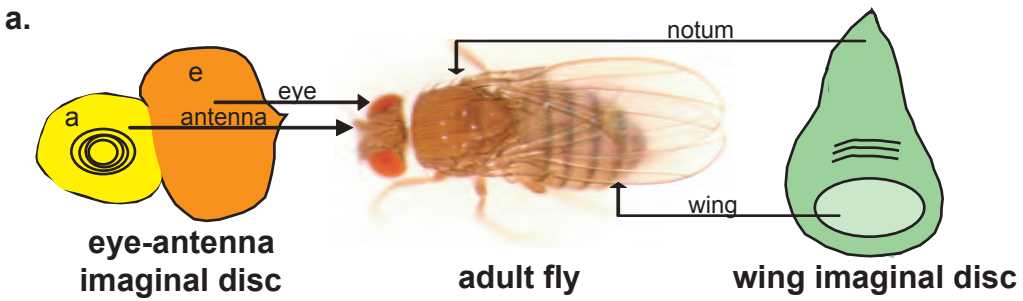
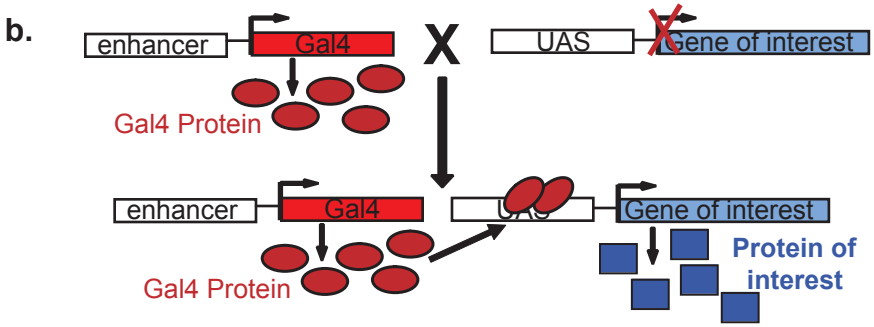
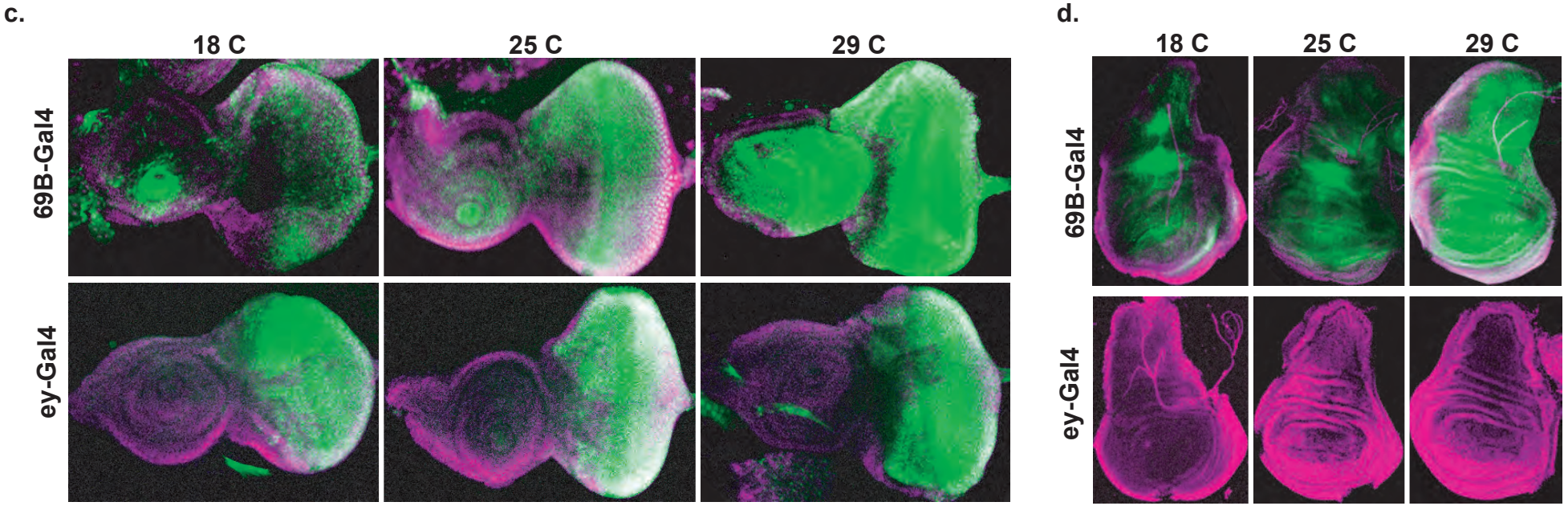






