

# **Inui et al. Supplementary Information**

Including three Supplementary Figures and two Supplementary Tables

## **Manuscript TITLE:**

**Rapid generation of mouse models with defined point mutations by the CRISPR/Cas9 system**

## **Authors list**

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## Supplementary Figure legend

### Supplementary Figure S1. Position of Sf-1 gRNA3, ssODN1, 4, 5 and their substitution target sites

A schematic illustration showing the locations of the Sf-1 gRNA3 and ssODNs, along with the mouse *Sf-1* locus. The blue bar and the letter indicate the position of the gRNA target with the red bar highlights the PAM sequences. ssODNs are shown in green letters at the corresponding position to the *Sf-1* locus. Red letters indicate the substitution target sites in the *Sf-1* locus and corresponding mismatched nucleotides in the ssODNs.

## **Supplementary Figure S2. The definition of mutation and substitution**

The representative sequences of the *Sox9* locus determined by direct sequencing. The top panel shows the wild type sequence without any overlapping peaks (categorized as “WT”). The second panel shows overlapped peaks at defined nucleotides (categorized as “monoallelic substitution”). The third panel shows continuous overlapping peaks (categorized as “indel”). The bottom panel shows peak overlapping at both defined nucleotides and in the following sequence (categorized as “monoallelic substitution + indel”). The targeted substitutions were indicated by red arrows and a pink background color, whereas overlapping peaks caused by random indels are indicated with a red background color.

### **Supplementary Figure S3. Off-target analysis of Sf-1 gRNAs**

(A) The sequences and positions of possible off-target sites for Sf-1 gRNAs.

OT: Off-target site.

(B) The representative results of the sequence analysis of the off-target sites. Direct sequencing of the PCR-amplified putative off-target sites from mice received the designated substitution (c.274 C>T) are shown as representative results. The possible off-target sequences were shown at the fourth to twenty-sixth bases, as indicated by the darker background color.

# Inui et al. Supplementary Figure S1

## Mouse *Sf-1* locus

5'...TTTGGAAAAGATCTGTGGCAGACACTTGGGAAGGTAGAACCCTGACAGACTGCTCCTGCCACAGCTGTGGTGTGATCGAATGCGGGTGGCCGGAAACAAGTTGGGCCCATGTACAAGAGAGACCGGGCCTTGAAGCAGCAGAA...3'  
3'...AAACCCTTTCTAGACACCGTCTGTCAACCTTCCATCTTGGACTGTCTGACGAGGACGGGTGTGACACGACAGACTAGCTTACGCCCCACCGGCTTGTTCAAACCCGGGTACATGTTCTCTGGCCCGAACTTCGTCTCT...5'

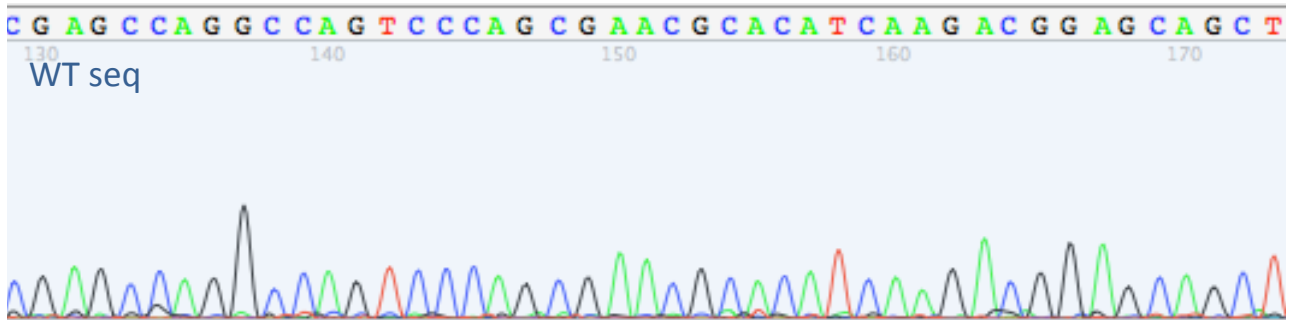
target of *Sf-1* gRNA3 PAM

5' - GACTGCTCCTGCCACAGCTGTGCGTGTGATCGAATGCGGGTGGCTGGAAACAAGTTGGGCCCATGTACAAGAGAGACCGGGCCTTGAAGCAGCAGAA-3' ssODN1  
5' - GACTGCTCCTGCCACAGCTGTGCGTGTGATCGAATGCGGGTGGCCGGAAACAAGTTGGGCCCATGTAACAAGAGAGACCGGGCCTTGAAGCAGCAGAA-3' ssODN4  
5' - GACTGCTCCTGCCACAGCTGTGTGTGCTGATCGAATGCGGGTGGCCGGAAACAAGTTGGGCCCATGTACAAGAGAGACCGGGCCTTGAAGCAGCAGAA-3' ssODN5

