Supplementary Information

Table S1. Oligonucleotide sequence and efficiency for *GGTA*, *β2M* and *CIITA* genes.

Table S2. Primers used for genotyping *GGTA1*, *β2M* and *CIITA* genes.

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Figure SI legends

Table S1. Oligonucleotide sequence and efficiency for *GGTA*, *β2M* and *CIITA* genes.

Name	Spacer + PAM (5'-3')	Mutation efficiency
sgGGTA-1	tggttatggtcacgacctct**ggg**	0.00%
sgGGTA-2	ctggttatggtcacgacctc**tgg**	15.00%
sgGGTA-3	aggtcgtgaccataaccaga**tgg**	30.00%
sgGGTA-4	gccaaacagaaaattaccgt**ggg**	29.41%
sgGGTA-5	atggaaggctccagtggtat**ggg**	88.24%
sgGGTA-6	taagtgccttcccataccac**tgg**	88.24%
sgGGTA-7	tcatggtggatgatatctcc**agg**	100.00%
sgGGTA-8	agaggacccagctctatcaa**agg**	12.50%
sgGGTA-9	ttgagatcaagtccgagaag**agg**	100.00%
sgGGTA-10	ctctcgtaggtgaactcgtc**agg**	5.56%
sgGGTA-11	ctgacgagttcacctacgag**agg**	0.00%
sgGGTA1-2	acgagttcacctacgagagg**cgg**	0.00%
sgGGTA-13	ggactccttccgcctctcgt**agg**	92.31%
sgGGTA-14	agaggaagtccacctcgtgc**tgg**	23.08%
sgGGTA-15	cttctgcatggacgtggatc**agg**	76.92%
sgGGTA-16	cctgtagctgagccaccgac**tgg**	26.32%
sgGGTA-17	ccagtcggtggctcagctac**agg**	15.79%
sgGGTA-18	aggatgtgccttgtaccacc**agg**	36.84%
sgβ2M-1	acagtgagagcagcccgagc**agg**	11.11%
sgβ2M-2	gagcaggaccaaggccacga**ggg**	77.78%
sgβ2M-3	ctcgggctgctctcactgtc**tgg**	44.44%
sgβ2M-4	tcgtggccttggtcctgctc**ggg**	35.00%

sgβ2M-5	gctgctctcactgtctggcc**tgg**	40.00%
sgβ2M-6	agcaggaccaaggccacgag**ggg**	55.00%
sgβ2M-7	tgagttcactcctaacgctg**tgg**	0.00%
sgβ2M-8	ctatactgatccacagcgtt**agg**	0.00%
sgβ2M-9	gagtaaacctgaaccttcgg**ggg**	0.00%
sgβ2M-10	tttggctttccgttttccgc**tgg**	0.00%
sgβ2M-11	caatttcaatctggggcgga**tgg**	0.00%
sgβ2M-12	tcacgccacccagcggaaaa**cgg**	0.00%
sgCIITA-1	tcagctgctgacggggcgaa**ggg**	0.00%
sgCIITA-2	tgctgacggggcgaagggac**tgg**	0.00%
sgCIITA-3	tggtggagccaggccggtct**ggg**	0.00%
sgCIITA-4	actttgggaggctatggaca**ggg**	0.00%
sgCIITA-5	caggccggtctggggactt**ggg**	10.00%
sgCIITA-6	ctttgggaggctatggacag**ggg**	0.00%
sgCIITA-7	gactttgggaggctatggac**agg**	15.00%
sgCIITA-8	ctggtggagccaggccggtc**tgg**	0.00%
sgCIITA-9	tgacggggcgaagggactgg**tgg**	60.00%
sgCIITA-10	tttgggaggctatggacagg**ggg**	63.16%
sgCIITA-11	ggtggagccaggccggtctg**ggg**	5.26%
sgCIITA-12	ggggaggtcagctgctgacg**ggg**	52.63%

Table S2. Primers used for genotyping *GGTA1*, *β2M* and *CIITA* genes.

