Decreased expression of *lethal giant larvae* causes ovarian follicle cell outgrowth in the *Drosophila Scutoid* mutant

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Abstract

Snail, a zinc-finger transcription factor, controls the process of epithelial-mesenchymal transition, and ectopic expression of this protein may produce cells with stem cell properties. Because the effect of Snail expression in ovarian epithelial cells remains unclear, we generated *Drosophila* ovarian follicle stem cells (FSCs) with homozygous *Scutoid* (*Sco*) mutation. The *Sco* mutation is a reciprocal transposition that is known to induce ectopic Snail activity. We found that *Sco* mutant FSCs showed excess proliferation and high competitiveness for niche occupancy, and the descendants of this lineage formed outgrowths that failed to enter the endocycle. Surprisingly, such phenotypes were not rescued by suppressing Snail expression, but were completely restored by supplying *Lethal giant larvae* (*Lgl*). The *lgl* allele is a cell polarity gene that is often mutated in the genome. Importantly, *Sco* mutants survived in a complementation test with *lgl*. This result was probably obtained because the *Sco*-associated *lgl* allele appears to diminish, but not ablate *lgl* expression. While our data do not rule out the possibility that the *Sco* mutation disrupts a regulator of *lgl* transcription, our results strongly suggest that the phenotypes we found in *Sco* mutants are more closely associated with the *lgl* allele than ectopic Snail activity.

**Introduction**

Epithelial-mesenchymal transition (EMT) is a highly conserved process in which immotile epithelial cells lose cell polarity and adhesion capability, becoming migratory mesenchymal cells [1]. Snail induces EMT by transcriptionally repressing E-cadherin [2, 3]. Recent data have shown that overexpression of Snail in tumor cell lines induces cell invasion, and cancer stem cell properties [4, 5]. We explored whether Snail dysregulation is sufficient to induce EMT, or a similar process, in non-cancerous epithelial cells, such as those derived from the *Drosophila* follicle cell lineage.

The *Drosophila* ovary is an excellent model by which to study the biology of epithelial cells [6]. Each ovary carries 15 to 20 ovarioles (Fig 1A), which are the functional units that continuously produce eggs [7]. The anterior-most structure of the ovariole, named the germarium, houses two or three germline stem cells (GSCs) at its tip. The immediate GSC progeny, called
a cystoblast, divides four times to produce a 16-cell germline cyst. This germline cyst is then surrounded by prefollicle cells and buds off from the germarium to become an egg chamber, which passes through 14 different stages and finally develops into a mature egg. Prefollicle cells are derived from two follicle stem cells (FSCs) that are located on opposite sides of the junction between the 2a and 2b regions of the germarium [8, 9]. Shortly after surrounding the germline cyst, prefollicle cells differentiate into stalk cells, polar cells, and follicle cells. Stalk cells link egg chambers, while two polar cells located at the anterior and posterior poles of the egg chamber function to control follicle cell fate and anterior-posterior axis determination. The follicle cells form a polarized epithelium around each egg chamber [8]. Before stage 6, follicle cells undergo a mitotic cycle that includes the complete set of G1, S, G2 and M phases [10], whereas around the beginning of stage 7, the follicle cells enter an endocycle, which includes only the G and S phases [11].

In our previous study, overexpression of snail in the follicle cell lineage by the UAS/GAL4 system only resulted in increased proliferation of FSCs and mild outgrowth of stalk cells [12]. However, the increased activity of Snail in this model may not be sufficient to drive EMT in follicle cells, as E-cadherin expression was only slightly reduced [12]. The dominant Scutoid (Sco) mutation is a reciprocal transposition of two small regions and results in ectopic Snail activity (plane A and B in S1 Fig) [13]. In this study, we generated FSCs that were homozygous for the Sco mutation and traced them and their progeny. We found that these Sco mutant FSCs were hyperproliferative, resulting in outgrowth and increased niche occupancy. Sco mutant follicle cells also did not enter the endocycle and lost cell polarity. However, none of the phenotypes were rescued by suppressing Snail activity. To our surprise, follicle cell defects could be completely rescued by exogenous expression of lethal giant larvae (lgl), a cell polarity gene that is often spontaneously mutated. Our results indicate that ectopic Snail activity is not responsible for the observed outgrowth of Sco mutant follicle cells, and does not drive EMT in ovarian epithelial cells. However, lgl may be a second-site allele that is associated with the Sco mutant and substantially contributes to the observed phenotypes. Importantly, this allele could not be identified by simple complementation test.

