

Appendix A. Results of the electrophoresis of total RNA and RT-PCR products. A) Electrophoresis results of total RNA, where 1 represents the RNA sample and M is the marker, and three bands can be seen. B) Results of the electrophoresis of RT-PCR products of the TLR4 gene. M represents the marker and 1 represents the RT-PCR product. C) Electrophoresis of the RT-PCR product of TLR4-ASV that primer pair designed in the each side of exon 2. It can be seen that 167 bp was skipped in TLR4-ASV as compared with TLR4-ALL.

1	ATGAAAACTTGTATTCAAGGTCTGGCTGGTTCAAAAATCAATC
1	M K T C I Q G L A G S K I N Q L V L G E F K N E R N L E S F «
91	GACAAATCTGTCCTGGAGGAACTGTGCAATTTGACCCTTGAACAGTTCCCGGATAGCACACTTTGGTGAATTCCCAGATGATGTTTCTGAC
31	D K S V L E E L C N L T L E Q F R I A H F G E F P D D V S D
181	TTATTTAATTGTTTGGCAAATGCCTCTGTGATTTCTCTGTTGAGTCTGAATTTACATGGTCTAGAAGCCCTTCCTAATGATTTCAGGTGG
61	L F N C L A N A S V I S L L S L N L H G L E A L P N D F R W
271	CAACACTTAGAAGTGGTTAACTGTAAACTTAAACAATTTCCTGCATTGAAGTTCAACTCTCTCAAAAAGTTTGTTT
91	Q H L E V V N C K L K Q F P A L K F N S L K K F V F K D N K
361	CATATGCACACTTTTACTGAAATTAACCTGCCAAACCTTCAGTTTCTAGATCTCAGTGGAAATCACTTGAGCTTTAAAGGTTGCTGTTCT
121	H M H T F T E I N L P N L Q F L D L S G N H L S F K G C C S
451	CACAATGAATTTGGGACAACCAAACTGAAGCATTTAGATCTGAGCTTCAATGAAATCATTACCATGAAGTCAAACTTCATGGGCTTAGAG
151	H N E F G T T K L K H L D L S F N E I I T M K S N F M G L E
541	CAACTAGAATACCTGGATTTCCAGCATTCCAGTTTGAAACAGGCTAATGACTTTTCCATATTCCTATCACTCAGAAACCTCCATTACCTT
181	Q L E Y L D F Q H S S L K Q A N D F S I F L S L R N L H Y L
631	GATATTTCTTATACTAACATCCACGTTGTCTTCCGTGGCATTTTTGCTGGCTTGGTCAGCCTCCAAACCTTGAAAATGGCTGGC
211	D I S Y T N I H V V F R G I F A G L V S L Q T L K M A G N S
721	TTTCAGAACAACTTGCTTOCTGATGTCTTCACAGATCTGACTAACTTAATCCTCCTGGAOCTCTCTAAGTGTCAACTGGAACAGGTATOC
241	F Q N N L L P D V F T D L T N L I L L D L S K C Q L E Q V S
811	CAGAGGGCATTTCATTCOCTCOCTAGGCTTCAGGTGCTAAATATGAGTCACAACAGACTCTTGTTCTTTGATACACTTCCTTATAAACCA
271	Q R A F H S L P R L Q V L N M S H N R L L F F D T L P Y K P
901	CTCCACTCCCTCAGGATTCTGGATTGCAGTTACAATCTTATCGTGGCCTCCAAGGAACAAGAACTACAGCATCTGCCAAGGAGCCTAGCT
301	LH S L R I L D C S Y N L I V A S K E Q E L Q H L P R S L A
991	TTTTTAAATCTCACTAAGAATGACTTTTCTTGTGCGTGTGAACACCAGACTTTCTTGCAGTGGGTCAAGGACCAGAAGCAGCTCCTTGTG
331	F L N L T K N D F S C A C E H Q T F L Q W V K D Q K Q L L V
1081	GGAGCTGAGCAAATGGTATGTACACAACCTTTAGAAATGCAGGACCTGOCTGTGCTGAGTTTCAGGAAOGOCAOCTGTCAGATAAGCGAG
361	G A E Q M V C T Q P L E M Q D L P V L S F R N A T C Q I S E
1171	GCCGTCATTAGTGCGTCAGTTCTCACCTTOCTOCTGGTATCTGTGGCCAGGAATCCTGGTCTATAAGTTCTATTTOCACTTGTTGCTTTTT
391	A V I S A S <u>V L T F L L V S V A G I L V Y K F Y F H L L L</u> F
1261	GTGGGCTGCAAAAAATATGGCAGAGGTGAAAGCA <u>OCTATGACGCCTTTGTTATCTACTCCAGOCAGGAOGAAGACTGGGTGAGGAATGAA</u>
421	V G C K K Y G R G E S <mark>T Y D A F V I Y S S Q D E D W V R N E</mark>
1351	CTGGTAAAGAACTTGGAGGAGGGGGGGGGCCCTTTTCATCTCTGCCTTCACTACAGAGACTTCATTCCCGGTGTGGCCATCGCTGCTAAC
451	L V K N L E E G V P F H L C L H Y R D F I P G V A I A A N
1441	ATCATCCAGGAAGGTTTOCACAAAAGTCCGGAAGGTTATTGTCGTGGTGTOCCAGCACTTCATACAGAGOCGATGGTGTATCTTTGAGTAT
481	I I Q E G F H K S R K V I V V V S Q H F I Q S R W C I F E Y
1531	GAGATTGCCCAGACCTGCCAGTTTCTGAGGAGTCATGCCGGCATCATCTTCATCGTCCTGCAGAAGTTGGAGAAGTCCCTGCTGCGGCAG
511	E I A Q T W Q F L R S H A G I I F I V L Q K L E K S L L R Q
1621	CAGGTGGAGCTGTATOGOCTTCTCAGCAGGAATAOCTAOCTGGAGTGGGAGGACAGOGTOCTGGGGGGGGCACATCTTCTGGAGAOGACTC
541	Q V E L Y R L L S R N T Y L E W E D S V L G R H I F W R R L
1711	$\underline{A}\underline{A}\underline{G}\underline{A}\underline{A}\underline{G}\underline{C}\underline{T}\underline{G}\underline{G}\underline{T}\underline{G}\underline{G}\underline{T}\underline{G}\underline{G}\underline{A}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}G$
571	<u>K K A L L D G</u> K P W S P E G T E D S E S N Q H D T T A F T *

