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(54) Title: THERAPY USING A FACTOR XII INHIBITOR IN A NEUROTRAUMATIC DISORDER

(57) Abstract: The present invention relates to the use of a direct Factor XII (FXII) inhibitor in the treatment of a neurotraumatic disorder resulting from a traumatic injury of the brain (traumatic brain injury, TBI) or the spinal cord (spinal cord injury, SCI).

CSL BEHRING GMBH**5 Therapy using a Factor XII inhibitor in a neurotraumatic disorder**

This application relates to the use of a direct Factor XII (FXII) inhibitor in the treatment of a neurotraumatic disorder selected from a spinal cord injury (SCI) and a traumatic brain injury (TBI).

10

Two converging pathways for coagulation exist that are triggered by either "extrinsic" (vessel wall) or "intrinsic" (blood-borne) components of the vascular system. The "intrinsic" or contact activation pathway is initiated when Factor XII (FXII, Hageman factor) comes into contact with negatively charged surfaces in a reaction involving high molecular weight 15 kininogen and plasma kallikrein. Factor XII is a serine protease and, once activated (FXIIa), it further activates circulating FXII in a positive feedback reaction (directly or via activation of prekallikrein). FXIIa also activates Factor XI and blood coagulation proceeds in a reaction cascade involving the activation of further factors by limited proteolysis culminating in the generation of thrombin, which converts plasma fibrinogen to fibrin and activates 20 platelets.

15

The kallikrein-kinin system (KKS) is also initiated by blood coagulation factor XII (FXII, Hageman factor) and plays an important role in the regulation of vascular permeability and edema formation (*Leeb-Lundberg et al. (2005) Pharmacol. Rev.; 571:27-77*). The activation 25 of the KKS was recently proven also in stroke patients (*Wagner et al. (2002) J. Neurol. Sci.; 202:75-76*). Kinins (e. g. bradykinin, kallidin) constitute the end products of the KKS. Kinins are highly active proinflammatory peptide hormones which are released by kallikreins from their precursors, kininogens, during various kinds of tissue injury including brain ischemia. The cellular effects of kinins are mediated by two different bradykinin receptors, B1R and 30 B2R. Activation of these receptors triggers inflammatory processes in the target organ such as the release of proinflammatory cytokines or the attraction of immune cells as well as increased vascular permeability.

20

Traumatic brain injury (TBI) is a devastating neurological condition and can be defined as 35 brain damage resulting from rapid movement of the brain within the skull or direct injury to

the brain and/or nerve roots due to a traumatic event causing immediate mechanical disruption of brain tissue/nerve roots and delayed pathogenic events. It is a heterogeneous disorder that can vary in the type of brain injury, distribution of brain damage and mechanisms of damage. The traumatic event is often caused by traffic or sport accidents, 5 and is the leading cause of death and disability in adolescent and young males (Tagliaferri *et al.*, 2006, *Acta Neurochir (Wien)*, 148(3):255-68). TBI constitutes approximately 20% of all traumas, and has a very high disease-associated spending (\$60 billion in the United States in 2000). Further, treatment options for TBI are very limited (Faul *et al.*, 2007, *Journal of Trauma-Injury Infection & Critical Care*, 63(6), 1271-8; Steudel *et al.*, 2005, *Acta 10 Neurochir (Wien)*, 147(3):231-42); at present, the only effective method to treat severe TBI is to prevent its occurrence. Although several phase-II clinical trials have shown favorable effects of therapeutic compounds (Narayan *et al.*, 2002, *J Neurotrauma*, 19(5):503-57), unfortunately all the compounds have failed to clearly show efficacy in phase-III trials (Doppenberg *et al.*, 2004, *Neurosurg Anesthesiol.*, 16(1):87-94). Despite numerous clinical 15 trials, attempts to find a safe and effective neuroprotective agent have all failed (Kabadi *et al.*, 2014, *International journal of molecular sciences* 15:1216-1236; Menon *et al.*, 2015, *Nature reviews Neurology* 11:71-72).

20 The primary brain damage that occurs due to an outside force causes irreversible mechanical disruption of brain tissue. In the sequel, secondary injury processes contribute to the exacerbation of traumatic brain damage. The primary brain tissue damage can be diffuse or focal, whereby the circumstances of injury determine the relative degree to which diffuse and focal trauma develops. While focal trauma is associated with brain tissue contusion, vascular injury, and hemorrhage, accompanied by ischemia, diffuse brain injury 25 is characterized by diffuse axonal injury. Key contributing factors to the brain damage are inflammation, metabolic disturbances and cerebrovascular dysfunction which further propagates injury-induced tissue ischemia and brain edema due to breakdown of the blood-brain-barrier (BBB) (Schlosberg *et al.* (2010) *Nat Rev Neurol.*; 6:393-403; Donkin and Vink (2010) *Curr. Opinion in Neurol.*; 23:293-299).

30 Beyond well-characterized injury processes like excitotoxicity, inflammation and blood-brain barrier damage, thrombus formation in the cerebral microcirculation probably contributes to secondary brain damage by causing peri-contusional ischemia and reducing regional cerebral blood flow (Schwarzmaier *et al.*, 2010, *J Neurotrauma* 27:121-130). In clinical TBI,

intracerebral vessel occlusion with subsequent ischemia worsens the outcome (Stein *et al.*, 2002, *J Neurosurg* 97:1373-1377; Stein *et al.*, 2004, *Neurosurgery* 54:687-691; Harhangi *et al.*, 2008, *Acta Neurochir (Wien)* 150:165-175; Maegele, 2013, *Transfusion* 53 Suppl 1:28S-37S). However, a potential use of conventional anticoagulants in TBI patients is 5 discussed controversially. Some studies support a net beneficial effect of anticoagulation following TBI by reducing the risk of thromboembolic events (Dudley *et al.*, 2010, *J Neurotrauma* 27:2165-2172; Albrecht *et al.*, 2014, *JAMA internal medicine* 174:1244-1251) and improving outcome parameters (Stutzmann *et al.*, 2002, *CNS Drug Rev* 8:1-30; Kim *et al.*, 2014, *J Emerg Trauma Shock* 7:141-148) while other studies report a detrimental effect 10 of anticoagulation (Peck *et al.*, 2014, *The journal of trauma and acute care surgery* 76:431-436) due to an increased risk of intracranial hemorrhages that also occur frequently after TBI.

The kallikrein-kinin system (KKS) is implicated in multiple pathological states (Leeb-Lundberg *et al.* (2005) *Pharmacol. Rev.*; 571:27-77), and represents an attractive therapeutic target in TBI. Kinins, liberated by the kallikreins, are proinflammatory peptides that mediate their effects via activation of two G-protein-coupled receptors (GPCR), kinin receptor B1 (B1R) and B2 (B2R) (Leeb-Lundberg *et al.*, 2005, *Pharmacol. Rev.*; 571:27-77; (Albert-Weissenberger *et al.* (2013) *Progr. Neurobiol.*; 101-102:65-82). Kinins play an 15 important role in regulating vascular permeability, edema formation, transendothelial cell migration, and inflammation in different organs following injury (Leeb-Lundberg *et al.*, 2005, *Pharmacol. Rev.*; 571:27-77). Moreover, the KKS is linked to the plasmatic coagulation 20 cascade via factor XII (FXII, Hageman factor). All constituents of the KKS have been identified in the rodent and human brain (Albert-Weissenberger *et al.* (2013) *Progr. Neurobiol.*; 101-102:65-82), and their expression is upregulated following brain injury 25 (Ongali *et al.* (2006) *J. Neurotrauma* 23, 696-707; Raslan *et al.*, (2010) *J. Cereb. Blood Flow Metab.* 30, 1477-1486; Trabold *et al.* (2010), *J. Cereb. Blood Flow Metab.* 30, 130-139.). Recently in mice, blockade of B1R, but not B2R, was shown to reduce blood-brain- 30 barrier damage and edema formation in experimental models of focal cerebral ischemia (Austenat *et al.* (2009) *Stroke*; 40:285-293) and traumatic brain injury (Albert-Weissenberger *et al.* (2012) *J. Cereb. Blood Flow Metab.*; 32:1747-56; Raslan *et al.* (2010) *J. Cereb. Blood Flow Metab.*; 30:1477-1486) suggesting functional relevance of the KKS on 35 brain edema formation in the acute phase of ischemic stroke and traumatic brain injury (Albert-Weissenberger *et al.* (2013) *Progr. Neurobiol.*; 101-102:65-82).

In studies preventing the activation of KKS via inhibition of FXII, which is activated physiologically upon contact with negatively charged surfaces (contact activation), neuropathological outcome following acute experimental stroke was investigated
5 (Hagedorn *et al.* (2010) *Circulation*; 121:1510-1517; Kleinschnitz *et al.* (2006) *JEM*; 203(3):513).

WO 2006/066878 discloses for the first time in general the use of a FXII inhibitor in treating or preventing venous or arterial thrombosis without being associated with abnormal
10 bleeding (hemostasis).

Hagedorn *et al.* ((2010) *Circulation*; 121:1510-1517) discloses the treatment and prevention of occlusive arterial thrombus formation by recombinant human albumin Infestin-4, a FXII inhibitor, while leaving hemostasis fully intact. Furthermore rHA-Infestin-4 was protective in
15 a murine model of ischemic stroke.

EP 2 623 110 A1 discloses FXII inhibitors for the treatment of neurological inflammatory disorders. Although the term "neurological inflammatory disease" refers to a condition with an inflammation of one or more areas of brain or spinal cord this disorder is not linked to
20 traumatic brain injury or spinal cord injury.

WO 2014/135694 (published on 12 September 2014) discloses a contact activation system selected from C1 esterase inhibitor, a kallikrein inhibitor and a FXII inhibitor, for use in the treatment and/or prevention of remote ischemia-reperfusion injury (IRI). Although such a
25 remote IRI may include disruption of blood brain barrier, the mentioned diseases and effects (cerebral edema, stroke, increased intracranial pressure and inflammation of neuronal tissue) as well as the remote IRI itself are not unambiguously linked to traumatic brain injury or spinal cord injury.

30 WO 2011/069090 discloses the treatment of a disease or condition associated with FXII activation by administering a phosphatidylserine binding agent, i.e. an inhibitor of an activator of FXII. It is mentioned that inhibition of FXII activation can e.g. be useful in preventing and treating (neurogenic) shock caused by e.g. spinal cord trauma. Although WO 2011/069090 also mentions shortly that a phosphatidylserine binding agent can be

combined with an anti-FXII antibody the efficacy of a direct FXII inhibitor (alone or in combination) in spinal cord trauma or in traumatic brain injury is unproven.

In summary there is state of the art disclosing the use of a FXII inhibitor either in particular 5 thrombotic indications or inflammatory diseases or neurological conditions. But none of these documents is related to a disease with the pathomechanism which is relevant in neurotraumatic disorders. Additionally, specific therapeutic interventions for traumatic brain injury are lacking in the state of the art and there is still a pressing demand to identify innovative pathomechanism-based concepts for effective therapies, in particular in view of 10 the controversial discussions of anticoagulant treatment in TBI patients.

Hence, in traumatic brain injury, it is apparent that there still exists a need for an improved 15 medication for the treatment of a traumatic brain injury. Therefore, it is an object of the present invention to satisfy such a need. Thus, the technical problem underlying the present invention was to provide alternative and/or improved means and methods for successfully targeting traumatic brain injury that form the basis or may allow the development of more satisfactory therapeutics for the treatment of traumatic brain injury.

The solution to this technical problem is achieved by providing the embodiments 20 characterized in the claims.

Accordingly, disclosed herein are therapies comprising the use of at least one direct Factor 25 XII inhibitor (FXII inhibitor) in the treatment of a neurotraumatic disorder. The neurotraumatic disorder is resulting from a traumatic injury of the central nervous system and can be selected from spinal cord injury and traumatic brain injury, and therapy can comprise administering an effective amount of at least one FXII inhibitor (e.g., rHA-Infestin-4 or anti-FXII antibody).

In other words, the inventors have discovered that a traumatic brain injury or a spinal cord 30 injury occurring after an initial traumatic injury of the central nervous system, in particular a traumatic edema, could be treated and/or prevented by the administration of a direct FXII inhibitor. Accordingly, in general the present invention relates to a direct FXII inhibitor for use in a method of treating a traumatic injury and/or treating or preventing the formation and/or reducing the size of a primary edema of the central nervous system (CNS) in a

subject wherein the subject, preferably a human subject, has or has had at least one disorder selected from the group consisting of traumatic brain injury, and spinal cord injury.

The inventors have found that pharmacological inhibition of FXII with a direct FXII inhibitor

5 minimizes trauma-induced microvascular thrombus formation after traumatic brain injury and improves functional outcome such as better motor function, reduced brain lesion volume, and diminished neurodegeneration without increasing the risk of abnormal intracerebral bleedings.

10 In some embodiments, the at least one FXII inhibitor can comprise a wild type Infestin-4 polypeptide sequence (SEQ ID NO: 1) or an Infestin-4 sequence harboring 1-5 amino acid mutations outside of N-terminal amino acid positions 2-13 of SEQ ID NO: 1 and/or a homology of at least 70%, 80%, 85%, 90%, 95%, 98%, or 99% to SEQ ID NO: 1 and retaining six conserved cysteine residues from SEQ ID NO: 1. In some embodiments, the

15 at least one FXII inhibitor can comprise a wild-type SPINK-1 polypeptide sequence (SEQ ID NO: 2), or a wild-type SPINK-1 polypeptide sequence in which N-terminal amino acid positions 2-13 have been replaced with the N-terminal amino acids 2-13 of SEQ ID NO: 1; and optionally further modified to contain 1-5 additional amino acid mutations that increase the homology of the polypeptide sequence to the sequence of SEQ ID NO: 1, and/or a

20 homology of at least 70%, 80%, 85%, 90%, 95%, 98%, or 99% to SEQ ID NO: 2 and retaining six conserved cysteine residues from SEQ ID NO: 2. In certain embodiments, the at least one FXII inhibitor can comprise an anti-FXII antibody. In some embodiments, the at least one FXII inhibitor can be linked to a fusion partner (e.g., a half-life enhancing polypeptide) either directly or via a linker. In some embodiments the FXII inhibitor is specific

25 to FXII, FXIIa and/or the activation of FXII.

In various embodiments, the FXII inhibitor is administered immediately after the neurotraumatic injury of a patient or after a patient develops the neurotraumatic disorder, or it is administered prophylactically. The FXII inhibitor can be administered once, or multiple

30 times (e.g., as a repeated prophylactic treatment, or as a repeated treatment during or following the traumatic injury resulting in the neurotraumatic disorder).

In some embodiments, a kit is provided, comprising at least one FXII inhibitor, instructions for using the kit in the treatment of a neurotraumatic disorder, and optionally, at least one

further therapeutically active compound or drug, wherein the further therapeutically active compound is not C1 inhibitor.

Brief Description of the Drawings

5

Figure 1 shows a reduced lesion size in rHA-Infestin-4 treated wild-type (WT) mice or FXII^{-/-} mice compared to NaCl-treated or WT controls, respectively, 24h after trauma induction. TTC stainings of brain slices gained from male and female mice were analyzed for their lesion volume. FXII deficiency or inhibition reduces lesion sizes 24h after trauma induction.

10

Figure 2 shows preservation of the blood-brain-barrier (BBB) integrity 24h after trauma induction in FXII^{-/-} mice compared to WT controls. BBB integrity was analyzed by the extent of Evans Blue extravasation in the lesioned hemisphere determined by photometry and the brain water content as a measure of brain edema in the lesioned hemisphere.

15

Figure 3 shows the preservation of the blood-brain-barrier (BBB) integrity 24h after trauma induction in rHA-Infestin-4 treated mice compared to NaCl-treated controls. BBB integrity was analyzed by the extent of Evans Blue extravasation in the lesioned hemisphere determined by photometry and the relative protein expression of the BBB structural protein Occludin. The brain water content is a measure of brain edema in the lesioned hemisphere.

20

Figure 4 shows the decrease in thrombus formation and dampened thrombotic processes 24h after trauma induction. The relative protein expression of Fibrin/Fibrinogen was analyzed via Western blotting and is reduced in FXII^{-/-} mice compared to WT controls. The ratio of occluded vessels to open vessels in the lesioned hemisphere was analyzed histologically via H&E staining of brain tissue and a reduced ratio of occluded to open vessels was found in rHA-Infestin-4 treated mice compared to NaCl-treated controls.

25

Figure 5 shows protection from inflammatory processes in FXII^{-/-} mice 24h after trauma induction. The quantification of macrophage infiltration into lesioned hemisphere was analyzed with immunohistochemical staining; the relative gene expression of proinflammatory cytokines TNF α and Interleukin-1 β was measured in FXII^{-/-} mice compared to WT controls and sham-operated mice.

Figure 6 shows that inflammatory processes are dampened in rHA-Infestin-4 treated animals 24h after trauma induction. The relative gene expression of proinflammatory cytokines TNF α and Interleukin-1 β was measured in rHA-Infestin-4 treated compared to NaCl-treated and sham-operated mice.

5

Figure 7 shows reduction in lesion size in FXII $^{-/-}$ mice compared to WT controls 3d after trauma induction. Lesion volumes were measured in TTC-stained brain slices and show protection from tissue damage for both male and female FXII $^{-/-}$ mice.

10 Figure 8 shows reduction of the blood-brain-barrier (BBB) damage 3d after trauma induction in FXII $^{-/-}$ mice compared to WT controls. BBB integrity was analyzed by the extent of Evans Blue extravasation in the lesioned hemisphere determined by photometry.

15 Figure 9 shows intracerebral platelet accumulation and thrombosis as pathologic features of traumatic brain injury (after weight drop injury and cryolesion).

Figure 10 shows diminishment of intracerebral platelet accumulation on day 7 after weight drop injury in FXII $^{-/-}$ mice.

20 Figure 11 shows diminishment of intracerebral platelet accumulation on day 1 and day 3 after cryolesion FXII $^{-/-}$ mice.

25 Figure 12 shows improvement of FXII-deficiency in the outcome after weight drop injury by FXII $^{-/-}$ mice developing a significantly lower neurological severity score than wild-type mice and FXII $^{-/-}$ mice reconstituted with human FXII on day 3 and on day 7 after diffuse brain trauma.

30 Figure 13 shows improvement of FXII-deficiency in the outcome after cryolesion by FXII $^{-/-}$ mice showing significantly reduced lesion volumes compared to WT mice and showing a significantly diminished number of apoptotic neurons in FXII $^{-/-}$ mice when compared to WT controls.

Figure 14 shows diminishment of intracerebral platelet accumulation and improvement of the outcome after weight drop brain trauma by pharmacological inhibition of FXII with rHA-Infestin-4.

5 Figure 15 shows diminishment of intracerebral platelet accumulation and protection from focal brain trauma by pharmacological inhibition of FXII with rHA-Infestin-4.

Figure 16 shows genetic deficiency and pharmacological inhibition of FXII does not lead to hemorrhages after cryolesion.

10 Figure 17 shows reduction of immune cell infiltration 24 hours and 3 days after injury induction.

Detailed Description of certain Embodiments

15 The embodiments of the application pertain to methods comprising administering a direct Factor XII (FXII) inhibitor to a patient to treat a neurotraumatic disorder selected from a spinal cord injury and a traumatic brain injury. In some embodiments, this therapy can interact with multiple pathways underlying the pathophysiology of the treated diseases, e.g. 20 thrombo-inflammation, cytotoxic and vascular brain edema, microvascular perfusion deficit due to vasospasms and microthrombus formation, damage to the microvascular endothelium and components of the blood-brain barrier, potentially providing more effective treatment to a broader range of patient populations compared to the treatment therapies in the prior art.

25 **Definitions**

30 In this application, the use of the singular (such as "a" or "the") includes the plural unless specifically stated otherwise. Also in this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", are not limiting. Any range described here will be understood to include the endpoints and all values between the endpoints.

The section headings are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents or portions of documents cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose.

5 To the extent publications and patents or patent applications incorporated by reference contradict the invention contained in the specification, the specification will supersede any contradictory material.

As used herein, a “FXII inhibitor” refers to an inhibitor of either or both of Factor XII (prior to 10 activation, i.e. its zymogen) and activated Factor XII (FXIIa) as well as to the activation of FXII. FXII inhibitors encompass functional variants and fragments of the wild-type inhibitor. A functional variant or fragment is a molecule that retains at least 50% (e.g., 50, 60, 70, 80, 90, 95, 99, or 100%, or any percentage in between) of the ability of the wild-type molecule to inhibit FXII, FXIIa or the activation of FXII.

15 The term “direct FXII inhibitor”, as used herein, refers to an inhibitor that acts via contact (e.g., binding) with FXII (or FXIIa), i.e., the FXII inhibitor binds to FXII and/or FXIIa and inhibits its activity and/or activation. In contrast, an indirect inhibitor may act without contacting FXII (or FXIIa) protein. For example, antisense RNA can be used to decrease 20 expression of the FXII gene, or a small molecule can inhibit the effects of FXIIa via interactions with downstream FXIIa reaction partners like Factor XI; these do not interact directly with the FXII protein. Thus, an indirect inhibitor, in contrast to a direct inhibitor, acts upstream or downstream from the FXII protein. Some examples of direct inhibitors are presented below. In some embodiments, the FXII inhibitors are non-endogenous inhibitors; 25 that is, they are not inhibitors that occur naturally in the human or animal body. In some embodiments, the FXII inhibitors are specific to FXII or FXIIa, in particular specific to human FXII or FXIIa as discussed below.

30 A “neurotraumatic disorder”, as used herein, refers to a traumatic injury of the central nervous system (CNS), selected of a spinal cord injury and a traumatic brain injury. Preferably the neurotraumatic disorder is a primary traumatic brain edema, which is an edema occurring during the initial insult or shortly or immediately (i.e. within minutes) after the insult. Accordingly, a neurotraumatic disorder or an edema of CNS refers to any direct brain or spinal cord swelling i.e. the swelling occurs immediately after the initial injury. It is

initially a vasogenic edema resulting from increased water diffusion over the damaged blood brain barrier but later also a cytotoxic edema resulting from abnormal water uptake by injured brain cells. A neurotraumatic disorder further implies direct neural injury (apoptosis, axonal damage), local brain tissue energy deficit caused by microvascular
5 damage and thrombo-inflammatory processes that occur immediately and last days to months after the insult.

Preferably the neurological disorder according to the invention initially occurs within a few hours after the initial injury (i.e. when the external force injures the CNS) and can persist for
10 weeks. In some embodiments the neurological disorder of CNS appears within 30 minutes or within 1, 2, 3, 4, 5, 6, 12 or 24 h after the initial insult or at any time in between.

Preferably the neurotraumatic disorder according to the invention initially occurs within a few hours after the initial injury (i.e. when the external force injures the CNS) and can persist for weeks. In some embodiments the neurotraumatic disorder of CNS appears within 30 minutes or within 1, 2, 3, 4, 5, 6, 12 or 24 h after the initial insult or at any time in between.
15

The term "traumatic brain injury" ("TBI") refers to a brain damage resulting from rapid movement of the brain within the skull due to a traumatic event causing immediate mechanical disruption of brain tissue and delayed pathogenic events. It is a heterogeneous disorder that can vary in the type of brain injury, distribution of brain damage and mechanisms of damage. A symptom of the TBI, which appears very often, is a traumatic brain edema. The traumatic event is often caused by traffic or sport accidents.
20

25 The term "spinal cord injury" (SCI) as used herein refers to any injury to the spinal cord that is caused by trauma instead of disease. Depending on where the spinal cord and nerve roots are damaged, the symptoms can vary widely, from pain to paralysis to incontinence. Spinal cord injuries are described at various levels of "incomplete", which can vary from having no effect on the patient to a "complete" injury which means a total loss of neurological/organ function. Often the spinal cord injury is associated with a spinal cord edema. Spinal cord injuries have many causes, but are typically associated with major trauma from e.g. motor vehicle accidents, falls, sports injuries, and violence.
30

The term "traumatic brain edema" or "brain edema" refers to any post-injury swelling of the brain, i.e. the swelling occurs within a short period of time after the initial injury of the CNS. The term "traumatic spinal cord edema" or "spinal cord edema" refers to any post-injury swelling of the spinal cord, i.e. the swelling occurs within a short period of time after the initial injury.

As used here, the terms "treat" and "treating" encompass preventing, inhibiting, eliminating, delaying the onset, slowing, lessening, reducing the severity, or ameliorating at least one sign, symptom, or aspect of a disorder or disease. Treating does not require a complete elimination of symptoms, in that it encompasses but is not equivalent to "cure" or "curing". In some embodiments, a patient can be treated to prevent a disorder, meaning either administering therapy to a subject known to be at risk for developing a neurotraumatic disorder selected from a spinal cord injury and a traumatic brain injury. In some embodiments, "treat" or "treating" can also include ameliorating the effects of a disorder or disease. The terms "ameliorating" and "ameliorating the effects" mean that some aspect of the disorder or disease that produces an impairment of one or more patient function is improved. For example, treating a traumatic brain injury can include preventing a traumatic brain injury, ameliorating the effects of the traumatic brain injury, or reducing the severity of the traumatic brain injury (as measured, e.g., by neurological function or brain imaging).

As used herein, a "patient" is any human or animal that has, has had, or is likely to develop a neurotraumatic disorder selected from a spinal cord injury and a traumatic brain injury and who could benefit from the administration of a FXII inhibitor. The administration of a FXII inhibitor can be by any known method of delivering a pharmaceutical or therapeutic agent to a patient, including, without limitation, parenteral administration (e.g., subdural, subcutaneous, intravenous, intra-arterial, intramuscular, intrathecal, intranasal, intratracheal, inhalative, and/or intraperitoneal injection), oral, and/or rectal administration, as well as administration by instillation, spray application, and/or infusion techniques. In certain embodiments, the administration can be done intravenously, subcutaneously or intrathecally.

