

Efficient generation of gene-modified pigs via injection of zygote with Cas9/sgRNA

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C1-1	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----AACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----GGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC </pre>	(WT, 0/14) (-24, 8/14) (-9, 6/14)
C1-2	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGC-----ACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----GGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC </pre>	(WT, 0/15) (-16, 7/15) (-9, 8/15)
C1-4	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATG-----TACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC ----- (-89bp) -----GCAACGCCACGCGCTGGCTC ----- (-104bp,+270bp) -----GCAACGCCACGCGCTGGCTC </pre>	(WT, 0/13) (-9, 1/13) (-89, 10/13) (-104, +270, 2/13)
C2-1	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGCTCCA-----GCTC catggagccctggaattgagccagcgtggcgcttgacagggccgagccgtac GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----AACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGCTCC----- (-412bp) ----- </pre>	(WT, 0/14) (-42, +56, 5/14) (-24, 5/14) (-412, 4/14)
C2-3	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGCTCCATG-----TGCACGCCACGCGCTGGCTC ggtcca GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----TCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGT-----GGCCCTGTGCAACGCCACGCGCTGGCTC </pre>	(WT, 0/14) (-24, +7, 3/14) (-12, 2/14) (-9, 9/14)
C2-4	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----GGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTG-----CTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC +535bp </pre>	(WT, 0/13) (-9, 12/13) (-6, +535, 1/13)
C2-5	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTC-----TACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC t GCCGCCGCCACCCTGGCCGTGGGCTCCATG-----TACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----AACGCCACGCGCTGGCTC </pre>	(WT, 0/12) (-13, 6/12) (+1, 2/12) (-9, 3/12) (-24, 1/12)
C2-6	<pre> GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCGTCTACGGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCT-----GGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----AACGCCACGCGCTGGCTC GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGC-----GGCTCGGCCCTGTGCAACGCCACGCGCTGGCTC at GCCGCCGCCACCCTGGCCGTGGGCTCCATGTGCGGCG-----CCCTGTGCAACGCCACGCGCTGGCTC +509bp </pre>	(WT, 0/13) (-28, 3/13) (-24, 2/13) (-9, +2, 7/13) (-12, +509, 1/13)

Figure S1 Sequences of the modified *NpcIII* alleles detected in the live founder pigs

Sequencing results of the modified *NpcIII* alleles detected in the live founder pigs. PCR products of the targeted region of *NpcIII* were amplified from all the founder pigs and sequenced. The PAM sequences are highlighted in green; the targeting sequences in red; the mutations in blue, lower case; deletions (-), and insertions (+). N/N indicates positive colonies out of total sequenced.

