Synaptotagmin-IV Modulates Synaptic Function and LTP by Regulating BDNF Release
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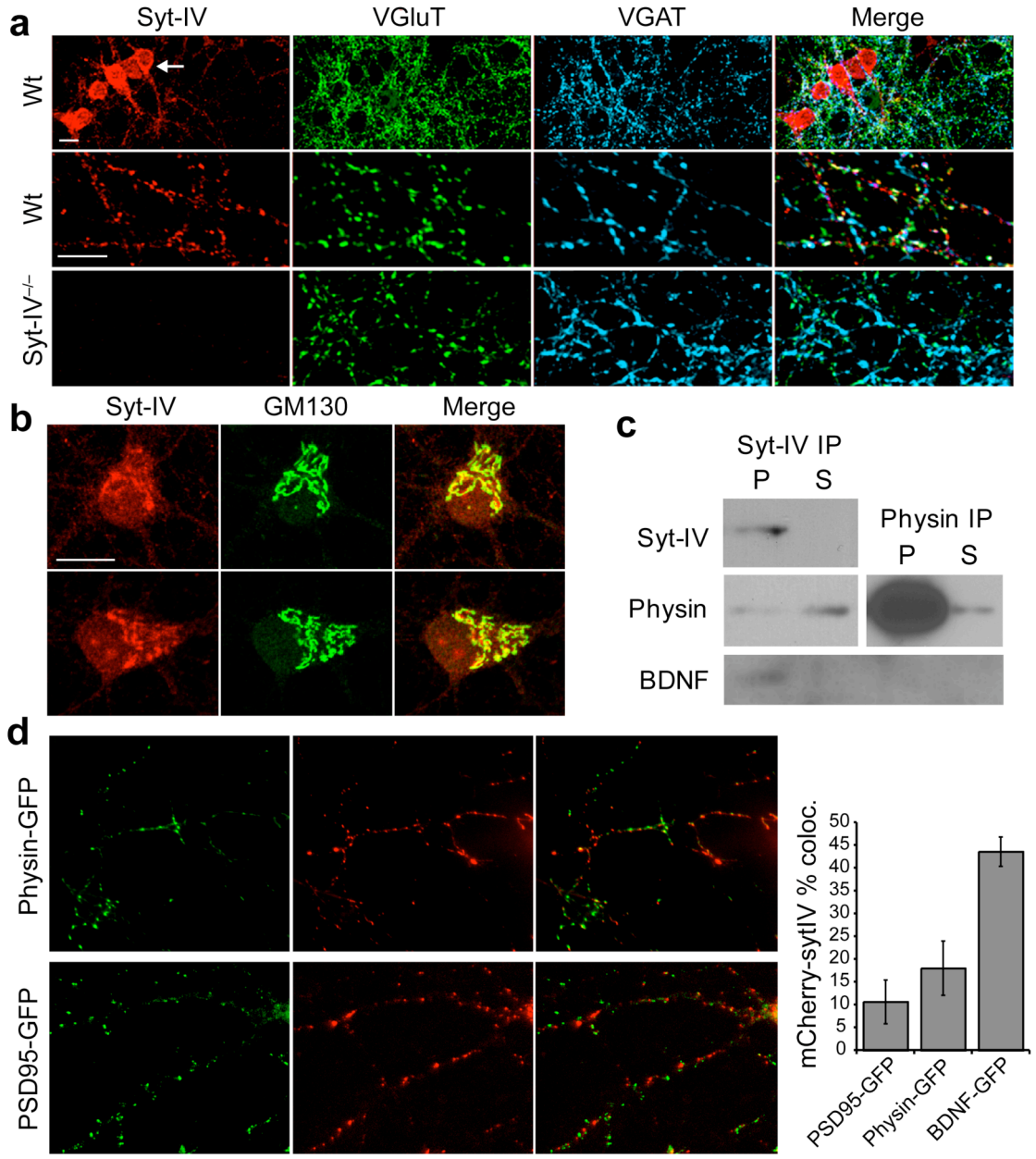
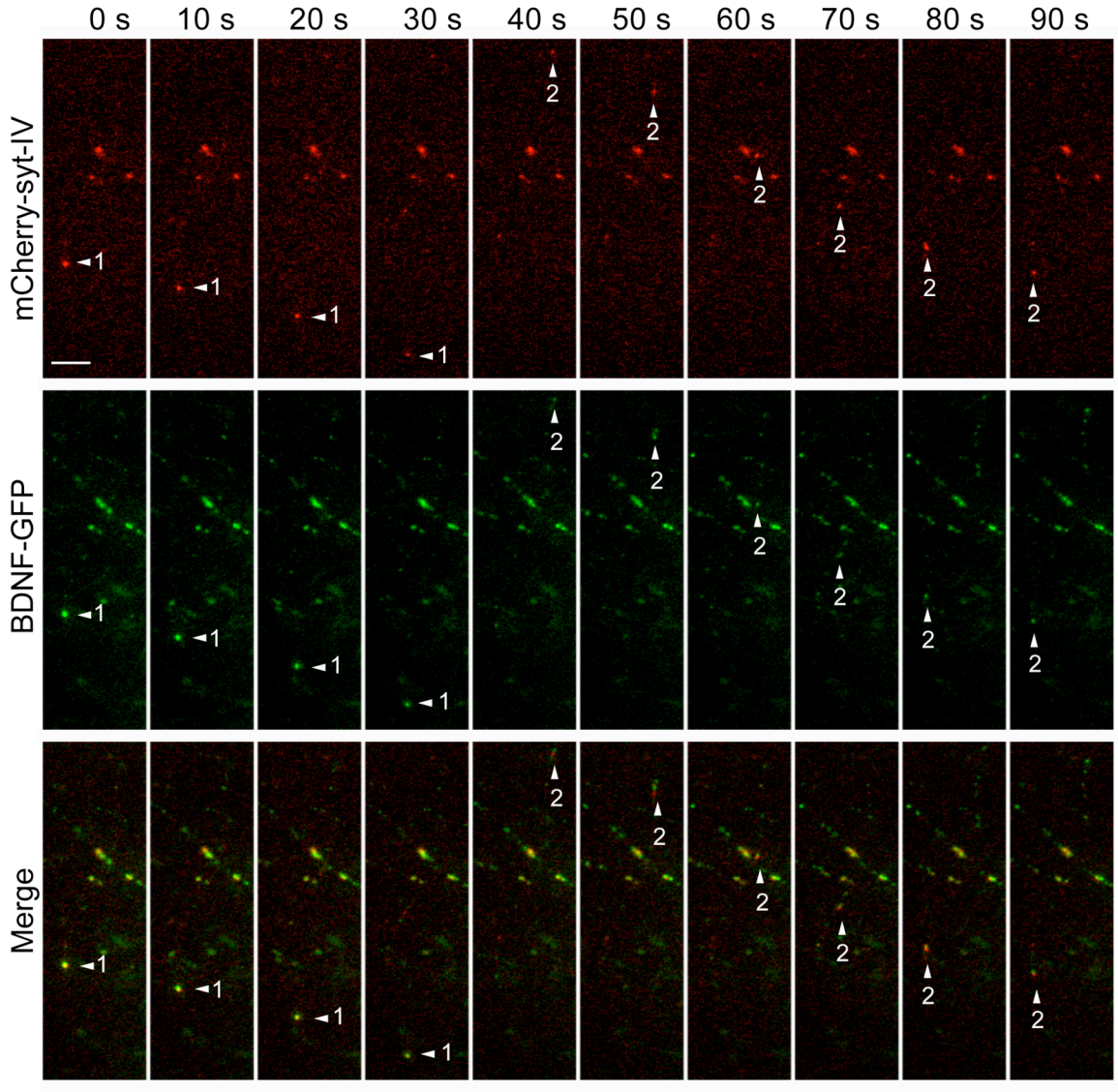
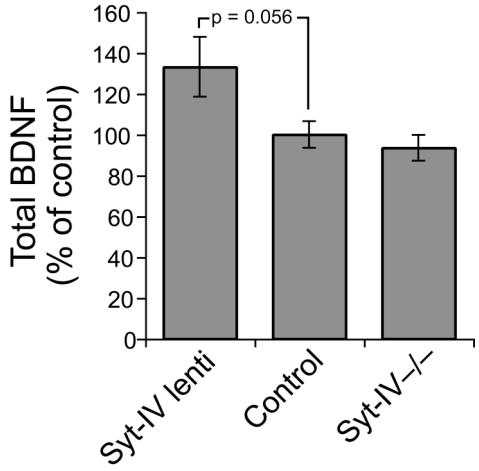


Supplemental Figure 1- Syt-IV localization.
