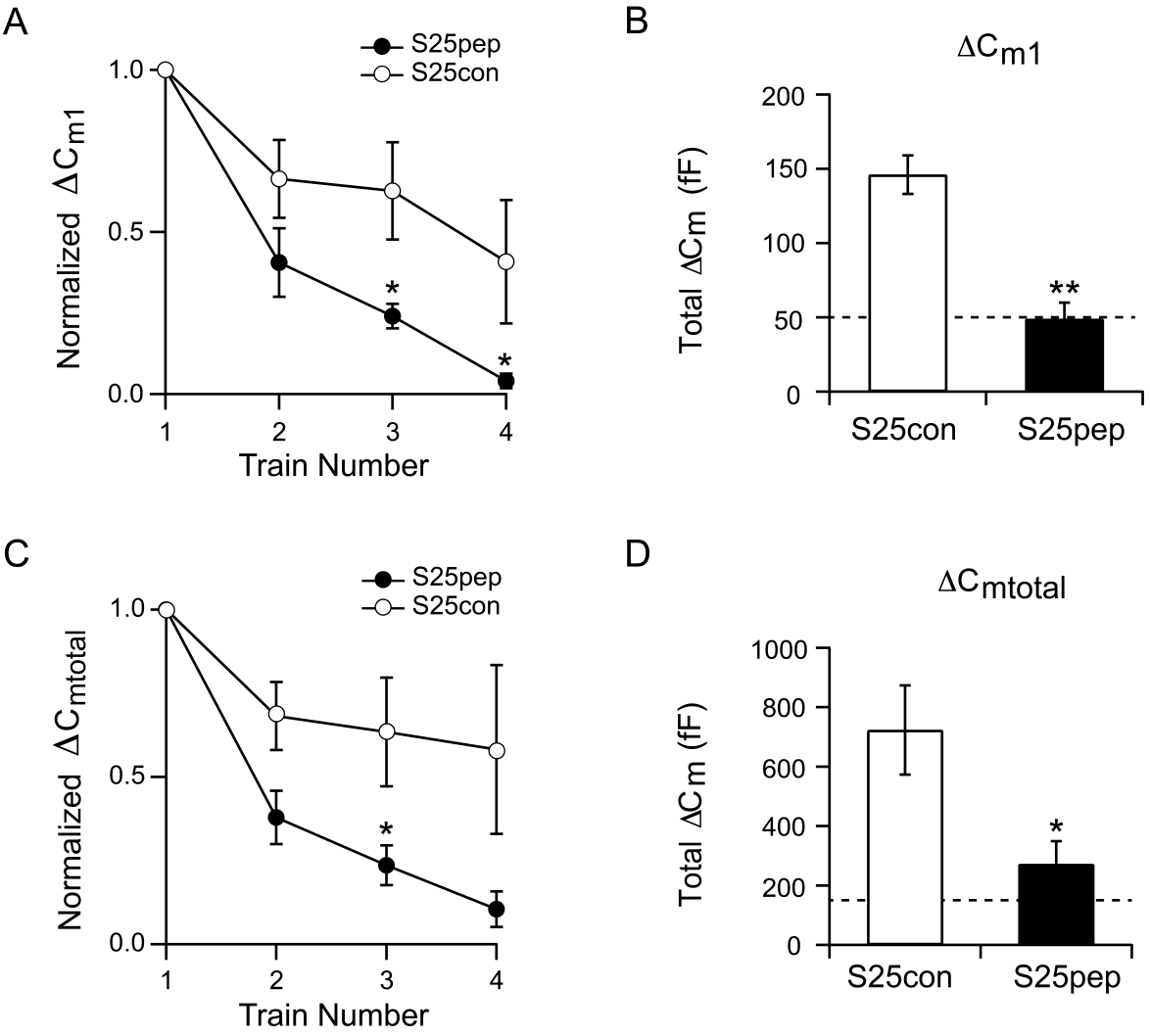

Figure S1. The SNAP-25 derived peptide S25pep spares the initial RRP and RP, but inhibits the recruitment of new vesicles to the fusion-competent state.

A. The normalized ΔC_{m1} obtained evoked by each of four stimulus trains applied at one minute intervals in bipolar cell synaptic terminals treated with either S25pep (filled circles) or S25con (open circles) is shown as a function of train number. S25pep inhibits refilling of the RRP relative to the scrambled control peptide S25con (for train 3, $p<0.04$ and for train 4, $p< 0.05$). Data were normalized to the ΔC_{m1} obtained from train 1. **B.** The cumulative total ΔC_{m1} evoked by the four stimulus trains was significantly reduced ($p<0.002$) in the presence of S25pep (filled bars) relative to S25con (open bars). Dotted line denotes 50fF. **C.** The normalized ΔC_{mtotal}

evoked by each of four successive stimulus trains applied at one minute intervals to bipolar cell synaptic terminals treated with either S25pep (filled circles) or S25con (open circles) is shown as a function of train number. There was a trend for S25pep to inhibit refilling of the RP relative to the scrambled control peptide S25con (for train 2, $p<0.08$; for train 3 $p\leq0.05$ and for train 4, $p\leq0.08$) that was supported by a difference between S25pep and S25con terminals in the slope of the relationship between ΔC_{mtotal} and train number ($p<0.03$, mixed effect model). Data were normalized to the ΔC_{mtotal} obtained from train 1. **D.** The cumulative total capacitance increase evoked by the four stimulus trains, total C_{mtotal}, exceeded 600fF in the presence of the scrambled control peptide S25con. By contrast, total ΔC_{mtotal} was significantly reduced in the presence of S25pep ($p<0.04$). Dotted line denotes 150fF. For all, S25pep terminals, n=4, and for S25con terminals, n=3 (trains 1-3) and n=2 (train 4). Data are expressed as mean ± s.e.m. Methods used to obtained these data were virtually identical to those described in the main text with the exception that the first stimulus train was given three minutes after achieving the whole-terminal recording configuration and the SNAP-25 peptides were not fluorescently-labeled. SNAP-25 peptides were derived from the C-terminal SNARE domain of SNAP25 and used at a nominal concentration of 250μM. The sequence for the SNAP25 peptide, S25pep, was NH2-IMEKADSNKTRIDEANQRATKMLGSG-OH. The sequence for S25con, the scrambled control peptide, was NH2-KNKGTSDEGSDIMQKAILNEARMTRA-OH.