As used herein, an "antibody" includes any polypeptide comprising a functional antigen-binding site, including immunoglobulins and antigen-binding parts or fragments thereof. A functional antigen-binding part or fragment is a molecule that retains at least 50% (e.g., 50,

60, 70, 80, 90, 95, 99, or 100%, or any percentage in between) of the ability of the full-length antibody to bind to and inhibit the antigen. The term antibody includes but is not limited to polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, fully human, camelized, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, back-mutated, and CDR-grafted antibodies. The term also includes antibody fragments such as Fab, F(ab')2, Fv, scFv, Fd, dAb, VHH (also referred to as nanobodies), and other antibody fragments or variants that retain antigen-binding function, including bi-specific or multi-specific antibodies. An antibody can be of any isotype, including IgA, IgD, IgE, IgG, and IgM. As used herein, an "antigen" is a target molecule that is capable of being bound by an antibody. As used herein, the term "antigen-binding site" refers to the part of an antibody molecule capable binding to or complementary to a part or all of an antigen.

I. Neurotraumatic Disorders

15 In some embodiments, a FXII inhibitor is disclosed and can be used in methods of treating a neurotraumatic disorder resulting from a traumatic injury of the central nervous system selected from a spinal cord injury and a traumatic brain injury. The methods can comprise administering to a subject in need thereof at least one FXII inhibitor. Accordingly, the invention also provides one or more pharmaceutical compositions comprising at least one 20 FXII inhibitor in pharmaceutically acceptable excipients or carriers for use in treating neurotraumatic disorders resulting from a traumatic injury of the central nervous system and selected from a spinal cord injury and a traumatic brain injury. Similarly, the invention also provides the use of one or more compositions comprising at least one FXII inhibitor in the preparation of a medicament for treating a neurotraumatic disorder selected from a 25 spinal cord injury and a traumatic brain injury. The at least one FXII inhibitor can be used alone or additional therapeutic compounds can also be administered.

A neurotraumatic disorder treated using the methods and compositions disclosed herein can include a spinal cord injury (SCI) or traumatic brain injury (TBI). Preferably the 30 neurotraumatic disorder treated using the methods and compositions disclosed herein is TBI.

A traumatic brain injury (TBI), also known as intracranial injury, according to the present invention occurs when an external force traumatically injures the brain. TBI can be

classified based on severity, mechanism (closed or penetrating head injury), or other features (e.g. occurring in a specific location or over a widespread area) and can cause a host of physical, cognitive, social, emotional, and behavioral effects, and outcome can range from complete recovery to permanent disability or death. Traumatic brain injury is

5 defined as damage to the brain resulting from external mechanical force, such as rapid acceleration or deceleration, impact, blast waves, or penetration by a projectile. Brain function is temporarily or permanently impaired and structural damage may or may not be detectable with current technology.

10 The most common causes of TBI include violence, transportation accidents, construction, and sport accidents. Motor bike accidents are major causes, increasing in significance. In children aged two to four, falls are the most common cause of TBI, while in older children traffic accidents compete with falls for this position. TBI is the third most common injury to result from child abuse. Abuse causes 19% of cases of pediatric brain trauma, and the
15 death rate is higher among these cases.

20 The type, direction, intensity, and duration of forces all contribute to the characteristics and severity of TBI. Forces that may contribute to TBI include angular, rotational, shear and translational forces. Even in the absence of an impact, significant acceleration or deceleration of the head can cause TBI; however in most cases a combination of impact and acceleration is probably to blame. Forces involving the head striking or being struck by something, termed *contact* or *impact loading*, are the cause of most focal injuries, and movement of the brain within the skull, termed *noncontact* or *inertial loading*, usually causes diffuse injuries. Damage may occur directly under the site of impact, or it may occur
25 on the side opposite the impact. A TBI according to the invention can be caused by a diffuse injury and/or by a focal injury.

30 Treatment with at least one FXII inhibitor can be done prophylactically to prevent a neurotraumatic disorder selected from a spinal cord injury and a traumatic brain injury. In most cases the treatment is done after the traumatic injury, i.e. treatment is administered immediately or at some time point after the traumatic injury of the central nervous system occurred resulting in a neurotraumatic disorder selected from a spinal cord injury and a traumatic brain injury. For example, treatment can be administered for the first time directly

after the external force injures the central nervous system, or up to about 1 hour, or up to about 2, or even up to 24 hours, or even up to 3 days.

In preferred embodiments, treatment is begun immediately after, or less than about 12 hours after the initial occurrence of the neurotraumatic disorder. In some embodiments, treatment is begun up to about 30 minutes, or up to about 1 hour, up to about 2, or even up to about 24 hours. In some embodiments, treatment is administered immediately after, or about 5, 10, 20, 30, 40, or 50 minutes, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours following the occurrence of the external force which traumatically injures the brain or the spinal cord (or at any time point in between). In some embodiments, treatment is administered as soon as possible after the occurrence of the initial injury, and preferably less than 1 hour, less than 6 hours, or less than 24 hours following injury. In some embodiments, treatment occurs no later than about 24 hours following initial injury, or no more than about 6 hours following injury, or no more than about 1 hour following injury.

In some embodiments, administration of at least one FXII inhibitor can be done prophylactically to prevent initial injury in a patient at risk for a neurotraumatic disorder. Prophylactic treatment can be done in a single dose or in repeated doses. Periodic doses can be administered for a set duration of time, for example over the duration of a course of treatment.

In some embodiments, administration of at least one FXII inhibitor can be done repeatedly e.g. to treat a patient at risk for a neurotraumatic disorder or to treat a patient in a more effective manner. Such treatment can be done in multiple doses, for example in two, three, four, five, or more doses in a repeated way, such as a dose every 1 hour, every 2 hours, every 4 hours, every 6 hours, every 12 hours, or any time period in between.

Administration of at least one FXII inhibitor (e.g., to a patient in need of treatment) may occur in a single dose or in repeated administrations, and in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier and/or additive as part of a pharmaceutical composition. Therefore, in certain embodiments, the at least one FXII inhibitor is administered (i) once, each as a separate injection or infusion, or in a single combined injection or infusion, (ii) in multiple doses, for

example in two, three, four, five, or more doses, each as an injection or infusion, or (iii) as an infusion or application. The infusion/application can be administered over a period of time, preferably over a period of 1 minute to 24 hours, or 10 minutes to 12 hours, or 10 minutes to 6 hours, or 10 minutes to 5 hours, or 10 minutes to 4 hours, or 10 minutes to 3 hours, or 10 minutes to 2 hours, or 10 minutes to 1 hour (or any time period in between). The administration can be done in a repeated manner after the insult until the symptoms of the neurotraumatic injury disappear, i.e. administration of at least one FXII inhibitor can occur in repeated administrations for days to months, e.g. for one day, two days, three days, four days, five days, six days, one week, two weeks, four weeks, two months.

10

The composition comprising at least one FXII inhibitor may be administered to a patient in a therapeutically effective amount. Generally, a therapeutically effective amount may vary with the subject's age, general condition, and gender, as well as the severity of the medical condition in the subject. The dosage may be determined by a physician and adjusted, as necessary, to suit the observed effects of the treatment.

A therapeutically effective dose of the at least one FXII inhibitor may depend on many factors such as, e.g., the exact indication, formulation, or mode of administration, and may be determined in preclinical and clinical trials for each respective indication. For example, 20 in one embodiment, the dose of FXII inhibitor is about 0.01, 0.1, 1, 50, 100, 200, 500, or 1000 mg/kg bodyweight, or any dose in between, or ranging from about 0.01-1000 mg/kg, about 0.1-1000 mg/kg, about 1-1000 mg/kg, about 1-500 mg/kg, about 10-200 mg/kg, about 10-100 mg/kg, about 50-500 mg/kg, about 50-200 mg/kg, or about 100-200 mg/kg, or any dose range in between. In certain embodiments, the FXII inhibitor is rHA-Infestin-4. In 25 some embodiments, when rHA-Infestin-4 is used, the dose may range between about 0.01 and 1000 mg/kg body weight, or between about 1 and 1000 mg/kg, or between about 1 and 500 mg/kg, or between about 50 and 500 mg/kg (or any dose range in between).

In some embodiments, the FXII inhibitor can be an anti-FXII antibody. In those 30 embodiments involving a therapeutically effective dose of an anti-FXII antibody, a therapeutically effective dose is a dose that brings about a positive therapeutic effect in the patient or subject requiring the treatment. A therapeutically effective dose can be in the range of about 0.001 to 100 mg/kg body weight, or from about 0.01 to 100 mg/kg, from about 0.01 to 50 mg/kg, from about 0.1 to 30 mg/kg, from about 0.1 to 10 mg/kg, from

about 0.1 to 5 mg/kg, from about 0.1 to 2 mg/kg or from about 0.1 to 1 mg/kg, or any dose range in between. For example, a therapeutically effective dose may be a dose that inhibits FXIIa activity in the subject by at least about 50%, or at least 60%, 70%, 80%, 90%, 95%, 99% or 100% (or any percentage in between).

5

The exact therapeutically effective dose of the FXII inhibitor may be determined by the person skilled in the art by routine experiments and does not involve any surprising steps.

10 In certain embodiments the at least one FXII inhibitor is administered at a concentration that produces a reduction in at least one symptom of the neurotraumatic disorder.

15 The administered pharmaceutical compositions may comprise at least one FXII inhibitor as the sole active compound, or may be delivered in combination with one or more additional compounds, compositions, or biological materials. Examples of additional compounds include steroids, in particular cortisone.

20 In some embodiments, the effects of treatment with at least one FXII inhibitor on a neurotraumatic disorder can be monitored by measuring the extent of tissue damage and/or edema. Methods for measuring the extent of tissue damage can include, e.g., histology, biochemical, colorimetric and immunological assays (Evans Blue-extravasation, TTC staining, Western Blot, RT-PCR of inflammatory mediators), measurement of neurological function or brain imaging (MRT, CT, PET). In some embodiments, the tissue damage causes loss of neurological function that can be measured using assessment of neurological function (Neuroscore).

25

In certain embodiments of the disclosed methods, patients should be treated according to the established standards of care for their clinical presentation.

II. Factor XII Inhibitors

30

As discussed above the terms "Factor XII" and "FXII" each refer to either or both of Factor XII (e.g., the zymogen or precursor form of the peptide) and activated Factor XII (FXIIa). Thus, "FXII inhibitors" can include inhibitors of either or both of FXII and FXIIa (also termed αFXIIa) as well as the activation of FXII, including the FXIIa cleavage products FXIIa alpha

and FXIIa beta (also termed FXIIIf). Further, anti-FXII antibodies include antibodies that bind to and inhibit either or both of FXII and FXIIa. The term "FXII inhibitor" is also meant to include an inhibitor of FXII that is linked to a half-life extending polypeptide, which in some embodiments includes a linker. Examples of FXII inhibitors that can be used include rHA-

5 Infestin-4, SPINK-1, anti-FXII antibodies, including modified versions/fragments of these proteins that retain the ability to inhibit the activation of FXII. In an embodiment, the FXII inhibitor is SPINK-1 or a modified SPINK-1. In an embodiment, the FXII inhibitor is Infestin-4 or a modified Infestin-4 or an anti-FXII antibody.

10 The FXII inhibitor is a direct inhibitor of FXII. The term "direct" inhibitor means an inhibitor that acts via contact (e.g., binding) with FXII (or FXIIa), i.e., the FXII inhibitor binds to FXII and/or FXIIa and inhibits its activity and/or activation. In contrast, an indirect inhibitor may act without contacting FXII (or FXIIa) protein. For example, antisense RNA can be used to decrease expression of the FXII gene, or a small molecule can inhibit the effects of FXIIa
15 via interactions with downstream FXIIa reaction partners like Factor XI; these do not interact directly with the FXII protein. Thus, an indirect inhibitor, in contrast to a direct inhibitor, acts upstream or downstream from the FXII protein. Some examples of direct inhibitors are presented below. In some embodiments, the FXII inhibitors are non-
20 endogenous inhibitors; that is, they are not inhibitors that occur naturally (endogenously) in the respective human or animal body. In some embodiments the FXII inhibitor is not a FXII inhibitor like e.g. C1 inhibitor.

In one embodiment the FXII inhibitor is a specific FXII inhibitor, preferably a specific FXIIa inhibitor.

25 A specific FXII inhibitor refers to an inhibitor which inhibits plasmatic serine proteases or other endogenous proteins other than FXII and/or FXIIa less than or equal to 25% if used in a molar ratio of 1:1. In other words: a specific FXII/FXIIa inhibitor inhibits plasmatic serine proteases other than FXII and/or FXIIa less than or equal to 25% when said inhibitor is
30 used in a molar ratio of 1:1 of the respective plasmatic serine protease to said inhibitor. Preferably the FXII inhibitor inhibits plasmatic serine proteases other than FXII and/or FXIIa less than or equal to 20%, preferably less than or equal to 15%, preferably less than or equal to 10%, preferably less than or equal to 5%, preferably less than or equal to 1% if used in a molar ratio of 1:1. For example, a specific FXII mAb inhibits the plasmatic serine

protease FXIa by only 5%, wherein the molar ratio of FXIa to said mAb is 1:1 whereas the same FXII mAb inhibits FXIIa by at least 80%, preferably at least 90%.

In one embodiment of the invention one other plasmatic serine protease is inhibited by
5 more than 50% if used in a molar ratio of 1:1 of the respective plasmatic serine protease to
said inhibitor.

In another embodiment of the invention two other plasmatic serine proteases are inhibited
by more than 50% if used in a molar ratio of 1:1 of the respective plasmatic serine protease
10 to said inhibitor.

In yet another embodiment the FXII inhibitor is a human FXII inhibitor, including a
humanized monoclonal antibody, preferably a fully human monoclonal antibody.

15 "Homology" as used herein refers to the percentage number of amino acids that are
identical or constitute conservative substitutions. Homology may be determined using
sequence comparison programs such as GAP (Deveraux et al., 1984, Nucleic Acids
Research 12, 387-395), which is incorporated herein by reference. In this way sequences
of a similar or substantially different length to those cited herein could be compared by
20 insertion of gaps into the alignment, such gaps being determined, for example, by the
comparison algorithm used by GAP.

In one embodiment the pharmaceutical formulation administered comprises the FXII
inhibitor as the only active substance, i.e. the FXII inhibitor is not used in combination with
25 another active agent. Preferably the FXII inhibitor is not used in combination with a
phosphatidylserine binding agent, e.g. a modified annexin V composition.

A. Infestin-4

30 In one embodiment, the application provides a FXII inhibitor comprising infestin domain 4
(referred to as "Infestin-4"). Infestins are a class of serine protease inhibitors derived from
the midgut of the hematophagous insect, *Triatoma infestans*, a major vector for the parasite
Trypanosoma cruzi, known to cause Chagas disease (Campos ITN et al. 32 *Insect
Biochem. Mol. Bio.* 991-997, 2002; Campos ITN et al. 577 *FEBS Lett.* 512-516, 2004; WO

2008/098720). This insect uses these inhibitors to prevent coagulation of ingested blood. The infestin gene encodes 4 domains that result in proteins that can inhibit different factors in the coagulation pathway. In particular, domain 4 encodes a protein (Infestin-4) that is a strong inhibitor of FXIIa. Infestin-4 has been administered in mice without resulting in 5 bleeding complications (WO 2008/098720; *Hagedorn et al., Circulation 2010; 121:1510-17*).

In various embodiments, a FXII inhibitor comprises Infestin-4. The term "Infestin-4," as used herein, encompasses variants or fragments of the wild-type peptide that retain the 10 ability to inhibit FXII. In some embodiments, the Infestin-4 is chosen for its ability to inhibit FXIIa. In certain embodiments, the Infestin-4 comprises a variant of Infestin-4, wherein the variant comprises Infestin domain 4, and optionally, Infestin domains 1, 2, and/or 3. In one embodiment, the Infestin-4 is a (His)₆-tagged Infestin-4 construct. In another embodiment, the Infestin-4 is a fusion protein comprising a fusion partner, such as a half-life enhancing 15 polypeptide (e.g., albumin, an Fc domain of an IgG, or PEG), bound to infestin-4. In some embodiments, a linker connects the fusion partner to Infestin-4. In various embodiments, the Infestin-4 is the rHA-Infestin-4 protein described in Hagedorn et al. (*Circulation 2010; 117:1153-60*). In one embodiment, a composition comprises albumin bound to the rHA-Infestin-4 protein described in Hagedorn et al. (*Circulation 2010; 117:1153-60*) by a flexible 20 linker. In certain embodiments, other Infestin-4 inhibitors of FXII are used, examples of which are described in WO 2008/098720 and Hagedorn et al. (*Circulation 2010; 117:1153-60*) both of which are incorporated by reference in their entirety.

An example of a wild type Infestin-4 sequence is presented in SEQ ID NO: 1: 25 EVRNPCACFRNYVPVCGSDGKTYGNPCMLNCAAQTKVPGLKLVHEGRC.

As used here, the term "variant" of Infestin-4 refers to a polypeptide with one or more amino acid mutation, wherein "mutation" is defined as a substitution, a deletion, or an addition, to the wild type Infestin-4 sequence (SEQ ID NO: 1). The term "Infestin-4" 30 encompasses these Infestin-4 variants. The term "variant" of Infestin-4 also includes fragments of the wild type or a mutated Infestin-4 sequence. In various embodiments, the one or more mutations to the wild type Infestin-4 sequence do not substantially alter the functional ability of the polypeptide to inhibit FXII. In some embodiments, the one or more mutations do not completely or substantially remove the ability of the polypeptide to inhibit

FXII (e.g., the variant retains at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or more of the inhibitory ability of wild type Infestin-4). Further examples of such variants are provided below.

5 In one embodiment, an Infestin-4 variant comprises the amino acid sequence VRNPCACFRNYV (SEQ ID NO: 20, which are residues 2-13 of SEQ ID NO: 1) from the amino terminal of the wild type Infestin-4 sequence. In certain embodiments, the variant can comprise residues 2-13 of SEQ ID NO: 1 and also comprises at least one, and optionally up to five, amino acid mutations, as compared to the wild type Infestin-4

10 sequence, outside residues 2-13 of SEQ ID NO: 1. In some embodiments, the variant retains six conserved cysteine residues from the wild type Infestin-4 sequence, and/or a homology of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, to the wild type Infestin-4 sequence. In some embodiments, a variant of Infestin-4 comprises the conserved N-terminal region amino

15 acids 2-13 of the wild type Infestin-4 sequence, and at least one, and optionally up to five, amino acid mutations outside these conserved N-terminal amino acids, resulting in differences from the wild type Infestin-4 sequence. As used here, the term “outside the N-terminal amino acids” of an Infestin variant refers to any amino acid along the polypeptide chain of the variant other than the contiguous stretch of amino acids that comprises the

20 sequence of SEQ ID NO: 20: VRNPCACFRNYV, which are amino acids 2-13 from SEQ ID NO: 1.

In another embodiment, an Infestin-4 variant comprises six conserved cysteine residues and/or has a homology of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, to the wild type Infestin-4 sequence. In one embodiment, the six conserved cysteine residues are amino acids at positions 6, 8, 16, 27, 31, and 48 of the wild type Infestin-4 sequence, SEQ ID NO: 1. In one embodiment, the variant comprises the final conserved cysteine at position 48. In other embodiments, the exact positions of the cysteine residues, and relative positions to each other, may change from positions 6, 8, 16, 27, 31, and 48 of the wild type Infestin-4 sequence due to insertions or deletions in the Infestin-4 variant. Nevertheless, in these embodiments, an Infestin-4 variant comprises all six cysteines and/or may share 70%, 75%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, or any percentage in between homology to the wild type Infestin-4 sequence. In some embodiments, the Infestin-4 variant retains amino

acids 2-13 from SEQ ID NO: 1 as well as all six cysteine residues, and may share 70%, 75%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, or any percentage in between homology to the wild type Infestin-4 sequence.

- 5 In some embodiments, the Infestin-4 variant comprises SEQ ID NO: 21: DSLGREVRNPCA. In some embodiments, this sequence is added at or near the N-terminus of a fragment or full length wild type Infestin-4 sequence and derives from the human protein SPINK-1.
- 10 In some embodiments, an Infestin-4 variant comprises a fusion construct between wild-type Infestin-4 or a variant Infestin-4 and human albumin (referred to as “HA”). In some embodiments, the HA is a recombinant protein (referred to as “rHA”). In certain embodiments, the Infestin-4 and HA proteins are joined directly, or via a linker polypeptide.
- 15 In one embodiment, the FXII inhibitor comprises a variant of the wild type Infestin-4 polypeptide sequence, wherein the variant comprises the N-terminal amino acids 2-13 of SEQ ID NO: 1; at least one, and optionally up to five, amino acid mutations outside the N-terminal amino acids; six conserved cysteine residues; and/or homology of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 20 99%, to the wild type Infestin-4 sequence.

In various embodiments, a variant of Infestin-4 is provided that retains the ability to inhibit FXII. The functional inhibitory activity may be assessed, for example, through *in vitro* and/or *in vivo* characterization, including direct assays to test inhibition of FXII enzyme activity, 25 prolonged coagulation time (e.g., activated partial thromboplastin time, aPTT), clinical clotting tests that address the intrinsic pathway of coagulation, or *in vivo* methods that evaluate coagulation.

Further examples of Infestin-4 variants are SPINK-1 mutants, which are described below.

30

B. SPINK-1 mutants

In various embodiments, the at least one FXII inhibitor can comprise a human protein with high similarity to Infestin-4. One example of a human protein with high similarity to Infestin-

4 is SPINK-1, a Kazal-type serine protease inhibitor expressed in the pancreas (also known as pancreatic secretory trypsin inhibitor, PSTI). The Kazal-type serine protease inhibitor family is one of numerous families of known serine protease inhibitors. Many similar proteins from different species have been described (*Laskowski M and Kato I, 49 Ann. 5 Rev. Biochem. 593-626, 1980*).

An example of a wild type SPINK-1 sequence is presented in SEQ ID NO: 2
DSLGRREAKCYNELNGCTKIYDPVCGTDGNTYPNECVLCFENRKRTSILIQKSGPC.

10 In various embodiments a wild-type SPINK-1 sequence (e.g., SEQ ID NO: 2) is used as the FXII inhibitor. The term "SPINK-1" also encompasses functional variants and fragments of SPINK-1 that substantially retain the ability to inhibit FXII, and in some embodiments, these SPINK-1 variants are used as the FXII inhibitors. For example, different variants of the wild-type sequence may be generated in order to increase the homology of the SPINK-1 15 sequence to Infestin-4. In one embodiment, SPINK-1 is mutated to comprise N-terminal amino acids 2-13 of SEQ ID NO: 1.

In one embodiment, a variant SPINK-1 comprises an N-terminal portion of a wild type Infestin-4 sequence (e.g., amino acids 2-13 of SEQ ID NO: 1), and optionally, at least one, 20 two, three, four, or five additional amino acid mutations outside the N-terminal amino acids that increase the homology of the variant to the wild type Infestin-4 sequence. In another embodiment, a variant SPINK-1 comprises six conserved cysteine residues and has a homology of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, to the wild type SPINK-1 sequence and, optionally, has 25 been mutated to increase the homology of the variant to the wild type Infestin-4 sequence. In some embodiments, the SPINK-1 variant also comprises an N-terminal portion of a wild type Infestin-4 sequence (e.g., amino acids 2-13 of SEQ ID NO: 1). A mutation may comprise a substitution, a deletion, and/or an addition. A mutation that is "outside the N-terminal amino acids" refers to one or more mutations in any amino acids along the 30 polypeptide chain of the variant other than the contiguous stretch of amino acids that comprises the sequence VRNPCACFRNYV (SEQ ID NO: 20), i.e., amino acids 2-13 of SEQ ID NO: 1. The term "variant" includes fragments of a SPINK-1 or mutated SPINK-1 sequence.

In some embodiments, the six conserved cysteine residues of SPINK-1 may be the amino acids at positions 9, 16, 24, 35, 38, and 56 of the wild type SPINK-1 sequence (e.g., SEQ ID NO: 2). In one embodiment, the variant comprises the final cysteine of the wild type SPINK-1 sequence (i.e., the cysteine at position 56 of SEQ ID NO: 2). In some 5 embodiments, the six cysteines are not mutated but the exact positions of the cysteines, and relative positions to each other, may change from positions 9, 16, 24, 35, 38, and 56 of the wild type SPINK-1 sequence due to insertions and/or deletions elsewhere in the SPINK-1 variant. Nevertheless, in these embodiments, a SPINK-1 variant comprises all six cysteines.

10

In some embodiments, the six cysteines of SPINK-1 are not mutated, but SPINK-1 is mutated to comprise an N-terminal portion of a wild type Infestin-4 sequence (e.g., amino acids 2-13 of SEQ ID NO: 1), and/or to have a homology of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, to the 15 wild type SPINK-1 sequence, and/or to include one, two, three, four, or five mutations in the SPINK-1 sequence outside the N-terminal amino acids. For example, a SPINK-1 variant may comprises the N-terminal amino acids 2-13 of SEQ ID NO: 1; at least one, and up to five, amino acid mutations outside the N-terminal amino acids that increase the homology of the variant to the wild type Infestin-4 sequence; six conserved cysteine residues from a 20 wild-type SPINK-1 sequence; and/or a homology of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, to the wild type SPINK-1 sequence.

In some embodiments, variants of SPINK-1 substantially retain their ability to inhibit FXII 25 (e.g., the variants retain at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or more of the inhibitory activity of wild type SPINK-1).

