Supplemental data
Figure 1

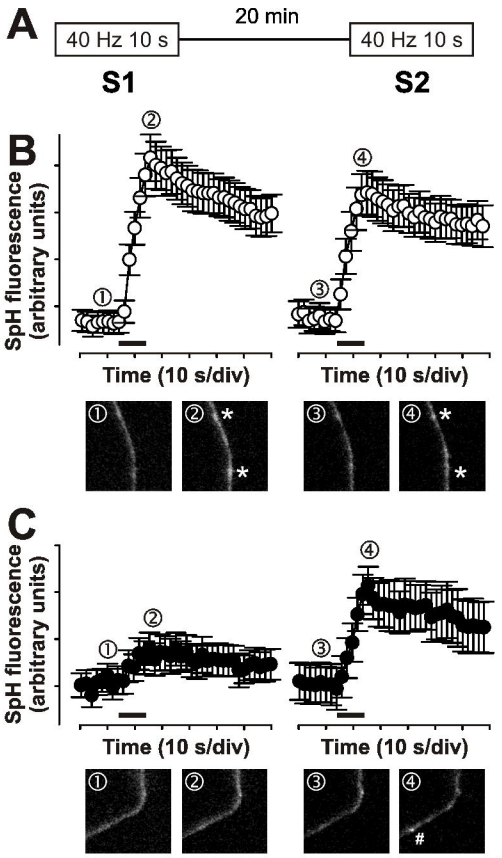

Figure 1. Detection of silent and active synapse populations with SynaptopHluorin (SpH).

Cerebellar granule neurons were transfected with SpH, a pH-dependent GFP variant fused to the luminal domain of vesicle associated membrane protein (VAMP), which displays an increase in fluorescence upon exposure to the extracellular medium during synaptic vesicle exocytosis (Miesenbock *et al*, 1998, *Nature* 394(6689):192-5). The scheme in *A* outlines the stimulation protocol, where transfected neurons were subjected to two stimulations of 40 Hz for 10 s (black bars) with a 20 min resting period between them. SpH fluorescence was measured during the train of stimulation and responses averaged for active (*B*) and silent (*C*) synapses. Representative images taken at the indicated times show the increase in fluorescence for individual active (*) and silent (#) synapses.

Supplemental data
Figure 2

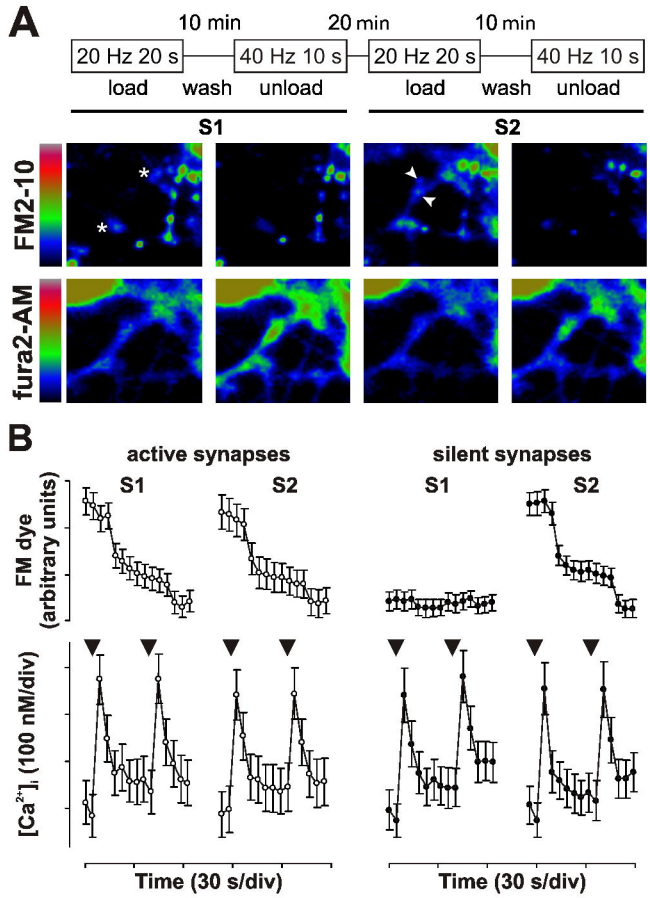

Figure 2. Silent synapses have evoked Ca^{2+} influx comparable to active synapses.

A, cerebellar granule neurons were loaded with 1 μM fura-2AM and then subjected to the S1,S2 FM2-10 loading/unloading protocol from Figure 2A. During the unloading stimulation, fluorescence images were captured at the emission wavelengths of both FM2-10 and fura-2AM. Asterisks indicate active synapses, arrowheads indicate silent synapses. *B*, fluorescence traces from a single representative experiment (mean ± SEM, n>50 synapses) are shown for FM2-10 and fura-2AM (340/380 nm ratio) in active (O) and silent synapses (●). Arrowheads indicate the timing of the 40 Hz 10 s unloading stimuli.