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(54) Title: MEANS AND METHODS TO TREAT INFLAMMATION

(57) Abstract: The present invention relates to a composition comprising MMP8 and TNFR1 inactivating antigen binding polypeptides, preferably antigen binding polypeptides comprising antibodies; it relates further to the use of such composition to treat inflammation, such as but not limited to systemic inflammatory response syndrome, sepsis, LPS induced inflammation, renal ischemia/reperfusion injury, ventilation induced lung injury, periodontal inflammation, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, ankylosing spondylitis, Lyme arthritis and osteoarthritis.

Means and methods to treat inflammation

The present invention relates to a composition comprising MMP8 and TNFR1 inactivating antigen binding polypeptides, preferably antigen binding polypeptides comprising antibodies; it relates further to the use of such composition to treat inflammation, such as but not limited to systemic inflammatory response syndrome, sepsis, LPS induced inflammation, renal ischemia/reperfusion injury, ventilation induced lung injury, periodontal inflammation, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, ankylosing spondylitis, Lyme arthritis and osteoarthritis.

Severe sepsis and septic shock are important causes of morbidity and mortality in intensive care units (Angus & van der Poll, New Engl J Med 2013; Gotts & Matthay, BMJ (Clinical research ed), 2016; Fleischmann et al., Current estimates and limitations. Am J Respir Crit Care Med, 2016). Current treatment of sepsis patients is limited and focuses mainly on supportive measures (Schuerholz et al. Minerva anestesiologica, 2008;74:181-195). Although many therapeutics have been clinically evaluated, no specific treatment is currently approved to treat the heterogeneous population of sepsis patients (Heming et al., Expert opinion on emerging drugs, 2016; van der Poll, Crit Care, 2016).

The pro-inflammatory cytokine tumor necrosis factor (TNF) is one of the first to be released in response to infection (Blackwell & Christman, Br J Anaesthesia, 1996), and some authors have suggested that the use of TNF inhibitors improves survival rates in animal models of sepsis, but numerous clinical trials failed to demonstrate a statistically significant benefit (Lv et al., Int J Clin Practice, 2014).

TNF signals through two receptors; pro-inflammatory signaling is believed to happen via TNFR1, whereas TNFR2 signaling is believed to be involved in immune regulation and maintaining homeostasis. Since anti-TNF treatment is associated with side-effects that might be due to blockage of TNFR1 as well as TNFR2 signals, we believe that selective TNFR1 inhibition might be a better and safer approach than inhibition of TNF itself (Van Hauwermeiren et al., Cytokine & growth factor reviews, 2011). It has been shown that TNFR1 deficiency protects against death after injection of moderate LPS doses (Vandenbroucke et al., EMBO Mol Med, 2013), but not against very high doses (Rothe et al., Circ Shock, 1994).

Nevertheless, in the TNF-induced lethal shock model, TNFR1-deficiency completely protects against TNF lethality by preventing TNF-induced gut permeability (Van Hauwermeiren et al., J Clin Invest, 2013; Van Hauwermeiren et al., Mucosal Immunol, 2014).

Matrix metalloproteinase 8 (MMP8) is a Zn^{2+}-dependent endopeptidase belonging to a structurally related group of MMPs that are considered important modulators of immunity and involved in several inflammatory diseases (Vandenbroucke & Libert, Nature Rev Drug Discovery, 2014). MMP8 was proposed as a potential drug target for treatment of sepsis and multiple studies with sepsis patients reported correlations between increased MMP8 levels, higher mortality rates, increased organ failure and high plasma C-reactive protein (CRP) levels (Martin et al, Scientific Rep, 2014). In mouse models of sepsis, it was also shown that genetic and pharmacological inhibition of MMP8 improved survival.

Additionally, we showed that at the level of the brain barrier located in the choroid plexus (CP), called the blood-cerebrospinal fluid (CSF) barrier, MMP8 was upregulated after LPS-induced endotoxemia and involved in the LPS-induced disruption of the blood-CSF barrier (Vandenbroucke et al., J Neurosci, 2012).

We are the first who thoroughly investigated the potential of combining both MMP8 and TNFR1 inhibition and we can show that our approach leads to an unexpected improvement of sepsis outcome compared to single inhibition. We found that both TNFR1 and MMP8 play mainly detrimental roles during sepsis and show that both TNFR1 and MMP8 inhibition has a cumulative effect in human sepsis patients as well as in mouse models. Even more beneficial we can provide a single construct capable to inhibit both MMP8 and TNFR1. Our pioneering work opens the road for a novel treatment approach for inflammatory diseases and particularly for sepsis and LPS induced inflammation commonly known to be hardly druggable.

Brief description of the figures

FIGURE 1 A-E: sTNFR1 and MMP8 levels are increased in human sepsis patients and the levels were correlated with disease severity. Blood was withdrawn daily of sepsis patients after meeting the inclusion criteria and after admission in the intensive care unit (ICU) during 7 consecutive days. (A-B) MMP8 and sTNFR1 levels were determined daily in plasma of sepsis patients with Luminex technology and ELISA, respectively and compared with the levels in healthy controls. (C-E) Scatterplots of sTNFR1 (C) and MMP8 (D) plasma levels versus SOFA score, and sTNFR1 versus MMP8 plasma (E) determined in sepsis patients. Bars represent mean ± SEM and n=13 sepsis patients and n=6 healthy controls.

FIGURE 2 A-B: MMP8 and TNFR1 deficiencies leads to elevated expression of Tnfrsf1a and Mmp8, respectively, eight hours after LPS injection. Wild type (WT), TNFR1-/- and MMP8-/- mice were injected with 10 mg/kg LPS (LD100) or PBS (untreated) and organs were analyzed after 8 h. (A) Fold change of

Tnfrsf1a gene expression in WT and MMP8-/- mice compared to untreated mice determined in ileum, choroid plexus and lung. (B) Fold change of Mmp8 gene expression in WT and TNFR1-/- mice compared to untreated mice determined in ileum, choroid plexus and lung. qPCR data were normalized to stable housekeeping genes. Bars represent mean ± SEM and n = 6–7 mice/group (untreated n = 7, WT n = 7, MMP8-/- n = 7, TNFR1-/- n = 6).

FIGURE 3 A-D: Dual deficiency of MMP8 and TNFR1 synergistically protects against endotoxemia. Wild type (WT, -■-), MMP8-/- (-○-), TNFR1-/-(-●-)and DKO (-□-) mice were injected intraperitoneally with LD100 of LPS (A-B) or with 6 x LD100 of LPS (60 mg/kg) (C-D). Survival and rectal body temperature were monitored. Body temperature and bars present mean ± SEM, n = 2–27 mice/group. (A-B) -■- WT (n = 7), -○- MMP8-/- (n = 7), -●-TNFR1-/- (n = 6), and -□- DKO (n = 12) (C-D) WT (n = 2), MMP8-/- (n = 12), TNFR1-/- (n = 6), and DKO (n = 27).
Survival curves were compared with Mantel-Cox test and Luminex data were analyzed with a Mann-Whitney test. * for $0.01 \leq p < 0.05$; ** for $0.001 \leq p < 0.01$; *** for $0.001 \leq p$ 0.0001, and **** for $p <$ 0.0001.

FIGURE 4 A-I: Dual MMP8/TNFR1 inhibition preserves the intestinal and blood–cerebrospinal fluid barrier and reduces local and systemic inflammation after endotoxemia. Wild type (WT), MMP8-/-, TNFR1-/- and DKO mice were injected intraperitoneally with LD100 of LPS or with PBS (untreated). Mice were euthanized 8 h after LPS injection. (A) Permeability of the intestinal barrier was assessed by measuring leakage of fluorescently labeled dextran from the intestines into the blood circulation. (B-C) Fold change in mRNA gene expression of Il6 and Mmp13 was determined in ileum. (D) Permeability of the blood-CSF (BCSFB) was assessed by measuring leakage of fluorescently labeled dextran from the blood into the CSF during endotoxemia. (E-F) Fold change in mRNA gene expression of Il6 and Nos2 was determined in the choroid plexus (CP). (G-I) Luminex technology was used to measure the cytokines IL6, IL17A and the chemokine MCP1 in plasma as indicators of systemic inflammation. qPCR data were normalized to stable housekeeping genes. Bars represent mean ± SEM, n = 4–10 mice/group.

FIGURE 5 A-D: Dual ablation of MMP8 and TNFR1 synergistically protects against sepsis induced by cecal ligation and puncture (CLP). Male wild type (WT, -■-), MMP8-/- (-○-), TNFR1-/-(-●-) and DKO (-□-) mice were subjected to CLP surgery and treated with broad-spectrum antibiotics 9 and 24 h after CLP, and mortality was monitored. Sham-treated mice underwent the same surgical procedure but without ligation and puncture. Serum and plasma were collected and analyzed 6 h after CLP. Survival (A) and rectal body temperature (B) were monitored for 10 days.

C-D: Plasma IL6 levels (C) and KC levels in peritoneal lavage fluid (D) were measured with the Luminex technology. (n=12 sham; n=12 WT; n=6 MMP8-/-; n=11 TNFR1-/-; n=10 DKO). The experiment is done twice and pooled results are shown.

Body temperature data represent bars mean ± SEM, WT, MMP8-/-, TNFR1-/-, DKO, n = 5–28 mice/group. Survival curves were analyzed with a Mantel-Cox test and Luminex data were analyzed with a two-tailed Mann-Whitney test. * for $0.01 \leq p < 0.05$; ** for $0.001 \leq p < 0.01$; *** for $0.001 \leq p$ 0.0001, and **** for $p < 0.0001$.

FIGURE 6 A-H: Dual ablation of MMP8 and TNFR1 leads to moderate neutrophil efflux from blood to the peritoneum with maintenance of bacterial clearance in peritoneum and blood. Male wild type (WT,), MMP8-/- ( ), TNFR1-/-( ) and DKO ( ) mice were subjected to CLP. Sham-treated mice underwent the same surgical procedure but without ligation and puncture. Six or 24 h after CLP, blood and peritoneal lavage fluid (PLF) were collected for analysis. (A-D) The absolute number of white blood cells (WBC) and neutrophils in circulation (A-B) and in PLF (C-D) 6 h after CLP, determined with Hemavet hematology analyzer. (E-F) Blood and PLF were collected 6 and 24 h after CLP and total bacterial counts were determined and expressed as colony-forming units (CFU) per peritoneal cavity. (G-H) Chemokines KC and MIP2 in PLF were measured by Luminex technology. Bars represent mean ± SEM, WT, MMP8-/-, TNFR1-/-, DKO, n = 6–12 mice/group.

PLF= peritoneal lavage fluid; CFU = colony forming units

FIGURE 7 A-F: In endotoxemia, simultaneous therapeutic inhibition of TNFR1 and MMP8 with biologicals is significantly more protective against lethality than single inhibition. Survival (A) and body temperature (B) were monitored in TNFR1-/- mice that were injected intraperitoneally (i.p.) with LPS (LD75 in TNFR1-/- mice) and with 500 µg anti-MMP8 Nanobody ( ) (Nb) or control Nb ( ). This injection was repeated 24 h after LPS injection (n = 7 mice/group). Survival (C) and body temperature (D) were monitored in MMP8-/- mice that were injected i.p. with LPS (LD100 in MMP8-/- mice) and 400 µg anti-TNFR1 ( ) or PBS ( ) (n = 5–6 mice/group). Survival (E) and body temperature (F) were monitored in WT mice treated with anti-MMP8 Nb and/or anti-TNFR1 Ab ( ) or PBS ( ) and injected with LPS (LD75 in WT mice). After 24 h, mice received a second injection of both treatments. Mice treated only with anti-MMP8 Nb or anti-TNFR1 Ab received an additional PBS injection after 24 h. Body temperature data represent mean ± SEM (B-D) or mean only (F), anti-MMP8 Nb, Nb Alb-Ctrl-Ctrl, anti-TNFR1 Ab, PBS, anti-MMP8 Nb + anti-PBS Nb, PBS + PBS n = 6–17 mice/group.

Body temperatures represent mean ± SEM (B, D, H) or mean only (F). Survival curves were analyzed with the Wilcoxon test. * for $0.01 \leq p < 0.05$; ** for $0.001 \leq p < 0.01$.

FIGURE 8 A-B: Structure and gene construct of Nb 70-alb-14. (A) Construction of Nb 70-alb-14. At the N-terminal end of the construct, the C-terminal end of Nb 70 is linked to the N-terminal end of Nb Alb via a [G4S]3 linker. The C-terminal end of Nb Alb is then linked to the N-terminal end of Nb 14 and this Nb is linked to the His6-tag at the C-terminus. (B) Nucleotide sequence of Nb 70-Alb-14. The Nb sequences are shown in bold, the sequence of the [G4S]3 linker is underlined and the His6-tag is shown in italic.

FIGURE 9 a-d: Binding Nb 70-alb-14 to hTNFR1, albumin, mMMP8_CD and mMMP8_FL. To determine the binding affinity of Nb 70-Alb-14 to hTNFR1 (a), albumin (b), the catalytic domain of mouse MMP8 (mMMP8_CD) (c) and the full length MMP8 (mMMP8_FL) (d), the respective ELISAs were performed. In all ELISAs, binding affinity of Nb 70-Alb-14 was compared with the relevant monovalent Nb: Nb 70 for hTNFR1 binding, Nb 14 for MMP8 binding and Nb Alb for albumin binding. A serial 0.2 dilution was applied starting at 6 µM (a) or 1 µM (b-d). Nb Alb-Ctrl-Ctrl, an irrelevant control Nanobody; 50%: Nanobody concentration that binds 50%.of the substrate. All ELISAs were done in triplicates and data are represented as mean ± S.E.M.

FIGURE 10 a-c: Surface plasmon resonance affinity measurement on immobilized hTNFR1, mMMP8_CD and mMMP8_FL. Surface plasmon resonance sensorgram of Nb 70-alb-14 on immobilized hTNFR1 (a), mouse MMP8 catalytic domain (mMMP8_CD) (b), mouse MMP8 full length (mMMP8_FL) (c). The adjusted sensorgram overlays show binding of Nb 70-alb-14 applied in a dilution series ranging from 500 nM to 2.01 nM or 250 nM to 0.97 nM to immobilized hTNFR1 or MMP8. Dotted lines show global fitting of the binding data to a two-state binding model. Surface plasmon resonance analyses were performed in duplicate.

FIGURE 11 a-c: Inhibition of TNF/TNFR1 and MMP8 activity by Nb70-alb-14. (a) HEK-2 blue inhibition assay with Nb 70-alb-14 and monovalent equivalents, in which cells were preincubated with a serial 0.2 Nb dilution starting at 5 µM. (b-c) The EnzChek collagenase assay was used to determine inhibitory capacity of Nb 70-alb-14 for the mouse catalytic domain of MMP8 (mMMP8_CD). (b) Change in fluorescence (RLU) in function of time as a measure of MMP8 activity. Higher Nb 70-alb-14 concentrations lead to reduced increment of fluorescence over time. (c) Percentage of MMP8 activity in function of Nb/MMP8 ratio. RLU: Relative light units; The HEK-2 blue assays were performed in triplicates, data represent mean ± S.E.M.

FIGURE 12 a-d: Serum half life of Nb 70-alb-14 and in vivo efficacy of Nb 70-alb-14 in endotoxemia and CLP.

A: Serum half-life of Nb 70-alb-14 was determined after a single injection of 1000 µg of Nb 70-alb-14 in wild type (WT) mice. Serum concentrations were determined with a hTNFR1 ELISA (n =10).

B: Serum half-life of Nb 70-alb-14 was determined in wild type C57BL/6J mice (n=9) after multiple injections of 100 µg of Nb 70-alb-14 where the first injection was followed by 2 consecutive injections after 10 h and 24 h. Serum concentrations were determined by a hTNFR1 ELISA.

C: Mice were subjected to cecal ligation and puncture (CLP) and injected with 500 µg Nb 70-alb-14(n=14) or PBS (n=14), directly after the procedure, after 24h and after 48h. Lethality was followed over time. Data represent mean ± S.E.M.

D: Wild type (WT) mice and mice that have a human TNFR1 transgene in a TNFR1-background (hTNFR1 Tg mice) were subjected to cecal ligation and puncture (CLP). Mice were treated with broad-spectrum antibiotics 9 and 24 h after CLP, and mortality was monitored. Lethality between the two mouse lines was compared to exclude differences in sensitivity between the WT and hTNFR1 mice. (n =11 WT, n =17 hTNFR1 Tg mice; p=0.61). This experiment was done twice.

FIGURE 13 A-I: sTNFR1, MMP8 and IL6 plasma levels are increased in human sepsis patients and the levels were correlated with each other and with disease severity

Blood was withdrawn daily of sepsis patients after meeting the inclusion criteria and after admission in the intensive care unit (ICU) during maximally 7 consecutive days or until the end of their stay in ICU.

A-C: MMP8, sTNFR1 and IL6 levels were determined daily in plasma of sepsis patients with Luminex technology (MMP8) and ELISA and were compared with the levels in healthy controls.

D-H: Scatterplots of sTNFR1 (D,F,H) and log(MMP8) (E,G,H) plasma levels versus SOFA score (F,G) and log(IL6) plasma levels (D,E), and sTNFR1 versus log(MMP8) plasma (H) determined in sepsis patients.

I: Fold induction of *MMP8* and *TNFRSF1A* in whole blood-derived RNA determined in sepsis patients and compared to expression levels determined in healthy controls (=reference set to 1).

In A-C, data represent min-to-max box and whiskers. MMP8 and sTNFR1 plasma levels and *MMP8* and *TNFRSF1A* expression levels were tested with one-way ANOVA, adjusted for multiple comparisons and compared to the healthy controls. MMP8 and IL6 plasma levels were log-transformed. Correlations were tested against zero using a two-sided t-test. n=13 sepsis patients; n=6 healthy controls. * for $0.01 \leq p < 0.05$; ** for $0.001 \leq p < 0.01$; *** for $0.001 \leq p$ 0.0001, and **** for $p < 0.0001$.

FIGURE 14 A-D: *Mmp8* and *Tnfrsf1a* expression is upregulated after endotoxemia and cecal ligation and puncture (CLP) in different organs

A-B: Wild type (WT) mice were injected with 10 mg/kg LPS (LD100) or PBS (untreated) and organs were analyzed after 8 h. Relative gene expression determined by qPCR of *Mmp8* (A) and *Tnfrsf1a* (B) in WT compared to untreated mice determined in liver, choroid plexus (CP) and ileum (n=7 untreated, n=7 LPS for all organs). The experiment is done twice.

C-D: Wild type (WT) mice were subjected to cecal ligation and puncture (CLP). Sham-treated mice underwent the same surgical procedure but without ligation and puncture. Organs were collected and analyzed 6 h after CLP. Relative gene expression of *Mmp8* (C) and *Tnfrsf1a* (D) in WT compared to untreated mice determined in liver, lung, choroid plexus (CP) and brain (liver n=4 sham and n=5 CLP; lung n=8 sham and n=12 CLP; CP n=6 sham and n=6 CLP, brain n=6 sham and n=6 CLP). (*Mmp8* liver p=0.04 lung p=0.0003, CP p=0.002, brain p=0.04: *Tnfrsf1a* liver p=0.03, lung p=0.0005, CP p=0.004). The experiment is done twice.

qPCR data were normalized to stable housekeeping genes determined by GeNorm. Data represent min-to-max box and whiskers. Statistics between untreated/sham and treated mice were calculated with a two-tailed Mann-Whitney test. * for $0.01 \leq p < 0.05$; ** for $0.001 \leq p < 0.01$; *** for $0.001 \leq p$ 0.0001, and **** for $p < 0.0001$.

FIGURE 15 A-C: Therapeutic simultaneous abrogation of TNFR1 and MMP8 in cecal ligation and puncture (CLP).

A-B: hTNFR1 Tg mice were subjected to cecal ligation and puncture (CLP) under lethal conditions (75% ligation and 2 punctures with 21G needle). Mice were treated with an intraperitoneal injection of 1 mg Nb 70-alb-14 or PBS directly after the CLP surgery and the treatment was repeated after 9, 24 and 48 h. All mice were treated with antibiotics 9 and 24 h after the CLP procedure. Survival (A) and rectal body temperature (B) were monitored for 10 days. (n=18 Nb 70-alb-14, 28% survival; n=16 PBS, 19% survival; p=0.02). This experiment was performed four times and results were pooled.

C: hTNFR1 Tg mice were subjected to cecal ligation and puncture (CLP) under sublethal conditions (50% ligation and 1 puncture with 21G needle). Mice were treated as in A.

Body temperatures represent mean ± SEM (B). Survival curves were analyzed with the Wilcoxon test. * for $0.01 \leq p < 0.05$.

Figure 16: Dual ablation of MMP8 and TNFR1 reduces systemic chemokine levels after endotoxemia.

A-F: Wild type (WT), $MMP8^{-/-}$, $TNFR1^{-/-}$ and double knockout (DKO) mice were injected intraperitoneally with a lethal LPS dose or PBS (untreated). Mice were euthanized 8 h after LPS injection and plasma was collected. Luminex technology was used to determine cytokine levels of IL6 (A) and IL17a (B) and

chemokine levels of MCP1 (C), KC (D), MIP1b (E) and RANTES (F) in plasma. (n = 9 untreated; n = 10 WT; n = 9 $MMP8^{-/-}$; n = 8 $TNFR1^{-/-}$; n = 7 DKO).

Bars represent mean ± SEM, data were analyzed with a two-tailed Mann-Whitney test, significance levels are indicated * for 0.01 ≤ p < 0.05; ** for 0.001 ≤ p < 0.01; *** for 0.001 ≤ p 0.0001, and **** for p < 0.0001.

Detailed description

The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes. Where the term "comprising" is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

The following terms or definitions are provided solely to aid in the understanding of the invention. Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook et al., Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Press, Plainsview, New York (2012); and Ausubel et al., Current Protocols in Molecular Biology (Supplement 114), John Wiley & Sons, New York (2016), for definitions and terms of the art. The definitions provided herein should not be construed to have a scope less than understood by a person of ordinary skill in the art.

An "antigen binding polypeptide" according to the invention typically refers to a polypeptide, which has affinity and specificity comparable to an immunoglobulin, such as an antibody.

