

**NGG**: PAM    **N**: mismatch  
**piwi-HDR-sgRNA-left**  
CGGACATGACCAAATGGAC**CCGG**  
CGGACATGACCAAAT**TGACTCGG** potential off-target site L1  
CGGACATGACCAGAT**CGACCGG** potential off-target site L2  
CGGACATGACCAAAT**CGAGTCGG** potential off-target site L3  
CGGACATGACCAGAT**CGACTAGG** potential off-target site L4  
CGGACATGACCAGAT**CGACTCAG** potential off-target site L5

**piwi-HDR-sgRNA-right**  
TATCGATTGCAAGTTGGGG**TAGG**  
TATCGATTGCAA**ATTGTGCTGG** potential off-target site R1  
TATCG**TTT**GCAAGTTG**CTATCAG** potential off-target site R2

**Figure S6. No off-target effect detected using the CRISPR system to generate *piwi* mutations.** Flies from six successful *piwi*<sup>HDR-mCherry</sup> mutation strains are tested. The two 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. 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.