

TCTGGGTGGCATAATCGAGGAGAATCGCTTTTATATTGTGGCCCCAAAGGATATTGTGG wild-type

TCTGGGTGGCATAATCGAGGAGAATCGCTT-----TTGTGGCCCCAAAGGATATTGTGG [-5]
TCTGGGTGGCATAATCGAGGAGAAT-----TGGCCCCAAAGGATATTGTGG [-13]
TCTGGGTGGCATAATCGAGGAGAATCGCTTTTttATATTGTGGCCCCAAAGGATATTGT [-2]
TCTGGGTGGCATAATCGAGGAGAATCGCTTT-ATATTGTGGCCCCAAAGGATATTGTGG [-1]
TCTGGGTGGCATAATCGAGGAGAATCGC-----TTGTGGCCCCAAAGGATATTGTGG [-7]
TCTGGGTGGCATAATCGAGGAGAATCGC-----TGTGGCCCCAAAGGATATTGTGG [-8]

Fig S6 Targeted indel mutations induced by Cas9/gRNA at the *CG18028* (*light*) locus in *Drosophila* (F₁)

GGATTTTCGTGGTTTGGGTCGTATATACAAGGGACTGGCCAATCCGGATCATGGGGAT wild-type

GGATTTTCGTGGTTTGGGTCGTATATACAAG-----cga----- [-30, +4]
GGATTTTCGTGGTTTGGGTCGTATATACAA-----GGCCCAATCCGGATCATGGGGAT [-6]
GGATTTTCGTGGTTTGGGTCGTATATACAA-----TGGCCAATCCGGATCATGGGGAT [-5]
GGATTTTCGTGGTTTGGGTCGTATATACAA-----CTGGCCAATCCGGATCATGGGGAT [-4]
GGATTTTCGTGGTTTGGGTCGTATATACAA-----GACTGGCCAATCCGGATCATGGGGAT [-2]
GGATTTTCGTGGTTTGGGTCGTATATACA---ggg---GGCCCAATCCGGATCATGGGGAT [-7, +3]
GGATTTTCGTGGTTTGGGTCGTATAT-----ga-----CCCAATCCGGATCATGGGGAT [-12, +2]
GGATTTTCGTGGTTTGGGTCGTATAT-----TGGCCAATCCGGATCATGGGGAT [-9]
GGATTTTCGTGGTTTGGGTCGTAT-----GGCCCAATCCGGATCATGGGGAT [-12]
GGATTTTCGTGGTTTGGGTCGTAT---cagg---GACTGGCCAATCCGGATCATGGGGAT [-8, +4]
GGATTTTCGTGGTTTGG-----ccaat---GACTGGCCAATCCGGATCATGGGGAT [-15, +5]
GGATTTTCGTGGT-----GACTGGCCAATCCGGATCATGGGGAT [-19]
GGATTTTCGTG-----GCCCAATCCGGATCATGGGGAT [-26]
GGATTTTCG-----cca-----GACTGGCCAATCCGGATCATGGGGAT [-23, +4]
-----caggac-----CTGGCCAATCCGGATCATGGGGAT [-116, +6]
-----catgetttggg-----TCATGGGGAT [-53, +11]
-----GACTGGCCAATCCGGATCATGGGGAT [-94]

Fig S7 Targeted indel mutations induced by Cas9/gRNA at the *CG17629* (*kl-3*) locus in *Drosophila* (F₀)

CTATCAAATTGCAGAGATGGGGGCTTATCGGTACATGCAGGAAC TTTATAGGAAGAAGC wild-type

CTATCAAATTGCAGAGATGGGGGCTTATCGGTA-----CAGGAAC TTTATAGGAAGAAGC [-4]
CTATCAAATTGCAGAGATGGGGGCTTATCGGT-----GCAGGAAC TTTATAGGAAGAAGC [-4]
CTATCAAATTGCAGAGATGGGGGCTTATCGGT-----AGGAAC TTTATAGGAAGAAGC [-6]
CTATCAAATTGCAGAGATGGGGGCTTATCGG-----CAGGAAC TTTATAGGAAGAAGC [-6]
CTATCAAATTGCAGAGATGGGGGCT-----ATGCAGGAAC TTTATAGGAAGAAGC [-9]
CTATCAAATTGCAGAGATGGGGGCT-----GCAGGAAC TTTATAGGAAGAAGC [-11]
CTATCAAATTGCAGAGATGGGGG-----ATGCAGGAAC TTTATAGGAAGAAGC [-11]
CTATCAAATTGCAGAGAT-----aa-----GCAGGAAC TTTATAGGAAGAAGC [-18, +2]
CTATCAAATTGCAGA-----at-----TGCAGGAAC TTTATAGGAAGAAGC [-20, +2]
CTATCAAATTGCA-----GGAAC TTTATAGGAAGAAGC [-26]
-----CAGGAAC TTTATAGGAAGAAGC [-50]
CTATCAAATTGCAGAGATGGGGGCTTATCG-----caa----- [-37, +3]
CTATCAAATTGCAGAGATGGGGGCTTATCGGTACaggTGCAGGAAC TTTATAGGAAGAAGC [+2]
CTATCAAATTGCAGAGATGGGGGCTTATCGGTACtttatcggGCAGGAAC TTTATAGAGC [-2, +7]