Table S1. Identification of potential off-target events.

Location	Start to end	Target gene	Genes upstream/downstream	Binding sequence[a]	No. of mismatches	Primers for sequencing	Primer sequence	Mutation
chrX	143201730~143201767 or 142894820~142894857	F8 intron or No	FUNDC2/DKC1 or CLIC2/VBP1	TGgCCTGcCCcGCCGCCTACGGGgAGcCcCTGCAGGCC	6	off1-F/R	gtcgagcaagctgaagaagc/ctgcatgcgcgtgaagac	ND
chr3	99610680~99610717	No	EPCAM/SOCS5	AGCCTGggGtGCCcTCCCCTAAGCGGCaGGCCAGGgCA	6	off2-F/R	ccactgctgttaccacgtca/gatctgggggtggacagtta	ND
chr3	41635838~41635874	IGFACS	NUBP2/MRPS34	CGgCCTGGCCAGCCtgGAGCCGcAGGCGCTGCtGGgC	6	off3-F/R	acgacgactacacggacga/gctgcaggtaggtcagcttg	ND
chr5	15812400~15812436 or 15728736~15728772	No	TUBA1B/AQP6	GGCCgGgAGgGCCTTgGGGAGGgGcCTGGCCAGGACG	6	off4-F/R	atacccattggtgggagtga/agtcaaaatggcccctctct	ND
chr8	33353489~33353525	No	CHRNA9/UCHL1	GaaCTGCAGCtCaTTCAGCTTGCtGCaGGCCAGGACC	6	off5-F/R	ctgggtgtttcagtgcctct/actcctcccctagctctgc	ND
chr14	152907141~152907178	No	GLRX3/MIR202	CGgCTGCAGCGaCgTCCAGGAGGCtGCTGcCCAGGgCG	6	off6-F/R	gctcgtgtgccctgagac/gtctggagaccatccaggag	ND
chr18	7180238~7180274	No	NOS3/TRY	GGTCCTGGCCAGaCcCCGAAAcAAGGaGCTGCAatCT	6	off7-F/R	cagggatcaaacccacattc/gggcaggtccagagatatga	ND
chr9	150358911~150358947	*GRB10* intron	DDC	GGgCggGGaCAGCCGCCCCAAGAcGGCGCTcCAGGCG	6	off8-F/R	gtcctctaagccagcgtgtc/ccgtgcaatatttgggacata	ND

[a] Lower-case letters denote the mismatched nucleotides with the ZFN target site.

Table S2. Optimization of transfection conditions using different amounts of ZFN mRNA or plasmids.

Type of ZFN	Cell Viability	Amount of ZFN[a]	Efficiency of Gene Editing[b]
mRNA	little	4 μg	0
mRNA	little	2 μg	0
plasmid	little	4 μg	1.2%
plasmid	little	3 μg	0
plasmid	little	2 μg	2.6%
plasmid	little	1 μg	1.8%
plasmid	normal	0.5μg	11.8%
plasmid	normal	0.3μg	0

[a] The amount denotes each ZFN construct used for transfection in the ZFN set pair.

[b] One hundred colonies were chosen for sequencing, and ZFN-mediated gene editing efficiency was calculated as the number of mutant colonies divided by the total colonies.

Table S3. Summary of the cloning and generation of *PKD1*$^{+/-}$ pigs.

Cell line	Recipient	Pregnant	Live Birth	Stillbirth	Genotype
96	3	2	10	2	*PKD1* $^{TGCT\ ins/+}$ (6) [a]
63	3	1	7	0	*PKD1* $^{T\ ins/+}$ (6)
60	3	1	1	2	*PKD1* $^{TGCT\ ins/+}$(1)
77	4	1	2	1	*PKD1*$^{+/+}$ [b]
Total	13	5	20	5	

[a] Parenthesed number indicates the number of mutant pigs. [b] Cell line 77 only gave rise to WT piglets rather than piglets with compound heterozygous *PKD1*.

