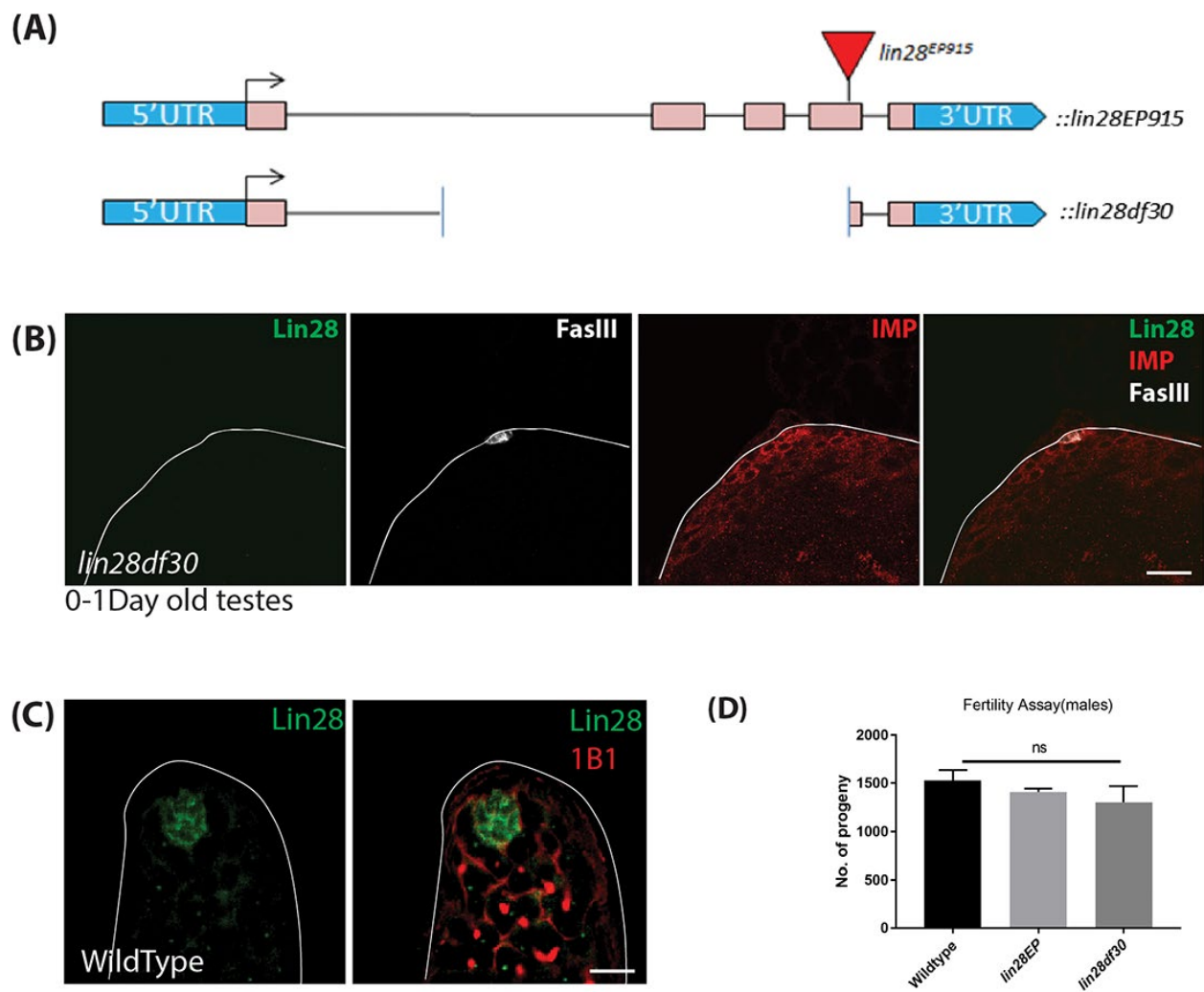
