Supplementary Figure Legends

Supplementary Figure 1. PH3 staining reveals, that the high number of SSCs that is caused by EcR overexpression is not due to fusome breakdown.

(A) Cells in developing germline cysts that are connected via a fusome are dividing simultaneously as shown here by PH3 mitotic marker. **(B)** The SSCs that are observed upon exogenous *hsEcR.A* expression do not have their division synchronized. Red, Adducin+LaminC; blue, DAPI; and green PH3**.**

Supplementary Figure 2. Tai expression in escort cells.

(A-B) To confirm the specificity of Tai antibody staining in escort cells we generated *tai* loss of function somatic clones *(hs Flp*; tai^{61G1} *FRT40A/UbiGFP FRT40A)* and observed that Tai staining is diminished in *tai* mutant cells. Compare levels of antibody staining in *tai* mutant escort cell (white arrows) and sister clones (green arrows).

Red, Taiman; blue, DAPI; and green, GFP.

Supplementary Figure 3.

Tai is not required for progressive oocyte development and GSC maintenance.

(A) tai^{61G1} loss of function clones in the germarium do not affect the steady production of egg chambers, showing that loss of Tai does not affect GSC division or oocyte differentiation.

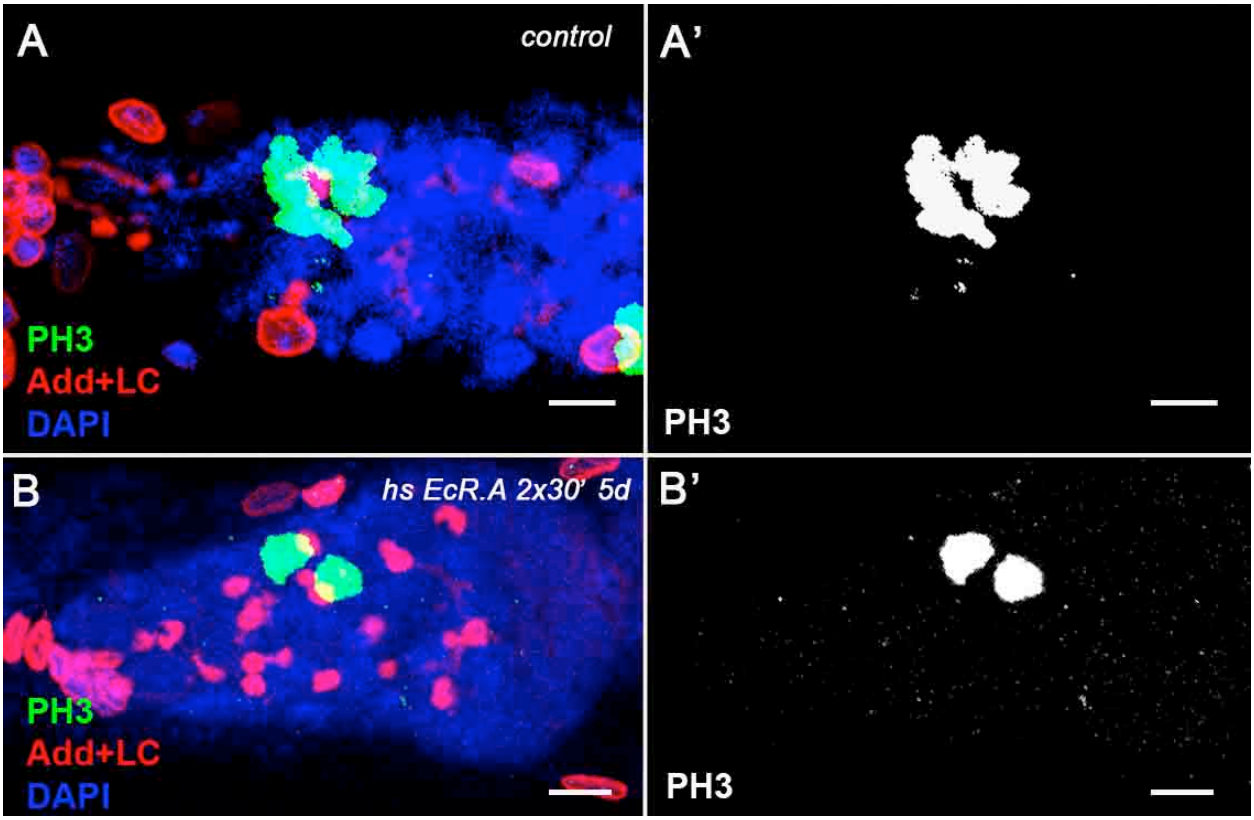
(B) tai^{61G1} or tai^{k15101} mutations do not affect the maintenance of GSC compared to parental GSC clones.

