

Figure S1

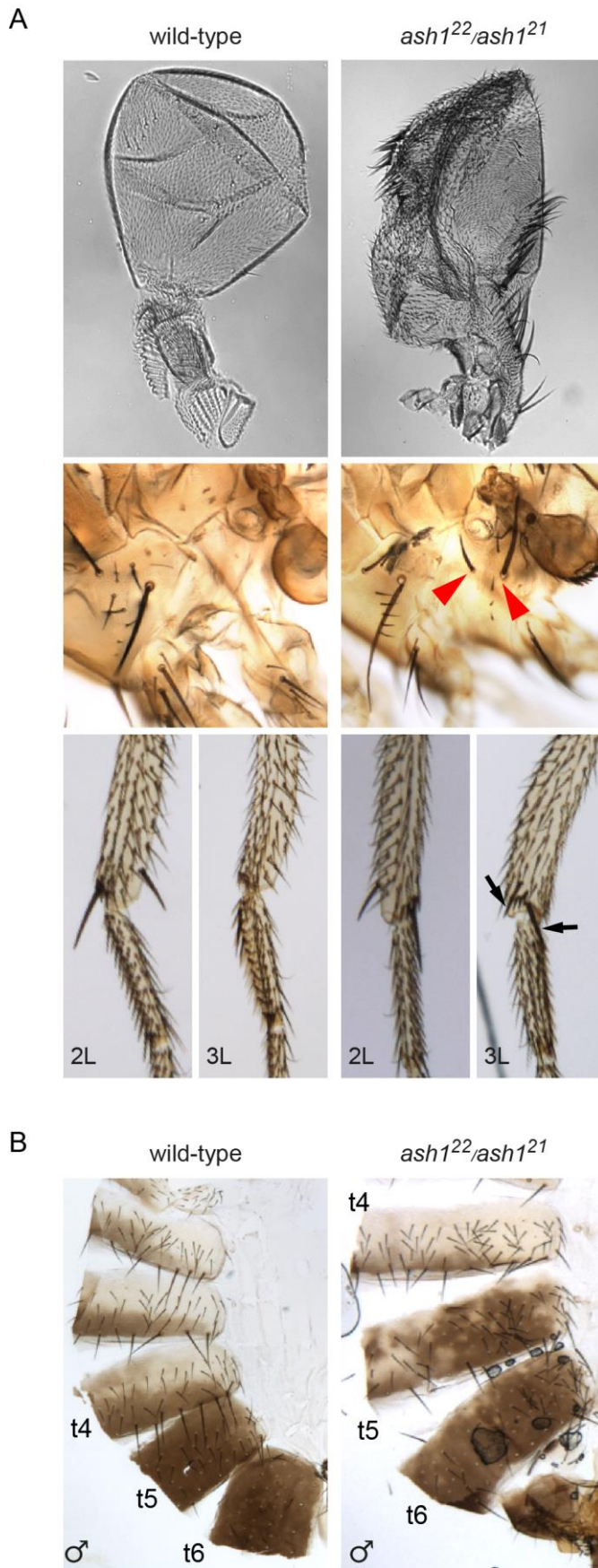


Figure S1. Homeotic transformation in *ash1* mutant flies. Characteristic homeotic phenotypes of *ash1* loss of function are caused by ectopic repression of *Ubx* and *Abd-B* genes. **(A)** Loss of *Ubx* expression leads to homeotic transformation of the third thoracic (T3) to second thoracic (T2) segment. This includes partial transformation of haltere to wing, characterized by the appearance of bristles on the haltere, appearance of hypopleural bristles on T3 (red arrowheads), and the third leg (3L) to second leg (2L) transformation manifested by the appearance of apical and pre-apical bristles on the third leg of *ash1²²/ash1²¹* animals (black arrows). **(B)** Erroneous repression of *Abd-B* leads to transformation of posterior abdominal segments to more anterior fate. This is visible by the partial loss of pigmentation in tergites 5 (t5) and 6 (t6).

