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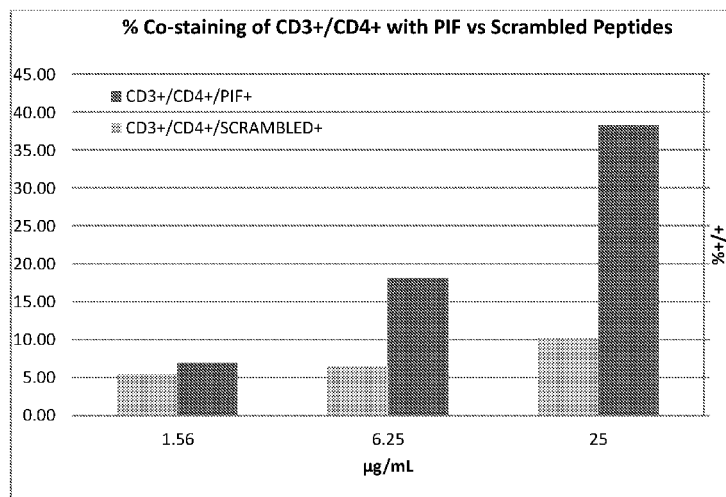


FIG. 1

(57) Abstract: The disclosure relates to
a pharmaceutical composition compris-
ing any one or combination of PIF pep-
tides or analogs or pharmaceutically
acceptable salts thereof. Methods of
treating endometriosis using the one or
a combination of PIF peptide or ana-
logs thereof or pharmaceutically ac-
ceptable salts thereof is also disclosed.



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PEPTIDES AND METHODS OF TREATING ENDOMETRIOSIS USING THE SAME

5 FIELD

The present disclosure generally relates to compositions and methods for the diagnosis and treatment of endometriosis. The disclosure also relates to pre-implantation factor (PIF) mutants and methods of treatment, such as treating endometriosis.

10 BACKGROUND

Endogenous Pre-Implantation Factor (PIF) is a 15 amino acid peptide (MVRIKPGSANKPSDD, SEQ ID NO:1) expressed by the embryo/fetus and placenta and is present in circulation of viable mammals throughout pregnancy starting post fertilization, playing a critical determining role to create and maternal tolerance without immune suppression. PIF exerts broad neurotrophic and neuroprotective effects. PIF regulates immunity, inflammation and transplant acceptance. By creating a favorable maternal milieu PIF specifically reduces neural damage while it promotes neural development, protecting against maternal adverse environment. PIF precisely targets proteins in the embryo to reduce oxidative stress and protein misfolding. *In vivo* PIF reduces spontaneous and LPS induced pregnancy loss by decreasing the pro-inflammatory response in the placenta.

It has been observed that PIF and synthetic PIF analogs (sPIF) have immune modulatory properties and such peptides are useful in the prevention and/or treatment of various immune-mediated diseases, including, but not limited to, endometriosis, a chronic inflammatory condition that affects reproductive age women. Compositions and methods for diagnosing, treating and/or preventing endometriosis are provided herein.

SUMMARY

The present disclosure relates to a method of treating or preventing endometriosis in a subject in need thereof, the method comprising administering to the subject at least one pre-implantation factor (PIF) peptide, a mimetic thereof, an analog thereof, or a pharmaceutically acceptable salt thereof.

In some embodiments, the step of administering to the subject at least one PIF peptide, a mimetic thereof, an analog thereof, or a pharmaceutically acceptable salt thereof comprises administering a therapeutically effective dose of the at least one PIF molecule, an

analog thereof, or a pharmaceutically acceptable salt thereof.

In some embodiments, the step of administering to the subject at least one PIF peptide, a mimetic thereof, an analog thereof, or a pharmaceutically acceptable salt thereof comprises administering a therapeutically effective dose of the PIF peptide, an analog thereof, or pharmaceutically acceptable salt thereof from about 0.001 mg/kg to about 200 mg/kg.

In some embodiments, the step of administering to the subject at least one PIF peptide, a mimetic thereof, an analog thereof, or a pharmaceutically acceptable salt thereof comprises administering a therapeutically effective dose of the PIF peptide, an analog thereof, or pharmaceutically acceptable salt thereof from about 0.5 mg/kg to about 5 mg/kg.

In some embodiments, the at least the PIF peptide, a mimetic thereof, an analog thereof, or pharmaceutically acceptable salt thereof comprises a chemical targeting moiety and/or a radioactive moiety.

In some embodiments, the at least one inhibitor of nuclear translocation of beta-catenin or pharmaceutically acceptable salt thereof comprises at least one radioactive moiety comprising at least one or a combination of the following isotopes: ^2H , ^3H , ^{13}C , ^{14}C , ^{15}N , ^{16}O , ^{17}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , and ^{36}Cl .

In some embodiments, the method further comprises administering at least one analgesic and/or one anti-inflammatory compound.

In some embodiments, the method further comprises administering at least one analgesic and or one anti-inflammatory compound before, after, or simultaneously with the administration of a therapeutically effective dose of at least one PIF peptide, an analog thereof or pharmaceutically acceptable salt thereof.

In some embodiments, the therapeutically effective dose is from about 1.0 mg/kg to about 5.5 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is from about 1.0 mg/kg to about 5.0 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is from about 1.0 mg/kg to about 4.5 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is from about 1.0 mg/kg to about 4.0 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is from about 1.0 mg/kg to about 3.5 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective

dose. In some embodiments, the therapeutically effective dose is administered subcutaneously, intravenously, intraperitoneally, topically, orally, sublingually, intranasally, or intramuscularly. In some embodiments, the therapeutically effective dose is administered once a week, twice a week, three times a week, four times a week, or five days per week, and optionally, wherein the dose is administered once in two, three, four or five days in succession. In some embodiments, the therapeutically effective dose is administered once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, once every eleven weeks, or once every twelve weeks. In some embodiments, the therapeutically effective dose is administered once a month, twice a month, three times a month, four times a month, or five times a month. In some embodiments, the therapeutically effective dose is administered once a year, twice a year, three times a year, four times a year, five times a year, six times a year, seven times a year, eight times a year, nine times a year, ten times per year, or eleven times per year.

In some embodiments, the PIF peptide comprises SEQ ID NO:1, SEQ ID NO:2, and/or SEQ ID NO:3. In some embodiments, the PIF peptide comprises SEQ ID NO:4 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:5 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:6 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:7 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:8 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:9 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:10 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:11 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:12 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:13 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:14 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:15 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:16 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:17 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:18 or a pharmaceutically acceptable salt thereof. In some

embodiments, the PIF peptide comprises SEQ ID NO:19 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:20 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:21 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:22 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:23 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:24 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:25 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:26 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:27 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:28 or a pharmaceutically acceptable salt thereof. In some embodiments, the PIF peptide comprises SEQ ID NO:29 or a pharmaceutically acceptable salt thereof.

In a further embodiment, a compound of the formula R_1 - R_2 - R_3 - R_4 (SEQ ID NO:31) is provided, wherein R_1 is Pro or a mimetic of Pro, R_2 is Gly or a mimetic of Gly, R_3 is Ser or a mimetic of Ser, and R_4 is Ala or a mimetic of Ala. In alternative embodiments, the compound may comprise one or more of up to 11 additional amino acid residues. Embodiments include those peptides derived from pre-implantation embryos that induces TH2 type cytokines like IL-10 synthesis or secretion from lymphocytes or other white blood cells and pharmacophores that binds specifically to PIF receptors (such but not limited to PGSA (A)VRKPGSANKPSDD or (Q)VRKPGSANKPSDD) or by substituting with D amino acids or by adding PEG. Preferably such peptides are from pre-implantation embryos and increases TH2/TH1 ratio through increased number of lymphocytes containing the desired cytokines and or by preferential secretion or TH2 over TH1 cytokines into the media. Such pre-implantation embryo-derived peptide may be used to cause a shift from pro-inflammatory to anti-inflammatory activities in lymphocytes. In a further embodiment, the pharmaceutical composition comprises a compound of the formula MVRK (SEQ ID NO: 32).

In some embodiments, the pharmaceutically acceptable carrier is sterile and pyrogen-free water or aqueous buffer, such as saline or Lactated Ringer's solution.

In some embodiments, the therapeutically effective dose is about 1.0 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 2.0 mg/kg, wherein kg is

kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 3.0 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 4.0 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some
5 of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 0.2 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 0.3 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some
10 embodiments, the therapeutically effective dose is about 0.4 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 0.5 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 0.6 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some
15 embodiments, the therapeutically effective dose is about 0.7 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose. In some embodiments, the therapeutically effective dose is about 0.8 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

20 The present disclosure also relates to a pharmaceutical composition comprising (i) a therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof; and (ii) a pharmaceutically acceptable carrier.

In some embodiments, the composition further comprises a therapeutically effective dose of one or a plurality of active agents.

25 In some embodiments, the one or plurality of active agents is one or a combination of compounds chosen from: an anti-inflammatory compound, alpha-adrenergic agonist, antiarrhythmic compound, analgesic compound, and an anesthetic compound, or a hormone therapy.

In some embodiments, the therapeutically effective dose of one or a combination of
30 PIF peptide or mimetics thereof or thereof analogs thereof or pharmaceutically acceptable salts thereof is about 1.0 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or mimetics thereof or analogs thereof or pharmaceutically acceptable salts

thereof is about 2.0 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 3.0
5 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 4.0
10 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, wherein the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.2 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

15 In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.3 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, the therapeutically effective dose of one or a combination of
20 PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.4 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.5
25 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.6
30 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.7 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

In some embodiments, wherein the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.8 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

5 In some embodiments, the therapeutically effective dose of one or a combination of PIF peptide or analogs thereof or pharmaceutically acceptable salts thereof is about 0.9 mg/kg, wherein kg is kilograms of the subject and mg is milligrams of the therapeutically effective dose.

10 In some embodiments, the composition further comprises one or a plurality of stem cells. In some embodiments, the stem cell is an autologous stem cell.

In some embodiments, the pharmaceutical composition is administered via parenteral injection, subcutaneous injection, intravenous injection, intramuscular injection, intraperitoneal injection, transdermally, orally, buccally, ocular routes, intravaginally, by inhalation, by depot injections, or by implants.

15 In some embodiments, the compositions further comprise one or a combination of active agents chosen from: an anti-inflammatory compound, alpha-adrenergic agonist, antiarrhythmic compound, analgesic compound, and an anesthetic compound.

In some embodiments, the one or combination of active agents is selected from Table Y.

20 The present disclosure also relates to a method of improving the clinical outcome in a subject suffering with, diagnosed with or suspected of having endometriosis comprising administering to the subject at least one pharmaceutical composition comprising: pre-implantation factor (PIF) peptide, an analog thereof, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

25 In some embodiments, methods of diagnosing endometriosis in a human subject are provided. In some embodiments, the methods comprises measuring pre-implantation factor (PIF) protein or mRNA expression levels from an endometrial tissue sample from the subject; and comparing the PIF protein expression levels from the endometrial sample to the PIF expression levels in a control normal sample; wherein the patient is diagnosed with
30 endometriosis if the PIF expression levels from the endometrial sample are greater than the PIF expression levels from the control normal sample.

In some embodiments, methods of treating endometriosis in a human subject are provided. In some embodiments, the methods comprise measuring pre-implantation factor (PIF) protein or mRNA expression levels from a endometrial sample from the subject;

comparing the PIF protein or mRNA expression levels from the endometrial sample to the PIF expression levels in a control normal sample, wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial sample are greater than the PIF expression levels from the control normal sample; and administering to the subject at least one
5 pharmaceutical composition comprising a therapeutically effective amount of a PIF peptide, mimetics thereof, analogs thereof, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of
10 these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or
15 group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a graph showing the dose dependent increase in the binding of PIF with
20 CD4+/CD25+ cells, in contract to a control scrambled-FITC-PIF.

FIG. 2 depicts a graph showing the dose dependent increase in the binding of PIF with CD4+/CD25+/FoxP3+ cells.

FIGs. 3A - 3D depict results showing that PIF imparts epithelial ectopic endometria. FIG. 3A depicts PIF staining in epithelial and stromal cells. FIG. 3B depicts graphs showing the
25 semi-quantitative evaluation of the PIF positive staining from FIG. 3A. FIG. 3C depicts a graph showing the cell viability with increasing sPIF concentration in epithelial cells compared to controls. FIG. 3D depicts a graph showing the cell viability with increasing sPIF concentration in stromal cells.

FIG. 4A depicts results of a global gene array from sPIF treated epithelial ectopic cells
30 lines. FIG. 4B depicts a heat map analysis of T-cell receptor genes identified in FIG. 4A.

FIGs. 5A- 5E depict results showing that PIF interacts with FoxP3 positive cells. FIG. 5A depicts FoxP3 and PIF staining in eutopic and ectopic tissues. FIG. 5B depicts graphs showing the semi-quantitative evaluation of the PIF positive staining from FIG. 5A. FIG. 5C
35 depicts the dose dependent increase in the binding of FITC-PIF to CD4+/CD25+/FoxP3+ cells using flow cytometry. FIG. 5D depicts a graph showing the cell viability with increasing sPIF concentration in epithelial cells with TNF α pretreatment. FIG. 5E depicts a cartoon showing the hypothesis that PIF re-expresses in the epithelial compartment of ectopic endometria resulting

in the recruitment of FoxP3+ cells into the stromal compartment creating a positive feed-back loop leading to cellular survival.

DETAILED DESCRIPTION

5 Before the present compositions and methods are described, it is to be understood that this disclosure is not limited to the particular molecules, compositions, methodologies or

protocols described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present disclosure which will be limited only by the appended claims. It is understood that these embodiments are not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It also is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present embodiments or claims. The compositions described herein may include D amino acids, L amino acids, a racemic backbone of D and L amino acids, or any mixture thereof at each residue. That is, at each position, the residue may be a D amino acid residue or a L-amino acid residue and each position can be independently D or L of each other position, unless context dictates otherwise.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present disclosure, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated by reference. Nothing herein is to be construed as an admission that the disclosure is not entitled to antedate such disclosure by virtue of prior disclosure.

As used herein, the phrase "in need thereof" means that the animal or mammal has been identified or suspected as having a need for the particular method or treatment. In some embodiments, the identification can be by any means of diagnosis or observation. In any of the methods and treatments described herein, the animal or mammal can be in need thereof.

As used herein, the term "subject," "individual" or "patient," used interchangeably.

As used herein, the terms "a" or "an" means that "at least one" or "one or more" unless the context clearly indicates otherwise. It must also be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth.

As used herein, the term "about" means that the numerical value is approximate and small variations would not significantly affect the practice of the disclosed embodiments. Where a numerical limitation is used, unless indicated otherwise by the context, "about" means the numerical value can vary by 10% and remain within the scope of the disclosed

embodiments. Where a numerical value is used with the term "about" the numerical value without the term "about" is also disclosed and can be used without the term "about."

As used herein, the terms "comprising" (and any form of comprising, such as "comprise", "comprises", and "comprised"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include"), or "containing" (and any form of containing, such as "contains" and "contain"), are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

As used herein, the phrase "integer from X to Y" means any integer that includes the endpoints. That is, where a range is disclosed, each integer in the range including the endpoints is disclosed. For example, the phrase "integer from X to Y" discloses about 1, 2, 3, 4, or 5 as well as the range from about 1 to about 5.

As used herein, the phrase "therapeutically effective amount" means the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response that is being sought in a tissue, system, animal, individual or human by a researcher, veterinarian, medical doctor or other clinician. The therapeutic effect is dependent upon the disorder being treated or the biological effect desired. As such, the therapeutic effect can be a decrease in the severity of symptoms associated with the disorder and/or inhibition (partial or complete) of progression of the disorder, or improved treatment, healing, prevention or elimination of a disorder, or side-effects. The amount needed to elicit the therapeutic response can be determined based on the age, health, size and sex of the subject. Optimal amounts can also be determined based on monitoring of the subject's response to treatment.

As used herein, the terms "treat," "treated," or "treating" can refer to therapeutic treatment and/or prophylactic or preventative measures wherein the object is to slow down (lessen) an undesired physiological condition, disorder or disease, or obtain beneficial or desired clinical results. For purposes of the embodiments described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of extent of condition, disorder or disease; stabilized (i.e., not worsening) state of condition, disorder or disease; delay in onset or slowing of condition, disorder or disease progression; amelioration of the condition, disorder or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder or disease. Treatment can also include eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. Thus, "treatment of

endometriosis " means an activity that prevents, alleviates or ameliorates any of the primary phenomena or secondary symptoms associated with the endometriosis.

This application describes compounds and methods of administering those compounds to a subject in need thereof. In some embodiments, "preimplantation factor" or "PIF" may also refer to synthetic PIF-1, which replicates the native peptide's effect and exerts potent immune modulatory effects on activated peripheral blood mononuclear cell (PBMC) proliferation and cytokine secretion, acting through novel sites on PBMCs and having an effect which is distinct from known immunosuppressive drugs. In some embodiments, "preimplantation factor" or "PIF" or "PIF analog" refers to an amino acid selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO: 31, SEQ ID NO:32, or peptidomimetics or mutants thereof, and combinations thereof that are about 75, 80, 81, 82, 83, 84 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% homologous to any such amino acid. In some embodiments, the terms "PIF," "PIF peptide," or "PIF analog" refers to an amino acid sequence of Table 1 or any peptidomimetics, mutant or analog thereof, that is from about 75% to about 100% homologous to any sequence of Table 1, optionally fused or unfused to one or more other amino acid sequences at its carboxy and/or its amino terminal ends. Pharmaceutical compositions of the present disclosure relate to any or all of the compounds or PIF peptides disclosed herein or their respective pharmaceutically effective salts or polymorphs.

Without being bound by any particular theory, the compounds described herein may act as agonists of PIF-mediated signal transduction via the receptor or receptors of PIF. Thus, these compounds modulate signaling pathways that provide significant therapeutic benefit in the treatment of, but not limited to, endometriosis. The compounds of the present disclosure may exist in unsolvated forms as well as solvated forms, including hydrated forms. The compounds of the present disclosure also are capable of forming both pharmaceutically acceptable salts, including but not limited to acid addition and/or base addition salts.

Furthermore, compounds of the present disclosure may exist in various solid states including an amorphous form (non-crystalline form), and in the form of clathrates, prodrugs, polymorphs, bio-hydrolyzable esters, racemic mixtures, non-racemic mixtures, or as purified stereoisomers including, but not limited to, optically pure enantiomers and diastereomers. In general, all of these forms can be used as an alternative form to the free base or free acid

forms of the compounds, as described above and are intended to be encompassed within the scope of the present disclosure.

A “polymorph” refers to solid crystalline forms of a compound. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties. Different physical properties include, but are not limited to stability (e.g., to heat or light), compressibility and density (important in formulation and product manufacturing), and dissolution rates (which can affect bioavailability). Different physical properties of polymorphs can affect their processing. The disclosure relates to a polymorph of any of the disclosed PIF peptides.

As noted above, the compounds of the present disclosure can be administered, *inter alia*, as pharmaceutically acceptable salts, esters, amides or prodrugs. The term “salts” refers to inorganic and organic salts of compounds of the present disclosure. The salts can be prepared in situ during the final isolation and purification of a compound, or by separately reacting a purified compound in its free base or acid form with a suitable organic or inorganic base or acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts, and the like. The salts may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to, ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. See, for example, S. M. Berge, et al., “Pharmaceutical Salts,” J Pharm Sci, 66: 1-19 (1977). The term “salt” refers to acidic salts formed with inorganic and/or organic acids, as well as basic salts formed with inorganic and/or organic bases. Examples of these acids and bases are well known to those of ordinary skill in the art. Such acid addition salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable acids may be of utility in the preparation and purification of the compound in question. Salts include those formed from hydrochloric, hydrobromic, sulphuric, phosphoric, citric, tartaric, lactic, pyruvic, acetic, succinic, fumaric, maleic, methanesulphonic and benzenesulphonic acids.

In some embodiments, salts of the compositions comprising either a PIF or PIF analog or PIF mutant may be formed by reacting the free base, or a salt, enantiomer or racemate thereof, with one or more equivalents of the appropriate acid. In some

embodiments, pharmaceutical acceptable salts of the present disclosure refer to analogs having at least one basic group or at least one basic radical. In some embodiments, pharmaceutical acceptable salts of the present disclosure comprise a free amino group, a free guanidino group, a pyrazinyl radical, or a pyridyl radical that forms acid addition salts. In some embodiments, the pharmaceutical acceptable salts of the present disclosure refer to analogs that are acid addition salts of the subject compounds with (for example) inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid, or amino acids such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, salicylic acid, 4-aminosalicylic acid, aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethane-sulfonic acid, or aromatic sulfonic acids, for example benzene-, p-toluene- or naphthalene-2-sulfonic acid. When several basic groups are present mono- or poly-acid addition salts may be formed. The reaction may be carried out in a solvent or medium in which the salt is insoluble or in a solvent in which the salt is soluble, for example, water, dioxane, ethanol, tetrahydrofuran or diethyl ether, or a mixture of solvents, which may be removed in vacuo or by freeze drying. The reaction may also be a metathetical process or it may be carried out on an ion exchange resin. In some embodiments, the salts may be those that are physiologically tolerated by a patient. Salts according to the present disclosure may be found in their anhydrous form or as in hydrated crystalline form (i.e., complexed or crystallized with one or more molecules of water).

Examples of pharmaceutically acceptable esters of the compounds of the present disclosure include C₁-C₈ alkyl esters. Acceptable esters also include C₅-C₇ cycloalkyl esters, as well as arylalkyl esters such as benzyl. C₁-C₄ alkyl esters are commonly used. Esters of compounds of the present disclosure may be prepared according to methods that are well known in the art. Examples of pharmaceutically acceptable amides of the compounds of the present disclosure include amides derived from ammonia, primary C₁-C₈ alkyl amines, and secondary C₁-C₈ dialkyl amines. In the case of secondary amines, the amine may also be in the form of a 5 or 6 membered heterocycloalkyl group containing at least one nitrogen atom. Amides derived from ammonia, C₁-C₃ primary alkyl amines and C₁-C₂ dialkyl secondary amines are commonly used. Amides of the compounds of the present disclosure may be

prepared according to methods well known to those skilled in the art.

"Administering" when used in conjunction with a therapeutic means to administer a therapeutic directly into or onto a target tissue or to administer a therapeutic to a patient whereby the therapeutic positively impacts the tissue to which it is targeted. Thus, as used
5 herein, the term "administering", when used in conjunction with PIF, can include, but is not limited to, providing PIF peptide into or onto the target tissue; providing PIF peptide systemically to a patient by, e.g., intravenous injection whereby the therapeutic reaches the target; providing PIF peptide in the form of the encoding sequence thereof to the target (e.g., by so-called gene-therapy techniques). "Administering" a composition may be accomplished
10 by parenteral, oral or topical administration.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of
15 administration upon a mammal without the production of undesirable physiological effects such as nausea, dizziness, rash, or gastric upset. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a subject for therapeutic purposes.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of
20 administration upon a mammal without the production of undesirable physiological effects such as nausea, dizziness, rash, or gastric upset. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a subject for therapeutic purposes.

As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, prevent or improve a subject with endometriosis or at risk of developing
25 endometriosis. Examples of such therapeutics, include, but are not limited to, the PIF peptides described herein.

