Developmental and Comparative Immunology 60 (2016) 127–138

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Identification and characterization of toll-like receptors (TLRs) in the Chinese tree shrew (*Tupaia belangeri chinensis*)

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ARTICLE INFO

Article history:
Received 30 January 2016
Accepted 23 February 2016
Available online xxx

Keywords:
TLRs
Tree shrew
Innate immunity
HCV
Animal model

ABSTRACT

In mammals, the toll-like receptors (TLRs) play a major role in initiating innate immune responses against pathogens. Comparison of the TLRs in different mammals may help in understanding the TLR-mediated responses and developing of animal models and efficient therapeutic measures for infectious diseases. The Chinese tree shrew (*Tupaia belangeri chinensis*), a small mammal with a close relationship to primates, is a viable experimental animal for studying viral and bacterial infections. In this study, we characterized the *TLRs* genes (*tTLRs*) in the Chinese tree shrew and identified 13 putative *TLRs*, which are orthologs of mammalian *TLR1-TLR9* and *TLR11-TLR13*, and *TLR10* was a pseudogene in tree shrew. Positive selection analyses using the Maximum likelihood (ML) method showed that *tTLR8* and *tTLR9* were under positive selection, which might be associated with the adaptation to the pathogen challenge. The mRNA expression levels of *tTLRs* presented an overall low and tissue-specific pattern, and were significantly upregulated upon Hepatitis C virus (HCV) infection. *tTLR4* and *tTLR9* underwent alternative splicing, which leads to different transcripts. Phylogenetic analysis and TLR structure prediction indicated that tTLRs were evolutionarily conserved, which might reflect an ancient mechanism and structure in the innate immune response system. Taken together, TLRs had both conserved and unique features in the Chinese tree shrew.

1. Introduction

The innate immune system is the first line of defense against microbial infections or endogenous danger signals and relies on a large family of pathogen-recognition receptors (PRRs), which identify molecular motif on pathogenic microorganisms (Akira et al., 2001; Janeway and Medzhitov, 2002). Four classes of PRRs have been reported, including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and cytoplasmic DNA sensors. Among them, the first identified and best characterized sensors were TLRs, which play a key role in the mammalian innate immune system to detect various types of pathogens (Akira et al., 2001; Janeway and Medzhitov, 2002). TLRs act as a bridge between the innate immunity and the adaptive immunity (Akira et al., 2006; Beutler et al., 2006; Medzhitov, 2001). Moreover, TLRs are one of the most ancient and conserved components of the immune system, and present in both invertebrates and vertebrates. Human has 10 functional TLR family members (*TLR1-TLR10*), whereas mouse has 12 *TLRs* (*TLR1-TLR9* and *TLR11-TLR13*) (Roach et al., 2005). TLRs are type I transmembrane proteins, with numerous extracellular leucine-rich repeats (LRRs; which are responsible for recognizing pathogen-associated molecular patterns [PAMPs]), a single transmembrane domain, and a cytoplasmic Toll/interleukin-1 receptor (TIR) domain that recruits the adapter protein MyD88 or TRIF (Jin and Lee, 2008).

Tree shrews (*Tupaia belangeri*) are squirrel-like, rat-sized animals inhabiting in the tropical shrubs or forests of South and

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http://dx.doi.org/10.1016/j.dci.2016.02.025

Southeast Asia (Fuchs and Corbach-Söhle, 2010) and South China (Peng et al., 1991). Recently, we confirmed that the tree shrew has a genetically close relationship with primates based on genome analysis (Fan et al., 2013; Xu et al., 2012). For several decades, tree shrew has attracted increasing attention in biomedical research; there are many efforts to establish animal models for human diseases, including infectious diseases, metabolic diseases, neurological and psychiatric disorders, and cancers (Cao et al., 2003; Wang et al., 2012; Xu et al., 2013). In particular, tree shrew has been shown to be susceptible to a wide range of human pathogenic viruses and bacterial mimic (Xu et al., 2013), including hepatitis B virus (HBV) (Kock et al., 2001; Yan et al., 1984, 1996), hepatitis C virus (HCV) (Amako et al., 2010), herpes simplex virus (HSV) (Li et al., 2015; Rosen et al., 1985) and bacterial infection (Li et al., 2012).

There is growing interest in targeting TLRs for the development of therapeutics (Hennessy et al., 2010; Ulevitch, 2004). A comprehensive characterization of the molecular conservation and specificity of the TLRs family will help to answer why tree shrew is susceptible to viral and bacterial infections and will contribute to animal model study and drug intervention. In this study, we identified and characterized TLRs orthologs of the Chinese tree shrew, and determined the structure of functionally important domains. We constructed a phylogenetic tree of the mammalian TLRs and analyzed the evolutionary dynamics and selective pressure on the tTLRs. Furthermore, tTLRs expression patterns in different tissues of adult Chinese tree shrews and in primary liver-derived cells in response to HCV infection were investigated. Our results indicated the conservation and specificity of TLRs between tree shrew and human and between tree shrew and mouse.

2. Materials and methods

2.1. Experimental animals

Chinese tree shrews were introduced from the experimental animal core facility of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). After lethally anesthetized by diethyl ether, seven different tissues (including heart, liver, spleen, lung, kidney, intestine and brain) of tree shrew were quickly dissected, immediately frozen in liquid nitrogen and were stored at −80 °C. All efforts were made to minimize the suffering of animals and the study protocol was approved by the Institutional Animal Care and Use Committee of KIZ, CAS.

2.2. RNA isolation and expression quantification

Total RNA was extracted from seven tissues of Chinese tree shrews using RNAsimple Total RNA Kit (TIANGEN, Beijing) according to the manufacturer's instruction. Around 2 μg total RNA of high quality (with an A260/A280 ratio of 1.8–2.0) was used to synthesize cDNA by using oligo-dT_{18} primer and M-MLV reverse transcriptase (Promega, USA). Real-time quantitative PCR (RT-qPCR) was performed using SYBR green Premix Ex Taq II (TaKaRa, Dalian) supplemented with gene specific primers (Table S1) on a MyIQ2 Two-Color Real-Time PCR Detection system (Bio-Rad, USA), as described previously (Xu et al., 2015; Yu et al., 2014).

2.3. Cloning of tTLRs

All primers were designed based on the predicted 13 *tTLRs* (*TLR1-TLR13*) sequences of tree shrew retrieved from the Ensemble (http://www.ensembl.org/index.html) and the genome sequence of Chinese tree shrew (Fan et al., 2013, 2014) (Table S1). In order to get a relatively intact mRNA sequence, rapid amplification of cDNA ends (RACE) was used to amplify the 5′ UTR and 3′ UTR using the SMARTer RACE cDNA Amplification Kit (Clontech, USA) and 3′ Full RACE Core Set Ver.2.0 (TaKaRa, Japan), respectively. Purified PCR products were cloned into the PMD 19-T simple vector (TaKaRa, Dalian). Five positive clones of each insert were sequenced to get a consensus sequence for the insert.

2.4. Phylogenetic analysis

To infer the phylogenetic position of the Chinese tree shrew based on the tTLRs sequences, we retrieved TLRs protein sequences of 10 species from GenBank (Table S2). Both the coding DNA sequences (CDS) and amino acid sequences were used for phylogenetic analyses. The protein sequences were aligned by Muscle 3.8 (Edgar, 2004). The maximum likelihood (ML) trees were reconstructed using Raxml 8.0.0 (Stamatakis, 2014) with the PROTGAMMA option and the BLOSUM62 as amino acid substitution mode. The neighbor-joining (NJ) trees were reconstructed using MEGA6 (Tamura et al., 2013) with Poisson as the model. Accuracies and statistical tests of phylogenetic trees were measured by bootstrap method with 1000 replications.

2.5. Modeling analysis of the positively selected sites

The TLRs sequences of Human, Macaca, Chinese Tree shrew, Rat, Mouse, Dog; Table S2) were used to test for possible selective pressure in the Chinese tree shrew lineage by using the maximum-likelihood analyses implemented in the phylogenetic analysis by maximum likelihood (PAML) package (Yang, 2007). To detect positively selected sites on the branch leading to tree shrew, we used the branch-site model (Zhang et al., 2005) with fixed foreground branch $\omega_2 = 1$ and non-fixed foreground branch ω_2, which is used to test whether a gene has undergone positive selection on a foreground branch. Finally, likelihood ratio test (LRT) was performed on the following model pairs to test whether a proportion of sites in the sequence provided statistically significant support for $\omega > 1$ on foreground branches (Yang, 2007).

2.6. Viruses and cells

The liver-derived cells were established using the same approach for isolating the primary renal cells as previously described (Xu et al., 2015). Briefly, single cell suspension was plated on cell culture plates. 6 h after plating, medium were changed to primary hepatocytes maintenance medium (Williams' medium E supplemented with 10% FBS, 1 × insulin-transferrin-selenium, 5 ng/mL EGF, 10 ng/mL HGF, 2% DMSO, 1 × penicillin/streptomycin [Invitrogen]). Cells were cultured at 37 °C in 5% CO_2 with regular medium change every 2 days.

The HCV (JFH-1) was propagated in Huh 7.5.1 cells. The 50% tissue culture infective dose ($TCID_{50}$) of HCV was determined by green fluorescence and the titers were calculated by the Reed-Muench method (Reed and Muench, 1938). Primary tree shrew liver-derived cells were infected with HCV (multiplicity of infection [MOI] = 10) for 6 h, then switched to fresh medium after two washes with PBS. The mRNA expression levels of *tTLRs* were analyzed at 48 h and 72 h after HCV infection.

2.7. Statistical analysis

For measurement of mRNA expression levels of *tTLRs* in primary tree shrew liver-derived cells with and without HCV infection, each assay was independently performed three times to validate the consistency of the result. Data were presented as mean ± SEM. Statistical analysis was performed using GraphPad software

(GraphPad Software, La Jolla, CA, USA) with the unpaired Student's *t*-test.

3. Results

3.1. Identification of 12 TLRs in the Chinese tree shrew

In mammals, 13 *TLRs* (10 in human and 12 in mouse) have been reported (Takeda et al., 2003). According to known *TLR1-TLR9* sequences of human and mouse in GenBank and the tree shrew genome sequence generated by our own (Fan et al., 2013, 2014), we first amplified cDNA that was synthesized on total RNA extracted from tree shrew spleen and obtained the full-length of the tree shrew *tTLR1-tTLR9* genes (Table 1. The *in silico* prediction for domains using the SMART program (http://smart.embl-heidelberg.de/) (Letunic et al., 2015; Schultz et al., 1998) showed that tTLR1-tTLR9 have typical TLR structures: a trans-membrane protein with multiple LRR motifs in the extracellular domain, a single-span transmembrane segment, and a cytoplasmic signaling domain homologous to the TIR domain (Fig. 1). *TLR11*, *TLR12* and *TLR13* were present in mouse but became pseudogenes in human (Guan et al., 2010; Roach et al., 2005). We searched the tTLR11-tTLR13 orthologs using mouse TLR proteins (TLR11: GenBank accession number NP_991388; TLR12: NP_991392; TLR13: NP_991389) in the tree shrew genome (Fan et al., 2013). Analysis of the putative open reading frame of tTLR11-tTLR13 predicted the existence of proteins with 933, 909, 950 amino acids, respectively. All had the hallmarks of the mammalian TLRs (Fig. 1) (Table 1).

To define the evolutionary relationship of the Chinese tree shrew and other mammalian TLRs (Table S2), we constructed phylogenetic trees using the ML method based on the canonical protein sequences. Each *TLR* gene from different mammalian species was grouped together. Six major TLR families/clades were recognized in the tree (Fig. 2). The TLR1 family/clade I was composed of TLR1, TLR2, TLR6, and TLR10; the TLR7 family/clade II contained TLR7, TLR8 and TLR9; and TLR11 family/clade III contained TLR11, TLR12 and TLR13; and the other three families/clades contained TLR3, TLR4, and TLR5, respectively. Similar clustering pattern of TLRs was observed when the cytoplasmic TIR domains were used for the phylogenetic analysis (Fig. S1). However, the clustering pattern in the ML tree was inconsistent with the species tree in our previous study (Fan et al., 2013). In particular, tree shrew had a distant affinity with primates (Fig. 2). In comparison with human and mouse, the composition of tTLRs was more similar to that of mouse (Fig. 1), but the tree shrew did not show a closer relationship to mouse in the ML tree for TLR1-TLR9 (Fig. 2). Different phylogenetic relationships between the tree shrew and other mammalian TLRs might reflect the imprints of different challenges from viral and bacterial pathogens (Ariffin and Sweet, 2013).

3.2. Pseudogenization of tTLR10

The *TLR10* gene was present in human but became a pseudogene in mouse (Hasan et al., 2005). We performed a blast search using human (NP_001017388) and rat (NP_001139507) *TLR10* against the tree shrew genome. The full-length cDNA of human *TLR10* had a coding region (2436 bp) that encodes 811 amino acids. However, there was only partial *TLR10* sequence matched in tree shrew genomic sequence (*TLR10*-like sequence, *tTLR10ψ*). We obtained the full-length of *tTLR10ψ* transcript which had a length of 1240 bp (including a poly-A tail; Table 1) via 5′ and 3′ RACE. We reconstructed the conserved syntenies involving *TLR10* and the nearby genes: *TLR1*, *TLR6*, and *TLR10* were adjacent to each other in human, rat and mouse genomic sequences (Fig. 3A). The gene orientations in the Chinese tree shrew were similar to those three species, reinforcing the notion that *tTLR10ψ* was a true ortholog. Interesting, there was a 58 bp fragment at the 5′-end proximal to *tTLR10ψ*, which is identical to 5′-UTR of *tTLR1* (Fig. 3A). Moreover, the transcripts of *tTLR10ψ* and *tTLR1* complied with the splicing rule and were GT-AG introns. These results suggested that *tTLR10ψ* and *tTLR1* might share the same promoter. Tandem repeats are unstable regions of the genome where frequent insertions and deletions of nucleotides might take place. We performed the repeat masking (http://www.repeatmasker.org/) for the genomic region covering *tTLR10ψ* to identify the repeats and observed that the tree shrew genome has a large amount of tree shrew-specific repeats, including Tu-I, Tu-II and Tu-III (tRNA-derived SINE family) between *tTLR1* and *tTLR10ψ* genes (Fig. 3B) (Fan et al., 2013; Nishihara et al., 2002). We speculated that *tTLR10ψ* became a pseudogene due to these repeats. We further analyzed the basal expression level of *tTLR10ψ*. The *tTLR10ψ* had a low basal expression and was not widely expressed. Spleen had a relatively high *tTLR10ψ* mRNA expression, whereas heart and small intestine had no detectable expression (Fig. 4A). Taken together, Chinese tree shrew *TLR10* might have become a pseudogene, or this reflected the ancestral status of the *TLR* genes in tree shrew relative to primates.

3.3. Adaptive evolution of tTLR genes in the tree shrew lineage

TLRs were under strong selection for both maintenance and adaptation of function (Roach et al., 2005). They are candidate molecules to examine how natural selection molds innate immunity receptors. To understand the evolutionary dynamics and selective pressure on the *tTLR* genes in the tree shrew, we calculated

Table 1
Information of the full-length Toll-like receptors in the Chinese tree shrew.

Gene	Full length mRNA, bp	5′-UTR, bp	CDS, bp (peptide, aa)	3′-UTR, bp	GenBank number
tTLR1	2884	126	2397 (798)	361	KT354316
tTLR2	2759	252	2355 (784)	152	KT354317
tTLR3	3394	278	2718 (905)	398	KT354318
tTLR4	3551	163	2526 (841)	862	KT354319
tTLR5	2928	74	2534 (861)	268	KT354320
tTLR6	3324	166	2391 (796)	767	KT354321
tTLR7	4218	209	3153 (1050)	856	KT354322
tTLR8	3221	81	3096 (1031)	44	KT354323
tTLR9[a]	3179	89	3090 (1029)	–	KT354324
tTLR10ψ[b]	1240	–	–	–	KT946778
tTLR11	3849	189	2802 (933)	858	KT354325
tTLR12	3071	97	2730 (909)	244	KT354326
tTLR13	3286	100	2853 (950)	333	KT354327

[a] We failed to obtain the 3′-UTR of the *tTLR9* gene by 3′ RACE.
[b] *tTLR10ψ* - a pseudogene of *tTLR10*.

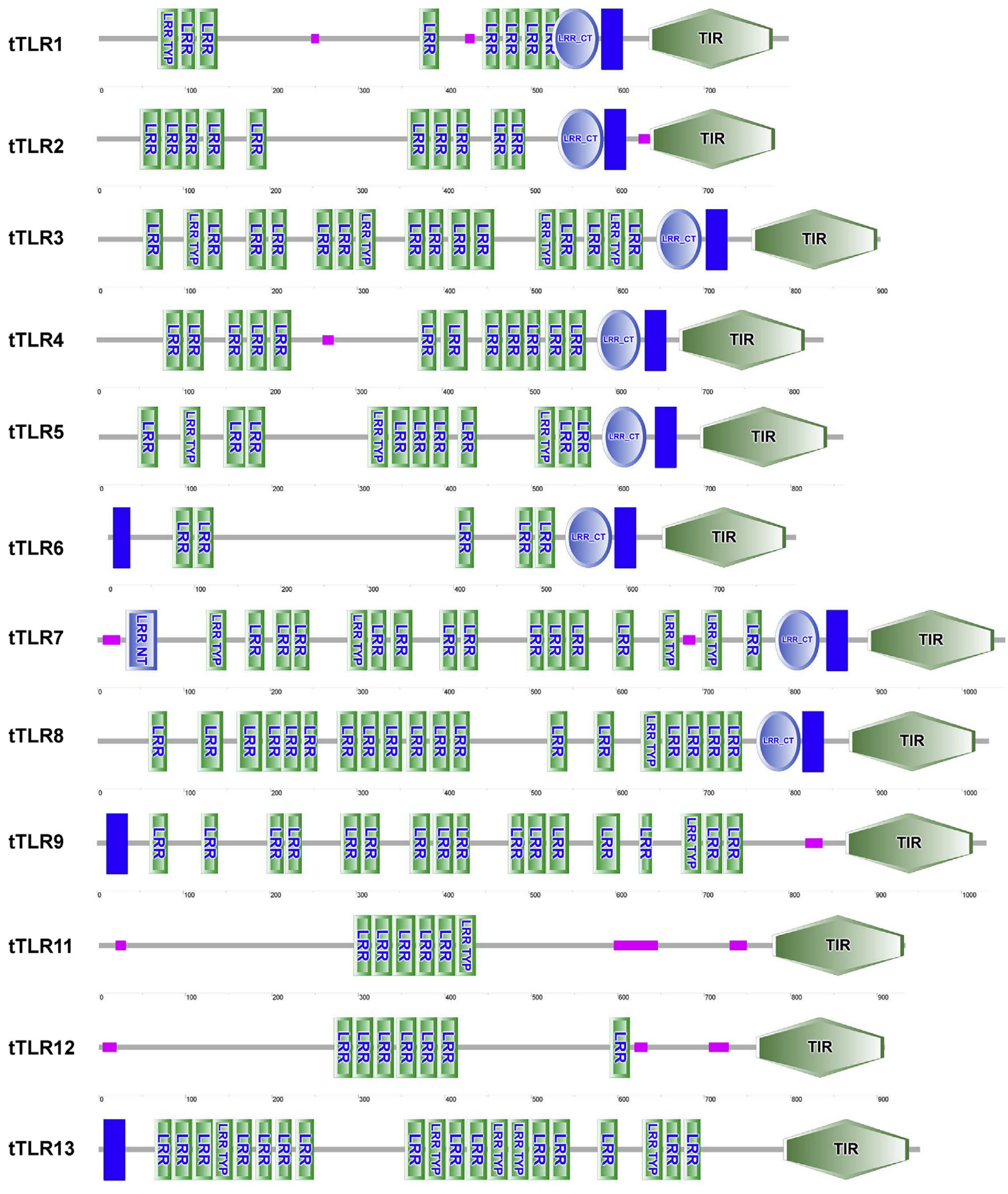

Fig. 1. Schematic domain organization of tree shrew TLR1-TLR9 and TLR11- TLR13. The domain organization of each TLR was predicted from the amino acid sequences using the SMART (http://smart.embl-heidelberg.de/). Similar to previously reported mammalian TLR proteins, all tTLRs have a LRR (leucine-rich repeat) repeat in the N-terminal region, followed by transmembrane region (mandarin blue pane) and the TIR (Toll/IL-1 receptor) domain at C-terminal end. NT: N-(amino) terminal. CT: C-(carboxyl) terminal. Pink pane: Low complexity region. LRR TYP: Leucine-rich repeats, typical (most populated) subfamily. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the average non-synonymous substitution/synonymous substitution rate (dN/dS, also referred to as ω) for the *tTLR1-tTLR9* genes using the maximum-likelihood analyses implemented in the PAML package (Yang, 2007). As shown in Table 2), only *tTLR8* and *tTLR9* genes were found to experience a positive selection (*tTLR8*, $P = 0.0013$; *tTLR8*, $P = 1.71 \times 10^{-5}$). In details, eight codons in *tTLR8* and 19 codons in *tTLR9* showed evidence for positive selection (Table 2). To gain insight into functional potential of positively selected sites (PSSs), we analyzed the location of these sites under positive selection. The TLRs sequences for PSSs were obtained from six species (human, macaca, Chinese tree shrew, rat, mouse, dog) (Fig. S2), so we first determined the equivalent positions of positively sites in humans and tree shrews (Table S3). These PSSs were located in the following domains: LRR10 (residue 357), LRR13 (residue 415), Z-loop (residues 465 and 481), LRR16 (residue 551), LRR17 (residue 552) and LRR25 (residue 572) in tTLR8 (Fig. S3); LRRNT (residues 63, 64, and 81), LRR5 (residues 220 and 221), LRR6 (residue 240), LRR10 (residue 336), LRR13 (residue 431), Z-loop

Table 2
Analysis of the positive selection for the tree shrew TLR1-TLR9 genes.

Foreground	lnL[a] (null)	np[b]1	lnL[a] (alternative)	np[b]2	2ΔlnL[c]	*p*-value	Positively selected sites[d] (BEB analysis[e])	Parameters
TLR1	−8146.171464	14	−8146.133309	15	0.07631	0.782361704	27 E 0.523; 143 S 0.530; 505 S 0.550;	p0 = 0.65072 p1 = 0.31429 p2a = 0.02359 p2b = 0.01139 w0 = 0.10761 w1 = 1.00000 w2 = 1.55808
TLR2	−8912.23616	14	−8912.23616	15	0	1	773 E 0.526; 785 S 0.562;	p0 = 0.66479 p1 = 0.33032 p2a = 0.00327 p2b = 0.00162 w0 = 0.09491 w1 = 1.00000 w2 = 1.00000
TLR3	−8812.143991	14	−8811.394157	15	1.499668	0.220722453	373 S 0.692;	p0 = 0.72925 p1 = 0.21969 p2a = 0.03924 p2b = 0.01182 w0 = 0.07701 w1 = 1.00000 w2 = 1.00000
TLR4	−9208.529758	14	−9208.529758	15	0	1	392 S 0.587;	p0 = 0.52775 p1 = 0.47225 p2a = 0.00000 p2b = 0.00000 w0 = 0.05652 w1 = 1.00000 w2 = 1.00000
TLR5	−10069.72076	14	−10068.1124	15	3.216702	0.07289035	244 E 0.846; 272 S 0.914; 289 I 0.910; 492 G 0.512;	p0 = 0.68442 p1 = 0.28867 p2a = 0.01893 p2b = 0.00798 w0 = 0.08972 w1 = 1.00000 w2 = 4.88892
TLR6	−8337.346939	14	−8337.346919	15	4.00E-05	0.994953769	24H 0.682; 48 D 0.750; 247 Q 0.674; 373 Q 0.638; 517 S 0.763; 588 V 0.514;	p0 = 0.68566 p1 = 0.31434 p2a = 0.00000 p2b = 0.00000 w0 = 0.11845 w1 = 1.00000 w2 = 1.00000
TLR7	−9558.074143	14	−9556.81585	15	2.516586	0.112654242	48 I 0.719; 242 E 0.661; 290 T 0.656; 649 N 0.724; 677 N 0.760; 924H 0.709;	p0 = 0.74091 p1 = 0.24136 p2a = 0.01337 p2b = 0.00436 w0 = 0.07071 w1 = 1.00000 w2 = 3.75110
TLR8	−10869.68025	14	−10864.48474	15	10.391008	0.001266305	357 P 0.868; 415 N 0.836; 465 S 0.723; 481 E 0.779; 551 P 0.926; 552H 0.894; 572 S 0.670; 772 I 0.537;	p0 = 0.69506 p1 = 0.28650 p2a = 0.01306 p2b = 0.00538 w0 = 0.07243 w1 = 1.00000 w2 = 11.25579
TLR9	−10851.28542	14	−10842.04346	15	18.483922	1.71E-05	40 Q 0.733; 63 P 0.942; 64H 0.740; 81 S 0.978*; 220 L 0.770; 221 G 0.697; 240 R 0.598; 336 V 0.693; 431 Q 0.509; 500 T 0.531; 511 N 0.819; 540 V 0.871; 543 S 0.978*; 549 S 0.847; 682 S 0.538; 768 V 0.617; 817 K 0.791; 831 A 0.926; 889 R 0.616;	p0 = 0.72710 p1 = 0.23385 p2a = 0.02955 p2b = 0.00950 w0 = 0.05133 w1 = 1.00000 w2 = 8.54739

[a] lnL: log-likelihood value.
[b] np:Number of parameters.
[c] 2ΔlnL: Twice the difference of ln(likelihood) values (2ΔlnL) between the two models compared.
[d] The amino acid positions refers to the aligned sequences of six species in supplementary Fig. S2.
[e] BEB analysis: Bayes Empirical Bayes analysis (Yang et al., 2005).

(residue 500), LRR15 (residue 511), LRR16 (residues 540, 543, and 549), LRR22 (residue 682), LRR25 (residue 740) and LRRCT (residues 817 and 831) in tTLR9 (Fig. S4). The equivalent positions of PSSs in tree shrew were also mainly located in the predicted LRR domain (Fig. S5). We further located these PSSs based on the three dimensional crystallographic structures of unliganded and ligand-induced activated human TLR8 and TLR9 proteins (Ohto et al., 2015; Tanji et al., 2013). The dimerization of human TLR8 in the unliganded or ligand states was associated with the LRR domain interactions, including LRR11-LRR14, LRR16-LRR18 (Tanji et al., 2013). The LRRNT-LRR10 and LRR20-LRR22 domains in TLR9 recognized CpG-DNA (Ohto et al., 2015). As shown in Fig. S6, these PSSs in tTLR8 and tTLR9 were all located in the defined functional regions that might affect dimerization and pathogen recognition.

3.4. Expression analysis of the tTLR genes

To assess mRNA expression of *tTLRs*, we performed RT-qPCR analyses for different tree shrew tissues. As shown in Fig. 4A, all *tTLR* genes were highly expressed in spleen compared to other tissues. The mRNA expression levels of *tTLRs* were moderate in liver, lung and kidney. In general, *tTLR3*, *tTLR4*, *tTLR6*, *tTLR11* and *tTLR12* had a relatively lower basal expression level than the other *tTLRs*. There was a tissue-specific expression pattern for *tTLR11* and *tTLR12*: *tTLR11* mRNA was only expressed in spleen and liver tissue, whereas no detectable *tTLR12* mRNA level was found in heart, kidney and small intestine tissue. The *tTLR5* had a strong mRNA expression in liver but a relatively low mRNA expression in spleen (Fig. 4A).

We further retrieved the normalized mRNA expression information of human and mouse from BioGPS (www.biogps.org), with an intention to compare *TLR* gene basal expression in different tissues of human, mouse and tree shrew (Fig. 4B). Unfortunately, the data of human spleen tissue were unavailable in BioGPS. Based on the relative abundance of *TLR* mRNA expression in human whole blood according to BioGPS, we speculated that the expression level of human *TLRs* should be high in spleen tissue. Comparison of *TLR* gene expression profiles of different tissues showed a relative conservation of tissue distribution in general (Fig. 4B). We also found some exceptions: *TLR4* and *TLR9* had the highest basal mRNA expression in tree shrew spleen tissue, whereas in mouse *TLR4* presented the highest mRNA expression in heart tissue and *TLR9* had the highest mRNA expression in liver tissue (Fig. 4B). Differences in cell-specific expression of human and mouse TLR orthologous were common (Ariffin and Sweet, 2013; Rehli, 2002).

