Supplemental Figure 1. Example graphic showing 3DMA automated spine detection. The bottom panel shows an example dendrite that has been reconstructed from forty 0.2 μm optical sections acquired with a 100X objective (1.4NA). The top panel shows the 3DMA graphical output of these stacks of images in which the dendritic backbone is in black, the central axis of the dendrite is designated as a grey line, and the detected spines are shown in color (colors are randomly assigned). 3DMA detection and categorization of spines proceeds in 4 main phases: segmentation, construction of the medial axis, dendritic backbone extraction, and spine detection. First, we chose a single threshold intensity for neuron detection: voxels having an intensity above this threshold were assigned to the neuron phase and the remaining voxels were assigned to the background phase. The same threshold intensity was applied to all images acquired for a single experiment in which 10 sections from a wild type and 10 sections from a *synGAP*$^{+/-}$ littermate were analyzed. Next, the medial axis of the neuron was detected using an erosion-based algorithm. The medial axis is composed of voxels from the neuron phase which are equidistant from voxels on the neuron surface. At this point, we manually trimmed spurious paths that were not part of the dendrite by using a minimum length criterion. After the medial axis was constructed and trimmed, the dendritic backbone was extracted from the medial axis. Finally, 3DMA detected the spine components. Any dendritic phase component that did not contain a dendritic backbone but had a center of mass within a specified distance from a nearby backbone was identified as a detached spine head. Attached spines were detected as local protrusions from a dendritic backbone. 3DMA then performed a final merging algorithm to identify spines with more than one attached or detached spine component. At this point, we manually edited protrusions that were erroneously detected as spines. Once the spines were

detected, 3DMA calculated the length and head volume of each spine and then categorized the spines as *stubby*, *thin*, or *mushroom* based on the ratio of these measurements.