Supplementary information for

Multiple sgRNAs with overlapping sequences enhance CRISPR/Cas9-mediated knock-in efficiency

Authors

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This file includes

1. Figure S1~S2: Schematic for experiment, sequence information of ssODN and sgRNAs, and result for genotyping.
2. Table S1~S2: ssODN and primer sequences used in this study

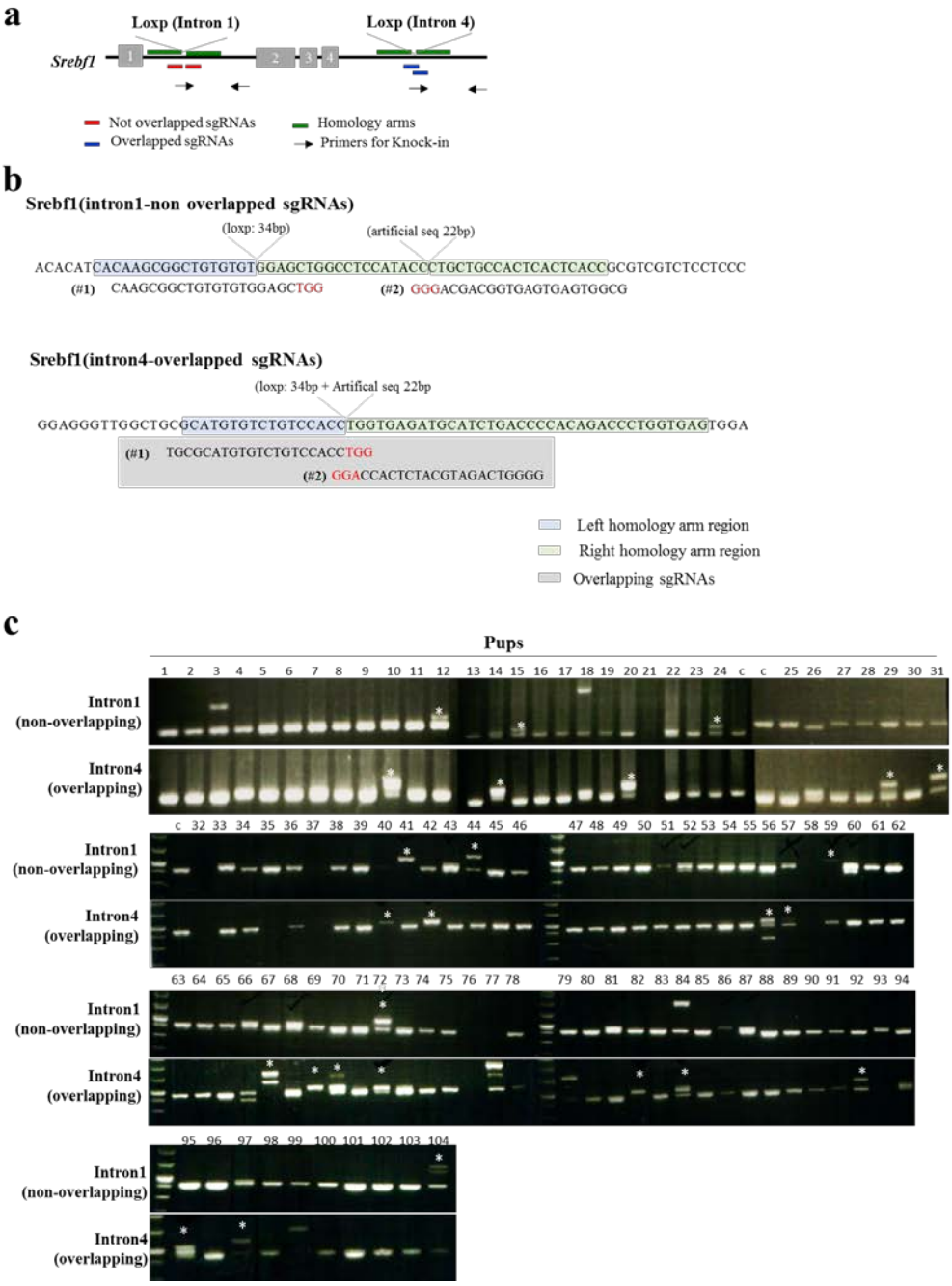

Figure S1. loxP sequence KI in zygotes with non-overlapping or overlapping sgRNAs into intron 1 or 4, respectively of the mouse *srebf1* locus. (a) A schematic diagram of the strategy for KI loxP sequence with ssODN consisting of homology arms and loxP into either intron 1 or intron 4 with non-overlapping or overlapping sgRNAs respectively to the mouse *Srebf1* locus. Non-overlapping sgRNAs targeting intron 1 were indicated as red bars, overlapping sgRNAs targeting intron 4 were indicated as blue bars and the position of primer sets for KI analysis (black arrows) were shown. **(b)** Non-overlapping and overlapping sgRNA sequences targeting intron 1 or 4 and their PAM (red letters). **(c)** Genotyping results of pups microinjected with non-overlapping or overlapping sgRNAs targeting intron 1 or 4 respectively of mouse *Srebf1* locus, Cas9-mRNA and ssODN by PCR with specific primers (see Table 1). * indicates KI.