"MMP8" refers to Matrix metalloproteinase-8, also known as collagenase-2 or neutrophil collagenase and was originally believed to be expressed only by neutrophils. More recently, it has become clear that MMP8 can be expressed in a wide range of cells, such as epithelial cells, fibroblasts and macrophages, mainly during inflammatory conditions (Van Lint and Libert, 2006). Inactive MMP8 is stored in the intracellular granules of neutrophils and is released upon activation to ensure rapid availability of MMP8 at inflammatory sites. The effect of MMP8 expression on cancer progression and its association with several inflammatory disorders has been described (Van Lint and Libert, 2006).

The term "MMP8 inactivating antigen binding polypeptide" as used herein, means that the antigen binding polypeptide significantly reduces the MMP8 activity as measured in an EnzCheck® fluorescein-labeled DQ gelatin conjugate test (Invitrogen). The skilled practitioner knows methods to investigate the ability of an antigen binding polypeptide to reduce the MMP8 activity. Said antigen binding polypeptide can be any antigen binding polypeptide known to the person skilled in the art, such as but not limited to proteins, antibodies, heavy chain antibodies (hcAb), single domain antibodies (sdAb), variable domain of camelid heavy chain antibody (VHH) variable domain of the new antigen receptor (VNAR), engineered CH2 domains (nanoantibodies; Dimitrov, 2009), minibodies (Tramontano et al., 1994), and alphabodies (WO 2010066740). Among others, non-limiting examples of MMP8 inactivating antigen binding polypeptides can be found in WO2012059513. All of these MMP8 inactivating polypeptides can be applied in the aspects and embodiments of this invention.

"TNFR1" refers to Tumor necrosis factor receptor 1. Most of the biological activities of TNF are initiated by binding to TNFR1. For further explanations on TNFR1 regulation the reader is referred to Van Hauwermeiren, F. et al., Cytokine & growth factor reviews, 2014.

The term "TNFR1 inactivating antigen binding polypeptide" as used herein, means that the antigen binding polypeptide significantly reduces the TNFR1 activity as measured in an HEK-2 blue assay (Invitrogen). Methods to investigate the ability of an antigen binding polypeptide to reduce the TNFR1 activity are well-known to the skilled practitioner. Said antigen binding polypeptide can be any antigen binding polypeptide known to the person skilled in the art, such as but not limited to proteins, antibodies, heavy chain antibodies (hcAb), single domain antibodies (sdAb), variable domain of camelid heavy chain antibody (VHH) variable domain of the new antigen receptor (VNAR), engineered CH2 domains (nanoantibodies; Dimitrov, 2009), minibodies (Tramontano et al., 1994), and alphabodiesTM (WO 2010066740). For non-limiting examples of TNFR1 inactivating antigen binding polypeptides the

reader is referred to WO2008149148, WO2008113515 and Steeland S et al., J Biol Chem. 2015. All of these TNFR1 inactivating polypeptides can be applied in the aspects and embodiments of this invention.

The term "specificity" refers to the ability of a polypeptide to bind preferentially to one antigenic target versus a different antigenic target and does not necessarily imply high affinity.

The term "affinity" refers to the degree to which a polypeptide binds to an antigen in such a way that the equilibrium of antigen and polypeptide shifts toward the presence of a complex formed by their binding. Thus, where an antigen and a polypeptide are combined in relatively equal concentration, a polypeptide of high affinity will bind to the available antigen in such a way that the equilibrium shifts toward high concentration of the resulting complex.

As used herein, the term "potency" is a measure of the biological activity of a polypeptide. Potency of an agent can be determined by any suitable method known in the art. Cell culture based potency assays are often the preferred format for determining biological activity since they measure the physiological response elicited by the agent and can generate results within a relatively short period of time. Various types of cell based assays, based on the mechanism of action of the product, can be used, including but not limited to proliferation assays, cytotoxicity assays, reporter gene assays, cell surface receptor binding assays and assays to measure induction/inhibition of functionally essential protein or other signal molecule (such as phosphorylated proteins, enzymes, cytokines, cA P and the like), all well-known in the art. Results from cell based potency assays can be expressed as "relative potency" as determined by comparison of the polypeptides of the invention to the response obtained for the corresponding reference polypeptides, optionally further comprising an irrelevant polypeptide, such as Nb Alb-Ctrl-Ctrl (cf. examples section).

The term “antibody” as used herein, refers to an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Such an antibody is commonly composed of 4 chains, 2 heavy- and 2 light chains, and is thus tetrameric. An exception thereto are camel antibodies that are composed of heavy chain dimers and are devoid of light chains, but nevertheless have an extensive antigen- binding repertoire.

Antibodies of the present invention can thus exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and F(ab)2, as well as single chain antibodies and humanized

antibodies (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, NY; Wörn and Plückthun, 2001; Koerber et al., 2015.).

A “VHH” according to the invention refers to a variable domain of a heavy chain camelid antibody. Camelid antibodies, and the VHH derived sequences are known to the person skilled in the art. Camelid antibodies have been described, amongst others in WO9404678 and in WO2007118670.

An antibody usually has both variable and constant regions whereby the variable regions are mostly responsible for determining the specificity of the antibody and will comprise complementarity determining regions (CDRs).

The term "complementarity determining region" or "CDR" refers to a variable loop within the variable regions of either heavy or light chains and contains the amino acid sequences capable of specifically binding to antigenic targets. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure. Such regions are also referred to as "hypervariable regions." The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable regions of the heavy and light chains of all canonical antibodies each have 3 CDR regions, each non- contiguous with the others for the respective light and heavy chains. The accepted CDR regions have been described by Kabat et al. (1991).

According to the invention, the MMP8 inactivating antigen binding protein and the TNFR1 inactivating antigen binding protein are present in one molecule. “Present in one molecule” according to the invention refers to attachment of the MMP8 inactivating antigen binding protein and the TNFR1 inactivating antigen binding protein. Attachment can be achieved in several ways as known in the art. For example, the MMP8 inactivating antigen binding protein and the TNFR1 inactivating antigen binding protein can be covalently bound to each other. As an alternative approach, the MMP8 inactivating antigen binding protein and the TNFR1 inactivating antigen binding protein are present in one molecule and are coupled by a linker. Optionally, a linker sequence can be used. An expert in this field knows how to choose an appropriate linker in between the various types of linkers known in the art. A further antigen binding protein can also be used as linker sequence to combine the MMP8 inactivating antigen

binding protein and the TNFR1 inactivating antigen binding protein are present in one molecule; one such example is described further herein.

A “nucleic acid” as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, and RNA. It also includes known types of modifications, for example, methylation, “caps” substitution of one or more of the naturally occurring nucleotides with an analog.

The term “vector”, as used herein, includes any vector known to the skilled person, including plasmid vectors, cosmid vectors, phage vectors, such as lambda phage, viral vectors, such as adenoviral, AAV or baculoviral vectors, or artificial chromosome vectors such as bacterial artificial chromosomes (BAC), yeast artificial chromosomes (YAC), or P1 artificial chromosomes (PAC). Said vectors include expression as well as cloning vectors. Expression vectors comprise plasmids as well as viral vectors and generally contain a desired coding sequence and appropriate DNA sequences necessary for the expression of the operably linked coding sequence in a particular host organism (e.g., bacteria, yeast, plant, insect, or mammal) or in in vitro expression systems. Cloning vectors are generally used to engineer and amplify a certain desired DNA fragment and may lack functional sequences needed for expression of the desired DNA fragments.

According to the invention, a “host cell” refers to a cell which is suitable for transformation with a vector comprising a nucleic acid of the disclosure. Said host cell can be any host cell known to the person skilled in the art and includes, but is not limited to bacterial cells, fungal cells including yeast cells, insect cells and mammalian cells. Particularly host cells, which can be used for the production of MMP8 and/or TNFR1 inactivating antigen binding polypeptides are envisaged herein.

The term “inflammatory disease” is known to the person skilled in the art. The list of inflammatory diseases includes, but is not limited to systemic inflammatory response syndrome, sepsis, LPS induced inflammation, renal ischemia/reperfusion injury, ventilation induced lung injury, periodontal inflammation, rheumatoid arthritis, multiple sclerosis, ankylosing spondylitis, Lyme arthritis and osteoarthritis.

A “pharmaceutical composition” refers to a composition comprising the MMP8 and TNFR1 inactivating antigen binding polypeptides, nucleic acids and vectors as referred to herein in a pharmaceutically

effective amount and a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is preferably a carrier that is relatively non-toxic and innocuous to a patient at concentrations consistent with effective activity of the active ingredient so that any side effects ascribable to the carrier do not vitiate the beneficial effects of the active ingredient. It is likely that the MMP8 and TNFR1 inactivating antigen binding polypeptides, nucleic acids and vectors according to the invention are administered as a combination therapy in combination with at least one further anti-inflammatory agent. Explicitly within the scope of the present application is the combined administration of the MMP8 and TNFR1 inactivating antigen binding polypeptides, nucleic acids and vectors as disclosed herein with anti-infectious agents. Typically, these anti-infectious reagents are antibiotics.

Such a pharmaceutical composition can be utilized to achieve the desired pharmacological effect by administration to a patient in need thereof. A patient, for the purpose of this invention, is a mammal, particularly a human, in need of treatment for the particular condition or disease. Also within the scope of the present invention are veterinary applications. A pharmaceutically effective amount of polypeptides, nucleic acids and vectors of the invention and a pharmaceutically acceptable carrier is preferably that amount which produces a result or exerts an influence on the particular condition being treated.

It is an object of the invention to provide therapeutic agents, particularly pharmaceutical compositions, inactivating both Matrix Metallo-Proteinase 8 (MMP8) and Tumor necrosis factor receptor 1 (TNFR1). Such therapeutic agents can be useful in the treatment of inflammatory diseases.

This is achieved, according to a first aspect, by providing a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a Tumor necrosis factor receptor 1 (TNFR1) inactivating antigen binding polypeptide. A series of candidate MMP8 inactivating antigen binding polypeptides and TNFR1 inactivating antigen binding polypeptides to be included in such composition have been described hereinabove. More specific MMP8 inactivating antigen binding polypeptides and TNFR1 inactivating antigen binding polypeptides to be included in such composition are described hereafter.

In an embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody.

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH.

In a further embodiment of the invention a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

SEQ ID NOs 1 to 3 refer to the sequence of the CDRs of the MMP8 inactivating antigen binding polypeptide:

SEQ ID NO: 1: GFTLDYYNIG

SEQ ID NO: 2: CISSSGGRTNYADSVKG

SEQ ID NO: 3: CMATTEGYEYDY

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

SEQ ID NOs 4 to 6 refer to the sequence of the CDRs of the TNFR1 inactivating antigen binding polypeptide:

SEQ ID NO: 4: VRTFSSYAMG

SEQ ID NO: 5: AISWSGGSTYYADSVKG

SEQ ID NO: 6: LHEDLYEYDY

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of

said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

According to a particular embodiment, a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule.

In an embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide,

wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody.

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH.

In a further embodiment of the invention a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in

SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in

SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further aspect, the invention relates to an isolated polypeptide comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding

polypeptide, and to compositions, such as pharmaceutical compositions, comprising such isolated polypeptide.

In one embodiment, such isolated polypeptide may be comprising a MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment, such isolated polypeptide may be comprising a TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID Nos.

In yet another embodiment, such isolated polypeptide may be comprising a MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID Nos; and may be comprising a TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID Nos. One non-limiting example of such isolated polypeptide is one comprising the amino acid sequence depicted in SEQ ID NO: 7 (except for 6xHis-tag this is the amino acid sequence encoded by the nucleotide sequence of Figure 8B).

According to a particular embodiment, a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity.

SEQ ID NO: 7:

QVQLQESGGGLVQAGGPLRLSCAASVRTFSSYAMGWFRQAPGKEREFVAAISWSGGSTYY
ADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAVLHEDLYEYDYWGQGTQVTVSSG
GGGSGGGGSGGGGSQVQLQESGGGLVQPGGSLRLSCEASGFTFSRFGMTWVRQAPGKGVE
WVSGISSLGDSTLYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCTIGGSLNPGG
QGTQVTVSSGGGGSGGGGSGGGGSQVQLQESGGGLVQPGGSLRLSCTASGFTLDYYNIGW
FRQAPGKERERVSCISSSGGRTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTGVYYC

AHCMATTEGYEYDYWGQGTQVTVS

SEQ ID NO: 7 depicts a tri-specific nanobody construct with the structure anti-TNFR1- anti-Albumine-anti-MMP8. The different building blocks of this construct are linked by a (G4S)3- linker. Based on the present disclosure and the underlying example section the skilled practitioner can modify the structure of this construct according to the actual needs. Modified constructs also fall under the scope of the present application and the invention should not be limited by the structure or particular sequence of the specific construct as disclosed.

In an embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said antigen binding polypeptides comprise an antibody .

In another embodiment of the invention a composition is provided comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH.

In a further embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a

CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are

present in one molecule and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ

ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

According to a particular embodiment, a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity.

In an embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said antigen binding polypeptides comprise an antibody. An exemplary nucleotide sequence is the one defined by SEQ ID NO: 28.

In another embodiment of the invention a nucleic acid is provided encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH.

In a further embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein

CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective

SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding

polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

According to a particular embodiment, a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity.

In an embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of composition comprising a Matrix Metallo-Proteinase 8 (MMP8)

inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said antigen binding polypeptides comprise an antibody.

In another embodiment of the invention a vector is provided comprising a nucleic acid encoding at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH.

In a particular embodiment, the composition, nucleic acid and vector according to the invention as described above are provided for use as a medicament.

In another particular embodiment, the composition, nucleic acid and vector according to the invention as described above are provided for use to treat an inflammatory disease.

In a preferred embodiment, the composition, nucleic acid and vector according to the invention as described above are provided for use to treat an inflammatory disease, wherein said inflammatory disease is selected from the list of diseases consisting of systemic inflammatory response syndrome, sepsis, LPS induced inflammation, renal ischemia/reperfusion injury, ventilation induced lung injury, periodontal inflammation, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, ankylosing spondylitis, Lyme arthritis and osteoarthritis.

In a particularly preferred embodiment, the composition, nucleic acid and vector according to the invention as described above are provided for use to treat an inflammatory disease, wherein said inflammatory disease is sepsis.

In another particularly preferred embodiment, the composition, nucleic acid and vector according to the invention as described above are provided for use to treat an inflammatory disease, wherein said inflammatory disease is LPS induced inflammation.

In a particular embodiment, a pharmaceutical composition is provided comprising the composition, nucleic acid and vector according to the invention as described above and a pharmaceutically acceptable carrier.

In another particular embodiment, a kit is provided comprising the composition, nucleic acid and vector according to the invention as described above.

In a further embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding

polypeptide and a TNFR1 inactivating antigen binding polypeptide is provided wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and

wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence

depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in

SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In a further embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein

comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In another embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

In one embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule and wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH and wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs and wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.

According to a particular embodiment, a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide

of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity.

In an embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said antigen binding polypeptides comprise an antibody.

In another embodiment of the invention a host cell is provided comprising a nucleic acid or a vector comprising said nucleic acid, wherein said nucleic acid encodes at least one antigen binding polypeptide of a composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity, wherein said antigen binding polypeptides comprise an antibody, wherein said antibody is a VHH.

In one embodiment of the invention, said antigen binding protein is derived from a camelid antibody, preferably from a heavy chain camelid antibody, devoid of light chains, such as a variable domain of a heavy chain camelid antibody (VHH).

Preferably, said VHH comprises a sequence selected from the group consisting of SEQ ID N° 1 – SEQ ID N°6.

In one embodiment a composition is provided comprising a MMP8 inactivating antigen binding polypeptide, a TNFR1 inactivating antigen binding polypeptide and at least one antigen binding polypeptide directed against a third target.

Accordingly, the present invention also relates to a trispecific or multispecific polypeptide, comprising or essentially consisting of at least three binding moieties, such as three immunoglobulin single variable domains (ISVs), wherein at least one of said at least three binding moieties is directed against a first target, at least one of said at least three binding moieties is directed against a second target and at least a third binding moiety increasing half-life, such as e.g. an Albumin binder.

As will be clear from the further description above and herein, the polypeptides of the invention can be used as "building blocks" to form polypeptides of the invention, e.g., by suitably combining ISVs with other groups, residues, moieties or binding units, in order to form compounds or constructs as described herein (such as, without limitations, bi-/tri-/tetra-/ multivalent polypeptides and bi-/tri-/tetra-/multispecific polypeptides of the invention described herein) which combine within one molecule one or more desired properties or biological functions. Examples of ways in which the valence and specificity of polypeptides of the invention can be adapted and examples of such polypeptides with adapted valence and specificity will be clear to the skilled person based on the disclosure herein; and such bi-/tri-/tetra-/ multivalent polypeptides and bi-/tri-/tetra-/multispecific polypeptides form a further aspect of the invention.

The process of designing/selecting and/or preparing the composition comprising a MMP8 inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide of the invention, starting from an amino acid sequence of the invention, is also referred to herein as "formatting" said amino acid sequence of the invention; and an amino acid of the invention that is made part of a compound or polypeptide of the invention is said to be "formatted" or to be "in the format of said compound or polypeptide of the invention". Examples of ways in which an amino acid sequence of the invention can be formatted and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted polypeptides form a further aspect of the invention. Moreover, examples of ways in which the antigen binding affinity of polypeptides of the invention can be adapted and examples of such polypeptides with adapted antigen binding affinities will be clear to the skilled person based on the disclosure herein; and such polypeptides with adapted antigen binding affinities form a further aspect of the invention.

Among others, non-limiting examples of MMP8 inactivating antigen binding polypeptides can be found in WO2012059513.

For non-limiting examples of TNFR1 inactivating antigen binding polypeptides the reader is referred to WO2008149148, WO2008113515 and Steeland S et al., J Biol Chem. 2015.

In a specific, but non-limiting aspect of the invention, which will be further described herein, the polypeptides of the invention have an increased half-life in serum (as further described herein). More specifically, the half-life of ISVs according to the invention is increased when compared to the immunoglobulin single variable domain from which they have been derived. For example, an

immunoglobulin single variable domain of the invention may be linked (chemically or otherwise) to one or more groups or moieties that extend the half-life (such as PEG), so as to provide a derivative of an amino acid sequence of the invention with increased half-life.

Some examples of such methods to increase the half-life of polypeptides of the invention are known to the skilled person and are further explained based on the disclosure herein, and for example comprise polypeptides of the invention that have been chemically modified to increase the half-life thereof (for example, by means of pegylation); polypeptides of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin); or polypeptides of the invention which comprise at least one amino acid sequence of the invention that is linked to at least one moiety (and in particular at least one amino acid sequence) which increases the half-life of the amino acid sequence of the invention. Examples of polypeptides of the invention which comprise such half-life extending moieties or immunoglobulin single variable domains for example include, without limitation, polypeptides in which the one or more immunoglobulin single variable domains of the invention are suitably linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, domain antibodies, immunoglobulin single variable domains that are suitable for use as a domain antibody, single domain antibodies, immunoglobulin single variable domains that are suitable for use as a single domain antibody, "dAb"'s, immunoglobulin single variable domains that are suitable for use as a dAb, or Nanobodies that can bind to serum proteins such as serum albumin (such as human serum albumin), serum immunoglobulins such as IgG, or transferrin; reference is made to the further description and references mentioned herein); polypeptides in which an amino acid sequence of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more immunoglobulin single variable domains of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins, such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489, WO2008/068280, WO2009/127691 and PCT/EP2011/051559.

Generally, the polypeptides of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence of the invention per se. For example, the polypeptides of the invention with increased half-life may have a half-life e.g., in humans that is increased with more than 1 hours, preferably more than 2 hours, more preferably more

than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In a preferred, but non-limiting embodiment of the invention, such polypeptides of the invention have a serum half-life e.g. in humans that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In another preferred, but non-limiting embodiment of the invention, such polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more.

Usually, for easy of expression and production, a polypeptide of the invention will be a linear polypeptide. However, the invention in its broadest sense is not limited thereto. For example, when a polypeptide of the invention comprises three or more building blocks, ISVs or Nanobodies, it is possible to link them by use of a linker with three or more "arms", which each "arm" being linked to a building block, ISV or Nanobody, so as to provide a "star-shaped" construct. It is also possible, although usually less preferred, to use circular constructs.

According to the invention, polypeptides are linked to one or more serum proteins or fragments thereof in such a way that linkage leads to an increased half-life of the polypeptides. The effect of different shapes of the construct can be tested during routine experimentation and non-limiting examples of ways in which the polypeptides can be linked are illustrated in the present disclosure; such linked polypeptides with an increased half-life form a further aspect of the invention.

The compounds or polypeptides of the invention can generally be prepared by a method which comprises at least one step of suitably linking the one or more immunoglobulin single variable domains of the invention to the one or more further groups, residues, moieties or binding units, optionally via the one or more suitable linkers, so as to provide the compound or polypeptide of the invention. Polypeptides of the invention can also be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a polypeptide of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide of the invention. Such methods can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

Also within the scope of the present invention are compounds or constructs, which comprise or essentially consist of one or more derivatives as described herein, and optionally further comprise one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers. In one embodiment, said one or more other groups, residues, moieties or binding units are immunoglobulin single variable domains.

In the compounds or constructs described above, the one or more polypeptides of the invention and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, moieties or binding units are immunoglobulin single variable domains, the linkers may also be immunoglobulin single variable domains, so that the resulting compound or construct is a fusion protein or fusion polypeptide.

In the polypeptides of the invention, the two or more building blocks, ISVs or Nanobodies and the optionally one or more polypeptides and one or more other groups, drugs, agents, residues, moieties or binding units may be directly linked to each other (as for example described in WO 99/23221) and/or may be linked to each other via one or more suitable spacers or linkers, or any combination thereof. Suitable spacers or linkers for use in multivalent and multispecific polypeptides will be clear to the skilled person, and may generally be any linker or spacer used in the art to link amino acid sequences. Preferably, said linker or spacer is suitable for use in constructing polypeptides that are intended for pharmaceutical use.

Some particularly preferred spacers include the spacers and linkers that are used in the art to link antibody fragments or antibody domains. These include the linkers mentioned in the general background art cited above, as well as for example linkers that are used in the art to construct diabodies or ScFv fragments (in this respect, however, it should be noted that, whereas in diabodies and in ScFv fragments, the linker sequence used should have a length, a degree of flexibility and other properties that allow the pertinent VH and VL domains to come together to form the complete antigen-binding site, there is no particular limitation on the length or the flexibility of the linker used in the polypeptide of the invention, since each nanobody by itself forms a complete antigen-binding site).

