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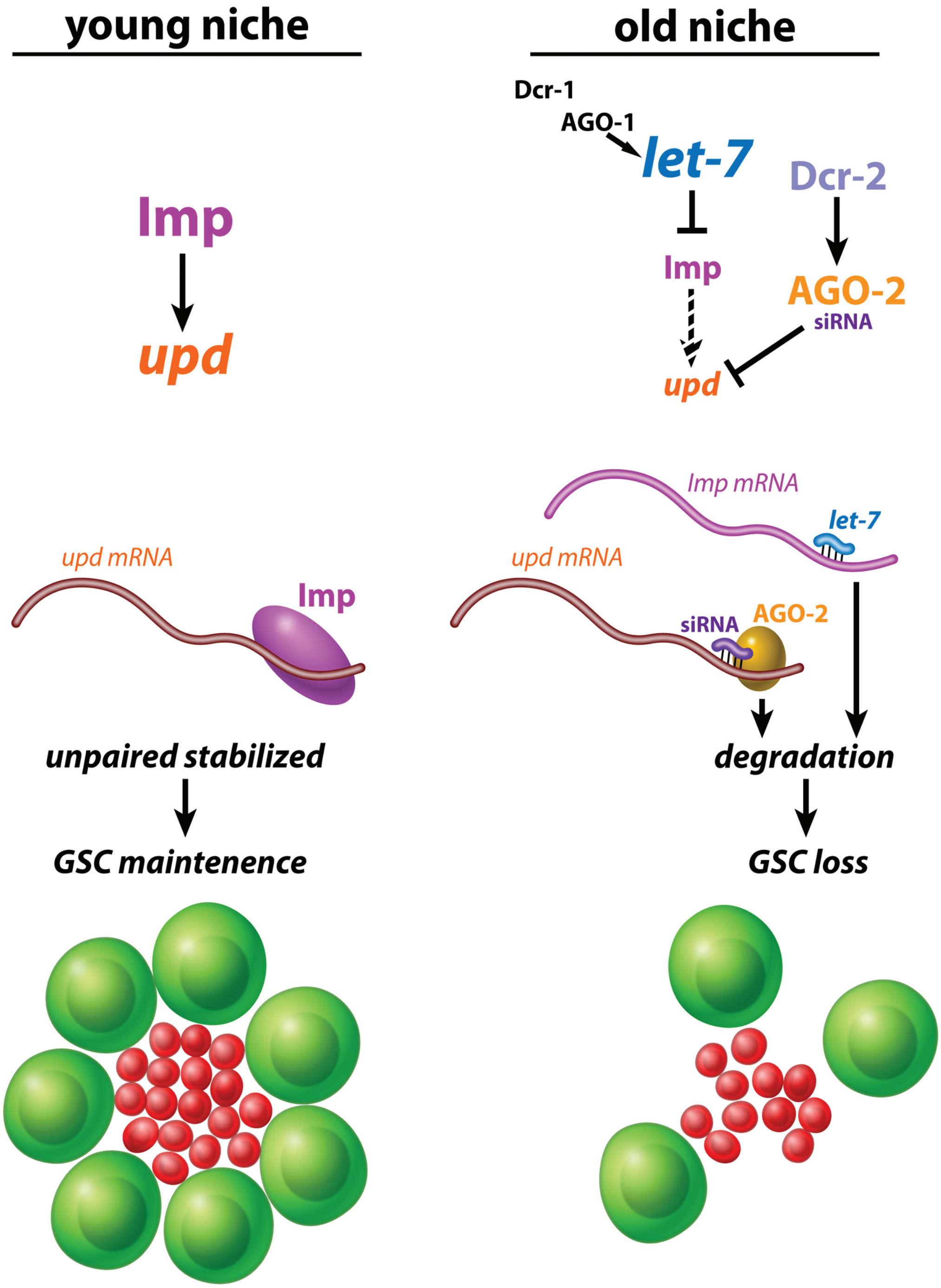

Figure S1: A proposed mechanism for aging of the *Drosophila* testis stem cell niche. In young adults, Imp binds to sequences primarily in the first 33 nucleotides of *upd* 3′UTR to protect against siRNA binding, thereby stabilizing *upd*. In older adults, expression of *let-7* in hub cells increases, leading to a decline in Imp expression in hub cells. Loss of Imp exposes *upd* mRNA to Dcr-2/AGO2-mediated degradation via siRNAs, resulting in reduced *upd* mRNA, a decline in niche function, and a loss of GSCs.

