Efficient base editing for multiple genes/loci in pigs using base editors

Supplementary Information

Xie et al. 2019

DMD DTL-E4: DTL-E11:	5'-TTTTTTTTATCTTTCAGGTGCTTCAGGAAGAACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3' 5'-TTTTTTTTATCTTTCAGGTGCTTTAGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3' 5'-TTTTTTTATCTTTCAGGTGCTTTAGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3'	Wild-type (13/20) (6/20)	(Q493STOP) (Q493STOP)
TYR	5'-GAGGTGATGGGAGTCCCTGTGGCCCAGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	Wild-type	
DTL-E4:	5'-GAGGTGATGGGAGTCCCTGTGGG TT AGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	(13/20)	(G27G, Q28STOP)
	5'-GAGGTGATGGGAGTCCCTGTGGGACAGCTCTTAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	(7/20)	(G27G, S30L)
DTL-E11:	5'-GAGGTGATGGGAGTCCCTGTGG TT AGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	(10/20)	(G27G, Q28STOP)
LMNA	5'-AGCAGCTCGGGAGCCCAGGTGGGCGGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	Wild-type	
DTL-E4:	5'-AGCAGCTCGGGAGCCCAGGTGGG T GGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	(4/20)	(G608G)
	5'-AGCAGCTCGGGAGCCCAGGTGGG T GGAT T CATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	(4/20)	(G608G, S610F)
DTL-E11:	5'-AGCAGCTCGGGAGCCCAGGTGGG T GGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	(20/20)	(G608G)
b RAG1 R12I-E3: R12I-E17	5'-AGGGGAAACCCTCGCT <mark>GGAGCAA</mark> TCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAG 5'-AGGGGAAACCCTCGCTGGAG T AATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAG 5'-AGGGGAAACCCTCGCTGGAG T AATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAG 5'-AGGGGAAACCCTCGCTGGAG T AAT T TCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAG	-3' Wild- -3' (5/20 -3' (8/20 -3' (3/20	type) (Q61STOP)) (Q61STOP)) (Q61STOP,S62F)
RAG2	5' - CCCACTGTCAGAAAATGTCACTACAGATGATAACAGTTGGTAATAACATGGCCTTAATT	-3' Wild-	type
<i>R12I-</i> E3:	5' - CCCACTGTCAGAAAATGT T ACTA T AGATGATAACAGTTGGTAATAACATGGCCTTAATT	-3' (3/20) (S2L,Q4STOP)
<i>R12I</i> -E17	5'-CCCACTGTCAGAAAATGTCACTA T AGATGATAACAGTTGGTAATAACATGGCCTTAATT	-3' (10/2	0) (Q4STOP)
	5' - CCCACTGTCAGAAAATGT T A T A T AGATGATAACAGTTGGTAATAACATGGCCTTAATT	-3' (10/2	0) (S2L,L3L,Q4STOP)
IL2RG R12I-E4: R12I-E17	5'-CTACCAAACATTTGTTGTCCAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCAC 5'-CTACCAAACATTTGTTGTTTAGCTTTAGGACCCACGGGAACCCAGGAGGCAGGACCCAC 5'-CTACCAAACATTTGTTGTTTAGTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCAC 5'-CTACCAAACATTTGTTGTTTAGTTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCAC	-3' Wild- -3' (4/20 -3' (15/2 -3' (5/20	<pre>type) (V154V,Q155STOP,L156L,Q157STOP) 0) (V154V,Q155STOP)) (V154V,Q155STOP,C156F)</pre>

Supplementary Fig. 1 Simultaneous <u>b</u>ase editing of DMD, TYR and LMNA, or RAG1, RAG2

and *IL2RG* in porcine embryos. a and b T-cloning and Sanger sequencing to detect base editing of DTL-E4, E17 (a), and R12I-E3, E17 (b). The sequences of sgRNAs are marked in yellow and the PAM sites sequences are labeled in green. The targeted sequence is underlined.

