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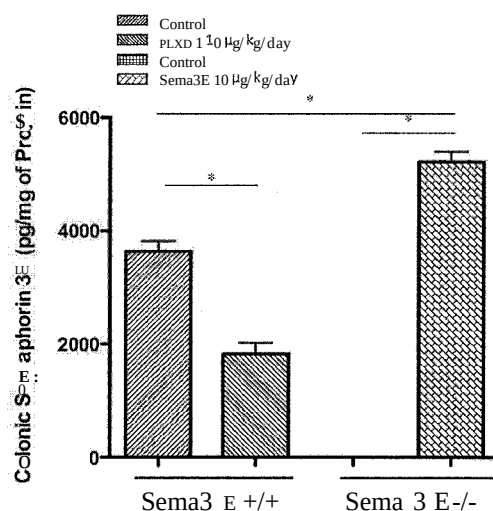


Fig. 12

(57) Abstract: Provided herein are methods for treating an inflammatory bowel disease in a subject. In one embodiment the method includes administering to a subject in need thereof an effective amount of a composition that includes a sema3E polypeptide, or a polynucleotide that encodes a sema3E polypeptide. The subject may have, or be at risk of having, an inflammatory bowel disease. Examples of inflammatory bowel diseases that may be treated using the methods disclosed herein include Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's disease, and indeterminate colitis. Also provided herein are methods for evaluating treatment options for a subject having inflammatory bowel disease.

METHODS FOR TREATING INFLAMMATORY BOWEL DISEASE

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 61/820,916, filed May 8, 2014, which is incorporated by reference herein.

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BACKGROUND

Inflammatory bowel diseases (IBD) are idiopathic chronic, recurrent intestinal disorders of complex pathogenesis, which include Crohn's disease (CD) and ulcerative colitis (UC). The estimated prevalence in Canada is around 500/100 000 persons (Bernstein et al., 2000, Am J Gastroenterol, 95:677-83). These diseases often present in adolescence or young adulthood and hence affected individuals have a long burden of disease with significant psychosocial, physical and economic impacts. In Canada, IBD represents a public health issue due to their impact on patient quality of life (estimated costs: \$1.7 billions) (Bernstein et al, 2000, Am J Gastroenterol, 95:677-83, CCFC. Annual Report. CCFC 2008).

The etiopathogenesis of IBD is multifactorial, involving an aberrant immune response to some environmental antigen in genetically predisposed individuals. The apparent therapeutic beneficial effect of biological therapy (tumor necrosis factor-(TNF-a-neutralizing antibody) (Han et al., 2007, Gut, 56:73-81), corticosteroids and thiopurines underscores the importance of the dysregulated immune response. However, some patients are resistant to these drugs, and all of these therapeutic agents have adverse side effects (Domenech, 2006, Digestion, 73 Suppl 1:67-76, Ananthakrishnan et al., 2010, J Clin Gastroenterol, 44:272-279). For the most ill patients monoclonal antibodies to TNF-a are used. These agents are expensive: require indefinite use for maximal effectiveness, and the concern for infectious and potentially even malignant complications (Vermeire et al., 2007, Aliment Pharmacol Ther 2007;25:3-12) limits the enthusiasm for introducing these agents earlier in the treatment paradigm.

It has been shown that a complex network of events at molecular, cellular, and tissue levels underlie inflammation and remodeling that eventually lead to development of IBD symptoms. Cell proliferation, migration and angiogenesis are cardinal cellular events that are tightly regulated by various mediators and mechanisms in physiological conditions. However, environmental antigens exposure impairs regulatory mechanisms in IBD patients leading to pathological features including gut inflammation and angiogenesis.

Functional alteration of angiogenesis and enhanced inflammatory cell recruitment have been described previously in patients with IBD (Rutella et al., 2011, J Cell Mol Med, 15:625-34). Semaphorins originally identified as axon guidance factors, are implicated in angiogenesis, and recent studies indicate that several members of the semaphorin family modulate various phases of immune response in conditions such as chronic obstructive pulmonary disease and arthritis (Catalano 2010, J Immunol, 185:6373-6383, Favre et al., 2003, Am J Physiol Heart Circ Physiol 2003;285:H1917-38). More recently, attention has been given to Semaphorin3E (Sema3E) that inhibits tumor angiogenesis and cell migration.

Semaphorins are type of signaling molecules, originally described as axonal guidance molecules (Pasterkamp and Kolodkin, 2003, Curr Opin Neurobiol 2003;13:79-89). There are 8 classes of semaphorins, of which 3 (classes 1-2) are expressed in invertebrates and 5 (classes 3-7) are widely expressed in vertebrates tissues, class 5 is found in both and V is specific to viruses (Tamagnone and Comoglio, 2000, Trends Cell Biol 2000;10:377-83, Yazdani and Terman, 2006, Genome Biol, 7:211). Of the classes 3-7, all are membrane-tethered except for the secreted class III semaphorins, which consist of 7 members (Sema3A-G), displaying a 35–52% protein homology (Yazdani and Terman, 2006, Genome Biol, 7:211), whereas classes 4-7 are transmembrane proteins that can also serve as receptors and interact with other transmembrane molecules (Yazdani and Terman, 2006, Genome Biol, 7:211, Kruger et al., 2005, Nat Rev Mol Cell Biol, 6:789-800). Membrane-bound vertebrate semaphorins bind directly to plexins. Similar to other semaphorins, the class III semaphorins have a domain that interacts with binding domains of receptor complexes consisting of a PlexinA but also Neuropilins (Nrp) (Gu et al., 2005, Science 2005;307:265-268). Two Nrps have been described to date, Nrpl and Nrp2, although Nrp2 has several transmembrane isoforms and the two Nrps have several soluble forms. Nrpl binds semaphorins 3A, 3B, 3C, and 3D, whereas NRP2 binds semaphorins 3F, 3B, 3C, and 3D (Geretti et al., 2008, Angiogenesis, 11:31-39, Neufeld et al., 2002, Trends Cardiovasc Med

2002;12:13-19). Semaphorin 3E (Sema3E, SemaH, or Coll-5) is one of the vertebrate secreted class 3 semaphorins. It has been shown that Sema3E plays a critical roles in axon pathfinding (Steinbach et al., 2002, Exp Cell Res, 279:52-61) and vascular patterning (Adams et al., 2010, Cold Spring Harb Perspect Biol, 2:a001875) during development. Unlike other class 3 semaphorins, Sema3E directly interacts with its receptor (PlexinD1), but does not bind Nrpl (Gu et al., 2005, Science 2005;307:265-268). However, gating of Sema3E-PlexinD1 complex by Nrpl switches repulsive signals to attractive ones (Chauvet et al., 2007, Neuron, 56:807-22, Bellon et al., 2010, Neuron, 66:205-19). Proteolytic cleavage of Sema3E leads to generation of a p61 kDa fragment which converts the repelling anti-migratory effect of Sema3E to an attracting pro-migratory one (Christensen et al., 2005, Cancer Res, 65:6167-77). This isoform is predominantly produced in metastatic cells and is involved in tumor progression (Christensen et al., 2005, Cancer Res, 65:6167-77, Casazza et al., 2010, J Clin invest, 120:2684-98). Silencing either Sema3E or PlexinD1 gene expression generates knockout mice with excessive and disorganized vascular growth and branching, indicating the importance of this ligand-receptor pair for vascular guidance (Gu et al., 2005, Science 2005;307:265-268, Gitler et al., 2004, Dev Cell, 7:107-116).

Emerging studies reveal anti-migratory and anti-proliferative effects of Sema3E on chemokine-stimulated thymocytes and VEGF-treated endothelial cells respectively (Choi et al., 2008, Immunity, 29:888-98, Moriya et al. 2010, Circ Res, 106:391-8). Sema3E has been shown to inhibit basal and platelet-derived growth factor -induced proliferation and migration of human airway smooth muscle cells (Movassagh et al, 2014, J Allergy Clin Immunol, 133(2):560-567). Sema3E has also been shown to suppress angiogenesis through rapid disassembly of integrin-mediated adhesive structures leading to inhibition of endothelial cells adhesion to the extracellular matrix. Anti-angiogenic effects of Sema3E are mediated by R-Ras inactivation and ADP-ribosylation factor 6, affecting the status of activation of integrins and their intracellular trafficking, respectively (Sakurai et al., 2010, Mol Cell Biol, 30:3086-98).

SUMMARY OF THE APPLICATION

Provided herein are uses of a sema3E polypeptide. In one embodiment, the use is in the preparation of a medicament for an inflammatory bowel disease. In one embodiment, the use is

for treating an inflammatory bowel disease. In one embodiment, the use is in the preparation of a medicament for an inflammatory bowel disease, wherein the sema3E polynucleotide encodes a sema3E polypeptide. In one embodiment, the use is for treating an inflammatory bowel disease, wherein the sema3E polynucleotide encodes a sema3E polypeptide. A medicament for an inflammatory bowel disease may include a pharmaceutically acceptable carrier.

Also provided herein are methods for using a sema3E polypeptide described herein. In one embodiment, the method includes treating an inflammatory bowel disease in a subject, including administering to a subject in need thereof an effective amount of a composition including a sema3E polypeptide. In one embodiment, the method includes treating a subject having, or at risk of having, inflammatory bowel disease, including administering to a subject in need thereof a composition including sema3E polypeptide, wherein the subject has decreased inflammatory markers in stool, decreased pathological lesions associated with inflammatory bowel disease, or a combination thereof, when compared to the subject before the administering. In one embodiment, the method includes treating an inflammatory bowel disease in a subject, including administering to a subject in need thereof an effective amount of a composition including a sema3E polynucleotide, wherein the sema3E polynucleotide encodes a sema3E polypeptide. In one embodiment, the method includes treating a subject having, or at risk of having, inflammatory bowel disease, including administering to a subject in need thereof a composition including sema3E polynucleotide, wherein the subject has decreased inflammatory markers in stool, decreased pathological lesions associated with inflammatory bowel disease, or a combination thereof, when compared to the subject before the administering, wherein the sema3E polynucleotide encodes a sema3E polypeptide.

In one embodiment, the subject is a human. In one embodiment, the method further includes administering a therapeutic compound, such as aminosalicylate, a corticoid, an immunosuppressive compound, a therapeutic antibody, an antibiotic, a thiopurine, a methotrexate, or a combination thereof. In one embodiment, the subject has, or at risk of having, an inflammatory bowel disease, such as Crohn's disease or ulcerative bowel disease.

In one embodiment, the sema3E polypeptide used in one of the uses or methods described herein includes a KRRXRR consensus site, wherein X is any amino acid. In one embodiment, the sema3E polypeptide further includes an RXXR consensus site, wherein X is any amino acid. In one embodiment, the sema3E polypeptide further includes a second RXXR

consensus site, wherein X is any amino acid. In one embodiment, the sema3E polypeptide includes a Sema domain. In one embodiment, the sema3E polypeptide further includes one or more domains selected from a cystine rich domain, an immunoglobulin domain, and a short basic domain. In one embodiment, the sema3E polypeptide further includes a cystine rich domain and an immunoglobulin domain. In one embodiment, the sema3E polypeptide includes an amino acid sequence having at least 80% identity with SEQ ID NO:2. In one embodiment, the inflammatory bowel disease is selected from Crohn's disease and ulcerative colitis. In one embodiment, the sema3E polypeptide is a fusion polypeptide. In one embodiment, the sema3E polypeptide has activity when determined by ability to bind to Plexin DL, or ability to inhibit neutrophil migration towards IL-8. In one embodiment, the sema3E polynucleotide is present in a vector, such as a viral vector.

Also provided herein are methods for evaluating treatment options for a subject having inflammatory bowel disease. In one embodiment, the method includes obtaining a biological sample from the subject, measuring the level of sema3E polypeptide in the biological sample, and comparing the level of sema3E polypeptide in the biological sample with the level of sema3E polypeptide in a control biological sample obtained from a healthy subject, wherein the presence of a decreased level of sema3E polypeptide compared to the control biological sample indicates the subject may be treated with a sema3E polypeptide. In one embodiment, the biological sample includes tissue from the gastrointestinal tract of the subject. In one embodiment, the method further includes administering to the subject a sema3E polypeptide or a fragment thereof. In one embodiment, the subject is a human.

The term "and/or" means one or all of the listed elements or a combination of any two or more of the listed elements.

The words "preferred" and "preferably" refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the invention.

The terms "comprises" and variations thereof do not have a limiting meaning where these terms appear in the description and claims.

Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

For any method disclosed herein that includes discrete steps, the steps may be conducted in any feasible order. And, as appropriate, any combination of two or more steps may be conducted simultaneously.

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Effects of lack of semaphorin (Sema) 3E on myeloperoxidase (MPO) activity. Sema3E^{+/+} (WT) and Sema3E^{-/-} (KO) mice were given dextran sulfate sodium (DSS) 5% solution in the drinking water to induce colitis (n=6). Control mice received water without DSS. WT were treated with PLXD1 Fc recombinant protein (10 µg/kg/day, i.p., n=3; R&D Systems) for 6 days, starting one day before induction. Sema3E^{-/-} (KO) mice were treated with Sema3E Fc Ig recombinant protein (10 µg/kg/day, i.p., n=3; R&D Systems) for 6 days starting one day before induction. Each value represents mean±SEM from 5 mice. *Significantly different (p<0.05, Student t-test).

Figure 2: Effects of lack of Semaphorin (Sema) 3E in gut in the development of DSS-colitis. Sema3E^{+/+} (WT) and Sema3E^{-/-} (KO) mice were given 5% DSS solution in the drinking water to induce colitis. Control mice received water without DSS. A: (a) Light micrograph of hematoxylin- and eosin-stained colonic section from Sema3E^{+/+} mouse on day 5 post-DSS. (b, c) Light micrograph of hematoxylin- and eosin-stained colonic section of Sema3E^{-/-} mouse on day 5 post-DSS. Magnification 100 and 400X. B: Histological Score. Each value represents

mean±SEM from 6 mice. *Significantly higher ($p<0.05$) in DSS-treated $\text{Sema3E}^{-/-}$ mice compared to DSS-treated $\text{Sema3E}^{+/+}$ mice.

Figure 3: Effects of the lack of Semaphorin (Sema) 3E on pro-inflammatory cytokines (Tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6 and IL12-p40) in dextran sulfate

sodium (DSS)-induced colitis. $\text{Sema3E}^{+/+}$ (WT) and $\text{Sema3E}^{-/-}$ (KO) mice were given 5% DSS solution in the drinking water to induce colitis and were sacrificed on day 5 post-DSS ($n=6$).

WT were treated with PLXD1 Fc recombinant protein (10 $\mu\text{g/kg/day}$, i.p., $n=3$; R&D Systems) for 6 days, starting one day before induction. $\text{Sema3E}^{-/-}$ (KO) mice were treated with Sema3E Fc Ig recombinant protein (10 $\mu\text{g/kg/day}$, i.p., $n=3$; R&D Systems) for 6 days starting one day

before induction. A: TL-1 β levels in colonic tissues, B: TNF- α levels in colonic tissues, C: IL-6 levels in colonic tissues, and D: IL-12P40 levels in colonic tissues. Each value represents

mean±SEM from 6 mice. *Significantly different ($p<0.05$, Student t-test). Figure 4: Sema3E

inhibits significantly human neutrophil migration towards IL-8 *in vitro*. 0.5×10^6 cells were added to the upper chamber while different concentrations of Sema3E were added to the upper chamber

in the presence or absence of IL-8/CXCL8 added to the bottom chamber. After one hour incubation at 37°C, migrated cells to the bottom well was collected and subjected to cell count using flow cytometry ($n=3$). *** $P<0.001$, ** $P<0.01$ compared to IL-8 alone.

Figure 5: Sema3E inhibits significantly human neutrophil migration towards IL-8 *in vitro*.

0.5×10^6 cells were added to the upper chamber while different concentrations of Sema3E

were added to the upper chamber in the presence or absence of IL-8/CXCL8 added to the bottom chamber. After one hour incubation at 37°C, migrated cells to the bottom well was collected and subjected to cell count using flow cytometry ($n=3$). *** $P<0.001$, ** $P<0.01$ compared to IL-8 alone.

Figure 6: Human neutrophil express plexin D1 receptor both at the mRNA and protein level. Peripheral blood neutrophil from healthy donors ($n=4$) were purified by Ficoll and dextran method were analyzed by RT-PCR (A) and flow cytometry using anti-human plexin D1 Alexa Fluor conjugated mAb (D). PBMC was used as positive control (E). The purity of neutrophil preparations was confirmed by flow cytometry using marker of neutrophil CD16/Fc γ RIII (B) and Wright Giemsa staining (C).

Figure 7: Effects of lack of semaphorin (Sema) 3E on interleukin (IL)-12p40, interferon (TNF)- γ level from splenic cells. $\text{Sema3E}^{+/+}$ (WT) and $\text{Sema3E}^{-/-}$ (KO) mice were given dextran

sulfate sodium (DSS) 5% solution in the drinking water to induce colitis (n=8) . Control mice received water without DSS (H2O). Isolated splenocytes. **A:** Interleukin (IL)-12p40 production from splenocytes. **B:** (INF)- γ production from splenocytes. Each value represents mean $\pm$ SEM from 5 mice. # significantly higher (p<0.05) in non-colitic Sema3E^{-/-} mice compared to non-colitic Sema3E^{+/+} mice; *significantly higher (p<0.05, Student t-test) in DSS-treated Sema3E^{-/-} mice compared to DSS-treated Sema3E^{+/+} mice.

Figure 8: Effects of semaphorin (Sema) 3E on splenic CD11c⁺ cells cytokine production in the context of dextran sulfate sodium (DSS)-induced colitis. **A:** Interleukin (IL)-12p40 production from dendritic cells. Splenic CD11c⁺ cells were isolated from wild-type (WT) and Sema3E^{-/-} (KO). Splenic CD11c⁺ cells were incubated *ex vivo* Sema3E Fc Immunoglobulin recombinant protein (10 nM). IL-12p40 was measured in media at 24h following treatments. Values are shown as means $\pm$ SEM, 3 independent experiments with 4 mice per group. ^aP<0.05 as compared to WT group, ^bP<0.05, n=7. One or two way ANOVA followed by the Tukey-Kramer multiple comparisons *post hoc* analysis

Figure 9: Effects of lack of semaphorin (Sema) 3E on bone marrow-derived cell (BMDC) isolated from non-colitic semaphorin (Sema) 3E deficient mouse (KO) treated with GM-CSF: Intracellular IL-12 production is higher in the absence of Sema3E in BMDC. Values are shown as means $\pm$ SEM, 3 independent experiments with 4 mice per group

Figure 10: Semaphorin (Sema) 3E down regulates dendritic cell (DC) differentiation, migration and antigen uptake. **A:** *In vitro* stimulation of bone marrow progenitor cells isolated from Sema3E^{-/-} (KO) mice with GM-CSF induced more DC differentiation compared to wild type (WT) mice according to CD11c⁺ staining at the same condition. **B:** Basal and chemokine-induced migration of bone marrow-derived cell (BMDC) from Sema3E KO mice is significantly higher than BMDC from WT mice. **C:** Bone marrow progenitor cells isolated from Sema3E KO mice with GM-CSF have higher antigen uptake capacity which is decreased upon Sema3E treatment. Values are shown as means $\pm$ SEM, 3 independent experiments with 4 mice per group. *P<0.05, n=7, Student t-test.