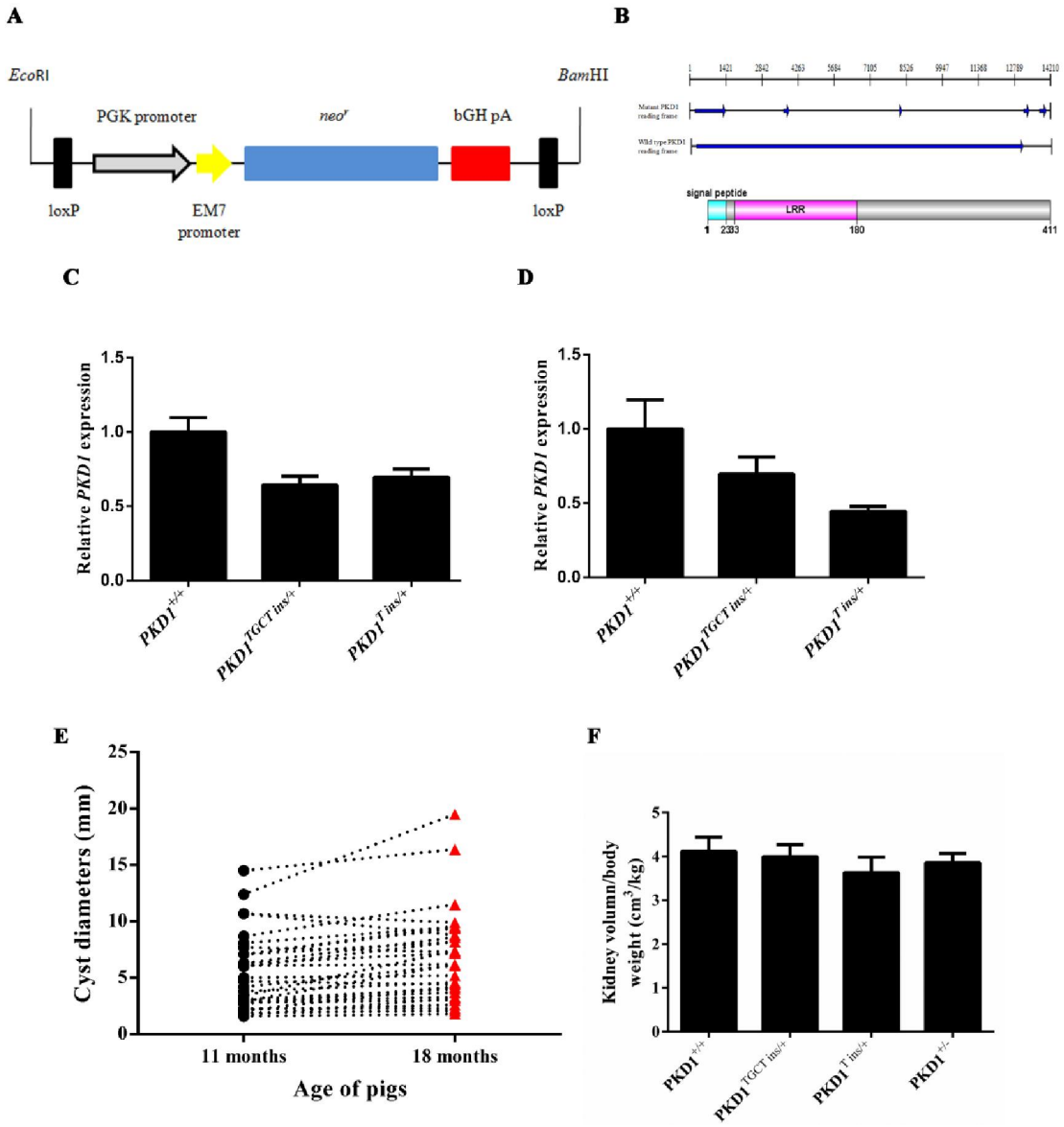
Table S4. The *PKD1* mutant alleles can be passed through germline transmission.

Genotype of F0 pigs	Identification No. of F0 pigs	Identification No. of sows	Number of live birth	Number of mutant F1 piglets	Number of WT F1 piglets
$PKD1^{TGCT}_{ins/+}$	436	353	7	4	3
	436	343	4	2	2
	435	366	8	5	3
	436	166	5	3	2
	491	288	10	1	9
	436	293	7	3	4
	Total		41	18	23
$PKD1^{T\ ins/+}$	488	152	9	7	2
	488	265	7	6	1
	487	333	7	4	3
	Total		23	17	6