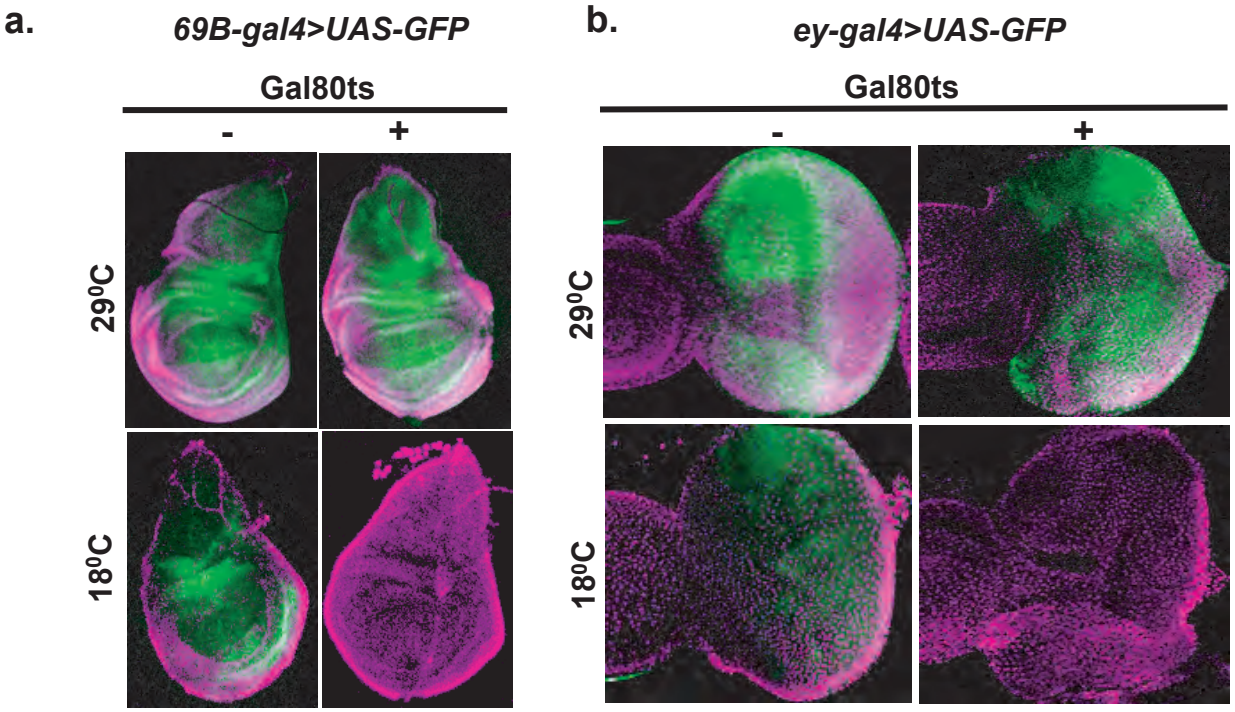
Supplementary Figure 1. Expression patterns of ey-gal4 and 69B-gal4 in wing and eye imaginal discs

