Table S1 Detailed results of the *in vitro* electroporation in the presence of ribonucleoprotein (RNP) targeted toward the exon 4 of murine *GGTA1*.

Name of single embryos[1]	Pattern of mutations in exon 4 of *GGTA1*[2]	Sequence at exon 4 of porcine *GGTA1*[3]
#1	Mosaic	AATAATGAANAG TGGTTCTGTCAATGCTGCTTGTCTCAAC
#2	Bi; Ins	AATAATGAATGTTTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#3	Bi; Del/Ins	AATAATGAATGTTCTGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#4	Bi; Ins	AATAATGAATGTTTTCATTTAATGTTTAATGTTTTCATTTAATGAATGTTGAAGGAAGAGTGGTTCTGTC AATGCTGCTTGTCTCAAC
#5	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#6	Bi; Del/Ins	AATAATGAATGTGAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#7	Bi; Ins	AATAATGAATGTTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#8	Bi; LD	AATAATGAATGGNGGAAGAATGATGGTTTCGGTCA
#9	Bi; LD	AAAATGAAGAGAGGGAAAGAGGGTTTCATTCTGCG
#10	-	-
#11	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#12	Bi; Del	AATAATGAATGTGAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAACTG
#13	-	-
#14	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#15	Bi; Del	AATAATGAATGTGAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC

#16	Bi; Del	AATAATGAAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#17	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#18	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#19	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#20	Bi; Ins	AATAATGAATGTTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#21	Bi; Del/Ins	AATAATGAATGAAAGGGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#22	Mosaic	AATAATGAATGT N AAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAACTG
#23	Bi; Del	AATAATGAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAACTG
#24	Bi; Del	AATAATGAATGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAACTG
#25	Bi; Del	AATAATGAATAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAACTG
#26	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#27	Bi; Del/Ins	AATAATGAATGGAAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#28	Bi; Del/Ins	AATAATGAATGAAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#29	Bi; Del	AATAATGAATGTAAAGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#30	Normal	AATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#31	Bi; Del/Ins	AATAATGAATGGAAAAGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#32	Mosaic	AATAATGATTGTNNAAGGGCTAGNGGTTCTGNCTGTGCTGCTTGTGTCAACTG
#33	Bi; Ins	AATAATGAATGTTATTCATTCATTATATTAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC
#34	Bi; Del	AATAATGAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAAC

[1]*In vitro* electroporation (EP) was performed on porcine parthenotes in the presence of RNP targeted toward porcine *GGTA1*. The treated parthenotes were cultured until reaching the blastocyst stage. The resulting single blastocysts were subjected to molecular biology analysis to detect the possible mutations in the target area of *GGTA1*. Among the 34 blastocysts obtained, genomic DNA could not be extracted from sample #10 and #13 blastocysts. The parthenotes numbered #1 to #19 are the samples electroporated 4 times, while those numbered #20 to #34 are the samples electroporated 7 times.

[2]Mutations that occurred in the exon 4 of *GGTA1* are defined as Ins [insertion of nucleotide(s)], Del [deletion of nucleotide(s)], Del/Ins [deletion of nucleotide(s) and subsequent insertion of nucleotide(s)], Mosaic (comprising several mutations in one embryo), LD [large deletion (at least 20 bp) immediately next to the translation initiation site (ATG)], and Normal (exhibiting no mutation in either of the alleles in an embryo). Bi: bi-allelic KO mutations.

[3]Sequences of the exon 4 of *GGTA1,* including the ATG site (boxed). The PAM site is underlined. The nucleotides depicted in red are those inserted, replaced, mixed (represented by N), or derived from the downstream sequence.

Table S2 Nucleotide sequences of a region spanning a sequence recognized by gRNA (targeted to GGTA1) in sub-clones from #2, #6, #1, #22 and #32 samples1.

Name of fetuses	Mode of mutations[2]	Mode of mutations in sub-clones[2]	Sequence (5'-3')/ PAM	Rate
#2	Bi	Ins	ATAATGAATGTTTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTT	6/6 (100%)
#6	Bi	Del/Ins	ATAATGAATGTGAGGAAGAGTGGTTCTGTCAATGCTGCTT	6/6 (100%)
#1	Mosaic	Wild-type	ATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTT	1/9 (11%)
		Del[3]	AAAAGAGTGGTTCTGTCAATGCTGCTT	7/9 (78%)
		Replac[4]	ATAATGAATGTCAAAGGACGAGTGGTTCTGTCAATGCTGCTT	1/9 (11%)
#22	Mosaic	Wild-type	ATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTT	3/6 (50%)
		Replac[5]	ATAATGAATTTTAAAGGAAGAGTGGTTCTGTCAATGCTGCTT	1/6 (17%)
		Replac[6]	ATAATGAATGGCAAAGGGGTACTGGCTCTGCCGCTGCTGCTT	1/6 (17%)
		Replac[7]	ATAATGAATGTCAAAGGAAAAGAGGGTCTGTCAATGCTGCTT	1/6 (17%)
#32	Mosaic	Wild-type	ATAATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTT	5/16 (31%)
		Del[8]	AAAAGAGTGGTTCTGTCAATGCTGCTT	7/16 (44%)
		Del[9]	AAAAAGTGAATGGCAAAGGGATACTGGTTCTGCCGCTG	1/16 (6%)

Replac[10]	ATAATGATTGTCAAAGGGCTGCTTGTTCTGTCTGTGCTGCTT	2/16 (13%)
Replac[11]	ATAATGATTGTCAAAGGGCTGCTTGGCTCGTCTGTGCTGCTT	1/16 (6%)

1. 1Sub-cloning was performed using TA cloning system. The samples sub-cloned are those with bi-allelic KO phenotype (Bi) for #2 and #6, and those with mosaic mutations, which had "N" in the sequence recognized by gRNA (see Suppl. Table 1).
2. 2Mode of mutations are classified as Bi or mosaic (including multiple alleles).
3. 318-bp deletion, including ATG.
4. 4Replacement (A to C) immediately below the PAM.
5. 5Replacement (GTC to TTT) immediately below ATG.
6. 6Replacement (TCAAAGGAAGAGTGGTTCTGTCAA to GCAAAGGGGTACTGGCTCTGCCGC) including the PAM.
7. 7Replacement (GAGT to AAGAG) immediately below the PAM.
8. 818-bp deletion, including ATG.
9. 9Over 30-bp deletion, including ATG and PAM.
10. 10Replacement (AAGAGTGGTTCTGTCAA to GCTGCTTGTTCTGTCTG) immediately below the PAM.
11. 11Replacement (AAGAGTGGTTCTGTCAA to GCTGCTTGGCTCGTCTG) immediately below the PAM.

Abbreviations: Ins, insertion; Del, deletion; Replac, replacement.