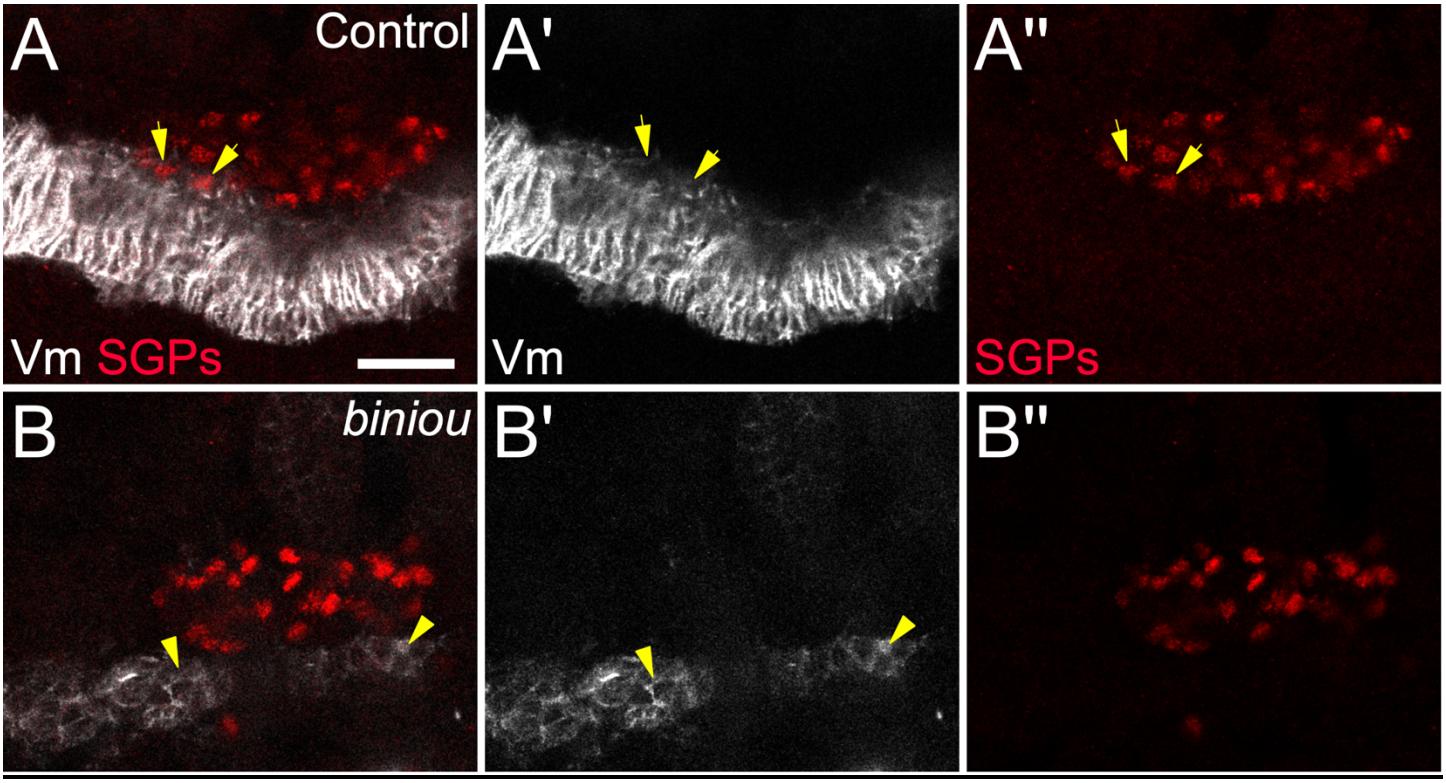

Figure S1. *biniou* mutant SGPs do not contact Vm precursors, Related to Figure 1. SGPs (Traffic jam, red) that are still coalescing, and neighboring Vm (Fas3, white) in (**A**) control and (**B**) *biniou[R22]* mutant embryos. (**A**) Arrows indicate two SGP nuclei that are directly adjacent to Fas3 positive Vm tissue. In (**B**), there is less Fas3 positive tissue, expected for *biniou* mutants. Vm tissue is near the gonad, but not contacting SGP nuclei (arrowheads). (**A',B'**) Fas3. (**A'',B''**) Tj. Scale bar, 20 um.

