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Figures and figure supplements

Endocrine remodelling of the adult intestine sustains reproduction in *Drosophila*

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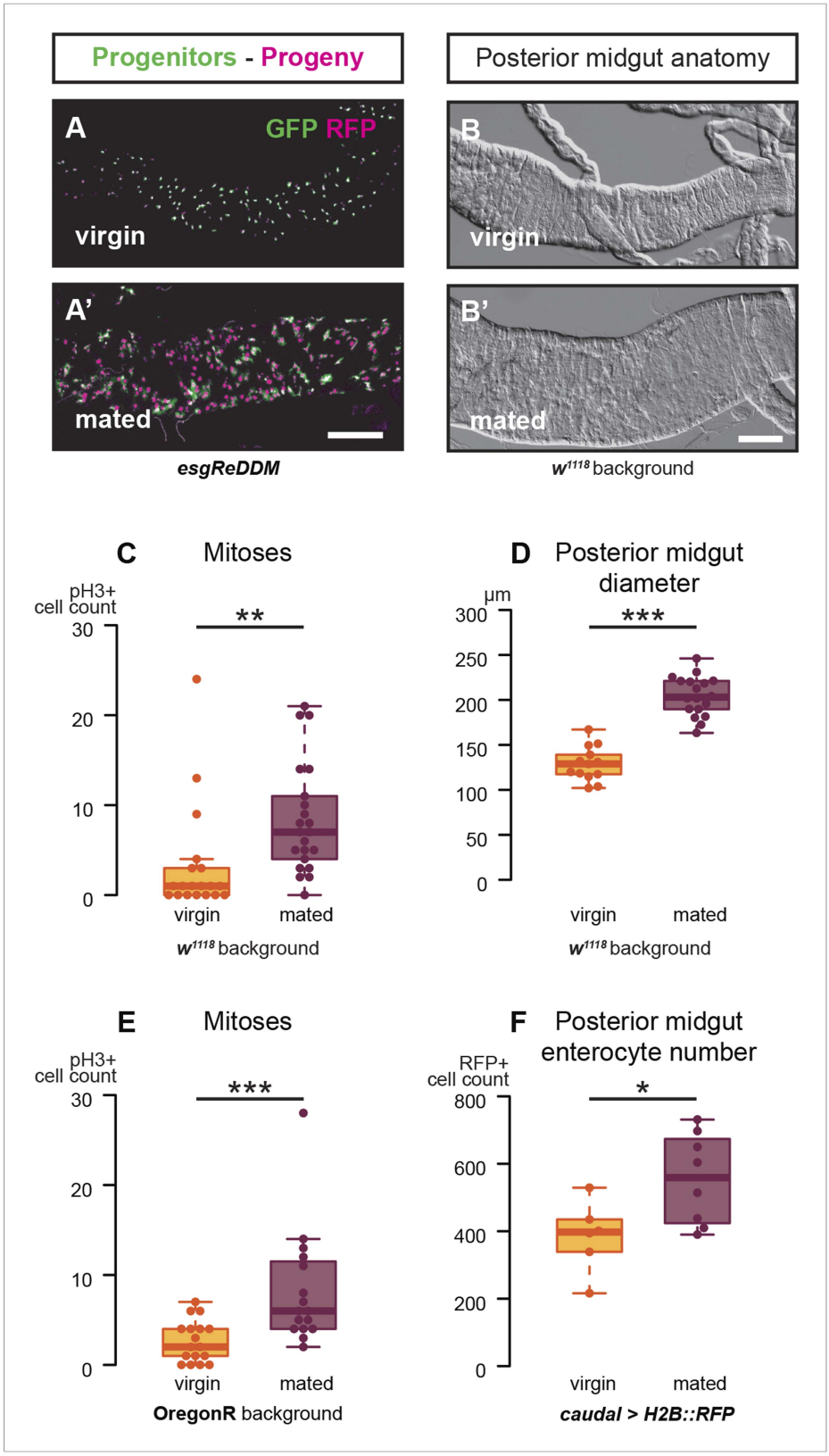

Figure 1. Mating increases ISC proliferation and gut size. (**A**, **A'**) Using the *esgReDDM* tracer (***Antonello et al., 2015***), intestinal progenitors (intestinal stem cells (ISCs) and enteroblasts) are labelled with GFP and RFP, whereas the postmitotic progeny (enterocytes (ECs) and enteroendocrine cells) that these progenitors give rise to in

Figure 1. continued on next page

Figure 1. Continued

a defined time window is labelled with RFP only (see Supplemental Information for additional details). At 3 days after mating, the posterior midgut of mated flies contains more newly generated postmitotic progeny (**A**) compared to age-matched virgins (**A'**). It has also become visibly larger (**B**, **B'**). At this time point, these guts also have a higher number of nuclei marked by the mitotic marker pH3 in both w^{1118} and OregonR backgrounds (**C**, $p = 0.008$, and **E**, $p < 0.001$, negative binomial GLM), although the proliferation increase is transient (data not shown). The size increase is quantified in the posterior midgut by measuring midgut diameter (**D**, $p < 0.001$, t-test) and counting the number of cells labelled by the EC marker *caudal-Gal4* (**F**, $p = 0.02$, t-test). See ***Table 1*** for full genotypes.

