Supplementary Table 1: The fly alleles used in this study.

Fly allele	Source	Stock number
w^{1118}- *mdg4*-TR (attP2 &attP40)	This paper	N/A
w^{1118}- *mdg4*-PC (attP2 &attP40)	This paper	N/A
w^{1118}- *mdg4*-NC (attP2 &attP40)	This paper	N/A
w^{1118}-*3S18*-TR (attP2)	This paper	N/A
w^{1118}-*412*-TR (attP2 &attP40)	This paper	N/A
w^{1118}-*Blood*-TR (attP2 &attP40)	This paper	N/A
w^{1118}-*Burdock*-TR (attP2 &attP40)	This paper	N/A
w^{1118}-*Copia*-TR (attP2 &attP40)	This paper	N/A
w^{1118}-*Mdg1*-TR (attP2 &attP40)	This paper	N/A
w; ac5c-gal4/CyO; mdg4-TR/TM3,*Sb,Ser*	This paper	N/A
w; ac5c-gal4/CyO; Tub-gal80^{ts}/TM3,*Sb*	This paper	N/A
w; ac5c-gal4/CyO:gfp	This paper	N/A
w; tjgal4/CyO; mdg4-TR/TM3,*Sb*	This paper	N/A
w^{1118}; sh-*white* (attp2)	Senti *et al., Genes & Dev.,* 2015	N/A
w^{1118}; sh-*piwi* (attp2)	Senti *et al., Genes & Dev.,* 2015	N/A
w^{1118}*; iso2; sh-white*/TM6B,*Tb,Hu*	This paper	N/A
w^{1118}*; iso2; sh- mdg4*-1/TM6B,*Tb,Hu*	This paper	N/A
w^{1118}*; iso2; sh- mdg4*-2/TM6B,*Tb,Hu*	This paper	N/A
w^{1118}*; iso2; sh- mdg4*-3/TM6B,*Tb,Hu*	This paper	N/A
w^{1118}*; iso2; sh- mdg4*-4/TM6B,*Tb,Hu*	This paper	N/A
w^{1118}*; iso2; sh- mdg4*-5/TM6B,*Tb,Hu*	This paper	N/A
w^{1118}*; iso2; sh- mdg4*-6/TM6B,*Tb,Hu*	This paper	N/A

w^{1118}*; iso2; sh-* *mdg4*-7/TM6B,*Tb,Hu*	This paper	N/A
sh-dcr2	BDSC	CAT#: 33656
sh-ago2	BDSC	CAT#: 34799
sh-pelle	BDSC	CAT#: 41935
sh-relish	BDSC	CAT#: 33661
sh-dSTING	VDRC	CAT#: 4031
dcr-2^{L811fsX}/*Cy*O	Lee *et al., Cell,* 2004	N/A
w^{1118}*; If*/*Cy*O*; iso3*	VDRC	CAT#: 313596
w^{1118}*; iso2; Dr*/TM6B,*Tb,Hu*	VDRC	CAT#: 313597

Supplementary Table 2: Fly genotypes for each figure.

