

Extended Data Figure 10 | DVB in hermaphrodites does not show neurite branching upon *gar-3b::ChR2::yfp* activation or NRX-1 or NLG-1 manipulation. a, Confocal images of  $lim-6^{int4}::wCherry$  and Ex[gar-3b::ChR2::yfp] expression in day 1 hermaphrodites showing DVB axon projection after activation with retinal (488-nm light for  $3 \times 15$  s every 45 min for 4.5 h). b, Confocal images of  $lim-6^{int4}::wCherry$  or  $lim-6^{int4}::gfp$  in control, nrx-1(wy778), nlg-1(ok259), and  $Ex[lim-6^{int4}::gfp::nrx-1^{LONG}]$  hermaphrodites at day 3. c, Quantification of the percentage of hermaphrodites with DVB axon abnormalities or

neurites (in almost all cases, a single neurite off the axon just posterior to the pre-anal ganglion) in day 1 control and Ex[gar-3b::ChR2::yfp] with activation, day 3 control, nrx-1(wy778), nlg-1(ok259), and  $\text{Ex}[lim\text{-}6^{int4}::gfp::nrx\text{-}1^{LONG}]$  worms. n shows number of worms, data points represent average percentage for each replicate of multiple hermaphrodites. 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,  $10\,\mu\text{m}$ .