Results and discussion

Sco mutant FSCs exhibit increased proliferation and extended lifespan

To determine whether forcing Snail activity induces EMT in the follicle cell lineage, we used a FLP-mediated recombination technique to generate GSCs homozygous for Sco (Fig 1B). The Sco mutant cells could be recognized by the absence of GFP (Fig 1C–1F) and their localization in the tissue. FSCs lack specific molecular markers, and therefore cannot be unambiguously identified. However, these cells can be indirectly recognized based on their location at the border of germarial regions 2a and 2b, where FasIII, a marker for prefollicle cells, is weakly expressed. FSCs are the cells immediately anterior to the FasIII-positive prefollicle cells [9, 14]. In addition, prefollicle cells eventually differentiate and leave the germarium after three to four days [14], while FSCs are retained. Therefore, beginning at one week after clone induction (ACI), the presence of prefollicle cell clones (GFP-negative) can be an indicator of the existence of GFP-negative FSCs.

We first examined the maintenance of Sco mutant FSCs by assessing the percentage of germaria carrying Sco mutant prefollicle cell clones over time (Fig 1G and S1 Table). At three weeks ACI, 46% of FRT40 control germaria (n = 205) retained at least one wild-type control FSC that was generated during the first week (Fig 1G). This result indicates that up to 54% of FSCs had undergone natural turnover in the controls, which is consistent with an earlier report that the half-life of FSCs is two to three weeks [15]. However, 83% of Sco mutant FSCs
Fig 1. Sco FSCs exhibit extended lifespan, enhanced proliferation, and increased competitiveness for niche occupancy. (A) Schematic of the Drosophila ovariole. The anterior-most structure of the ovariole, the germarium, contains germ cells that are enveloped by prefollicle cells (light green), to form egg chambers. The prefollicle cells are derived from two FSCs (yellow), which are located at the 2a/2b boundary of the germarium. Follicle cells of egg chambers up to stage (S) 6 (light blue) undergo mitotic cycles, while follicle cells of egg chambers after stage 7 (orange) enter the endocycle. (B) Mitotic recombination was used to generate Sco FSCs. Females were generated, carrying a wild-type allele linked to a marker gene (GFP) in trans with the Sco allele. FLP-mediated recombination between FRT sites during mitotic division generated a homozygous Sco FSC that could be identified by the absence of GFP. (C-F) Control (Ctrl) (C and E) and Sco mosaic germaria (D and F) shown at one week (W) after clone induction (ACI): GFP (green, wild-type cells), FasIII (red, follicle cell lineages), DAPI (blue, DNA), and Edu (white in E and F, indicating proliferating cells). Solid and empty triangles indicate GFP-positive Sco FSCs. Decreased lgl expression results in follicle cell outgrowth in Drosophila Sco mutants.
remained in Sco mutant mosaic germaria \( n = 163 \) at three weeks ACI, suggesting that Sco mutant FSCs have a prolonged lifespan. We also examined the division rate of Sco mutant FSCs using a mitotic marker, phospho-Histone3 (PH3), to label cells in M phase, and a DNA replication marker, EdU incorporation, to show cells in S phase (Fig 1E and 1F). We did not observe any PH3 positive FSCs in control mock or mutant mosaic germaria at one week ACI (control \( n = 183 \), mutant \( n = 252 \); S1 Table), probably due to the short duration of mitosis. These results are consistent with those of a previous report [16]. However, at the same time-point, the frequency of EdU positive Sco mutant FSCs in Sco mutant mosaic germaria was 2.3-fold greater than that of control FSCs in mock mosaic germaria (Fig 1H; 13.1% for control, \( n = 67 \) vs. 29.5% for Sco mutant, \( n = 121 \)), indicating that homozygous Sco mutant FSCs underwent rapid division.