Red, Adducin+LaminC; blue, DAPI; and green, GFP.

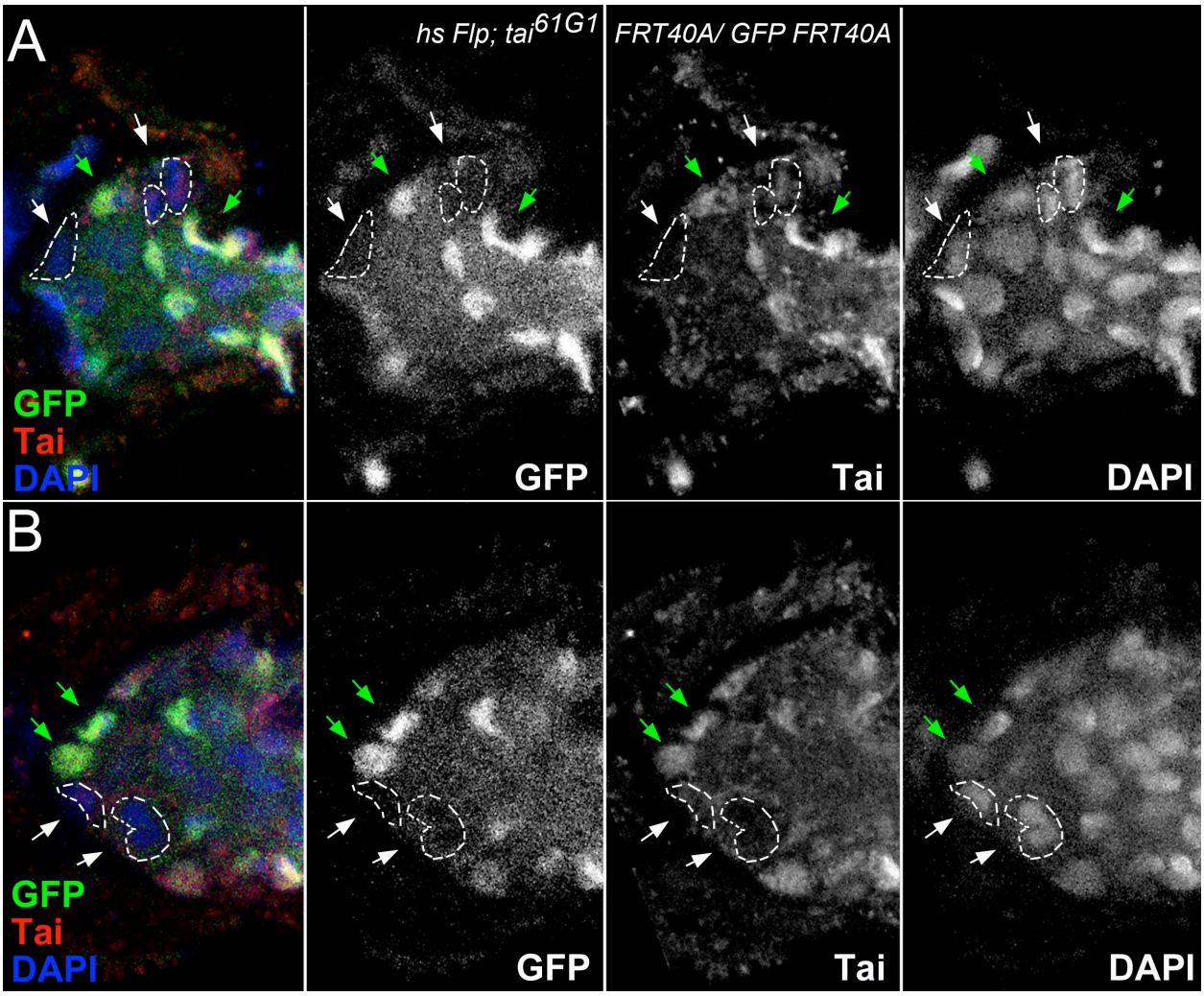
Supplementary Figure 4. *ptcGal4* and *bab1Gal4*, the drivers used in this study drive *UAS lacZ* expression in the somatic cells of the germarium