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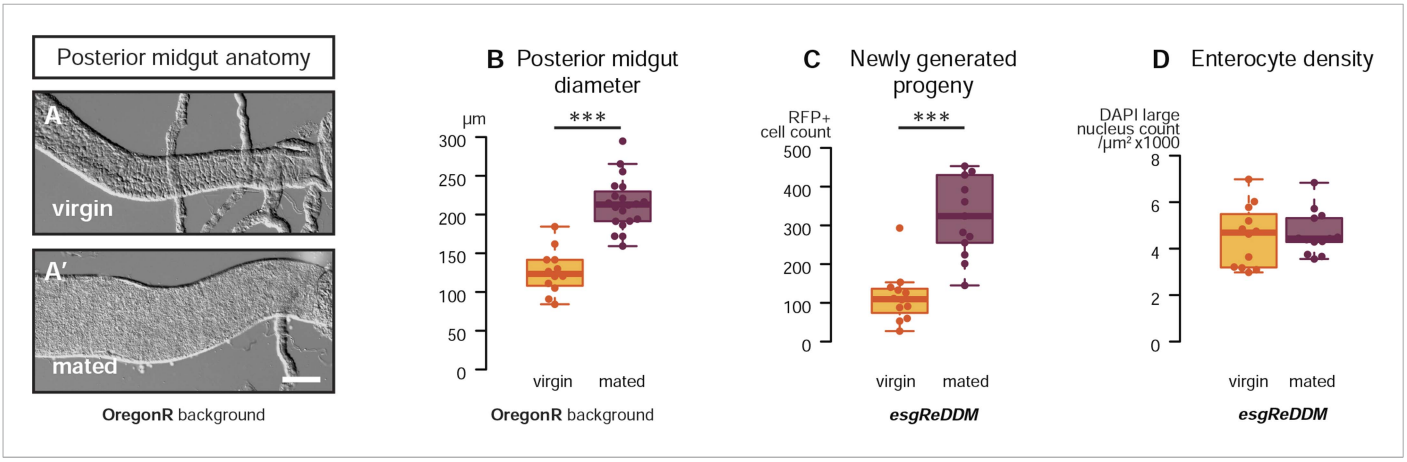

Figure 1—figure supplement 1. Mating re-sizes the Drosophila gut. The increase in gut size at 3 days after mating is also measurable (**A**, **A'**) and significant (**B**, $p < 0.001$, t-test) in the OregonR background. The *esgReDDM* tracing system reveals that mated guts contain more cells generated in the last 7 days if the fly had been mated in that time (**C**, $p < 0.001$, t-test) than if it had not. The size increase is not due to stretching of the tissue, as the density of nuclei in the posterior midgut remains the same (**D**, $p = 0.77$, t-test). See ***Table 1*** for full genotypes.

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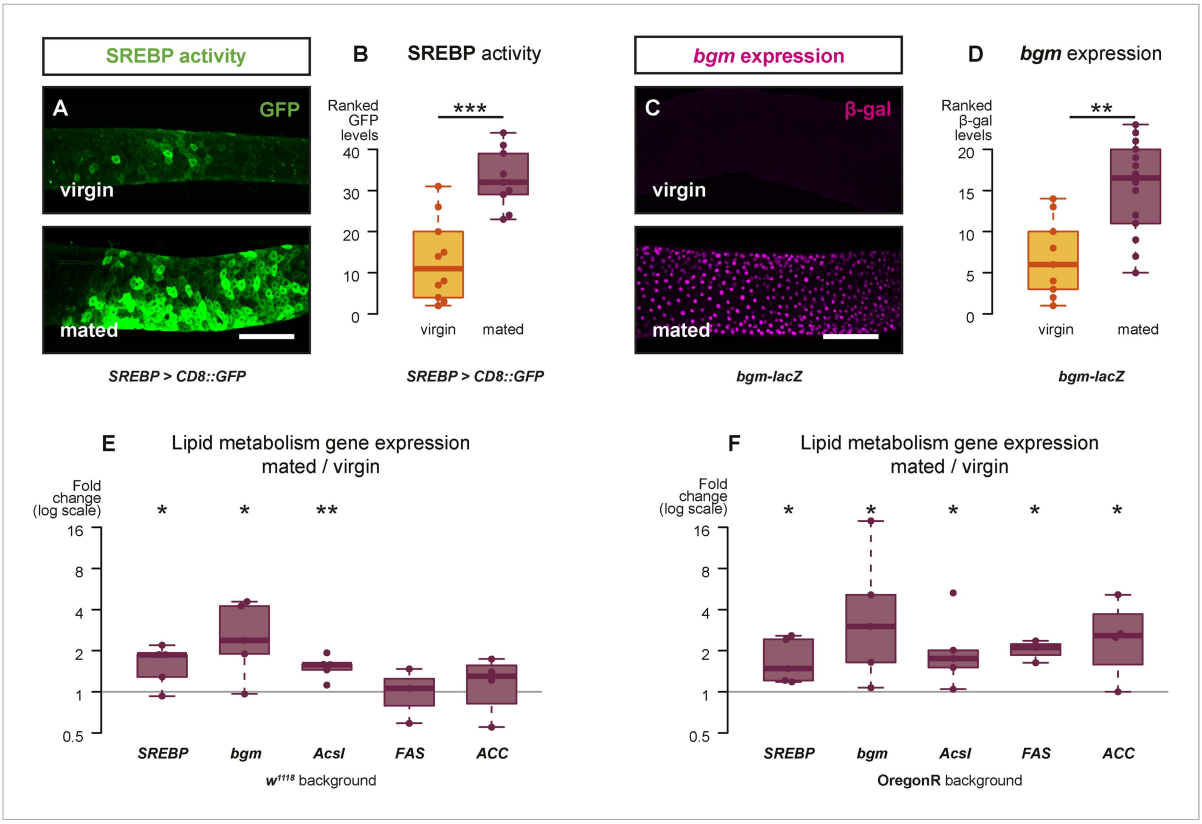