Interestingly, we also observed that the proportion of Sco mutant mosaic germaria, in which all FSCs were mutant (i.e. two GFP-negative FSCs), increased from 10% \( (n = 193 \) germaria) at one week to 32% \( (n = 163 \) germaria) by three weeks ACI (Fig 1I and S1 Table). In FRT40A mock mosaic germaria, a much smaller increase was observed (one week ACI: 6.2%, \( n = 176 \) germaria vs. three week ACI: 13.3%, \( n = 155 \) germaria). This small increase was probably due to natural loss, which arose from replacement of opposite GFP-negative FSCs with GFP-positive FSCs from the same germarium (Fig 1I). These results indicate that Sco mutant FSCs are more competitive for niche occupancy and tend to replace wild-type FSCs. Furthermore, it is possible that their progeny exhibit increased chance of migrating across the germ-line to compete in the FSC niche [9].

**Sco** mutant follicle cells form outgrowths that fail to enter the endocycle

We noticed that progeny derived from Sco mutant FSCs, including stalk cell precursors (generated from prefollicle cell intermediates) and follicle cells (which cover egg chambers), formed outgrowths. Compared to the control, the number of stalk cell precursors in Sco mutants increased from 10% \( (n = 193 \) germaria) at one week to 32% \( (n = 163 \) germaria) by three weeks ACI (Fig 1I and S1 Table). In FRT40A mock mosaic germaria, a much smaller increase was observed (one week ACI: 6.2%, \( n = 176 \) germaria vs. three week ACI: 13.3%, \( n = 155 \) germaria). This small increase was probably due to natural loss, which arose from replacement of opposite GFP-negative FSCs with GFP-positive FSCs from the same germarium (Fig 1I). These results indicate that Sco mutant FSCs are more competitive for niche occupancy and tend to replace wild-type FSCs. Furthermore, it is possible that their progeny exhibit increased chance of migrating across the germ-line to compete in the FSC niche [9].
Fig 2. *Sco* follicle cells are hyperproliferative and do not enter the endocycle. Control (Ctrl) (A, C, F and H) and *Sco* mosaic ovarioles (B, D, G and I) at one week (1W) after clone induction (ACI) are labeled with GFP (green, wild-type cells) and FasIII (gray, membranes of follicle cell lineages) in A and B, phospho-Histone 3 (PH3, gray, mitotic marker) and DAPI (blue, DNA) in C, D, F and G, and Cyclin B (CycB, gray, G2/M phase marker) in H and I. Wild-type cells are outlined by yellow dashed lines. The scale bar in A is 10 μm, and scale bars in C, F and H are 20 μm. (A and B) *Sco* mosaic ovarioles contain stalk cell overgrowths that are completely composed of excessive numbers of irregularly-shaped *Sco* cells, as compared to the control. (C and D) *Sco* follicle cells formed multiple layers in stage (S) 4 and 6 egg chambers. (E) Percentage (%) of mosaic ovarioles exhibiting PH3 signal in GFP-negative follicle cell clones. The number of ovarioles analyzed is shown above each bar. (F and G) *Sco* follicle cells of the stage 8 egg chamber formed multiple layers, and continued to undergo mitosis. F’ and G’ show the...
G2/M phase marker [17], is normally only expressed in follicle cells until stage 6 (Fig 2H, n = 11). However, Cyclin B expression was detected in Sco mutant follicle cells of all the stage 7 egg chamber (Fig 2I, n = 23), indicating that Sco mutant follicle cells fail to switch from a mitotic cycle to an endocycle.

Interestingly, all Sco mutant follicle cells lost cell polarity, as evidenced by dysregulated E-cadherin (E-cad; Fig 3A, n = 30, and B, n = 27), Disc large (Dlg; Fig 3C, n = 11, and D, n = 19) and Atypical protein kinase C (aPKC; Fig 3E, n = 5, and F, n = 7) expression. The loss of all of these factors may also contribute to the failure of the mitotic-endocycle transition [18, 19]. E-cad and Dlg are polarity genes that are mainly enriched in apical-lateral and lateral domains of follicular cells, respectively [19–21]. However, expression of E-cad and Dlg was not restricted to specific subcellular domains in Sco mutant follicle cells (Fig 3A–3D). aPKC is mainly expressed in the apical domain of follicle cells [19], but similar to the other two markers, it was not restricted to a specific domain of Sco mutant follicle cells (Fig 3E and 3F). In addition, Notch signaling is a key factor in the control of the mitotic-endocycle transition [10, 22, 23]. In mitotic follicle cells of stage 1 to 6 egg chambers, Cut is expressed and suppresses early entry into the endocycle. After stage 6, follicle cells receive Delta from germ cells and activate Notch signaling, which upregulates expression of Hindsight to suppress Cut expression and initiate entry into the endocycle (Fig 4A). We found that Notch signaling, as monitored by the E(spl)m7-lacZ Notch reporter [24], was not activated in the outer layer of all Sco mutant follicle cells (Fig 4B, n > 20, an C, n > 20). Consequently, Cut expression was retained (Fig 4D, n > 20, and E, n > 20), and Highlight expression was absent from the outer layer of all Sco mutant follicle cells of stage 7 egg chambers (Fig 4F, n > 20 and G, n > 20). To summarize, we observed that Sco mutant FSCs lost cell polarity, formed outgrowths and failed to undergo the mitosis-endocycle transition. Together, these observations strongly suggest that EMT, or an EMT-like process, occurs within Sco mutant cells.