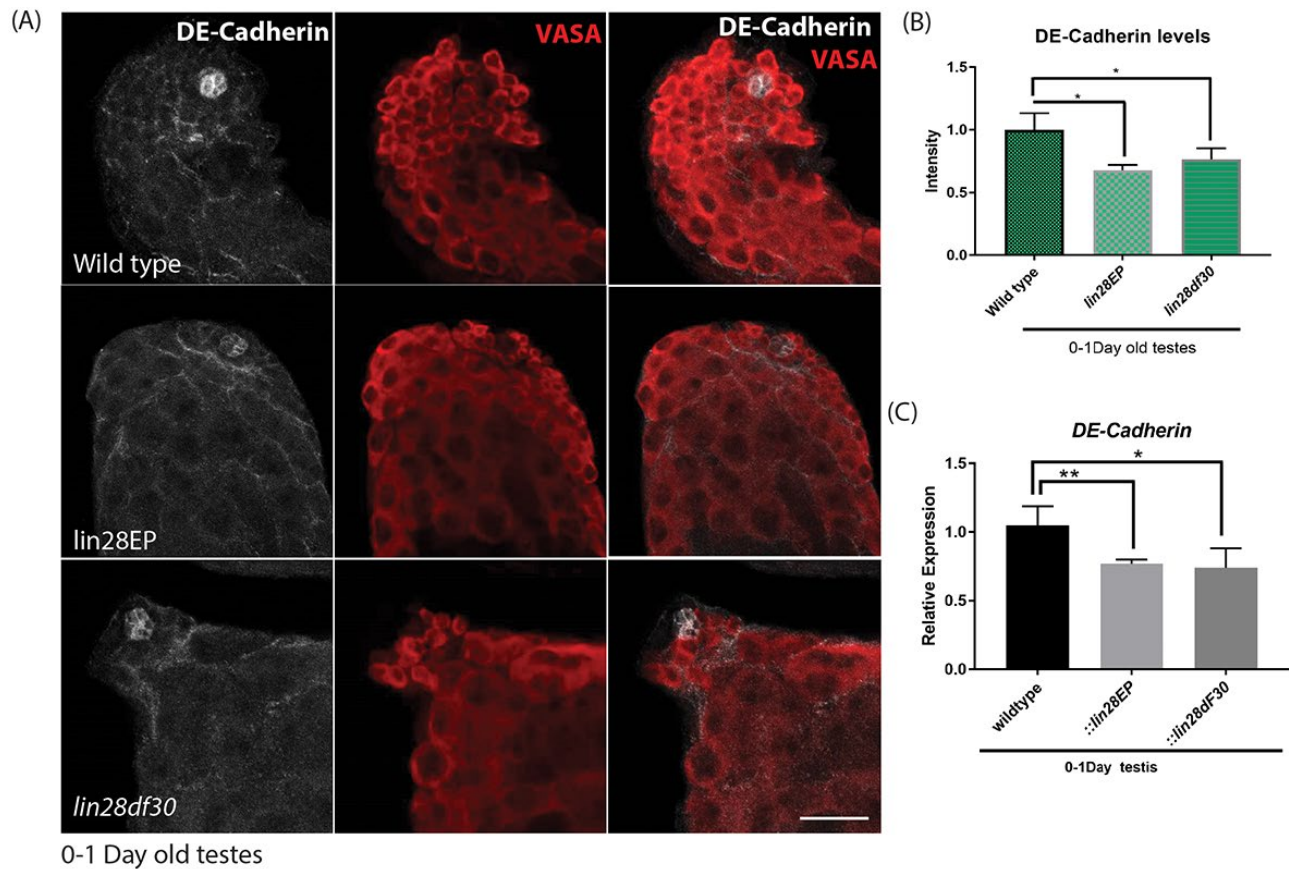


