SUPPLEMENTARY TEXT

Tracheal defects

fga phenotypes were also analyzed in the tracheal system, the organ responsible for oxygen delivery in the fly. In *fga* homozygous mutant alleles, we observed defects in tracheal liquid absorption apparent at the onset of the 1st larval instar (Fig S2), when the luminal fluid secreted by tracheal cells should normally be cleared and replaced by air (Manning & Krasnow, 1993). The penetrance of tracheal Air Filling Defects (AFD) was different for each of the *fga* alleles (Fig S2G) and broadly correlated with the extent of Sima protein accumulation in normoxia (Fig 1B, in the main text). To test if the accumulation of Sima protein observed in *fga* mutants might be the cause of AFD, we used a series of *GAL4* drivers to direct Sima overexpression to the tracheal system. Overexpression of Sima in all tracheal cells caused AFD, with phenotypes essentially indistinguishable from the ones observed in *fga* mutants (Fig S2C). When Sima ectopic expression was specifically induced in particular tracheal cell types, such as "fusion" or "terminal" cells, AFD were restricted to these cells (Fig S2D,E), indicating that the effect is cell-autonomous. In order to better visualize the cells forming liquid-filled tracheal tubes, a membrane-tagged EGFP was co-expressed together with Sima. As shown in figure S2E, terminal cells in this situation looked normal, indicating that overexpression of Sima did not affect the basic shape of these cells. Analysis of the tracheal system of *fga*^{1} *sima*^{07607} double homozygous larvae revealed that AFD did not develop, these individuals being phenotypically normal (Fig S2F). Taken together these results strongly suggest that AFD in *fga* mutants are due to over-accumulation of Sima protein in tracheal cells. The mechanism by which over-accumulation of Sima provoke AFD is unclear; as

tracheal development relies on other bHLH-PAS proteins such as Trachealess (Wilk *et al*, 1996), over-accumulation of Sima in the tracheae may cause either over-expression of endogenous tracheal genes or a dominant negative effect over the common bHLH-PAS partner subunit, Tango (Sonnenfeld *et al*, 1997). As an additional observation, tracheal terminal branches, that were shown to sprout in response to environmental oxygen levels (Jarecki *et al*, 1999), looked normal in *fga*^{1} *sima*^{07607} early 1st instar larvae (not shown), suggesting that initial formation of terminal branches does not depend on Sima at early developmental stages.

SUPPLEMENTARY REFERENCES

Jarecki J, Johnson E, Krasnow MA (1999) Oxygen regulation of airway branching in Drosophila is mediated by branchless FGF. *Cell* **99:** 211-20

Manning G & Krasnow MA (1993) Development of the Drosophila Tracheal System. In: Bate M & Martinez Arias A (eds), *The Development of Drosophila melanogaster* pp 609-685 Cold Spring Harbor Laboratory Press.

Sonnenfeld M, Ward M, Nystrom G, Mosher J, Stahl S, Crews S (1997) The Drosophila tango gene encodes a bHLH-PAS protein that is orthologous to mammalian Arnt and controls CNS midline and tracheal development. *Development* **124:** 4571-82

Wilk R, Weizman I, Shilo BZ (1996) trachealess encodes a bHLH-PAS protein that is an inducer of tracheal cell fates in Drosophila. *Genes Dev* **10:** 93-102