For example, a linker may be a suitable amino acid sequence, and in particular amino acid sequences of between 1 and 50, preferably between 1 and 30 amino acid residues. Some preferred examples of such

amino acid sequences include gly-ser linkers, for example of the type (glyxsery)z, such as (for example (gly ser)3 or (gly3ser2)3, as described in WO 99/42077 and the (G4S)3, GS30, GS15, GS9 and GS7 linkers described in the applications by Ablynx mentioned herein (see for example WO 06/040153 and WO 06/122825), as well as hinge-like regions, such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences (such as described in WO 94/04678). The preferred linker is (G4S)3. Some other linkers are poly-alanine (such as AAA), as well as the linkers GS30 (SEQ ID NO: 85 in WO 06/122825) and GS9 (SEQ ID NO: 84 in WO 06/122825). Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in polypeptides for pharmaceutical use. For instance, poly(ethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026.

It is encompassed within the scope of the invention that the length, the degree of flexibility and/or other properties of the linker(s) used (although not critical, as it usually is for linkers used in ScFv fragments) may have some influence on the properties of the final polypeptide of the invention, including but not limited to the affinity, specificity or avidity for a chemokine, or for one or more of the other antigens. Based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments. For example, in multivalent polypeptides of the invention that comprise building blocks, ISVs or Nanobodies directed against a first and second target, the length and flexibility of the linker are preferably such that it allows each building block, ISV or Nanobody of the invention present in the polypeptide to bind to its cognate target, e.g. the antigenic determinant on each of the targets. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

It is also within the scope of the invention that the linker(s) used confer one or more other favourable properties or functionality to the polypeptides of the invention, and/or provide one or more sites for the formation of derivatives and/or for the attachment of functional groups (e.g. as described herein for the derivatives of the Nanobodies of the invention). For example, linkers containing one or more charged amino acid residues can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments. Finally, when two or more linkers are used in the polypeptides of the invention, these linkers may be the same or different. Again, based on the disclosure herein, the skilled person will be able to determine the

optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

It will be appreciated that the order of the first building block and the second building block on the polypeptide (orientation) can be chosen according to the needs of the person skilled in the art, as well as the relative affinities which may depend on the location of these building blocks in the polypeptide, and whether the polypeptide comprises a linker, is a matter of design choice. However, some orientations, with or without linkers, may provide preferred binding characteristics in comparison to other orientations. For instance, the order of the first and the second building block in the polypeptide of the invention can be (from N-terminus to C-terminus): (i) first building block (e.g. a first ISV such as a first Nanobody) - [linker] -second building block (e.g. a second ISV such as a second Nanobody); or (ii) second building block (e.g. a second ISV such as a second Nanobody) - [linker] -first building block (e.g. a first ISV such as a first Nanobody); (wherein the linker is optional). All orientations are encompassed by the invention, and polypeptides that contain an orientation that provides desired binding characteristics can be easily identified by routine screening, for instance as exemplified in the examples section.

A further embodiment of the invention is a nucleic acid encoding a MMP8 inactivating antigen binding and/or a TNFR1 inactivating antigen binding polypeptide according to the invention. In a preferred embodiment, said nucleic acid is operably linked to a promoter, thereby allowing expression of the MMP8 and/or TNFR1 inactivating polypeptide in a selected host. “Operably linked” refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A promoter sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the promoter sequence. “Promoter sequence” as used here refers to a functional DNA sequence unit that, when operably linked to a coding sequence and possibly placed in the appropriate inducing conditions, is sufficient to promote transcription of said coding sequence.

In still another embodiment of the invention a host cell transformed with a vector comprising a nucleic acid according to the invention is provided. Said host cell can be any host cell known to the person skilled in the art and includes, but is not limited to bacterial cells, fungal cells including yeast cells, insect cells and mammalian cells. Preferably said host cell is a bacterial cell or a yeast cell.

Still another aspect of the invention is the use of a host cell according to the invention, for the production of a MMP8 and/or TNFR1 inactivating antigen binding polypeptide. Said production will normally

comprise (a) the cultivation of the host cell (b) creating conditions allowing the expression of the nucleic acid encoding the MMP8 and/or TNFR1 inactivating antigen binding polypeptides – either in parallel with the cultivation or at the end of the cultivation (c) isolating the MMP8 and/or TNFR1 inactivating antigen binding polypeptide. The MMP8 and/or TNFR1 inactivating antigen binding polypeptide can be secreted in the medium, or it may remain intracellular, requiring disruption of the host cell for the isolation of the MMP8 and/or TNFR1 inactivating antigen binding polypeptide.

Pharmaceutical composition- Route of administration

In one embodiment, a pharmaceutical composition is provided comprising the polypeptides, nucleic acids and vectors according to the invention and a pharmaceutically acceptable carrier. Such a pharmaceutical composition can be utilized to achieve the desired pharmacological effect by administration to a patient in need thereof.

A patient, for the purpose of this invention, is a mammal, particularly a human, in need of treatment for the particular condition or disease.

The terms “treatment” or “treating” or “treat” can be used interchangeably and are defined by a therapeutic intervention that slows, interrupts, arrests, controls, stops, reduces, or reverts the progression or severity of a sign, symptom, disorder, condition, or disease, but does not necessarily involve a total elimination of all disease-related signs, symptoms, conditions, or disorders. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

Therefore, the present invention includes pharmaceutical compositions that are comprised of a pharmaceutically acceptable carrier and a pharmaceutically effective amount of polypeptides, nucleic acids and vectors of the invention and a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is preferably a carrier that is relatively non-toxic and innocuous to a patient at concentrations consistent with effective activity of the active ingredient so that any side effects ascribable to the carrier do not vitiate the beneficial effects of the active ingredient. A pharmaceutically effective amount of polypeptides, nucleic acids and vectors of the invention and a pharmaceutically acceptable carrier is preferably that amount which produces a result or exerts an influence on the particular condition being treated. The polypeptides, nucleic acids and vectors of the invention and a pharmaceutically acceptable carrier can be administered with pharmaceutically acceptable carriers well known in the art using any effective conventional dosage form, including immediate, slow and timed release preparations, and can be administered by any suitable route such as any of those commonly

known to those of ordinary skill in the art. For therapy, the pharmaceutical composition of the invention can be administered to any patient in accordance with standard techniques. The administration can be by any appropriate mode, including orally, parenterally, topically, nasally, ophthalmically, intrathecally, intracerebroventricularly, sublingually, rectally, vaginally, and the like. Still other techniques of formulation as nanotechnology and aerosol and inhalant are also within the scope of this invention. The dosage and frequency of administration will depend on the age, sex and condition of the patient, concurrent administration of other drugs, counter-indications and other parameters to be taken into account by the clinician.

The pharmaceutical composition of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use.

When prepared as lyophilization or liquid, physiologically acceptable carrier, excipient, stabilizer need to be added into the pharmaceutical composition of the invention (Remington's Pharmaceutical Sciences 22th edition, Ed. Allen, Loyd V, Jr. (2012). The dosage and concentration of the carrier, excipient and stabilizer should be safe to the subject (human, mice and other mammals), including buffers such as phosphate, citrate, and other organic acid; antioxidant such as vitamin C, small polypeptide, protein such as serum albumin, gelatin or immunoglobulin; hydrophilic polymer such as PVP, amino acid such as amino acetate, glutamate, asparagine, arginine, lysine; glycose, disaccharide, and other carbohydrate such as glucose, mannose or dextrin, chelate agent such as EDTA, sugar alcohols such as mannitol, sorbitol; counterions such as Na+, and /or surfactant such as as TWEEN™, PLURONICS™ or PEG and the like.

The preparation containing pharmaceutical composition of this invention should be sterilized before injection. This procedure can be done using sterile filtration membranes before or after lyophilization and reconstitution.

The pharmaceutical composition is usually filled in a container with sterile access port, such as an i.v. solution bottle with a cork. The cork can be penetrated by hypodermic needle.

In one embodiment, the polypeptides, nucleic acids and vectors according to the invention are provided for use as a medicament. Said medicament is needed in a therapeutically effective amount. One of ordinary skill in the art will recognize that the potency and, therefore, an "effective amount" can vary

for the polypeptides, nucleic acids and vectors of the present invention. One skilled in the art can readily assess the potency of the polypeptides, nucleic acids and vectors according to the invention.

The dosage and concentration can be adjusted according to the actual situation as the effective amount needed will depend upon the severity of the disease and the general state of the patient's health. One skilled in the art knows how to choose proper dosage and injection means according to the actual situation.

Furthermore, the skilled clinician will be able to determine the appropriate dosing interval to treat a disease. The animal experiments presented in the example section disclose credible instructions on how to determine an effective amount for the treatment of a human. Further on, the principle for adjusting between different species such as mice and human can be seen in Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" in Toxicokinetics and New Drug Development, Yacobi et al.; Pergamon Press, New York 1989, pp.42-96.

Treatment performed using the compositions described herein is considered "effective" if one or more symptoms are reduced, relative to such symptoms present before treatment, or relative to such symptoms in an individual (human or model animal) not treated with such composition or other suitable control. Symptoms will obviously vary depending upon the disease or disorder targeted, but can be measured by an ordinarily skilled clinician or technician. Such symptoms can be measured, for example, by monitoring the level of one or more biochemical indicators of the disease or disorder (e.g., levels of an enzyme or metabolite correlated with the disease, affected cell numbers, etc.), by monitoring physical manifestations (e.g., inflammation, etc.), or by an accepted clinical assessment scale. A sustained (e.g., one day or more, preferably longer) reduction in disease or disorder symptoms by one or more points on a given clinical scale is indicative of "effective" treatment. Similarly, prophylaxis performed using a composition as described herein is "effective" if the onset or severity of one or more symptoms is delayed, reduced or abolished relative to such symptoms in a similar individual (human or animal model) not treated with the composition.

The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Modifications and variation of the above-described embodiments of the invention are possible without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore understood that, within the scope of the claims and their equivalents, the invention may be practiced

otherwise than as specifically described. The invention will now be further described by means of the following non-limiting preferred aspects, examples and figures.

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove.

Examples

Example 1: MMP8 and sTNFR1 plasma levels are elevated in human severe sepsis and septic shock.

After admission to the intensive care unit (ICU), thirteen patients were enrolled within 24 h after meeting the criteria for severe sepsis or septic shock defined at the consensus conference of 2001 (Levy et al. 2003, Intensive Care Med 29:530-538) and after a signed informed consent was obtained from the patient itself or a legal representative. Recently, a new consensus definition has been published (Singer et al. 2016, JAMA 315:801-810) but they were published after the initiation of the study so we applied the old definition. Patients' characteristics such as initial SOFA score, site of infection, the presence of septic shock and treatments were summarized in **Table 1**.

Table 1 Clinical characteristics of patients including in the study

Patient number	SOFA score @ time of ICU admission	Site of infection	Septic shock?	Treatment
1	12	Perforation bowel, complication after surgery (draining liver abscess)	Yes	Vancocin/Flagyl/Meronem/Ciproxine/Zyvoxid
2	8	Pancreatitis	SIRS	Tazocin
3	11	Small bowel perforation (no fecal peritonitis), 4 quadrant peritonitis (whole peritoneum infected, not local), complication after surgery	Yes	NA
4	14	Endocarditis	Yes	Meronem, Zyvoxid, Tavanic, Pentrexyl
5	10	Steatohepatitis + colon perforation	Yes	Meronem, Tazocin, Vancocin, Erythrocine, Tavanic, Eusaprim
6	6	Bilateral pneumonia	Yes	Augmentin, Biclar, Tazocin, Augmentin
7	6	Trauma by surgery, pancreatitis, necrosectomy	Yes	Meronem, Vancocin, Ciproxine
8	9	Urinary tract infection	Yes	Zyvoxid, Augmentin
9	10	Pneumonia	Severe sepsis	Tazocin, Eusaprim
10	11	Pneumonia	Yes	Cefazoline, Zinacef IV

Patient number	SOFA score @ time of ICU admission	Site of infection	Septic shock?	Treatment
11	7	Pneumonia	NA	Augmentin, Biclar, Solumedrol
12	13	Pneumonia	Yes	Augmentin, Biclar, Meronem, Negaban
13	6	Infection with unknown focus	Yes	Tazocin, Flagyl, Tavanic
NA: Not available				

Male or female patients ≥ 18 years of age were included after meeting two of following criteria of severe sepsis: hyper- or hypothermia (>38°C or < 36°C); heart rhythm > 90/min; respiratory rate > 20/min and leukocytosis or leukopenia (> 12000/mm^3 or < 4000/mm^3), in addition to a suspected or present source of infection and elevated lactate levels (> 12 mg/dl) OR urinary output < 0.5 ml/kg/h during > 2 h despite adequate fluid resuscitation OR acute lung injury with PaO_2/FiO_2 < 250 in the absence of pneumonia as an infection source OR acute lung injury with PaO_2/FiO_2 < 200 in the presence of pneumonia as an infection source OR thrombocytopenia (< 100000/µl) OR coagulopathy (INR > 1.5). Patients with septic shock were included when they fulfilled the criteria of severe sepsis in addition to persistent hypotension despite adequate fluid resuscitation (systolic pressure < 90 mmHg or reduction of > 40 mmHg compared to baseline) OR the need for vasopressors despite adequate fluid resuscitation.

Patients were excluded after meeting the following criteria: age < 18 year, the use of immunosuppressive medication and patients with HIV, hematological malignancies, liver cirrhosis or chronic kidney insufficiency.

From patients meeting the inclusion criteria, blood was withdrawn within 24 h of diagnosis (day 1) and from then every day until the end of their stay in ICU. The CRP levels and sequential organ failure (SOFA) score were determined daily. Blood was collected in heparin-coated vials for other cytokine analysis or in EDTA-coated vials to determine whole blood RNA expression. Control patients had blood withdrawn at ambulatory centers. Thirteen patients and six healthy controls were included in this study. Real-time qPCR was performed (see further) on total RNA was isolated from whole blood using the NucleoSpin® RNA blood kit (Macherey-Nagel) according to manufacturer's guidelines. Bio-Plex and ELISA were performed on plasma to determine MMP8, IL6 and sTNFR1 levels (see further). To demonstrate a link between MMP8 and sTNFR1 levels on the one hand and disease severity on the other hand, we correlated MMP8 and sTNFR1 levels with the CRP, the SOFA score and IL6 levels (see Statistics). MMP8 and sTNFR1 levels were also correlated with each other.

We could show that both MMP8 and sTNFR1 protein levels were increased in plasma of sepsis patients compared to healthy controls already from day one of ICU admission and they remained elevated the following days (**Figure 1A-B, Figure 13A-B**). To demonstrate a link between MMP8 and sTNFR1 levels on the one hand and disease severity on the other hand, we measured IL6 and CRP plasma levels in those patients and correlated them with each other. This revealed that MMP8 and sTNFR1 levels were positively correlated with IL6 (**Figure 13C**) and CRP levels but also with the sequential organ failure assessment (SOFA) score, an objective score that describes the incidence and severity of organ dysfunction in critically ill patients (Vincent JL, et al., *Intensive care medicine* 1996;22:707-710) (**Figure 1C-D**). More importantly, MMP8 and sTNFR1 levels were also correlated with each other (**Figure 1E**). Also *MMP8* and *TNFRSF1A* mRNA expression in whole blood-derived RNA of sepsis patients was increased on day 1 after ICU admission compared to healthy control patients (data not shown) but no correlation was found between expression levels and disease severity, except for *TNFRSF1A* and CRP (data not shown).

By measuring IL6 plasma levels in the sepsis patients, it was shown that the levels on day 1 were elevated but not significantly due to high interpatient variability. They were the highest at day 2 in ICU and then they gradually decrease although they remain significantly higher than in healthy controls. We also determined plasma IL1β levels but these did not exceed the detection limit (data not shown). The levels of sTNFR1, MMP8 and IL6 follow the same pattern and we correlated them with each other and with disease severity. Our data revealed that TNFR1 and MMP8 plasma levels were positively correlated with IL6 (r 0.53 and 0.59, **Figures 13D and E, Table 2**) and CRP levels (r 0.39 and 0.53), and also with the sequential organ failure assessment (SOFA) score (r 0.58 and 0.51), an objective score that describes the incidence and severity of organ dysfunction in critically ill patients (Vincent et al., 1996, Intensive Care Med 22:707-710) (Figures 13F and G, **Table 2**). As expected, IL6 plasma levels were also correlated with the SOFA score (r 0.47, p=0.0013) (**Table 2**). More importantly, MMP8 and sTNFR1 levels were also correlated with each other (r 0.66, **Figure 13H**). Also TNFRSF1A mRNA expression in whole blood-derived RNA of sepsis patients was significantly increased and MMP8 mRNA expression tended to be increased from the first day after ICU admission compared to healthy control patients (**Figure 13I**) but no correlation was found between mRNA levels and disease severity, except for TNFRSF1A and CRP (**Table 2**).

Table 2 Pearson correlation coefficients for inflammatory markers and disease score in sepsis patients

	Log([MMP8])	[sTNFR1]	Log(*MMP8*)	*TNFRSF1A*	Log([IL6])	CRP	SOFA
Log([MMP8])							
[sTNFR1]	**0.659**						
	<0.0001						
Log(*MMP8*)	0.334	0.286					
	0.027	0.0600					
TNFRSF1A	0.382	0.279	0.323				
	0.011	0.067	0.032				
Log([IL6])	**0.589**	**0.527**	0.367	0.276			
	<0.0001	**<0.0001**	0.014	0.069			
CRP	**0.532**	0.387	0.466	**0.574**	0.350		
	0.00020	0.0095	0.0014	**<0.0001**	0.020		
SOFA	**0.510**	**0.582**	0.287	0.00465	**0.469**	**0.473**	
	0.00035	**<0.0001**	0.059	0.976	**0.0013**	**0.0012**	

Upper values: correlation coefficients *r*; lower values: corresponding p-value;
Correlations with correlation value > 0.4 and p-values ≤ 0.005 in bold

Collectively, these data indicate that increments in both TNFR1 and MMP8 are features of sepsis, meaning that those two molecules together may form interesting markers of sepsis severity and/or drug targets to treat sepsis.

Example 2: Increased *Tnfrsf1a* and *Mmp8* expression during endotoxemia compensate for the loss of MMP8 and TNFR1 in deficient mice.

Since we found that in human sepsis patients MMP8 and sTNFR1 plasma levels are positively correlated with sepsis severity and with each other, we studied the interaction of the TNFR1 and MMP8 pathways in endotoxemia. Therefore, we investigated *Tnfrsf1a* and *Mmp8* expression levels in organs of wildtype (WT) mice and compared them with those in $MMP8^{-/-}$ and $TNFR1^{-/-}$ mice. Eight hours after LPS-injection, we observed that $MMP8^{-/-}$ mice display a more pronounced *Tnfrsf1a* upregulation in ileum, choroid plexus and lung compared to WT mice (**Figure 2A**). Inversely, $TNFR1^{-/-}$ mice display increased *Mmp8*

expression in ileum and lung, but not in choroid plexus (**Figure 2B**). These data are also depicted in **Figures 14 A-B**.

Next, we studied whether *Tnfrsf1a* and *Mmp8* expression is also increased in septic mice, using the cecal ligation and puncture (CLP) model (**Figures. 14C-D**). Six hours after CLP, *Mmp8* expression was significantly increased in liver, lung, CP and brain compared to sham-treated mice (**Figure 14C**). Also *Tnfrsf1a* expression was increased in multiple organs e.g. liver, lung and CP, but not in brain (**Figure 14D**). These results clearly show the involvement of the two mediators in the pathology of sepsis.

Together, these data suggest that loss of MMP8 or TNFR1 leads to increased expression of *Tnfrsf1a* or *Mmp8*, respectively, and that this might reflect a compensation mechanism that arises by abrogation or inhibition of only one single pathway. Consequently, deficiency or inhibition of both MMP8 and TNFR1 might provide stronger protection against endotoxemia-induced lethality compared to single deficiency.

Example 3: <u>Combined genetic deficiency of MMP8 and TNFR1 leads to superior protection against endotoxemia.</u>

To investigate whether abrogation of both MMP8 and TNFR1 could increase protection in endotoxemia, we generated double knockout (DKO) mice by crossing $MMP8^{-/-}$ and $TNFR1^{-/-}$ mice and analyzed their susceptibility to endotoxemia. Initially, all mice were injected with a dose of LPS which is LD_{100} for WT mice, and both $MMP8^{-/-}$ and $TNFR1^{-/-}$ mice showed partial but significant protection (survival rate of 57% and 83%, respectively). DKO mice, however, were completely protected (**Figure 3A-B**). To further investigate the magnitude of protection, all mice were injected with 6 x LD_{100} of WT mice. As shown in **Figure 3C-D**, DKO mice were highly resistant (survival rate of 81,5%) to hypothermia and death induced by this LPS dose, which is in sharp contrast to the WT and single deficient mice, all of which died 24 to 30 h after challenge. Eight hours after LPS, extremely high systemic levels of pro-inflammatory cytokines, such as IL6 and IL17A, were found in serum of WT mice (**Figure 16 A-B**). IL6 levels in single KO mice were not significantly lower compared to WT mice but they were completely reduced in the DKO mice. Levels of IL17a were already lower in the single KO mice, and the same trend was seen in the DKO mice. Similarly, secretion of chemokines like MCP1, KC, RANTES and MIP1b (**Figure 16 C-F**) was reduced in DKO mice compared to WT mice while this was not always the case in the single deficient mice. Altogether, this shows that deficiency in both TNFR1 and MMP8 leads to superior protective patterns by completely blunting the systemic inflammation compared to single KO mice.

To further explore this remarkable and robust protective phenotype, all four strains were injected with the LD_{100} LPS dose in further experiments, followed by detailed analysis of different organs.

Example 4: Both MMP8 and TNFR1 are involved in the increased permeability of intestinal and blood-cerebrospinal fluid barriers during endotoxemia.

To understand the mechanisms behind the extreme resistance of DKO mice to lethal endotoxemia, we studied intestinal and blood-CSF barrier permeability during endotoxemia. We found that LPS induced intestinal permeability in WT mice but less so in $MMP8^{-/-}$ and DKO mice. We found no protection in $TNFR1^{-/-}$ mice at this level (**Figure 4A**). At the level of the blood-CSF barrier, an additive protective effect was observed in the DKO mice (**Figure 4D**).

Example 5: MMP8/TNFR1 double deficiency blunts local and systemic inflammation during endotoxemia.

