

ADDITIONAL FILES 1

TABLES

Table S1. Summary of sampled specimens and identification of haplotypes.

ID	Species	Country	Province	Sex	LBR _{TLR4} variants	LBR _{TLR7} variants	Hap Exon 3 <i>Tlr4</i>	Hap Exon 3 <i>Tlr7</i>	GenBank Acc. <i>Tlr4/Tlr7</i>
C0443	<i>Bandicota indica</i>	Cambodia	Mondolkiri	F	Bain1_Bain2	Rattus sp.	bainh5_bainh6	bainh1	KC811609/KC811705
L0276	<i>Bandicota indica</i>	Lao PDR	Champasak	M	Bain3	Rattus sp.	bainh1_bainh2	bainh2	KC811610/KC811706
R4000	<i>Bandicota indica</i>	Thailand	Kalasin	M	Bain1	Rattus sp.	bainh4	bainh3	KC811611/KC811707
R5313	<i>Bandicota indica</i>	Thailand	Nan	M	Bain3	Rattus sp.	bainh3	bainh4	KC811612/KC811708
C0709	<i>Bandicota savilei</i>	Cambodia	Mondolkiri	F	Basa2	Basa	basah1	basah2	KC811613/KC811709
L0331	<i>Bandicota savilei</i>	Lao PDR	Champasak	F	Basa1_Basa2	Basa	basah2_basah3	basah1_basah2	KC811614/KC811710
R4141	<i>Bandicota savilei</i>	Thailand	Phrae	M	Basa3	Basa	basah4	basah2	KC811615/ KC811711
R5475	<i>Bandicota savilei</i>	Thailand	Buriram	F	Basa1	Basa	basah5_basah6	basah2	KC811616/ KC811712
C0333	<i>Berylmys berdmorei</i>	Cambodia	Sihanouk	M	Bebe	Be sp.	bebeh2	bebeh1	KC811617/ KC811713
C0481	<i>Berylmys berdmorei</i>	Cambodia	Mondolkiri	M	Bebe	Be sp.	bebeh1	bebeh2	KC811618/ KC811714
L0006	<i>Berylmys berdmorei</i>	Lao PDR	Luang Prabang	M	Bebe	Be sp.	bebeh3_bebah4	bebeh3	KC811619/ KC811715
R3441	<i>Berylmys berdmorei</i>	Thailand	Loei	M	Bebe	Be sp.	bebeh3	bebeh4	KC811620/ KC811716
L0151	<i>Berylmys bowersi</i>	Lao PDR	Luang Prabang	F	Bebo	Be sp.	beboh4_bebah6	beboh1_bebah4	KC811621/ KC811717
R4400	<i>Berylmys bowersi</i>	Thailand	Loei	M	Bebo	Be sp.	beboh1	beboh4	KC811622/ KC811718
R4804	<i>Berylmys bowersi</i>	Thailand	Loei	M	Bebo	Be sp.	beboh2_bebah3	beboh4	KC811623/ KC811719
R5410	<i>Berylmys bowersi</i>	Thailand	Nan	F	Bebo	Be sp.	beboh5_bebah6	beboh2_bebah3	KC811624/ KC811720
C0421	<i>Leopoldamys edwardsi</i>	Cambodia	Mondolkiri	M	Leed	Le sp.	leedh4_leedh5	leedh3	KC811625/ KC811721
R4070	<i>Leopoldamys edwardsi</i>	Thailand	Loei	M	Leed	Le sp.	leedh6_leedh7	leedh1	KC811626/ KC811722
R4276	<i>Leopoldamys edwardsi</i>	Thailand	Phrae	F	Leed	Le sp.	leedh1_leedh3	leedh2	KC811627/ KC811723
R4296	<i>Leopoldamys edwardsi</i>	Thailand	Phrae	F	Leed	Le sp.	leedh1_leedh2	leedh2	KC811628/ KC811724
R4350	<i>Leopoldamys edwardsi</i>	Thailand	Phrae	?	x	Le sp.	x	leedh2	x/ KC811725
R5057	<i>Leopoldamys edwardsi</i>	Thailand	Loei	M	x	Le sp.	x	leedh2	x/ KC811726
R4477	<i>Leopoldamys neilli</i>	Thailand	Phrae	F	Lene	Le sp.	leneh2_leneh4	leneh1	KC811629/ KC811727
R4486	<i>Leopoldamys neilli</i>	Thailand	Phrae	F	Lene	Le sp.	leneh1_leneh4	leneh1	KC811630/ KC811728
R4527	<i>Leopoldamys neilli</i>	Thailand	Loei	F	Lene	Le sp.	leneh4	leneh2	KC811631/ KC811729
R4530	<i>Leopoldamys neilli</i>	Thailand	Loei	M	Lene	Le sp.	leneh3_leneh4	leneh2	KC811632/ KC811730
R3033	<i>Leopoldamys sabanus</i>	Thailand	Kanchanaburi	F	Lesa	Le sp.	lesah1_lesah2	lesah1_lesah2	KC811633/ KC811731
R3111	<i>Leopoldamys sabanus</i>	Thailand	Kanchanaburi	M	Lesa	Le sp.	lesah1_lesah2	lesah2	KC811634/ KC811732
C0118	<i>Maxomys surifer</i>	Cambodia	Sihanouk	M	Masu1	x	masuh5_masuh7	x	KC811635/ x
C0478	<i>Maxomys surifer</i>	Cambodia	Mondolkiri	M	Masu2	x	masuh1_masuh2	x	KC811636/ x
L0274	<i>Maxomys surifer</i>	Lao PDR	Champasak	F	Masu1	x	masuh6	x	KC811637/ x
R4099	<i>Maxomys surifer</i>	Thailand	Loei	M	Masu3_Masu4	x	masuh3_masuh4	x	KC811638/x
C0423	<i>Mus caroli</i>	Cambodia	Mondolkiri	M	Muca2	Mus sp.	mucah3	mucah4	KC811639/ KC811733
L0014	<i>Mus caroli</i>	Lao PDR	Luang Prabang	F	Muca1	Mus sp.	mucah2	mucah1	KC811640/ KC811734
L0211	<i>Mus caroli</i>	Lao PDR	Luang Prabang	M	Muca1	Mus sp.	mucah3_mucah4	mucah1	KC811641/ KC811735
L0275	<i>Mus caroli</i>	Lao PDR	Champasak	M	Muca3	Mus sp.	mucah1_mucah3	mucah2	KC811642/ KC811736
R5642	<i>Mus caroli</i>	Thailand	Buriram	M	x	Mus sp.	x	mucah3	x/ KC811737
R4864	<i>Mus cervicolor</i>	Thailand	Loei	F	Muce1_Muce2	Mus sp.	muceh4_muceh5	muceh1	KC811643/ KC811738
R5644	<i>Mus cervicolor</i>	Thailand	Buriram	M	Muce1_Muce2	Mus sp.	muceh1_muceh2	muceh2	KC811644/ KC811739
R5666	<i>Mus cervicolor</i>	Thailand	Buriram	F	x	Mus sp.	x	muceh2	x/ KC811740
R5671	<i>Mus cervicolor</i>	Thailand	Buriram	F	Muce2	Mus sp.	muceh3_muceh2	muceh3	KC811645/ KC811741
L0103	<i>Mus cookii</i>	Lao PDR	Luang Prabang	F	Muco1	Mus sp.	mucoh3	mucoh3	KC811646/ KC811742
L0178	<i>Mus cookii</i>	Lao PDR	Luang Prabang	M	Muco1	Mus sp.	mucoh3	mucoh3	KC811647/ KC811743
R4106	<i>Mus cookii</i>	Thailand	Loei	F	Muco1_Muco2	Mus sp.	mucoh1_mucoh2	mucoh1_mucoh2	KC811648/ KC811744

