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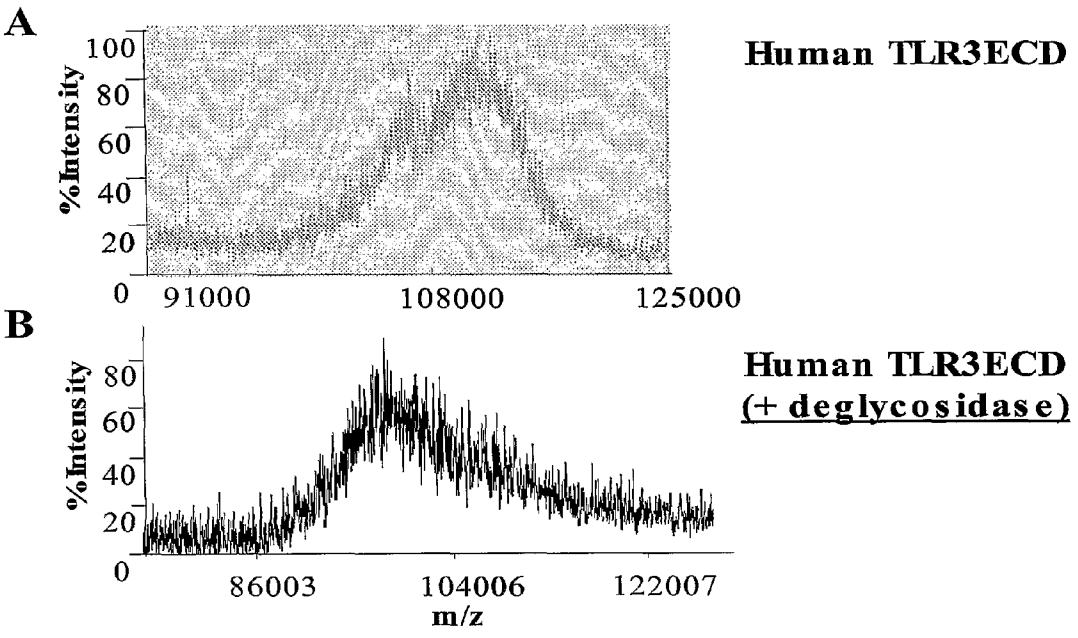
(54) **Title:** TLR3 GLYCOSYLATION SITE MUTEINS AND METHODS OF USE




(57) **Abstract:** TLR3 glycosylation site muteins, nucleic acids encoding the muteins, and methods of modulating TLR3 activity in a cell are disclosed.

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# TLR3 GLYCOSYLATION SITE MUTEINS AND METHODS OF USE

## Field of the Invention

The present invention relates to TLR3 glycosylation site muteins, nucleic acids encoding the muteins, and methods of modulating TLR3 activity in a cell.

## Background of the Invention

Pathologies associated with inflammatory conditions represent a significant challenge in health care and can be painful, debilitating and lethal. For example, sepsis and sepsis-associated conditions affect more than 750,000 people annually in the U.S. with mortality rates of 28-50%, resulting in 215,000 annual deaths (Natanson *et al.*, *Crit. Care Med. 26*:1927-1931 (1998); Angus *et al.*, *Crit. Care Med. 29*:1303-1310 (2001)). Other inflammatory conditions such as the inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis affect more than 1 million people per year in the U.S. (Hanauer *et al.*, *Rev. Gastroenterol. Disord. 3*:81-92 (2003)).

Inflammatory pulmonary conditions affecting lung function such as chronic obstructive pulmonary disease (COPD), asthma and lung infections also affect significant numbers of people in the U.S. COPD, for example, affects an estimated 10 million adult Americans and the prevalence is rising (Mapel *et al.*, *Manag. Care Interface 17*:61-66 (2004)). Pathologies associated with these inflammatory conditions and exacerbations of these conditions have significant health and economic impacts.

Exacerbation in pulmonary diseases such as asthma and COPD is characterized by the worsening of symptoms and a decline in lung function. Viral infections are associated with exacerbations of many pulmonary diseases (Johnston, *Am. J. Respir. Crit. Care Med. 152*: S46-52 (1995); Bandi *et al*, *FEMS Immunol. Med. Microbiol. 37*: 69-75 (2003)) and are believed to be a major cause of exacerbations. Secretion of pro-inflammatory cytokines in the lungs following viral infection represents a

crucial step in promoting the inflammatory response in various lung diseases (Gern *et al.*, *Am. J. Respir. Cell. Mol. Biol.* *28*:731-737 (2003); Panina-Bordignon *et al.*, *Curr. Opin. Pulm. Med.* *9*:104-110 (2003)).

Recognition of microbial antigens by the host immune system is mediated through innate immune receptors, whose activation represents an important step in the initiation of an inflammatory response. Toll-Like Receptors (TLR) represent a family of innate immune receptors that play a crucial role in mediating an immune response to foreign antigens. TLR3, for example, is a mammalian pattern recognition receptor that recognizes double-stranded (ds) RNA as well as the synthetic ds RNA analog poly-riboinosinic-ribocytidylic acid (poly I:C), (Alexopoulou *et al.*, *Nature 413*: 732-238 (2001)). Moreover, TLR3 has been shown to recognize endogenous ligands such as mRNA released from necrotic cells (Kariko *et al.*, *J. Biol. Chem. 26*: 12542-12550 (2004)) indicating that necrotic cell death at inflammation sites may contribute to activation of TLR3. A full-length human TLR3 amino acid sequence and encoding polynucleotide sequence is shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Activation of TLR3 by the ds viral RNA analog poly(I:C) or by endogenous mRNA ligands induces secretion of pro-inflammatory cytokines and chemokines, a finding that indicates TLR3 activation modulates disease outcome during infection-associated inflammation. Thus, TLR3 ligation *in vivo* is thought to occur in the context of viral infection (Tabeta *et al.*, *Proc. Natl. Acad. Sci. USA 101*:3516-3521 (2004)) or necrosis associated with inflammation (Kariko *et al.*, *J. Biol. Chem. 26*: 12542-12550 (2004)). Overall, these data demonstrate that ligation of TLR3 initiates cascades of phosphorylation and transcriptional activation events that result in the production of numerous inflammatory cytokines that are thought to contribute to innate immunity (reviewed by Takeda and Akira, *J. Derm. Sci. 34*:73-82 (2004)). Further, these data suggest that sustained TLR3 activation can be a critical component in the modulation of

infection-associated inflammatory diseases. Published data lend support to this hypothesis as shown by findings that associate over-production of pro-inflammatory cytokines with systemic inflammatory response syndrome, infection-associated acute cytokine storms (reviewed by Van Amersfoort *et al.*, *Clin. Microbiol. Rev. 16*: 379-414 (2003)) and immune-mediated chronic conditions such as rheumatoid arthritis (reviewed by Miossec *et al.*, *Curr. Opin. Rheumatol. 16*:218-222 (2004)) and inflammatory bowel diseases (reviewed by Ogata and Hibi, *Curr. Pharm. Des. 9*: 1107-1113 (2003)).

Importantly, it is becoming clear that TLR3 activity also plays a significant role in conditions such as inflammatory bowel disease symptoms, sepsis, cytokine, chemokine, and growth factor mediated lung pathologies and pulmonary inflammatory conditions resulting from increased inflammatory cell infiltration into lung tissues. However, it has been unclear what, if any, effect TLR3 glycosylation has on TLR3 activity and TLR3 activity mediated conditions.

Thus, a need exists to understand the effect of TLR3 glycosylation on TLR3 activity and exploit this information to develop compositions and methods that effectively modulate TLR3 activity.

## Brief Description of the Drawings

Fig. 1 panel (A) shows MALDI-TOF mass spectra of hTLR3 extracellular domain (ECD) and panel (B) shows MALDI-TOF mass spectra of hTLR3 ECD treated with deglycosidases.

Fig. 2 shows the effect of N-glycosylation with tunicamycin onpoly(I:C) induced activation of hTLR3 signaling in HEK293 cells.

Fig. 3 shows the effect of ECD N-glycosylation by mutagenesis of N247, N252, or N413 in hTLR3 (SEQ ID NO: 2) on activation of hTLR3 signaling in cells. Panel (A) is an alignment of possible N-glycosylation sites from selected hTLR3 homologs; panels (B) and (C) are hTLR3 activation assays

performed with the indicated hTLR3 mutants at 10 μg/ml (B) or 2.5 μg/ml (C) of the hTLR3 ligand poly(I:C).

Fig. 4 shows the effect of hTLR3 ECD N-glycosylation by mutagenesis of N247, N252, or N662 of hTLR (SEQ ID NO: 2) on poly(I:C) induced activation of hTLR3 signaling in cells.

## Summary of the Invention

One aspect of the invention is a peptide chain comprising an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 6 and at least one mutation within 3 amino acid residues of a position aligning to N221, N226, N387, or N636 of the amino acid sequence SEQ ID NO: 6.

Another aspect of the invention is a peptide chain comprising at least one mutation within 3 amino acid residues of position N221, N226, N387, or N636 of the amino acid sequence SEQ ID NO: 6.

Another aspect of the invention is a peptide chain comprising the amino acid sequence SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, or SEQ ID NO: 20.

Another aspect of the invention is a method of modulating TLR3 activity in a cell comprising decreasing TLR3 glycosylation in the cell.

## Detailed Description of the Invention

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though fully set forth.

The term "homolog" means protein sequences having between 40% and 100% sequence identity to a reference sequence. Homologs of hTLR3 include peptide chains from other species that have between 40% and 100% sequence identity to a known hTLR3 sequence. The terms "TLR3 homolog" and "TLR3" are used interchangeably throughout the specification and claims.

The term "peptide chain" as used herein means a molecule comprising at least two naturally or non-naturally occurring amino acid residues linked by peptide bonds.

The term "TLR3 activity" as used herein refers to any activities occurring as a result of ligand binding to a cell surface TLR3 homolog or that are mediated, in whole or in part, by at least one TLR3 homolog peptide chain.

The compositions and methods of the invention are useful in modulating the activity of TLR3 homologs in cells both *in vitro* and *in vivo*. In particular, the compositions and methods of the invention can be used to attenuate *in vivo* TLR3 dependent signaling and biological processes associated with TLR3 activity in conditions such as inflammatory bowel disease symptoms, sepsis, cytokine, chemokine, and growth factor mediated lung pathologies and pulmonary inflammatory conditions resulting from increased inflammatory cell infiltration into lung tissues.

One aspect of the invention is a peptide chain comprising an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 6 and at least one mutation within 3 amino acid residues of a position aligning to N221, N226, N387, or N636 of the amino acid sequence SEQ ID NO: 6. Percent identity between two peptide chains can be determined by alignment using the default settings of the BLASTP 2.2.12 [Aug-07-2005] algorithm with low complexity filtering turned off and using SEQ ID NO: 6 as the BLASTP query sequence. The amino acid sequences of SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, and SEQ ID NO: 20 are exemplary of such peptide chains. Such peptide chains may comprise additional sequences in addition to the mutagenized mature form ECD TLR3 homolog. Such additional sequences may be, for example, signal peptides such as a native signal peptide or the native intracellular and transmembrane domains of the homolog. Those skilled in the art will recognize other such additional sequences such as affinity tag sequences or other sequences that facilitate TLR activity, function or purification.

Such peptide chains may be readily made by determining the percent identity between a TLR3 homolog and SEQ ID NO: 6 as described above, selecting a homolog with at least 75% identity to SEQ ID NO: 6, identifying a position aligning to N221, N226, N387, or N636 of SEQ ID NO: 6, and introducing at least one mutation within 3 residues of the position identified. Importantly, the core consensus glycosylation site motif is the 3 amino acid residue Asn-X-Ser/Thr motif. Such mutations may be substitutions, deletions, or insertions and can be generated using *in vitro* and *in vivo* mutagenesis techniques well know in the art. Such peptide chains may also comprise additional mutations incorporated into the TLR3 homolog that do not occur within 3 amino acid residues of a position aligning to N221, N226, N387, or N636 of the amino acid sequence SEQ ID NO: 6.

