

Supplementary Materials for **Somatic cell reprogramming-free generation of genetically modified pigs**

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Supplementary Text

All animal care and experiments were carried out in accordance with the Guidelines for Animal Experiments of Tokushima University and the National Institute of Agrobiological Sciences. This study was approved by the Ethics Committee of Tokushima University for Animal Research (Approval number: T28-21) and the Safety Management Section of National Institute of Agrobiological Sciences (Approval number: H18-038).

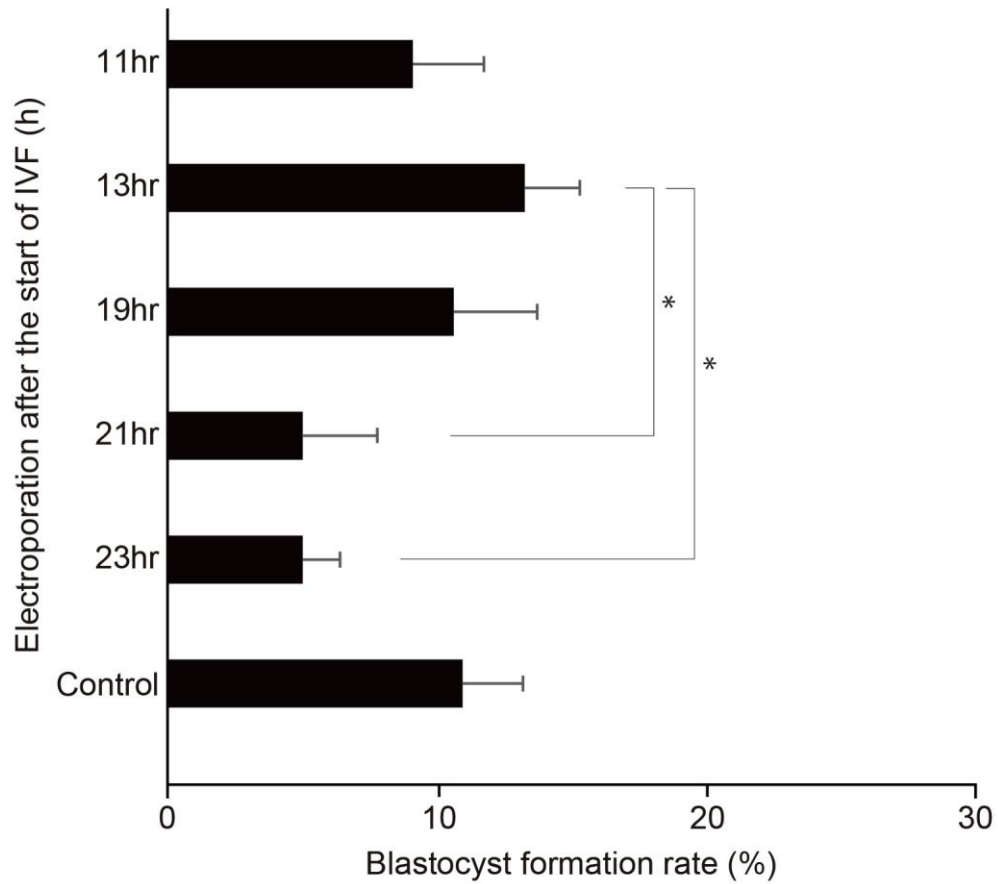


fig. S1. Optimization of the time period between IVF initiation and electroporation. Presumptive zygotes were electroporated with *Cas9* mRNA and *FGF10* guide RNA at 11, 13, 19, 21, and 23 h after IVF initiation. After incubation of the electroporated zygotes for 7 d, the blastocyst formation rates were determined. * $P < 0.05$ using one-way ANOVA. Error bars, mean \pm s.e.m.

