USE OF TOLL-LIKE RECEPTOR AGONIST FOR TREATING CANCER

FIELD OF THE INVENTION

[0001] This invention relates to methods of treating cancer in Toll-Like Receptor-expressing tissues, and to methods of protecting the liver from the effects of a liver toxicity, using a TLR agonist.

BACKGROUND OF THE INVENTION

[0002] Interaction between members of the death receptor family and their cognate ligands induces apoptosis controlling the homeostasis of cell populations in tissues, particularly in the immune system. Although many tumor cell types are sensitive to death ligands, activation of Fas signaling also induces massive apoptosis in the liver leading to organ failure and death precluding its use for systemic anticancer therapy. Fas ligand is a 40 kDa physiological agonist of Fas signaling expressed on activated lymphocytes and many tumor cells which can also be secreted through metalloproteinase-mediated cleavage and kill the sensitive cells in autocrine and paracrine manner. Fas is a transmembrane receptor expressed on activated lymphocytes, variety of tissues and tumor cells. Fas signaling plays crucial role in regulation of the immune system by triggering autocrine suicide or paracrine death (apoptosis), suppressing immune reaction by eliminating activated lymphocytes. Upon binding, it induces p53 independent cell death through extrinsic pathway of apoptosis engaging DISC formation, caspase-8 and 10, and intrinsic (mitochondrial) apoptosis activating caspase-8 and Bid cleavage, and cytochrome release. Both apoptotic pathways lead to activation of caspase-3 and 7. Mitochondrial apoptosis is regulated by pro- and anti-apoptotic Bcl2 family members. In tumor cells, Fas signaling is often found deregulated either by absence of Fas receptor, or by constitutive activation of NF-kB resulting in the expression of anti-apoptotic genes, such as c-Flip, Bcl-2, Bcl-xL. C-Flip, an NF-kB responsive gene, has been demonstrated to inhibit caspase-8 and Fas mediated apoptosis in tumors (REFs, Kataoka et al 2000).

[0003] Upon discovery of p53 independent apoptotic mechanism through Fas, TRAIL and TNFα death receptor signaling, they seemed to be promising targets for anti-cancer therapy since tumor cells usually have impaired p53 function. A severe hepatotoxicity, however, is induced by death

receptor ligands. This has hampered development of these anti-cancer therapies. While Fas agonists cause liver damage and TNF-a induces strong inflammation in liver, lungs and other organs, TRAIL is the least toxic in humans. TRAIL has therefore received more attention than other agonists for the clinical application for an anticancer treatment. Many tumors, however, are not sensitive to TRAIL therapy. Several approaches to resolve death receptor toxicity issue are currently undertaken, most of which are aimed to increase tumor sensitivity by blockage of NF-kB activity and increasing receptor expression thus reducing the amount of drug necessary for the effective therapy. Another direction is to localize the drug delivery to the tumors to minimize toxic effects on distant organs. To date, there is no reliable approach to the prevention of toxicity (including liver injury) that would allow the systemic application of death receptor agonists in clinical trials. Accordingly, there is a need in the art for methods of preventing the undesirable effects of death receptors when they are used to treat cancer. In particular, there is a need to protect the liver from these undesirable effects. There is also a need for protecting the liver from liver toxicities in general.

[0004] TLRs are found to be expressed on both epithelial and endothelial cells as well as immunocytes. At present, thirteen TLRs have been identified in mammals. Upon receptor stimulation, several common signaling pathways get activated such as NF-kB, AP-1, PI3K/AKT and mitogen-activated protein kinases (MAPK) leading to increased survival, stimulation of cell proliferation and the secretion of many cytokines with chemotactic and pro-inflammatory functions. Induction of TLR in cancer cells can be used to treat cancer, however, the distribution of different TLRs varies significantly among the various organs and cell types. This affects the cytokine profile and extent of the inflammatory response of cells. Accordingly, there is a need in the art for cancer immunotherapeutic methods that do not depend on the presence of TLR5 expression.

SUMMARY OF THE INVENTION

[0005] Provided herein is a method of treating cancer in a mammal, which may comprise administering to a mammal in need thereof of Toll-Like Receptor (TLR) agonist. Also provided is a method of reducing cancer recurrence in a mammal, which may comprise administering to a mammal in need thereof a TLR agonist. The cancer may be present in a tissue that expresses TLR. The cancer may be a metastasis or tumor regrowth.

[0006] The TLR agonist may be flagellin. The cancer may not express TLR, which may be TLR5. The tissue may be liver, lung, bladder, or intestinal. The cancer may be metastatic. The cancer may be melanoma, colon, breast, prostate, or a hematological malignancy, which may be lymphoma. The cancer may be tumor.

[0007] The agent may be administered as a monotherapy. The mammal may not be receiving a combination therapy. The mammal may also not be receiving chemotherapy or radiation therapy, but may be treated surgically. The mammal may have sufficient innate immunity, which may be at a level that is equivalent to the level required for eligibility for a first or subsequent round of chemotherapy. The mammal may have a white blood cell count within the range of normal, or may have a white blood cell count indicative of mild-immunosuppression. The TLR agonist may be administered to the mammal before, after or concurrent with removal of a tumor. The TLR agonist may be administered during tumor removal.

[0008] Further provided herein is a method of treating cancer in a mammal, which may comprise administering to a mammal in need thereof a FAS agonist and a TLR agonist, which may be flagellin. The FAS agonist may be a FAS agonist antibody. The cancer may be metastatic, and may be a tumor. The cancer may not express a TLR. The cancer may have metastasized to an invaded tissue that expresses TLR. The invaded tissue may be liver, bladder, lung, or intestinal.

[0009] Also provided herein is a method of protecting liver tissue in a mammal from the effects of a liver toxicity, which may comprise administering to a mammal in need thereof a TLR agonist. The toxicity may be a FAS ligand, a FAS agonistic antibody, TNFα, acetaminophen, alcohol, a viral infection of the liver, or a chemotherapeutic agent. The toxicity may also be a Salmonella infection, which may be from *Salmonella typhimurium*. The TLR agonist may be flagellin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Figure 1 shows the domain structure of bacterial flagellin. The Ca backbone trace, hydrophobic core distribution and structural information of F41. Four distinct hydrophobic cores that define domains D1, D2a, D2b and D3. All the hydrophobic side-chain atoms are displayed with the Ca backbone. Side-chain atoms are color coded: Ala, yellow; Leu, Ile or Val, orange; Phe and Tyr, purple (carbon atoms) and red (oxygen atoms). c, Position and region of various structural features in the amino-acid sequence of flagellin. Shown are, from top to bottom: the

F41 fragment in blue; three b-folium folds in brown; the secondary structure distribution with a-helix in yellow, b-structure in green, and b-turn in purple; tic mark at every 50th residue in blue; domains D0, D1, D2 and D3; the axial subunit contact region within the proto-element in cyan; the well-conserved amino-acid sequence in red and variable region in violet; point mutations in F41 that produce the elements of different supercoils. Letters at the bottom indicate the morphology of mutant elements: L (D107E, R124A, R124S, G426A), L-type straight; R (A449V), R-type straight; C (D313Y, A414V, A427V, N433D), curly33.

[0011] Figure 2 shows a schematic of Salmonella flagellin domains, its fragments, and its interaction with TLR5. Dark bars denote regions of the flagellin gene used to construct fragments comprising A, B, C, A' and B'.

[0012] Figure 3 depicts flagellin derivatives. The domain structure and approximate boundaries (amino acid coordinates) of selected flagellin derivatives (listed on the right). FliC flagellin of Salmonella dublin is encoded within 505 amino acids (aa).

[0013] Figure 4 shows the nucleotide and amino acid sequence for the following flagellin variants: AA' (SEQ ID NO: 7-8), AB' (SEQ ID NO: 9-10), BA' (SEQ ID NO: 11-12), BB' (SEQ ID NO: 13-14), CA' (SEQ ID NO: 15-16), CB' (SEQ ID NO: 17-18), A (SEQ ID NO: 19-20), B (SEQ ID NO: 21-22), C (SEQ ID NO: 23-24), GST-A' (SEQ ID NO: 25-26), GST-B' (SEQ ID NO: 27-28), AA'n1-170 (SEQ ID NO: 29-30), AA'n1-163 (SEQ ID NO: 33-34), AA'n54-170 (SEQ ID NO: 31-32), AA'n54-163 (SEQ ID NO: 335-36), AB'n1-170 (SEQ ID NO: 37-38), AB'n1-163 (SEQ ID NO: 39-40), AA'n1-129 (SEQ ID NO: 41-42), AA'n54-129 (SEQ ID NO: 43-44), AB'n1-129 (SEQ ID NO: 45-46), AB'n54-129 (SEQ ID NO: 47-48), AA'n1-100 (SEQ ID NO: 49-50), AB'n1-100 (SEQ ID NO: 51-52), AA'n1-70 (SEQ ID NO: 53-54) and AB'n1-70 (SEQ ID NO: 55-56). The pRSETb leader sequence is shown in Italic (leader includes Met, which is also amino acid 1 of FliC). The N terminal constant domain is underlined. The amino acid linker sequence is in Bold. The C terminal constant domain is underlined. GST, if present, is highlighted.

[0014] Figure 5 shows a comparison of amino acid sequences of the conserved amino (Fig. 5A) and carboxy (Fig. 5B) terminus from 21 species of bacteria. The 13 conserved amino acids important for TLR5 activity are shown with shading. The amino acid sequences are identified by their accession numbers from TrEMBL (first letter = Q) or Swiss-Prot (first letter = P).

[0015] Figure 6 shows the sequence of human TLR5.

[0016] Figure 7 NF-kB activation in vivo in response to CBLB502 and LPS injections. A. Background and NF-kB dependent luciferase expression in BALB/c-Tg(IκBα-luc)Xen reporter mice was detected by noninvasive imaging 2 hs after the treatment with CBLB502 (0.2 mg/kg). B. NF-kB dependent luciferase expression in liver, small intestine (ileum part), colon, spleen, kidneys, lungs and heart was assessed in the reporter mice 2 hs after s.c. injections of 100 μl of either PBS, CBLB502 (0.2 mg/kg) or LPS (1 mg/kg). Luciferase activity normalized per μg of the protein extract was detected in 3 mice in each group. Bars represent average +/- s.d. C. The dynamics of NF-kB nuclear translocation (p65) indicative of the bioactivity of agonists LPS and CBLB502 in liver from NIH-Swiss mice injected s.c either with CBLB502 or LPS. Control mice were injected with PBS. Tissue samples were obtained 20, 40 and 60 min after the treatments, processed into paraffin blocks. Nuclear translocation of p65 in primary mouse hepatocytes isolated from NIH-Swiss mice (D) and human hepatocytes purchased from (BD Biosciences) (E) was detected after in vitro treatment with CBLB502 (100 ng/ml) or LPS (1 μg/ml) for indicated period of time. Control hepatocytes remained intact. P65 was stained with green fluorescence, cytokeratin-8 with red fluorescence and nuclei with non-specific Dapi blue staining. Pictures are taken at x20 magnification. Arrows indicate Kupffer and endothelial cells determined based on morphological criteria.

[0017] Figure 8 shows CBLB502 protection from Fas mediated hepatotoxicity. A. Survival of NIH-Swiss mice after i.p. injection of 4 μg of anti-Fas antibodies alone or in combination with CBLB502 (1 μg/ mouse) injected 30 min, 2 hours and 6 hours prior antibodies. In parenthesis are the numbers of mice per each treatment. B. Protection of livers from anti-Fas antibody toxicity. Apoptosis in livers 5 hours after injections of anti-Fas antibodies was detected using TUNEL technique. C. Tissue morphology with H&E staining revealed necrotic damge to livers by anti-Fas antibody injections and protection by CBLB502. D. Hemorrhage in liver was detected using erythrocyte autofluorescence (rhodamine channel, red), mouse IgG control (Cy5-conjugated anti-mouse IgG antibody, pceudocolored in purple) and DAPI nuclei (blue). E. Caspase-3/7 activity in liver samples of NIH-Swiss mice was determined in tissue protein lysates 5 hours after injection of 3 μg anti-Fas antibody with or without CBLB502 thirty minute pre-treatment. N=3. Bars represent average +/- s.d. F. Alanine aminotransferase (ALT) accumulation in blood serum of NIH-Swiss mice was detected 5 hours after anti-Fas antibody injections with or without CBLB502. N=3. Bars represent average +/- s.d. G. Caspase-8 activity in liver samples of NIH-

Swiss mice was determined in tissue protein lysates 5 hours after injection of 3 μg anti-Fas antibody with or without CBLB502 thirty minute pre-treatment. N=3. Bars represent average +/- s.d. .

[0018] Figure 9 shows regulation of apoptosis-related factors by CBLB502 in liver and its effect on Fas-mediated antitumor activity in CT-26 tumor model. Inhibition of caspase-8 (A) and Bid (B) cleavage by CBLB502 detected in liver isolated from C57BL/6 mice 2 hours after anti-Fas antibody injections (5 μg) alone or in combination with CBLB502 by western blot. C. RNA expression of Bcl2A1B, Bcl2A1D, IER-3, Fos, Jun and JunB genes in livers of intact mice and treated with CBLB502 for 30 min and 2 hours was detected by RT-PCR. GAPDH was used as a control to monitor the induction of gene expression. D. Mice with s.c. growing CT-26 tumors were injected either with single anti-Fas antibodies (4 μg/mouse) and CLB502 or their combination. Control mice ("intact") received PBS in replace of CBLB502 and antibodies. In parenthesis are the numbers of tumors in each group. The results represent the average tumor volumes (m +/- standard error). (*) – The difference between intact and combination treatment groups is significant ($p<0.05$). E. Mice were treated with anti-Fas antibodies alone or in combination with CBLB502 on day 5 after intrasplenic injection of luciferase expressing CT-26 tumor cells. Tumor growth in livers was determined using Xenogen IVIS Imaging System on the days 10, 15, 17, 22, 28 and 40 after tumor cell inoculation. Images of 3 mice from each group taken on day 15 are presented. The difference between proportions of mice with tumor-free livers in CBLB502-treated and control groups reaches statistical significance ($p<0.05$) on days indicated by asterisks. F. Migration and infiltration of immunocytes (arrows) into tumor nodules grown in liver of mice 5 hrs post treatment with CBLB502.

[0019] Figure 10. Dynamics of NF-kB activation in different organs after injections with CBLB502 (5 μg, s.c.) or LPS (20 μg, s.c.). Mice were euthanized 2, 6, 24 and 48 hours later by CO2 inhalation. Luciferase activity in protein extracts from liver, large intestine, kidneys and lungs was normalized per μg of the protein extract and average values were calculated per organ. Luciferase fold induction was calculated as ratio between average luciferase activity in protein extract from organs of the TLR agonist treated mice and that obtained in the extracts from the corresponding organs of the PBS injected control mice (3 mice/ group). Bars represent fold induction as average ± s.e.

[0020] Figure 11. NF-kB dependent luciferase expression in primary culture of mouse hepatocytes isolated from luciferase reporter mice and treated in vitro for 3 hours with CBLB502 (100 ng/ml), LPS (5 μg/ml) or PBS control. Then hepatocytes were rinsed with PBS and collected in cell lysis buffer (Promega). Luciferase activity in the protein supernatants was determined by Promega reporter system and normalized per μg of the protein extract. Bars represent luciferase units (mean ± s.d.).

[0021] Figure 12. H&E staining of liver samples from NIH-Swiss mice treated with CBLB502, anti-Fas antibodies (3 μg) or their combination obtained at different time-points after the treatment. Samples of livers were obtained 5, 12 and 26 hours after injections of anti-Fas antibodies, fixed in 10% formalin, embedded in paraffin and stained for tissue morphology with hematoxilin and eosin.

[0022] Figure 13. TLR5 expression on B16 and CT-26 cells. A. Expression of mRNA encoding TLR5 and GAPDH (as a control) genes in B16 and CT-26 cells were determined by RT-PCR. A region of mouse TLR5 mRNA was amplified using primers specific for the mouse TLR5 gene: forward (5'-AGTCCCCCAGCTCCAGTTTC-3') and reverse (5'-GGAGCCCCCTAGCAGTG AGT-3'). B. NF-kB activation in B16 and CT-26 tumor cells in response to CBLB502, mouse TNFα and LPS was tested using luciferase reporter assay. Data represent luciferase units (mean ± s.d).

[0023] Figure 14. Shows TLR5 expresion in CT-26 tumor cells and A20 lymphoma cells. For Figs. 14A and C, total RNA was extracted from CT-26 and B16 tumor cells (Fig. 14A) and CT-26 and A20 cells (Fig. 14C) using TRIzol reagent as described in the main text of the manuscript. The primers for TLR5 were designed using LaserGene software (DNASTAR, Inc., Madison, WI). A region of mouse TLR5 mRNA (GenBank Accession No. NM_016928.2) was amplified using primers specific for the mouse TLR5 gene: forward (5'-AGTCCCCCAGCTCCAGTTTC-3') and reverse (5'-GGAGCCCCCTAGCAGTGAGT-3'). GAPDH was used as a control to monitor the induction of gene expression. cDNAs were synthesized using SuperscriptTM II Reverse Transcriptase and oligo(dT)12-18 primer (Invitrogen, Carlsbad, CA). B. An *in vitro* luciferase assay for NF-kB activation in B16 (TLR5 positive) and CT-26 TLR5 negative) tumor cells was performed.

[0024] Figure 15 shows the dynamics of TLR5 positive HCT116 tumor growth in athymic nude mice after CBLB502 or PBS (no treatment) treatments (0.2 mg/kg, s.c., days 1, 2, 3), n=6-10.

[0025] Figure 16 shows 293-TLR5 tumor growth in athymic nude mice after CBLB502 or PBS (no treatment) treatments (0.2 mg/kg, s.c., days 1, 2, 3), n=6-10.

[0026] Figure 17 shows the dynamics of xenogenic A549 tumor growth in athymic nude mice during 2 courses of CBLB502 vs. PBS (control) treatments (days 1, 2, 3, 14, 15 and 16), n=6-10. Antitumor activity of colon HCT116 adenocarcinoma s.c. Grown as a xenograft in athymic mice. HCT116 were injected s.c. into 2 flanks of 8 athymic nude mice (0.5x106 /100 ml of PBS) to induce tumors. When tumors became of about 3-5 mm in diameter (by day 6 after injections) mice were randomly distributed into 2 groups, 5 mice for CBLB502 treated group and 3 mice in PBS control group.

[0027] Figure 18 shows the rate of SCCVII orthotopic tumor growth in syngenic C3H mice after CBLB502 or PBS (no treatment) treatments (0.1 mg/kg, s.c.days 1, 2, 3) to reach 400 mm^3 tumor size, n=6-10. Right figure represents the amount of days needed for tumors to reach 400 mm3 volume with and without treatment with CBLB502.

[0028] Figure 19. Fischer rats with s.c. growing syngeneic Ward colon tumors were treated with CBLB502 (0.2 mg/kg) was administered by i.p. once a day for three days.

[0029] Figure 20 shows the dynamics of xenogenic A549-shV and A549-shTLR5 tumor growth in athymic nude mice after CBLB502 or PBS (control) treatments (days 1, 2, 3). Statistical difference between tumor volumes on days 2, 4, 6 and 8 observed in A549-shV tumors ($p<0.05$), n=9-14. Right figure demonstrates NF-kB dependent induction of luciferase reporter expression in A549-shV and A549-shTLR5 in response to CBLB502 treatment.

[0030] Figure 21 shows the dynamics of H1299 (control) and H1299-TLR5 tumor growth in athymic nude mice after CBLB502 or PBS (control) treatments (days 1, 2, 3), n=6. Right figure demonstrates IL-8 production in response to CBLB502 treatment as indicative of TLR5 function in H1299-TLR5 cells.

[0031] Figure 22 shows that the bladder strongly responds to CBLB502.

[0032] Figure 23 shows that CBLB502 treatment delays tumor appearance and growth in livers, even in tumors that do not express TLR5.

[0033] Figure 24 shows CBLB502 protection from Fas mediated hepatotoxicity.

[0034] Figure 25 shows that the liver is protected from TNFα and LPS toxicity by CBLB502.

[0035] Figure 26 shows that CBLB502 protects the lungs from TNF and LPS toxicity.

[0036] Figure 27 shows that CBLB502 protects mice from legal oral administration of Salmonella.

[0037] Figure 28 shows that irinotecan abrogates the antitumor effect of flagellin (CBLB502).

DETAILED DESCRIPTION

[0038] The inventors have made the surprising discovery that the provision of a Toll-Like Receptor (TLR) agonist, such an agonist of TLR5 like flagellin, can effectively inhibit the growth of and reduce cancer cells, even when the cells do not express TLR5. The TLR agonist may be particularly useful in treating liver, bladder, lung, and intestinal cancers, whether primary or metastatic, as well as cancer affecting other TLR5-positive tissues. The TLR agonist can also be used to treat cancers that originate in tissues other than the liver, bladder, lung, intestinal, and other TLR5-positive tissues, but metastasize to these tissues. Even though the metastatic cancer cells do not express TLR5, the cancer may nonetheless be treatable with the TLR agonist when the cancer has metastasized to TLR5-expressing tissues such as the liver. While not being bound by theory, the idea implemented in this invention is that TLR agonists effectively reduce or kill cancer cells affecting a tissue that has a strong innate immunity system, thereby obviating the need for any pre-existing expression of TLR5 in the cancer cells. Unexpectedly, by providing a TLR agonist, the innate immune system is sufficiently triggered so as to treat cancers that are devoid of TLR5 expression. Thus, TLR5 does not need to be provided to the cancer cells in order for the TLR agonist to effectively reduce or kill cancer cells.

[0039] The inventors have also made the surprising discovery that a TLR agonist can protect the liver from a liver toxicity. For example, death ligands and activators of FAS-mediated apoptosis, such as FAS ligand and anti-FAS agonistic antibodies, can induce does-dependent hepatotoxicity. Administering the TLR agonist can protect the liver against such toxicities. This unexpected property of TLR agonists allows it be combined with FAS agonists or TNF for cancer treatment, such that the adverse of effects of the FAS agonist or TNF are reduced or prevented.

