Appendix

The Grainyhead/LSF transcription factor GRH-1 is rhythmically required for molting

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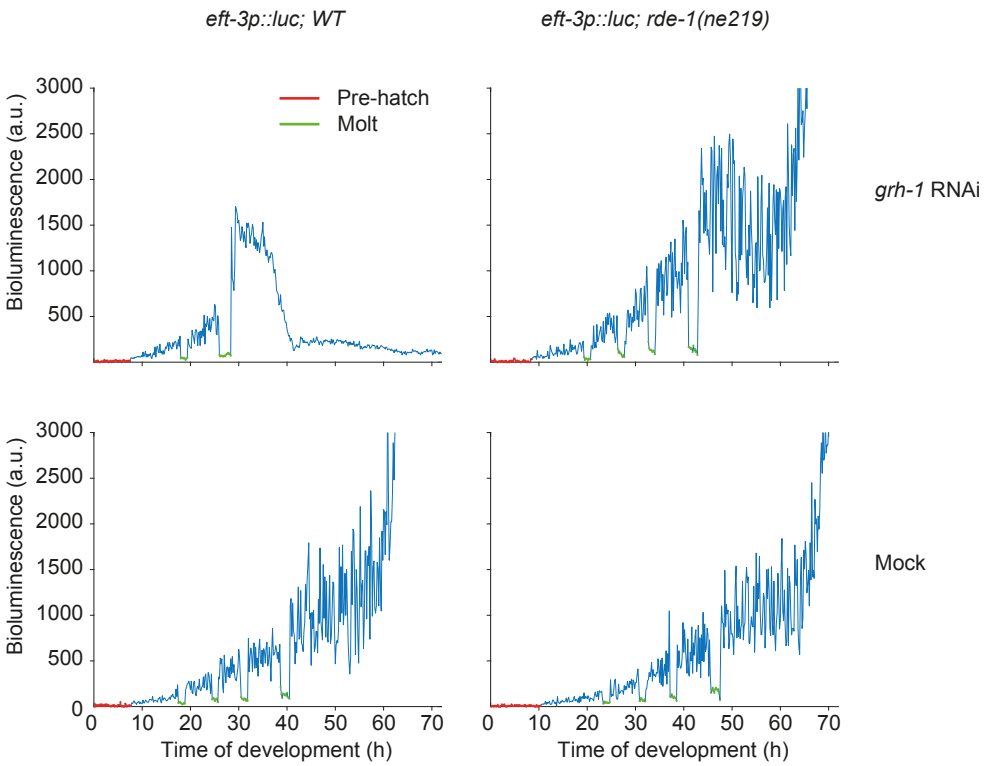
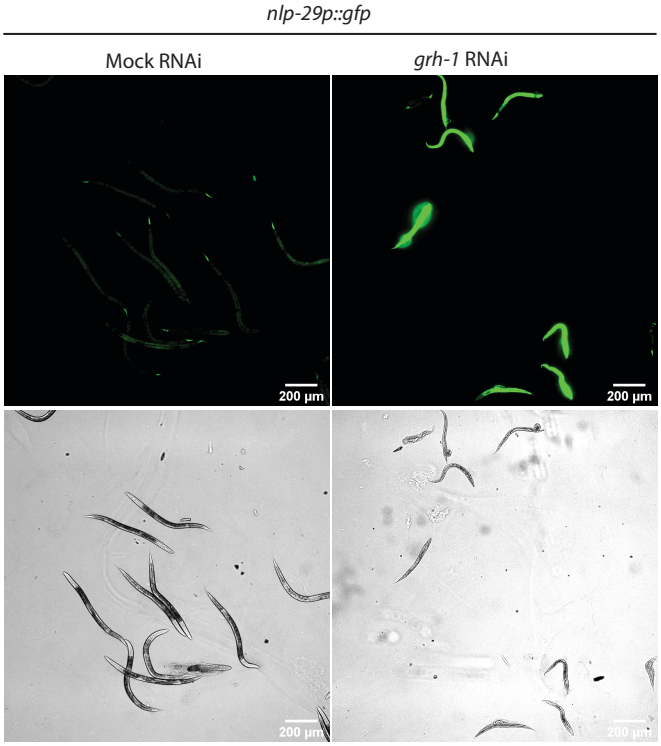