	Supple	ementary Fig. 2		
а		DMD	TYR	LMNA
-		GCTTCAGGAAGACCTAGAGCAG	G GTGGCCAGCTCTCAGGCAGGGG	GTGGGCGGATCCATCTCCT CTGG
	wт	MMMMMMMW	N MMMMMMM	M M M M M M M M M M M M M M M M M M M
	A3A-DTL-E6		1 Mit Man March	MMMMMMM
_	A3A-DTL-E7	, www.www.	M Manmaham	MMMMMMM
b		RAG1	RAG2	IL2RG
		GGAGCAATCTCCAGCAGTC CT GG	GTCACTACAGATGATAACAGTTGG G	TCCAGCT CCAGGACCCACG G G
	WT	Mmmmmh	mannhamm	MMMMMMM
1	\3A-R12I-E4	Mannann	mmmmmml	ManManan
, C	\3A-R12I-E8	Mamman	minimum l	Mannamm
-	DMD DTL-E6: DTL-E7:	5' -TTTTTTTTATCTTTCAGGT GCTT<u>CAG</u> 5' -TTTTTTTTATCTTTCAGGTGCTT T AG 5' -TTTTTTTTATCTTTCAGGTGCTT T AG	GAAGACCTAGAGCAGGAACAAGTCAGGGTCA GAAGACCTAGAGCAGGAACAAGTCAGGGTCA GAAGACCTAGAGCAGGAACAAGTCAGGGTCA	ATTC-3' Wild-type ATTC-3' (14/20) (Q493STOP) ATTC-3' (12/20) (Q493STOP)
	<i>TYR</i> <i>DTL</i> -E6:	5' -GAGGTGATGGGAGTCCCT <mark>GTGGC<u>CA</u> 5' -GAGGTGATGGGAGTCCCTGTGGTTA 5' -GAGGTGATGGGAGTCCCTGTGGCGA</mark>	<u>CTCTCAGGCAGGGG</u> TTCCTGTCAGGACATC GCTCTCAGGCAGGGGTTCCTGTCAGGACATC GCTCT C AGGCAGGGGTTCCTGTCAGGACATC	ATTC-3' Wild-type ATTC-3' (11/20)(G27G,Q28STOP) ATTC-3' (1/20)(G27G,S30L)
	DTL-E7:	5'-GAGGTGATGGGAGTCCCTGTGGC T A	GCTCTCAGGCAGGGGTTCCTGTCAGGACATC.	ATTC-3' (2/20) (G27G,Q28STOP)
_	<i>LMNA DTL-</i> E6: <i>DTL-</i> E7:	5'-AGCAGCTCGGGAGCCCAG GTGGGCG 5'-AGCAGCTCGGGAGCCCAGGTGGG T G 5'-AGCAGCTCGGGAGCCCAGGTGGG T G	SATCCATCTCCTCTGGCTCCTCCGCCTCCAG GATCCATCTCCTCTGGCTCCTCCGCCTCCAG GATCCATCTCCTCTGGCTCCTCCGCCTCCAG	TGTC-3' Wild-type TGTC-3' (11/20)(G608G) TGTC-3' (4/20) (G608G)
d	RAG1 R12I-E4: R12I-E8:	5'-AGGGGAAACCCTCGCT <mark>GGAGCAATCTC</mark> 5'-AGGGGAAACCCTCGCTGGAG T AATCTC 5'-AGGGGAAACCCTCGCTGGAG T AATCTC	CAGCAGTCCTGGACAAGCCTGGTGGTCAGAAG- CAGCAGTCCTGGACAAGCCTGGTGGTCAGAAG- CAGCAGTCCTGGACAAGCCTGGTGGTCAGAAG-	3' Wild-type 3' (3/20) (Q61STOP) 3' (10/20) (Q61STOP)
	RAG2 R12I-E4:	5'-CCCACTGTCAGAAAATGTCACTACAGA 5'-CCCACTGTCAGAAAATGTTATTATAGA	TGATAACAGTTGGTAATAACATGGCCTTAATT- TGATAACAGTTGGTAATAACATGGCCTTAATT-	3' Wild-type 3' (10/20) (S2L,L3L,Q4STOP) 2' (0/20) (S2L,O4STOP)
	<i>R12I-</i> E8:	5' -CCCACTGTCAGAAAATGTTATATAGA 5' -CCCACTGTCAGAAAATGTTATATAGA 5' -CCCACTGTCAGAAAATGTTACTATAGA	TGATAACAGI IGGIAATAACAIGGCCI TAATT- TGATAACAGTTGGTAATAACATGGCCTTAATT- TGATAACAGTTGGTAATAACATGGCCTTAATT-	3' (10/20) (S2L,Q4STOP) 3' (7/20) (S2L,Q4STOP)
	IL2RG R12I-E4: R12I-E8:	5'-CTACCAAACATTTGTTGTTGTCCAGCTCCA 5'-CTACCAAACATTTGTTGTTTAGCTCCA 5'-CTACCAAACATTTGTTGTTTAGCTCCAG	GGACCCACGGGAACCCAGGAGGCAGGACCCAC- GGACCCACGGGAACCCAGGAGGCAGGACCCAC- GACCCACGGGAACCCAGGAGGCAGGACCCAC-3	3' Wild-type 3' (4/20) (V154V,Q155STOP) ' (9/20) (V154V,Q155STOP)
e		5 - CIACCAAACATTTGTTGT TT AGCT TT A	GGAUUUAUGGAAUUUAGGAGGUAGGACCCAC-	

No. of embryos injected	No. of blastocysts (%)	No. of blastocysts detected	No. of I single ger	plastocyst ne base ec	ts with diting (%)	No. of blas ba	tocysts with ase editing (⁶	No. of blastocysts with three genes base editing (%)	
			DMD	TYR	LMNA	DMD and TYR	TYR and LMNA	DMD and LMNA	DMD, TYR and LMNA
100	20 (24)	10	0	0	0	1 (10.0)	0	2 (20.0)	5 (50.0)
			RAG1	RAG2	IL2RG	RAG1 and RAG2	RAG1 and IL2RG	RAG2 and IL2RG	RAG1, RAG2 and IL2RG
100	25 (25)	12	1 (8.3%)	0	1 (8.3%)	1 (8.3%)	0	1 (8.3%)	5 (41.7)

Supplementary Fig. 2 hA3A-BE3-mediated multiple genes base editing in porcine embryos. a and b Sanger sequencing results of the target sites in A3A-DTL-E6, E7 (a) and A3A-R12I-E4, E6 (b). Target base editing was labeled with red arrows. c and d T-cloning and Sanger sequencing of base editing of A3A-DTL-E6, E7 (c) and A3A-R12I-E4, E6 (d). PAM sites are highlighted in green; target sequences are red. e Summary of multiple sites base editing by hA3A-BE3 in porcine embryos

