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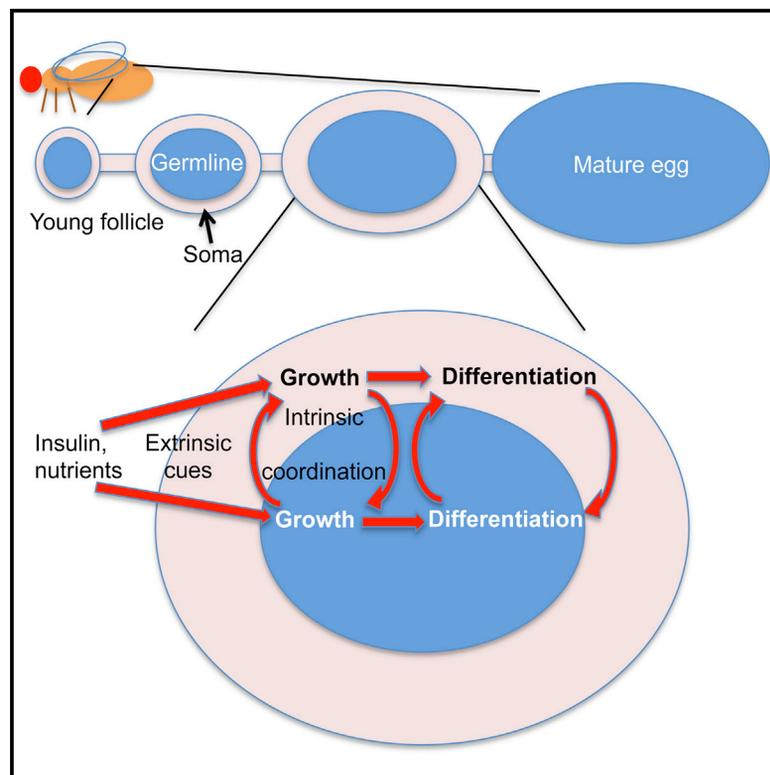
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Cell Reports

Tight Coordination of Growth and Differentiation between Germline and Soma Provides Robustness for *Drosophila* Egg Development

Graphical Abstract



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In Brief

The development of adjacent tissues often needs to be coordinated. Here, Vachias et al. show that, in each *Drosophila* ovarian follicle, somatic cells and germ cells intrinsically synchronize their growth and differentiation in relation to each other. Classical growth pathways such as PI3K or TORC1 also influence differentiation timing but only respond to extrinsic cues. This multidimensional intrinsic coordination ensures high robustness of the developmental steps to produce mature oocytes with high reproducibility.

Highlights

Somatic follicular epithelium and germline cyst control each other's growth

This bidirectional control also ensures control of cell differentiation timing

PI3K and TORC1 control differentiation but are not regulated by the adjacent tissue

This mechanism ensures high robustness of egg development



Tight Coordination of Growth and Differentiation between Germline and Soma Provides Robustness for *Drosophila* Egg Development

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SUMMARY

Organs often need to coordinate the growth of distinct tissues during their development. Here, we analyzed the coordination between germline cysts and the surrounding follicular epithelium during *Drosophila* oogenesis. Genetic manipulations of the growth rate of both germline and somatic cells influence the growth of the other tissue accordingly. Growth coordination is therefore ensured by a precise, two-way, intrinsic communication. This coordination tends to maintain constant epithelial cell shape, ensuring tissue homeostasis. Moreover, this intrinsic growth coordination mechanism also provides cell differentiation synchronization. Among growth regulators, PI3-kinase and TORC1 also influence differentiation timing cell-autonomously. However, these two pathways are not regulated by the growth of the adjacent tissue, indicating that their function reflects an extrinsic and systemic influence. Altogether, our results reveal an integrated and particularly robust mechanism ensuring the spatial and temporal coordination of tissue size, cell size, and cell differentiation for the proper development of two adjacent tissues.

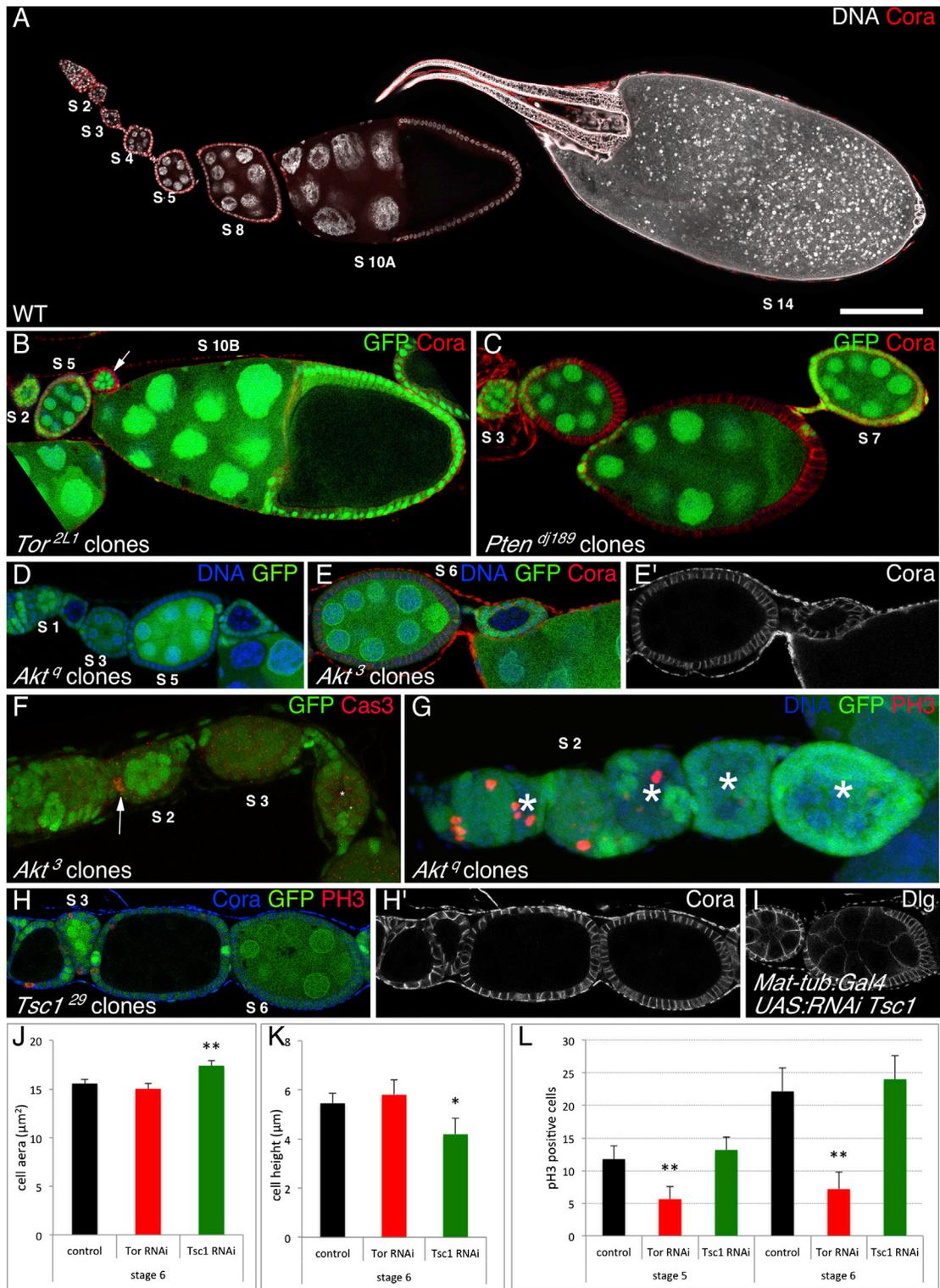
INTRODUCTION

In each cell, growth regulation needs to integrate several inputs, like nutrient availabilities and the spatiotemporal developmental pattern, to ensure the robust development of multicellular organisms (Lander, 2011). On the one hand, this integration of growth with development is extensively studied in order to understand how the whole body or specific tissues respond at the cellular level to systemic signals and how the production of these signals is developmentally controlled (Lloyd, 2013). On the other hand, the intrinsic control of a single tissue size by developmental signals such as local production of morphogens is also highly debated (Baena-Lopez et al., 2012). However, in between these two conceptual approaches of growth control, the development

of complex organs often requires the coordinated growth of distinct tissues in contact with each other. How such coordination between adjacent tissues is established is still poorly understood.

Drosophila oogenesis offers a genetically tractable model to tackle this question because it involves two tissues completely independent in origin; i.e., the soma and germline. Oogenesis takes place in a structure called the ovariole (Figure 1A). At the anterior tip of each ovariole, the germarium contains germline and somatic stem cells. From this germarium bud follicles formed by a 16-cell germline cyst with 15 endoreplicative nurse cells and one oocyte, surrounded by a monolayer of epithelial somatic cells: the follicular cells (fc). Follicles rapidly grow with a massive increase in volume. During this growth there is also a long list of developmental steps, including cell identity changes and morphogenetic events that lead to the production of a mature egg at the posterior of the ovariole (Horne-Badovinac and Bilder, 2005). On the basis of size and morphological aspects, follicle development has been subdivided into 14 different stages (Spradling, 1993). Growing follicles are attached to each other in such a way that an older, and therefore bigger, follicle is always posterior of the next younger and smaller one.

Whereas the final size of the eggs appears to be independent of nutritional conditions, their production rate is highly influenced by food availability. This includes the control of the germ stem cell division rate by Insulin/phosphatidylinositol-4-5 bisphosphate 3-kinase (PI3K) and the Target of rapamycin (Tor) signaling pathways (LaFever and Drummond-Barbosa, 2005; LaFever et al., 2010; Sun et al., 2010). However, the speed of all subsequent steps of oogenesis is also regulated and the same Insulin and Tor pathways influence tissue growth cell-autonomously both in the germline and the soma (Drummond-Barbosa and Spradling, 2001; LaFever et al., 2010; Sun et al., 2010). These results could suggest that growth of the germline and soma are independently and extrinsically controlled by nutrients and systemic signals. However, several studies have hinted at the existence of an intrinsic growth coordination between germline and somatic cells during follicle development (Maines et al., 2004; Wang and Riechmann, 2007; LaFever et al., 2010; Sun et al., 2010). Nonetheless, this coordination has never been carefully studied.