Based on our finding that *Tnfrsf1a* expression was increased in the intestine and choroid plexus of $MMP8^{-/-}$ mice and *Mmp8* expression was increased in the intestine of $TNFR1^{-/-}$ mice (**Figure 2A-B**), we decided to investigate the degree of local inflammation in both ileum and choroid plexus. We found that in both organs, simultaneous removal of MMP8 and TNFR1 had an additive protective effect on inflammatory gene induction. Induction of *Il6* expression in both organs, *Mmp13* expression in the ileum and *Nos2* expression in the choroid plexus were significantly reduced in the single KOs, but induction was completely prevented in DKO mice (**Figure 4B-C and E-F**). Similar findings were found in lung, liver, spleen and cortex/hypothalamus (data not shown). Moreover, eight hours after LPS injection, extremely high systemic levels of pro-inflammatory cytokines, such as IL6 and IL17A, were found in serum of WT mice, and these levels were much reduced in DKO mice (**Figure 4G-H**). Similarly, secretion of chemokines MCP1 (**Figure 4I**), KC, RANTES, MIP1a and MIP1b (data not shown) was reduced in DKO mice while this was not always the case in single KO mice. Altogether, this shows that deficiency in both TNFR1 and MMP8 leads to superior protective patterns in gene expression and cytokine release compared to single KO mice.

Example 6: Combined deficiency of MMP8 and TNFR1 leads to superior protection against CLP and is accompanied by reduced systemic inflammation and liver damage.

Since combined inhibition of MMP8 and TNFR1 confers strong protection against endotoxemia, we verified the results using the CLP model, which is a model of septic peritonitis and is more representative of human polymicrobial sepsis (Dejager L et al., *Trends in microbiology* 2011;19:198-208). We previously reported that $MMP8^{-/-}$ mice have a significantly better outcome than WT mice after CLP (Vandenbroucke RE et al., *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2012;32:9805-

9816). However, the outcome of TNFR1$^{-/-}$ mice in the CLP model is more an issue of controversy, thus it is likely that the TNF/TNFR1 axis plays a mediatory role in the model without being a good drug target to increase survival (Ebach DR et al., *Shock* 2005;23:311-318). As shown in **Figure 5 A-C**, DKO mice subjected to CLP were significantly protected against hypothermia and lethality compared to WT and single KO mice (survival rate of 24% in WT, 20% in TNFR1-/-, 40% in MMP8-/- and 76% in DKO mice) and also reduced serum IL6 levels were observed in those mice. Strikingly, though TNFR1$^{-/-}$ mice displayed reduced hypothermia for a period of 72 h after initiation of CLP compared to WT mice, they eventually died at a similar rate as WT mice. These data demonstrate that the combination of TNFR1 and MMP8 deficiency is needed to obtain protection against CLP-induced death. The protection in the DKO mice is accompanied by lower systemic IL6 levels and also in the peritoneal lavage fluid chemokine levels like KC were significantly lower in DKO mice than in WT or single KO mice (**Figure 5 C-D**).

Example 7: <u>Double deficiency abrogates neutrophil efflux and protects against neutrophil depletion in CLP</u>

Because of the promising phenotype of the DKO mice in the CLP model, we continued to document the resistance of DKO mice in more detail. First, we examined the effects of dual deficiency on the innate immune response during the initial hyperinflammatory state. We observed that early after CLP, blood neutrophil levels significantly decreased in WT and TNFR1$^{-/-}$ mice but not in MMP8$^{-/-}$ and DKO mice (**Figure 6A-B**). In WT mice, neutrophils accumulated in the peritoneal fluid lavage (PFL). Conversely, in TNFR1$^{-/-}$ mice the reduced number of blood neutrophils could not be explained by an efflux to the peritoneum (**Figure 6C-D**). In MMP8$^{-/-}$ and DKO mice, there was only a minor WBC influx and no significant neutrophil influx in the peritoneum after CLP.

To address the biological effect of the presence or absence of this blood-to-peritoneum neutrophil flux, we investigated the bactericidal effect of the neutrophils in blood and PFL 6 and 24 h after CLP. On the one hand, we found that neutrophil efflux in WT mice was associated with increased bacterial content in blood, despite more efficient bacterial clearance in the peritoneum. On the other hand, clearance of bacteria in the peritoneum was diminished in MMP8$^{-/-}$ and TNFR1$^{-/-}$ mice and intermediate in DKO mice, though neutrophil numbers were similar (**Figure 6D-F**). Strikingly, blood of MMP8$^{-/-}$ mice contained more bacteria than that of DKO mice, and this was not correlated with the number of neutrophils, as in both genotypes there was no neutrophil efflux from blood to the peritoneum (**Figure 6B,D,E**). This may point towards a failed bacterial clearance in the blood of MMP8$^{-/-}$ mice. We next investigated neutrophil-attracting chemokines in PFL to understand the reduced neutrophil influx to the peritoneum in the DKOs and respective single KOs. Surprisingly, TNFR1$^{-/-}$ and MMP8$^{-/-}$ mice did not have lower levels of neutrophil

chemokines KC and MIP2 in PFL than WT mice (**Figure 6G-H**), pointing to an inefficient chemokine response in those mice as the reduced number of neutrophils in DKO mice could be explained by a reduction in PFL chemokines. Collectively, these data suggest that the protection seen in DKO mice can be attributed to a better control of intraperitoneal bacteria, as it is known that failure of this control ultimately leads to shock and death because of an overwhelming innate immune response (Lee MO et al., *Journal of leukocyte biology* 2011;89:423-432). Additionally, as the neutrophil load in blood and peritoneum remained balanced in DKO mice, enough bacterial clearance in both blood and peritoneum is maintained, eventually protecting against severe bacterial sepsis (Secher T et al., *J Immunol* 2009;182:7855-7864).

Example 8: <u>Blocking TNFR1 and MMP8 simultaneously with antibodies provides stronger protection than single treatments and reproduces the DKO phenotype after endotoxemia.</u>

To determine whether combining TNFR1 and MMP8 inhibition is useful as a new therapeutic strategy, we used a commercially available anti-mouse TNFR1 monoclonal antibody (Ab) and a specific in-housed generated anti-MMP8 Nanobody (Nb) (Demeestere D et al., *Molecular therapy : the journal of the American Society of Gene Therapy* 2016) in several therapeutic setups. First, TNFR1$^{-/-}$ mice were injected with the anti-MMP8 Nb and LPS (LD_{75} in TNFR1$^{-/-}$ mice). This treatment completely protected against hypothermia and death (**Figure 7 A-B**). Next, we injected MMP8$^{-/-}$ mice with anti-TNFR1 Ab and a lethal dose of LPS. Again, though Ab-treated mice experienced severe hypothermia, the survival rates were significantly higher (20%) compared to vehicle-treated mice (0%) (**Figure 7 C-D**). As final proof-of-concept, we tested whether the combination of both therapies could improve survival during endotoxemia in WT mice compared to the single treatments and injection of control vehicle.

Ultimately, we co-injected WT mice with the combined treatment of anti-TNFR1 Ab and anti-MMP8 Nb which markedly diminished mortality and protected against hypothermia (100% survival) compared to single anti-MMP8 therapy (36% survival) or no treatment (44% survival) (**Figure 7 E-F**). Combined therapy also seemed to perform better than single anti-TNFR1 therapy (71% survival).

Materials and Methods

Mice: C57BL/6J wildtype (WT), C57BL/6J TNFR1 (TNFR1-/-) and C57BL/6J MMP8 deficient (MMP8-/-) and C57BL/6J TNFR1-/-MMP8-/- mice (DKO) mice were used. TNFR1-/- and MMP8-/- mice are described by M. Rothe (Rothe J et al., Nature 1993;364:798-802) and Balbin (Balbin M et al., Nature genetics 2003;35:252-257), respectively. DKO mice were generated by intercrossing TNFR1-/- and MMP8-/- mice. All mice were housed in SPF conditions with 12-12 h light and dark cycles and free access to food and

water. We used age- (8-12 weeks) and gender-matched mice. All experiments were approved by the animal ethics committee of Ghent University.

Patients: The clinical study protocol was approved by the ethics committee of the University Hospital of Ghent. Patient selection and sample collection was conducted as described in online supplement.

Endotoxemia model: Mice were injected intraperitoneally (i.p.) with lipopolysaccharide (LPS) from Salmonella enterica serotype abortus equi (Sigma). The doses corresponded to LD100 (10 mg/kg) or 6 x LD100 for WT mice (60 mg/kg) or the lethal dose for TNFR1$^{-/-}$ and MMP8$^{-/-}$ mice (20 mg/kg). Control mice were injected with PBS. Rectal body temperature was followed after challenge. Body temperature of dead mice is set to 24°C as long as the remaining living mice form the same group are monitored. Organs and blood were isolated 8 h after injection of 10 mg/kg LPS for further analysis.

CLP procedure: Mice were subjected to CLP for induction of polymicrobial, peritoneal sepsis according to Wichterman & Chaudry 1980 (J Surgic Res 29:189-201). Sepsis was induced in isoflurane-anesthetized mice by 75% ligation of the cecum, followed by a single through-and-through puncture with a 21-Gauge needle, extruding a small amount of cecal contents as described previously (Vandenbroucke RE et al. 2012, J Neurosci 32:9805-9816). During lethality studies, mice were injected i.p. with broad-spectrum antibiotics (25 mg/kg ceftriaxone and 12.5 mg/kg metronidazole, Sigma) 9 h and 24 h after the CLP procedure. Sham-operated mice underwent the same procedure but without ligation and puncture.

The cecum of mice subjected to sublethal CLP was 50% ligated and punctured only once. Rectal body temperature was followed after challenge during several days. Body temperature of dead mice is set to 24°C as long as the remaining living mice from the same group are monitored. After 6 h or 24 h, blood and organs were collected and peritoneal lavage was performed with 4 ml of ice-cold PBS.

Therapeutic inhibition of MMP8 and TNFR1 in endotoxemia: MMP8-/- and TNFR1-/- mice were injected i.p. with 10 mg/kg Armenian hamster anti-TNFR1 antibody (R&D) or 25 mg/kg trivalent anti-MMP8 Nanobody (Demeestere D et al., Molecular therapy : the journal of the American Society of Gene Therapy 2016), respectively, and an LD100 injection of LPS. Control mice were treated with control Nb (Steeland S, The Journal of biological chemistry 2014) or PBS. Similarly, LPS-injected WT mice were treated with 10 mg/kg anti-TNFR1 antibody, 25 mg/kg anti-MMP8 Nanobody, a combination of both, or with vehicle.

Statistics: Statistics were performed using GraphPad Prism (GraphPad Software, Inc.). Survival curves were compared with a Mantel-Cox test. Bars represent mean ± SEM. Data were analyzed with a Mann-Whitney test unless mentioned differently. Pearson correlations were calculated between the variants. Variants showing a non-normal distribution (visual inspection) were log-transformed. Correlations were tested against zero using a two-sided t-test. Significance levels are indicated * for $0.01 \leq p < 0.05$; ** for $0.001 \leq p < 0.01$; *** for $0.001 \leq p$ 0.0001, and **** for $p < 0.0001$.

Real-time qPCR: Tissue sample, except choroid plexus, were isolated and stored in RNALater (Ambion). Choroid plexus was snap-frozen. RNA was isolated using the InviTrap Spin Universal RNA Mini kit (Isogen Life Science) for liver and the RNeasy Mini Kit (Qiagen) for all other organs. RNA concentration was measured using the Nanodrop 1000 (Thermo Scientific) and cDNA was synthesized by the iScript cDNA Synthesis Kit (Bio-Rad). qPCR was performed with the Light Cycler 480 system (Roche) using Sensifast Bioline Mix (Bio-Line). Expression levels were normalized to the expression of the two most stable housekeeping genes, which were determined for each organ using geNorm (Vandesompele et al. 2002, Genome Biol 3:research0034): liver, hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) and ribosomal protein (*Rpl*); ileum, ribosomal protein (*Rpl*) and *Gapdh*; lung: *Rpl* and *Hprt*; brain: *Hprt* and *Ubc*; and choroid plexus (CP): *Gapdh* and *Rpl*. For qPCRs on whole blood-derived mRNA of sepsis patients, housekeeping genes were 60S acidic ribosomal protein P0 (*RPLP0)* and 60S ribosomal protein L13a (*RPL13A*).

Primer sequences

Mmp8: forward 5'-ATTCCCAAGGAGTGTCCAAGC-3' (SEQ ID NO:8); reverse 5'-TGATTGTCATATCTCCAGCACTGG-3' (SEQ ID NO:9);

Tnfrsf1a: forward 5'-CCGGGAGAAGAGGGATAGCTT-3' (SEQ ID NO:10); reverse 5'-TCGGACAGTCACTCACCAAGT-3' (SEQ ID NO:11);

MMP8: forward 5'-TTTTGATGCCGAAGAAACATGGA-3' (SEQ ID NO:12), reverse 5'-GTGAGCGAGCCCCAAAGAA-3' (SEQ ID NO:13);

TNFRSF1A: forward 5'-CCTCAGGGGTTATTGGACTGG-3' (SEQ ID NO:14), reverse 5'-GGGGACACACACTATCTCTCT-3' (SEQ ID NO:15);

Gapdh: forward 5'-TGAAGCAGGCATCTGAGGG-3' (SEQ ID NO:16), reverse 5'-CGAAGGTGGAAGAGTGGGAG-3' (SEQ ID NO:17);

Ubc: forward 5'-AGGTCAAACAGGAAGACAGAC GTA-3' (SEQ ID NO:18), reverse 5'-TCACACCCAAGAACAAGCACA-3' (SEQ ID NO:19);

Hprt: forward 5'-AGTGTTGGATACAGG CCAGAC-3' (SEQ ID NO:20), reverse 5'-CGTGATTCAAATCCCTGAAGT-3' (SEQ ID NO:21);

Rpl: forward 5'-CCTGCTGCTCTCAAGGTT-3' (SEQ ID NO:22), reverse 5'-TGGTTGTCACTGCCTCGTACTT-3' (SEQ ID NO:23);

RPLP0: forward 5'-CATGCTCAACATCTCCCCCTTCTCC-3' (SEQ ID NO:24), reverse 5'-GGGAAGGTGTAATCCGTCTCCACAG-3' (SEQ ID NO:25);

RPL13A: forward 5'-CCTGGAGGAGAAGAGGAAAGAGA-3' (SEQ ID NO:26), reverse 5'-TTGAGGACCTCTGTGTATTTGTCAA-3' (SEQ ID NO:27).

Plasma cytokine, chemokine and MMP8 determination: Levels of cytokines and chemokines were measured in plasma and peritoneal lavage fluid using a multiplex method (Bio-Plex Pro-assay Bio-Rad). MMP8 levels in human heparin plasma were detected with the Luminex Fluorokine Map Multiplex Human MMP Assay (R&D Systems). All techniques were performed according to the manufacturer's guidelines.

ELISA: Soluble human TNFR1 and IL6 levels were determined in human heparin plasma with the human TNFRSF1A ELISA Pair set (Sino Biological Inc.), the human IL6 ready-set-go kit (eBioscience) according to the manufacturer's guidelines.

The following examples focus on the design and use of a trispecific construct capable of inhibition of both TNFR1 and MMP8.

Example 9: ***Binding affinity of Nb 70-alb-14.***

We characterized the binding affinity of Nb 70-alb-14 to human TNFR1, mouse MMP8 and albumin with ELISA and surface plasmon resonance (SPR). First, via ELISA we determined the affinity of Nb 70-alb-14 to human TNFR1 (hTNFR1) and to the catalytic domain of MMP8 (mMMP8_CD) (**Figure 9 a and c,** respectively), but as the full length of MMP8 is present *in vivo*, we also verified the affinity to the full length domain of MMP8 (mMMP8_FL) (**Figure 9 d**). We compared the affinities with the affinity of the respective monovalent Nbs: Nb 70, Nb 14 and Nb Alb. As shown in **Figure 9 a and c**, the binding affinities of Nb 70-alb-14 to hTNFR1 and mMMP8_CD are completely preserved compared to the affinity of Nb 70 (K_d 122.8 and 94.49 nM respectively). The affinity of Nb70-alb-14 to MMP8_FL was even ameliorated compared to Nb14 (**Figure 9d**). The affinity of Nb70-alb-14 for mouse albumin was lower than that of Nb ctrl (a trivalent Nb consisting of two irrelevant Nbs coupled to Nb Alb, or monovalent Nb Alb, respectively) (**Figure 9b**). The generation of a trispecific Nb also did not really alter the binding affinity to MMP8: binding to the catalytic domain of MMP8 (K_d Nb 70-alb-14 1.482 nM and Nb 14 0.66 nM) was completely preserved and the affinity of Nb 70-alb-14 to full-length MMP8 even increased compared to Nb 14 (K_d 0.9458 and 6.925 nM, respectively) (**Table 3**). The affinity of Nb 70-alb-14 for mouse albumin was lower than that of Nb Alb-Ctrl-Ctrl and of Nb Alb (25.89 nM, 3.55 nM and 0.8249 nM, respectively).

Surface plasmon analysis (SPR) analysis confirmed the trends we observed with the ELISA with only some slight differences (**Table 3** and **Figure 10**). Nb 70-alb-14 has fast kinetics of association to immobilized hTNFR1 (K_{a1} 7.523E4 1/Ms) with an overall equilibrium affinity of 75.4 nM (**Figure 10a**) but to MMP8_CD and MMP8_FL the association kinetics are a little bit slower (K_{a1} 5.074E4 1/Ms and K_{a1} 3.140E4 1/Ms, respectively) (**Figure 10b-c**). Nevertheless, their overall equilibrium affinities (K_D) are in the nanomolar

range, 293.5 nM to MMP8_CD and 164.7 nM to MMP8_FL. Strikingly, the association kinetics to MMP8_CD are better than to MMP8_FL, but the K_D to MMP8_FL is better which is good as *in vivo* mainly MMP8_FL is present.

Table 3

Equilibrium constant (K_D) of Nb 70-alb-14, Nb 14 and Nb 70 determined by surface plasmon resonance (SPR)[1] and K_d by ELISA to immobilized mouse catalytic domain MMP8 (mMMP8_CD), mouse full length domain (mMMP8_FL), human TNFR1 (hTNFR1) and albumin. *NA means not applicable*

	SPR-analysis[1]			***ELISA[2]***			
	mMMP8_CD K_D (nM)	*mMMP8_FL K_D (nM)*	*hTNFR1 K_D (nM)*	*mMMP8_CD K_d (nM)*	*mMMP8_FL K_d (nM)*	*hTNFR1 K_d (nM)*	*Mouse Albumin K_d (nM)*
Nb 70-alb-14	*294*	*189*	*153*	*1.482*	*0.9458*	*122.8*	*25.89*
Nb 14	*228*	*165*	*NA*	*0.66*	*6.925*	*NA*	*NA*
Nb 70	*NA*	*NA*	*38.71*	*NA*	*NA*	*94.49*	*NA*

Example 10: ***Inhibition capacity Nb 70-A-14 of MMP8 and TNFR1.***

First, we used HEK-2 blue cells to determine the ability of Nb 70-alb-14 to inhibit the TNF/hTNFR1 signaling cascade. In this assay, TNF/hTNFR1 signaling activates NF-κB (Wajant H and Scheurich P, *The FEBS journal* 2011;278:862-876) resulting in the transcription and secretion of the reporter protein alkaline phosphatase, which can be detected by purple/blue coloration of the HEK-2 blue detection medium. We used this system in the presence of increasing concentrations of Nb 70-alb-14 and its monovalent respective Nbs: Nb 70 and Nb14. **Figure 11a** shows that the inhibition capacity of the monovalent Nb 70 is higher than that of Nb 70-alb-14 (IC_{50} 868.3 nM vs 1670 vs). This indicates that Nb 70 needs to be in a certain conformation to inhibit the TNF/TNFR1 signaling pathway and that this conformation is altered by the creation of our trivalent construct resulting in an almost 2-fold reduction in inhibitory activity.

Next, the inhibitory capacity of the MMP8-inhibiting unit of Nb 70-alb-14 was determined by the DQ gelatin test (**Figure 11b**). DQ gelatin is a substrate for MMP8 that contains a quenched fluorophore which is released when gelatin is cleaved. First Nb 70-alb-14 was incubated with mMMP8_CD for 30 min after which DQ gelatin was added. The reduced change in fluorescence over time is a measure for the inhibitory capacity of Nb 70-alb-14 for mMMP8_CD. **Figure 11b, left** shows that increased amounts of Nb 70-alb-14 reduce the fluorescence over time. The higher the Nb/MMP8 ratio (w/w), the more MMP8

activity is reduced. Nb 70-alb-14 has an IC_{50} of 4.91 µM which is 5.5 times better than the IC_{50} of Nb 14 (26.97739 µM). This indicates that in the case of MMP8 inhibition the generation of a trivalent construct ameliorates its inhibition capacities.

Example 11: ***Analysis of the serum half-life of Nb 70-alb-14.*** Serum half-life of Nb 70-alb-14 was determined after a single intraperitoneal (i.p.) injection of 1000 µg Nb 70-alb-14 in wild type C57BL/6J mice. Blood was taken at various time points for seven consecutive days and serum concentrations of Nb70-alb-14 were determined using hTNFR1 ELISA. After i.p. injection, Nb70-alb-14 is absorbed through the peritoneum into the bloodstream (absorption phase) and Nb 70-alb-14 serum levels reach a maximal concentration (C_{max}) after 8 h of 48.2 ng/µl and has an area under the curve (AUC) of 2115+/-323 (**Figure 12a**). Once C_{max} is reached, Nb 70-alb-14 shows biphasic elimination, starting with a distribution and elimination phase that quickly decreases serum Nb 70-alb-14 concentration, followed by slower elimination from the circulation. In the end, Nb70-alb-Nb14 as a T1/2 of 43.5h. Multiple injections of 100 µg Nb 70-alb-14 (first injection followed by 2 consecutive injections after 10 h and 24 h) results in a peak concentration 300 ng/µl after 28 h (**Figure 12b**). After reaching C_{max} the serum levels decrease more steadily than the serum levels decrease after the C_{max} when only a single injection is given. Twenty hours after the C_{max} has been reached, the concentrations were halved.

Example 12: ***In vivo inhibition in CLP.***

We subjected mice to a clinically relevant model of human peritonitis, namely cecal ligation and puncture (CLP). Mice were treated with 500 µg Nb 70-alb-14 immediately after the surgery, and after 24 h and 48 h. In this model, mice are slightly protected and have a delay of mortality and the final mortality rates were lower after treatment. However, further research is needed to see whether survival could be enhanced with a higher dose and a more frequent dosing of Nb 70-alb-14 (**Figure 12 C**).