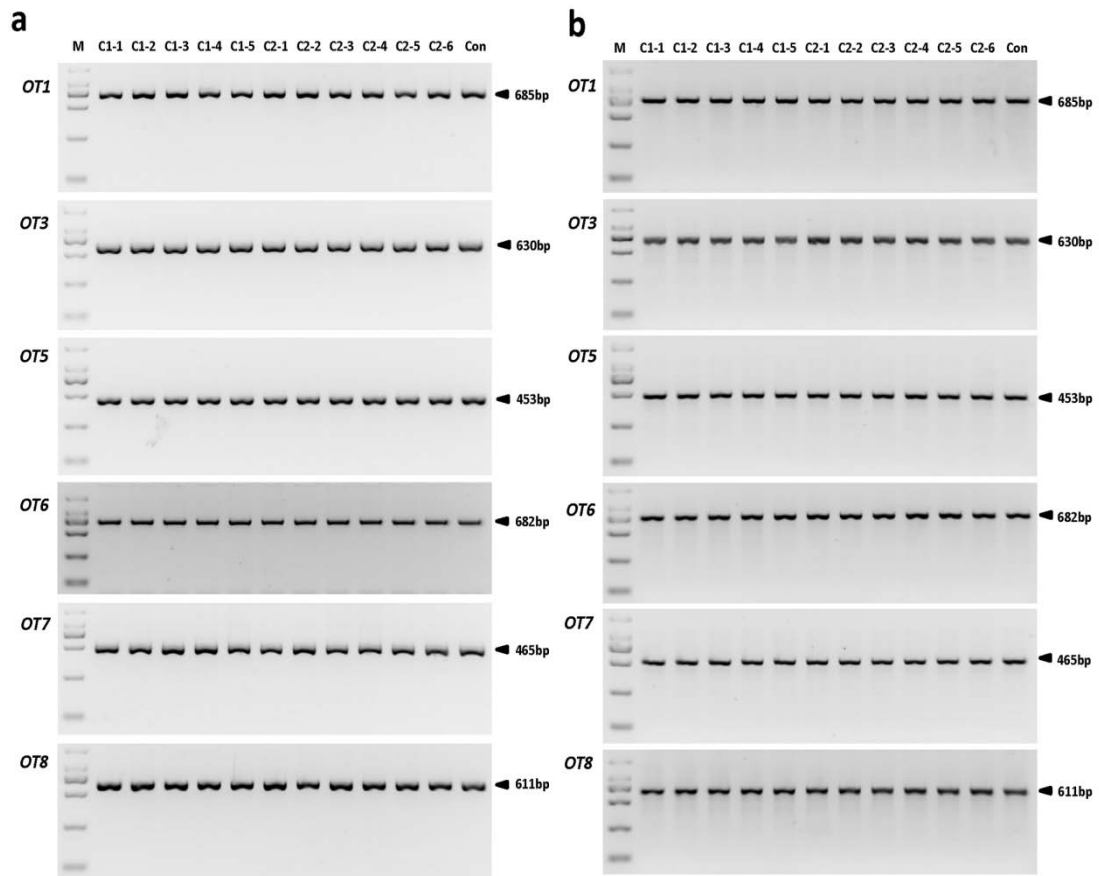


Figure S2 Detection of the *Npc111* sgRNA:Cas9-mediated off-target cleavages *in vivo*

(a) PCR products of the potential off-target sites of *Npc111* sgRNA:Cas9 from founder pigs.

A total of 8 potential off-target sites most homologous to *Npc111* sgRNA were named OT1 to OT8. OT1, 3, 5~8 were subjected to PCR amplification using genomic DNA from founders.

(b) Detection of *Npc111* sgRNA:Cas9-mediated off-target cleavage by T7EN1 cleavage assay. All PCR products from (a) were subjected to T7EN1 cleavage assay.

Supplementary Table 1 List of putative off-target sites homologous to *NpcIII*-sgRNA

Position	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	N	G	G	Location				
pNpc1L1	G	G	C	T	C	C	A	T	G	T	G	C	G	G	C	G	T	C	T	A	C	G	G	Chro.	Strand	Start	End	
OT1	G	G	C	T	C	C	A	T	G	T	G	C	A	G	A	G	G	C	T	A	C	A	G	1	+	55561919	55561942	
OT2	G	G	C	T	C	T	G	T	T	T	G	C	G	G	C	G	T	C	T	A	A	A	G	6	-	151976910	151976933	
OT3	G	G	C	T	G	C	A	T	G	T	G	C	G	G	C	G	T	A	T	G	G	A	G	7	+	53582684	53582707	
OT4	G	G	C	C	C	C	G	T	G	C	G	C	A	G	C	G	T	C	T	A	C	A	G	8	-	631994	632017	
OT5	G	G	C	T	C	C	A	C	C	T	G	C	G	G	C	G	T	A	T	A	G	A	G	9	+	8212187	8212210	
OT6	G	G	C	T	C	C	A	T	G	G	G	C	G	G	C	G	C	C	T	C	C	G	G	10	+	44897687	44897710	
OT7	T	G	C	T	C	C	A	T	G	C	C	C	T	G	C	G	T	C	T	A	C	A	G	16	-	82099593	82099616	
OT8	G	G	C	T	A	C	A	T	G	T	G	C	T	G	C	G	T	C	T	T	C	A	G	18	-	23387983	23388006	

PAM is shaded in pink, NAG is highlighted. Bases identical to sgRNA are shaded in red.

Supplementary Table 2 Oligonucleotides for generating *Npc111*-sgRNA expression vector

<i>Npc111</i> -sgRNA top strand	5'-TAGGCTCCATGTGCGGCGTCTA
<i>Npc111</i> -sgRNA bottom strand	5'-AAACTAGACGCCGCACATGGAG

Supplementary Table 3 Primers for genotyping and amplifying Cas9/sgRNA-targeted*Npc111* fragment

Name	Sequence	Amplicon
pNpc111 For	5'-TGCATTGCCACAACACCTGCAGC	648
pNpc111 Rev	5'-TGCTAAGGAGGCTGTAGGTGGAG	

Supplementary Table 4 Sequences of primers for PCR amplification of *Npc111*

sgRNA:Cas9 off-target sites

Name	Sequence	Amplicon
OT1 For	5'-GGAGACACCTTGAGACTTCAGA	685
OT1 Rev	5'-CCAGGAACCTTGTGAGGAATGC	
OT2 For	5'-ATCTGGTCGTCTGTCTTAGGTT	681
OT2 Rev	5'-GCACAGGTATTCTCATCACAATC	
OT3 For	5'-AGACTGCCAGCAGACAACCAA	630
OT3 Rev	5'-GAGCCGCATCTGTGACCTACA	
OT4 For	5'-AGGCAACAGTTGGCATCCATAAC	757
OT4 Rev	5'-TGAGATGAAGACGGAGCGACTC	
OT5 For	5'-CTATGAAGACAGTGAATGACTC	453
OT5 Rev	5'-CAGGATAGAAGTTCCCGACAT	
OT6 For	5'-TTGAGTGAGTGTGAATGTTCTG	682
OT6 Rev	5'-CAGTCCAAAGCATAAACCTAATC	
OT7 For	5'-GCTTCCFTTCCGACTGCCTATGT	465
OT7 Rev	5'-GGTGTGCTGCTCGTGCCCTAAT	
OT8 For	5'-TGGAGACCTGACTTCATCTAGG	611
OT8 Rev	5'-GGCATCTGAGATAGGAGCATTC	