а

Blastocysts	C to T	C to G	WT	Ratio of base editing
pol-E1	57.8%	29.5%	12.7	87.3%
pol-E2	70.8%	20.5%	8.7%	91.3%
pol-E3	78.9%	21%	0	100%
pol-E4	35.3%	11.8%	52.9%	47.1%
pol-E5	72.9%	13.5%	13.6%	86.4%
pol-E6	71.0%	18.0%	11.0%	89.0%
pol-E7	42.5%	25.6%	31.9%	68.1%
pol-E8	40.0%	22.5%	37.5%	62.5%
pol-E9	54.2%	24.5%	21.3%	78.7%
pol-E10	54.4%	32.6%	13.0%	87.0%
pol-E11	48.2%	30.2%	22.6%	78.4%
pol-E12	44.7%	20.9%	34.4%	65.6%
pol-E13	29.5%	46.8%	23.7%	76.3%
pol-E14	46.8%	26.3%	26.9%	73.1%
pol-E15	55.2%	25.9%	18.9%	81.1%
pol-E16	58.0%	17.1%	24.9%	75.1%
pol-E17	63.9%	21.3%	14.8%	85.2%
pol-E18	56.5%	25.3%	18.2%	81.8%
pol-E19	65.5%	19.8%	14.7%	85.3%
pol-E20	61.7%	16.5%	21.8%	78.2%
No. of embryos inje	cted No. of blast	ocysts (%) No. of	blastocysts detected	No. of blastocysts with po gene base editing (%)
100	20 (2	0%)	20	20 (100%)

Supplementary Fig. 3 Base editing of the pol gene in porcine embryos. a and b Detailed

information concerning base editing efficiency of each injected porcine embryo.

a DTL mutant cell colonies

DMD DTL-C1: DTL-C23: DTL-C37:	5'-TTTTTTTATCTTTCAGGTGCTTCAGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3' 5'-TTTTTTTATCTTTCAGGTGCTTTAGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3' 5'-TTTTTTTATCTTTCAGGTGCTTTAGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3' 5'-TTTTTTTATCTTTCAGGTGCTTCAGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3' 5'-TTTTTTTATCTTTCAGGTGCTTTAGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTC-3'	Wild-type (Q493STOP) (Q493STOP) (Q493STOP)
TYR	5'-GAGGTGATGGGAGTCCCTGTGGCCAGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	Wild-type
DTL-C1:	5'-GAGGTGATGGGAGTCCCTGTGG TT AGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	(G27G, Q28STOP)
DTL-C23:	5'-GAGGTGATGGGAGTCCCTGTGGCCAGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	
	5'-GAGGTGATGGGAGTCCCTGTGGC T AGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	(Q28STOP)
DTL-C37:	5'-GAGGTGATGGGAGTCCCTGTGGCCAGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	
	5'-GAGGTGATGGGAGTCCCTGTGGG TT AGCTCTCAGGCAGGGGTTCCTGTCAGGACATCATTC-3'	(G27G, Q28STOP)
LMNA	5'-AGCAGCTCGGGAGCCCAGGTGGGCGGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	Wild-type
DTL-C1:	5'-AGCAGCTCGGGAGCCCAGGTGGGCGGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	
	5'-AGCAGCTCGGGAGCCCAGGTGGG T GGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	(G609G)
DTL-C23:	5'-AGCAGCTCGGGAGCCCAGGTGGGCGGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	
	5'-AGCAGCTCGGGAGCCCAGGTGGG T GGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	(G609G)
DTL-C37:	5'-AGCAGCTCGGGAGCCCAGGTGGG T GGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	(G609G)
	5'-AGCAGCTCGGGAGCCCAGGTGGG T GGATCCATCTCCTCTGGCTCCTCCGCCTCCAGTGTC-3'	(G609G)

b *R121* mutant cell colonies

RAG1	5'-AGGGGAAACCCTCGCT GGAG CAA TCTCCAGCAGTCCTGG CCAGGTGGTCAGAAGTCAGC-3'	Wild-type
R12I-C22	5'-aggggaaaccctcgctggag T aatctccagcagtcctggacaagcctggtcggtcagaagtcagc-3'	(Q61STOP)
R12I-C29	5'-aggggaaaccctcgctggagcaatctccagcagtcctggacaagcctggtcagaagtcagc-3'	Wild-type
R12I-C56	5'-aggggaaaccctcgctggagcaatctccagcagtcctggacaagcctggtcagaagtcagc-3'	Wild-type
R12I-C108	5'-aggggaaaccctcgctggagcaatctccagcagtcctggacaagcctggtcagaagtcagc-3'	Wild-type
	5'-aggggaaaccctcgctggag T aatctccagcagtcctggacaagcctggtggtcagaagtcagc-3'	(Q61STOP)
RAG2	5'-CCCACTGTCAGAAAATGTCACTACAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3'	Wild-type
R12I-C22	5'- CCCactgtcagaaaatgtcattaa Agatgataacagttggtaataacatggccttaattcagcc-3'	(L3L, Q4K)
	$\texttt{5'-CCCACTGTCAGAAAATGT}{\textbf{TAT}} \texttt{TAT} \texttt{AGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3'}$	(S2L, L3L, Q4STOP)
R12I-C29	5'-CCCACTGTCAGAAAATGT T ACTACAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3'	(S2L)
	$\texttt{5'-CCCACTGTCAGAAAATGT}{\textbf{T}}{\textbf{A}}{\textbf{T}}{\textbf{T}}{\textbf{A}}{\textbf{C}}{\textbf{A}}{\textbf{G}}{\textbf{A}}{\textbf{C}}{\textbf{A}}{\textbf{T}}{\textbf{A}}{\textbf{C}}{\textbf{A}}{A$	(S2L, L3L)
R12I-C56	$\texttt{5'-CCCACTGTCAGAAAATGT}{\textbf{T}}{\textbf{A}}{\textbf{T}}{\textbf{T}}{\textbf{A}}{\textbf{C}}{\textbf{A}}{\textbf{G}}{\textbf{A}}{\textbf{C}}{\textbf{A}}{A$	(S2L, L3L)
	5'- CCCACTGTCAGAAAATGT TAT TAT AGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3'	(S2L, L3L, Q4STOP)
R12I-C108	$\texttt{5'-CCCACTGTCAGAAAATGT}{\textbf{TAT}} \texttt{A} \texttt{T} \texttt{A} \texttt{T} \texttt{A} \texttt{G} \texttt{A} \texttt{G} \texttt{A} \texttt{G} \texttt{G} \texttt{G} \texttt{G} \texttt{A} \texttt{A} \texttt{A} \texttt{A} \texttt{C} \texttt{A} \texttt{G} \texttt{G} \texttt{C} \texttt{C} \texttt{T} \texttt{A} \texttt{A} \texttt{C} \texttt{A} \texttt{G} \texttt{C} \texttt{C} \texttt{C} \texttt{A} \texttt{A} \texttt{C} \texttt{A} \texttt{G} \texttt{C} \texttt{C} \texttt{C} \texttt{C} \texttt{A} \texttt{A} \texttt{C} \texttt{C} \texttt{C} \texttt{C} \texttt{C} \texttt{C} \texttt{C} C$	(S2L, L3L, Q4STOP)
IL2RG	5'-CTACCAAACATTTGTT GTC CAG CTCCAGGACCCACGGG AACCCAGGAGGCAGGACCCACAGACG-3'	Wild-type
R12I-C22	$\texttt{5'-CTACCAAACATTTGTTGTTTAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-\texttt{3'}$	(V154V, Q155STOP)
R12I-C29	5'-ctaccaaacatttgttgtccagctccaggacccacgggaacccaggaggcaggacccacagacg-3'	Wild-type
	$\texttt{5'-CTACCAAACATTTGTTGTTTAGCTCCAGGACCCACGGGAACCCAGGAGGACGCAGGACCCACAGACG-\texttt{3'}$	(V154V, Q155STOP)
R12I-C56	$\texttt{5'-CTACCAAACATTTGTTGTCCAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-\texttt{3'}}$	Wild-type
	$\texttt{5'-CTACCAAACATTTGTTGTTTAGCTTTAGGACCCACGGGAACCCAGGAGGACGCACGACGACG-\texttt{3'}$	(V154V, Q155STOP, L156L, Q157STOP)
R12I-C108	5'-CTACCAAACATTTGTTGT TT AGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3'	(V154V, Q155STOP)

