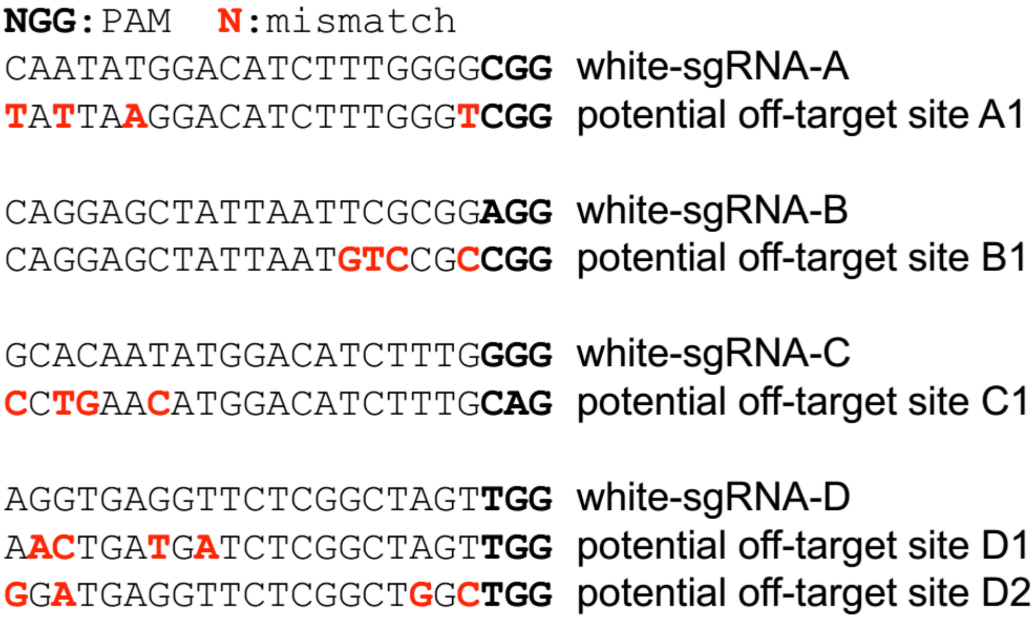

Figure S4. No off-target effect detected using the transgenic Cas9 nickase flies to generate heritable mutations. F1 flies from three groups of successful *white* mutations using Cas9^{D10A} flies and pairs of sgRNAs are tested. Four sgRNA on-target sequences and their corresponding potential off-target 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.