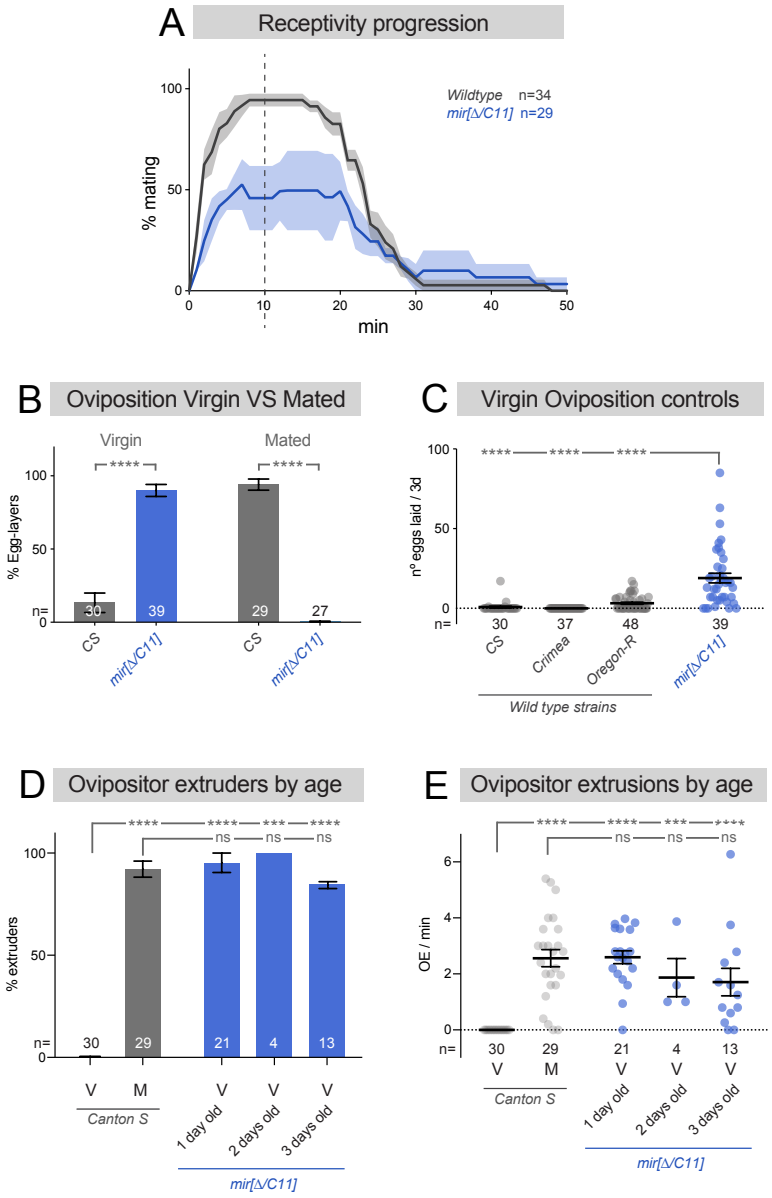

Supplementary Figure 1 (related to Figure 1). Extended behavioral analysis of *mir-iab-4/8* mutants. (A) Temporal analysis of sexual receptivity, assessed by the fraction of individual females that are copulating at a given timepoint. The 10 minute timepoint (dotted line) was used subsequently to summarize and compare multiple genotypes or manipulations, shown in Figure 1C. (B) Qualitative oviposition in wildtype and *mir[Δ/C11]* flies. Egg-laying is uncoupled from internal state. (C) Quantitative egg-laying counts across several wild type strains. Qualitative (D) and quantitative (E) representation of ovipositor extrusions. Overall levels of male rejection by extruding the ovipositor is maximal in young virgins (1 day old). Fisher's exact test for qualitative analysis (B, D), and Mann-Whitney non parametric test for quantitative analysis (C, E). ns=not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Error bars and shaded area = SEM.

