

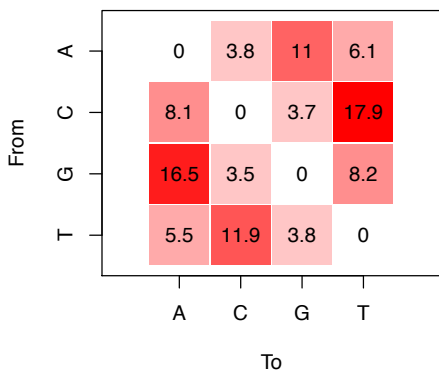
Supplemental Figure 1: CRISPR-dependent mutations favor transitions over transversions. Heat maps represent the percentage of specific point mutation nucleotide changes detected in CRISPR-treated animals. For both animals, most mutations were G to A and C to T mutations.

a

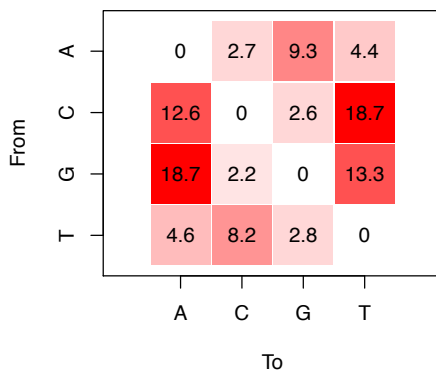
F03

Variants by nucleotide

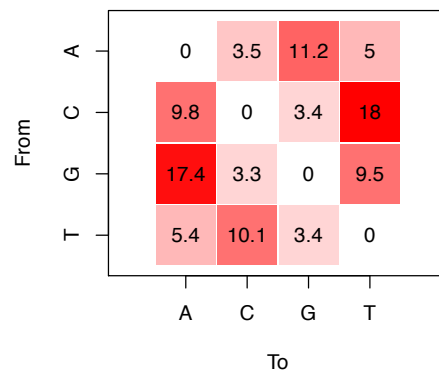
Mutect



Lofreq



Strelka

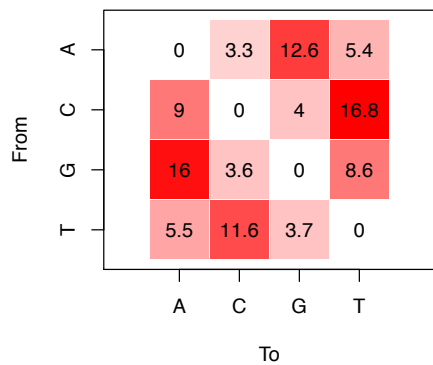


b

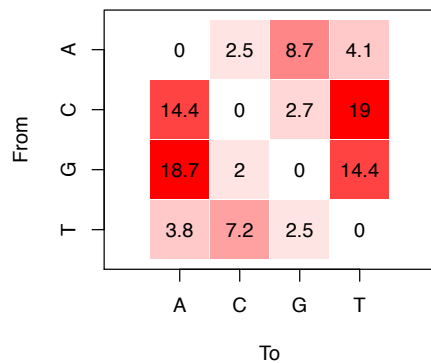
F05

Variants by nucleotide

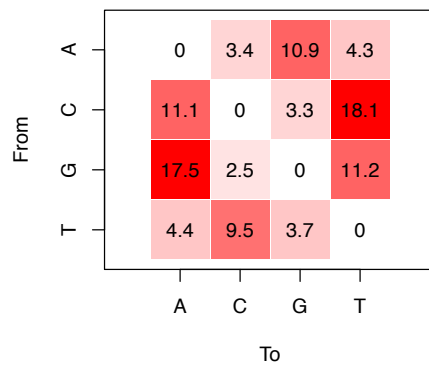
Mutect



Lofreq

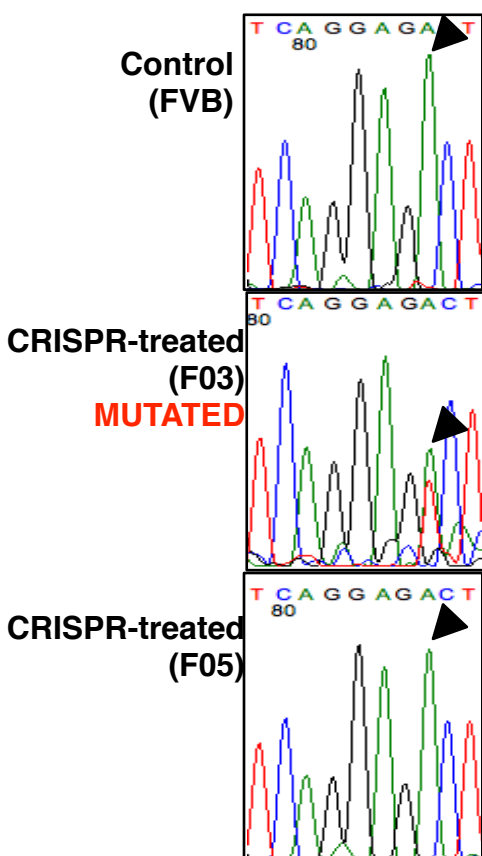


