Supporting Information

Hsu and Drummond-Barbosa 10.1073/pnas.0809144106

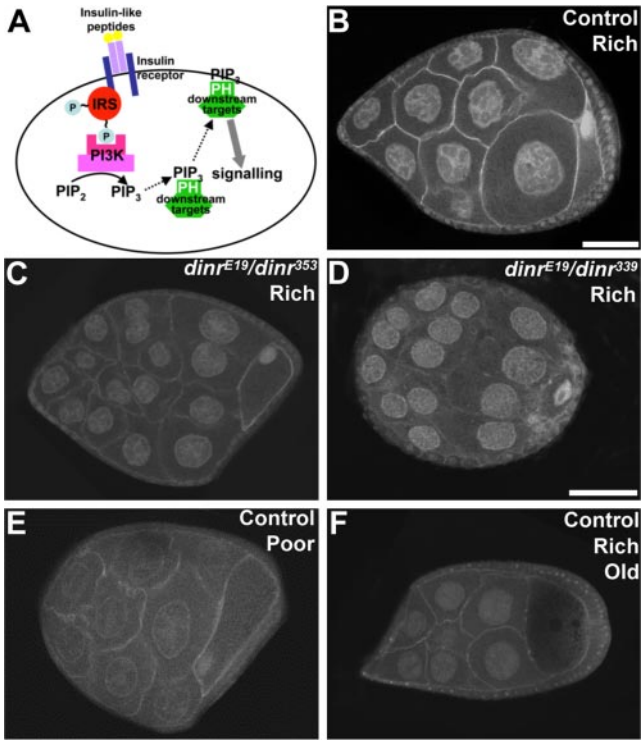

Fig. S1. Insulin signaling levels in the ovary are reduced under a poor diet or in older females. (*A*) Stimulation of the insulin receptor results in insulin receptor substrate (IRS) phosphorylation and phosphoinositide 3-kinase (PI3K) activation. PI3K-generated phosphatidylinositol (3, 4, 5)-trisphosphate binds specifically to the pleckstrin homology (PH) domain–containing proteins, recruiting them to the membrane. The PH domain–GFP fusion (tGPH) reporter becomes enriched in cell membranes upon PI3K activation. The enrichment of the tGPH reporter at the plasma membrane of germ cells in egg chambers of 1-week-old control females (*B*) is decreased in $dinr^{E19}/dinr^{353}$ females (*C*) and virtually abolished in $dinr^{E19}/dinr^{339}$ females (*D*) on a protein-rich diet, reflecting the relative severity of these *dinr* mutant combinations. (*E*) Reduced tGPH in germ cell membranes of wild-type females kept on a protein-poor diet for 2 days. (*F*) Reduced tGPH in germ cell membranes of 4-week old wild-type females on a rich diet. The ratio of the average tGPH intensity in the membrane region vs. the cytoplasmic region was determined using Microsoft ImageJ software: 1-week-old (rich diet), 1.56 ± 1.19 ($n = 20$); 4-week-old (rich diet), 1.07 ± 0.11 ($n = 27$, $P < 0.001$); rich diet (1-week-old), 1.51 ± 0.17 ($n = 13$); poor diet (1-week old), 1.18 ± 0.12 ($n = 14$, $P < 0.001$). *n*, number of germ cells quantitatively analyzed. *P* values were calculated using the Student's *t* test. (*B*, *D*, *E*, and *F* are at the same magnification. Scale bars: *B*, 50 μm; *D*, 40 μm.)

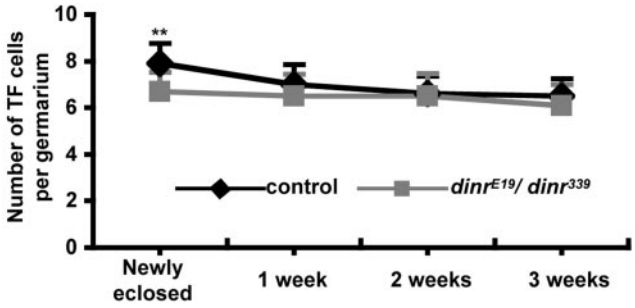

Fig. S2. Impaired insulin signaling does not result in increased rate of terminal filament (TF) cell loss. The number of TF cells per germarium in control and $dinr^{339}/dinr^{E19}$ germaria at different ages is similar, except for a slight reduction in *dinr* mutants at eclosion. Consistent with that, we do not detect an effect of diet on the number of TF cells (H.-J.H. and D. D.B., unpublished data). Degeneration of previtellogenic egg chambers is also not observed in females on a poor diet or mutant for *dinr* (H.-J.H. and D. D.B., unpublished data), further underscoring that the effect of diet and insulin signaling on cap cell number is specific. $^{**}P < 0.01$.