Another aspect of the invention is a peptide chain comprising at least one mutation within 3 amino acid residues of position N221, N226, N387, or N636 of the amino acid sequence SEQ ID NO: 6. The amino acid sequences of SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20 are exemplary of such peptide chains. Such mutations may be substitutions, deletions, or insertions and can be generated using *in vitro* and *in vivo* mutagenesis techniques well know in the art. Such peptide chains may also comprise additional sequences as discussed above.

Another aspect of the invention is a peptide chain comprising the amino acid sequence SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, or SEQ ID NO: 20. Such peptide chains may also comprise additional sequences as discussed above.

In one embodiment the invention provides a nucleic acid encoding a peptide chain of the invention.

In another embodiment the invention provides a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, or SEQ ID NO: 19.

The nucleic acids of the invention may be made using *in vivo* or *in vitro* techniques known by those skilled in the art. Such nucleic acids may comprise DNA and RNA. Additionally, such nucleic acids may comprise additional nucleic acid sequences or be conjugated to another class of molecules. For example, such nucleic acids may be inserted into vector nucleic acids or supplied to a cell or system to produce expression of a peptide chain encoded by the nucleic acid. Such cells or systems may be eukaryotic cells, prokaryotic cells, archael cells, or cell free *in vitro* systems such as *in vitro* coupled transcription and translation systems. Techniques for peptide chain expression, introducing nucleic acids into vectors and supplying nucleic acids to cells, and cells or systems suitable for expression of peptide chains encoded by a nucleic acid are well know to those skilled in the art.

Another aspect of the invention is a method of modulating TLR3 activity in a cell comprising decreasing TLR3 glycosylation in the cell using techniques such as gene inactivation or transcript targeting (*e.g.* siRNA). TLR3 glycosylation may decreased, for example, by providing a cell with small molecules that inhibit TLR3 glycosylation such as tunicamycin, providing TLR3 homologs in which a glycosylation site has been modified so that it can no longer be glycosylated, over expressing glycolytic enzymes in the cell, and deceasing or inactivating expression of glycoslyases in the cell. Antibody molecules or antibody fragments may also be used to decrease TLR3 glycosylation and activity. Such antibody molecules or fragments may block ligand or receptor multimerization by binding to the region of cell surface TLR3 containing residue N221, N226, N387 or N636 of SEQ ID NO: 6. In the methods of the invention both N-glycosylation and O-glycosylation of TLR3 homologs may be targeted to decrease TLR3 glycosylation.

One aspect of the invention is a method of modulating TLR3 activity in a cell comprising providing tunicamycin to the cell. Tunicamycin can be provided to a cell *in vitro* via the culture

media (at a concentration of about 0.2 to 0.5 µg/ml or *in vivo* via intravenous injection for example. Those skilled in the art will recognize many other methods and routes by which tunicamycin can be provided to a cell *in vitro* or *in vivo*.

In another embodiment the invention provides a method of modulating TLR3 activity in a cell comprising providing a peptide chain of the invention to the cell. Peptide chains may be provided to a cell by, for example, supplying the peptide chain to the fluid or tissue surrounding a cell, introducing an exogenous nucleic acid expressing the peptide chain into the cell, microinjection of the peptide chain into the cell, or fusing a cell with a second cell or vesicle containing the peptide chain. Those skilled in the art will recognize other means by which to provide a peptide chain to a cell.

In another embodiment the invention provides a method of modulating TLR3 activity in a cell comprising providing a nucleic acid of the invention to the cell. The nucleic acids of the invention may be provided to a cell through the use of viral, plasmid, cellular, or vesicular vectors, microinjection, naturally occurring nucleic acid uptake or competency, conjugation to molecule capable of entering a cell or by any other technique known to those skilled in the art by which nucleic acids may be introduced into a cell.

In another embodiment of the invention the nucleic acid is provided to the cell by transfection. Transfection of nucleic acids into a cell may be accomplished by a variety of techniques such as electroporation, chemical shock, lipofection, vescicle fusion, and other techniques known by those skilled in the art.

The present invention will now be described with reference to the following specific, non-limiting examples.

## Example 1

## Glycosylation of hTLR3 Extracellular Domain

Mass spectroscopic analysis performed on the purified, soluble ECD of hTLR3 recombinantly expressed in *Homo sapiens* derived HEK293 cells (ATCC® number: CRL-1573™) revealed a high degree of charge heterogeneity among ionized hTLR3 ECD fragment species (Fig. 1A) and an average ion fragment mass of 110 kD. Treatment of recombinantly expressed hTLR3 ECD with a mixture of deglycosidases specific for O-linked and N-linked glycosylation followed by mass spectroscopic analysis revealed that deglycosidase treatment decreased the average ion fragment mass to 94 kD (Fig. 1B). Additionally, incubation of hTLR3 ECD with N-acetylneuraminic acid (NANAse), O-glycosidase DS, peptide N-Glycosidase F (PNGase F), or a cocktail containing all of these enzymes decreased glycoprotein specific staining of SDS-PAGE resolved hTLR3 ECD. Together these results indicate that the hTLR3 ECD is both N- and O-glycosylated.

hTLR3 ECD for mass spectroscopic and glycoprotein specific SDS-PAGE analyses were prepared as follows. First, a cDNA (SEQ ID NO: 1) encoding the full-length *Homo sapiens* TLR3 protein (SEQ ID NO: 2) and identical to accession number U88879 was cloned into pcDNA3.1. Next, a cDNA fragment encoding the hTLR3 ECD (SEQ ID NO: 3) and consisting of amino acids 1-703 of full length hTLR3 (SEQ ID NO: 4) was cloned into pcDNA3.1. This cDNA fragment was cloned into pcDNA3.1 in frame and on the 5′ side of a cDNA encoding a hexahistidine affinity tag. The resulting plasmid encodes a recombinant hTLR3 ECD comprising a C-terminal hexahistidine tag.

hTLR3 ECD encoded by this plasmid was expressed, processed, and secreted by transiently transfected HEK293 cells. Cells were transfected and cultured using standard methods. The recombinant hTLR3 ECD with carboxy terminal hexahistidine affinity tag was purified from cell culture supernatant using a Ni-NTA resin and was further purified by ion exchange chromatography using

standard methods. The majority of hTLR3 ECD protein produced by this process is predicted to lack the first 26 amino terminal, signal peptide residues due to post-translational proteolytic processing and secretion. The hTLR3 ECD protein purified comprised amino acid residues 27 to 703 (SEQ ID NO: 6) of full length hTLR3 (SEQ ID NO: 2).

Mass spectroscopic analyses of purified, recombinant hTLR3 ECD were performed as follows. First, samples were concentrated with a Nanosep™ centrifugation concentrator (Pall Corp., East Hills, NY), desalted using C-18 Zip Tips (Millipore Corp., Billerica, MA), and eluted with 50% acetonitrile/0.1% trifluoroacetic acid. 1 µL of each sample was then co-crystallized with the matrix in 2,5-dihydroxybenzoic acid in acetonitrile/water (50:50) containing 0.1% trifluoroacetic acid (TFA). MALDI-TOF experiments were then performed using standard methods and recorded using an ABI Voyager-DE™ STR mass spectrometer. To examine glycosylation, 1 µg of the purified hTLR3 ECD protein was incubated with a mixture of N-glycanase, O-glycanase, and sialidase at 37°C for 24 h. Products of this incubation were then analyzed by MALDI-TOF spectroscopy as described above.

Preparation of purified hTLR3 ECD for SDS-PAGE resolution and glycoprotein specific visualization was performed as follows. First, samples containing recombinantly expressed hTLR3 ECD alone, hTLR3 ECD incubated with N-acetylneuraminic acid (NANAse), hTLR3 ECD incubated with O-glycosidase DS, peptide N-Glycosidase F (PNGase F), or a cocktail containing all of these enzymes and hTLR3 ECD were prepared. Samples containing equal amounts of hTLR3 ECD were then resolved by SDS-PAGE on a 4-12% gradient gel using standard methods and glycoprotein was visualized with the glycoprotein specific SYPRO Ruby protein gel stain (Invitrogen, Inc., Carlsbad, CA).

## Example 2

## Effect of N-Glycosylation on Poly(I:C) Induced Activation of hTLR3 Signaling in Cells

In these experiments, TLR3 signaling was assayed using the pNF-κB-Luciferase (Stratagene, Inc., Carlsbad, CA) reporter gene construct transiently transfected by standard methods into HEK293 cells. This reporter construct comprised an NF-κB responsive DNA element linked to a luciferase reporter gene. Activation of TLR3 by poly(I:C) ligand increases NF-κB activity and results in activation of NF-κB responsive genes such as the luciferase reporter gene. HEK293 cells transfected with pNF-κB-Luciferase were transiently co-transfected with the control vector pHRL-TK constitutively producing a luciferase protein derived from *Renilla*. A cDNA encoding full-length hTLR3 was also transiently co-transfected, using standard methods, into HEK-293 cells transfected with the luciferase reporter gene.

After transfection, cells were treated with non-toxic doses of tunicamycin as shown in Fig. 2. Tunicamycin inhibits N-linked glycosylation by inhibiting the attachment of N-acetylglucoasamine which is the first sugar residue attached to a peptide chain during glycosylation. Treated or control HEK293 cells were then incubated with 10 μg/ml of the hTLR3 ligand poly(I:C) (PIC) as indicated in Fig. 2.

After treatment, luciferase expressed from pNF-κB-Luciferase and pHRL-TK was assayed using standard methods. Data was expressed as a "luciferase ratio" equal to the pNF-κB-Luciferase activity normalized to pHRL-TK luciferase activity. Results are presented as scatter plots of data collected from 9 individual samples and is representative of 3 independently conducted, identical experiments.

## Example 3

## Effect of hTLR3 ECD N-Glycosylation Inhibition on Poly(I:C) Induced Activation of hTLR3 Signaling in Cells

Surprisingly, two potential N-glycosylation sites, N247 and N413, of the many possible sites within the hTLR3 sequence of SEQ ID NO: 2 were found to play a critical role in hTLR3 signaling (Fig. 3C). Additionally, two other potential N-glycosylation sites in SEQ ID NO: 2, N252 and N662, were also found to play an important role in hTLR signaling (Fig. 3B and Fig. 4). Positions N247, N252, N413, and N662 in SEQ ID NO: 2 are respectively equivalent to N221, N226, N387, and N636 of SEQ ID NO: 6.

The hTLR3 ECD has several potential N-linked glycosylation sites, based on the presence of the N-glycosylation motif Asn-X-Ser/Thr (Fig. 3A) in various TLR3 homologs. Only five of these potential N-linked glycosylation sites were conserved between *Homo sapiens, Pan troglodytes, Canis familiaris, Bos taurus, Rattus norvegicus* and *Mus musculus* TLR3 homologs (Fig. 3A) as assessed by standard CLUSTALW alignment. The five conserved sites in SEQ ID NO: 2 were N57, N196, N247, N275, and N413. Four other potential N-glycosylation sites in SEQ ID NO: 2 varied between the various TLR3 homologs examined (Fig. 3A). These four other potential, albeit non-conserved, N-glycosylation sites were N252, N265, N291, N507, N636, and N662 of SEQ ID NO: 2.

The asparagines residues within these potential N-linked glycosylation sites in the hTLR3 ECD were individually mutated using standard methods to alanine residues (Fig. 3 and Fig. 4). Four additional asparagine residues present in the hTLR3 ECD that do not contain a match to the Asn-X-Ser/Thr N-glycosylation motif in any TLR3 homolog were also mutated as a control. These residues were N70, N124, and N388 of full length hTLR3 (SEQ ID NO: 2). All mutagenesis was performed on cDNAs encoding full-length hTLR3 in order to produce full-length, processed, and secreted hTLR3 with a mutagenized ECD. Mutated positions in SEQ

ID NO: 2 are indicated in the figures by identification of the position of the alanine substitution (*e.g.* N70 means N70 has been replaced with an alanine residue).