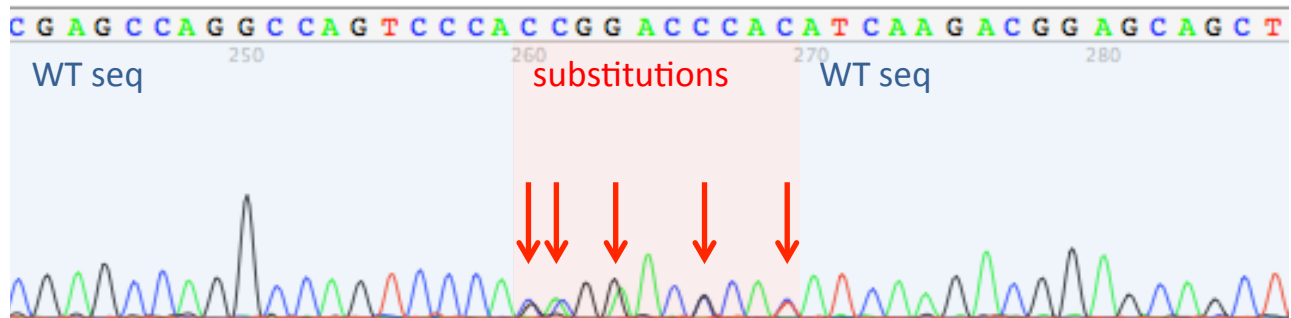
ssODNs (HDR templates)

# Inui et al. Supplementary Figure S2

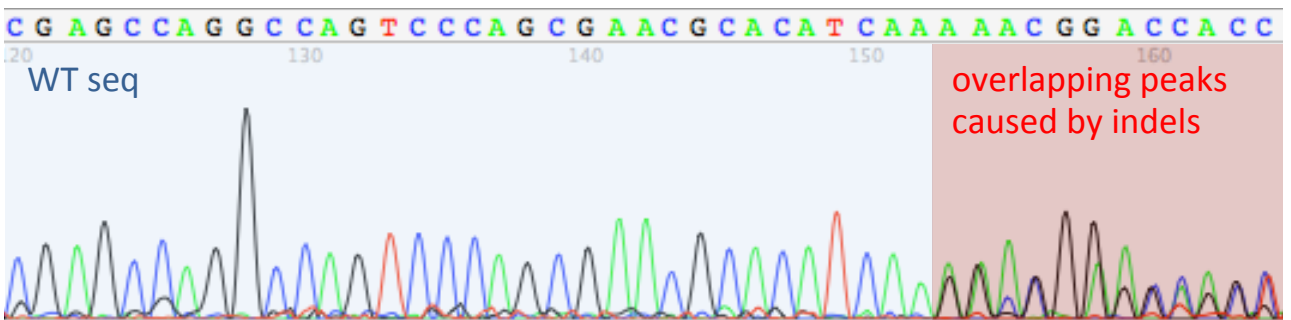
Mouse No. 29 wild type sequence ("WT")