Figure S2

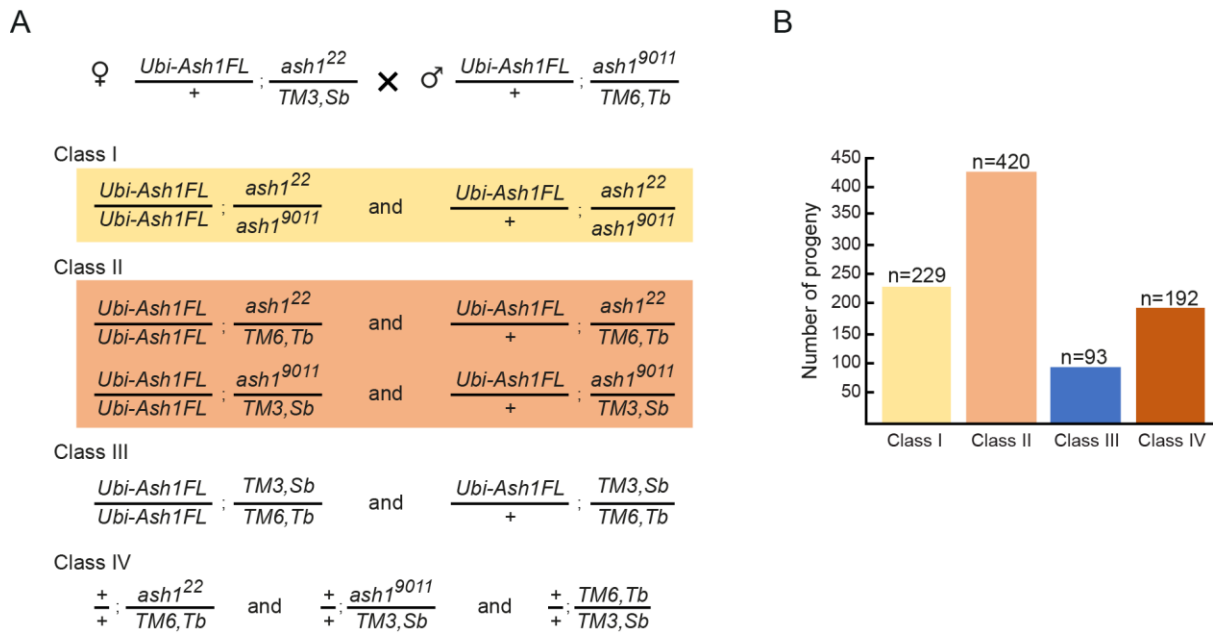
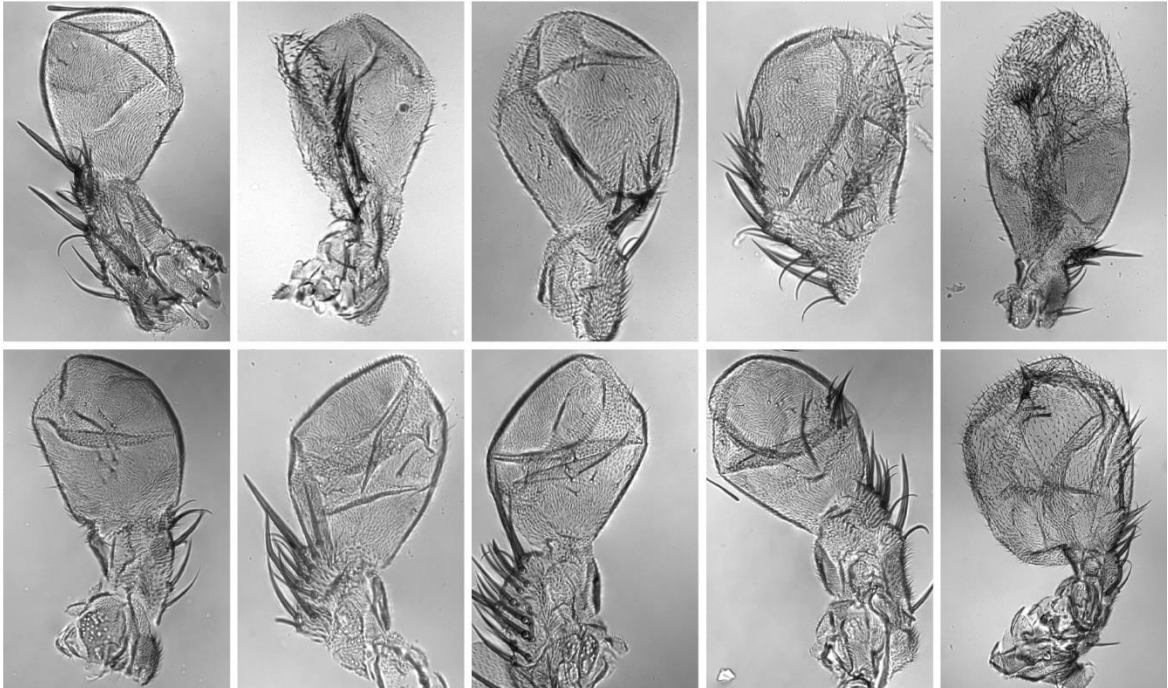


