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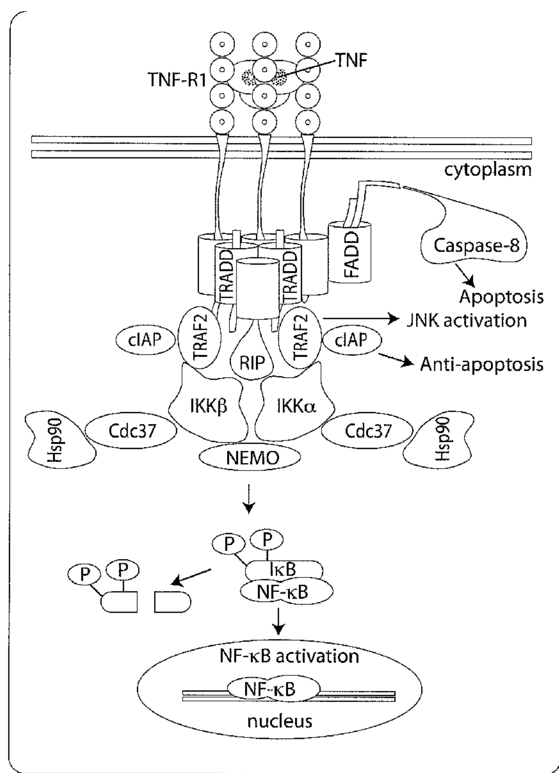

Fig. 1

(57) Abstract: The application discloses SPINT1 and TM-PRS S4 polypeptides having TNFα modulating activity and compositions and uses thereof.

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MODULATORS OF TNFα-DEPENDENT SIGNALING PATHWAYS AND USES THEREOF

FIELD OF THE DISCLOSURE

[0001] Embodiments of the present disclosure are directed to inhibitors of TNFα and methods of use thereof.

BACKGROUND OF THE DISCLOSURE

[0002] **Biological functions of TNFα**. TNFα is a pleiotropic pro-inflammatory cytokine. In concert to other members of the cytokine family, TNFα plays a critical role in the host immune system which provides defense from variety of environmental pathogens, including viruses and bacteria. As pro-inflammatory cytokine, TNFα is primarily involved in the acute phase of immune responses. TNFα can be produced in response to injury or infection by a broad variety of cell types, but mainly by macrophages. Newly synthesized TNFα molecules form stable homotrimers which may exist in both membrane-integrated and soluble forms (Pennica *et al.*, 1984; Tang *et al.*, 1996; reviewed in Locksley *et al.*, 2001). The membrane bound form of TNFα exerts its signaling function via direct cell-cell contacts. Soluble TNFα is released from the membrane by proteolytic cleavage that is mediated by the TNFα converting enzyme (Black *et al.*, 1997). Once released into a bloodstream, along with other cytokines, TNFα may cause numerous physiological responses. Most significantly, it promotes chemotaxis of neutrophiles attracting them to the sites of primary infection and stimulating phagocytosis. Additionally, released TNFα initiates various systemic effects by influencing function of hypothalamus. Such effects include fewer and loss of appetite and are largely mediated by the ability of TNFα to stimulate secretion of corticotropin releasing hormone and production inflammatory prostaglandin PGE2.

[0003] Under normal, pathogen-free conditions, TNFα participates in a variety of additional physiological processes, most importantly in a day-to-day tissue remodeling and wound healing. These functions of TNFα are mediated by its ability to induce apoptosis in certain cell types, to promote proliferation in others, and to inhibit cell differentiation. These processes, however, require proper and timely regulation of TNFα expression. Loss of proper

regulation of TNFα may lead to development of serious pathological conditions including septic shock, chronic inflammation, autoimmunity, osteoporosis, cancer and diabetes.

[0004] **TNFα-mediated cell signaling**. (reviewed in Chen and Goeddel, 2002). On the cellular level, TNFα signaling is mediated by two distinct receptors, TNFαR1 and TNFαR2. TNFαR1 is constitutively expressed in most cell types, and can be fully activated by both the membrane-bound and soluble forms of TNFα. In contrast, TNFαR2 is only found in cells of the immune system and responds to the membrane-bound form of the TNFα. It is commonly thought that the binding of TNFα to TNFαR1 initiates most biological effects of TNFα, while the role of TNFαR2 in TNFα signaling remains largely under-investigated. Upon contact with their ligand, TNFα receptors undergo a conformational change triggering dissociation of the inhibitory protein silencer, SODD, from the intracellular domain of the receptor and recruitment of the adaptor protein TRADD. TRADD recruits additional adapter proteins to the receptor complex, TRAF2, RIP and FADD, which serve as signaling branching points for activation of three major pathways: NF-κB pathway, MAPK pathway and apoptotic pathway.

[0005] **Activation of transcription factor NF-κB by TNFα.** The NF-κB family of transcription factors comprises five members, NF-κB1, NF-κB2, c-Rel, RelA and RelB (reviewed in Luo *et al.*, 2005). These proteins demonstrate constrained DNA binding specificities provided by Rel homology domain which their share, and can form homo- and heterodimers, with the most frequent being the RelA/NF-κB1 (p65/p50) heterodimer. In resting (unstimulated) cells, NF-κB proteins complex with inhibitory factors, IkBα, IkBβ and IkBε, and reside in the cytoplasm in an inactive form. NF-κB activation requires several consecutive steps including phosphorylation, ubiquitination and degradation of the inhibitory IkB proteins. The protein kinase activity which initiates this chain of events is possessed by a multi-protein complex termed IkB kinase (IKK). IKK comprises two catalytic subunits, IKKα and IKKβ, and one adapter subunit, IKKγ. Once recruited to TNFαR1, TRAF2 and RIP proteins form transient complex with IKK which also include additional factors, such as Hsp90 and Cdc37 (Chen *et al.*, 2002). As a result, the multi-component protein kinase IKK becomes activated, enabling initial phosphorylation and then dissociation and degradation of the IκBs. This allows NF-κB to translocate to the nucleus and activate transcription of target genes. While translocation to the nucleus is essential step in NF-κB activation, several reports

have suggested that some additional, posttranslational modifications of the NF-κB are required to achieve its maximum transcriptional activity. To this end, at least three distinct serine residues within p65/RelA protein may become phosphorylated in response to TNF$\tilde{\alpha}$. Ser-276, located in the Rel homology domain, can be phosphorylated by the catalytic subunit of protein kinase A (PKAc) as well as by mitogen- and stress-activated protein kinase-1 (MSK1). In addition, Ser-529 or Ser-536, located in the transactivation domain, can be phosphorylated by casein kinase II (CK II) or by the IKKβ in response to TNFα. These inducible modifications provide additional level of regulation of NF-κB pathway by TNFα. Among transcriptional targets of NF-κB is a vast array of genes involved in cell survival, proliferation, pro- and anti- inflammatory response, and anti-apoptotic factors. Interestingly, some NF-κB-dependent genes encode inhibitors of the TNFα/NF-κB pathway, including IkBα and A20. Thus, by inducing these genes' expression, NF-κB creates negative feedback circuitry which effectively limits extend and duration of its own activation.

[0006] Activation of the MAPK pathway by TNFα. Among the three major MAPK cascades, TNFα strongly induces the stress-related JNK pathway which leads to activation of transcription factor c-Jun (Brenner *et al.*, 1989). Upon recruitment to TNFαR1, TRAF2 activates the JNK-inducing upstream kinases, such as extracellular signal regulated kinase kinase MEKK1 and apoptosis stimulated kinase ASK1. This activates a cascade of additional kinases that ultimately leads to activation of c-Jun-NH_2 terminal kinase, JNK. Activated JNK translocates to the nucleus and phosphorylates c-Jun as well some additional transcription factors whereby increasing their trans-activation potential. The JNK pathway is involved in cell differentiation, proliferation, and is generally pro-apoptotic.

[0007] Induction of cell death signaling by TNFα. In addition to NF-κB and MAPK, TNFαR1 is involved in death signaling. TNFα-induced pathway of cell death is mediated by death domains (DDs) of TNFαR1 and TRADD. It is thought that after its initial assembly, TRAF2-TRADD-RIP1 complex dissociates from TNFαR1, allowing Fas-associated death domain (FADD) adaptor to interact with either DDs of TNFαR1 or TRADD (Micheau and Tschopp, 2003). This interaction results in recruitment and self-activation of cysteine protease procaspase-8/10, and subsequent cleavage of the downstream effector caspases, triggering cell apoptosis. The apoptotic signaling transduced by TNFα is, however, often

counteracted by the transcriptional activity of NF-κB, which induces expression of anti-apoptotic proteins, such as inhibitor of caspese-8 $FLIP_L$ (Micheau and Tschopp, 2003).

[0008] TNFα/NF-κB pathway as a pharmaceutical target. Because the TNFα/NF-κB pathway is implicated in so many pathological conditions, the ability to specifically modulate its activity has been a long standing goal of pharmacology. To this end two major classes of therapeutic agents are being developed: TNFα blockers and NF-κB inhibitors.

[0009] NF-κB inhibitors. Since many adverse effects of deregulated TNFα signaling are mediated thru transcription factor NF-κB, several intracellular components of the TNFα/NF-κB pathway, including IKKs and NF-κB itself have become attractive potential targets for pharmacological intervention. It has been found that many traditionally used medications such as sulfasalazine, aspirin and other NSAIDs, glucocorticoids as well as some naturally-derived products, such as trans-resveratrol, curcumin and 1-acetoxychavicol acetate, exhibit inhibitory effects on NF-κB activity (reviewed in Luo *et al.*, 2005). These inhibitors however have never been systematically selected as NF-κB inhibitors, and therefore are not specific to TNFα/NF-κB signaling. They generally would reduce, to some extent, NF-κB activation in response to a variety of stimuli including IL-1β, bacterial inducers of toll-like receptors (TLRs), viruses and growth factors, while also affecting many other cellular functions. Frequently, their inhibitory effects are seen only at very high concentrations that may approach or even exceed cytotoxic levels.

[0010] Several new low molecular weight pharmacological inhibitors of NF-κB pathway have been recently identified thru systematic screens of compound libraries (reviewed in Roshak *et al.*, 2002). For example, PS1145, a specific inhibitor of IKKβ, has been shown to induce apoptosis in multiple myeloma cells with constitutively active NF-κB. AS602868, which also has been selected for its ability to block IKKβ, strongly potentiated TNFα-induced apoptosis. Another inhibitor, BMS-345541 efficiently blocked TNFα-induced NF-κB transcription *in vitro* and *in vivo* by inhibiting both IKKα and IKKβ subunits of IKK. Two additional compounds, BAY 11-7082 and BAY 11-7085 block NF-κB signaling by inhibiting TNFα-induced phosphorylation and degradation of IkBα.

[0011] Meanwhile, by using hypothesis driven approaches and based on known protein components of the NF-κB signaling pathway, several research groups have designed small peptide molecules capable of inhibiting NF-κB function thru dominant-negative interactions. Thus, SN50 peptide comprising the nuclear localization signal of NF-κB1/p50 inhibits NF-κB activity by interfering with its translocation through the nuclear pore (Lin *et al.*, 1995). Another peptide, NBD, comprises NEMO-biding domain that is essential for interaction of IKKγ with IKKα and -β. The NBD peptide interferes with proper assembly of IKK complex thereby inhibiting NF-κB activation pathway (May *et al.*, 2000). Finally, two additional peptides, P1 and P6, comprising amino acids surrounding Ser-276 and Ser-529 and -536 phosphorylation sites of RelA/p65, have been also shown to inhibit TNFα-induced NF-κB activation *in vivo* by blocking TNFα-induced phosphorylation and nuclear translocation of RelA/p65 while not affecting upstream components of the pathway, such as IkBα degradation and TNFα-induced activation of c-Jun (Takada *et al.*, 2004).

[0012] **TNFα blockers** comprise either monoclonal anti-TNFα antibodies (found in drugs marketed as Infliximab and Adalimumab) that bind specifically to human TNFα and neutralize its function, or fusion proteins (such as Etanercept) that act as a soluble decoy receptor for TNFα and inhibit the binding of TNFα to its endogenous receptor (reviewed in Chang and Girgis, 2007). Approved clinical indications for the use of TNFα blockers include various severe forms of rheumatologic indications (such as active rheumatoid arthritis, ankylosing spondylitis, polyarticular juvenile idiopathic arthritis and psoriatic arthritis), and gastrointestinal indications (Crohn's disease), which are refractory to conventional therapies.

[0013] Loss of proper regulation of tumor necrosis factor alpha (TNFα)-dependent signaling has been associated with a vast array of serious pathological conditions including autoimmune diseases, diabetes, osteoporosis and cancer. Thus, identification of modulators of TNFα function has been a long standing goal of academic research and drug discovery. TNFα exerts most of its functions through activation of transcription factor NF-κB. Thus many efforts are focused on development of pharmacological agents targeting proteins involved into TNFα/NF-κB signaling pathway.

[0014] Throughout this description, including the foregoing description of related art, any and all publicly available documents described herein, including any and all U. S. patents, are

specifically incorporated by reference herein in their entirety. The foregoing description of related art is not intended in any way as an admission that any of the documents described therein, including pending United States patent applications, are prior art to embodiments of the present disclosure. Moreover, the description herein of any disadvantages associated with the described products, methods, and/or apparatus, is not intended to limit the disclosed embodiments. Indeed, embodiments of the present disclosure may include certain features of the described products, methods, and/or apparatus without suffering from their described disadvantages.

SUMMARY OF THE DISCLOSURE

[0015] The present invention provides for inhibitors of a TNFα pathway identified by a genetic screening technology. Considering a critical role of TNFα/NF-κB pathway in inflammation, as well as acute and chronic diseases related to abnormal regulation of inflammatory responses, this discovery will be useful for creating new anti-inflammatory reagents, drugs and(or) therapies. This inhibitors will be useful for the modulation of other TNFα and NF-κB regulated processes under both normal and pathological conditions.

[0016] Inhibitors of TNFα/NF-κB pathway disclosed in this application represent plasma membrane bound proteins that likely target the TNFα receptor complex. In this respect, SPINT1 and (or) TMPRSS4-specific drug candidates may represent valuable alternatives for currently used TNFα blockers. Furthermore, simultaneous targeting of TNFα (with described anti-TNFα drugs) and TNFα receptor (with SPINT1 and/or TMPRSS4 –derived recombinant proteins) may provide a complementary strategy for efficient inhibition of TNFα signaling.

[0017] The compounds of the present invention are useful *in vitro* as unique tools for understanding the biological role of molecules involved in the TNFα/NF-κB pathway. The antagonists of the present invention are also useful in the development of other compounds that bind and/or inhibit TNFα because the peptide antagonists of the present invention provide important information on the relationship between structure and activity that will facilitate such development.

[0018] The antagonists of the present invention can also be used in assays as probes for determining the expression of molecules involved in the TNFα/NF-κB pathway. Such assays

may be useful, for example, for determining the degree of cellular inflammatory response to tissue infection or injury. Typically, the cells under study are exposed to the peptides or peptidomimetics of the present invention and reacted cells are visualized (*e.g.*, after wash, cell sorting, affinity chromatography, immunohistochemistry, autoradiography *etc.*).

[0019] The compounds of the present invention can be administered to a subject to completely or partially inhibit the effects of TNFα *in vivo*. Thus the methods of the present invention are useful in the therapeutic treatment of TNFα related disorders. For example, the compositions of the present invention can be administered in a therapeutically effective amount to treat symptoms related to inappropriate production of TNFα or inappropriate response to TNFα.

[0020] According to some embodiments, there is provided a pharmaceutical composition comprising a therapeutically effective amount of a SPINT1 polypeptide or derivative, variants, or fragments thereof, and a pharmaceutically acceptable excipient.

[0021] According to some embodiments, there is provided a pharmaceutical composition comprising a therapeutically effective amount of a TMPRSS4 polypeptide or derivative, variants, or fragments thereof, and a pharmaceutical acceptable excipient.

[0022] According to some embodiments, there is provided a polypeptide having 95% sequence identity to the human spint1 deletion variants and having TNFα inhibitory activity.

[0023] According to some embodiments, there is provided a polypeptide having 95% sequence identity to the mouse spint1 deletion variants and having TNFα inhibitory activity.

[0024] According to some embodiments, there is provided a polypeptide having 98% sequence identity to the human spint1 deletion variant and having TNFα inhibitory activity.

[0025] According to some embodiments, there is provided a polypeptide having 98% sequence identity to the mouse spint1 deletion variant and having TNFα inhibitory activity.

[0026] According to some embodiments, there is provided a method for treating a TNFα associated disease comprising administrating a subject in need thereof with the compositions or compounds described herein.

[0027] According to some embodiments, there is provided a method for screening for compounds that have TNFα modulating activity comprising contacting a cell expressing the SPINT1 and TMPRSS4 polypeptides of the present invention with a test agent and detecting whether TNFα activity is affected.

[0028] According to some embodiments, there is provided a method for screening for compounds that have SPINT1 and TMPRSS4 polypeptide binding activity comprising contacting a SPINT1 and TMPRSS4 polypeptide of the present invention with a test agent and detecting whether the SPINT1 and TMPRSS4 polypeptide binds to the test agent.

Brief Description of the Drawings

[0029] For a better understanding of the present invention, reference is made to the following description, taken in conjunction with the accompanying drawings, in which like reference characters refer to like parts throughout.

[0030] Figure 1. Illustration of TNFα-mediated cellular signaling.

[0031] Figure 2A. DNA sequence and open reading frame (ORF) of cDNA inserts coding for a SPINT1 deletion variant isolated by pLCX-MGC-3'del library screening. SalI and PacI are vector-derived flanking restriction sites. See SEQ ID NO: 1 and SEQ ID NO: 2.

[0032] Figure 2B. DNA sequence and open reading frame (ORF) of cDNA inserts coding for a TMPRSS4 isolated by pLCX-MGC-3'del library screening. EcoRI and PacI are vector-derived flanking restriction sites. See SEQ ID NO: 3 and SEQ ID NO: 4.

[0033] Figure 3. TMPRSS4 and SPINT1 cDNA variants identified by pLCX-MGC-3'del library screening inhibit TNFα induced NF-κB activation. Activity of NF-κB was assayed in HEK293 cells co-transfected with NF-κB-dependent luciferase reporter gene construct 4xkB-Luc and pSV40-SEAP reporter plasmids along with: - pLCX-MGC-3'del (a plasmid pool containing original/non-selected 3' deletion cDNA library), - pLCX-Ctrl (vector control

plasmid), - pLCX-IkBaSR (pLCX-based IkBa Super Repressor construct), - pLCX-IkBa-del (pLCX-based 3' cDNA deletion coding for a C-termial truncation of IkBa that produces a strong dominant negative phenotype), - pLCX-IkBa(wt) (pLCX-based wild type IkBa expression construct), - pLCX-TMPRSS4 (pLCX-based 3' cDNA deletion coding for full-length TMPRSS4 protein) or - pLCX-SPINT1-del (pLCX-based 3' cDNA deletion coding for a C-termial truncation of SPINT1 that produces a strong dominant negative phenotype).

[0034] Transfected cells were stimulated with 3 ng/ml of TNFα for 0 (no stimulation control), 4 or 16 hrs. Activity of luciferase reporter in control and TNFα-stimulated samples was normalized to SEAP activity measured at 0 hrs. Data were expressed as average of three experiments +/- standard deviation.

[0035] Figure 4. Stable expression of SPINT1-del and TMPRSS4 inhibits TNFα induction of NF-κB. Pools of HEK293kB-EGFP reporter cells stably overexpressing identified cDNA deletion variant of SPINT1 (SPINT1-del), a frame-shift mutant version of SPINT1-del ((fs)-SPINT1-del) and full length TMPRSS4 were treated with TNFα (3ng/ml) for 16 hrs. in parallel with a vector control cell poll (Ctrl). NF-κB activity was monitored through FACS analysis of EGFP expression. NOTE: Basal EGFP profiles for analyzed cDNA clones were similar to the basal profile of control cells and, for clarity, they have been omitted.

[0036] Figure 5. Stable expression of SPINT1 and TMPRSS4 cDNA clones inhibits TNFα dependent NF-κB activity in the presence of high TNFα concentrations. Pools of HEK293kB-EGFP reporter cells stably overexpressing cDNA deletion variants of IkBa (IkBa-del), IKKg (IKKg-del), SPINT1 (SPINT1-del) and full length TMPRSS4 were treated with increasing TNFα concentrations (0, 0.3, 3, and 30 ng/ml) for 16 hrs. in parallel with a vector control cell poll (Ctrl). NF-κB activity was monitored through FACS analysis of EGFP expression. Figure 5 shows representative data for the highest TNFα concentration tested.

[0037] Figure 6. Stable expression of SPINT1 and TMPRSS4 clones inhibits TNFα/NF-κB pathway but does not inhibit IL-1b or flagellin induced NF-κB activity. Pools of HEK293kB-EGFP reporter cells stably overexpressing cDNA deletion variants of IkBa (IkBa-del), IKKg (IKKg-del), SPINT1 (SPINT1-del) and full length TMPRSS4 were treated

with TNFα (3 ng/ml) (B), IL-1b (10 ng/ml) (C) or flagellin (100 ng/ml) (D) for 16 hrs. in parallel with vector control cell poll (Ctrl). NF-κB activity was monitored through FACS analysis of EGFP expression. Basal EGFP profiles for tested cell pools are shown in (A).

[0038] Figure 7. Serine proteinase inhibitors: Pefabloc and aprotinin reverse inhibition of TNFα/NF-κB pathway in cells expressing TMPRSS4 but not SPINT1. Pools of HEK293kB-EGFP reporter cells stably overexpressing cDNA deletion variants of SPINT1 (SPINT1-del) and full length TMPRSS4 were treated with TNFα (3 ng/ml) for 16 hrs. in parallel with vector control cell poll (Vect.Ctrl.). NF-κB activity was monitored through FACS analysis of EGFP expression. Basal EGFP profiles for tested cell pools are shown in (A, C, E) for Vect.Ctrl., TMPRSS4 and SPINT1-del, respectively. EGFP profiles of TNFα treated reporter cell pools are shown in (B, D, F) for Vect.Ctrl., TMPRSS4 and SPINT1-del, respectively.

[0039] Figure 8. Engineered human spint1 deletion variant that lacks spint1 intracellular domain (h-spint1-dID) inhibits TNFα induced NF-κB activation. Activity of NF-κB was assayed in HEK293 cells co-transfected with NF-κB-dependent luciferase reporter gene construct 4xkB-Luc and pSV40-SEAP reporter plasmids along with constructs expressing: h-spint1_FL: wild type human spint1, h-spint1_dID: human spint1 without intracellular domain; a.a. 1-468 of h-spint1 ORF, h-spint1_dTM: human spint1 without intracellular domain and transmembrane domain; a.a. 1-443 of h-spint1 ORF, m-spint1-d1604: mouse spint1 deletion variant identified in the screening without intracellular domain; includes a.a. 1-468 of mouse spint1 ORF + C-teminal vector derived sequence: PLIN.

[0040] Transfected cells were stimulated with 3 ng/ml of TNFα for 24 hrs. Activity of luciferase reporter in control and TNFα-stimulated samples was normalized to SEAP activity measured at 0 hrs. Data were expressed as average of three experiments +/- standard deviation.

[0041] Figure 9. Mouse spint1 domains (SEQ ID NO: 5).

[0042] Figure 10. Human spint1 domains (SEQ ID NO: 6).

[0043] Figure 11A-B. Amino acid sequence of a mouse (SEQ ID NO: 7) and human (SEQ ID NO: 8) spint1 deletion variant exhibiting anti-TNFα activity.

[0044] Figure 12. Alignment of mouse (SEQ ID NO: 7) and human (SEQ ID NO: 8) spint1 deletion variants exhibiting anti-TNFα activity (including predicted signal peptide sequences .

[0045] Figure 13. Alignment of mouse (SEQ ID NO: 9) and human (SEQ ID NO: 10) spint1 deletion variants exhibiting anti-TNFα activity (excluding predicted signal peptide sequences).

[0046] Figures 14A and 14B. TMPRSS4 dependent shedding of TNFα receptor I (TNFRI). (a) Control PE413 cells and the cells ectopically expressing TMPRSS4 were cultured in serum-free DMEM media in the presence or absence of TNFα (3ng/ml) for 24 hours. Cell lysates and conditioned media collected from the respective cell samples were analyzed by Western blot with antibodies specific to the extracellular part of hTNFRI. (b) Control PE413 cells and the cells ectopically expressing TMPRSS4 were cultured for 24 hours in serum-free DMEM media with the indicated concentrations of serine protease inhibitors. Conditioned media collected from the respective cells samples were analyzed by Western blot with antibodies specific to the extracellular part of hTNFRI.

[0047] Figure 15. Alignment of mouse (SEQ ID NO: 5) spint1 ORF (top sequence) and human (SEQ ID NO: 6) spint1 ORF (bottom sequence).