Appendix Figure S1: *grh-1* RNAi causes aberrant progression through development
Representative raw luminescence trace of single animals grown at 20°C for 72 hours. Left column showing wild-type animals and right column RNAi-deficient animals (*rde-1(ne219)*). Animals in upper panel were grown in the presence of *grh-1* RNAi and animals in the lower panel were grown in the presence of mock (empty vector) RNAi. Pre-hatch is indicated in red and molts in green.

A



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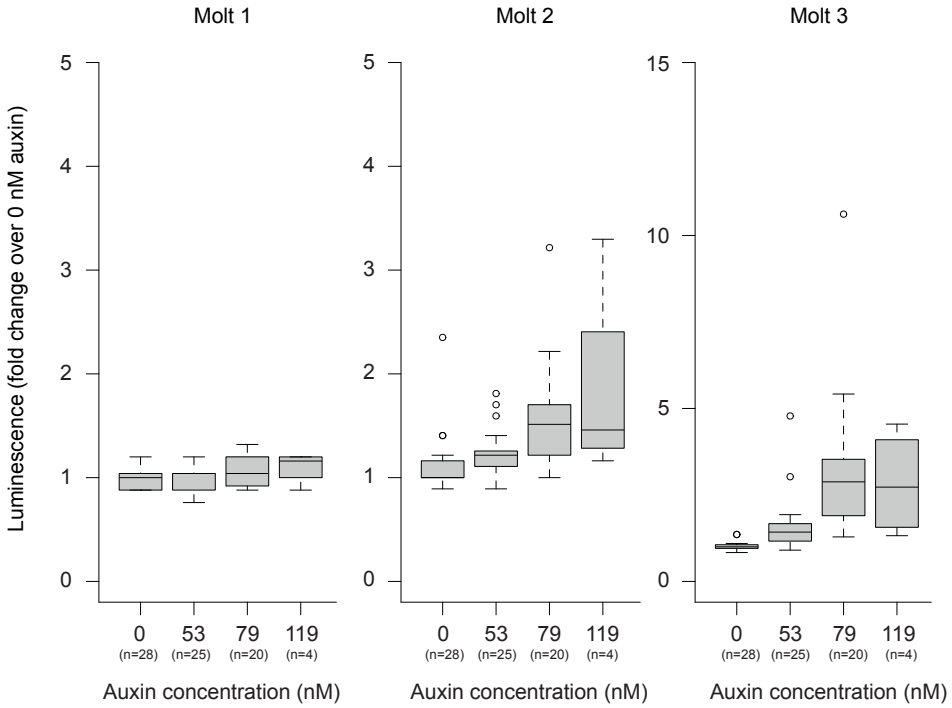
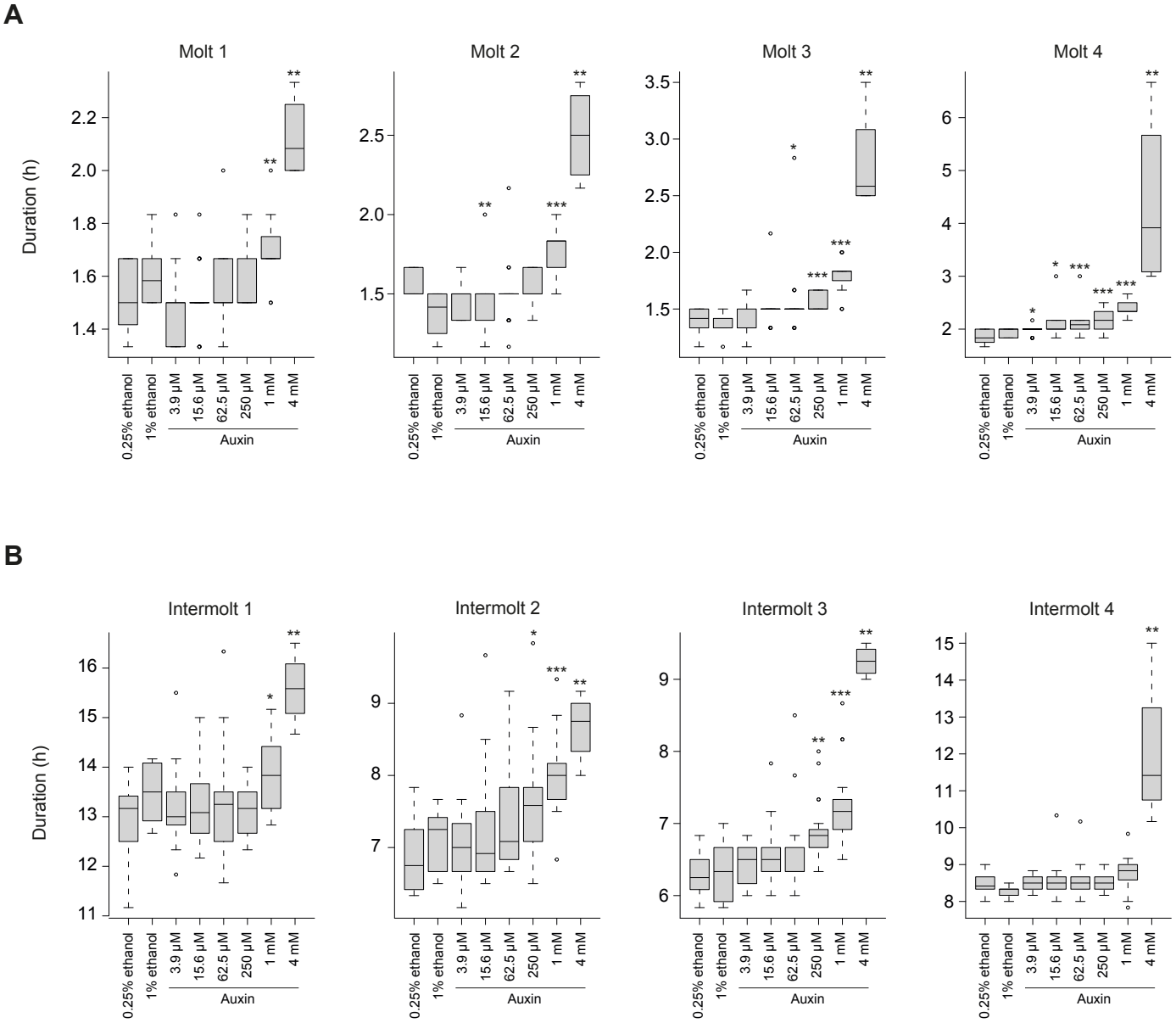