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RESULTS

Somatic Growth and Germline Growth Control Each Other

We first aimed to genetically manipulate somatic growth and look at its impact on germline growth. For this, we generated clones of *fc* that are mutant for cell growth regulators, like components of the Tor and Insulin pathways, which are normally cell-autonomously required, and we asked whether this clone had an impact on germline growth. The impact on follicle growth is easily observable as a given follicle should be smaller than the one placed to its posterior and bigger than the one to its anterior. Mutant cells for *Akt* or *Tor* usually generated only small clones and have no visible impact on germline growth (Figure S1B). However, when the clone is large enough to cover the germline cyst fully or nearly fully, the whole follicle remains much smaller than it should be, indicating a non-cell-autonomous effect of these mutants from the soma to the germline (Figure 1B). We performed the opposite experiments by inducing somatic clones for the *Pten* (or *Tsc1*, not shown) mutation that are growing faster than wild-type (WT) cells. In this case, the whole follicle, including the germline, grows faster than a follicle without clones (Figure 1C). This effect appears to be linked to the size of the clones because small clones do not have visible consequences (Figure S1C). Moreover, comparison of adjacent follicles containing mutant clones confirms that clone size is correlated with its effect on germline growth (Figure S1D). However, we did not try to statistically link the size of the clones and the size of the follicle because of the probably complex growth kinetics of these follicles and the difficulty in estimating the size of the somatic clones in 3D (Figure S1A). We did not find a correlation of this non-cell-autonomous effect with the position of the clones or whether they include the pairs of polar cells, which are the only subdifferentiated follicular cell type during early stages (Figure S1E). Finally, the effect on germline growth is homogenous in all the cells on the basis of the observation of their size or the size of their nucleus. The effect of the soma on the germline is therefore not limited to the cells in contact with the mutant somatic cells. Thus, the overall somatic tissue growth rate is able to either accelerate or slow down germline tissue growth.

We then asked whether manipulating germline growth would have an effect on the surrounding somatic cells. We first slowed down germline cyst growth by producing mutant germline clones for *Akt*. Follicles containing mutant cysts have approximately the WT size when forming, but their growth is almost completely blocked afterward (Figure 1D). The growth of the whole somatic

tissue is also decreased in this context (Figure 1E). We induced germline RNAi against *Tor* from stage 3 and quantified the effect of reduced germline growth on somatic cells in follicles with a size corresponding to stage 6. In comparison to WT follicles, there is no significant change in cell surface, cell height, and therefore cell volume (red bars on Figures 1J, 1K, and S1F). This indicates that the follicular epithelium tightly adjusts its growth to the size of the germline and maintains the shape of its cells. We then asked whether this slower growth of the tissue is due to reduced cell growth and proliferation or alternative mechanisms. In normal conditions, apoptotic *fc* are very rarely observed (Figure S1G). Likewise, we did not observe more cleaved caspase-3 positive cells when the germline is mutant for *Akt* or when *Tor* germline knockdown is induced from stage 3 (Figures 1F and S1G). Moreover, we never observed delaminating cells from these follicles. Observation of an ovariole in which most of the germline cysts are mutant for *Akt* reveals that although *fc* still divide in the youngest follicles, proliferation stops rapidly (Figure 1G). Moreover, the number of dividing cells is greatly reduced in germline RNAi against *Tor* compared with WT in follicles with a length that normally corresponds to stages 5 and 6 (Figure 1L). Together, these data indicate that the follicular epithelium size can precisely adjust to that of the germline by a reduction in cell growth and proliferation.

We performed the reverse experiment by accelerating germline growth with mutants of the tuberous sclerosis complex *Tsc1* and *Tsc2*. In both cases, mutant follicles become as big or even slightly bigger than the next older WT one, although the phenotype is not as dramatic as when the growth of somatic cells is genetically increased (Figure 1H). However, when follicles bud from the germarium, *Tsc* mutant cysts already have the size of a WT stage 4, and it is therefore unclear whether these mutants can promote faster growth of the germline cyst after follicle formation. We therefore induced RNAi against *Tsc1* in the germline after follicle formation. However, we were unable to detect a significant increase in cell proliferation (green bar on Figure 1L). Moreover, cells tend to slightly increase their planar cell area and to reduce cell height, keeping the cell volume fairly constant (green bars on Figures 1J, 1K, and S1F). Thus, the cells are stretched to some extent (Figure S1H). We also observed that germline RNAi against *Tsc1* frequently produces holes in the epithelium (Figure 1I). These results suggest that accelerating germline cell growth does not induce a detectable increase in somatic growth and, as a consequence, causes a mechanical tension that can lead to a slight stretching and a continuity breakdown of the follicular epithelium.

(C) Large somatic *Pten*^{dj189} clones accelerate germline growth.

(D) Ovariole with young *Akt*^{d3} germline clone just after germarium (left) and older germline clone (right), showing reduced growth.

(E) Part of an ovariole with an *Akt*^{d3} germline clone and stained for Cora (red and F').

(F) Whole z-projection showing the absence of Cas3 (red) staining in *fc* surrounding an *Akt*^{d3} mutant germline clone (asterisk). Arrow points to a Cas3 positive cell on a WT follicle.

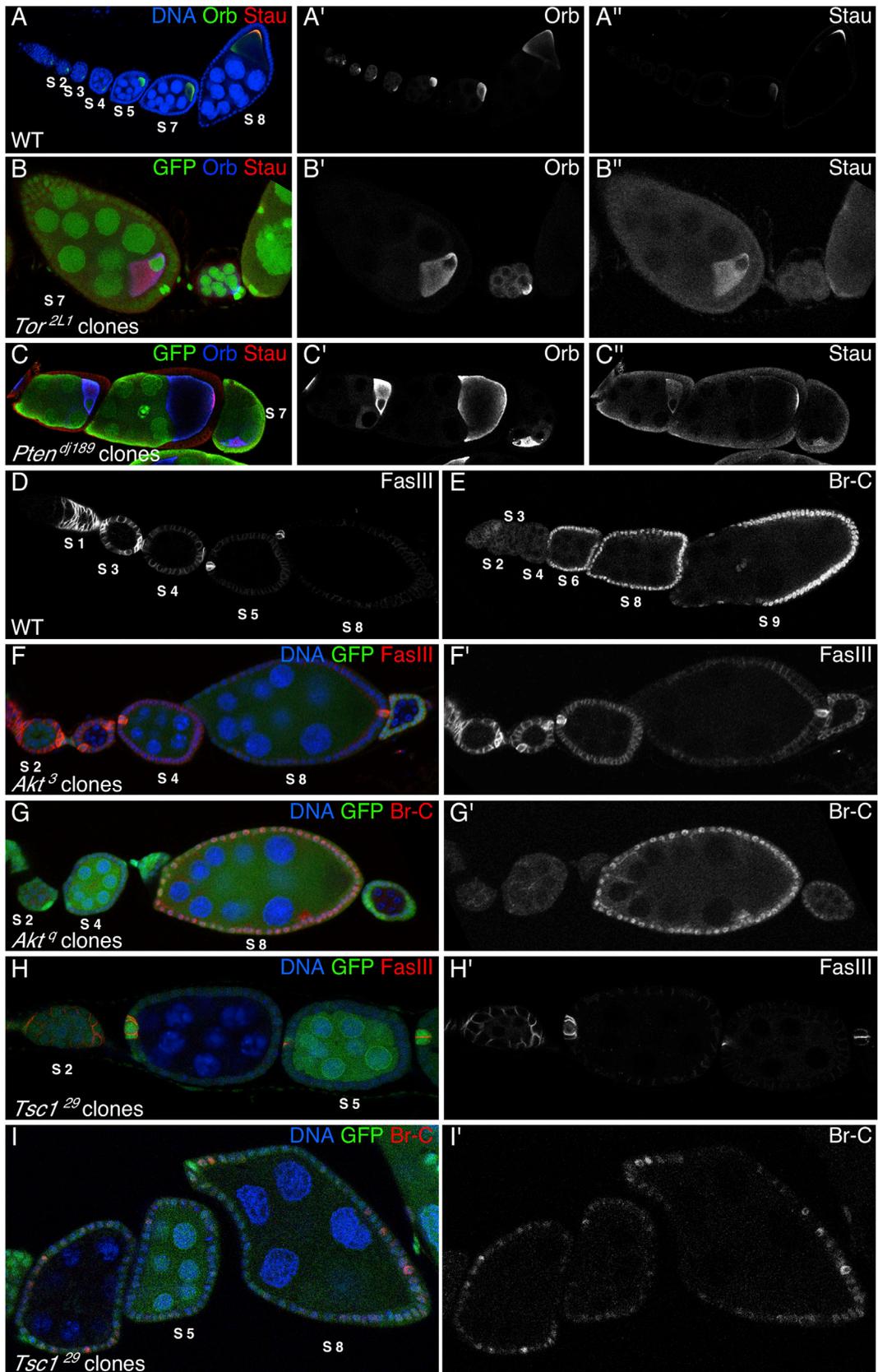
(G) Whole z-projection showing the absence of pH3 (red) staining in *fc* surrounding older *Akt*^{d3} mutant germline clones (asterisks).

(F and G) The z-projection allows seeing all the wild-type *fc* surrounding the mutant clones but masks the absence of GFP in the clones.

(H) Part of an ovariole stained for pH3 (red) and for Cora (red and H') with two *Tsc1*^{d29} germline clones.

(I) Example of a hole in the epithelium induced by germline *Tsc1* RNAi.

(J–L) Quantification of somatic cell area (J), cell height (K), and number of mitotic cells (L) in control conditions or germline RNAi against the indicated genes and for follicles of a length corresponding to a stage 5 or stage 6 (*p < 0.01, **p < 0.001).



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Nonetheless, these data show that the germline is able to control somatic growth, though the ability of somatic cells to grow faster may be limiting. It therefore appears that growth coordination between the soma and the germline is ensured through a bidirectional intrinsic communication.

Non-Cell-Autonomous Effect of Growth on Differentiation Timing

We then asked whether affecting the growth rate of a tissue could non-cell-autonomously affect the developmental timing of the adjacent tissue, using developmental markers and morphological criteria. For the germline we used oo18-RNA binding protein (Orb) and Staufen (Stau) (Lantz et al., 1994; St Johnston et al., 1991; Figures 2A' and 2A''). We also took into account the size of the oocyte compared with the total size of the germline cyst, which progressively increases during follicle development. Somatic clones for *tor* reducing germline growth also induce a delay in oocyte expansion, in Orb relocalization, and in Stau expression (Figure 2B). Strikingly, when *fc* mutant for *Pten* induce faster germline growth, they also induce its faster development. For instance, if the faster growing follicle has the size of a putative stage 9, it also displays all the developmental germline criteria of this stage: oocyte size starts to massively increase compared with nurse cells, Orb is mainly relocalized to the anterior corners of the oocyte, whereas Stau accumulates at the posterior pole (Figure 2C). Thus, somatic growth rate is able to control germline developmental timing.