Figure 11: Differential expression of semaphorin (Sema)3E in inflamed colon. Colon were obtained from C57B1/6 colitic mice treated with **A:** dextran sulfate sodium (DSS) for 5 days (5%) or with **B:** 2,4-dinitrobenzenesulfonic acid (DNBS) 4mg in ethanol (Etho) 30% and from non-colitic mice. Analyzed by ELISA using laboratory basic protocol (USCNK, life Science

Inc.). Each value represents mean $\pm$ SEM from n>5 mice. *Significantly lower (p<0.05, Student t-test).

Figure 12: Level of colonic Sema 3E in Wild-type and Sema3E Ko mice after treatment with PLDX1 soluble receptor or Sema3E recombinant protein. Each value represents mean $\pm$ SEM from 3 mice. *Significantly lower (p<0.05, Student t-test) in DSS-treated Sema3E^{+/+} mice compared to DSS-treated Sema3E^{+/-} mice and significantly higher (p<0.05, Student t-test) in DSS-treated Sema3E^{-/-} mice compared to DSS-treated Sema3E^{+/-} mice.

Figure 13: Effects of lack of semaphorin (Sema) 3E treatment on myeloperoxidase (MPO) activity. Sema3E^{+/+} (WT) mice were given dextran sulfate sodium (DSS) 5% solution in the drinking water to induce colitis (n=6). Control mice received water without DSS. WT with Sema3E Fc Ig recombinant protein (10 μ g/kg/day, i.p., n=3; R&D Systems) for 6 days starting one day before induction. Each value represents mean $\pm$ SEM from 5 mice. *Significantly different (p<0.05, Student t-test).

Figure 14: Effects of Semaphorin (Sema) 3E treatment on pro-inflammatory cytokines (Tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6 and IL12-p40) in dextran sulfate sodium (DSS)-induced colitis. Sema3E^{+/+} (WT) mice were given 5% DSS solution in the drinking water to induce colitis and were sacrificed on day 5 post-DSS (n=6). Control mice received water without DSS. WT were treated with Sema3E Fc Ig recombinant protein (10 μ g/kg/day, i.p., n=3; R&D Systems) for 6 days starting one day before induction. A: IL-1 β levels in colonic tissues, B: TNF- α levels in colonic tissues, C: IL-6 levels in colonic tissues, and D: IL-12P40 levels in colonic tissues. Each value represents mean $\pm$ SEM from 6 mice. *Significantly different (p<0.05, Student t-test).

Figure 15: A; Amino acid sequences of Semaphorin 3E from *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*. B; Multiple sequence alignment of SEQ ID NO:1, 3, and 5. "*" refers to identical amino acids in the consensus sequence; ":" refers to conserved amino acids in the consensus sequence.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Recent work has implicated plexins and semaphorins in the regulation of the immune system (Walzer et al., 2005, Int Immunol 17:943-50, Wong et al., 2003, Nat Immunol, 4:891-

898). Several plexins and semaphorins are expressed by both naive and activated immune cells. PlexinD1 and Sema3E are expressed in the thymus and by B cells (Choi et al., 2008, *Immunity*, 29:888-98). The wide distribution of plexins and semaphorins across immune system cells and environments suggest that they function in immune system development and response, especially in immune cell trafficking and immune cell-cell interactions (Choi et al., 2008, *Immunity*, 29:888-98, Takamatsu et al., 2010, *Nat Immunol* 11:594-600). The thymus is a primary lymphoid organ that supports T cell differentiation and selection, chemokines, sphingosine-1-phosphates and adhesion molecules (Takahama, 2006, *Nat Rev Immunol* 6:127-35) are crucially involved in this process, thus, chemorepellent molecules, such as semaphorins, play a role in the sequential movement of thymocytes in the thymus. Secreted semaphorin, Sema3E, has been shown to participate in thymocyte development (Choi et al., 2008, *Immunity*, 29:888-98) and is expressed by thymic epithelial cells in the medulla of the thymus, and CD4⁺CD8⁺ thymocytes express high amounts of plexin-D1. Positively-selected CD69⁺ cells migrate through the cortex to the corticomedullary junction in a chemokine CC receptor (CCR) 9-dependent manner and Sema3E inhibits CCR9-mediated migration into the medulla. Accumulating evidence also shows that PlexinD1 is involved in the various phases of physiological and pathological immune responses by regulating cell-cell communications in a contact-dependent or -independent manner. PlexinD1 is necessary for the normal humoral immunity associated with B cells (Holl, 2012, *PLoS One*;7:e43333). PlexinD1 expression in B cells increases upon activation and in PlexinD1^{-/-} mice the number in the marginal zone in the spleen is reduced. To date, other types of semaphorins have been implicated in neutrophil regulation; for example Sema7A triggers the inflammatory response by activation of monocytes and macrophages at sites of inflammation (Suzuki et al., 2007, *Nature* 446:680-4), but also drives neutrophils at these sites (Morote-Garcia et al., 2012, *Proc Natl Acad Sci U S A* 2012;109:14146-51), thus enhancing an immune response.

The inventors have found that sema3E inhibits inflammatory responses in experimental colitis, and can be used to treat intestinal diseases. As will be appreciated by one of skill in the art, the use of Sema3E to treat intestinal diseases such as ulcerative colitis is highly surprising. It is completely unexpected that a protein known to inhibit tumor angiogenesis and treat asthma by inhibiting airway smooth muscle cell migration or proliferation (Soussi Gounni, WO

201 2/1 31477) can be used to treat inflammatory bowel disease. Furthermore, it is unexpected that an immune cell can be regulated directly in this manner.

The results of this study clearly indicate that the absence of Sema3E results in an increased susceptibility to experimental colitis. Without intending to be limited by theory, this deleterious effect may be related to the neutrophil cell population.

The present invention includes isolated polypeptides having semaphorin 3E activity. As used herein, the term "polypeptide" refers broadly to a polymer of two or more amino acids joined together by peptide bonds. The term "polypeptide" also includes molecules which contain more than one polypeptide joined by a disulfide bond, or complexes of polypeptides that are joined together, covalently or noncovalently, as multimers (e.g., dimers, tetramers). Thus, the terms peptide, oligopeptide, enzyme, and protein are all included within the definition of polypeptide and these terms are used interchangeably. It should be understood that these terms do not connote a specific length of a polymer of amino acids, nor are they intended to imply or distinguish whether the polypeptide is produced using recombinant techniques, chemical or enzymatic synthesis, or is naturally occurring. An "isolated" polypeptide is one that has been removed from a cell. For instance, an isolated polypeptide is a polypeptide that has been removed from the cytoplasm of a cell, and many of the polypeptides, nucleic acids, and other cellular material of its natural environment are no longer present. Polypeptides that are produced by recombinant, enzymatic, or chemical techniques are considered to be isolated and purified by definition, since they were never present in a cell. A "purified" polypeptide is one that is at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components of a cell.

Whether a polypeptide has semaphorin 3E activity may be determined by *in vitro* or *in vivo* assays. In one embodiment, semaphorin 3E activity refers to the ability of a polypeptide to bind Plexin D1 with an apparent K_D of <2 nM in a functional ELISA. For instance, when recombinant human Plexin D1 (such as catalog number 4160-PD, R&D Systems, Minneapolis, MN) is coated at 5 $\mu\text{g/mL}$, a polypeptide having semaphorin 3E activity will bind with an apparent K_D of <2 nM.

In one embodiment, semaphorin 3E activity refers to the ability of a polypeptide to inhibit neutrophil migration towards IL-8. Methods for measuring neutrophil migration towards IL-8 are known to the skilled person and routine. In one embodiment, semaphorin 3E activity

refers to the ability of a polypeptide to inhibit, in male C57BL/6 mice having DSS-induced colitis, the signs of colitis as measured by myeloperoxidase activity, colonic pro-inflammatory cytokine level, and/or histological score.

A polypeptide having semaphorin 3E activity is referred to herein as a sema3E polypeptide. An example of a sema3E polypeptide is depicted at SEQ ID NO:2, which is amino acids 25-766 of SEQ ID NO: 1 (SEQ ID NO: 1 is available through the Genbank database at accession number AAI44339.1). Another example of a sema3E polypeptide is depicted at SEQ ID NO:4, which is amino acids 26-766 of SEQ ID NO:3 (SEQ ID NO:3 is available through the Genbank database at accession number NP_035478.2). Another example of a sema3E polypeptide is depicted at SEQ ID NO:6, which is amino acids 26-766 of SEQ ID NO:5 (SEQ ID NO:5 is available through the Genbank database at accession number NP_001 100049.1). Other examples of sema3E polypeptides include amino acids 25-775 of SEQ ID NO:1, amino acids 26-775 of SEQ ID NO:3, and amino acids 26-775 of SEQ ID NO:5.

In one embodiment a sema3E polypeptide appears as an 87 kDa polypeptide on SDS-PAGE under reducing conditions. In one embodiment a sema3E polypeptide has an apparent molecular weight of 61 kDa or 25 kDa polypeptide on SDS-PAGE under reducing conditions. In one embodiment, a sema3E polypeptide migrates under non-reducing conditions as an oligomer with two 61 kDa polypeptides. In one embodiment, a sema3E polypeptide migrates under non-reducing conditions as an oligomer with one 61 kDa polypeptide and one 25 kDa polypeptide. In one embodiment a sema3E polypeptide is a monomer, and in one embodiment a sema3E polypeptide is a multimer, either a homomultimer or a heteromultimer. A multimer may be a dimer, a trimer, a tetramer, etc. In one embodiment a sema3E polypeptide may be a dimer, either a homodimer (such as, but not limited to, two 61 kDa polypeptides or two 25 kDa polypeptides) or a heterodimer (such as, but not limited to, a 61 kDa polypeptide a 25 kDa polypeptide).

Other examples of sema3E polypeptides of the present invention include those that are structurally similar to the amino acid sequence of SEQ ID NO:2, 4, or 6. Structural similarity of two polypeptides can be determined by aligning the residues of the two polypeptides (for example, a candidate polypeptide and a reference polypeptide described herein) to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper

order. A reference polypeptide may be a polypeptide described herein, such as SEQ ID NO:2, 4, or 6. A candidate polypeptide is the polypeptide being compared to the reference polypeptide. A candidate polypeptide may be isolated, for example, from a cell of an animal, such as a mouse, a rat, or a primate, such as a human, or can be produced using recombinant techniques, or
 5 chemically or enzymatically synthesized. A candidate polypeptide may be inferred from a nucleotide sequence present in the genome of an animal cell.

Unless modified as otherwise described herein, a pair-wise comparison analysis of amino acid sequences can be carried out using the Blastp program of the blastp suite-2sequences search algorithm, as described by Tatiana et al., (*FEMS Microbiol Lett*, 174, 247-250 (1999)), and
 10 available on the National Center for Biotechnology Information (NCBI) website. The default values for all blastp suite-2sequences search parameters may be used, including general parameters: expect threshold=10, word size=3, short queries=on; scoring parameters: matrix = BLOSUM62, gap costs=existence:11 extension: 1, compositional adjustments=conditional
 15 compositional score matrix adjustment. Alternatively, polypeptides may be compared using the BESTFIT algorithm in the GCG package (version 10.2, Madison WI).

In the comparison of two amino acid sequences, structural similarity may be referred to by percent "identity" or may be referred to by percent "similarity." "Identity" refers to the presence of identical amino acids. "Similarity" refers to the presence of not only identical amino acids but also the presence of conservative substitutions. A conservative substitution for an
 20 amino acid in a polypeptide described herein may be selected from other members of the class to which the amino acid belongs. For example, it is known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can be substituted for another amino acid without altering the activity of a protein, particularly in regions of the protein that are not directly
 25 associated with biological activity. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and tyrosine. Polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.
 30 Conservative substitutions include, for example, Lys for Arg and vice versa to maintain a positive charge; Glu for Asp and vice versa to maintain a negative charge; Ser for Thr so that a

free -OH is maintained; and Gin for Asn to maintain a free -NH₂. A high degree of identity is evident throughout the amino acid sequence of sema3E polypeptides. SEQ ID NO:1, 3, and 5 are shown in Figure 15B in a multiple protein alignment. Identical and conserved amino acids are marked in the consensus sequence with "*" and ":", respectively.

5 In one embodiment, a sema3E polypeptide includes consensus sites KRRXRR and RXXR, where X is any amino acid (Gherardi et al., 2004, Curr. Op. Struct. Biol., 14:669-678). In one embodiment, a sema3E polypeptide includes two RXXR consensus sites, and in one embodiment, a sema3E polypeptide includes one RXXR consensus site, where the carboxy-terminal RXXR consensus site is not present. In one embodiment, a sema3E polypeptide
10 includes consensus site KRRXRR. In one embodiment, a sema3E polypeptide includes amino acids corresponding to a Sema domain, a cystine rich domain, an Ig domain, and a short basic domain (Gherardi et al., 2004, Curr. Op. Struct. Biol., 14:669-678). In one embodiment, a sema3E polypeptide includes amino acids corresponding to a Sema domain, a cystine rich domain, and an immunoglobulin (Ig) domain.

15 Thus, as used herein, a sema3E polypeptide includes those with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence similarity to a reference amino acid sequence.

20 Alternatively, as used herein, a sema3E polypeptide includes those with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to a reference amino acid sequence.

25 A sema3E polypeptide having structural similarity the amino acid sequence of SEQ ID NO:2, 4, or 6 has semaphorin 3E activity. In one embodiment, a sema3E polypeptide may be a dimer, such as a homodimer or a heterodimer.

The present invention also includes polypeptides having a length of less than SEQ ID NO:2, 4, or 6. For instance, a polypeptide of the present invention may include a sequence
30 having a deletion of 1 or more amino acids from the amino terminal end, the carboxy terminal end, or a combination thereof, of SEQ ID NO:2, 4, or 6, or a polypeptide having structural

similarity to SEQ ID NO: 2, 4, or 6. In one embodiment, a polypeptide may have a deletion of amino acids that is, is at least, or is no greater than 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues.

In one embodiment, a sema3E polypeptide may include other amino acid residues at the amino-terminal end, the carboxy-terminal end, or both the amino- and carboxy-terminal ends, of a sema3E polypeptide disclosed herein or a polypeptide having structural similarity with sema3E polypeptide disclosed herein. In one embodiment, the additional amino acids may be heterologous amino acids. As used herein, "heterologous amino acids" refers to amino acids that are not normally or naturally found flanking the sequence depicted at, for instance, SEQ ID NO:2, SEQ ID NO:2, or SEQ ID NO:6. Such a polypeptide that includes, for instance, SEQ ID NO:2, SEQ ID NO:2, or SEQ ID NO:6 and heterologous amino acids may be referred to as a fusion polypeptide. In one embodiment, the additional amino acid sequence may be useful for purification of the fusion polypeptide by affinity chromatography. Various methods are available for the addition of such affinity purification moieties to proteins. Representative examples include, for instance, polyhistidine-tag (His-tag) and maltose-binding protein (see, for instance, Hopp et al. (U.S. Pat. No. 4,703,004), Hopp et al. (U.S. Pat. No. 4,782,137), Sgarlato (U.S. Pat. No. 5,935,824), and Sharma (U.S. Pat. No. 5,594,115)). In one embodiment, the additional amino acid sequence may be a carrier polypeptide. The carrier polypeptide may be used to increase the immunogenicity of the fusion polypeptide to increase production of antibodies that specifically bind to a polypeptide of the invention. The invention is not limited by the types of carrier polypeptides that may be used to create fusion polypeptides. Examples of carrier polypeptides include, but are not limited to, keyhole limpet hemacyanin, bovine serum albumin, ovalbumin, mouse serum albumin, rabbit serum albumin, and the like. In another embodiment, the additional amino acid sequence may be a fluorescent polypeptide (e.g., green, yellow, blue, or red fluorescent proteins) or other amino acid sequences that can be detected in a cell, for instance, a cultured cell, or a tissue sample that has been removed from an animal. In one embodiment, a polypeptide disclosed herein is fused to an FC region of an immunoglobulin molecule, such as human IgG1. If a polypeptide of the present invention includes an additional amino acid sequence not normally or naturally associated with the polypeptide, the additional amino acids are not considered when percent structural similarity to a reference amino acid sequence is determined.

Polypeptides of the present invention can be produced using recombinant DNA techniques, such as an expression vector present in a cell. Such methods are routine and known in the art. The polypeptides may also be synthesized *in vitro*, e.g., by solid phase peptide synthetic methods. The solid phase peptide synthetic methods are routine and known in the art.

5 A polypeptide produced using recombinant techniques or by solid phase peptide synthetic methods can be further purified by routine methods, such as fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on an anion-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, gel filtration using, for example, Sephadex G-75, or ligand affinity. Such methods
10 may also be used to isolate a polypeptide of the present invention from a cell.

The present invention also includes polynucleotides. In one embodiment, a polynucleotide encodes a polypeptide described herein. Also included are the complements of such polynucleotide sequences. A polynucleotide encoding a polypeptide having semaphorin 3E activity is referred to herein as a sema3E polynucleotide.

15 As used herein, the term "polynucleotide" refers to a polymeric form of nucleotides of any length, either ribonucleotides, deoxynucleotides, peptide nucleic acids, or a combination thereof, and includes both single-stranded molecules and double-stranded duplexes. A polynucleotide can be obtained directly from a natural source, or can be prepared with the aid of recombinant, enzymatic, or chemical techniques. Preferably, a polynucleotide of the present
20 invention is isolated. An "isolated" polynucleotide is one that has been removed from a cell. For instance, an isolated polynucleotide is a polynucleotide that has been removed from a cell and many of the polypeptides, nucleic acids, and other cellular material of its natural environment are no longer present. Polynucleotides that are produced by recombinant, enzymatic, or chemical techniques are considered to be isolated and purified by definition, since they were never present
25 in a cell.