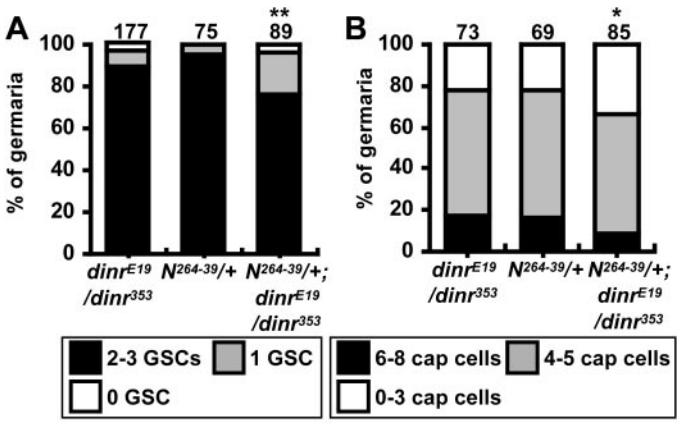

Fig. S3. *dinr* and *Notch* genetically interact to maintain cap cell and GSC numbers. Number of GSCs (*A*) or cap cells (*B*) per germarium in weak hypomorphic $dinr^{E19}/dinr^{353}$ mutants, null N^{264-39} heterozygotes, and combined $N^{264-39}/+$; $dinr^{E19}/dinr^{353}$ 1-week-old females, showing dominant enhancement of $dinr^{E19}/dinr^{353}$ by N^{264-39}. The number of germaria analyzed is shown above each bar. $*P < 0.05$, $**P < 0.01$.

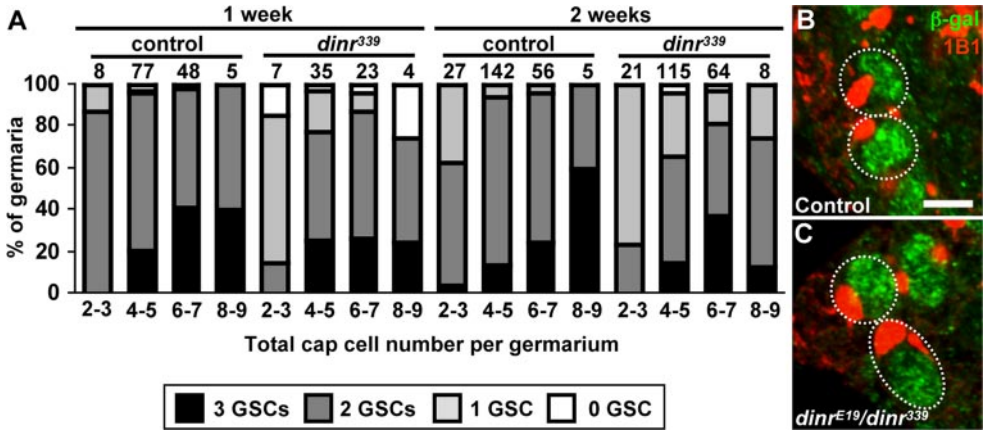

Fig. S4. The presence of *dinr* mutant cap cells within the cap cell population increases the rate of GSC loss independent of BMP signals. (*A*) Distribution of GSC number per germarium in relation to total cap cell number in mosaic germaria from control (in which β-gal–negative wild-type cap cells are present within the cap cell population) or $dinr^{339}$ (in which β-gal–negative $dinr^{339}$ mutant cap cells are present within the cap cell population) mosaic females at 1 or 2 weeks after eclosion. In germaria containing control β-gal–negative cap cells, the total cap cell number is roughly proportional to the GSC number; however, the cap cell–to-GSC ratio is not fixed, suggesting variability in cap cell potential to maintain GSCs. Side-by-side comparisons for any given total cap cell number within the population of germaria analyzed suggest that the number of GSCs tends to be smaller if $dinr^{339}$ cap cells are present within that population. The number of germaria showing each total cap cell population size is shown above the bars. GSCs in *Dad-lacZ* $dinr^{E19}$/*TM3* (*B*, control) and *Dad-LacZ* $dinr^{E19}/dinr^{339}$ (*C*) germaria labeled with β-gal (green) and 1B1 (red, fusome) show similar levels of BMP signaling. Dashed ovals, GSCs. (Scale bar, 5 μm.)

