

Supplementary information

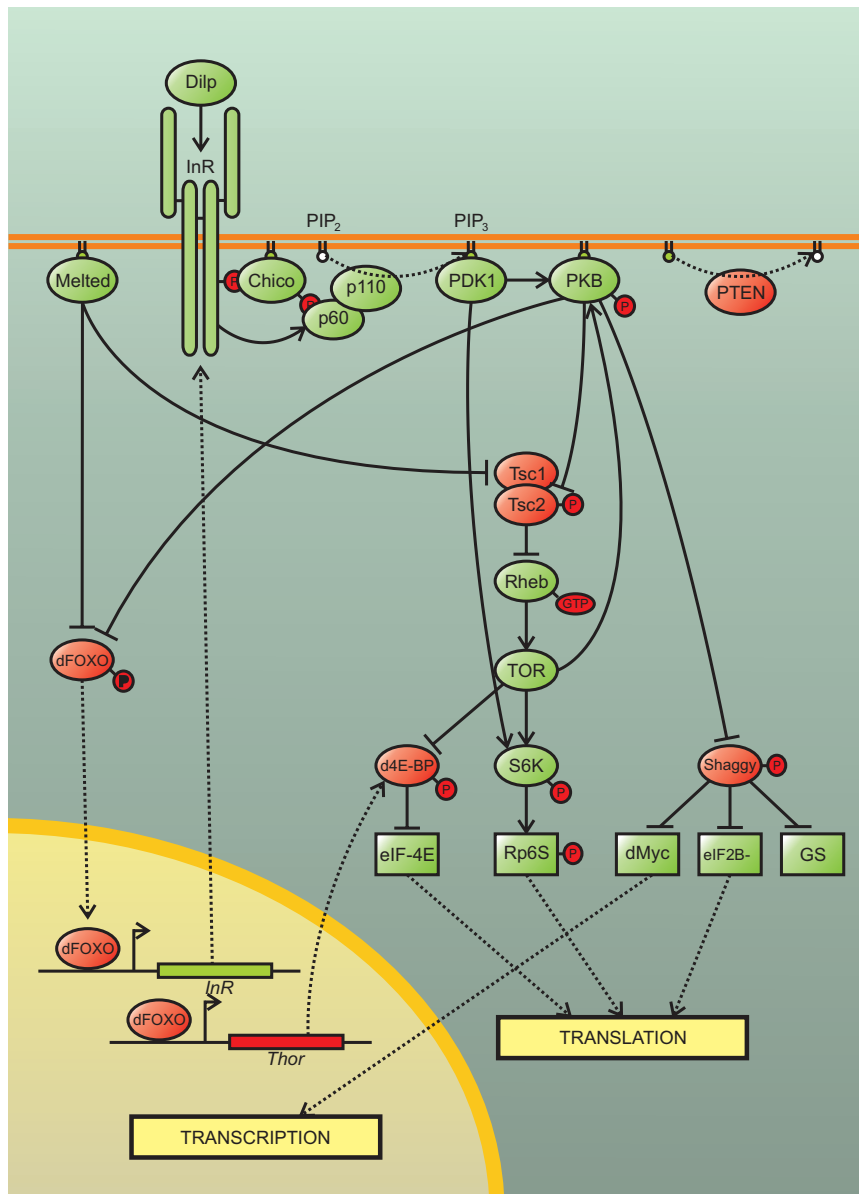


Figure S1. Insulin/TOR pathway in *D. melanogaster*. In the presence of insulin, the insulin receptor (InR) undergoes autophosphorylation providing docking sites for a protein complex; this complex catalyzes the phosphorylation of the membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP₂) to phosphatidylinositol-3,4,5-trisphosphate (PIP₃). The secondary messenger PIP₃ recruits a series of pleckstrin homology domain-containing proteins to the plasma membrane including PKB, which becomes phosphorylated at the plasma membrane. The phosphorylated form of PKB phosphorylates downstream elements, thus unleashing a reactions cascade that activates a series of effectors, including transcription factors and proteins involved in translation and in anabolic metabolism. Activatory and inhibitory elements are represented in green and in red, respectively. Solid lines indicate physical protein-protein interactions (PPIs). Activating and inhibiting interactions are represented by arrows and by lines ending in 'T', respectively. The pathway final effector proteins are represented by rectangles. Recently, Bai *et al.* have shown that, in mammalian cells, Rheb activates mTOR by binding FKBP38 (an inhibitor of mTOR) (Bai *et al.* 2007). Even though there is a putative FKBP38 ortholog in the *D. melanogaster* genome (*CG5482*), currently it is unknown if this gene is really involved in the IT pathway. Furthermore, previous analysis suggested that the activation of mTOR by Rheb takes place through direct Rheb-TOR interaction (Long *et al.* 2005). Therefore, we have not considered in the analyses the *CG5482* gene. Including this gene in the analyses, however, does not change the main conclusions of our work.