[0048] Figures 16 A - F. Functional validation of SPINT1-d1604 and TMPRSS4 as inhibitors of TNFα/NF-κB pathway **(A)** Inhibitory effects of wild type (wt) SPINT1, SPINT1-d1604 and a frame-shifted (fs) variant of SPINT1-d1604 on TNFα/NF-κB pathway were compared in a transient transfection assay performed as described above. **(B)** NF-κB/GFP reporter cells stably transduced with a vector control construct, SPINT1-d1604 - or TMPRSS4 expression construct were used for RT Q-PCR analysis of endogenous NF-κB regulated targets (TNFα and IκBα); quantitation of TGFβ1 mRNA was used as a negative control. Total RNA was isolated for each experimental group (prepared in quadruplicates) at 0, 2 and 6 hrs. after treatment with TNFα (10 ng/ml). RT-Q-PCR measurements of TGFα1, IκBα and TNFα mRNA levels were normalized to that of GAPDH mRNA and expressed as mean values (+/- standard deviation). **(C)** Status of IκBα protein phosphorylation and degradation in was assessed in NF-κB/GFP cells stably transduced with a vector control

construct, SPINT1-d1604 - or TMPRSS4 expression construct. A Western blot from a representative experiment was probed with anti-phospho- IκBα (P- IκBα) anti-IκBα and anti-β-tubulin antibodies (the latest used as protein loading control). Expression of NF-κB dependent GFP reporter in response to TNFα [3 ng/ml] and IL-1β [100 u/ml] was analyzed in vector control NF-κB/GFP cells **(D),** SPINT1-d1604 **(E)** - and TMPRSS4-expressing cells **(F).** The cells were stimulated with cytokines for 18 hrs and the NF-kB dependent induction GFP reporter was analyzed by FACS.

DETAILED DESCRIPTION OF THE INVENTION

[0049] For the purposes of promoting an understanding of the embodiments described herein, reference will be made to preferred embodiments and specific language will be used to describe the same. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention.

[0050] This invention pertains to novel methods for modulating TNFα function. The invention also pertains to new means of modulating TNFα/NF-κB, IL1-R/NF-κB and to TLR5/NF-κB signaling pathways. The invention is based on a discovery that ectopic expression of variants of mouse SPINT1 cDNA results in inhibition of TNFα-inducible activation of transcription factor NF-κB. The invention is also based on a discovery that ectopic expression of full length cDNA encoding human TMPRSS4 protein results in inhibition of TNFα-inducible activation of transcription factor NF-κB. As SPINT1 and TMPRSS4 proteins are evolutionary conserved and share significant degree of similarity across mammalian species, the methods of current invention equally apply to any of mammalian SPINT1 and TMPRSS4 protein homologs and orthologs.

[0051] In one embodiment the invention provides new method of modulating function of TNFα, by using the SPINT1 and TMPRSS4 cDNA variants of the present invention. In this embodiment, the modulation of TNFα activity is achieved by ectopic expression of the cDNA variants of the present invention and their derivatives in a biological system of interest, including a cell, a tissue, an organ, or an organism of interest. The ectopic expression can be achieved by using any available methods for delivery and expression of genes in a biological system. These methods include, but not limited to: transient and stable transfection-reagent

mediated techniques, retrovirus-mediated gene transfer, adenovirus-mediated gene transfer, hydrodynamic gene delivery, electroporation and other methods known to the knowledgeable in the art of molecular cell biology and medicine.

[0052] In a second embodiment, the invention provides new methods for modulating TNFα function, by using SPINT1 and TMPRSS4 proteins and variants, derivatives, fragments, mimetics, and homologues thereof. In this embodiment, in addition to ectopic expression of the corresponding cDNAs, the modulation of TNFα activity is achieved by providing the biological system of interest with purified or synthesized peptides or polypeptides that encompass full length or partial regions of SPINT1 or TMPRSS4 proteins. Various techniques that are available for purification or synthesis of proteins of interest can be used here, including recombinant protein production in eukaryotic and prokaryotic cells, affinity-based, size-based, charge-based protein purification methods, and protein synthesizers. According to this embodiment, the purified or synthesized proteins can be delivered into the biological system of interest by variety of methods, including simple addition to the tissue medium, systemic and local injection (intravenous (iv), intraperitoneal (ip) and subcutaneous (sc) injections), micro-injection, electrophoresis and by any other method known to the knowledgeable in the art of molecular cell biology and medicine.

[0053] In a third embodiment, the invention provides new methods for modulating TNFα function, by using reagents that modulate expression of function of endogenous SPINT1 or TMPRSS4 proteins. Various reagents exist or can be produced for this purpose, including small molecular weight inhibitors of SPINT1 or TMPRSS4, antibodies against SPINT1 or TMPRSS4, siRNA and anti-sense oligonucleotides designed to diminish expression of SPINT1 or TMPRSS4 genes and other reagents known to the knowledgeable in the art of molecular cell biology and medicine. According to this embodiment, the reagents can be delivered into the biological system of interest by variety of methods, including simple addition to the tissue medium, systemic and local injection (iv, ip and sc injections), micro-injection, electrophoresis and by any other method known to the knowledgeable in the art of molecular cell biology and medicine.

[0054] In a fourth embodiment, the invention provides new methods for treatment of disease condition by using the described new methods of modulating TNFα function.

According to this embodiment, the disease condition is any pathological condition associated with TNFα function, including, but not limited to inflammation, autoimmunity, cancer and any other condition that is or will be recognized as being associated with TNFα function. According to this embodiment, the treatment comprises administration of pharmacological reagents that modulate function of SPINT1 or TMPRSS4 proteins. The pharmacological reagents are not limited to, but may include any reagents described above. Various vehicles and methods of delivery of the pharmacological reagents exist, including oral tablets, injectable solutions, liposomes, porous beads, nanoparticles and others known to the knowledgeable in the art of molecular cell biology and medicine.

SPINT1 and TMPRSS4 polypeptides

[0055] According to some embodiments, the present invention provides for amino acid sequences derived from SPINT1 and TMPRSS4 that have the ability to modulate TNFα activity. According to some embodiments, the protein or peptides possess the ability to modulate TNFα/NF-κB, IL1-R/NF-κB and TLR5/NF-κB signaling pathways. According to some embodiments, the polypeptides of the present invention modulate the TNFα/NF-κB signaling pathway. According to other embodiments, the polypeptides of the present invention are inhibitors of TNFα activity.

[0056] According to some embodiments, the present invention provides SPINT1 and TMPRSS4 polypeptides that have the ability to modulate TNFα activity. Preferably, the protein or peptides possess the ability to modulate TNFα/NF-κB, IL1-R/NF-κB and to TLR5/NF-κB signaling pathways. The present application is directed to SPINT1 and TMPRSS4 polypeptides at least 80%, 85%, 88%, 95%, 96%, 97%, 98% or 99% identical to the SPINT1 and TMPRSS4 amino acid sequences disclosed herein. The present application is directed to fragments, derivative, variants, homologues, and mimetics of the SPINT1 and TMPRSS4 amino acid sequences disclosed herein having TNFα modulating activity, preferably TNFα inhibitory activity.

Fragments

[0057] The term “fragment” or “subsequence” refers to a protein or polypeptide that consists of a continuous subsequence of the amino acid sequence of a protein or peptide and

includes naturally occurring fragments such as splice variants and fragments resulting from naturally occurring *in vivo* protease activity. Such a fragment may be truncated at the amino terminus, the carboxy terminus, and/or internally (such as by natural splicing). Such fragments may be prepared with or without an amino terminal methionine. The term “fragment” includes fragments, whether identical or different, from the same protein or peptide, with a contiguous amino acid sequence in common or not, joined together, either directly or through a linker. Such fragments may comprise at least 5 to about 50 contiguous amino acids that are identical to the amino acid sequence of the present invention. According to some embodiments, the SPINT1 and TMPRSS4 peptides or fragments comprise at least 7 to about 50 contiguous amino acids that are identical to the amino acid sequence of the present invention. This includes, but is not limited to, the following: at least 7 to about 50 contiguous amino acids; at least 7 to about 45 contiguous amino acids; at least 7 to about 40 contiguous amino acids; at least 7 to about 35 contiguous amino acids; at least 7 to about 30 contiguous amino acids; at least 7 to about 25 contiguous amino acids; at least 7 to about 20 contiguous amino acids; at least 7 to about 18 contiguous amino acids; at least 7 to about 15 contiguous amino acids; at least 7 to about 12 contiguous amino acids; at least 8 to about 25 contiguous amino acids; at least 9 to about 25 contiguous amino acids; at least 10 to about 25 contiguous amino acids; at least 12 to about 25 contiguous amino acids; at least 15 to about 25 contiguous amino acids; at least 10 to about 50 contiguous amino acids; at least 15 to about 50 contiguous amino acids; at least 18 to about 50 contiguous amino acids; at least 20 to about 50 contiguous amino acids; at least 22 to about 50 contiguous amino acids; at least 25 to about 50 contiguous amino acids; at least 30 to about 50 contiguous amino acids; and at least 40 to about 50 contiguous amino acids.

[0058] According to some embodiments, the SPINT1 and TMPRSS4 peptides or fragments comprise at least 5, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 50 contiguous amino acids that are identical to the SPINT1 and TMPRSS4 amino acid sequences disclosed herein. According to some embodiments, the SPINT1 and TMPRSS4 peptides or fragments are 95%, 96%, 97%, 98% or 99% identical to the contiguous amino acids of SPINT1 and TMPRSS4 amino acid sequences. For example, the 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, *etc.* amino acids may substituted or

deleted while maintaining the desired activity of the derived SPINT1 and TMPRSS4 peptides. Preferably, the amino acid substitutions are conservative amino acid substitutions.

[0059] The term “variant” refers to a protein or polypeptide in which one or more amino acid substitutions, deletions, and/or insertions are present as compared to the amino acid sequence of an protein or peptide and includes naturally occurring allelic variants or alternative splice variants of an protein or peptide. The term “variant” includes the replacement of one or more amino acids in a peptide sequence with a similar or homologous amino acid(s) or a dissimilar amino acid(s). There are many scales on which amino acids can be ranked as similar or homologous. (Gunnar von Heijne, Sequence Analysis in Molecular Biology, p. 123-39 (Academic Press, New York, N.Y. 1987.) Preferred variants include alanine substitutions at one or more amino acid positions. Other preferred substitutions include conservative substitutions that have little or no effect on the overall net charge, polarity, or hydrophobicity of the protein. Conservative substitutions are set forth in the table below. According to some embodiments, the SPINT1 and TMPRSS4 polypeptides have at least 80%, 85%, 88%, 95%, 96%, 97%, 98% or 99% sequence identity with the amino acid sequences of the preferred embodiments.

Conservative Amino Acid Substitutions

Basic:	arginine lysine histidine
Acidic:	glutamic acid aspartic acid
Uncharged Polar:	glutamine asparagine serine threonine tyrosine
Non-Polar:	phenylalanine tryptophan cysteine glycine alanine valine praline methionine leucine isoleucine

[0060] The table below sets out another scheme of amino acid substitution:

Original Residue	Substitutions
Ala	Gly;Ser
Arg	Lys
Asn	Gln;His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Ala;Pro
His	Asn;Gln
Ile	Leu;Val
Leu	Ile;Val
Lys	Arg;Gln;Glu
Met	Leu;Tyr;Ile
Phe	Met;Leu;Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp;Phe
Val	Ile;Leu

[0061] Other variants can consist of less conservative amino acid substitutions, such as selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions that in general are expected to have a more significant effect on function are those in which (a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, *e.g.*, seryl or threonyl, is substituted for (or by) a hydrophobic residue, *e.g.*, leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any other residue; (d) a residue having an electropositive side chain, *e.g.*, lysyl, arginyl, or histidyl, is substituted for (or by) a residue having an electronegative charge, *e.g.*, glutamyl or aspartyl; or (e) a residue having a bulky side chain, *e.g.*, phenylalanine, is substituted for (or by) one not having such a side chain, *e.g.*, glycine. Other variants include those designed to either generate a novel glycosylation and/or phosphorylation site(s), or those designed to delete an existing glycosylation and/or phosphorylation site(s). Variants include at least one amino acid

substitution at a glycosylation site, a proteolytic cleavage site and/or a cysteine residue. Variants also include proteins and peptides with additional amino acid residues before or after the protein or peptide amino acid sequence on linker peptides. The term "variant" also encompasses polypeptides that have the amino acid sequence of the proteins/peptides of the present invention with at least one and up to 25 (*e.g.*, 5, 10, 15, 20) or more (*e.g.*, 30, 40, 50, 100) additional amino acids flanking either the N-terminal or C-terminal end of the amino acid sequence.

[0062] According to some embodiments, the SPINT1 peptides or fragments at least comprise a sequence derived from the transmembrane domain (*e.g.*, SEQ ID NO: 11)

[0063] According to some embodiments, the SPINT1 peptides or fragments at least comprise a sequence derived from the transmembrane domain and can also further comprise a sequence derived from the extracellular domain of SPINT1. For example, the SPINT1 peptides or fragments at least comprise a sequence derived from the transmembrane domain and optionally at least 5, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 50, at least 100, at least 200, at least 300, *etc.* up to 444 amino acids of the N-terminus region.

[0064] According to some embodiments, the SPINT1 peptides or fragments at least comprise a sequence derived from the transmembrane domain and optionally at least 5, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 50, at least 100, at least 200, at least 300, *etc.* amino acids of the extracellular domain.

[0065] According to some embodiments, the SPINT1 peptides or fragments at least comprise of at least 5, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 50, at least 100, at least 200, at least 300, etc amino acids of the extracellular domain.

[0066] Specific examples of human SPINT1 peptides are provided in the table below. One of ordinary skill in the art will recognize that the equivalent peptides may be obtained using the mouse SPINT1 amino acid sequence or by using the guidance provided herein for obtaining SPINT1 peptides, fragments, derivatives, variants, *etc.* The "X" represents any

amino acid or none. Preferably, the "X" represents a conservative amino acid or an alanine or glycine.

human SPINT1 peptides	SEQ ID NO
MAVAVFLVICIVVVVAIL	SEQ ID NO: 11
MAVAVXLVICIVVVVAIL	SEQ ID NO: 12
MAVAVXLVICXVVVVAXL	SEQ ID NO: 13
MXVXVFLVICIVVVVXIL	SEQ ID NO: 14
XAVAVXXVICIVVVVAIL	SEQ ID NO: 15
XAVAVFLVICIVVVVAXL	SEQ ID NO: 16
MAVAVFXVICIVVXVAIL	SEQ ID NO: 17
MAVAVFXVICIVXVVAIX	SEQ ID NO: 18
XXVXVFLVICIVVVVAXX	SEQ ID NO: 19
VAVFLVICIVVVV	SEQ ID NO: 20
VAVFLVICIVVVVAIL	SEQ ID NO: 21
AVAVFLVICIVVVVAIL	SEQ ID NO: 22
MAVAVFLVICIVVVVAIL	SEQ ID NO: 23
MAVAVFLVICIVVVVAI	SEQ ID NO: 24
MAVAVFLVICIVVVVA	SEQ ID NO: 25
REIPIPSTGSVEMAVAVFLVICIVVVVAI L	SEQ ID NO: 26
PIPSTGSVEMAVAVFLVICIVVVVAI L	SEQ ID NO: 27
STGSVEMAVAVFLVICIVVVVAI L	SEQ ID NO: 28
SVEMAVAVFLVICIVVVVAI L	SEQ ID NO: 29
VEMAVAVFLVICIVVVVAIL	SEQ ID NO: 30
ADCLNSFTAGVPGFVLDTNASVSNGATFLESPTVRRGWDCV RACCTTQNCNLALVELQPDRGEDAIAACFLINCLYEQNFVC KFAPREGFINYLTREVYRSYRQLRTQGFGGSGIPKAWAGIDL KVQPQEPLVLKDVENTDWRLLRGDTDVRVERKDPNQVEL WGLKEGTYLFQLTVTSSDHPEDTANVTVTVLSTKQTEDYCL ASNKVGRCRGSFPRWYYDPTEQICKSFVYGGCLGNKNNYL REEECILACRGVQGPSMERRHPVCSGTCQPTQFRCSNGCCID SFLECDDTPNCPDASDEAACEKYTSGFDELQRIHFPSDKGHC VDLPDTGLCKESIPRWYYNPFSEHCARFTYGGCYGNKNNFE EEQQCLESCRGISKKDVF	SEQ ID NO: 31
ADCLNSFTAGVPGFVLDTNASVSNGATFLESPTVRRGWDCV RACCTTQNCNLALVELQPDRGEDAIAACFLINCLYEQNFVC KFAPREGFINYLTREVYRSYRQLRTQGFGGSGIPKAWAGIDL KVQPQEPLVLKDVENT	SEQ ID NO: 32
DWRLLRGDTDVRVERKDPNQVELWGLKEGTYLFQLTVTSS DHPEDTANVTVTVLSTKQTEDYCLASNKVGRCRGSFPRWY YDPTEQICKSFVYGGCLGNKNNYLREEECILACRGVQGPSM ERRHPVCSGTCQPTQFRCS	SEQ ID NO: 33

human SPINT1 peptides	SEQ ID NO
NGCCIDSFLECDDTPNCPDASDEAACEKYTSGFDELQRIHFP SDKGHCVDLPDTGLCKESIPRWYYNPFSEHCARFTYGGCYG NKNNFEEEQQCLESCRGISKKDVF	SEQ ID NO: 34
ADCLNSFTAGVPGFVLDTNASVSNGATFLESPTVRRGWDCV RACCTTQNCNLALVELQPDRGEDAIAACF	SEQ ID NO: 35
LINCLYEQNFVCKFAPREGFINYLTREVYRSYRQLRTQGFGG SGIPKAWAGIDLKVQPQEPLVLKDVENT	SEQ ID NO: 36
DWRLLRGDTDVRVERKDPNQVELWGLKEGTYLFQLTVTSS DHPEDTANVTVTVLSTKQTEDYCLASNKVG	SEQ ID NO: 37
RCRGSFPRWYYDPTEQICKSFVYGGCLGNKNNYLREEECIL ACRGVQGPSMERRHPVCSGTCQPTQFRCS	SEQ ID NO: 38
NGCCIDSFLECDDTPNCPDASDEAACEKYTSGFDELQRIHFP SDKGHCVDLPDTGLCKESIPRWYYNPFS	SEQ ID NO: 39
EHCARFTYGGCYGNKNNFEEEQQCLESCRGISKKDVF	SEQ ID NO: 40
ADCLNSFTAGVPGFVLDTNASVSNGATFLESPTVR	SEQ ID NO: 41
RGWDCVRACCTTQNCNLALVELQPDRGEDAIAACF	SEQ ID NO: 42
LINCLYEQNFVCKFAPREGFINYLTREVYRSYRQL	SEQ ID NO: 43
RTQGFGGSGIPKAWAGIDLKVQPQEPLVLKDVENT	SEQ ID NO: 44
DWRLLRGDTDVRVERKDPNQVELWGLKEGTYLFQL	SEQ ID NO: 45
TVTSSDHPEDTANVTVTVLSTKQTEDYCLASNKVG	SEQ ID NO: 46
RCRGSFPRWYYDPTEQICKSFVYGGCLGNKNNYLR	SEQ ID NO: 47
EEECILACRGVQGPSMERRHPVCSGTCQPTQFRCS	SEQ ID NO: 48
NGCCIDSFLECDDTPNCPDASDEAACEKYTSGFDE	SEQ ID NO: 49
LQRIHFPSDKGHCVDLPDTGLCKESIPRWYYNPFS	SEQ ID NO: 50
EHCARFTYGGCYGNKNNFEEEQQCLESCRGISKKDVF	SEQ ID NO: 51
ADCLNSFTAGVPGFVLDTNASVSN	SEQ ID NO: 52
GATFLESPTVRRGWDCVRACCTTQ	SEQ ID NO: 53
NCNLALVELQPDRGEDAIAACF	SEQ ID NO: 54
LINCLYEQNFVCKFAPREGFINYL	SEQ ID NO: 55
TREVYRSYRQLRTQGFGGSGIPKA	SEQ ID NO: 56
WAGIDLKVQPQEPLVLKDVENT	SEQ ID NO: 57
DWRLLRGDTDVRVERKDPNQVELW	SEQ ID NO: 58
GLKEGTYLFQLTVTSSDHPEDTAN	SEQ ID NO: 59
VTVTVLSTKQTEDYCLASNKVG	SEQ ID NO: 60
RCRGSFPRWYYDPTEQICKSFVYG	SEQ ID NO: 61
GCLGNKNNYLREEECILACRGVQG	SEQ ID NO: 62
PSMERRHPVCSGTCQPTQFRCS	SEQ ID NO: 63
NGCCIDSFLECDDTPNCPDASDEA	SEQ ID NO: 64
ACEKYTSGFDELQRIHFPSDKGHC	SEQ ID NO: 65

human SPINT1 peptides	SEQ ID NO
VDLPDTGLCKESIPRWYYNPFS	SEQ ID NO: 66
EHCARFTYGGCYGNKNNFEEEQQC	SEQ ID NO: 67
LESCRGISKKDVF	SEQ ID NO: 68

[0067] According to some embodiments, the peptides, peptide derivatives, or peptidomimetics of the present invention may be represented by the following formula: X1-X2-X3, wherein X1 represents an amino acid sequence of 0 to 50 amino acids in length (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40 or 50, *etc.*), X2 is a Spint1 or TMPRSS4 polypeptide sequence according to the present invention, and X3 represents an amino acid sequence of 0 to 50 amino acids in length (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40 or 50, *etc.*)

Homologue

[0068] The term "homologue" refers to a protein that is at least 80 percent identical in its amino acid sequence of the proteins of the present invention as determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. The degree of similarity or identity between two proteins can be readily calculated by known methods, including but not limited to those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo H. and Lipman, D., SIAM, J. Applied Math., 48:1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs.

[0069] Preferred computer program methods useful in determining the identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., *et al.*, Nucleic Acids Research, 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA, Atschul, S. F. *et al.*, J. Molec. Biol., 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., *et al.*, NCBI

NLM NIH Bethesda, Md. 20894; Altschul, S., *et al.*, J. Mol. Biol., 215: 403-410 (1990). By way of example, using a computer algorithm such as GAP (Genetic Computer Group, University of Wisconsin, Madison, Wis.), the two proteins or polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the “matched span”, as determined by the algorithm).

[0070] A gap opening penalty (which is calculated as 3x (times) the average diagonal; the “average diagonal” is the average of the diagonal of the comparison matrix being used; the “diagonal” is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually {fraction (1/10)} times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff *et al.* in: Atlas of Protein Sequence and Structure, vol. 5, supp.3 for the PAM250 comparison matrix; see Henikoff *et al.*, Proc. Natl. Acad. Sci USA, 89:10915-10919 for the BLOSUM 62 comparison matrix) also may be used by the algorithm. The percent identity then is calculated by the algorithm. Homologues will typically have one or more amino acid substitutions, deletions, and/or insertions as compared with the comparison protein or peptide, as the case may be.

Fusion protein

[0071] The term “fusion protein” refers to a protein where one or more peptides are recombinantly fused or chemically conjugated (including covalently and non-covalently) to a protein such as (but not limited to) an antibody or antibody fragment like an Fab fragment or short chain Fv. The term “fusion protein” also refers to multimers (*i.e.* dimers, trimers, tetramers and higher multimers) of peptides. Such multimers comprise homomeric multimers comprising one peptide, heteromeric multimers comprising more than one peptide, and heteromeric multimers comprising at least one peptide and at least one other protein. Such multimers may be the result of hydrophobic, hyrdrophilic, ionic and/or covalent associations, bonds or links, may be formed by cross-links using linker molecules or may be linked indirectly by, for example, liposome formation.

Peptide Derivatives and Peptidomimetics

[0072] The term "peptide mimetic" or "mimetic" refers to biologically active compounds that mimic the biological activity of a peptide or a protein but are no longer peptidic in chemical nature, that is, they no longer contain any peptide bonds (that is, amide bonds between amino acids). Here, the term peptide mimetic is used in a broader sense to include molecules that are no longer completely peptidic in nature, such as pseudo-peptides, semi-peptides and peptoids. Examples of peptide mimetics in this broader sense (where part of a peptide is replaced by a structure lacking peptide bonds) are described below. Whether completely or partially non-peptide, peptide mimetics according to the embodiments provide a spatial arrangement of reactive chemical moieties that closely resemble the three-dimensional arrangement of active groups in the peptide on which the peptide mimetic is based. As a result of this similar active-site geometry, the peptide mimetic has effects on biological systems that are similar to the biological activity of the peptide.

[0073] The peptide mimetics of the embodiments are preferably substantially similar in both three-dimensional shape and biological activity to the peptides described herein. Examples of methods of structurally modifying a peptide known in the art to create a peptide mimetic include the inversion of backbone chiral centers leading to D-amino acid residue structures that may, particularly at the N-terminus, lead to enhanced stability for proteolytic degradation without adversely affecting activity. An example is given in the paper "Tritriated D-ala^1-Peptide T Binding", Smith C. S. *et al.*, Drug Development Res., 15, pp. 371-379 (1988). A second method is altering cyclic structure for stability, such as N to C interchain imides and lactames (Ede *et al.* in Smith and Rivier (Eds.) "Peptides: Chemistry and Biology", Escom, Leiden (1991), pp. 268-270). An example of this is given in conformationally restricted thymopentin-like compounds, such as those disclosed in U.S. Pat. No. 4,457,489 (1985), Goldstein, G. *et al.*, the disclosure of which is incorporated by reference herein in its entirety. A third method is to substitute peptide bonds in the peptide by pseudopeptide bonds that. confer resistance to proteolysis.