SK1515	<i>Mus domesticus</i>	France	Saint Jean-et-Royans	F	Mus sp.	Mus sp.	mudoh5	mudoh3	KC811649/ KC811745
SU3770	<i>Mus domesticus</i>	Turkey	Izmir	F	Mus sp.	Mus sp.	mudoh2_mudoh5	mudoh1_mudoh3	KC811650/ KC811746
M273	<i>Mus domesticus</i>	Syria	Palmyra	M	Mus sp.	Mus sp.	mudoh4_mudoh5	mudoh1	KC811651/ KC811747
MISC352	<i>Mus domesticus</i>	Egypt	Sabha Oasis	M	Mus sp.	Mus sp.	mudoh1_mudoh3	mudoh2	KC811652/ KC811748
JPC2821	<i>Mus musculus</i>	Czech rep.	Buskovice	F	Mus sp.	Mus sp.	mumuh3	mumuh2	KC811653/ KC811749
SK843	<i>Mus musculus</i>	Germany	Lindhorst	M	Mumu2	Mus sp.	mumuh4	mumuh2	KC811654/ KC811750
SU5218	<i>Mus musculus</i>	Russia	Shkili	F	Mumu2	Mus sp.	mumuh4	mumuh1_mumuh2	KC811655/ KC811751
360	<i>Mus musculus</i>	Romania	Botosani	M	Mumu2	Mus sp.	mumuh3_mumuh4	mumuh3	KC811656/ KC811752
SU5209	<i>Mus musculus</i>	Ukraine	Primorskoe	M	Mus sp._Mumu1	Mus sp.	mumuh1_mumuh2	mumuh1	KC811657/ KC811753
C0322	<i>Niviventer fulvescens</i>	Cambodia	Sihanouk	F	Nifu1	Nifu	nifuh1_nifuh2	nifuh2	KC811658/ KC811754
C0430	<i>Niviventer fulvescens</i>	Cambodia	Mondolkiri	F	x	Nifu	x	nifuh5_nifuh8	x/ KC811755
L0273	<i>Niviventer fulvescens</i>	Lao PDR	Champasak	F	Nifu1	Nifu	nifuh3_nifuh4	nifuh6_nifuh7	KC811659/ KC811756
R4071	<i>Niviventer fulvescens</i>	Thailand	Loei	M	Nifu1_Nufu2	Nifu	nifuh5_nifuh6	nifuh1	KC811660/ KC811757
R4497	<i>Niviventer fulvescens</i>	Thailand	Phrae	F	Nifu1	Nifu	nifuh3_nifuh7	nifuh3_nifuh4	KC811661/ KC811758
L0052	<i>Rattus andamanensis</i>	Lao PDR	Luang Prabang	F	Raan1	Rattus sp.	raanh1	raanh1_raanh2	KC811662/ KC811759
L0149	<i>Rattus andamanensis</i>	Lao PDR	Luang Prabang	F	Raan2_Raan3	Rattus sp.	raanh2_raanh3	raanh1_raanh2	KC811663/ KC811760
R2953	<i>Rattus andamanensis</i>	Thailand	Kanchanaburi	F	Raan3	Rattus sp.	raanh4_raanh5	raanh3_raanh4	KC811664/ KC811761
R3087	<i>Rattus andamanensis</i>	Thailand	Kanchanaburi	M	Raan3	Rattus sp.	raanh4_raanh5	raanh3	KC811665/ KC811762
C0014	<i>Rattus argentiventer</i>	Cambodia	Sihanouk	M	Raan1	Rattus sp.	raarh5	raarh1	KC811666/ KC811763
C0048	<i>Rattus argentiventer</i>	Cambodia	Sihanouk	F	Raan1	Rattus sp.	raarh3_raarh5	raarh1_raarh2	KC811667/ KC811764
C0104	<i>Rattus argentiventer</i>	Cambodia	Sihanouk	M	Raan1	Rattus sp.	raarh2	raarh1	KC811668/ KC811765
R5674	<i>Rattus argentiventer</i>	Thailand	Buriram	F	Raan1	Rattus sp.	raarh1	raarh1	KC811669/ KC811766
R5679	<i>Rattus argentiventer</i>	Thailand	Buriram	M	Raar	Rattus sp.	raarh4	raarh1	KC811670/ KC811767
C0278	<i>Rattus exulans</i>	Cambodia	Sihanouk	M	Raex1	Raex	raexh1_raexh3	raexh5	KC811671/ KC811768
C0353	<i>Rattus exulans</i>	Cambodia	Mondolkiri	F	Raex3	Raex	raexh1_raexh2	raexh2_raexh5	KC811672/ KC811769
L0217	<i>Rattus exulans</i>	Lao PDR	Champasak	F	Raex1	Raex	raexh7	raexh4_raexh5	KC811673/ KC811770