Plasmids encoding and expressing the mutant hTLR3 cDNA constructs described above were individually transfected into HEK293T cells. Cells transfected with plasmids encoding individual, mutant hTLR3 molecules were also simultaneously transfected with the pNF-κB-Luciferase reporter and pHRL-TK luciferase control vector. Transfections and cell culture were performed using standard methods.

Transfected and control HEK293 cells were then incubated with 10 μg/ml (Fig. 3B and Fig. 4) or 2.5 μg/ml (Fig. 3C) of the hTLR3 ligand poly(I:C) (PIC) as indicated to assess the effect of the various hTLR3 mutations on hTLR3 signaling. After treatment luciferase expressed from pNF-κB-Luciferase and pHRL-TK was assayed using standard methods. Data was expressed as a "luciferase ratio" as described above. Results are presented as scatter plots of data collected from 6 individual samples.

The present invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

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<212> PRT
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Arg Leu Pro Ala Ala Asn Phe Thr Arg Tyr Ser Gln Leu Thr Ser Leu
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Asp Val Gly Phe Asn Thr Ile Ser Lys Leu Glu Pro Glu Leu Cys Gln
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Lys Leu Pro Met Leu Lys Val Leu Asn Leu Gln His Asn Glu Leu Ser
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Gln Leu Ser Asp Lys Thr Phe Ala Phe Cys Thr Asn Leu Thr Glu Leu
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His Leu Met Ser Asn Ser Ile Gln Lys Ile Lys Asn Asn Pro Phe Val
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Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu Lys Ser Glu Glu Leu Asp
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Leu Cys Leu Glu Leu Ala Asn Thr Ser Ile Arg Asn Leu Ser Leu Ser
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Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr Thr Phe Leu Gly Leu Lys
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385 390 395 400
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660 665 670
Ser His Tyr Leu Cys Asn Thr Pro Pro His Tyr His Gly Phe Pro Val
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690 695 700
Phe Phe Met Ile Asn Thr Ser Ile Leu Leu Ile Phe Ile Phe Ile Val
705 710 715 720

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Gln Phe Glu Tyr Ala Ala Tyr Ile Ile His Ala Tyr Lys Asp Lys Asp
755 760 765
Trp Val Trp Glu His Phe Ser Ser Met Glu Lys Glu Asp Gln Ser Leu
770 775 780
Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Phe Glu Leu
785 790 795 800
Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe Val
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Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Lys Arg Phe Lys Val
820 825 830
His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile Ile
835 840 845
Leu Val Phe Leu Glu Glu Ile Pro Asp Tyr Lys Leu Asn His Ala Leu
850 855 860
Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp Pro
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| Phe | Gly | Met | Leu | Cys | Ala | Ser | Ser | Thr | Thr | Lys | Cys | Thr | Val | Ser | His |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Glu | Val | Ala | Asp | Cys | Ser | His | Leu | Lys | Leu | Thr | Gln | Val | Pro | Asp | Asp |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Leu | Pro | Thr | Asn | Ile | Thr | Val | Leu | Asn | Leu | Thr | His | Asn | Gln | Leu | Arg |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Arg | Leu | Pro | Ala | Ala | Asn | Phe | Thr | Arg | Tyr | Ser | Gln | Leu | Thr | Ser | Leu |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Asp | Val | Gly | Phe | Asn | Thr | Ile | Ser | Lys | Leu | Glu | Pro | Glu | Leu | Cys | Gln |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Lys | Leu | Pro | Met | Leu | Lys | Val | Leu | Asn | Leu | Gln | His | Asn | Glu | Leu | Ser |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Gln | Leu | Ser | Asp | Lys | Thr | Phe | Ala | Phe | Cys | Thr | Asn | Leu | Thr | Glu | Leu |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| His | Leu | Met | Ser | Asn | Ser | Ile | Gln | Lys | Ile | Lys | Asn | Asn | Pro | Phe | Val |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Lys | Gln | Lys | Asn | Leu | Ile | Thr | Leu | Asp | Leu | Ser | His | Asn | Gly | Leu | Ser |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Ser | Thr | Lys | Leu | Gly | Thr | Gln | Val | Gln | Leu | Glu | Asn | Leu | Gln | Glu | Leu |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Leu | Leu | Ser | Asn | Asn | Lys | Ile | Gln | Ala | Leu | Lys | Ser | Glu | Glu | Leu | Asp |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Ile | Phe | Ala | Asn | Ser | Ser | Leu | Lys | Lys | Leu | Glu | Leu | Ser | Ser | Asn | Gln |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Ile | Lys | Glu | Phe | Ser | Pro | Gly | Cys | Phe | His | Ala | Ile | Gly | Arg | Leu | Phe |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Gly | Leu | Phe | Leu | Asn | Asn | Val | Gln | Leu | Gly | Pro | Ser | Leu | Thr | Glu | Lys |
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| Leu | Cys | Leu | Glu | Leu | Ala | Asn | Thr | Ser | Ile | Arg | Asn | Leu | Ser | Leu | Ser |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Asn | Ser | Gln | Leu | Ser | Thr | Thr | Ser | Asn | Thr | Thr | Phe | Leu | Gly | Leu | Lys |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Trp | Thr | Asn | Leu | Thr | Met | Leu | Asp | Leu | Ser | Tyr | Asn | Asn | Leu | Asn | Val |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Val | Gly | Asn | Asp | Ser | Phe | Ala | Trp | Leu | Pro | Gln | Leu | Glu | Tyr | Phe | Phe |
| | 290 | | | | | 295 | | | | | 300 | | | | |

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Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile Asp Asp Phe Ser Phe Gln
340 345 350
Trp Leu Lys Cys Leu Glu His Leu Asn Met Glu Asp Asn Asp Ile Pro
355 360 365
Gly Ile Lys Ser Asn Met Phe Thr Gly Leu Ile Asn Leu Lys Tyr Leu
370 375 380
Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg Thr Leu Thr Asn Glu Thr
385 390 395 400
Phe Val Ser Leu Ala His Ser Pro Leu His Ile Leu Asn Leu Thr Lys
405 410 415
Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala Phe Ser Trp Leu Gly His
420 425 430
Leu Glu Val Leu Asp Leu Gly Leu Asn Glu Ile Gly Gln Glu Leu Thr
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Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile Phe Glu Ile Tyr Leu Ser
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Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn Ser Phe Ala Leu Val Pro
465 470 475 480
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Leu Trp Lys His Ala Asn Pro Gly Gly Pro Ile Tyr Phe Leu Lys Gly
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Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Phe Asp Glu
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Ile Pro Val Glu Val Phe Lys Asp Leu Phe Glu Leu Lys Ile Ile Asp
580 585 590
Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro Ala Ser Val Phe Asn Asn
595 600 605
Gln Val Ser Leu Lys Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr Ser
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Val Glu Lys Lys Val Phe Gly Pro Ala Phe Arg Asn Leu Thr Glu Leu
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Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ala Trp
645 650 655
Phe Val Asn Trp Ile Asn Glu Thr His Thr Asn Ile Pro Glu Leu Ser
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35 40 45
Ser Gln Leu Thr Ser Leu Asp Val Gly Phe Asn Thr Ile Ser Lys Leu
50 55 60
Glu Pro Glu Leu Cys Gln Lys Leu Pro Met Leu Lys Val Leu Asn Leu
65 70 75 80
Gln His Asn Glu Leu Ser Gln Leu Ser Asp Lys Thr Phe Ala Phe Cys
85 90 95
Thr Asn Leu Thr Glu Leu His Leu Met Ser Asn Ser Ile Gln Lys Ile
100 105 110

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Glu Asn Leu Gln Glu Leu Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu
145                 150                 155                 160
Lys Ser Glu Glu Leu Asp Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu
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Glu Leu Ser Ser Asn Gln Ile Lys Glu Phe Ser Pro Gly Cys Phe His
            180                 185                 190
Ala Ile Gly Arg Leu Phe Gly Leu Phe Leu Asn Asn Val Gln Leu Gly
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Pro Ser Leu Thr Glu Lys Leu Cys Leu Glu Leu Ala Asn Thr Ser Ile
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Arg Asn Leu Ser Leu Ser Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr
225                 230                 235                 240
Thr Phe Leu Gly Leu Lys Trp Thr Asn Leu Thr Met Leu Asp Leu Ser
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Tyr Asn Asn Leu Asn Val Val Gly Asn Asp Ser Phe Ala Trp Leu Pro
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Arg Ser Phe Thr Lys Gln Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile
305                 310                 315                 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Cys Leu Glu His Leu Asn Met
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Glu Asp Asn Asp Ile Pro Gly Ile Lys Ser Asn Met Phe Thr Gly Leu
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Ile Asn Leu Lys Tyr Leu Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg
        355                 360                 365
Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu His
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Ile Leu Asn Leu Thr Lys Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala
385                 390                 395                 400
Phe Ser Trp Leu Gly His Leu Glu Val Leu Asp Leu Gly Leu Asn Glu
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Ile Gly Gln Glu Leu Thr Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile
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Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn
        435                 440                 445
Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
    450                 455                 460
Ala Leu Lys Asn Val Asp Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg
465                 470                 475                 480
Asn Leu Thr Ile Leu Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn
                485                 490                 495
Asp Asp Met Leu Glu Gly Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln
            500                 505                 510
His Asn Asn Leu Ala Arg Leu Trp Lys His Ala Asn Pro Gly Gly Pro
        515                 520                 525
Ile Tyr Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
    530                 535                 540
Ser Asn Gly Phe Asp Glu Ile Pro Val Glu Val Phe Lys Asp Leu Phe
545                 550                 555                 560
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Glu Leu Lys Ile Ile Asp Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro
565 570 575
Ala Ser Val Phe Asn Asn Gln Val Ser Leu Lys Ser Leu Asn Leu Gln
580 585 590
Lys Asn Leu Ile Thr Ser Val Glu Lys Lys Val Phe Gly Pro Ala Phe
595 600 605
Arg Asn Leu Thr Glu Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
610 615 620
Cys Glu Ser Ile Ala Trp Phe Val Asn Trp Ile Asn Glu Thr His Thr
625 630 635 640
Asn Ile Pro Glu Leu Ser Ser His Tyr Leu Cys Asn Thr Pro Pro His
645 650 655
Tyr His Gly Phe Pro Val Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp
660 665 670
Ser Ala Pro Phe Glu
675

<210> 7
<211> 2031
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial cDNA sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutation at N221.

<400> 7
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ccggcggcga actttacccg ctatagccag ctgaccagcc tggatgtggg ctttaacacc 180
attagcaaac tggaaccgga actgtgccag aaactgccga tgctgaaagt gctgaacctg 240
cagcataacg aactgagcca gctgagcgat aaaaccttg cgttttgcac caacctgacc 300
gaactgcatc tgatgagcaa cagcattcag aaaattaaaa acaacccgtt tgtgaaacag 360
aaaaacctga ttaccctgga tctgagccat aacggcctga gcagcaccaa actgggcacc 420
caggtgcagc tggaaaacct gcaggaactg ctgctgagca acaacaaaat tcaggcgctg 480
aaaagcgaag aactggatat ttttgcgaac agcagcctga aaaaactgga actgagcagc 540
aaccagatta aagaatttag cccgggctgc tttcatgcga ttggccgcct gtttggcctg 600
tttctgaaca acgtgcagct gggcccgagc ctgaccgaaa aactgtgcct ggaactggcg 660
gcgaccagca ttcgcaacct gagcctgagc aacagccagc tgagcaccac cagcaacacc 720
acctttctgg gcctgaaatg gaccaacctg accatgctgg atctgagcta taacaacctg 780
aacgtggtgg gcaacgatag ctttgcgtgg ctgccgcagc tggaatattt ttttctggaa 840
tataacaaca ttcagcatct gtttagccat agcctgcatg gcctgtttaa cgtgcgctat 900
ctgaacctga aacgcagctt taccaaacag agcattagcc tggcgagcct gccgaaaatt 960
gatgatttta gctttcagtg gctgaaatgc ctggaacatc tgaacatgga agataacgat 1020
attccgggca ttaaaagcaa catgtttacc ggcctgatta acctgaaata tctgagcctg 1080
agcaacagct ttaccagcct gcgcaccctg accaacgaaa cctttgtgag cctggcgcat 1140
agcccgctgc atattctgaa cctgaccaaa aacaaaatta gcaaaattga aagcgatgcg 1200
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ctgcgccgcg tggcgctgaa aaacgtggat agcagcccga gcccgtttca gccgctgcgc 1440
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tttgattgca cctgcgaaag cattgcgtgg tttgtgaact ggattaacga aacccatacc 1920
aacattccgg aactgagcag ccattatctg tgcaacaccc cgccgcatta tcatggcttt 1980
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<210> 8
<211> 677
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial amino acid sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutation at N221.

