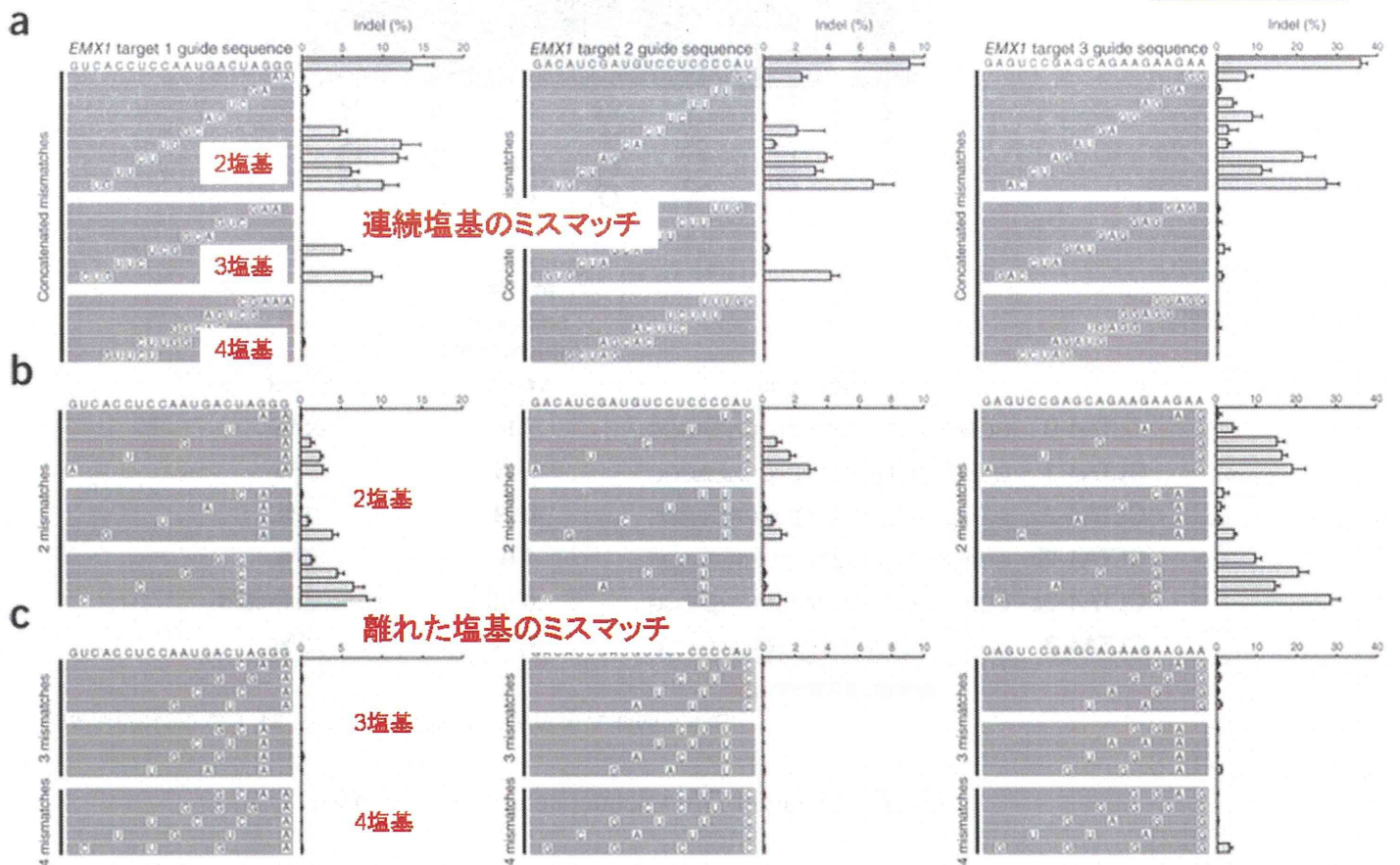


On- and off-target mutations induced by RGNs designed to endogenous human genes

Target	Site name	Sequence	Indel mutation frequency (%) ± s.e.m.			Gene	
			U2OS.EGFP	HEK293	K562		
Target 1 (VEGFA site 1) GC含量70%	T1	GGGTGGGGGAGTTTGCTCCTGG	26.0 ± 2.9	<u>10.5</u> ± 0.07	3.33 ± 0.42	VEGFA	on-target
	OT1-3	GGATGGAGGGAGTTTGCTCCTGG	25.7 ± 9.1	<u>18.9</u> ± 0.77	2.93 ± 0.04	IGDCC3	off-target
	OT1-4	GGGAGGGTGAGTTTGCTCCTGG	9.2 ± 0.8	8.32 ± 0.51	N.D.	LOC116437	
	OT1-6	CGGGGGAGGGAGTTTGCTCCTGG	5.3 ± 0.2	3.67 ± 0.09	N.D.	CACNA2D	
	OT1-11	GGGGAGGGGAAGTTTGCTCCTGG	17.1 ± 4.7	8.54 ± 0.16	N.D.		
Target 2 (VEGFA site 2) GC含量80%	T2	GACCCCTCCACCCCGCCTCCGG	50.2 ± 4.9	<u>38.6</u> ± 1.92	15.0 ± 0.25	VEGFA	on-target
	OT2-1	GACCCCTCCACCCCGCCTCCGG	14.4 ± 3.4	33.6 ± 1.17	4.10 ± 0.05	FMN1	off-target
	OT2-2	GGGCCCTCCACCCCGCCTCTGG	20.0 ± 6.2	15.6 ± 0.30	3.00 ± 0.06	PAX6	
	OT2-6	CTACCCCTCCACCCCGCCTCCGG	8.2 ± 1.4	15.0 ± 0.64	5.24 ± 0.22	PAPD7	
	OT2-9	GCCCCACCCACCCCGCCTCTGG	50.7 ± 5.6	<u>30.7</u> ± 1.44	7.05 ± 0.48	LAMA3	
	OT2-15	TACCCCTCCACCCCGCCTCTGG	9.7 ± 4.5	6.97 ± 0.10	1.34 ± 0.15	SPNS3	
	OT2-17	ACACCCCTCCACCCCGCCTCAGG	14.0 ± 2.8	12.3 ± 0.45	1.80 ± 0.03		
	OT2-19	ATTCCCTCCACCCCGCCTCAGG	17.0 ± 3.3	19.4 ± 1.35	N.D.	HDLBP	
	OT2-20	CCCCACCCACCCCGCCTCAGG	6.1 ± 1.3	N.D.	N.D.	ABLIM1	
	OT2-23	CGCCTCCACCCCGCCTCCGG	44.4 ± 6.7	<u>28.7</u> ± 1.15	4.18 ± 0.37	CALY	
	OT2-24	CTCCCCACCCACCCCGCCTCAGG	62.8 ± 5.0	<u>29.8</u> ± 1.08	21.1 ± 1.68		