As a final proof-of-concept, we subjected hTNFR1 transgenic mice (Steeland et al., unpublished results) to severe CLP with high mortality or to sublethal CLP with lower mortality to reproduce the sepsis lethality rates in human (data not shown). First, the sensitivity of hTNFR1 Tg mice in cecal ligation and puncture (CLP) was analyzed: because the TNFR1-binding unit Nb 70 only binds and inhibits human TNFR1, we relied on human TNFR1 transgenic (hTNFR1 Tg) mice as we described previously (Steeland et al., unpublished results). To ensure that the hTNFR1 Tg mice behave similarly as WT mice in the CLP model, we subjected them to CLP and confirmed a similar sensitivity as in WT mice (**Figure 12 D).**

Mice were treated with Nb 70-alb-14 according to the following treatment scheme based on their serum half-life (T1/2). hTNFR1 Tg mice were treated with 1 mg Nb 70-alb-14 or PBS directly after the CLP surgery and after 9 h, 24 h and 48 h. Mice treated with Nb 70-alb-14 experienced reduced hypothermia compared to mice treated with PBS (**Figure 15 A**). Eventually, mortality rates were reduced when mice subjected to severe CLP were treated with Nb 70-alb-14 compared to PBS treated mice (survival rate of 28% *vs*. 19%, **Figure 15 B**), but also when they were subjected to less severe CLP (survival rate of 75% *vs.* 50%, data not shown). This shows that simultaneous inhibition MMP8 and TNFR1 is useful and can protect against sepsis.

Material and methods

Generation of Nb 70-alb-14 expressed in Pichia pastoris. We generated Nb 70-alb-14 consisting of Nb 70 (a Nb that binds human tumor necrosis factor receptor 1 (hTNFR1) and inhibits the TNF/hTNFR1 signaling) (Steeland S et al., *J Biol Chem* 2014). Nb 14 (a Nb that inhibits human and mouse matrix metalloproteinase 8 activity) (Demeestere D et al, *Molecular therapy : the journal of the American Society of Gene Therapy* 2016) and an anti-albumin Nb, linked to each other with flexible $[G_4S]_3$ linkers. The C-terminal end of Nb 70 is linked to the N-terminal end of the anti-Alb Nb and its C-terminal end to the N-terminal end of Nb 14, followed by a his_6-tag (**Figure 8a-b**).

The different VHH building blocks of Nb 70-alb-14 were designed in the pUC57, with the Nb 14 VHH gene at the N-terminal end and the His_6-tag at the C-terminal end and the construct was ordered by GenScript. The yeast *Pichia pastoris* (Strain GS115) expression system was used to express Nb 70-alb-14 and to allow expression in *P. pastoris*, the sequence was cloned into the pAOXZalfa vector and transformed in wild type GS115 *P. pastoris*. After selection of an appropriate expression clone, Nb 70-alb-14 was produced in 6 L of culture medium in baffled shake flasks (Schoonooghe S et al., *Methods Mol Biol* 2012;907:325-340). The medium was isolated by centrifugation at 18,000 x g for 30 min at 4°C and diafiltered against 20 mM NaH_2PO_4 pH 7.5, 500 mM NaCl, 20 mM imidazole and 1 mM PMSF. After loading, the column was washed with 20 column volumes of the same buffer in the presence of 0.1% empigen as detergent. Before elution, the column was equilibrated with 5 column volumes of equilibration buffer without detergent. The Nbs were first eluted with 20 mM NaH2PO4 pH 7.5, 20 mM NaCl, 50 mM imidazole and 1 mM PMSF, and then with 400 mM imidazole in the same buffer. The eluate was diluted 20 times with 25 mM sodium acetate pH 5.5 and loaded on a Source 15S column (GE Healthcare) to remove LPS and other contaminants. After equilibration, the Nbs were eluted by a linear gradient over 20 column volumes of NaCl from 0 to 1000 mM in 25 mM sodium acetate pH 5.5. Finally, the recombinant protein was injected on a Superdex 75 gel filtration column with PBS as running solution. The obtained fractions

were analyzed with Coomassie stained SDS-PAGE gels and anti-His western blots. Protein concentration was measured by the Micro-BCA assay (Pierce). LPS levels were determined using an EndoSafe-PTS assay (Charles River) that makes use of LAL reagents in a FDA-licensed disposable test cartridge with a handheld reader for real-time endotoxin testing. The LPS concentration was < 0.5 EU/ml.

ELISA. To determine the binding affinity of the purified Nbs, we performed different ELISAs. Microtiter half-area plates (Nunc) were coated overnight at 4°C with 50 ng of the substrate of interest in Tris-buffered saline (TBS) per well. Binding affinity was determined for the following substrates: hTNFR1 (PeproTech, 210-07), mMMP8_CD (2), full length mouse MMP8 (mMMP8_FL; R&D systems, 2904-MP-010) and mouse albumin (Sigma, A3559). Residual protein binding sites were blocked for 1 h at room temperature with TBS supplemented with 0.05% Tween 20 (TBST) and 5% BSA. Next, Nbs were added to the wells at the indicated concentrations in TBST and 2.5% BSA, and incubated for 1 h. A Nb with unrelated specificity was used as negative control: a trivalent Nb consisting of two cAbBcII10 Nbs, which is a control Nb targeting anti-β-lactamase (Conrath KE et al., *Antimicrobial agents and chemotherapy* 2001;45:2807-2812), coupled to an anti-albumin Nb, called Nb "Alb-Ctrl-Ctrl". Bound Nbs were detected with a mouse anti-His antibody (1:1000) (AbD SeroTec, MCA1396) followed by anti-mouse IgG1-HRP (1:2000) (GE Healthcare, NA931). Absorption at 450 nm was measured after adding the peroxidase substrate 3,30,5,50-tetra-methylbenzidine (TMB) (BD OptEIA) followed by stopping buffer (1 M H_2SO_4). The background signal at 595 nm was subtracted.

Surface Plasmon Resonance (SPR) analysis. Nb affinity for hTNFR1 and mMMP8_CD and mMMP8_FL was determined by Surface Plasmon Resonance (SPR) analysis using BiaCore T200. Human sTNFR1 (PeproTech, 210-07), mMMP8_CD (Demeestere D et al, *Molecular therapy : the journal of the American Society of Gene Therapy* 2016) or mMMP8_FL diluted in NaAc pH 4 (hTNFR1) or pH 5, respectively was chemically immobilized on a CM5 sensor chip using NAS/EDC until a RU of 581, 452.4 or 733.4, respectively was obtained at 25°C. Binding experiments were performed at 25°C in HBS (10 mM Hepes pH 7.5, 150 mM NaCl, 3.5 mM EDTA and 0.005% Tween 20) at a flow rate of 20 µl/min and a 2-fold dilution series of Nb was applied ranging from 250 nM to 1 nM. Between applying different Nb concentrations, the chip was regenerated during 300 s at a flow rate of 30 µl/min with 25 mM NaOH and 500 mM NaCl (hTNFR1 coating) or with 20 mM NaOH (MMP8 coating), without any detectable effect on the binding capacity of hTNFR1 or MMP8. A blank uncoated channel was used as an online reference during all injections. To analyze the results and determine kinetics of association (K_a) and dissociation (K_d), the sensograms were fitted by subtracting the signal of the reference flow cell using BiaCore T200

software. The curves of Nb 70-A-14 were fitted with a bivalent binding model. Curves were analyzed using BiaCore's evaluation software and interpreted visually.

Inhibition of hTNFR1 signaling via HEK-2 blue assay. The HEK-2 blue assay is a colorimetric assay in which HEK-2 blue cells are engineered with multiple genes from the TLR2 pathway (Invitrogen). HEK-2 blue cells stably express optimized alkaline phosphatase under the control of an inducible promoter, and the enzyme is secreted upon induction of the transcription factors NF-κB. Reaction of the enzyme with the HEK-2 blue detection medium can be determined by colorimetry. HEK-2 blue cells in detection medium were seeded at 50,000 cells per well in a 96-well plate (Invitrogen). After 3 h, cells were incubated for 30 min with a serial dilution of Nb starting at 5000 µM at 37°C. Next, 100 IU/ml hTNF was added and incubation continued for 18 h at 37°C. Absorption of the culture medium was measured at 655 nm with a plate reader. GraphPad Prism 6.0 was used to determine IC_{50} values based on a non-linear regression model and a dose-response inhibition equation.

Inhibitory capacity of the anti-MMP8 Nanobodies. The inhibitory capacity of the different Nbs for MMP8 was investigated using the EnzChek Gelatinase/Collagenase Assay Kit (Molecular Probes, Life technologies, E12055) according to the manufacturer's instructions. In short, DQ gelatin (1:4) was incubated with recombinant mMMP8_CD after pre-incubation of 30 min with increasing amounts of Nb. Changes in fluorescence over time by cleavage of DQ-gelatin were followed for 2 h at 37°C in a fluorescence microplate reader ($\lambda_{ex}/\lambda_{em}$ = 495/515 nm) (Fluostar Omega). Activity of mMMP8_CD and inhibitory capacity of the Nbs was determined by the changes in fluorescence over time. Plotting the activity in function of the logarithmic Nb concentration gives a sigmoidal shaped curve. A nonlinear regression was used to determine the IC50 value with GraphPad Prism 6.0.

Mice. In this study, wild type C57BL/6J mice and hTNFR1$^{Tg/Tg}$mTNFR1$^{-/-}$ transgenic mice were used. The generation of the transgenic mice is described in Steeland et al, in preparation. Shortly, a BAC-construct containing the *TNFRSF1A* gene was injected *via* pronucleus injection into zygotes of heterozygous *Tnfrsf1a* mice. Via a breeding program, mice were obtained that are full *Tnfrsf1a* knock-out and homozygous for *TNFRSF1A*. All mice were bred in the specific pathogen free (SPF) facility of the Inflammation Research Center (IRC, Belgium) in a controlled environment (12-hour light-dark cycle; 20°C) with food and water *ad libitum*.

Pharmacokinetics. To determine the *in vivo* pharmacokinetic properties of Nb 70-alb-14 eight-week-old female C57BL/6J mice were intraperitoneally (i.p.) injected with Nb 70-alb-14. Blood was sampled via

retro-orbital blood collection at different time points (1, 8, 10, 24, 30, 48 and 72 h) after a single Nb 70-alb-14 injection (1000 µg) or multiple injections (100 µg at t=0, 10 and 24h). Blood was stored overnight at 4°C and supernatant was collected from clotted blood and centrifuged at 14,000 g for 15 min at 4°C. Nb serum concentrations were determined by hTNFR1 ELISA as described above.

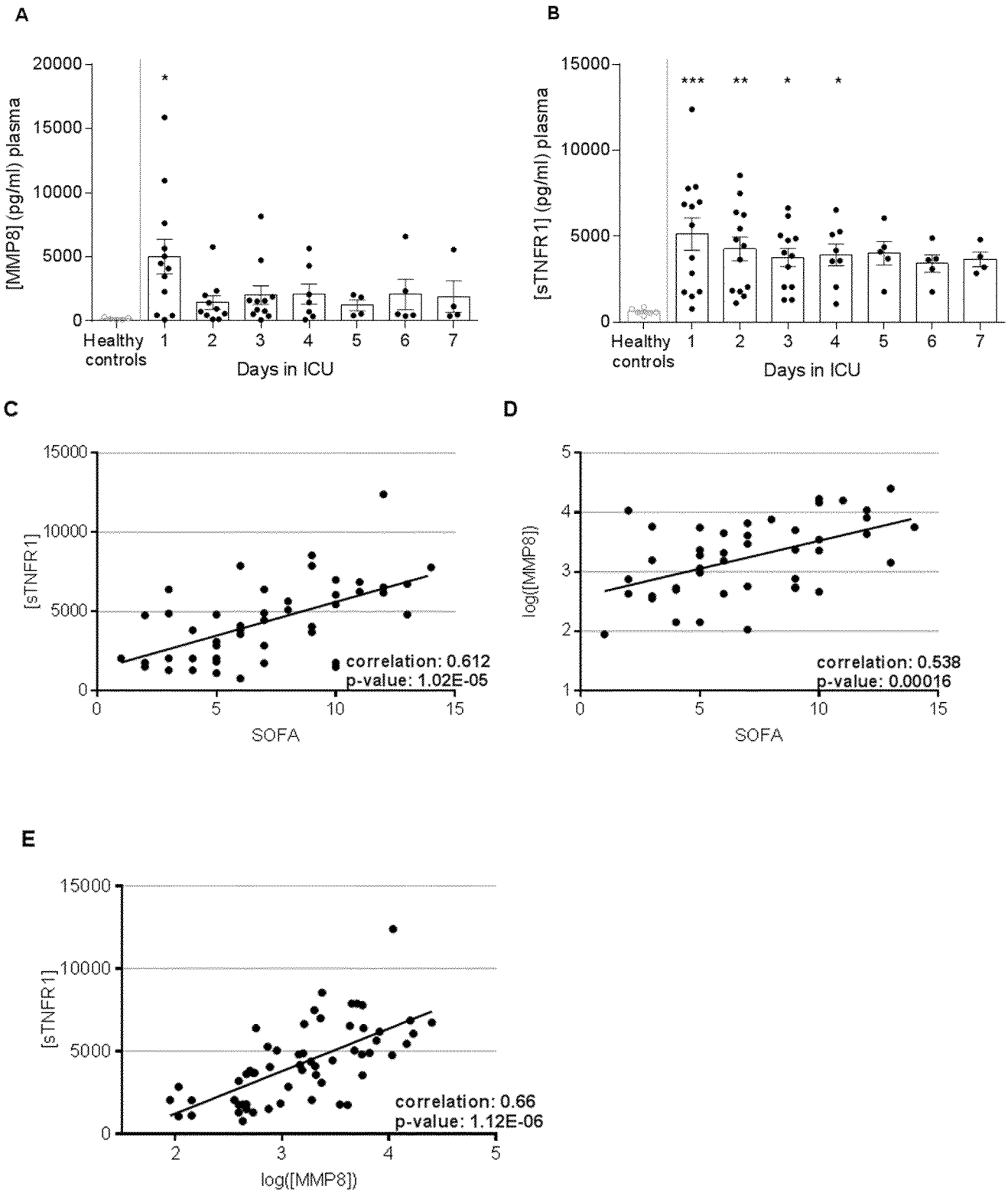
CLP procedure. Mice were subjected to CLP for induction of polymicrobial, peritoneal sepsis. Sepsis was induced in isoflurane-anesthetized mice by 75%/50% ligation of the cecum, followed by a double/single puncture with a 21-Gauge needle (lethal/sublethal model, respectively), extruding a small amount of cecal contents as described previously (Vandenbroucke RE et al., J Neurosci 32:9805-9816). Mice were injected i.p. with broad-spectrum antibiotics (25 mg/kg ceftriaxone and 12.5 mg/kg metronidazole, Sigma) 9 h and 24 h after the CLP procedure. Mice were injected i.p. right after, 24 h and 48 h after the CLP surgery with 500 µg/200 µl Nb 70-alb-14 or with 200 µl D-PBS. Rectal body temperature and lethality were followed after challenge.

Claims

1. A composition comprising a Matrix Metallo-Proteinase 8 (MMP8) inactivating antigen binding polypeptide and a Tumor necrosis factor receptor 1 (TNFR1) inactivating antigen binding polypeptide.
2. A composition according to claim 1, wherein said antigen binding polypeptides comprise an antibody.
3. A composition according to claim 2, wherein said antibody is a VHH.
4. A composition according to any one of claims 1 to 3, wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.
5. A composition according to any one of claims 1 to 4, wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.
6. A composition according to any one of claims 1 to 5, wherein said MMP8 inactivating antigen binding polypeptide and said TNFR1 inactivating antigen binding polypeptide are present in one molecule.
7. A composition according to claim 6 depicted in SEQ ID NO: 7 or a variant thereof with at least 90% sequence identity.
8. An isolated polypeptide comprising a MMP8 inactivating antigen binding polypeptide and a TNFR1 inactivating antigen binding polypeptide.
9. The isolated polypeptide according to claim 8 wherein said MMP8 inactivating antigen binding polypeptide comprises a CDR1 sequence depicted in SEQ ID NO: 1, a CDR2 sequence depicted in SEQ ID NO: 2 and a CDR3 sequence depicted in SEQ ID NO: 3 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID NOs.
10. The isolated polypeptide according to claim 8 wherein said TNFR1 inactivating antigen binding protein comprises a CDR1 sequence depicted in SEQ ID NO: 4, a CDR2 sequence depicted in SEQ ID NO: 5 and a CDR3 sequence depicted in SEQ ID NO: 6 and wherein CDR1, CDR2 and/or CDR3 have a 1-, 2- or 3-amino acid difference with any of said foregoing respective SEQ ID Nos.
11. The isolated polypeptide according to claim 8 comprising the amino acid sequence depicted in SEQ ID NO: 7.
12. A nucleic acid encoding at least one antigen binding polypeptide according to any one of claims 1 to 11.
13. A vector comprising a nucleic acid according to claim 12.

14. A host cell comprising a nucleic acid according to claim 12 or a vector according to claim 13.
15. A composition according to any one of claims 1 to 7, an isolated polypeptide according to any of claims 8 to 11, a nucleic acid according to claim 12 or a vector according to claim 13 for use as a medicament.
16. A composition according to any one of claims 1 to 7, an isolated polypeptide according to any of claims 8 to 11, a nucleic acid according to claim 12 or a vector according to claim 13 for use to treat an inflammatory disease.
17. A composition according to any one of claims 1 to 7, an isolated polypeptide according to any of claims 8 to 11, a nucleic acid according to claim 12 or a vector according to claim 13 for use to treat an inflammatory disease, wherein said inflammatory disease is selected from the list of diseases consisting of systemic inflammatory response syndrome, sepsis, LPS induced inflammation, renal ischemia/reperfusion injury, ventilation induced lung injury, periodontal inflammation, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, ankylosing spondylitis, Lyme arthritis and osteoarthritis.
18. A pharmaceutical composition comprising a composition according to any one of claims 1 to 7, an isolated polypeptide according to any of claims 8 to 11, a nucleic acid according to claim 8 or a vector according to claim 9 and a pharmaceutically acceptable carrier.
19. A kit comprising a composition according to any one of claims 1 to 7, an isolated polypeptide according to any of claims 8 to 11, a nucleic acid according to claim 8 or a vector according to claim 9.

Figure 1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10000
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*
Healthy controls
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2
3
4
5
6
7
Days in ICU
B
[sTNFR1] (pg/ml) plasma
15000
10000
5000
0
***
**
*
*
Healthy controls
1
2
3
4
5
6
7
Days in ICU
C
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SOFA
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Figure 2