Supplementary Fig. 4 Simultaneous base editing of *DMD*, *TYR*, and *LMNA* or *RAG1*, *RAG2*, and *IL2RG* genes in porcine fibroblast cells. a and b T-cloning and Sanger sequencing of base editing of *DMD*, *TYR*, and *LMNA* in individual cell colony 1#, 23#, 37# (a), and *RAG1*, *RAG2*, and *IL2RG* in cell colony 22#, 29#, 56#, 108# (b).

S	Supple	emer	ntary	Fig. 5	5					
а	•••	с с т т г		MD		TYR		LMNA		
A	WT \3A-DTL-C8	M								
ļ	\3A-DTL-C1	₩ ° ₩			AM AWY		MM IMM			
b			RAG	1 		RAG2		IL2RG		
	WT	Mhm			M MANA			MMMMM		
A	3A-R12I-C8	M	w MMM	MMM		MMMM		MMMMM		
A3/	A-R12I-C10		www	www	NIA MMM	MMM/M/	MM MAN			
C	<i>DMD</i> <i>DTL</i> -C8: <i>DTL</i> -C10:	5'-TTT 5'-TTT 5'-TTT 5'-TTT 5'-TTT	TTTTATCT TTTTTATCT TTTTTATCT	FTCAGGT <mark>GC</mark> FTCAGGTGC FTCAGGTGC FTCAGGTGC	TT <u>CAG</u> GAAGACCTA TT T AGGAAGACCTA TTCAGGAAGACCTA TTTAGGAAGACCTA	GAGCAGCAACAAGT GAGCAGGAACAAGT GAGCAGGAACAAGT GAGCAGGAACAAGT	CAGGGTCAATTC-3' CAGGGTCAATTC-3' CAGGGTCAATTC-3' CAGGGTCAATTC-3'	Wild-type (Q493STOP) Wild-type (Q493STOP)		
	TYR DTL-C8:	5'-GAG 5'-GAG 5'-GAG	GTGATGGG GTGATGGG GTGATGGG	AGTCCCT GT AGTCCCTGT AGTCCCTGT	GGC <u>CAG</u> CTCTCAGG GG TT AGCTCTCAGG GG TG AGCTCT T AGG	CAGGGGTTCCTGTC. CAGGGGTTCCTGTC. CAGGGGTTCCTGTC.	AGGACATCATTC-3' AGGACATCATTC-3' AGGACATCATTC-3'	Wild-type (13/20) (G27G, Q28STOF (7/20) (G27G, S28E)		
	DTL-CIU: LMNA DTL-C8:	5'-GAG 5'-GAG 5'-AGC 5'-AGC 5'-AGC	GTGATGGG GTGATGGG AGCTCGGG AGCTCGGG AGCTCGGG	AGTCCCTGT AGTCCCTGT AGCCCAG <mark>GT</mark> AGCCCAGGT AGCCCAGGT	GGCCAGCTCTCAGG GGC T AGCTCTCAGG GGGCGGATCCATCT GGGCGGATCCATCT GGG T GGATCCATCT	CAGGGGTTCCTGTC CAGGGGTTCCTGTC CCTCTGGCTCCTCC CCTCTGGCTCCTCC CCTCTGGCTCCTCC	AGGACATCATTC-3' AGGACATCATTC-3' GCCTCCAGTGTC-3' GCCTCCAGTGTC-3' GCCTCCAGTGTC-3'	Wild-type (Q28STOP) Wild-type Wild-type (G608G)		
_	DTL-C10	: 5'-AG 5'-AGC	CAGCTCGGG AGCTCGGG	GAGCCCAGG AGCCCAGGT	TGGGCGGATCCATC GGG T GGATCCATCT	TCCTCTGGCTCCTC CCTCTGGCTCCTCC	CGCCTCCAGTGTC-3 GCCTCCAGTGTC-3'	' Wild-type (G608G)		
d	RAG1 R12I-C8:	5'-AG 5'-AG 5'-AG	GGGAAACC GGGAAACC GGGAAACC	CTCGCT <mark>GG</mark> CTCGCTGG CTCGCTGG	AG <u>CAA</u> TCTCCAGC AGCAATCTCCAGC AG T AATCTCCAGC	AGTCCTGGACAAG AGTCCTGGACAAG AGTCCTGGACAAG	CCTGGTGGTCAGAA CCTGGTGGTCAGAA CCTGGTGGTCAGAA	G-3' Wild-type G-3' Wild-type G-3' (Q61STOP)		
	<i>K121</i> -C10	: 5'-AG 5'-AG	GGGAAACC GGGAAACC	CTCGCTGG	AGCAATCTCCAGC AG T AAT T TCCAGC	AGTCCTGGACAAG AGTCCTGGACAAG	CCTGGTGGTCAGAA CCTGGTGGTCAGAA	G-3' WIId-type G-3' (Q61STOP)		
	RAG2 5'-CCCACTGTCAGAAAATGTCACTACAGATGATAACAGTTGGTAATAACATGGCCTTAATT-3' Wild-type R12I-C8: 5'-CCCACTGTCAGAAAATGTTATTATAGATGATAACAGTTGGTAATAACATGGCCTTAATT-3' (S2L, L3L, Q4STOP) 5'-CCCACTGTCAGAAAATGTCACTATAGATGATAACAGTTGGTAATAACATGGCCTTAATT-3' (Q4STOP) 812I-C10: 5'-CCCACTGTCAGAAATGTCACTATAGATGATAACAAGTTGGTAATAACATGGCCTTAATT-3' (Q4STOP)									
