Developmental Cell, Volume *56*

Supplemental information

Proliferative stem cells maintain quiescence of their niche by secreting the Activin inhibitor Follistatin

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Table S1: Hub cell number in the analyzed genotypes, Related to Figures 1-5 and STAR methods

#	Figure	Genotype	Treatment	Average	SE	n	p
1	1I	*tj > +*	10 days	11.83	±0.53	18	
2	1I	*tj > Dp-RNAi (Vienna), GFP*	10 days	2.10	±0.43	31	$1e^{-16}$ (vs #1)
3	1I	*tj > Dp-RNAi (TRiP)*	10 days	9.14	±0.96	14	0.0232 (vs #1)
4	1I	*tj > Dp-RNAi, Dp*	10 days	4.93	±0.86	15	0.0075 (vs #2)
5	1I	*tj > E2f1-RNAi*	10 days	7.29	±0.64	17	$5e^{-6}$ (vs #1)
6	1I	*tj > Rbf^{280}*	10 days	5.00	±1.50	12	0.0008 (vs #1)
7	1J	*tj, hh-$GAL80^{TS}$ > +*	10 days	10.56	±0.32	27	
8	1J	*tj, hh-$GAL80^{TS}$ > Dp-RNAi, LacZ*	10 days	4.00	±0.76	8	$2e^{-5}$ (vs #7)
9	1J	*tj, hh-$GAL80^{TS}$ > Dp-RNAi, Dp*	10 days	8.36	±0.35	33	0.0004 (vs #8)
10	2D	*tj > LacZ, LacZ*	0 days	10.92	±0.30	49	
11	2D	*tj > LacZ, LacZ*	14 days	9.69	±0.54	22	
12	2D	*tj > Fs-RNAi, LacZ*	0 days	6.06	±0.48	32	
13	2D	*tj > Fs-RNAi, LacZ*	14 days	1.11	±0.28	28	$2e^{-15}$ (vs #11)
14	2D	*tj > Fs-RNAi, Fs*	0 days	7.52	±0.71	31	
15	2D	*tj > Fs-RNAi, Fs*	14 days	4.56	±0.68	34	$3e^{-5}$ (vs #13)
16	2D	*tj > LacZ, Fs*	0 days	10.49	±0.30	37	
17	2D	*tj > LacZ, Fs*	14 days	10.58	±0.43	31	0.20 (vs #11)
18	2E	*Fs^{null} -/-*	0 days	9.39	±0.85	18	
19	2E	*Fs^{null} -/-*	4 weeks	2.16	±0.63	31	$6e^{-8}$ (vs #18)
20	2J	*tj, hh-$GAL80^{TS}$ > +*	10 days	10.56	±0.32	27	
21	2J	*tj, hh-$GAL80^{TS}$ > Dp-RNAi, LacZ*	10 days	4.00	±0.76	8	$2e^{-5}$ (vs #20)
22	2J	*tj, hh-$GAL80^{TS}$ > Dp-RNAi, Fs*	10 days	10.88	±0.45	17	$4e^{-6}$ (vs #21)
23	3B	*upd^{TS} > +*	0 days	11.17	±0.74	23	
24	3B	*upd^{TS} > +*	1 week	10.33	±0.73	15	
25	3B	*upd^{TS} > +*	2 weeks	10.62	±0.58	13	
26	3B	*upd^{TS} > +*	3 weeks	10.13	±0.85	8	
27	3B	*upd^{TS} > +*	4 weeks	7.68	±0.38	84	
28	3B	*upd^{TS} > babo^{QD}*	0 days	9.88	±0.68	16	0.2032 (vs #23)
29	3B	*upd^{TS} > babo^{QD}*	1 week	6.25	±0.62	16	0.0002 (vs #24)
30	3B	*upd^{TS} > babo^{QD}*	2 weeks	5.18	±0.47	28	$8e^{-8}$ (vs #25)
31	3B	*upd^{TS} > babo^{QD}*	3 weeks	2.69	±0.47	35	$7e^{-6}$ (vs #26)
32	3B	*upd^{TS} > babo^{QD}*	4 weeks	0.60	±0.28	30	$7e^{-28}$ (vs #27)
33	4E	*upd^{TS} > NLAP*	4 weeks	7.68	±0.48	41	
34	4E	*upd^{TS} > esg^{NLAP}*	4 weeks	10.24	±0.50	38	0.0004 (vs #33)
41	5D	*tj > LacZ*	0 days	10.92	±0.30	49	
42	5D	*tj > LacZ*	4 weeks	9.14	±0.27	66	
43	5D	*tj > Fs*	0 days	10.49	±0.30	37	
44	5D	*tj > Fs*	4 weeks	10.45	±0.38	42	0.0058 (vs #42)

45	5E	*upd^{TS} > LacZ*	0 days	10.64	±0.42	45	
46	5E	*upd^{TS} > LacZ*	4 weeks	7.56	±0.34	84	
47	5E	*upd^{TS} > smox-RNAi*	0 days	11.79	±0.40	19	
48	5E	*upd^{TS} > smox-RNAi*	4 weeks	9.45	±0.33	47	0.0007 (vs #46)
49	5E	*upd^{TS} > babo-RNAi*	0 days	11.45	±0.45	38	
50	5E	*upd^{TS} > babo-RNAi*	4 weeks	10.15	±0.58	26	0.0042 (vs #46)
51	5E	*upd^{TS} > daw-RNAi-HMS01110*	0 days	12.97	±0.41	33	
52	5E	*upd^{TS} > daw-RNAi-HMS01110*	4 weeks	11.13	±0.32	45	$2e^{-34}$ (vs #46)
53	5E	*upd^{TS} > daw-RNAi-HMJ03135*	0 days	12.24	±0.34	29	
54	5E	*upd^{TS} > daw-RNAi-HMJ03135*	4 weeks	10.48	±0.45	46	0.0010 (vs #46)
55	S2E	*tj^{TS} > +*	0 days	11.88	±0.33	17	
56	S2E	*tj^{TS} > +*	3 days	12.05	±0.33	21	
57	S2E	*tj^{TS} > +*	7 days	11.63	±0.50	8	
58	S2E	*tj^{TS} > +*	10 days	11.69	±0.29	35	
59	S2E	*tj^{TS} > +*	20 days	10.76	±0.47	17	
60	S2E	*tj^{TS} > Dp-RNAi*	0 days	11.18	±0.38	28	0.1686 (vs #55)
61	S2E	*tj^{TS} > Dp-RNAi*	3 days	9.79	±0.68	14	0.0076 (vs #56)
62	S2E	*tj^{TS} > Dp-RNAi*	7 days	5.00	±1.59	6	0.0074 (vs #57)
63	S2E	*tj^{TS} > Dp-RNAi*	10 days	2.31	±0.47	39	$6e^{-20}$ (vs #58)
64	S2E	*tj^{TS} > Dp-RNAi*	20 days	0.03	±0.03	30	$1e^{-13}$ (vs #59)
65	S2E	*tj^{TS} > E2f1-RNAi*	0 days	10.61	±0.78	18	0.2699 (vs #55)
66	S2E	*tj^{TS} > E2f1-RNAi*	3 days	9.58	±0.55	17	0.0083 (vs #56)
67	S2E	*tj^{TS} > E2f1-RNAi*	7 days	7.00	±1.04	11	0.0013 (vs #57)
68	S2E	*tj^{TS} > E2f1-RNAi*	10 days	4.24	±0.36	29	$6e^{-16}$ (vs #58)
69	S2E	*tj^{TS} > E2f1-RNAi*	20 days	0.50	±0.33	22	$2e^{-17}$ (vs #59)
70	S2F	*eyaA3 > +*	10 days	11.25	±0.33	32	
71	S2F	*eyaA3 > Dp-RNAi*	10 days	1.88	±0.33	43	$7e^{-31}$ (vs #70)
72	S2F	*C587 > +*	10 days	11.82	±0.27	35	
73	S2F	*C587 > Dp-RNAi*	10 days	0.46	±0.17	52	$9e^{-42}$ (vs #72)
74	S2F	*fng^{TS} > +*	0 days	12.50	±0.56	12	
75	S2F	*fng^{TS} > +*	10 days	12.57	±0.72	7	
76	S2F	*fng^{TS} > +*	20 days	12.62	±0.29	37	
77	S2F	*fng^{TS} > Dp-RNAi*	0 days	11.93	±0.64	14	0.6416 (vs #74)
78	S2F	*fng^{TS} > Dp-RNAi*	10 days	7.40	±0.32	9	0.0001 (vs #75)
79	S2F	*fng^{TS} > Dp-RNAi*	20 days	4.59	±0.60	32	$9e^{-16}$ (vs #76)
80	S2F	*fng^{TS} > E2f1-RNAi*	10 days	9.00	±0.42	16	0.0015 (vs #75)