3.5. Alternative splicing transcripts of the tTLRs

Members of the Toll-like receptor signaling pathway were

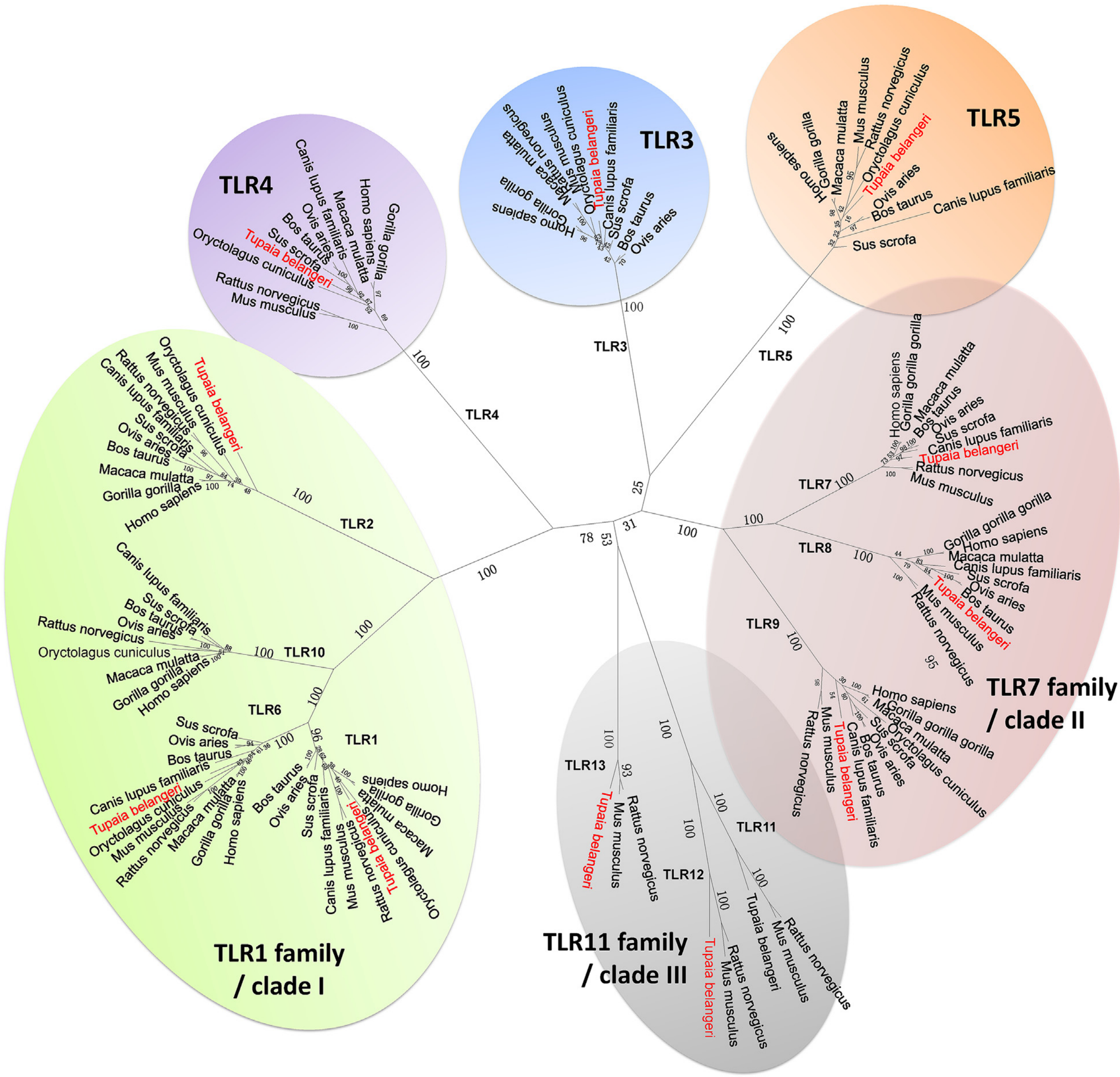

Fig. 2. Maximum likelihood tree of the mammalian TLRs based on the predicted amino acid sequences. Phylogenetic analysis were reconstructed using Raxml 8.0.0 (Stamatakis, 2014) with the PROTGAMMA option and BLOSUM62 as amino acid substitution mode. Bootstrap values based on 1000 replicates are indicated on each branch. The sequences of 11 species, including *Homo sapiens* (human), *Gorilla gorilla* (gorilla), *Macaca mulatta* (Rhesus Macaque), *Mus musculus* (mouse), *Rattus norvegicus* (Rat), *Bos Taurus*(Cattle), *Sus scrofa* (pig), *Canis lupus familiaris* (dog), *Ovis aries* (sheep), *Oryctolagus cuniculus* (rabbit), and *Tupaia belangeri chinensis* (Chinese tree shrews) were analyzed. GenBank accession numbers of these sequences were listed in Table S2. We did not include rabbit TLR7 and TLR8 in the phylogenetic analysis, as neither mRNA nor genomic sequences of rabbit TLR7 could be retrieved from available data sets, and rabbit TLR8 was a pseudogene (Astakhova et al., 2009).

highly alternatively spliced, producing a large number of proteins with the potential to functionally alter inflammatory outcomes (Wells et al., 2006). Each *TLR* gene has numerous alternatively spliced variants (Carpenter et al., 2014), especially for *TLR4* (Iwami et al., 2000; Jaresova et al., 2007). We also detected potential *TLR* mRNA transcripts in the Chinese tree shrew tissues. We found a transcript of *tTLR4* (*tTLR4-sv1*), which was resulted from the alternative splicing of exon 4 of the *TLR4* gene in tree shrew spleen (Fig. 5). Two transcripts (*tTLR9-sv1*, lacking partial exon 2 and exon3; *tTLR9-sv2*, lacking partial exon 2) were recognized in tree shrew spleen tissue (Fig. 5). *tTLR4-sv1* had a 432 bp deletion in exon 4, while *tTLR9-sv1* and *tTLR9-sv2* had a 1173 bp and 1032 bp deletion in exons 2 and 3, respectively. Although these transcripts had a full open reading frame, it is unknown whether the transcripts could be successfully translated *in vivo*.

3.6. Alteration of tTLRs expression in response to HCV infection

Many attempts had used tree shrew to create animal models for studying HCV infection and pathogenesis (Amako et al., 2010; Barth et al., 2005; Guitart et al., 2005; Xu et al., 2007). To explore the potential role of the tTLRs-mediated signaling pathway in this process, we assessed the *tTLRs* mRNA expression level in response to HCV infection in tree shrew primary liver-derived cells. We found that the *tTLR2*, *tTLR4* and *tTLR8* genes had a significantly increased mRNA expression after HCV infection for 48 h or even later (Fig. 6); the *tTLR3* mRNA level was down-regulated at 72 h post infection; and mRNA expression of the other *tTLR* genes had no

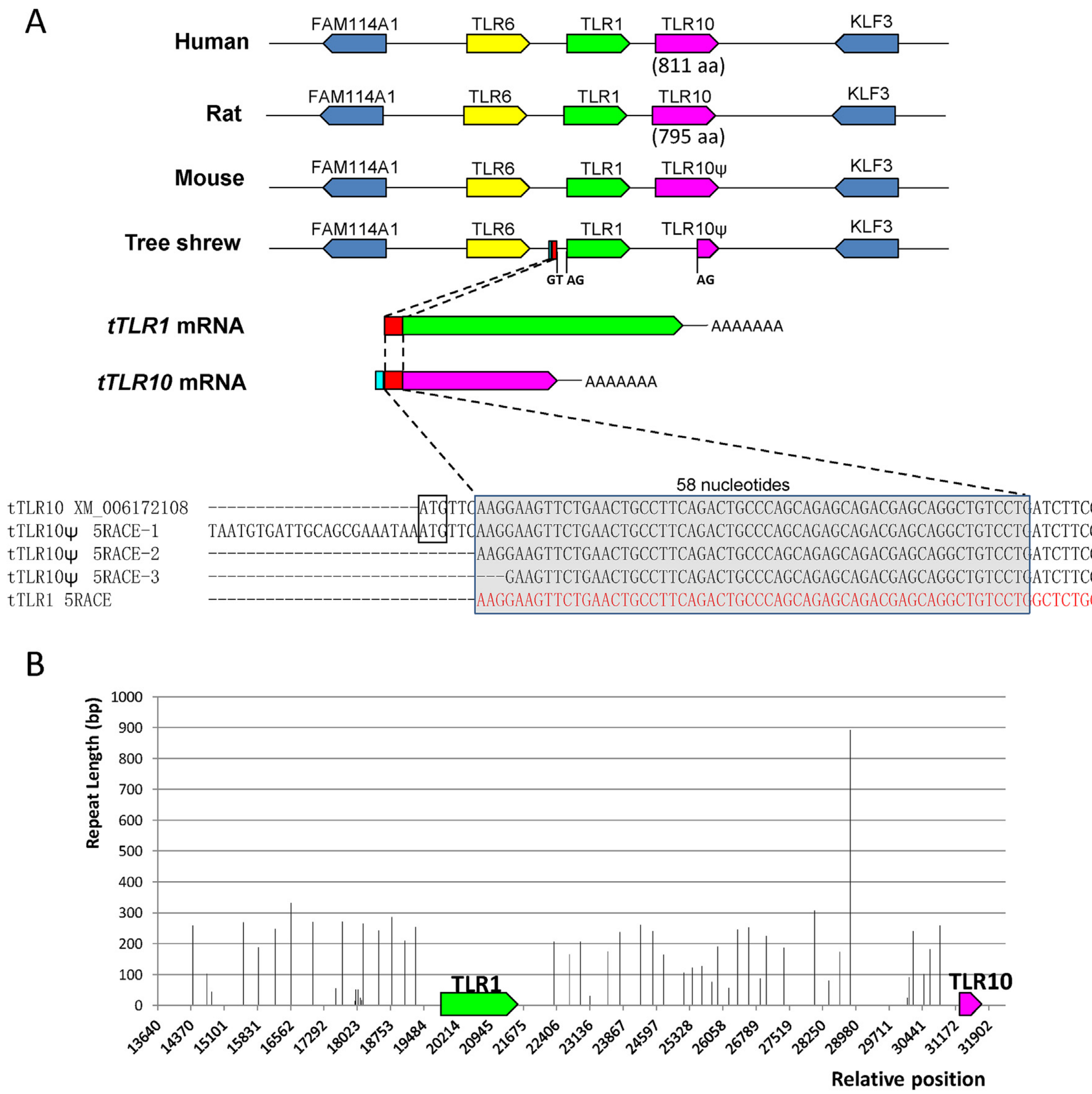

Fig. 3. Gene structure and location of *tTLR1* and *tTLR10* in the genome. (A) Conserved synteny around the *TLR10* gene in the genomic sequences of the Chinese tree shrew, human, mouse and rat (data are retrieved from the Ensembl website [http://www.ensembl.org/] and the tree shrew genome generated in our previous study (Fan et al., 2013)). There is a fragment with identical 58 bp in the 5′ proximal sequences of the *tTLR10* and *tTLR1* mRNAs (Red box). Dark light box indicated 58-bp identical in all 5′ RACE sequences for *tTLR10* and *tTLR1*. Three 5′ RACE clones for *tTLR10ψ* were shown here. *tTLR1* 5′ RACE sequence was marked by red. (B) Map of tree shrew genomic region containing *tTLR1* and *tTLR10*. Interspersed repeat features were annotated based on repeatmasker (http://www.repeatmasker.org/). Vertical line indicated the position and length of tandem repeats. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

change. The exact role of the mRNA expression alterations of *tTLR2, tTLR3, tTLR4*, and *tTLR8* in HCV infection in primary liver-derived cells remains to be determined.

4. Discussion

TLR family plays an important role in response to the pathogen challenges and is highly conserved in vertebrates (Roach et al., 2005). The discovery of TLRs and their signaling pathways provides new opportunities for drug intervention to manipulate immune response (Hennessy et al., 2010; Rauta et al., 2014). The tree shrew has been proposed as an alternative experimental animal to primates in biomedical research (Xu et al., 2013; Zheng et al., 2014). However, the existence of TLRs homolog in tree shrew has not been well determined so far. In this study, we characterized TLR1-TLR13 homologs in the Chinese tree shrew and identified 13 TLRs (tTLR1-tTLR13). These tTLRs had a high structural similarity to mammalian TLRs. Phylogenetic clustering pattern of these genes further supported the conserved status of tTLRs. However, tTLRs also exhibited some distinct features which were likely derived from evolutionary pressure.

One unique feature for tTLRs is that tTLR10 underwent pseudogenization due to the tandem repeats (Fig. 3). We searched the TLR10 homolog in the Malayan flying lemur (*Galeopterus variegatus*) genome, which has a close relationship to tree shrew (Murphy et al., 2001), and we confirmed the presence of a TLR10 homolog in Malayan flying lemur (authors' unpublished data). Thus, we hypothesized that the *TLR10* deficiency in tree shrew might have occurred after the divergence of tree shrew from Malayan flying lemur. Analysis of the genomic sequence revealed that the mouse *TLR10* gene was a nonfunctional gene, with numerous gaps and insertions, and the TIR domain was replaced by a retrovirus-like sequence in mouse after the separation of the mouse and rat lineages (Hasan et al., 2005). On this point, the tree

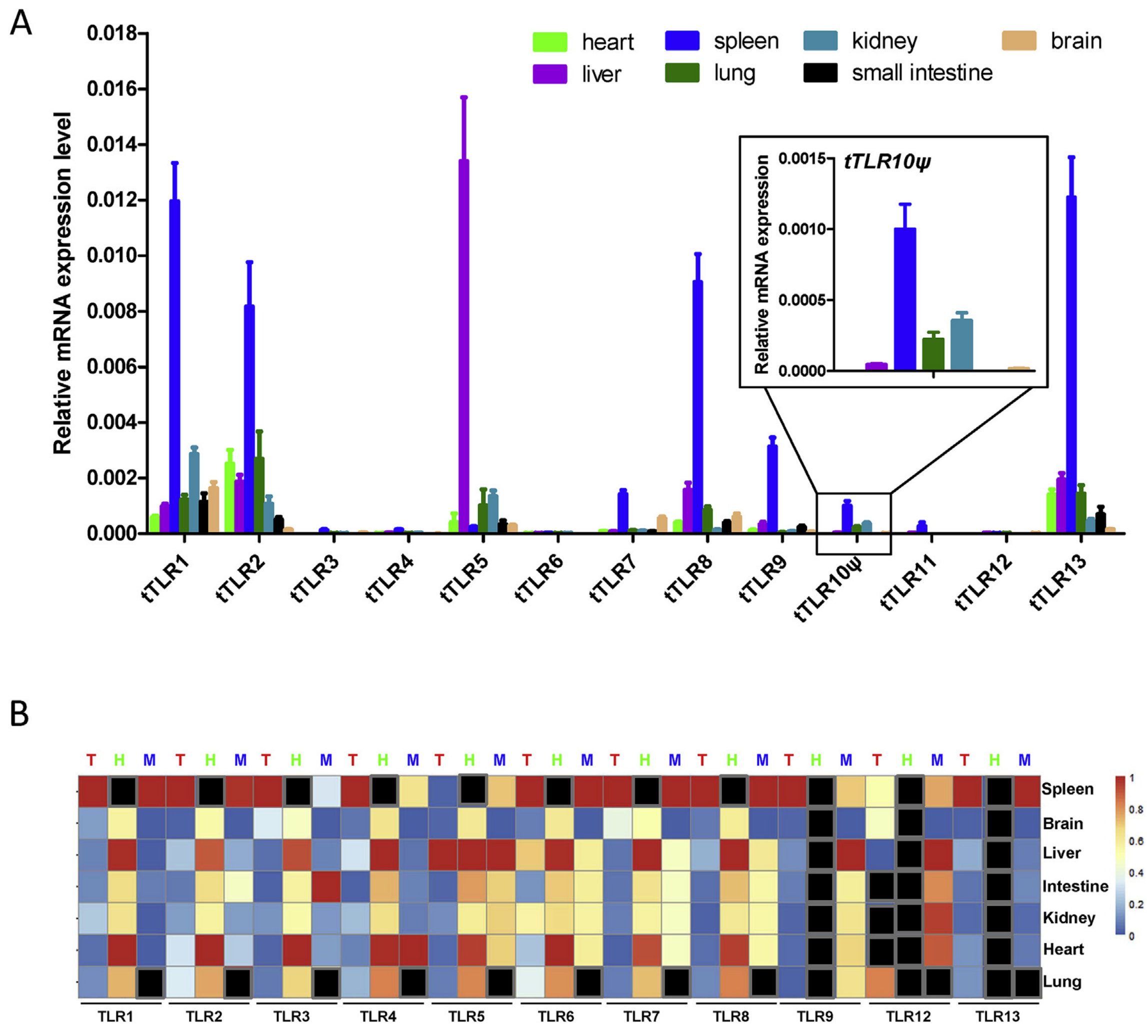

Fig. 4. Characterization of the *tTLRs* mRNA expression profile. (A) Quantitative real-time PCR analysis of *tTLR1*-*tTLR13* mRNA expression in seven tissues of the Chinese tree shrew (N = 10). The graphs showed the mean ± SEM. value of each tissue sample from different animals. The *β-actin* was used for quantification of *tTLRs* mRNA. (B) Heat map showing the basal *TLRs* gene expression in seven tissues of human (H), mouse (M) and tree shrew (T). Human and mouse *TLRs* gene expression data were taken from BioGPS (www.biogps.org). Black boxes indicated missing information in human or mouse.

shrew resembles rodents, instead of primates for TLRs composition.

Since the initial identification of human *TLR10* (Chuang and Ulevitch, 2001), accumulating genetic studies showed that human *TLR10* was associated with a variety of diseases (Guirado et al., 2012; Lazarus et al., 2004; Morgan et al., 2012), but the mechanisms remain unknown. Recent studies showed that TLR10 played a role in innate immune response to influenza virus infection (Lee et al., 2014) and *Helicobacter pylori* infection (Nagashima et al., 2015), and might be the first TLR receptor with inhibitory properties (Oosting et al., 2014). The pseudogenization of *TLR10* in the Chinese tree shrew might have an unknown biological significance. Although pseudogene has been presumed to be “non-functional” due to the loss of protein-coding capacity, it might play a regulatory role in modulating the expression of their parental or non-parental genes using its transcript (Guo et al., 2014; Johnsson et al., 2013; Muro et al., 2011; Pink et al., 2011). We found that *tTLR10* could be transcribed into RNA in spleen, kidney, lung, liver and brain tissues (Fig. 4A). Although at a very low level, we could not exclude the possibility that *tTLR10ψ* might have a regulatory effect. For instance, it may act as a decoy factor for microRNAs targeting to TLRs or other genes, similar to the *PTEN* pseudogene (Johnsson et al., 2013). Further experimental work should be carried out to clarify this issue.

Previous studies had documented that purifying selection as the major force driving TLRs evolution, presumably for preserving a well-established biological function (Barreiro et al., 2009; Mukherjee et al., 2009). Viral TLRs (TLR3, TLR7, TLR8, and TLR9) were under stronger functional constraint than non-viral TLRs (TLR1, TLR2, TLR4, TLR5, and TLR6), because viral TLRs had a balancing role in maintaining their function to recognize viral nucleic acids but avoiding autoimmunity at the same time (Wlasiuk and Nachman, 2010). The viral TLRs were not expected to accumulate non-synonymous substitutions as this might affect their functional integrity (Babik et al., 2015). TLRs might undergo positive selection due to co-evolutionary dynamics with their microbial molecules (Wlasiuk and Nachman, 2010). The strongest evidence for positive selection has been reported for non-viral TLRs, such as TLR4 and TLR1 (Nakajima et al., 2008; Wlasiuk and Nachman, 2010). In our study, the *tTLR8* and *tTLR9* genes were found to undergo positive selection (Table 2), which was inconsistent with previous studies that viral TLRs were under a strong purifying selection than non-viral TLRs (Alcaide and Edwards, 2011; Barreiro et al., 2009; Wlasiuk and Nachman, 2010). Interesting, non-viral TLRs showed no evidence of positive selection in the Chinese tree shrew. The presence of the positive selection signature in tTLR8 and tTLR9 could be resulted from ancient functional adaptation (Jann

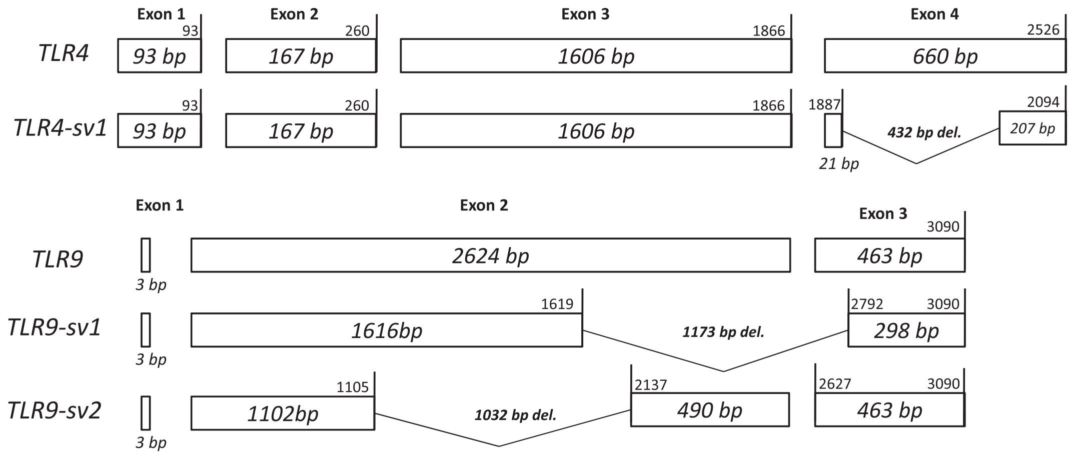

Fig. 5. Schematic gene structures of *tTLR4* and *tTLR9* mRNA and their transcripts. Exons were indicated as boxes. Broken lines indicated alternative splicing of exons in the *tTLR* transcripts. The alternative splicing transcripts were marked by "-sv".

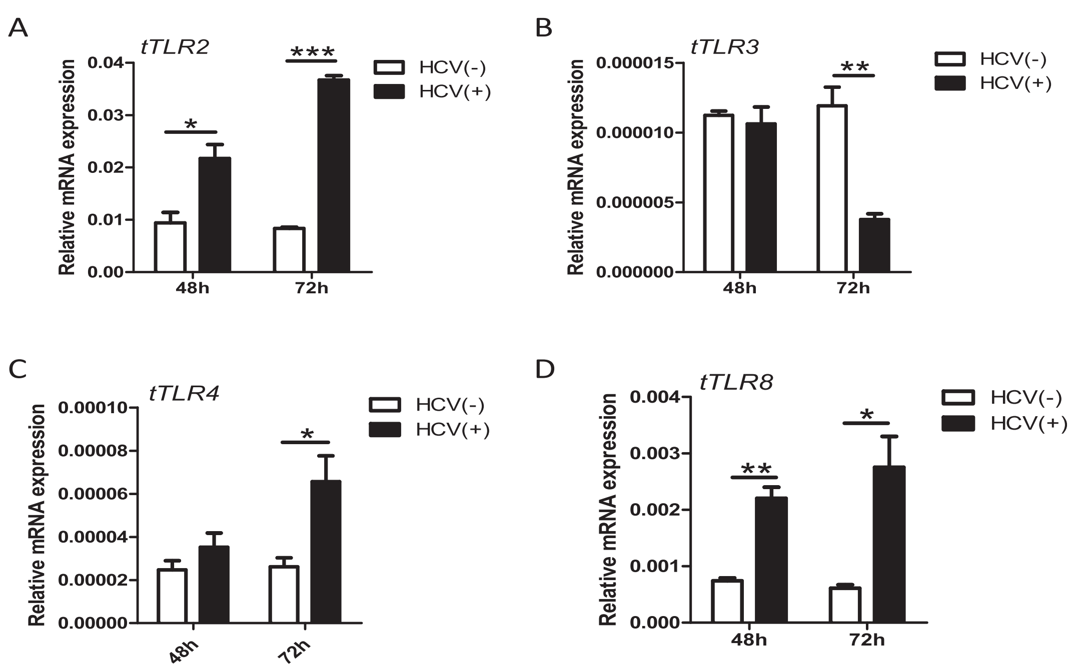

Fig. 6. mRNA expression levels of *tTLR* in the tree shrew primary liver-derived cells upon HCV infection. Cells were infected with JFH1 HCV at an MOI of 10. *tTLR2* (A), *tTLR3* (B), *tTLR4* (C), and *tTLR8* (D) mRNA expression levels were analyzed by RT-qPCR at 48 h and 72 h post-infection. Results were normalized to the β-actin. The data were representative of three independent experiments and were presented as mean ± SEM. *P < 0.05, **P < 0.001, two-tailed unpaired Student's *t*-test.

et al., 2008). There was an abundance of PSSs in tTLR8 and tTLR9, and all these sites were mainly located in the LRR domains (Fig. S3, S4 and S5), which usually have a higher rate of evolution than that of the TIR domains (Mikami et al., 2012). In mammals, TLR8 was implicated in recognizing single-strand RNA (Heil et al., 2004) and TLR9 had been shown to response to unmethylated CpG DNA (Bauer et al., 2001). The reason why the *TLR8* and *TLR9* genes in the tree shrew lineage were under positive selection is unknown. To maintain a role of specific PAMP recognition could be a possible cause. In addition, tree shrew lost RIG-I (DDX58) in its genome (Fan et al., 2013), which was one of the two families of PRRs (TLRs and RLRs) that recognize viral nucleic acids, this event might also be linked with the positive selection on tTLR8 and tTLR9.

To search for potential ligands of *tTLRs*, we made the following attempts: (1) determination of mRNA expression levels of *tTLR1*-*tTLR9* in primary tree shrew renal cells in response to stimulation by different agonists for human and mouse *TLR1*-*TLR9*, such as Pam3CysSerLys4 (Pam3CSK4), heat-killed *Listeria monocytogenes* (HKLM), Poly(I:C), lipopolysaccharide (LPS), Flagellin, Pam2CGDPKHPKSF (FSL-1), Imiquimod (R837), ssRNA, and CpG DNA (ODN2006); (2) determination of mRNA expression levels of *tTLR3* in primary tree shrew renal cells infected with different human viruses, including Sendai virus, Vesicular stomatitis virus, Avian influenza virus, Newcastle disease virus, Herpes simplex virus-1. Unfortunately, we did not obtain useful information to characterize potential function of these tTLRs (authors' unpublished data). Two possible reasons may account for this result. First, in contrast to the ubiquitous RLRs, TLRs are mainly displayed on antigen presenting cells (APCs) such as dendritic cells (DCs) and prime the adaptive immunity such as B cell and T cell responses (Kawai and Akira, 2010). The analysis of tree shrew primary renal cells might not be proper to show the effect of these agonists.

Second, there are differences in ligand specificity for human and other animal TLR orthologs. Previous studies had reported species-specific ligand recognition patterns by TLRs (Ariffin and Sweet, 2013; Werling et al., 2009). For example, chicken, human and mouse TLR5 could be discriminated by different flagellins (Andersen-Nissen et al., 2007; Keestra et al., 2008). Non-rodent TLR8 could be activated by ssRNA and small synthetic ligands, whereas rodent TLR8s failed to be activated by non-rodent ligands (Govindaraj et al., 2011; Zhu et al., 2009). Another limitation of the current cellular assay is that we only analyzed the mRNA level of *tTLRs*. It may be worthwhile to investigate how different agonists activate the NF-κB signaling pathway in tree shrew macrophages (Shi et al., 2011).

Upon the HCV infection in primary liver-derived cells, the mRNA levels for *tTLR2*, *tTLR3*, *tTLR4* and *tTLR8* had a significant change (Fig. 6). HCV infection is limited to humans and chimpanzees, which hindering HCV research and development of drugs and vaccines. Primary human hepatocytes (PHHs) were believed to maximally imitate the *in vivo* infection of HCV (Steinmann and Pietschmann, 2013), but there is an extreme lack of donors. Tree shrew is emerging as a potential animal model for investigating the HCV infection (Xu et al., 2013). In our study, we examined the mRNA expression of *TLRs* in tree shrew primary liver-derived cells after HCV infection. Altered mRNA levels for *tTLR2*, *tTLR4* and *tTLR8* (Fig. 6) indicated that HCV could trigger the innate response in tree shrew primary liver-derived cells. TLRs served as host PRRs to detect HCV PAMPs (Yang and Zhu, 2015) and HCV could be sensed by TLR3, TLR7 and TLR8 in cell cultures (Lee et al., 2015; Metz et al., 2013). HCV infection induced the expression of TLR4 in human (Machida et al., 2006). HCV core protein and NS3 protein have been shown to trigger cellular activation through the TLR2-mediated pathway in monocytes (Dolganiuc et al., 2004). Our results indicated that tree shrew primary liver-derived cells exhibited a similar expression change of TLRs with human hepatocytes in response to HCV infection. In addition, we observed that the *tTLR3* mRNA was obviously inhibited in the late phase of acute HCV infection (Fig. 6). This result might be caused by the NS3/4A cleavage of TRIF and suppression of the TLR3 signaling (Li et al., 2005). The precise mechanism of the HCV activated signaling of TLRs in tree shrew needs further investigate.

In short, we characterized the *TLR1-TLR13* genes in the Chinese tree shrew and confirmed the conservation of the tTLRs structure and function in this species. The current knowledge about tTLRs may stimulate future efforts to identify the ligands of these TLRs, which will advance comparative immunology research and will contribute to the development of animal model for infectious disease and vaccines.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

We are grateful to the members in Yao's laboratory for helpful discussion and Xiao-Hong Liu for technical assistance. This study was supported by the National Natural Science Foundation of China (U1402224) and Yunnan Province (2013FB071).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dci.2016.02.025.

