Supplementary materials:

Table S1. Sequences of primers used to clone *MARK4* cDNA and construct vectors.

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
MARK4	GCGGGAAGAAATCAAAGAGGC	TAGGTGGCGGTCACTTCGTTGT
HPRT	CAGTCAACGGGCGATATAAAAGT	CCAGTGTCAATTATATCTTCAACAATCA
CLONE-1	TTAGATATCCCCAGTCCCCTGGGACCCGGA	TAAGAATTCAGGCGATGCCAAAGGGAGACC
GSP-5	----------	TCTTCTTCCTCCTGCCGCTTGCGCTCCCGC
GSP-3	CGGCTCCCCTTCATCCAGCACAGCCAGAA	----------
2235/3007-p	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAGTTTAAACGTGTGGGGTATTTACAAGGA
871/1467-p	GTCTCGAGTTCAGCAATGAGTTCACAC	TAGCGGCCGCGCTGGAGCTTGTTCCATT
2235/2508-2482Wt-p	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAGTTTAAACTGGAGATGCGGGTGACGA
2235/2508-2482Mut-p	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAGTTTAAACTGGAGATGCGGGTGACGAGG GAAGTCGGGGCCAGGGCGGTG
2235/2502-p	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAAGTTTAAACCGGGTGACGAGGG
2434/2533-2502Mut-p	ATTGCGATCGCGGCCTGCGGGGAGTTCTC TTCGGCCGCGTGG	TAAGTTTAAACGGCGGCTCAGAGCTCGA GGTCGTTGGAGATCCGGGTGAC
2434/2533-2502Wt-p	ATTGCGATCGCGGCCTGCGGGGAG TTCTCTTCGGCCGCGTGG	TAAGTTTAAACGGCGGCTCAGAGCTCGA GGTCGTTGGAGATGCGGGTGAC
2434/2533-R1-p	ATTGCGATCGCGGCCTGCGGGGAGTTCTC TTCCGCCGCGTGGCGGGCACCGCCCTGGC CTTCCGCACCCTCGTCAC	TAAGTTTAAACGGCGGCTCAGAGCTCGAGG TCGTTGGAGATGCGGGTGACGAGGGGTGAC GAGGGTGCGGA
2434/2533-R2-p	ATTGCGATCGCGGCCTGCGGGGAGTTCTC TTCCGCCGCGTGGCGGGCACCGCCCTGGC CTTCCGCACCCTCGTCAC	TAAGTTTAAACGGCGGCTCAGAGCTCGAGG TCGTTGGAGGTGACGAGGGATGCGGGTGAC GAGGGTGCGGA

Table S2. Sequence of primers used for SNP identification and genotyping.

Primer	Target region	Forward (5'-3')	Reverse (5'-3')	Product size (bp)
P1	Exon1 (from 51th bp)	TTCATAGAGCACGTTGGGAGACC	AGGGATGCCTGAAACTGGTTCTC	552
P2	Exon 2-3	AGAACGAGGGCTGTCTGTGAAAGG	CAGAGTCTCCTCCCTTCTGCCTG	412
P3	Exon 4-5	CCGGCCATTGTAGTGAAGCTCT	ACCCGGACTCAAGCCACACC	361
P4	Exon 6	TTCCCACTGAGGCCAAATTGG	GGACACATGGAGCCATTGGAG	481
P5	Exon 7	TCCACTTTGGGATGAGGACAGG	TCCTGGGCAGAAGAGGTGTCTG	461
P6	Exon 8-9	GAGCTCCTGTCACTTCTGTTGGG	TTGGCTCCGCTCTGCGTGAC	764
P7	Exon 10-11	AGTACGAGATGCGGAAACAAGTGG	CAGTTTCTAACGCATTCCTGAGCC	638
P8	Exon 12	TGTGATTTATGTGGCACCTCTGG	CAGGGTGCGAAAAATGTGACC	508
P9	Exon 13	CCAAGGTCTCCCGTGATAGCCTT	TCCCTCACCGAAGAGCACCTG	297
P10	Exon 14	GGGTAGCTGGAGGTTTCTGATCTCA	CCGTTTAGAGGGATCGAGGGTAAC	570
P11	Exon 15	TGGACTCCCAGCCCTCCATAG	AAAGGCTGGTGCTCAGGAATGG	425
P12	Exon16	AGGAGGCTGGGAAATGGAGTCTC	AACGGAGGCGATGCCAAAGG	601
P13	Exon16	CTCGTCACCCGCATCTCCAA	CACCCAAAGCGGTGGTTTCT	1107
P14	2581	TAACCTCTTCCTCTTCCTCGCCCCC TTCGCTGCA	CCTCAGTTTCCCTTTCTGC	287

Table S3. Genotypic and allelic frequencies for the g.2581A>G *MARK4* polymorphism in eight pig breeds.