To observe the effect of the reverse experiment (i.e., whether genetic manipulation of the germline growth affects follicular cell differentiation) somatic developmental markers as Fasciclin III (FasIII) and Broad Complex (Br-C) were used (Ruohola et al., 1991; Buszczak et al., 1999; Figures 2D and 2E). By comparing with anterior and younger WT follicles, we observed that reducing germline growth rate results in maintenance of high FasIII level and an absence of Br-C expression (Figures 2F and 2G). Nonetheless, Br-C expression, although delayed by a slower germline growth, can be expressed at normal levels in a follicle that has not reached the size of a WT stage 5 follicle (Figure S2; Maines et al., 2004). This indicates that somatic differentiation is strongly delayed by reduced germline growth but not completely blocked. Even so, morphological observation of such follicles never reveals the presence of border cells, stretched cells, or columnar cells, which normally appears at stage 9. When the germline is mutated for *Tsc1*, FasIII and Br-C disappear and appear slightly earlier than in a WT follicle, respectively (Figures 2H and 2I). Typically, in the case of Br-C this can be observed by the fact that a mutant follicle usually shows similar intensity staining to the next older one, even if the latter is a stage 5, when its expression normally begins. In this respect, these differentiation defects are highly reminiscent of the growth defects previously

observed. Together, these data indicate that follicular cell differentiation timing is strongly influenced by germline growth rate. Thus, growth coordination also affects differentiation in a bidirectional manner.

TORC1 and PI3K Pathways Cell-Autonomously Influence Differentiation Timing

We hypothesized that modulation of growth signals was responsible for the coordination revealed in all these experiments. If so, mutations affecting the pathway relaying these signals should cell-autonomously reproduce the effect of the coordination on developmental timing. Therefore, we conducted a “pathway candidate” approach by testing whether small mutant clones would affect expression of somatic developmental markers. The pathways that we tested are EGFR-Ras, Hippo, Insulin-PI3K, and TORC1.

The EGFR-Ras pathway is well known for its patterning function in *fc* but has not been described as affecting follicle growth (Horne-Badovinac and Bilder, 2005). Nonetheless, this pathway is involved earlier during gonad development in coordinating the number of primordial germ cells with the number of somatic cells (Gilboa and Lehmann, 2006). We saw no significant effect of *ras* mutation on FasIII expression level suggesting that this pathway is not involved in the coordination process (Figure S3A). It has also been shown that the Hippo pathway plays a role in a specific subpopulation of *fc* (Meignin et al., 2007; Polesello and Tapon, 2007). Even so, the complexity of the events controlling Yorkie activity (homolog of YAP) may have masked a more general function, and we therefore looked at *yorkie* mutant clones (Yu and Guan, 2013). We found, for instance, that Br-C was either not affected or expressed slightly sooner than in WT cells, thus not correlated with *yorkie* impact on cell proliferation (Figure S3B). We concluded that the Hippo pathway is not required for the coordination.

We next tested whether FasIII expression was affected by small clones mutant for *Pten* and *Akt*, leading to a gain and a loss of activity of the PI3K pathway, respectively. In both cases, we observed a clear effect, with a FasIII downregulation in *Pten* (in 8 out of 10 clones between stages 4 and 8) and an upregulation in *Akt* mutant cells (in 3 out of 4 clones) compared with surrounding WT cells (Figures 3A and 3C). Similarly, Br-C expression also appears sooner in *Pten* mutant cells and later in *Akt* clones (Figures 3B and 3D). Thus, the PI3K pathway strongly influences the timing of differentiation in somatic cells in a cell-autonomous manner. Recent data indicate that Tor Complex 1 (TORC1) is an important effector of PI3K pathway in the ovary (Pallares-Cartes et al., 2012). *Tor* null or strong hypomorphic (*Tor*^{2L1}) mutations also affect FasIII expression (in 4 out of 4 and in 9 out of 10 clones, respectively) (Figure S3C). However, this mutation does not allow discrimination between the

Figure 2. Tissue Differentiation Is Controlled by the Growth of the Adjacent Tissue

(A) WT ovariole stained for DNA (blue) Orb (green and A') and Stau (red and A'').
 (B) Effect of large somatic *Tor*^{2L1} clone (not green, right) on Orb (blue and B') and Stau (red and B'') compared with a younger WT follicle (left).
 (C) Effect of two large somatic *Pten*^{dl189} clones (not green, left and middle) on Orb (blue and C') and Stau (red and C'') compared with an older WT follicle (right).
 (D and E) WT ovarioles stained for FasIII (D) and Br-C (E).
 (F and G) *Akt* mutant germline clones at the posterior ends of the ovarioles (not green) stained for DNA (blue) and FasIII (F, red and F') or Br-C (G, red and G').
 (H and I) *Tsc1*²⁹ mutant germline clones (not green) stained for DNA (blue) and FasIII (H, red and H') or Br-C (I, red and I').

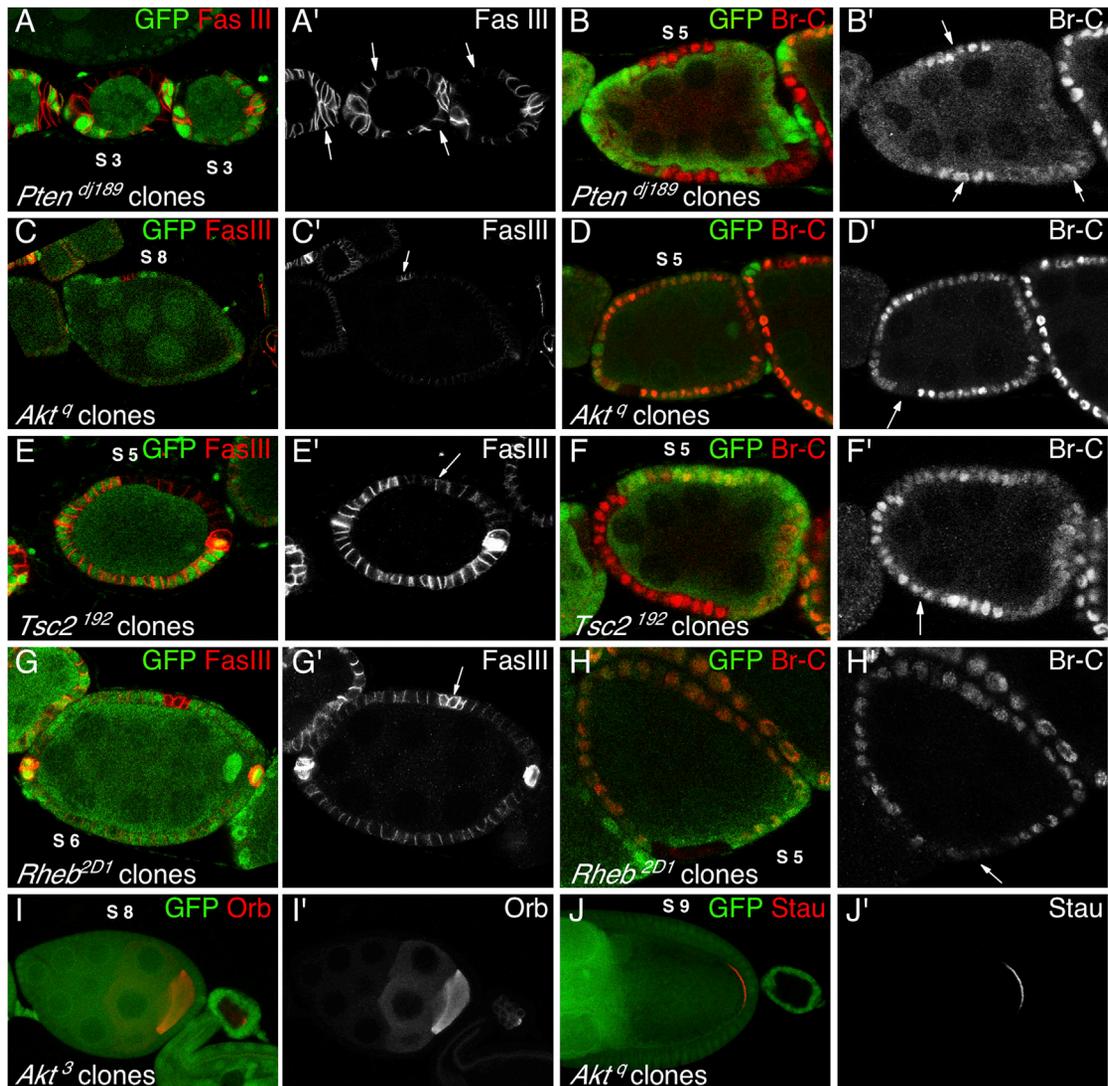


Figure 3. PI3K and TORC1 Influence Cell Differentiation Timing

(A and B) Small *Pten*^{dj189} clones stained for FasIII (red and A') or Br-C (red and B').

(C and D) Small *Akt*^q clones stained for FasIII (red and C') or Br-C (red and D').

(E and F) Small *Tsc2*¹⁹² clones stained for FasIII (red and E') or Br-C (red and F').

(G and H) Small *Rheb*^{2D1} clones stained for FasIII (red and G') or Br-C (red and H').

(I) Ovary containing a *Akt*³ germline clone and stained for Orb (I' and red).