Examples of SPINK-1 variants that can be used in the methods disclosed herein include: K1: DSLGREVRNPCACFRNYVPVCGTDGNTYPNECVLCFENRKRQTSILIQKSGPC; 30 K2: DSLGREVRNPCACFRNYVPVCGTDGNTYGNECMLCAENRKRQTSILIQKEGPC; and K3: DSLGREVRNPCACFRNYVPVCGTDGNTYGNECMLNCAENRKRQTSILIQKEGPC (SEQ ID NOS: 3, 4, and 5, respectively).

In some embodiments, further amino acid substitutions can be made outside of the N-terminus relative to K1 in order to increase homology to Infestin-4. In the case of the SPINK-1 variant K3, five amino acid substitutions increase homology to Infestin-4. In certain embodiments, a SPINK-1 variant may share 70%, 75%, 80%, 85%, 90%, 91%, 5 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% (or any percentage in between) homology with the wild type SPINK-1 sequence.

C. FXII Antibodies

10 In various embodiments, the FXII inhibitor is an anti-FXII antibody that binds to FXII and/or to FXIIa and inhibits or reduces FXII activation and/or activity. The term “anti-factor XII antibody” encompasses full length antibodies and functional fragments thereof (e.g., antigen binding fragments such as Fab, F(ab)₂, Fv, and scFv). The term also encompasses polyclonal and monoclonal antibodies and antibodies of any of the isotypes such as IgM, 15 IgD, IgA, IgG, and IgE, and any subclass thereof, such as IgG₁. The antibody may be from a mammalian species such as human, mouse, rat, rabbit, goat, hamster, or monkey. In some embodiments, the antibody may be humanized or CDR-grafted. In some embodiments, the antibody may be mutated or modified to alter immunogenicity, half-life, and/or to impart other advantageous properties associated with a therapeutic antibody. In 20 one embodiment, the antibody is an anti-FXII antibody that binds to an epitope on the heavy chain or light chain of FXII, such as a neutralizing epitope. In some embodiments, the antibody may be conjugated to a polypeptide, nucleic acid, or small molecule. An “anti-FXII antibody” also includes antibodies that bind to and/or inhibit either or both of the zymogen of FXII and the activated protein (FXIIa), including the FXIIa alpha and FXIIa beta 25 cleavage fragments. In some embodiments, the antibody binds specifically to FXIIa or the alpha or beta chain fragments of FXIIa.

In some embodiments, the anti-FXII antibody can bind to and inhibit FXIIa activation and/or activity. Anti-FXII antibodies have been described, for example, in WO 2006/066878, and in 30 *Ravon et al., Blood 86: 4134-43 (1995)*, the disclosures of which are hereby incorporated by reference in their entirety. Other monoclonal antibodies (mAbs) to human Factor XII include the B7C9 mAb described by Pixley et al. (*J Biol Chem* 1987; 262, 10140-45); a mAb described by Small et al. (*Blood* 1985; 65:202-10); the monoclonal antibodies F1 and F3 described by Nuijens et al. (*J. Biol. Chem.* 1989; 264:12941-49); the B6F5, C6B7, and

D2E10 monoclonal antibodies against the light chain of FXII described in WO89/11865; a monoclonal antibody that selectively binds FXIIa- β over FXII described in WO90/08835; and the anti-FXII antibody OT-2 described in WO91/17258, the disclosures of which are hereby incorporated by reference in their entirety.

5

Additional anti-Factor XII monoclonal antibodies are described in WO 2013/014092, which is incorporated herein by reference in its entirety. In some embodiments, the antibodies may have a more than 2 fold higher binding affinity to human Factor XIIa-beta than to inactivated human FXII and may be capable of inhibiting the amidolytic activity of human

10 Factor XIIa.

Table 1.

Region	Amino acid sequence
VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYIMQWVRQAPGK GLEWVSGIRPSGGTTVYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCARALPRSGYLYSPHYYYYALDVWGGTGTVSS (SEQ ID NO: 6)
VL	QSELTQPPSASGTPGQRTISCGSSSNIGRNYVYQQVPGTA PKLLIYSNNQRPSGVPDRFSGSKSGTSASLVISGLRSEDEADYYC AAWDASLRGVFGGGTKLTVLG (SEQ ID NO: 7)
HCDR 1 (<i>Kabat</i> 31–35)	KYIMQ (SEQ ID NO: 8)
HCDR 2 (<i>Kabat</i> 50–65)	GIRPSGGTTVYADSVKG (SEQ ID NO: 9)
HCDR 3 (<i>Kabat</i> 95–102)	ALPRSGYLYSPHYYYYALDV (SEQ ID NO: 11)
LCDR 1 (<i>Kabat</i> 24–34)	SGSSSNIGRNYVY (SEQ ID NO: 13)
LCDR 2 (<i>Kabat</i> 50–56)	SNNQRPS (SEQ ID NO: 14)
LCDR 3 (<i>Kabat</i> 89–97)	AAWDASLRGV (SEQ ID NO: 15)

In certain embodiments, an anti-FXII antibody comprises the heavy chain variable region (VH) and light chain variable region (VL) sequences presented in Table 1. In some embodiments, an anti-FXII antibody comprises the HCDR1, HCDR2, and HCDR3, and/or comprises the VCDR1, VCDR2, and VCDR3 shown in Table 1. Antibody 3F7 as described 5 in WO 2013/014092 A1 is an example of such an antibody.

In some embodiments, the antibody has one or more of the following features: (a) binds 10 human FXII; (b) comprises a heavy chain variable (VH) region which is more than 85% identical to the sequence of SEQ ID NO: 6, such as more than 90%, 95%, 98%, or 99% identical; (c) comprises a light chain variable (VL) region which is more than 85% identical to the sequence of SEQ ID NO: 7, such as more than 90%, 95%, 98%, or 99% identical; (d) comprises heavy chain CDR1 at least 80% identical to the sequence of SEQ ID NO: 8, such as more than 85%, 90%, 95%, 98%, or 99% identical, and/or heavy chain CDR2 at least 60% identical with SEQ ID NO: 9, such as more than 70%, 80%, 85%, 90%, 95%, 15 98%, or 99% identical, and/or heavy chain CDR3 at least 80% identical to the sequence of SEQ ID NO: 11, such as more than 85%, 90%, 95%, 98%, or 99% identical; (e) comprises light chain CDR1 at least 50% identical to SEQ ID NO: 13, such as more than 60%, 70%, 80%, 85%, 90%, 95%, 98%, or 99% identical, and/or at least 50% identical to light chain CDR2 of SEQ ID NO: 14, such as more than 60%, 70%, 80%, 85%, 90%, 95%, 98%, or 20 99% identical, and/or at least 50% identical to light chain CDR3, with the sequence A-X₁-W-X₂-X₃-X₄-X₅-R-X₆-X₇ (SEQ ID NO: 16), such as more than 60%, 70%, 80%, 85%, 90%, 95%, 98%, or 99% identical; wherein X₁ can be A or S, X₅ can be L or V, and the other X_n's can be any amino acid; (f) binds human Factor XIIa-beta with a K_D of better than 10⁻⁸M; (g) 25 competes with Infestin-4 for binding to human Factor XIIa-beta; or (h) is a human IgG or functional variant thereof, preferably human IgG4 or a functional variant thereof.

In certain embodiments, the anti-FXII antibody is an IgG antibody that binds human FXII/FXIIa and comprises (a) a VH region comprising heavy chain CDR1 as set forth in SEQ ID NO: 8, heavy chain CDR2 as set forth in SEQ ID NO: 10, and heavy chain CDR3 30 as set forth in SEQ ID NO: 12; and/or (b) a VL region comprising light chain CDR1 as set forth in SEQ ID NO: 13, light chain CDR2 as set forth in SEQ ID NO: 14, and light chain CDR3 as set forth in SEQ ID NO: 16. A heavy chain CDR2 can comprise the sequence GIX₁X₂X₃X₄X₅X₆TVYADSVKG (SEQ ID NO: 10), wherein X₁ is R, N or D, X₂ is P, V, I, or M; X₃ is S, P, or A; X₄ is G, L, V, or T; X₅ can be any amino acid, preferably X₅ is G, Y, Q, K, R,

N, or M; and X₆ is T, G, or S. A heavy chain CDR3 can comprise the sequence ALPRSGYLX₁X₂X₃X₄YYYYYALDV (SEQ ID NO: 12), wherein X₁ is I, M or V; X₂ is S or K; X₃ is P, K, T, or H; and X₄ is H, N, G, or Q. A light chain CDR3 can comprise the sequence AX₁WX₂X₃X₄X₅RX₆X₇ (SEQ ID NO: 16), wherein X₁ is A or S; X₂ is D, Y, E, T, W, E, or S; 5 X₃ is A, N, I, L, V, P, Q, or E; X₄ is S, D, P, E, Q, or R; X₅ is L or V; X₆ is G, L, or K; and X₇ is V, A, D, T, M, or G.

In other embodiments, the anti-FXII antibody is a fragment of an IgG antibody that binds 10 human FXII/FXIIa and comprises (a) a VH region comprising heavy chain CDR1 as set forth in SEQ ID NO: 8, heavy chain CDR2 as set forth in SEQ ID NO: 9, and heavy chain CDR3 as set forth in SEQ ID NO: 11; and/or (b) a VL region comprising light chain CDR1 as set forth in SEQ ID NO: 13, light chain CDR2 as set forth in SEQ ID NO: 14, and light chain CDR3 as set forth in SEQ ID NO: 15.

15 In various embodiments, the anti-FXII antibody is an affinity matured, chimeric, CDR grafted, or humanized antibody, or a functional antigen binding fragment thereof. In some embodiments, the anti-FXII antibody is chosen from the affinity matured (relative to 3F7) antibodies VR115, VR112, VR24, VR110, and VR119 (SEQ ID NOs for HCDR 1-3 and LCDR1-3 of these antibodies are shown below in Table 2).

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Table 2.

mAb	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
3F7	8	9	11	13	14	15
VR119	8	10	11	13	14	15
VR112	8	10	11	13	14	15
VR115	8	10	11	13	14	15
VR24	8	9	11	17	14	15
VR110	8	10	11	13	14	15

As noted above, SEQ ID NO: 10 is a degenerate sequence. VR119 comprises SEQ ID NO: 10 wherein X₁ is N, X₂ is V, X₃ is P; X₄ is L, X₅ Y; and X₆ is G. VR112 comprises SEQ ID NO: 10 wherein X₁ is N, X₂ is V, X₃ is P, X₄ is V, X₅ is Q, and X₆ is G. VR115 comprises SEQ ID NO: 10 wherein X₁ is D, X₂ is I, X₃ is P, X₄ is T, X₅ is K, and X₆ is G. VR110

comprises SEQ ID NO: 10 wherein X₁ is D, X₂ is M, X₃ is P, X₄ is T, X₅ is K, and X₆ is G. VR24 comprises a unique LCDR1: SGSSEMTVHHYVY (SEQ ID NO: 17).

5 In embodiments involving antibody CDRs, CDR's are defined according to the KABAT numbering system (Kabat et al., Sequences of proteins of immunological interest, 5th ed. U.S. Department of Health and Human services, NIH, Bethesda, MD. (1991)).

10 In some embodiments, the antibody is an anti-FXII monoclonal antibody or antigen-binding fragment thereof that inhibits human FXIIa-alpha, e.g., in an in vitro FXIIa amidolytic activity assay (WO 2013/014092), by more than 40%, more than 50%, or more than 60%, when used at a molar ratio of 1:0.2 of FXIIa-alpha to antibody. In some embodiments, the antibody or antigen binding fragment thereof inhibits human Factor XIIa-alpha by more than 15 80%, more than 85%, or more than 90%, when used at a molar ratio of 1:0.5 of FXIIa-alpha to antibody. In one embodiment, the antibody achieves complete or nearly complete (e.g., 95%, 96%, 97%, 98%, 99%, or greater) inhibition of human FXIIa-alpha when used at a molar ratio of 1:0.5. In one embodiment, the antibody or antigen binding fragment thereof has an affinity for human FXIIa that is at least approximately comparable to that of antibody 3F7.

20 **D. FXII Inhibitors linked to HLEPs**

Another aspect of the application provides FXII inhibitors linked to fusion partners, such as 25 half-life enhancing polypeptides (HLEPs) or molecules such as PEG. In one embodiment, FXII inhibitors are small proteins. Therefore, rapid renal clearance (as is observed for other small proteins) can be expected (Werle M and Bernkop-Schnurch A, *Amino Acids* 2006; 30:351-367). One way to address a short plasma half-life of a polypeptidic compound is to inject it repeatedly or via continuous infusion. Another approach is to increase the intrinsic plasma half-life of the polypeptide itself. For example, in one embodiment, FXII inhibitors are linked to half-life extending proteins.

30 A "half-life enhancing polypeptide" is a polypeptide fusion partner that may increase the half-life of the FXII inhibitor *in vivo* in a patient or in an animal. Examples include albumin and immunoglobulins and their fragments, such as Fc domains, or derivatives, which may be fused to a FXII inhibitor directly or via a cleavable or non-cleavable linker. Ballance et al.

(WO 2001/79271) described fusion polypeptides comprising a multitude of different therapeutic polypeptides fused to human serum albumin.

The terms "albumin" and "serum albumin" encompass human albumin (HA) and variants thereof, the full mature form of which is disclosed herein (SEQ ID NO: 19), as well as albumin from other species and variants thereof. As used herein, "albumin" refers to an albumin polypeptide or amino acid sequence, or an albumin variant, having one or more functional activities (e.g. biological activities) of albumin. In certain embodiments, albumin is used to stabilize or prolong the therapeutic activity of a FXII inhibitor. The albumin may be derived from any vertebrate, especially any mammal, for example human, monkey, cow, sheep, or pig. Non-mammalian albumin can also be used and includes, but is not limited to, albumin from chicken and salmon. The albumin portion of the albumin-linked polypeptide may be from a different animal than the therapeutic polypeptide portion. See WO 2008/098720 for examples of albumin fusion proteins, incorporated herein by reference in its entirety.

In one embodiment, an albumin variant is at least 10, 20, 40, 50, 60, or at least 70 amino acids long (or any length in between) or may include 15, 20, 25, 30, 50 or more contiguous amino acids (or any number in between) from a human albumin (HA) sequence (e.g., SEQ ID NO: 19), or may include part or all of specific domains of HA. An albumin variant may include an amino acid substitution, deletion, or addition, either conservative or non-conservative substitution, wherein such changes do not substantially alter the active site, or active domain, which confers the therapeutic activities of the half-life enhancing polypeptides. These variants may share homology of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% (or any percentage in between).

In some embodiments, the albumin variant is a fragment and may comprise at least one whole domain of albumin and/or fragments of those domains, for example domains 1 (amino acids 1-194 of SEQ ID NO: 19), 2 (amino acids 195-387 of SEQ ID NO: 19), 3 (amino acids 388-585 of SEQ ID NO 19), 1 + 2 (1-387 of SEQ ID NO: 19), 2 + 3 (195-585 of SEQ ID NO: 19) or 1 + 3 (amino acids 1-194 + amino acids 388-585 of SEQ ID NO: 19). Each domain is itself made up of two homologous subdomains namely residues 1-105, 120-194, 195-291, 316-387, 388-491 and 512-585, of SEQ ID NO: 19, with flexible inter-subdomain linker regions comprising residues Lys106 to Glu119, Glu292 to Val315 and

Glu492 to Ala511. Thus, in some embodiments, the albumin variant comprises at least one whole subdomain of albumin.

In certain embodiments, other proteins that are structurally or evolutionarily related to albumin ("albumin family proteins") may be used as HLEPs, including, but not limited to alpha-fetoprotein (WO 2005/024044; *Beattie and Dugaiczyk*, 20 *Gene* 415-422, 1982), afamin (*Lichenstein et al.* 269 (27) *J. Biol. Chem.* 18149-18154, 1994), and vitamin D binding protein (*Cooke and David*, 76 *J. Clin. Invest.* 2420-2424, 1985). The genes encoding these proteins represent a multigene cluster with structural and functional similarities mapping to the same chromosomal region in humans, mice, and rats. The structural similarity of the albumin family members suggests that they can be used as HLEPs. For example, alpha-fetoprotein has been claimed to extend the half-life of an attached therapeutic polypeptide *in vivo* (WO 2005/024044). Thus, in some embodiments, these proteins, or variants thereof, that may be capable of stabilizing or prolonging therapeutic activity, can be used as HLEPs linked to FXII or FXIIa and may be derived from any vertebrate, especially any mammal, for example human, monkey, cow, sheep, or pig, or non-mammal including but not limited to, hen or salmon. In some embodiments, variants may comprise 10 or more amino acids in length, or may comprise about 15, 20, 25, 30, 50 or more contiguous amino acids of the respective protein sequence from which they are derived, or may include part or all of specific domains of the respective proteins. Albumin family member fusion proteins may include naturally occurring polymorphic variants.

In certain embodiments, mono- or poly- (e.g., 2-4) polyethylene glycol (PEG) moieties may be used as fusion partners and may extend *in vivo* half-lives. PEGylation may be carried out by any of the pegylation reactions available. Exemplary methods for preparing pegylated protein products can generally include (a) reacting a polypeptide with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby the protein becomes attached to one or more PEG groups; and (b) obtaining the reaction product(s). There are a number of PEG attachment methods. See, for example, EP 0 401 384; *Malik et al.*, *Exp. Hematol.*, 20:1028-1035 (1992); *Francis, Focus on Growth Factors*, 3(2):4-10 (1992); EP 0 154 316; EP 0 401 384; WO 92/16221; WO 95/34326; U.S. Pat. No. 5,252,714, incorporated herein by reference in their entirety.

In some embodiments, an immunoglobulin (Ig) may be used as an HLEP. The term “immunoglobulin” encompasses functional fragments and variants thereof, such as an Fc region or one or more Ig constant domains. In some embodiments, the Ig comprises an Fc region or portions of the immunoglobulin constant domain(s). The constant region may be 5 that of an IgM, IgG, IgD, IgA, or IgE immunoglobulin. In some embodiments, the therapeutic polypeptide portion is connected to the Ig via the hinge region of the antibody or a peptide linker, which may be cleavable. Several patents and patent applications describe the fusion of therapeutic proteins to immunoglobulin constant regions to extend the therapeutic protein’s half-life *in vivo* (US 2004/0087778, WO 2005/001025, WO 10 2005/063808, WO 2003/076567, WO 2005/000892, WO 2004/101740, US 6,403,077), incorporated herein by reference. Therefore, in some embodiments, immunoglobulin regions (e.g., Fc domains, Fc fragments of immunoglobulins, and variants thereof) are used as HLEPs. In some embodiments, inhibitors of FXII can be fused to Fc domains or portions of immunoglobulin constant regions as HLEPs. In some embodiments, these 15 fusion proteins are prepared as recombinant molecules expressed in prokaryotic or eukaryotic host cells, such as bacteria, yeast, plant, animal (including insect) or human cell lines or in transgenic animals (WO 2008/098720, incorporated herein by reference).

An example of a SPINK mutant-Fc fusion protein, the SPINK-K2-Fc fusion protein, is 20 described in WO 2008/098720.

E. Linkers

In various embodiments, an intervening peptidic linker may be introduced between a 25 therapeutic polypeptide and a HLEP. In one embodiment, a cleavable linker is introduced, particularly if the HLEP has the potential to interfere with the therapeutic polypeptide’s specific activity, e.g. by steric hindrance. In certain embodiments, the linker is cleavable by enzymes involved in coagulation, such as coagulation proteases of the intrinsic, extrinsic, or common coagulation pathway. Coagulation proteases of the intrinsic pathway include proteases in the contact activation pathway, e.g., FXIIa, FXIa, or FIXa. In one embodiment, the linker is cleaved by FXIIa. Proteases of the extrinsic pathway include proteases in the tissue factor pathway, for example, FVIIa. Proteases of the common pathway include proteases involved in the conversion of fibrinogen to fibrin, for example, FXa, FIIa, and FXIIIa.

III. Pharmaceutical Compositions

In any of the various aspects of the invention, the FXII inhibitor may have a purity of greater

5 than 80%, or greater than 95%, 96%, 97%, 98%, or 99%. In one embodiment, the FXII inhibitor may have a pharmaceutically pure state that is greater than 99.9% pure with respect to contaminating macromolecules, such as other proteins and nucleic acids, and may be free of infectious and pyrogenic agents.

10 In certain embodiments, a pharmaceutical composition can comprise at least one additive such as a filler, bulking agent, buffer, stabilizer, or excipient. Some exemplary pharmaceutical formulation techniques are described, e.g., in the 2005 Physicians' Desk Reference, Thomson Healthcare: Montvale, NJ, 2004; Remington: The Science and Practice of Pharmacy, 20th ed., Gennaro et al., Eds. Lippincott Williams & Wilkins:

15 Philadelphia, PA, 2000; Kibbe et al. Handbook of Pharmaceutical Excipients, 3rd ed., Pharmaceutical Press, 2000. Pharmaceutical additives include, e.g., mannitol, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. In certain embodiments, the pharmaceutical compositions may 20 also contain pH buffering reagents and wetting or emulsifying agents. In further embodiments, the compositions may contain preservatives and/or stabilizers. Pharmaceutical compositions may be formulated in lyophilized or stable soluble form. Polypeptides may be lyophilized by a variety of procedures known in the art.

25 In certain embodiments, a pharmaceutical composition comprising at least one FXII inhibitor is prepared for use in treating a neurotraumatic disorder selected from spinal cord injury and traumatic brain injury. For example, if a powder or lyophilized form of FXII inhibitor (e.g., by freeze drying) is provided and an aqueous pharmaceutical is desired, the powder can be dissolved by mixing with aqueous components of the pharmaceutical 30 formulation and stirred using suitable techniques such as vortexing or gentle agitation. Alternatively, if an aqueous pharmaceutical is desired and the FXII inhibitor is already in aqueous form, the components can be directly combined prior to administration. In certain embodiments, FXII inhibitor is provided in lyophilized form and combined with aqueous

pharmaceutical components (e.g., additional active components or inactive components such as fillers, stabilizers, solvents or carriers) prior to administration.

5 The formulation of pharmaceutical compositions may vary depending on the intended route of administrations and other parameters (see, e.g., Rowe et al., *Handbook of Pharmaceutical Excipients*, 4th ed., APhA Publications, 2003). In some embodiments, the pharmaceutical composition may be a lyophilized cake or powder. The lyophilized composition may be reconstituted for administration by intravenous injection, for example with Sterile Water for Injection, USP. In other embodiments, the composition may be a 10 sterile, non-pyrogenic solution. In still further embodiments, the composition is delivered in powder form, in a pill or tablet.

15 Formulations of the FXII inhibitor may be delivered to the patient by any pharmaceutically suitable means of administration. For example, the compositions can be administered systemically, such as parenterally, or intrathecally. Parenteral formulations may be administered intravenously or subcutaneously, either in bolus form or as an infusion, according to known procedures. Preferred liquid carriers, which are well known for parenteral use, include sterile water, saline, aqueous dextrose, sugar solutions, ethanol, 20 glycols, and oils. For systemic use, the therapeutic protein(s) may be formulated for an intravenous line or an arterial line. The formulations may be administered continuously by infusion or by bolus injection.

25 Tablets and capsules for oral or rectal administration may contain conventional excipients such as binding agents, fillers, lubricants, or wetting agents, etc. Oral or rectal liquid preparations may be in the form of aqueous or oily suspensions, solutions, emulsions, syrups, elixirs or the like, or may be presented as a dry product for reconstitution with water or other suitable vehicle prior to use. Such liquid preparations may contain conventional additives, such as suspending agents, emulsifying agents, non-aqueous vehicles, and preservatives. Some formulations encompass slow release systems, such as a patch.

30 Also disclosed are kits for the treatment of a neurotraumatic disorder selected from spinal cord injury and traumatic brain injury. In certain embodiments, the kits comprise (a) at least one FXII inhibitor; (b) instructions for use in the treatment of a neurotraumatic disorder selected from spinal cord injury and traumatic brain injury, and optionally (c) at least one

further therapeutically active ingredient wherein the further therapeutically active ingredient is not C1 inhibitor.

5 The components of the kit may be contained in one or different containers such as one or more vials. The at least one FXII inhibitor may be in liquid or solid form (e.g. after lyophilization). If in liquid form, the at least one FXII inhibitor may comprise additives such as stabilizers and/or preservatives such as proline, glycine, or sucrose or other additives.

10 In certain embodiments, the kit may contain additional compounds such as therapeutically active compounds or drugs that are to be administered before, at the same time, or after administration of the at least one FXII inhibitor, wherein the further therapeutically active ingredient is not C1 inhibitor. Examples of such compounds include vitamins, antibiotics, anti-viral agents, etc. In other embodiments, a steroid, in particular cortisone, can be included with the kit.

15 In various embodiments, instructions for use of the kit can include directions to use the kit components in the treatment of a neurotraumatic disorder selected from spinal cord injury and traumatic brain injury. The instructions may further contain information regarding how to prepare (e.g., dilute or reconstitute, in the case of freeze-dried protein) the at least one FXII inhibitor. The instructions may further include guidance regarding the dosage and/or frequency of administration.

The Figures show:

25 Figure 1 shows reduction of lesion size in rHA-Infestin-4 treated or FXII^{-/-} mice compared to NaCl-treated or wild-type (Bl/6) controls, respectively, 24h after trauma induction. TTC stainings of brain slices gained from male and female mice were analyzed for their lesion volume. FXII deficiency or inhibition reduces lesion sizes 24h after trauma induction. (A) 30 Lesion volumes in male wild-type and FXII^{-/-} mice (n=19), (B) in female mice (n=4 for Bl/6 and n=5 for FXII^{-/-}) and (C) in rHA-Infestin-4 treated animals and controls (n=14 for rHA-Infestin-4 and n=13 for controls). ***P<0.001, **P<0.01, unpaired, two-tailed Student's t-test, mean+SEM.