81	S2F	*fng^{TS} > E2f1-RNAi*	20 days	7.33	±0.68	12	3e^{-6} (vs #76)
82	S3F	*nos* > +	0 days	11.70	±0.56	10	
83	S3F	*nos* > +	10 days	12.83	±1.28	12	
84	S3F	*nos > Fs-RNAi*	0 days	11.76	±0.61	17	0.9382 (vs #82)
85	S3F	*nos > Fs-RNAi*	10 days	12.25	±0.57	24	0.9793 (vs #83)
86	S3H	*upd^{TS} > +, Fs^{null} / +*	0 days	10.71	±0.41	21	
87	S3H	*upd^{TS} > +, Fs^{null} / +*	2 weeks	10.20	±0.47	30	
88	S3H	*upd^{TS} > +, Fs^{null} / +*	4 weeks	9.40	±0.96	10	
89	S3H	*upd^{TS} > Fs, Fs^{null} / +*	0 days	10.24	±0.38	21	
90	S3H	*upd^{TS} > Fs, Fs^{null} / +*	2 weeks	9.92	±0.58	12	
91	S3H	*upd^{TS} > Fs, Fs^{null} / +*	4 weeks	8.79	±0.35	19	
92	S3H	*upd^{TS} > +, Fs$^{null\ -/-}$*	0 days	9.71	±0.52	17	
93	S3H	*upd^{TS} > +, Fs$^{null\ -/-}$*	2 weeks	6.92	±0.48	38	
94	S3H	*upd^{TS} > +, Fs$^{null\ -/-}$*	4 weeks	6.20	±0.59	11	
95	S3H	*upd^{TS} > Fs, Fs$^{null\ -/-}$*	0 days	9.95	±0.60	19	
96	S3H	*upd^{TS} > Fs, Fs$^{null\ -/-}$*	2 weeks	9.71	±0.39	28	3e^{-5} (vs #93)
97	S3H	*upd^{TS} > Fs, Fs$^{null\ -/-}$*	4 weeks	9.36	±0.75	19	0.0028 (vs #94)
98	S3I	*fng > +, Fs^{null} / +*	4 weeks	9.14	±1.09	14	
99	S3I	*fng > Fs, Fs^{null} / +*	4 weeks	8.07	±0.89	14	
100	S3I	*fng > +, Fs$^{null\ -/-}$*	4 weeks	5.00	±0.84	11	
101	S3I	*fng > Fs, Fs$^{null\ -/-}$*	4 weeks	8.29	±0.53	24	0.0034 (vs #100)
102	S3J	*Fs$^{GFSTF.1}$ / Fs^{MI11350}*	0 days	11.64	±0.48	36	
103	S3J	*Fs$^{GFSTF.1}$ / Fs^{MI11350}*	4 weeks	11.14	±0.30	37	0.3751 (vs #102)
104	S3J	*Fs$^{TG4.1}$ / Fs^{MI}-GAL4*	0 days	11.39	±0.37	18	
105	S3J	*Fs$^{TG4.1}$ / Fs^{MI}-GAL4*	4 weeks	5.64	±0.67	39	6e^{-10} (vs #104)
106	S3J	*Fs$^{GFSTF.1}$ / Fs$^{TG4.1}$*	0 days	10.40	±0.46	25	
107	S3J	*Fs$^{GFSTF.1}$ / Fs$^{TG4.1}$*	4 weeks	7.88	±0.76	32	0.0067 (vs #106)
108	S3J	*Fs$^{GFSTF.1}$ / Fs^{MI}-GAL4*	0 days	10.32	±0.58	19	
109	S3J	*Fs$^{GFSTF.1}$ / Fs^{MI}-GAL4*	4 weeks	9.55	±0.37	51	0.2744 (vs #108)
110	S3J	*Fs^{MI}-GAL4 / Fs^{MI11350}*	0 days	13.21	±0.40	39	
111	S3J	*Fs^{MI}-GAL4 / Fs^{MI11350}*	4 weeks	11.48	±0.79	25	0.0576 (vs #110)
112	S3J	*Fs$^{TG4.1}$/ Fs^{MI11350}*	0 days	11.47	±0.63	15	
113	S3J	*Fs$^{TG4.1}$/ Fs^{MI11350}*	4 weeks	3.41	±0.43	66	2e^{-11} (vs #112)

Table S2: Genotypes, Related to Figures 1-5

Figure	Full genotype	Abbreviation used in Figure
1A,I	*yw / Y; tj-GAL4 / +;*	*tj > +*
1B,1	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722);*	*tj > Dp-RNAi*
1C,E,G	*yw, hs-Flp, tub>GAL4, UAS-nlsGFP / Y; FRT^{42D} tub>GAL80 / FRT ^{42D};*	Control clones
1D,F,H	*yw, hs-Flp, tub>GAL4, UAS-nlsGFP / Y; FRT^{42D} tub>GAL80 /FRT ^{42D} Dp^{a3};*	*Dp^{a3}* clones
1I	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722); UAS-GFP / +*	*tj > Dp-RNAi (GD), GFP*
1I	*yw / Y; tj-GAL4 / +; UAS-Dp-RNAi (TRiP JF02519) / +*	*tj> Dp-RNAi (TRiP)*
1I	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722); UAS-Dp / +*	*tj > Dp-RNAi (GD), Dp*
1I	*yw / Y; tj-GAL4 / +: UAS-E2f1-RNAi / +*	*tj > E2f1-RNAi*
1I	*yw / Y; tj-GAL4 / +: UAS-Rbf^{280} / +*	*tj > Rbf^{280}*
1J	*yw / Y; tj-GAL4 / +: hh-GAL80 / +*	*tj, hh-GAL80 > +*
1J	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722); hh-GAL80 / UAS-LacZ*	*tj, hh-GAL80 > Dp-RNAi, LacZ*
1J	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722); hh-GAL80 / UAS-Dp*	*tj, hh-GAL80 > Dp-RNAi, Dp*
2B,D	*w / Y; tj-GAL4 / UAS-LacZ; UAS-LacZ / UAS-Dicer-2*	*tj > LacZ, LacZ*
2C,D	*w / Y; tj-GAL4 / UAS-LacZ; UAS-Fs-RNAi / UAS-Dicer-2*	*tj > Fs-RNAi, LacZ*
2D	*w / Y; tj-GAL4 / UAS-Fs; UAS-Fs-RNAi / UAS-Dicer-2*	*tj > Fs-RNAi, Fs*
2D	*w /Y; tj-GAL4 / UAS-Fs; UAS-LacZ / UAS-Dicer-2*	*tj > LacZ, Fs*
2E,F	*w /Y; Fs^{null} / Fs^{null}*	*Fs^{null} -/-*
2F	*Oregon-R*	*wt*
2G,I	*w / Y; Fs-GAL4 / UAS-LacZ; UAS-GFP / +*	*Fs > GFP, LacZ*
2H,I	*w / Y; Fs-GAL4 / UAS-Dp-RNAi; UAS-GFP / +*	*Fs > GFP, Dp-RNAi*
2J	*yw / Y; tj-GAL4 / +; hh-GAL80 / +*	*tj, hh-GAL80 > +*
2J	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722); hh-GAL80 / UAS-LacZ*	*tj, hh-GAL80 > Dp-RNAi, LacZ*
2J	*yw/Y; tj-GAL4/UAS-Dp-RNAi (GD 12722); hh-GAL80 / UAS-Fs*	*tj, hh-GAL80 > Dp-RNAi, Fs*
3A	*w / Y; ; babo$^{fTRG00444.sfGFP-TVPTBF}$*	*babo-GFP*
3B	*upd-GAL4, tub-GAL80^{TS} / Y*	*upd^{TS} > +*
3B,C	*upd-GAL4, tub-GAL80^{TS} / Y;; UAS-babo^{QD} / +*	*upd^{TS} > babo^{QD}*
3D,F	*upd-GAL4, tub-GAL80^{TS} / Y; ubiP63E-FRT-STOP-FRT-GFP, UAS-Flp / +; +*	*upd^{TS} > lineage*
3E,F	*upd-GAL4, tub-GAL80^{TS} / Y; ubiP63E-FRT-STOP-FRT-GFP, UAS-Flp / +; UAS-babo^{QD} / +*	*upd^{TS} > babo^{QD}, lineage*
4B,D	*C587-GAL4 / Y; QUAS-Flp / +; act>y[+]>LHV2-86Fb, 13XlexAop2-myr::GFP / hh-QF*	*c587 > +, hh > lineage*