A "therapeutically effective amount" or "effective amount" or "physiologically relevant amount" of a composition is an amount calculated to achieve a desired effect, *i.e.*, to effectively inhibit or reduce symptoms and/or complications associated with endometriosis.
30 Effective amounts of compounds of the present disclosure can objectively or subjectively reduce or decrease the severity or frequency of symptoms associated with endometriosis. The specific dose of a compound administered according to this disclosure to obtain therapeutic and/or prophylactic effects will, of course, be determined by the particular circumstances surrounding the case, including, for example, the compound administered, the route of

administration, and the condition being treated. The compounds are effective over a wide dosage range and, for example, dosages per day will normally fall within the range of from about 0.01 mg/kg to about 100 mg/kg, more preferably about 0.1 mg/kg to about 1 mg/kg. In some embodiments, the therapeutically effective dose of PIF or PIF analog or peptide is
5 about 0.1mg/kg, 0.2mg/kg, 0.3mg/kg, 0.4mg/kg, 0.5mg/kg, 0.6mg/kg, 0.7mg/kg, 0.8mg/kg, 0.9mg/kg, and 1mg/kg, where "mg" is milligram of PIF analog or peptide "kg" is kilogram of the subject.

In some embodiments, the pharmaceutical compositions comprise a therapeutically effective amount of PIF peptide or analog but the composition is free of SEQ ID NO:1 or a
10 pharmaceutically acceptable salt thereof.

It will be understood that the effective amount administered will be determined by the physician in the light of the relevant circumstances including the condition to be treated, the choice of compound to be administered, and the chosen route of administration, and therefore the above dosage ranges are not intended to limit the scope of the disclosure in any way. A
15 therapeutically effective amount of compound of this disclosure is typically an amount such that when it is administered in a physiologically tolerable excipient composition, it is sufficient to achieve an effective systemic concentration or local concentration in the tissue. In some embodiments, the term "therapeutically effective amount" as used herein, refers to that amount of active compound or pharmaceutical agent that elicits the biological or
20 medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that which may treat or alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. However, it is to be understood that the
25 total daily usage of the compounds and compositions described herein may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight,
30 general health, gender and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidentally with the specific compound employed; and like factors well known to the researcher, veterinarian, medical doctor or other clinician of ordinary skill.

It is also appreciated that the therapeutically effective amount, whether referring to monotherapy or combination therapy, is advantageously selected with reference to any toxicity, or other undesirable side effect, that might occur during administration of one or more of the compounds described herein. Further, it is appreciated that the co-therapies
5 described herein may allow for the administration of lower doses of compounds that show such toxicity, or other undesirable side effect, where those lower doses are below thresholds of toxicity or lower in the therapeutic window than would otherwise be administered in the absence of a co-therapy.

As used herein, the term "composition" generally refers to any product comprising the
10 specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts. It is to be understood that the compositions described herein may be prepared from isolated compounds described herein or from salts, solutions, hydrates, solvates, and other forms of the compounds described herein. It is also to be understood that the compositions may be
15 prepared from various amorphous, non-amorphous, partially crystalline, crystalline, and/or other morphological forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various hydrates and/or solvates of the compounds described herein. Accordingly, such pharmaceutical compositions that recite compounds described herein are to be understood to include each of, or any combination of, the various
20 morphological forms and/or solvate or hydrate forms of the compounds described herein.

Illustratively, compositions may include one or more carriers, diluents, and/or excipients. The compounds described herein, or compositions containing them, may be formulated in a therapeutically effective amount in any conventional dosage forms appropriate for the methods described herein. The compounds described herein, or
25 compositions containing them, including such formulations, may be administered by a wide variety of conventional routes for the methods described herein, and in a wide variety of dosage formats, utilizing known procedures (see generally, Remington: The Science and Practice of Pharmacy, (21st ed., 2005)).

The terms "treat," "treated," or "treating" as used herein refers to both therapeutic
30 treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of

the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes
5 prolonging survival as compared to expected survival if not receiving treatment.

"Immune-modulating" refers to the ability of a compound of the present disclosure to alter (modulate) one or more aspects of the immune system. The immune system functions to protect the organism from infection and from foreign antigens by cellular and humoral
10 mechanisms involving lymphocytes, macrophages, and other antigen-presenting cells that regulate each other by means of multiple cell-cell interactions and by elaborating soluble factors, including lymphokines and antibodies, that have autocrine, paracrine, and endocrine effects on immune cells.

"Auto-immune disease" refers to various diseases that arise from an abnormal
15 immune response of the body against substances and tissues normally present in the body. This may be restricted to certain organs or involve a particular tissue in different places. A large number of auto-immune diseases are recognized, including, but not limited to, Hashimoto's thyroiditis, pernicious anemia, Addison's disease, type I (insulin dependent) diabetes, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's
20 syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, and Grave's disease, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, auto-immune hemolytic anemia, auto-immune hepatitis, auto-immune inner ear disease, auto-immune lymphoproliferative syndrome (ALPS), auto-immune thrombocytopenic purpura (ATP), Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis,
25 chronic fatigue syndrome immune deficiency syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, cicatricial pemphigoid, cold agglutinin disease, CREST syndrome, Crohn's disease, Dego's disease, dermatomyositis, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Guillain-Barre syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA nephropathy,
30 juvenile arthritis, Meniere's disease, mixed connective tissue disease, pemphigus vulgaris, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomenon, rheumatic fever, sarcoidosis, scleroderma, stiff-man syndrome, Takayasu

arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, and Wegener's granulomatosis.

"Inflammatory response" or "inflammation" is a general term for the local accumulation of fluid, plasma proteins, and white blood cells initiated by physical injury, infection, or a local immune response. Inflammation is an aspect of many diseases and disorders, including but not limited to diseases related to immune disorders, viral infection, arthritis, autoimmune diseases, collagen diseases, allergy, asthma, pollinosis, and atopy. Inflammation is characterized by rubor (redness), dolor (pain), calor (heat) and tumor (swelling), reflecting changes in local blood vessels leading to increased local blood flow which causes heat and redness, migration of leukocytes into surrounding tissues (extravasation), and the exit of fluid and proteins from the blood and their local accumulation in the inflamed tissue, which results in swelling and pain, as well as the accumulation of plasma proteins that aid in host defense. These changes are initiated by cytokines produced by activated macrophages. Inflammation is often accompanied by loss of function due to replacement of parenchymal tissue with damaged tissue (e.g., in damaged myocardium), reflexive disuse due to pain, and mechanical constraints on function, *e.g.*, when a joint swells during acute inflammation, or when scar tissue bridging an inflamed joint contracts as it matures into a chronic inflammatory lesion.

"Anti-inflammatory" refers to the ability of a compound to prevent or reduce the inflammatory response, or to soothe inflammation by reducing the symptoms of inflammation such as redness, pain, heat, or swelling. Inflammatory responses can be triggered by injury, for example injury to skin, muscle, tendons, or nerves. Inflammatory responses can also be triggered as part of an immune response. Inflammatory responses can also be triggered by infection, where pathogen recognition and tissue damage can initiate an inflammatory response at the site of infection. Generally, infectious agents induce inflammatory responses by activating innate immunity. Inflammation combats infection by delivering additional effector molecules and cells to augment the killing of invading microorganisms by the front-line macrophages, by providing a physical barrier preventing the spread of infection, and by promoting repair of injured tissue. "Inflammatory disorder" is sometimes used to refer to chronic inflammation due to any cause.

It is understood that the terms "immune disorder" and "inflammatory response" are not exclusive. It is understood that many immune disorders include acute (short term) or chronic (long term) inflammation. It is also understood that inflammation can have immune aspects and non-immune aspects. The role(s) of immune and nonimmune cells in a particular

inflammatory response may vary with the type of inflammatory response, and may vary during the course of an inflammatory response. Immune aspects of inflammation and diseases related to inflammation can involve both innate and adaptive immunity. Certain diseases related to inflammation represent an interplay of immune and nonimmune cell interactions, for example intestinal inflammation (Fiocchi et al., 1997 Am J Physiol Gastrointest Liver Physiol 273: G769-G775), pneumonia (lung inflammation), or glomerulonephritis.

As used herein, "conservative" amino acid substitutions may be defined as set out in Tables A, B, or C below. The PIF compounds of the disclosure include those wherein conservative substitutions (from either nucleic acid or amino acid sequences) have been introduced by modification of polynucleotides encoding polypeptides of the disclosure. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. In some embodiments, the conservative substitution is recognized in the art as a substitution of one nucleic acid for another nucleic acid that has similar properties, or, when encoded, has similar binding affinities. Exemplary conservative substitutions are set out in Table A.

Table A -- Conservative Substitutions I

Side Chain Characteristics	Amino Acid
Aliphatic	
Non-polar	G A P I L V F
Polar - uncharged	C S T M N Q
Polar - charged	D E K R
Aromatic	H F W Y
Other	N Q D E

Alternately, conservative amino acids can be grouped as described in Lehninger, (Biochemistry, Second Edition; Worth Publishers, Inc. NY, N.Y. (1975), pp. 71-77) as set forth in Table B.

Table B -- Conservative Substitutions II

Side Chain Characteristic	Amino Acid
Non-polar (hydrophobic)	
Aliphatic:	A L I V P .
Aromatic:	F W Y

	Sulfur-containing:	M
	Borderline:	G Y
	Uncharged-polar	
	Hydroxyl:	S T Y
5	Amides:	N Q
	Sulfhydryl:	C
	Borderline:	G Y
	Positively Charged (Basic):	K R H
	Negatively Charged (Acidic):	D E
10	Alternately, exemplary conservative substitutions are set out in Table C.	

Table C -- Conservative Substitutions III

	Original Residue	Exemplary Substitution
	Ala (A)	Val Leu Ile Met
15	Arg (R)	Lys His
	Asn (N)	Gln
	Asp (D)	Glu
	Cys (C)	Ser Thr
	Gln (Q)	Asn
20	Glu (E)	Asp
	Gly (G)	Ala Val Leu Pro
	His (H)	Lys Arg
	Ile (I)	Leu Val Met Ala Phe
	Leu (L)	Ile Val Met Ala Phe
25	Lys (K)	Arg His
	Met (M)	Leu Ile Val Ala
	Phe (F)	Trp Tyr Ile
	Pro (P)	Gly Ala Val Leu Ile
	Ser (S)	Thr
30	Thr (T)	Ser
	Trp (W)	Tyr Phe Ile
	Tyr (Y)	Trp Phe Thr Ser
	Val (V)	Ile Leu Met Ala

It should be understood that the inhibitors described herein are intended to include nucleic acids and, where the inhibitors include polypeptide, polypeptides bearing one or more insertions, deletions, or substitutions, or any combination thereof, of amino acid residues as well as modifications other than insertions, deletions, or substitutions of amino acid residues.

As used herein, the terms "peptide," "polypeptide" and "protein" are used interchangeably and refer to two or more amino acids covalently linked by an amide bond or non-amide equivalent. The peptides of the disclosure can be of any length. For example, the peptides can have from about two to about 100 or more residues, such as, about 4 to about 15, about 12 to about 15, about 8 to about 18, about 18 to about 25, about 15 to about 50, about 50 to about 75, or about 75 to about 100 or more amino acids in length. Preferably, peptides are from about 4 to about 18 residues in length. The peptides of the disclosure also include l- and d-isomers, and combinations of l- and d-isomers. The peptides can include modifications typically associated with posttranslational processing of proteins, for example, cyclization (e.g., disulfide or amide bond), phosphorylation, glycosylation, carboxylation, ubiquitination, myristylation, or lipidation. In some embodiments, the compositions or pharmaceutical compositions of the disclosure relate to analogs of any PIF sequence set forth in Table 1 that share no less than about 70%, about 75%, about 79%, about 80%, about 85%, about 86%, about 87%, about 90%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% homology with any one or combination of PIF sequences set forth in Table 1. In some embodiments, PIF or PIF peptide may refer to an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or a functional fragment thereof that is about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to any such amino acid sequence. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ ID. NO: 20. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ ID. NO: 21. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ ID. NO: 22. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%,

86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ

ID. NO: 23. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%,

86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ

5 ID. NO: 24. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%,

86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ

ID. NO: 25. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%,

10 86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ

ID. NO: 26. In some embodiments, PIF may refer to an amino acid sequence comprising, consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%,

86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ

ID. NO: 27. In some embodiments, PIF may refer to an amino acid sequence comprising,

15 consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%,

86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ

ID. NO: 28. In some embodiments, PIF may refer to an amino acid sequence comprising,

consisting essentially of, or consisting of a sequence that is at least 70%, 75%, 80%, 85%,

86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to SEQ

20 ID. NO: 29. In some embodiments, the PIF mutant comprises a sequence selected from:

XVZIKPGSANKPSD, XVZIKPGSANKPS XVZIKPGSANKP XVZIKPGSANK

XVZIKPGSAN, XVZIKPGSA, XVZIKPGS, XVZIKPG, XVZIKP, XVZIK, XVZI, XVZ

wherein X is a non-natural amino acid or a naturally occurring amino acid. In some

embodiments, the PIF mutant comprises a sequence selected from: XVZIKPGSANKPSD,

25 XVZIKPGSANKPS XVZIKPGSANKP XVZIKPGSANK XVZIKPGSAN, XVZIKPGSA,

XVZIKPGS, XVZIKPG, XVZIKP, XVZIK, XVZI, XVZ wherein X is a non-natural amino

acid or a naturally occurring amino acid except that X is not methionine if Z is arginine, and

Z is not arginine if X is methionine. In some embodiments, the PIF analog or mutant is

synthetic or synthetically made.

30 Peptides disclosed herein further include compounds having amino acid structural and functional analogs, for example, peptidomimetics having synthetic or non-natural amino acids (such as a norleucine) or amino acid analogues or non-natural side chains, so long as the mimetic shares one or more functions or activities of compounds of the disclosure. The compounds of the disclosure therefore include "mimetic" and "peptidomimetic" forms. As

used herein, a "non-natural side chain" is a modified or synthetic chain of atoms joined by covalent bond to the α -carbon atom, β -carbon atom, or γ -carbon atom which does not make up the backbone of the polypeptide chain of amino acids. The peptide analogs may comprise one or a combination of non-natural amino-acids chosen from: norvaline, tert-butylglycine, phenylglycine, He, 7-azatryptophan, 4-fluorophenylalanine, N-methyl-methionine, N-methyl-valine, N-methyl-alanine, sarcosine, N-methyl-tert-butylglycine, N-methyl-leucine, N-methyl-phenylglycine, N-methyl-isoleucine, N-methyl-tryptophan, N-methyl-7-azatryptophan, N-methyl-phenylalanine, N-methyl-4-fluorophenylalanine, N-methyl-threonine, N-methyl-tyrosine, N-methyl-valine, N-methyl-lysine, homocysteine, and Tyr;

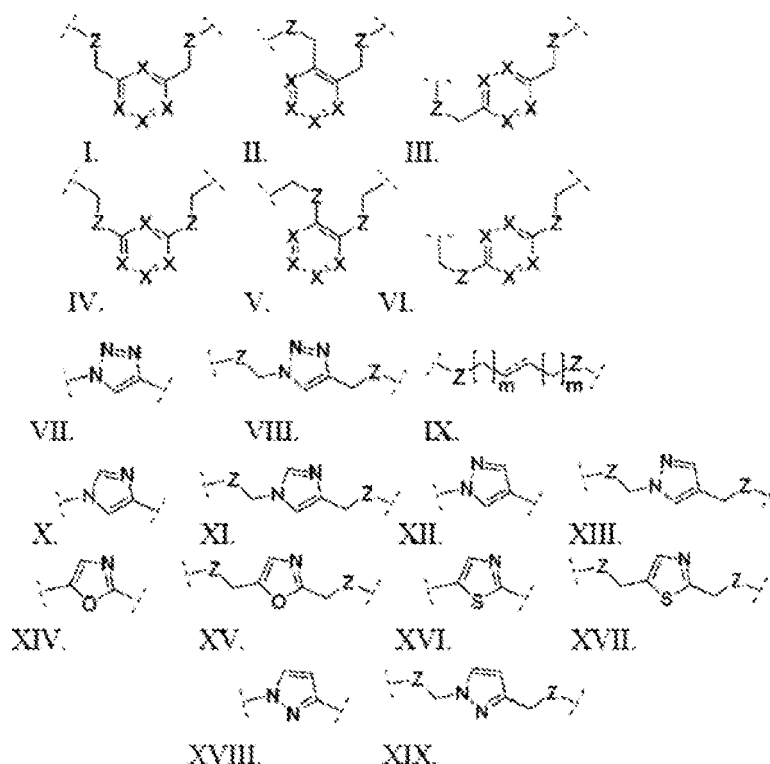
Xaa2 is absent, or an amino acid selected from the group consisting of Ala, D-Ala, N-methyl-alanine, Glu, N-methyl-glutamate, D-Glu, Gly, sarcosine, norleucine, Lys, D-Lys, Asn, D-Asn, D-Glu, Arg, D-Arg, Phe, D-Phe, N-methyl-phenylalanine, Gin, D-Gln, Asp, D-Asp, Ser, D-Ser, N-methyl-serine, Thr, D-Thr, N-methyl-threonine, D-Pro D-Leu, N-methyl-leucine, D-Ile, N-methyl-isoleucine, D-Val, N-methyl-valine, tert-butylglycine, D-tert-butylglycine, N-methyl-tert-butylglycine, Trp, D-Trp, N-methyl-tryptophan, D-Tyr, N-methyl-tyrosine, 1-aminocyclopropanecarboxylic acid, 1-aminocyclobutanecarboxylic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclohexanecarboxylic acid, 4-aminotetrahydro-2H-pyran-4-carboxylic acid, aminoisobutyric acid, (5)-2-amino-3-(1H-tetrazol-5-yl)propanoic acid, Glu, Gly, N-methyl-glutamate, 2-amino pentanoic acid, 2-amino hexanoic acid, 2-amino heptanoic acid, 2-amino octanoic acid, 2-amino nonanoic acid, 2-amino decanoic acid, 2-amino undecanoic acid, 2-amino dodecanoic acid, octylglycine, tranexamic acid, aminovaleric acid, and 2-(2-aminoethoxy)acetic acid. The natural side chain, or R group, of an alanine is a methyl group. In some embodiments, the non-natural side chain of the composition is a methyl group in which one or more of the hydrogen atoms is replaced by a deuterium atom. Non-natural side chains are disclosed in the art in the following publications: WO/2013/172954, WO2013123267, WO/2014/071241, WO/2014/138429, WO/2013/050615, WO/2013/050616, WO/2012/166559, US Application No. 20150094457, Ma, Z., and Hartman, M.C. (2012). In Vitro Selection of Unnatural Cyclic Peptide Libraries via mRNA Display. In J.A. Douthwaite & R.H. Jackson (Eds.), *Ribosome Display and Related Technologies: Methods and Protocols* (pp. 367-390). Springer New York., all of which are incorporated by reference in their entireties.

The terms "mimetic," "peptide mimetic" and "peptidomimetic" are used interchangeably herein, and generally refer to a peptide, partial peptide or non-peptide

molecule that mimics the tertiary binding structure or activity of a selected native peptide or protein functional domain (*e.g.*, binding motif or active site). These peptide mimetics include recombinantly or chemically modified peptides, as well as non-peptide agents such as small molecule drug mimetics, as further described below. The term "analog" refers to any

5 polypeptide comprising at least one α -amino acid and at least one non-native amino acid residue, wherein the polypeptide is structurally similar to a naturally occurring full-length PIF protein and shares the biochemical or biological activity of the naturally occurring full-length protein upon which the analog is based. In some embodiments, the compositions, pharmaceutical compositions and kits comprise a peptide or peptidomimetic sharing share no
10 less than about 70%, about 75%, about 79%, about 80%, about 85%, about 86%, about 87%, about 90%, about 93%, about 94% about 95%, about 96%, about 97%, about 98%, about 99% homology with any one or combination of PIF sequences set forth in Table 1; and wherein one or a plurality of amino acid residues is a non-natural amino acid residue or an amino acid residue with a non-natural sidechain. In some embodiments, peptide or peptide
15 mimetics are provided, wherein a loop is formed between two cysteine residues. In some embodiments, the peptidomimetic may have many similarities to natural peptides, such as: amino acid side chains that are not found among the known 20 proteinogenic amino acids, non-peptide-based linkers used to effect cyclization between the ends or internal portions of the molecule, substitutions of the amide bond hydrogen moiety by methyl groups (N-
20 methylation) or other alkyl groups, replacement of a peptide bond with a chemical group or bond that is resistant to chemical or enzymatic treatments, N- and C-terminal modifications, and conjugation with a non-peptidic extension (such as polyethylene glycol, lipids, carbohydrates, nucleosides, nucleotides, nucleoside bases, various small molecules, or phosphate or sulfate groups). As used herein, the term "cyclic peptide mimetic" or "cyclic
25 polypeptide mimetic" refers to a peptide mimetic that has as part of its structure one or more cyclic features such as a loop, bridging moiety, and/or an internal linkage. As used herein, the term "bridging moiety" refers to one or a series of bonded atoms that covalently link one or a plurality of amino acid side chains to one another within an amino acid sequence.

In some embodiments, peptide or peptide mimetics are provided, wherein the loop
30 comprises a bridging moiety selected from the group consisting of:



wherein each X is independently N or CH, such that no ring contains more than 2 N; each Z is independently a bond, NR, O, S, CH₂, C(O)NR, NRC(O), S(O)_vNR, NRS(O)_v; each m is independently selected from 0, 1, 2, and 3; each v is independently selected from 1 and 2; each R is independently selected from H and C₁-C₆; and each bridging moiety is connected to the peptide by independently selected C₀-C₆ spacers.

In some embodiments, the PIF peptides of the disclosure are modified to produce peptide mimetics by replacement of one or more naturally occurring side chains of the 20 genetically encoded amino acids (or D amino acids) with other side chains, for instance with groups such as alkyl, lower alkyl, cyclic 4-, 5-, 6-, to 7 membered alkyl, amide, amide lower alkyl, amide di (lower alkyl), lower alkoxy, hydroxy, carboxy and the lower ester derivatives thereof, and with 4-, 5-, 6-, to 7 membered heterocyclics. For example, proline analogs can be made in which the ring size of the proline residue is changed from 5 members to 4, 6, or 7 members. Cyclic groups can be saturated or unsaturated, and if unsaturated, can be aromatic or nonaromatic. Heterocyclic groups can contain one or more nitrogen, oxygen, and/or sulphur heteroatoms. Examples of such groups include the furazanyl, furyl, imidazolidinyl, imidazolyl, imidazolyl, isothiazolyl, isoxazolyl, morpholinyl (*e.g.* morpholino), oxazolyl, piperazinyl (*e.g.* 1-piperazinyl), piperidyl (*e.g.* 1-piperidyl, piperidino), pyranyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrrolidinyl (*e.g.* 1-

pyrrolidinyl), pyrrolinyl, pyrrolyl, thiadiazolyl, thiazolyl, thienyl, thiomorpholinyl (*e.g.* thiomorpholino), and triazolyl. These heterocyclic groups can be substituted or unsubstituted. Where a group is substituted, the substituent can be alkyl, alkoxy, halogen, oxygen, or substituted or unsubstituted phenyl. Peptidomimetics may also have amino acid residues that have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties.

In a further embodiment a compound of the formula $R_1-R_2-R_3-R_4-R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}$, wherein R_1 is Met or a mimetic of Met, R_2 is Val or a mimetic of Val, R_3 is Arg or a mimetic of Arg, or any amino acid, R_4 is Ile or a mimetic of Ile, R_5 is Lys or a mimetic of Lys, R_6 is Pro or a mimetic of Pro, R_7 is Gly or a mimetic of Gly, R_8 is Ser or a mimetic of Ser, R_9 is Ala or a mimetic of Ala, R_{10} is Asn or a mimetic of Asn, R_{11} is Lys or a mimetic of Lys, R_{12} is Pro or a mimetic of Pro, R_{13} is Ser or a mimetic of Ser, R_{14} is Asp or a mimetic of Asp and R_{15} is Asp or a mimetic of Asp is provided. In a further embodiment, a compound comprising the formula $R_1-R_2-R_3-R_4-R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}$, wherein R_1 is a mimetic of the naturally occurring residue at position 1 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R_2 is a mimetic of the naturally occurring residue at position 2 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R_3 is a mimetic of the naturally occurring residue at position 3 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R_4 is a mimetic of the naturally occurring residue at position 4 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R_5 is a mimetic of the naturally occurring residue at position 5 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R_6 is a mimetic of the naturally occurring residue at position 6 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R_7 is a mimetic of the naturally occurring residue at position 7 of SEQ ID

NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₈ is a mimetic of the naturally occurring residue at position 5 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₉ is a mimetic of the naturally occurring residue at position 9 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₁₀ is a mimetic of the naturally occurring residue at position 10 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₁₁ is a mimetic of the naturally occurring residue at position 11 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₁₂ is a mimetic of the naturally occurring residue at position 12 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₁₃ is a mimetic of the naturally occurring residue at position 13 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₁₄ is a mimetic of the naturally occurring residue at position 14 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences; wherein R₁₅ is a mimetic of the naturally occurring residue at position 15 of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 or the residue at that position of such sequences.