R1805	<i>Rattus exulans</i>	Thailand	Bangkok	M	Raex1	Raex	raexh5_raexh6	raexh3	KC811674/ KC811771
R4103	<i>Rattus exulans</i>	Thailand	Loei	M	Raex2	Raex	raexh4_raexh7	raexh1	KC811675/ KC811772
L0277	<i>Rattus sakeratensis</i>	Lao PDR	Champasak	F	Rasa	Rattus sp.	rasah5_rasah6	rasah2	KC811676/ KC811773
R0237	<i>Rattus sakeratensis</i>	Thailand	Ratchaburi	F	Rasa	Rattus sp.	rasah1_rasah6	rasah1_rasah5	KC811677/ KC811774
R1015	<i>Rattus sakeratensis</i>	Thailand	Nakhon Ratchasima	M	Rasa	Rattus sp.	rasah3_rasah5	rasah3	KC811678/ KC811775
R4402	<i>Rattus sakeratensis</i>	Thailand	Loei	F	Rasa	Rattus sp.	rasah4_rasah5	rasah5	KC811679/ KC811776
R4568	<i>Rattus sakeratensis</i>	Thailand	Phrae	M	Rasa	Rattus sp.	rasah2	rasah4	KC811680/ KC811777
L0180	<i>Rattus nitidus</i>	Lao PDR	Luang Prabang	F	Rani	Rano-Rani	ranih5	ranih1	KC811681/ KC811778
L0191	<i>Rattus nitidus</i>	Lao PDR	Luang Prabang	M	Rani	Rano-Rani	ranih3_ranih5	ranih1	KC811682/ KC811779
L0192	<i>Rattus nitidus</i>	Lao PDR	Luang Prabang	F	Rani	Rano-Rani	ranih1_ranih4	ranih1	KC811683/ KC811780
L0196	<i>Rattus nitidus</i>	Lao PDR	Luang Prabang	M	x	Rano-Rani	x	ranih1	x/ KC811781
R4846	<i>Rattus nitidus</i>	Thailand	Loei	M	Rani	Rano-Rani	ranih1_ranih2	ranih1	KC811684/ KC811782
C0141	<i>Rattus norvegicus</i>	Cambodia	Sihanouk	F	Rano	Rano-Rani	ranoh1	ranoh1_ranoh3	KC811685/ KC811783
C0210	<i>Rattus norvegicus</i>	Cambodia	Sihanouk	F	Rano	Rano-Rani	ranoh1	ranoh2_ranoh3	KC811686/ KC811784
C0211	<i>Rattus norvegicus</i>	Cambodia	Sihanouk	F	Rano	Rano-Rani	ranoh1	ranoh3	KC811687/ KC811785
C0224	<i>Rattus norvegicus</i>	Cambodia	Sihanouk	F	Rano	Rano-Rani	ranoh1	ranoh3	KC811688/ KC811786
C0028	<i>Rattus tanezumi R3</i>	Cambodia	Sihanouk	F	Rata7_Rata8	Rattus sp.	ratah1_ratah7	ratah7_ratah8	KC811689/ KC811787
C0250	<i>Rattus tanezumi R3</i>	Cambodia	Sihanouk	F	Rati_Rata9	Rattus sp.	ratah4_ratah5	ratah1	KC811690/ KC811788
C0477	<i>Rattus tanezumi R3</i>	Cambodia	Mondolkiri	M	Rata9	Rattus sp.	ratah6	ratah4	KC811691/ KC811789
L0313	<i>Rattus tanezumi R3</i>	Lao PDR	Champasak	F	x	Rattus sp.	x	ratah11_ratah12	x/ KC811790
L0242	<i>Rattus tanezumi R3</i>	Lao PDR	Champasak	F	Rata9	Rattus sp.	ratah6_ratah8	ratah5_ratah6	KC811692/ KC811791
R5051	<i>Rattus tanezumi R3</i>	Thailand	Loei	F	Rata10_Rata11	Rattus sp.	ratah2_ratah3	ratah2_ratah3	KC811693/ KC811792
NK37	<i>Rattus rattus</i>	Senegal	?	F	Rara	Rattus sp.	rarah1	rarah1	KC811694/ KC811793
R197	<i>Rattus rattus</i>	Guadeloupe	?	?	Rara	Rattus sp.	rarah1	rarah1	KC811695/ KC811794
R2	<i>Rattus rattus</i>	Rio	?	?	Rara	Rattus sp.	rarah1	rarah1	KC811696/ KC811795
L0100	<i>Rattus tanezumi R2</i>	Lao PDR	Luang Prabang	F	Rata1_Rata2	Rattus sp.	ratah10_ratah11	ratah9_ratah10	KC811697/ KC811796