(a) Image of syt-IV, and VGluT and VGAT, to mark excitatory and inhibitory synapses, respectively. The majority of syt-IV signal is in the Golgi (top panel, arrow). Syt-IV is also in neuronal processes (lower panels). Syt-IV$^{-/-}$ neurons were used to control for specificity of the syt-IV antibody; gain levels were set such that no background staining was visible in knockout neurons. (b) Image of syt-IV and GM130, to mark the Golgi. Gain was set such that the Golgi was clearly visible, so syt-IV in processes appears dim. (c) Immuno-organelle isolation using anti-syt-IV and anti-synaptophysin to IP vesicles from hippocampal culture lysates. Supernatant represents 5% of total material (5μg protein) for syt-IV IP and BDNF blot of physin IP, and 2.5% of total material (2.5 μg protein) for physin blot of physin IP. Pellet (P) represents 90% of IP material for syt-IV IP and 30% of IP material for physin IP. (d) Axon of a neuron co-transfected with mCherry-syt-IV and physin-GFP, to mark synaptic vesicles. (e) Dendrites of a neuron co-transfected with mCherry-syt-IV and PSD95-GFP, to mark post-synaptic sites. (f) Fraction of mCherry-syt-IV signal colocalized with GFP for the indicated fusion proteins (n = 12 cells; 3 cultures; error bars indicate SEM). Scale bar is 10 μm in all panels.



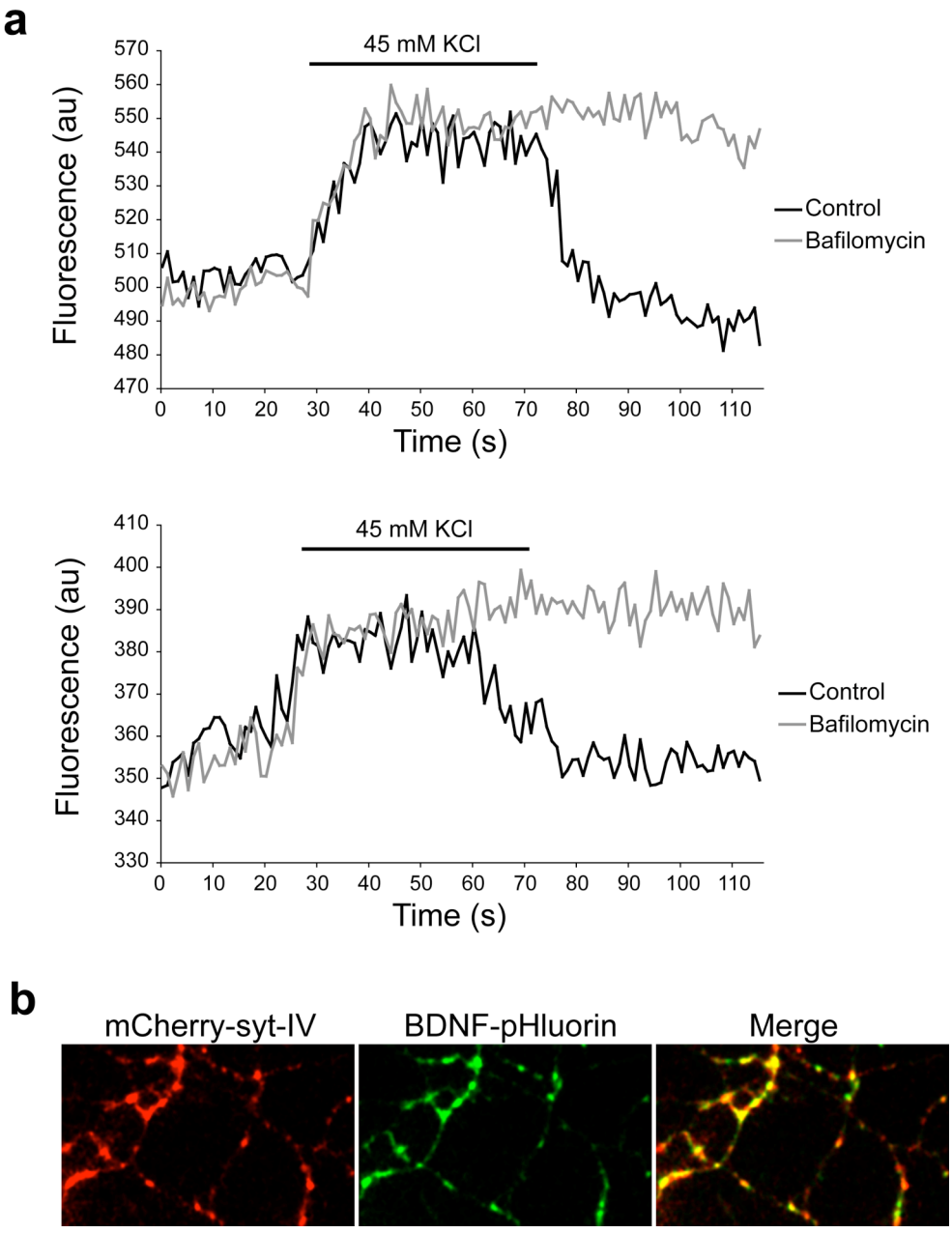
Supplemental Figure 2- mCherry-syt-IV and BDNF-GFP containing vesicles traffic rapidly together.

mCherry-syt-IV and BDNF-GFP traffic together in co-transfected hippocampal neurons. Two mobile vesicles, labeled 1 and 2, are shown moving through the field of view. Alternating images of mCherry-syt-IV and BDNF-GFP were acquired sequentially with 500 msec intervals, thus during periods of fast movement (e.g. at 50 s and 90 s) the mCherry-syt-IV signal appears "ahead" of the BDNF-GFP signal in the merged images. Approximately 50% of vesicles were mobile and traveled in anterograde and retrograde directions at speeds up to 2 μm/sec. Vesicle movement was saltatory, with an average of four pauses/ changes in direction per minute. Scale bar is 5 μm.

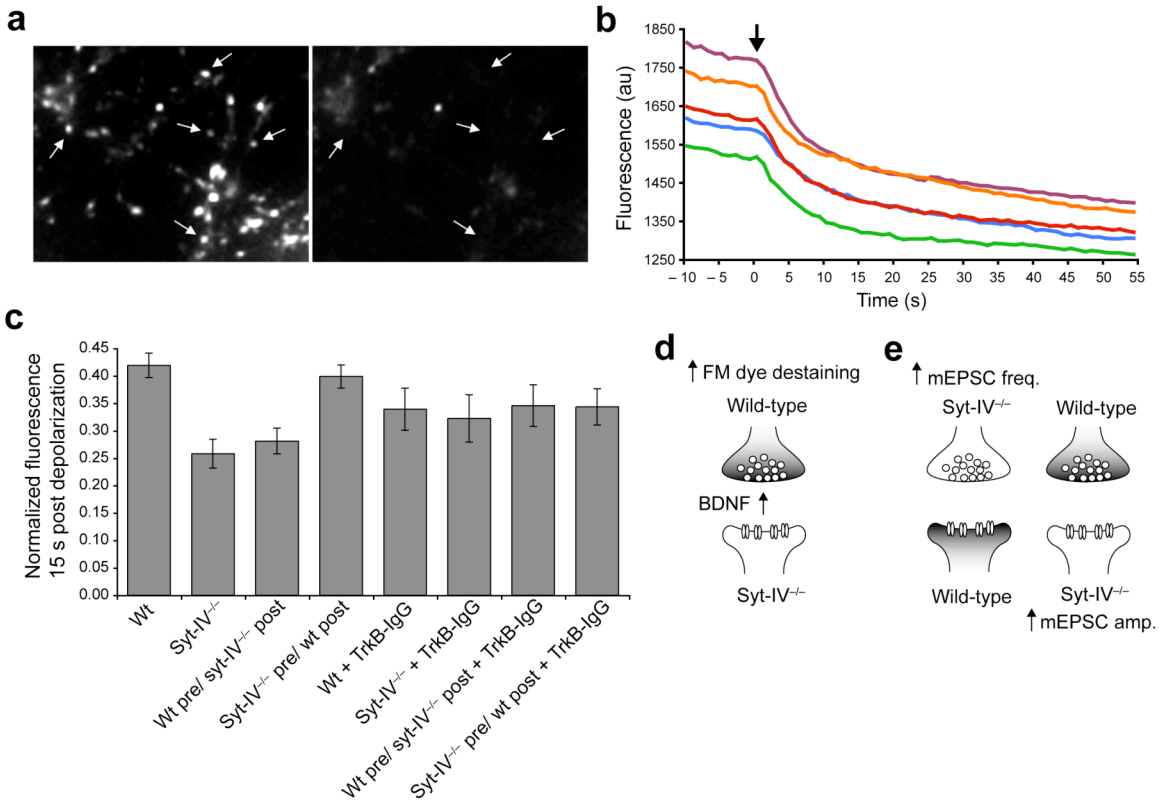


Supplemental Figure 3- Total BDNF in syt-IV over-expressing, syt-IV knockout and control neurons.