Figure 2. Mating changes the activity and/or expression of lipid metabolism genes in the intestine. At 3 days after mating, increased expression of a reporter that replicates the transcriptional regulation and post-translational modification of sterol regulatory element-binding protein (SREBP) is apparent in the posterior midgut (**A**, **A'**, quantified in **B**, $p < 0.001$, Mann–Whitney test). A *bgm* transcriptional reporter is also increased specifically in the ECs of the posterior midgut following mating (**C**, **C'**, quantified in **D**, $p = 0.002$, Mann–Whitney test). Transcript abundance of *SREBP*, *bgm*, and the *SREBP* targets *Acyl-CoA synthetase long-chain (Acsl)*, *Fatty acid synthase (FAS)*, and *Acetyl-CoA carboxylase (ACC)* is increased by mating in either one or both of the w^{1118} and OregonR backgrounds (**E** w^{1118}: $p = 0.02$ *SREBP*, $p = 0.02$ *bgm*, $p = 0.005$ *Acsl*, $p = 0.5$ *FAS*, $p = 0.3$ *ACC*; **F** OregonR: $p = 0.02$ *SREBP*, $p = 0.03$ *bgm*, $p = 0.03$ *Acsl*, $p = 0.01$ *FAS*, $p = 0.04$ *ACC*, paired one-tailed t-test). See ***Table 1*** for full genotypes.

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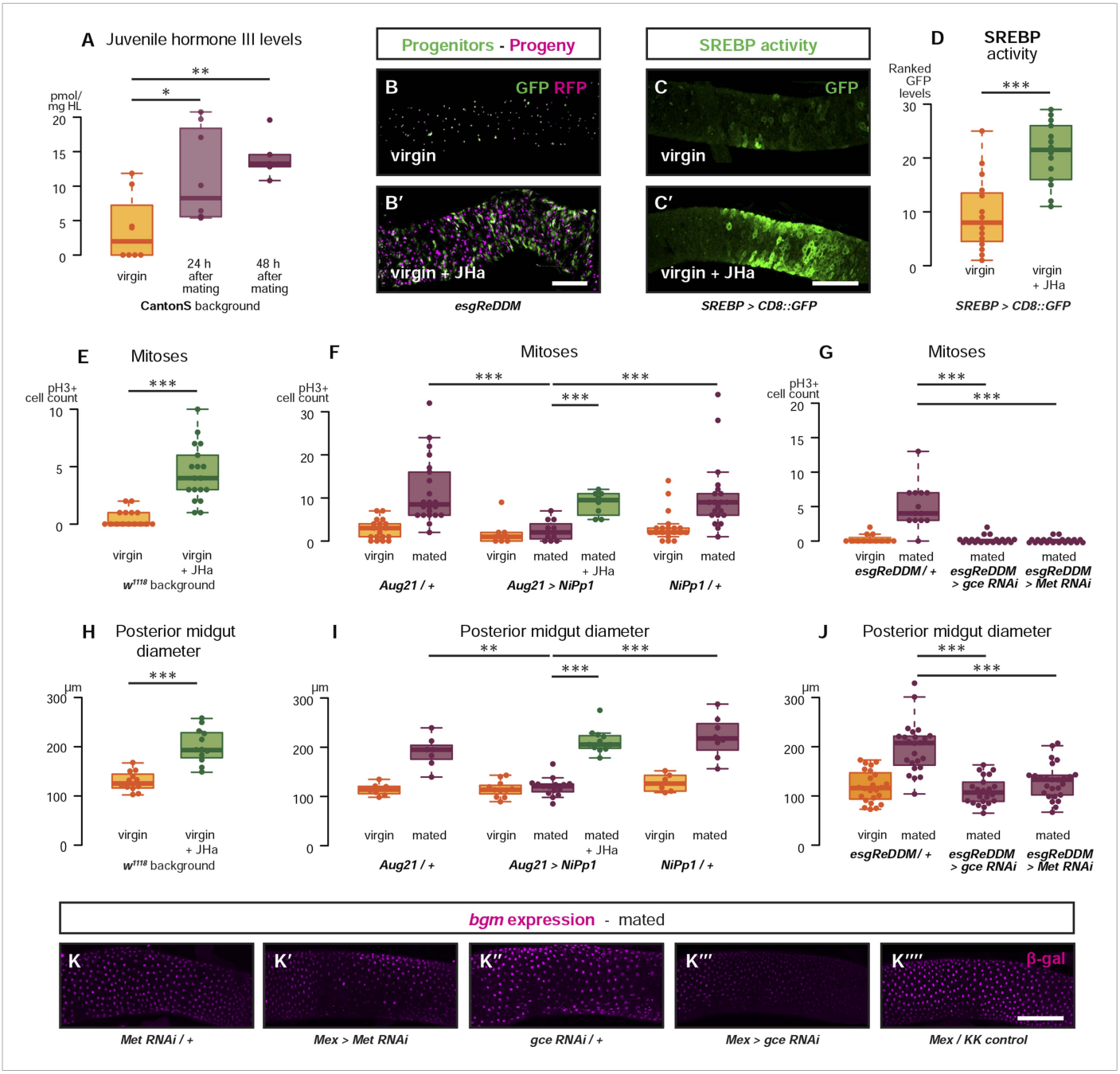