Strelka

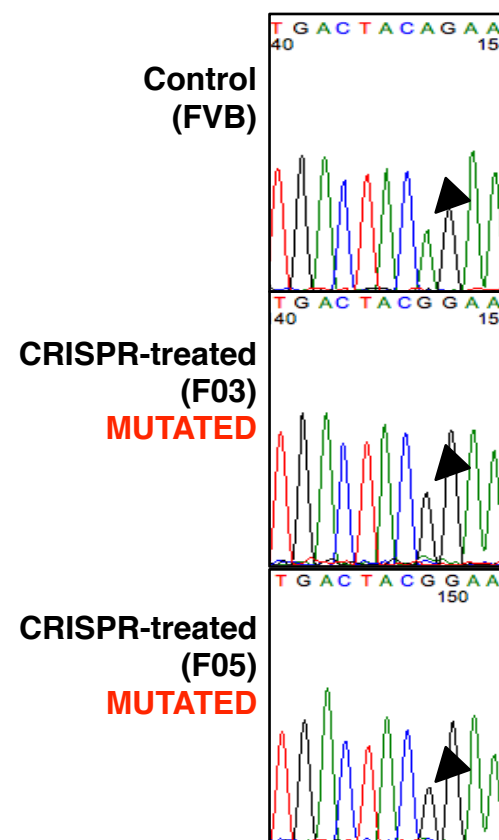


Supplemental Figure 2. Sanger sequencing confirms CRISPR-induced mutants detected by WGS. **a.** Example of a coding SNV confirmed to be heterozygous in F03. F05 is wildtype as is reported in Supplemental Tables 1 and 2. **b.** Example of an intronic SNV confirmed in both mice as reported in Supplemental Table 5. **c.** Example of an SNV in a snoRNA confirmed in both mice as reported in Supplemental Table 5.

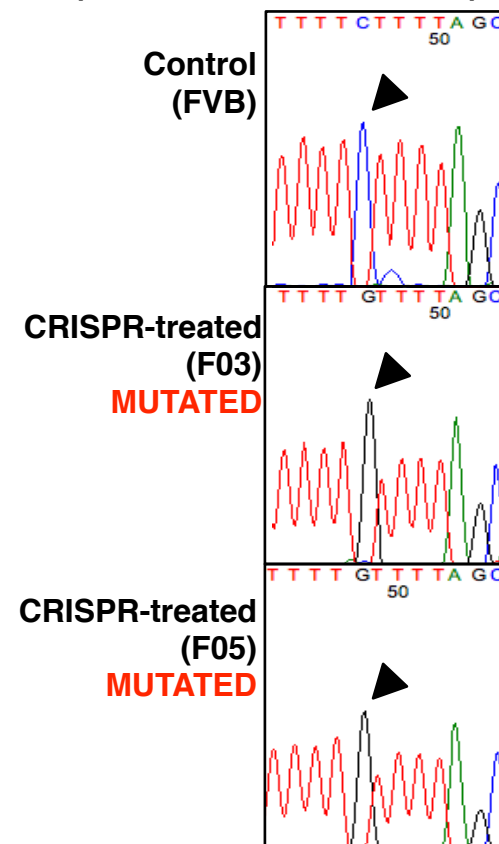
a Coding SNV: *Olf1166*
(p.Arg305Ser/c.915A>T)



b Intronic SNV: 5530601H04RiK
(chrX:105068204, A>G)



c snoRNA SNV: Gm23529
(chr12: 33455323, A>G)