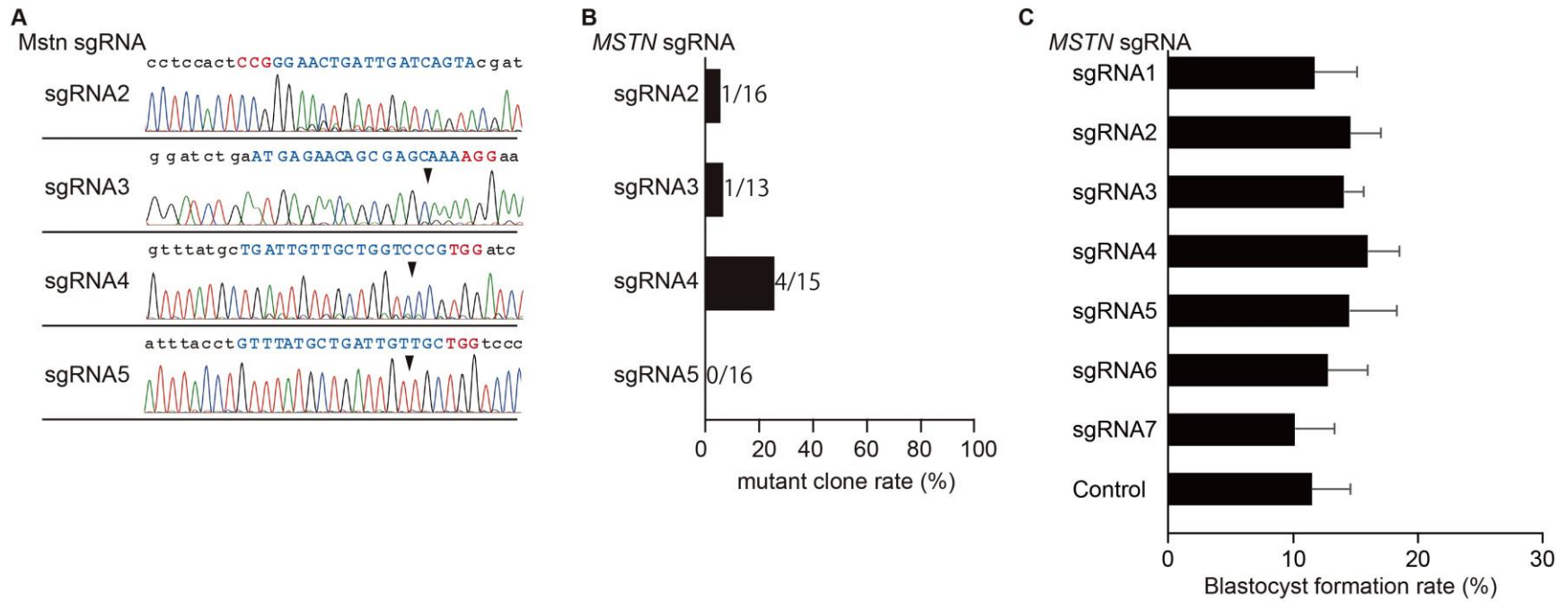


fig. S2. Genome editing of the *MSTN* gene. (A) Genomic *MSTN* sequences in the blastocysts formed from electroporated zygotes. (B) Frequency of mutations detected in the PCR amplicons. (C) Blastocyst formation rates of the electroporated zygotes. Error bars, mean \pm s.e.m.

table S1. Sequence of the *MSTN* target region in piglets. The nucleotides in blue represent target sequences, while those in red represent PAM sequences.

piglet	<i>MSTN</i> sgRNA	Genome sequence	indels	frequency
	sgRNA1 target	ctgatcAATCAGTTCCCGGAGTGGAGGagc		
#1		ctgatcAATCAGTTCCCGGAGTGGAGGagc	none	9/16
		ctgatcAATCAGTTCCCGGAGGAGGAGTTCTGGAGGagc	+10bp	7/16
#5		ctgatcAATCAGTTCCCGGAGTGGAGGagc	none	11/14
		ctgatcAATCAGTTCCCGGAG-----Gagc	-5bp	3/14
#6		ctgatcAATCAGTTCCCGGAGTGGAGGagc	none	8/14
		ctgatcAATCAGTTCCCGGAGATGGAGGagc	+1bp	6/14
	sgRNA7 target	aagGAAAATGTGGAAAAAGAGGGGctg		
#2		aagGAAAATGTGGAAA---AGGGGctg	-3bp (inframe)	5/15
		aagGAAAAT--GGAAAATGAGGGGctg	-2bp, m1bp	4/15
		aagGAAAATGTGGAAAAAGAGGGGctg	+1bp	3/15
		aagGAAAATGTGGAAAAAGAGGGGctg	none	3/15
#7		a-----AGGGGctg	-18bp (inframe)	7/15
		aagGAAAATGTGGAAAAAGAGGGGctg	+1bp	6/15
		aagGAAAATGTG-----GAGGGGctg	-6bp (inframe)	1/15
		aagGAAAATGTGGAAAAAGAGGGGctg	none	1/15
#8		aagGAAAATGTGGAAAAAGAGGGGctg	+1bp	7/13
		aagGAAAATGTGGAAAAAGAGGGGctg	none	6/13
#9		aagGAAAATGT-----GGGctg	+10bp	10/12
		aagGAAAATGTGGAAAAAGAGGGGctg	none	2/12
#10		aagGAAAATGTGGAAAAAGAGGGGctg	none	15/15

table S2. Off-target analysis of the piglets by deep-sequencing.