Name	Sequence(5'-3')	Length
D-Pig-GGTA1-F1	GCTGCAATGTCACACCACCAG	498bp
D-Pig-GGTA1-R1	CTGAGGTGGGAAAGAGTTGACCC	
D-Pig-GGTA1-F2	CTGGGGCTAAAATACAGTCAGGATTC	376bp
D-Pig-GGTA1-R2	CCTCTCCACTAATTCAGACATGACATG	
D-Pig-GGTA1-F3	CAGCAACAGACGTCTCTCATCCAG	619bp
D-Pig-GGTA1-R3	CACTCCTGAGTGATGTTTAGAACCTG	
D-Pig-GGTA1-F4	CTCCTCTAGAAATCCCAGAGGTTAC	461bp
D-Pig-GGTA1-R4	CCTGGCCAAACGGAATGTAGG	
D-Pig-B2M-F1	GACGACATACAGAGAGTGAATCCACC	463bp
D-Pig-B2M-R1	CGAGCCGGGAAGGGAAACTTG	
D-Pig-B2M-F2	GTCCCTCTTTCTAACCTGCTCG	306bp
D-Pig-B2M-R2	CAGAGTTAGCGCCCGGAGTG	
D-Pig-B2M-F3	GGAAGCTCATTTGGCCTGAAGG	596bp
D-Pig-B2M-R3	CACTGTCTCAGGCACTGTGCC	
D-Pig-B2M-F4	CCTGGCTTTAGACACAGGTAGC	440bp
D-Pig-B2M-R4	GATGCAGATGCTTTTAGGTCAGAGCAG	
D-Pig-CIITA-F1	GATGGGGAAATCAAGGCTCCGG	539bp

D-Pig-CIITA-R1	CCCCATTGAGGTGAGATTCTCC	
D-Pig-CIITA-F2	CCCTCTCCTCTCTCCAGACTCC	369bp
D-Pig-CIITA-R2	GATCACAACTTGCTGGTCCTGC	

Table S3. Transplantation information of *GGTA1*$^{-/-}$*β2M*$^{-/-}$*CIITA*$^{-/-}$ triple gene knockout pigs.

Cell line	Surrogate ID	Embryo number	Born number
21	1700706	300	Execute was 65 days pregnant
21	1714304	298	Execute was 95 days pregnant
21	1709804	251	Born 4 Piglets
21	1721303	272	Abortion
3	1713704	225	Born 4 Piglets

Table S4. Physiological index of the neonatal *GGTA1*$^{-/-}$*β2M*$^{-/-}$*CIITA*$^{-/-}$ triple gene knockout pigs

pig ID	sex	weight(kg)	height (cm)	length (cm)	chest circumference(cm)	hipline (cm)
20180914-3-4	male	0.29	12	16	16	7
20180926-21-1	male	0.68	14	20	19	10
20180926-21-2	male	0.56	13	18	18	10
20180926-21-3	male	0.42	13	17	17	9
20180926-21-4	male	0.3	12	16	15	7

Figure SI legends

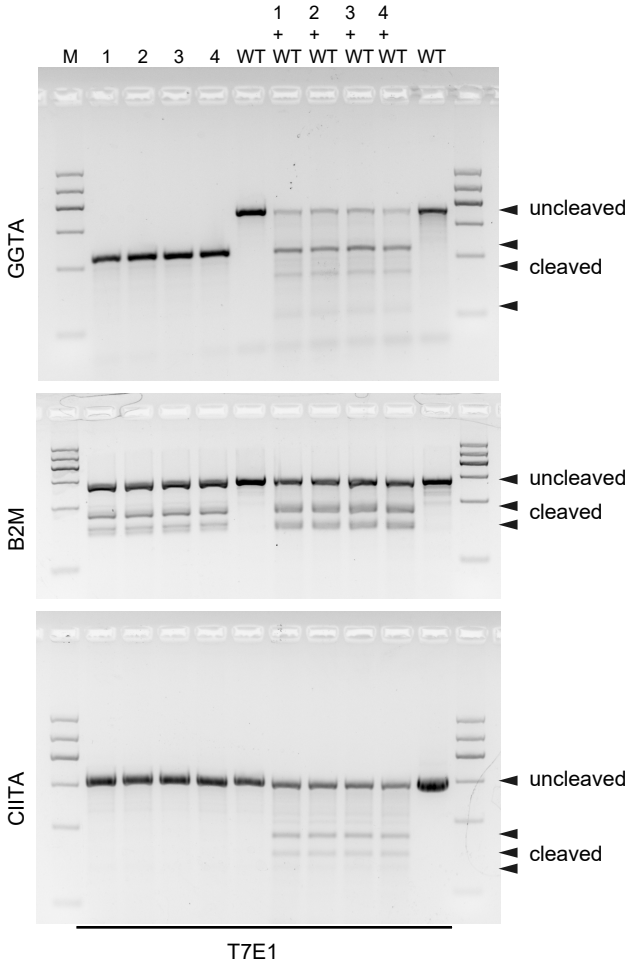
Figure S1. The pigs derived from SCNT targeting of *GGTA*/*β2M*/*CIITA* genes were identified by T7EI-based genotyping assay. Four born animals carried mutations.

Figure S2. The phenotype of GBC-3KO pigs. (A) RT-PCR was used to detect the expression of SLA-DR in the pigs' hearts and kidneys. (B) Expression of α-Gal, SLA-I, and SLA-II in

splenocytes derived from WT and GBC-3KO neonatal pigs was detected by flow cytometry. (C) T cell subtype (CD4 and CD8) percentage from neonatal GBC-3KO/WT pig PBMCs and from fetal GBC-3KO/WT pig splenocytes. The CD4 and CD8 T cells were gated from CD45 and CD3 double-positive living cells.