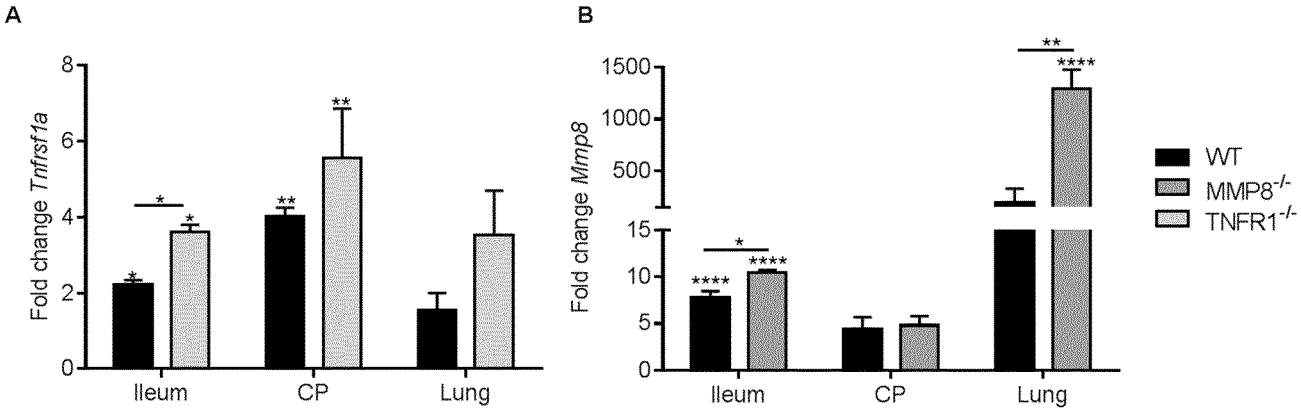


Figure 3

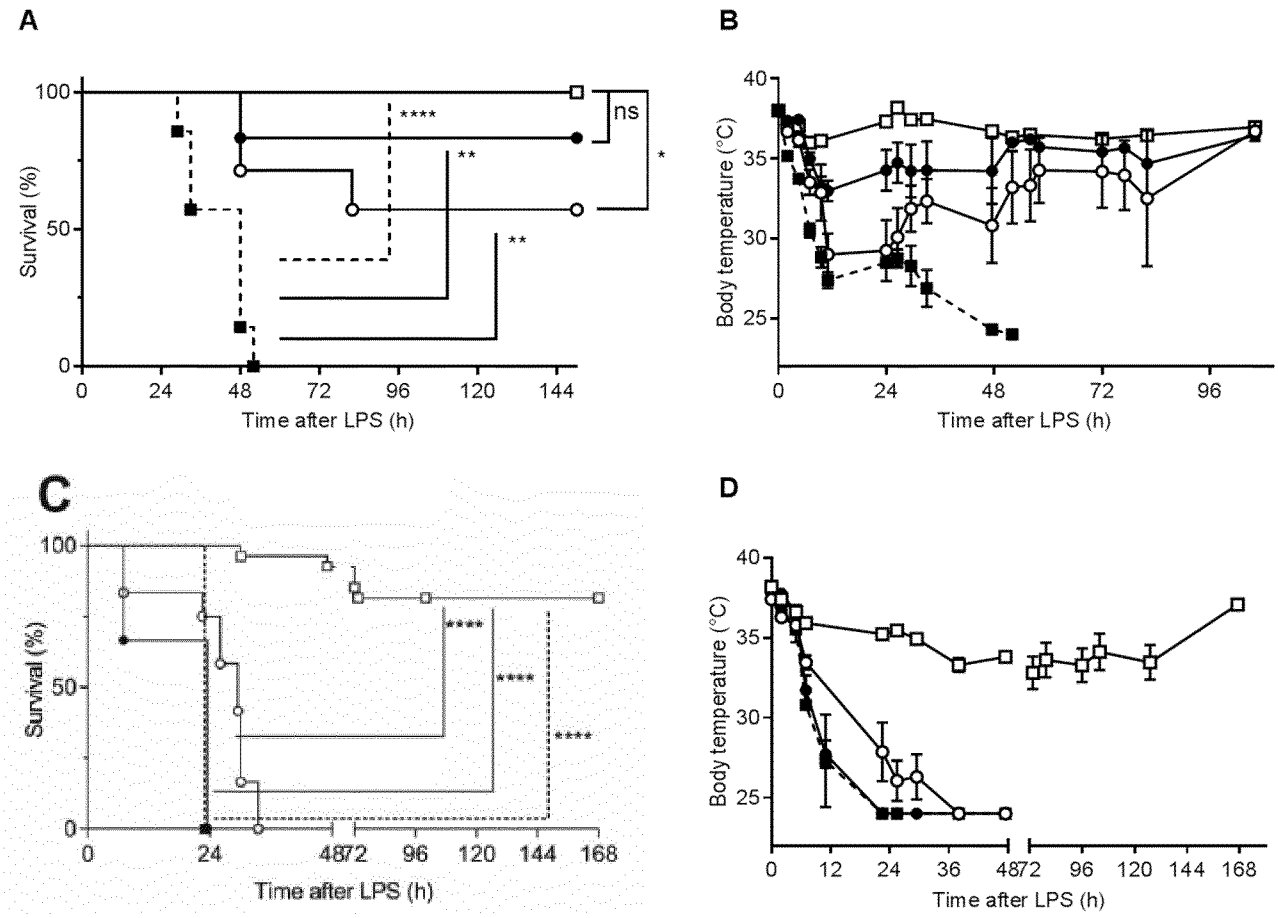


Figure 4

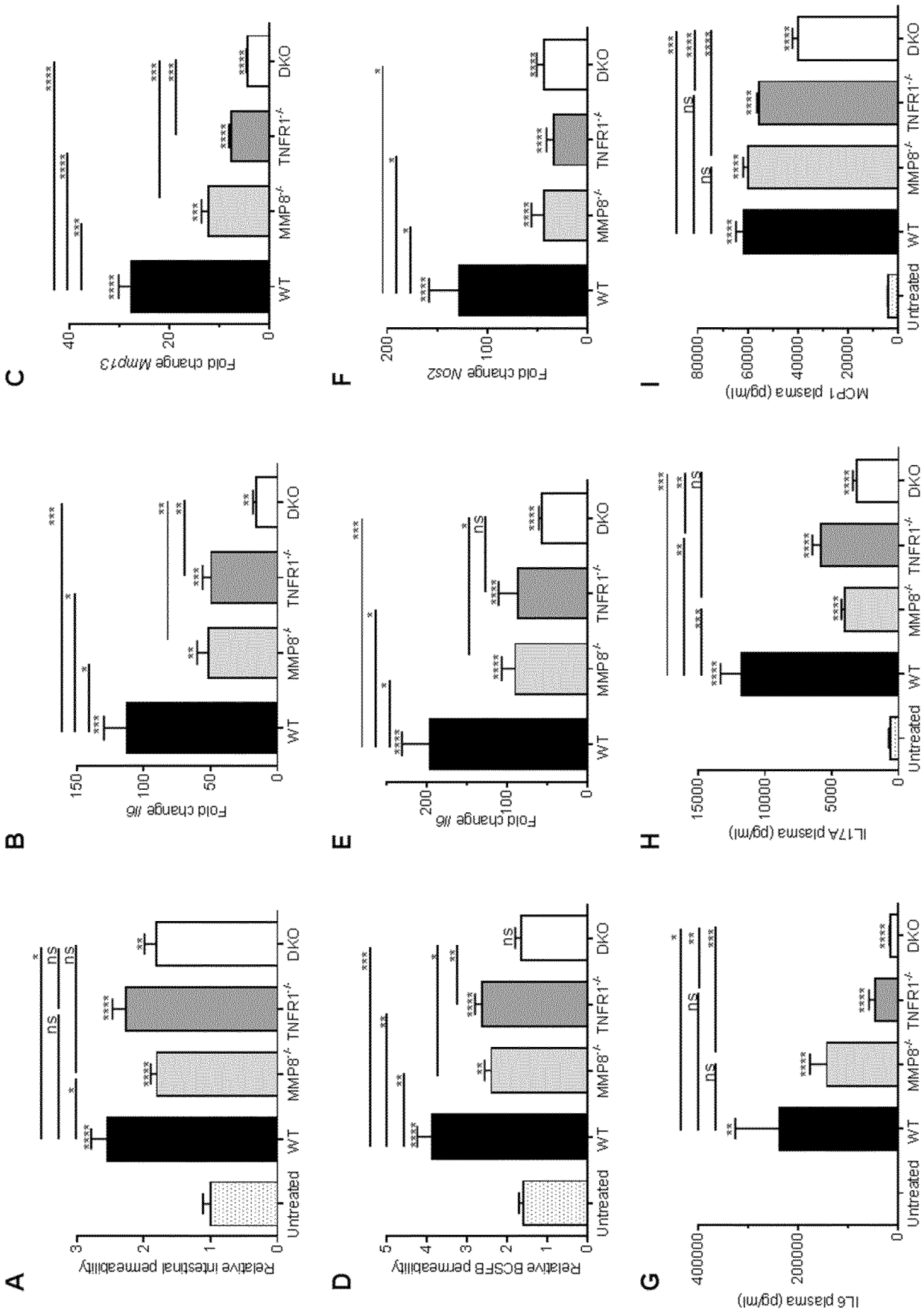

A
Relative intestinal permeability
B
Fold change Il6
C
Fold change Mmp13
D
Relative BCSFB permeability
E
Fold change Il6
F
Fold change Nos2
G
IL6 plasma (pg/ml)
H
IL17A plasma (pg/ml)
I
MCP1 plasma (pg/ml)
Untreated
WT
MMP8-/-
TNFR1-/-
DKO
ns

Figure 5

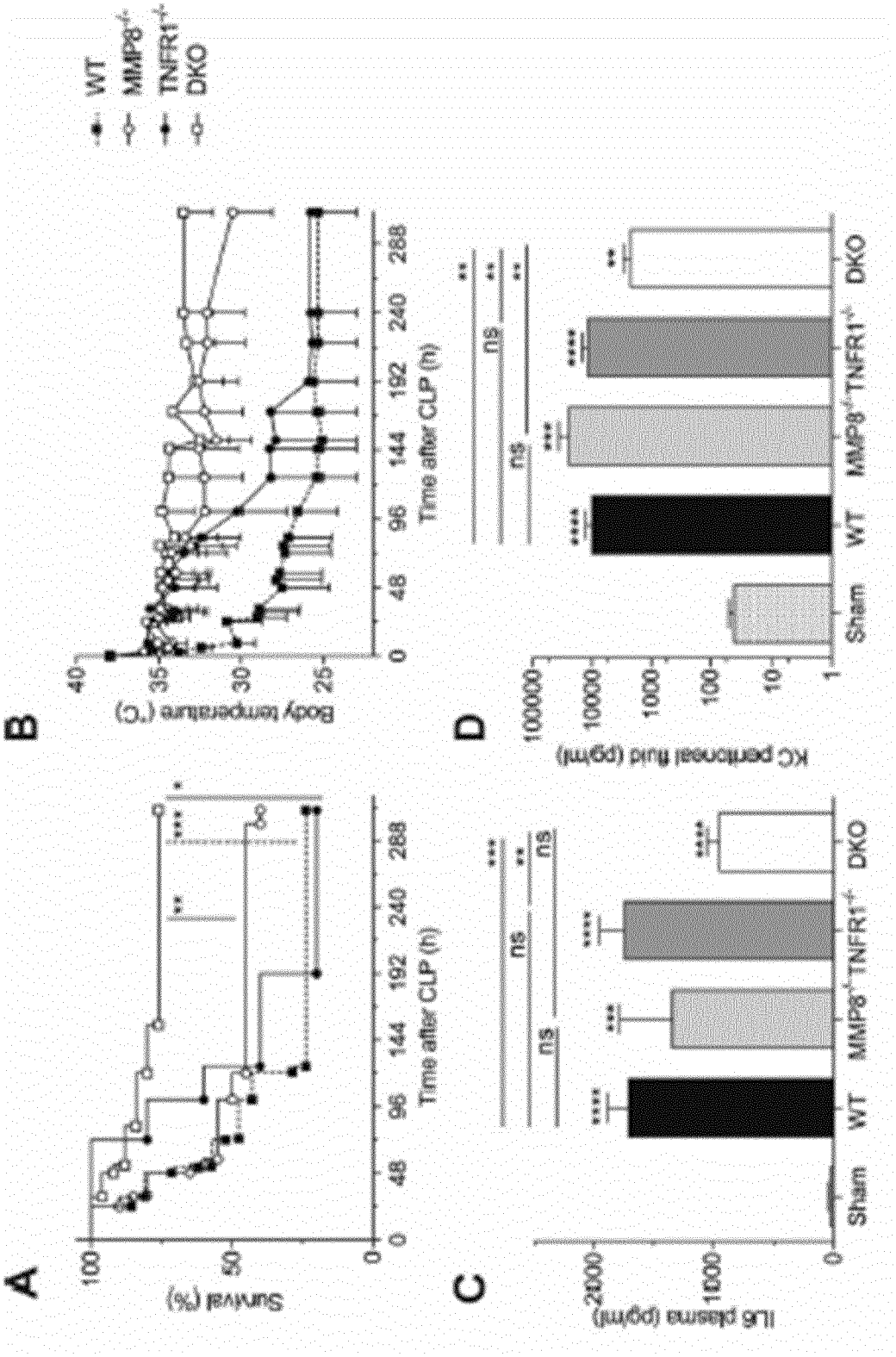

A
Survival (%)
Time after CLP (h)
B
Body temperature (°C)
Time after CLP (h)
WT
MMP8-/-
TNFR1-/-
DKO
C
IL6 plasma (pg/ml)
Sham
WT
MMP8-/-TNFR1-/-
DKO
D
KC peritoneal fluid (pg/ml)
Sham
WT
MMP8-/-TNFR1-/-
DKO

Figure 6

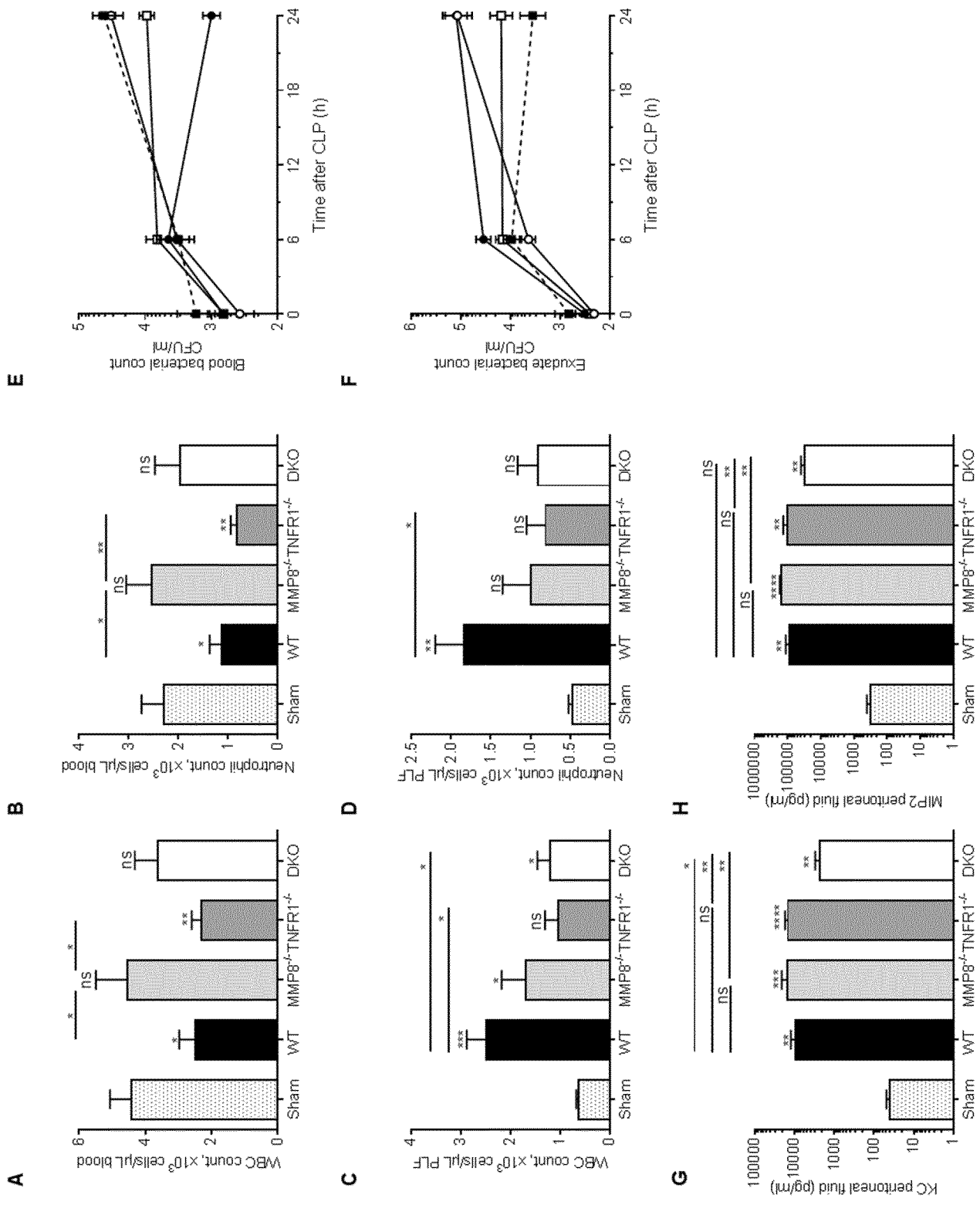

A
WBC count, x10³ cells/µL blood
B
Neutrophil count, x10³ cells/µL blood
C
WBC count, x10³ cells/µL PLF
D
Neutrophil count, x10³ cells/µL PLF
E
Blood bacterial count CFU/ml
Time after CLP (h)
F
Exudate bacterial count CFU/ml
Time after CLP (h)
G
KC peritoneal fluid (pg/ml)
H
MIP2 peritoneal fluid (pg/ml)
Sham
WT
MMP8-/-TNFR1-/-
DKO
ns

Figure 7

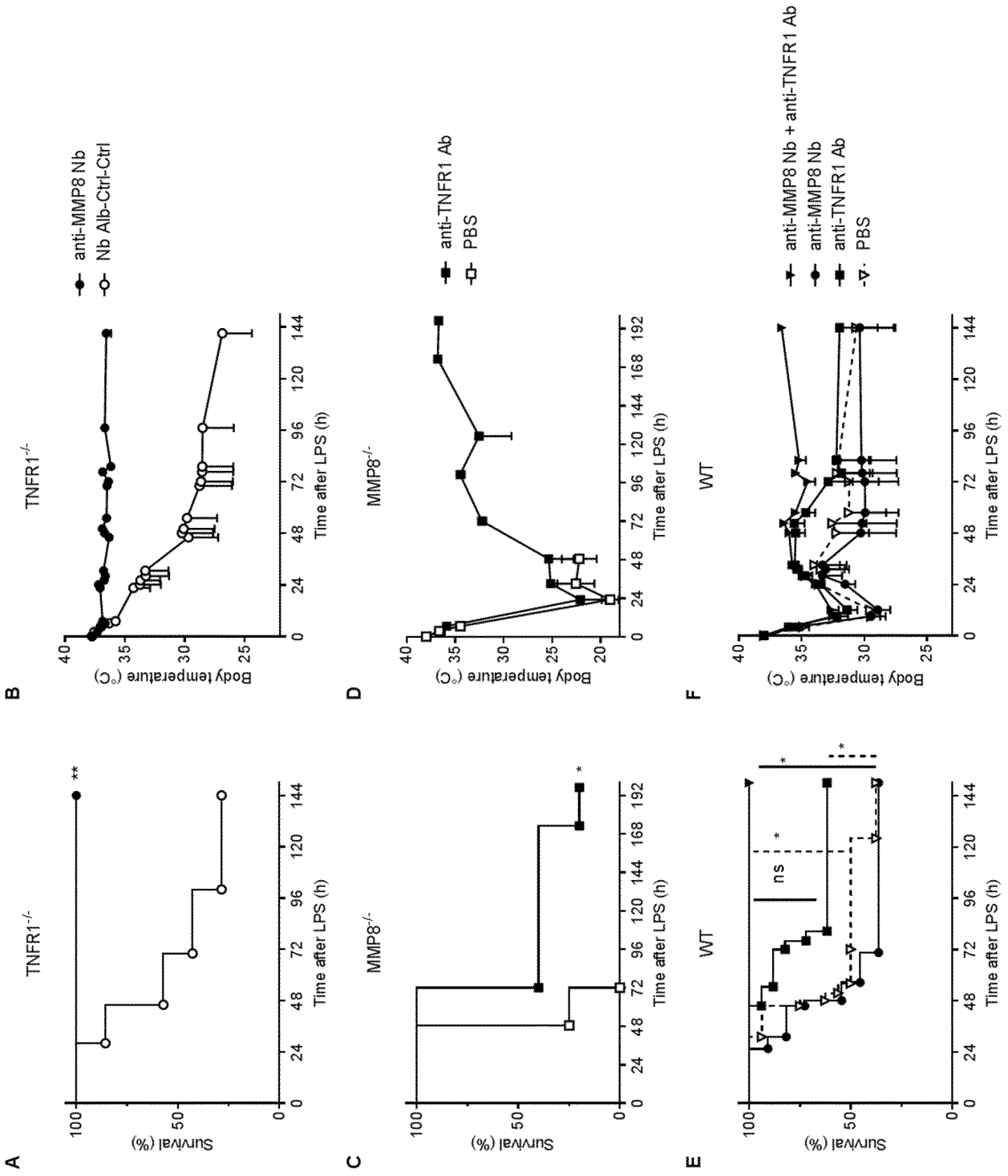
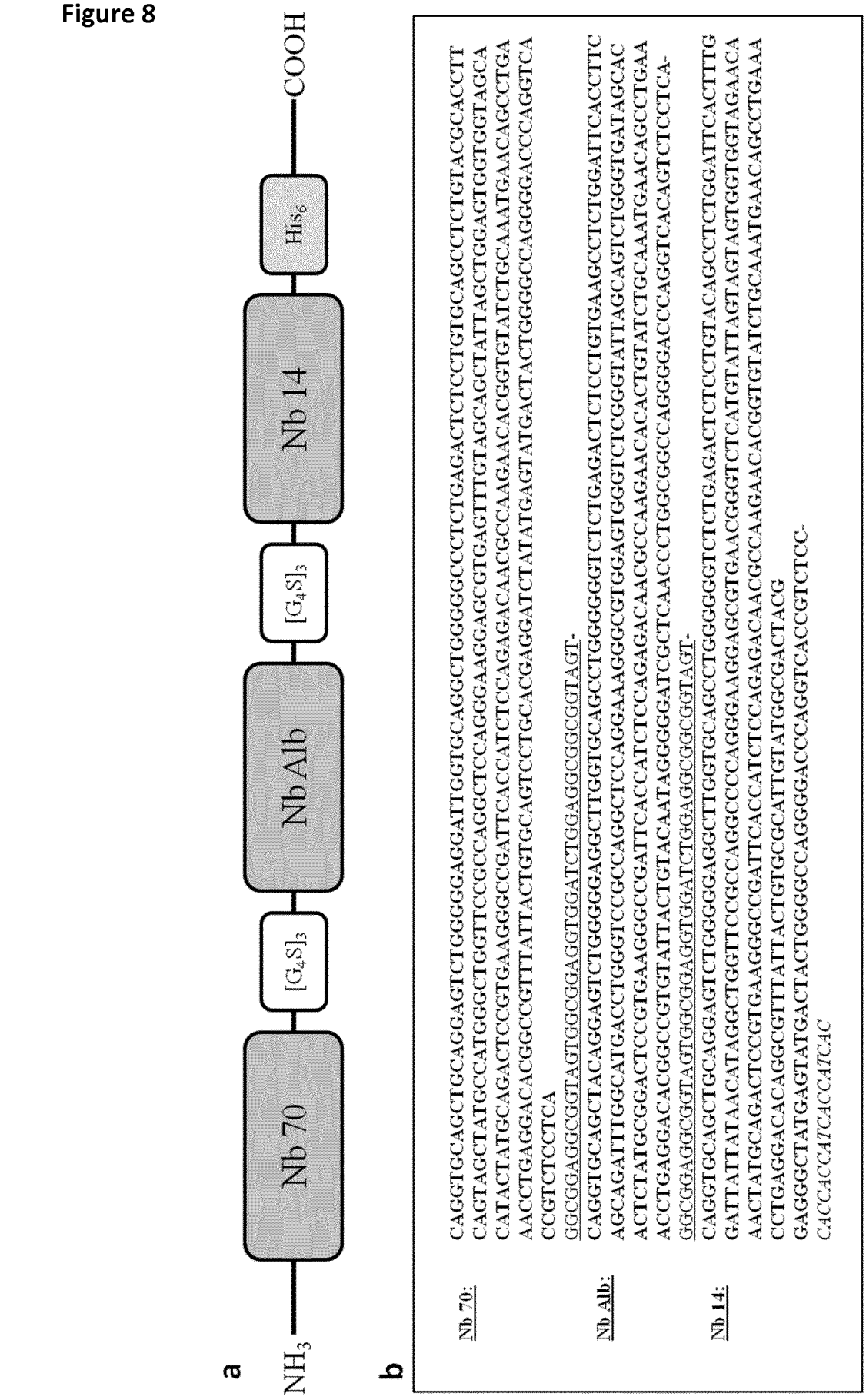

A
TNFR1-/-
Survival (%)
Time after LPS (h)
B
TNFR1-/-
anti-MMP8 Nb
Nb Alb-Ctrl-Ctrl
Body temperature (°C)
Time after LPS (h)
C
MMP8-/-
Survival (%)
Time after LPS (h)
D
MMP8-/-
anti-TNFR1 Ab
PBS
Body temperature (°C)
Time after LPS (h)
E
WT
ns
Survival (%)
Time after LPS (h)
F
WT
anti-MMP8 Nb + anti-TNFR1 Ab
anti-MMP8 Nb
anti-TNFR1 Ab
PBS
Body temperature (°C)
Time after LPS (h)