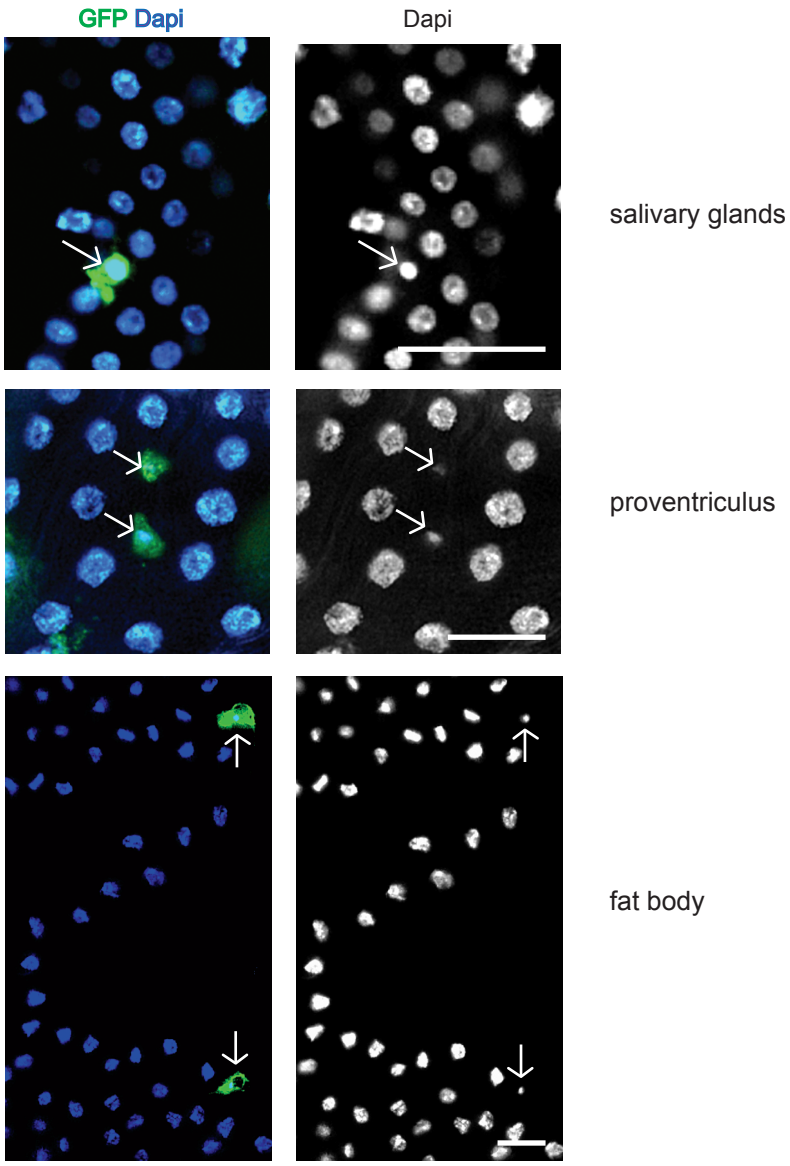
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1- Effect of Sima accumulation in different endoreplicative tissues. In endoreplicative tissues, the DNA content correlates with cell size and thus, smaller nuclei correspond to smaller cells (see Fig 3E in the main text for an example). To assess the role of Sima in cell growth, the “flip-out” technique was used to direct the expression Sima to random cells that could be recognized because they simultaneously expressed GFP. Cells were analyzed in different endoreplicative tissues such as the fat body, salivary glands and proventriculus; 71% of the nuclei of Sima-expressing cells

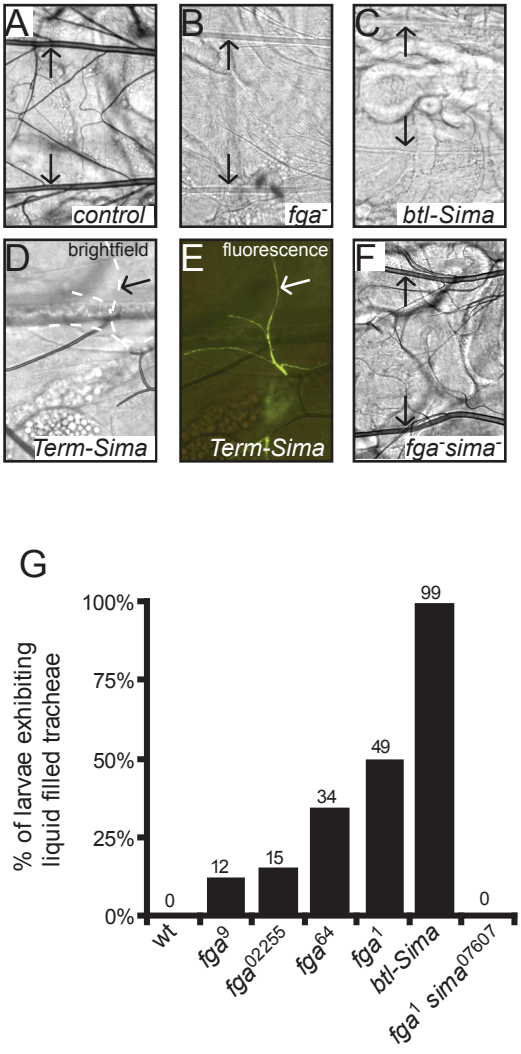
(arrows) were clearly smaller than control nuclei from neighboring GFP-negative cells (N>70). Scale bar, 50μm.

