



Extended Data Figure 8 | NLG-1 expression decreases from day 1 to day 3. **a**, Confocal images of *nlg-1::nlg-1::gfp* in males at days 1, 3, and 5. Example regions of interest for measurements taken from single planes: blue, dorsal spicule muscles; red, pre-anal ganglion; magenta, DVB. **b**, **c**, Quantification of fluorescence intensity of *nlg-1::nlg-1::gfp* in males at days 1, 3, and 5 reported as a ratio of mean fluorescence in dorsal spicule muscles (**b**) or pre-anal ganglion (**c**) normalized to background of DVB, which has little to undetectable expression. Dorsal spicule muscles refer to the gubernacular retractor, gubernacular erector, anterior oblique, and anal depressor. **d**, Confocal images of *nlg-1::nlg-1::gfp* in day 3 males

as follows: control, *nlg-1(ok259)*, *nlg-1(ok259)* with overnight GABA exposure, *nlg-1(ok259)* with 3-day GABA exposure, and *nxr-1(wy778)*. **e**, **f**, Quantification of fluorescence intensity of *nlg-1::nlg-1::gfp* in day 1 and 3 male worms as follows: control, *nlg-1(ok259)*, *nxr-1(wy778)*, day 3 *nlg-1(ok259)* with overnight GABA exposure, and *nlg-1(ok259)* with 3-day GABA exposure, as a ratio of mean fluorescence in dorsal spicule muscles (**e**) or pre-anal ganglion (**f**) normalized to background of DVB. 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 μ m.