R1831	<i>Rattus tanezumi</i> R2	Thailand	Nakhon Sri Thammarat	F	Rata3_Rata4	Rattus sp.	ratah15_ratah16	ratah9	KC811698/ KC811797
R3560	<i>Rattus tanezumi</i> R2	Thailand	Samui	F	Rati_Rata5	Rattus sp.	ratah13_ratah14	ratah13	KC811699/ KC811798
R4377	<i>Rattus tanezumi</i> R2	Thailand	Loei	M	Rata1_Rata6	Rattus sp.	ratah9_ratah12	ratah9	KC811700/ KC811799
Li0249	<i>Rattus tiomanicus</i>	Sumatra	Minas	M	Rati	x	ratih1_ratih2	x	KC811701/ x
Li0258	<i>Rattus tiomanicus</i>	Sumatra	Minas	M	Rati	x	ratih3_ratih6	x	KC811702/ x
Li0259	<i>Rattus tiomanicus</i>	Sumatra	Minas	M	Rati	x	ratih4_ratih6	x	KC811703/ x
Li0315	<i>Rattus tiomanicus</i>	Sumatra	Minas	?	Rati	Rattus sp.	ratih5_ratih6	ratih1	KC811704/ KC811800

NOTE. - **ID** - identification of specimens; **LBR_{TLR4}** and **LBR_{TLR7}** **variants** - variants of ligand-binding region, double indication mean heterozygote specimen; **Hap_Exon3_Tlr4** and **Tlr7**- alleles of exon 3 for each species, double indication means heterozygote specimen; haplotypes used for all analysis are in bold; **x** - sequence not complete; **?** - no exact information; **GenBank Acc.** - Gen Bank Accession numbers.

Table S2. Primer description.

Primer ID	Sequence of primers 5' – 3'	Function
rTLR4-F	AGT TTA TCA TCA CTG YA GCA AG	amplification
rTLR4-XF2	CCC AAT TGA CTC CAT TCA AGC CC	amplification
rTLR4-XF3	CCC TCA GGA CTC TTG ATT GCA G	amplification
rTLR4-R-1	ATT CTC CCA AGA TCA ACC GAT G	amplification
rTLR4-R-3	CTG KTC CTT GAC CCA CTG C	amplification
rTLR4-R	AGA RMC CCA GRT GAR CTG TAG CAT T	amplification
rTLR7-F	AAG ACC YRT GTT GYT TAG TTT TAA TAA TG	amplification
rTLR7-1F	CAG ATT AGA CCT GGA AGC TTT AGT G	amplification
rTLR7-4F	TCT TGA CCT TGG CAC TAA CTT CAT A	amplification
rTLR7-5F	CCA TTG GCC AAA CTC TTA ATG G	sequencing
rTLR7-6F	GGT GAT AAC AGA TAC TTG GAC TTC T	sequencing
rTLR7-7F	CTG GCC ACT GAT GTG ACT TGT	sequencing
rTLR7-2R	GTT AGC CTC AAG GCT CAG AAG	amplification
rTLR7-9R	TAT CGG AAA TAG TGT AAG GCC TCA AG	amplification
rTLR7-R	AGA AAG AAR TTA TCK TCT ATC AGT CTC	amplification

Table S3. Residues binding to LPS in TLR4 based on knowledge of 3D-crystallography in human predicted by Park et al. 2009.

Position in rodent alignment	Numbering in human sequence	Function of AA	Residue variety in rodents
<u>263</u>	hTLR4_R264	LPS (Charge interaction with phosphates)	positively charged R, K
<u>339</u>	hTLR4_K341	LPS (Charge interaction with phosphates)	positively charged R, H, uncharged NH2 residue Q
<u>360</u>	hTLR4_K362	LPS (Charge interaction with phosphates)	positively charged K, R
<u>386</u>	hTLR4_K388	LPS (Charge interaction with phosphates)	positively charged R, uncharged hydrophilic T, small uncharged hydrophilic S, uncharged hydrophobic I
434	hTLR4_Q436	LPS (Hydrogen bond)	uncharged R, K
438	hTLR4_F440	LPS, MD-2 (Hydrophobic interaction)	uniformly aromatic hydrophobic F
442*	hTLR4_L444	LPS, MD-2 (Hydrophobic interaction)	aliphatic hydrophobic L, aromatic polar hydrophobic Y
461	hTLR4_F463	LPS, MD-2 (Hydrophobic interaction)	uniformly aromatic hydrophobic F

