

Supplemental Figure 3

a	crRNA	DNA	Chromosome	Position	Direction	#Bulge type	Mismatches	Bulge Size	Offtarget gene
1	TTTGTCTCGTATACCGCAAANGG	TTTGTCTCGTATACCGCAAAGGG	chr12	100209858	-	X	0	0	
2	ACGCAACATCCAGTGCAAGCNNG	ACGCAACATCCAGTGCAAGCTGG	chr12	100207399	+	X	0	0	
3	GAGTTGGTCCCCCGTCAGAGNNG	GAGTTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	X	0	0	
4	TTTGTCTCGTATACCGCAAANGG	TTTGTCTCGTATACCGC-AAAGG	chr12	100209859	-	RNA	0	1	
5	TTTGTCTCGTATACCGCAAANGG	TTTGTCTCGTATACCGCA-AAAG	chr12	100209859	-	RNA	0	1	
6	TTTGTCTCGTATACCGCAAANGG	T-TGTCCTCGTATACCGCAAAGGG	chr12	100209858	-	RNA	0	1	
7	TTTGTCTCGTATACCGCAAANGG	TT-TGTCCTCGTATACCGCAAAGGG	chr12	100209858	-	RNA	0	1	
8	AC--GCAACATCCAGTGCAAGCNNG	ACACGCAACATCCAGTGCAAGCTGG	chr12	100207397	+	DNA	0	2	
9	A--CGCAACATCCAGTGCAAGCNNG	ACACGCAACATCCAGTGCAAGCTGG	chr12	100207397	+	DNA	0	2	
10	GAGTTGGTCCCCCGTCAGAGNNG	G--TTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	RNA	0	2	
11	TTTGTCTCGTATACCGCAAANGG	T--GTCTCGTATACCGCAAAGGG	chr12	100209858	-	RNA	0	2	
12	ACGCAACATCCAGTGCAAGCNNG	c-GCAACATCCAGTGCAAGCTGG	chr12	100207400	+	RNA	1	1	
13	GAGTTGGTCCCCCGTCAGAGNNG	a-GTTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	RNA	1	1	
14	TT-TGTCTCGTATACCGCAAANGG	gTTTGTCTCGTATACCGCAAAGGG	chr12	100209858	-	DNA	1	1	
15	T-TGTCTCGTATACCGCAAANGG	gTTTGTCTCGTATACCGCAAAGGG	chr12	100209858	-	DNA	1	1	
16	TTT-GTCTCGTATACCGCAAANGG	gTTTGTCTCGTATACCGCAAAGGG	chr12	100209858	-	DNA	1	1	
17	A-CGCAACATCCAGTGCAAGCNNG	cACGCAACATCCAGTGCAAGCTGG	chr12	100207398	+	DNA	1	1	
18	TTTGTCTCGTATACCGCAAANGG	TTTGTCTCGTATACCG-cAAAGG	chr12	100209859	-	RNA	1	1	
19	TTTGTCTCGTATACCGCAAANGG	TTg-TCTCGTATACCGCAAAGGG	chr12	100209858	-	RNA	1	1	
20	G-AGTTGGTCCCCCGTCAGAGNNG	aGAGTTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	DNA	1	1	
21	GCTTGCACCTGGATGTTGCGTNNG	GCTTGCACCTGGgTGT--GTGGG	chr8	39376664	+	RNA	1	2	Intergenic
22	GCTTGCACCTGGATGTTGCGTNNG	GCTTGCACCTGGgTGTGT--TGGG	chr8	39376664	+	RNA	1	2	Intergenic
23	T--TTGTCTCGTATACCGCAAANGG	gTTTGTCTCGTATACCGCAAAGGG	chr12	100209858	-	DNA	1	2	
24	G--AGTTGGTCCCCCGTCAGAGNNG	tAGAGTTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	DNA	1	2	
25	ACGCAACATCCAGTGCAAGCNNG	g--CAACATCCAGTGCAAGCTGG	chr12	100207401	+	RNA	1	2	
26	ACGCAACATCCAGTGCAAGCNNG	gC--AACATCCAGTGCAAGCTGG	chr12	100207401	+	RNA	1	2	
27	GA--TTGGTCCCCCGTCAGAGNNG	tAGAGTTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	DNA	1	2	
28	ACG--CAACATCCAGTGCAAGCNNG	ACaCGCAACATCCAGTGCAAGCTGG	chr12	100207397	+	DNA	1	2	
29	CTCTGACGGGGGACCAA--CTCNNG	CTCTGACGGGGGACCAACTCTaTGG	chr12	100209718	+	DNA	1	2	
30	GAGTTGGTCCCCCGTCAGAGNNG	Gt-TGTCCTCGTATACCGCAAAGG	chr12	100209715	-	RNA	1	2	
31	ACGC--AACATCCAGTGCAAGCNNG	ACaCGCAACATCCAGTGCAAGCTGG	chr12	100207397	+	DNA	1	2	
32	TTTGTCTCGTATACCGCAAANGG	Tg--TCTCGTATACCGCAAAGGG	chr12	100209858	-	RNA	1	2	
33	TTTGTCTCGTATACCGCAAANGG	TgT--CTCGTATACCGCAAAGGG	chr12	100209858	-	RNA	1	2	
34	CTCTGACGGGGGACCAAC--TCNNG	CTCTGACGGGGGACCAACTCTaTGG	chr12	100209718	+	DNA	1	2	
35	GAG--TTGGTCCCCCGTCAGAGNNG	tAGAGTTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	DNA	1	2	