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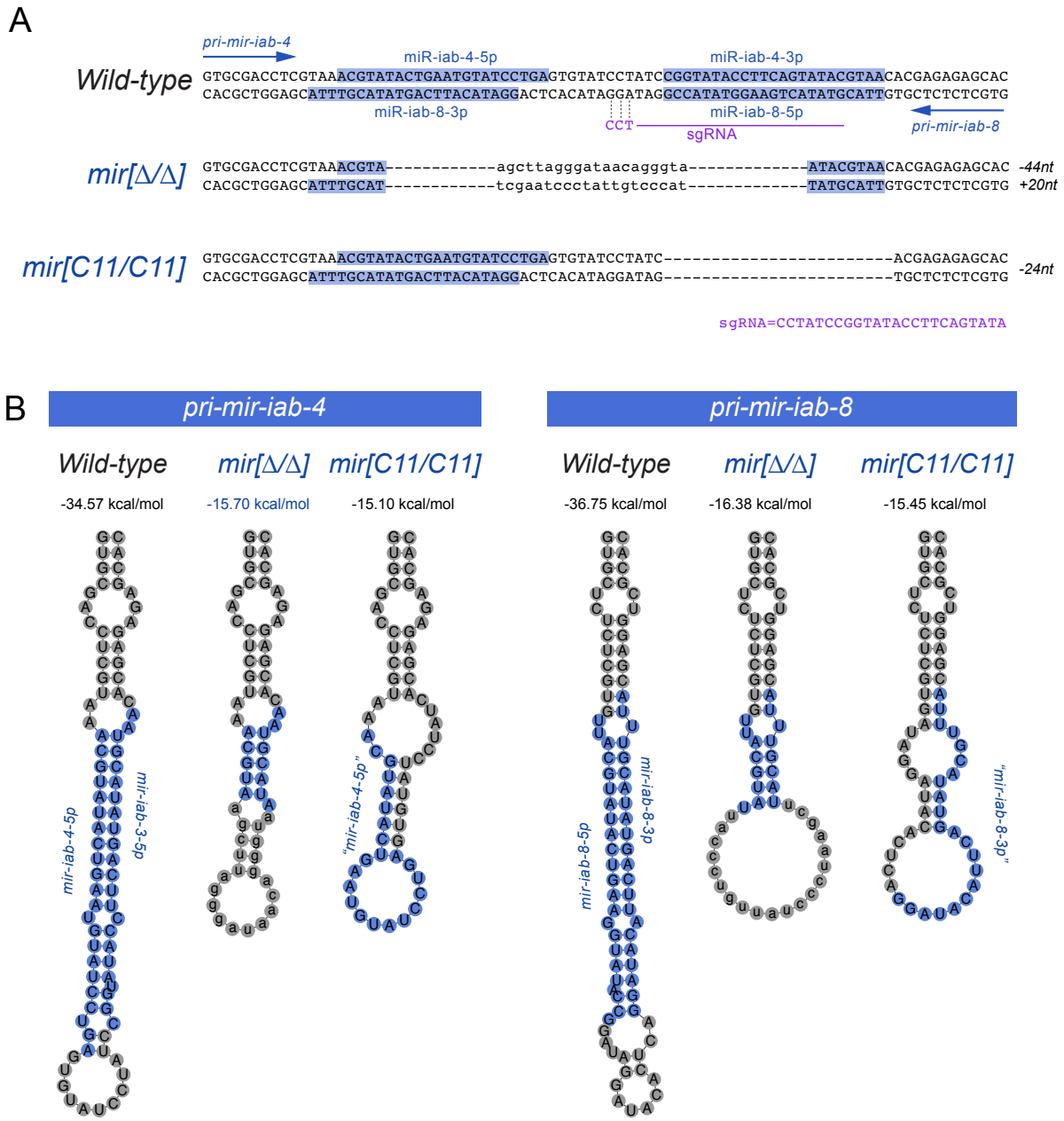
Supplementary Figure 1



Supplementary Figure 2 (related to Figure 1). *mir-iab-4/8* mutant alleles. (A) Wildtype, *mir[Δ/Δ]* and *mir[C11/C11]* sequence. (B) RNAfold predictions of each allele. The newly made *mir[C11]* allele deletes miR-iab-8-5p (miR-iab-4-3p) and does not adopt a pre-miRNA-like hairpin structure. Blue= *mir-iab-4/8* species in each allele. Magenta= sgRNA used to mutagenize *mir-iab-4/8 locus*. Low-case letters: inserted nucleotides. nt=nucleotides.

