Table S1. Efficiency of Generation of Reporter Embryos by Cytoplasm and Pronuclear Injection, Related to Figure 2 and Table 1

Donor	Dose of Cas9/sgRNA (ng/μl)	Dose of Donor vector (ng/μl)	Injected zygotes	Targeted Blastocysts / Total
One-step injection				
Nanog-mCherry	100/50 (Cyto)	500(Cyto)	186	1/81
Nanog-mCherry	100/50 (Cyto)	200(Cyto)	1262	86/936
Nanog-mCherry	100/50 (Cyto)	50(Cyto)	402	7/308
Nanog-mCherry	100/50 (Cyto)	10(Cyto)	333	1/278
Oct4-GFP	100/50 (Cyto)	200(Cyto)	345	47/254
Nanog-mCherry	5/2.5 (Nuc)	10 (Nuc)	98	7/75
Oct4-GFP	5/2.5 (Nuc)	10 (Nuc)	105	13/72
Two-step injection				
Nanog-mCherry	100/50 (Cyto)	50 (Nuc)	45	0/0
Nanog-mCherry	100/50 (Cyto)	10 (Nuc)	91	1/34
Nanog-mCherry	100/50 (Cyto)	2 (Nuc)	85	1/68

Cas9 mRNA, sgRNAs targeting *Nanog*, or *Oct4*, and double stranded donor vectors were injected into cytoplasm or pronuclei of zygotes. In one-step injection, the RNA and the DNA were simultaneously injected into the cytoplasm or pronucleus. In two-step injection, Cas9/sgRNA were injected into the cytoplasm followed 2 hours later by pronuclear injection of the DNA vector. Targeted blastocysts were identified by fluorescence of reporters.

Cyto, cytoplasm; Nuc, nucleus.

Table S2. Mosaicism in Targeted Mice, Related to Figure S2 and Tables 1 and 2

Donor		Mosaic / Total targeted
Nanog-Cherry	Mice	1/7
	ESCs	2/6
	Total	3/13
Oct4-EGFP	Mice	0/3
	ESCs	1/3
	Total	1/6
Mecp2-L2 + Mecpe-R1	Male	11/28
	Female	9/21
	Total	20/49[a]

Targeted mice or ESCs were identified by RFLP, Southern bolt or Sequencing. The frequency of mosaicism in targeted mice was determined by fluorescent reporter or Southern blot analysis.

[a]These 49 mice contain at least one loxP integration.

