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.
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.

<table>
<thead>
<tr>
<th></th>
<th>F0 #1</th>
<th>F0 #2</th>
<th>F0 #3</th>
<th>F0 #4</th>
<th>F0 #5</th>
<th>Total (#1 to #5)</th>
<th>vas-cas9/+ control</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2 <em>ebony</em> fly count</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td>7</td>
<td>37</td>
<td>121</td>
</tr>
<tr>
<td>F2 non-<em>ebony</em> fly count</td>
<td>96</td>
<td>103</td>
<td>89</td>
<td>139</td>
<td>97</td>
<td>524</td>
<td>0</td>
</tr>
<tr>
<td>Fraction of non-<em>ebony</em></td>
<td>92.3%</td>
<td>94.5%</td>
<td>94.7%</td>
<td>92.7%</td>
<td>93.3%</td>
<td>93.4%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>
Materials and Methods

Construction of CATCHA plasmid and verification
gRNA sequence targeting \textit{cas9} was generated by annealing oligos \textit{cas9\_gRNA\_F} (CTTCGGCTACGCCGGCTACATTGA) and \textit{cas9\_gRNA\_R} (AAACTCAATGTAGCCGGCGTAGCC). The resulting product was ligated into BbsI-digested plasmid pU6-BbsI-chiRNA\textsuperscript{1} (Addgene ID #45946), followed by transformation into TOP10 Chemically Competent \textit{E. coli} (Life Technologies). Positive pU6-cas9-chiRNA colonies were confirmed by sequencing.

The homology arm (1042 bp) for the downstream of the \textit{cas9} cleaved site were amplified by PCR using primers \textit{cas9C\_infus\_F} (GGGGATCCACTAGTTGACGGCGGAGCCAGC) and \textit{cas9C\_infus\_R} (TGGCGGCCGCTCTAGCTTTCTGGATGTCCTCT), from template plasmid pHsp70-Cas9\textsuperscript{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 \textit{cas9} cleaved site was amplified by PCR using primers \textit{cas9\_N\_Kpn1F} (ATGGTACCGCAAGAAATTCAAGGTGCTG) and \textit{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”.

\textit{Drosophila} stocks
\textit{w[1118]; PBac[y\textsuperscript{+mDint2}=vas-Cas9]\textsuperscript{VK00027\textsuperscript{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 \textit{wingless} and \textit{ebony} were gifts from Fillip Port\textsuperscript{3}.

CATCHA stocks were confirmed by PCR with primers \textit{cas9\_F\_seq} (CTGAGCGCCTCTATGATC) and pU6\_\textit{R\_seq} (AACTAGTGATCCCCCG) (blue primers in Fig. 1a, left). A 705-bp band was yielded from CATCHA positive stock. \textit{cas9\_F\_seq} (CTGAGCGCCTCTATGATC) and \textit{cas9\_R\_seq} (TCTCATCCCTCGGTACAGT) (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\textit{cas9\_R\_seq} primer (TGGT\textit{CAGCTCGTTATACACGGGTG}). The sequencing results are deposited at Harvard Dataverse.

\textbf{Supplementary Information}
Supplementary References