Figure 3. Systemic JH secreted after mating acts directly in the intestinal epithelium to drive reproductive remodelling. Circulating juvenile hormone (JH) is elevated after mating in the haemolymph of female flies (**A**, p = 0.02 at 24 hr, p = 0.002 at 48 hr, t-test with Holm's correction). Increased tissue renewal (**B**, **B′**) and SREBP activation (**C**, **C′**, quantified in **D**, p < 0.001, Mann–Whitney test) are apparent following a 3-day dietary supplementation with JH analogue (JHa). JHa treatment is sufficient to increase mitoses (**E**, p < 0.001, negative binomial GLM) and size (**H**, p < 0.001, t-test) of the posterior midgut. Conversely, when the endogenous JH source is genetically ablated by means of *Aug21* > *NiPp1* (***Yamamoto et al., 2013***), the proliferation and size increase that follow mating are abolished, although they can be reinstated by feeding JHa (proliferation **F**, p < 0.001 between *Aug21*/+ and *Aug21* > *NiPp1* mated, p < 0.001 between *Aug21* > *NiPp1* and *NiPp1*/+ mated, p < 0.001 between *Aug21* > *NiPp1* and *Aug21* > *NiPp1* + JHa mated; all relevant comparisons between virgins are not significant, negative binomial GLM with Holm's correction; gut diameter **I**, p = 0.002 between *Aug21*/+ and *Aug21* > *NiPp1* mated, p < 0.001 between *Aug21* > *NiPp1* and *NiPp1*/+ mated, p < 0.001 between *Aug21* > *NiPp1* and *Aug21* > *NiPp1* + JHa mated; all relevant comparisons between virgins are not significant, t-test with Holm's correction). Downregulation of either *gce* or *Met* in adult progenitors abrogates post-mating proliferation (**G**, p < 0.001 between *esgReDDM*/+ and *esgReDDM* > *gce RNAi* mated, p < 0.001 between *esgReDDM*/+ and *esgReDDM* > *Met RNAi* mated, negative binomial GLM with Holm's correction) and gut size increase (**J**, p < 0.001 between *esgReDDM*/+ and *esgReDDM* > *gce RNAi* mated, p < 0.001 between *esgReDDM*/+ and *esgReDDM* > *Met RNAi* mated, t-test with Holm's correction). The upregulation of *bgm*

Figure 3. continued on next page

Figure 3. Continued

reporter upon mating is abolished by the downregulation of *gce*, but not *Met*, in ECs using the EC-specific driver *Mex-Gal4* (**K**–**K**′′′′). See ***Table 1*** for full genotypes.