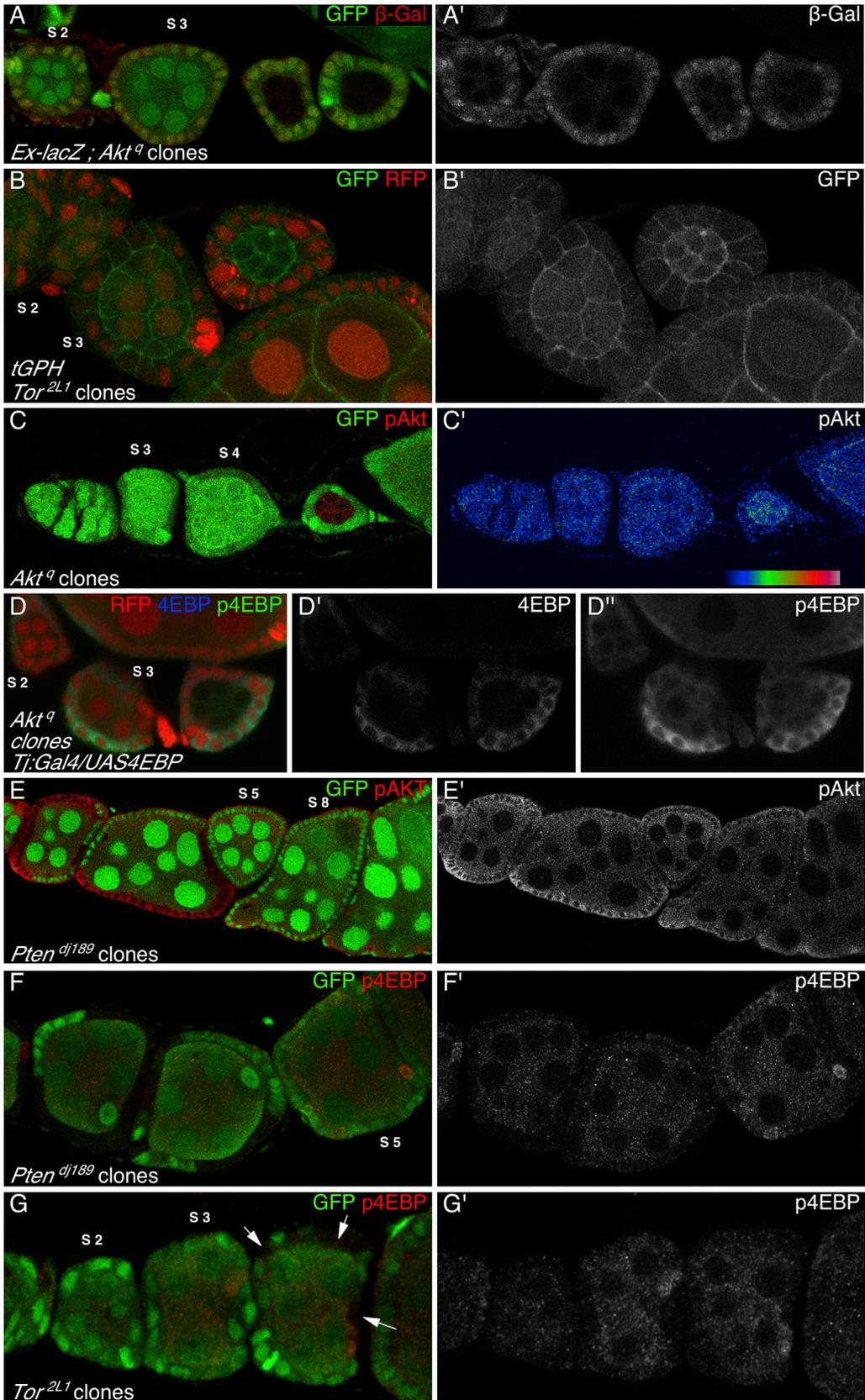
(J) Ovary containing a *Akt*^q germline clone and stained for Stau (J' and red).

Mutant cells are marked by the absence of GFP (green) (A–J). Arrows indicate position of the clones (A–H).

activity of TORC1 and TORC2, another growth regulating complex containing Tor protein (Laplanche and Sabatini, 2012). Nonetheless, mutant clones for *sin1*^{e3756}, a specific component of TORC2, do not affect FasIII expression, suggesting that Tor effect relies on TORC1 activity (Figure S3D). We tried to confirm the central role of TORC1 in this process by looking at mutations affecting upstream negative or positive specific regulators of TORC1, *Tsc2* and *Rheb*, respectively. Both affect developmental timing cell-autonomously as can be observed by the expression level of FasIII and Br-C (Figures 3E–3H). However, blocking TORC1 activity delays, but does not completely block, the

appearance of the Br-C marker (Figure S3E). Nonetheless, TORC1 activity ensures synchronization cell-autonomously between growth and differentiation in the soma.

We also performed similar experiments in the germline, looking at germline differentiation markers when mutated for *Akt*. We observed that Orb is expressed, but at low level, and is localized as in very young stages, even when the mutant follicle is posterior to a stage 8 (Figure 3I). Stau expression is blocked in comparison with younger WT follicles (Figure 3J). Therefore, mutants affecting PI3K activity also influence the developmental timing in the germline in a cell-autonomous manner. Therefore,



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both germline and soma differentiation show a tight synchronization with growth, and this coordination can be achieved cell-autonomously.

TORC1, PI3K, and Hippo Are Not Regulated by the Growth of the Adjacent Tissue

If a specific growth signaling pathway regulates the coordination, then its activity should respond to the effect of coordination between tissues. Therefore, we looked in fc for activity readout of these pathways when germline growth was genetically manipulated. EGFR-ras transcriptional readout is already described as expressed in specific populations of fc and at specific stages, suggesting that it cannot be generally controlled in all cells by the coordination process (Ghiglione et al., 1999). The expression of Expanded-lacZ, a transgene used as a reporter for Hippo pathway activity, is not modified in a follicle with a germline clone for *Akt* compared with a WT follicle, confirming that this pathway is not involved in the coordination between the germline and the soma (Figure 4A).

We then looked at a reporter of Insulin/PI3K pathway activity; i.e., at the cortical localization of a GFP reporter (tGPH) that binds phosphatidylinositol phosphate 3 (PIP3), which is the direct product of PI3K activity. Slower germline growth clones did not modify the pattern of this reporter in fc when compared with WT follicles of the same size (Figure 4B). We also looked at the phosphorylation level of Akt on Ser505, a classical downstream readout for this pathway, and did not observe any change in its level (Figures 4C and S4A). So although PI3K pathway modulation can mimic coordination, its activity is not regulated by this process in somatic cells.

We then tried to find a reliable readout for TORC1 activity by looking at phosphorylation of a direct target. Phospho-4EBP antibodies have been shown to work in immunofluorescence in *Drosophila* (Cheng et al., 2011). However, 4EBP is not expressed in fc (Figure S4B). We therefore induced ectopic clonal 4EBP overexpression in the fc, and it clearly allows detection of the protein and its phosphorylated form (Figures S4C and S4D). We then produced germline *Akt* clones in flies where 4EBP is ectopically expressed in all fc with Tj:Gal4, and we compared the levels of phospho4EBP and total 4EBP. We did not observe a change of phospho4EBP in somatic cells when the germline is mutant for *Akt* (Figure 4D). From this experiment, we concluded that TORC1 activity is not controlled in the soma by the rate of germline growth.

Finally, we performed reciprocal experiments for the germline by looking at pAkt and p4EBP when somatic clones for *Pten* or *Tor* influenced germline growth. However, none of them shows a difference compared with surrounding WT follicles (Figures 4E to 4G), whereas cell autonomous effect on these markers in

the germline can be easily spotted (Figures 4C, S4E, and S4F). Therefore, germline growth control by the somatic cells is not achieved by any of these canonical growth pathways.

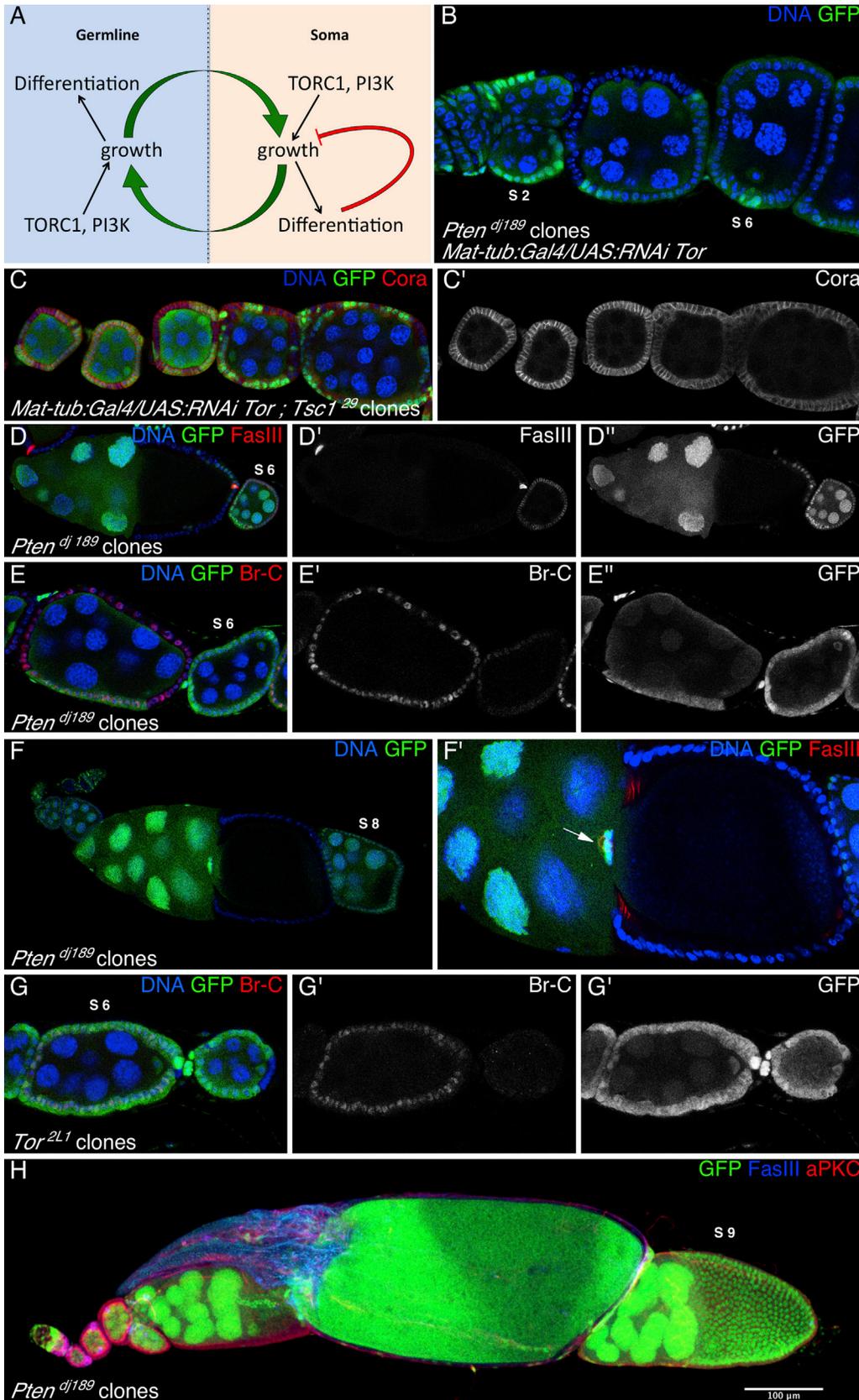
Coordination Allows Robust Egg Development

