

**Figure S1** Genetic schemes for mutant screening. (A) A screening scheme for screening *ebony* mutants. In order to detect the mutant phenotype of *ebony*, an autosomal gene, in the F1 generation, Cas9/RNA complex-injected G0 flies were crossed with *ebony* heterozygous adults with double-balanced chromosome D/e, Ser, TM3. Since *ebony* is linked to the Ser, TM3 balancer, only adults showing the serrated wing phenotype (also heterozygous for *ebony*) were monitored in the F1

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generation to see if they exhibited the *ebony* phenotype. This indicates that the *ebony* gene in the homologous chromosome descended from injected G0 adults carried the mutation induced by the injection of Cas9/RNA complex. (B) Screening scheme for *singed* mutants. In order to detect the mutant phenotype of *singed*, an X chromosome-linked gene, in the F1 generation, Cas9/RNA complex-injected G0 female flies were crossed with wild-type flies and F1 males were screened for the *singed* phenotype.