Table S3. Off-Target Analysis, Related to Figures 1, 2, 3 and Tables 1 and 2

Site name	Sequence	Indel mutation frequency (Mutant/Total)	Coordinate
Target_Sox2_Stop	TGCCCCTGTCGCACATGTGAGGG	/	chr3: 34550278-34550300
OT1_Sox2_Stop	T**a**CCC**t**TGT**t**GCACATGTGAAGG	0/10	chr4: 126636377-126636399
OT2_Sox2_Stop	T**t**CCC**a**TGT**a**GCACATGTGAGGG	0/10	chr14: 58830941-58830963
OT3_Sox2_Stop	T**c**CCCCTGTC**a**CACATGTG**g**TGG	0/10	chr1: 136641174-136641196
OT4_Sox2_Stop	TGC**a**CCTGT**g**GCACATGTG**g**GGG	0/10	chr9: 69081892-69081914
OT5_Sox2_Stop	TGCC**a**C**a**GT**t**GCACATGTGAGGG	0/10	chr1: 130633965-130633987
OT6_Sox2_Stop	TGCC**a**CTGT**t**GCA**a**ATGTGAGGG	0/10	chr18: 61611640-61611662
OT7_Sox2_Stop	TGCC**t**CTGTC**a**CA**g**ATGTGACGG	0/10	chr5: 136841014-136841036
OT8_Sox2_Stop	TGCC**t**CTGTC**t**CACATGTG**c**TGG	0/10	chr4: 141162434-141162456
OT9_Sox2_Stop	TGCC**t**CTGTCGC**t**CATG**g**GATGG	0/10	chr9: 48224429-48224451
OT10_Sox2_Stop	TGCCC**a**TGTC**c**CACATG**g**GATGG	0/10	chr7: 72596616-72596638
OT11_Sox2_Stop	TGCCCCT**c**T**gt**CACATGTGAAGG	0/10	chr18: 56473819-56473841
OT12_Sox2_Stop	TGCCCCTGTC**at**ACATGTG**g**AGG	0/10	chr6: 98776389-98776411
OT13_Sox2_Stop	TGCCCCTGTC**t**C**c**CATGTG**c**TGG	0/10	chr4: 148868089-148868111
Target_Nanog_Stop[a]	CGTAAGTCTCATATTTCACCTGG	/	chr6: 122663559-122663581
OT1_Nanog_Stop	**t**GTAAGTCTCATATTTCACCTGG	7/9[b]	chrX: 87128718-87128740
OT2_Nanog_Stop	C**cc**AAGTCTCAT**t**TTTCACCAGG	0/9	chr14: 21598653-21598675
OT3_Nanog_Stop	**ga**TAAG**ga**TCATATTTCACCCGG	0/9	chrX:88301349-88301371
OT4_Nanog_Stop	**t**G**c**AA**t**T**t**TCATATTTCACCTGG	0/9	chr12:71841888-71841910
OT5_Nanog_Stop	**g**GT**c**A**tc**CTCATATTTCACCAGG	0/9	chr11:13346951-13346973
OT6_Nanog_Stop	**t**GT**t**A**t**TC**a**CATATTTCACCTGG	0/9	chr6:13888078-13888100
OT7_Nanog_Stop	**t**GT**g**AGT**ag**CATATTTCACCTGG	0/9	chr18:41037112-41037134
OT8_Nanog_Stop	**t**GTAA**a**T**aa**CATATTTCACCCGG	0/9	chrX:70168441-70168463
OT9_Nanog_Stop	C**a**TA**gag**CTCATATTTCACCAGG	0/9	chr4:80388067-80388089
Target_Oct4_Stop	GCTCAGTGATGCTGTTGATCAGG	/	chr17:35647634-35647656
OT1_ Oct4_Stop	G**t**TCAGTGATGCTGTTGATCTGG	4/6[b]	chr3:129137779-129137801
OT2_ Oct4_Stop	G**tc**CAGTGATGCTGTTGATCAGG	0/6	chr14:17938758-17938780
OT3_ Oct4_Stop	**c**CTCA**c**TGA**a**GCTGTTGATCAGG	0/6	chr16:77843697-77843719
OT4_ Oct4_Stop	**a**CTCAGTGAT**t**CTG**c**TGATCTGG	0/6	chr11:52953191-52953213
OT5_ Oct4_Stop	G**gg**CAGTGATGCTGTTGA**c**CAGG	0/6	chr14:119915346-119915368
OT6_ Oct4_Stop	G**g**TCAG**a**GATGCTG**a**TGATCAGG	0/6	chr1:100129231-100129253
OT7_ Oct4_Stop	GCT**t**AGTGATGCTGT**ac**ATCTGG	0/6	chr3:39458512-39458534
OT8_ Oct4_Stop	GCTCA**a**TGAT**a**CT**t**TTGATCTGG	0/6	chr5:16781367-16781389
OT9_ Oct4_Stop	GCTCA**a**TGAT**t**CTGTTGAT**t**GGG	0/6	chr4:35112775-35112797
OT10_ Oct4_Stop	GCTCAG**g**GATGC**a**GTTG**t**TCTGG	0/6	chr4:25918134-25918156
OT11_ Oct4_Stop	GCTCAGTGATGCTGT**ctg**TCTGG	0/6	chr6:138212621-138212643