References

Akira, S., Takeda, K., Kaisho, T., 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2, 675–680.
Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell. 124, 783–801.
Alcaide, M., Edwards, S.V., 2011. Molecular evolution of the toll-like receptor multigene family in birds. Mol. Biol. Evol. 28, 1703–1715.
Amako, Y., Tsukiyama-Kohara, K., Katsume, A., Hirata, Y., Sekiguchi, S., Tobita, Y., Hayashi, Y., Hishima, T., Funata, N., Yonekawa, H., Kohara, M., 2010. Pathogenesis of hepatitis C virus infection in *Tupaia belangeri*. J. Virol. 84, 303–311.
Andersen-Nissen, E., Smith, K.D., Bonneau, R., Strong, R.K., Aderem, A., 2007. A conserved surface on toll-like receptor 5 recognizes bacterial flagellin. J. Exp. Med. 204, 393–403.
Ariffin, J.K., Sweet, M.J., 2013. Differences in the repertoire, regulation and function of toll-like receptors and inflammasome-forming nod-like receptors between human and mouse. Curr. Opin. Microbiol. 16, 303–310.
Astakhova, N.M., Perelygin, A.A., Zharkikh, A.A., Lear, T.L., Coleman, S.J., MacLeod, J.N., Brinton, M.A., 2009. Characterization of equine and other vertebrate TLR3, TLR7, and TLR8 genes. Immunogenetics 61, 529–539.
Babik, W., Dudek, K., Fijarczyk, A., Pabijan, M., Stuglik, M., Szkotak, R., Zielinski, P., 2015. Constraint and adaptation in newt toll-like receptor genes. Genome Biol. Evol. 7, 81–95.
Barreiro, L.B., Ben-Ali, M., Quach, H., Laval, G., Patin, E., Pickrell, J.K., Bouchier, C., Tichit, M., Neyrolles, O., Gicquel, B., Kidd, J.R., Kidd, K.K., Alcais, A., Ragimbeau, J., Pellegrini, S., Abel, L., Casanova, J.L., Quintana-Murci, L., 2009. Evolutionary dynamics of human Toll-like receptors and their different contributions to host defense. PLoS Genet. 5, e1000562.
Barth, H., Cerino, R., Arcuri, M., Hoffmann, M., Schurmann, P., Adah, M.I., Gissler, B., Zhao, X., Ghisetti, V., Lavezzo, B., Blum, H.E., von Weizsacker, F., Vitelli, A., Scarselli, E., Baumert, T.F., 2005. Scavenger receptor class B type I and hepatitis C virus infection of primary tupaia hepatocytes. J. Virol. 79, 5774–5785.
Bauer, S., Kirschning, C.J., Hacker, H., Redecke, V., Hausmann, S., Akira, S., Wagner, H., Lipford, G.B., 2001. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. Proc. Natl. Acad. Sci. U.S.A 98, 9237–9242.
Beutler, B., Jiang, Z., Georgel, P., Crozat, K., Croker, B., Rutschmann, S., Du, X., Hoebe, K., 2006. Genetic analysis of host resistance: toll-like receptor signaling and immunity at large. Annu. Rev. Immunol. 24, 353–389.
Cao, J., Yang, E.B., Su, J.J., Li, Y., Chow, P., 2003. The tree shrews: adjuncts and alternatives to primates as models for biomedical research. J. Med. Primatol. 32, 123–130.
Carpenter, S., Ricci, E.P., Mercier, B.C., Moore, M.J., Fitzgerald, K.A., 2014. Post-transcriptional regulation of gene expression in innate immunity. Nat. Rev. Immunol. 14, 361–376.
Chuang, T., Ulevitch, R.J., 2001. Identification of hTLR10: a novel human toll-like receptor preferentially expressed in immune cells. Biochim. Biophys. Acta 1518, 157–161.
Dolganiuc, A., Oak, S., Kodys, K., Golenbock, D.T., Finberg, R.W., Kurt-Jones, E., Szabo, G., 2004. Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation. Gastroenterology 127, 1513–1524.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
Fan, Y., Huang, Z.-Y., Cao, C.-C., Chen, C.-S., Chen, Y.-X., Fan, D.-D., He, J., Hou, H.-L., Hu, L., Hu, X.-T., Jiang, X.-T., Lai, R., Lang, Y.-S., Liang, B., Liao, S.-G., Mu, D., Ma, Y.-Y., Niu, Y.-Y., Sun, X.-Q., Xia, J.-Q., Xiao, J., Xiong, Z.-Q., Xu, L., Yang, L., Zhang, Y., Zhao, W., Zhao, X.-D., Zheng, Y.-T., Zhou, J.-M., Zhu, Y.-B., Zhang, G.-J., Wang, J., Yao, Y.-G., 2013. Genome of the Chinese tree shrew. Nat. Commun. 4, 1426.
Fan, Y., Yu, D., Yao, Y.-G., 2014. Tree shrew database (TreeshrewDB): a genomic knowledge base for the Chinese tree shrew. Sci. Rep. 4, 7145.
Fuchs, E., Corbach-Söhle, S., 2010. Tree Shrews. The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals. Wiley-Blackwell, pp. 262–275.
Govindaraj, R.G., Manavalan, B., Basith, S., Choi, S., 2011. Comparative analysis of species-specific ligand recognition in toll-like receptor 8 signaling: a hypothesis. PLoS One 6, e25118.
Guan, Y., Ranoa, D.R., Jiang, S., Mutha, S.K., Li, X., Baudry, J., Tapping, R.I., 2010. Human TLRs 10 and 1 share common mechanisms of innate immune sensing but not signaling. J. Immunol. 184, 5094–5103.
Guirado, M., Gil, H., Saenz-Lopez, P., Reinboth, J., Garrido, F., Cozar, J.M., Ruiz-Cabello, F., Carretero, R., 2012. Association between C13ORF31, NOD2, RIPK2 and TLR10 polymorphisms and urothelial bladder cancer. Hum. Immunol. 73, 668–672.
Guitart, A., Riezu-Boj, J.I., Elizalde, E., Larrea, E., Berasain, C., Aldabe, R., Civeira, M.P., Prieto, J., 2005. Hepatitis C virus infection of primary tupaia hepatocytes leads to selection of quasispecies variants, induction of interferon-stimulated genes and NF-kappaB nuclear translocation. J. Gen. Virol. 86, 3065–3074.
Guo, X., Lin, M., Rockowitz, S., Lachman, H.M., Zheng, D., 2014. Characterization of human pseudogene-derived non-coding RNAs for functional potential. PLoS One 9, e93972.
Hasan, U., Chaffois, C., Gaillard, C., Saulnier, V., Merck, E., Tancredi, S., Guiet, C., Briere, F., Vlach, J., Lebecque, S., Trinchieri, G., Bates, E.E., 2005. Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells,

which activates gene transcription through MyD88. J. Immunol. 174, 2942–2950.
Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., Bauer, S., 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303, 1526–1529.
Hennessy, E.J., Parker, A.E., O'Neill, L.A., 2010. Targeting toll-like receptors: emerging therapeutics? Nat. Rev. Drug Discov. 9, 293–307.
Iwami, K.I., Matsuguchi, T., Masuda, A., Kikuchi, T., Musikacharoen, T., Yoshikai, Y., 2000. Cutting edge: naturally occurring soluble form of mouse toll-like receptor 4 inhibits lipopolysaccharide signaling. J. Immunol. 165, 6682–6686.
Janeway Jr., C.A., Medzhitov, R., 2002. Innate immune recognition. Annu. Rev. Immunol. 20, 197–216.
Jann, O.C., Werling, D., Chang, J.S., Haig, D., Glass, E.J., 2008. Molecular evolution of bovine toll-like receptor 2 suggests substitutions of functional relevance. BMC Evol. Biol. 8, 288.
Jaresova, I., Rozkova, D., Spisek, R., Janda, A., Brazova, J., Sediva, A., 2007. Kinetics of toll-like receptor-4 splice variants expression in lipopolysaccharide-stimulated antigen presenting cells of healthy donors and patients with cystic fibrosis. Microbes Infect. 9, 1359–1367.
Jin, M.S., Lee, J.O., 2008. Structures of the toll-like receptor family and its ligand complexes. Immunity 29, 182–191.
Johnsson, P., Ackley, A., Vidarsdottir, L., Lui, W.O., Corcoran, M., Grander, D., Morris, K.V., 2013. A pseudogene long-noncoding-RNA network regulates PTEN transcription and translation in human cells. Nat. Struct. Mol. Biol. 20, 440–446.
Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat. Immunol. 11, 373–384.
Keestra, A.M., de Zoete, M.R., van Aubel, R.A., van Putten, J.P., 2008. Functional characterization of chicken TLR5 reveals species-specific recognition of flagellin. Mol. Immunol. 45, 1298–1307.
Kock, J., Nassal, M., MacNelly, S., Baumert, T.F., Blum, H.E., von Weizsacker, F., 2001. Efficient infection of primary tupaia hepatocytes with purified human and woolly monkey hepatitis B virus. J. Virol. 75, 5084–5089.
Lazarus, R., Raby, B.A., Lange, C., Silverman, E.K., Kwiatkowski, D.J., Vercelli, D., Klimecki, W.J., Martinez, F.D., Weiss, S.T., 2004. TOLL-like receptor 10 genetic variation is associated with asthma in two independent samples. Am. J. Respir. Crit. Care Med. 170, 594–600.
Lee, J., Tian, Y., Chan, S.T., Kim, J.Y., Cho, C., Ou, J.H., 2015. TNF-alpha induced by hepatitis C virus via TLR7 and TLR8 in hepatocytes supports interferon signaling via an autocrine mechanism. PLoS Pathog. 11, e1004937.
Lee, S.M., Kok, K.H., Jaume, M., Cheung, T.K., Yip, T.F., Lai, J.C., Guan, Y., Webster, R.G., Jin, D.Y., Peiris, J.S., 2014. Toll-like receptor 10 is involved in induction of innate immune responses to influenza virus infection. Proc. Natl. Acad. Sci. U. S. A. 111, 3793–3798.
Letunic, I., Doerks, T., Bork, P., 2015. SMART: recent updates, new developments and status in 2015. Nucleic Acids Res. 43, D257–D260.
Li, K., Foy, E., Ferreon, J.C., Nakamura, M., Ferreon, A.C., Ikeda, M., Ray, S.C., Gale Jr., M., Lemon, S.M., 2005. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the toll-like receptor 3 adaptor protein TRIF. Proc. Natl. Acad. Sci. U. S. A. 102, 2992–2997.
Li, L., Li, Z., Wang, E., Yang, R., Xiao, Y., Han, H., Lang, F., Li, X., Xia, Y., Gao, F., Li, Q., Fraser, N.W., Zhou, J., 2015. HSV-1 infection of tree shrews differs from that of mice in the severity of acute infection and viral transcription in the peripheral nervous system. J. Virol. 90, 790–804.
Li, S.A., Lee, W.H., Zhang, Y., 2012. Two bacterial infection models in tree shrew for evaluating the efficacy of antimicrobial agents. Zool. Res 33, 1–6.
Machida, K., Cheng, K.T., Sung, V.M., Levine, A.M., Foung, S., Lai, M.M., 2006. Hepatitis C virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon and interleukin-6. J. Virol. 80, 866–874.
Medzhitov, R., 2001. Toll-like receptors and innate immunity. Nat. Rev. Immunol. 1, 135–145.
Metz, P., Reuter, A., Bender, S., Bartenschlager, R., 2013. Interferon-stimulated genes and their role in controlling hepatitis C virus. J. Hepatol. 59, 1331–1341.
Mikami, T., Miyashita, H., Takatsuka, S., Kuroki, Y., Matsushima, N., 2012. Molecular evolution of vertebrate Toll-like receptors: evolutionary rate difference between their leucine-rich repeats and their TIR domains. Gene 503, 235–243.
Morgan, A.R., Lam, W.J., Han, D.Y., Fraser, A.G., Ferguson, L.R., 2012. Genetic variation within TLR10 is associated with Crohn's disease in a New Zealand population. Hum. Immunol. 73, 416–420.
Mukherjee, S., Sarkar-Roy, N., Wagener, D.K., Majumder, P.P., 2009. Signatures of natural selection are not uniform across genes of innate immune system, but purifying selection is the dominant signature. Proc. Natl. Acad. Sci. U. S. A. 106, 7073–7078.
Muro, E.M., Mah, N., Andrade-Navarro, M.A., 2011. Functional evidence of post-transcriptional regulation by pseudogenes. Biochimie 93, 1916–1921.
Murphy, W.J., Eizirik, E., O'Brien, S.J., Madsen, O., Scally, M., Douady, C.J., Teeling, E., Ryder, O.A., Stanhope, M.J., de Jong, W.W., Springer, M.S., 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294, 2348–2351.
Nagashima, H., Iwatani, S., Cruz, M., Jimenez Abreu, J.A., Uchida, T., Mahachai, V., Vilaichone, R.K., Graham, D.Y., Yamaoka, Y., 2015. Toll-like receptor 10 in *Helicobacter pylori* infection. J. Infect. Dis. 212, 1666–1676.
Nakajima, T., Ohtani, H., Satta, Y., Uno, Y., Akari, H., Ishida, T., Kimura, A., 2008. Natural selection in the TLR-related genes in the course of primate evolution. Immunogenetics 60, 727–735.
Nishihara, H., Terai, Y., Okada, N., 2002. Characterization of novel Alu- and tRNA-related SINEs from the tree shrew and evolutionary implications of their origins. Mol. Biol. Evol. 19, 1964–1972.
Ohto, U., Shibata, T., Tanji, H., Ishida, H., Krayukhina, E., Uchiyama, S., Miyake, K., Shimizu, T., 2015. Structural basis of CpG and inhibitory DNA recognition by Toll-like receptor 9. Nature 520, 702–705.
Oosting, M., Cheng, S.C., Bolscher, J.M., Vestering-Stenger, R., Plantinga, T.S., Verschueren, I.C., Arts, P., Garritsen, A., van Eenennaam, H., Sturm, P., Kullberg, B.J., Hoischen, A., Adema, G.J., van der Meer, J.W., Netea, M.G., Joosten, L.A., 2014. Human TLR10 is an anti-inflammatory pattern-recognition receptor. Proc. Natl. Acad. Sci. U. S. A. 111, E4478–E4484.
Peng, Y.Z., Ye, Z.Z., Zou, R.J., Wang, Y.X., Tian, B.P., Ma, Y.Y., Shi, L.M., 1991. Biology of Chinese Tree Shrews (*Tupaia Belangeri Chinensis*). Yunnan Science and Technology Press, Kunming, China.
Pink, R.C., Wicks, K., Caley, D.P., Punch, E.K., Jacobs, L., Carter, D.R., 2011. Pseudogenes: pseudo-functional or key regulators in health and disease? RNA 17, 792–798.
Rauta, P.R., Samanta, M., Dash, H.R., Nayak, B., Das, S., 2014. Toll-like receptors (TLRs) in aquatic animals: signaling pathways, expressions and immune responses. Immunol. Lett. 158, 14–24.
Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent endpoints. Am. J. Epidemiol. 27, 493–197.
Rehli, M., 2002. Of mice and men: species variations of toll-like receptor expression. Trends Immunol. 23, 375–378.
Roach, J.C., Glusman, G., Rowen, L., Kaur, A., Purcell, M.K., Smith, K.D., Hood, L.E., Aderem, A., 2005. The evolution of vertebrate toll-like receptors. Proc. Natl. Acad. Sci. U. S. A. 102, 9577–9582.
Rosen, A., Gelderblom, H., Darai, G., 1985. Transduction of virulence in herpes simplex virus type 1 from a pathogenic to an apathogenic strain by a cloned viral DNA fragment. Med. Microbiol. Immunol. 173, 257–278.
Schultz, J., Milpetz, F., Bork, P., Ponting, C.P., 1998. SMART, a simple modular architecture research tool: identification of signaling domains. Proc. Natl. Acad. Sci. U. S. A. 95, 5857–5864.
Shi, Z., Cai, Z., Sanchez, A., Zhang, T., Wen, S., Wang, J., Yang, J., Fu, S., Zhang, D., 2011. A novel toll-like receptor that recognizes vesicular stomatitis virus. J. Biol. Chem. 286, 4517–4524.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Steinmann, E., Pietschmann, T., 2013. Cell culture systems for hepatitis C virus. Curr. Top. Microbiol. Immunol. 369, 17–48.
Takeda, K., Kaisho, T., Akira, S., 2003. Toll-like receptors. Annu. Rev. Immunol. 21, 335–376.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Tanji, H., Ohto, U., Shibata, T., Miyake, K., Shimizu, T., 2013. Structural reorganization of the toll-like receptor 8 dimer induced by agonistic ligands. Science 339, 1426–1429.
Ulevitch, R.J., 2004. Therapeutics targeting the innate immune system. Nat. Rev. Immunol. 4, 512–520.
Wang, J., Zhou, Q.X., Lv, L.B., Xu, L., Yang, Y.X., 2012. A depression model of social defeat etiology using tree shrews. Zool. Res. 33, 92–98.
Wells, C.A., Chalk, A.M., Forrest, A., Taylor, D., Waddell, N., Schroder, K., Himes, S.R., Faulkner, G., Lo, S., Kasukawa, T., Kawaji, H., Kai, C., Kawai, J., Katayama, S., Carninci, P., Hayashizaki, Y., Hume, D.A., Grimmond, S.M., 2006. Alternate transcription of the toll-like receptor signaling cascade. Genome Biol. 7, R10.
Werling, D., Jann, O.C., Offord, V., Glass, E.J., Coffey, T.J., 2009. Variation matters: TLR structure and species-specific pathogen recognition. Trends Immunol. 30, 124–130.
Wlasiuk, G., Nachman, M.W., 2010. Adaptation and constraint at toll-like receptors in primates. Mol. Biol. Evol. 27, 2172–2186.
Xu, L., Chen, S.Y., Nie, W.-H., Jiang, X.-L., Yao, Y.-G., 2012. Evaluating the phylogenetic position of Chinese tree shrew (*Tupaia belangeri chinensis*) based on complete mitochondrial genome: implication for using tree shrew as an alternative experimental animal to primates in biomedical research. J. Genet. Genomics. 39, 131–137.
Xu, L., Yu, D., Peng, L., Fan, Y., Chen, J., Zheng, Y.-T., Wang, C., Yao, Y.-G., 2015. Characterization of a MAVS ortholog from the Chinese tree shrew (*Tupaia belangeri chinensis*). Dev. Comp. Immunol. 52, 58–68.
Xu, L., Zhang, Y., Liang, B., LÜ, L.-B., Chen, C.-S., Chen, Y.-B., Zhou, J.-M., Yao, Y.-G., 2013. Tree shrews under the spot light: emerging model of human diseases. Zool. Res. 34, 59–69 in Chinese.
Xu, X., Chen, H., Cao, X., Ben, K., 2007. Efficient infection of tree shrew (*Tupaia belangeri*) with hepatitis C virus grown in cell culture or from patient plasma. J. Gen. Virol. 88, 2504–2512.
Yan, R.Q., Su, J.J., Chen, Z.Y., Liu, Y.G., Gan, Y.Q., Zhou, D.N., 1984. A preliminary study on experimental infection of human hepatitis B virus in adult tree shrews. J. Guangxi Med. Univ. 1, 10–15 in Chinese.
Yan, R.Q., Su, J.J., Huang, D.R., Gan, Y.C., Yang, C., Huang, G.H., 1996. Human hepatitis B virus and hepatocellular carcinoma. I. Experimental infection of tree shrews with hepatitis B virus. J. Cancer Res. Clin. Oncol. 122, 283–288.
Yang, D.R., Zhu, H.Z., 2015. Hepatitis C virus and antiviral innate immunity: who wins at tug-of-war? World J. Gastroenterol. 21, 3786–3800.
Yang, Z., 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24, 1586–1591.
Yang, Z., Wong, W.S., Nielsen, R., 2005. Bayes empirical bayes inference of amino acid sites under positive selection. Mol. Biol. Evol. 22, 1107–1118.

Yu, D., Xu, L., Liu, X.-H., Fan, Y., Lü, L.-B., Yao, Y.-G., 2014. Diverse Interleukin-7 mRNA transcripts in Chinese tree shrew (*Tupaia belangeri chinensis*). PLoS One 9, e99859.
Zhang, J., Nielsen, R., Yang, Z., 2005. Evaluation of an improved branch-site likelihood method for detecting positive selection at the molecular level. Mol. Biol. Evol. 22, 2472–2479.
Zheng, Y.-T., Yao, Y.-G., Xu, L., 2014. Basic biology and disease models of Chinese tree shrews. Yunnan Science and Technology Press, Kunming, China page 1–475.
Zhu, J., Brownlie, R., Liu, Q., Babiuk, L.A., Potter, A., Mutwiri, G.K., 2009. Characterization of bovine Toll-like receptor 8: ligand specificity, signaling essential sites and dimerization. Mol. Immunol. 46, 978–990.

Supplementary materials

Table S1. Primers used in this study.

Gene	Usage	Primer sequence (5'-3')
TLR1	5' RACE	ACTCCTTGCATATGGGCAGGGCATC
	5' RACE nest	AAAACACTGAGATCAAGATGCT
	3' RACE	CAAGCACTTAGACCTTTCGTTT
	3' RACE nest	GCTAGATGATAACCAATGTTCC
	Full-length amplification, Forward	CATCTGTACCTGGACGCTCTGA
	Full-length amplification, Reverse	AAAGACAAGCATCCCCAATAAG
	Sequencing, Forward	TCTCACCCATATTCCCCAAGAC
	Sequencing, Reverse	ATTCTAAGTATGTCCTTCGTGC
	Sequencing, Reverse	AGTCCTCCACCACCCCTCTT
	Real time, Forward	CCTCCTACCACTCTCCAA
	Real time, Reverse	TCCAAGTATTCCAATTCCTGAT
TLR2	5' RACE	GGACCTCAGACTTCTGGGCTCATAGCTC
	5' RACE nest	GGACCGGAGCACCAGAGACTTGAG
	3' RACE	TGTCTGCACAAGCGCGACTTCGTC
	3' RACE nest	GGAGCGAGTGGTGCAAGTACGAG
	Full-length amplification, Forward	TACTGTGGGCCAGGCACC
	Full-length amplification, Reverse	CTGTCTGTCCGATGCGCA
	Full-length nest amplification, Forward	CTCTGTCCTGTGACGCCAGT
	Full-length nest amplification, Reverse	CTCGTCGAAGAGGCGGAAGT
	Sequencing, Forward	CCTGACTTCCTTGAGATT
	Sequencing, Forward	ACCTGATCCTTCGCATGAGACC
	Sequencing, Forward	TGGCCCGAGAAGCTGGAA
	Sequencing, Reverse	CGTGAATGTGTAACTGTT
	Sequencing, Reverse	CTCACGAAGTTCTCCGAGAGCACGA
	Sequencing, Reverse	TGAAGGCCAGAAAGTCAC
	Real time F	CCTGACTTCCTTGAGATT
	Real time R	CGTGAATGTGTAACTGTT
TLR3	5' RACE	CTCCTGCCCTGTGAGTTCTTGCCCA
	5' RACE nest	TTTAGATGACCCAACCAAGAG
	3' RACE	ACTGACTGTCTTGGATGGAGGCT
	3' RACE nest	CACTTGCTCATTCTCCTTTACTC
	Full-length amplification, Forward	TATGAACGGCCAGAGTACGGAA
	Full-length amplification, Reverse	CCTTCTGGTTCGGGACCCTAAT
	Sequencing, Reverse	ACAAACCAGGCAATGCTTTCAC
	Sequencing, Reverse	GAACTGCTCTGGCTGTCTGTCTA
	Real time F	CTTCGTCATACTGCTCATC
	Real time R	CTCTGGCTGTCTGTCTAT
TLR4	5' RACE	GTTCTCGGTTGAGGATGGGATG
	5' RACE nest	TTGATAGTCCAGAAAAGGCTCCCAGGC
	3' RACE	TTTGACTCACTCCTCCATCTTC
	3' RACE nest	TGGAGTTGTATCGCCTTCTTAG

	Full-length amplification, Forward	AGTTCCAGCCTGTCATGTTCGC
	Full-length amplification, Reverse	GTGGAACCATTCAGTATTTGTC
	Full-length nest amplification, Forward	GGTTCCTAACATTACTTACCAAT
	Full-length nest amplification, Reverse	GGTTTACCATCCAGGAGGGCTTT
	Sequencing, Forward	TTTAGCATTCTTAGATGACTCCC
	Sequencing, Reverse	TTTGAAGCCACTATGCGGTTAA
	Real time F	GTTCAGTCTCCGTGTCTT
	Real time R	AAGGTCAAGTCAGTCAGATT
TLR5	5’ RACE	CTGCTCCAGGAAGGGGAAAGA
	5’ RACE nest	GGGATAGTCCTTGAAAAGCATCTGGGTG
	3’ RACE	TGTGAATGTGAACTTAGTGCTT
	3’ RACE nest	AGTTTTCCCTTTTCATCTTCTTC
	Full-length amplification, Forward	AGCTGCGGGGAGGAGCGAGTC
	Full-length amplification, Reverse	CCATTATGCTAACTGCTGGTCC
	Full-length nest amplification, Forward	GATGGTCAGATTGCCTTGTATCG
	Full-length nest amplification, Reverse	TTTCTTTTTTTACTAAGTGTCG
	Sequencing, Forward	TGCTGGGTTTGGCTTCCGTA
	Sequencing, Reverse	GGCTGTAAGACTGATATGTGGC
	Real time F	TCCAAGCATACCTGATAT
	Real time R	TAGCCTGTTCTCTGATAA
TLR6	5’ RACE	TCTCCACCCAGAGGCAATTTCCCTC
	5’ RACE nest	TTGAAAAATGCTGTCTGTGAAA
	3’ RACE	CATATCGGATACTCCTTTCATAC
	3’ RACE nest	CGTGGAGAATATCGTCAACTG
	Full-length amplification, Forward	AGATCTGCTTCCAATCGCACAC
	Full-length amplification, Reverse	TCATAATGGCACCACTCACTCTG
	Sequencing, Reverse	CAGAGGAGGGTCATGGTCACAGC
	Real time F	TCCACATTAGTTAGATTAGAGA
	Real time R	GAGTCCATCAGATTCCAA
TLR7	5’ RACE	AACAACGAGGGCAGTTTCCACTTAGGTC
	5’ RACE nest	ATAATAACAGTTTTGACCCAGG
	3’ RACE	CTTGGCAACTCTCATGCTCTGC
	3’ RACE nest	GACCTAAGTGGAAACTGCCCTCG
	Full-length amplification, Forward	TGTGGACTGCACCGACAAGCA
	Full-length amplification, Reverse	AAAATCTAAGCAGCCAGGTGTC
	Sequencing, Reverse	AGAGATAAGAAAGCAGCAACTA
	Sequencing, Reverse	GTCATTGTGGTTCATCATTAGTTT
	Sequencing, Reverse	GAGCTGGAGGAACTTGGACTT
	Real time F	CGGCTTGACTTACTCTACT
	Real time R	AATGGCTGTTGCTACTTATATC
TLR8	5’ RACE	GCTCCAGAGGCTATTTCTTGTA
	5’ RACE	GGGTCAAAGTTGGTATCTGCTGAGCGTG
	3’ RACE	AATGCAACAACCTTCGACTACA
	3’ RACE	AAACCTGACCCAACTTCGTTA
	Full-length amplification, Forward	TTTCTCTTCTCCACGCACCTAC
	Full-length amplification, Reverse	CATTGCTTCGCATTTTTATTAT
	Sequencing, Reverse	TGAGCCAGGGCAGTCAACATA
	Sequencing, Reverse	TACAGATCCGCTGCCGTAGCCG

	Real time F	CTGTGGAATGCTGAAGAC
	Real time R	TGGAATACGCTGAAGGTTA
TLR9	5’ RACE	TGGGCTCCGTGTGCACCAACTCGATG
	5’ RACE	TGTAGCCCGGCATGGAGTAGATGA
	3’ RACE	GGACTGGCTACCCGGCAAAACGCTCTTC
	3’ RACE	TGACGCCCGCCGCTCCCGCTACGTGCGG
	Full-length amplification, Forward	GAGCGGGAGACCATCGAGT
	Full-length amplification, Reverse	TGAGTGCCTGCTCTGCCC
	Full-length nest amplification, Forward	CTTCTTATTCATAGACGGCAAC
	Full-length nest amplification, Reverse	GCACTGGGCTGGCTATTC
	Sequencing, Forward	GCGTCTTCGGGAACTTCT
	Sequencing, Forward	ATGCCCTGCCCTATGACG
	Sequencing, Reverse	TGAAGTTGTGGCCTACGC
	Sequencing, Reverse	GGAGATAGAGCCGCTGCA
	Sequencing, Reverse	AGAGGGTTGGCGGTCACAT
	Real time F	TCGCTCAAGTACAACAAC
	Real time R	GTAGGACAACAGCAGATAC
TLR10	5’ RACE	TGACGGCACCCAGGAAGATCAGGAC
	5’ RACE	TTAGCTGGGCTGAGAATTAAGTTCA
	3’ RACE	ACTTTGTTCCCGGCCAGAGTGTCAT
	3’ RACE	CAGAGTGAGTGGTGCCGTTATGAGC
	Full-length amplification, Forward	GAGAATGAATGAACCCACAATC
	Full-length amplification, Reverse	CCAGAAGGCCACATTTAT
	Sequencing, Forward	TGCCTCCGCTTTGATCTG
	Real time F	CTTTGTCCAGAGTGAGTG
	Real time R	GGATTCCAGTAAGATGAGAA
TLR11	5’ RACE	CAGGCAACAGGGGACATGATGCTCAAAG
	5’ RACE	GGATCTGAGGGGCTCCAAGGCATCTG
	3’ RACE	TGACGCCCGCCGCTCCCGCTACGTGCGG
	3’ RACE	CTTGACCAGGGACAACCGCCACTTCTAT
	Full-length amplification, Forward	GTGTGACAGACGGCAGAG
	Full-length amplification, Reverse	TTGAGGGCACAGAATCTT
	Full-length nest amplification, Forward	CCCGAGCTCATGGTGGAGGAAAGATTCTC
	Full-length nest amplification, Reverse	TCATCGGATAGAGGTAAC
	Sequencing, Forward	TGAGCTGGGCCTGGACTG
	Sequencing, Forward	TGCAGCACCTTTCCTTTGAGCATCATGTC
	Sequencing, Forward	ACCTCACTGAACCTCCTGGGCACTTATT
	Sequencing, Reverse	CTGGGGGATGCCTTCTGT
	Sequencing, Reverse	CCCATCGATATCCTCTCTCTCTTGTCCAG
	Real time F	TTCTCAACTCTGAATGGA
	Real time R	AGGTGAAGGTAATATCTGA
TLR12	5’ RACE	CAGATCAGGAAGAGGCGGGAGACCA
	5’ RACE	AGGACAGAAGGAGAGCCGCTGAGAC
	3’ RACE	CTGGTGGTCTTCCTGGAGCCGATCT
	3’ RACE	GCATGTGGCCCAAGCAAGATGAAAG
	Full-length amplification, Forward	GAGCGGGAGGCGGGATCG
	Full-length amplification, Reverse	CACACCACCTCCGGGCTG
	Full-length nest amplification, Forward	CTGTCCTGCCACGGGATGCTTTCTCCAACTTC

