Supporting Information

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SI Materials and Methods

Genetic Screen. Fly culture and crosses were carried out at 25 °C unless otherwise specified. Canton-S was used as the WT strain. A series of Bloomington deficiency kit lines were crossed with *UAS-eiger^{regg1}; GMR-GAL4/TM3* transgenic flies, and the F1 progenies were examined for suppression of the small eye phenotype. If a chromosomal deficiency region contained a gene that functions downstream of Eiger, the Eiger-induced small eye phenotype should be suppressed. More than 80% of the *Drosophila* genome was analyzed in the primary screen. The responsible regions for phenotype suppression were narrowed down by using small, overlapping deficiencies. The candidates for responsible genes were then screened by examining publicly available mutants. To obtain images of the adult eye, flies were frozen at −80 °C for 5 min, and photographs were taken and processed with an Olympus SZX16 camera with Dynamic Eye REAL software (Mitani).

Fly Food. To avoid variations in energy supply from the fly food, we used freshly cooked fly food according to the following recipe in all of the experiments: 400 g dry yeast (Oriental Dry Yeast), 400 g corn flour (Nippn), and 80 g agar (Kishida Chemical) were mixed and boiled well in 9 L water. Subsequently, 1,000 g glucose (Nakalai Tesque) were dissolved in 1 L hot water, and added to the mixture; 30 mL propionic acid (Nakalai Tesque) and 50 mL 10% butyl p-hydroxybenzoic acid in 70% ethanol were added before aliquotting.

Histology. Eye discs from third instar larvae were stained by standard immunohistochemical procedures using a rabbit anti-phospho-JNK monoclonal antibody (1:100; Cell Signaling) or rabbit anti-cleaved caspase 3 antibody (1:100; Cell Signaling). The secondary antibodies were 555 Alexa anti-mouse IgG (1:1,000; Molecular Probes) and 555 Alexa anti-rabbit IgG (1:1,000; Molecular Probes). The stained samples were mounted with antifade reagent (Slow Fade Gold; Invitrogen). For acridine orange staining, dissected eye discs were incubated with 1.6 μM acridine orange (Sigma) solution for 2 min (1). After a brief rinse with PBS, the samples were analyzed by fluorescence microscopy.

Dihydroethidium Staining. Eye discs of third instar larvae were dissected in PBS and then stained in 5 μM dihydroethidium (Invitrogen)/PBS solution for 5 min at room temperature. The discs were then briefly rinsed with PBS two times and washed with PBS for 5 min. The fluorescence was analyzed under a fluorescence microscope.

Monitoring of Cellular Reactive Oxygen Species Production by a gstD-GFP Reporter. The eye discs of transgenic flies containing a gstD-GFP reporter construct were dissected and stained using an anti-GFP antibody (1:100; MBL). The secondary antibody was 488 Alexa anti-rabbit IgG (1:1,000; Molecular Probes). The samples were mounted with VECTASHIELD mounting medium (Vector Laboratories). The number of GFP-positive clusters was counted under a fluorescence microscope.

ATP Assay. The amount of ATP in the eye antenna imaginal discs was analyzed using an ATP determination kit (A22066; Molecular Probes). In brief, the discs were dissected in PBS, and each disc was separately placed into 1× reporter lysis buffer (Promega). The samples were immediately heat-shocked at 95 °C to inactivate ATPase and then quickly frozen. The tissue lysates were centrifuged at 15,300 × *g* for 5 min, and the supernatant was subjected to the ATP assay. The luminofluoresence was measured by a Lumat LB9507 (Berthold).

Detailed Genotypes of the Animals Used in Figures.

Fig. 1A: WT (Canton-S)
Fig. 1B: *w; UAS-eiger^{regg1}/UAS-lacZ; GMR-GAL4/+*
Fig. 1C: *w; GMR-GAL4/UAS-lacZ; GMR-rpr/+*
Fig. 1D: *w; GMR-hid/UAS-lacZ; GMR-GAL4/+*
Fig. 1E: *w; UAS-eiger^{regg1}/+; GMR-GAL4/UAS-p35*
Fig. 1F: *w; GMR-GAL4/+; GMR-rpr/UAS-p35*
Fig. 1G: *w; GMR-hid/+; GMR-GAL4/UAS-p35*
Fig. 1H: *w; UAS-lacZ/GMR-GAL4*
Fig. 1I: *w; UAS-eiger^{regg1}/GMR-GAL4*
Fig. 1J: *w; GMR-hid/+*
Fig. 1K: *yw eyFLP/+; GMR-hid l(3)* FRT2A/FRT2A*
Fig. 1L: *yw eyFLP/+; GMR-hid l(3)* FRT2A/dronc$^{\Delta A8}$ FRT2A*
Fig. 1M: *yw eyFLP/+; GMR-GAL4, UAS-eiger^{8}/+; GMR-hid, l(3)* FRT2A/FRT2A*
Fig. 1N: *yw eyFLP/+; GMR-GAL4, UAS-eiger^{8}/+; GMR-hid, l(3)* FRT2A/ dronc$^{\Delta A8}$ FRT2A*

