Kamimura et al., http://www.jcb.org/cgi/content/full/jcb.201207036/DC1

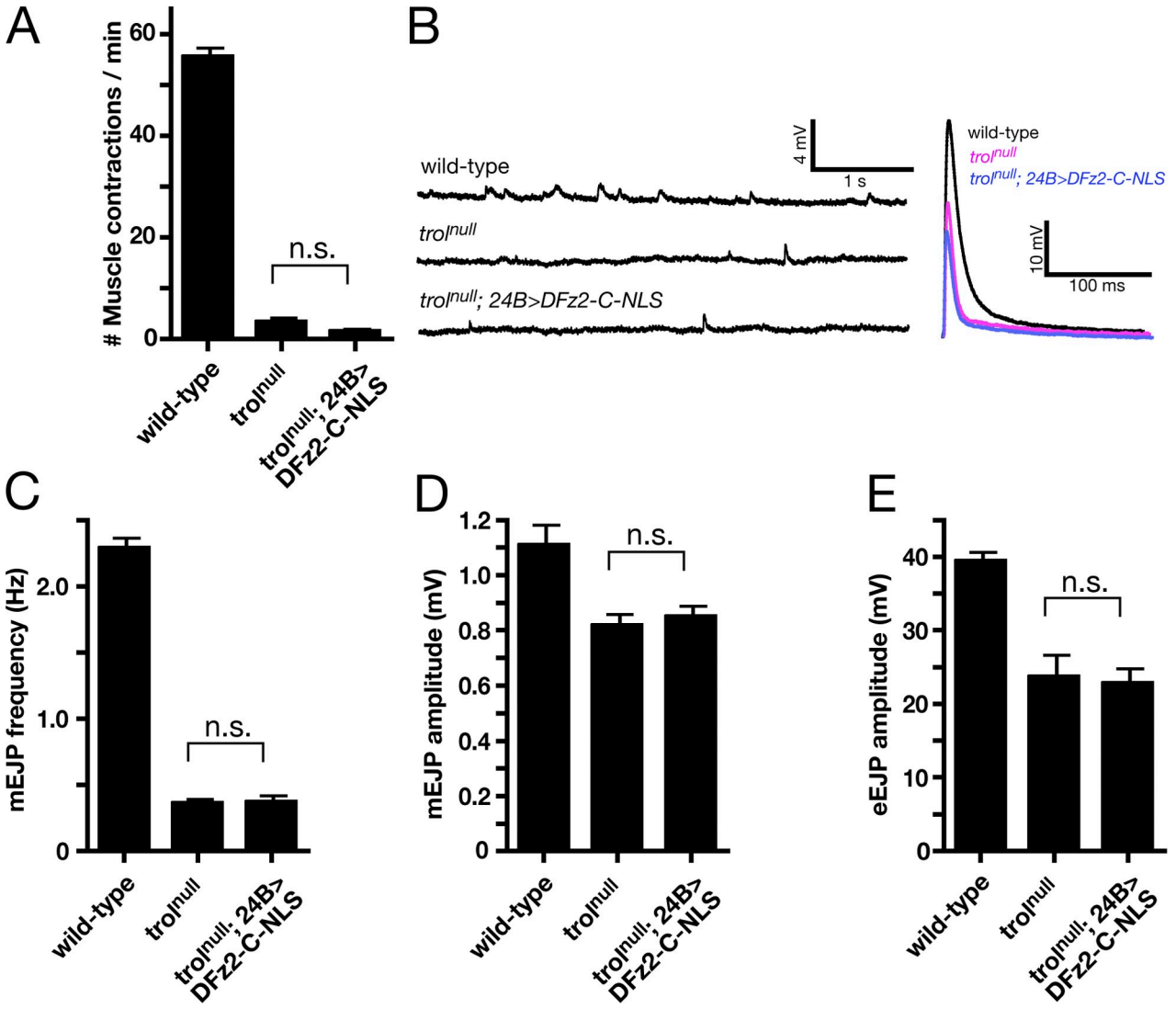

Figure S1. **Activation of postsynaptic Wg signaling did not rescue the locomotion and electrophysiological defect of *trol* mutant larvae.** (A) Locomotion of wild-type, *trol*^{null}, and *trol*^{null}*; 24B>DFz2-C–NLS* third-instar larvae. (B) Representative traces of mEJP (left) and eEJP (right) of wild-type, *trol*^{null}, and *trol*^{null}*; 24B>DFz2-C–NLS* animals. (C–E) Bar graphs show the mEJP frequency (C) and amplitude (D) and eEJP amplitude (E) of the indicated genotypes. Expression of *DFz2-C–NLS* in muscle cells did not affect these physiological defects of *trol*^{null} larvae. Error bars represent SEM (n = 10).

