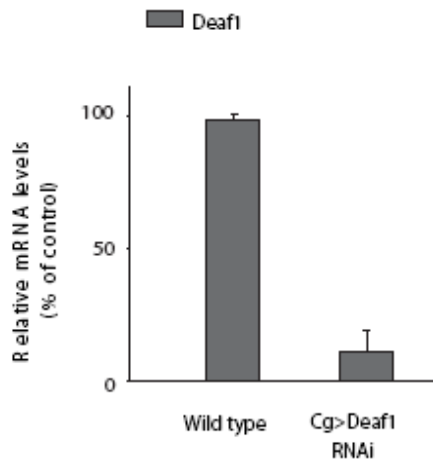
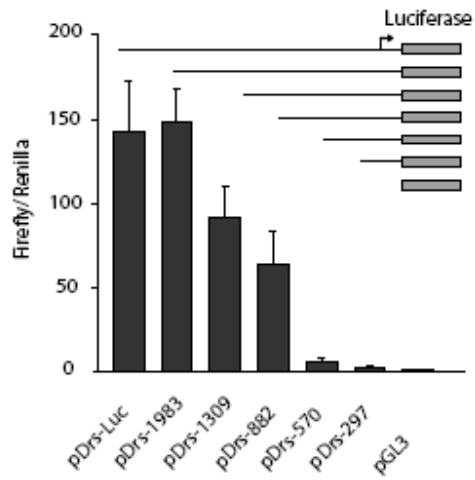


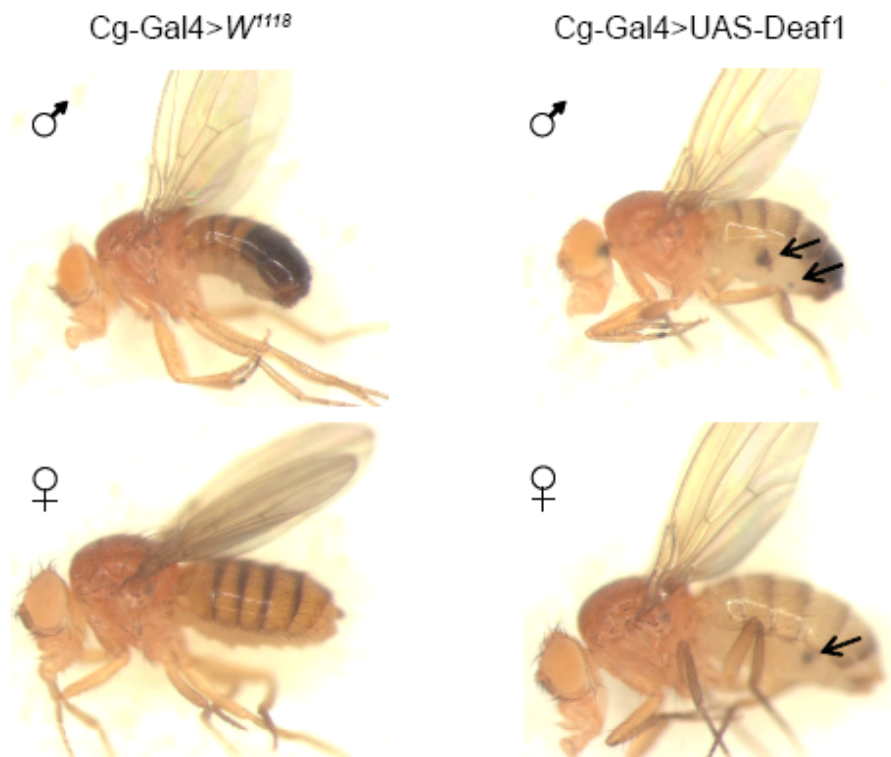
Online Supplementary Material



Online Supplementary Fig. 1. Analysis of UAS-Deaf1 RNAi efficiency. Adult fat bodies of 5 day old flies expressing the Deaf1 RNAi construct (Cg>Deaf1 RNAi) were dissected to assess Deaf1 mRNA levels. *Deaf1* mRNA levels in wild-types were set to 100%. Error bars indicate two technical replicates. Experiment was independently reproduced twice.