	<pre>IL2RG 5'-CTACCAAACATTTGTTGTCCAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCAC-3' Wild-type R12I-C8: 5'-CTACCAAACATTTGTTGTCCAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCAC-3' Wild-type 5'-CTACCAAACATTTGTTGTTGTTGTTGTTGTCAGGACCCACGGGAACCCAGGAGGCAGGACCCAC-3' (V154V 01555T0P)</pre>									
	<i>R12I-</i> C10	: 5'-CT 5'-CT	АССАААСА АССАААСА	TTTGTTGT. TTTGTTGT	CCAGCTCCAGGAC TT AGCTCCAGGAC	CCACGGGAACCCA CCACGGGAACCCA	GGAGGCAGGACCCA GGAGGCAGGACCCA	C-3' Wild-type C-3' (V154V,Q155STOP)		
e	No. of screened colonies	No. of gene	colonies wi base editi	ith single ng (%)	No. of colonie	s with two genes I	base editing (%)	No. of colonies with three genes base editing (%)		
		DMD	TYR	LMNA	DMD and TYR	TYR and LMNA	DMD and LMNA	DMD, TYR and LMNA		
	20	0 RAG1	0 RAG2	0 IL2RG	1 (5.0) <i>RAG1</i> and <i>RAG2</i>	1 (5.0) <i>RAG1</i> and	3 (15.0) RAG2 and	11 (55.0) RAG1, RAG2 and IL2RG		
	20	2 (10.0)	2 (10.0)	1 (5.0)	0	0	2 (10.0)	8 (40.0)		

Supplementary Fig. 5 hA3A-BE3-mediated multiple genes base editing in porcine somatic cells. a and b Sanger sequencing results of the target sites in cell colony A3A-DTL-C8, C10 (a) and A3A-R12I-C8, C10 (b). c and d T-cloning and Sanger sequencing of base editing of A3A-DTL-C8, C10 (c) and A3A-R12I-C8, C10 (d). e Summary of multiple sites base editing by hA3A-BE3 in porcine somatic cells

	a	WT								pol -C30														
	b					•																		
	[,	Val (V)		(Gln(Q)		A	Arg(R)			Leu(L)			lle (I)		G	iln(Q)		G	iln(Q)			
		G	Т	т	с	Α	Α	Α	G	Α	Т	Т	Α	Α	т	С	С	Α	Α	С	Α	G	G	G
	T	0.0	100.0	100.0	84.9	0.0	0.0	0.0	0.0	0.0	100.	0 100.0	0.0	0.0	100.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pol- C30	C	0.0	0.0	0.0	13.2	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	98.0	100.0	0.0	0.0	100.0	0.0	100.0	100.0	100.0
	Δ	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0
	~1																							
	т	0.0	100.0	100.0	84.0	0.0	0.0	0.0	0.0	0.0	100.	0 100.0	0.0	0.0	100.0	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pol-	c	0.0	0.0	0.0	13.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	97.6	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
C87	G	100.0	0.0	0.0	2.4	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
	A	0.0	0.0	0.0	0.0	100.0	100.0	100.0	0.0	100.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0

С

Gene	No. of screened colonies	No. of colonies with base editing (<50%)	No. of colonies with base editing (≥50%)			
pol	155	40 (25.8%)	59 (38.1%)			

Supplementary Fig. 4 Base editing of the *pol* gene in porcine somatic cells. a Photograph of selected *pol*-inactivated and WT colony. Scale bars: $20 \,\mu\text{m}$. b Summary of the deep sequencing of the on-target sites of the *pol* gene in cell colony 30# and 87#. c Summary of base editing efficiency of selected cell colonies.