Genotype	Figure
mdg4-TR: w^{1118}- *mdg4*-TR (attP2) *mdg4*-PC: w^{1118}- *mdg4*-PC (attP2) *mdg4*-NC: w^{1118}- *mdg4*-NC (attP2)	Figure 1b
mdg4-TR: w^{1118}- *mdg4*-TR (attP2)	Figure 1c
w^{1118}- *mdg4*-TR (attP2)	Figure 2a, b and d (Different stages)
Positive control: *w**; *tj-Gal4*; sh-*piwi* Negative control: *w**; *ac5c-Gal4*; sh- *mdg4*-1	Figure 2d
Lab strain-1: *w**; ac5c-Gal4/CyO; *mdg4*-TR Lab strain-2: *w**; ac5c-Gal4/CyO; tub-Gal80^{ts}	Figure 3
sh-white: *w*; ac5c-Gal4; mdg4*-TR/sh-*white* *sh- mdg4*: *w*; ac5c-Gal4; mdg4*-TR/sh- *mdg4*-1	Figure 4a; Extended Data Fig. 4
sh-white: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*white* *sh- mdg4*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh- *mdg4*-1	Figure 4b and 4c
sh-white: *w*; ac5c-Gal4; mdg4*-TR/sh-*white* *sh- mdg4*: *w*; ac5c-Gal4; mdg4*-TR/sh- *mdg4*-1	Figure 5a and b
sh-white: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*white* *sh- mdg4*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh- *mdg4*-1	Figure 5c and d
sh-white: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*white* *sh- mdg4*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh- *mdg4*-1 sh-*relish*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*relish* sh-*dcr-2*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*dcr-2* sh-*ago-2*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*ago-2* sh-*pelle*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*pelle*	Figure 6a, d and e
sh-white: *w*; ac5c-Gal4; mdg4*-TR/sh-*white* sh-*relish*: *w**; *ac5c-Gal4*; *mdg4*-TR/sh-*relish*	Figure 6b and c
sh-white: *w*; ac5c-Gal4;* sh-*white* *sh- mdg4*: *w*; ac5c-Gal4;* sh- *mdg4*-5 *sh-relish*: *w*; ac5c-Gal4;* sh-*relish*	Figure 7a and b
sh-white: *w*; ac5c-Gal4;* sh-*white* *sh-dSTING*: *w*; ac5c-Gal4; dSTING*	Figure 8a and b
sh-white: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*white* *sh-dSTING*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*dSTING*	Figure 8c and d
3S18: w^{1118}-*3S18*-TR (attP2) *412*: w^{1118}-*412*-TR (attP2 and attP40) *Mdg1*: w^{1118}-*Mdg1*-TR (attP2 and attP40) *blood*: w^{1118}-*blood*-TR (attP2 and attP40) *I-element*: w^{1118}-*I-element*-TR (attP2 and attP40) *Copia*: w^{1118}-*Copia*-TR (attP2 and attP40) *Doc*: w^{1118}-*Doc*-TR (attP2 and attP40) *Burdock*: w^{1118}-*Burdock*-TR (attP2 and attP40) *mdg4*: w^{1118}- *mdg4*-TR (attP2 and attP40)	Extended Data Fig. 1b
mdg4-TR: w^{1118}- *mdg4*-TR (attP2) *mdg4*-PC: w^{1118}- *mdg4*-PC (attP2)	Extended Data Fig. 1c

mdg4-NC: *w*^{1118}- *mdg4*-NC (attP2)	
w^{1118}- *mdg4*-TR (attP2)	Extended Data Fig. 2b and c
w^{1118}- *mdg4*-TR (attP2)	Extended Data Fig. 3b and c (Different tissues)
PC: *w*^{1118}- *mdg4*-PC (attP2) NC: *w*^{1118}- *mdg4*-NC (attP2)	Extended Data Fig. 3c
*w**; ac5c-Gal4/CyO; tub-Gal80ts	Extended Data Fig. 4
sh-white: *w*; ac5c-Gal4;* sh-*white* *sh- mdg4*-X: *w*; ac5c-Gal4;* sh- *mdg4*-X	Extended Data Fig. 5a, 5d and 5e
sh-white: *w*; ac5c-Gal4; mdg4*-TR/sh-*white* *sh- mdg4*-1: *w*; ac5c-Gal4; mdg4*-TR/sh- *mdg4*-1	Extended Data Fig. 5b and c
*w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/UAS-GFP	Extended Data Fig. 6b
sh-white: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*white* *sh- mdg4*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh- *mdg4*-1	Extended Data Fig. 6c and d
sh-white: *w*; ac5c-Gal4; mdg4*-TR/sh-*white* *sh- mdg4*: *w*; ac5c-Gal4; mdg4*-TR/sh- *mdg4*-1	Extended Data Fig. 7a, b and c
sh-white: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*white* *sh- mdg4*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh- *mdg4*-1	Extended Data Fig. 7d
sh-white: *w*; ac5c-Gal4; mdg4*-TR/sh-*white* sh-*relish*: *w**; *ac5c-Gal4*; *mdg4*-TR/sh-*relish*	Extended Data Fig. 8a and b
sh-white: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*white* sh-*relish*: *w**; *ac5c-Gal4*; tub-*Gal80*^{ts}/sh-*relish*	Extended Data Fig. 8c and d
dcr2^{L811fsx}/ *dcr2*^{L811fsx}	Extended Data Fig. 8e
sh-white: *w*; ac5c-Gal4;* sh-*white* *sh- mdg4*: *w*; ac5c-Gal4;* sh- *mdg4*-1	Extended Data Fig. 8f
sh-white: *w*; ac5c-Gal4;* sh-*white* *sh- mdg4*: *w*; ac5c-Gal4;* sh- *mdg4*-5 *sh-relish*: *w*; ac5c-Gal4;* sh-*relish*	Extended Data Fig. 9a
w^{1118}- *mdg4*-TR (attP2)	Extended Data Fig. 9b
sh-white: *w*; ac5c-Gal4;* sh-*white* *sh- mdg4*: *w*; ac5c-Gal4;* sh- *mdg4*-5	Extended Data Fig. 9c

Supplementary Table 3a: Primers used for RT-PCR and RT-qPCR.