Figure 2 shows preservation of blood-brain-barrier (BBB) integrity 24h after trauma induction in FXII^{-/-} mice compared to wild-type controls (Bl/6). (A) Extent of Evans Blue extravasation in the lesioned hemisphere determined by photometry (n=6 for FXII^{-/-} and n=8 for Bl/6). (B) Brain water content as a measure of brain edema in the lesioned hemisphere (n=8 for FXII^{-/-} and n=9 for Bl/6). *P<0.05, unpaired, two-tailed Student's t-test, mean+SEM.

Figure 3 shows preservation of the blood-brain-barrier (BBB) 24h after trauma induction in rHA-Infestin-4 treated mice compared to NaCl-treated controls. BBB integrity was analyzed by the (A) extent of Evans Blue (EB) extravasation in the lesioned hemisphere determined by photometry (n=5) and (B) brain water content as a measure of brain edema in the lesioned hemisphere (n=8 for rHA-Infestin-4 and n=9 for controls). (C) Relative protein expression of tight junction protein Occludin (n=5) **P<0.01, *P<0.05, unpaired, two-tailed Student's t-test, mean+SEM.

Figure 4 shows the decrease in thrombus formation and dampened thrombotic processes 24h after trauma induction. (A) Relative protein expression of Fibrin/Fibrinogen was analyzed via Western blotting and is reduced in FXII^{-/-} mice compared to wild-type controls (Bl/6; n=3). (B) The ratio of occluded vessels to open vessels in the lesioned hemisphere was determined in H&E stained brain slices (n=5, 5 slices per animal). Representative stainings (lower panel) with occluded vessels (arrow) and open vessels (asterisk). An improved ratio was found in rHA-Infestin-4 treated mice compared to NaCl-treated controls. **P<0.01, *P<0.05, unpaired, two-tailed Student's t-test, mean+SEM.

Figure 5 shows protection from inflammatory processes in FXII^{-/-} mice 24h after trauma induction. (A) The quantification of macrophage infiltration into lesioned hemisphere was analyzed with immunohistochemical staining against CD11b+ macrophages/ microglia at Day 1 and compared to wild-type controls (Bl/6; n=4). Relative gene expression of proinflammatory cytokines TNF α (B) and Interleukin-1 β (C) was measured in FXII^{-/-} mice compared to wild-type controls (Bl/6) and sham-operated mice in the ipsilateral cortices (ipsi) after trauma induction (n=5). ***P<0.001, **P<0.01, * P<0.05, compared with Bl/6, #P<0.01, #P<0.05 compared with sham operated animals, unpaired, two-tailed Student's t-test (A), 1-way ANOVA followed by Bonferroni post-hoc test, mean+SEM.

Figure 6 shows proinflammatory cytokines in rHA-Infestin-4 treated compared to NaCl-treated and sham-operated mice. Inflammatory processes are dampened in rHA-Infestin-4 treated animals 24h after trauma induction. Relative gene expression for proinflammatory cytokines TNF α (A) and Interleukin-1 β (B) in the ipsilateral cortices (ipsi; n=3) are shown.

5 #P<0.05 compared with sham operated animals, 1-way ANOVA followed by Bonferroni post-hoc test, mean+SEM.

Figure 7 shows reduction in lesion size in FXII $^{-/-}$ mice compared to wild-type controls (Bl/6) 3d after trauma induction. FXII-deficient mice are protected from tissue damage at Day 3.

10 Lesion volumes were measured in TTC-stained brain slices in male (n=9 for FXII $^{-/-}$ and n=14 for Bl/6) and female mice (n=3). **P<0.01, unpaired, two-tailed Student's t-test, mean+SEM.

Figure 8 shows reduction of the blood-brain-barrier (BBB) damage 3d after trauma

15 induction in FXII $^{-/-}$ mice compared to wild-type controls (Bl/6). BBB integrity is preserved in FXII $^{-/-}$ mice at Day 3 after trauma induction. Extent of Evans Blue extravasation in the lesioned hemisphere was determined by photometry (n=4 for Bl/6 and n=5 for FXII $^{-/-}$). ***P<0,001, unpaired, two-tailed Student's t-test, mean+SEM.

20 Figure 9 shows intracerebral platelet accumulation and thrombosis are pathologic features of traumatic brain injury. (A) Representative immunohistological staining for the platelet marker glycoprotein Ib (GPIb) of human traumatized brain tissue shows marked intravascular platelet deposition (arrow; non-occluded vessel is indicated by an asterisk; scale bar represents 100 μ m). (B) Representative hematoxylin and eosin staining from a 25 mouse brain section of the lesioned hemisphere on day 7 after weight drop injury shows occluded vessels (arrows; scale bar represents 100 μ m). (C) The cerebral blood flow over the right parietal cortex (impact area) decreases significantly within 7 days. (E) Representative hematoxylin and eosin staining from a mouse brain section of the lesioned hemisphere on day 1 after cryolesion shows occluded vessels (arrow; scale bar represents 30 100 μ m).

Figure 10 shows intracerebral platelet accumulation on day 7 after weight drop injury is diminished in factor XII-deficient (FXII $^{-/-}$) mice. (A) Calculation of the thrombosis index from brain sections stained with hematoxylin and eosin shows that occluded vessels were

decreased in FXII^{-/-} mice when compared with wild-type (WT) mice and FXII^{-/-} mice reconstituted with human FXII (FXII^{-/-}/hFXII) (n=5 per group, ***P<0.001, **P<0.01). (B) Analysis of immunofluorescence stainings using glycoprotein Ib (GPIb) and CD31 antibodies reveals marked reduction in intravascular platelet depositions on day 7 after injury induction 5 in FXII^{-/-} mice when compared with WT mice and FXII^{-/-}/hFXII mice (n=4 per group, ***P<0.001). (C) Western Blot analysis using a GPIb antibody confirms that platelets accumulate to a smaller extent in FXII^{-/-} mice when compared to WT controls or sham-operated mice. Bands were quantified by densitometry in relation to β -actin control. Lower 10 panel shows two representative blots of each group (n=5 per group, ***P<0.001, **P<0.01; AU=arbitrary units).

Figure 11 shows intracerebral platelet accumulation on day 1 (d1) and day 3 (d3) after cryolesion is diminished in factor XII-deficient (FXII^{-/-}) mice. (A) Calculation of the thrombosis index from brain sections stained with hematoxylin and eosin shows that 15 occluded vessels are decreased in FXII^{-/-} mice when compared with wild-type (WT) mice and FXII^{-/-} mice reconstituted with human FXII (FXII^{-/-}/hFXII) (n=5 per group, ***P<0.001, **P<0.01). (B) Western Blot analysis using a glycoprotein Ib (GPIb) antibody confirms that platelets accumulate to a smaller extent in FXII^{-/-} mice when compared to WT controls or sham-operated mice (Sham). Bands were quantified by densitometry in relation to β -actin 20 control (n=5 per group, *P<0.05). (C) Analysis of immunofluorescence stainings using GPIb and CD31 antibodies reveals marked reduction in intravascular platelet depositions in FXII^{-/-} mice when compared with WT mice and FXII^{-/-}/hFXII mice.

Figure 12 shows Factor XII (FXII)-deficiency improves outcome after weight drop injury. 25 FXII-deficient (FXII^{-/-}) mice develop a significantly lower neurological severity score (NSS) than wild-type (WT) mice and FXII^{-/-} mice reconstituted with human FXII (FXII^{-/-}/hFXII) on day 3 and on day 7 after diffuse brain trauma. One hour (day 0) and 1 day after trauma, animals displayed similar neurological deficits (n=10-13 per group, *P<0.05).

Figure 13 shows Factor XII (FXII)-deficiency improves outcome after cryolesion. (A) Lesion 30 volumetry after 2,3,5-triphenyltetrazolium chloride (TTC) staining of brain sections from male wild-type (WT) mice, FXII-deficient (FXII^{-/-}) mice, FXII^{-/-} mice reconstituted with human FXII (FXII^{-/-} + hFXII), female wild-type (WT fem) mice and female FXII^{-/-} (FXII^{-/-} fem) mice was performed on day 1 (d1) and day 3 (d3) after focal brain trauma. Male and female FXII^{-/-}

$^{/\sim}$ mice show significantly reduced lesion volumes when compared to WT mice. The beneficial effect of FXII-deficiency can be reverted by application of human FXII (hFXII) (n=7 per group, **P<0.01, *P<0.05). (B) Serial coronal T2-weighted gradient echo MR images show hyperintense lesions on d1 and day 7 (d7) after trauma induction in WT mice and FXII $^{/\sim}$ mice. Hypointense areas indicating intracerebral hemorrhage are absent in both groups. Lower panel shows two representative brain slices per group and time-point. MRI-based lesion volumetry (upper panel) confirms the development of smaller lesions in FXII $^{/\sim}$ mice (n=8-9 per group, *P<0.05). (C) Neuronal apoptosis is diminished in FXII $^{/\sim}$ mice. Panel shows the number of TUNEL-positive neurons per brain slice in the injured hemisphere on d1 and on d3. The number of apoptotic neurons is significantly diminished in FXII $^{/\sim}$ mice when compared to WT controls (n=4 per group, ***P<0.001, *P<0.05, scale bar represents 50 μ m).

Figure 14 shows pharmacological inhibition of factor XII (FXII) with rHA-Infestin-4 diminishes intracerebral platelet accumulation and improves outcome after weight drop brain trauma. (A) Occluded vessels are more abundant in vehicle-treated mice and when compared to mice treated with rHA-Infestin-4. This finding is confirmed by calculating the thrombosis index from brain sections stained with hematoxylin and eosin on day 7 showing a highly significant decrease of occluded vessels in mice treated with rHA-Infestin-4 (n=5 per group, ***P<0.001). (B) Analysis of immunofluorescence stainings using glycoprotein Ib (GPIb) and CD31 antibodies reveals marked reduction in intravascular platelet depositions on day 7 after injury induction when mice were treated with rHA-Infestin-4 (n=4 per group, ***P<0.001). (C) Mice treated with rHA-Infestin-4 develop a significantly lower neurological severity score (NSS) than vehicle-treated (Vehicle) mice on day 3 and on day 7 after brain trauma. One hour (day 0) and 1 day after brain trauma, animals displayed similar neurological deficits (n=13 per group, *P<0.05).

Figure 15 shows pharmacological inhibition of factor XII with rHA-Infestin-4 diminishes intracerebral platelet accumulation and provides protection from focal brain trauma. (A) Occluded vessels are more abundant in vehicle-treated animals when compared to rHA-Infestin-4-treated mice as determined by calculating the thrombosis index on day 1 (d1) and on day 3 (d3) after injury induction showing a highly significant increase of occluded vessels in vehicle-treated animals (n=4 per group, ***P<0.001, **P<0.01, scale bar represents 100 μ m). (B) Analysis of immunofluorescence stainings using glycoprotein Ib

(GPIb) and CD31 antibodies reveals marked reduction in intravascular platelet aggregation on d1 and d3 after injury induction when mice were treated with rHA-Infestin-4 (n=4 per group, ***P<0.001). **(C)** Lesion volumetry after 2,3,5-triphenyltetrazolium chloride (TTC) staining of brain sections of vehicle-treated mice and mice treated with rHA-Infestin-4 was performed on d1 and d3 after focal brain trauma. Mice treated with rHA-Infestin-4 are substantially protected from brain trauma (n=7 per group, **P<0.01, *P<0.05). **(D)** The number of TUNEL-positive neurons per brain slice was assessed after immunolabeling for the neuronal marker NeuN and subjection to TUNEL assay to detect apoptosis. The number of apoptotic neurons is significantly diminished in rHA-Infestin-4-treated mice when compared to vehicle-treated controls on d1 and d3 (n=4 per group, **P<0.01, *P<0.05).

Figure 16 shows genetic deficiency and pharmacological inhibition of factor XII does not lead to hemorrhages after cryolesion. Upper panel shows representative brain slices stained with 2,3,5-triphenyltetrazolium chloride (TTC) of sham-operated mice, FXII-deficient mice, mice treated with rHA-Infestin-4 and mice deficient for FXI. Lower panel shows the amount of hemoglobin in the lesioned hemispheres of sham-operated mice (Sham), wild-type (WT) mice, FXII-deficient (FXII^{-/-}) mice, vehicle-treated (Vehicle) mice, mice treated with rHA-Infestin-4 and FXI-deficient (FXI^{-/-}) mice one day after trauma induction. Hemoglobin concentrations in the groups with FXII-inhibition remain at the level of sham-operated animals, FXI^{-/-} mice show highly significantly increased amounts of hemoglobin (n=4-5 per group, ns=not significant, ***P<0.001).

Figure 17 shows reduction of immune cell infiltration 24 hours and 3 days after injury induction. The amount of Ly.6B.2-positive neutrophils was determined immunohistochemically in FXII^{-/-}-mice and rHA-Infestin-4-treated mice in comparison with WT or NaCl-treated controls, respectively.

The examples illustrate the invention. The example is intended to illustrate and in no way limit the present disclosure. Other embodiments of the disclosed compositions and methods will be apparent to those skilled in the art from consideration of the specification and practice of the compositions and methods disclosed herein.

5

Example 1:**Methods:**

6-week old C57Bl/6 wild-type mice (Bl/6) and FXII-deficient mice (FXII^{-/-}) were subjected to 10 experimental focal TBI using a cortical cryogenic lesion model. For pharmacological inhibition of activated FXII, wild-type mice were treated with rHA-Infestin-4 (200mg/kg i.v.) 1h after trauma induction. Lesion size was determined by volumetry from brain slices stained with 2,3,5-triphenyltetrazolium chloride (TTC). To assess blood-brain-barrier (BBB) damage, intracerebral Evans Blue (EB) extravasation was measured by photometry and 15 the wet-to-dry weight ratio was calculated for measurement of brain water content (=edema). Western Blot (WB) analysis and immunohistochemical (IHC) stainings were performed to assess protein expression of tight junction proteins and fibrin/fibrinogen. The local inflammatory response after TBI was analyzed by PCR and histology.

20 **Results:**

24h after trauma induction, a significant reduction in lesion size could be observed in FXII^{-/-} mice as well as in rHA-Infestin-4-treated wild-type mice when compared with controls. Less thrombus formation within the brain vasculature as well as preserved BBB integrity could be identified as underlying mechanisms. Moreover, FXII inhibition dampened the local 25 inflammatory response after TBI. Furthermore, reduction in lesion size and preservation of BBB integrity could be observed in FXII^{-/-} mice 3 days after trauma induction when compared with controls.

Conclusion:

30 Blocking of FXII protects from TBI by reducing 'thrombo-inflammation'. Therefore, inhibition of FXII is a promising strategy to combat TBI and other neurological, preferably neurotraumatic disorders.

Example 2:**Materials and methods:****5 Animals**

A total of 124 (110 male and 14 female) C57Bl/6N (wild-type) mice, 55 (41 male and 14 female) FXII-deficient (FXII^{-/-}) mice (Pauer *et al.*, 2004, *Thromb Haemost* 92:503-508), and 5 male FXI-deficient (FXI^{-/-}) mice (Gailani *et al.*, 1997, *Blood Coagul Fibrinolysis* 8:134-144) were used in this study. Mice were housed in groups of five to nine with free access to food and water and a 12-hour light/12-hour dark cycle. In this study, all experiments were approved by institutional and regulatory authorities and were conducted in accordance with the EU Directive 2010/63/EU and the ARRIVE criteria (Kilkenny *et al.*, 2012, *Osteoarthritis Cartilage* 20:256-260).

15 Cortical cryolesion model

Cortical cryolesion was induced as described previously (Albert-Weissenberger *et al.*, 2014, *Frontiers in cellular neuroscience* 8:269). Briefly, 6 week old mice were anesthetized with intraperitoneal injections of ketamine (0.1 mg/g) and xylazine (0.005 mg/g). After restraining the mouse head in a stereotactic frame (TSE systems) surgery was performed on the right parietal cortex after exposing the skull through a scalp incision. A copper cylinder with a tip diameter of 2.5 mm was filled with liquid nitrogen (-196 °C) and placed on the right parietal cortex (coordinates from the bregma: 1.5 mm caudal, 1.5 mm lateral) for 90 s. Sham-operated animals underwent the same surgical procedure without cooling of the copper cylinder.

25

Weight drop model

Experimental closed head injury was performed as previously described (Albert-Weissenberger *et al.*, 2012, *J Cereb Blood Flow Metab* 32:1747-1756; Albert-Weissenberger *et al.*, 2012, *Exp Transl Stroke Med* 4:1). Briefly, after the induction of isoflurane anesthesia, spontaneously breathing 10 to 16 week old mice were placed in a stereotactic frame and the skull was exposed by a midline longitudinal scalp incision. After the identification of the impact area a weight with a silicone-covered blunt tip was dropped with a final impact of 0.01 J. Sham-operation included anesthesia and exposure of the skull but without weight drop injury. The neurobehavioral status of mice was assessed by the

neurological severity score (NSS), a composite score including tasks on motor function, alertness and physiological behavior with lower scores indicating less deficits. Mice were evaluated 1 hour, 1 day, 3 days, and 7 days after weight drop injury. Personnel who performed functional assays were blinded to the experimental groups.

5

Pharmacological treatment

One hour after induction of focal cryolesion or diffuse weight drop injury, wild-type mice received a single intravenous injection of the specific FXII-inhibitor rHA-Infestin-4 (CSL Behring, Marburg, Germany) at a dose of 200 mg/kg body weight. Control animals received equal volumes of 0.9 % sodium chloride (vehicle). Intravenous injection of 2 µg/g body weight human FXII (hFXII) (Sekisui Diagnostics, ADG412H) 1 h after injury induction and continually every 72 h resulted in reconstitution of FXII^{-/-} mice.

Determination of lesion size after cortical cryolesion

15 Twenty-four hours or 3 days after cryolesion, mice were sacrificed; the brains were quickly removed and cut in five 1 mm thick coronal sections using a mouse brain slice matrix (Harvard Apparatus). The slices were stained for 20 min at 37 °C with 2 % 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich) in PBS to visualize the lesion. Lesion volumes were calculated by volumetry (ImageJ software, National Institutes of Health, 20 USA) in a blinded fashion.

Magnetic resonance imaging

30 MRI was performed repeatedly 1 day and 7 days after cryolesion on a 3 Tesla unit (Vision; Siemens) under anesthesia with ketamine (0.1 mg/g) and xylazine (0.005 mg/g). The protocol included a coronal T2-weighted sequence (slice thickness 2 mm), and a blood-sensitive coronal T2-weighted gradient echo CISS sequence (Constructed Interference in Steady State; slice thickness 1 mm). Lesion volumes were calculated by planimetry of the hyperintense area on high-resolution CISS images. CISS images were additionally examined for possible intracerebral bleeding.

30

Laser Doppler flowmetry

Laser Doppler flowmetry (Moor Instruments) was used to monitor regional cerebral blood flow over the right parietal cortex (impact area). Cerebral blood flow was measured serially at baseline (before injury induction), 1 hour, 3 and 7 days after injury induction.

Histology and immunohistochemistry

Cryo-embedded mouse brains were cut into 10- μ m-thick or 15- μ m-thick slices (cortical cryolesion model and weight drop model, respectively) using a cryostat (Leica). For 5 assessment of the thrombosis index hematoxylin and eosin staining was performed according to standard procedures. Stainings were examined in a blinded fashion and the number of occluded (N_{occ}) and non-occluded (N_{open}) blood vessels within the lesioned hemispheres was counted in every tenth brain slice under a 20-fold magnification. The thrombosis index was calculated using the following equation: $(N_{\text{occ}}/(N_{\text{open}}+N_{\text{occ}})) \times 100$. To 10 assess platelet aggregates within the vessels in the human brain, paraffin-embedded sections were stained against glycoprotein Ib (GPIb; abcam, ab102647) according the manufacturer's protocol and then counter-stained with hematoxylin to visualize all nuclei. For immunofluorescence stainings, the following primary antibodies were applied: anti- 15 GPIb (1:100, kind gift from Prof. Nieswandt, Rudolf-Virchow-Zentrum, Würzburg), anti-CD31 (Bio-Rad Laboratories, MCA2388GA, 1:100), anti-NeuN (Millipore, MAB377, 1:1000). As secondary antibodies, Cy2 anti-rat (Dianova, 122-225-167, 1:100), Cy3 anti-rat 20 (Dianova, 712-165-150, 1:100) and DyLight 488 anti-mouse (abcam, ab96871, 1:100) were used. Neuronal apoptosis was assessed using a TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) *in situ* Cell Death Detection Kit (Roche, Basel, 25 Switzerland) according to the manufacturer's instructions. Numbers of apoptotic neurons were determined from three fields at a 40-fold magnification from the lesioned hemisphere of two brain slices under a Nikon microscope Eclipse 50i equipped with the DS-U3 DS camera control unit and the NIS-Elements software (Nikon, Düsseldorf, Germany). To assess platelet aggregates two brain slices per animal were quantified. For quantitative analysis we used sections from near-identical brain regions for better comparison between 25 groups.

Western Blot analysis

Immunoreactivity for GPIb (anti-GPIb, 1:500, kind gift from Prof. Nieswandt, Rudolf- 30 Virchow-Zentrum, Würzburg) in lesioned cortices was detected by Western Blot analysis as previously described (Langhauser *et al.*, 2012, *Blood*, 120(19):4082-92). Densitometric analysis of GPIb was performed in a blinded way using the ImageJ software (National Institutes of health, USA) with β -Actin (Dianova, A5441, 1:500000) as loading control to normalize the levels of GPIb detected.

Quantification of intracerebral hemorrhage

Hemoglobin concentration in brain parenchyma that correlates to the extent of hemorrhage was determined spectrophotometrically (Choudhri *et al.*, 1997, *Stroke* 28:2296-2302).

5 Twenty-four hours after trauma animals were sacrificed and the brains were removed. The lesioned hemispheres were sonified for 60 s in 1.5 ml ice-cooled water and afterwards centrifuged at 4 °C for 30 min. One ml of Drabkin's solution was added to 250 µl of the supernatant and incubated at room temperature for 15 min. The absorbance was measured at 540 nm (MultiskanEX, Thermo Scientific, Waltham, MA).

10

Experimental design

Numbers of animals necessary to detect a standardized effect size on lesion volumes ≥ 0.2 on day 1 after cortical cryolesion or NSS ≥ 0.2 on day 1 after weight drop injury, respectively, were determined via a priori sample size calculation with the following

15 assumptions: $\alpha = 0.05$, $\beta = 0.2$, mean, and standard deviation (G*Power 3.0.10). Mice have been randomly assigned to treatment groups (block randomization after cryolesion and to achieve balanced groups stratified randomization after weight drop injury). To avoid bias, experiments have been performed and analyzed in a blinded fashion.

20 **Statistics**

All results were expressed as mean \pm SEM except for the NSS scales which are depicted as scatter plots including median with the 25 % percentile and the 75 % percentile given in brackets in the text. For statistical analysis PrismGraph 5.0 software package (GraphPad Software) was used. Data were tested for Gaussian distribution with the Kolmogorov

25 Smirnov test and in case of measuring the effects of two factors simultaneously analyzed by two-way ANOVA with post hoc Bonferroni correction for multivariate analyses or in case of non-parametric data (NSS) Kruskal Wallis test with post hoc Dunns correction. In case of measuring the effect of one factor, one-way ANOVA with post hoc Bonferroni correction was applied. If only two groups were compared, unpaired, two-tailed Student's t test was 30 performed. P values <0.05 were considered statistically significant.

Results:**Microvascular thrombosis is a common pathological feature in traumatic brain injury**

Firstly, we analyzed a human brain sample obtained after a fatal case of TBI, showing that

5 platelets accumulate in the microvessels of the traumatized brain (Figure 9 A). Consequently, we closely mimicked human TBI in mice using a weight drop model resulting in a predominantly diffuse brain trauma. Hematoxylin and eosin stainings of injured brain tissue from wild-type mice showed numerous occlusions of vessel lumina (Figure 9 B). Accordingly, we found intravascular accumulation of platelets. Interestingly, the cerebral
10 blood flow at the impact area slightly decreased over time with a significantly reduced cerebral blood flow on day 7 after weight drop injury (Figure 9 C). When focal brain trauma was induced by cortical cryolesion numerous occluded vessels and intravascular accumulations of GPIb-positive platelets were found in cortical brain tissue on day 1 and 3 after injury induction (Figures 1 D). These results strongly support the hypothesis that
15 microvascular thrombosis is a common pathological feature in TBI.

Factor XII contributes to microvascular thrombosis in traumatic brain injury

To assess the impact of FXII on intracerebral thrombus formation after TBI, we first

20 analyzed contusioned brain tissue of FXII-deficient mice in comparison with wild-type mice or FXII-deficient mice that were reconstituted with intravenous injections of hFXII (FXII^{-/-}/hFXII). On day 7 after weight drop injury, histological analysis of hematoxylin and eosin-stained brain sections demonstrated fewer occluded cerebral microvessels in FXII^{-/-} mice when compared with wild-type or FXII^{-/-}/hFXII mice (Figure 10 A). We consistently detected less intravascular GPIb-positive platelet accumulations in brains of FXII-deficient mice
25 (Figure 10 B). Furthermore, Western Blot analyses confirmed that the amount of platelets was significantly diminished in brain tissue of FXII-deficient mice (Figure 10 C). Similar to weight drop injury, fewer thrombus-occluded brain vessels (Figure 11 A), less platelet accumulations in the brain vasculature (Figure 11 C), and a decreased amount of platelets in the brain tissue (Figure 11 B) was detected one and three days after focal cryolesion in
30 FXII^{-/-} mice when compared with wild-type mice.