In some embodiments, the pharmaceutical composition comprising the formula R₁-R₂-R₃-R₄-R₅-R₆-R₇-R₈-R₉-R₁₀-R₁₁-R₁₂-R₁₃-R₁₄-R₁₅-R₁₆-R₁₇-R₁₈, wherein R₁ is Ser or a mimetic of Ser, R₂ is Gly or a mimetic of Gly, R₃ is Ile or a mimetic of Ile, R₄ is Val or a mimetic of Val, R₅ is Ile or a mimetic of Ile, R₆ is Tyr or a mimetic of Tyr, R₇ is Gln or a mimetic of Gln, R₈ is Tyr or a mimetic of Tyr, R₉ is Met or a mimetic of Met, R₁₀ is Asp or a mimetic of Asp, R₁₁ is Asp or a mimetic of Asp, R₁₂ is Arg or a mimetic of Arg, R₁₃ is Tyr or

a mimetic of Tyr, R₁₄ is Val or a mimetic of Val, R₁₅ is Gly or a mimetic of Gly, R₁₆ is Ser or a mimetic of Ser, R₁₇ is Asp or a mimetic of Asp and R₁₈ is Leu or a mimetic of Leu; and a compound comprising the formula R₁-R₂-R₃-R₄-R₅-R₆-R₇-R₈-R₉, wherein R₁ is Val or a mimetic of Val, R₂ is Ile or a mimetic of Ile, R₃ is Ile or a mimetic of Ile, R₄ is Ile or a mimetic of Ile, R₅ is Ala or a mimetic of Ala, R₆ is Gln or a mimetic of Gln, R₇ is Tyr or a mimetic of Tyr, R₈ is Met or a mimetic of Met, and R₉ is Asp or a mimetic of Asp is provided. In some embodiments, R₃ is not Arg or a mimetic of Arg.

A variety of techniques are available for constructing peptide mimetics with the same or similar desired biological activity as the corresponding native but with more favorable activity than the peptide with respect to solubility, stability, and/or susceptibility to hydrolysis or proteolysis (see, *e.g.*, Morgan & Gainor, Ann. Rep. Med. Chem. 24,243-252,1989).

Certain peptidomimetic compounds are based upon the amino acid sequence of the peptides of the disclosure. Often, peptidomimetic compounds are synthetic compounds having a three dimensional structure (*i.e.* a "peptide motif") based upon the three-dimensional structure of a selected peptide. The peptide motif provides the peptidomimetic compound with the desired biological activity, *i.e.*, binding to PIF receptors, wherein the binding activity of the mimetic compound is not substantially reduced, and is often the same as or greater than the activity of the native peptide on which the mimetic is modeled. Peptidomimetic compounds can have additional characteristics that enhance their therapeutic application, such as increased cell permeability, greater affinity and/or avidity and prolonged biological half-life.

Peptidomimetic design strategies are readily available in the art (see, *e.g.*, Ripka & Rich, Curr. Op. Chem. Bioi. 2,441-452,1998; Hruby et al., Curr. Op.Chem. Bioi. 1,114-119,1997; Hruby & Baise, Curr.Med. Chem. 9,945-970,2000). One class of peptidomimetics a backbone that is partially or completely non-peptide, but mimics the peptide backbone atom-for atom and comprises side groups that likewise mimic the functionality of the side groups of the native amino acid residues. Several types of chemical bonds, *e.g.*, ester, thioester, thioamide, retroamide, reduced carbonyl, dimethylene and ketomethylene bonds, are known in the art to be generally useful substitutes for peptide bonds in the construction of protease-resistant peptidomimetics. Another class of peptidomimetics comprises a small non-peptide molecule that binds to another peptide or protein, but which is not necessarily a structural mimetic of the native peptide. Yet another class of peptidomimetics has arisen from combinatorial chemistry and the generation of massive chemical libraries. These generally comprise novel templates which, though structurally unrelated to the native peptide, possess

necessary functional groups positioned on a nonpeptide scaffold to serve as "topographical" mimetics of the original peptide (Ripka & Rich, 1998, *supra*).

A list of PIF peptides are provided below in Table 1. Antibodies to various PIF peptides and scrambled PIF peptides are also provided.

5

Table 1. PIF Peptides

(SEQ ID NO)	Peptide	Amino Acid Sequence
SEQ ID NO:1 isolated native, matches region of Circumsporozoite protein (Malaria)	nPIF-1 ₁₅	MVRIKPGSANKPSDD
SEQ ID NO:2 isolated native, matches region of Circumsporozoite protein (Malaria)	nPIF -1 _(15-alter)	MVRIKYGSYNNKPSD
SEQ ID NO:3 isolated native, matches region of Circumsporozoite protein (Malaria)	nPIF-1 ₍₁₃₎	MVRIKPGSANKPS
SEQ ID NO:4 isolated native, matches region of Circumsporozoite protein (Malaria)	nPIF-1 ₍₉₎	MVRIKPGSA
SEQ ID NO:5 synthetic, scrambled amino acid sequence from region of Circumsporozoite protein Malaria	scrPIF-1 ₁₅	GRVDPSNKSMPKDIA
SEQ ID NO:6 isolated native, matches region of human retinoid and thyroid hormone receptor-SMRT	nPIF-2 ₍₁₀₎	SQAVQEHA
SEQ ID NO:7 isolated native, matches region of human retinoid and thyroid hormone receptor (SMRT)	nPIF-2 ₍₁₃₎	SQAVQEHA
SEQ ID NO:8 synthetic, scrambled amino acid sequence from region of human retinoid and thyroid hormone receptor SMRT	scrPIF-2 ₍₁₃₎	EVAQHSQASTMNG
SEQ ID NO:9	scrPIF-2 ₍₁₄₎	GQASSAQMNSTGVH
SEQ ID NO:10 isolated native, matches region of Rev Trans	nPIF-3 ₍₁₈₎	SGIVIQYMD
SEQ ID NO:11 synthetic, scrambled amino acid sequence from region of Circumsporozoite protein Malaria	Neg control for negPIF-1 ₍₁₅₎	GMRELQRSANK
SEQ ID NO:12 isolated native, matches region of Rev Trans	nPIF-4 ₍₉₎	VIIAQYMD
antibody of native isolated nPIF-1 ₁₅	AbPIF-1 ₍₁₅₎	
(SEQ ID NO:13)	sPIF-1 ₍₁₅₎	MVRIKPGSANKPSDD

synthetic, amino acid sequence from region of Circumsporozoite protein Malaria		
(SEQ ID NO:14) synthetic, amino acid sequence from of human retinoid and thyroid hormone receptor SMRT	sPIF-2 ₍₁₃₎	SQAVQEHA STMNG
(SEQ ID NO:15) synthetic, amino acid sequence from region of Circumsporozoite protein Malaria	sPIF-3 ₍₁₈₎	SGIVYQYMD DRYVGS DL
(SEQ ID NO: 16) synthetic, amino acid sequence from region of Circumsporozoite protein Malaria	sPIF-1 ₍₉₎	MVRIKPGSA
antibody of native isolated nPIF-2 ₍₁₃₎	AbPIF-2 ₍₁₃₎	
antibody of native isolated nPIF -3 ₍₁₈₎	AbPIF-3 ₍₁₈₎	
(SEQ ID NO: 17) Synthetic	sPIF-4 ₍₉₎	VIIIAQYMD
SEQ ID NO: 18 Synthetic	sPIF-1 ₍₅₎	MVRIK
SEQ ID NO: 19 Synthetic	sPIF-1 ₍₄₎	PGSA
SEQ ID NO: 20	PIF (-3)	MVXIKPGSANKPSDD
SEQ ID NO: 21	PIF (-1)	XVRIKPGSANKPSDD
SEQ ID NO: 22	PIF (-1, -3)	XVXIKPGSANKPSDD
SEQ ID NO: 23	PIF (-6)	MVRIKXGSANKPSDD
SEQ ID NO: 24	PIF (-4)	MVRXKPGSANKPSDD
SEQ ID NO: 25	PIF (-2)	MXRIKPGSANKPSDD
SEQ ID NO: 26	mut1	MVRIKEGSANKPSDD
SEQ ID NO: 27	mut3	MVRGKPGSANKPSDD
SEQ ID NO: 28	mut4	MERIKPGSANKPSDD
SEQ ID NO: 29	mut5	AVRIKPGSANKPSDD
n= native, s= synthetic, scr =scrambled, same AA, ()= number of AA, Ab=antibody, X = any amino acid, except arginine		

In some embodiments of the present disclosure, a PIF peptide (or analog) is provided.

In some embodiments, the PIF analog binds or associates with human insulin degrading enzyme (IDE – SEQ ID NO:30) at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% or

- 5 higher than native or wild-type PIF sequences. In some embodiments, the PIF analog may have a binding affinity for insulin degrading enzyme (IDE) that has at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% or higher than native or wild-type PIF sequences. In some embodiments, the PIF analog may have a binding affinity for insulin degrading enzyme that has from about 1% to about 30% or higher than the affinity native or wild-type PIF sequences
- 10 have for IDE. In some embodiments, the PIF analog may have a binding affinity for insulin degrading enzyme that has from about 1% to about 10% or higher than the affinity native or

wild-type PIF sequences have for IDE. In some embodiments, the PIF analog may have a binding affinity for insulin degrading enzyme that has from about 1% to about 20% or higher than the affinity native or wild-type PIF sequences have for IDE. In some embodiments, the PIF analog may have a binding affinity for insulin degrading enzyme that has from about 10% to about 20% or higher than the affinity native or wild-type PIF sequences have for IDE. Such PIF peptides in therapeutically effective amounts may be useful for treating any of the diseases or disorder disclosed herein.

IDE sequence:

MRYRLAWLLHPALPSTFRSVLGARLPPPERLCGFQKKTYSKMNNPAIKRIGNHITKSP
 10 EDKREYRGLELANGIKVLLISDPTTDKSSAALDVHIGSLSDPPNIAGLSHFCEHMLFLG
 TKKYPKENEYSQFLSEHAGSSNAFTSGEHTNYYFDVSHEHLEGALDRFAQFFLCPLF
 DESCKDREVNADVSEHEKNVMNDAWRLFQLEKATGNPKHPFSKFGTGKNKYTLETR
 PNQEGIDVRQELLKFHSAYYSSNLMAVCVLGRESLDDLTLNVVKLFSEVENKNVPLP
 EFPEHPFQEEHLKQLYKIVPIKDIRNLYVTFFIPDLQKYYKSNPGHYLGHLIGHEGPGS
 15 LLSELKSKGWVNTLVGGQKEGARGFMFFIINVDLTEEGLLHVEDIILHMFQYIQLRA
 EGPQEWVFQECKDLNAVAFRFKDKERPRGYTSKIAGILHYPLEEVLTAEYLLEEFR
 PDLIEMVLDKLRPENVRVAIVSKSFEGKTDRTTEWYGTQYKQEAIPDEVIKKWQNAD
 LNGKFKLPTKNEFIPTNFEILPLEKEATPYPALIKDTAMSKLWFKQDDKFFLPKACLN
 FEFSPFAYVDPLHCNMAYLYLELLKDSLNEYAYAAELAGLSYDLQNTIYGMYSV
 20 KGYNDKQPILLKKIIEKMATFEIDEKRFEIIEAYMRSLNNFRAEQPHQHAMYLRLL
 MTEVAWTKDELKEALDDVTLPLRLKAFIPQLLSRLHIEALLHGNITKQAALGIMQMVE
 DTLIEHAHTKPLLPSQLVRYREVQLPDRGWVYQQRNEVHNNGIEIYYQTDMQSTS
 ENMFLELFCQIISEPCFNTLRTKEQLGYIVFSGPRRANGIQGLRFIIQSEKPPHYLESRV
 EAFLITMEKSIEDMTEEAFQKHIQALAIRRLDKPKKLSAECAKYWGEIISQQYNFDRD
 25 NTEVAYLKTTLTKEDIKFYKEMLAVDAPRRHKVSVHVLAREMDSCPVVGEFPCQNDI
 NLSQAPALPQPEVIQNMTEFKRGLPLFPLVKPHINFMAAKL (SEQ ID NO:30).

In another embodiment, a pharmaceutical composition comprising a PIF peptide is provided. In preferred embodiments, the pharmaceutical composition comprises a therapeutically effective amount of a PIF peptide or a pharmaceutically acceptable salt thereof.

In another embodiment, a method of treating endometriosis is provided. In a preferred embodiment, the method comprises administering an effective amount of a PIF peptide to a subject in need thereof. In a further embodiment, a method for treating endometriosis comprising administering an effective amount of a PIF peptide in combination with one or

more immunotherapeutic, anti-epileptic, diuretic, or blood pressure controlling drugs or compounds to a subject in need thereof is provided. Such a combination may enhance the effectiveness of the treatment of either component alone, or may provide less side effects and/or enable a lower dose of either component.

5 In some embodiments, a PIF peptide is provided. Such PIF peptides may be useful for treating or ameliorating immune-mediated disorders, such as autoimmune diseases, such as endometriosis. The PIF peptide can be, for example, as described herein.

 In some embodiments, a pharmaceutical composition comprising a PIF peptide or a pharmaceutically acceptable salt thereof is provided. In preferred embodiments, the
10 pharmaceutical composition comprises a therapeutically effective amount of a PIF peptide or a pharmaceutically acceptable salt thereof.

 For therapeutic treatment of the specified indications, a PIF peptide may be administered as such, or can be compounded and formulated into pharmaceutical compositions in unit dosage form for parenteral, transdermal, rectal, nasal, local intravenous
15 administration, or, preferably, oral administration. Such pharmaceutical compositions are prepared in a manner well known in the art and comprise at least one active PIF peptide associated with a pharmaceutically carrier. The term "active compound", as used throughout this specification, refers to at least one compound selected from compounds of the formulas or pharmaceutically acceptable salts thereof.

20 In such a composition, the active compound is known as "active ingredient." In making the compositions, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier that may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid, or liquid material that acts as a vehicle, excipient or medium for the active ingredient. Thus, the
25 composition can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, emulsion, solutions, syrups, suspensions, soft and hard gelatin capsules, sterile injectable solutions, and sterile packaged powders.

 The terms "pharmaceutical preparation" or "pharmaceutical composition" includes preparations suitable for administration to mammals, e.g., humans. When the compounds of
30 the present disclosure are administered as pharmaceuticals to mammals, e.g., humans, they can be given per se or as a pharmaceutical composition containing, for example, from about 0.1 to about 99.5% of active ingredient in combination with a pharmaceutically acceptable carrier.

 The phrase "pharmaceutically acceptable carrier" is art recognized and includes a

pharmaceutically acceptable material, composition or vehicle, suitable for administering compounds of the present disclosure to mammals. The carriers include, for example, liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, which is incorporated herein by reference in its entirety. In some embodiments, the pharmaceutically acceptable carrier is sterile and pyrogen-free water. In some embodiments, the pharmaceutically acceptable carrier is Ringer's Lactate, sometimes known as lactated Ringer's solution.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, .alpha.-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present disclosure include those suitable for oral, nasal, topical, buccal, sublingual, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient that can be combined with a

carrier material to produce a single dosage form will generally be that amount of the compound that produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate alginates, calcium salicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, tragacanth, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium stearate, water, and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

For oral administration, a compound can be admixed with carriers and diluents, molded into tablets, or enclosed in gelatin capsules. The mixtures can alternatively be dissolved in liquids such as 10% aqueous glucose solution, isotonic saline, sterile water, or the like, and administered intravenously or by injection.

The local delivery of inhibitory amounts of active compound for the treatment of immune disorders can be by a variety of techniques that administer the compound at or near the targeted site. Examples of local delivery techniques are not intended to be limiting but to be illustrative of the techniques available. Examples include local delivery catheters, site specific carriers, implants, direct injection, or direct applications, such as topical application.

Local delivery by an implant describes the surgical placement of a matrix that contains the pharmaceutical agent into the affected site. The implanted matrix releases the pharmaceutical agent by diffusion, chemical reaction, or solvent activators.

For example, in some aspects, the disclosure is directed to a pharmaceutical composition comprising a PIF peptide, and a pharmaceutically acceptable carrier or diluent, or an effective amount of pharmaceutical composition comprising a PIF peptide.

The compounds of the present disclosure can be administered in the conventional manner by any route where they are active. Administration can be systemic, topical, or oral. For example, administration can be, but is not limited to, parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, oral, buccal, ocular routes, intravaginally, by inhalation, by depot injections, or by implants. Thus, modes of

administration for the compounds of the present disclosure (either alone or in combination with other pharmaceuticals) can be, but are not limited to, sublingual, injectable (including short-acting, depot, implant and pellet forms injected subcutaneously or intramuscularly), or by use of vaginal creams, suppositories, pessaries, vaginal rings, rectal suppositories, intrauterine devices, and transdermal forms such as patches and creams.

Specific modes of administration will depend on the indication. The selection of the specific route of administration and the dose regimen is to be adjusted or titrated by the clinician according to methods known to the clinician in order to obtain the optimal clinical response. The amount of compound to be administered is that amount which is therapeutically effective. The dosage to be administered will depend on the characteristics of the subject being treated, e.g., the particular mammal or human treated, age, weight, health, types of concurrent treatment, if any, and frequency of treatments, and can be easily determined by one of skill in the art (e.g., by the clinician).

Pharmaceutical formulations containing the compounds of the present disclosure and a suitable carrier can be solid dosage forms which include, but are not limited to, tablets, capsules, cachets, pellets, pills, powders and granules; topical dosage forms which include, but are not limited to, solutions, powders, fluid emulsions, fluid suspensions, semi-solids, ointments, pastes, creams, gels and jellies, and foams; and parenteral dosage forms which include, but are not limited to, solutions, suspensions, emulsions, and dry powder; comprising an effective amount of a polymer or copolymer of the present disclosure. It is also known in the art that the active ingredients can be contained in such formulations with pharmaceutically acceptable diluents, fillers, disintegrants, binders, lubricants, surfactants, hydrophobic vehicles, water soluble vehicles, emulsifiers, buffers, humectants, moisturizers, solubilizers, preservatives and the like. The means and methods for administration are known in the art and an artisan can refer to various pharmacologic references for guidance. For example, *Modern Pharmaceuticals*, Banker & Rhodes, Marcel Dekker, Inc. (1979); and *Goodman & Gilman's The Pharmaceutical Basis of Therapeutics*, 6th Edition, MacMillan Publishing Co., New York (1980) can be consulted.

The compounds of the present disclosure can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. The compounds can be administered by continuous infusion subcutaneously over a predetermined period of time. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as

suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

For oral administration, the compounds can be formulated readily by combining these compounds with pharmaceutically acceptable carriers well known in the art. Such carriers
5 enable the compounds of the disclosure to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by adding a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include,
10 but are not limited to, fillers such as sugars, including, but not limited to, lactose, sucrose, mannitol, and sorbitol; cellulose preparations such as, but not limited to, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as, but not limited to, the cross-
15 linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores can be provided with suitable coatings. For this purpose, concentrated sugar solutions can be used, which can optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the
20 tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include, but are not limited to, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active
25 ingredients in admixture with filler such as, e.g., lactose, binders such as, e.g., starches, and/or lubricants such as, e.g., talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers can be added. All formulations for oral administration should be in dosages suitable for such administration.

30 For buccal administration, the compositions can take the form of, e.g., tablets or lozenges formulated in a conventional manner.

For administration by inhalation, the compounds for use according to the present disclosure are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g.,

dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds of the present disclosure can also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds of the present disclosure can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection.

Depot injections can be administered at about 1 to about 6 months or longer intervals. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

In transdermal administration, the compounds of the present disclosure, for example, can be applied to a plaster, or can be applied by transdermal, therapeutic systems that are consequently supplied to the organism.

Pharmaceutical compositions of the compounds also can comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as, e.g., polyethylene glycols.

For parenteral administration, analog can be, for example, formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques. For example, a parenteral composition suitable for administration by injection is prepared by dissolving 1.5% by weight of analog in 0.9% sodium chloride solution.

The present embodiments also relate to routes of administration include intramuscular, sublingual, intravenous, intraperitoneal, intrathecal, intravaginal, intraurethral,

intradermal, intrabuccal, via inhalation, via nebulizer and via subcutaneous injection. Alternatively, the pharmaceutical composition may be introduced by various means into cells that are removed from the individual. Such means include, for example, microprojectile bombardment and liposome or other nanoparticle device.

5 Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In solid dosage forms, the analogs are generally admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, starch, or other generally regarded as safe (GRAS) additives. Such dosage forms can also comprise, as is normal practice, an additional substance other than an inert diluent, e.g., lubricating agent such as
10 magnesium stearate. With capsules, tablets, and pills, the dosage forms may also comprise a buffering agent. Tablets and pills can additionally be prepared with enteric coatings, or in a controlled release form, using techniques known in the art.

 Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions and syrups, with the elixirs containing an inert diluent
15 commonly used in the art, such as water. These compositions can also include one or more adjuvants, such as wetting agent, an emulsifying agent, a suspending agent, a sweetening agent, a flavoring agent or a perfuming agent.

 In some embodiments, the compounds and compositions described are used to treat a patient suffering from, or susceptible to endometriosis and an autoimmune disease.

20 One of skill in the art will recognize that the appropriate dosage of the compositions and pharmaceutical compositions may vary depending on the individual being treated and the purpose. For example, the age, body weight, and medical history of the individual patient may affect the therapeutic efficacy of the therapy. Further, a lower dosage of the composition may be needed to produce a transient cessation of symptoms, while a larger dose may be
25 needed to produce a complete cessation of symptoms associated with the disease, disorder, or indication. A competent physician can consider these factors and adjust the dosing regimen to ensure the dose is achieving the desired therapeutic outcome without undue experimentation. It is also noted that the clinician and/or treating physician will know how and when to interrupt, adjust, and/or terminate therapy in conjunction with individual patient response.
30 Dosages may also depend on the strength of the particular analog chosen for the pharmaceutical composition.

 The dose of the composition or pharmaceutical compositions may vary. The dose of the composition may be once per day. In some embodiments, multiple doses may be administered to the subject per day. In some embodiments, the total dosage is administered in

at least two application periods. In some embodiments, the period can be an hour, a day, a month, a year, a week, or a two-week period. In an additional embodiment of the invention, the total dosage is administered in two or more separate application periods, or separate doses over the course of about an hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 or more hours. a day, a month, a year, a week, or a two-week period.

In some embodiments, subjects can be administered the composition in which the composition is provided in a daily dose range of about 0.0001 mg/kg to about 5000 mg/kg of the weight of the subject. The dose administered to the subject can also be measured in terms of total amount of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof administered per day. In some embodiments, a subject is administered from about 0.001 to about 3000 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered up to about 2000 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered up to about 1800 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered up to about 1600 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered up to about 1400 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered up to about 1200 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered up to about 1000 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered up to about 800 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per day. In some embodiments, a subject is administered from about 0.001 milligrams to about 700 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per dose. In some embodiments, a subject is administered up to about 700 milligrams of PIF peptide or PIF analog per dose. In some embodiments, a subject is administered up to about 600 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per dose. In some embodiments, a subject is administered up to about 500 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per dose. In some embodiments, a subject is administered up to about 400 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per dose. In some embodiments, a subject is administered up to about 300 milligrams of PIF peptide or PIF

analog or pharmaceutically acceptable salt thereof per dose. In some embodiments, a subject is administered up to about 200 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per dose. In some embodiments, a subject is administered up to about 100 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per dose. In some embodiments, a subject is administered up to about 50 milligrams of PIF peptide or PIF analog or pharmaceutically acceptable salt thereof per dose.