Whereas *bab1Gal4* (*UAS lacZ/+; bab1Gal4/+***)** drives expression in CpCs (pink arrowheads), ECs and FCs **(A),** *ptcGal4* (*UAS lacZ/ptcGal4)* is only active in ECs and FCs, but not in the CpCs (pink arrowheads, **B)**.

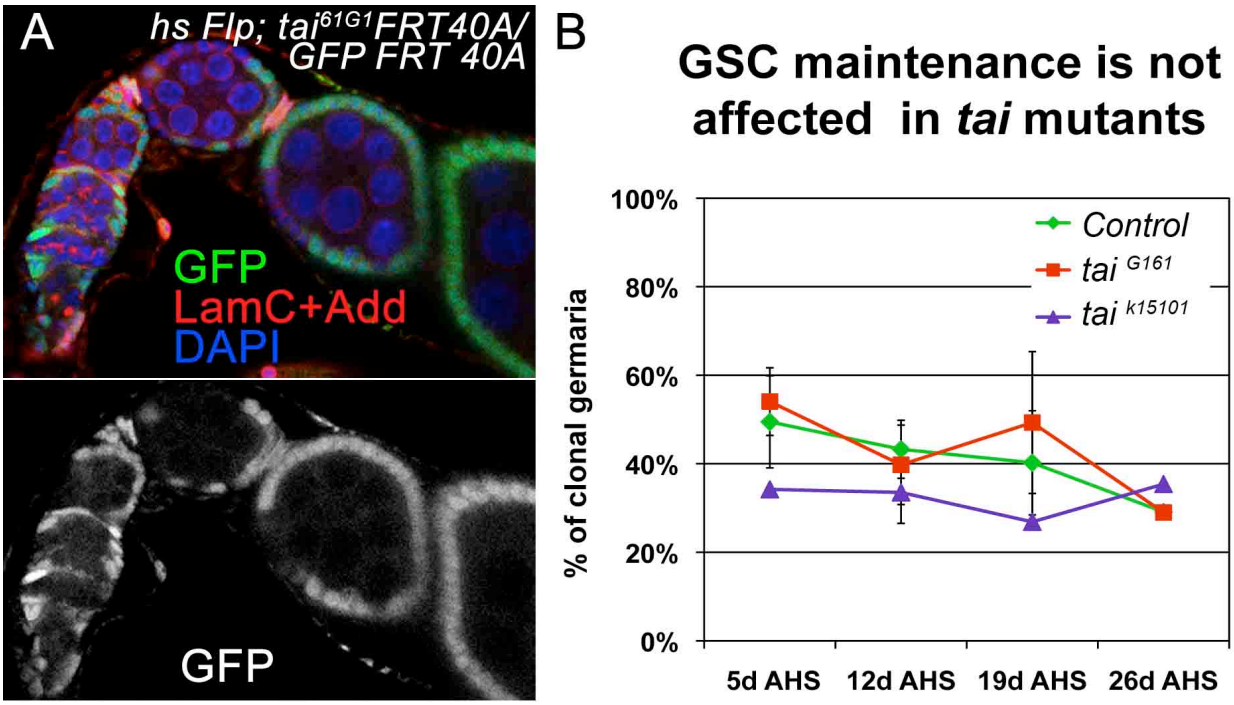
(A, B) are projections of optical sections assembled through the germarial tissue. Red, Adducin+LaminC; blue, DAPI; and green, β-Galactosidase**.**