Online Supplementary Fig. 3. Identification of a minimal *Drs* enhancer sequence. Enhancer elements of the *Drs* promoter ranging from +50 to -1983, -1309, -882, -570 and -297 were cloned upstream of a luciferase gene in the pGL3 vector (Promega). All reporter constructs were transiently transfected with Toll^{ALLR} and *Renilla* coreporter to test for Luciferase expression. Experiment was independently reproduced twice. Error bars account for the standard deviation of quadruplicates.



Online Supplementary Fig. 4. Overexpression of Deaf1 in adult fatbody and hemocytes induces melanotic tumors. Flies carrying UAS-Deaf1 construct (7), were crossed with the Cg-Gal4 driver and kept at 29°C. Arrows indicate melanotic tumors. ^{w¹¹¹⁸} flies crossed with Cg-Gal4 individuals served as the negative control.

Supplemental Methods

Large scale RNAi screening

Hemocyte derived *Drosophila* SL2 cells were maintained in Schneider's medium (Invitrogen) supplemented with 10% fetal calf serum (PAA) and penicillin-streptomycin (Invitrogen). Cells were grown at 25°C at sub-confluent densities. All screening experiments were performed in 96-well tissue culture plates (Microtest Tissue Culture Plate 96 well Flat Bottom, Falcon). Screening plates were pre-loaded with an average of 1µg dsRNA per well in 10µl of 1mM Tris pH7 with a Biomek FX robot (Beckmann-Coulter). To transfect *EGFR-Toll*, *Drs-luc* and *pIz-RL* reporter constructs, 5 million SL2 cells were seeded per 35mm dish (Cellstar, Greiner) and cultured for 24h. Then 0.7µg of h*EGFR-Toll* (1), 0.7 µg *Drs-luc* plasmid DNA (2) and 0.7µg *pIz-Rluc* (*pIZ-RL*, Renilla luciferase expression plasmid, Invitrogen) were co-transfected using Effectene (Qiagen) according to manufacturer's instructions. Sixteen hours after transfection, cells were resuspended in serum free medium, adjusted to 10,000 cells per µl and 50µl were subsequently seeded per well of the 96-well tissue culture plates containing the dsRNA using a liquid dispensing unit (Multidrop, Thermo Labsystems). After 45 minutes of starvation, 50µl of complete medium was added to each well. Cells were incubated in sealed assay plates at 25°C. Ninety-six hours after RNAi treatment, cells were stimulated by adding recombinant human EGF (Biomol) to a final concentration of 0.2ng/µl. Sixteen hours after induction, cells were lysed and the lysate was split to read Firefly and *Renilla* luciferase activities in separate assay plates (white 96-well LIA Plates, Greiner) using a Mithras LB940 plate reader (Berthold Technologies). The screen was performed in duplicate.

Computational analysis

To identify candidate genes that significantly increase or decrease Toll pathway activity, the ratio of Firefly and *Renilla* raw luciferase results were normalized by median centering of each 96well plate. z-scores were calculated as the number of median-adjusted standard deviation (mad) that a particular well differed from the median of the 96well plate. All calculations were performed on the R-biostatistical platform using cellHTS package (3). To minimize false negatives, we applied a set of low-stringency criteria to generate a list of candidate genes. First, we filtered dsRNA treatments with z-scores > 2.5 for negative regulators or <-2.5 for positive regulators, respectively. Treatments that showed a high variability between duplicates were excluded (standard deviation higher than 50% of the replicates average). We also filtered against previously identified cell viability modifiers that show a phenotype in cultured *Drosophila* cells (4). We further excluded genes that showed phenotypes in other screens (M.B., unpublished observations). These filtering steps led to a final list of approximately 32 candidates (Supplementary table 1).