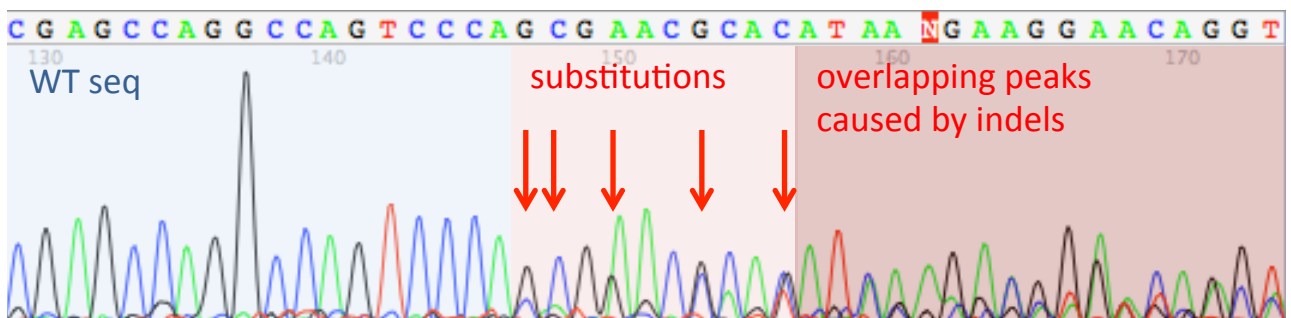
Mouse No. 4 monoallelic K-to-K substitution ("substituted")



Mouse No. 36 with indel ("mutated")



Mouse No. 27 monoallelic substitution + indel ("substituted")