In some embodiments, subjects can be administered the composition in which the composition comprising a PIF peptide or PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dose range of about 0.0001 mg/kg to about 5000 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 450 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 400 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 350 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 300 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 250 mg/kg of the weight of the subject. In some embodiments, the composition comprising PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 200 mg/kg of the weight of the subject. In some embodiments, the composition comprising PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 150 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 100 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 50 mg/kg of the weight of the subject. In some embodiments, the composition comprising PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 25 mg/kg of the weight of the subject.

In some embodiments, the composition comprising a PIF peptide or a PIF analog or

pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 10 mg/kg of the weight of the subject. In some embodiments, the composition comprising PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 5 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 2.0 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 3.0 mg/kg of the weight of the subject. In some embodiments, the composition comprising PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 1 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF peptide or a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 0.1 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 0.01 mg/kg of the weight of the subject. In some embodiments, the composition comprising a PIF analog or pharmaceutically acceptable salt thereof is administered in a daily dosage of up to about 0.001 mg/kg of the weight of the subject. The dose administered to the subject can also be measured in terms of total amount of a PIF peptide or PIF analog administered per day.

In some embodiments, a subject in need thereof is administered from about 1 ng to about 500 µg of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 1 ng to about 10 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 10 ng to about 20 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 10 ng to about 100 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 100 ng to about 200 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 200 ng to about 300 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 300 ng to about 400 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 400 ng to about 500 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 500 ng to about 600 ng of analog or pharmaceutically salt thereof

per day. In some embodiments, a subject in need thereof is administered from about 600 ng to about 700 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 800 ng to about 900 ng of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 900 ng to about 1 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 1 μ g to about 100 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 100 μ g to about 200 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 200 μ g to about 300 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 300 μ g to about 400 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 400 μ g to about 500 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 500 μ g to about 600 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 600 μ g to about 700 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 800 μ g to about 900 μ g of analog or pharmaceutically salt thereof per day. In some embodiments, a subject in need thereof is administered from about 900 μ g to about 1 mg of analog or pharmaceutically salt thereof per day.

In some embodiments, a subject in need thereof is administered from about .0001 to about 3000 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered up to about 2000 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered up to about 1800 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered up to about 1600 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered up to about 1400 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered up to about 1200 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered up to about 1000 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered up to about 800 milligrams of a PIF peptide or PIF

analog or pharmaceutically salt thereof per day. In some embodiments, a subject is administered from about 0.0001 milligrams to about 700 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 700 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 600 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 500 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 400 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 300 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 200 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 100 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 50 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 25 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 15 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 50, 60, 70, 80, 90, or 100 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose.

In some embodiments, a subject is administered up to about 10 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 5 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 1 milligram of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 0.1 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 0.001 milligrams of a PIF peptide or PIF analog or pharmaceutically salt thereof per dose.

The dose administered to the subject can also be measured in terms of total amount of a PIF peptide or PIF analog or pharmaceutically salt thereof administered per ounce of liquid prepared. In some embodiments, the PIF peptide or PIF analog or pharmaceutically salt

peptide or PIF analog or pharmaceutically salt thereof is at a concentration of about 0.2 grams per ounce of solution. In some embodiments, the PIF peptide or PIF analog or pharmaceutically salt thereof is at a concentration of about 0.1 grams per ounce of solution. In some embodiments, the PIF peptide or PIF analog or pharmaceutically salt thereof is at a concentration of about 0.01 grams per ounce of solution. In some embodiments, the PIF peptide or PIF analog or pharmaceutically salt thereof is at a concentration of about 0.001 grams per ounce of solution prepared. In some embodiments, the PIF peptide or PIF analog or pharmaceutically salt thereof is at a concentration of about 0.0001 grams per ounce of solution prepared. In some embodiments, the PIF peptide or PIF analog or pharmaceutically salt thereof is at a concentration of about 0.00001 grams per ounce of solution prepared. In some embodiments, the PIF peptide or PIF analog or pharmaceutically salt thereof is at a concentration of about 0.000001 grams per ounce of solution prepared.

Dosage may be measured in terms of mass amount of analog per liter of liquid formulation prepared. One skilled in the art can increase or decrease the concentration of the analog in the dose depending upon the strength of biological activity desired to treat or prevent any above-mentioned disorders associated with the treatment of subjects in need thereof. For instance, some embodiments of the invention can include up to 0.00001 grams of analog per 5 mL of liquid formulation and up to about 10 grams of analog per 5 mL of liquid formulation. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:1. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:2. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:3. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:4. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:5. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:6. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:7. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:8. In some

embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:9. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:10. In some
5 embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:11. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:12. In some
10 embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:13. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:14. In some
15 embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:15. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:16. In some
20 embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:17. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:18. In some
25 embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:19. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:20. In some
30 embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:21. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:22. In some
embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:23. In some
embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:24. In some
embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:25. In some

embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:26. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:27. In some
5 embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:28. In some embodiments, the pharmaceutical compositions comprising a PIF analog or any of such compositions in any of the disclosed methods are free of SEQ ID NO:29.

In some embodiments the pharmaceutical compositions of the claimed invention
10 comprises at least one or a plurality of active agents other than the PIF peptide, analog or pharmaceutically acceptable salt thereof. In some embodiments the active agent is covalently linked to the PIF peptide or PIF analog disclosed herein optionally by a protease cleavable linker (including by not limited to Pro-Pro or Cituline-Valine di- α -amino acid linkers). In some embodiments, the one or plurality of active agents is one or a combination of
15 compounds chosen from: an anti-inflammatory compound, alpha-adrenergic agonist, antiarrhythmic compound, analgesic compound, and an anesthetic compound.

Table Y

Examples of anti-inflammatory compounds include:

20 aspirin
celecoxib
diclofenac
diflunisal
etodolac
25 ibuprofen
indomethacin
ketoprofen
ketorolac nabumetone
naproxen
30 oxaprozin
piroxicam
salsalate
sulindac
tolmetin
35

Examples of alpha-adrenergic agonists include:

Methoxamine
Methylnorepinephrine
Midodrine

Oxymetazoline
Metaraminol
Phenylephrine
Clonidine (mixed alpha2-adrenergic and imidazoline-II receptor agonist)
5 Guanfacine, (preference for alpha2A-subtype of adrenoceptor)
Guanabenz (most selective agonist for alpha2-adrenergic as opposed to imidazoline-II)
Guanoxabenz (metabolite of guanabenz)
Guanethidine (peripheral alpha2-receptor agonist)
Xylazine,
10 Tizanidine
Medetomidine
Methyldopa
Fadolmidine
Dexmedetomidine

15 Examples of antiarrhythmic compound include:

Amiodarone (Cordarone, Pacerone)
Bepiridil Hydrochloride (Vascor)
Disopyramide (Norpace)
20 Dofetilide (Tikosyn)
Dronedarone (Multaq)
Flecainide (Tambocor)
Ibutilide (Corvert)
Lidocaine (Xylocaine)
25 Procainamide (Procan, Procanbid)
Propafenone (Rythmol)
Propranolol (Inderal)
Quinidine (many trade names)
Sotalol (Betapace)
30 Tocainide (Tonocarid)

Examples of analgesic compound include:

codeine
hydrocodone (Zohydro ER),
35 oxycodone (OxyContin, Roxicodone),
methadone
hydromorphone (Dilaudid, Exalgo),
morphine (Avinza, Kadian, MSIR, MS Contin), and
fentanyl (Actiq, Duragesic)

40 Examples of anesthetic compounds include:

Desflurane
Isoflurane
Nitrous oxide
45 Sevoflurane
Xenon

The compounds of the present disclosure can also be administered in combination with other active ingredients, such as, for example, adjuvants, or other compatible drugs or compounds where such combination is seen to be desirable or advantageous in achieving the desired effects of the methods described herein.

5 The methods disclosed herein can be used with any of the compounds, compositions, preparations, and kits disclosed herein.

 In some embodiments, methods of diagnosing endometriosis in a human subject are provided. In some embodiments, the method comprises measuring pre-implantation factor (PIF) protein or mRNA expression levels from an endometrial tissue sample from the subject;
10 and comparing the PIF protein expression levels from the endometrial sample to the PIF expression levels in a control normal sample; wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial sample are greater than the PIF expression levels from the control normal sample. In some embodiments, the endometrial sample is an endometrial epithelial tissue sample .

15 In some embodiments, the sample is a biopsy sample. That is the endometrial sample can, for example, be obtained by a biopsy.

 Methods of measuring PIF protein expression levels can be performed by any method. For example, in some embodiments, the measuring of PIF protein expression levels comprises contacting the sample with an anti-PIF antibody and detecting bound anti-PIF
20 antibody to PIF in the sample. The detection can be by staining methods or other type methods using modified antibodies to visualize and/or quantitate the amount of PIF in the sample. The amount of the bound antibody can be converted into the amount of PIF present in the sample by comparing to controls, which is routine in the art. In some embodiments, the amount of bound antibody indicates the PIF protein expression levels. The sample can also be stained
25 for PIF protein expression levels using available protocols. As a non-limiting example, the method can comprise staining the sample with an anti-PIF antibody that binds to PIF in the sample. In some embodiments, instead of measuring protein levels, the mRNA levels encoding PIF are measured. Accordingly, in some embodiments, the measuring of PIF mRNA expression levels comprises contacting the sample with a probe that binds to PIF
30 mRNA and detecting the bound probe in the sample. The probe can be detected by performing amplification methods, such as RT-PCR, PCR, and the like. In some embodiments, the probe is linked to fluorescent or radioactive moiety and the probe is detected by measuring the fluorescence or radioactive signal to determine the presence or absence of PIF in the sample.

In some embodiments, a method of treating endometriosis is combined with a method of diagnosing endometriosis. In some embodiments, the method comprises measuring pre-implantation factor (PIF) protein or mRNA expression levels from an endometrial sample from the subject; comparing the PIF protein or mRNA expression levels from the endometrial sample to the PIF expression levels in a control normal sample, wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial sample are greater than the PIF expression levels from the control normal sample; and administering to the subject at least one pharmaceutical composition comprising a therapeutically effective amount of a PIF peptide, mimetics thereof, analogs thereof, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. In some embodiments, the endometrial sample is an endometrial epithelial tissue sample. In some embodiments, the endometrial tissue sample is a biopsy sample. The PIF protein expression levels and the PIF mRNA expression levels can be measured, for example, according to the methods described herein.

In some embodiments, if the PIF expression levels in the endometrial epithelial tissue sample are detectable or greater than as compared to the control, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 1% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 5% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 10% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 15% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 20% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 25% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 30% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 50% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the

PIF expression levels in the endometrial epithelial tissue sample are at least 100% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 200% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 300% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 400% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 500% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the PIF expression levels in the endometrial epithelial tissue sample are at least 1000% greater than the PIF expression levels in the control sample, the patient is diagnosed with endometriosis. In some embodiments, the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 10% greater than the PIF expression levels from the control normal sample. In some embodiments, the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 50% greater than the PIF expression levels from the control normal sample. In some embodiments, the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 100% greater than the PIF expression levels from the control normal sample. In some embodiments, the patient is with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 200% greater than the PIF expression levels from the control normal sample. For the avoidance of doubt, a control sample is a control normal sample unless otherwise indicated by context.

The disclosure also relates to methods for treating endometriosis comprising administering an effective amount of the compositions described herein to a subject in need thereof.

In an embodiment, the composition is administered once a day to a subject in need thereof. In another embodiment, the composition is administered every other day, every third day or once a week. In another embodiment, the composition is administered twice a day. In still another embodiment, the composition is administered three times a day or four times a day. In a further embodiment, the composition is administered at least once a day for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks. In still a further embodiment, the composition is

administered at least once a day for a longer term such as at least 4, 6, 8, 10, 12 or 24 months. Administration in some embodiments includes but is not limited to a dosage of 10-50 mg of composition at a frequency of minimum 1, 2, 3 or 4 times per day. Optionally, administration continues until all symptoms are resolved and cleared by medical personnel via standardized testing such as SCAT 2.

In some embodiments, the composition is administered at least once a day until the condition has ameliorated to where further treatment is not necessary. In another embodiment, the composition is administered until all symptoms of endometriosis are resolved. In further embodiments, the composition is administered for at least 1, 2, 3, 6, 8, 10 or 12 or 24 months after the subject is asymptomatic.

The compositions of the present disclosure are useful and effective when administered to treat endometriosis. The amount of each component present in the composition will be the amount that is therapeutically effective, i.e., an amount that will result in the effective treatment of the condition (e.g., endometriosis) when administered. The therapeutically effective amount will vary depending on the subject and the severity and nature of the injury and can be determined routinely by one of ordinary skill in the art.

In some embodiments, the disclosure relates to a method of treating or preventing any of the indications set forth in US Pat. Nos. 8,222,211, 7,723,289, 7,723,290, 8,454,967, 9,097,725, (each of which are incorporated by reference in their entireties) comprising administering compositions or pharmaceutical compositions comprising any one or plurality of PIF peptides, analogs, or pharmaceutically acceptable salts thereof disclosed herein.

In some methods, the disclosure relates to a method of stimulating the differentiation and/or proliferation of stem cells in a subject in need thereof comprising administering compositions or pharmaceutical compositions comprising any one or plurality of PIF peptides, analogs, or pharmaceutically acceptable salts thereof disclosed herein.

In some embodiments, the disclosure relates to any of the methods disclosed in US Pat. Nos. 7,273,708, 7,695,977, 7,670,852, 7,670,851, 7,678,582, 7,670,850, 8,012,700 (each of which are incorporated by reference in their entireties) comprising administering compositions or pharmaceutical compositions comprising any one or plurality of PIF peptides, analogs, or pharmaceutically acceptable salts thereof disclosed herein.

This disclosure also incorporates by reference in their entireties US Pat. Nos. 7,789,289, 7,723,290, 8,222,211, and 8,454,967.

In some embodiments, the disclosure relates to a method of treating endometriosis by administering at least one or a plurality of compositions disclosed herein comprising PIF peptide, an analog thereof, or a pharmaceutically acceptable salt thereof.

5 In some embodiments, the disclosure relates to a method of endometriosis by administering a therapeutically effective amount or dose of one or a plurality of compositions disclosed herein comprising at least one PIF peptide, an analog thereof, or a pharmaceutically acceptable salt thereof.

10 In some embodiments, the disclosure relates to a method of treating endometriosis by administration of a pharmaceutical composition comprising a therapeutically effective amount or dose of at least one PIF peptide, an analog thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

15 In some embodiments, the disclosure relates to a pharmaceutical composition comprising a therapeutically effective amount or dose of at least one PIF peptide, an analog thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier for the treatment of endometriosis.

20 In some embodiments, the disclosure relates to the use of a therapeutically effective amount or dose of any one or plurality of compositions disclosed herein comprising at least one PIF peptide, an analog thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier in the manufacture of a medicament for the treatment of endometriosis.

In some embodiments, the disclosure relates to the use of a pharmaceutical composition comprising a therapeutically effective amount or dose at least one PIF peptide, an analog thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier in the manufacture of a medicament for the endometriosis.

25 In some embodiments, the disclosure relates to a method of inducing an immunomodulation effect in a subject in need thereof, when subject has been or is suspect of having endometriosis.

Kits

30 According to some embodiments of the invention, the formulation may be supplied as part of a kit. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:1. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide

and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:2. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof

5 comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:3. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:4. In some embodiments, the kit comprises comprising a

10 PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:5. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof

15 comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:6. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:7. In some embodiments, the kit comprises comprising a

20 PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:8. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof

25 comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:9. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:10. In some embodiments, the kit comprises comprising a

30 PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:11. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof

comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:12. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:13. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:14. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:15. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:16. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:17. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:18. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:19. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:20. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:21. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least

70% homologous to SEQ ID NO:22. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:23. In some embodiments, the kit
5 comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:24. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or
10 pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:25. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:26. In some embodiments, the kit
15 comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:27. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or
20 pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:28. In some embodiments, the kit comprises comprising a PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof, the PIF peptide and/or a PIF analog or pharmaceutically acceptable salt thereof comprises a non-natural amino acid or is at least 70% homologous to SEQ ID NO:29. In another embodiment, the kit
25 comprises a pharmaceutically acceptable salt of an analog with a rehydration mixture. In another embodiment, the pharmaceutically acceptable salt of an analog are in one container while the rehydration mixture is in a second container. The rehydration mixture may be supplied in dry form, to which water or other liquid solvent may be added to form a suspension or solution prior to administration. Rehydration mixtures are mixtures designed to
30 solubilize a lyophilized, insoluble salt of the invention prior to administration of the composition to a subject takes at least one dose of a purgative. In another embodiment, the kit comprises a pharmaceutically acceptable salt in orally available pill form.

The kit may contain two or more containers, packs, or dispensers together with instructions for preparation and administration. In some embodiments, the kit comprises at

least one container comprising the pharmaceutical composition or compositions described herein and a second container comprising a means for delivery of the compositions such as a syringe. In some embodiments, the kit comprises a composition comprising an analog in solution or lyophilized or dried and accompanied by a rehydration mixture. In some
5 embodiments, the analog and rehydration mixture may be in one or more additional containers.

 The compositions included in the kit may be supplied in containers of any sort such that the shelf-life of the different components are preserved, and are not adsorbed or altered by the materials of the container. For example, suitable containers include simple bottles that
10 may be fabricated from glass, organic polymers, such as polycarbonate, polystyrene, polypropylene, polyethylene, ceramic, metal or any other material typically employed to hold reagents or food; envelopes, that may consist of foil-lined interiors, such as aluminum or an alloy. Other containers include test tubes, vials, flasks, and syringes. The containers may have two compartments that are separated by a readily removable membrane that upon removal
15 permits the components of the compositions to mix. Removable membranes may be glass, plastic, rubber, or other inert material.

 Kits may also be supplied with instructional materials. Instructions may be printed on paper or other substrates, and/or may be supplied as an electronic-readable medium, such as a floppy disc, CD-ROM, DVD-ROM, zip disc, videotape, audio tape, or other readable
20 memory storage device. Detailed instructions may not be physically associated with the kit; instead, a user may be directed to an internet web site specified by the manufacturer or distributor of the kit, or supplied as electronic mail.

 In another embodiment, a packaged kit is provided that contains the pharmaceutical formulation to be administered, i.e., a pharmaceutical formulation containing PIF peptide
25 and/or a PIF analog or pharmaceutically acceptable salt thereof, a container (e.g., a vial, a bottle, a pouch, an envelope, a can, a tube, an atomizer, an aerosol can, etc.), optionally sealed, for housing the formulation during storage and prior to use, and instructions for carrying out drug administration in a manner effective to treat any one or more of the indications disclosed herein. The instructions will typically be written instructions on a
30 package insert, a label, and/or on other components of the kit.

 Depending on the type of formulation and the intended mode of administration, the kit may also include a device for administering the formulation (e.g., a transdermal delivery device). The administration device may be a dropper, a swab, a stick, or the nozzle or outlet of an atomizer or aerosol can. The formulation may be any suitable formulation as described

herein. For example, the formulation may be an oral dosage form containing a unit dosage of the active agent, or a gel or ointment contained within a tube. The kit may contain multiple formulations of different dosages of the same agent. The kit may also contain multiple formulations of different active agents. The kit may contain a number of pharmaceutically effective dosages in separate containers or syringes necessary to treat one or more symptoms of endometriosis. in some embodiments, the kit contains about 1, 2, 3, 4, or 5 or more dosages in 1, 2, 3, 4, or five containers (such as a syringe), that enable administration of any of the dosages into the subject in need thereof.

The present kits will also typically include means for packaging the individual kit components, i.e., the pharmaceutical dosage forms, the administration device (if included), and the written instructions for use. Such packaging means may take the form of a cardboard or paper box, a plastic or foil pouch, etc.

According to some embodiments, the kits are provided that can be used for diagnosing subjects with endometriosis. The kits can, for example, comprises reagents that are used to detect PIF in the endometrial sample. These reagents can be buffers, antibodies to PIF, secondary antibodies, stains, probes to PIF mRNA and the like. The kit can also comprise instructions for performing methods described herein. In some embodiments, the kit also comprises reagents for processing the endometrial sample before detecting PIF expression.

This disclosure and embodiments illustrating the method and materials used may be further understood by reference to the following non-limiting examples.

Example 1: PIF expression in endometriosis

Endometriosis is a chronic benign disease characterized by the presence of endometrial tissue, both stromal and epithelial components, outside the uterine cavity, affecting women in their reproductive age (Giudice LC 2010). Endometriosis lesions can be found mostly in the pelvis, where the most common sites are the ovaries, anterior and posterior cul-de-sac, broad and utero-sacral ligaments, uterus, fallopian tubes, sigmoid colon and appendix (Olive et al 1993). At the moment the most accepted hypothesis regarding its pathogenesis is the retrograde menstruation, even though stem cell origin as well as its genetic cause are gaining attention (Macer et al 2012).

Several evidences have shown the presence of putative stem cells in the human endometrium as well as in other mammals (Taylor 2004, Du et al 2007, Kao et al 2011, Hafnagel et al 2015), and it has been showed that stem cells for the endometrium may be

involved in endometriosis pathogenesis: apart from their of endometrial origin they may be mesenchymal stem cells derived from bone marrow.

Pre-Implantation Factor or PIF is a fifteen amino acid linear peptide secreted by viable human, bovine and murine embryos (Stamatkin et al 2011, Stamatkin et al 2011b, Barnea et al 2012, Ramu et al 2013). In singly cultured bovine and murine embryos increased levels of PIF in the media correlate with embryos development whereas PIF is absent in non-viable embryos (Stamatkin et al 2011). PIF plays an essential role in human pregnancy, as it primes the endometrium for implantation, promotes trophoblast invasion and regulates systemic immune response (Barnea et al 2012, Paidas et al 2010, Barnea et al 2012b, Duzyj et al 2010). Relevant to PIF's immune regulatory features, translational aspects to treatment of non-pregnant autoimmune and transplantation models were documented (Weiss et al 2011, Weiss et al 2012, Azar et al 2013). PIF also promotes trophoblast invasion, and orchestrates maternal systemic immune response (Barnea et al 2012, Duzyj et al, Roussev et al 2013). Pathway analysis in autoimmunity and transplantation models demonstrate that sPIF, administered as a single agent to non-pregnant mice, acts by reducing oxidative stress and protein misfolding (Weiss et al 2011, Weiss et al 2012, Paidas et al 2012, Azer et al 2013, Shainer et al 2013).

Tregs are derived from the CD4 lineage of T cells and are produced naturally in the thymus, express IL-10 receptor (CD25+) and the forkhaed box P3 transcription factor (Foxp3+). CD4+ T cells become Tregs by the Foxp3 expression induced by the cytokines increase in the microenvironment. Activated Tregs suppress the response of effector T cell indirectly by inhibiting the dendritic cells or the other antigen presenting cells (APC) from triggering effector T cell proliferation (Tang et al 2006). Tregs induce immune tolerance through the production of IL-10 and Transforming Growth Factor- β , anti-inflammatory cytokines or Th2, which inhibit T helper cell activation. (von Boemer et al 2006). The absence or depletion of Tregs leads to multi-systemic autoimmunity in mice and humans (Bruckner et al 2010). It has been reported that Tregs are critical for tumor growth, since they may provide an immunologically protected micro environment (Bergman et al 2007, Strauss et al 2007, Bergmann et al 20011, Wang et al 2012). The presence of Tregs in eutopic and ectopic endometrium of women affected by endometriosis has been reported (Budiu et al 2009, Berbic et al 2010).