	Full-length nest amplification, Reverse	CTTTCATCTTGCTTGGGC
	Sequencing, Forward	GTGGGCTCCAATAGGCTC
	Sequencing, Forward	TGTTCCAGGGCCTACAGA
	Sequencing, Forward	GCGGTCTCTGGCATGAAG
	Sequencing, Forward	TGCCCAAGCTAGAGGTGC
	Sequencing, Reverse	CTCAAAGTCCCGCTCAGG
	Real time F	ATGTCTTTCCAGTCTTAC
	Real time R	AAGGTCTTCGAGATATTC
TLR13	5’ RACE	TTCCACTCCAGTCGTAAGTCCACC
	5’ RACE	CCCCAAAAGGCCCTCTTACCAATC
	3’ RACE	AAATGCCATCAACACCAGCCGTAAA
	3’ RACE	TTGGGTAATAAAACTGTGGAGAAAG
	Full-length amplification, Forward	CCCGAGCTCATGGCTTCAAACAGCTTCCT
	Full-length amplification, Reverse	GGCATCGATCTCAGCCACAATTAGCTGTG
	Sequencing, Forward	GCTCAGATCTAAGGCCGTCAAGTTCT
	Sequencing, Forward	CTTACCTACATAATCTTGACCTGGCATACAAC
	Sequencing, Reverse	GAGTTTCATAAATGGAGGGGAGTGCAAGGTCT
	Sequencing, Reverse	GTGGTTACTGACTACACACAAAGTTTTACGG
	Real time F	TGAATGGTGTAGGCTTGA
	Real time R	GTCGGTGGTAACTGGATA
β-actin	Real time F	ATTTTGAATGATCAGCCACC
	Real time R	AGGTAAGCCCTGGCTGCCTC

Table S2. 12 species used in the phylogenetic analyses

Protein	Species	GenBank accession number
TLR1	*Homo sapiens*	XP_011512047.1
	Mus musculus	NP_001263374.1
	Rattus norvegicus	NP_001165591.1
	Bos taurus	NP_001039969.1
	Sus scrofa	NP_001026945.1
	Macaca mulatta	NP_001123896.1
	Canis lupus familiaris	NP_001139615.1
	Gorilla gorilla	NP_001266513.1
	Ovis aries	NP_001128532.1
	Oryctolagus cuniculus	XP_002709316.1
	Tupaia belangeri chinensis	KT354316
TLR2	*Homo sapiens*	NP_003255.2
	Mus musculus	NP_036035.3
	Rattus norvegicus	NP_942064.1
	Bos taurus	NP_776622.1
	Sus scrofa	NP_998926.1
	Macaca mulatta	NP_001123897.1
	Canis lupus familiaris	NP_001005264.2
	Gorilla gorilla	NP_001266693.1
	Ovis aries	NP_001041696.1
	Oryctolagus cuniculus	NP_001076250.1
	Tupaia belangeri chinensis	KT354317
TLR3	*Homo sapiens*	NP_003256.1
	Mus musculus	NP_569054.2
	Rattus norvegicus	NP_942086.1
	Bos taurus	NP_001008664.1
	Sus scrofa	NP_001090913.1
	Macaca mulatta	NP_001031762.1
	Canis lupus familiaris	XP_005630024.1
	Gorilla gorilla	NP_001266681.1
	Ovis aries	NP_001129400.1
	Oryctolagus cuniculus	NP_001075688.1
	Tupaia belangeri chinensis	KT354318
TLR4	*Homo sapiens*	NP_612564.1
	Mus musculus	NP_067272.1
	Rattus norvegicus	NP_062051.1
	Bos taurus	NP_776623.5
	Sus scrofa	NP_001106510.2
	Macaca mulatta	NP_001032169.1
	Canis lupus familiaris	NP_001002950.2
	Gorilla gorilla	NP_001266512.1

	Ovis aries	NP_001129402.1
	Oryctolagus cuniculus	NP_001076201.1
	Tupaia belangeri chinensis	KT354319
TLR5	*Homo sapiens*	NP_003259.2
	Mus musculus	NP_058624.2
	Rattus norvegicus	NP_001139300.1
	Bos taurus	NP_001035591.1
	Sus scrofa	NP_001116674.1
	Macaca mulatta	NP_001123901.1
	Canis lupus familiaris	NP_001184105.1
	Gorilla gorilla	NP_001266608.1
	Ovis aries	NP_001129398.1
	Oryctolagus cuniculus	XP_008266592.1
	Tupaia belangeri chinensis	KT354320
TLR6	*Homo sapiens*	NP_006059.2
	Mus musculus	NP_035734.3
	Rattus norvegicus	NP_997487.1
	Bos taurus	NP_001001159.1
	Sus scrofa	NP_998925.1
	Macaca mulatta	NP_001123902.1
	Canis lupus familiaris	XP_005618690.1
	Gorilla gorilla	NP_001266567.1
	Ovis aries	NP_001129399.1
	Oryctolagus cuniculus	XP_008273269.1
	Tupaia belangeri chinensis	KT354321
TLR7	*Homo sapiens*	NP_057646.1
	Mus musculus	NP_001277684.1
	Rattus norvegicus	NP_001091051.1
	Bos taurus	NP_001028933.1
	Sus scrofa	NP_001090903.1
	Macaca mulatta	NP_001123898.1
	Canis lupus familiaris	NP_001041589.1
	Gorilla gorilla	XP_004063841.1
	Ovis aries	NP_001128531.1
	Tupaia belangeri chinensis	KT354322
TLR8	*Homo sapiens*	NP_057694.2
	Mus musculus	NP_573475.2
	Rattus norvegicus	NP_001094479.1
	Bos taurus	NP_001029109.1
	Sus scrofa	NP_999352.1
	Macaca mulatta	NP_001123899.1
	Canis lupus familiaris	XP_003435496.1
	Gorilla gorilla	XP_004063842.1
	Ovis aries	NP_001129401.1

	Tupaia belangeri chinensis	KT354323
TLR9	*Homo sapiens*	NP_059138.1
	Mus musculus	NP_112455.2
	Rattus norvegicus	NP_937764.1
	Bos taurus	NP_898904.1
	Sus scrofa	NP_999123.1
	Macaca mulatta	NP_001123903.1
	Canis lupus familiaris	NP_001002998.1
	Gorilla gorilla	XP_004034320.1
	Ovis aries	NP_001011555.1
	Oryctolagus cuniculus	XP_008258980.1
	Tupaia belangeri chinensis	KT354324
TLR10	*Homo sapiens*	NP_001017388.1
	Rattus norvegicus	NP_001139507.1
	Bos taurus	NP_001070386.1
	Sus scrofa	NP_001025705.1
	Macaca mulatta	NP_001123906.1
	Canis lupus familiaris	NP_001166598.1
	Gorilla gorilla	NP_001266468.1
	Ovis aries	NP_001129397.1
	Oryctolagus cuniculus	NP_001284430.1
	Tupaia belangeri chinensis	KT946778
TLR11	*Mus musculus*	NP_991388.2
	Rattus norvegicus	NP_001138251.2
	Tupaia belangeri chinensis	KT354325
TLR11	*Mus musculus*	NP_991392.1
	Rattus norvegicus	NP_001102152.1
	Tupaia belangeri chinensis	KT354326
TLR11	*Mus musculus*	NP_991389.1
	Rattus norvegicus	XP_008771576.1
	Tupaia belangeri chinensis	KT354327

Table S3. Positively selected sites in tTLR8 and tTLR9 and their locations in three dimensional TLR8 and TLR9 protein structures.

Protein	**Position Codon** [a]	**Equivalent codon in human**	**Equivalent codon in tree shrew**
TLR8	357P	355P	328D
	415N	413N	386R
	465S	463S	436L
	481E	478E	451N
	551P	548P	521R
	552H	549H	522G
	572S	569T	542Q
	772I	769I	741L
TLR9	40Q	16Q	16W
	63P	39P	39D
	64H	40H	40P
	81S	57S	57K
	220L	196L	195A
	221G	197G	196N
	240R	216R	215Q
	336V	312V	311T
	431Q	407Q	409P
	500T	472T	472K
	511N	483N	483T
	540V	512V	512P
	543S	515S	515K
	549S	521T	521Q
	682S	654S	654G
	768V	740V	740I
	817K	789K	789S
	831A	803A	803Q
	889R	859R	859L

[a] The amino acid positions refers to the aligned sequences of 6 species in Figure S2.

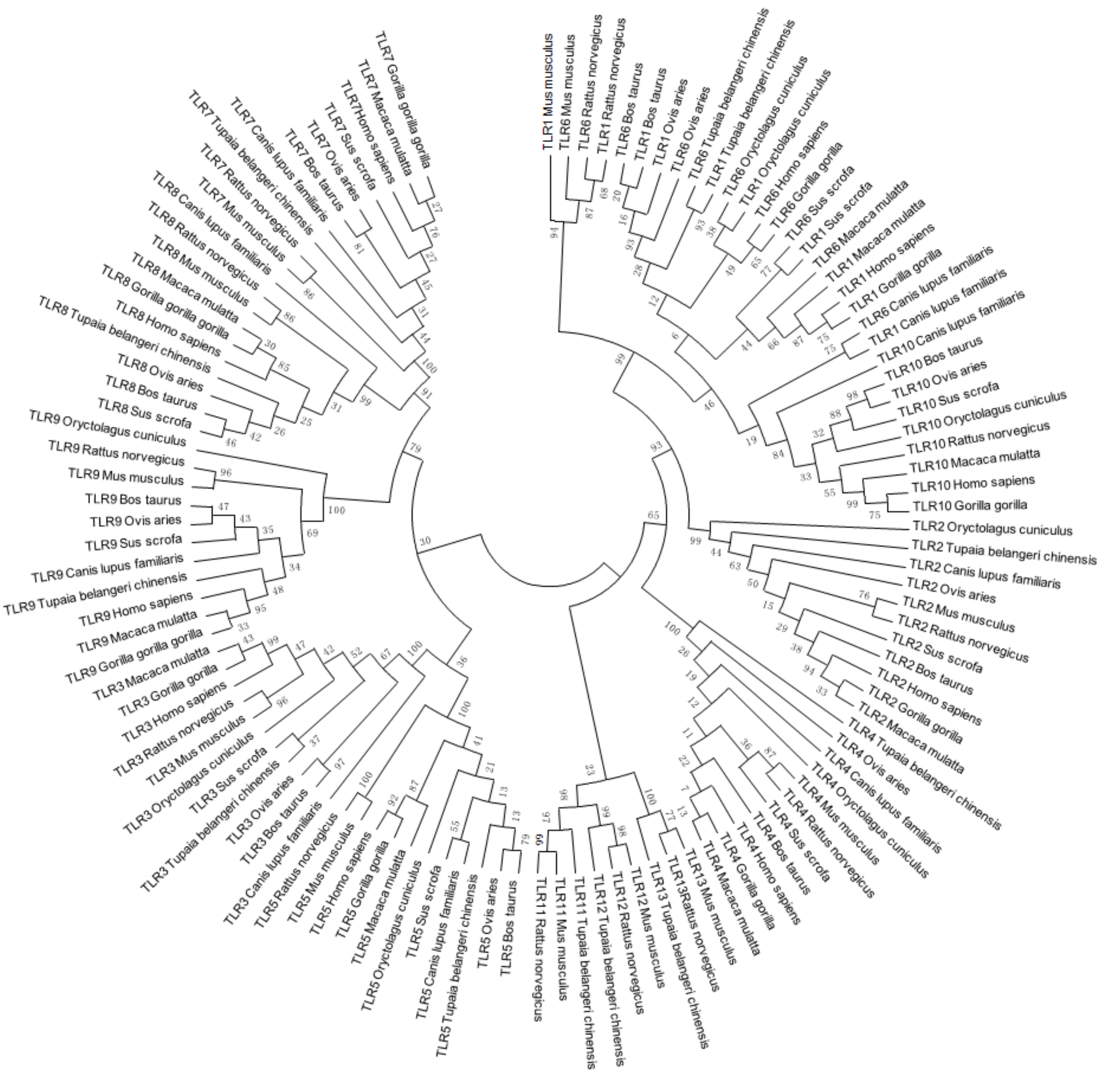

Figure S1. Phylogenetic trees of tTLRs based on amino acid alignments of the TIR domain. Bootstrap values base on 1000 replicates are indicated on each branch. The neighbor-joining (NJ) trees were reconstructed using MEGA6 (Tamura et al. 2013) with Poisson as the model. 11 representative TIR domains were defined by the GenBank information and the SMART webserver. The sequences include *Homo sapiens* (human), *Gorilla gorilla* (gorilla), *Macaca mulatta* (Rhesus Macaque), *Mus musculus* (mouse), *Rattus norvegicus* (Rat), *Bos Taurus* (Cattle), *Sus scrofa* (pig), *Canis lupus familiaris* (dog), *Ovis aries* (sheep), *Oryctolagus cuniculus* (rabbit), and *Tupaia belangeri chinensis* (Chinese tree shrews).

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TLR1          1                                                                                                                                                          150
human         ----MTSIFHFAIIFMLILQIRIQLSEESEFLVDRSKNGLIHVPKDLSQKTTILNISQNYISELWTSDILSLSKLRILIISHNRIQYLDISVFKFNQELEYLDLSHNKLVKISCHPTVNLKHLDLSFNAFDALPICKEFGNMSQLKFLGL
treeshrew     ----MMSIFHXIITFIVILEIRIQLSNETEVLVNRSKTDLTHIPQDLPLETTTLDMSHNYISELQTSVLLPLSNLRILILSHNSIQHLDLSVFEFNQELEYLDVSHNKLGSLSCHPTVNLKHLDLSFNAFDALPICKEFGNMLQLKFLGL
mouse         MTKPNSLIFYCIIVLGLTL-MKIQLSEECELIIKRPNANLTRVPKDLPLQTTTLDLSQNNISELQTSDILSLSKLRVLIMSYNRLQYLNISVFKFNTELEYLDLSHNELKVILCHPTVSLKHLDLSFNAFDALPICKEFGNMSQLQFLGL
rhesus        ----MTSIFHFAIIFMLTLQIRIQLSEESEFLVDRSKNSLIHVPKDLSQKTTILNISQNYISELWTSDILSLSKLRILIISHNRLQYLDISVFKFNQELEYLDLSHNKLAKISCHPTVNLKHLDLSFNAFDALPICKEFGNMSQLKFLGL
dog           MMKTNPSIFQFAIIFILILEIRIQLSEESDFLVNRSKAGLFHIPKDLSLKTTILDISQNYISELQTSDILSLSKLRILIVSYNRIQYLDISVFKFNQELEYLDLSHNELGRISCHPTVNLKHLDLSFNAFDDLPICKEFGNMSQLEFLGL
rat           MTKTQSTIFYCIVVLGLIL-IKIQLSEESELIIKRPNANLTRVPKDLPLQTTTLDVSQNNISELQTSDILLLSKLRVFIMSYNRLQYLNISVFKFNTELEYLDLSHNELRLISCHATADLKHLDLSFNAFDALPICKEFGNLSQLQFLGL
                                                                                                                                                                         300
human         STTHLEKSSVLPIAHLNISKVLLVLGETYGEKEDPEGLQDFNTESLHIVFPTNKEFHFILDVSVKTVANLELSNIKCVLEDNKCSYFLSILAKLQTNPKLSNLTLNNIETTWNSFIRILQLVWHTTVWYFSISNVKLQGQLDFRDFDYSG
treeshrew     SASQLQKXSMLPIAHLNISKLLLVLGETYGKKXDPGSLQGINTESLHIVFPTEKEFHYILDVSVSTIESLELSNIKCVLDDNQCSHFLHVLSKLQRNPRLSNLSLNNIETTWNSFMKILQSIWNTTIEYFSISNVKLQGQLDFTDFNYFD
mouse         SGSRVQSSSVQLIAHLNISKVLLVLGDAYGEKEDPESLRHVSTETLHIVFPSKREFRFLLDVSVSTTIGLELSNIKCVLEDQGCSYFLRALSKLGKNLKLSNLTLNNVETTWNSFINILQIVWHTPVKYFSISNVKLQGQLAFRMFNYSD
rhesus        STTHLEKSTVLPIAHLNISKVLLVLGEHYGDKEDPEGLQNFNTESLHIVFPTSKEFNFILDVSVRTVANLELSNIKCVLEDNECSYFLNILAKLQTNPKLSSLTLNNIETTWNSFIRILQLVWHTTVWYFSISNVKLQGQLDFRDFDYSG
dog           SATQLQKSSMLPIASLHIRKVLLVLGDTYGKKEDPESLQKLNTESLHIVFPIRKEFSFTLDVSVSTAVSLELSNIKCVPDGHGWSYFQNVLSKLQKNSRLSSLTLNNIETTWNFFIMLLQLVWHTSIEYFSISNVKLQGYPDFRDFDYSD
rat           SGSQIQNSSVQLIAHLNISKVLLVLGDTYGEKEDPKCLQHISTETLHIVFPSKREFHFLLDMSVSTAISLELSNIKCVLEDKNCSYFLGTLERLRKTQRLSNLTLNNVDTTWNSFINILQLVWHTPVKSFSISNVKLKGHFNFRRFHYSD
                                                                                                                                                                         450
human         TSLKALSIHQVVSDVFGFPQSYIYEIFSNMNIKNFTVSGTRMVHMLCPSKISPFLHLDFSNNLLTDTVFENCGHLTELETLILQMNQLKELSKIAEMTTQMKSLQQLDISQNSVSYDEKKGDCSWTKSLLSLNMSSNILTDTIFRCLPPR
treeshrew     TSLKTLSIQQVVSDVFNFPQSKIYKIFSNMNIQNFTVSGTRMIHMLCPSQISPFLYLDFSNNLLTDTVFENCRSLTKLKTFILRINQLKKLTNIVHMTKEMKSLQQLDISQNSIRYDDNEEVCFWTKSLLNLNMSSNVLTDSVFSCLPPK
mouse         TSLKALSIHQVVTDVFSFPQSYIYSIFANMNIQNFTMSGTHMVHMLCPSQVSPFLHVDFTDNLLTDMVFKDCRNLVRLKTLSLQKNQLKNLENIILTSAKMTSLQKLDISQNSLRYSDGGIPCAWTQSLLVLNLSSNMLTGSVFRCLPPK
rhesus        TSLKALSVHQVVSDVFNFPQRDIYEIFSNMNIKNFTVSGTRMIHMVCPSKISPFLHLDFSNNLLTDTVFENCGHLTELETLILQMNQLKELSKIAEMTTRMKSLQQLDISQNSVSYDEKKGDCSWTKSLLSLNMSSNILTDTIFKCLPPR
dog           TSLKALSIHQVVSNAFNLPQSYIYKIFSNMNIQNFTVSGTHMVHMVCPSQISPFLHLDFSNNLLTDIVFKNCRNLIKLETLSLQMNQLKELASIAQMTNEMKSLQQLDISQNSLRYDENEGNCSWTRSLLSLNMSSNILTDSVFRCLPPK
rat           TSLRALSIHQVVTDVFSFPQSNIYSIFSNMNIQSFTVSGTRMVHMLCPDQISPFLYLDFTDNLLTDIVFEDCRNLIRLKTLSLQKNQLKTLENIILMSMEMTSLQKLDISQNSLRYSDAGSPCSWTQSLLVLNLSSNMLTDSVFRCLPPK
                                                                                                                                                                         600
human         IKVLDLHSNKIKSIPKQVVKLEALQELNVAFNSLTDLPGCGSFSSLSVLIIDHNSVSHPSADFFQSCQKMRSIKAGDNPFQCTCELGEFVKNIDQVSSEVLEGWPDSYKCDYPESYRGTLLKDFHMSELSCNITLLIVTIVATMLVLAVT
treeshrew     IKILDLHKNRIQSIPKGVLQLESLQELNIAFNSLADLPGCGTFSSLSVLILDHNVVSHPSADFFQSCQKIRSLKAGNNPFQCTCELREFIKNIGQVPRGVVEDWPDSYKCDYPESYKGTPLKDFHPSQLSCNTALLIVTVGATMLLLTVT
mouse         VKVLDLHNNRIMSIPKDVTHLQALQELNVASNSLTDLPGCGAFSSLSVLVIDHNSVSHPSEDFFQSCQNIRSLTAGNNPFQCTCELRDFVKNIGWVAREVVEGWPDSYRCDYPESSRGTALRDFHMSPLSCDTVLLTVTIGATMLVLAVT
rhesus        IKVLDLHSNKIKSIPKQVIKLEALQELNVAFNSLTDLPGCGSFSSLSVLIIDHNSVSHPSADFFQSCQKMRSIKAGNNPFQCTCELREFIKNIEQVSSEVVEGWPDSYKCDYPESYRGTPLKDFHMSELSCNITLLIVTIGATMLVLAVT
dog           VKVLDLHDNRIRSIPKPIMKLEDLQELNVASNSLAHFPDCGTFNRLSVLIIDSNSISNPSADFLQSCHNIRSISAGNNPFQCTCELREFVQSLGQVASKVVEGWPDSYKCDSPENYKGTLLKDFHVSPLSCNTTLLLVTIGVAVLVFTVT
rat           VKVLDLHNNRIVSISKDVTHLQALQELNVASNFLTDLPGCGAFSSLSVLVIDHNSVSHPSSDFFQSCQNIRSITAGNNPFRCTCELREFVKNIGQASREVVEGWPDSYRCDYPDSIKGTPLQDFHMSPLSCDTILLTVTIGATLLLLAAI
                                                                                                                                                                         750
human         VTSLCSYLDLPWYLRMVCQWTQTRRRARNIPLEELQRNLQFHAFISYSGHDSFWVKNELLPNLEKEGMQICLHERNFVPGKSIVENIITCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEGSNSLILILLEPIPQYSIPSSYHKL
treeshrew     MTLLCIYLDLPWYLRMVFQWTQTRRRARNLPLEELQRTLQFHAFISYSGHDSAWVKNELVPNLEKEDVRICLHERNFVPGKSIVENIVNCIEKSYKSIFVLTPNFVQSEWCHYELYFAHHNLFHAGSDNLILILLEPIPQYSIPSSYHKL
mouse         GAFLCLYFDLPWYVRMLCQWTQTRHRARHIPLEELQRNLQFHAFVSYSGHDSAWVKNELLPNLEKDDIQICLHERNFVPGKSIVENIINFIEKSYKSIFVLSPHFIQSEWCHYELYFAHHNLFHEGSDNLILILLAPIPQYSIPTNYHKL
rhesus        VTFLCIYLDLPWYLRMVCQWTQTRRRARNVPLEELQRNLQFHAFISYSGHDSFWVKNELLPNLEKEGMQICLHERNFVPGKSIVENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEGSNNLILILLEPIPQYSIPSSYHKL
dog           VTALCIYFDLPWYLRMVFQWTQTRRRARNTPLENLQRTIQFHAFISYSGHDSAWVKSELLPNLEKEELRICLHERNFIPGKSIVENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEGSNNLILILLEPIPQYSIPSSYHKL
rat           GASLCLYFDLPWYLRMLWQWTQTRHRARNIPLEELQRNLQFHAFVSYSGHDSAWVKNELLPNLEKDDIRVCLHERNFVPGKSIVENIIHFIEKSYKSIFVLSPHFIQSEWCHYELYFAHHNLFHEGSDNLILILLEPIPQYSIPTNYHKL

human         KSLMARRTYLEWPKEKSKRGLFWANLRAAINIKLTEQAKK------------
treeshrew     KSLMARRTXLEWPKEKSKRGLFWVNLRAAINIKLMERATEVSHTSNYSHPSS
mouse         KTLMSRRTYLEWPTEKNKHGLFWANLRASINVKLVNQAEGTCYTQQ------
rhesus        KNLMARRTYLEWPKEKSKHGLFWANLRAAINIKLTEQAKK------------
dog           KNLMAQRTYLEWPKEKSKHGLFWANLRASINIKLREQAKK------------
rat           KTLMARRTYLEWPTEKSKHGLFWANLRASINVKLVNQAEATCYTQQ------
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TLR2

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                1                                                                                                                                                    150
treeshrew       MPHAWWTVWTLGAAISLFREGARGQV-TLSCDASGVCDGRSRSLRSIPSGLTAAVRSLDLSDNNITHVGDRDLQRCVNLKSLVLRSSGINTIDEDAFSPLVSLELLDLSYNHLLKLSSSWFRSLTSLRFLYLLGNPYKTLGETSLFSHLT
rhesus          MPHTLWMVWVLGVIISLSKEESSNQA-SLSCDHNGICKGSSGSLNSIPSVLTEAVKCLDLSNNRITYISNSDLQRYVNLQALVLTSNGINTIEEDSFSSLGRLEHLDLSYNYLSNLSSSWFKPLSSLKFLNLLGNPYKTLGETSLFSHLT
rat             MLQALWLFWILMAVIGLSREGHSAQA-SLSCDAAGVCDGSSRSFTSIPSGLTANTKKLDLSFNKITYIGHGDLRACVNLRVLTLESSGINTIEGDAFYSLGSLEHLDLSNNHLSSLSSSWFRPLSSLKYLNLMGNPYRTLGETSLFSNLT
mouse           MLRALWLFWILVAITVLFSKRCSAQE-SLSCDASGVCDGRSRSFTSIPSGLTAAMKSLDLSFNKITYIGHGDLRACANLQVLMLKSSRINTIEGDAFYSLGSLEHLDLSDNHLSSLSSSWFGPLSSLKYLNLMGNPYQTLGVTSLFPNLT
dog             MSRVLWTLWVLGAVTNLSKEEAPDQSSSLSCDPTGVCDGRSRSLNSMPSGLTAAVRSLDLSNNEITYIGNSDLRDCVNLKALRLESNGINTIEEESFFSLWSLEHLDLSYNLLSNLSSSWFRPLSSLKFLNLLGNPYKSLGETPLFSQLT
human           MPHTLWMVWVLGVIISLSKEESSNQA-SLSCDRNGICKGSSGSLNSIPSGLTEAVKSLDLSNNRITYISNSDLQRCVNLQALVLTSNGINTIEEDSFSSLGSLEHLDLSYNYLSNLSSSWFKPLSSLTFLNLLGNPYKTLGETSLFSHLT
                                                                                                                                                                     300
treeshrew       RLQILSVGNSYTFTELQRKDFAGLTSLKELEIDASSLQSYEPRSLRSIENISHLILRMRRPLSLLEIFDDLLSSVEHLELRDTYLDTFRFSKLFIREKNPLLKKLTFRNVEITDDSFSELAKLLFYASRLSDVEFDDCTLNGVGDFSVSA
rhesus          KLRILRVGNMDTFTKIQRKDFAGLTFLEELEIDASDLQSYEPKSLKSIQNVSHLILHMKQHILLLEIFVDLTSSVECLELRDTDLNTFHFSELSTGETNSLIKKFTFRNVKITDESLFQVMKLLSQISGLLELEFDDCTLNGVGDFRGSD
rat             NLQNLRVGNVDTFSEIRRIDFAGLTSLNELEIQVLSLGNYESRSLQSIRDIYHLTLHLSESAFLLGIFADILSSVRYLELRDTNLARFQFSELSVDEINSPMKKLAFRNADLTDKSFNELLKLLRYILELMEVEFDHCTLNGVGNFNPSE
mouse           NLQTLRIGNVETFSEIRRIDFAGLTSLNELEIKALSLRNYQSQSLKSIRDIHHLTLHLSESAFLLEIFADILSSVRYLELRDTNLARFQFSPLPVDEVSSPMKKLAFRGSVLTDESFNELLKLLRYILELSEVEFDDCTLNGLGDFNPSE
dog             NLRILKVGNIYSFTEIQDKDFAGLTFLEELEIDASNLQRYEPKSLKSIQNISYLALRMKQPVLLVEIFVDLSSSLKHLELRDTHLDTFHFSEASINETHTLVKKWTFRNVKVTDRSFTEVVRLLNYVSGVLEVEFEDCTLYGLGDFDIPD
human           KLQILRVGNMDTFTKIQRKDFAGLTFLEELEIDASDLQSYEPKSLKSIQNVSHLILHMKQHILLLEIFVDVTSSVECLELRDTDLDTFHFSELSTGETNSLIKKFTFRNVKITDESLFQVMKLLNQISGLLELEFDDCTLNGVGNFRASD
                                                                                                                                                                     450
treeshrew       LSKVKDSGKIETLTVRRLHIPKFYLFYDLSSIYSLTENVKRITIENSKVFLVPCSLSRCLTSLEYLDLSENLMVEEYLENSACEEAWPSLQTLILRQNHLTLLEKTGEVLLTLKNLTSLDVSKNSFHSMPETCRWPEKLERLNLSSTRLR
rhesus          NDRVIDPGKVETLTIRRLHIPQFYSFNDLSTLYPLTERVKRITVENSKVFLVPCLLSRHLKSLEYLDLSENLMVEEYLKNSACEDAWPSLQTLILRQNHLASLGKTGETLLTLKNLTNLDISKNTFHYMPETCQWPEKMKYLNLSSTRIH
rat             SDVVRELGKVETVTIRSLHIPQFYLFYDLSTVYSLLEKVKRITVENSKVFLVPCSFSQHLKSLEFLDLSENLMVEEYLKNSACEGGWPSLQSLVLSQNHLRSIRKTAEILLTLKNLTALDISKNSFQPMPDSCQWPGKMRFLNLSSTGIQ
mouse           SDVVSELGKVETVTIRRLHIPQFYLFYDLSTVYSLLEKVKRITVENSKVFLVPCSFSQHLKSLEFLDLSENLMVEEYLKNSACKGAWPSLQTLVLSQNHLRSMQKTGEILLTLKNLTSLDISRNTFHPMPDSCQWPEKMRFLNLSSTGIR
dog             VDKIKNIGQIETLTVRRLHIPHFYSFYDMSSIYSLTEDVKRITVESSKVFLVPCSLSQHLKSLEYLDLSDNLMVEEYLRNSACQHAWPLLQTLILRQNRLKSLEKTGETLLTLKNLVNLDISKNNYLSMPETCQWPEKLKCLNLSDTRMQ
human           NDRVIDPGKVETLTIRRLHIPRFYLFYDLSTLYSLTERVKRITVENSKVFLVPCLLSQHLKSLEYLDLSENLMVEEYLKNSACEDAWPSLQTLILRQNHLASLEKTGETLLTLKNLTNIDISKNSFHSMPETCQWPEKMKYLNLSSTRIH
                                                                                                                                                                     600
treeshrew       GVTPCLPRTLAVLDLSDNELTAFSLLLPQLRELYISKNKLKMLPAASAFPSLLVMKVSRNTITAFSKEQLDSFRQLQTLEAGDNSFICSCDFLAFMQAQPAPTQALGGWPNTYVCDSPSHLRGQLVRDARLSPSECHKVALVSGVCCALC
rhesus          SVTGCIPKTLEILDISNNNLNLFSLNLPQLKELYISRNKLMTLPDASLLPMLLVLKISRNTITTFSKEQLDSFHTLKTLEAGGNNFICSCEFLSFTQEQQALAKVLADWPANYLCDSPSHVRGQRVQDVRLSVSECHRAALVSGMCCALF
rat             AVKTCIPQTLEVLDVSNNNLDSFSLFLPRLQELYISRNKLKTLPEASLFPVLQVMKIRENAISTFSKDQLGSFPKLETLEAGDNHFICSCELLSFILERPALVHVLVDWPDSYLCDSPPRLHGQRLQDARPSVLECHQAALVSGVCCALL
mouse           VVKTCIPQTLEVLDVSNNNLDSFSLFLPRLQELYISRNKLKTLPDASLFPVLLVMKIRENAVSTFSKDQLGSFPKLETLEAGDNHFVCSCELLSFTMETPALAQILVDWPDSYLCDSPPRLHGHRLQDARPSVLECHQAALVSGVCCALL
dog             SITRCIPQTLEILDVSNNNLESFSLILPQLKELSISRNKLKTLPDASFLPTLQIMRISRNTINAFSKEQLDSFHRLQTLEAGGNNFLCSCEFLSFTQEQQALAGLLVGWPEDYLCHSPSYVRGQRVGTARLPASECHRTALVAAVCCVLL
human           SVTGCIPKTLEILDVSNNNLNLFSLNLPQLKELYISRNKLMTLPDASLLPMLLVLKISRNAITTFSKEQLDSFHTLKTLEAGGNNFICSCEFLSFTQEQQALAKVLIDWPANYLCDSPSHVRGQQVQDVRLSVSECHRTALVSGMCCALF
                                                                                                                                                                     750
treeshrew       LLLLLTVGLCHRFHGLWYLRMTWAWLQAKRKPRRAPARPICYDAFVSYSERDAGWVEDLLVRELERGDAPLRLCLHKRDFVPGKWIIDNIIDSIERSRKTVFVLSENFVRSEWCKYELDFSHFRLFDENDDAAVLVLLEPLEKKAIPQRF
rhesus          LLILLMGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPNRDICYDAFVSYSERDAYWVENLMVQELENFNPPFKLCLHKRDFIPGKWIIDNIIDSIEKSHKTVFVLSENFVKSEWCKYELDFSHFRLFDENNDAAILVLLEPIEKKAIPQRF
rat             LLILLLGALCYHFHGLWYLRMMWAWLRAKRKPKKAPCRDLCYDAFVSYSEQDSYWVENLMVQQLENSDPPFKLCLHKRDFVPGKWIIDNIIDSIEKSHKTVFVLSENFVRSEWCKYELDFSHFRLFDENNDAAILVLLEPIEKKAIPQRF
mouse           LLILLVGALCHHFHGLWYLRMMWAWLQAKRKPKKAPCRDVCYDAFVSYSEQDSHWVENLMVQQLENSDPPFKLCLHKRDFVPGKWIIDNIIDSIEKSHKTVFVLSENFVRSEWCKYELDFSHFRLFDENNDAAILVLLEPIERKAIPQRF
dog             LLVLLTAGACHHFHGLWYLRMLWAWLQAKRKPRKAPSRDVCYDAFVSYSEHDSYWVENLLVQKLEHFNPPFKLCLHKRDFIPGKWIIDNIIDSIEKSHKTIFVLSENFVKSEWCKYELDFSHFRLFDENNDAAILILLEPIEKKAIPQRF
human           LLILLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPSRNICYDAFVSYSERDAYWVENLMVQELENFNPPFKLCLHKRDFIPGKWIIDNIIDSIEKSHKTVFVLSENFVKSEWCKYELDFSHFRLFDENNDAAILILLEPIEKKAIPQRF