In summary, we observed that the injury-induced microvascular thrombosis and brain damage could be reproduced in FXII-deficient mice that were reconstituted by the administration of hFXII. This proves for the first time that activation of the intrinsic

coagulation pathway by FXII plays a role in posttraumatic cerebral thrombus formation. Consequently, we conclude that FXII contributes to microvascular thrombosis independently of the nature of TBI.

5 **Factor XII deficiency results in a better outcome after traumatic brain injury**

To evaluate the pathological significance of reduced intracerebral thrombosis in FXII-deficient mice, we next determined the impact of FXII-deficiency on functional outcome after weight drop injury. Trauma severity at early stages (1 h and 1 day after injury induction) was comparable in all groups, 3 days after weight drop injury FXII^{-/-} mice had 10 recovered significantly better than wild-type or FXII^{-/-}/hFXII mice (median NSS [25th percentile, 75th percentile]: 4.0 [3.0, 4.0] in wild-type mice and 3.5 [3.0, 4.0] in FXII^{-/-}/hFXII mice vs 2.0 [1.0, 3.0] in FXII^{-/-} mice, P<0.05, respectively; Figure 12). Importantly, the better 15 neurological outcome in FXII^{-/-} mice was persistent until day 7 (median NSS [25th percentile, 75th percentile]: 3.0 [2.0, 3.0] in wild-type mice and 2.5 [2.0, 3.0] in FXII^{-/-}/hFXII mice vs 1.0 [1.0, 2.5.0] in FXII^{-/-} mice, P<0.05, respectively; Figure 12).

We next evaluated the impact of FXII-deficiency on cortical lesion volume and neurodegeneration on day 1 and 3 after cryolesion. In male mice, FXII-deficiency resulted 20 in significantly reduced lesion volumes on day 1 and 3 as assessed by TTC-staining of brain sections (Figure 13 A). As gender has a significant impact on the clinical outcome following TBI (Wright et al., 2014), we subjected female mice to cryolesion. FXII-deficiency in female mice also resulted in significantly smaller brain lesions on day 1 and 3 when compared with wild-type mice (Figure 13 A). These observations were corroborated by studies using brain MRI showing that FXII-deficiency resulted in sustained reduction in 25 lesion size after focal brain injury (Figure 13 B). Reduction in lesion volume was accompanied with significantly diminished neuronal apoptosis in mice deficient for FXII on day 1 after cryolesion when compared with control mice (Figure 13 C). An even more pronounced difference in the amount of apoptotic cells was observed on day 3 after cryolesion (Figure 13 C).

30

Pharmacological inhibition of Factor XII results in reduced microvascular thrombosis and a better outcome after traumatic brain injury

To test the efficacy of pharmacological FXII inhibition for treatment of pathological thrombosis in TBI, we administered the selective inhibitor of activated FXII, rHA-Infestin-4,

200 mg/kg body weight intravenously and monitored microvascular thrombosis, functional outcome and lesion volumes in mice subjected to weight drop injury or cryolesion. Detailed analysis of hematoxylin and eosin-stained brain sections and immunohistochemistry visualizing platelets and endothelium showed that fewer thrombi occluded cerebral 5 microvessels in rHA-Infestin-4-treated mice when compared with vehicle-treated mice in both TBI models (Figures 14 A, 14 B, 15 A, and 15 B).

The diminished thrombus formation in rHA-Infestin-4-treated mice was associated with better neurological outcome. While after weight drop injury the initial severity of 10 neurological deficits (1 h and day 1) was comparable between the treatment groups ($P>0.05$), 3 and 7 days after trauma rHA-Infestin-4-treated mice had recovered significantly better than vehicle-treated mice (day 3: median NSS [25th percentile, 75th percentile]: 2.0 [2.0, 3.0] in rHA-Infestin-4-treated mice vs 3.0 [3.0, 3.0] in vehicle-treated mice, $P<0.05$; day 7: median NSS [25th percentile, 75th percentile]: 2.0 [1.5, 2.5] in rHA-Infestin-4-treated 15 mice vs 3.0 [2.0, 5.0] in vehicle-treated mice, $P<0.05$; Figure 14 C). After cryolesion, lesion volume and neurodegeneration on day 1 and 3 was reduced in rHA-Infestin-4-treated mice when compared with vehicle-treated mice. For both readouts the protective effect of rHA-Infestin-4 was even more pronounced on day 3 than on day 1 after cryolesion (Figures 7 C and 7 D).

20 Similar to FXII-deficiency, acute treatment of mice with rHA-Infestin-4 prevents from pathological thrombus formation in the cerebral microcirculation in both TBI models. The decrease in thrombus formation is associated with a better neurological outcome preserved at later stages after brain trauma (day 3, day 7), less brain damage, and less 25 neurodegeneration after weight drop injury and cryolesion injury, respectively. As a single administration of rHA-Infestin-4 after brain trauma seems sufficient for protection against injury deterioration. In summary, pharmacological inhibition of FXII results in reduced microvascular thrombosis and a better outcome after experimental TBI.

30 **FXII deficiency or inhibition does not increase the risk of intracerebral bleedings**

To proof the safety of FXII inhibition after brain trauma with regard to abnormal cerebral bleedings, we determined the extent of hemorrhage in the lesioned brain hemispheres of FXII^{-/-} mice, rHA-Infestin-treated mice, and their respective controls. After cryolesion, neither FXII-deficient mice nor mice treated with rHA-Infestin-4, showed increased levels of

hemorrhages (Figure 16). To validate this result, we also determined the extent of hemorrhage in FXI^{-/-} mice as FXI-deficiency is associated with increased post-traumatic bleedings in humans (Rosenthal *et al.*, 1955, *Blood* 10:120-131). In these mice we observed increased hemorrhages in the lesioned brain hemispheres that can also be seen 5 macroscopically (Figure 16). Moreover, MRI scans of FXII-deficient mice and wild-type controls showed no signs of bleeding either.

CSL BEHRING GMBH**5 Claims**

1. A direct inhibitor of Factor XII (FXII) for the use of treating a neurotraumatic disorder resulting from a traumatic injury of the brain (traumatic brain injury) or the spinal cord (spinal cord injury) wherein the FXII inhibitor is not C1 esterase inhibitor.

10

2. A FXII inhibitor for use according to claim 1, wherein the FXII inhibitor comprises

(i) the wild type Infestin-4 polypeptide sequence (SEQ ID NO: 1), or a polypeptide sequence comprising:

15

(a) SEQ ID NO: 1 modified to contain 1-5 amino acid mutations outside of N-terminal amino acid positions 2-13 of SEQ ID NO: 1;

and/or

20

(b) a homology of at least 70%, 80%, 85%, 90%, 95%, 98%, or 99% to SEQ ID NO: 1 and retaining six conserved cysteine residues from SEQ ID NO: 1;

25

(ii) a wild-type SPINK-1 polypeptide sequence (SEQ ID NO: 2), or a polypeptide sequence comprising:

30

(a) SEQ ID NO: 2 mutated to replace N-terminal amino acid positions 2-13 with the N-terminal amino acids 2-13 of SEQ ID NO: 1; and optionally further modified to contain 1-5 additional amino acid mutations that increase the homology of the polypeptide sequence to the sequence of SEQ ID NO: 1;

and/or

35

(b) a homology of at least 70%, 80%, 85%, 90%, 95%, 98%, or 99% to SEQ ID NO: 2 and retaining six conserved cysteine residues from SEQ ID NO: 2;

5 (iii) one of SPINK-1 mutants K1, K2, or K3 (SEQ ID NOS: 3, 4, or 5);
and/or
(iv) an anti-FXII antibody.

10 3. A FXII inhibitor for use according to claim 2, wherein the anti-FXII antibody comprises
(i) (a) a VH region comprising heavy chain CDR1 as set forth in SEQ ID NO: 8, heavy chain CDR2 as set forth in SEQ ID NO: 10, and heavy chain CDR3 as set
15 forth in SEQ ID NO: 12;
and/or
(b) a VL region comprising light chain CDR1 as set forth in SEQ ID NO: 13, light chain CDR2 as set forth in SEQ ID NO: 14, and light chain CDR3 as set forth in SEQ ID NO: 16.;

20 or
25 (ii) (a) a VH region comprising heavy chain CDR1 as set forth in SEQ ID NO: 8, heavy chain CDR2 as set forth in SEQ ID NO: 9, and heavy chain CDR3 as set forth in SEQ ID NO: 11;
and/or
30 (b) a VL region comprising light chain CDR1 as set forth in SEQ ID NO: 13, light chain CDR2 as set forth in SEQ ID NO: 14, and light chain CDR3 as set forth in SEQ ID NO: 15;

35 or

(iii) a VH region comprising SEQ ID NO: 6 and a VL region comprising SEQ ID NO: 7.

5 4. A FXII inhibitor according to any one of claims 2-3, wherein the anti-FXII antibody is an IgG antibody.

5. A FXII inhibitor for use according to any one of claims 1-4, wherein the at least one FXII inhibitor is linked to a fusion partner comprising PEG or a half-life enhancing 10 polypeptide selected from albumin, afamin, alpha-fetoprotein, vitamin D binding protein, human albumin, an immunoglobulin, and an Fc of an IgG.

15 6. A FXII inhibitor for use according to claim 5, wherein the half-life enhancing polypeptide is linked to the FXII inhibitor via a linker.

7. A FXII inhibitor for use according to any one of claims 5-6, wherein the FXII inhibitor is a fusion protein comprising human albumin joined to a FXII inhibitor via a linker peptide.

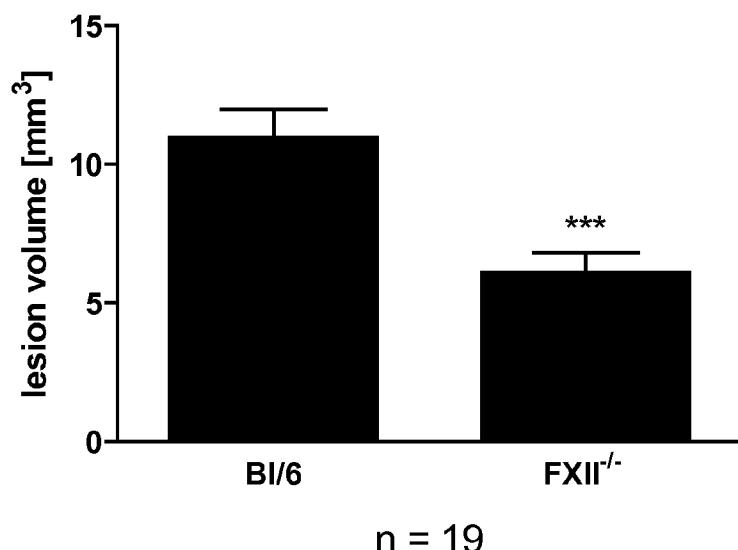
20 8. A FXII inhibitor for use according to any one of the claims 1-7, wherein the FXII inhibitor is administered intravenously or subcutaneously or intrathecal.

9. A FXII inhibitor for use according to any one of the claims 1-8, wherein the FXII inhibitor is administered (i) in a single dose as an injection or an infusion, (ii) in 25 multiple doses, each as an injection or an infusion, or (iii) as a continuous infusion or application.

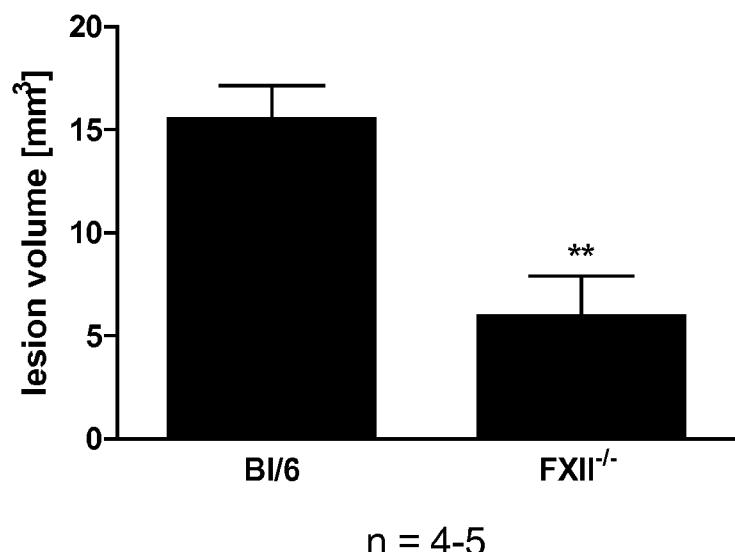
10. A FXII inhibitor for use according to any one of the claims 1-9, wherein the FXII inhibitor is administered at a concentration ranging from about 0.01 to about 1000 30 mg/kg body weight, or preferentially from about 1 to about 500 mg/kg.

11. A FXII inhibitor for use according to any one of claims 1-10, wherein the FXII inhibitor is administered after the traumatic injury.

12. A FXII inhibitor for use according to claim 11, wherein the first administration of the FXII inhibitor takes place immediately after the traumatic injury, or up to 24 hours, preferably up to 12 hours, more preferably up to 6 hours after the traumatic injury.
- 5 13. A FXII inhibitor for use according to any one of claims 1-12, wherein the FXII inhibitor is administered at least once, at least twice, at least three times, at least four times, or at least five times.
- 10 14. A kit for use in the treatment of a neurotraumatic disorder selected from a spinal cord injury and a traumatic brain injury, comprising:
 - (a) at least one direct FXII inhibitor;
 - (b) instructions for using the kit in the treatment of a neurotraumatic disorder resulting from a traumatic injury of the brain (traumatic brain injury) or the spinal cord (spinal cord injury); and
 - (c) optionally, at least one further therapeutically active compound or drug; wherein neither the FXII inhibitor nor the further therapeutically active compound or drug is C1 esterase inhibitor.
- 15 20 15. The kit according to claim 14, wherein the further therapeutically active compound or drug is a steroid, in particular cortisone.

Figure 1**A****male**

n = 19

B**female**

n = 4-5

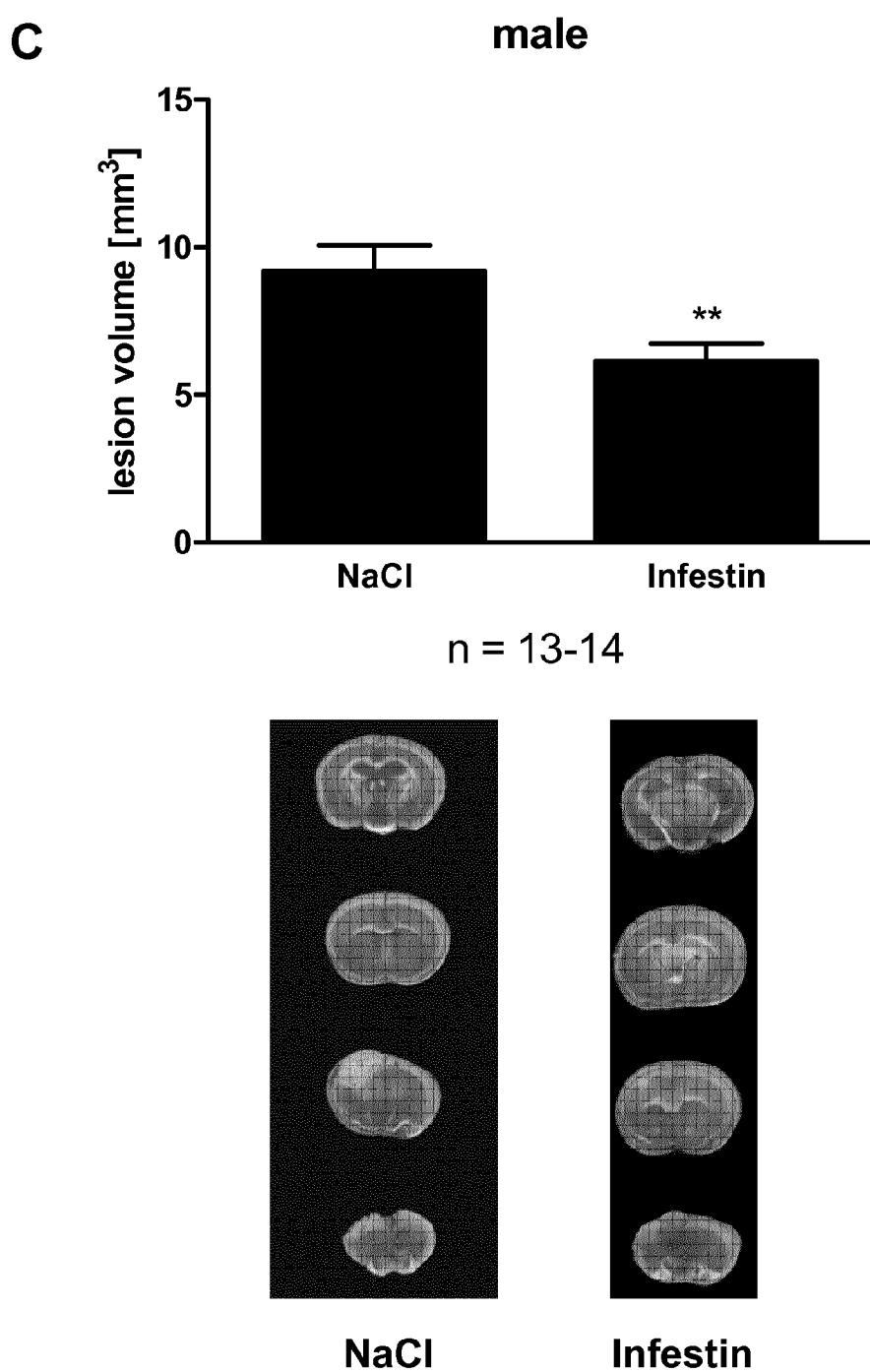
Figure 1

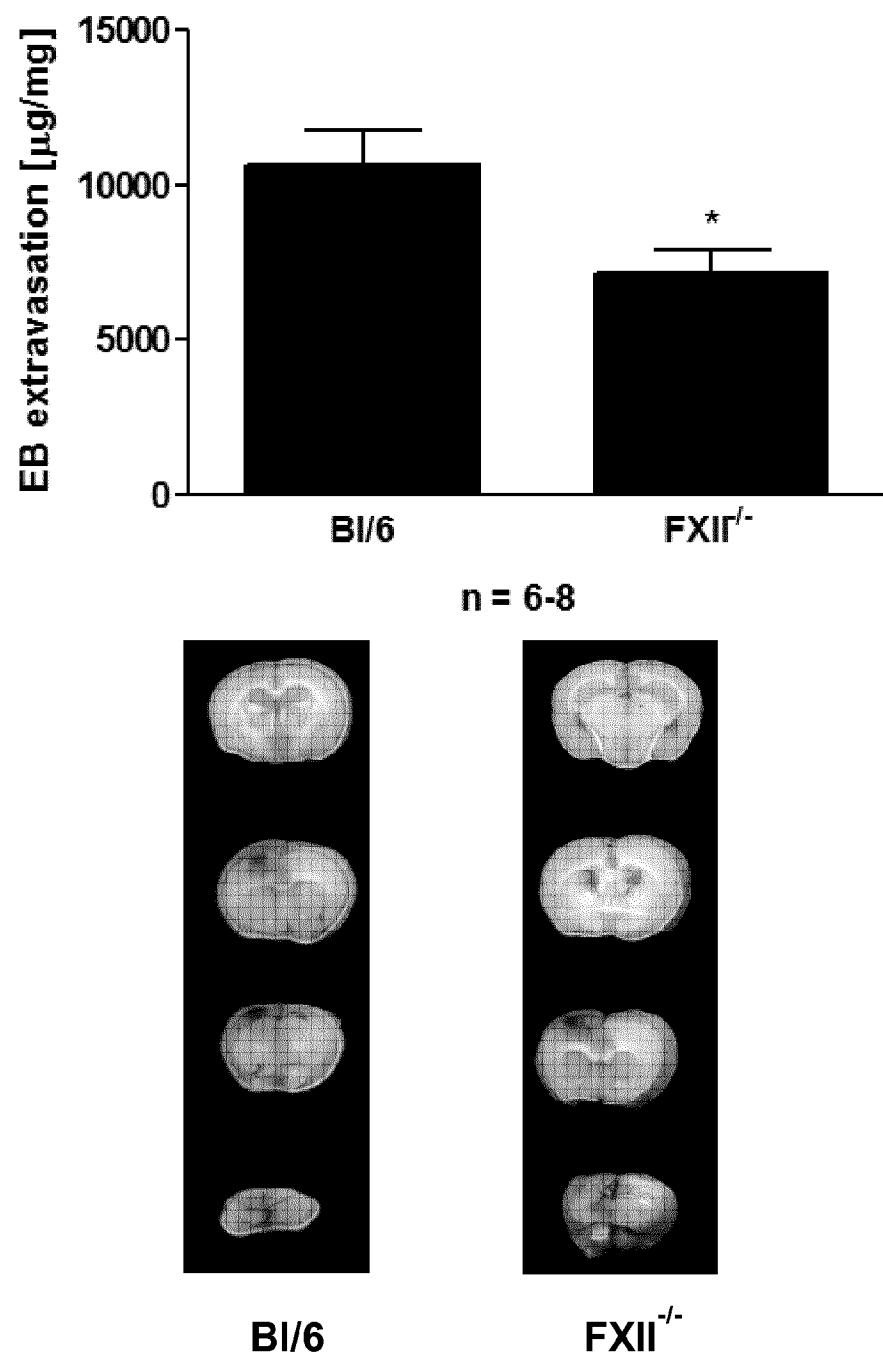
Figure 2**A**

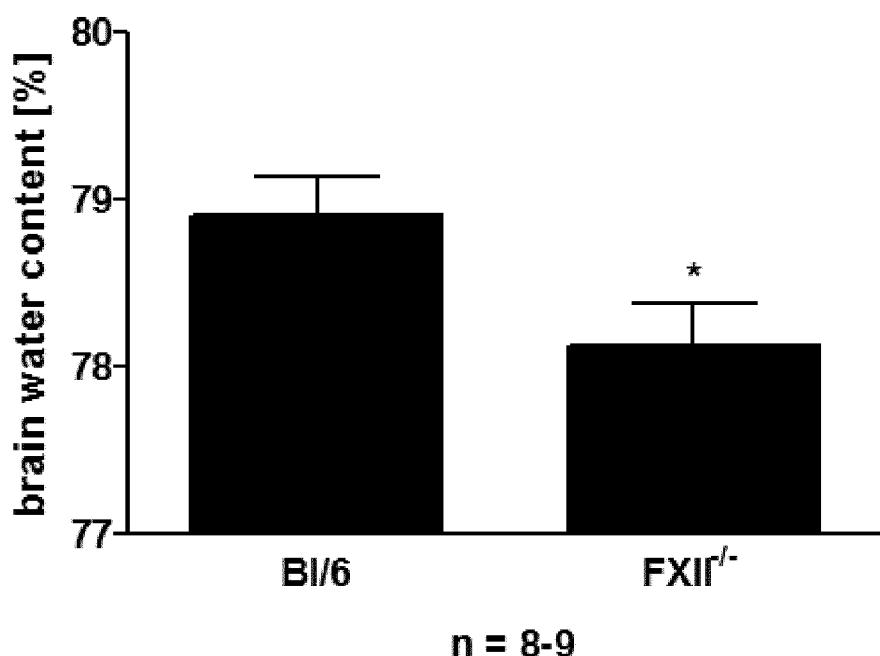
Figure 2**B**

Figure 3

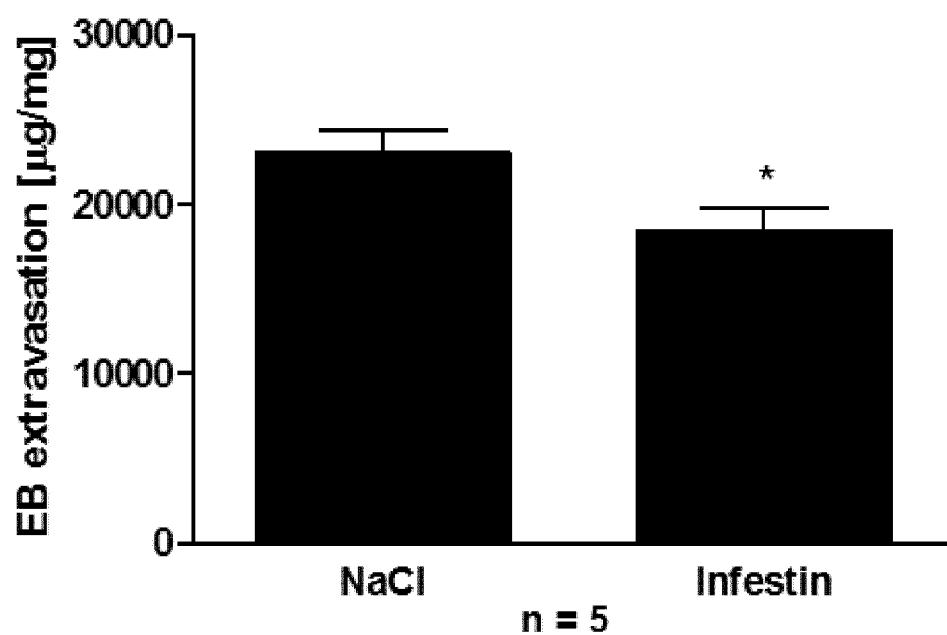
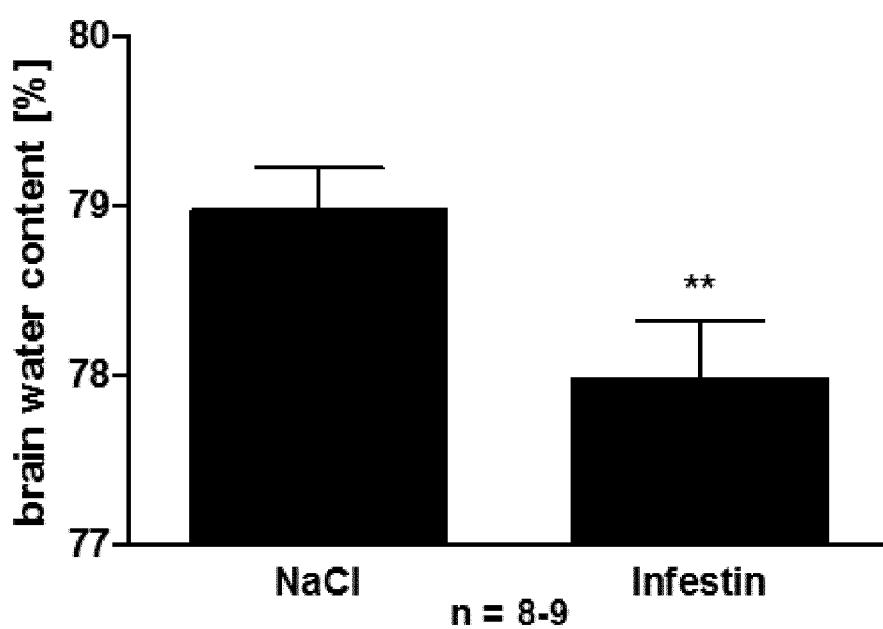
A**B**