piglet		Genome sequence	mismatch	locus (strand)	frequency
	sgRNA6 target	gcTGTTCTCATTTCAGATCCA CGG			
#4	Off-target candidates	OT4 aaTGTTCTCATGCAGATCCAGGG	1bp	chr16: 3,206,113-3,206,133 (-)	2195/2209 (99%)
		OT5 tgCTTTCTCATTTCAGATCCATGG	2bp	chr11: 75,202,772-75,202,792 (-)	2168/2181 (99%)
		OT6 gtTTTCTCATTTCAGATCC TAGG	2bp	chr1:200,634,452-200,634,472 (-)	2734/2754 (99%)
	sgRNA7 target	agGAAAATGTGGAAAAAGAG GGG			
#8	Off-target candidates	OT1 aaGAAAATATGGAAAAAGAGAGG	1bp	chr3:96,400,245-96,400,265 (+)	2896/2924 (99%)
		OT2 atGAAAATGTGAAAAAGAGAGG	1bp	chr7:115,969,797-115,969,817 (-)	2843/2861 (99%)
		OT3 atGAAAAC TTGGAAAAAGAGAGG	2bp	chr7:46,099,859-46,099,879 (-)	3025/3047 (99%)

table S3. Sequence analysis of the *MSTN* gene in piglets #4 and #8 by deep sequencing.

piglet		Genome sequence	indels	frequency
#4	sgRNA6 target	cgcTGTTCTCATTTCAGATCCA CGGgac		
		cgcTGTTCTCATTTCAGAT TCCACGGgac	+1bp	1486/2560 (58%)
		cgcTGTTCTCATTTCAGA GAACACGGgac	+1bp, m2bp	954/2560 (38%)
		cgcTGTTCTCATTCA----- GGgac	-7bp	50/2560 (2%)
#8	sgRNA7 target	aagGAAAATGTGGAAAAAGAG GGGctg		
		aagGAAAATGTGGAAAAA AGAGGGGctg	+1bp	1391/2849 (49%)
		aagGAAAATGTGGAAAAAGAGGGGctg	none	1438/2849 (50%)

table S4. Oligonucleotide sequences used to generate sgRNA.

	Forward oligos	Reverse oligos
Fgf10 sgRNA	TAGGAAAAGGAGCTCCCAGGAG	AAACCTCCTGGGAGCTCCTTTT
Mstn sgRNA1	TAGGCCACTCCGGGAACTGATT	AAACCCACTCCGGGAACTGATT
Mstn sgRNA2	TAGGTACTGATCAATCAGTTCC	AAACGGAAGCTGATTGATCAGTA
Mstn sgRNA3	TAGGATGAGAACAGCGAGCAA	AAACTTTGCTCGCTGTTCTCAT
Mstn sgRNA4	TAGGTGATTGTTGCTGGTCCCG	AAACCGGGACCAGCAACAATCA
Mstn sgRNA5	TAGGGTTTATGCTGATTGTTGC	AAACGCAACAATCAGCATAAAC
Mstn sgRNA6	TAGGTGTTCTCATTCAGATCCA	AAACTGGATCTGAATGAGAACA
Mstn sgRNA7	TAGGGAAAATGTGGAAAAGAG	AAACCTCTTTTTCCACATTTTC

table S5. Oligonucleotide sequences used for off-target analysis.

	common sequence	specific sequence
forward primer	sgRNA6 target	ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	OT1	ACCTGTTTATGCTGATTGTTGCT
	OT2	GTGATCTTCACCTGCGCAG
	OT3	GTGCTCAGCCCTGACTCAG
	sgRNA7 target	TCGGAGAGACGTTCCCCTG
	OT4	TGGTCCCGTGGATCTGAATG
	OT5	AGGAATGGCCCTAGAAAAGGC
OT6	CCAGACTTCCTATTTTCCCTCAACA	
reverse primer	sgRNA6 target	CAGGCGAATTTAATCAGGCCA
	OT1	GTGTTTTGTCTCCACATACATGCA
	OT2	CTGACGGCACTGGAACCTT
	OT3	GGCCTAAAATCCTGAGGCCT
	sgRNA7 target	GATGGACGCCCTCTGAATG
	OT4	GTGTTTTGTCTCCACATACATGCA
	OT5	TGTGGATCTGAGCATGAAAGG
OT6	TGTGGAAGATGAATTGTTTTAAAGCT	
		TGCATTATGTTACAGAATTTGGACT