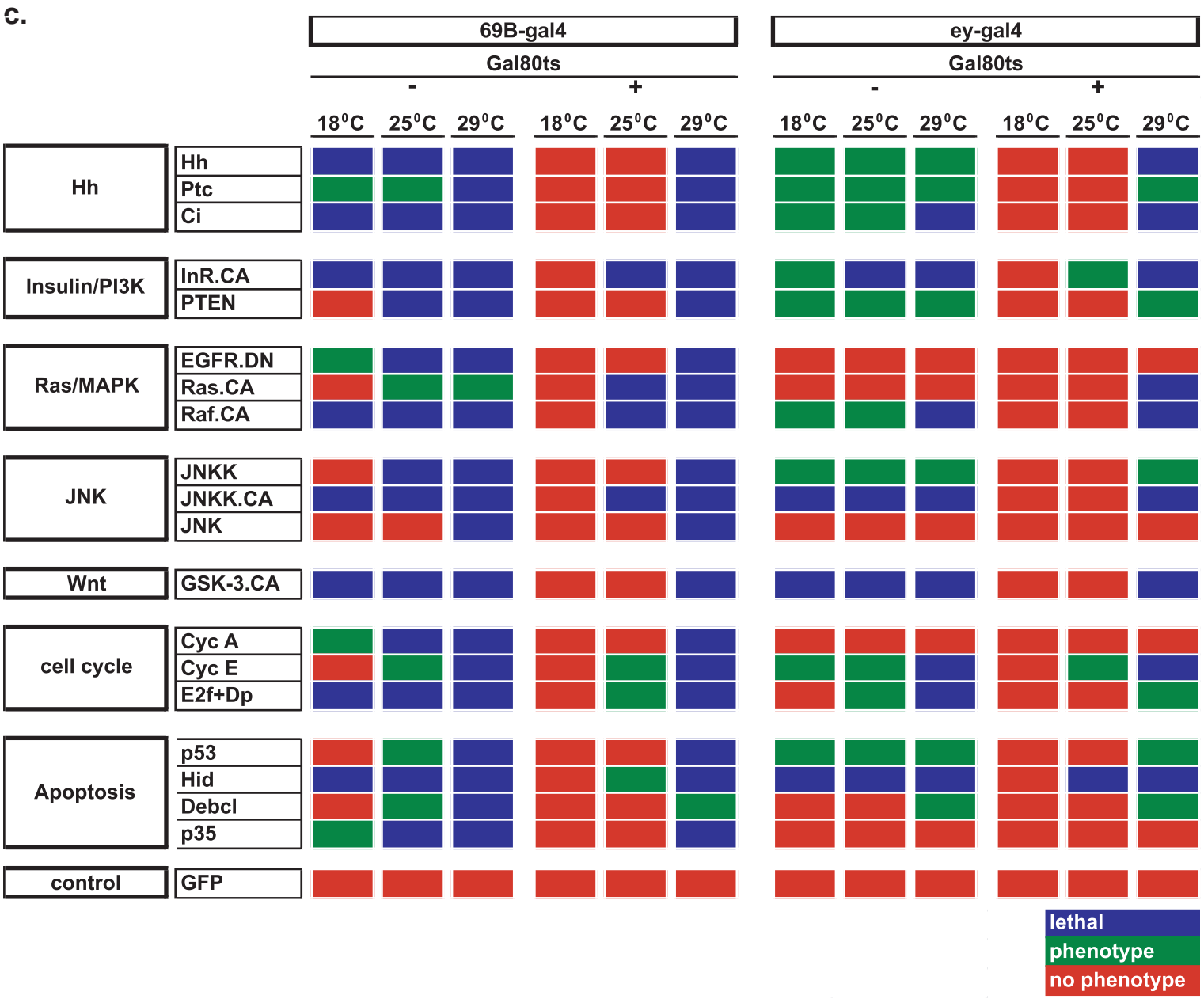
Supplementary Figure 2. Compounds selested to test for activity in Drosophila by feeding