Appendix B Nucleotide and deduced amino acid sequence of TLR4 alternative splicing variant in Bama miniature pigs. Complete sequence of the full-length TLR4 alternative splicing variant presented and translated amino acid sequence is shown under the nucleotide sequence. Numbers to the left of each row refer to nucleotides. Leucine-rich repeat (LRR) domains are shaded in grey. The initiation codon (atg) is underlined. The predicted transmembrane segment is double underline. The translational stop site is indicated by an asterisk. TIR motif is shown by a long box.

BAMA_TLR4_ASV Homo_sapiens_TLR4 Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2. Sus_scrofa_TLR4ASV3.	MIPRIRLAVATIPANAFLSCD. MIPRIRLAVATIPANAFLSCLRSESWDPC MIPRIRLAVATIPANAFLSCLRSESWDPC	VQVVPNISYQCWELNFYKIPDNIPTS VQVVPNISYQCWELNFYKIPDNIPTS	LRAGTLA SVKILDLSFNVLSHLDSNSFS SVKILDLSFNVLSHTAIASPA	CRCEI SFPELQVLDLSRCEI FQNCRC	0 0 33 90 81
BAMA_TLR4_ASV Homo_sapiens_TLR4.seq Sus_scrofa_TLR4_ASV1 Sus_scrofa_TLR4_ASV2 Sus_scrofa_TLR4_ASV3	QTIDDDAYQGLNYLSTLTLTGNPIQSLAL QTIDDDAYQGLNYLSTLTLTGNPIQSLAL WIYFDVKFRQLTMTGNPIQSLAL	GAFSGLPSLQKLVAVETNLASLEDFF GAFSGLPSLQKLVAVETNLASLEDFF GAFSGLPSLQKLVAVETNLASLEDFF	PIGHLKTLKELNVAHNHIHSF PIGHLKTLKELNVAHNHIHSF PIGHLKTLKELNVAHNHIHSF	KLPEYFSNLPNLEHL KLPEYFSNLPNLEHL KLPEYFSNLPNLEHL	0 123 180 165
BAMA_TLR4_ASV Homo_sapiens_TLR4 Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2 Sus_scrofa_TLR4ASV3	MPLENLSLD DLSKNKIENIYHEHSQVLHQVPLLNLSLD DLSKNKIENIYHEHSQVLHQVPLLNLSLD DLSKNKIENIYHEHSQVLHQVPLLNLSLD	LSLNPMNFIQPGAFKEIRLHKLTLR LSLNPLNFIEPGAFNKIRLNGLTLR LSLNPLNFIEPGAFNKIRLNGLTLR LSLNPLNFIEPGAFNKIRLNGLTLR	MKTCIQGLAGSKI NFDSLNVMKTCIQGLAGEV SNFNSSDVMKTCIQGLAGSKI SNFNSSDVMKTCIQGLAGSKI SNFNSSDVMKTCIQGLAGSKI	NCSVLGEFENDENLE HRIVLGEFENDENLE NCIVLGEFENDENLE NCIVLGEFENDENLE NCIVLGEFENDENLE NCIVLGEFENDENLE	28 70 213 270 255
