

Supplemental material

Abbineni et al., <https://doi.org/10.1085/jgp.201812182>

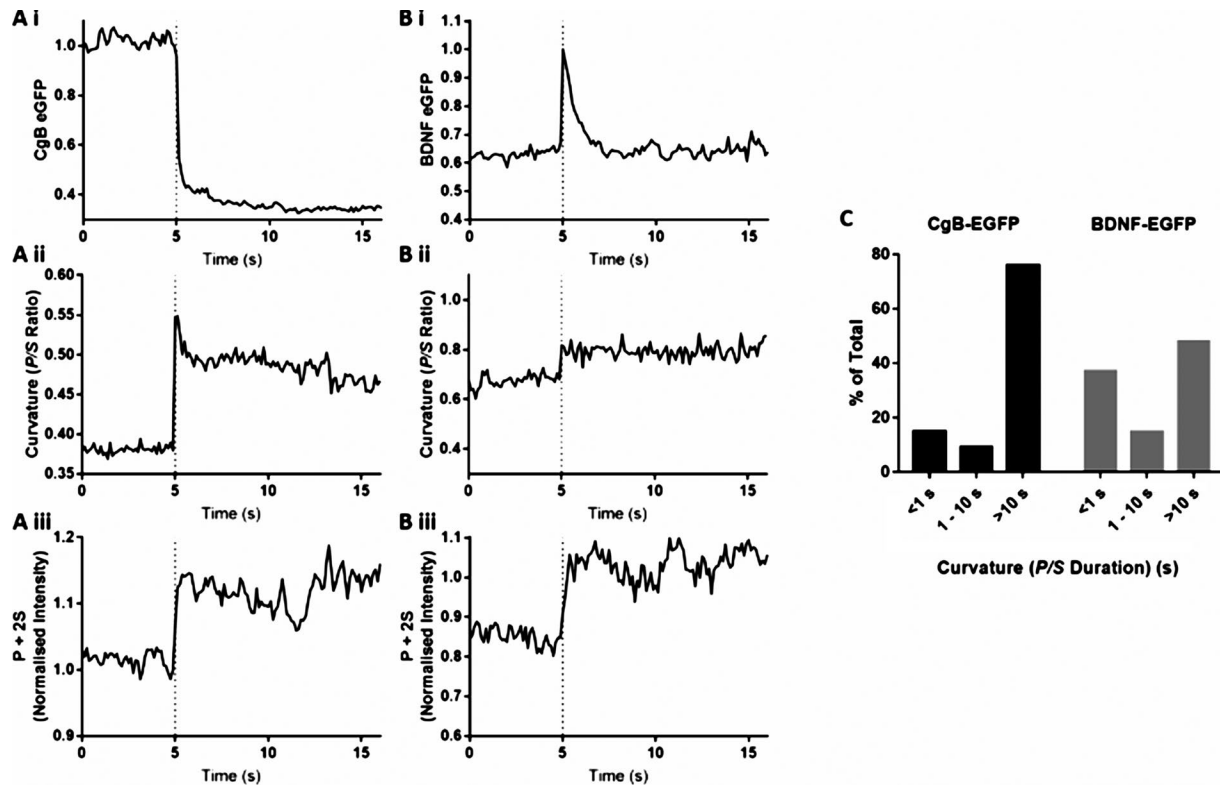


Figure S1. **CgB and BDNF restrict fusion pore expansion.** (A and B) Chromaffin cells were transfected to express CgB-EGFP or BDNF-EGFP. After stimulation of the cells with elevated K^+ , discharge of luminal proteins from individual chromaffin granules was monitored using TIRF microscopy (A i and B i). pTIRF was used to simultaneously monitor fusion pore expansion (A ii, A iii, B ii, and B iii). (C) The length of time *P/S* was elevated was calculated as described in Materials and methods for $n = 86$ CgB-EGFP granules and $n = 67$ BDNF-EGFP granules and binned into three categories.

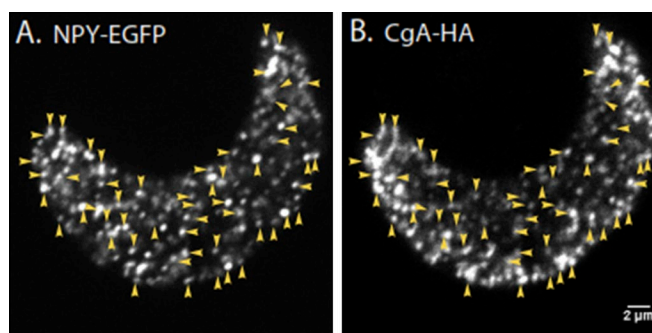


Figure S2. **Coexpressed NPY-EGFP and CgA-HA colocalize in granules.** (A and B) Chromaffin cells transfected with NPY-EGFP + CgA-HA in the experiment shown in Fig. 9 were fixed, incubated with antibody to the HA epitope, and imaged with confocal microscopy. Yellow arrowheads indicate some examples of puncta that are positive for both NPY-EGFP (A) and CgA-HA (B). To be considered colocalized, both images must have a punctate immunofluorescence that is at least $2\times$ background. For the sake of clarity, all instances of colocalization have not been marked. Fraction of NPY-GFP-containing puncta/cell that also have some CgA-HA = $97.4 \pm 1.1\%$ (mean \pm SEM). $n = 1,066$ puncta in nine cells. Fraction of CgA-HA-containing puncta/cell that also have NPY-GFP = $90.5 \pm 1.3\%$. Scale bar = $2 \mu\text{m}$.

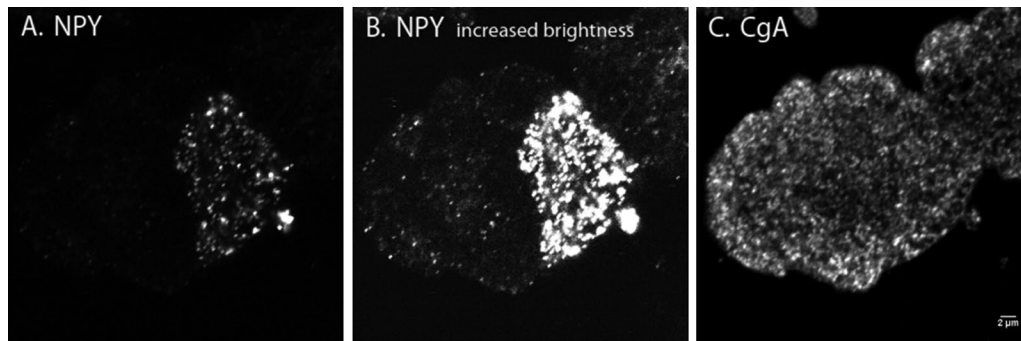


Figure S3. **Identification of cells containing high and low amounts of endogenous NPY and endogenous CgA.** Cultured bovine chromaffin cells were fixed with 4% paraformaldehyde and permeabilized with methanol. Cells were incubated with antibodies to NPY and bovine CgA, followed by Alexa Fluor-labeled anti-mouse and anti-rabbit secondary antibodies, and then visualized by confocal microscopy (as shown in Fig. 7). **(A)** Endogenous NPY is strongly expressed in one chromaffin cell. Note that there are several other chromaffin cells in the field that express CgA but barely detectable NPY. **(B)** The brightness of the NPY image in A was enhanced to show low levels of NPY. The fluorescence of NPY in the dimmer cell is less than 10% that of the brighter cell. **(C)** Endogenous CgA in the same field as in A and B. Typically ~4% of cultured bovine chromaffin cells strongly express NPY.