Appendix Figure S2: GRH-1 depletion impairs cuticular integrity

A, *grh-1* RNAi induces expression of *nlp-29p::gfp*, a sensor of cuticle integrity. Synchronized *nlp-29p::gfp* L1 stage larvae were grown at 20˚C on *grh-1* or mock (empty vector) RNAi plates . After 32 h, when ~50% of *grh-1* RNAi animals showed the characteristic bursting phenotype, viable animals were picked and imaged on a Z1 epifluorescence microscrope at 5 x magnification. Top panels: fluorescence, 100 ms exposure time, bottom panels: brightfield. Note the smaller size of *grh-1* relative to mock RNAi-treated animals, reflecting slowed development. Scale bars = 200 µm.

B, Fold change in median luminescence during the timepoints annotated as molts in viable *grh-1::aid* animals exposed to low amounts of auxin relative to vehicle control (see Figure 4 for details on auxin titration). In the boxplots, the horizontal line represents the median, hinges extend to first and third quartiles and the whiskers extend up to 1.5*IQR (interquartile range). Data beyond that limit is plotted as dots.

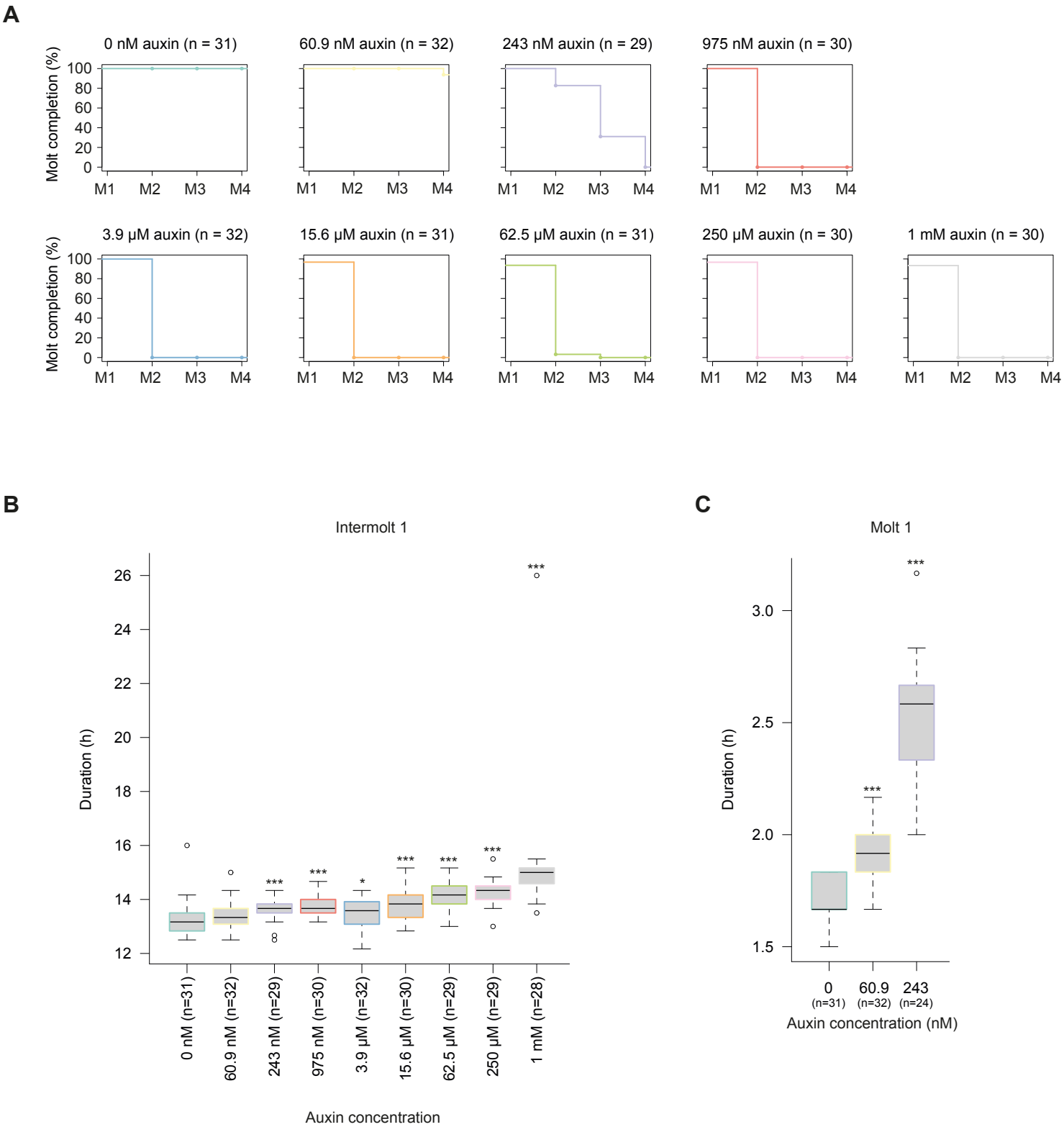


Appendix Figure S3: Effects of auxin in animal lacking a degron

A, Boxplots showing duration of each molt for indicated concentrations of auxin in *eft-3p::TIR1; eft-3p::luc* animals (HW1984), which lack the aid tag on grh-1. Differences relative to 0.25% ethanol (0 nM auxin) for 3.9 µM – 1mM auxin and relative to 1% ethanol (0 nM auxin) for 4 mM auxin are indicated. Note that concentrations ≤ 250 µM yield at most minor extensions of molt durations. At 4 mM auxin, most animals fail to complete M4.