pathway	compound	Dose (μM)*	IC50 in mammailan cells**	mechanism of action(MoA)
Hh	AY 9944 dihydrochloride	15	13 nM	inhibits cholesterol biosynthesis/cellular transport
	SANT-1	15	20nM	smo antagonist
	Hh(Ant)-1	6		smo antagonist
	Ibudilast	15	10-53 mM	phosphodiesterase inhibitor, raises cAMP levels
	Forskolin	15	41 nM	activates adenylyl cyclase, raises cAMP levels
insulin/PI3K	BVT 948	15	0.09-1.7 μM	phosphatase inhibitor, enhances insulin signaling
	Demethylasterriquinone B1	30	3-6 μM	activates the insulin receptor
	Deguelin	3	10 nM	PI3-K inhibitor
	Wortmannin	15	2-4 nm	PI3-K inhibitor
	LY 294002	7.5	0.3-1 μM	PI3-K inhibitor
	10-DEBC HCl	30	2-6 μM	akt/PTB inhibitor
EGFR/MAPK	AG 494	15	0.7 μM	EGFR inhibitor
	AG 99	30	10 μM	EGFR inhibitor
	GW 5074	30	9 nM	cRaf1 inhibitor
	SL 327	30	0.8-0.22 μM	MEK inhibitor
	UO126	30	0.06-0.07 μM	MEK inhibitor
	PD 98059	7.5	2-7 μM	MEK inhibitor
JNK	SP 600125	30	40-90 nM	JNK inhibitor
Wnt	SB 216763	30	100 nM	GSK-3 inhibitor
cell cycle	NSC 95397	15	500 nM	cdc25 inhibitor
	Kenpaullone	30	0.4 μM	CDK inhibitor
	Aminopurvalanol	30	0.02-0.03 μM	CDK inhibitor
	Olomoucine	15	7 μM	CDK inhibitor
apoptosis	pifithrin-α hydrobromide	30	10 μM	p53 inhibitor
	cisplatin	15	0.18-7.7 μM	blocks DNA synthesis & induces apoptosis
	embelin	30	4-6 μM	XIAP inhibitor
	Z-VAD-FMK	6	0.0015-5.8 mM	caspase inhibitor

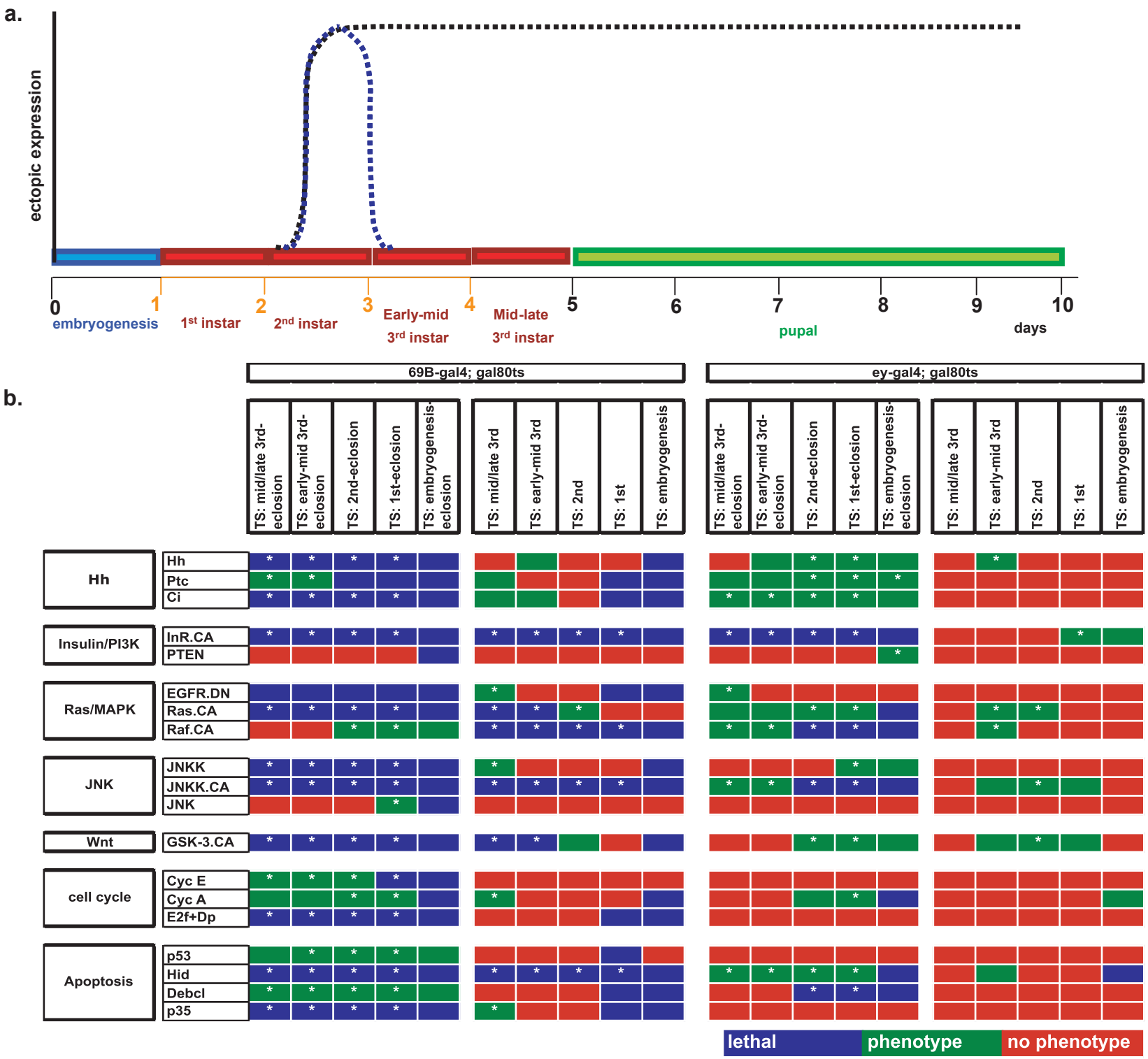
*:concentrations are calculated assuming uniform distribution within food

