Supplementary data

Production of biallelic CMP-Neu5Ac hydroxylase knock-out pigs

Deug-Nam Kwon*[1], Kiho Lee*[2], Man-Jong Kang*[3], Yun-Jung Choi[1], Chankyu Park[1], Jeffrey J Whyte[2], Alana N Brown[2], Jae-Hwan Kim[4], Melissa Samuel[2], Jiude Mao[2], Kwang-Wook Park[2,5], Clifton N Murphy[2], Randall S. Prather[2†], and Jin-Hoi Kim[1†]

[1]Department of Animal Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea
[2]Division of Animal Science, University of Missouri-Columbia, Columbia, Missouri

[3]Department of Animal Science, Chonnam National University, Gwangju 500-757, Republic of Korea

[4]CHA Stem Cell Institute, Graduate School of Life Science and Biotechnology, Pochon CHA University, Seoul 135-907, Republic of Korea

[5]Department of Animal Science and Technology, Sunchon National University, Suncheon, Jeonnam 540-742, Republic of Korea

Supplementary data include 5 tables and 2 figures

Supplementary table 1. Primer sets used to construct donor DNA with a minimum homology length in the ZFN mediated gene targeting

Donor		Forward primer	Reverse primer	PCR product
780 bp	Left	GCgcggccgcCCACTCTCTATTTGGTGGCT	GCgaattc GGAGTTTCTTCCTTTCTG	789 bp
	Right	CGaagcttCCTACAACCCAGAATTTACTGC	CGctcgagAACAGGGACCTGCCAAGAGGCC	763 bp
240 bp	Left	GCgcggccgcGTCTTTGACTAGTTTGGGAT	GCgaattc GGAGTTTCTTCCTTTCTG	240 bp
	Right	CGaagcttCCTACAACCCAGAATTTACTGC	CGctcgagACACTCTTGTATTTAATTTT	240 bp
200 bp	Left	GCgcggccgcAAAGCCACTAATGACAAGGAGC	GCgaattc GGAGTTTCTTCCTTTCTG	192 bp
	Right	CGaagcttCCTACAACCCAGAATTTACTGC	CGctcgagTTCAGGCTGCTTGTTAAAAT	200 bP
160 bp	Left	GCgcggccgcTTCTGCATCACTCAACTGTC	GCgaattc GGAGTTTCTTCCTTTCTG	160 bp
	Right	CGaagcttCCTACAACCCAGAATTTACTGC	CGctcgagTCATTCTTTCTCATTTCATG	160bp
76 bp	Left	GCgcggccgcGAGAAGACTAATCCAAACCC	GCgaattc GGAGTTTCTTCCTTTCTG	76 bp
	Right	CGaagcttCCTACAACCCAGAATTTACTGC	CGctcgagTATCCAGCCATACTTGTCTG	76 bp
Primer A&B		TCGTGCTTTACGGTATCGCCGCTCCCGATT	AAGACTCCCACTTTAAAGGGTGGTGTGTAG	1.9 kb

Supplementary table 2. Primer sets used for screening and genotyping KO pigs

Primers	Sequences (5’-3’)
Neo 3-1	TCGTGCTTTACGGTATCGCCGCTCCCGATT
ScS5	CCCTTCCATCCCACCCGTCCTCATCCTTAC
ScAS3	AAGACTCCCACTTTAAAGGGTGGTGTGTAG
CMAH-F	TCCAAACCCTGTCATTCCAGAGGA
CMAH-R	ACTCTCTGTTTTCAGGCTGCTTGTT

Supplementary table 3. Primer sets used to identify no integration of ZFN constructs in the genome of KO pigs.

Primers	Sequences (5’-3’)
FokIF1	CGGACGGAGCAATTTATACT
FokIR1	CCACCATTCATTAGGGTTGA
HPRTF1	GACTAGCATTCCTACTGCTTGCTG
HPRTR1	CCATGCTACTCAGGACAAGTTGAC

Supplementary table 4. Primer sets used to identify off-targeting.

OFF-TARGET GENE LOCI: CMAH ZFN MUTAGENESIS				
Gene	**Abbrivi ation**	**Forward primer**	**Reverse primer**	**Pro duct**

ArfGAP with GTPase domain, ankyrin repeat and PH domain 1	AGAP1	AGCTTGTGGATTTGCGTCTT	CCCCTTCCCTTTGCTTATTG	763 bp
Transient receptor potential cation channel, subfamily M, member 7	TRPM7	TCAGTTTTCCAGAGGCTGGT	CTTTTGCAACTTGGCTGAGA	211 bp
Nonhomologous end-joining factor 1	NHEJ1	GGAATAGCCAAGTGGGATGA	TCACAGTGGGATCACCTTCA	574 bp
Tousled-like kinase 2	TLK2	GGCTGCTCTGAGTGAGGAAA	CTGCGTCTGACTGGAATGAA	430 bp
Tropomodulin 2	TMOD2	TCAACCTGCAGTGTCTCTGG	GCACAAGGCTCCAAGTTCTC	220 bp
Estrogen receptor 1	ESR1	GGGTCCAAATCAAGTGAGGA	ATGAAGCCAGGCAAGAAAAA	239 bp
A kinase (PRKA) anchor protein 13	AKAP13	ACGTGACCTTTGCTGTGTTG	TCAGATCCTCCTTGGCAAAC	334 bp
NME/NM23 nucleoside diphosphate kinase 2 (NME2)	NME2	ATGCTGCTGATCTCGTTGTG	TAGTGCCTCCTTCCCTAGCA	351 bp

