Protein Mobility within Secretory Granules

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SUPPLEMENTAL FIGURES

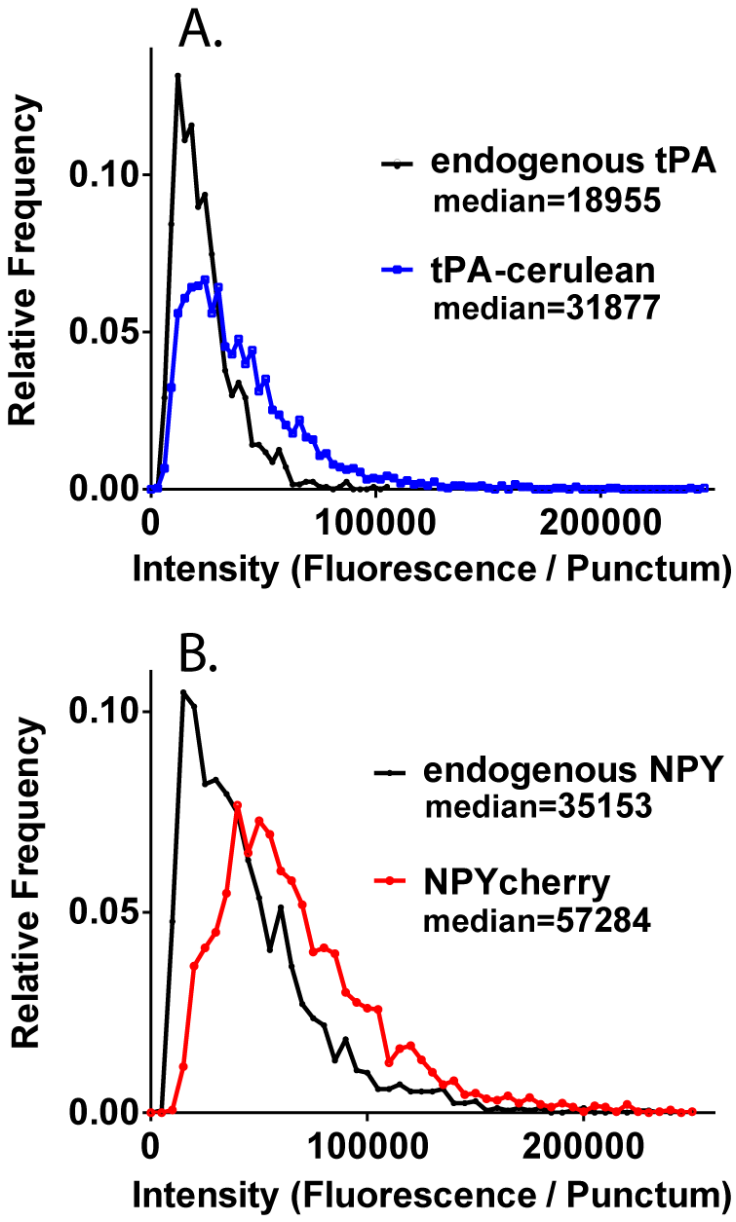

Fig. S1. Frequency distribution of the fluorescence intensity of (A) tPA- or (B) NPY-containing puncta in transfected and non-transfected cells. Chromaffin cells expressing tPA-cerulean (A) and NPY-mcherry (B) were fixed, permeabilized, and incubated with antibodies to human tPA and human NPY respectively, as in Materials and Methods. Transfected cells were identified by the appearance of tPA-cerulean or NPY-mcherry; neighboring non-transfected cells lacking a cerulean or cherry signal were used to determine levels of the endogenous proteins. Endogenous tPA, n=19 cells, 1270 puncta; tPA-cerulean, n=21 cells, 2534 puncta; endogenous NPY, n=26 cells, 1698 puncta; NPY-mcherry, n=31 cells, 2869 puncta.

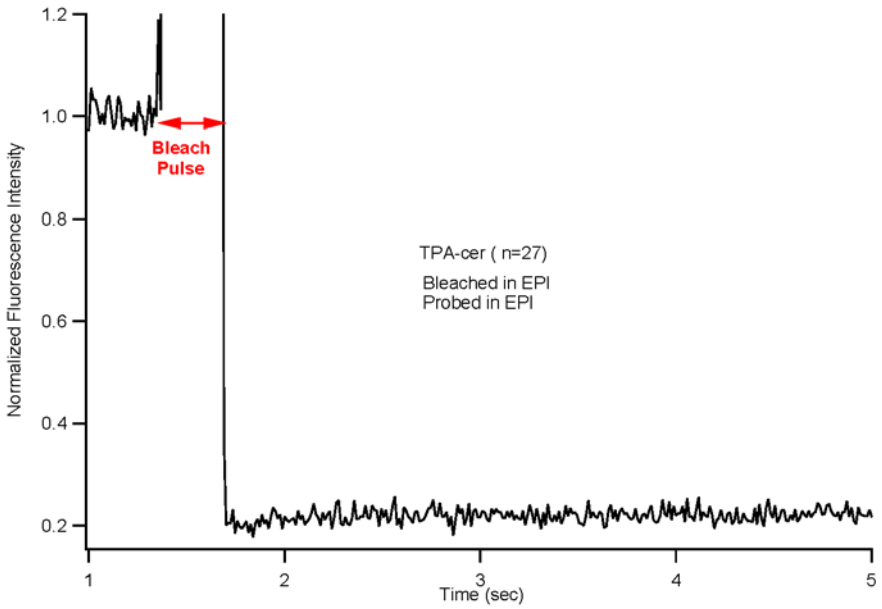

Fig. S2. Absence of significant reversible bleaching of cerulean under experimental conditions. Cells expressing tPA-cer were bleached with a 442 nm laser in EPI excitation for 300 ms. The time-dependent fluorescence intensity of each granule (n=27) was recorded in EPI. The intensity traces were first converted to normalized form $F'(t)$ according to Eq. (1), so that the resulting fluorescence immediately after the bleach was zero. The normalized fluorescence of all the tPA-cer granules were then averaged and plotted according to Eq.(2), so that the ordinate at time prior to the bleach is 1.0 and immediately after the bleach equaled the average, 0.2 . The recovery is 3% of the bleach fraction, much smaller than a typical recovery for the TIR bleach/TIR probe protocol.