treeshrew       CRLRRVMNTRTYLEWPAAEAEQPAFWASLRATLQG
rhesus          CKLRKIMNTKTYLEWPMDEARQEGFWVNLRAAIKS
rat             CKLRKIMNTKTYLEWPLDEGQREVFWANLRTAIKS
mouse           CKLRKIMNTKTYLEWPLDEGQQEVFWVNLRTAIKS
dog             CKLRKIMNTKTYLEWPTDDAQQEGFWLNLRTAIKS
human           CKLRKIMNTKTYLEWPMDEAQREGFWVNLRAAIKS
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TLR3        1                                                                                                                                                  150
rat         MKGRSSYLIYSFGGLLSLWILVVSSTNQCTVRYNVADCSHLKLTHIPDDLPSNITVLNLTHNQLRGLPPANFTRYSQLALLDAGFNSISKLEPELCQILPLLKVLNLQHNELSQISDQTFAFCTNLTELHLMSNSIRKIKSNPFKNQKSL
dog         MSQSLLYHIYSFLGLLPFWILCTSSTNKCVVRHEVADCSHLKLTQVPDDLPANITVLNLTHNQLRRLPPANFTRYSQLTILDGGFNSISKLEPELCQKLPLLEILNLQHNELSHLSDQTFVFCVNLTELHLMSNSIKIIQNNPFRSLKNL
human       MRQTLPC-IYFWGGLLPFGMLCASSTTKCTVSHEVADCSHLKLTQVPDDLPTNITVLNLTHNQLRRLPAANFTRYSQLTSLDVGFNTISKLEPELCQKLPMLKVLNLQHNELSQLSDKTFAFCTNLTELHLMSNSIQKIKNNPFVKQKNL
treeshrew   MRQSLPSYIYSFMGLLSFCILCASSTNKCVVRREVADCSHLKLTQVPNDLPTNITVLNLTHNQLRRLPSANLTRYNRLTVLDGGFNSISKLEPELCQKLPLLQVLDLRHNELSQLSDKTFSFCTNLTELNLMSNPIQKIQNNPFKNQKNL
mouse       MKGCSSYLMYSFGGLLSLWILLVSSTNQCTVRYNVADCSHLKLTHIPDDLPSNITVLNLTHNQLRRLPPTNFTRYSQLAILDAGFNSISKLEPELCQILPLLKVLNLQHNELSQISDQTFVFCTNLTELDLMSNSIHKIKSNPFKNQKNL
rhesus      MRQTLPY-TYFWWGLLPFGMLCASSTNKCTVSQEVADCSHLKLTQVPDDLPTNITVLNLTHNQLRRLPAANFTRYSQLTILDVGFNSISKPEPELCQKLPMLKVLNLQHNELSQLSDKTFAFCMNLTELHSLSHSIQKIKNNPFVKQKNL
                                                                                                                                                               300
rat         IKLDLSRNGLSSTKLGTGVQLENLQELLLAKNKIFALRSEELDFLGNSSLQKLDLSSNPLKEFSPGCFHAIGKLFVLLLNNAQLNLNLTEKLCWELSNTSIQNLSLANNQLLATSNSTFSGLKQTNLTSLDLSYNSLRYVGNDAFSWLPH
dog         VKLDLSHNGLSSTKLGSQLQLENLQELLLSNNKINVLRREELDFLGNSSLEKLELSSNPIKEFSPGCFHAIGKLFGLSLNNVQLNPSLTENLCLELSNTSIQNLSLSNTQLHRTSNMTFLGLKHTNLTMLDLSHNNLNVIENNSFVWLPH
human       ITLDLSHNGLSSTKLGTQVQLENLQELLLSNNKIQALKSEELDIFANSSLKKLELSSNQIKEFSPGCFHAIGRLFGLFLNNVQLGPSLTEKLCLELANTSIRNLSLSNSQLSTTSNTTFLGLKWTNLTMLDLSYNNLNVVGNDSFAWLPQ
treeshrew   IKLDLSHNGLSSTKLGTQVQLENLQELRLSNNKISALRREELDFLGNSSLKILELSSNQIKEFSPGCFHAIGKLFGLFLNNAQLSSSLIEKLCLELSDTNIQSLSLSNNQLYRTSNMTFSGLKQTNLSMLDLSHNSLNVIGNNSFVWLPH
mouse       IKLDLSHNGLSSTKLGTGVQLENLQELLLAKNKILALRSEELEFLGNSSLRKLDLSSNPLKEFSPGCFQTIGKLFALLLNNAQLNPHLTEKLCWELSNTSIQNLSLANNQLLATSESTFSGLKWTNLTQLDLSYNNLHDVGNGSFSYLPS
rhesus      ITLDLSHNGLSSIKLGTQVQMENLQELLLSNNKIQALKSEELGILANSSLKKLELSSNQIKEFSPGCFHAIGRSLGLFLNNVQLGPRLTEKLCLELANTSVRNLSLSNSQLSTTSNTTFLGLKWTNLTMLDLSHNNLNVVGNDSFAWLPQ
                                                                                                                                                               450
rat         LKYLSLEYNNIQSLTPHSFRGLSNLRYLSLKRAFTKQSVALASHPNIDDFSFQWLKCLEHLNMDDNTIPGIKSNTFTGLVSLKYLSLSKTFTGLQTLTNETFVSLTHSPLLTLNLTKNHISKIASGTFSWLGQLRILDLGLNEIEQELTG
dog         LEYFLLEYNNIEHLFSHSFYGLLNVRYLDLKRSFAKQSTSLASHPRIDDFSFQWLKCLQYLNMEDNYFAGIKSNMFTGLIKLKHLSLSNSFTSLQTLTNETFLSLAQSPLITLNLTKNKISKIESGAFSWLGHLQVLDLGLNEIGQELTG
human       LEYFFLEYNNIQHLFSHSLHGLFNVRYLNLKRSFTKQSISLASLPKIDDFSFQWLKCLEHLNMEDNDIPGIKSNMFTGLINLKYLSLSNSFTSLRTLTNETFVSLAHSPLHILNLTKNKISKIESDAFSWLGHLEVLDLGLNEIGQELTG
treeshrew   LEYFSLEYNNIEHLSSHSFYGLVNVRNLNLKRSFTKQSISLTSFPKIDDFSFQWLKCLEYLNMEDNNMAGIRPNMFTGLTNLKYLSLSNAFTSLRTLTNETFSSLAHSPLLTLNLTKNKILKIESGAFSWLGHLKVLDLGLNEIGQELTG
mouse       LRYLSLEYNNIQRLSPRSFYGLSNLRYLSLKRAFTKQSVSLASHPNIDDFSFQWLKYLEYLNMDDNNIPSTKSNTFTGLVSLKYLSLSKTFTSLQTLTNETFVSLAHSPLLTLNLTKNHISKIANGTFSWLGQLRILDLGLNEIEQKLSG
rhesus      LEYFFLEYNNIQHLLSHSLHGLFNVRYLNLKRSFTKQSISLASLPKIDDFSFRWLTCLEHLNMEDNDISGIKSNMFTGLINLKYLSLSNSFTSLQTLTNETFVSLAHSPLHILNLTKNKISKIESGAFSWLGHLEVLDLGLNEIGQELTG
                                                                                                                                                               600
rat         QEWRGLGNIFEIYLSYNKYLQLTSKSFTLVPSLQRLMLRRVALKSVDISPSPFRPLYNLTILDLSNNNIANLNEDLLEGLENLEILDFQHNNLARLWKHANPGGPVNFLKGLSHLHILNLESNGLDEIPVKVFKNLFELKSINLGLNNLN
dog         QEWRGLENIVEIYLSYNKYLQLTSSSFALIPSLRRLMLRRTALRNVDSSPSPFHPLRNLNILDLSNNNIANINDELLEGLEKLEILDMQHNNLARLWKHANPGGPVHFLKGLSHLHILNLESNGFDEIPAEVFKGLSELKSIDLGLNNLN
human       QEWRGLENIFEIYLSYNKYLQLTRNSFALVPSLQRLMLRRVALKNVDSSPSPFQPLRNLTILDLSNNNIANINDDMLEGLEKLEILDLQHNNLARLWKHANPGGPIYFLKGLSHLHILNLESNGFDEIPVEVFKDLFELKIIDLGLNNLN
treeshrew   QEWRGLENIFEIYLSYNKYVELTATSFASISSLQRLMLRRVTLKNVASSPSPFHPLRNLTILDLSNNNIANINDELLEGLERLEVLDLQHNNLARLWKHANPGGPVHFLKGLYHLHVLNLESNGFDEIPAEAFKDLPELQRLRLGLNNLN
mouse       QEWRGLRNIFEIYLSYNKYLQLSTSSFALVPSLQRLMLRRVALKNVDISPSPFRPLRNLTILDLSNNNIANINEDLLEGLENLEILDFQHNNLARLWKRANPGGPVNFLKGLSHLHILNLESNGLDEIPVGVFKNLFELKSINLGLNNLN
rhesus      QEWSGLENIFEIYLSYNKYLQLTKNSFALVRSLQRLMLRRVALKNVDCSPSPFQPLGNLTILDLSNNNIANINDDMLEGLEKLEILDLQHNNLARLWKHANPGGPVYFLKGLSHLHILNLESNGFDEIPVEVFKDLSELKIIDLGLNNLN
                                                                                                                                                               750
rat         TLLPSIFDDQTSLRSLNLQKNLITSVEKSVFGPAFHNLNSLDMSFNPFDCTCESIAWFVTWLNQTHTNIPELSTHYLCNTPQRYHGLPVKLFDTSSCKDSAPFQLLFIINTSTLLTFILAVLLIHFEGWRISFYWNVSVHRILGFKEIDA
dog         IFPSSLFNDQVSLKSLNLQKNLITSVEKNVFGPAFRNLSNLDMSFNPFDCTCESIAWFVNWINSTHTNISELSSHYLCNTPPQYHGFPVMLFDISPCKDSAPFEIFFIINTSVLLTFIFIVLLIHFEGWRISFYWNVSVHRILGFKEIDK
human       TLPASVFNNQVSLKSLNLQKNLITSVEKKVFGPAFRNLTELDMRFNPFDCTCESIAWFVNWINETHTNIPELSSHYLCNTPPHYHGFPVRLFDTSSCKDSAPFELFFMINTSILLIFIFIVLLIHFEGWRISFYWNVSVHRVLGFKEIDR
treeshrew   ILPPSVFDHQVSLTSLSLQKNLITSVEKNVFWSAFKNLRDLDMSSNPFDCTCESIAWFVSWINKTHTNIPDLSSHYLCNTPRQYHGFPAMLFDISPCKDSAPFELLFMISTSMLSIFIFVILLIHFEGWRISFYWNVSVHRVLGFKEIDR
mouse       KLEPFIFDDQTSLRSLNLQKNLITSVEKDVFGPPFQNLNSLDMRFNPFDCTCESISWFVNWINQTHTNISELSTHYLCNTPHHYYGFPLKLFDTSSCKDSAPFELLFIISTSMLLVFILVVLLIHIEGWRISFYWNVSVHRILGFKEIDT
rhesus      TLPASVFDNQVSLKSLNLQKNLITSVEKKVFGPAFRNLSNLDMRFNPFDCTCESIAWFVNWISKTHANIPELSSHYLCNTPPHYHGFPVRLFDTSSCKDSAPFELFFIINTSILLICIFVVLLIHFEGWRISFYWNVSVHRVLGFKEIDR
                                                                                                                                                               900
rat         QGEQFEYTAYIIHAQKDRDWVWEHFSPMEEQDQSLKFCLEERDFEAGVLGLEAIVNSIKRSRKIIFVITHHLLKDPLCRRFKVHHAVQQAIEQNLDSIILIFLQNIPDYKLNHALCLRRGMFKSHCILNWPIQKERINAFHHKLQVALGS
dog         QPEQFEYAAYIIHAYKDRDWVWEHFSPMEEKDETLKFCLEERDFEAGVLELESIINSIKKSRKTIFVITQHLLKDPLCKRFKVHQAVQQAIEQNLESIILIFLEEIPDYKLNHALCLRRGMFKSHCILNWPVQKERVNAFHHKLQVALGS
human       QTEQFEYAAYIIHAYKDKDWVWEHFSSMEKEDQSLKFCLEERDFEAGVFELEAIVNSIKRSRKIIFVITHHLLKDPLCKRFKVHHAVQQAIEQNLDSIILVFLEEIPDYKLNHALCLRRGMFKSHCILNWPVQKERIGAFRHKLQVALGS
treeshrew   QPEQFEFAAYIIHAHKDRDWVWEHFSPMEEKDRTLKFCLEERDFEAGVLELEAIVNSIKRSRKIIFVITQHLLRDPLCKRFKVHHAVQQAIEQNLDSIILIFLEEIPDHKLNHALCLRRGMFKSHCILNWPVQKERINAFHHKLQVALGS
mouse       QAEQFEYTAYIIHAHKDRDWVWEHFSPMEEQDQSLKFCLEERDFEAGVLGLEAIVNSIKRSRKIIFVITHHLLKDPLCRRFKVHHAVQQAIEQNLDSIILIFLQNIPDYKLNHALCLRRGMFKSHCILNWPVQKERINAFHHKLQVALGS
rhesus      QTEQFEYAAYIIHAHKDKDWVWEHFSSMEKEDQSLKFCLEERDFEAGVFELEAIVNSIKRSRKIIFIITHHLLKDPLCKRFKVHHAVQQAIEQNLDSIILIFLEEIPDYKLNHALCLRRGMFKSHCILNWPVQKERIGAFHHKLQVALGS

rat         RNSAH
dog         RNSIH
human       KNSVH
treeshrew   RNSVH
mouse       RNSAH
rhesus      KNSVH
```

```
TLR4       1                                                                                                                                                    150
mouse      MMPPWLLARTLIMAL-FFSCLTPGSLNPCIEVVPNITYQCMDQKLSKVPDDIPSSTKNIDLSFNPLKILKSYSFSNFSELQWLDLSRCEIETIEDKAWHGLHHLSNLILTGNPIQSFSPGSFSGLTSLENLVAVETKLASLESFPIGQLI
rat        MMPLLHLAGTLIMAL-FLSCLRPGSLNPCIEVLPNITYQCMDQNLSKIPHDIPYSTKNLDLSFNPLKILRSYSFSNFSQLQWLDLSRCEIETIEDKAWHGLNQLSTLVLTGNPIKSFSPGSFSGLTNLENLVAVETKMTSLEGFHIGQLI
dog        MMSPTRLVGILIPAMAFLSCLRPESWDPCMQVVANTTYQCMELNLSKIPNNIPTSTEKLDLSFNPLRHLGSHCFSNFPKLQVLDLSRCEIQVIEDDAYQGLNHLSILILTGNPIQRLFPRAFSGLSSLKTLVAKETKLTSLEDFPIGHLK
human      MMSASRLAGTLIPAMAFLSCVRPESWEPCVEVVPNITYQCMELNFYKIPDNLPFSTKNLDLSFNPLRHLGSYSFFSFPELQVLDLSRCEIQTIEDGAYQSLSHLSTLILTGNPIQSLALGAFSGLSSLQKLVAVETNLASLENFPIGHLK
rhesus     MTSALRLAGTLIPAMAFLSCVRPESWEPCVEVVPNITYQCMELKFYKIPDNIPFSTKNLDLSFNPLRHLGSYSFLRFPELQVLDLSRCEIQTIEDGAYQSLSHLSTLILTGNPIQSLALGAFSGLSSLQKLVAVETNLASLENFPIGHLK
treeshrew  MMPPXRLXGTLIPAMAFLSCLKPESWEPCVXVVPNITYQCMEVNLYKIPDNIPSSTENLDLSFNPLRYLGNRNFSKFPELQVLDLSRCDIQAIEDDAYWGLNHLSTLILTGNPIQHLGLGAFSGLSNLQKLVAVETNLDSLENFPIGHLK
                                                                                                                                                                300
mouse      TLKKLNVAHNFIHSCKLPAYFSNLTNLVHVDLSYNYIQTITVNDLQFLRENPQVNLSLDMSLNPIDFIQDQAFQGIKLHELTLRGNFNSSNIMKTCLQNLAGLHVHRLILGEFKDERNLEIFEPSIMEGLCDVTIDEFRLTYTNDFSDDI
rat        SLKKLNVAHNLIHSFKLPEYFSNLTNLEHVDLSYNYIQTISVKDLQFLRENPQVNLSLDLSLNPIDSIQAQAFQGIRLHELTLRSNFNSSNVLKMCLQNMTGLHVHRLILGEFKNERNLESFDRSVMEGLCNVSIDEFRLTYINHFSDDI
dog        TLKELNVAHNLIHSFKLPAYFSNMPNLENVDLSNNKIQNIYREDLQVLHHMPLLNLSLDLSLNPLYFIQPGSFKEIKLHKLTLRSNFNSTDVMKTFIQGLAGLKINQLVLGEFKNERKLESFDNSLLEGLCNLTIEKFRIAYFDSFSKDT
human      TLKELNVAHNLIQSFKLPEYFSNLTNLEHLDLSSNKIQSIYCTDLRVLHQMPLLNLSLDLSLNPMNFIQPGAFKEIRLHKLTLRNNFDSLNVMKTCIQGLAGLEVHRLVLGEFRNEGNLEKFDKSALEGLCNLTIEEFRLAYLDYYLDDI
rhesus     TLKELNVAHNLIQSFKLPEYFSNLTNLEHLDLSSNKIQNIYCKDLQVLHQMPLSNLSLDLSLNPINFIQPGAFKEIRLHKLTLRSNFDDLNVMKTCIQGLAGLEVHRLVLGEFRNERNLEEFDKSSLEGLCNLTIEEFRLTYLDYYLDNI
treeshrew  TLKELNVAHNLIHSFKIPGYFSNLPNLEYLDLSNNKIRNIFHEDVQVLHQMPLLNLSLEISLNPIDFIQPSAFNGIRLYGLTLRNNFNSTNIMKTCIQGLAGLEVHQLVLGEFRNERNIENFNKSSLEGLCNLTIGEFHLAFLDDSPDNT
                                                                                                                                                                450
mouse      VK-FHCLANVSAMSLAGVSIKYLEDVPKHFKWQSLSIIRCQLKQFPTLDLPFLKSLTLTMNKGSISFKKVALPSLSYLDLSRNALSFSGCCSYSDLGTNSLRHLDLSFNGAIIMSANFMGLEELQHLDFQHSTLKRVTEFSAFLSLEKLL
rat        YN-LNCLANISAMSFTGVHIKHIADVPRHFKWQSLSIIRCHLKPFPKLSLPFLKSWTLTTNREDISFGQLALPSLRYLDLSRNAMSFRGCCSYSDFGTNNLKYLDLSFNGVILMSANFMGLEELEYLDFQHSTLKKVTEFSVFLSLEKLL
dog        TNLFNQLVNISAISLAHLYLDTPKYLPKNLRWQRLEIVNCNLEQFPAWELDSLKEFVLTSNKGMNTFADMKMESLEFLDLSRNRLSFKTCCSHSDFGTTRLKHLDLSFNEIITMSSNFLGLEQLEYLDLQHSSLKQASDFSVFLSLRNLR
human      IDLFNCLTNVSSFSLVSVTIERVKDFSYNFGWQHLELVNCKFGQFPTLKLKSLKRLTFTSNKGGNAFSEVDLPSLEFLDLSRNGLSFKGCCSQSDFGTTSLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSEFSVFLSLRNLI
rhesus     IDLFNCLANVSSFSLVSVSIKRVEDFSYNFRWQHLELVNCKFEQFPTLELESLKRLTFTANKGGNAFSEVDLPSLEFLDLSRNGLSFKGCCSQSDFGTTSLKYLDLSFNDVITMSSNFLGLEKLEHLDFQHSNLKQMSQFSVFLSLRNLI
treeshrew  IDLFNCLANVSAISLVSLYLNNLEGLPRQVRWQSLELIHCNYKHFPSLAISSLKRFVFTANKGGDTFTEVVLPSLEYLDLSGNGLSFKSCCDHTDLGTSKLKYLDMSFNGVIIMSSNFMGLERLEYLDFQHSTLKHVNDFPVFLSLKNLL
                                                                                                                                                                600
mouse      YLDISYTNTKIDFDGIFLGLTSLNTLKMAGNSFKDNTLSNVFANTTNLTFLDLSKCQLEQISWGVFDTLHRLQLLNMSHNNLLFLDSSHYNQLYSLSTLDCSFNRIETSKGI-LQHFPKSLAFFNLTNNSVACICEHQKFLQWVKEQKQF
rat        YLDISYTNTKIDFDGIFLGLISLNTLKMAGNSFKDNTLSNVFTNTTNLTFLDLSKCQLEQISRGVFDTLYRLQLLNMSHNNLLFLDPSHYKQLYSLRTLDCSFNRIETSKGI-LQHFPKSLAVFNLTNNSVACICEYQNFLQWVKDQKMF
dog        YLDISYTRTEVAFQGIFDGLVSLEVLKMADNSFPDNSLPNIFKGLTNLTILDLSRCHLERVSQESFVSLPKLQEINMSHNSLLSLDTLAYEPLLSLQILDCSFNRIVAFKEQGQQHFPSNLVSLNLTRNNFACDCEHQSFLQWVKDHRQL
human      YLDISHTHTRVAFNGIFNGLSSLEVLKMAGNSFQENFLPDIFTELRNLTFLDLSQCQLEQLSPTAFNSLSSLQVLNMSHNNFFSLDTFPYKCLNSLQVLDYSLNHIMTSKKQELQHFPSSLAFLNLTQNDFACTCEHQSFLQWIKDQRQL
rhesus     YLDISHTHTRVAFNGIFDGLLSLKVLKMAGNSFQENFLPDIFTDLKNLTFLDLSQCQLEQLSPTAFDTLNKLQVLNMSHNNFFSLDTFPYKCLPSLQVLDYSLNHIMTSNNQELQHFPSSLAFLNLTQNDFACTCEHQSFLQWIKDQRQL
treeshrew  YLDISYTHIRVVFLGIFDGLFSLRVLKMAGNSFLNNLLPNIFTNLTDLTFLDLTHCQLEGVSPMAFDSLLHLQSLNMSHNHLLVLDTAPYKHLQSLQVLDCSFNRIVASKGQELQHFPSKLTLLNLTQNEFACTCEHQGFLQWVKDQRRL
                                                                                                                                                                750
mouse      LVNVEQMTCATPVEMNTSLVLDFNNSTCYMYKTIISVSVVSVIVVSTVAFLIYHFYFHLILIAGCKKYSRGESIYDAFVIYSSQNEDWVRNELVKNLEEGVPRFHLCLHYRDFIPGVAIAANIIQEGFHKSRKVIVVVSRHFIQSRWCIF
rat        LVNVEQMKCASPIDMKASLVLDFTNSTCYIYKTIISVSVVSVLVVATVAFLIYHFYFHLILIAGCKKYSRGESIYDAFVIYSSQNEDWVRNELVKNLEEGVPRFQLCLHYRDFIPGVAIAANIIQEGFHKSRKVIVVVSRHFIQSRWCIF
dog        LVEVEQMVCAKPLDMKDMPLLSFRNATCQRSKTIISVSVFTVLMVSLVAVLAYKFYFHLMLLAGCKRYNRGESTYDAFVIYSSQDEDWVRNELVKNLEEGVPPFQLCLHYRDFIPGVAIAANIIQEGFYKSRKVIVVVSQHFIQSRWCIF
human      LVEVERMECATPSDKQGMPVLSL-NITCQMNKTIIGVSVLSVLVVSVVAVLVYKFYFHLMLLAGCIKYGRGENIYDAFVIYSSQDEDWVRNELVKNLEEGVPPFQLCLHYRDFIPGVAIAANIIHEGFHKSRKVIVVVSQHFIQSRWCIF
rhesus     LVEAERMECATPSDKQGMPVLSL-NITCQMNKTIIGVSVFSVLVVSVVAVLVYKFYFHLMLLAGCINYGRGENIYDAFVIYSSQDEDWVRNELVKNLEEGVPPFQLCLHYRDFIPGVAIAANIIHEGFHKSRKVIVVVSQHFIQSRWCIF
treeshrew  LVEAEQMICATPSDMQHMPVLSFRNATCQVSKTTISVSVLSVLVVSAVVVLVYKFYFHLMLLAGCKKYGRGESTYDAFVIYSSQDEDWVRNELVRNLEEGVPSFQLCLHYRDFIPGVAIAANIIQEGFHKSRKVIVVVSQHFIQSRWCIF

mouse      EYEIAQTWQFLSSRSGIIFIVLEKVEKSLLRQQVELYRLLSRNTYLEWEDNPLGRHIFWRRLKNALLDGKASNPEQTAEE----EQETATWT
rat        EYEIAQTWQFLSSRSGIIFIVLEKVEKSLLRQQVELYRLLSRNTYLEWEDNALGRHIFWRRLKKALLDGKALNPDETSEE----EQEATTLT
dog        EYEIAQTWQFLSSRAGIIFIVLQKVEKSLLRQQVELYRLLSRNTYLEWEDSVLGRHIFWRRLRKALLDGKPWSPEGTEDAPQNLQVDASTKS
human      EYEIAQTWQFLSSRAGIIFIVLQKVEKTLLRQQVELYRLLSRNTYLEWEDSVLGRHIFWRRLRKALLDGKSWNPEGTVGT--GCNWQEATSI
rhesus     EYEIAQTWQFLSSRAGIIFIVLQKVEKTLLRQQVELYRLLSRNTYLEWEDSVLGQHIFWRRLRKALLDGRSWNPE-TVGT--GCN-------
treeshrew  EYEIAQTWQFLSSRAGIIFIVLQKLEKSLLQQQVELYRLLSRNTYLEWEDNALGRHIFWRRLRKALLDGKPWSPEAAADA-ENCQQEATTAT
```