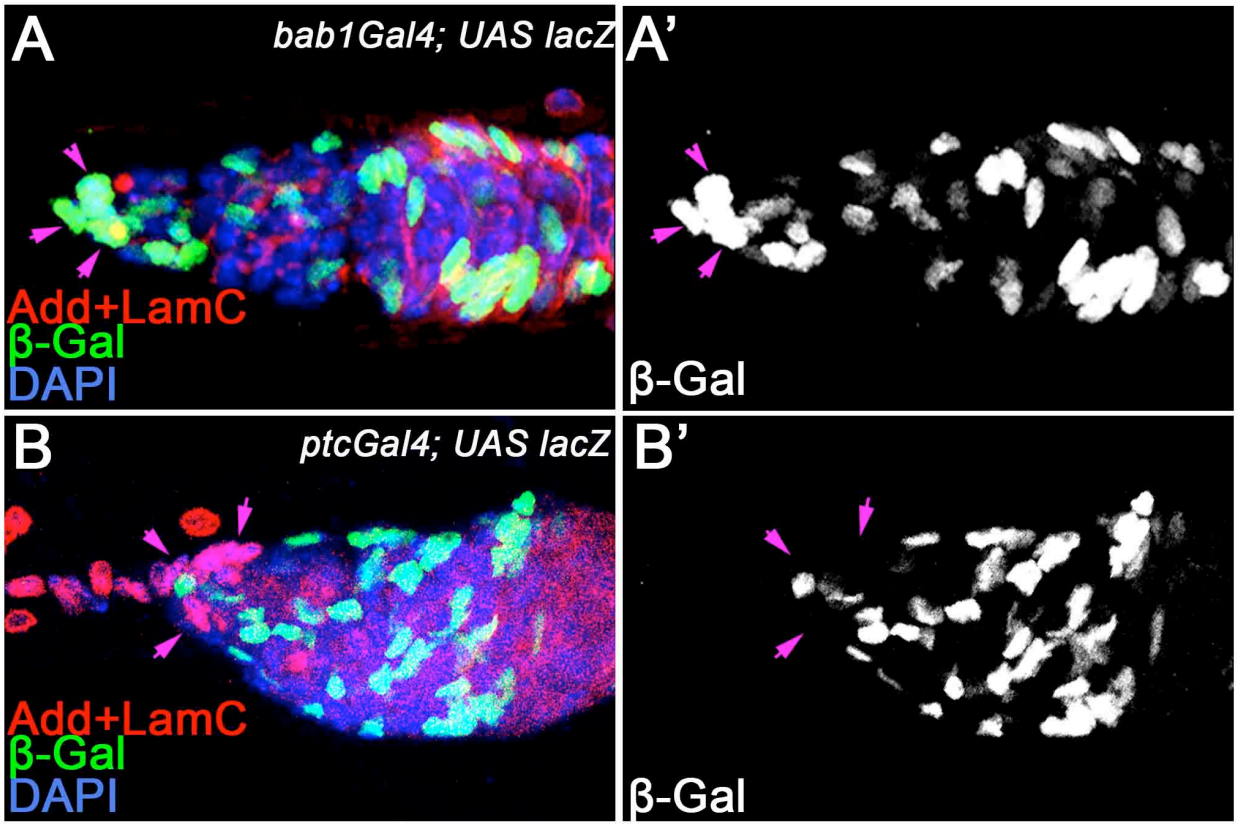
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Supplementary Table S1. Loss of function of ecdysone receptor co-activator *tai* increases number of niche and germline stem cells

