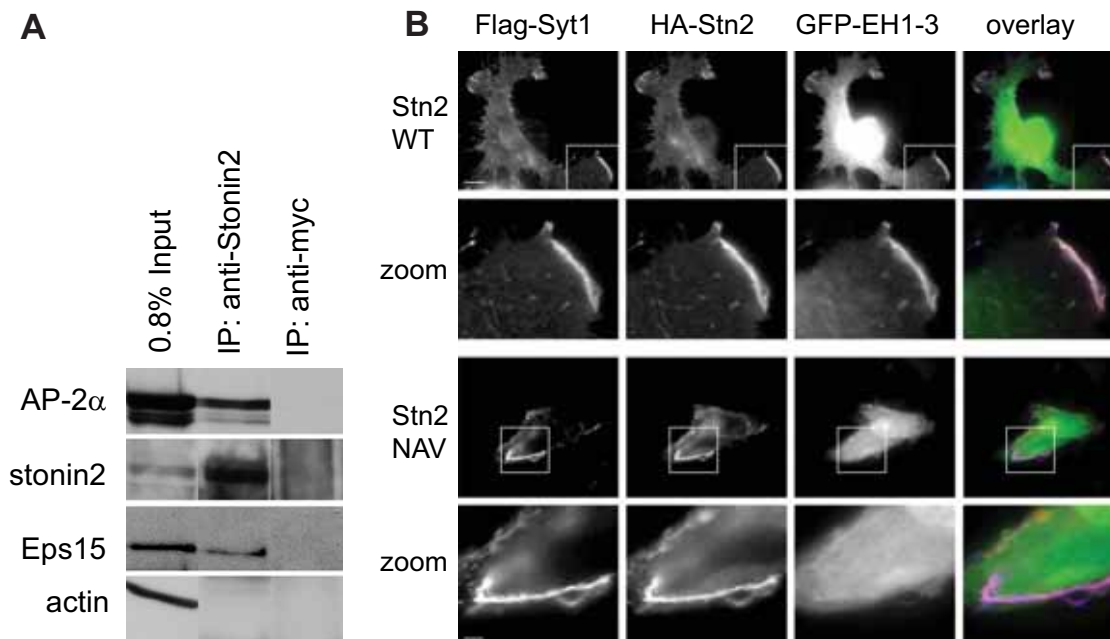


Supplementary Figure 1: Complex formation of Stonin2 and Eps15 in living cells.



(A) Stonin2 co-immunoprecipitates with both AP-2 and Eps15. Anti-stonin2- or c-myc-antibody coupled protein A/G beads were incubated with Triton X-100-extracted rat brain homogenate. Immunoprecipitated proteins were detected by immunoblotting for AP-2 $\alpha$ , stonin2, Eps15, or actin as a control. 0.8% of the input was loaded as standard.

(B) NIE-115 cells were co-transfected with lumenally Flag-tagged synaptotagmin1 (Syt1), HA-tagged stonin2 WT (HA-Stn2) or stonin2 NAV mutant (Stn2 NAV) and GFP-EH1-3. Surface synaptotagmin1 was labeled by anti-Flag antibodies (blue). Following permeabilization stonin2 was immunostained using antibodies directed against stonin2 (red). GFP EH1-3 is depicted in green. Synaptotagmin1 is known to recruit stonin2 to the plasma membrane in NIE-115 cells. Stonin2 WT in turn is then able to recruit GFP-EH1-3 to the plasma membrane as well, while in the presence of the stonin2 NAV mutant GFP-EH1-3 is not detected at the plasma membrane. The scale bar is 10 $\mu$ m. The boxed areas are enlarged in the panel underneath.