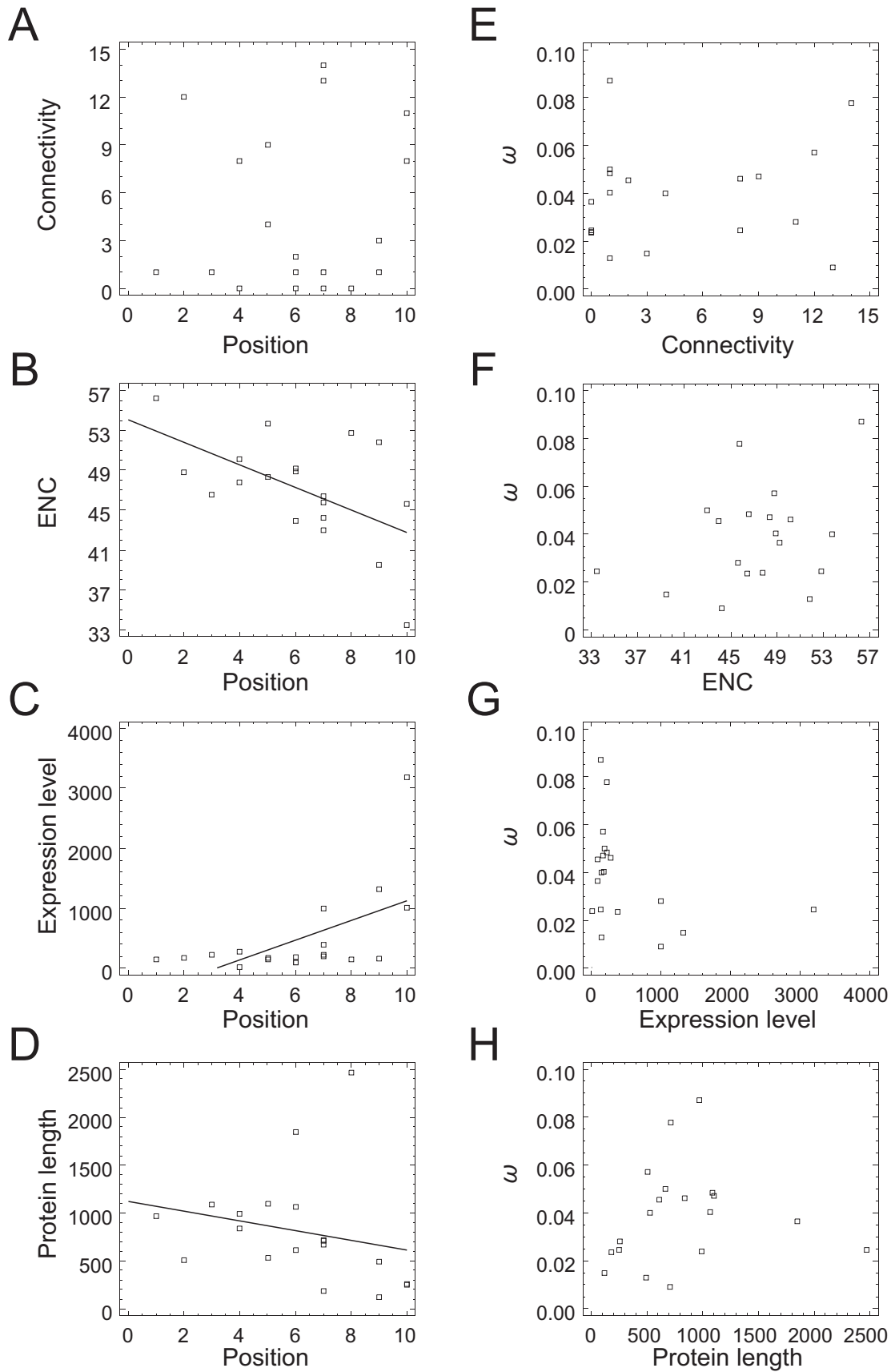


Figure S2. Correlations between different factors (connectivity, codon bias, gene expression levels and protein length) and either the element position in the insulin/TOR pathway (A–D) or the ω values (E–H). Regression lines are represented only for the significant correlations.

