



Supplementary Figure 4. Analyses of SHANK3 mutations in rat hippocampal neuronal cultures. **(a)** Western Blot analysis of the GFP constructs revealed similar sizes of Shank3 WT and fusion proteins carrying point mutations (R12C, R300C). The frame-shift mutation (InsG3680) results in a truncation of the protein. **(b, c)** Compared to the WT protein, the truncated Shank3 molecule (missing the C-terminal SAM domain), is evenly distributed in dendrites and does not cluster at synapses (synaptic clustering ratio, SCR, 0.8) as revealed by immunostaining (IH) against the presynaptic marker molecule Bassoon. In contrast, the two other mutated Shank3 proteins have the potential to cluster (SCR \leq 0.35). However, only about one third of these clusters are Bassoon positive (S/B = Shank3/Bassoon ratio, arrows, $^{***} \leq 0.001$).