<400> 8

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| Lys | Cys | Thr | Val | Ser | His | Glu | Val | Ala | Asp | Cys | Ser | His | Leu | Lys | Leu |
| 1 | | | | 5 | | | | 10 | | | | | | 15 | |
| Thr | Gln | Val | Pro | Asp | Asp | Leu | Pro | Thr | Asn | Ile | Thr | Val | Leu | Asn | Leu |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Thr | His | Asn | Gln | Leu | Arg | Arg | Leu | Pro | Ala | Ala | Asn | Phe | Thr | Arg | Tyr |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Ser | Gln | Leu | Thr | Ser | Leu | Asp | Val | Gly | Phe | Asn | Thr | Ile | Ser | Lys | Leu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Glu | Pro | Glu | Leu | Cys | Gln | Lys | Leu | Pro | Met | Leu | Lys | Val | Leu | Asn | Leu |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Gln | His | Asn | Glu | Leu | Ser | Gln | Leu | Ser | Asp | Lys | Thr | Phe | Ala | Phe | Cys |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Thr | Asn | Leu | Thr | Glu | Leu | His | Leu | Met | Ser | Asn | Ser | Ile | Gln | Lys | Ile |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Lys | Asn | Asn | Pro | Phe | Val | Lys | Gln | Lys | Asn | Leu | Ile | Thr | Leu | Asp | Leu |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Ser | His | Asn | Gly | Leu | Ser | Ser | Thr | Lys | Leu | Gly | Thr | Gln | Val | Gln | Leu |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Glu | Asn | Leu | Gln | Glu | Leu | Leu | Leu | Ser | Asn | Asn | Lys | Ile | Gln | Ala | Leu |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Lys | Ser | Glu | Glu | Leu | Asp | Ile | Phe | Ala | Asn | Ser | Ser | Leu | Lys | Lys | Leu |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Glu | Leu | Ser | Ser | Asn | Gln | Ile | Lys | Glu | Phe | Ser | Pro | Gly | Cys | Phe | His |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Ala | Ile | Gly | Arg | Leu | Phe | Gly | Leu | Phe | Leu | Asn | Asn | Val | Gln | Leu | Gly |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Pro | Ser | Leu | Thr | Glu | Lys | Leu | Cys | Leu | Glu | Leu | Ala | Ala | Thr | Ser | Ile |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Arg | Asn | Leu | Ser | Leu | Ser | Asn | Ser | Gln | Leu | Ser | Thr | Thr | Ser | Asn | Thr |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Thr | Phe | Leu | Gly | Leu | Lys | Trp | Thr | Asn | Leu | Thr | Met | Leu | Asp | Leu | Ser |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Tyr | Asn | Asn | Leu | Asn | Val | Val | Gly | Asn | Asp | Ser | Phe | Ala | Trp | Leu | Pro |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Gln | Leu | Glu | Tyr | Phe | Phe | Leu | Glu | Tyr | Asn | Asn | Ile | Gln | His | Leu | Phe |
| | | 275 | | | | | 280 | | | | | 285 | | | |

Ser His Ser Leu His Gly Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys
290 295 300
Arg Ser Phe Thr Lys Gln Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile
305 310 315 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Cys Leu Glu His Leu Asn Met
325 330 335
Glu Asp Asn Asp Ile Pro Gly Ile Lys Ser Asn Met Phe Thr Gly Leu
340 345 350
Ile Asn Leu Lys Tyr Leu Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg
355 360 365
Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu His
370 375 380
Ile Leu Asn Leu Thr Lys Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala
385 390 395 400
Phe Ser Trp Leu Gly His Leu Glu Val Leu Asp Leu Gly Leu Asn Glu
405 410 415
Ile Gly Gln Glu Leu Thr Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile
420 425 430
Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn
435 440 445
Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
450 455 460
Ala Leu Lys Asn Val Asp Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg
465 470 475 480
Asn Leu Thr Ile Leu Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn
485 490 495
Asp Asp Met Leu Glu Gly Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln
500 505 510
His Asn Asn Leu Ala Arg Leu Trp Lys His Ala Asn Pro Gly Gly Pro
515 520 525
Ile Tyr Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
530 535 540
Ser Asn Gly Phe Asp Glu Ile Pro Val Glu Val Phe Lys Asp Leu Phe
545 550 555 560
Glu Leu Lys Ile Ile Asp Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro
565 570 575
Ala Ser Val Phe Asn Asn Gln Val Ser Leu Lys Ser Leu Asn Leu Gln
580 585 590
Lys Asn Leu Ile Thr Ser Val Glu Lys Lys Val Phe Gly Pro Ala Phe
595 600 605
Arg Asn Leu Thr Glu Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
610 615 620
Cys Glu Ser Ile Ala Trp Phe Val Asn Trp Ile Asn Glu Thr His Thr
625 630 635 640
Asn Ile Pro Glu Leu Ser Ser His Tyr Leu Cys Asn Thr Pro Pro His
645 650 655
Tyr His Gly Phe Pro Val Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp
660 665 670
Ser Ala Pro Phe Glu
675

<210> 9
<211> 2031
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial cDNA sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutation at N387.

<400> 9
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gatgatctgc cgaccaacat taccgtgctg aacctgaccc ataaccagct gcgccgcctg 120
ccggcggcga actttacccg ctatagccag ctgaccagcc tggatgtggg ctttaacacc 180
attagcaaac tggaaccgga actgtgccag aaactgccga tgctgaaagt gctgaacctg 240
cagcataacg aactgagcca gctgagcgat aaaaccttg cgttttgcac caacctgacc 300
gaactgcatc tgatgagcaa cagcattcag aaaattaaaa acaacccgtt tgtgaaacag 360
aaaaacctga ttaccctgga tctgagccat aacggcctga gcagcaccaa actgggcacc 420
caggtgcagc tggaaaacct gcaggaactg ctgctgagca acaacaaaat tcaggcgctg 480
aaaagcgaag aactggatat ttttgcgaac agcagcctga aaaaactgga actgagcagc 540
aaccagatta aagaatttag cccgggctgc tttcatgcga ttggccgcct gtttggcctg 600
tttctgaaca acgtgcagct gggcccgagc ctgaccgaaa aactgtgcct ggaactggcg 660
aacaccagca ttcgcaacct gagcctgagc aacagccagc tgagcaccac cagcaacacc 720
acctttctgg gcctgaaatg gaccaacctg accatgctgg atctgagcta taacaacctg 780
aacgtggtgg gcaacgatag ctttgcgtgg ctgccgcagc tggaatattt ttttctggaa 840
tataacaaca ttcagcatct gtttagccat agcctgcatg gcctgtttaa cgtgcgctat 900
ctgaacctga aacgcagctt taccaaacag agcattagcc tggcgagcct gccgaaaatt 960
gatgatttta gctttcagtg gctgaaatgc ctggaacatc tgaacatgga agataacgat 1020
attccgggca ttaaaagcaa catgtttacc ggcctgatta acctgaaata tctgagcctg 1080
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aacctgacca ttctggatct gagcaacaac aacattgcga acattaacga tgatatgctg 1500
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aaaaaagtgt ttggcccggc gtttcgcaac ctgaccgaac tggatatgcg ctttaacccg 1860
tttgattgca cctgcgaaag cattgcgtgg tttgtgaact ggattaacga aacccatacc 1920
aacattccgg aactgagcag ccattatctg tgcaacaccc cgccgcatta tcatggcttt 1980
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<210> 10
<211> 677
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial amino acid sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutation at N387.

<400> 10
Lys Cys Thr Val Ser His Glu Val Ala Asp Cys Ser His Leu Lys Leu
1 5 10 15

Thr Gln Val Pro Asp Asp Leu Pro Thr Asn Ile Thr Val Leu Asn Leu
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Thr His Asn Gln Leu Arg Arg Leu Pro Ala Ala Asn Phe Thr Arg Tyr
35 40 45
Ser Gln Leu Thr Ser Leu Asp Val Gly Phe Asn Thr Ile Ser Lys Leu
50 55 60
Glu Pro Glu Leu Cys Gln Lys Leu Pro Met Leu Lys Val Leu Asn Leu
65 70 75 80
Gln His Asn Glu Leu Ser Gln Leu Ser Asp Lys Thr Phe Ala Phe Cys
85 90 95
Thr Asn Leu Thr Glu Leu His Leu Met Ser Asn Ser Ile Gln Lys Ile
100 105 110
Lys Asn Asn Pro Phe Val Lys Gln Lys Asn Leu Ile Thr Leu Asp Leu
115 120 125
Ser His Asn Gly Leu Ser Ser Thr Lys Leu Gly Thr Gln Val Gln Leu
130 135 140
Glu Asn Leu Gln Glu Leu Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu
145 150 155 160
Lys Ser Glu Glu Leu Asp Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu
165 170 175
Glu Leu Ser Ser Asn Gln Ile Lys Glu Phe Ser Pro Gly Cys Phe His
180 185 190
Ala Ile Gly Arg Leu Phe Gly Leu Phe Leu Asn Asn Val Gln Leu Gly
195 200 205
Pro Ser Leu Thr Glu Lys Leu Cys Leu Glu Leu Ala Asn Thr Ser Ile
210 215 220
Arg Asn Leu Ser Leu Ser Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr
225 230 235 240
Thr Phe Leu Gly Leu Lys Trp Thr Asn Leu Thr Met Leu Asp Leu Ser
245 250 255
Tyr Asn Asn Leu Asn Val Val Gly Asn Asp Ser Phe Ala Trp Leu Pro
260 265 270
Gln Leu Glu Tyr Phe Phe Leu Glu Tyr Asn Asn Ile Gln His Leu Phe
275 280 285
Ser His Ser Leu His Gly Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys
290 295 300
Arg Ser Phe Thr Lys Gln Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile
305 310 315 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Cys Leu Glu His Leu Asn Met
325 330 335
Glu Asp Asn Asp Ile Pro Gly Ile Lys Ser Asn Met Phe Thr Gly Leu
340 345 350
Ile Asn Leu Lys Tyr Leu Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg
355 360 365
Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu His
370 375 380
Ile Leu Ala Leu Thr Lys Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala
385 390 395 400
Phe Ser Trp Leu Gly His Leu Glu Val Leu Asp Leu Gly Leu Asn Glu
405 410 415
Ile Gly Gln Glu Leu Thr Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile
420 425 430
Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn
435 440 445
Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
450 455 460

Ala Leu Lys Asn Val Asp Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg
465 470 475 480
Asn Leu Thr Ile Leu Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn
485 490 495
Asp Asp Met Leu Glu Gly Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln
500 505 510
His Asn Asn Leu Ala Arg Leu Trp Lys His Ala Asn Pro Gly Gly Pro
515 520 525
Ile Tyr Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
530 535 540
Ser Asn Gly Phe Asp Glu Ile Pro Val Glu Val Phe Lys Asp Leu Phe
545 550 555 560
Glu Leu Lys Ile Ile Asp Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro
565 570 575
Ala Ser Val Phe Asn Asn Gln Val Ser Leu Lys Ser Leu Asn Leu Gln
580 585 590
Lys Asn Leu Ile Thr Ser Val Glu Lys Lys Val Phe Gly Pro Ala Phe
595 600 605
Arg Asn Leu Thr Glu Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
610 615 620
Cys Glu Ser Ile Ala Trp Phe Val Asn Trp Ile Asn Glu Thr His Thr
625 630 635 640
Asn Ile Pro Glu Leu Ser Ser His Tyr Leu Cys Asn Thr Pro Pro His
645 650 655
Tyr His Gly Phe Pro Val Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp
660 665 670
Ser Ala Pro Phe Glu
675

<210> 11
<211> 2031
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial cDNA sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains alanine (A) substitution mutations at N221 and N387.