	OT2-29	TGCCCTCCACCCCGCCTCTGG	13.8 ± 5.2	N.D.	N.D.	ACLY	
	OT2-34	AGGCCCTCCACCCCGCCTCAGG	2.8 ± 1.5	N.D.	N.D.		
	Target 3 (VEGFA site 3) GC含量60%	T3	GGTGAGTGAGTGTGTGCGTGTGG	49.4 ± 3.8	<u>35.7</u> ± 1.26	27.9 ± 0.52	
OT3-1		GGTGAGTGAGTGTGTGTGAGG	7.4 ± 3.4	8.97 ± 0.80	N.D.	(abParts)	off-target
OT3-2		AGTGAGTGAGTGTGTGTGGGG	24.3 ± 9.2	<u>23.9</u> ± 0.08	8.9 ± 0.16	MAX	
OT3-4		GCTGAGTGAGTGTATGCGTGTGG	20.9 ± 11.8	<u>11.2</u> ± 0.23	N.D.		
OT3-9		GGTGAGTGAGTGCGTGCGGTGG	3.2 ± 0.3	2.34 ± 0.21	N.D.	TPCN2	
OT3-17		GTTGAGTGAATGTGTGCGTGAGG	2.9 ± 0.2	1.27 ± 0.02	N.D.	SLIT1	
OT3-18		TGTGCGTGAGTGTGTGCGTGAGG	13.4 ± 4.2	12.1 ± 0.24	2.42 ± 0.07	COMDA	
OT3-20		AGAGAGTGAGTGTGTGCATGAGG	16.7 ± 3.5	7.64 ± 0.05	1.18 ± 0.01		

Fu et al, Nature Biotechnology, 31, 822 (2013)より改変

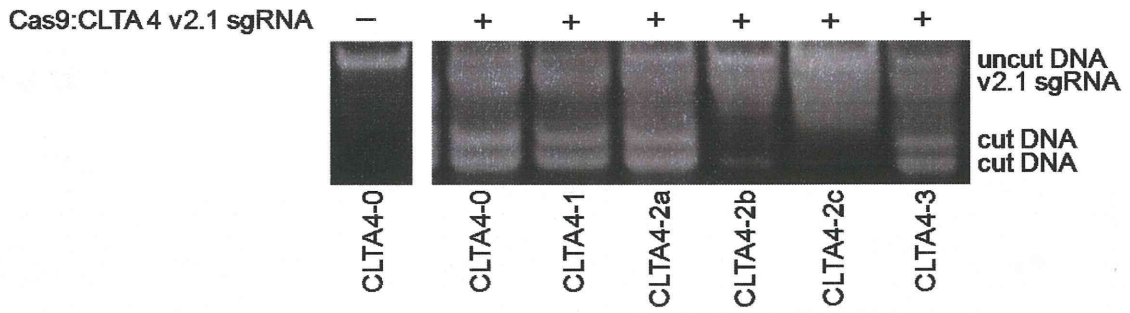
Fig.9 Single-nucleotide specificity of Cas9



ポイント: 3塩基以上のミスマッチはほとんど起きないため、2塩基ミスマッチ以内がゲノム上に存在しないように設計すればよい (GC含量が高く75%以上とか、PAM distal領域に集中した時には3-4塩基ミスマッチもあるが、設計で回避可能)

Hsu et al, Nature Biotechnology, 31, 827 (2013)より改変

Fig.10 Single-nucleotide specificity of Cas9

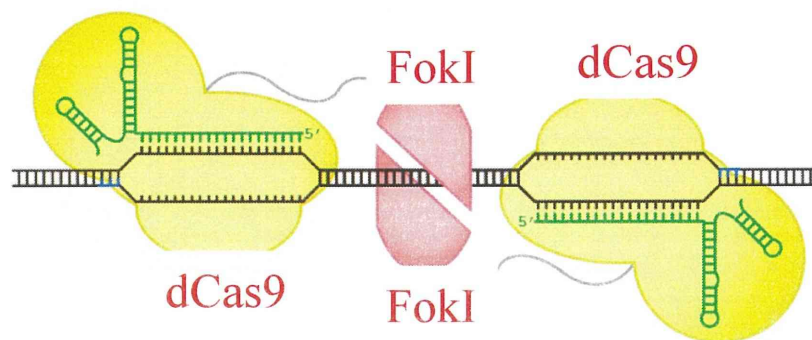


	sequence	<i>In vitro</i> selection enrichment value	% cut
CLTA4-0	GCAGATGTAGTGT ^{TT} TCCACAGGG	7.9	85%
CLTA4-1	GaAGATGTAGTGT ^{TT} TCCACAGGG	27.5	84%
CLTA4-2a	GaAGATGTAGTGT ^{TT} TCCAC ^t GGG	43.9	79%
CLTA4-2b	GCAGATG ^g AG ^g GT ^{TT} TCCACAGGG	1.0	35%
CLTA4-2c	GCAGATGTAGTGT ^{Ta} CCAG ^g AGGG	0.064	none detected
CLTA4-3	G ^g gGATGTAGTGT ^{TT} TCCAC ^t GGG	95.9	72%

