## Supplemental Information

Rhesus iPSC Safe Harbor Gene-Editing Platform

for Stable Expression of Transgenes

**in Differentiated Cells of All Germ Layers**

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SUPPLEMENTARY MATERIAL
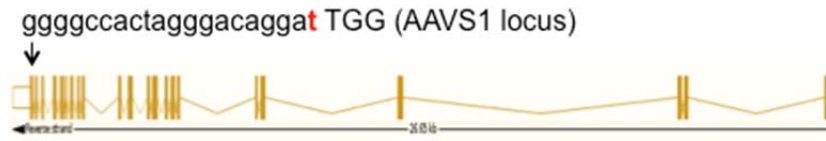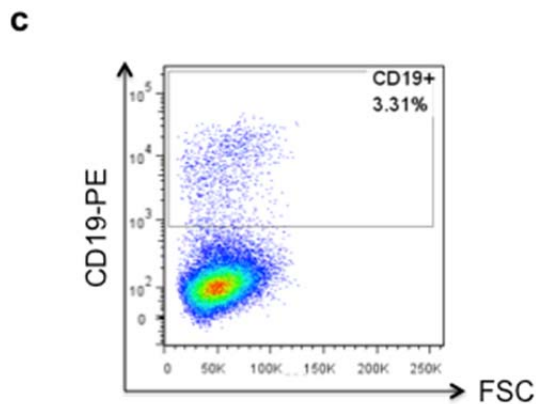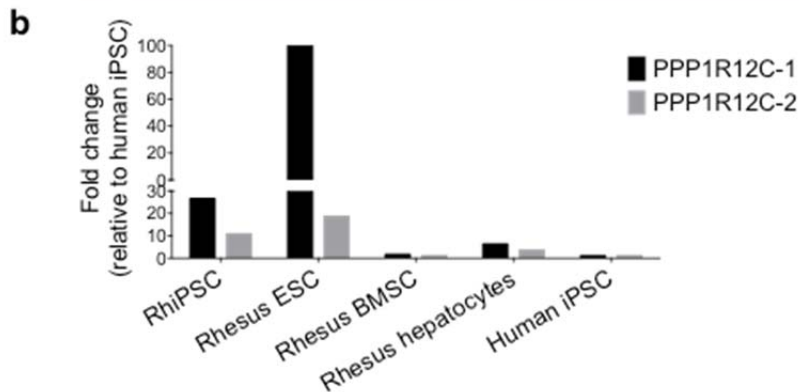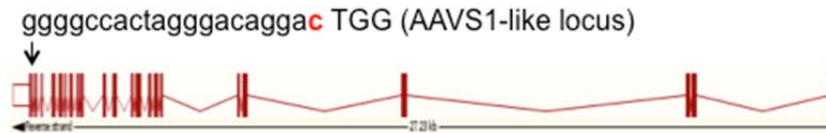
Figure S1 CRISPR/Cas9-mediated targeting of rhesus macaque AAVS1
(**a**) Comparison of structure of the *PPP1R12C* gene and the AAVS1 site in the human and rhesus macaque genome. Small letters represent gRNA target sequences while capital letters indicate PAM sequences. Note that the last base pair of the gRNA target sequence is different between human and rhesus. (**b**) The rhesus *PPP1R12C* gene is expressed in various rhesus macaque cell types, including induced pluripotent stem cells (iPSC), embryonic stem cells (ESC), bone-marrow stromal cells (BMSC), and primary hepatocytes. Two different primer sets (PPP1R12C-1 and -2) were used to confirm the pattern of gene expression. (**c**) CD19 expression in 293T cells after transfection of the hΔCD19 donor plasmid

Figure S2 On-target mutations in non-targeted alleles
The AAVS1 locus in a non-targeted allele of CRISPR/Cas9-edited clones was sequenced. Parental RhiPSC clones were highlighted in bold. The clone with intact non-targeted allele without any mutation is highlighted in red. The boxes indicate the 20 bp gRNA-targeting sequence and the 3 bp protospacer-associated motif or PAM (Chromosome 19:61115591-61115613). Red highlight indicates mismatches compared to the rhesus macaque reference sequence.