```
TLR5        1                                                                                                                                                    150
dog         --------------MGRQLGRALGLLLVAGAVAAASCCVADGRRALYRSCNLSQVPPVPS-TTEILLLSFNYIRAVTRASFPLLERLQLLELGTQQTPFSVDREAFRNLPNLRTLDLGNSRVDFLHPDAFQGLPHLQELRLFACGLSDVV
human       --------------MGDHLDLLLGVVLMAGPVFGIPSCSFDGRIAFYRFCNLTQVPQVLN-TTERLLLSFNYIRTVTASSFPFLEQLQLLELGSQYTPLTIDKEAFRNLPNLRILDLGSSKIYFLHPDAFQGLFHLFELRLYFCGLSDAV
mouse       MDAEFPHAPHFSRIMACQLDLLIGVIFMASPVLVISPCSSDGRIAFFRGCNLTQIPWILNTTTERLLLSFNYISMVVATSFPLLERLQLLELGTQYANLTIGPGAFRNLPNLRILDLGQSQIEVLNRDAFQGLPHLLELRLFSCGLSSAV
treeshrew   --------------MGNHLDXLLGMLLMASPVFGIPSCSSDGQIALYRFCNLTEIPQVLN-STERLLLSFNYIRTVTTTSFPFLEQLWLLELGTQFTPLTINKEAFRHLPNLRILDLGKSQIDFLHPDAFQGLSHLFELRLFFCGLSDAV
rhesus      --------------MGDHLDLLLGVVLVASPVFGFPSCSFDGRIAFYRFCNLTQVPQVLN-TTERLLLSFNYIRTVTVSSFPFLEQLQLLELGNQYTPLTIDKEAFRNLPNLRILDLGSSQIYFLHPDAFQGLFHLFELRLYFCGLSDAV
rat         MWCFYSLFSH--RIMAYQLDLLIGVVFMASPVLEMSPCFSDGRIALFRGCNLTQIPWVLN-TTERLLLSFNYISTVVTTSFPLLEQLLLLELGTQYARLTIGQEAFRNLPNLRILDLGQSQIEVLNPDAFQGLPHLFELRLFDCGLSNAV
                                                                                                                                                                 300
dog         LTDGYFRNLGALLRLDLSKNQIGSLELHASFRELGSLRSVDFSLNRIPAACEQGLRPLQGKALSLLNLAANGLYSRAPVDWGRCGNPFRNVVLETLDVSNNGWTADVTGNVTRAIGGSQISSLVLAHHIMGQGFGFRNIRDPDRSTFAGL
human       LKDGYFRNLKALTRLDLSKNQIRSLYLHPSFGKLNSLKSIDFSSNQIFLVCEHELEPLQGKTLSFFSLAANSLYSRVSVDWGKCMNPFRNMVLEILDVSGNGWTVDITGNFSNAISKSQAFSLILAHHIMGAGFGFHNIKDPDQNTFAGL
mouse       LSDGYFRNLYSLARLDLSGNQIHSLRLHSSFRELNSLSDVNFAFNQIFTICEDELEPLQGKTLSFFGLKLTKLFSRVSVGWETCRNPFRGVRLETLDLSENGWTVDITRNFSNIIQGSQISSLILKHHIMGPGFGFQNIRDPDQSTFASL
treeshrew   LKDGYFRNLNSLTRLDLSKNKIRSLHLHPSFQELNSLQSIDFSLNQIVTVCESELKPLQGKMLSSLNLRANNLYSRVLVDWAKCMNPFRKMVLNTLDVSGNGWSVDITRNFSNAVNGSQISNLILAHHTMGAGFGFRNLKDPDQNTFAGL
rhesus      LKNGYFRNLKSLTRLDLSKNQIRSLYLHPSFGKLNSLKSIDFSSNQIFLVCEHELEPLQGKMLSFFSLAANNLYSRVSVDWGKCMNPFRNMVLETLDVSGNGWTVDITGNFSNAISKSQAFSLILAHHIMGAGFGFHNIKDPDQNTFAGL
rat         LRDAYFRNLNSLARLDLSANEIHSLHLHSSFQELSSLSDINFSFNRIFTLCEDELQPLQGRTLSFFGLKSTSLFSRVFVDWEACRNPLRGIRLETLDLSENGWTAAILGNFSHTIQGSHISSLILTYHIMGSGFGFQNIKDPDQSTFASL
                                                                                                                                                                 450
dog         AGSSVLRLDLSHGFVFSLNARLFEVLGDLKLLDLAHNKINRIAGEAFHGLGSVQVLNLSHNLLGELYDSDFSGLAEVAYIDLQHNHIGIIQDQTFRFLGALRTLDLRDNALKTVSFVPSIDTIFLGNNKLETVSHMDLTASFLELSDNRL
human       ARSSVRHLDLSHGFVFSLNSRVFETLKDLKVLNLAYNKINKIADEAFYGLDNLQVLNLSYNLLGELYSSNFYGLPKVAYIDLQKNHIAIIQDQTFKFLEKLQTLDLRDNALTTIHFIPSIPDIFLSGNKLVTLPKINLTANLIHLSENRL
mouse       ARSSVLQLDLSHGFIFSLNPRLFGTLKDLKMLNLAFNKINKIGENAFYGLDSLQVLNLSYNLLGELYNSNFYGLPRVAYVDLQRNHIGIIQDQTFRLLKTLQTLDLRDNALKAIGFIPSIQMVLLGGNKLVHLPHIHFTANFLELSENRL
treeshrew   ARSLVIHLDLSHGFIFSLNSQSFKTLKDLEVLNLAYNKINKIADGAFYGLNNLQVLNLSFNLLGELYNSNFYGLPNIVYIDLQSNHIGIIQDQTFRFLEKLNTLDLQDNALKTISFIPSIPDIFLGGNKLVTLPHISLTANFIQLSENRL
rhesus      ARSSVRHLDLSHGFIFSLNSRVFETLQDLQVLNLAYNKINKIAVEAFYGLDNLQVLNLSYNLLGELYSSNFYGLPKVAYIDLQKNHIGIIQDQTFKFLENLQTLDLRDNALTTIHFIPSIPDIFLSGNKLVTLSEINLTANFIHLSENRL
rat         ARSSVLQLDLSHGYIFSLNPRLFETLKDLKKLNLAFNKINKISDYAFHGLDSLQILNLSYNLLGELYNSNFYGLPSIAYLDLQRNHIGIIQDRTFRLLKKLQTLDLRDNALKTIGFIPSVQMVLLGSNKLTHLPHVRFTANFIELSENGL
                                                                                                                                                                 600
dog         EDLGDLYSLLRVPALQVLILNRNRLSACRGGH---GPTGSVGPERLFLGSNMLQLAWETGRCWDVFRGLPRLRVLHLNHNYLAALPPGLLRDLTALRGLDLSANRLSTLSRGDLPAALEVLDVSRNQLLSLDPGLLAPLRAVDLTHNKFI
human       ENLDILYFLLRVPHLQILILNQNRFSSCSGDQ---TPSENPSLEQLFLGENMLQLAWETELCWDVFEGLSHLQVLYLNHNYLNSLPPGVFSHLTALRGLSLNSNRLTVLSHNDLPANLEILDISRNQLLAPNPDVFVSLSVLDITHNKFI
mouse       ENLSDLYFLLRVPQLQFLILNQNRLSSCKAAH---TPSENPSLEQLFLTENMLQLAWETGLCWDVFQGLSRLQILYLSNNYLNFLPPGIFNDLVALRMLSLSANKLTVLSPGSLPANLEILDISRNQLFSPDPALFSSLRVLDITHNEFV
treeshrew   EKLDDVYFLLQVPQLRILILKQNRLSFCNQNHPPYPPSENLHLEKLFLGENMLQLAWETGFCWDIFKGLSRLQILYLNDNYLNFLPPGVFQDLTALRGLSLSSNRLVFLSPDDLPANLEILDISRNQLLSPDPNLFVSLSALDITHNKFI
rhesus      ENLDILYFLLRVPHLQILILNQNRLSSCSGAQ---TPSENPSLEQLFLGGNMLQLAWETQLCWDVFEGLSNLQVLYLNNNYLNSLPPGVFSHLTALKRLSLNSNRLTVLSHNDLPANLEILDISGNQLLAPDPDLFVSLSVLDITHNKFI
rat         ENLSDLYFLLRIPGLQFLILNQNRLSSCSNVD--YAPSQNLSLEQLFLAENMLQLAWETGLCWDIFKGLSRLQILYLNNNYLNFLPPGIFNGLVALRMLSLSANRLTMLSPGSLPANLEILDISRNQLFSPDPGLFSSLRALDITHNEFI
                                                                                                                                                                 750
dog         CGCELRPLVRWLNRTNVTVFGSRADVRCAYPSLLAGTPLSSVSMEGCDDEEALRTLTFSLFIFSTVGVTLFLLAVLVAAKLRGLCFLCYKAARRLLPAGPAEDGAPDAYQYDAYLCFSGRDFEWVQRALLRHLDAQYSSRNRLNLCFEER
human       CECELSTFINWLNHTNVTIAGPPADIYCVYPDSFSGVSLFSLSTEGCDEEEVLKSLKFSLFIVCTVTLTLFLMTILTVTKFRGFCFICYKTAQRLVFKDHPQGTEPDMYKYDAYLCFSSKDFTWVQNALLKHLDTQYSDQNRFNLCFEER
mouse       CNCELSTFISWLNQTNVTLFGSPADVYCMYPNSLLGGSLYNISTEDCDEEEAMRSLKFSLFILCTVTLTLFLVITLVVIKFRGICFLCYKTIQKLVFKDKVWSLEPGAYRYDAYFCFSSKDFEWAQNALLKHLDAHYSSRNRLRLCFEER
treeshrew   CECELSAFISWLNQTNVTIFGSPADIYCTYPDSFFGVSLYSISTEGCDEEEVFKSMKFSLFIFFTVTLTLFLMIILIVTKLRGVCFTCYKTVQGLMFKNHPPGTESGRYRYDAYLCFSSKDFEWVQNALLKHLDAQYSDQNRFNLCFEER
rhesus      CECTLSTFIHWLNHTNVTIAGPPADIHCVYPDSLSGVSLFSLSTEACDEEEVLKSLKFSLFIVCTVTLTLFLMTILIVTKFRGFCFICYKTAQRLVFKYHPQGTEPDTYKYDAYLCFSSKDFAWVQNALLKHLDTQYSDQNRFNLCFEER
rat         CDCELSTFIVWLNQTNVTLFGSPADVYCMYPNSLLGSSLYNISTKDCDEEEAVRSLNFSLFILCTVTLTLFLVITLIVTKFRGICFLCFKTIQKLMFKGKFRNPEPSAYRYDAYFCFSSKDFEWAQNALLKHLDAQYSSQNRLRLCFEER

dog         DFVPGREHIANIQDAVWSSRKVVCLVSRHFLRDGWCLEAFAAARSRCASHLDGALVLVVVGSLSQYQLRRHPAIGGFVRQRRYLRWPEDLQDVGWFLDTLSRHILQEQRGARGDGGIPLRTVAAVA
human       DFVPGENRIANIQDAIWNSRKIVCLVSRHFLRDGWCLEAFSYAQGRCLSDLNSALIMVVVGSLSQYQLMKHQSIRGFVQKQQYLRWPEDFQDVGWFLHKLSQQILKKEKEKKKDNNIPLQTVATIS
mouse       DFIPGENHISNIQAAVWGSRKTVCLVSRHFLKDGWCLEAFRYAQSRSLSDLKSILIVVVVGSLSQYQLMRHETIRGFLQKQQYLRWPEDLQDVGWFLDKLSGCILKEEKGKKRSSSIQLRTIATIS
treeshrew   DFVPGENHVTNIQDAVWSSRKIICLVSRHFLRDGWCLEAFDYAQSRCLSDLNNVLIMVVVGSLSQYQLMKHQSLRGFVQKQQYLRWPEDVQDVGWFLNKLSRHLVKKERGKKKDSDIRLQTVSTIS
rhesus      DFVPGENHIANIQDAIWNSRKIVCLVSRHFLRDGWCLEAFSYAQGRCLSDLNSALIMVVVGSLSQYQLMKHQSIRGFVQKQPYLRWPEDLQDVGWFLHKLSQQILKKEKEKKKDSNIPLRTVATIS
rat         DFIPGENHISNIQAAVWGSRKTVCLVSRHFLKDGWCLEAFRYAQSRCLSDLKRVLIVVVVGSLPQYQLMRHETIRGFLQKQQYLRWPEDLQDVDWFLDKLSGCILKEEKGKKRSSPIQLRTIRTVS
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TLR6	1 150
mouse	MVKSLWDSLCNMSQDRKPIVGSFHFVCALALIVGSMTPFSNELESMVDYSNRNLTHVPKDLPPRTKALSLSQNSISELRMPDISFLSELRVLRLSHNRIRSLDFHVFLFNQDLEYLDVSHNRLQNISCCPMASLRHLDLSFNDFDVLPVC
treeshrew	-----------MTKNKELAVRIVCFMCIVTMIAAATNQFCNESGLSVCRSNIGLTRIPKDLSPVIEDLDVSQNDITELQASDLSFLSRLKVLRVSYNRIQQLDLSVFKFNHDLEYLDLSHNQLRRISCYALMSCKHLDLSFNDFDALPIC
rat	MVKSLWDSLCNMSQDREPIVESFHFVCTLALIVGSMTQFSDEFESVVDYSNKNLTHVPKDLSPSTKSLSLSQNSISDLQMSDISFLSELRVLRLSHNRIRRLDFGVFLLNRDLEYLDVSHNQLQNISCCPMVNLKHLDLSFNDFEVLPVY
human	-----------MTKDKEPIVKSFHFVCLMIIIVGTRIQFSDGNEFAVDKSKRGLIHVPKDLPLKTKVLDMSQNYIAELQVSDMSFLSELTVLRLSHNRIQLLDLSVFKFNQDLEYLDLSHNQLQKISCHPIVSFRHLDLSFNDFKALPIC
rhesus	-----------MTKDKEPVVKSFHFVCLMIIIVGTRIQFSDGSEFAVDKSKRGLTHVPKDLPPKTKVLDMSHNYIAELQVSDISFLSELKVLRLSHNKIQLLDLSVFKFNQDLEYLDLSHNQLQKISCHPIMSFRHLDLSFNDFEALPIC
dog	IIQNLYILMCIMIKDKDSITGSFHFVYIVTLIVGTIIQFSDESEFTVDMSNMNLTHVPEDLPPKTKILDMSQNNISELHLSDMSYLSGLKILRISHNRIWWLDFSIFKFNQDLEYLDLSYNQLRNMSCHLIRSLKHLDLSFNDFHVLPIC
	300
mouse	KEFGNLTKLTFLGLSAAKFRQLDLLPVAHLHLSCILLDLVSYHIKGGETESLQIPNTTVLHLVFHPNSLFSVQVNMSVNALGHLQLSNIKLNDENCQRLMTFLSELTRGPTLLNVTLQHIETTWKCSVKLFQFFWPRPVEYLNIYNLTIT
treeshrew	EEFGNLTQLSFLGLSARKLQQLDLLPIAHLHLSYILLELGGYYVKENGRESLQILNTKTLHLVFHPNTLFFVQVNISVNTLGCLQLTNIKLNDKNCXVFIEFLSELTRGPTLLNVTLYHIETTWKCLVSIFQFLWPKPVEYLNIYNLTII
rat	KEFGNLMKLSFLGLSAAKFRQLDLLPISHLHLSCVLLDLVNYQIKDGETESLQVPNTNVLHLVFHPNSLFSVQVNISVNALGCLQLSNIKLNDKNCQSLIIFLSELTRGPTLLNLTLQHIETNWKCFVRLLQFLWPRPVEYLNIYNLTIT
human	KEFGNLSQLNFLGLSAMKLQKLDLLPIAHLHLSYILLDLRNYYIKENETESLQILNAKTLHLVFHPTSLFAIQVNISVNTLGCLQLTNIKLNDDNCQVFIKFLSELTRGSTLLNFTLNHIETTWKCLVRVFQFLWPKPVEYLNIYNLTII
rhesus	KQFGNLSQLNFLGLSAMKLQKLDLLPIAHLHLSYILLDLRNYYIKENETESLQILNAKTLHLVFHPTSLFSIQVNISVNTLGCLQLTNIKLNDDNCQVFIKFLLELTRGPTLLNFTLNHIETTWKCLVRVFQFLWPKPVEYLNIYNLTII
dog	KEFGNLTQLQFLGLSATKLRQLDLLPIAHLHLSYILLDLQGYYAKESEKGSLQILDTKTLHLVFHPNQLFSVQANMLVNNLGCLQLTNIKLNNDNCQVLIQFLSELTRGPTLLNFTLQHVKTTWKCLVRIFKFLWPKPVQYLNIYNLTIV
	450
mouse	ERIDREEFTYSETALKSLMIEHVKNQVFLFSKEALYSVFAEMNIKMLSISDTPFIHMVCPPSPSSFTFLNFTQNVFTDSVFQGCSTLKRLQTLILQRNGLKNFFKVALMTKNMSSLETLDVSLNSLNSHAYDRTCAWAESILVLNLSSNM
treeshrew	EDIQKENFTYXKTTLKALXIEHVKNRVFIFSQTVLYRVFSEMNIVMLTISDTPFIHMLCPQAPSTFKFLNFTRNVFTDSIFQKCSTLVRLETLILQKNGLKDLYKVGLMTKDMPSLEILDVSWNSLESDGLEGNCLWVESIVMLDLSSNM
rat	ESISRETFIYVETVLKSLKIEHVTNQVFLFVKDALYSVFAEMNIRMLTLSDTPFIHMVCPEFPSTFAFLNFTQNVFTDSIFQGCSTLKRLETLILQRNGLKNLFKVALMTKTMSSLETLDVSLNSLNSHVYDRTCAWAESIRVLNLSSNV
human	ESIREEDFTYSKTTLKALTIEHITNQVFLFSQTALYTVFSEMNIMMLTISDTPFIHMLCPHAPSTFKFLNFTQNVFTDSIFEKCSTLVKLETLILQKNGLKDLFKVGLMTKDMPSLEILDVSWNSLESGRHKENCTWVESIVVLNLSSNM
rhesus	ESIHEEDFTYSKTTLKALKIEHITNQVFIFSQTALYTVFSEMNIMMLTISDTPFIHMLCPRAPSTFKFLNFTQNVFTDSIFEKCSTLVKLETLILQKNGLKDLFKVGLMTKDMPSLEILDVSWNSLESGRHRENCTWVESIVVLNLSSNI
dog	ESINKEYIHYPKTALKALTIEHVKNEVFLFSQTALYTIFSEMNIMMLTISDTPFIHMLCPPPSNTFKFLNFTQNVFTDSVFQSCSHLVRLETLILRKNKLKDLYKVGLMTKHMTSLEILDVSVNSLEYDRYDGNCTWVGSIVVLNLSSNI
	600
mouse	LTGSVFRCLPPKVKVLDLHNNRIMSIPKDVTHLQALQELNVASNSLTDLPGCGAFSSLSVLVIDHNSVSHPSEDFFQSCQNIRSLTAGNNPFQCTCELRDFVKNIGWVAREVVEGWPDSYRCDYPESSKGTALRDFHMSPLSCDTVLLTV
treeshrew	LTDSVFSCLPPXVKVLDLHKNRIQSIPKGVMQXESLQELNVAFNSLADLPGCXTFSSLSVLXLDHNXVSHPSADFFQSCQKIRSXKAGNNPFQCTCELREFIKNIGQVPRGVVEDWPDSYKCDYPESYKGXXLKDFHPSQLSCNTVLLIV
rat	LSDSVFRCLPPKVKVLDLHNNRIVSIPKDVTHLQALQELNVASNFLTDLPGCGAFSSLSVLVIDHNSVSHPSSDFFQSCQNIRSITAGNNPFRCTCELREFVKNIGQASREVVEGWPDSYRCDYPDSIKGTPLQDFHMSPLSCDTILLTV
human	LTDSVFRCLPPRIKVLDLHSNKIKSVPKQVVKLEALQELNVAFNSLTDLPGCGSFSSLSVLIIDHNSVSHPSADFFQSCQKMRSIKAGDNPFQCTCELREFVKNIDQVSSEVLEGWPDSYKCDYPESYRGSPLKDFHMSELSCNITLLIV
rhesus	LTDSVFRCLPPRIKVLDLHNNKIKSIPKQVVKLEALQELNVAFNSLTDLPGCGSFSSLSVLIIDHNSVSHPSADFFQSCQKMRSIKAGNNPFQCTCELREFVKNIEQVSSEVVEGWPDSYKCDYPESYRGTPLKDFHMSELSCNITLLII
dog	LTDSVFRCLPPKVKVLDLHDNRIRSIPKPIMKLEDLQELNVASNSLAHFPDCGTFNRLSVLIIDSNSISNPSADFLQSCHNIRSMSAGNNPFQCTCELREFVQSLGQVASKVVEGWPDSYKCDSPENYKGTLLKDFHVSPLSCNTTLLLV
	750
mouse	TIGATMLVLAVTGAFLCLYFDLPWYVRMLCQWTQTRHRARHIPLEELQRNLQFHAFVSYSEHDSAWVKNELLPNLEKDDIRVCLHERNFVPGKSIVENIINFIEKSYKAIFVLSPHFIQSEWCHYELYFAHHNLFHEGSDNLILILLEPI
treeshrew	XVGATMLLLAVTMTLLXIYLDLPWYLRMMFQWTQTRRRARNLPLEELQRTLQFHAFIXYSXHDSAWVKNXLVPXLEKEDVRICLHERNFVPGKSIVENIVNCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHAGSDNLILILLXPI
rat	TIGATLLLLAAIGASLCLYFDLPWYLRMLWQWTQTRHRARNIPLEELQRNLQFHAFVSYSEHDSAWVKNELLPNLEKDDIRVCLHERNFVPGKSIVENIIHFIEKSYKSIFVLSPHFIQSEWCHYELYFAHHNLFHEGSDNLILILLEPI
human	TIGATMLVLAVTVTSLCIYLDLPWYLRMVCQWTQTRRRARNIPLEELQRNLQFHAFISYSEHDSAWVKSELVPYLEKEDIQICLHERNFVPGKSIVENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEGSNNLILILLEPI
rhesus	TIGATMLVLAVTVTFLCIYLDLPWYLRMVCQWTQTRHRARNVPLEELQRNLQFHAFISYSEHDSAWVKNELVPYLEKEGMQVCLHERNFVPGKSIVENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEGSNNLILILLEPI
dog	TIGVAVLVFTVTVTALCIYFDLPWYLRMVFQWTQTRRRARNTPLEELQRTIQFHAFISYSEHDSAWVKNELVPCLEKEELRICLHERNFIPGKSIVENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEGSNNLILILLEPI
mouse	LQNNIPSRYHKLRALMAQRTYLEWPTEKGKRGLFWANLRASFIMKLALVNED-DVKT
treeshrew	PQNSIPNKYHKLRALMTQRTYLEWPKEKSKRGLFWANIRAAFNMKLTLGTENKDEKT
rat	QQNNIPSRYHKLRALMAQRTYLEWPIEKGKRGLFWANLRASFIMKLALVNEN-DVKT
human	PQNSIPNKYHKLKALMTQRTYLQWPKEKSKRGLFWANIRAAFNMKLTLVTENNDVKS
rhesus	PQNSIPNKYHKLRALMTQRTYLQWPKEKSKRGLFWANIRATFNVKLTLVTENNDVKS
dog	PQNCIPSKYHKLRALMTQRTYLEWPKEKSKHGLFWANIRAAFNMKLTLIAENNNAE-

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dog MVFPMWTLKRQFFILLNIILISKLLGARWFPKTLPCDVSLDAPKAHVIVDCTDKHLTEIPGGIPSNATNLTLTINHIPGISPASFHQLDYLVEIDFRCNCIPVRLGPKDHLCTRRPQIKPRSFSSLTYLKSLYLDGNQLLEIPEGLPPSL
rat MVFPMWTLKRQSFIFLNMILVSRVLGFRWYPKTLPCDVSLDSTNTHVIVDCTDKHLTEIPEGIPTNTTNLTLTINHIPSISPDSFHRLKHLEELDLRCNCVPILLGSKANVCTKRLQIRPGSFSGLSDLKSLYLDGNQLLEIPQDLPSSL
rhesus MMFPVWTLKRQILILFNIILISKLLGARWFPKTLPCDVTLDVSKNHVIVDCTDKHLTEIPGGIPTNTTNLTLTINHIPDISPASFHRLVHLVEIDFRCNCVPIRLGSKSNMCPRRLQIKPRSFSGLTYLKSLYLDGNQLLEIPQGLPPSL
treeshrew MVFPMWILKRLFLILFNIILISKFLGARWFPKTLPCDVTLDEPMTHVSVDCTDKHLKEIPEGIPTNTTNLTLTINHIPDISPASFQRLDRLVEIDFRCNCVPIRLGPKNNVCTRRLQIKPKSFSKLSNLRSLYLDGNQLVEIPKDLPPNL
human MVFPMWTLKRQILILFNIILISKLLGARWFPKTLPCDVTLDVPKNHVIVDCTDKHLTEIPGGIPTNTTNLTLTINHIPDISPASFHRLDHLVEIDFRCNCVPIPLGSKNNMCIKRLQIKPRSFSGLTYLKSLYLDGNQLLEIPQGLPPSL
mouse MVFSMWTRKRQILIFLNMLLVSRVFGFRWFPKTLPCEVKVNIPEAHVIVDCTDKHLTEIPEGIPTNTTNLTLTINHIPSISPDSFRRLNHLEEIDLRCNCVPVLLGSKANVCTKRLQIRPGSFSGLSDLKALYLDGNQLLEIPQDLPSSL

300

dog ELLSLEANSIFSIMKNNLTELTNIERLYLGQNCYFRNPCNVSFFIEKDAFLSLKNLKLLSLKDNNITYVPTTLPSTLTELYLYNNAIAKIQEDDFNNLNQLRILDLSGNCPRCYNVPFPCTPCENNSPLQIHESAFDALTELQVLRLHSN
rat QLLSLEANNIFSITKENLSELVNIESLYLGQNCYYRNPCNVSYSIEKDAFLVMKNLKVLSLKDNNVTAVPTILPPNLLELYLYNNIIKRIQEHDFNKLSQLQVLDLSGNCPRCYNVPYPCTPCENNSPLQIHDNAFDSLTELKVLRLHSN
rhesus QLLSLEANNIFSIRKENLTELANIEILYLGQNCYYRNPCYVSYSIEKDAFLNLTKLKVLSLKDNNVTTVPTVLPSTLTELYLYNNMIAEIQEDDFNNLNQLQILDLSGNCPRCYNAPFPCTPCKNNSPLQIPVNAFDALTELKVLRLHSN
treeshrew RLLSLEANNIFSIMKENLTELTNIEILYLGQNCYYRNPCNVSFLIEKNAFISLKNLKVLSLKXNNVTDVPTVLPSTLTELYLHNNIIAKIQRDDFKNLNQLQILDLSGNCPRCYNVPFPCTPCENNSPLQIDEHAFDSLEKLKVLXLHSN
human QLLSLEANNIFSIRKENLTELANIEILYLGQNCYYRNPCYVSYSIEKDAFLNLTKLKVLSLKDNNVTAVPTVLPSTLTELYLYNNMIAKIQEDDFNNLNQLQILDLSGNCPRCYNAPFPCAPCKNNSPLQIPVNAFDALTELKVLRLHSN
mouse HLLSLEANNIFSITKENLTELVNIETLYLGQNCYYRNPCNVSYSIEKDAFLVMRNLKVLSLKDNNVTAVPTTLPPNLLELYLYNNIIKKIQENDFNNLNELQVLDLSGNCPRCYNVPYPCTPCENNSPLQIHDNAFNSLTELKVLRLHSN

450

dog SLQRVPQRWFKNIKKLKELDLSQNFLAKEIGDAKFLYLLHDLVQLDLSFNYELQVYRAALNLSDAFSSLKNLKVLRIKGYVFKELSSHHLSPLQSLTNLEVLDLGTNFIKIADLSIFEQFKTLKVIDLSMNKISPSGDSGEVGFCSSTRT
rat SLQHVPAEWFKNMSNLQELDLSQNYLAREIEEAKFLNSLPNLVQLDLSFNYELQVYHASITLPHSLSSLTKLKNLYIKGYVFKELKDSSLSVLHNLSNLEVLDLGTNFIKIADLNIFQQFENLKFIDLSVNKISPSEESREVGLCPNAQT
rhesus SLQHVPPRWFKNINNLQELDLSQNFLAKEIGDAKFLHFLPNLIQLDLSFNFELQVYRASMNLSQAFSSLKSLKILRIRGYVFKELKSFNLSPLHNLQNLEVLDLGTNFIKIANLSMFKQFKRLKVIDLSVNKISPSGDSSEVGFCSNART
treeshrew SLQHVPKEWFKNIRNLQELDLSQNFLAKEIGDAEFLKYLPNLVQLDLSFNYELQVYRAFINLSCTFSSLKNLKILRLKGYVFKELKSLNLSPLRDLLNLEVLDLGTNFIKIADLSMFKQFERLKVIDLSVNKISPSGDSSEAGFCSNTRT
human SLQHVPPRWFKNINKLQELDLSQNFLAKEIGDAKFLHFLPSLIQLDLSFNFELQVYRASMNLSQAFSSLKSLKILRIRGYVFKELKSFNLSPLHNLQNLEVLDLGTNFIKIANLSMFKQFKRLKVIDLSVNKISPSGDSSEVGFCSNART
mouse SLQHVPPTWFKNMRNLQELDLSQNYLAREIEEAKFLHFLPNLVELDFSFNYELQVYHASITLPHSLSSLENLKILRVKGYVFKELKNSSLSVLHKLPRLEVLDLGTNFIKIADLNIFKHFENLKLIDLSVNKISPSEESREVGFCPNAQT