B, Boxplots showing duration of each intermolt for indicated concentrations of auxin in the animals shown in (A). Note that concentrations ≤ 250 µM yield at most minor extensions of intermolt durations.

P-values were determined relative to vehicle by a Wilcoxon rank sum test. * p<0.05, ** p<0.01, *** P<0.001; otherwise non-significant.



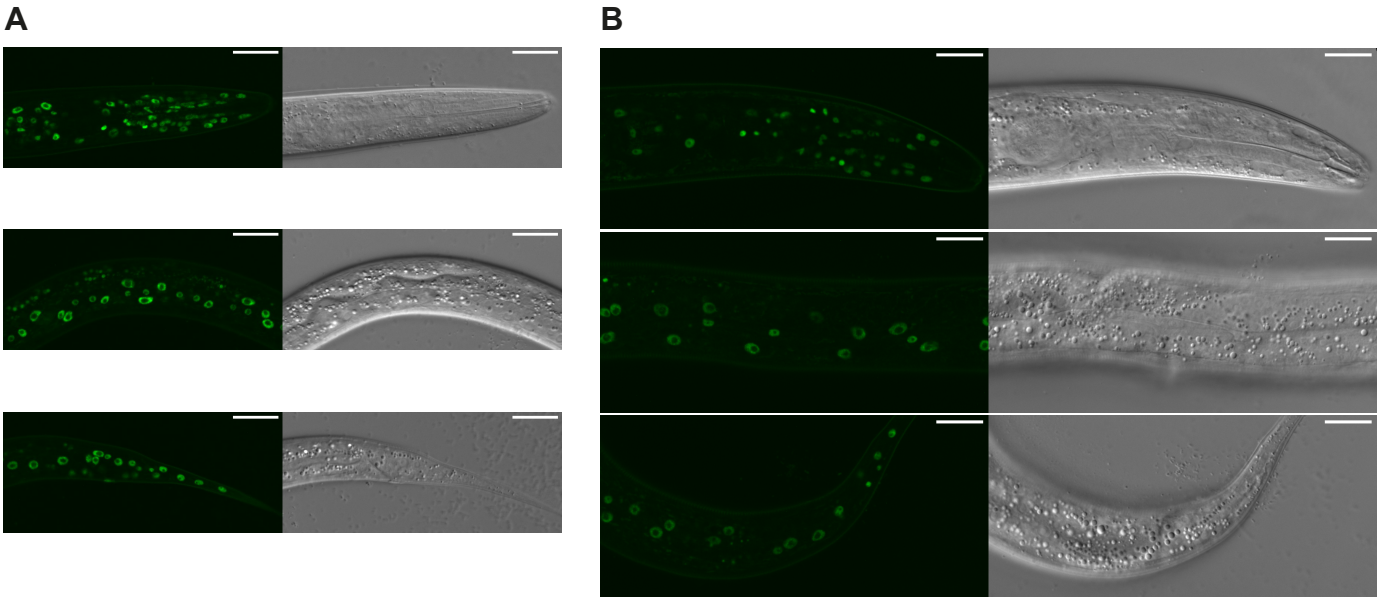
Appendix Figure S4: Titration of auxin concentration for *grh-1::aid* animals

A, Quantification of the percentage of *grh-1(xe135); eft-3p::luc; eft-3p::tir1* animals (HW2434) that enter each of four molts upon increasing concentrations of auxin. Note that for some concentrations, M1 does not start at 100%, i.e., some animals initiated I1 but M1 was not observed.

