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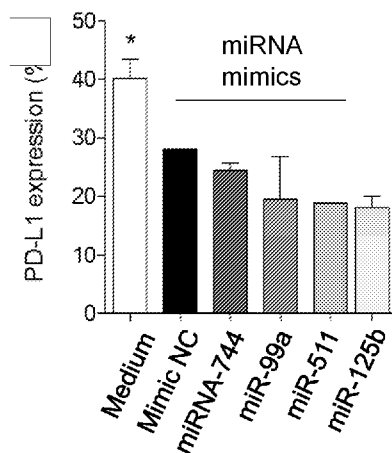
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(54) Title: TREATMENT OF TYPE 1 DIABETES AND AUTOIMMUNE DISEASES OR DISORDERS

Fig. 23B



(57) Abstract: Described herein are methods and compositions for producing modified, PD-L1 expressing hematopoietic stem cells, and uses thereof. Aspects of the invention relate to modulating the expression of micro RNA that controls the expression of PD-L1 in the hematopoietic stem cell. Methods for modulating the expression of micro RNA include, e.g., introducing to the cell a nucleic acid encoding a given micro RNA, or an agent that inhibits a given micro RNA.

TREATMENT OF TYPE 1 DIABETES AND AUTOIMMUNE DISEASES OR DISORDERS**CROSS-REFERENCE TO RELATED APPLICATION**

[0001] This Application claims benefit under 35 U.S.C. § 119(e) of the U.S. Provisional Application No 62/562,111 filed Sept 22, 2017; and U.S. Provisional Application No 62/663,367 filed April 27, 2018, the contents of each of which are incorporated herein by reference in their entireties.

FIELD OF THE DISCLOSURE

[0002] This invention relates to treatment or autoimmune diseases or disorders.

BACKGROUND

[0003] Since the search for feasible and safe immunological approaches to re-establish tolerance toward islet auto-antigens and preserve β -cell function in type 1 diabetes (T1D) began, very little progress has been made (1). However, most immunotherapies tested thus far are simply broadly immunosuppressive and are not clearly linked to any immunological abnormalities detected in T1D (2). Thus, the development of an effective treatment that is specific for an autoimmune diseases and disorder, e.g., T1D, is essential.

SUMMARY

[0004] Immunologically-based clinical trials performed thus far have failed to cure type 1 diabetes (T1D), in part because these approaches were nonspecific. Transplantation of hematopoietic stem and progenitor cells (HSPCs) has been recently offered as a therapy for T1D. Interestingly, transcriptomic profiling of HSPCs revealed that these cells are deficient in PD-L1 in the T1D NOD mouse model. It was therefore sought to determine whether genetic/pharmacological restoration of this defect would cure T1D.

[0005] Genetically engineered (or pharmacologically modulated) HSPCs overexpressing PD-L1 (PD-L1 Tg HSPCs) inhibited the autoimmune response *in vitro*, reverted diabetes in newly hyperglycemic NOD mice *in vivo*, and homed to the pancreas of hyperglycemic NOD mice. The PD-L1 expression defect was confirmed in human HSPCs in T1D patients as well, and pharmacologically modulated human HSPCs also inhibited the autoimmune response *in vitro*. Targeting a specific immune checkpoint defect in HSPCs thus contributes to establishing a therapeutic for T1D.

[0006] One aspect of the invention described herein provides an *ex vivo* method of producing a population of modified, PD-L1+ expressing hematopoietic stem cells (HSCs) comprising modulating the expression of miRNAs controlling the expression of PD-L1 in the HSCs.

[0007] In one embodiment of any aspect, the modulation of the expression of miRNAs is increasing the expression of miRNA or decreasing the expression of miRNA.

[0008] In one embodiment of any aspect, the miRNA expression is increased by introducing an exogenous copy of a nucleic acid encoding the miRNA for the expression of the miRNA in the cell.

[0009] In one embodiment of any aspect, the miRNA expression is decreased by an agent that inhibits the expression of the miRNA. Exemplary agents include, but are not limited to an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.

[0010] In one embodiment of any aspect, the exogenous copy is introduced by a vector, such as a viral vector.

[0011] In one embodiment of any aspect, the agent is introduced into the HSC by a vector, such as a viral vector.

[0012] In one embodiment of any aspect, the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

[0013] In one embodiment of any aspect, the modified, PD-L1+ expressing HSCs carries an exogenous copy of a nucleic acid encoding a miRNA selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

[0014] Another aspect of the invention described herein provides a population of modified hematopoietic stem cells (HSCs) in which the modified cells have increased PD-L1 expression compared to control, non-modified cells, wherein the cells carry an exogenous copy of a nucleic acid encoding a miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA, for example, an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.

[0015] In one embodiment of any aspect, the HSC cells are mammalian HSC cells. In one embodiment of any aspect, the mammalian HSC cells are human HSC cells.

[0016] In one embodiment of any aspect, prior to the modification, the HSCs are obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood. In one embodiment of any aspect, the HSCs are obtained from mobilized peripheral blood.

[0017] In one embodiment of any aspect, the HSCs are obtained from a healthy individual.

[0018] In one embodiment of any aspect, the HSCs are obtained from an individual with a diagnosed disease or disorder. In one embodiment of any aspect, the diagnosed disease or disorder is an autoimmune disease or disorder. In one embodiment of any aspect, the autoimmune disease or disorder is Type 1 diabetes (T1D).

[0019] In one embodiment of any aspect, the HSC cells are *ex vivo* cultured before or after or both before and after the modification of the PD-L1 expression.

[0020] In one embodiment of any aspect, the HSC cells are cryopreserved prior to or after or both prior to and after the modification of the PD-L1 expression. In one embodiment of any aspect, the modified HSC cells are cryopreserved prior to use.

[0021] In one embodiment of any aspect, the HSC cells are produced by a method comprising: (a) contacting a sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA; (b) *ex vivo* culturing the resultant modified cells from the contacting; and (c) establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1. In one embodiment, the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

[0022] In one embodiment of any aspect, the population of modified HSCs described herein are produced by any of the methods described herein.

[0023] Another aspect of the invention described herein provides a composition of modified HSCs comprising of any of the HSCs described herein.

[0024] Another aspect of the invention described herein provides a method of treating Type 1 Diabetes or an immune disease or disorder or cancer treatment in a host in need thereof comprising administering or *ex vivo* contacting an effective amount of an agent that modulates the expression of miRNAs controlling the expression of PD-L1 in the HSCs in a cell to a host.

[0025] In one embodiment of any aspect, the cell is a progenitor cell. In one embodiment of any aspect, the progenitor cell is a hematopoietic progenitor cell.

[0026] In one embodiment of any aspect, the agent is a vector comprising a nucleic acid sequence that miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

[0027] In one embodiment of any aspect, the vector is a virus.

[0028] Another aspect of the invention described herein provides a method of treating an autoimmune disorder or for cancer immune therapy (aka cancer therapy) in a subject in need thereof

comprising administering to a subject a composition comprising any of the hematopoietic stem cells described herein. In one embodiment of any aspect, the autoimmune disorder is Type 1 diabetes (T1D).

[0029] In one embodiment of any aspect, the HSCs are autologous to the recipient subject.

[0030] In one embodiment of any aspect, the HSCs are non-autologous and allogenic to the recipient subject.

[0031] In one embodiment of any aspect, the HSCs are non-autologous and xenogeneic to the recipient subject.

[0032] Another aspect of the invention described herein provides a method of modulating an immune response (e.g., for autoimmune disease, or for cancer immune therapy) in a subject comprising: administering or transplanting any of the modified HSC's described herein, of a composition thereof, to a subject.

[0033] Another aspect of the invention described herein provides a method of modulating an immune response in a subject comprising: (a) providing a population of hematopoietic stem cells (HSCs); (b) contacting sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA; (c) *ex vivo* culturing the resultant modified cells from the contacting; (d) establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1; and (e) transplanting said population of PD-L1+ expressing HSCs into a recipient subject, thereby modulating the immune response in the recipient subject.

[0034] In one embodiment of any aspect, the population of HSCs is obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood. In one embodiment of any aspect, wherein the population of HSCs is obtained from mobilized peripheral blood.

[0035] In one embodiment of any aspect, the population of HSCs autologous to the recipient subject. In one embodiment of any aspect, the population of HSCs allogeneic to the recipient subject. In one embodiment of any aspect, the population of HSCs is xenogeneic to the recipient subject.

[0036] In one embodiment of any aspect, the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

[0037] Another aspect of the invention described herein provides a composition comprising any of the PD-L1 expressing hematopoietic stem cells described herein in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the

delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0038] Another aspect of the invention described herein provides a composition comprising any of the PD-L1 expressing hematopoietic stem cells described herein for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0039] Another aspect of the invention described herein provides a population of any of the PD-L1 expressing hematopoietic stem cells described herein for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0040] Another aspect of the invention described herein provides a population of any of the PD-L1 expressing hematopoietic stem cells described herein for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

Definitions

[0041] As used herein, the term “comprising” means that other elements can also be present in addition to the defined elements presented. The use of “comprising” indicates inclusion rather than limitation.

[0042] The term "consisting of" refers to methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0043] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the disclosure.

[0044] 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 administration to or upon a mammal without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like. A pharmaceutically acceptable carrier will not promote the raising of an immune response to an agent with which it is admixed, unless so desired. The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be

limited based on formulation. Typically, such compositions are prepared as injectable either as liquid solutions or suspensions, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified or presented as a liposome composition. The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients include, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient. The therapeutic composition of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like. Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions. The amount of an active agent used in the methods described herein that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field of art. For example, a parenteral composition suitable for administration by injection is prepared by dissolving 1.5% by weight of active ingredient in 0.9% sodium chloride solution.

[0045] In one embodiment, the “pharmaceutically acceptable” carrier does not include *in vitro* cell culture media.

[0046] In one embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Specifically, it refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without

excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0047] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations, and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed. (Mack Publishing Co., 1990). The formulation should suit the mode of administration.

[0100] A "subject," as used herein, includes any animal that exhibits a symptom of a monogenic disease, disorder, or condition that can be treated with the gene therapy vectors, cell-based therapeutics, and methods disclosed elsewhere herein. In preferred embodiments, a subject includes any animal that exhibits symptoms of a disease, disorder, or condition of the hematopoietic system, e.g., a hemoglobinopathy, that can be treated with the gene therapy vectors, cell-based therapeutics, and methods contemplated herein. Suitable subjects (e.g., patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably, human patients, are included. Typical subjects include animals that exhibit aberrant amounts (lower or higher amounts than a "normal" or "healthy" subject) of one or more physiological activities that can be modulated by gene therapy.

[0101] A subject can be an adult subject, e.g., >18 years of age, or a pediatric subject, e.g., < 18 years of age. A subject can have been diagnosed with having a disease or disorder (e.g., an autoimmune disease or disorder), can be at risk of having (e.g., exhibit at least one risk factor), or does not have, or is not at risk of having a disease or disorder. A subject can have been previously treated for a disease or disorder, or is currently being treated for a disease or disorder.

[0102] In one embodiment, as used herein "treatment" or "treating," includes any beneficial or desirable effect on the symptoms or pathology of a disease or pathological condition, e.g., autoimmune disease (e.g., type I diabetes), and may include even minimal reductions in one or more measurable

markers of the disease or condition being treated. In another embodiment, treatment can involve optionally either the reduction or amelioration of symptoms of the disease or condition, or the delaying of the progression of the disease or condition. "Treatment" does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof.

[0103] In one embodiment, as used herein, "prevent," and similar words such as "prevented," "preventing" etc., indicate an approach for preventing, inhibiting, or reducing the likelihood of the occurrence or recurrence of, a disease or condition. In another embodiment, the term refers to delaying the onset or recurrence of a disease or condition or delaying the occurrence or recurrence of the symptoms of a disease or condition. In another embodiment, as used herein, "prevention" and similar words includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to onset or recurrence of the disease or condition.

[0048] As used herein, the term "amount" refers to "an amount effective" or "an effective amount" of a virus or transduced therapeutic cell to achieve a beneficial or desired prophylactic or therapeutic result, including clinical results.

[0049] A "therapeutically effective amount" of a virus or transduced therapeutic cell may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the stem and progenitor cells to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the virus or transduced therapeutic cells are outweighed by the therapeutically beneficial effects. The term "therapeutically effective amount" includes an amount that is effective to "treat" a subject (e.g., a patient).

[0050] As used herein, the terms "administering," refers to the placement of a nucleic acid or agent that controls expression of PD-L1 into a subject by a method or route which results in at least partial localization of the agent at a desired site, the HSC described herein. The nucleic acid or agent can be administered by any appropriate route which results in an effective treatment in the subject.

[0051] As used herein, "cultured," or "culturing" refers to maintaining a cell population in conditions (e.g., type of culture medium, nutrient composition of culture medium, temperature, pH, O₂ and/or CO₂ percentage, humidity level) suitable for growth.

[0052] The term "decrease", "reduce", or "inhibit" are all used herein to mean a decrease by a reproducible statistically significant amount. In some embodiments, "decrease", "reduce" or "inhibit" typically means a decrease by at least 10% as compared to a reference level (e.g. the absence of a given treatment) and can include, for example, a decrease by at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99% , as well as a 100% decrease.

[0053] The terms “increase”, “enhance”, or “activate” are all used herein to mean an increase by a reproducible statistically significant amount. In some embodiments, the terms “increase”, “enhance”, or “activate” can mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, a 20 fold increase, a 30 fold increase, a 40 fold increase, a 50 fold increase, a 6 fold increase, a 75 fold increase, a 100 fold increase, etc. or any increase between 2-fold and 10-fold or greater as compared to a reference level. In the context of a marker, an “increase” is a reproducible statistically significant increase in such level.

[0054] As used herein, “programmed death ligand-1 (PD-L1)” refers to a type I transmembrane protein that functions to suppress the immune system during particular events in a subject, such as pregnancy, tissue allografts, disease states (e.g., hepatitis), and presence of an autoimmune disease. PD-L1 sequences are known for a number of species, e.g., human PD-L1, also known as cluster of differentiation 274 (CD274) and B7 homolog 1 (B7-H1), (NCBI Gene ID: 29126) polypeptide (e.g., NCBI Ref Seq NP_001254635.1) and mRNA (e.g., NCBI Ref Seq NM_001267706.1). PD-L1 can refer to human PD-L1, including naturally occurring variants, molecules, and alleles thereof. PD-L1 refers to the mammalian PD-L1 of, e.g., mouse, rat, rabbit, dog, cat, cow, horse, pig, and the like.

[0055] As used herein, the term "DNA" is defined as deoxyribonucleic acid. The term "polynucleotide" is used herein interchangeably with "nucleic acid" to indicate a polymer of nucleosides. Typically, a polynucleotide is composed of nucleosides that are naturally found in DNA or RNA (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine) joined by phosphodiester bonds. However, the term encompasses molecules comprising nucleosides or nucleoside analogs containing chemically or biologically modified bases, modified backbones, etc., whether or not found in naturally occurring nucleic acids, and such molecules may be preferred for certain applications. Where this application refers to a polynucleotide it is understood that both DNA, RNA, and in each case both single- and double-stranded forms (and complements of each single-stranded molecule) are envisioned. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one nucleic acid strand of a denatured double-stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double-stranded DNA.

[0056] As used herein, the terms “protein” and “polypeptide” are used interchangeably herein to refer to a polymer of amino acids. A peptide is a relatively short polypeptide, typically between about 2 and 60 amino acids in length. Polypeptides used herein typically contain amino acids such as the 20 L-amino acids that are most commonly found in proteins. However, other amino acids and/or amino acid

analogues known in the art can be used. One or more of the amino acids in a polypeptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a fatty acid group, a linker for conjugation, functionalization, etc. A polypeptide that has a non-polypeptide moiety covalently or noncovalently associated therewith is still considered a "polypeptide." Exemplary modifications include glycosylation and palmitoylation. Polypeptides can be purified from natural sources, produced using recombinant DNA technology or synthesized through chemical means such as conventional solid phase peptide synthesis, etc.

[0057] As used herein, "modulating" refers to altering (e.g., increasing or reducing) of the function of a miRNA, e.g., that controls PD-L1. This can be accomplished by directly altering the production of the miRNA itself in the cell, or alternatively by altering the miRNA function/activity. The function/activity for a given miRNA can be reduced, for example by directly inhibiting the miRNA itself or otherwise targeting that miRNA for degradation. miRNA function/activity can be increased, for example by directly upregulating the miRNA itself or otherwise targeting that miRNA for upregulation, or activation. As such, an agent useful in the present invention for modulation is one that alters miRNA expression, or alters miRNA function or activity. Modulation of a given miRNA can also be accomplished, e.g., by alteration of an upstream factor that induces, positively regulates, or inhibits miRNA expression or miRNA function/activity. As such, another useful agent for modulation is an agent that inhibits or increases such an upstream factor by methods that correspond to those described for a given miRNA.

[0058] The terms "microRNA" or "miRNA" are used interchangeably and these are endogenous RNAs, some of which are known to regulate the expression of protein-coding genes at the posttranscriptional level. Endogenous microRNA are small RNAs naturally present in the genome which are capable of modulating the productive utilization of mRNA. The term artificial microRNA includes any type of RNA sequence, other than endogenous microRNA, which is capable of modulating the productive utilization of mRNA. MicroRNA sequences have been described in publications such as Lim, et al., *Genes & Development*, 17, p. 991-1008 (2003), Lim et al *Science* 299, 1540 (2003), Lee and Ambros *Science*, 294, 862 (2001), Lau et al., *Science* 294, 858-861 (2001), Lagos-Quintana et al, *Current Biology*, 12, 735-739 (2002), Lagos Quintana et al, *Science* 294, 853-857 (2001), and Lagos-Quintana et al, *RNA*, 9, 175-179 (2003), which are incorporated by reference. Multiple microRNAs can also be incorporated into a precursor molecule. Furthermore, miRNA-like stem-loops can be expressed in cells as a vehicle to deliver artificial miRNAs or the purpose of modulating the expression of endogenous genes through the miRNA pathway.

[0059] The term "vector", as used herein, refers to a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral. The term "vector" encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but

is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, artificial chromosome, virus, virion, etc.

[0060] As used herein, the term "viral vector" refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain a nucleic acid encoding a polypeptide as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring nucleic acids into cells either *in vitro* or *in vivo*. Numerous forms of viral vectors are known in the art.

[0061] As used herein, the term "expression vector" refers to a vector that directs expression of an RNA or polypeptide (e.g., a polypeptide encoding miRNA that controls PD-L1) from nucleic acid sequences contained therein linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification. The term "expression" refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing.

[0062] A vector can be integrating or non-integrating. "Integrating vectors" have their delivered RNA/DNA permanently incorporated into the host cell chromosomes. "Non-integrating vectors" remain episomal which means the nucleic acid contained therein is never integrated into the host cell chromosomes. Examples of integrating vectors include retroviral vectors, lentiviral vectors, hybrid adenoviral vectors, and herpes simplex viral vector.

[0063] One example of a non-integrative vector is a non-integrative viral vector. Non-integrative viral vectors eliminate the risks posed by integrative retroviruses, as they do not incorporate their genome into the host DNA. One example is the Epstein Barr oriP/Nuclear Antigen-1 ("EBNA1") vector, which is capable of limited self-replication and known to function in mammalian cells. As containing two elements from Epstein-Barr virus, oriP and EBNA1, binding of the EBNA1 protein to the virus replicon region oriP maintains a relatively long-term episomal presence of plasmids in mammalian cells. This particular feature of the oriP/EBNA1 vector makes it ideal for generation of integration-free iPSCs. Another non-integrative viral vector is adenoviral vector and the adeno-associated viral (AAV) vector.

[0064] Another non-integrative viral vector is RNA Sendai viral vector, which can produce protein without entering the nucleus of an infected cell. The F-deficient Sendai virus vector remains in the cytoplasm of infected cells for a few passages, but is diluted out quickly and completely lost after several passages (e.g., 10 passages).

[0065] Another example of a non-integrative vector is a minicircle vector. Minicircle vectors are circularized vectors in which the plasmid backbone has been released leaving only the eukaryotic promoter and cDNA(s) that are to be expressed.

[0066] Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. It should be understood that this disclosure is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[0067] Definitions of common terms in molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3), (2015 digital online edition at merckmanuals.com, Robert S. Porter *et al.* (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis *et al.*, Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entireties. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0068] Unless otherwise stated, the present invention was performed using standard procedures known to one skilled in the art, for example, in Michael R. Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012); Davis *et al.*, Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (1986); Current Protocols in Molecular Biology (CPMB) (Fred M. Ausubel, *et al.* ed., John Wiley and Sons, Inc.), Current Protocols in Immunology (CPI) (John E. Coligan, *et al.*, ed. John Wiley and Sons, Inc.), Current Protocols in Cell Biology (CPCB) (Juan S. Bonifacino *et al.* ed., John Wiley and

Sons, Inc.), Culture of Animal Cells: A Manual of Basic Technique by R. Ian Freshney, Publisher: Wiley-Liss; 5th edition (2005), Animal Cell Culture Methods (Methods in Cell Biology, Vol. 57, Jennie P. Mather and David Barnes editors, Academic Press, 1st edition, 1998), Methods in Molecular biology, Vol.180, Transgenesis Techniques by Alan R. Clark editor, second edition, 2002, Humana Press, and Methods in Molecular Biology, Vol. 203, 2003, Transgenic Mouse, edited by Marten H. Hofker and Jan van Deursen, which are all herein incorporated by reference in their entireties.

[0069] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[0070] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The term “about” when used in connection with percentages will mean $\pm 1\%$.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] FIGS 1A-1V presents data that show PD-L1 is downregulated in HSPCs from NOD mice. (FIGS 1A, 1B). Transcriptomic profiling of Sca-1+Lineage-c-kit+ cells (KLS) obtained from bone marrow of NOD and C57BL/6 mice showed reduced PD-L1 expression. (FIG. 1C) Bar graph representing mRNA expression of PD-L1 as measured by qRT-PCR in Lin-c-kit+ (KL) cells, collected from bone marrow of C57BL/6 and NOD mice. All samples were run in triplicate and normalized to expression of the housekeeping gene GAPDH. (FIG. 1D-1K) Representative flow cytometric analysis and quantitative bar graphs confirming the PD-L1 defect in 4 populations of HSPCs in C57BL/6 and NOD mice. (FIG. 1L-1N) Representative flow cytometry and quantitative bar graphs of PD-L1 expression in KL cells obtained from the bone marrow of C57BL/6 and NOD mice at different ages. (FIG. 1O-1Q) Representative flow cytometric analysis and quantitative bar graphs of PD-L1 expression in KL cells and in other non-progenitor cells from bone marrow or spleen respectively obtained from C57BL/6 and NOD mice. (FIG. 1R1-1R3, 1S1-1S3, and 1T) Confocal imaging and quantification of bone marrow sections of C57BL/6 and NOD mice for c-kit (shown in red) and PD-L1 (shown in green) staining; the quantification of the orange-stained bone marrow element was performed by ImageJ. Histology magnification, 63x. Scale bar, 40 μm . (FIG. 1U, 1V) Western blotting and quantitative bar graphs confirming reduced PD-L1 protein expression in KL cells obtained from bone marrow of C57BL/6 and NOD mice, with GAPDH used as an internal control. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least n=3 mice and two independent experiments. *P<0.05; **P<0.01; ***P<0.001.

[0072] FIGs 2A-2L presents data that show the mechanism of PD-L1 downregulation in NOD HSPCs. (FIG. 2A) Bar graphs depicting percentage of PD-L1+ cells within Lin-c-kit+ (KL) cells isolated from bone marrow of C57BL/6 and NOD mice and cultured for 3 days in normal glucose, in 20 mM or in 35 mM high glucose. (FIG. 2B) Similar proliferation rates of CFSE-labeled KL cells obtained from C57BL/6 and NOD bone marrow were evident at baseline or after 1 and 3 days of culture. (FIG. 2C) The frequency of apoptosis of KL cells obtained from bone marrow of C57BL/6 and NOD mice was higher at baseline in NOD mice as compared to C57BL/6 mice, while the opposite was observed after 1 and 3 days of culture. (FIG. 2D) MA-plot for GWAS analysis performed in Sca-1+Lineage-c-kit+ cells (KLS) obtained from bone marrow of C57BL/6 compared to NOD mice. (FIG. 2E, 2F) List of miRNAs significantly upregulated (FIG. 2E) and downregulated (FIG. 2F) in KLS cells obtained from the bone marrow of NOD as compared to C57BL/6 mice. (FIG. 2G) miRNAs network controlling PD-L1 gene expression generated with Mouse Genome Informatics MGI. (FIG. 2H, 2I) KL cells obtained from bone marrow of NOD mice were cultured in the presence of miR1905 inhibitor and WT (untreated KL) were used as controls. qRT-PCR of miR-1905 (FIG. 2H) and qRT-PCR of PD-L1 (FIG. 2I) are shown. (FIG. 2J, 2K) Western blotting and quantitative bar graphs of PD-L1 protein expression in KL cells obtained from bone marrow of NOD mice cultured in the presence of miR-1905 inhibitor and WT (untreated KL) were used as controls, with GAPDH was used as an internal control. (FIG. 2L) Methylation status of the PD-L1 locus in KLS obtained from the bone marrow of C57BL/6 and NOD mice. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least n=3 mice and two independent experiments. *P<0.05; **P<0.01; ***P<0.001.

[0073] FIGs 3A-3V presents data that show genetically engineered PD-L1.Tg KL cells abrogate the autoimmune response *in vitro* and revert diabetes in hyperglycemic NOD mice *in vivo*. (FIG. 3A, 3B, 3C) Representative flow cytometric analysis and quantitative bar graph of Lin-c-kit+ (KL) cells obtained from bone marrow of NOD mice pre- and post-transduction with PD-L1 lentivirus showed upregulation of PD-L1. (FIG. 3D, 3E) Confocal imaging of KL cells obtained from bone marrow of NOD mice pre- and post-lentiviral PD-L1 transduction confirmed PD-L1 upregulation. Magnification 63x. Scale bar, 50 μ m. (FIG. 3F, 3G) MA-plot and PD-L1 fold change for gene expression level in KL cells obtained from bone marrow of NOD mice transduced with PD-L1 lentivirus as compared to mock-transduced KL cells, demonstrating PD-L1 upregulation. (FIG. 3H, 3I) Representative flow cytometric analysis and quantitative bar graph of IFN- γ +CD4+ T cells isolated from NOD-BDC2.5 TCR Tg mice stimulated with BDC2.5 peptide in the presence of DCs (Control) or upon co-culture with untransduced KL cells (WT), PD-L1.Tg KL cells (at different ratios) or with PD-L1.Tg KL cells pre-treated with anti-PD-L1 blocking mAb. (FIG. 3J, 3K) Representative flow cytometric analysis and quantitative bar graph of IFN- γ +CD8+ T cells isolated from NOD-8.3 TCR Tg mice stimulated with IGRP peptide in the presence of DCs (Control), or upon co-culture with WT KL cells, PD-L1.Tg KL cells (at different ratios) or with PD-L1.Tg KL cells pre-treated with PD-L1 blocking mAb. (FIG. 3L, 3M) Representative flow

cytometric analysis and quantitative bar graph of IFN- γ +CD4⁺ T cells isolated from normoglycemic NOD mice stimulated with soluble anti-CD3/anti-CD28 (Control), or upon co-culture with WT KL cells, PD-L1.Tg KL cells (at different ratios), or with PD-L1.Tg KL cells pre-treated with PD-L1 blocking mAb. PD-L1.Tg KL cells strongly abrogate the CD4/CD8- restricted autoimmune response and anti-CD3/CD28-dependent T cell stimulation *in vitro*. (FIG. 3N1-3N6) Representative immunohistochemical H&E analysis and CD3/insulin staining in serial pancreatic islet tissue sections from PD-L1.Tg KL cell-treated or untreated newly hyperglycemic NOD mice showing the protection conferred by PD-L1.Tg KL. Histology magnification 20x. Scale bar, 200 μ m. (FIG. 3O, 3P, 3Q, 3R) Newly hyperglycemic NOD mice were treated with WT KL cells, with PD-L1.Tg KL cells, with doxycycline or were left untreated. PD-L1.Tg KL cells reverted diabetes in newly hyperglycemic NOD mice. (FIG. 3S) Insulinitis score confirmed the protection offered by PD-L1.Tg KL cell treatment, which resulted in fewer infiltrated islets in PD-L1.Tg KL cell-treated NOD mice. (FIG. 3T) PD-L1.Tg KL cell-treated NOD mice were regularly immunocompetent in an ovalbumin re-challenge test. (FIG. 3U) Immunophenotypic analysis of lymphocytes isolated from spleens by flow cytometry showed an increased percentage of FoxP3⁺ regulatory T cells in PD-L1.Tg KL cell-treated NOD mice as compared to untreated NOD mice. (FIG. 3V) Quantification of IFN- γ -producing cells (with number of spots normalized for background) in an *ex vivo* assay, in which splenocytes were challenged with islet peptides (BDC2.5, IGRP, GAD-65 and insulin) after 40 days following treatment in newly hyperglycemic PD-L1.Tg KL cell-treated NOD mice or in untreated hyperglycemic NOD mice. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least n=3 mice and two independent experiments. *P<0.05; **P<0.01; ***P<0.001. #p<0.05 vs. all; §p<0.05 vs. all.

[0074] FIGs 4A-4V presents data that show genetically engineered PD-L1.Tg KL cells traffic to the pancreas in hyperglycemic NOD mice. (FIG. 4A, 4B) PD-L1.Tg KL cells induced greater late apoptosis in autoreactive CD4⁺ and CD8⁺ T cells as compared to WT KL cells. (FIG. 4C, 4D) Representative flow cytometric analysis for lymphoid and myeloid markers and quantitative bar graphs of isolated KL cells before and after lentiviral transduction. (FIG. 4E, 4F, 4H, 4I) Representative flow cytometric analysis and quantitative bar graphs of ZsGreen+PD-L1.Tg KL cells in the pancreas of hyperglycemic and normoglycemic NOD mice at 1, 7 days and 14 days after treatment with PD-L1.Tg KL cells. ZsGreen+PD-L1.Tg KL cells traffic efficiently to the pancreas of hyperglycemic NOD, with no evidence of their presence in the pancreas of normoglycemic NOD. (FIG. 4G, 4J) Quantification of ZsGreen mRNA in the pancreas of hyperglycemic and normoglycemic NOD mice by qRT-PCR after treatment with PD-L1.Tg KL cells showed the presence of ZsGreen mRNA in the pancreata of hyperglycemic NOD mice, but not in those of normoglycemic NOD mice. (FIG. 4K, 4M) Bar graphs depicting flow cytometric quantification of ZsGreen+PD-L1.Tg KL cells and (FIG. 4L, 4N) quantification of ZsGreen mRNA by qRT-PCR in the bone marrow of hyperglycemic and normoglycemic NOD mice, after treatment with PD-L1.Tg KL cells, showed the presence of

ZsGreen+PD-L1.Tg KL cells in the bone marrow of hyperglycemic and normoglycemic NOD mice. (FIG. 4O, 4Q) Bar graphs for flow cytometric quantification of ZsGreen+PD-L1.Tg KL cells and (FIG. 4P, 4R) quantification of ZsGreen mRNA by qRT-PCR in the spleen of hyperglycemic and normoglycemic NOD mice showed ZsGreen+PD-L1.Tg KL cells in the spleen of both groups of NOD mice. (FIG. 4S1-4S4, 4T1-4T4) Confocal imaging of pancreatic sections obtained from normoglycemic or hyperglycemic NOD mice after 1, 7 and 14 days after treatment with ZsGreen+PD-L1.Tg KL cells. ZsGreen+ cells are evident in the pancreata of hyperglycemic, but not in those of normoglycemic, NOD mice. Magnification 63x in all confocal images, Scale bar, 20 μ m (FIG. 4S1-4S4 and 4T1, 4T3, 4T4); Scale bar, 5 μ m (FIG. 4T2). (FIG. 4U, 4V) Luminescent images of NOD mice adoptively transferred with Luciferase+PD-L1.Tg KL cells after 24 hours and 7 days of treatment, showing the rapid disappearance from peripheral blood of Luciferase+PD-L1.Tg KL cells. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least n=3 mice and two independent experiments. *P<0.05; **P<0.01; ***P<0.001.

[0075] FIGs 5A-5U presents data that show pharmacologically modulated KL cells (pKL) abrogate the autoimmune response *in vitro*. (FIG. 5A-5C) Results of screening of small molecules tested for their ability to upregulate PD-L1 (MFI) on mobilized CD34+ cells obtained from healthy controls, the 3-color coding shown in (FIG. 5C) represents lowest PD-L1 MFI values (orange), median PD-L1 MFI values (yellow) and highest PD-L1 MFI values (green). (FIG. 5D, 5E) PD-L1 expression (mRNA and MFI) fold change was quantified for the indicated component, alone or in combination. (FIG. 5F-5H) Representative flow cytometric analysis and quantitative bar graph of PD-L1 expression on Lin-c-kit+ (KL) cells from NOD mice pre- and post-pharmacologic modulation with the indicated small molecules, alone or in combination. (FIG. 5I, 5J) Confocal imaging of KL cells pre- and post-modulation with small molecules, showing DAPI (in blue) and PD-L1 (in red) staining. Magnification 63x. Scale bar, 50 μ m. (FIG. 5K, 5L) MA-plot and fold change for gene expression in KL cells obtained from bone marrow of NOD mice and pKL as compared to unmodulated KL cells (Vehicle-KL cells, WT). (FIG. 5M, 5N) Representative flow cytometric analysis and quantitative bar graph for IFN- γ +CD4+ T cells isolated from NOD-BDC2.5 TCR Tg mice and stimulated with BDC2.5 peptide in the presence of DCs (Controls) or upon co-culture with unmodulated KL (WT), pKL cells (at different ratios) or with pKL cells pre-treated with anti-PD-L1 blocking mAb. (FIG. 5O) Bar graph for flow cytometric quantification of IFN- γ +CD4+ T cells after co-culture of CD4+ T cells isolated from NOD-BDC2.5 TCR Tg mice stimulated with BDC2.5 peptide in the presence of DCs (Control) or upon co-culture with unmodulated KL cells (WT), with pKL cells, or with PD-L1.Tg KL cells, or with CD4+CD25+ T regulatory cells. (FIG. 5P, 5Q) Representative flow cytometric analysis and quantitative bar graph for IFN- γ +CD8+ T cells isolated from NOD-8.3 TCR Tg mice and stimulated with IGRP peptide in the presence of DCs (Control) or upon co-culture with unmodulated KL cells (WT), pKL cells, (at different ratios) or pKL cells pre-treated with anti-PD-L1 blocking mAb. (FIG. 5R) Bar graph for flow cytometric quantification

of IFN- γ +CD8⁺ T cells after co-culture of CD8⁺ T cells isolated from NOD-8.35 TCR Tg mice stimulated by IGRP peptide in the presence of DCs (Control) or upon co-culture with unmodulated KL cells (WT), with pKL cells, or with PD-L1.Tg KL cells, or with CD4⁺CD25⁺ T regulatory cells. (FIG. 5S, 5T) Representative flow cytometric analysis and quantitative bar graph for IFN- γ +CD4⁺ T cells isolated from NOD mice and stimulated with soluble anti-CD3/anti-CD28 (Control) or upon co-culture with unmodulated KL cells (WT), with pKL cells (at different ratios), or with pKL cells pre-treated with PD-L1 blocking/neutralizing mAb. pKL strongly abrogate the CD4/CD8-restricted autoimmune response and anti-CD3/anti-CD28-dependent T cell stimulation *in vitro*. (FIG. 5U) Bar graph depicting flow cytometric quantification of IFN- γ +CD4⁺ T cells within CD4⁺CD25⁺ T cells isolated from NOD mice and stimulated with soluble anti-CD3/anti-CD28 (Control) or upon co-culture with WT, with pKL, or with PD-L1.Tg KL cells, or with CD4⁺CD25⁺ T regulatory cells. PD-L1.Tg KL cells - and pKL to a lesser extent - appear as potent as regulatory T cells in inhibiting the CD4/CD8-restricted autoimmune response and anti-CD3/anti-CD28-dependent T cell stimulation. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least two independent experiments. *P<0.05; **P<0.01; ***P<0.001. #p<0.05 vs. all; §p<0.05 vs. all.

[0076] FIGs 6A-6k present data that show pharmacologically modulated KL cells (pKL) revert hyperglycemia in NOD mice *in vivo*. (FIG. 6A) In newly hyperglycemic NOD mice, treatment with pKL reverted diabetes. (FIG. 6B) Kaplan-Meier curve showing reversal of diabetes in newly hyperglycemic NOD mice with different treatments. (FIG. 6C) Quantification of IFN- γ -producing cells in an *in vitro* assay, in which splenocytes were challenged with islet peptides after treatment with pKL, normalized for background. (FIG. 6D) Immunophenotype of lymphocytes isolated from spleen of pKL-treated newly hyperglycemic NOD mice showed an increase in the percentage of FoxP3⁺ regulatory T cells. (FIG. 6E-6G) Bar graph showing the levels of pro- (IL-2/IL-6) and anti-inflammatory (IL-4) cytokines as measured by Luminex in the serum of untreated or pKL-treated newly hyperglycemic NOD mice at baseline and at 7 days after treatment. (FIG. 6H) pKL-treated NOD mice were regularly immunocompetent upon ovalbumin re-challenge. (FIG. 6I) Insulinitis score is reduced in pKL-treated newly hyperglycemic NOD mice. (FIG. 6J1-6J6) Representative immunohistochemical H&E analysis and CD3/insulin staining in serial pancreatic islet tissue sections from pKL-treated or untreated NOD mice showing the protection conferred by pKL. Histology magnification 20x. Scale bar, 200 μ m. (FIG. 6K) Schematic representing the defect in PD-L1 in HSPCs in T1D, as well as the effect of PD-L1 genetic/pharmacological restoration. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least n=3 mice and two independent experiments. *P<0.05; **P<0.01; ***P<0.001; # P<0.05 vs. all.

[0077] FIGs 7A-7Z present data that show the PD-L1 defect is evident in HSPCs from T1D patients. (FIG. 7A, 7B, 7C) Representative flow cytometric and quantitative bar graph of PD-L1+CD34⁺ cells from patients with T1D as compared to healthy controls (n=10 from each group). (FIG. 7D, 7E)

Western blot analysis and (FIG. 7F) qRT-PCR confirmed the PD-L1 defect in CD34+ cells from T1D patients (n=3 in each group). (FIG. 7G1-7G3) and (FIG. 7H1-7H3 and 7I) Confocal imaging and quantitative bar graph of bone marrow sections obtained from T1D patients and healthy controls showing CD34 (red) and PD-L1 (green) staining; the quantification of the orange-stained bone marrow element was performed by ImageJ. Histology magnification, 63x; scale bar 40 μ m. (FIG. 7J) Bar graph depicting the percentage of PD-L1 on CD34+ cells obtained from peripheral blood of T1D patients or healthy controls at baseline or cultured for 3 days in normal glucose, in 20 mM or in 35 mM high glucose (n=5 samples from each group). (FIG. 7K) CFSE-based proliferation assay of peripheral CD34+ cells obtained from T1D and healthy control patients at baseline and after 1 and 3 days of culture. (FIG. 7L) The rate of apoptosis of CD34+ cells was higher in T1D patients at baseline as compared to healthy controls, while no differences were evident after 1 and 3 days of culture. (FIG. 7M) Table of human miRNAs, discovered by bioinformatic approach, involved in the regulation of PD-L1 expression. (FIG. 7N) qRT-PCR showed differentially expressed miRNA in human CD34+ cells obtained from T1D patients as compared to controls. (FIG. 7O) No difference in the DNA methylation status of the PD-L1 gene promoter was evident in peripheral CD34+ cells obtained from T1D patients as compared to healthy controls. (FIG. 7P, 7Q, 7R) Representative flow cytometric and quantitative bar graph of PD-L1 expression on peripheral CD34+ cells from T1D patients pre- and post-pharmacologic-modulation with a small molecule, either alone or in combination. (FIG. 7S, 7T) Confocal imaging of PD-L1 expression on CD34+ cells from T1D patients pre- and post-pharmacologic-modulation. Magnification, 63x. Scale bar, 50 μ m. (FIG. 7U) PD-L1 expression fold change in pharmacologically-modulated CD34+ (pCD34+) after 24 hours and in vehicle-treated CD34+ cells as assessed by RT-PCR. (FIG. 7V, 7W) Unmodulated CD34+ and pCD34+ cells abrogate the IFN- γ autoimmune response towards insulin-associated 2 (FIG. 7I-7A2) autoantigen *in vitro*, as measured via the quantification of IFN- γ -producing cells. (FIG. 7X, 7Y) An anti-PD-L1 blocking mAb abrogated the immunological effect of pCD34+ cells *in vitro*. (FIG. 7Z) Unmodulated CD34+ and pCD34+ cells did not abrogate the anti-CD3/CD28-stimulated PBMC response *in vitro* as measured via the quantification of IFN- γ -producing cells. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least two independent experiments. *P<0.05; **P<0.01; ***P<0.001. #p<0.05 vs. all.

[0078] FIGs 8A-8M present data that show costimulatory molecules other than PD-L1 (i.e. PD-L2 and PD-1). (FIG. 8A-8F) Other molecules are scarcely present in hematopoietic stem progenitor cells isolated from the bone marrow of NOD and C57BL/6 mice. (FIG. 8G-8I) PD-L1 expression on other relevant immune cells (ILC1, ILC2, ILC3, early myeloid CD11b⁺ cells and NKT cells) obtained from bone marrow of NOD and C57BL/6 mice. (FIG. 8J-8M) PD-L1 expression on other relevant immune cells (CD4⁺CD25⁺, CD4 effector T cells, CD4 memory T cells, CD8⁺ T cells, CD8 effector T cells, CD8 memory T cells, CD4⁺CD25⁺FoxP3⁺ T regulatory cells, NKT cells and early myeloid CD11b⁺ cells) obtained from

spleen of NOD and C57BL/6 mice. Data are expressed as mean $\pm$ standard error of the mean (SEM) unless otherwise specified. Data are representative of at least two independent experiments. * P <0.05; ** P <0.01; *** P <0.001. # P <0.05 vs. all.