The aim of the study was to evaluate the possible expression of PIF and FOX-P3 in the ectopic endometrium in order to evaluate the expression of these antigens in the ectopic

endometrium, and identify cells that are able to modulate immune competent cells response in endometriotic lesions.

Material and Methods

5 Synthetic PIF15 (MVRKPGSANKPSDD) and a scrambled peptide same amino acid sequence but in random order (GRVDPSNKSMPKDIA) were synthesized by solid-phase peptide synthesis (Peptide Synthesizer, Applied Biosystems) employing Fmoc (9-fluorenylmethoxycarbonyl) chemistry at Bio-Synthesis, Inc. (Lewisville, TX). Final purification was carried out by reversed-phase HPLC and identity was verified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and amino acid analysis
10 at >95% purity. Fluorescein labeled FITC-PIF and scrambled PIF (FITC- PIFscr) were also generated as previously reported (Barnea AJOG 2012). Anti-PIF monoclonal antibody against MVRKPGSANKPSDD was generated in (Genway, SanDiego, CA). et al 2012).

Patient tissue material: Tissue specimens were obtained from 25 women who
15 underwent laparoscopic surgery for severe endometriosis according to the revised criteria of the American Society Reproductive Medicine. The surgical procedures were carried out in CERM-Hungaria Institute, Rome, Italy, from September 2014 through April 2015. The project had the approval of the Hospital's Ethical Committee. Samples were obtained from the ectopic endometrium, ovarian endometriomas and peritoneal implants. A total of 25
20 eutopic endometria, 25 ovarian endometriomas and 10 peritoneal implants were collected from patients. Furthermore, the endometria of 10 healthy women in different phases of the menstrual cycle were used as controls. Biopsy samples were fixed in 4% neutral buffered formalin overnight and were subsequently paraffin embedded.

Before performing immunohistochemistry sections of the tissues were stained with
25 eosin and hematoxylin to select tissues with ectopic epithelial cells. Serial sections of the same selected samples 5µm thick were used for immunohistochemistry. Commercially available monoclonal antibodies were used for the detection of PIF (Bioincept, LLC) and FOXP3 (Santa Cruz, Santa Cruz, CA, USA). Immunohistochemistry was performed according to Hsu et al 1981. Briefly, tissue sections were dewaxed and rehydrated
30 conventionally, after quenching the endogenous peroxidase by incubation with 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature sections were exposed to a non-immune block with normal horse serum for 30 minutes at room temperature. Incubation with the first antibody was carried out at 4° C overnight with a dilution of 1 to 100 for the monoclonal mouse anti-human PIF and with a dilution of 1 to 50 for the monoclonal

mouse anti-human FOXP3. Thereafter tissue sections were labeled with the avidin-biotin-peroxidase detection system Vectastain (Vector Laboratories, Burlingthor VT, USA). Each step was followed by meticulous washing with PBS. Finally, 3,3'-diaminobenzidine (DAB) and/or 3-amino-9-ethylcarbazole (AEC) were used as chromogens for single or double staining. Counterstaining was performed with hematoxylin. The positive controls were PIF and FOXP3 tissues. Negative controls were performed by replacing the primary antibody with mouse immunoglobulin at the same concentration as the primary antibody. In order to perform the statistical analysis of immunostaining for each protein, a semi-quantitative analysis of specific staining was performed using an HSCORE system according to Mc Carty et al. 1985. The HSCORE was calculated using the following equation: $HSCORE = \sum P_i(i+1)$, where i is the intensity of staining with a value of 1, 2, or 3 (weak, strong or very strong respectively) and P_i is the percentage of stained cells for each intensity, varying from 0% to 100%. For all samples, ten microscopic fields were counted by two of the authors independently in each slide.

The intra-observer and inter-observer coefficient of variation were 3.4% and 4.2% respectively. Three slides of each sample were observed for both antigens tested under the microscope and each observer was blind to which sample it was. The slides were numbered progressively by a technician, who reported on a separate worksheet the number of the slide and the corresponding name of the patient or control. Only after the slide was analyzed by the two different observers and scored for the HSCORE each value was reported on the worksheet against the corresponding name of the patient for each slide number. The HSCORE analysis was performed separately for the component of endometrium, glandular and stromal cells, and for the ectopic tissue (each observer performed 4 different HSCOREs for each slide).

All data are reported as a mean + standard deviation. Statistical analysis was performed by SPSS statistical package (Chicago, IL USA), using the Mann-Whitney Sum Rank test as appropriate.

FITC-PIF Flow Cytometry Studies: Non-pregnant infertile and first trimester pregnant patients at Millenova Immunology Laboratories who were undergoing fertility treatments signed a standard informed consent (CARI, Institute, Chicago) as we have previously reported 17,18. All experiments were performed in accordance with the guidelines and regulations of CARI, Institute, Chicago and with the approval from the Institutional Review Board of the University of Illinois at Chicago in March 2006, Dr. R. Roussev, PI. The blood was drawn as part of their work-up process with the use of excess specimen without

identifiers. We reported on FITC-PIF binding to CD14+ and CD3+, cells in both pregnant and non-pregnant patients.¹⁷ In addition binding to activated CD4+ cells were documented. Whether PIF targets regulatory T-cells was examined by using specific anti-CD4+, anti-CD25+ and anti-FoxP3+ antibodies. Binding was compared with scrambled-FITC-PIF used as a negative control. Following separation using Ficoll-Hypaque, PBMC were isolated. Binding to Isotype control served as negative controls. Two- three color staining was done using conventional techniques. Fluorescence measurements (20,000-50,000 gated events per sample) were performed in a Coulter® Epics® XL™ Flow Cytometer using System II software for data acquisition and analysis (Beckman Coulter, Inc., Miami, FL).

Results

In all eutopic endometria of patients with endometriosis and in healthy controls neither epithelial and stromal cells were negative for PIF specific staining. The specific staining for FOX-P3 was observed with low intensity in epithelial cells of eutopic endometrium, and in few stromal cells (putative Treg cells), regardless whether patients or controls. The percentage of cells expressing this antigen was 9.2±2.1% of stromal cells and 32.8±0.9% in the glands, with an HSCORE of 78±7 in epithelial cells and 26±4 in stromal cells. No differences were observed among the endometria of women with and without endometriosis

In the ectopic tissue of endometriotic lesions, 25 ovarian cyst walls and 10 peritoneal implants, we observed the expression of specific staining for PIF mostly in the epithelial cells, even though not in all cells and glands, but with a segmental pattern, in almost samples examined. The stromal cells did not showed specific staining for PIF. The specific staining for PIF was observed in 65.9±9.4% of epithelial cells with an HSCORE of 138±49, whereas in the stromal cells the specific staining for PIF was not observed.

The specific staining for FOX-P3 was observed with low intensity in epithelial cells of ectopic endometrium, and in few stromal cells (putative Treg cells), even though in larger amount than in eutopic samples. The percentage of stromal cells expressing this antigens was (17.2± 3.2%) with a HSCORE of 114±35. The percentage of ectopic epithelial cells showing specific staining for FOX-P3 was (12.4±5.6%) with a HSCORE of (67±11). All data set are reported in Table 2. The cells showing specific staining for FoxP3 observed in the stroma (putative Treg cells) were mostly localized in the stoma surrounding the ectopic glandular cells expressing PIF such as shown by the double-staining immune histochemistry.

Table 2: Immunohistochemical findings for PIF and FOXP3 in eutopic and ectopic endometrial samples

	% of cells	Positivity	HSCORE
PIF			
Eutopic endometrium			
Epithelia cells	0	-	-
Stromal cells	0	-	-
Ectopic endometrium			
Epithelia cells	65.9 ± 9.4	++	138 ± 49
Stromal cells	0	-	-
FOXP3			
Eutopic endometrium			
Epithelia cells	3.8 ± 0.9	+	78 ± 7
Stromal cells	13.2 ± 2.1	±	26 ± 4
Ectopic endometrium			
Epithelia cells	12.4 ± 1.5	+	67 ± 11
Stromal cells	17.2 ± 3.2	++	114 ± 35

Co-expression of PIF and Tregs FoxP3⁺ cells was found in ectopic endometrium.

- 5 Therefore whether PIF also interacts with these subset of cells in PBMC was examined. Flow cytometry data showed that in non-pregnant women there is a dose dependent increase in the binding to CD4⁺/CD25⁺ cells, which is in contrast to scrambled-FITC-PIF used as control which binding was minimal (FIG. 1). Further experiments examined the specific interaction of FITC-PIF with FoxP3⁺ cells. Data showed that FITC-PIF targets those specific systemic
 10 immune cells in a dose dependent manner (FIG. 2). This reflects possible PIF induced Tregs regulation.

Discussion

- In this study we showed for the first time that ectopic endometrial epithelial cells
 15 express PIF, which in contrast it was not found in the epithelial layer of eutopic endometrium,. Furthermore, PIF expression, evident only in the epithelial layer, was co-expressed with FoxP3⁺, an antigen expressed by regulatory T cells, which expression was not specific in the ectopic endometrium since it was found also in eutopic tissues. Noteworthy,

stromal cells in ectopic endometrium expressing FoxP3 (putative Treg cells) were preferentially observed in proximity of ectopic epithelial cells expressing PIF, in a way that suggest as PIF may target systemic FoxP3+ immune cells to engraft endometriotic lesions to play their role locally, downregulating the inflammatory process and immune response to ectopic endometrium. Our flow cytometry data indicated that FITC-PIF targets CD4+/CD25+/FoxP3+ systemic immune cells confirming partially a functional link between PIF and regulation of Treg cells in establishing a local immune privilege in endometriosis lesions.

The presence of Tregs in eutopic and ectopic endometrium of women affected by endometriosis has been reported (Budiu et al 2009, Berbic et al 2010). The mRNA of FoxP3, specific of Tregs, seems to be increased in ectopic endothelial tissue (Budiu et al 2009). Furthermore, it has been reported that the percentage of Tregs cells was significantly decreased in the peripheral blood in women with endometriosis as compared to health controls, whereas Treg cells percentage was increased in the peritoneal fluid of these patients with respect to controls. (Olkonska-Truchanowicz et al 2013). Our study evidenced for the first time the presence of Treg cells in the endometriotic lesion and their number increase in relation with PIF expression by epithelial cell lineage, suggesting a role for these cells in determining an immune privilege for endometriosis, such as similarly we previously reported for Fas-FasLigand system (Sbracia et al 2015). The role of these cells in several diseases has been highlighted, and in particular autoimmune disorders might be due to a dysregulation of T regulatory cells function, in particular of CD4+-CD25+-FoxP3+ regulatory cells, which play a key role in the suppression of many types of effector cells such as macrophages, NK cells, dendritic cells, cytotoxic T cells (Sathaguchi et al 2008, Berbic & Fraser 2011).

The observation that PIF is highly and specifically expressed in the ectopic endometrial epithelial cells is reported for the first time. We confirmed that expression of PIF is not expressed by normal eutopic endometrium but only in the ectopic tissue. Such reflects that PIF-like molecule re-expression is conditioned by the abnormal cells likely multipotent stems cells which also in certain cases lead to development of this diseases as well as the endometroid cancer. The lack of expression in stroma while the high expression being in the epithelial layer supports interaction mostly with rapidly proliferating cells that may have even an oncogenic potential. Under close observation of the cells expressing PIF it shows the presence of several large and even abnormal looking nuclei reflecting their possible hyperplastic features. As cells become more hyperplastic they tend to resemble stem cells which we showed that PIF regulates. Testing effect of PIF on singly cultured bovine embryo

was shown to promote their development when added in culture. In addition the dependence of murine embryos on endogenously secreted PIF was demonstrated since addition of an anti-PIF antibody severely blocked cultured embryos development. Thus PIF and stem cells appear to have a symbiotic relationship. Whether in the case of endometriosis PIF re-emergence to regulate the non-pregnant stems cells is plausible. This stems of the fact that PIF was shown effective in promoting both semi and allogenic stems cells transplantation in a murine graft vs host disease model. Thus confirming also in vivo the close link and the direct effect of PIF on healthy stem cells engraftment irrespective whether they are matched or not. Importantly in this study the beneficial graft vs leukemia was preserved strongly indicating the protective effect of PIF against tumors development. Thus the ensemble of the data support the view that PIF expression in the ectopic endometrium may reflect a protect effect against the localized inflammation.

The expression of FoxP3+ by epithelial cells was analyzed showing a heterogenous expression both in normal and ectopic endometrium with no differences in intensity of expression between ectopic and eutopic epithelial cells. This suggests that in contrast to PIF, FoxP3, which is a transcription factor, is more likely to have a role in regulate epithelial transition and differentiation instead that reflecting a reaction to the inflammatory process. Whether PIF interacts with the immune suppressive Treg cells was examined. This stems from the fact that these cells expression appears to increase close to implantation therefore they are associated with development of immune tolerance for the embryo. The observation that PIF specifically targets those cells combined their co-localization supports a possible regulatory role systemically as shown both in vitro and in vivo setting in CD4+ cells. It also raises the possibility that similar action also takes place locally on the site of endometriosis. Indeed a number of preclinical models have documented that PIF has both a local and systemic integrated protective effect.

Our findings seem depict a possible biological scenario where endometriotic stem cells, which in turn may originate the disease, with the onset of inflammatory reaction to endometriotic implants by the host tissues, such as ovary or peritoneum, to protect themselves express and produce PIF to recall cells like Treg in order to induce an immune privileged environment. These findings may suggest a potential role for PIF not only in the pathogenesis of endometriosis, but also a potential target for a biological treatment of this diseases.

Example 2: PIF in endometriosis: a potential role in inducing immune privilege

Endometriosis is a chronic inflammatory condition characterised by the growth of endometrial epithelial and stromal cells outside the uterine cavity. Besides Sampson's theory of retrograde menstruation, endometriosis pathogenesis is related to the privileged inflammatory microenvironment in these lesions. One of the pivotal factors are T regulatory FoxP3⁺ expressing T cells (Tregs). PreImplantation factor (PIF) is a peptide essential for pregnancy recognition and development. Besides immune modulatory function the synthetic PIF analog (sPIF) was successfully tested in multiple animal models. We report that PIF re-expresses in the epithelial ectopic cells in close proximity to FoxP3⁺ stromal cells. We provide evidence that PIF interacts with FoxP3⁺ cells and modulates cell viability diversely depending on cell source and presence of inflammatory mediators. Our finding represent a novel PIF-based mechanism in endometriosis, thus holding potential of novel therapeutics.

Material and methods

Production and labelling of synthetic PIF. Synthetic PIF₁₅ (MVRKPGSANKPSDD) and a scrambled peptide sequence with the same amino acids in random order (GRVDPSNKSMPKDIA) were synthesized by solid-phase peptide synthesis (Peptide Synthesizer, Applied Biosystems) employing Fmoc (9-fluorenylmethoxycarbonyl) chemistry at Bio-Synthesis, Inc. (Lewisville, TX, USA). Final purification was carried out by reversed-phase HPLC and identity was verified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and amino acid analysis at >95% purity. Fluorescein labeled FITC-PIF and scrambled PIF (FITC-PIFscr) were also generated as previously reported (Barnea et al, 2012a; Barnea et al, 2012c). Anti-PIF monoclonal antibody against MVRKPGSANKPSDD was generated in (Genway, SanDiego, CA, USA).

Immunohistochemistry. Tissue specimens were obtained from 25 women who underwent laparoscopic surgery for severe endometriosis according to the revised criteria of the American Society Reproductive Medicine. The surgical procedures were carried out in CERM-Hungaria Institute, Rome, Italy, from September 2014 through April 2015. The project had the approval of the Hospital's Ethical Committee. Samples were obtained from the ectopic endometrium, ovarian endometriomas and peritoneal implants. A total of 25 eutopic endometria, 25 ovarian endometriomas and 10 peritoneal implants were collected from patients. Furthermore, the endometria of 10 healthy were used as controls. Biopsy samples were fixed in 4% neutral buffered formalin overnight and were subsequently paraffin embedded.

Before performing immunohistochemistry (IHC) tissue sections were stained with

eosin and hematoxylin to select tissue with ectopic epithelial cells. Serial sections of 5µm thick were used for IHC. Commercially available monoclonal antibodies were used for the detection of PIF (BioIncept, LLC, NJ, USA) and Foxp3 (number: sc-53876, Santa Cruz, CA, USA). IHC was performed according to manuals instructions. Briefly, tissue sections were dewaxed and re-hydrated and endogenous peroxidase activity quenched by incubation with 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature. Sections were exposed to a non-immune block with normal horse serum for 30 minutes at room temperature. Incubation with the first antibody was carried out at 4° C overnight with a dilution of 1 to 100 for the monoclonal mouse anti-human PIF and with a dilution of 1 to 50 for the monoclonal mouse anti-human Foxp3. Thereafter tissue sections were labelled with the avidin-biotin-peroxidase detection system Vectastain (Vector Laboratories, Burlingthor VT, USA). Each step was followed by meticulous washing with PBS. Finally, 3,3'-diaminobenzidine (DAB) and/or 3-amino-9-ethylcarbazole (AEC) were used as chromogens for single or double staining. Counterstaining was performed with hematoxylin. The positive controls were PIF and Foxp3 tissues. Negative controls were performed by replacing the primary antibody with mouse immunoglobulin at the same concentration as the primary antibody.

A semi-quantitative statistical analysis of specific staining was performed using an HSCORE system (McCarty et al, 1985). The HSCORE was calculated using the following equation: $HSCORE = \sum Pi(i+1)$, where i is the intensity of staining with a value of 1, 2, or 3 (weak, strong or very strong respectively) and Pi is the percentage of stained cells for each intensity, varying from 0% to 100%. For all samples, ten microscopic fields were counted by two observers blinded to the different groups. Three slides of each sample were analysed. The HSCORE analysis was performed separately for the component of endometrium, glandular and stromal cells, and for the ectopic tissue (each observer performed 4 different HSCOREs for each slide). The intra-observer and inter-observer coefficient of variation were 3.4% and 4.2% respectively.

FITC-PIF Flow Cytometry. Non-pregnant infertile and first trimester pregnant patients at Millenova Immunology Laboratories who were undergoing fertility treatments signed a standard informed consent (CARI, Institute, Chicago, usa). All experiments were performed in accordance with the guidelines and regulations of CARI, Institute, Chicago and with the approval from the Institutional Review Board of the University of Illinois at Chicago in March 2006, Dr. R. Roussev, PI. The blood was drawn as part of their work-up process with the use of excess specimen without identifiers. We reported on FITC-PIF binding to

CD14⁺ and CD3⁺ cells in both pregnant and non-pregnant patients (Barnea et al, 2012b). In addition binding to activated CD4⁺ cells were documented. Whether PIF targets regulatory T-cells was examined by using specific anti-CD4⁺, anti-CD25⁺ and anti-FoxP3⁺ antibodies (BD, Pharmingen, USA). Binding was compared with scrambled-FITC-PIF used as a negative control. Following separation using Ficoll-Hypaque, peripheral blood mononuclear cells (PBMC) were isolated. Binding to Isotype control served as negative controls. Two- three color staining was done using conventional techniques. Fluorescence measurements (20,000-50,000 gated events per sample) were performed in a Coulter[®] Epics[®] XL[™] Flow Cytometer using System II software for data acquisition and analysis (Beckman Coulter, Inc., Miami, FL, USA).

Isolation and culture of in vitro cell models. After ethical approval and informed consent was obtained endometrial biopsies were collected via soft curette (Pipelle de Cornier, Laboratoire CCD, France) and stored in RNAlater at -80°C from women undergoing laparoscopic surgery at the University Hospital Bern, Switzerland as described previously (Santi et al, 2011). The pelvic cavity was examined and any endometriotic lesions were removed and the presence of endometriosis confirmed via histological investigation. Endometriosis biopsies were collected from women both with (n = 4) and without (n = 5) endometriosis. All surgeries were performed during the proliferative phase of the menstrual cycle. Primary endometrial stromal cells (ESC) from women with and without endometriosis were prepared via a collagenase digestion and size exclusion protocol as described previously (McKinnon et al, 2013).

Isolated ESC were maintained in Iscoves's modified Eagle medium (IMEM) (Invitrogen) supplemented with 10% fetal calf serum (fcs) (Invitrogen) and 1% antibiotic/antimycotic (Invitrogen). Immortalized epithelial cell lines were kindly provided by Professor Kyo, Kanazawa, Japan and were isolated from eutopic endometrium, EM E6/E7 (Kyo et al, 2003) and an ectopic endometriomas, EM'osis, (Bono et al, 2012). Epithelial cells were cultured in Dulbecco's modified Eagles medium (DMEM) (invitrogen) with 10% fcs and 1% antibiotic/antimycotic.

Analysis of sPIF influence on cell viability. For the MTS assay cell were plated into 96 well plates at a density of approximately 6×10^3 /well. Sixteen hours prior to treatments cells were changed into a reduced serum media (0.5% FCS). sPIF was prepared by diluting into phosphate buffered saline (PBS) at a final concentration of 300nM. Subsequent 1:3 serial dilutions (111nM, 37nM, 12.3nM and 4.12nM) were prepared for a dose-response assay. Treatment was performed for a total of 48 hours with the treatment media replaced after the

first 24 hours. Cell viability was measured with the CellTiter 96 Aqueous Solution Cell Proliferation Assay (Promega, Madison, Wisconsin, USA). A control (without sPIF) was included for each experiment and all subsequent values expressed as a percent of control. For assay including TNF α recombinant human TNF α (R&D, Cat No; 210-TA, Minneapolis, MN, USA) was diluted into PBS at 100ng/ml and included in treatment media.

Whole transcriptome expression array. For whole transcriptome expression array analysis EM'osis cells were plated into 6 well plates at approximately 2×10^5 /well and grown until approximately 80% confluent. Sixteen hours prior to treatment cells were transferred to 0.5 % FCS media to synchronize cell cycles. Treatment with 100nM PIF was performed for 48 hours with the treatment media replenished after 24 hours. After treatment period cells were lysed in Qiazol lysis buffer (Qiagen) and RNA isolated using the RNeasy mini kit (Qiagen), as per the manufacturer's instructions. RNA quantity was measured via the Nanodrop 2000 (Witec) and quality via the Bioanalyser 2000 (Agilent). RNA was considered of sufficient quality if RNA integrity number (RIN) was above 9.8. A final concentration for all samples was approximately 200ng/ml. 6 RNA samples were analysed using the Affymetrix platform according to the manual's instructions (GeneChip® Human Transcriptome Array 2.0 and miRNA Array, Affymetrix).

Statistical Analysis. IHC data are reported as a mean $\pm$ standard deviation. Statistical analysis was performed by SPSS statistical package (Chicago, IL USA), using the Mann-Whitney Sum Rank test as appropriate. Analysis of cell viability in the *in vitro* cell models in response to PIF treatment was performed with a two-way analysis of variance (ANOVA) test with a post-hoc Sidak's multiple comparison test to determine if cell viability was increased by treatment conditions from control. Significance was considered a value for $p < 0.05$ and analysis performed with Graphpad Prism 6. The raw microarray data was background-corrected, normalized using the RMA method as implemented in the R/Bioconductor package affy (Gautier et al, 2004). Probe sets were redefined using the alternative chip definition file mogene20stmmmentrezgcdf (Dai et al, 2005). Differential gene expression was calculated using the moderated t-test as described previously and implemented in the R/Bioconductor package limma (Ritchie et al, 2015).

The Pathway analysis: The output of limma was used to perform gene set enrichment analysis (GSEA) using the SetRank method. The key principle of this algorithm is that it discards gene sets that have initially been flagged as significant, if their significance is only due to the overlap with another gene set. It calculates the p-value of a gene set using the ranking of its genes in the ordered list of p-values as calculated by limma. The following

databases were searched for significant gene sets: BIOCYC, Gene Ontology, KEGG, Pathway Interaction Database, REACTOME, and WikiPathways.