Figure S3. Activation of human T cells. (A) The route of CD4 T cell-gated and CD8 T cell-gated is shown. (B) Representative data of CFSE density dilution in CD3 populations is shown. (C) Comparison between WT and GBC-3KO piglet with respect to the CFSE density dilution in CD3 population (n(GBC-3KO)=6, n(WT)=3). (D) Percentage of $CD25^+$ cells in the CD3 population of the lymphocytes stimulated by GBC-3KO or WT splenocytes. (E) Comparison of the percentage of $CD25^+$ cells in the CD3 population that were stimulated by the irradiated splenocytes from WT or GBC-3KO fetal pigs. Plot graphs represent the mean ± S.D. Statistical analysis was done using *t*-test. * $P<0.05$.

Figure S1


M 1 2 3 4 WT 1 + WT 2 + WT 3 + WT 4 + WT WT
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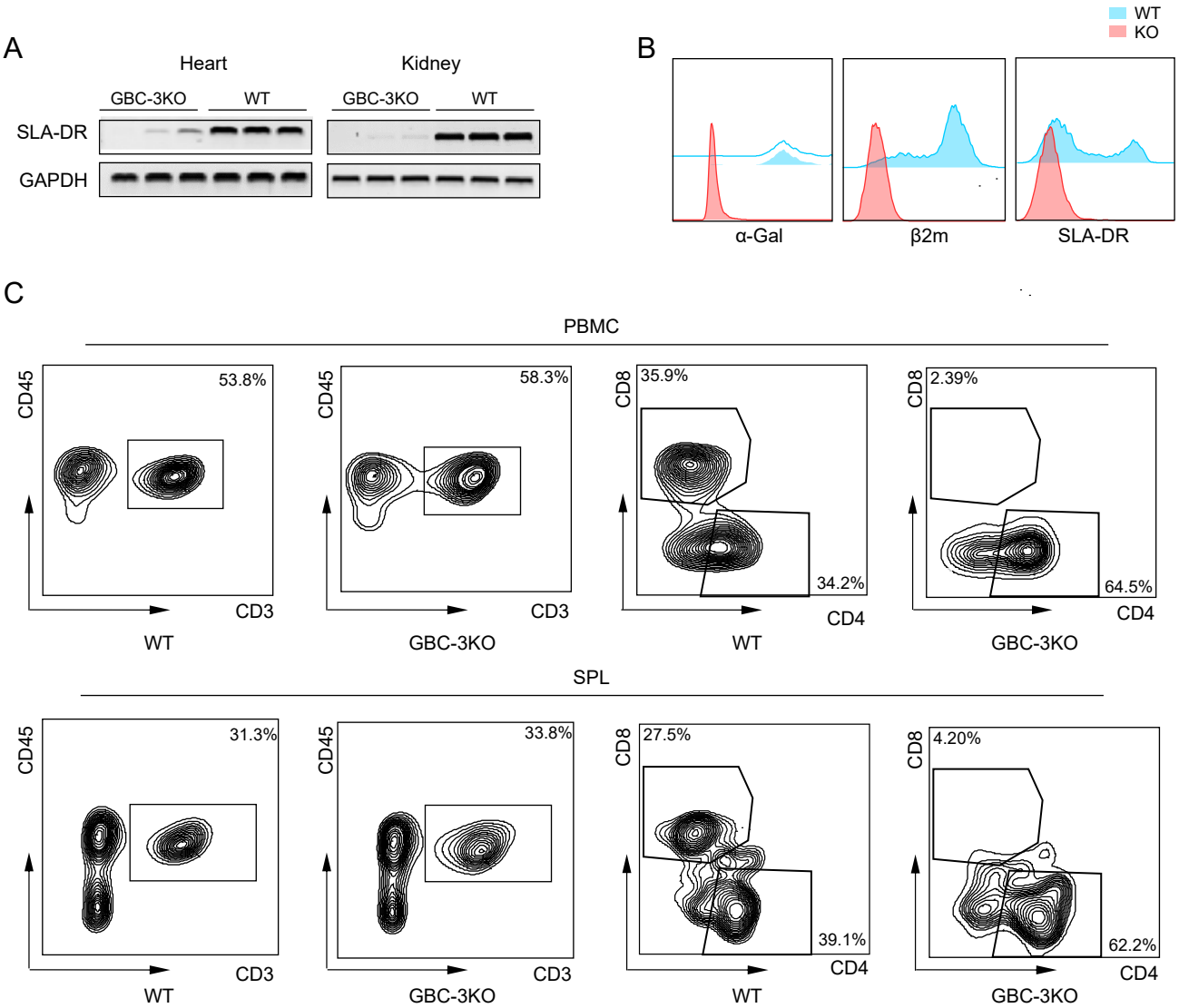
uncleaved
cleaved
B2M
uncleaved
cleaved
CIITA
uncleaved
cleaved
T7E1