The transgenic RNAi flies used in the experiments in columns a–n of Fig. 2 were *UAS-lacZ-inverted repeat* (*IR*; a), *UAS-CPTI-IR^{VDRC4046}* (b), *UAS- cyt.c-d-IR^{VDRC17129}* (c), *UAS-walrus-IR^{VDRC44378}* (d), *UAS- pgk-IR^{VDRC33798}* (e), *UAS-SdhA-IR^{VDRC110440}* (f), *UAS- PDH-IR^{VDRC40410}* (g), *UAS-GAPDH1-IR^{VDRC31631}* (h), *UAS-GAPDH2-IR^{VDRC23645}* (i), *UAS-acon-IR^{VDRC11767}* (j), *UAS-Idh-IR^{VDRC42915}* (k), *UAS-Indy-IR^{VDRC9981}* (l), *UAS-mtacp1-IR^{VDRC43503}* (m), and *UAS-Mdh-IR^{VDRC27535}* (n).

Fig. 3 A and A′ : *w; UAS-lacZ/+; GMR-GAL4/+*
Fig. 3 B and B′ : *w; UAS-eiger^{regg1}/UAS-lacZ-IR; GMR-GAL4/+*
Fig. 3 C and C′ : *w; UAS-eiger^{regg1}/+; GMR-GAL4/UAS-CPTI-IR^{VDRC4046}*
Fig. 3 D and D′ : *w; UAS-eiger^{regg1}/+; GMR-GAL4/UAS-pgk-IR^{VDRC33798}*
Fig. 3 E and E′ : *w; UAS-eiger^{regg1}/UAS-cyt.c-d-IR^{VDRC17129}; GMR-GAL4/+*
Fig. 4 A and A′ : *w; gstD-GFP, GMR-GAL4/UAS-lacZ*
Fig. 4 B and B′ : *w; gstD-GFP, GMR-GAL4/UAS-eiger^{wk}*
Fig. 4 C and C′ : *w; gstD-GFP, GMR-GAL4/UAS-eigerst*
Fig. 4 D and D′ : *w; gstD-GFP, GMR-GAL4/UAS-eigerst, bsk^{1}*
Fig. 4 E and E′ : *w; GMR-hid/gstD-GFP*
Fig. 5 A, E, and E′ : *yw, eyFLP1/+; act > y^{+} > Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B*
Fig. 5 B, F, and F′ : *yw, eyFLP1/+; act > y^{+} > Gal4, UAS-GFP/ UAS-lacZ-IR; FRT82B, Tub-Gal80/FRT82B, scrib^{1}*
Fig. 5 C, G, and G′ : *yw, eyFLP1/+; act > y^{+} > Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/UAS-CPTI-IR^{VDRC4046} FRT82B, scrib^{1}*
Fig. 5 D, H, and H′ : *yw, eyFLP1/+; act > y+ > Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/UAS-pgk-IR^{VDRC33798} FRT82B, scrib^{1}*

The genotypes of the control animals in which *CPTI-* or *pgk-*RNAi was induced in the WT clone in Fig. 5 *I* and *J* were *yw, eyFLP1/+; act > y+ > Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/ UAS-CPTI-IR^{VDRC4046} FRT82B* (*CPTI*), and *yw, eyFLP1/+; act >*

y+ > Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/UAS-pgk-IR^{VDRC33798} *FRT82B* (*pgk*), respectively.

Abbreviations in Fig. 2 are as follows: acon, aconitase; CPTI, carnitine palmitoyltransferase I; cyt.c-d, cytochrome *c*-distal; Idh, Isocitrate dehydrogenase; Indy, I'm not dead yet; IR, inverted repeat; Mdh, malate dehydrogenase; mtacp1, mitochondrial acyl carrier protein 1; Pgk, phosphoglycerate kinase; Sdh, succinate dehydrogenase.

1. Miron M, et al. (2001) The translational inhibitor 4E-BP is an effector of PI(3)K/Akt signalling and cell growth in *Drosophila*. *Nat Cell Biol* 3:596–601.