[0074] The term "derivative" refers to a chemically modified protein or polypeptide that has been chemically modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques, as for example, by

addition of one or more polyethylene glycol molecules, sugars, phosphates, and/or other such molecules, where the molecule or molecules are not naturally attached to wild-type proteins. Derivatives include salts. Such chemical modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given protein or polypeptide. Also, a given protein or polypeptide may contain many types of modifications.

[0075] Modifications can occur anywhere in a protein or polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, Proteins--Structure And Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., "Posttranslational Protein Modifications: Perspectives and Prospects," pgs. 1-12 in Posttranslational Covalent Modification Of Proteins, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter *et al.*, Meth. Enzymol. 182:626-646 (1990) and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging," Ann. N.Y. Acad. Sci. 663: 48-62 (1992).

[0076] Also included by the present invention are other types of peptide derivatives containing additional chemical moieties not normally part of the peptide, provided that the derivative retains the desired functional activity of the peptide. Examples of such derivatives include (i) N-acyl derivatives of the amino terminal or of another free amino group, wherein

the acyl group may be an alkanoyl group (*e.g.*, acetyl, hexanoyl, octanoyl), an aroyl group (*e.g.*, benzoyl) or a blocking group such as F-moc (fluorenylmethyl-O--CO--); (ii) esters of the carboxy terminal or of another free carboxy or hydroxyl group; (iii) amide of the carboxy terminus or of another free carboxyl group produced by reaction with ammonia or with a suitable amine; (iv) phosphorylated derivatives; (v) derivatives conjugated to an antibody or other biological ligand and other types of derivatives.

[0077] The term "derivatives" include chemical modifications resulting in the protein or polypeptide becoming branched or cyclic, with or without branching. Cyclic, branched and branched circular proteins or polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well. A cyclic derivative containing an intramolecular disulfide bond may be prepared by conventional solid phase synthesis while incorporating suitable S-protected cysteine or homocysteine residues at the positions selected for cyclization such as the amino and carboxy termini. Following completion of the chain assembly, cyclization can be performed either (1) by selective removal of the S-protecting group with a consequent on-support oxidation of the corresponding two free SH-functions, to form a S--S bonds, followed by conventional removal of the product from the support and appropriate purification procedure; or (2) by removal of the peptide from the support along with complete side chain deprotection, followed by oxidation of the free SH-functions in highly dilute aqueous solution.

[0078] The cyclic derivative containing an intramolecular amide bond may be prepared by conventional solid phase synthesis while incorporating suitable amino and carboxyl side chain protected amino acid derivatives, at the position selected for cyclization. The cyclic derivatives containing intramolecular --S-- alkyl bonds can be prepared by conventional solid phases while incorporating an amino acid residue with a suitable amino-protected side chain, and a suitable S-protected cysteine or homocysteine residue at the position selected for cyclization.

[0079] Systematic substitution of one or more amino acids of a consensus sequence with D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) may be used to generate more stable peptides. Thus, a peptide derivative or peptidomimetic of the present invention may be all L, all D or mixed D, L peptide. In a preferred embodiment, the peptides consist of

all D-amino acids. The presence of an N-terminal or C-terminal D-amino acid increases the *in vivo* stability of a peptide since peptidases cannot utilize a D-amino acid as a substrate.

[0080] Substitution of unnatural amino acids for natural amino acids in a subsequence of the peptides can also confer resistance to proteolysis. Such a substitution can, for instance, confer resistance to proteolysis by exopeptidases acting on the N-terminus. Such substitutions have been described and these substitutions do not affect biological activity. Examples of non-naturally occurring amino acids include α,α-disubstituted amino acids, N-alkyl amino acids, lactic acids, C-α-methyl amino acids, and β-methyl amino acids. Amino acid analogs useful in the present invention may include but are not limited to β-alanine, norvaline, norleucine, 4-aminobutyric acid, orithine, hydroxyproline, sarcosine, citrulline, cysteic acid, cyclohexylalanine, 2-aminoisobutyric acid, 6-aminohexanoic acid, t-butylglycine, phenylglycine, o-phosphoserine, N-acetyl serine, N-formylmethionine, 3-methylhistidine and other unconventional amino acids. Furthermore, the synthesis of peptides with unnatural amino acids is routine and known in the art.

[0081] One other effective approach to confer resistance to peptidases acting on the N-terminal or C-terminal residues of a peptide is to add chemical groups at the peptide termini, such that the modified peptide is no longer a substrate for the peptidase. One such chemical modification is glycosylation of the peptides at either or both termini. Certain chemical modifications, in particular N-terminal glycosylation, have been shown to increase the stability of peptides in human serum. Other chemical modifications which enhance serum stability include, but are not limited to, the addition of an N-terminal alkyl group, consisting of a lower alkyl of from 1 to 20 carbons, such as an acetyl group, and/or the addition of a C-terminal amide or substituted amide group. In particular the present invention includes modified peptides consisting of peptides bearing an N-terminal acetyl group and/or a C-terminal amide group.

Pharmaceutical Compositions

[0082] The present invention relates to a method for inhibiting TNFα. In view of the importance of TNFα function in numerous pathways and conditions in animals, the peptides,

peptide derivatives and peptidomimetics of the present invention are useful in the treatment of conditions or diseases associated with TNFα.

[0083] Therefore, methods of the present invention comprise administering to a subject in need thereof or at risk of being in need thereof an effective amount of a peptide, peptide derivative or peptidomimetic, or a composition comprising a peptide, peptide derivative or peptidomimetic to a subject, to inhibit TNFα biological activity. In one embodiment, an effective amount of a therapeutic composition comprising a peptide or peptide derivative thereof and a suitable pharmaceutical carrier is administered to a subject to inhibit TNFα biological activity to prevent, ameliorate symptoms or treat a disorder, disease or condition related to abnormal signaling through TNFα a pathway. In one embodiment, the subject is an animal. In another embodiment, the subject is a mammal, and preferably a human.

[0084] The peptides, peptide derivatives and peptidomimetics of the present invention are used in the treatment, prophylaxis or amelioration of symptoms in any disease condition or disorder where the inhibition of TNFα biological activity might be beneficial.

[0085] Compositions within the scope of the present invention should contain the active agent (*e.g.* peptide, peptide derivative or peptidomimetic) in an amount effective to achieve the desired therapeutic effect while avoiding adverse side effects. Pharmaceutically acceptable preparations and salts of the active agent are within the scope of the present invention and are well known in the art. For the administration of polypeptide antagonists and the like, the amount administered should be chosen so as to avoid adverse side effects. The amount of the therapeutic or pharmaceutical composition which is effective in the treatment of a particular disease, disorder or condition will depend on the nature and severity of the disease, the target site of action, the patient's weight, special diets being followed by the patient, concurrent medications being used, the administration route and other factors that will be recognized by those skilled in the art. The dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the patient. Typically, 0.001 to 100 mg/kg/day will be administered to the subject. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems. For example, in order to obtain an effective mg/kg dose for

humans based on data generated from rat studies, the effective mg/kg dosage in rat is divided by six.

[0086] Various delivery systems are known and can be used to administer peptides, peptide derivatives or peptidomimetics or a pharmaceutical composition of the present invention. The pharmaceutical composition of the present invention can be administered by any suitable route including, intravenous or intramuscular injection, intraventricular or intrathecal injection (for central nervous system administration), orally, topically, subcutaneously, subconjunctivally, or via intranasal, intradermal, sublingual, vaginal, rectal or epidural routes.

[0087] Other delivery systems well known in the art can be used for delivery of the pharmaceutical compositions of the present invention, for example via aqueous solutions, encapsulation in microparticles, or microcapsules.

[0088] In yet another embodiment, the pharmaceutical compositions of the present invention can be delivered in a controlled release system. In one embodiment polymeric materials can be used, in another embodiment, a pump may be used.

[0089] Compounds of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled to a class of biodegradable polymers useful in achieving controlled release of the drug, non-limiting examples, include: polylactic acid, polyorthoesters, cross-linked amphipathic block copolymers and hydrogels, polyhydroxy butyric acid and polydihydropyrans.

[0090] As mentioned above, pharmaceutical compositions of the present invention comprise a peptide, peptide derivative or peptidomimetic combined with a pharmaceutically acceptable carrier. The term carrier refers to diluents, adjuvants, excipients such as a filler or a binder, a disintegrating agent, a lubricant a silica flow conditioner a stabilizing agent or vehicles with which the peptide, peptide derivative or peptidomimetic is administered. Such pharmaceutical carriers include sterile liquids such as water and oils including mineral oil, vegetable oil (*e.g.*, peanut oil, soybean oil, sesame oil, canola oil), animal oil or oil of synthetic origin. Aqueous glycerol and dextrose solutions as well as saline solutions may also

be employed as liquid carriers of the pharmaceutical compositions of the present invention. Of course, the choice of the carrier depends on the nature of the peptide, peptide derivative or peptidomimetic, its solubility and other physiological properties as well as the target site of delivery and application. For example, carriers that can penetrate the blood brain barrier are used for treatment, prophylaxis or amelioration of symptoms of diseases or conditions (*e.g.* inflammation) in the central nervous system. Examples of suitable pharmaceutical carriers are described in Remington: The Science and Practice of Pharmacy by Alfonso R. Gennaro, 2003, 21st edition, Mack Publishing Company.

[0091] Further pharmaceutically suitable materials that may be incorporated in pharmaceutical preparations of the present invention include absorption enhancers, pH regulators and buffers, osmolarity adjusters, preservatives, stabilizers, antioxidants, surfactants, thickeners, emollient, dispersing agents, flavoring agents, coloring agents and wetting agents.

[0092] Examples of suitable pharmaceutical excipients include, water, glucose, sucrose, lactose, glycol, ethanol, glycerol monostearate, gelatin, rice, starch, flour, chalk, sodium stearate, malt, sodium chloride and the like. The pharmaceutical compositions of the present invention can take the form of solutions, capsules, tablets, creams, gels, powders, sustained release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides (see Remington: The Science and Practice of Pharmacy by Alfonso R. Gennaro, 2003, 21.sup.th edition, Mack Publishing Company). Such compositions contain a therapeutically effective amount of the therapeutic composition, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulations are designed so as to suit the mode of administration and the target site of action (*e.g.*, a particular organ or cell type).

[0093] The pharmaceutical compositions of the present invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those that form with free amino groups and those that react with free carboxyl groups. Non-toxic alkali metal, alkaline earth metal and ammonium salts commonly used in the pharmaceutical industry include sodium, potassium, lithium, calcium, magnesium, barium, ammonium, and protamine zinc salts, which are prepared by methods well known in the art. The term also includes non-toxic

acid addition salts, which are generally prepared by reacting the compounds of the present invention with suitable organic or inorganic acid. Representative salts include the hydrobromide, hydrochloride, valerate, oxalate, oleate, laureate, borate, benzoate, sulfate, bisulfate, acetate, phosphate, tysolate, citrate, maleate, fumarate, tartrate, succinate, napsylate salts and the like.

[0094] Examples of fillers or binders that may be used in accordance with the present invention include acacia, alginic acid, calcium phosphate (dibasic), carboxymethylcellulose, carboxymethylcellulose sodium, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, dextrin, dextrates, sucrose, tylose, pregelatinized starch, calcium sulfate, amylose, glycine, bentonite, maltose, sorbitol, ethylcellulose, disodium hydrogen phosphate, disodium phosphate, disodium pyrosulfite, polyvinyl alcohol, gelatin, glucose, guar gum, liquid glucose, compressible sugar, magnesium aluminum silicate, maltodextrin, polyethylene oxide, polymethacrylates, povidone, sodium alginate, tragacanth, microcrystalline cellulose, starch, and zein. Another most preferred filler or binder consists of microcrystalline cellulose.

[0095] Examples of disintegrating agents that may be used include alginic acid, carboxymethylcellulose, carboxymethylcellulose sodium, hydroxypropylcellulose (low substituted), microcrystalline cellulose, powdered cellulose, colloidal silicon dioxide, sodium croscarmellose, crospovidone, methylcellulose, polacrilin potassium, povidone, sodium alginate, sodium starch glycolate, starch, disodium disulfite, disodium edathamil, disodium edetate, disodiumethylenediaminetetraacetate (EDTA) crosslinked polyvinylpyrollidines, pregelatinized starch, carboxymethyl starch, sodium carboxymethyl starch and microcrystalline cellulose.

[0096] Examples of lubricants include calcium stearate, canola oil, glyceryl palmitostearate, hydrogenated vegetable oil (type I), magnesium oxide, magnesium stearate, mineral oil, poloxamer, polyethylene glycol, sodium lauryl sulfate, sodium stearate fumarate, stearic acid, talc, zinc stearate, glyceryl behapate, magnesium lauryl sulfate, boric acid, sodium benzoate, sodium acetate, sodium benzoate/sodium acetate (in combination) and DL leucine.

[0097] Examples of silica flow conditioners include colloidal silicon dioxide, magnesium aluminum silicate and guar gum. Another most preferred silica flow conditioner consists of silicon dioxide.

[0098] Examples of stabilizing agents include acacia, albumin, polyvinyl alcohol, alginic acid, bentonite, dicalcium phosphate, carboxymethylcellulose, hydroxypropylcellulose, colloidal silicon dioxide, cyclodextrins, glyceryl monostearate, hydroxypropyl methylcellulose, magnesium trisilicate, magnesium aluminum silicate, propylene glycol, propylene glycol alginate, sodium alginate, carnauba wax, xanthan gum, starch, stearate(s), stearic acid, stearic monoglyceride and stearyl alcohol.

[0099] The present invention also provides for modifications of peptides or peptide derivatives such that they are more stable once administered to a subject (*i.e.*, once administered it has a longer half-life or longer period of effectiveness as compared to the unmodified form). Such modifications are well known to those skilled in the art to which this invention pertain (*e.g.*, polyethylene glycol derivatization a.k.a. PEGylation, microencapsulation, *etc.*).

Nucleotide Sequences

[00100] According to some embodiments, the present invention provides nucleic acid molecules encoding SPINT1 and TMPRSS4 proteins or peptides that have the ability to modulate TNFα activity. Preferably, the protein or peptides possess the ability to modulate TNFα/NF-κB, IL1-R/NF-κB and to TLR5/NF-κB signaling pathways. The present application is directed to nucleic acid molecules at least 80%, 85%, 88%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences described herein. By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a TNFα modulating polypeptides of the present invention is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per 100 nucleotides of the reference nucleotide sequence encoding the TNFα modulating polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be

deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[00101] The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the polynucleotides of the present invention is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50, 100, 150, 200, 250, 300, 350, 400, or 450 nt in length are also useful. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequences described herein. Generating such DNA fragments would be routine to the skilled artisan. For example, restriction endonuclease cleavage or shearing by sonication could easily be used to generate fragments of various sizes. Alternatively, such fragments could be generated synthetically.

[00102] Due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequences disclosed herein will encode a polypeptides of the present invention. In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assays. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptides having the desired activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (*e.g.*, replacing one aliphatic amino acid with a second aliphatic amino acid).

Antibodies

[00103] The present invention also provides for antibodies the SPINT1 and TMPRSS4 polypeptides of the present invention. According to some embodiments, the antibodies of the present invention target the transmembrane and extracellular domains of SPINT1 and TMPRSS4 or like sequences. Antibodies to the SPINT1 and TMPRSS4 polypeptides of the present invention may be obtained or prepared using any conventional method of making antibodies.

[00104] The SPINT1 and TMPRSS4 antibodies may be either monoclonal, polyclonal, or a mixture of monoclonal and/or polyclonal antibodies. The SPINT1 and TMPRSS4 antibodies may comprise whole antibody or antigen-binding fragments thereof, such as Fv, $F(ab')_2$ and Fab. Antibody fragments may be prepared by cleavage of the intact protein, *e.g.* by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the $F(ab')_2$ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

[00105] SPINT1 and TMPRSS4 antibodies can be produced as a single chain, instead of the normal multimeric structure. Single chain antibodies are described in Jost *et al.,* 269 J. BIOL. CHEM. 26267-73 (1994), incorporated herein by reference, and others. DNA sequences encoding the variable region of the heavy chain and the variable region of the light chain are ligated to a spacer encoding at least about 4 amino acids of small neutral amino acids, including glycine or serine. The protein encoded by this fusion allows assembly of a functional variable region that retains the specificity and affinity of the original antibody.

[00106] SPINT1 and TMPRSS4 antibodies can be produced by use of Ig cDNA for construction of chimeric immunoglobulin genes (Liu *et al.,* 84 PROC. NATL. ACAD. SCI. 3439 (1987) and 139 J. IMMUNOL. 3521 (1987), incorporated herein by reference. mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Patent 4,683,195 and U.S. Patent 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of

human constant regions genes may be found in Kabat *et al.*, "Sequences of Proteins of Immunological Interest" N.I.H. PUBLICATION NO. 91-3242 (1991). Human C region genes are readily available from known clones. The chimeric, humanized antibody is then expressed by conventional methods.

[00107] The SPINT1 and TMPRSS4 antibodies may be labeled according to standard methods known in the art. For example, antibodies can be labeled with detectable labels such as enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Enzyme labels include, but are not limited to, horseradish peroxidase, alkaline phosphatase, β-galactosidase, acetylcholinesterase, and combinations thereof. Prosthetic groups include, but are not limited to, streptavidin, biotin, avidin, and combinations thereof. Fluorescent material includes, but is not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, phycoerythrin, and combinations thereof. Luminescent material includes, but is not limited to, luminol. Bioluminescent material includes, but is not limited to, luciferase, luciferin, aequorin, and combinations thereof. Radioactive material includes, but is not limited to, ^{125}I, ^{131}I, ^{35}S, and ^{3}H.

Methods of screening

[00108] The present invention provides for methods of screening for compounds (*e.g.*, small molecules, macromolecules, *etc.*) that have TNFα modulating activity. According to some embodiments, there is provided a method for screening for compounds that have TNFα modulating activity comprising contacting a cell expressing the SPINT1 and TMPRSS4 polypeptides of the present invention with a test agent and detecting whether TNFα activity is affected. According to some embodiments, the detecting step comprises detecting whether the TNFα/NF-κB, IL1-R/NF-κB and to TLR5/NF-κB signaling pathways have been modulated (*e.g.*, activated or inhibited).

[00109] According to some embodiments, there is provided a method for screening for compounds that have SPINT1 and TMPRSS4 polypeptide binding activity comprising contacting a SPINT1 and TMPRSS4 polypeptide of the present invention with a test agent and detecting whether the SPINT1 and TMPRSS4 polypeptide binds to the test agent.

Definitions

[00110] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only not intended to be limiting. Other features and advantages of the invention will be apparent from the following detailed description and claims.

[00111] Biological system: any vertebrate cell, cell population, tissue, organ or organism.

[00112] TNFα signaling: any chain of molecular events initiated by TNFα, including but not limited to the NF-κB pathway, the MAPK pathway, or cell death signaling. TNFα function: any biological process mediated (*e.g.*, activated or inhibited) by TNFα under normal or pathological conditions, including but not limited to immune response to bacterial, fungal, viral and parasitic infection; cell proliferation, differentiation and apoptosis at sites of tissue injury of inflammatory and non-inflammatory origin.

[00113] TNFα associated disease: any pathological condition in which monitoring or modulation of TNFα level or activity is used for prognosis, prophylaxis or therapy. Examples of acute TNFα associated diseases include, but are not limited to, septic shock, adult respiratory distress disorder, gastrointestinal necrosis, acute renal tube necrosis, adrenal hemorrhage and disseminated intravascular coagulation. Examples of chronic TNFα associated diseases include but are not limited to cachexia, anorexia, hepatosplenomegaly, subendocardial inflammation, insulin resistance, rheumatoid arthritis and multiple sclerosis. Gene specific reagents: reagents that can be designed or generated by the skilled in the art based on nucleotide or amino acid sequence of a disclosed gene or related nucleotide or amino acid sequences including nucleotide or amino acid sequences of alternative transcript variants of a disclosed gene, single nucleotide polymorphism variants of a disclosed gene and other natural or engineered sequences significantly homologous (orthologous or paralogous)

to the sequence of a disclosed gene. Examples of gene specific reagents include: nucleotide sequence derived reagents, DNA or RNA oligonucleotides, oligonucleotide probes, siRNA molecules, other recombinant DNA or RNA constructs containing complete or partial fragments of nucleotide sequence of a disclosed gene or related nucleotide sequences as well as other reagents that can interact with (*e.g.* directly bind) DNA or RNA sequences of a disclosed gene, gene transcripts or related nucleotide sequences; and amino acid sequence derived reagents: proteins or peptides comprising complete or partial amino acid sequences encoded by a disclosed gene or related nucleotide sequences; reagents mimicking structure of interaction domains of such proteins or peptides; reagents that can specifically interact with such proteins or peptides including, but not limited to engineered interaction partners of such proteins or peptides: antibodies, peptides or small molecule reagents directly binding disclosed proteins or peptides (*e.g.* synthetic substrates or inhibitors of proteins that posses enzymatic activities) or physiological interaction partners of disclosed proteins or peptides: *e.g.* proteins identified through their direct binding to disclosed proteins or peptides that modulate disclosed biological function(s) or natural(physiological) substrates or inhibitors of proteins that posses enzymatic activities.

[00114] Delivery method: any method that can be employed by one skilled in the art to introduce a gene specific reagent into a biological system. Examples of delivery methods for gene specific reagents include but are not limited to DNA transfection (lipofection, electroporation, nucleoporation), viral gene delivery methods or direct injection of DNA, RNA or proteins molecules into a biological system.

[00115] Pharmaceutically acceptable carrier: refers to a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredients of the compound and which is not toxic for the host (*e.g.*, patient) to whom it is administered.

[00116] Therapeutically or pharmaceutically effective amount: refers herein to the amount of composition of the present invention sufficient to induce a desired effect. Such result can be alleviation or reduction of the signs, symptoms or causes of the disease or any other desired alteration of the target physiological system. For example, in the case of inflammatory diseases (*e.g.*, arthritis and inflammation) a typical result will involve decrease in inflammatory and immunological responses.

[00117] The terms "molecule", "compound", "agent" or "ligand" are used interchangeably and broadly to refer to natural, synthetic or semi-synthetic molecules or compounds. The term "molecule" therefore denotes for example chemicals, macromolecules, and the like. Non-limiting examples of molecules include peptides, antibodies, carbohydrates and pharmaceutical agents. The agents can be selected and screened by a variety of means including random screening, rational selection and by rational design using for example protein or ligand modeling methods such as computer modeling. As will be understood by the person of ordinary skill, macromolecules having non-naturally occurring modifications are also within the scope of the term "molecule". For example, peptidomimetics, well known in the pharmaceutical industry and generally referred to as peptide analogs can be generated by modeling as mentioned above. Similarly, in a preferred embodiment, the polypeptides of the present invention are modified to enhance their stability. It should be understood that in most cases this modification should not alter the biological activity of the polypeptide.

[00118] Purified: refers to a compound or compounds having been separated from a component of the composition in which it was originally contained. Thus, for example, a "purified peptide" or a "purified composition of peptides" has been purified to a level not found in nature. A "substantially pure" compound is a compound that is lacking in most other components (*e.g.*, 30, 40, 50, 60, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 100% free of contaminants). By opposition, the term "crude" means compounds that have not been separated from the components of the original composition in which it was present. For the sake of brevity, the units (*e.g.* 66, 67 . . . 81, 82, . . . 91, 92% . . .) have not been specifically recited but are considered nevertheless within the scope of the present invention.

[00119] Short peptide: is intended to mean a sequence of about 6-25 amino acids.

[00120] As used throughout this disclosure, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a composition” includes a plurality of such compositions, as well as a single composition, and a reference to “a therapeutic agent” is a reference to one or more therapeutic and/or pharmaceutical agents and equivalents thereof known to those skilled in the art, and so forth. Thus, for example, a reference to ”a host cell” includes a plurality of such host cells, and a

reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

[00121] The following examples are illustrative, but not limiting, of the methods and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in therapy and that are obvious to those skilled in the art are within the spirit and scope of the embodiments.

EXAMPLES

[00122] It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also provided within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention. While the claimed invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made to the claimed invention without departing from the spirit and scope thereof. Thus, for example, those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

Example 1. Identification of novel inhibitors of TNFα.

[00123] Several NF-κB inhibitors were isolated by using a genetic screening technology. Majority of NF-κB inhibitors encoded by identified cDNA variants represented known NF-κB pathway genes: NFKBIA, NFKBIB, NFKBIE and IKBKG (Table 1). Additional cDNA clones inhibiting TNFα/NF-κB signaling coded for Kunitz-type membrane-associated serine protease inhibitor deletion variant (SPINT1, Fig. 2A) and full length trans-membrane serine protease 4 (TMPRSS4, Fig. 2B). Neither of these proteins has been previously known as regulators of TNFα/NF-κB pathway (Table 1).

[00124] Table 1. cDNA variants identified through expression cloning and functional selection in TNFα/NF-κB reporter cell line.

GeneBank number/ symbol	Gene name
NM_020529 NFKBIA	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha
NM_003639 IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma
NM_010908 Nfkbib [m]	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, beta
NM_010907 Nfkbia [m]	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha
NM_004556 NFKBIE	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon
NM_019894 TMPRSS4	Transmembrane protease, serine 4
NM_002503 NFKBIB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
NM_016907 SPINT1	Serine protease inhibitor, Kunitz type 1

Example 2. Validation of SPINT1 and TMPRSS4 cDNA variants as inhibitors of TNFα inducible activation of NF-κB.

