OMTN, Volume *24*

Supplemental information

CRISPR/Cas9-mediated correction of *MITF* homozygous point mutation in a Waardenburg syndrome 2A pig model

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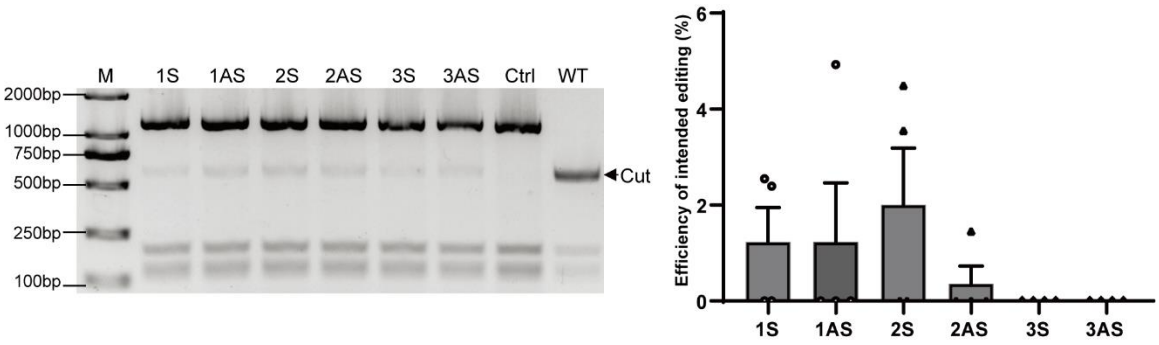

Figure S1. The comparison of correction efficiency with different sgRNAs and ssODNs in *MITF*$^{L247S/L247S}$ fibroblast cells. A representative genotyping picture of transfected cells by PCR and *DraI* digestion was shown on the left, and the quantitative efficiency of intended mutations evaluated by Hi-TOM was presented as Mean ± SEM (n = 4 from four independent experiments) on the right. Sequences with the intended c.740 C>T correction and at least one introducing mutation in the donor DNA was counted as corrected events. 1~3 stands for sgRNA1~3 illustrated in Fig. 1B, S stands for the sense ssODN (ssODN-S), and AS stands for the antisense ssODN (ssODN-AS). Small deletions or insertions to the genomic TTTCAAAA sequence in *MITF* may result in TTTAAA sequence that could be cut by *DraI* but could not be counted as corrected events. Therefore, the estimated correction efficiency from the left genotyping picture may be higher than the actual correction efficiency.

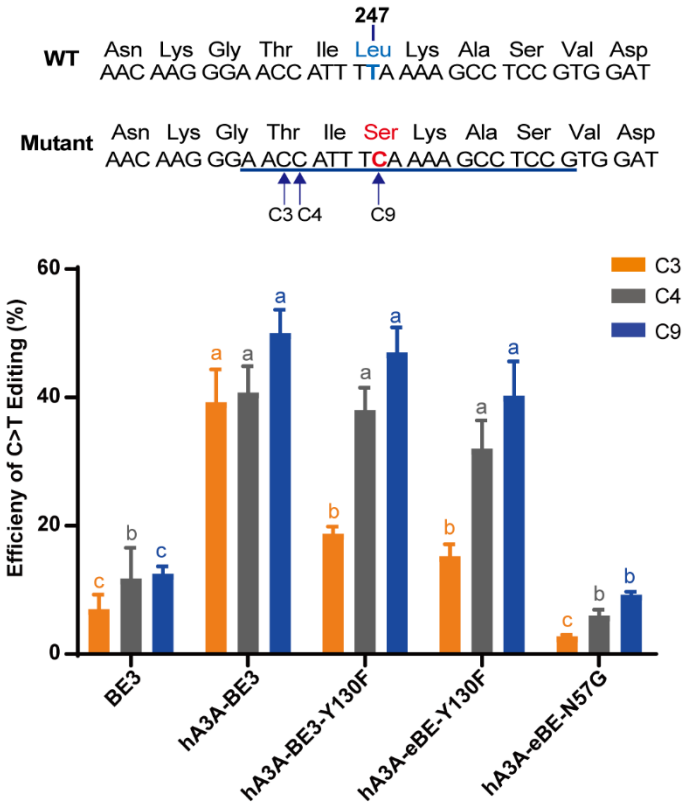

Figure S2. The comparison of C>T editing efficiency with different base editors. The targeted L247S (c.740 T>C) mutation was illustrated for BE editing. Efficiency of C>T editing was analyzed with EditR after Sanger sequencing of the PCR products from the transfected cells. The three Cs in the editing window was shown, counting the end distal to the PAM as position 1. Data are represented as the Mean ± SEM (n = 4 from four independent experiments). a, b, c Values with no letter in common within the same C column (C3, C4 or C9) are significantly different, $P<0.05$.