[0079] FIGs 9A-9I present data that show generation and characteristics of PD-L1.Tg KL cells. (FIG. 9A) Schematic representation of the genetic approach employed to generate PD-L1.Tg KL cells by lentiviral transduction. (FIG. 9B, 9C) Quantification of ZsGreen mRNA in the pancreatic lymph nodes of hyperglycemic and normoglycemic NOD mice by qRT-PCR after treatment with PD-L1.Tg KL cells. (FIG. 9D, 9E) Representative flow cytometric analysis and quantitative bar graphs of GFP⁺ KL cells in the pancreas of hyperglycemic NOD mice at 1, 7 and 14 days after treatment with WT KL cells isolated from bone marrow cells of normoglycemic NOD Luciferase⁺ GFP⁺ mice. (FIG. 9F, 9G) Few CD11c⁺ZsGreen⁺ cells and CD11b⁺ZsGreen⁺ cells were observed in the pancreas and PLN (FIG. 9H, 9I) of adoptively transferred NOD mice. Data are expressed as mean $\pm$ standard error of the mean (SEM) unless otherwise specified. Data are representative of at least two independent experiments.

[0080] FIGs 10A and 10B present data that show immunotyping of pharmacologically-modulated KL cells. (FIG. 10A-10B) Representative flow cytometric analysis and quantitative bar graph of positive and negative costimulatory molecules, (CD40, CD80, CD86, ICOSL, PD-1) and of select pro-inflammatory and anti-inflammatory cytokines (IFN- γ , IL-10, IL-4) in pharmacologically-modulated KL cells (pKL) from NOD mice as compared to unmodulated KL-Veh cells isolated from the bone marrow of normoglycemic NOD mice. Data are expressed as mean $\pm$ standard error of the mean (SEM) unless otherwise specified. Data are representative of at least two independent experiments. * P <0.05; ** P <0.01; *** P <0.001.

[0081] FIGs 11A-11E present data that show analysis of IFN- γ ⁺CD4⁺ T cells. (FIG. 11A) Representative flow cytometric analysis of IFN- γ ⁺CD4⁺ T cells isolated from NOD-BDC2.5 TCR Tg mice stimulated with BDC2.5 peptide in the presence of DCs (Control) or upon co-culture with unmodulated KL cells (WT), with pharmacologically-modulated KL cells (pKL), with PD-L1.Tg KL cells or with CD4⁺CD25⁺ T regulatory cells. (FIG. 11B) Representative flow cytometric analysis of IFN- γ ⁺CD8⁺ T cells isolated from NOD-8.3 TCR Tg mice stimulated with IGRP peptide in the presence of DCs (Control) or upon co-culture with unmodulated KL cells (WT), with pKL cells, with PD-L1.Tg KL cells, or with CD4⁺CD25⁺ T regulatory cells. (FIG. 11C) Representative flow cytometric analysis of IFN- γ ⁺CD4⁺ T cells isolated from normoglycemic NOD mice and stimulated with soluble anti-CD3/anti-CD28 (Control) or upon co-culture with unmodulated KL cells (WT), with pKL, with PD-L1.Tg KL cells or with CD4⁺CD25⁺ T regulatory cells. (FIG. 11D, 11E) Treatment with anti-PD-1 stimulating mAb (clone P1M2) as previously described did not delay diabetes onset in pre-diabetic 10-week-old NOD mice, nor did it revert diabetes in newly hyperglycemic NOD mice. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least two independent experiments.

[0082] FIGs 12A-12N present data that show gene expression of PD-L1⁺CD34⁺ cells. (FIG. 12A) Quantification of PD-L1⁺CD34⁺ cells by flow cytometry in long-standing or new-onset T1D patients and in healthy controls (CTRL). (FIG. 12B, 12C, 12D) PD-L1 expression on other relevant immune cells in patients with T1D and in healthy controls. (FIG. 12E-12N) Other costimulatory molecules are scarcely present in CD34⁺ cells isolated from peripheral blood of healthy controls or from long-standing or new-onset T1D patients. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least two independent experiments. * P <0.05; ** P <0.01; *** P <0.001. # P <0.05 vs. all.

[0083] FIGs 13A-13B present data that show analysis of pharmacologically-modulated CD34⁺ cells. (FIG. 13A-13B) Representative flow cytometric analysis and quantitative bar graph of positive and negative costimulatory molecules, (CD40, CD80, CD86, ICOSL, PD-1) and of select pro-inflammatory and anti-inflammatory cytokines (IFN- γ , IL-10, IL-4) in human pharmacologically-modulated CD34⁺ cells (pCD34⁺) as compared to unmodulated CD34⁺ cells isolated from peripheral blood of T1D patients (n=3). Data are expressed as mean $\pm$ standard error of the mean (SEM) unless otherwise specified. Data are representative of at least two independent experiments.

[0084] FIGs 14A-14B present data that show Scattered dot plot (FIG. 14A) and fold change (FIG. 14B) representing transcriptomic profiling of immune-related molecules in pCD34⁺ cells as compared to unmodulated CD34⁺ cells isolated from peripheral blood of T1D patients (n=3). Data are expressed as mean $\pm$ standard error of the mean (SEM) unless otherwise specified. Data are representative of at least two independent experiments.

[0085] FIGs 15A-15C present data that CD34⁺ cell mobilization with Plerixafor does not increase the percentage of peripheral PD-L1⁺CD34⁺ cells in T1D patients, and decreases the frequency of these cells in healthy controls. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least two independent experiments. * P <0.05; ** P <0.01; *** P <0.001. # P <0.05 vs. all

[0086] FIGs 16A-16D show nonmyeloablative autologous hematopoietic stem cell transplantation (AHSCT) preserved pancreatic β cell function in patients newly diagnosed with T1D. (FIG. 16A) Line graph showing 2-hour peak-stimulated C-peptide levels after a mixed meal tolerance test following AHSCT, at 12 and 24 months of follow-up. (FIG. 16B) Correlation between the number of HSPCs injected during AHSCT and 2-hour peak-stimulated C-peptide levels evaluated at 6 months of follow-up. (FIG. 16C, 16D) Correlation between the number of hematopoietic stem cells (HSPCs) injected during AHSCT and exogenous insulin requirement units (EIR) evaluated at 8 months (C) and 12 months (D) of follow-up. All data are expressed as mean $\pm$ SEM. All parameters examined were statistically significantly different when comparing baseline values vs. those at 6, 8 and 12 months. * P <0.05, ** P <0.001. Abbreviations: AHSCT, autologous hematopoietic stem cell transplantation; UI, units of insulin; EIR, exogenous insulin requirement units.

[0087] FIGs 17A-17J show prostaglandins enhance PD-L1 expression on human CD34⁺ cells. (FIG. 17A-17B) Results of screening of a prostaglandin small molecule library tested for the ability to upregulate PD-L1 (MFI) on CD34⁺ cells obtained from T1D patients. The schematic of the experimental design is shown in (A). The 3-color coding shown in (FIG. 17B) represents lowest PD-L1 MFI values (blue), median PD-L1 MFI values (pink) and highest PD-L1 MFI values (red). (FIG. 17C-17D) Representative flow cytometric analysis and quantitative bar graph of PD-L1 expression on CD34⁺ cells from T1D patients pre- and post-pharmacologic modulation with PGE₂ as compared to CD34⁺ cells obtained from healthy controls. (FIG. 17E) PD-L1 and CXCR4 expression (mRNA) fold change was quantified for CD34⁺ cells pre- and post-modulation with PGE₂. (FIG. 17F) Migration assay using CD34⁺ cells pre- and post-modulation with PGE₂. (FIG. 17G) Confocal imaging of CD34⁺ cells pre- and post-modulation with PGE₂, showing DAPI (in blue) and PD-L1 (in green) staining. 63x magnification. Scale bar, 50 μ m. (FIG. 17H) IDO-1 expression (mRNA) fold change was quantified for CD34⁺ cells pre- and post-modulation with PGE₂. (FIG. 17I-17J) Representative flow cytometric analysis (FIG. 17I) and quantitative bar graph (FIG. 17J) of PD-L1 expression on CD34⁺ cells from T1D patients pre- and post-pharmacologic modulation with 4 small molecule PGE₂ agonists. All data are expressed as mean $\pm$ SEM. * P <0.05, ** P <0.001. Abbreviations: MFI, mean fluorescence intensity.

[0088] FIGs 18A-18J show effects of human PGE₂-modulated and cytokine-treated CD34⁺ cells. (FIG. 18A-18B) Sustained and robust upregulation of PD-L1 upon culture for 7 days with PGE₂ and a cocktail of cytokines (heparin, human SCF, human TPO, human FGF-1, IGFBP2, and Angptl3) on CD34⁺ cells obtained from T1D patients as compared to those from healthy controls. (FIG. 18C) Effect of PGE₂ pulsing on CD34⁺ cells cultured for 0, 24h, 72h and 6 days on PD-L1 expression on CD34⁺ cells. (FIG. 18D-18E) Bar graphs showing an increase in the number of PD-L1⁺ CD34⁺ cells and fold increase in cell number after 7 days of culture with PGE₂ supplemented with cytokines. (FIG. 18F-18G) Upregulation of PD-L1 expression following culture with PGE₂ (shown as percentage) on CD34⁺ cells was not altered by the freeze/thawing process. (FIG. 18H) PGE₂-modulated CD34⁺ cells abrogate the IFN- γ autoimmune response to insulin-associated 2 (I-A2) autoantigen *in vitro*, as measured via the quantification of IFN- γ -producing cells in an Elispot assay; Diftavax refers to a vaccine including immunization against tetanus toxoid, difteria and hemophilus. (FIG. 18I) PGE₂-modulated CD34⁺ cells and PGE₂-modulated CD34⁺ cells cultured for 7 days in STFIA media abrogate the IFN- γ autoimmune response towards insulin-associated 2 (I-A2) autoantigen *in vitro*, as measured via the quantification of IFN- γ -producing cells in an Elispot assay, even when added at low dose. (FIG. 18J) PGE₂-modulated CD34⁺ cells abrogate the anti-CD3/CD28-stimulated PBMC response *in vitro* as measured via the quantification of IFN- γ -producing cells in an Elispot assay. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least two independent experiments. * P <0.05; ** P <0.01. Abbreviations: SCF, stem cell factor; TPO, thrombopoietin; hFGF-1, human fibroblast growth factor 1;

IGFBP2, insulin-like growth factor-binding protein 2; Angptl3, angiopoietin-like 3; PBMCs, peripheral blood mononuclear cells.

[0089] FIGs 19A-19G show the profile of murine KL cells. (FIG. 19A-19B) Representative flow cytometric analysis and quantitative bar graph of PD-L1 expression on Lineage⁻c-kit⁺ (KL) cells from NOD mice pre- and post-pharmacologic modulation with PGE₂. (FIG. 19C-19D) Representative flow cytometric analysis and quantitative bar graph of CXCR4 expression on KL cells from NOD mice pre- and post-pharmacologic modulation with PGE₂. (FIG. 19E) Quantitative bar graph of flow cytometric expression of positive and negative costimulatory molecules (CD40, CD80, CD86, PD-L1, PD-L2 and PD-1) and of select pro-inflammatory and anti-inflammatory cytokines (IFN- γ , IL-10, IL-4) in PGE₂-modulated KL cells from NOD mice as compared to those modulated with a selected PGE₂ clinical grade agonist (dmPGE₂) and compared to unmodulated KL cells isolated from the bone marrow of normoglycemic NOD mice. (FIG. 19F) Flow cytometric expression of selected chemokine receptors (CXCR4, CCR2, CCR4, CCR5, CCR6, CCR7, CCR8, and CXCR3) in PGE₂-modulated KL cells from NOD mice as compared to those modulated with a selected PGE₂ clinical grade agonist (dmPGE₂) and to unmodulated KL cells isolated from the bone marrow of normoglycemic NOD mice. (FIG. 19G) Quantitative bar graph of flow cytometric expression of chemokines receptors (CCR2, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR3 and CXCR4) in PGE₂-modulated KL cells from NOD mice as compared to those modulated with a selected PGE₂ clinical grade agonist (dmPGE₂) and compared to unmodulated KL cells isolated from the bone marrow of normoglycemic NOD mice. Data are expressed as mean $\pm$ standard error of the mean (SEM). Data are representative of at least two independent experiments. * P <0.05; ** P <0.01; *** P <0.001. Abbreviations: KL, Lineage⁻c-kit⁺ cells.

[0090] FIGs 20A-20E show the effects of murine KL cells. (FIG. 20A-20B) Representative flow cytometric analysis (FIG. 20A) and quantitative bar graph (FIG. 20B) for IFN- γ ⁺CD4⁺T cells isolated from NOD-BDC2.5 TCR Tg mice and stimulated with BDC2.5 peptide in the presence of DCs (Control) or upon co-culture with unmodulated KL or with PGE₂-modulated KL cells (at different ratios). (FIG. 20C) Quantitative bar graph of the percentage of CD4⁺T cells upon coculture with KL or PGE₂-modulated KL cells. (FIG. 20D) Representative flow cytometric analysis of GFP⁺PGE₂-modulated KL cells in the PLN of treated NOD mice following 24 hours of treatment with GFP⁺PGE₂-modulated KL cells, demonstrating that they traffic to the PLN following adoptive transfer into NOD mice. (FIG. 20E) Representative flow cytometric analysis of GFP⁺PGE₂-modulated KL cells in the pancreas of NOD mice 24 hours post-infusion with GFP⁺PGE₂-modulated KL cells, demonstrating the surface phenotype of GFP⁺ cells. Abbreviations: KL, Lineage⁻c-kit⁺ cells; PGE₂-KL, PGE₂-modulated KL cells; HSPCs, hematopoietic stem and progenitor cells; GFP, green fluorescent protein.

[0091] FIGs 21A-21D show PD-1 expression on murine T cells. (FIG. 21A-21B) Representative flow cytometric analysis (FIG. 21A) and quantitative bar graph (FIG. 21B) for PD-1⁺CD4⁺

T cells isolated from splenocytes of NOD mice as compared those from C57BL/6 mice. (FIG. 21C-21D) Representative flow cytometric analysis (FIG. 21C) and quantitative bar graph (FIG. 21D) for PD-1⁺CD8⁺ T cells isolated from splenocytes of NOD mice as compared those from C57BL/6 mice.

[0092] FIGs 22A-22D show targeting miRNAs downregulates PD-L1 in human cancer MDA-MB-231 cells. (FIG. 22A) Changes in miRNA-targeted genes levels as an effect of miRNA targeting in MDA-MB-231 cells; each targeted gene is indicated along with its targeting miRNA in parenthesis. (FIG. 22B) Bar graph depicting mRNA expression of PD-L1 in miRNA mimic- and anti-miR-treated cells by real-time PCR. Data are normalized by GAPDH mRNA levels. (FIG. 22C) Representative western blot pattern of MDA-MB-231 cells treated with the shown miRNA mimic or anti-miR. (FIG. 22D) Quantitative bar graphs of western blotting on miRNA mimic- and anti-miR-treated cells. All data in FIG. 22A, 22B, and 22D are normalized to the corresponding RNA mimic or anti-miR negative control. Abbreviations: Mimic NC, miRNA mimic negative control; Anti-miR NC, Anti-miR negative control.

[0093] FIGs 23A-23D show targeting miRNAs decrease the proportion of PD-L1-expressing MDA-MB-231 cells. (FIG. 23A and 23C) FACS histograms showing a lower number of cells expressing PD-L1 in miRNA mimic (FIG. 23A) or anti-miR (FIG. 23B) treated cells as compared to their negative controls). The percentage of untreated MDA-MB-231 cells expressing PD-L1 is also shown. (FIG. 23B and 23C) Bar graph representing the percentage of cells expressing PD-L1 in the untreated sample, or in cells exposed to a miRNA mimic (FIG. 23B) or anti-miR (FIG. 23D) treatment. Abbreviations: Mimic NC, miRNA mimic negative control; Anti-miR NC, Anti-miR negative control.

DETAILED DESCRIPTION

[0094] The present invention relates to methods and compositions directed at modulating a hematopoietic stem cell, or a population thereof, and uses thereof for the purpose of, e.g., treating and/or preventing a disease or disorder (e.g., an autoimmune disease or cancer), inducing an immune response, etc.

[0095] The present invention is based in part on the discovery that hematopoietic stem cells obtained from mice models of type 1 diabetes have a deficit of PD-L1 expression compared to control mice. Data presented herein show that hematopoietic stem cells overexpressing PD-L1 (PD-L1.Tg HSPCs) inhibited the autoimmune response in *in vitro* assays. In addition, transplantation of PD-L1.Tg HSPCs was found to be capable of reverting the onset of diabetes in newly hyperglycemic NOD mice *in vivo*, and PD-L1.Tg HSPCs homed to the pancreas of hyperglycemic NOD mice.

[0096] These findings were confirmed in human patient having a diagnosis of type 1 diabetes. Hematopoietic stem cells obtained from subjects having type 1 diabetes also have a deficit of PD-L1 expression compared to hematopoietic stem cells obtained from a healthy subject, or a subject who has not been diagnosed with type 1 diabetes. Modified hematopoietic stem cells that were pharmacologically modulated to have increased expression of PD-L1 were able to inhibit the autoimmune response *in vitro*. It

is specifically contemplated herein that compositions and cell populations comprising modified, PD-L1 expressing hematopoietic stem cells can be used in the treatment, prevention, or the delay of onset of an autoimmune disease, such as type 1 diabetes.

[0097] Other embodiments of the invention described herein are directed to the use of compositions and modified hematopoietic stem cell populations described herein for reducing the immune response in a subject, and in the prevention and delay of an allogenic tissue or organ transplant rejection.

[0098] The disclosure described herein, in a preferred embodiment, does not concern a process for cloning human beings, processes for modifying the germ line genetic identity of human beings, uses of human embryos for industrial or commercial purposes or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes.

[0099] It is also envisioned that the methods described herein can be used as prophylaxis treatment, e.g., to prevent the onset of an autoimmune disease, such as type 1 diabetes.

Hematopoietic stem cells

[0001] Methods and compositions described herein comprise the use of modified hematopoietic stem cells. Hematopoietic tissues contain cells with long-term and short-term regeneration capacities, and committed multipotent, oligopotent, and unipotent progenitors. Endogenous hematopoietic stem cells can be found in a variety of tissue sources, such as the bone marrow of adults, which includes femurs, hip, ribs, sternum, and other bones, as well as umbilical cord blood and placenta, and mobilized peripheral blood. Endogenous hematopoietic stem cells can be obtained directly by removal from, for example, the hip, using a needle and syringe, or from the blood following pre-treatment with cytokines, such as G-CSF (granulocyte colony-stimulating factors), that induce cells to be released from the bone marrow compartment. However, such methods yield varying amounts of hematopoietic stem cells, which are oftentimes not enough for use in treatment options.

[0100] Accordingly, “hematopoietic stem cells,” as the terms are used herein, encompass all multipotent cells capable of differentiating into all the blood or immune cell types of the hematopoietic system, including, but not limited to, myeloid cells (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NKT-cells, NK-cells), and which have multi-lineage hematopoietic differentiation potential and sustained self-renewal activity.

[0101] In one embodiment, a hematopoietic stem cell is a mammalian hematopoietic stem cell. In one embodiment, a mammalian hematopoietic stem cell is a human hematopoietic stem cell.

[0102] In one embodiment, a hematopoietic stem cell is obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood, peripheral blood, or mobilized peripheral blood. Methods for obtaining hematopoietic stem cells are known in the art.

[0103] In one embodiment, a hematopoietic stem cell is obtained from a healthy individual, an individual with a diagnosed disease or disorder, or an individual with a diagnosed autoimmune disease or disorder, e.g., type 1 diabetes.

[0104] In various aspects of the invention, modified hematopoietic stem cells are administered to a recipient subject in need thereof. In one embodiment, the hematopoietic stem cells are autologous to the recipient subject. As used herein, “autologous” refers to a hematopoietic stem cell obtained from the same subject, e.g., the recipient subject.

[0105] In one embodiment, the hematopoietic stem cells are non-autologous and allogenic to the recipient subject. In one embodiment, the hematopoietic stem cells are non-autologous and xenogeneic to the recipient subject. As used herein, “non-autologous and allogenic” refers to a hematopoietic stem cell obtained from a different subject, e.g., not the recipient subject, that is a genetic match for the recipient subject. As used herein, “non-autologous and xenogeneic” refers to a hematopoietic stem cell obtained from a different subject, e.g., not the recipient subject, that is a not the same species as the recipient subject.

Populations of modified, PDL1+ expressing hematopoietic stem cells

[0106] One aspect of the invention is a cell population comprising any of the modified hematopoietic stem cells described herein. In various aspects, the hematopoietic stem cells are PD-L1 expressing hematopoietic stem cells. Programmed death-ligand 1 (PD-L1 is a transmembrane protein that functions to suppress the immune system in particular events such as pregnancy, tissue allografts, autoimmune disease, and hepatitis. Binding of PD-L1 to its receptor programmed death-1 (PD-1) transmits an inhibitory signal that reduces the proliferation of T cells and can induce apoptosis.

[0107] In one embodiment, the expression level of PD-L1 in a modified, PD-L1 expressing hematopoietic stem cell is increased by at least 2-fold, by at least 3-fold, by at least 4-fold, by at least 5-fold, by at least 6-fold, by at least 7-fold, by at least 8-fold, by at least 9-fold, by at least 10-fold or more as compared to an appropriate control, or by at least 10%, by at least 20%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, by at least 100% or more as compared to a control, non-modified hematopoietic stem cell. The expression level of PD-L1 on hematopoietic stem cells can be detected by assessing the protein or mRNA of PD-L1 on an isolated population of hematopoietic stem cells, e.g., via western blotting or PCR-based assays (e.g., qPCR), respectively.

[0108] A hematopoietic stem cell, or population thereof, can be isolated via, e.g., using flow cytometry to determine if a hematopoietic stem cell -specific marker is present or absent. Non-limiting examples of markers specific for human hematopoietic stem cell-specificity include cKit/CD117-positive, CD34-positive, CD59- positive, CD38-negative, and Thy1/CD90-positive. Hematopoietic stem cell lack expression of mature blood cell markers and are thus called Lin-negative.

[0109] One aspect of the invention is an *ex vivo* method of producing a population of modified, PD-L1+ expressing hematopoietic stem cells comprising modulating the expression of at least a miRNA that controls the expression of PD-L1 in the hematopoietic stem cell. In one embodiment, the modulation of the expression of miRNAs is increasing the expression of miRNA or decreasing the expression of miRNA.

[0110] Another aspect of the invention is a population of modified hematopoietic stem cells (HSCs) in which the modified cells have increased PD-L1 expression compared to control, non-modified cells, wherein the cells carry an exogenous copy of a nucleic acid encoding a miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.

[0111] In one embodiment, the hematopoietic stem cells are *ex vivo* cultured before, or after, or both before and after, the modification of the PD-L1 expression.

[0112] In one embodiment, the hematopoietic stem cells are cryopreserved prior to, or after, or both prior to and after, the modification of the PD-L1 expression. In one embodiment, the hematopoietic stem cells are cryopreserved prior to use (e.g., to be modified, or to be administered to a subject). Methods for cryopreservation are known in the art and can be performed by a skilled practitioner. Cryopreserved hematopoietic stem cells maintain their function and pluripotency.

[0113] In one embodiment, the hematopoietic stem cells comprised in the population of hematopoietic stem cells are produced by a method comprising: (a) contacting a sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA; (b) *ex vivo* culturing the resultant modified cells from the contacting; and (c) establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified hematopoietic stem cell expressing PD-L1.

[0114] Another aspect of the invention is a population of any of the PD-L1 expressing hematopoietic stem cells described herein for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the

onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0115] Yet another aspect of the invention described herein provides a population of any of the PD-L1 expressing hematopoietic stem cells described herein for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0002] In one embodiment, a population described herein is a pure population. Used in this context, “pure” refers to a population of cells substantially similar consisting of a single cell type. The cell population, in one embodiment can be mixed, including a hematopoietic stem cells and a second, different cell type. A pure cell population can be isolated using standard techniques known in the art.

miRNA

[0116] Methods and compositions described herein comprise modulating the expression of miRNAs that control the expression of PD-L1. microRNAs are small non-coding RNAs with an average length of 22 nucleotides. These molecules act by binding to complementary sequences within mRNA molecules, usually in the 3' untranslated (3'UTR) region, thereby promoting target mRNA degradation or inhibited mRNA translation. The interaction between microRNA and mRNAs is mediated by what is known as the “seed sequence”, a 6–8-nucleotide region of the microRNA that directs sequence-specific binding to the mRNA through imperfect Watson–Crick base pairing. More than 900 microRNAs are known to be expressed in mammals. Many of these can be grouped into families on the basis of their seed sequence, thereby identifying a “cluster” of similar microRNAs. A miRNA can be expressed in a cell, e.g., as naked DNA. A miRNA can be encoded by a nucleic acid that is expressed in the cell, e.g., as naked DNA or can be encoded by a nucleic acid that is contained within a vector. A miRNA can be an endogenous miRNA or an artificial miRNA.

[0117] In one embodiment, the miRNA that modulates expression of PD-L1 is miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, or miR-374c-5p.

[0118] In one embodiment, miRNA expression is increased, e.g., by introducing an exogenous copy of a nucleic acid encoding the miRNA (e.g., a nucleic acid encoding miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, or miR-374c-5p). In one embodiment, introducing a nucleic acid encoding the miRNA results in the expression of the miRNA in the cell.

[0119] In one embodiment, the level of the miRNA is increased by at least 2-fold, by at least 3-fold, by at least 4-fold, by at least 5-fold, by at least 6-fold, by at least 7-fold, by at least 8-fold, by at

least 9-fold, by at least 10-fold or more as compared to an appropriate control, or by at least 10%, by at least 20%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, by at least 100% or more as compared to an appropriate control. As used herein, an “appropriate control” refers to a similarly or identically treated cell or population thereof that an exogenous miRNA is not introduced to. Levels of a miRNA can be measured, e.g., by measuring the miRNA in a total RNA sample via, e.g., microarray or PCR-based screening (e.g., quantitative RT-CPR (q-PCR)).

[0120] In one embodiment, a modified, PD-L1+ expressing HSCs carries an exogenous copy of a nucleic acid encoding a miRNA selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

[0121] In one embodiment, the miRNA is decreased by an agent that inhibits expression the miRNA. An agent can be an antagomir of the miRNA, an anti-miRNA oligonucleotide that binds the miRNA, an antisense oligonucleotide to the miRNA, or a locked nucleic acid that anneals the miRNA.

[0122] As used herein, an “antagomir” refers to a small synthetic RNA having complementarity to a specific microRNA target (e.g., a miRNA that controls PD-L1 expression), with either mispairing at the cleavage site or one or more base modifications to inhibit cleavage. Antagomirs are single stranded, double stranded, partially double stranded and hairpin structured chemically modified oligonucleotides that target a microRNA. Preferably, an antagomir featured in the invention includes a nucleotide sequence sufficiently complementary to hybridize to a miRNA target sequence of about 12 to 25 nucleotides, preferably about 15 to 23 nucleotides

[0123] As used herein, an “antisense oligonucleotide” refers to a synthesized nucleic acid sequence that is complementary to a DNA or mRNA sequence, such as that of a microRNA. Antisense oligonucleotides are typically designed to block expression of a DNA or RNA target by binding to the target and halting expression at the level of transcription, translation, or splicing. Antisense oligonucleotides of the present invention are complementary nucleic acid sequences designed to hybridize under cellular conditions to a given target (e.g., a miRNA to be decreased). Thus, oligonucleotides are chosen that are sufficiently complementary to the target, i.e., that hybridize sufficiently well and with sufficient specificity in the context of the cellular environment, to give the desired effect.

[0124] As used herein, a “locked nucleic acid” refers to a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively “locks” the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to, e.g., siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. et al., (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. et al., (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. et al., (2003) *Nucleic Acids Research* 31(12):3185-3193).

[0003] In another embodiment, an “agent” can be any chemical, entity or moiety, including without limitation synthetic and naturally-occurring proteinaceous and non-proteinaceous entities. In some embodiments, an agent is nucleic acid, nucleic acid analogues, proteins, antibodies, peptides, aptamers, oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNAzymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof etc. In certain embodiments, agents are small molecule having a chemical moiety. For example, chemical moieties included unsubstituted or substituted alkyl, aromatic, or heterocyclyl moieties including macrolides, leptomycins and related natural products or analogues thereof. Compounds can be known to have a desired activity and/or property, or can be selected from a library of diverse compounds.

[0004] Such an agent can take the form of any entity which is normally not present or not present at the levels being administered in the cell. Agents such as chemicals; small molecules; nucleic acid sequences; nucleic acid analogues; proteins; peptides; aptamers; antibodies; or fragments thereof, can be identified or generated for use to downmodulate or upmodulate SIRT1 or SIRT2.

[0005] Agents in the form of nucleic acid sequences designed to specifically inhibit miRNA expression are particularly useful. Such a nucleic acid sequence can be RNA or DNA, and can be single or double stranded, and can be selected from a group comprising; nucleic acid encoding a protein of interest, oligonucleotides, nucleic acid analogues, for example peptide-nucleic acid (PNA), pseudo-complementary PNA (pc-PNA), etc. Such nucleic acid sequences include, for example, but are not limited to, nucleic acid sequence encoding proteins, for example that act as transcriptional repressors, antisense molecules, ribozymes, small inhibitory nucleic acid sequences, for example but are not limited to RNA interference, short hairpin RNA, silencing RNA, etc.

[0006] The agent can be a molecule from one or more chemical classes, e.g., organic molecules, which may include organometallic molecules, inorganic molecules, genetic sequences, etc. Agents may also be fusion proteins from one or more proteins, chimeric proteins (for example domain switching or homologous recombination of functionally significant regions of related or different molecules), synthetic proteins or other protein variations including substitutions, deletions, insertion and other variants.

[0125] In one embodiment, the level of the miRNA is decreased by at least 10%, by at least 20%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, by at least 100% or more as compared to an appropriate control. As used herein, an “appropriate control” refers to a similarly or identically treated cell or population thereof that an agent is not introduced to. Levels of a miRNA can be measured as described above.

[0126] In one embodiment, a nucleic acid for use of an agent as described herein is comprised within a vector, e.g., a viral vector.

[0127] In the various embodiments described herein, it is further contemplated that variants (naturally occurring or otherwise), alleles, homologs, conservatively modified variants, and/or conservative substitution variants of any of the particular polypeptides described are encompassed. As to amino acid sequences, one of ordinary skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid and retains the desired activity of the polypeptide. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles consistent with the disclosure.

[0128] A given amino acid can be replaced by a residue having similar physiochemical characteristics, e.g., substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, e.g. control of PD-L1 expression.

[0129] Amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); (4) basic: Lys (K), Arg (R), His (H). Alternatively, naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Particular conservative substitutions include, for example; Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

[0130] In some embodiments, a polypeptide described herein (or a nucleic acid encoding such a polypeptide) can be a functional fragment of one of the amino acid sequences described herein. As used herein, a “functional fragment” is a fragment or segment of a peptide which retains at least 50% of the wildtype reference polypeptide’s activity according to an assay known in the art or described below herein. A functional fragment can comprise conservative substitutions of the sequences disclosed herein.

[0131] In some embodiments, a polypeptide described herein can be a variant of a polypeptide or molecule as described herein. In some embodiments, the variant is a conservatively modified variant. Conservative substitution variants can be obtained by mutations of native nucleotide sequences, for example. A "variant," as referred to herein, is a polypeptide substantially homologous to a native or reference polypeptide, but which has an amino acid sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions or substitutions. Variant polypeptide-encoding DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, but that encode a variant protein or fragment thereof that retains activity of the non-variant polypeptide. A wide variety of PCR-based site-specific mutagenesis approaches are known in the art and can be applied by the ordinarily skilled artisan.

[0132] A variant amino acid or DNA sequence can be at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, identical to a native or reference sequence. The degree of homology (percent identity) between a native and a mutant sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g. BLASTp or BLASTn with default settings).

[0133] Alterations of the native amino acid sequence can be accomplished by any of a number of techniques known in the art. Mutations can be introduced, for example, at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites permitting ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered nucleotide sequence having particular codons altered according to the substitution, deletion, or insertion required. Techniques for making such alterations are well established and include, for example, those disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, January 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462, which are herein incorporated by reference in their entireties. Any cysteine residue not involved in maintaining the proper conformation of a polypeptide also can be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) can be added to a polypeptide to improve its stability or facilitate oligomerization.

Introducing a nucleic acid or agent

[0134] Various aspects of the invention described herein comprise introducing a nucleic acid or agent described herein to a cell, e.g., a hematopoietic stem cell, to modulate the expression of a miRNA

that controls expression of PD-L1. Agents that act on the cell internally (e.g., nucleic acid encoding miRNA, or an antagomir) may be introduced in a form readily taken up by the cell when contacted to the cell (e.g., in a formulation which facilitates cellular uptake and delivery to the appropriate subcellular location). In one embodiment, the nucleic acid or agent is in a formulation in which it is readily taken up by the cell so that it can exert its effect. In one embodiment, the nucleic acid or agent is applied to the media, where it contacts the cell and produces its modulatory effects. In one embodiment, a nucleic acid or an agent is introduced to a cell via culturing the cell in a medium comprising the nucleic acid or agent.

[0135] As used herein, “introducing” refers to an effective amount of, e.g., a nucleic acid or an agent, that enters a cell or population thereof, and properly functions, e.g., modulates the expression of a miRNA that controls expression of PD-L1. Delivery can be done using any technique known in the art. Exemplary techniques include, but are not limited to transduction, nucleofection, electroporation, direct injection, or transfection. Effective introducing of a nucleic acid or an agent (e.g., a nucleic acid encoding a miRNA, or an antagomir which targets a miRNA) can be assessed by measuring miRNA levels of the intended miRNA target as described herein above. Effective introducing of an agent can additionally be measured by assessing the biological function of the intended target (e.g., miRNA) of the nucleic acid or agent, e.g., via assessing the expression of PD-L1, e.g., by western blotting to measure its protein levels.

[0136] It is understood that the optimal method for delivery can vary based on the type of agent, and can be determined by a skilled practitioner.

Hematopoietic stem cell transplant

[0137] Methods described herein are directed at transplanting modified, PD-L1 expressing hematopoietic stem cells into a subject. Transplantation of hematopoietic stem cells has become the treatment of choice for a variety of inherited or malignant diseases. The donor and the recipient can be a single individual or different individuals, for example, autologous or allogeneic transplants, respectively. When allogeneic transplantation is practiced, regimes for reducing implant rejection and/or graft vs. host disease, as well known in the art, should be undertaken. Such regimes are currently practiced in human therapy. The cell populations selected can also be depleted of T lymphocytes, which may be useful in the allogeneic and haploidentical transplants setting for reducing graft-versus-host disease.

[0138] Most advanced regimes are disclosed in publications by Slavin S. et al., e.g., J Clin Immunol 2002;22:64, and J Hematother Stem Cell Res 2002; 11:265, Gur H. et al. Blood 2002;99:4174, and Martelli MF et al, Semin Hematol 2002;39:48, which are incorporated herein by reference.

[0139] Methods for administering bone marrow transplants to a subject are known in the art and are described in medical textbooks, e.g., Whedon, M.B. (1991) Whedon, M.B. "Bone Marrow Transplantation: Principles, Practice, and Nursing Insights", Boston: Jones and Bartlett Publishers. Bone marrow cells from a healthy patient can be removed, preserved, and then replicated and re-infused should the patient develop an illness which either destroys the bone marrow directly or whose treatment

adversely affects the marrow. If the patient is receiving his or her own cells, this is called an autologous transplant; such a transplant has little likelihood of rejection.

[0140] Exemplary methods of administering stem cells to a subject, particularly a human subject, include injection or transplantation of the cells into target sites in the subject. The induced HSCs can be inserted into a delivery device which facilitates introduction, by injection or transplantation, of the cells into the subject. Such delivery devices include tubes, e.g., catheters, for injecting cells and fluids into the body of a recipient subject. The tubes additionally have a needle, e.g., a syringe, through which the cells of the invention can be introduced into the subject at a desired location. The stem cells can be inserted into such a delivery device, e.g., a syringe, in different forms. For example, the cells can be suspended in a solution, or alternatively embedded in a support matrix when contained in such a delivery device.

[0141] As used herein, the term "solution" includes a pharmaceutically acceptable carrier or diluent in which the cells of the invention remain viable. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is known in the art. The solution is preferably sterile and fluid to the extent that easy syringability exists.

[0142] Preferably, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. Solutions of the invention can be prepared by incorporating stem cells as described herein in a pharmaceutically acceptable carrier or diluent and, as required, other ingredients enumerated above, followed by filtered sterilization.

Autoimmune disease or disorder

[0143] One aspect of the invention is a method of treating Type 1 Diabetes or an immune disease or disorder or cancer treatment in a host in need thereof comprising administering or *ex vivo* contacting an effective amount of a nucleic acid or an agent that modulates the expression of miRNAs controlling the expression of PD-L1 in the HSCs in a cell to a host.

[0144] In one embodiment, the cell is a progenitor cell. In another embodiment, the cell is a hematopoietic progenitor cell.

[0145] In one embodiment of any aspect, the autoimmune disorder is Type 1 diabetes.

[0146] As used herein, an "autoimmune disease or disorder" is characterized by the inability of one's immune system to distinguish between a foreign cell and a healthy cell. This results in one's immune system targeting one's healthy cells for programmed cell death. In various embodiments, the autoimmune disease is type 1 diabetes. Non-limiting examples of additional autoimmune disease or

disorder include inflammatory arthritis, mellitus, multiples sclerosis, psoriasis, inflammatory bowel diseases, SLE, and vasculitis, allergic inflammation, such as allergic asthma, atopic dermatitis, and contact hypersensitivity, rheumatoid arthritis, multiple sclerosis (MS), systemic lupus erythematosus, Graves' disease (overactive thyroid), Hashimoto's thyroiditis (underactive thyroid), chronic graft v. host disease, hemophilia with antibodies to coagulation factors, celiac disease, Crohn's disease and ulcerative colitis, Guillain-Barre syndrome, primary biliary sclerosis/cirrhosis, sclerosing cholangitis, autoimmune hepatitis, Raynaud's phenomenon, scleroderma, Sjogren's syndrome, Goodpasture's syndrome, Wegener's granulomatosis, polymyalgia rheumatica, temporal arteritis/giant cell arteritis, chronic fatigue syndrome (CFS), psoriasis, autoimmune Addison's Disease, ankylosing spondylitis, Acute disseminated encephalomyelitis, antiphospholipid antibody syndrome, aplastic anemia, idiopathic thrombocytopenic purpura, Myasthenia gravis, opsoclonus myoclonus syndrome, optic neuritis, Ord's thyroiditis, pemphigus, pernicious anaemia, polyarthritis in dogs, Reiter's syndrome, Takayasu's arteritis, warm autoimmune hemolytic anemia, Wegener's granulomatosis and fibromyalgia (FM).

[0147] In one embodiment, the hematopoietic stem cells described herein are co-administered with at least one additional autoimmune disease or disorder therapy.

Cancer treatment

[0148] Another aspect of the invention is a method of treating an autoimmune disorder or for cancer immune therapy (e.g., cancer therapy) in a subject in need thereof comprising administering to a subject a composition comprising any of the hematopoietic stem cells described herein. In one embodiment, the cancer is a carcinoma, a melanoma, a sarcoma, a myeloma, a leukemia, or a lymphoma. As used herein, “cancer” refers to a hyperproliferation of cells that have lost normal cellular control, resulting in unregulated growth, lack of differentiation, local tissue invasion, and metastasis. Cancers are classified based on the histological type (e.g., the tissue in which they originate) and their primary site (e.g., the location of the body the cancer first develops), and can be carcinoma, a melanoma, a sarcoma, a myeloma, a leukemia, or a lymphoma. “Cancer” can also refer to a solid tumor. As used herein, the term “tumor” refers to an abnormal growth of cells or tissues, e.g., of malignant type or benign type. “Cancer” can be metastatic, meaning the cancer cells have disseminated from its primary site of origin and migrated to a secondary site.

[0149] In one embodiment, the hematopoietic stem cells described herein are co-administered with at least one other anti-cancer therapy. Exemplary anti-cancer therapies include chemotherapy, radiation therapy, chemo-radiation therapy, immunotherapy, hormone therapy, and stem cell therapy. In one embodiment of any aspect described herein, the immunotherapy is a tumor vaccine, a chimeric antigen receptor T cell (CAR T cell), an adoptive T cell therapy (e.g., adoptive CD4⁺ or CD8⁺ effector T cell therapy), an adoptive natural killer (NK) cell therapy, or an adoptive NK T cell therapy. In one embodiment, the anticancer treatment is an antagomir or a miRNA mimic described herein. In one

embodiment, the antagomir or a miRNA mimic described herein are delivered via injection. In one embodiment, the antagomir or a miRNA mimic is selected from Table 12. In one embodiment, antagomir or a miRNA mimic has a sequence selected from Table 12. In one embodiment, antagomir or a miRNA mimic consists of or consists essentially of a sequence selected from Table 12.

Modulating an immune response

[0150] One aspect of the invention is a method of modulating an immune response (e.g., for autoimmune disease, or for cancer immune therapy) in a subject comprising: (a) providing a population of hematopoietic stem cells (HSCs); (b) contacting sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA; (c) transplanting said population of PD-L1+ expressing HSCs into a recipient subject, thereby modulating the immune response in the recipient subject.

[0151] Another aspect of the invention is a method of modulating an immune response in a subject comprising: (a) providing a population of hematopoietic stem cells (HSCs); (b) contacting sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA; (c) *ex vivo* culturing the resultant modified cells from the contacting; (d) establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1; and (e) transplanting said population of PD-L1+ expressing HSCs into a recipient subject, thereby modulating the immune response in the recipient subject.

[0152] As used herein, “modulating an immune response” can be increasing an immune response, or decreasing an immune response. An immune response can be, for example, raising antibodies to the population of PD-L1+ expressing HSCs or provoking an allergic or inflammatory response. One of skilled in the art would know how to determine if any given population of PD-L1+ expressing HSCs provokes such a response.

Compositions

[0153] One aspect of the invention is a composition comprising any of the modified hematopoietic stem cells described herein. In another aspect is a composition comprising any of the populations of modified hematopoietic stem cells described herein.

