

Simultaneous targeting of linked loci in mouse embryos using base editing

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Supplementary Figure legends

Supplementary Fig. 1 Simultaneous targeting of two linked genomic loci using CRISPR/Cas9 genome editing. **(a)** Summary of data obtained from founder mice in which 18 kb, 9 kb, and 22 kb loci between two sites were targeted by Cas9 and two sgRNAs, respectively. The sgRNA sequences are underlined and PAM sites are highlighted in brown. Deletions are shown as dash lines. The frequency column illustrates the percentage of large deletions obtained from mutant mice generated by simultaneous injection with two sgRNAs by CRISPR/Cas9 genome editing. **(b)** List of studies on CRISPR/Cas9 genome editing of the mouse germline and summary of large deletions caused by CRISPR/Cas9 and multiple sgRNA injection into mouse zygotes.

Supplementary Fig. 2 The enhancer landscape of the *Csn2* and *Csn1s2a* locus using ChIP-seq analysis for transcription factor binding (STAT5, GR, ELF5 and MED1) and the presence of H3K27ac marks.

Supplementary Fig. 3 Whole genome sequence analysis. **(a)** Number of predicted off-targets for two sgRNAs. **(b)** Number of total SNPs and indels as well as the number of mutations located at potential off-target sites identified in two base-edited mice, a female (F885) and a male (M888).