Figure 3

C

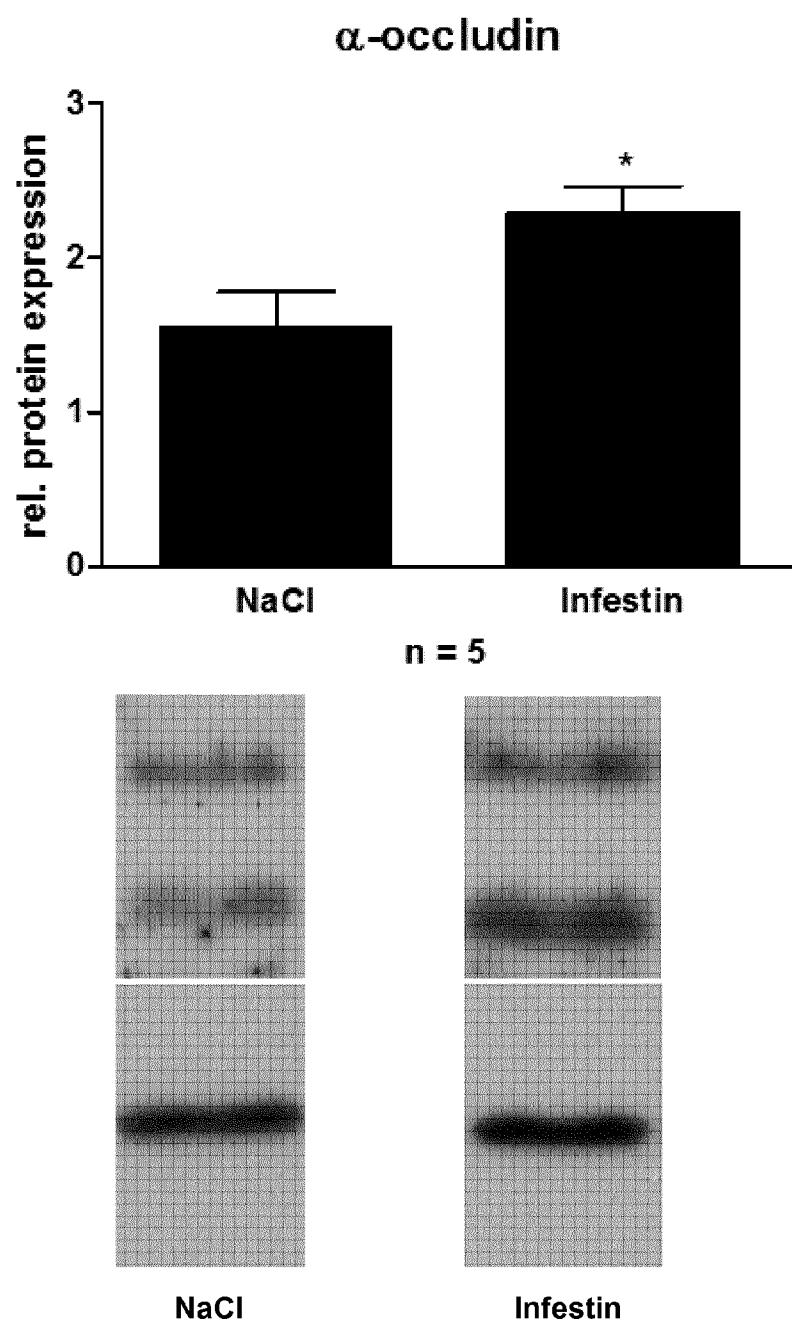


Figure 4

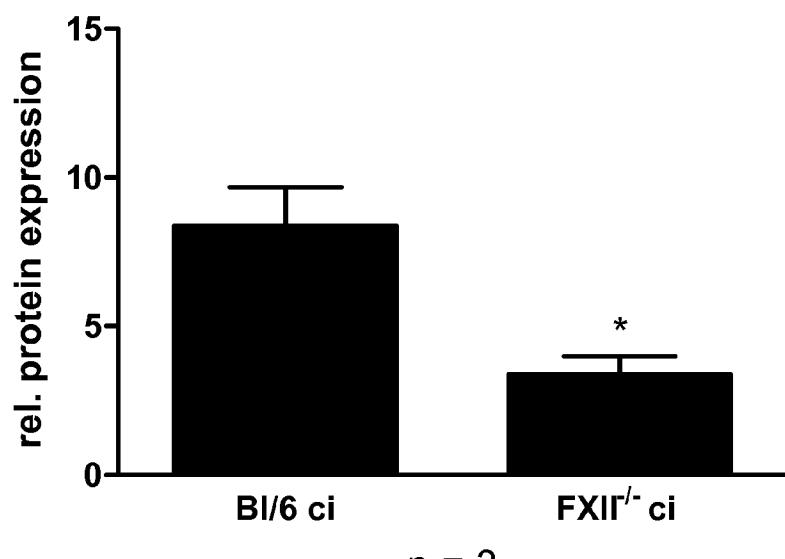
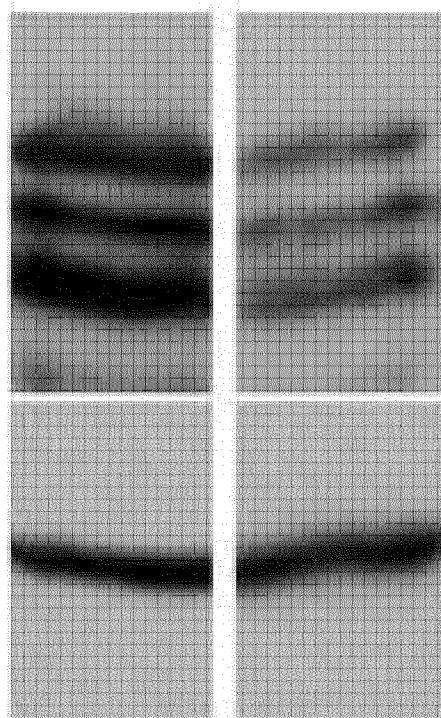
A α -fibrin(ogen) $n = 3$ BI/6 $\text{FXII}^{-/-}$

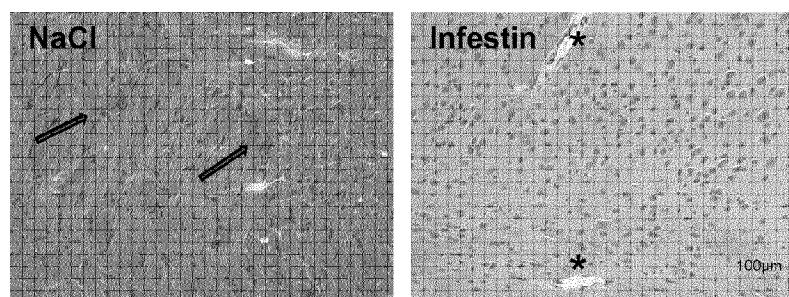
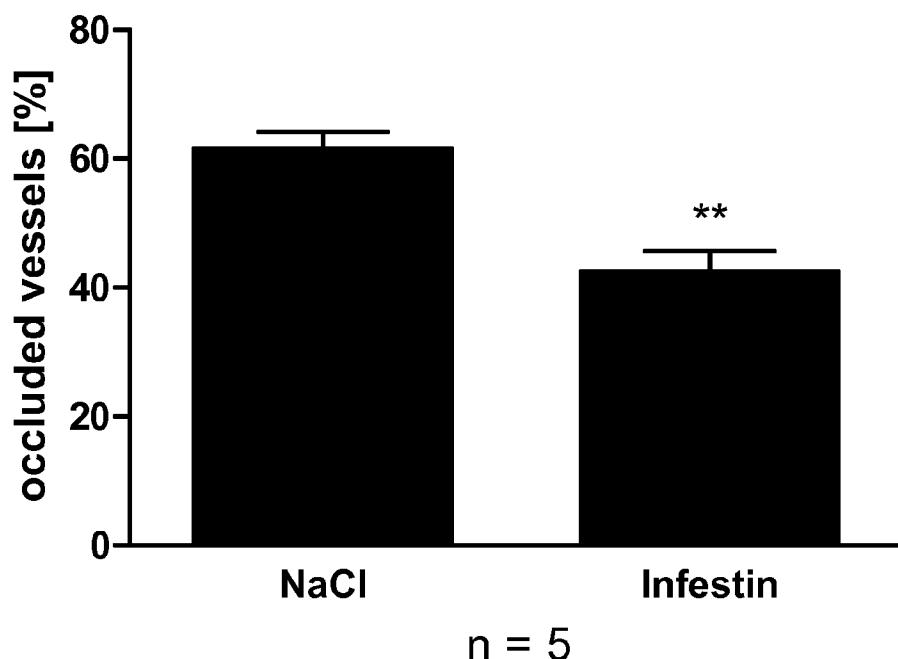
Figure 4**B**

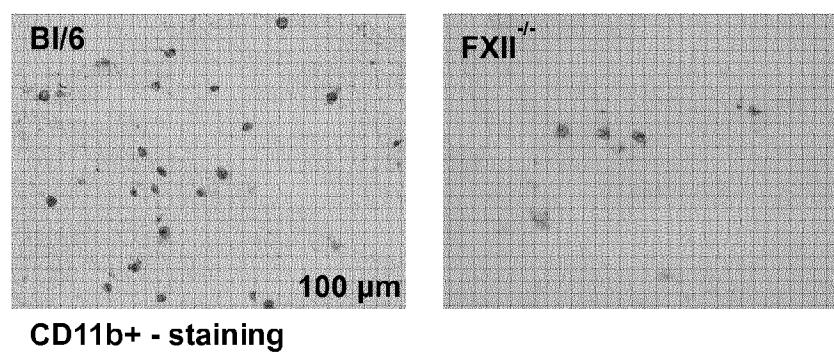
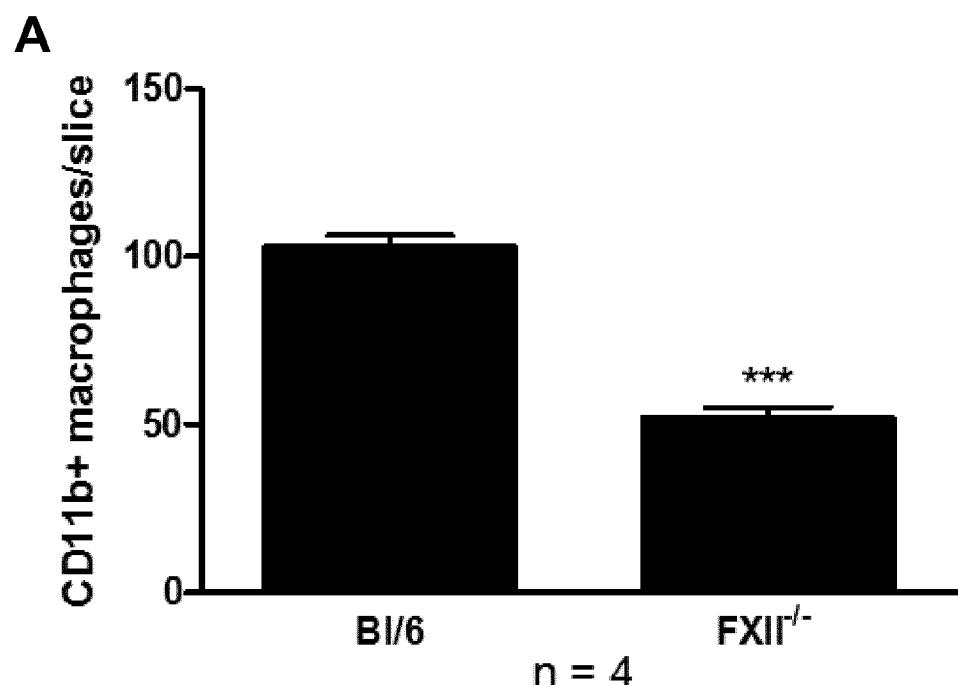
Figure 5

Figure 5

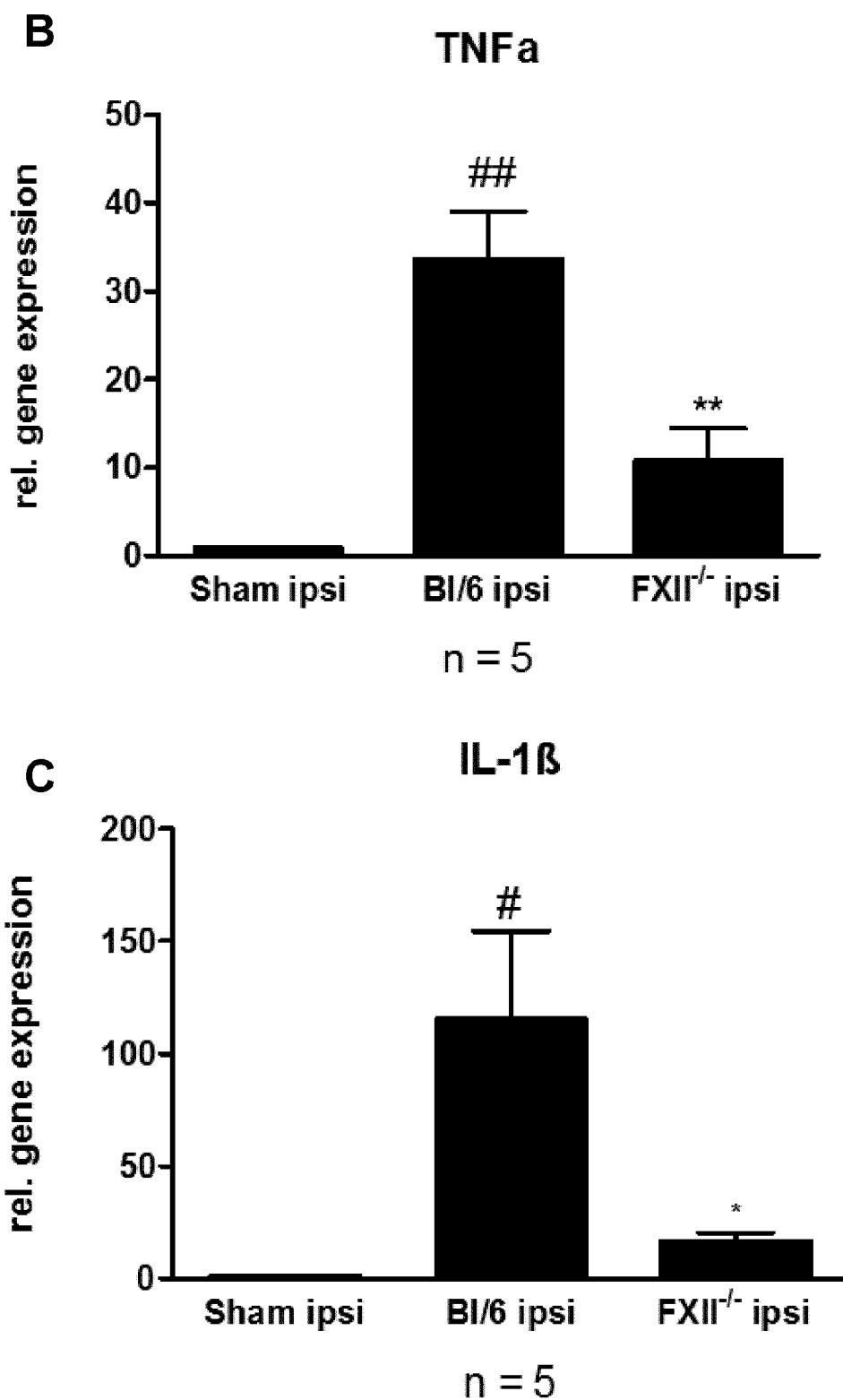


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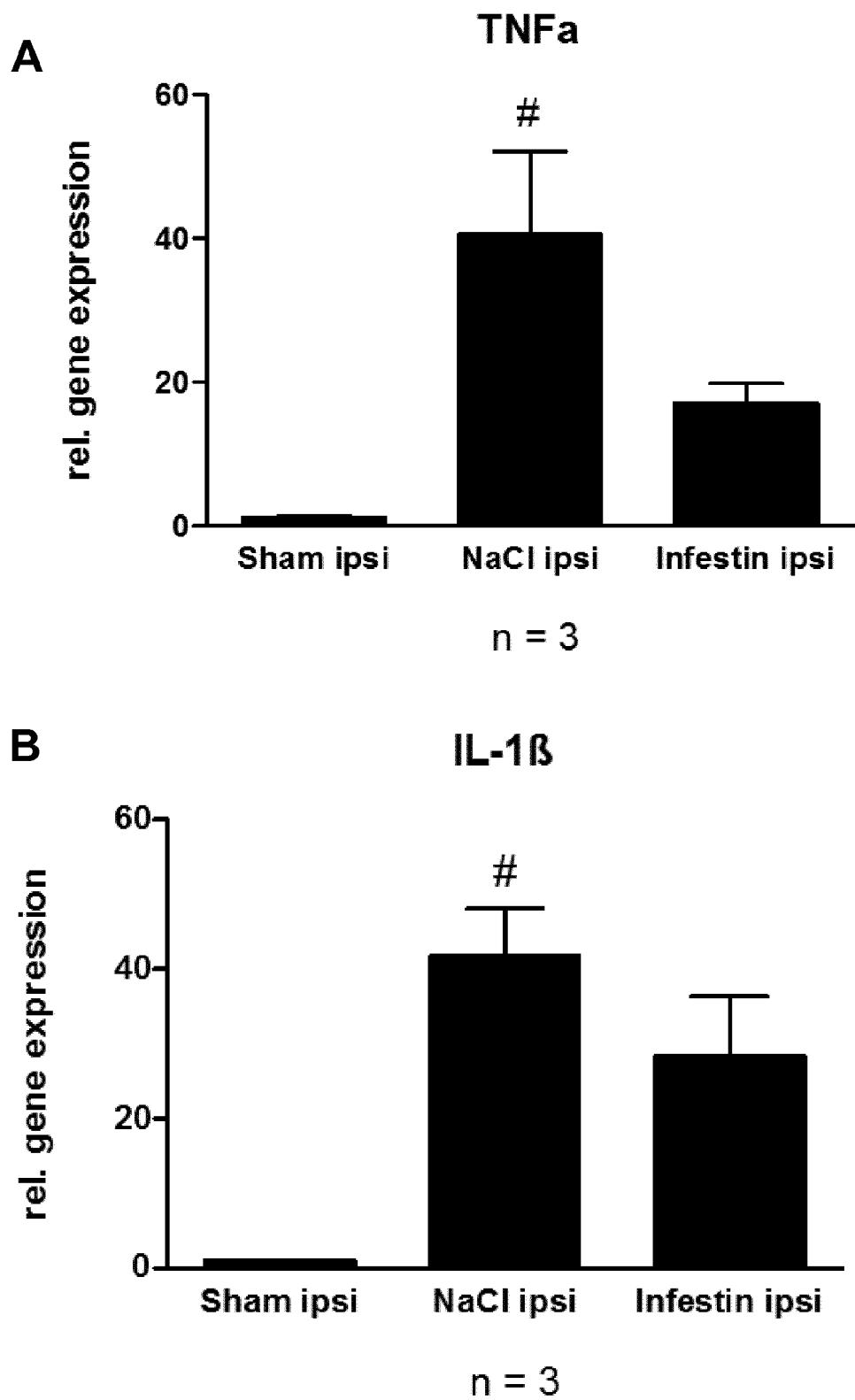


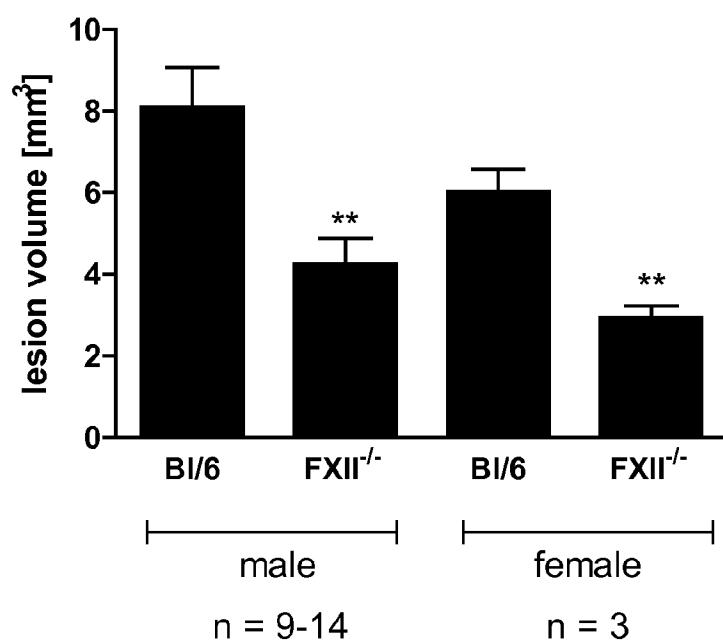
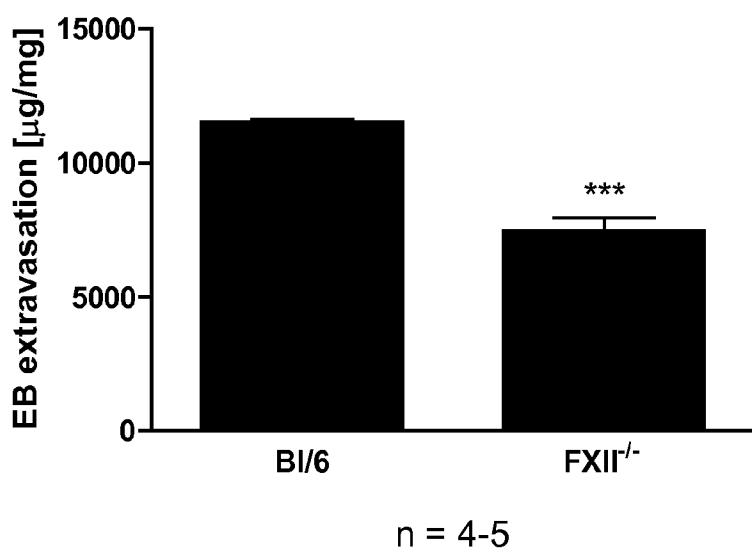
Figure 7**Figure 8**

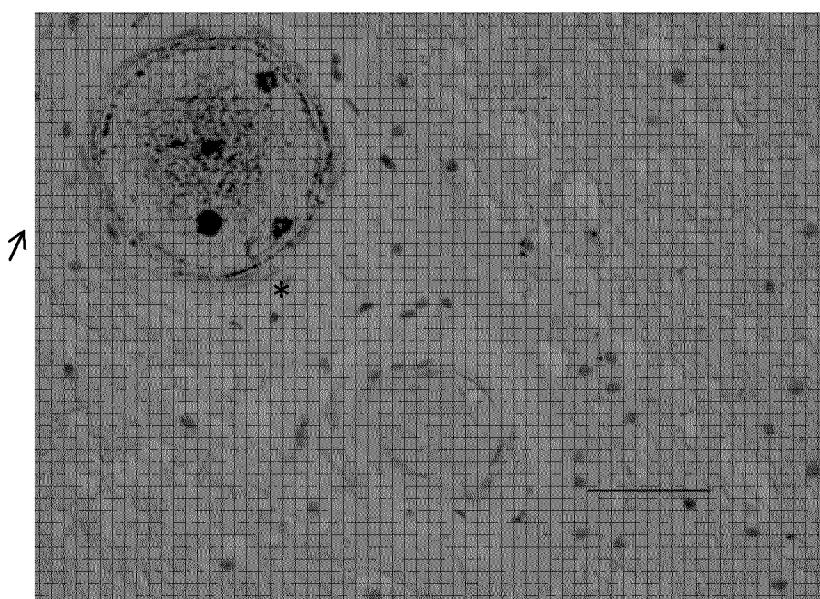
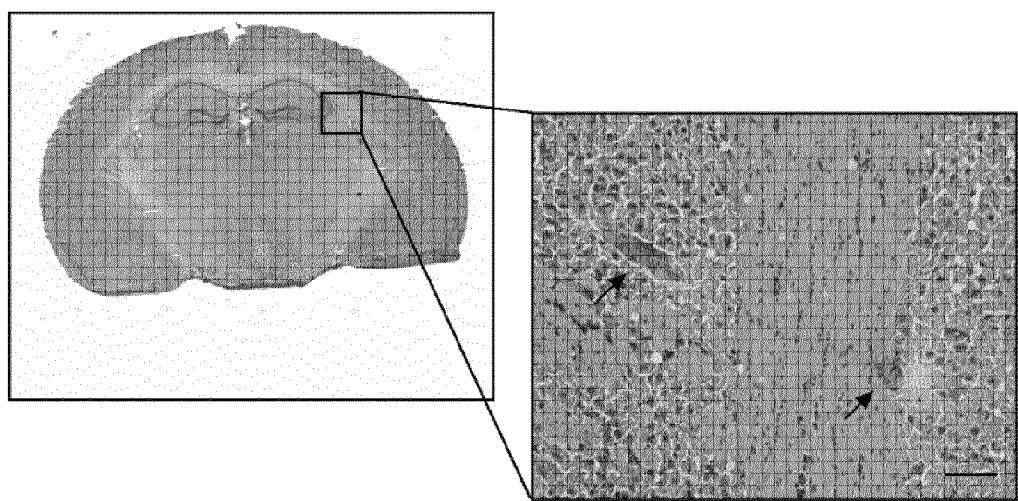
Figure 9**A****B**

