

NGG:PAM **N**:mismatch
 CAATATGGACATCTTTGGGG**CGG** white-sgRNA-A
TATTAAGGACATCTTTGGGTCGG**** potential off-target site A1

 CAGGAGCTATTAATTCGCGG**AGG** white-sgRNA-B
 CAGGAGCTATTAAT**GTC**CG**CCGG** potential off-target site B1

 GCACAATATGGACATCTTTG**GGG** white-sgRNA-C
CTGAACATGGACATCTTTG**CAG** potential off-target site C1

 AGGTGAGGTTCTCGGCTAGT**TGG** white-sgRNA-D
AACTGATGATCTCGGCTAGT**TGG** potential off-target site D1
GGATGAGGTTCTCGGCT**GGCTGG** potential off-target site D2

Figure S4. No off-target effect detected using the transgenic Cas9 nickase flies to generate heritable mutations. F1 flies from three groups of successful *white* mutations using Cas9^{D10A} flies and pairs of sgRNAs are tested. Four sgRNA on-target sequences and their corresponding potential off-target sites are listed. Genomic regions that have no more than four mismatches to the on-target and a neighboring PAM are sequenced from eight F1 white-eyed flies for each group. No mutations were detected at the potential off-targets. Mismatches between the potential off-targets and the targeted region are shown in red. The PAM sequences are in bold type.