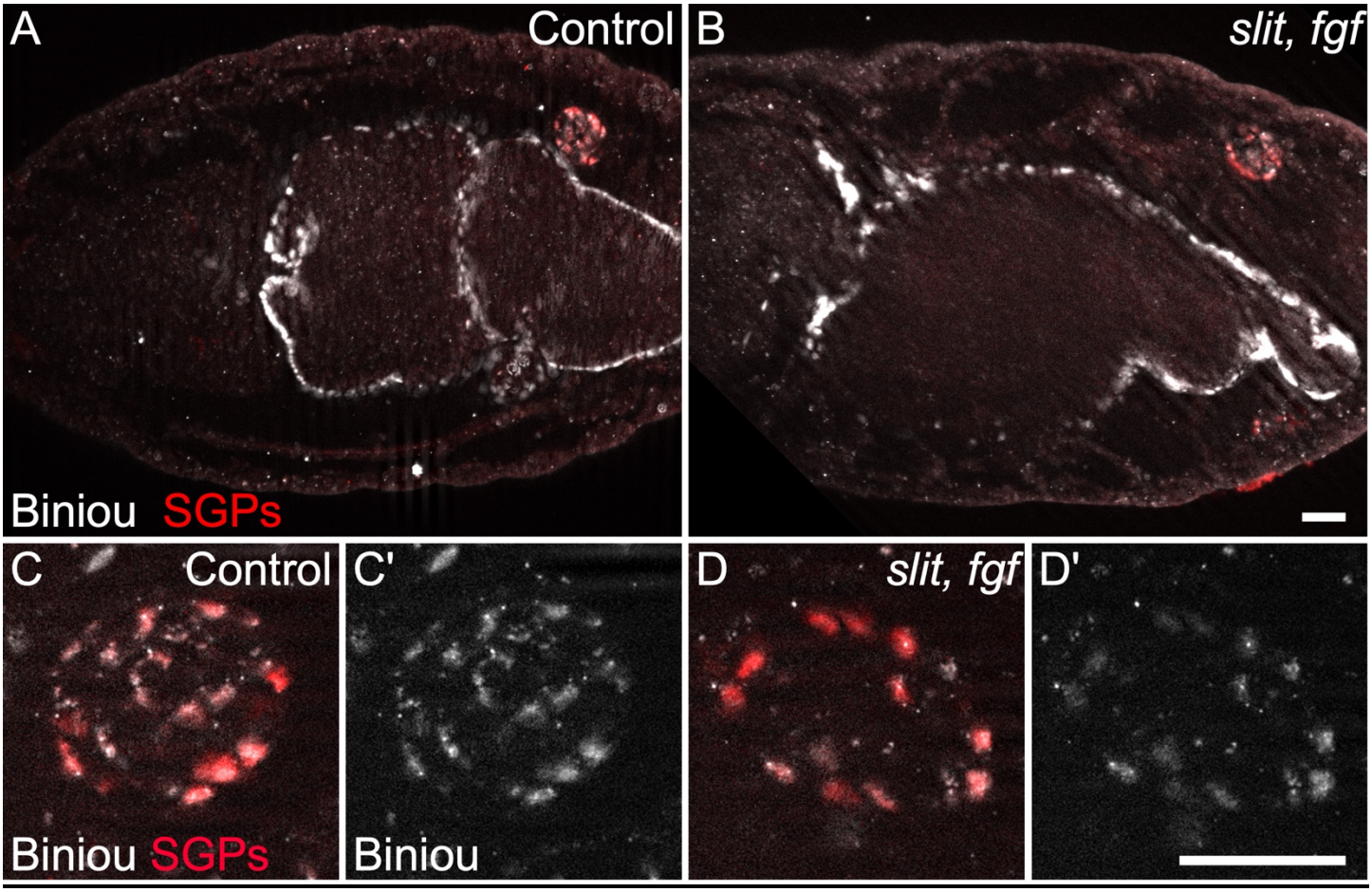

Figure S2. SGPs express *biniou*, Related to Figure 1. Stage 16 (**A-B**) embryos, and (**C-D**) gonads immunostained for Biniou (white) and Traffic jam (red, SGPs). SGPs from (**A,C**) wild type or (**B,D**) combined mutants with *slit,* and *pyr* and *ths* removed (*fgf*) express *biniou*. Prime panels, Biniou. Scale bars, 20 um.

