Table S2 Assessment of somatic and germ line mutagenesis mediated by *U6:3-gRNA* transgenes targeting pigmentation genes

gRNA spacer sequence[a]	Plasmid ID	Target gene	% phenotypically mutant cuticle per animal[b]	% germ line transmission of non-functional alleles, mean ± SD[c]	No. in-frame mutations /functional alleles analyzed
GCGATATAGTTGGAGCCAGC	*pFP407*[d]	*y*	100	100	nd
gATTCGTCACTGTTCCCCGC	*pFP570*	*y*	100	100	nd
gCTGTTGGAGTCGAACACTT	*pFP566*	*y*	80 - 100	99 ± 2	nd
gCAAAGTGTTCGACTCCAAC	*pFP553*	*y*	100	100	nd
gAGGACCAAGCTCTGGCTAG	*pFP552*	*y*	80 - 100	93 ± 7	nd
gTGGCCATCTGGAAGGCTGG	*pFP545*	*e*	100	100	nd
gATCGAGTCCACGAAGGTTA	*pFP578*	*e*	100	100	nd
gCAGCAGTATGTGGTGAATG	*pFP507*	*e*	80 - 100	99 ± 1	nd
gTCTACACCTCGGGCAGTAC	*pFP573*	*e*	100	99 ± 1	nd
GCACGAGAGCATCCTCAAT	*pFP576*	*e*	100	97 ± 5	nd
GTTGGAGCGTTATAAGATC	*pFP574*	*e*	40 - 80	96 ± 6	nd
gGTGGGTCTCGGCCACCAGG	*pFP527*	*e*	40 - 80	90 ± 13	2/3
gAGATGCGGTGCAGAGCTCT	*pFP529*	*e*	80 - 100	87 ± 12	3/3
gGTGTGCATGCAGCCGTCGG	*pFP548*	*e*	80-100	84 ± 10	2/3
GGCTCCAATCTGCTCTCAG	*pFP575*	*e*	80 - 100	81 ± 7	2/2
gCTGACTGGGCGCCATTCCC	*pFP514*	*e*	40 - 80	80 ± 15	3/3
gTCCTGCAGCCAAACAGCGA	*pFP530*	*e*	40 - 80	75 ± 5	3/3
gCCTTGACGATCGACAATTG	*pFP547*	*e*	40 - 80	70 ± 23	3/3
gTTCCCTGGCCCGTAGTGCT	*pFP510*	*e*	40 - 80	65 ± 21	3/3
gCTTCGAGGAGCAGCAGCTG	*pFP546*	*e*	40 - 80	45 ± 31	8/8*
gCGCACGCTCGTTCATCTGG	*pFP519*	*e*	80 - 100	40 ± 4	0/6
gACCATCGCTGTTTGGCTGC	*pFP549*	*e*	40 - 80	35 ± 9	0/6
gCACAATTGTCGATCGTCAA	*pFP544*	*e*	5 - 40	13 ± 4	0/7
			% area of eye with sepia pigmentation, mean ± SD[e]		
gTTCTGCCCATTTGCCCAAC	*pFP556*	*se*	96 ± 1		nd
GATGCACCCGTTGGGCAAAT	*pFP567*	*se*	100		nd
GCACGCACATCATGAGTAA	*pFP564*	*se*	67 ± 3		2/2
gGGCGAAGGATACCATCTTC	*pFP563*	*se*	61 ± 2		2/3
gTCTTCGGGAACATCCGGCA	*pFP557*	*se*	59 ± 1		3/3
GCACATCATGAGTAACGGC	*pFP562*	*se*	57 ± 4		3/3
gTGCCGGATGTTCCCGAAGA	*pFP558*	*se*	25 ± 7		2/3
GCAGAAGCGCATCGAGTAC	*pFP561*	*se*	7 ± 3		0/3
gCCACTAGGCTCACCCATGC	*pFP565*	*se*	0**		nd

a, gRNAs for each gene are listed in the same order as in Figure 3; nucleotides in lower case are mismatched to the genomic target; b, estimate based on observation of > 20 flies of each genotype; c, from scoring pigmentation of progeny of three independent crosses of *act-cas9 gRNA* flies to a homozygous *e* mutant strain. At least 125 progeny were analyzed for each genotype; nd = not determined; d, *gRNA-y* transgene first described in Port *et al*. 2014; e, mean of estimates by three independent observers blind to the genotype of each fly (based on observation of > 20 flies per genotype per researcher); *, gRNA target site with suspected microhomology bias in non-homologous end joining; all eight analyzed flies had deletions of either six or nine base pairs, consistent with the microhomology around the cut site. **, gRNA (designed using the *Drosophila* reference genome) contained a single nucleotide polymorphism to the *se* sequence in our experimental strains.