Results

5 *PIF imparts epithelial ectopic endometria.* To determine the role of PIF in endometriosis, tissue samples were obtained from women with or without endometriosis including the ectopic endometrium, ovarian endometriomas and peritoneal implants during the proliferative and secretory phases. We could not detect PIF positive cells in both epithelial and stromal cells of healthy controls (FIGs. 3A and 3B). However, we detected PIF
10 positive cells in the epithelial compartment of ectopic endometria documenting for the first time re-expression of PIF outside of pregnancy. We hypothesized that PIF re-expression may be induced as a protective mechanism so we tested the effect of sPIF using an in-vitro system next.

To begin to elucidate the potential role of PIF in endometriosis we used both
15 epithelial cell lines isolated from eutopic endometrium (EM E6/E7) (Kyo et al, 2003) and ectopic endometriomas (EM'osis) (Bono et al, 2012) and primary endometrial stromal cells (ESC) from women with and without endometriosis. We tested sPIF treatment in ascending dose in epithelial cells first. Indeed, sPIF treatment resulted in a significantly decreased cell viability compared to control (FIG. 3C solid line). Furthermore and supporting PIF's role
20 during embryo implantation, sPIF increased the viability of eutopic endometrium cells as well (FIG. 3D dashed line) (Barnea et al, 2015). We tested sPIF effects in stromal cells next. In line with our previous reports and observation in human tissues (FIG. 3B), sPIF treatment did not alter stromal cell viability (Barnea et al, 2012a). Together, both PIF re-expression and sPIF treatment impart epithelial but not stromal endometriotic cells significantly suggesting a
25 novel pathogenesis of endometriosis. To further dissect the underlying mechanisms, we performed a global gene array from sPIF treated ectopic endometrial epithelial cells next.

sPIF modulates T-cell receptor signalling. Having a screening approach in mind, we performed a global gene array from sPIF's treated epithelial ectopic cell lines as sPIF imparts those cells (FIG. 4A). In line with previous reports (Paidas et al, 2010), sPIF treatment
30 resulted in modulation of multiple signaling pathways. We detected significant changes in channels activity pathways with cation and potassium channels activity being crucial. It seems that these modifications are central to sPIF functions especially as one of previously identified sPIF targets is the potassium channel (Chen et al, 2016). Another interesting observation are the changes in multiple pathways involved in neuronal development,

plasticity, and protection. Again, this is in line with previous reports of sPIF neuroprotective effects (Mueller et al, 2015; Mueller et al, 2014; Weiss et al, 2012b). Interestingly, we detected changes in T-cell receptor signalling as well. This is of potential interest as involvement of T cell signalling in the pathogenesis of endometriosis was reported previously (Berbic & Fraser, 2011; Sakaguchi et al, 2008). Therefore, we performed a detailed heat map analysis and detected multiple genes being modulated by sPIF treatment (FIG. 4B). Of special interest is the FoxP3 gene as FoxP3⁺ Tregs fail to decline during the secretory phase in endometriotic lesions which may lead to their survival and subsequent establishment (Berbic & Fraser, 2011). Given the importance of FoxP3 in the pathogenesis of endometriosis (Berbic & Fraser, 2011; Berbic et al, 2010), we aimed to confirm PIF interactions with FoxP3 signalling next.

PIF interacts with FoxP3 positive cells. We stained FoxP3 and PIF in human samples and detected FoxP3⁺ cells in both eutopic and ectopic tissues (FIGs. 5A and 5B).

Interestingly, we detected increased number of FoxP3⁺ cells in the ectopic endometria which was especially evident during the secretory phase (Berbic et al, 2010; Budiu et al, 2009). As hypothesized, FoxP3⁺ cells in the stromal compartment of the ectopic endometria was in close proximity to the positive PIF cells in the epithelial compartment (FIG. 5B). This observation suggests that the increased FoxP3 expression may be due to the re-expression of PIF in these lesions. To support the PIF and FoxP3⁺ interactions, we tested PIF's effects in well-defined system of peripheral non-pregnant blood mononuclear cells (PBMCs). Using flow cytometry, we documented a dose dependent increase in the binding of FITC-PIF to CD4⁺/CD25⁺/FoxP3⁺ cells (Fig.5C) confirming this specific interaction.

Finally, we aimed to detect sPIF effects in the inflammatory endometriotic environment. Notably, multiple chemokines affect FoxP3⁺ cells function and FoxP3 cells regulate the cellular survival of ectopic endometria (Li et al, 2014). We decided to test sPIF effect on cell viability in the presence of TNF α as TNF α was identified as one of the pivotal chemokines involved in endometriosis and cell death (Han et al, 2015). Indeed, in the presence of TNF α sPIF's specific effect on cell viability in epithelial ectopic cells was abolished suggesting the pivotal involvement of TNF α (FIG. 5D) in sPIF's mediated effects. This observation supports the notion of a disturbed balance between pro-inflammatory and anti-inflammatory factors in endometriosis affecting the cellular survival (Tagashira et al, 2009).

Together, we hypothesize that PIF re-expresses in the epithelial compartment of ectopic endometria resulting in the recruitment of FoxP3⁺ cells into the stromal

compartment. The additional influx of chemokines and pro-inflammatory factors such as TNF α result in divergent sPIF effect on these cells creating a positive feed-back loop. It leads to cellular survival which again contribute to a significant control of the immune privilege in the endometriotic microenvironment (FIG. 5E). Thus, PIF may be a crucial factor contributing to the pathogenesis of endometriosis on the local level. However, the immune modulatory effect of sPIF applied peripherally as a therapeutic option may lead to a decreased inflammatory response on the global level, leading to the recovery of proper immune balance on the local level and finally cell death of ectopic endometria. Studies investigating this hypothesis are currently underway.

Discussion

Our study showed for the first time the presence of FoxP3⁺ cells in the endometriotic lesion in close proximity to PIF expressing epithelial cells. The presence of Tregs in eutopic and ectopic endometrium of women with endometriosis has been reported previously (Berbic et al, 2010; Budiu et al, 2009). FoxP3 mRNA is increased in ectopic endothelial tissue and the percentage of Tregs is significantly decreased in the peripheral blood of women with endometriosis, compared to healthy controls. However in the peritoneal fluid the Treg percentage is increased (Olkowska-Truchanowicz et al, 2013). This discrepancy suggests a differential immune modulatory system response on the local and global level. Given that sPIF imparts ectopic endometrial cells and interacts with FoxP3⁺ cells, we hypothesize that PIF re-expression may determine an immune privilege for endometriotic lesions. A similar role was reported for Fas-FasLigand system previously (Sbracia et al, 2016). Notably, in autoimmune disorders CD4⁺/CD25⁺/FoxP3⁺ dependent suppression of effector cells (macrophages, natural killer, dendritic, and cytotoxic T cells) was reported (Berbic & Fraser, 2011; Sakaguchi et al, 2008). As FoxP3⁺ cells were present in ectopic and eutopic epithelial cells, we hypothesize that FoxP3 may regulate epithelial transition and differentiation as a transcription factor instead of reflecting a reaction to the inflammatory process as in case of TNF α (Han et al, 2015).

Recent evidence suggest that apoptotic epithelial cells control Treg survival and abundance (Nakahashi-Oda et al, 2016). Such a role for PIF would be plausible as cellular expression increases close to implantation and thus it is associated with the development of immune tolerance for the embryo (Barnea et al, 2012a; Duzyj et al, 2010). The observation that PIF specifically targets those cells combined with their co-localization support a potential regulatory function, as shown in both in vitro and in vivo setting in CD4⁺ cells. It also raises

the possibility that similar action takes place locally at the site of endometriosis. Indeed a number of preclinical models have documented that PIF has both a local and global integrated protective effects (Barnea et al, 2015). Furthermore, a potential for a bi-directional communication between PIF expressing epithelial cells and Treg cells exists. PIF increased cell viability of epithelial cells derived from the eutopic endometrium and decreased cell viability of cells derived from ectopic tissue (FIGs. 3C and 3D). These results indicate that PIF may have local paracrine effects on the epithelial cells within the endometriotic lesion. Furthermore, this effect may be modulated by the inflammatory microenvironment as in case of TNF α (FIG. 5E). The whole genome transcriptome analysis showed a significant influence on the expression of T cell receptor signalling pathways raising the possibility that not only does the paracrine effects of PIF influence the survival of ectopic epithelial cells, but that it also influences gene expression and the ability of these epithelial cells to respond to the immune regulating effects of infiltrating Tregs.

The variation in response to PIF between the eutopic derived and ectopic derived epithelial cells is also of interest. Although the pathogenesis of endometriosis is still not resolved, the notion that ectopic endometriotic cells have an inherent characteristic that leads to implantation is intriguing (Macer & Taylor, 2012). Such as a pathological alteration may be due to the ectopic environment or may have the origin in stem cells (Macer & Taylor, 2012). Since embryo development largely dependent on PIF and sPIF promotes cultured embryos and stem cells development support such a premise (Shainer et al, 2016; Stamatkin et al, 2011a; Stamatkin et al, 2011b). Although hypothetical the idea that a peptide such as PIF may be the missing link between the role of stems cells and immune responses in the pathogenesis of endometriosis is intriguing (Berbic & Fraser, 2011; Han et al, 2015; Macer & Taylor, 2012). Lastly, endometriotic derived PIF may influence the symptomology of endometriosis as well. sPIF is a well described neuroprotective compound (Barnea et al, 2015). Recent evidence suggest that the pain experienced by endometriosis sufferers occurs through an interaction with endometriotic associated nerve fibers detected in close proximity to the lesions (McKinnon et al, 2012; McKinnon et al, 2015). The neuroprotective effects of epithelia derived PIF may stimulate an enhanced nerve presence and influence the interpretation of pain in endometriosis. On the other hand peripheral sPIF and the ensuing reduced neuroinflammation may limit such neurotropic pain as well (Mueller et al, 2014; Weiss et al, 2012b).

In summary, our report suggest a possible biological scenario where endometriotic cells, as a possible lesion source, combined with the stimulation of the inflammatory reaction

to endometriotic implants, express and produce PIF to recall Treg cells in order to induce an immune privileged environment (to protect themselves). The presence of both PIF and Treg cells in the microenvironment may modulate the endometriotic lesions regulating survival and development, especially in reaction to inflammatory agents such as TNF α . Our findings suggest a potential role for PIF not only in the pathogenesis of endometriosis, but also sPIF as a potential compound for a biological treatment of endometriosis in the future.

Example 3: sPIF has similar binding to lymphocytes compared to standard PIF.

The sPIF sequences provided herein (SEQ ID NOs: 20-29) are non-naturally occurring mutants of PIF. In order to determine if they sPIF sequences provided herein have similar properties to PIF, FITC-PIF assays was performed to examine sPIF binding to monocytes. SEQ ID NO: 27 and SEQ ID NO: 29 were compared to natural PIF in binding to CD11b (macrophage) and CD19 (B cell) monocytes.

Table 3: PIF v sPIF binding

PIF type	CD11b %	CD19 %
SEQ ID NO: 27	35.48%	5.60%
control PIF	27.94%	3.23%
SEQ ID NO: 29	33.54%	5.90%
control PIF	27.11%	4.34%

The sPIF sequences SEQ ID NOs: 27 and 29 showed higher binding affinity to both CD11b and CD19 monocytes. This result lends support to the idea that the sPIF sequences provided here (SEQ ID NOs: 20-29) may bind to PIF receptors at least as well as, if not better than, native PIF.

Example 4: sPIF used as therapeutic in endometriosis animal model

To test whether sPIF may be effective for human treatment, an animal model described in Greaves et al., Am J Pathol. 2014 Jul;184(7):1930-9, will be used prior to primate experimentation. That reference is incorporated by reference in its entirety, however, pertinent components of the experiment are disclosed below to describe how induction of an endometriotic condition in mice can be studied to test a potential treatment for human use.

Animals

Mature (approximately 8-week-old) female C57BL/6 mice will be purchased from an animal supplier and will be allowed to acclimate for 1 week before surgery. Some experiments may be performed using transgenic Cfs1r-EGFP mice (MacGreen) that were originally generated as described in detail elsewhere. Two heterozygous enhanced GFP+ males and two heterozygous enhanced GFP+ females were cross-bred with wild-type (WT) C57BL/6 mice to form a breeding colony and were bred under standard conditions. Offspring will be genotyped and classified as either MacGreen or WT. Mice will be maintained on standard chow and water available ad libitum and will be housed in environmentally controlled facilities illuminated between 7:00 am and 7:00 pm. All the animal procedures will be performed in accordance with legal requirements related to animal care and under licensed approval from the appropriate ethical authorities.

Mouse Model of Endometriosis

Menstruation will be induced in adult donor mice (approximately 8 weeks of age) using a protocol developed in-house set forth in PLoS One. 2014;9:e86378. In brief, ovariectomized mice (day 1) will be primed with s.c. injections of 100 ng of estradiol-17 β (days 7 to 9), treated with progesterone (P4; Sigma-Aldrich, Dorset, UK) delivered via a SILASTIC implant (Dow Corning Corp, Midland, MI) from days 13 to 19, and injected with 5 ng of estradiol-17 β in sesame oil on days 13, 14, and 15. Decidualization will be induced in one uterine horn using 20 μ L of oil (day 15), and endometrial tissue in the process of being shed from the decidualized horn will be recovered from mice culled on day 19, 4 hours after P4 withdrawal (removal of pellet) by opening the horn longitudinally in a petri dish and scraping the tissue away from the myometrial layer using a scalpel. The tissue mass will be suspended in 0.2 mL of PBS and passed through a 19-gauge needle before being injected i.p. into anesthetized recipient mice (approximately 8 weeks of age) that had been previously ovariectomized and implanted with an estradiol-17 β (Sigma-Aldrich) SILASTIC implant. Tissue from one decidualized donor horn will be used to inoculate each recipient mouse (approximately 40 mg tissue/0.2 mL PBS per mouse). Three weeks after i.p. injection, recipient mice will be culled (photographs of the body cavity taken and were taken the lesions carefully dissected from surrounding tissue) and tissues will be either fixed in 4% normal buffered formaldehyde for histologic analysis or placed in RNA Save (GeneFlow Ltd, Lichfield, UK) for RNA extraction. In total, endometriosis will be induced in about 18 mice. Of these, 10 mice will be used in the MacGreen reciprocal transfers: MacGreen donor and WT recipient (n = 6) and WT donor and MacGreen recipient (n = 4).

Endometrial tissues will be fixed to slides and stained using known immunostaining procedures. Briefly, after fixation, mouse lesions will be stained using H&Eosin; only lesions containing identifiable stromal and epithelial compartments will be used for further analysis. Single-color IHC analysis will be performed according to standard protocols^{24,26} with citrate antigen retrieval followed by blocking endogenous peroxidase with 3% H₂O₂ in methanol. A streptavidin-biotin block (Vector Laboratories, Peterborough, UK) will be performed, and any nonspecific staining will be reduced using species-specific blocking solution (1:4 serum in Tris-buffered saline + 5% bovine serum albumin). Incubation with the appropriate dilution of primary antibody will be performed overnight at 4°C in blocking solution (Table 2). Biotinylated secondary antibodies (dilution 1:500) will be diluted in blocking solution and incubated at room temperature for 30 minutes. A streptavidin–horseradish peroxidase conjugate (dilution 1:1000; Sigma-Aldrich) will be diluted in Tris-buffered saline and incubated at room temperature for 1 hour, followed by visualization using ImmPACT diaminobenzidine peroxidase substrate (Vector Laboratories). For anti-CD31 IHC analysis, nonspecific epitopes will be blocked with Bloxall blocking solution (Dako UK Ltd, Ely, UK), and the secondary antibody will be detected using a streptavidin–alkaline phosphatase conjugate and visualized using PermaRed (Dako UK Ltd). Images will be captured using AxioVision software version 4.8.2.0 (Carl Zeiss Ltd, Cambridge, UK) or a similar system and a Provis microscope (Olympus America Inc., Center Valley, PA). Dual immunofluorescence was performed as previously herein with a secondary F(ab) polyclonal antibody to IgG (horseradish peroxidase), and antibody detection will be performed using a TSA system kit (PerkinElmer, Waltham, MA) labeled with either Cy3 (red) or fluorescein (green). For detection of the second antigen, sections will be microwaved for 2.5 minutes in boiling citrate buffer, and the second primary antibody was applied overnight at 4°C. Secondary antibody will be detected as before with an appropriate TSA system, and sections will be counterstained with DAPI (dilution 1:500). Slides will be mounted in PermaFluor medium (Thermo Fisher Scientific, Waltham, MA). Sections will be evaluated using an LSM 710 confocal microscope and ZEN 2009 software (Carl Zeiss Ltd) or another microscope of a similar likeness.

List of Antibodies Used in IHC Analysis

Antibody specificity (supplier)	Raised in	Used on	Dilution
ERβ [AbD Serotec (#MCA1974S)]	Mouse	Human	1:50

ER β [Santa Cruz Biotechnology (#SC-8974)]	Rabbit	Mouse	1:500
ER α (Vector Laboratories)	Mouse	Mouse	1:50
CD31 (Dako)	Rabbit	Mouse	1:800
Cytokeratin [Sigma-Aldrich (#C2562)]	Mouse	Mouse	1:2000
Vimentin [Cell Signaling Technology Inc. (#5741)]	Rabbit	Mouse	1:600
GFP [Invitrogen Molecular Probes (#A11122)]	Rabbit	Mouse	1:250
Biotinylated anti-mouse (Vector Laboratories)	Goat		1:500
Biotinylated anti-rabbit (Vector Laboratories)	Goat		1:500
Horseradish peroxidase–conjugated streptavidin (Dako)	NA		1:200

Variable	Mouse
Sample size (No.)	about 18
Recovery rate (%)	
Number of lesions (mean $\pm$ SD)	
Lesions with glands and stroma (%)	

Mice will be divided into two sets: those left treated with PIF and those left untreated. Immunohistochemistry will reveal the experimental factors disclosed in the table to the left.

- 5 In mice that are treated with one or more PIF peptides, we anticipate that recovery rate of the animals will improve, lesion size will decrease, and the number of lesions will decrease as compared to mice untreated with PIF peptide. By using this study we can confirm that induction of a menses-like event in mice was associated with the presence of macrophages in the uterus and that lesions formed in mice contained tissue-resident macrophages and a
- 10 proinflammatory microenvironment. We expect that therapeutically effective levels of PIF peptide or their pharmaceutically acceptable salts will be able to lessen the severity of the endometriotic state induced in the mice.

Example 5: sPIF used as therapeutic for treatment of human endometriosis.

- 15 To test whether one or more PIF peptides will be effective to treat endometriosis, sPIF will be administered to healthy female patients and/or female patients diagnosed with

endometriosis. The purpose of this study is to examine the safety and tolerability of synthetic PreImplantation Factor (sPIF) in female patients with endometriosis. PIF apparently initiates both maternal tolerance preventing the loss/rejection of the fetus. Synthetic PIF (sPIF) successfully translates PIF endogenous properties to treatment of pregnant and non-pregnant immune disorders. sPIF was found to be effective in preclinical models of autoimmunity and transplantation (published). In FDA mandated safety studies in humans (Phase I clinical trial), sPIF administration to subjects diagnosed with autoimmune hepatitis followed by observation period demonstrated that at doses of sPIF ranging from 0.1 mg/kg and 1.0 mg/kg were tolerated safely. This study using sPIF a dose dependent design will evaluate the safety, tolerability and pain assessment of patients with endometriosis. The primary endpoint will be the total pain burden (dysmenorrhoea, deep dyspareunia and non-menstrual pain) rated on a daily basis by the patient using standard pain scales and analgesic intake as the outcome measures. Secondary endpoints will include the volume of the endometriotic nodule assessed clinically and on transvaginal ultrasound, the amount of pelvic tenderness on clinical examination and the presence of other endometriotic lesions at laparoscopy.

Arms	Assigned Interventions
Active Comparator: SAD Normal sPIF Within each cohort (at least 3 patients/cohort; at least 9 subjects in total), patients with normal uterine function tests will be randomized in a 2:1 ratio (active drug : placebo) to receive a single dose of sPIF or placebo as follows: Cohort 1: single dose 0.1 mg/kg sPIF Day 1 given subcutaneously (SQ) Cohort 2: single dose 0.5 mg/kg sPIF Day 1 given SQ Cohort 3: single dose 1.0 mg/kg sPIF Day 1 given SQ	Drug: sPIF Other Name: synthetic PreImplantation Factor in Ringer's lactate
Placebo Comparator: SAD Normal Placebo Within each cohort (at least 3 patients/cohort; at least 9 subjects in total), patients with normal uterine function will be randomized in a 2:1 ratio (active drug : placebo) to receive a single dose of sPIF or placebo as follows: Cohort 1: single dose placebo Day 1 given SQ Cohort 2: single dose placebo Day 1 given SQ Cohort 3: single dose placebo Day 1 given SQ	Drug: Placebo Other Name: Ringer's lactate to mimic sPIF solution for injection

Active Comparator: SAD Abnormal sPIF Within each cohort (at least 3 patients/cohort; at least 9 subjects in total), subjects with abnormal uterine function will be randomized in a 2:1 ratio (active drug : placebo) to receive a single dose of sPIF or placebo as follows: Cohort 1: single dose 0.1 mg/kg sPIF Day 1-5 given SQ Cohort 2: single dose 0.5 mg/kg sPIF Day 1-5 given SQ Cohort 3: single dose 1.0 mg/kg sPIF Day 1-5 given SQ	Drug: sPIF Other Name: synthetic PreImplantation Factor
Placebo Comparator: single ascending dose (SAD) Abnormal LFTs Placebo Within each cohort (at least 3 patients/cohort; at least 9 subjects in total), subjects with abnormal uterine function tests will be randomized in a 2:1 ratio (active drug : placebo) to receive a single dose of sPIF or placebo as follows: Cohort 1: single dose placebo Day 1-5 given SQ Cohort 2: single dose placebo Day 1-5 given SQ Cohort 3: single dose placebo Day 1-5 given SQ	Drug: Placebo Other Name: Ringer's lactate to mimic sPIF solution for injection
Active Comparator: multiple ascending dose (MAD) Normal sPIF Within each cohort (at least 3 patients/cohort), subjects with normal uterine function tests will be randomized in a 2:1 ratio (active drug : placebo) to multiple doses of sPIF administered subcutaneously once a day for 5 consecutive days (Days 1 to 5): Cohort 1: 0.1 mg/kg sPIF Days 1-5 given SQ Cohort 2: 0.5 mg/kg sPIF Days 1-5 given SQ Cohort 3: 1.0 mg/kg sPIF Days 1-5 given SQ	Drug: sPIF Other Name: synthetic PreImplantation Factor
Placebo Comparator: MAD Normal Placebo Within each cohort (at least 3 patients/cohort), subjects with normal liver function tests will be randomized in a 2:1 ratio (active drug : placebo) to multiple doses of placebo administered subcutaneously once a day for 5 consecutive days (Days 1 to 5): Cohort 1: placebo Days 1-5 given SQ Cohort 2: placebo Days 1-5 given SQ Cohort 3: placebo Days 1-5 given SQ	Drug: Placebo Other Name: Ringer's lactate to mimic sPIF solution for injection

Active Comparator: MAD Abnormal sPIF Within each cohort (at least 3 patients/cohort), subjects with abnormal uterine function tests will be randomized in a 2:1 ratio (active drug : placebo) to multiple doses of sPIF administered subcutaneously once a day for 5 consecutive days (Days 1 to 5): Cohort 1: 0.1 mg/kg sPIF Days 1-5 given SQ Cohort 2: 0.5 mg/kg sPIF Days 1-5 given SQ Cohort 3: 1.0 mg/kg sPIF Days 1-5 given SQ	Drug: sPIF Other Name: synthetic PreImplantation Factor
Placebo Comparator: MAD Abnormal Placebo Within each cohort (at least 3 patients/cohort), subjects with abnormal uterine function tests will be randomized in a 2:1 ratio (active drug : placebo) to multiple doses of placebo administered subcutaneously once a day for 5 consecutive days (Days 1 to 5): Cohort 1: placebo Days 1-5 given SQ Cohort 2: placebo Days 1-5 given SQ Cohort 3: placebo Days 1-5 given SQ	Drug: Placebo Other Name: Ringer's lactate to mimic sPIF solution for injection

After a sufficient observation period, the patients will be evaluated for changes in volume of the endometriotic nodule assessed clinically and/or on transvaginal ultrasound, degree of pelvic tenderness on clinical examination and the presence of other endometriotic lesions at laparoscopy. We expect that sPIF-treated cohorts will experience less pain than untreated patients presenting with abnormal uterine dysfunction. We also expect that sPIF-treated cohorts will experience reduced volume of endometriotic nodules, less tenderness and fewer lesions or lesions with decreased size as compared non treated subjects.