Breed	Number	Genotype	Number	Genotype frequency				Allele	
				AA	AG	GG		A	G
Jinhua	33	AA	0	0.00	0.03	0.97	Number	1	65
		AG	1				Frequency	0.02	0.98
		GG	32						
Neijiang	25	AA	0	0.00	0.08	0.92	Number	2	48
		AG	2				Frequency	0.04	0.96
		GG	23						
Erhualian	13	AA	0	0.00	0.08	0.92	Number	1	25
		AG	1				Frequency	0.04	0.96
		GG	12						
Wuzhishan	32	AA	0	0.00	0.03	0.97	Number	1	63
		AG	1				Frequency	0.02	0.98
		GG	31						
Bamei	29	AA	0	0.00	0.28	0.72	Number	8	50
		AG	8				Frequency	0.14	0.86
		GG	21						
Tibet	26	AA	0	0.00	0.08	0.92	Number	2	50
		AG	2				Frequency	0.04	0.96
		GG	24						
Duroc	277	AA	87	0.31	0.52	0.17	Number	318	236
		AG	144				Frequency	0.57	0.43
		GG	46						
Yorkshire	30	AA	24	0.80	0.20	0.00	Number	54	6
		AG	6				Frequency	0.90	0.10
		GG	0						

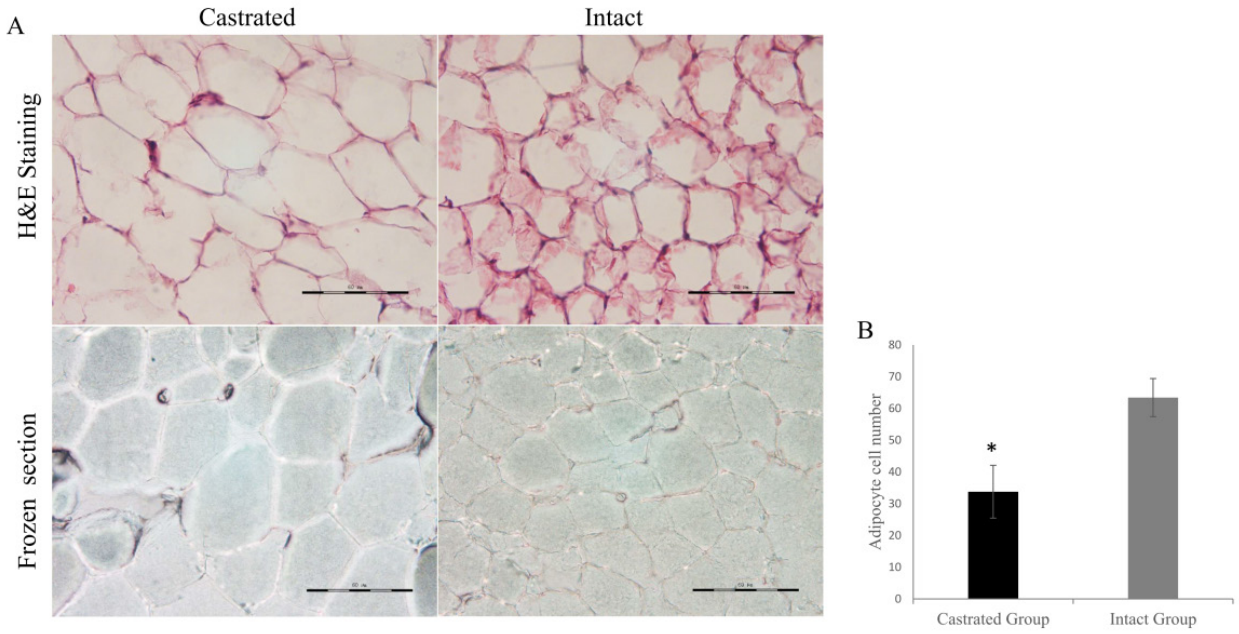

Figure S1. Histological analysis of differences in adipocyte size in back fat tissue obtained from castrated and intact male pigs. (A) Observation of adipocyte size with H&E staining. (B) Statistical analysis of adipocyte number per visual field. *, $p<0.05$.