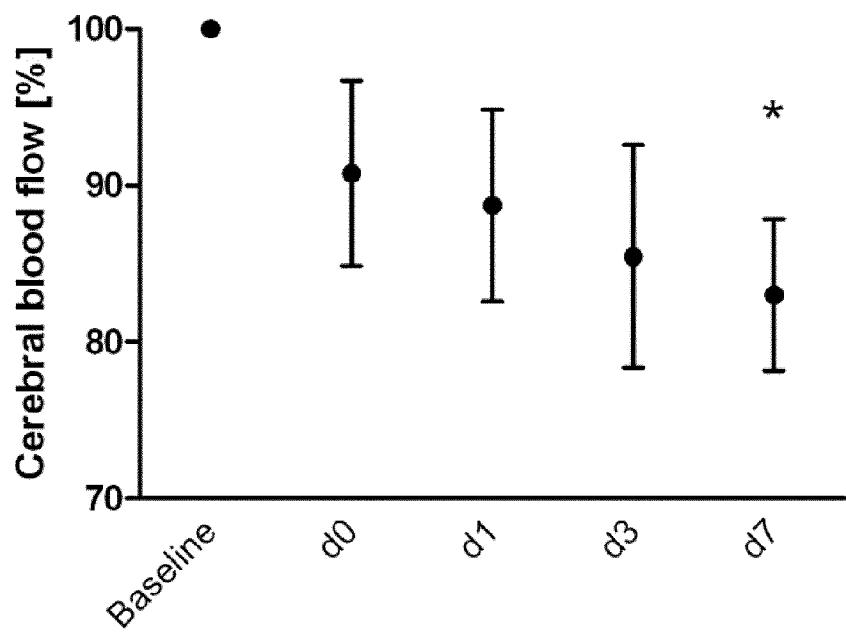
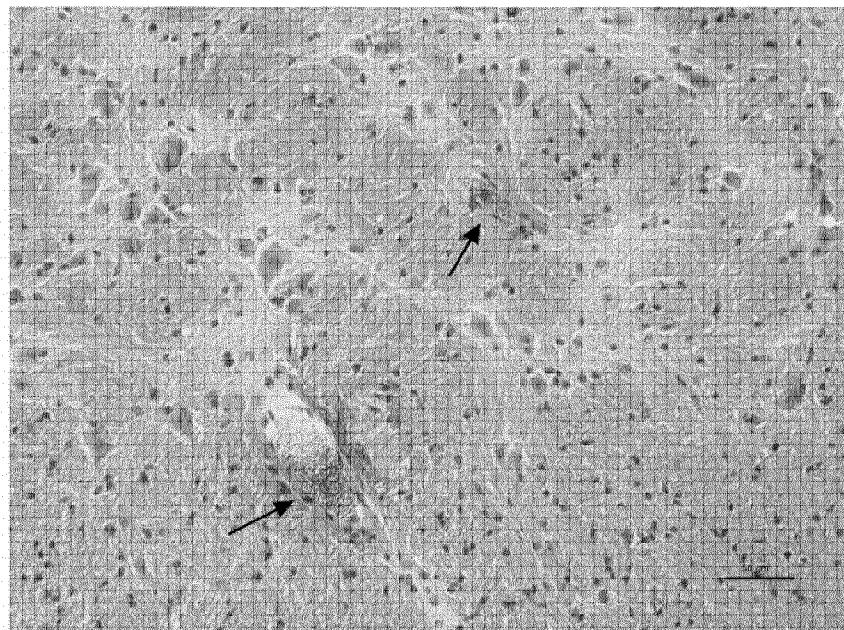
Figure 9**C****D**

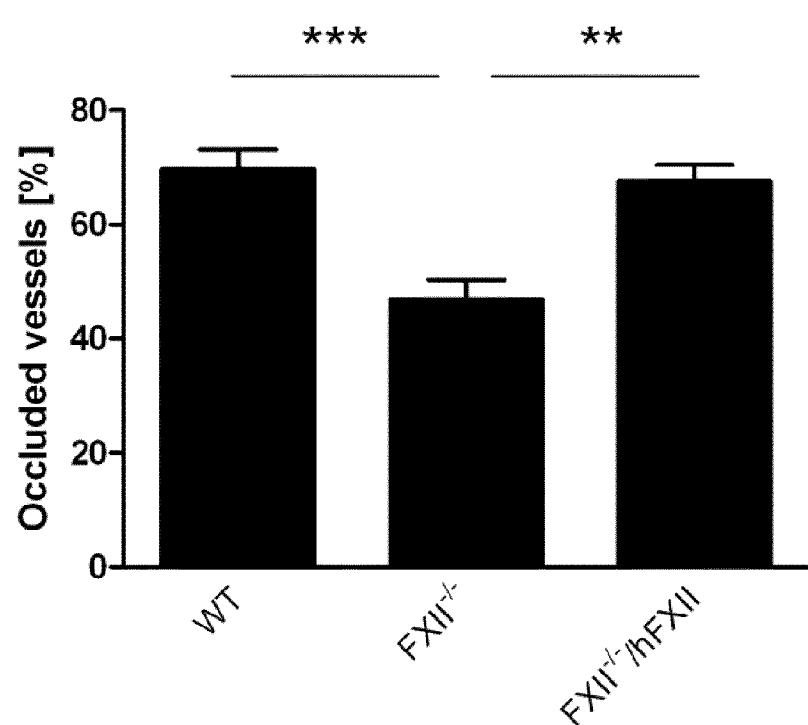
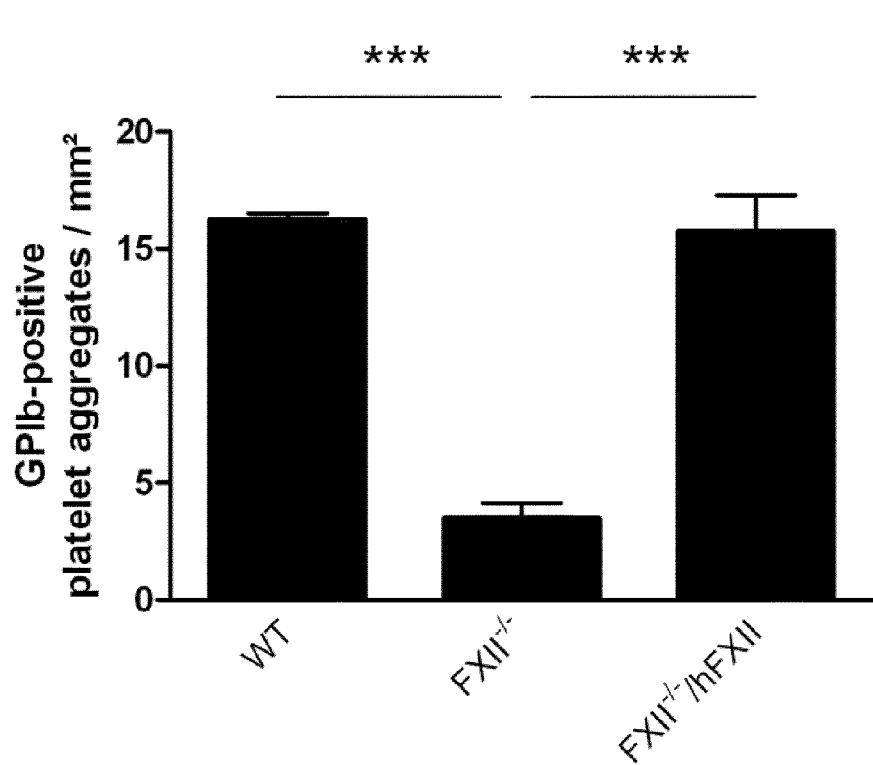
Figure 10**A****B**

Figure 10

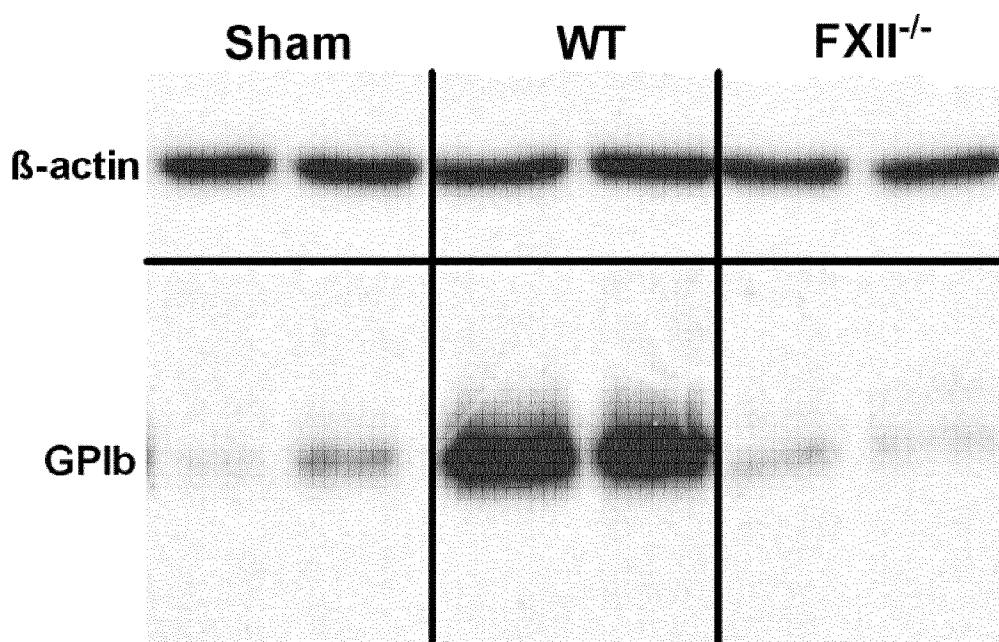
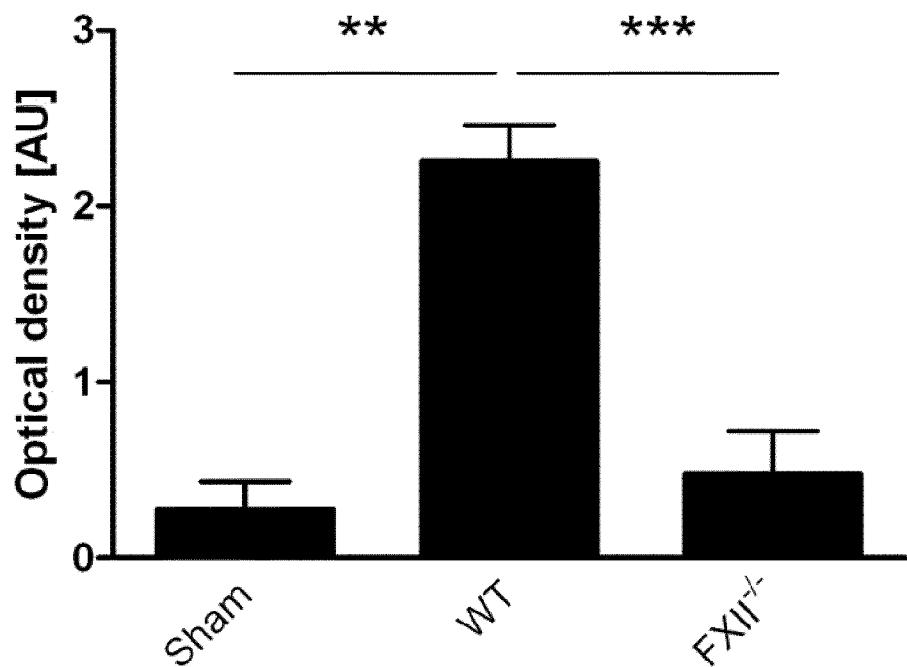
C

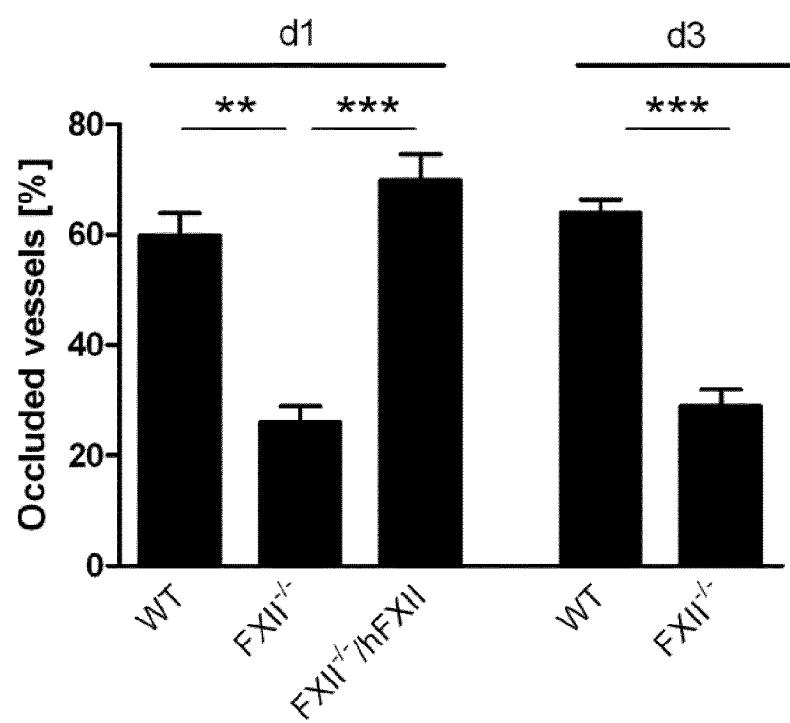
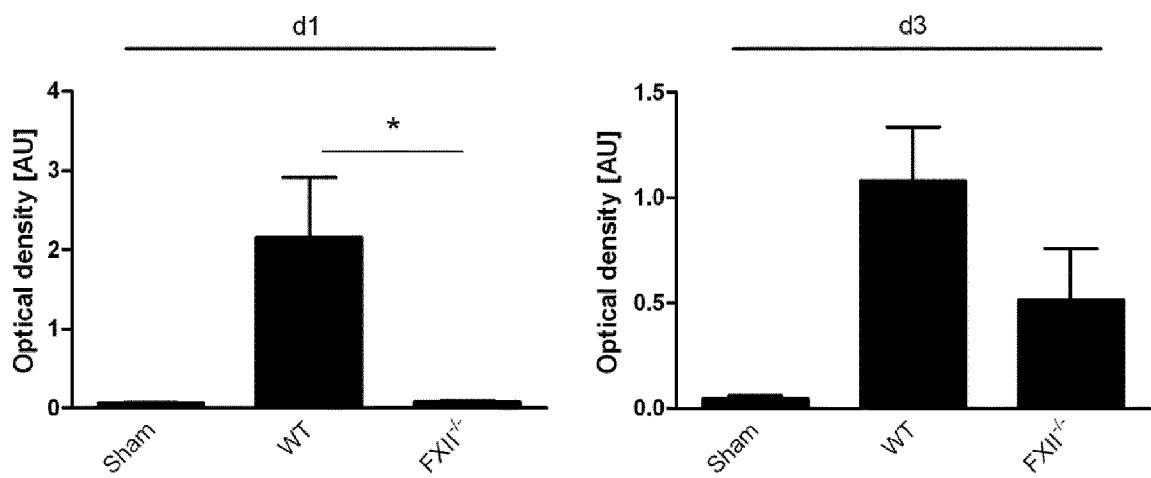
Figure 11**A****B**

Figure 11

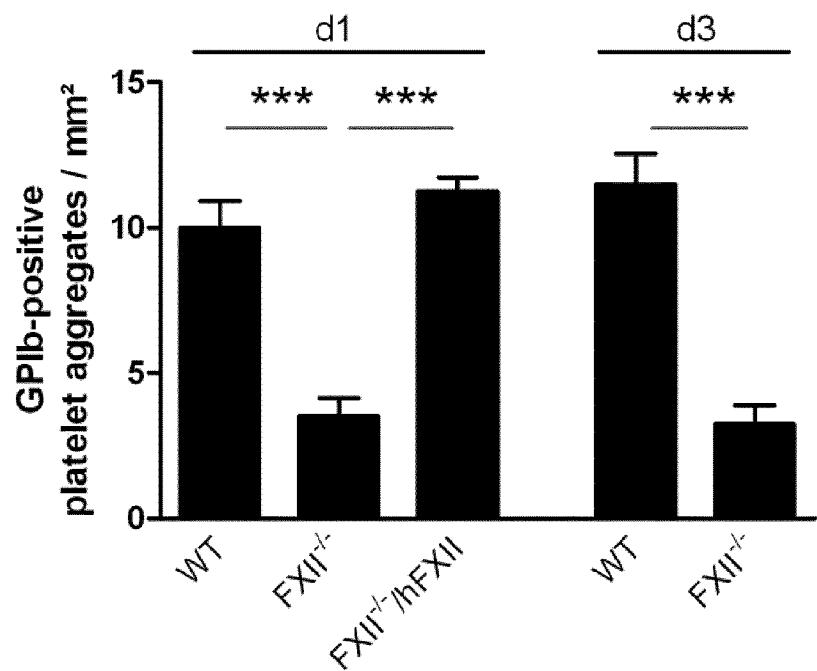
C

Figure 12

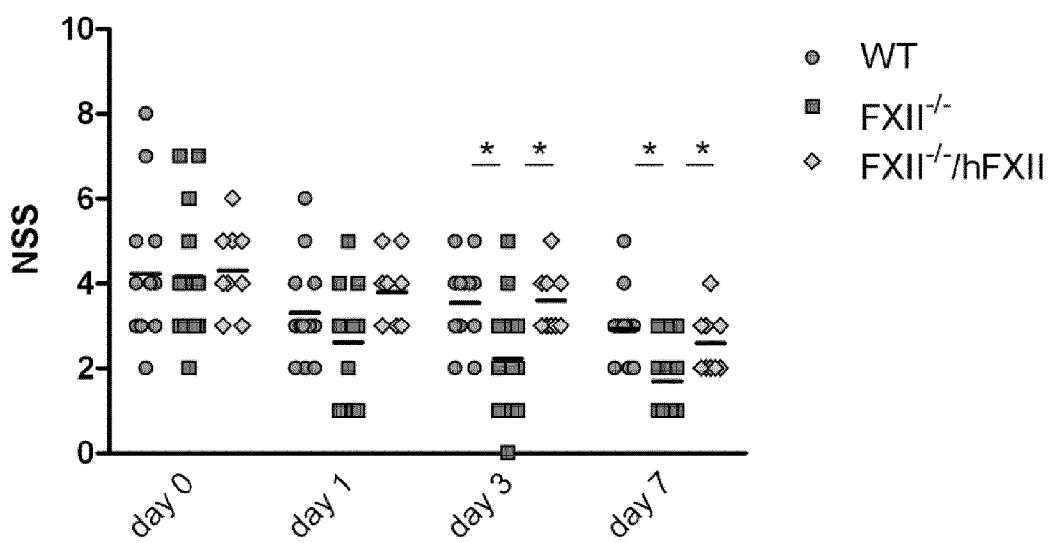


Figure 13

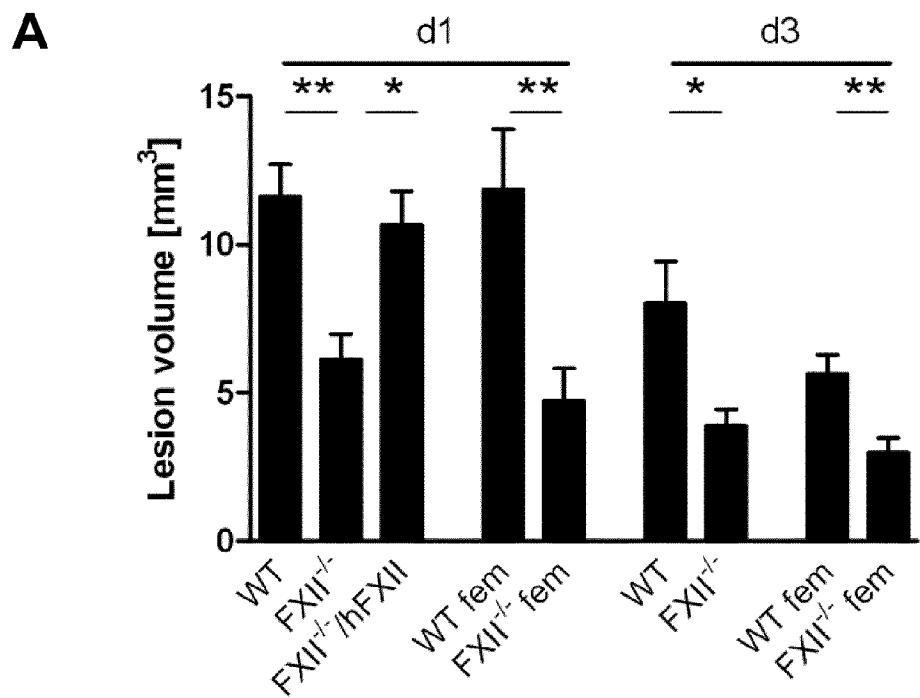


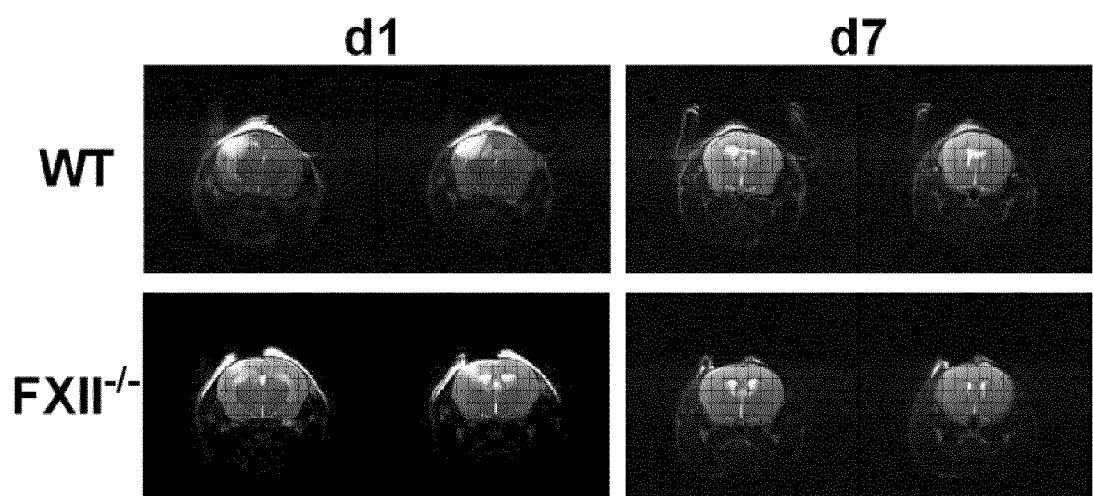
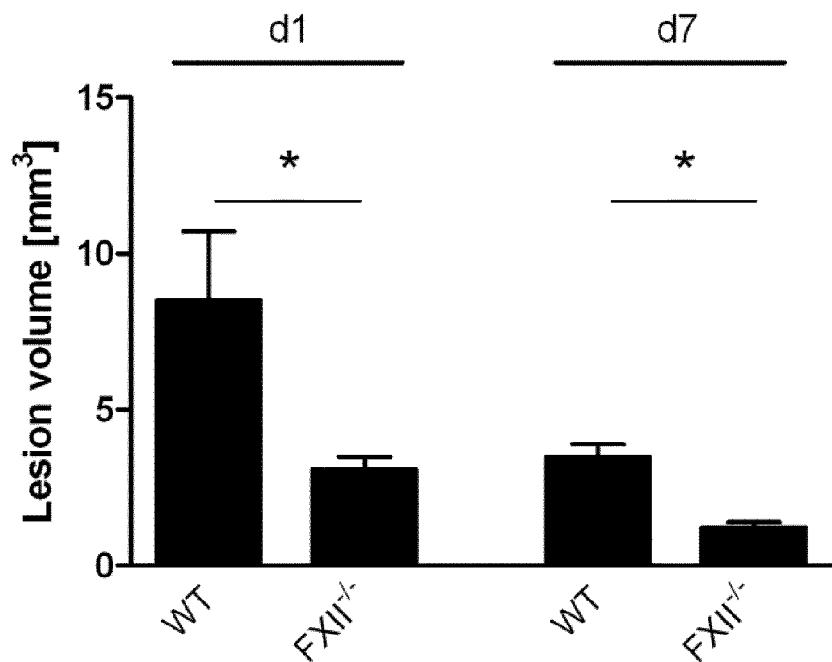
Figure 13**B**

