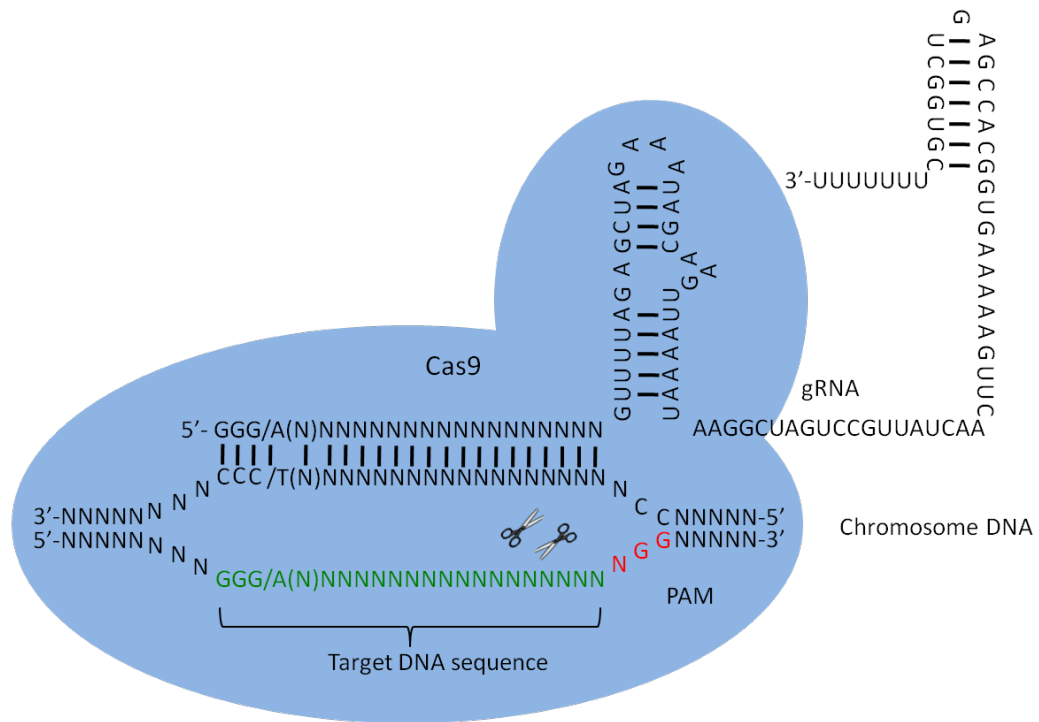
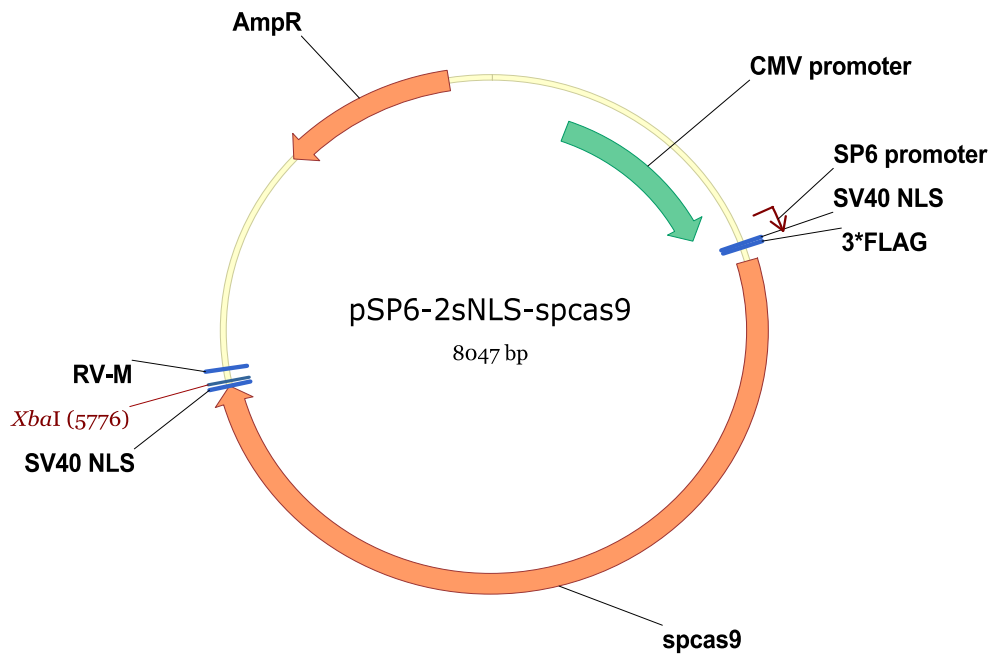