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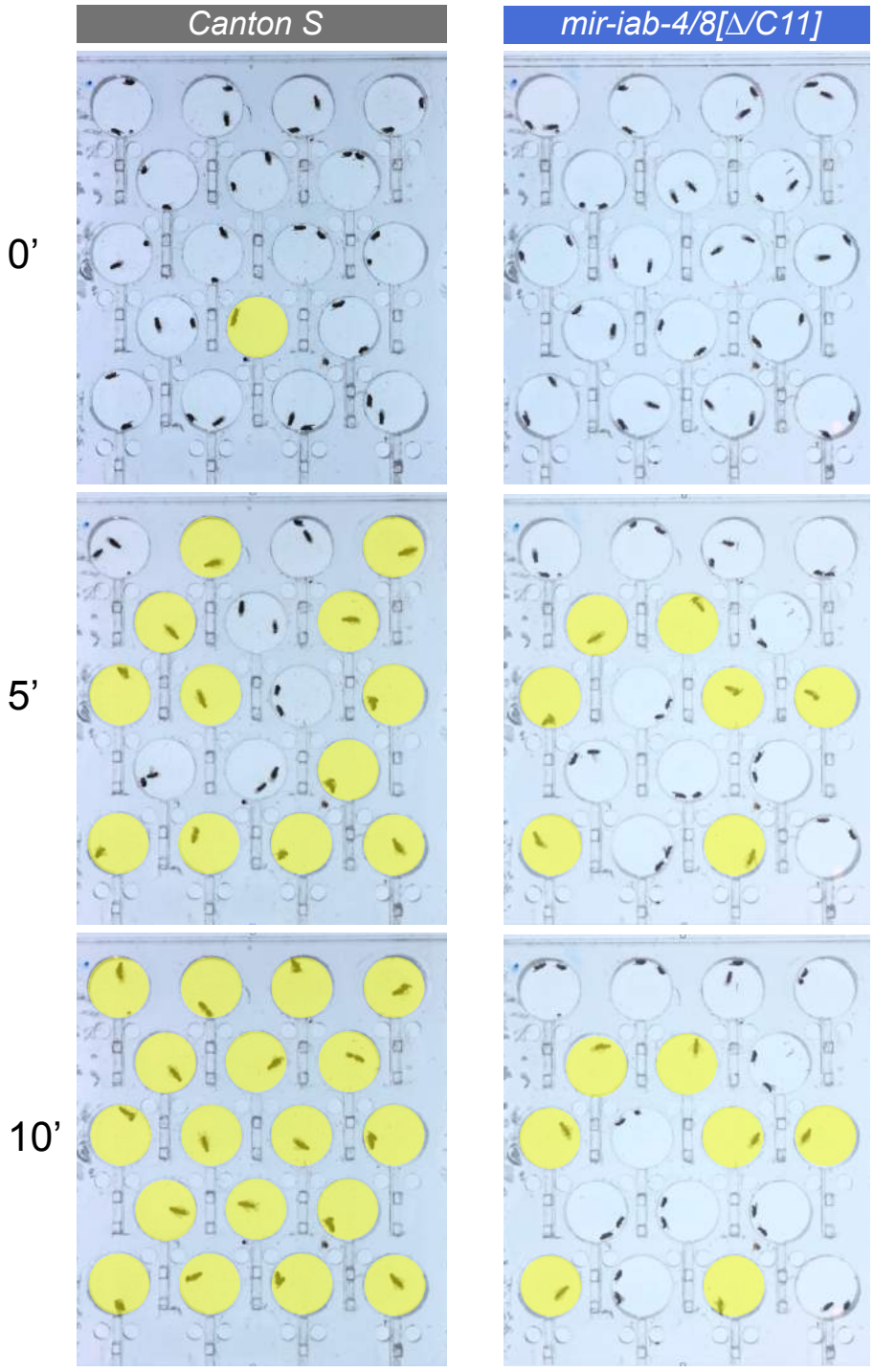
Supplementary Figure 2



Supplementary Figure 3 (related to Figure 1). Receptivity dynamics in *mir-iab-4/8* mutants. Time lapse shots from movies of 18 *wildtype* (*Canton-S*) and 18 *mir[Δ/C11*] female flies. In all cases males are *Canton-S*. Mating couples are marked in yellow. Compared to control, the copulation success of *mir[Δ/C11]* mutants is markedly reduced.