Figure S2. Expression from the *Ubi-p63E* promoter provides enough transgenic Ash1 protein. (A) Crossing scheme. Flies heterozygous for the full-length *Ash1* transgene under the control of *Ubi-p63E* promoter (*Ubi-Ash1FL*) on the second chromosome and heterozygous for the *ash1²²* allele on the third chromosome were crossed to flies heterozygous for the same *Ash1* transgene and *ash1⁹⁰¹¹* allele. **(B)** The viability test. If the *Ash1* transgene fully complements the lethality of the *ash1²²/ash1⁹⁰¹¹* mutants, the number of the Class I progeny is expected to be half of that of Class II. Lower than expected numbers of flies in Class III and Class IV are due to reduced fitness of flies with balancer chromosomes. (n = number of flies counted per phenotypic class).

Figure S3

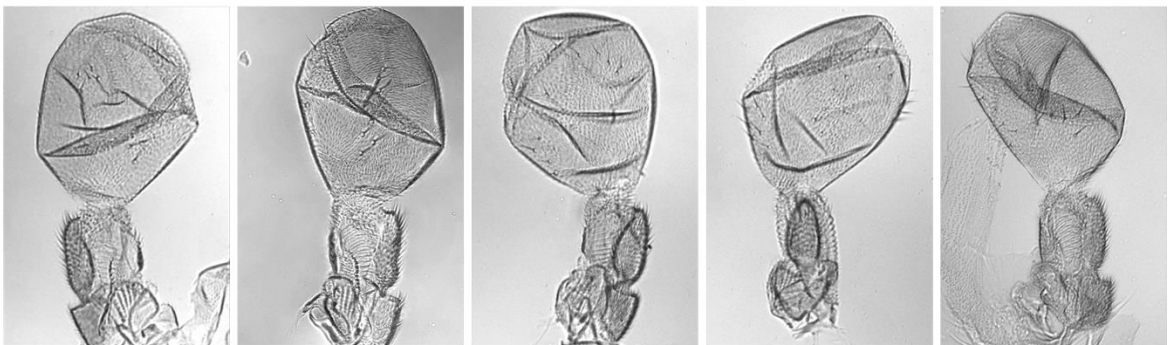
A

Mutant halteres



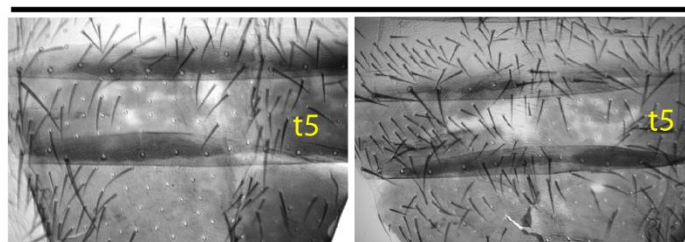
B

wild-type halteres



C

Weak



D

Strong

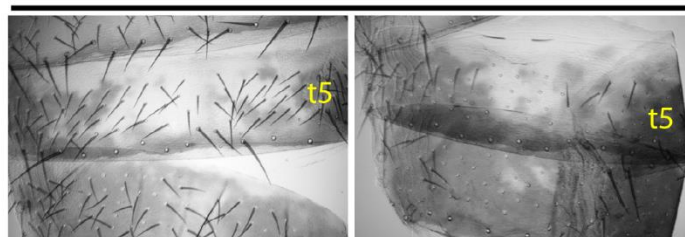


Figure S3. Examples of homeotic transformations illustrating the grading scale of Figures 4D and 8C. (A) Partial haltere to wing transformations ranging from mild (upper left corner) to severe (lower right corner). Note the appearance of bristles and changes in haltere shapes. (B) Wild-type halteres as a control. (C) Weak transformation of the abdomen in male flies is manifested by partial loss of pigmentation in tergite 5 (t5). (D) Strong transformation of the abdomen is characterized by greater area of t5 that lacks pigmentation.

Table S1. The list of oligonucleotides.