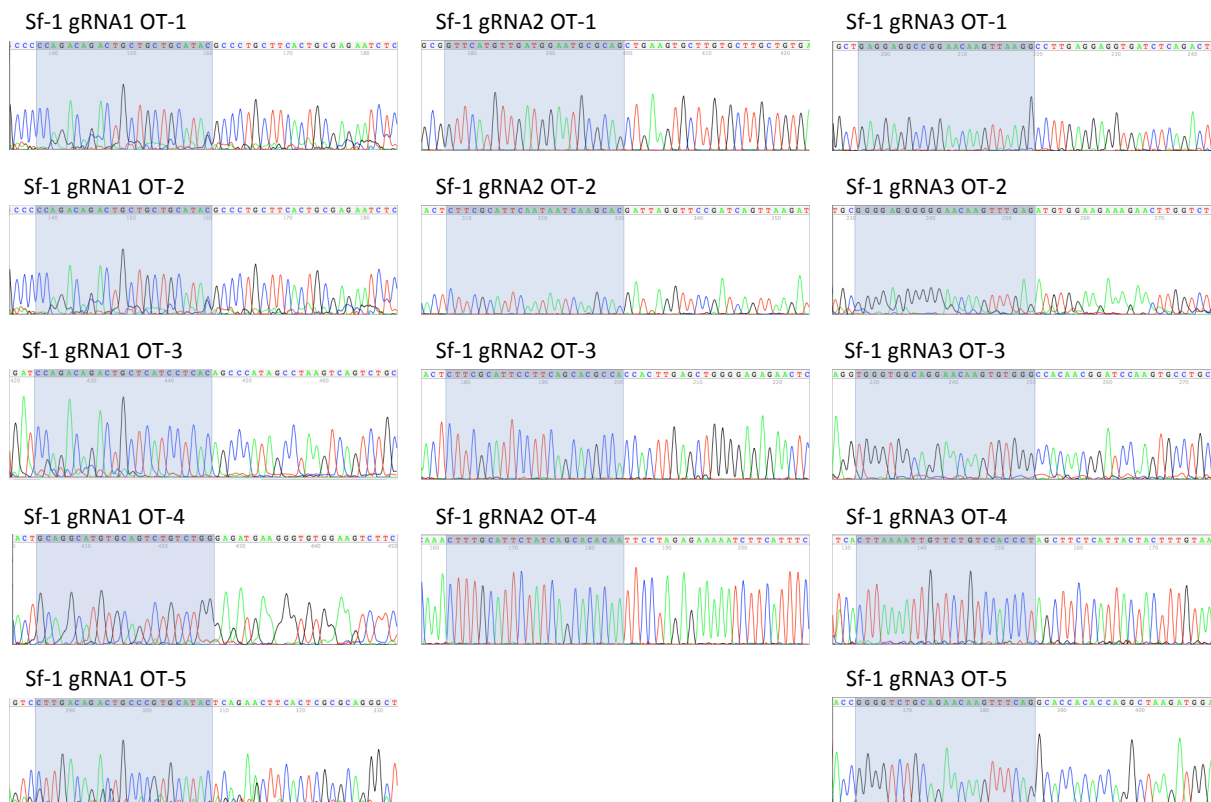


# Inui et. al Supplementary Figure S3

## A

	chromosome	position	strand	sequence	# mismatches
Sf-1-gRNA1 target seq.	chr2	38,564,062-38,564,084	+	<b>GTGGGCAGGAGCAGTCTGTCAGG</b>	0
Sf-1-gRNA1 OT-1	chr7	148,612,950-148,612,972	-	GCAGCCAGGAGCAGTCTGTCTGG	3
Sf-1-gRNA1 OT-2	chr4	116,778,470-116,778,492	+	GTATGCAGCAGCAGTCTGTCTGG	3
Sf-1-gRNA1 OT-3	chr11	51,842,777-51,842,799	+	GTGAGGATGAGCAGTCTGTCTGG	3
Sf-1-gRNA1 OT-4	chr8	87,831,623-87,831,645	+	GCAGGCATGTGCAGTCTGTCTGG	4
Sf-1-gRNA1 OT-5	chr6	22,330,472-22,330,494	-	GTATGCACGGGCAGTCTGTCAAG	4
Sf-1-gRNA2 target seq.	chr2	38,564,035-38,564,057	-	<b>GTGCGTGCTGATCGAATGCCGGG</b>	0
Sf-1-gRNA2 OT-1	chr15	82,000,998-82,001,020	+	GTTTCATGTTGATGGAATGCCGAG	4
Sf-1-gRNA2 OT-2	chr13	93,916,133-93,916,155	-	GTGCTTGATTATTGAATGCCAAG	4
Sf-1-gRNA2 OT-3	chr13	55,723,514-55,723,536	-	TGGCGTGCTGAAGGAATGCCAAG	4
Sf-1-gRNA2 OT-4	chr15	17,258,555-17,258,577	+	TTGTGTGCTGATAGAATGCAAG	4
Sf-1-gRNA3 target seq.	chr2	38,564,016-38,564,038	-	<b>GGGGTGGCCGGAACAAGTTTGGG</b>	0
Sf-1-gRNA3 OT-1	chr8	49,395,258-49,395,280	+	GAGGAGGCCGGAACAAGTTAAGG	3
Sf-1-gRNA3 OT-2	chr12	106,627,747-106,627,769	-	GGGGAGGGGGAACAAGTTTGGG	3
Sf-1-gRNA3 OT-3	chr2	166,995,191-166,995,213	-	TGGGTGGCAGGAACAAGTTGGG	3
Sf-1-gRNA3 OT-4	chr2	23,010,345-23,010,367	+	AGGGTGGACAGAACAATTTAAG	4
Sf-1-gRNA3 OT-5	chr17	89,072,909-89,072,931	-	GGGGTTCGAGAACAAGTTTCAG	4

## B



Supplementary Table S1. Summary of experiments comparing various positions of the point mutations

Experiment	Microinjection		Survival of zygotes			Result of genotyping				Ratio of genotype	
	gRNA	ssODN	2 cell embryo / injected zygotes	Transferred	Genotyped	Monoallelic substitution (+indel)	Biallelic substitution (+indel)	Indel	WT	Mouse with substituted alleles (%)	Mouse with mutated alleles (%)
4	3	1	157/217	144	54	10 (7)	6 (5)	28	10	16 (29.6)	44 (81.5)
5	3	4	136/142	136	25	3 (2)	1 (0)	16	5	5 (20.0)	24 (82.8)
6	3	5	108/110	108	17	0 (0)	0 (0)	16	1	0 (0)	16 (94.1)



**Supplementary Table S2. Summary of off-target effects by Sf-1 gRNAs**

<b>Target sites</b>	<b>Mouse with mutated alleles / genotyped (%)</b>
Sf-1-gRNA1 OT-1	0/1 (0)
Sf-1-gRNA1 OT-2	0/1 (0)
Sf-1-gRNA1 OT-3	0/1 (0)
Sf-1-gRNA1 OT-4	0/1 (0)
Sf-1-gRNA1 OT-5	0/1 (0)
Sf-1-gRNA2 OT-1	0/9 (0)
Sf-1-gRNA2 OT-2	0/9 (0)
Sf-1-gRNA2 OT-3	0/9 (0)
Sf-1-gRNA2 OT-4	0/9 (0)
Sf-1-gRNA3 OT-1	0/16 (0)
Sf-1-gRNA3 OT-2	0/16 (0)
Sf-1-gRNA3 OT-3	0/16 (0)
Sf-1-gRNA3 OT-4	0/16 (0)
Sf-1-gRNA3 OT-5	0/16 (0)