**:references for IC50 values and MoA can be found at www.tocris.com

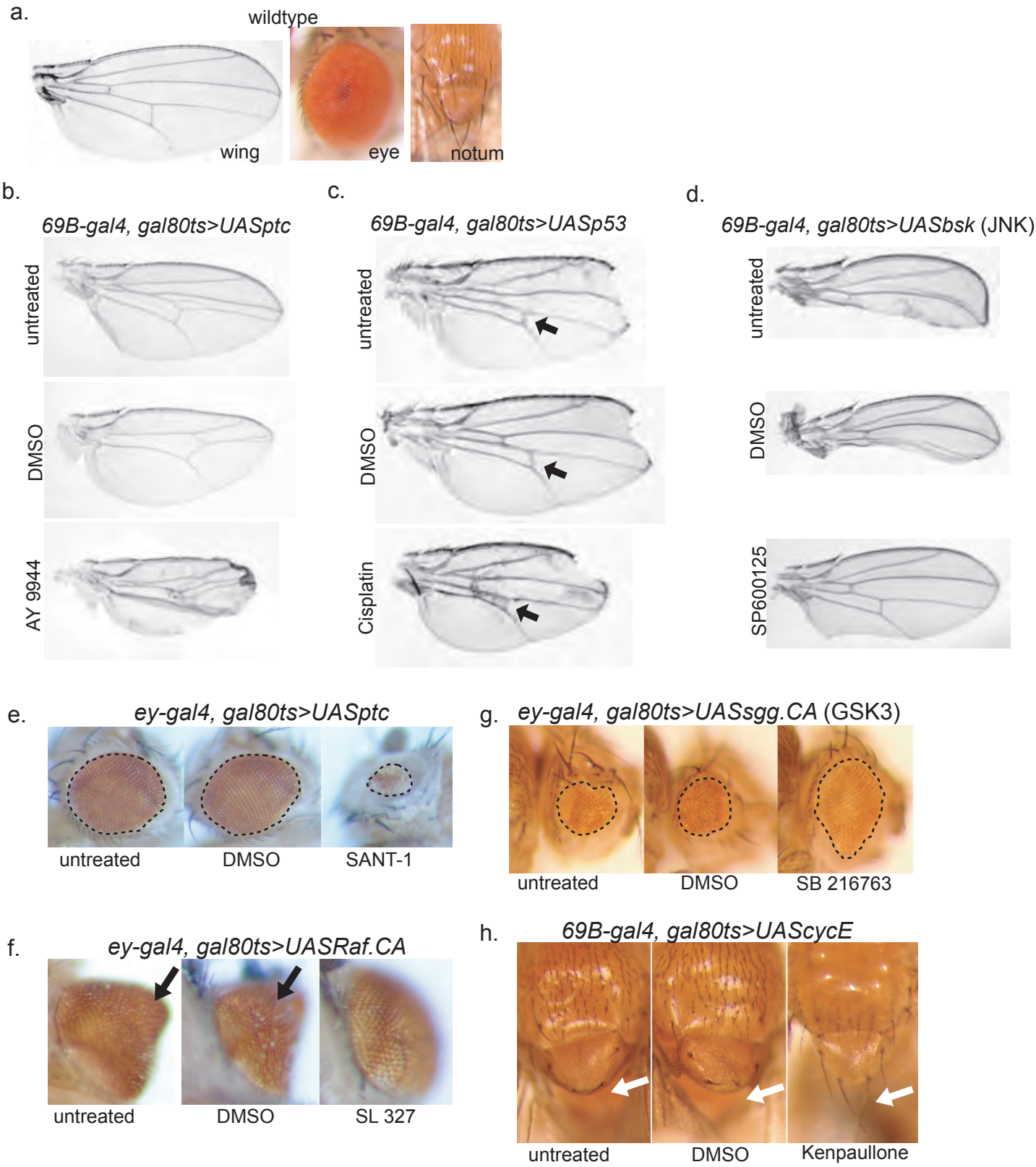