Oligo name	sequence
ASH1_seq3	CCAGGAGTAAGCGACGGCAG
ASH1_seq4	TGATGAACCAGCGTAAAGAAACCC
ASH1_seq5	AATGGGAAATTGAACGCAGAGGC
ASH1_seq6	ACAACAGCCGTGATTGATGAGC
ASH1_seq7	ATCCTACCGAGCACGATCCC
ASH1_seq8	ACTAAGCTACCCATTGCGAAGGG
ASH1_seq9	AAAAGATACGCAGATGCAAGGCC
ASH1_seq12	TTTCGTCAGAGGAGGAACCTGG
ASH1_seq13	ATTGAGGCCTCTCCTGATGAGG
ASH1_seq14	TCCAGTGGGATTAGTAAATCGGAC
deltaAT_A	GGTGCATCAGCAGAGAGCTC
deltaAT_B	CTGTTCCTAGGAACAGCTGTTTGGACTAGGG
deltaAT_C	CAGGAACGTTCTGCCCCAGGAAACTC
deltaAT_D	CAGTATCGTCTGCTGAGCTCTTC
deltaAT_E	GCCTGGGCTGCAGCC
deltaAT_F	CTTCAGCTCTAGCTGGTGTGTTGAGGACGC
deltaAT_G	CAGCTAGAGCTGAAGCTGCACCTCAACC
deltaAT_H	CACTGACTCTGCGTATACATTGGTG
deltaAT_I	GCTCTTCTGGCCTTATCTCAGGTTCCG
deltaAT_J	TAAGGCCAGAAGAGCCACCACCCACAC
deltaBAH_A	CCAAAGCTGACATCGATGCG
deltaBAH_B	GGTTGGCGCCGGACTCATCCTTGATGG
deltaBAH_C	AGTCCGGCGCCAACCATCCAGCCTGC
deltaBAH_D	ATCTCGATTACTCGAGTGTTGAGTTG
deltaPHD_A	CAGAAGGCCAAAAGCATGCTC
deltaPHD_B	CCACTTCATCCTCATCAGGAGAGGCCTC
deltaPHD_C	ATGAGGATGAAGTGGATCGAGAAATTCCCC
deltaPHD_D	TGACCAACTCAATGGGTACCAC
deltaSET_A	ATCCGCACCAATGTATACGCAG
deltaSET_B	AGGGATTGACGGCATGTCGCTGGATCTTC
deltaSET_C	ATGCCGTCAATCCCTCAGAGGGTCAGCC
deltaSET_D	CCGCCTGCCGTTGAGC
poSET-CRSP-1.1	CTTCGCTTGGCCTCCACAGCAGGA
poSET-CRSP-1.2	AAACTCCTGCTGTGGAGGCCAAGC
SET-CRSP-1.1	CTTCGTTATCGACGGTCAGCGGAT
SET-CRSP-1.2	AAACATCCGCTGACCGTCGATAAC

Table S2. The list of antibodies.

Antigen	Host	Reference/company/catalogue #	IF	ChIP	WB
Abd-B	mouse monoclonal	DHSB, 1A2E9	1:10	-	-
GFP	mouse monoclonal	Thermofisher, MA5-15256	1:200	-	-
BEAF-32	mouse monoclonal	DHSB, anti-BEAF	1:5	-	-
Ash1	rabbit polyclonal	Kahn at al., 2016	1:500	1:100	-
Ash1	rabbit polyclonal	against aa 2156-2217, gift from V.Pirrotta	-	-	1:500
anti-rabbit Alexa-488	goat	Invitrogen, # A-11008	1:500	-	-
anti-mouse Alexa-555	goat	Abcam, # ab150114	1:500	-	-
anti-rabbit (Fc) AP conjugated	goat	Promega, #S3731	-	-	1:10000

Table S3. The list of amplicons used in ChIP-qPCR

Amplicon name	Oligonucleotides	5'-3' sequence
rap	rap.1.1	TCTTCACGTCGTCGATTGCCG
	rap.1.2	CAGACGAGTAAGAAGCAGCGGGAC
eIF4a	eIF4a.1.1	AACTTGCGCGAGGAGTTGCTG
	eIF4a.1.2	TGACATCGCGACCCCTCACAC
emc	emc.1.1	GATCCAGATATCCTCGCCCAGC
	emc.1.2	CCACAAGAGTGTTGGGCGTTTG
hth	hth.1.1	TGTGTTTTTTGCGACGGCAATTTG
	hth.1.2	GCCGCGGAGGGAATTTCTTTC
eff	eff.1.1	AAATGGCGCTGTCTCCTTCTGC
	eff.1.2	TGTAAGAGAGAGGGAGGGGAGCC
Su(z)2	Su(z)2-2.1	CGTGCCGGTCGAACTGTTAT
	Su(z)2-2.2	TCCTTAGCCCGCTTTCTGTT
noc	noc-exp-1.1	AGATTTCCGCGCACGATTCCTC
	noc-exp-1.2	TTCCGTTGGCATTGTTTCAGCACC
Taf4	Taf4-ex1.1	AGCCCGCAGCCGTGTTTGAT
	Taf4-ex1.2	CGTGCTGCGGCGGAGGTTTA
intergenic	3R-nc-1.1	TTGCGGAGCGTGAGCGGAAG
	3R-nc-1.2	AAGCCAGGAGAACGGCACGC