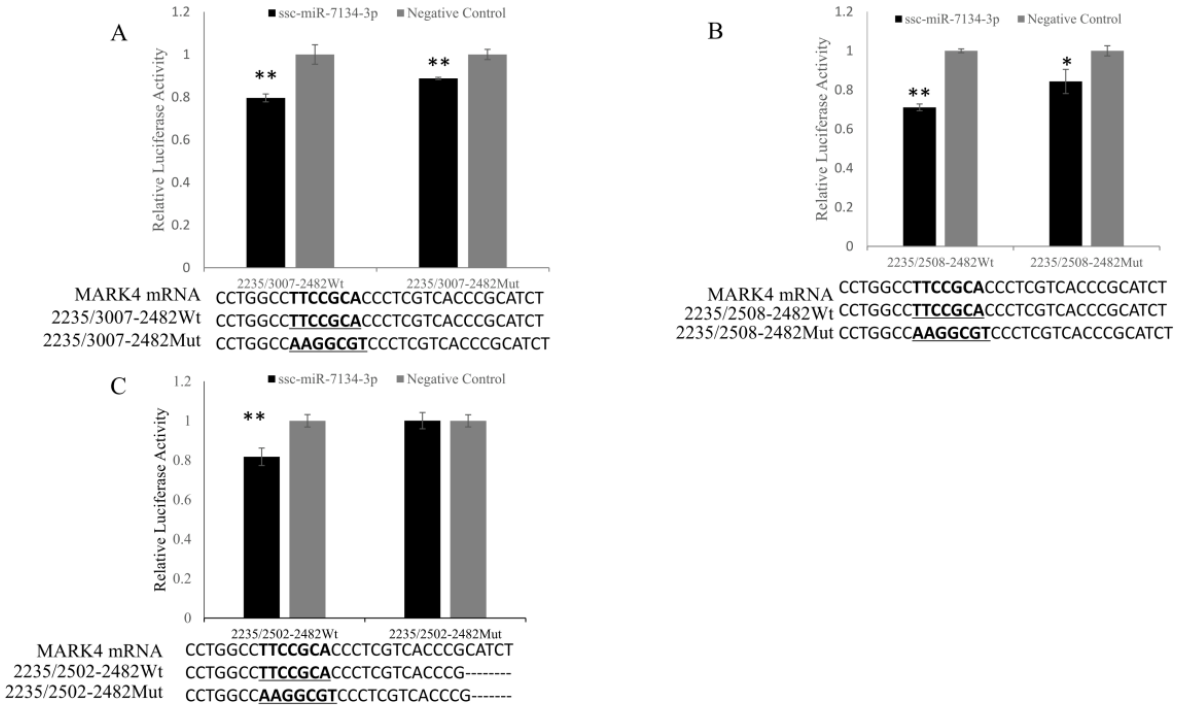

Figure S2. Detection of ssc-miR-7134-3p target site on *MARK4* using the dual-luciferase system. (A-C) The assay was implemented with fragments containing the region spanning 2235 to 3007 (A), 2235 to 2508 (B), and 2235 to 2502 (C). The bold characters indicate the location of the mutation. All relative luciferase values were normalized to negative controls. **, $p<0.01$; *, $p<0.05$.

Figure S3

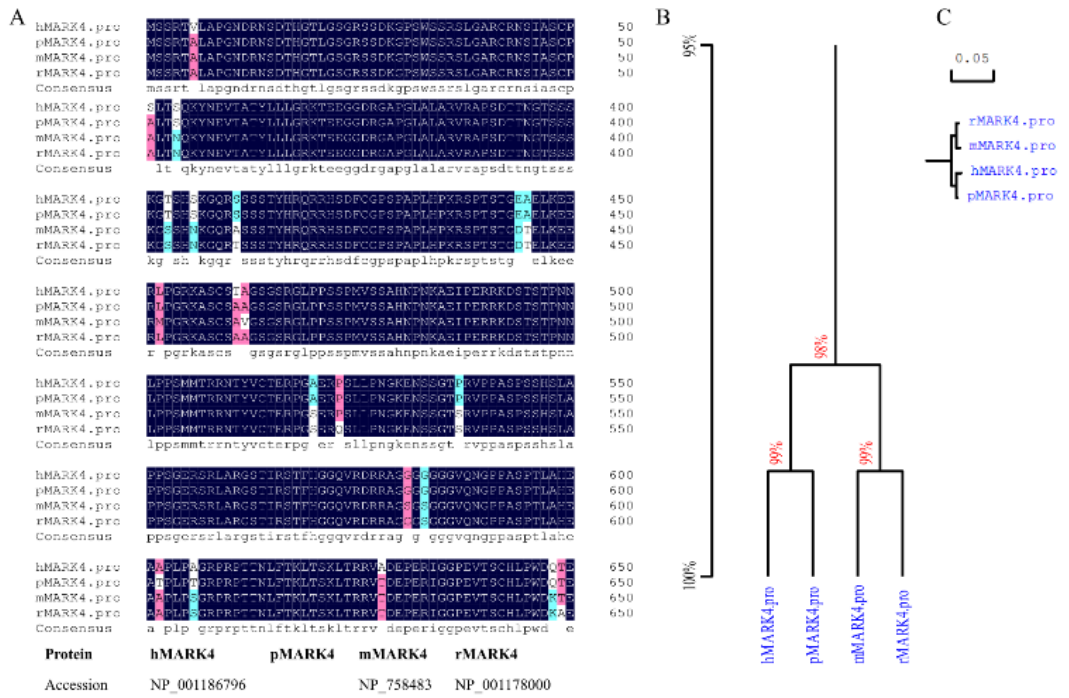

Figure S3. Alignment and phylogenetic analysis of MARK4 amino acid sequences from different species. (A) MARK4 sequence alignment. Identical amino acids are shadowed in black. The regions from residue 51 to 399, and from residue 651 to 752 are identical and are not shown. (B) Homology of MARK4 amino acids among four species. (C) Phylogenetic analysis based on the MARK4 amino acid sequence. The analysis was performed with human (h), pig (p), mouse (m) and rat (r) sequences.