Given the amino acid sequence of any one of the sema3E polypeptides described herein, a person of ordinary skill in the art can determine the full scope of polynucleotides that encode that amino acid sequence using conventional, routine methods. In one embodiment, a sema3E polynucleotide may have a nucleotide sequence encoding a polypeptide having the amino acid
30 sequence shown at amino acids 25-766 of SEQ ID NO:1, amino acids 25-775 of SEQ ID NO:1, a polypeptide having sequence similarity with 25-766 of SEQ ID NO:1 or amino acids 25-775 of

SEQ ID NO:1. It should be understood that a polynucleotide encoding a sema3E polypeptide represented by, for instance, amino acids 25-766 of SEQ ID NO: 1 is not limited to a single nucleotide sequence, but includes the class of polynucleotides encoding such a polypeptide as a result of the degeneracy of the genetic code. For example, a naturally occurring nucleotide sequence encoding amino acids 25-766 of SEQ ID NO: 1 is but one member of the class of nucleotide sequences encoding such a polypeptide. The class of nucleotide sequences encoding a selected polypeptide sequence is large but finite, and the nucleotide sequence of each member of the class may be readily determined by one skilled in the art by reference to the standard genetic code, wherein different nucleotide triplets (codons) are known to encode the same amino acid.

As used herein, the terms "coding region" and "coding sequence" are used interchangeably and refer to a nucleotide sequence that encodes a polypeptide and, when placed under the control of appropriate regulatory sequences expresses the encoded polypeptide. The boundaries of a coding region are generally determined by a translation start codon at its 5' end and a translation stop codon at its 3' end. A "regulatory sequence" is a nucleotide sequence that regulates expression of a coding sequence to which it is operably linked. Non-limiting examples of regulatory sequences include promoters, enhancers, transcription initiation sites, translation start sites, translation stop sites, and transcription terminators. The term "operably linked" refers to a juxtaposition of components such that they are in a relationship permitting them to function in their intended manner. A regulatory sequence is "operably linked" to a coding region when it is joined in such a way that expression of the coding region is achieved under conditions compatible with the regulatory sequence.

A sema3E polynucleotide of the present invention may include heterologous nucleotides flanking the open reading frame encoding the semaphorin 3E polynucleotide. As used herein, "heterologous nucleotides" refers to a nucleotide sequence that is not normally or naturally found flanking a semaphorin 3E open reading frame in a cell. Typically, heterologous nucleotides may be at the 5' end of the coding region, at the 3' end of the coding region, or the combination thereof. Examples of heterologous nucleotides include, but are not limited to, a regulatory sequence. The number of heterologous nucleotides may be, for instance, at least 10, at least 100, or at least 1000.

A polynucleotide of the present invention can be present in a vector. A vector is a replicating polynucleotide, such as a plasmid, phage, or cosmid, to which another polynucleotide

may be attached so as to bring about the replication of the attached polynucleotide. Construction of vectors containing a polynucleotide of the invention employs standard ligation techniques known in the art. See, e.g., Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989). A vector can provide for further cloning (amplification of the polynucleotide), i.e., a cloning vector, or for expression of the polynucleotide, i.e., an expression vector. The term vector includes, but is not limited to, plasmid vectors, viral vectors, cosmid vectors, transposon vectors, and artificial chromosome vectors. Examples of viral vectors include, for instance, adenoviral vectors, adeno-associated viral vectors, lentiviral vectors, retroviral vectors, and herpes virus vectors. A vector may be replication-proficient or replication-deficient. A vector may result in integration into a cell's genomic DNA. Typically, a vector is capable of replication in a host cell, for instance a mammalian and/or a bacterial cell, such as *E. coli*.

Selection of a vector depends upon a variety of desired characteristics in the resulting construct, such as a selection marker, vector replication rate, use in gene transfer into cells of the respiratory tract, and the like. Suitable host cells for cloning or expressing the vectors herein are prokaryotic or eukaryotic cells. Suitable eukaryotic cells include mammalian cells, such as murine cells and human cells. Suitable prokaryotic cells include eubacteria, such as gram-negative organisms, for example, *E. coli*.

An expression vector optionally includes regulatory sequences operably linked to the polynucleotide of the present invention. An example of a regulatory sequence is a promoter. A promoter may be functional in a host cell used, for instance, in the construction and/or characterization of a Sema3E polynucleotide, and/or may be functional in the ultimate recipient of the vector. A promoter may be inducible, repressible, or constitutive, and examples of each type are known in the art. A polynucleotide of the present invention may also include a transcription terminator. Suitable transcription terminators are known in the art.

Polynucleotides of the present invention can be produced *in vitro* or *in vivo*. For instance, methods for *in vitro* synthesis include, but are not limited to, chemical synthesis with a conventional DNA/RNA synthesizer. Commercial suppliers of synthetic polynucleotides and reagents for *in vitro* synthesis are well known. Methods for *in vitro* synthesis also include, for instance, *in vitro* transcription using a circular or linear expression vector in a cell free system.

Expression vectors can also be used to produce a polynucleotide of the present invention in a cell, and the polynucleotide may then be isolated from the cell.

The present invention is also directed to compositions including one or more polypeptides or polynucleotides described herein. Such compositions typically include a pharmaceutically acceptable carrier. As used herein "pharmaceutically acceptable carrier" includes, but is not limited to, saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Additional compounds can also be incorporated into the compositions.

A composition may be prepared by methods well known in the art of pharmacy. In general, a composition can be formulated to be compatible with its intended route of administration. A formulation may be solid or liquid. Administration may be systemic or local. In some aspects local administration may have advantages for site-specific, targeted disease management. Local therapies may provide high, clinically effective concentrations directly to the treatment site, with less likelihood of causing systemic side effects.

Examples of routes of administration include parenteral (e.g., intravenous, intradermal, subcutaneous, intraperitoneal, intramuscular), enteral (e.g., oral or rectal), and topical (e.g., epicutaneous, inhalational, transmucosal) administration. Appropriate dosage forms for enteral administration of the compound of the present invention may include tablets, capsules or liquids. Appropriate dosage forms for parenteral administration may include intravenous administration. Appropriate dosage forms for topical administration may include nasal sprays, metered dose inhalers, dry-powder inhalers or by nebulization.

Solutions or suspensions can include the following components: a sterile diluent such as water for administration, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates; electrolytes, such as sodium ion, chloride ion, potassium ion, calcium ion, and magnesium ion, and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. A composition can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Compositions can include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline. A composition is typically sterile and, when suitable for injectable use, should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile solutions can be prepared by incorporating the active compound (e.g., a polypeptide or polynucleotide described herein) in the required amount in an appropriate solvent with one or a combination of ingredients such as those enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a dispersion medium and other ingredients such as from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation that may be used include vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

For enteral administration, a composition may be delivered by, for instance, nasogastric tube, enema, colonoscopy, or orally. Oral compositions may include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier. Pharmaceutically compatible binding agents can be included as part of the composition. The tablets, pills, capsules, troches and the like may contain any of the following ingredients, or compounds of a similar nature: a binder

such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the active compounds may be delivered in the form of an aerosol spray, a nebulizer, or an inhaler, such as a nasal spray, metered dose inhaler, or dry-powder inhaler.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds may be formulated into ointments, salves, gels, or creams as generally known in the art. An example of transdermal administration includes iontophoretic delivery to the dermis or to other relevant tissues.

The active compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

The active compounds may be prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques. The materials can also be obtained commercially. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art. Delivery reagents such as lipids, cationic lipids, phospholipids, liposomes, and microencapsulation may also be used.

In one embodiment, an active compound may be associated with a targeting group. As used herein, a "targeting group" refers to a chemical species that interacts, either directly or indirectly, with the surface of a cell, for instance with a molecule present on the surface of a cell,

e.g., a receptor. The interaction can be, for instance, an ionic bond, a hydrogen bond, a Van der Waals force, or a combination thereof. Examples of targeting groups include, for instance, saccharides, polypeptides (including hormones), polynucleotides, fatty acids, and catecholamines. Another example of a targeting group is an antibody. The interaction between the targeting group and a molecule present on the surface of a cell, e.g., a receptor, may result in the uptake of the targeting group and associated active compound. Cells that may be targeted include, but are not limited to, cell of the gastrointestinal tract such as macrophages, CD4+ and CD8+ T cells, NKT cells, mast cells, intraepithelial T cells, T regulatory cells, but also enterochromaffin cells, paneth cells, and goblet cells.

When a polynucleotide is introduced into cells using a suitable technique, the polynucleotide may be delivered into the cells by, for example, transfection or transduction procedures. Transfection and transduction refer to the acquisition by a cell of new genetic material by incorporation of added polynucleotides. Transfection can occur by physical or chemical methods. Many transfection techniques are known to those of ordinary skill in the art including, without limitation, calcium phosphate DNA co-precipitation, DEAE-dextrin DNA transfection, electroporation, naked plasmid adsorption, cationic liposome-mediated transfection (commonly known as lipofection), use of glycoconjugates and polyplexes, targeting serpin-enzyme complex receptors, and polyethyleneimine. Transduction refers to the process of transferring nucleic acid into a cell using a DNA or RNA virus.

A polynucleotide described herein may be used in combination with other agents assisting the cellular uptake of polynucleotides, or assisting the release of polynucleotides from endosomes or intracellular compartments into the cytoplasm or cell nuclei by, for instance, conjugation of those to the polynucleotide. The agents may be, but are not limited to, peptides, especially cell penetrating peptides, protein transduction domains, and/or dsRNA-binding domains which enhance the cellular uptake of polynucleotides (Dowdy et al., US Published Patent Application 2009/0093026, Eguchi et al., 2009, *Nature Biotechnology* 27:567-571, Lindsay et al., 2002, *Curr. Opin. Pharmacol.*, 2:587-594, Wadia and Dowdy, 2002, *Curr. Opin. Biotechnol.* 13:52-56. Gait, 2003, *Cell. Mol. Life Sci.*, 60:1-10). The conjugations can be performed at an internal position at the oligonucleotide or at a terminal positions either the 5'-end or the 3'-end.

Toxicity and therapeutic efficacy of such active compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the ED₅₀ (the dose therapeutically effective in 50% of the population

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. Examples of animal models include, but are not limited to, dextran sulfate sodium (DSS)-induced colitis (Neurath et al., 1995, J Exp Med 182:1281-90; Ghia et al., 2007, J Clin Invest 118:2209-18; Ghia et al., 2007, Am J Physiol Gastrointest Liver Physiol 293:G71 1-8; Ghia et al, 2008, Am J Physiol Gastrointest Liver Physiol 294:770-777), the 2,4-dinitrobenzenesulfonic acid (DNBS) model which mimics some features of CD (Elson et al., 1995, 109:1344-67), and a spontaneous model of ileal inflammation that bears a very close resemblance to CD, known as SAMPI/YitFc (Pizarro et al., 2003, Trends Mol Med 9:218-22; Rivera-Nieves et al., 2003, Gastroenterology 124:972-82). The dosage of such active compounds lies preferably within a range of concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For an active compound used in the methods of the invention, it may be possible to estimate the therapeutically effective dose initially from cell culture assays. A dose may be formulated in animal models to achieve a concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of signs and/or symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

The compositions can be administered one or more times per day to one or more times per week, including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with an effective amount of a polynucleotide or a polypeptide can include a single treatment or can include a series of treatments. In one embodiment, the treatment is administered for a period of time of at least 1 day, at least 5 days, at least 10 days, or at least 20 days. In one embodiment, the treatment is administered for a period of time of no greater than 60 days, no greater than 40 days, or no greater than 30 days.

The present invention includes methods for using the polypeptides and polynucleotides disclosed herein. In one embodiment, a method includes contacting a cell with an effective amount of a sema3E polypeptide. In one embodiment, the contacting is under conditions suitable for allowing the sema3E polypeptide to interact with the surface of the cell, such as with a
5 PlexinD1 molecule. In one embodiment, the contacting is under conditions suitable for introduction of a sema3E polypeptide into the cell. In another embodiment, a method includes contacting a cell with an effective amount of a sema3E polynucleotide. In one embodiment, the contacting is under conditions suitable for introduction of a scma3E polynucleotide into the cell.

Conditions that are "suitable" for an event to occur, such as introduction of a polypeptide
10 into a cell, or "suitable" conditions are conditions that do not prevent such events from occurring. Thus, these conditions permit, enhance, facilitate, and/or are conducive to the event. As used herein, an "effective amount" relates to a sufficient amount of a sema3E polypeptide or a sema3E polynucleotide to provide the desired effect. For instance, in one embodiment an "effective amount" is an amount effective to alter certain characteristics of cells, such as
15 neutrophils. Examples of characteristics include, but are not limited to, migration. An example of migration is migration towards IL-8. The method may result in decreasing migration of the cell. In one embodiment, a cell is considered to have a decrease in migration if there is a statistically significant decrease in either migration compared to a control not contacted with the sema3E polypeptide or the sema3E polynucleotide. In one embodiment, a cell is considered to have a
20 decrease in migration if there is a decrease in proliferation or migration of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% compared to a control not contacted with the sema3E polypeptide or the sema3E polynucleotide.

A cell that may be used in the methods described herein may be *ex vivo* or *in vivo*. As used herein, "ex vivo" refers to a cell that has been removed from the body of an animal. *Ex vivo*
25 cells include, for instance, primary cells (e.g., cells that have recently been removed from a subject and are capable of limited growth in tissue culture medium), and cultured cells (e.g., cells that are capable of long term culture in tissue culture medium). Examples of primary cells include cells normally present in an animal's gastrointestinal tract, including, but not limited to, immune cells (such as neutrophils and macrophages, CD4 and CD8+ T cells, NKT cells, mast
30 cells, intraepithelial T cells, T regulatory cells), and also enterochromaffin cells, paneth cells, and goblet cells. Examples of cultured cells include, but are not limited to, neutrophils and

macrophages, CD4 and CD8+ T cells, NKT cells, mast cells, intraepithelial T cells, T regulatory cells), and also enterochromaffin cells, paneth cells, and goblet cells. Control cells may be obtained from the ATCC and may be cultured according to methods known in the art. Control cells may also be obtained from tissue samples through, for example, biopsy. As used herein, "in vivo" refers to a cell that is present within an animal. A cell that may be used in the methods described herein may be a mammalian cell, such as, for instance, mouse, rat, primate (e.g., monkey, human), or a dog, a sheep, a guinea pig, or a horse.

The present invention also includes methods for treating certain diseases. In one embodiment, a method includes treating a disease in a subject, where a subject in need thereof is administered an effective amount of a composition that includes a sema3E polypeptide or a sema3E polynucleotide. The subject may be a mammal, such as a member of the family Muridae (a murine animal such as rat or mouse), a primate, (e.g., monkey, human), a dog, a sheep, a guinea pig, or a horse. As used herein, the term "disease" refers to any deviation from or interruption of the normal structure or function of a part, organ, or system, or combination thereof, of a subject that is manifested by a characteristic symptom or clinical sign. Diseases include inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's disease, and indeterminate colitis.

As used herein, the term "symptom" refers to subjective evidence of disease or condition experienced by the patient and caused by disease. As used herein, the term "clinical sign," or simply "sign," refers to objective evidence of a disease present in a subject. Symptoms and/or signs associated with diseases referred to herein and the evaluation of such signs are routine and known in the art. A symptom and/or sign may be localized to, for instance, a subject's gastrointestinal tract, or a combination thereof. Whether a subject has a disease, and whether a subject is responding to treatment, may be determined by evaluation of signs associated with the disease.

In one embodiment, the method also includes evaluating a subject for signs associated with the disease after the Sema3E polypeptide is administered. Such an evaluation may be used to determine the status of disease in the subject, and/or determine if the treatment has resulted in a reduction of the disease. The evaluating may be done at various intervals after the

administering, such as 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 1 month, 2 months, 3 months, and so on, after the administering.

In one embodiment, a disease treated using a method of the present invention is an inflammatory bowel disease, such as Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's disease, and indeterminate colitis. Signs of inflammatory bowel disease, may include, but are not limited to, abdominal pain, vomiting, diarrhea, rectal bleeding, intestinal cramps, weight loss, and anemia.

In one embodiment, the methods include contacting cells of a subject's gastrointestinal tract with an effective amount of a composition that includes a sema3E polypeptide or a sema3E polynucleotide. The method may result in decreasing migration of cells, such as neutrophils. In one embodiment, the subject may be suffering from, or at risk of suffering from, inflammatory bowel disease.

In one embodiment, provided herein are methods for decreasing the risk of developing colonic carcinoma. Without intending to be limited by theory, low amounts of Sema3E in the context of chronic inflammation of the colon and/or ileum may perpetuate the inflammatory process and lead in the long-term to the development of colonic cancer. The method includes identifying a subject at risk of developing colonic carcinoma. In one embodiment, a subject may have gastrointestinal dysplasia (a pre-cancerous lesion). The method includes administering to a subject in need thereof an effective amount of a composition that includes a sema3E polypeptide or a sema3E polynucleotide. Patients with inflammatory bowel disease have been reported to have an increased risk of colon carcinoma (Nieminen et al., 2014, Int J Cancer, 134(1): 198-196), and risk factors include the severity of inflammation, a family history of bowel cancer, and disease duration (Mason and Siegel, 2013, Inflamm. Bowel Dis., 19:1306-21). By regulating Sema3E, it may be possible to decrease colonic inflammation and diminish the risk of developing colonic carcinoma.

Treatment of a disease can be prophylactic or, alternatively, can be initiated after the development of a disease. Treatment that is prophylactic, for instance, initiated before a subject manifests signs of a disease, is referred to herein as treatment of a subject that is "at risk" of developing a disease. An example of a subject that is at risk of developing a disease is a person having a risk factor. Examples of risk factors include genetic susceptibilities including, but not limited to, coding mutations in JTNI, SLC22A4, NDD2, ATGI6L1, XBP1, NOD2, CARD9,

FCGR2A, MST1, ERAP2, IL23R, TNFSF15, IL7R, IL27, GPX1, H5PA5, UTS2, PEX13, THADA, AND GCKR. Examples of other risk factors include age, ethnicity, diet, family history, and parasite exposure. Treatment can be performed before, during, or after the occurrence of the diseases described herein. Treatment initiated after the development of a disease may result in decreasing the severity of the signs of the disease, or completely removing the signs. An "effective amount" may be an amount effective to alleviate one or more symptoms and/or signs of the disease. In one embodiment, an effective amount is an amount that is sufficient to effect a reduction in a symptom and/or sign associated with a disease. A reduction in a symptom and/or a sign is, for instance, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% in a measured sign as compared to a control, a non-treated subject, or the subject prior to administration of the sema3E polypeptide. It will be understood, however, that the total daily usage of the compositions and formulations as disclosed herein will be decided by the attending physician within the scope of sound medical judgment. The exact amount required will vary depending on factors such as the type of disease being treated.