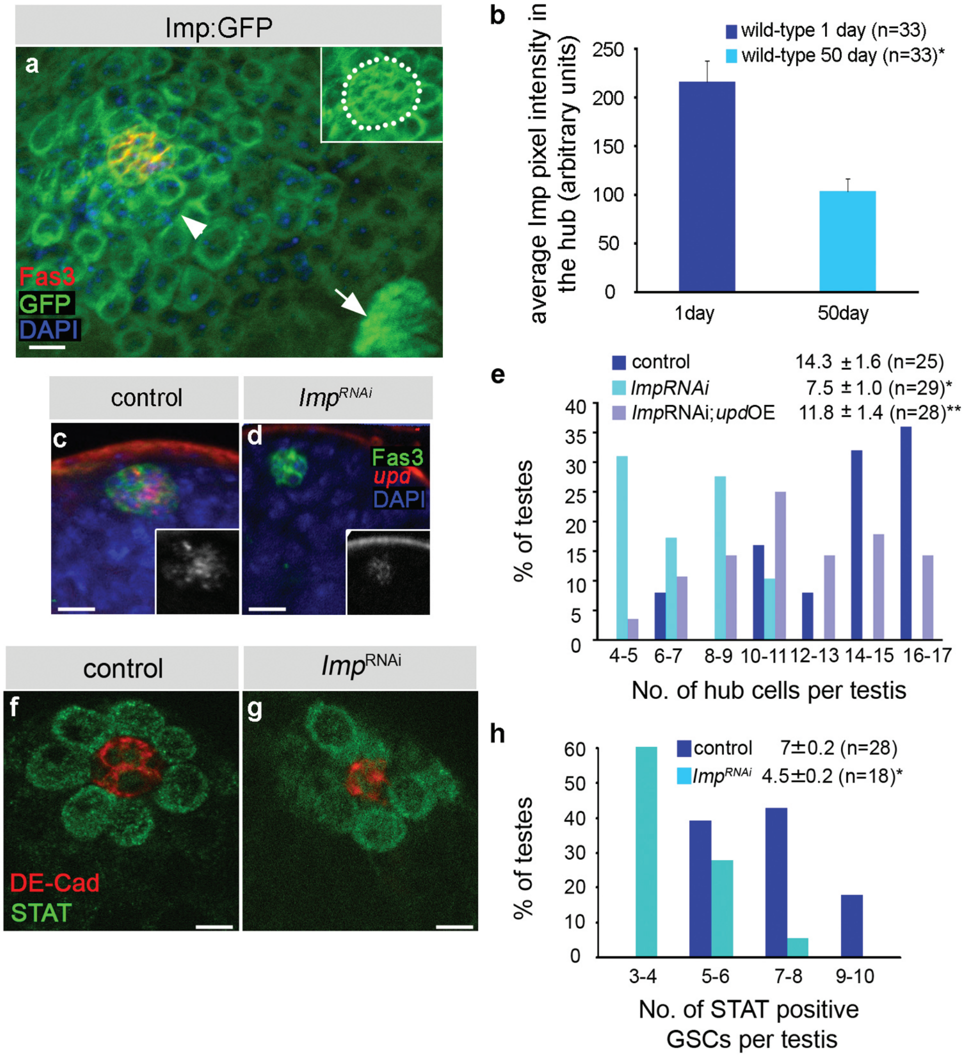

Figure S2: Imp expression and effects on *upd* mRNA. a, Testis from a 1-day old adult with Imp:GFP (green) Fas3 (red) and DAPI (blue). Imp:GFP is expressed in the hub (outlined, inset), in GSCs (arrowhead) and at the base of sperm bundles (arrow). **b**, Densitometric analysis of Imp staining in the hub from 1 or 50 day old males, shows ~ 50% decrease in aged males. **c**-**d**, Dual labeling for Fas3 (green) and for *upd* mRNA (red) from 10-day old flies from control, **c** (*w*$^-$,*upd*GAL4,UAS-*gfp* outcrossed to *w*^{1118}) or *Imp*RNAi, **d** (*w*$^-$,*upd*GAL4,UAS-*gfp;*UAS-*ImpRNAi). ***e** Distribution of the number of hub cells in testes from 10-day old: control (dark blue), *Imp*RNAi (light blue) and *Imp*RNAi; *upd*OE (purple) (*w*$^-$,*upd*GAL4,UAS-*gfp;*UAS-*ImpRNAi* UAS*Imp*^{T21}). **f-g,** Testis from 10-day old flies immunolabeled for STAT (green) and DE-Cadherin (red) from: control, **f** (*w*$^-$,*upd*GAL4,UAS-*gfp* outcrossed to *w*^{1118}) or *Imp*RNAi,**g** (*w*$^-$,*upd*GAL4,UAS-*gfp;*UAS-*ImpRNAi)* **h**, Distribution of the number of STAT positive GSCs as in **f**,**g**. Note reduction of STAT positive GSCs and *upd* signal upon loss of Imp (insets in **c**, **d**). Densitometric quantification of *upd* signal in hubs of **c**: control 112±8 (n=14) and **d**: *Imp*RNAi 59±2 (n=14) revealed a 47% decrease in *upd* levels when Imp levels are reduced in the hub.