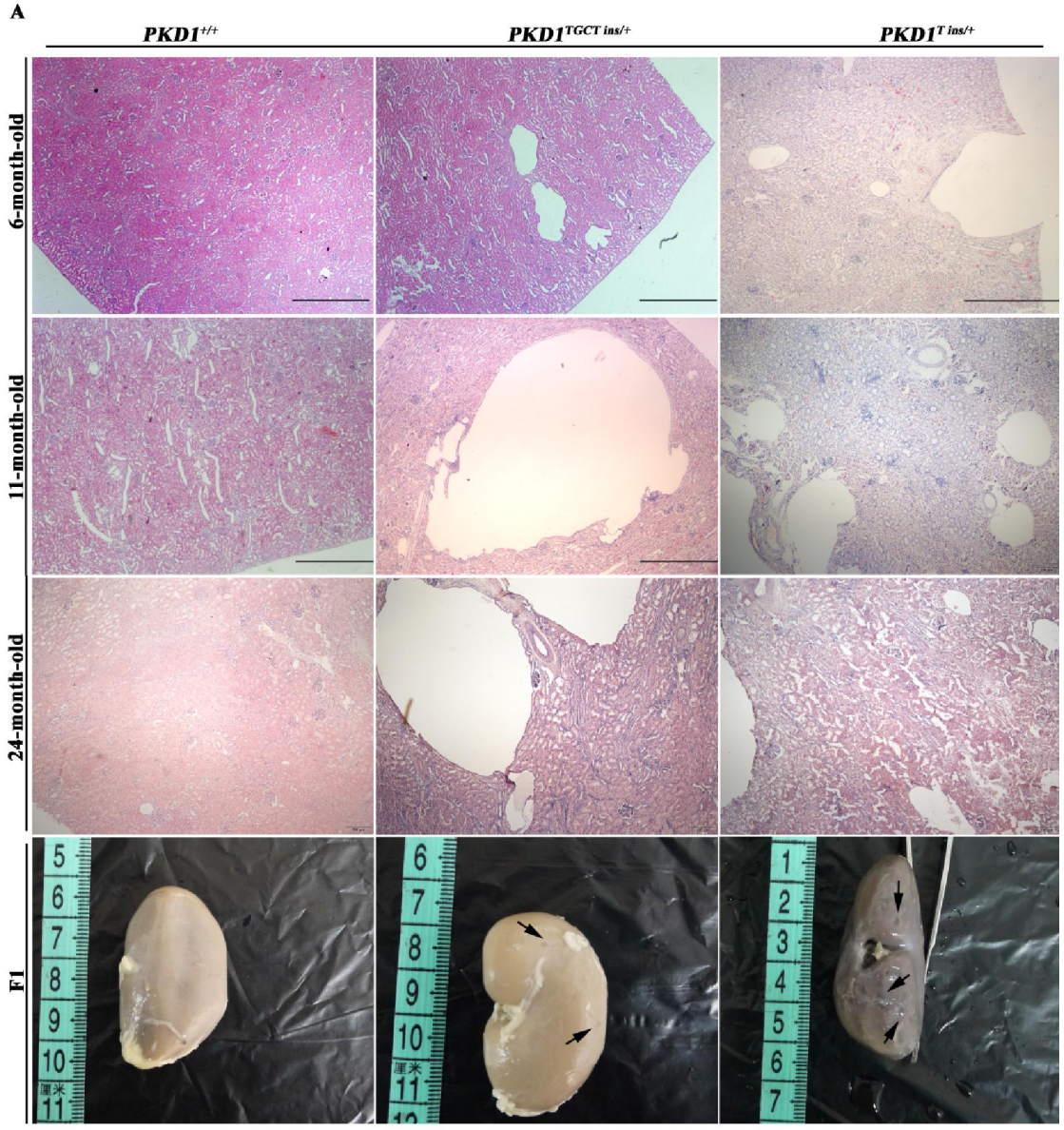
Table S5. CT analysis of 11-month-old PKD1+/- pigs.