a M11-10-GFP-21   **b** M11-10-hΔCD19-7
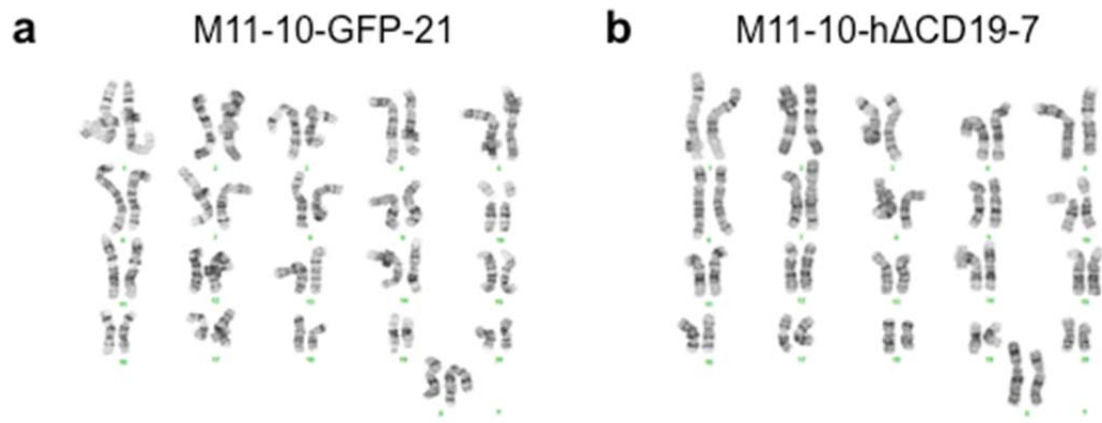
Figure S3 CRISPR/Cas9-edited clones maintained normal karyotype, representative examples are shown.

a

Animal ID	RhiPSC clone	Predicted off-target site									
		1	2	3	4	5	6	7	8	9	10
ZG15	ZG15-M11-10-CD19-7										
	ZG15-M11-10-CD19-22								-19		
	ZG15-M11-10-GFP-5					*			+1		
	ZG15-M11-10-GFP-11										
	ZG15-M11-10-GFP-20										
	ZG15-M11-10-GFP-21										
ZG32	ZG32-3-4-CD19-11					-48			-5		
	ZG32-3-4-GFP-10								+212		
ZH26	ZH26-HS41-CD19-3					-9			**		
	ZH26-HS41-CD19-7										