LMNA+/+

LMNA^{G608G/+}



Supplementary Fig. 7 Photographs of the $LMNA^{+/+}$ and $LMNA^{G608G/+}$ cell colonies. $LMNA^{G608G/+}$ cell colony exhibited premature senescence phenotypes. Scale bars: 20 µm.

	G608G
357-1	
357-2	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM
357-3	MMMMMMMMMMMMMMMMMMMMMMMM
357-4	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM
357-5	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM
357-6	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM
357-7	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM
357-8	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM
357-9	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM

Supplementary Fig. 8 Sanger sequencing results of LMNA^{G608G} mutant piglets



Supplementary Fig. 9 *LMNA* c.1824C > T mutations in the heart, liver, spleen, lung and kidney of 357-8 and 357-9 piglets were detected by Sanger sequencing

Pig <i>LMI</i>	VA OT-1	OT-2	OT-3	OT-4	OT-5	OT-6	OT-7
WT							
357-1							
357-2							
357-3							
357-4							
357-5							
357-6							
357-7							
357-8							
357-9		Mmmmmm	Momm	Mmmmm	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM	Malan

Supplementary Fig. 15 Off-target analysis of *LMNA^{G608G}* mutant piglets.

DMD mutant colonies

	TTTTTATCTTTCAGGT GCTT<u>CAG</u>GAAGACCTAGAGCAGG AACAAGTCAGGGTCAATTCTCTCAC	Wild-type
<i>DMD</i> -C3:	TTTTTATCTTTCAGGTGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(∆18)
	TTTTTATCTTTCAGGTGCTT T AGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(Q493STOP)
<i>DMD</i> -C17:	${\tt TTTTTATCTTTCAGGTGCTTCAGGAAGAACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC}$	Wild-type
	TTTTTATCTTTCAGGTGCTT T AGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(Q493STOP)
<i>DMD</i> -C36:	TTTTTATCTTTCAGGTGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(∆18)
	TTTTTATCTTTCAGGTGCTT T AGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(Q493STOP)
<i>DMD</i> -C59:	TTTTTATCTTTCAGGTGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(∆18)
	TTTTTATCTTTCAGGTGCTT T AGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(Q493STOP)
<i>DMD</i> -C67:	${\tt TTTTTATCTTTCAGGTGCTTCAGGAAGAACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC}$	Wild-type
	TTTTTATCTTTCAGGTGCTT T AGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(Q493STOP)
<i>DMD</i> -C87:	TTTTTATCTTTCAGGTGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(∆18)
	TTTTTATCTTTCAGGTGCTT T AGGAAGACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC	(Q493STOP)
<i>DMD</i> -C92:	${\tt TTTTTATCTTTCAGGTGCTTCAGGAAGAACCTAGAGCAGGAACAAGTCAGGGTCAATTCTCTCAC}$	Wild-type
	TTTTTATCTTTCAGGTGCTT T A G G A G G A G G G G G G G G	(Q493STOP)

Supplementary Fig. 11 Genotype of selected cell colonies for SCNT to generate *DMD* heterozygous piglets.



Supplementary Fig. 12 Genotype of newborn *DMD* mutant piglet. a and b T-cloning and Sanger sequencing of base editing of *DMD* in newborn T62-1-1 piglet.

R12I mutant piglets

RAG1 5'-AGGGGAAACCCTCGCTGGAG <u>CAA</u> TCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAGTCAGC-3' Wild-type
A632-1 5'-AGGGGAAACCCTCGCTGGAGTAATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAGTCAGC-3' Q61STOP
A632-2 5'-AGGGGAAACCCTCGCTGGAGCAATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAGTCAGC-3' Wild-type
5'-AGGGGAAACCCTCGCTGGAGTAATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAGTCAGC-3' (Q61STOP)
A632-3 5'-AGGGGAAACCCTCGCTGGAGCAATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAGTCAGC-3' Wild-type
A633-1 5'-AGGGGAAACCCTCGCTGGAGCAATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAGTCAGC-3' Wild-type
A633-2 5'-AGGGGAAACCCTCGCTGGAGCAATCTCCAGCAGTCCTGGACAAGCCTGGTGGTCAGAAGTCAGC-3' Wild-type
RAG2 5'-CCCACTGTCAGAAAATGTCACTA <u>CAG</u> ATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' Wild-type
A632-1 5'-CCCACTGTCAGAAAATGTCA <mark>T</mark> TA <mark>A</mark> AGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' L3L, Q4K
5'-CCCACTGTCAGAAAATGTTATTATAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L, L3L, Q4STOP
A632-2 5'-CCCACTGTCAGAAAATGTTATTATAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L, L3L, Q4STOP
A632-3 5'-CCCACTGTCAGAAAATGTTACTACAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L
5'-CCCACTGTCAGAAAATGTTATTACAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L, L3L
A633-1 5'-CCCACTGTCAGAAAATGTTATTACAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L, L3L
5'-CCCACTGTCAGAAAATGTTATTATAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L, L3L, Q4STOP
A633-2 5'-CCCACTGTCAGAAAATGTTATTACAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L, L3L
5'-CCCACTGTCAGAAAATGTTATTATAGATGATAACAGTTGGTAATAACATGGCCTTAATTCAGCC-3' S2L, L3L, Q4STOP
IL2RG 5'-CTACCAAACATTTGTTGTCCAGGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' Wild-type
A632-1 5'-CTACCAAACATTTGTTGTTTAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' V154V, Q155STOP
A632-2 5'-CTACCAAACATTTGTTGTTTAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' V154V, Q155STOP
A632-3 5'-CTACCAAACATTTGTTGTCCAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' Wild-type
5'-CTACCAAACATTTGTTGTTTAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' V154V, Q155STOP
A633-1 5'-CTACCAAACATTTGTTGTCCAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' Wild-type
5'-CTACCAAACATTTGTTGTTTAGGTTTAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' V154V, Q155STOP, L156L, Q157STOP
A633-2 5'-CTACCAAACATTTGTTGTCCAGCTCCAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' Wild-type
5'-CTACCAAACATTTGTTGTTTAGGTTTAGGACCCACGGGAACCCAGGAGGCAGGACCCACAGACG-3' V154V, Q155STOP, L156L, Q157STOP

Supplementary Fig. 13 Genotype of newborn RAG1, RAG2, and IL2RG mutant piglets.