1. Definitions.

[0040] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the

singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0041] For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

[0042] "Administer" may mean a single dose or multiple doses of an agent or agent.

[0043] "Analog" may mean, in the context of a peptide or polypeptide, a peptide or polypeptide comprising one or more non-standard amino acids or other structural variations from the conventional set of amino acids.

[0044] "Antibody" may mean an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, or derivatives thereof, including Fab, F(ab')2, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody may be a monoclonal antibody, polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom. The antibody may also be a chimeric antibody. The antibody may be derivatized by the attachment of one or more chemical, peptide, or polypeptide moieties known in the art. The antibody may be conjugated with a chemical moiety.

[0045] A "derivative" may mean a peptide or polypeptide different other than in primary structure (amino acids and amino acid analogs). Derivatives may differ by being glycosylated, one form of post-translational modification. For example, peptides or polypeptides may exhibit glycosylation patterns due to expression in heterologous systems. If at least one biological activity is retained, then these peptides or polypeptides are derivatives according to the invention. Other derivatives may include fusion peptides or fusion polypeptides having a covalently modified N- or C-terminus, PEGylated peptides or polypeptides, peptides or polypeptides associated with lipid moieties, alkylated peptides or polypeptides, peptides or polypeptides linked via an amino acid side-chain functional group to other peptides, polypeptides or chemicals, and additional modifications as would be understood in the art.

[0046] A "fragment" may mean a portion of a reference peptide or polypeptide.

[0047] A "homolog" may mean a peptide or polypeptide sharing a common evolutionary ancestor.

[0048] A "leader sequence" may be a nucleic acid encoding any peptide sequence that is linked and translated with a peptide or polypeptide of interest to allow the peptide or polypeptide of interest be properly routed through a eukaryotic cell's endoplasmic reticulum and Golgi complexes for the purposed of extracellular secretion from the cell's membrane. The leader peptide sequence may be derived from alkaline phosphatase. The leader sequence may have a DNA sequence comprising atgctgctgctgctgctgctgctgggcctgaggctacagctct ccctgggc.

[0049] A "liposome" may mean a tiny bubble (vesicle) made out of the same material as a cell membrane. A liposome be filled with drugs and used to deliver drugs for cancer and other diseases. A liposome may be filled with a vector. A liposome membrane may be made of phospholipids, which are molecules that have a head group and a tail group. The head of the liposome may be attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water. The tails may be repelled by water, and line up to form a surface away from the water. The lipids in the plasma membrane may be chiefly phospholipids like phosphatidylethanolamine and phosphatidylcholine. Liposomes may be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine), or of pure surfactant components like DOPE (dioleoylphosphatidylethanolamine).

[0050] A "peptide" or "polypeptide" may mean a linked sequence of amino acids and may be natural, synthetic, or a modification or combination of natural and synthetic.

[0051] "Substantially identical" may mean that a first and second amino acid sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%,or 99% over a region of 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 amino acids .

[0052] "Treating," "treatment," or "to treat" each may mean to alleviate, suppress, repress, eliminate, prevent or slow the appearance of symptoms, clinical signs, or underlying pathology of a condition or disorder on a temporary or permanent basis. Preventing a condition or disorder involves administering a agent of the present invention to a subject prior to onset of the disease. Suppressing a condition or disorder involves administering a agent of the present invention to a subject after induction of the condition or disorder but before its clinical appearance. Repressing the condition or disorder involves administering a agent of the present invention to a subject after clinical appearance of the disease.

[0053] A "variant" may mean means a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Representative examples of "biological activity" include the ability to bind to a toll-like receptor and to be bound by a specific antibody. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte *et al., J. Mol. Biol.* 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hyrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

[0054] A "vector" may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, a yeast or a mammalian artificial chromosome. A vector may be a RNA or DNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

2. Toll-like Receptor Agonist

[0055] Provided herein is a TLR agonist. The TLR agonist may be a PAMP, which may be conserved molecular product derived from a pathogen. The pathogen may be a Gram-positive bacterium, Gram-negative bacterium,, fungus, or virus. The TLR agonist may be a damage-associated molecular pattern (DAMP) ligand, which may be an endogenous molecule released from injured or dying cells. A DAMP or PAMP may initiate an immune response through TLR signals and recruit adapter molecules within the cytoplasm of cells in order to propagate a signal. The TLR agonist may be an agonist for the TLR, which may be a ligand from the following in Table 1:

Table 1 TLRs and Ligands

TLR	**Ligand DAMP**	**Ligand PAMP**
TLR1		Triacyl lipoproteins
TLR2	Heat Shock proteins	Peptidoglycan
	HMGB1 (high mobility group box 1—amphoterin)	Lipoprotein
		Lipoteichoic acid
		Zymosan
TLR3	Self dsRNA	Viral dsRNA
TLR4	Heat shock proteins	Heat shock proteins
	Fibrinogen	Lipopolysaccharides
	Heparan sulfate	RSV fusion protein
	Fibronectin	MMTV (Mouse mammary tumor virus) envelope proteins
	Hyaluronic acid	Paclitaxel
	HMGB1	
TLR5		flagellin
TLR6		Lipoteichoic acid
		Triacyl lipoproteins
		zymosan
TLR7/TLR8	Self ssRNA	Viral ssRNA
TLR9	Self DNA	Bacterial and viral DNA
TLR10		
TLR11		Profilin

[0056] The TLR agonist may be a fragment, variant, analog, homology or derivative of a PAMP or DAMP that binds a TLR and induces TLR-mediated activity, such as activation of NF-κB activity. The TLR agonsist fragment, variant, analog, homolog, or derivative may be at least 30-99% identical to amino acids of a TLR-agonist and induce TLR-mediated activity.

[0057] The TLR agonist may target a TLR such as TLR-5. The TLR agonist may be an agonist of TLR-5 and stimulate TLR-5 activity. The TLR agonist may be an anti-TLR5 antibody or other small molecule. The TLR agonist may be flagellin.

[0058] The flagellin may also be a flagellin or flagellin-related polypeptide. The flagellin may be from any source, including a variety of Gram-positive and Gram-negative bacterial species. The flagellin may be a flagellin polypeptide from any Gram-positive or Gram-negative bacterial species including, but not limited to, a flagellin polypeptide disclosed in U.S. Pat. Pub. No. 2003/000044429, the contents of which are fully incorporated herein by reference. For example, the flagellin may have an amino acid sequence from a bacterial species depicted in Figure 7 of U.S. Patent Publication No. 2003/0044429. The nucleotide sequences encoding the flagellin polypeptides listed in Figure 7 of U.S. 2003/0044429 are publicly available at sources including the NCBI Genbank database. The flagellin may also be a flagellin peptide corresponding to an Accession number listed in the BLAST results shown in Fig. 25 of U.S. Patent Pub. 2003/000044429, or a variant thereof. The flagellin may also be a flagellin polypeptide as disclosed in U.S. Patent Appl. Publication No. 2009/0011982, the contents of which are fully incorporated herein. The flagellin maybe any one of a flagellin polypeptide as disclosed in Figures 3 and 4 herein.

[0059] The flagellin may be a fragment, variant, analog, homology or derivative of a flagellin that binds TLR5 and induces TLR5-mediated activity, such as activation of NF-κB activity. A fragment, variant, analog, homolog, or derivative of flagellin may be at least 30-99% identical to amino acids of a flagellin that binds TLR5 and induces TLR5-mediated activity.

[0060] The flagellin may be from a species of Salmonella, a representative example of which is S.dublin (encoded by GenBank Accession Number M84972). The flagellin related-polypeptide may be a fragment, variant, analog, homolog, or derivative of M84972, or combination thereof, that binds to TLR5 and induces TLR5-mediated activity, such as activation of NF-kB activity. A fragment, variant, analog, homolog, or derivative of flagellin may be obtained by rational-based design based on the domain structure of Flagellin and the conserved structure recognized by TLR5.

[0061] The flagellin may comprise at least 10, 11, 12, or 13 of the 13 conserved amino acids shown in Fig. 2 (positions 89, 90, 91, 95, 98, 101, 115, 422, 423, 426, 431, 436 and 452). The flagellin may be at least 30-99% identical to amino acids 1 174 and 418 505 of M84972. Fig. 26

of U.S. Patent Appl Publication No. 2009/0011982, the contents of which are fully incorporated herein, lists the percentage identity of the amino- and carboxy-terminus of flagellin with known TLR-5 stimulating activity, as compared to M84972.

[0062] The flagellin may be the major component of bacterial flagellum. The flagellin may be composed of three domains (Fig. 1). Domain 1 (D1) and domain 2 (D2) may be discontinuous and may be formed when residues in the amino terminus and carboxy terminus are juxtaposed by the formation of a hairpin structure. The amino and carboxy terminus comprising the D1 and D2 domains may be most conserved, whereas the middle hypervariable domain (D3) may be highly variable. Studies with a recombinant protein containing the amino D1 and D2 and carboxyl D1 and D2 separated by an Escherichia coli hinge (ND1-2/ECH/CD2) indicate that D1 and D2 may be bioactive when coupled to an ECH element. This chimera, but not the hinge alone, may induce IkBa degradation, NF-kB activation, and NO and IL-8 production in two intestinal epithelial cell lines. The non-conserved D3 domain may be on the surface of the flagellar filament and may contain the major antigenic epitopes. The potent proinflammatory activity of flagellin may reside in the highly conserved N and C D1 and D2 regions (See Figure 1).

[0063] The flagellin may induce NF-kB activity by binding to Toll-like receptor 5 (TLR5). The TLR may recognize a conserved structure that is particular to the flagellin. The conserved structure may be composed of a large group of residues that are somewhat permissive to variation in amino acid content. Smith et al., Nat Immunol. 4:1247-53 (2003), the contents of which are incorporated herein by reference, have identified 13 conserved amino acids in flagellin that are part of the conserved structure recognized by TLR5. The 13 conserved amino acids of flagellin that may be important for TLR5 activity are shown in Fig. 2.

[0064] Numerous deletional mutants of flagellin have been made that retain at least some TLR5 stimulating activity. The flagellin may be such a deletional mutant, and may be a deletional mutant disclosed in the Examples herein. The flagellin may comprise a sequence translated from GenBank Accession number D13689 missing amino acids 185-306 or 444-492, or from GenBank Accession number M84973 missing amino acids 179-415, or a variant thereof.

[0065] The flagellin may comprise transposon insertions and changes to the variable D3 domain. The D3 domain may be substituted in part, or in whole, with a hinge or linker polypeptide that allows the D1 and D2 domains to properly fold such that the variant stimulates TLR5 activity. The variant hinge elements may be found in the *E.coli* MukB protein and may have a sequence

as set forth in International Application No. PCT/US10/51646, filed on October 6, 2010, the contents of which are incorporated herein by reference.

[0066] The flagellin as described above may further comprise a leader sequence. The flagellin further comprising a leader sequence may be CBLB502S.

3. Agent

[0067] This invention also relates to an agent comprising a therapeutically effective amount of a TLR agonist. The agent may be a polypeptide. The agent may also be a vector. The vector may comprise a nucleic acid encoding the TLR agonist. The vector may be capable of transducing mammalian cells. The vector may be delivered into a mammalian cell by a virus or liposome related vector system. The virus vector system may be an adenovirus or a cytomegalovirus.

[0068] The agent may be a liposome harboring the vector. The liposome maybe capable of transducing mammalian cells and delivering the vector for expression.

[0069] The agent may be a drug formulation that activates a TLR, thereby exposing tumor or infected cells to the host immune system imitating the situation of a massive penetration through the intestinal wall. The agent may be delivered systematically in solution for administration such as intramuscularly. The agent may be a drug formulation that expresses the TLR agonist in the form of a nano-particle, which may carry a functional agonist to the cell surface of a mammalian cell.

[0070] The agent may be a pharmaceutical agent comprising the drug formulation described above, which may be produced using methods well known in the art. The agent may also comprise a coagent.

[0071] The vector may comprise a nucleic acid encoding flagellin. The vector may be capable of expressing flagellin using a strong promoter. The expression vector may further comprise a leader sequence cloned upstream of the gene encoding the TLR agonist. The drug formulation may be an adenovirus expressing:

[0072] the TLR agonist, delivered systematically in solution for administration, such as intramuscularly; or

[0073] the TLR agonist, expressed in the form of nano-particles carrying functional TLR agonist, such as flagellin, which may be derived from CBLB502, on their surface. The nano-particle may be on the basis of a bacteriophage T7, or fully formed to retain its biological activity. The nano-formulation may provide for dose-dependent, NF-κB-responsive reporter

activation, and may result in cell internalization by endocytosis for effective immunization approach (Mobian AP-A).

a. Administration

[0074] Administration of the agents using the method described herein may be systemically, orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, or combinations thereof. Parenteral administration includes, but is not limited to, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intrathecal, and intraarticular. Administration may also be subcutaneous, intravenous, via intra-air duct, or intra-tumoral. For veterinary use, the agent may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The agents may be administered to a human patient, cat, dog, large animal, or an avian.

[0075] The agent may be administered as a monotherapy or simultaneously or metronomically with other treatments, which may be a surgery or removal of a tumor. The term "simultaneous" or "simultaneously" as used herein, means that the agent and other treatment be administered within 48 hours, preferably 24 hours, more preferably 12 hours, yet more preferably 6 hours, and most preferably 3 hours or less, of each other. The term "metronomically" as used herein means the administration of the agent at times different from the other treatment and at a certain frequency relative to repeat administration.

[0076] The agent may be administered at any point prior to another treatment including about 120 hr, 118 hr, 116 hr, 114 hr, 112 hr, 110 hr, 108 hr, 106 hr, 104 hr, 102 hr, 100 hr, 98 hr, 96 hr, 94 hr, 92 hr, 90 hr, 88 hr, 86 hr, 84 hr, 82 hr, 80 hr, 78 hr, 76 hr, 74 hr, 72 hr, 70 hr, 68 hr, 66 hr, 64 hr, 62 hr, 60 hr, 58 hr, 56 hr, 54 hr, 52 hr, 50hr, 48 hr, 46 hr, 44 hr, 42 hr, 40 hr, 38 hr, 36 hr, 34 hr, 32 hr, 30 hr, 28 hr, 26 hr, 24 hr, 22 hr, 20 hr, 18 hr, 16 hr, 14 hr, 12 hr, 10 hr, 8 hr, 6 hr, 4 hr, 3 hr, 2 hr, 1 hr, 55 mins., 50 mins., 45 mins., 40 mins., 35 mins., 30 mins., 25 mins., 20 mins., 15 mins, 10 mins, 9 mins, 8 mins, 7 mins., 6 mins., 5 mins., 4 mins., 3 mins, 2 mins, and 1 mins. The agent may be administered at any point prior to a second treatment of the agent including about 120 hr, 118 hr, 116 hr, 114 hr, 112 hr, 110 hr, 108 hr, 106 hr, 104 hr, 102 hr, 100 hr, 98 hr, 96 hr, 94 hr, 92 hr, 90 hr, 88 hr, 86 hr, 84 hr, 82 hr, 80 hr, 78 hr, 76 hr, 74 hr, 72 hr, 70 hr, 68 hr, 66 hr, 64 hr, 62 hr, 60 hr, 58 hr, 56 hr, 54 hr, 52 hr, 50hr, 48 hr, 46 hr, 44 hr, 42 hr, 40 hr, 38 hr,

36 hr, 34 hr, 32 hr, 30 hr, 28 hr, 26 hr, 24 hr, 22 hr, 20 hr, 18 hr, 16 hr, 14 hr, 12 hr, 10 hr, 8 hr, 6 hr, 4 hr, 3 hr, 2 hr, 1 hr, 55 mins., 50 mins., 45 mins., 40 mins., 35 mins., 30 mins., 25 mins., 20 mins., 15 mins., 10 mins., 9 mins., 8 mins., 7 mins., 6 mins., 5 mins., 4 mins., 3 mins, 2 mins, and 1 mins.

[0077] The agent may be administered at any point after another treatment including about 1min, 2 mins., 3 mins., 4 mins., 5 mins., 6 mins., 7 mins., 8 mins., 9 mins., 10 mins., 15 mins., 20 mins., 25 mins., 30 mins., 35 mins., 40 mins., 45 mins., 50 mins., 55 mins., 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 14 hr, 16 hr, 18 hr, 20 hr, 22 hr, 24 hr, 26 hr, 28 hr, 30 hr, 32 hr, 34 hr, 36 hr, 38 hr, 40 hr, 42 hr, 44 hr, 46 hr, 48 hr, 50 hr, 52 hr, 54 hr, 56 hr, 58 hr, 60 hr, 62 hr, 64 hr, 66 hr, 68 hr, 70 hr, 72 hr, 74 hr, 76 hr, 78 hr, 80 hr, 82 hr, 84 hr, 86 hr, 88 hr, 90 hr, 92 hr, 94 hr, 96 hr, 98 hr, 100 hr, 102 hr, 104 hr, 106 hr, 108 hr, 110 hr, 112 hr, 114 hr, 116 hr, 118 hr, and 120 hr. The agent may be administered at any point prior after a second treatment of the agent including about 120 hr, 118 hr, 116 hr, 114 hr, 112 hr, 110 hr, 108 hr, 106 hr, 104 hr, 102 hr, 100 hr, 98 hr, 96 hr, 94 hr, 92 hr, 90 hr, 88 hr, 86 hr, 84 hr, 82 hr, 80 hr, 78 hr, 76 hr, 74 hr, 72 hr, 70 hr, 68 hr, 66 hr, 64 hr, 62 hr, 60 hr, 58 hr, 56 hr, 54 hr, 52 hr, 50hr, 48 hr, 46 hr, 44 hr, 42 hr, 40 hr, 38 hr, 36 hr, 34 hr, 32 hr, 30 hr, 28 hr, 26 hr, 24 hr, 22 hr, 20 hr, 18 hr, 16 hr, 14 hr, 12 hr, 10 hr, 8 hr, 6 hr, 4 hr, 3 hr, 2 hr, 1 hr, 55 mins., 50 mins., 45 mins., 40 mins., 35 mins., 30 mins., 25 mins., 20 mins., 15 mins., 10 mins., 9 mins., 8 mins., 7 mins., 6 mins., 5 mins., 4 mins., 3 mins, 2 mins, and 1 mins.

b. Formulation

[0078] The method may comprise administering the agent. Agents provided herein may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients may be binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers may be lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants may be potato starch and sodium starch glycollate. Wetting agents may be sodium lauryl sulfate. Tablets may be coated according to methods well known in the art.

[0079] Agents provided herein may also be liquid formulations such as aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The agents may also be formulated as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain additives such as suspending agents, emulsifying agents, nonaqueous vehicles and preservatives. Suspending agent may be sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents may be lecithin, sorbitan monooleate, and acacia. Nonaqueous vehicles may be edible oils, almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives may be methyl or propyl p-hydroxybenzoate and sorbic acid.

[0080] Agents provided herein may also be formulated as suppositories, which may contain suppository bases such as cocoa butter or glycerides. Agents provided herein may also be formulated for inhalation, which may be in a form such as a solution, suspension, or emulsion that may be administered as a dry powder or in the form of an aerosol using a propellant, such as dichlorodifluoromethane or trichlorofluoromethane. Agents provided herein may also be formulated as transdermal formulations comprising aqueous or nonaqueous vehicles such as creams, ointments, lotions, pastes, medicated plaster, patch, or membrane.

[0081] Agents provided herein may also be formulated for parenteral administration such as by injection, intratumor injection or continuous infusion. Formulations for injection may be in the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulation agents including, but not limited to, suspending, stabilizing, and dispersing agents. The agent may also be provided in a powder form for reconstitution with a suitable vehicle including, but not limited to, sterile, pyrogen-free water.

[0082] Agents provided herein may also be formulated as a depot preparation, which may be administered by implantation or by intramuscular injection. The agents may be formulated with suitable polymeric or hydrophobic materials (as an emulsion in an acceptable oil, for example), ion exchange resins, or as sparingly soluble derivatives (as a sparingly soluble salt, for example).

c. Dosage

[0083] The method may comprise administering a therapeutically effective amount of the agent to a patient in need thereof. The therapeutically effective amount required for use in therapy varies with the nature of the condition being treated, the length of time desired to activate TLR

activity, and the age/condition of the patient. In general, however, doses employed for adult human treatment typically are in the range of 0.001 mg/kg to about 200 mg/kg per day. The dose may be about 1 mg/kg to about 100 mg/kg per day. The desired dose may be conveniently administered in a single dose, or as multiple doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day. Multiple doses may be desired, or required.

[0084] The dosage may be at any dosage such as about 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 25 mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 225 mg/kg, 250 mg/kg, 275 mg/kg, 300 mg/kg, 325 mg/kg, 350 mg/kg, 375 mg/kg, 400 mg/kg, 425 mg/kg, 450 mg/kg, 475 mg/kg, 500 mg/kg, 525 mg/kg, 550 mg/kg, 575 mg/kg, 600 mg/kg, 625 mg/kg, 650 mg/kg, 675 mg/kg, 700 mg/kg, 725 mg/kg, 750 mg/kg, 775 mg/kg, 800 mg/kg, 825 mg/kg, 850 mg/kg, 875 mg/kg, 900 mg/kg, 925 mg/kg, 950 mg/kg, 975 mg/kg or 1 mg/kg.

d. Monotherapy

[0085] The agent may be administered as a monotherapy, under which the agent is not administered together with any other type of cancer treatment, such as chemotherapy, radiation therapy, another biological therapy, or other combination therapies; provided that "monotherapy" may include administration of the agent together with surgical treatment. The agent may be administered in combination with a surgery, which may be tumor removal. The agent may be administered prior to, together with, or after the surgery. The agent may be administered during the surgery.