Figure 8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His6
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b
Nb 70:
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CAGTAGCTATGCCATGGGCTGGTTCCGCCAGGCTCCAGGGAAGGAGCGTGAGTTTGTAGCAGCTATTAGCTGGAGTGGTGGTAGCA
CATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTGA
AACCTGAGGACACGGCCGTTTATTACTGTGCAGTCCTGCACGAGGATCTATATGAGTGATATGACTACTGGGGCCAGGGGACCCAGGTCA
CCGTCTCCTCA
GGCGGAGGCGGTAGTGGCGGAGGTGGATCTGGAGGCGGCGGTAGT-
Nb Alb:
CAGGTGCAGCTACAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGGTCTCTGAGACTCTCCTGTGAAGCCTCTGGATTCACCTTC
AGCAGATTTGGCATGACCTGGGTCCGCCAGGCTCCAGGAAAGGGCGTGGAGTGGGTCTCGGGTATTAGCAGTCTGGGTGATAGCAC
ACTCTATGCGGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACACTGTATCTGCAAATGAACAGCCTGAA
ACCTGAGGACACGGCCGTGTATTACTGTGCTCAACCCTGGCGGCCAGGGGACCCAGGTCACAGTCTCCTCA-
GGCGGAGGCGGTAGTGGCGGAGGTGGATCTGGAGGCGGCGGTAGT-
Nb 14:
CAGGTGCAGCTGCAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGGTCTCTGAGACTCTCCTGTACAGCCTCTGGATTCACTTTG
GATTATTATAACATAGGCTGGTTCCGCCAGGCCCCAGGGAAGGAGCGTGAACGGGTCTCATGTATTAGTAGTGGTGGTAGAACA
AACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTGAAA
CCTGAGGACACAGGCGTTTATTACTGTGCGCATTGTATGGCGACTACG
GAGGGCTATGAGTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCC-
CACCACCATCACCATCAC

Figure 9

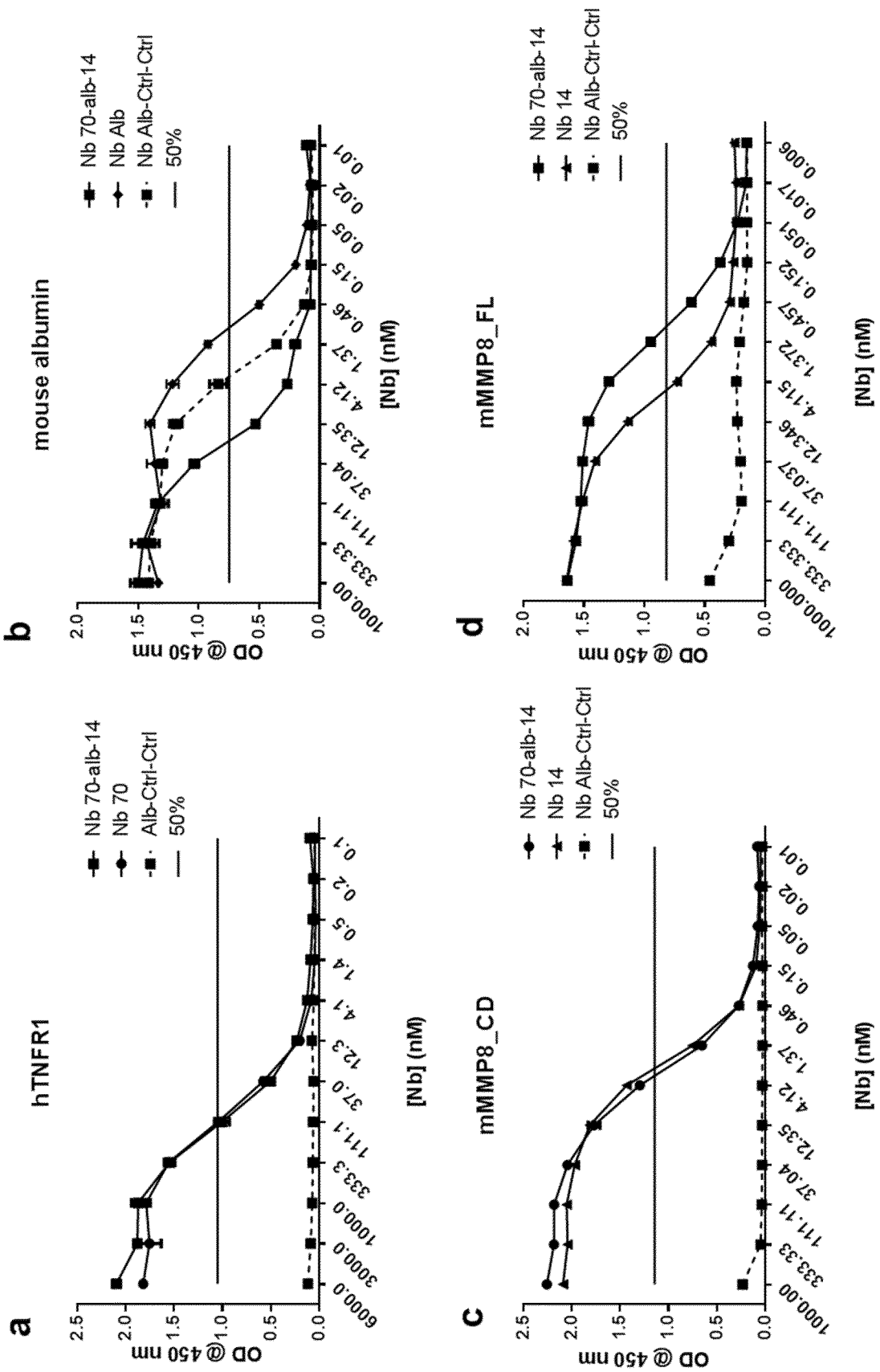

a
hTNFR1
Nb 70-alb-14
Nb 70
Alb-Ctrl-Ctrl
50%
OD @ 450 nm
[Nb] (nM)
b
mouse albumin
Nb 70-alb-14
Nb Alb
Nb Alb-Ctrl-Ctrl
50%
OD @ 450 nm
[Nb] (nM)
c
mMMP8_CD
Nb 70-alb-14
Nb 14
Nb Alb-Ctrl-Ctrl
50%
OD @ 450 nm
[Nb] (nM)
d
mMMP8_FL
Nb 70-alb-14
Nb 14
Nb Alb-Ctrl-Ctrl
50%
OD @ 450 nm
[Nb] (nM)

Figure 10

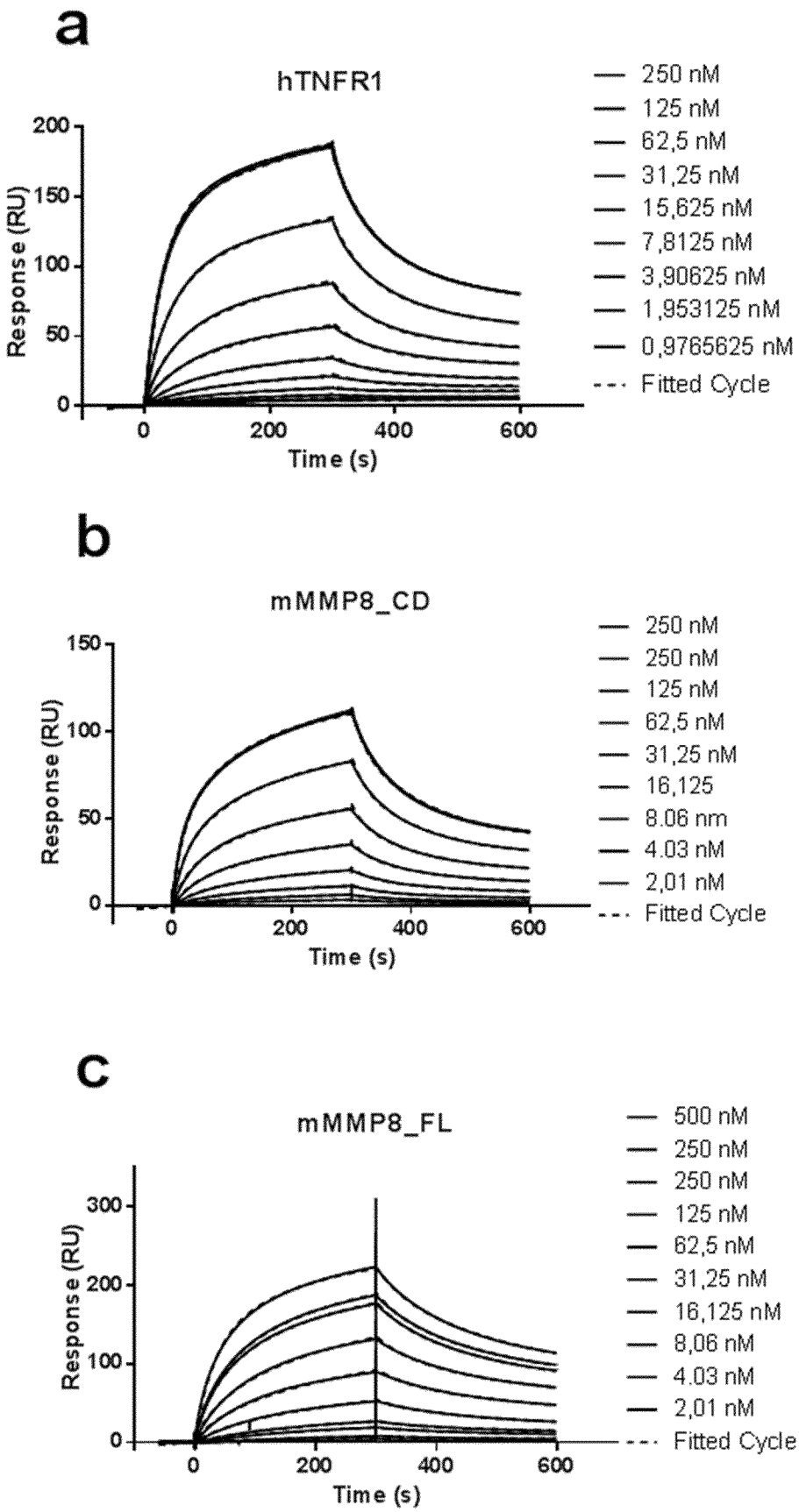

a
hTNFR1
Response (RU)
Time (s)
250 nM
125 nM
62,5 nM
31,25 nM
15,625 nM
7,8125 nM
3,90625 nM
1,953125 nM
0,9765625 nM
Fitted Cycle
b
mMMP8_CD
Response (RU)
Time (s)
250 nM
250 nM
125 nM
62,5 nM
31,25 nM
16,125
8.06 nm
4.03 nM
2,01 nM
Fitted Cycle
c
mMMP8_FL
Response (RU)
Time (s)
500 nM
250 nM
250 nM
125 nM
62,5 nM
31,25 nM
16,125 nM
8,06 nM
4.03 nM
2,01 nM
Fitted Cycle

Figure 11

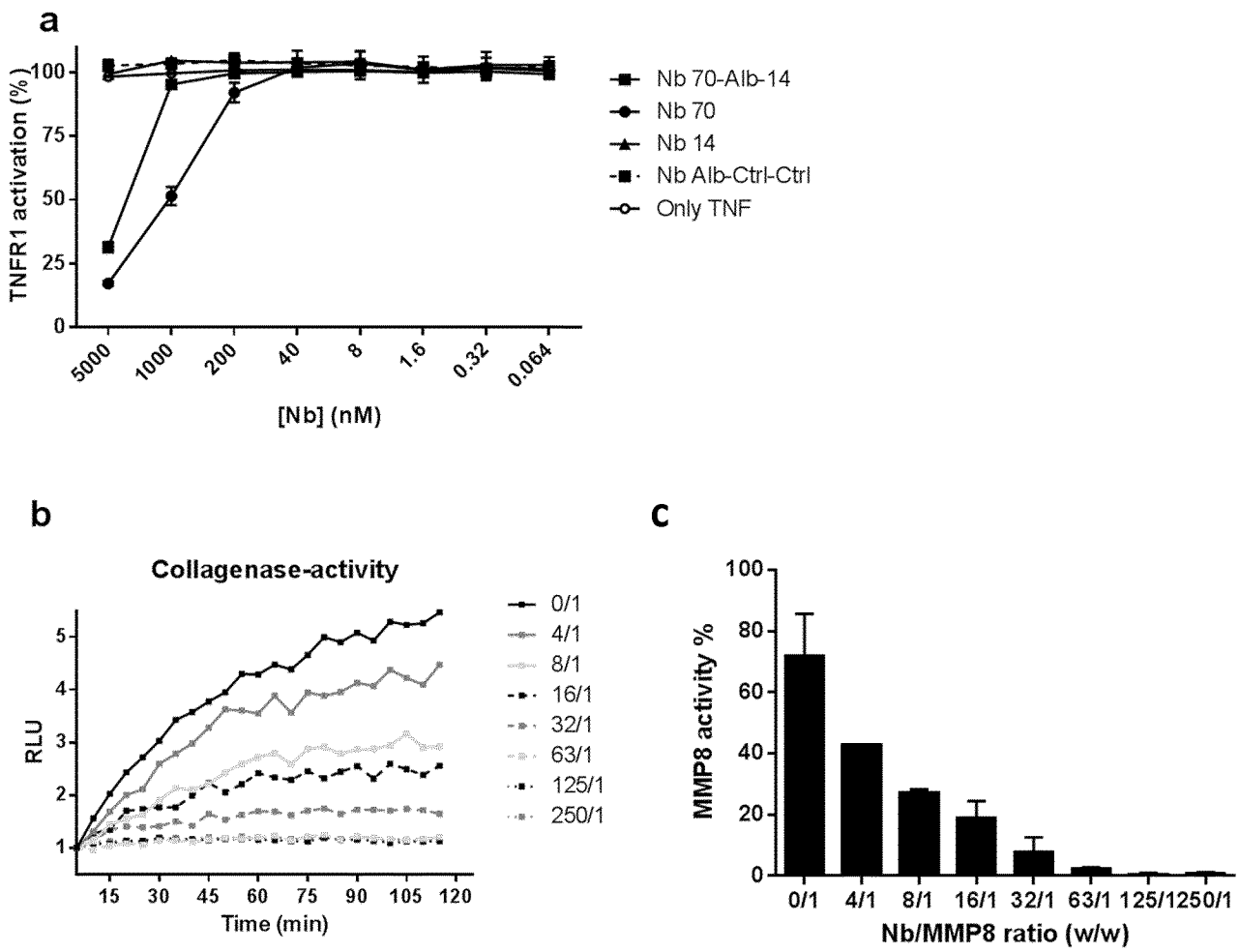

a
TNFR1 activation (%)
100
75
50
25
0
5000
1000
200
40
8
1.6
0.32
0.064
[Nb] (nM)
Nb 70-Alb-14
Nb 70
Nb 14
Nb Alb-Ctrl-Ctrl
Only TNF
b
Collagenase-activity
RLU
5
4
3
2
1
15
30
45
60
75
90
105
120
Time (min)
0/1
4/1
8/1
16/1
32/1
63/1
125/1
250/1
c
MMP8 activity %
100
80
60
40
20
0
0/1
4/1
8/1
16/1
32/1
63/1
125/1250/1
Nb/MMP8 ratio (w/w)

Figure 12

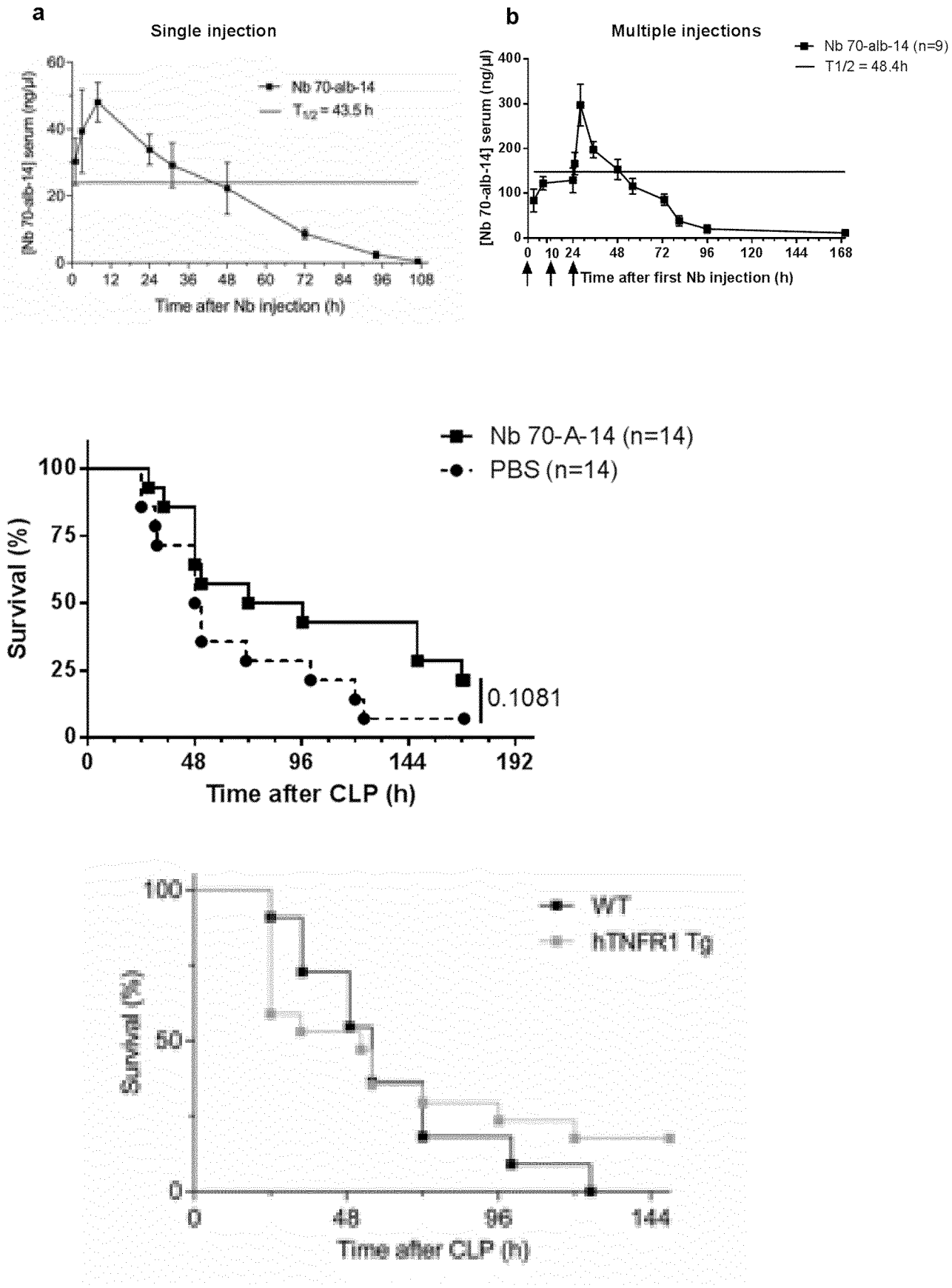

a
Single injection
[Nb 70-alb-14] serum (ng/µl)
Nb 70-alb-14
T1/2 = 43.5 h
Time after Nb injection (h)
b
Multiple injections
Nb 70-alb-14 (n=9)
T1/2 = 48.4h
[Nb 70-alb-14] serum (ng/µl)
Time after first Nb injection (h)
C
Nb 70-A-14 (n=14)
PBS (n=14)
Survival (%)
0.1081
Time after CLP (h)
D
WT
hTNFR1 Tg
Survival (%)
Time after CLP (h)

Figure 13

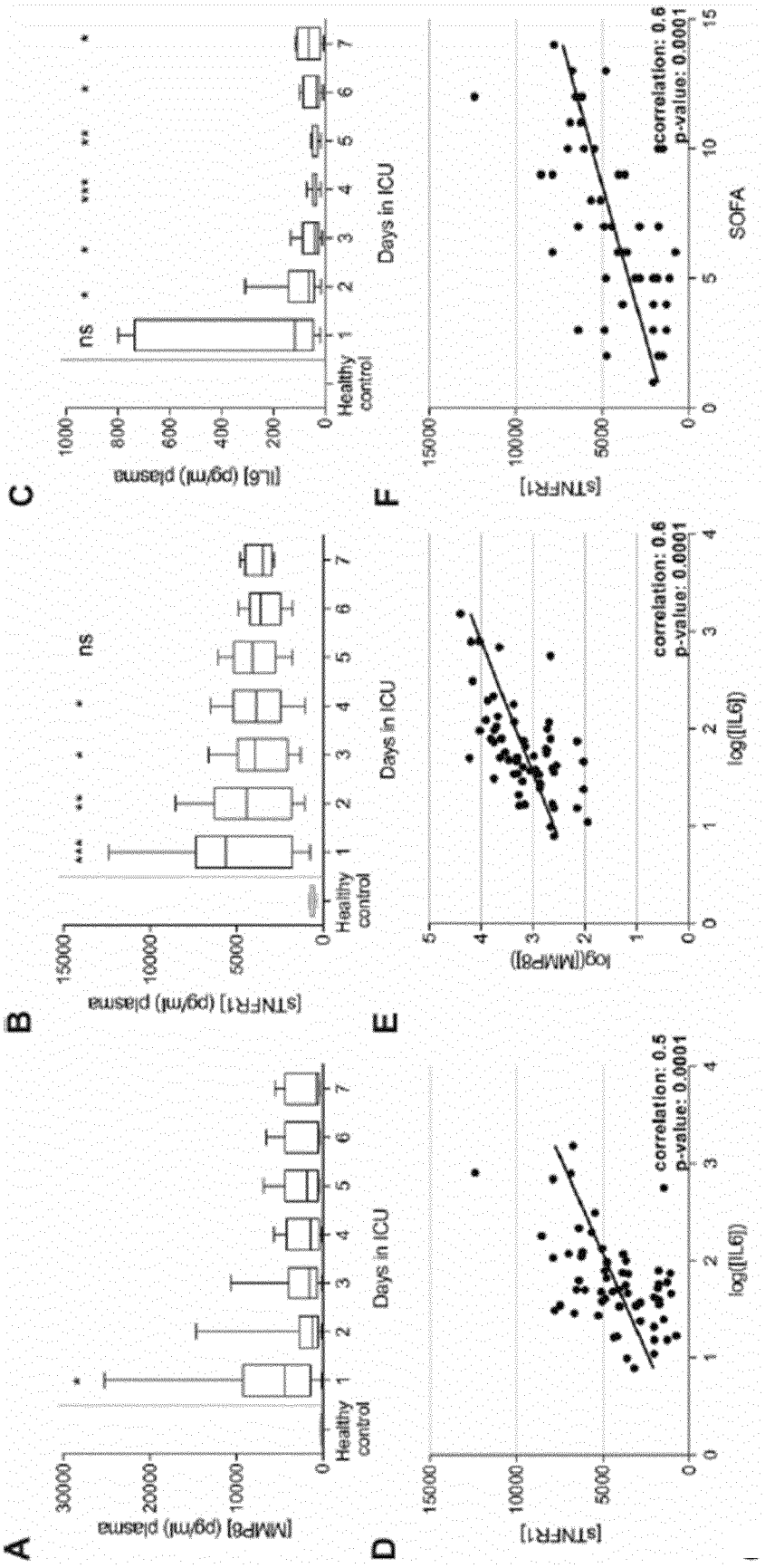

A
B
C
D
E
F
[MMP8] (pg/ml) plasma
[sTNFR1] (pg/ml) plasma
[IL6] (pg/ml) plasma
Healthy control
Days in ICU
ns
correlation: 0.5
p-value: 0.0001
correlation: 0.6
p-value: 0.0001
log([IL6])
log([MMP8])
[sTNFR1]
SOFA