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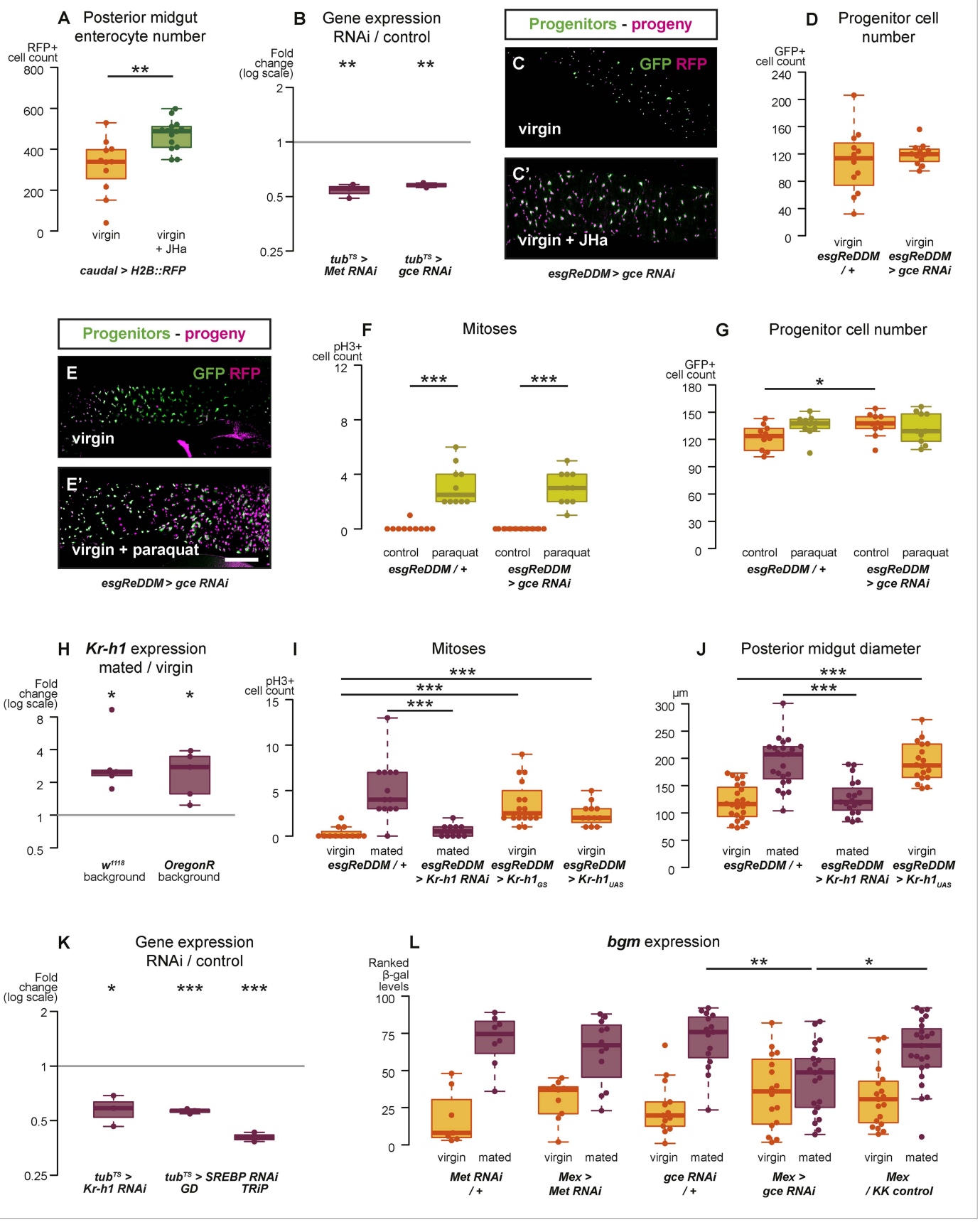
A Posterior midgut enterocyte number
RFP+ cell count
800
600
400
200
0
**
virgin
virgin + JHa
caudal > H2B::RFP
B Gene expression RNAi / control
Fold change (log scale)
2
1
0.5
0.25
** **
tubTS > Met RNAi
tubTS > gce RNAi
Progenitors - progeny
C GFP RFP
virgin
C'
virgin + JHa
esgReDDM > gce RNAi
D Progenitor cell number
GFP+ cell count
240
200
160
120
80
40
0
virgin esgReDDM / +
virgin esgReDDM > gce RNAi
Progenitors - progeny
E GFP RFP
virgin
E'
virgin + paraquat
esgReDDM > gce RNAi
F Mitoses
pH3+ cell count
12
8
4
0
*** ***
control paraquat control paraquat
esgReDDM / +
esgReDDM > gce RNAi
G Progenitor cell number
GFP+ cell count
180
150
120
90
60
30
0
*
control paraquat control paraquat
esgReDDM / +
esgReDDM > gce RNAi
H Kr-h1 expression mated / virgin
Fold change (log scale)
8
4
2
1
0.5
* *
w1118 background
OregonR background
I Mitoses
pH3+ cell count
15
10
5
0
*** *** ***
virgin mated mated virgin virgin
esgReDDM / +
esgReDDM > Kr-h1 RNAi
esgReDDM > Kr-h1GS
esgReDDM > Kr-h1UAS
J Posterior midgut diameter
µm
300
250
200
150
100
50
0
*** ***
virgin mated mated virgin virgin
esgReDDM / +
esgReDDM > Kr-h1 RNAi
esgReDDM > Kr-h1UAS
K Gene expression RNAi / control
Fold change (log scale)
2
1
0.5
0.25
* *** ***
tubTS > Kr-h1 RNAi
tubTS > SREBP RNAi GD
tubTS > SREBP RNAi TRiP
L bgm expression
Ranked β-gal levels
100
75
50
25
0
** *
virgin mated
Met RNAi / +
Mex > Met RNAi
gce RNAi / +
Mex > gce RNAi
Mex / KK control