<400> 11
aaatgcaccg tgagccatga agtggcggat tgcagccatc tgaaactgac ccaggtgccg 60
gatgatctgc cgaccaacat taccgtgctg aacctgaccc ataaccagct gcgccgcctg 120
ccggcggcga actttacccg ctatagccag ctgaccagcc tggatgtggg ctttaacacc 180
attagcaaac tggaaccgga actgtgccag aaactgccga tgctgaaagt gctgaacctg 240
cagcataacg aactgagcca gctgagcgat aaaaactttg cgttttgcac caacctgacc 300
gaactgcatc tgatgagcaa cagcattcag aaaattaaaa acaacccgtt tgtgaaacag 360
aaaaacctga ttaccctgga tctgagccat aacggcctga gcagcaccaa actgggcacc 420
caggtgcagc tggaaaacct gcaggaactg ctgctgagca acaacaaaat tcaggcgctg 480
aaaagcgaag aactggatat ttttgcgaac agcagcctga aaaaactgga actgagcagc 540
aaccagatta aagaatttag cccgggctgc tttcatgcga ttggccgcct gtttggcctg 600
tttctgaaca acgtgcagct gggcccgagc ctgaccgaaa aactgtgcct ggaactggcg 660
gcgaccagca ttcgcaacct gagcctgagc aacagccagc tgagcaccac cagcaacacc 720
acctttctgg gcctgaaatg gaccaacctg accatgctgg atctgagcta taacaacctg 780
aacgtggtgg gcaacgatag ctttgcgtgg ctgccgcagc tggaatattt ttttctggaa 840
tataacaaca ttcagcatct gtttagccat agcctgcatg gcctgtttaa cgtgcgctat 900

ctgaacctga aacgcagctt taccaaacag agcattagcc tggcgagcct gccgaaaatt 960
gatgatttta gctttcagtg gctgaaatgc ctggaacatc tgaacatgga agataacgat 1020
attccgggca ttaaaagcaa catgtttacc ggcctgatta acctgaaata tctgagcctg 1080
agcaacagct ttaccagcct gcgcaccctg accaacgaaa cctttgtgag cctggcgcat 1140
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tttgattgca cctgcgaaag cattgcgtgg tttgtgaact ggattaacga aacccatacc 1920
aacattccgg aactgagcag ccattatctg tgcaacaccc cgccgcatta tcatggcttt 1980
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<210> 12
<211> 677
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial amino acid sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains alanine (A) substitution mutations at N221 and N387.

<400> 12

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| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Thr | Gln | Val | Pro | Asp | Asp | Leu | Pro | Thr | Asn | Ile | Thr | Val | Leu | Asn | Leu |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Thr | His | Asn | Gln | Leu | Arg | Arg | Leu | Pro | Ala | Ala | Asn | Phe | Thr | Arg | Tyr |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Ser | Gln | Leu | Thr | Ser | Leu | Asp | Val | Gly | Phe | Asn | Thr | Ile | Ser | Lys | Leu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Glu | Pro | Glu | Leu | Cys | Gln | Lys | Leu | Pro | Met | Leu | Lys | Val | Leu | Asn | Leu |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Gln | His | Asn | Glu | Leu | Ser | Gln | Leu | Ser | Asp | Lys | Thr | Phe | Ala | Phe | Cys |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Thr | Asn | Leu | Thr | Glu | Leu | His | Leu | Met | Ser | Asn | Ser | Ile | Gln | Lys | Ile |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Lys | Asn | Asn | Pro | Phe | Val | Lys | Gln | Lys | Asn | Leu | Ile | Thr | Leu | Asp | Leu |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Ser | His | Asn | Gly | Leu | Ser | Ser | Thr | Lys | Leu | Gly | Thr | Gln | Val | Gln | Leu |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Glu | Asn | Leu | Gln | Glu | Leu | Leu | Leu | Ser | Asn | Asn | Lys | Ile | Gln | Ala | Leu |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Lys | Ser | Glu | Glu | Leu | Asp | Ile | Phe | Ala | Asn | Ser | Ser | Leu | Lys | Lys | Leu |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Glu | Leu | Ser | Ser | Asn | Gln | Ile | Lys | Glu | Phe | Ser | Pro | Gly | Cys | Phe | His |
| | | | 180 | | | | | 185 | | | | | 190 | | |

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Ala Ile Gly Arg Leu Phe Gly Leu Phe Leu Asn Asn Val Gln Leu Gly
        195                 200                 205
Pro Ser Leu Thr Glu Lys Leu Cys Leu Glu Leu Ala Ala Thr Ser Ile
    210                 215                 220
Arg Asn Leu Ser Leu Ser Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr
225                 230                 235                 240
Thr Phe Leu Gly Leu Lys Trp Thr Asn Leu Thr Met Leu Asp Leu Ser
                245                 250                 255
Tyr Asn Asn Leu Asn Val Val Gly Asn Asp Ser Phe Ala Trp Leu Pro
            260                 265                 270
Gln Leu Glu Tyr Phe Phe Leu Glu Tyr Asn Asn Ile Gln His Leu Phe
        275                 280                 285
Ser His Ser Leu His Gly Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys
    290                 295                 300
Arg Ser Phe Thr Lys Gln Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile
305                 310                 315                 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Cys Leu Glu His Leu Asn Met
                325                 330                 335
Glu Asp Asn Asp Ile Pro Gly Ile Lys Ser Asn Met Phe Thr Gly Leu
            340                 345                 350
Ile Asn Leu Lys Tyr Leu Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg
        355                 360                 365
Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu His
    370                 375                 380
Ile Leu Ala Leu Thr Lys Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala
385                 390                 395                 400
Phe Ser Trp Leu Gly His Leu Glu Val Leu Asp Leu Gly Leu Asn Glu
                405                 410                 415
Ile Gly Gln Glu Leu Thr Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile
            420                 425                 430
Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn
        435                 440                 445
Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
    450                 455                 460
Ala Leu Lys Asn Val Asp Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg
465                 470                 475                 480
Asn Leu Thr Ile Leu Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn
                485                 490                 495
Asp Asp Met Leu Glu Gly Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln
            500                 505                 510
His Asn Asn Leu Ala Arg Leu Trp Lys His Ala Asn Pro Gly Gly Pro
        515                 520                 525
Ile Tyr Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
    530                 535                 540
Ser Asn Gly Phe Asp Glu Ile Pro Val Glu Val Phe Lys Asp Leu Phe
545                 550                 555                 560
Glu Leu Lys Ile Ile Asp Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro
                565                 570                 575
Ala Ser Val Phe Asn Asn Gln Val Ser Leu Lys Ser Leu Asn Leu Gln
            580                 585                 590
Lys Asn Leu Ile Thr Ser Val Glu Lys Lys Val Phe Gly Pro Ala Phe
        595                 600                 605
Arg Asn Leu Thr Glu Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
    610                 615                 620
Cys Glu Ser Ile Ala Trp Phe Val Asn Trp Ile Asn Glu Thr His Thr
625                 630                 635                 640
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Asn Ile Pro Glu Leu Ser Ser His Tyr Leu Cys Asn Thr Pro Pro His
645 650 655
Tyr His Gly Phe Pro Val Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp
660 665 670
Ser Ala Pro Phe Glu
675

<210> 13
<211> 2031
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial cDNA sequence derived from the Mus musculus TLR3 ECD which lacks a signal peptide and contains alanine (A) substitution mutations at N221, N226, N387, and N636.

<400> 13
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gatgatctgc cgagcaacat taccgtgctg aacctgaccc ataaccagct gcgccgcctg 120
ccgccgacca actttacccg ctatagccag ctggcgattc tggatgcggg ctttaacagc 180
attagcaaac tggaaccgga actgtgccag attctgccgc tgctgaaagt gctgaacctg 240
cagcataacg aactgagcca gattagcgat cagacctttg tgttttgcac caacctgacc 300
gaactggatc tgatgagcaa cagcattcat aaaattaaaa gcaacccgtt taaaaaccag 360
aaaaacctga ttaaactgga tctgagccat aacggcctga gcagcaccaa actgggcacc 420
ggcgtgcagc tggaaaacct gcaggaactg ctgctggcga aaaacaaaat tctggcgctg 480
cgcagcgaag aactggaatt tctgggcaac agcagcctgc gcaaactgga tctgagcagc 540
aacccgctga aagaatttag cccgggctgc tttcagacca ttggcaaact gtttgcgctg 600
ctgctgaaca acgcgcagct gaacccgcat ctgaccgaaa aactgtgctg ggaactgagc 660
gcgaccagca ttcaggcgct gagcctggcg aacaaccagc tgctggcgac cagcgaaagc 720
acctttagcg gcctgaaatg gaccaacctg acccagctgg atctgagcta taacaacctg 780
catgatgtgg gcaacggcag ctttagctat ctgccgagcc tgcgctatct gagcctggaa 840
tataacaaca ttcagcgcct gagcccgcgc agcttttatg gcctgagcaa cctgcgctat 900
ctgagcctga aacgcgcgtt taccaaacag agcgtgagcc tggcgagcca tccgaacatt 960
gatgatttta gctttcagtg gctgaaatat ctggaatatc tgaacatgga tgataacaac 1020
attccgagca ccaaaagcaa cacctttacc ggcctggtga gcctgaaata tctgagcctg 1080
agcaaaacct ttaccagcct gcagaccctg accaacgaaa cctttgtgag cctggcgcat 1140
agcccgctgc tgaccctggc gctgaccaaa aaccatatta gcaaaattgc gaacggcacc 1200
tttagctggc tgggccagct gcgcattctg gatctgggcc tgaacgaaat tgaacagaaa 1260
ctgagcggcc aggaatggcg cggcctgcgc aacatttttg aaatttatct gagctataac 1320
aaatatctgc agctgagcac cagcagcttt gcgctggtgc cgagcctgca gcgcctgatg 1380
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gaaggcctgg aaaacctgga aattctggat tttcagcata acaacctggc gcgcctgtgg 1560
aaacgcgcga acccgggcgg cccggtgaac tttctgaaag gcctgagcca tctgcatatt 1620
ctgaacctgg aaagcaacgg cctggatgaa attccggtgg gcgtgtttaa aaacctgttt 1680
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gatgatcaga ccagcctgcg cagcctgaac ctgcagaaaa acctgattac cagcgtggaa 1800
aaagatgtgt ttggcccgcc gtttcagaac ctgaacagcc tggatatgcg ctttaacccg 1860
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aacattagcg aactgagcac ccattatctg tgcaacaccc cgcatcatta ttatggcttt 1980
ccgctgaaac tgtttgatac cagcagctgc aaagatagcg cgccgtttga a 2031

<210> 14
<211> 677

<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial amino acid sequence derived from the Mus musculus TLR3 ECD which lacks a signal peptide and contains alanine (A) substitution mutations at N221, N226, N387, and N636.