4. Method for Treating Cancer

[0086] Provided herein is a method for treating cancer, which may be present in a tissue that expresses a TLR such as TLR5, by administering to a mammal in need thereof the agent. The cancer may be a tumor or a metastatic cancer. The cancer may also be present in liver, bladder, lung, or intestinal tissue, and also may have originated in another type of tissue such as colon, breast, or prostate. The cancer may also be melanoma or a hematological malignancy such as lymphoma. The cancer may also be any cancer that has metastasized to a TLR-expressing tissue, such as liver, lung, bladder, intestine, or other TLR-expressing tissue. The cancer may be a TLR-negative cancer, and thus lack expression of a Toll-Like Receptor. The cancer may lack both endogenous and exogenous expression of the Toll-Like Receptor. The method may

comprise a step of not providing the Toll-Like Receptor to the cancer, which may include not providing the Toll-Like Receptor either exogenously or endogenously. The cancer may lack any and all Toll-Like Receptor expression.

a. Toll-Like Receptor

[0087] The Toll-Like Receptor (TLR) may recognize molecules that are conserved molecular products derived from pathogens that include Gram-positive, Gram-negative bacteria, fungi, and viruses, but are distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs). The TLR may also recognize endogenous molecules released from injured or dying cells, collectively referred to as damage-associated molecular pattern (DAMPs). A PAMP or DAMP may be a TLR agonist as further described below. The TLR may be a fragment, variant, analog, homolog or derivative that recruits adapter molecules within the cytoplasm of cells in order to propagate a signal. The TLR may be from a human or other mammalian species such as rhesus monkey, mouse, or rat. The TLR may be at least 30-99% identical to a TLR that recruits adapter molecules within the cytoplasm of cells in order to propagate a signal.

[0088] The TLR may be one of the between ten and fifteen types of TLR that are estimated to exist in most mammalian species. The TLR may be one of the 13 TLR (named simply TLR1 to TLR13) that have been identified in humans and mice together, or may be an equivalent form that has been found in other mammalian species. The TLR may be one of the 11 members (TLR1-TLR11) that have been identified in humans.

[0089] The TLR may ordinarily be expressed by different types of immune cells, and may be located on the cell surface or in the cell cytoplasm. The TLR may ordinarily be expressed on cancer cells. The TLR may ordinarily be expressed by normal epithelial cells in the digestive system, normal keratinocytes in the skin, alveolar and bronchial epithelial cells, and epithelial cells of the female reproductive tract. These cells lining an organ may be the first line of defense against invasion of microorganisms, and TLRs ordinarily expressed in epithelial cells may have a crucial role in the regulation of proliferation and apoptosis.

[0090] The TLR may not be expressed by the cancer cells. The TLR-negative cancer cells may not express any TLR mRNA, may not express any TLR protein, or may not express any functional TLR protein. The TLR protein may not function due to reduced ability to bind a TLR ligand or reduced ability to transmit downstream signals triggered by ligand binding. The TLR-

negative cancer cells may also have reduced levels of TLR mRNA, protein, or TLR function. The reduction may be 100%, or by more than 99.9%, 99%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50%, as compared to a normal cell from the tissue from which the cancer cell originated, or as compared to another, known TLR-expressing cell type. The TLR-expressing cell may be a normal cell or a tumor cell, such as a tumor cell line or tumor xenograft.

[0091] The TLR ordinarily expressed on cancer cells may upregulate the NF-κB cascade and produce anti-apoptotic proteins that contribute to carcinogenesis and cancer cell proliferation.

[0092] Four adapter molecules of TLRs are known to be involved in signaling. These proteins are known as myeloid differentiation factor 88 (MyD88), Tirap (also called Mal), Trif, and Tram. The adapters activate other molecules within the cell, including certain protein kinases (IRAK1, IRAK4, TBK1, and IKKi) that amplify the signal, and ultimately lead to the induction or suppression of genes that orchestrate the inflammatory response. TLR signaling pathways during pathogen recognition may induce immune reactions via extracellular and intracellular pathways mediated by MyD88, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and mitogen-associated protein kinase (MAPK). In all, thousands of genes are activated by TLR signaling, and collectively, the TLR constitute one of the most pleiotropic, yet tightly regulated gateways for gene modulation.

[0093] TLRs together with the Interleukin-1 receptors form a receptor superfamily, known as the "Interleukin-1 Receptor/Toll-Like Receptor Superfamily." All members of this family have in common a so-called TIR (Toll-IL-1 receptor) domain. Three subgroups of TIR domains may exist. Proteins with subgroup I TIR domains are receptors for interleukins that are produced by macrophages, monocytes and dendritic cells and all have extracellular Immunoglobulin (Ig) domains. Proteins with subgroup II TIR domains are classical TLRs, and bind directly or indirectly to molecules of microbial origin. A third subgroup of proteins containing TIR domains (III) consists of adaptor proteins that are exclusively cytosolic and mediate signaling from proteins of subgroups 1 and 2. The TLR may be a fragment, variant, analog, homolog or derivative that retains either a subgroup I TIR domain, subgroup II TIR domain, or subgroup III TIR domain.

[0094] The TLR may function as a dimer. For example, although most TLRs appear to function as homodimers, TLR2 forms heterodimers with TLR1 or TLR6, each dimer having a different ligand specificity. The TLR may also depend on other co-receptors for full ligand sensitivity,

such as in the case of TLR4's recognition of LPS, which requires MD-2. CD14 and LPS Binding Protein (LBP) are known to facilitate the presentation of LPS to MD-2.

(1) TLR1

[0095] The TLR may be TLR1, which recognizes PAMPs with a specificity for gram-positive bacteria. TLR1 has also been designated as CD281.

(2) TLR5

[0096] The TLR may be Toll-Like Receptor 5. The protein encoded by the TLR5 may play a fundamental role in pathogen recognition and activation of innate immunity. TLR5 may recognize PAMPs that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. TLR5 may recognize bacterial flagellin, a principal component of bacterial flagella and a virulence factor. The activation of the TLR5 may mobilize the nuclear factor NF-κB and stimulate tumor necrosis factor-alpha production.

(3) Cancer type

[0097] The cancer may be a primary cancer or a metastatic cancer. The primary cancer may be an area of cancer cells at an originating site that becomes clinically detectable, and may be a primary tumor. In contrast, the metastatic cancer may be the spread of a disease from one organ or part to another non-adjacent organ or part. The metastatic cancer may be caused by a cancer cell that acquires the ability to penetrate and infiltrate surrounding normal tissues in a local area, forming a new tumor, which may be a local metastasis.

[0098] The metastatic cancer may also be caused by a cancer cell that acquires the ability to penetrate the walls of lymphatic and/or blood vessels, after which the cancer cell is able to circulate through the bloodstream (thereby being a circulating tumor cell) to other sites and tissues in the body. The metastatic cancer may be due to a process such as lymphatic or hematogeneous spread. The metastatic cancer may also be caused by a tumor cell that comes to rest at another site, re-penetrates through the vessel or walls, continues to multiply, and eventually forms another clinically detectable tumor. The metastatic cancer may be this new tumor, which may be a metastatic (or secondary) tumor.

[0099] The metastatic cancer may be caused by tumor cells that have metastasized, which may be a secondary or metastatic tumor. The cells of the metastatic tumor may be like those in the original tumor. As an example, if a breast cancer or colon cancer metastasizes to the liver, the

secondary tumor, while present in the liver, is made up of abnormal breast or colon cells, not of abnormal liver cells. The tumor in the liver may thus be a metastatic breast cancer or a metastatic colon cancer, not liver cancer.

[0100] The metastatic cancer may have an origin from any tissue. The metastatic cancer may originate from melanoma, colon, breast, or prostate, and thus may be made up of cells that were originally skin, colon, breast, or prostate, respectively. The metastatic cancer may also be a hematological malignancy, which may be lymphoma. The metastatic cancer may invade a tissue such as liver, lung, bladder, or intestinal. The invaded tissue may express a TLR, while the metastatic cancer may or may not express a TLR.

b. Combination

[0101] The method may also comprise co-administration of the TLR agonist with an anti-cancer therapy. The anti-cancer therapy may be FAS ligand, a FAS agonistic antibody, TNFα, a TNFα agonistic antibody, TRAIL, or a TRAIL agonistic antibody. The TLR5 agonist may be used to sensitize the cancer to the anti-cancer therapy. The method may also be combined with other methods for treating cancer, including use of an immunostimulant, cytokine, or chemotherapeutic. The immunostimulant may be a growth hormone, prolactin or vitamin D.

5. Method of reducing cancer recurrence

[0102] Also provided herein is a method of reducing cancer recurrence, comprising administering to a mammal in need thereof a TLR agonist. The cancer may be or may have been present in a tissue that either does or does not express TLR, such as TLR5. The cancer, tissue, TLR, mammal, and agent may be as described above. The method may also prevent cancer recurrence. The cancer may be an oncological disease.

[0103] The cancer may be a dormant tumor, which may result from the metastasis of a cancer. The dormant tumor may also be left over from surgical removal of a tumor. The cancer recurrence may be tumor regrowth, a lung metastasis, or a liver metastasis.

6. Mammal

[0104] The mammal may have a fully-functional immune system, and may not be immunocompromised. The mammal may also have a level of immunity that is equivalent to the level sufficient to make the mammal eligible for a first or second round a chemotherapy. The mammal may not have a low white blood cell count, which may be chemotherapy-induced. The low white blood cell count may be caused by the loss of healthy cells during chemotherapy. The

loss may be an expected side effect of a chemotherapy drug. The low white blood cell count may be a severe immunosuppression caused by chemotherapy. The low white blood cell count may compromise the antitumor effect of the agent. The low white blood cell count may be restored 7-14 days after a chemotherapy treatment.

[0105] The mammal may have a white blood cell count that is within a normal range. The mammal may also have a white blood cell count that is indicative of mild immunosuppression. The mammal may have not received chemotherapy treatment for 7-14 days, or at least 14 days. The mammal may also have total white blood cell count of at least 3000 or 3500 cells/ml of whole blood; a granulocyte count of at least 1800 or 2100 cells/ml of whole blood; or an albumin level of at least 3.0 or 3.5 g/100 ml of whole blood. The white blood cell count, granulocyte count, or albumin level may also fall within +/- 5%, 10%, 20%, 30%, 40%, or 50% of these levels.

7. Method of protecting liver

[0106] As discussed above, anti-cancer treatments that trigger apoptosis through FAS, TRAIL, and TNFα death receptor signaling, such as death ligands, can cause severe liver toxicity. Thus, the use of molecules such as FAS, TRAIL, and TNFα as anti-cancer treatments has been limited, despite the efficacy of these molecules in targeting cancer cells. Accordingly, also provided herein is a method of protecting liver tissue in a mammal from the effects of a liver toxicity. The liver may be protected by administering the agent to the mammal. The death receptor signaling agonist may be FAS, TRAIL, or TNFα. The death ligand may be a liver toxicity. The FAS, TRAIL, or TNFα may be used as an anti-cancer agent.

[0107] The liver toxicity may also be a Salmonella infection, which may be from *Salmonella typhimurium.* The agent may also be used to protect against liver toxicity that may be FAS-mediated. The toxicity may also be FAS ligand, a FAS agonistic antibody, TNFα, acetaminophen, alcohol, a viral infection of the liver, or a chemotherapeutic agent. The agent may be administered to the mammal.

Example 1

An agonist of TLR5 protects liver from hepatotoxicity

[0108] CBLB502, which is a pharmacologically optimized TLR5 agonist, is a powerful radioprotectant due to, at least in part, inhibition of apoptosis in radiosensitive tissues. CBLB502

was tested for liver protection from Fas-mediated apoptosis. The following examples demonstrate that upon stimulation with CBLB502 the TLR5 pathway is active in liver hepatocytes of mice and humans leading to NF-kB-dependent induction of genes encoding anti-apoptotic proteins. Pretreatment of mice with CBLB502 protected them from lethal doses of Fas agonistic antibodies, reduced Fas-induced elevation of liver enzymes in the blood, caspase activity in liver extracts and preserved liver tissue integrity. CBLB502 did not protect tumors in syngeneic melanoma and colon carcinoma mouse models. These observations support the use of Fas agonists for cancer treatment under the protection of a TLR5 agonist, such as CBLB502.

[0109] NF-kB response was compared in different organs after administration of TLR5 agonist CBLB502 and TLR4 agonist LPS, another known activator of NF-kB. CBLB502 was found to induce fast direct activation of NF-kB in hepatocytes, while LPS activation of NF-kB in hepatocytes was mediated through different types of cells. The following data thus also demonstrate that pre-treatment with CBLB502 can reduce Fas-mediated hepatotoxicity during anti-cancer therapy in mice. The approaches described below are based on the increasing the resistance of normal tissues to damaging side effects through activation of NF-kB signaling by toll-like receptor-5 (TLR5) agonist CBLB502 derived from flagellin of *Salmonella typhimurium*.

1. Determination of NF-k activation in vivo in response to TLR4 and TLR5 agonists.

[0110] NF-kB response was investigated in different organs of mice to TLR5 agonist CBLB502 in comparison with bacterial LPS acting through TLR4. NF-kB dependent luciferase reporter Xenogen mouse model in which luciferase transgene is expressed under the control of NFkB-dependent natural promoter of IkBα gene (Zhang N, et al, 2005). Upon administration of NFkB-activating agents, luciferase activity was increased in cells and tissues that respond to a given agent. Using noninvasive Xenogen imaging system and ex vivo luciferase reporter assay, detected strong activation of NF-kB in liver of mice was detected 2 hours after s.c. injection of CBLB502 (Figure 7A). The quantitative analysis of NF-kB activation in different organs revealed that in comparison with LPS, CBLB502 induced much stronger activation of NF-kB in liver, similar high NF-kB activation level in the intestine, while less NF-kB activity was found in spleen, bone marrow, kidney and lungs (Figure 7B). The dynamics of NF-kB induced luciferase reporter activity was similar for both TLR agonists with the activation profile peaking approximately two hours after injection, reduced at the six hour time point and effectively undetectable 24 hours post-injection (Figure 10).

[0111] Immunohistochemical staining of mouse liver samples for p65 translocation to the nuclei revealed that CBLB502 directly activated NF-kB in hepatocytes as early as 20 min after injection with no response of Kupffer and endothelial cells yet (Figure 7C). By 1 h after CBLB502 injection, all liver cells including Kupffer cells and endothelium cells demonstrated nuclear accumulation of p65 suggesting overlap of primary and secondary effects with subsequent activation of NF-kB by paracrine mechanisms. In contrast, LPS-activated NF-kB nuclear translocation in hepatocytes occurred significantly later. The activation of NF-kB was observed first in Kupffer and endothelial cells followed by the engagement of hepatocytes about 1 h after LPS administration.

[0112] Primary hepatocyte cultures (murine and human) treated with CBLB502, but not with LPS, demonstrated NF-kB translocation to the nuclei (Figure 7D, E). CBLB502 mediated NF-kB activation was confirmed by NF-kB dependent luciferase expression with murine hepatocyte cell culture, while LPS did not induce NF-kB activation in this cells (Figure 11). Small level of NF-kB activation found in LPS-treated hepatocytes was more likely due to contamination of primary hepatocyte culture with other stromal liver cells.

[0113] These results show that hepatocytes express TLR5 but not TLR4 allowing CBLB502 to directly activate NF-kB in hepatocytes while LPS initially activates other cell types (immune and/or stromal) and only later indirectly activates hepatocytes as a secondary event.

2. CBLB502 protection from Fas mediated hepatotoxicity

[0114] As it has been demonstrated, the anti-Fas antibodies can induce dose-dependent hepatotoxicity and rapidly kill mice by inducing apoptosis, liver tissue necrosis and hemorrhage (Ogasawara J et al, Nature 1993, Nishimura et al 1997). Thus, NF-kB activation in hepatocytes induced by TLR5 agonist CBLB502 may protect liver from Fas mediated apoptosis. In NIH-Swiss mice, 4 μg of anti-Fas antibodies (clone Jo2) injected i.p. induced massive apoptosis, necrosis and hemorrhage in liver (Figure 8B, C and D) killing mice within first 1-2 days after antibody injections (Figure 8A). Pathomorphological examination of CBLB502-treated mice in dynamics compared to intact control mice showed that their livers had slight vacuolization of the hepatocytes (Figure 12). The examination of mice injected with sub-lethal dose of anti-Fas antibodies (3 μg/ mouse) in dynamics revealed pronounced apoptosis of the hepatocytes around the portal tracts with better preserved cells adjacent to the terminal (central) venues, most pronounced at 5 hrs and diminishing with time (12 and 24 hours post-injection). In the livers of

mice treated with CBLB502 and anti-Fas antibodies the changes were minimal and the hepatocytes looked close to normal – only slight vacuolization and single apoptotic cells were visible.

[0115] CBLB502 injected mice had much less damage to the liver that deflected in better overall survival after injections of about than 80% of NIH-Swiss mice when injected 30 min before anti-Fas antibodies (Figure 8A). All mice survived when CBLB502 was injected 2 hours before antibodies. The protection level then declined by 6 hours time-point of pre-treatment.

[0116] Two and three μg of anti-Fas antibodies induced only transient liver toxicity in NIH-Swiss mice, caspase 3/7 activation in the liver and alanine aminotransferase (ALT) secretion in the blood (Figure 8E, F). Both tests showed significant reduction of liver damage induced by anti-Fas antibodies if mice were pre-treated with CBLB502. Interestingly, Balb/c and C57Bl/6 mice appeared to be less sensitive to anti-Fas antibodies than NIH-Swiss mice. Four μg of anti-Fas antibodies, the lethal dose for NIH-Swiss mice, induced only transient caspase 3/7 activation in BALB/c and C57Bl/6 mice which was successfully prevented by CBLB502 injection 30 min before antibodies (Figure 13).

[0117] These data support the hypothesis that TLR5 mediated NF-kB activation in hepatocytes can be an indicator and a measure of increased resistance to Fas-mediated toxicity.

3. Suppression of pro-apoptotic and induction of anti-apoptotic factors by CBLB502 in liver.

[0118] Caspases 3 and 7 are downstream targets of both intrinsic (mitochondrial) and extrinsic (caspase) Fas-mediated apoptosis signaling. Upon activation of the receptor, first caspase-8 becomes phosphorylated and cleaved leading to activation of mitochondrial apoptotic mechanism acting through cleavage of pro-apoptotic Bid protein and cytochrome release (Lou et al 1998). Therefore we examined whether CBLB502 suppresses this mechanism.

[0119] Western blot analysis of liver protein extracts for both caspases-8 and Bid demonstrated much less cleavage of these proteins in mice injected with combination of CBLB502 and anti-Fas antibodies in comparison with a single injection of anti-Fas antibodies (Figure 9A, B). Consistently, caspase 8 activation was reduced to a background level, as indicated by using fluorigenic substrate assay (Fig. 8F).

[0120] The fact that the protection of mice from Fas-mediated hepatotoxicity by CBLB502 is increased with time with maximum peaking at 30 min-2 hours suggests the existing of pre-conditioning events in hepatocytes. Among the numerous of cytokines and anti-apoptotic factors,

the up-regulation of two anti-apoptotic bcl2 family members bcl2A1B and bcl2A1D (Chao and Korsmeyer, 1998, Arikawa et al 2006) was found in livers by RNA array hybridization 30 min and 2 hours after CBLB502 administration that was confirmed by RT-PCR (Figure 9C). CBLB502 also quickly induced RNA expression of another anti-apoptotic protein immediate early response protein IER-3 (Figure 9C, IEX-1 is an alternative name) that was shown suppressing the production of reactive oxygen species and mitochondrial apoptotic pathway (Shen et al 2009). RT-PCR analysis of liver samples revealed the induction of IER-3 RNA expression by CBLB502 already 30 min after administration with significant increase by 2 hours. Several proteins of MAPK pathway were found up-regulated in livers of CBLB502 treated mice. It was demonstrated that activation of MAPK pathway in tumors mediates the resistance of these cells to Fas receptor apoptosis (REF). The up-regulation of Jun, Jun-B and Fos gene expressions directly correlated with mouse survival after anti-Fas antibody injections followed the pre-treatment with CBLB502 suggesting their possible role in CBLB502 mediated protection from Fas hepatotoxicity.

4. Effect of CBLB502 on Fas-mediated antitumor activity

[0121] LPS is not a good candidate for clinical application, since it induces strong inflammation in many organs and can be directly cytotoxic through FADD/caspase-8 apoptotic pathway (REFs). CBLB502 in its turn has been tested in mice, non-human primates and human healthy volunteers and found to be a rather mild inducer of short-lasting inflammation. When evaluating a tissue protecting compounds, there is always possibility that by reducing toxic side effects it can also make tumor cells more resistant and jeopardize the efficacy of antitumor therapy. The in vivo antitumor effect of combination treatment with CBLB502 and anti-Fas antibodies was tested in CT-26 colon carcinoma mouse model of s.c. growing tumors and experimental liver metastases. This tumor model was used in a recently published study applying FasL-expressing *S. typhimurium*, total attenuated bacteria, to deliver FasL to the tropic tumors and to induce Fas mediated antitumor effect (Loeffler et al 2008). CT-26 tumor cells and A20 lymphoma cells do not express TLR5, as determined by RT-PCR and a NF-kB dependent luciferase reporter assay (Figure 14). Here, tumor-bearing mice were treated with anti-Fas antibodies alone or combination of recombinant CBLB502 given twice 24 hs and 1 h before a single injection of anti-Fas antibodies (4 μg/ mouse, Figure 9D). The volumes of s.c. growing tumors in treated mice were compared with tumors growing in the intact mice. CT-26 tumors were found to be

rather resistant to the toxic but not lethal dose of anti-Fas antibodies (Figure 9D). Pre-treatment with CBLB502 slightly sensitized tumors to anti-Fas antibodies reflecting in growth-inhibitory tumor response. Fas mediated antitumor effect was tested in the experimental model of liver metastases induced by intrasplenic injection of luciferase expressing CT-26 tumor cells followed by splenectomy. Hepatic tumor growth was assessed using Xenogen luciferase imaging every 4-6 days after the treatment. Mice remained free from liver tumor growth were counted at each imaging procedure (Figure 9E). The results demonstrate significant delay of tumor appearance (Figure 9G) and growth in livers by both treatments, anti-Fas antibody alone or given after pre-treatment with CBLB502. The increased sensitivity of TLR5 negative CT-26 tumors to combination treatment with anti-Fas and CBLB502 suggests the activation of antitumor immune response against CT-26 tumors. Indeed, the immunohistochemical analysis of liver sample with CT-26 tumors taken 24 hours after anti-Fas/CBLB502 treatment revealed the accumulation of neutrophils in inside and around of tumor nodules (Figure 9F). Thus, CBLB502 does not protect tumors from anti-Fas antibodies toxicity and can even slightly enhance Fas mediated antitumor effect against CT-26 tumors. The simultaneous protection of normal liver tissue from Fas mediated toxicity may allow increasing the amount of the Fas agonist reaching complete prevention of liver metastases and the therapeutic effect against s.c. growing tumors.