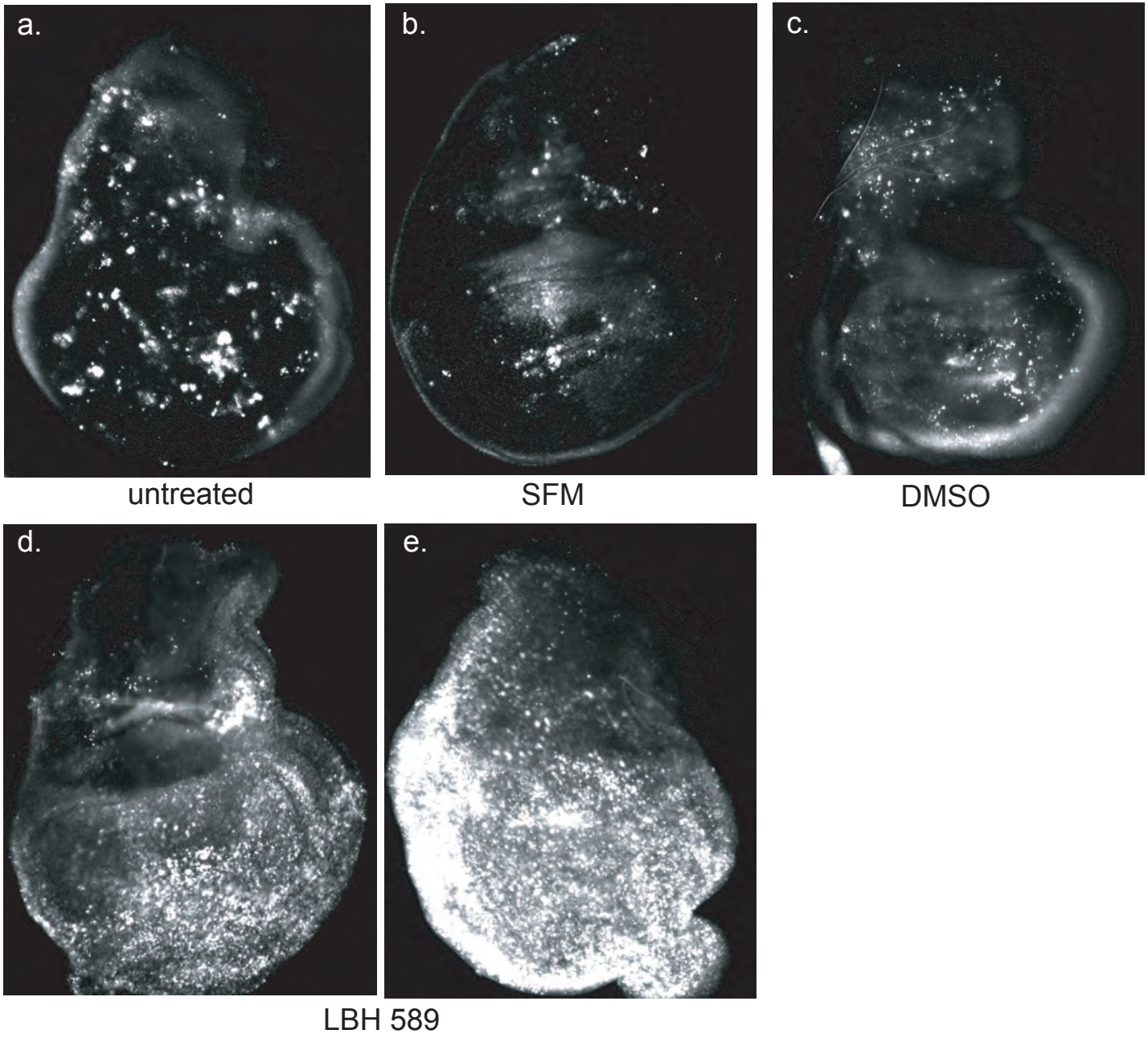
Supplementary Figure 3. Temporal control of ectopic expression using gal80ts