BAMA_TLR4_ASV Homo_sapiens_TLR4 Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2 Sus_scrofa_TLR4ASV3	SFDKSVLBELCNLTUECFRUAHFGEHEDD KFDKSALECLCNLTUEEFRUANLDYNUDD SFDKSVLBELCNLTUEEFRUAHFGEHEDD SFDKSVLBELCNLTUEEFRUAHFGEHEDD SFDKSVLBELCNLTUEEFRUAHFGEHEDD	VSDLFNCIANASVISULSINLHGLA I DDLFNCLTIVSSFSIVSVTIERVKU VSDLFNCLTIVSSFSIVSVTIERVKU VSDLFNCLANASVISULSINLRGLA VSDLFNCLANASVISULSINLRGLA VSDLFNCLANASVISULSINLRGLA	NIPNIFRWGHLENVNCKLROF DFSYR-GWGHLENVNCKLGOF LIPNIFRWGHLENVNCKLGOF NIPNIFRWGHLENVNCKLGOF LIPNIFRWGHLENVNCKLGOF	FALKENSLEXEVEKD FOLKLESLERLEETS FALKENSLEXEVERD FALKENSLEXEVERD FALKENSLEXEVERD	118 160 303 360 345
BAMA_TLR4_ASV Homo_sapiens_TLR4 Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2 Sus_scrofa_TLR4ASV3	LKRI NYHMHT TEIN EN LEFKE NYGGNA SEVDI SELDISKN GISKG NYHMHT TEIN EN LEFKE NYHMHT TEIN EN LEFKE NYHMHT TEIN EN LEFKE NYHMHT TEIN EN LEFKE NYHMHT TEIN EN LEFKE	LKR2 CCSHNEFGTINENHDDESENETITM CCSQSDEGTISENYDDESENGTIN CCSHNEFGTINENHDDESENETITM CCSHNEFGTINENHDDESENETITM CCSHNEFGTINENHDDESENETITM CCSHNEFGTINENHDDESENETITM	SNAVGLEQLAYLDFQHSGL SNAUGLEQLAFLDFQHSGL SNAUGLEQLAFLDFQHSSL SNAVGLEQLAYLDFQHSSL SNAVGLEQLAYLDFQHSSL SNAUGLEQLAYLDFQHSSL 1005	CANDESTELSTRNLH CMSEFSWEISTRNLT CANDESTELSTRNLH CANDESTELSTRNLH CANDESTELSTRNLH	208 250 393 450 435
BAMA_TLR4_ASV. Homo_sapiens_TLR4. Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2 Sus_scrofa_TLR4ASV3	VLDISVINIK WHIGHAGUSUUTIKAA YLDISH BIRMARNOHNGISUUTIKAA YLDISH BIRMARNOHNGIAGUSUUTIKAA YLDISVINIK WHIGHAGUSUUTIKAA YLDISVINIK WHIGHAGUSUUTIKAA YLDISVINIK WHIGHAGUSUUTIKAA	CASEGNILED FOR THE INTELLOS CASEGNILED FOR THE INTELLOS	CQLEQVSQRAFHSUPRLQVI CQLEQVSQRAFHSUPRLQVI CQLEQVSQRAFHSUPRLQVI CQLEQVSQRAFHSUPRLQVI CQLEQVSQRAFHSUPRLQVI	NMSHNRLLFFDTUFY NMSHNNFFSUDTFPY NMSHNRLLFUDTUFY NMSHNRLLFUDTUFY NMSHNRLLFUDTUFY	298 340 483 540 525