Table S1. Insulin signaling is required for GSC maintenance

		Percentage of germaria														
		Newly enclosed			1 week			2 weeks			3 weeks			4 weeks		
Genotypes	Diet*	2 or 3 GSCs	1 GSC	0 GSC	2 or 3 GSCs	1 GSC	0 GSC	2 or 3 GSCs	1 GSC	0 GSC	2 or 3 GSCs	1 GSC	0 GSC	2 or 3 GSCs	1 GSC	0 GSC
yw	R	95	5 (176)†	0	97	2 (496)	1	83	15 (273)	2	62	30 (498)	8	34	46 (247)	20
	P		n.d.		86	12 (112)	2	69	27 (71)	4	31	43 (108)‖	26	21	53 (146)‖	26
dinr^{353} or dinr^{E19}/TM3	R	94	6 (85)	0	92	7 (60)	2	81	17 (90)	2		n.d.			n.d.	
dinr^{339} or dinr^{E19}/TM3	R	95	3 (229)	2	96	4 (198)	1	93	6 (106)	1		n.d.			n.d.	
dinr^{353} dinr^{E19}	R	85	14 (141)	2	91	6 (177)	3	81	14 (195)	5	50	34 (72)	16		n.d.	
dinr^{339}/dinr^{E19}	R	80	19 (172)	1	57	29 (317)‖	14	46	36 (172)‖	18	33	47 (147)	20	18	55 (78)	27
Notch$^{264–39}$/+	R		n.d.		95	5 (75)	0		n.d.			n.d.			n.d.	
Notch$^{264–239}$/+; dinr^{353}/dinr^{E19}	R		n.d.		76	20 (89)§	4		n.d.			n.d.			n.d.	
chico^{1}/CyO	R	92	5 (123)	3	95	5 (127)	0	81	17 (66)	1		n.d.			n.d.	
chico^{1}	R	85	15 (63)	0	79	12 (120)‖	9	65	29 (77)‡	6		n.d.			n.d.	
hh-lacZ/+	R	99	1 (166)	0	97	3 (146)	0	75	23 (147)	2		n.d.			n.d.	
hh-lacZ dinr^{339}/+	R	96	4 (107)	0	90	8 (140)	2	76	18 (112)	6	67	27 (127)	6		n.d.	
hh-lacZ dinr^{339}/dinr^{E19}	R	79	20 (101)‖	1	54	37 (178)‖	8	57	37 (277)‖	6	41	31 (145)‖	28		n.d.	
	P		n.d		61	37 (96)	2		n.d			n.d			n.d	
c587-GAL4/+	R	99	(71)	0		n.d.			n.d.		57	38 (191)	5	19	53 (145)	29
UAS- dilp2/+	R	97	3 (90)	0		n.d.			n.d.		65	26 (291)	9	42	38 (110)	20
c587-GAL4/+; UAS-dilp2/+	R	98	2 (175)	0		n.d.			n.d.		80	15 (413)§	5	78	19 (361)‖	3
	P		n.d.			n.d.			n.d.		85	15 (122)‡	0	50	38 (108)	12
c587-GAL4/+; UAS-dilp2/+; hh-lacZ/+	R	97	3 (115)	0	96	4 (110)	0	91	9 (119)	0	76	21 (176)§	3	64	32 (133)‖	4
c587-GAL4/+; CyO/+; dinr^{E19}GAL80^{ts}/dinr^{339}hhLacZ	R29		n.d.		56	14 (86)	30		n.d.			n.d.			n.d.	
c587-GAL4/+; UAS-inr^{WT}/+; dinr^{E19}GAL80^{ts}/dinr^{339}hhLacZ	R29		n.d.		95	5 (122)‖	0		n.d.			n.d.			n.d.	
c587-GAL4/+; CyO/+; dinr^{E19} or dinr^{339}hhLacZ/TM3	R		n.d.		97	3 (39)	0		n.d.			n.d.			n.d.	
c587-GAL4/+; CyO/+; dinr^{E19}/dinr^{339}hhLacZ	R		n.d.		75	21 (108)	3		n.d.			n.d.			n.d.	
c587-GAL4/+; UAS-N^{act}/+; dinr^{E19}/dinr^{339}hhLacZ	R		n.d.		97	3 (60)‖	0		n.d.			n.d.			n.d.	