600

dog SVEGHAPQVLETLHYFRYDEYARSCRFKNKETPSFLPFNKDCYMYGGQTLDLSRNNIFFIKSSDFQHLSFLKCLNLSGNTIGQTLNGSEFQPLVELKYLDFSNNRLDLLYSTAFEELRKLEVLDISSNSHYFQSEGITHMLNFTKNLKVLK
rat SVDWHGPQVLEALHYFRYDEYARSCRFKNKEPPTFLPLNADCHTYGKTLDLSRNNIFFIKPSDFKHLSFLKCLNLSGNAIGQTLNGSELQPLRELRYLDFSNNRLDLLYSTAFEELQNLEILDLSSNSHYFQAEGITHMLNFTKKLRHLE
rhesus SVESYEPQVLEQLYYFRYDKYARSCRFKNKE-ASFTSVNESCYKYGGQTLDLSKNSIFFIKSSDFQHLSFLKCLNLSGNLISQTLNGSEFQPLAELRYLDFSNNRLDLLHSTAFEELRKLEVLDISSNSHYFQSEGITHMLNFTKNLKVLQ
treeshrew PVESRGPQFFEALHYFRYDEYARSCRSKDKEMPSLLPFNEECYEYGGQTLDLSKNNIFFIKPSDFQNLSFLKCLNLSGNSIGQTLNGSEFQPLVELKYLDFSNNRLDLLYSTAFEELHNLEILDISSNSHYFQSEGITHMLNFTKNLKFLK
human SVESYEPQVLEQLHYFRYDKYARSCRFKNKE-ASFMSVNESCYKYGGQTLDLSKNSIFFVKSSDFQHLSFLKCLNLSGNLISQTLNGSEFQPLAELRYLDFSNNRLDLLHSTAFEELHKLEVLDISSNSHYFQSEGITHMLNFTKNLKVLQ
mouse SVDRHGPQVLEALHYFRYDEYARSCRFKNKEPPSFLPLNADCHIYGGQTLDLSRNNIFFIKPSDFQHLSFLKCLNLSGNTIGQTLNGSELWPLRELRYLDFSNNRLDLLYSTAFEELQSLEVLDLSSNSHYFQAEGITHMLNFTKKLRLLD

750

dog KLMMNNNDIATSTSRTMESESLKILEFRGNHLDVLWRDGDNRYLKFFKNLLNLEELDISENSLSFLPSGVFDGMPPNLKTLSLVKNGLKSFHWERLQYLKNLETLDLSYNELKIVPERLYNCSRSLKKLILKYNQIRQLTKHFLQDAFQL
rat KLMMNDNDISTSASRTMESESLRVLEFRGNHLDVLWRDGDNRYLDFFKNLLNLEELDISRNSLNSVPPGVFEGMPPNLTTLSLAKNGLRSFSWGRLQLLKHLKNLDLSHNQLTTVPARLANCSKSLTKLILNHNQIRQLTKYFLEDALQL
rhesus KLMMNDNDISSSTSRTMESESLRTLEFRGNHLDVLWRDGDNRYLQLFKNLLKLEELDISKNSLSFLPSGVFDGMPPNLKNLSLAKNGLKSFIWEKLRYLKNLETLDLSHNQLTTVPERLSNCSRSLKNLILKNNQIRSLTKYFLQDAFQL
treeshrew KLMMNHNDISTSVSRNMESKSLRTLEFRGNHLDILWRDGDNRYLQFFRELQNLEELDISENSLSFLPPGVFNGMPPQLKNLSLAKNALKSFNWGKLQELKNLETLDLSHNQLTTVPERLSNCXRSXKRLILRKNQIRHLTKYFXQDAFQL
human KLMMNDNDISSSTSRTMESESLRTLEFRGNHLDVLWREGDNRYLQLFKNLLKLEELDISKNSLSFLPSGVFDGMPPNLKNLSLAKNGLKSFSWKKLQCLKNLETLDLSHNQLTTVPERLSNCSRSLKNLILKNNQIRSLTKYFLQDAFQL
mouse KLMMNDNDISTSASRTMESDSLRILEFRGNHLDVLWRAGDNRYLDFFKNLFNLEVLDISRNSLNSLPPEVFEGMPPNLKNLSLAKNGLKSFFWDRLQLLKHLEILDLSHNQLTKVPERLANCSKSLTTLILKHNQIRQLTKYFLEDALQL

900

dog RYLDLSSNKIQIIQKTSFPENVLNNLEMLLLHHNRFLCTCDAVWFVWWVNHTEVTIPYLATDVTCVGPGAHKGQSVVSLDLYTCELDLTNLVLFSFSLSLALFLMVITTANHLYFWDVWYSYHYCKAKIKGYRRLKSLDSCYDAFVVYDT
rat RYLDISSNKIQVIQKTSFPENVLNNLNMLLLHHNRFLCNCDAVWFVWWVNHTDVTIPYLATDVTCAGPGAHKGQSVISLDLYTCELDLTNLILFSVSISSVLFLMIVMTTSHLFFWDMWYIYYFWKAKIKGYQHLQSMESCYDAFIVYDT
rhesus RYLDLSSNKIQMIQKTSFPENVLNNLKMLLLHHNRFLCTCDAVWFVWWVNHTEVTIPYLATDVTCVGPGAHKGQSVISLDLYTCELDLTNLILFSLSISVSLFLMVMMTASHLYFWDVWYIYHFCKAKIKGYQRLISPDCCYDAFIVYDT
treeshrew RYLDLSSNKIQIIQKTSFPENVLNNLEKLLLHHNRFLCTCDAVWFVWWVNHTEVTIPYLATEVTCVGPGAHKGQSVVSLDMYTCELDLTNLILFSLSVSVALFLMVVMTTSHLYFWDVWYSYHFCKAKIKGYQRLMSQNCCYDAFIVYDT
human RYLDLSSNKIQMIQKTSFPENVLNNLKMLLLHHNRFLCTCDAVWFVWWVNHTEVTIPYLATDVTCVGPGAHKGQSVISLDLYTCELDLTNLILFSLSISVSLFLMVMMTASHLYFWDVWYIYHFCKAKIKGYQRLISPDCCYDAFIVYDT
mouse RYLDISSNKIQVIQKTSFPENVLNNLEMLVLHHNRFLCNCDAVWFVWWVNHTDVTIPYLATDVTCVGPGAHKGQSVISLDLYTCELDLTNLILFSVSISSVLFLMVVMTTSHLFFWDMWYIYYFWKAKIKGYQHLQSMESCYDAFIVYDT

1050

dog KDPAVTEWVLDELVAKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMTNKYAKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPLQKSKFLQLRKRLCKSSVLEWPRNPQAHPYFWQCLKNALATDNHVTYSQVFKETV
rat KNSAVTEWVLQELVVKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSRKTVFVMTQKYAKTESFKMAFYLSHQRLMDEKVDVIILIFLEKPLQKSKFLQLRKRLCSSSVLEWPTNPQAHPYFWQCLKNALTTDNHVAYSQMFKETV
rhesus KDPAVTEWVLAELVAKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMTDKYAKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPFQKSKFLQLRKRLCGSSVLEWPTNPQAHPYFWQCLKNALATDNHVAYSQVFKETV
treeshrew EDPAVTEWVLDELVAKLEDPREKSFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMTNKYSKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPLQKSKFLQLRKRLCGSSVLEWPTNPQAHPYFWQSLKNSLAMDNHVAYSQVFXETV
human KDPAVTEWVLAELVAKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMTDKYAKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPFQKSKFLQLRKRLCGSSVLEWPTNPQAHPYFWQCLKNALATDNHVAYSQVFKETV
mouse KNSAVTEWVLQELVAKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMTQKYAKTESFKMAFYLSHQRLLDEKVDVIILIFLEKPLQKSKFLQLRKRLCRSSVLEWPANPQAHPYFWQCLKNALTTDNHVAYSQMFKETV