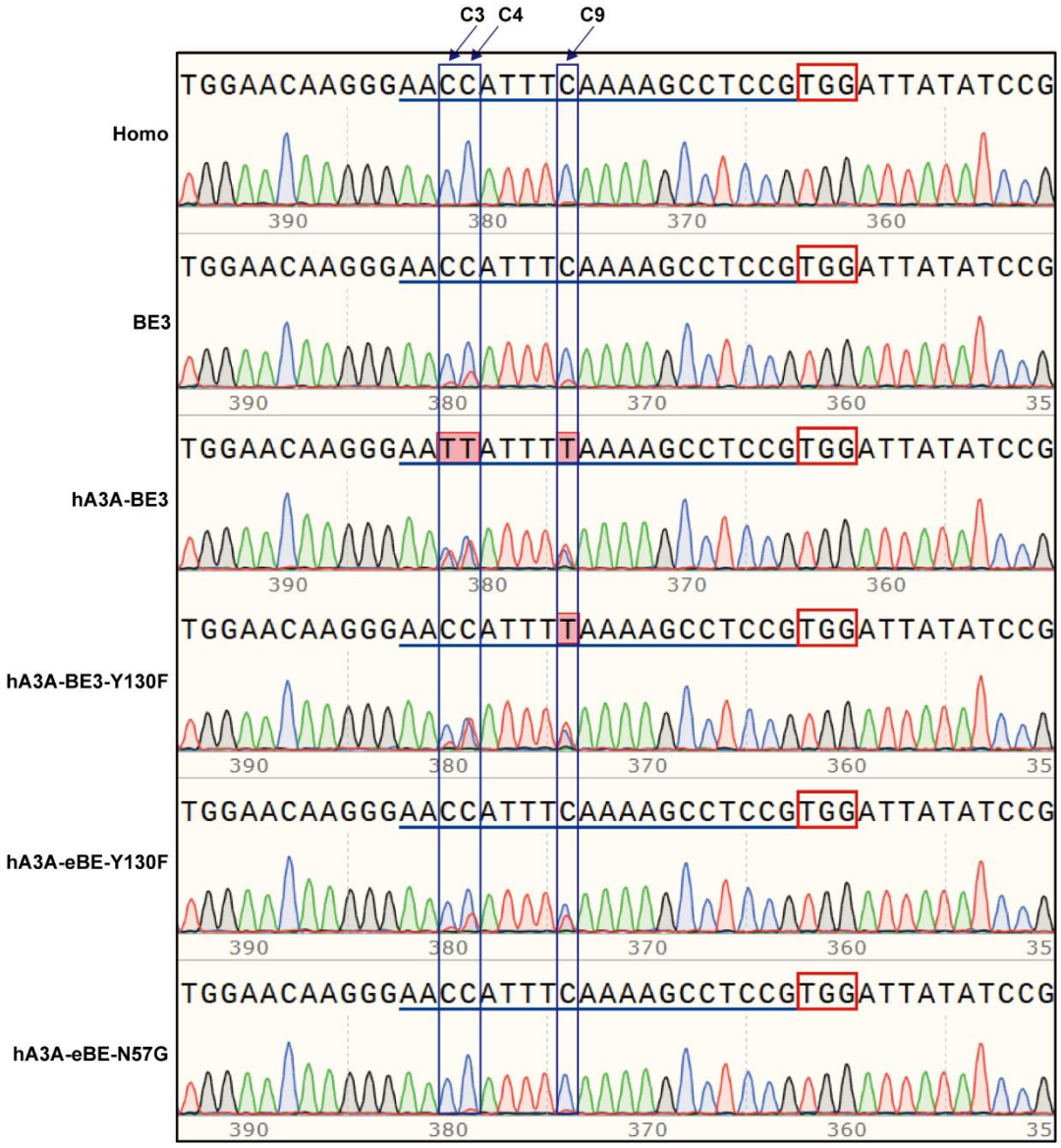

Figure S3. The representative Sanger sequencing diagrams of the *MITF* amplicons in fibroblast cells transfected with different base editors. The sgRNA sequences are underlined, the PAM sequences of the sgRNA are shown in red rectangle, and the edited Cs are indicated by rectangles and arrows. Homo stands for the *MITF*$^{L247S/L247S}$ fibroblast cells.

