

**Aminopeptidase N-null neonatal piglets are protected from
transmissible gastroenteritis virus but not porcine epidemic diarrhea
virus**

Lei Luo^{a,d,1}, Shaohua Wang^{a,1}, Lin Zhu^{b,1}, Baochao Fan^b, Tong Liu^a, Lefeng Wang^a,
Panpan Zhao^a, Yanna Dang^a, Pei Sun^d, Jianwen Chen^d, Yunhai Zhang^d, Xinjian Chang^b,
Zhengyu Yu^b, Huanan Wang^a, Rongli Guo^b, Bin Li^{b,c,2}, and Kun Zhang^{a,2}

Figure Legends

Fig. S1. APN-null piglets are resistant to TGEV infection. (A) Macroscopic examination of TGEV-challenged wild-type (WT) and APN-null (KO) piglets. (B) TGEV viral RNA genome copies in fecal samples were quantified by real-time PCR.

Fig. S2. APN-null piglets are still susceptible to PEDV infection. (A) Macroscopic examination of PEDV-challenged wild-type (WT) and APN-null (KO) piglets. (B) The ratio of villus height to crypt depth was similar between APN-null pigs (n=7) and WT pigs (n=10). (C and D) PEDV antigen was quantified in pig tissues (C) and fecal samples (D) by ELISA. Data in (B-D) were shown as mean $\pm$ SEM.

Table S1.

Summary of somatic cell nuclear transfer results using APN-null porcine fibroblast cells as donors.

Table S2.

Summary of embryo transfer experiments from APN-null cloned embryos.

Table S3.

Genotypes of cloned piglets from APN-null porcine fibroblast cells. K1-K3, K11, K12, K14 and K19: 7 APN-null piglets used for PEDV challenge experiments. K4, K5, K16, K17, K18 and K20: 6 APN-null piglets used for TGEV challenge experiments.

Table S4.

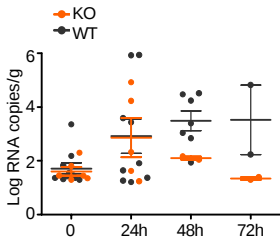
Fecal shedding in TGEV-challenged wildtype (WT) and APN-null (KO) piglets.

A

WT



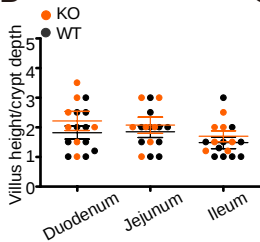
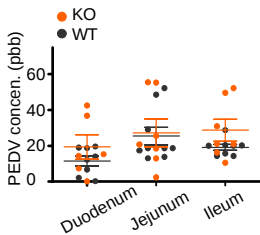
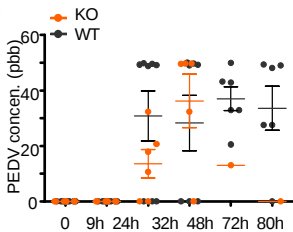
KO

**B**

A

WT

KO

**B****C****D**

Date of nuclear transfer	No. oocytes cultured	No. matured oocyte (%)	Type of embryos	No. embryos cultured	No. cleaved embryos (%)	Blastocyst rate (%)
Jan 11, 2018	1674	982 (58.7%)	PA	44	38 (86.4%)	8 (18.2%)
			SCNT	21	20 (95.2%)	9 (42.9%)
Jan 18, 2018	1691	1001 (59.2%)	PA	73	70 (95.9%)	52 (71.2%)
			SCNT	22	19 (86.4%)	9 (40.9%)

Parthenogenetic activation was performed as a control of oocyte quality. PA: parthenogenetic activated oocytes; SCNT: somatic cell nuclear transfer

Surrogate sow ID	No. embryos transferred	Pregnancy (d28)	Pregnancy (d50)	Piglets at birth	Birth weight	Note
CRMY01416801344	176	Yes	Yes	3	1.06kg, 0.46kg, 0.64kg	One piglet died in day 1
CRMY01416801381	176	Yes	No	0	—	Spontaneous abortion
CRMY01416801362	176	Yes	Yes	3	0.68kg, 0.5kg, 0.74kg	One piglet died in day 1
LLMY01416028366	176	No	No	0	—	—
CRMY01416801499	176	Yes	Yes	4	1.44kg, 1.14kg, 1.22kg, 1.6kg	—
YYMY01416670602	176	Yes	No	0	—	Spontaneous abortion
LLMY01416314510	202	Yes	Yes	4	1.4kg, 0.82kg, 1.0kg, 0.98kg	One dead fetus
CRMY01416801607	203	Yes	Yes	2	NA	—
LLMY01416030204	202	Yes	Yes	3	1.2kg, 0.72kg, 1.16kg	—
LLMY01416027910	203	Yes	Yes	2	0.92kg, 0.94kg	One piglet died in day 1
Summary	1866	9/10 (90%)	7/10 (70%)	21/1866 (1.13%)		