Gene	Sequence	Experiment
mdg4-gag (Full length of mRNA)	F: 5′-AGACAATCGGCCATATACGC-3′ R: 5′-TTTCCAATCCTTGCCTCAAC-3′	RT-PCR
mdg4-env (Spliced mRNA)	F: 5′- GGCTCATTGCCGTTAAACAT-3′ R: 5′- CAAGAGGTCACGCTGTTCAA-3′	RT-PCR
GFP reporter	F: 5′- CGGGACTACTCCGGTAAACA-3' R: 5′- CTAGGCACACCGAAACGACT-3'	RT-PCR
DCV-RT (4235-4863)	F: 5′- CGACTCGTACTGGGGATTGT-3′ R: 5′- AGGAAATCCTGGTGACGTTG-3′	RT-PCR
DCV-RT (3133-4328)	F: 5′- GTTGCCTTATCTGCTCTG-3′ R: 5′- CGCATAACCATGCTCTTCTG-3′	RT-PCR
DCV-RTQ	F: 5′- CAGGACACCCTCTCTGCTTC-3′ R: 5′- CCGCAGTGGTACTAGGGGTA-3′	RT-qPCR
relish	F: 5′- TACAAGAGCGAGATGCATGG-3′ R: 5′- ATAAATTGCGCCACATAGCC-3′	RT-qPCR
sting	F: 5′- TCCCGATGAGATGTTTGTCA-3′ R: 5′- GCCAGGTACCCGACAAGTTA-3′	RT-qPCR
dcr2	F: 5′- GTTCCGCTTTGGTCAACAAT-3′ R: 5′- AGTCGGCTGAACATCAGCTT-3′	RT-qPCR
ago2	F: 5′- CACCATTGTGCATCCTAACG-3′ R: 5′- CATGTGGCACAGGTTGTAGG-3′	RT-qPCR
pelle	F: 5′- CGGAAAACGAGACGAAGAAG-3′ R: 5′- GGCCCATCTAGTCGGTAACA-3′	RT-qPCR
rp49	F: 5′- CCGCTTCAAGGGACAGTATCTG-3′ R: 5′- ATCTCGCCGCAGTAAACGC-3′	RT-PCR & RT-qPCR

Supplementary Table 3b: Primers used for constructing plasmids:

Construct	Sequence
mdg4-rpsL-neo (*mdg4*-TR & *mdg4*-TR-NC)	F: 5′-CTTATTAAGGGGAGGGTGGTCCACAAGGACAACCT CAAGTAGACATTCCCGGCCTGGTGATGATGGCGGGATCG-3′ R: 5′-TACTACAGTGCTCGGATTTTCCTTGACATAACTTAC TACCTAACTGTAGATCAGAAGAACTCGTCAAGAAGGCG-3′
mdg4-TR & *mdg4*-TR-NC	F: 5′-CTTATTAAGGGGAGGGTGGTCCACAAGGACAACC TCAAGTAGACATTCCCGGCGCGCCGGTACCGAAT-3′ R: 5′-TACTACAGTGCTCGGATTTTCCTTGACATAACTTA CTACCTAACTGTAGATATGCCGAATGGGCATTTATTG-3′
412-rpsL-neo	F: 5′-AACTTATATTTTCCTTAATCATTTACACAAATTTTCC ATACACTACGTAT GGCCTGGTGATGATGGCGGGATCG-3′ R: 5′-TGTTTTTGTTTGAACAACAATAGTTGATTTTATAATGC AAAGATAAAAATTCAGAAGAACTCGTCAAGAAGGCG-3′
412-TR	F: 5′-AACTTATATTTTCCTTAATCATTTACACAAATTTTC CATACACTACGTATGGCGCGCCGGTACCGAAT-3′ R: TGTTTTTGTTTGAACAACAATAGTTGATTTTATAATG CAAAGATAAAAATTATGCCGAATGGGCATTTATTG-3′
Blood-rpsL-neo	F: 5′-CATAATAAAACTTAAATAACGGCCTGATCAGCCAA AACAATATAACAAAGGGCCTGGTGATGATGGCGGGATCG-3′ R: 5′-AAAAAATATGTATGTATTTTGAATTAATAAAAATTCG ATTATGTCTATGTTCAGAAGAACTCGTCAAGAAGGCG -3′
Blood-TR	F: 5′-CATAATAAAACTTAAATAACGGCCTGATCAGCCAA AACAATATAACAAAGGGCGCGCCGGTACCGAAT-3′ R: 5′-AAAAAATATGTATGTATTTTGAATTAATAAAAATTC GATTATGTCTATGTTATGCCGAATGGGCATTTATTG-3′
Burdock-rpsL-neo	F: 5′-TACTGAAACGACGAACTGAATAATATCTGCCATCAGA CGCCAACCAGAGTGGCCTGGTGATGATGGCGGGATCG-3′ R: 5′- TGCTGATGTTGGTTGAACTAGTTGACCATCAAAACGTA TGTGTTGAACGCTCAGAAGAACTCGTCAAGAAGGCG -3′
Burdock-TR	F: 5′- TACTGAAACGACGAACTGAATAATATCTGCCATCA GACGCCAACCAGAGTGGCGCGCCGGTACCGAAT-3′ R: 5′- TGCTGATGTTGGTTGAACTAGTTGACCATCAAAACG TATGTGTTGAACGCTATGCCGAATGGGCATTTATTG-3′