4C,D	*C587-GAL4 / Y; QUAS-Flp /UAS-Dp-RNAi (GD 12722); act>y[+]>LHV2-86Fb, 13XlexAop2-myr::GFP / hh-QF*	*c587 > Dp-RNAi, hh > lineage*
4E	*upd-GAL4, tub-GAL80^{TS} / Y; UAS-NLAP / + ; UAS-babo^{QD} / +*	*NLAP (control)*
4E	*upd-GAL4, tub-GAL80^{TS} / Y; UAS-esg^{NLAP} / + ; UAS-babo^{QD} / +*	*esg^{NLAP}*
5A,B,C	*w / Y; Fs-GAL4 / + ; UAS-GFP / +*	*Fs > GFP*
5D	*w / Y; tj-GAL4 / UAS-LacZ; UAS-Dicer-2 / +*	*tj-GAL4 > LacZ*
5D	*w / Y; tj-GAL4 / UAS-Fs; UAS-Dicer-2 / +*	*tj-GAL4 > Fs*
5E	*upd-GAL4, tub-GAL80^{TS} / Y; UAS-LacZ / +*	*upd^{TS} > LacZ*
5E	*upd-GAL4, tub-GAL80^{TS} / Y; UAS-smox-RNAi / +*	*upd^{TS} > smox-RNAi*
5E	*upd-GAL4, tub-GAL80^{TS} / Y; UAS-babo-RNAi / +*	*upd^{TS} > babo-RNAi*
5E	*upd-GAL4, tub-GAL80^{TS} / Y; UAS-daw-RNAi^{HMS01110} / +*	*upd^{TS} > daw-RNAi-HMS01110*
5E	*upd-GAL4, tub-GAL80^{TS} / Y; UAS-daw-RNAi^{HMJ03135} / +*	*upd^{TS} > daw-RNAi-HMJ03135*
5F	*yw / Y; daw^{MI05383}*	*daw-GFP*
5G	*w / Y; daw^{NP4661}-GAL4 / + ; UAS-GFP.nls*	*daw^{NP4661} > GFP*
5H	*Oregon-R*	*wt*
S1A,B	*yw, hs-Flp, tub-GAL4, UAS-nlsGFP / Y; FRT^{42D} tub-GAL80 / FRT^{42D} Dp^{a4}*	*Dp^{a4} clones*
S1B	*yw, hs-Flp, tub-GAL4, UAS-nlsGFP / Y; FRT^{42D} tub-GAL80 / FRT^{42D};*	*FRT^{42D} (control)*
S1B,C,E	*yw, hs-Flp, tub-GAL4, UAS-nlsGFP / Y; FRT^{42D} tub-GAL80 / FRT^{42D} Dp^{a3};*	*Dp^{a3} clones*
S1D	*yw, hs-Flp, tub-GAL4, UAS-nlsGFP / Y; UAS-Dp-RNAi (GD 12722) / +; FRT^{82B} tub-GAL80 / FRT^{82B}*	*Dp-RNAi clones*
S1F	*yw, hs-Flp, tub-GAL4, UAS-nlsGFP / Y; ; FRT^{82B} tub-GAL80 / FRT^{82B} E2f1^{729}*	*E2f1 clones*
S1G	*yw, hs-Flp, tub-GAL4, UAS-nlsGFP / Y; ; FRT^{82B} tub-GAL80 / FRT^{82B}*	*FRT^{82B} (control)*
S2A	*yw / Y; tj-GAL4 / +; hh^{P30} / +*	*tj > +; hh-LacZ*
S2B	*upd^{PD1} / Y; tj-GAL4 / +;*	*tj > +; upd-LacZ*
S2C	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722); hh^{P30} / +*	*tj > Dp-RNAi; hh-LacZ*
S2D	*upd^{PD1} / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722)*	*tj > Dp-RNAi; upd-LacZ*
S2E	*yw / Y; tj-GAL4 / +; tub-GAL80^{TS} / +*	*tj^{TS} > +*
S2E	*yw / Y; tj-GAL4 / UAS-Dp-RNAi (GD 12722); tub-GAL80^{TS} / +*	*tj^{TS} > Dp-RNAi*
S2E	*yw / Y; tj-GAL4 / +; tub-GAL80^{TS} / UAS-E2f1-RNAi*	*tj^{TS} > E2f1-RNAi*
S2F	*yw, hs-Flp / Y; eyaA3-GAL4, M5-4-LacZ / +;*	*eyaA3 > +*
S2F	*yw, hs-Flp / Y; eyaA3-GAL4, M5-4-LacZ / UAS-Dp-RNAi (GD 12722);*	*eyaA3 > Dp-RNAi*
S2F	*C587-GAL4 / Y; ; Chd64-GFP / +*	*C587 > +*
S2F	*C587-GAL4 / Y; UAS-Dp-RNAi (GD 12722) / +; Chd64-GFP / +*	*C587 > Dp-RNAi*
S2F	*yw, hs-Flp / Y; tub-GAL80^{TS} / +; fng-GAL4 / +*	*fng^{TS} > +*
S2F	*yw, hs-Flp / Y; tub-GAL80^{TS} / UAS-Dp-RNAi (GD 12722); fng-GAL4 / +*	*fng^{TS} > Dp-RNAi*