Snail and Noc do not account for the multiple-layered phenotype of Sco mutant follicle cells

The Sco mutant chromosome is induced by the transposition of two DNA fragments, causing fusion of the snail and no ocelli (noc, encoding a zinc finger protein belonged to the NET family) genes [13]. It has been shown that the phenotype of mechano-bristle loss in the thorax and eyes of Sco heterozygous flies can be rescued by decreasing expression of snail or increasing expression of noc [13], indicating that Snail is ectopically expressed while Noc expression is reduced in Sco mutants. We therefore hypothesized that these two genes may be responsible for the phenotype we observed in the Sco mutant follicle cell lineage. To test this possibility, we knocked down snail in FSCs and their progeny that were homozygous for Sco mutations using the FLP/FRT system under the control of c587-GAL4, which is expressed in the follicle lineage (S2 Fig). However, knockdown of snail did not prevent Sco mutant follicle cells from forming multiple layers (Fig 5A to 5D; control: n = 10, Sco: n = 10, and Sco with SnailRNAi: n = 10). We then attempted to phenocopy the Sco mutation by knocking down noc expression in the follicle cell lineage using 109–30 GAL4 (Fig 5E and 5G), which is expressed in FSCs and early follicle cells, or GR1 GAL4 (Fig 5F and 5H), which is expressed from stage 3 to stage 10 follicle cells [25, 26]. However, neither the elimination of noc (Fig 5G, n > 20, and H, n > 20), nor
Fig 3. Sco follicle cells lose cell polarity and are delaminated. (A-F) One-week (w)-old control (ctrl) (A, C and E) and Sco mosaic (B, D and F) stage (S) 6 and 7 egg chambers: GFP (green, wild-type cells) and E-cadherin (gray, E-cad) in A and B; Disc large (gray, Dlg) in C and D; aPKC (E and F); DAPI (blue, DNA). A’ and B’ show E-cad channel only; C’ and D’ show Dlg channel only; E’ and F’ show aPKC only. Dashed lines indicate follicle cell clones. Inserts are enlarged images from the area indicated by asterisks. Scale bar, 20 μm. The genotype of A, C and E is hs-flp/+; ubi-gfp FRT40A/FRT40A, of B, D and F is hs-flp/+; ubi-gfpFRT40A/ScoFRT40A.

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Fig 4. Notch signaling is disturbed in the outer layers of Sco follicle cells. (A) Notch signaling is required for the transition of follicle cells from the mitotic phase to endocycle phase [10]. In mitotic follicle cells (up to stage 6), Notch signaling activity is low because germ cells produce low amounts of Delta (a Notch ligand, shown as yellow triangles). In addition, Cut is expressed in follicle cells to suppress the mitosis-endocytosis transition. After stage 7, Notch signaling is activated to promote Hindsight expression, which suppresses Cut expression and thereby permits the mitosis-endocytosis transition. Red squares indicate Notch receptors. (B-G) One-week (w)-old control (ctrl) (B, D and F) and Sco mutant mosaic egg chambers (C, E and G) at stages (S) 6 and 7: GFP (green, wild-type cells), E(spl)m7-lacZ (gray, a Notch signaling reporter) in B and C, Cut (gray) in D and E, Hindsight (gray) in F and G. Expression of E(spl)m7-lacZ is decreased in ectopic layers of Sco mutant follicle cells located far from the germline at the anterior and posterior poles. In the control, Cut is mainly expressed in stage 6 follicle cells and is downregulated in stage 7 follicle cells. However, Cut expression is weaker in the inner layer as compared to the outer layer of Sco mutant follicle cells. In contrast, Hindsight expression is stronger in the inner layer as compared to the outer layer of Sco follicle cells at stage 7, indicating a non-cell autonomous effect of Sco on the mitosis-endocycle transition. Arrows show the boundary between germ cells and follicle cells. Asterisks indicate outer layers of ectopic follicle cells. Scale bar, 20 μm. The genotype of B, D and F is hs-flp/+; ubi-gfpFR40A/FR40A, of C, E and G is hs-flp/+; ubi-gfpFR40A/ScoFRT40A.