Allele	*x tai^{G161}*			*x tai ^{k15101}*		
	# of CpCs AVE±SEM	**# of GSCs AVE±SEM**	**# of germaria analyzed**	**# of CpCs AVE±SEM**	**# of GSCs AVE±SEM**	**# of germaria analyzed**
	p (compared to *tai^{G161}/ w^{1118}*)			**p (compared to *tai ^{k15101}/ w^{1118}*)**		
Control, w^{1118}	6.56±0.24	2.44±0.13	25	5.63±0.22	2.11±0.08	27
tai ^{G161}	lethal			7.10±0.28 $p=2.08x10^{-4}$	3.45±0.20 $p=1.52x10^{-7}$	31
tai ^{01315}	10.50±0.61 $p=6.20x10^{-11}$	4.00±0.25 $p=6.30x10^{-11}$	11	6.33±0.53 p=0.16	3.22±0.32 $p=2.55x10^{-6}$	9
tai ^{EY11718}	7.40±0.51 p=0.16	3.67±0.26 $p=4.49x10^{-3}$	5	N/A		
tai ^{KG02309}	9.40±0.70 p=0.02	3.40±0.54 $p=2.34x10^{-5}$	10	N/A		
tai ^{BG02711}	10.56±0.60 $p=1.11x10^{-9}$	5.11±0.39 $p=1.60x10^{-8}$	10	N/A		
EcR^{Q50st}	8.40±0.78 $p=5.08x10^{-3}$	3.70±0.21 $p=1.30x10^{-5}$	10	7.86±0.93 $p=1.31x10^{-3}$	3.71±0.61 $p=3.88x10^{-5}$	7
usp^{4}	9.00±0.85 $p=9.68x10^{-4}$	3.67±0.27 $p=3.30x10^{-5}$	12	8.07±0.38 $p=6.70x10^{-7}$	2.79±0.19 p=0.10	14
usp ^{EP1193}	8.13±0.31 $p=4.50x10^{-3}$	3.50±0.51 $p=1.05x10^{-3}$	8	5.92±0.36 p=0.48	2.25±0.13 p=0.36	12

p-value was calculated using the two tailed Students t-test.

Supplementary Table S2. **Reduction of ecdysone signaling via *ecd1^{ts}* mutation or dominant negative EcR forms increases the number of single spectrosome cells and delays cyst differentiation**

Genotype	Number of CpCs Ave ± SEM	Number of GSCs Ave ± SEM	Number of SSCs Ave ± SEM	Number of Cysts Ave ± SEM	Ratio Cyst/SSC Ave ± SEM	# of analyzed germaria
w ^{1118} 5d at 29°C	5.78±0.25	2.18±0.12	3.91±0.31	4.82±0.38	1.30±0.12	11
ecd $^{4210\ ts}$ 1d at 29°C	5.71±0.30 (p=0.89)	2.19±0.19 (p=0.98)	9.81±1.12 ($p=2.38\times10^{-4}$)***	4.63±0.87 (p=0.86)	0.63±0.13 ($p=1.76\times10^{-3}$)**	16
ecd $^{4210\ ts}$ 3-5d at 29°C	5.77±0.17 (p=0.98)	1.80±0.19 (p=0.26)	7.47±0.36 ($p=1.58\times10^{-6}$)***	5.80±0.46 (p=0.23)	0.84±0.08 ($p=4.16\times10^{-3}$)**	30
ecd $^{4210\ ts}$ 7d at 29°C	6.00±0.22 (p=0.59)	1.62±0.20 (p=0.04)*	7.23±0.99 (p=0.01)*	3.85±0.47 (p=0.15)	0.66±0.10 ($p=6.61\times10^{-4}$)***	15
hs-Gal4-usp.LBD hs 30' 1-3d	6.20±0.30 (p=0.47)	3.20±0.37 ($p=4.73\times10^{-3}$)**	7.20±0.74 ($p=2.34\times10^{-4}$)***	5.40±0.51 (p=0.39)	0.78±0.10 (p=0.02)*	5
hs-Gal4-EcR.LBD hs 30' 1-3d	6.29±0.18 (p=0.23)	2.75±0.31 (p=0.08)	7.13±0.72 ($p=3.00\times10^{-4}$)***	4.86±0.38 (p=0.95)	0.67±0.05 ($p=1.30\times10^{-3}$)**	8

p-value was calculated using the two tailed Students t-test. *p<0.05, **p<0.005. ***p<0.0005