赤字は、ミスマッチ塩基

Pattanayak V *et al*, *Nature Biotechnology*, 31, 839 (2013)より改変

Fig.11 Off-target DNA sequence and cut ratio (%)



Tsai SQ, *et al*, *Nature Biotechnology*, doi:10.1038/nbt.2908 (2014)より改変

Fig.12 Off-target DNA sequence and cut ratio (%)

Deletions:

```

CTTGATGCCGTTCTTCTGGTCATCCTCATCCTGATAAACTGCAAAGAAGTGTGCGGCCATGATA wt
CTTGATGCCGTTCTTCTGGTCATCCTCATCCTGA-AAACTGCAAAGAAGTGTGCGGCCATGATA -1
CTTGATGCCGTTCTTCTGGTCATCCTCATC-----TAAACTGCAAAGAAGTGTGCGGCCATGATA -4
CTTGATGCCGTTCTTCTGGTCATCCTCATCCTGA-----CAAAGAAGTGTGCGGCCATGATA -7
CTTGATGCCGTTCTTCTGGTCATCCTC-----ACTGCAAAGAAGTGTGCGGCCATGATA -10
CTTGATGCCGTTCTTCTGGT-----CTGATAAACTGCAAAGAAGTGTGCGGCCATGATA -10
CTTGATGCCGTTCTTCTGGTCATCCTCATCCTGA-----AACTTGTGCGGCCATGATA -13
CTTGATGCCGTTCTTCTGGTCATCC-----AAGAAGTGTGCGGCCATGATA -19
CTTGATGCCGTTCTTCTGGTC-----AAAGAAGTGTGCGGCCATGATA -22

```

Insertions:

```

CTTGATGCCGTTCTTCTGGTCATCCTCATCCTGATAAACTGCAAAGAAGTGTGCGGCCATGATA wt
CTTGATGCCGTTCTTCTGGTCATCCTCtltttGccg-ACTGCAAAGAAGTGTGCGGCCATGATA +2
TGCCGTTCTTCTGGTCATCCTCATCCTGAtctgaggAACTGCAAAGAAGTGTGCGGCCATGATA +5

```

Gaj T, et al, *Nature Methods*, 9, 805 (2012)より
(-はdeletionの数、+はinsertionの数を示す)

Fig.13-1 Indel pattern of the cleavage site by ZFN

(a) **noggin TALEN**

```

TCCTAGTGAAAACCTACCACTGGTGGACCTTATTGAGCATCCGGATCCTA
TCCTAGTGAAAACCTA-----GGAGGAGAGACTTGGAG (Δ274)
GTCTTCCTG-----//-----GGAGGAGAGACTTGGAG (Δ197)
-----CCTTATTGAGCATCCGGATCCTA (Δ57)
TCCTAG-----TTGAGCATCCGGATCCTA (Δ26)
TCCTAGTGAAAACCTAC-----TGAGCTCCGGATCCTA (Δ17,+1)
TCCTAGTGAAAACCT-----TATTGAGCATCCGGATCCTA (Δ16)
TCCTAGTGAAA-----ACCTTATTGAGCATCCGGATCCTA (Δ15)
TCCTAGTGAAAACCTACCAC-----TGAGCATCCGGATCCTA (Δ13)
TCCTAGTGAAAACCTACC-----TATTGAGCATCCGGATCCTA (Δ11)
TCCTAGTGAAA-----CTA-----GGAtgCTTATTGAGCATCCGGATCCTA (Δ11,+2)
TCCTAGTGAAAACCTACCAC-----TATTGAGCATCCGGATCCTA (Δ9)
TCCTAGTGAAAACCTACC-----ACCTTATTGAGCATCCGGATCCTA (Δ8)
TCCTAGTGAAAACCTACCCTGG-----TATTGAGCATCCGGATCCTA (Δ7)
TCCTAGTGAAAACCTACCtc-----ACCTTATTGAGCATCCGGATCCTA (Δ7,+1)
TCCTAGTGAAAACCTACCACcCT-----ACCTTATTGAGCATCCGGATCCTA (Δ5,+1)
TCCTAGTGAAAACCTACCACtaccactaccACCTTATTGAGCATCCGGA (Δ5,+10)

```

(b) **noggin ZFN**

```

GGACCTTATTGAGCATCCGGATCCTATCTATGATCCCAAGGAGAAGGATCTT
ACCTACCAC-----//-----AT-----CCTAGGGACCAG (Δ332)
CTTATTGAGCAT-----//-----CCACTTTGACCCCA (Δ68)
GGACCTTATTGAG-----CAAGGAGAAGGATCTT (Δ23)
GGACCTTATTGAGCATCCGGAT-----CAAGGAGAAGGATCTT (Δ14)
GGACCTTATTGAGaA-----CCT-TC-----TCCCAAGGAGAAGGATCTT (Δ14,+1)