Cloning of Deaf1 full length and deletion constructs

cDNA from reverse transcribed total RNA served as template to amplify Deaf1 in a PCR reaction using AccuPrime™ Taq DNA Polymerase High Fidelity (Invitrogen) following manufacturer's instructions. Fragments generated by combinations of forward primers EcoRI-CTATCGTTGGACCGCAGAATAAGTG, EcoRI-CTAGTATGCGTCTGCTGCGACG-ACGAAGC and reverse primers XbaI- GGAATGATCGTCGATCAACAGC, XbaI-GTCCGCGTGCACCTTGACAC were subcloned into EcoRI and XbaI sites of pAc5.1V5/His expression vector (Invitrogen).

***Drosomycin* promoter luciferase reporters**

All *Drs* promoter sequences were subcloned into NheI/NcoI sites of pGL3-basic luciferase reporter plasmid (Promega). To generate *Drs* reporters of different length we combined 3' primer NcoI-TGGAAAAGGTTCTCACGGAG with 5' primer NheI-GTTTCAGCGGAGAGTTCCAG (pDrs-1983), NheI-GGCAATCTTAGCAACAACGA (pDrs-1309), NheI-CGGTCGTTTCGAATTTTGTT (pDrs-882), NheI-CCGTGCATTC-TTAAAGCAA (pDrs-570) and NheI-CGAATCATTTTTGCTGGACA (pDrs-297).

Site directed mutagenesis

To introduce mutations in TTCG sites of the construct pDrs-882 we used the QuickChange® II Site-Directed Mutagenesis kit (Stratagene) according to manufacturer's instructions. Site location, mutated sequence and primers are given in Supplementary table 3.

Cloning of a *Deaf1* *in-vivo* hairpin RNAi construct

XbaI-GCCCTGTCCGTCTCTTACA and XbaI-TCCTTTTGC-AGAACCTCGAT primers were used to amplify a 517bp long fragment of exon three of *Deaf1* for *in vivo* RNAi. The PCR generated amplicon was subcloned into NheI and SpeI sites of the pWiz plasmid (5) to generate an inverted repeat separated by the white intron 2. The resulting plasmid, pWiz-*Deaf1*-RNAi, was first tested in cell culture for RNAi efficiency and then injected into *w*¹¹¹⁸ embryos to generate transgenic flies.