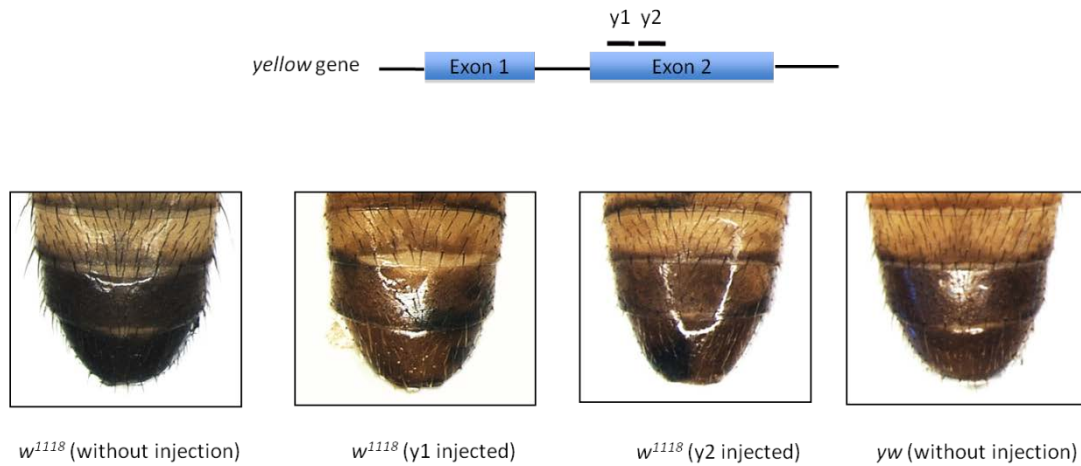
**A**



**B**



C



D

### y1-indels

ACCGCATTAAAGTGGATGAGTGTGGTCCGGCTGTGGGTTTTGGACACTGGAACCGTGGGCA wild-type

ACCGCATTAAAGTGGATGAGTGTGGT-----TTTGGACACTGGAACCGTGGGCA [-11]  
 ACCGCATTAAAGTGGATGAGTGTG-----TGGGTTTTGGACACTGGAACCGTGGGCA [-8]  
 ACCGCATTAAAGTGGATGAGTGTGGTCCG-----TTTTGGACACTGGAACCGTGGGCA [-7]  
 ACCGCATTAAAGTGGATGAGTGTGGTCCGGCTGtttTGGGTTTTGGACACTGGAACCGTGG [+3]  
 ACCGCATTAAAGTGGATGAGTGTGGTCCGGCtttgggttttgtgTGTGGGTTTTGGACACT [+13]

### y2-indels

GAGTGTGGTCCGGCTGTGGGTTTTGGACACTGGAACCGTGGGCATCGGCAATACCACCACT wild-type

GAGTGTGGTCCGGCTGTGGGTTTTGGACACTGGA-CCGTGGGCATCGGCAATACCACCACT [-1]  
 GAGTGTGGTCCGGCTGTGGGTTTTGGACAC-----CGTGGGCATCGGCAATACCACCACT [-6]  
 GAGTGTGGTCCGGCTGTGGGTTTTGGACAC-----GTGGGCATCGGCAATACCACCACT [-7]  
 GAGTGTGGTCCGGCTGTGGGTTTTGGACACTGG-----GCATCGGCAATACCACCACT [-8]  
 GAGTGTGGTCCGGCTGTGGGTTTTGGACACTGGAcacCCGTGGGCATCGGCAATACCACCA [1, +3]

**Fig S1** Targeted indel mutations mediated by Cas9/gRNA at the *yellow* locus. (A) Schematic illustration of Cas9/gRNA system used in our study. The transcription of Cas9 flanked by two NLS is driven by the SP6 promoter, whereas the transcription of gRNA is driven by the T7 promoter. gRNA is designed to target the genome sequence of the standard form of 5'-GG(G/A)-N<sub>17/18</sub>-NGG-3' at the 5' of PAM (NGG). (B) The physical map of the pSP6-2sNLS-spcas9 plasmid used in our study. (C) Images showing the mosaic *yellow* phenotype at the abdomen of Cas9/gRNA-induced *yellow* mutants. y1: y1-gRNA; y2: y2-gRNA. (D) Representative DNA sequencing results of the PCR products from F<sub>0</sub> individual flies showing indel mutations induced by Cas9/gRNA at the targeted *yellow* locus. The wild-type DNA sequence is shown on the top line with the target site underlined and the PAM sequence highlighted in red. Deletions are shown as red dashes and insertions as highlighted in blue and lowercase letters. The change of DNA length (in nucleotides) caused by each mutation is indicated on the right of each sequence (+, insertion; -, deletion). Note that some alterations have both insertions and deletions of nucleotides and in these cases the alterations are enumerated in the brackets.