Figure S2


A
Heart
Kidney
GBC-3KO
WT
GBC-3KO
WT
SLA-DR
GAPDH
B
WT
KO
α-Gal
β2m
SLA-DR
C
PBMC
CD45
53.8%
CD3
WT
CD45
58.3%
CD3
GBC-3KO
CD8
35.9%
34.2%
CD4
WT
CD8
2.39%
64.5%
CD4
GBC-3KO
SPL
CD45
31.3%
CD3
WT
CD45
33.8%
CD3
GBC-3KO
CD8
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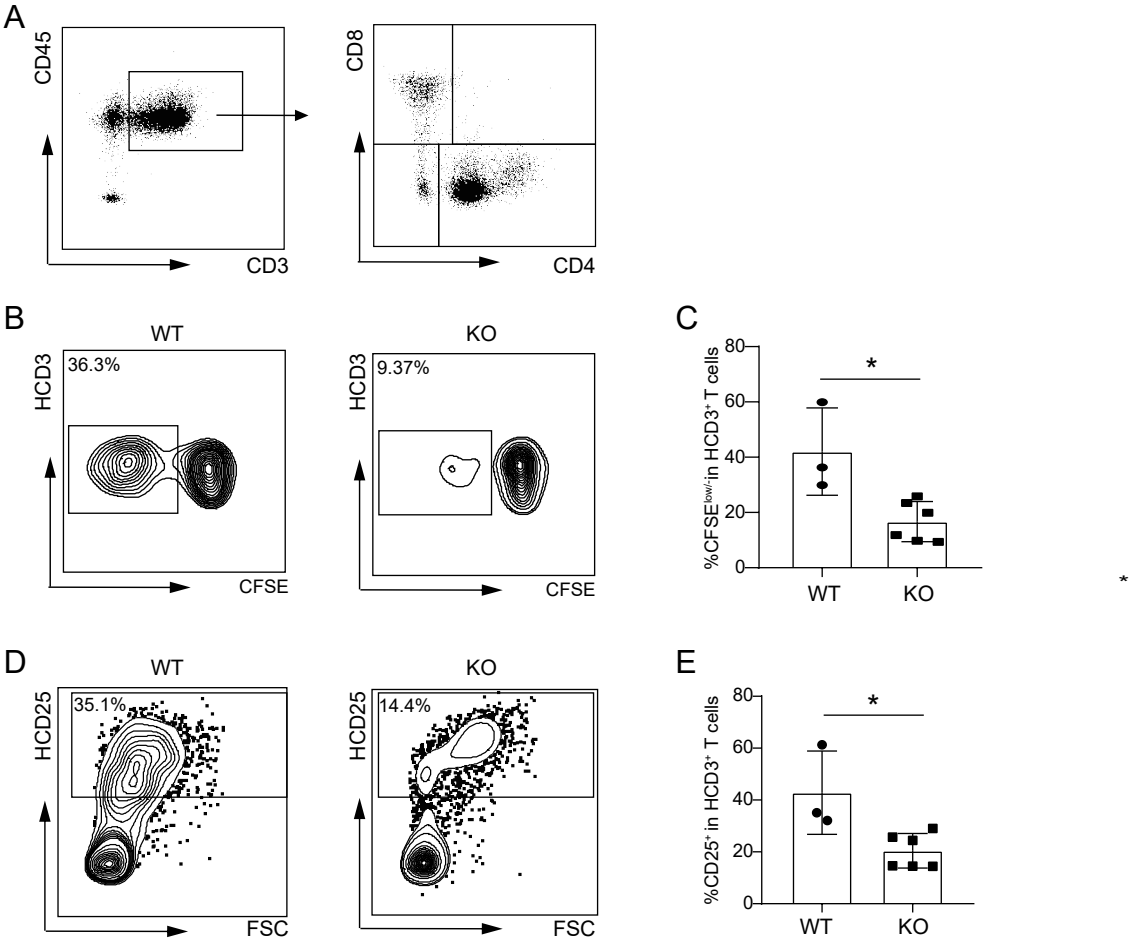
39.1%
CD4
WT
CD8
4.20%
62.2%
CD4
GBC-3KO

Figure S3


A
CD45
CD3
CD8
CD4
B
WT
KO
HCD3
36.3%
9.37%
CFSE
C
%CFSE^low/- in HCD3+ T cells
80
60
40
20
0
*
WT
KO
D
HCD25
35.1%
14.4%
FSC
E
%CD25+ in HCD3+ T cells

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