S2F	*yw, hs-Flp / Y; tub-GAL80^{TS} / +; fng-GAL4 / UAS-E2f1 RNAi*	*fng^{TS}> E2f1-RNAi*
S3B,C,E	*w / Y; tj-GAL4 / + ; UAS-Fs-RNAi / UAS-Dicer-2*	*tj > Fs-RNAi*
S3E	*w / Y; tj-GAL4 / + ; UAS-Dicer-2 / +*	*tj > +*
S3E	*Oregon-R*	*wt*
S3E,G	*w / Y; Fs^{null} / Fs^{null}*	*Fs$^{null\ -/-}$*
S3F	*w / Y; nos-GAL4-VP16 / + ; +*	*nos > +*
S3F	*w / Y; nos-GAL4-VP16 / + ; UAS-Fs-RNAi / +*	*nos > Fs-RNAi*
S3H	*upd-GAL4, tub-GAL80^{TS} / Y; Fs^{null} / + ; +*	*upd^{TS} > +, Fs^{null} / +*
S3H	*upd-GAL4, tub-GAL80^{TS} / Y; Fs^{null} / + ; UAS-Fs / +*	*upd^{TS} > Fs, Fs^{null} / +*
S3H	*upd-GAL4, tub-GAL80^{TS} / Y; Fs^{null} / Fs^{null} ; +*	*upd^{TS}> +, Fs$^{null\ -/-}$*
S3H	*upd-GAL4, tub-GAL80^{TS} / Y; Fs^{null} / Fs^{null} ; UAS-Fs / +*	*upd^{TS}> Fs, Fs$^{null\ -/-}$*
S3I	*w / Y; Fs^{null} / + ; fng-GAL4 / +*	*fng > +, Fs^{null} / +*
S3I	*w / Y; Fs^{null} / + ; fng-GAL4 / UAS-Fs*	*fng > Fs, Fs^{null} / +*
S3I	*w/ Y; Fs^{null} / Fs^{null} ; fng-GAL4 / +*	*fng > +, Fs$^{null\ -/-}$*
S3I	*w / Y; Fs^{null} / Fs^{null} ; fng-GAL4 / UAS-Fs*	*fng > Fs, Fs$^{null\ -/-}$*
S3J	*w / Y; Fs$^{GFSTF.1}$ / Fs^{MI11350}*	*Fs$^{GFSTF.1}$ / Fs^{MI11350}*
S3J	*w / Y; Fs$^{TG4.1}$ / Fs^{MI}-GAL4*	*Fs$^{TG4.1}$ / Fs^{MI}-GAL4*
S3J	*w / Y; Fs$^{GFSTF.1}$ / Fs$^{TG4.1}$*	*Fs$^{GFSTF.1}$ / Fs$^{TG4.1}$*
S3J	*w / Y; Fs$^{GFSTF.1}$ / Fs^{MI}-GAL4*	*Fs$^{GFSTF.1}$ / Fs^{MI}-GAL4*
S3J	*w / Y; Fs^{MI}-GAL4 / Fs^{MI11350}*	*Fs^{MI}-GAL4 / Fs^{MI11350}*
S3J	*w / Y; Fs$^{TG4.1}$/ Fs^{MI11350}*	*Fs$^{TG4.1}$/ Fs^{MI11350}*
S4A,C	*yw / Y; tj-GAL4/Fs$^{MI04308\text{-}GFSTF.1\ (Fs\text{-}GFP)}$;*	*tj > +*
S4B,C	*yw / Y; tj-GAL4/ Fs$^{MI04308\text{-}GFSTF.1\ (Fs\text{-}GFP)}$; UAS-E2f1-RNAi / +*	*tj > E2f1-RNAi*
S5B,C	*C587-GAL4 / Y; QUAS-Flp / +; ubiP63E-FRT-STOP-FRT-GFP / hh-QF*	+ (in B) *C587 > +, hh > lineage (GTRACE)* (in C)
S5B,D	*C587-GAL4 / Y; QUAS-Flp / UAS-Dp-RNAi (GD 12722); ubiP63E-FRT-STOP-FRT-GFP / hh-QF*	*Dp-RNAi* (in b) *C587 > Dp-RNAi, hh > lineage (GTRACE)* (in D)
S5E	*C587-GAL4 / Y; QUAS-Flp / +; act>y[+]>LHV2-86Fb, 13xlexAop2-myr::GFP / hh-QF*	*C587 > +, hh > lineage (FlexAmp)*
S5F,G	*C587-GAL4 / Y; QUAS-Flp / UAS-Dp-RNAi (GD 12722); act>y[+]>LHV2-86Fb, 13xlexAop2-myr::GFP / hh-QF*	*C587 > Dp-RNAi, hh > lineage (FlexAmp)*
S6A	*w / Y; daw^{NP6274}-GAL4, UAS-LacZ / + ; UAS-GFPnls*	*daw^{NP6274} > GFP*
S6B	*w / Y; ; babo$^{fTRG00444.sfGFP\text{-}TVPTBF}$*	*babo-GFP*
S6C	*w / Y; ; Actβ-GAL4 / UAS-GFPnls*	*Actβ-GAL4 > GFP*
S6D	*w / Y; ; Actβ$^{fTRG00506.sfGFP\text{-}TVPTBF}$*	*Actβ-GFP*
S6E	*w / Y; myo-GAL4 / UAS-GFPnls*	*myo-GAL4 > GFP*
S6F	*w / Y; ; myo$^{fTRG00161.sfGFP\text{-}TVPTBF}$*	*myo-GFP*
S6G	*w / Y; ; put$^{fTRG00910.sfGFP\text{-}TVPTBF}$*	*put-GFP*

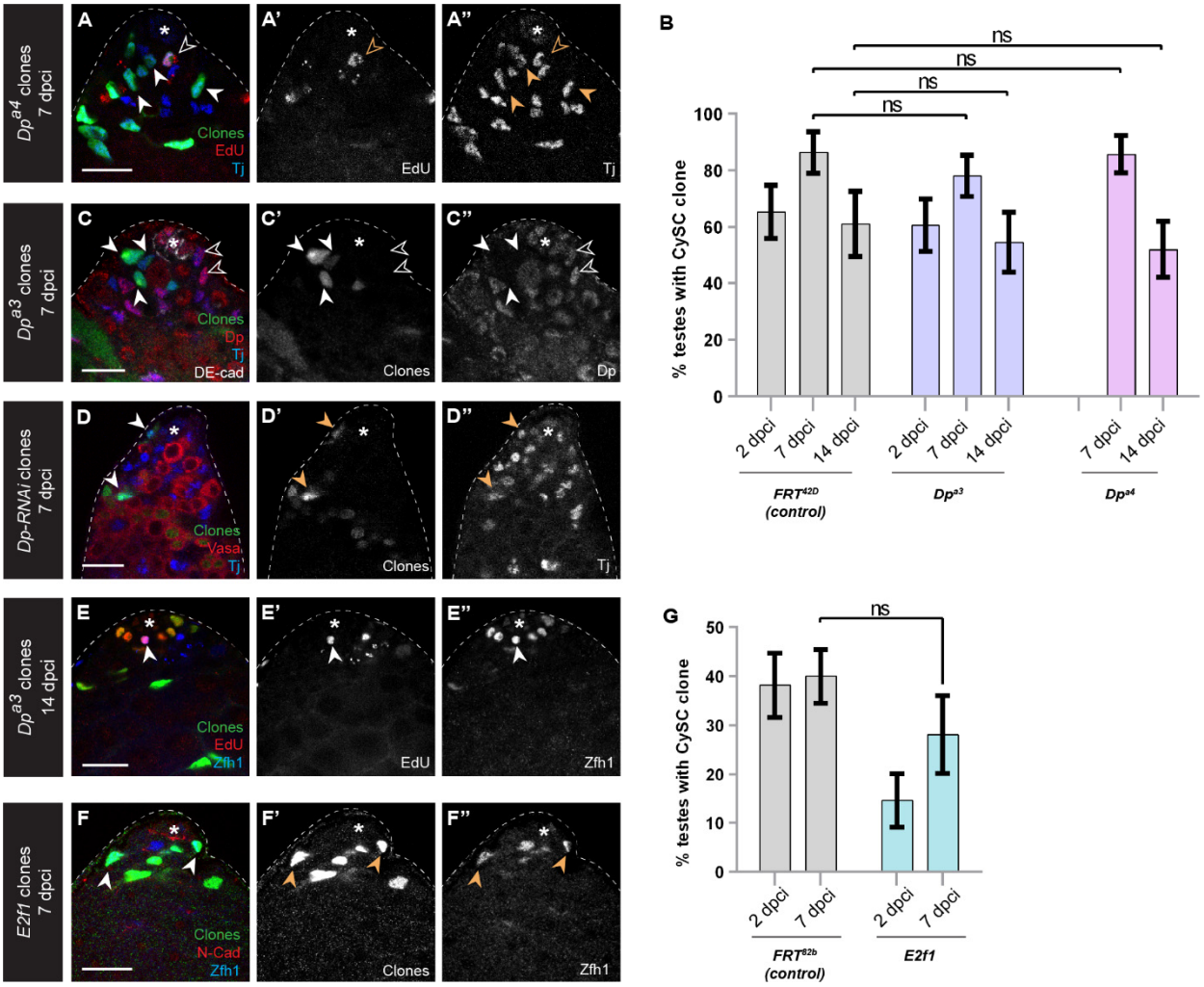

Figure S1: CySCs lacking *Dp* can self-renew and produce daughter cells that differentiate, Related to Fig. 1