Target_Mecp2_L2	CCCAAGGATACAGTATCCTAGGG	/	chrX: 71282802-71282824
OT1_Mecp2_L2	CCCAAGGAT**g**C**tt**TATCCTAAGG	0/10	chr8: 121441299-121441321
OT2_Mecp2_L2	CCCA**t**GGATA**g**AGTA**g**CCTAAGG	0/10	chr15: 55526927-55526949
OT3_Mecp2_L2	CCCAAG**a**ATACAGT**g**T**g**CTAAGG	0/10	chr4: 83371755-83371777
OT4_Mecp2_L2	CCCAAGGA**c**ACAG**g**ATCC**c**AAGG	0/10	chr17: 27887352-27887374
Target_Mecp2_R1	AGGAGTGAGGTCTAGTACTTGGG	/	chrX: 71282103-71282125
OT1_Mecp2_R1	AGG**g**GTGAG**t**TCTAGTACTTCGG	0/10	chr13: 48912459-48912481
OT2_Mecp2_R1	**t**GGAGTGAGGTCT**t**GTACTTGGG	1/10[b]	chr12: 15404584-15404606
OT3_Mecp2_R1	AGGAGT**ct**GG**g**CTAGTACTTGGG	0/10	chr6: 115474148-115474170
OT4_Mecp2_R1	A**a**GAGTGAGG**g**CTA**c**TACTTTGG	0/10	chr9: 74128922-74128944
OT5_Mecp2_R1	AGGAGTGAGGT**g**T**t**GT**g**CTTAGG	0/10	chr9: 69065264-69065286
OT6_Mecp2_R1	AGGAGTGAGGTCT**g**G**at**CTTGGG	0/10	chr5: 137155304-137155326
OT7_Mecp2_R1	AGGAGTGAGG**aa**TAGTA**a**TTGGG	0/10	chr5: 53385487-53385509
OT8_Mecp2_R1	AGGAG**g**GAGGTCT**c**GTA**a**TTTGG	0/10	chr10: 94407719-94407741
OT9_Mecp2_R1	AGGAGTG**g**GGTCTAGT**c**CT**c**AGG	0/10	chr1: 38087440-38087462
OT10_Mecp2_R1	AGGAGT**a**AGGTCTAGTAC**ca**AGG	0/10	chr4: 13635404-13635426

Mismatches from the on-target sequence are lower-case, boldface and underlined. Indel mutation frequencies in targeted mice or ESCs were calculated by Suveryor assay. Coordinate in which sites were located are shown. OT, off-target; /, not tested.

[a]Nanog OT1 and 2 contain 3bp mismatches; OT3 to 9 contain 4bp mismatches lying in PAM distal region.

[b]PCR products were cloned and sequenced to confirm off-target mutations.

Table S4. Oligonucleotides Used In This Study

Oligonucleotides used for cloning sgRNA expression vector

Gene target	Direction	Sequence (5’ to 3’)
Tet1	F	caccggctgctgtcagggagctca
	R	aaactgagctccctgacagcagcc
Tet2	F	caccgaaagtgccaacagatatcc
	R	aaacggatatctgttggcactttc
Sox2	F	caccgtgcccctgtcgcacatgtga
	R	aaactcacatgtgcgacaggggcac
Nanog	F	caccgcgtaagtctcatatttcacc
	R	aaacggtgaaatatgagacttacgc
Oct4	F	caccgctcagtgatgctgttgatc
	R	aaacgatcaacagcatcactgagc
Mecp2 L1	F	caccgttgggccccagcttgaccca
	R	aaactgggtcaagctggggcccaac
Mecp2 L2	F	caccgcccaaggatacagtatccta
	R	aaactaggatactgtatccttgggc
Mecp2 R1	F	caccgaggagtgaggtctagtactt
	R	aaacaagtactagacctcactcctc
Mecp2 R2	F	caccgtttggtggtggattaggtct
	R	aaacagacctaatccaccaccaaac

Oligonucleotides used for RFLP analysis and PCR genotyping.

Gene target	Direction	Sequence (5’ to 3’)
Tet1	F	ttgttctctcctctgactgc
	R	tgattgatcaaataggcctgc
Tet2	F	cagatgcttaggccaatcaag
	R	agaagcaacacacatgaagatg
Sox2	SF	acatgatcagcatgtacctcc
	SR	taatttggatgggattggtgg
V5	V5F	acatgggcaagcccatcc
Mecp2 L1,L2	LF	aatgtgccactttaacagcac
	LR	ttctgatgtttctgctttgcc
Mecp2 R1,R2	RF	aagcatgagccactacaacc
	RR	cttgctcagaagccaaaacag

Oligonucleotides used for making template for in vitro transcription

Template	Direction	Sequence (5’ to 3’)
Cas9	F	taatacgactcactatagggagaatggactataaggaccacgac
	R	gcgagctctaggaattcttac
Tet1	F	ttaatacgactcactataggctgctgtcagggagctc