*-38, -162; **-5, -13, -23

b

Figure S4 Off-target analysis of CRISPR/Cas9 system
(a) Summary of off-target analysis in six RhiPSC clones. The top 10 potential off-target sites were sequenced. Red: Insertions/deletions (indels) were found at the off-target site with the respective size of indel(s) denoted. White: No mutation was found at the off-target site. **(b)** After off-target PCR, TOPO cloning was performed to identify exact sequence of the indels. Representative sequencing results of off-target sites five and eight are from clones ZH26-HS41-CD19-3 and ZG15-M11-10-CD19-22, respectively. The boxes indicate the 20 bp off-target sequence and the 3 bp PAM sequence.
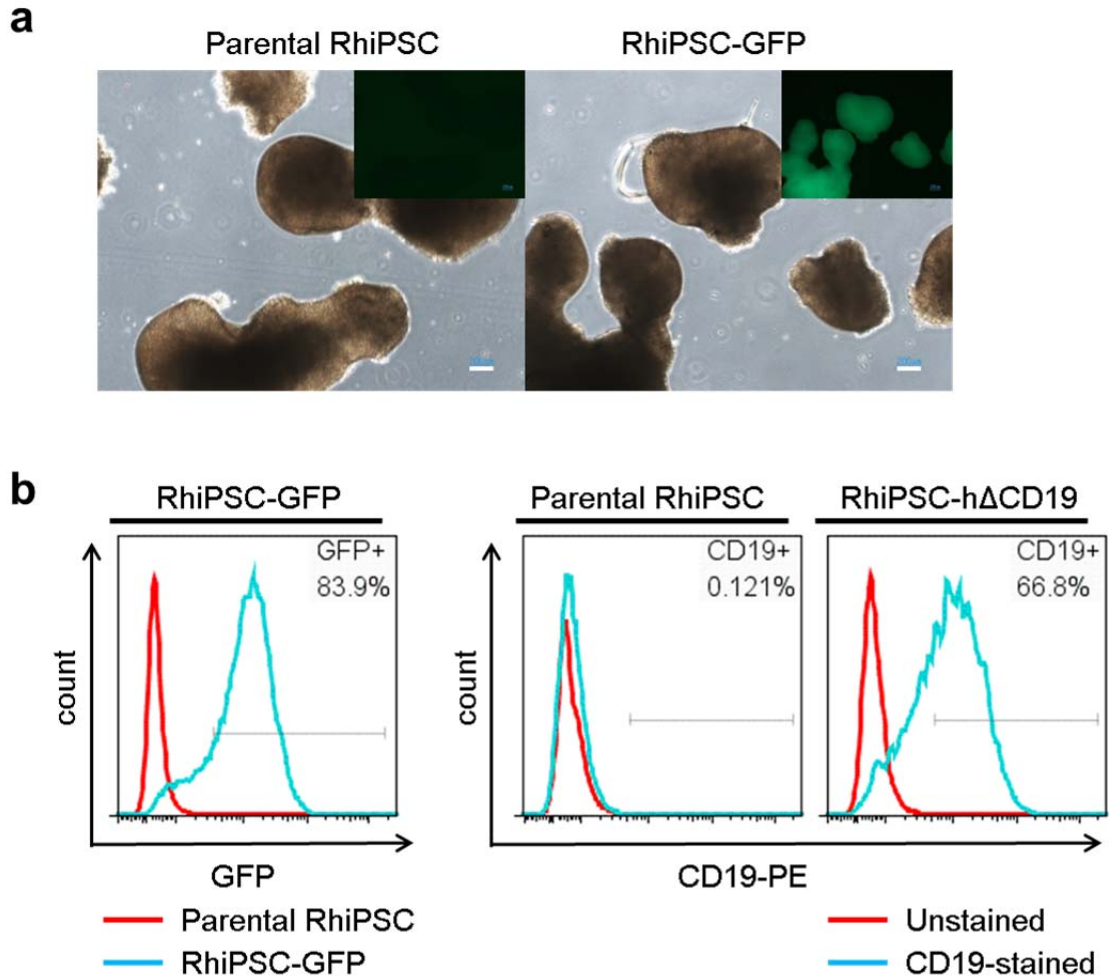
Figure S5 Stable transgene expression in CRISPR/Cas9-edited clones after *in vitro* spontaneous differentiation