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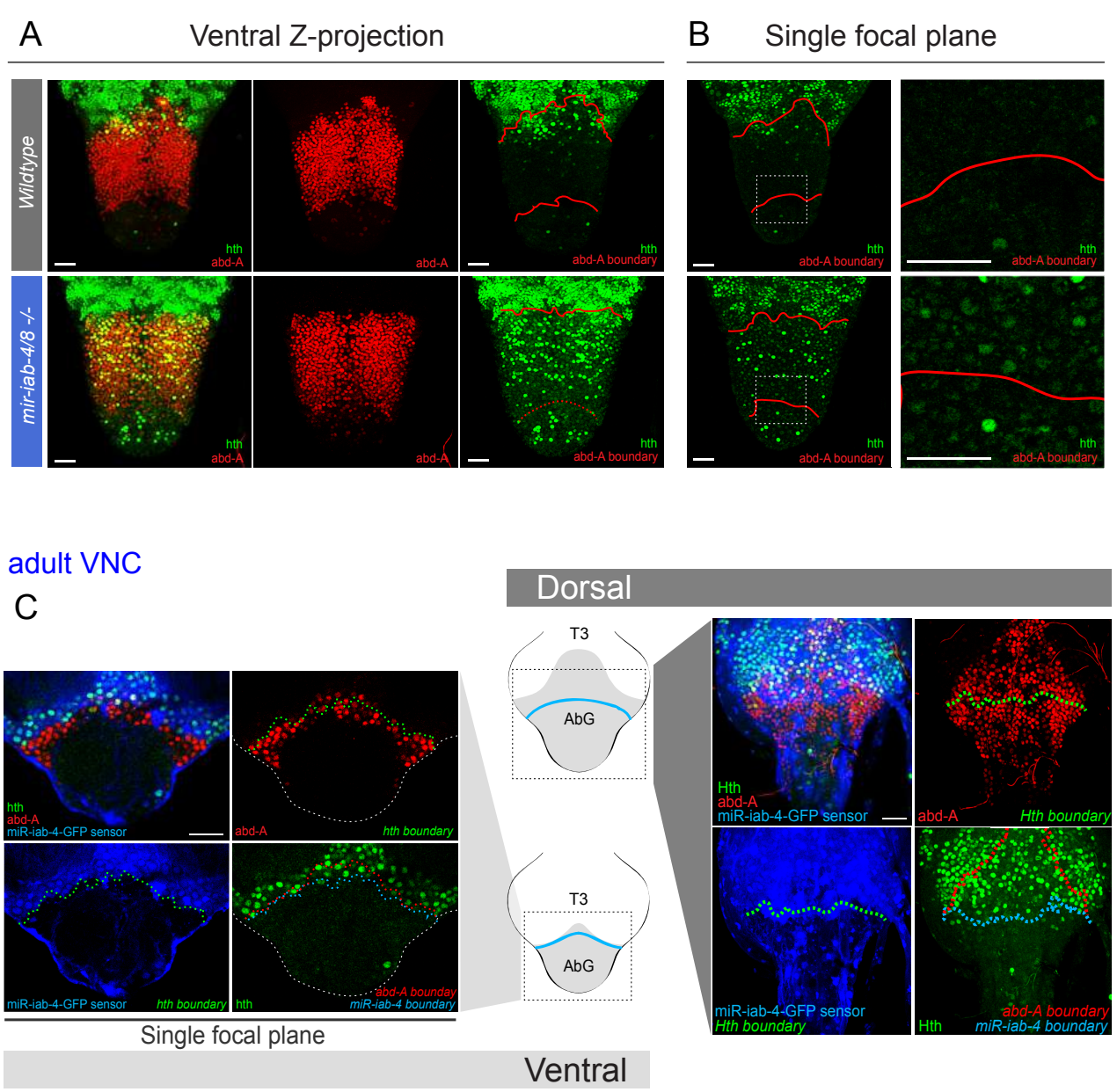
Supplementary Figure 3