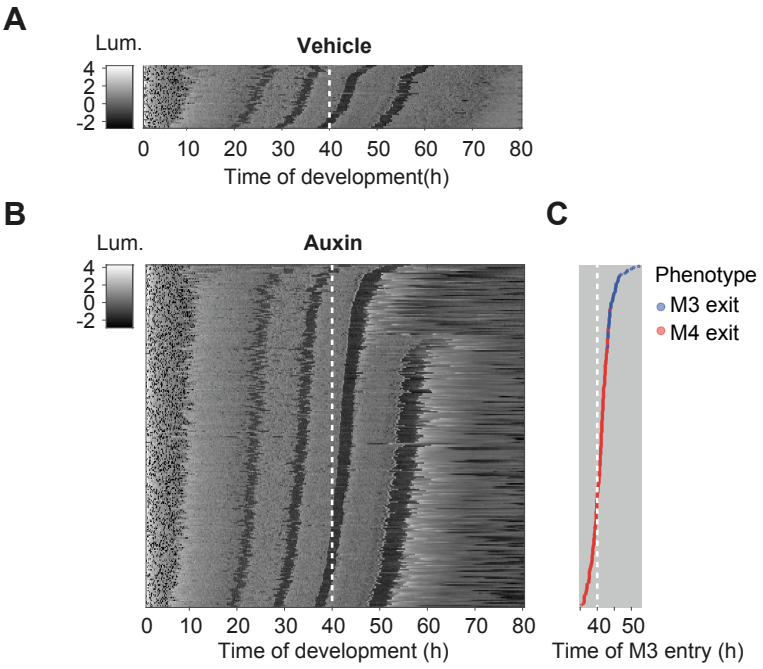
B, Boxplot showing the duration of the first intermolt of animals treated with indicated concentrations of auxin. Animals from (A) that failed to progress development beyond M1 were excluded from quantification.

C, Boxplot showing the duration of M1 of animals treated with indicated concentrations of auxin. Animals from (A) that failed to progress development beyond M1 were excluded from quantification.

In the boxplots, the horizontal line represents the median, hinges extend to first and third quartiles and the whiskers extend up to 1.5*IQR (interquartile range). Data beyond that limit is plotted as dots. P-values were determined relative to vehicle (0 nM) by a Wilcoxon rank sum test. * p<0.05, ** p<0.01, *** P<0.001; otherwise non-significant.



Appendix Figure S5: GRH-1-GFP accumulation is reduced in adults
A,B, Confocal fluorescence microscopy (left) and DIC (right) images of *grh-1::gfp::3xflag* (HW2603) animals at the L2 larval (A) and young adult (B) stages, respectively, reveals reduced *grh-1* expression in various tissues in young adults. Scale bars: 20 µm.



Appendix Figure S6: Molt exit phenotype is dependent on the time of GRH-1 depletion

A,B, Heatmap showing trend-correct luminescence (Lum, arbitrary units) of *grh-1(xe135); eft-3p::luc; eft-3p::tir1 animals* (HW2434) treated with vehicle (0.25% ethanol, A) or 250 µM auxin (B) at 40 hours (white dashed line). Black intensities correspond to low luminescence occurring during lethargus (molt). Embryos of various stages were left to hatch and develop during the assay. Luminescence traces are sorted by entry into molt 3 (M3) such that traces of early hatched animals are at the bottom and those of late hatched animals are at the top.

C, Plot of phenotype onset over time of auxin application relative to entry into molt 3 (M3 entry) of animals shown in (B). Dots represent individual animals from (B), sorted by time of entry into M3 and colored according to whether the last observed molt in the luminescence trace was M3 (M3 exit phenotype; blue) or M4 (M4 exit phenotype; red). Time of auxin addition is indicated by a white dashed line; dots to the left represent animals that entered M3 before auxin application, dots to the right represent animals that entered M3 after auxin application.