<400> 14

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Gln Cys Thr Val Arg Tyr Asn Val Ala Asp Cys Ser His Leu Lys Leu
 1               5                  10                  15
Thr His Ile Pro Asp Asp Leu Pro Ser Asn Ile Thr Val Leu Asn Leu
            20                  25                  30
Thr His Asn Gln Leu Arg Arg Leu Pro Pro Thr Asn Phe Thr Arg Tyr
        35                  40                  45
Ser Gln Leu Ala Ile Leu Asp Ala Gly Phe Asn Ser Ile Ser Lys Leu
    50                  55                  60
Glu Pro Glu Leu Cys Gln Ile Leu Pro Leu Leu Lys Val Leu Asn Leu
65                  70                  75                  80
Gln His Asn Glu Leu Ser Gln Ile Ser Asp Gln Thr Phe Val Phe Cys
                85                  90                  95
Thr Asn Leu Thr Glu Leu Asp Leu Met Ser Asn Ser Ile His Lys Ile
            100                 105                 110
Lys Ser Asn Pro Phe Lys Asn Gln Lys Asn Leu Ile Lys Leu Asp Leu
        115                 120                 125
Ser His Asn Gly Leu Ser Ser Thr Lys Leu Gly Thr Gly Val Gln Leu
    130                 135                 140
Glu Asn Leu Gln Glu Leu Leu Leu Ala Lys Asn Lys Ile Leu Ala Leu
145                 150                 155                 160
Arg Ser Glu Glu Leu Glu Phe Leu Gly Asn Ser Ser Leu Arg Lys Leu
                165                 170                 175
Asp Leu Ser Ser Asn Pro Leu Lys Glu Phe Ser Pro Gly Cys Phe Gln
            180                 185                 190
Thr Ile Gly Lys Leu Phe Ala Leu Leu Leu Asn Asn Ala Gln Leu Asn
        195                 200                 205
Pro His Leu Thr Glu Lys Leu Cys Trp Glu Leu Ser Ala Thr Ser Ile
    210                 215                 220
Gln Ala Leu Ser Leu Ala Asn Asn Gln Leu Leu Ala Thr Ser Glu Ser
225                 230                 235                 240
Thr Phe Ser Gly Leu Lys Trp Thr Asn Leu Thr Gln Leu Asp Leu Ser
                245                 250                 255
Tyr Asn Asn Leu His Asp Val Gly Asn Gly Ser Phe Ser Tyr Leu Pro
            260                 265                 270
Ser Leu Arg Tyr Leu Ser Leu Glu Tyr Asn Asn Ile Gln Arg Leu Ser
        275                 280                 285
Pro Arg Ser Phe Tyr Gly Leu Ser Asn Leu Arg Tyr Leu Ser Leu Lys
    290                 295                 300
Arg Ala Phe Thr Lys Gln Ser Val Ser Leu Ala Ser His Pro Asn Ile
305                 310                 315                 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Tyr Leu Glu Tyr Leu Asn Met
                325                 330                 335
Asp Asp Asn Asn Ile Pro Ser Thr Lys Ser Asn Thr Phe Thr Gly Leu
            340                 345                 350
Val Ser Leu Lys Tyr Leu Ser Leu Ser Lys Thr Phe Thr Ser Leu Gln
        355                 360                 365
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Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu Leu
370 375 380
Thr Leu Ala Leu Thr Lys Asn His Ile Ser Lys Ile Ala Asn Gly Thr
385 390 395 400
Phe Ser Trp Leu Gly Gln Leu Arg Ile Leu Asp Leu Gly Leu Asn Glu
405 410 415
Ile Glu Gln Lys Leu Ser Gly Gln Glu Trp Arg Gly Leu Arg Asn Ile
420 425 430
Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Ser Thr Ser
435 440 445
Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
450 455 460
Ala Leu Lys Asn Val Asp Ile Ser Pro Ser Pro Phe Arg Pro Leu Arg
465 470 475 480
Asn Leu Thr Ile Leu Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn
485 490 495
Glu Asp Leu Leu Glu Gly Leu Glu Asn Leu Glu Ile Leu Asp Phe Gln
500 505 510
His Asn Asn Leu Ala Arg Leu Trp Lys Arg Ala Asn Pro Gly Gly Pro
515 520 525
Val Asn Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
530 535 540
Ser Asn Gly Leu Asp Glu Ile Pro Val Gly Val Phe Lys Asn Leu Phe
545 550 555 560
Glu Leu Lys Ser Ile Asn Leu Gly Leu Asn Asn Leu Asn Lys Leu Glu
565 570 575
Pro Phe Ile Phe Asp Asp Gln Thr Ser Leu Arg Ser Leu Asn Leu Gln
580 585 590
Lys Asn Leu Ile Thr Ser Val Glu Lys Asp Val Phe Gly Pro Pro Phe
595 600 605
Gln Asn Leu Asn Ser Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
610 615 620
Cys Glu Ser Ile Ser Trp Phe Val Asn Trp Ile Ala Gln Thr His Thr
625 630 635 640
Asn Ile Ser Glu Leu Ser Thr His Tyr Leu Cys Asn Thr Pro His His
645 650 655
Tyr Tyr Gly Phe Pro Leu Lys Leu Phe Asp Thr Ser Ser Cys Lys Asp
660 665 670
Ser Ala Pro Phe Glu
675

<210> 15
<211> 2031
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial cDNA sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutation at N226.

<400> 15
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gatgatctgc cgaccaacat taccgtgctg aacctgaccc ataaccagct gcgccgcctg 120
ccggcggcga actttacccg ctatagccag ctgaccagcc tggatgtggg ctttaacacc 180

attagcaaac tggaaccgga actgtgccag aaactgccga tgctgaaagt gctgaacctg 240
cagcataacg aactgagcca gctgagcgat aaaacctttg cgttttgcac caacctgacc 300
gaactgcatc tgatgagcaa cagcattcag aaaattaaaa acaacccgtt tgtgaaacag 360
aaaaacctga ttaccctgga tctgagccat aacggcctga gcagcaccaa actgggcacc 420
caggtgcagc tggaaaacct gcaggaactg ctgctgagca acaacaaaat tcaggcgctg 480
aaaagcgaag aactggatat ttttgcgaac agcagcctga aaaaactgga actgagcagc 540
aaccagatta aagaatttag cccgggctgc tttcatgcga ttggccgcct gtttggcctg 600
tttctgaaca acgtgcagct gggcccgagc ctgaccgaaa aactgtgcct ggaactggcg 660
aacaccagca ttcgcgcgct gagcctgagc aacagccagc tgagcaccac cagcaacacc 720
acctttctgg gcctgaaatg gaccaacctg accatgctgg atctgagcta taacaacctg 780
aacgtggtgg gcaacgatag ctttgcgtgg ctgccgcagc tggaatattt ttttctggaa 840
tataacaaca ttcagcatct gtttagccat agcctgcatg gcctgtttaa cgtgcgctat 900
ctgaacctga aacgcagctt taccaaacag agcattagcc tggcgagcct gccgaaaatt 960
gatgatttta gctttcagtg gctgaaatgc ctggaacatc tgaacatgga agataacgat 1020
attccgggca ttaaaagcaa catgtttacc ggcctgatta acctgaaata tctgagcctg 1080
agcaacagct ttaccagcct gcgcaccctg accaacgaaa cctttgtgag cctggcgcat 1140
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aaatatctgc agctgacccg caacagcttt gcgctggtgc cgagcctgca gcgcctgatg 1380
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aacattccgg aactgagcag ccattatctg tgcaacaccc cgccgcatta tcatggcttt 1980
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<210> 16
<211> 677
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial amino acid sequence derived from the
Homo sapiens hTLR3 ECD which lacks a signal
peptide and contains an alanine (A) substitution
mutation at N226.

<400> 16

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lys | Cys | Thr | Val | Ser | His | Glu | Val | Ala | Asp | Cys | Ser | His | Leu | Lys | Leu |
| 1 | | | | 5 | | | | 10 | | | | | 15 | | |
| Thr | Gln | Val | Pro | Asp | Asp | Leu | Pro | Thr | Asn | Ile | Thr | Val | Leu | Asn | Leu |
| | | | 20 | | | | | 25 | | | | 30 | | | |
| Thr | His | Asn | Gln | Leu | Arg | Arg | Leu | Pro | Ala | Ala | Asn | Phe | Thr | Arg | Tyr |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Ser | Gln | Leu | Thr | Ser | Leu | Asp | Val | Gly | Phe | Asn | Thr | Ile | Ser | Lys | Leu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Glu | Pro | Glu | Leu | Cys | Gln | Lys | Leu | Pro | Met | Leu | Lys | Val | Leu | Asn | Leu |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Gln | His | Asn | Glu | Leu | Ser | Gln | Leu | Ser | Asp | Lys | Thr | Phe | Ala | Phe | Cys |
| | | | | 85 | | | | | 90 | | | | | 95 | |

Thr Asn Leu Thr Glu Leu His Leu Met Ser Asn Ser Ile Gln Lys Ile
100 105 110
Lys Asn Asn Pro Phe Val Lys Gln Lys Asn Leu Ile Thr Leu Asp Leu
115 120 125
Ser His Asn Gly Leu Ser Ser Thr Lys Leu Gly Thr Gln Val Gln Leu
130 135 140
Glu Asn Leu Gln Glu Leu Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu
145 150 155 160
Lys Ser Glu Glu Leu Asp Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu
165 170 175
Glu Leu Ser Ser Asn Gln Ile Lys Glu Phe Ser Pro Gly Cys Phe His
180 185 190
Ala Ile Gly Arg Leu Phe Gly Leu Phe Leu Asn Asn Val Gln Leu Gly
195 200 205
Pro Ser Leu Thr Glu Lys Leu Cys Leu Glu Leu Ala Asn Thr Ser Ile
210 215 220
Arg Ala Leu Ser Leu Ser Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr
225 230 235 240
Thr Phe Leu Gly Leu Lys Trp Thr Asn Leu Thr Met Leu Asp Leu Ser
245 250 255
Tyr Asn Asn Leu Asn Val Val Gly Asn Asp Ser Phe Ala Trp Leu Pro
260 265 270
Gln Leu Glu Tyr Phe Phe Leu Glu Tyr Asn Asn Ile Gln His Leu Phe
275 280 285
Ser His Ser Leu His Gly Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys
290 295 300
Arg Ser Phe Thr Lys Gln Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile
305 310 315 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Cys Leu Glu His Leu Asn Met
325 330 335
Glu Asp Asn Asp Ile Pro Gly Ile Lys Ser Asn Met Phe Thr Gly Leu
340 345 350
Ile Asn Leu Lys Tyr Leu Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg
355 360 365
Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu His
370 375 380
Ile Leu Asn Leu Thr Lys Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala
385 390 395 400
Phe Ser Trp Leu Gly His Leu Glu Val Leu Asp Leu Gly Leu Asn Glu
405 410 415
Ile Gly Gln Glu Leu Thr Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile
420 425 430
Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn
435 440 445
Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
450 455 460
Ala Leu Lys Asn Val Asp Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg
465 470 475 480
Asn Leu Thr Ile Leu Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn
485 490 495
Asp Asp Met Leu Glu Gly Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln
500 505 510
His Asn Asn Leu Ala Arg Leu Trp Lys His Ala Asn Pro Gly Gly Pro
515 520 525
Ile Tyr Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
530 535 540

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Ser Asn Gly Phe Asp Glu Ile Pro Val Glu Val Phe Lys Asp Leu Phe
545                 550                 555                 560
Glu Leu Lys Ile Ile Asp Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro
                565                 570                 575
Ala Ser Val Phe Asn Asn Gln Val Ser Leu Lys Ser Leu Asn Leu Gln
            580                 585                 590
Lys Asn Leu Ile Thr Ser Val Glu Lys Lys Val Phe Gly Pro Ala Phe
        595                 600                 605
Arg Asn Leu Thr Glu Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
    610                 615                 620
Cys Glu Ser Ile Ala Trp Phe Val Asn Trp Ile Asn Glu Thr His Thr
625                 630                 635                 640
Asn Ile Pro Glu Leu Ser Ser His Tyr Leu Cys Asn Thr Pro Pro His
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Tyr His Gly Phe Pro Val Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp
            660                 665                 670
Ser Ala Pro Phe Glu
        675
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<210> 17
<211> 2031
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial cDNA sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutation at N636.

<400> 17

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ccggcggcga actttacccg ctatagccag ctgaccagcc tggatgtggg ctttaacacc 180
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aaaaaagtgt ttggcccggc gtttcgcaac ctgaccgaac tggatatgcg ctttaacccg 1860
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aacattccgg aactgagcag ccattatctg tgcaacaccc cgccgcatta tcatggcttt 1980
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<212> PRT
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<223> Artificial amino acid sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutation at N636.

