

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	-----	TCTTCTTCTCCTGCCGCTTGCGCTCCCCG
GSP-3	CGGCTCCCCTTCATCCAGCACAGCCAGAA	-----
2235/3007-p	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAGTTTAAACGTGTGGGGTATTTACAAGGA
871/1467-p	GTCTCGAGTTCAGCAATGAGTTCACAC	TAGCGGCCGCGCTGGAGCTTGTTCATT
2235/2508-	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAGTTTAAACTGGAGATGCGGGTGACGA
2482Wt-p		
2235/2508-	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAGTTTAAACTGGAGATGCGGGTGACGAGG
2482Mut-p		GAAGTCGGGGCCAGGGCGGTG
2235/2502-p	TTGCGATCGCCCGATTCCCCTGGAGTGT	TAAGTTTAAACCGGGTGACGAGGG
2434/2533-	ATTGCGATCGCCGCTGCGGGGAGTTCTC	TAAGTTTAAACGCGGCTCAGAGCTCGA
2502Mut-p	TTCGGCCGCGTGG	GGTCGTTGGAGATCCGGGTGAC
2434/2533-	ATTGCGATCGCGCCCTGCGGGGAG	TAAGTTTAAACGCGGCTCAGAGCTCGA
2502Wt-p	TTCTCTTCGGCCGCGTGG	GGTCGTTGGAGATGCGGGGTGAC
2434/2533-	ATTGCGATCGCGCCCTGCGGGGAGTTCTC	TAAGTTTAAACGCGGCTCAGAGCTCGAGG
R1-p	TTCCGCCGCGTGGCGGGCACCGCCCTGGC	TCGTTGGAGATGCGGGGTGACGAGGGGTGAC
	CTCCGCACCCTCGTCAC	GAGGGTGCGGA
2434/2533-	ATTGCGATCGCGCCCTGCGGGGAGTTCTC	TAAGTTTAAACGCGGCTCAGAGCTCGAGG
R2-p	TTCCGCCGCGTGGCGGGCACCGCCCTGGC	TCGTTGGAGGTGACGAGGGATGCGGGGTGAC
	CTCCGCACCCTCGTCAC	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 51 th 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	CCAAGTCTCCCGTGATAGCCTT	TCCCTCACCGAAGAGCACCTG	297
P10	Exon 14	GGGTAGCTGGAGGTTTCTGATCTCA	CCGTTTAGAGGGATCGAGGGTAAC	570
P11	Exon 15	TGGACTCCAGCCCTCCATAG	AAAGGCTGGTGCTCAGGAATGG	425
P12	Exon16	AGGAGGCTGGGAAATGGAGTCTC	AACGGAGGCGATGCCAAAGG	601
P13	Exon16	CTCGTCACCCGCATCTCAA	CACCCAAAGCGGTGGTTTCT	1107
P14	2581	TAACCTCTTCCTCTTCCTCGCCCC 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						