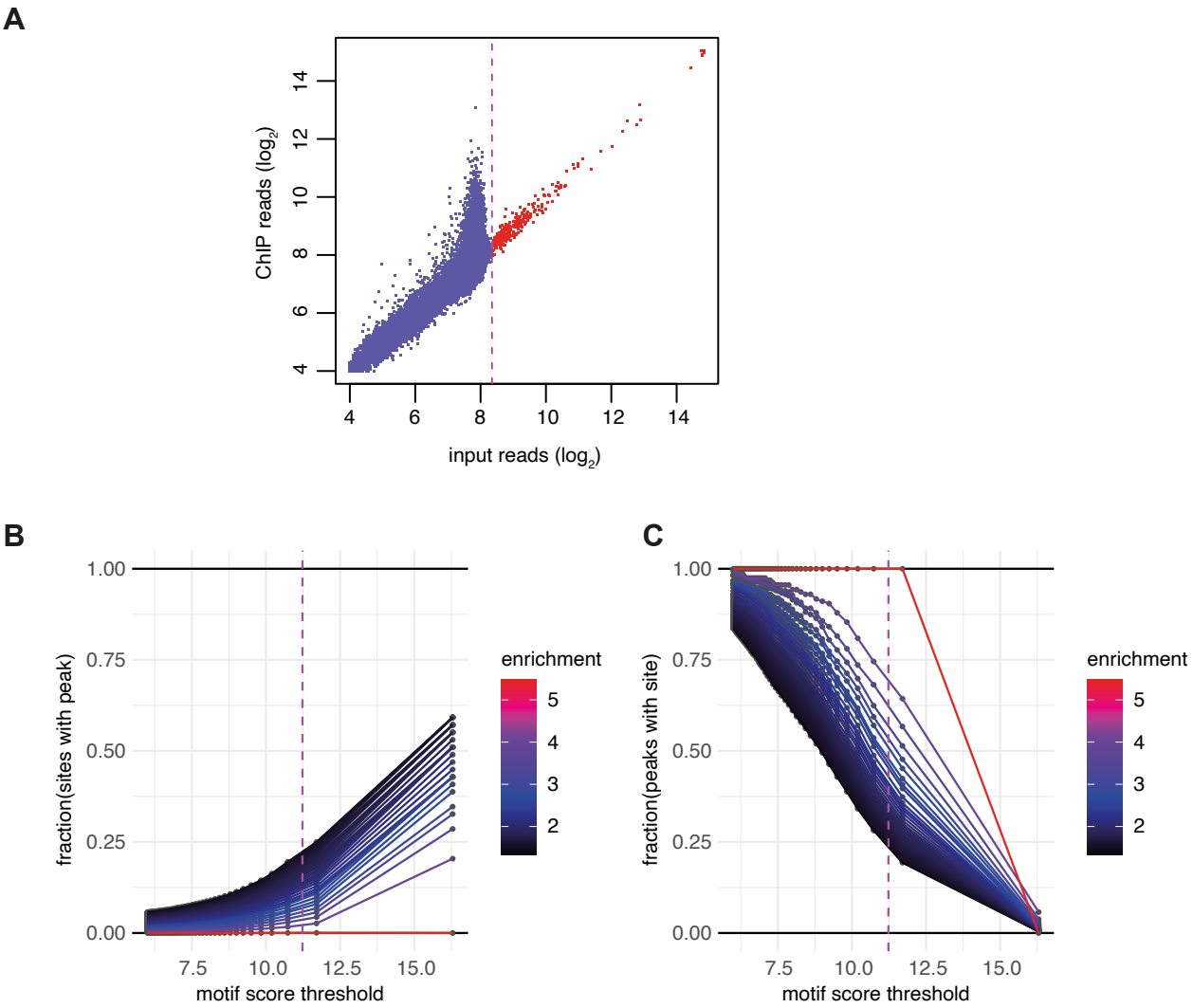


Figure S7: GRH-1 ChIP-seq analysis

A, Scatter plot of transcript reads in GRH-1 ChIP-seq versus input samples (experiment 1, 500 mM salt) reveals "overmapped" reads (red) that are excluded from further analysis as indicated by the dashed line.

B, Fraction of motif occurrences ("sites") within GRH-1 ChIP-seq peaks as a function of motif score threshold and for various enrichment (ChIP over input fold enrichment) cut-offs. A dashed line indicates the default motif score cut-off used by HOMER (Heinz et al., 2010).

C, Fraction of peaks containing motif occurrences ("sites") within GRH-1 ChIP-seq peaks as a function of motif score threshold for various enrichment (ChIP over Input fold enrichment) cut-offs. A dashed line indicates the default motif score cut-off used by HOMER (Heinz et al., 2010).

References

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell *38*, 576-589.