<400> 18

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| 1 | | | | 5 | | | | 10 | | | | | | 15 | |
| Thr | Gln | Val | Pro | Asp | Asp | Leu | Pro | Thr | Asn | Ile | Thr | Val | Leu | Asn | Leu |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Thr | His | Asn | Gln | Leu | Arg | Arg | Leu | Pro | Ala | Ala | Asn | Phe | Thr | Arg | Tyr |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Ser | Gln | Leu | Thr | Ser | Leu | Asp | Val | Gly | Phe | Asn | Thr | Ile | Ser | Lys | Leu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Glu | Pro | Glu | Leu | Cys | Gln | Lys | Leu | Pro | Met | Leu | Lys | Val | Leu | Asn | Leu |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Gln | His | Asn | Glu | Leu | Ser | Gln | Leu | Ser | Asp | Lys | Thr | Phe | Ala | Phe | Cys |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Thr | Asn | Leu | Thr | Glu | Leu | His | Leu | Met | Ser | Asn | Ser | Ile | Gln | Lys | Ile |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Lys | Asn | Asn | Pro | Phe | Val | Lys | Gln | Lys | Asn | Leu | Ile | Thr | Leu | Asp | Leu |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Ser | His | Asn | Gly | Leu | Ser | Ser | Thr | Lys | Leu | Gly | Thr | Gln | Val | Gln | Leu |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Glu | Asn | Leu | Gln | Glu | Leu | Leu | Leu | Ser | Asn | Asn | Lys | Ile | Gln | Ala | Leu |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Lys | Ser | Glu | Glu | Leu | Asp | Ile | Phe | Ala | Asn | Ser | Ser | Leu | Lys | Lys | Leu |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Glu | Leu | Ser | Ser | Asn | Gln | Ile | Lys | Glu | Phe | Ser | Pro | Gly | Cys | Phe | His |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Ala | Ile | Gly | Arg | Leu | Phe | Gly | Leu | Phe | Leu | Asn | Asn | Val | Gln | Leu | Gly |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Pro | Ser | Leu | Thr | Glu | Lys | Leu | Cys | Leu | Glu | Leu | Ala | Asn | Thr | Ser | Ile |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Arg | Asn | Leu | Ser | Leu | Ser | Asn | Ser | Gln | Leu | Ser | Thr | Thr | Ser | Asn | Thr |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Thr | Phe | Leu | Gly | Leu | Lys | Trp | Thr | Asn | Leu | Thr | Met | Leu | Asp | Leu | Ser |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Tyr | Asn | Asn | Leu | Asn | Val | Val | Gly | Asn | Asp | Ser | Phe | Ala | Trp | Leu | Pro |
| | | | 260 | | | | | 265 | | | | | 270 | | |

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Ser His Ser Leu His Gly Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys
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Arg Ser Phe Thr Lys Gln Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile
305                 310                 315                 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Cys Leu Glu His Leu Asn Met
                325                 330                 335
Glu Asp Asn Asp Ile Pro Gly Ile Lys Ser Asn Met Phe Thr Gly Leu
            340                 345                 350
Ile Asn Leu Lys Tyr Leu Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg
        355                 360                 365
Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu His
    370                 375                 380
Ile Leu Asn Leu Thr Lys Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala
385                 390                 395                 400
Phe Ser Trp Leu Gly His Leu Glu Val Leu Asp Leu Gly Leu Asn Glu
                405                 410                 415
Ile Gly Gln Glu Leu Thr Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile
            420                 425                 430
Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn
        435                 440                 445
Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
    450                 455                 460
Ala Leu Lys Asn Val Asp Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg
465                 470                 475                 480
Asn Leu Thr Ile Leu Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn
                485                 490                 495
Asp Asp Met Leu Glu Gly Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln
            500                 505                 510
His Asn Asn Leu Ala Arg Leu Trp Lys His Ala Asn Pro Gly Gly Pro
        515                 520                 525
Ile Tyr Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
    530                 535                 540
Ser Asn Gly Phe Asp Glu Ile Pro Val Glu Val Phe Lys Asp Leu Phe
545                 550                 555                 560
Glu Leu Lys Ile Ile Asp Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro
                565                 570                 575
Ala Ser Val Phe Asn Asn Gln Val Ser Leu Lys Ser Leu Asn Leu Gln
            580                 585                 590
Lys Asn Leu Ile Thr Ser Val Glu Lys Lys Val Phe Gly Pro Ala Phe
        595                 600                 605
Arg Asn Leu Thr Glu Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
    610                 615                 620
Cys Glu Ser Ile Ala Trp Phe Val Asn Trp Ile Ala Glu Thr His Thr
625                 630                 635                 640
Asn Ile Pro Glu Leu Ser Ser His Tyr Leu Cys Asn Thr Pro Pro His
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Ser Ala Pro Phe Glu
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<213> Artificial Sequence

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<223> Artificial cDNA sequence derived from the Homo sapiens hTLR3 ECD which lacks a signal peptide and contains an alanine (A) substitution mutations at N221, N226, N387, and N636.

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ccggcggcga actttacccg ctatagccag ctgaccagcc tggatgtggg ctttaacacc 180
attagcaaac tggaaccgga actgtgccag aaactgccga tgctgaaagt gctgaacctg 240
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gatgatttta gctttcagtg gctgaaatgc ctggaacatc tgaacatgga agataacgat 1020
attccgggca ttaaaagcaa catgtttacc ggcctgatta acctgaaata tctgagcctg 1080
agcaacagct ttaccagcct gcgcaccctg accaacgaaa cctttgtgag cctggcgcat 1140
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tttagctggc tgggccatct ggaagtgctg gatctgggcc tgaacgaaat tggccaggaa 1260
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aacattccgg aactgagcag ccattatctg tgcaacaccc cgccgcatta tcatggcttt 1980
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<210> 20
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35 40 45
Ser Gln Leu Thr Ser Leu Asp Val Gly Phe Asn Thr Ile Ser Lys Leu
50 55 60
Glu Pro Glu Leu Cys Gln Lys Leu Pro Met Leu Lys Val Leu Asn Leu
65 70 75 80
Gln His Asn Glu Leu Ser Gln Leu Ser Asp Lys Thr Phe Ala Phe Cys
85 90 95
Thr Asn Leu Thr Glu Leu His Leu Met Ser Asn Ser Ile Gln Lys Ile
100 105 110
Lys Asn Asn Pro Phe Val Lys Gln Lys Asn Leu Ile Thr Leu Asp Leu
115 120 125
Ser His Asn Gly Leu Ser Ser Thr Lys Leu Gly Thr Gln Val Gln Leu
130 135 140
Glu Asn Leu Gln Glu Leu Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu
145 150 155 160
Lys Ser Glu Glu Leu Asp Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu
165 170 175
Glu Leu Ser Ser Asn Gln Ile Lys Glu Phe Ser Pro Gly Cys Phe His
180 185 190
Ala Ile Gly Arg Leu Phe Gly Leu Phe Leu Asn Asn Val Gln Leu Gly
195 200 205
Pro Ser Leu Thr Glu Lys Leu Cys Leu Glu Leu Ala Ala Thr Ser Ile
210 215 220
Arg Ala Leu Ser Leu Ser Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr
225 230 235 240
Thr Phe Leu Gly Leu Lys Trp Thr Asn Leu Thr Met Leu Asp Leu Ser
245 250 255
Tyr Asn Asn Leu Asn Val Val Gly Asn Asp Ser Phe Ala Trp Leu Pro
260 265 270
Gln Leu Glu Tyr Phe Phe Leu Glu Tyr Asn Asn Ile Gln His Leu Phe
275 280 285
Ser His Ser Leu His Gly Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys
290 295 300
Arg Ser Phe Thr Lys Gln Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile
305 310 315 320
Asp Asp Phe Ser Phe Gln Trp Leu Lys Cys Leu Glu His Leu Asn Met
325 330 335
Glu Asp Asn Asp Ile Pro Gly Ile Lys Ser Asn Met Phe Thr Gly Leu
340 345 350
Ile Asn Leu Lys Tyr Leu Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg
355 360 365
Thr Leu Thr Asn Glu Thr Phe Val Ser Leu Ala His Ser Pro Leu His
370 375 380
Ile Leu Ala Leu Thr Lys Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala
385 390 395 400
Phe Ser Trp Leu Gly His Leu Glu Val Leu Asp Leu Gly Leu Asn Glu
405 410 415
Ile Gly Gln Glu Leu Thr Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile
420 425 430
Phe Glu Ile Tyr Leu Ser Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn
435 440 445

Ser Phe Ala Leu Val Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val
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Ala Leu Lys Asn Val Asp Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg
465 470 475 480
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485 490 495
Asp Asp Met Leu Glu Gly Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln
500 505 510
His Asn Asn Leu Ala Arg Leu Trp Lys His Ala Asn Pro Gly Gly Pro
515 520 525
Ile Tyr Phe Leu Lys Gly Leu Ser His Leu His Ile Leu Asn Leu Glu
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Ser Asn Gly Phe Asp Glu Ile Pro Val Glu Val Phe Lys Asp Leu Phe
545 550 555 560
Glu Leu Lys Ile Ile Asp Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro
565 570 575
Ala Ser Val Phe Asn Asn Gln Val Ser Leu Lys Ser Leu Asn Leu Gln
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595 600 605
Arg Asn Leu Thr Glu Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr
610 615 620
Cys Glu Ser Ile Ala Trp Phe Val Asn Trp Ile Ala Glu Thr His Thr
625 630 635 640
Asn Ile Pro Glu Leu Ser Ser His Tyr Leu Cys Asn Thr Pro Pro His
645 650 655
Tyr His Gly Phe Pro Val Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp
660 665 670
Ser Ala Pro Phe Glu
675

## CLAIMS

1. A peptide chain comprising an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 6 and at least one mutation within 3 amino acid residues of a position aligning to N221, N226, N387, or N636 of the amino acid sequence SEQ ID NO: 6.

2. A peptide chain comprising at least one mutation within 3 amino acid residues of position N221, N226, N387, or N636 of the amino acid sequence SEQ ID NO: 6.

3. A peptide chain comprising the amino acid sequence SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, or SEQ ID NO: 20.

4. A nucleic acid encoding the peptide chain of claim 1, 2 or 3.

5. The nucleic acid of claim 4 comprising the nucleic acid sequence of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, or SEQ ID NO: 19.