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           1                                                                                                                                                      150
mouse      -----------------MENMPPQSWILTCFCLLSSGTSAIFHKANYSRSYPCDEIRHNSLVIAECNHRQLHEVPQTIGKYVTNIDLSDNAITHITKESFQKLQNLTKIDLNHNAKQQH----PNENKNGMNITEGALLSLRNLTVLLL
treeshrew  --------------------MSLQSSILTCLFLLTSGSCEFFIERNYFRSYPCDEKRQNDSIIAECNXLRLQEVXQTVGKYVTELDLSDNFITHITNESFQGLQSLTKINLNHNSANLR---NP--NKNGMNITDGAFLNLKNLRELLL
rhesus     MKESSLQNSSCSLGKESKKENMFLQSSMLTCLFLLIPGSCELCPEENFSRSYPCEEKRQNHCVIAECSNRRLREVPQTVGKYVTELDLSDNFITHITNESFQGLQNLTKINLNHNPNVQRQNGNPGMQSNGLNITDGAFLNLKNLRELLL
dog        ------------------ENMSPRSLVLTCLFLLISDSYEFVTKANYSRSYPCDERRQNGSVIAECNGRRLQEVPQTVGKYVTVLDLSDNYITHITNESFHGLQNLTKINLNHNANPQHLSENPD-NKNGMNITDGAFLNLQNLNQLLL
human      -----------------MENMFLQSSMLTCIFLLISGSCELCAEENFSRSYPCDEKKQNDSVIAECSNRRLQEVPQTVGKYVTELDLSDNFITHITNESFQGLQNLTKINLNHNPNVQHQNGNPGIQSNGLNITDGAFLNLKNLRELLL
rat        --------------------MSPQSWILTCFCLLSSGTSAVFLKGNFSRSYPCDEKRHNALVTAECNHRQLHEVPQTIGKYVTDVDLSDNTIMHITNESFQKFRNLTKINLNHNAKQQH----PNENKNGMNITEGAFLSLRNLTELLL
                                                                                                                                                                  300
mouse      EDNQLYTIPAGLPESLKELSLIQNNIFQVTKNNTFGLRNLERLYLGWNCYF--KC-NQTFKVEDGAFKNLIHLKVLSLSFNNLFYVPPKLPSSLRKLFLSNAKIMNITQEDFKGLENLTLLDLSGNCPRCYNAPFPCTPCKENSSIHIHP
treeshrew  EDNQLIKIPTGLPESLRELSLIQNNIVLVTKKDTLGLKKLQSLYLGWNCYF--DC-NKTFHIEEGTFENLTDLRVLSLSFNHLYHVPPKLLISLRKLFLSNTNIKNITEEDFKGLRNLRLLDLSGNCPRCFNAPFPCKPCEKDASIQIQP
rhesus     EDNQLPQIPSGLPESLTELSLIQNNIYNITKEGISRLINLKYLYLAWNCYFNKVC-EKT-NIEDGVFETLTNLELLSLSFNSLSHVPPKLPSSLRKLFLSNTQIKYIGEEDFKGLINLTLLDLSGNCPRCFNAPFPCVPCDGGASINIDR
dog        EDNQLYQIPAGLPGSLKELSLIQNNIIWVTKKNTSGLTNLERLYLSWNCYFGNNCNNKTFNIEDGTFESLTNLEVLSLSFNKLVHVPPKLPSSLKELYLSNAKIKIISQEDFKGLRNLRVLDLSGNCPRCFNAPFPCTPCEGGASIQIHP
human      EDNQLPQIPSGLPESLTELSLIQNNIYNITKEGISRLINLKNLYLAWNCYFNKVC-EKT-NIEDGVFETLTNLELLSLSFNSLSHVPPKLPSSLRKLFLSNTQIKYISEEDFKGLINLTLLDLSGNCPRCFNAPFPCVPCDGGASINIDR
rat        EDNQLYTIPAGLPESLKELSLIQNNIFQVTKNNTFGLRNLERLYLGWNCYF--KC-NQIFKVEDGAFNNLINLKLLSLSFNNLFSVPPKLPSSLSKLFLSNAKISTITQEDFKGLEHLILLDLSGNCPRCFNAPFPCESCNLSASIRIHP
                                                                                                                                                                  450
mouse      LAFQSLTQLLYLNLSSTSLRTIPSTWFENLSNLKELHLEFNYLVQEIASGAFLTKLPSLQILDLSFNFQYKEYLQFINISSNFSKLRSLKKLHLRGYVFRELKKKHFEHLQSLPNLATINLGINFIEKIDFKAFQNFSKLDVIYLSGNRI
treeshrew  LAFQNLTQLRYLNLSSTSLRTICATWFDNMPHLKVLHLEFNYLIQEIASGAFLTKLDYLETLDLSFNYVKTEYPQYINISKNFSKLRHLKSLHLRGYVFQKLRKEDFQPLMNLSRLKTINLGINFIKQIDFTLFQQFSNLKIIYLSENRI
rhesus     FAFQNLTQLQYLNLSSTSLRKINAAWFKNMPHLKVLDLEFNYLVGEIASGEFLTMLPRLEILDLSFNYIKGSYPQHINISKNFSKLLSLRALHLRGYVFQELRKDDFQPLMQLPNLSTINLGINFIKQIDFNLFQNFPNLEIIYLSENRI
dog        LAFQTLTELRYLNLSSTSLRKIPATWFDNMRNLKVLHLEFNYLVDEIASGEFLTKLPVLEILDLSYNYVKAKYPKYINISHNFSSLKLLQALHLRGYVFQELRAGDFEPLMGLSNLKTINLGVNFIKQINFTLFQNFPNLSIIYLSENRI
human      FAFQNLTQLRYLNLSSTSLRKINAAWFKNMPHLKVLDLEFNYLVGEIASGAFLTMLPRLEILDLSFNYIKGSYPQHINISRNFSKLLSLRALHLRGYVFQELREDDFQPLMQLPNLSTINLGINFIKQIDFKLFQNFSNLEIIYLSENRI
rat        LAFQNLTQLRFLNLSSTSLRTIPSTWFDNLTNLKELHLEFNYLVQEIASGAFLTKLPSLQTLDLSFNFIHKEYLQYITISPNFSMLRSLRKLHLKGYVFRELKKEHFKPLQNLPNLTTINLGINFIEKIDFKAFQDFPNLKVIYLSGNRI
                                                                                                                                                                  600
mouse      ASVLDGT-----DYSSWRNRLRKPLSTDDDEFDPHVNFYHSTKPLIKPQCTAYGKALDLSLNNIFIIGKSQFEGFQDIACLNLSFNANTQVFNGTEFSSMPHIKYLDLTNNRLDFDDNNAFSDLHDLEVLDLSHNAHYFSIAGVTHRLGF
treeshrew  SPLVNDIRQNNTNGLSFQSHTLKTRSADT-NFDPHSNFYHRTTPLIKPQCTAYGKSLDLSLNSIFFIGEKQFEGFTDIACLNLSSNGNGQVLHGNEFSAMRGVKYLDLTNNRLDFDDDKTLQDLPYLEVLDLSYNAHYFRIAGVTHRLGF
rhesus     SPLVKDTRQSYANSSSFQRHILKRRSTDF-EFDPHSNFYHFTRPLIKPQCAAYGKALDLSLNNIFFIGPNQFENLPDIACLNLSANSNAQVLSGTEFSAIPHVKYLDLTNNRLDFDNASALTELSDLEVLDLSYNSHYFRIAGVTHHLEF
dog        SPLVNDIRQNEVNGSSSQRHVLKPRSADM-EFDPHSNFYHNTHPLIKPQCTVYGKALDLSLNSIFFIGREQFEAFHDIACLNLSSNGNGQVLHGNEFSAVPHIKYLDLTNNRLDFDDDNALSDLPELEVLDLSYNAHYFRIAGVTHRLGF
human      SPLVKDTRQSYANSSSFQRHIRKRRSTDF-EFDPHSNFYHFTRPLIKPQCAAYGKALDLSLNSIFFIGPNQFENLPDIACLNLSANSNAQVLSGTEFSAIPHVKYLDLTNNRLDFDNASALTELSDLEVLDLSYNSHYFRIAGVTHHLEF
rat        ASVIDGT-----DHSSWRNRLRKPLSTDYDEFDPHMNFYHSTEPLIKPQCTTYGKALDLSLNNIFVIGKSQFEGFQDIACLNLSFNANGQVLNGTEFSSMPHIKYLDLTNNRLDFDDNQTFSDLHDLEVLDLSHNAHYFSIAGVTHRLGF
                                                                                                                                                                  750
mouse      IQNLINLRVLNLSHNGIYTLTEESELKSISLKELVFSGNRLDRLWNANDGKYWSIFKSLQNLIRLDLSYNNLQQIPNGAFLNLPQSLQELLISGNKLRFFNWTLLQYFPHLHLLDLSRNELYFLPNCLSKFAHSLETLLLSHNHFSHLPS
treeshrew  IQNLTQLKVLNLSYNSIYTLTE-YELKSLSLEELVFSGNRLDLLWNAEDGRYITIFKGLVNLTRLDISFNNLQRIPDEAFLNLPQNLTKLYINDNMLNFFNWTLLQYFPQLHLLDLSRNKLSLVTHSLSTFTTSLQKLLLSQNRISHLPS
rhesus     IQNFTNLKVLNLSHNNIYTLTDKYNLESKSLVELVFSGNRLDILWNDDDNRYISIFKGLTNLTKLDLSLNKLKHIPNEAFLNLPASLTELHINDNMLKFFNWTLLQQFPHLQLLDLRGNKLFFLTDSLSDFTSSLQTLLLSHNRISHLPS
dog        IQNLTQLKVLNLSHNSIYTLTE-QDLRSVSLEELVFSGNRLDILWNAEGDKYWKIFTRLRNLTRLDLSLNNLRRIPNEAFLNLPQSLTQLYIKNNALNFFNWTLLQEFPRLQVLDLSGNRLSSITNSLSKFTSSLQTLLLHRNRISHLPA
human      IQNFTNLKVLNLSHNNIYTLTDKYNLESKSLVELVFSGNRLDILWNDDDNRYISIFKGLKNLTRLDLSLNRLKHIPNEAFLNLPASLTELHINDNMLKFFNWTLLQQFPRLELLDLRGNKLLFLTDSLSDFTSSLRTLLLSHNRISHLPS
rat        IQNLIKLKVLNLSHNGIYTLTDEYKLQSKSLKELVFSGNRLDRLWNANDGKYWSIFTSLETLTRLDLSYNNLQQIPNEAFLNLPQSLQELHINDNRLRFFNWTLLQYFPHLHVLDLGRNELYFLTNCLSKFTHSLKTLLLNHNHFSHLPA
                                                                                                                                                                  900
mouse      GFLSEARNLVHLDLSFNTIKMINKSSLQTKMKTNLSILELHGNYFDCTCDISDFRSWLDENLNITIPKLVNVICSNPGDQKSKSIMSLDLTTCVSDTTAAVLFFLTFLTTSMVMLAALVHHLFYWDVWFIYHMCSAKLKGYRTSSTSQTF
treeshrew  GFLSGASSLVHLDLRSNLLRMLNKSTLQTKTTTNLAVLELGRNPLDCTCDIGDFQSWMDENPNITIPRLIDVICDSPGDQRGKSIVSLELTTCVSDTIAAVLFFFTFFITIMVMLTALAHHLFYWDVWFIYHVCLAKVKGYRSLSTSQTF
rhesus     GFLSEVSSLMHLDLSSNLLKTINKSALETKTTTNLCILELHGNPFECTCDIGDFRRWMDEHLNVTIPRLVDVICASPGDQRGKSIVSLELTTCVSDVTAVILFFFTFFITTMVMLTALAHHLFYWDVWFIYNVCLAKVKGYRSLSTSQTF
dog        SFLSEASSLIHLDLSSNLLKMINKSTLQTKTNTSLAILELGRNPFDCTCDIGDFRRWMDENLNVTIPRLTDVICSSPGDQRGKSIVSLELTTCISDTLAAVLCIFTSFITVTVMLAALGHHWFYWDVWFIYHVCLAKVKGYRSVSTSQTF
human      GFLSEVSSLKHLDLSSNLLKTINKSALETKTTTKLSMLELHGNPFECTCDIGDFRRWMDEHLNVKIPRLVDVICASPGDQRGKSIVSLELTTCVSDVTAVILFFFTFFITTMVMLAALAHHLFYWDVWFIYNVCLAKVKGYRSLSTSQTF
rat        GFLSEARNLVYLDLSFNTIKMINKSSLQTETKTNLSVLDLQGNHFDCTCDISDFRSWLEENPHVRIPRLVDVICSNPGDQRWKSVMSLDLTTCVSDTTAAILFFFTFLTTSTVLLAALVHHLFYWDVWFIYHMCSAKLRGYRSSSTSQTF
                                                                                                                                                                  1050
mouse      YDAYISYDTKDASVTDWVINELRYHLEESEDKSVLLCLEERDWDPGLPIIDNLMQSINQSKKTIFVLTKKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQYSQYLRLRQRICKSSILQWPNNPKAENLFWQSLKNVVLTENDSR
treeshrew  YDAYISYDTKDASVTDWVINELRYHLEESEEKNVLLCLEERDWDPGLAIIDNVMQSINQSKKTIFVLTKKYAKNWNFKTAXYLALQRLMDENMDVIIFIXLEPVLQHXQYLRLRQRICKSSILQWPENPKAEGLFWQSXKNVXLTENNSR
rhesus     YDAYISYDTKDASVTDWVINELRYHLEESQDKNVLLCLEERDWDPGLAIIDNLMQSINQSKKTVFVLTKKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQHSQYLRLRQRICKSSILQWPDNPKAEGLFWQTLRNVVLTENDSR
dog        YDAYVSYDTKDASVTDWVINELRFHLEESEGKNVLLCLEERDWDPGLAIIDNLMQSINQSKKTIFVLTKEYAQNWNFKTAFYLALQRLMDENMDVIIFILLEPVLQHSQYLRLRQRICKSSILQWPDNPKAEGLFWQSLKNVVLTENDSR
human      YDAYISYDTKDASVTDWVINELRYHLEESRDKNVLLCLEERDWDPGLAIIDNLMQSINQSKKTVFVLTKKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQHSQYLRLRQRICKSSILQWPDNPKAEGLFWQTLRNVVLTENDSR
rat        YDAYISYDTKDASVTDWVINELRYHLEESEDKSVLLCLEERDWDPGLPIIDNLMQSINQSKKTIFVLTKKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQYSQYLRLRQRICKSSILQWPNNPKAENLFWQSLKNVVLTENDSR
```

mouse	YDDLYIDSIRQY
treeshrew	YNNLYXDSIKQY
rhesus	YNNMYVDSIKQ-
dog	YNNLYVDSIKQY
human	YNNMYVDSIKQY
rat	YDNLYIDSIRQY

TLR9

	1 … 150
human	-----------------------MGFCRSALHPLSLLVQAIMLAMTLALGTLPAFLPCELQPHGLVNCNWLFLKSVPHFSMAAPRGNVTSLSLSSNRIHHLHDSDFAHLPSLRHLNLKWNCPPVGLSPMHFPCHMTIEPSTFLAVPTLE
rhesus	MLYSSCKSRLLDSVEQDFHLEIAKKGFCCSALHPLSLLVQAMVLATTLALGTLPAFLPCELQPHGLVNCNWLFLKSVPHFSAAAPRGNVTSLSLSSNRIHHLHDSDFARLPSLRRLNLKWNCPPVGLSPMHFPCHMTIEPSTFLAVPTLE
dog	-----------------------MGPCRGALHPLSLLVQAAALALALAQGTLPAFLPCELQPHGLVNCNWLFLKSVPRFSAAAPRGNVTSLSLYSNRIHHLHDYDFVHFVHLRRLNLKWNCPPASLSPMHFPCHMTIEPNTFLAVPTLE
mouse	-----------------------MVLRRRTLHPLSLLVQAAVLAETLALGTLPAFLPCELKPHGLVDCNWLFLKSVPRFSAAASCSNITRLSLISNRIHHLHNSDFVHLSNLRQLNLKWNCPPTGLSPLHFSCHMTIEPRTFLAMRTLE
rat	-----------------------MVLCRRTLHPLSLLVQAAMLAEALALGTLPAFLPCELKPHGLVDCNWLFLKSVPHFSAAEPRSNITSLSLIANRIHHLHNLDFVHLPNVRQLNLKWNCPPPGLSPLHFSCRMTIEPKTFLAMRMLE
treeshrew	-----------------------MGPCSSALQPLSLLVWAAVLAVGLGLGTLPAFLPCEFRDPGLVNCNWLFLKSVPRFK-AASRNNITSLSLLSNRIHHLHDSDFAHLPNLRRLNLKWNCPPAGLSPMHFPCHMTIERNTFLAVPTLE
	300
human	ELNLSYNNIMTVPALPKSLISLSLSHTNILMLDSASLAGLHALRFLFMDGNCYYKNPCRQALEVAPGALLGLGNLTHLSLKYNNLTVVPRNLPSSLEYLLLSYNRIVKLAPEDLANLTALRVLDVGGNCRRCDHAPNPCMECPRHFPQLH
rhesus	ELNLSYNSITTVPALPKSLISLSLSHTNILVLDSDSLASLHSLRFLFMDGNCYYKNPCRQELEVAPGALLGLGNLTHLSLKYNNLTVVPRNLPSSLEYLLLSYNRIIKLAPEDLANLTALRVLDVGGNCRRCDHAPNPCMECPRHFPQLH
dog	DLNLSYNSITTVPALPSSLVSLSLSRTNILVLDPATLAGLYALRFLFLDGNCYYKNPCQQALQVAPGALLGLGNLTHLSLKYNNLTVVPRGLPPSLEYLLLSYNHIITLAPEDLANLTALRVLDVGGNCRRCDHARNPCRECPKGFPQLH
mouse	ELNLSYNGITTVPRLPSSLVNLSLSHTNILVLDANSLAGLYSLRVLFMDGNCYYKNPCTGAVKVTPGALLGLSNLTHLSLKYNNLTKVPRQLPPSLEYLLVSYNLIVKLGPEDLANLTSLRVLDVGGNCRRCDHAPNPCIECGQKSLHLH
rat	ELNLSYNGITTVPRLPSSLTNLSLSHTNILVLDASSLAGLHSLRVLFMDGNCYYKNPCNGAVNVTPDAFLGLSNLTHLSLKYNNLTEVPRQLPSSLEYLLLSYNLIVKLGPEDLANLTSLRVLDVGGNCRRCDHAPDLCTECRQKSLDLH
treeshrew	ELNLSYNGISTVPALPSSLVFLSLSRTNILTLGPASLAGLHSLRFLFIDGNCYYKNPCGRALEVAPGALANLSNLTRLSLKYNNXTAVPQNLPPSLEYLLLSYNHIVKLAPQDLANLTALRVLDVGGNCRRCDHARNPCVECPLGFPQLH
	450
human	PDTFSHLSRLEGLVLKDSSLSWLNASWFRGLGNLRVLDLSENFLYKCITKTKAFQGLTQLRKLNLSFNYQKRVSFAHLSLAPSFGSLVALKELDMHGIFFRSLDETTLRPLARLPMLQTLRLQMNFINQAQLGIFRAFPGLRYVDLSDNR
rhesus	PDTFSHLSRLEGLVLKDSSLSWLNASWFRGLGNLRVLDLSENFLYKCITKTKAFQGLTQLRQLNLSFNYHKRVSFAHLSLAPSFGSLVALKELDMHGIFFRSLDETTLRPLAHLPVLQTLRLQMNFISQAQLGIFRAFPGLRYVDLSDNR
dog	PNTFGHLSHLEGLVLRDSSLYSLDPRWFHGLGNLMVLDLSENFLYDCITKTKAFYGLARLRRLNLSFNYHKKVSFAHLHLASSFGSLLSLQELDIHGIFFRSLSKTTLQSLAHLPMLQRLHLQLNFISQAQLSIFGAFPGLRYVDLSDNR
mouse	PETFHHLSHLEGLVLKDSSLHTLNSSWFQGLVNLSVLDLSENFLYESITHTNAFQNLTRLRKLNLSFNYRKKVSFARLHLASSFKNLVSLQELNMNGIFFRLLNKYTLRWLADLPKLHTLHLQMNFINQAQLSIFGTFRALRFVDLSDNR
rat	PQTFRHLSHLEGLVLKDSSLHSLNSKWFQGLVNLSVLDLSENFLYESINKTSAFQNLTRLRKLDLSFNYCKKVSFARLHLASSFKSLVSLQELNMNGIFFRLLNKNTLRWLAGLPKLHTLHLQMNFINQAQLSVFSTFRALRFVDLSNNR
treeshrew	PYTFSHLSHLEGLVLKDNSLYSLNATWFHGLDNLTTLDLSENFLYDCINKTTAFRSLARLRKLNLAFNYQKGMSISHLHLAPSFGNLTSLQELDMHGIFFHSLSKTTLQLLARLPSLQTLHLEMNFITQAPLSVFGNFSSLRFVDLSNNR
	600
human	ISGAS--ELTATMGEA--DGGEKVWLQPGDLAPAPVDTPSSEDFRPNCSTLNFTLDLSRNNLVTVQPEMFAQLSHLQCLRLSHNCISQAVNGSQFLPLTGLQVLDLSHNKLDLYHEHSFTELPRLEALDLSYNSQPFGMQGVGHNFSFVA
rhesus	ISGAS--ELTATMQEV--VGGEKVWLQPGDLAPAPVDTPSSEDFRPNCSTLNFTLDLSRNNLVTVRPEMFAQLSHLQCLRLSHNCISQAVNGSQFLPLTSLQVLDLSHNKLDLYHEHSFTELPRLEALDLSYNSQPFGMQGVGHNFSFVA
dog	ISGAA--EPAAATGEVEADCGERVWPQSRDLALGPLGTPGSEAFMPSCRTLNFTLDLSRNNLVTVQPEMFVRLARLQCLGLSHNSISQAVNGSQFVPLSNLRVLDLSHNKLDLYHGRSFTELPRLEALDLSYNSQPFSMRGVGHNLSFVA
mouse	ISGPS--TLSEATPEE-ADDAEQEELLSADPHPAPLSTPASKNFMDRCKNFKFTMDLSRNNLVTIKPEMFVNLSRLQCLSLSHNSIAQAVNGSQFLPLTNLQVLDLSHNKLDLYHWKSFSELPQLQALDLSYNSQPFSMKGIGHNFSFVT
rat	ISGPP--TLSRVAPEK-ADEAEKGVPWPASLTPALPSTPVSKNFMVRCKNLRFTMDLSRNNLVTIKPEMFVNLSHLQCLSLSHNCIAQAVNGSQFLPLTNLKVLDLSYNKLDLYHSKSFSELPQLQALDLSYNSQPFSMQGIGHNFSFLA
treeshrew	ISGVSKRKPAAATGEA---DSKEVWVQSQDFAPAPLEAPRSKDFMQNCSKSSFTLDLSRNTLVTVRPEMFEGLAHLQCLRLSYNCIAQTPSGKEFRPLQSLRVLDLSHNKLDLYNEHSFTELPCLEALDLSYNSQPFGMQGVGHNFSFVT
	750
human	HLRTLRHLSLAHNNIHSQVSQQLCSTSLRALDFSGNALGHMWAEGDLYLHFFQGLSGLIWLDLSQNRLHTLLPQTLRNLPKSLQVLRLRDNYLAFFKWWSLHFLPKLEVLDLAGNQLKALTNGSLPAGTRLRRLDVSCNSISFVAPGFFS
rhesus	HLRTLRHLSLAHNNIHSQVSQQLCSTSLRALDFSGNALGRMWAEGDLYLHFFQGLSGLIWLDLSQNRLHTLLPHTLDKLPKSLQVLHLRDNYLAFFKWGNLIHLPKLKVLDLAGNQLKALTNGSLPAGTRLRRLDVSCNSISFVDPGFFS
dog	QLPALRYLSLAHNGIHSRVSQQLRSASLRALDFSGNTLSQMWAEGDLYLRFFQGLRSLVQLDLSQNRLHTLLPRNLDNLPKSLRLLRLRDNYLAFFNWSSLALLPKLEALDLAGNQLKALSNGSLPNGTQLQRLDLSGNSIGFVVPSFFA
mouse	HLSMLQSLSLAHNDIHTRVSSHLNSNSVRFLDFSGNGMGRMWDEGGLYLHFFQGLSGLLKLDLSQNNLHILRPQNLDNLPKSLKLLSLRDNYLSFFNWTSLSFLPNLEVLDLAGNQLKALTNGTLPNGTLLQKLDVSSNSIVSVVPAFFA
rat	NLSRLQNLSLAHNDIHSRVSSRLYSTSVEYLDFSGNGVGRMWDEEDLYLYFFQDLRSLIHLDLSQNKLHILRPQNLNYLPKSLTKLSFRDNHLSFFNWSSLAFLPNLRDLDLAGNLLKALTNGTLPNGTLLQKLDVSSNSIVFVVPAFFA
treeshrew	RLRNLSNLSLAHNNIHSRVSPRLCSTSLQNLDFSGNSLSRMWAEGDLYLNFFHDLTNLCQLDLSQNNLHTLLPRTLARLPKGLQRLYLRDNYLAFFNWSSLAFLSELQELDLAGNQLKALANGSLPNGTKLHTLDLSSNSISFVVPGFFA
	900
human	KAKELRELNLSANALKTVDHSWFGPLASALQILDVSANPLHCACGAAFMDFLLEVQAAVPGLPSRVKCGSPGQLQGLSIFAQDLRLCLDEALSWDCFALSLLAVALGLGVPMLHHLCGWDLWYCFHLCLAWLPW--RGRQSGRDEDALPY
rhesus	KAKELRELNLSANALKTVDPSWFGPLASALQILDVSANPLHCACGAAFIDFLLEVQAAVPGLPSRVKCGSPGQLQGLSIFAQDLRLCLDEALSWDCFTLSLLSVALGLGVPMLHHLCGWDLWYCFHLCLAWLPW--RGRQSGQGEDALPY
dog	LAVRLRELNLSANALKTVEPSWFGSLAGALKVLDVTANPLHCACGATFVDFLLEVQAAVPGLPSRVKCGSPGQLQGRSIFAQDLRLCLDEALSWVCFSLSLLAVALSLAVPMLHQLCGWDLWYCFHLCLAWLPR--RGRR--RGVDALAY
mouse	LAVELKEVNLSHNILKTVDRSWFGPIVMNLTVLDVRSNPLHCACGAAFVDLLLEVQTKVPGLANGVKCGSPGQLQGRSIFAQDLRLCLDEVLSWDCFGLSLLAVAVGMVVPILHHLCGWDVWYCFHLCLAWLPLLARSR---RSAQTLPY
rat	LAVELKEVNLSHNILKTVDRSWFGPIVMNLTVLDVSSNPLHCACGAPFVDLLLEVQTKVPGLANGVKCGSPRQLQGRSIFAQDLRLCLDDVLSRDCFGLSLLAVAVGTVLPLLQHLCGWDVWYCFHLCLAWLPLLTRGR---RSAQALPY
treeshrew	LAQNLQVLNLSDNFLMTIEPSWFGSLANNLKILDVTANPLHCACGAVFVDFLLELQNKVPGLPGRVSCGGPGQLQGRSIFQQDLRLCLDEALSWDCFGLSLLVVALGLVVPVLHHLCGWDLWYCFYLCQAWLPR--WGL---RGADALPY
	1050
human	DAFVVFDKTQSAVADWVYNELRGQLEECRGRWALRLCLEERDWLPGKTLFENLWASVYGSRKTLFVLAHTDRVSGLLRASFLLAQQRLLEDRKDVVVLVILSPDGRRSRYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGMALTRDNHHF
rhesus	DAFVVFDKTQSAVADWVYNELRGQLEERRGRWALRLCLEERDWLPGKTLFENLWASVYGSRKTLFVLAHTDRVSGLLRASFLLAQQRLLEDRKDVVVLVILSPDGRRSRYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGMALTRDNHHF
dog	DAFVVFDKAQSSVADWVYNELRVQLEERRGRRALRLCLEERDWVPGKTLFENLWASVYSSRKTLFVLARTDRVSGLLRASFLLAQQRLLEDRKDVVVLVILCPDAHRSRYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGTALTRDNRHF
mouse	DAFVVFDKAQSAVADWVYNELRVRLEERRGRRALRLCLEDRDWLPGQTLFENLWASIYGSRKTLFVLAHTDRVSGLLRTSFLLAQQRLLEDRKDVVVLVILRPDAHRSRYVRLRQRLCRQSVLFWPQQPNGQGGFWAQLSTALTRDNRHF
rat	DAFVVFDKAQSAVADWVYNELRVRLEERRGRRALRLCLEDRDWLPGQTLFENLWASIYGSRKTLFVLAHTDKVSGLLRTSFLLAQQRLLEDRKDVVVLVILRPDAHRSRYVRLRQRLCRQSVLFWPHQPNGQGSFWAQLSTALTRDNHHF
treeshrew	DAFVVFDKAQSAVADWVYNELRGRLEERRGRRALRLCLEERDWLPGKTLFENLWASVYGSHKTLFVLAHTDRVSGLLRAGFLLAQQRLLEDRMDVVVLVILRPDARRSRYVRLRQRLCRHSVLLWPHQPGGQGRFWAQLGTALTRDNRHF

human	YNRNFCQGPTAE
rhesus	YNRNFCQGPTAE
dog	YNQNFCRGPTTA
mouse	YNQNFCRGPTAE
rat	YNRNFCRGPTAE
treeshrew	YNQNFCRGPAAE

Figure S2. Sequence alignment of tTLR1-tTLR9 amino acid sequences from 6 mammalian species (human, rat, mouse, dog, tree shrew, macaque; Table S2). Positively selected sites of tTLR8 and tTLR9 are marked in red.

Signal sequence
```
hTLR8   MENMFLQSSMLTCIFLLISGSCELCA
mTLR8   MENMPPQSWILTCFCLLSSGTSAIFH
tTLR8   ---MSLQSSILTCLFLLTSGSCEFFI
```
LRRNT
```
hTLR8   EENFSRSYPCDEKKQNDSVIAECSNRRLQEVPQTVGK   63
mTLR8   KANYSRSYPCDEIRHNSLVIAECNHRQLHEVPQTIGK
tTLR8   ERNYFRSYPCDEKRQNDSIIAECNXLRLQEVXQTVGK
```
LRR1
```
hTLR8   YVTELDLSDNFITHITNESFQGLQ   87
mTLR8   YVTNIDLSDNAITHITKESFQKLQ
tTLR8   YVTELDLSDNFITHITNESFQGLQ
```
LRR2
```
hTLR8   NLTKINLNHNPNVQHQNGNPGIQSNGLNITDGAFLNLK   125
mTLR8   NLTKIDLNHNAKQQH----PNENKNGMNITEGALLSLR
tTLR8   SLTKINLNHNSANLR---NP--NKNGMNITDGAFLNLK
```
LRR3
```
hTLR8   NLRELLLEDNQLPQIPSGLPE   146
mTLR8   NLTVLLLEDNQLYTIPAGLPE
tTLR8   NLRELLLEDNQLIKIPTGLPE
```
LRR4
```
hTLR8   SLTELSLIQNNIYNITKEGISRLI   170
mTLR8   SLKELSLIQNNIFQVTKNNTFGLR
tTLR8   SLRELSLIQNNIVLVTKKDTLGLK
```
LRR5
```
hTLR8   NLKNLYLAWNCYFNKVCEKT-NIEDGVFETLT   201
mTLR8   NLERLYLGWNCYF--KCNQTFKVEDGAFKNLI
tTLR8   KLQSLYLGWNCYF-DCNKTFHIEEGTFENLTT
```
LRR6
```
hTLR8   NLELLSLSFNSLSHVPPKLPS   222
mTLR8   HLKVLSLSFNNLFYVPPKLPS
tTLR8   DLRVLSLSFNHLYHVPPKLLI
```
LRR7
```
hTLR8   SLRKLFLSNTQIKYISEEDFKGLI   246
mTLR8   SLRKLFLSNAKIMNITQEDFKGLE
tTLR8   SLRKLFLSNTNIKNITEEDFKGLR
```
LRR8
```
hTLR8   NLTLLDLSGNCPRCFNAPFPCVPCDGGASINIDRFAFQNLT   287
mTLR8   NLTLLDLSGNCPRCYNAPFPCTPCKENSSIHIHPLAFQSLT
tTLR8   NLRLLDLSGNCPRCFNAPFPCKPCEKDASIQIQPLAFQNLT
```
LRR9
```
hTLR8   QLRYLNLSSTSLRKINAAWFKNMP   311
mTLR8   QLLYLNLSSTSLRTIPSTWFENLS
tTLR8   QLRYLNLSSTSLRTICATWFDNMP
```
LRR10
```
hTLR8   HLKVLDLEFNYLVGEIASGAFLTMLP   337
mTLR8   NLKELHLEFNYLVQEIASGAFLTKLP
tTLR8   HLKVLHLEFNYLIQEIASGAFLTKLD
```
LRR11
```
hTLR8   RLEILDLSFNYIKGSYPQHINISRNFSKLL   367
mTLR8   SLQILDLSFNFQYKEYLQFINISSNFSKLR
tTLR8   YLETLDLSFNYVKTEYPQYINISKNFSKLR
```
LRR12
```
hTLR8   SLRALHLRGYVFQELREDDFQPLMQLP   394
mTLR8   SLKKLHLRGYVFRELKKKHFEHLQSLP
tTLR8   HLKSLHLRGYVFQKLRKEDFQPLMNLS
```
LRR13
```
hTLR8   NLSTINLGINFIKQIDFKLFQNFS   418
mTLR8   NLATINLGINFIEKIDFKAFQNFS
tTLR8   RLKTINLGINFIKQIDFTLFQQFS
```
LRR14
```
hTLR8   NLEIIYLSENRISPLVKDTRQSY   441
mTLR8   KLDVIYLSGNRIASVLDGT----
tTLR8   NLKIIYLSENRISPLVNDIRQNN
```
Z-loop
```
hTLR8   ANSSSFQRHIRKRRSTDF-EFDPHSNFYHFTRPLIKPQCAA   481
mTLR8   -DYSSWRNRLRKPLSTDDDEFDPHVNFYHSTKPLIKPQCTA
tTLR8   TNGLSFQSHTLKTRSADT-NFDPHSNFYHRTTPLIKPQCTA
```
LRR15
```
hTLR8   YGKALDLSLNSIFFIGPNQFENLP   505
mTLR8   YGKALDLSLNNIFIIGKSQFEGFQ
tTLR8   YGKSLDLSLNSIFFIGEKQFEGFT
```
LRR16
```
hTLR8   DIACLNLSANSNAQVLSGTEFSAIP   530
mTLR8   DIACLNLSFNANTQVFNGTEFSSMP
tTLR8   DIACLNLSSNGNGQVLHGNEFSAMR
```
LRR17
```
hTLR8   HVKYLDLTNNRLDFDNASALTELS   554
mTLR8   HIKYLDLTNNRLDFDDNNAFSDLH
tTLR8   GVKYLDLTNNRLDFDDDKTLQDLP
```
LRR18
```
hTLR8   DLEVLDLSYNSHYFRIAGVTHHLEFIQNFT   584
mTLR8   DLEVLDLSHNAHYFSIAGVTHRLGFIQNLI
tTLR8   YLEVLDLSYNAHYFRIAGVTHRLGFIQNLT
```
LRR19
```
hTLR8   NLKVLNLSHNNIYTLTDKYNLESK   608
mTLR8   NLRVLNLSHNGIYTLTEESELKSI
tTLR8   QLKVLNLSYNSIYTLTE-YELKSL
```
LRR20
```
hTLR8   SLVELVFSGNRLDILWNDDDNRYISIFKGLK   639
mTLR8   SLKELVFSGNRLDRLWNANDGKYWSIFKSLQ
tTLR8   SLEELVFSGNRLDLLWNAEDGRYITIFKGLV
```
LRR21
```
hTLR8   NLTRLDLSLNRLKHIPNEAFLNLPA   664
mTLR8   NLIRLDLSYNNLQQIPNGAFLNLPQ
tTLR8   NLTRLDISFNNLQRIPDEAFLNLPQ
```
LRR22
```
hTLR8   SLTELHINDNMLKFFNWTLLQQFP   688
mTLR8   SLQELLISGNKLRFFNWTLLQYFP
tTLR8   NLTKLYINDNMLNFFNWTLLQYFP
```
LRR23
```
hTLR8   RLELLDLRGNKLLFLTDSLSDFTS   712
mTLR8   HLHLLDLSRNELYFLPNCLSKFAH
tTLR8   QLHLLDLSRNKLSLVTHSLSTFTT
```
LRR24
```
hTLR8   SLRTLLLSHNRISHLPSGFLSEVS   736
mTLR8   SLETLLLSHNHFSHLPSGFLSEAR
tTLR8   SLQKLLLSQNRISHLPSGFLSGAS
```
LRR25
```
hTLR8   SLKHLDLSSNLLKTINKSALETKTTT   762
mTLR8   NLVHLDLSFNTIKMINKSSLQTKMKT
tTLR8   SLVHLDLRSNLLRMLNKSTLQTKTTT
```
LRR26
```
hTLR8   KLSMLELHGNPFE   775
mTLR8   NLSILELHGNYFD
tTLR8   NLAVLELGRNPLD
```
LRRCT
```
hTLR8   CTCDIGDFRRWMDEHLNVKIPRLVDVICASPGDQRGKSIVSLELTTCVSDVT   827
mTLR8   CTCDISDFRSWLDENLNITIPKLVNVICSNPGDQKSKSIMSLDLTTCVSDTT
tTLR8   CTCDIGDFQSWMDENPNITIPRLIDVICDSPGDQRGKSIVSLELTTCVSDTI
```

Figure S3. Sequence alignment of human (h), mouse (m) and tree shrew (t) TLR8 on the basis of human TLR8 structure (Tanji et al., 2013). Sequence alignments were displayed for each LRR module. Positively selected sites were indicated by red.

Signal sequence
```
hTLR9  MGFCRSALHPLSLLVQAIMLAMTLA                 25
mTLR9  MVLRRRTLHPLSLLVQAAVLAETLA
tTLR9  MGPCSSALQPLSLLVWAAVLAVGLG
```
LRRNT
```
hTLR9  LGTLPAFLPCELQPHGLVNCNWLFLKSVPHFSMAAPRG    63
mTLR9  LGTLPAFLPCELKPHGLVDCNWLFLKSVPRFSAAASCS
tTLR9  LGTLPAFLPCEFRDPGLVNCNWLFLKSVPRFK-AASRN
```
LRR1
```
hTLR9  NVTSLSLSSNRIHHLHDSDFAHLP                  87
mTLR9  NITRLSLISNRIHHLHNSDFVHLS
tTLR9  NITSLSLLSNRIHHLHDSDFAHLP
```
LRR2
```
hTLR9  SLRHLNLKWNCPPVGLSPMHFPCHMTIEPSTFLAVP      123
mTLR9  NLRQLNLKWNCPPTGLSPLHFSCHMTIEPRTFLAMR
tTLR9  NLRRLNLKWNCPPAGLSPMHFPCHMTIERNTFLAVP
```
LRR3
```
hTLR9  TLEELNLSYNNIMTVPALPK                      143
mTLR9  TLEELNLSYNGITTVPRLPS
tTLR9  TLEELNLSYNGISTVPALPS
```
LRR4
```
hTLR9  SLISLSLSHTNILMLDSASLAGLH                  167
mTLR9  SLVNLSLSHTNILVLDANSLAGLY
tTLR9  SLVFLSLSRTNILTLGPASLAGLH
```
LRR5
```
hTLR9  ALRFLFMDGNCYYKNPCRQALEVAPGALLGLG          199
mTLR9  SLRVLFMDGNCYYKNPCTGAVKVTPGALLGLS
tTLR9  SLRFLFIDGNCYYKNPCGRALEVAPGALANLS
```
LRR6
```
hTLR9  NLTHLSLKYNNLTVVPRNLPS                     220
mTLR9  NLTHLSLKYNNLTKVPRQLPP
tTLR9  NLTRLSLKYNNXTAVPQNLPP
```
LRR7
```
hTLR9  SLEYLLLSYNRIVKLAPEDLANLT                  244
mTLR9  SLEYLLVSYNLIVKLGPEDLANLT
tTLR9  SLEYLLLSYNHIVKLAPQDLANLT
```
LRR8
```
hTLR9  ALRVLDVGGNCRRCDHAPNPCMECPRHFPQLHPDTFSHLS  284
mTLR9  SLRVLDVGGNCRRCDHAPNPCIECGQKSLHLHPETFHHLS
tTLR9  ALRVLDVGGNCRRCDHARNPCVECPLGFPQLHPYTFSHLS
```
LRR9
```
hTLR9  RLEGLVLKDSSLSWLNASWFRGLG                  308
mTLR9  HLEGLVLKDSSLHTLNSSWFQGLV
tTLR9  HLEGLVLKDNSLYSLNATWFHGLD
```
LRR10
```
hTLR9  NLRVLDLSENFLYKCITKTKAFQGLT                334
mTLR9  NLSVLDLSENFLYESITHTNAFQNLT
tTLR9  NLTTLDLSENFLYDCINKTTAFRSLA
```
LRR11
```
hTLR9  QLRKLNLSFNYQKRVSFAHLSLAPSFGSLV            364
mTLR9  RLRKLNLSFNYRKKVSFARLHLASSFKNLV
tTLR9  RLRKLNLAFNYQKGMSISHLHLAPSFGNLT
```
LRR12
```
hTLR9  ALKELDMHGIFFRSLDETTLRPLARLP               391
mTLR9  SLQELNMNGIFFRLLNKYTLRWLADLP
tTLR9  SLQELDMHGIFFHSLSKTTLQLLARLP
```
LRR13
```
hTLR9  MLQTLRLQMNFINQAQLGIFRAFP                  415
mTLR9  KLHTLHLQMNFINQAQLSIFGTFR
tTLR9  SLQTLHLEMNFITQAPLSVFGNFS
```
LRR14
```
hTLR9  GLRYVDLSDNRISGAS--ELT                     434
mTLR9  ALRFVDLSDNRISGPS--TLS
tTLR9  SLRFVDLSNNRISGVSKRKPA
```
Z-loop
```
hTLR9  ATMGEA--DGGEKVWLQPGDLAPAPVDTPSSEDFRPNCST  472
mTLR9  EATPEE-ADDAEQEELLSADPHPAPLSTPASKNFMDRCKN
tTLR9  AATGEA---DSKEVWVQSQDFAPAPLEAPRSKDFMQNCSK
```
LRR15
```
hTLR9  LNFTLDLSRNNLVTVQPEMFAQLS                  496
mTLR9  FKFTMDLSRNNLVTIKPEMFVNLS
tTLR9  SSFTLDLSRNTLVTVRPEMFEGLA
```
LRR16
```
hTLR9  HLQCLRLSHNCISQAVNGSQFLPLT                 521
mTLR9  RLQCLSLSHNSIAQAVNGSQFLPLT
tTLR9  HLQCLRLSYNCIAQTPSGKEFRPLQ
```
LRR17
```
hTLR9  GLQVLDLSHNKLDLYHEHSFTELP                  545
mTLR9  NLQVLDLSHNKLDLYHWKSFSELP
tTLR9  SLRVLDLSHNKLDLYNEHSFTELP
```
LRR18
```
hTLR9  RLEALDLSYNSQPFGMQGVGHNFSFVAHLR            575
mTLR9  QLQALDLSYNSQPFSMKGIGHNFSFVTHLS
tTLR9  CLEALDLSYNSQPFGMQGVGHNFSFVTRLR
```
LRR19
```
hTLR9  TLRHLSLAHNNIHSQVSQQLCST                   598
mTLR9  MLQSLSLAHNDIHTRVSSHLNSN
tTLR9  NLSNLSLAHNNIHSRVSPRLCST
```
LRR20
```
hTLR9  SLRALDFSGNALGHMWAEGDLYLHFFQGLS            628
mTLR9  SVRFLDFSGNGMGRMWDEGGLYLHFFQGLS
tTLR9  SLQNLDFSGNSLSRMWAEGDLYLNFFHDLT
```
LRR21
```
hTLR9  GLIWLDLSQNRLHTLLPQTLRNLPK                 653
mTLR9  GLLKLDLSQNNLHILRPQNLDNLPK
tTLR9  NLCQLDLSQNNLHTLLPRTLARLPK
```
LRR22
```
hTLR9  SLQVLRLRDNYLAFFKWWSLHFLP                  677
mTLR9  SLKLLSLRDNYLSFFNWTSLSFLP
tTLR9  GLQRLYLRDNYLAFFNWSSLAFLS
```
LRR23
```
hTLR9  KLEVLDLAGNQLKALTNGSLPAGT                  701
mTLR9  NLEVLDLAGNQLKALTNGTLPNGT
tTLR9  ELQELDLAGNQLKALANGSLPNGT
```
LRR24
```
hTLR9  RLRRLDVSCNSISFVAPGFFSKAK                  725
mTLR9  LLQKLDVSSNSIVSVVPAFFALAV
tTLR9  KLHTLDLSSNSISFVVPGFFALAQ
```
LRR25
```
hTLR9  ELRELNLSANALKTVDHSWFGPLAS                 750
mTLR9  ELKEVNLSHNILKTVDRSWFGPIVM
tTLR9  NLQVLNLSDNFLMTIEPSWFGSLAN
```
LRR26
```
hTLR9  ALQILDVSANPLH                             763
mTLR9  NLTVLDVRSNPLH
tTLR9  NLKILDVTANPLH
```
LRRCT
```
hTLR9  CACGAAFMDFLLEVQAAVPGLPSRVKCGSPGQLQGLSIFAQDLRLCLDEALSWDC  818
mTLR9  CACGAAFVDLLLEVQTKVPGLANGVKCGSPGQLQGRSIFAQDLRLCLDEVLSWDC
tTLR9  CACGAVFVDFLLELQNKVPGLPGRVSCGGPGQLQGRSIFQQDLRLCLDEALSWDC
```

Figure S4. Sequence alignment of human (h), mouse (m) and tree shrew (t) TLR9 on the basis of human TLR9 structure (Ohto et al., 2015). Sequence alignments were displayed for each LRR module. Positively selected sites were indicated by red.

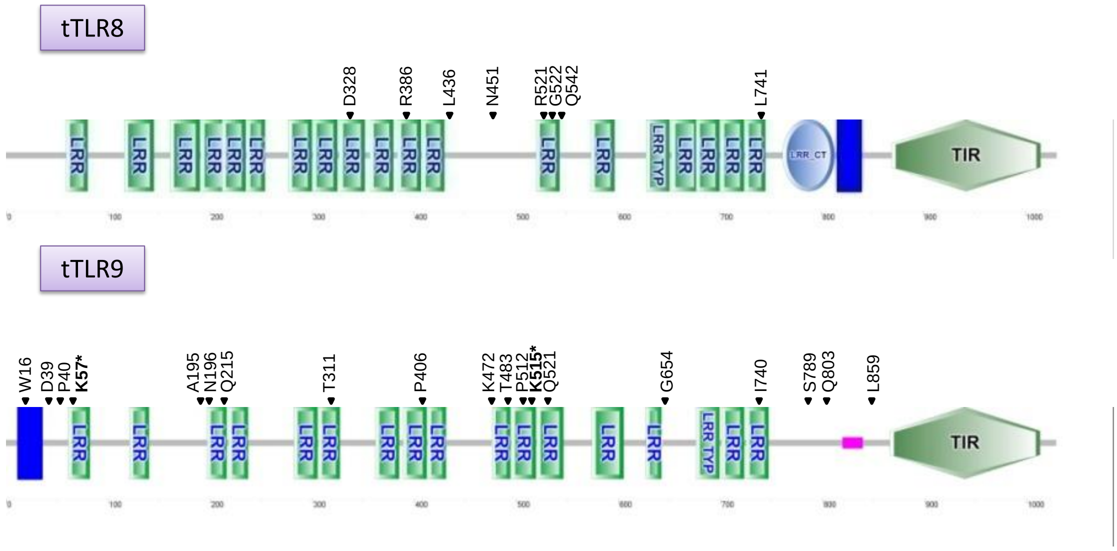

Figure S5. Diagram illustrating domain structures of the tTLR8 and tTLR9 and their positively selected sites in the Chinese tree shrew. tTLR8 and tTLR9 have LRR repeat in the N-terminal region, transmembrane region (mandarin blue pane), and the TIR (Toll/IL-1 receptor) domain at C-terminal end. NT: N-(amino) terminal. CT: C-(carboxyl) terminal. The low complexity region was marked in pink. LRR TYP: leucine-rich repeats, typical (most populated) subfamily. Positively selected sites are marked in black triangle.

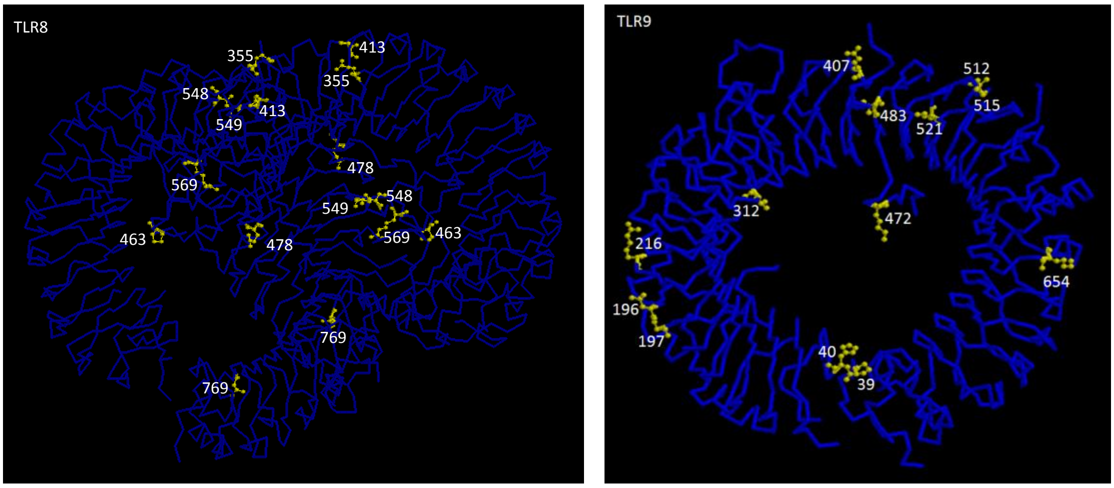

Figure S6. Positively selected sites in the three dimensional structures of TLR8 (Tanji et al., 2013) and TLR9 (Ohto et al., 2015). Positively selected sites equivalent codons in human were colored in yellow.

Supplementary references

Ohto, U., Shibata, T., Tanji, H., Ishida, H., Krayukhina, E., Uchiyama, S., Miyake, K., Shimizu, T., 2015. Structural basis of CpG and inhibitory DNA recognition by Toll-like receptor 9. Nature. 520, 702-705.

Tanji, H., Ohto, U., Shibata, T., Miyake, K., Shimizu, T., 2013. Structural reorganization of the Toll-like receptor 8 dimer induced by agonistic ligands. Science. 339, 1426-1429.