[0154] One aspect of the invention is a composition comprising any of the PD-L1 expressing hematopoietic stem cells described herein in the prevention or treatment of an autoimmune disease or

disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0155] Another aspect of the invention is a composition comprising any of the PD-L1 expressing hematopoietic stem cells described herein for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0156] Yet another aspect of the invention is a population of any of the PD-L1 expressing hematopoietic stem cells described herein for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

Administration

[0157] Methods and compositions described herein are directed at the treatment or prevention of an autoimmune disease or disorder, e.g., type I diabetes. In one embodiment, the modified hematopoietic stem cells described herein are administered to a subject having a diagnosed autoimmune disease to treat the disease in the subject. In one embodiment, the modified hematopoietic stem cells described herein are administered to a subject at risk of having a diagnosed autoimmune disease to prevent the disease in the subject.

[0158] Methods and compositions described herein are directed at the treatment or prevention of cancer, e.g., as a cancer immune therapy. In one embodiment, the modified hematopoietic stem cells described herein are administered to a subject having a cancer to treat the disease in the subject. In one embodiment, the modified hematopoietic stem cells described herein are administered to a subject at risk of having cancer to prevent the disease in the subject. In one embodiment, the modified hematopoietic stem cells described herein may be administered as part of a bone marrow or cord blood transplant in an individual that has or has not undergone bone marrow ablative therapy. In one embodiment, the modified hematopoietic stem cells described herein are administered in a bone marrow transplant to an individual that has undergone chemoablative or radioablative bone marrow therapy.

[0159] In one embodiment, a dose of modified hematopoietic stem cells is delivered to a subject intravenously. In one embodiment, modified hematopoietic stem cells are intravenously administered to a subject.

[0160] In particular embodiments, subjects receive a dose of modified hematopoietic stem cells of about 1×10^5 cells/kg, about 5×10^5 cells/kg, about 1×10^6 cells/kg, about 2×10^6 cells/kg, about 3×10^6 cells/kg, about 4×10^6 cells/kg, about 5×10^6 cells/kg, about 6×10^6 cells/kg, about 7×10^6 cells/kg, about 8×10^6 cells/kg, about 9×10^6 cells/kg, about 1×10^7 cells/kg, about 5×10^7 cells/kg, about 1×10^8 cells/kg, or more in one single intravenous dose. In certain embodiments, patients receive a dose of genetically modified cells, e.g., hematopoietic stem cells, of at least 1×10^5 cells/kg, at least 5×10^5 cells/kg, at least 1×10^6 cells/kg, at least 2×10^6 cells/kg, at least 3×10^6 cells/kg, at least 4×10^6 cells/kg, at least 5×10^6 cells/kg, at least 6×10^6 cells/kg, at least 7×10^6 cells/kg, at least 8×10^6 cells/kg, at least 9×10^6 cells/kg, at least 1×10^7 cells/kg, at least 5×10^7 cells/kg, at least 1×10^8 cells/kg, or more in one single intravenous dose.

[0161] In an additional embodiment, subjects receive a dose of modified hematopoietic stem cells of about 1×10^5 cells/kg to about 1×10^8 cells/kg, about 1×10^6 cells/kg to about 1×10^8 cells/kg, about 1×10^6 cells/kg to about 9×10^6 cells/kg, about 2×10^6 cells/kg to about 8×10^6 cells/kg, about 2×10^6 cells/kg to about 8×10^6 cells/kg, about 2×10^6 cells/kg to about 5×10^6 cells/kg, about 3×10^6 cells/kg to about 5×10^6 cells/kg, about 3×10^6 cells/kg to about 4×10^8 cells/kg, or any intervening dose of cells/kg.

[0162] In various embodiments, the methods of the invention provide more robust and safe gene therapy than existing methods and comprise administering a population or dose of modified hematopoietic stem cells comprising about 5% transduced cells, about 10% transduced cells, about 15% transduced cells, about 20% transduced cells, about 25% transduced cells, about 30% transduced cells, about 35% transduced cells, about 40% transduced cells, about 45% transduced cells, or about 50% transduced cells, to a subject.

[0163] In some embodiment, the administered hematopoietic stem cell differentiates into a blood cell following transplantation into a subject. In some embodiments of all aspects, the HSC is committed to the blood lineage following transplantation into a subject. Differentiation of HSCs to fully differentiated blood cells is believed to be an irreversible process under normal physiological conditions. Hematopoietic lineage specification takes place within the bounds of strict lineal relationships: for example, megakaryocyte progenitors give rise to megakaryocytes and ultimately platelets, but not to any other blood lineages. A HSC can differentiate into all blood cell types. Non-limiting examples of blood cells that a HSC can differentiate into include a myeloid progenitor, a lymphoid progenitor, a megakaroblast, a promegakarocyte, a megakaryocyte, a thrombocyte, a proerythroblast, a basophilic erythroblast, a polychromatic erythroblast, an orthochromatic erythroblast, a polychromatic erythrocyte, an erythrocyte, a myeloblast, a B. promyelocyte, a B. myelocyte, a B. metamyelocyte, a B. band, a Basophil, a N. promyelocyte, a N. myelocyte, a N. metamyelocyte, a N. band, a neutrophil, an E. promyelocyte, an E. myelocyte, an E. metamyelocyte, an E. band, an eosinophil, a monoblast, a promonocyte, a monocyte,

a macrophage, a myeloid dendritic cell, a lymphoblast, a prolymphocyte, a small lymphocyte, a B lymphocyte, a T lymphocyte, a plasma cell, a large granular lymphocyte, and a lymphoid dendritic cell.

[0164] Various studies have reported successful mammalian dosing using complementary nucleic acid sequences. For example, Esau C., et al., (2006) *Cell Metabolism*, 3(2):87-98 reported dosing of normal mice with intraperitoneal doses of miR-122 antisense oligonucleotide ranging from 12.5 to 75 mg/kg twice weekly for 4 weeks. The mice appeared healthy and normal at the end of treatment, with no loss of body weight or reduced food intake. Plasma transaminase levels were in the normal range (AST $\frac{3}{4}$ 45, ALT $\frac{3}{4}$ 35) for all doses with the exception of the 75 mg/kg dose of miR-122 ASO, which showed a very mild increase in ALT and AST levels. They concluded that 50mg/kg was an effective, non-toxic dose. Another study by Krützfeldt J., et al., (2005) *Nature* 438, 685-689, injected anatgomirs to silence miR-122 in mice using a total dose of 80, 160 or 240 mg per kg body weight. The highest dose resulted in a complete loss of miR-122 signal. In yet another study, locked nucleic acids ("LNAs") were successfully applied in primates to silence miR-122. Elmen J., et al., (2008) *Nature* 452, 896-899, report that efficient silencing of miR-122 was achieved in primates by three doses of 10 mg kg⁻¹ LNA-antimiR, leading to a long-lasting and reversible decrease in total plasma cholesterol without any evidence for LNA-associated toxicities or histopathological changes in the study animals.

[0007] In various embodiments, the modified hematopoietic stem cells described herein are administered in combination with at least one additional therapeutic (e.g., an autoimmune therapy, or an anti-cancer therapy). Administered "in combination," as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder (e.g., an autoimmune disease, or cancer) and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered. The treatment described herein (e.g., modified hematopoietic stem cells described herein, or compositions comprising modified hematopoietic stem cells described herein) and the at least one

additional therapy can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the treatment described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed. The treatment described herein and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The treatment described herein can be administered before another treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

Formulation

[0165] Therapeutic compositions or pharmaceutical compositions can be formulated for passage through the blood-brain barrier or direct contact with the endothelium. In some embodiments, the compositions can be formulated for systemic delivery. In some embodiments, the compositions can be formulated for delivery to specific organs, for example but not limited to the liver, spleen, the bone marrow, and the skin. Therapeutic compositions or pharmaceutical compositions can be formulated for aerosol application by inhalation the lung. Alternatively, the therapeutic compositions or pharmaceutical compositions can also be formulated for a transdermal delivery, e. g. a skin patch. Therapeutic compositions or pharmaceutical compositions can be enteric coated and formulated for oral delivery. Therapeutic compositions or pharmaceutical compositions can be encapsulated in liposomes or nanoparticles and formulated for slow sustained delivery *in vivo*. Alternatively, the therapeutic compositions or pharmaceutical compositions can be formulated for targeted delivery, eg., encapsulated in liposomes or nanoparticles that are designed and feature targeting moiety to on the liposomes or nanoparticles.

[0166] The modified hematopoietic stem cells, and the compositions described herein can be administered by any known route. By way of example, the modified hematopoietic stem cells and the compositions described herein can be administered by a mucosal, pulmonary, topical, or other localized or systemic route (e.g., enteral and parenteral). The modified hematopoietic stem cells may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

[0167] Routes of administration include, but are not limited to aerosol, direct injection, intradermal, transdermal (e.g., in slow release polymers), intravitreal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, topical, oral, transmucosal, buccal, rectal, vaginal, transdermal, intranasal and parenteral routes. "Parenteral" refers to a route of administration that is generally associated with injection, including but not limited to intraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intrahepatic, intrarogan, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular,

subcutaneous, transmucosal, or transtracheal. Any other therapeutically efficacious route of administration can be used, for example, infusion or bolus injection, absorption through epithelial or mucocutaneous linings, or by gene therapy wherein a DNA molecule encoding the therapeutic protein or peptide is administered to the patient, e.g., via a vector, which causes the protein or peptide to be expressed and secreted at therapeutic levels *in vivo*. In various embodiments, administration can be inhaled in to the lung via aerosol administration, e.g. with nebulization. Administration also can be systemic or local. Intratumoral delivery is also included.

[0168] For example, the modified hematopoietic stem cells can be administered as a formulation adapted for passage through the blood-brain barrier or direct contact with the endothelium. In some embodiments, the modified hematopoietic stem cells can be administered as a formulation adapted for systemic delivery. In some embodiments, the modified hematopoietic stem cells can be administered as a formulation adapted for delivery to specific organs, for example but not limited to the liver, spleen, the bone marrow, and the skin.

[0169] In addition, the modified hematopoietic stem cells described herein can be administered together with other components of biologically active agents, such as pharmaceutically acceptable surfactants (e.g., glycerides), excipients (e.g., lactose), carriers, diluents and vehicles.

[0170] The modified hematopoietic stem cells described herein can be administered therapeutically to a subject prior to, simultaneously with (in the same or different compositions) or sequentially with the administration of at least one other cancer therapy. For example, the additional cancer therapy is radiation or chemotherapy or proton therapy. The modified hematopoietic stem cells described herein can be administered as adjunctive and/or concomitant therapy to a cancer therapy.

[0171] For parenteral (e.g., intravenous, subcutaneous, intramuscular) administration, modified hematopoietic stem cells described herein can be 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 non-aqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can 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.

[0172] The dosage administered to a subject will vary depending upon a variety of factors, including the pharmacodynamic characteristics of the particular antagonists, and its mode and route of administration; size, age, sex, health, body weight and diet of the recipient; nature and extent of symptoms of the disease being treated, kind of concurrent treatment, frequency of treatment, and the effect desired.

[0173] Usually a daily dosage of active ingredient can be about 0.01 to 500 milligrams per kilogram of body weight. Ordinarily 1 to 40 milligrams per kilogram per day given in divided doses 1 to

6 times a day or in sustained release form is effective to obtain desired results. The active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition. Second or subsequent administrations can be administered at a dosage which is the same, less than or greater than the initial or previous dose administered to the individual.

[0174] A second or subsequent administration is preferably during or immediately prior to relapse or a flare-up of the disease or symptoms of the disease, e.g., an autoimmune disease. For example, second and subsequent administrations can be given between about one day to 30 weeks from the previous administration. Two, three, four or more total administrations can be delivered to the individual, as needed.

[0175] The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, e.g., an autoimmune disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0176] Efficacy testing can be performed during the course of treatment using the methods described herein. Measurements of the degree of severity of a number of symptoms associated with a particular disease or disorder, e.g., an autoimmune disease, are noted prior to the start of a treatment and then at later specific time period after the start of the treatment.

[0177] For example, when treating an autoimmune disease such as type 1 diabetes, the unintentional weight loss, frequent urination, and blurred vision are symptoms that, e.g., occur at the onset of disease. Unintentional weight loss, frequent urination, and blurred vision are noted before and after a treatment. The severity of unintentional weight loss, frequent urination, and blurred vision after the treatment are compared to those before the treatment. A decrease in the unintentional weight loss, frequent urination, and blurred vision indicate that the treatment is effective in reducing the severity of the disease, thereby decreasing unintentional weight loss, frequent urination, and blurred vision.

[0178] The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, e.g., an autoimmune disease, previous treatments, the general health and/or age of the subject, and other diseases present. The dose levels can also depend on whether modified hematopoietic stem cells encompassed by the disclosure can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as known in the art, or as described herein. Preferred dosages for a modified hematopoietic stem cells are readily determinable by those of skill in the art by a variety of means.

[0179] This invention is further illustrated by the following example which should not be construed as limiting. The contents of all references cited throughout this application, as well as the figures and table are incorporated herein by reference.

[0180] Those skilled in the art will recognize, or be able to ascertain using not more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[0181] All patents and publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0182] The present invention can be defined in any of the following numbered paragraphs:

1. An ex vivo method of producing a population of modified, PD-L1+ expressing hematopoietic stem cells (HSCs), the method comprising modulating the expression of miRNAs controlling the expression of PD-L1 in the HSCs.
2. The method of paragraph 1, wherein the modulation of the expression of miRNAs is increasing the expression of miRNA or decreasing the expression of miRNA.
3. The method of paragraph 1 or 2, wherein the miRNA expression is increased by introducing an exogenous copy of a nucleic acid encoding the miRNA for the expression of the miRNA in the cell.
4. The method of method of paragraph 1 or 2, wherein the miRNA expression is decreased by an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.
5. The method of paragraph 3, wherein the exogenous copy is introduced by way of a vector, such as a viral vector.
6. The method of paragraph 4, wherein the agent is introduced into the HSC by a vector, such as a viral vector.
7. The method of any of paragraphs 1-6, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.
8. The method of any of paragraphs 1-7, wherein the modified, PD-L1+ expressing HSCs carries an exogenous copy of a nucleic acid encoding a miRNA selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

9. A population of modified hematopoietic stem cells (HSCs) in which the modified cells have increased PD-L1 expression compared to control, non-modified cells, where the cells carry an exogenous copy of a nucleic acid encoding a miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.

10. The population of modified HSCs of paragraph 9, wherein the HSC cells are mammalian HSC cells.

11. The population of modified HSCs of paragraph 10, wherein the mammalian HSC cells are human HSC cells.

12. The population of modified HSCs of any of paragraphs 9-11, wherein prior to the modification, the HSCs are obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood.

13. The population of modified HSCs of any of paragraphs 9-11, wherein the HSCs are obtained from mobilized peripheral blood.

14. The population of modified HSCs of any of paragraphs 9-13, wherein the HSCs are obtained from a healthy individual.

15. The population of modified HSCs of any of paragraphs 9-13, wherein the HSCs are obtained from an individual with a diagnosed disease or disorder.

16. The population of modified HSCs of paragraph 15, wherein the diagnosed disease or disorder is an autoimmune disease or disorder.

17. The population of modified HSCs of paragraph 16, wherein the autoimmune disease or disorder is Type 1 diabetes (T1D).

18. The population of modified HSCs of any of paragraphs 9-17, wherein the HSC cells are ex vivo cultured before or after or both before and after the modification of the PD-L1 expression.

19. The population of modified HSCs of any of paragraphs 9-17, wherein the HSC cells are cryopreserved prior to or after or both prior to and after the modification of the PD-L1 expression.

20. The population of modified HSCs of any of paragraphs 9-17, wherein the modified HSC cells are cryopreserved prior to use.

21. The population of modified HSCs of any of paragraphs 9-20, wherein the HSC cells are produced by a method comprising:

a. contacting a sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA;

- b. ex vivo culturing the resultant modified cells from the contacting; and
- c. establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1.

22. The population of modified HSCs of any paragraphs 9-21, the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

23. The population of modified HSCs of any of paragraphs 9-20 that is produced by the method paragraphs of 1-8.

24. A composition of modified HSCs comprising of HSCs of any of paragraphs 1-23.

25. A method of treating Type 1 Diabetes or an immune disease or disorder or cancer treatment in a host in need thereof, the method comprising administering or ex vivo contacting an effective amount of an agent that modulates the expression of miRNAs controlling the expression of PD-L1 in the HSCs in a cell to a host.

26. The method of paragraph 25, wherein the cell is a progenitor cell.

27. The method of paragraph 26, wherein the progenitor cell is a hematopoietic progenitor cell.

28. The method of paragraph 27, wherein the agent is a vector comprising a nucleic acid sequence that miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

29. The method of paragraph 28, wherein the vector is a virus.

30. A method of treating an autoimmune disorder or for cancer immune therapy (aka cancer therapy) in a subject in need thereof, the method comprising administering to a subject a composition comprising the hematopoietic stem cells in any of the preceding paragraphs.

31. The method of paragraph 30, wherein the autoimmune disorder is Type 1 diabetes (T1D).

32. The method of paragraph 30 or 31, wherein the HSCs are autologous to the recipient subject.

33. The method of paragraph 30 or 31, wherein the HSCs are non-autologous and allogenic to the recipient subject.

34. The method of paragraph 30 or 31, wherein the HSCs are non-autologous and xenogeneic to the recipient subject.

35. A method of modulating an immune response (e.g., for autoimmune disease, or for cancer immune therapy) in a subject comprising:

- a. providing a population of hematopoietic stem cells (HSCs);
- b. contacting sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA;
- c. transplanting said population of PD-L1+ expressing HSCs into a recipient subject, thereby modulating the immune response in the recipient subject.

36. A method of modulating an immune response in a subject comprising:

- a. providing a population of hematopoietic stem cells (HSCs);
- b. contacting sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA;
- c. ex vivo culturing the resultant modified cells from the contacting;
- d. establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1; and
- e. transplanting said population of PD-L1+ expressing HSCs into a recipient subject, thereby modulating the immune response in the recipient subject.

37. The method of paragraph 35 or 36, wherein the population of HSCs is obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood.

38. The method of any of paragraphs 35-37, wherein the population of HSCs is obtained from mobilized peripheral blood.

39. The method of any of paragraphs 35-37, wherein the population of HSCs autologous to the recipient subject.

40. The method of any of paragraphs 35-37, wherein the population of HSCs allogeneic to the recipient subject.

41. The method of any of paragraphs 35-37, wherein the population of HSCs is xenogeneic to the recipient subject.

42. The method of any of paragraphs 35-41, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

43. The method of any of the preceding paragraph, wherein the vector is a virus.

44. A composition comprising the PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

45. A composition comprising the PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

46. A population of PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

47. A population of PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

[0183] The present invention can be further defined in any of the following numbered paragraphs:

1. An ex vivo method of producing a population of modified, PD-L1+ expressing hematopoietic stem cells (HSCs), the method comprising modulating the expression of miRNAs controlling the expression of PD-L1 in the HSCs.
2. The method of paragraph 1, wherein the modulation of the expression of miRNAs is increasing the expression of miRNA or decreasing the expression of miRNA.
3. The method of paragraph 1 or 2, wherein the miRNA expression is increased by introducing an exogenous copy of a nucleic acid encoding the miRNA for the expression of the miRNA in the cell.
4. The method of method of paragraph 1 or 2, wherein the miRNA expression is decreased by an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.

5. The method of paragraph 3, wherein the exogenous copy is introduced by way of a vector, such as a viral vector.
6. The method of paragraph 4, wherein the agent is introduced into the HSC by a vector, such as a viral vector.
7. The method of any of paragraphs 1-6, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.
8. The method of any of paragraphs 1-7, wherein the modified, PD-L1+ expressing HSCs carries an exogenous copy of a nucleic acid encoding a miRNA selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.
9. The method of any of paragraphs 1-7, wherein the agent is as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA
10. A population of modified hematopoietic stem cells (HSCs) in which the modified cells have increased PD-L1 expression, where the cells carry an exogenous copy of a nucleic acid encoding a miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA.
11. The population of modified HSCs of paragraph 10, wherein the agent is an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.
12. The population of modified HSCs of paragraph 10, wherein the HSC cells are mammalian HSC cells.
13. The population of modified HSCs of paragraph 12, wherein the mammalian HSC cells are human HSC cells.
14. The population of modified HSCs of any of paragraphs 10-13, wherein prior to the modification, the HSCs are obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood.
15. The population of modified HSCs of any of paragraphs 10-13, wherein the HSCs are obtained from mobilized peripheral blood.
16. The population of modified HSCs of any of paragraphs 10-15, wherein the HSCs are obtained from a healthy individual.
17. The population of modified HSCs of any of paragraphs 10-15, wherein the HSCs are obtained from an individual with a diagnosed disease or disorder.
18. The population of modified HSCs of paragraph 17, wherein the diagnosed disease or disorder is an autoimmune disease or disorder.

19. The population of modified HSCs of paragraph 18, wherein the autoimmune disease or disorder is Type 1 diabetes (T1D).
20. The population of modified HSCs of any of paragraphs 10-19, wherein the HSC cells are ex vivo cultured before or after or both before and after the modification of the PD-L1 expression.
21. The population of modified HSCs of any of paragraphs 10-19, wherein the HSC cells are cryopreserved prior to or after or both prior to and after the modification of the PD-L1 expression.
22. The population of modified HSCs of any of paragraphs 10-19, wherein the modified HSC cells are cryopreserved prior to use.
23. The population of modified HSCs of any of paragraphs 10-22, wherein the HSC cells are produced by a method comprising:
 24. contacting a sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA;
 25. ex vivo culturing the resultant modified cells from the contacting; and
 26. establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1.
27. The population of modified HSCs of any paragraphs 10-22, the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.
28. The population of modified HSCs of any of paragraphs 10-22 that is produced by the method paragraphs of 1-8.
29. A composition of modified HSCs comprising of HSCs of any of paragraphs 1-25.
30. A method, the method comprising administering or ex vivo contacting an effective amount of an agent that modulates the expression of miRNAs controlling the expression of PD-L1 in the HSCs in a cell to a host.
31. The method of paragraph 27, wherein the cell is a progenitor cell.
32. The method of paragraph 28, wherein the progenitor cell is a hematopoietic progenitor cell.
33. The method of paragraph 29, wherein the agent is a vector comprising a nucleic acid sequence that miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the

miRNA or a locked nucleic acid that anneals to miRNA, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

34. The method of paragraph 30, wherein the vector is a virus.

35. The method of paragraph 27, wherein the method is used to treat type 1 diabetes, an autoimmune disease, or cancer.

36. A method of treating an autoimmune disorder or cancer in a subject in need thereof, the method comprising administering to a subject a composition comprising the hematopoietic stem cells in any of the preceding paragraphs.

37. The method of paragraph 33, wherein the autoimmune disorder is Type 1 diabetes (T1D).

38. The method of paragraph 33 or 34, wherein the HSCs are autologous to the recipient subject.

39. The method of paragraph 33 or 34, wherein the HSCs are non-autologous and allogenic to the recipient subject.

40. The method of paragraph 33 or 34, wherein the HSCs are non-autologous and xenogeneic to the recipient subject.

41. A method of modulating an immune response in a subject comprising, administering or transplanting the cell of paragraphs 10-22, or administering the composition of paragraph 26.

42. A method of modulating an immune response in a subject comprising:

43. providing a population of hematopoietic stem cells (HSCs);

44. contacting sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA;

45. ex vivo culturing the resultant modified cells from the contacting;

46. establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1; and

47. transplanting said population of PD-L1+ expressing HSCs into a recipient subject, thereby modulating the immune response in the recipient subject.

48. The method of paragraph 38 or 39, wherein the population of HSCs is obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood.

49. The method of any of paragraphs 38-40, wherein the population of HSCs is obtained from mobilized peripheral blood.

50. The method of any of paragraphs 38-40, wherein the population of HSCs autologous to the recipient subject.

51. The method of any of paragraphs 38-40, wherein the population of HSCs allogeneic to the recipient subject.

52. The method of any of paragraphs 38-40, wherein the population of HSCs is xenogeneic to the recipient subject.

53. The method of any of paragraphs 38-44, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

54. The method of paragraph 39, wherein the agent is an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.

55. The method of any of the preceding paragraph, wherein the vector is a virus.

56. A composition comprising the PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

57. A composition comprising the PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

58. A population of PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

59. A population of PD-L1 expressing hematopoietic stem cells of any one of the preceding paragraphs for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

EXAMPLES

[0184] EXAMPLE 1

[0185] Recently, Voltarelli et al. evaluated the safety and efficacy of autologous hematopoietic stem and progenitor cell (HSPC) transplantation in combination with Thymoglobulin plus cyclophosphamide as induction in newly diagnosed T1D patients (3). The latest multicenter analysis on 65 newly diagnosed T1D patients treated with autologous HSPC transplantation achieved insulin independence in nearly 60% of treated patients (4), suggesting that HSPCs may be a therapeutic option for selected T1D patients. Interestingly, HSPCs are endowed with immunoregulatory properties, which have been shown to be linked to the expression of the immune checkpoint PD-L1 (or CD274) (5). PD-L1 is the ligand for the inhibitory programmed death 1 (PD-1) receptor, expressed primarily on activated T cells (6). Crosslinking of PD-L1 and PD-1 inhibits T cell activation and favors their exhaustion/apoptosis (7); indeed, mice deficient in PD-L1/PD-1 develop accelerated diabetes (6). PD-L1⁺ HSPCs play an important endogenous immunoregulatory role, capable of eliminating autoreactive T cells but eventually becoming defective in T1D.

Materials and Methods

[0186] *Human studies* - T1D patients and healthy patients matched for age and gender were enrolled (Table 8). The study presented herein was conducted in accordance with Institutional Review Board approval (BCH 3851).

[0187] *In vitro human studies* - Isolated human CD34⁺ HSCs were stimulated for 24h with hIFN- β , hIFN- γ and Poly[I:C]. PD-L1 expression was evaluated before and after culture by different techniques (qRT-PCR, FACS, confocal imaging). PBMCs isolated from T1D patients, were cultured for 2 days in the presence of IA-2 peptide. Cells were plated with or without CD34⁺ or pharmacologically-modulated CD34⁺ cells. hIFN- γ spots were counted using an Elispot Reader.

[0188] *Animal studies* - Animal studies were conducted in NOD and C57BL/6 mice; all the mice were used according to institutional guidelines and animal protocol were approved by the Boston Children's Hospital Institutional Animal Care and Use Committee.

[0189] *In vitro murine studies* - Murine bone marrow KL cells were transduced with PD-L1 lentivirus and 24 hours after transduction PD-L1 expression was evaluated by multiple techniques (qRT-PCR, FACS, confocal imaging). *In vitro* assays were performed by co-culturing KL-PD-L1.Tg KL cells, unmodulated KL cells, or pKL with CD4⁺CD25⁻/CD8⁺ T cells extracted from splenocytes of NOD BDC2.5 TCR Tg mice or 8.3 TCR Tg NOD mice in the presence of islet mimotope peptides.

[0190] *In vivo interventional murine studies* - Newly diabetic NOD mice were treated with PD-L1.Tg KL cells, unmodulated KL cells, or pKL, and glycemia was monitored daily. Mechanistic studies were conducted on different groups of treated NOD mice and compared to untreated NOD mice (ELISPOT, flow cytometry, Luminex).

[0191] *Statistical analysis* - Statistical analysis was performed using the unpaired Student t test. A two-sided value of $P \leq 0.05$ was considered statistically significant. Kaplan-Meier curve analysis with the Wilcoxon test was used to analyze the development of diabetes in mice. For multiple comparisons, one-way ANOVA followed by Bonferroni post-test between the group of interests and all other groups was used. All graphs were generated using GraphPad Prism software version 5.0b (GraphPad Software, Inc., La Jolla, CA). All statistical tests were performed at the 5% significance level.

Results

[0192] *A defect in PD-L1 is evident in HSPCs from NOD mice*

[0193] In order to identify any defects in immunoregulatory molecules in HSPCs derived from NOD mice, broad transcriptomic profiling of immune-related molecules in murine HSPCs was performed. Sca-1+Lineage-c-kit+ cells, (KLS, or murine HSPCs) obtained from normoglycemic NOD mice had decreased PD-L1 transcripts as compared to HSPCs obtained from C57BL/6 mice (Fig. 1A-1B and Table 3). Measurement of PD-L1 mRNA expression by RT-PCR confirmed reduction in NOD HSPC as well (Fig. 1C). A range of techniques was next used to demonstrate the defect in PD-L1 expression in a variety of bone marrow HSPCs, including KLS cells, Lineage-c-kit+ (KL) cells and long-term repopulating HSPCs (CD41-CD48-CD150+ cells and CD244-CD48-CD150+) and compared it to the expression observed in NOR and C57BL/6 mice (Fig. 1D-1K). The overall PD-L1 defect is primarily confined to NOD mice (Fig. 1D-1K). It was then sought to explore any association of the PD-L1 defect in HSPCs with age or disease status. A slight decline in the number of KL-PD-L1+ cells was identified in both strains with progressive age, but again with a clear defect in NOD mice (Fig. 1L-1N). Other costimulatory molecules were evaluated as well, and no major significant differences were observed in HSPCs (Fig. 8A-8F), indicating the uniqueness of the PD-L1 defect. The PD-L1 defect was primarily confined to HSPCs in NOD mice, although other bone marrow-derived myeloid immune cells were slightly deficient in PD-L1 expression (i.e. F4/80+ cells, CD11b+ cells) (Fig. 1O-1Q, Fig. 8G-8M). In order to understand the extent of the PD-L1 defect within the HSPC niche, bone marrow tissues were analyzed using confocal imaging. Fewer c-kit+PD-L1+ cells were observed in samples obtained from NOD as compared to C57BL/6 control mice (Fig. 1R₁-1R₃, 1S₁-1S₃, and 1T). Western blotting confirmed reduced PD-L1 protein expression on KL cells obtained from NOD bone marrow compared to C57BL/6 bone marrow (Fig. 1U-1V). Data presented herein confirmed the existence of a defect in PD-L1 expression in HSPCs in NOD mice.

[0194] *The PD-L1 defect is associated with an altered network of PD-L1-related miRNAs*

[0195] In order to better understand the mechanism behind the PD-L1 defect in HSPCs of NOD mice, a series of *in vitro* experiments were performed. The effect of high glucose on PD-L1 expression was tested and the existence of any HSPC survival defect that could explain the deficiency in PD-L1 was then evaluated. Isolated KL cells from NOD and C57BL/6 mice were cultured for 3 days in high glucose

and no particular pattern that would indicate the existence of a high glucose-associated effect on PD-L1 expression was observed, although the fact that the observed PD-L1 defect may be caused by a metabolic derivative of high glucose cannot be excluded (Fig. 2A). No differences in the proliferation rate, and a slight difference in the percentage of apoptotic cells, were detected among KL cells from NOD or C57BL/6 mice (Fig. 2B-2C). When extending the investigation into analysis of gene expression profiles, affymetrix microarray analysis revealed 48 microRNAs (miRNAs) differentially expressed in KLS cells of NOD and C57BL/6 mice (Fig. 2D-2F and data not shown). Data related to upregulated and downregulated miRNAs observed in the GWAS study performed on KLS cells obtained from NOD and C57BL/6 mice are shown as an MA-Plot in Fig. 2D and listed in Fig. 2E and 2F. Multiple databases and bioinformatics tools (Mouse Genome Informatics-MGI, DIANA-microT-CDS, [<http://microrna.gr/microT-CDS>] and <http://www.mousemine.org>) enabled the identification of ~330 miRNAs predicted to target the PD-L1 gene and revealed a comprehensive miRNA network associated with PD-L1 (Fig. 2G). Interestingly, 14 miRNAs appeared both key players in controlling PD-L1 expression and altered in KLS cells obtained from NOD mice (Fig. 2E-2G). Next, to test the proof-of-concept that an altered miRNAs network may have influenced PD-L1 expression on HSPCs, miR-1905, one of the miRNAs found altered in NOD HSPCs, was silenced in isolated KL extracted from BM of NOD mice (Fig. 2H-2I). Although silencing one miRNA may influence other target genes, miR-1905 was chosen as a target due to the fact that it was the only mature miRNA among the six identified as relevant to PD-L1. Furthermore, miR-1905 was confirmed as having the higher miTG score or prediction score by the online software “DIANA TOOLS/microT-CDS”, thus indicating a higher probability of affecting PD-L1 expression as compared to other miRNAs on the list. Indeed, miR-1905 antagomir increased the expression of PD-L1 transcripts and protein in HSPCs (Fig. 2I-2K). An altered network of miRNAs may be responsible for PD-L1 reduced expression in HSPCs. Finally, it was demonstrated that the absence of any difference in the methylation status of the PD-L1 promoter in KLS cells from NOD mice, which could have accounted for the PD-L1 defect (Fig. 2L).

[0196] *Genetically engineered NOD HSPCs abrogate the autoimmune response in vitro*

[0197] The effect of a genetic engineering approach to overcome the PD-L1 defect in NOD HSPCs was next tested. murine KL cells were genetically engineered *ex vivo* to generate PD-L1.Tg KL cells (Fig. 3A-3C) from normoglycemic NOD mice by using 3rd generation self-inactivating lentiviral vectors (Lv), a technique which can be use *in vivo* because of its high efficiency and low risk of genotoxicity (Fig. 9A) (8). Immunofluorescence and genome wide analysis of these PD-L1.Tg KL cells confirmed the increase in PD-L1 expression compared to Mock-Lv-transduced KL cells (Fig. 3D-3G and Table 4). The immunoregulatory properties of PD-L1.Tg KL cells was then explored in an autoimmune setting *in vitro*. PD-L1.Tg KL cells generated from normoglycemic NOD mice were co-cultured at different ratios with T cells (1:1, 1:5 and 1:10) with CD11c+ DCs (dendritic cells) and BDC2.5 transgenic CD4+CD25- T cells in the presence of the CD4-restricted islet mimotope peptide BDC2.5. A

significant decrease in the percentage of IFN- γ +CD4+ T cells, as quantified by flow cytometry, was evident when naïve T cells were co-cultured with PD-L1.Tg KL cells as compared to those cultured alone or co-cultured with untransduced KL cells (Fig. 3H-3I). The gating strategy was determined using nonreactive isotype-matched control mAbs in each culture conditions, in which 99% of non-reactive cells were excluded. When PD-L1.Tg KL cells were pre-cultured at a ratio of 1:1 to T cells with an anti-PD-L1 blocking mAb, the aforementioned immunoregulatory effect was severely hampered (Fig. 3H-3I). The robust and PD-L1-dependent immunoregulatory properties of PD-L1.Tg KL cells were confirmed using a CD8-restricted peptide-based assay (Fig. 3J-3K) as well as an assay not specific to the autoimmune setting (anti-CD3/anti-CD28 stimulation [Fig. 3L-3M]), thus confirming that PD-L1 transgenic HSPCs abrogate the autoimmune response *in vitro*.

[0198] *Genetically engineered NOD HSPCs revert hyperglycemia in vivo*

[0199] In order to evaluate the immunoregulatory properties of PD-L1.Tg KL cells *in vivo*, newly hyperglycemic NOD mice were adoptively transferred intravenously with 3×10^6 PD-L1.Tg KL cells (Fig. 3P), and received doxycycline in water (2mg/ml) till the completion of the study, or with 3×10^6 untransduced KL cells (Fig. 3R), respectively. PD-L1.Tg KL cells successfully reverted hyperglycemia in 100% of treated hyperglycemic NOD mice with nearly 30% of treated mice remaining normoglycemic in the long-term, while none of the untreated hyperglycemic NOD mice (Fig. 3O) or those treated with doxycycline (Fig. 3Q) reverted to normoglycemia. When untransduced KL cells were used, 1 hyperglycemic NOD mouse reverted to normoglycemia and 1 showed a mild transient improvement of glycemic levels (Fig. 3R). Pathology of the pancreas of PD-L1.Tg KL cell-treated hyperglycemic NOD mice revealed reduced islet infiltration, with fewer CD3+ cells, preserved insulin staining and improved insulinitis score as compared to hyperglycemic untreated NOD (Fig. 3N₁-3N₆ and 3S). It was then evaluated whether immunocompetence was preserved in NOD mice during treatment with PD-L1.Tg KL cells. PD-L1.Tg KL cell-treated NOD mice, untransduced KL cell-treated NOD mice and untreated NOD mice were immunized at day 14 after the onset of hyperglycemia with ovalbumin, and after 3 days splenocytes were harvested and re-challenged *in vitro* with ovalbumin. Treated NOD mice were capable of mounting a regular immune response to ovalbumin similar to other groups tested and were thus immunocompetent (Fig. 3T). Immunophenotyping of PD-L1.Tg KL cell-treated hyperglycemic NOD mice showed at day 14 after treatment a 2-fold increase in the percentage of FoxP3+ regulatory CD4+ T cells as compared to untreated mice (Fig. 3U), while no changes were observed in the percentage of IFN- γ + and IL-17+ CD4+/CD8+ T cells (data not shown). Furthermore, a reduction in IFN- γ -producing cells was evident in PD-L1.Tg KL cell-treated as compared to untreated hyperglycemic NOD mice in an *ex vivo* assay, when splenocytes were challenged with islet peptides at day 40 following treatment (Fig. 3V). BDC2.5 transgenic CD4+CD25- T cells appear to be more susceptible to the effect of PD-L1 upregulation as compared to IGRP transgenic CD8+ T cells. To

understand the mechanism by which PD-L1.Tg KL cells exert their immunosuppressive effects on autoreactive T cells, an apoptosis assay was performed *in vitro* during a diabetogenic autoimmune response by coculturing IGRP Tg CD8⁺ T cells from NOD 8.3 (stimulated with the islet peptide IGRP) or CD4⁺ CD25⁻ T cells from NOD BDC2.5 (stimulated with the islet peptide BDC2.5) in the presence of PD-L1.Tg KL or WT KL cells. PD-L1.Tg KL cells induced cell death of autoreactive CD4⁺ and CD8⁺ T cells, while only a minor effect was observed, primarily on autoreactive CD8⁺ T cells, when WT KL cells were added (Fig. 4A-4B). If a possible conversion into myeloid suppressive cells may partially explain the immunoregulatory effects of PD-L1.Tg KL cells was then explored. Interestingly, while after transduction the presence of T/B cell markers was very scant, myeloid markers were strongly expressed (Fig. 4C-4D).

[0200] *Genetically engineered HSPCs traffic to the pancreas in hyperglycemic NOD mice*

[0201] To explore the fate of infused PD-L1.Tg KL cells in NOD mice, a set of tracking experiments were performed in the pancreas, spleen, pancreatic draining lymph nodes (PLN) and bone marrow using the tracer ZsGreen, present on the vector used to transduce PD-L1.Tg KL cells. PD-L1.Tg KL cells were adoptively transferred into normoglycemic and hyperglycemic NOD mice, and tissues were harvested at days 1, 7 and 14 post-infusion. ZsGreen⁺ cells and ZsGreen mRNA expression were quantified in all tissues by flow cytometry and qRT-PCR, respectively. PD-L1.Tg KL cells preferentially trafficked to the pancreas once infused into hyperglycemic NOD (Fig. 4E-4G), while they homed to a lesser extent to the PLN, bone marrow and spleen (Fig. 4K-4L, 4O-4P, and 9B). Conversely, in normoglycemic NOD mice, PD-L1.Tg KL cells scarcely trafficked to the pancreas, to the spleen or to the PLN (Fig. 4H-4J, 4Q-4R and 8C), but instead preferentially homed to the bone marrow (Fig. 4M-4N). Confocal imaging confirmed that ZsGreen⁺ cells were absent in the pancreata of normoglycemic NOD mice (Fig. 4S₁-4S₄), while they were detectable in the pancreata of hyperglycemic NOD mice, particularly at day 1 after PD-L1.Tg KL cell infusion (Fig. 4T₁-4T₄). Although preferential homing of PD-L1.Tg KL cells was observed to the bone marrow (and perhaps spleen) in hyperglycemic NOD mice based on ZsGreen transcript quantification by qRT-PCR, flow cytometry and confocal imaging consistently showed trafficking of PD-L1.Tg KL cells to the pancreas. A tracking experiment was also performed using GFP⁺ KL cells (WT, untransduced KL cells) infused into treated hyperglycemic NOD mice and examined these mice by flow cytometry at days 1, 7 and 14; results showed no migration into the pancreas of the treated hyperglycemic NOD mice (Fig. 9D-9E). Moreover, the chemokine receptor profile of PD-L1.Tg KL cells were explored in order to determine which chemokine receptors are more likely involved in the homing of transgenic HSPCs. Interestingly, results showed that CXCR4 is the most expressed and possibly the most relevant chemokine receptor for PD-L1.Tg KL cell trafficking (Table 7). Bioluminescence imaging of NOD mice adoptively transferred with Luciferase+PD-L1.Tg KL cells showed a rapid disappearance of Luciferase+PD-L1.Tg KL cells from the peripheral blood (Fig. 4U-4V). It was also determined whether PD-L1.Tg KL cells differentiated after their infusion into NOD mice.

Tracking studies revealed a predominant transformation into myeloid cells (e.g., CD11b) into the PNL of treated mice (Fig. 9F-9I). These PDL-1 expressing myeloid cells may interact with autoreactive CD4 and CD8 T cells contributing to their death.