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Fig 5. Snail and Noc do not account for the multiple-layered phenotype of Sco follicle cells. (A-D) One-week-old control (ctrl) mosaic (A), Sco mosaic (B) and Sco mosaic ovarioles with snail knockdown (C and D). (E-J) One-week (W)-old control (ctrl) (E and F), 10930>nocRNAi (G), GR1> nocRNAi (H), 10930>snail & gfp (I), and 10930>snail & nocRNAi ovarioles (J); FasIII (green, follicle cell lineages), Traffic Jam (Tj) (gray, follicle cells) in I and J, and DAPI (blue, DNA). Arrows in I and J indicated overexpression of Snail increase cell number in the stalk that connects two egg chambers. The scale bar is 20 μm. The genotype of A is c587-GAL4/UAS-flp; ubi-gfpFR40A/FRT40A, of B, C, and D is c587-GAL4/UAS-flp; ScoFRT40A/ubi-gfpFRT40A.

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exogenous expression of snail with decreased noc expression could cause the formation of multiple layers of follicle cells (Fig 5I, n > 20, and J, n > 20). Notably, overexpression of snail did induce increased cells numbers in the stalk that connects two egg chambers in approximately 30% of the ovarioles (arrows in Fig 5I, n > 20, and G, n > 20). Therefore, the phenotype caused by the Sco chromosome is complex and not fundamentally reliant on snail and noc, suggesting that the EMT-like phenotype may require the disruption of a tumor suppressor gene.

Lethal giant larvae controls follicle cell homeostasis in the Sco stock

The Sco allele is located on the left arm of Chromosome II, which also contains lgl. This gene is frequently found to be spontaneously mutated in Drosophila stocks [27], due to its location as the second protein-coding gene downstream of the sub-telomeric region of chromosome 2L [28]. Mutations in lgl have been shown to produce long stayed FSCs [29], multiple-layered follicle cells [30] and failure to enter the endocycle [10, 18]. To determine if the Sco stock we used contains an lgl mutation, which could explain the previously described phenotypes, we first performed a complementation test. We crossed Sco FRT40A/CyO flies with flies heterozygous for lgl<sup>t</sup>, a null allele [31], balanced by CyO. We then examined the generation of Sco FRT40A/lgl<sup>t</sup> flies. Because lgl<sup>t</sup> is homozygous lethal, we should not obtain Sco FRT40A/lgl<sup>t</sup> flies if the chromosome carries both Sco FRT40A and a strong lgl mutation. The ratio of three genotypes we obtained from the progeny were as follows: Sco FRT40A/CyO, 2 ± 4%; lgl<sup>t</sup>/CyO, 57 ± 2%; and Sco FRT40A/lgl<sup>t</sup>, 41 ± 6% (333 flies were analyzed for each test). Since we clearly observed that Sco FRT40A/lgl<sup>t</sup> flies were frequently produced, the result suggested that the Sco phenotypes we observed in FSCs and their progeny may not be due to the mutation of lgl. However, it remained possible that this test may not detect a weak lgl allele. To test this, we generated the Sco mutant follicle cell lineage expressing lgl-gfp, using the Mosaic Analysis with Repressible Marker (MARCM) technique (Fig 6A). Surprisingly, the multiple-layered phenotype of Sco mutant follicle cell clones was completely rescued by exogenous expression of Lgl (Fig 6B, n = 13, and C, n = 24). In addition, we did not observe an increased number of stalk precursors in Sco mutant plus Lgl overexpression mosaic ovarioles, indicating that Lgl supplementation also rescues the Sco mutant stalk cell phenotypes. Consistent with this rescue effect, expression of lgl in the ovaries carrying Sco mutant follicle cell lineages (FPKM value: 31) was less than half of that in the control ovaries (FPKM value: 69, P<0.0001), as analyzed by RNASeq. These results strongly suggest that a second allele mutation in lgl accounts for the phenotypes that we observed in the Sco mutant follicle cell lineage. However, we cannot rule out the possibility that the Sco mutation may directly or indirectly disrupt some regulator of lgl expression.