(a) Representative images of embryoid bodies (EBs) derived from parental RhiPSC (left) and edited clones (right) on 15 days of differentiation. Scale bars, 200 μm **(b)** Flow analysis confirmed stable transgene expression in EBs from both RhiPSC-GFP (left) and RhiPSC-hΔCD19 (right) clones.
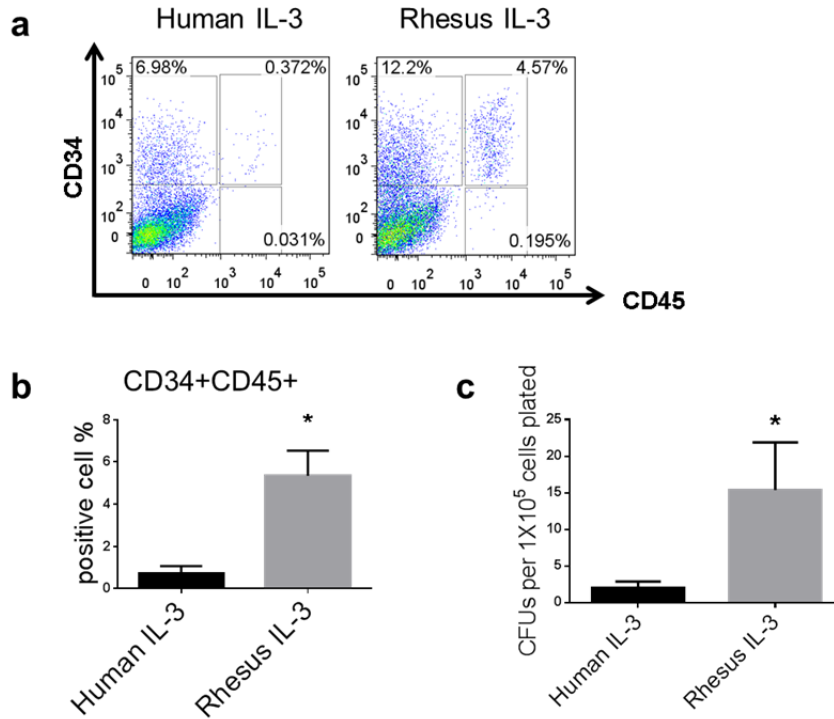
Figure S6 Effect of rhesus IL-3 versus human IL-3 on RhiPSC hematopoietic differentiation
(a) CD34+CD45+ cells were detected at the end of differentiation by flow cytometry. Representative flow cytometry plot comparing hematopoietic differentiation from RhiPSC with human versus rhesus IL-3 at a concentration of 10 ng/ml is shown. **(b)** A summary graph of flow cytometry analysis from 3 independent experiments. **(c)** Number of CFU colonies from CD34+CD45+ cells differentiated using either human or rhesus IL-3. In all bar graphs, the final result given for a sample represents the mean and standard error of the mean. Student's t-tests, *P < 0.05, n=3
