



Extended Data Figure 7 | NLG-1 expression in multiple male sex muscles rescues *nlg-1* mutant DVB neurite phenotype. Silencing spicule circuit or exposure to exogenous GABA does not reduce DVB neurites in *nlg-1* mutant males. **a–c**, Confocal images of DVB (*lim-6^{int4}::wCherry*) (**a**), and quantification of total neurite outgrowth (**b**) and number of neurite junctions (**c**) in control, *nlg-1(ok259)*, *nlg-1(ok259); nlg-1^P::nlg-1:gfp*, and *nlg-1^P::nlg-1:gfp* day 3 males. **d, e**, Quantification of total neurite outgrowth (**d**) and number of neurite junctions (**e**) in control or *nlg-1(ok259)* mutant males with or without tissue-specific NLG-1 expression. Expression patterns for rescue promoters: *lim-6^{int4}* in DVB; *gar-3b* in SPC and spicule protractor muscles; *unc-103F* in SPC, PCA,

PCB and other neurons; *unc-103E* in male sex muscles; *flp-13* in spicule retractor muscles and CP6. **f–h**, Confocal images (**f**) of *lim-6^{int4}::wCherry* and *Ex[gar-3b::HisCl::gfp]* in day 5 male worms, with total neurite length (**g**) and number of neurite junctions (**h**) of *nlg-1(ok259)* worms with or without 10 mM histamine overnight, *nlg-1(ok259); gar-3b::HisCl::gfp* worms with or without 10 mM histamine overnight, and *nlg-1(ok259)* worms with 30 mM GABA overnight. Dot represents one worm; magenta bar, median; boxes, quartiles; one-way ANOVA and post-hoc Tukey HSD, *P* values shown above plots, bold shows significance (*P* < 0.05), scale bars, 10 μm.