Copia-rpsL-neo	F: 5′-CATATTTTGTTACAATGATCTGATCGGGTTTTTCTGGG TTTTCCCCGTATGGCCTGGTGATGATGGCGGGATCG-3′ R: 5′- GGTGGTGTGCATTCTGGGAAGTGTTAACTGATCCAG CATTTGCTGCGAGGTCAGAAGAACTCGTCAAGAAGGCG-3′
Copia-TR	F: 5′- CATATTTTGTTACAATGATCTGATCGGGTTTTTCTGGG TTTTCCCCGTATGGCGCGCCGGTACCGAAT-3′ R: 5′- GGTGGTGTGCATTCTGGGAAGTGTTAACTGATCCAG CATTTGCTGCGAGGTATGCCGAATGGGCATTTATTG-3′
Mdg1-rpsL-neo	F: 5′-CATAAAGATAGGTTAAAAATTTTTAATTCATAATACA TTTTGTTTGGTTGGGCCTGGTGATGATGGCGGGATCG-3′ R: 5′- TGGTTTTTTATTTGTGGTTTTTTATTTGTGGTTTTTTAT TTGTGGTTGGCTCAGAAGAACTCGTCAAGAAGGCG-3′
Mdg1-TR	F: 5′- CATAAAGATAGGTTAAAAATTTTTAATTCATAATAC ATTTTGTTTGGTTGGGCGCGCCGGTACCGAAT-3′ R: 5′- TGGTTTTTTATTTGTGGTTTTTTATTTGTGGTTTTTTAT TTGTGGTTGGCTATGCCGAATGGGCATTTATTG-3′
mdg4-TR-PC (Fragment 1)	F: 5′- CCTGCAGGTCGACTCTAGAGA GTTAACAACTAACAATGTATTG-3′ R: 5′- CCGGCGCGCCGGGAATGTCTACTTGAGG-3′
mdg4-TR-PC (Fragment 2)	F: 5′- AGACATTCCCGGCGCGCCGGTACCGAAT -3′ R: 5′- TAACTGTAGATATGCCGAATGGG CATTTATTGGTTTATTAGATTGGC-3′
mdg4-TR-PC (Fragment 3)	F: 5′- ATTCGGCATATCTACAGTTAGGTAGTAAGTTATG-3′ R: 5′- ATTCGAGCTCGGTACCCGGGAA TTATATAAGTTCCAATAGGTCC-3′
3S18-TR (Fragment 1)	F: 5′- ATTCGGCATACGAGCTTGCCCAGTACTTTTC-3′ R: 5′- ATTCGAGCTCGGTACCCGGGATATTTGAGAAGT TTGACTCGTTTATGTTATC-3′
3S18-TR (Fragment 2)	F: 5′- TAATTGTCCGGGCGCGCCGGTACCGAAT -3′ R: 5′- GGCAAGCTCGTATGCCGAATGGGCATTTATTG GTTTATTAGATTGGC-3′