Supplementary Figure 4 (related to Figure 4). Extended characterization of Hth expression: I. (A, B) Hth immunostaining (green) in larval VNCs. (A) Hth is derepressed in abdominal segments (Abd-A, red) of *mir[Δ/C11]* female larvae (Z-projections). (B) Mild levels of Hth accumulation are detected in nearly all cells of mutant VNCs. Along the dorsoventral axis, Hth is mutually exclusive with miR-iab-4 and miR-iab-8 (inferred from activity *tub-GFP-sensors*), but not with Abd-A (C). Scalebar= 25µm.

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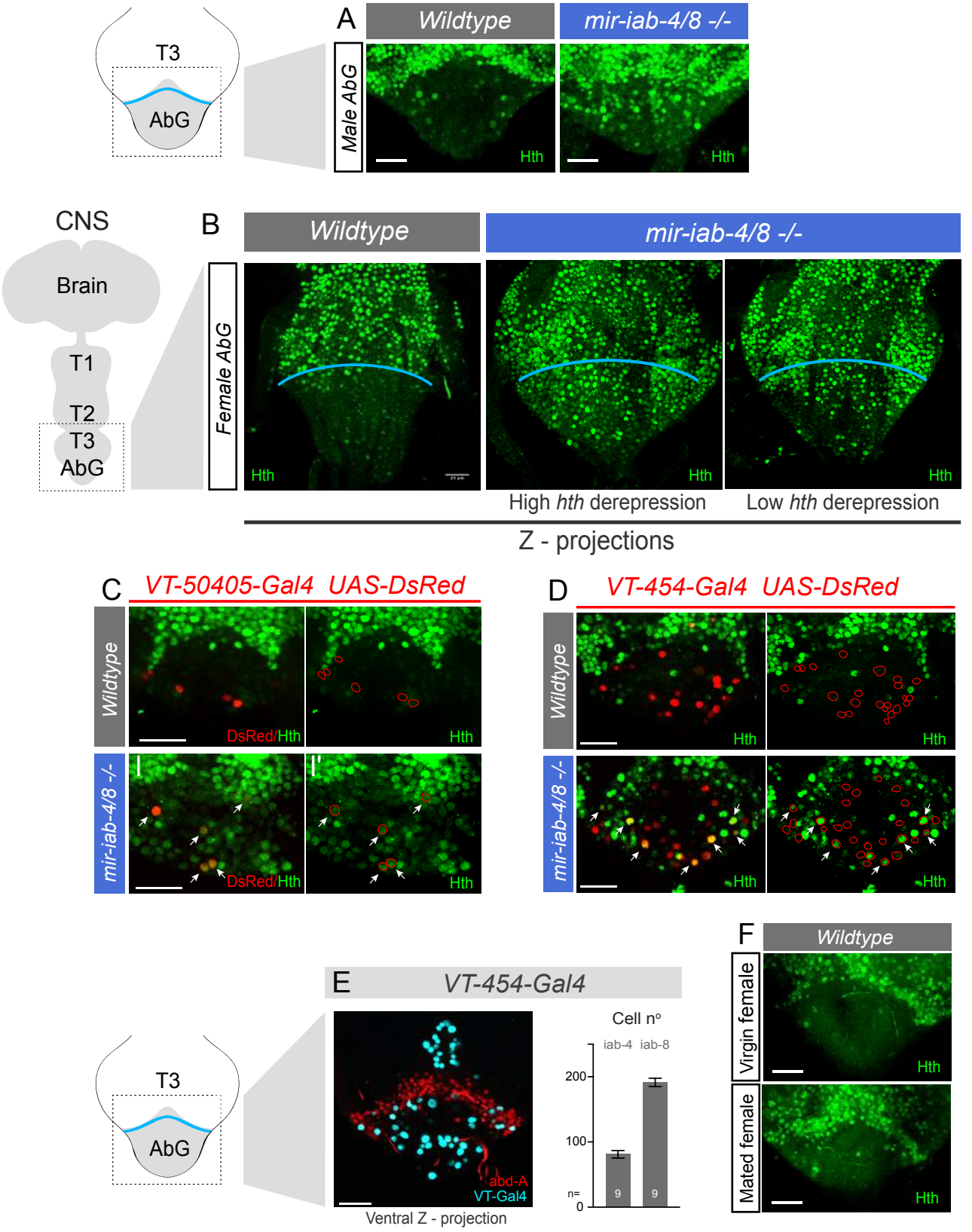
Supplementary Figure 4