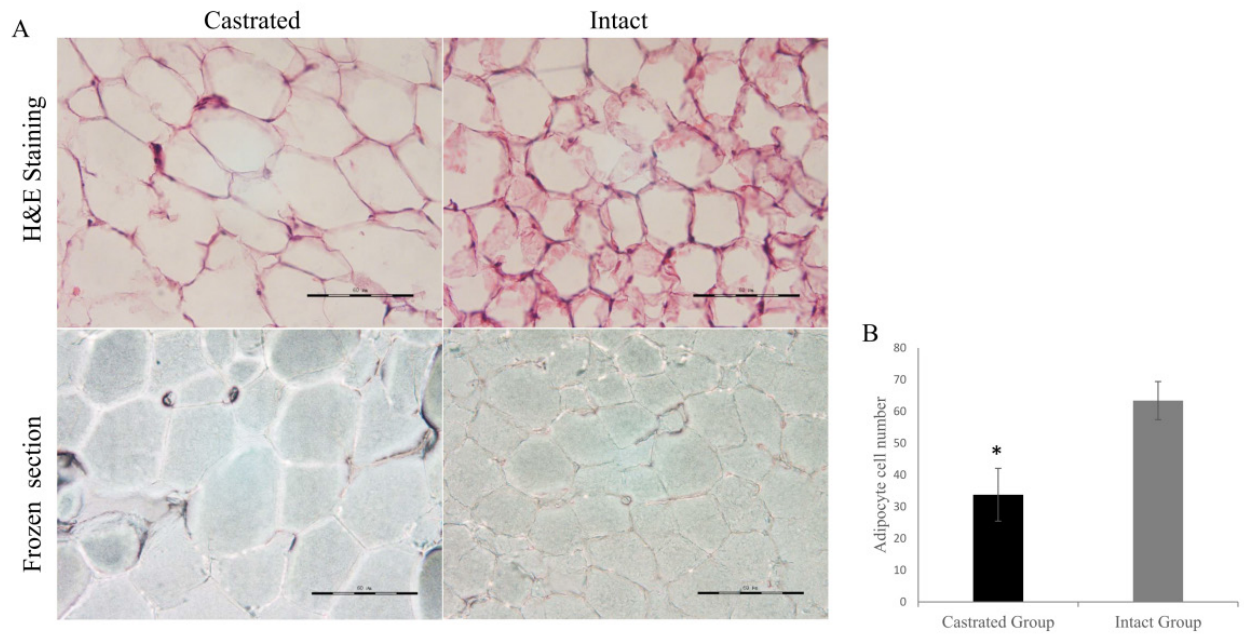


Figure S1. Histological analysis of differences in adipocyte size in back fat tissue obtained from castrated and intact male pigs. (A) Observation of adipocyte size with H&E staining. (B) Statistical analysis of adipocyte number per visual field. *, $p < 0.05$.

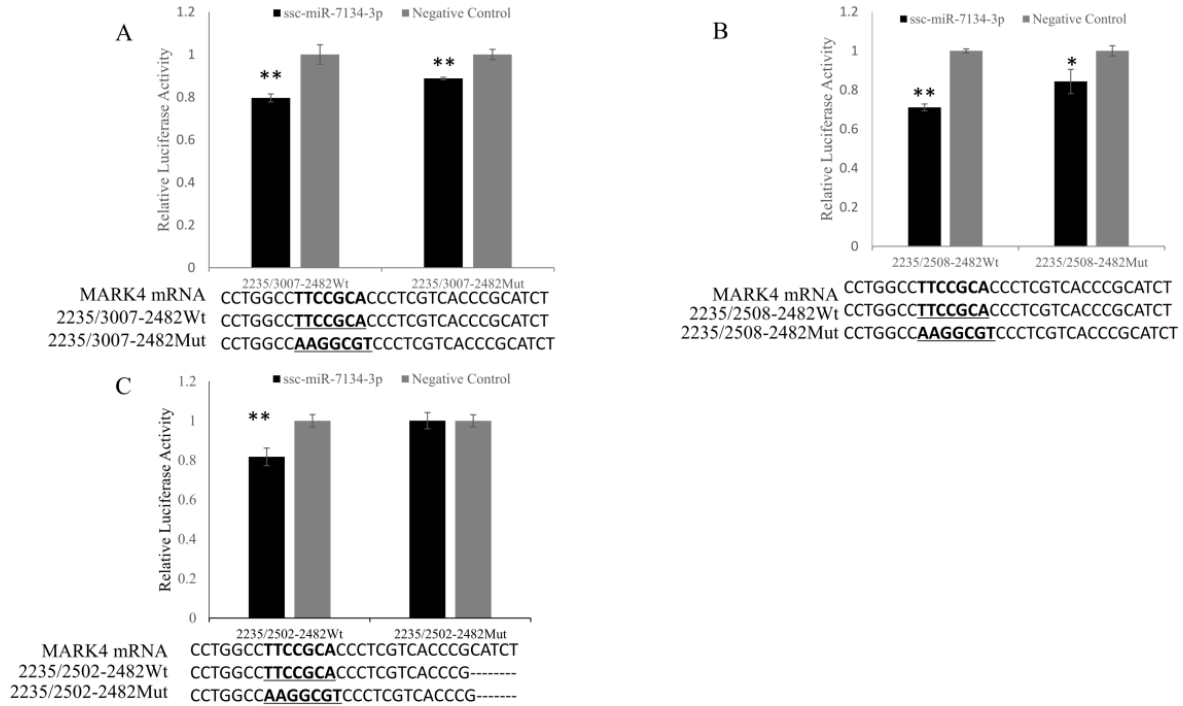


Figure S2. Detection of ssc-miR-7134-3p target site on *MARK4* using the dual-luciferase system. (A-C) The assay was implemented with fragments containing the region spanning 2235 to 3007 (A), 2235 to 2508 (B), and 2235 to 2502 (C). The bold characters indicate the location of the mutation. All relative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