Table S1. Genes involved in the *D. melanogaster* insulin/TOR signaling pathway.

Gene	Accession number	Protein	Number of isoforms	Chosen isoform	Protein length	Chromosome	Position
<i>Akt1</i>	CG4006	PKB	2	A	530	3R	11,927,872
<i>chico</i>	CG5686	Chico	1	A	968	2L	10,244,329
<i>dm</i>	CG10798	dMyc	1	A	717	X	3,274,192
<i>eIF2B-ε</i>	CG3806	eIF2B-ε	1	A	669	X	1,815,720
<i>eIF-4E</i>	CG4035	eIF4E-1,2	2	A	259	3L	9,393,583
<i>eIF4E-3</i>	CG8023	eIF4E-3	1	A	244	3L	8,223,001
<i>eIF4E-4</i>	CG10124	eIF4E-4	1	A	229	3L	6,658,069
<i>eIF4E-5</i>	CG8277	eIF4E-5	1	A	232	3L	7,888,543
<i>eIF4E-6</i>	CG1442	eIF4E-6	1	A	173	3R	24,852,462
<i>eIF4E-7</i>	CG32859	eIF4E-7	1	A	429	X	1,053,493
<i>4EHP</i>	CG33100	eIF4E-8	1	A	223	3R	19,911,879
<i>foxo</i>	CG3143	dFOXO	2	B	613	3R	9,899,992
<i>gig</i>	CG6975	Tsc2	1	A	1847	3L	20,127,198
<i>melt</i>	CG8624	Meltd	2	A	992	3L	7,134,079
<i>Pi3K21B</i>	CG2699	p60	1	A	506	2L	300,531
<i>Pi3K92E</i>	CG4141	p110	1	A	1088	3R	16,457,356
<i>Pk61C</i>	CG1210	PDK1	4	D	836	3L	136,821
<i>Pten</i>	CG5671	PTEN	3	D	514	2L	10,258,031
<i>Rheb</i>	CG1081	Rheb	1	A	182	3R	1,395,392
<i>RpS6</i>	CG10944	RpS6	3	C	251	X	7,794,475
<i>S6k</i>	CG10539	S6k	1	A	490	3L	5,798,515
<i>sgg</i>	CG2621	Shaggy	6	D	1067	X	2,561,010
<i>step</i>	CG11628	Step	2	B	488	2L	21,744,219
<i>Thor</i>	CG8846	d4E-BP	1	A	117	2L	3,479,004
<i>Tor</i>	CG5092	TOR	1	A	2470	2L	13,008,859
<i>Tsc1</i>	CG6147	Tsc1	1	A	1100	3R	19,958,219
<i>CG6904^a</i>	CG6904	GS ^a	2	A	709	3R	10,969,512

^aPutative ortholog of the glucogen synthase.

Table S2. Copy number of the insulin/TOR signaling pathway genes in 12 *Drosophila* genomes

Gene	Dmel	Dsim	Dsec	Dyak	Dere	Dana	Dpse	Dper	Dwil	Dmoj	Dvir	Dgri
<i>Akt1</i>	1	1	1	1	1	1	1	1	2 ^a	1	1	1
<i>chico</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>dm</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>elF2B-ε</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>elF-4E</i>	1	1	1	1	1	1	1	1	4	1+1 ^b	2	2
<i>elF4E-3</i>	1	1	1	1	1	1	2	2	1	1	1	2
<i>elF4E-4</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>elF4E-5</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>elF4E-6</i>	1	1	1	1	1	0	0	0	0	0	0	0
<i>elF4E-7</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>4EHP</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>foxo</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>gig</i>	1	1	1	2	1	1	1	1	1	1	1	1
<i>melt</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>Pi3K21B</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>Pi3K92E</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>Pk61C</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>Pten</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>Rheb</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>RpS6</i>	1	1	1	1	1	2	2+1 ^b	2+1 ^b	1	1	1+2 ^b	1
<i>S6k</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>sgg</i>	1	1	1	1	1	1	1	1	1	1+1 ^b	1	1
<i>step</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>Thor</i>	1	1	1	1	1	1	1	1	2	2 ^a	2 ^a	2 ^a
<i>Tor</i>	1	1	1	1	1	1	1	1	2 ^a	1	1	1
<i>Tsc1</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>CG6904</i>	1	1	1	1	1	1+1 ^{ab}	1	1	1	1	1	1

Dmel, *D. melanogaster*; Dsim, *D. simulans*; Dsec, *D. sechellia*; Dyak, *D. yakuba*; Dere, *D. erecta*; Dana, *D. ananassae*; Dpse, *D. pseudoobscura*; Dper, *D. persimilis*; Dwil, *D. willistoni*; Dmoj, *D. mojavensis*; Dvir, *D. virilis*; Dgri, *D. grimshawi*.