Animal ID	Sequence	Description
K1, K2, K3, K4, K5	Allele 1: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACA-----//-----AGGGCAAAAGCA TCGTCGCTT	Δ130bp
	Allele 2: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACAGC CGCCAC+++++//+++++CaaC gTGacgCtGA G+++++//+++++CAcGtCGcc CA+++++ACCaccAC aGCCgcCaCA CAACGCTGTT GCCTGATTCC TACAACGTGA CGCTGAGACC CTACCTCACT CCCAACGCGG ATGGCCTGTA CATCTTCAAG	+43bp, mismatch18bp
K11, K20	Allele 1: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACAGC CGCctgattc cT----- aCAA-- CGTG acgCtGagAC --CCTACctc actCGCTGTT GCCTGATTCC TACAACGTGA CGCTGAGACC CTACCTCACT CgCtg----- tTGCCTGTA CATCTTCAAG	Δ18bp, mismatch 22bp
	Allele 2: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACA-----//-----ACGCTGTT GCCTGATTCC TACAACGTGA CGCTGAGACC CTACCTCACT CCCAACGCGG ATGGCCTGTA CATCTTCAAG	Δ54bp
K12, K17, K19	Allele 1: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACAGC CGCCaCaacg Tga+++GcCtAcA aCgtGaCGct G++++AAC+tGacgC tGCCTAC+++++//+++++CtA CAACgt++cGcT GCCT+++++//+++++GcTgCC TACAACGTGA CGCTGAGACC CTACCTCACT CCCAACGCGG ATGGCCTGTA CATCTTCAAG	+52bp, mismatch25bp
	Allele 2: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACAGC CGCCATCACC TTGGACCAGA GCAAGCCGTG GAACCGGTAC CGCCTACC-A CAACGCTGTT GCCTGATTCC TACAACGTGA CGC-----//-----GG ATGGCCTGTA CATCTTCAAG	Δ26bp
K14, K16, K18	Allele 1: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACAGC CGCCATCACC TTGGACCAGA GCAAGCCGTG GAACCGGTAC CGCCTACCCA --ACGCTGTT GCCTGATTCC TACAACGTGA CGCTGAGACC CTACCTCACT CCCAACGCGG ATGGCCTGTA CATCTTCAAG	Δ2bp
	Allele 2: GCCGAGCATG TCCCCAGGC CCCACGTCG CCCACCATCA CCACCACAGC CGCCATCACC TTGGACCAGA GCAAGCCGTG GAACCGGTAC CGCCTACCCA CAACaacGTg aGgc-----tgACGTGA CGCTGAGACC CTACCTCACT CCCAACGCGG ATGGCCTGTA CATCTTCAAG	Δ8bp, mismatch 9bp

Piglet ID	Fecal shedding (TGEV), ppb, by hours post inoculation							
	0	12h	24h	36h	48h	56h	72h	80h
KO4	0	0	0	0	1.33618	1.358652	9.729438	0.637303
KO5	0.190112	0.212584	0.212584	0.212584	0.619326	0.601348	7.983371	0.605843
KO16	0	ND	2.213115§					
KO17	0	ND	0§					
KO18	0	ND	2.382172	7.185792	0§			
KO20	0	ND	0	4.26932	0§			
WT6	0	0	4.113708	4.113708	14.00135	13.86652	0.477753	0.477753
WT7	0.073258	0	13.70022	13.89124§				
WT8	0.142921	0.354157	13.89124	13.56539§				
WT9	0.459775	0.354157	13.82157	13.5227	0.354157	5.75191§		
WT10	0	0.282247	0.477753	13.74517	0.477753	13.93843§		
WT16	0	ND	6.73668	7.317281	10.00342	9.986339	8.604714§	
WT17	0	ND	6.466872	2.184085	6.355874	11.20325§		
WT18	0	ND	0.495219	ND	7.28323§			
WT19	0	ND	1.331967	4.91291	0	8.911901§		
WT20	0	ND	9.465505§					

KO4-20: APN-null piglets; WT7-20: Wild-type piglets; ppb, parts per billion; ND, not determined; -, no result as pig euthanized; §, euthanized.