Flies were cultured under a protein-rich diet at room temperature (22–23° C), except where indicated (R29, flies were cultured at 29° C), and food was changed daily until dissection.

*A protein-rich diet (R) consists of standard medium supplemented with wet yeast paste, whereas a protein-poor diet (P) consists of 5% molasses.

†Total number of germaria analyzed is shown in parentheses.

‡Significant difference relative to controls: $P < 0.05$.

§Significant difference relative to controls: $P < 0.01$.

‖Significant difference relative to controls: $P < 0.001$.

n.d., not determined.

Table S2. Insulin signaling controls cap cell number

		Percentage of germaria																	
		Newly eclosed			2 days			1 week			2 weeks			3 weeks			4 weeks		
Genotypes	Diet*	≥6 cap cells	4–5 cap cells	3–0 cap cells	≥6 cap cells	4–5 cap cells	0–3 cap cells	≥6 cap cells	4–5 cap cells	0–3 cap cells	≥6 cap cells	4–5 cap cells	0–3 cap cells	≥6 cap cells	4–5 cap cells	0–3 cap cells	≥6 cap cells	4–5 cap cells	0–3 cap cells
yw	R	23	58 (125)†	19	12	66 (97)	22	9	78 (204)	13	9	66 (163)	25	7	42 (189)	51	5	32 (109)	63
	P		n.d.			n.d.		17	56 (109)	27	8	31 (65)‖	62		n.d.		0	25 (56)‡	75
dinr^{353}/dinr^{E19}	R	10	65 (71)	25	23	59 (78)	18	17	61 (73)	22	5	54 (117)	41	15	31 (72)	54		n.d.	
dinr^{339}/dinr^{E19}	R		n.d.			n.d.		9	57 (118)	34		n.d.			n.d.			n.d.	
Notch$^{264–39}$/+	R		n.d.			n.d.		16	62 (69)	22		n.d.			n.d			n.d.	
Notch$^{264–39}$/+; dinr^{353}/dinr^{E19}	R		n.d.			n.d.		8	60 (85)‡	32		n.d.			n.d			n.d.	
chico^{1}/CyO	R		n.d.			n.d.		36	55 (53)	9		n.d.			n.d			n.d.	
chico^{1}	R		n.d.			n.d.		11	56 (57)‖	33		n.d.			n.d			n.d.	
hh-lacZ/+	R	7	66 (165)	27	8	60 (192)	31	6	71 (145)	23	6	66 (146)	27	1	46 (249)	53		n.d.	
hh-lacZ dinr^{339}/+	R	8	72 (107)	19	14	63 (120)	23	21	61 (141)	18	12	54 (103)	34	3	57 (115)	41		n.d.	
hh-lacZ dinr^{339}/ dinr^{E19}	R	8	59 (101)	33	3	43 (108)‖	54	6	44 (98)‖	50	3	44 (93)‖	53	0	33 (75)‖	67		n.d.	
c587-GAL4/+	R	29	69 (70)	3		n.d.			n.d.			n.d.		8	49 (90)	43		n.d.	
UAS-dilp2/+	R	16	60 (68)	24		n.d.			n.d.			n.d.		2	31 (118)	67		n.d.	
c587-GAL4/+; UAS-dilp2/+	R	27	65 (125)	8		n.d.			n.d.			n.d.		16	72 (145)‖	12	23	63 (104)	14
c587-GAL4/+; UAS- dilp2/+; hh-LacZ/+	R	22	65 (122)	13	20	68 (66)	12	30	67 (109)	3	26	62 (122)	12	13	65 (167)‖	22	7	72 (128)	21
c587-GAL4/+; CyO/+; dinr^{E19} or dinr^{339}hhLacZ/TM3	R		n.d			n.d		65	31 (52)	4		n.d			n.d			n.d	
c587-GAL4/+; CyO/+; dinr^{E19}/dinr^{339}hhLacZ	R		n.d			n.d		22	55 (105)	23		n.d			n.d			n.d	
c587-GAL4/+; UAS-N^{act}/+; dinr^{E19}/dinr^{339}hhLacZ	R		n.d			n.d		88	12 (59)‖	0		n.d			n.d			n.d	

*See Table S1. Flies were cultured at room temperature (22–23° C), except where indicated (R29, flies were cultured at 29° C), and food was changed daily until dissection.

†Total number of germaria analyzed is shown in parentheses.

‡Significant difference relative to controls: $P < 0.05$.

‖Significant difference relative to controls: $P < 0.001$.

n.d., not determined.