[00125] We next examined the ability of SPINT1 and TMPRSS4 cDNA variants identified in our screening to inhibit TNFα-dependent activation of transcription factor NF-κB. To this end, the cDNA variants were transiently transfected into parental reporter cells along with NF-κB-dependent luciferase reporter gene constructs. Luciferase activity was measured in un-stimulated cells and in cells stimulated with TNFα. We found that expression of both SPINT1 and TMPRSS4 cDNA variants resulted in reduced NF-κB induction in response to TNFα as compared to that in control samples, in which either empty pLCX vector or entire (not functionally selected) MGC deletion cDNA library were co-transfected with the NF-κB-dependent luciferase reporter gene construct (Fig. 3). Inhibitory effects of SPINT1 and TMPRSS4 cDNA variants were also compared to those of three different forms of model NF-κB inhibitory protein IkBα, wild type IkBa, mutant non-degradable form of IkBα (IkBa super repressor) and 3'-deletion variant of IkBα that also has been identified in our screening. We found that, in these experiments, inhibitory effects of both SPINT1 and TMPRSS4 cDNA variants on NF-κB activation were stronger than that of wt form of IkBα (Fig. 3).

[00126] To assess whether stable expression of SPINT1 and TMPRSS4 cDNA variants also inhibits induction of NF-κB by TNFα we generated HEK293kB-EGFP reporter cell line with integrated NF-κB-EGFP reporter construct, thus enabling monitoring of NF-κB status by FACS-based measurement of EGFP fluorescence in individual cells. The HEK293kB-EGFP cells were stably transduced with recombinant retroviruses containing CMV-cDNA-IRES-puro^R expression cassettes co-expressing either SPINT1 or TMPRSS4 cDNA variants with puro^R gene that facilitates an effective puromycin (2 ug/ml) selection. Additional retroviral constructs expressing IkBα and IKKγ deletion variants (also isolated during MGC cDNA library screening) were used as positive controls for NF-κB inhibition. Empty vector and a retroviral construct with a frame-shift variant of SPINT1 were generated to provide negative controls for the experiments. Data in Fig. 4 show FACS-generated EGFP profiles of HEK293kB-EGFP cell pools (vector control, SPINT1-del, TMPRSS4 and frame-shift SPINT1-del variant) following stimulation with TNFα. We found that both SPINT1 and TMPRSS4 cDNA variants, but not frame-shift SPINT1-del variant, strongly inhibited TNFα dependent accumulation of EGFP-positive cells. The inhibition was evident over broad range of TNFα concentrations (Fig. 5).

Example 3. Specificity of SPINT1 and TMPRSS4 cDNA variants towards inhibition of TNFα signaling.

[00127] NF-κB transcription can be induced by variety of pro-inflammatory stimuli, including stimuli that activate IL-R1 and TLR signaling pathways. To assess specificity of SPINT1 and TMPRSS4 cDNA variants towards TNFα/NF-κB signaling pathway, we investigated whether activation of NF-κB by IL-1β and flagellin (specific ligand of toll like receptor 5) was also impaired in the cells stably expressing SPINT1 and TMPRSS4 cDNA variants. We found that, in contrast to TNFα, neither IL-1β- nor flagellin-dependent NF-κB activation was inhibited by stable expression of SPINT1 and TMPSS4 cDNA variants (Fig. 6). Therefore, we concluded that expression of SPINT1 and TMPRSS4 cDNA variants specifically affected TNFα/NF-κB signaling pathway. Interestingly, TMPRSS4 significantly increased NF-κB activity in cells subjected to IL-1β or flagellin, suggesting a broader role of this protein in coordinating NF-κB dependent responses under inflammatory conditions.

Example 4. Effect of small molecule inhibitors and protein inhibitors of serine proteases on SPINT1 and TMPRSS4 mediated inhibition of TNFα signaling.

[00128] Two lines of evidence indicate that TNFα receptor complex may be a target of SPINT1 and TMPRSS4 mediated inhibition of TNFα/NF-κB pathway: **1.** Both SPINT1 and TMPRSS4 represent membrane associated proteins containing large extracellular domains with defined biochemical activities. **2.** SPINT1 and TMPRSS4 mediated NF-κB inhibition is TNFα specific since NF-κB pathway is still activated by IL-1β or flagellin in cells ectopically expressing SPINT1 or TMPRSS4. Known biological functions of SPINT1 (a serine proteinase inhibitor) and TMPRSS4 (a serine proteinase) point out to their involvement in proteolysis of membrane-associated and/or extracellular protein substrates. Therefore we decided to test if broad spectrum class specific serine protease inhibitors: Pefabloc (AEBSF, 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride) and aprotinin (a well characterized Kunitz-type protein inhibitor of serine proteases) may affect the phenotype of cells expressing SPINT1 and TMPRSS4. We found out that both tested serine proteinase inhibitors were able to reverse TMPRSS4 mediated inhibition of TNFα/NF-κB pathway but neither of them had an effect on NF-κB activity in cells overexpressing SPINT1 (**Fig. 7**). Based on this observation, proteolytic processing of a component/components of TNFα receptor complex (*e.g.* TNFR1, TNFα receptor subunit 1) may represent a mechanism of TMPRSS4 dependent inhibition of TNFα signaling pathway. In contrast, SPINT1 may block function of TNFα pathway through an alternative mechanism that presumably does not require proteolysis *e.g.*, via a direct interaction with TNFα receptor complex.

[00129] To validate TMPRSS4 as the anti-TNFα agent, we assessed its effect on the status of TNFα receptor I, TNFRI. To do so, we prepared protein samples from either control cells or from cells stably expressing TMRSS4, as well as from corresponding aliquots of conditioned growth media. Subsequent Western blot analysis with antibodies against the extracellular part of TNFα receptor I demonstrated that expression of TMPRSS4 induced release of a ~ 30 kDa TNFRI fragment into the growth media, while reducing the amount of TNFRI in the corresponding cell lysates **(Figure 14a)**. Similar effects were observed in the presence of TNFα stimulation. The anti-TNFRI antibody immunoreactive band was not detectable in the media samples collected from the control PE413 cells, suggesting that the

expression of TMPRSS4 was absolutely required to facilitate the release of a soluble form of TNFRI under these experimental conditions.

[00130] Since TMPRSS4-dependent inhibition of TNFα signaling could be reversed by serine protease inhibitors (aprotinin and Pefabloc) added to the cell culture media, we tested the effects of these compounds on the release of soluble form of TNFRI from PE413-TMPRS4 cells **(Figure 14b)**. Consistent with our previous data, both protease inhibitors block secretion of a soluble ~ 30 kDa TNFRI fragment to the cell culture media in a dose-dependent manner. These data further indicate that TMPRSS4 directly or indirectly mediates proteolysis and inactivation of TNFα receptor, thus inhibiting TNFα signaling.

[00131] It is noteworthy that these data imply a dual mechanism of TMPRSS4-dependent anti-TNFα activity. First, shedding of TNFRI from the cell surface renders TMPRSS4-expressing cells refractory to the TNFα stimulation. Second, secretion of a soluble TNFRI may lead to the local sequestering of TNFα molecules (through TNFα- soluble TNFRI interactions) and preventing TNFα from binding to functional TNFα receptor complexes present on the cells, regardless of their TMPRSS4 activity.

Example 5. Analysis of spint1 domains involved in inhibition of TNFα signaling.

[00132] Initially identified spint1 deletion variant (spint1_d1604) represents a C-terminal truncation of the wild type mouse spint1 that lacks intracellular domain (ID) but retains complete transmembrane (TM) and extracellular domains. We tested whether full length human spint1 protein as well as engineered deletion variants without the intracellular domain (h-spint1_dID; includes a.a. 1-468 of h-spint1 ORF) or without both intracellular and transmembrane domains (h-spint1_dTM; includes a.a. 1-443 of h-spint1 ORF) may function as inhibitors of TNFα dependent NFkB activation (see Fig. 8). Transient expression of h-spint1_dID largely reproduced NFkB inhibition observed for m-spint1_d1604. In contrast, full length human spint1 only marginally inhibited this pathway, whereas h-spint1_dTM acted as a weak agonist of TNFα dependent induction of NFkB. These data indicate that engineered molecules including spint1 transmembrane and extracellular domains (but excluding intracellular domain) represent biological reagents with strong anti-TNFα activity.

[00133] Inhibition of TNFα signaling was confirmed for both human and mouse spint1 derived deletion variants. The level of homology between human and mouse spint1 ORF is ~88%, see Figure 15.

Example 6. Identification and validation of SPINT1 and TMPRSS4 as novel modulators of TNFA signaling.

[00134] A deletion variant of mouse Kunitz-type membrane-associated serine protease inhibitor, Spint1-d1604, and another one encoding full-length human trans-membrane serine protease 4 (TMPRSS4) were confirmed to inhibit the TNFA induced activation of NF-kB reporter (Fig. 16). The inhibitory effects of both cDNA variants were stronger than that of cDNA encoding wild type form of IkBα used as a positive control for NF-KB pathway inhibition.

[00135] Wild type SPINT1 (also known as HAI-1) is produced as a type 1 transmembrane protein. The SPINT1-d1604 variant comprises 3'-terminal deletion removing almost the entire region coding for a short C-terminal intracellular domain of the protein, while retaining the transmembrane and extracellular regions.

[00136] To assess the importance of this deletion for manifestation of the observed phenotype, we also tested the ability of wt SPINT1 to influence TNFA-inducible NF-KB transcription. A reporter gene assay (Fig 16A) showed that transient overexpression of full-length SPINT1 cDNA also inhibited the TNFA/NF-kB activation (50% inhibition, as compared to empty vector control). However, this inhibitory effect was significantly weaker than that of SPINT1-d1604 (Fig. 16A). In addition, a frame shift mutation that disrupts ORF of SPINT1-d1604, completely abolished the inhibition (Fig.16A), strongly suggesting that synthesis of the truncated SPINT1 protein was required to inhibit TNFA/NF-KB signaling.

[00137] To assess the effects of stable expression of SPINT1-d1604 and TMPRSS4 on the TNFA dependent NF-KB activity, NF-KB/GFP cells (with integrated NF-κB-GFP reporter construct) were transduced with recombinant retroviruses containing either SPINT1-d1604 or TMPRSS4 expression cassettes, stimulated with TNFA and analyzed by flow cytometry. We

found that both cDNA variants strongly inhibited NF-KB dependent accumulation of GFP positive cells over a broad range of TNFA concentrations ((Fig. 16D-F).

[00138] We next examined effects of SPINT1-d1604 and TMPRSS4 on endogenous transcriptional targets of TNFA/NF-KB pathway. To this end, the ability of TNFA to activate expression of two classical NF-KB-dependent genes, one encoding TNFA itself and another one encoding IκBα, was assessed by quantitative real time PCR. We found that levels of IkBα and TNFA mRNA were significantly (10- and 20-fold respectively) increased by TNFA in control cells, but not in the cells stably expressing SPINT1-d1604 and TMPRSS4 (Fig. 16B).

[00139] Since the primary mechanism whereby TNFA activates transcription factor NF-KB includes phosphorylation and degradation of IκBα protein, we examined status of IKBA in SPINT1-d1604 and TMPRSS4-expressing cells treated with TNFA. Western blot analysis showed that both phosphorylation and degradation of IKBA was impaired in SPINT1-d1604 and TMPRSS4-expressing cells (Fig. 16C). These data indicated that both SPINT1-d1604 and TMPRSS4 blocked TNFA/NF-KB signaling events upstream of IκB kinase (IKK) complex activation.

[00140] While the invention has been described with reference to particularly preferred embodiments and examples, those skilled in the art recognize that various modifications may be made to the invention without departing from the spirit and scope thereof.

[00141] All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety.

References

[00142] 1: Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase

disintegrin that releases tumour-necrosis factor-alpha from cells. Nature. 1997 Feb 20;385(6618):729-33.

[00143] 2: Brenner DA, O'Hara M, Angel P, Chojkier M, Karin M. Prolonged activation of jun and collagenase genes by tumour necrosis factor-alpha. Nature. 1989 Feb 16;337(6208):661-3.

[00144] 3: Chang J, Girgis L. Clinical use of anti-TNFαlpha biological agents--a guide for GPs. Aust Fam Physician. 2007 Dec;36(12):1035-8.

[00145] 4: Chen G, Goeddel DV. TNFαR1 signaling: a beautiful pathway. Science. 2002 May 31;296(5573):1634-5. Review.

[00146] 5: Chen G, Cao P, Goeddel DV. TNFαinduced recruitment and activation of the IKK complex require Cdc37 and Hsp90. Mol Cell. 2002 Feb;9(2):401-10.

[00147] 6: Hoffmann A, Levchenko A, Scott ML, Baltimore D. The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. Science. 2002 Nov 8;298(5596):1241-5.

[00148] 7: Lin YZ, Yao SY, Veach RA, Torgerson TR, Hawiger J. Inhibition of nuclear translocation of transcription factor NF-kappa B by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. J Biol Chem. 1995 Jun 16;270(24):14255-8.

[00149] 8: Locksley RM, Killeen N, Lenardo MJ. The TNFαand TNFαreceptor superfamilies: integrating mammalian biology. Cell. 2001 Feb 23;104(4):487-501. Review.

[00150] 9: Luo JL, Kamata H, Karin M. IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. J Clin Invest. 2005 Oct;115(10):2625-32. Review.

[00151] 10: May MJ, D'Acquisto F, Madge LA, Glöckner J, Pober JS, Ghosh S. Selective inhibition of NF-kappaB activation by a peptide that blocks the interaction of NEMO with the IkappaB kinase complex. Science. 2000 Sep 1;289(5484):1550-4.

[00152] 11: Micheau O, Tschopp J. Induction of TNFαreceptor I-mediated apoptosis via two sequential signaling complexes. Cell. 2003 Jul 25;114(2):181-90.

[00153] 12: Nelson DE, Ihekwaba AE, Elliott M, Johnson JR, Gibney CA, Foreman BE, Nelson G, See V, Horton CA, Spiller DG, Edwards SW, McDowell HP, Unitt JF, Sullivan E Grimley R, Benson N, Broomhead D, Kell DB, White MR. Oscillations in NF-kappaB signaling control the dynamics of gene expression. Science. 2004 Oct 22;306(5696):704-8.

[00154] 13: Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature. 1984 Dec 20-1985 Jan 2;312(5996):724-9.

[00155] 14: Pierce JW, Lenardo M, Baltimore D. Oligonucleotide that binds nuclear factor NF-kappa B acts as a lymphoid-specific and inducible enhancer element. Proc Natl Acad Sci U S A. 1988 Mar;85(5):1482-6.

[00156] 15: Roshak AK, Callahan JF, Blake SM. Small-molecule inhibitors of NF-kappaB for the treatment of inflammatory joint disease. Curr Opin Pharmacol. 2002 Jun;2(3):316-21. Review.

[00157] 16: Shyamala V, Khoja H, Anderson ML, Wang JX, Cen H, Kavanaugh WM. High-throughput screening for ligand-induced c-fos mRNA expression by branched DNA assay in Chinese hamster ovary cells. Anal Biochem. 1999 Jan 1;266(1):140-7.

[00158] 17: Suter-Crazzolara C, Klemm M, Reiss B. Reporter genes. Methods Cell Biol. 1995;50:425-38. No abstract available.

[00159] 18: Takada Y, Singh S, Aggarwal BB. Identification of a p65 peptide that selectively inhibits NF-kappa B activation induced by various inflammatory stimuli and its role in down-regulation of NF-kappaB-mediated gene expression and up-regulation of apoptosis. J Biol Chem. 2004 Apr 9;279(15):15096-104. Epub 2004 Jan 7.

[00160] 19: Tang P, Hung M-C, Klostergaard J. Human pro-tumor necrosis factor is a homotrimer. Biochemistry. 1996 Jun 25;35(25):8216-25.

[00161] 20: Wang J, Shen L, Najafi H, Kolberg J, Matschinsky FM, Urdea M, German M. Regulation of insulin preRNA splicing by glucose. Proc Natl Acad Sci U S A. 1997 Apr 29;94(9):4360-5.

What is claimed is:

1. A pharmaceutical composition comprising a therapeutically effective amount of a SPINT1 polypeptide or derivative, variants, or fragments thereof, and a pharmaceutically acceptable excipient.

2. A pharmaceutical composition comprising a therapeutically effective amount of a TMPRSS4 polypeptide or derivative, variants, or fragments thereof, and a pharmaceutical acceptable excipient.

3. A polypeptide having 95% sequence identity to the human spint1 deletion variants and having TNFα inhibitory activity.

4. A polypeptide having 95% sequence identity to the mouse spint1 deletion variants and having TNFα inhibitory activity.

5. A polypeptide having 98% sequence identity to the human spint1 deletion variant and having TNFα inhibitory activity.

6. A polypeptide having 98% sequence identity to the mouse spint1 deletion variant and having TNFα inhibitory activity.

7. A method of treating a TNFα associated disease comprising administrating a subject in need thereof with the compositions of any one of claims 1 to 6.

8. A method for screening for compounds that have TNFα modulating activity comprising contacting a cell expressing the SPINT1 and TMPRSS4 polypeptides of the present invention with a test agent and detecting whether TNFα activity is affected.

9. A method for screening for compounds that have SPINT1 and TMPRSS4 polypeptide binding activity comprising contacting a SPINT1 and TMPRSS4 polypeptide of the present invention with a test agent and detecting whether the SPINT1 and TMPRSS4 polypeptide binds to the test agent.

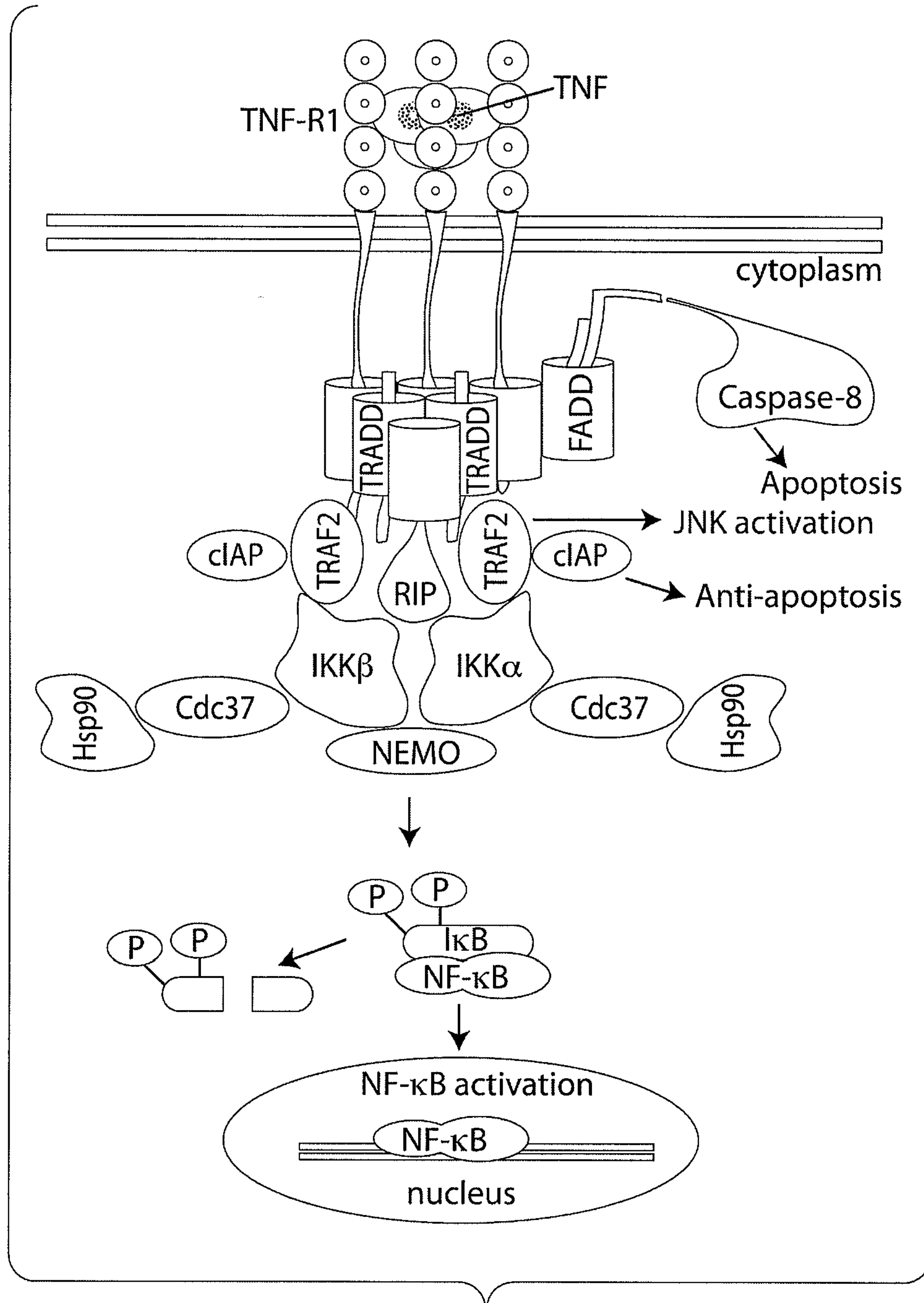

Fig. 1

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     SalI
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1    GTCGACCCACGCGTCCGGTCACAAGCACCCTCGGAACCCGGAGGCTCGCACTGTGAAGGTGACCCCCGCCTCGGGGAGGAGGGCGACGGCCCCTGGGAGC
     CAGCTGGGTGCGCAGGCCAGTGTTCGTGGGAGCCTTGGGCCTCCGAGCGTGACACTTCCACTGGGGGCGGAGCCCCTCCTCCCGCTGCCGGGGACCCCTCG
     MetAlaGlyArgArgLeuAlaArgAlaSerIleSerAlaValGlyValTrpLeuLeuCysAlaLeuGlyLeuGlnAlaThrGluAlaGluLeuProSer·
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
101  ACGATGGCGGGCCGCCGCCTGGCGCGGGCCAGCATCTCTGCGGTCGGCGTATGGCTCCTTTGCGCGCTTGGCCTCCAGGCCACCGAGGCCGAGCTGCCGT
     TGCTACCGCCCGGCGGCGGACCGCGCCCGGTCGTAGAGACGCCAGCCGCATACCGAGGAAACGCGCGAACCGGAGGTCCGGTGGCTCCGGCTCGACGGCA
     ·SAlaProAlaGluLeuProGlyGlyAlaAlaCysLeuSerArgPheThrSerGlyValProSerPheValLeuAspThrGluAlaSerValSerAsnGly·
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
201  CCGCACCCGCTGAGCTTCCCGGGGGAGCCGCCTGCTTGAGCCGCTTTACCAGCGGGGTGCCGTCCTTTGTGCTGGACACCGAAGCCTCAGTCAGCAACGG
     GGCGTGGGCGACTCGAAGGGCCCCCTCGGCGGACGAACTCGGCGAAATGGTCGCCCCACGGCAGGAAACACGACCTGTGGCTTCGGAGTCAGTCGTTGCC
     ·AlaThrPheLeuGlySerProThrAlaArgArgGlyTrpAspCysValArgSerCysCysThrThrGlnAsnCysAsnLeuAlaLeuValGluLeuGln
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
301  GGCCACCTTCCTGGGTTCCCCGACGGCGCGCCGCGGCTGGGACTGCGTGCGCTCCTGCTGCACCACTCAGAACTGCAACCTGGCGCTGGTGGAGCTGCAG
     CCGGTGGAAGGACCCAAGGGGCTGCCGCGCGGCGCCGACCCTGACGCACGCGAGGACGACGTGGTGAGTCTTGACGTTGGACCGCGACCACCTCGACGTC
     ProAspGlyGlyGluAspAlaIleSerAlaCysPheLeuMetAsnCysLeuTyrGluGlnAsnPheValCysLysPheAlaProLysGluGlyPheIleAsn·
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
401  CCAGACGGCGGCGAGGACGCCATCTCTGCCTGCTTCCTCATGAACTGCCTGTACGAGCAGAACTTCGTGTGCAAGTTCGCTCCGAAGGAGGGCTTCATCA
     GGTCTGCCGCCGCTCCTGCGGTAGAGACGGACGAAGGAGTACTTGACGGACATGCTCGTCTTGAAGCACACGTTCAAGCGAGGCTTCCTCCCGAAGTAGT
     ·ATyrLeuThrGlnGluLeuTyrArgSerTyrArgGluLeuArgThrArgGlyPheGlyGlySerArgIleProArgIleTrpMetGlyIleAspLeuLys·
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
501  ACTATCTTACCCAGGAGCTTTACCGCTCCTACCGCGAGCTGCGGACTCGAGGCTTTGGAGGGTCCCGGATCCCTCGAATCTGGATGGGCATAGACTTGAA
     TGATAGAATGGGTCCTCGAAATGGCGAGGATGGCGCTCGACGCCTGAGCTCCGAAACCTCCCAGGGCCTAGGGAGCTTAGACCTACCCGTATCTGAACTT
     ·ValGlnLeuGlnLysProLeuValLeuAsnGluAlaAspAsnThrAspTrpHisLeuLeuGlnGlyAspSerAspValArgValGluArgLysArgPro
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
601  GGTCCAGCTGCAGAAGCCCCTGGTACTGAACGAGGCAGACAACACAGACTGGCACCTACTGCAAGGTGACAGTGATGTCAGAGTAGAGAGAAAACGTCCG
     CCAGGTCGACGTCTTCGGGGACCATGACTTGCTCCGTCTGTTGTGTCTGACCGTGGATGACGTTCCACTGTCACTACAGTCTCATCTCTCTTTTGCAGGC
     GluGluValGluLeuTrpGlyLeuLysGluGlyThrTyrLeuPheGlnLeuThrArgThrAspSerAspGlnProGluGluThrAlaAsnLeuThrIleThr·
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Fig. 2A-1