From all these experiments, we can propose a simple model in which growth of one tissue controls growth and the developmental program in the adjacent tissue (Figure 5A). A simple prediction of this model is that growth activity must be affected in both tissues to block the coordination, because if it is affected in a single tissue then the second will adjust its growth accordingly, as we have generally observed in all our experiments so far. To further test the robustness of this mechanism, we generated somatic *Pten* or *Tsc1* clones in ovarioles where *Tor* knock-down was induced in the germline. Under these conditions, *Pten* or *Tsc1* mutant clones do not induce faster germline growth as on a WT germline (Figures 5B and 5C). Therefore, in this experiment we blocked the ability of the germline to respond to faster somatic growth. However, the follicular epithelium shows no evidence of abnormal cell shape or cell overproliferation despite the presence of large *Pten* or *Tsc1* clones. This indicates that fc in which the PI3K or TORC1 pathway is overactivated do not overcome their growth control by the germline and are therefore still responsive to the coordination process. First, this provides genetic confirmation that these pathways are not directly involved in the growth coordination of the soma with the germline. Second, it also illustrates the developmental robustness provided by this coordination.

Another prediction of such a model would be that in the case of a large mutant somatic clone influencing germline growth, this growth defect should influence back developmental timing of the WT somatic cells. In our previous experiments, we observed that small *Pten* somatic clones that do not induce visible effects on the germline do not influence FasIII and Br-C expression levels in WT somatic cells, even in cells that directly contact the clone. However, *Pten* somatic clones large enough to increase germline growth influence, as predicted, somatic expression of FasIII or Br-C in all fc, independently of their mutant or WT genotype (Figures 5D and 5E). Moreover, we observed that WT border cells migrate properly according to the apparent stage of the follicle (Figures 5F and 2C) (Spradling, 1993). Similarly, somatic clones for *Tor* influence expression of Br-C when they have an effect on germline growth (Figure 5G). This non-cell-autonomous effect of soma on soma is observed only when an effect on germline growth is also seen. Moreover, when it occurs, it is never limited to the WT somatic cells close to the mutant clone. These two points argue against a direct signal coming from these mutant cells toward somatic WT cells, but strongly suggest that this effect is dependent on a relay coming from the germline. It

Figure 4. No Regulation of PI3K, TORC1, and Hippo Pathways by the Adjacent Tissue

- (A) Ovariole expressing Ex:LacZ with *Akt^{fl}* germline clones (not green) and stained for DNA (blue) and β -galactosidase (red and A').
 (B) Ovariole expressing tGPH (green) with an *Akt^{fl}* germline clone (not red).
 (C) pAkt staining of an ovariole containing an *Akt^{fl}* germline clone (not green). (C') A color intensity scale is used. Note that it is normal that pAkt is high in the mutant cells because of the nature of *Akt^{fl}* allele as explained in Kockel et al. (2010).
 (D) Ovariole with ectopic expression of 4EBP in fc and with an *Akt^{fl}* germline clone and stained for 4EBP (blue and D') and p4EBP (red and D'').
 (E) Ovarioles stained with pAkt (red and E') containing large *Pten^{dl189}* somatic clones with an impact on germline growth.
 (F and G) Ovarioles stained with p4EBP (red and F', G') containing large *Pten^{dl189}* (F) or *Tor^{2L1}* (G) somatic clones with an impact on germline growth.



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also indicates that faster germline growth promotes faster differentiation of subpopulations of fc.

Finally, the scheme that we propose would be incomplete without a way of stopping follicle growth. In WT conditions it is not yet known how final egg size is controlled, but it seems likely that it depends on the differentiation program taking place in each follicle. Therefore, growth controls developmental timing that in turn might be able to block growth when a follicle reaches its final size. We observed that follicles containing somatic *Pten* clones can give rise to a mature egg with a normal morphological aspect in the middle of an ovariole indicating that all the developmental steps were accomplished faster but correctly (Figure 5H). It therefore suggests that all aspects of somatic and germline development are influenced by the coordination process. Particularly, these eggs retain an overall normal size, indicating that the mutant clones do not drive uncontrolled growth and that a developmental program is still able to normally block follicle growth “on time.” This observation is in favor of a negative feedback of the developmental program on follicle growth that ensures their synchronization until a mature egg is formed.

DISCUSSION

Several Levels of Coordination Provide Robustness

From the work that we have presented in this article, several main conclusions can be drawn. First, in each follicle, growth is intrinsically coordinated between the two tissues. Second, this growth control tends to optimize cell shape in the epithelium. This is likely to be representative of the development of many epithelia where cell shape must be maintained because it is essential for the function of the tissue. In the third place, growth control has a very important impact on differentiation timing in each tissue. Furthermore, several growth pathways can cell-autonomously influence differentiation rate but are not regulated by the adjacent tissue, indicating that they only respond to extrinsic cues. Finally, as a whole, this study reveals the robustness of the spatiotemporal pattern allowing the production of mature eggs with a normal shape and a normal size. At least two examples based on *Pten* somatic clones can illustrate this robustness. WT border cells migrate perfectly “on time” in a follicle in which mutant somatic cells have induced a faster germline development. Second, a WT looking mature egg can be found in the middle of an ovariole, suggesting that all developmental steps have been faster but correctly orchestrated. This robustness probably reflects the fact that final egg size is constant, that most of the developmental steps have to occur at a specific size, and that differentiation is able to block growth when the definitive egg size is reached. These observations raise

the question as to how the differentiation program regulates growth and especially growth arrest in each follicle.

Our results indicate a two-way communication between the germline and the soma to ensure their coordination. We also observed that somatic cells can influence other somatic cells but, importantly, that such an effect depends on the relay of the germ cells. This result suggests that coordination is achieved by different signals depending on the tissue. The soma and germline could communicate via the secretion of growth factors controlling the adjacent tissue, though we excluded obvious candidates. An alternative explanation would be that, as it is proposed in mammals, the two tissues are interdependent for specific metabolites, although it would be independent of TORC1, a classical sensor of metabolic activity (Su et al., 2009). Finally, an attractive hypothesis would be that growth regulation between the soma and the germline depends on a mechanical steady state. Germline growth creates a tension on the fc, leading to the proposal that this tension could trigger epithelial growth (Wang and Riechmann, 2007). If so, it would also mean that fc provide a mechanical strain limiting germline growth. The mechanical control of growth in epithelial cells is usually devoted to the Hippo pathway, which is not involved here (Halder et al., 2012, Rauskolb et al., 2014). Thus, our work does not allow favoring one or the other of these nonexclusive mechanisms.

Altogether, our results highlight several dimensions of coordination between cell growth, cell shape, and cell identity and all this between two distinct tissues. These different functional links offer a highly robust program in space and time. The relevance for such robustness has been very recently highlighted because it probably confers the reproducibility on embryonic development (Petkova et al., 2014). Since usual pathways controlling growth are not involved in this two-way communication, this multidimensional coordination will be a useful framework for identifying molecular actors ensuring tissue homeostasis in the recurrent context of the development of two adjacent tissues.

EXPERIMENTAL PROCEDURES

Genetics

Detailed genotype and heat-shock conditions corresponding to each picture is given in Table S1.

Immunostaining and Imaging

Ovaries were dissected in Schneider medium and subsequently fixed with 4% paraformaldehyde for 20 min before manual separation of the ovarioles. After blocking and permeabilization with PBS with 0.5% BSA and 0.2% Triton X-100 (PBT), ovaries were incubated in PBT containing primary or secondary antibodies at 4°C overnight. Antibodies are described in Table S2. Hoechst was

Figure 5. A Model for Growth Coordination

- (A) Scheme of the mutual control of growth and differentiation between germline and somatic cells.
 (B–H) Mutant cells are marked by the absence of GFP (green).
 (B and C) Large somatic *Pten*^{dj189} (B) or *Tsc1*²⁹ (C) clones do not accelerate germline growth when *Tor* RNAi is induced in the germline. The mutant epithelial cells do not show aberrant proliferation (no multilayers) or shape.
 (D and E) Large somatic *Pten*^{dj189} clones induce premature disappearance of FasIII (D) or appearance of Br-C (E) in WT somatic cells of the same follicle.
 (F and F' higher magnification) Large somatic *Pten*^{dj189} clone inducing premature migration of WT border cells (arrow).
 (G) A large somatic *Tor*^{2L1} clone delaying the appearance of Br-C in WT somatic cells.
 (H) Large somatic *Pten*^{dj189} clone inducing the precocious formation of a mature egg of a normal size.

used to stain DNA and Alexa568-phalloidin for F-actin. A Tyramide Signal Amplification fluorescein kit was used to detect biotinylated α 4EBP1. Images were taken on a Leica SP5 confocal microscope. Cell segmentation and measurements were performed on follicles stained with Cora using Imaris. The cell area was automatically determined after cell segmentation on planar view images using a homemade macro. For each follicle, cell height was measured on six independent positions on a confocal plane including the polar cells. Figures were assembled using ScientiFig (Aigouy and Mirouse, 2013).

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2014.09.035>.

AUTHOR CONTRIBUTIONS

C.V., C.F., and V.M. designed experiments. C.V. and C.F. performed the experiments where somatic growth and germline growth are genetically modified, respectively. P.P and O.B. provided quantification tools. C.V., C.F., and V.M. wrote the article.