6. A method of modulating TLR3 activity in a cell comprising decreasing TLR3 glycosylation in the cell.

7. A method of modulating TLR3 activity in a cell comprising providing tunicamycin to the cell.

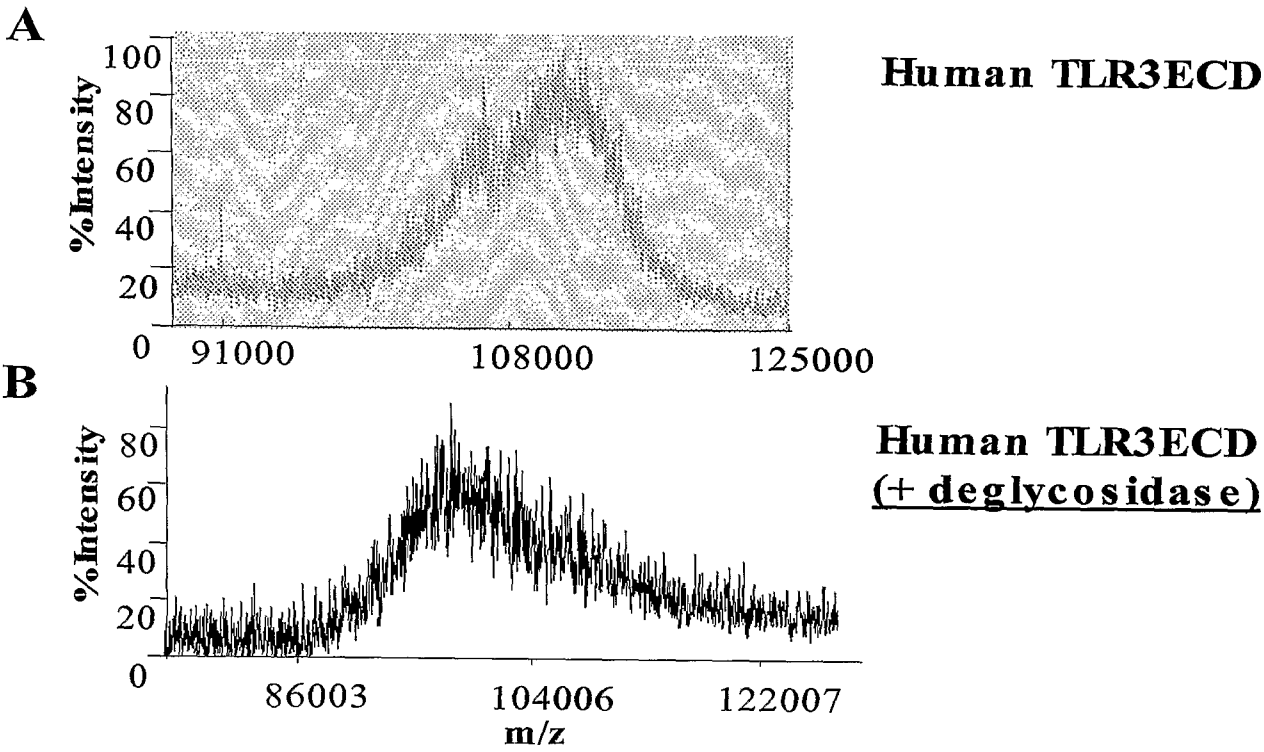
8. The method of claim 7 wherein the tunicamycin concentration outside the cell is about 0.2 µg/ml to 0.5 µg/ml.

9. A method of modulating TLR3 activity in a cell comprising providing the peptide chain of claim 1, 2, or 3 to the cell.

10. A method of modulating TLR3 activity in a cell comprising providing the nucleic acid of claim 4 to the cell.

11. The method of claim 10 wherein the nucleic acid is provided to the cell by transfection.

Fig. 1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80
60
40
20
0
%Intensity
91000
108000
125000
Human TLR3ECD
B
80
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20
0
%Intensity
86003
104006
122007
m/z
Human TLR3ECD
(+ deglycosidase)

Fig. 2

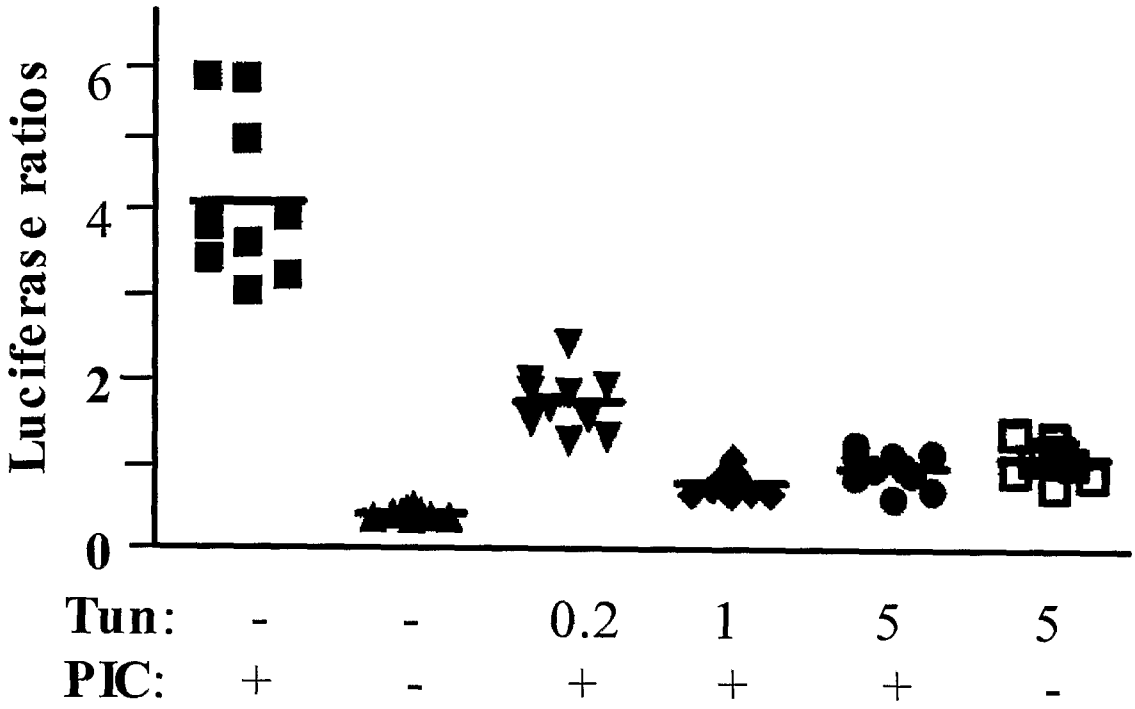
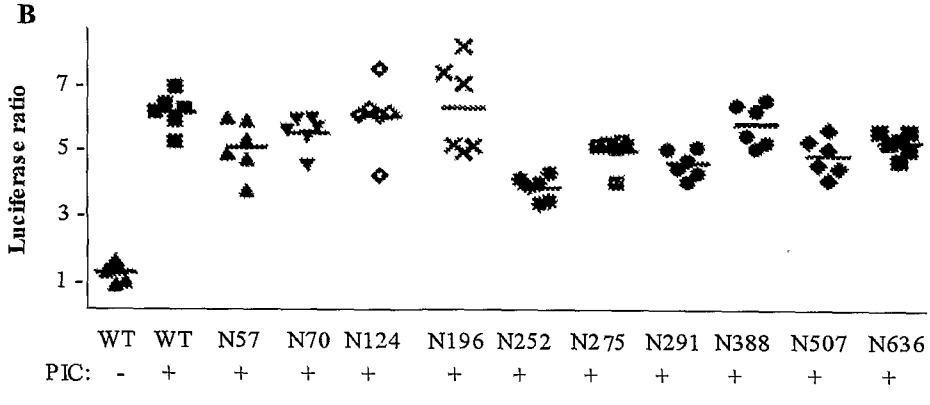


Fig. 3

**A**

| Species: | N57 | N70 | N124 | N196 | N247 | N252 | N265 | N275 | N291 | N388 | N413 | N507 | N636 | N662 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Homo | NLT | NTI | NSI | NSS | NLS | NSQ | NTT | NLT | NDS | NSF | NLT | NLT | NLT | NET |
| Pan | NLT | NTI | NSI | NSS | NLS | NSQ | NTT | NLT | NDS | NSF | NLT | NLT | NLT | NET |
| Canis | NLT | NSI | NSI | NSS | NLS | NTQ | NMT | NLT | NNS | NSF | NLT | NLN | NLS | NST |
| Bos | NLT | NSI | NSI | NSS | NLS | SNQ | HTT | NLT | NDS | NSF | NLT | NLV | NLS | NIT |
| Rattus | NLT | NSI | NSI | NSS | NLS | NNQ | NST | NLT | NDA | KTF | NLT | NLT | NLN | NQT |
| Mus | NLT | NSI | NSI | NSS | NLS | NNQ | EST | NLT | NGS | KTF | NLT | NLT | NLN | NQT |

**B**




**C**

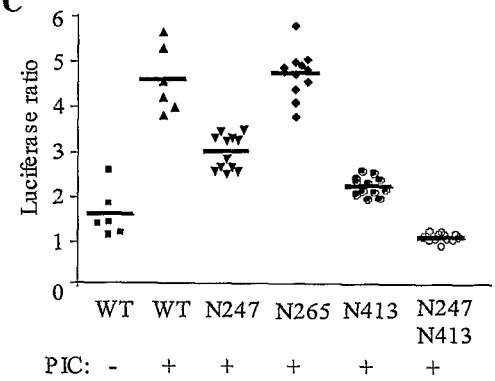
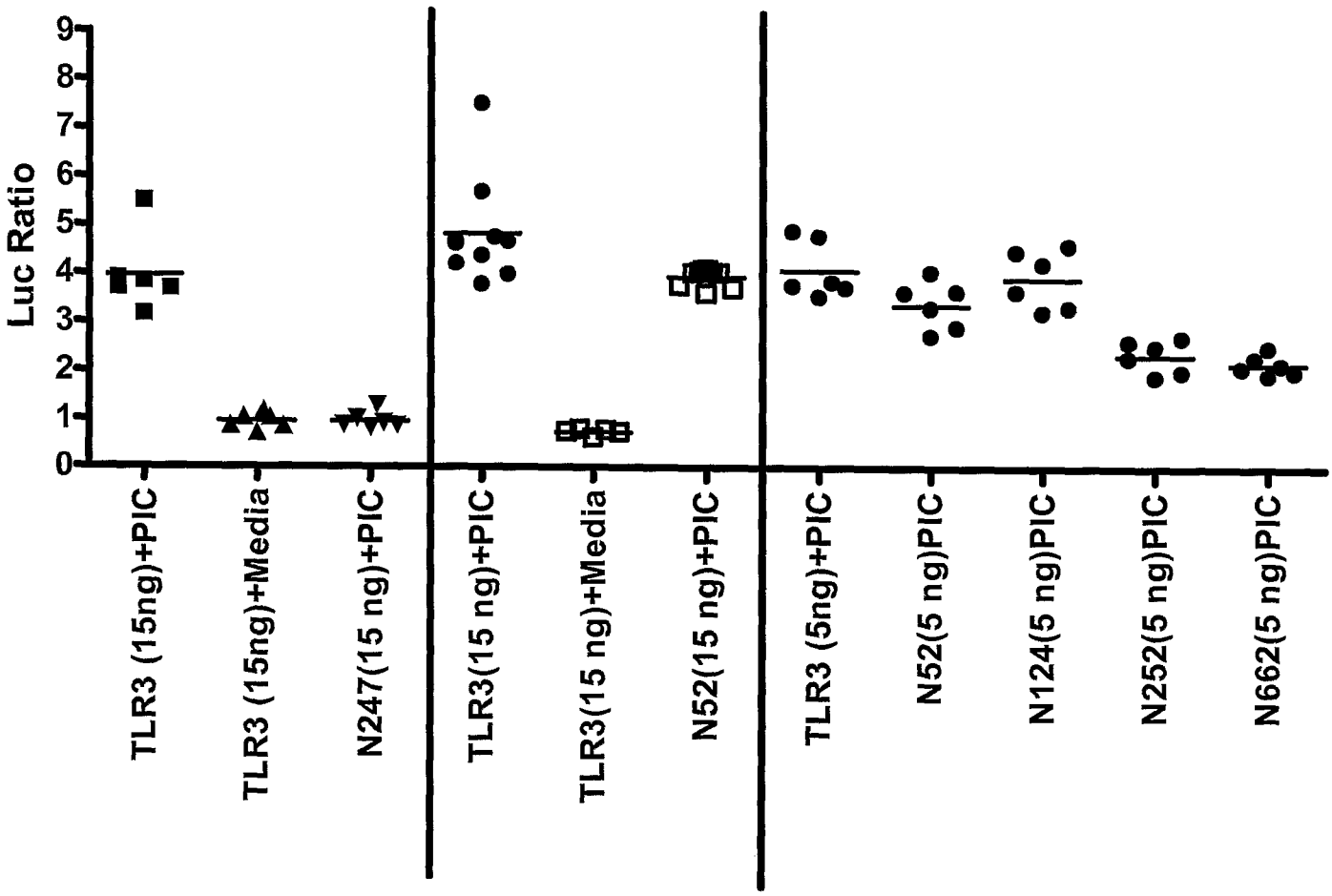


Fig. 4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1
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3
4
5
6
7
8
9
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TLR3 (15ng)+Media
N247(15 ng)+PIC
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N52(5 ng)PIC
N124(5 ng)PIC
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