^aCopies located in tandem in the same genomic scaffold.

^bPseudogene, DNA sequence with a frameshift or covering less than 40% of the *D. melanogaster* ortholog.

Table S3. Phylogenetic analysis by maximum likelihood of the insulin/TOR pathway genes

Gene	ℓ_{M0}	ℓ_{FR}	ℓ_{M1a}	ℓ_{M2a}	ℓ_{M3}	ℓ_{M7}	ℓ_{M8}	$2(\ell_{FR}-\ell_{M0})$	$2(\ell_{M2a}-\ell_{M1a})$	$2(\ell_{M8}-\ell_{M7})$	$2(\ell_{M3}-\ell_{M0})$
<i>Akt1</i>	-3,233.47	-3,219.85	-3,194.43	-3,191.90	-3,191.72	-3,197.52	-3,191.72	27.25 ***	5.06	11.60 **	83.51 ***
<i>chico</i>	-7,055.41	-7,046.34	-7,003.48	-7,003.48	-6,991.73	-6,993.51	-6,992.98	18.14 *	0.00	1.06	127.35 ***
<i>dm</i>	-3,620.02	-3,611.08	-3,569.89	-3,569.89	-3,564.14	-3,565.58	-3,565.39	17.89 **	0.00	0.39	111.77 ***
<i>eIF2B-ε</i>	-4,679.84	-4,673.55	-4,656.33	-4,655.25	-4,654.48	-4,662.72	-4,655.32	12.60	2.16	14.80 ***	50.74 ***
<i>eIF-4E</i>	-1,368.58	-1,365.22	-1,360.96	-1,360.96	-1,358.49	-1,358.49	-1,358.49	6.73	0.00	0.00	20.19 ***
<i>eIF4E-3</i>	-2,059.13	-2,049.09	-2,035.92	-2,035.92	-2,035.00	-2,035.98	-2,035.18	20.08 **	0.00	1.60	48.26 ***
<i>eIF4E-4</i>	-1,565.39	-1,561.53	-1,558.78	-1,558.78	-1,555.94	-1,556.55	-1,556.55	7.72	0.00	0.00	18.90 ***
<i>eIF4E-5</i>	-1,449.04	-1,427.93	-1,446.09	-1,446.09	-1,444.33	-1,444.42	-1,444.42	42.22 ***	0.00	0.00	9.43
<i>eIF4E-6^a</i>	—	—	—	—	—	—	—	—	—	—	—
<i>eIF4E-7</i>	-1,965.06	-1,918.53	-1,951.28	-1,951.28	-1,950.66	-1,953.51	-1,951.06	93.05 ***	0.00	4.89	28.79 ***
<i>4EHP</i>	-1,311.36	-1,294.92	-1,310.00	-1,310.00	-1,309.86	-1,309.98	-1,309.88	32.88 ***	0.00	0.21	2.99
<i>foxo</i>	-3,429.60	-3,420.59	-3,420.45	-3,420.45	-3,420.21	-3,420.33	-3,420.22	18.02 **	0.00	0.23	18.79 ***
<i>gig</i>	-13,110.05	-13,103.01	-13,074.83	-13,074.60	-13,063.34	-13,065.68	-13,063.42	14.08	0.48	4.51	93.42 ***
<i>melt</i>	-6,518.80	-6,515.25	-6,496.18	-6,496.18	-6,494.46	-6,496.07	-6,494.49	7.11	0.00	3.17	48.67 ***
<i>Pi3K21B</i>	-3,562.99	-3,557.21	-3,538.34	-3,538.34	-3,531.28	-3,531.84	-3,531.62	11.57	0.00	0.44	63.42 ***
<i>Pi3K92E</i>	-7,852.60	-7,843.01	-7,822.97	-7,822.97	-7,810.25	-7,810.43	-7,810.43	19.20 **	0.00	0.00	84.71 ***
<i>Pk61C</i>	-4,576.43	-4,573.15	-4,567.55	-4,567.55	-4,564.51	-4,564.72	-4,564.57	6.55	0.00	0.30	23.85 ***
<i>Pten</i>	-3,599.14	-3,588.06	-3,570.06	-3,569.09	-3,569.09	-3,571.22	-3,569.10	22.16 **	1.94	4.24	60.09 ***
<i>Rheb</i>	-1,244.78	-1,239.81	-1,244.20	-1,244.20	-1,242.16	-1,242.25	-1,242.25	9.93	0.00	0.00	5.23
<i>RpS6</i>	-1,383.46	-1,377.25	-1,374.83	-1,374.40	-1,374.40	-1,376.67	-1,374.43	12.42	0.85	4.48	18.12 ***
<i>S6k</i>	-2,769.89	-2,765.16	-2,769.88	-2,769.86	-2,769.86	-2,769.94	-2,769.91	9.48	0.04	0.06	0.07
<i>sgg</i>	-4,760.04	-4,757.31	-4,728.18	-4,728.18	-4,727.92	-4,728.22	-4,728.00	5.47	0.00	0.44	64.23 ***
<i>step</i>	-3,534.06	-3,521.30	-3,516.42	-3,516.42	-3,513.24	-3,513.79	-3,513.56	25.52 ***	0.00	0.46	41.65 ***
<i>Thor</i>	-799.33	-797.34	-799.33	-799.33	-799.33	-799.49	-799.49	3.96	0.00	0.00	0.00
<i>Tor</i>	-15,824.18	-15,789.18	-15,754.01	-15,754.01	-15,747.90	-15,751.67	-15,747.39	69.99 ***	0.00	8.57 *	152.56 ***
<i>Tsc1</i>	-7,365.47	-7,356.64	-7,348.84	-7,348.84	-7,342.41	-7,343.34	-7,342.67	17.66 **	0.00	1.34	46.12 ***
<i>CG6904</i>	-4,305.75	-4,297.50	-4,297.89	-4,297.89	-4,296.97	-4,298.61	-4,297.56	16.50 *	0.00	2.09	17.56 ***