[0122] Materials and Methods

[0123] Mice

[0124] NIH-Swiss female mice were purchased from NCI (Frederick, MD), BALB/c and C57Bl/6 female mice were purchased from Jackson Laboratory (Bar Harbor, ME). All mice were used in the experiments at the age of 10-14 weeks old. Balb/C-Tg(IκBα-luc)Xen mice with NF-kB inducible luciferase reporter gene were originally purchased from Xenogen (Alameda, CA) and bred in our domestic colony.

[0125] Reagents

[0126] CBLB502, a bacterial flagellin derivative, was obtained from Cleveland BioLabs, Inc. Bacterial lipopolysacharide (LPS) from Escherichia coli 055:B5 was purchased from Sigma. Purified agonistic hamster anti-mouse Fas antibodies, clone Jo2, were purchased from BD Biosciences.

[0127] Analysis of NF-κB activation in vivo using NF-kB reporter mouse model

[0128] BALB/c-Tg(IκBα-luc)Xen reporter mice were injected s.c. with CBLB502 (0.2 mg/kg). The induction of NF-kB by CBLB502 was detected by noninvasive in vivo imaging 2 hours after the treatment (Fig. 1A). Mice were injected with D-luciferin (3 mg/ 100 μl, i.p., Promega), immediately anesthetized with isofluorane and images were taken using Xenogen IVIS Imaging System 100 series. To quantify the results, samples of liver, lungs, kidney, spleen, heart and intestine from NF-kB reporter mice injected s.c. with 100 μl of either PBS, CBLB502 (0.2 mg/kg) or LPS (1 mg/kg) were obtained 2, 6 and 24 h after injections (Fig. 7B, 10). Tissue samples were covered with lysis buffer containing proteinase inhibitor cocktail (according to manufacture's recommendation, Calbiochem) to get 100 mg tissue per 1 ml lysis buffer. This was followed by homogenization and centrifugation at 14,000 rpm for 10 min at 4C. Luciferase activity was measured in 20 μl of samples immediately after adding 30 μl of luciferin reagent (Bright-Glo Luciferase Assay System, Promega). Luciferase activity was normalized per g of the protein extract. Luciferase fold induction was calculated as ratio between average luciferase units in livers of the TLR ligand treated mice and that obtained from PBS injected control mice.

[0129] Immunohistochemical staining for p65 translocation.

[0130] P65 localization was detected in livers isolated from NIH-Swiss mice injected s.c either with CBLB502 (0.04 mg/kg) or LPS (1 mg/kg). Control mice were injected with PBS. Tissue samples were obtained 20, 40 and 60 min after the treatments, processed into paraffin blocks. All liver tissues were stained with rabbit polyclonal antibody against NF-kB p65 and rat monoclonal antibody against cytokeratin 8 followed by appropriate secondary fluorochrome-conjugated antibodies (p65 – green, cytokeratin-8 – red). The same staining was performed on the plates with primary mouse hepatocytes isolated from EGTA (0.5mM in PBS) perfused liver tissues of NIH-Swiss mice followed by collagenase digestion and with human hepatocyte culture purchased from (BD Biosciences). Both types of hepatocytes were treated in vitro with CBLB502 (100 ng/ml) or LPS (1 μg/ml) for indicated period of time. Control hepatocytes remained intact. Pictures were taken at x20 magnification (Fig. 7C, D, E).

[0131] Survival assay

[0132] NIH-Swiss mice were injected i.p. with 2, 3, 4, and 5 μg of anti-Fas antibodies in 200 μl of PBS to determine a 100% lethal dose that was found to be 4 μg/ mouse for this mouse strain. Then CBLB502 (0.04 mg/kg, s.c.) was injected s.c. 30 min, 2 hours and 6 hours before 4 μg of

anti-Fas antibodies (i.p.) (Fig. 8A). Usually death from anti-Fas hepatotoxicity occurs during first 1-2 days after antibody injections. Mouse survival was observed and recorded during 30 days.

[0133] TUNEL staining of apoptotic cells in liver

[0134] Apoptosis in the liver of NIH-Swiss mice five hours after injections with CBLB502 (s.c., 0.04 mg/kg) or PBS 30 min before anti-Fas antibodies was detected in paraffin-embedded specimens. Apoptotic cells were stained by the indirect terminal deoxynucleotidyl transferase mediated deoxyuridine tri-phosphate nick end labeling (TUNEL) method with TUNEL POD kit (Roche Applied Science) (Fig. 8B).

[0135] Histological assessment of liver morphology

[0136] Liver specimens were collected from NIH-Swiss mice five hours (Fig. 2C) or in dynamics of 5, 12 and 26 hours (Fig. S3) after anti-Fas antibody injections with or without pre-treatment with CBLB502 (0.04 mg/kg) 30 minutes before antibodies. Mice that were not treated ("intact") were used as controls. Tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned and processed with H&E staining.

[0137] Histological staining of liver for hemorrhage

[0138] Paraffin sections were stained with antibody against mouse IgG conjugated with Cy5 [Jackson Immunoresearch, pseudo-colored in purple] and mounted with ProLong Gold anti-fade reagent with DAPI[Invitrogen, blue nuclear stain]. Erythrocytes were fisualized in red channel by red autofluorescence. (Figure 2D). Images were captured under AxioImager Z1 fluorescent microscope (Zeiss) equipped with AxioCam HRc 13 megapixel digital camera using Axio Vision software (rel. 4.6.3)

[0139] Caspase activation

[0140] Livers were cut to small pieces and homogenized with a tissue grinder (Bullet Blender, NextAdvance) in the buffer (10mM Hepes, 0.4mM EDTA, 0.2% CHAPS, 2% glycerol), supplemented with 2mM DTT. All steps were performed on ice. Liver homogenates were centrifuged for 20 min at 13,000 xg, and supernatant was stored at -20°C. Caspase activities were determined by incubation of liver homogenate (containing 50 μg of total protein) with 50 μM of the fluorogenic substrate acetyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-aminomethylcoumarin (Ac-DEVD-amc) (ENZO, LifeSciences) in 200 μl cell-free system buffer containing 10 mM HEPES, 0.4mM EDTA, 0.2% CHAPS, 2% glycerol and 2 mM DTT. The release of fluorescent amc was measured after at time 0 and 2 hours of incubation at 37°C by fluorometry (Ex: 355,

Em: 485) (Victor3, PerkinElmer). Data are shown as the difference between twp and zero hours (Figure 8E).

[0141] Detection of alanine-aminotransferase (ALT) in the serum of anti-Fas antibody -treated mice with and without CBLB502 injections

[0142] NIH-Swiss mice (3 per group) were injected s.c. with 1 μg CBLB502 30 min before anti-Fas antibodies. The alanine aminotransferase (ALT) presence in mouse serum was determined using commercial enzyme assays according to the manufacturer's instructions (Stanbio Laboratory, Boerne, TX, USA). Absorbance at 340nm was measured at 60 second interval (ΔA/minute). (Figure 8F)

[0143] Western blot analysis

[0144] Total protein was isolated from treated and untreated mouse liver using RIPA buffer (Sigma-Aldrich St. Louis, MO) supplemented with protease inhibitor cocktail (Sigma-Aldrich St. Louis, MO). The protein extracts were separated by electrophoresis in denaturing 4 to 20% polyacrylamide Novex gels (Invitrogen, Carlsbad, CA) and transferred to nylon polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore Billerica MA). The following antibodies were used: Caspase-8 antibody (Calbiochem, Darmstadt, Germany), anti-BID (AbCam, Cambridge MA). Horseradish peroxidase (HRP)-conjugated secondary anti-rabbit and anti-mouse antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). (Figure 9A and 9B)

[0145] RNA analysis

[0146] Total RNA was extracted from treated and untreated mouse livers using TRIzol reagent according to manufacturer instructions (Invitrogen, Carlsbad, CA). To eliminate any eventual contamination with genomic DNA, isolated RNAs were treated with DNaseI (Invitrogen, Carlsbad, CA). cDNAs were synthesized by using SuperScriptTM II Reverse Transcriptase and oligo(dT)12-18 primer (Invitrogen, Carlsbad, CA), according to manufacturer instructions. RNA expression of Bcl2A1B, Bcl2A1D, IER-3, Fos, Jun and JunB genes in livers of intact mice and treated with CBLB502 and LPS for 30 min and 2 hours was detected by RT-PCR. GAPDH was used as a control to monitor the induction of gene expression. The primers were designed using LaserGene software (DNASTAR, Inc., Madison, WI) and then UCSC Genome Browser In-Silico PCR website was used to check for locating primers. Primers specific for the IER3 gene (GenBank Accession No. NM_133662.2) (sense 5'-ACTCGCGCAACCATCTCCACAC-3' and

antisense 5'-CTCGCACCAGGTACCCATCCAT-3'), Bcl2A1B gene (GenBank Accession No. NM_007534.3) (sense 5'-TAGGTGGGCAGCAGCAGTCA-3' and antisense 5'-CTCCATTCCGCCGTATCCAT-3'), Bcl2A1D gene (GenBank Accession No. NM_007536.2) (sense 5'-TCTAGGTGGGCAGCAGCAGTC-3' and antisense 5'-ATTCCGCCGTATCCATTCTCC-3'), Jun (GenBank Accession No. NM_010591.2) (sense 5'-TGAAGCCAAGGGTACACAAGAT-3' and antisense 5'-GGACACCCAAACAAACAAACAT-3'), Fos (GenBank Accession No. NM_010234.2) (sense 5'-GAGCGCAGAGCATCGGCAGAAG-3' and antisense 5'-TTGAGAAGGGGCAGGGTGAAGG-3'), JunB (GenBank Accession No. NM_008416.2) (sense 5'-AGCCCTGGCAGCCTGTCTCTAC-3' and antisense 5'-GTGATCACGCCGTTGCTGTTGG-3') and GAPDH gene (sense 5'-ACCACAGTCCATGCCATCAC-3' and antisense 5'-TCCACCACCATGTTGCTGTA-3') were used. Amplification of cDNA was done for 20-30 cycles using specific primer pairs for each gene (Figure 3C).

[0147] Experimental therapy of CT-26 tumor-bearing mice

[0148] The effect of CBLB502 on the sensitivity of tumors to anti-Fas antibodies was analyzed using two models of syngenic colon adenocarcinoma CT-26 tumor: 1) CT-26 s.c. growing tumors, and 2) Experimental liver metastatic model of CT-26 tumors. CT-26 cells were transduced with lentiviral vector carrying luciferase gene under CMV promoter for constitutive expression of luciferase. Tumors were induced by s.c. injections of CT-26 tumor cells (2.5x105/ 100 μl) in both flanks of BALB/c mice. When the tumors reached about 4-5 mm in diameter, the mice were randomly divided into three groups and treatment was initiated. One group of mice was injected i.p. with anti-Fas antibodies (4 μg/mouse), another was treated with CBLB502 (1 μg/mouse) 24 h and 1 h before anti-Fas antibody injection (4 μg/mouse). Control mice ('intact') received PBS injections s.c. and i.p. in replace of CBLB502 and antibodies. Tumor volumes were measured every second day using calipers and calculated by formula: $V=\Pi/6*a2*b$, where $a<b$. Survival was followed for 2 weeks when experiment was terminated due to large tumors in the control group (Fig. 9D). Statistical difference between tumor volumes was estimated using ANOVA one-way analysis of variances ($p<0.05$). For the development of liver tumor growth, CT-26 tumor cells (2x105 /50 μl) were injected directly into spleen followed by splenectomy 5 min later. Mice were treated with anti-Fas antibodies and combination of CBLB502 with

antibodies the same way as described for s.c. tumors starting on day 5 after tumor cell inoculation. Noninvasive bioluminescent imaging of mice anesthetized with isoflurane and injected with D-luciferin (3 mg/ 100 μl, i.p.) was performed using Xenogen IVIS Imaging System 100 series on the days 14, 17, 22 and 28 after tumor cell injection. Mice were sacrificed when tumor growth in liver was determined. Statistical comparison of liver tumor-free curves was done using log-rank (Mantel-Cox) test ($p<0.05$) (Fig. 9G).

Example 2

[0149] Antitumor activity of CBLB502 on colon HCT116 adenocarcinoma s.c. growth in xenogenic model of athymic mice. HCT116 were injected s.c. into 2 flanks of 8 athymic nude mice (0.5x106 /100 μl of PBS) to induce tumors. When tumors became of about 3-5 mm in diameter (by day 6 after injections) mice were randomly distributed into 2 groups, 5 mice for CBLB502 treated group and 3 mice in PBS control group. Suppression of tumor growth was determined in CBLB502 treated mice. Data are shown in Figure 15.

Example 3

[0150] Antitumor activity of CBLB502 on 293-TLR5 s.c. tumor growth in xenogenic model of athymic mice. Tumor cells were injected s.c. into 2 flanks of 10 athymic nude mice ($2x10^6$ /100 μl of PBS) to induce tumors. When tumors became of about 3-5 mm in diameter (by day 7 after injections) mice were randomly distributed into 2 groups, 5 mice for CBLB502 treated group and 5 mice in PBS control group. Suppression of tumor growth was found in CBLB502 treated mice. Data are shown in Figure 16.

Example 4

[0151] Antitumor activity of CBLB502 on A549 adenocarcinoma s.c. growth in xenogenic model of athymic mice. The original A549 cells (ATCC, CLL-185) were injected s.c. into 2 flanks of 8 athymic nude mice ($0.5x10^6$ /100 μl of PBS) to induce tumors. When tumors became of about 3-5 mm in diameter (by day 6 after injections) mice were randomly distributed into 2 groups, 5 mice for CBLB502 treated group and 3 mice in PBS control group. A549 tumor-bearing mice were injected with either CBLB502 (1 μg/ mouse) or PBS three times with a 24-hr time interval. In the PBS injected control group of mice, tumor volumes gradually and regularly increased. On the other hand, the CBLB502 injected mice expressed inhibited tumor growth

during the first several days after injections and then tumor growth restored. The second round of CBLB502 injections 2 weeks after the first treatment (days 14, 15 and 16) induced analogous tumor growth inhibition for approximately 1-2 weeks before the restart of tumor growth. As a result, by the end of the experiment the sizes of the A549 tumors differed significantly in the two groups of mice, being much smaller in CBLB502 treated vs. PBS treated mice. Data are shown in Figure 17.

Example 5

[0152] Antitumor effect of CBLB502 on syngenic orthotopically (s.c.) growing squamous cell carcinoma SCCVII tumors. The rate of SCCVII orthotopic tumor growth in syngenic C3H mice after CBLB502 or PBS (no treatment) treatments (0.1 mg/kg, s.c.days 1, 2, 3) to reach 400 mm^3 tumor size, n=6-10. The x-axis in Figure 18 represents the amount of days needed for tumors to reach 400 mm^3 volume with and without treatment with CBLB502. Data are shown in Figure 18.

Example 6

[0153] Antitumor activity of CBLB502 in Fischer rats bearing s.c. advanced Ward colorectal carcinoma. CBLB-502 was administered by i.p. once a day for 5 days (0.2 mg/kg x 5 doses) initiated 5 days after tumor transplantation into 4 rats. Control 4 rats received PBS injection as a vehicle control. Tumor weight was measured daily. Complete response (tumor complete disappearance) was observed in 3 rats treated with CBLB502 (Figure 19). The fourth rat in this group had tumor growth similar to rats in the control group.

Example 7

[0154] The effect of CBLB502 injections on A549 tumors differing in TLR5 expression (A549-shTLR5 vs. A549-shV). In order to suppress TLR5 expression, A549 cells expressing Firefly luciferase gene under the control of NF-kB promoter (Cellecta, Mountain View, CA) were transduced with lentiviral pLKO1-puro vector expressing shRNA specific to human TLR5 gene [CCG-GCC-TTG-CCT-ACA-ACA-AGA-TAA-ACT-CGA-GTT-TAT-CTT-GTT-GTA-GGC-AAG-GTT-TTT-G] or control empty vector (shV, Sigma-Aldrich, St. Louis, MO). After puromycin selection, A549-shV and A549-shTLR5 cells were tested for NF-kB activation in response to CBLB502 treatment using luciferase reporter assay according to manufacture

protocol (Promega, Cat#E4530, Madison, WI). Then A549-shV and A549-shTLR5 cells (1x106 /100 μl of PBS) were injected s.c. into 2 flanks of 20 athymic nude mice to induce tumors. Mice bearing s.c. growing A549-shV and A549-shTLR5 tumor xenografts (5 mice per group) were treated with either CBLB502 or PBS acting as control The results demonstrate that the repeated administration of CBLB502 alone led to a reduction in tumor growth rates in the A549-shV (TLR5-expressing) tumor xenografts demonstrating a direct tumor suppressive effect of the drug. As shown for A549 derived tumors, this effect was TLR5 dependent since TLR5 knockdown elicited by lentiviral transduction of shRNA against human TLR5 rendered the A549 tumors no longer sensitive to the direct antitumor effect of CBLB502. Data are shown in Figure 20.

Example 8

[0155] The effect of CBLB502 injections on H1299 tumors differing in TLR5 expression (H1299-control vs. H1299-TLR5). In order to induce TLR5 expression, H1299 cells (originally TLR5 negative) were transduced with lentriviral construct expressing human TLR5 gene. The functional activity of TLR5 was checked by IL-8 production in response to CBLB502 treatment. Then both tumor cell types ($1x10^6$ /100 μl of PBS) were injected s.c. into 2 flanks of athymic nude mice to induce tumors. Similar to A549 model described above, mice bearing were treated with either CBLB502 or PBS acting as control. The results demonstrate that the repeated administration of CBLB502 alone led to a reduction in tumor growth rates only in H1299-TLR5 (TLR5-expressing) tumor xenografts demonstrating a direct tumor suppressive effect of the drug. As shown for the control H1299 (TLR5-negative) tumor growth was not affected CBLB502 treatment. Data are shown in Figure 21.

Example 9

[0156] This example demonstrates that bladder tissue is a strong responder to CBLB502. The experiment was conducted as described as described above for liver tissues. NF-kB dependent luciferase expression in liver, small intestine (ileum part), colon, spleen, kidneys, lungs and heart was assessed in the reporter mice 2 hs after s.c. injections of 100 μl of either PBS, CBLB502 (0.2 mg/kg) or LPS (1 mg/kg). Luciferase activity normalized per μg of the protein extract was detected in 3 mice in each group. The data are shown in Figure 22.

Example 10

[0157] Table 2 shows the spectrum of genes transcriptionally activated by CBLB502 in target organs of mice (bladder results are shown). Genes that are strongly upregulated in bladders of mice treated with CBLB502, 1 and 3 hrs post-injection, are clustered according to their function. The largest group consists of chemokines, cytokines and their receptors indicative of activation of innate immunity mobilizing mechanisms.

Example 11

[0158] CT-26 tumor cells, which do not express TLR5, were injected s.c. into syngenic BALB/c mice to induce tumors. Tumor bearing mice were treated with CBLB502 (0.04 mg/kg, s.c.) given twice 24 hour apart. The volumes of s.c. growing tumors in treated mice were compared with tumors growing in the intact mice. Pre-treatment with CBLB502 did not have any effect on tumor growth. Then CT26 tumor growth was tested in the experimental model of liver metastases induced by intrasplenic injection of luciferase expressing CT-26 tumor cells (Figures 23B and C) and A20 lymphoma cells (Figure 23D) followed by splenectomy. Hepatic tumor growth was assessed using Xenogen luciferase imaging every 4-6 days after the treatment. Mice remained free from liver tumor growth were counted at each imaging procedure. The results demonstrate prevention of tumor growth and significant delay of tumor appearance in livers by CBLB502 treatment in both tumor models. The difference between CBLB502 treated and control groups in liver tumor models (B, C, D) is significant (log rank $p<0.05$). The data are shown in Figure 23.

Example 12

[0159] CBLB502 protection from Fas mediated hepatotoxicity. A. Survival of NIH-Swiss mice after i.p. injection of 4 μg of anti-Fas antibodies alone or in combination with CBLB502 (1 μg/ mouse) injected 30 min, 2 hours and 6 hours prior antibodies. In parenthesis are the numbers of mice per each treatment. B. Protection of livers from anti-Fas antibody toxicity. Apoptosis in livers 5 hours after injections of anti-Fas antibodies was detected using TUNEL technique. Tissue morphology with H&E staining revealed necrotic damage to livers by anti-Fas antibody injections and protection by CBLB502. Hemorrhage in liver was detected by erythrocyte infiltration in tissue, mouse IgG control (purple) and DAPI nuclei (blue). Data are shown in Figure 24.

