

**Figure S5.** Overexpression of RAB5 has marginal effects on homotypic fusion of CHRN carriers. TA muscles were cotransfected with RAB5-GFP and either T145A or T145E. Nine days later, CHRN were marked with BGT-AF647. Twenty-four hours later, muscles were analyzed with in vivo imaging. (**A**) Representative maximum-z projections displaying signals of BGT-AF647 (CHRN), RAB5-GFP (RAB5), T145E fused to mCherry (T145E), and the overlay of all signals of 2 neighboring fibers. In the overlay panel, CHRN, RAB5, and T145E signals are shown in white/gray, green, and red, respectively. Scale bar: 20 µm. Note that the left muscle fiber expresses RAB5-GFP at much lower amounts compared to the right fiber. (**B**) Quantification of CHRN puncta per NMJ. For each transfection condition, fibers were sorted into RAB5-GFP high and low expressing. Corresponding CHRN vesicle numbers per fiber were then grouped accordingly. Shown are mean values ± S.E.M. (n-values: T145E + RAB5 co-transfection: n = 11 fibers for RAB5-GFP high, n = 14 fibers for RAB5-GFP low; T145A + RAB5 cotransfection: n = 12 fibers for RAB5-GFP high, n = 16 fibers for RAB5-GFP low. Statistical significance was probed using ANOVA. (**C**) Diffraction-limited distribution of CHRN vesicle sizes in untransfected muscles or muscles cotransfected with RAB5-GFP and T145A or T145E, as indicated. Four muscles per condition were analyzed.