[0202] *Pharmacologically modulated HSPCs abrogate the autoimmune response in vitro*

[0203] In order to offer an alternative approach to gene therapy, the feasibility of pharmacological modulation of PD-L1 was explored. Agents, either alone or in combination, that are capable of upregulating PD-L1 in human CD34⁺ cells were tested (Fig. 5A-5C) and identified as being able to robustly upregulate PD-L1 (Fig. 5D-5E). The use of these agents in combination are further described in, e.g., Nasr, M. B., et al. Sci. Transl. 9, eaam7543 (2017), which is incorporated herein by reference in its entirety. The ability of the agent, alone or in combination, to upregulate PD-L1 in murine KL cells isolated from normoglycemic NOD mice was then confirmed, thus generating pharmacologically modulated KL cells (pKL cells) (Fig. 5F-5L, and Tables 5 and 6). The expression of costimulatory molecules, e.g., pro-inflammatory and anti-inflammatory cytokines, were then analyzed by flow cytometry, which demonstrated upregulation of IL-4, PD-1, CD80, CD86 and ICOSL in pKL cells as compared to unmodulated-KL (KL-Veh) cells, with CD40 comparatively downregulated in pKL cells (Fig. 10A-10B). The immunoregulatory properties of pKL cells were explored in an autoimmune setting *in vitro*. pKL cells generated from normoglycemic NOD mice were co-cultured at ratios of 1:1, 1:5 and 1:10 to T cells with CD11c⁺ DCs and BDC2.5 transgenic CD4⁺CD25⁻ T cells in the presence of BDC2.5 peptide. Quantification by flow cytometry revealed a pronounced and significant decrease in the percentage of IFN- γ ⁺CD4⁺ T cells when pKL cells were added to the assay as compared to when unmodulated KL cells were used (Fig. 5M-5N). Immunoregulation was determined to be PD-L1-dependent by pre-culturing pKL cells at a ratio of 1:1 to T cells with an anti-PD-L1 blocking mAb, which resulted in a dramatic reduction in the immunoregulatory effect (Fig. 5M-5N). These effects and PD-L1 dependency were confirmed in CD8-dependent (Fig. 5P-5Q) and in non-autoimmune-specific anti-CD3/anti-CD28 (Fig. 5S-5T) assays. Finally, the immunosuppressive effects of PD-L1.Tg KL cells, pKL cells and unmodulated KL cells (WT) were compared with that of CD4⁺CD25⁺ regulatory T cells similarly obtained from normoglycemic NOD mice. In the 3 aforementioned CD4- and CD8-restricted autoimmune and anti-CD3/anti-CD28 assays, PD-L1.Tg KL cells and pKL cells exerted robust immunoregulatory properties almost comparable or higher to those obtained with CD4⁺CD25⁺ regulatory T cells; indeed, this was particularly evident for PD-L1.Tg KL cells (Fig. 5O, 5R, 5U, and 11A-11C). A less pronounced effect was observed when unmodulated KL (WT) were added to the assays. Pharmacologically modulated HSPCs are thus endowed with immunoregulatory properties and abrogate the autoimmune response *in vitro*.

[0204] *Pharmacologically modulated HSPCs revert hyperglycemia in vivo*

[0205] In order to evaluate the effect of pKL cells *in vivo*, newly hyperglycemic NOD mice were adoptively transferred with 3x10⁶ pKL cells (Fig. 6A). pKL cells successfully reverted diabetes in

40% of treated newly hyperglycemic NOD mice, with 30% remaining normoglycemic until the completion of the study at day 40. Kaplan-Meier analysis showed a greater effect of PD-L1.Tg KL cells in reverting hyperglycemia in NOD mice, as compared to pKL cells (Fig. 6B). Quantification of IFN- γ -producing cells in an *ex vivo* assay where splenocytes were challenged with islet peptides at day 40 (BDC2.5, IGRP, GAD-65 and insulin) revealed a reduction in IFN- γ -producing cells in pKL cell-treated hyperglycemic NOD mice (Fig. 6C). Immunophenotyping of treated NOD mice showed at day 40 post-treatment a marked increase in FoxP3⁺ regulatory CD4⁺ T cells (Fig. 6D) and a reduction in the percentage of IFN- γ +CD4⁺ and IFN- γ +IL-17+CD8⁺ T cells (data not shown). A reduction in the peripheral levels of pro-inflammatory (IL-2/IL-6) (Fig. 6E-6F) and an increase in anti-inflammatory (IL-4) (Fig. 6G) cytokines were evident in pKL cell-treated NOD mice. whether immunocompetence was maintained during treatment of NOD mice with pKL cells was evaluated, in the same manner as using PD-L1.Tg KL cells. pKL cell-treated NOD mice were capable of mounting a regular immune response to ovalbumin once immunized and re-challenged *in vitro* with ovalbumin similar to untreated NOD mice and were thus immunocompetent (Fig. 6H). Pathology of the pancreas of pKL cell-treated NOD mice revealed mild infiltration of the islets, with preserved insulin staining and reduced insulinitis score as compared to untreated hyperglycemic NOD mice (Fig. 6I and 6J₁-6J₆). Finally, the effects of a PD-1 signaling mAb capable of mimicking PD-L1/PD-1 cross-linking was tested. PD-1 mAb treatment did not delay diabetes onset in pre-diabetic NOD mice nor did it revert diabetes in newly hyperglycemic NOD mice (Fig. 11D, 11E). It is possible that the mAb used was unable to reach the inflamed islets within the pancreas, which conversely could be accessed by modified HSPCs because of their CXCR4 expression, which allows the trafficking of HSPCs into inflamed areas that release high levels of SDF-1. Without wishing to be bound by a particular theory, a working hypothesis, in which an altered network of miRNAs is responsible for the PD-L1 defect in HSPCs in T1D, is proposed. The genetic and pharmacological restoration of this PD-L1 defect in HSPCs in T1D generates a new pool of PD-L1⁺ HSPCs, which once adoptively transferred traffic to the pancreas and are able to delete autoreactive T cells via a PD-L1/PD-1-dependent mechanism, thus reverting hyperglycemia (Fig. 6K).

[0206] *The PD-L1 defect is evident in human HSPCs from T1D patients*

[0207] To assess whether patients with T1D displayed defects in HSPCs similar to those observed in the preclinical models presented herein, PD-L1 expression was analyzed on CD34⁺ cells isolated from peripheral blood (Table 8). In line with the findings in NOD mice presented herein, fewer PD-L1⁺CD34⁺ cells were detectable in T1D patients as compared to healthy controls (Fig. 7A-7C). Interestingly, the defect was evident in newly diagnosed T1D patients as well, when it is unlikely there is any effect of high glucose (Fig. 12A). Western blot and PCR analysis confirmed reduced PD-L1 protein and mRNA expression in CD34⁺ cells obtained from T1D patients as compared to those obtained from healthy controls (Fig. 7D-7F). Other relevant immune cells were not deficient in PD-L1 (Fig. 12B-12D),

thus confirming that the PD-L1 defect is mainly restrained to HSPCs in T1D patients. Interestingly, PD-L2 was expressed on CD34+ cells from T1D patients at higher levels as compared to controls; however, PD-L2 has been described to be less relevant for T1D onset as shown by the lack of effect when knocking down PD-L2 in NOD mice (Fig. 12E-12I). PD-1 expression was slightly reduced by MFI in T1D patients compared to healthy controls (Fig. 12J-12N). Finally, a reduced number of PD-L1+CD34+ cells in the bone marrow of T1D patients was confirmed by confocal imaging as compared to healthy controls (Fig. 7G₁-7G₃; 7H₁-7H₃ and 7I). Data presented herein confirmed the existence of a defect in PD-L1 expression in HSPCs of T1D patients.

[0208] *The altered miRNA network is also evident in human HSPCs*

[0209] In order to understand the immunological basis of the PD-L1 defect in human HSPCs, *in vitro* experiments similar to those performed in mice were performed. Any potential high glucose-associated effect on PD-L1 expression on CD34+ cells, or small differences, if any, in the proliferation and apoptosis rate in CD34+ cells obtained from T1D patients and controls were not found (Fig. 7J-7L). Bioinformatic analysis of miRNAs showed a number of miRNA species involved in controlling PD-L1 expression (Fig. 7M). qRT-PCR analysis of several relevant miRNAs confirmed that a number of miRNA species were differentially expressed in human CD34+ cells obtained from T1D patients as compared to controls (Fig. 7N), with no differences observed in the methylation status of the promoter of the PD-L1 gene (Fig. 7O). In line with preclinical findings, an altered miRNA network is evident in HSPCs of T1D patients.

[0210] *Pharmacologically modulated human HSPCs abrogate the autoimmune response ex vivo*

[0211] The effect of overcoming PD-L1 deficiency in human HSPCs was tested by using the same agents described herein above (e.g., tested in NOD mice). As shown by flow cytometric analysis, confocal imaging and qRT-PCR, modulation of CD34+ cells with an agent, alone or in combination, upregulated PD-L1 expression in human CD34+ cells obtained from T1D patients (pCD34+) as compared to unmodulated CD34+ cells (Fig. 7P-7U). Immunophenotyping and transcriptome profiling of pharmacologically modulated CD34+ cells (pCD34+) (Fig. 13A, 13B, Fig. 14A-14B, and Table 9) confirmed specific PD-L1 upregulation in pCD34+ cells as compared to unmodulated CD34+ cells. To study the *ex vivo* immunoregulatory functions of pCD34+, CD34-depleted PBMCs were co-cultured with unmodulated CD34+ or pCD34+ in the presence of insulin-associated autoantigen-2 (I-A2), and the number of IFN- γ -producing cells was quantified in an ELISPOT assay. Interestingly, the addition of unmodulated CD34+ or pCD34+ resulted in a significant decrease in the number of IFN- γ -producing cells as compared to those obtained when PBMCs were cultured without unmodulated CD34+/pCD34+ in the presence of the islet peptide IA-2 (Fig. 7V-7W). The suppression was more pronounced when pCD34+ were added, indicating that pCD34+ are endowed with greater immunoregulatory activity than unmodulated CD34+ (Fig. 7V-7W). To further confirm that the immunosuppressive effect exerted by pCD34+ was primarily due to PD-L1 expression, pCD34+ were pre-cultured in the presence of an anti-

PD-L1 blocking mAb and then tested in the autoimmune assay. Ab-mediated PD-L1 blockade hampered the immunoregulatory effect exerted by pCD34+ (Fig. 7X-7Y). This strongly confirms that pCD34+ are endowed with PD-L1-dependent regulatory properties *ex vivo*. Surprisingly, the effects of pCD34+ were not confirmed in a non-autoimmune-specific anti-CD3/anti-CD28 assay (Fig. 7Z). Finally, the effect of plerixafor-mediated mobilization, used in clinic (5, 9), was analyzed on PD-L1 expression in CD34+ cells obtained from 5 T1D patients and 8 healthy controls (Table 10). While the percentage and absolute number of CD34+ cells significantly increased, the percentage of CD34+PD-L1+ cells remained stable in T1D patients but decreased after mobilization in healthy controls, highlighting that CD34+ cell mobilization may have failed in previous clinical trials due to PD-L1 downregulation (Fig. 15A-15C).

Discussion

[0212] T1D is regarded as one of the most aggressive autoimmune diseases and requires life-long exogenous insulin administration. Efforts to halt β -cell decline or stall chronic complications are ongoing (10-13); however, the immunotherapies tested thus far have failed, mostly due to their lack of specificity as well as the fact that they are usually simply adopted from other settings (e.g. kidney transplantation) (14-16). The need for more T1D-tailored therapies led the inventors to explore the existence of immune checkpoint abnormalities unique to the disease. Various pieces of evidence led to the hypothesis that an HSPC-specific PD-L1 defect may be involved in the onset of T1D and that the resolution of this defect may provide a cure for the disease. First of all, the expansion and reinfusion of autologous HSPCs was the most potent therapy in reverting hyperglycemia in T1D patients (4); secondly, there is a strong link between the PD-L1 defect and T1D (6, 17), and finally, PD-L1 is a key player in HSPC immunobiology, such that the lack of PD-L1 reduces the ability of HSPCs to abrogate the immune response (5). Indeed, while immunosuppressant treatment alone (i.e. ATG) failed to preserve β -cell function in recent onset T1D, HSPCs plus immunosuppressant were successful in the Voltarelli trial. This result indicates that either there is a synergistic effect between HSPCs and immunosuppression or that the PD-L1 defect prevents HSPCs from being fully effective in their suppression. Transcriptomic profiling, flow cytometric analysis, RT-PCR and direct analysis of bone marrow showed a reduction in PD-L1 expression in HSPCs in both NOD mice and T1D patients. While high glucose, altered HSPC survival or epigenetic abnormalities cannot account for the impaired PD-L1 expression, gene expression profiling unveiled abnormalities in the HSPC miRNA network in T1D that may be responsible for the PD-L1 defect. Therefore, a genetic approach to overcome the PD-L1 defect was developed and PD-L1.Tg HSPCs were generated to test their ability to affect the autoimmune response *in vitro* and *in vivo*. These PD-L1+ HSPCs successfully abrogated the autoimmune response *in vitro*. The use of an anti-PD-L1 blocking antibody impeded the observed effect of PD-L1.Tg HSPCs, thus confirming that HSPC immunoregulatory properties are PD-L1-dependent. Notably, PD-L1.Tg HSPCs described herein successfully converted all treated hyperglycemic NOD mice to normoglycemia, with suppression of the autoimmune response. Tracking studies suggested that PD-L1.Tg HSPCs preferentially homed to the

inflamed pancreas, due to substantial CXCR4 expression, which is in line with the CXCL12 shown to be released by inflamed pancreatic islets (18). Once in the pancreas, PD-L1.Tg HSPCs may induce apoptosis of autoreactive T cells. The recent progress in the field of gene therapy (19) provides a basis for the potential use of the aforementioned genetic approach in T1D as well. Interestingly and clinically relevant, pharmacologically modulated HSPCs also exhibited immunoregulatory effects, as they markedly abrogated CD4/CD8-restricted autoimmune responses *in vitro* and partially reverted diabetes in newly hyperglycemic NOD mice. The human data parallel the preclinical findings, confirming the presence of the PD-L1 defect in human CD34+ cells. Results described herein have 2 major implications. Firstly, a novel and important path involved in the onset of T1D was identified, and the PD-L1 defect in HSPCs have a permissive role on the generation of autoreactive T cells (20). The study described herein thus provides key insight into the potential role of miRNAs in the regulation of PD-L1 expression of HSPCs and potentially of T1D pathogenesis. Secondly, expression of PD-L1 in HSPCs can be used as a novel tool for targeted immunotherapy in T1D, which appears more efficacious than mAbs and also appears to be safe (21, 22). In conclusion, data presented herein has discovered a novel mechanism involved in the onset of T1D, whose correction may provide an immunological tool to be used to cure T1D.

[0213] The references cited herein and throughout the specification are incorporated herein by reference in their entirety.

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[0215] EXAMPLE 2

[0216] Encouraging results of previous pilot trials suggest that autologous hematopoietic stem and progenitor cell transplantation (AHSCT) may be a relevant alternative therapeutic option to immunosuppressive drugs in the treatment of several refractory autoimmune disorders (1, 2). Over 3,000 transplants using AHSCT have been performed worldwide with a very high safety profile (2, 3). It was recently demonstrated that AHSCT could induce long-term, drug free and symptoms-free remission in patients newly diagnosed with type 1 diabetes (T1D). Insulin independence was achieved in nearly 60% of treated subjects at 6 months, with 40% showing sustained insulin-free remission over 4 years following the procedure (4). The aim behind the use of AHSCT is to suppress autoreactive immune cells, while allowing for de novo generation of a naïve immune compartment tolerant to pancreatic β cells antigens (5), thus preventing T cell infiltration into targeted organs (6). AHSCT trials showed that in treated patients, an overall resetting of the immune system toward a “regulatory”-like T cell landscape was evident, with an increase in CD4+Foxp3+ Tregs (7). Unfortunately, the use of immunosuppression during AHSCT limits the potential use of this therapy in T1D to experimental conditions, due to patients’ potential exposure to adverse effects. Interestingly, the immunoregulatory properties of HSPCs seem to be linked to their expression of the immune checkpoint-signaling molecule PD-L1 (or CD274) (8, 9). They further express CXCR4, which allows HSPCs to traffic to inflamed area/sites of injuries (10). Unlike mesenchymal or embryonic stem cells, which are associated with the potential development of tumorigenesis and formation of ectopic tissue (5, 11-13), HSPCs have been safely used for years (14-16). Several studies suggested that PGE2 might have anti-inflammatory effects through inhibition of several pro-inflammatory cytokines (17). Others have demonstrated that the endogenous anti-inflammatory role of PGE2 is mainly mediated through its receptor EP4, thereby inhibiting macrophage derived-pro-inflammatory chemokines production during atherogenesis (18, 19). While others have mainly studied in depth the mechanism by which PGE2 can control inflammation and demonstrated that PGE2 plays its regulatory role by limiting T cell activation thereby impairing T cell arrest and inhibiting T cells interactions with DCs (20). Previous reports have introduced and identified PGs as potential HSPCs enhancing candidates capable of inducing/improving their long-term maintenance and engraftment faculties (21). Without wishing to be bound by a particular theory, it was hypothesized that enhancing the immunoregulatory properties of HSPCs using pharmacological modulation with small molecules may create a novel powerful immunoregulatory tool for the treatment of T1D.

Methods and Materials

[0217] Human studies

[0218] Study population included in the AHSCT clinical trial. Two cohorts consisting of 36 T1D patients were enrolled in the AHSCT (autologous hematopoietic stem cell transplantation) program and were also enrolled in 3 independent clinical trials as previously described (6). Auto-antibodies were analyzed on serum by RIA (for IAA) and ELISA (for IA-2A, GAD, Znt8) according to the standard of care clinical procedure. The study was performed in accordance with Institutional Review Board committee approval of each participant Institution, informed consent was provided by all individuals. All baseline demographic and clinical characteristics of the study population are reported in Table 1.

[0219] Study population included in the PG-library screening. Blood samples were obtained from long lasting T1D patients (n=24) and healthy controls (CTRL) (n=5) in accordance with Institutional Review Board committee approval of San Raffaele Hospital and of Boston children's Hospital (BCH 3851); informed consent was provided by all individuals included in the present study. Baseline characteristics of the study population are summarized in Table 2. Peripheral blood mononuclear cells (PBMCs) isolated from 20 ml blood samples using Lymphoprep (Stem Cell Technologies, Cambridge, MA) were frozen in freezing medium (RPMI 1640 20% FBS and 8% DMSO) and stored at -80 °C. After thawing, PBMCs were recovered in culturing medium consisting of RPMI 1640 (Life Technologies, Carlsbad, CA) supplemented with 10% FBS, 2 mM L-glutamine (Life Technologies), 100 U/ml penicillin (Life Technologies), for 48 h, and CD34+ cells were then isolated using a CD34 Positive Isolation Kit (Miltenyi Biotec, San Diego, CA) according to the manufacturer's instructions.

[0220] Pharmacological modulation of human CD34+ cells. 1×10^5 of isolated human CD34+ HSPCs (purity 99%) were cultured in 200 μ l of StemSpan SFEM II media (STEMCELL Technologies Inc., Cambridge, MA, USA), and each compound in the Prostaglandin Screening Library II (Cayman Chemicals, Ann Arbor, MI), was added individually at day 0 and at day 1 at a concentration of 10 μ M as previously reported by the inventors and others (9, 21). In another assay, isolated CD34+ cells from freshly isolated human PBMCs or from cryopreserved PBMCs, and processed as described earlier, were cultured in the presence of a cocktail of cytokines containing: 10 μ g/ml heparin (STEMCELL Technologies Inc., Cambridge, MA, USA), 10 ng/ml human SCF (Miltenyi Biotec, San Diego, CA), 20 ng/ml human TPO (Miltenyi Biotec, San Diego, CA), 10 ng/ml human FGF-1 (Miltenyi Biotec, San Diego, CA), 100 ng/ml IGFBP2 (R&D Systems, Inc., Minneapolis, MN), and 500 ng/ml Angptl3 (R&D Systems, Inc., Minneapolis, MN). PGE2 (PromoKine, PromoCell GmbH, Germany) was added by pulsing the culture at 0, 24h, 72h and 6 days with 2 μ l of diluted PGE2 (10 μ M). Cells were cultured for 7 days at 37°C in 5% CO₂, and CD34+ cells were then subjected to FACS analysis and were run on a FACSCelesta™ (Becton Dickinson, Franklin Lakes, NJ). Data were analyzed using FlowJo software version 8.7.3 (Treestar, Ashland, OR). The different cytokines used here and their related concentration as well as the choice of the incubation timing was used as previously reported in the literature (22).

[0221] qRT-PCR. RNA was extracted from CD34⁺ cells using Direct-zol™ RNA Kits (Zymo, Irvine, CA, USA) and Trizol Reagent (Invitrogen Carlsbad, CA), RNA quality was assessed by Multiskan™ GO Microplate spectrophotometer and the ratios of absorbance at 260 nm and 280nm were assessed for all the samples. Only samples with RNA ratios within 1.9 were included in the present study. cDNA synthesis was made from purified total RNA by reverse transcription using High capacity cDNA Reverse Transcription RETROscript® Kit (Thermo Fisher Scientific, Waltham, MA, USA) followed by a pre-amplification using Taqman PreAmp Kit (Applied Biosystems) according to the manufacturer's instructions. qRT-PCR analysis was performed using TaqMan assays (Life Technologies, Grand Island, NY) containing PCR primers and TaqMan probes according to the manufacturer's instructions. Normalized expression values were determined using the ΔC_t method. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) data were normalized for the expression of GAPDH. qRT-PCR reactions were performed in triplicate in a 96-well format using an Applied Biosystems 7900HT fast real-time PCR instrument. Relative expression was calculated using the comparative threshold cycle method as previously described (23, 24). For two-group comparisons, a Student's t test was employed. Reported below are the main characteristics of the primers used:

[0222] Table 11. Main characteristics of the primers used for qPCR.

Gene Symbol	Assay ID	Refseq Accession #	Band Size (bp)	Reference Position
CD274 (PD-L1)	Hs01125299_m1	NM_001267706.1	89	441
CD184 (CXCR4)	Hs00237052_m1	NM_001008540.1	153	973
IDO1	Hs00984148_m1	NM_0022164.5	66	651
GAPDH	Hs99999905_m1	<u>NM_001289746.1</u>	122	229

[0223] Human ELISPOT assay. An ELISPOT assay was used to measure the number of IFN- γ -producing cells according to the manufacturer's protocol (BD Biosciences, San Jose, CA) as previously shown by the inventors (2). 1x10⁶ PBMCs isolated from T1D patients were cultured for 2 days in the presence of IA-2 peptide (Thermo Fisher Scientific GmbH, Germany) (100 μ g/ml) in RPMI medium supplemented with 10% FBS. At 24h after stimulation, 500 μ l of medium was added to the culture. Cells were collected at day 2 and added to plates coated with anti-IFN- γ antibody (eBioscience, Thermo Fisher Scientific, Waltham, MA USA) with or without PGE2-modulated CD34⁺ cells at ratios of 1:2 or 1:10 or 1:32 in RPMI medium supplemented with 10% FBS. Spots were counted using an A.EL.VIS Elispot

Reader (A.EL.VIS GmbH, Hannover, Germany) or on an Immunospot Reader (C.T.L. Cellular Technology Ltd, Cleveland, OH.).

[0224] Immunofluorescence and confocal microscopy. Regulatory CD34+ (PGE2-modulated) cells and unmodulated CD34+ cells isolated from peripheral blood of healthy controls were fixed in 4% PFA for 1h at 4C, washed 3 times for 20 min in PBS, and cells were counterstained with blue fluorescent DAPI (1:10000, BioLegend, San Diego, CA) and anti-human PD-L1 (BD Biosciences). Cells were photographed under a 63x objective. Images were captured on a Leica SP5X system with an upright DM6000 microscope and A1R confocal microscope (Nikon Instruments, Melville, NY). Histology was evaluated by at least two expert pathologist (9).

[0225] Migration assay. Transwell migration assays were performed on PGE2-modulated HSPCs compared to unmodulated HSPCs in the presence of 0 to 50 ng/ml SDF-1 (R&D Systems, Minneapolis, MN). In brief, cells were suspended in 0.5% BSA Phenol Red-Free RPMI and plated in the upper chambers of an HTS-Transwell-96-well permeable support plate (Corning, Acton, MA) and incubated at 37°C in 5% CO₂ for 2 hours. After 2 hours incubation, migrated cells were counted using BD TruCount (BD Biosciences) by flow cytometry.

[0226] Murine studies

[0227] Mice. Female NOD/ShiLtJ (NOD) or non-obese diabetic mice (NOD) which is the commonly used model for autoimmune type 1 diabetes studies, NOD.FVB-Tg (CAG-luc,-GFP)L2G85Chco/FathJ (Luciferase NOD) mice which exhibit a widespread expression of the two cell tracers eGFP and firefly luciferase directed by the CAG promoter allowing thus an easily tracking of the cells and NOD.CgTg (TcrBDC2.5,TcrbBDC2)1Doi/DoiJ (BDC2.5 NOD) mice which has the particularity to carry a rearranged TCR α and δ genes from a diabetogenic T cell clone, BDC2.5 and is commonly used in vitro autoimmune assays; were purchased from the Jackson Laboratory (Bar Harbor, Maine). All mice were housed under specific pathogen-free conditions at an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International)-accredited facility at Boston Children's Hospital. Institutional guidelines and protocols were approved and adhered to the Institutional Animal Care and Use Committee (IACUC).

[0228] Murine regulatory KL cell modulation. Murine bone marrow KL (c-Kit+Lineage-) cells were isolated using magnetic beads and MACS® separation columns (Miltenyi Biotec, San Diego, CA) and ~ 2x10⁵ cells were plated in a U-bottomed 96-well plate with 200 μ l of stem cell medium, Stemspan-SFEMII (STEMCELL Technologies, Cambridge, MA) and PGE2 (PromoKine, PromoCell GmbH, Germany) was added at day 0 and day 1, at a concentration of 10 μ M.

[0229] Flow cytometric analysis and intracellular cytokine staining. Flow cytometry was performed to analyze surface expression markers of PGE2-modulated HSPCs and dmPGE2 (16, 16-dimethyl PGE2)-modulated HSPCs. Anti-mouse PD-L1, PD-L2, PD-1, CD40, CD80, CD86, CD4, CD8,

Ly-6G (Gr-1), B220, CD3, CXCR4, CCR2, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR3, IL-4, IL-10 and IFN- γ were purchased from BD Biosciences, eBioscience (San Diego, CA) and BioLegend. The following antibodies corresponded to isotype controls for the murine antibodies above: PE mouse IgG1, κ isotype ctrl, Armenian hamster IgG; APC mouse IgG2b, κ isotype ctrl, Armenian hamster IgG. Cells were subjected to FACS analysis and were run on a FACSCalibur™ (Becton Dickinson). Data were analyzed using FlowJo software version 8.7.3 (Treestar).

[0230] Intracellular staining for flow cytometry. Naïve CD4+CD25- T cells (5×10^5) were isolated from BDC2.5 TCR tg mice with a negative selection strategy using a CD4+ CD25+ Regulatory T cell isolation kit (Miltenyi Biotec) and were stimulated with BDC2.5 peptides and CD11c+ dendritic cells (DCs) (2.5×10^5) previously isolated using CD11c+ mAb-coated microbeads. DCs were added at a 1:2 ratio to T cells and were co-cultured with PGE2-modulated KL cells at ratios of 1:1, 5:1 and 10:1 (ratio of T cells to PGE2-modulated KL cells) or alone (controls) or with untransduced KL cells for 24 hours in RPMI 10% FBS in a humidified incubator at 37°C, 5% CO₂. After incubation, cells were collected, washed and plated in RPMI 10% FBS, then stimulated with 50 ng/ml PMA (Sigma Aldrich, St. Louis, MO), 750 ng/ml ionomycin (Sigma Aldrich) and the protein transport inhibitor BD GolgiStop (6 μ l per 6 ml of RPMI as recommended by the manufacturer, BD Biosciences) for 5h in a humidified incubator at 37°C, 5% CO₂. After incubation, cells were collected, washed, stained for surface marker CD4 APC markers (i.e CD4 APC), followed by washing and permeabilization using the BD Cytofix/Cytoperm Kit (BD Biosciences) and staining with anti-mouse IFN- γ (eBioscience). Finally, CD4+ IFN- γ + cells were assessed by flow cytometric analysis.

[0231] Pancreas digestion and preparation for flow cytometry. Pancreata were collected in ice-cold IMDM medium, cut into small pieces, and digested with Collagenase D for 1h at 37°C, with DNase I added after 30 minutes. Digested pancreata were passed through a 70- μ m cell strainer to obtain single cell suspensions and then analyzed by flow cytometry. For tracking GFP+ cells, biotinylated anti-GFP (BD Biosciences) was used at 20 ug/ml followed by staining with APC-conjugated streptavidin (BD Biosciences).

[0232] Statistical analysis. Statistical analysis was performed using an unpaired Student's t test. A two-sided value of $p \leq 0.05$ was considered statistically significant. All graphs were generated using GraphPad Prism software version 5.0b (GraphPad Software, Inc., La Jolla, CA). All statistical tests were performed at the 5% significance level.

Results

[0233] AHSCT improves β cell function in treated T1D patients. Two cohorts consisting of 36 T1D patients were enrolled in the AHSCT (autologous hematopoietic stem cell transplantation) program and were also enrolled in 3 independent clinical trials as previously described (6). All baseline demographic and clinical characteristics of the study population are reported in Table 1. The patient

group was predominantly male (27 males and 9 females) with a mean age of 22.4 years and a short history of disease duration (within 6 weeks of diagnosis), confirmed by the presence of autoantibodies to islet peptides (glutamic acid decarboxylase antibodies [anti-GAD] were detected in 86% of patients, while other autoantibodies were detected in 17% of patients). Most of the patients studied (67%) had no previous history of diabetic ketoacidosis/ketosis. The mean body mass index (BMI) of patients at diagnosis was 20.7 ± 0.5 (kg/m²±SEM), and their mean glycated haemoglobin of (HbA1c) was 86.6 ± 6.4 (mmol/mol±SEM). All patients underwent a stem cell mobilization protocol as previously described (6) with cyclophosphamide (2 g/m²) and granulocyte colony-stimulating factor (G-CSF) (5–10 µg/kg) daily, beginning the day after cyclophosphamide administration (6). A mean dose of $5.8 \pm 0.8 \times 10^6$ /kg cryopreserved CD34+ cells was administered as a single infusion at day 0 (6). All patients showed improvement in β cell function, as revealed by an increase in C-peptide levels over time, which reached a persistent and stable median value >2.5 ng/mL at 12 months of follow-up and lasted until 24 months after treatment (Fig. 16A). Interestingly, compared to pre-AHSCT treatment levels, 2-hour postprandial peak-stimulated C-peptide levels increased significantly at 6 months post-AHSCT treatment and reached ~2.7 ng/ml at 24 months of follow-up (Fig. 16A). Furthermore, a significant positive correlation, albeit weak, was found between the number of CD34+ cells infused and C-peptide levels at 6 months after treatment (Fig. 16B). Statistical analysis revealed a significant negative correlation between the number of CD34+ cells injected during AHSCT and exogenous insulin requirement units (EIR) evaluated at 8 months (Fig. 16C) and 12 months (Fig. 16D) of follow-up. T1D patients treated with autologous hematopoietic stem cell transplantation showed an overall improvement in glycometabolic control and maintenance of β cell function.

[0234] Prostaglandin library screening. PGE2 has been described as a small molecule known to enhance the homing and engraftment of HSPCs. It was therefore sought to screen all known types of prostaglandins using the Prostaglandin Screening Library II, which contains 64 small molecules. Each small molecule contained in the library was first screened for its capacity to upregulate PD-L1 in human CD34+ cells isolated from T1D patients (Fig. 17A-17B). CD34+ cells isolated from T1D patients were cultured in StemSpan SFEMII and pulsed with each PG (prostaglandin) small molecule contained in the aforementioned library at a concentration of 10µM at 24 and 48h. Using FACS analysis, the MFI (mean fluorescence intensity) of PD-L1 expression was calculated following treatment with each small molecule used to modulate CD34+ cells as compared to untreated/unmodulated CD34+ cells (Fig. 17B). A heat map was generated depicting the degree to which each PG small molecule affected PD-L1 expression on CD34+ cells (Fig. 17B), thus allowing for the evaluation of PG candidates. Based on its ability to modulate PD-L1 expression, PGE2 was selected as a candidate for further study; in addition, PGE2 has been described in the literature and has been tested in clinical trials as a potential therapy for enhancing HSPC engraftment following cord blood transplantation (21). Isolated CD34+ cells from healthy control patients (CTRL) and from T1D patients were cultured in StemSpan SFEMII and pulsed

with 10 μ M of PGE2 at 24 hours and 48 hours. The effect of pharmacological modulation was first tested with PGE2 by FACS analysis, and the data revealed a slight increase in PD-L1 expression, although not significant, in cultured CD34+ cells from T1D and healthy control patients, with the latter showing a higher percentage of PD-L1 expression as compared to cells from T1D patients (Fig. 17C-17D). This pattern was further confirmed by confocal imaging, in which PD-L1 surface expression was upregulated in PGE2-treated CD34+ cells as compared to untreated (Fig. 17G). Similar results were obtained by RT-PCR, which demonstrated a slight increase (although not significant) in PD-L1 expression in CD34+ cells upon modulation with PGE2 (Fig. 17E). Notably, RT-PCR showed a 2-fold increase of CXCR4 gene expression, a protein required for the homing of HSPCs, in PGE2-treated CD34+ cells as compared to untreated (Fig. 17E). It was therefore sought to perform a migration assay in order to assess the homing properties of PGE2-treated CD34+ cells (Fig. 17F). The data confirmed a substantial increase in the homing potential of PGE2-treated CD34+ cells (Fig. 17F). The expression of another relevant immunoregulatory protein, IDO-1, remained unchanged post-pharmacologic modulation with PGE2 (Fig. 17H). The 4 PG small molecules (16,16-dimethyl PGE2, 16,16-dimethyl PGE2 4-[4-acetamidobenzamido] phenyl ester, 6-keto PGE1 and 20-ethyl PGE2) that showed the strongest capacity to upregulate PD-L1 were next selected based on the results obtained from library screening (Fig. 17I-17J). By a PGE2-analog and two competitive inhibitors of 15-hydroxy PG dehydrogenase (PGDH), which possess a prolonged half-life in vivo (16,16-dimethyl PGE2, 16,16-dimethyl PGE2 4-(4-acetamidobenzamido) phenyl ester and 20-ethyl PGE2). FACS analysis of PD-L1 protein expression following pharmacologic modulation with these 4 PGs (Fig. 17I-17J) demonstrated robust upregulation of PD-L1 expression. These results were further confirmed by RT-PCR, which showed a marked upregulation of PD-L1 mRNA following pharmacological modulation with 16,16-dimethyl PGE2, 16,16-dimethyl PGE2 4-(4-acetamidobenzamido) phenyl ester and 20-ethyl PGE2 (data not shown).

[0235] PGE2 highly augments PD-L1 expression in human HSPCs when supplemented with hematopoietic cytokine. In order to improve the strategy used for HSPC expansion and to enhance the function of PGE2-modulated HSPC, hematopoietic cytokines (SCF, TPO, FGF-1, IGFBP-2 and Angptl-3 proteins) known as a potent cocktail for HSPC maintenance, were added into the established culture conditions (22). Isolated CD34+ cells (HSPCs) obtained from T1D patients and from healthy controls were cultured using StemSpan SFEMII supplemented with the aforementioned human stem cell growth factors (STFIA medium) and pulsed with PGE2 (10 μ M) at 24 hours, 96 hours and at 7 days at 37 °C 5% CO₂. PD-L1+ HSPCs were then quantified by FACS analysis at different time points post-culture. After 7 days, a ~5-fold increase in the percentage of PD-L1+CD34+ cells were evident in human HSPCs obtained from T1D, with a similar albeit much less pronounced increase in the percentage of PD-L1+CD34+ cells obtained from healthy control patients (~2-fold increase) (Fig. 18A-18E). It was next determined whether freezing/cryopreservation has any effect on PD-L1 expression, by comparing freshly isolated HSPCs with frozen HSPCs, after 7 days of culture using STFIA media pulsed with PGE2 (Fig.

18F-18G). Sustained and conserved PD-L1 expression was observed pre- and post-cryopreservation, indicating that storage of HSPCs has no detrimental impact on their ex vivo expansion.

[0236] PGE2-modulated human HSPCs abrogate the autoimmune response ex vivo. To study the ex vivo immunoregulatory effects of PGE2 modulation as well as whether cytokine treatment enhances these effects, an autoimmune assay was performed using unmodulated CD34+ cells, PGE2-modulated CD34+ cells, or PGE2-modulated HSPCs cultured for 7 days in STFIA medium. CD34-depleted PBMCs were co-cultured with control CD34+ cells (unmodulated), PGE2-modulated CD34+ cells or STFIA medium-cultured PGE2-modulated human CD34+ cells in the presence of insulin-associated autoantigen-2 (I-A2) peptide at different cell ratios (1:2; 1:8 and 1:32 CD34+ cells to PBMCs), and the number of IFN- γ -producing cells was quantified using an ELISPOT assay (Fig. 18H-18I). Interestingly, addition of PGE2-modulated human CD34+ cells resulted in a significant decrease in the number of IFN- γ -producing cells (Fig. 18H), indicating that PGE2-modulated CD34+ cells are endowed with immunoregulatory activity ex vivo. Addition of PGE2-modulated CD34+ cells cultured for 7 days in STFIA medium showed a further abrogation of the autoimmune response, and this effect was observed even when cells were added at a very low ratio (1:32) to PBMCs (Fig. 18I). The effects of PGE2-modulated CD34+ cells cultured for 7 days in STFIA media were further confirmed in a nonautoimmune-specific anti-CD3/anti-CD28 assay (Fig. 18J).

[0237] Murine PGE2-modulated HSPCs abrogate the autoimmune response in vitro. The feasibility of pharmacological modulation of PD-L1 with PGE2 was next explored in murine HSPCs. FACS analysis showed an upregulation of PD-L1 post-PGE2 modulation in KL (c-Kit+Lineage-) cells isolated from bone marrow of NOD mice (Fig. 19A-19B). Interestingly, another protein, CXCR4, primary involved in the homing of HSPCs, was markedly upregulated (Fig. 19C-19D). The expression of costimulatory molecules as well as pro-inflammatory and anti-inflammatory cytokines by flow cytometry, was then analyzed, which demonstrated upregulation of PD-L2, PD-1, CD80 and CD40 in PGE2-modulated KL cells as compared to unmodulated KL cells. The expression of these molecules in KL cells modulated with dmPGE2 (known as 16, 16-dimethyl PGE2, a molecule which exerts a prolonged effect in vivo as compared to PGE2) was similarly upregulated (Fig. 19E). Moreover, the chemokine receptor profile of PGE2-modulated KL cells as compared to dmPGE2-modulated KL cells and unmodulated KL cells was explored in order to assess which chemokines are potentially involved in the homing of PGE2-modulated and dmPGE2-modulated KL cells. Consistent with the results in Fig. 19C, CXCR4 was the most expressed chemokine in both groups of KL cells treated with PGE2 and dmPGE2 (Fig. 19F). The immunoregulatory properties of PGE2-modulated KL cells was next explored in an autoimmune setting in vitro. PGE2-modulated KL cells generated from normoglycemic NOD mice were co-cultured at ratios of 1:1, 1:5 and 1:10 (KL cells to T cells) with CD11c+ DCs and BDC2.5 transgenic CD4+CD25- T cells in the presence of BDC2.5 peptide. Quantification by flow cytometry revealed a pronounced and significant decrease in the percentage of IFN- γ +CD4+ T cells when PGE2-

modulated KL cells were added to the assay as compared to when unmodulated KL cells were used (Fig. 20A-20B). Indeed, PGE2-modulated KL cells exerted a robust immunoregulatory effect even if added at low ratios (1:5 PGE2-treated KL cell to T cells). A less pronounced effect was observed when unmodulated KL were added to the assays. Interestingly, the percentage of activated CD4+CD25+ T cells declined upon coculture with KL or PGE2-modulated KL cells (Fig. 20C). PGE2-modulated HSPCs are thus endowed with immunoregulatory properties and are capable of abrogating the autoimmune response in vitro.

[0238] Adoptively transferred murine PGE2-modulated HSPCs traffic to inflamed areas. To examine the trafficking properties of GFP+ PD-L1-expressing KL cells in an in vivo inflammatory setting, a set of tracking experiments was performed in NOD mice. Following infusion of GFP+ KL cells extracted from the bone marrow of Luciferase NOD-GFP mice and treated with PGE2 as previously described, the pancreas and pancreatic draining lymph nodes (PLN) of NOD mice were harvested at 24 hours. GFP+ cells were quantified in the aforementioned tissues by flow cytometry and were detectable in the PLN (Fig. 20D) and in the pancreata of NOD mice (Fig. 20E). The phenotype of the GFP+ cells after adoptive transfer and after homing to the pancreata of NOD mice showed Gr-1 expression, indicative of myeloid lineage, while very few cells were CD3+ (Fig. 20E). These GFP+ PD-L1-expressing cells might probably interact with autoreactive CD4 and CD8 T cells.

Discussion

[0239] The prospect of successful cell therapy has recently gained greater footing in the medical landscape in the past 2 years with the arrival of many cell-based products. Recently, many AHSCT-related clinical trials have demonstrated a beneficial effect in the treatment of several autoimmune diseases, and AHSCT is now considered one of the few therapies capable of reversing T1D in humans (6, 14, 25-27). In this study described herein, preservation of β cell function following AHSCT was observed, as most patients included in the study population exhibited a sustained and adequate postprandial C-peptide response. The majority of these patients achieved and maintained peak-stimulated C-peptide levels higher than 0.6 ng/ml for at least 2 years of follow-up. Sustained C-peptide secretion is known to be associated with reduced prevalence (~30%) of hypoglycemic events and with a slower progression of diabetes complications, as reported by the DCCT Trial (28). Several patients also experienced reversal of the disease or a decrease in the exogenous insulin daily requirement. Although these are very encouraging results, many investigators have reported various complications and adverse effects associated with AHSCT in T1D patients, primarily related to the effects of immunosuppression (6). Some patients experience only temporary remission, and thus achieving prolonged remission of the disease remains the foremost goal for future clinical trials. Recently, much progress has been made with regard to the identification of small molecules and growth factors capable of both enhancing HSPC proliferation (15, 16) and further expanding the immunomodulatory subsets of HSPCs, in order to capitalize on their immunosuppressive properties. Interestingly, a screening study performed in zebrafish

embryos showed that prostaglandin E2 (PGE2) enhances HSPC expansion and facilitates HSPC engraftment after bone marrow transplantation (21). Investigating and determining the effects of ex vivo modulation of HSPCs with PGE2 in an autoimmune setting may provide insight with regard to how to robustly enhance their immunoregulatory properties. The screening results performed on ~64 known prostaglandins (PGs) allowed the selection of 4 PGs, which are analogs to PGE2 and which show induce relatively high upregulation of PD-L1 expression on human CD34+ cells. It was therefore sought to test the ability of PGE2-modulated HSPCs to affect the autoimmune response in vitro. Compared to unmodulated HSPCs, HSPCs overexpressing PD-L1 successfully abrogated the human autoimmune response in vitro. Next, it was sought to explore whether refining the ex vivo culture approach by including a cocktail of potent cytokines important for HSPC maintenance and extending the length of culture to 7 days could enhance the effects observed. Importantly, this approach remarkably enhanced the immunoregulatory properties of HSPCs and induced more pronounced PD-L1 expression. This expression appeared to be stable, unaffected by the freeze/thaw process, and resulted in a potent abrogation of the autoimmune response by modulated HSPCs, even when added at a very low ratio to T cells. Paralleling the human data, these preclinical murine studies also confirmed that PGE2-modulated HSPCs similarly exhibited immunoregulatory effects, as they markedly abrogated CD4-restricted autoimmune responses in vitro. In vivo tracking studies indicated that PGE2-modulated HSPCs home to the inflamed pancreas and PLN of NOD mice, most likely due to their substantial expression of CXCR4 (9). Based on the data herein, ex vivo expansion strategies with PGE2 combined with hematopoietic cytokines could generate a novel immunoregulatory HSPC-based approach potentially useful in the treatment of autoimmune T1D, without the detrimental effect of immunosuppressive agent toxicity, which is observed with standard immunotherapy. The recent discovery that a pre-established suicide genetic system may control survival and prevent toxicity of HSPCs undergoing ex vivo expansion will implement their use in clinical settings, allowing for easier manipulation of HSPCs and for a cell therapy-based approach in immune-mediated disorders (29).