Figure S3 A

hMARK4.pro	MSSRTFLAPGNDRNEDTHGTLGGGR99DKGPGMSSRSLGARCNRSTASCF	50		
pMARK4.pro	MSSRTFLAPGNDRNEDTHGTLGGGR99DKGPGMSSRSLGARCNRSTASCF	50		
mMARK4.pro	MSSRTFLAPGNDRNEDTHGTLGGGR99DKGPGMSSRSLGARCNRSTASCF	50		
rMARK4.pro	MSSRTFLAPGNDRNEDTHGTLGGGR99DKGPGMSSRSLGARCNRSTASCF	50		
Consensus	msart flapgndrnaahgtlqsggrnsdkgpawasrrlgsrerniascp			
hMARK4.pro	SLNRSKYNEVTA ⁵¹ YLLLRKSTREGGDRGAPGLALA ³⁹⁹ AVRAPSD ⁴⁰⁰ TLNRTSSS	400		
pMARK4.pro	SLNRSKYNEVTA ⁵¹ YLLLRKSTREGGDRGAPGLALA ³⁹⁹ AVRAPSD ⁴⁰⁰ TLNRTSSS	400		
mMARK4.pro	SLNRSKYNEVTA ⁵¹ YLLLRKSTREGGDRGAPGLALA ³⁹⁹ AVRAPSD ⁴⁰⁰ TLNRTSSS	400		
rMARK4.pro	SLNRSKYNEVTA ⁵¹ YLLLRKSTREGGDRGAPGLALA ³⁹⁹ AVRAPSD ⁴⁰⁰ TLNRTSSS	400		
Consensus	lt qkynevtstylllgrkteeggdrgappglalazvrapsdttngtssz			
hMARK4.pro	KNSSTKQGR ⁴⁵⁰ SSSTYHQRS ⁴⁵⁰ SDPCGSPALLPKRSPT ⁴⁵⁰ Q ⁴⁵⁰ SLKDA	450		
pMARK4.pro	KNSSTKQGR ⁴⁵⁰ SSSTYHQRS ⁴⁵⁰ SDPCGSPALLPKRSPT ⁴⁵⁰ Q ⁴⁵⁰ SLKDA	450		
mMARK4.pro	KNSSTKQGR ⁴⁵⁰ SSSTYHQRS ⁴⁵⁰ SDPCGSPALLPKRSPT ⁴⁵⁰ Q ⁴⁵⁰ SLKDA	450		
rMARK4.pro	KNSSTKQGR ⁴⁵⁰ SSSTYHQRS ⁴⁵⁰ SDPCGSPALLPKRSPT ⁴⁵⁰ Q ⁴⁵⁰ SLKDA	450		
Consensus	ky st kqgr ssttyhqrs ⁴⁵⁰ sdpcgspalhpkrspstq slkee			
hMARK4.pro	LINDKASQSA ⁵⁰⁰ SSGSRGLDFSSPMVSSAHNTNKASIPERRKDSSTPNN	500		
pMARK4.pro	LINDKASQSA ⁵⁰⁰ SSGSRGLDFSSPMVSSAHNTNKASIPERRKDSSTPNN	500		
mMARK4.pro	LINDKASQSA ⁵⁰⁰ SSGSRGLDFSSPMVSSAHNTNKASIPERRKDSSTPNN	500		
rMARK4.pro	LINDKASQSA ⁵⁰⁰ SSGSRGLDFSSPMVSSAHNTNKASIPERRKDSSTPNN	500		
Consensus	r pcrkascz gsgarglppsspmvssahnpkaielperkdstatpnn			
hMARK4.pro	LPPSMTPRRNTYVCTERDPS ⁵⁵⁰ QGL ⁵⁵⁰ PNKENS ⁵⁵⁰ SGTV ⁵⁵⁰ RVPPAS ⁵⁵⁰ PSHS ⁵⁵⁰ LA	550		
pMARK4.pro	LPPSMTPRRNTYVCTERDPS ⁵⁵⁰ QGL ⁵⁵⁰ PNKENS ⁵⁵⁰ SGTV ⁵⁵⁰ RVPPAS ⁵⁵⁰ PSHS ⁵⁵⁰ LA	550		