Although the present invention has been described in considerable detail with reference to certain preferred embodiments thereof, other versions are possible. Therefore the spirit and scope of the appended claims should not be limited to the description and the preferred versions contain within this specification. Any patent applications or other journal articles disclosed herein are incorporated by reference in their entireties.

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CLAIMS

1. A method of treating endometriosis in a subject in need thereof, the method comprising administering to the subject at least one pharmaceutical composition comprising:
 - a therapeutically effective amount of a pre-implantation factor (PIF) peptide, mimetics thereof, analogs thereof, or a pharmaceutically acceptable salt thereof; and
 - a pharmaceutically acceptable carrier.
2. The method of claim 1, wherein the therapeutically effective dose is about 1.0 mg/kg to about 4.0 mg/kg.
3. The method of claim 1, wherein the therapeutically effective dose is about 0.2 mg/kg to about 0.8 mg/kg.
4. The method of any one of claims 1-3, wherein the PIF peptide is selected from the group consisting of: SEQ ID NO: 13, SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29, or pharmaceutically acceptable salt thereof.
5. The method of any one of claims 1-4, wherein the PIF peptide, or mimetic, analog or pharmaceutically acceptable salt thereof comprises a chemical targeting moiety and/or a radioactive moiety.
6. The method of any one of claims 1-5, wherein the method further comprises administering at least one analgesic and/or one anti-inflammatory compound.
7. The method of any one of claims 1-6, wherein the pharmaceutical composition further comprises a therapeutically effective dose of one or more of an anti-inflammatory compound, alpha-adrenergic agonist, antiarrhythmic compound, analgesic compound, and an anesthetic compound.

8. The method of any one of claims 1-7, wherein the pharmaceutical composition is administered via parenteral injection, subcutaneous injection, intravenous injection, intramuscular injection, intraperitoneal injection, transdermally, orally, buccally, ocular routes, intravaginally, by inhalation, by depot injections, or by implants.
9. A method of diagnosing endometriosis in a human subject, the method comprising: measuring pre-implantation factor (PIF) protein or mRNA expression levels from an endometrial tissue sample from the subject; and
comparing the PIF protein expression levels from the endometrial sample to the PIF expression levels in a control normal sample;
wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial sample are greater than the PIF expression levels from the control normal sample.
10. A method of treating endometriosis in a human subject, the method comprising:
measuring pre-implantation factor (PIF) protein or mRNA expression levels from a endometrial sample from the subject;
comparing the PIF protein or mRNA expression levels from the endometrial sample to the PIF expression levels in a control normal sample,
wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial sample are greater than the PIF expression levels from the control normal sample; and
administering to the subject at least one pharmaceutical composition comprising a therapeutically effective amount of a PIF peptide, mimetics thereof, analogs thereof, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.
11. The method of claim 9 or 10, wherein the endometrial sample is an endometrial epithelial tissue sample.
12. The method of claim 9 or 10, wherein the endometrial tissue sample is a biopsy sample.
13. The method of claim 9 or 10, wherein the measuring of PIF protein expression levels comprises contacting the sample with an anti-PIF antibody and detecting bound anti-PIF antibody to PIF in the sample.

14. The method of claim 13, wherein the amount of bound antibody indicates the PIF protein expression levels.
15. The method of claim 9 or 10, wherein the measuring of PIF protein expression levels comprises staining the sample with an anti-PIF antibody that binds to PIF in the sample.
16. The method of claim 9 or 10, wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 10% greater than the PIF expression levels from the control normal sample.
17. The method of claim 9 or 10, wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 50% greater than the PIF expression levels from the control normal sample.
18. The method of claim 9 or 10, wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 100% greater than the PIF expression levels from the control normal sample.
19. The method of claim 9 or 10, wherein the patient is diagnosed with endometriosis if the PIF expression levels from the endometrial epithelial sample are at least 200% greater than the PIF expression levels from the control normal sample.
20. The method of claim 9 or 10, wherein the measuring of PIF mRNA expression levels comprises contacting the sample with a probe that binds to PIF mRNA and detecting the bound probe in the sample.