Supplementary Figure 5 (related to Figure 4). Extended characterization of Hth expression: II. (A) Hth expression in AbG of wildtype and *mir-iab-4/8* mutant males is similar to their female counterparts. (B) Hth accumulation in wildtype and two *mir-iab-4/8* mutant female VNCs, illustrating variability of derepression in *mir-iab-4/8* nulls. (C,D) Hth expression relative to VT-switch neuron populations. Only showing the AbG boxed in A. Some *VT-454* labeled neurons show especially high accumulation of Hth protein (arrows). (E) Z-projection of *454-Gal4* VT-switch neurons (cyan) co-stained with Abd-A (red). Number of VT-switch+ cells per VNC domain (*iab-4* vs. *iab-8*). (F) Copulation does not affect Hth expression levels in the female AbG. Scalebar= 25µm. Bars in E correspond to SEM.

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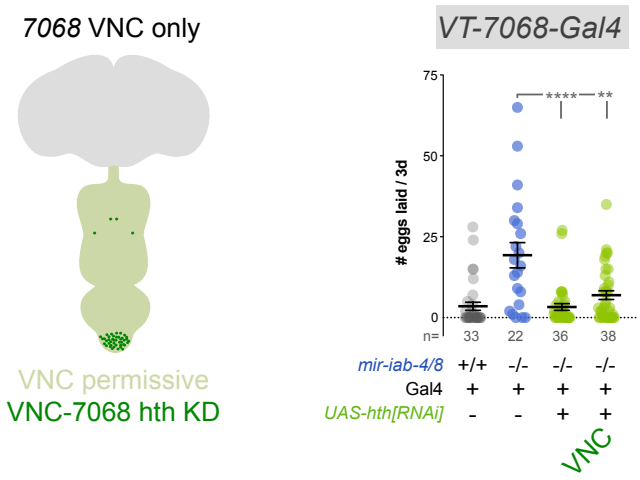
Supplementary Figure 5



Supplementary Figure 6 (related to Figure 5). VNC specific *hth[RNAi]* rescues. Restriction of *hth* knockdown to the *VT-7068* neurons in VNC in *OTD-flp, tub>stop>Gal80 /UAS-hth-[RNAi]; 7068-Gal4, mir-C11/Δmir* flies, is sufficient to revert significantly the elevated egg-laying observed in *mir-iab-4/8* mutants. Mann-Whitney non parametric test, ** p<0.01, **** p<0.0001. Error bars= SEM. Wildtype flies are *Canton-S* strain, *mir-iab-4/8 -/-* mutants=*mir[Δ/C11]* transheterozygotes.