[0240] Table 1. Baseline demographic and clinical characteristics of patients with T1D treated with autologous non-myeloablative hematopoietic stem cell transplantation in two AHCST cohorts.

Patient characteristics	
Number of patients included	n=36
Age (years±SEM)	22.4±0.9
Gender (M/F)	27/9
BMI (kg/m ² ±SEM)	20.7±0.5
HbA1c (mmol/mol ±SEM)	86.6±6.4
C-peptide (ng/mL ±SEM)	0.73±0.06
Autoantibodies	(% of patients)

<i>GAD</i>	86
<i>Other (IAA, IA-2A, ICA)</i>	17
DKA or DK history	(% of patients)
<i>No DKA/DK</i>	67
<i>DKA</i>	28
<i>DK</i>	5

[0241] Abbreviations used in Table 1. T1D, type 1 diabetes; AHSCT, autologous hematopoietic stem cell transplantation; BMI, body mass index; GAD, glutamic acid decarboxylase autoantibodies; ICA, islet cell cytoplasmic autoantibodies; IA2A, insulinoma-2-associated autoantibodies; IAA, insulin autoantibodies; DKA, diabetic ketoacidosis; DK, diabetic ketosis.

[0242] Table 2. Clinical characteristics of patients with T1D and of healthy controls included in the PGs library screening.

Patient characteristics

Number of patients included	n=24
Age (years±SEM)	58.2±11.6
Gender (M/F)	16/8
BMI (kg/m ² ±SEM)	20.7±0.5
EIR (UI)	18.3 ± 5.4
Concomitant treatment	Levothyroxine (n=8) Statin (n=5)
Healthy control characteristics	
Number of individuals included	n=5
Age (years±SEM)	40.8±6.4
Gender (M/F)	2/3

[0243] Abbreviations used in Table 2. T1D, type 1 diabetes; BMI, body mass index; EIR, exogenous insulin requirements.

[0244] EXAMPLE 3

[0245] *Material and Methods*

[0246] *In vitro studies*

[0247] *miRNA mimic/anti-miR transfection.* MDA-MB-231 breast cancer cells were cultured in DMEM high glucose medium (Gibco, Thermo Fisher Scientific; Waltham, MA USA) supplemented with 10% heat-inactivated fetal calf serum (Gibco, Thermo Fisher Scientific), 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. 2×10^5 cells were seeded in each well of a 6-well plate and transfected with 10 pmol of the miRNA mimic or miRNA mimic negative control, or 100 pmol of the anti-miR or anti-miR negative control (all from Exiqon, Qiagen) using the Lipofectamine RNAiMAX transfection reagent (Invitrogen, Thermo Fisher Scientific) in a final culture medium volume of 2 ml, following manufacturer's instructions. Details (e.g., the targeted miRNA and nucleotide sequence) on the used miRNA mimics and anti-miRs are displayed herein in Table 12. Forty-eight hours after transfection, cells were collected from each well for RNA extraction, cell lysate preparation and FACS analysis. RNA was extracted using Direct-zol RNA miniprep plus (Zymo research, Irvine, CA, USA) and RNA quality was checked and then retro-transcribed using RETROscript® Kit (Fisher Scientific) following manufacturer's instructions. Cell lysates were obtained in RIPA buffer (50 mmol/l Tris-HCl, pH 8.0, 1% Triton-x, 0.5% sodium deoxycholate, 0.1% SDS, 150 mmol/l sodium chloride) with protease inhibitor cocktail (Roche).

[0248] Table 12. List of miRNA mimics and anti-miRs and targeted miRNAs.

Targeting agent	Targeted miRNA	Sequence	Concentration
miRNA mimic	hsa-miR-125b-5p	UCCCUGAGACCCUAACUUGUGA	5 nM
miRNA mimic	hsa-miR-511-3p	AAUGUGUAGCAAAAGACAGA	5 nM
miRNA mimic	hsa-miR-99a	AACCCGUAGAUCGGAUCUUGUG	5 nM
miRNA mimic	hsa-miR-744-5p	CUGUUGCCACUAACCUCAACCU	5 nM
miRNA Mimic, Negative Control	-	UCACCGGGUGUAAAUCAGCUUG	5 nM
Anti-miR	hsa-miR-599	GUUUGAUAAACUGACACAAC	50 nM
Anti-miR	hsa-miR-206	CACACACUCCUUAUACAUUCC	50 nM
Anti-miR	hsa-miR-26b-5p	CUAUCCUGAAUUACUUGA	50 nM
Anti-miR, Negative Control	-	UAACACGUCUAUACGCCCA	50 nM

[0249] *qRT-PCR.* qRT-PCR for PD-L1 analysis was performed on retro-transcribed cDNA using SYBR® Green dye (Life Technologies, Thermo Fisher Scientific). Amplification was performed on a QuantStudio S6 Real Time PCR system (Thermo Fisher Scientific). To confirm a successful transfection, the mRNA level of genes known to be controlled by the targeted miRNAs were evaluated by SYBR® Green dye (Life Technologies, Thermo Fisher Scientific) according to the manufacturer's instructions. Normalized expression values were determined using the $\Delta\Delta C_t$ method in treated as compared to negative treated samples, using GAPDH mRNA as endogenous reference. The miRNA-

targeted genes tested, along with forward and reverse primer sequences used for their amplification, are listed in Table 13.

[0250] Table 13. Gene IDs and qPCR primer sequences of miRNA targets used as positive controls for miRNA mimic/anti-miR transfection.

Gene/ Targeting miRNA	Targeted gene	Primer Sequence	
		Forward:	Reverse:
PD-L1	-	5'-GCAAAGTGATACACATTTGGAGGA-3'	5'-CCCCGATGAACCCCTAAACC-3'
		5'-GCAAAGTGATACACATTTGGAGGA-3'	5'-CCCCGATGAACCCCTAAACC-3'
hsa-miR-125b-5p	NEU1	5'-GCACATCCAGAGTTCCGAGT-3'	5'-CAGGGTTGCCAGGGATGAAT-3'
		5'-GCACATCCAGAGTTCCGAGT-3'	5'-CAGGGTTGCCAGGGATGAAT-3'
hsa-miR-99a-5p	MTOR	5'-GGGCCATCCGGAATTTTTTG-3'	5'-TCGTGCTCTGAATTGAGGTGT-3'
		5'-GGGCCATCCGGAATTTTTTG-3'	5'-TCGTGCTCTGAATTGAGGTGT-3'
hsa-miR-744-5p hsa-mir-599	MYC	5'-CGTCCTCGGATTCTCTGCTC-3'	5'-TGTTCTCCTCAGAGTCGCT-3'
		5'-CGTCCTCGGATTCTCTGCTC-3'	5'-TGTTCTCCTCAGAGTCGCT-3'
hsa-miR-511-3p	IGF1R	5'-CCTGACATGCTGTTTGAAGTGA-3'	5'-GCTTGTTCTCCTCGCTGTAGT-3'
		5'-CCTGACATGCTGTTTGAAGTGA-3'	5'-GCTTGTTCTCCTCGCTGTAGT-3'
hsa-miR-206	VEGFA	5'-CTTGCTGCTCTACCTCCACC-3'	5'-TGAAGTTCACCACTTCGTGATG-3'
		5'-CTTGCTGCTCTACCTCCACC-3'	5'-TGAAGTTCACCACTTCGTGATG-3'
hsa-miR-26b-5p	RB1	5'-CTGAAGGAAGCAACCCTCCT-3'	5'-TCGAGTAGAAGTCATTTCTGCCA-3'
		5'-CTGAAGGAAGCAACCCTCCT-3'	5'-TCGAGTAGAAGTCATTTCTGCCA-3'
Endogenous reference gene	GAPDH	5'-GTGAACCATGAGAAGTATGACAAC-3'	5'-CATGAGTCCTTCCACGATACC-3'
		5'-GTGAACCATGAGAAGTATGACAAC-3'	5'-CATGAGTCCTTCCACGATACC-3'

[0251] *Western blot.* Protein concentration in MDA-MB-231 cell lysates was measured. Fifteen micrograms of total proteins were electrophoresed on 8-16% gradient SDS-PAGE gels and blotted onto PVDF membrane (Bio-Rad, Hercules, CA, USA). Blots were then stained with Ponceau S. Membranes were blocked for 1 h in 5% non-fat dry milk in TBST (Tris [10 mmol/l], NaCl [150mmol/l]), 0.1% Tween-20, 5% non-fat dry milk, pH 7.4 at 25°C) and then incubated for 12 h with a polyclonal rabbit anti-human PD-L1 antibody (Santa Cruz Biotechnology, Dallas, TX, USA) diluted 1:200 or with a monoclonal rabbit anti-β-tubulin antibody (Abcam, Cambridge, UK) diluted 1:10,000 in TBS–5% milk at 4°C, washed four times with TBS–0.1% Tween-20, then incubated with a peroxidase-labeled mouse anti-rabbit IgG secondary antibody diluted 1:80,000 (Sigma-Aldrich, Saint Louis, MO, USA) in TBS–5% milk for 1 h, and finally washed with TBS–0.1% Tween-20. The resulting bands were visualized using Clarity Max western ECL substrate (Bio-Rad, Hercules, CA, USA) on a Uvitec Mini HD9 (Cleaver Scientific, Rugby, Warwickshire, UK) image documentation system. Finally, for the quantification of western blot, images of PVDF membranes were analyzed by ImageJ software to quantify size and strength of protein bands.

[0252] *Flow cytometric analysis.* Flow cytometry was performed to analyze PD-L1 surface expression on miRNA mimic-treated MDA-MB-231 cells. BV421 labelled anti-human PD-L1 (BD

Biosciences) was used to stain the cells. Background staining was determined using BV421 labelled mouse IgG1, nonreactive isotype-matched control antibody with gates positioned to exclude 99% of non-reactive cells. Cells were subjected to FACS analysis and were run on a FACSCelesta™ (Becton Dickinson). Data were analyzed using FlowJo software version 8.7.3 (Treestar).

Results

[0253] *miRNA targeting decreases PD-L1 in human cancer cells.* To determine if targeting the miRNA network decreases PD-L1 expression in human cancer setting, PD-L1-expressing MDA-MB-231 human breast cancer cells were transfected with a set of miRNA mimics or anti-miRs to either increase or decrease the function of a set of miRNAs not previously known to affect PD-L1 (miR-125b, miR-99a, miR-744, miR-599, miR-511, miR-206, miR-26b). The effect of treatment with miRNA mimics and anti-miRs on the percentage of PD-L1-expressing cells, as well as on PD-L1 mRNA and protein levels, was investigated. miRNA mimic/anti-miR treated-cells were compared to corresponding mimic/anti-miR negative control-treated cells by flow cytometric analysis, Real Time PCR and western blot to assess percentage of PD-L1-expressing cells, PD-L1 mRNA, and PD-L1 protein levels, respectively. A successful miRNA mimic/anti-miR transfection was confirmed by the detection of changed mRNA levels of the validated miRNA targets NEU1 (for miR-125b mimic), MTOR (for miR-99a mimic), MYC (for miR-744 mimic and miR-599 anti-miR), IGF1R (for miR-511 mimic), VEGFA (for miR-206 anti-miR) and RB1 (for miR-26b anti-miR) in treated cells (Fig. 22A). Exposure of MDA-MB-231 cells to both miRNA mimics and anti-miRs resulted in decreased levels of both PD-L1 mRNA (Fig. 22B) and protein (Fig. 22C and 22D) as compared to the corresponding negative controls, with the only exception of miR-599 anti-miR, for which an increase of PD-L1 protein was observed. Lysates of miR-511 mimic and miR-206 anti-miR treated cells were not available for western blot analysis. Furthermore, FACS analysis showed that all the used miRNA mimics and anti-miRs induced a decreased proportion of PD-L1-expressing cells as compared to controls (Fig. 23A-23D). Overall, these findings show that PD-L1 expression can be decreased in cancer cells by targeting miR-125b, miR-99a, miR-744, miR-511, miR-206, miR-26b, indicating that targeting the miRNA network controlling PD-L1 can be employed as a tool to regulate PD-L1 expression in cancer cells.

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[000248] Table 3. Transcriptomic profiling of murine KLS cells. List of differentially expressed pro-/anti-inflammatory genes identified by transcriptomic profiling in Sca-1⁺Lineage⁻c-kit⁺ (KLS) cells from NOD as compared to those obtained from C57BL/6 mice.

Table 3		
Refseq	Symbol	p value
NM_007722	Ackr3	0.112734
NM_009645	Aicda	0.517745
NM_009741	Bcl2	0.453924
NM_009743	Bcl2l1	0.352899
NM_011333	Ccl2	0.032665
NM_016960	Ccl20	0.301623
NM_009137	Ccl22	0.501808
NM_020279	Ccl28	0.305028
NM_013652	Ccl4	0.00679
NM_013653	Ccl5	0.682931
NM_009912	Ccr1	0.986653
NM_007721	Ccr10	0.416696
NM_009915	Ccr2	0.052858
NM_009916	Ccr4	0.593762
NM_009917	Ccr5	0.24604
NM_007719	Ccr7	0.066286
NM_009913	Ccr9	0.005054
NM_021893	Cd274	0.003313
NM_007778	Csf1	0.142109
NM_009969	Csf2	0.832481
NM_009971	Csf3	0.560767
NM_009843	Ctla4	0.07252
NM_008176	Cxcl1	0.130166
NM_021274	Cxcl10	0.275381
NM_019494	Cxcl11	0.69816
NM_021704	Cxcl12	0.019921
NM_009140	Cxcl2	0.484508
NM_009141	Cxcl5	0.419449
NM_008599	Cxcl9	0.278309
NM_178241	Cxcr1	0.464608
NM_009909	Cxcr2	0.448862
NM_009910	Cxcr3	0.753073
NM_009911	Cxcr4	0.940543
NM_007551	Cxcr5	0.461243
NM_010113	Egf	0.564223
NM_007912	Egfr	0.745452

NM_010177	Fasl	0.855735
NM_054039	Foxp3	0.305028
NM_010259	Gbp2b	0.50175
NM_010370	Gzma	0.827176
NM_013542	Gzmb	0.208598
NM_010380	H2-D1	0.008824
NM_001001892	H2-K1	0.001135
NM_010431	Hif1a	0.472615
NM_008324	Ido1	0.095082
NM_008337	Ifng	0.347564
NM_010512	Igf1	0.487476
NM_010548	Il10	0.295336
NM_008351	Il12a	0.011179
NM_008352	Il12b	0.209679
NM_008355	Il13	0.152192
NM_008357	Il15	0.039649
NM_010552	Il17a	0.461524
NM_010554	Il1a	0.653187
NM_008361	Il1b	0.479829
NM_008362	Il1r1	0.030932
NM_008366	Il2	0.00208
NM_016971	Il22	0.247782
NM_031252	Il23a	0.418581
NM_021283	Il4	0.005864
NM_010558	Il5	0.349687
NM_031168	Il6	0.187398
NM_008390	Irf1	0.221717
NM_013598	Kitl	0.913051
NM_010798	Mif	0.327861
NM_010849	Myc	0.647938
NM_010851	Myd88	0.295548
NM_008689	Nfkb1	0.498591
NM_010927	Nos2	0.967604
NM_008798	Pdcd1	0.34361
NM_011198	Ptgs2	0.259837
NM_009263	Spp1	0.455322
NM_009283	Stat1	0.118395
NM_011486	Stat3	0.133445
NM_011577	Tgfb1	0.910619
NM_011905	Tlr2	0.951419
NM_126166	Tlr3	0.278306

NM_021297	Tlr4	0.685418
NM_133211	Tlr7	0.016282
NM_031178	Tlr9	0.08124
NM_013693	Tnf	0.056009
NM_009425	Tnfsf10	0.000973
NM_011640	Trp53	0.947607
NM_009505	Vegfa	0.203475
NM_007393	Actb	0.915067
NM_009735	B2m	0.006167
NM_008084	Gapdh	0.084597
NM_010368	Gusb	0.315452
NM_008302	Hsp90ab1	0.882837
SA_00106	MGDC	0.305028
SA_00104	RTC	0.385845
SA_00104	RTC	0.417732
SA_00104	RTC	0.317356
SA_00103	PPC	0.047277
SA_00103	PPC	0.49981
SA_00103	PPC	0.212719

[000249] Table 4. Genome-wide expression analysis of Tg KL cells. List of differentially expressed genes identified by genome-wide expression analysis (GWAS) performed on Tg.KL cells as compared to Mock.KL cells isolated from NOD mice ($p < 0.05$).

Transcript Cluster ID	Tg	Mock	Fold Change	Gene Symbol	Description
TC1900000441.MM.1	14.12	5.77	327.59	Cd274 (PD-L1)	CD274 antigen
TC0600000577.MM.1	13.41	5.09	320.41	Gpmb	glycoprotein (transmembrane) mb
TC1200002584.MM.1	14.28	6.83	174.57	LOC544905	Ig heavy chain V region; immunoglobulin heavy variable V9-3
TC0900003098.MM.1	15.11	8.16	123.97	Camp	cathelicidin antimicrobial peptide
TC0300000811.MM.1	17.5	11	90.29	S100a8	S100 calcium binding protein A8 (calgranulin A)
TC120000647.MM.1	12.62	6.29	80.58	Ighv8-12	Ig heavy chain V region; immunoglobulin heavy variable V8-12
TC0700003570.MM.1	10.4	4.54	57.92	Anpep	alanyl (membrane) aminopeptidase
TC0900001461.MM.1	15.04	9.26	54.98	Ngp	neutrophilic granule protein
TC1500001728.MM.1	13.74	7.97	54.58	Ly6a	lymphocyte antigen 6 complex, locus A (lys6a), mRNA.

TC1000003214.MM.1	16.38	10.61	54.48	Lilrb4	leukocyte immunoglobulin-like receptor, subfamily B, member 4
TC0400001645.MM.1	5.82	11.6	-54.6	Rhd	Rh blood group, D antigen
TSU nmapped00000051.mm.1	11.85	17.78	-61.14	Ahsp	alpha hemoglobin stabilizing protein
TC0600000664.mm.1	7.43	13.67	-75.45	Aqp1	aquaporin 1
TC0400003418.mm.1	6.47	13.34	-117.38	Ermap	erythroblast membrane-associated protein
TC0500003382.mm.1	6.2	13.48	-155.47	Cldn13	claudin 13
TC0300001667.mm.1	11	18.4	-169.05	Car1	carbonic anhydrase 1
TC1700000817.mm.1	6.47	14.3	-228.24	Rhag	Rhesus blood group-associated A glycoprotein
TC0300000094.MM1	9.67	17.85	-290.12	Gm5843	predicted gene 5843; carbonic anhydrase 1 (car1) pseudogene

[000250] Table 5. Chemokine receptors expression in different groups of KL cells. List of differentially expressed chemokine receptors in PD-L1.Tg KL cells, pharmacologically-modulated KL cells (pKL) and unmodulated-KL cells (KL-Veh) isolated from bone marrow of normoglycemic NOD mice. Data are expressed as mean $\pm$ standard error (SEM). *mean statistically significant vs. others.

Chemokine receptors	Expression on KL-Veh (%)	Expression on pKL (%)	Expression on PD-L1 Tg.KL (%)	P value
CCR2	6.1 $\pm$ 0.1	5.2 $\pm$ 0.0	2.9 $\pm$ 0.1*	0.02
CCR4	1.4 $\pm$ 0.1	3.1 $\pm$ 0.2	11.6 $\pm$ 1.1*	0.02
CCR5	0.7 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	ns
CCR6	2.1 $\pm$ 0.3	8.6 $\pm$ 0.7*	5.3 $\pm$ 0.6	0.02
CCR7	2.7 $\pm$ 0.3	0.2 $\pm$ 0.0	0.7 $\pm$ 0.3	ns
CCR8	2.0 $\pm$ 0.4	9.8 $\pm$ 0.4	2.0 $\pm$ 0.1	ns
CXCR3	0.2 $\pm$ 0.1	0.0 $\pm$ 0.0	1.7 $\pm$ 0.8	ns
CXCR4	41.7 $\pm$ 0.3	37.0 $\pm$ 2.5	61.6 $\pm$ 1.9*	0.004
S1PR1	65.4 $\pm$ 0.7	66.3 $\pm$ 0.8	64.6 $\pm$ 0.4	ns

[000251] Table 6. Genome-wide expression analysis of pKL cells: up-regulated genes. List of upregulated genes identified by genome wide expression analysis (GWAS) performed on pharmacologically-modulated KL cells (pKL) as compared to vehicle-treated KL cells isolated from normoglycemic NOD mice ($p < 0.05$).

Table 6					
Transcript Cluster ID	pKL	KL	Fold Change	ANOVA p-value	Gene Symbol
TC1800000609.mm.1	15.67	4.57	2202.84	0.033892	F830016B08Rik
TC0500002755.mm.1	13.77	3.24	1472.96	0.009558	Cxcl9
TC0300003133.mm.1	15.32	5.7	785.65	0.02577	Ifi44
TC1400002869.mm.1	14.03	5.31	421.89	0.021385	Phf11d
TC0500002895.mm.1	13.79	5.11	408.6	0.005609	Gbp10
TC1800000610.mm.1	13.45	4.87	382.79	0.015108	Iigp1
TC1800000607.mm.1	12.57	4.06	366.25	0.030559	Gm5970
TC1400002162.mm.1	14.88	6.45	344.7	0.007689	Phf11b
TC1800000606.mm.1	13.01	4.79	298.3	0.0158	Gm4951
TC0200005290.mm.1	14.14	6	282.59	0.019226	Zbp1
TC0500003731.mm.1	13.87	5.78	273.4	0.023606	Gbp4
TC0500001240.mm.1	13.77	5.74	260.57	0.012623	Oasl1
TC0600001369.mm.1	16.86	9.07	221.84	0.026909	Usp18
TC1400001182.mm.1	12.78	5.13	202.12	0.00179	Irg1
TC1100001240.mm.1	12.24	4.76	178.73	0.011603	
TC0800000659.mm.1	12.87	5.42	174.56	0.014638	Ddx60
TC1600002148.mm.1	12.07	4.68	167.59	0.028162	Mx1
TC0300001447.mm.1	12.98	5.6	166.73	0.031497	Gbp2
TC1400002161.mm.1	12.09	4.8	156.37	0.019668	Phf11a
TC0500002756.mm.1	11.45	4.17	155.68	0.003655	Cxcl10
TC1800001396.mm.1	12.7	5.45	152.28	0.030824	Gm4841
TC1700002816.mm.1	12.05	4.88	144.54	0.019777	Cfb
TC0500003730.mm.1	13.01	5.84	144.23	0.005339	Gbp9
TC1900001422.mm.1	11.51	4.46	132.76	0.004877	Gm14446
TC0100000744.mm.1	13.46	6.46	127.73	0.010464	Csprs
TC0700004530.mm.1	13.15	6.18	125.77	0.046568	Irf7
TC1600001092.mm.1	12.21	5.35	115.89	0.020935	Mx2
TC0500003175.mm.1	14.31	7.47	115.12	0.037819	Oas2
TC0300001444.mm.1	12.51	5.69	113.12	0.007548	Gbp2b
TC1100004265.mm.1	16.51	9.8	104.91	0.010256	Igtp
TC0100000727.mm.1	15.45	8.76	103.3	0.013707	Csprs
TC1200000225.mm.1	15.53	8.9	99.2	0.03583	Cmpk2
TC0500002896.mm.1	11.45	4.84	97.56	0.012491	Gbp11
TC0500003729.mm.1	11.74	5.27	88.47	0.025759	Gbp8
TC1100001241.mm.1	12.46	6.01	87.02	0.017125	Slfn4
TC0100003590.mm.1	11.67	5.4	77.1	0.034339	BC094916
TC1900001166.mm.1	10.13	4.01	69.53	0.04386	
TC0700003946.mm.1	14.6	8.49	68.84	0.00128	Trim30d
TC1000002383.mm.1	11.94	5.95	63.71	0.025344	Gstt1

TC1000001994.mm.1	11.77	5.87	59.87	0.030488	Fam26f
TC0300001446.mm.1	12.18	6.29	59.67	0.012022	Gbp3
TC1700002823.mm.1	12.27	6.4	58.8	0.02762	H2-T10
TC1900001123.mm.1	13.1	7.23	58.21	0.025695	AW112010
TC1900000500.mm.1	13.22	7.36	58.19	0.047266	Ifit2
TC1100000683.mm.1	13.37	7.53	57.31	0.036366	
TC1500001729.mm.1	12.61	6.81	55.91	0.043783	Ly6c1
TC0300000161.mm.1	11.44	5.74	51.86	0.042309	Tnfsf10
TC0100001574.mm.1	10	4.38	49.2	0.005685	Fcgr4
TC1900000217.mm.1	15.44	9.82	49.06	0.001199	Ms4a4c
TC0100000730.mm.1	13.71	8.1	48.53	0.017127	
TC0100000739.mm.1	13.71	8.1	48.53	0.017127	
TC0100000748.mm.1	13.71	8.1	48.53	0.017127	
TC0100000753.mm.1	13.71	8.1	48.53	0.017127	
TC0100002706.mm.1	13.71	8.1	48.53	0.017127	
TC0500003459.mm.1	13.71	8.1	48.53	0.017127	
TC1_GL456211_random00000012.mm.1	13.71	8.1	48.53	0.017127	
TC1_GL456211_random00000017.mm.1	13.71	8.1	48.53	0.017127	
TC1_GL456212_random00000002.mm.1	13.71	8.1	48.53	0.017127	
TC1_GL456212_random00000012.mm.1	13.71	8.1	48.53	0.017127	
TC1_GL456221_random00000006.mm.1	13.71	8.1	48.53	0.017127	
TC1_GL456221_random00000023.mm.1	13.71	8.1	48.53	0.017127	
TC0600003503.mm.1	14.7	9.36	40.32	0.011732	Herc6
TC1100003382.mm.1	12.25	6.93	39.94	0.019584	Slfn8
TC1100003779.mm.1	13.06	7.75	39.72	0.007905	Dhx58
TC0700003947.mm.1	11.2	5.92	39.01	0.04821	
TC0700003942.mm.1	13.05	7.82	37.6	0.014688	Trim30c
TC0100003599.mm.1	13.19	7.97	37.41	0.035123	Ifi202b
TC1100003383.mm.1	13.5	8.27	37.41	0.016144	
TC0600002114.mm.1	14.64	9.5	35.1	0.03847	Parp12
TC1000001836.mm.1	9.09	3.97	34.81	0.00462	
TC1100001236.mm.1	9.9	4.79	34.44	0.042429	Slfn5
TC0700003941.mm.1	11.07	5.97	34.32	0.015197	Trim30b
TC1_GL456212_random00000008.mm.1	12.66	7.65	32.31	0.015784	
TC0100000375.mm.1	14.92	9.92	31.9	0.00176	Stat1
TC1100003381.mm.1	10.8	5.81	31.89	0.017379	
TC0100000758.mm.1	11.84	6.85	31.69	0.0269	Gm7592
TC1100002003.mm.1	15.08	10.1	31.45	0.049647	Rnf213
TC0200003630.mm.1	12.8	7.88	30.33	0.040556	Ifih1
TC1700002789.mm.1	13.47	8.56	30.08	0.028461	H2-Q7
TC0100000743.mm.1	11.75	6.85	29.89	0.019143	Gm7609

TC1200001552.mm.1	15.89	11.02	29.38	0.020888	Rsad2
TC0300001445.mm.1	15.83	11	28.46	0.012716	Gbp7
TC0500003436.mm.1	11.14	6.36	27.47	0.048814	A630081J09Rik
TC1700002788.mm.1	13.31	8.57	26.72	0.012048	H2-Q8
TC0100002711.mm.1	11.32	6.6	26.48	0.040411	Gm2635
TC1000001596.mm.1	14.86	10.2	25.3	0.00199	Stat2
TC1700002822.mm.1	10.35	5.78	23.9	0.040511	H2-T22
TC0700004009.mm.1	14.63	10.07	23.5	0.041694	Gm8995
TC1100002641.mm.1	12.07	7.54	23.2	0.003131	Irgm1
TC0700002644.mm.1	9.95	5.46	22.4	0.041784	Axl
TC0700003943.mm.1	13.98	9.51	22.09	0.02924	Trim30a
TC0100003891.mm.1	13.67	9.25	21.46	0.002795	Ifi203
TC1800000612.mm.1	9.36	4.94	21.42	0.021876	BC023105
TC0300002659.mm.1	11.72	7.31	21.28	0.047584	Mov10
TC0100000766.mm.1	14.19	9.79	21.08	0.017049	Sp100
TC0700004002.mm.1	12.92	8.54	20.72	0.035247	Gvin1
TC1600001556.mm.1	12.71	8.37	20.24	0.04905	Parp14
TC0100002709.mm.1	8.09	3.77	19.99	0.036287	
TC0900003335.mm.1	13.51	9.19	19.89	0.007649	Uba7
TC0100003595.mm.1	13.21	8.94	19.25	0.035899	Gm16340
TC0500001349.mm.1	11.47	7.21	19.14	0.041335	Oas1b
TC0200001222.mm.1	13.04	8.84	18.48	0.024467	Ube216
TC1100003380.mm.1	9	4.83	17.96	0.030712	Mir7679
TC0700003996.mm.1	12.59	8.43	17.88	0.016158	Gm4070
TC0400002108.mm.1	10.6	6.47	17.43	0.035926	AW011738
TC0600003231.mm.1	8.16	4.09	16.87	0.002582	Klrk1
TC0100000760.mm.1	8.29	4.31	15.78	0.017207	
TC1700000621.mm.1	12.25	8.32	15.29	0.001634	Tap1
TC0800001109.mm.1	8.93	5	15.21	0.041757	
TC0100003890.mm.1	13.91	10.04	14.62	0.013585	Mndal
TC0100001634.mm.1	7.98	4.11	14.58	0.010466	Pydc3
TC0500002757.mm.1	7.56	3.78	13.67	0.049141	Cxcl11
TC0500002843.mm.1	11.18	7.46	13.18	0.047259	Hpsc
TC1_GL456211_random00000020.mm.1	9.74	6.02	13.16	0.007954	
TC1_GL456221_random00000003.mm.1	9.74	6.02	13.16	0.007954	
TC1_GL456221_random00000015.mm.1	9.74	6.02	13.16	0.007954	
TC1900000441.mm.1	12.09	8.38	13.06	0.038552	Cd274
TC1400001087.mm.1	9.09	5.4	12.95	0.02631	
TC1700002526.mm.1	11.31	7.63	12.87	0.017876	Xdh
TC0700004003.mm.1	10.8	7.15	12.54	0.022983	RP24-196H4.1
TC0700001520.mm.1	10.7	7.05	12.49	0.008379	Trim34b

TC1600002101.mm.1	7.75	4.13	12.27	0.007698	
TC1100004292.mm.1	10.63	7.01	12.26	0.047885	9930111J21Rik1
TC1100002648.mm.1	10.38	6.76	12.25	0.034066	9930111J21Rik2
TC1100004266.mm.1	9.16	5.56	12.14	0.013088	Irgm2
TC0700003850.mm.1	9.8	6.21	12.07	0.013493	Il18bp
TC0400000464.mm.1	10.61	7.05	11.78	0.012918	Melk
TC1100000496.mm.1	9.4	5.86	11.61	0.010226	Ifi47
TC1_GL456211_random00000003.mm.1	9.71	6.18	11.54	0.027189	
TC0100002718.mm.1	13.51	9.98	11.51	0.03166	Gm7281
TC0100000736.mm.1	9.6	6.08	11.5	0.047915	Gm10553
TC1000001989.mm.1	12.08	8.56	11.42	0.00509	
TC1600001580.mm.1	10.86	7.38	11.17	0.015284	Cd86
TC0600001481.mm.1	9.7	6.23	11.14	0.046489	Parp11
TC1400002120.mm.1	12.38	8.91	11.07	0.006457	Gzmb
TC1_GL456210_random00000003.mm.1	16.02	12.57	10.95	0.020413	
TC1100003314.mm.1	16.29	12.84	10.93	0.010389	Lgals9
TC0100000735.mm.1	16.35	12.91	10.89	0.017303	Gm2389
TC0700003998.mm.1	10.55	7.11	10.86	0.037839	Gm8979
TC0200005181.mm.1	12.11	8.67	10.86	0.018902	Znfx1
TC0200005013.mm.1	14.62	11.23	10.48	0.039543	Samhd1
TC1_GL456210_random00000011.mm.1	15.94	12.56	10.46	0.001016	
TC0100003550.mm.1	8.93	5.56	10.36	0.046786	Slamf7
TC1600000500.mm.1	10.13	6.77	10.28	0.016439	Parp9
TC0700004007.mm.1	12.16	8.8	10.27	0.018557	Gm21884
TC0100000757.mm.1	13.48	10.15	10.11	0.039387	Gm2427
TC1100000209.mm.1	11.2	7.87	10.03	0.003163	
TC1400002870.mm.1	11.33	8.03	9.87	0.014639	Setdb2
TC1900000246.mm.1	11.08	7.78	9.85	0.030917	Mpeg1
TC0200003528.mm.1	11.01	7.74	9.62	0.024083	Nmi
TC0100000765.mm.1	13.56	10.3	9.6	0.02966	Sp140
TC0700001116.mm.1	11.34	8.11	9.37	0.040369	Isg20
TC1700001902.mm.1	10.92	7.72	9.2	0.009689	Psmb9
TC0500003083.mm.1	8.17	4.97	9.13	0.025155	Gm13822
TC0200003234.mm.1	12.35	9.21	8.86	0.023196	Gfi1b
TC0700003948.mm.1	11.54	8.39	8.85	0.018418	Gm16464
TC1700000684.mm.1	10.36	7.24	8.69	0.011494	H2-Q4
TC1700002778.mm.1	12.14	9.03	8.66	0.038873	Tapbp
TC1100000206.mm.1	8.71	5.65	8.33	0.031788	2610024D14Rik
TC1000001347.mm.1	8.21	5.17	8.22	0.013768	4933412E12Rik
TC0700003945.mm.1	8.67	5.64	8.19	0.005147	
TC0100003320.mm.1	11.45	8.43	8.12	0.036118	Tor3a

TC1900000502.mm.1	7.26	4.24	8.09	0.008644	I830012O16Rik
TC0100000742.mm.1	11.89	8.93	7.81	0.032576	Gm6264
TC1600000936.mm.1	9.75	6.79	7.75	0.049237	Mir155
TC0700004008.mm.1	8.7	5.76	7.68	0.005904	Gm4759
TC1000001988.mm.1	11.92	8.98	7.66	0.009241	Zufsp
TC1100000205.mm.1	10.3	7.37	7.63	0.045181	Peli1
TC1300001772.mm.1	9.68	6.75	7.6	0.037364	Gm6093
TC0800000817.mm.1	8.31	5.38	7.57	0.017572	Hsh2d
TC0100002707.mm.1	11.47	8.57	7.48	0.011384	C130026I21Rik
TC0300001667.mm.1	14.49	11.59	7.44	0.041085	Carl
TC1900001388.mm.1	9.57	6.7	7.3	0.038598	Asah2
TC1500001727.mm.1	8.11	5.26	7.19	0.005322	Ly6i
TC1700001892.mm.1	11.66	8.82	7.19	0.019862	H2-K1
TC1700002820.mm.1	9.99	7.18	7.02	0.000582	C920025E04Rik
TC0900000599.mm.1	7.43	4.62	7	0.02757	Il18
TC1_GL456221_random00000019.mm.1	8.64	5.85	6.93	0.040922	Csprs
TC1100003385.mm.1	9.36	6.61	6.73	0.026339	Slfn10-ps
TC0800002842.mm.1	12.67	9.95	6.57	0.025686	Psmb10
TC1100001657.mm.1	11.54	8.84	6.52	0.010344	Ifi35
TC0600002342.mm.1	12.82	10.16	6.31	0.029025	Nt5c3
TC1900000219.mm.1	8.23	5.58	6.25	0.014976	Ms4a4b
TC1700001993.mm.1	9.52	6.87	6.25	0.028963	H2-T24
TC0100002726.mm.1	9.14	6.52	6.14	0.044593	Gm10552
TC1700002790.mm.1	14.52	11.91	6.11	0.011267	H2-L
TC1900000141.mm.1	9.64	7.03	6.1	0.013319	Pla2g16
TC0900001431.mm.1	10.49	7.91	6	0.020383	Shisa5
TC0200004599.mm.1	7.92	5.35	5.92	0.034776	Il1a
TC0300001706.mm.1	11.08	8.55	5.76	0.036011	Mir7007
TC1700001890.mm.1	12.29	9.77	5.76	0.035396	H2-K2
TC1700002558.mm.1	12.06	9.54	5.76	0.037361	Eif2ak2
TC0300002506.mm.1	10.05	7.54	5.69	0.000266	Fcgr1
TC0X00002476.mm.1	10.39	7.92	5.57	0.02738	Mtcp1
TC1_GL456211_random00000010.mm.1	10.53	8.07	5.51	0.023212	LOC100041057
TC0600001536.mm.1	11.94	9.48	5.5	0.027498	Gabarapl1
TC1700002819.mm.1	8.98	6.54	5.42	0.044742	H2-T23
TC0700003993.mm.1	8.14	5.7	5.42	0.042643	9130208D14Rik
TC1700001962.mm.1	11.49	9.07	5.35	0.000925	
TC0100001600.mm.1	10.02	7.65	5.16	0.011155	Slamf1
TC0200005472.mm.1	9.99	7.63	5.14	0.017924	Eng
TC1_GL456210_random00000005.mm.1	12.18	9.83	5.08	0.006966	
TC1_GL456211_random00000005.mm.1	12.18	9.83	5.08	0.006966	

TC1_GL456211_random00000026.mm.1	12.18	9.83	5.08	0.006966	
TC1_GL456221_random00000004.mm.1	12.18	9.83	5.08	0.006966	
TC0600001530.mm.1	12.44	10.1	5.04	0.004085	Clec2d
TC0900002389.mm.1	11.87	9.55	5.03	0.018171	Pml
TC0500003460.mm.1	11.43	9.1	5.02	0.017354	Gm15753
TC1000001757.mm.1	8.81	6.49	5.01	0.027153	
TC0900001339.mm.1	8.73	6.41	5	0.025804	Tlr9
TC0300001704.mm.1	14.26	11.95	4.97	0.00845	Cpa3
TC0700004515.mm.1	13.87	11.56	4.97	0.045279	Ifitm3
TC1500001489.mm.1	10.89	8.6	4.89	0.028208	
TC1900001461.mm.1	7.99	5.72	4.83	0.000898	Myof
TC0600001770.mm.1	10.29	8.02	4.81	0.027721	
TC0100002725.mm.1	10.95	8.7	4.73	0.027147	Sp110
TC1000001830.mm.1	7.35	5.11	4.72	0.037833	Pde7b
TC0200001838.mm.1	18.6	16.37	4.71	0.028402	B2m
TC0100002712.mm.1	9.15	6.92	4.68	0.031925	
TC0700003995.mm.1	8.49	6.27	4.68	0.02515	
TC0600000065.mm.1	7.42	5.2	4.67	0.025429	
TC1600000553.mm.1	7.82	5.6	4.64	0.002505	Cd80
TC0700002414.mm.1	10.39	8.18	4.63	0.038205	Sepw1
TC1300001081.mm.1	10.43	8.27	4.47	0.024993	F2rl2
TC0300000769.mm.1	10.79	8.66	4.37	0.03856	Adar
TC0200002588.mm.1	10.96	8.83	4.36	0.015075	Rnf114
TC1100000850.mm.1	10.03	7.92	4.33	0.004073	Sco1
TC0700004001.mm.1	8.23	6.16	4.22	0.038356	
TC0200004539.mm.1	10.22	8.15	4.19	0.042964	Hdc
TC1900000327.mm.1	6.34	4.3	4.11	0.027442	Trpm6
TC1700000623.mm.1	8.94	6.9	4.11	0.00929	Tap2
TC0400001834.mm.1	8.2	6.18	4.08	0.015535	Gm13086
TC1000000449.mm.1	8.36	6.33	4.07	0.023957	
TC0300000949.mm.1	14.62	12.62	4.01	0.049253	Txnip
TC0500002623.mm.1	7.26	5.25	4.01	0.002767	Kdr
TC1700001290.mm.1	9.49	7.51	3.95	0.029325	
TC0500000171.mm.1	9.23	7.26	3.93	0.036859	Fgl2
TC0100003037.mm.1	14	12.03	3.92	0.040946	Cd55
TC1100002747.mm.1	6.21	4.24	3.91	0.012157	Gm12216
TC1100001386.mm.1	12.18	10.22	3.9	0.025164	Trim25
TC0300002718.mm.1	8.92	6.97	3.86	0.035023	Csf1
TC1700001578.mm.1	8.4	6.48	3.79	0.022937	Zfp945
TC1300000009.mm.1	8.36	6.44	3.78	0.015383	Asb13
TC0X00003360.mm.1	9.05	7.15	3.75	0.016777	Tlr7