SUPPLEMENTARY MATERIAL

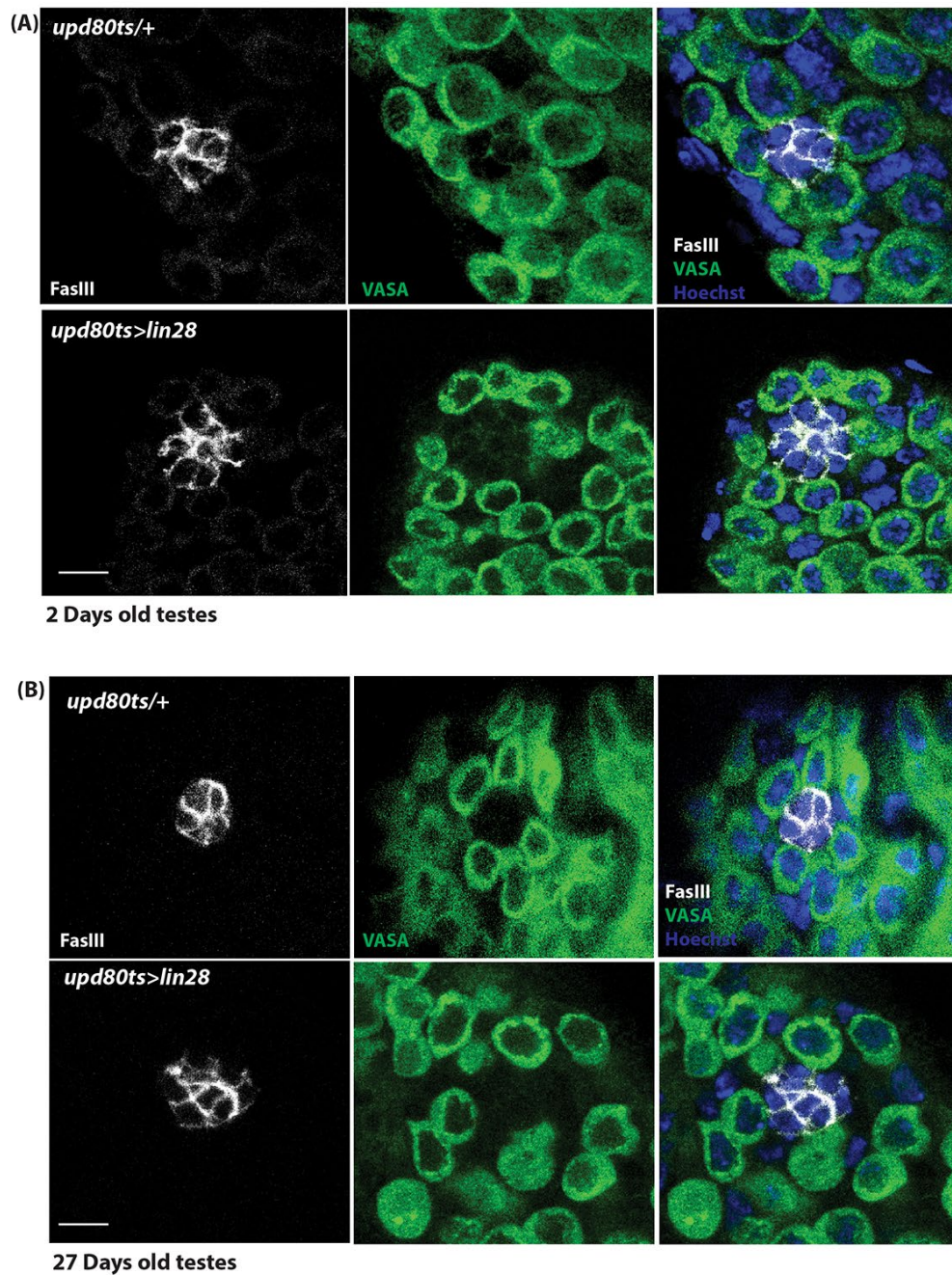
Supplementary Figures



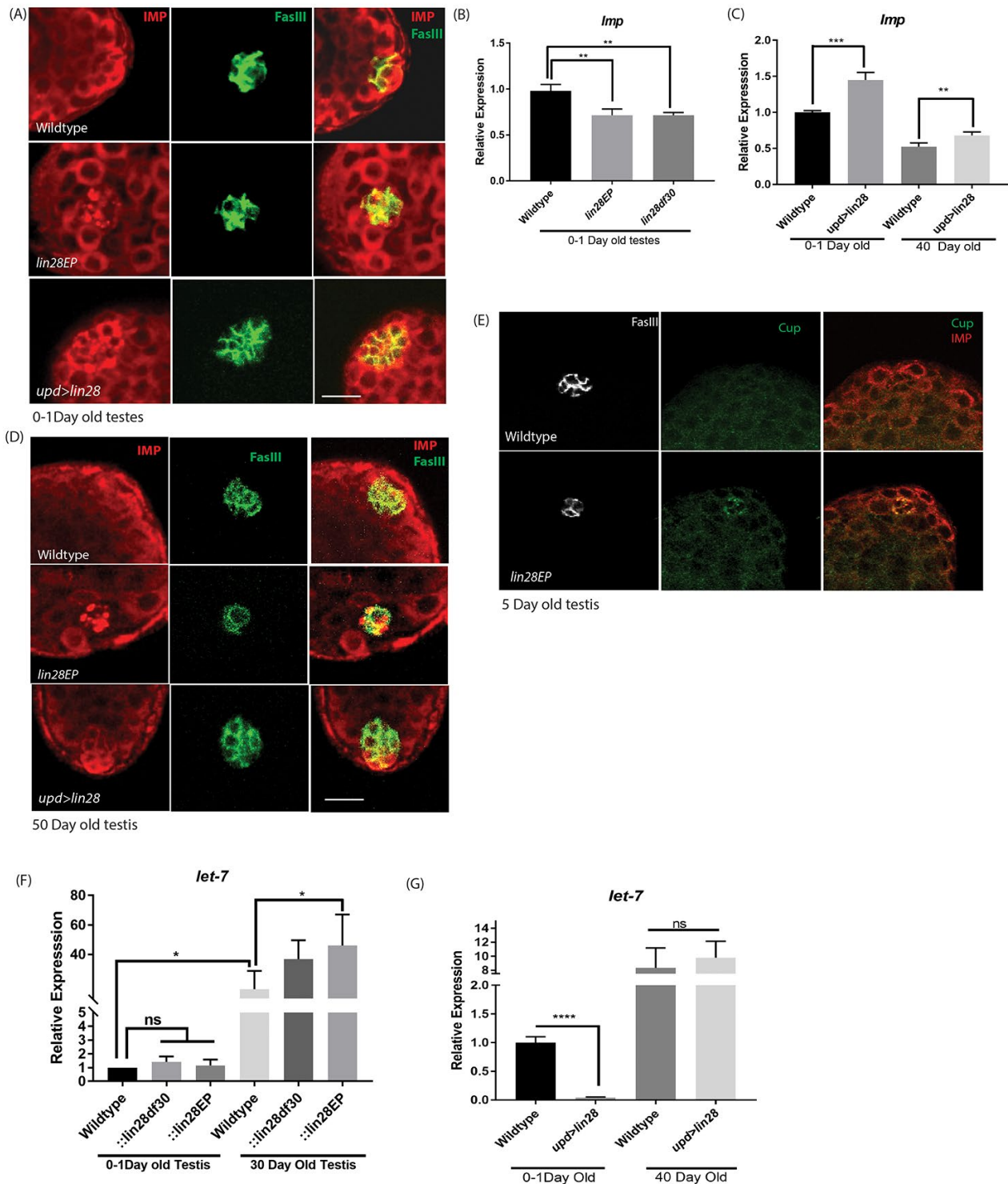
Supplementary Figure S1. Expression of Lin28. (A) A cartoon showing the P-element insertion site in the *lin28* gene locus along with the deletion mutant of *lin28*. (B) Expression of Lin28 in *lin28^{df30}* showing complete loss of Lin28 expression (C). Expression of Lin28 in wildtype testes to show the specificity of antibody. (D) Fertility assay showing no significant change in number of progeny compared to WT vs mutants. Scale bar 10µm.



