Novel ganglioside-mediated entry of botulinum neurotoxin serotype D into neurons"
(JBC/2011/254086)
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Supplemental Figure 1

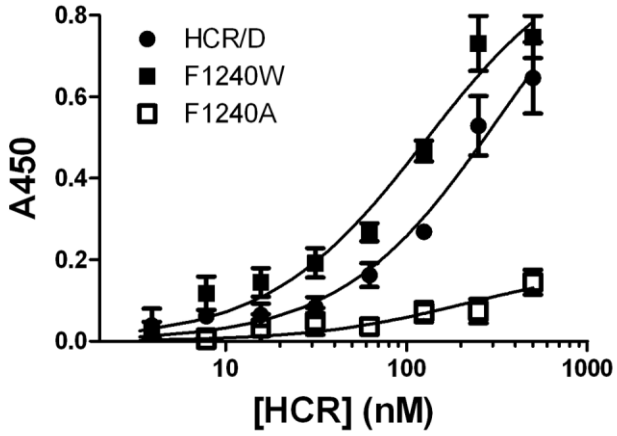

Fig S1. Gangloside binding mediated by F1240 is conserved with W substitution. GT1b (0.5ug/well) was immobilized on 96-well microtiter plate. HCR/D, F1240W, and F1240A were bound for 1 hr at 4°C. Bound HCR was detected using anti-FLAG antibody and HRP- conjugated secondary mAb and detection by using Ultra-TMB. The reaction was stopped with sulfuric acid and absorbance was read at 450 nm. Data were generated in triplicate and shown as described in the Experimental Procedures.

Supplemental Figure 2
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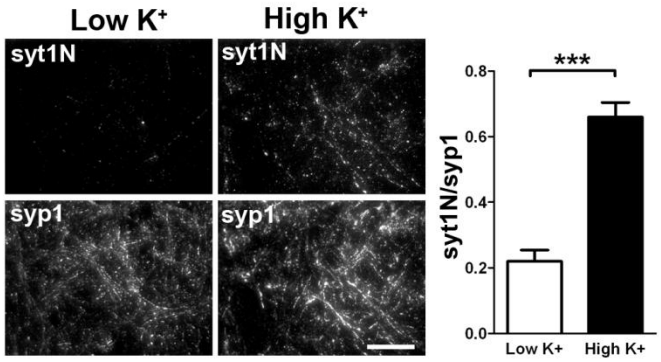

Fig S2. High potassium triggers exocytosis of synaptic vesicles. An N-terminal monoclonal synaptotagmin antibody labeled with Oyster 650 (syt1N) was incubated with rat E18 primary cortical neurons for 5 min at 37°C in 56 mM K^+ (high) or 5.6mM K^+ (low) buffer. Cells were fixed, permeabilized, and stained for synaptophysin1. Representative immunofluorescence images are shown. Scale bar = 20μm. Quantification of syt1N was normalized to syp1 signal and averaged from five fields; ***, P<0.001.

Supplemental Figure 3
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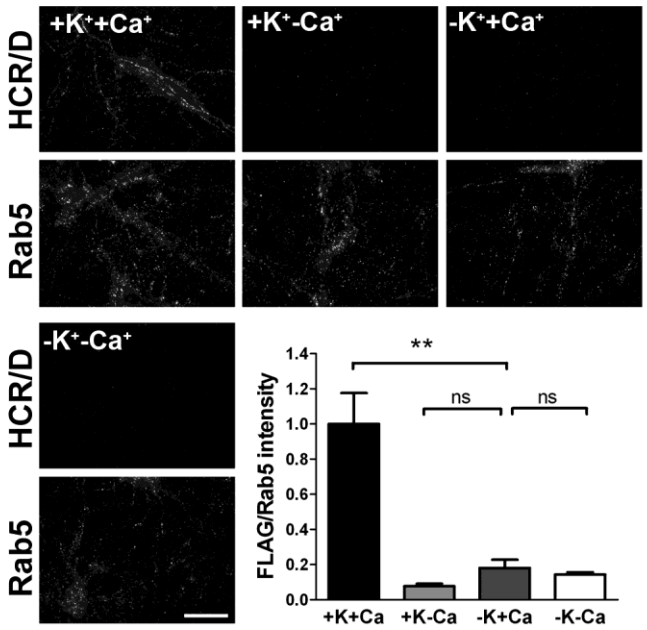

Fig S3. Uncoupling membrane depolarizing with SV cycling inhibits HCR/D entry. HCR/D was incubated with rat E18 primary cortical neurons for 5 min at 37°C in $+K^{+}$,$+Ca^{++}$, $+K^{+}$,$-Ca^{++}$, $-K^{+}$,$+Ca^{++}$, or $-K^{+}$,$-Ca^{++}$ buffer. Cells were fixed, permeabilized, and stained for FLAG-HCR and Rab5. Representative immunofluorescence images are shown. Scale bar = 20μm. Quantification of FLAG-HCR was normalized to Rab5 signal and averaged from five fields; **, $P<0.01$ and ns, not significant.