

AACTTCAGCAGGTGCGGATTTCTGATTACGCGGTACGGGACCTCAGCGTGTGATTCCCC wild-type

AACTTCAGCAGGTGCGGATTTCTGATTACG---TACGGGGACCTCAGCGTGTGATTCCCC [-3]  
AACTTCAGCAGGTGCGGATTTCTGATTACG-----CGGGGACCTCAGCGTGTGATTCCCC [-5]  
AACTTCAGCAGGTGCGGATTTCTGATTACGC-----GGGACCTCAGCGTGTGATTCCCC [-6]  
AACTTCAGCAGGTGCGGATTTCTGATTA-----CGTGTGATTCCCC [-19]  
AACTTCAGCAGGTGCGGATTTCTGATTAC----- [-36]  
AACTTCAGCAGGTGCGGATTTCTGATTAC-----GCGTGTGATTCCCC [-17]  
AACTTCAGCAGGTGCGGATTTCTGATTA-----CGGGGACCTCAGCGTGTGATTCCCC [-7]  
AACTTCAGCAGGT-----ACGGGGACCTCAGCGTGTGATTCCCC [-21]  
AACTTCAGCAGGTGCGGATTTCTGATTACGCGtagGGGGACCTCAGCGTGTGATTCCCC [-4, +4]  
AACTTCAGCAGGTGCGGATTTCTGATTACGCGgTACGGGGACCTCAGCGTGTGATTCCC [+1]  
AACTTCAGCAGGTGCGGATTTCTGATTACGCGgattagTACGGGGACCTCAGATTCCCC [+6]  
AACTTCAGCAGGTGCGGATTTCTGATTACGCtgaagtceggggtccccGTACGGGGACCTC [-1, +16]

**Fig S3** Targeted indel mutations induced by Ca9/gRNA at the *CG14251 (K81)* locus in *Drosophila* (F<sub>1</sub>)

CTCGGAGTTGGTCCAGGATCAGACGAGCCGCTTCTGGAGAGCCTAATGGATGTGCGCCT wild-type

CTCGGAGTTGGTCCAGGATCAGACGAGCCGCT-CTGGAGAGCCTAATGGATGTGCGCCT [-1]  
CTCGGAGTTGGTCCAGGATCAGACGAGCCG-TTCTGGAGAGCCTAATGGATGTGCGCCT [-1]  
CTCGGAGTTGGTCCAGGATCAGACGAGCC-CTTCTGGAGAGCCTAATGGATGTGCGCCT [-1]  
CTCGGAGTTGGTCCAGGATCAGACGAGCC-----TGGAGAGCCTAATGGATGTGCGCCT [-5]  
CTCGGAGTTGGTCCAGGATCAGACGAGCC-----TAATGGATGTGCGCCT [-14]  
CTCGGAGTTGGTCCAGGATCAGACGAGCC-----GCCTAATGGATGTGCGCCT [-11]  
CTCGGAGTTGGTCCAGGATCAGAC-----GAGCCTAATGGATGTGCGCCT [-14]  
CTCGGAGTTGGTCCAGGATCAGCA-----GAGAGCCTAATGGATGTGCGCCT [-13]  
CTCGGAGTTGGTCCAGGATC-----TGGAGAGCCTAATGGATGTGCGCCT [-15]  
CTCGGAGTTGG-----AGAGCCTAATGGATGTGCGCCT [-27]  
-----AGAGCCTAATGGATGTGCGCCT [-67]  
CTCGGAGTTGGTCCAGGATCAGACGAG--a-----GAGAGCCTAATGGATGTGCGCCT [-9, +1]  
CTCGGAGTTGGTCCAGGATCA--ggacgagcc-----GGAGAGCCTAATGGATGTGCGCCT [-15, +9]  
CTCGGAGTTGGTCCAGGATCAGACGAGCCtgatccaggatcaTCTGGAGAGCCTAATG [-2, +13]  
CTCGGAGTTGGTCCAGGATCAGACGAGCCagaTCTGGAGAGCCTAATGGATGTGCGCC [-2, +3]  
CTCGGAGTTGGTCCAGGATCAGACGATcctggaTTCTGGAGAGCCTAATGGATGTGCGC [-5, +7]

**Fig S4** Targeted indel mutations induced by Cas9/gRNA at the *CG3708* locus in *Drosophila* (F<sub>1</sub>)

CATTGTCAGCAGTTGGATGTCGATTTCAATGTCCAGGTCCACCTTGGCGGTCGTGAGCT wild-type

CATTGTCAGCAGTTGGATGTCGATTTCAAT-TCCAGGTCCACCTTGGCGGTCGTGAGCT [-1]  
CATTGTCAGCAGTTGGATGTCGATTTCC-----CAGGTCCACCTTGGCGGTCGTGAGCT [-6]

**Fig S5** Targeted indel mutations induced by Cas9/gRNA at the *CG9652* locus in *Drosophila* (F<sub>1</sub>)