(**A**) CySC clones (green) mutant for Dp^{a4} can remain in the niche for at least 7 days after clone induction (dpci). Arrowheads mark the CySC clones and the open arrowhead a CySC clone positive for EdU (red, A'). Tj (blue, A") labels CySCs and early cyst cells.

(**B**) Graph indicating the clone recovery rates at 2, 7 and 14 dpci for control clones (labeled FRT^{42D}, gray bars, n=26, 22, 18, respectively), Dp^{a3} mutant clones (blue bars, n=28, 32, 22, respectively), and Dp^{a4} mutant clones (purple bars, n=28, 25 respectively). ns = not significant, assessed by Fisher's exact test. Error bars represent SEM.

(**C**) A Dp^{a3} mutant clone (green, C') at 7 dpci, labeled with an antibody against Dp protein (red, C"). Mutant CySCs (closed arrowheads) lack detectable Dp. Neighboring wild type CySCs, marked with open arrowheads, express Dp protein (C").

(**D**) CySC clones expressing the same RNAi construct targeting Dp as in Fig. 1 (arrowheads) can be recovered at 7 dpci. GFP (green) marks the clones. Tj (blue) marks CySCs and early cyst cell. Vasa (red) labels the germline.
(**E**) A Dp^{a3} mutant clone (green) at 14 dpci labeled with EdU. The clone contains several Zfh1-positive (blue, D"), EdU-positive (red, D') CySCs (arrowhead shows one such CySC).
(**F**) CySC clones (green) mutant for *E2f1* can be recovered after 7 days. (The arrowheads show two such CySCs). Zfh1 (blue) marks CySCs and early cyst cells. N-Cad (red) marks the hub.
(**G**) Graph indicating the clone recovery rates at 2 and 7 dpci for control clones (labeled FRT^{82B}, gray bars, n=35 and 80, respectively) and *E2f1* mutant clones (blue bars, n=41 and 32, respectively). ns = not significant, assessed by Fisher's exact test. Error bars represent SEM.
The hub is shown with an asterisk in A, C, D, E, F.
Scale bar = 20 μM.

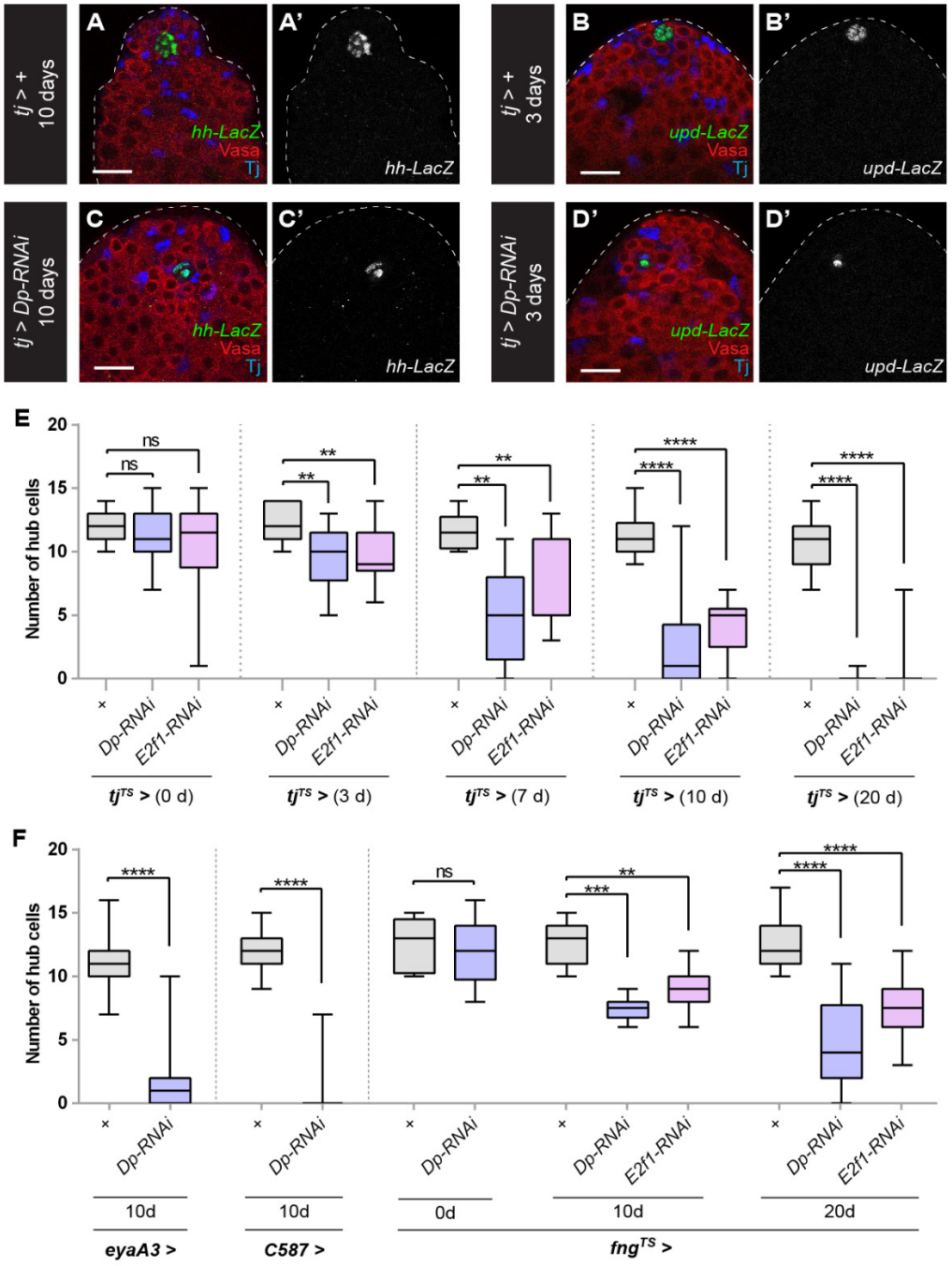

Figure S2: Hub cells are lost when Dp/E2f1 complex activity is depleted from CySCs, Related to Fig. 1.

(**A-D**) Hub cells in control *tj* > + testes (A,B) express *hh-LacZ* and *upd-LacZ*, but the number of cells expressing these markers is greatly reduced in *tj* > *Dp-RNAi* testes (C,D) after 10 and 3 days at 29ºC, respectively.