The polypeptides and/or polynucleotides described herein may also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. Therapeutic compounds useful for the treatment of the diseases described herein are known and used routinely. Therapeutic compounds may include, but are not limited to, Aminosalicylates (such as mesalazine or 5-aminosalicylic acid), corticoids (such as oral prednisolone, prednisonm budesonide or intravenous hydrocortisnem methylprednisolone or topical suppositories, foam or liquid anemas include hydrocortisone, prednisolone, metasulphobenzoate, betamethasone, budesonide), immunosuppressive compounds (such as ciclosporin or tacrolimus), chimeric monoclonal therapy (an anti-TNF monoclonal antibody such as Infleximab), antibiotics (metronidazole, ciprofloxacin), thipopurines (azathioprine or mercaptopurin), or methotrexate.

The present invention also includes methods for diagnosing whether a subject has, or is at risk of having, an inflammatory bowel disease. The method may include measuring the expression of a sema3E polypeptide present in a biological sample. In one embodiment, the biological sample includes a cell present in, or explanted from, the gastrointestinal tract of a subject. In one embodiment, the biological sample includes a fluid from the gastrointestinal tract

of a subject, such as the colon or ileum. In one embodiment, the biological sample includes a combination of fluid and cells from the gastrointestinal tract of a subject. A decrease of semaphorin 3E in the biological sample compared to a control biological sample indicates the subject has, or is at risk of having, an inflammatory bowel disease. For instance, a decrease of semaphorin 3E in the cell relative to a control cell indicates the subject has, or is at risk of having, an inflammatory bowel disease. Optionally, the method also includes further evaluation of symptoms and/or signs of the disease in the subject; however, altered sema3E levels may be detected when the disease is in a preclinical stage and subject does not present with symptoms and/or signs of an inflammatory bowel disease. Optionally, the method also includes obtaining a biological sample from the subject. Optionally, the method further includes counseling the subject regarding treatment options and/or treating the subject.

Also provided are methods for evaluating treatment options for a subject having inflammatory bowel disease. For instance, such a method may indicate that treatment with a sema3E polypeptide or a sema3E polynucleotide is appropriate. The method includes measuring the expression of a sema3E polypeptide in a biological sample. In one embodiment, the biological sample includes a cell present in, or explanted from, the gastrointestinal tract of a subject. In one embodiment, the biological sample includes a fluid from the gastrointestinal tract of a subject, such as the colon or ileum. In one embodiment, the biological sample includes a combination of fluid and cells from the gastrointestinal tract of a subject. A decrease of semaphorin 3E in the biological sample compared to a control biological sample indicates the subject has, or is at risk of having, an inflammatory bowel disease. For instance, a decrease of semaphorin 3E in the cell relative to a control cell indicates the subject has, or is at risk of having, inflammatory bowel disease. Optionally, the method also includes further evaluation of symptoms and/or signs of the disease in the subject; however, altered sema3E levels may be detected when the disease is in a preclinical stage and subject does not present with symptoms and/or signs of an inflammatory bowel disease. Optionally, the method also includes obtaining a biological sample from the subject. Optionally, the method further includes evaluating treatment options and/or counseling the subject regarding treatment options and/or treating the subject.

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from a subject, including but not limited to, for example, cells, and tissues such as biopsy samples, from a gastrointestinal tract, such as macrophages, CD4 and CD8+ T cells, NKT cells, mast cells,

intraepithelial T cells, T regulatory cells, enterochromaffin cells, paneth cells, and goblet cells. Biological samples also include explants and primary and/or transformed cell cultures derived from patient tissues. A biological sample can be provided by removing a sample of cells or a fluid from a subject, but can also be accomplished by using previously isolated cells (e.g.,
5 isolated by another person, at another time, and/or for another purpose). Methods for measuring the amount of polypeptides such as semaphorin 3E are known in the art and are routine. Such methods include, for instance, Western immunoblot, ELISA, immunoprecipitation, or immunohistochemistry. Western immunoblot and immunoprecipitation are generally used with *ex vivo* cells, and immunohistochemistry is generally used with *in vivo* or *ex vivo* cells. Antibody
10 to semaphorin 3E is commercially available.

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

Example 1

Inflammatory bowel diseases (IBD) are idiopathic, chronic intestinal disorders of complex pathogenesis. The etiopathogenesis of IBD is multifactorial, involving an aberrant
20 immune response to some environmental antigen in genetically predisposed individuals. Current treatments are expensive and require indefinite use yet many IBD patients remain poorly controlled. Functional alteration of angiogenesis and enhanced inflammatory cell recruitment have been described previously in patients with IBD. Semaphorin was originally identified as an axon guidance factor implicated in angiogenesis and recent studies indicate that several members
25 of the semaphorin family can modulate various phases of immune responses in conditions such as chronic obstructive pulmonary disease and arthritis. Recently, Semaphorin 3E (Sema3E) and its receptors plexinD1 (PLXD1) have emerged as an essential axis involved in some immunoinflammatory responses. However, the role of sema3E in IBD is not known. We determined whether the absence of Sema3E alters inflammation in mice with colitis, the
30 mediating role of neutrophil signaling. An experimental model of colitis was used in C57BL/6 mice to mimic ulcerative colitis. The lack of Sema3E increased mucosal inflammation associated

with increased colonic pro-inflammatory cytokine secretion. Moreover, the absence of Sema3E increased the colonic myeloperoxidase activity (a marker of polymorphonuclear neutrophil infiltration). IL-8-induced migration of human neutrophils was inhibited by Sema3E. Sema3E plays a counter-inflammatory role in acute colitis via a neutrophil-dependent mechanism. The identification of a sema3E pathway would open new therapeutic avenues for treating acute exacerbations of IBD.

As will be appreciated by one of skill in the art, much of the recent progress in the understanding of immunity has been achieved by the study of experimental animal models of intestinal inflammation. Although these models do not represent the complexity of human disease and do not replace studies with patient material, they are valuable tools for studying many important disease aspects that are difficult to address in humans. The most widely used and characterized experimental model of UC is the DSS-induced colitis, which was developed by administration of DSS in the drinking water. DSS induces a very reproducible acute colitis characterized by superficial inflammation and infiltrations.

Although previous observations clearly implicate macrophages (Ananthakrishnan et al., 2010, J Clin Gastroenterol, 44:272-279, Catalano 2010, J Immunol, 185:6373-6383) and T cells (Brannigan et al., 2000, Shock 13:361-6) in the context of colitis, it is not clear whether changes in neutrophils response play a role in regulating gut inflammation by Sema3E signaling. Our data demonstrate that the lack of Sema3E increased the number of neutrophils. Inflammation in IBD is characterized by mucosal recruitment of variety of inflammatory cells including T lymphocytes, macrophages, dendritic cells, neutrophils, and plasma cells. The persistent release of inflammatory mediators promotes adhesion, migration and activation of immune and inflammatory cells and causes tissue damage. A hallmark of IBD is a marked accumulation of myeloid cells particularly neutrophils and inflammatory monocytes. Histology (Morson BC, Dawson IMP. Gastrointestinal Pathology. Blackwell Scientific Publication. Oxford 1989) and radioisotope-labelled leukocyte scans (Saverymuttu et al., 1986, Gastroenterology 90:1 121-8, Segal, 1981, Lancet 2:230-2) show infiltration of leukocyte, particularly neutrophils, into the inflamed intestinal mucosa of patients with IBD (Carlson et al., 2002, Gut 50:501-6, Izzo et al., 1992, Am J Gastroenterol 87:1447-52). The accumulation of neutrophils in the intestinal mucosa may be a result both from increased recruitment of neutrophils and from defective apoptosis (Brannigan et al, 2000, Shock 13:361-6).

Neutrophils are key cells of the innate immune system and perform a key role in activation of immune responses and generation of gut inflammation. Neutrophils are released and migrate from the bone marrow towards site of inflammation under various chemoattractant factors stimuli. Generally, the recruitment of neutrophils from the blood into inflamed tissue is regulated by various chemotactic factors. A number of different neutrophil chemotactic agents have been shown to be overproduced in mucosa affected by UC, including IL-8 (Raab et al., Gut 34:1203-6), TNF- α (Tsukada et al, 20002, Am J Gastroenterol 97:2820-8), macrophage inflammatory protein 1 α (Banks et al., 2003, J Pathol 199:28-35), and leukotriene B4 (Jupp et al, 2007, Inflamm Bowel Dis 13:537-46). Under inflammatory circumstances, the production of these proteins occurs by epithelial cells and various immune cells, but neutrophils are also a major source of proinflammatory mediators including cytokines (IL-8, IL-6, IL- β and TNF- α) (Hatanaka et al., 2006, Clin Exp Immunol 146:443-7), collectively, selective depletion or adhesion blockade of neutrophil by monoclonal antibody suppresses DSS-colitis (Kuhl et al., 2007, Gastroenterology 133:1882-92, Natsui et al., 1997, J Gastroenterol Hepatol 12:801-8).

Despite strong evidence of the pathophysiological role of neutrophils in colitis, little is known about the key mechanisms that regulate neutrophils cell migration to the gut inflamed tissue. What role Sema3E plays in neutrophil recruitment to the gut is unknown.

Our findings prompt close consideration of the relationship between Sema3E and disease activity in patients with IBD and have important clinical relevance. IBD patients might be selected for novel treatment strategies including Sema3E protein, polynucleotides that encode Sema3E protein.

Methods

Animals. Male C57BL/6 (7-9 weeks old) were obtained from the University of Manitoba breeding facility (Winnipeg, Canada) and maintained in the animal care facility under specific pathogen-free conditions. No differences in food intake or body weight were observed between the groups. All experiments were approved by the University of Manitoba animal ethics committee (10-073) and conducted under the Canadian guidelines for animal research.

Induction of DSS colitis. DSS (molecular weight, 40 kilodaltons: ICN Biomedicals Inc) was added to the drinking water in a final concentration of 5% (wt/vol) for 5 days (Casazza et al., 2010, J Clin Invest, 120:2684-98, Gitler et al, 2004, Dev Cell, 7:107-16). Controls were all time-

matched and consisted of mice that received normal drinking water only. Mean DSS consumption was noted per cage each day.

Characterization of inflammation. Disease activity index (DAI) and macroscopic scores and colonic damage were determined using a previously described scoring system for DSS colitis (Ananthakrishnan et al., 2010, J Clin Gastroenterol, 44:272-279, Sakurai et al., 2010, Mol Cell Biol, 30:3086-98). Foimalin-fixed colon segments coming from the splenic flexure were stained with hematoxylin-eosin (Ananthakrishnan et al., 2010, J Clin Gastroenterol, 44:272-279). Myeloperoxidase (MPO) activity was determined following an established protocol (Wong et al., 2003, Nat Immunol, 4:891-898). Serum C-reactive protein (CRP) and cytokine levels were determined using an ELISA commercial kit (R&D Systems, Minneapolis, Minnesota, USA).

Statistical analysis. Results are presented as means $\pm$ SEM. Statistical analysis was performed using one or two way ANOVA followed by the Tukey-Kramer multiple comparisons post hoc analysis and a p value of <0.05 considered significant with $n=8$ to 12 depending on the groups tested (Prism 4, GraphPad).

Results

Reduced availability of Sema3E in gut decreased the severity of DSS-induced colitis

Administration of DSS in wild-type (Sema3E^{+/+}) mice induced colitis characterized by weight loss and frequent stools which was evident by day 3 post-DSS. In Sema3E^{-/-} mice, the onset of DSS-colitis was shortened as reflected in the DAI. The DAI was significantly higher in Sema3E^{-/-} mice compared to the Sema3E^{+/+} mice on days 3, 4 and 5 post-DSS. In addition, the macroscopic and histological scores were significantly increased in Sema3E^{-/-} mice as compared to that in Sema3E^{+/+} mice on day 5 after DSS treatment (Figure 2). This increase in the severity of DSS-induced colonic inflammation in Sema3E^{-/-} mice was associated with up-regulation in MPO activity in colonic tissues (Figure 1), and in the amount of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, and IL-12p40) in colon (Figure 3).

Along the same lines, IL-8-induced migration of human neutrophils was inhibited by Sema3E (Figure 4, 5) as neutrophils constitutively express plexin D1 receptor. We confirmed the presence of the Plexin D1 receptor on human neutrophil (Figure 6). These observations suggest that lack of Sema3E in Sema3E^{-/-} mice increased the severity of DSS-induced colitis and that

Sema3E plays a suppressive role in the intestinal inflammatory response at least by reducing neutrophil migration.

Summary

Based on these data we hypothesize that Sema3E induces a tonic inhibitory influence in the development of colonic inflammation potentially *via* an effect on immune cells involving cell activation, migration and survival.

Example 2

Dendritic cells (DC) and gut inflammation. DC are found throughout the spleen, mesenteric lymph nodes (MLN), the intestine where they are present at the level of the lamina propria, Peyer's patches and lymphoid follicles (Iwasaki, 2007, Annu Rev Immunol 25:381-418; Johansson and Kelsall, 2005, Semin Immunol 17:284-94.) They are present in a so-called 'immature' state and are unable to stimulate T cells. Although these DC lack the requisite accessory signals for T cell activation, such as CD40, they are extremely well equipped to capture antigens (Ag). Once primed, they migrate to the secondary lymphoid compartments (MLN or spleen) to present Ag-peptide complexes to naive CD4⁺ T cells and CD8⁺ cytotoxic T cells to initiate pathology or tolerance via an increase in expression of major histocompatibility complex (MHC) 2, CD40, CD80 and CD86 co-stimulatory molecules. In inflammatory bowel disease (IBD), the persistent release of inflammatory mediators promotes adhesion, migration and activation of immune and inflammatory cells and causes tissue damage. IBD is characterized by mucosal recruitment of a variety of immune inflammatory cells including DC. A hallmark of IBD is a marked accumulation of myeloid cells particularly monocytes and DC. Increased numbers of MDC8⁺ monocytes, which are the precursors of mucosal DC populations, are found in patients with IBD and, hence, anti-TNF treatment results in reduced DC activation (de Baey et al., 2003, J Immunol 170:5089-94; de Baey et al., 2001, Eur J Immunol 31:1646-55) Moreover, in IBD DC are increased within the lamina propria and peripheral blood; and DC isolated from peripheral blood monocytes show increased abilities to stimulate immune responses (Keda et al., 2001, Eur J Gastroenterol Hepatol 13:841-50; Murakami et al., 2002, Clin Exp Immunol 128:504-510; Yeung et al., 2000, Gut 47:215-27). As such, selective depletion or adhesion

blockade of DC by Diphtheria Toxin or anti-IL-12 treatment suppress DSS-colitis (Abe et al., 2007, Proc Natl Acad Sci USA 104:17022-7; Berndt et al., 2007, Immunol 179:6255-62). Migration of DC into inflamed tissues is regulated by various chemotactic factors (including CCL21, McNamee et al, 2013, Gut 62:53-62.; Middel et al., 2006, Gut 55:220-7, TNF, Tsukada et al, 2002, Am J Gastroenterol 97:2820-8, and leukotriene B4, Jupp et al., 2007, Inflamm Bowel Dis 13:537-46.) shown to be overproduced in IBD affected mucosa or in experimental colitis. Under inflammatory circumstances, these factors are produced by epithelial cells and various immune cells. Signals such as lipopolysaccharide (LPS), TNF- α and CD₄₀-L stimulate development of mature DC defined by a well-characterized surface phenotype and effector profile (IL-12 production) that results in type 1 helper (Th1) T cell priming function. Recently, PLXD1 receptors have been shown to be expressed on immature or mature myeloid CD11c⁺ DC. Taken together, these observations raise the possibility that some of the immunomodulatory effects of Sema3E may be partly mediated by altered DC function, and this can take place in lymphoid organs. Despite strong evidence of the pathophysiological role of DC in colitis, little is known about the key mechanisms that regulate DC migration to inflamed gut tissues. What role Sema3E plays in DC recruitment to the gut is unknown. The goal of this study was to investigate the role of Sema3E/PLXD1 axis in the recruitment, survival of DC in the gut.

Despite strong evidence of the pathophysiological role of dendritic cells in colitis, little is known about the key mechanism that regulate dendritic cell migration to the gut inflamed tissue. What role Sema3E plays any role in dendritic cell recruitment to the gut is unknown.

The results shown herein prompt close consideration of the relationship between Sema3E and disease activity in patients with IBD and have important clinical relevance. IBD patients might be selected for novel treatment strategies including Sema3E protein, polynucleotides that encode Sema3E protein.

Methods

Animals. Male C57BL/6 (7-9 weeks old) were obtained from the University of Manitoba breeding facility (Winnipeg, Canada) and maintained in the animal care facility under specific pathogen-free conditions. No differences in food intake or body weight were observed between the groups. All experiments were approved by the University of Manitoba animal ethics committee (10-073) and conducted under the Canadian guidelines for animal research.

Induction of DSS colitis. DSS (molecular weight, 40 kilodaltons: ICN Biomedicals Inc) was added to the drinking water in a final concentration of 5% (wt/vol) for 5 days (Casazza et al., 2010, J Clin Invest, 120:2684-98, Gitler et al, 2004, Dev Cell, 7:107-16). Controls were all time-matched and consisted of mice that received normal drinking water only. Mean DSS consumption was noted per cage each day.

