

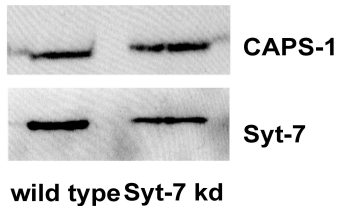
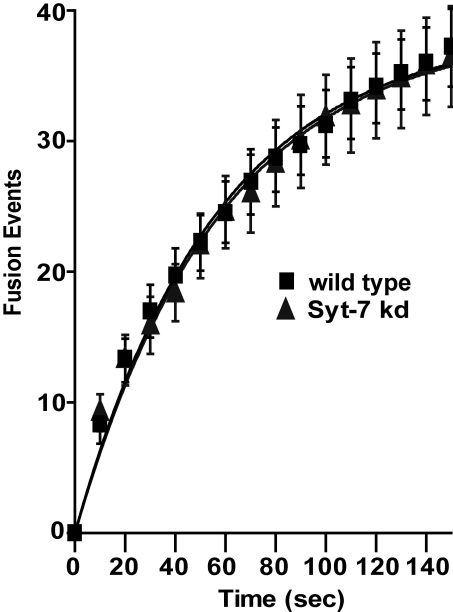
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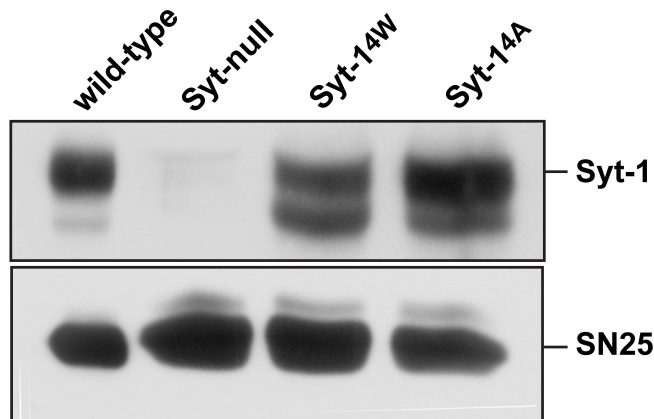
Supplemental Fig. 1. Knock down of Syt-7 does not affect evoked dense-core vesicle exocytosis in PC12 cells. Exocytic events were monitored as changes in ANF-EGFP fluorescence in 10s intervals and plotted cumulatively in wild-type cells and cells transfected with a Syt-7-directed shRNA plasmid. Western blotting and densitometry indicated that the Syt-7 shRNA plasmid reduced Syt-7 levels by at least 60%. CAPS-1 is shown as a loading control.

Supplemental Fig. 2. Syt-1^{4A} and Syt-1^{4W} are re-expressed and appropriately targeted to dense-core vesicles in Syt-1/9-null PC12 cells. **(A)** Western blotting was used to compare Syt-1 levels in wild-type cells and Syt-1/9-null cells versus null cells re-expressing Syt-1^{4W} or Syt-1^{4A}. 10 µg of cell lysate was analyzed per lane with immunoblotted SNAP-25 as a loading control. **(B)** Immunocytochemistry with Syt-1 antibody was used to localize Syt-1^{4W} and Syt-1^{4A} re-expressed in Syt-null PC12 cells. The re-expressed proteins exhibited a punctate distribution resembling that of chromogranin B (CgB), a dense-core vesicle protein. The Syt-1 antibody exhibited a degree of non-specific staining evident in the Syt-null cells that did not co-localize with CgB.

Supplemental Fig. 3. **(A)** ANF-EGFP is expressed and targeted to dense-core vesicles similarly in wild-type or Syt-1 mutant-expressing cells. Distribution shows the vesicle ANF-EGFP fluorescence (background subtracted) for 150 vesicles determined by TIRF microscopy in wild-type, Syt-1^{RK}-expressing or Syt-1^{4W}-expressing cells. Mean (\pm SE) fluorescence values were 3093 ± 182 for wild-type, 2699 ± 168 for Syt-1^{RK}, and 3025 ± 173 for Syt-1^{4W}, which were not significantly different. **(B)** BDNF-EGFP is expressed and targeted to dense-core vesicles similarly in wild-type or Syt-1 mutant-expressing cells. Distribution shows the vesicle BDNF-EGFP fluorescence (background subtracted) for 100 vesicles determined by TIRF microscopy in wild-type, Syt-1^{RK}-expressing or Syt-1^{4W}-expressing cells. Mean (\pm SE) fluorescence values were 2063 ± 163 for wild-type, 2106 ± 176 for Syt-1^{RK}, and 2417 ± 178 for Syt-1^{4W}, which were not significantly different. **(C)** Syt-1 mutants do not affect the number of plasma membrane-proximal vesicles. Dense-core vesicles containing ANF-EGFP were counted in cell footprints by TIRF microscopy and

normalized to calculated footprint area (mean \pm SE). Wild-type cells (n=20) did not differ significantly from Syt-1/9-null cells that expressed Syt-1^{RK} (n=18), Syt-1^{RK/KK} (n=15), Syt-1^{4A} (n=10) or Syt-1^{4W} (n=10) mutants.



A**B**