TC1900000479.mm.1	5.99	4.08	3.75	0.039146	
TC1600000366.mm.1	6.19	4.29	3.74	0.03604	
TC0100001016.mm.1	10.01	8.12	3.7	0.013276	Zcchc2
TC0800000899.mm.1	8.65	6.77	3.68	0.042293	Inpp4b
TC1700001294.mm.1	8.69	6.83	3.63	0.004013	
TC1600000861.mm.1	12.06	10.21	3.61	0.040244	Usp25
TC0800001132.mm.1	9.63	7.8	3.56	0.016797	Usb1
TC0600002655.mm.1	13.46	11.63	3.56	0.04435	
TC0400004170.mm.1	8.11	6.3	3.53	0.026668	Agm
TC1700001154.mm.1	9.78	7.97	3.51	0.038357	Ehd3
TC0500001563.mm.1	6.17	4.38	3.47	0.034593	
TC1700001953.mm.1	11.06	9.29	3.42	0.038875	Lst1
TC0X00003014.mm.1	5.43	3.65	3.42	0.018916	1700008I05Rik
TC0600001689.mm.1	11.05	9.28	3.4	0.012891	Cmas
TC0100002721.mm.1	6.56	4.81	3.38	0.036097	Gm2666
TC1300001448.mm.1	9.98	8.23	3.36	0.038657	Lgals8
TC1300000586.mm.1	7.66	5.92	3.35	0.017667	Fbxw17
TC1300000904.mm.1	11.84	10.11	3.33	0.00674	Erap1
TC1300000607.mm.1	8.77	7.04	3.33	0.035086	Gadd45g
TC1300000651.mm.1	13.69	11.95	3.32	0.034161	
TC0600002204.mm.1	7.32	5.59	3.31	0.026402	
TC1300000364.mm.1	6.42	4.7	3.29	0.038593	Serpinb9
TC0200000222.mm.1	9.05	7.35	3.26	0.035532	Ii15ra
TC0600001769.mm.1	12.07	10.38	3.22	0.022163	2810474O19Rik
TC0400001149.mm.1	6.68	5.01	3.18	0.038712	Gm12737
TC0200004525.mm.1	6	4.34	3.15	0.023572	Fbn1
TC0800002728.mm.1	6.46	4.81	3.13	0.007505	9330175E14Rik
TC1900000527.mm.1	12.39	10.75	3.11	0.021719	5-Mar
TC0100001645.mm.1	9.75	8.12	3.11	0.015455	
TC1100002565.mm.1	6.13	4.52	3.05	0.025796	Mir146
TC1200002083.mm.1	11.85	10.25	3.02	0.022646	Arel1
TC1000001661.mm.1	9.16	7.57	3.01	0.049749	Tespa1
TC0200002157.mm.1	8.58	7	2.99	0.032755	Slc24a3
TC1200001972.mm.1	7.41	5.84	2.98	0.021739	Plek2
TC1500001828.mm.1	15.86	14.29	2.96	0.008521	Csf2rb2
TC0200003885.mm.1	6.41	4.86	2.94	0.042931	P2rx3
TC1300002126.mm.1	11.22	9.66	2.94	0.04887	Zcchc6
TC1100001011.mm.1	8.68	7.13	2.93	0.044604	P2rx1
TC1100000286.mm.1	12.44	10.89	2.93	0.0351	Pnpt1
TC0800000026.mm.1	11.38	9.83	2.93	0.018003	Gm16589
TC1400000574.mm.1	15.32	13.77	2.92	0.012036	Pnp

TC0400001296.mm.1	11.94	10.4	2.92	0.035937	
TC1800000225.mm.1	12.66	11.12	2.92	0.035417	
TC0200000219.mm.1	6.37	4.83	2.91	0.02699	Gm10851
TC1600002076.mm.1	7.72	6.18	2.91	0.011258	
TC1400002393.mm.1	10.06	8.53	2.88	0.049086	Gm20290
TC0500000488.mm.1	12.32	10.79	2.88	0.023557	Lap3
TC0200002544.mm.1	7.96	6.44	2.87	0.005866	Cd40
TC0100003863.mm.1	8.73	7.22	2.85	0.036155	Gm16026
TC1200001367.mm.1	10.18	8.67	2.84	0.040108	Mfsd2b
TC0500002530.mm.1	8.85	7.35	2.83	0.019968	
TC0900000412.mm.1	9.25	7.76	2.82	0.006484	Vwa5a
TC1100002310.mm.1	10.99	9.49	2.82	0.028384	Aftph
TC0200002159.mm.1	7.68	6.19	2.81	0.045268	
TC0900000556.mm.1	6.57	5.08	2.81	0.021967	
TC0600002959.mm.1	8.67	7.19	2.79	0.001312	
TC0300000491.mm.1	9.37	7.92	2.73	0.049625	P2ry1
TC1000001834.mm.1	5.85	4.4	2.73	0.020095	
TC1300001173.mm.1	6.78	5.33	2.72	0.026748	Adamts6
TC0X00000888.mm.1	8.42	6.98	2.72	0.034242	Pcyl1b
TC1700002774.mm.1	9.71	8.27	2.71	0.047043	Cmtr1
TC0900002305.mm.1	9.88	8.45	2.71	0.024009	Atm
TC0900003285.mm.1	7.21	5.77	2.7	0.001551	
TC0900002770.mm.1	10.5	9.07	2.68	0.016343	Fam46a
TC0700000253.mm.1	10.37	8.94	2.68	0.032837	
TC1100001256.mm.1	6.32	4.91	2.66	0.007858	Ccl4
TC1300002140.mm.1	7.64	6.23	2.66	0.045738	4930486L24Rik
TC0100001944.mm.1	12.05	10.64	2.66	0.048457	Vcpip1
TC0500000867.mm.1	8.03	6.62	2.66	0.019599	
TC0900001205.mm.1	7.52	6.11	2.66	0.044051	
TC1700000496.mm.1	15.09	13.68	2.65	0.004944	Cdkn1a
TC0200002495.mm.1	6.99	5.58	2.65	0.011567	
TC0600002778.mm.1	12.05	10.65	2.65	0.027712	
TC0300002459.mm.1	7.9	6.5	2.63	0.025766	A730011C13Rik
TC1_GL456211_random00000004.mm.1	9.92	8.53	2.62	0.012504	LOC100041034
TC0200001552.mm.1	9.31	7.92	2.62	0.038212	Cd59a
TC1000001266.mm.1	12.71	11.32	2.61	0.024981	Ppp1r12a
TC0100002575.mm.1	10.67	9.29	2.61	0.03673	
TC1700001944.mm.1	6.34	4.96	2.59	0.031813	Ly6g6f
TC0600000236.mm.1	8.43	7.06	2.59	0.034276	Irf5
TC0700004274.mm.1	8.79	7.42	2.59	0.025573	Mir7058
TC1400002377.mm.1	5.14	3.77	2.58	0.048682	

TC0700001794.mm.1	5.82	4.46	2.57	0.024086	Il21r
TC1100000211.mm.1	9.75	8.39	2.56	0.018215	Vps54
TC0100003313.mm.1	9.7	8.35	2.56	0.043727	Tor1aip1
TC1400002091.mm.1	12.57	11.22	2.55	0.008749	Psme2
TC0100002840.mm.1	6.48	5.13	2.55	0.01609	Pdcd1
TC1_GL456211_random00000025.mm.1	5.83	4.48	2.55	0.025483	
TC0600002425.mm.1	5.77	4.43	2.54	0.031805	Il23r
TC0800000819.mm.1	12.8	11.46	2.52	0.000685	
TC1700000745.mm.1	8.08	6.76	2.5	0.018503	Trim26
TC1900000899.mm.1	14.68	13.36	2.5	0.034296	
TC1700001884.mm.1	6.39	5.09	2.46	0.048092	BC051226
TC1200000807.mm.1	7.8	6.5	2.46	0.001571	
TC1000002385.mm.1	9.69	8.4	2.45	0.039158	Gstt2
TC0900002907.mm.1	6.62	5.34	2.44	0.008331	4930579K19Rik
TC1300000481.mm.1	5.27	3.99	2.43	0.025689	Edn1
TC1300000913.mm.1	9.28	8.01	2.41	0.015618	
TC1100001219.mm.1	10.59	9.34	2.38	0.049656	Ccl2
TC0700004532.mm.1	5.69	4.44	2.38	0.044542	Sct
TC0700000638.mm.1	5.37	4.13	2.38	0.042329	
TC1200000616.mm.1	6.2	4.95	2.38	0.00096	
TC0700004358.mm.1	6.03	4.79	2.37	0.028665	Cox6a2
TC1500000555.mm.1	4.96	3.72	2.37	0.007562	Ly6f
TC0600001839.mm.1	11.55	10.31	2.37	0.014185	Asns
TC1300002265.mm.1	10.02	8.78	2.37	0.038477	
TC0200000130.mm.1	6.64	5.4	2.36	0.043822	Gm22775
TC0X00001912.mm.1	7.95	6.72	2.35	0.005259	LOC100503338
TC1100003460.mm.1	16.53	15.31	2.32	0.008403	
TC0600000565.mm.1	7.36	6.15	2.31	0.018207	Gimap9
TC1200002127.mm.1	11.37	10.16	2.31	0.012406	Snw1
TC1000001267.mm.1	7.27	6.06	2.31	0.0038	
TC1100001197.mm.1	7.59	6.39	2.3	0.000667	Rnf135
TC1100001302.mm.1	5.28	4.08	2.3	0.030762	Tbx2
TC0600002099.mm.1	11.42	10.22	2.3	0.032149	Zc3hav1
TC0500001569.mm.1	7.7	6.5	2.3	0.040885	Wbscr27
TC1200000615.mm.1	6.72	5.52	2.3	0.036392	
TC1500001452.mm.1	7.67	6.47	2.3	0.013432	
TC0200000006.mm.1	10.1	8.91	2.29	0.016705	Dclre1c
TC0400002945.mm.1	7.42	6.24	2.28	0.000941	Ttc39b
TC0200001940.mm.1	6.61	5.44	2.25	0.040691	Zc3h6
TC1400001105.mm.1	15.05	13.88	2.25	0.007915	Elf1
TC0200004545.mm.1	10.93	9.77	2.24	0.003885	Sppl2a

TC0200000112.mm.1	6.36	5.2	2.24	0.031442	Mir669g
TC0100003204.mm.1	8.23	7.08	2.23	0.031135	Rgs2
TC0100001356.mm.1	7.77	6.61	2.23	0.041636	Fam129a
TC0400002429.mm.1	5.41	4.25	2.23	0.002153	
TC0600001771.mm.1	5.5	4.34	2.23	0.006018	
TC0900000719.mm.1	11.53	10.38	2.22	0.009584	Ubl7
TC0500002532.mm.1	6.94	5.79	2.22	0.00558	
TC0X00002710.mm.1	6.3	5.16	2.21	0.028038	Slc7a3
TC0900001206.mm.1	11.1	9.95	2.21	0.00976	
TC0100002194.mm.1	8.29	7.15	2.2	0.00368	
TC0900002758.mm.1	10.66	9.53	2.2	0.046655	
TC1700002002.mm.1	5.51	4.38	2.19	0.040245	Gm17782
TC0700004604.mm.1	8.28	7.15	2.19	0.023801	Ctnn
TC1800000029.mm.1	8.45	7.32	2.19	0.010248	Zeb1
TC0100000268.mm.1	9.09	7.97	2.18	0.042451	Mirpl30
TC0X00000742.mm.1	11.29	10.17	2.17	0.00221	Brcc3
TC1700000719.mm.1	6.03	4.91	2.17	0.004692	Gm6034
TC1500001815.mm.1	5.32	4.21	2.16	0.033271	Apol10b
TC1200002267.mm.1	11.99	10.88	2.16	0.014938	Ddx24
TC1500000641.mm.1	15.59	14.49	2.15	0.023305	Csf2rb
TC1300000735.mm.1	7.55	6.44	2.15	0.023455	Dapk1
TC1400002384.mm.1	4.54	3.43	2.15	0.010404	Mir687
TC1200000942.mm.1	6.99	5.89	2.15	0.034228	
TC0200003474.mm.1	9.14	8.04	2.15	0.005798	
TC0300002588.mm.1	7.23	6.12	2.15	0.008456	
TC1300001391.mm.1	8.03	6.93	2.15	0.026504	
TC1700001293.mm.1	7.12	6.02	2.15	0.042284	
TC0400002428.mm.1	7.87	6.77	2.14	0.013225	
TC0800001442.mm.1	7.77	6.67	2.14	0.021125	
TC1100000411.mm.1	11.07	9.98	2.13	0.018225	Gm12139
TC0600002487.mm.1	13.24	12.15	2.13	0.000812	Vamp5
TC0500003376.mm.1	9.17	8.09	2.12	0.00039	Lat2
TC0100001172.mm.1	13.91	12.82	2.12	0.020701	Ctse
TC0500001777.mm.1	4.85	3.76	2.12	0.014205	
TC0500000843.mm.1	15.41	14.33	2.11	0.017981	Pf4
TC1200002359.mm.1	10.4	9.31	2.11	0.033725	Wars
TC0200000164.mm.1	6.42	5.35	2.11	0.027569	Gm26156
TC1600000498.mm.1	5.22	4.14	2.11	0.030253	
TC0300000003.mm.1	5.99	4.91	2.1	0.03131	
TC0500000994.mm.1	5.23	4.16	2.1	0.010794	
TC1300001023.mm.1	5.91	4.83	2.1	0.001388	

TC1100000628.mm.1	6.5	5.44	2.09	0.014181	Gm24198
TC0200005471.mm.1	6.81	5.75	2.09	0.0015	Akl
TC1300001020.mm.1	8.4	7.34	2.09	0.011944	Ssbp2
TC0300000468.mm.1	8.06	7	2.09	0.010343	
TC1500000270.mm.1	6.83	5.77	2.09	0.017331	
TC0800002023.mm.1	9.77	8.72	2.08	0.045585	Leprotl1
TC0X00002685.mm.1	6.04	4.98	2.08	0.02035	Eda2r
TC0400000637.mm.1	4.85	3.79	2.08	0.030688	
TC1100000015.mm.1	7.39	6.34	2.07	0.00803	Selm
TC1700002347.mm.1	7.39	6.34	2.07	0.014085	Dennd1c
TC0900001863.mm.1	8.54	7.49	2.07	0.036296	Keap1
TC1100003161.mm.1	5.27	4.21	2.07	0.027467	Aspa
TC1400000503.mm.1	9.59	8.55	2.06	0.014882	
TC1100001688.mm.1	10.73	9.69	2.05	0.020977	Grn
TC0200000114.mm.1	6.05	5.02	2.05	0.016774	Mir669j
TC1400002392.mm.1	17.11	16.08	2.05	0.04811	Itm2b
TC0800001728.mm.1	6.29	5.25	2.05	0.006067	Grtp1
TC1300002562.mm.1	5.37	4.33	2.05	0.010288	
TC1000002056.mm.1	8.82	7.79	2.04	0.038519	Cep57l1
TC0100001160.mm.1	5.8	4.77	2.04	0.014266	
TC0300002850.mm.1	5.36	4.33	2.04	0.010207	
TC1200001729.mm.1	4.73	3.71	2.03	0.021443	Gpr33
TC1800001193.mm.1	5.84	4.82	2.03	0.049228	Hbegf
TC0900001909.mm.1	5.52	4.49	2.03	0.003412	
TC1000000160.mm.1	7.59	6.58	2.02	0.001282	Ahi1
TC1000000989.mm.1	7.25	6.23	2.02	0.035004	
TC0500001597.mm.1	6.65	5.64	2.01	0.049027	Rasa4
TC1100002242.mm.1	6.85	5.84	2.01	0.039136	Gm12664
TC0500002588.mm.1	7.41	6.4	2.01	0.024596	
TC1300002159.mm.1	14.3	13.3	2.01	0.025612	
TC1400002108.mm.1	6.2	5.19	2	0.033401	Cma1
TC0200001448.mm.1	7.03	6.03	2	0.024966	Trp53i11
TC0X00003314.mm.1	5.9	4.9	2	0.014412	Mir3473a
TC1300000321.mm.1	10.26	9.26	2	0.044679	Gm23729

[000252] Table 7. Genome-wide expression analysis of pKL cells: down-regulated genes. List of downregulated genes identified by genome wide expression analysis (GWAS) performed on pharmacologically-modulated KL cells (pKL) as compared to Vehicle treated-KL cells isolated from bone marrow of normoglycemic NOD mice ($p < 0.05$).

Table 7					
Transcript Cluster ID	pKL	KL	Fold Change	ANOVA p-value	Gene Symbol
TC1300000691.mm.1	7.76	14.79	-130.98	0.029904	Tgfbi

TC0200000245.mm.1	7.52	14.18	-101.07	0.007806	Mrc1
TC0300002684.mm.1	7.6	13.33	-53.03	0.014861	Chil3
TC0500003399.mm.1	6.8	12.25	-43.79	0.007984	Ccl24
TC1100000941.mm.1	7.53	12.55	-32.37	0.039087	Mgl2
TC1100001267.mm.1	10.88	15.6	-26.38	0.033053	Wfdc21
TC0800002403.mm.1	9.58	13.65	-16.71	0.013546	Ifi30
TC0700000275.mm.1	8.14	12.08	-15.34	0.023592	Pglyrp1
TC1500000389.mm.1	7.53	11.18	-12.52	0.018949	Nov
TC1200001742.mm.1	7.41	10.95	-11.6	0.012045	Egln3
TC0300000807.mm.1	7.44	10.97	-11.54	0.028013	S100a4
TC0500002753.mm.1	8.65	12.18	-11.51	0.011324	Naaa
TC1100000942.mm.1	6.91	10.24	-10.06	0.03963	Clec10a
TC0700001793.mm.1	10.86	14.13	-9.7	0.023945	Il4ra
TC0700002611.mm.1	5.31	8.58	-9.68	0.033875	Cd177
TC0900000598.mm.1	4.36	7.64	-9.67	0.027364	Plet1
TC1000002479.mm.1	7.64	10.85	-9.2	0.002719	Prss57
TC1800000050.mm.1	7.59	10.77	-9.07	0.045047	Fabp5l2
TC0200004450.mm.1	8.56	11.72	-8.96	0.047326	6330405D24Rik
TC0500002320.mm.1	7.7	10.81	-8.62	0.033116	Prom1
TC1900001562.mm.1	6.58	9.67	-8.49	0.006597	Scd1
TC0700002128.mm.1	9.16	12.11	-7.73	0.000892	Tarm1
TC0200002426.mm.1	7.24	10.1	-7.3	0.047526	Lbp
TC0900000702.mm.1	13.17	16.02	-7.24	0.048689	Gm6166
TC1700000558.mm.1	13.66	16.49	-7.15	0.015241	
TC1300002716.mm.1	4.17	6.99	-7.07	0.026316	
TC1500001580.mm.1	4.98	7.69	-6.53	0.029423	
TC0200000246.mm.1	5.64	8.34	-6.49	0.029152	Mir511
TC0600001397.mm.1	6.09	8.76	-6.34	0.018648	Clec4a1
TC1000000352.mm.1	7.85	10.51	-6.3	0.015573	
TC0600003220.mm.1	5.47	8.09	-6.17	0.013027	Gm15987
TC1800001048.mm.1	9.89	12.51	-6.15	0.047342	B4galt6
TC1900000375.mm.1	7.59	10.2	-6.08	0.047738	
TC0500001487.mm.1	5.05	7.64	-6.02	0.032158	Glt1d1
TC0700000005.mm.1	6.4	8.99	-6.01	0.014874	Myadm
TC0600002965.mm.1	7.74	10.3	-5.9	0.040346	Plxnd1
TC0M00000005.mm.1	11.53	14.07	-5.8	0.027548	mt-Tm
TSUnmapped00000176.mm.1	11.47	13.99	-5.73	0.035519	Snora73b
TC0700001432.mm.1	7.28	9.78	-5.66	0.008242	Pde2a
TC0100002413.mm.1	6.75	9.23	-5.56	0.01916	Raph1
TC1100003236.mm.1	4.92	7.39	-5.51	0.040352	Fam101b
TC0400003669.mm.1	11.54	13.88	-5.07	0.012962	Snora73b
TC0700002635.mm.1	6.18	8.47	-4.9	0.00224	Ceacam1
TC1900001554.mm.1	6.58	8.86	-4.87	0.034279	Gm24336
TC1300001218.mm.1	4.61	6.86	-4.75	0.036265	
TC0X00002266.mm.1	7.37	9.57	-4.59	0.045966	Mmgt1
TC1900000819.mm.1	7.7	9.87	-4.51	0.018173	Atrnl1
TC0700001953.mm.1	5.32	7.48	-4.48	0.009431	
TC0700002636.mm.1	6.74	8.88	-4.41	0.031972	Ceacam2
TC1200001323.mm.1	8.65	10.77	-4.37	0.022741	
TC1300002717.mm.1	4.95	7.06	-4.31	0.002954	Itgal
TC1400001972.mm.1	16.73	18.83	-4.28	0.021974	Gm3601

TC0100000030.mm.1	7.84	9.92	-4.25	0.004376	Gm9826
TC0200002581.mm.1	6.43	8.51	-4.25	0.04173	Gm23201
TC0200001959.mm.1	9.04	11.11	-4.19	0.013974	Sirpa
TC1500002067.mm.1	4.83	6.89	-4.18	0.014017	Cpne8
TC1100003057.mm.1	5.64	7.67	-4.07	0.0291	Gm25835
TC0800002027.mm.1	6.98	9	-4.05	0.047665	Gm6100
TC1900001561.mm.1	5.15	7.17	-4.05	0.040968	
TC1200001013.mm.1	4.9	6.91	-4.02	0.027239	
TC0600001634.mm.1	5.18	7.17	-4	0.013773	Ptpro
TC1300001215.mm.1	4.5	6.49	-3.98	0.011273	
TC0200001960.mm.1	7.28	9.27	-3.96	0.04494	
TC1800000498.mm.1	10.27	12.23	-3.92	0.016258	Eno1b
TC0200001120.mm.1	11.53	13.5	-3.9	0.010776	Gm13669
TC0900000126.mm.1	8.54	10.49	-3.88	0.024277	Slc36a4
TC1500000747.mm.1	4.97	6.91	-3.85	0.008532	3-Sep
TC0200003608.mm.1	5.28	7.22	-3.83	0.042554	
TC1200001521.mm.1	6.27	8.21	-3.83	0.033233	
TC1300001451.mm.1	6.28	8.2	-3.78	0.029912	Gpr137b-ps
TC0700002828.mm.1	13.28	15.19	-3.77	0.027086	Gpi1
TC1300002477.mm.1	12.37	14.28	-3.76	0.006205	Plp2
TC0X00000989.mm.1	6	7.9	-3.73	0.042717	
TC1200001321.mm.1	3.9	5.79	-3.71	0.000654	
TC0600003383.mm.1	4.94	6.82	-3.68	0.004168	St8sia1
TC0300000555.mm.1	10.05	11.92	-3.65	0.027119	Mfsd1
TC0100001550.mm.1	8.62	10.48	-3.64	0.048934	Aldh9a1
TC1200000166.mm.1	6.55	8.4	-3.62	0.038281	
TC1600000698.mm.1	4.17	6.02	-3.58	0.012831	Retnla
TC0400001062.mm.1	5.45	7.28	-3.56	0.049629	Tctex1d1
TC0200003512.mm.1	5.02	6.85	-3.56	0.0347	Rnd3
TC0100002075.mm.1	11.95	13.76	-3.53	0.048288	Gm10222
TC0X00002193.mm.1	7.2	9.02	-3.52	0.016505	
TC1500001495.mm.1	4.61	6.41	-3.5	0.010156	
TC1100001251.mm.1	5.77	7.57	-3.48	0.026464	1700020L24Rik
TC0900002113.mm.1	7.38	9.18	-3.47	0.039865	Sorl1
TC0M00000003.mm.1	13.15	14.94	-3.46	0.040803	mt-Tv
TC0600001399.mm.1	5.17	6.94	-3.4	0.044745	Clec4a4
TC1300001295.mm.1	10.9	12.67	-3.4	0.026549	
TC1300001461.mm.1	6.07	7.82	-3.37	0.00105	Gpr137b
TC1900001095.mm.1	12.48	14.23	-3.36	0.013942	Fads2
TSUnmapped00000030.mm.1	7.07	8.81	-3.36	0.013742	H60a
TC0100001424.mm.1	11.45	13.19	-3.34	0.021423	Gm15428
TC1600001559.mm.1	9.61	11.36	-3.34	0.007514	Fam162a
TC0500003395.mm.1	6.92	8.66	-3.33	0.019694	Hip1
TC0200003843.mm.1	5.88	7.61	-3.31	0.04474	
TC0900001043.mm.1	6.3	8.02	-3.31	0.01661	
TC0900000204.mm.1	9.58	11.3	-3.28	0.002249	Ldlr
TC1600002080.mm.1	9.78	11.49	-3.26	0.028475	Tmem50b
TC0900001042.mm.1	10.33	12.03	-3.25	0.02713	Elov15
TC1800000626.mm.1	6.38	8.08	-3.25	0.020747	Mir6983
TC0900000816.mm.1	6.39	8.08	-3.23	0.036367	Snape5
TC1000000968.mm.1	8.2	9.9	-3.23	0.004532	

TC0200002138.mm.1	4.61	6.29	-3.21	0.045668	
TC1700002776.mm.1	4.89	6.56	-3.19	0.028144	Pram1
TC0500000260.mm.1	9.31	10.96	-3.14	0.037644	Insig1
TC1000001406.mm.1	9.89	11.54	-3.14	0.047448	
TC1000001407.mm.1	9.89	11.54	-3.14	0.047448	
TC1000002951.mm.1	9.89	11.54	-3.14	0.047448	
TC1000002952.mm.1	9.89	11.54	-3.14	0.047448	
TC0100001325.mm.1	5.7	7.35	-3.13	0.019617	
TC0100001326.mm.1	5.7	7.35	-3.13	0.019617	
TC0100001327.mm.1	5.7	7.35	-3.13	0.019617	
TC0200001569.mm.1	5.7	7.35	-3.13	0.019617	
TC0200001615.mm.1	5.7	7.35	-3.13	0.019617	
TC0800000456.mm.1	5.7	7.35	-3.13	0.019617	
TC0X00000800.mm.1	5.7	7.35	-3.13	0.019617	
TC0X00001094.mm.1	5.7	7.35	-3.13	0.019617	
TC0X00001200.mm.1	5.7	7.35	-3.13	0.019617	
TC1000001319.mm.1	5.7	7.35	-3.13	0.019617	
TC1000002465.mm.1	5.7	7.35	-3.13	0.019617	
TC1200000168.mm.1	5.7	7.35	-3.13	0.019617	
TC1200001486.mm.1	5.7	7.35	-3.13	0.019617	
TC1200001838.mm.1	5.7	7.35	-3.13	0.019617	
TC1200002489.mm.1	5.7	7.35	-3.13	0.019617	
TC1600000722.mm.1	5.7	7.35	-3.13	0.019617	
TC1800001393.mm.1	5.7	7.35	-3.13	0.019617	
TC1900001143.mm.1	5.7	7.35	-3.13	0.019617	
TC0700003657.mm.1	8.3	9.93	-3.11	0.002626	Fah
TC0X00001780.mm.1	11.55	13.19	-3.11	0.005311	Plp2
TC1400000186.mm.1	6.67	8.31	-3.11	0.019209	
TC1600001280.mm.1	13.9	15.53	-3.1	0.026114	Sdf2l1
TC0800003031.mm.1	8.29	9.9	-3.05	0.04969	Cotl1
TC1500000658.mm.1	14.68	16.28	-3.04	0.031283	Lgals1
TC0800000394.mm.1	12.52	14.13	-3.04	0.029628	Gsr
TC1100003398.mm.1	5.21	6.81	-3.03	0.016931	
TC0X00002486.mm.1	7.77	9.36	-3.02	0.029594	Gm14708
TC1800001585.mm.1	6.21	7.8	-3.02	0.046751	
TC0700001761.mm.1	3.66	5.25	-3.01	0.010671	
TC0700002718.mm.1	6.05	7.64	-3	0.036272	Kcnk6
TC0900000659.mm.1	10.15	11.73	-3	0.036766	Idh3a
TC0400001974.mm.1	11.96	13.54	-2.99	0.006804	Eno1
TC0800000172.mm.1	9.86	11.44	-2.99	0.014044	Agpat5
TC1400002487.mm.1	7.78	9.35	-2.98	0.039485	Rgcc
TC0100002047.mm.1	6.57	8.15	-2.98	0.038828	
TC0700004320.mm.1	11.23	12.8	-2.96	0.023586	Dctpp1
TC1200001644.mm.1	10.46	12.02	-2.96	0.040646	Bzw2
TC1000003223.mm.1	10.89	12.46	-2.96	0.025374	
TC0500000544.mm.1	3.37	4.92	-2.94	0.02258	
TC0300002357.mm.1	6.68	8.23	-2.93	0.029787	Il6ra
TC0900002115.mm.1	9.7	11.25	-2.93	0.049392	Sc5d
TC0700001951.mm.1	6.93	8.48	-2.92	0.049509	Dock1
TC0300002181.mm.1	5.59	7.14	-2.92	0.046454	
TC0500001338.mm.1	7.07	8.61	-2.9	0.004893	Slc8b1

TC0100002453.mm.1	9.88	11.41	-2.9	0.045259	Gm11605
TC0900001597.mm.1	6.39	7.92	-2.89	0.049425	Gm26448
TC0600002518.mm.1	7.5	9.03	-2.88	0.01998	Gm9008
TC1800000794.mm.1	9.45	10.97	-2.87	0.01314	Acaa2
TC0X00000938.mm.1	7.96	9.47	-2.85	0.038624	Gm14814
TC0800002905.mm.1	12.47	13.98	-2.84	0.012589	Hp
TC1500000640.mm.1	9.18	10.68	-2.83	0.019595	Ncf4
TC0700000561.mm.1	11.38	12.87	-2.83	0.032301	Tyrbp
TC0300002040.mm.1	12.61	14.1	-2.81	0.044252	Gm5537
TC1100002030.mm.1	7.2	8.69	-2.81	0.003933	Slc25a10
TC1100001264.mm.1	5.17	6.67	-2.81	0.020795	Wfdc17
TC1100000120.mm.1	6.51	7.99	-2.8	0.008736	Abca13
TC0200003164.mm.1	6.66	8.14	-2.8	0.022038	Tmem141
TC0X00001766.mm.1	6.27	7.75	-2.8	0.027341	Cln5
TC0800002618.mm.1	6.24	7.72	-2.8	0.009507	Neto2
TC0600001853.mm.1	7.72	9.19	-2.76	0.02348	
TC0700001613.mm.1	6.19	7.65	-2.75	0.026888	Gm24888
TC1000001680.mm.1	6.78	8.24	-2.75	0.039079	
TC1600001723.mm.1	3.82	5.28	-2.75	0.048147	
TC0100003655.mm.1	4.63	6.09	-2.74	0.022664	
TC1400002589.mm.1	7.53	8.98	-2.74	0.031384	
TC0500000470.mm.1	11.42	12.86	-2.71	0.009054	Gm7879
TC1900000698.mm.1	5.22	6.66	-2.71	0.045093	Gm24610
TC1400000573.mm.1	7.81	9.26	-2.71	0.033055	Apex1
TC1000002500.mm.1	9.51	10.95	-2.71	0.041088	Uqcr11
TC0400002744.mm.1	7.01	8.45	-2.71	0.034871	Ptgr1
TC0700002951.mm.1	5.62	7.05	-2.7	0.014363	Siglece
TC1700000436.mm.1	2.87	4.3	-2.69	0.047439	
TC1600000629.mm.1	6.25	7.67	-2.68	0.017855	Gm15711
TC0700001629.mm.1	10.12	11.54	-2.68	0.03238	Gm10087
TC1000001601.mm.1	9.17	10.58	-2.67	0.022969	Ankrd52
TC0200005080.mm.1	11.66	13.08	-2.67	0.037848	Gm11451
TC1800000291.mm.1	9.74	11.16	-2.67	0.030243	Kif20a
TC0M00000026.mm.1	7.8	9.21	-2.67	0.045539	
TC0100002050.mm.1	7.47	8.88	-2.66	0.037695	
TC0400003424.mm.1	10.75	12.15	-2.65	0.031072	Ldha-ps2
TC0900001337.mm.1	8.19	9.59	-2.64	0.024297	Twf2
TC0X00000218.mm.1	3.67	5.07	-2.64	0.033216	Timp1
TC0300001874.mm.1	8.25	9.64	-2.63	0.008446	Gm12565
TC0700001929.mm.1	8.74	10.13	-2.63	0.017324	
TC1600000867.mm.1	4.18	5.58	-2.63	0.029319	
TC0200003093.mm.1	10.83	12.23	-2.62	0.026518	Gm13339
TC0M00000011.mm.1	11.98	13.37	-2.62	0.016305	ND3
TC1900000693.mm.1	5.93	7.31	-2.61	0.007529	Tmem180
TC0700004221.mm.1	8.24	9.62	-2.61	0.037146	Gga2
TC1700002179.mm.1	7.42	8.8	-2.6	0.020789	Srf
TC0600002128.mm.1	8.6	9.98	-2.6	0.028135	
TC1800000015.mm.1	7.7	9.07	-2.58	0.016668	9430020K01Rik
TC0600000954.mm.1	9.29	10.66	-2.57	0.038542	Npm3-ps1
TC0200002744.mm.1	4.81	6.18	-2.57	0.00691	
TC1300001641.mm.1	6.16	7.52	-2.56	0.029897	Hfe

TC0600002137.mm.1	5.83	7.18	-2.56	0.001745	E330009J07Rik
TC1900000996.mm.1	7.87	9.23	-2.56	0.039557	Gm19505
TC1000000937.mm.1	8.73	10.08	-2.55	0.005197	Chst11
TC1100001276.mm.1	8.58	9.93	-2.55	0.032203	Acaca
TC0X00002393.mm.1	10.15	11.51	-2.55	0.025864	BC023829
TC0900003182.mm.1	11.74	13.08	-2.54	0.039627	Stt3b
TC0200001741.mm.1	8.67	10.01	-2.54	0.007079	Bub1b
TC1900000549.mm.1	8.44	9.78	-2.54	0.011511	Slc35g1
TC0100002867.mm.1	4.64	5.98	-2.54	0.006226	
TC1100001191.mm.1	6.58	7.91	-2.51	0.029317	Gm11203
TC1300000655.mm.1	11.69	13.02	-2.51	0.040755	Prelid1
TC0500003651.mm.1	8.87	10.19	-2.51	0.006544	Gm8615
TC0700002168.mm.1	4.74	6.06	-2.5	0.040905	Tmem150b
TC1400001187.mm.1	5.56	6.88	-2.49	0.025689	
TC0M00000022.mm.1	7.52	8.83	-2.48	0.001574	mt-Tn
TC1200001262.mm.1	9.25	10.56	-2.48	0.035405	Adssl1
TC0700002918.mm.1	11.4	12.71	-2.47	0.046731	Gm5593
TC0200005454.mm.1	8.4	9.7	-2.46	0.000598	Rgs19
TC0X00000611.mm.1	6.09	7.38	-2.46	0.022132	Gm5638
TC0500003393.mm.1	9.17	10.47	-2.46	0.029129	Pom121
TC0400002914.mm.1	7.28	8.57	-2.45	0.036657	Gm11247
TC0X00002338.mm.1	6.44	7.74	-2.45	0.038796	Gm14672
TC1700000403.mm.1	8.9	10.19	-2.44	0.00035	Mrpl28
TC1700002392.mm.1	6.98	8.26	-2.43	0.006052	Gm17228
TC0800000126.mm.1	12.88	14.16	-2.43	0.038551	Tfdp1
TC0200005014.mm.1	15.46	16.74	-2.43	0.0137	
TC0400001414.mm.1	8.48	9.75	-2.42	0.044399	Gm12902
TC1100000892.mm.1	10.19	11.45	-2.41	0.005458	Aurkb
TC0900000544.mm.1	6.48	7.75	-2.4	0.041745	Bace1
TC0300001453.mm.1	15.63	16.9	-2.4	0.040965	Gm2574
TC0100001283.mm.1	6.53	7.8	-2.4	0.013301	
TC1600001969.mm.1	5.16	6.42	-2.4	0.024569	
TC1000000566.mm.1	7.08	8.33	-2.39	0.029289	Lrrc20
TC1000000986.mm.1	3.77	5.03	-2.39	0.019359	
TC1000003148.mm.1	10.84	12.09	-2.38	0.00236	Esyt1
TC0X00002165.mm.1	8.48	9.72	-2.37	0.033833	Aifm1
TC1800000486.mm.1	7.25	8.5	-2.37	0.045632	
TC1900001082.mm.1	6.21	7.45	-2.36	0.015246	
TC0900000854.mm.1	10.07	11.3	-2.35	0.046986	2810417H13Rik
TC1500000714.mm.1	3.97	5.2	-2.35	0.032305	
TC1200001397.mm.1	7.32	8.55	-2.34	0.035373	Rhob
TC1100004220.mm.1	7.41	8.62	-2.33	0.028282	Pcyt2
TC0500001940.mm.1	8.17	9.38	-2.32	0.007802	Gm7332
TC1400000887.mm.1	7.7	8.92	-2.32	0.027441	Wdfy2
TC1900001207.mm.1	9.64	10.86	-2.32	0.027025	Gm10819
TC1700000836.mm.1	5.05	6.26	-2.31	0.0418	Cyp39a1
TC0200002839.mm.1	6.41	7.62	-2.31	0.017308	
TC0900002102.mm.1	5.35	6.55	-2.3	0.010426	Gm16096
TC0500001764.mm.1	10.72	11.92	-2.3	0.004861	Arpc1b
TC1200000682.mm.1	5.29	6.49	-2.3	0.025133	Plekkg3
TC0900001638.mm.1	8.96	10.16	-2.3	0.030878	Abhd5

TC0200002577.mm.1	11.58	12.78	-2.3	0.049908	Cse11
TC0400002236.mm.1	10.89	12.08	-2.29	0.046116	Otud6b
TC0500001780.mm.1	5.56	6.75	-2.29	0.038693	
TC1000001917.mm.1	6.08	7.27	-2.27	0.001674	Gm8709
TC1900000560.mm.1	10.03	11.21	-2.27	0.04384	Hells
TC0700001180.mm.1	5.02	6.2	-2.27	0.009952	Gm25907
TC1400000306.mm.1	8.88	10.05	-2.26	0.045389	Tkt
TC1600001079.mm.1	7.95	9.13	-2.26	0.030564	Wrb
TC1500000163.mm.1	9.24	10.41	-2.25	0.018882	Gm2862
TC1400001314.mm.1	9.6	10.76	-2.25	0.040265	Ipo5
TC0600001974.mm.1	7.32	8.49	-2.24	0.028322	Impdh1
TC1000000015.mm.1	9.19	10.36	-2.24	0.047707	Mthfd11
TC0400000501.mm.1	10.42	11.58	-2.24	0.015172	Ncbp1
TC1900000941.mm.1	9.52	10.68	-2.23	0.013103	Dpp3
TC0400004097.mm.1	7.62	8.77	-2.22	0.010273	Kcnab2
TC0200002990.mm.1	6.26	7.4	-2.22	0.002564	Ptpla
TC0100002077.mm.1	16.17	17.32	-2.22	0.03879	
TC0300002960.mm.1	5.79	6.94	-2.22	0.029371	
TC0300003225.mm.1	6.15	7.29	-2.21	0.002687	LOC100038947
TC0700000931.mm.1	6.19	7.33	-2.21	0.029119	Siglech
TC1200001077.mm.1	12.35	13.49	-2.21	0.040395	Vrk1
TC1100003640.mm.1	12.59	13.73	-2.21	0.046699	Kpnb1
TC1400002400.mm.1	10.85	12	-2.21	0.04487	Gm6984
TC1500000663.mm.1	4.79	5.92	-2.2	0.013327	Gcat
TC1800001024.mm.1	10.82	11.95	-2.2	0.01406	Gm7665
TC0200004665.mm.1	6.97	8.11	-2.2	0.017652	Rassf2
TC0800002941.mm.1	9.01	10.15	-2.2	0.022672	Glg1
TC0900000071.mm.1	3.24	4.38	-2.2	0.010571	
TC0X00001393.mm.1	5.23	6.37	-2.19	0.046434	Gm15079
TC1300001140.mm.1	6.13	7.26	-2.19	0.047889	Ccdc125
TC1100003407.mm.1	7.01	8.14	-2.19	0.033461	Tada2a
TC0700002782.mm.1	10.29	11.43	-2.19	0.044209	Usf2
TC0200001703.mm.1	5	6.13	-2.19	0.019328	
TC1100004085.mm.1	7.39	8.5	-2.17	0.01079	Gm11702
TC1300002052.mm.1	10.22	11.34	-2.17	0.004026	Rab24
TC1400000354.mm.1	5.04	6.15	-2.17	0.011034	Gm7734
TC1500001899.mm.1	4.18	5.3	-2.17	0.044475	Snord43
TC0200004718.mm.1	8.28	9.4	-2.17	0.023127	Gm14063
TC1300002594.mm.1	7.09	8.2	-2.17	0.042397	Nln
TC1000002988.mm.1	7.66	8.77	-2.17	0.029317	Gm9046
TC0500001380.mm.1	11.57	12.69	-2.17	0.023899	Ppp1cc
TC0400002593.mm.1	8.94	10.06	-2.17	0.035282	Gm12444
TC0500000523.mm.1	3.9	5.02	-2.17	0.00857	
TC0X00003430.mm.1	7.95	9.06	-2.16	0.032947	Arhgap4
TC1200000675.mm.1	10.59	11.7	-2.16	0.011722	Mthfd1
TC0400000870.mm.1	5.68	6.79	-2.16	0.031655	
TC1600000880.mm.1	4.84	5.95	-2.16	0.004801	
TC0600001398.mm.1	9.18	10.28	-2.15	0.024866	Clec4a3
TC1100002046.mm.1	12.49	13.6	-2.15	0.045829	Hmgal-rs1
TC0500003626.mm.1	5.41	6.51	-2.15	0.039179	Flt3
TC1900000127.mm.1	13.12	14.22	-2.14	0.017541	Ppp1r14b

