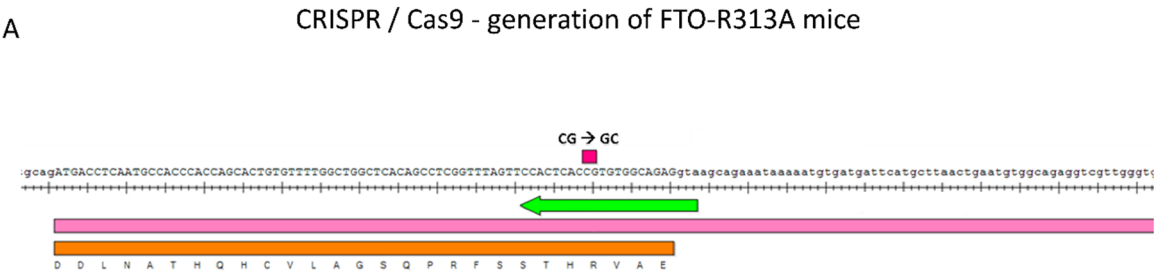
A



B

Control for off-target effects

potential off-target locus 8_1:

TACTTCTGCTACACGGTGGGCAG	0.9	3MMs [4:10:19]	NM_023395	chr8:-122211870		

Animal ID	Geno-type	TOPO clone	sequence	ok?
2e	WT	A	-	
		B	tgcagcttctaggtgacttggcagattttgcctgcggaaggcatatattcatccctaatgttccccaaataat ctgcccaccgtgtagcagaagtaagctactttatcaggcgtgagccacagagagtcgtgatccttgagagg caagac = correct	☑
3d	WT	A	correct	☑
		B	correct	☑
3e	WT	A	correct	☑
		B	correct	☑
3g	HOM	A	-	
		B	correct	☑
3h	HOM	A	correct	☑
		B	correct	☑
3i	HOM	A	correct	☑
		B	correct	☑

potential off-target locus 8_2:

TTCCTCTGTGACACGGTGTGGAG	0.3	4MMs [2:9:10:19]	NM_176940	chr8:-75191268		

Animal ID	Geno-type	TOPO clone	sequence	ok?
2e	WT	A	CTTGGCATATGGGCTGCCACTGCCCCCAGCCCAGGTCCTGGAGGCCCACAGCAGGGTGG GCCATTTCTTCCATACTCTCCTCCACACCGTGTCACAGAGGAACTTTGAGTCCCTGGTGTT GCTCCTGGACTCCGTGGATGACCTGGACTCCATATGCCATTCTCCGAGGGTCTCTTGGCT GCCCCTCAAGTGTCCTCCAAGGGTGCACCTCATCCTCTCTACATGCTCAGGCCAGCAGGT TTTACATAACCTGCAACAGACCCTCAAGGACCCCAGCACCTACTGGGAGGTGAAGGCCCT GTCTGGAAGTCAAGGCCAGGAGTTCATACAGCTGCTGCTGGCTGCAGAAGAAGGATGC TGAGCCCGGGTCAGAGAGATGTCCTCTGGGCCAGCCTCCCAGAGTGTGGCCACCCAGGG AGGCTAAGGCTGGCCTTGAGGAGGCCCGGAAGTGGGCCTCCTTCACTGTGCCAGTGCC CTTGGCCACTACAGCAGAGGAAGCCACACACCAGCTCTGTATAGCCTGGAGGAGACAC ATGGGGCACTGCTGGTGGCTCATGTACTGGGCTATATTGTGT = correct	☑
		B	mini preparation DNA concentration too low	
		C	correct	☑
		D	correct	☑
		E	short sequencing read	
3d	WT	A	correct	☑
		B	deletion that spans vector and insert	
3e	WT	A	correct	☑
		B	no read	
3g	HOM	A	correct	☑
		B	sequ correct but read 50 bp too short	
3h	HOM	A	correct	☑
		B	correct	☑
3i	HOM	A	correct	☑
		B	correct	☑

Supp. Figure 1. Generation of the FTO-R313A allele.

A. Sequence at the genomic locus of the Fto gene, on mouse chromosome 8. Out of several mRNA guides used for the CRISPR approach, the successful guide (green) was the one binding on top of the mutation site. Shown in pink is the stretch of sequence that was injected as single strand DNA to serve as donor for mutation by homologous repair. Shown in orange is Fto exon 5, with the translated amino acids shown below. CG→GC is the sequence change introduced to result in the Arg313Ala mutation.

B. At the top, potential off-target sites, determined using Zhang Lab CRISPR design tool (http://crispr.mit.edu/). From left to right: sequence at locus; likeliness-score for off-target mutation; number & position of mismatches compared to guide / gene ID / chromosomal location. The tables below show an overview of the respective control checks. Each locus was checked for several animals, both wildtype (WT) controls, to show the locus is correctly detected, and HOM R313A/R313A animals. For each animal, several clones were amplified, sub-cloned with a TOPO PCR cloning kit (Thermo Fisher) and sequenced (TOPO clones A,B etc). Sequences shown are the sequences amplified and sub-cloned, the guide RNA binding position is marked in red. None of the successful sequencings uncovered any mutation, indel or other damage to the sites.