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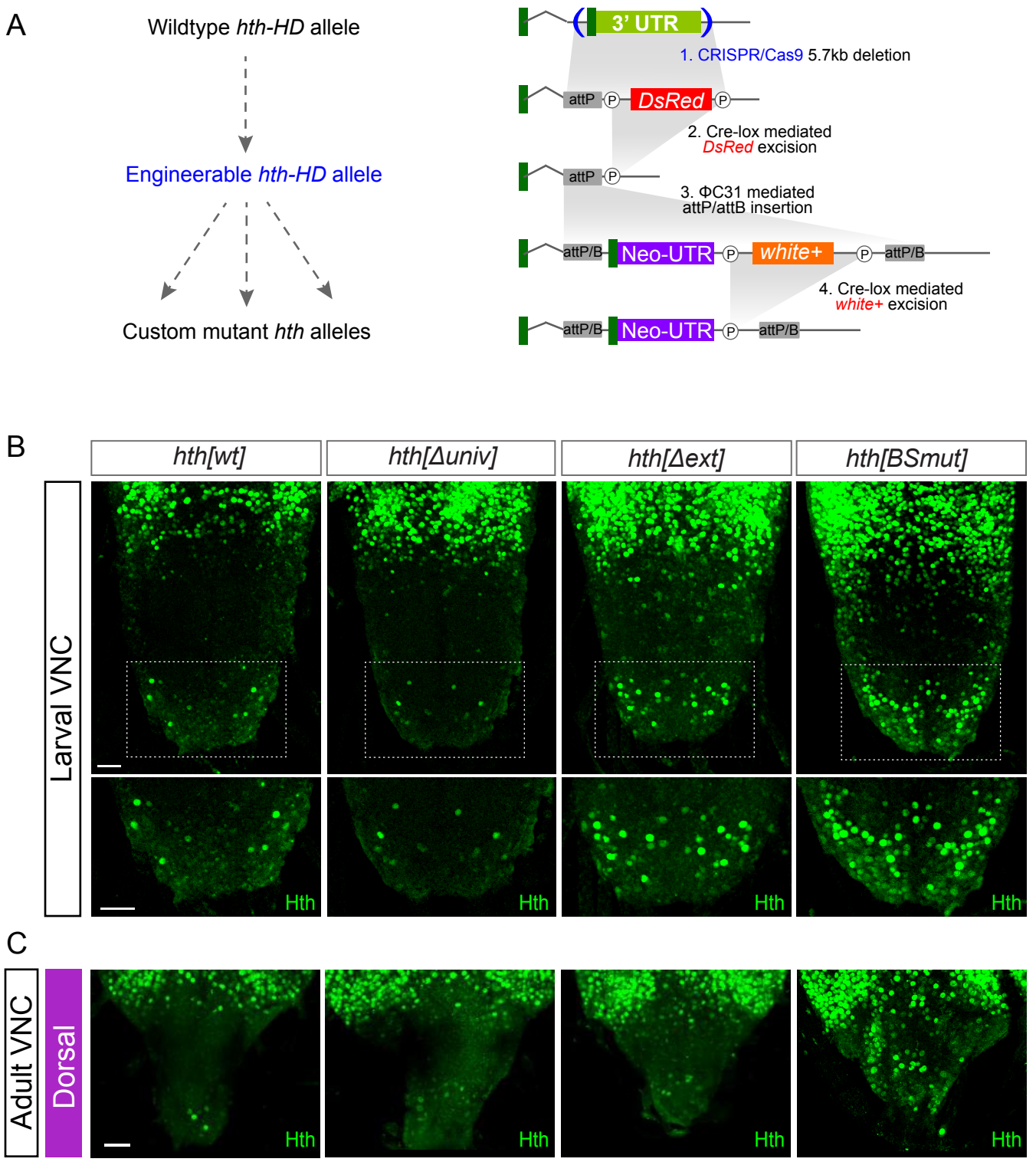
Supplementary Figure 6



Supplementary Figure 7 (related to Figure 6). Hth engineering pipeline and patterning in *hth-HD* 3´UTR alleles. (A) CRISPR/ΦC31 approach to generate the 3´UTR engineering platform in *hth-HD* isoform. Hth pattern in the entire abdominal region of larval VNCs (B) and the dorsal region of adult AbG of *hth-HD* 3' UTR alleles. Scale bar= 50µm.-

