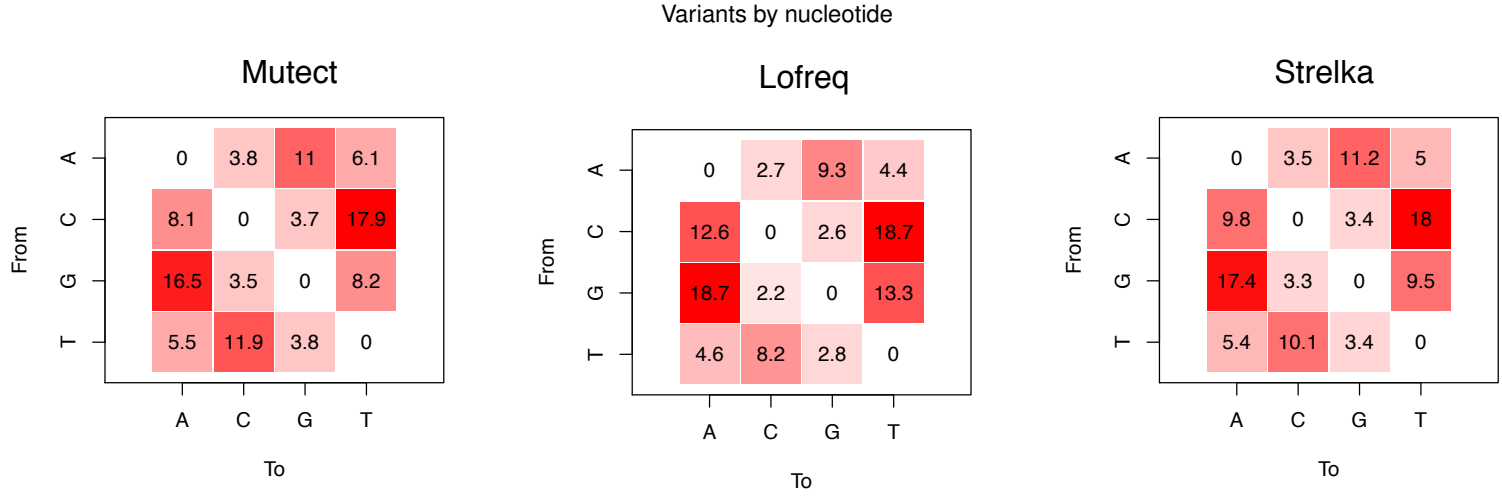
Supplemental Figure 1: CRISPR-dependent mutations favor transitions over transversions. Heat maps represent the percentage of specific point mutation nucleotide changes detected in CRISPR-treated animals. For both animals, most mutations were G to A and C to T mutations.