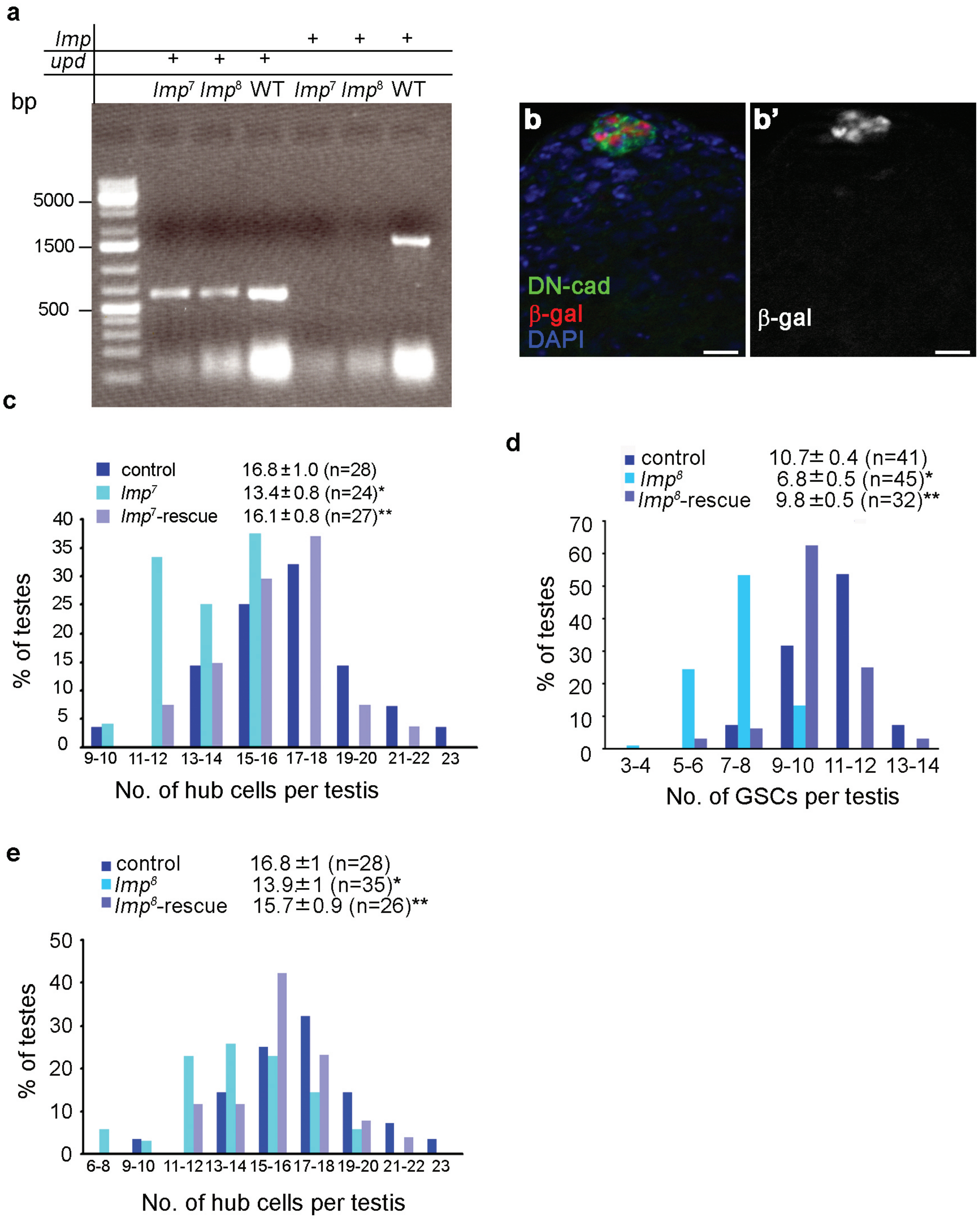

Figure S3: Loss of *Imp* results in reduced *upd* and GSCs. a, PCR for control, *Imp*^{7} and *Imp*^{8} hemizygous L3 larvae, with primers for *upd* (control) or *Imp* shows loss of *Imp* in *Imp*^{7} and *Imp*^{8} larvae. **b,** Expression pattern of *8-156*GAL4 driver in the testes (w; *8-156*GAL4; UAS*lacZ*^{nls}), DN-cadherin (green), β-Galactosidase (red) and DAPI (blue). (**b'**) Note driver activity of 8-156GAL4 in somatic hub cells **c,** Distribution of the number of Fas3$^+$/DE-cad$^+$ hub cells in L3 male gonads: control (EP(X)760 - dark blue), *Imp*^{7} (light blue) and *Imp*^{7}-rescue (*Imp*^{7} ; *8-156*GAL4; UAS*Imp*^{T21}, purple). **d-e**, Distribution of the number of GSCs (**d**) and hub cells (**e**) in testes from control (EP(X)760, dark blue), *Imp*^{8} (light blue) and *Imp*^{8}-rescued (*Imp*^{8}; *8-156*GAL4; UAS*Imp*^{T21}, purple) flies.

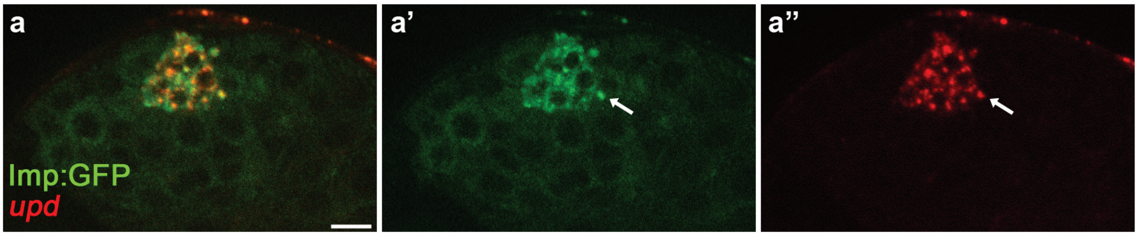

Figure S4: Imp co-localizes with *upd in vivo.* a-a", High magnification view of Imp:GFP (green) expression in the testis from a 1-day old adult, labeled for GFP (green) and FISH for *upd* mRNA (red). (**a**) Merged image. Notice co-localization of *upd* mRNA and Imp:GFP protein in hub cells. Germ cells are out of the plane of focus.