Supplementary Table S3. Adult EcR overexpression causes germline differentiation delay in the germarium that can be recovered by supplying 20E

Genotype	Condition	exp	Number of CpCs Ave ± SEM	Number of GSCs Ave ± SEM	Number of SSCs Ave ± SEM	Number of Cysts Ave ± SEM	Ratio Cyst/SSC Ave ± SEM	# of analyzed germaria
w^{1118}	control 25°C	I	6.33±0.20	2.88±0.23	4.5±0.38	4.63±0.46	1.09±0.16	8
	20E 25°C	I	5.78±0.19 [a](p=0.27)	2.26±0.24 [a](p=0.14)	3.84±0.32 [a](p=0.24)	4.16±0.24 [a](p=0.34)	1.34±0.23 [a](p=0.51)	19
hsEcR.A/+	control 25°C	I	6.00±0.22 [a](p=0.53)	2.67±0.19 [a](p=0.50)	4.80±0.31 [a](p=0.84)	5.31±0.27 [a](p=0.20)	1.13±0.16 [a](p=0.91)	15
		II	5.58±0.26	2.42±0.15	4.50±0.38	5.76±0.28	1.35±0.11	12
		III	5.67±0.24	2.22±0.22	4.00±0.33	5.44±0.44	1.44±0.16	9
	20E 25°C	I	6.40±0.31 [a](p=0.91)	2.50±0.17 [a](p=0.19)	4.50±0.65 [a](p=1.00)	5.40±0.40 [a](p=0.22)	1.57±0.33 [a](p=0.25)	10
		II	6.43±0.36	2.20±0.13	3.80±0.42	6.40±0.27	1.89±0.22	10
		III	5.67±0.27	2.44±0.18	5.44±0.73	5.33±0.33	1.27±0.29	9
w^{1118}	control heat shock	I	5.90±0.28 [a](p=0.44)	2.50±0.27 [a](p=0.32)	4.50±0.56 [a](p=1.00)	5.80±0.36 [a](p=0.06)	1.49± 0.22 [a](p=0.18)	10
		III	6.13±0.26	2.30±0.15	4.90±0.55	5.80±0.44	1.32±0.19	10
hsEcR.A/+	heat shock	I	5.70±0.21 [b](p=0.57) [c](p=0.36)	2.90±0.28 [b](p=0.31) [c](p=0.48)	11.0±0.75 [b]($p=1.68\times10^{-6}$)*** [c]($p=5.24\times10^{-9}$)***	2.30±0.67 [b]($p=2.14\times10^{-4}$)*** [c]($p=1.94\times10^{-4}$)***	0.19±0.05 [b]($p=1.95\times10^{-5}$)*** [c]($p=1.49\times10^{-4}$)***	10
		II	6.09±0.28 [c](p=0.20)	2.82±0.18 [c](p=0.10)	13.8±1.40 [c]($p=1.79\times10^{-6}$)***	2.6±0.29 [c]($p=4.33\times10^{-7}$)***	0.17±0.03 [c]($p=1.99\times10^{-9}$)***	11
		III	5.91±0.23 [c](p=0.48)	3.15±0.19 [c]($p=4.93\times10^{-3}$)**	13.46±1.26 [c]($p=5.72\times10^{-6}$)***	5.69±0.54 [c](p=0.74)	0.50±0.10 [c]($p=3.39\times10^{-5}$)***	13
	20E heat shock	I	5.9±0.35 [d](p=0.63)	3.6±0.22 [d](p=0.06)	10.2±0.87 [d](p=0.50)	4.70±0.50 [d](p=0.01)*	0.50±0.08 [d]($p=4.53\times10^{-3}$)**	10
		II	6.00±0.21 [d](p=0.80)	3.00±0.15 [d](p=0.44)	12.5±0.67 [d](p=0.44)	3.86±0.35 [d](p=0.02)*	0.33±0.04 [d](p=0.01)*	14
		III	5.92±0.22 [d](p=0.98)	2.46±0.14 [d]($p=7.96\times10^{-3}$)*	9.62±0.74 [d]($p=1.45\times10^{-2}$)*	7.15±0.30 [d]($p=2.52\times10^{-2}$)*	0.79±0.07 [d]($p=2.00\times10^{-2}$)*	13