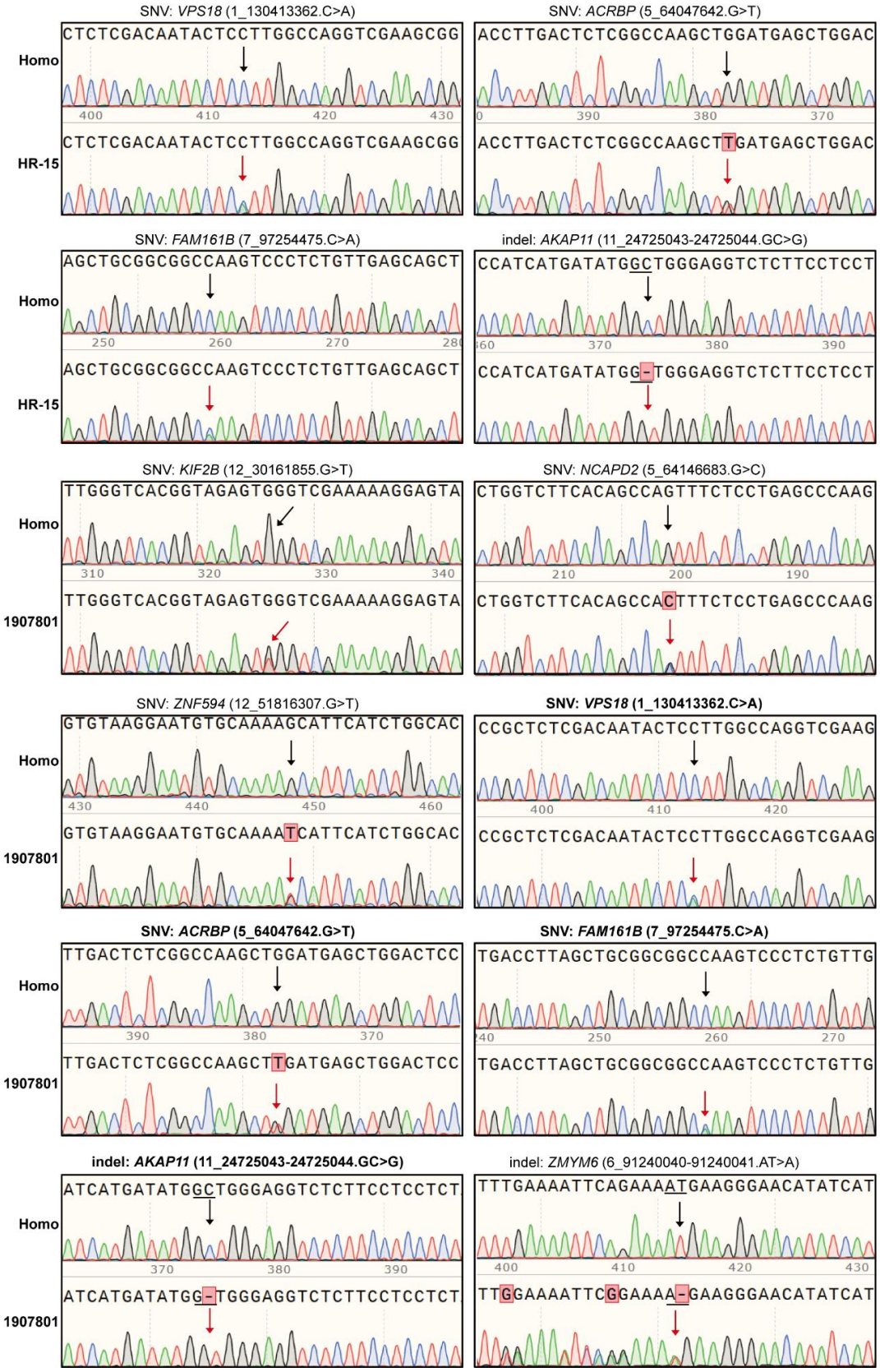

Figure S4. Representative Sanger sequencing diagrams of SNVs and indels detected by WGS in

colony HR-15 and HR-15 derived pig #1907801. The SNVs and indels detected simultaneously in HR-15 and 1907801 was shown in bold in sample 1907801. The detected mutations were indicated in red arrows, as compared with the original base(s), shown in black arrows.