Figure 13 - continued

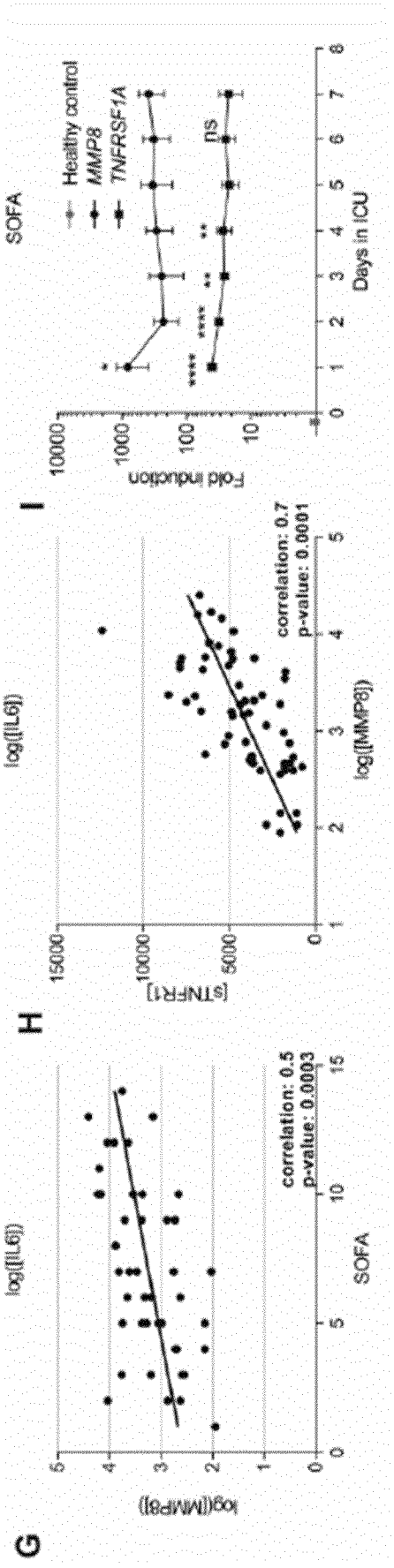


Figure 14

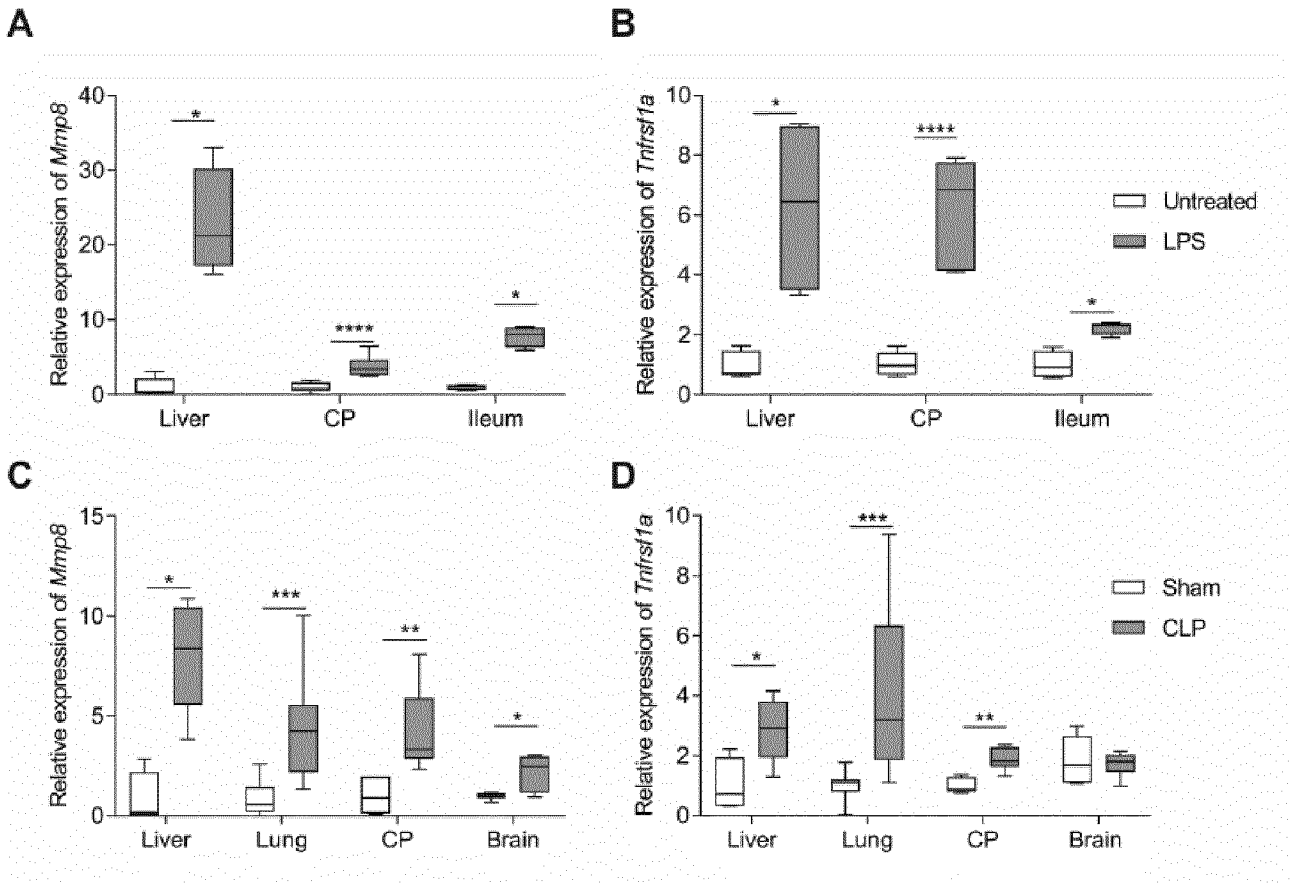
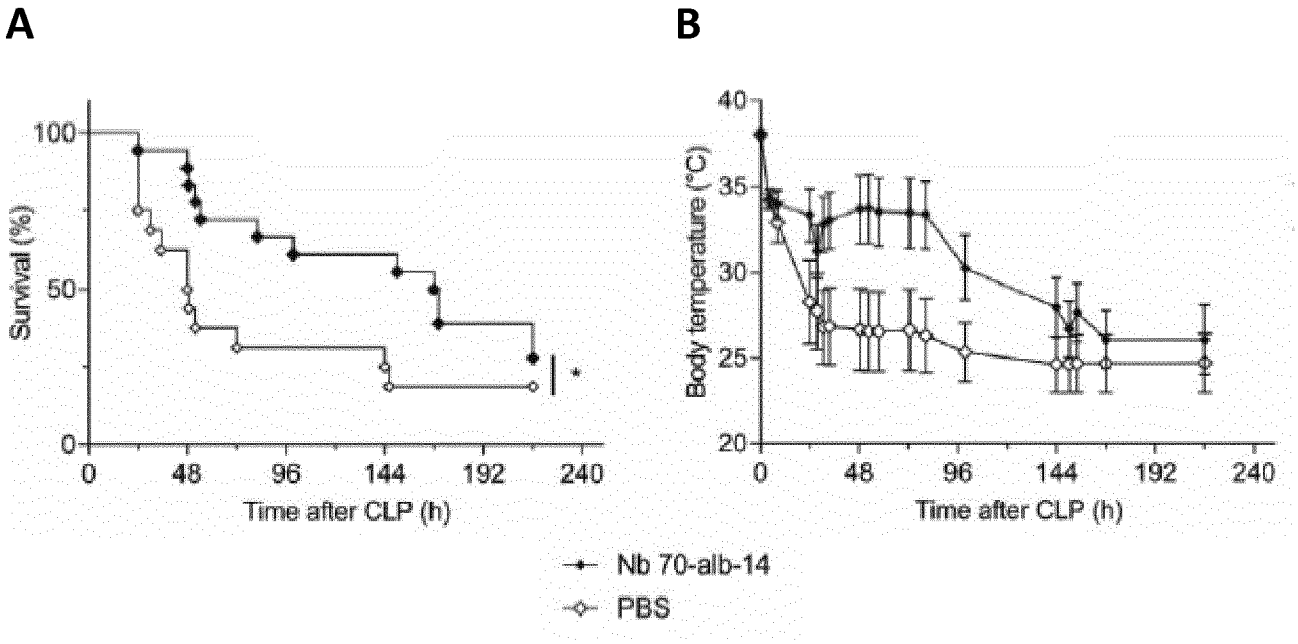

A
B
C
D
Relative expression of Mmp8
Relative expression of Tnfrsf1a
Liver
CP
Ileum
Lung
Brain
Untreated
LPS
Sham
CLP

Figure 15


A
B
Survival (%)
100
50
0
0
48
96
144
192
240
Time after CLP (h)
*
Body temperature (°C)
40
35
30
25
20
Time after CLP (h)
Nb 70-alb-14
PBS

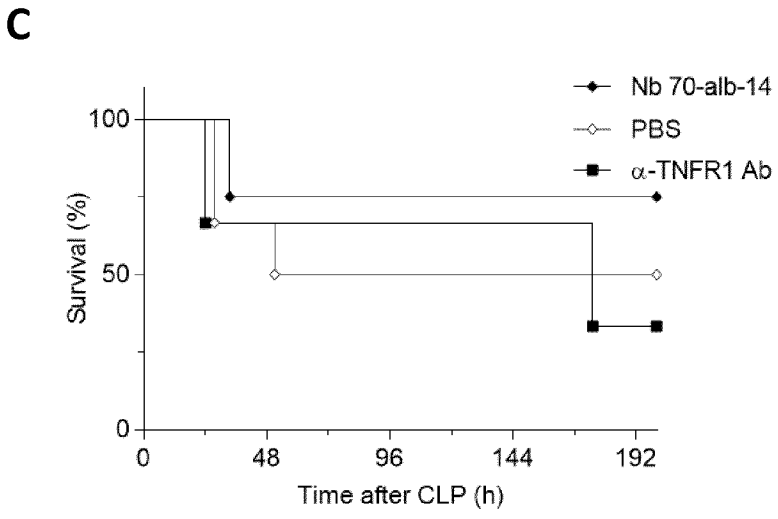
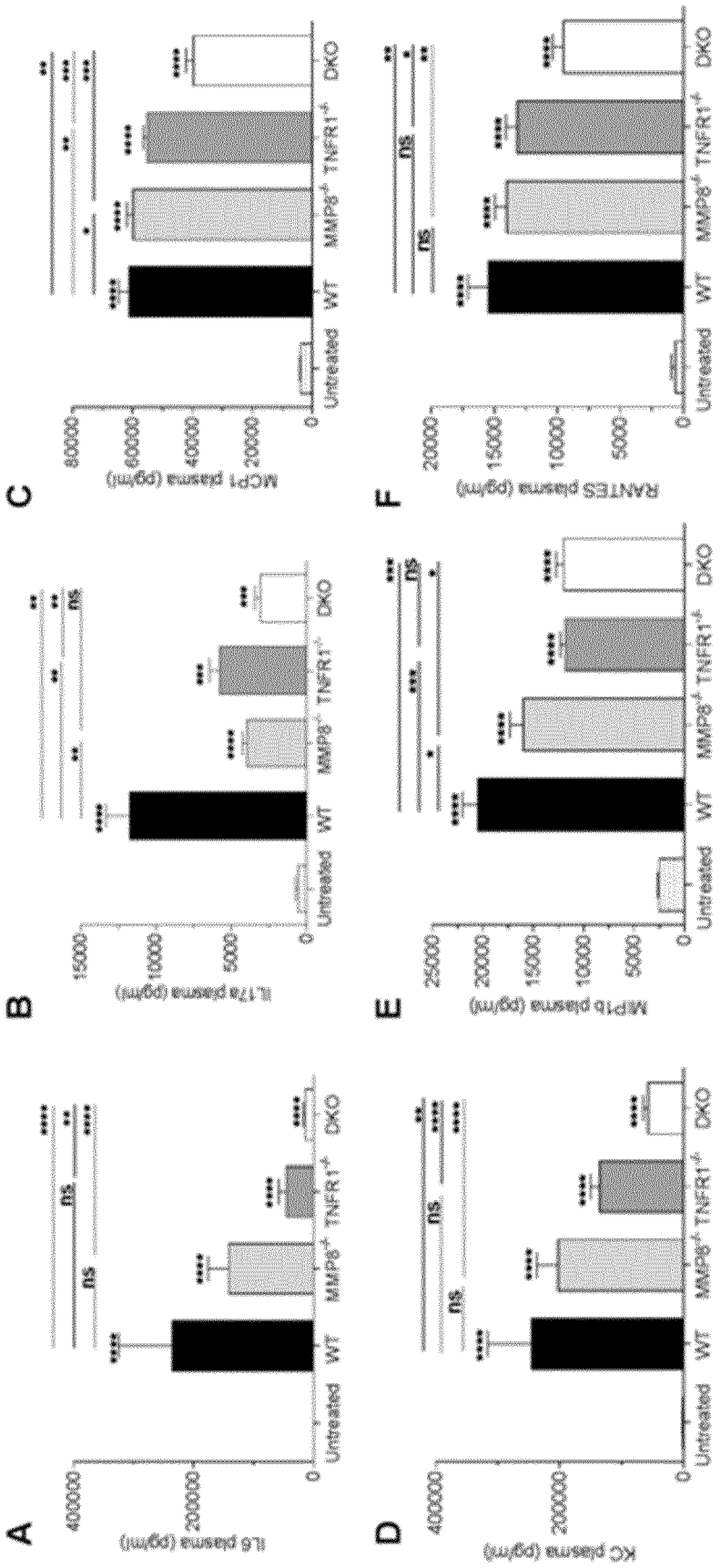

C
Nb 70-alb-14
PBS
α-TNFR1 Ab
Survival (%)
100
50
0
0
48
96
144
192
Time after CLP (h)

Figure 16


A
IL6 plasma (pg/ml)
B
IL17a plasma (pg/ml)
C
MCP1 plasma (pg/ml)
D
KC plasma (pg/ml)
E
MIP1b plasma (pg/ml)
F
RANTES plasma (pg/ml)
Untreated
WT
MMP8-/-
TNFR1-/-
DKO
ns

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/076427

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/40 C07K16/28 A61K39/395 A61K31/135
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2002/155113 A1 (CHUN MIYOUNG [US] ET AL) 24 October 2002 (2002-10-24) [0072],[0085],[0239-0260],[0277]; claims 5-7,18-21	1-19
Y	T. SECHER ET AL: "Crucial Role of TNF Receptors 1 and 2 in the Control of Polymicrobial Sepsis", THE JOURNAL OF IMMUNOLOGY, vol. 182, no. 12, 3 June 2009 (2009-06-03) , pages 7855-7864, XP055436517, US ISSN: 0022-1767, DOI: 10.4049/jimmunol.0804008 page 7862 - page 7863; figures 3,8	1-19

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2017	23/01/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Klee, Barbara

4

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/149148 A2 (DOMANTIS LTD [GB]; JESPERS LAURENT [GB]; PUPECKA MALGORZATA [GB]; TOML) 11 December 2008 (2008-12-11) claims 39, 52-55	1-19
Y	US 2006/002935 A1 (BREWIS NEIL D [GB] ET AL) 5 January 2006 (2006-01-05) claims 45-157	1-19
Y	EP 1 972 637 A1 (UNIV STUTTGART [DE]) 24 September 2008 (2008-09-24) claims 1-20	1-19
Y	WO 2012/059513 A1 (VIB VZW [BE]; UNIV GENT [BE]; LIBERT CLAUDE [BE]; DEJONCKHEERE ELINE [) 10 May 2012 (2012-05-10) page 3 - page 4; claims 1-11	1-19
Y	WO 2012/122464 A1 (UNIV NEW YORK [US]; LIU CHUAN-JU [US]) 13 September 2012 (2012-09-13) [0069]; claims 1-20	1-19
Y	US 2013/022605 A1 (BREWIS NEIL D [GB] ET AL) 24 January 2013 (2013-01-24) claims 242-254	1-19
Y	SOPHIE STEELAND ET AL: "Generation and Characterization of Small Single Domain Antibodies Inhibiting Human Tumor Necrosis Factor Receptor 1", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 290, no. 7, 23 December 2014 (2014-12-23), pages 4022-4037, XP055436881, ISSN: 0021-9258, DOI: 10.1074/jbc.M114.617787 page 4023 - page 4035	3-11

4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/076427

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002155113 A1	24-10-2002	US 2002155113 A1	24-10-2002
		WO 02089843 A1	14-11-2002
WO 2008149148 A2	11-12-2008	AR 066847 A1	16-09-2009
		AR 066848 A1	16-09-2009
		AR 066850 A1	16-09-2009
		AU 2008259514 A1	11-12-2008
		AU 2008259515 A1	11-12-2008
		AU 2008259516 A1	11-12-2008
		AU 2008259590 A1	11-12-2008
		BR PI0812795 A2	02-12-2014
		CA 2683791 A1	11-12-2008
		CA 2683823 A1	11-12-2008
		CA 2688433 A1	11-12-2008
		CA 2688434 A1	11-12-2008
		CA 2688447 A1	11-12-2008
		CA 2688456 A1	11-12-2008
		CL 2008001673 A1	21-11-2008
		CL 2008001674 A1	21-11-2008
		CL 2008001676 A1	21-11-2008
		CN 101778865 A	14-07-2010
		CN 101802004 A	11-08-2010
		CN 101883788 A	10-11-2010
		CN 104311663 A	28-01-2015
		CO 6251321 A2	21-02-2011
		CO 6251322 A2	21-02-2011
		CR 11194 A	28-06-2010
		CR 11195 A	24-05-2010
		CY 1116762 T1	15-03-2017
		DK 2162467 T3	24-08-2015
		DO P2009000268 A	28-02-2010
		EA 200901300 A1	30-06-2010
		EA 200901491 A1	30-08-2010
		EA 200901494 A1	30-06-2010
		EA 200901495 A1	29-10-2010
		EA 201100546 A1	30-12-2011
		EP 2158218 A2	03-03-2010
		EP 2162465 A2	17-03-2010
		EP 2162466 A2	17-03-2010
		EP 2162467 A2	17-03-2010
		EP 2162468 A2	17-03-2010
		EP 2164867 A2	24-03-2010
		EP 2746290 A2	25-06-2014
		EP 2746291 A2	25-06-2014
		ES 2546943 T3	30-09-2015
		HK 1138022 A1	18-12-2015
		HR P20151024 T1	06-11-2015
		HU E025899 T2	30-05-2016
		JP 5325211 B2	23-10-2013
		JP 5444215 B2	19-03-2014
		JP 2010529841 A	02-09-2010
		JP 2010530220 A	09-09-2010
		JP 2010530364 A	09-09-2010
		KR 20100018040 A	16-02-2010
		KR 20100021642 A	25-02-2010
		KR 20100040841 A	21-04-2010
		KR 20100041746 A	22-04-2010
		MA 31403 B1	03-05-2010

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/076427

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		MA 31668 B1	01-09-2010
		NZ 581372 A	24-02-2012
		PE 03222009 A1	20-04-2009
		PE 03232009 A1	20-04-2009
		PE 07632009 A1	10-07-2009
		PT 2162467 E	29-10-2015
		SG 182141 A1	30-07-2012
		SG 182151 A1	30-07-2012
		SI 2162467 T1	30-11-2015
		TW 200902551 A	16-01-2009
		TW 200907128 A	16-02-2009
		TW 200911831 A	16-03-2009
		TW 200911832 A	16-03-2009
		TW 200911834 A	16-03-2009
		TW 200918088 A	01-05-2009
		US 2009148437 A1	11-06-2009
		US 2010247515 A1	30-09-2010
		US 2010266616 A1	21-10-2010
		US 2011256122 A1	20-10-2011
		US 2012134982 A1	31-05-2012
		WO 2008149148 A2	11-12-2008
		WO 2008149149 A2	11-12-2008
		WO 2008149150 A2	11-12-2008
US 2006002935 A1	05-01-2006	AU 2005291017 A1	13-04-2006
		CA 2583417 A1	13-04-2006
		EP 1841452 A2	10-10-2007
		EP 2371390 A2	05-10-2011
		ES 2471943 T3	27-06-2014
		JP 5030782 B2	19-09-2012
		JP 2008515420 A	15-05-2008
		US 2006002935 A1	05-01-2006
		WO 2006038027 A2	13-04-2006
EP 1972637 A1	24-09-2008	AT 514714 T	15-07-2011
		CA 2680085 A1	25-09-2008
		CN 101679520 A	24-03-2010
		EP 1972637 A1	24-09-2008
		EP 2121759 A2	25-11-2009
		ES 2368764 T3	22-11-2011
		JP 5534819 B2	02-07-2014
		JP 2010521508 A	24-06-2010
		PL 1972637 T3	31-01-2012
		US 2010150916 A1	17-06-2010
		US 2013244341 A1	19-09-2013
		WO 2008113515 A2	25-09-2008
WO 2012059513 A1	10-05-2012	CA 2816912 A1	10-05-2012
		EP 2635606 A1	11-09-2013
		US 2014037646 A1	06-02-2014
		WO 2012059513 A1	10-05-2012
WO 2012122464 A1	13-09-2012	US 2014045756 A1	13-02-2014
		WO 2012122464 A1	13-09-2012
US 2013022605 A1	24-01-2013	NONE	