Adult *hsEcR.A/+* or w^{1118} flies were treated as indicated. Heat shocks were performed twice per day for 30 min each. 1 µM Ecdysone (20E) was diluted in 5% Ethanol. For control 5% Ethanol was used.

p-value was calculated using the two tailed Students t-test. *p<0.05. **p<0.005. ***p<0.0005
a Compared to w^{1118} flies that were kept without heat shocks on 5% Ethanol for control.
b Compared to w^{1118} flies that were heat shocked for control.
c Compared to *hsEcR.A/+* flies of the respective experiment that were kept without heat shocks on 5% Ethanol for control.
d Compared to *hsEcR.A/+* flies of the respective experiment where overexpression of EcR.A was induced via daily heat shocks on 5% Ethanol.

Supplementary Table S4. Ecdysone receptor co-activator *tai* is not required for germline stem cell maintenance

Genotype	Experiment	% of germaria with clonal GSCs				Average GSC loss per day ±SD, %	GSCs half-life, days
		Time-point I (5d after hs)	Time-point II (12d after hs)	Time-point III (19d after hs)	Time-point IV (26d after hs)		
Control, parental hsFLP; FRT40A /FRT40A GFP	Exp I	56.8% n=44	35.7% n=28	28.3 % n=32	ND	1.74±2.82%	≥ 3 weeks
	Exp II	42.1% n=38	46.8% n=47	51.6% n=31	ND		
	Exp III	ND	47.3% n=55	40.9% n=66	29.1% n=86		
tai^{61G1} hsFLP; FRT40A tai^{61G1}/FRT40A GFP	Exp I	48.7% n=37	30.8% n=26	46.2% n=13	ND	1.83±2.08%	≥ 3 weeks
	Exp II	59.5% n=37	48.7% n=39	66.7% n=15	ND		
	Exp III	ND	39.8% n=103	35.1% n=57	29.0% n=100		
tai^{k15101} hsFLP; FRT40A tai^{k15101}/FRT40A GFP	Exp I	34.2% n=38	38.5% n=39	ND	ND	-1.07±1.68%	≥ 3 weeks
	Exp III	ND	28.6% n=77	26.9% n=67	35.4% n=48		

n=number of germaria analyzed

GSC loss per day=(% of clonal GSC at time-point 1 -% of clonal GSC at time-point 2)x100%/ % of clonal GSC at time-point 1/elapsed time

GSCs half-life=elapsed time x log[2]/log[% of clonal germaria at time-point1/% of clonal germaria at time-point2]

Supplementary Table S5. Ecdysone signaling alteration in soma causes germline differentiation delay

Genotype	days on 29°C	Number of CpCs Ave ± SEM	Number of GSCs Ave ± SEM	Number of SSCs Ave ± SEM	Number of Cysts Ave ± SEM	Ratio Cyst/SSC Ave ± SEM	# of analyzed germaria
Control*	*	6.19±0.19	2.15±0.15	4.00±0.31	4.82±0.27	1.40±0.12	33
tubGal80^{ts}/+; UAS EcR RNAi^{97}/ bab1Gal4	7d	6.16±0.29 (p=0.94)	2.42±0.26 (p=0.34)	4.79±0.36 (p=0.11)	3.89±0.25 (p=0.03)*	0.88±0.09 (p=5.18x10^{-3})**	19
tubGal80^{ts}/+; UAS EcR RNAi^{97}/ bab1Gal4	21d	6.93±0.32 (p=0.09)	2.21±0.21 (p=0.82)	8.57±0.83 (p=7.84x10^{-3})**	4.57±0.37 (p=0.60)	0.62±0.09 (p=3.41x10^{-4})***	14
tubGal80^{ts}/+; UAS EcR RNAi^{104} /bab1Gal4	7d	6.36±0.27 (p=0.67)	2.75±0.30 (p=0.06)	5.00±0.55 (p=0.11)	3.92±0.34 (p=0.07)	0.92±0.17 (p=0.04)*	12
tubGal80^{ts}/+; UAS EcR RNAi^{104}/ bab1Gal4	15d	5.86±0.44 (p=0.52)	2.07±0.16 (p=0.76)	11.0±3.90 (p=0.01)*	3.55±0.57 (p=0.04)*	0.55±0.11 (p=3.34x10$^{-4)}$***	14
ptcGal4/+; UAS EcR RNAi^{97}/ tubGal80^{ts}	14d	6.47±0.36 (p=0.54)	2.80±0.34 (p=0.05)	6.00±0.59 (p=2.30x10^{-3})*	NC	NC	14
ptcGal4/+; UAS EcR RNAi^{97}/ tubGal80^{ts}	21d	6.38±0.26 (p=0.671)	2.63±0.18 (p=0.150)	6.25±0.59 (p=1.86x10^{-3})*	NC	NC	8