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REFERENCES

- Aigouy, B., and Mirouse, V. (2013). ScientiFig: a tool to build publication-ready scientific figures. *Nat. Methods* *10*, 1048.
- Baena-Lopez, L.A., Nojima, H., and Vincent, J.P. (2012). Integration of morphogen signalling within the growth regulatory network. *Curr. Opin. Cell Biol.* *24*, 166–172.
- Buszczak, M., Freeman, M.R., Carlson, J.R., Bender, M., Cooley, L., and Segraves, W.A. (1999). Ecdysone response genes govern egg chamber development during mid-oogenesis in *Drosophila*. *Development* *126*, 4581–4589.
- Cheng, L.Y., Bailey, A.P., Leever, S.J., Ragan, T.J., Driscoll, P.C., and Gould, A.P. (2011). Anaplastic lymphoma kinase spares organ growth during nutrient restriction in *Drosophila*. *Cell* *146*, 435–447.
- Drummond-Barbosa, D., and Spradling, A.C. (2001). Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* *231*, 265–278.
- Ghiglione, C., Carraway, K.L., 3rd, Amundadottir, L.T., Boswell, R.E., Perrimon, N., and Duffy, J.B. (1999). The transmembrane molecule kekkon 1 acts in a feedback loop to negatively regulate the activity of the *Drosophila* EGF receptor during oogenesis. *Cell* *96*, 847–856.
- Gilboa, L., and Lehmann, R. (2006). Soma-germline interactions coordinate homeostasis and growth in the *Drosophila* gonad. *Nature* *443*, 97–100.
- Halder, G., Dupont, S., and Piccolo, S. (2012). Transduction of mechanical and cytoskeletal cues by YAP and TAZ. *Nat. Rev. Mol. Cell Biol.* *13*, 591–600.
- Horne-Badovinac, S., and Bilder, D. (2005). Mass transit: epithelial morphogenesis in the *Drosophila* egg chamber. *Dev. Dyn.* *232*, 559–574.
- Kockel, L., Kerr, K.S., Melnick, M., Brückner, K., Hebrok, M., and Perrimon, N. (2010). Dynamic switch of negative feedback regulation in *Drosophila* Akt-TOR signaling. *PLoS Genet.* *6*, e1000990.
- LaFever, L., and Drummond-Barbosa, D. (2005). Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*. *Science* *309*, 1071–1073.
- LaFever, L., Feoktistov, A., Hsu, H.J., and Drummond-Barbosa, D. (2010). Specific roles of Target of rapamycin in the control of stem cells and their progeny in the *Drosophila* ovary. *Development* *137*, 2117–2126.
- Lander, A.D. (2011). Pattern, growth, and control. *Cell* *144*, 955–969.
- Lantz, V., Chang, J.S., Horabin, J.I., Bopp, D., and Schedl, P. (1994). The *Drosophila* orb RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. *Genes Dev.* *8*, 598–613.
- Laplanche, M., and Sabatini, D.M. (2012). mTOR signaling in growth control and disease. *Cell* *149*, 274–293.
- Lloyd, A.C. (2013). The regulation of cell size. *Cell* *154*, 1194–1205.
- Maines, J.Z., Stevens, L.M., Tong, X., and Stein, D. (2004). *Drosophila* dMyc is required for ovary cell growth and endoreplication. *Development* *131*, 775–786.
- Meignin, C., Alvarez-Garcia, I., Davis, I., and Palacios, I.M. (2007). The salvador-warts-hippo pathway is required for epithelial proliferation and axis specification in *Drosophila*. *Curr. Biol.* *17*, 1871–1878.
- Pallares-Cartes, C., Cakan-Akdogan, G., and Teleman, A.A. (2012). Tissue-specific coupling between insulin/IGF and TORC1 signaling via PRAS40 in *Drosophila*. *Dev. Cell* *22*, 172–182.
- Petkova, M.D., Little, S.C., Liu, F., and Gregor, T. (2014). Maternal origins of developmental reproducibility. *Curr. Biol.* *24*, 1283–1288.
- Polesello, C., and Tapon, N. (2007). Salvador-warts-hippo signaling promotes *Drosophila* posterior follicle cell maturation downstream of notch. *Curr. Biol.* *17*, 1864–1870.
- Rauskolb, C., Sun, S., Sun, G., Pan, Y., and Irvine, K.D. (2014). Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex. *Cell* *158*, 143–156.
- Ruohola, H., Bremer, K.A., Baker, D., Swedlow, J.R., Jan, L.Y., and Jan, Y.N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* *66*, 433–449.
- Spradling, A.C. (1993). Developmental genetics of oogenesis. In *The Development of Drosophila Melanogaster*, M. Bate and A. Martinez-Arias, eds. (Plainview: Cold Spring Harbor Laboratory Press), pp. 1–70.
- St Johnston, D., Beuchle, D., and Nüsslein-Volhard, C. (1991). Staufén, a gene required to localize maternal RNAs in the *Drosophila* egg. *Cell* *66*, 51–63.
- Su, Y.Q., Sugiura, K., and Eppig, J.J. (2009). Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. *Semin. Reprod. Med.* *27*, 32–42.
- Sun, P., Quan, Z., Zhang, B., Wu, T., and Xi, R. (2010). TSC1/2 tumour suppressor complex maintains *Drosophila* germline stem cells by preventing differentiation. *Development* *137*, 2461–2469.
- Wang, Y., and Riechmann, V. (2007). The role of the actomyosin cytoskeleton in coordination of tissue growth during *Drosophila* oogenesis. *Curr. Biol.* *17*, 1349–1355.
- Yu, F.X., and Guan, K.L. (2013). The Hippo pathway: regulators and regulations. *Genes Dev.* *27*, 355–371.

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Supplemental Information

**Tight Coordination of Growth and Differentiation
between Germline and Soma Provides Robustness
for *Drosophila* Egg Development**

Caroline Vachias, Cornelia Fritsch, Pierre Pouchin, Olivier Bardot, and Vincent Mirouse

Inventory of Supplemental Information

- Figures S1 to S4 related to fig 1 to 4
- Supplementary figure legends
- Supplemental tables S1 and S2 related to experimental procedures.

Figure S1

A

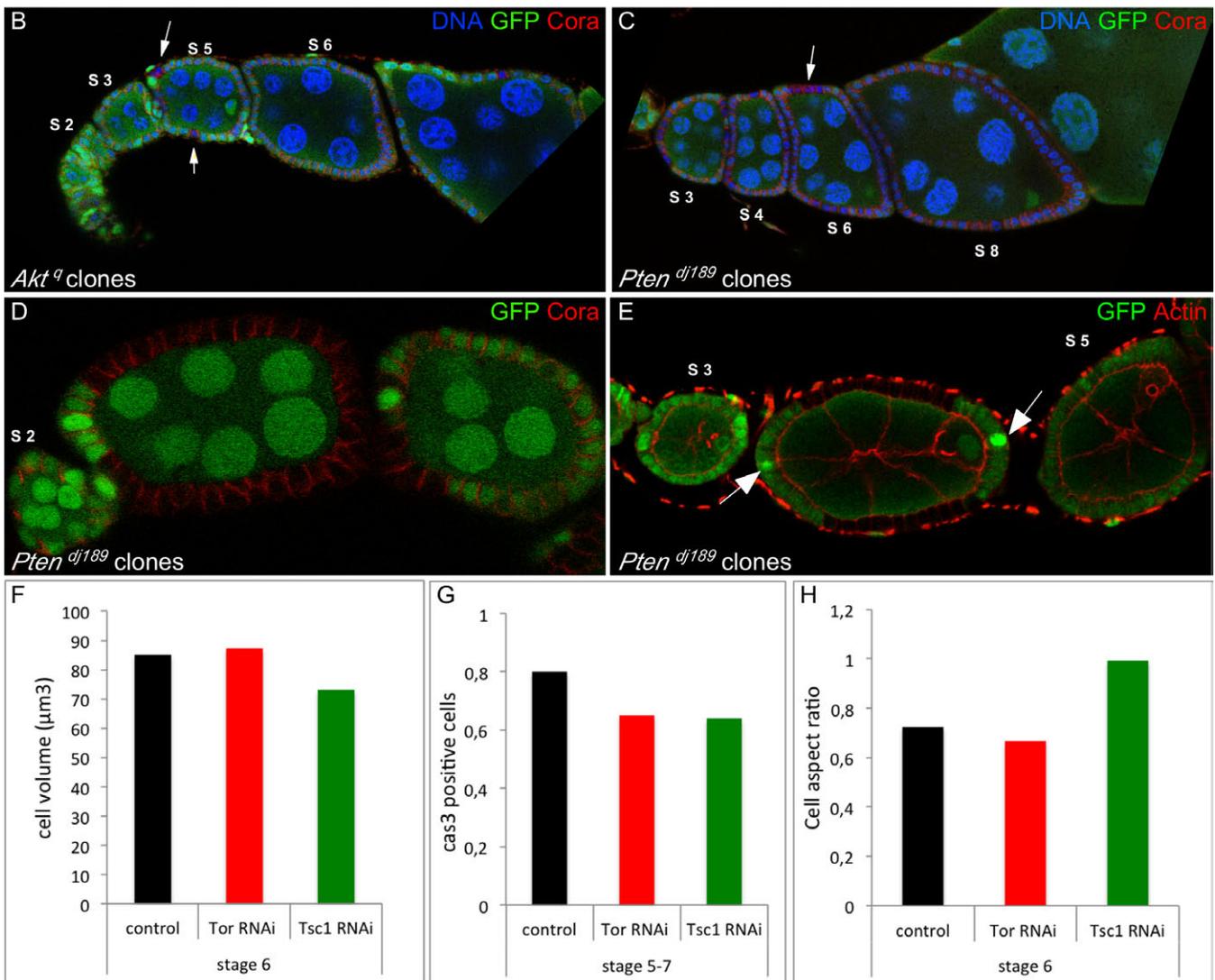
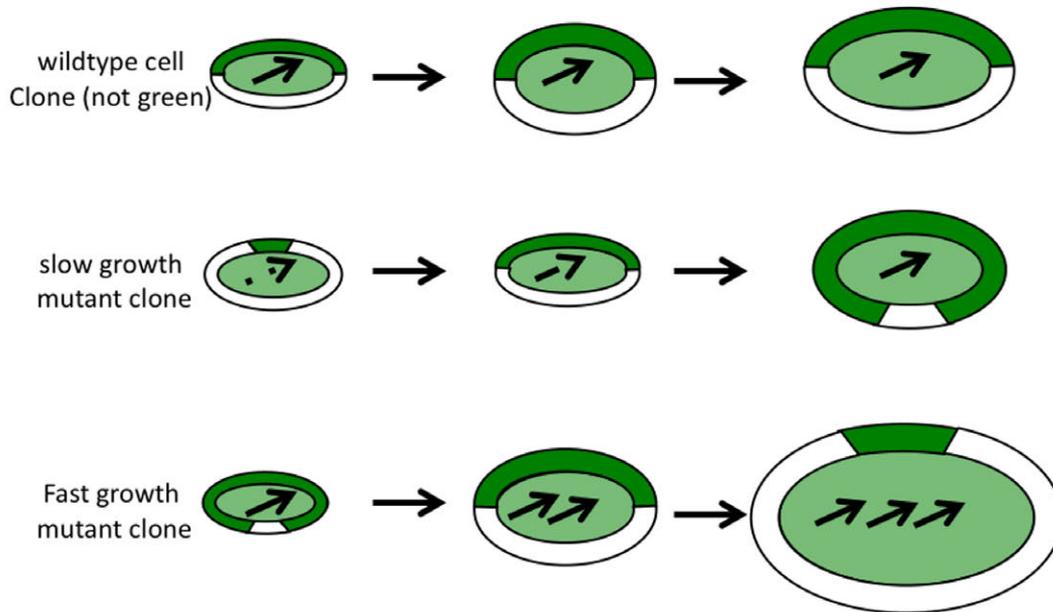