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701   GAAGAAGTGGAGCTGTGGGGACTTAAGGAGGGGACCTACCTGTTCCAACTGACAAGAACTGACTCAGACCAACCAGAGGAAACAGCCAACCTGACTATCA
      CTTCTTCACCTCGACACCCCTGAATTCCTCCCCTGGATGGACAAGGTTGACTGTTCTTGACTGAGTCTGGTTGGTCTCCTTTGTCGGTTGGACTGATAGT
      ·TValLeuThrAlaLysGlnThrGluAspTyrCysLeuAlaSerTyrLysValGlyArgCysArgGlySerPheProArgTrpTyrTyrAspProLysGlu·

801   CTGTGCTAACTGCCAAGCAGACAGAAGATTATTGCCTCGCATCCTACAAGGTGGGCCGCTGCCGGGGCTCCTTCCCACGCTGGTACTACGACCCCAAGGA
      GACACGATTGACGGTTCGTCTGTCTTCTAATAACGGAGCGTAGGATGTTCCACCCGGCGACGGCCCCGAGGAAGGGTGCGACCATGATGCTGGGGTTCCT
      ·GlnIleCysLysSerPheThrPheGlyGlyCysLeuGlyAsnLysAsnAsnTyrLeuArgGluGluGluCysMetLeuAlaCysLysAspValGlnGly

901   ACAGATCTGCAAAAGTTTTACTTTTGGAGGTTGCCTGGGCAACAAGAACAACTACCTTCGTGAGGAAGAGTGCATGCTAGCCTGCAAGGATGTGCAAGGA
      TGTCTAGACGTTTTCAAAATGAAAACCTCCAACGGACCCGTTGTTCTTGTTGATGGAAGCACTCCTTCTCACGTACGATCGGACGTTCCTACACGTTCCT
      IleSerProLysArgHisHisProValCysSerGlySerCysHisAlaThrGlnPheArgCysSerAsnGlyCysCysIleAspGlyPheLeuGluCysAsp·

1001  ATCTCACCGAAAAGGCACCATCCAGTGTGCTCCGGCAGCTGCCATGCCACCCAGTTCCGCTGTAGCAATGGCTGCTGTATCGATGGCTTCCTGGAGTGTG
      TAGAGTGGCTTTTCCGTGGTAGGTCACACGAGGCCGTCGACGGTACGGTGGGTCAAGGCGACATCGTTACCGACGACATAGCTACCGAAGGACCTCACAC
      ·AAspThrProAspCysProAspGlySerAspGluAlaThrCysGluLysTyrThrSerGlyPheAspGluLeuGlnAsnIleHisPheLeuSerAspLys·

1101  ATGACACGCCAGACTGCCCTGATGGCTCCGACGAGGCCACCTGTGAAAAATACACCAGCGGCTTTGATGAGCTTCAGAATATCCATTTCCTCAGTGACAA
      TACTGTGCGGTCTGACGGGACTACCGAGGCTGCTCCGGTGGACACTTTTTATGTGGTCGCCGAAACTACTCGAAGTCTTATAGGTAAAGGAGTCACTGTT
      ·GlyTyrCysAlaGluLeuProAspThrGlyPheCysLysGluAsnIleThrArgTrpTyrTyrAsnProPheSerGluArgCysAlaArgPheThrTyr

1201  AGGGTACTGTGCAGAGCTGCCAGACACTGGGTTTTGCAAGGAGAACATCACCCGCTGGTATTACAACCCATTCAGTGAACGCTGTGCCCGATTCACCTAT
      TCCCATGACACGTCTCGACGGTCTGTGACCCAAAACGTTCCTCTTGTAGTGGGCGACCATAATGTTGGGTAAGTCACTTGCGACACGGGCTAAGTGGATA
      GlyGlyCysTyrGlyAsnLysAsnAsnPheGluGluGluGlnGlnCysLeuGluSerCysArgGlyIleSerLysLysAspValPheGlyLeuArgArgGlu·
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Fig. 2A-2

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1301  GGTGGTTGCTATGGGAACAAGAACAACTTTGAGGAGGAACAGCAGTGCCTTGAGTCCTGCCGTGGCATCTCCAAGAAGGATGTGTTTGGTCTTCGGAGGG
      CCACCAACGATACCCTTGTTCTTGTTGAAACTCCTCCTTGTCGTCACGGAACTCAGGACGGCACCGTAGAGGTTCTTCCTACACAAACCAGAAGCCTCCC
      ~~~~~~~~~~~~~~~~~~

      ·GGlySerIleProThrValGlySerAlaGluValAlaIleAlaValPheLeuValIleCysIleIleValValLeuThrIleLeuGlyTyrCysPhePhe·
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1401  AAGGCTCCATTCCCACTGTAGGCTCCGCTGAGGTAGCTATTGCTGTATTTCTGGTCATCTGCATCATAGTGGTCTTAACCATCCTGGGCTACTGTTTCTT
      TTCCGAGGTAAGGGTGACATCCGAGGCGACTCCATCGATAACGACATAAAGACCAGTAGACGTAGTATCACCAGAATTGGTAGGACCCGATGACAAAGAA
                                                                                      PacI
                                                                                    ~~~~~~~~

      ·LysAsnProLeuIleAsnSTOP
      ~~~~~~~~~~~~~~~~~~~
1501  CAAGAACCCCTTAATTAACTAGTTAA
      GTTCTTGGGGAATTAATTGATCAATT
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Fig. 2A-3

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    EcoRI
    ~~~~~~
  1 GAATTCGGCACGAGGGAGAGGCAGCAGCTTGCTCAGCGGACAAGGATGCTGGGCGTGAGGGACCAAGGCCTGCCCTGCACTCGGGCCTCCTCCAGCCAGT
    CTTAAGCCGTGCTCCCTCTCCGTCGTCGAACGAGTCGCCTGTTCCTACGACCCGCACTCCCTGGTTCCGGACGGGACGTGAGCCCGGAGGAGGTCGGTCA
101 GCTGACCAGGGACTTCTGACCTGCTGGCCAGCCAGGACCTGTGTGGGGAGGCCCTCCTGCTGCCTTGGGGTGACAATCTCAGCTCCAGGCTACAGGGAGA
    CGACTGGTCCCTGAAGACTGGACGACCGGTCGGTCCTGGACACACCCCTCCGGGAGGACGACGGAACCCCACTGTTAGAGTCGAGGTCCGATGTCCCTCT
                                               MetLeuGlnAspProAspSerAspGlnProLeuAsnSerLeuAspValLysProLeuArgLysProArgIleProMet
201 CCGGGAGGATCACAGAGCCAGCATGTTACAGGATCCTGACAGTGATCAACCTCTGAACAGCCTCGATGTCAAACCCCTGCGCAAACCCCGTATCCCCATG
    GGCCCTCCTAGTGTCTCGGTCGTACAATGTCCTAGGACTGTCACTAGTTGGAGACTTGTCGGAGCTACAGTTTGGGGACGCGTTTGGGGCATAGGGGTAC
    GluThrPheArgLysValGlyIleProIleIleIleAlaLeuLeuSerLeuAlaSerIleIleIleValValValLeuIleLysValIleLeuAspLysTyr·
301 GAGACCTTCAGAAAGGTGGGGATCCCCATCATCATAGCACTACTGAGCCTGGCGAGTATCATCATTGTGGTTGTCCTCATCAAGGTGATTCTGGATAAAT
    CTCTGGAAGTCTTTCCACCCCTAGGGGTAGTAGTATCGTGATGACTCGGACCGCTCATAGTAGTAACACCAACAGGAGTAGTTCCACTAAGACCTATTTA
    ·TTyrPheLeuCysGlyGlnProLeuHisPheIleProArgLysGlnLeuCysAspGlyGluLeuAspCysProLeuGlyGluAspGluGluHisCysVal·
401 ACTACTTCCTCTGCGGGCAGCCTCTCCACTTCATCCCGAGGAAGCAGCTGTGTGACGGAGAGCTGGACTGTCCCTTGGGGGAGGACGAGGAGCACTGTGT
    TGATGAAGGAGACGCCCGTCGGAGAGGTGAAGTAGGGCTCCTTCGTCGACACACTGCCTCTCGACCTGACAGGGAACCCCCTCCTGCTCCTCGTGACACA
    ·LysSerPheProGluGlyProAlaValAlaValArgLeuSerLysAspArgSerThrLeuGlnValLeuAspSerAlaThrGlyAsnTrpPheSerAla
501 CAAGAGCTTCCCCGAAGGGCCTGCAGTGGCAGTCCGCCTCTCCAAGGACCGATCCACACTGCAGGTGCTGGACTCGGCCACAGGGAACTGGTTCTCTGCC
    GTTCTCGAAGGGGCTTCCCGGACGTCACCGTCAGGCGGAGAGGTTCCTGGCTAGGTGTGACGTCCACGACCTGAGCCGGTGTCCCTTGACCAAGAGACGG
    CysPheAspAsnPheThrGluAlaLeuAlaGluThrAlaCysArgGlnMetGlyTyrSerSerLysProThrPheArgAlaValGluIleGlyProAspGln·
601 TGTTTCGACAACTTCACAGAAGCTCTCGCTGAGACAGCCTGTAGGCAGATGGGCTACAGCAGCAAACCCACTTTCAGAGCTGTGGAGATTGGCCCAGACC
    ACAAAGCTGTTGAAGTGTCTTCGAGAGCGACTCTGTCGGACATCCGTCTACCCGATGTCGTCGTTTGGGTGAAAGTCTCGACACCTCTAACCGGGTCTGG
    ·GAspLeuAspValValGluIleThrGluAsnSerGlnGluLeuArgMetArgAsnSerSerGlyProCysLeuSerGlySerLeuValSerLeuHisCys·
701 AGGATCTGGATGTTGTTGAAATCACAGAAAACAGCCAGGAGCTTCGCATGCGGAACTCAAGTGGGCCCTGTCTCTCAGGCTCCCTGGTCTCCCTGCACTG
    TCCTAGACCTACAACAACTTTAGTGTCTTTTGTCGGTCCTCGAAGCGTACGCCTTGAGTTCACCCGGGACAGAGAGTCCGAGGGACCAGAGGGACGTGAC
    ·LeuAlaCysGlyLysSerLeuLysThrProArgValValGlyGlyGluGluAlaSerValAspSerTrpProTrpGlnValSerIleGlnTyrAspLys
801 TCTTGCCTGTGGGAAGAGCCTGAAGACCCCCCGTGTGGTGGGTGGGGAGGAGGCCTCTGTGGATTCTTGGCCTTGGCAGGTCAGCATCCAGTACGACAAA
    AGAACGGACACCCTTCTCGGACTTCTGGGGGGCACACCACCCACCCCTCCTCCGGAGACACCTAAGAACCGGAACCGTCCAGTCGTAGGTCATGCTGTTT
    GlnHisValCysGlyGlySerIleLeuAspProHisTrpValLeuThrAlaAlaHisCysPheArgLysHisThrAspValPheAsnTrpLysValArgAla·
```

Fig. 2B-1

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 901 CAGCACGTCTGTGGAGGGAGCATCCTGGACCCCCACTGGGTCCTCACGGCAGCCCACTGCTTCAGGAAACATACCGATGTGTTCAACTGGAAGGTGCGGG
     GTCGTGCAGACACCTCCCTCGTAGGACCTGGGGGTGACCCAGGAGTGCCGTCGGGTGACGAAGTCCTTTGTATGGCTACACAAGTTGACCTTCCACGCCC
                                                                                   EcoRI
                                                                                   ~~~~~~
     ·AGlySerAspLysLeuGlySerPheProSerLeuAlaValAlaLysIleIleIleIleGluPheAsnProMetTyrProLysAspAsnAspIleAlaLeu·

1001 CAGGCTCAGACAAACTGGGCAGCTTCCCATCCCTGGCTGTGGCCAAGATCATCATCATTGAATTCAACCCCATGTACCCCAAAGACAATGACATCGCCCT
     GTCCGAGTCTGTTTGACCCGTCGAAGGGTAGGGACCGACACCGGTTCTAGTAGTAGTAACTTAAGTTGGGGTACATGGGGTTTCTGTTACTGTAGCGGGA
     ·MetLysLeuGlnPheProLeuThrPheSerGlyThrValArgProIleCysLeuProPhePheAspGluGluLeuThrProAlaThrProLeuTrpIle
1101 CATGAAGCTGCAGTTCCCACTCACTTTCTCAGGCACAGTCAGGCCCATCTGTCTGCCCTTCTTTGATGAGGAGCTCACTCCAGCCACCCCACTCTGGATC
     GTACTTCGACGTCAAGGGTGAGTGAAAGAGTCCGTGTCAGTCCGGGTAGACAGACGGGAAGAAACTACTCCTCGAGTGAGGTCGGTGGGGTGAGACCTAG
     IleGlyTrpGlyPheThrLysGlnAsnGlyGlyLysMetSerAspIleLeuLeuGlnAlaSerValGlnValIleAspSerThrArgCysAsnAlaAspAsp·
1201 ATTGGATGGGGCTTTACGAAGCAGAATGGAGGGAAGATGTCTGACATACTGCTGCAGGCGTCAGTCCAGGTCATTGACAGCACACGGTGCAATGCAGACG
     TAACCTACCCCGAAATGCTTCGTCTTACCTCCCTTCTACAGACTGTATGACGACGTCCGCAGTCAGGTCCAGTAACTGTCGTGTGCCACGTTACGTCTGC
     ·AAlaTyrGlnGlyGluValThrGluLysMetMetCysAlaGlyIleProGluGlyGlyValAspThrCysGlnGlyAspSerGlyGlyProLeuMetTyr·
1301 ATGCGTACCAGGGGGAAGTCACCGAGAAGATGATGTGTGCAGGCATCCCGGAAGGGGGTGTGGACACCTGCCAGGGTGACAGTGGTGGGCCCCTGATGTA
     TACGCATGGTCCCCCTTCAGTGGCTCTTCTACTACACACGTCCGTAGGGCCTTCCCCCACACCTGTGGACGGTCCCACTGTCACCACCCGGGGACTACAT
     ·GlnSerAspGlnTrpHisValValGlyIleValSerTrpGlyTyrGlyCysGlyGlyProSerThrProGlyValTyrThrLysValSerAlaTyrLeu
1401 CCAATCTGACCAGTGGCATGTGGTGGGCATCGTTAGCTGGGGCTATGGCTGCGGGGGCCCGAGCACCCCAGGAGTATACACCAAGGTCTCAGCCTATCTC
     GGTTAGACTGGTCACCGTACACCACCCGTAGCAATCGACCCCGATACCGACGCCCCCGGGCTCGTGGGGTCCTCATATGTGGTTCCAGAGTCGGATAGAG
     AsnTrpIleTyrAsnValTrpLysAlaGluLeuSTOP
1501 AACTGGATCTACAATGTCTGGAAGGCTGAGCTGTAATGCTGCTGCCCCTTTGCAGTGCTGGGAGCCGCTTCCTTCCTGCCCTGCCCACCTGGGGATCCCC
     TTGACCTAGATGTTACAGACCTTCCGACTCGACATTACGACGACGGGGAAACGTCACGACCCTCGGCGAAGGAAGGACGGGACGGGTGGACCCCTAGGGG
                                         PacI
                                       ~~~~~~~~
1601 CAAAGTCAGACACAGAGCAAGAGTCCCCTTAATTAACTAGTTAA
     GTTTCAGTCTGTGTCTCGTTCTCAGGGGAATTAATTGATCAATT
```