mMARK4.pro	LPPSMTPRRNTYVCTERDPS ⁵⁵⁰ QGL ⁵⁵⁰ PNKENS ⁵⁵⁰ SGTV ⁵⁵⁰ RVPPAS ⁵⁵⁰ PSHS ⁵⁵⁰ LA	550		
rMARK4.pro	LPPSMTPRRNTYVCTERDPS ⁵⁵⁰ QGL ⁵⁵⁰ PNKENS ⁵⁵⁰ SGTV ⁵⁵⁰ RVPPAS ⁵⁵⁰ PSHS ⁵⁵⁰ LA	550		
Consensus	lppsmtprrntyvcterpg er slpngknsstz rvpvppaspsahla			
hMARK4.pro	PPSGGRSLARG ⁶⁰⁰ STIRST ⁶⁰⁰ ITGGQVRRRAS ⁶⁰⁰ SSGGVQNG ⁶⁰⁰ FPAS ⁶⁰⁰ PTLAA ⁶⁰⁰	600		
pMARK4.pro	PPSGGRSLARG ⁶⁰⁰ STIRST ⁶⁰⁰ ITGGQVRRRAS ⁶⁰⁰ SSGGVQNG ⁶⁰⁰ FPAS ⁶⁰⁰ PTLAA ⁶⁰⁰	600		
mMARK4.pro	PPSGGRSLARG ⁶⁰⁰ STIRST ⁶⁰⁰ ITGGQVRRRAS ⁶⁰⁰ SSGGVQNG ⁶⁰⁰ FPAS ⁶⁰⁰ PTLAA ⁶⁰⁰	600		
rMARK4.pro	PPSGGRSLARG ⁶⁰⁰ STIRST ⁶⁰⁰ ITGGQVRRRAS ⁶⁰⁰ SSGGVQNG ⁶⁰⁰ FPAS ⁶⁰⁰ PTLAA ⁶⁰⁰	600		
Consensus	ppsggrslargstiratitggqvrrray g gggvqngppasptlae			
hMARK4.pro	SLPLSRFRFT ⁶⁵⁰ NLTKLTSALIRV ⁶⁵⁰ QCPDRIGGPEVT ⁶⁵⁰ QCHLPW ⁶⁵⁰ Q ⁶⁵⁰	650		
pMARK4.pro	SLPLSRFRFT ⁶⁵⁰ NLTKLTSALIRV ⁶⁵⁰ QCPDRIGGPEVT ⁶⁵⁰ QCHLPW ⁶⁵⁰ Q ⁶⁵⁰	650		
mMARK4.pro	SLPLSRFRFT ⁶⁵⁰ NLTKLTSALIRV ⁶⁵⁰ QCPDRIGGPEVT ⁶⁵⁰ QCHLPW ⁶⁵⁰ Q ⁶⁵⁰	650		
rMARK4.pro	SLPLSRFRFT ⁶⁵⁰ NLTKLTSALIRV ⁶⁵⁰ QCPDRIGGPEVT ⁶⁵⁰ QCHLPW ⁶⁵⁰ Q ⁶⁵⁰	650		
Consensus	a plp ssprrftnltkltsalirv qcpdriggpevtqchlpw q			
Protein	hMARK4	pMARK4	mMARK4	rMARK4
Accession	NP_001186796		NP_758483	NP_001178000

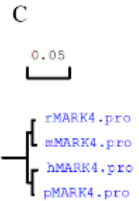
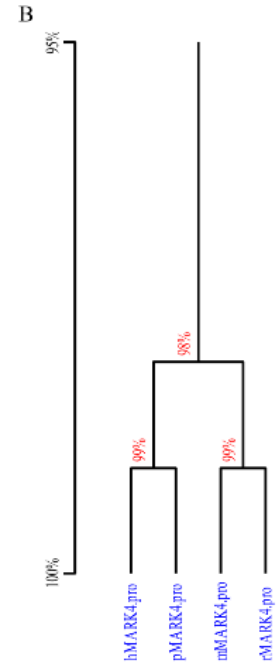


Figure S3. Alignment and phylogenetic analysis of MARK4 amino acid sequences from different species. (A) MARK4 sequence alignment. Identical amino acids are shadowed in black. The regions from residue 51 to 399, and from residue 651 to 752 are identical and are not shown. (B) Homology of MARK4 amino 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.