Figure S2

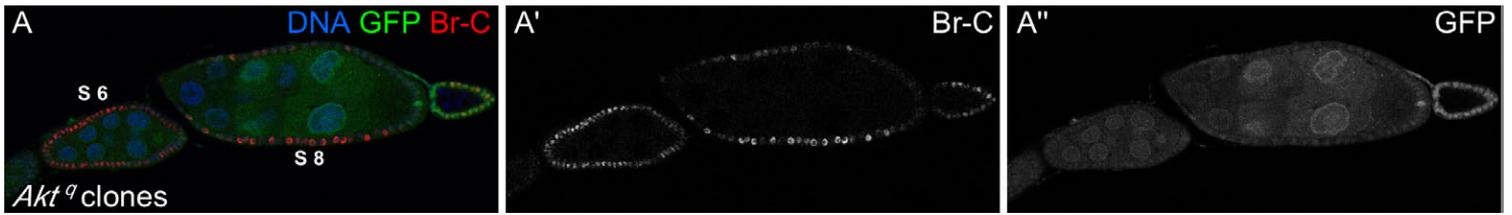


Figure S3

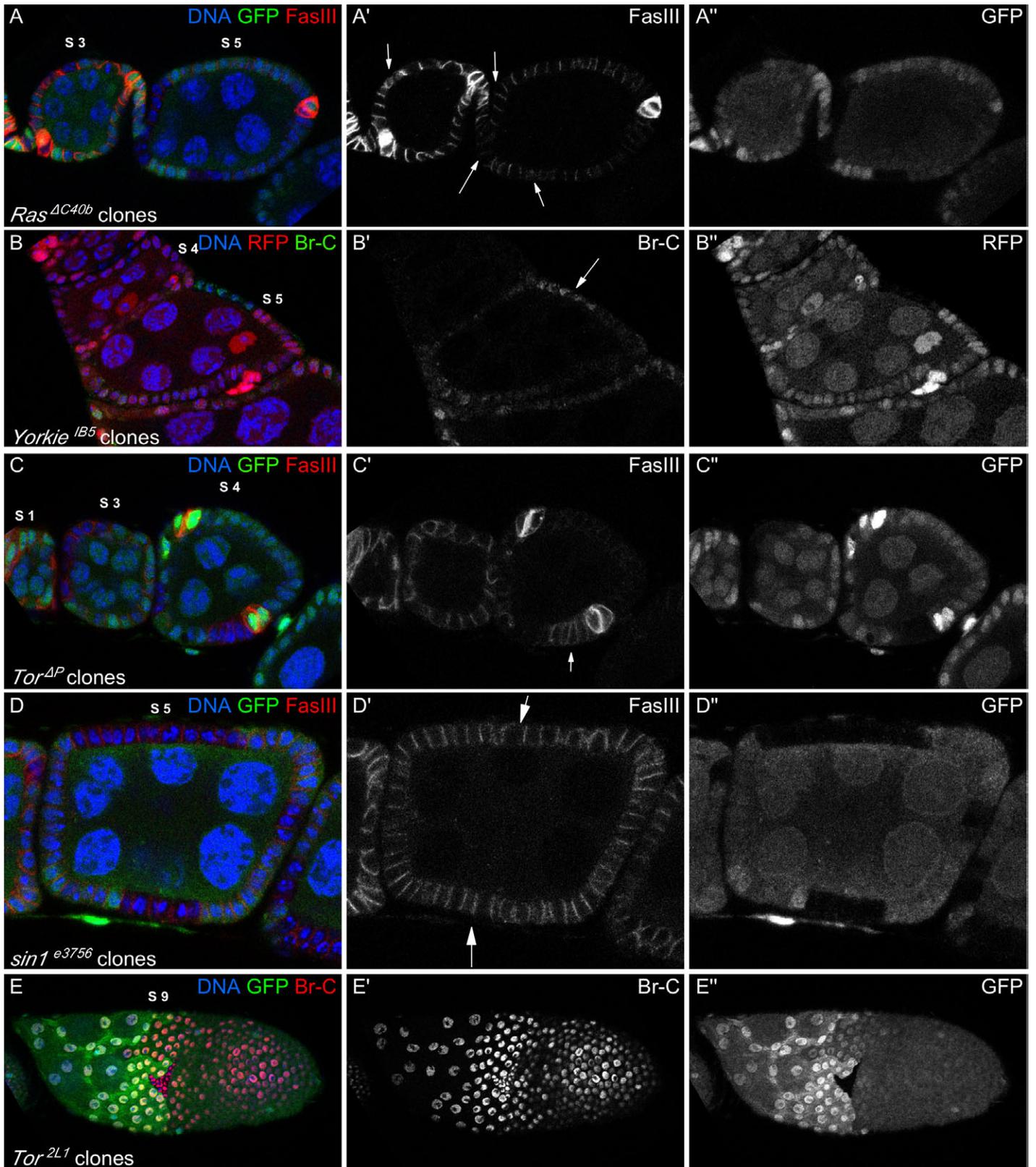
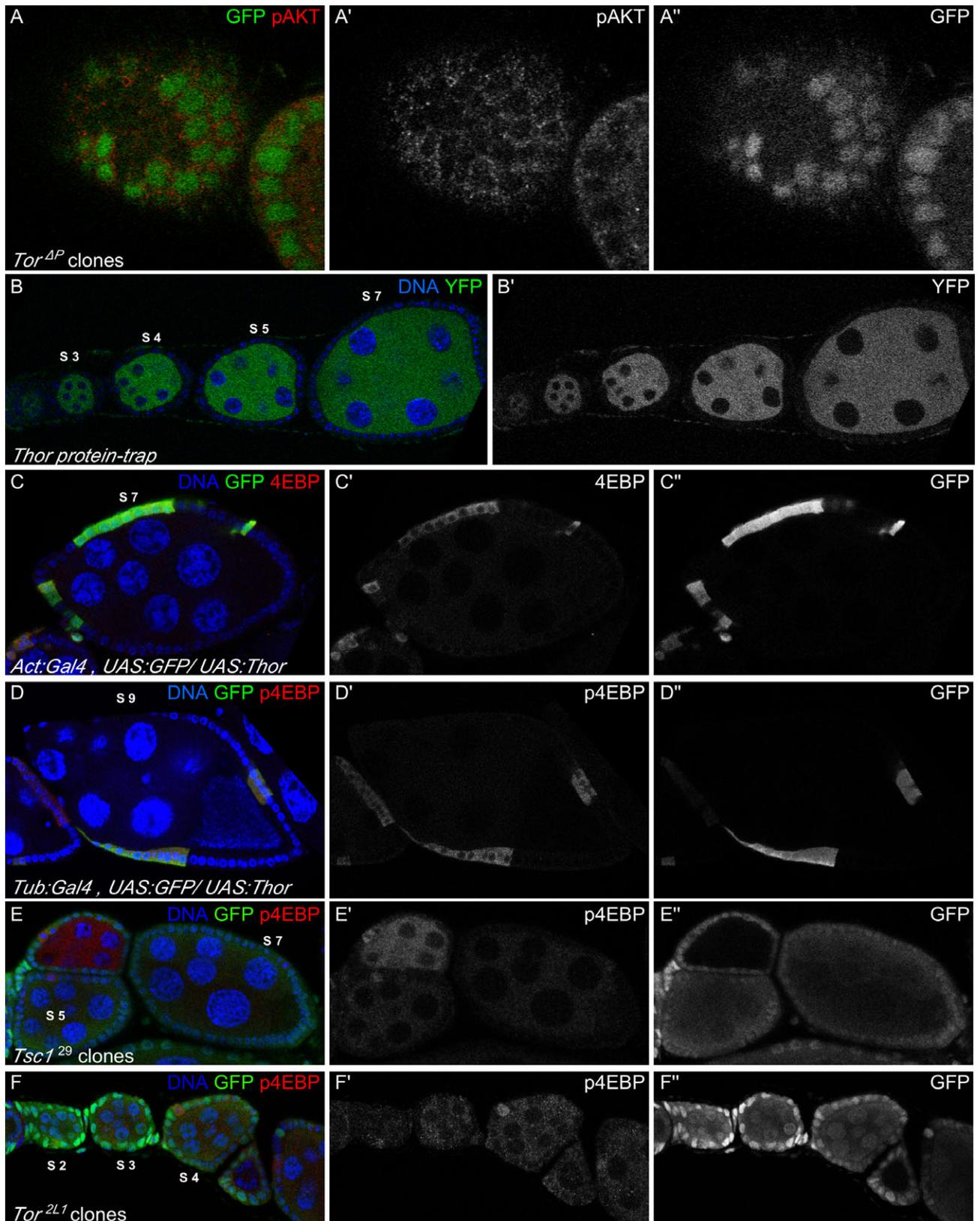


Figure S4



Supplementary figure legends

Figure S1 (related to Figure 1)

- A) Scheme illustrating the kinetics of fast or slow growing mutant clones and their impact on germline growth.
- B) Small *Akt^q* clones (arrow) with no visible effect on germline growth
- C) Small *Pten^{dj189}* clones (arrow) with no visible effect on germline growth
- D) *Pten* effect on germline growth depends on clone size
- E) *Pten^{dj189}* clone that does not contain polar cells (bright green, arrows)
- F) Calculation of follicular cell volume surrounding control, *Tor* or *Tsc1* RNAi germline
- G) Quantification of Cas3 expressing cells per follicle surrounding control, *Tor* or *Tsc1* RNAi germline (n>30 for each genotype)
- H) Calculation of cell stretching by the ratio of area square root over height

Figure S2 (related to Figure 2)

Example of a follicle with a germline *Akt^q* clone (right) that expresses Br-C whereas it should not according to its size.