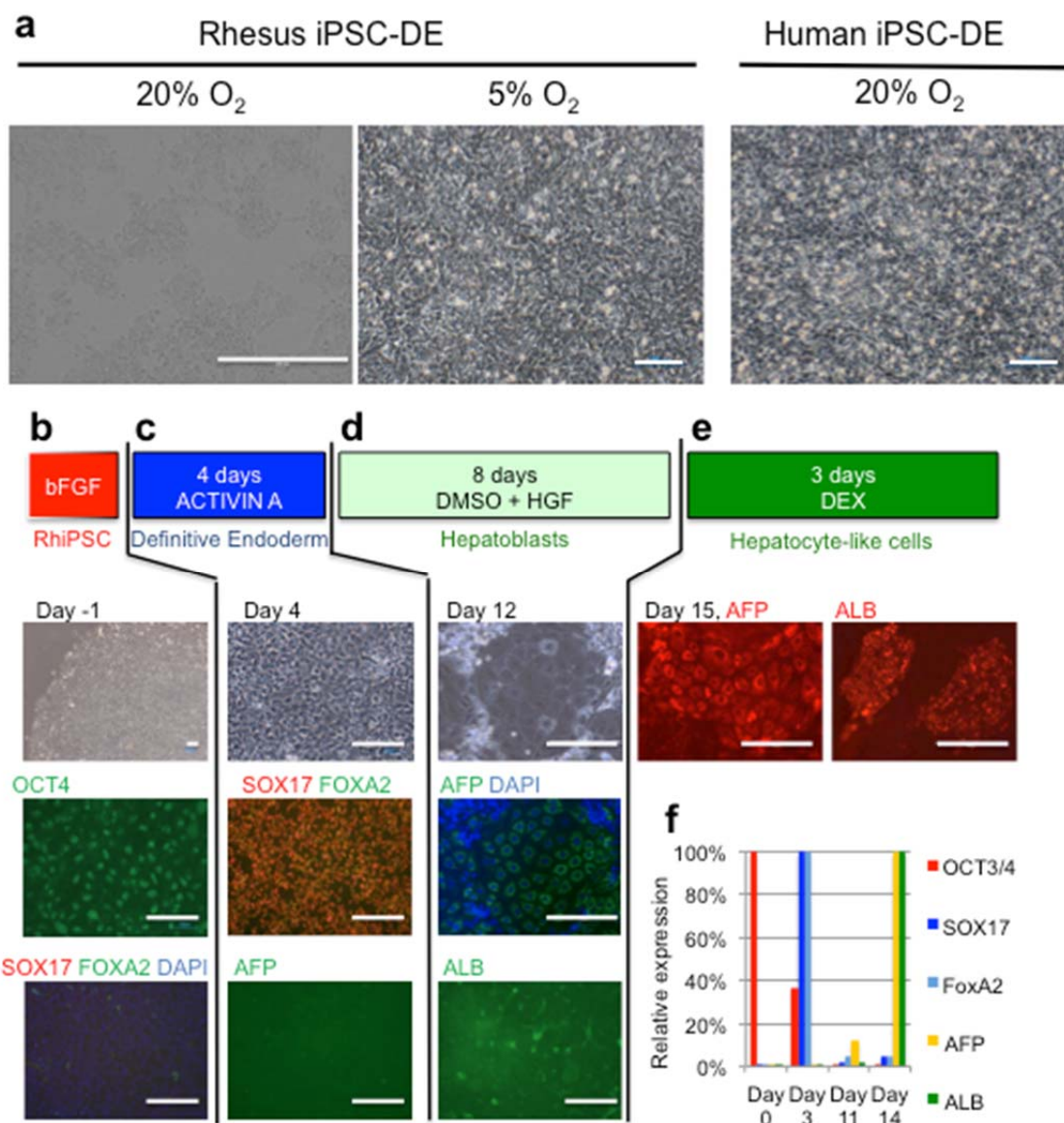
Figure S7 Establishment of hepatic differentiation protocol for RhiPSC
(a) 5% O_2 hypoxic culture was required for definitive endoderm (DE) induction from RhiPSC **(b)** Undifferentiated RhiPSCs before hepatic differentiation **(c)** After 4 days, RhiPSC-DE cells expressed SOX17 and FOXA2, but not AFP. **(d)** By Day 12, AFP+ hepatoblasts were derived. **(e)** After dexamethasone treatment, ALB+ hepatocyte-like cells were observed. **(f)** RT-PCR analysis of relevant transcripts during differentiation. Scale bars, 200 μm.
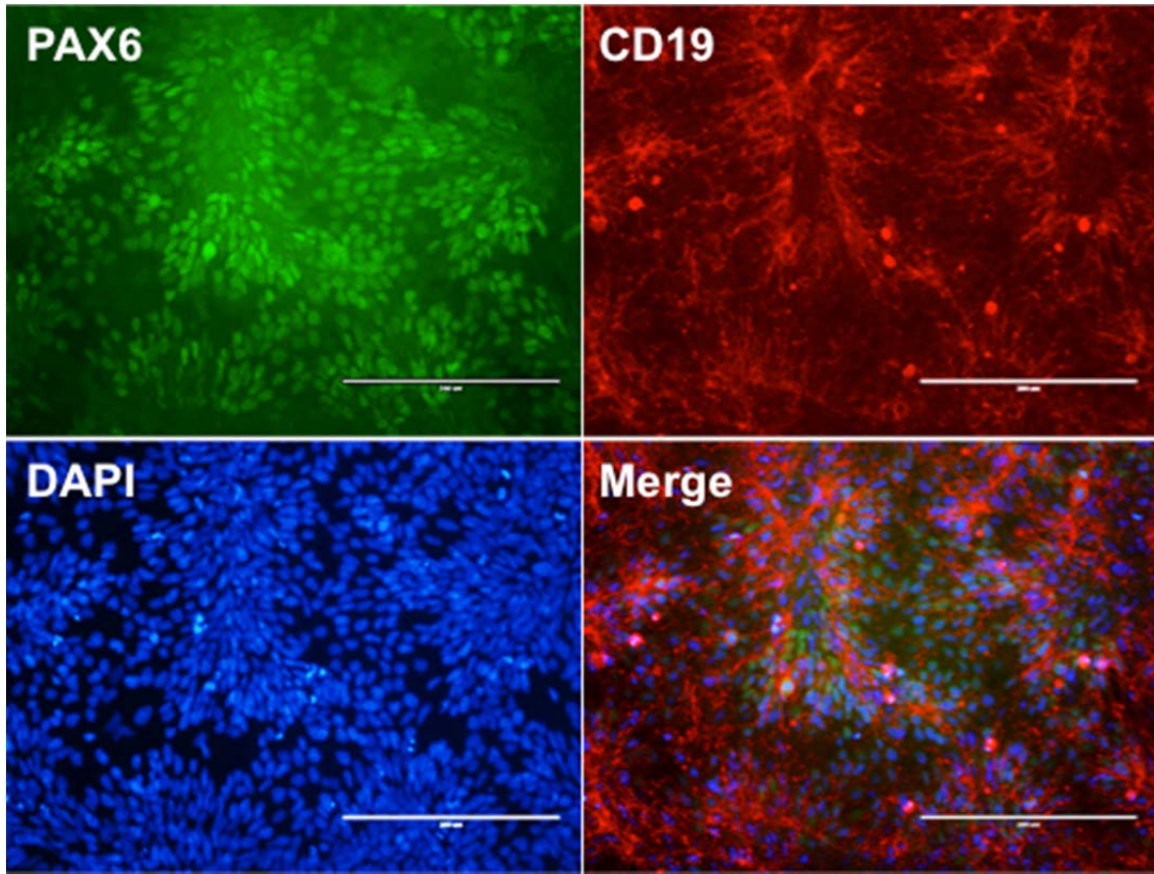
Figure S8 Ectodermal differentiation from edited RhiPSCs
RhiPSC-hΔCD19-7-derived neural stem cells co-expressed early neural marker, Pax6 and the transgene (hΔCD19). Scale bars, 200 μm.