(**E**) Graph of the number of hub cells in *tj*^{TS} > + (gray bars, n=17, 21, 8, 35, 17 at 0, 3, 7, 10, 20 days, respectively), *tj*^{TS} > *Dp-RNAi* (purple bars, n=28, 14, 6, 39, 30 at 0, 3, 7, 10, 20 days, respectively); *tj*^{TS} > *E2f1-RNAi* (pink bars, n=18, 17, 11, 29, 22 at 0, 3, 7, 10, 20 days, respectively). Flies were reared at 18ºC until eclosion and then reared at 29ºC for the indicated time periods.

(**F**) Graph of the number of hub cells in control testes (gray bars, n=32 for *eyaA3* > +, n=35 for *C587* > +, n=12, 7, and 37 for *fng*^{TS} > + at 0, 10 and 20 days, respectively), or in testes with knockdown of *Dp* (purple bars, n=43 for *eyaA3* > *Dp-RNAi,* n=52 for *C857* > *Dp-RNAi,* n=14, 9, 32 for *fng*^{TS} > *Dp-RNAi* at 0, 10, and 20 days, respectively) or *E2f1-RNAi* (pink bars, n=16 and 12 for *fng*^{TS} > *E2f1-RNAi* at 10 and 20 days, respectively) in *eya* >, *C587* >, or *fng*^{TS} > at the indicated time periods.

Error bars represent the data range. **** P < 0.0001; *** P < 0.001; ** P < 0.01; ns = not significant, as assessed by Student's t-test. See Supplementary Table 1 for exact P values. Scale bar = 10 μM.

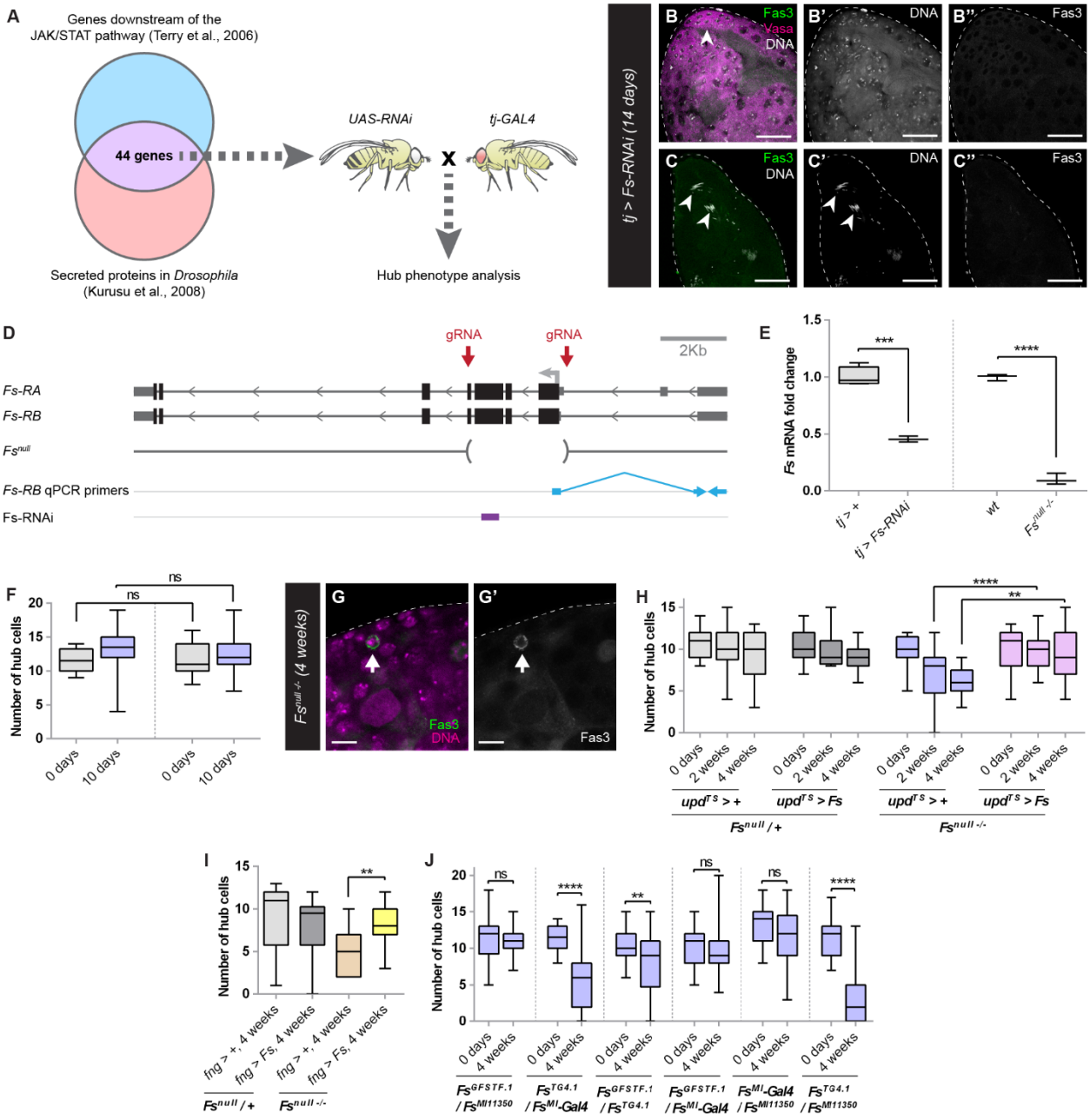

Figure S3: *Fs* loss-of-function results in hub cell loss, Related to Fig. 2.

(**A**) Schematic of the screen performed to identify secreted factors from CySCs influencing hub fate. Briefly, we cross-referenced genes enriched in testis stem cells with the list of *Drosophila* secreted proteins. We obtained flies carrying RNAi lines targeting the resulting 44 genes and crossed them to a *tj-GAL4* line, and scored for phenotypes on hub cells.

(**B, C**) Representative confocal images of testes from *tj > Fs-RNAi* at 2 weeks of adulthood. Note the absence of Fas3-positive hub cells in both images. Arrowhead in (B) indicates a

differentiating 16-cell spermatogonia. Arrowhead in (C) indicates canoe stage spermatids that normally are located basally. Due to the absence of any mitotic cells, these differentiated cells are observed more apically. Vasa (magenta) labels germ cells in (B); Fas3 (green) marks hub cells and Topro (white) marks DNA.

(**D**) Model of the *Fs* genomic locus and the two predicted *Fs* isoforms (*Fs-RA* and *Fs-RB*). The first line below the genomic locus shows the approximate extent of the deletion spanning coding exons 1-4 in the Fs^{null} allele created with gDNAs in the positions shown with red arrows. The location of the primers used for qPCR and the sequence targeted by the RNAi is shown below. Gray boxes represent non-coding exons and black boxes the coding exons.

(**E**) Left panel: expression of *Fs* mRNA in control *tj* > + (gray bar) *and tj* > *Fs-RNAi* (blue bar) as measured by qRT-PCR (n=4). Right panel: expression of *Fs* mRNA in wild (wt) type testes (gray bar) and Fs^{null} mutant testes (blue bar) as measured by qRT-PCR (n=4).

(**F**) Number of hub cells in control *nos* > + (gray bar, n=10 and 12 at 0 and 10 days, respectively) *and nos* > *Fs-RNAi* (blue bar, n=12 and 17 at 0 and 10 days, respectively) at 0 and 10 days of adulthood.

(**G**) Representative confocal image of a Fs^{null} testis at 4 weeks of adulthood. Fas3 (green) staining reveals only one hub cell (arrow) remaining in these testes. Topro (magenta) marks DNA.

(**H**) Graph of number of hub cells at different time points of adulthood in testes carrying the hub cell driver *upd-GAL4* in an heterozygous Fs^{null}/+ (light gray bars) or homozygous Fs^{null}/Fs^{null} (blue bars) background; or in testes in which *upd-GAL4* drives *UAS-Fs* in an heterozygous Fs^{null}/+ (dark gray bars) or homozygous Fs^{null}/Fs^{null} (purple bars) background. See Supplementary Table 1 for n values.