DMD

	OT-1	OT-2	OT-3	OT-4	OT-5
	CCTGCTCTAGGTCTTCCTGTCCC	GCTGCTGGAAGACCTAGATCTGG	GGTTCAAGAAGACCCAGAGCAGG	CCTGCTGGAGGTCTTCCAGAAGC	GCTTCAGAAAGACATAGAGATGG
WT					
T62-1-1#			Amahamahan		
	OT-6	OT-7	8-TO		OT-10
WT					
T62-1-1#	CCAGCACTAGGTGTTCCTGAAGG	CCTGCTCTCAGTGTTCCTGAAGC	CCTACTCTCGGTCTCCCTGAAGC	GTTTCAGGAAGACCTGCAGCAGG	GCTACAGGCTGACCTAGAGCTGG
102 1 1/		<u></u>	MMMMMMM		
	OI-11 CCTGCTCTCGGTCATCCTGGAGC	OI-12 GCTTCAGGGAGCCCAAGAGCTGG	OI-13 CCTGCTCCTGGTCTTCCTGAAGG	OI-14 CCTACAGGAAGATCTAGAGCAGG	CCAGCTGCAGGTCATCCTGAAGC
WT					
T62-1-1#					
	OT-16				
WT					
T62-1-1#		GCTTCAGGAACACCTGGAACTGG	CCAGCTCTAGGTCCTCCTGAGCC		
	OT-21 CCTGCAGGAAGACCAAGAGCCGG				
wт	Amanananan				
	CCTGCAGGAAGACCAAGAGCCGG				
T62-1-1#	A.M.M.M.M.M.M.M.M.M.M.M.M.M.M.M.M.M.M.M				

Supplementary Fig. 14 Off-target analysis of DMD mutant piglet (T62-1-1).

RAG1

GGA	OT-1 Agcaagatccagcagtcc	OT-2 gcagctatccccagcagtcc	OT-3	OT-4	OT-5	OT-6	OT-7	c
wт 📈	han han han ha	Mana Mana Mana Mana Mana Mana Mana Mana	MMMM	MMMMMMMM	. M.M.M.M.M.	MMMMMMMM	MAMMAMM 200 210	M
GGA	AGCAAGATCCAGCAGTCC	GCAGCTATCCCCAGCAGTCC	GGACTGCTGCAGATTTGTCC	GGACTGCTGGAGCTTTCTGG	GGACTGCTGGTGCTTGCTTT	GGACTGCTGGAGAAAGCTCT	GTGGTAATTTCCAGC <mark>G</mark> GTC	c
A632-1	Minhanne	Maralan Maralan	mmmmm	and	Ammanna	Mahahaha	. MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM	M
GGA	AGCAAGATTCAGCAGTCC	GCAGCTATCCCCAGCAGTCC	GGACTGCTGCAGATTTGTCC	GGACTGCTGGAGCTTTCTGG	GGACTGCTGGTGCTTGCTTT	GGACTGCTGGAGAAAGCTCT	GTGGTAATTTCCAGC <mark>G</mark> GTC	с
A632-2	Ann 180 190	Man	AMAMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	<u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>		AMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	MAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
A622.2	AGCAAGATCCAGCAGTCC	GCAGCTATCCCCAGCAGTCC	GGACTGCTGCAGATTTGTCC	GGACTGCTGGAGCTTTCTGG	GGACTGCTGGTGCTTGCTT	GGACTGCTGGAGAAAGCTCT	GTGGTAATTTCCAGC	c
A032-3	MMM 190	MAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	MAMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM	AAMAMAMAMAMAMA	AMAMAMAMAMA	AMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	MAMMAMAAAAA	M
4633-1	AGCAAGATCCAGCAGTCC	GCAGCTATCCCCAGCAGTCC	GGACTGCTGCAGATTTGTCC	GGACTGCTGGAGCTTTCTGG	GGACTGCTGGTGCTTGCTTT	GGACTGCTGGAGAAAGCTCT	GTGGTAATTTCCAGCGGTC	c
	hunn	MAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	. My Man My Mark	170 MMMMMMM	Mymmym		. AMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Δ
A622.2	AGCAAGATCCAGCAGTCC	GCAGCTATCCCCAGCAGTCC	GGACTGCTGCAGATTTGTCC	GGACTGCTGGAGCTTTCTGG	GGACTGCTGGTGCTTGCTTT	GGACTGCTGGAGAAAGCTCT	GTGGTAATTTCCAGC	c
A033-2 M	180 190		MMMMM	70 180	1 AMAMAMAMA 340 350	AMAMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AM200 MAAAAA210	M
R	AG2							
TCG	OT-1 gctacagatgttaacagt	OT-2 CCAGGACAGATGATAACAGT	OT-3	OT-4	OT-5 ACTGTTATCAGCAGTAGTAC	OT-6 actgttatcagcggtattaa	OT-7	OT-8 CTACTACAGATGATAACATT
wт 👧	mmmm	mmmm	AMAMAMAMAMA	mmmm	mannan	As Amaran March	mahannahan	Amman
TCG	GCTACAGATGTTAACAGT	CCAGGACAGATGATAACAGT	TTACTACTGATGATAACAGG	TGCCTACAGATGATAACAGC	ACTGTTATCAGCAGTAGTAC	ACTGTTATCAGCGGTATTAA	ACTGTTATCACCTGTGTAGA	CTACTACAGATGATAACATT
A632-1 👧	mmmmm	mmmm	amanahanna	mannahaman	mahammanam	mmmmmm	mannanan	mmmmm
тсс	GCTACAGATGTTAACAGT	CCAGGACAGATGATAACAGT	TTACTACTGATGATAACAGG	TGCCTACAGATGATAACAGC	ACTGTTATCAGCAGTAGTAC	ACTGTTATCAGCGGTATTAA	ACTGTTATCACCTGTGTAGA	CTACTACAGATGATAACATT
A632-2 MA	mmm	mmmm	Amanahamana	mmmmmm	mann	mannon	mahannahan	montan
TCG	GCTACAGATGTTAACAGT	CCAGGACAGATGATAACAGT	TTACTACTGATGATAACAGG	TGCCTACAGATGATAACAGC	ACTGTTATCAGCAGTAGTAC	ACTGTTATCAGCGGTATTAA	ACTGTTATCACCTGTGTAGA	CTACTACAGATGATAACATT
A632-3	MMMMMMMM	M.M.M.M.M.	AMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AMAMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	MM 250 250	Mahalana Maha	200 AMAMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AAM100000000000000000000000000000000000
TCG	GCTACAGATGTTAACAGT	CCAGGACAGATGATAACAGT	TTACTACTGATGATAACAGG	TGCCTACAGATGATAACAGC	ACTGTTATCAGCAGTAGTAC	ACTGTTATCAGCGGTATTAA	ACTGTTATCACCTGTGTAGA	CTACTACAGATGATAACATT
					٨			