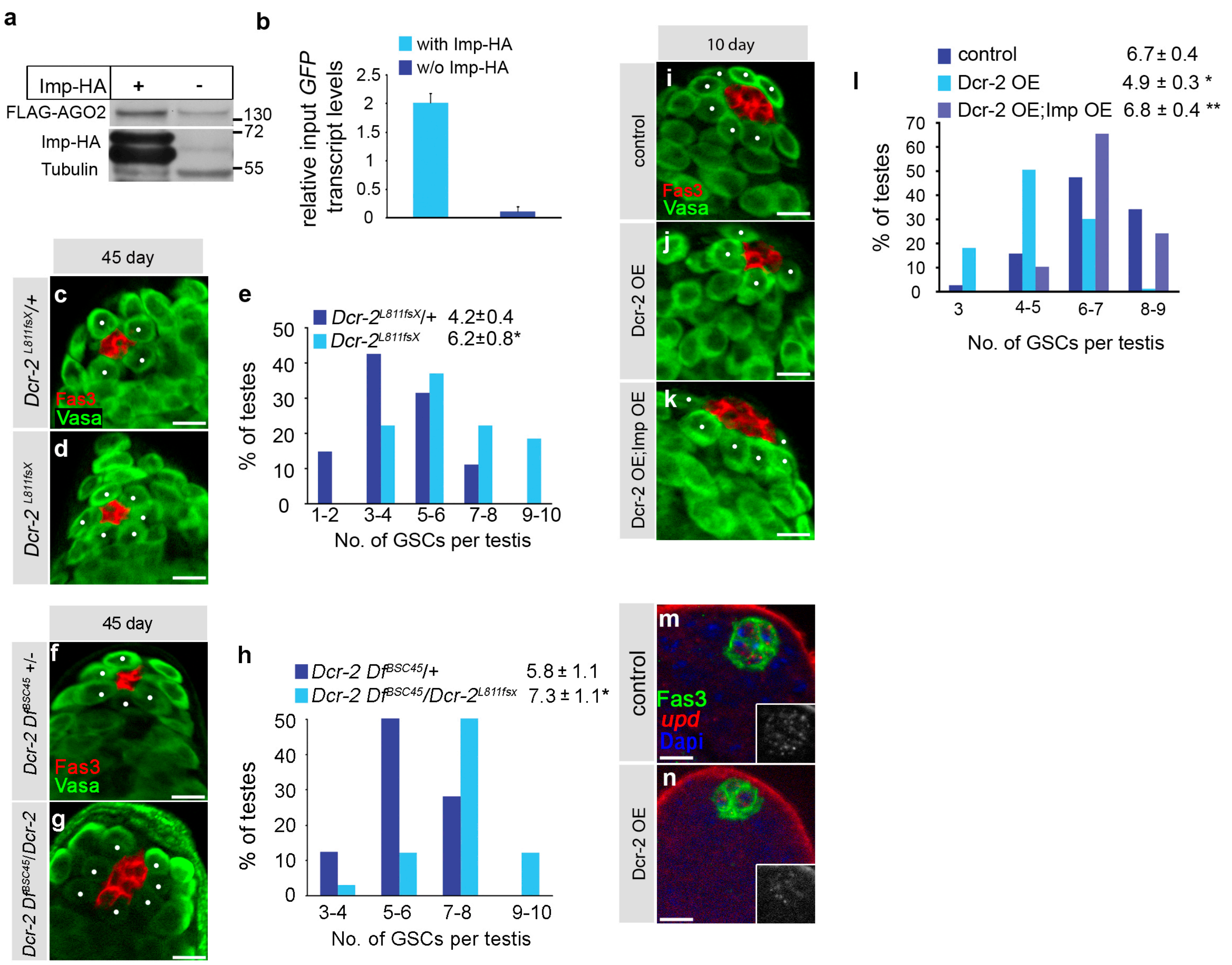

Figure S5: The siRNA machinery regulates *upd* levels and GSCs.
a-b,FLAG-AGO2 RIP from S2 cells stably expressing FLAG-AGO2 and transiently expressing *gfp-upd3'UTR* with (light blue) or without (dark blue) Imp-HA. (**a**),Immunoblot showing input levels of FLAG-AGO2, Imp-HA and Tubulin; (**b**) qRT-PCR showing input *gfp* levels normalized to *rp49.* **c-d** GSCs number in heterozygous (**c**, *Dcr-2*^{L811fsX}/+) and *Dcr-2* homozygous (**d**,*Dcr-2*^{L811fsX}) 45-day old males. **e,** Distribution of GSCs, as in **c** (n= 54) and **d** (n=28).**f-g,** Testes from 45-day old heterozygous, control (**f**, *Df(2R)BSC45/+*) and *Dcr-2* null, mutant (**g**, *Df(2R)BSC45/Dcr-2* ^{L811fsX}) males co-labeled with Vasa (green) and Fas3 (red). **h**, GSC distribution per testis as in **f** (n=32) and **g** (n=33). **i-k**,Testes from 10-day old males of genotypes: **(i)** *w*$^-$*,upd*GAL4,UAS-*gfp* outcrossed to *w*^{1118}; **(j)***w*$^-$*,upd*GAL4,UAS-*gfp*;UAS-*Dcr-2;* **(k)***w*$^-$*,upd*-GAL4,UAS-*gfp*;UAS-*Dcr-2;*UAS-*Imp*^{T21}. **l**, Distribution of GSCs, as in **i** (n=38), **j** (n=83) and **k** (n=29). **m-n**, Dual labeling for Fas3 (green) and *upd* mRNA (red) of testis from 10-day control (**m**, *w*$^-$*, upd*GAL4, UAS-*gfp* outcrossed to *w*^{1118}) or *Dcr-2*OE in the hub (**n**, *w*$^-$*, upd*GAL4, UAS-*Dcr-2*) flies. Note reduction of *upd* when *Dcr-2* is overexpressed (**n**, inset). **e,l** The average number ± 95% confidence interval is shown. Asterisks denote a statistically significant difference (*) from controls (**) from Dcr2 overexpression (**l**) (student's t-test p<0.01). GSC: white dots. Scale bars, 10*μ*m.