Figure 3—figure supplement 1. Continued

Figure 3—figure supplement 1. Intestinal JH signalling is relayed through Kr-h1 and underlies mating-dependent intestinal growth and gene expression phenotypes. JHa application in virgin females results in a net growth of the gut, as shown by the increase in *caudal*-marked cells (**A**, p = 0.004, t-test). The JH signalling pathway can be targeted using RNAi constructs against the receptors *Met* and *gce*, which decrease transcript abundance compared to a tub^{ts} control when expressed globally in larvae for 3 hr at 29˚C (**B**, p = 0.002 for *Met*, p = 0.005 for *gce*, paired one-tailed t-test). Consequently, using *esgReDDM* to specifically knockdown *gce* in adult intestinal progenitor cells abolishes the proliferative effect of JHa application (**C**, **C'**), but does not reduce the number of progenitors after 7 days of downregulation (**D**, p > 0.05, t test). Progenitors in which *gce* is downregulated can still proliferate normally to replenish a gut damaged by a 24 hr application of the toxin paraquat (**E**, **E'** with quantification of mitoses in **F**, p < 0.001 for both *esgReDDM/+* and *esgReDDM > gce RNAi*, p > 0.05 for all other relevant comparisons, t test with Holm's correction) and the number of progenitors is not reduced by this treatment (**G**, p = 0.04 between *esgReDDM/+* untreated control and *esgReDDM > gce RNAi* untreated control, p > 0.05 for all other relevant comparisons, t test with Holm's correction). The transcription factor *Kruppel homolog 1* (*Kr-h1*), a well-established effector of JH responses, is transcriptionally upregulated after 3 days of mating (**H**, p = 0.02 in w^{1118}, p = 0.02 in OregonR, paired two-tailed t-test). *Kr-h1* function is necessary and sufficient for the re-sizing of the gut after mating, as its downregulation in intestinal progenitors through RNA interference using *esgReDDM* prevents the increase in proliferation (**I**, p < 0.001 between *esgReDDM/+* and *esgReDDM > Kr-h1 RNAi* mated, negative binomial GLM with Holm's correction) and gut size (**J**, p < 0.001 between *esgReDDM/+* and *esgReDDM > Kr-h1 RNAi* mated, t-test with Holm's correction) typically observed after 7 days of mating, while overexpression of *Kr-h1* constructs from the same cells recapitulates the effect of mating in virgins (proliferation, **I**, p < 0.001 between *esgReDDM/+* and *esgReDDM > Kr-h1$_{GS}$* virgin, p < 0.001 between *esgReDDM/+* and *esgReDDM > Kr-h1$_{UAS}$* virgin, negative binomial GLM with Holm's correction; gut size **J**, p < 0.001 between *esgReDDM/+* and *esgReDDM > Kr-h1$_{UAS}$* virgin, t-test with Holm's correction). RNAi constructs against *Kr-h1* and *SREBP* are effective in downregulating these genes; they decrease transcript abundance compared to a tub^{ts} control when expressed globally in larvae for 3 hr at 29˚C (**K**, p = 0.02 for *Kr-H1*, p < 0.001 for both *SREBP* constructs, paired one-tailed t-test). Downregulating *gce* constitutively from ECs using *Mex-Gal4* significantly suppresses the transcriptional increase of the lipid metabolism gene *bgm* upon mating, as indicated by the intensity ranking of a *gce* reporter (**L**, p = 0.004 between *gce RNAi/+* and *Mex > gce RNAi*, p = 0.02 between *Mex > gce RNAi* and *Mex/KK control*, Mann–Whitney test with Holm's correction; relevant comparisons with *Met RNAi* are not significant). See ***Table 1*** for full genotypes.