Fig. 2B-2

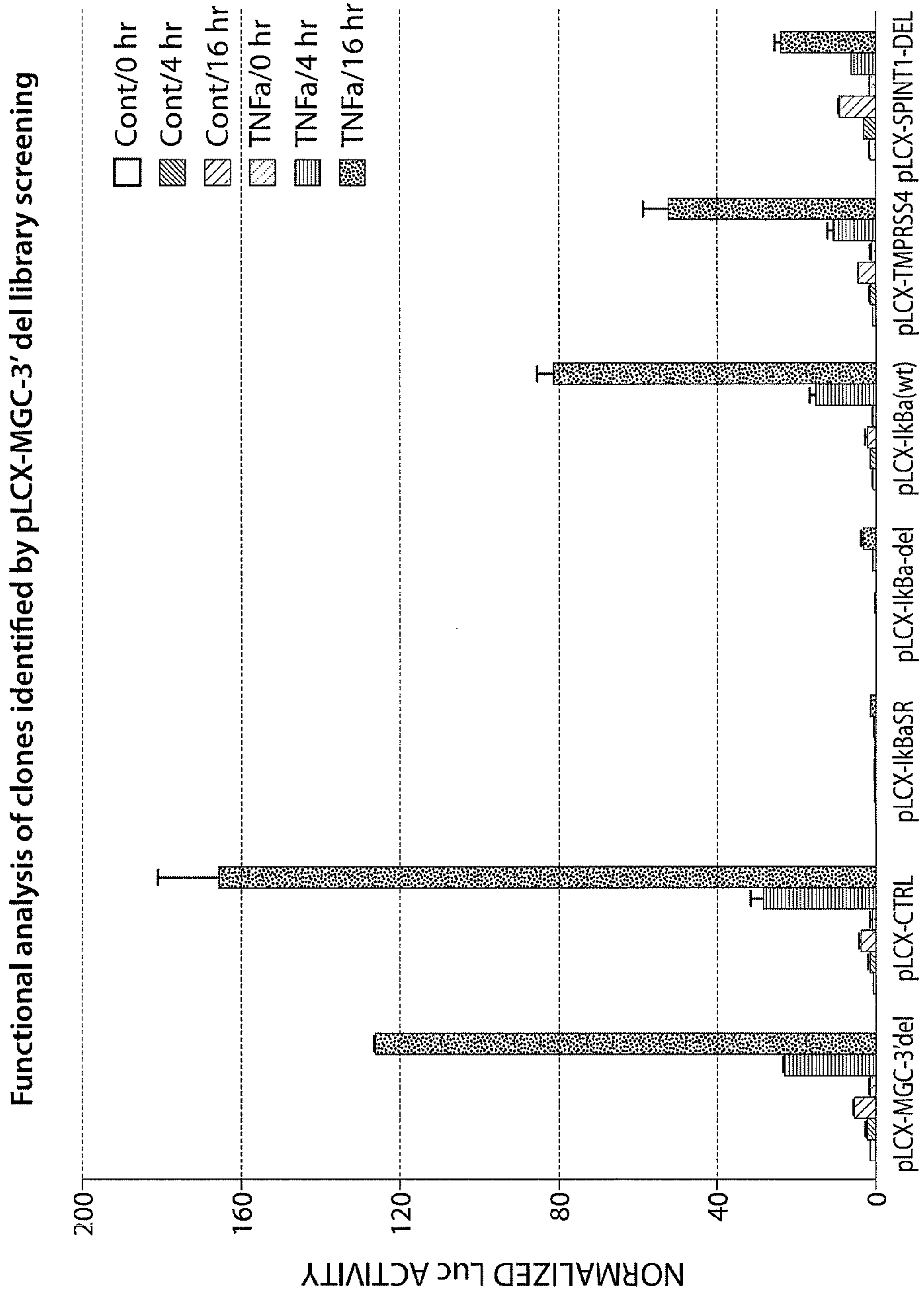

Fig. 3

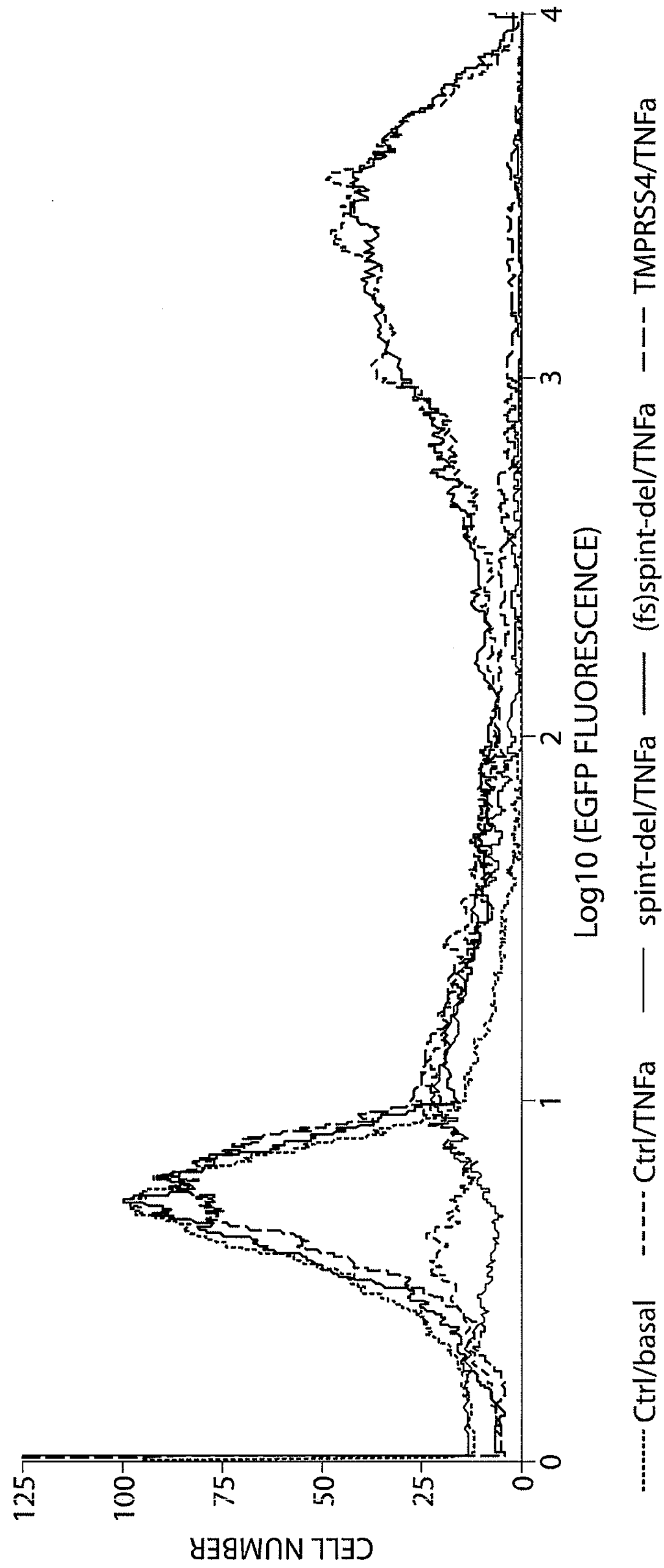

Fig. 4

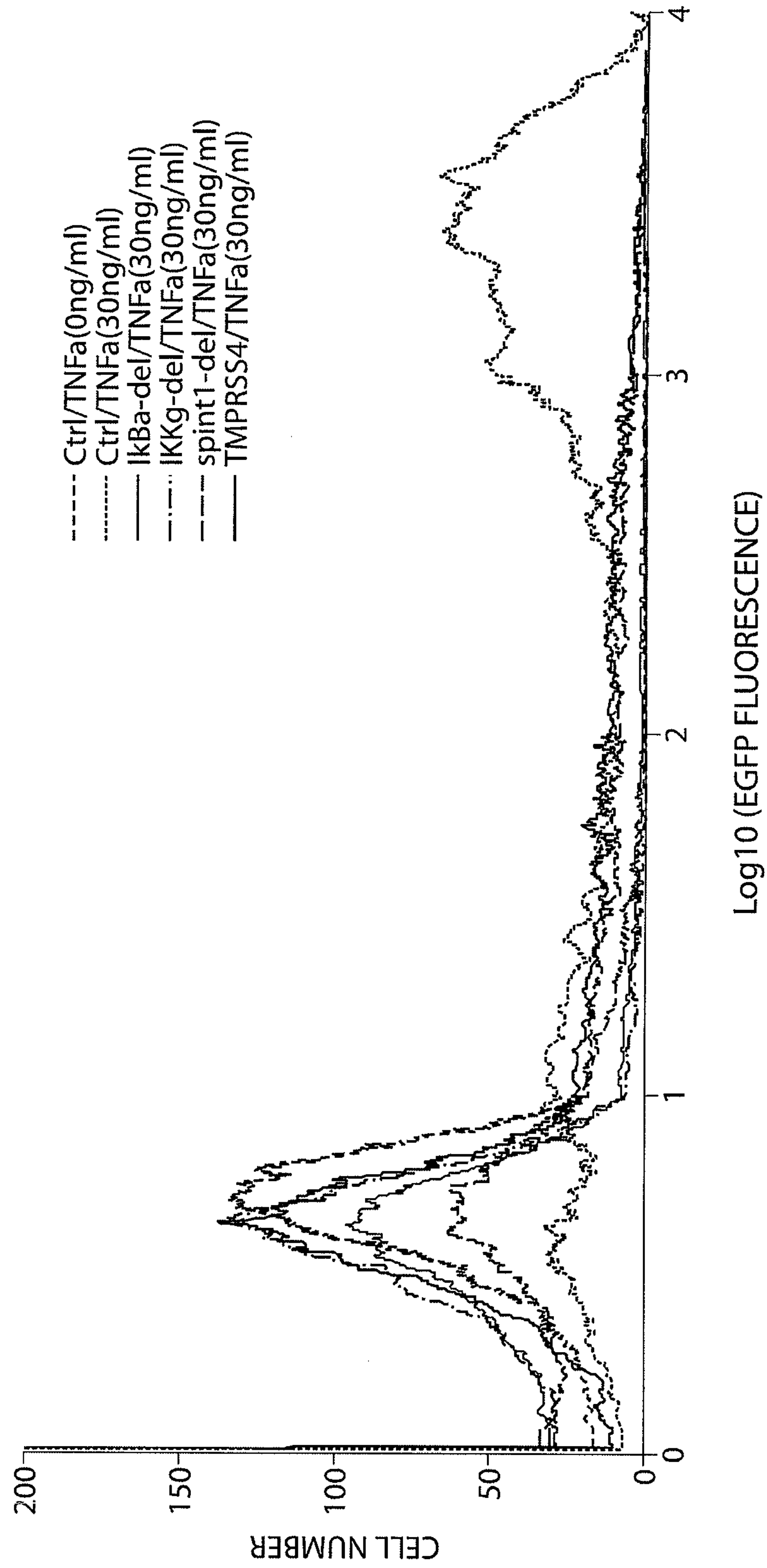

Fig. 5

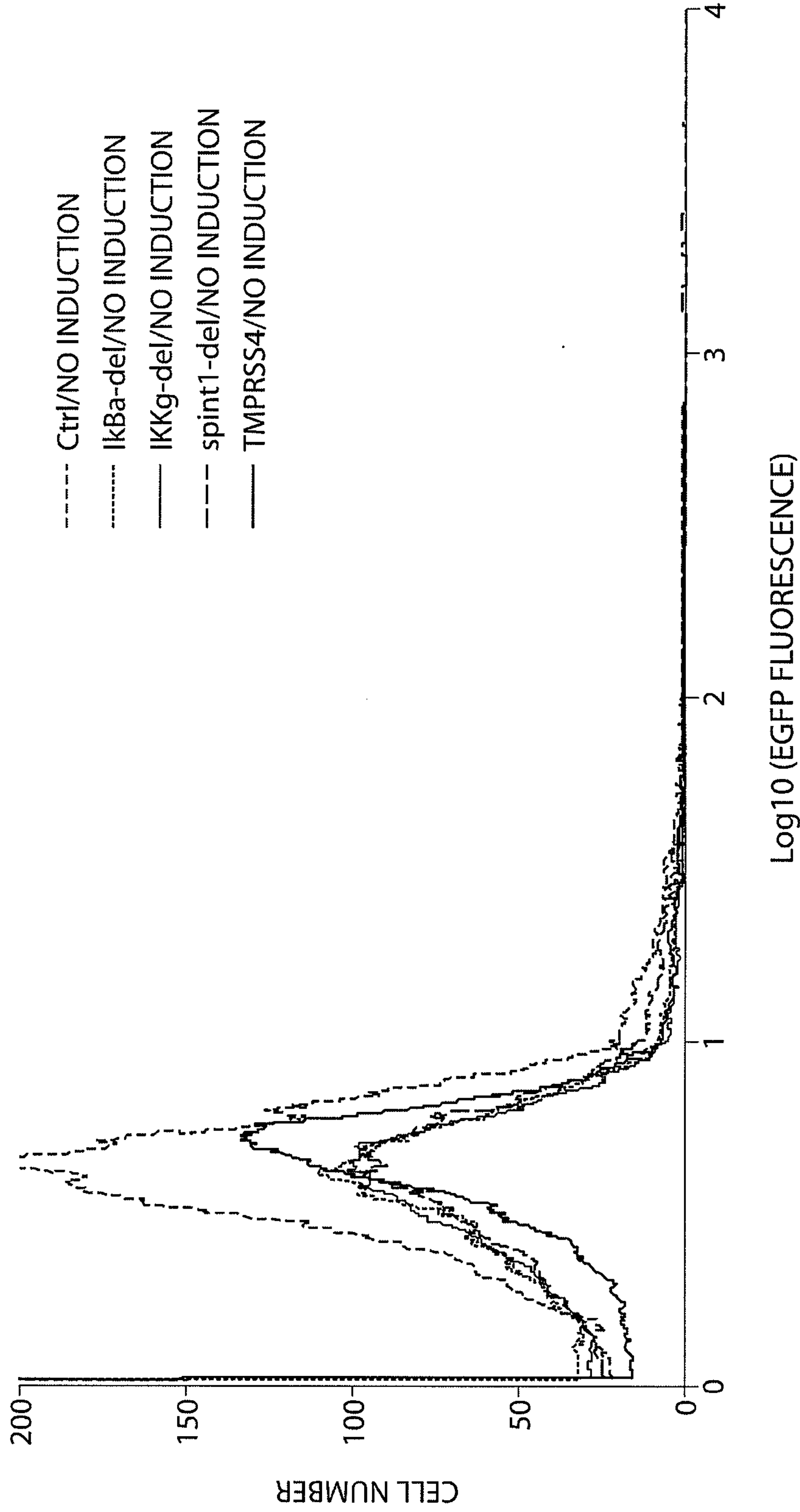

Fig. 6A

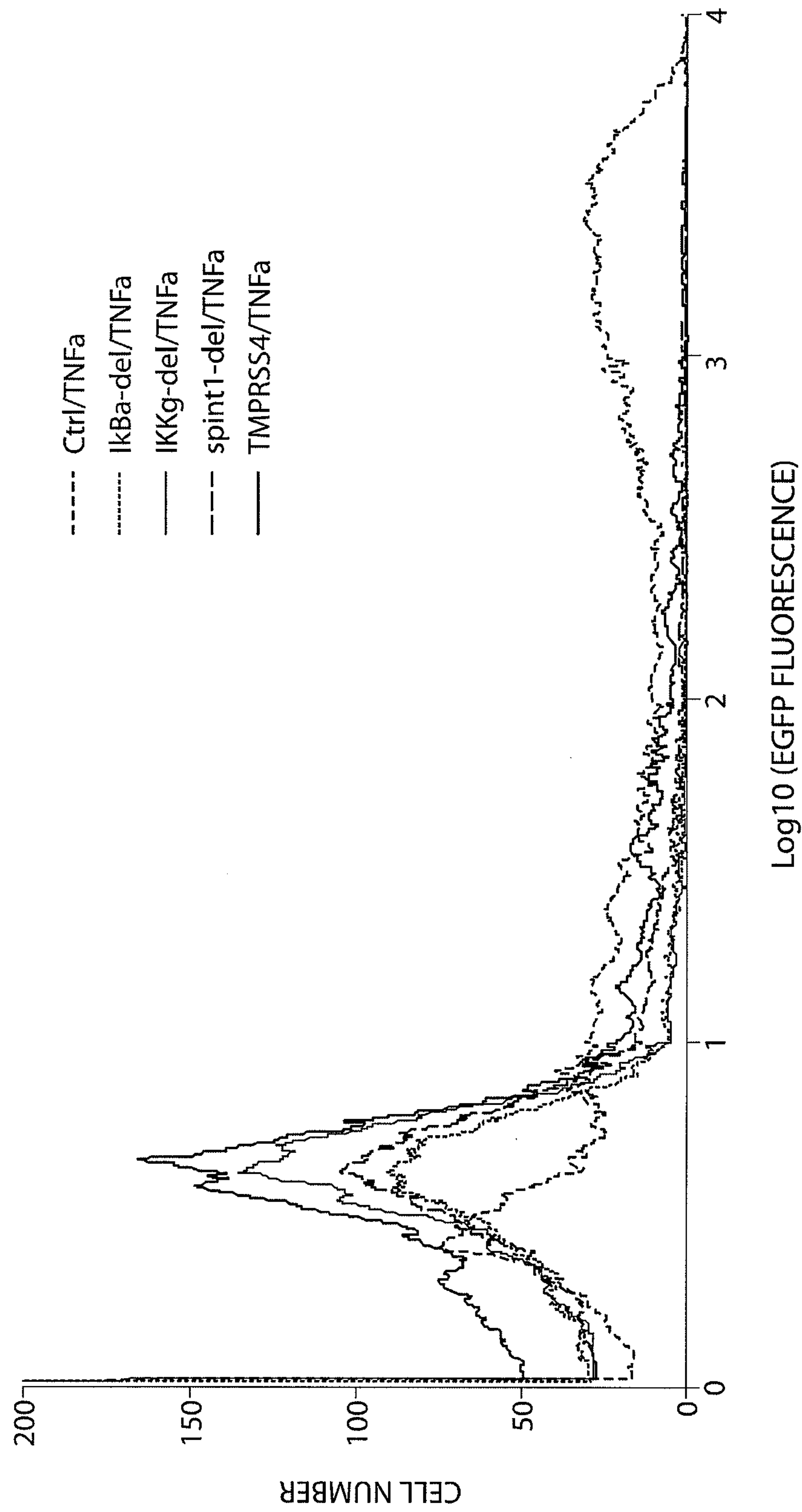

Fig. 6B

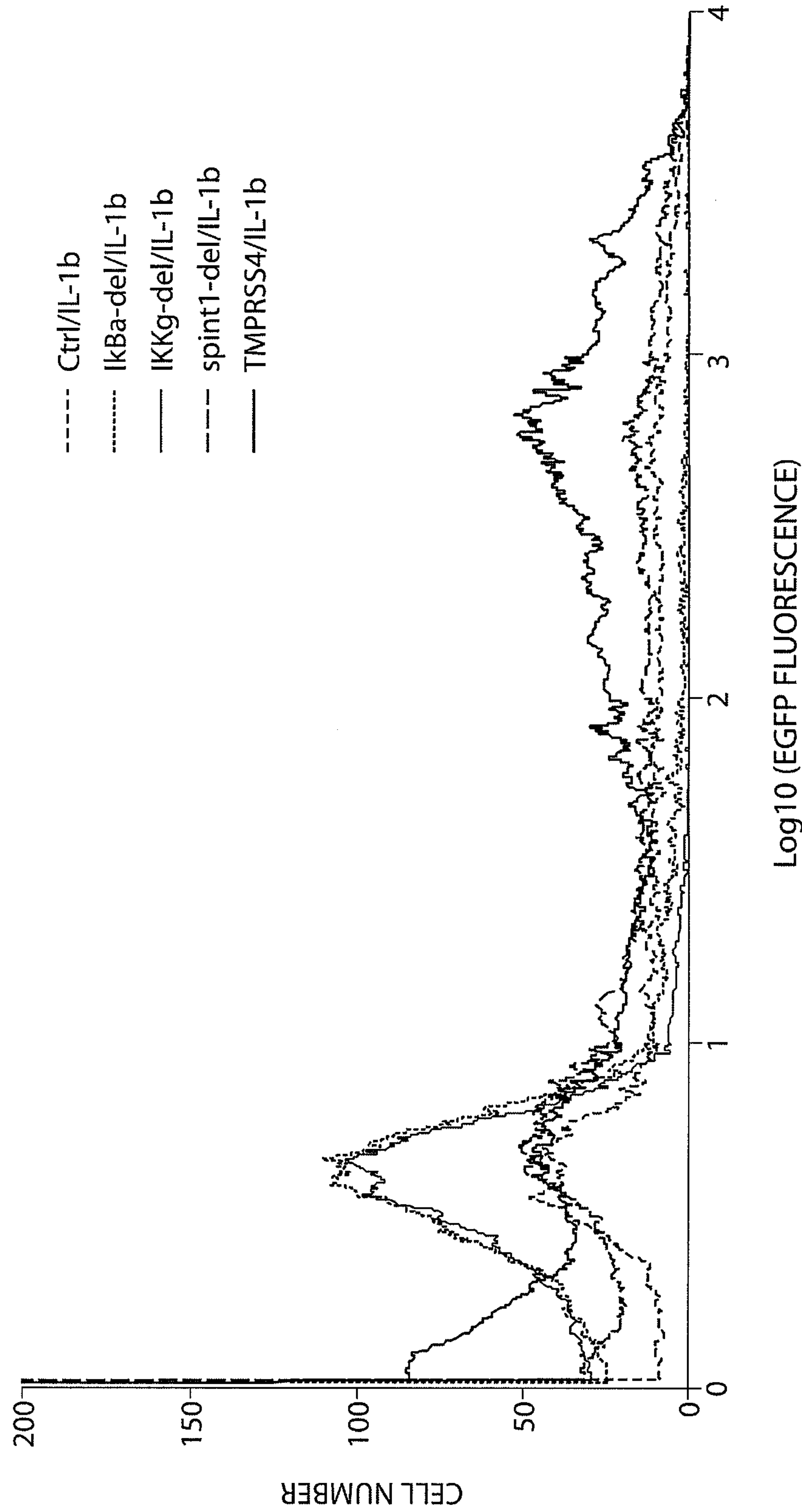

Fig. 6C

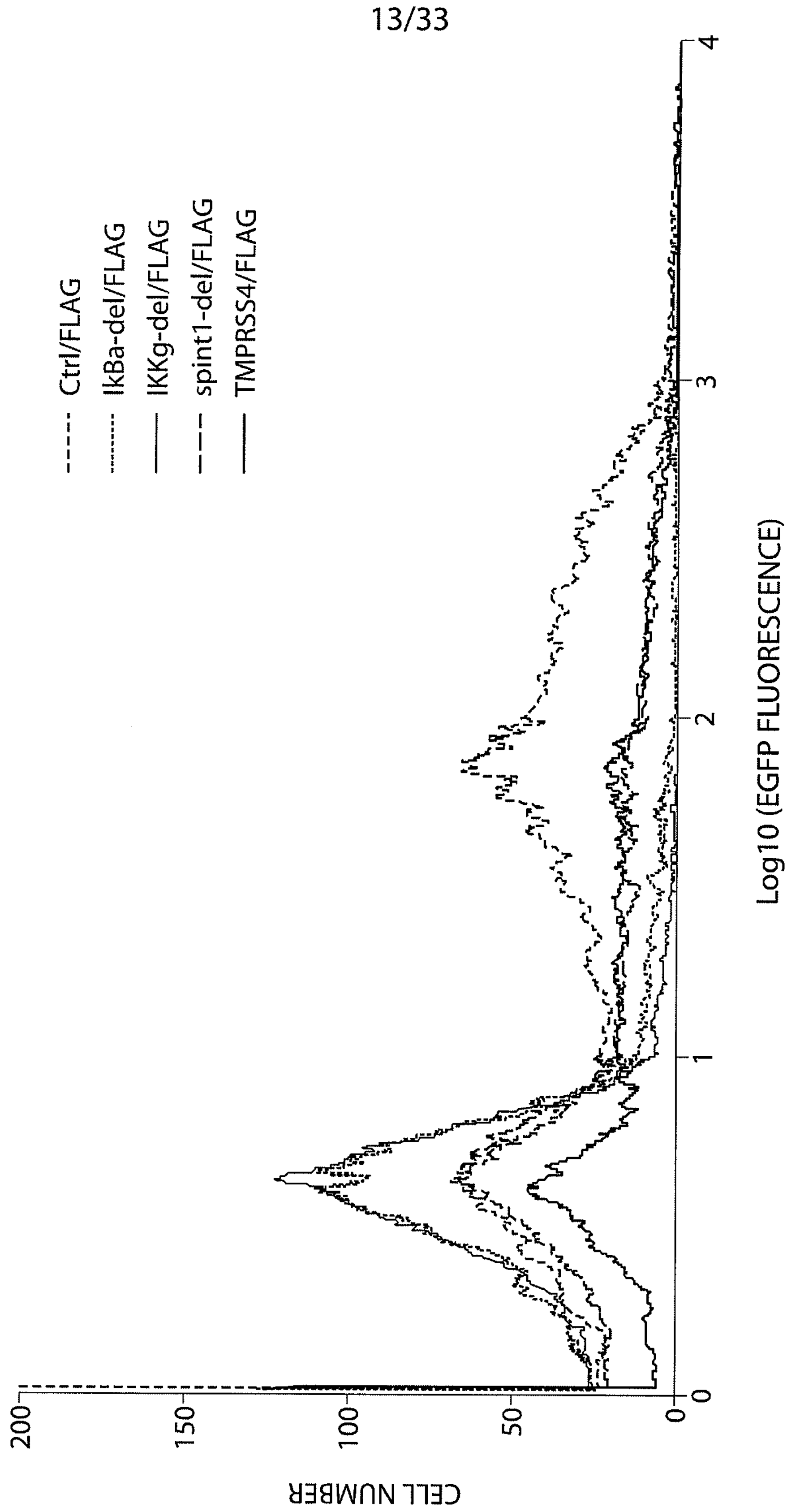

Fig. 6D

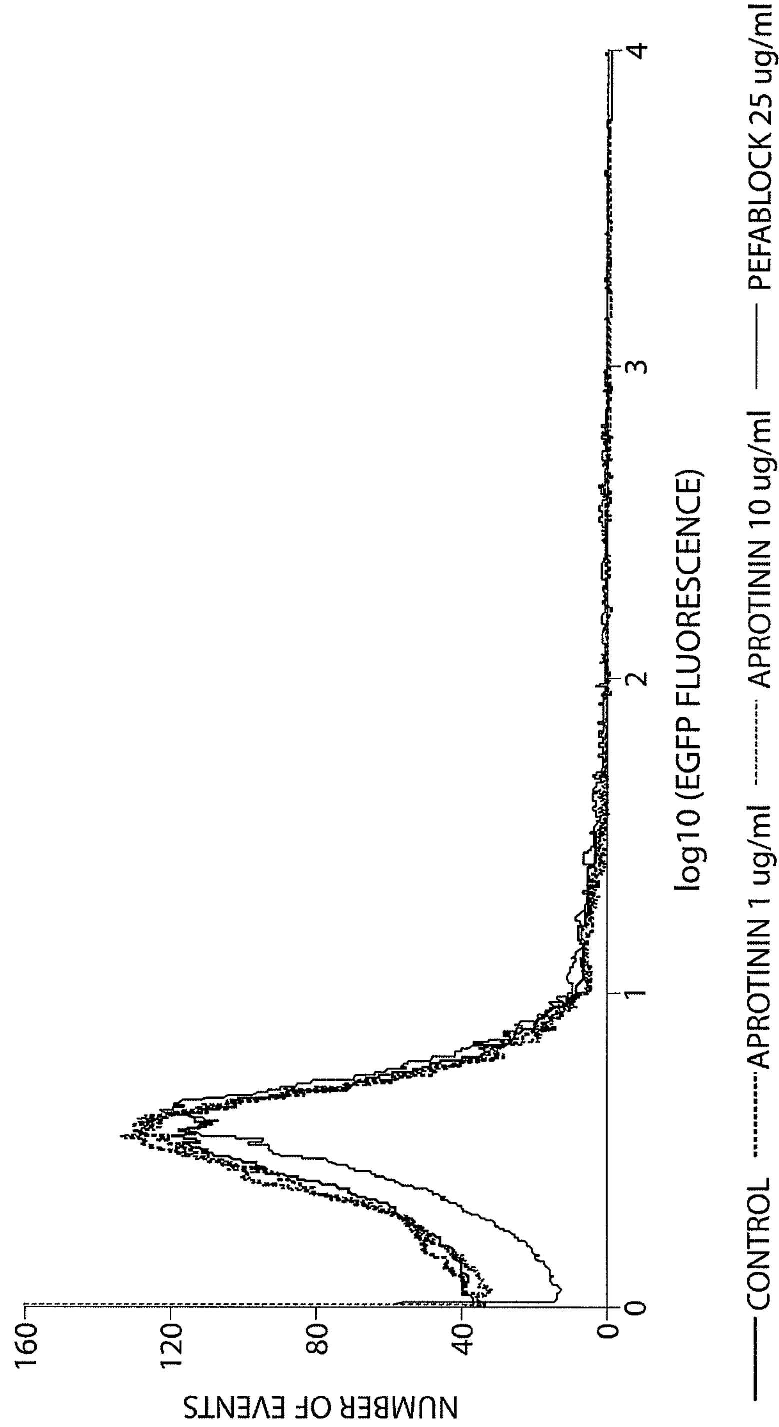

Fig. 7A

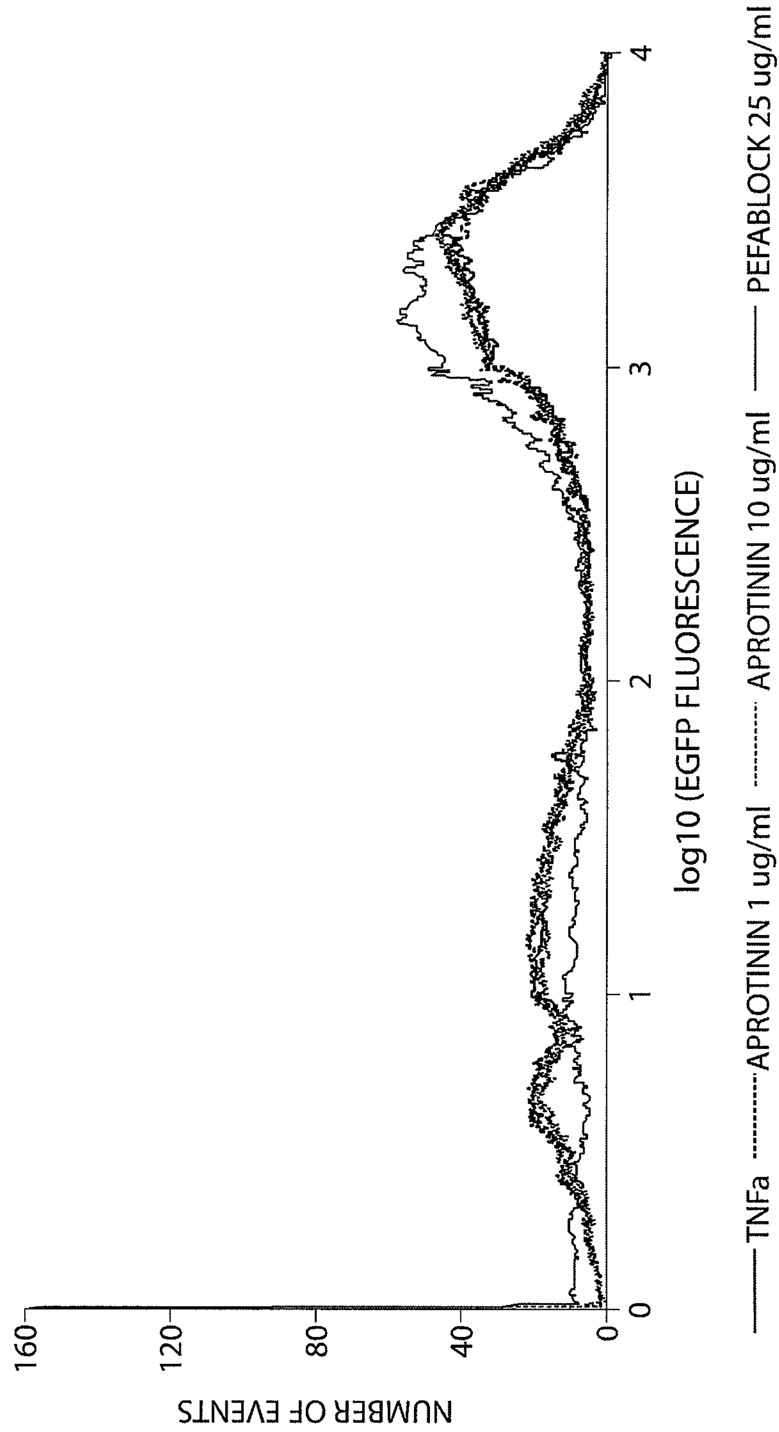

Fig. 7B

Effect of serine proteinase inhibitors on basal NF-kB activity in PE413-TMPRSS4 cells