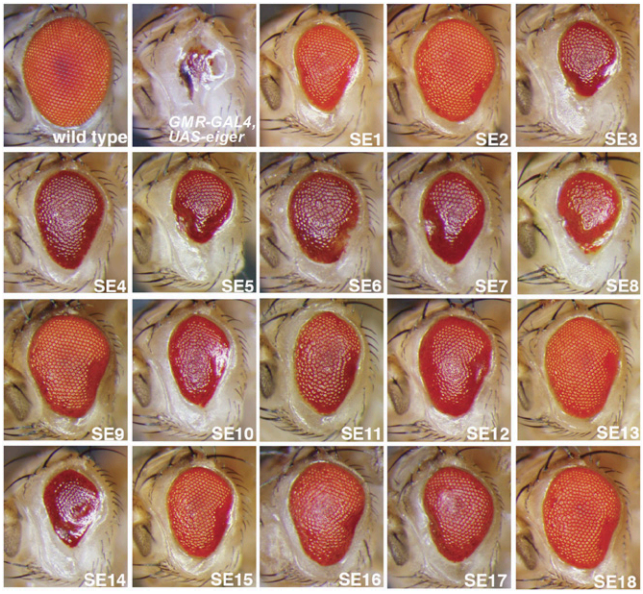

Fig. S1. Suppression of the Eiger-induced small eye phenotype by suppressors of Eiger (SE) alleles identified in the screen. Light micrographs of adult fly eyes. For all flies except WT (Canton-S) and *GMR-GAL4, UAS-eiger* (*UAS-eiger*^{regg1}*/+; GMR-GAL4/ +*), the genotype was *UAS-eiger*^{regg1}*/+; GMR-GAL4/+* on the heterozygous background for the deficiencies mentioned in the panels. The name, cytology of the deficiency region, and Bloomington stock number for each line are summarized in Table S1.

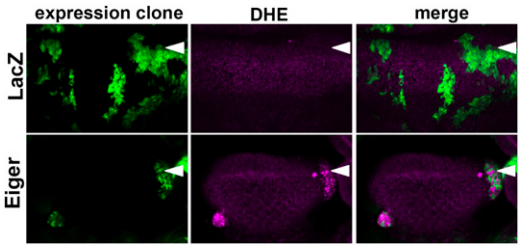

Fig. S2. Activation of Eiger signaling induces the production of superoxide. Eye discs were stained with dihydroethidium (DHE), which is an indicator of superoxide (O_2^-). DHE-positive cells were detected in the clones in which Eiger was overexpressed. Genotypes were *yw, eyFLP1/+; act > y*$^+$ *> Gal4, UAS-GFP/UAS-lacZ; FRT82B, Tub-Gal80/FRT82B* (*Upper*) and *yw, eyFLP1/+; act > y*$^+$ *> Gal4, UAS-GFP/UAS-eiger*^{12}*; FRT82B, Tub-Gal80/FRT82B* (*Lower*), respectively. Arrowheads indicate the morphogenetic furrow. Anterior is to the top.

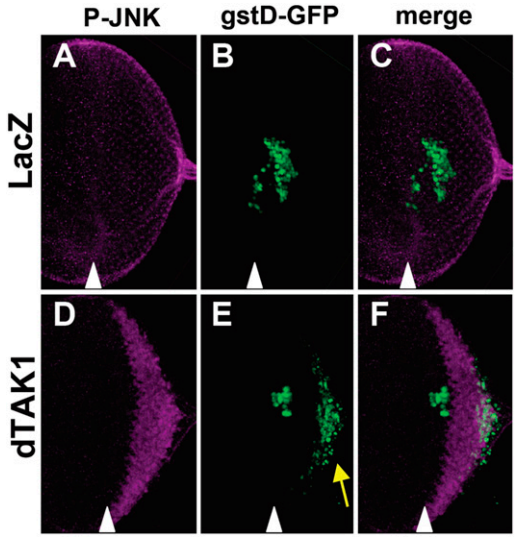

Fig. S3. Ectopic expression of *dTAK1* increases the gstD-GFP signal. Eye discs were dissected, and the gstD-GFP signal was examined. The activation of JNK signaling was detected by the immunostaining of anti-phospho-JNK. Genotypes were as follows: *w; GMR-GAL4, gstD-GFP/UAS-lacZ* (*A*–*C*) and *w; GMR-GAL4, gstD-GFP/UAS-dTAK1* (*D*–*F*). Anterior is to the left. Arrowheads indicate the morphogenetic furrow. Yellow arrow indicates the gstD-GFP signal, which was induced by the overexpression of dTAK1.