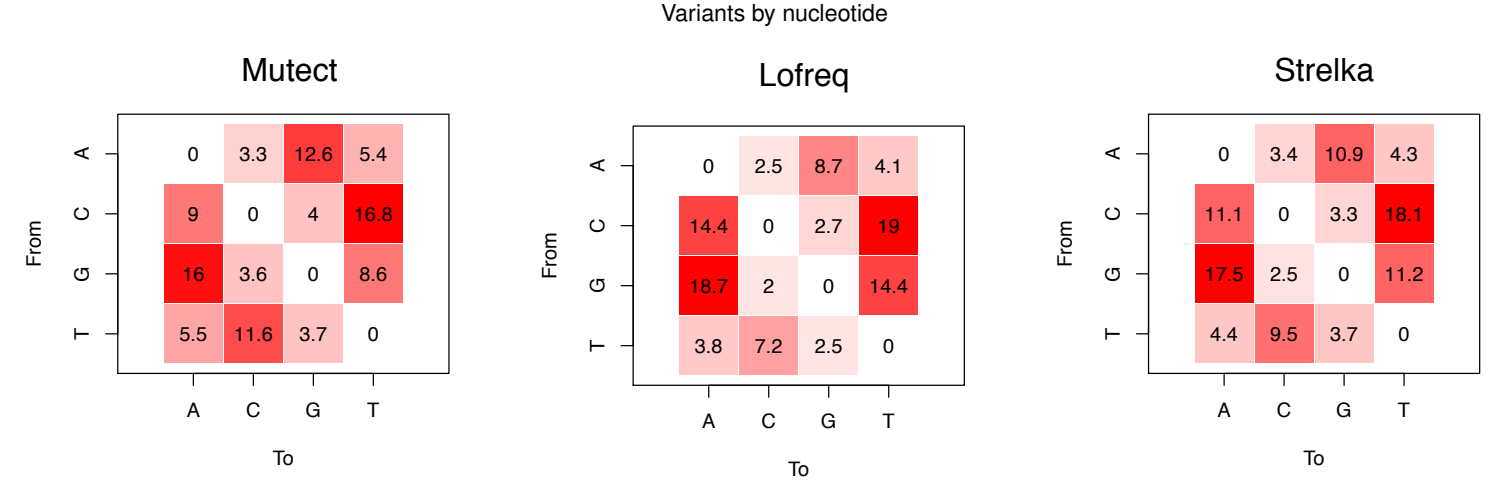
a

F03

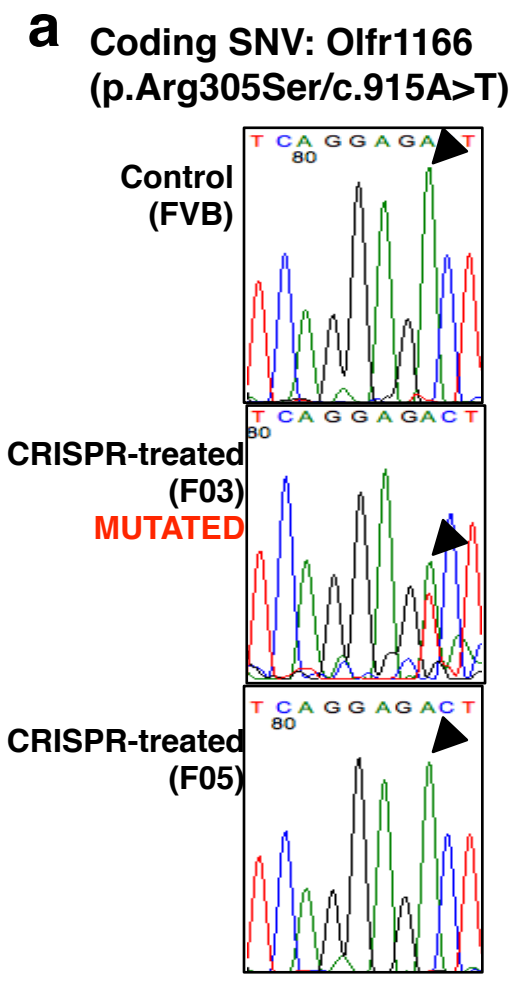


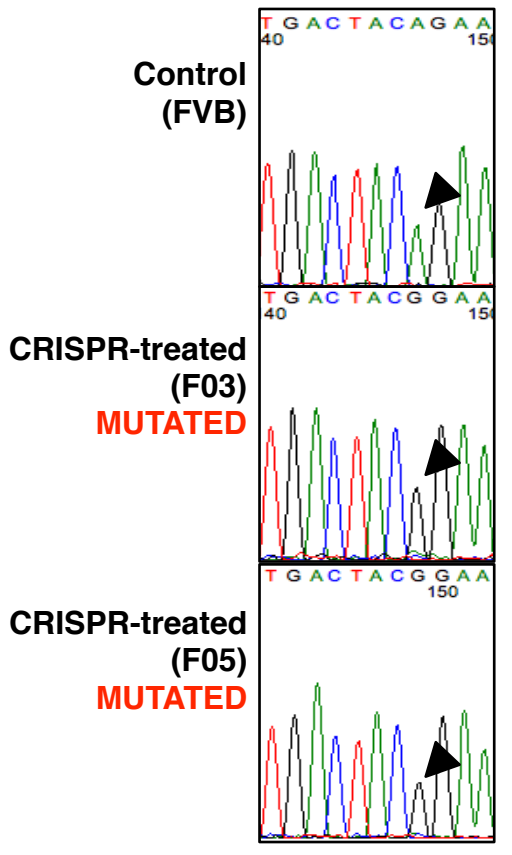
b

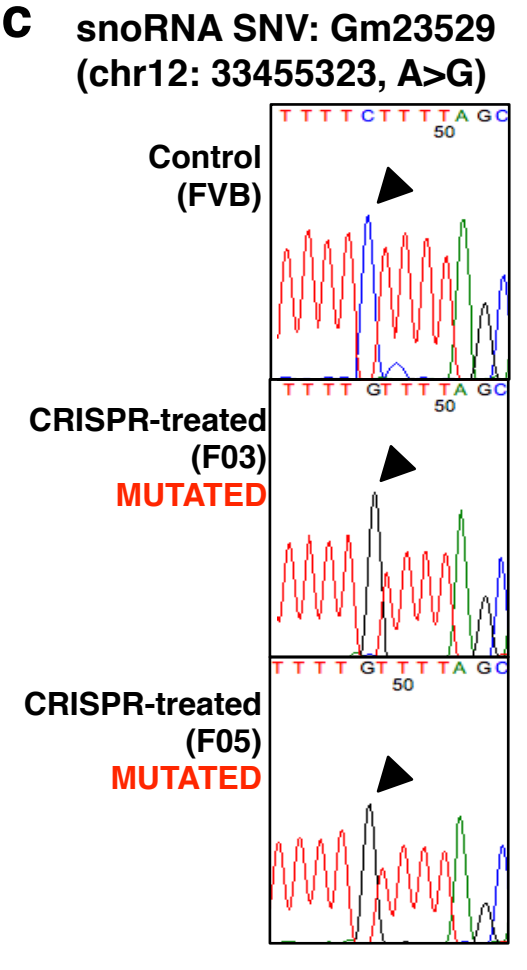
F05



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.