ℓ_i , log-likelihood of the observed data under the evolutionary model *i*. *, $P < 0.05$; **, statistically significant values at a false discovery rate (FDR) of 0.05. ***, statistically significant values under the Bonferroni correction.

^aAnalysis not conducted since there is no orthologous copy in *D. ananassae*.

Table S4. Connections between the insulin/TOR pathway and other pathways in *D. melanogaster*.

IT pathway element	Pathway or molecule	Input/output	Effect on IT pathway	Kind of evidence	References
p60	Decapentaplegic pathway	input	activation	m	(Higaki and Shimokado 1999; Martin-Castellanos and Edgar 2002)
p110	Ras pathway	input	activation	d	(Orme et al. 2006)
Tsc2 and/or TOR	amino acids	input	activation	d	(Gao et al. 2002)
Rheb	TCTP	input	activation	d	(Hsu et al. 2007)
eIF-4E	LK6	input	activation	d	(Arquier et al. 2005; Reiling et al. 2005)
dMyc	Ras pathway	input	activation	d	(Prober and Edgar 2002)
p60	Sus1	input	inhibition	d	(Wittwer et al. 2005)
Tsc1	Scylla/Charybdis	input	inhibition	d	(Reiling and Hafen 2004)
S6K	PP2A	input	inhibition	d	(Bielinski and Mumby 2007)
dMyc	Archipelago	input	inhibition	d	(Moberg et al. 2004)
dMyc	Dco	input	inhibition	d	(Galletti et al. 2007)
dMyc	Wingless pathway	input	inhibition	d	(Johnston et al. 1999; Quinn et al. 2004)
dFOXO	JNK pathway	input	inhibition	d	(Wang et al. 2005)
Shaggy	Hedgehog pathway	input/output	activation	d	(Jia et al. 2002; Price and Kalderon 2002)
Shaggy	Wingless pathway	input/output	no effect	d	(Noordermeer et al. 1994)
InR	Dock pathway	output	activation	d	(Song et al. 2003)
PKB	RSK	output	activation	d	(Rintelen et al. 2001)
S6K	Sima/HIF-1	output	activation	d	(Dekanty et al. 2005)
PKB	Trh	output	activation	d	(Jin et al. 2001)
PKB	sugar metabolism	output	activation	m	(Rulifson et al. 2002)
PKB	lipid metabolism	output	activation	d	(Vereshchagina and Wilson 2006)
PKB	Sima/HIF-1	output	activation	d	(Dekanty et al. 2005)
PKB	ERK pathway	output	activation	d	(Kim et al. 2004)
PKB	apoptosis	output	inhibition	m	(Scanga et al. 2000)

d, interaction observed in *D. melanogaster*; m, interaction observed in mammals that may also exist in *D. melanogaster* as judged from some indirect evidence.

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