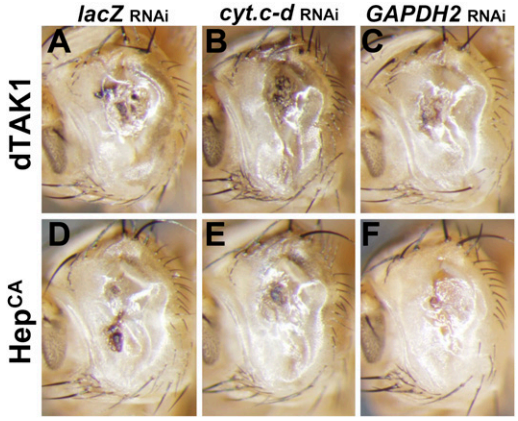

Fig. S4. Down-regulation of *cyt.c-d* or *GAPDH2* does not suppress the dTAK1- or Hep^{CA}-induced eye phenotype. Light micrographs of transgenic flies. Genotypes were as follows: *w; GMR-GAL4, UAS-lacZ-IR/+; UAS-dTAK1/+* (*A*), *w; GMR-GAL4, UAS-cyt.c-d-IR*^{VDRC17129}*/+; UAS-dTAK1/+* (*B*), *w; GMR-GAL4, UAS-GAPDH2-IR* ^{VDRC23645}*/+; UAS-dTAK1/+* (*C*), *w; GMR-GAL4, UAS-lacZ-IR/+; UAS-hep*^{CA}*/+* (*D*), *w; GMR-GAL4, UAS-cyt.c-d-IR*^{VDRC17129}*/+; UAS-hep*^{CA} */+* (*E*), and *w; GMR-GAL4, UAS-GAPDH2-IR* ^{VDRC23645}*/+; UAS-hep*^{CA}*/+* (*F*). Anterior is to the left. Animals were raised at 18 °C to avoid lethality.

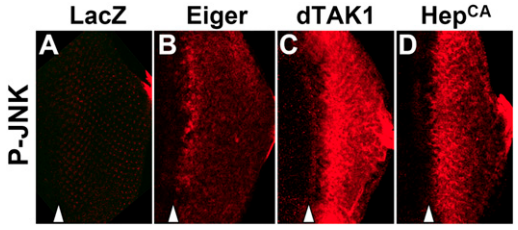

Fig. S5. Overexpression of dTAK1 or Hep^{CA} strongly activates JNK. Eye discs were dissected, and the activation of JNK signaling was detected by the immunostaining of anti-phospho-JNK. Genotypes were as follows: *w; GMR-GAL4/UAS-lacZ* (*A*), *w; GMR-GAL4/ UAS-eiger*^{regg1} (*B*), *w; GMR-GAL4/ UAS-dTAK1* (*C*), and *w; GMR-GAL4/ UAS-hep*^{CA} (*D*). Anterior is to the left. Arrowheads indicate the morphogenetic furrow.

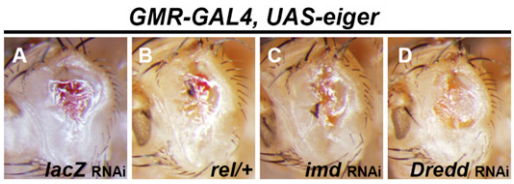

Fig. S6. Lack of a genetic interaction between Eiger-induced cell death and Imd-related genes. Genotypes were as follows: *w; UAS-eiger^{regg1}/UAS-lacZ-IR; GMR-GAL4/+* (*A*), *w; UAS-eiger^{regg1}/+; GMR-GAL4/rel^{E20}* (*B*), *w; UAS-eiger^{regg1}/UAS-Imd-IR$^{5516R-1}$; GMR-GAL4/+* (*C*), and *w; UAS-eiger^{regg1}/UAS-Dredd-IR$^{7486R-2}$; GMR-GAL4/+* (*D*).