Figure S3 (related to Figure 3)

A) to G) mutant cells are marked by the absence of GFP (green and right panel). Arrows indicate position of the clones.

- A) Small *ras85D^{ΔC40b}* clones stained for DNA (blue) and FasIII (red and A')
- B) Small *yki^{B5}* clones stained for DNA (blue) and Br-C (red and B')
- C) Small *Tor^{ΔP}* clones stained for DNA (blue) and FasIII (red and C')
- D) Small *Sin1^{e3756}* clones stained for DNA (blue) and FasIII (red and D')
- E) Small *Tor^{2L1}* clones stained for Br-C (red and G')

Figure S4 (related to Figure 4)

- A) Small *Tor^{ΔP}* clones stained for pAkt (red and A')
- B) Thor (4EBP) YFP protein trap expression pattern
- C) and D) Clonal overexpression of Thor marked with GFP (green and right panel) and stained for 4EBP (red in C and C') or p4EBP (red in D and D') and DNA (blue)
- E) and F) Ovarioles containing a germline clone mutant for *Tsc1²⁹* (E) or *Tor^{2L1}* (F) (not green and right panel) and stained for p4EBP (Red and E' and F')

Supplemental table 1: exact genotype and specific conditions for each experiment

"3 HS, 8 days after HS" means a 1 hour heat-shock on three consecutive days, dissection eight days after the first heat-shock. For some mutants several HS were performed to increase the probability to obtain clones

Figure	Genotype	Conditions
Figure 1		
Fig1A	<i>Oregon wild-type strain</i>	
Fig1B	<i>y,w,HS:flp122/+; FRT40A,Tor^{2L1}/ FRT40A, ubi:GFP</i>	1HS, 8 days after HS
Fig1C	<i>y,w,HS:flp122/+; FRT40A, Pten^{dj189}/ FRT40A, ubi:GFP</i>	1HS, 5 days after HS
Fig1D,G	<i>y,w,HS:flp122/+ ;; FRT82B, Akt^q/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Fig1E,F	<i>y,w,HS:flp122/+ ;; FRT82B, Akt³/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Fig1H	<i>y,w,HS:flp122/+ ;; FRT82B, Tsc1²⁹/ FRT82B, ubi:GFP</i>	1HS, 7 days after HS
Fig1I	<i>Mattub-Gal4/+; UAS-Tsc1^{GL00012}/+</i>	Crosses were raised at 18°C and adult progeny were put 36 hours at 25°C
Fig1J-L	<i>Mattub-Gal4/+ (control)</i>	
	<i>Mattub-Gal4/+; UAS-Tsc1^{GL00012}/+</i>	
	<i>Mattub-Gal4/+; UAS-Tor^{GL00156}/+</i>	
Figure 2		
Fig2A,D,E	<i>Oregon wild-type strain</i>	
Fig2B	<i>y,w,HS:flp122/+; FRT40A,Tor^{2L1}/ FRT40A, ubi:GFP</i>	2HS, 8 days after HS
Fig2C	<i>y,w,HS:flp122/+; FRT40A,Pten^{dj189}/ FRT40A, ubi:GFP</i>	1HS, 6 days after HS
Fig2F	<i>y,w,HS:flp122/+;; FRT82B,Akt³/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Fig2G	<i>y,w,HS:flp122/+;; FRT82B,Akt^q/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Fig2H,I	<i>y,w,HS:flp122/+;; FRT82B,Tsc1²⁹/ FRT82B, ubi:GFP</i>	1HS, 6 days after HS
Figure 3		
Fig3A, B	<i>y,w,HS:flp122/+; FRT40A,Pten^{dj189}/ FRT40A, ubi:GFP</i>	1HS, 3 days after HS
Fig3C, D	<i>y,w,HS:flp122/+;; FRT82B, Akt^q/ FRT82B, ubi:GFP</i>	1HS, 3 days after HS
Fig3E, F	<i>y,w,HS:flp122/+;; FRT80B, Tsc2¹⁹²/ FRT80B, ubi:GFP</i>	1HS, 3 days after HS
Fig3G, H	<i>y,w,HS:flp122/+;; FRT82B, Rheb^{2D1}/ FRT82B, ubi:GFP</i>	1HS, 7 days after HS
Fig3I	<i>y,w,HS:flp122/+;; FRT82B,Akt³/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Fig3J	<i>y,w,HS:flp122/+;; FRT82B,Akt^q/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Figure 4		
Fig4A	<i>y,w,HS:flp122/+; expanded-lacZ⁶⁹⁷/+ ; FRT82B,Akt^q/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Fig4B	<i>y,w,HS:flp122/+; FRT40A,Tor^{2L1}/ FRT40A, ubi:RFP ; tGPH-4/+</i>	2HS, 10 days after HS
Fig4C	<i>y,w,HS:flp122/+;; FRT82B,Akt^q/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Fig4D	<i>y,w,HS:flp122/+;Tj:Gal4/ UAS:Thor-wt; FRT82B,Akt^q/ FRT82B, ubi:GFP</i>	1HS, 7 days after HS
Fig4E	<i>y,w,HS:flp122/+; FRT40A,Pten^{dj189}/ FRT40A,</i>	1HS, 7 days after HS

	<i>ubi:GFP</i>	
Fig4F	<i>y,w,HS:flp122/+; FRT40A,Pten^{dj189}/ FRT40A, ubi:GFP</i>	1HS, 6 days after HS
Fig4G	<i>y,w,HS:flp122/+; FRT40A,Tor^{2L1}/ FRT40A, ubi:GFP</i>	1HS, 6 days after HS
Figure 5		
Fig5B	<i>y,w,HS:flp122/+; FRT40A,Pten^{dj189}/ FRT40A, ubi:GFP ; Mat-tub:Gal4/ UAS-Tor^{GL00156}</i>	1 HS, 5 days after HS 2 days at 30°C
Fig5C	<i>y,w,HS:flp122/+; Mat-tub:Gal4/+; FRT82B,Tsc1²⁹, UAS-Tor^{GL00156}/ FRT82B, ubi:GFP</i>	1 HS, 5 days after HS
Fig5D-F, H	<i>y,w,HS:flp122/+; FRT40A,Pten^{dj189}/ FRT40A, ubi:GFP</i>	1 HS, 6 days after HS
Fig5G	<i>y,w,HS:flp122/+; FRT40A,Tor^{2L1}/ FRT40A, ubi:GFP</i>	2 HS, 7 days after HS
Figure S1		
FigS1B	<i>y,w,HS:flp122/+;; FRT82B, Akt^q/ FRT82B, ubi:GFP</i>	2 HS, 3 days after HS
FigS1C	<i>y,w,HS:flp122/+; FRT40A, Pten^{dj189}/ FRT40A, ubi:GFP</i>	1HS, 2 days after HS
FigS1D, E	<i>y,w,HS:flp122/+; FRT40A, Pten^{dj189}/ FRT40A, ubi:GFP</i>	1HS, 3 days after HS
FigS1F-H	<i>Mattub-Gal4/+ (control)</i>	Crosses were raised at 18°C and adult progeny were put 36 hours at 25°C
	<i>Mattub-Gal4/+ ; UAS-Tsc1^{GL00012}/+</i>	
	<i>Mattub-Gal4/+ ; UAS-Tor^{GL00156}/+</i>	
Figure S2		
FigS2A	<i>y,w,HS:flp122/+;; FRT82B,Akt^q/ FRT82B, ubi:GFP</i>	3HS, 8 days after HS
Figure S3		
FigS3A	<i>y,w,HS:flp122/+;; FRT82B,Ras85D^{ΔC40b}/ FRT82B, ubi:GFP</i>	1HS, 5 days after HS
FigS3B	<i>y,w,HS:flp122/+; FRT42D, Yorkie^{iB5}/ FRT82B, ubi:RFP</i>	1HS, 3 days after HS
FigS3C	<i>y,w,HS:flp122/+; FRT40A,Tor^{ΔP}/ FRT40A, ubi:GFP</i>	2HS, 5 days after HS
FigS3D	<i>y,w,HS:flp122/+; FRT42D, Sin1^{e3756}/ FRT42D, ubi:GFP</i>	2HS, 8 days after HS
FigS3E	<i>y,w,HS:flp122/+; FRT40A,Tor^{2L1}/ FRT40A, ubi:GFP</i>	2HS, 7 days after HS
Figure S4		
FigS4A	<i>y,w,HS:flp122/+; FRT40A,Tor^{ΔP}/ FRT40A, ubi:GFP</i>	2HS, 5 days after HS
FigS4B	<i>Thor^{CPT1001137}</i>	
FigS4C	<i>y,w,HS:flp122/+ ;Act:FRT-stop-FRT-Gal4, UAS:GFP/ UAS:Thor-wt</i>	1HS, 5 days after HS
FigS4D	<i>y,w,HS:flp122/+ ;Tub:FRT-stop-FRT-Gal4, UAS:GFP/ UAS:Thor-wt</i>	1HS, 5 days after HS
FigS4E	<i>y,w,HS:flp122/+ ;; FRT82B, Tsc1²⁹/ FRT82B, ubi:GFP</i>	1HS, 6 days after HS
FigS4F	<i>y,w,HS:flp122/+; FRT40A,Tor^{2L1}/ FRT40A, ubi:GFP</i>	1HS, 6 days after HS

Supplemental table 2 : list of antibodies used for immunostaining

Antigen	Origin	Name or reference	Dilution
FasIII	Developmental Studies Hybridoma Bank (DHSB)	7G10	1:1000
Br-C	DHSB	25E9D7	1:200
Orb	DHSB	4H8 and 6H4	1:100
Cora	DHSB	615-16	1:250
cleaved caspase3	Cell Signaling	#9661	1:500
PhosphoS505-Akt	Cell Signaling	#4054	1:100
phosphoT37T46-4EBP1	Cell Signaling	#2855	1:800
phosphoT37T46-4EBP1 biotinylated	Cell Signaling	#3929	1:100
Non-phosphoT46-4EBP1	Cell Signaling	#4923	1:800
GFP	Abcam	#5450	1:1000
β -Galactosidase	Capell	#55976	1:1000
Stau	D. St Johnston lab		1:1000
rabbit phosphoS10-PH3	Millipore		1:2000