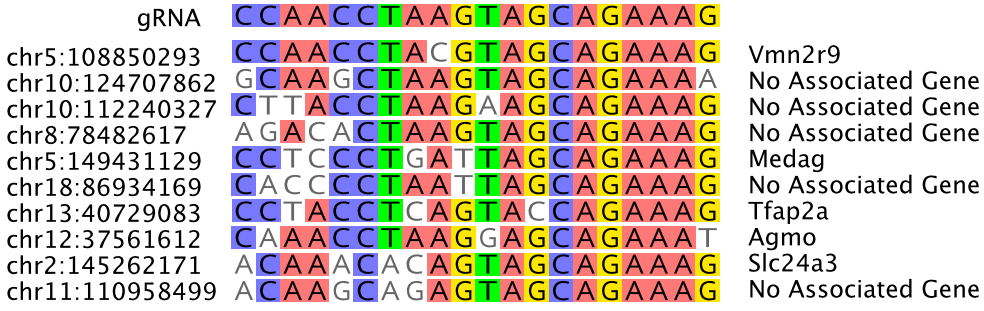




Supplemental Figure 3: Sequence alignment of guide RNA to actual off-target regions does not show significant homology. a. The top 10 predicted off-target regions predicted *in silico,* by Benchling, aligned to the gRNA. Sequences are 80-95% homologous to the gRNA. **b.** Regions surrounding 10 selected 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)

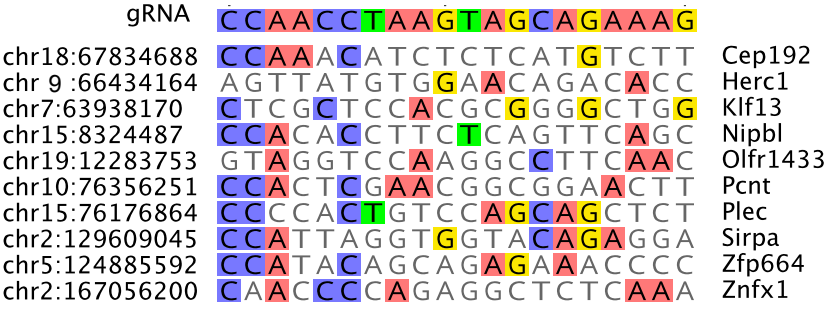
a

Top 10-Predicted Off-Target Sites:



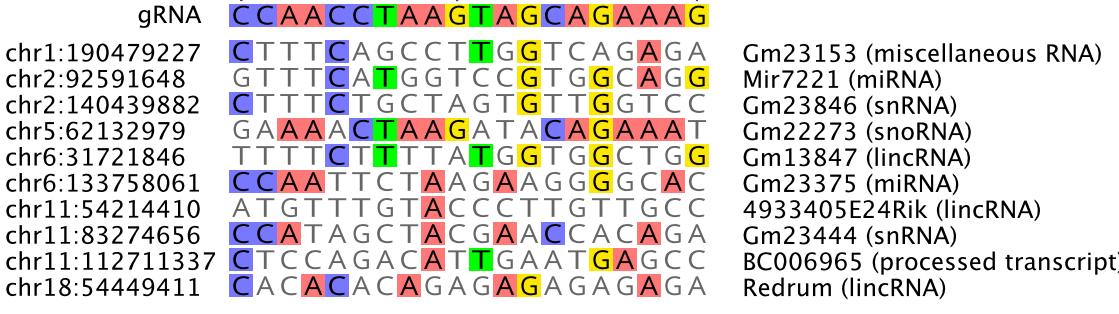
b

10 True off-target site SNVs in Coding Regions



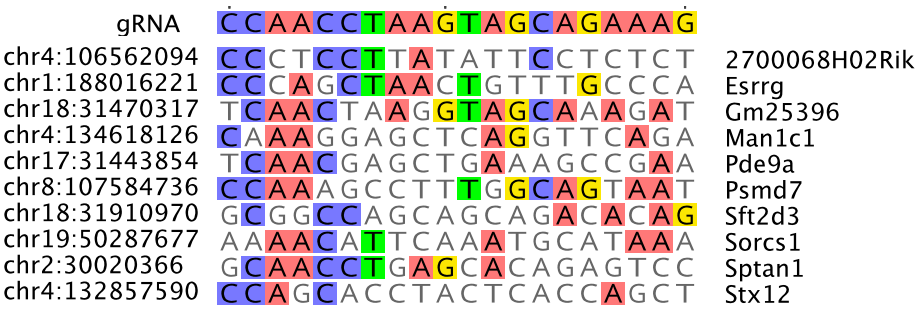
c

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



d

10 True off-target site Indels



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.