(**I**) Graph of number of hub cells at different time points of adulthood in testes carrying the somatic cell driver *fng-GAL4* in a heterozygous Fs^{null}/+ (light gray bar, n=14) or homozygous Fs^{null}/Fs^{null} (dark gray bar, n=11) background; or in testes in which *fng-GAL4* drives *UAS-Fs* in a heterozygous Fs^{null}/+ (brown bar, n=14) or homozygous Fs^{null}/Fs^{null} (yellow bar, n=24) background.

(**J**) Graph of number of hub cells at 0 and 4 weeks of adulthood in heteroalleic combinations of the indicated *Fs* hypomorphic mutant alleles. See Supplementary Table 1 for n values.

Error bars represent the data range. **** P < 0.0001; *** P < 0.001; ** P < 0.01; ns = not significant, assessed by Student's t-test.

Scale bar = 50 μM.

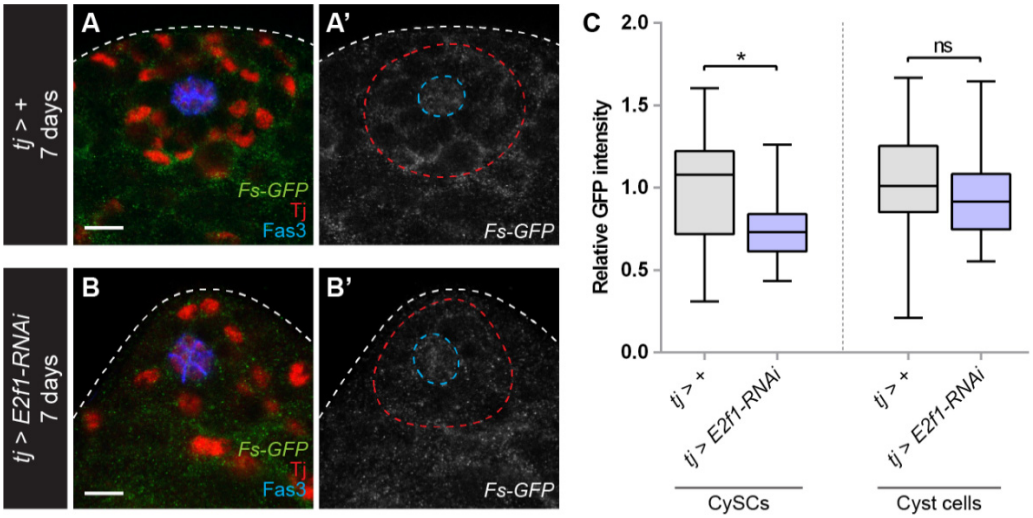

Figure S4: Depletion of *E2f1* from the cyst lineage causes a significant decrease in Fs protein in the testis stem cell microenvironment, Related to Fig. 2.

(**A, B**) Representative confocal images of the expression pattern of a Fs protein trap (Fs^{GFP} in green) in control *tj* > + testis (A) and a *tj* > *E2f1-RNAi* testis in which E2f1 was depleted from the cyst cell lineage for 7 days (B). Tj (red) marks CySCs and early cyst cells and Fas3 (blue) marks hub cells. Dashed blue line indicates cluster of hub cells. Red dashed line indicates CySCs within 1 cell diameter of the hub.

(**C**) Graph of relative GFP intensity in *tj* > + testes (gray bars, n=15) or *tj* > *E2f1-RNAi* testes (blue bars, n=23) of CySCs (i.e., the area between the dashed blue and red lines in A,B) and in cyst cells (i.e., the area basal to the red dashed line in A,B).

Error bars represent the data range. * $P < 0.05$; ns = not significant, assessed by Student's t-test.

Scale bar = 10 μM.

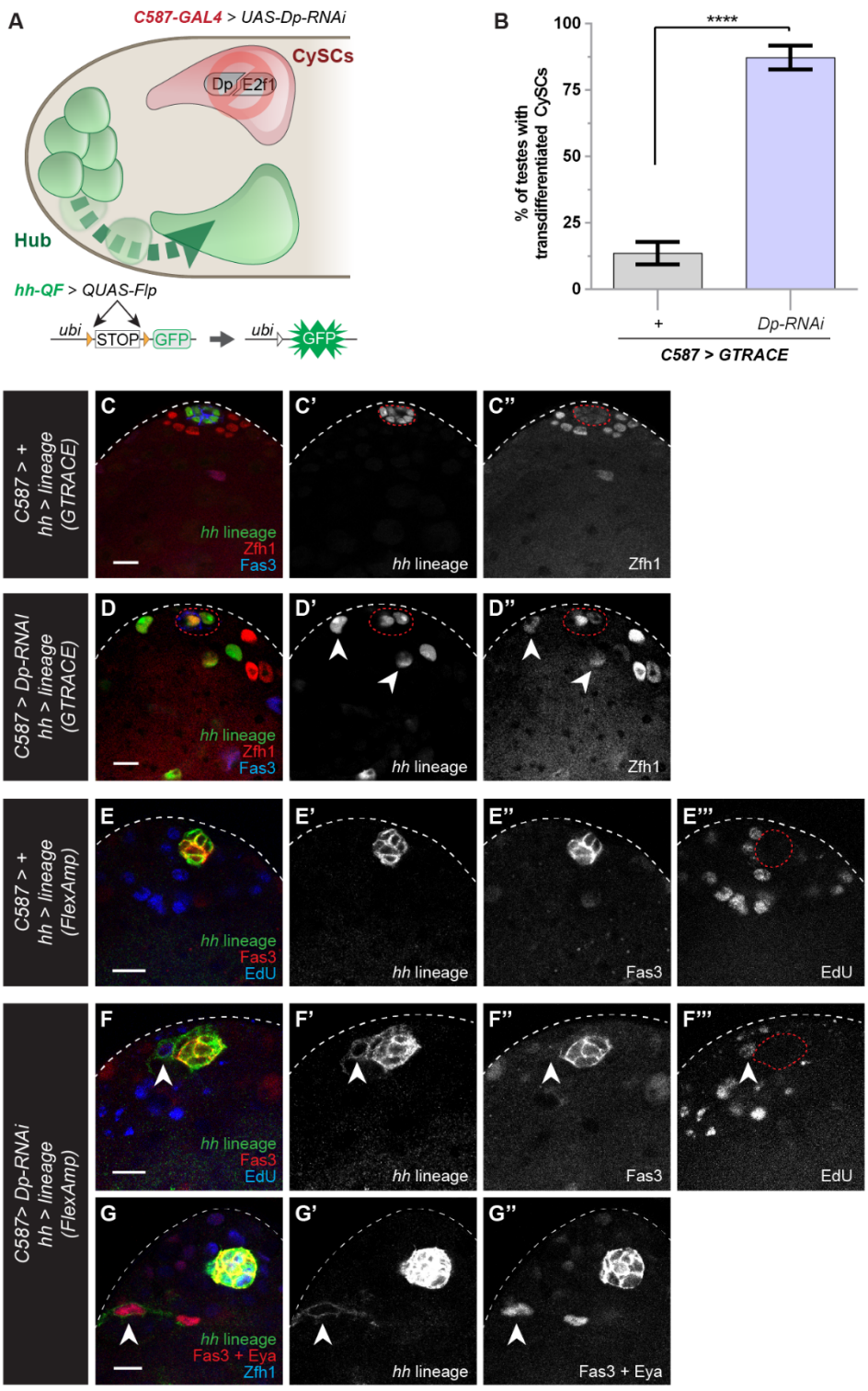

Figure S5: Depletion of *Dp* from the cyst lineage causes transdifferentiation of hub cells into CySCs, Related to Fig 4.