Control*: *tubGal80^{ts}; UAS GFP/TM6, tubGal80^{ts}; UAS GFP/bab1Gal4, tubGal80^{ts}; UAS EcR RNAi ^{97}/TM6, tubGal80^{ts}; bab1Gal4/CyO;* analyzed at different time points

The expression of *EcR RNAi* during larval development is lethal. Therefore we used the *tubGal80^{ts}* system. Flies were raised at 18°C where *tubGal80^{ts}* suppresses the expression of Gal4. Transferring the adult flies to 29°C caused Gal4 and therefore *UAS EcR RNAi* expression in the soma. NC: Counting of cysts not possible due to strong morphological abnormalities. p-value was calculated using two tailed Students t-test. *p<0.05, **p<0.005. ***p<0.0005

Supplementary Table S6. Overexpression of *EcR* during niche development in larval somatic ovarian cells causes an increase in the number of CpCs

Genotype		Number of CpCs Ave ± SEM	Number of SSCs Ave ± SEM	# of analyzed germaria
ptcGal4 X	control *UAS lacZ*	5.70±0.34	4.20±0.25	10
	UAS EcR.A	7.47±0.32 [a](p=1.63x10^{-3})**	4.47±0.47 [a](p=0.68)	17
	UAS EcR.B1	6.14±0.32 [a](p=0.41)	4.00±0.35 [a](p=0.72)	22
bab1Gal4 X	control *UAS lacZ*	5.91±0.29	4.91±0.37	11
	UAS EcR.A	9.31±0.42 [a](p=3.72x10^{-5})*** [b](p=7.08x10^{-3})*	7.38±0.49 [a](p=7.34^{-3})** [b](p=3.91^{-4})***	32
	UAS EcR.B1	9.29±0.53 [a](p=5.73x10^{-5})*** [b](p=7.10x10^{-6})***	5.94±0.37 [a](p=0.07) [b](p=5.99x10^{-4})**	17
W^{1118} X	control *UAS EcR.A*	6.36±0.24	5.73±0.57	11
	control *UAS EcR.B1*	6.83±0.50	5.00±1.00	12

UAS EcR.A/bab1Gal4 and *UAS EcR.B1/bab1Gal4* express exogenous EcR in the CpCs. *ptcGal4/+; UAS EcR.A/+ and ptcGal4/+; UAS EcR.B1/+* express exogenous *EcR* in the other somatic ovarian cells, but not in CpCs (for expression patterns see Supplementary Figure S4). The stem cell marker pMad was used to confirm GSC identitiy if CpC number was increased. a *ptcGal4/+; UAS EcR/+* and *UAS EcR/bab1Gal4* were compared to *ptcGal4/+; UAS lacZ/+* or *bab1Gal4/UAS lacZ* respectively. b *UAS EcR.A* or *B1* driven by bab1Gal4 were compared to the *UAS EcR.A* or *B1* driven by *ptcGal4.* The p-value was calculated using two tailed Student´s t-test. *p<0.05, **p<0.005. ***p<0.0005.