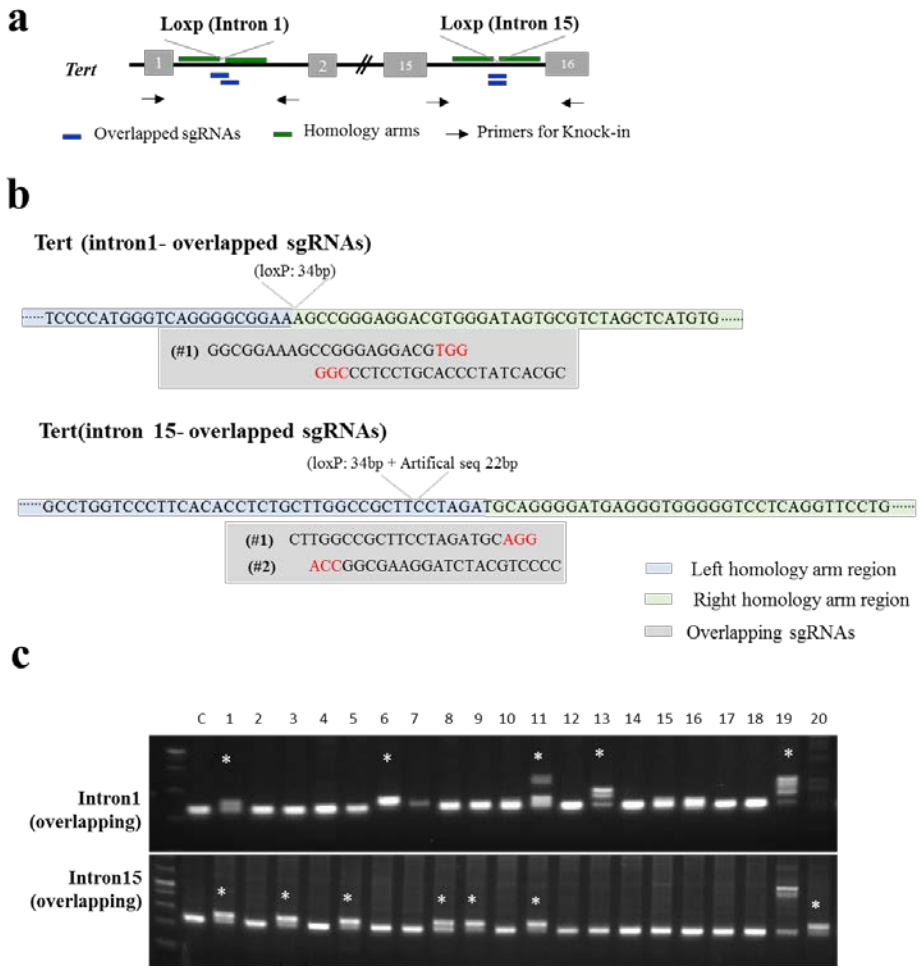

Figure S2. loxP sequence KI in zygotes with overlapping sgRNAs into intron 1 and 15 of the mouse *tert* locus. (a) A schematic diagram of the strategy for KI loxP sequence with ssODN consisting of homology arms and loxP into either intron 1 or intron 15 with overlapping sgRNAs at the mouse *Tert* locus. Overlapping sgRNAs targeting intron 1 and 15 of the mouse *tert* locus were indicated as blue bars and the position of primer sets for KI analysis (black arrows) were shown. **(b)** Overlapping sgRNA sequences targeting intron 1 or 15 and their PAM (red letters). **(c)** Genotyping results of pups microinjected with overlapping sgRNAs targeting intron 1 or 15 of the mouse *Tert* locus, Cas9-mRNA and ssODN by PCR with specific primers (see Table 1). * indicates KI.

Table S1. ssODN used in this study.