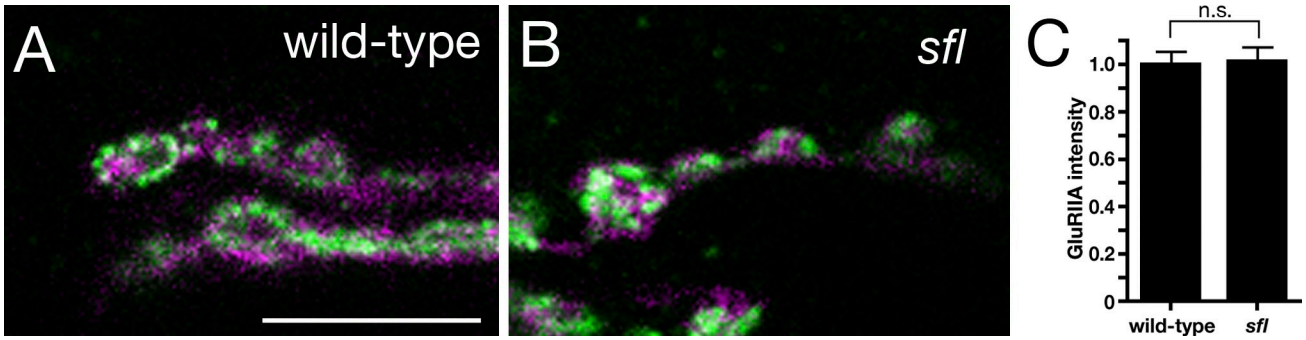

Figure S2. ***sfl* mutations did not affect the glutamate receptor localization at postsynaptic sites.** (A and B) Synaptic regions of wild-type and sfl^{9B4}/sfl^{03844} animals were stained with the antibodies against HRP (magenta) and GluRIIA (green). Bar, 10 µm. (C) Bar graph shows the GluRIIA intensity at boutons of wild-type and sfl^{9B4}/sfl^{03844} animals. Loss of sulfate groups of HS did not affect the GluRIIA levels at postsynaptic sites. Error bars represent SEM (n = 20).

