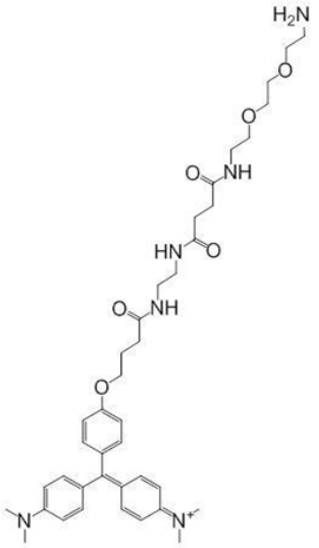
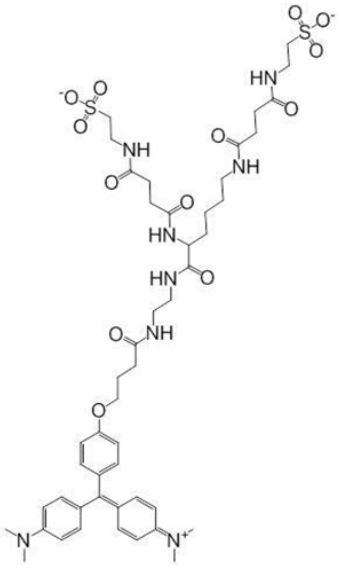
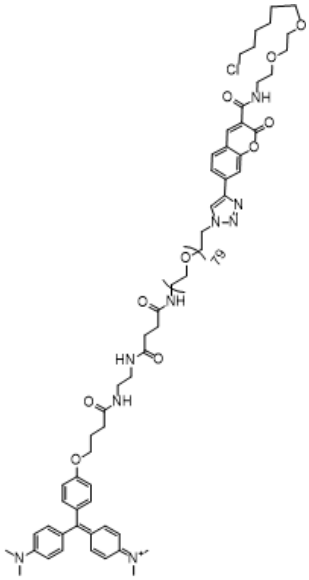
Fig. S1. MG derivative chemical structures and MWs.



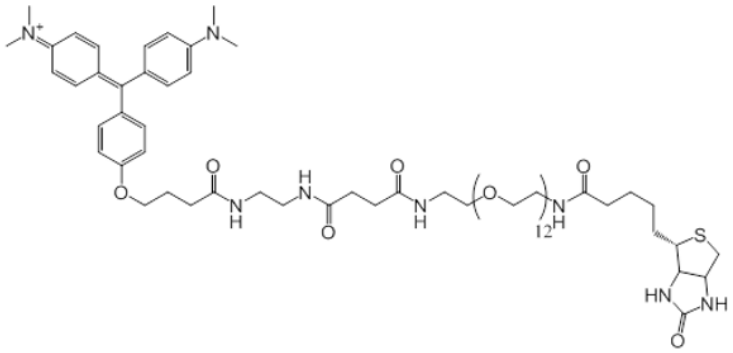
MG-2p, 704



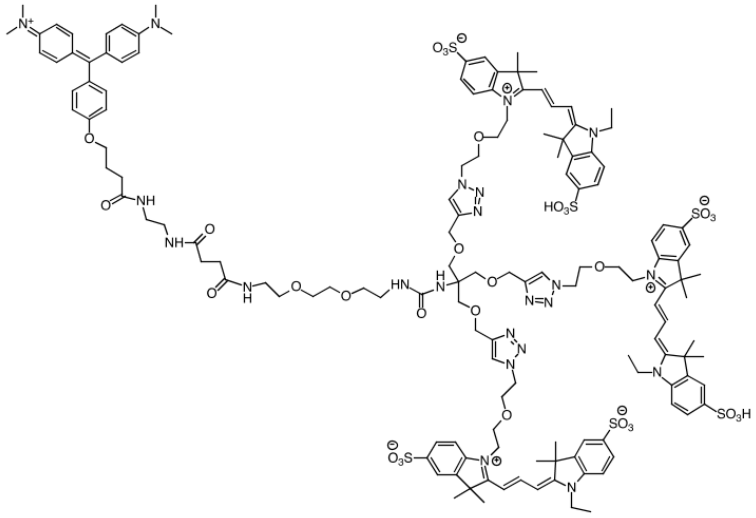
MG-B tau, 1015



MG-80p-halo, 4478



MG-12p-biotin, 1327



MG-2p(Cy3)$_3$, 2853

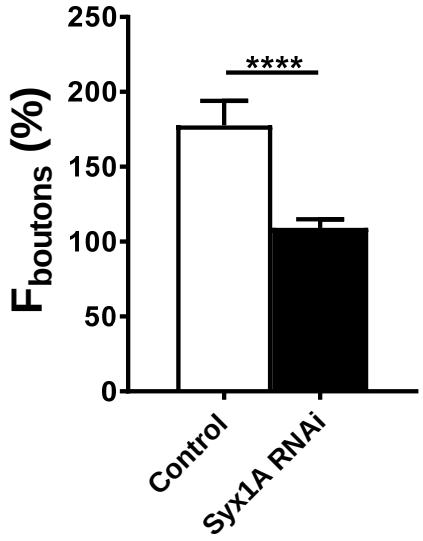


Fig. S2. Knockdown of Syntaxin1A (Syx1A) immunofluorescence.

Data derived from muscle 13 type 1s boutons from 3 animals for each genotype (n was 22 boutons for the Control animals and 30 boutons for the RNAi animals). ****p < 0.0001. To sensitively detect knockdown of expression of Syx1A protein, w; ShakB>FAP; {PZ}Syx1A06737/TM6B, Tb animals were crossed to y1 v1; P{TRiP.JF01829}attP2 (syx1A RNAi) to generate ShakB>FAP; {PZ}Syx1A06737/ y1 v1; P{TRiP.JF01829}attP2 larvae that are heterozygous for a P insertion in syx1A and express syx1A RNAi in type 1s boutons. ShakB>FAP; {PZ}Syx1A06737/TM6B, Tb larvae served as a control. For the RNAi-Syx1A knockdown, experimental and control larvae were fixed for 30 minutes in 4% paraformaldehyde in PBS, rinsed, stained with anti-syx1A antibody 8C3 at 1:50 dilution (Developmental Hybridoma Bank) in PBT with 10% normal donkey serum (Jackson Immunoresearch) overnight at 4C, washed, then stained with Cy3 donkey anti-mouse antibody at 1:400 dilution (Jackson Immunoresearch) and FITC anti-HRP at 1:100 dilution (Jackson Immunoresearch) in PBT with 10% normal donkey serum for 6 hours at room temperature, washed, postfixed 10 minutes in 4% paraformaldehyde in PBS for 10 minutes and mounted on slides with Vectashield (Vector Labs).

Movie S1. Activity-evoked FAP response.

Images were acquired at 1 Hz. 30 Hz stimulation was initiated at the 10 second time point.

Movie S2. Movement of FAP-labeled DCVs in a bouton.

Movie S3. Retrograde traffic of a FAP-labeled DCV.

Images were acquired at 1 Hz.

Movie S4. Spontaneous FAP events.

Images were acquired at 0.2 Hz in 0 Ca^{2+} saline containing the Ca^{2+} chelator EGTA.