Primer name	ssODN	PCR size (bp)
	GGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTCTTCCCTCGT	homology arm (45 nt)
	ATAACTTCGTATAATGTATGCTATACGAAGTTAT	loxp (34 nt)
Rosa26	TTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCAT	Multiple cloning site (42 nt)
	ATAACTTCGTATAGGATACTTTATACGAAGTTAT	loxp (34 nt)
	GATCTGCAACTCCAGTCTTTCTAGAAGATGGGCGGGAGTCTTCTG	homology arm (45 nt),
	GCTACTTCTAGGCTCCTGAAGAACCCGGTGGCTAAGTGTTGGGTGGA	homology arm (48 nt)
	TGCTGACTTAGTGCTCTATAGCTGAAG	homology arm with mutation in PAM sequence
Upf1 (intron 1)	ATAACTTCGTATAGCATACATTATACGAAGTTAT	loxP (34 nt)
	TTCCTGGGGTGATGATTAAGCTGTGCATACTCAGGCTAGTCAGG	homology arm with mutation in PAM sequence
	TCTCTGTGGTGGGACTTCAGTGCAGACATCCTATGCTGTGACTTTGAG	homology arm (48 nt) with 3’ end
	AGCGGCTGTGTGT	homology arm (13 nt)
	ATAACTTCGTATAATGTATGCTATACGAAGTTAT	loxP (34 nt)
Srebf1 (intron 1)	GGAGCTGGCCTCCATACC	homology arm (18 nt)
	CGTCGCCGTCCAGCTCGACCAG	artificial sequence to screen (22nt)
	CTGCTGCCACTCACTCACC	homology arm (19nt)
	GGTTGGCTGCGCATGTGTCTGTCCACC	homology arm (27 nt)
Srebf1 (intron 4)	ATAACTTCGTATAATGTATGCTATACGAAGTTATTGGCACGAACTCCAGCAGGACCATG	loxP and artificial sequence (59 nt)
	TGAGATGCATCTGACCCCACAGACCCTGGTGAG	homology arm (33 nt)
	GGGGCTCACAGCCTCCACCTGCCGACCTTTCCTTCCACCAGGTGGGCCTCCAGGCGGGATCCCCATGGGTCAGGGGCGGAA	homology arm (80 nt)
Tert (intron 1)	ATAACTTCGTATAATGTATGCTATACGAAGTTAT	loxP (34 nt)
	AGCCGGGAGGACGTGGGATAGTGCGTCTAGCTCATGTGTCAAGACCCTCTTCTCCTTACCAGGTGTCATCCCTGAAAGA	homology arm (79 nt)

Tert (intron 15)	AGCCGGGAGGACGTGGGATAGTGCGTCTAGCTCATGTGTCAAGACCCTCTTCTCCTTACCAGGTGTCATCCCTGAAAGA	homology arm (79 nt)
	TAACTTCGTATAGCATACATTATACGAAGTTATA	loxP sequence (34 t)
	TGCAGGGGATGAGGGTGGGGGTCCTCAGGTTCCTGTCTTGCTTCTGTTTCCAGATTGTCAAGCTTGAGGTCTTTGTGTG	homology arm (79 nt)
Morc2a	TGAGAAAGTAATGCTATTTTTTTTCCCTCTTGTTAACCATTTGAGGTGAT	homology arm (50 nt)
	GCTGCTAGTGTGATACAATTTGGGAAAT(**T**)AGCGAAGAGGACGCCTGAATCGACTCAAATTGGGCA	insert with silent mutation (65 nt)
	GTATGGCAATGGATTAAAATCGTACGTATATTGGAAGCCTAGCATAGGGC	homology arm (50 nt)
Adora2b (overlapping)	CTCTTCCATCCAGCCCTGGCCAAGGACAAGCCCAAGTGGGTGATGAATGTGGCCATCCTCCTGTC	homology arm (65 nt)
	ACATGCCAATTCTGTGGTCAACCCGATCGTATATGCGTACCGTAACCGTGGCTTCC	insert with silent mutation (56 nt)
	GCTACAGTTTCCACAAGATCATCTCCAGATATGTTCTCTGCCAGGCCGAGACCAAGGGTGGGAGT	homology arm (65 nt)
Adora2b (non-overlapping)	AATGTGGCCATCCTCCTGTCACATGCCAATTCAGTTGTCAACCCCATTGTCTATGCCTACAGGAA	homology arm (65 nt)
	CCGAGGCTTCCGCTACTCCTTCCACAAAATAATAAGTCGGTATGTGTTATGCCAAGCCGAAACCAAAGGT	insert with silent mutation (70 nt)
	GGGAGTGGGCAGGCTGGGGCGCAGTCTACTCTCAGTCTGGGCTTATGACCTAGGCTCTGGCCTTT	homology arm (65 nt)

Table S2. Primers used for HDR analysis.

Primer name	Forward	Reverse	PCR size (bp)
Rosa26	CTCTCCCAAAGTCGCTCT	ACTACCTATCCTCCCATTTTCC	495
Upf1 (intron 1)	TTGGGGGGATGCTGACTT	AGGTTGTGCCTTATGGTGAG	237
Upf1-deep seq	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGTTGTGCCTTATGGTGAG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGAGGTGTCTGCTCCTATC	250
Upf1 (intron 2)	CACACACACACCCAGCAA	GGTGGAAAGGACGACTAGG	272
Morc2a	TGTGTTTGCCAGGTCTTGT	GGCGTCCTCTTCGCTAAT	393
Morc2a-deep seq	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGTGTACAGCCATGCTTG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCAAGAATCCACCATCTG	253
Srebf1 (intron 1)	GTGTGCTATGCTGGGGATGT	GCTCTCAGGAGAGTTGGCAC	355
Srebf1 (intron 4)	CAGACTCACTGCTGCTGACA	ACCGGTAGCGCTTCTCAATG	368
Tert (intron 1)	GCCCAATGCCTAGTGTGC	GCACGTTTCTCTCGTTGCG	272
Tert (intron 15)	CACTCAAGATCCCTGCCTGG	CCTTAGCCTGCTTGGGTCAA	269
Adora2b	AGGACAAGCCCAAGTGGGTG	CCTAGGTCATAAGCCCAGAC	212