Figure 13

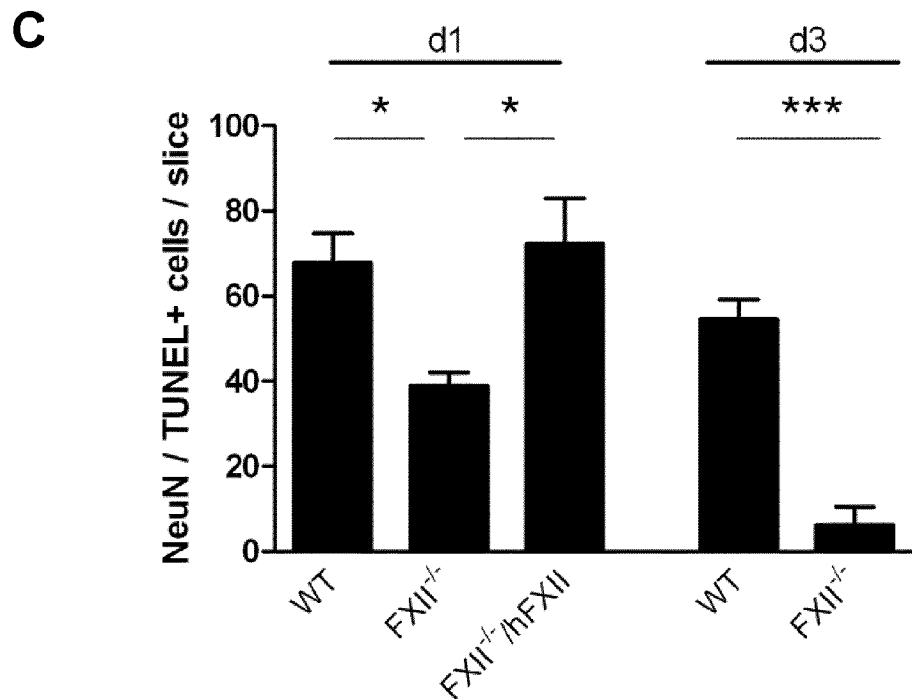


Figure 14

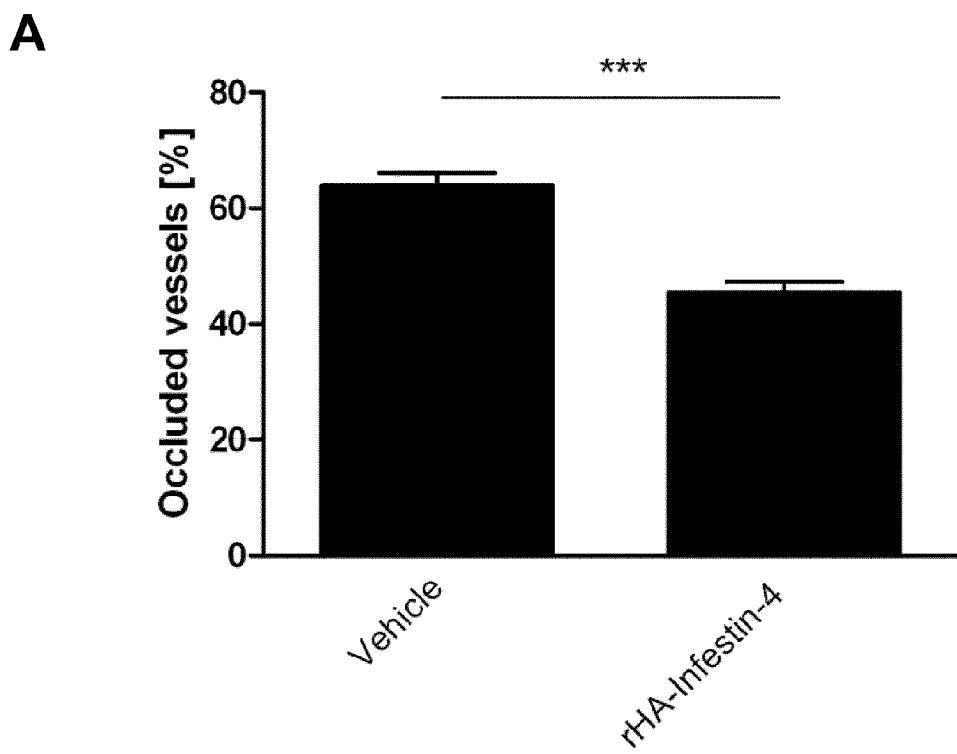


Figure 14

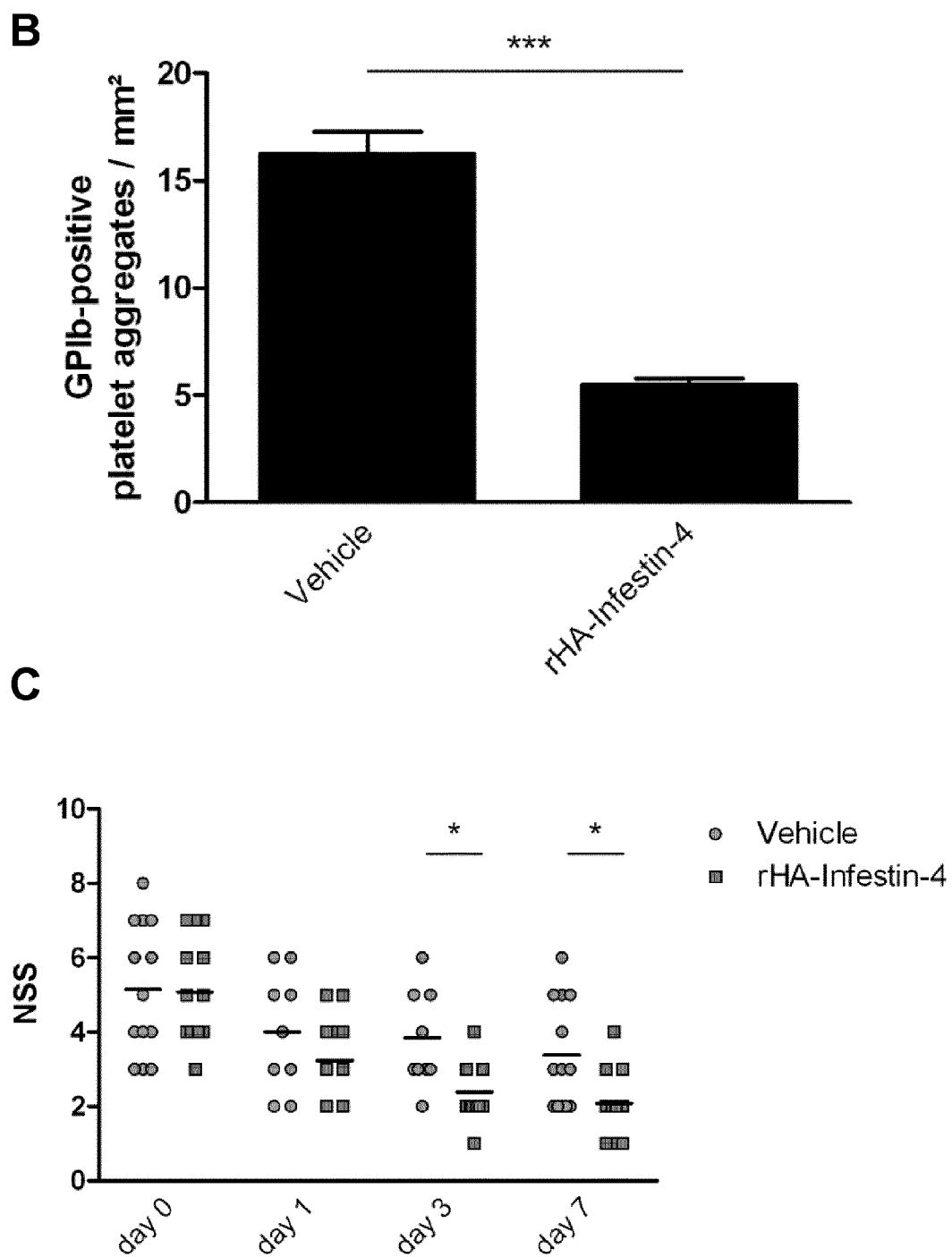


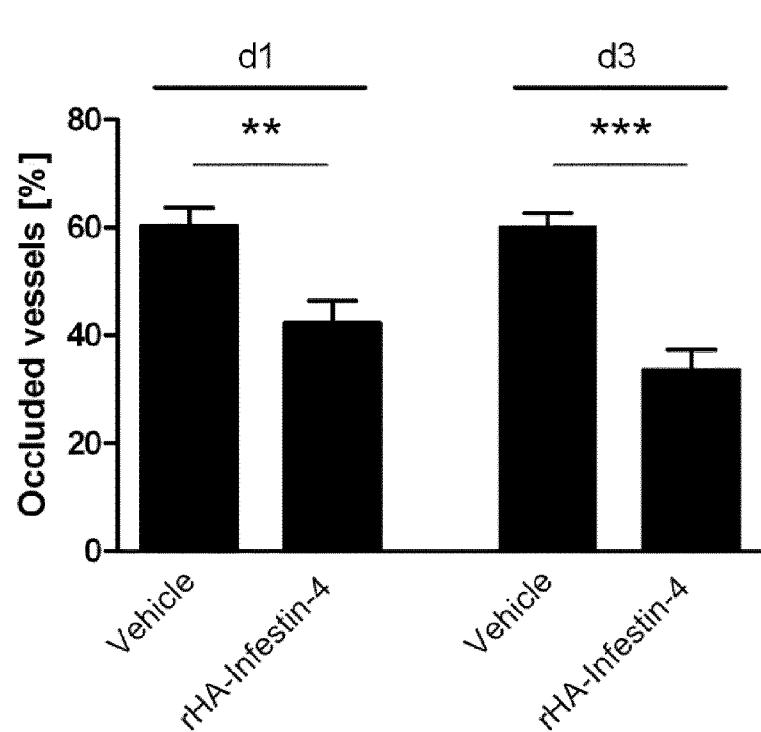
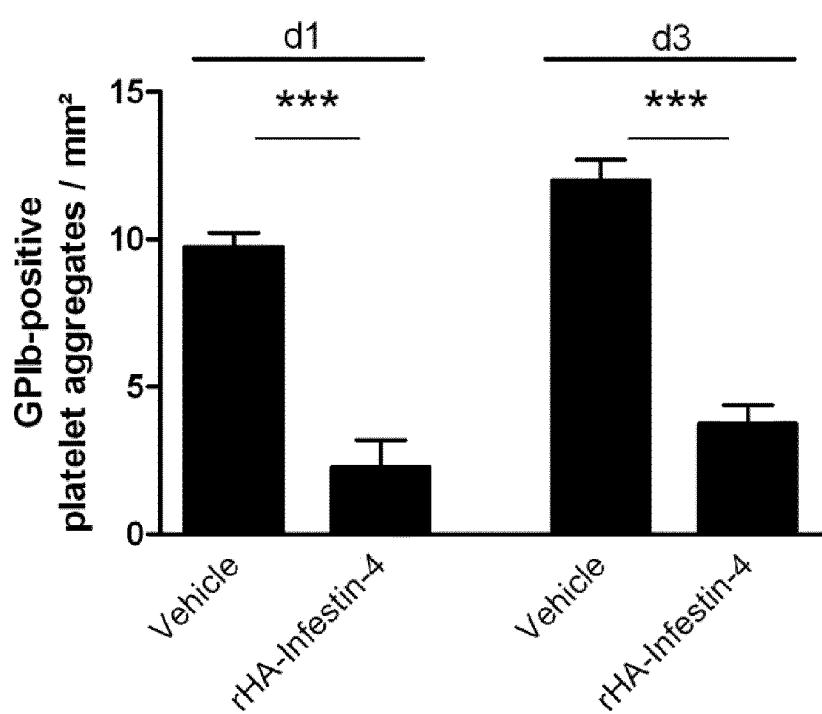
Figure 15**A****B**

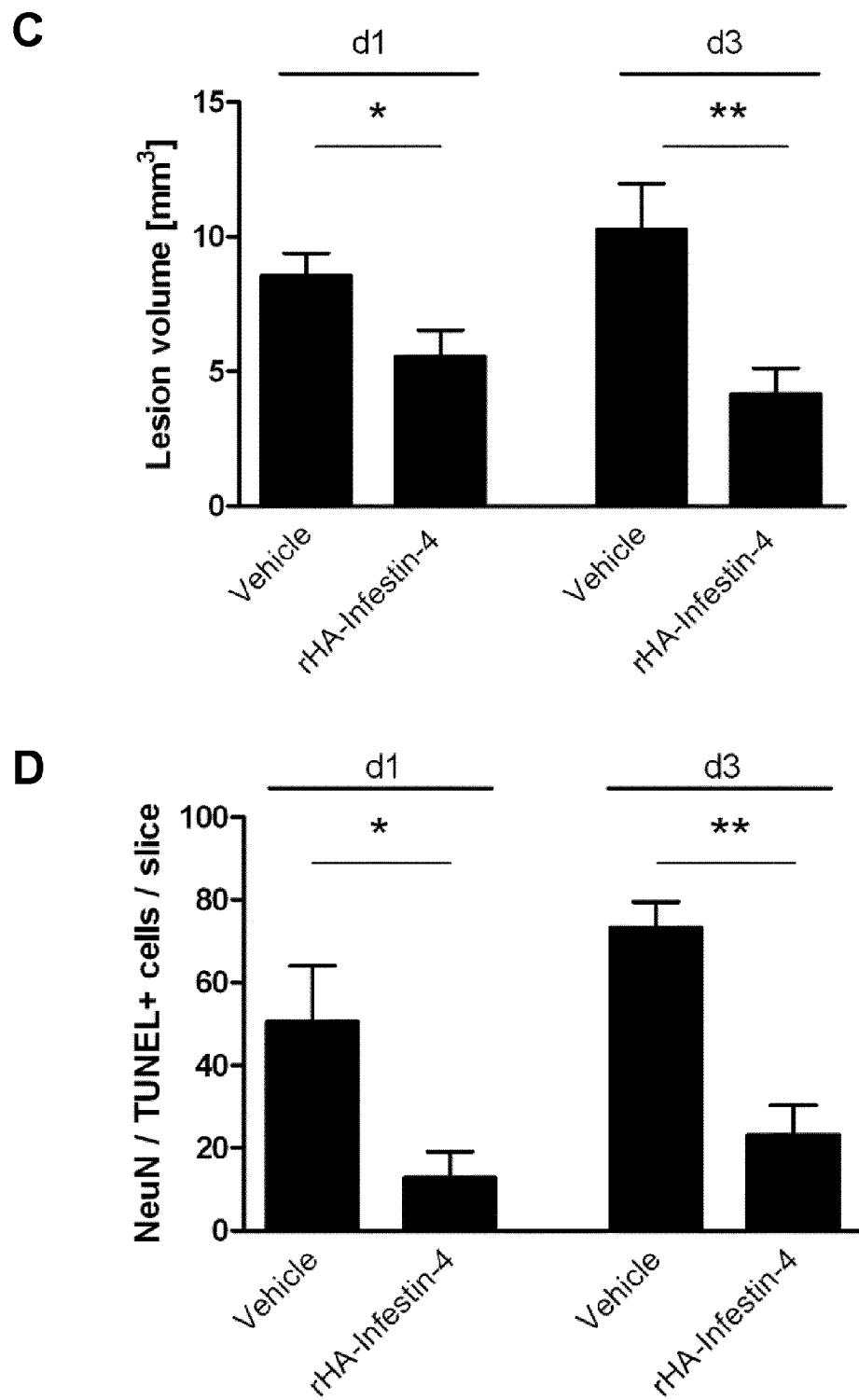
Figure 15

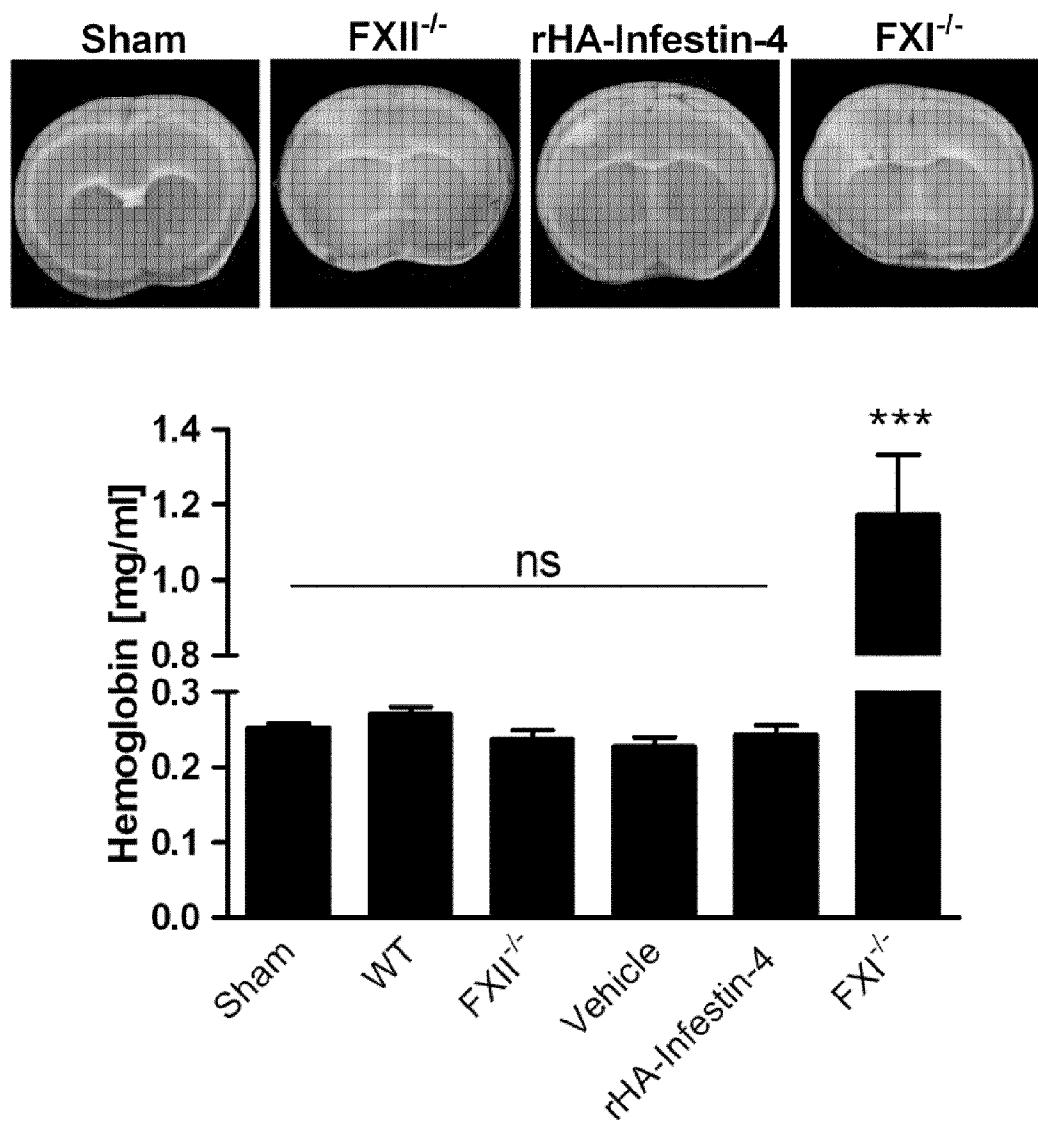
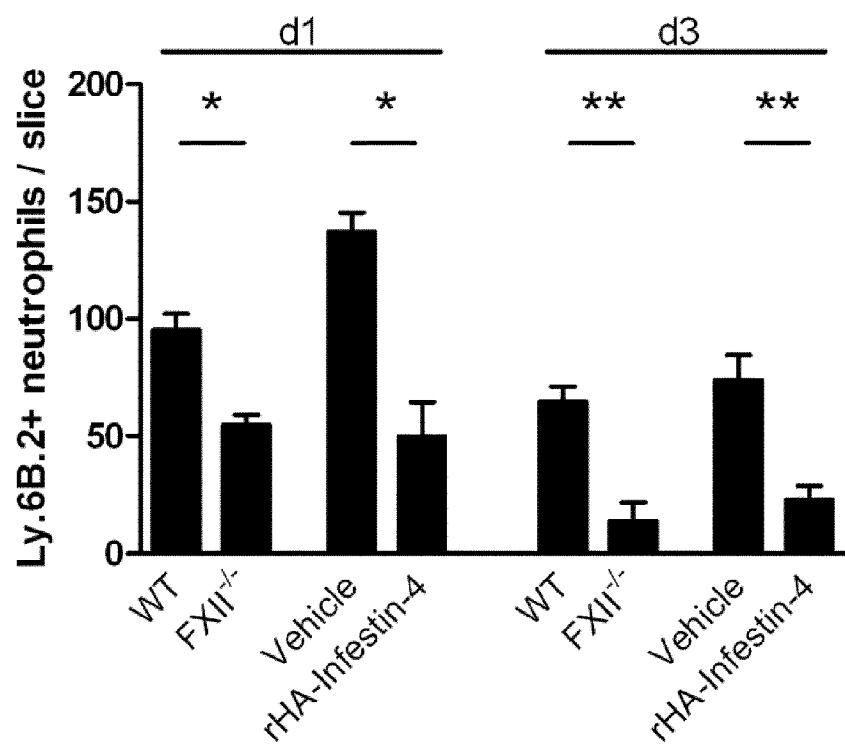
Figure 16

Figure 17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2015/063760

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed:
 - in the form of an Annex C/ST.25 text file.
 - on paper or in the form of an image file.
 - b. furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. furnished subsequent to the international filing date for the purposes of international search only:
 - in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/063760

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/57 A61K39/395 A61P25/00
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)
A61K C07K

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, WPI Data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	<p>WO 2014/135694 A1 (CSL BEHRING AG [CH]) 12 September 2014 (2014-09-12) page 11, line 27 - page 12, line 4 page 13, line 3 - line 26 page 14, line 22 - line 30 page 18, line 16 - page 31, line 2 page 32, line 12 - line 28 page 35, line 22 - line 24 page 42, line 16 - line 29 claims</p> <p>-----</p> <p style="text-align: center;">-/-</p>	1-14

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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Date of the actual completion of the international search	Date of mailing of the international search report
29 September 2015	06/10/2015
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Fey-Lamprecht, F

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/063760

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 623 110 A1 (CSL BEHRING GMBH [DE]; CSL LTD [AU]; UNIV WUERZBURG J MAXIMILIANS [DE]) 7 August 2013 (2013-08-07) paragraph [0014] - paragraph [0024] paragraph [0030] paragraph [0034] paragraph [0049] - paragraph [0097] examples -----	1-13
Y	paragraph [0033] paragraph [0038] - paragraph [0041] paragraph [0046] paragraph [0054] - paragraph [0057] paragraph [0071] - paragraph [0073] paragraph [0090] paragraph [0101] paragraph [0131] paragraph [0140] paragraph [0151] claims -----	1-15
X	WO 2011/069090 A1 (ALAVITA PHARMACEUTICALS INC [US]; ALLISON ANTHONY [US]; RINGOLD GORDON) 9 June 2011 (2011-06-09) paragraph [0033] paragraph [0038] - paragraph [0041] paragraph [0046] paragraph [0054] - paragraph [0057] paragraph [0071] - paragraph [0073] paragraph [0090] paragraph [0101] paragraph [0131] paragraph [0140] paragraph [0151] claims -----	1,2,5,6, 8-13
Y	INA HAGEDORN ET AL: "Factor XIIa Inhibitor Recombinant Human Albumin Infestin-4 Abolishes Occlusive Arterial Thrombus Formation Without Affecting Bleeding", CIRCULATION, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 121, 6 April 2010 (2010-04-06), pages 1510-1517, XP002715877, ISSN: 0009-7322, DOI: 10.1161/CIRCULATIONAHA.109.924761 [retrieved on 2010-03-22] the whole document -----	1-15
X	WO 2006/066878 A1 (NIESWANDT BERNHARD [DE]; RENNE THOMAS [DE]) 29 June 2006 (2006-06-29) cited in the application page 5, line 12 - line 22 claims -----	1-15
X, P	WO 2014/207199 A1 (CSL BEHRING GMBH [DE]) 31 December 2014 (2014-12-31) claims -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2015/063760

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 2014135694	A1 12-09-2014	AU 2014224599	A1 20-08-2015	CA 2901225	A1 12-09-2014
		WO 2014135694	A1 12-09-2014		
EP 2623110	A1 07-08-2013	AU 2013200498	A1 15-08-2013	CA 2862903	A1 08-08-2013
		CN 104080470	A 01-10-2014	EP 2623110	A1 07-08-2013
		EP 2809336	A1 10-12-2014	JP 2015511221	A 16-04-2015
		KR 20140117660	A 07-10-2014	US 2014378653	A1 25-12-2014
		WO 2013113774	A1 08-08-2013		
WO 2011069090	A1 09-06-2011	NONE			
WO 2006066878	A1 29-06-2006	AU 2005318464	A1 29-06-2006	BR PI0519349	A2 20-01-2009
		CA 2591786	A1 29-06-2006	CN 101102819	A 09-01-2008
		DK 1830924	T3 21-05-2013	EP 1830924	A1 12-09-2007
		EP 2272565	A2 12-01-2011	ES 2407965	T3 17-06-2013
		JP 5118492	B2 16-01-2013	JP 2008525342	A 17-07-2008
		KR 20070092303	A 12-09-2007	US 2008254039	A1 16-10-2008
		US 2012148688	A1 14-06-2012	US 2013164301	A1 27-06-2013
		WO 2006066878	A1 29-06-2006		
WO 2014207199	A1 31-12-2014	NONE			