Example 13

[0160] Liver protection from TNF-alpha and LPS toxicity. A. Caspases 3/7 were detected 5 hours after injections of TNF-a or LPS and lipis oxidation (indicative of inflammation damage) was detected 24 hours post injection in mice with and without CBLB502 treatment 30 min before TPS/ TNF-a. Caspase activation and lipid oxidation in lungs induced by TNF (1 mg/mouse) was prevented by CBLB502 injection. LPS (10 mg/kg) induced damaging effect was completely abolished by CBLB502 injection 30 min before LPS. Data normalized by protein concentration, 24 hours after the treatment, n=3. It was no caspase activation (5 hours after TNF injections) and much less lipid oxidation (24 hours post-TNF injections as indicative of inflammatory damage) in livers of mice if CBLB502 was injected 30 min before h-TNF. B. Immunohistochemical analysis (H&E staining) confirmed the preservation of liver integrity by CBLB502 injection before TNF-a. Compared to the intact control, the liver of the TNF-treated mice showed vacuolization of the hepatocytes that is slightly more pronounced periportally and is dose-dependent (more severe in TNF 0.4 mg/mouse). In the livers of mice treated with CBLB502 and TNF 0.2 mg or 0.4 mg/mouse, the changes were minimal and the hepatocytes were close to normal though slight vacuolization was still visible. Data are shown in Figure 25.

Example 14

[0161] Lung protection from TNF-a and LPS toxicity. Compared to intact control, the lungs of the TNF-treated mice showed reactive proliferation of alveolar cells, hyperemia , interstitial edema and exudates in alveoli leading to reduction of the air spaces and the alteration was dose-dependent (more severe in TNF 400). In the lungs of mice treated with CBLB502 and TNF 200 ng or 400 ng, the changes were minimal. The morphology was close to normal though slight thickening of alveolar walls was still visible (Figure 26B). It was almost normal level of lipid oxidation (indicative of inflammatory damage) in lungs of mice if CBLB502 was injected 30 min before LPS (10 mg/kg) or h-TNF (0.05 mg/kg) (Figure 26A). Data are shown in Figure 26.

Example 15

[0162] Protection of mice from lethal oral Salmonella typhimurium administration by CBLB502 injections. Conditions of the experiments are shown in Figure 27.

Example 16

[0163] This examples demonstrates that irinotecan abrogates the antitumor effect of flagellin. The data are shown in Figure 28. Fischer rats with s.c. growing syngeneic Ward colon tumors were treated with CBLB502 (0.2 mg/kg), which was administered by i.p. once a day for three days. Irinotecan (200 mg/kg) was injected i.v. 30 min after each CBLB502 injection. PBS was used as a vehicle control (Fig. 28A). CBLB502 rescued rats from Irinotecan toxicity with no interference with irinotecan antitumor activity (Fig. 28B). The antitumor effect of CBLB502, however, was not observed in irinotecan-treated rats (Fig. 28C). This demonstrates that the antitumor effect of CBLB502 requires sufficient innate immunity levels.

Table 2

Chemokines Cytokines and their receptors								
	control	flic 1	LPS 1	1 час критерий				3 часа
CXCL2	1	4175.5	466.8	4175.5	-1.6	92.4	113.7	92.4
CXCL10	1	3477.1	751.9	3477.1	6.1	304	18.9	49.8
CCL2	1	1460.8	623.6	1460.8	-0.6	247.6	-0.4	248.9
CXCL1	21.6	21314.9	13985.8	986.8	73.3	18247.6	5458.7	248.9
CCL20	1	521.4	16.1	521.4				
CXCL2	1	240.2	8.9	240.2				
CCL7	25.2	3738.3	2575.3	148.3	1.1	664.1	218.3	10.7
CCL4	14.6	613.2	3296.7	42.0	28.2	1002	3630.4	35.5
CCL3	1.8	74.2	350.5	41.2				
CXCL9	11.6	185.6	94.5	16.0	2.8	389.7	481.3	139.2
CCL4	11.1	99.3	446.7	8.9	28.2	1002	3630.4	14.2
CXCL16	263.2	2048.1	546.6	7.8	36.8	524.2	386.1	14.2
CXCL15	1	34.5	14.1	34.5				
CCL5					19	480	658.7	25.3
CCL19					1.9	44.6	35.2	23.5
CXCL12					-6.3	14.9	14.4	14.9
CCL25					26.5	224.5	280.1	8.5
CCL1	1	4.2	-2.5	4.2				
CCL11	192.8	616.4	795.2	3.2				
CCL11	149.7	462.1	565.6	3.1				
CCL17	13.7	126.5	114.3	9.2	3.5	540.1	373.7	154.3
CCL19	7.6	18.4	9.8	2.4				
CCL2	1	1460.8	623.6	1460.8				
CCL20	1	521.4	16.1	521.4				
CCL22	1	3.9	7.7	3.9				
CCL26	1	4.2	0.7	4.2				
CCL28	1	3.7	-1.2	3.7				
CCL3	1.8	74.2	350.5	41.2				
CCL4	14.6	613.2	3296.7	42.0				
CCL4	11.1	99.3	446.7	8.9				
CCL7	25.2	3738.3	2575.3	148.3				
CCL7	12.3	1453.5	1195.4	118.2				
CCR3	1	2.3	2.8	2.3				
CCR5	5.9	12.1	-6.2	2.1				
CCR5	11.5	22.7	11	2.0				
CCR6	1	3.8	4.6	3.8	-4.7	17.5	5.8	17.5
CCRL2	62.7	610.6	224.9	9.7				
CLCF1	4.6	23.2	4.9	5.0				
CNTFR	1	9.1	-1.1	9.1				

Table 2

CNTFR	14.5	29.8	21.8	2.1				
CX3CL1	216.7	1081.9	328.9	5.0	183.2	1471.3	440.5	8
CXADR (CAR)	22.2	59.4	52.7	2.7				
CXCL1	21.6	21314.9	13985.8	986.8				
CXCL10	1	3477.1	751.9	3477.1				
CXCL15	1	34.5	14.1	34.5				
CXCL16	30.4	371.9	61.3	12.2				
CXCL16	263.2	2048.1	546.6	7.8				
CXCL17	1	7.7	4.5	7.7				
CXCL2	1	4175.5	466.8	4175.5				
CXCL2	1	240.2	8.9	240.2				
CXCL9	11.6	185.6	94.5	16.0				
CXCR5	1.3	4.7	14	3.6				
FAS	138.7	967.8	288.9	7.0				
FAS	396.2	1802.5	730.6	4.5				
FASL	3.6	10.2	1.1	2.8				
FPR2	14.2	96.4	187.3	6.8	0.1	954.1	1765.8	954.1
IER3	1494.5	12012.4	8339.1	8.0				
LIF	1	213.4	13.8	213.4				
LIFR	1	6	4.7	6.0				
DARC	131.2	360.4	277	2.7	87.9	1102.6	2605.8	12.5
CMKLR1					-7.3	8.9	10.4	8.9
FPR2					0.1	954.1	1765.8	954.1
EBI3					21.5	297.3	208.5	13.8
Interleukines and their receptors								
IL7R					1.6	24.9	43.8	15.6
IL8RA					-2	10.7	3.4	10.7
IL9R					0.5	9.5	3.1	9.5
IL17C	1	887.3	31.8	887.3				
LIF	1	213.4	13.8	213.4				
IL1RN	1	52.8	45.2	52.8	51.3	729.5	538.5	14.2

Table 2

IL1F5					-4.2	8	3.8	8.0
IL1R1					0.1	10	22.1	10.0
IL1R2					51.8	397.6	281.7	7.7
IL10	1	19.5	26.3	19.5				
IL10RA	15.5	34.3	29.5	2.2				
IL10RB	1.5	14	15.2	9.3				
IL12A					-1.2	32.2	10.3	32.2
IL12RB1	1	14.7	7.2	14.7	-0.5	13.7	10.9	13.7
IL12RB1	7.1	21.7	7.9	3.1				
IL13					11.4	111.9	258.5	9.8
IL13RA2					-4.2	12.4	121.7	12.4
IL15	4.8	10.6	8.6	2.2				
IL15RA	7.4	38.1	19	5.1				
IL15RA	5.1	24.8	14.3	4.9				
IL15RA	19.2	47.1	32	2.5				
IL16	1	2	4	2.0				
IL17A	1	5.4	2.2	5.4				
IL17B					-2.5	30.8	-4.5	30.8
IL17C	1	887.3	31.8	887.3	-0.2	8.6	1.7	8.6
IL17RA	26.1	72.2	37.1	2.8				
IL17RB					0.2	38.1	10	38.1
IL18BP					20	143.7	283	7.2
IL18R1					57.4	857.8	892.9	14.9
IL18RAP	1.3	24.3	0.1	18.7				
IL18RAP	7.6	18.4	19.1	2.4				
IL19					-0.7	26.7	95.4	26.7
IL1A	1	14.9	43.7	14.9				
IL1A	2.8	35.5	72.3	12.7				
IL1B	20.7	437.9	1023.3	21.2				
IL1RAP	6.1	11.9	5	2.0				
IL1RL1	1	22.3	13.1	22.3				
IL1RN	1	52.8	45.2	52.8	-2.7	34.4	0.9	34.4
IL1RN	1.9	6	2.9	3.2	51.3	729.5	538.5	10.3
IL1RN	51.5	158.5	118.8	3.1	-2.9	10.3	-1	14.2
IL2RA					-5.8	8.7	18.1	8.7
IL2RB					4.8	49	-2.2	10.2
IL2RG					1.2	12.9	3	10.8
IL20RA					4.8	34.2	21	7.1
IL20RB	1.6	6	7.7	3.8	-1.1	11.9	-3.4	11.9
IL20RB	2.5	8.5	5.3	3.4				
IL21R	1	7.5	3.3	7.5	5.8	65	65.4	11.2
IL21R	6.5	13	6.6	2.0				
IL22RA1	1.2	8	3.1	6.7				
IL23A	2.2	10.5	-2.1	4.8				
IL27	1	5.2	5	5.2				
IL28RA	1.6	15.3	11.1	9.6				
IL28RA	134.8	279.3	180	2.1				
IL2RG	1	7	6.1	7.0				

Table 2

IL31RA	1	4.5	7.6	4.5				
IL33	150.4	352.9	222	2.3				
IL4I1	28.9	1891	152.4	65.4	46.8	4000	741.7	85.5
IL5	1	2.5	0.4	2.5	-0.7	9.2	3.4	9.2
IL6	1.1	11.8	37.4	10.7	0.4	12	59.6	12.0
IL6RA					37.1	387.1	798.5	10.4
ILF2					0.9	17.4	8	17.4
ILTIFB					0.8	20.5	10.4	20.5
Growth factors								
AIF1					-5.2	8.3	11	8.3
ARID5A					25.1	259.4	302.6	10.3
EGR4	1	231.2	65.7	231.2				
CSF2	1	202.5	17.1	202.5	0	10.4	4	10.4
CD70	1	54.7	9.8	54.7	0.6	520.2	67.5	520.2
AREG	8	1299.1	256.3	162.4	11.5	692.1	255.8	60.2
CSF3-(GCSF)	1	35.4	15.6	35.4				
CSF1	49.7	486	238.9	9.8	33.1	509.2	397.4	15.4
CSF2	1	202.5	17.1	202.5				
CSF2RB	1.2	6.6	-2.9	5.5				
CSF3	1.3	6	12.8	4.6				
CSF3-(GCSF)	1	35.4	15.6	35.4				
CSF3R	1	2.3	0.3	2.3				
EGR1	1824.5	6578.8	6395	3.6				
EGR2	31.1	224.1	157.4	7.2				
EGR3	58.6	1126.3	1120.8	19.2				
EGR4	1	231.2	65.7	231.2				
FGF10	3.5	8.8	8.3	2.5				
FGF12	1	5.3	-5.8	5.3				
FGF12	4.8	11.7	10.7	2.4				
FGF13	4.9	15.2	6.9	3.1				
FGF15	6.6	18.4	5.2	2.8				
FGF17	1.1	3	2.1	2.7				
FGF22	1.7	13.6	17.7	8.0				
FGF3	1	2.3	9.7	2.3				
FGFBP3	1	6.4	-2.5	6.4				
FGFR1OP	11.8	31.7	16	2.7				
FGFR2	11.1	24.6	19.5	2.2				
GDF15	8.5	173.4	22.8	20.4				
HBEGF	72.3	1118.3	246.3	15.5				
IGFBP3	120.1	301.3	192.4	2.5				
NELL2					-4.9	24.9	10.3	24.9
BDNF					0.4	19.2	18.6	19.2
TGFBR1					6.6	73.4	137.8	11.1
NGFR					20.3	177.1	52.3	8.7

Table 2

PAMP-recognizing molecules and other immune receptors								
PTX3	1	309.8	525.7	309.8	-1.6	68.6	4.4	44.6
CLEC1A	5.8	12.5	9.5	2.2				
CLEC2D	1547.2	4521	2234.1	2.9				
CLEC2E	1	3.3	-1.4	3.3				
CLEC4D	12.3	61.7	114.5	5.0				
CLEC4E	1	4.6	11.6	4.6				
CLEC7A	5.4	11	13.2	2.0				
CLECSF9	12.1	34.7	52.9	2.9				
PGLYRP	10	34.2	9.4	3.4				
PGLYRP1	21.7	429.5	28	19.8				
PTX3	1	309.8	525.7	309.8				
PVR	13.8	99.3	31.1	7.2				
PVR	52.1	262.7	144.2	5.0				
PVR	30.7	95.4	52.2	3.1				
PVRL1	40.7	107.6	52.4	2.6				
PVRL2	70.7	207.8	96.9	2.9				
S100A8	34.5	288.9	32.6	8.4				
S100A9	23.6	139.9	20.6	5.9				
SAA1	2.9	5.7	16.3	2.0				
SAA3	125.6	500	1208.9	4.0				
ICOSL	25.3	106.7	30	4.2				
KLRG2	273.2	695.9	458.4	2.5				
KLRI1	2.1	14.5	12.1	6.9				
PRR, their adaptors and other receptor of innate immunity								
SFTPD					-7.5	23.4	84.8	23.4
SAA3					69.9	3183.4	2571.7	45.5
PGLYRP1					40.8	2012.5	255	49.3
KLRD1					5.8	107.8	171.1	18.6
HCST					26.4	198.3	202.3	7.5
FCGR4					37.6	1168.4	2200.4	31.1
MYD88	75.8	312.2	174.2	4.1				2.955748
								6.730455
NOD2	5.3	37.6	17.2	7.1	2	30.3	7.8	15.15
								0.402163
TLR1								**5.1**
TLR2	372.6	7545.6	2270.3	20.3				**4.5**
TLR3								**9.1**
TLR5								**2.0**
TLR6								2.804973
								0.353293

Table 2

TLR7								**2.5**
LY96					332.8	2474.1	1466.9	7.4
IRAK3	109.4	314.9	231.3	2.9	101.8	941.3	1132.4	9.2
IRAK4	1	10.5	7	10.5				
Anti-microbial proteins								
ZBP1					27.5	210.1	419.6	7.6
WFDC12					3.8	1134.8	3.3	298.6
S100A8					0	897.8	661.5	897.8
S100A9					-0.7	1021.2	1620.9	1021.2
RSAD2					154	5347.3	3471.3	34.7
REG3G					-3.3	857.6	471.2	857.6
MX2					21.9	959.8	671.8	43.8
LYZL4					24.8	215.1	-8.4	8.7
LTF					-0.5	272	184	272.0
DMBT1					-4.3	69.9	1	69.9
CRP					0.6	210.5	1721	210.5
CHI3L1					6.8	843.8	257.1	124.1
HAMP	1	117.6	6.2	117.6	-7.2	27.2	8.9	27.2
LCN2	7.7	492.4	173.8	63.9	9.2	1256.1	511.3	136.5
CHI3L1	11.8	128.2	46.5	10.9	6.8	843.8	257.1	124.1
DEFB18					-5.7	11.7	-5.4	11.7
DEFB20					-0.5	22.1	22	22.1
DEFB26					2.4	30.1	29.5	12.5
DEFB34					-2.2	7.1	-5.3	7.1
DEFB36					-0.7	8.3	-1.3	8.3
DEFB38					-6.9	7.6	-2.9	7.6
DEFB50					-5.4	8.6	6	8.6
DEFCR5					-2.6	10.9	-4.7	10.9
DEFCR-RS1					-1.2	8	3.9	8.0
DEFCR-RS12					-0.7	11.9	16.9	11.9
DEFCR-RS12					-6	11.6	5.4	11.6
DEFCR-RS2					-2.8	9.4	-3.1	9.4
DEFB23	1	3.5	-1.9	3.5				
DEFB30	1	2.4	-1	2.4				
DEFB5	7.4	16.4	12.4	2.2				
DEFCR6	3.7	7.6	6.8	2.1				
C7	27.4	57.7	20.1	2.1				
CFB					24.2	396	682.4	16.4
C1QL2					-4.5	18.3	12.1	18.3
C9	1	4.2	2.2	4.2	-8	15.4	17.8	15.4
CAMP	1.2	7.5	12.4	6.3				
IRGM1	1	3.6	3.4	3.6	1.2	32.2	-4.3	4.1
MX2	20	42.6	91.4	2.1	21.9	959.8	671.8	43.82648
REG3G	1	81.2	3.9	81.2	-3.3	857.6	471.2	259.8
WFDC12	1	238.5	-2	238.5	3.8	1134.8	3.3	298.6316
LCN10					-0.7	49.8	50.7	49.8
LCN3					-1.3	36.5	0	36.5

Table 2

CAMP					2.4	56.3	43	23.5
APOM					1	21.7	2.5	21.7
APOOL					-8	8.9	-8.2	8.9
C1QL2					-4.5	18.3	12.1	18.3
CD								
CD14	570.3	12181.7	2137.5	21.4				
CD160					-1.1	70.5	96.2	70.5
CD177	1	5.7	-5.4	5.7	0.3	23.2	3.9	23.2
CD177					9.9	110.9	3.6	11.2
CD19	2.1	6.9	5.7	3.3				
CD200R1	1	4.1	-4.9	4.1				
CD200R2					-4.5	7.6	12.1	7.6
CD207	1.2	5.8	-2.4	4.8				
CD207	1	3.1	2.4	3.1				
CD247	1	2.9	3.6	2.9				
CD27					-2.9	11.3	4.4	11.3
CD274					108	1070.2	398.3	9.9
CD28	1.2	2.7	1.1	2.3				
CD209C					2	24.2	93.6	12.1
CD209D					-3.6	35.5	54.7	35.5
CD209E					-8.5	7.1	11.5	7.1
CD300LB	1	2.7	-2.6	2.7				
CD33	13.5	27.4	26.4	2.0				
CD3E					17.2	122.3	21.2	7.1
CD4	1.2	9.3	9.1	7.8				
CD40	52.6	460.8	212.7	8.8	54.8	1419.4	775.7	25.9
CD40	15.2	108.4	44.9	7.1	15.9	193.1	101.3	12.1
CD44	31.3	106.2	42.3	3.4	-2.6	27.8	19	27.8
CD44	13.5	32.8	11.1	2.4	-1.7	22.8	0.2	22.8
CD44	305.7	725.2	505.5	2.4	171.5	1251.9	596.8	7.3
CD44	66.4	133.3	77.7	2.0				
CD52	1	2.1	-4.3	2.1				
CD53	10	20.6	19.4	2.1				
CD6	1	5.5	2.9	5.5	-2	7.4	17.5	7.4
CD6	1	3.3	-6.2	3.3				
CD6	3.2	7.2	-1	2.3				
CD6	6.7	13.1	13.5	2.0				
CD69	4	100.3	112.3	25.1	9.7	97.2	269.3	10.0
CD7					1.3	10.5	8.9	8.1
CD70	1	54.7	9.8	54.7	0.6	520.2	67.5	520.2
CD79B	1	3.9	4.6	3.9				
CD80	8.5	31.5	16.2	3.7				
CD80	3.1	7.1	3.5	2.3				
CD83	45.7	771.8	486	16.9				
CD86	3.2	5.8	-4.3		3.5	59.4	30.3	17.0
CD8B1					-1	30.2	-5.1	30.2
CD96	1	5.3	4.1	5.3				

Table 2

Transcription and proliferation								
NEO1					9.8	69.3	42.6	7.1
NFIC					12.3	136.2	297.6	11.1
POU2F2					11	161.3	164.9	14.7
POU3F1					6.5	140.6	83.1	21.6
SBNO2					54	765.1	860.1	14.2
SIRT6					17.7	182.1	195.4	10.3
GTF3C6					4.2	84.2	106.1	20.0
GEMIN5					11.3	93.2	157.1	8.2
FIP1L1					-2.1	57.9	10.3	57.9
FAP					5	51.7	2.4	10.3
CHD1					27.5	295.2	529.8	10.7
BACH1					21.7	159	299.5	7.3
BARX2					15.6	234.6	144.6	15.0
ATF3	31.6	690.5	276.5	21.9				
ATF4	43.8	115	71	2.6				
BATF	17.2	150.8	57.3	8.8	21.9	185.7	460.4	8.5
BAZ1A	27.6	93.8	44	3.4				
CCND2	1.6	7.9	6.3	4.9				
CCNL	30.3	67.6	38.5	2.2				
CCNYL1	30.5	103.3	71.9	3.4				
CDK2	29.1	67.9	52	2.3				
CDK3	4	8.8	4.5	2.2				
CDK5R1	24.2	110.2	42.6	4.6				
CDK6	4.1	27.3	19.5	6.7				
CDK7	1.5	3.8	-1.2	2.5				
CDKN1A	147.1	374.4	226.9	2.5				
CDKN1A	370.5	730.6	423	2.0				
CDKN2B	1231.3	2751.7	1575.8	2.2				
EGLN3	406	2015	751.8	5.0	-1.3	19.3	-4.6	19.3
EGLN3	3.2	11.4	1.1	3.6				
EIF4E1B					-2.4	18.9	4.9	18.9
ELF3	#REF!	3525.2	875.2	14.4				
ETS1	1	22.7	7.7	22.7				
FKHL18	63.6	336.2	133.8	5.3	52.6	431.6	355	8.2
FOSB	92.3	738.1	332.9	8.0				
FOSL2	4.6	32.2	8.5	7.0	0.4	11.7	-0.1	11.7
FOSL2	4.7	20.4	6.8	4.3	6.7	70.5	61	10.5
FOXA3	1	13.2	6.6	13.2	3.2	100.6	0.9	31.4
FOXB1	1	3.8	9.2	3.8				
FOXD2	1	5.6	4.7	5.6	1.2	10.3	40.5	8.6
FOXE1					-0.4	8.8	-2.9	8.8
FOXF2					-1.2	10.3	20.6	10.3
FOXF1A	400.7	960.3	831.7	2.4				
FOXI2	1	2.6	-3.8	2.6				
FOXJ3	9.2	20.3	13.9	2.2				
FOXJ3	5.7	12.2	9	2.1				