TC0X00001053.mm.1	4.96	6.06	-2.14	0.03298	Gm9673
TC0600001203.mm.1	5.06	6.15	-2.13	0.025935	Bhlhe40
TC1600001497.mm.1	9.66	10.76	-2.13	0.031144	Pak2
TC0200002146.mm.1	9.45	10.55	-2.13	0.001687	Pet117
TC1100002250.mm.1	11.01	12.1	-2.13	0.043678	Gm12013
TC0900000280.mm.1	4.5	5.59	-2.13	0.01833	
TC0900000974.mm.1	5.04	6.13	-2.13	0.011146	
TC1800000844.mm.1	14.06	15.15	-2.13	0.014757	
TC1000002043.mm.1	11.28	12.36	-2.12	0.04902	Amd2
TC1500002221.mm.1	10.97	12.06	-2.12	0.047627	Cers5
TC1100003095.mm.1	8.44	9.52	-2.12	0.010272	Pelp1
TC0100002049.mm.1	5.01	6.1	-2.12	0.011683	
TC0700004157.mm.1	6.28	7.36	-2.11	0.036125	Pik3c2a
TC0800002313.mm.1	9	10.07	-2.11	0.015395	Msmo1
TC0100002053.mm.1	5.63	6.71	-2.11	0.030669	
TC1100000796.mm.1	5.05	6.13	-2.11	0.027278	
TC1100000545.mm.1	7.14	8.21	-2.1	0.001149	Gm12197
TC0200004840.mm.1	8.74	9.81	-2.1	0.005878	Gm14121
TC0X00003122.mm.1	9.52	10.59	-2.1	0.026645	Gm15067
TC1000002188.mm.1	4.33	5.4	-2.1	0.005777	
TC1600001155.mm.1	7.48	8.54	-2.09	0.034311	Nagpa
TC0800002558.mm.1	8.75	9.82	-2.09	0.003445	Cd97
TC0700001714.mm.1	14.47	15.53	-2.09	0.045773	Gm5601
TC0500001462.mm.1	7.01	8.06	-2.08	0.036902	Bri3bp
TC0200001784.mm.1	6.04	7.1	-2.08	0.040705	
TC0800002840.mm.1	8.15	9.21	-2.08	0.017559	
TC1300001227.mm.1	5.42	6.48	-2.08	0.037621	
TC0400002193.mm.1	10.8	11.85	-2.07	0.020666	Gm11814
TC1400000529.mm.1	14.76	15.82	-2.07	0.010369	Gm3534
TC0200003141.mm.1	7.98	9.03	-2.07	0.037823	Ssna1
TC0400000516.mm.1	7.82	8.87	-2.07	0.033794	Gm12424
TC0900001424.mm.1	5.11	6.15	-2.06	0.030186	Celsr3
TC1100000802.mm.1	8.12	9.16	-2.06	0.032038	Trpv2
TC1100004270.mm.1	6.7	7.75	-2.06	0.008766	Tlcd2
TC0800002413.mm.1	10.39	11.44	-2.06	0.021425	Mir7067
TC0300000439.mm.1	4.98	6.02	-2.06	0.007132	
TC0600001731.mm.1	4.98	6.02	-2.06	0.007132	
TC0600002537.mm.1	4.98	6.02	-2.06	0.007132	
TC0600003306.mm.1	4.98	6.02	-2.06	0.007132	
TC0900002567.mm.1	4.98	6.02	-2.06	0.007132	
TC0X00000379.mm.1	4.98	6.02	-2.06	0.007132	
TC0X00003344.mm.1	4.98	6.02	-2.06	0.007132	
TC1400001128.mm.1	4.98	6.02	-2.06	0.007132	
TC1400002462.mm.1	5.19	6.24	-2.06	0.025107	
TC1400002705.mm.1	4.98	6.02	-2.06	0.007132	
TC1500000481.mm.1	4.98	6.02	-2.06	0.007132	
TC1800000768.mm.1	4.98	6.02	-2.06	0.007132	
TC1800001570.mm.1	4.98	6.02	-2.06	0.007132	
TC1900001634.mm.1	4.98	6.02	-2.06	0.007132	
TC0500000459.mm.1	5.86	6.89	-2.05	0.000222	Cpeb2
TC0900002020.mm.1	9.57	10.6	-2.05	0.049948	Gm10105

TC1100000093.mm.1	11.68	12.71	-2.05	0.014066	Ogdh
TC1300001212.mm.1	3.84	4.88	-2.05	0.022614	
TC0300000904.mm.1	15.37	16.39	-2.04	0.04309	Hmgbl-ps5
TC1000001856.mm.1	13.89	14.92	-2.04	0.028687	Hmgbl-ps8
TC1700002191.mm.1	10.25	11.28	-2.04	0.02939	Rpl7l1
TC1000002985.mm.1	9.89	10.92	-2.04	0.041065	Gm9040
TC1000002986.mm.1	9.89	10.92	-2.04	0.041065	Gm9044
TC1700002318.mm.1	7.73	8.76	-2.03	0.032179	Dpp9
TC0500002923.mm.1	10.84	11.86	-2.03	0.0458	Gm8152
TC0900002638.mm.1	12.97	13.99	-2.03	0.023276	RP24-282D16.5
TC0200005145.mm.1	6.49	7.51	-2.03	0.023624	Slc35c2
TC1100000592.mm.1	8.51	9.53	-2.03	0.008323	Vdac1
TC0700001412.mm.1	8.28	9.3	-2.03	0.044622	Cox20-ps
TC0100000408.mm.1	4.42	5.44	-2.03	0.020475	
TC0600001734.mm.1	10.61	11.63	-2.02	0.020979	Med21
TC1000000056.mm.1	4.84	5.85	-2.02	0.002652	Zc3h12d
TC0200003240.mm.1	16.42	17.44	-2.02	0.026298	Gm13394
TC0200003109.mm.1	6.34	7.36	-2.02	0.021129	Hnmt
TC0800000142.mm.1	11.36	12.37	-2.02	0.014776	Gm3160
TC0X00000874.mm.1	6.39	7.41	-2.02	0.003215	Gm14778
TC1900000188.mm.1	11.28	12.29	-2.02	0.049538	Fads1
TC1700002656.mm.1	7.28	8.29	-2.02	0.04045	Lrpprc
TC1500000677.mm.1	6.98	8	-2.02	0.014575	Cbyl
TC0X00002643.mm.1	11.87	12.88	-2.02	0.045167	Eif2s3x
TC1200000033.mm.1	13.17	14.18	-2.01	0.017567	Gm6682
TC0600000417.mm.1	5.4	6.41	-2.01	0.031195	Trbv1
TC1100004215.mm.1	14.22	15.22	-2	0.035288	P4hb
TC1100002965.mm.1	15.05	16.05	-2	0.035967	Hmgbl-ps3
TC1700002186.mm.1	10.25	11.25	-2	0.010558	Ppp2r5d

[000253] Table 8. Characteristics of patients enrolled in the study. Baseline demographic characteristics of patients enrolled in the study. Data are expressed as mean $\pm$ standard error (SEM).

	CTRL (n=12)	T1D (n=12)	New-onset T1D (n=12)
Sex (M/F)	4/8	6/6	4/8
Age (years)	34.8 $\pm$ 2.3	39.6 $\pm$ 3.1	10.9 $\pm$ 1.1
Years of T1D	N/A	22.3 $\pm$ 3.8	N/A
HbA1c % (mmol/mol)	5.0 $\pm$ 0.05 (31 $\pm$ 0.5)	9.2 $\pm$ 0.4 (78 $\pm$ 4.0)	12.3 $\pm$ 0.4 (111 $\pm$ 4.9)
EIR (UI)	N/A	42.0 $\pm$ 4.1	N/A

Concomitant Treatments	N/A	Levothyroxine (n=3) Statin (n=2)	N/A
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[000254] Table 9. Characteristics of patients enrolled in the plerixafor mobilization study. Baseline demographic characteristics of patients enrolled in the plerixafor mobilization study. Data are expressed as mean $\pm$ standard error (SEM)

	CTRL (n=8)	T1D (n=5)	P value
Age (years)	44.1 $\pm$ 5.1	39.0 $\pm$ 3.9	0.494
Male/Female		4/1	Ns
Weight (kg)	76.7 $\pm$ 6.7	76.2 $\pm$ 6.1	0.961
Height (cm)	174.8 $\pm$ 1.84	174.4 $\pm$ 4.6	0.913
BMI (kg/mq)	25.0 $\pm$ 2.1	25.0 $\pm$ 1.5	0.988
HbA1c (%)	5.7 $\pm$ 0.2	7.9 $\pm$ 0.3	0.026
Duration (Years)	0.0	20.8 $\pm$ 4.9	Ns
Hypertension (%)	37.5	0	0.139
Retinopathy (%)	0.0	20.0	0.742
Microalbuminuria (%)	0.0	0.0	Ns
Neuropathy (%)	0.0	0.0	Ns
Atherosclerosis (%)	0.0	20.0	Ns
White blood cells (10 ⁹ per liter)	7.1 $\pm$ 0.9	6.1 $\pm$ 0.6	Ns

[000255] Table 10. Transcriptome of pCD34+ cells. List of upregulated and downregulated inflammatory and costimulation-related genes identified by transcriptome profiling in pharmacologically-modulated CD34+ cells as compared to unmodulated-CD34+ cells obtained from T1D patients. Genes with statistically significant differences ($p < 0.05$) are in italics.

Table 10		
Refseq	Symbol	p value
NM_000022	ADA	0.225567

NM_020661	AICDA	0.195329
NM_000038	APC	0.351487
NM_000633	BCL2	0.099101
NM_000057	BLM	0.417912
NM_013314	BLNK	0.556359
NM_002983	CCL3	0.188691
NM_001295	CCR1	0.430142
NM_001123396	CCR2	0.48641
NM_001837	CCR3	0.234087
NM_005508	CCR4	0.530672
NM_000579	CCR5	0.651288
NM_001766	CD1D	0.669927
NM_001767	CD2	0.457163
NM_001242	CD27	0.975865
NM_014143	<i>CD274</i>	0.028999
NM_025240	CD276	0.537183
NM_006139	CD28	0.650923
NM_000732	CD3D	0.464723
NM_000733	CD3E	0.79027
NM_000073	CD3G	0.288999
NM_000616	CD4	0.512874
NM_001250	CD40	0.600445
NM_000074	CD40LG	0.891166
NM_001777	CD47	0.982858
NM_014207	CD5	0.548741
NM_006137	CD7	0.410343
NM_005191	CD80	0.170296
NM_004356	CD81	0.811353
NM_006889	CD86	0.382565
NM_001768	CD8A	0.263857
NM_004931	CD8B	0.400975
NM_000758	CSF2	0.630803
NM_002996	CX3CL1	0.337314
NM_001504	CXCR3	0.351979
NM_003467	CXCR4	0.373218
NM_001716	CXCR5	0.365898
NM_001935	DPP4	0.764763
NM_001964	EGR1	0.367139
NM_000043	FAS	0.568721
NM_000639	FASLG	0.189251
NM_014009	FOXP3	0.334684
NM_015259	ICOSLG	0.804737
NM_000619	IFNG	0.936207
NM_000572	IL10	0.645899
NM_000641	IL11	0.391528
NM_000882	IL12A	0.428351
NM_002187	IL12B	0.453057
NM_005535	<i>IL12RB1</i>	0.023026
NM_001559	IL12RB2	0.701111
NM_002188	IL13	0.291112
NM_000585	IL15	0.183724

NM_001562	IL18	0.972391
NM_003855	IL18R1	0.179383
NM_000576	IL1B	0.26186
NM_000586	IL2	0.24441
NM_000417	IL2RA	0.311566
NM_000588	IL3	0.820972
NM_000589	IL4	0.352994
NM_000418	IL4R	0.223243
NM_000879	IL5	0.849461
NM_000600	IL6	0.295894
NM_000880	IL7	0.601403
NM_000584	CXCL8	0.075554
NM_002460	IRF4	0.32968
NM_002286	LAG3	0.420723
NM_005356	LCK	0.437734
NM_003188	MAP3K7	0.310046
NM_005931	MICB	0.307045
NM_021950	MS4A1	0.290522
NM_006153	NCK1	0.419349
NM_000625	NOS2	0.386605
NM_002838	PTPRC	0.217912
NM_000448	RAG1	0.518868
NM_003821	RIPK2	0.395143
NM_003745	<i>SOCS1</i>	0.032789
NM_000660	TGFB1	0.110908
NM_003263	TLR1	0.519406
NM_003264	TLR2	0.102702
NM_138554	TLR4	0.447099
NM_006068	TLR6	0.306693
NM_017442	TLR9	0.850156
NM_003807	TNFSF14	0.289412
NM_005428	VAV1	0.83807
NM_001101	ACTB	0.169224
NM_004048	B2M	0.812532
NM_002046	GAPDH	0.297957
NM_000194	HPRT1	0.986777
NM_001002	RPLP0	0.382779
SA_00105	HGDC	0.374447
SA_00104	RTC	0.488473
SA_00104	RTC	0.829614
SA_00104	RTC	0.290938
SA_00103	PPC	0.565867
SA_00103	PPC	0.386582
SA_00103	PPC	0.394285

CLAIMS

1. An *ex vivo* method of producing a population of modified, PD-L1+ expressing hematopoietic stem cells (HSCs), the method comprising modulating the expression of miRNAs controlling the expression of PD-L1 in the HSCs.
2. The method of claim 1, wherein the modulation of the expression of miRNAs is increasing the expression of miRNA or decreasing the expression of miRNA.
3. The method of claim 1 or 2, wherein the miRNA expression is increased by introducing an exogenous copy of a nucleic acid encoding the miRNA for the expression of the miRNA in the cell.
4. The method of method of claim 1 or 2, wherein the miRNA expression is decreased by an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.
5. The method of claim 3, wherein the exogenous copy is introduced by way of a vector, such as a viral vector.
6. The method of claim 4, wherein the agent is introduced into the HSC by a vector, such as a viral vector.
7. The method of any of claims 1-6, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.
8. The method of any of claims 1-7, wherein the modified, PD-L1+ expressing HSCs carries an exogenous copy of a nucleic acid encoding a miRNA selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.
9. The method of any of claims 1-7, wherein the agent is as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA
10. A population of modified hematopoietic stem cells (HSCs) in which the modified cells have increased PD-L1 expression, where the cells carry an exogenous copy of a nucleic acid encoding a miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA.
11. The population of modified HSCs of claim 10, wherein the agent is an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.

12. The population of modified HSCs of claim 10, wherein the HSC cells are mammalian HSC cells.
13. The population of modified HSCs of claim 12, wherein the mammalian HSC cells are human HSC cells.
14. The population of modified HSCs of any of claims 10-13, wherein prior to the modification, the HSCs are obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood.
15. The population of modified HSCs of any of claims 10-13, wherein the HSCs are obtained from mobilized peripheral blood.
16. The population of modified HSCs of any of claims 10-15, wherein the HSCs are obtained from a healthy individual.
17. The population of modified HSCs of any of claims 10-15, wherein the HSCs are obtained from an individual with a diagnosed disease or disorder.
18. The population of modified HSCs of claim 17, wherein the diagnosed disease or disorder is an autoimmune disease or disorder.
19. The population of modified HSCs of claim 18, wherein the autoimmune disease or disorder is Type 1 diabetes (T1D).
20. The population of modified HSCs of any of claims 10-19, wherein the HSC cells are *ex vivo* cultured before or after or both before and after the modification of the PD-L1 expression.
21. The population of modified HSCs of any of claims 10-19, wherein the HSC cells are cryopreserved prior to or after or both prior to and after the modification of the PD-L1 expression.
22. The population of modified HSCs of any of claims 10-19, wherein the modified HSC cells are cryopreserved prior to use.
23. The population of modified HSCs of any of claims 10-22, wherein the HSC cells are produced by a method comprising:
 - a. contacting a sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA;
 - b. *ex vivo* culturing the resultant modified cells from the contacting; and
 - c. establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1.

24. The population of modified HSCs of any claims 10-22, the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

25. The population of modified HSCs of any of claims 10-22 that is produced by the method claims of 1-8.

26. A composition of modified HSCs comprising of HSCs of any of claims 1-25.

27. A method, the method comprising administering or *ex vivo* contacting an effective amount of an agent that modulates the expression of miRNAs controlling the expression of PD-L1 in the HSCs in a cell to a host.

28. The method of claim 27, wherein the cell is a progenitor cell.

29. The method of claim 28, wherein the progenitor cell is a hematopoietic progenitor cell.

30. The method of claim 29, wherein the agent is a vector comprising a nucleic acid sequence that miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA such as an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.

31. The method of claim 30, wherein the vector is a virus.

32. The method of claim 27, wherein the method is used to treat type 1 diabetes, an autoimmune disease, or cancer.

33. A method of treating an autoimmune disorder or cancer in a subject in need thereof, the method comprising administering to a subject a composition comprising the hematopoietic stem cells in any of the preceding claims.

34. The method of claim 33, wherein the autoimmune disorder is Type 1 diabetes (T1D).

35. The method of claim 33 or 34, wherein the HSCs are autologous to the recipient subject.

36. The method of claim 33 or 34, wherein the HSCs are non-autologous and allogenic to the recipient subject.

37. The method of claim 33 or 34, wherein the HSCs are non-autologous and xenogeneic to the recipient subject.

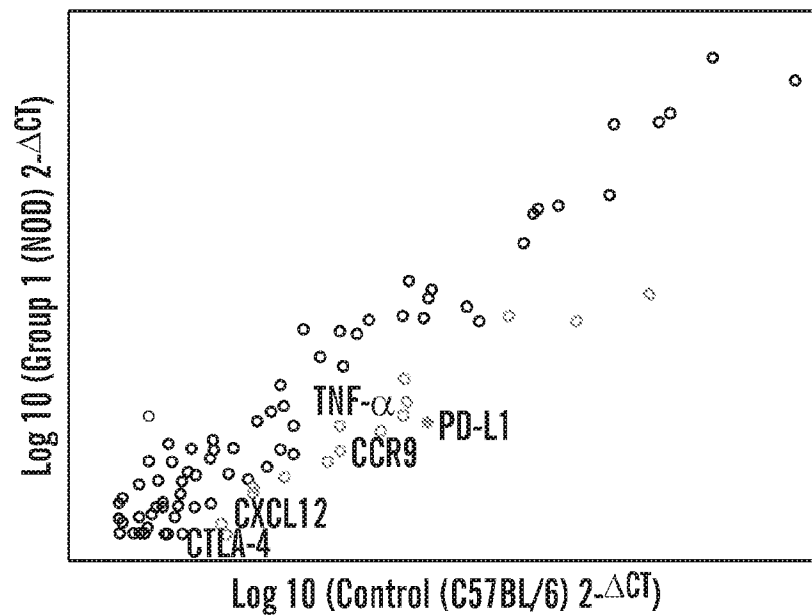
38. A method of modulating an immune response in a subject comprising, administering or transplanting the cell of claims 10-22, or administering the composition of claim 26.
39. A method of modulating an immune response in a subject comprising:
- providing a population of hematopoietic stem cells (HSCs);
 - contacting sample of HSCs with a vector carrying an exogenous copy of a nucleic acid encoding miRNAs that controls the expression of programmed cell death-1 receptor ligand (PD-L1) or an agent that inhibits the expression of the miRNA;
 - ex vivo* culturing the resultant modified cells from the contacting;
 - establishing the expression of PD-L1 on the modified HSCs, thereby producing a population of modified HSCs cells expressing PD-L1; and
 - transplanting said population of PD-L1+ expressing HSCs into a recipient subject, thereby modulating the immune response in the recipient subject.
40. The method of claim 38 or 39, wherein the population of HSCs is obtained from the bone marrow, umbilical cord, amniotic fluid, chorionic villi, cord blood, placental blood or peripheral blood.
41. The method of any of claims 38-40, wherein the population of HSCs is obtained from mobilized peripheral blood.
42. The method of any of claims 38-40, wherein the population of HSCs autologous to the recipient subject.
43. The method of any of claims 38-40, wherein the population of HSCs allogeneic to the recipient subject.
44. The method of any of claims 38-40, wherein the population of HSCs is xenogeneic to the recipient subject.
45. The method of any of claims 38-44, wherein the miRNA is selected from the group consisting of miR-4282, miR-7853, miR-7853-5p, miR-105, miR-105-5p, miR-224, miR-224-3p, miR-4279, miR-522, miR-522-3p, miR-374c, and miR-374c-5p.
46. The method of claim 39, wherein the agent is an antagomir of the miRNA, an anti-miRNA oligonucleotide to the miRNA, an antisense oligonucleotide to the miRNA or a locked nucleic acid that anneals to miRNA.
47. The method of any of the preceding claim, wherein the vector is a virus.
48. A composition comprising the PD-L1 expressing hematopoietic stem cells of any one of the preceding claims for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk

of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

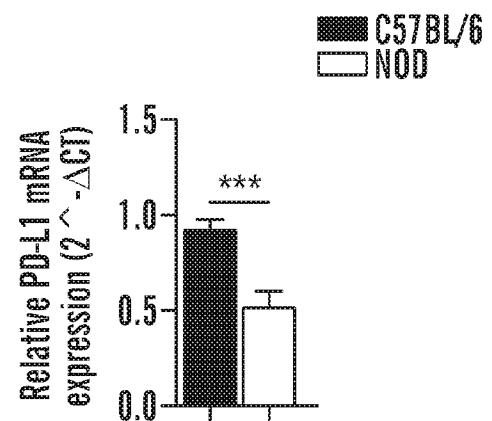
49. A composition comprising the PD-L1 expressing hematopoietic stem cells of any one of the preceding claims for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

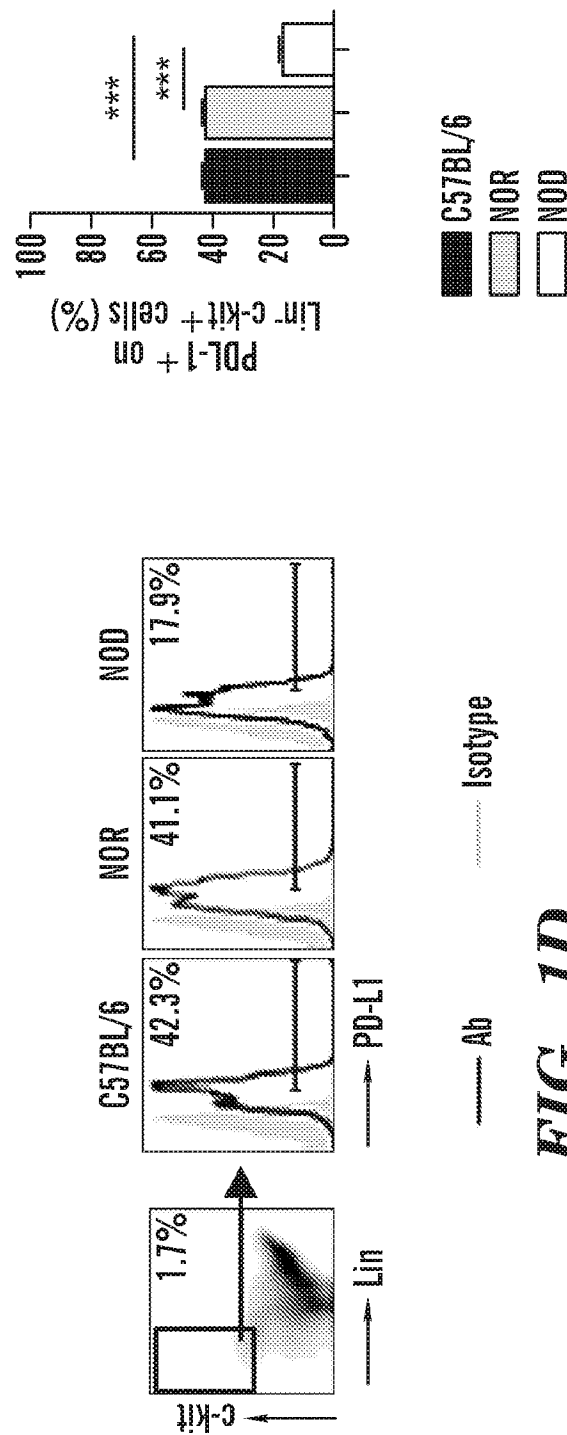
50. A population of PD-L1 expressing hematopoietic stem cells of any one of the preceding claims for use in the prevention or treatment of an autoimmune disease or disorder, for use in suppressing an immune response in a subject, for use in the delay of the onset of T1D in a subject at risk of developing T1D, for use in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

51. A population of PD-L1 expressing hematopoietic stem cells of any one of the preceding claims for the manufacture of medicament for use in the prevention or treatment of an autoimmune disease or disorder, in the suppression of an immune response in a subject, in the delay of the onset of T1D in a subject at risk of developing T1D, in the prevention and delay of an allogenic tissue or organ transplant rejection, and for the treatment of T1D in adult and pediatric subjects.

**FIG. 1A**

Gene Symbol	P-value	Fold change
CD274 (PD-L1)	0.003313	-14.4402
CCR9	0.005054	-8.6234
CTLA-4	0.072520	-7.2386
CXCL12	0.019921	-5.3904
TNF-α	0.056009	-2.5213

FIG. 1B**FIG. 1C**



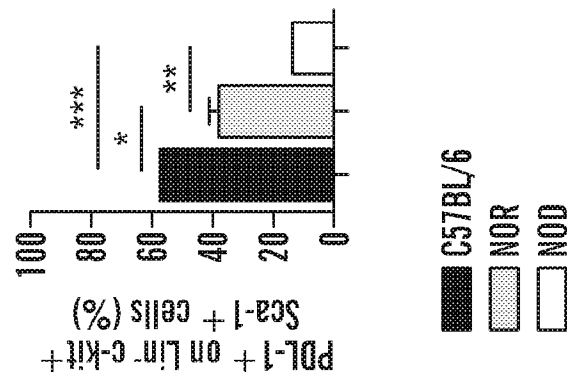


FIG. 1G

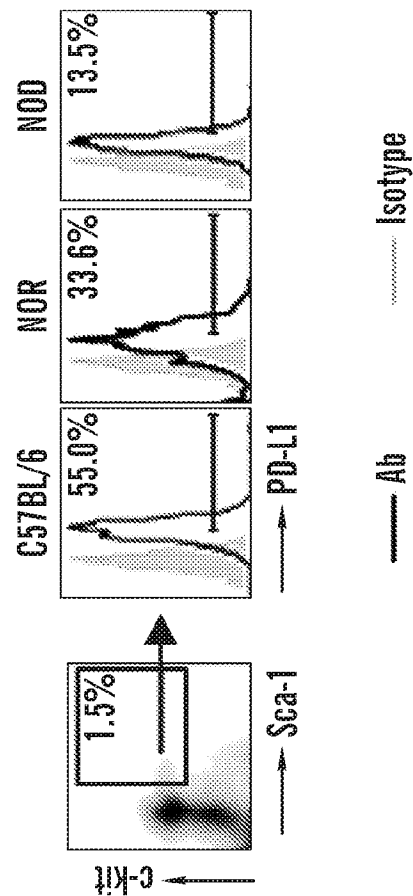
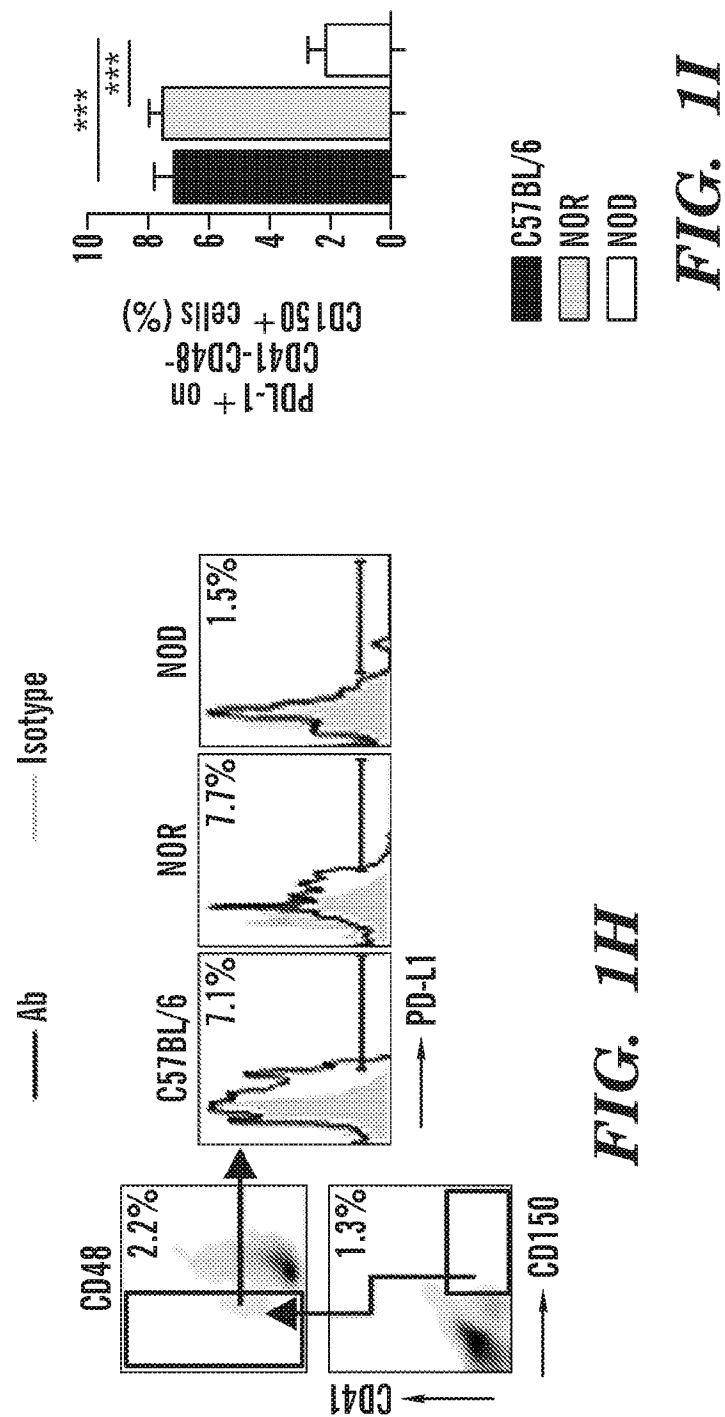


FIG. 1F



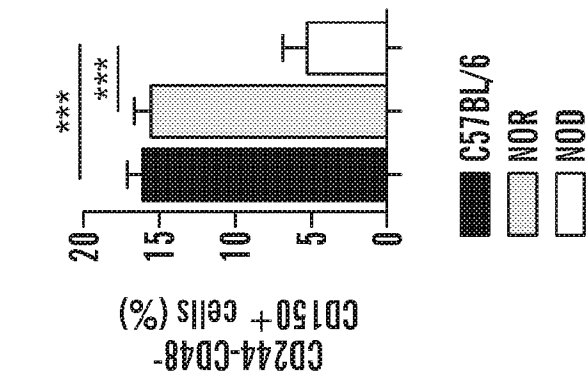


FIG. 1K

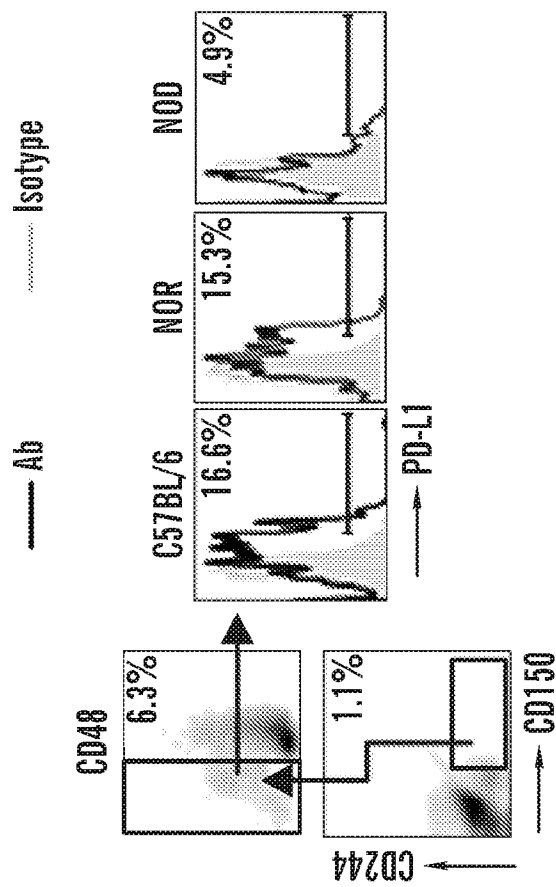


FIG. 1J

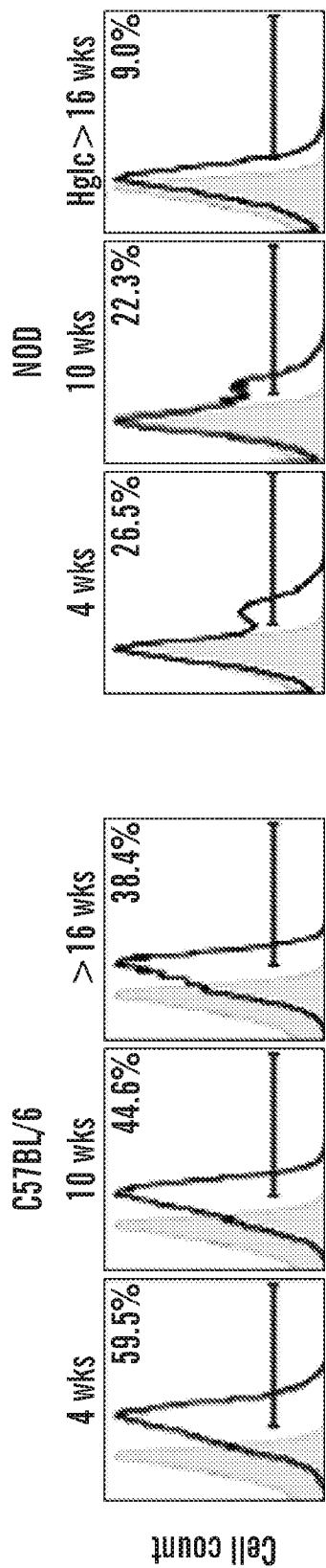


FIG. 1L

FIG. 1M

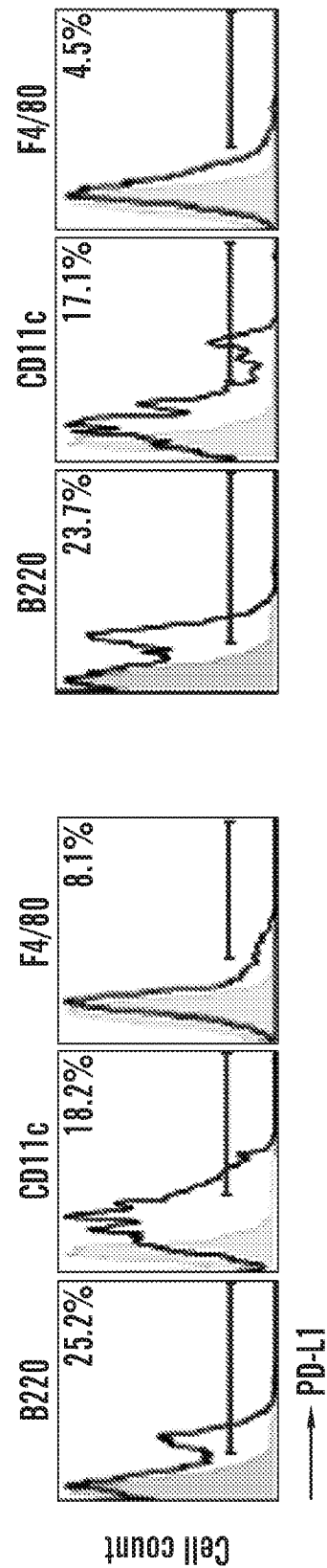
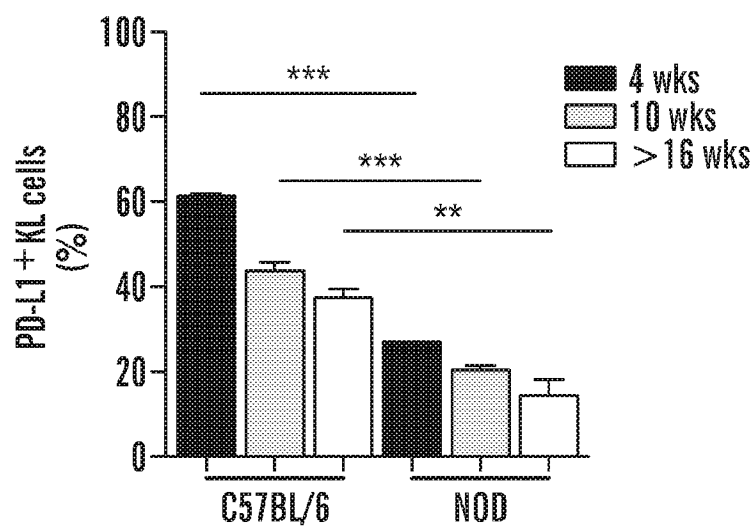
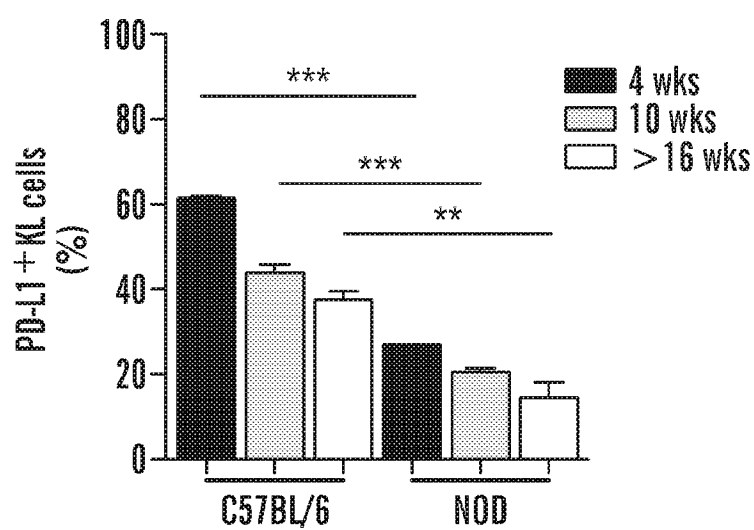


