

**Figure S3.** Number of GFP-MAP1LC3B and CHRN double-positive puncta does not increase disproportionately in the presence of chloroquine. TA muscles were transfected with GFP-MAP1LC3B. Nine days later, CHRN were labeled with BGT-AF647. One day later, in vivo imaging was performed. During the 5 days before imaging, mice received either saline (**A**) or chloroquine (**B**) injections. (**A-B**) Upper panels depict maximum-z projections of representative fluorescence signals of BGT-AF647 (CHRN), GFP-MAP1LC3B (MAP1LC3B), and overlays of both (overlay). Lower panels show details of single optical slices of the boxed regions in the upper panels. In overlay panels, BGT-AF647 and GFP signals are displayed in white/gray and green, respectively. Green and red arrowheads indicate CHRN puncta colocalizing or not colocalizing with GFP-MAP1LC3B, respectively. To better visualize CHRN-positive vesicles, zoom and overlay panels were contrast enhanced. Scale bars: 20 µm. (**C**) Quantitative analysis of CHRN puncta per NMJ. Mean ± S.E.M. (n = 6 for each condition; statistical significance was probed using t-test; \* P < 0.05). (**D**) Colocalization analysis of GFP-MAP1LC3B and CHRN double-positive puncta per NMJ. Mean ± S.E.M. (n = 6 for both conditions; statistical significance was probed using t-test. P = 0.42).