```

(c) **ets1 TALEN**

```

TTACTCTGAAAGGAGTGGACTTTCAGAAGTCTSTATGAGCGGAGCAGCA
TTACTCTGAAAGGAGTGGACTTT----- (Δ403)
-----TATGAGCGGAGCAGCA (Δ400)
TTACTCTGAAAG-----GAGCGGAGCAGCA (Δ25)
TTACTCTGAAAGGA-----GTATGAGCGGAGCAGCA (Δ19)
TTACTCTGAAAGGAG-----cGTATGAGCGGAGCAGCA (Δ18,+1)
TTACTCTGAAAGGAGTGG-----ATGAGCGGAGCAGCA (Δ17)
TTACTCTGAAAGGAGTGG-----CTGTATGAGCGGAGCAGCA (Δ12)
TTACTCTGAAAGGAGT-----GTCTGTATGAGCGGAGCAGCA (Δ12)
TTACTCTGAAAGGAGTGG-----GcTCTGTATGAGCGGAGCAGCA (Δ12,+1)
TTACTCTGAAAGGAGTGG-----GTTCTGTATGAGCGGAGCAGCA (Δ11)
TTACTCTGAAAGGAGT-----AGTTCGTATGAGCGGAGCAGCA (Δ11)
TTACTCTGAAAGGAGTGGAC-----TCTGTATGAGCGGAGCAGCA (Δ10)
TTACTCTGAAAGGAGTGG-----AGcTCTGTATGAGCGGAGCAGCA (Δ10,+1)
TTACTCTGAAAGGAGTGGAC-----TTCTGTATGAGCGGAGCAGCA (Δ9)
TTACTCTGAAAGGAGT-----ACT-----cAGTTCGTATGAGCGGAGCAGCA (Δ8,+1)
TTACTCTGAAAGGAGTGGACT-----GTTCTGTATGAGCGGAGCAGCA (Δ7)
TTACTCTGAAAGGAGTGGACTTT-----TTCTGTATGAGCGGAGCAGCA (Δ7)
TTACTCTGAAAGGAGTGGACTTT-----GTTCTGTATGAGCGGAGCAGCA (Δ6)
TTACTCTGAAAGGAGTGGAC-----AAGTTCGTATGAGCGGAGCAGCA (Δ6,+1)
TTACTCTGAAAGGAGTGGACTTT-----AtGTTaTGTATGAGCGGAGCAGCA (Δ6,+2)
TTACTCTGAAAGGAGTGGACTTT-----tGTTCTGTATGAGCGGAGCAGCA (Δ5,+1)
TTACTCTGAAAGGAGTGGACTgT-----AAGTTCGTATGAGCGGAGCAGCA (Δ4,+1)

```

Lei Y, et al, *PNAS*, 109, 17484 (2012)より改変
(Δはdeletionの数、+はinsertionの数を示す)

Fig.13-2 Indel pattern of the cleavage site by ZFN and TALEN

(a) **EMX1 site 1 full-length gRNA**

```
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAgGGCTCCCATCACATCAACCGGTGG wild-type x35
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGAG > Δ202
<-----> Δ115
GAA > Δ94
<-----> Δ78
GAAGCTGGAGG > Δ72
GAAGCTGGA > GG Δ56
GAAGCTGGAGGAGGAAGGGCCTGA > GTGG Δ39
GAAGCTGGAGGAG > GAAGGGCTCCCATCACATCAACCGGTGG Δ26 x2
GAAGCTGGAGGAGGAAGGGCCTGAGT > CCATCACATCAACCGGTGG Δ22
GAAGCTGGAGGAGGAAGGGCCTGAG > TCCCATCACATCAACCGGTGG Δ21 x3
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGAG > CATCACATCAACCGGTGG Δ18
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGA > GCTCCCATCACATCAACCGGTGG Δ14
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGC > AGAAGGGCTCCCATCACATCAACCGGTGG Δ6 x3
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGC > AGAAGAAGGGCTCCCATCACATCAACCGGTGG Δ3 x3
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGA > AAGAAGGGCTCCCATCACATCAACCGGTGG Δ2 x2
GAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGA > ACAAGAAGGGCTCCCATCACATCAACCGGT +2
```

(b) **VEGFA site 3 full-length gRNA**

```
GAGGACGTGTGTGTCTGTGTGGGTGAGTGTGCGCTGtGGGGTTGAGGGTGTGGAGCGGGGA wild-type x35
GAGGACGTGTGTGTCTGTGTG > Δ117
GAGGACGTGTGTGTTGG > Δ84
GAGGACGTGTGTGTCTGTGTG > Δ75
GAGGACGTGTGTGTCTGTGTG > Δ49
GAGGACGTGTGTGTCTGTGTGGGTGAGTGTG > Δ43
GAGGACGTGTGTGTCTGTGTGGGTGAGTG > Δ40
GAGGACGTGTGTGTCTGTGTGAGT > GGGGA Δ39
GAGGACGTGTGTGTCTGTGTGGGTGAGTGTGNNG > Δ37
GAGGACGTGTGTGTCTGTGTGGGTGAGT > GAGNNGNGN Δ30 x2
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAG > TGGGGCGGGGA Δ25
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTG > TGGAGCGGGGA Δ23
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGT > GTTGGAGCGGGGA Δ22
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGT > GTTGGAGCGGGGA Δ20
GAGGACGTGTGTGTCTGTGTGGGTGAGT > TGAGGGTGTGGAGCGGGGA Δ20 x2
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGT > GGGCGTTGGAGCGGGGA Δ18
GAGGACGTGTGTGTCTGTGTGGGTGA > NNGTGGGGTTGAGGGTGTGGAGCGGGGA Δ12
GAGGACGTGTGTGTCTGTGTGGGTGAGTGA > GTGTGGGGTTGAGGGCGTTGGAGCGGGGA Δ8 x3
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGT > GGGTTGAGGGCGTTGGAGCGGGGA Δ7
GAGGACGTGTGTGTCTGTGTGG-TGAGTGAGTGTGT > GGGGTGAGGGTGTGGAGCGGGGA Δ6
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGT > GNGTGGGGTTGAGGGTGTGGAGCGGGGA Δ6 x5
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGT > GTGTGGGGTTGAGGGTGTGGAGCGGGGA Δ4
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGTG > TGGGGTTGAGGGTGTGGAGCGGGGA Δ3

GAGGANNGTGTGTCTGTGTGGGTGAGTGAGTGTGTG > TGGGTGAGTGAGTGTGTGCTGTGGGGTTG +20
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGTG > CGTGTGGGGTTGAGGGTGTGGAGCGG +3
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGTG > AAAGTGTGGGGTTGAGGGTGTGGAGCGG +3
GAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGTG > GCGGGGTGAGGGTGTGGAGCGG +2
```

Fu Y, et al, *Nature Biotechnology*, **32**, 279 (2014)より改変

(Δはdeletionの数、+はinsertionの数, xは頻度を示す)