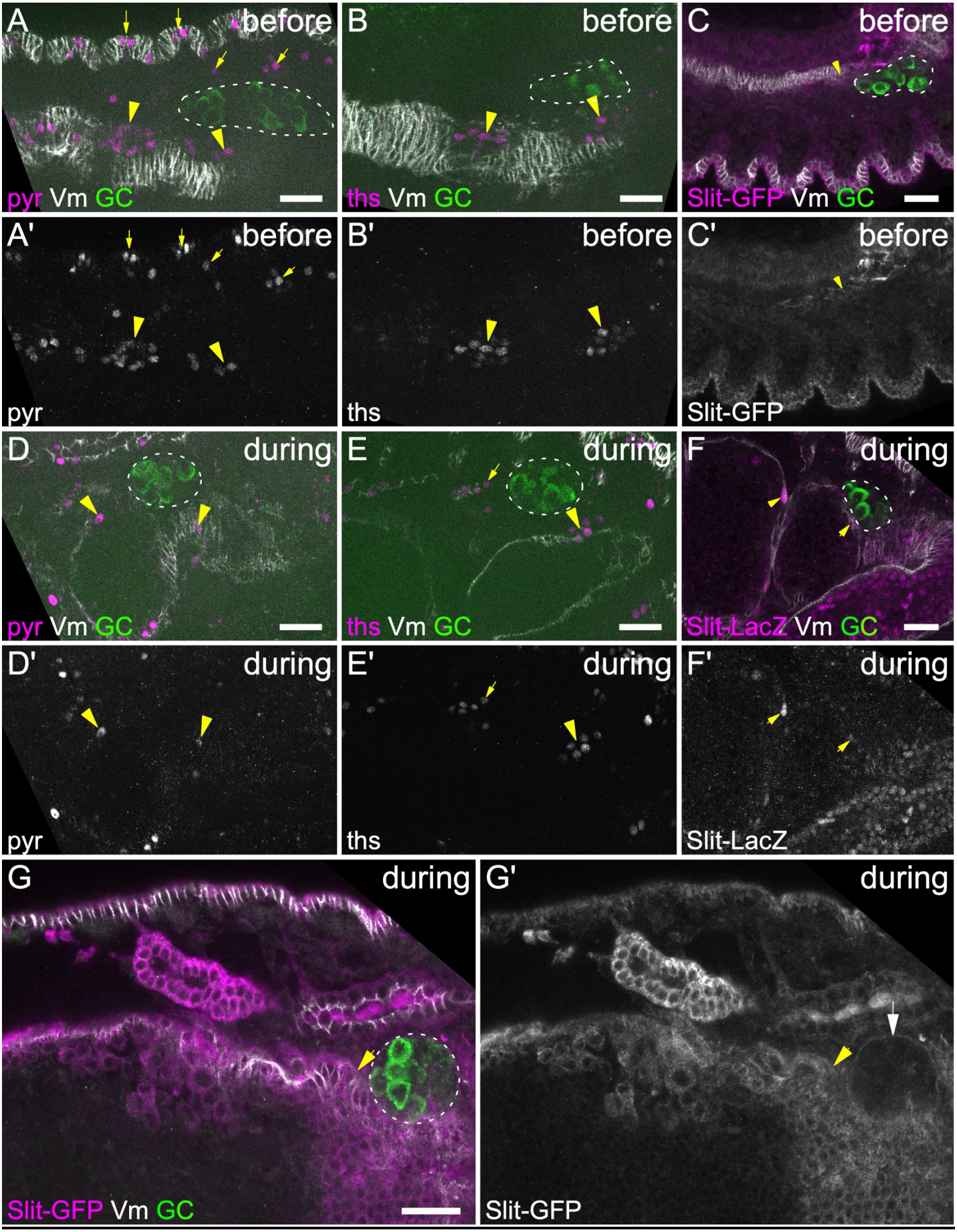
A
before
pyr Vm GC
B
before
ths Vm GC
C
before
Slit-GFP Vm GC
A'
before
pyr
B'
before
ths
C'
before
Slit-GFP
D
during
pyr Vm GC
E
during
ths Vm GC
F
during
Slit-LacZ Vm GC
D'
during
pyr
E'
during
ths
F'
during
Slit-LacZ
G
during
Slit-GFP Vm GC
G'
during
Slit-GFP

Figure S3. FGF and Slit are expressed in visceral mesoderm, Related to Figure 2. (**A-G**) Embryos immunostained for Vasa (green, germ cells), and Fas3 (white) to show visceral mesoderm and other tissues, including overlying epidermis, and tubular hindgut and foregut. FGF or Slit expression in magenta, or (**A'-G'**) white alone. (**A,D**) Embryos express *pyr*-Gal4 driving UAS-red Stinger (magenta) to show FGF ligand expression (**A**) before or (**D**) during niche assembly. (**A, A'**) Arrowheads show *pyr*-expressing cells within Vm. Arrows reveal a metameric pattern of Pyr in overlying epidermis and some underlying mesoderm. At this stage SGPs are intermingled with germ cells where little or no Pyr is detectable. (**D, D'**) Pyr is clearly detectable among Vm cells (arrowheads) and the hindgut, but not among germ cells. (**B,E)** Embryos express *ths*-Gal4 driving UAS-red Stinger (magenta) to show expression of the second, redundant FGF ligand. As with *pyr*, we observe expression in the Vm (arrowheads) and some neighboring mesodermal cells (arrows), but not among germ cells. (**C,F**) Embryo expressing Slit::GFP or Slit-LacZ (magenta) (**C**) before or (**F**) during niche assembly. (**C, C**') Arrowheads indicate clusters of Vm cells expressing Slit::GFP including those near germ cells. Slit expression is also observed in cells neighboring the gonad that do not derive from visceral mesoderm. (**F, F'**) Occasional Slit-expressing Vm cells are still present (arrow), but little or no expression is detectable among germ cells. (**G, G'**) Slit-GFP protein accumulates near gonadal extra-cellular matrix (ECM, white arrow) during niche assembly. Nearby Vm cells expressing Slit are indicated with a yellow arrow. Scale bars, 20 um.

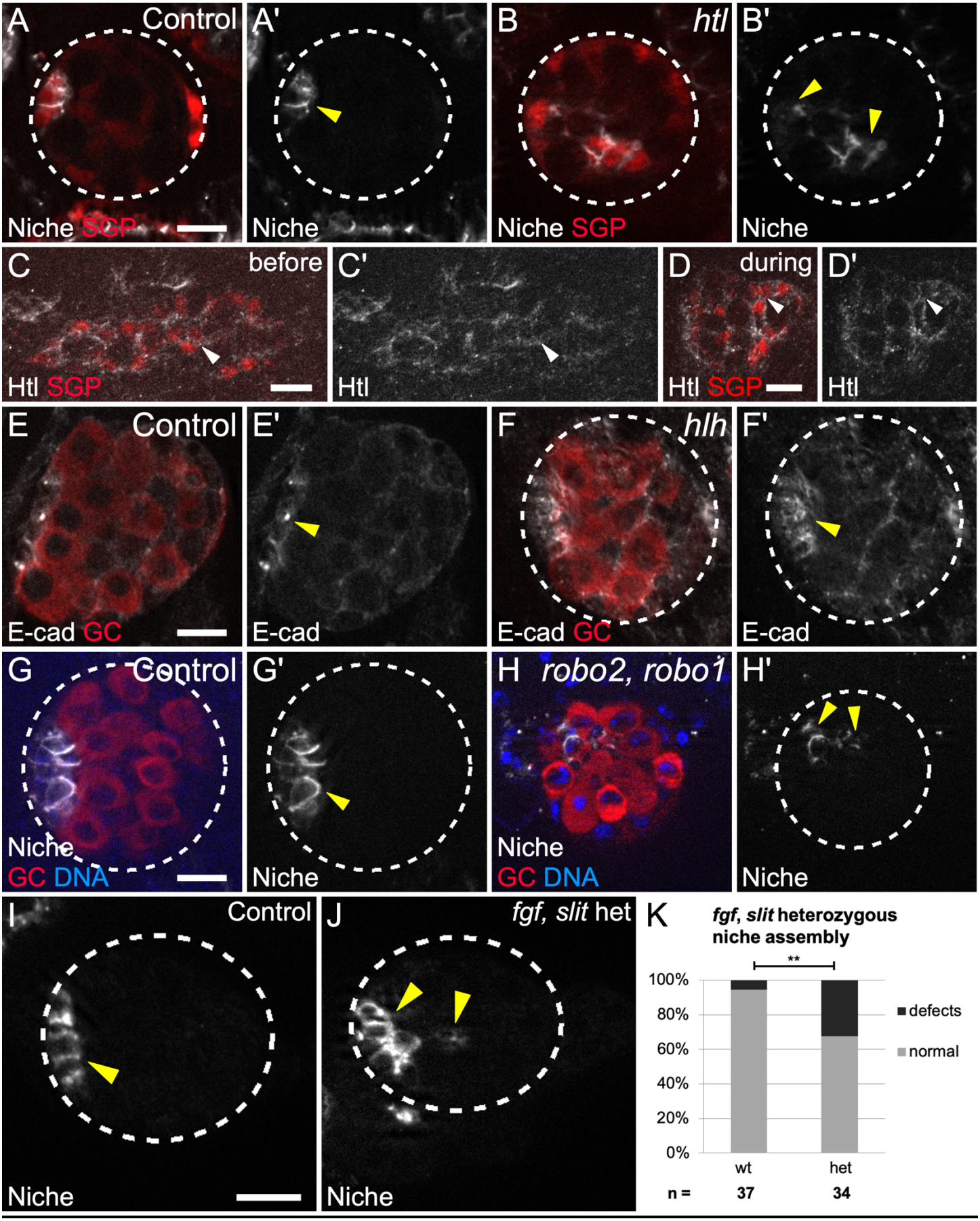
A
Control
Niche SGP
A'
Niche
B
htl
Niche SGP
B'
Niche
C
before
Htl SGP
C'
Htl
D
during
Htl SGP
D'
Htl
E
Control
E-cad GC
E'
E-cad
F
hlh
E-cad GC
F'
E-cad
G
Control
Niche
GC DNA
G'
Niche
H
robo2, robo1
Niche
GC DNA
H'
Niche
I
Control
Niche
J
fgf, slit het
Niche
K
fgf, slit heterozygous
niche assembly
**
100%
80%
60%
40%
20%
0%
defects
normal
wt
het
n =
37
34

Figure S4. FGF and Slit receptors and dosage are important for niche assembly, Related to Figure 2.