36	CTCTGACGGGGGACCAACT--CNNG	CTCTGACGGGGGACCAACTCTaTGG	chr12	100209718	+	DNA	1	2	
37	CTCTGACGGGGGACCAACTCNNG	CTCTGA--GGGGICCAACTCAGG	chr10	62679358	-	RNA	1	2	Stox1 intron
38	TTTGTCTCGTATACCGCAAANGG	TT--TCTCGTATAcGCAAAAGG	chrX	134330263	+	RNA	1	2	Cenpi intron
39	TTTGTCTCGTATACCGCAAANGG	TTT--CTCGTATAcGCAAAAGG	chrX	134330263	+	RNA	1	2	Cenpi intron
40	GAGTTGGTCCCCCGTCAGAGNNG	cAGTTGGTCCCCCG-aAGAGTGG	chr8	121767423	+	RNA	2	1	Jph3 intron
41	GCTTGCACCTGGATGTTGCGTNNG	GCTTGCgCTtGATGTTGC-TCGG	chr8	71465346	-	RNA	2	1	Mrpl34 exon
42	GAGTTGGTCCCCCGTCAGAGNNG	GAG-TGaTCCiCCGTcAGAGGGG	chr8	89063789	+	RNA	2	1	Intergenic
43	GAGTTGGTCCCCCGTCAGAGNNG	GAGT-GaTCCiCCGTcAGAGGGG	chr8	89063789	+	RNA	2	1	Intergenic
44	ACGCAACATCCAGTGCAAGCNNG	AgaCAACATCCAGTG-AAGCTGG	chr8	76542388	-	RNA	2	1	Intergenic
45	ACGCAACATCCAGTGCAAGCNNG	AgGCAACATcAGTG-AAGCAGG	chr8	124262803	+	RNA	2	1	Galnt2 intron
46	GAGTTGGTCCCCCGTCAGAGNNG	GAGTcGGTCctCCGT-AGAGTGG	chr8	71545799	-	RNA	2	1	Mvb12a exon
47	GAGTTGGTCCCCCGTCAGAGNNG	cAGTTGGTCCCCCGa-AGAGTGG	chr8	121767423	+	RNA	2	1	Jph3 intron
48	ACGCAACATCCAGTGCAAGCNNG	ACtCAAgATCCAGT-CAAGCTGG	chr8	104115432	+	RNA	2	1	Cdh5 intron
49	TTTGTCTCGTATACCGCAAANGG	TTgt-TCTCGTATACCGCAAAGGG	chr12	100209858	-	RNA	2	1	
50	GAGTTGGTCCCCCGTCAGAGNNG	ag-TTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	RNA	2	1	
51	ACGCAACATCCAGTGCAAGCNNG	cg-CAACATCCAGTGCAAGCTGG	chr12	100207400	+	RNA	2	1	
52	TTT-TCTCGTATACCGCAAANGG	gTTtGTCTCGTATACCGCAAAGGG	chr12	100209858	-	DNA	2	1	
53	GA-TTGGTCCCCCGTCAGAGNNG	agAGTTGGTCCCCCGTCAGAGAGG	chr12	100209715	-	DNA	2	1	
54	TTTGTCTCGTATACCGCAAANGG	TTTGTCTCGTATACC-gcAAAGG	chr12	100209859	-	RNA	2	1	
55	CTCTGACGGGGGACCAACTCNNG	CTtTGACtGGGG-CCAActCAGG	chr12	76602542	+	RNA	2	1	Sptb intron
56	AC-GCAACATCCAGTGCAAGCNNG	caCGCAACATCCAGTGCAAGCTGG	chr12	100207398	+	DNA	2	1	
57	ACGCAACATCCAGTGCAAGCNNG	AaGCAACATCCAGT-CAAGaTGG	chr3	126820828	+	RNA	2	1	Camk2d intron
58	TTTGCGGTATACGAGACAAANGG	TTTGaGGTATAC-AGACAAtTGG	chr3	72454474	+	RNA	2	1	Intergenic
59	CTCTGACGGGGGACCAACTCNNG	CTCTGAC-GGGGACgAgCTCGGG	chr3	146121860	-	RNA	2	1	Mcoln3 exon
60	CTCTGACGGGGGACCAACTCNNG	CTCTGACG-GGGACgAgCTCGGG	chr3	146121860	-	RNA	2	1	

[illegible]

Supplemental Figure 3. CRISPR gRNA off-target analysis. (a) CRISPR RGEN Tools was used to analyze Cas9 off-target sites using all the 6 gRNAs used in this study as input sequences. Original output file was sorted by mismatch numbers and bulge size. Top 60 positions shown here. Given that most of the top targets of gRNAs belonged to the intended *Calm1* locus (shaded in grey), these were exempted from Sanger sequencing analysis. In order to perform more genome-wide analysis, several genomic locations from the top of the list were selected (Red) (b) PCR amplification of the genomic loci followed by Sanger sequencing. Shaded pink regions are potential off-target sites which showed no alteration in *Calm1*^{ΔL/ΔL}. Numbers next to the genotypes (either WT or ΔL) indicate mouse identification number. Related to Figure 3.