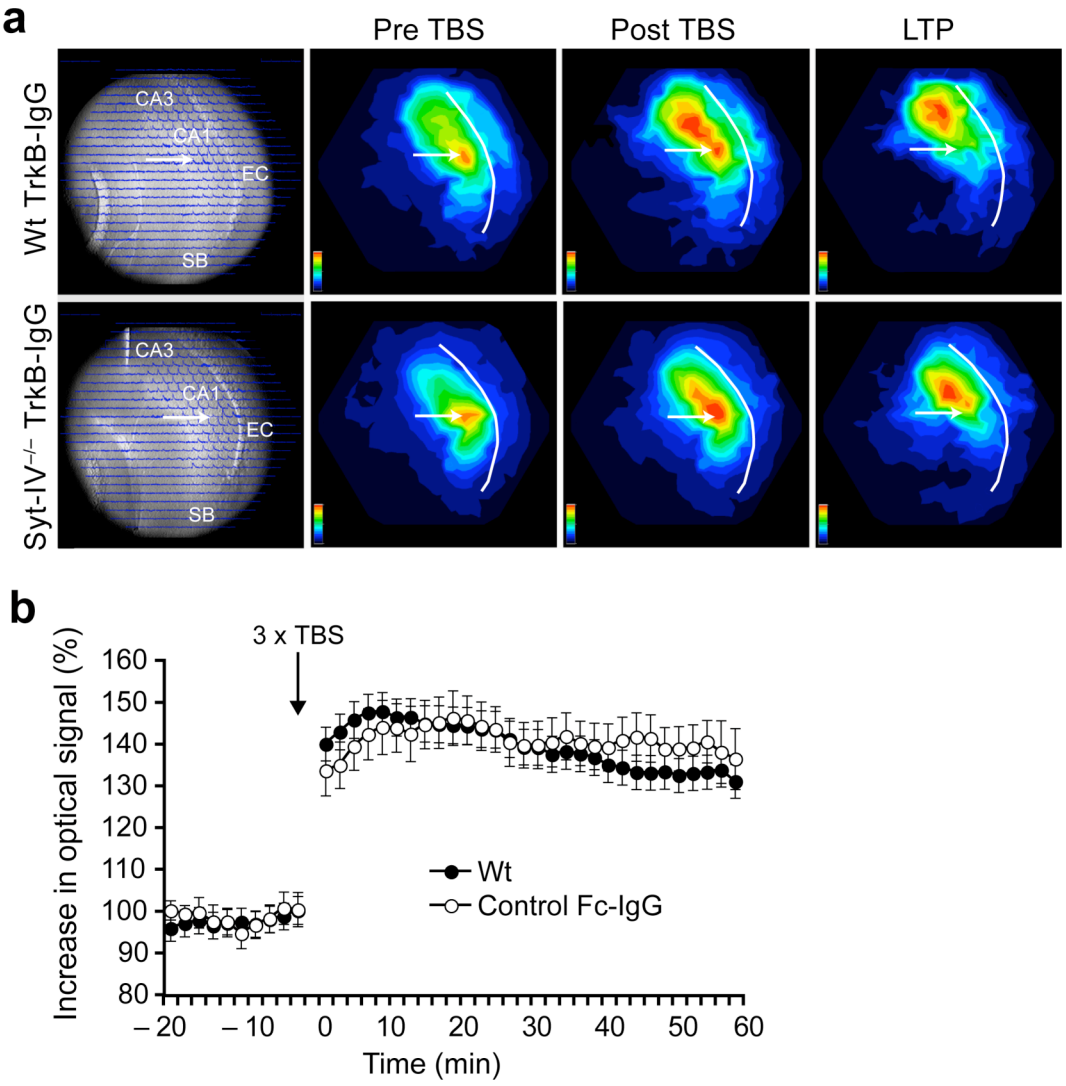
Total (intra- and extracellular) BDNF in syt-IV over-expressing, control, and syt-IV knockout neurons, expressed as percent of control (n = 5 different neuronal cultures for each condition; 3-4 duplicate wells for each culture; error bars indicate SEM). Significance was determined by a Student's t-test where * = $p < 0.05$, and one way ANOVA where $p = 0.055$).