Supplementary Figure 4. Genetic manipulation of pathway activity at various times during development



Supplementary Figure 5. Examples of phenotypes modified by feeding compounds



Supplementary Figure 6. ex vivo wing imaginal disc incubation assay

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Expression patterns of ey-gal4 and 69B-gal4 in wing and eye imaginal discs. **a.** Diagram of larval wing and eye-antenna imaginal discs. Regions of eye-antenna imaginal disc that give rise to adult antennae and eye are shown in yellow and orange, respectively. Wing imaginal disc gives rise to the adult wing (wing pouch, in light green) as well as the notum with sensory bristles. **b.** Outline of the Gal4-UAS system. Ectopic expression is achieved by bringing together two separate elements in the same fly: a Gal4 construct, where Gal4 expression is driven by a tissue specific promoter of choice, and a UAS construct, where a gene of interest is cloned downstream of UAS, the GAL4 binding site. When these two elements are together, Gal4 protein is able to bind to the UAS sequence and activate expression of the gene cloned downstream of it. This way, expression of the gene of choice is activated in all the cells that express Gal4 **c,d.** Expression domains of *69B-Gal4* and *ey-Gal4* in wing and eye-antenna imaginal discs are visualized by inducing GFP expression from a *UAS-GFP* transgene by each Gal4 line. Eye **(c)** and wing **(d)** imaginal discs of 3rd instar larvae were dissected, fixed and counterstained with Hoechst to label nuclei (magenta). GFP expression (green) indicates domains of Gal4 expression. Temperature dependence of Gal4 activity is evident when expression level at 18^{0}C is compared to that of at 29^{0}C. *69B-Gal4* is expressed throughout the wing and eye-antenna imaginal disc. *ey-Gal4* is strongly expressed in the eye imaginal disc with very low levels of expression in parts of the antenna imaginal disc. No expression was detectable in the wing disc.

Fig. S2. Compounds selected to test for activity in Drosophila by feeding. Mechanism of action and/or cellular targets of compounds selected to test for activity are indicated.

Compound concentrations listed indicate those of the solutions mixed in with the food. Uniform diffusion of the compounds throughout the food will result in a 300-fold dilution of the compound, therefore, assuming uniform distribution, compound concentrations that developing larvae are exposed to will range from 3-30 μM.

Fig. S3. Temporal control of ectopic expression using Gal80ts. Flies bearing a Gal80ts insertion in combination with *69B-gal4* or *ey-gal4* were generated and the ability of Gal80ts to inhibit Gal4 activity was tested in wing and eye imaginal discs using GFP as a read-out **(a,b)** and in adult flies using phenotypic read-outs **(c)**. Wing **(a)** and eye **(b)** imaginal discs from larvae expressing GFP under the control of *69B-gal4* and *ey-gal4,* respectively, in the presence or absence of Gal80ts at18^0C and 29^0C were dissected and counterstained with Hoechst to label nuclei (magenta). In the presence of Gal80ts, GFP expression (green) was only observed in discs of larvae raised at 29^0C, the restrictive temparature for Gal80ts. Even at this temperature, GFP level was lower in the presence of Gal80ts, indicating that not all of Gal80ts was inactivated. No GFP expression was detectable at 18^0C. In the absence of Gal80ts, GFP expression was detectable at both temperatures, albeit at lower levels at 18^0C. **c.** Ectopic expression of wildtype, dominant negative (DN) or constitutively active (CA) versions of pathway components belonging to Hh, Insulin/PI3K, EGFR/MAPK, JNK, Wnt, cell cycle and apoptosis (19 UAS lines total and *UAS-GFP* as control) was induced using *69B-gal4* and *ey-gal4* in the presence or absence of Gal80ts at 18, 25 and 29^0C and progeny were scored for phenotypes (blue: lethality, green: phenotype, red: no effect). At 29^0C, majority of lines lead to phenotypes or lethality similar to those obtained in the absence of Gal80ts. In many cases, effects observed in the presence of Gal80ts were weaker (weaker phenotypes or lethality at a

later developmental stage), consistent with a lower level of expression observed in the presence of Gal80ts **(a)**. At 25^0C, a smaller number of phenotypes/lethality were observed compared to 29^0C, as only a fraction of the Gal80ts pool would be inactivated at this intermediate temperature.

