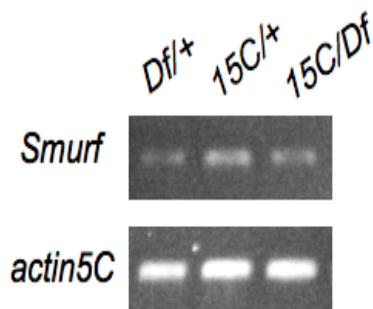


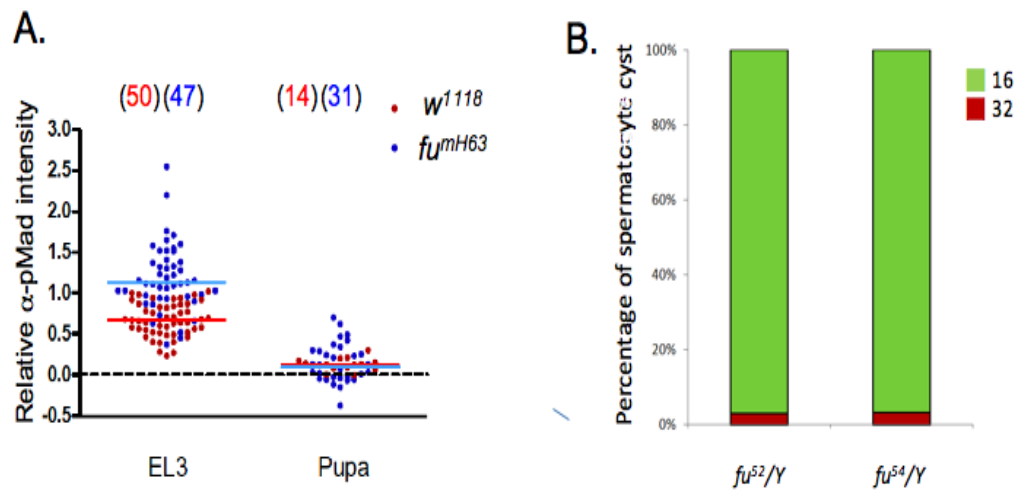
Supplementary Fig. 1. pMad expression patterns in first instar larvae.

Confocal images of L1 gonads immunostained for pMad (green). Germ cells are marked by anti-Vasa staining (red). (Asterisk) Hub. (A) Very low levels of pMad in testes of 0-4 h after hatching. (B) pMad expression clearly detected in the anterior germ cells in testes of 4-8 h after hatching. (C) pMad expression in germ cells restricted to hub-proximal cells in testes of 20-24 h after hatching.



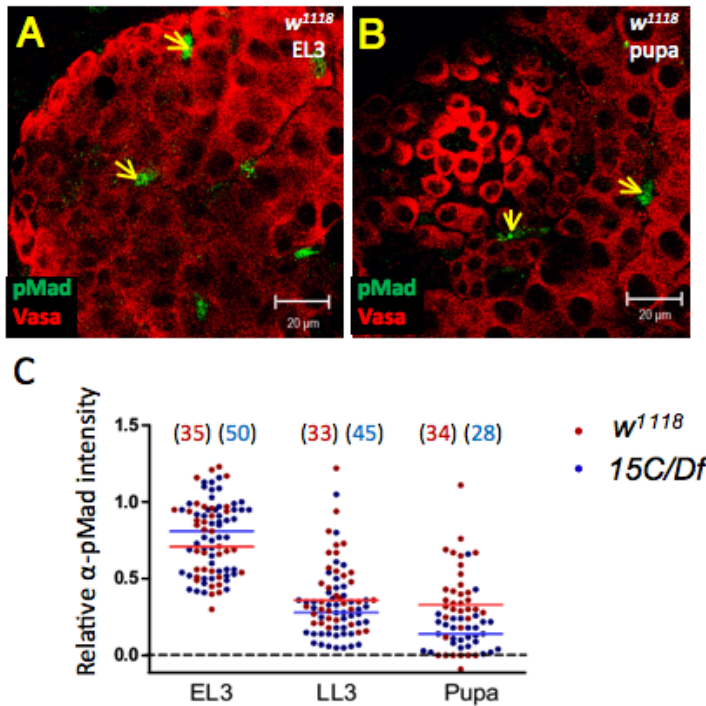
Supplementary Fig. 2. *Smurf* mRNA expressed in *Smurf^{15C}/Df7149* mutant testes.

RT-PCR analysis of *Smurf* mRNA expression in adult testes of *Df7149/+* (lane 1), *Smurf^{f15C}/+* (lane 2), and *Smurf^{f15C}/Df7149* (lane 3). *actin5C* was used as an internal control.



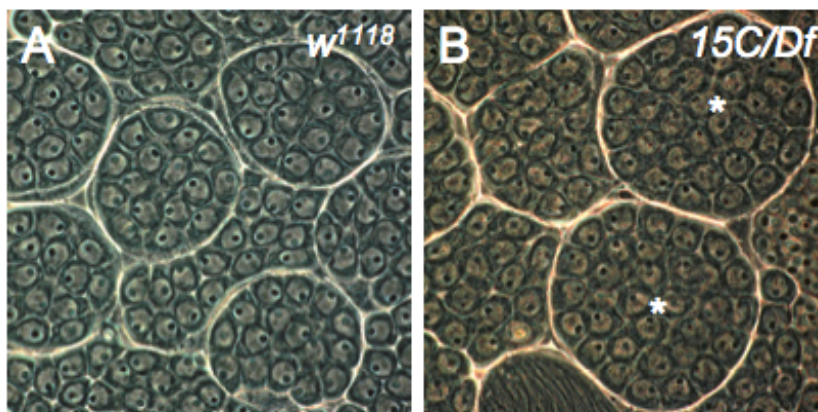
Supplementary Fig. 3: fused is not required for pMad downregulation from EL3 to early pupae.