Isolation of splenic CD11c⁺ cells and culture. Isolation of splenic CD11c⁺ cells and culture. 5 days post-activation associated DSS colitis, the spleens were digested in 2mg/mf¹ collagenase D (Roche Diagnostics, Meylan, France) in RPMI 1640 (Life Technologies, Grand Island, NY) for 30 minutes at 37°C. EDTA (Sigma) at 5mM was added during the last 5 minutes to disrupt DC-T cell complexes, and the cell suspension was filtered. Total splenocytes after RBC lysis with ACK lysis buffer (150mMNH₄Cl, 10mM KHC0₃, 0.1mM EDTA; Life Technologies) were incubated with CD11c⁺ microbeads (Miltenyi Biotec, Auburn, CA) for 15 minutes at 48°C. The cells were then washed, resuspended in cell separation buffer (Dulbecco's Phosphate-Buffered Saline [D-PBS] without Ca²⁺ and Mg²⁺ containing 2% FBS and 2mM EDTA, (Life Technologies) and passed through magnetic columns (Miltenyi Biotec) for positive selection. After passing consecutively through two columns, the collected splenic CD11c⁺ cell preparations showed greater than 95% purity. Splenic CD11c⁺ cell were cultured in complete RPMI 1640 medium (Life Technologies) containing 10% heat-inactivated FBS, 25mg/ml⁻¹ gentamicin, 2mM L-glutamine in 12-well plates at 1.10⁶ cells/well for 24hrs, and the supernatants were measured for IL-12p40, by ELISA (R&D Systems). Cell culture groups were treated with recombinant Sema3E (10ng/ml) for 24 hours.

Statistical analysis. Results are presented as means ±SEM. Statistical analysis was performed using one or two way ANOVA followed by the Tukey-Kramer multiple comparisons post hoc analysis and a p value of <0.05 considered significant with n=8 to 12 depending on the groups tested (Prism 4, GraphPad).

Results

To gain further insight into the cellular mechanisms mediating these effects on colitis we studied the role of splenocytes and more specifically splenic CD11c⁺ cells. IL-12p40 levels in cultured splenocytes supernatant from non-colitic Sema3E^{-/-} mice (Figure 7) were significantly increased as compared to non-colitic Sema3E^{+/+} mice. The level of INF-g was not affected.

Moreover, IL-12p40 and INF-g levels in cultured splenocytes supernatant from colitic Sema3E^{-/-} mice were significantly increased as compared to colitic Sema3E^{+/-} mice (Figure 7).

Along the same line, an approach targeting more specifically the splenic CD11c⁺ cells and IL-12p40 demonstrated an increase of IL-12p40 in Sema3E^{-/-} mice compared to Sema3E^{+/-} in non-colitic and colitic condition (Figure 8). We confirmed the higher release of IL-12p40 from bone marrow isolated from Sema3E^{-/-} mice (Figure 9).

Splenic CD11c⁺ cells incubated *ex vivo* Sema3E Fc Immunoglobulin recombinant protein (10 nM) demonstrated a significant decrease of IL-12p40 after 24h, in non-colitic and colitic Sema3E^{-/-} and Sema3E^{+/-} mice (Figure 8).

Moreover, *in vitro* stimulation of bone marrow progenitor cells isolated from Sema3E^{-/-} (KO) mice with GM-CSF induced more DC differentiation compared to wild type (WT) mice according to CD11c⁺ staining at the same condition. In addition, basal and chemokine-induced migration (CCL-21) of bone marrow-derived cell (BMDC) from Sema3E KO mice was significantly higher than BMDC isolated from WT mice. Finally, bone marrow progenitor cells isolated from Sema3E KO mice and stimulated with GM-CSF have higher antigen uptake capacity which is decreased upon Sema3E treatment (Figure 10).

Summary

Based on these data we hypothesize that Sema3E induces a tonic inhibitory influence in the development of colonic inflammation potentially *via* an effect on immune cells involving cell activation, migration and survival related to dendritic cells.

Example 3

Methods

Animals. Male C57BL/6 (7-9 weeks old) were obtained from the University of Manitoba breeding facility (Winnipeg, Canada) and maintained in the animal care facility under specific pathogen-free conditions. No differences in food intake or body weight were observed between the groups. All experiments were approved by the University of Manitoba animal ethics committee (10-073) and conducted under the Canadian guidelines for animal research.

Induction of DSS colitis. DSS (molecular weight, 40 kilodaltons: ICN Biomedicals Inc)

was added to the drinking water in a final concentration of 5% (wt/vol) for 5 days (Casazza et al., 2010, J Clin Invest, 120:2684-98, Gitler et al, 2004, Dev Cell, 7:107-16). Controls were all time-matched and consisted of mice that received normal drinking water only. Mean DSS consumption was noted per cage each day.

5 Induction of DNBS study. Mice were anaesthetized using Isoflurane® (Abbott, Toronto, Canada). PE-90 tubing (10 cm long; ClayAdam, Parispany, NJ) that was attached to a tuberculin syringe (BD, Mississauga, Canada) was inserted 3.5 cm into the colon. Colitis was induced by administration of 100µl of 4mg of DNBS solution (ICN Biomedical Inc. Aurora, OH) in 30% ethanol (Sigma, Mississauga, Canada) and left for 3 days. For the DNBS study mice
10 were supplied with 6% sucrose in their drinking water to prevent dehydration.

Sema 3E levels. Colonic samples were homogenized in 700µl of Tris- HC1 buffer containing protease inhibitors (Sigma, Mississauga, Canada). Samples were centrifuged for 30 min, and the supernatant was frozen at -80°C until assay. Commercial ELISA were used to determine Sema3E levels (R&D Systems, Minneapolis, MN).

15 Treatments. WT were treated with PLXD1 Fc recombinant protein (10 µg/kg/day, i.p., n=3; R&D Systems) for 6 days, starting one day before induction. Sema3E^{-/-}(KO) mice were treated with Sema3E Fc Ig recombinant protein (10 µg/kg/day, i.p., n=3; R&D Systems) for 6 days starting one day before induction.

Characterization of inflammation. Disease activity index (DAI) and macroscopic scores
20 and colonic damage were determined using a previously described scoring system for DSS colitis (Ananthakrishnan et al., 2010, J Clin Gastroenterol, 44:272-279, Sakurai et al, 2010, Mol Cell Biol, 30:3086-98). Formalin-fixed colon segments coming from the splenic flexure were stained with hematoxylin-eosin (Ananthakrishnan et al., 2010, J Clin Gastroenterol, 44:272-279). Myeloperoxidase (MPO) activity was determined following an established protocol (Wong et al.,
25 2003, Nat Immunol, 4:891-898). Serum C-reactive protein (CRP) and cytokine levels were determined using an ELISA commercial kit (R&D Systems, Minneapolis, Minnesota, USA).

Statistical analysis. Results are presented as means ±SEM. Statistical analysis was performed using one or two way ANOVA followed by the Tukey-Kramer multiple comparisons post hoc analysis and a p value of <0.05 considered significant with n=8 to 12 depending on the
30 groups tested (Prism 4, GraphPad).

Results

Using two different models of colitis we have demonstrated a significant decrease of Sema3E within the colon in colitic conditions (Figure 11).

Administration of PLXD 1 Fc recombinant protein in wild-type (Sema3E^{+/+}) mice increased significantly the colitis. This increase in the severity of DSS-induced colonic inflammation in WT treated mice was associated with up-regulation in MPO activity in colonic tissues (Figure 1), and in the amount of pro-inflammatory cytokines (TNF-a, IL-1 β , IL-6, and IL-12p40) in colon (Figure 3) .

Administration of Sema3E Fc Ig recombinant protein to Sema3E^{-/-} mice significantly decreased the colitis. This decrease in the severity of DSS-induced colonic inflammation in Sema3E^{-/-} mice treated mice was associated with down-regulation in MPO activity in colonic tissues (Figure 1), and in the amount of pro-inflammatory cytokines (TNF-a, IL-1 β , IL-6, and IL-12p40) in colon (Figure 3) .

Efficacy of the treatment was verified by assessing the level of Sema3E within the colon. Wild-type (Sema3E^{+/+}) mice treated with PLXD1 Fc recombinant protein, the level of coloninc Sema3E was significantly decreased, conversely, in Sema3E^{-/-} mice treated with Sema3E Fc Ig recombinant the level of Smea3E was significantly restored (Figure 12).

Administration of Sema3E to colitic wild-type (Sema3E^{+/+}), demonstrated a significant decrease of MPO activity (Figure 13) and pro-inflammatory cytokine (Figure 14).

Summary

Based on these data we hypothesize that Sema3E induces a tonic inhibitory influence in the development of colonic inflammation.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the claims.

The complete disclosure of all patents, patent applications, and publications, and electronically available material (including, for instance, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid sequence submissions in, e.g., SwissProt, PIR, PRF,

PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference in their entirety. Supplementary materials referenced in publications (such as supplementary tables, supplementary figures, supplementary materials and methods, and/or supplementary experimental data) are likewise incorporated by reference in their entirety.

5 In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one
10 skilled in the art will be included within the invention defined by the claims.

Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary
15 depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope
20 of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. All numerical values, however, inherently contain a range necessarily resulting from the standard deviation found in their respective testing measurements.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

25

What is claimed is:

1. Use of a sema3E polypeptide in the preparation of a medicament for an inflammatory bowel disease.
2. Use of a composition comprising a sema3E polypeptide and a pharmaceutically acceptable carrier for treating an inflammatory bowel disease.
3. Use of a sema3E polynucleotide in the preparation of a medicament for an inflammatory bowel disease, wherein the sema3E polynucleotide encodes a sema3E polypeptide.
4. Use of a composition comprising a sema3E polynucleotide and a pharmaceutically acceptable carrier for treating an inflammatory bowel disease, wherein the sema3E polynucleotide encodes a sema3E polypeptide.
5. The use of claim 1, 2, 3, or 4 wherein the sema3E polypeptide comprises a KRRXRR consensus site, wherein X is any amino acid.
6. The use of claim 5 wherein the sema3E polypeptide further comprises an RXXR consensus site, wherein X is any amino acid.
7. The use of claim 6 wherein the sema3E polypeptide further comprises a second RXXR consensus site, wherein X is any amino acid.
8. The use of claim 1, 2, 3, or 4 wherein the sema3E polypeptide comprises a Sema domain.
9. The use of claim 8 wherein the sema3E polypeptide further comprises one or more domains selected from a cystine rich domain, an immunoglobulin domain, and a short basic domain.

10. The use of claim 8 wherein the sema3E polypeptide further comprises a cystine rich domain and an immunoglobulin domain.
11. The use of claim 1, 2, 3, or 4 wherein the sema3E polypeptide comprises an amino acid sequence having at least 80% identity with SEQ ID NO:2, wherein the sema3E polypeptide has sema3E activity, wherein the sema3E activity is binding Plexin D1 with an apparent K_D of <2 nM.
12. The use of claim 1, 2, 3, or 4 wherein the inflammatory bowel disease is selected from Crohn's disease and ulcerative colitis.
13. The use of claim 1, 2, 3, or 4 wherein the sema3E polypeptide is a fusion polypeptide.
14. The use of claim 1, 2, 3, or 4 wherein the sema3E polypeptide has activity when determined by ability to bind to Plexin D1, or ability to inhibit neutrophil migration towards IL-8.
15. The use of claim 3 or 4 wherein the sema3E polynucleotide is present in a vector.
16. The use of claim 15 wherein the vector is a viral vector.
17. A method for treating an inflammatory bowel disease in a subject, comprising administering to a subject in need thereof an effective amount of a composition comprising a sema3E polypeptide.
18. A method for treating a subject having, or at risk of having, inflammatory bowel disease, comprising:
administering to a subject in need thereof a composition comprising sema3E polypeptide, wherein the subject has decreased inflammatory markers in stool, decreased pathological lesions

associated with inflammatory bowel disease, or a combination thereof, when compared to the subject before the administering.

19. A method for treating an inflammatory bowel disease in a subject, comprising administering to a subject in need thereof an effective amount of a composition comprising a sema3E polynucleotide, wherein the sema3E polynucleotide encodes a sema3E polypeptide.

20. A method for treating a subject having, or at risk of having, inflammatory bowel disease, comprising:

administering to a subject in need thereof a composition comprising sema3E polynucleotide, wherein the subject has decreased inflammatory markers in stool, decreased pathological lesions associated with inflammatory bowel disease, or a combination thereof, when compared to the subject before the administering, wherein the sema3E polynucleotide encodes a sema3E polypeptide.

21. The method of any of claims 17, 18, 19, or 20 wherein the subject is a human.

22. The method of any of claims 17, 18, 19, or 20 wherein the method further comprises administering a therapeutic compound.

23. The method of claim 22 wherein the therapeutic compound is selected from an aminosalicylate, a corticoid, an immunosuppressive compound, a therapeutic antibody, an antibiotic, a thiopurine, a methotrexate, or a combination thereof.

24. The method of claim 17 wherein the subject has, or at risk of having, an inflammatory bowel disease.

25. The method of claim 24 wherein the inflammatory bowel disease is selected from Crohn's disease or ulcerative bowel disease.

26. The method of any of claims 17, 18, 19, or 20 wherein the sema3E polypeptide comprises a KRRXRR consensus site, wherein X is any amino acid.
27. The method of claim 26 wherein the sema3E polypeptide further comprises an RXXR consensus site, wherein X is any amino acid.
28. The method of claim 27 wherein the sema3E polypeptide further comprises a second RXXR consensus site, wherein X is any amino acid.
29. The method of any of claims 17, 18, 19, or 20 wherein the sema3E polypeptide comprises a Sema domain.
30. The method of claim 29 wherein the sema3E polypeptide further comprises one or more domains selected from a cystine rich domain, an immunoglobulin domain, and a short basic domain.
31. The use of claim 29 wherein the sema3E polypeptide further comprises a cystine rich domain and an immunoglobulin domain.
32. The method of any of claims 17, 18, 19, or 20 wherein the sema3E polypeptide comprises an amino acid sequence having at least 80% identity with SEQ ID NO:2, wherein the sema3E polypeptide has sema3E activity, wherein the sema3E activity is binding Plexin D1 with an apparent K_D of <2 nM.
33. The method of any of claims 17, 18, 19, or 20 wherein the sema3E polypeptide is a fusion polypeptide.
34. The method of any of claims 17, 18, 19, or 20 wherein the sema3E polypeptide has activity when determined by ability to bind to Plexin D1, or ability to inhibit neutrophil migration towards IL-8

35. The use of claim 19 or 20 wherein the sema3E polynucleotide is present in a vector.
36. The use of claim 35 wherein the vector is a viral vector.
37. A method for evaluating treatment options for a subject having inflammatory bowel disease comprising:
obtaining a biological sample from the subject;
measuring the level of sema3E polypeptide in the biological sample; and
comparing the level of sema3E polypeptide in the biological sample with the level of sema3E polypeptide in a control biological sample obtained from a healthy subject, wherein the presence of a decreased level of scma3E polypeptide compared to the control biological sample indicates the subject may be treated with a sema3E polypeptide.
38. The method of any of claim 37 wherein the biological sample comprises tissue from the gastrointestinal tract of the subject.
39. The method of any of claim 37 further comprising administering to the subject a sema3E polypeptide or a fragment thereof.
40. The method of claim 37 wherein the subject is a human.

