

Table S3 Computed gRNA efficiency scores and 3' 6nt GC content

gRNA spacer sequence ^a	Plasmid ID	Target gene	E-CRISPR efficacy score ^{b,c}	DRSC efficiency score ^{b,d}	Broad Institute gRNA score ^{b,e}	3' 6nt GC content (%)	Phenotypic efficiency score ^g
<i>GTATCTGTATCAAAGGACA</i>	<i>pFP505</i>	<i>Dhc64C</i>	36.7	3.91	0.584	50	100
<i>GATAAGCTTGAGGCACCGC</i>	<i>pFP508</i>	<i>Dhc64C</i>	35.0	4.99	0.108	83	100
<i>GCTTGAGGCACCGCTGGAT</i>	<i>pFP511</i>	<i>Dhc64C</i>	34.8	7.72	0.004	50	100
<i>GACTACAATCCTCTGATGA</i>	<i>pFP516</i>	<i>Dhc64C</i>	49.3	2.89	0.073	33	100
<i>GAAAAGCGCGAGTCGCCAG</i>	<i>pFP535</i>	<i>Dhc64C</i>	40.0	9.90	0.449	83	100
<i>GATCTGCTGCACAGCAGGT</i>	<i>pFP541</i>	<i>Dhc64C</i>	28.1	3.57	0.581	67	100
<i>GCGAGTTTGCAGCTTCTTA</i>	<i>pFP504</i>	<i>Dhc64C</i>	42.9	3.64	0.013	17	100
<i>GACTGTCTGGATATCACCA</i>	<i>pFP532</i>	<i>Dhc64C</i>	41.8	2.07	0.741	50	100
<i>GATGCTAACGCTATCGAGG</i>	<i>pFP518</i>	<i>Dhc64C</i>	36.9	5.70	0.411	67	80
<i>gTTGAGACCTCCGCAAGG</i>	<i>pFP551</i>	<i>Lis1</i>	33.0	7.08	0.397	67	100
<i>GATCGGCCTCCTTGCGGA</i>	<i>pFP554</i>	<i>Lis1</i>	31.7	7.90	0.272	67	100
<i>GCCGAGGTATCCACTCGCC</i>	<i>pFP555</i>	<i>Lis1</i>	32.1	4.62	0.089	83	100
<i>gCAGAGAAGTTCTCCCTGAC</i>	<i>pFP569</i>	<i>Lis1</i>	55.3	5.06	0.222	67	100
<i>gCAAACGGACGCCTGGCGAG</i>	<i>pFP550</i>	<i>Lis1</i>	33.1	8.77	0.301	83	80
<i>gGCAGTCTCAGCCAAGTCGA</i>	<i>pFP513</i>	<i>wg</i>	47.3	3.96	0.529	50	100
<i>gAGAAATCCGGAAGGGGCCG</i>	<i>pFP515</i>	<i>wg</i>	42.5	3.68	0.353	100	20
<i>GGGCAAACAGAAATCCGGAA</i>	<i>pFP533</i>	<i>wg</i>	47.8	5.94	0.636	67	20
<i>GCAGCAGTCTCAGCCAAGTCG</i>	<i>pFP411^f</i>	<i>wg</i>	44.3	4.98	0.201	50	20
<i>gTTTCTGTTTGCCCTCGACT</i>	<i>pFP506</i>	<i>wg</i>	38.1	4.46	0.157	50	20
<i>gGGGCAAACAGAAATCCGGA</i>	<i>pFP521</i>	<i>wg</i>	55.3	3.84	0.449	67	20
<i>GACTGCTGCCGCCGCTGCAC</i>	<i>pFP542</i>	<i>wg</i>	47.6	6.40	0.021	67	20
<i>gACAGAAATCCGGAAGGGGC</i>	<i>pFP538</i>	<i>wg</i>	49.5	5.95	0.068	83	15
<i>gACCATACTGGAGAACCTGAG</i>	<i>pFP410</i>	<i>wls</i>	27.8	6.04	0.414	67	100
<i>GCTGCTAAGTGATATTCGCCA</i>	<i>pFP412</i>	<i>wls</i>	40.5	5.09	0.627	67	100
<i>gGGCCGCAAGCTGTCCATAT</i>	<i>pFP512</i>	<i>wls</i>	40.5	6.77	0.018	33	100
<i>gGACATGGCCGGCGGGCAGG</i>	<i>pFP525</i>	<i>wls</i>	45.4	6.62	0.071	83	100
<i>gACCTGGCAGAGCAGCAAAG</i>	<i>pFP522</i>	<i>wls</i>	44.6	4.13	0.391	50	100
<i>gAGCAAAGTGCCACCAATA</i>	<i>pFP526</i>	<i>wls</i>	39.0	3.35	0.065	33	100
<i>gGGACAGCTTGCGGCCACTC</i>	<i>pFP531</i>	<i>wls</i>	19.1	5.07	0.056	67	95
<i>gGTTTCCTGCTGTAATCACG</i>	<i>pFP534</i>	<i>wls</i>	54.1	7.93	0.886	67	90
<i>gCACCAATATGGACAGCTTG</i>	<i>pFP509</i>	<i>wls</i>	38.5	4.00	0.512	50	90
<i>gATCCCAGTGACGCGAGAGG</i>	<i>pFP523</i>	<i>wls</i>	51.1	6.00	0.434	67	90
<i>gCCAGGTGCGCAGGTCACG</i>	<i>pFP520</i>	<i>wls</i>	41.1	7.12	0.396	67	60
<i>gAGGTGTTGTGCTTCCTGCT</i>	<i>pFP540</i>	<i>wls</i>	38.2	6.81	0.068	67	60
<i>gTTCTGCCCATTGCCCCAAC</i>	<i>pFP556</i>	<i>se</i>	55.8	4.94	0.115	67	96
<i>GATGCACCCGTTGGGCAAAT</i>	<i>pFP567</i>	<i>se</i>	64.2	5.62	0.433	33	100
<i>GCACGCACATCATGAGTAA</i>	<i>pFP564</i>	<i>se</i>	43.7	6.17	0.537	33	67
<i>gGGCGAAGGATACCATCTTC</i>	<i>pFP563</i>	<i>se</i>	41.6	4.98	0.019	33	61
<i>gTCTTCGGGAACATCCGGCA</i>	<i>pFP557</i>	<i>se</i>	41.8	7.49	0.034	83	59
<i>GCACATCATGAGTAACGGC</i>	<i>pFP562</i>	<i>se</i>	42.9	5.77	0.270	67	57
<i>gTGCCGGATGTTCCCGAAGA</i>	<i>pFP558</i>	<i>se</i>	40.1	4.10	0.107	50	25
<i>GCAGAAGCGCATCGAGTAC</i>	<i>pFP561</i>	<i>se</i>	53.9	4.30	0.115	50	7
<i>gCCACTAGGCTCACCCATGC</i>	<i>pFP565</i>	<i>se</i>	42.9	6.54	0.024	67	0