BAMA_TLR4_ASV Homo_sapiens_TLR4 Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2 Sus_scrofa_TLR4ASV3	FUESCRILDOS NLIVASREGELCHER KCINSLOVENSLNEINTSTRGELCHER NEUESCRILDOS NLIVASREGELCHER NEUESCRILDOS NLIVASREGELCHER REUESCRILDOS NLIVASREGELCHER Transmehrane Dom	SLAFLNLTENDFSCACEHOTFLOWVE SLAFLNLTGNDACTCEHOSFLOWT SLAFLNLTENDFSCACEHOTFLOWVE SLAFLNLTENDFSCACEHOTFLOWVE SLAFLNLTENDFSCACEHOTFLOWVE SLAFLNLTENDFSCACEHOTFLOWVE SLAFLNLTENDFSCACEHOTFLOWVE	BRKGLLVGABONVO TORGLVEVENEGATESDR DOKOLVGABONVOTOFLEN DOKOLVGABONVOTOFLEN DOKOLVGABONVOTOFLEN	GDIEVLSERNATCGI GGNEVLSLN.IICCM GDIEVLSERNATCGI GDIEVLSERNATCGI GDIEVLSERNATCGI Box2	388 429 573 630 615
BAMA_TLR4_ASV Homo_sapiens_TLR4 Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2 Sus_scrofa_TLR4ASV3.	SEAN ISASVIITEI UVSNAGTI VVKFYFHI NKTII GUSVISVU VVN VAN VVKFYFHI SEAN ISASVIITEI UVSNAGTI VVKFYFHI SEAN ISASVIITEI UVSNAGTI VVKFYFHI SEAN ISASVIITEI UVSNAGTI VVKFYFHI	LLEYGGRYYGRGESI YDAFV IYSSQI LLAGG XYGRGESI YDAFV IYSSQI LLYGGXYYGRGESI YDAFV IYSSQI LLYGGXYYGRGESI YDAFV IYSSQI LLYGGXYYGRGESI YDAFV IYSSQI	DE DWVRNELVKNLEEGVPPF DE DWVRNELVKNLEEGVPPF DE DWVRNELVKNLEEGVPPF DE DWVRNELVKNLEEGVPPF DE DWVRNELVKNLEEGVPPF	LCLHYRDFIPGVAIA LCLHYRDFIPGVAIA LCLHYRDFIPGVAIA LCLHYRDFIPGVAIA LCLHYRDFIPGVAIA	478 519 663 720 705
BAMA_TLR4_ASV. Homo_sapiens_TLR4 Sus_scrofa_TLR4ASV1 Sus_scrofa_TLR4ASV2 Sus_scrofa_TLR4ASV3.	ANIICEGFHKSRKVIVVVSQHFIQSRWCI ANII <mark>H</mark> EGFHKSRKVIVVVSQHFIQSRWCI ANIICEGFHKSRKVIVVVSQHFIQSRWCI ANIICEGFHKSRKVIVVVSQHFIQSRWCI ANIICEGFHKSRKVIVVVSQHFIQSRWCI	FEYEIAQTWQFLESEAGIIFIVLQAT FEYEIAQTWQFLESEAGIIFIVLQAT FEYEIAQTWQFLESEAGIIFIVLQA FEYEIAQTWQFLESEAGIIFIVLQAT FEYEIAQTWQFLESEAGIIFIVLQAT	EKSLLRQQVELYRLLSRNTY EKSLLRQQVELYRLLSRNTY EKSLLRQQVELYRLLSRNTY EKSLLRQQVELYRLLSRNTY EKSLLRQQVELYRLLSRNTY	REWEDSVLGR HIFWR LEWEDSVLGR HIFWR LEWEDSVLGR HIFWR LEWEDSVLGR HIFWR LEWEDSVLGR HIFWR	568 609 753 810 795
BAMA_TLR4_ASV. Homo_sapiens_TLR4. Sus_scrofa_TLR4_ASV1. Sus_scrofa_TLR4_ASV2 Sus_scrofa_TLR4_ASV3.	RLEKALLDGHEWSPEGTEDSESNOHDT FA RLEKALLDGHEWSPEGTEDSESNOHDT FA RLEKALLDGHEWSPEGTEDSESNOHDT FA RLEKALLDGHEWSPEGTEDSESNOHDT FA RLEKALLDGHEWSPEGTEDSESNOHDT FA				598 639 783 840 825

