



**Extended Data Figure 3 | DVB inhibits expulsion-associated spicule protraction at day 3.** Laser ablation of DVB and channelrhodopsin expression in DVB and spicule protraction circuit. **a**, Confocal images of male worm with *lim-6<sup>int4</sup>::wCherry* and *lim-6<sup>int4</sup>::HisCl1::gfp* at day 3. **b**, Quantification of the percentage of expulsion steps with spicule protraction for day 1 control, day 3 control, day 3 control + histamine, and day 3 *lim-6<sup>int4</sup>::HisCl1::gfp* + histamine males. **c**, Time between consecutive expulsion steps for day 1 control, day 3 control, day 3 control + histamine, and day 3 *lim-6<sup>int4</sup>::HisCl1::gfp* + histamine males

(+ histamine is on 10 mM histamine plates; 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)). **d**, Confocal images of male worms with or without laser ablation of DVB at day 1 or 2, visualized with *lim-6<sup>int4</sup>::gfp*. **e**, Confocal images of DVB (*lim-6<sup>int4</sup>::wCherry*) expressing channelrhodopsin at day 1 and 5, *Ex[lim-6<sup>int4</sup>::ChR2::yfp]*. **f**, Confocal images of DVB (*lim-6<sup>int4</sup>::wCherry*) and spicule circuit expressing channelrhodopsin at day 1 and 5, *Ex[gar-3b::ChR2::yfp]*. *n* > 10 for **d–f**. Scale bars, 10  $\mu$ m.