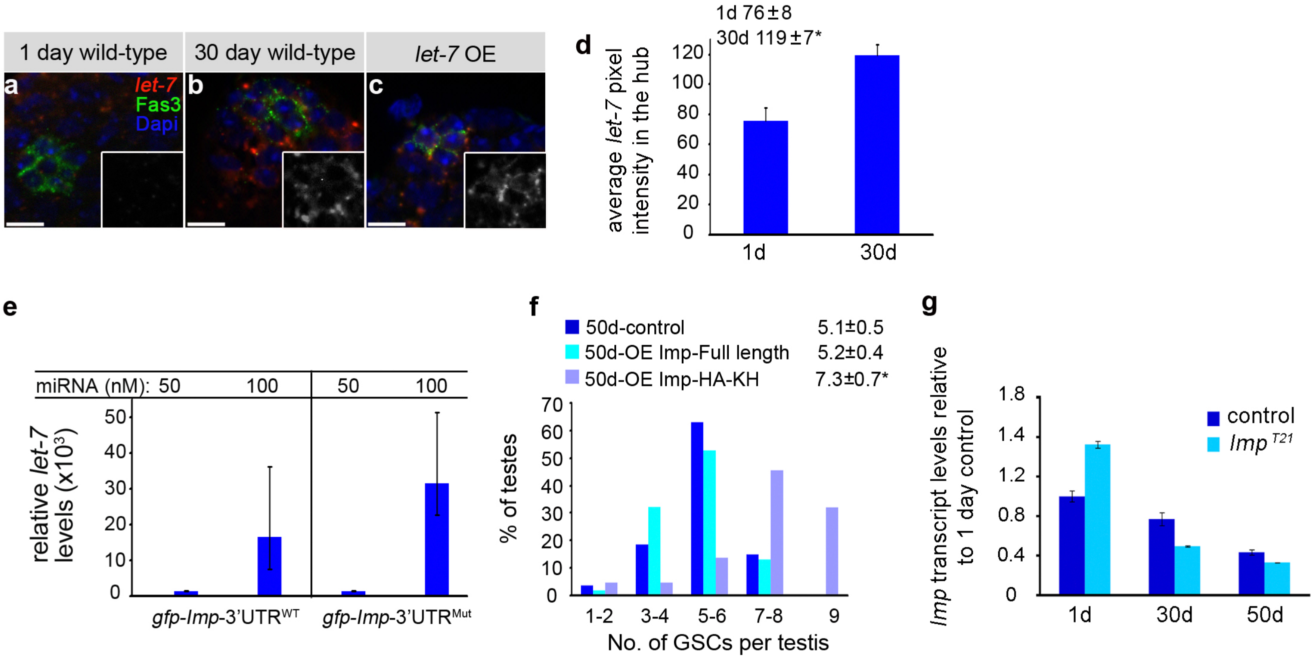

Figure S6: *Imp* is targeted by the *let-7* miRNA in the testis

a-c, Dual labeling with Fas3 (green), let-7 (red, insets) and DAPI (blue) of testes from 1 (**a**) and 30 (**b**) day-old wild-type flies, and from (**c**) 1-day old fly overexpressing *let-7* in the hub (*upd*GAL4; UAS-*let-7*$^{[701.12.9]}$). **d**, Quantification of let-7 signal in the hub shows increased expression in aged flies, 1day (n=13) and 30day (n=24).**e**, qRT-PCR for mature *let-7* expression in S2 cells, relative to *bantam*. **f**, Distribution of GSCs in testes from 50-day old controls (dark blue, n=27): *w,upd*GAL4,UAS-*gfp* outcrossed to *w*^{1118} , Imp Full length (light blue, n=53): *w*$^-$*,upd*GAL4*,*UAS-*gfp;*UAS*Imp*^{T21} or Imp with a truncated 3'UTR (purple, n=41): *w,upd*GAL4;UAS-*Imp*$^{HA\text{-}KH1\text{-}3\text{-}3}$. **g**,qRT-PCR showing relative Imp mRNA in testes from 1-, 30- and 50-day old control flies (w-, updGAL4, UASgfp outcrossed to w1118, dark blue) or flies overexpressing Imp with a full length 3'UTR (imp T21) in hub cells (w-, updGAL4, UASgfp; UASImpT21 flies, light blue). Imp overexpression in young (1 day-old) flies was not maintained with age. **d,f** The average number ± 95% confidence interval is shown. Asterisks denote a statistically significant difference (*) from controls (student's t-test *p<0.01). Scale bars, 10*μ*m.