(**A-B**) Stage 17 gonads expressing *six4*-nls-eGFP (SGPs, red) immunostained for Fas3 (niche cells, white). (**A',B'**) Fas3 alone. (**A**) Wild type gonads have a single anterior niche, while (**B**) *htl* receptor mutants exhibit dispersed niche cell aggregates. (**C,D**) Htl-mCherry expressing gonads (**C**) before and (**D**) during assembly immunostained for RFP (Htl, white) and TJ antibody (SGPs, red). Neighboring SGPs show Htl localization at cell boundaries. (**C'D'**) Htl alone. (**E,F**) Stage 17 gonads stained with Vasa (red, germ cells) and E-cadherin (white, enriched in the niche). (**E',F'**) Ecad alone. Both (**E**) controls and (**F**) *hlh54f* cvm mutants have an anterior niche. However, *hlh54f* mutant niches do not accumulate Fas3 (not shown). (**G,H**) Stage 17 (**G**) control or (**H**) *robo2, robo1* gonads immunostained for Vasa (germ cells, red), Fas3 (niche cells, white), and Hoechst (DNA, blue). (**G',H'**) Fas3 alone. (**I-J**) Stage 17 gonads stained for Vasa (germ cells, not shown), and Fas3 (niche cells, white). (**I**) Controls have a single anterior niche, in contrast to (**J**) *slit*, *FGF* heterozygotes which show dispersed niche cells. **(K)** Quantification of niche assembly in *slit*, *fgf* heterozygotes ($p = 0.005$, Fisher's exact test). In all panels: yellow arrowheads, niche cells; dotted lines, gonad boundaries; scale bars, 10 um.