Supplemental Figure 3: Sequence alignment of guide RNA to actual off-target regions does not show significant homology. a. The top 10 predicted off-target regions predicted *in silico*, by Benchling, aligned to the gRNA. Sequences are 80-95% homologous to the gRNA. **b.** Regions surrounding 10 selected experimentally-observed SNVs in coding regions aligned to the gRNA. All regions were observed in both CRISPR-treated mice. Sequences are 15-45% homologous to the gRNA. **c.** Regions surrounding 10 selected experimentally-observed SNVs in non-coding region aligned to the gRNA. All regions were observed in both CRISPR-treated mice. Sequences are 5-65% homologous to the gRNA. **d.** Regions surrounding 10 selected experimentally observed indels in both coding and non-coding regions aligned to the gRNA. All regions were observed in both CRISPR-treated mice. Sequences are 25-65% homologous to the gRNA. (SNVs: single nucleotide variants, Indels: insertions/deletions)

a Top 10-Predicted Off-Target Sites:

gRNA	CCAACCTTAAGTAGCAGAAAG	
chr5:108850293	CCAACCTTACGTAGCAGAAAG	Vmn2r9
chr10:124707862	GCAAGCTTAAGTAGCAGAAAG	No Associated Gene
chr10:112240327	CTTACCTTAAGAAGCAGAAAG	No Associated Gene
chr8:78482617	AGAACAATAAGTAGCAGAAAG	No Associated Gene
chr5:149431129	CTTCCCTGATTAGCAGAAAG	Medag
chr18:86934169	CACCCCTAATTAGCAGAAAG	No Associated Gene
chr13:40729083	CTTACCTCAGTACAGAAAG	Tfap2a
chr12:37561612	CAAACTTAAGGAGCAGAAAT	Agmo
chr2:145262171	ACAAACAACAGTAGCAGAAAG	Slc24a3
chr11:110958499	CAAGCAGAGTAGCAGAAAG	No Associated Gene

b 10 True off-target site SNVs in Coding Regions

gRNA	CCAACCTTAAGTAGCAGAAAG	
chr18:67834688	CCAAACATCTCTCATGTCTT	Cep192
chr 9 :66434164	AGTTATGTGGAAACAGACACC	Herc1
chr7:63938170	CTGCCTCCACGCGGGCTGG	Klf13
chr15:8324487	CCAACA CTTTCAGTTCCAGC	Nipbl
chr19:12283753	GTAGGTCCAAAGGCCCTTCAA	Olfr1433
chr10:76356251	CCACTCGAAACGGCGGAACCT	Pcnt
chr15:76176864	CCCCACGTGTCCAGCAGCTCT	Plec
chr2:129609045	CCATTAGGTGGTACAGAGGA	Sirpa
chr5:124885592	CCATACAGCAGAGAAACCCC	Zfp664
chr2:167056200	CAACC CAGAGGCTCTCAA	Znfx1

c 10 True off-target site SNVs in Non-coding Regions

gRNA	CCAACCTTAAGTAGCAGAAAG	
chr1:190479227	CTTTTCAGCCTTGGTTCAGAGA	Gm23153 (miscellaneous RNA)
chr2:92591648	GTTTTCA TGGTCCGTGGCAGG	Mir7221 (miRNA)
chr2:140439882	CTTTTCGTAGTGTGGTCC	Gm23846 (snRNA)
chr5:62132979	GAAACCTTAAGATACAGAAAT	Gm22273 (snoRNA)
chr6:31721846	TTTTCTTTTAA TGGTGGCTGG	Gm13847 (lincRNA)
chr6:133758061	CCAAATTCTAAGAAAGGGGAC	Gm23375 (miRNA)
chr11:54214410	ATGTTTGTACCCTTGTGTC	4933405E24Rik (lincRNA)
chr11:83274656	CCATAGCTACGAACACAGAG	Gm23444 (snRNA)
chr11:112711337	CTCCAGACATTTGAATGAGCC	BC006965 (processed transcript)
chr18:54449411	CACACACAGAGAGAGAGAGA	Redrum (lincRNA)