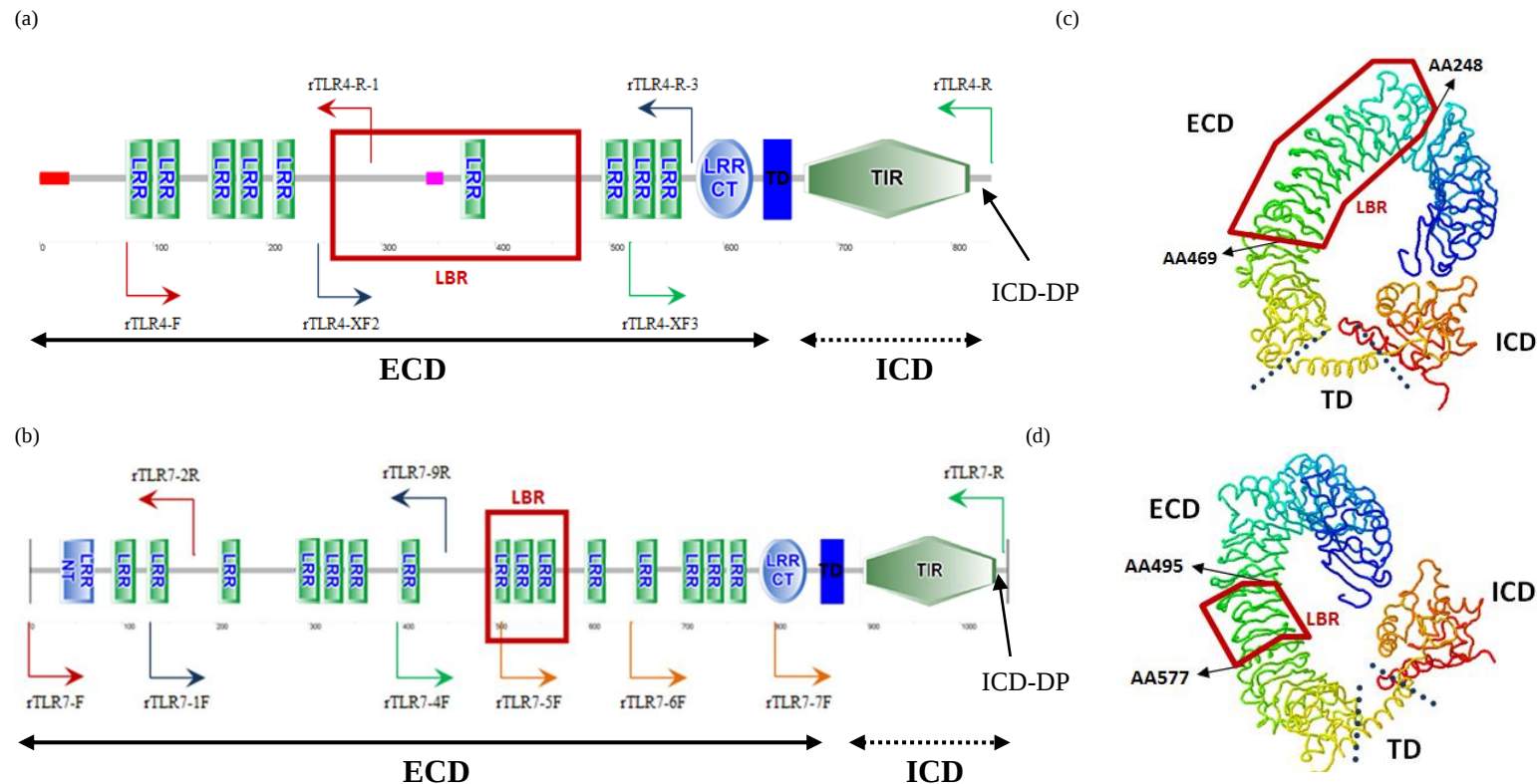
NOTE. - Variable sites detected by CONSURF are underlined; * indicates sites identified by MEME.

Table S4. Potential residues binding ssRNA predicted by Wei et al. 2009.

Position in rodent alignment	Numbering in human sequence	Function of AA	Residue variety in rodents
503	hTLR7_K502	Potential ligand binding residue	uniformly positively charged R
505	hTLR7_S504	Potential ligand binding residue	uniformly uncharged NH ₂ N
527	hTLR7_G526	Potential ligand binding residue	uniformly tiny G
532	hTLR7_Q531	Potential ligand binding residue	uniformly uncharged NH ₂ Q
552	hTLR7_N551	Potential ligand binding residue	uniformly uncharged NH ₂ N
554	hTLR7_R553	Potential ligand binding residue	uniformly positively charged R
557	hTLR7_L556	Potential ligand binding residue	uniformly aliphatic hydrophobic L
576	hTLR7_S575	Potential ligand binding residue	uniformly small uncharged hydrophilic S
579	hTLR7_H578	Potential ligand binding residue	uniformly positively charged aromatic H

FIGURES

Figure S1. Protein structure of TLR4 (a, c) and TLR7 (b, d) identified by SMART (<http://smart.embl-heidelberg.de/>) (a, b) and CONSURF (c, d). SMART (a, b) identified following types of domains: **LRR** - Leucine rich repeat; **LRRCT** - Leucine rich repeat C-terminal domain; **TIR** - TIR domain, **Fulfilled blue box (TD)** - transmembrane domain; **LRRNT** - Leucine rich repeat N-terminal domain. **Red box** - LBR (from AA248 to AA469 for TLR4 and from AA495 to AA597 for TLR7). **ECD** - extracellular domain is represented by solid black double arrow; **ICD** - intracellular domain is represented by dashed double arrow. Distal part of ICD (**ICD-DP**) is indicated by a simple solid arrow. Positions of forward and reverse primers used for amplification are shown by arrows. Arrows of same color indicates primer pairs. Description of crystallographic structure (c, d) **LBR** is represented by red polygon; **TD** is present between two dashed lines. To the right from TD is **ICD**, to the left is **ECD**.