Supplemental Figure 4- Syt-IV vesicles undergo exocytosis during depolarization with 45 mM KCl, and endocytosis following repolarization with 5 mM KCl.
(a) Sample traces of fluorescence change for large dendritic pHluorin-syt-IV events during depolarization with 45 mM KCl and repolarization with 5 mM KCl (time period of incubation with 45 mM KCl is indicated), in control conditions (black trace), and following two minute incubation with bafilomycin to inhibit the vesicular H^+ transporter and block re-acidification of newly endocytosed vesicles (gray trace). Two dendritic pHluorin-syt-IV event sites are shown (upper and lower graph), with each site imaged in control conditions, and again following bafilomycin treatment. (b) Image of a hippocampal neuron co-expressing mCherry-syt-IV and BDNF-pHluorin.



Supplemental Figure 5- FM dye destaining of hippocampal neurons and effect of syt-IV. (a) Image of boutons in a hippocampal culture loaded with FM1-43 prior to destaining (left panel), and following complete destaining 60 seconds after addition of 45 mM KCl to depolarize neurons (right panel). Arrows indicate examples of boutons selected for analysis that have undergone complete destaining. (b) Destaining curves (prior to normalization) of the boutons shown in panel a. Arrow indicates addition of 45 mM KCl. (c) Cross comparison of FM dye destain rates between the indicated conditions. A time where the vertical distances were largest in the FM destain curves (t = 15 s post depolarization) was chosen, and the normalized fluorescence value at this time for all eight traces was plotted for comparison between conditions (n = 16 coverslips; 4 neuron cultures, 10 boutons per coverslip for each condition; error bars indicate SEM). (d) Loss of post-synaptic syt-IV increases the rate of FM dye destaining from presynaptic terminals via BDNF. (e) Loss of presynaptic syt-IV increases mEPSC frequency, and loss of post-synaptic syt-IV increases mEPSC amplitude.



Supplemental Figure 6- BDNF blockade reduces LTP to the same level in wild-type and syt-IV knockouts.

(a) Left panels show syt-IV knockout and wild-type hippocampal slices with voltage-sensitive dye optical signals from each of 464 photodiodes overlayed. CA1, CA3, subiculum (SB), entorhinal cortex (EC), and stimulating electrode (arrow) are indicated. Slices were incubated with 4 μg/ml TrkB-IgG scavenger for 2-3 hours prior to voltage imaging experiments. Middle panels show color-coded amplitude maps of the voltage-sensitive dye optical signal detected in wild-type and syt-IV knockout slices pre and post theta burst stimulation (TBS), normalized to maximum amplitude in each case. Right panels are color-coded LTP maps generated by subtracting the optical signal pre TBS from the optical signal post TBS, and normalized to maximum amplitude increase for comparison between syt-IV knockout and wild-type. (b) Time course of LTP (n = 7 wild-type slices, and 5 wild-type slices incubated with control Fc-IgG; error bars indicate SEM). Incubation of slices in 4 μg/ml control Fc-IgG for 2-3 hours prior to voltage imaging experiments had no effect on LTP. The stimulation current was 25 μA for all experiments.