Fig.13-3 Indel pattern of the cleavage site by CRISPR/Cas9

(c) **Target 4 (EMX1): (EMX1のoff-targetサイト)**

OT4-1

ACCTGTACATCTGCACAAGATTGCCTTTACTCCATG	CCTTTCTTCTTCTGCTCTAACTCT	GACAATC	Wild-type x20
-----	-----	ATC	Δ64
ACCTGTACATCTGCACAAGATTGCCTTTACTCC	-----	ACAATC	Δ28
ACCTGTACATCTGCACAAGATTGCCTTTACTCCAT	-----	ACTCTGACAATC	Δ20
ACCTGTACATCTGCACAAGATTGC	-----	CTTCTGCTCTAACTCTGACAATC	Δ20
ACCTGTACATCTGCACAAGATTGCCTTTACTCCATGCCTTTCT	-----	CAATC	Δ19
ACCTGTACATCTGCACAAGATTGCCTTTACTCCA	-----	TGCTCTAACTCTGACAATC	Δ14
TCCTGTACATCTGCACAAGATTGCCTTTACTCC	-----	CTTCTTCTGCTCTAACTCTGACAATC	Δ8

(d) **Target 3 (VEGFA Site 3): (VEGFA site3のoff-targetサイト)**

OT3-2

GAGTGAGAGAGCGAGTGAGTG	AGTGAGTGAGTG	TGTGTGTGGGGG	GGACTCGGCTTGTGTGTGTCGG	Wild-type x14
GAGTGAGAGAGCGAGTGAGTGAGTGAGTGA	-----	GTGTGTGGGGGGGACTCGGCTTGTGTGTGTCGG		Δ4
GAGTGAGAGAGCGAGTGAGTGAGTGAGTGA	-----	GTGTGGGGGGGACTCGGCTTGTGTGTGTCGG		Δ6 x2

OT3-9

GTGTTGGGATGCGGGAGTG	GGTGAGTGAGTGCGGTGCGGGTGGC	GATGCAAGCGTGCGAATGCGTG	x173
GTGTTGGGATGCGGGA	-----	>	Δ80
GTGTTGGGATGC	-----	GCGTG	Δ50
GTGTTGGGATGCGGGAGTGGGTGAGTGA	-----	GTGGCGATGCAAGCGTGCGAATGCGTG	Δ10

GTGTTGGGATGCGGGAGTGGGTGAGTGAGTGCA	AACTGCGGGTGGCGATGCAAGCGTGCGAATGCGTG	+2
-----------------------------------	-------------------------------------	----

OT3-18

TTTCAAAGACAGTAGATCTTAAATGT	CCTCACGCACACTCACCCACAC	ATAAAAAGGTGGTAACTG	Wild-type x27
TTTCAAAGACAGTAGATCT	-----	TAAAAGGTGGTAACTG	Δ32
TTTCAAAGACAGTAGATCTTAAATGT	-----	CATAAAAAGGTGGTAACTG	Δ23
TTTCAAAGACAGTAGATCTTAAATGTCCT	-----	CACATAAAAAGGTGGTAACTG	Δ18 x4
TTTCAAAGACAGTAGATCTTAAATGTCCT	-----	CCACACATAAAAAGGTGGTAACTG	Δ15
TTTCAAAGACAGTAGATCTTAAATGTC	-----	CTCACCCACACATAAAAAGGTGGTAACTG	Δ12
TTTCAAAGACAGTAGATCTTAAATGTCCTCA	-----	CACACACTCACCCACACATAAAAAGGTGGTAACTG	Δ2

TTTCAAAGACAGTAGATCTTAAATGTCCTCAC	AGGCTGGAGTACAGTGGCATGATATCAGCTCACTGCAATCTCGGGCTCCCGGGTTCAAG	
CCATGCACACACTCACCCACACATAAAAAGGTGGTAAC		+63