sgRNA	R	aaaagcaccgactcggtgcc
Tet2 sgRNA	F	ttaatacgactcactataggaaagtgccaacagatatcc
	R	aaaagcaccgactcggtgcc
Sox2 sgRNA	F	ttaatacgactcactatagtgcccctgtcgcacatgtga
	R	aaaagcaccgactcggtgcc
Nanog sgRNA	F	ttaatacgactcactatagcgtaagtctcatatttcacc
	R	aaaagcaccgactcggtgcc
Oct4 sgRNA	F	ttaatacgactcactataggctcagtgatgctgttgatc
	R	aaaagcaccgactcggtgcc
Mecp2-L1 sgRNA	F	ttaatacgactcactatagttgggccccagcttgaccca
	R	aaaagcaccgactcggtgcc
Mecp2-L2 sgRNA	F	ttaatacgactcactatagcccaaggatacagtatccta
	R	aaaagcaccgactcggtgcc
Mecp2-R1 sgRNA	F	ttaatacgactcactatagaggagtgaggtctagtactt
	R	aaaagcaccgactcggtgcc
Mecp2-R2 sgRNA	F	ttaatacgactcactatagtttggtggtggattaggtct
	R	aaaagcaccgactcggtgcc

Oligonucleotides used for HDR-mediated repair through embryo injection

Gene target	**Sequence (5’ to 3’)**
Tet1-loxP	gaaaaaggcccatattatacacaccttggggcaggaccaagtgtggctgctgtcaggg agGAATTCataacttcgtataatgtatgctatacgaagttatctcatggagactaggt gaggaactctgcttcccgctaacccattcttcccggtgacctgg
Tet2-loxP	ctctgtgactataaggctctgactctcaagtcacagaaacacgtgaaagtgccaacag atGAATTCataacttcgtataatgtatgctatacgaagttatatccaggctgcagaat cggagaaccacgcccgagctgcagagcctcaagcaaccaaaagc
Sox2-v5	taccagagcggcccggtgcccggcacggccattaacggcacactgcccctgtcgcaca tgGGCAAGCCCATCCCCAACCCCCTGCTGGGCCTGGACAGCACCtgagggctggactg cgaactggagaaggggagagatttcaaagagatacaagggaattg
Mecp2-L1 -loxP	tgtttgaccaatatcaccagcaacctaaagctgttaagaaatctttgggccccagctt gaGCTAGCataacttcgtataatgtatgctatacgaagttatcccaaggatacagtat cctagggaagttaccaaaatcagagatagtatgcagcagccagg
Mecp2-L2 -loxP	ccagcaacctaaagctgttaagaaatctttgggccccagcttgacccaaggatacagt atGCTAGCataacttcgtataatgtatgctatacgaagttatcctagggaagttacca aaatcagagatagtatgcagcagccaggggtctcatgtgtggca
Mecp2-R1 -loxP	ccactcctctgtactccctggcttttccacaatccttaaactgaaggagtgaggtcta gtataacttcgtatagcatacattatacgaagttatGAATTCacttgggggtcattgg gctagactgaatatctttggttggtacccagacctaatccacca
Mecp2-R2 -loxP	ccaaaaaggctggacaccatgccttggttaaaatggaggaatgttttggtggtggatt agGAATTCataacttcgtataatgtatgctatacgaagttatgtctgggtaccaacca aagatattcagtctagcccaatgacccccaagtactagacctca

Oligonucleotides used for off-targeted analysis

Gene target	Direction	Sequence (5’ to 3’)
OT1_Sox2_Stop	F	atgacatgacctaagtaaaccc
	R	ctccactctgtactaggcac
OT2_Sox2_Stop	F	tgatggtttttggtgactgcc
	R	gacagatcatagatagaaaattg
OT3_Sox2_Stop	F	aaactgaggcacagagtctg
	R	gtgacgaagccactttgacc
OT4_Sox2_Stop	F	caccttaggttcatggcattc
	R	gatggatcagtgattaagagc
OT5_Sox2_Stop	F	accatgatggactgtaccatc
	R	catggacgtcattactagatg
OT6_Sox2_Stop	F	ttcctcgaagatgaaatgatt
	R	cagtgtgcagactctgagag
OT7_Sox2_Stop	F	atgtgccacacaaggcaggc
	R	gcaaaacctctgaaagttgac
OT8_Sox2_Stop	F	ttcctgtcctggcttccttc
	R	gcactagttgtcacgtgatg
OT9_Sox2_Stop	F	gactcagatttccaagccatg
	R	acatctctgagctctaagcc
OT10_Sox2_Stop	F	tgccatgtgctgtgttcacc
	R	ttgatatttaagacagggtctc
OT11_Sox2_Stop	F	gtaaggaatgtaagaactcttg
	R	aattctcaactgaggaatactg
OT12_Sox2_Stop	F	tctcagacagaaacgctgtg
	R	gacttgatatgccaggatgag
OT13_Sox2_Stop	F	agctgacagaagacgatgag
	R	taaacccaagcaaaggtcatg
OT1_Nanog_Stop	F	gctggtgagatggctcagtg
	R	gtcttaacctgcttatagcaac
OT2_Nanog_Stop	F	agatcccattacggatggttg
	R	ggacactcaccaatgtttgg
OT3_Nanog_Stop	F	tagattatctagtgtgttccac
	R	agtttcagtgctcagagcac
OT4_Nanog_Stop	F	gacactttctaagtgggcttg
	R	gttaagggacagtgaatatcc
OT5_Nanog_Stop	F	tcccatctaccctctgactg
	R	gcctgaagaaaagaaggtcc
OT6_Nanog_Stop	F	tctgaggtgagcaaagcatg
	R	aatccaccatgtcttccgtg
OT7_Nanog_Stop	F	caattttctcagtgaggtagg