Supplementary Table 1 Screening results

GeneID	CG	z-score ^a	dsRNA ID	Primer 1	Primer 2	Specificity / Efficiency / % ^b
Rel	CG11992	-11.19	HFA16819	CAGAGCGCTCGTACAAA	TCCTGTTTAAATTTCGAATAA	100 20
Myd88	CG2078	-10.15	HFA06815	ACTCGTGGCCCATCTCC	CTGGATATCGTGGCAGAC	100 5
Trf2	CG18009	-9.86	HFA18727	CTAGCGTAAACCAAGTTGA	GTAACGTGGACGCTATTCT	100 23
plf	CG5974	-8.22	HFA17026	GGCGTCAAGAACTCAA	CGAGGCTGATCTAAAC	100 12
tkk	CG1856	-7.68	HFA17091	ACAGCGATGGCGAAGGT	GACCCAGCAGCCTTGAAC	100 17
Deaf1	CG8567	-6.16	HFA11143	GATGTCATCCAGCAACAG	TGACTACCTGCTGCTC	100 9
TRIA-L	CG5930	-5.95	HFA16882	ATGCCAAGGCCAAGAAAG	ACAGGCATATGGATTGG	100 9
TRIA-S	CG5163	-5.46	HFA16883	TGAACTGTACCGCAACCC	AGAACTCGCGCTCTTG	79 15
M-2	CG8103	-5.33	HFA11222	CGGAGGCGAAGATGG	ACCCATTGGGAATGCCAG	100 7
bon	CG5206	-5.28	HFA16914	AGGTGCTGGTGGAAAAGAA	ACCGTCAACCCGAAAG	100 13
Eip93F	CG18389	-5.07	HFA16661	GGCATAATGAACGAGGGAC	CATCATCTCTGTATGCTAGAC	102 8
kay	CG15509	-4.99	HFA16977	CGGAAATACCTGAAATAAG	GCGCCGGCTTGAGA	100 7
CG1244	CG1244	-4.27	HFA08274	CAATTGAGACCAAGTATGG	GCGCATGATGGCCATT	100 13
Su(Hw)2-10	CG8068	-4.23	HFA07721	CATGCGATTTCCGCTCTT	GTAAGAAAGGCACCTCTTGG	100 12
Taf4	CG5444	-4.03	HFA11297	ATTGTGCAATACGTGGACC	GGCTGGGTTCTTTT	100 10
CG32180	CG32180	-3.96	HFA11160	CGAGTCAAAGTCTCTATAAT	TTGATCAAGCTCTCTGTAG	100 4
hay	CG8019	-3.2	HFA11354	TACCAACAATCCAGACATTAAT	ATCCAGCTTCAATGAGA	100 18
Fer1	CG33323	-3.04	HFA12555	CTGTACCGCAAGGGTGA	ACTGCCGCTGTGAAAA	100 11
CG3711	CG3711	-3.01	HFA18647	TCTTCGAGGCGCTAC	CCCAACCCGTAAGCCTTC	100 15
gce	CG6211	3.11	HFA19974	CAATAGTTCATATGGCGAGTC	GTTTTGGGTTACCATGCC	100 12
CG30426	CG30426	3.14	HFA04119	CGGTACACCAAGGAGATGG	CAAAATTGCTACGTCAACAC	100 13
CG31441	CG31441	3.14	HFA15723	TGCGAAAAAGCGGATAGT	GCTACAGAACCGCAAGC	100 19
CG7015	CG7015	3.19	HFA10761	AGTGGCGATCCGACC	CITCAGTAAAGTGGAAAAATAAAC	91 14
stw	CG3836	3.32	HFA11400	TTCGAGCGACCGATG AAG	AATATCGGAGAAAGGATCTG	100 21
pb	CG31481	3.33	HFA12629	ATCAAAAAGGAGCGGCT	GTTGGTCTGTTGTAGTGT	100 14
CG33936	CG33188	3.56	HFA15147	CGCTTAGAAAAAGCAACAG	ACTCTCTGCAAAACATCTCC	80 14
BhbVII	CG11494	3.73	HFA08151	TGCAATGGACAGTTGTTGATT	GCCGCTCAGGGGCT	100 7
CG33213	CG33213	4.07	HFA14487	CGCAACCGGCAAAAGGT	CCACTTGTTCTCCAGCG	100 9
CG32767	CG32767	4.14	HFA18386	CAGCAACATCATCTGCTAAC	TGTAGACAGGTGGTCTTG	100 10
Bk	CG6500	4.2	HFA19350	TGATGACTATGGACATACCCAAA	GCTTGCAGAGCATCAGGT	100 9
osa	CG7467	4.21	HFA17022	CGGTGGTGGGATGGTAG	CATTGAAAAGCGTGTCT	100 5
CG3726	CG3726	4.35	HFA18320	CCAGATGCGCCCAATC	CGTCTCGATCTCTCCAC	100 7
dalaio	CG7055	4.83	HFA18419	CCTCCAGGAGGACTAAA	CTCGACTAGCGTTGACTG	100 5
brm	CG5942	4.87	HFA11330	GTTTGGCTGTACAATAACAATC	ATGTTGGAGCAGGACTTAAAG	100 28

^a negative scores correspond to significant impairment of pathway activity. Positive scores correspond with hyperactivation. Calculation of Z-scores were performed using BioConductor package cellHTS (Boutros et al., 2006)

^b percentage of all possible 21 nucleotide long siRNA originating from dsRNA sequence which match completely to target gene sequence. (Arizman et al., 2005)

^c percentage of matching siRNAs which are efficient according to Reynolds criteria (Reynolds et al., 2004; Arizman et al., 2005).