Appendix C Multiple alignment of TLR4 alternative splicing variants from different species. TIR domains of TLR4 alternative splicing variants protein sequences were determined by the SMART program and aligned using ClustalW. Three conserved boxes (box 1, 2 and 3) are framed. The dotted lines indicate the amino acids that were skipped in TLR4 alternative splicing variants. LRR, leucine-rich

repeats.



Appendix D The predicted molecular characteristic of TLR4 alternative splicing in Bama miniature pigs. A) The three-dimensional structure of the predicted extracellular region of TLR4-ASV. The leucine-rich repeats (LRRs) are indicated in green, and the LRR-CT is indicated in red. B) Three-dimensional structure of the extracellular region of TLR4. The LRRs are indicated in green, and the LRR-CTs are indicated in red. The spliced region of TLR4 is indicated in blue. C) Prediction of the transmembrane region of TLR4-ASV. D) Prediction of conserved domain region of TLR4-ASV.



Least stable gene Most stable gene

Appendix E The expression stability of housekeeping genes. The results of qRT-PCR analyses in the in vitro cultured PBMCs from Bama miniature pigs were used to calculate stability using geNorm software. The relative stability (M) (Y-axis) is defined as a measure of gene expression stability, and higher M values indicate less stability. Note the range of M values (high to low), indicating the degree of variability between the least and most stable genes. ACTB: β -actin, RPL4: Ribosomal protein L4, TBP: TATA-box-binding protein, B2M: beta-2-microglobulin, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Gene	Sequence	Reference	Amplicon	Product	PCR
			Length (bp)	$T_m(^{\circ}C)$	efficiency (%)
TLR4-All	5'-TTCATACAGAGCCGATGGTG-3'	NM_001113039	154	88	0.9815
	5'- TCCTGCTGAGAAGGCGATA -3'				
TLR4-Classic	5'- CCACATCAGTCAAGATACTGGACC-3'	NM_001113039	168	89.5	0.9925
	5'- AAAGGCTCCCAGGGCTAAAC-3'				

Appendix F Primer sequences and amplicon characteristics in Bama miniature pigs.

TLR4-ALL was used to quantify the expression of TLR4, which contained the alternative splicing variant, and

TLR4-classic, in which TLR4 was expressed without splicing in exon 2.