Figures S2 and S3 (Phylogenetic trees) are placed in Additional files 2

Figures S4. Test of congruence between the presumably neutral and *Tlr* phylogenies (*Tlr4* (a), *Tlr7* (b) following JANE 4). Number at X axis represents costs of co-divergence. The red dashed line represents the cost observed in our data. The blue columns represent the random distributions of costs. Lower cost than random observed in our data signified higher congruence between species and gene topologies.

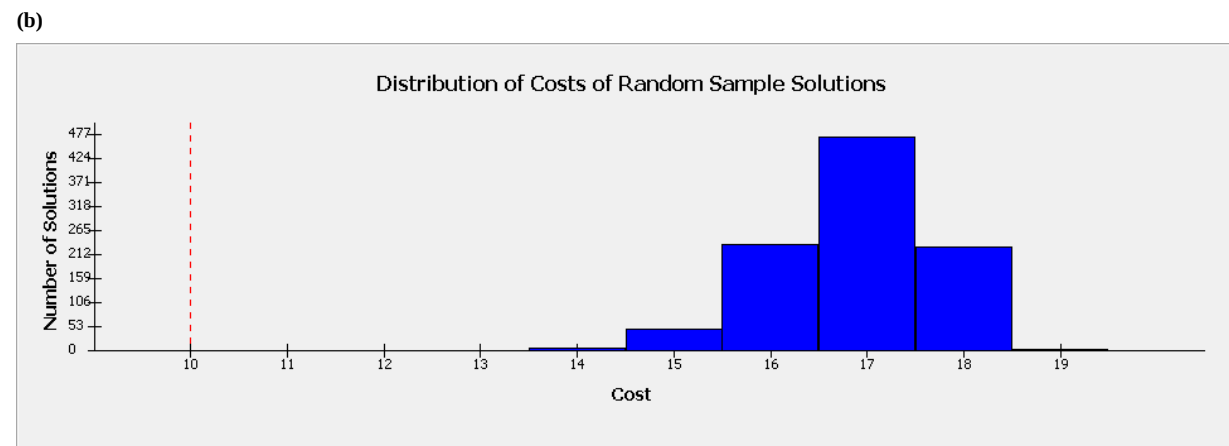
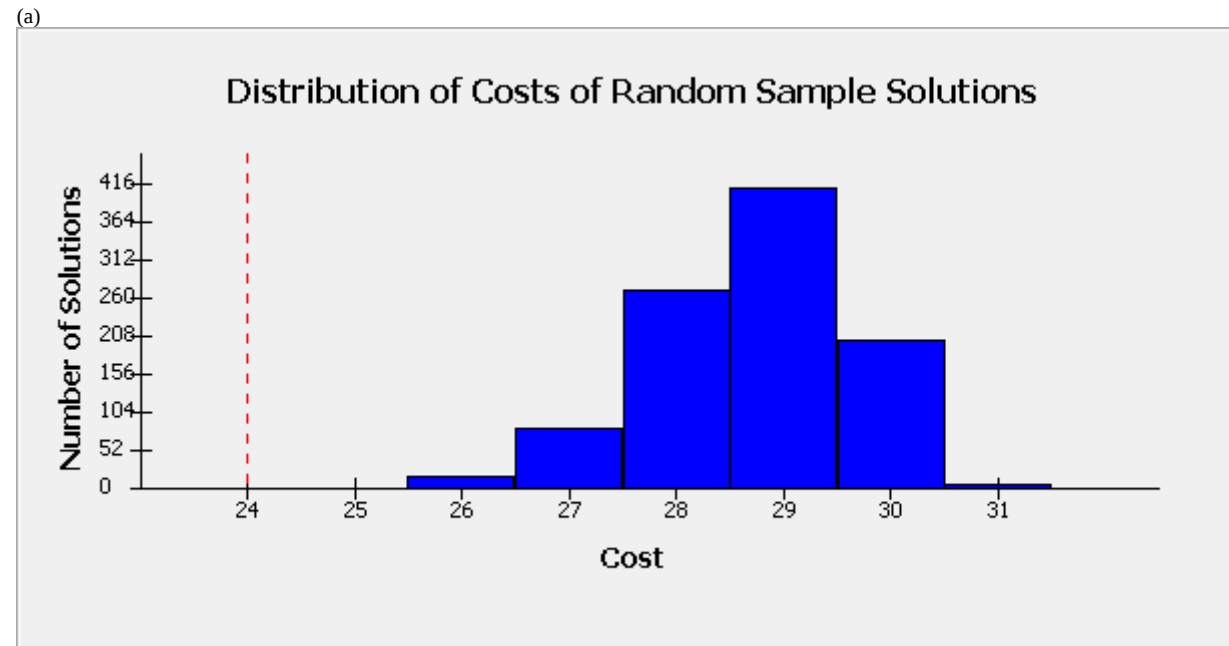
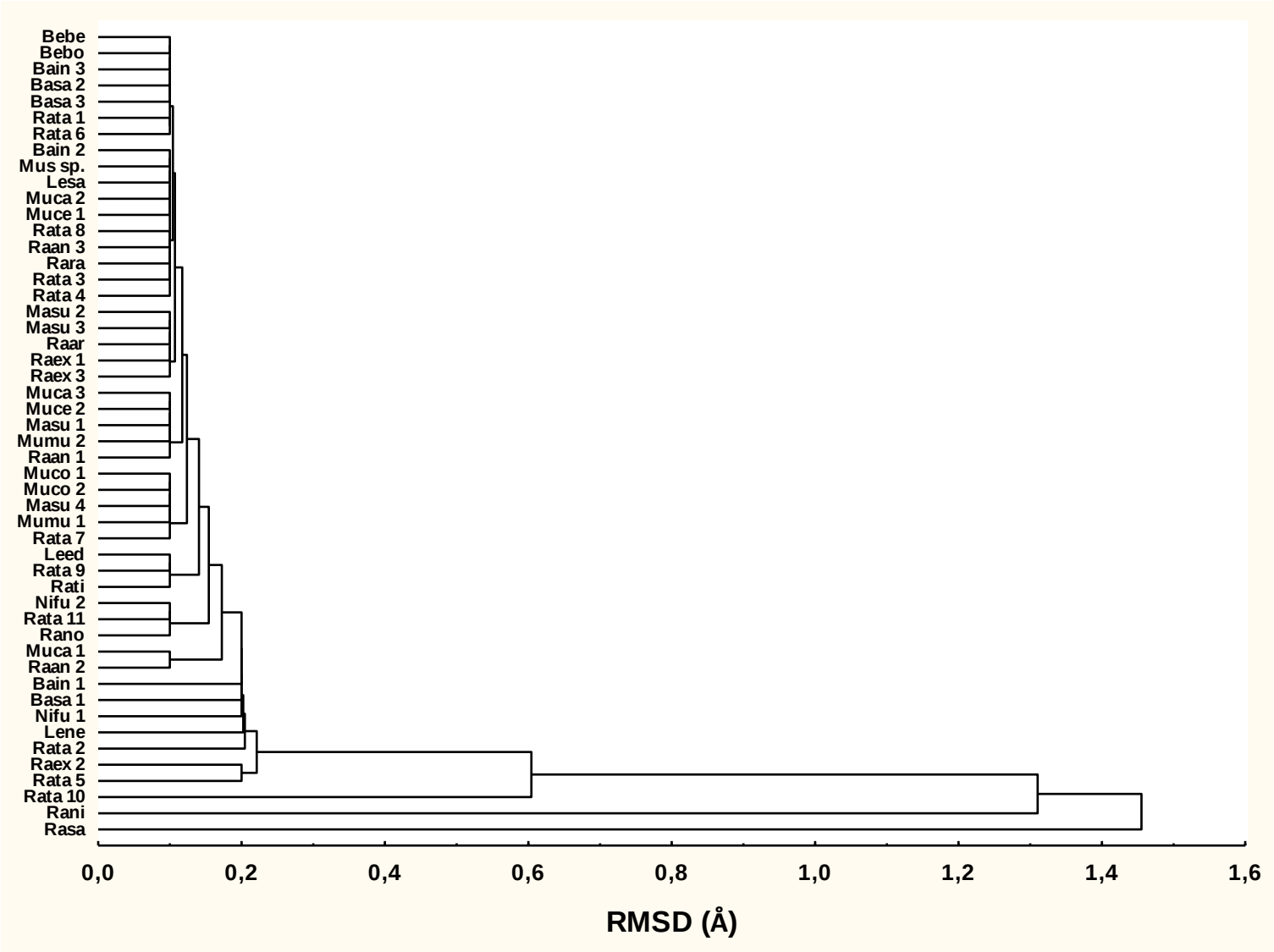