Fig.13-4 Indel pattern of the cleavage site by CRISPR/Cas9

Arabidopsis: mutations in 12 out of 25 sequenced clones

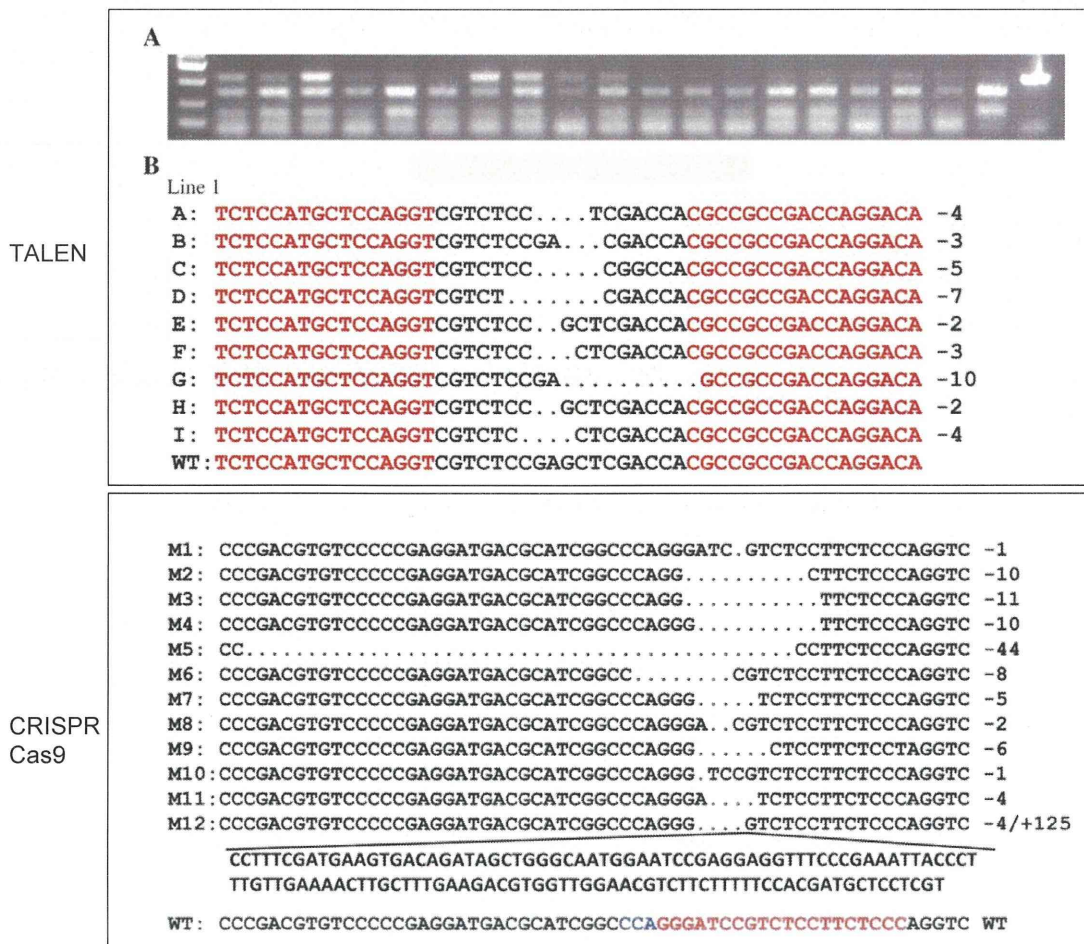
Sequence	In/Del	Freq.
CATGGAGCGCTTCAAGGTGCACATGGAGGACTAGTAAAGGAGAAGAAC		
CATGGAGCGCTTCAAGGTCCCATGGAGGACTAGTAAAGGAGAAGAAC	0 (-3,+3)	1x
CATGGAGCGCTTCAAGGTGCACATGGAGGACTAGTAAAGGAGAAGAAC	+1	1x
CATGGAGCGCTTCAAGGTGCA...GGAGGACTAGTAAAGGAGAAGAAC	-3	4x
CATGGAGCGCTTCAAGGTGCA...AGGACTAGTAAAGGAGAAGAAC	-5	3x
CATGGAGCGCTTCAAGGTGC...AGGACTAGTAAAGGAGAAGAAC	-6	2x
CATGGAGCGCT...GACTAGTAAAGGAGAAGAAC	-17	1x

Tobacco: mutations in 15 out of 28 sequenced clones

Sequence	In/Del	Freq.
CATGGAGCGCTTCAAGGTGCACATGGAGGACTAGTAAAGGAGAAGAAC		
CATGGAGCGCTTCAAGGTGCACATGGAGGACTAGTAAAGGAGAAGAAC	+1 (+1)	1x
CATGGAGCGCTTCAAGGTGCACATGGAGGACTAGTAAAGGAGAAGAAC	0 (-1,+1)	1x
CATGGAGCGCTTCAAGGTGCA...GGAGGACTAGTAAAGGAGAAGAAC	-3	4x
CATGGAGCGCTTCAAGGTG...TGGAGGACTAGTAAAGGAGAAGAAC	-4	1x
CATGGAGCGCTTCAAGGTGCA...GAGGACTAGTAAAGGAGAAGAAC	-4	1x
CATGGAGCGCTTCAAGGTGC...AGGACTAGTAAAGGAGAAGAAC	-5	3x
CATGGAGCGCTTCAAGGTGC...AGGACTAGTAAAGGAGAAGAAC	-6	3x
CATGGAGCGCTTCAAGGTGCA...AGGAGAAGAAC	-16	1x

Jian W, et al, *Nucleic Acid Research*, **41**, e188 (2013)より改変
 (-はdeletionの数、+はinsertionの数、xは頻度を示す)

Fig.14-1 Indel pattern of the cleavage site by CRISPR/Cas9 in Plants (Arabidopsis and Tobacco)



Lian Z, et al, *J. Genetics Genomics*, **41**, 63 (2014)より改変
 (-はdeletionの数、+はinsertionの数を示す)

Fig.14-2 Indel pattern of the cleavage site by TALEN and CRISPR/Cas9 in Plant (Zea mays)

CRISPR plasmid construct

target in genome 5'-gctaggctatatttoggatGNNNNNNNNNNNNNNNNNNNNNGGaaattcaccgcatta-3' (+)鎖
 3'-cgatccgatataaaagcctaCNNNNNNNNNNNNNNNNNNNNNCCttaagtggcgaat-5' (-)鎖