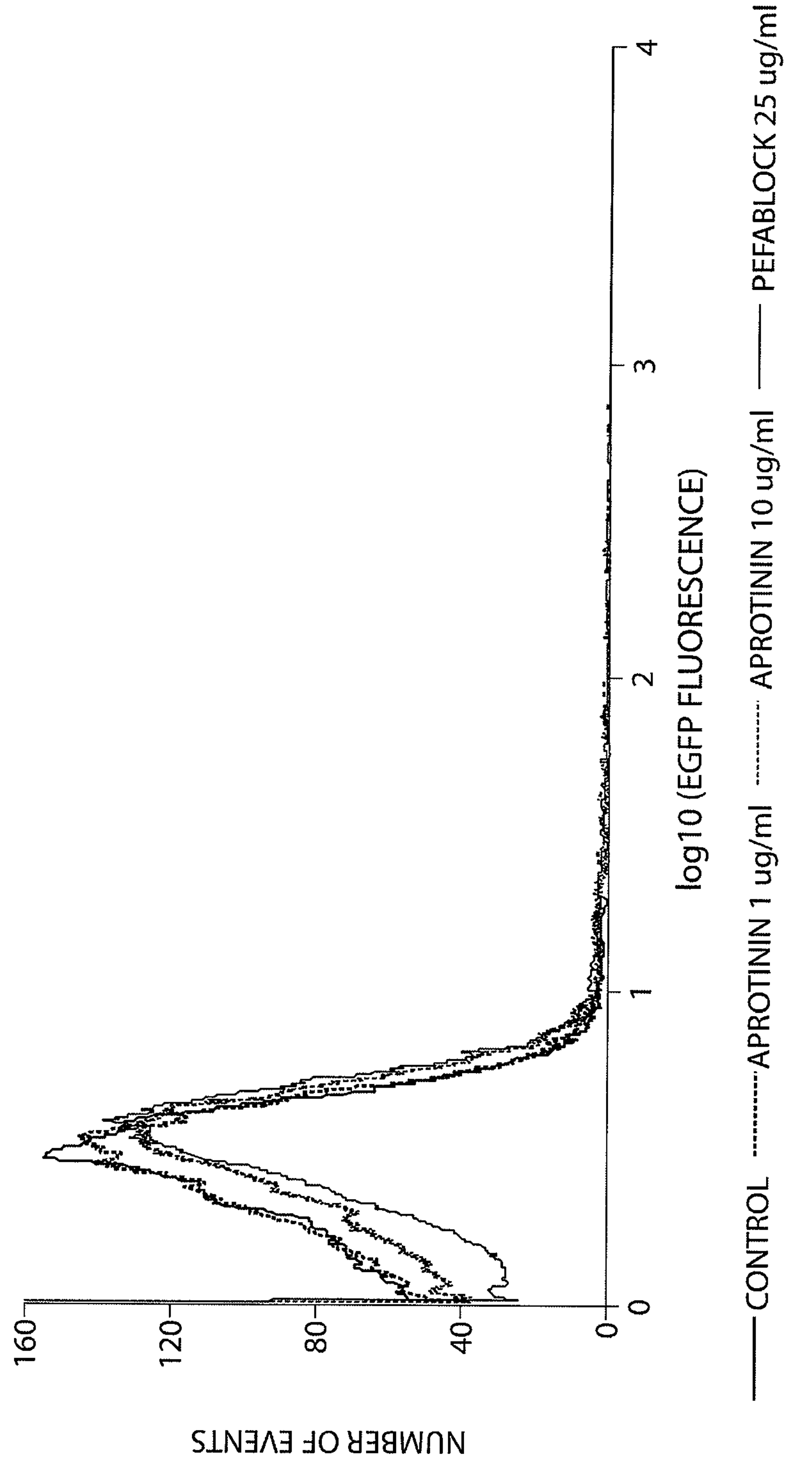

Fig. 7C

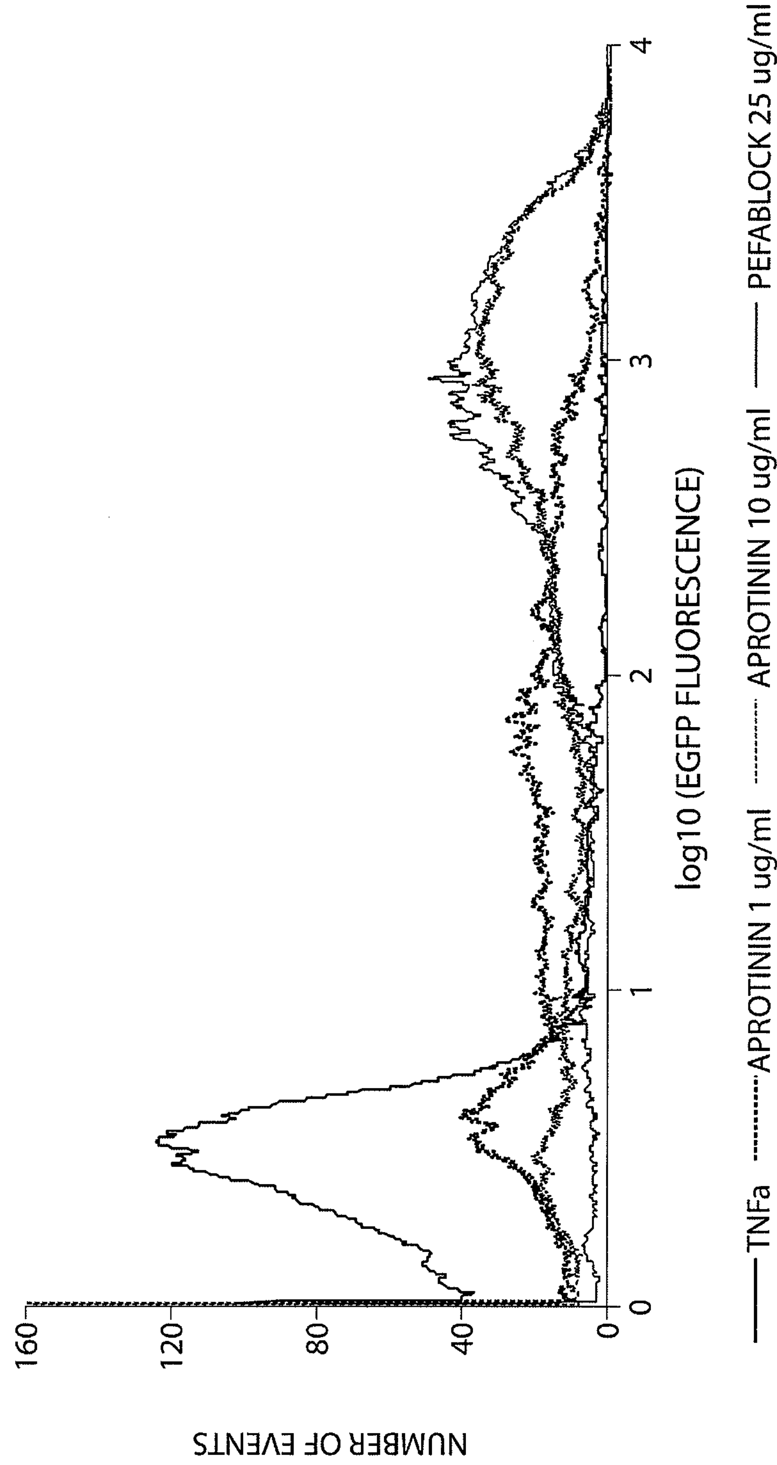

Fig. 7D

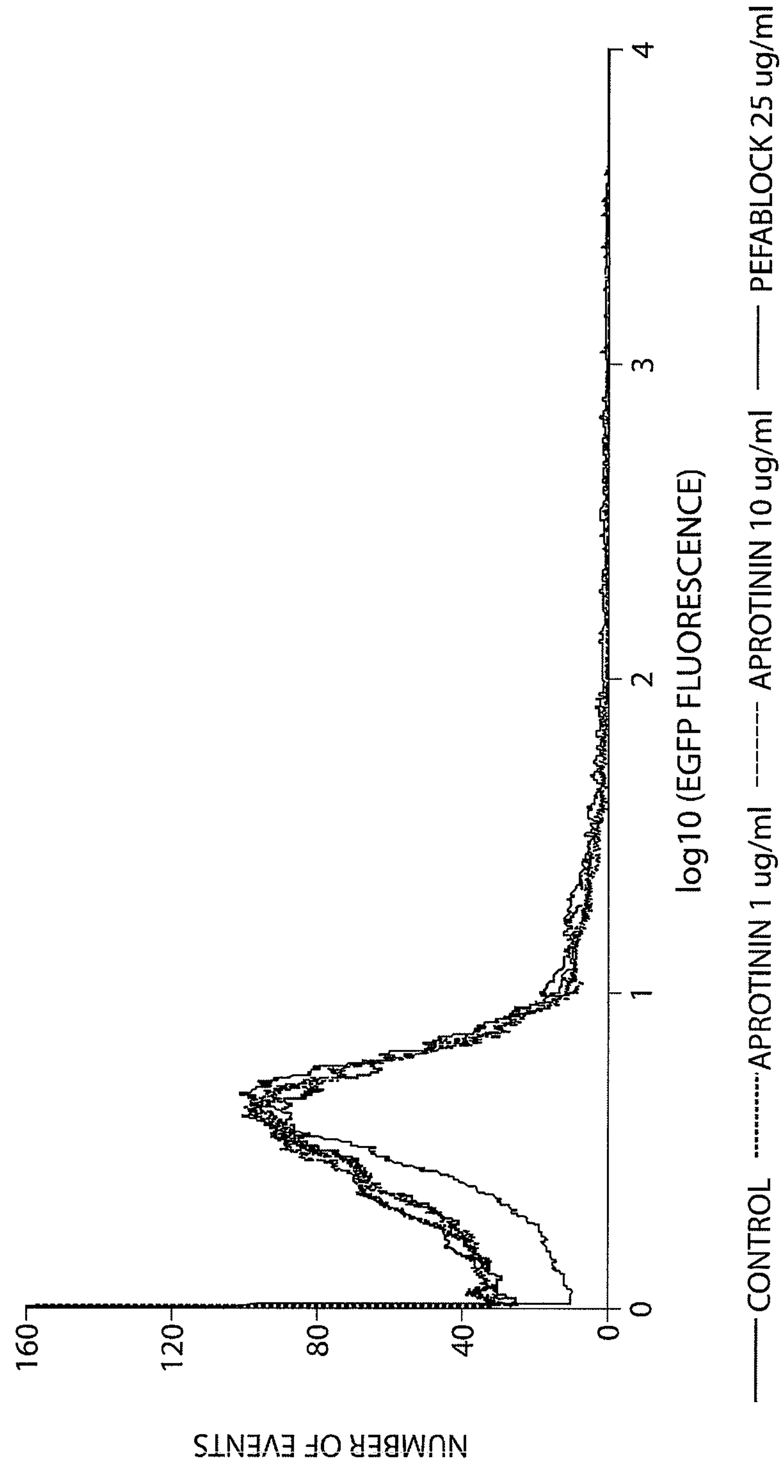

Fig. 7E

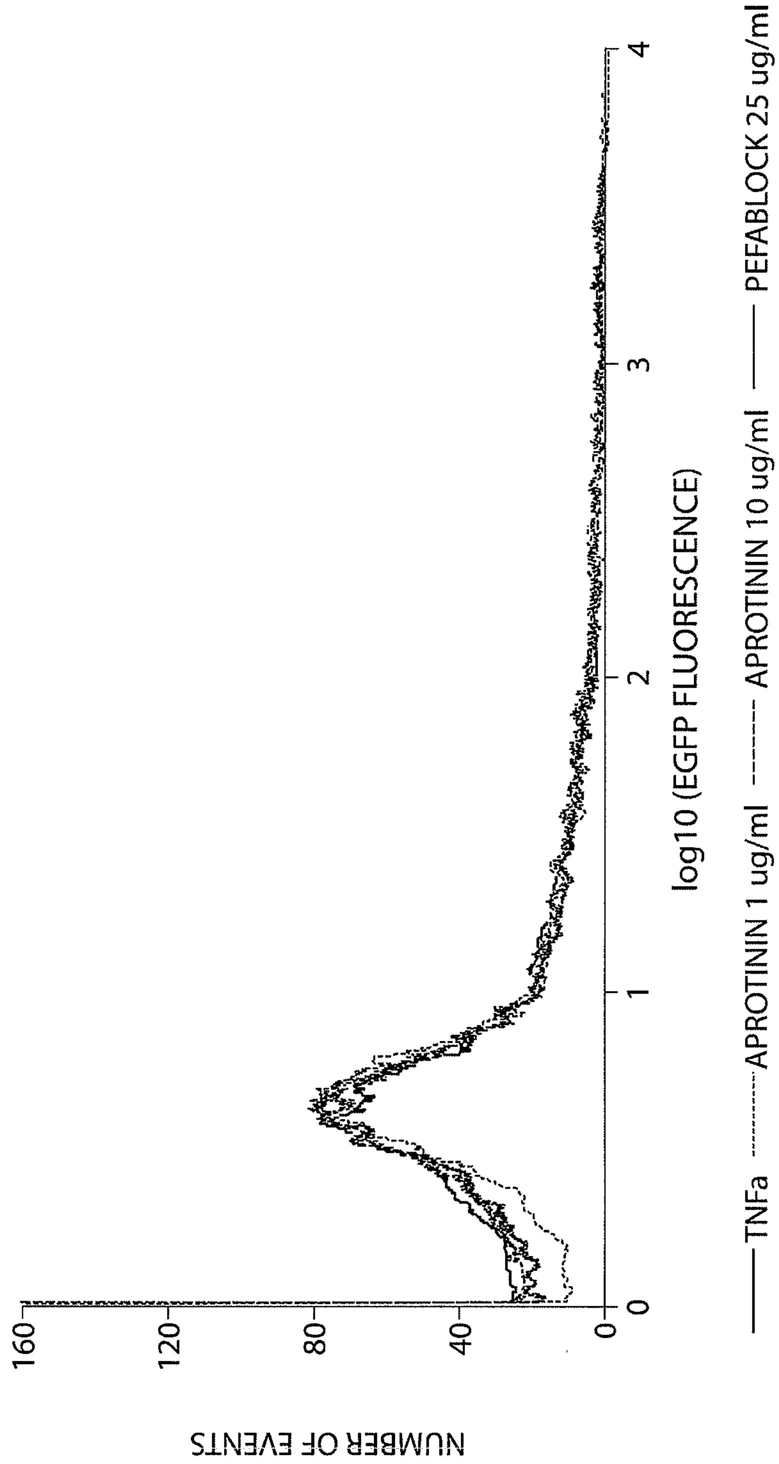

Fig. 7F

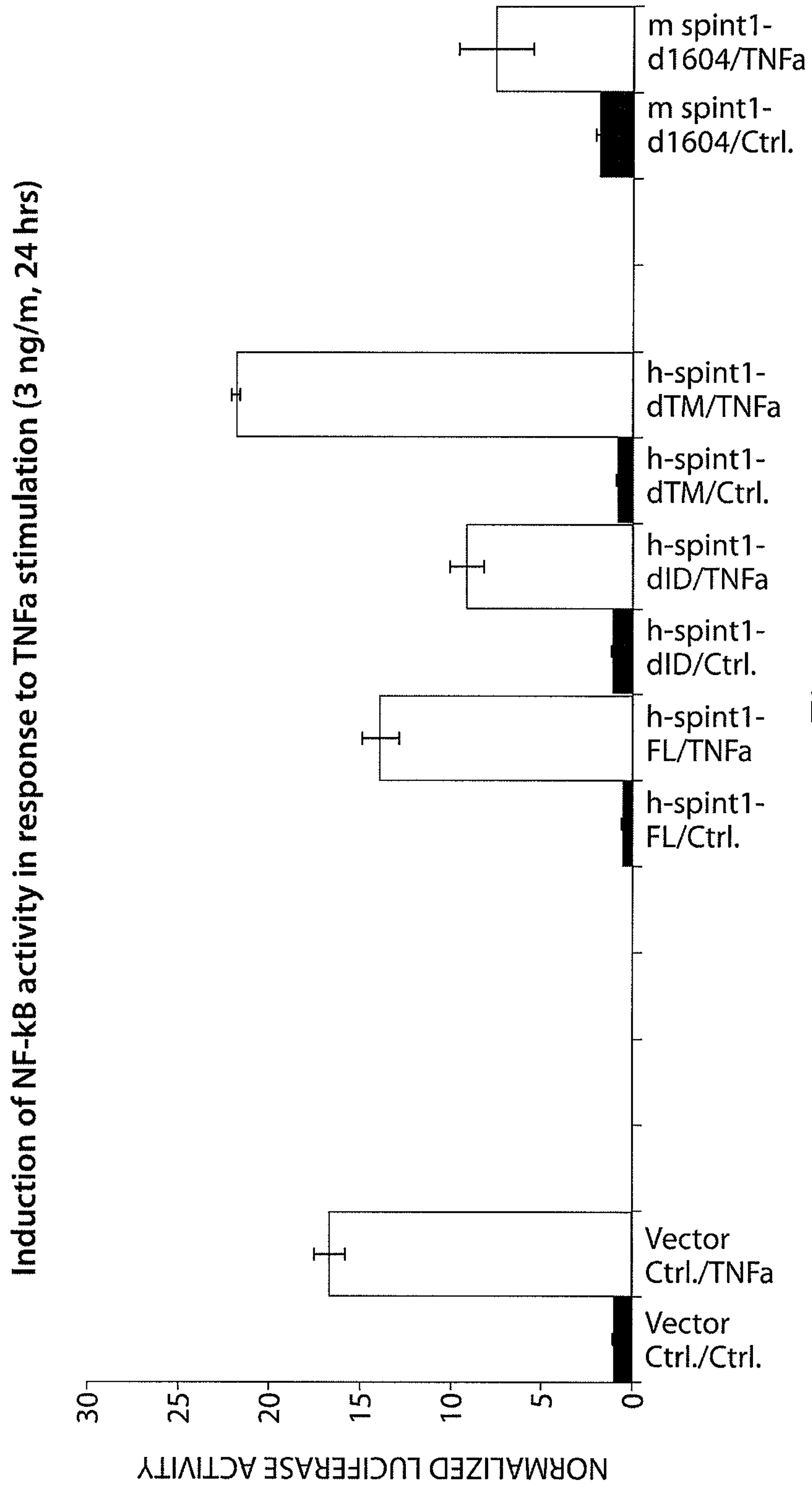

Fig. 8

```
Mouse spint1 domains:
Legend:
S – predicted signal peptide
O – extracellular domain
H - transmembrane helix (444-461)
I – intracellular domain

  mspint1 MAGRRLARAS ISAVGVWLLC ALGLQATEAE LPSAPAELPG GAACLSRFTS    50
          SSSSSSSSSS SSSSSSSSSS SSSSSSoooo oooooooooo o000000000

  mspint1 GVPSFVLDTE ASVSNGATFL GSPTARRGWD CVRSCCTTQN CNLALVELQP   100
          0000000000 0000000000 0000000000 0000000000 0000000000

  mspint1 DGGEDAISAC FLMNCLYEQN FVCKFAPKEG FINYLTQELY RSYRELRTRG   150
          0000000000 0000000000 0000000000 0000000000 0000000000

  mspint1 FGGSRIPRIW MGIDLKVQLQ KPLVLNEADN TDWHLLQGDS DVRVERKRPE   200
          0000000000 0000000000 0000000000 0000000000 0000000000

  mspint1 EVELWGLKEG TYLFQLTRTD SDQPEETANL TITVLTAKQT EDYCLASYKV   250
          0000000000 0000000000 0000000000 0000000000 0000000000

  mspint1 GRCRGSFPRW YYDPKEQICK SFTFGGCLGN KNNYLREEEC MLACKDVQGI   300
          0000000000 0000000000 0000000000 0000000000 0000000000

  mspint1 SPKRHHPVCS GSCHATQFRC SNGCCIDGFL ECDDTPDCPD GSDEATCEKY   350
          0000000000 0000000000 0000000000 0000000000 0000000000

  mspint1 TSGFDELQNI HFLSDKGYCA ELPDTGFCKE NIPRWYYNPF SERCARFTYG   400
          0000000000 0000000000 0000000000 0000000000 0000000000

  mspint1 GCYGNKNNFE EEQQCLESCR GISKKDVFGL RREGSIPTVG SAEVAIAVFL   450
          0000000000 0000000000 00000000oo oooooooooo oooHHHHHHH

  mspint1 VICIIVVLTI LGYCFFKNQR KEFHSPLHHP PPTPASSTVS TTEDTEHLVY   500
          HHHHHHHHHH Hiiiiiiiii iiiiiiIIII IIIIIIIIII IIIIIIIIII

  mspint1 NHTTQPL  507
          IIIIIII
```

Fig. 9

```
Human spint1 domains:
Legend:
S – predicted signal peptide
O – extracellular domain
H - transmembrane helix (444-461)
I – intracellular domain

  hspint1 MARARLAPAG IPAVALWLLC TLGLQGTQAG PPPAPPGLPA GADCLNSFTA    50
          SSSSSSSSSS SSSSSSSSSS SSSSSSoooo oooooooooo o000000000

  hspint1 GVPGFVLDTN ASVSNGATFL ESPTVRRGWD CVRACCTTQN CNLALVELQP   100
          0000000000 0000000000 0000000000 0000000000 0000000000

  hspint1 DRGEDAIAAC FLINCLYEQN FVCKFAPREG FINYLTREVY RSYRQLRTQG   150
          0000000000 0000000000 0000000000 0000000000 0000000000

  hspint1 FGGSGIPKAW AGIDLKVQPQ EPLVLKDVEN TDWRLLRGDT DVRVERKDPN   200
          0000000000 0000000000 0000000000 0000000000 0000000000

  hspint1 QVELWGLKEG TYLFQLTVTS SDHPEDTANV TVTVLSTKQT EDYCLASNKV   250
          0000000000 0000000000 0000000000 0000000000 0000000000

  hspint1 GRCRGSFPRW YYDPTEQICK SFVYGGCLGN KNNYLREEEC ILACRGVQGP   300
          0000000000 0000000000 0000000000 0000000000 0000000000

  hspint1 SMERRHPVCS GTCQPTQFRC SNGCCIDSFL ECDDTPNCPD ASDEAACEKY   350
          0000000000 0000000000 0000000000 0000000000 0000000000

  hspint1 TSGFDELQRI HFPSDKGHCV DLPDTGLCKE SIPRWYYNPF SEHCARFTYG   400
          0000000000 0000000000 0000000000 0000000000 0000000000

  hspint1 GCYGNKNNFE EEQQCLESCR GISKKDVFGL RREIPIPSTG SVEMAVAVFL   450
          0000000000 0000000000 00000000oo oooooooooo oooHHHHHHH

  hspint1 VICIVVVVAI LGYCFFKNQR KDFHGHHHHP PPTPASSTVS TTEDTEHLVY   500
          HHHHHHHHHH Hiiiiiiiii iiiiiiiIII IIIIIIIIII IIIIIIIIII

  hspint1 NHTTRPL  507
          IIIIIII
```

Fig. 10

Amino acid sequence of a mouse spint1 deletion variant exhibiting anti-TNFa activity (SEQ ID NO: 7):

```
MAGRRLARASISAVGVWLLCALGLQATEAELPSAPAELPGGAACLSRFTSGVPSFVLDTEASVS
NGATFLGSPTARRGWDCVRSCCTTQNCNLALVELQPDGGEDAISACFLMNCLYEQNFVCKFAPK
EGFINYLTQELYRSYRELRTRGFGGSRIPRIWMGIDLKVQLQKPLVLNEADNTDWHLLQGDSDV
RVERKRPEEVELWGLKEGTYLFQLTRTDSDQPEETANLTITVLTAKQTEDYCLASYKVGRCRGS
FPRWYYDPKEQICKSFTFGGCLGNKNNYLREEECMLACKDVQGISPKRHHPVCSGSCHATQFRC
SNGCCIDGFLECDDTPDCPDGSDEATCEKYTSGFDELQNIHFLSDKGYCAELPDTGFCKENIPR
WYYNPFSERCARFTYGGCYGNKNNFEEEQQCLESCRGISKKDVFGLRREGSIPTVGSAEVAIAV
FLVICIIVVLTILGYCFFKN
```

Fig. 11A

Amino acid sequence of a human spint1 deletion variant exhibiting anti-TNFa activity (SEQ ID NO: 8):

```
MARARLAPAGIPAVALWLLCTLGLQGTQAGPPPAPPGLPAGADCLNSFTAGVPGFVLDTNASVS
NGATFLESPTVRRGWDCVRACCTTQNCNLALVELQPDRGEDAIAACFLINCLYEQNFVCKFAPR
EGFINYLTREVYRSYRQLRTQGFGGSGIPKAWAGIDLKVQPQEPLVLKDVENTDWRLLRGDTDV
RVERKDPNQVELWGLKEGTYLFQLTVTSSDHPEDTANVTVTVLSTKQTEDYCLASNKVGRCRGS
FPRWYYDPTEQICKSFVYGGCLGNKNNYLREEECILACRGVQGPSMERRHPVCSGTCQPTQFRC
SNGCCIDSFLECDDTPNCPDASDEAACEKYTSGFDELQRIHFPSDKGHCVDLPDTGLCKESIPR
WYYNPFSEHCARFTYGGCYGNKNNFEEEQQCLESCRGISKKDVFGLRREIPIPSTGSVEMAVAV
FLVICIVVVVAILGYCFFKN
```

Fig. 11B

Alignment of mouse and human spint1 deletion variants exhibiting anti-TNFa activity (including predicted signal peptide sequences):

