Supplementary Figure 1  FM 4-64 loading and unloading of PH-GFP transfected and control synaptic boutons. These are representative results from 6 out of the 15 individual experiments which were pooled in compiling Fig. 4 of the main text. These data are provided to address any concern that excessive scatter in measurements of FM dye loading might somehow obscure direct effects of PH-GFP transfection on exocytosis that could lead to the misinterpretation of the residual dye staining as described in Fig 4b,c in the main text.

Neuronal cultures were loaded with FM 4-64 using a stimulation of 10 Hz for 30 sec. The top panel compares the initial FM load (arbitrary units) of transfected and control synapses. The lower panel compares the residual FM dye (fraction of the initial load) after an unloading stimulation of 10 Hz for 2 min in the same experiments. While PH-GFP transfected synapses always have higher residual FM (in 4 out of 6 experiments this is statistically significant with $P < 0.01$), the initial load is highly variable and both higher and lower FM loading of transfected synapses can be observed in individual experiments. When data are averaged across experiments, no significant differences are observed between the initial loads of transfected and control synaptic boutons (means of 712 vs. 716 a.u. for these 6 experiments). Thus, there is no correlation between the initial loading of synapses and their extent of unloading. This null result argues against an interpretation of the residual staining as the sign of a primary deficit in exocytosis. Asterisks indicate probabilities $P < 0.01$. Data are from 6 independent experiments with $n > 30$ for PH-GFP synapses and $n > 50$ for control synaptic boutons for each experiment.