	R	cttgttcagtgcattgctgc
OT8_Nanog_Stop	F	tctcttcagaaaagagtaggc
	R	gttggcaactgcactctgtg
OT9_Nanog_Stop	F	agctcatgcatgctgagctg
	R	aacttcaagtggaactgcttg
OT1_Mecp2_Left2	F	cacacacacactgaataaaatg
	R	aagctggctttgagcaggac
OT2_Mecp2_Left2	F	tagtcacttatgtttactcctc
	R	gtgatgccagcagttggcag
OT3_Mecp2_Left2	F	tcactttccctcagtactcc
	R	caagtatcattctctgaacaag
OT4_Mecp2_Left2	F	gaactttgagacagggtctc
	R	gacagagcagcttggccttc
OT1_Mecp2_Right1	F	gcagcaccagtggaatattac
	R	gcctattgatgaatctgccc
OT2_Mecp2_Right1	F	acagatgcagccactcacag
	R	gtccaagtcacttctcccac
OT3_Mecp2_Right1	F	tccgacaatggtttatgtctg
	R	agatactagcagtgcagctg
OT4_Mecp2_Right1	F	gttcctgctggttttgtttcg
	R	tagaccaatctacaaccacag
OT5_Mecp2_Right1	F	tgctgtgaaactcaggcatg
	R	cttctaagacaagccagaaag
OT6_Mecp2_Right1	F	cggcataaacctcccattag
	R	ctctgtgcttgtaaggcaaac
OT7_Mecp2_Right1	F	gccagacaataattcccaag
	R	ctgatattgctactgctaacc
OT8_Mecp2_Right1	F	ccattgtgaaagtgggatgc
	R	ggctgctctcgtaaacaaaac
OT9_Mecp2_Right1	F	gtcactctcatgtgcaggtg
	R	ctagcacttgggaagcaaatg
OT10_Mecp2_Right1	F	ctaatcacacttctacaagctg
	R	agagaggctccaattgttag
OT1_Oct4_Stop	F	accacactactcgatacctg
	R	gggtaatgcgctgagtggac
OT2_Oct4_Stop	F	atgatgtgaactaaggcaagg
	R	ccaagtaatacacctgcaatg
OT3_Oct4_Stop	F	gatctttccatcttctgagatc
	R	aatcagggactagacaaggc
OT4_Oct4_Stop	F	tggcaatgccagacactaag
	R	ttggatgctgcctcactgtc
OT5_Oct4_Stop	F	tatccgtggtatcataggttg

	R	tgtttgccaacatgctaaacg
OT6_Oct4_Stop	F	acaagtggattactaagggtg
	R	agtaggcttcttaccgatttc
OT7_Oct4_Stop	F	accatctgatgattgagtgag
	R	atttcaactacaaacttaatggc
OT8_Oct4_Stop	F	ttggcggagctagccttgag
	R	cctccctgtgcactaggaac
OT9_Oct4_Stop	F	ctttgtgttccattgtcaagc
	R	cagttcatccagtcttcttag
OT10_Oct4_Stop	F	aatccaacttgaccatttaagc
	R	caccttgccagctgtagac
OT11_Oct4_Stop	F	cactgcatgacatatagagag
	R	catggacttatgcacaaaagg