Fig. 1

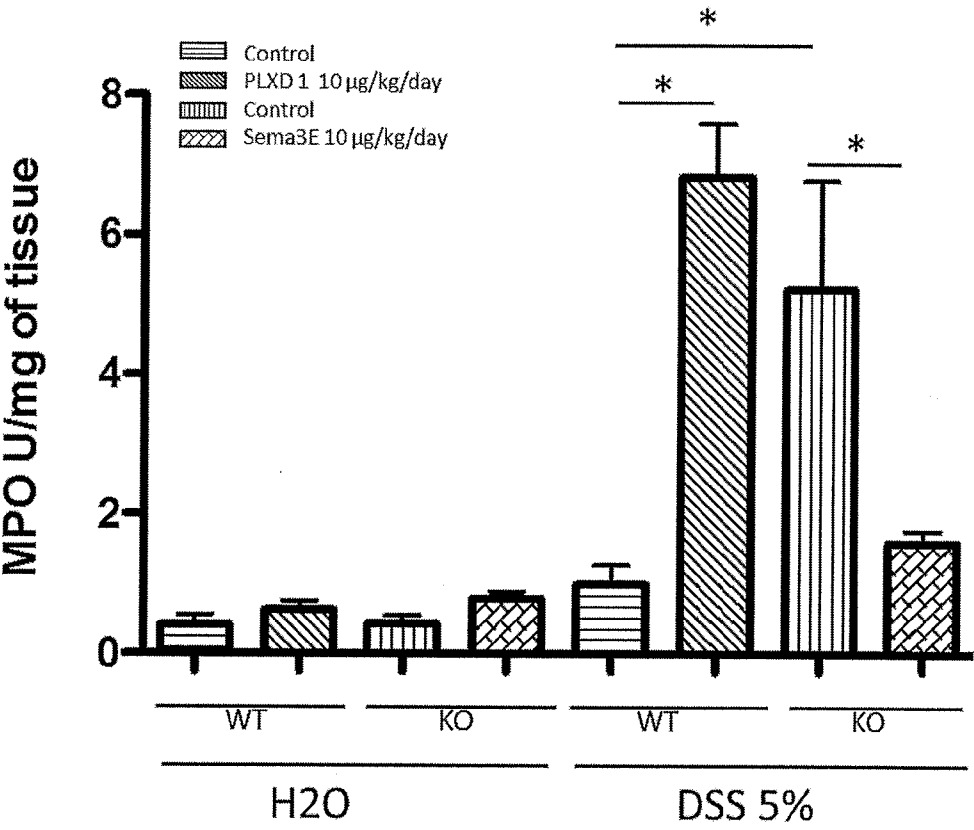


Fig. 2

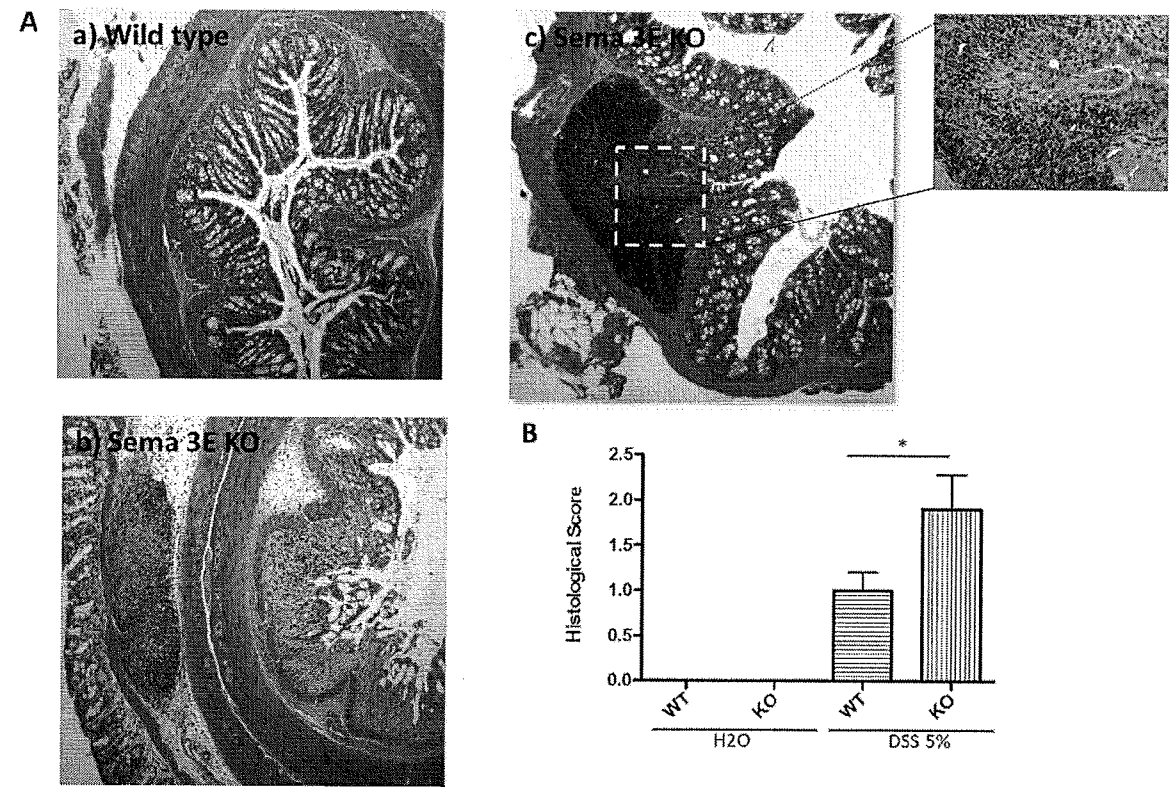


Fig. 3

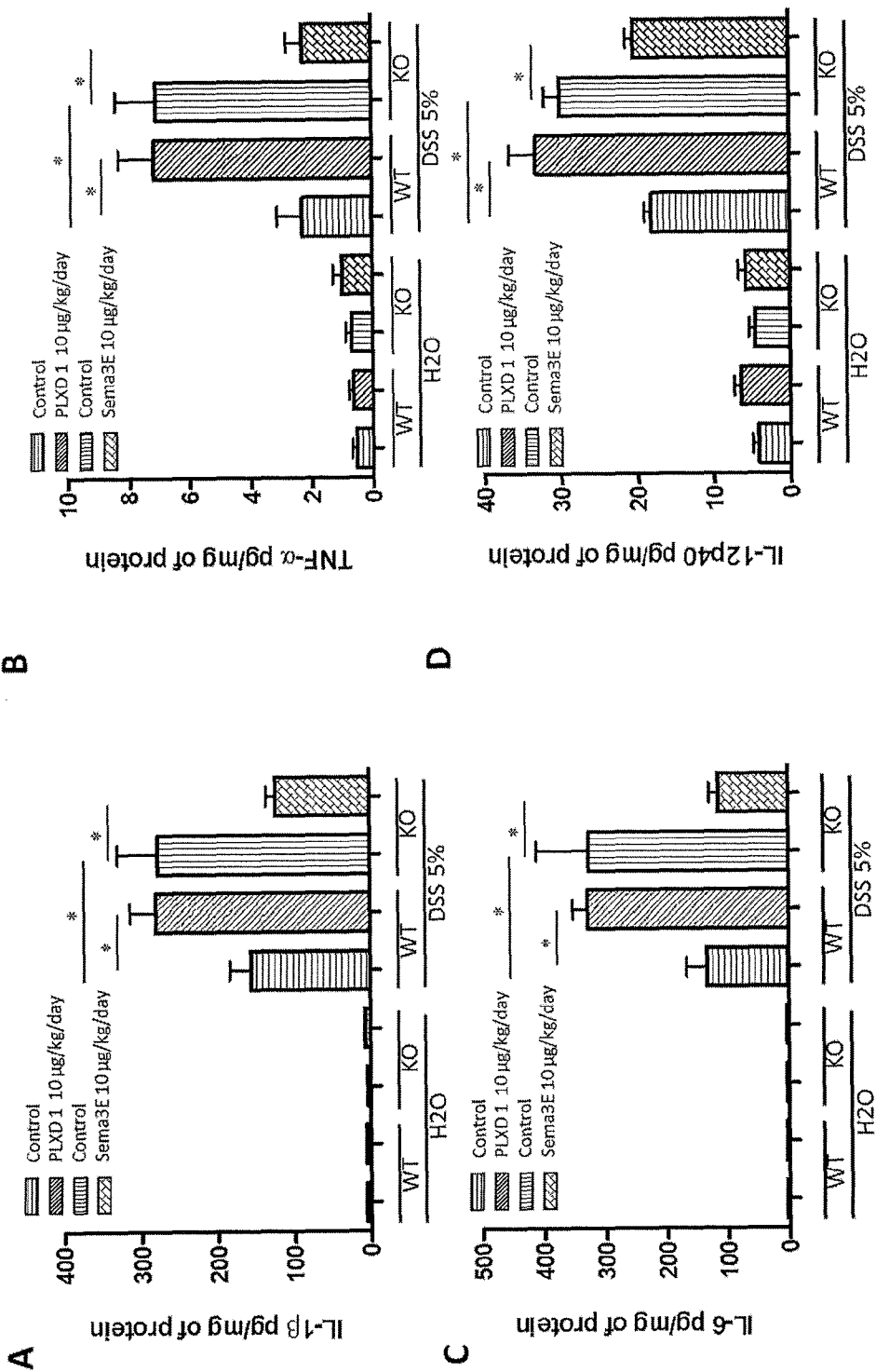


Fig. 4

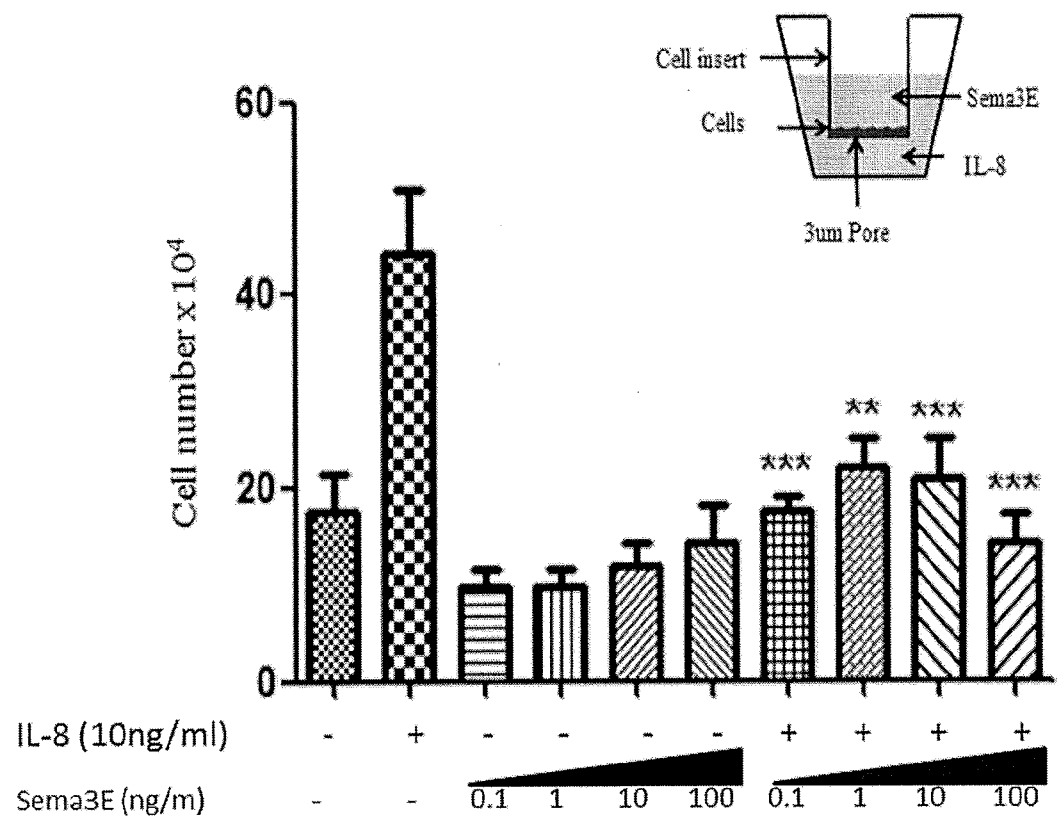


Fig. 5

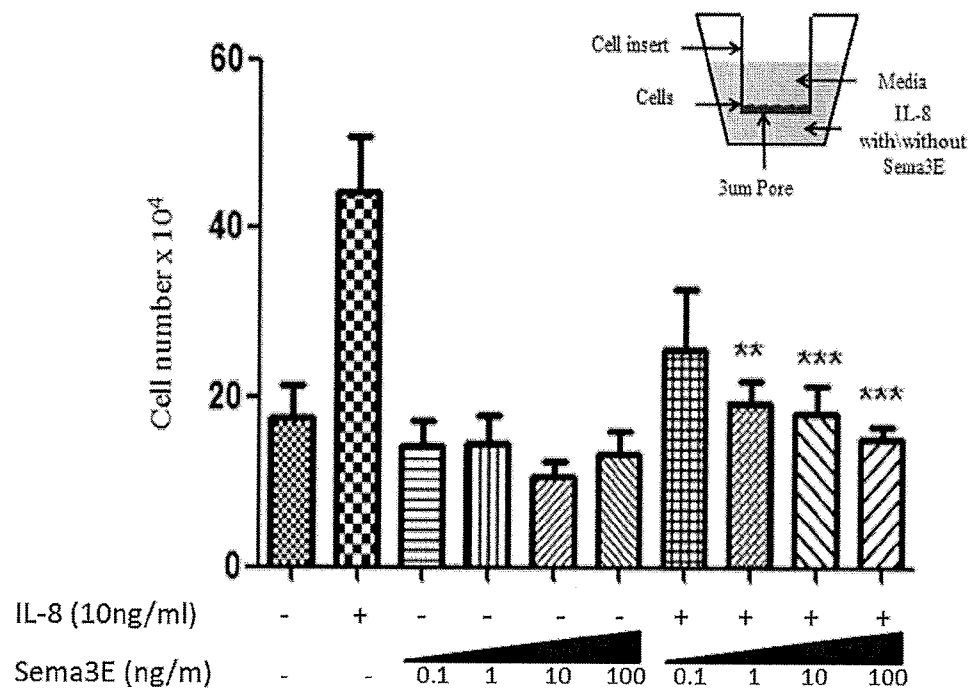


Fig. 6

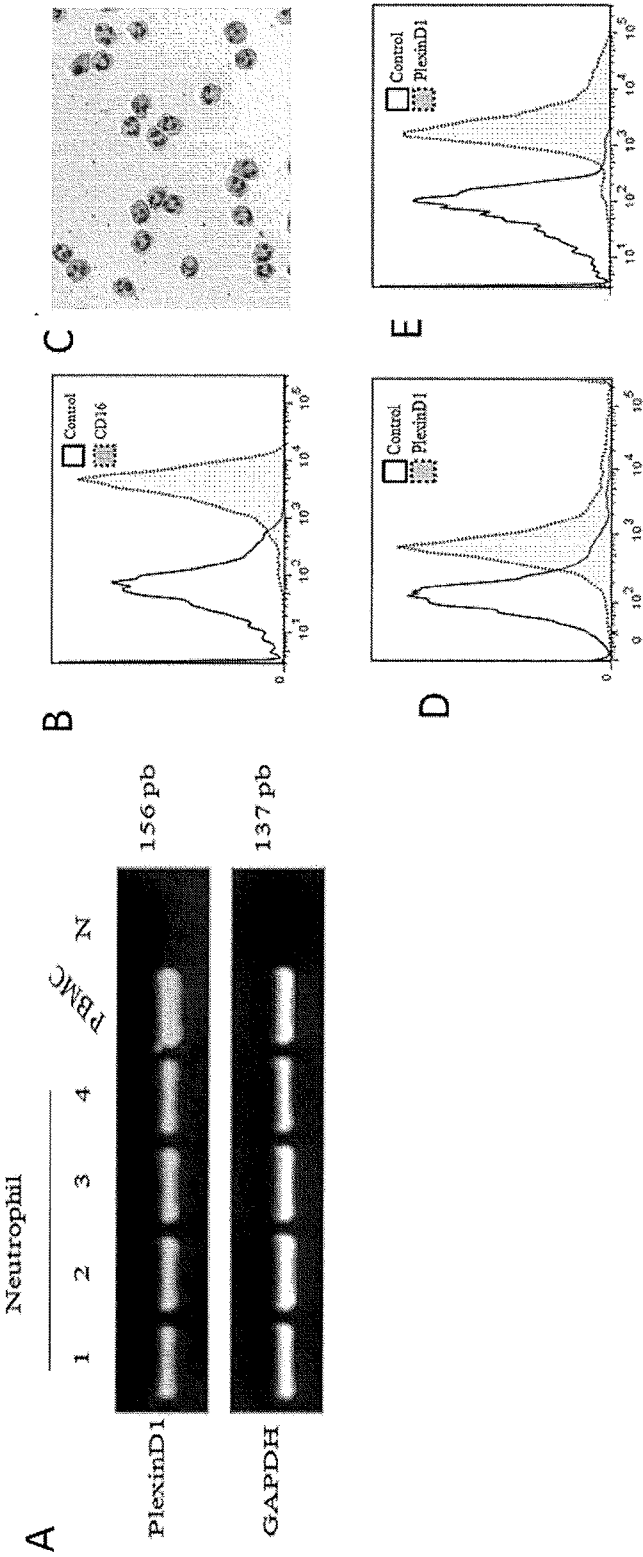


Fig. 7

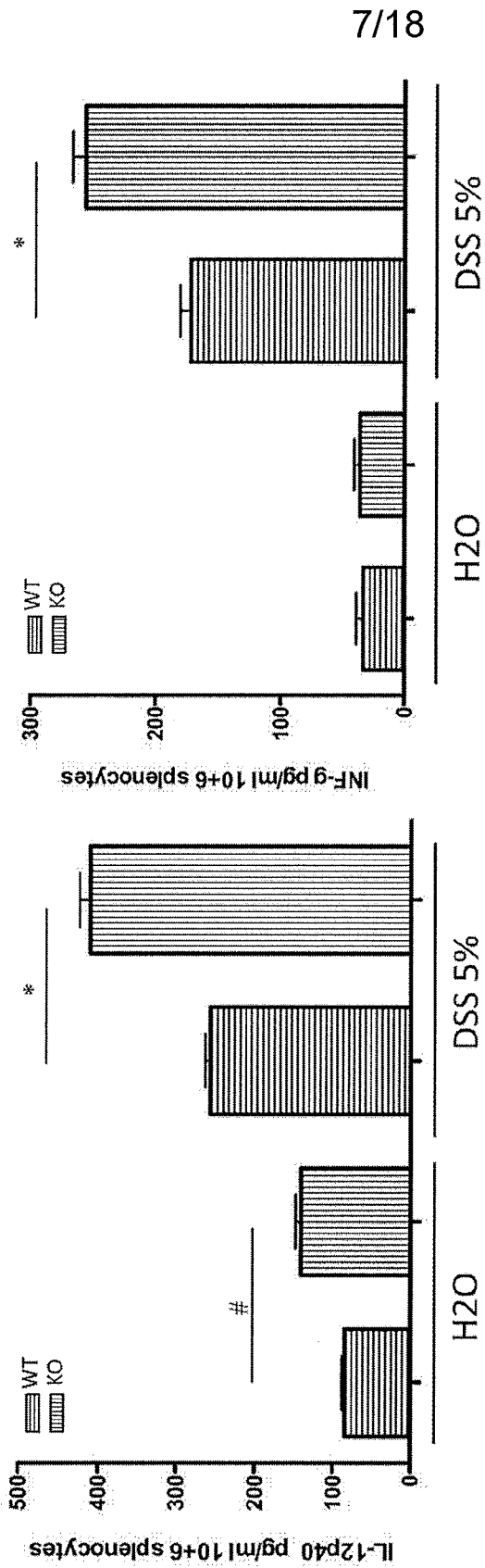
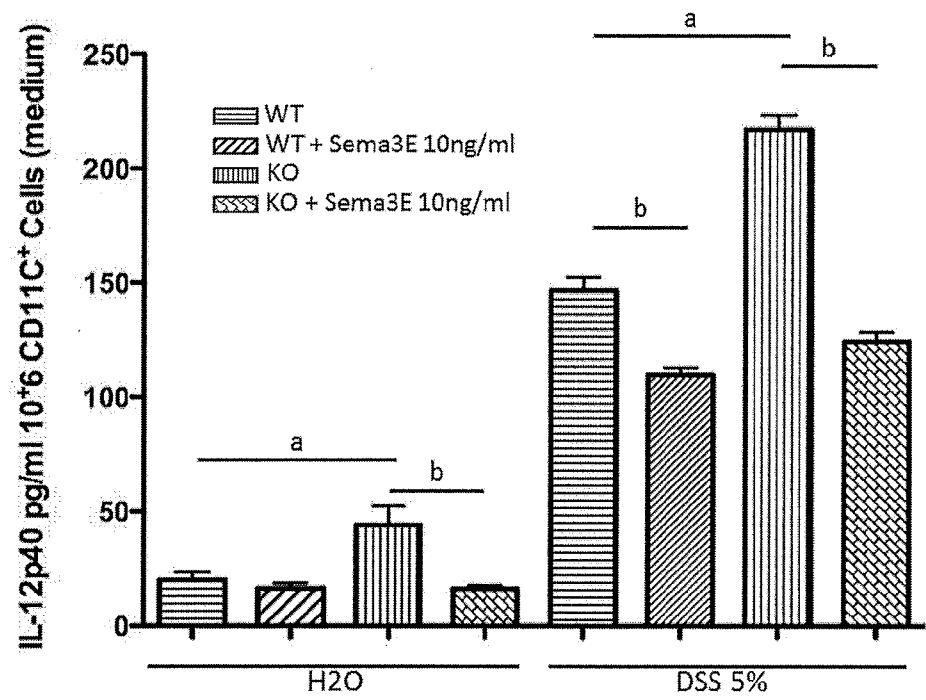
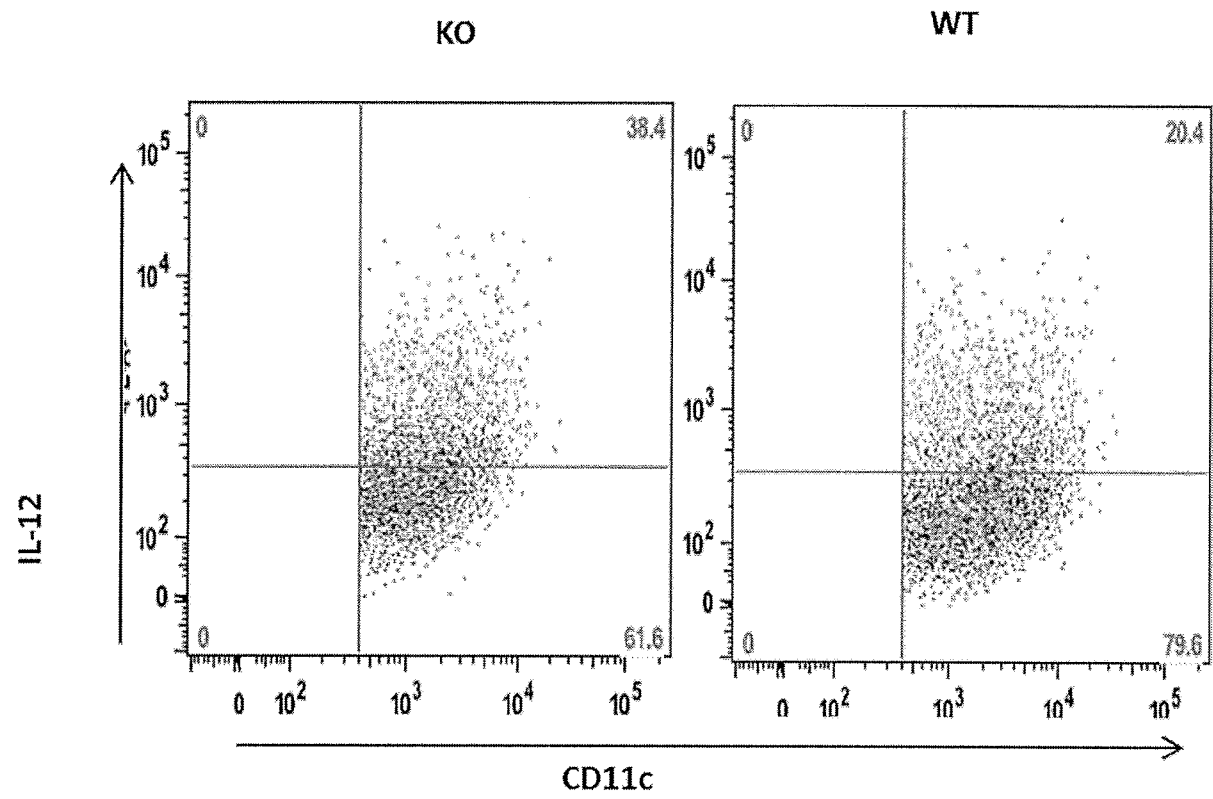