Table 2

FOXK1	2.9	7.7	5.2	2.7				
FOXK1	2.6	5.4	-1.3	2.1				
FOXL2	1	9.5	0.1	9.5	-1.6	13.3	4.4	13.3
FOXO4					-6.2	10.5	12.5	10.5
FOXO6	1	2.8	0.9	2.8	0.3	30.4	99.7	30.4
FOXP3					0.9	19.6	3.9	14.1
FOXP4	21	69.7	18.7	3.3				
G3BP1	350.1	898.7	467.6	2.6				
CXXC1	9.4	25.7	18.5	2.7				
GADD45A	1324.4	6076.1	2042.7	4.6				
GADD45A	903.7	3390.1	1019.9	3.8				
GADD45B	1	72.8	70.2	72.8	1.8	101.6	60.8	56.4
GADD45B	106.8	2799	1357.3	26.2				
GADD45G	498	1323.4	1534.7	2.7				
HIF1A	245.5	704.1	335.7	2.9				
HIF1AN					-3.2	23.9	41	23.9
JDP2	140.5	635.1	586.8	4.5				
JUN	128.1	714	553.2	5.6				
JUNB	994.9	2976.3	3927.9	3.0				
JUND1	72.6	227	113.1	3.1				
MAFF	2.5	72.5	17.3	29.0				
NF1	1	14.4	2.8	14.4				
NFATC1	6.6	58	11.3	8.8				
NFATC1	15.8	75.5	35.2	4.8				
NFATC1	152.8	550	312.6	3.6				
NFATC1	179	586.1	275.9	3.3				
NFATC2	1	8.4	9.3	8.4				
NFATC2	1	5.9	-2.3	5.9				
NFE2L2	403.6	1300.1	834.9	3.2				
KLF6	32.4	74.9	74.9	2.3				
MYC	48.9	160.6	153.1	3.3				
MYCT1	1	4.8	0.7	4.8				
SOX7	17	43.5	44.9	2.6				
SOX9	1	140.4	23.5	140.4				
SOX9	109.4	2159.4	462.5	19.7				
Apoptosis								
ANKHD1					35.7	320.8	506.2	9.0
AEN	15.1	41.5	16.7	2.7				
APBA1					0.3	14.8	7.9	14.8

Table 2

APBB1IP					-3.9	11.3	80	11.3
APH1A					0.1	140.8	158.1	140.8
APIP					15.4	119.7	190	7.8
BIRC2					25.6	222.3	277.1	8.7
BIRC3					-4.7	47.2	2.2	47.2
BIRC6					-0.9	9.4	-1.7	9.4
BBC3					-1.1	59.9	131.2	59.9
BCLAF1					-1.6	36.8	2	36.8
BCL10	1309.4	3434.1	1987.1	2.6				
BCL10	2.9	6.6	8.9	2.3				
BCL2A1B	60.8	330.2	280	5.4				
BCL2A1C	3.3	27.3	19.2	8.3				
BCL2A1D	1	15.8	7.6	15.8				
BCL2A1D	32.6	253.6	247.7	7.8				
BCL2L11	5	37.3	5.6	7.5				
BCL2L11	50.7	302.2	52.2	6.0				
BCL2L11	22.7	115.2	27.8	5.1				
BCL2L11	455	1485	306.3	3.3				
BCL3	32.9	610.6	270.4	18.6				
BCL6B	47	97.6	112.8	2.1				
BCL9	3.6	13.6	16.5	3.8				
BCL9L	1.3	21	12.7	16.2				
BCOR	209.5	980.2	226.5	4.7				
CASP4	125.3	740.5	278.1	5.9				
CASP8	404.5	845.1	530.3	2.1				
NUPR1	1049.6	3339.3	2496	3.2				
DNASE1L3					8.9	179.6	139.6	20.2
PDCD4					476.3	4862.7	12537.8	10.2
Kinases								
CSNK1G1	1	8	18.3	8.0	0.5	75.7	119.2	75.7
DUSP1	1719.3	4023.2	4344.7	2.3				
DUSP16	48.9	143.8	66.2	2.9				
DUSP13					-3	23.8	27.7	23.8
DUSP2	30.1	262.3	191.1	8.7	8.2	78.6	58	9.6
DUSP2	21.8	106.3	81.9	4.9	20.3	180.1	51.3	8.9
DUSP3	49.6	101.7	105.6	2.1				
DUSP3	40.1	81.1	125.2	2.0				
DUSP6	20.4	161.5	35.7	7.9				
DUSP6	50.9	174.7	46.3	3.4				
DUSP6	1275.3	3870.6	1335.9	3.0				
DUSP8	96.2	454.9	414.5	4.7				
MAP2K3	1864.1	4967	2371.6	2.7				
MAP3K2	2.5	6.8	7.2	2.7				
MAP3K7	34.9	74.5	51	2.1				
MAP3K8	52.5	618.6	432.5	11.8				
MAP4K5	2.6	5.8	2.4	2.2	0.5	8	8	8.0
MAPK11	122.6	248.5	139.9	2.0				
MAPK15	1	3.6	0.6	3.6				

Table 2

MAPK1IP1L	183.6	365	286.5	2.0				
MAPK6	477.4	986.2	578.6	2.1				
MAPK8	4.9	11	6.9	2.2				
MAPK8	1	2	0.5	2.0				
MAPK8IP3	1	14.7	7.8	14.7				
MAPK8IP3	2	5.6	7	2.8				
MAP2K1					15.1	120.9	127.1	8.0
MAP3K12					7.7	58.7	66.7	7.6
MAP3K4					0.2	8.9	17.1	8.9
MAP3K5					-2.5	19.4	12.2	19.4
MAP3K6					59.4	475.2	741.5	8.0
MAPK10					-1	12.9	40.2	12.9
MAPK7					0.7	61.3	1.4	61.3
MAPK8IP2					1.1	38	0.6	34.5
CKMT1					-1	61.6	144.9	61.6
CSNK1G1					0.5	75.7	119.2	75.7
DAPP1					21.1	236.3	160.8	11.2
DYRK1A					4.9	80.1	87.6	16.3
PIM1	92.9	227.5	130.4	16.7				
PIM1	434.4	1213.4	801.2	3.2				
PIM2	92.9	227.5	130.4	7.3				
PIM2	434.4	1213.4	801.2	2.1				
PLK2	92.9	227.5	130.4	2.4				
PLK3	434.4	1213.4	801.2	2.8				
ITPKC	405.2	1250.1	984.1	3.1				
RIPK2	86.5	856.8	300.4	9.9	79.7	819.8	152.4	10.3
Cathepsins								
CTSB	2.8	6.9	4.8	2.5				
CTSC	9.2	64	7.1	7.0	40	410.5	164.3	10.3
CTSC	7.9	37	5.3	4.7	7.8	60	0.3	7.7
CTSC	39.3	134.5	42.7	3.4	17.9	136.5	34.8	7.6
CTSJ	1.1	2.4	-3.6	2.2	0.9	11.9	3.9	11.9
LAMP2	2	21.3	14.4	10.7				
CTSM					-3.8	11.1	73.6	11.1
CTSR					10	78.6	88.2	7.9
Interferon and IFN-inducible genes								
IRF1	16.4	531.5	170.4	30.7				
GBP1	796	1974.1	1706.5	2.5				
GBP10	8.6	53.9	40.6	6.3				
GBP2	28.9	67.5	76.4	2.3				
GBP3	580.4	1978.1	1291.3	3.4				
GBP3	548.9	1582.2	1065.5	2.9				
GBP5	90.4	672	507.1	7.4				
IFI202B	14.6	51.4	60.1	3.5				

Table 2

IFI47	38.6	90.7	95.3	2.3				
IFITM1	3562.3	8026.1	5161.6	2.3	8.1	474	585.6	58.5
IFITM1					1023	7728.1	4581.7	7.6
IFITM5	1.3	20.3	10.7	15.6	-2.5	14.6	-1.9	14.6
IFITM6					-1.9	12.1	62.7	12.1
IFNE1	8.5	28	3.1	3.3				
IFNG	1	2.6	3.8	2.6				
IFNGR2	107.1	425.9	202.8	4.0				
IRF5	23.5	52.3	54.6	3.9				
IRF6	23.5	52.3	54.6	4.1	-5.1	15.4	-4.7	15.4
IRF6	23.5	52.3	54.6	2.0				
IRF7					15.4	230.7	207.4	15.0
IRF9	23.5	52.3	54.6	2.2				
PLSCR1	114.6	893.3	318.7	7.8				
PLSCR1	66.9	305.6	158.3	4.6				
SLFN2	18.4	252.7	69.5	13.7				
ISG20L1					19.9	159.7	166.4	8.0
IGTP					164.5	1836.5	1147	11.2
LOC100048583					-3.5	27.8	109.6	27.8
LOC100048583					-3.5	27.8	109.6	20.4
GVIN1					45.4	458.2	976.1	10.1
proteasome								
PSMB9					15.4	114.6	142.3	7.4
PSMD11					6.1	62.8	0.6	10.3
PSMD3					-5.7	15.5	132.5	15.5
Ubiquitine-associated								
CUL4A	1	3	-0.2	3.0				
IBRDC3	177	699	463.2	3.9				
LNX1	1.4	10.9	6.2	7.8				
UBD	17.4	85.8	20.6	4.9				
UBTD2	20.4	60.9	52.8	3.0				
USP2	6.2	15.6	20.9	2.5				
USP23	2.7	8.7	8.2	3.2	1.4	28.6	-1.8	20.4
USP25	6.9	15.6	18.6	2.3				
USP37	5.5	19.2	7.9	3.5				
USP38	22.9	45.5	27.7	2.0				
USP42	1	2.9	5.2	2.9				
USP48	4.8	17.1	13.8	3.6				
USP27X					-0.9	8.7	-1.5	8.7
USP29					-2.8	7	10.1	7.0
USP37					19	330.9	294.3	17.4
USP43					10.1	108.8	30.8	10.8
USP8					-2.4	27.9	5.3	27.9
USP9X					-3	37.8	115.3	37.8
CUL4A					0.5	22.4	6.2	22.4
UBD					13.4	446.1	278.5	33.3
LINCR	24.7	1194.7	109.8	48.4				

Table 2

DTX3L					166.3	1484.1	608.4	8.9
MARCH1					10.5	75.1	134.7	7.2
Actin-tubulin								
FLNB								
CCT5					288.6	2407.1	1216.1	8.3
CDC42EP1					13.6	110.1	113.6	8.1
CDC42EP5					2	52.5	48.1	26.3
CEP350					-2.3	54.4	167.6	54.4
ARPC4					19.9	155	138.7	7.8
ARPM1					-1.1	10	3.2	10.0
ACTB	3142.5	7089.1	6166.2	2.3				
ARC	16.7	320.9	83.6	19.2				
ABLIM3	1	4.8	7.9	#REF!	-5.9	17.4	13	17.4
ACTL7B					-2.4	13.6	0.9	13.6
ACTR6					-1.4	9.4	28.3	9.4
SCIN	54	597.9	49.6	11.1	59.7	1853	145.9	31.0
TUBA6	1429.6	2863.9	2070.6	2.0				
TUBB2B	940.9	2963.8	1731.2	3.1				
ER-transport								
DUOXA2	1	280.8	5.7	280.8	11.5	878	25.2	76.3
EHD1	131.3	725.6	270	5.5	115.1	808.1	615.6	7.0
G-proteins etc								
GPR109A	131.7	4123.4	1185.8	31.3				
GPRC5A	39.6	421.2	251.8	10.6				
RAI3	24.4	212.5	117.6	8.7				
LPAR2	32.4	221.4	19.3	6.8				
LPAR3	129.6	660.7	158.6	5.1				
RGS16	RGS16	387.9	372.6	13.4				
RND1	1.6	11	20.7	6.9				
RND3	157	806.7	435.3	5.1				
GPR84	1	65.4	70	65.4				
GNAS					-1	267.2	553.9	267.2
LPAR2					12.3	94.5	-3.7	7.7
Mucose generation and cell-to-cell connection								
KRT16	1	150	8.2	150.0				
HAS1	22.7	939.7	1378.5	41.4				
SPRR2G	1.8	70	11.3	38.9	9.6	817.1	19.2	85.1
PCDH10	1	38.4	7.4	38.4	-0.9	89	90.9	89.0
PCDH10					-1.4	9.9	11.2	9.9

Table 2

PCDHA11					1.4	42.8	14.2	30.6
PCDHA7					-1	10.6	-3.3	10.6
PCDHA7					6.1	50	98	8.2
PCDHB6					2	14.1	29.5	7.1
PCDHGA10					1.8	17.8	55.1	9.9
PCDHGA9					-0.5	11.8	0.7	11.8
PCDHGB6					-4	7	-8.3	7.0
PCDHGB7					-2.9	8.3	62.3	8.3
PCDHGB8					-3.1	16.3	7.8	16.3
AMIGO2	205.4	480.7	564.1	2.3				
CATNAL1	1.9	14.3	4.2	7.5				
CHST4	1	3.8	2.1	3.8				
CLDN7	91.3	187.6	110.1	2.1	104.5	794.3	479.9	7.6
CNFN	1	19.3	-10.6	19.3	-1.5	74.4	-4.8	74.4
COL11A1	1	4.7	1.5	4.7	0.5	15.2	59.2	15.2
COL11A1	1	2.6	0.3	2.6				
COL11A2	9.5	19.9	15.5	2.1				
COL19A1	1	4.5	-0.6	4.5	2.1	43.4	5.9	20.7
COL25A1	6.6	12.9	13.7	2.0				
COL2A1	2.3	5.8	11	2.5				
COL4A3	2.9	9.9	7.1	3.4				
COL5A3	6.6	21.6	11.4	3.3				
COL6A1	11.1	27.4	19.1	2.5				
COL9A1	1.9	3.9	-3.1	2.1				
COL9A2	2.3	12.2	9.8	5.3				
COL5A2					2.8	70	90.4	25.0
COLQ					-6.9	14.5	7	14.5
FAT1	6.9	28.2	31.2	4.1				
FLNB	329.5	729.2	391	2.2	301.2	2663.6	1356	4.4
GALNT3	1	26.3	-1.5	26.3				
GFPT1	1	14.5	6.6	14.5	0.9	16.6	8.4	16.6
GFPT1	3.6	14.4	5.6	4.0	14.6	105.7	33.8	7.2
GFPT2	155.1	1284.9	1257.2	8.3	97	1762.3	2584.6	18.2
GCA	9.4	24.9	20.6	2.6				
GJA10	2	7.3	12.3	3.7				
GJA8	1	4.9	2.6	4.9				
GJB1	7.3	14.8	13.7	2.0				
GJB2	26.5	58.6	38.3	2.2				
GJB2	364.8	770.8	479.5	2.1				
GJB2	33.3	68	46.7	2.0				
GJB4	1	16.5	6.2	16.5				
GJC2	1	3.5	3.6	3.5	1.1	13	45.5	11.8
GJD2	1	4.6	-1	4.6	-5	18.3	51.1	18.3
HAS1	22.7	939.7	1378.5	41.4				
HAS2	11	81	88.7	7.4				
HS3ST1	753.4	8916.3	1840.7	11.8				
HS6ST1	5.3	25.1	-0.2	4.7				

Table 2

HS6ST1	395.4	1824.5	349.2	4.6				
ITGA2	5.6	49.3	13.1	8.8				
ITGA5	157.4	332.9	164.5	2.1				
ITGA6	1	9	4.1	9.0				
ITGAD	1.5	3	-0.3	2.0	2.1	18.2	-5.4	8.7
ITGAE	1	10	8.4	10.0				
ITGAV	59.2	172.4	86.5	2.9				
ITGB2					2.7	53.1	8	19.7
ITGB6	180	620.7	249.4	3.4				
ITGB6	1253.2	3474.8	1568.1	2.8				
ITGB6	513.8	1370.7	645.1	2.7				
ITGP	1	10.6	4.8	10.6				
KRT14	254.8	1063	660.2	4.2				
KRT1-5	1	18.6	-2.1	18.6				
KRT16	1	150	8.2	150.0	1.2	148	16	124.3
KRT23	679.4	4234.8	720.2	6.2				
KRT36	6.5	60.5	2.5	9.3				
KRT17					7.7	103.2	65.5	13.4
KRT33B					-1.8	7.9	0.8	7.9
KRT35					-4.4	12.1	85.9	12.1
KRT82					-0.6	18.2	3.6	18.2
KRT84					0.8	8.1	20.4	8.1
KRT86					-1.7	12.9	4.6	12.9
KRTAP16-5					0.3	14.1	34.6	14.1
KRTAP3-2					-2.7	7.9	-5.9	7.9
KRTAP9-1					-1	7.7	49	7.7
KRTDAP					19.2	157.7	258.3	8.2
LOXL4	2.7	21.6	7.6	8.0				
SPRR2D	6.1	1052.3	-0.1	172.5	1.1	3215.9	14.1	2923.5
SPRR2E	1	245.5	-8.1	245.5	-1.9	772.1	-4.5	772.1
SPRR2F	14.4	137.8	9.1	9.6	5.9	2404.5	45.6	407.5
SPRR2G	1.8	70	11.3	38.9	9.6	817.1	19.2	85.1
CDCP1	5.8	43.7	21.8	7.5				
AMICA1					22.2	372.2	455	16.8
CDH2					10.2	74.1	10.8	7.3
LOXL4					43	469.9	373.6	10.9
NRXN2					6.5	78.9	153	12.1
Immune adhesion molecules								
VCAM1	5.4	68.1	34.9	12.6	0.8	45	-6.6	45.0
VCAM1	637.4	4462.9	3422.1	7.0	0.9	14.9	9.4	14.9
ICAM1	329.6	7607.4	4220.4	23.1	256.4	2069.1	705.5	8.1
SELP	36.5	225.9	1068.5	6.2	28	756.9	2978.1	27.0
SELL	4.3	10	6.6	2.3	8.8	84.1	26.8	9.6
CEACAM1					12	137.2	72.8	11.4
CEACAM1					42.4	303.9	59.4	7.2
CEACAM2					50.4	408.1	22	8.1
NF-kB and inflammation								

Table 2

REL	1	132.3	26.3	132.3				
NFKBIZ	147.6	6159.8	2878.9	41.7				
NFKBID	40.9	1493.2	362.5	36.5				
IKBKE	90.1	569	99.7	6.3				
NFIB	3.5	9	5.6	2.6				
NFKB1	20.5	151.3	76.4	7.4	7.4	69.9	144	9.4
NFKB1	561.3	2247	840.7	4.0				
NFKB2	1	6.8	-0.5	6.8				
NFKBIA	630	11386.8	6795.9	18.1	18.1	301.2	226.9	16.6
NFKBIA	27.7	338.8	182.1	12.2				
NFKBIB	19.8	200.7	46.8	10.1	17.9	264.1	221.6	14.8
NFKBID	40.9	1493.2	362.5	36.5	27.8	399	248.8	14.4
NFKBIE	8.1	180.2	29.6	22.2	60.2	536	169.6	
NFKBIE	60.2	731.7	136	12.2	164.2	3145.7	2250.7	3.9
NFKBIZ	147.6	6159.8	2878.9	41.7				19.2
REL	1	132.3	26.3	132.3				
RELA	1948.7	4768.8	2897.9	2.4				
RELB	106.2	1265.6	336	11.9				
HSP								
HSPA1A	64.7	744.8	102.8	11.5				
HSPA1A	35.2	239.8	53.7	6.8				
HSPA1A	1469.1	5421.2	1074.3	3.7				
HSPA1B	22.6	227.3	22.7	10.1				
HSPA1B	135	1184.7	193.7	8.8				
HSP90AA1					6.6	49.3	77.1	7.5
HSPA14					-11.8	8	-3.9	8.0
HSPB3					-0.4	19.8	44.3	19.8
COX4I2					22.2	236.9	358.2	10.7
LTB4R1					28.9	222	363.3	7.7
NKIRAS1					14.9	135.5	189.4	9.1
RIPK2					79.7	819.8	152.4	10.3
Ion-channel								
KCNE2	1.8	68.8	4.5	38.2				
MCOLN2	12.8	42.3	26.9	3.3	7.2	108.2	7.9	15.0
CYCS	33.1	66.2	43.6	2.0	10.2	116.5	193.5	11.4
CLIC4	121.4	459	228.6	3.8				
CLIC6					33.9	494.6	73.1	14.6
CACNA1F					1	10	4.1	10.0
CACNG3					-8.9	9.4	-0.8	9.4
CACNG5					-1.6	16.1	47.9	16.1
CACNG6					1	13	7.9	13.0
CLCA3					5.3	46.1	-4.1	8.7
CLCN3					1.3	10.7	-2.6	8.2
CLCNKA					-0.8	7.3	5.3	7.3
CLIC6					33.9	494.6	73.1	14.6

Table 2

KCNA10					2.3	20	6.5	8.7
KCNA7					0	14.2	-3.8	14.2
KCNAB1					-3.3	8.4	-4.2	8.4
KCNC2					-2.5	20.1	-0.5	20.1
KCND2					2.8	26.3	3.4	9.4
KCND3					-6.3	9	-0.1	9.0
KCNE1L					6.8	70.1	112.6	10.3
KCNH4					-0.8	9.4	13.3	9.4
KCNJ1					-3	8	6.4	8.0
KCNK13					23.7	216.8	298.4	9.1
KCNK15					0.5	7.7	40.7	7.7
KCNK9					-4.4	9.8	2.4	9.8
KCNQ2					-1.3	9.1	10.3	9.1
KCNQ2					-1.3	9.1	10.3	7.7
KCNQ5					-2.1	20.3	-1.2	20.3
KCNS3					-1	11.6	4.7	11.6
SCN2A1					1	14.8	-7	14.8
SCN8A					-8.8	13.5	16.4	13.5
SCNM1					9.4	172.1	333.6	18.3
SCNM1					26.5	214.3	284.7	8.1
TPCN1					-5.1	12.8	4.5	12.8
MCOLN2					7.2	108.2	7.9	
Histone and histone associated								
EHMT2	1	10.1	14	10.1				
DOT1L	16.5	56.1	42.6	3.4				
histocompatibility 2								
H2-Q5	10.7	171.7	59.7	16.0				
H2-Q7	124.9	313.3	141.1	2.5				
H2-Q7	30.4	73.3	42.7	2.4				
H2-Q8	17.2	96.9	34.2	5.6				
Ras-Rho related								
RAN								45.6