Table S1 Top 10 potential off-target sites

	Off-target Sequence	PAM	Chr	Position	Strand	MM Locations	# of MMs	Score	Overlapping Gene[a]
1	AGGGCCACTT GGGACAGGAC	TAG	4	77699284	-	1,10	2	7.414830508	None
2	GGCCCCACTA GGGACAGGAC	AAG	3	160355267	+	3,4	2	5.146703297	CADPS2[b]
3	GGGGCCAGTG GGGACAGGAC	AGG	13	127282774	-	8,10	2	5.028448276	None
4	GGCCCCATTA GGGACAGGAC	CAG	1	18536016	+	3,4,8	3	2.448888889	None
5	GGGACCATGA GGGACAGGAC	TGG	19	19145046	-	4,8,9	3	1.51751634	ENSMMUG0 0000003164[b]
6	GGGGGCAGCA GGGACAGGAC	TGG	17	93563191	-	5,8,9	3	1.482630907	None
7	GGAGCTAATA GGGACAGGAC	TGG	14	30064062	-	3,6,8	3	1.481577778	None
8	GGGAGCACTA AGGACAGGAC	CGG	7	46978018	-	4,5,11	3	1.446502058	ITGA11[b]
9	GGGAGCAGTG GGGACAGGAC	TGG	11	3181345	-	4,5,8,10	4	1.257112069	None
10	GGGGCCAGTG GGGACAGGAA	GGG	12	95806523	-	8,10,20	3	1.141953521	None

MM stands for Mismatch.

[a]Gene information was found using BLAT online analysis (www.ensembl.org).

[b]Off-target sequence is located in the intron of the corresponding gene.

Table S2 List of primers and probes used in this study

Assay	Gene/Target	Forward sequence (5'-3')	Reverse sequence (5'-3')
Gibson Assembly	Rhesus AAVS1 5' homology arm	tatgaccatgattacgccgccacctccttcaggttccagcttcct	tatgctatacgaagttatgcctgtccctagtggccccacggtggg
	Rhesus AAVS1 3' homology arm	agtcagtgagaatattgtttgactggtgacaaaaagccccatcct	taaaacgacggccagtgtttcggagcagggccttagggaagaggg
	hΔCD19	tcattttggcaaagaattgtatgccacctcctcgcctcctcttct	gcacctgaggagtgaattcattagaatctcctggtggggtcagtc
PCR screening for targeted integration		tcctgctttcactgacctgc	ggcttgtactcggtcatcctag
PPP1R12C expression	PPP1R12C-1 (primer #1)	aggtggtgcgcttcttggtg	tgttgacggcggcgatgttg
	PPP1R12C-2 (primer #2)	tggtgcgcttcttggtggag	tgttgacggcggcgatgttg
T7E1 assay	RhAAVS1	tgctttctttgcctggacac	tgatgcacagaggaacagtac
Off-target analysis	OT-1	agagagtgtgtaggtgggtatt	gcctcttggaatggtgctataa
	OT-2	aaagttcaaagtggagacagga	aagagcaagcagggtgttta
	OT-3	ccaggttagaggaggcataaac	ggaactgaagagatgcccaa
	OT-4	tttgaaccgtgctcctagc	gctggacttcaggcctattt
	OT-5	gctcatcttgagagagagaagaaa	gctgtagcgggttatttgaatg
	OT-6	atgtcacaagcgtgcctt	atccatgaagcctccaagatg
	OT-7	ttctggtggttacacttcattca	agggctctttgacagatcattac
	OT-8	ggttactgttcttagccactgt	gactcagggaatgcgtttct
	OT-9	tgtgaggtactgtgcctgga	gaaaggccattgcattagga
	OT-10	tccacctctcaggttcaagc	acccaaggaacctttgcttt

OT: predicted potential off-target locus