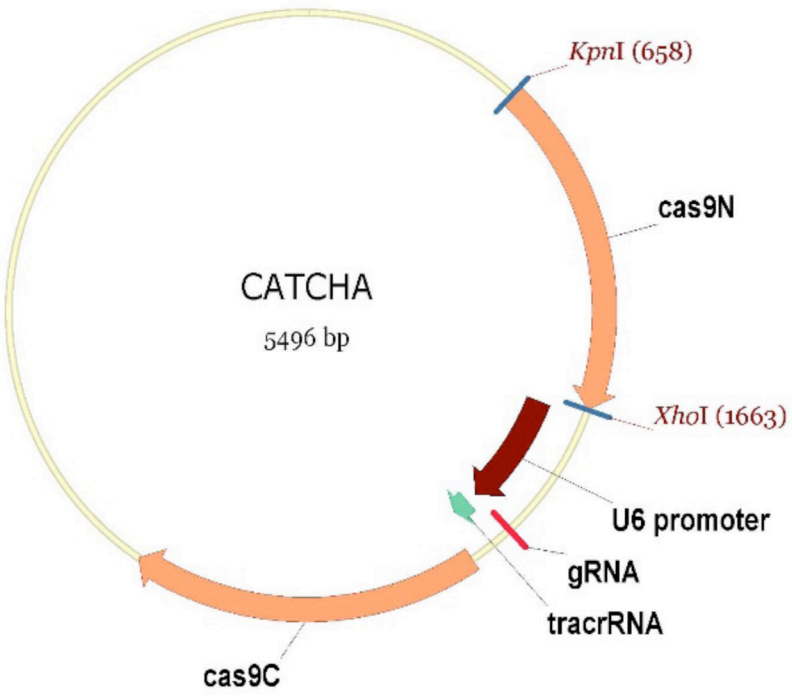
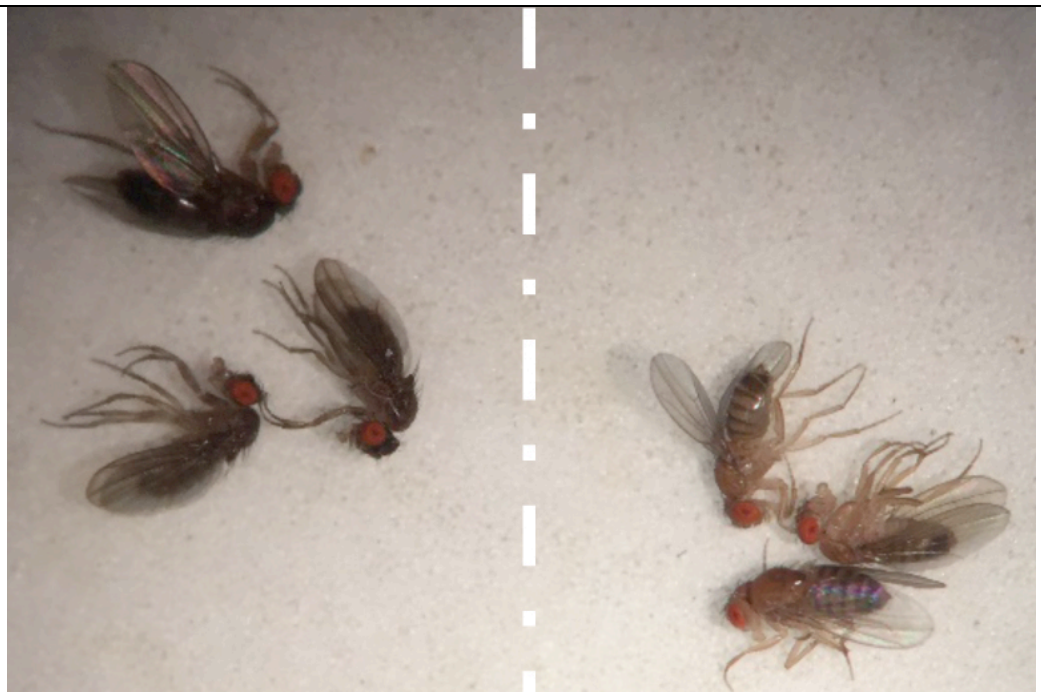


Supplementary Figure 1

Diagram for CATCHA construct.



Supplementary Figure 2

Representative view of ebony (left) and non-ebony (right) F2 flies from experiments described in Fig. 1c.