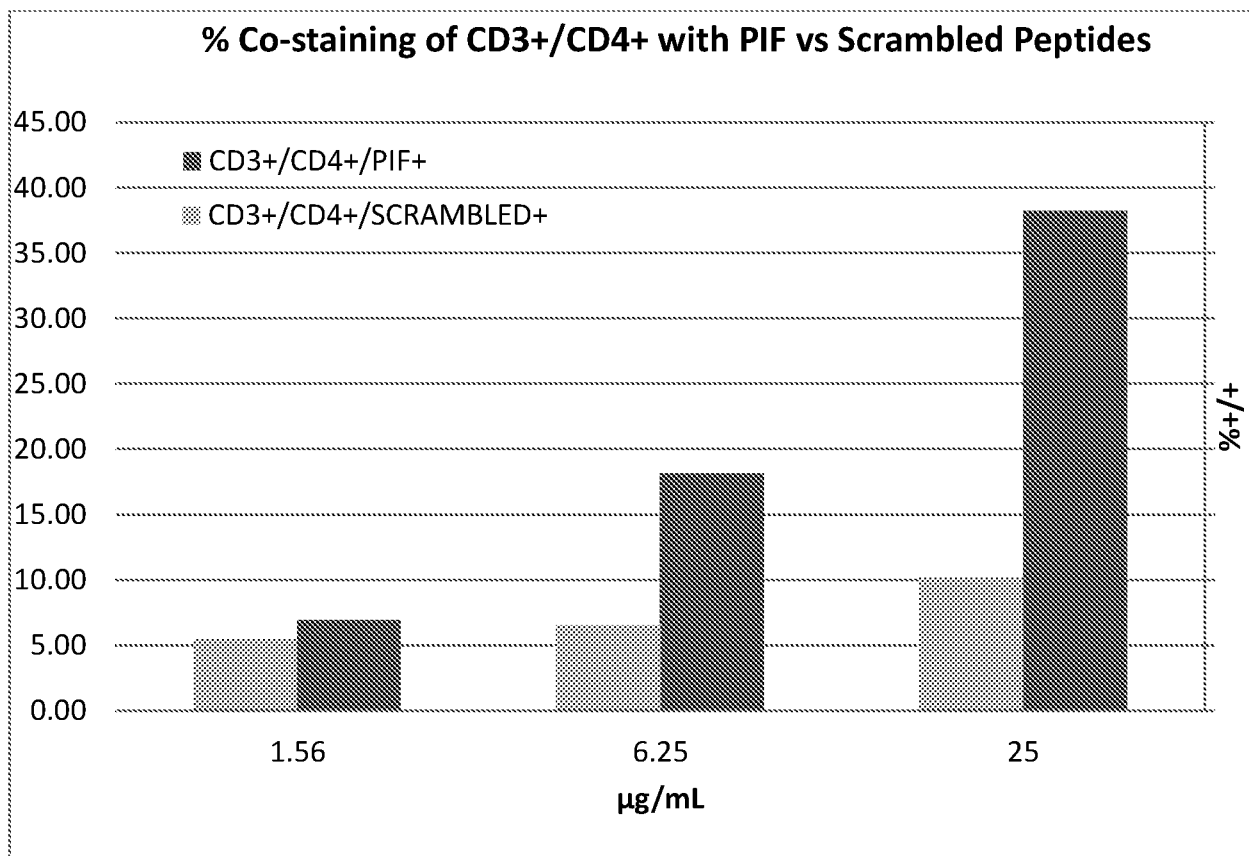


FIG. 1

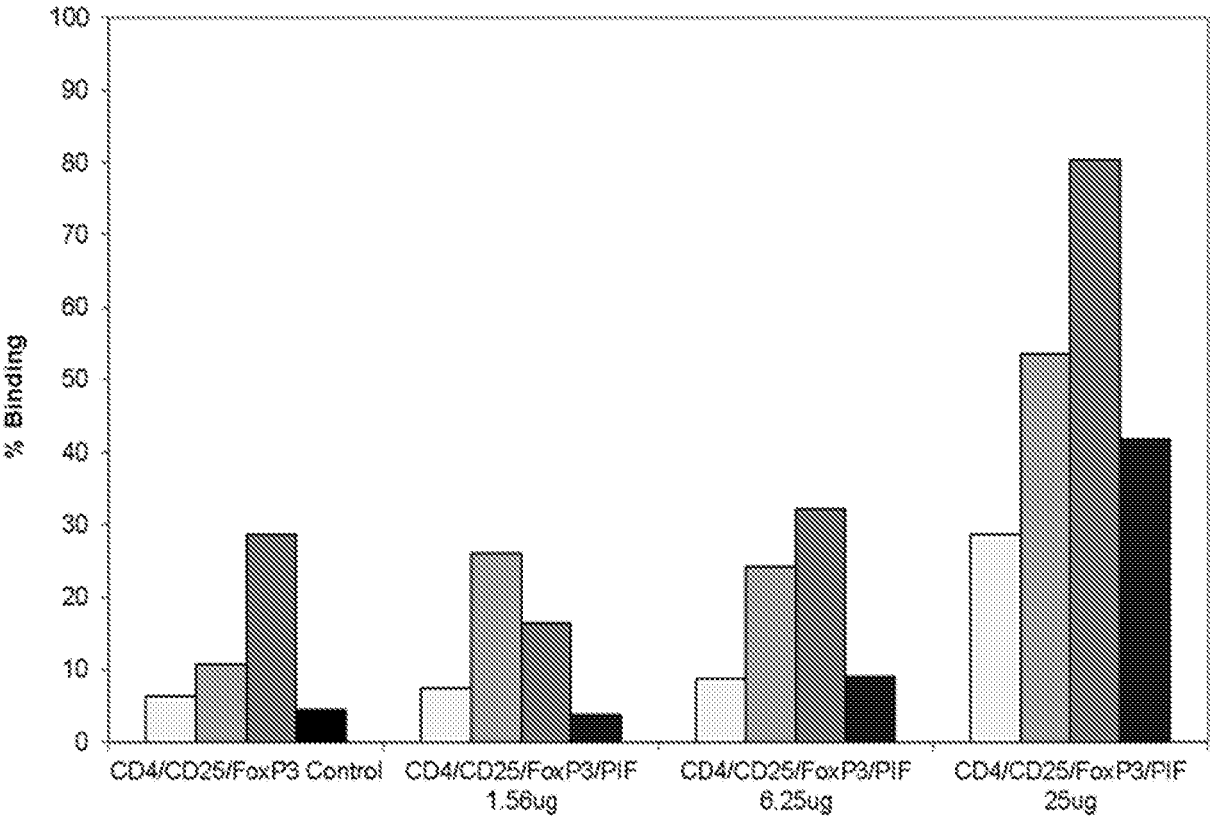


FIG. 2

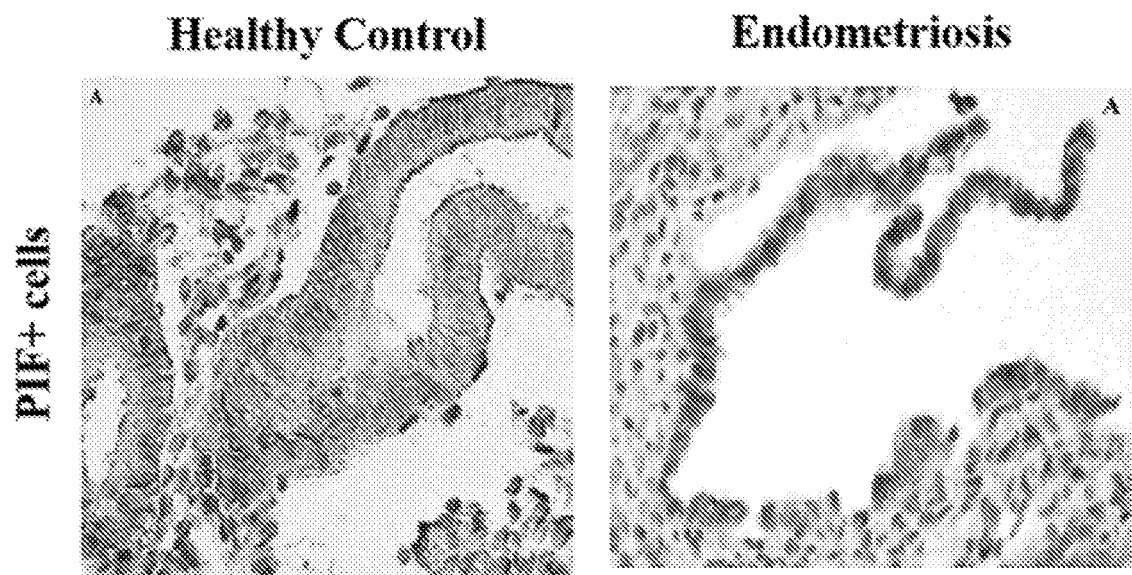


FIG. 3A

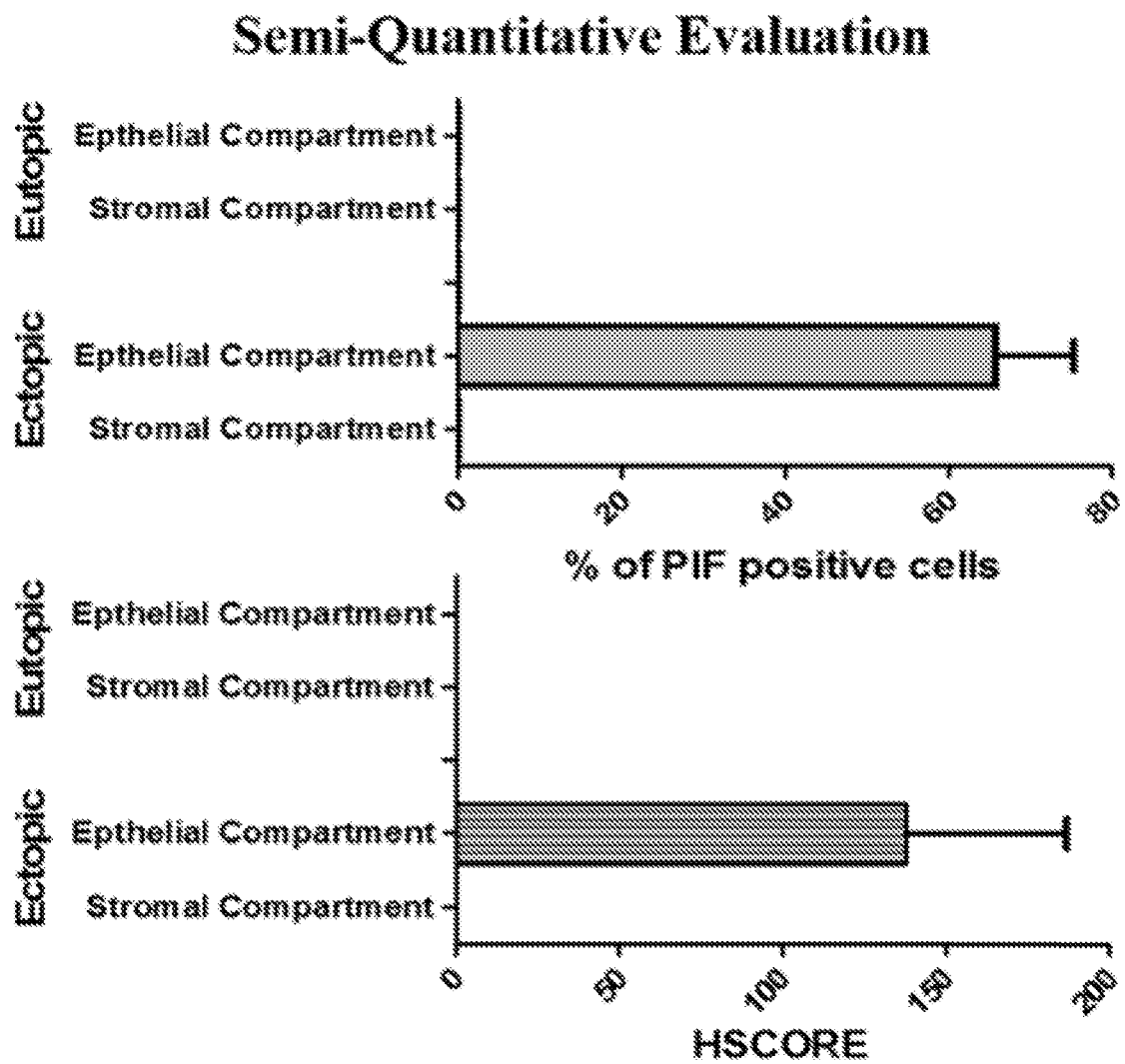


FIG. 3B

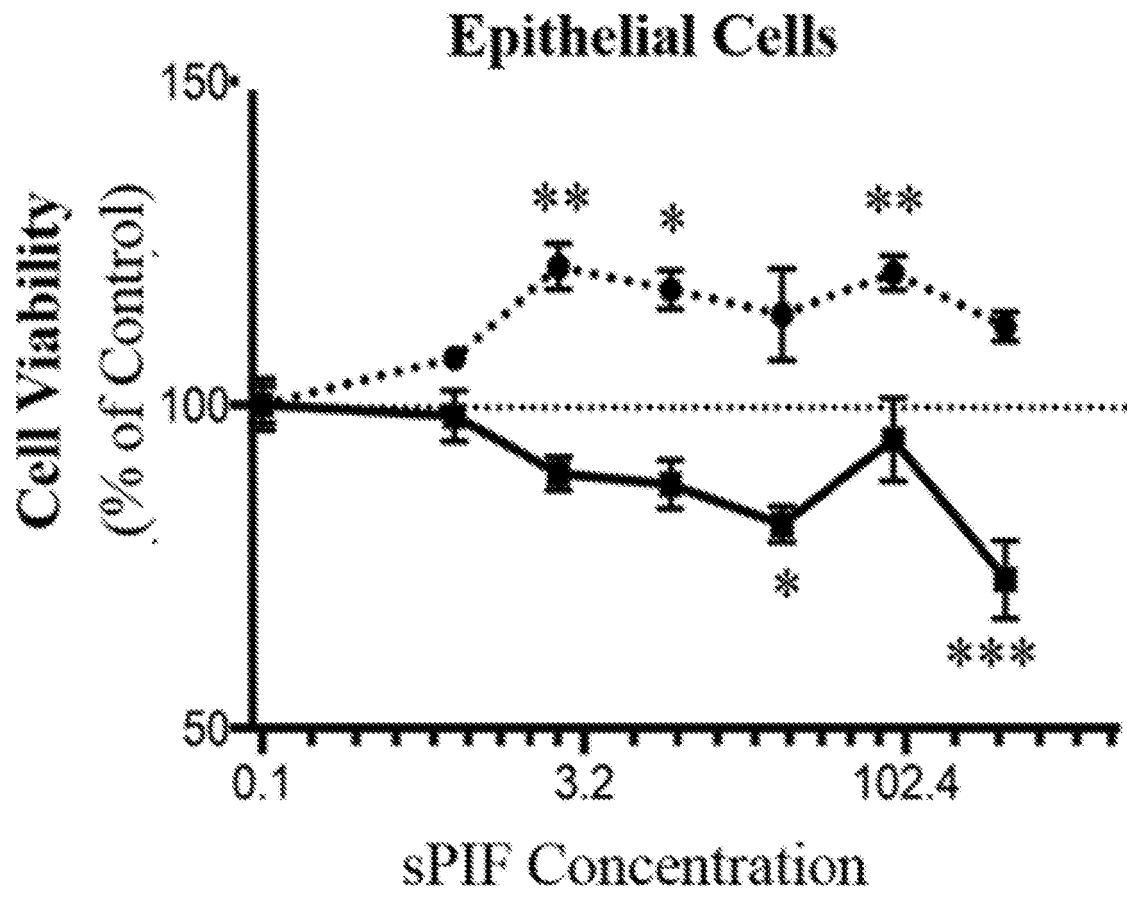


FIG. 3C

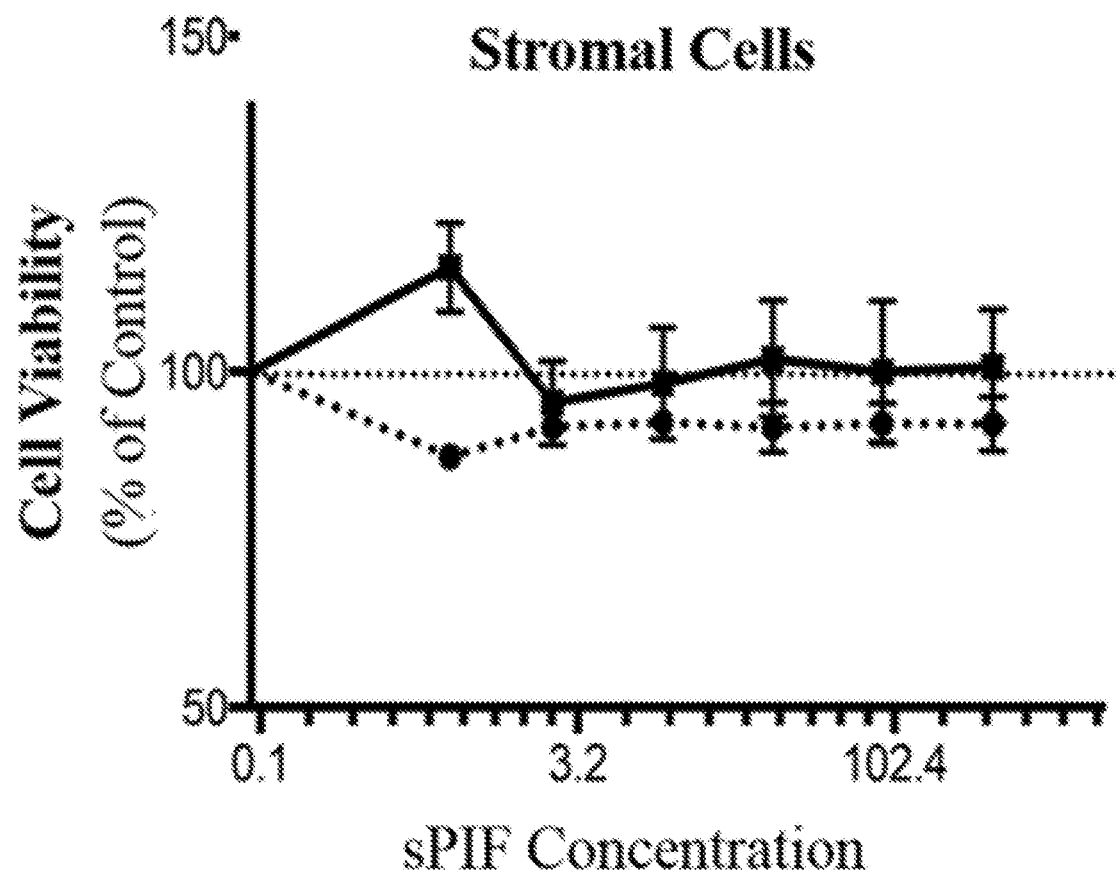


FIG. 3D

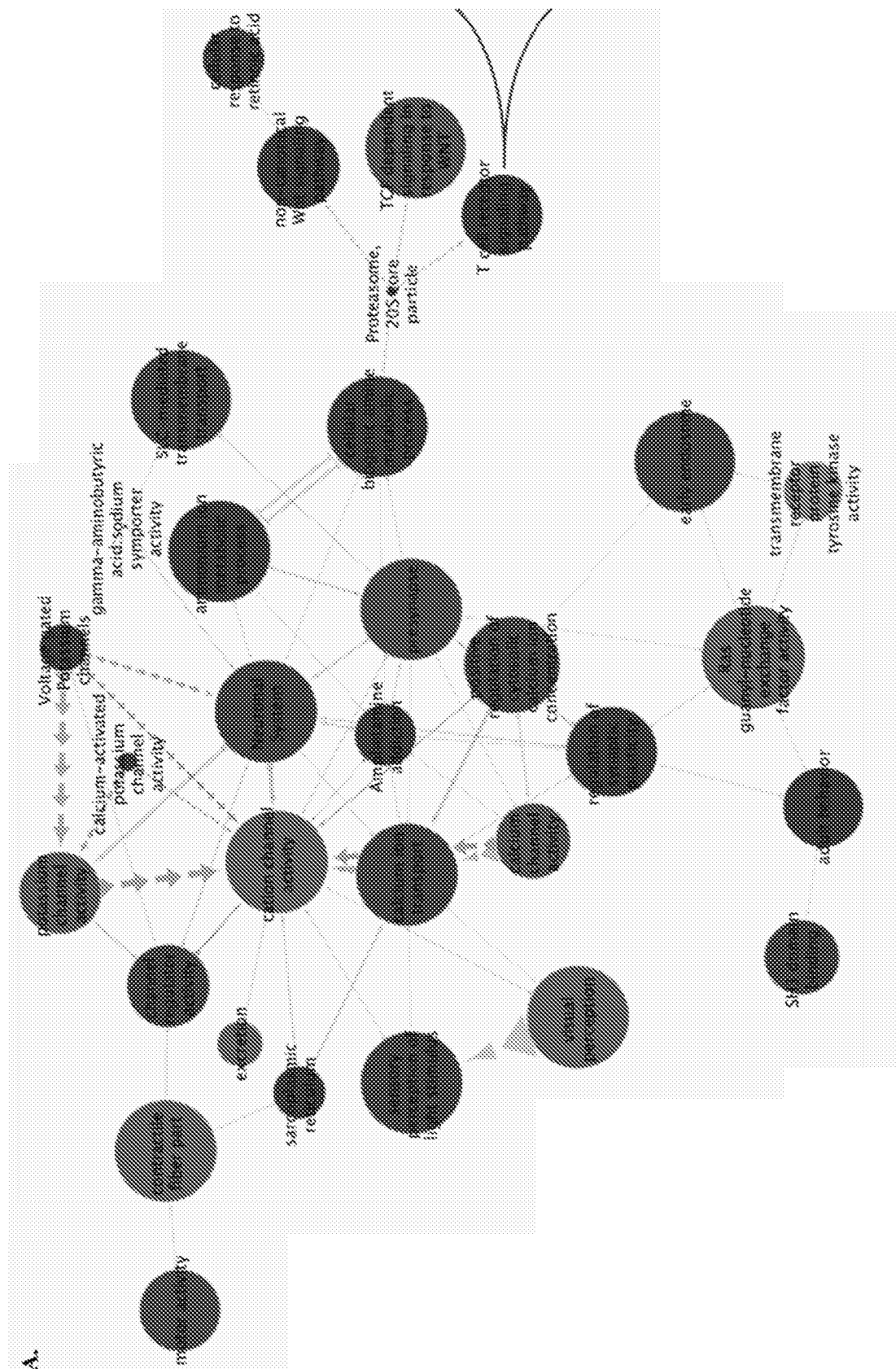


FIG. 4A

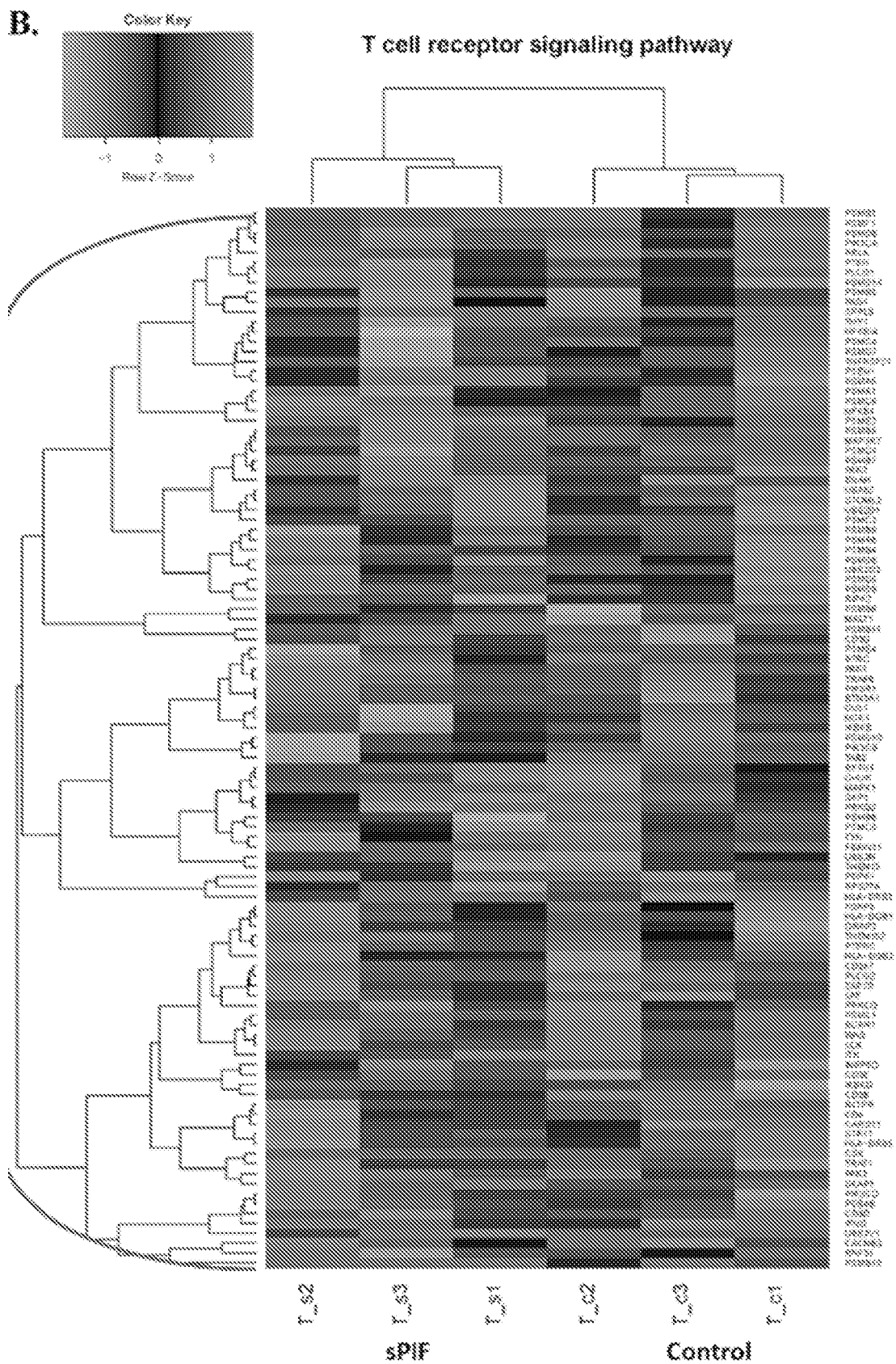


FIG. 4B

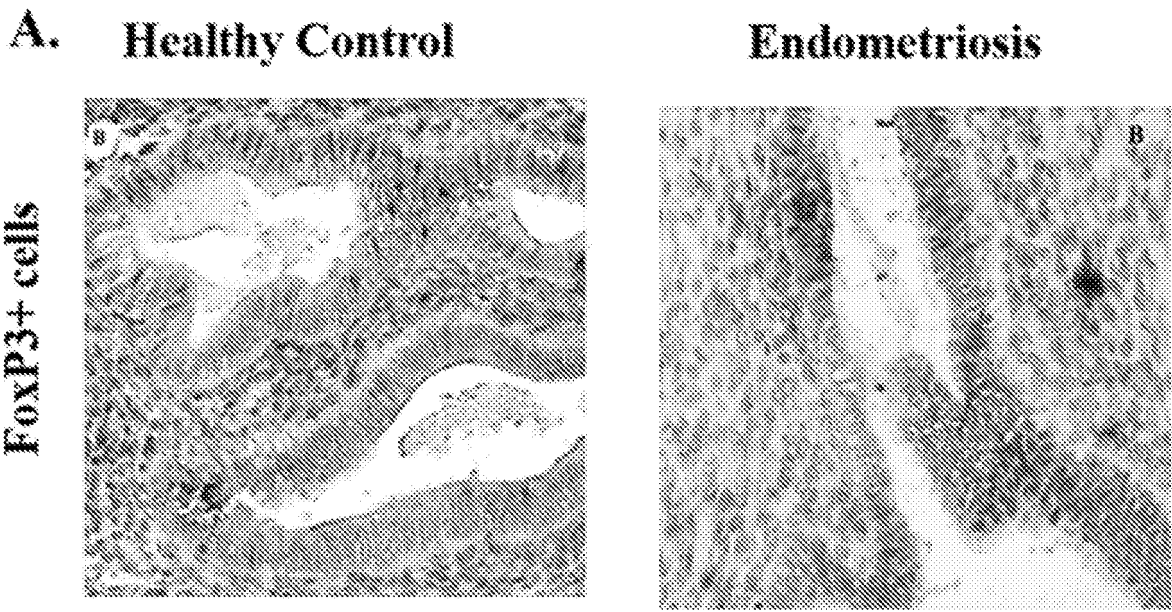


FIG. 5A

Semi-Quantitative Evaluation

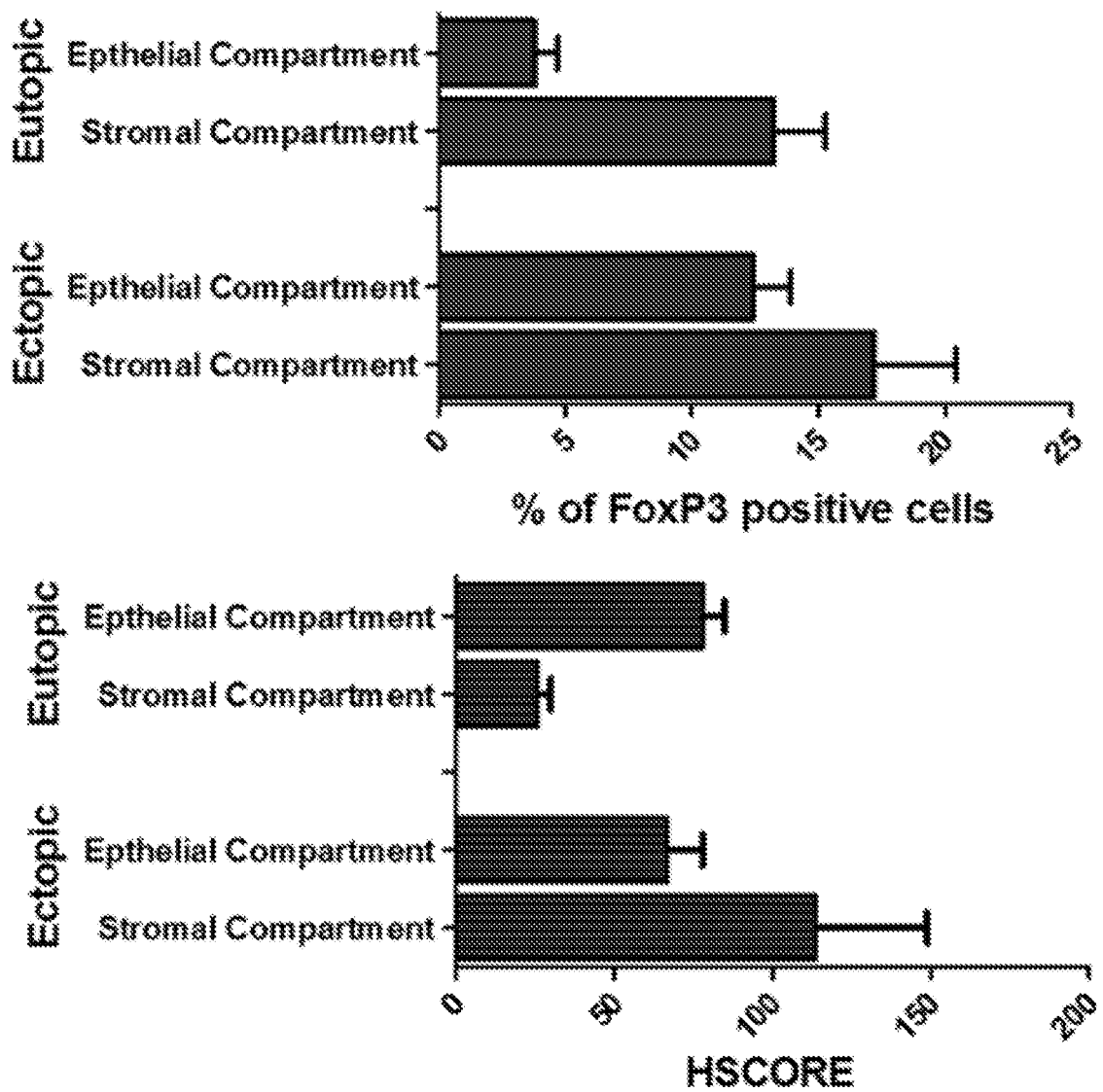


FIG. 5B

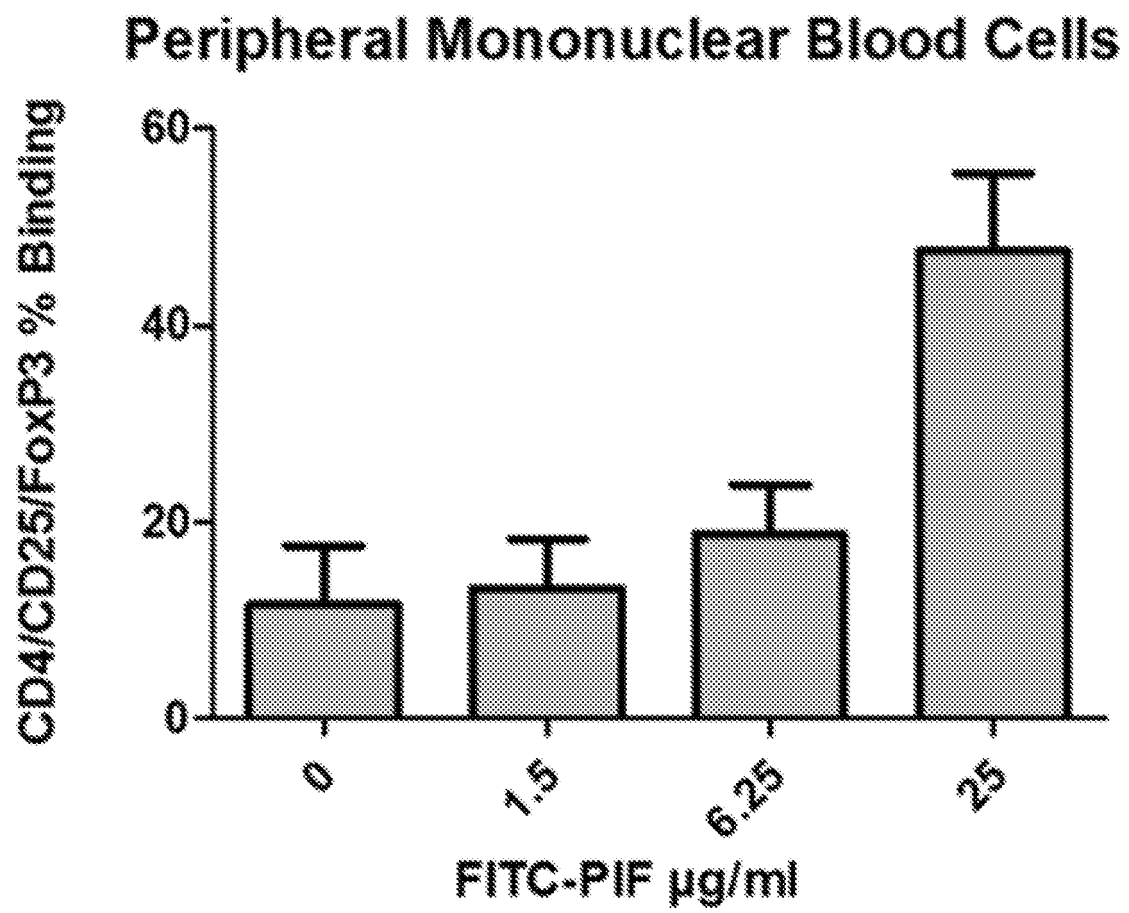


FIG. 5C

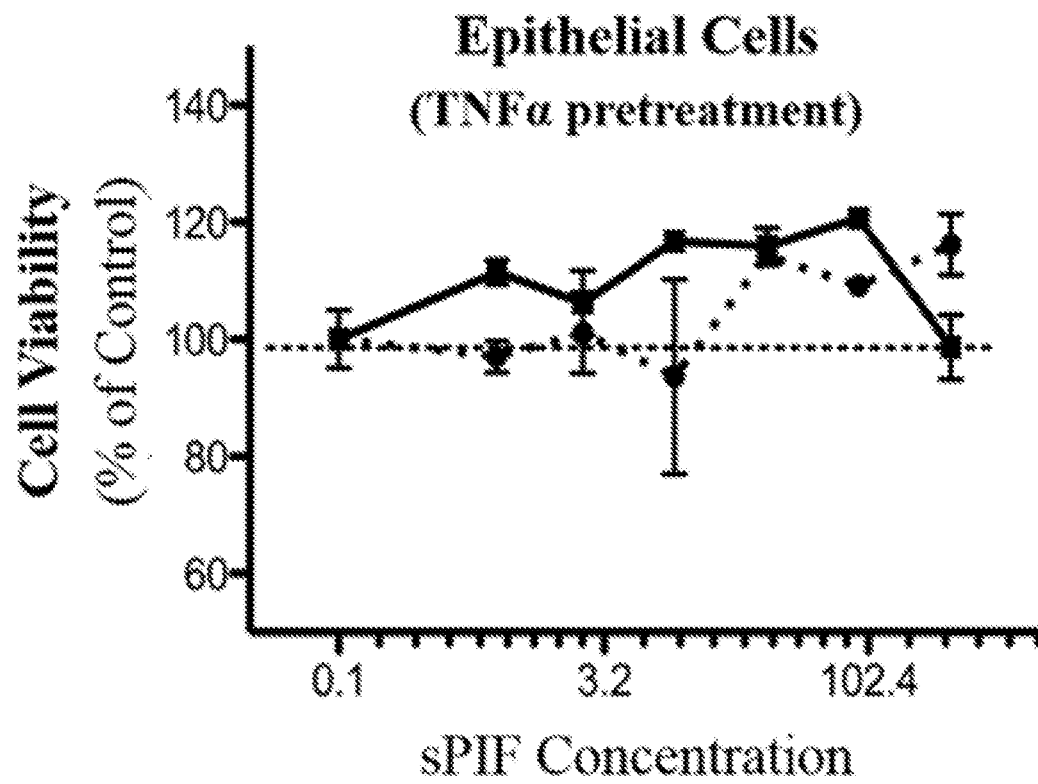


FIG. 5D

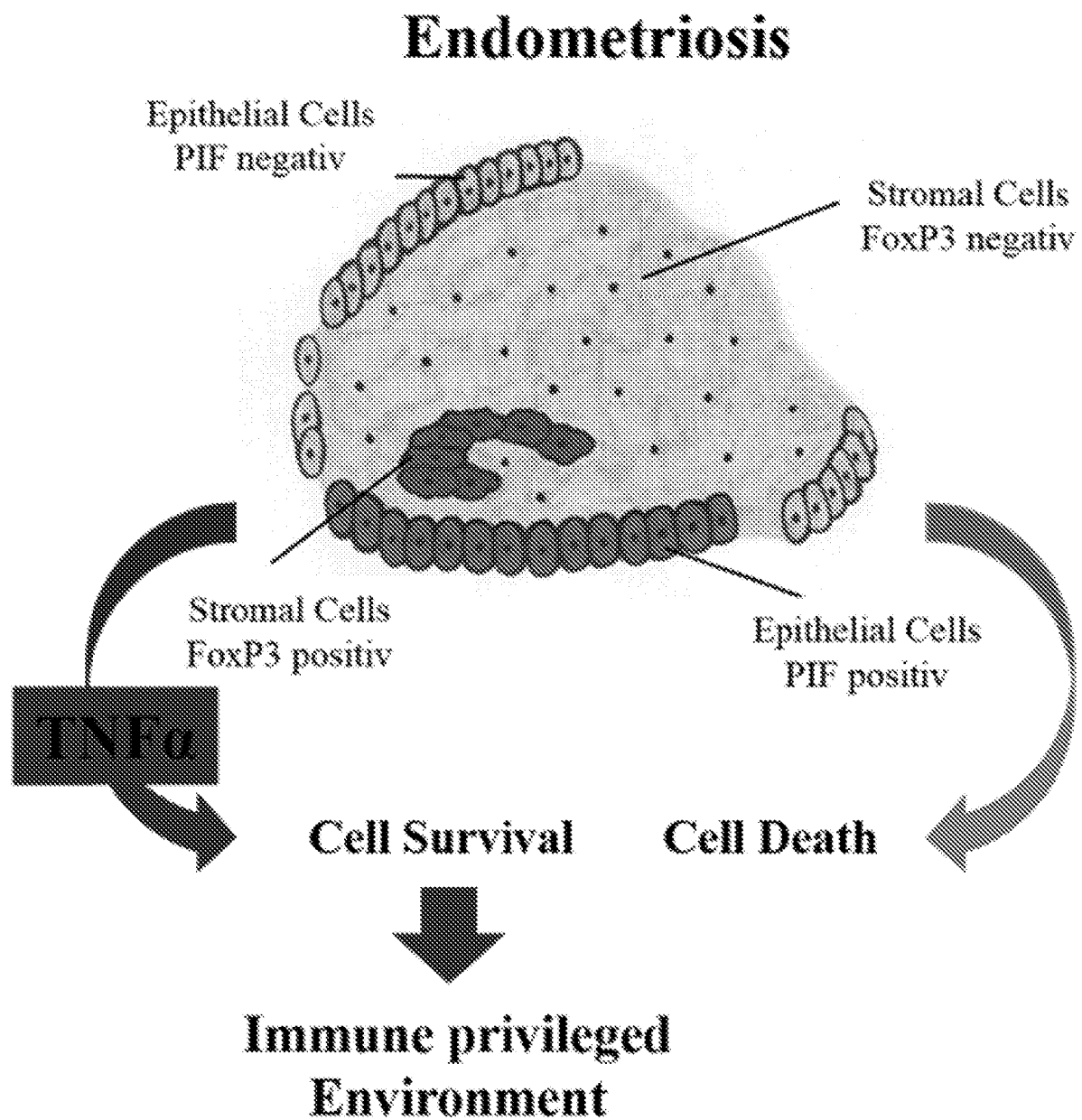


FIG. 5E