Following our observation that lgl supplementation can rescue the phenotype in Sco mutant FSC lineage cells, we carefully compared the phenotype of lgl mutant FSCs and their lineage that was reported by previous studies [18, 29] and the Sco mutants. This comparison revealed two major differences between lgl and Sco mutant FSCs and follicle cells. First, although both lgl and Sco mutant FSCs exhibit prolonged maintenance and increased competition for niche occupancy, the lgl mutation does not affect FSC proliferation, while the Sco mutation results in a two-fold increase in FSC proliferation. Second, although both lgl and Sco mutant follicle cells form multiple layers in egg chambers, 61% of lgl mutant mosaic ovarioles carry fused egg chambers that are not observed in Sco mutant mosaic ovarioles. We have previously demonstrated that Snail promotes FSC proliferation [12], suggesting that Sco mutant FSCs may exhibit a combined phenotype that results from high Snail and low Lgl activity.

It has been previously reported that a high frequency of lgl alleles exist in wild populations of Drosophila melanogaster, as well as the Bloomington second chromosome deficiency kit and the University of California at Los Angeles Bruinfly FRT40A-lethal P collection [27]. Here, we
also report that a weak lgl mutant allele may be associated with the Bloomington Sco stock. These results emphasize the need to routinely test second chromosome stocks for second-site alleles of lgl. Furthermore, it is important to note that simple complementation tests may not be enough to identify mutant lgl alleles.

**Materials and methods**

**Fly stocks and culture**

Flies were cultured at 22–25˚C on standard medium, unless otherwise indicated. w^1118^ was used as wild-type controls. Sco is a chromosome rearrangement mutant generated by X-ray-induced mutagenesis; ScoFRT40A was obtained from the Bloomington fly stock center (B
5759) [13, 32]. UAS-RNAi lines against snail (VDRC 50003) and noc (VDRC 108422) were obtained from the Vienna Drosophila RNAi Center; their efficiencies were described previously or tested here [33, 34]. c587-GAL4, 10930-GAL4, GR1-GAL4, and UAS-lgl-gfp have been previously described [24–26, 35, 36]. E(spl)m7-lacZ was used to monitor Notch signaling [33]. Food was changed daily until dissection. Other genetic elements are described in Flybase (http://flybase.bio.indiana.edu).

**Genetic mosaic analysis**

Mosaic clones were generated by Flipase (FLP)/FLP recognition target (FRT)-mediated mitotic recombination [37]. For conventional mosaic analysis, females of genotype hs-flp/+; ubi-GFPFRT40A/ScoFRT40A and hs-flp/+; ubiGFPFRT40A/FRT40A, c587, UAS-flp/+; ubiGFPFRT40A/ScoFRT40A were generated using standard crosses. For mosaic analysis with repressible marker (MARCM) [38], females of genotype hs-flp/+; tubGAL80FRT40A/ScoFRT40A; actFRT-CD2-FRT-GAL4 UAS-nlacZ/+; and hs-flp/+; tubGAL80FRT40A/ScoFRT40A; actFRT-CD2-FRT-GAL4 UAS-nlacZ/ UAS-lgl-gfp were generated. To generate conventional FSC clones, two-day-old female flies were subjected to heat shock at 37˚C for 1 h, twice a day for three days. For MARCM experiments, two-day-old female flies were heat shocked at 37˚C for 30 min. After heat shock, females were raised at 25˚C and received fresh food daily until dissection. Homozygous mutant cells were recognized by the absence of GFP in conventional mosaic analysis, but identified by the presence of LacZ in MARCM. We were unable to directly identify FSCs in MARCM experiments, due to the weak expression of LacZ in FSCs.