Supplementary Table 2 dsRNA template and qPCR primers

Gene	Primer 1	Primer 2	Probe library # ^a
T7 tagged primer for RNAi probe synthesis			
GFP	TAAATACGACTACTATAGGACCCCTGTGACCAACCCTGACCTAC	TAATACGACTCACTATAGGGGACCAATGTGATCGCGCTTCTCGT	
Myd88	TAATACGACTCACTATAGGGAACAACATCA.CAGTGGCCAAAAGTG	TAATACGACTCACTATAGGGGAGTAGATCGAAAAGGCATCCAGA	
Cactus	TAATACGACTCACTATAGGGAA.GGCAACAAGCAACAAGTGA	TAATACGACTCACTATAGGGCCTGA.TCCTCTTCTTCGTCCG	
Dif	TAATACGACTCACTATAGGGCCCAAAATTGGACCAC	TAATACGACTCACTATAGGGTGGCACTCATTTTCTGACTTA	
Dorsal	TAATACGACTCACTATAGGACTACA.ATCGTTTGCCAG	TAATACGACTCACTATAGGGTGATTTGTGGTCAGGTTCCA	
IMD	TAATACGACTCACTATAGGAGA.ATGTCAAAGCTCAGGAACCT	TAATACGACTCACTATAGGGAGAAATGCTGACCCGTTTTGCGCG	
Relish	TAATACGACTCACTATAGGTGGACACATGGATCGCTAA	TAATACGACTCACTATAGGCGGACTTGGGTTATTGATT	
Deaf1 1	TAATACGACTCACTATAGGTGATGAGGAAGAAGAGGAGGA	TAATACGACTCACTATAGGGTTCACATTATCTGCGGTCCA	
Deaf1 2	TAATACGACTCACTATAGGGATCGTCA.TCCAGCAACAG	TAATACGACTCACTATAGGGTGACTCACCTGCTTCGTC	
Deaf1 3	TAATA.CGACTCACTATAGGGCCCTGTCGTCCTTTTACA	TAATACGACTCACTATA.GGGGCCATCGTTGATGTTCA.CCAC	
Deaf1 4	TAATACGACTCACTATAGGGCTAAAAGT.CCTTGCAGTTCGGTT	TAATACGACTCACTATAGGGGCTTGCCTCAGTTC.AATA	
qPCR primer sequence			
RP49	CGGATCGATATGCTAAGCTGT	GCGCTTGTTCGATCCGTA	105
Drosomylin	TACTTGTTGCCCCCTCTTCG	GTATCTTCCGGACAGGCAGT	131
Diptericin	CCGAGTACCACCTCAATCT	ACTGCAAAGCCAAAACCATC	70
Deaf1	CCGAGGATGTTGTGAAAGAAG	ATGCGAACCTTGGTGTCC	19
			84

^a Primers for qPCR analysis were generated using the online tool at www.universalprobelibrary.com

Supplementary Table 3 Primers used to introduce point mutations.

Name	site location ^a	Wild Type sequence	mutated sequence	Primer 1	Primer 2
Drs-mutA	-23	CGAA	AGAG	GTGCACTATAAAGCTTCTCCTAGAGGTTCCCAAGCGACTTGTGGCTTGGGAACCTCTAGGAGAAGCTTCCACAAGTCCGATATAGTGCAC	ATATAGTGCAC
Drs-mutB	-88	TTCG	A GAG	GTATCATCATAAATTTTGTGATATACAGAGTTTATA GCCGATGGGTAGTCGGGTATAAACTCTGTATATCACCCGACTACGCATCGGC	ACAAAATTATGATGATAC
Drs-mutC	-140	CGAA	AGAG	CAAAGTAGTTCCCTACAGAGGGCCTATAAATGTG CATGTCCAGTCCACATTTATAGGCCCTCTGTAGGGGACTGCACATG	AACTACTTTCG
Drs-mutD	-169	TTCG	AGAG	GCAATGCTTTTTCGCTTAGAGATAAGCGTAAAGAACTACTTGTACCGCTTATCTCTAAAGCGTAAAGCCAAAGTAGTTCC	AAAAGCATTTGC
Drs-mutE	-182	TTCG	AGAG	CAAATGAGGCTCTAAGCAATGCTTAGAGCTTACG CGCTTATCGAAAAGCGTAAAGCTTAAAGCAATTGCTTCTTTTCGATAAGCG	AGAGCCCTCATTTG

^a location of TTCG motif relative to transcriptional start site of *Drosomycin*

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