3S18-TR (Fragment 3)	F: 5′- CCTGCAGGTCGACTCTAGAGTCAAGAAAATG TTTATAAAGCAATTGTTTG-3′ R: 5′- CCGGCGCGCCCGGACAATTACAGCGGTG-3′
sh- *mdg4*-1	F: 5′- CTAGCAGTCGATGTGCTTAGAGAGCAATAGTT ATATTCAAGCATATTGCTCTCTAAGCACATCGGCG-3′ R: 5′- AATTCGCCGATGTGCTTAGAGAGCAATATGCTT GAATATAACTATTGCTCTCTAAGCACATCGACTG-3′
sh- *mdg4*-2	F: 5′- CTAGCAGTGGGCGACGATAGAATATAATAGTT ATATTCAAGCATATTATATTCTATCGTCGCCCGCG-3′ R: 5′- AATTCGCGGGCGACGATAGAATATAATATGCTT GAATATAACTATTATATTCTATCGTCGCCCACTG-3′
sh- *mdg4*-3	F: 5′- CTAGCAGTGGGTTGACACTCCACACTTATAGTT ATATTCAAGCATATAAGTGTGGAGTGTCAACCGCG-3′ R: 5′- AATTCGCGGGTTGACACTCCACACTTATATGCTT GAATATAACTATAAGTGTGGAGTGTCAACCACTG-3′
sh- *mdg4*-4	F: 5′- CTAGCAGTGCAAGTTCAGTTGCAAGAAATAGTT ATATTCAAGCATATTTCTTGCAACTGAACTTGGCG-3′ R: 5′- AATTCGCGCAAGTTCAGTTGCAAGAAATATGCTT GAATATAACTATTTCTTGCAACTGAACTTGACTG-3′
sh- *mdg4*-5	F: 5′- CTAGCAGTGGGCTAGTGATAATAACTAATAGTT ATATTCAAGCATATTAGTTATTATCACTAGCCGCG-3′ R: 5′- AATTCGCGGGCTAGTGATAATAACTAATATGCTT GAATATAACTATTAGTTATTATCACTAGCCACTG-3′
sh- *mdg4*-6	F: 5′- CTAGCAGTGCGACAAACAGGGTAGTTAATAGTT ATATTCAAGCATATTAACTACCCTGTTTGTCGGCG-3′ R: 5′- AATTCGCGCGACAAACAGGGTAGTTAATATGCTT GAATATAACTATTAACTACCCTGTTTGTCGACTG-3′
sh- *mdg4*-7	F: 5′- CTAGCAGTGGTTCTTATTTACAATCAAATAGTT ATATTCAAGCATATTTGATTGTAAATAAGAACGCG-3′ R: 5′- AATTCGCGGTTCTTATTTACAATCAAATATGCTT GAATATAACTATTTGATTGTAAATAAGAACACTG-3′

Supplementary Table 4

Sample	Genotype	Sequencing platform	Number of reads (pairs)	Mean read length	Total bases	Mapping rate (%)	Source
RNA-Seq (illumina)							
embryo 0-2	*w**; ac5c-Gal4/CyO; tub-Gal80ts	illumina paired end	36,306,251	150	5,445,937,650	93.87	This study
embryo 2-8	*w**; ac5c-Gal4/CyO; tub-Gal80ts	illumina paired end	39,392,568	150	5,908,885,200	89.83	This study
L1	y; cn bw sp	illumina paired end	63,416,208	100	6,341,620,800	88.37	(Graveley et al., 2011)
L2	y; cn bw sp	illumina paired end	71,745,818	100	7,174,581,800	89.04	(Graveley et al., 2011)
L3	y; cn bw sp	illumina paired end	70,228,806	100	7,022,880,600	87.23	(Graveley et al., 2011)
pupa 0h	*w**; ac5c-Gal4/CyO; tub-Gal80ts	illumina paired end	33,146,684	150	4,972,002,600	61.47	This study
adult	*w**; ac5c-Gal4/CyO; tub-Gal80ts	illumina paired end	22,651,919	150	3,397,787,850	87.75	This study
Genome-Seq (Nanopore)							
acGal4	acGal4/cyo;Gypsy-TR-1/TM3,Sb	Nanopore GridION	733,441	6,308	4,626,248,460	99.62	This study
acGal4; tub-Gal80ts	acGal4/cyo;Gal80ts	Nanopore GridION	1,059,880	6,391	6,773,130,483	99.77	This study
DGRP379	DGRP-379	Nanopore MinION	878,451	8,449	7,421,727,828	80.76	(Ellison and Cao.,2020)
DGRP732	DGRP-732	Nanopore MinION	1,195,332	8,070	9,646,392,198	80.99	(Ellison and Cao.,2020)