Note: (-)鎖に設計する時は、向きに注意

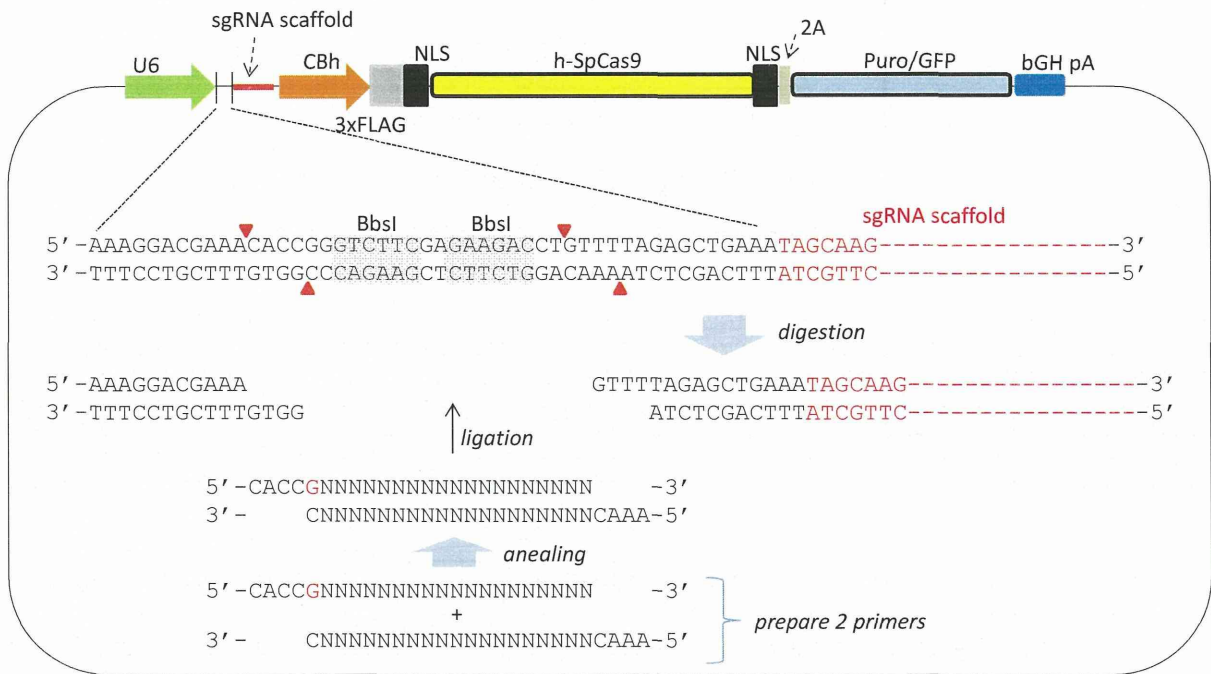
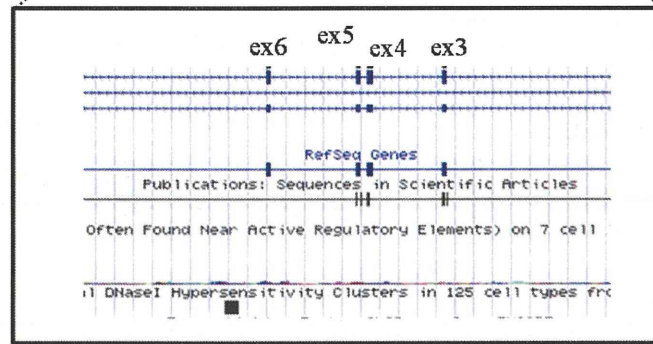
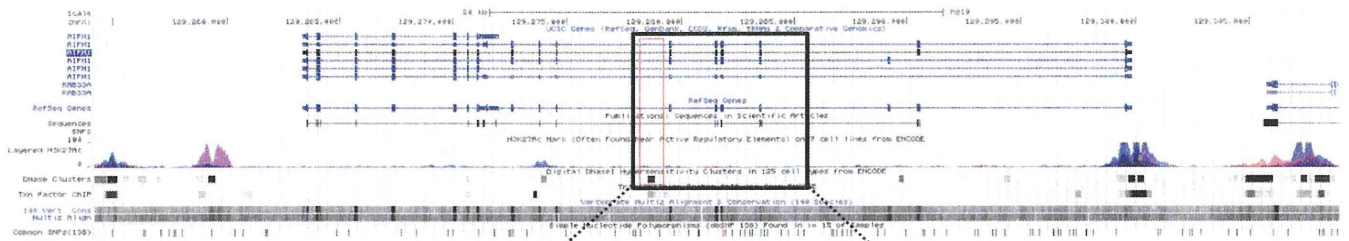


Fig.15 CRISPR/Cas9 plasmid we used in this study



ATTAGGGCTAGGAATATAGCTCAGTGGCCTGATATGCGTAAGGCCATAGGTAGTCTCTAGCTTCTAAATACATTAAACTAAGTTAAAGTCACTA
 ATAAATCCTTCTGCTATGTATTATAAAGATGAAAAGCAGAACTAGTCATAACTAAAATATCCTTCTCAATTGAAAGTATAGGGGAAAGAGTA
 TGCTTGAAATAAAACACTTTAGTGAAAATGATGTTTGACCCTTACTAATTATTAATTTGTTAGTGTCTTCTGAGTCAAGATTTCCACC
 CCCTTAGTTTAA
 AAAATGTAAACTATTATTGAAAAGGTGACTTACATTTGAAAGATAGTATACACCTAACCCATATCATCCGTGGCAAACCTTAAACATCTACCAT
 GAAGATGTATTTCCAGTTTCTACCTAGGCCATATAGAATGATTTTACAACCTAGCACCAGCATAGTCCAAAAGCCCTTTTGTGTTTATGCCATT
 TTTTACTTACTAAAGTTACATTTCTTTAATCTACTTTCTGAATTCAGTATCAGGAGAGTTCCTTACTGAGAGTCTCAGAAAACCTGATACATT
 TGATCTATTTTCAAGTATTTCTTCCCTTATCAGATATGTATAAAGTGGATAGTTTATTTCTTGACAAAAGAAGTCTTGCGTGGAGTTAAAGTTAGT

TALEN-VR-AIF-up-A

GCAGAGCGCTTGATTAGTATGCATGAAGCTCTGGGTTTCATCCTAGTACTGAAAAGTCAAATTCGCCTAGTAGTGTGGCATAAAGGAGCTAAC
 TAGCTCAATGTAGACAAAAGCTTCTCATTTAATGAAATGATGTTAGCCTATGTTATGATATAGCTGATCATGAAATAAAGTCTTCCATGTTGG
 GTCTACAGGCCTACAAGACTATTAAAGAAGACCAAAAAGATATAATGAAAGAATAATGGGATTAG
 GACTGTCACCAGAAGAGAAACAGAGAAGAGCCATTGCCTCTGGTAAGGACTCCCCTATGCTCTTCTGTGTGACT
 TGAGATTAATAAAGGAAAGTGATTAAGGTTGAGGTGCTATGATTTGCTAATATCCGTGTTGAAGTGTAAACAATGTGAGATGTGAAGCTTCTAGAAAC
 TATGGTTTTTACCCATAGCAGAGAGCAGTTTCCFAAAGTATTTGATTGGCATTTTAACCTTGAGAGTAAAATTAATATTGTGGGTACCTATCCTTT
 TTTTAATTTGTTTTATTTACATTCCAATGTTACCCCCACTTCTTGGTCCCCCTCCAAGAGTCTTTTACCCCATACCCTCTCCCCTTGGCTCTGAG