Supplementary Figure S2. Expression of DE-Cadherin. (A) Young testes stained with DE-Cadherin and VASA to show the loss in expression of DE-cadherin in the mutants. Scalebar 10 μ m. (B) Quantification of the intensity of DE-cadherin in hub area. WT n= 17; *lin28EP* n=22 ;*lin28df30* n=22. (C) RT-qPCR of young testes to show the reduction in *DE cadherin* in Lin28 mutant testes.



Supplementary Figure S3. Adult specific expression of Lin28 in hub cells leads to increase in hub cell number. (A) 2 days after shifting to permissive temperature at 29°C adult testes stained with hub specific marker, FasIII and GSC marker VASA, shows increase in number of hub cells in overexpression of Lin28. (B) Testis stained after 27 days at 29°C shows Lin28 is required for maintaining the hub cell number in adult specific manner. Scale 10µm.



Supplemental Figure S4. Expression of IMP and *let-7* does not play any role in the Lin28 mediated aging of hub cells.

(A) Young testes stained with IMP antibody showing the accumulation of IMP into granular structures in hub are in *lin28* mutant. (B) RT-qPCR of young testes showing loss of *Imp* transcripts in *lin28* mutants. (C) qRT-PCR for the expression of *Imp* transcripts in young and old testis, suggesting that over-expression of Lin28 leads to slight accumulation of *Imp* transcripts. (D) Old testes stained with IMP antibody showing the expression pattern of IMP in the hub cells. (E) 5 day old testes stained with Cup antibody along with IMP antibodies showing the co-localization of Cup and IMP into stress granules. (F) RT qPCR of young vs old testis to show that *let-7* is not affected by Lin28 in the testes. (G) RT-qPCR of young and old testis suggesting no effect when Lin28 is overexpressed in old testes. Scale bar 10µm.

Supplementary Tables

Table S1. Quantification of hub cell number.

Genotype	No. of Hub cells	No. of GSCs	No. of TJ positive cells (in apical hub region) excluding hub cells.	No. of testes analyzed
Young (0-1 Day)				
<i>W1118</i>	11.1 ± 0.2	9.05 ± 0.22	48.2 ± 0.44	N=55
:: <i>lin28EP</i>	6.53 ± 0.28	6.9 ± 0.25	34.7 ± 0.28	N=68
<i>Upd-GAL4>UASlin28</i>	13.6 ± 0.26	9.22 ± 0.27	44.8 ± 0.28	N=42
<i>Upd-GAL4>UAS-lin28; lin28EP</i>	10.3 ± 0.19	9.125 ± 0.26	-	N=17
Old (50 Days)				
<i>W1118</i>	7.6 ± 0.33	5.75 ± 0.29	22 ± 0.3	N=40
:: <i>lin28EP</i>	3 ± 0.30	3.3 ± 0.19	13 ± 0.28	N=60
<i>Upd-GAL4>UASlin28</i>	10.8 ± 0.36	7.4 ± 0.38	27.75 ± 0.32	N=38

Table S2. Quantification of Hub cell number.

Genotype	No. of Hub cells	No. of GSCs	No. of testes analyzed
Young (2 Days) @29°C			
<i>upd-80ts/+</i>	8.5 ± 0.33	7.8 ± 0.241	N=36
<i>upd-80ts>UAS-lin28</i>	11.43 ± 0.37	9.286 ± 0.28	N=42
Old (27 Days) @29°C			
<i>upd-80ts/+</i>	5.68 ± 0.95	4.385 ± 0.85	N=39
<i>upd-80ts>UAS-lin28</i>	10.69 ± 0.26	8.33 ± 0.36	N=48