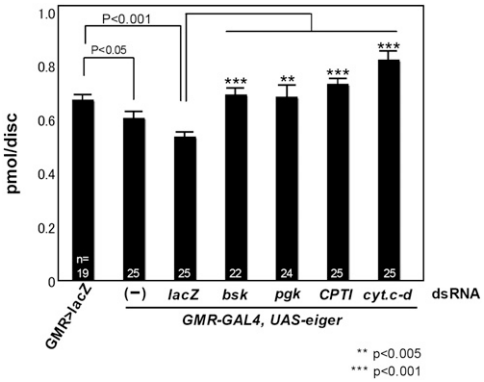

Fig. S7. Activation of Eiger signaling leads to a reduction in ATP. The eye antenna imaginal discs of third instar larvae were dissected and subjected to an ATP assay. The amount of ATP in individual eye antenna imaginal discs was analyzed separately. Genotypes: *UAS-lacZ/+; GMR-GAL4/+* (*GMR > lacZ*), *UAS-eiger^{regg1}/+; GMR-GAL4/+* (–), *UAS-eiger^{regg1}/UAS-lacZ-IR; GMR-GAL4/+* (*lacZ*), *UAS-eiger^{regg1}/+; GMR-GAL4/UAS-bsk-IR^{VDRC104569}* (*bsk*), *UAS-eiger^{regg1}/+; GMR-GAL4/UAS-pgk-IR^{VDRC33798}* (*pgk*), *UAS-eiger^{regg1}/+; GMR-GAL4/UAS-CPTI-IR^{VDRC4046}* (*CPTI*), and *UAS-eiger^{regg1}/UAS-cyt.c-d-IR^{VDRC17129}; GMR-GAL4/+* (*cyt.c-d*). All data are presented as the mean ± SEM (black bar) of the number of eye discs indicated by *n*. Asterisks indicate statistical significance determined by Student's *t* test. $^{**}P < 0.005$; $^{**}P < 0.001$.

Table S1. Eighteen deficiency lines that suppressed the Eiger-induced eye reduction

SE	BL	Allele	Small df	Responsible region	Allele	Candidate gene	(Predicted) molecular function
1	3,070	*Df(1)E128*	None	17C; 18A	see ref. 1	*wgn*	TNF receptor
2	967	*Df(1)C246*	None	11D-E; 12A01-02	*hep^{1}*	*hep*	JNKK
3	1,682	*Df(2R)or-BR6*	2	59F3; 60A8-16	*CG11299^{BG01215}*	*CG11299*	
4	2,471	*Df(2R)M60E*	None	60E02-03; 60E11-12	*zip^{1}*	*zip*	Cytoskeletal protein binding
5	2,583	*Df(2L)cact-255rv64*	1	36A8-9; 36D	*bln^{1}*	*cyt-c-d*	Electron transport
6	3,133	*Df(2L)dp-79b*	1	22A6-22B9	*CG18317^{BG02028}*	*CG18317*	Mitochondrial carrier
7	6,299	*Df(2L)BSC5*	None	26B1-2; 26D1-2			
8	1,541	*Df(3L)66C-G28*	1	66C07-10; 66C7-10		*Idh*	Isocitrate dehydrogenase
					Nmt^{j1C7}	*Nmt*	Glycylpeptide *N*-tetradecanoyltransferase
9	1,962	*Df(3R)p-XT103*	4	85A04-05; 85A06-11			
10	1,990	*Df(3R)Tpl10*	None	83C01-02; 84B02	*Scr^{17}*	*Scr*	Transcription factor
					ftz^{Ual2rv3}	*ftz*	Transcription factor
					zen^{2}	*zen*	Transcription factor
11	2,611	*Df(3L)vin5*	4	68C08-11; 68D6	*CG5946^{BG01087}*	*CG5946*	Cytochrome-b5 reductase
12	2,990	*Df(3L)Cat*	1	075B08; 075F01	*W^{05014}*	*hid*	Cell death induction
					l(3)j14E7^{j14E7}	*Indy*	Transporter
					CG6896^{BG01140}	*CG6896*	Phosphatase
13	3,011	*Df(3R)Cha7*	4	90F01-04; 91A1-2			
14	3,547	*Df(3R)L127*	None	99B05-06; 99E04-F01			
15	5,411	*Df(3L)Aprt-32*	1	62B07; 62B12			
16	4,431	*Df(3R)DG2*	4	90F01-04; 91A01-02			
17	1,045	*Df(2L)Mdh*	None	30D-F; 31F	*bsk^{1}, bsk^{2}*	*bsk*	JNK
					nmd^{k10909}	*Nmd* (*no mitochondrial derivative*)	ATPase, carrier
18	950	*Df(1)RA2*	None	7D18; 8A4			

The suppressor of Eiger (SE) number, Bloomington stock number (BL), deficiency name, number of overlapping small deficiencies, cytology of the determined responsible region, allele name that dominantly suppressed the Eiger-induced eye phenotype, predicted responsible gene of the allele, and molecular function of the putative responsible gene product are listed. Energy metabolism-related molecules are highlighted in red letters.

1. Kanda H, Igaki T, Kanuka H, Yagi T, Miura M (2002) Wengen, a member of the *Drosophila* tumor necrosis factor receptor superfamily, is required for Eiger signaling. *J Biol Chem* 277: 28372–28375.