Supplementary Fig. 1

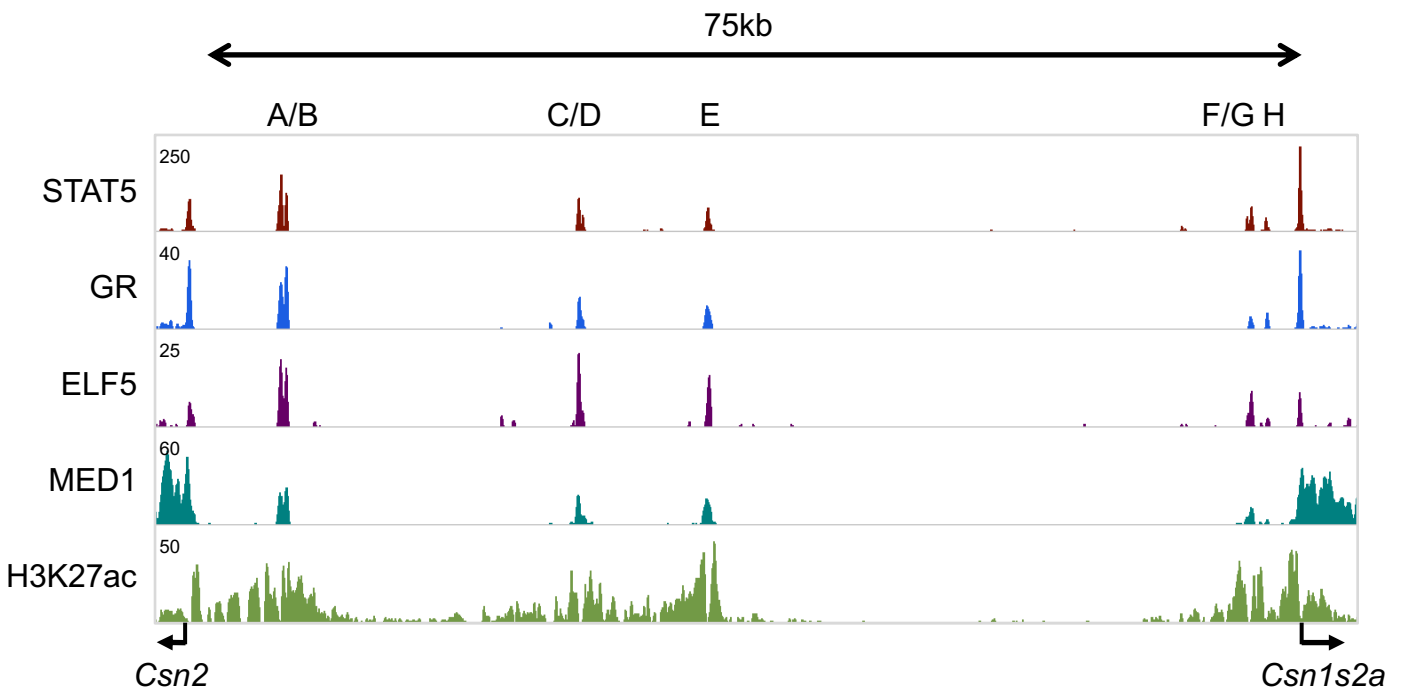
a

Target	Large deletion		Frequency
Wap-Ramp3 locus	WT	18kb ATATATGGTCAGCATATAAAG CCA GCAGACTTCCCAGATCTCCC..... CCT GCCCTCAGTTAGCTTCAGAC	11/14 (79%)
	F591	ATATA-----AGTTAGCTTCAGAC	
	F592	ATATATGGTCAGCATATAAA-----CTCAGTTAGCTTCAGAC	
	F593	ATATATGGTCAGCATATAAA-----AGTTAGCTTCAGAC	
	F595	ATATATGGTCAGCATATAAAGCCAGC-----TTCAGAC	
	F597	ATATA-----CTCAGTTAGCTTCAGAC	
	F598	ATATATGGTCAGCATATAAAGCCAG-----	
	M599	ATATATGGTCAGCATATAAAGCCA-----AGCTTCAGAC	
	M601	ATATATGGTCAGCATAT-----	
	M1482	ATATATGGTCAGCATATAAAGC-----TTAGCTTCAGAC	
	M1483	ATATATG-----	
	F1484	-----AGCTTCAGAC	
Csn super-enhancer locus	WT	9kb ACCT CCT GAGACCCTACATGCTTAGCG..... CCT GTCTAGTGGAAGAACACCCATCTAA	15/23 (65%)
	F6385	ACCTCCTG-----GGAAGAACACCCATCTAA	
	F6386	-----AACACCCATCTAA	
	M6390	-----CCCATCTAA	
	M6392	ACCTCCTG-----AACACCCATCTAA	
	M6393	ACCTCCTGA-----GAACACCCATCTAA	
	M6394	ACCTCTAACCTCCT-----	
	F6395	ACCTCCTGA-----TAGTGGAAGAACACCCATCTAA	
	F6396	ACCTCCTG-----	
	M6398	ACCTCCTGAG-----TAGTGGAAGAACACCCATCTAA	
	F6426	-----	
	F6427	-----	
	M6429	ACCTC-----TGGAAGAACACCCATCTAA	
	M6430	-----	
PMID 28561021 (D-1/2/3/4/5)	WT	22kb CTCTGAGTTTCCCATATGAACTC AGG GAGACCCA.....TATGTGT CCT TGGGTGTATGTAAGTAAGTGTGT	9/10 (90%)
	F2215	-----	
	F2216	CTCTGAGTTTCCCATAT-----TGTATGTAAGTAAGTGTGT	
	F2217	-----TATGTAAGTAAGTGTGT	
	F2218	CTCTGAG-----	
	M2219	-----TATGTGTCC-----	
	M2220	-----GTGTATGTAAGTAAGTGTGT	
	M2221	CTCTGAGTTTCCCATATG-----GTAAGTAAGTGTGT	
	M2222	-----	
	M2223	-----	

b

PMID	Large deletion	Frequency
26742453	9 kb	3/30 (10%)
23997119	10 kb	9/27 (33%)
25137067	23 kb	4/25 (16%)
25137067	23 kb	3/9 (33.3%)
25803037	65 kb	3/14 (21%)
26742453	65 kb	13/81 (16%)
26620761	95 kb	2/20 (10%)
27396308	0.5 M	6/27 (22%)

Supplementary Fig. 2



Supplementary Fig. 3

a

Base editor	Protospacer	PAM	Number of Potential off-targets
VQR-BE3	GAGTTCAAAGAAGGCAGGAA	AGAG	434
BE4	CTTCCTTGTTACACCCTTT	GGG	143

b

	F885	M888
Number of total SNPs	12,310	12,705
Number of total INDELs	182	172
Number of SNPs at potential off-target sites	0	0
Number of INDELs at potential off-target sites	0	0