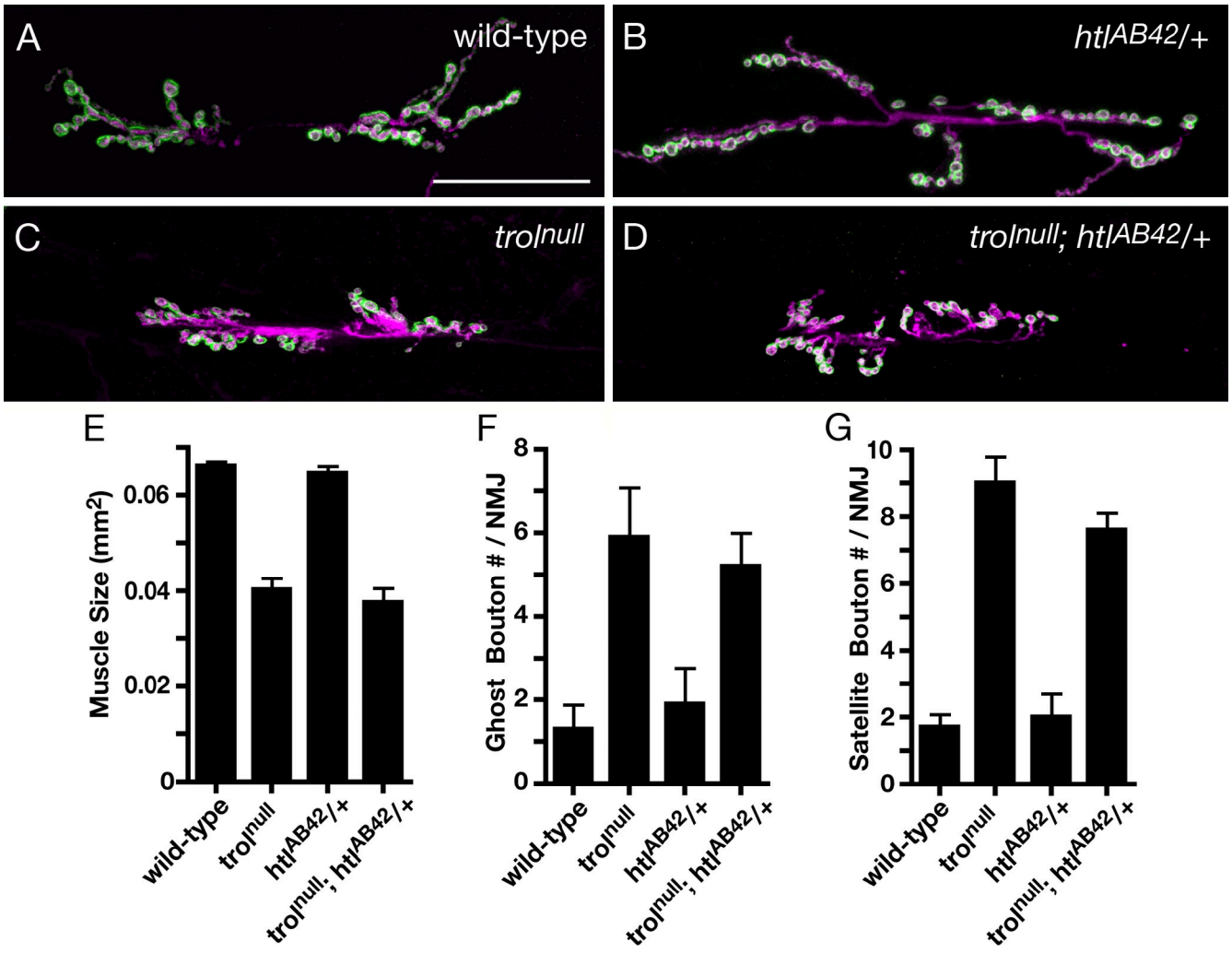

Figure S3. **Reduction of FGF signaling did not affect the NMJ morphologies of *trol* mutants.** (A–D) Larval NMJs of wild type (A), htl^{AB42}/+ heterozygotes carrying the null allele of *heartless* (B), $trol^{null}$ (C), and $trol^{null}$; htl^{AB42}/+ (D) were stained with the antibodies against HRP (magenta) and DLG (green). Bar, 50 µm. (E–G) Bar graphs show the muscle size and the numbers of ghost and satellite boutons in larval NMJ of the indicated genotypes. The heterozygous mutations of *htl* did not affect the *trol* NMJ phenotypes. Error bars represent SEM (n = 10).

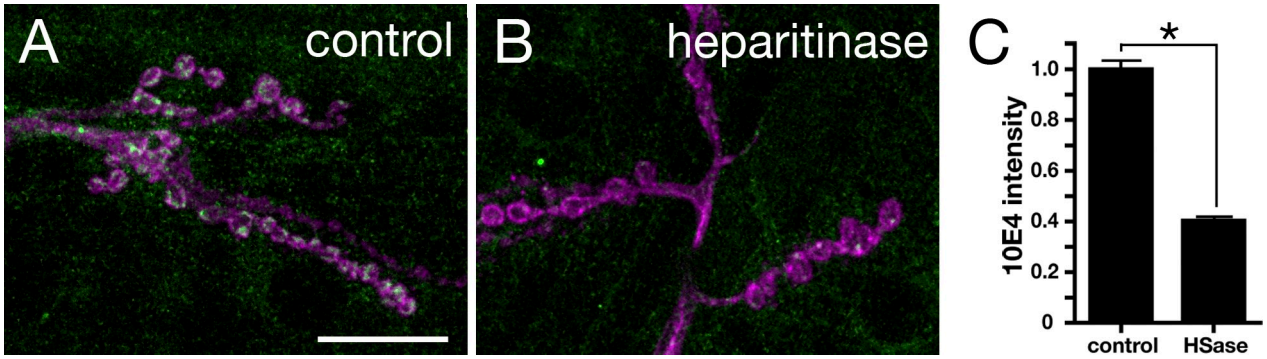

Figure S4. **Heparitinase treatment reduced the extracellular levels of HS at NMJ.** (A and B) Live body wall tissues of control and heparitinase-treated larvae were incubated with anti-HRP (magenta) and anti-HS (10E4; green) antibodies. Bar, 20 µm. (C) Bar graph shows the 10E4 signal intensity at boutons of control and heparitinase-treated larvae. Treatment of larval NMJ with heparitinase remarkably decreased the level of extracellular HS. Error bars represent SEM (n = 10; *, $P < 0.0001$). HSase, heparitinase.