GCGATATAGTTGGAGCCAGC	pFP407 ^f	y	61.1	5.60	0.276	83	100
gATTCGTCACTGTTCCCCGC	pFP570	y	53.7	7.28	0.304	100	100
gCTGTTGGAGTCGAACACTT	pFP566	y	34.1	4.56	0.338	33	99
gCAAAGTGTTCTCGACTCCAAC	pFP553	y	40.7	3.04	0.716	50	100
gAGGACCAAGCTCTGGCTAG	pFP552	y	47.7	6.90	0.029	67	93
gTGGCCATCTGGAAGGCTGG	pFP545	e	48.8	5.47	0.240	83	100
gATCGAGTCCACGAAGGTTA	pFP578	e	35.3	7.67	0.163	33	100
gCAGCAGTATGTGGTGAATG	pFP507	e	32.2	5.13	0.508	33	99
gTCTACACCTCGGGCAGTAC	pFP573	e	32.9	4.99	0.023	50	99
GCACGAGAGCATCCTCAAT	pFP576	e	34.6	3.96	0.164	33	97
GTTGGAGCGTTATAAGATC	pFP574	e	37.4	6.64	0.113	33	96
gGTGGGTCTCGGCCACCAGG	pFP527	e	42.1	5.99	0.408	67	90
gAGATGCGGTGCAGAGCTCT	pFP529	e	26.7	5.36	0.009	50	87
gGTGTGCATGCAGCCGTCGG	pFP548	e	44.0	5.42	0.045	83	84
GGCTCCAATCTGCTCTCAG	pFP575	e	29.5	5.21	0.175	50	81
gCTGACTGGGCGCCATTCCC	pFP514	e	39.6	4.39	0.018	50	80
gTCCTGCAGCCAAACAGCGA	pFP530	e	41.6	6.24	0.332	67	75
gCCTTGACGATCGACAATTG	pFP547	e	29.4	5.45	0.097	33	70
gTTCCTGGCCCGTAGTGCT	pFP510	e	42.5	9.58	0.074	50	65
gCTTCGAGGAGCAGCAGCTG	pFP546	e	29.2	5.70	0.114	67	45
gCGCACGCTCGTTCATCTGG	pFP519	e	45.1	6.08	0.545	50	40
gACCATCGCTGTTTGGCTGC	pFP549	e	52.2	6.18	0.027	83	35
gCACAATTGTCGATCGTCAA	pFP544	e	43.8	5.99	0.535	50	13

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, accessed in January 2015; c, www.e-crispr.org; d, <http://www.flyrnai.org/evaluateCrispr/>; e, <http://www.broadinstitute.org/rnai/public/analysis-tools/sgRNA-design>; f, gRNA transgene first described in Port *et al.* 2014; g, values reproduced from figure 2. For *Dhc64C*, *Lis1*, *wg* and *wls* the score is the percentage of animals that mimic the null mutant phenotype; for *se* the score is the percentage of phenotypically mutant eye tissue; for *y* and *e* the score is the mean germ line transmission rate of non-functional alleles (see Table S2 for more information on mutation frequencies for non-essential genes).