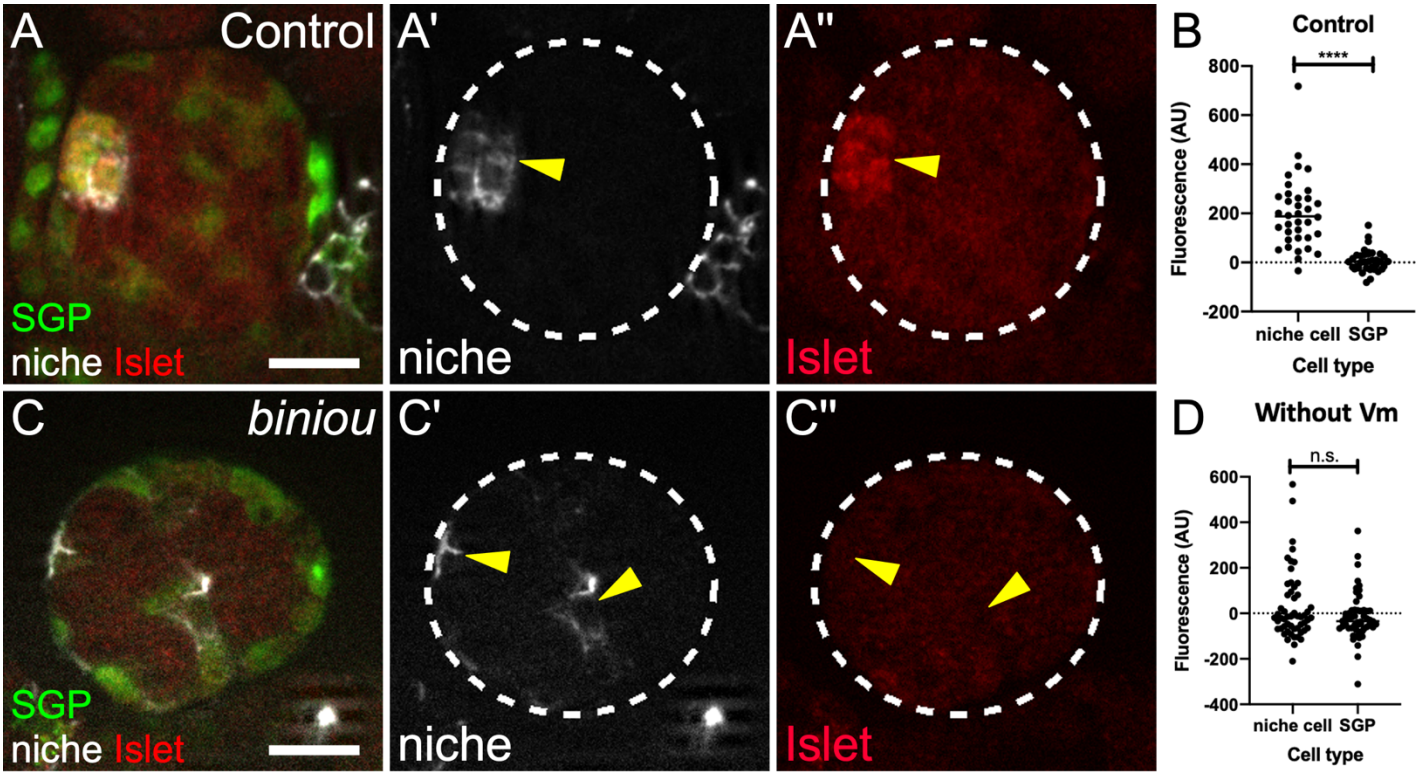

Figure S5. *islet* expression depends on *biniou*, Related to Figure 4. (**A,C**) Stage 17 *six4nls*GFP gonads immunostained for GFP (green, SGPs and other mesoderm), Fas3 (white, niche cells), and Islet (red). (**A',C'**) Fas3 alone (arrowhead). (**A'',C''**) Islet alone (arrowhead, niche cells). (**A**) Control gonads express Islet in niche cells, while (**C**) *biniou* mutants do not. (**B,D**) Islet accumulation in Fas3 positive niche cells, compared to non-niche SGPs in (**B**) control ($p < 0.0001$, Mann Whitney test) and (**D**) *biniou* mutants.