Table 2

RANBP3L								13.8
RANGAP1								14.0
CDC42EP2	732.4	2299.1	556.7	3.1				
CDGAP	1.9	7.7	7	4.1				
IRGQ	562.2	1304.5	491.8	2.3				
RAB20	4.9	27.8	29.5	5.7				
RAB32	452.5	1946	939.8	4.3				
RHOF	17.9	252.9	14	14.1	21	340.1	5.1	16.2
RAB1								8.7
RAB1B								15.4
RAB3B								9.5
RAB3IP2-PENDING								20.9
RAB5B								13.2
RAB7								12.4
RAP1A								11.7
RASAL1								7.0
RASGEF1A								14.6
RASGRP1								14.9
RASSF10								15.3
RASSF4								9.7
RASSF6								10.5
RASSF9								12.9
ARHGAP15								7.2
ARHGAP25								9.8
ARHGAP5								9.0
ARHGEF1								7.9
ARHGEF7								11.2
Protease-inhibitior								
SERPINA3F	1	19.9	-0.3	19.9				96.3
SERPINA3G	142.5	5320.8	1240.8	37.3				
SERPINA3H	31.7	2296.8	799.4	72.5				287.3
SERPINA3N	675	1797.8	1726.1	2.7				30.0
SERPINB1A	45.3	88.7	121.7	2.0				
SERPINB2	1	79.1	358.6	79.1				
SERPINA12								11.3
SERPINA1C								11.0
SERPINA3G								129.7
SERPINA3M								20.3
SERPINB3B								12.3
SERPINE2								11.1
SERPINF2								13.2
CST6					-4.4	107.1	74.5	107.1
STFA1					4.9	67.2	22.5	13.7
solute carrier family								

Table 2

SLC10A5					0.2	27.5	1.7	27.5
SLC10A6	78	434.6	617.3	5.6				
SLC10A6	30.9	155.7	163	5.0	77.3	592.2	321.4	7.7
SLC11A2	6.1	32.2	20.7	5.3	52.9	503.5	229.3	9.5
SLC11A2	60.3	147.6	106	2.4				
SLC12A1					5	70.3	94.4	14.1
SLC12A2					-6.7	7.4	-0.3	7.4
SLC13A3					1.3	10.1	3	7.8
SLC14A2					-4.4	29	23.9	29.0
SLC15A3	27.3	135.7	73.2	5.0	23.6	260.3	476.5	11.0
SLC16A3	5.1	14.5	7.3	2.8				
SLC16A5	54.2	183.9	66.3	3.4	45.2	417.1	80.3	9.2
SLC16A9	175	473.1	267.9	2.7	1.2	172.9	315.5	144.1
SLC17A3					6	62.8	9.2	10.5
SLC17A6					-2.9	8	-5.8	8.0
SLC1A4	194.9	460.9	191.4	2.4				
SLC25A25	195.8	956.8	660.4	4.9				
SLC2A6					62.8	3525.2	871.1	45.1
SLC22A3					-1.1	19.2	55.1	19.2
SLC22A4					2.4	52.3	-4.8	21.8
SLC22A6					-0.8	9.9	-2.3	9.9
SLC22A9					-8.3	24.4	-6.1	24.4
SLC24A5					-1	45.3	16	45.3
SLC25A2					0.7	10	4.6	10.0
SLC25A34					-4.9	7.5	-0.9	7.5
SLC25A4					0.9	8.1	-2.2	8.1
SLC25A40					-1	17.1	3.2	17.1
SLC25A40					-2.6	10.4	16.6	10.4
SLC26A3					-4.5	8.8	8.1	8.8
SLC26A4					3.1	45.5	10.6	14.7
SLC26A7					-9.7	15.9	5.1	15.9
SLC29A4					4.6	41.7	23.7	9.1
SLC2A2					-6.5	12.7	8.2	12.7
SLC2A6					62.8	3525.2	871.1	56.1
SLC30A3					2.3	52.9	2.1	23.0
SLC30A4					-0.3	34.6	57.5	34.6
SLC34A2					0.1	7.3	-3.2	7.3
SLC35D3					-4.1	12.3	-4.5	12.3
SLC35F4					-0.6	9.9	63.5	9.9
SLC36A3					0	34.6	5.4	34.6
SLC38A1					-0.5	18.3	13.7	18.3
SLC38A2					3.8	35	8.1	9.2
SLC39A8	29.3	77.6	105.2	2.6				
SLC39A8					-10.1	10.6	-2.6	10.6
SLC45A3	31	169	46	5.5				
SLC39A4					129.2	927.6	400.6	7.2
SLC39A5					-2	31.7	2.4	31.7
SLC3A2					-0.2	26.2	0.5	26.2
SLC45A2					0.3	16.2	18.4	16.2

Table 2

SLC4A1					21.3	212.3	266.8	10.0
SLC4A4					5.7	171.3	291.7	30.1
SLC5A1					37.8	307	107.7	8.1
SLC5A10					-2	28.6	-0.6	28.6
SLC5A2					-2.3	17.3	-1.1	17.3
SLC6A12					-0.3	8.7	64.1	8.7
SLC6A15					-6.3	40.3	5.6	40.3
SLC6A16					-6.5	8.2	3	8.2
SLC6A2					0.2	14.9	6.8	14.9
SLC6A4					-3.3	10.7	98.3	10.7
SLC7A11					2.7	29.1	13.1	10.8
SLC7A11					44.5	408.4	38.8	9.2
SLC7A2					-4.2	8.1	2.7	8.1
SLC7A9					-3	15.3	13	15.3
SLC8A2					0.8	8.6	15.2	8.6
SLC9A6					2.2	26.1	47.3	11.9
SLCO1A5					1.4	15.1	6.1	10.8
SLCO4A1					-2.7	180.6	39.5	180.6
SLCO6B1					-6.4	7.6	51.4	7.6
Suppressor of cytokine signaling								
SOCS1					4.7	56.2	61.4	12.0
SOCS2	209	1043.8	941.3	5.0				
SOCS3	177.3	4410.8	5007.3	24.9	52.5	2767.6	3346.5	52.7
SOCS4	69.3	211.4	94.8	3.1				
SOCS7					-3.2	7.9	4.1	7.9
SOCS7					-1.6	7.3	-6.7	7.3
olfactory receptor								
OLFR1					-3.2	7.1	1.6	7.1
OLFR100					2.7	19.9	8.7	7.4
OLFR1000					-5.2	8.3	7.5	8.3
OLFR101					-5.4	10.2	2.4	10.2
OLFR1015					**0.3**	**37.5**	**0.5**	**37.5**
OLFR1024					-4	20.4	-5	20.4
OLFR1038					-3.4	7.6	7.6	7.6
OLFR1040					1.7	13.9	15.2	8.2
OLFR1042					-1.1	19.6	8	19.6
OLFR1048					-1.1	12.5	-1.6	12.5
OLFR1056					0.7	16	4.2	16.0
OLFR1061					-1.9	11.1	8.5	11.1
OLFR1065					-1.2	15.3	17.3	15.3

Table 2

OLFR1085					**-2.4**	**27.6**	**107.2**	**27.6**
OLFR1102					1	12.7	0.5	12.7
OLFR1109					-4.1	16.4	7.3	16.4
OLFR1112					-5.9	20.7	3.3	20.7
OLFR1129					-7.3	7.3	-1.9	7.3
OLFR113					-5	31.6	37.9	31.6
OLFR1130					-1.1	16	4	16.0
OLFR1133					1.3	9.9	11.3	7.6
OLFR1138					-7.9	11.7	16.8	11.7
OLFR1148					-3.8	7.8	1.4	7.8
OLFR115					-1	11.3	14.6	11.3
OLFR1170					**-2**	**10.6**	**63.8**	**10.6**
OLFR1176					-3	8.9	4.5	8.9
OLFR1178					1.1	9.9	-4.8	9.0
OLFR1181					0.3	7.5	57.7	7.5
OLFR1183					-6	18	3.7	18.0
OLFR1186					-4.3	8	-2.6	8.0
OLFR1198					**3**	**52.2**	**70.4**	**17.4**
OLFR120					-5.7	15.5	96.7	15.5
OLFR1223					0.8	11.4	-10.5	11.4
OLFR1232					1.5	11.5	-1.3	7.7
OLFR1249					3.9	50.6	-1	13.0
OLFR1250					1.8	23.6	56.1	13.1
OLFR1260					-4.2	10.7	9.6	10.7
OLFR1278					-1.2	10.2	6.6	10.2
OLFR1288					-2.2	8.1	10.9	8.1
OLFR1309					-2.1	7.6	0.9	7.6
OLFR1320					-2.9	8.4	0.1	8.4
OLFR1323					-4.5	12.1	0.6	12.1
OLFR1331					1.3	11	31.5	8.5
OLFR1333					0.1	41.1	92	41.1
OLFR1337					-2.8	26.1	-5.2	26.1
OLFR1342					-4.2	10.8	49.8	10.8
OLFR1344					-7.4	9.4	27.9	9.4
OLFR1347					-10.5	8.1	5.3	8.1
OLFR1348					-3.5	10.7	3	10.7
OLFR1349					-0.5	13.2	4.7	13.2
OLFR1361					1.2	9.8	-0.3	8.2
OLFR1384					-1.8	45.5	19.6	45.5
OLFR1385					-6.9	12.3	11.6	12.3
OLFR1390					-0.4	24.7	82.9	24.7
OLFR1406					-3.1	7.1	-5.5	7.1
OLFR1412					3.3	49.4	4.3	15.0
OLFR1424					1.7	44.3	66	26.1
OLFR1436					0.9	16.6	40	16.6
OLFR1437					2.8	41.2	26.6	14.7
OLFR1443					-1	15.7	49.7	15.7
OLFR1444					-2.3	34.1	31.4	34.1
OLFR1453					1.4	13.2	38.9	9.4
OLFR1474					0.3	15.9	-4.6	15.9

Table 2

OLFR1475					0.1	15.9	39.3	15.9
OLFR148					-1.6	7.7	-2.9	7.7
OLFR1489					-1.5	22.2	16	22.2
OLFR1508					1	8.7	11.5	8.7
OLFR1513					-4.3	14.7	11.8	14.7
OLFR159					3.6	91.1	18.3	25.3
OLFR165					-1.9	14.4	-3.6	14.4
OLFR166					-0.5	9.2	2.5	9.2
OLFR167					-4.1	20.6	-1.7	20.6
OLFR168					-3.9	36.2	54.1	36.2
OLFR168					0.3	9	21.7	9.0
OLFR171					-3.2	14.2	5	14.2
OLFR176					-3	22.6	15.2	22.6
OLFR192					-3.6	20.7	60.7	20.7
OLFR257					-1.6	31.9	22	31.9
OLFR26					1	13.2	13.7	13.2
OLFR262					-1.9	8.3	4.8	8.3
OLFR270					**3.3**	**25.7**	**93.6**	**7.8**
OLFR272					**4.5**	**49.8**	**110**	**11.1**
OLFR293					-0.1	21.2	-3.4	21.2
OLFR31					0.2	14.9	24.6	14.9
OLFR312					-2.6	10.4	-6.3	10.4
OLFR313					0.7	15.7	58.4	15.7
OLFR351					-6.3	24	66.5	24.0
OLFR362					-8.3	20.3	30.4	20.3
OLFR376					0.7	8.3	-4.9	8.3
OLFR380					-3.8	9.1	-0.7	9.1
OLFR415					-0.3	20.3	17.7	20.3
OLFR429					-2.4	14.3	-0.4	14.3
OLFR432					-10.6	7.3	14.8	7.3
OLFR434					3	48.6	16.7	16.2
OLFR458					-2.5	21.4	36.6	21.4
OLFR464					-0.7	14.4	73	14.4
OLFR467					-2.2	15.1	16.4	15.1
OLFR469					3.1	36.4	39.3	11.7
OLFR470					-0.8	7.1	2.7	7.1
OLFR478					-3.7	13.7	6.3	13.7
OLFR48					3.9	46.6	-8.7	11.9
OLFR486					0.7	35.5	-3.7	35.5
OLFR490					-3.4	29.3	3.5	29.3
OLFR5					0.7	29.1	42.2	29.1
OLFR504					-5.3	11.2	56.6	11.2
OLFR510					-0.9	9.4	39.5	9.4
OLFR516					3.4	25.8	31.5	7.6
OLFR517					0	24.8	0.8	24.8
OLFR524					0.9	9.7	35.6	9.7
OLFR525					-3.9	16.2	6.2	16.2
OLFR530					-3.3	16.4	-3.8	16.4
OLFR535					-1.5	20.5	9.7	20.5
OLFR536					-0.4	9	0.7	9.0

Table 2

OLFR538					-0.5	10.7	54	10.7
OLFR558					-0.9	8.5	-3.8	8.5
OLFR56					-11.9	48.4	-6	48.4
OLFR566					-3.1	7.1	-2.5	7.1
OLFR57					-4.7	18.4	11.3	18.4
OLFR578					-2.8	8.8	25.8	8.8
OLFR597					0.7	9.2	74.5	9.2
OLFR606					2.8	26.5	-5.2	9.5
OLFR608					-4.6	13.5	18.5	13.5
OLFR616					-7.8	7.4	-3.6	7.4
OLFR618					6.6	54	78	8.2
OLFR619					-2.3	8.2	24	8.2
OLFR62					-5.9	15.3	29.7	15.3
OLFR630					-0.2	13	-1.7	13.0
OLFR638					-3.4	18.8	1.4	18.8
OLFR639					-1.4	12.2	11.7	12.2
OLFR64					-2.7	9.9	22.8	9.9
OLFR653					-1	8.4	15.7	8.4
OLFR654					0.9	21.2	-4.6	21.2
OLFR658					-1	12.1	14.4	12.1
OLFR659					3	42.3	69.1	14.1
OLFR665					2.3	48.3	83.9	21.0
OLFR672					-3.9	12.5	-1.6	12.5
OLFR677					-0.5	15.5	89.1	15.5
OLFR681					3.4	27.3	0.9	8.0
OLFR691					-6.2	22	-2.7	22.0
OLFR692					**1.4**	**18.9**	**64.2**	**13.5**
OLFR698					0.6	13.6	4	13.6
OLFR702					3	50.9	83	17.0
OLFR710					0.2	11.3	5.1	11.3
OLFR716					-0.3	13	-13.1	13.0
OLFR722					-0.1	8.3	23.6	8.3
OLFR725					-2.2	10.9	-0.9	10.9
OLFR731					0.6	8	0.5	8.0
OLFR744					-3.5	7.5	31	7.5
OLFR748					**-1.3**	**49.1**	**111.4**	**49.1**
OLFR76					0.9	8	-2.4	8.0
OLFR761					1.8	14.3	-3.3	7.9
OLFR781					0.5	29.6	1.3	29.6
OLFR786					2.8	67	65.3	23.9
OLFR796					2.8	22.8	62	8.1
OLFR800					-3.3	11.2	28.6	11.2
OLFR812					-3.8	7.9	-3.4	7.9
OLFR816					-7.5	13.2	-3.7	13.2
OLFR821					-5.9	9.1	5.4	9.1
OLFR824					-1.2	22.7	26.7	22.7
OLFR826					-2.6	7.5	11.5	7.5
OLFR828					-0.5	15.7	29.4	15.7
OLFR829					0.7	15.1	26.8	15.1
OLFR851					-0.7	10.5	1.6	10.5

Table 2

OLFR854					-4.2	17.5	-2.9	17.5
OLFR855					0.1	11.8	-2.6	11.8
OLFR868					-2.7	7.1	0.5	7.1
OLFR869					0	8.5	1.7	8.5
OLFR871					0.9	8	1	8.0
OLFR872					-1.5	14	26.5	14.0
OLFR881					-7.3	7.8	10.2	7.8
OLFR906					-5	61.4	-7.2	61.4
OLFR910					4.7	86	69.5	18.3
OLFR912					-4.8	8.9	-3.6	8.9
OLFR917					2.5	25.5	0.1	10.2
OLFR923					-2.6	27.1	13.6	27.1
OLFR951					0	14.9	14.1	14.9
OLFR974					-3.2	9.5	13.1	9.5
OLFR976					-4.4	12.3	3.9	12.3
OLFR980					-6.6	9.9	20.8	9.9
OLFR983					-1.8	20.6	13.3	20.6
OLFR987					-1.2	9.2	-4.2	9.2
OLFR99					0.9	10.9	19.2	10.9
OLFR995					-1.6	9.1	3.5	9.1
vomeronasal 1 receptor								
V1RA2					-5	11.3	76.5	11.3
V1RB9					-5.6	8.6	5.8	8.6
V1RC10					-10.7	9.5	-5.1	9.5
V1RC8					-4.1	9.9	18.4	9.9
V1RD12					0	13.2	34.5	13.2
V1RD2					3.1	38.7	38.3	12.5
V1RD20					-4.3	9.7	4.4	9.7
V1RD3					1.6	29	7.6	18.1
V1RE12					-3.1	33.6	-10.4	33.6
V1RE13					-6.9	19.6	-6.1	19.6
V1RF3					-0.3	9.6	2.4	9.6
V1RG3					-1	11.1	34.7	11.1
V1RH13					-6	9.3	20.1	9.3
V1RH21					-3.3	19.8	51	19.8
V1RI1					-5.8	13.3	-4.7	13.3
V1RJ3					-3.2	8.8	2.2	8.8
V1RL1					-3.9	8.1	35.2	8.1
Proteins associated with the cartilage								
CHST11					12.7	125.3	126.3	9.9
ASPN					0.2	14	1.8	14.0

Table 2

ACAN					0.7	16	7.6	16.0
ADAM1B					1.3	14.6	-2.9	11.2
ADAM22					-2.7	19.2	7.3	19.2
ADAM29					-1.3	14	3.3	14.0
ADAM32					0.4	58.5	162.5	58.5
ADAM7					1	8.5	12.1	8.5
ADAMTS1					2	19.9	46	10.0
ADAMTS17					-3.2	21.7	-2.6	21.7
ADAMTS4					19.5	1742.5	2573.8	89.4
ADAMTS9					-1.2	11.4	30.1	11.4
ADAMTSL1					6	50	38.6	8.3
MMP12					-1.4	46.1	1.2	46.1
MMP13					6.9	284.6	539.4	41.2
MMP20					-2.4	13.4	-2.5	13.4
MMP24					2.3	36.4	112.2	15.8
MMP24					-4.3	7.5	29	7.5
MMP27					-5.9	7.1	2.6	7.1
MMP3					3.7	54.5	3.9	14.7
MMP3					-0.6	9.9	16.1	9.9
TIMP1					326.5	3621.6	3232	11.1
TIMP1					383.6	3723.8	3477.1	9.7
TIMP3					11.1	87.9	142.7	7.9
Protocadherin								
PCDH10					-0.9	89	90.9	89.0
PCDH10					-1.4	9.9	11.2	9.9
PCDHA11					1.4	42.8	14.2	30.6
PCDHA7					-1	10.6	-3.3	10.6
PCDHA7					6.1	50	98	8.2
PCDHB6					2	14.1	29.5	7.1
PCDHGA10					1.8	17.8	55.1	9.9
PCDHGA9					-0.5	11.8	0.7	11.8
PCDHGB6					-4	7	-8.3	7.0
PCDHGB7					-2.9	8.3	62.3	8.3
PCDHGB8					-3.1	16.3	7.8	16.3
Prolactin family								

Table 2

PRL2C2					-3.4	20.1	6.9	20.1
PRL2C3					-3.5	8.2	21.6	8.2
PRL3D3					1	10.7	40.5	10.7
PRL4A1					-5.4	7.6	-2.5	7.6
PRL7A2					-6.8	7.6	-5.3	7.6
Prolactin receptor								
PRLR					22.5	173.6	257.9	7.7
TNF and TNF-related genes								
TNF	1	854.3	94.3	854.3	0.5	21.4	4.2	21.4
TNF	3	1170.4	143.3	390.1	-4.2	9.3	17.7	9.3
TNFAIP2	685	9076.5	1738.9	13.3				
TNFAIP2	951.4	10471.8	2463.2	11.0				
TNFAIP2	1876.9	15829.8	5120	8.4				
TNFAIP3	42.3	2753.3	1202.9	65.1	0.5	21.4	4.2	21.4
TNFSF10					-4.6	40.8	111.2	40.8
TNFRSF10B	20	104.7	28.5	5.2	-4.2	9.3	17.7	9.3
TNFRSF10B	12.1	41.2	21.9	3.4	-4.1	21.7	-5.7	21.7
TNFRSF12A	307.7	1962.6	1503	6.4				
TNFRSF1B	19.7	62.1	36.3	3.2				
TNFSF11	4.5	66.1	35.1	14.7				
TNFSF12-TNF	2.6	20.4	10.3	7.8				
TNFSF13B					-0.9	7.2	1.2	7.2
TNFSF14					-4.3	29.5	74	29.5
TNKS					3	84.9	116.2	28.3
TNFSF9								
TNIP1	9.4	40.4	8.4	4.3				
TNIP1	149.9	601.5	219.4	4.0				
TRAF1	12.6	52.6	22.7	4.2				
TRAF6	37.9	96.8	48.3	2.6				
BAT5					10.4	89.8	31.3	8.6
Nuclear receptor subfamily								
NR4A2	8.1	97.3	97.2	12.0				
NR4A3	1	30.7	25.5	30.7				
NR4A3	1	28.9	23.8	28.9				