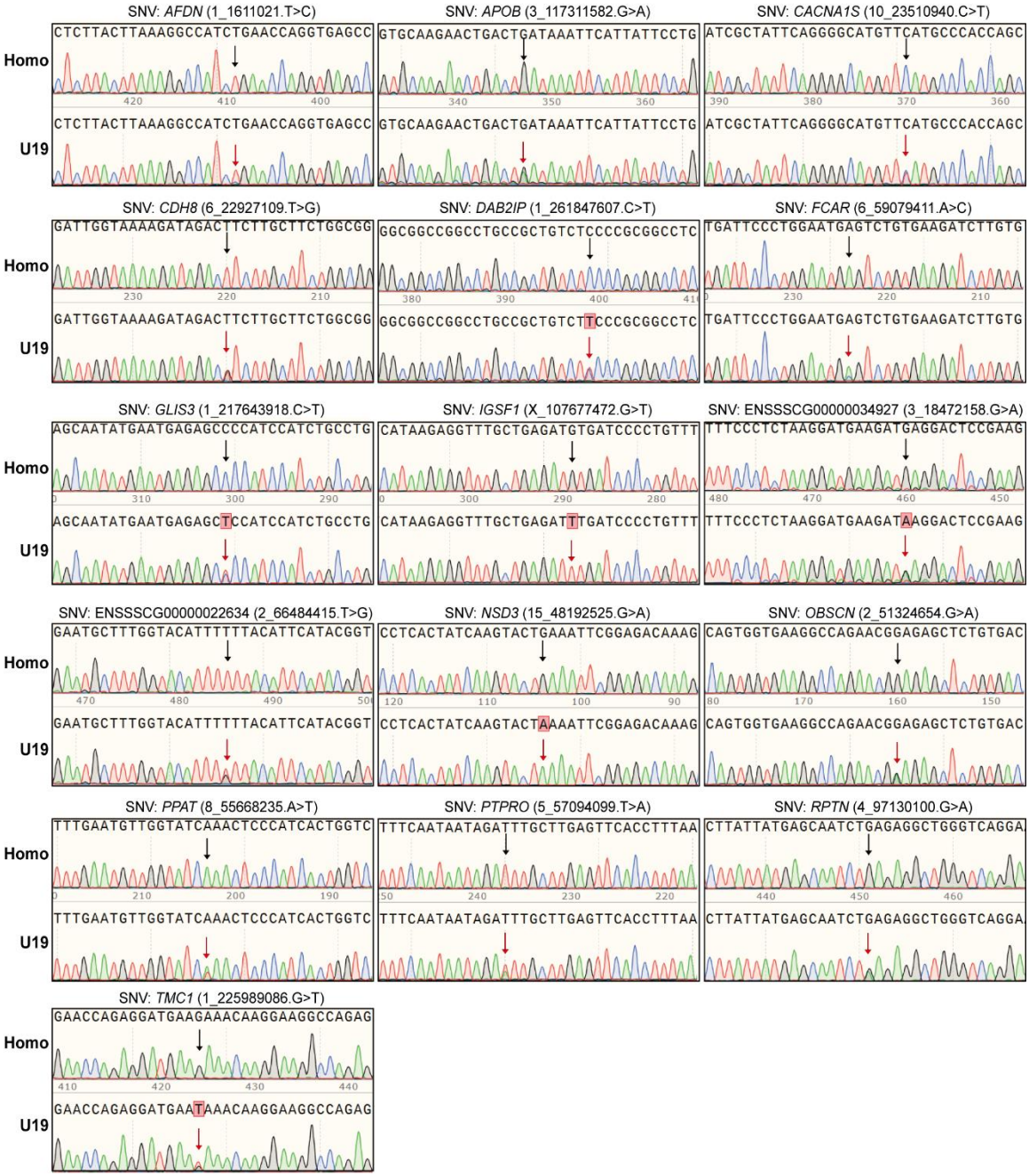

Figure S5. Representative Sanger sequencing diagrams of SNVs detected by WGS in colony U19. The detected mutations were indicated in red arrows, as compared with the original base(s), shown in black arrows.