Fig. S4. Genetic manipulation of pathway activity at various times during development. a. Drosophila development spans a 10 day period at 25^0C, beginning with embryogenesis, followed by three larval stages, and adult flies emerging at the end of a 5 day pupal stage. Larvae start eating after they hatch from embryos as 1st instar larvae and continue until mid-3rd instar stage (orange line), when they stop eating and prepare for pupariation. Development at 18^0C takes 20 days, with each developmental stage roughly doubled in time. For ectopic expression experiments, development was allowed to proceed at 18^0C until expression was induced by a temperature switch to 25^0C or 29^0C during 5 different stages: embryogenesis, 1st instar, 2nd instar, early-mid 3rd instar and mid-late 3rd instar. Ectopic expression was allowed for either 24 hours, followed by a switch back to 18^0C, or until the end of development. For simplicity, induction during 2nd larval instar is shown only. **b.** Summary of phenotypes obtained by ectopic expression during various developmental stages. UAS lines were crossed to *69B-gal4* and *ey-gal4* in the presence of Gal80ts for 2 days at 18, and switched to 29^0C at indicated developmental stages. Progeny from these crosses were scored for phenotypes (blue: lethality, green: phenotype, red: no effect). Inducing ectopic expression of the majority of UAS lines for 24 hours did not lead to any effect, yet, a high number of lethal, eye and wing phenotypes were observed when ectopic expression was allowed until the end of development.

Phenotypes selected as read-outs for compound feeding are marked with white stars (*) (see main text for selection criteria).

Fig S5. Examples of phenotypes modified by feeding compounds. **a.** Wildtype adult wing, eye and notum. **b-g.** Example eye, wing and bristle phenotypes that are modified by compound feeding. **b.** Ectopic expression of Ptc in the wing leads to small wings with central pattern elements missing (top panel). AY9944 enhances this phenotype, leading to smaller wings with stronger patterning defects (bottom panel). **c.** p53 overexpression in the wing discs leads to small wings with L4-L5 intervein area is reduced (arrow, top panel). This phenotype is enhanced by feeding Cisplatin, a compound that induces apoptosis by blocking DNA synthesis (bottom). **d.** Ectopic expression of Bsk (JNK) leads to wings with large notches occupying the entire posterior compartment (top panel). Wings from SP 600125 (JNK inhibitor) fed animals show a much weaker phenotype with small notches in the posterior compartment (bottom panel). **e.** Ptc overexpression in the eye leads to a mild rough eye phenotype (left panel), which is strongly enhanced by feeding the Smo inhibitor SANT-1 (right panel). **f.** Ectopic expression of activated Raf leads to bulgy and rough eyes associated with excessive folding of the eye tissue due to overproliferation (black arrows, left panel). This phenotype is strongly suppressed by feeding the MEK inhibitor SL327 (right panel). **g.** Ectopic expression of activated sgg (GSK3) gives rise to a strong small eye phenotype (left panel), which is suppressed by the GSK inhibitor SB 216763 (right panel). **h.** Ectopic expression of cycE in wing imaginal discs leads to the formation of short and thin sensory bristles on the notum (white arrows, left panel)), a phenotype which is suppressed by the CDK inhibitor kenpaullone (right panel).

Fig. S6. *ex vivo* wing imaginal disc incubation assay. Dissected wing imaginal discs (genotype: *w; tub-gal80ts/+; 69B-gal4/+*) are incubated in **(b)** Serum Free Medium (SFM), **(c)** 0.5% DMSO, or **(d,e)** 100mM LBH589 (pan histone deacetylase inhibitor) for 24 hours at room temperature and stained with acridine orange to detect cell death along with freshly dissected untreated discs **(a)**. Incubation alone or DMSO treatment is not toxic to wing imaginal disc cells. Consistent with the ability of LBH589 to induce apoptosis, discs treated with this compound show significantly more cell death.