Supplementary table 5. Primer sets used for detection of sialyltransferases mRNA expression

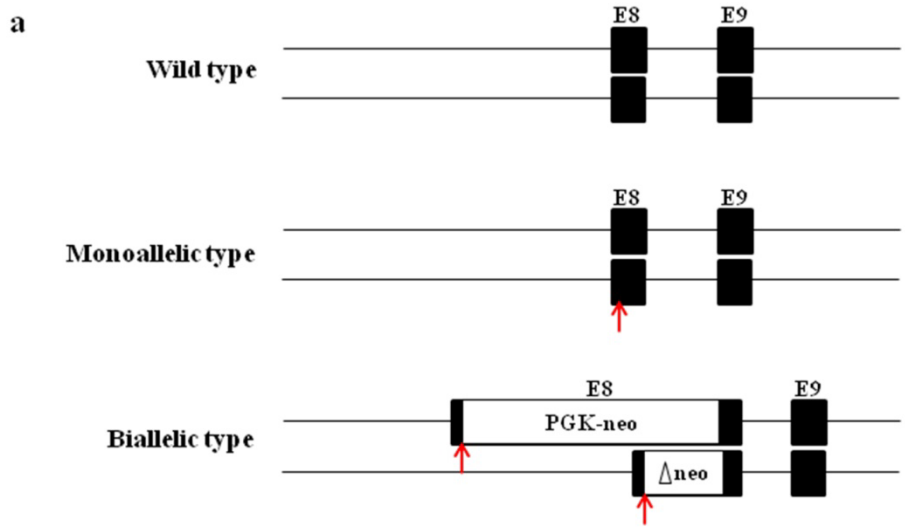
Antigen	Gene	Forward primer	Reverse primer	Product
H-D antigen	ST3Gal1	GCATCCTCTCCGTGATCTTC	CAAGATGGTTGTCACGTTGG	176
	ST3Gal2	AACCACCCACCATTTCATGT	ACTTCACTGGGGCATAGGTG	152
	ST3Gal3	GCTTCAAGTGGCAGGACTTC	ATGAGGCCATTGTTGAAAGG	193
	ST3Gal4	GCCATCACCAGCTATTCCAT	GTGGGCAGATTCAGGGTAGA	219
	ST6Gal1	TGTGTGACCAGGTGGATGTT	TCCCAAGCAGGTAGATGTCC	183
	ST6Gal2	ACCTGCCATGAAACCACACT	GGTCTCCAGGAAGGAGAAGG	151
Sialyl-Tn antigen	ST6GalNac2	GGCTGGTTCACCATGATTCT	AACACGGCCTTCTCAGTGAT	209
	ST6GalNac3	CTTCGAACTCACTATGGATAC	GAGCCAGACTGGACTCTGTCCT	467
	ST6GalNac6	ATGAGTAGCAACAAAGAGCAG	CCTGGGGCTTCTGCATCTTG	478
Tn antigen	GALNT1	CTTTCACTCGCTGTGAACCA	CTCTTCATTACGCCAGCACA	227
	GALNT2	CAGCCTCCCAGTCTGACTTC	AGCTGGGGGACTGTTTTCTT	179
	GALNT3	TTGGCCTTTGTGTAAAACCAG	TGCTTCAAGTCAAGTGGATTTC	239
	GALNT4	ATTCCAGTGGCATTCTGTCC	GACAGCTCGAGGTTCTCACC	177
	GALNT7	TCAGGGTCTGAAAGGCAGTT	TGTGGCAGTGCTTCAAAAAG	152

Supplementary Figure legend

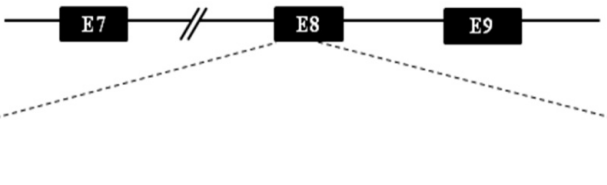
Supplementary Figure 1. (a) Genotyping of monoallelic and biallelic CMAH KO mutants. The red arrow indicates mutation region compared with wild type. (b) The target sequence of CMAH-ZFN and mutation region by non-homologous end joining in the pig CMAH locus.

The red arrow indicates an 1 bp insertion in monoallelic CMAH KO mutant. (c) The target sequence of CMAH-ZFN and mutation region by homologous recombination in the pig CMAH locus. The red arrow and under line indicate a donor DNA insertion site and a partial neo sequence in biallelic CMAH KO mutant.

