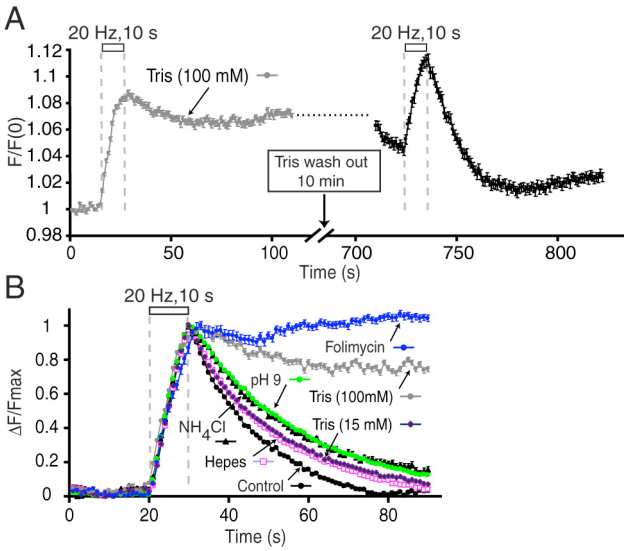

Supplementary Figure 5, The effect of different manipulations on the rate synaptic vesicle re-acidification in neuronal cell cultures prepared from transgenic mice expressing synaptophluorin in excitatory synapses. A, Plots showing the fluorescence increase upon 20 Hz-10 s stimulation in the presence of 100 mM Tris and after wash out of Tris for 10 min. Fluorescence values were normalized with respect to baseline. Fluorescence levels were maintained during 10 min wash out period. Note that after 2nd stimulation, the decrement in the fluorescence level is getting higher from the beginning of 2nd stimulation (n=4 coverslips). B, Plots showing the kinetics of fluorescence change (ΔF) in response to 20 Hz-10 s stimulation in the presence of 15 mM HEPES, 5 mM NH_4Cl, 67 nM folimycin, 15 and 100 mM Tris, and in alkaline Tyrode solution. All manipulations slowed the rate of re-acidification at neuronal cell cultures from spH21 transgenic mice ($p<0.05$ for 15 mM HEPES, 5 mM NH_4Cl, 15 mM Tris, alkaline pH experiments and $p<0.001$ for 67 nM folimycin and 100 mM Tris experiments, n=4-8 coverslips for each protocol). Time constants for re-acidification were 16.9 ± 1.7 s for controls, 24.5 ± 1.6 s for 15 mM Tris, 871.3 ± 313.4 s for 100 mM Tris, 28.2 ± 1.6 s for alkaline pH, 26.7 ± 3.1 s for NH_4Cl, 22.7 ± 1.3 s for 15 mM HEPES and 983.7 ± 214.4 s for folimycin experiments.