



**Supplemental Figure 1. Folimycin unquenches intraterminal spH.**

A. Terminal spH fluorescence as a fraction of initial fluorescence is plotted over time. 200 nM folimycin was added at the time point indicated by the arrow. Over the next few minutes the resting terminal fluorescence more than doubled before reaching a plateau. Data represent an average of four different preparations. Error bars indicate SEM.

B. Representative spH fluorescence traces collected during 100 Hz stimulation (duration indicated by bar) at an early time point (1 in panel A) or at a later time point after folimycin application (2 in panel A). Recovery due to endocytosis and reacidification is not reduced at time point 1 (black line). By time point 2 (gray line) recovery is blocked, but the unquenching of vesicle spH has reduced the fluorescence response to stimulation by 80-85%.