Fig. 8



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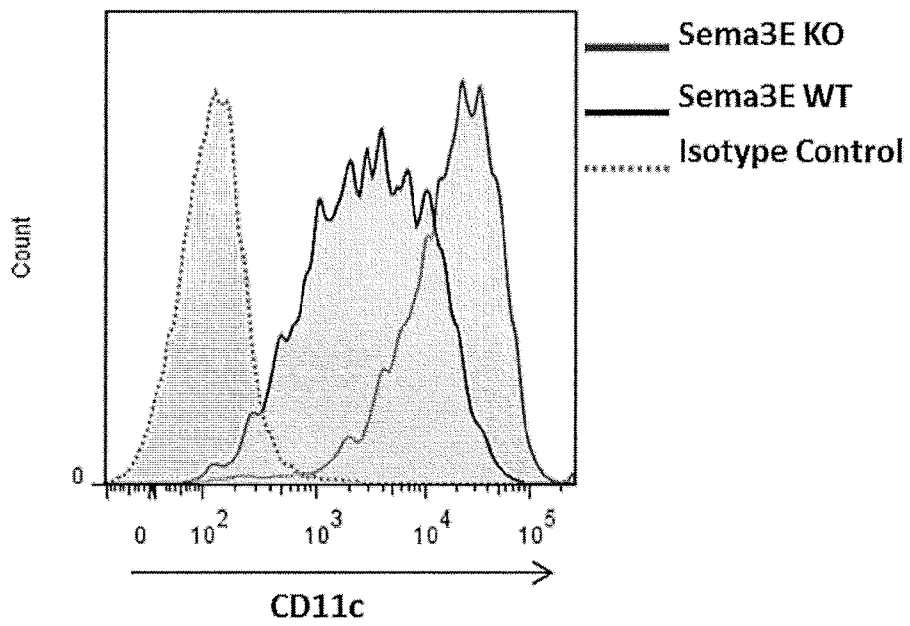
Fig. 9



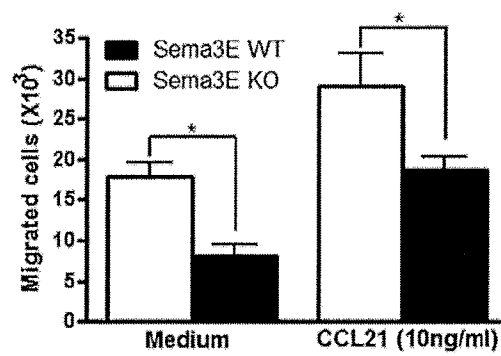
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Fig. 10-1

A



B



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Fig. 10-2

C

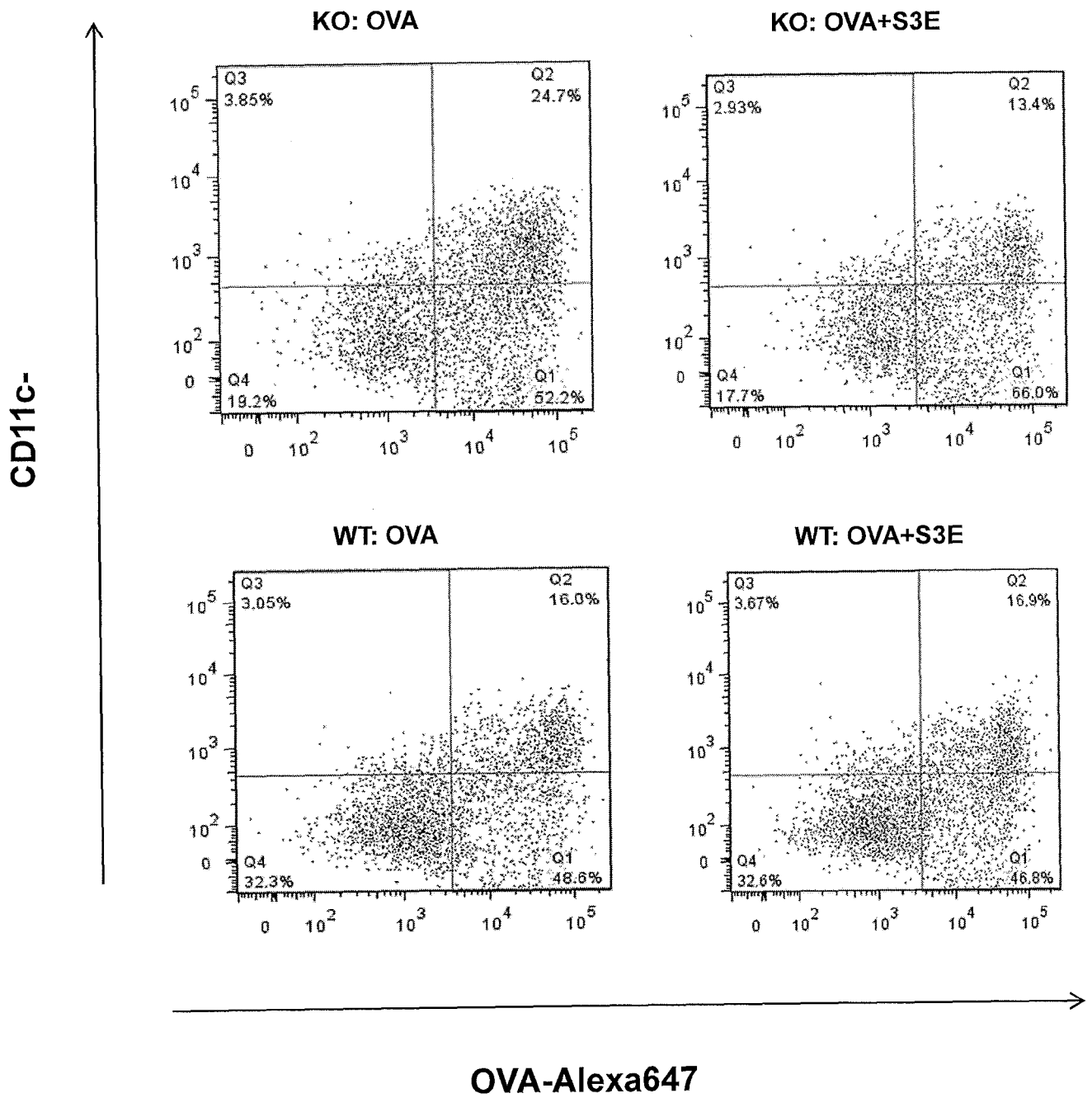
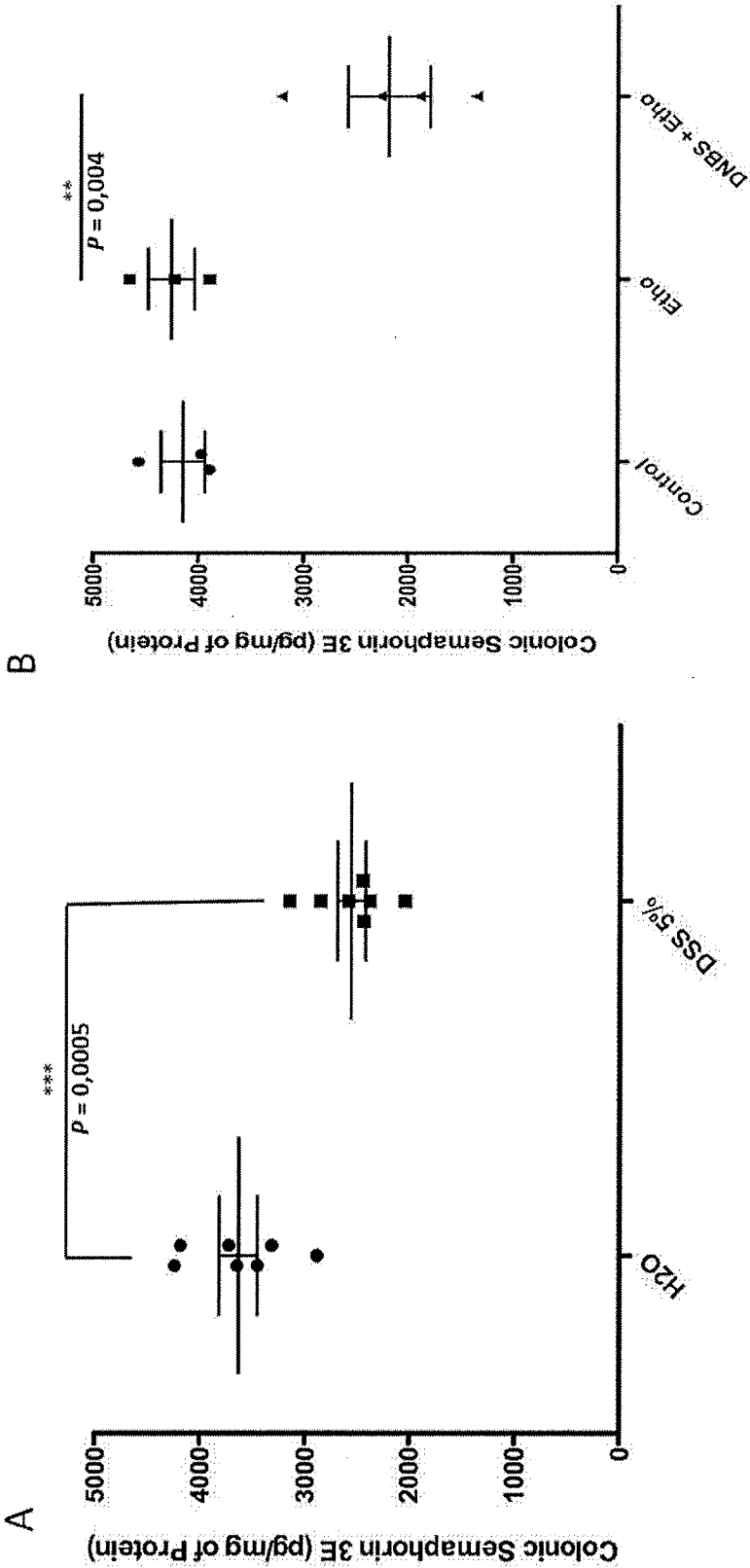
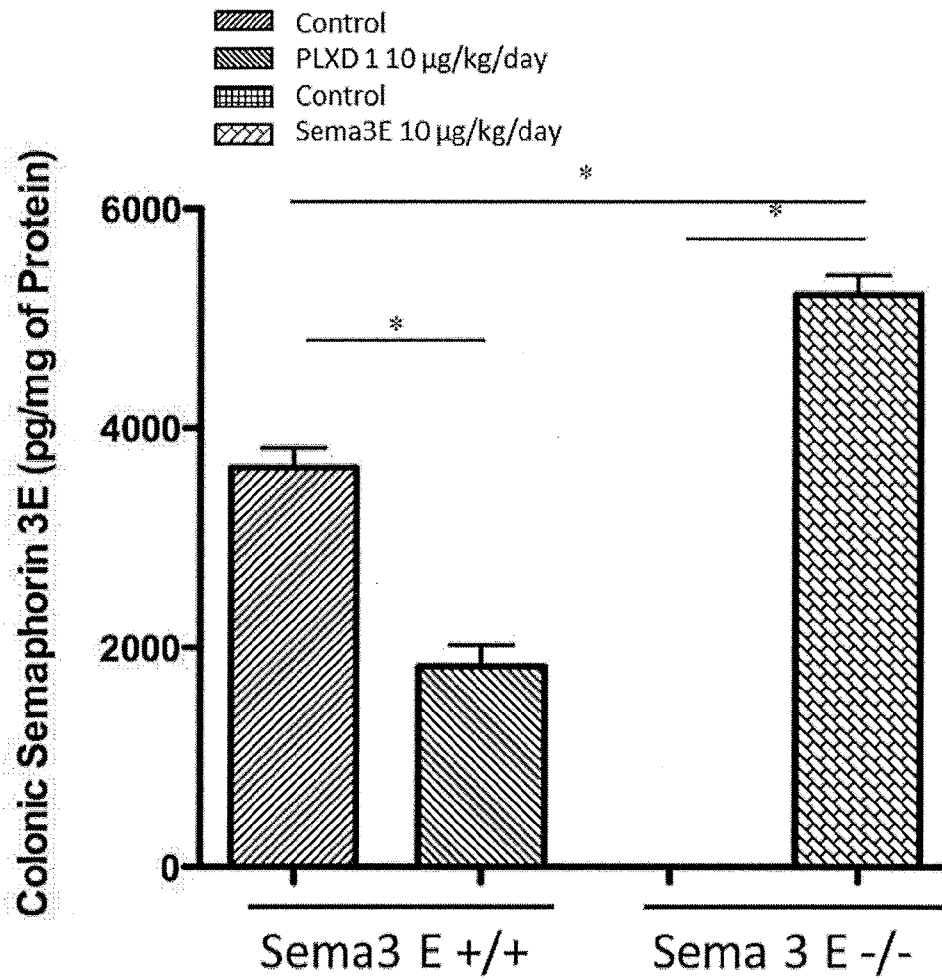