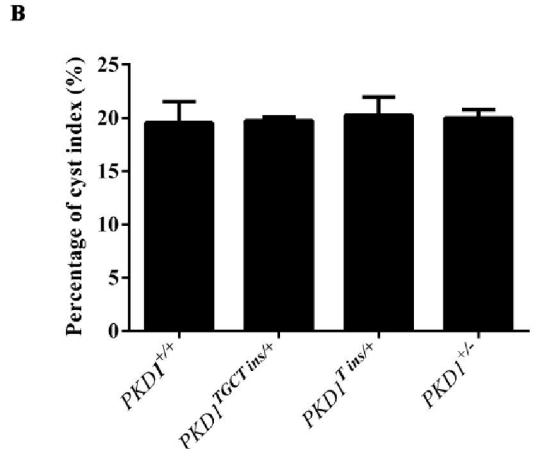
Id. No.	Number of cysts with designated diameter (mm) in right kidneys					Number of cysts with designated diameter (mm) in left kidneys				
	1.5-3	3-5	5-7	7-10	>10	1.5-3	3-5	5-7	7-10	>10
PKD1$^{TGCT\ ins/+}$										
434	1	3	2	1	2	1	3	2	2	0
435	2	0	2	0	0	1	2	0	0	0
436	2	2	2	1	1	1	4	2	4	4
463	0	4	2	2	5	0	3	1	3	2
491	0	3	1	0	2	0	2	1	0	0
PKD1$^{T\ ins/+}$										
487	4	1	1	2	0	4	0	1	0	0
488	1	5	2	1	1	1	3	0	2	0
490	0	0	0	0	1	3	0	4	0	1

Supplementary Figure S1. Construction and characterization of *PKD1*$^{+/-}$ pigs. (A) Schematic diagram of the ~1.9 kb neomycin resistance cassette digested from plasmid pL452 by *Bam*HI and *Eco*RI. (B) Schematic representations of WT and mutant *PKD1* gene reading frames and the predicted structure of truncated PC1 produced by mutant *PKD1* alleles. The blue arrows indicate the reading frames. The truncated PC1 fragment only preserves the signal peptide (blue box) and the LRR domain (purple box), which can be recognized by the 7e12 anti-PC1 antibody. (C) qRT-PCR analysis of 5' part of *PKD1* expression (mean ± SEM) in neonatal pig ear biopsies, showing that *PKD1*$^{TGCT\ ins/+}$ (n=6) and *PKD1*$^{T\ ins/+}$ (n=5) pigs had a reduced *PKD1* expression compared to WT pigs (n=6, * $P<0.05$). (D) qRT-PCR of 5' part of *PKD1* expression in neonatal kidneys, which also shows a reduction of *PKD1* in *PKD1*$^{+/-}$ pigs. Data obtained from three replicated experiments are presented as the mean ± SD. (E) Cyst diameters, determined by CT, increased with the age ($P=0.0002$). Black dots and red triangles indicate cysts in 11- and 18-month-old kidneys from three *PKD1*$^{+/-}$ pigs, respectively. The same cysts at the two stages are connected by dotted lines.(F) Kidney volumes, adjusted by the body weight of each pig, were determined by 3D reconstruction of CT results. The kidney volumes of each pig represent the mean value of both kidneys. Data are shown as mean ± SEM. (n=5 for *PKD1*$^{+/+}$ pigs, n=5 for *PKD1*$^{TGCT\ ins/+}$, n=3 for *PKD1*$^{T\ ins/+}$, n=8 for *PKD1*$^{+/-}$ pigs).


A
EcoRI
BamHI
PGK promoter
neo^r
bGH pA
loxP
EM7
promoter
loxP
B
signal peptide
LRR
1
233
180
411
C
Relative PKD1 expression
1.5
1.0
0.5
0.0
PKD1 +/+
PKD1 TGCT ins/+
PKD1 T ins/+
D
Relative PKD1 expression
1.5
1.0
0.5
0.0
PKD1 +/+
PKD1 TGCT ins/+
PKD1 T ins/+
E
Cyst diameters (mm)
25
20
15
10
5
0
11 months
18 months
Age of pigs
F
Kidney volumn/body weight (cm3/kg)
5
4
3
2
1
0
PKD1 +/+
PKD1 TGCT ins/+
PKD1 T ins/+
PKD1 +/-

Supplementary Figure 2. (A) The upper three panels are the representative histological images of pig kidneys at lower magnification. The lower panel shows the gross morphology of F1 piglet kidneys. The arrows indicate the renal cysts in KO pig kidneys. (B) The morphometric analysis of cyst index of pig kidneys.


A
PKD1+/+
PKD1TGCT ins/+
PKD1T ins/+
6-month-old
11-month-old
24-month-old
F1


B
Percentage of cyst index (%)
25
20
15
10
5
0
PKD1+/+
PKD1TGCT ins/+
PKD1T ins/+
PKD1+/-