	F0 #1	F0 #2	F0 #3	F0 #4	F0 #5	Total (#1 to #5)	*vas-cas9* /+ control
F2 *ebony* fly count	8	6	5	11	7	37	121
F2 non-*ebony* fly count	96	103	89	139	97	524	0
Fraction of non-*ebony*	92.3%	94.5%	94.7%	92.7%	93.3%	93.4%	0.0%

Supplementary Table 1. *ebony* phenotypic analysis for assessing CATCHA-induced *cas9* ablation.
Columns from F0 #1 to F0 #5 were from five experiments in parallel using five CATCHA stocks. Each CATCHA stock (F2 in **Fig. 1b**) originated from a single F1 male that is positive for CATCHA. In *vas-cas9*/+ control group, *vas-cas9*/+ flies were used as F1 females in experiment described in **Fig. 1c**, demonstrating the efficiency of *ebony* gRNA. All flies in the first column (highlighted in yellow) were molecularly genotyped in **Fig. 1c**, which revealed that the 8 *ebony* flies also carry either CATCHA or NHEJ-mediated indels. Thus, the fraction of non-*ebony* in F2 is likely an underestimate of ablation efficiency. The disruption of the *ebony* gene in these 8 flies may be caused by the following two factors. First, the maternal contribution of *cas9* mRNA and/or Cas9 protein can disrupt *ebony* in the zygote. Since *vas-cas9* is expressed in nurse cells, *cas9* mRNA and Cas9 protein can be deposited into fertilized eggs regardless of the latter's genotype. If CATCHA-mediated ablation occurs late in the germline, sufficient numbers of nurse cells may still carry functional *vas-cas9* and deposit maternal Cas9 to cleave *ebony* in the zygote. That 100% (rather than 50%) *vas-cas9*/+ progeny are *ebony* supports this interpretation.

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Materials and Methods

Construction of CATCHA plasmid and verification

gRNA sequence targeting *cas9* was generated by annealing oligos cas9_gRNA_F (CTTCGGCTACGCCGGCTACATTGA) and cas9_gRNA_R (AAACTCAATGTAGCCGGCGTAGCC). The resulting product was ligated into BbsI-digested plasmid pU6-BbsI-chiRNA[1] (Addgene ID #45946), followed by transformation into TOP10 Chemically Competent *E. coli* (Life Technologies). Positive pU6-cas9-chiRNA colonies were confirmed by sequencing.

The homology arm (1042 bp) for the downstream of the *cas9* cleaved site were amplified by PCR using primers cas9C_infus_F (GGGGATCCACTAGTTGACGGCGGAGCCAGC) and cas9C_infus_R (TGGCGGCCGCTCTAGCTTTCTGGATGTCCTCT), from template plasmid pHsp70-Cas9[1] (Addgene ID #45945), followed by PCR purification (Qiagen). In-Fusion (Clontech) was performed to insert the product into vector pU6-cas9-chiRNA at the Xba1 site. Transformation and colony confirmation were conducted as described above, yielding a pU6-cas9-chiRNA-cas9C plasmid.

The homology arm (1003 bp) for the upstream of the *cas9* cleaved site was amplified by PCR using primers cas9_N_Kpn1F (ATGGTACCGCAAGAAATTCAAGGTGCTG) and cas9_N_Xho1R (ATCTCGAGATGTAGCCGGCGTAGCCGTTCT). Products were purified and treated with Kpn1 and Xho1 restriction enzymes (New England Biolabs). Purified products were then ligated with pU6-cas9-chiRNA-cas9C vector digested by Kpn1 and Xho1. The final cas9N-pU6-cas9-chiRNA-cas9C plasmid (CATCHA construct, **Supplementary Fig. 1**) was verified by sequencing. Plasmid sequence is available at Harvard Dataverse (https://dataverse.harvard.edu/), with the title "Cas9-Triggered Chain Ablation (CATCHA) sequence materials".

Drosophila stocks

w[1118]; PBac{y[+mDint2]=vas-Cas9}VK00027[2] (Bloomington stock # 51324) was injected with the CATCHA construct. This strain also served as the allelic *vas-cas9* to test conversion efficiency of integrated CATCHA. Transgenic gRNAs against *wingless* and *ebony* were gifts from Fillip Port[3].

CATCHA stocks were confirmed by PCR with primers cas9_F_seq (CTGAGCGCCTCTATGATC) and pU6_R_seq (AACTAGTGGATCCCCCG) (blue primers in Fig. 1a, left). A 705-bp band was yielded from CATCHA positive stock. cas9_F_seq (CTGAGCGCCTCTATGATC) and cas9_R_seq (TCTCATTCCCTCGGTCACGT) (black primers in Fig. 1a, right) were used to obtain a 1221-bp band from CATCHA positive (HDR) flies, and a 654-bp band from flies carrying NHEJ alleles. The NHEJ alleles were sequenced using nest_cas9_R_seq primer (TGGTCAGCTCGTTATACACGGTG). The sequencing results are deposited at Harvard Dataverse.

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Supplementary References

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