Supplementary Figure 2. Chromatogram analysis of N-glycolylneuraminic acid (Neu5Gc) in wild type, monoallelic, and biallelic CMAH KO pig lysates. After isolation of the glycoproteins from each lysate of wild type, monoallelic, and biallelic CMAH KO pig, free Neu5Gc was liberated by weak acid treatment of glycoproteins. The Chromatographic peak of Neu5Ac and Neu5Gc from each sample was measured by using a calibration curve obtained for the DMB derivative of standard Neu5Ac and Neu5Gc: (a) Standard Neu5Gc, (b) Standard Neu5Ac, (c) Control pig-derived lysate, (d) Monoallelic CMAH KO pig-derived lysate, (e) Biallelic CMAH KO pig-derived lysate. Long red arrows indicate a peak of Neu5Gc and Neu5Ac, respectively. Short blue arrows indicate O-acotylated Neu5Gc and O-acotylated Neu5Ac, respectively.



b

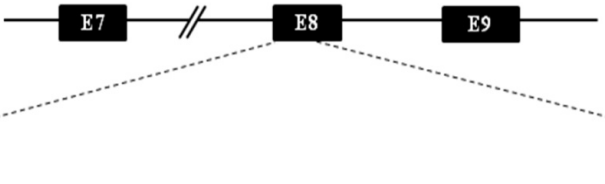


Exon 8

AGGAATGGAAAGCCCAATTCATTAAAACAGAAAGGAAGAAACTCCTGAACTACAA GGCTCGGCTGGTGAAGGACCTACAACCCAGAATT
AGGAATGGAAAGCCCAATTCATTAAAACAGAAAGGAAGAAACTCCTGAACTACAAAGGCTCGGCTGGTGAAGGACCTACAACCCAGAATT

A addition

c



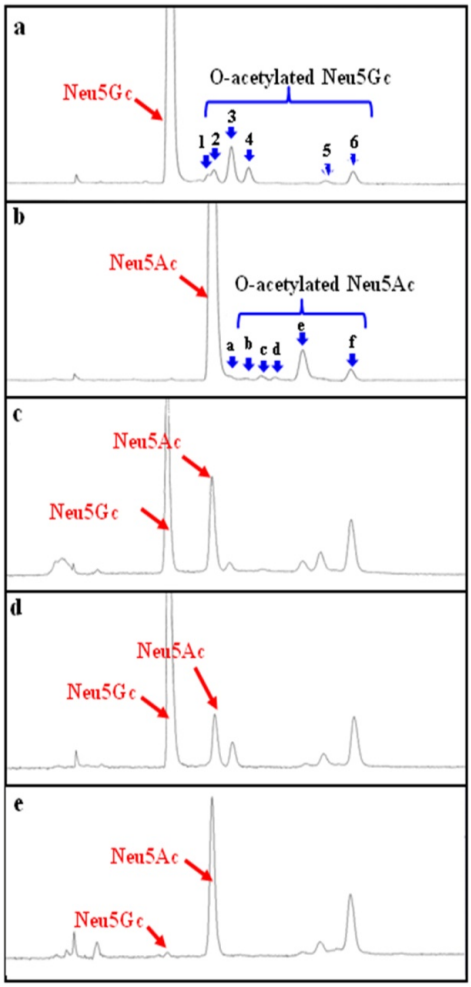
Exon 8

AGGAATGGAAAGCCCAATTCATTAAAACAGAAAGGAAGAAACTCCTGAACTACAAGGCTCGGCTGGTGAAGGACCTACAACCCAGAATT

Donor DNA insertion site

Donor DNA sequence: △neo

AGGAATGGAAAGCCCAATTCATTAAAACAGAAAGGAAGAAACTCCTACCAGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATT
CATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCCACCCGTGATATTGCTGAAGAGCTTGGCGGCGAAT
GGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA
GGGGATCCGCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATGGAAGTTTTTCCTGTCATACTTTGT
TAAGAAGGGTGAGAACAGAGTACCTACATTCTGAATGGAAGGATTGGAGCTACGGGGGTGGGGGTGGGGTGGGATTAGATAAATGCCTG
CTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAGTTGGATATCATAATTTAAACAAGCAAAACCAAATTAAGGGC
CAGCTCATTCCTCCCACTCATGATCTATAGATCCCTCGAGCCTACAACCCAGAATT



O-acetylated Neu5Gc

1. Neu5Gc7Ac
2. Neu5Gc8Ac
3. Neu5Gc9Ac
4. Neu5Gc7,8Ac_2
5. Neu5Gc7,9Ac_2
6. Neu5GC8,9Ac_2

O-acetylated Neu5Ac

a. Neu5,7Ac
b. Neu5,8Ac
c. Neu5,9Ac
d. Neu5,7,8Ac
e. Neu5,7,9Ac
f. Neu5,8,9Ac