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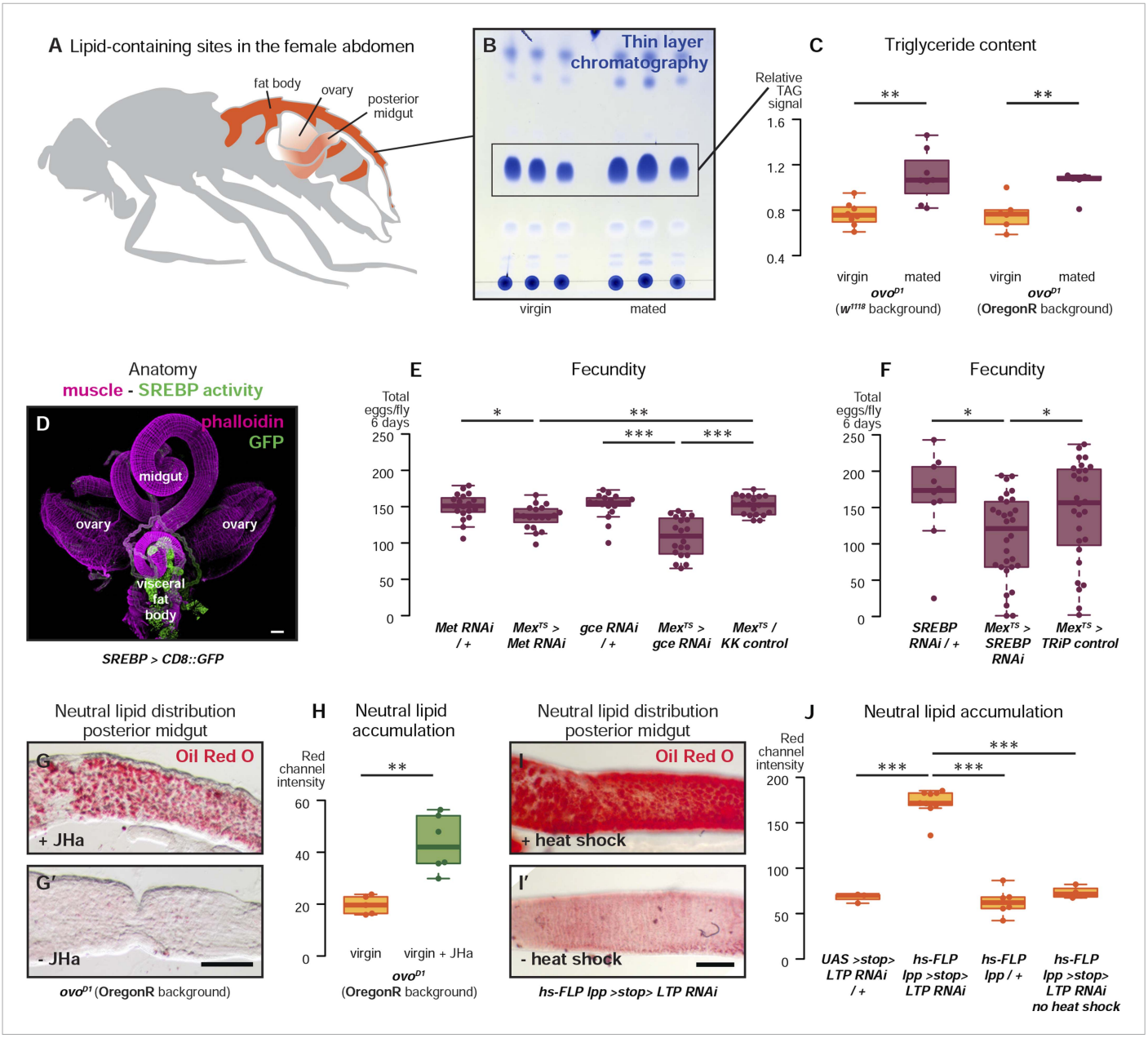

Figure 4. Metabolic remodelling of ECs by JH sustains reproduction. Lipid-harbouring tissues (fat body, posterior midgut, and ovary) are found in close proximity in the fly's abdomen (represented schematically in **A**, and in confocal microscopy in **D**). The amount of stored triglycerides (TAG) in the carcass of 3-day mated sterile female flies is increased compared to virgins (**B**, p = 0.003 in w^{1118}, p = 0.009 in OregonR, t-test), as quantified by thin-layer chromatography (**C**). Adult-specific downregulation of JH receptors *gce* and *Met* or *SREBP* in ECs reduces the total progeny produced by females in the 6 days following their first mating (**E**, p = 0.01 between *Met RNAi*/+ and Mex^{ts} > *Met RNAi*, p = 0.007 between Mex^{ts} > *Met RNAi* and Mex^{ts}/*KK* control, p < 0.001 between *gce RNAi*/+ and Mex^{ts} > *gce RNAi*, p = 0.007 between Mex^{ts}> *gce RNAi* and Mex^{ts}/*KK* control; **F** p = 0.04 between *SREBP RNAi*/+ and Mex^{ts} > *SREBP RNAi*, p = 0.04 between Mex^{ts} > *SREBP RNAi* and Mex^{ts}/*TRiP* control, t-test with Holm's correction). In the absence of the ovarian lipid sink in sterile ovo^{D1} virgin flies, treatment with JHa increases neutral lipid content, as revealed by Oil Red O staining, in the posterior midgut (**G**, **G'**, quantified in **H**: p = 0.002, t-test). Acute block of lipid export by heat-shock activation of *lpp > stop > LTP RNAi* (***Palm et al., 2012***) in virgin females results in heavy accumulation of neutral lipid in this gut region, further indicating that this midgut region provides a net source of lipid in adult flies (**I**, quantified in **J**: p < 0.001 between *LTP RNAi*/+ and *lpp > stop > LTP RNAi*, p < 0.001 between *lpp > stop > LTP RNAi* and *lpp > stop>*/+, p < 0.001 between *lpp > stop > LTP RNAi* and *lpp > stop > LTP RNAi* heat shock control, t-test with Holm's correction). See ***Table 1*** for full genotypes.