Table 2

NR5A1	1	6.5	4.6	6.5				
circadian clock								
CCRN4L					7.7	141.7	141.6	18.4
Golgi								
GCC2					6.2	317	209	51.1
COG8					7.5	165.3	246.6	22.0
GOLGA2					30.5	599.1	1711.7	19.6
antioxidant								
CP					48.1	715.1	581	14.9
CP					22.9	253.6	233.9	11.1
oncogenes								
VAV1					57.1	427.1	634	7.5
other tumor supressor								
H19					39.4	579.8	1096.1	14.7
immunomodulators								
LST1					97.1	721	772.9	7.4
chaperon								
ERAF					16	148.7	190	9.3
Unclassified								
PRF1					1.4	74.9	23.9	53.5
ADAMTS1	42	139.8	202.9	3.3				
ADAMTS1	8.9	21.2	24.1	2.4				
ADAMTS4	78	1034.4	945.4	13.3				
ADM	48.3	267.2	78.7	5.5				
AI987692	1346.8	2785.5	3643.1	2.1				
AIM1L	669.4	1706.5	1315.2	2.5				
AKAP1	1	2.5	4.8	2.2				
AKAP12	1202.9	2614.9	1811.3	2.2				
AKAP2	14.3	53.9	48.5	3.8				
AKAP2	100.2	247.1	193.1	2.5				
AKAP2	399.6	915.1	718.7	2.3				
AKAP4	1	5.6	0.7	5.6				
AKAP8	1	2.3	3	2.3				
AKR1B8	1874.5	5175.9	1788.2	2.8				
AMD2	96.7	197	157.4	2.0				
ANGPTL4	1	4.3	8.8	3.8				
ANKRD1	7.2	35.5	63.2	4.9				
ANKRD1	7.2	35.5	63.2	4.0				

Table 2

ANKRD11	1	6.8	3.4	6.8				
ANKRD2	1	3.5	-3.3	3.5				
ANKRD56	104.9	314.3	104.2	3.0				
ANKRD6	3.3	9.2	-0.3	2.8				
ANKRD6	1	2.5	-0.1	2.5				
ANKRD9	1	10.8	12.7	10.8				
ARG1	1	16.7	28.7	16.7				
ATP10D	374.9	773.6	447	2.1				
AXUD1	66.1	490.3	404.6	7.4				
B230378H13R	5.9	34.2	24.7	5.8				
BACH1	31.4	66.7	174.8	2.1				
BCAR3	5	29.7	10	5.9				
BDH1	1.9	10.6	0.3	5.6				
C330006D17R	2.9	18.2	44.5	6.3				
C330006P03R	207.3	618.3	403.5	3.0				
C730046C01R	16.7	35.2	13.5	2.1				
CCDC155	1	4.4	4	4.4				
CCDC21	17.5	36.2	12.8	2.1				
CCDC49	7.2	14.2	8.7	2.0				
CCDC85B	2.1	130.3	52.5	62.0				
CCDC89	1	4.7	5.1	4.7				
CCDC93	4.2	10.5	2.7	2.5				
CCDC94	84.3	203.1	121.4	2.4				
CCDC99	31.4	71.4	50.2	2.3				
CCT7	4	11.4	14.4	2.9				
CDC5L	1	7.5	17.2	7.5				
CDCA4	90.6	245.5	147.2	2.7				
CDCA5	6.2	18.3	16.2	3.0				
CLN5	1788.2	3680.5	2055.8	2.1				
COQ10B	489.8	1789.3	1122.3	3.7				
COQ10B	584.7	1758.4	1329.9	3.0				
CTPS	322.7	978.8	860.3	3.0				
CYR61	17.4	53.3	61.7	3.1				
DGAT2	1832.3	6757.6	1508	3.7				
DONSON	1.5	11.9	2.4	7.9				
DONSON	19.8	62.6	27.5	3.2				
EDG7	64.1	403.6	103	6.3				
EDG8	6.1	12	13.6	2.0				
ERRFI1	1168.7	4991.4	4278.4	4.3				
F2RL1	208.8	1549.4	350.7	7.4				
F2RL1	125.7	850.8	158	6.8				
FAR1	313.7	779.8	367.1	2.5				
GCH1	110	1580	555.6	14.4				
GM826	4.8	75.6	6.3	15.8				
GNL3	41.6	115.8	92.2	2.8				
GRWD1	211.7	456.4	345.7	2.2				

Table 2

GTLF3A	12.8	40.6	4.2	3.2				
HDC	47.5	137.9	190	2.9				
HP	56.6	508.4	240.5	9.0				
HP	39.2	334.8	190.8	8.5				
HP	128.6	938.3	494.3	7.3				
IDS	7.8	21.8	25.2	2.8				
IHH	1	23.2	-1.4	23.2				
INS1	5.2	11.8	8.2	2.3				
INSIG1	62.2	217.4	120.6	3.5				
INSIG1	83.1	221.6	159.4	2.7				
INSR	1	8.7	3.6	8.7				
IRG1	1	14.1	34.9	14.1				
IRG1	6.1	48.6	102.2	8.0				
IRGQ	1	13.6	4.9	13.6				
IRGQ	10.6	57.5	21.1	5.4				
IRX1	1	4.1	6.9	4.1				
IRX2	8.6	16.3	7.5	7.5				
IRX3	17.9	49.4	70.7	2.8				
IRX4	1	2	-7.1	2.0				
JMJD3	260.2	539	472.4	2.1				
KLHL25	48.3	383.8	131.5	7.9				
LDLR	48.5	136.3	108.9	2.8				
LIPK	7.4	50.6	13.5	6.8				
MARCKSL1	76.6	162.6	131.8	2.1				
MARCO	3.1	11.9	13.7	3.8				
MAT2A	33.7	107.7	132.4	3.2				
MAT2A	135.1	412.6	463.5	3.1				
MAT2A	60	173.3	214.4	2.9				
MAT2A	2963.8	5883.3	4696.7	2.0				
MATN4	2.5	6.8	6.6	2.7				
MCM10	1	9.3	3.7	9.3				
MCM10	5.9	42.9	25.5	7.3				
MCOLN2	12.8	42.3	26.9	3.3				
MFSD2	203.8	1933.3	339.4	9.5				
MID1	14.1	48.5	9.7	3.4				
MOBKL1A	2.9	15.9	3.6	5.5				
MOBKL2A	79	209.2	159.9	2.6				
MOGAT2	22.2	72.5	20.5	3.3				
MOGAT2	6.7	16	-3.8	2.4				
MPPED1	3	21.8	12	7.3				
MRGPRA2	5.5	20.2	0.4	3.7				
MT1	11776.7	23326.9	25806	2.0				
MT2	45.3	178.4	186.2	3.9				
MTMR14	59.2	173.6	111.9	2.9				
MTMR14	50.8	118.4	56.5	2.3				
MVD	423.3	1508.7	468.8	3.6				
MYBBP1A	97.9	191.9	157.2	2.0				
MYD116	256.8	1849	537.3	7.2				
MYOM2	4.2	27.9	15.6	6.6				

Table 2

NFXL1	1.9	7.8	4.8	4.1				
NFYA	14.6	44.3	19.2	3.0				
NGFB	8.6	61.8	37.5	7.2				
NGFB	19.7	86.7	61.4	4.4				
NHLRC3	13.4	30.5	27.3	2.3				
NLE1	1	15.7	18.3	15.7				
NOC3L	136.1	295	175.7	2.2				
NOL1	44.3	88.2	73.8	2.0				
NRG1	39.5	105.4	92.9	2.7				
NRIP3	8.8	30	20.5	3.4				
NSUN5	27.7	58.5	42.9	2.1				
NUAK2	132.7	507.5	460.4	3.8				
NUPR1	1049.6	3339.3	2496	3.2				
OASL1	4.9	25.2	57.1	5.1				
OASL1	4.4	16.9	41.2	3.8				
PCDH10	1	38.4	7.4	38.4				
PDE4A	6.2	20.2	13.1	3.3				
PDE4B	4.6	23.1	13.7	5.0				
PDE4B	1	4.9	2.1	4.9				
PDE4B	30.9	145.3	168.4	4.7				
PDE6G	2.3	4.7	5.7	2.0				
PDGFB	2.2	15.1	4.6	6.9				
PDGFB	120	249.1	168.8	2.1				
PELI1	314.1	658	421.6	2.1				
PELI3	1	11.7	-2.6	11.7				
PHLDA1	1471.3	3547	1808.2	2.4				
PIGM	1.5	12.6	11.5	8.4				
PIGN	3.5	9.5	11.2	2.7				
PIK3C2A	20.8	43.5	40.6	2.1				
PLCXD3	1.1	7.3	11.7	6.6				
PLEK	6.2	28.6	55.8	4.6				
PLEKHG2	24.9	63.3	88.1	2.5				
PLEKHG2	355	760.5	964.9	2.1				
PLEKHG2	598.3	1260.3	1525.7	2.1				
PMAIP1	19.1	60.4	32	3.2				
PMAIP1	165.7	381.4	177.7	2.3				
PMP22	232.3	460.7	379.6	2.0				
POF1B	460.9	953.8	732.6	2.1				
PPAN	22.3	48.7	45.6	2.2				
PRDM2	69.9	202.9	219	2.9				
PRDM2	114.9	251.5	296.5	2.2				
PRR7	34.5	767.7	178.9	22.3				
PRSS22	342.8	1971	767.7	5.7				
PRSS23	1	6.5	8.3	6.5				
PRSS27	27.7	100.2	114.8	3.6				
PTGES	44.1	239.1	96	5.4				
PTGES	194.3	757.7	372.2	3.9				
PTGFRN	25.8	53.6	32.9	2.1				

Table 2

PTPN12	19.7	60.3	30.8	3.1				
PTPN12	22.5	65.9	33.4	2.9				
PTPN12	33.1	74.1	34.1	2.2				
PTPN12	32.6	71.3	26	2.2				
PTPN2	57.9	217.3	86.2	3.8				
RAMP3	1.1	14.1	17.1	12.8				
RCAN1	34.6	371.1	242.1	10.7				
RDH10	17.9	64.4	21.9	3.6				
RCAN1	34.6	371.1	242.1	10.7				
RDH10	17.9	64.4	21.9	3.6				
RETNLG	1	35.3	-4.3	35.3				
RRP1B	4	12.4	6.7	2.5				
RUNX1	53.9	116.7	63.8	2.2				
SBNO2	70.3	319.7	167.2	4.5				
SFN	1052.7	2514.4	1776.1	2.4				
SGK1	1164.7	3136.8	2751.7	2.7				
SH3BP2	196.1	504.2	219.7	2.6				
SHROOM3	90.2	290.3	161.2	3.2				
SKIL	12.2	35.1	16.5	2.9				
SNAI3	48.8	1451.2	204.6	29.7				
SNF1LK	17.1	150.4	61.8	8.8				
SPSB1	660.2	2016.3	2569.9	3.1				
ST3GAL1	23.1	101.3	33.9	4.4				
STK35	17.8	60	25.6	3.4				
SYNGR2	361.8	726.5	650.7	2.0				
TAC1	1	17.8	19.5	17.8				
TAL1	1	13.9	13.9	13.9				
TAX1BP3	8.6	19.8	10.2	2.3				
TBC1D10A	212.5	553.3	285.6	2.6				
TBC1D10A	437.9	948.2	531.3	2.2				
TGIF1	31.2	134.8	25.2	4.3				
TGM1	1.4	17.6	28.1	12.6				
THBS1	20.6	110.9	169.3	5.4				
TIMP1	269	1445.7	1953.2	5.4				
TREX1	2.6	8.7	9.2	3.3				
TRIM21	6.2	18.5	16.6	3.0				
TRIM27	27	79.3	50.7	2.9				
TRPV4	58.1	183.4	73.2	3.2				
TRPV4	78.2	244	109.8	3.1				
TSSK6	5.7	38.2	8.8	6.7				
TTC39B	14.7	40.4	36.9	2.7				
UPP1	18.5	38	43.6	2.1				
VPS37B	915.9	2307.5	1154.6	2.5				
WDR4	37.6	89	68.5	2.4				
WDR43	371.1	803	494.6	2.2				
WDR46	3.9	8.2	-1.8	2.1				
WDR70	8.1	16.3	12	2.0				
WDR82	1	4.4	3.6	4.4				
WDR92	17.8	70.1	27.7	3.9				
WNT10B	7.6	45.7	29.1	6.0				

Table 2

WNT7B	133	353.4	187.2	2.7				
WSB1	540.6	1289.7	909.7	2.4				
YBX3	4821.9	17559.2	16672.8	3.6				
ZFP295	33.9	80.4	47	2.4				
ZFP36	901.1	6682.9	7114.2	7.4				
ZFP57	13.2	68.7	21.8	5.2				
ZFP607	55	114.5	60.6	2.1				
ZSWIM4	192.5	656.7	272	3.4				
ARMC1					7.3	96.4	94.6	13.2
CHD7					15.3	172.1	251.4	11.2
CHKA					208.4	2673	3980.2	12.8
CRISPLD2					334.1	2824.7	1873.8	8.5
EEF1A2					12.3	122.8	12.4	10.0
FABP5					41.1	435.8	309.8	10.6
FBN1					13.9	106.9	47.4	7.7
FFAR2					27.2	208.4	4.9	7.7
FGL1					-2.4	74.7	260.5	74.7
FGL1					5.6	291.4	550.8	52.0
FIBP					5.5	76.2	203.5	13.9
GFPT1					14.6	105.7	33.8	7.2
GFPT2					97	1762.3	2584.6	18.2
GIMAP5					8.7	62.1	109.7	7.1
GMIP					69.8	489.2	536.8	7.0
HRC					19.8	148.3	238.2	7.5
ITIH4					9.2	86.9	89.5	9.4
MT2					53.7	1757.4	1431.7	32.7
MTRF1					7.6	72.6	-5.3	9.6
PCNP					233.2	2364.7	3003.8	10.1
PHF11					14.4	139.4	576.2	9.7
PHF11					12.9	98.8	431.3	7.7
PIGR					10.4	153.5	102.4	14.8

Table 2

RGS16					32	858.6	429.7	26.8
RHBDL2					95.2	1060.7	1284.9	11.1
SCIN					59.7	1853	145.9	31.0
SLA					57.8	430.4	435.4	7.4
SLAMF8					38.1	326.6	404.5	8.6
SNTB2					18.6	134.7	243.7	7.2
SP5					15.1	184.8	272	12.2
SPINK8					14	123.3	161	8.8
SRR					253.8	3474.8	5827.9	13.7
SRR					150.9	1930.8	2623.3	12.8
TCOF1					78.8	675.2	1224.3	8.6
TIMELESS					40.9	411.4	684.6	10.1

Table 2

TIMM13					16	117.2	143.3	7.3
TSLP					4.1	64.1	118.5	15.6
TYKI					78.6	2634.1	3096.2	33.5
ATG4D					6.6	67.1	92.5	10.2
ATP5O					0.5	139.8	169.2	139.8
BACE2					28.8	262.8	229.6	9.1
BRIP1					9.5	109.1	101.8	11.5
CALCA					-0.2	197.2	195.9	197.2
CH25H					28.9	463.5	789.5	16.0
CRYBG3					12.6	94.6	159.7	7.5
DDAH1					11	117	45.1	10.6
EPSTI1					7.7	111.5	39.6	14.5
GNG13					15	110.1	281.5	7.3
LOC100047934					384.8	2880.7	2006.3	7.5
LOC100047963					69.1	989.1	857.8	14.3
LOC100048556					26.2	705.6	1454.3	26.9
LOC638301					100	1318.6	1562.7	13.2
NEK6					10.7	94.1	37.8	8.8
OSMR					-0.8	62.9	-1.7	62.9
PCNP					233.2	2364.7	3003.8	10.1
SCL0002368.1_75					304.6	2626.6	3372.7	8.6
STFA1					9.9	93.1	55	9.4
STFA2					4.9	114.2	115.8	23.3
APOLD1	1	23.1	147.7	23.1				
BC037703	1	36.7	-2.7	36.7				
CH25H	21	567	574.2	27.0				

Table 2

NOS2	8.8	101.4	11.2	11.5	0.1	108.8	-7.6	108.8
ZC3H12A	3.8	590.8	84.5	155.5	123.5	2475.7	671.7	20.0
PLAT	#REF!	5226.9	823.1	10.2				
PLAUR	83.4	679.9	377.2	8.2				
PSORS1C2	1	74.1	-8.7	74.1	-6.7	130.3	0	130.3
F10	2.2	7.2	63.5	**3.3**				
DLL1	56.1	324.4	149	5.8				
DLL4	1	6.6	8.2	6.6				
DLL4	1	3.9	1.1	3.9				
Unknown function								
A630077B13R	1.1	58	39.4	52.7				
A230065H16R	1	46.6	-6.3	46.6				
1190003J15RI	1581.5	4188	2241.8	2.6				
1500041J02RI	19	46.2	32.2	2.4				
2310014H01RI	2716.3	5339.4	3951.4	2.0				
2310016C08RI	319.3	2877.1	665.7	9.0				
2310016C08RI	319.3	2877.1	665.7	6.1				
2210008F06RI	1	26.1	1	26.1				
AI607873	73.4	188.4	182.8	2.6				
AI987692	1346.8	2785.5	3643.1	2.1				
AIM1L	27.2	76.5	33.5	2.3				
2310014H01RIK								
2310016C08RIK								
2310016C08RIK								
2310026J01RIK								
2310061F22RIK								
2410025L10RIK								
2810402K13RIK								
3830432E14RIK								
5033413D16RIK								
5530400B01RIK								
5830457O10RIK								
9930023K05RIK								
9930122J16RIK								
A130051J06RIK								
A130082M07RIK								
A230065H16RIK								
LOC10004623	266.4	882.3	962.3	3.3				

Table 2

LOC10004726(	129.2	851	168.6	6.6				
LOC10004733	42.2	87.6	88.8	2.1				
LOC10004777(	14.4	75.2	10.6	5.2				
LOC10004793	613	2978.1	1464.3	4.9				
LOC10004855	13.8	61.5	223.4	4.5				
LOC10004855	27	91.5	373.9	3.4				
LOC212399	254.5	965.7	432.3	3.8				
LOC240672	86.8	713.8	227.5	8.2				
LOC381140	360.4	724.8	865.7	2.0				
LOC638301	97.3	325.3	417.1	3.3				
D17H6S56E-3	4.3	15.9	10.5	3.7				
D17H6S56E-5	30.4	580	90.8	19.1				
D330008I21RI	16	167.9	28.4	10.5				
D7BWG0611E	1.3	27.1	8.8	20.8				
D930038O18R	2.4	17.2	7	7.2				
D930039D09R	1	11.1	3.7	11.1				

CLAIMS

1. A method of treating cancer in a mammal comprising administering to a mammal in need thereof a Toll-Like Receptor (TLR) agonist, wherein the cancer is present in a tissue that expresses TLR.

2. A method of reducing cancer recurrence in a mammal comprising administering to a mammal in need thereof a TLR agonist, wherein the cancer is present in a tissue that expresses TLR.

3. The method of claim 2, wherein the cancer recurrence is selected from the group consisting of a metastasis and tumor regrowth.

4. The method of any one of claims 1-3, wherein the TLR agonist is flagellin.

5. The method of any one of the preceding claims, wherein the cancer does not express TLR.

6. The method of any one of the preceding claims, wherein the TLR is TLR5.

7. The method of any one of the preceding claims, wherein the tissue is selected from the group consisting of liver, lung, bladder, and intestinal.

8. The method of any one of the preceding claims, wherein the cancer is metastatic.

9. The method of claim 8, wherein the cancer is selected from the group consisting of melanoma, colon, breast, prostate, and a hematological malignancy.

10. The method of any one of the preceding claims, wherein the cancer is a tumor.

11. The method of claim 9, wherein the hematological malignancy is lymphoma.

12. The method of any one of the preceding claims, wherein the agonist is administered as a monotherapy.

13. The method of any one of the preceding claims, wherein the mammal is not receiving a combination cancer therapy.

14. The method of claim 13, wherein the mammal is not receiving chemotherapy or radiation therapy.

15. The method of any one of the preceding claims, wherein the mammal has sufficient innate immunity.

16. The method of claim 15, wherein the sufficient immunity level is equivalent to the level required for eligibility for a first or subsequent round of chemotherapy.

17. The method of claim 15, wherein the mammal has a white blood cell count that is within the range of clinically normal.

18. The method of any one of the preceding claims, wherein the TLR agonist is administered to the mammal before, after, or concurrent with removal of a tumor.

19. A method of treating cancer in a mammal, comprising administering to a mammal in need thereof a FAS agonist and a TLR agonist.

20. The method of claim 19, wherein the TLR agonist is flagellin.

21. The method of claim 19, wherein the FAS agonist is a FAS agonist antibody.

22. The method of claim 19, wherein the cancer is metastatic.

23. The method of claim 22, wherein the cancer is a tumor.

24. The method of claim 22, wherein the metastatic cancer does not express a TLR, and wherein the cancer has metastasized to an invaded tissue that expresses TLR.

25. The method of claim 24, wherein the invaded tissue is selected from the group consisting of liver, bladder, lung, and intestinal.

26. A method of protecting liver tissue in a mammal from the effects of a liver toxicity comprising administering to a mammal in need thereof a Toll-Like Receptor agonist.

27. The method of claim 26, wherein the toxicity is selected from the group consisting of a FAS ligand, a FAS agonistic antibody, TNFα, acetaminophen, alcohol, a viral infection of the liver, and a chemotherapeutic agent.

28. A method of treating a Salmonella infection in a mammal, comprising administering to a mammal in need thereof a TLR agonist.

29. The method of claim 28, wherein the TLR agonist is flagellin.

30. The method of claim 28, wherein the Salmonella is *Salmonella typhimurium*.

31. The method of claim 28, wherein the infection is present in liver tissue.