(A) Column scatter graph of the relative α -pMad intensity in GSCs in EL3 and 0-4 hours APF testes from *w¹¹¹⁸* or *fu^{mH63}/Y* animals. Horizontal lines represent the median value. Numbers in the parenthesis are the numbers of GSC scored. (B) Percentage of 16- and 32-cell spermatocyte cysts in *fu⁵²/Y* and *fu⁵⁴/Y* 0-1 day adult testes.



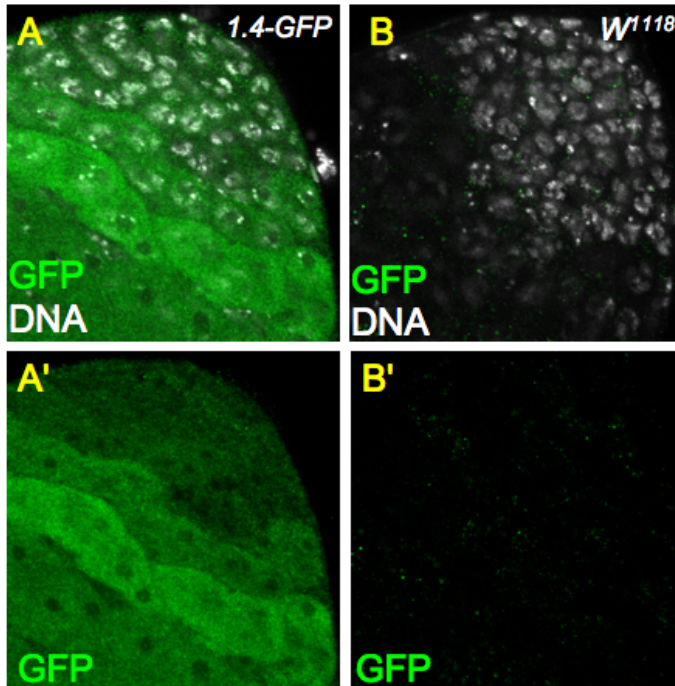
Supplementary Fig. 4: Smurf is not required in cyst cells to downregulate pMad.

(A and B) Developing *w¹¹¹⁸* testes immunostained for pMad (green), co-stained with anti-Vasa (red) antibodies. (Arrow) Cyst cell surrounding the germ cells entering spermatocyte differentiation. (C) Column scatter graph of the relative α -pMad intensity in GSCs in EL3, LL3 and 0-4 hours APF testes from *w¹¹¹⁸* or *Smurf^{f15C}/Df7149* animals. Horizontal lines represent the median value. Numbers in the parenthesis are the numbers of GSCs scored.

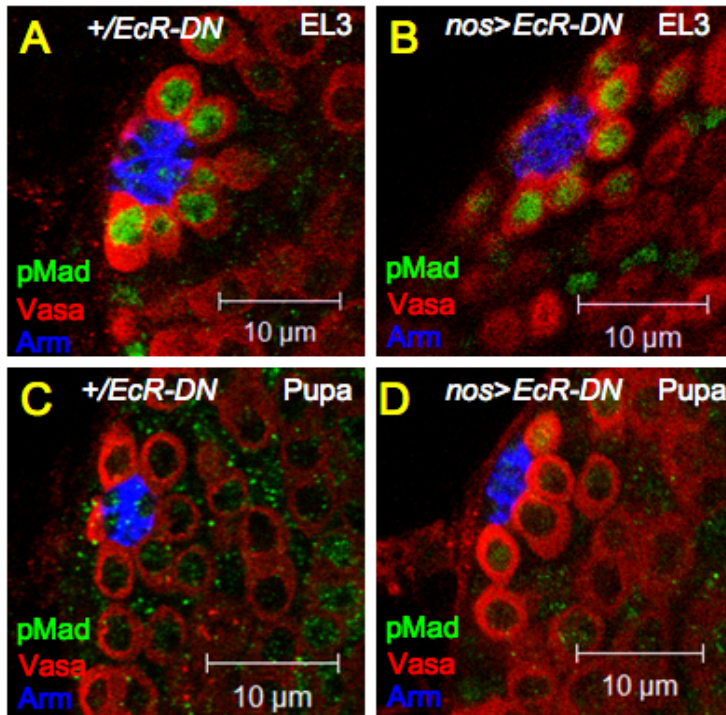


Supplementary Fig. 5: 32-cell cysts in *Smurf* mutant testes.

(A) Normal cysts of differentiating spermatocytes in w^{1118} adult testis, showing 16 cells in each cyst. (B) Differentiating spermatocyte cysts in $Smurf^{fl5C}/Df7149$ adult testis, showing cysts with 32 cells (asterisks).



Supplementary Fig. 6: Comparison of α -GFP immunofluorescence between w^{1118} and 1.4 -GFP testes. Testes immunostained with α -GFP antibody (green), co-stained with DNA-dye Hoechst 33324.



Supplementary Fig. 7: Ecdysone signaling is not required autonomously for pMad downregulation in GSCs. Developing testes immunostained for pMad (green), co-stained with anti-Vasa (red) and anti-Arm (blue) antibodies. Overexpression of $EcRB1^{.655F645A}$, the dominant-negative ecdysone receptor (EcR-DN), in early germ cells by *nos-Gal4* did not block pMad downregulation in GSCs from EL3 (B) to pupae (D).