U6-Rev-exon3mae (red arrow pointing to TTTTCATC)

U6-Rev-exon3mae2 (green arrow pointing to GCCTAGTAGTGTGGCATAAAGGAGCTAAC)

U6-Rev-exon3usiro (red arrow pointing to TTTTCATC)

U6-Rev-left-2 (blue arrow pointing to TTTTCATC)

AGGGTGATCTTCCCCCCCCAAACTCCCCCGTCAATCCTCCTTCTGTGGGGCATCAGTCTCTACAGGATTAGGCACATCCTTTCCCTCTGAAGCCA
 GCCAAGGCAGTCTCTGCTACATGAGTGCCAGGGCCCTGAGATCAGCCTATGATGCTTTTTGGTGGTGGCAGTCCCTGGGAGCTTCCAGGGGT
 CCAGGTTAGTTGACACCGTTGTTCTTCTATGGGGTGGCATCCACTCAGTCTTCCCCTAACTCTTTCATTGGGGTCCCCTGTGCTCAGAACAAATG
 CTCACCTGTGAGTATCTGCATCTGTCTCAGTCAGCTGCTGGTAGAGCCTCTCAGAGGACAGCCATGCTAGGCTCCTGCCTGAAAGTACACATAGCA
 TCAGTAATAGTCTGGTGGTGGTGCCTGCCATGGGATGAATCCTAACCTGGGCTGGCACTTGGTGGCCTTCCCTCAGTCTCTGCTCCATTTTT
 GTCTTTGCATTTCTTTTAGACAGGAACCATTTGGGTCAAGAAATTTGAAGGTAGGTTGATGATATTTGGTGAATGCTGGGAAAGATAGGAAAGCC
 ATTTTCCAATGACTAAACCTTTTCAATCACATTAATCCCTTGAATCTTTGGAGTTTGGAGTTGTTATAGATGTGTTTTCTGTAGCACAAAGCTC
 TAGCCTTCTATTGATTGAGTTGGATTTTCAAAGAGGAATACTTTAATCCTTTAATAGGCCTATAATCTGGGTAGGCGGTTGTATTGCTTTTTCG
 AATAATGCCTCCTTCTTACATGAACATTGTATAAATAATCCCAATTGACCTCCTAGGTGTTTCCCCTTTATGC

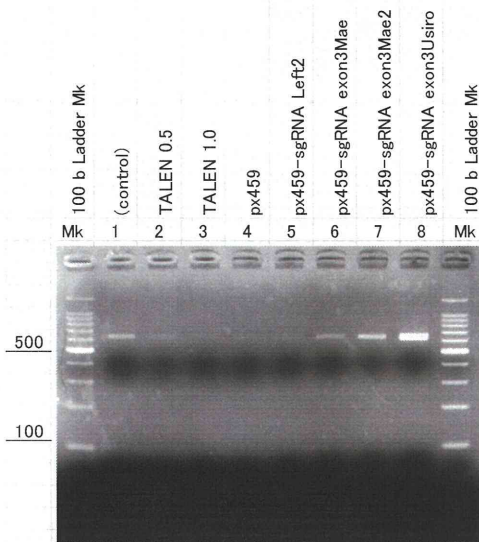
Fig.16 TALEN and CRISPR/Cas9 design targeted for AIFM1 exon3 region
 四角で囲んだ配列は、CRISPR標的配列、太字黒または茶で示した配列は
 TALEN標的配列を示す。大文字(緑)は、exon3を示す

• Platinum TALENのTarget: TTTTCATCCCTAGTACTG-AAAAGTCAAATTCGC-CTTAGTAGTGTTTGCATA

• CRSIPR/Cas9のTarget:

Name	Length	Start	End	Strand	Nucleotide sequence	GC% in spacer	Ranking	Match-start	Match-end
U6-Rev-exon3mae2	23	20	42	minus	GAATTTGACTTTTTCAGTACTAGG	30	100	135399601	135399625
U6-Rev-exon3mae	23	116	138	plus	GTTAGCCTATGTTATGATATAGG	30	100	23972	23996
U6-Rev-left2	23	288	310	minus	GTACACAGAAGAGACATAGGGG	45	100	23800	23824
U6-Rev-exon3usiro	23	419	441	minus	GGAAACTGCTCTCTGCTATGGGG	50	100	23669	23693

SURVEYOR assay 後



TALEN spacer (talen-VR-AIF-up-A)

SUR-PCR4_T1-2	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-3	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-13	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-24	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-9	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-15	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-19	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-20	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-22	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-5	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-18	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-4	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-23	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-11	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-10	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-7	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-16	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-6	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-8	110	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-17	109	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
PC12HS-tetoff10	111	TTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	220
SUR-PCR4_T1-33	111	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	220
SUR-PCR4_T1-42	111	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	220
SUR-PCR4_T1-27	110	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-35	109	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-30	110	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-31	110	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-29	110	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-39	110	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-32	110	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	219
SUR-PCR4_T1-36	109	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-25	109	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-37	108	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	217
SUR-PCR4_T1-45	108	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	217
SUR-PCR4_T1-44	108	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	217
SUR-PCR4_T1-26	108	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	217
SUR-PCR4_T1-38	107	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	216
SUR-PCR4_T1-48	108	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	217
SUR-PCR4_T1-34	109	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-28	109	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-46	109	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	218
SUR-PCR4_T1-43	108	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	217
SUR-PCR4_T1-47	108	CTTTGATTAGTATG	CATGAAG	CTCGGGTTT	CATCCCTAGTACT	GAAAAGTCAAATTCGC	CTTAGTAGTGT	TTGCATAAAGGAG	TAACTAGCTCAATGTAGACAAAAGCT	217

Fig.17 AIFM1遺伝子exon3前後intron領域を標的にデザインしたsgRNAと切断活性(SURVEYOR Assay), シークエンス解析