Supplementary Figure S2.- *sima* loss-of-function reverts tracheal phenotypes in *fga* mutants. (**A**) In wild type 1st instar larvae, dorsal tracheal trunks (arrows) are filled with air; in fga^{1} mutants (**B**) or in individuals overexpressing Sima through a *btl-Gal4* driver in the tracheae (**C**), defects in tracheal liquid clearance can be observed. Whereas air filled tubes can be visualized by bright field microscopy (**A**), liquid filled tubes are barely visible (**B-D**). Specific expression of Sima in tracheal terminal cells through a *Term-Gal4* driver caused liquid clearance defects specifically in these cells (arrow in **D**), indicating that the effect is cell autonomous. (**E**) Simultaneous expression of GFP in terminal cells shows that liquid-filled tracheal branches are intact. (**F**) Normal liquid clearance was fully restored in fga^{1} $sima^{07607}$ double homozygous mutant larvae (arrows). (**G**) Air filling is normal in 100% of wild type individuals but impaired with different penetrance in homozygotes for the different *fga* alleles or in individuals overexpressing Sima in the tracheae. In fga^{1} $sima^{07607}$ double homozygotes, air-filling defects do not occur (N>120).

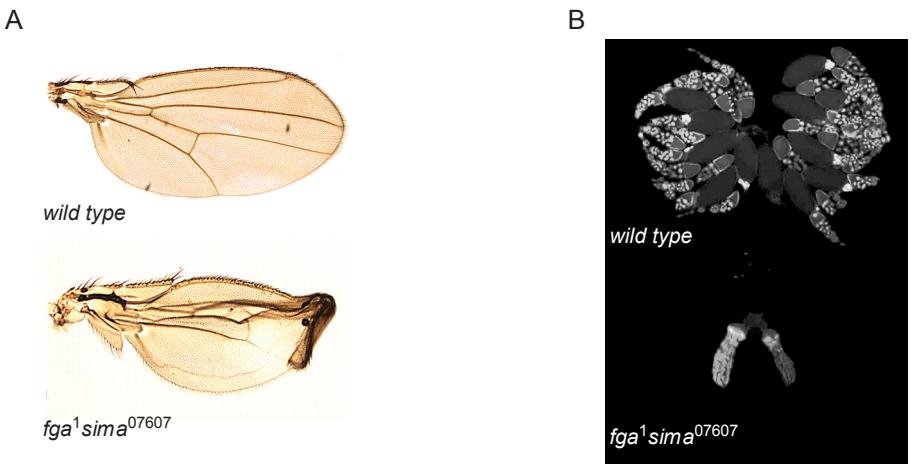
Supplementary Figure S3. Defects in wing and ovary development of fga^{1} $sima^{07607}$ double-mutant adults. **(A)** fga^{1} $sima^{07607}$ double mutants exhibit defects in the distal part of the wing 56/59). **(B)** fga^{1} $sima^{07607}$ females are sterile and display aberrant ovaries that do not develop beyond stage 4-6 of oogenesis; vitellogenic oocytes were never observed in double mutant females (7/7).



Supplementary Figure S1.- Effect of Sima accumulation in different endoreplicative tissues



Supplementary Figure S2.- *sima* loss-of-function reverts tracheal phenotypes in *fga* mutants.



Supplementary Figure S3. Defects in wings and ovaries of *fga*^{1} *sima*^{07607} double-mutant adults