Figure S5. Superimposition of structures, tree clustering diagrams based on linkage distance, (a) LBR_{TLR4} and (b) LBR_{TLR7}; individual LBR-variants often unify more species; description of LBR-variants labels is in the Table S1 under Hap_LBR_{TLR4} and Hap_LBR_{TLR7}.

(a)



(b)

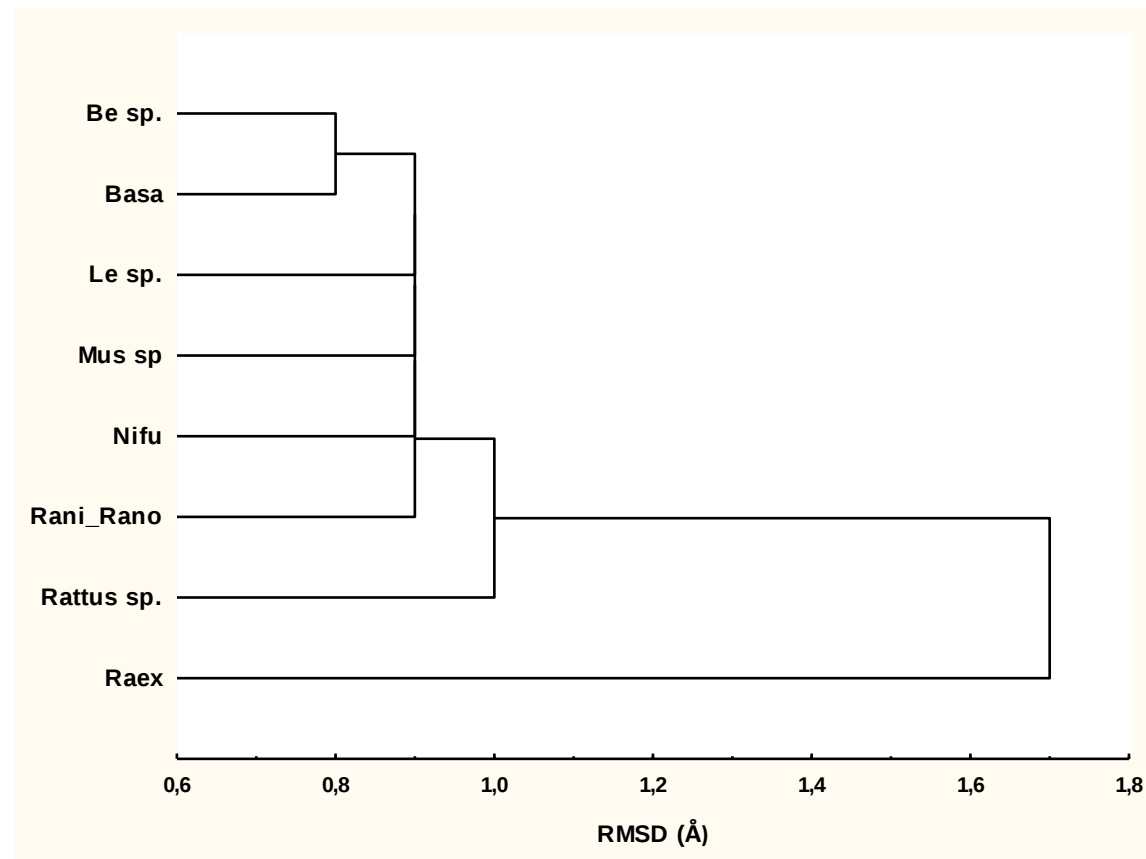
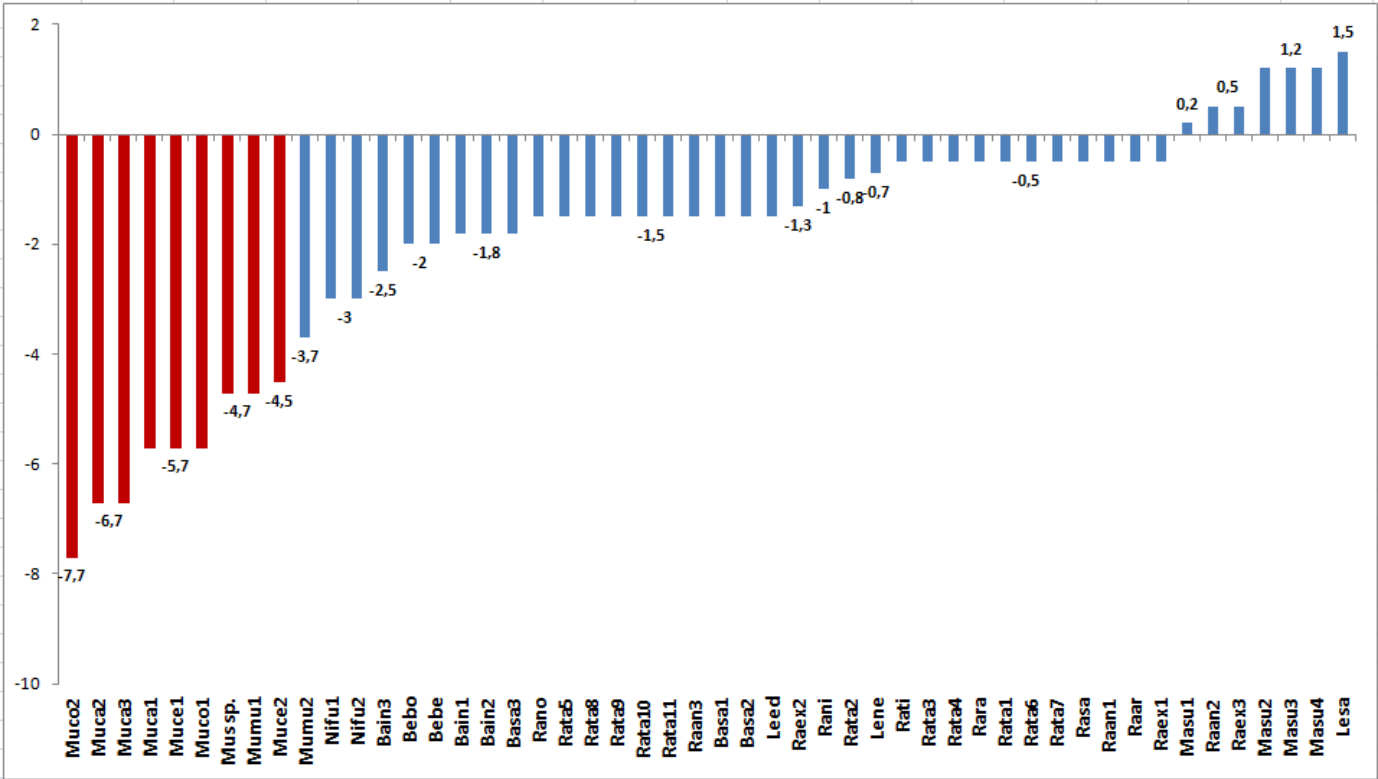


Figure S6. Analysis of LBR amino acid sequence charge at pH 7 (LRRFinder) for (a) LBR_{TLR4} and (b) LBR_{TLR7}, individual LBR-variants often unify more species; description of LBR-variants labels is in the Table S1 under Hap_LBR_{TLR4} and Hap_LBR_{TLR7}. Mouse species are in red, *Rattus* spp. and related genera are in blue.

(a)



(b)