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Supplementary Figure 7

Supplementary Table 1 (related to Figure 6). sgRNA guides and primers used for CRISPR-Cas9 engineered alleles:

sgRNA sequences for *hth-HD* deletion	**Coordinates**
Intron break:	
GAGGCCGTGTCAAAGCTTGC-GGG	chr3R: 6337918-6337932
GTGACTGTCACTCGGCCCGC-CGG	chr3R: 6337877-6337891
TTAAAGCAGAAAGCCGCCGG-CGG	chr3R: 6337869-6337883
GCAGTTGCCCGTTGATCCGC-AGG	chr3R: 6337807-6337821
GCTTGCGGGTAACATCGTCT-TGG	chr3R: 6337904-6337926
Downstream break:	
CGAGCTGGTGGCTCTCCCGC-TGG	chr3R: 6332418-6332432
AAAGTAATTACAGCCGCCGA-CGG	chr3R: 6332151-6332165
TGCCTATAATTAGTGGGCAG-TGG	chr3R: 6332216-6332230
Primers used to clone homology arms in pHD-attP-DsRed:	**Coordinates**
“Intronic” Homology Arm (Left):	
Fwd; AATATCGCATCGCCCCC	chr3R: 6338982-6339004
Rev; AGCTTTGACACGGCCTC	chr3R: 6337921-6337940
"Downstream" Homology Arm (Right):	
Fwd; GCGGCTGTAATTACTTTTACG	chr3R: 6332139-6332159
Rev; AATAAATGGCTTGACAAACGC	chr3R: 6330834-6330854
Primers used to verify *hth-HD[∆attP]* allele:	
Sequence	**Coordinates**
Fwd; AACACTGCGTAGAAAGGTCC	chr3R: 6339186-6339205
Rev; AGTGCATTAATGAGCCTCGG	chr3R: 6330700-6330719
Primers used to clone *hth-HD* 3’UTR alleles:	
Sequence	**Coordinates**
Intronic/exonic region for pRIV-w-CR/DS generation	
Fwd; TGCGGGTAACATCGTCTTGG	chr3R: 6337904-6337923
Rev; TTACAAGTGCTGGCCGTAGT	chr3R: 6337099-6337118
Intergenic region pRIV-w-CR/DS generation	
Fwd; CGTGAAAGTCTTTTTGAATTGTC	chr3R: 6333257-6333279
Rev; CGACGGACAATGCGGGG	chr3R: 6332160-6332179
wt-3’UTR (for *hth[wt]* allele)	chr3R: 6333280-6337098
Fwd; TCCTCGTAATCCGGCCC	chr3R: 6337079-6337098
Rev; ATTGGTTTTCATAATCTTTTATTTCAT	chr3R: 6333280-6333306
univ-3’UTR (for *hth[∆ext]* allele)	chr3R: 6335562-6337098
Fwd; TCCTCGTAATCCGGCCC	chr3R: 6337079-6337098

Rev; TTATTTTTTCAAATATTTTCTCATATATGC	chr3R: 6335560-6335589
ext-3'UTR (for *hth[Δuniv]* allele)	chr3R: 6333280-6335561
Fwd; AATCTTTCTTAGTGTTTTTATTTCAAT	chr3R: 6335535-6335561
Rev; ATTGGTTTTCATAATCTTTTATTTCAT	chr3R: 6333280-6333306
Design of miR-iab-4 and miR-iab-8 mutant binding sites for *hth[BSmut]*	
Wildtype sequence	Mutant sequence
miR-iab-4-5p	
GTATACG	GAAAACG
miR-iab-4-3p	
GTATACC	GTAAGCC
miR-iab-8-5p	
ATACGTA	AAACGTG
miR-iab-8-3p	
ATGTATC	AAGTAAC

Supplementary Table 2 (related to Figure 6). Oligonucleotide sequences used to generate Northern probes.

Probe	Forward primer (5'-3')	Reverse Primer (5'-3')
hth_universal_1 (exon)	CGCTGGTAGTACTCCCGGTCCACTGT	CCGGAGGGACCTGGATGCGGTGTATA
hth_universal_2 (3' UTR)	TCCAGGTCCCTCCGGATATG	TGCTTTTGTTATTTACGTTTAGGGT
hth_short_1	CGAAATGCCAATCGAGCGAA	CTTGGAAGCTCGTTTGTCGC
hth_short_2	GCACATGGTCCTATATTGGCG	TCTGCGTTCGGATTTGGATT