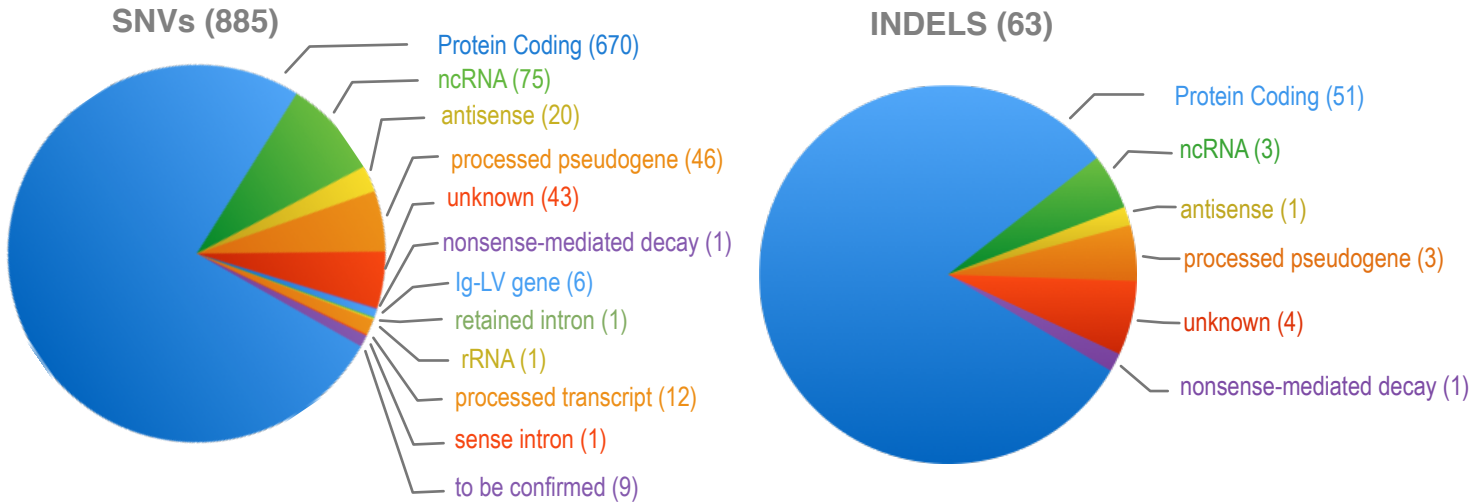
d 10 True off-target site Indels

gRNA	CCAACCTTAAGTAGCAGAAAG	
chr4:106562094	CCCTCCCTTATATTCCTCTCT	2700068H02Rik
chr1:188016221	CCCAAGCTAACCTGTTTGCCCA	Esrrg
chr18:31470317	TCAAATAAGTAGCAAAAGAT	Gm25396
chr4:134618126	CAAAAGGAGCTCAGGTTCAGA	Man1c1
chr17:31443854	TCAAACGAGCTGAAAGCCGAA	Pde9a
chr8:107584736	CCAAAGCCTTTGGCAGTAAT	Psmc7
chr18:31910970	GC GGCCAGCAGCAGACACAG	Sft2d3
chr19:50287677	AAAAACA TTTCAAATGCAATAA	Sorcs1
chr2:30020366	GCAACCTT GAGCA CAGAGTCC	Sptan1
chr4:132857590	CCAGCACCTACTCACCAAGCT	Stx12

Supplemental Figure 4: Off-target CRISPR mutations could cause unwanted phenotypes. Pie charts show the SNVs and indels detected in WGS of CRISPR-treated animals based on assigned biotype. Biotypes were assigned by SNPEff software (Cingolani P, et al. Fly, 2012.) Intragenic regions were not assigned a biotype.

a

F03



b

F05