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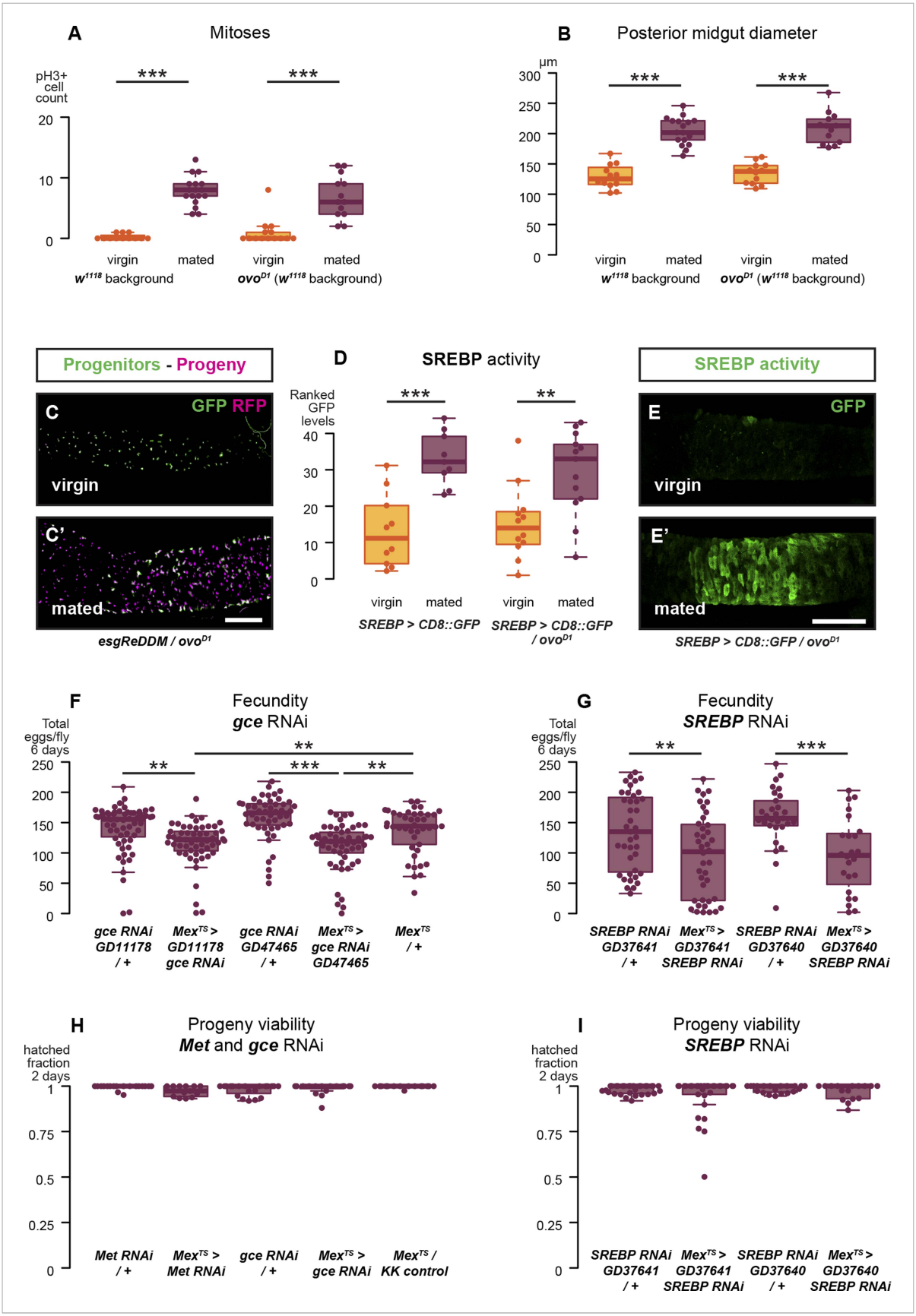

Figure 4—figure supplement 1. continued on next page

Figure 4figure supplement 1. Continued

Figure 4—figure supplement 1. Reproductive intestinal remodelling is uncoupled from germline demands and is needed to sustain reproduction. Sterile females carrying the ovo^{D1} mutation experience the post-mating increase in progenitor proliferation (**A**, $p < 0.001$ for w^{1118} and ovo^{D1}, negative binomial GLM, visualised in **C** and **C′** using the *esgReDDM* tracing system), gut size increase (**B**, $p < 0.001$ for w^{1118} and ovo^{D1}, t-test), and *SREBP* reporter activation (**D**, $p < 0.001$ for w^{1118} and $p = 0.005$ for ovo^{D1}, Mann–Whitney test, visualised in **E** and **E′**). The role of intestinal remodelling in enhancing reproductive capacity is confirmed with additional RNA interference lines against the JH receptor *gce* (chosen because of its larger effect in ***Figure 4E***; **F**, $p = 0.002$ between *GD11178/+* and Mex^{ts}> *GD11178*, $p = 0.008$ between Mex^{ts} > *GD11178* and Mex^{ts}/+, $p < 0.001$ between *GD47465/+* and Mex^{ts}> *GD47465*, $p = 0.003$ between Mex^{ts} > *GD11178* and Mex^{ts}/+, t-test with Holm's correction) and *SREBP* (**G**, $p = 0.008$ between *GD37641/+* and Mex^{ts} > *GD37641*, $p < 0.001$ between *GD37640/+* and Mex^{ts} > *GD37640*, t-test). Despite these effect on fecundity, eggs laid by *gce*, *Met*, or *SREBP* RNAi mothers are viable (**H** and **I**, mean hatched fraction >0.9 for all groups, $p > 0.05$ for all relevant comparisons, t-test). See ***Table 1*** for full genotypes.

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