Table S1. Embryo transfer results of corrected pigs from ssODN-mediated strategy

Recipient ID	Donor cells	No. of embryos transferred	Pregnant and develop to term	No. of piglets
1310701	Colony 27	250	Y	3 stillborn, 2 alive
1303602	Colony 27	250	Y	3 alive
1319202	Colony 27	231	Y	4 alive
1222101	Colony 27	300	N	
YJ160409	Colony 27	287	N	
1305105	Colony 27	246	N	
1234102	Colony 27	276	N	
1313605	Colony 27	250	N	
1234302	Colony 27	290	N	

Table S2. Embryo transfer results of corrected pigs from long donor DNA-mediated strategy

Recipient ID	Donor cell	No. of embryos transferred	Pregnant and develop to term	No. of piglets
1803502	HR-15 colony	270	Y	2 stillborn, 4 alive
WT180202	HR-15 colony	275	Y	1 stillborn
1807701	HR-15 colony	280	N	
1807401	HR-15 colony	245	N	
WT177504	HR-15 colony	300	Y	2 alive
1807002	HR-15 colony	275	N	

Table S3. Embryo development derived from *MITF*$^{L247S/L247S}$ and *MITF*$^{L247S/+}$ fibroblast cells after microinjection

Donor cell	No. of reconstructed embryos	Cleavage rate (%)	Blastocyst rate (%)
Hetero (*MITF*$^{L247S/+}$)	374	79.41 (297/374)	13.37 (50/374)
Homo (*MITF*$^{L247S/L247S}$)	379	78.36 (297/379)	18.21 (69/379)

Table S4. Overview of WGS reads

Sample	Raw reads	Clean reads	Raw Base (G)	Clean Base (G)	Error rate (%)	Coverage (>Q30)
Homo	789,114,709	787,919,487	236.73	236.38	0.03	92.53%
HR-15	790,559,880	789,735,106	237.17	236.92	0.03	92.42%
1907801	747,263,061	746,510,388	224.18	223.95	0.03	92.24%
U19	771,922,698	769,838,126	231.58	230.95	0.03	91.97%

Table S5. Sanger sequencing primers for validation of WGS results

See the file Table S5.xlsx

Table S6. Sanger sequencing validation of WGS results

See the file Table S6.xlsx

Table S7. Primers used in the study

Oligos for constructing long donor DNA plasmids

Primer name	Sequence (5'-3')	Product
HA-sgRNA3-F	CTTTCGGATATAATCCACGGAGGCCCTACCATTTCCTAGCAGT	1684 bp
HA-sgRNA4-R	TTTTTAGGACGCGAAGATCCAGGGCTGATCCAAACACTGAATT	
HA-sgRNA1-F	ATCCACGGAGGCTTTTGAAATGGCCCTACCATTTCCTAGCAGT	1684 bp
HA-sgRNA1-R	ATCCACGGAGGCTTTTGAAATGGGCTGATCCAAACACTGAATT	
HA-F2	CCCTACCATTTCCTAGCAGT	1638 bp
HA-R2	GCTGATCCAAACACTGAATT	
sgRNA3-mu-F	AGCGTCCGTGGATTATATCCGAAAGTTGC	
sgRNA3-mu-R	TTTAAAATGGTTCCCTTGTTCCAGCGCATG	
sgRNA4-mu-F	TCGTGGATCTTCGCGTCCTAAAAAAAAGTC	
sgRNA4-mu-R	ATATGGAGCATTCAATAGTCAGAGCATTCG	
sgRNA1-mu-F	AACGATTTTAAAAGCCTCCGTGGATTATATC	
sgRNA1-mu-R	CCCTTGTTCCAGCGCATGTCTCTGTGATG	

Oligos for genotyping

Primer name	Sequence (5'-3')	Product
M-HA-F1	GAGTGGTTTAGGGCATGAAG	1749 bp
M-HA-R1	CCACCCAGTCTATGGTATTTT	
MITF(8-g)-F	AGTATGAGCCTTGTCCAACCTTA	546 bp
MITF(8-g)-R	GCTGTAGTCTTTGCCATCTTTAG	
MITF-M-2F	CACAACTTGATTGAACGAAG	197bp
MITF-M-2R	AGCTTCTTCTGTCTGTTTTCA	
MITF-M-1F	AGCCCAAAGGCAGCAGGTAA	988 bp
MITF-M-1R	TCCGAGGTTGTTGTTGAAGGTG	
MITF-new-F	CCCACCAAGTACCACATACAGC	1565bp
MITF-new-R	AAGGAAGTCACAGGCATACAAAT	
MITF-Target-F1	ggagtgagtacggtgtgcTGGGCTGTAGTCTTTGCCAT	242bp
MITF-Target-R1	gagttggatgctggatggTCAAGCTCTTTTGCGCGTTG	

Oligos for *in vitro* transcription

Primer name	Sequence (5'-3')
sgRNA2-IVT-F	TAATACGACTCACTATAGGAACCATTTCAAAAGCCTCCGGTTTTAGAGCTAG AAATAGC
sgRNA2-IVT-R	TAATGCCAACTTTGTACAAGAAAG