Fig. 11



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Fig. 12



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Fig. 13

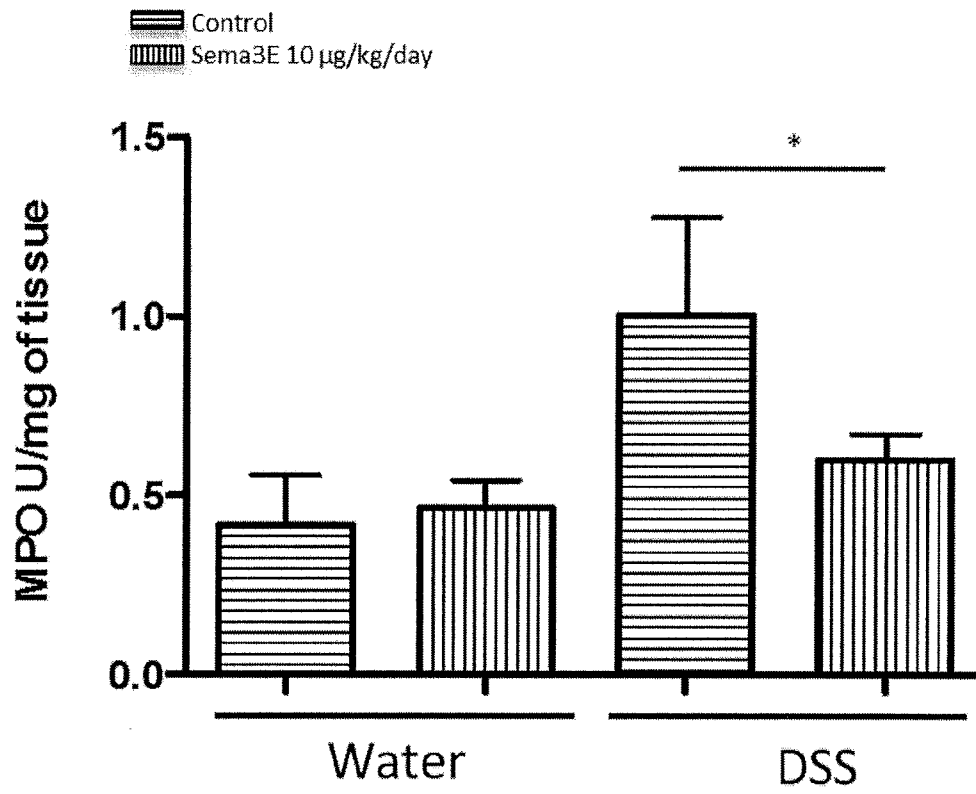
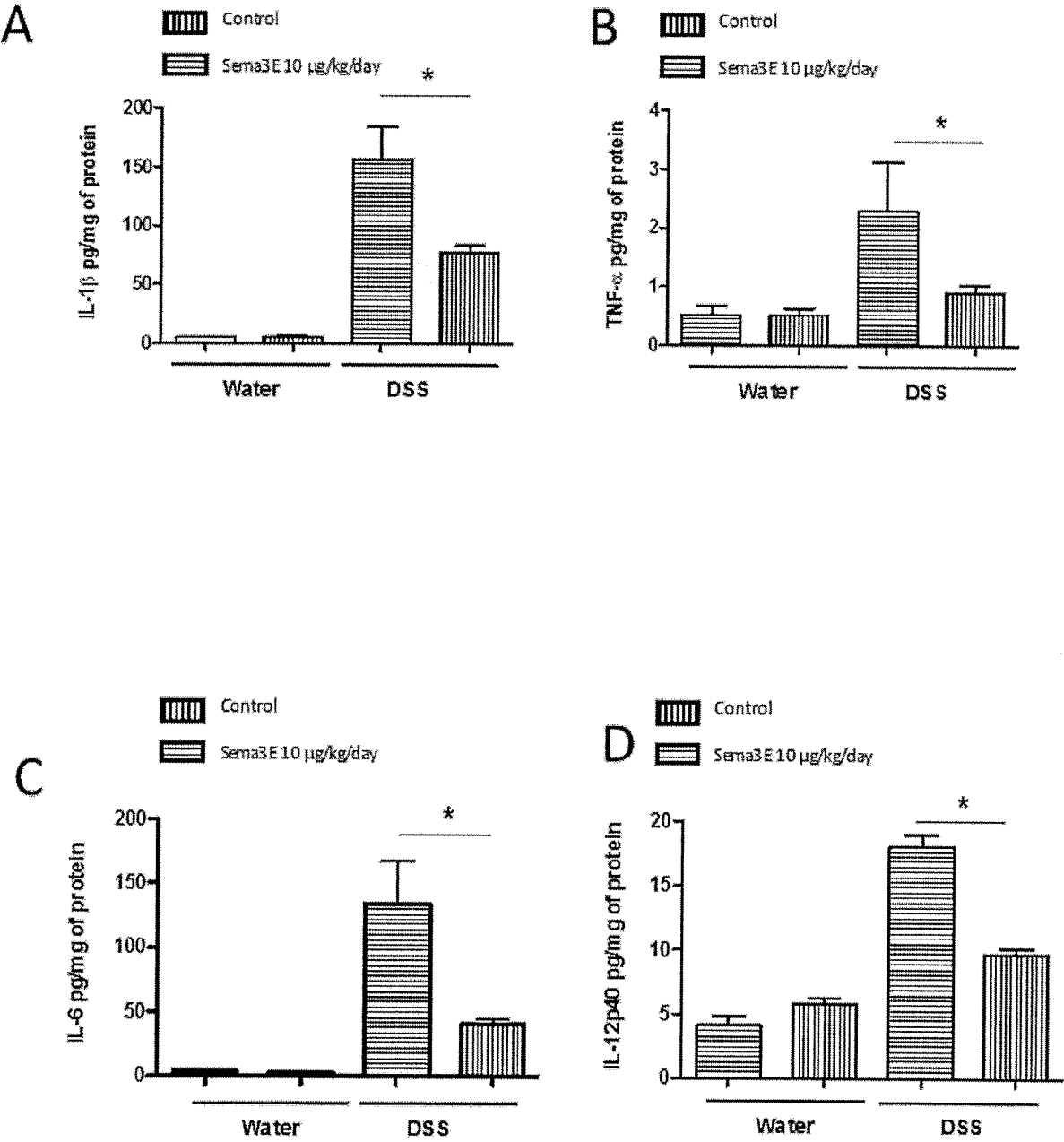


Fig. 14



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Fig. 15A

SEQ ID NO:1

>Homo sapiens

MASAGHIITLLLLWGYLLELWTGGHTADTTHPRLRLSHKELLNLNRTSIFHSPFGFLDLHTMLLDEYQERL
FVGGRDLVYSLSLERISDGYKEIHW PSTALKMEECIMKGKDAGECANYVRVLHHYNRTHLLTCGTGAFDP
VCAFIRVGYHLEDPLFHLESPRSEGRGRCPDFPSSSFISTLIGSELFAGLYSDYWSRDAIFRSMGPLA
HIRTEHDDERLLKEPKFVG SYMIPDNEDRDDNKVYFFFTEKALEAENNAHAIYTRVGRLCVNDVGGQRIL
VNKWSTRFLKARLVCSVPGMNGIDTYFDELEDVFLLPTRDHKNPVI FGLFN TTSNIFRGHAICVYHMSSIR
AAFNGPYAHKEGPEYHWSVYEGKVPYPRPGSCASKVNNGGRYGTTKDYPDDAIRFARSHPLMYQAIKPAHK
KPILVKTDGKYNLKQIAVDRVEAEDGQYDVLFIGT DNGIVLKVITIYNQEMESMEEVILEELQIFKDPVP
IISMEISSKRQQLYIGSASAVAQVRFHHC DMYGSACADCLARDPYCAWDGISCSRYYP TGHAKRRFRK
QDVRHGNAAQQCFCGQQFVG DALDK'TEEHLAYGIENNSTLLECTPRSLQAKVIWFVQKGRETRKEEVKTD
RVVKMDLGLLFLRLHKS DAGTYFCQTVEHSFVHTVRKITLEVVEEEKVEDMFNKDDEEDRHHRMPCPAQS
SISQGAKPWYKEFLQLIGYSNFQRVEEYCEKVWCTDRKRKKLKMSPSKWKYANPQEKRLRSKPEHYRLPR
HTLDS

SEQ ID NO:3

>Mus musculus

MAPAGHIITLLLLWGHLLLELWTPGHSANPSYPRLRLSHKELLELNRTSIFQSPPLGFLDLHTMLLDEYQERL
FVGGRDLVYSLNLERSVDGYREIYWPSTAVKVEECIMKGKDANECANYIRVLHHYNRTHLLTCATGAFDP
HCAFIRVGHHS EEP LFHLESHRSEGRGRCPDFPNSSFVSTLVGNELFAGLYSDYWGRDSAIFRSMGKLG
HIRTEHDDERLLKEPKFVG SYMIPDNEDRDDNKMYFFFTEKALEAENNAHTIYTRVGRLCVNDMGGQRIL
VNKWSTRFLKARLVCSVPGMNGIDTYFDELEDVFLLPTRDPKNPVI FGLFN TTSNIFRGHAVCVYHMSSIR
EAFNGPYAHKEGPEYHWSLYEGKVPYPRPGSCASKVNNGGKYGTTKDYPDDAIRFARMHPLMYQPIKPVHK
KPILVKTDGKYNLRLQIAVDRVEAEDGQYDVLFIGTDTGIVLKVITIYNQETEWMEEVILEELQIFKDPAP
IISMEISSKRQQLYIGSASAVAQVRFHHC DMYGSACADCLARDPYCAWDGISCSRYYP TGAHAKRRFR
QDVRHGNAAQQCFCGQQFVG DALDRTEERLAYGIESNSTLLECTPRSLQAKVIWFVQKGRDVRKEEVKTD
RVVKMDLGLLFLVRKSDAGTYFCQTVEHNFVHTVRKITLEVVEEHKVEGMFHKDHEEERHHKMPCPPLS
GMSQGTKPWYKEFLQLIGYSNFQRVEEYCEKVWCTDKKRKKLKMSPSKWKYANPQEKRLRSKAEHFRLPR
HTLLS

SEQ ID NO:5

>Rattus norvegicus

MAPTGHITLLLLWGHLLLELWTPGYTANPSYPRLRLSHKELLELNRTSIFQSPFGFLDLHTMLLDEYQERL
FVGGRDLVYSLNLERSVDGYREIYWPSTAVKVEECIMKGKDANECANYVRVLHHYNRTHLLTCATGAFDP
HCAFIRVGHHS EDP LFHLESHRSEGRGRCPDFPNSSFVSTLVGNELFAGLYSDYWGRDSAIFRSLGKLG
HIRTEHDDERLLKEPKFVG SYMIPDNEDPEDNKMYFFFTEKALEAENNAHTIYTRVGRLCVNDMGGQRIL
VNKWSTRFLKARLVCSVPGMNGIDTHFDELEDVFLLPTRDPKNPVI FGLFN TTSNIFRGHAVCVYHMSSIR
EAFNGPYAHKEGPEYHWSLYEGKVPYPRPGSCASKVNNGGKYGSTKDYPDDAIRFARMHPLMYQPIKPVHK
KPILVKTDGKYNLRLQIAVDRVEAEDGQYDVLFIGTDTGIVLKVITIYNQETEWMEEVILEELQIFKDPAP
IISMEISSKRQQLYIGSASAVAQVRFHHC DMYGSACADCLARDPYCAWDGISCSRYYP TGAHSKRFR
QDVRHGNAAQQCFCGQQFVG DALDRTEERLAYGIESNSTLLECTPRSLQAKVVWFVQKGRDVRKEEVKTD
RVVKMDLGLLFLVRKSDAGTYFCQTVEHSFVHTVRKITLEVVEEHKVEGMFHKDREEEKHHKMPCPPLT
GMSQGTKPWYKEFLQLIGYSNFQRVEEYCEKVWCTDKKRKKLKMSPSKWKYANPQEKRLRSKAEHFRLPR
HTLVS

H. sapiens	MASAGHIITLLLLWGYLLELWLTGGHTADTTHPRRLRLSHKELLELNRTSIFHSPFGFLDLHT
M. musculus	MAPAGHILTLLLLWGHLLLELWTPGHSANPSYPRLRLSHKELLELNRTSIFQSPGLGFLDLHT
R. norvegicus	MAPTGHILTLLLLWGHLLLELWTPGYTANPSYPRLRLSHKELLELNRTSIFQSPFGFLDLHT *:*:***:*****:***** *:*:*:*:*****:*****:*.**:*****
H. sapiens	MLLDEYQERLFVGGRDVLVYSLSLERISDGYKEIHW PSTALKMEECIMKGKDAGECANYVR
M. musculus	MLLDEYQERLFVGGRDVLVYSLNLERVSDGYREIYWPSTAVKVVEECIMKGKDANECANYIR
R. norvegicus	MLLDEYQERLFVGGRDVLVYSLNLERVSDGYREIYWPSTAVKVVEECIMKGKDANECANYVR *****:*****:*****:*****:*****:*****:*****:*****:*****:*
H. sapiens	VLHHYNRTHLLTCGTGAFDPVCAFI RVGYHLEDPLFHLES PRSERGRGRCPFPDPSSSFIS
M. musculus	VLHHYNRTHLLTCATGAFDPHCAFI RVGHHSEEP LFHLESHR SERGRGRCPFPDPNSSFVS
R. norvegicus	VLHHYNRTHLLTCATGAFDPHCAFI RVGHHSEDP LFHLESHR SERGRGRCPFPDPNSSFVS *****:*****:*****:*****:*****:*****:*****:*****:*
H. sapiens	TLIGSELFAGLYSDYWSRDAAI FRSMGPLAHIRTEHDDERLLKEPKFVG SYMIPDNEDRD
M. musculus	TLVGNELFAGLYSDYWG RDSAIFRSMGKLGHIRTEHDDERLLKEPKFVG SYMIPDNEDRD
R. norvegicus	TLVGNELFAGLYSDYWG RDSAIFRSLGKLGHIRTEHDDERLLKEPKFVG SYMIPDNEDPE *:*:*****:*****:*****:*****:*****:*****:*****:*****:*
H. sapiens	DNKVYFFFTEKALEAENNAHAIIYTRVGRLCVNDVGGQRILVNKWSTFLKARLVCSVPGMN
M. musculus	DNKMYFFFTEKALEAENNAHTIIYTRVGRLCVNDMGGQRILVNKWSTFLKARLVCSVPGMN
R. norvegicus	DNKMYFFFTEKALEAENNAHTIIYTRVGRLCVNDMGGQRILVNKWSTFLKARLVCSVPGMN ***:*****:*****:*****:*****:*****:*****:*****:*****
H. sapiens	GIDTYFDELEDVFLLPTRDHKNPVI FGLFN TTSNIFRGHAICVYHMSSIRA AFNGPYAHK
M. musculus	GIDTYFDELEDVFLLPTRDPKNPVI FGLFN TTSNIFRGHAVCVYHMSSIREAFNGPYAHK
R. norvegicus	GIDTHFDELEDVFLLPTRDPKNPVI FGLFN TTSNIFRGHAVCVYHMSSIREAFNGPYAHK ****:*****:*****:*****:*****:*****:*****:*****:*****
H. sapiens	EGPEYHWSVYEGKVPYPRPGSCASKVNGGRYGTTKDYPDDAIRFARSHPLMYQAIKPAHK
M. musculus	EGPEYHWSLYEGKVPYPRPGSCASKVNGGKYGTTKDYPDDAIRFARMHPLMYQPIKPVHK
R. norvegicus	EGPEYHWSLYEGKVPYPRPGSCASKVNGGKYGSTKDYPDDAIRFARMHPLMYQPIKPVHK *****:*****:*****:*****:*****:*****:*****:*****:*****
H. sapiens	KPILVKTDGKYNLKQIAVDRVEAEDGQYDVLFIGT DNGI VLKVITIYNQEMESMEEVILE
M. musculus	KPILVKTDGKYNLRLQ LAVDRVEAEDGQYDVLFIGTDTGIVLKVITIYNQETEWMEEVILE
R. norvegicus	KPILVKTDGKYNLRLQ LAVDRVEAEDGQYDVLFIGTDTGIVLKVITIYNQETEWMEEVILE *****:*****:*****:*****:*****:*****:*****:*****:*****
H. sapiens	ELQIFKDPVPIISMEISSKRQQLYIGSASAVAQVRFHHC DMYGSACADCC LARDPYCAWD
M. musculus	ELQIFKDPAPIISMEISSKRQQLYIGSASAVAQVRFHHC DMYGSACADCC LARDPYCAWD
R. norvegicus	ELQIFKDPAPIISMEISSKRQQLYIGSASAVAQVRFHHC DMYGSACADCC LARDPYCAWD *****:*****:*****:*****:*****:*****:*****:*****:*****
H. sapiens	GISC SRY YPTGTHAKRRFR RQDVRHGNA AQQCFGQQFVG DALDKTEEHLAYGIENNSTLL
M. musculus	GISC SRY YPTGAHAKRRFR RQDVRHGNA AQQCFGQQFVG DALDRTEERLAYGIESNSTLL
R. norvegicus	GISC SRY YPTGAHAKRRFR RQDVRHGNA AQQCFGQQFVG DALDRTEERLAYGIESNSTLL *****:*****:*****:*****:*****:*****:*****:*****:*****

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Fig. 15B-2

H. sapiens	ECTPRSLQAKVIWVQKGRRETRKEEVKTTDDRVRVKMDLGLLFLRLHKSDAGTYFCQTVEHS
M. musculus	ECTPRSLQAKVIWVQKGRDVRKEEVKTTDDRVRVKMDLGLLFLRVRKSDAGTYFCQTVEHN
R. norvegicus	ECTPRSLQAKVVWVQKGRDVRKEEVKTTDDRVRVKMDLGLLFLRVRKSDAGTYFCQTVEHS
	*****.:*****.:*****.:*****.
H. sapiens	FVHTVRKITLEVVEEEKVEDMFNKDDEEDRHHRMPCPAQSSISQGAKPWYKEFLQLIGYS
M. musculus	FVHTVRKITLEVVEEHKVEGMFHKDHEEERHHKMPCPPLSGMSQGTKPWYKEFLQLIGYS
R. norvegicus	FVHTVRKITLEVVEEHKVEGMFHKDREEEKHHKMPCPPLTGMSSQGTKPWYKEFLQLIGYS
	*****.***.**:** **:**:***. :.:***:*****
H. sapiens	NFQRVEEYCEKVVCTDRKRKKLKMSPSKWKYANPQEKRLRSKPEHYRLPRHTLDS
M. musculus	NFQRVEEYCEKVVCTDKKRKKLKMSPSKWKYANPQEKRLRSKAHFRLPRHTLLS
R. norvegicus	NFQRVEEYCEKVVCTDKKRKKLKMSPSKWKYANPQEKRLRSKAHFRLPRHTLVS
	*****.*****.*****.*****.*****.*****.*****.*****

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2014/061275

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **A61K 38/17** (2006.01) , **A61P 1/00** (2006.01) , **G01N 33/48** (2006.01)

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)
P C (2006.1) A61K, A61P, G01N

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

Electronic database(s) consulted during the international search (name of databases) and, where practicable, search terms used)
Questel, Scopus

Keywords: sema, semaphorin, sema3E, sema-3E, semaphorin3E, semaphorin-3E, IBD, inflammatory bowel disease, colitis, Crohn's

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US2009/0181035 A1 (WATTS, R. J. et al.) 16 M y 2009 (16-07-2009)	1-9, 12-30 and 33-36
Y	*paragraphs [0008], [0009], [0021]-[0023], [0041-0042], [0081], [0239], [0247], Examples 1-5*	10, 11, 31, 32 and 37-40
Y	WO2012/131477 A1 (GOUNNI, A. S.) 4 October 2012 (04-10-2012) *abstract, claims*	10, 11, 31, 32 and 37-40

 Further documents are listed in the continuation of Box C.

 See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
19 June 2014 (19-06-2014)

Date of mailing of the international search report
22 July 2014 (22-07-2014)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, CI 14 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer
Kerry Ferguson (819) 994-9451

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2014/061275**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2Xa) for the following reasons:

1. ☒ Claim Nos.: 17-40
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 17-40 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under Rule 39.1(iv) of the PCT. However, this Authority has carried out a search based on the alleged use of a composition comprising a sema3E polypeptide or a sema3E polynucleotide for treating an inflammatory bowel disease or for treating a subject at risk of having inflammatory bowel disease (claims 17-36) or the use of a sema3E polypeptide for evaluating treatment options for a subject having inflammatory bowel disease (claims 37-40).
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/IB2014/061275

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