

**Figure S7.** Evaluation of mCherry fusion construct behavior. TA muscles were either transfected with pcDNA3.1, pmCherry, ptagRFP1, pmCherry-SH3GLB1, or ptagRFP1-SH3GLB1 as indicated below each panel. Nine days after transfection, CHRN were marked with BGT-AF647. One day later, in vivo-imaging was performed. (**A**)CHRN signals of representative fibers in the NMJ region are depicted. Note extensive disassembly of NMJs and redistribution of CHRN into intracellular vesicles in the presence of pmCherry. This was not observed for pcDNA3.1 or ptagRFP1. (**B-C**) Maximum-z projections of BGT-AF647 (CHRN), SH3GLB1 fused to mCherry (SH3GLB1 in **B**), SH3GLB1 fused to RFP1 (SH3GLB1 in **C**), and overlays of these signals from representative fibers in the NMJ region are depicted. CHRN signals in “CHRN”-labeled and overlay panels are shown without and with contrast enhancement, respectively. In the overlay panels, CHRN and SH3GLB1 fluorescence signals are depicted in gray/white and red, respectively. Note that both SH3GLB1 fusion constructs lead to CHRN and SH3GLB1 signals with comparable size, distribution and number. Furthermore, SH3GLB1-mCherry did not induce any visible damage to NMJs as empty pmCherry vector did. (**D**) Quantitative analysis of CHRN puncta per NMJ. Mean ± S.E.M. (n = 3 muscles for pcDNA3.1 and n = 4 muscles for both SH3GLB1-mcherry and SH3GLB1-RFP1; statistical significance was probed using ANOVA; \*\* P < 0.01).