A633-1 manufalance under alle and a second alle and a second alle and a second a secon

IL2RG

A633-1

A633-2

	OT-1	OT-2	OT-3 gtgggtcctggagctggaat	OT-4	OT-5
WT	MMMM MMM	Mana Man Mana	170 160 160	Mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm	MMMMMM 430
	TTTCCAGCTCCAGGACCCAA	GGTCCAGATCCAGGACCCAC	GTGGGTCCTGGAGCTG <mark>A</mark> AAT	TGTACAGCTCCAGGACCCGC	GTTGGTCCTGGAGCTGGACT
A632-1	MM2000 200	Marra Agama Alama	annananananananananananananananananana	Mm Mm Marm	MMMMMMMM
	TTTCCAGCTCCAGGACCCAA	GGTCCAGATCCAGGACCCAC	GTGGGTCCTGGAGCT <mark>AA</mark> AAT	TGTACAGCTCCAGGACCCGC	GTTGGTCCTGGAGCTGGACT
A632-2	MAAAAAAA	Manna Mana Manaa		Man	<u></u>
	TTTCCAGCTCCAGGACCCAA	GGTCCAGATCCAGGACCCAC	GTGGGTCCTGGAGCTGGAAT	TGTACAGCTCCAGGACCCGC	GTTGGTCCTGGAGCTGGACT
A632-3	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM	Marana Marana Marana	AMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Margan Margan	20MmMalashamma
	TTTCCAGCTCCAGGACCCAA	GGTCCAGATCCAGGACCCAC	GTGGGTCCTGGAGCT <mark>AA</mark> AAT	TGTACAGCTCCAGGACCCGC	GTTGGTCCTGGAGCTGAACT
A633-1	MM AM AM	Margan Mars Mar Mar	AMMAAAMMAAAM	Manan Maran	MMMMMMMM
	TTTCCAGCTCCAGGACCCAA	GGTCCAGATCCAGGACCCAC	GTGGGTCCTGGAGCT <mark>AA</mark> AAT	TGTACAGCTCCAGGACCCGC	GTTGGTCCTGGAGCTGGACT
A633-2	MMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Mana Mana Mana	180 170	MAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	260 250
	ОТ-6	ОТ-7	ОТ-8	ОТ-9	OT-10
	AGCCCAGCCCCAGGACCCAC	TTTCCAGCCTCAGGACCCAC	GGTGCTGCTCCAGGACCCAC	GTGGGTCCTAGATCTGCACA	ATGGGTCCTGGAGCTGCTCA
WT	MMMMMM 210 220	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM	170 MARANA 180 MARAN	
	AGCCCAGCCCCAGGACCCAC	TTTCCAGCCTCAGGACCCAC	GGTGCTGCTCCAGGACCCAC	GTGGGTCCTAGATCTGCACA	ATGGGTCCTGGAGCTGCTCA
A632-1	MMMMMMM	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	MMMMMMM	MMMMM	MAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AGCCCAGCCCCAGGACCCAC	TTTCCAGCCTCAGGACCCAC	GGTGCTGCTCCAGGACCCAC	GTGGGTCCTAGATCTGCACA	ATGGGTCCTGGAGCTGCTCA
A632-2	MMMMMMMMM	AMAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	MMMMMMMMM	MAMMA MAAMAA	170 180
	AGCCCAGCCCCAGGACCCAC	TTTCCAGCCTCAGGACCCAC	GGTGCTGCTCCAGGACCCAC	GTGGGTCCTAGATCTGCACA	ATGGGTCCTGGAGCTGCTCA

Supplementary Fig. 15 Off-target analysis of RAG1, RAG2, and IL2RG mutant piglets.

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Supplementary Fig. 16 Photographs of the lungs from piglets A632-1, A633-1, and A633-2, died 49, 41, and 74 days after birth.



Supplementary Fig. 17 RT-PCR and Sanger sequencing of *IL2RG* mRNA in peripheral blood of A632-1, A632-2, A632-3, A633-1, A633-2, and WT piglets.