Score = 759 bits (1960), Expect = 0.0
Identities = 375/468 (80%), Positives = 412/468 (88%), Gaps = 0/468 (0%)

```
mspint1    1   MAGRRLARASISAVGVWLLCALGLQATEAELPSAPAELPGGAACLSRFTSGVPSFVLDTE   60
               MA  RLA A I AV +WLLC LGLQ T+A  P AP  LP GA CL+ FT+GVP FVLDT
hspint1    1   MARARLAPAGIPAVALWLLCTLGLQGTQAGPPPAPPGLPAGADCLNSFTAGVPGFVLDTN   60

mspint1   61   ASVSNGATFLGSPTARRGWDCVRSCCTTQNCNLALVELQPDGGEDAISACFLMNCLYEQN   120
               ASVSNGATFL SPT RRGWDCVR+CCTTQNCNLALVELQPD GEDAI+ACFL+NCLYEQN
hspint1   61   ASVSNGATFLESPTVRRGWDCVRACCTTQNCNLALVELQPDRGEDAIAACFLINCLYEQN   120

mspint1  121   FVCKFAPKEGFINYLTQELYRSYRELRTRGFGGSRIPRIWMGIDLKVQLQKPLVLNEADN   180
               FVCKFAP+EGFINYLT+E+YRSYR+LRT+GFGGS IP+ W GIDLKVQ Q+PLVL + +N
hspint1  121   FVCKFAPREGFINYLTREVYRSYRQLRTQGFGGSGIPKAWAGIDLKVQPQEPLVLKDVEN   180

mspint1  181   TDWHLLQGDSDVRVERKRPEEVELWGLKEGTYLFQLTRTDSDQPEETANLTITVLTAKQT   240
               TDW LL+GD+DVRVERK P +VELWGLKEGTYLFQLT T SD PE+TAN+T+TVL+ KQT
hspint1  181   TDWRLLRGDTDVRVERKDPNQVELWGLKEGTYLFQLTVTSSDHPEDTANVTVTVLSTKQT   240

mspint1  241   EDYCLASYKVGRCRGSFPRWYYDPKEQICKSFTFGGCLGNKNNYLREEECMLACKDVQGI   300
               EDYCLAS KVGRCRGSFPRWYYDP EQICKSF +GGCLGNKNNYLREEEC+LAC+ VQG
hspint1  241   EDYCLASNKVGRCRGSFPRWYYDPTEQICKSFVYGGCLGNKNNYLREEECILACRGVQGP   300

mspint1  301   SPKRHHPVCSGSCHATQFRCSNGCCIDGFLECDDTPDCPDGSDEATCEKYTSGFDELQNI   360
               S +R HPVCSG+C  TQFRCSNGCCID FLECDDTP+CPD SDEA CEKYTSGFDELQ I
hspint1  301   SMERRHPVCSGTCQPTQFRCSNGCCIDSFLECDDTPNCPDASDEAACEKYTSGFDELQRI   360
```

Fig. 12

```
mspint1  361  HFLSDKGYCAELPDTGFCKENIPRWYYNPFSERCARFTYGGCYGNKNNFEEEQQCLESCR  420
              HF SDKG+C +LPDTG CKE+IPRWYYNPFSE CARFTYGGCYGNKNNFEEEQQCLESCR
hspint1  361  HFPSDKGHCVDLPDTGLCKESIPRWYYNPFSEHCARFTYGGCYGNKNNFEEEQQCLESCR  420

mspint1  421  GISKKDVFGLRREGSIPTVGSAEVAIAVFLVICIIVVLTILGYCFFKN  468
              GISKKDVFGLRRE  IP+ GS E+A+AVFLVICI+VV+ ILGYCFFKN
hspint1  421  GISKKDVFGLRREIPIPSTGSVEMAVAVFLVICIVVVVAILGYCFFKN  468
```

Fig. 12 (con't)

Alignment of mouse and human spint1 deletion variants exhibiting anti-TNFa activity (excluding predicted signal peptide sequences):

Score = 728 bits (1880), Expect = 0.0
Identities = 358/442 (80%), Positives = 394/442 (89%), Gaps = 0/442 (0%)

```
mspint1   1    TEAELPSAPAELPGGAACLSRFTSGVPSFVLDTEASVSNGATFLGSPTARRGWDCVRSCC  60
               T+A  P AP  LP GA CL+ FT+GVP FVLDT ASVSNGATFL SPT RRGWDCVR+CC
hspint1   1    TQAGPPPAPPGLPAGADCLNSFTAGVPGFVLDTNASVSNGATFLESPTVRRGWDCVRACC  60

mspint1   61   TTQNCNLALVELQPDGGEDAISACFLMNCLYEQNFVCKFAPKEGFINYLTQELYRSYREL  120
               TTQNCNLALVELQPD GEDAI+ACFL+NCLYEQNFVCKFAP+EGFINYLT+E+YRSYR+L
hspint1   61   TTQNCNLALVELQPDRGEDAIAACFLINCLYEQNFVCKFAPREGFINYLTREVYRSYRQL  120

mspint1   121  RTRGFGGSRIPRIWMGIDLKVQLQKPLVLNEADNTDWHLLQGDSDVRVERKRPEEVELWG  180
               RT+GFGGS IP+ W GIDLKVQ Q+PLVL + +NTDW LL+GD+DVRVERK P +VELWG
hspint1   121  RTQGFGGSGIPKAWAGIDLKVQPQEPLVLKDVENTDWRLLRGDTDVRVERKDPNQVELWG  180

mspint1   181  LKEGTYLFQLTRTDSDQPEETANLTITVLTAKQTEDYCLASYKVGRCRGSFPRWYYDPKE  240
               LKEGTYLFQLT T SD PE+TAN+T+TVL+ KQTEDYCLAS KVGRCRGSFPRWYYDP E
hspint1   181  LKEGTYLFQLTVTSSDHPEDTANVTVTVLSTKQTEDYCLASNKVGRCRGSFPRWYYDPTE  240

mspint1   241  QICKSFTFGGCLGNKNNYLREEECMLACKDVQGISPKRHHPVCSGSCHATQFRCSNGCCI  300
               QICKSF +GGCLGNKNNYLREEEC+LAC+ VQG S +R HPVCSG+C  TQFRCSNGCCI
hspint1   241  QICKSFVYGGCLGNKNNYLREEECILACRGVQGPSMERRHPVCSGTCQPTQFRCSNGCCI  300

mspint1   301  DGFLECDDTPDCPDGSDEATCEKYTSGFDELQNIHFLSDKGYCAELPDTGFCKENIPRWY  360
               D FLECDDTP+CPD SDEA CEKYTSGFDELQ IHF SDKG+C +LPDTG CKE+IPRWY
hspint1   301  DSFLECDDTPNCPDASDEAACEKYTSGFDELQRIHFPSDKGHCVDLPDTGLCKESIPRWY  360
```

Fig. 13

```
mspint1    361  YNPFSERCARFTYGGCYGNKNNFEEEQQCLESCRGISKKDVFGLRREGSIPTVGSAEVAI  420
                YNPFSE CARFTYGGCYGNKNNFEEEQQCLESCRGISKKDVFGLRRE  IP+ GS E+A+
hspint1    361  YNPFSEHCARFTYGGCYGNKNNFEEEQQCLESCRGISKKDVFGLRREIPIPSTGSVEMAV  420

mspint1    421  AVFLVICIIVVLTILGYCFFKN  442
                AVFLVICI+VV+ ILGYCFFKN
hspint1    421  AVFLVICIVVVVAILGYCFFKN  442
```

Fig. 13 (con't)

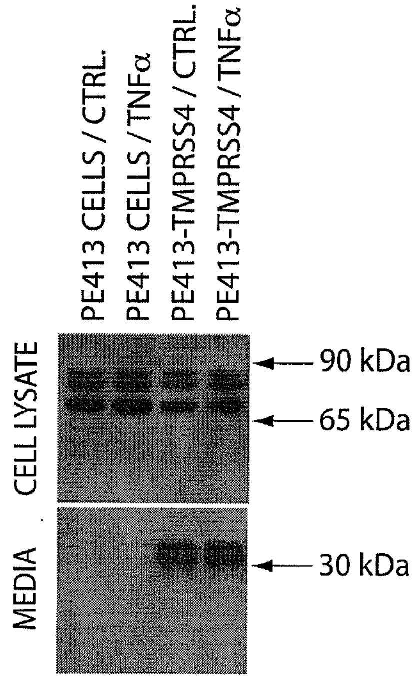

Fig. 14A

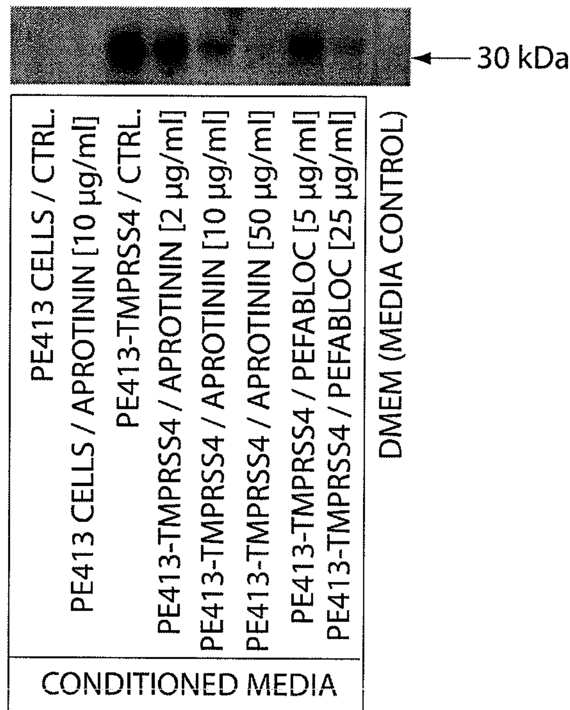

Fig. 14B

Alignment of mouse spint1 ORF (top sequence) and human spint1 ORF (bottom sequence).
Score = 887 bits (2291), Expect = 0.0
Identities = 409/507 (80%), Positives = 448/507 (88%), Gaps = 0/507 (0%)

```
Query  1    MAGRRLARASISAVGVWLLCALGLQATEAELPSAPAELPGGAACLSRFTSGVPSFVLDTE  60
            MA  RLA A I AV +WLLC LGLQ T+A  P AP  LP GA CL+ FT+GVP FVLDT
Sbjct  7    MARARLAPAGIPAVALWLLCTLGLQGTQAGPPPAPPGLPAGADCLNSFTAGVPGFVLDTN  66

Query  61   ASVSNGATFLGSPTARRGWDCVRSCCTTQNCNLALVELQPDGGEDAISACFLMNCLYEQN  120
            ASVSNGATFL SPT RRGWDCVR+CCTTQNCNLALVELQPD GEDAI+ACFL+NCLYEQN
Sbjct  67   ASVSNGATFLESPTVRRGWDCVRACCTTQNCNLALVELQPDRGEDAIAACFLINCLYEQN  126

Query  121  FVCKFAPKEGFINYLTQELYRSYRELRTRGFGGSRIPRIWMGIDLKVQLQKPLVLNEADN  180
            FVCKFAP+EGFINYLT+E+YRSYR+LRT+GFGGS IP+ W GIDLKVQ Q+PLVL + +N
Sbjct  127  FVCKFAPREGFINYLTREVYRSYRQLRTQGFGGSGIPKAWAGIDLKVQPQEPLVLKDVEN  186

Query  181  TDWHLLQGDSDVRVERKRPEEVELWGLKEGTYLFQLTRTDSDQPEETANLTITVLTAKQT  240
            TDW LL+GD+DVRVERK P +VELWGLKEGTYLFQLT T SD PE+TAN+T+TVL+ KQT
Sbjct  187  TDWRLLRGDTDVRVERKDPNQVELWGLKEGTYLFQLTVTSSDHPEDTANVTVTVLSTKQT  246

Query  241  EDYCLASYKVGRCRGSFPRWYYDPKEQICKSFTFGGCLGNKNNYLREEECMLACKDVQGI  300
            EDYCLAS KVGRCRGSFPRWYYDP EQICKSF +GGCLGNKNNYLREEEC+LAC+ VQG
Sbjct  247  EDYCLASNKVGRCRGSFPRWYYDPTEQICKSFVYGGCLGNKNNYLREEECILACRGVQGP  306

Query  301  SPKRHHPVCSGSCHATQFRCSNGCCIDGFLECDDTPDCPDGSDEATCEKYTSGFDELQNI  360
            S +R HPVCSG+C  TQFRCSNGCCID FLECDDTP+CPD SDEA CEKYTSGFDELQ I
Sbjct  307  SMERRHPVCSGTCQPTQFRCSNGCCIDSFLECDDTPNCPDASDEAACEKYTSGFDELQRI  366
```

Fig. 15

```
Query  361  HFLSDKGYCAELPDTGFCKENIPRWYYNPFSERCARFTYGGCYGNKNNFEEEQQCLESCR  420
            HF SDKG+C +LPDTG CKE+IPRWYYNPFSE CARFTYGGCYGNKNNFEEEQQCLESCR
Sbjct  367  HFPSDKGHCVDLPDTGLCKESIPRWYYNPFSEHCARFTYGGCYGNKNNFEEEQQCLESCR  426

Query  421  GISKKDVFGLRREGSIPTVGSAEVAIAVFLVICIIVVLTILGYCFFKNQRKEFHSPLHHP  480
            GISKKDVFGLRRE  IP+ GS E+A+AVFLVICI+VV+ ILGYCFFKNQRK+FH   HHP
Sbjct  427  GISKKDVFGLRREIPIPSTGSVEMAVAVFLVICIVVVVAILGYCFFKNQRKDFHGHHHHP  486

Query  481  PPTPASSTVSTTEDTEHLVYNHTTQPL  507
            PPTPASSTVSTTEDTEHLVYNHTT+PL
Sbjct  487  PPTPASSTVSTTEDTEHLVYNHTTRPL  513
```

Fig. 15 (con't)

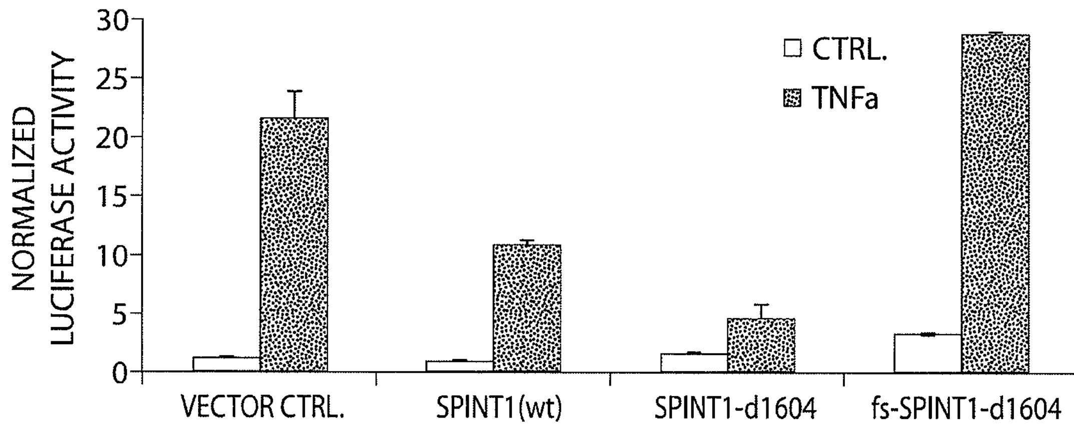

Fig. 16A

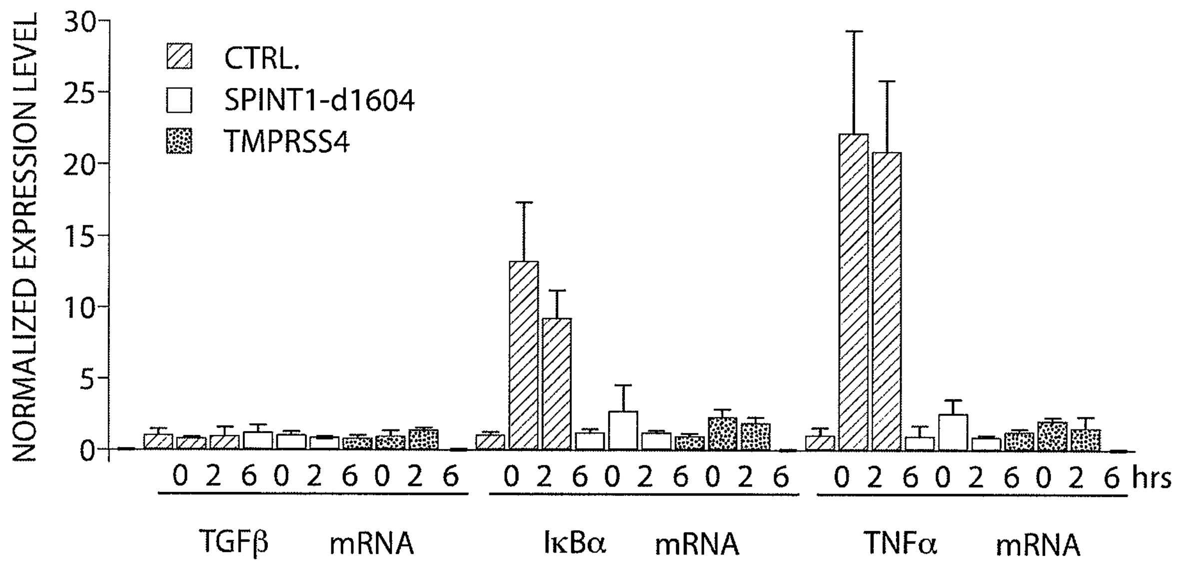

Fig. 16B

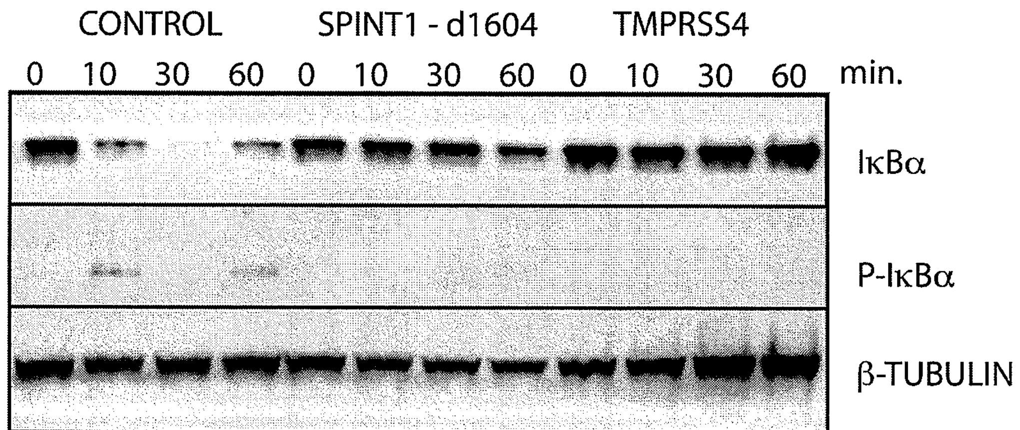

Fig. 16C

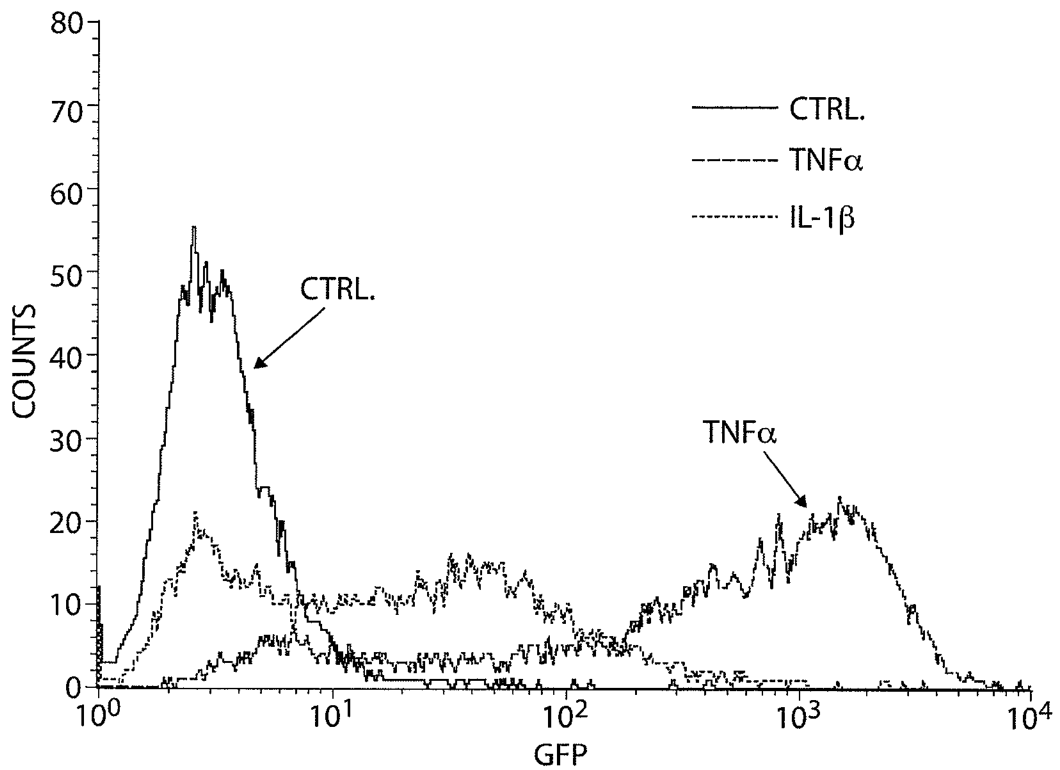

Fig. 16D

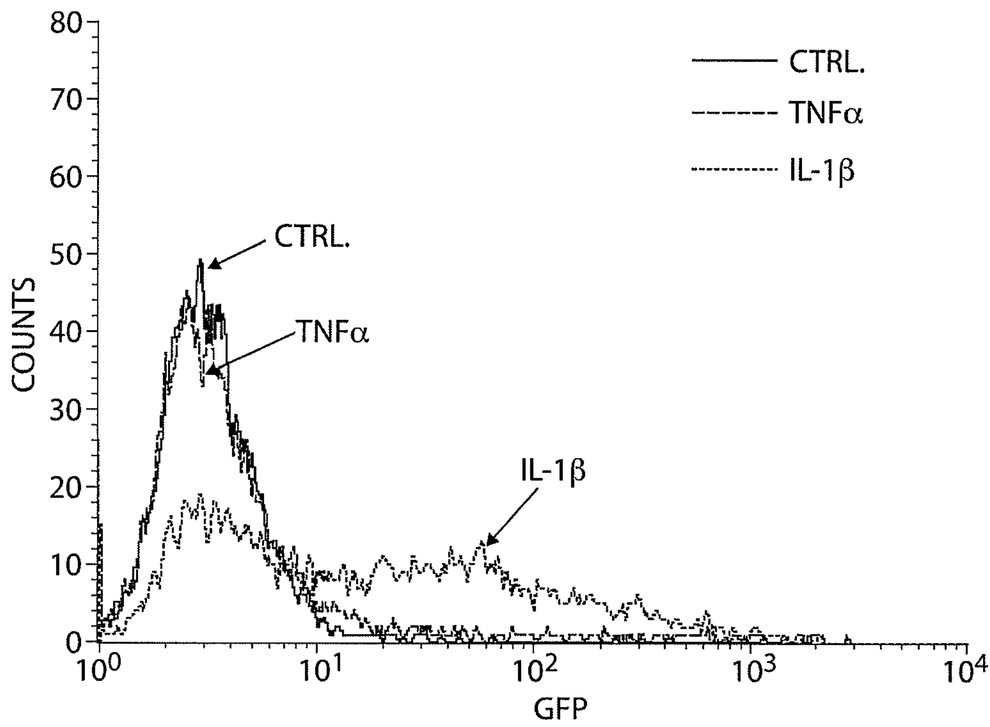

Fig. 16E

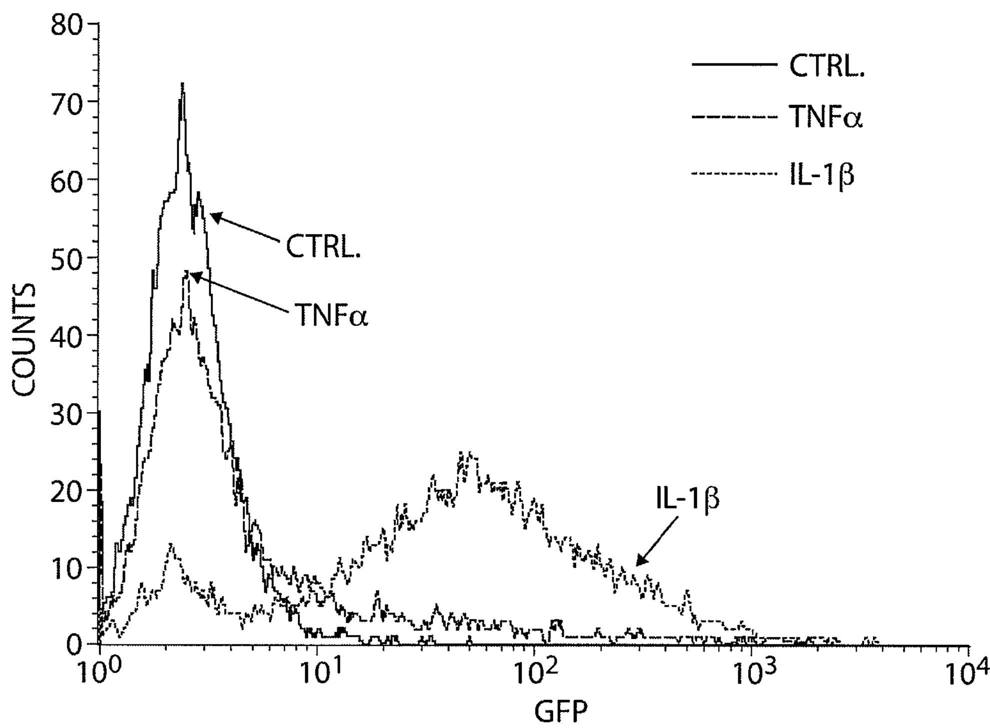

Fig. 16F