(**A**) Model indicating the alternative experimental design (referred to as “hh > lineage (GTRACE)”) to deplete Dp from CySCs while concomitantly lineage tracing hub cells using

independent binary expression systems, complementary to the design shown in Figure 4A (referred to as “hh > lineage (FlexAmp)”). Experimental data are shown in (B). To trace the lineage of hub cells, we used *hh-QF*, which is expressed in only hub cells, to induce *QUAS-FLP*. In turn, FLP recombines *FRT* sites in the *ubi>STOP>GFP* GTRACE transgene. This leads to the labeling of hub cells and their lineage with nuclear GFP. At the same time, *C587-GAL4* drives expression of a *Dp-RNAi* transgene, which depletes Dp from CySCs but not from hub cells.

(**B**) Graph showing the percentage of testes with hub lineage-positive CySCs in *C587* > + (gray bar, n=67) and *C587* > *Dp-RNAi* (blue bar, n=48) testes, using the experimental design indicated in (A).

(C, D) Example images of *C587* > + (C) and *C587* > *Dp-RNAi* (D) testes, using the experimental design indicated in (A). Only in the latter genotype can GFP (green)-positive cells from the hub lineage (D, arrowheads) be found outside the hub. Zfh1 (red) marks CySCs; Fas3 (blue) marks hub cells. A red dashed line outlines the hub.

(**E-G**) There are GFP (green)-positive cells lacking the hub cell marker Fas3 (red) (F-F’”, arrowhead) located outside the cluster of hub cells in a *C587* > *Dp-RNAi; hh* > *lineage* (*FLEXAMP*) testis but not in a control *C587* > *+; hh* > *lineage* (*FLEXAMP*) testis (E), following the experimental design indicated in **Fig. 4A**. These GFP-positive cells also incorporate EdU, showing that they undergo DNA replication (F’”, arrowhead) and differentiate into cyst cells, which express Eya and have thin, long membranes wrapping differentiating germ cell cysts (G-G”, arrowhead). A red dashed line outlines the hub. The *hh* lineage is green in (E-G). EdU is blue and Fas3 is red in (E,G). Fas and Eya are red and Zfh1 is blue in (G).

Error bars represent SEM. **** P < 0.0001, assessed by Fisher’s exact test.

Scale bar = 10 μM.

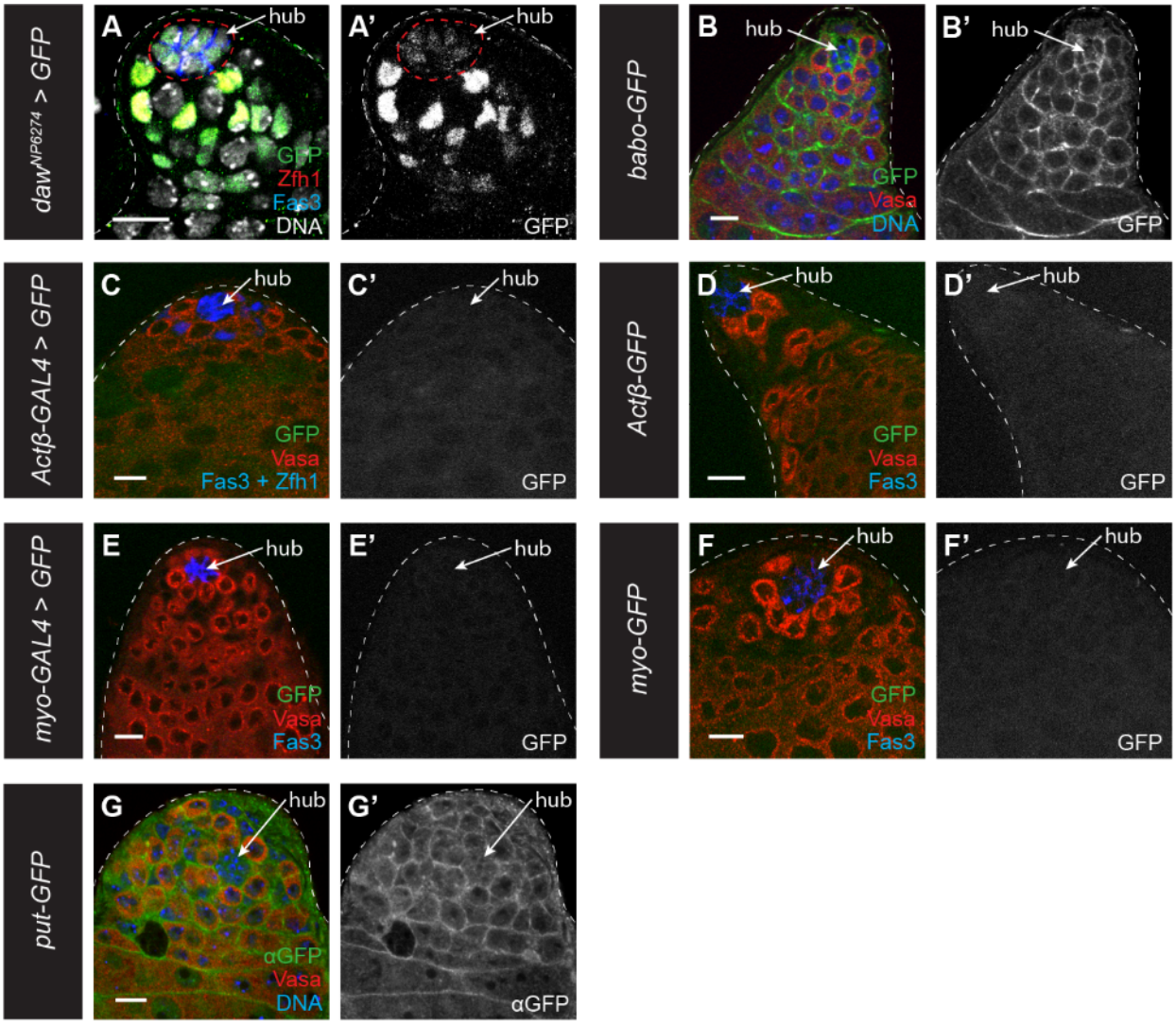

Figure S6: Expression pattern of Activin ligands and receptors in the *Drosophila* testis, Related to Fig. 5.

(A) A *daw-GAL4* enhancer trap driving *UAS-GFP* (GFP). The *GAL4* insertions drives GFP expression in Fas3-positive hub cells and Zfh1-positive CySCs. GFP is green; Fas3 is blue; Zfh1 is red; DAPI (white) marks DNA.

(B) Babo, as assessed by a fosmid *babo-GFP* transgene, is present at the surface of hub cells, CySCs and cyst cells and early germ cells. GFP is green; Vasa is red and Topro, which marks DNA, is blue.

(C) *Actβ-GAL4* driving expression of *UAS-GFP*. GFP is not detected in any cells of the testis stem cell niche. GFP is green; Vasa is red; Fas3 and Zfh1 are both blue.

(D) Actβ, as assessed by a fosmid *Actβ-GFP* transgene, is not detected in any cells in the testis stem cell niche. GFP is green; Vasa is red; and Fas3 is blue.
(E) *myo-GAL4* driving expression of *UAS-GFP*. GFP is not detected in any cells of the testis stem cell niche. GFP is green; Vasa is red; Fas3 is blue.
(F) Myo, as assessed by a fosmid *myo-GFP* transgene, is not detected in any cells in the testis stem cell niche. GFP is green; Vasa is red; and Fas3 is blue.
(G) The type II receptor Put is not detected in hub cells in the testis stem cell niche, as assessed by a fosmid *put-GFP* transgene. GFP is green; Vasa is red; and Fas3 is blue.
Scale bar = 10 μM.