**RNA sequencing analysis**

Thirty pairs of ovaries were collected and dissected from 1-week old female flies that were cultured at 25˚C. The genotypes of the flies were c587-GAL4, UAS-flp/+; ubi-gfp FRT40A/FRT40 or c587-GAL4, UAS-flp/+; ubi-gfp FRT40A/ScoFRT40A. Total RNA were extracted by Trizol reagent (Invitrogen, USA) according to the instructions. RNA was quantified at by absorbance at 260 nm using a ND-1000 spectrophotometer (Nanodrop Technology, USA) and quality was assessed using a Bioanalyzer 2100 (Agilent Technology, USA) with a RNA 6000 labchip kit (Agilent Technologies, USA). All RNAseq procedures were carried out according to the manufacturer’s protocol from Illumina. Library construction for all samples was accomplished with Agilent’s SureSelect Strand Specific RNA library Preparation Kit for 75SE (Single-End or Paired-End) sequencing on Solexa platform. The sequence was directly determined using sequencing-by-synthesis technology with a TruSeq SBS kit. Raw sequences were obtained from the Illumina Pipeline software bcl2fastq v2.0 and expected to generate 12.5M (million reads) per sample. The sequences were then filtered to obtain qualified reads. Trimmomatic software was implemented to trim or remove the reads according to the quality score. The gene expression level was calculated as FPKM (Fragment Per Kilobase of transcript per Million mapped reads). For differential expression analysis, CummeRbund was used to perform statistical analysis of gene expression profiles. The reference gene annotations were retrieved from Flybase. Data was deposited in the NCBI GEO under the accession number GSE43506.

**Immunostaining and fluorescence microscopy**

Immunostaining and EdU incorporation in ovarian tissue was performed as previously described [12, 33]. The following primary antibodies were used: mouse anti-Fasciclin III (Fas-III) (Developmental Studies Hybridoma Bank, DSHB, 1:50), Mouse anti-discs large (Dlg) (DSHB, 1:50), Mouse anti-cut (DSHB, 1:50), Mouse anti-hindsight (Hnt) (DSHB, 1:50), Rabbit
anti-Phospho H3 (Millipore, 1:500), Rabbit anti-GFP (Torrey Pines, 1:1000), Mouse anti-β-gal (Promega, 1:500). The following secondary antibodies were used: AlexFluro 488-, 563-, and 633-conjugated goat species-specific secondary antibody (Molecular Probes, 1:1000). EdU incorporation was performed with the Click-iT Edu imaging kit (Invitrogen). Samples were stained with 0.5 μg/ml DAPI (Sigma) and mounted in 80% glycerol with 20 μg/ml N-propyl gallate (Sigma), and analyzed using a Zeiss LSM 710 confocal microscope.

Egg chamber stages were characterized as previously described [39, 40]. The stage 1 egg chamber resides in region 3 of the germarium; stages 2–7 are characterized by polyploidization of the nurse cells and mitotic division of the follicle cells, as well as increases in the size of the egg chamber. Stage 7 egg chambers are obviously elongated in shape. At the molecular level, stage 6 and 7 are distinguished by the expression of mitotic markers, Notch reporter, Cut and Hnt. Mitotic markers and Cut are expressed in stage 6, but diminished in stage 7; Notch reporter and Hnt start to be expressed in stage 7. The oocyte begins to accumulate yolk at stage 8. At stage 9, most of the follicle cells change from their original cuboidal shape to a columnar shape and are located at the posterior half of the egg chamber.

Supporting information

S1 Fig. Sco mutant flies exhibit a lack of bristles, due to ectopic Snail activity. (A) Sco/+ flies lack anterior and posterior scutellar bristles. (B) Knockdown of snail using a UAS-RNAi line driven by act-GAL4 restores apical and basal scutellar bristles in Sco/+ flies. Asterisks indicate scutellum. (C-F) One-week-old stage 7 control (C and D) and Sco mutant mosaic egg chambers (E and F) with GFP (green, wild-type cells), FasIII (red, follicle cell lineage), and DAPI (blue, DNA). Dashed lines outline clones; scale bar, 20 μm. The genotype of flies in A are Sco FRT40A/CyO, B includes snailRNAi/+; Sco FRT40A/++; act-GAL4/++; C and D include hs-flp/+; ubigfp FRT40A/FRT40A, and E and F show hs-flp/++; ubi = gfp FRT40A/ScoFRT40A.

S2 Fig. c587-GAL4 is expressed in escort cell, FSCs and follicle cells of the early egg chamber. (A) 7-day-old c587>gfp germarium labeled with 1B1 (gray, fusomes and follicle cell membranes) and GFP (green). (A') the image shown the GFP channel only. Scale bar, 20 μm.

S1 Table. Sco mutant FSCs divide faster and persist longer than control FSCs.

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