FIG. 1O

FIG. 1P

7/76

**FIG. 1N****FIG. 1Q**

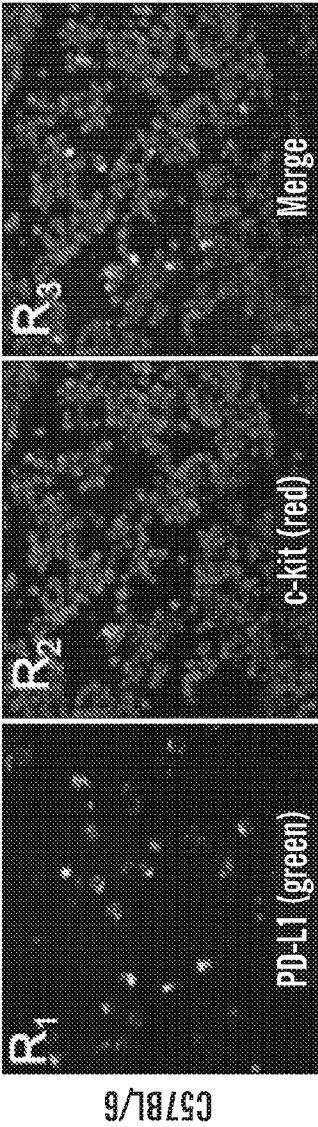


FIG. 1R1 FIG. 1R2 FIG. 1R3

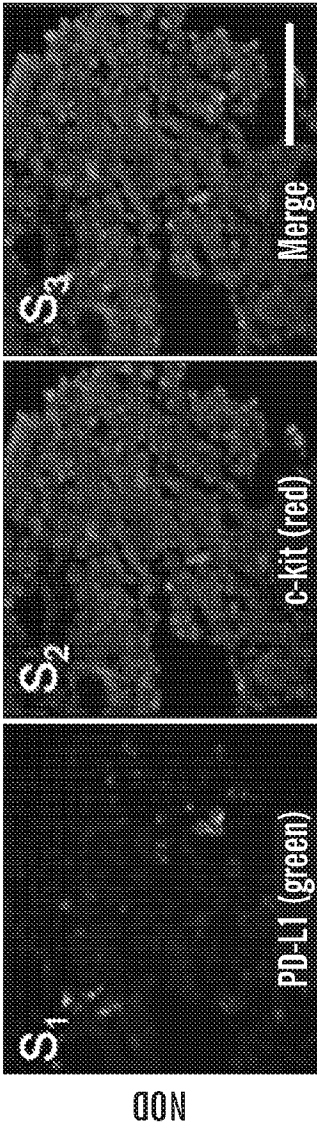


FIG. 1S1 FIG. 1S2 FIG. 1S3

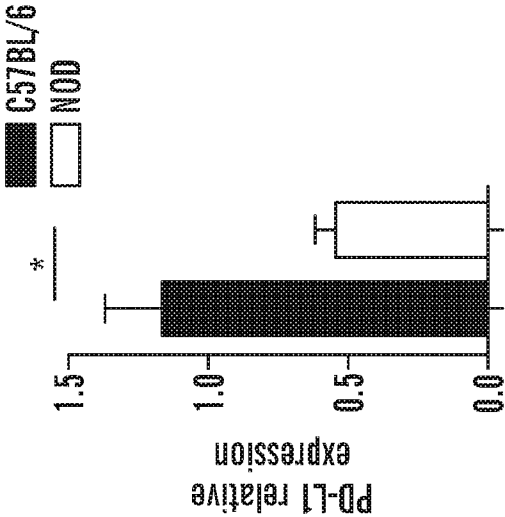


FIG. 1V



FIG. 1U

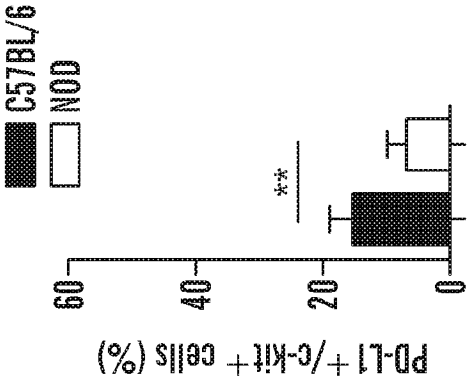


FIG. 1T

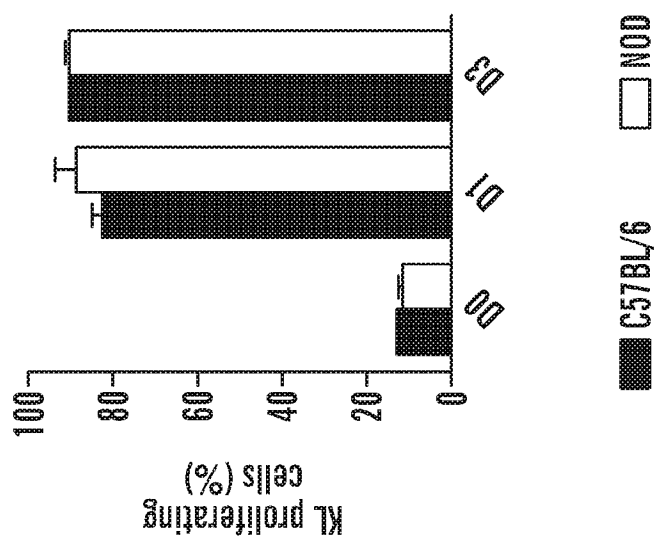


FIG. 2B

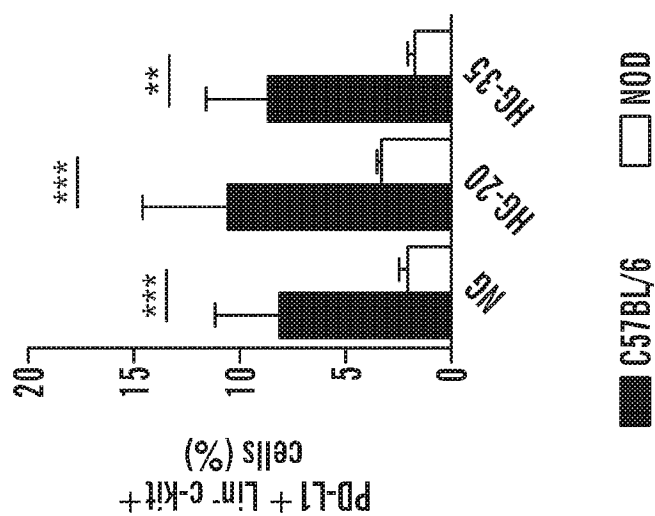


FIG. 2A

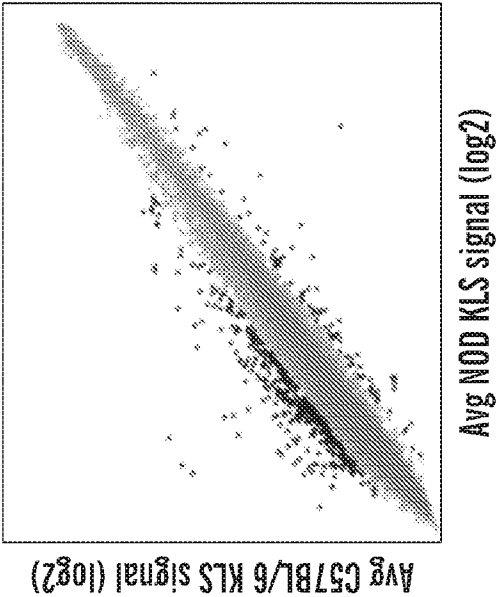


FIG. 2D

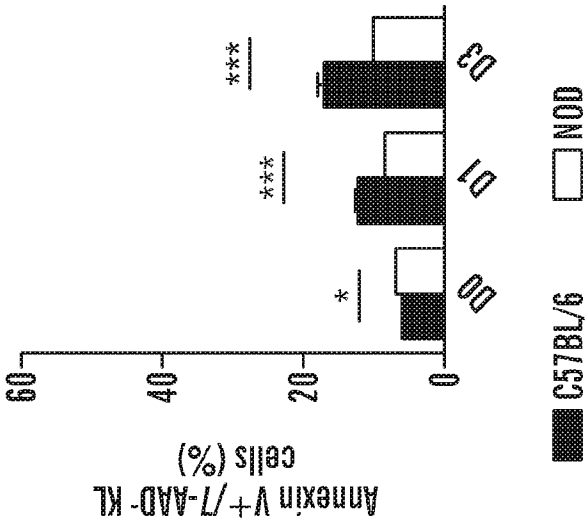


FIG. 2C

12/76

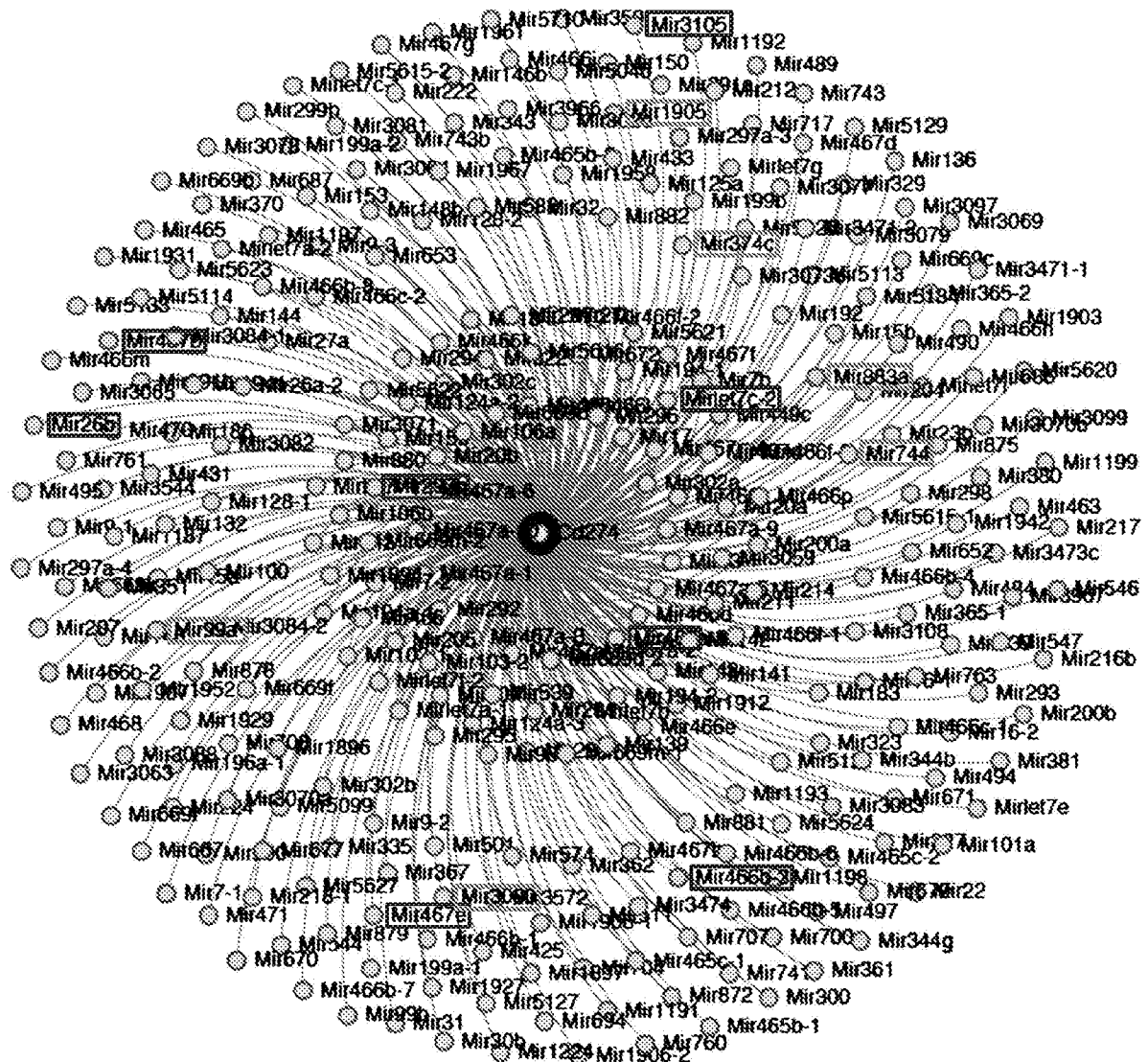
Gene Symbol	Fold Change in KLS (linear) (NOD vs. C57BL/6)	ANOVA p-value
Mir5103	1.15	0.000783
Mir6980	1.32	0.001285
Mir6403	1.22	0.002286
Mir1a-1	1.21	0.004198
Mir7003	1.20	0.007257
Mir744	1.15	0.009597
Mir3085	1.59	0.009612
Mir374c	1.20	0.009665
Mir1905	1.15	0.010847
Mir7069	1.20	0.015308
Mir6396	1.18	0.017188
Mirlet7j	1.19	0.017254
Mir99a	1.22	0.021452
Mir125b-1	1.11	0.024741
Mir3090	1.29	0.030353
Mir6991	1.43	0.034347
Mir7652	1.19	0.035569
Mir1934	1.43	0.037478
Mir697	1.20	0.038232
Mir7680	1.13	0.038836
Mir883a	1.31	0.042102
Mir6420	1.36	0.047160
Mir6947	1.25	0.049832

FIG. 2E

13/76

Gene Symbol	Fold Change in KLS (linear) (NOD vs. C57BL/6)	ANOVA p-value
Mir3970	-1.07	0.004263
Mir143hg	-1.08	0.005565
Mir3105	-1.21	0.006781
Mir297b	-1.64	0.012577
Mir26b	-1.17	0.013327
Mir6351	-1.04	0.017714
Mir105	-1.12	0.018491
Mir599	-1.19	0.019421
Mir487b	-1.12	0.019875
Mir206	-1.04	0.019993
Mir6374	-1.29	0.022005
Mir7078	-1.22	0.023855
Mir1935	-1.05	0.024732
Mir467c	-1.34	0.026936
Mir467e	-1.49	0.030203
Mir182	-1.09	0.032037
Mir6372	-1.07	0.034827
Mir6931	-1.73	0.036027
Mir7660	-1.43	0.042443
Mir344d-1	-1.06	0.042523
Mirlet7c-2	-1.04	0.043381
Mir1928	-1.12	0.045996
Mir1190	-2.10	0.046372
Mir466b-3	-1.14	0.049132
Mir7015	-1.33	0.049148

FIG. 2F

**FIG. 2G**

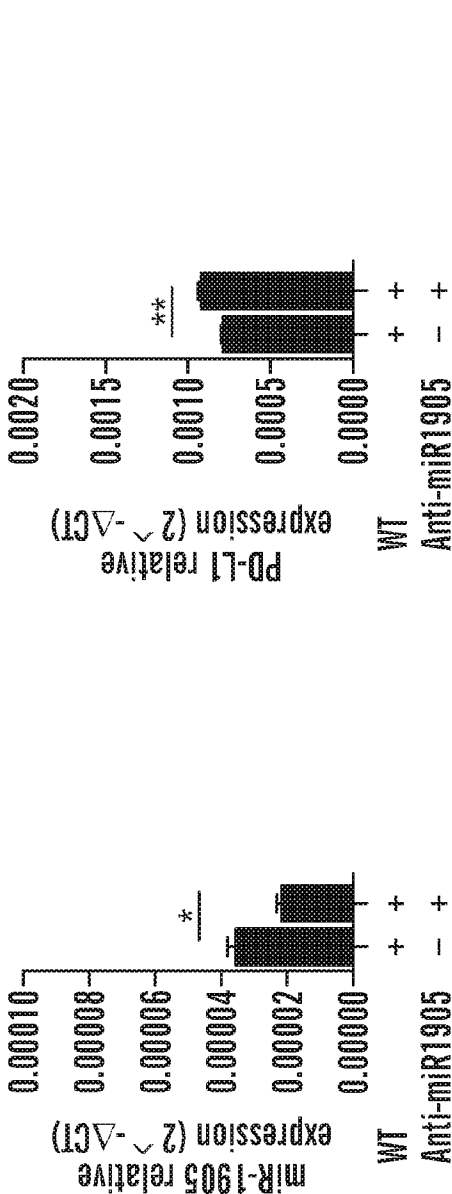


FIG. 2I

FIG. 2H

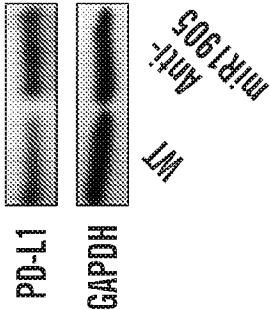


FIG. 2J

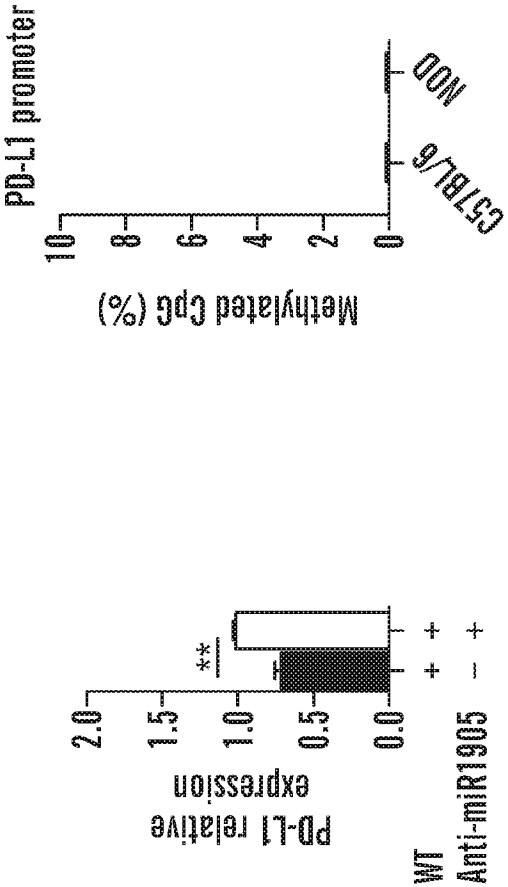


FIG. 2L

FIG. 2K

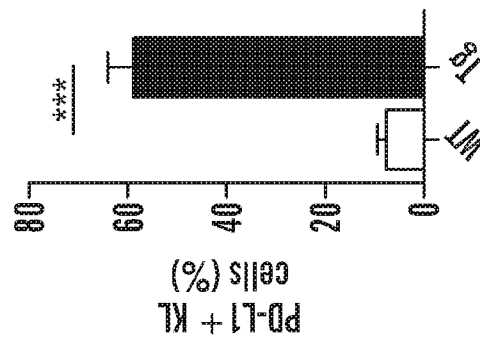


FIG. 3C

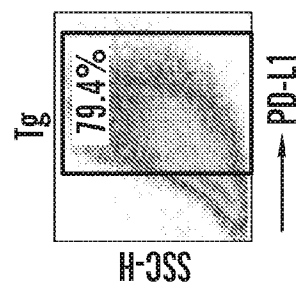


FIG. 3B

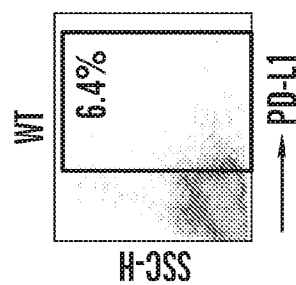


FIG. 3A

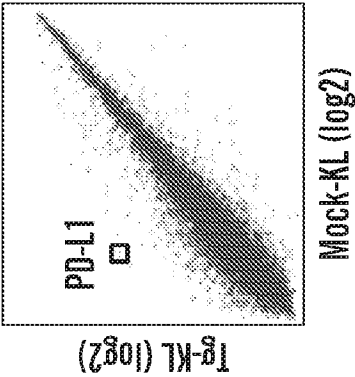


FIG. 3F

Gene Symbol	Ig	Mock	Fold Change
CD274 (PD-L1)	14.12	5.77	327.59

FIG. 3G

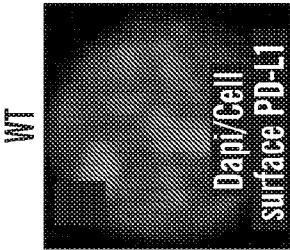


FIG. 3D

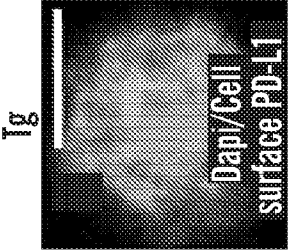


FIG. 3E

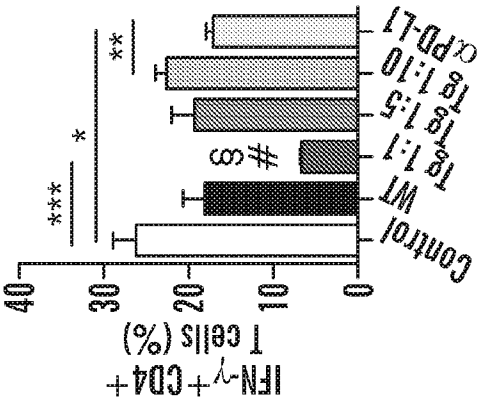


FIG. 3I

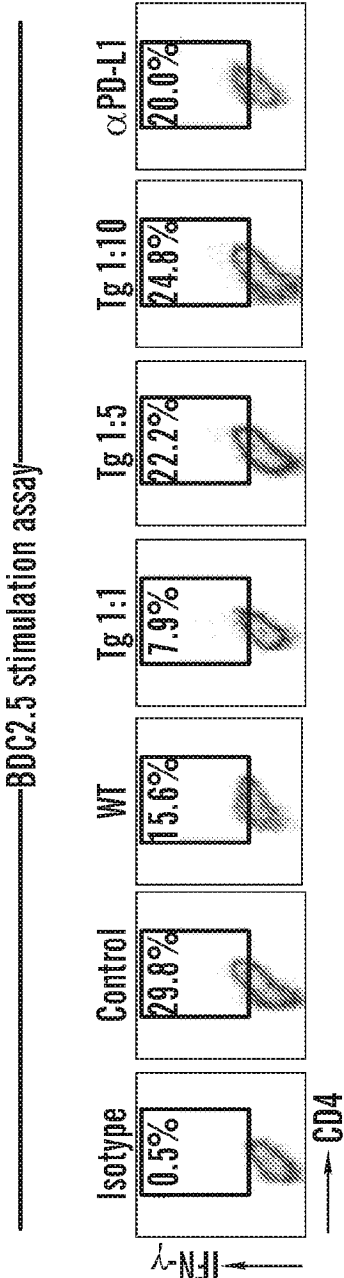


FIG. 3H

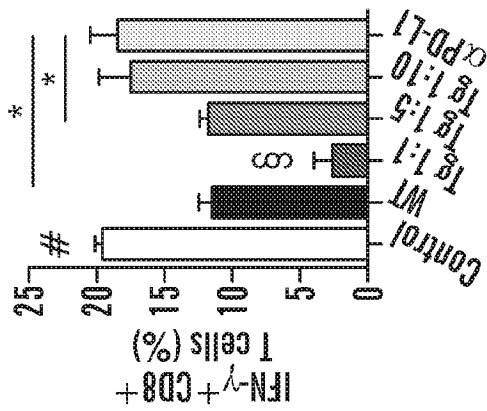


FIG. 3K

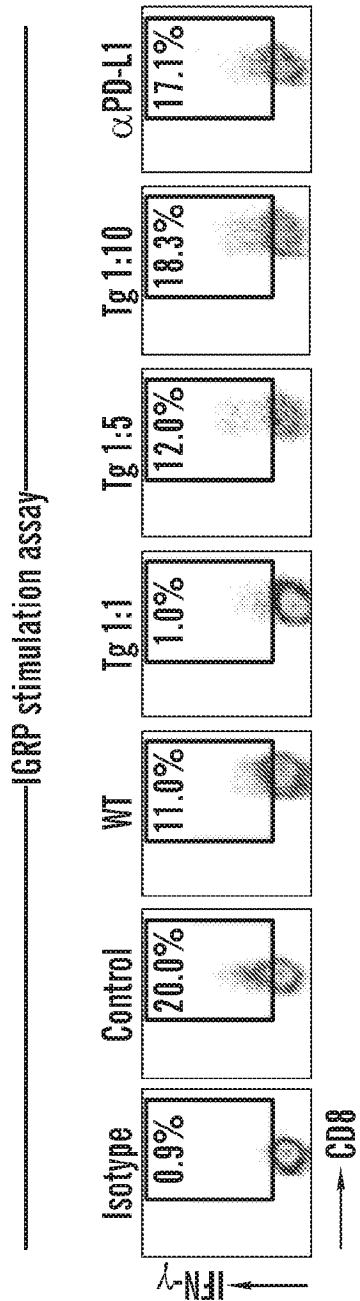


FIG. 3J

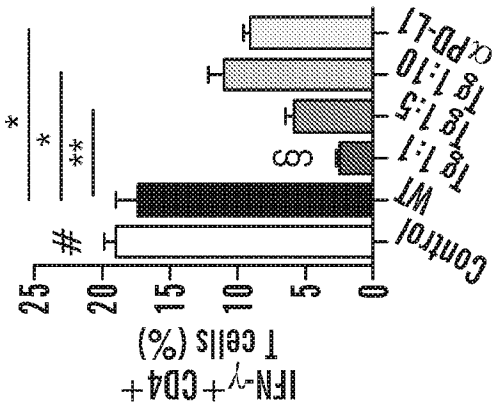


FIG. 3M

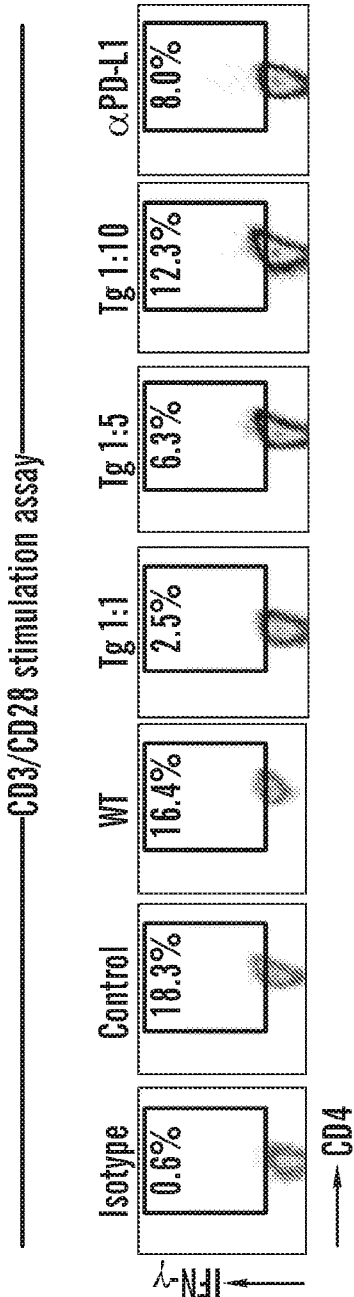
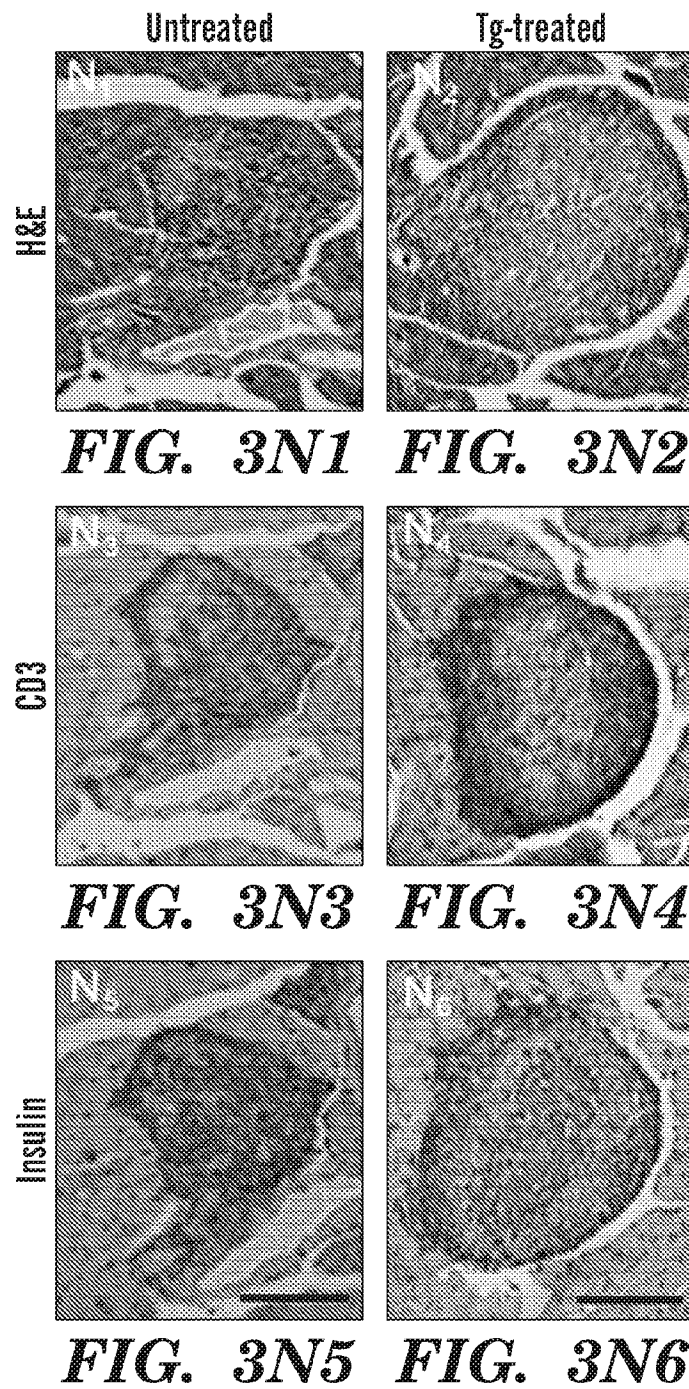
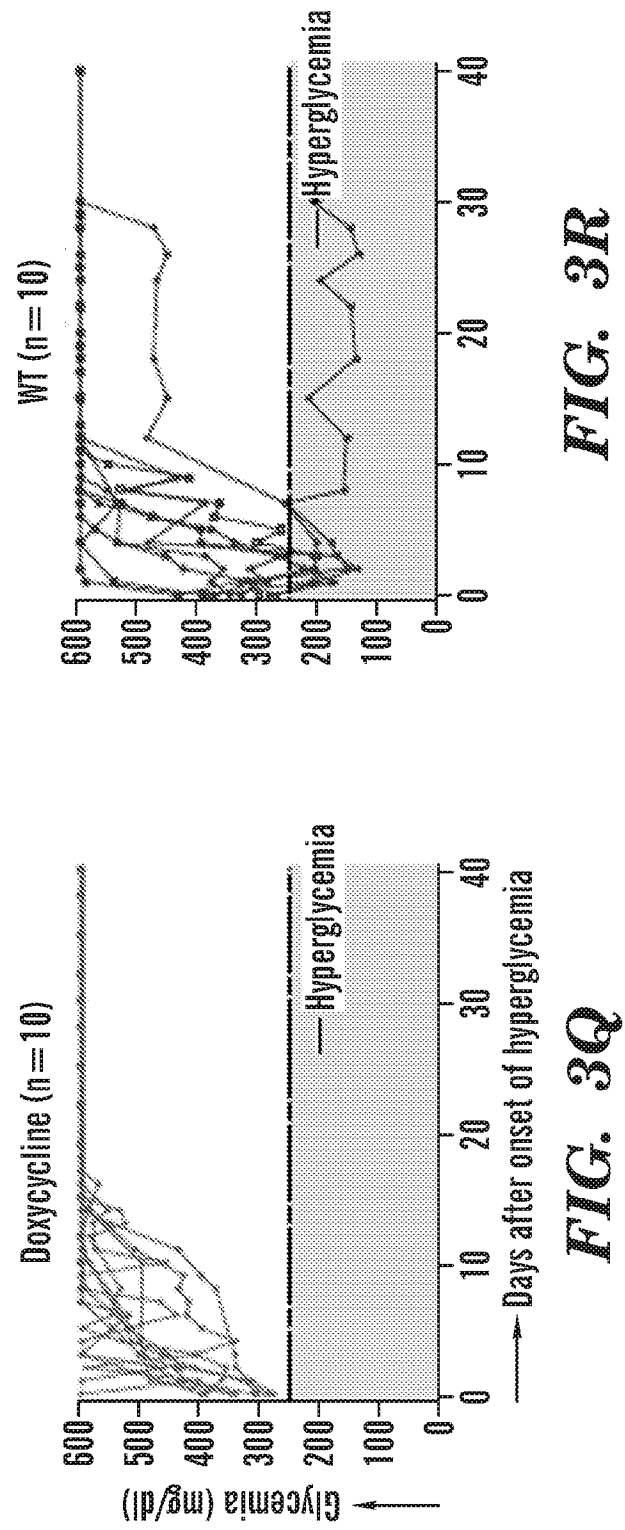
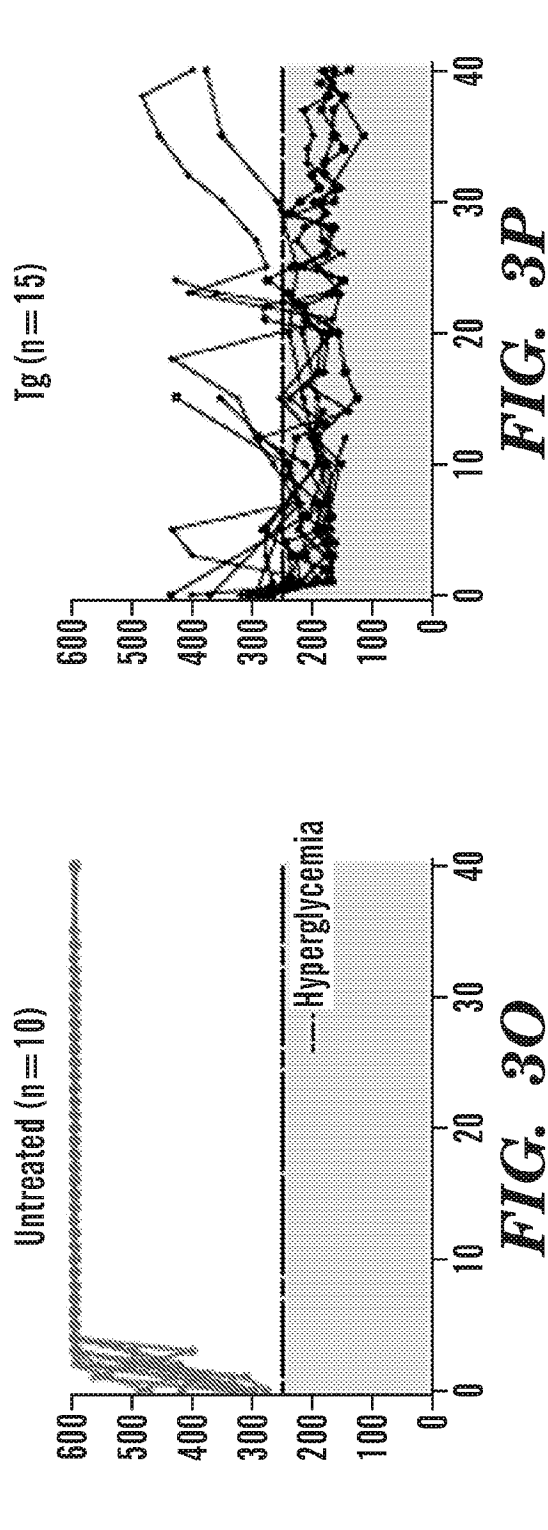


FIG. 3L





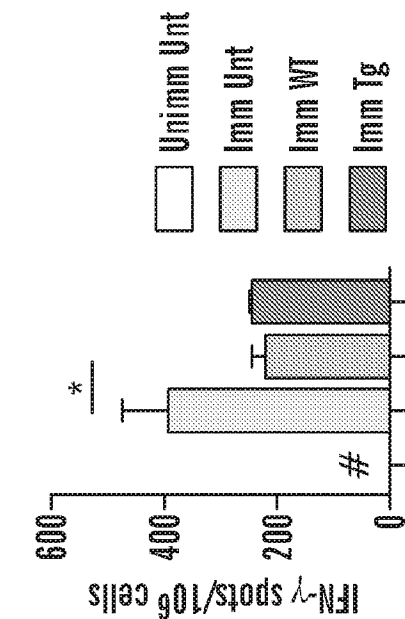


FIG. 3T

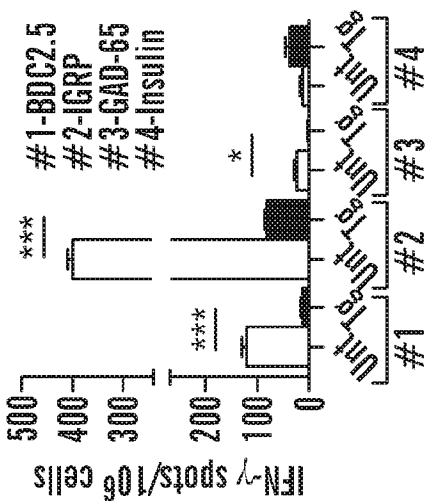


FIG. 3V

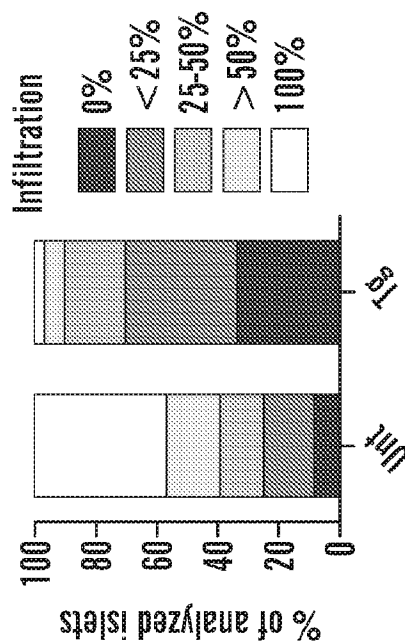


FIG. 3S

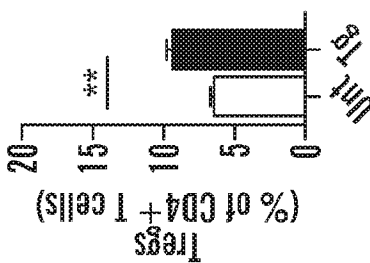
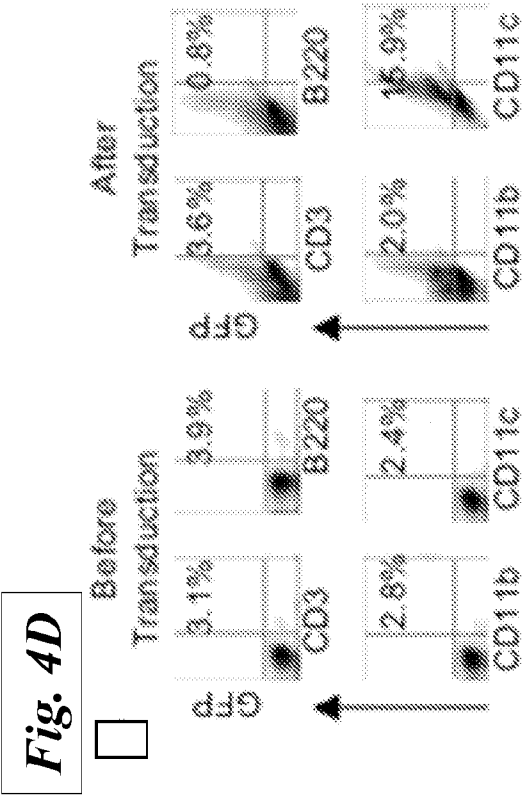
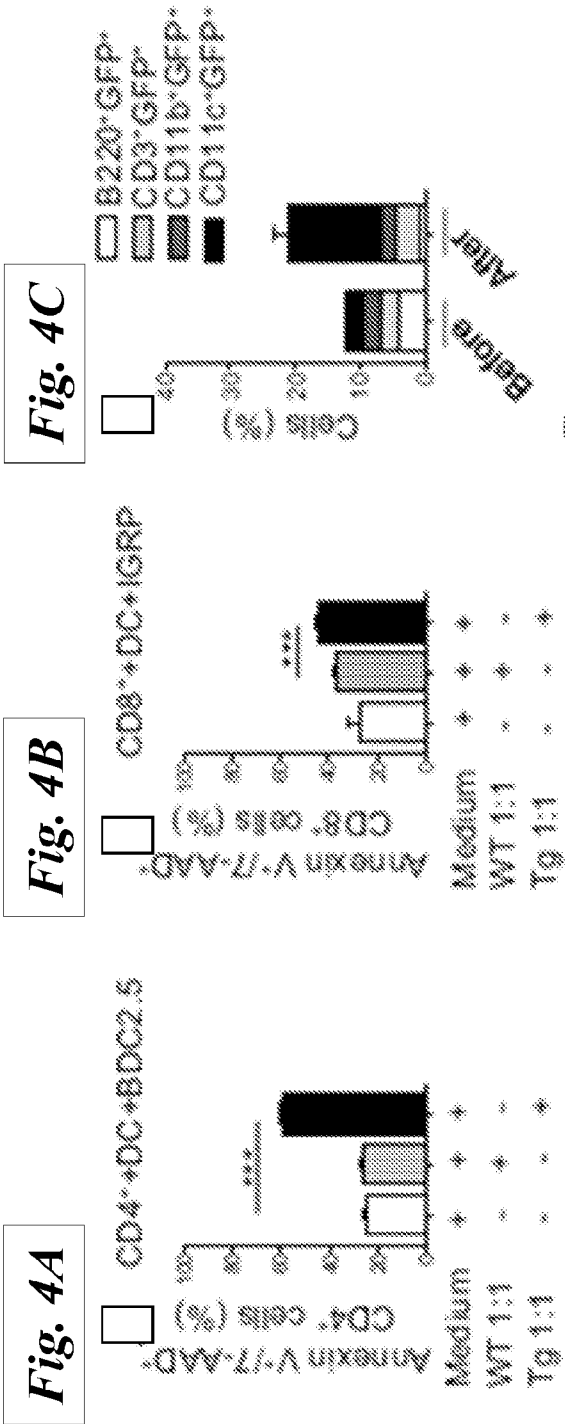


FIG. 3U



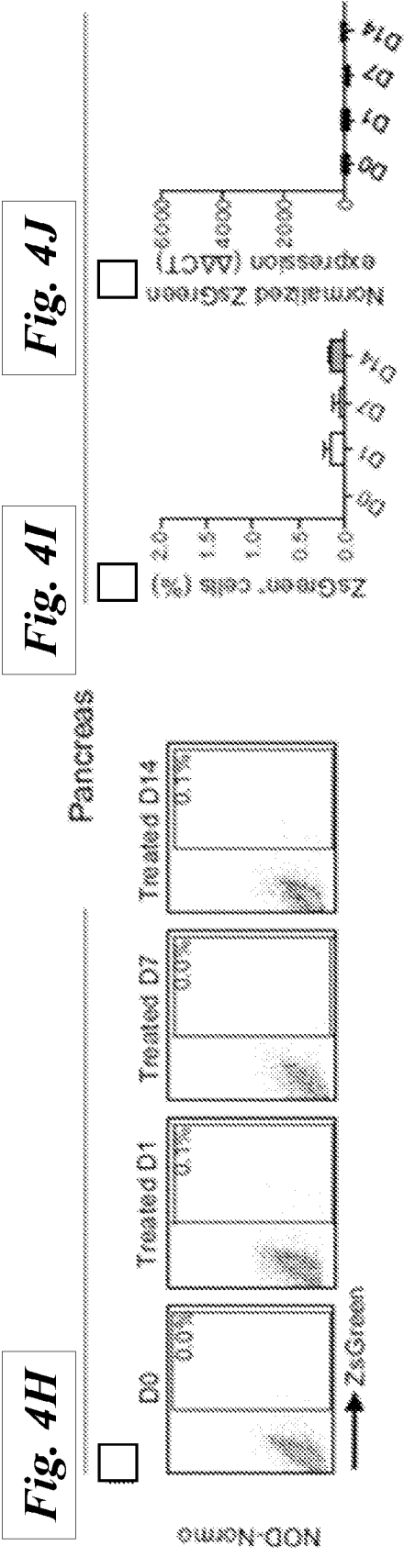
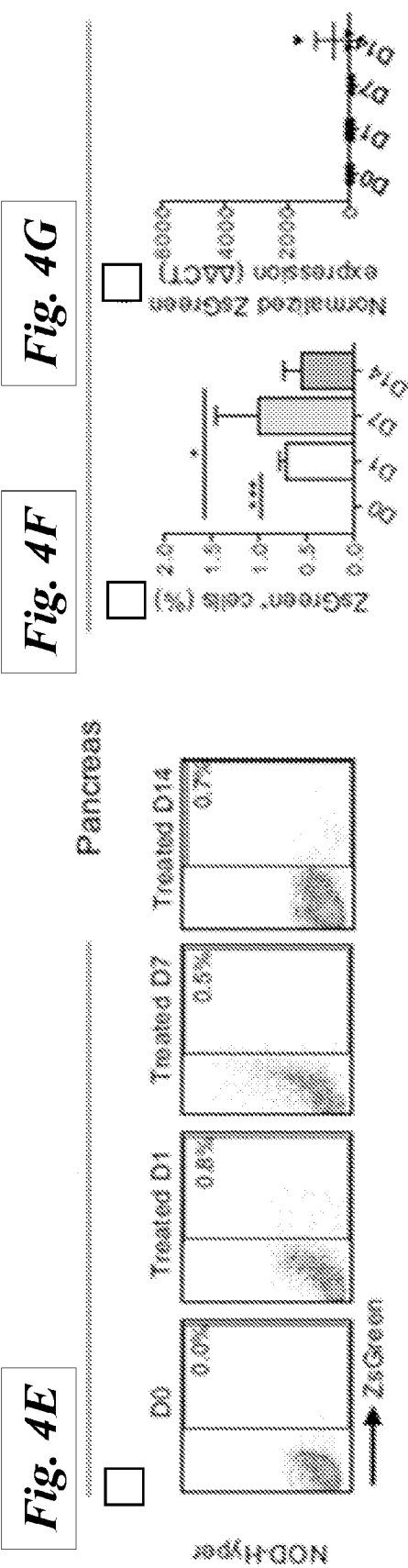


Fig. 4K

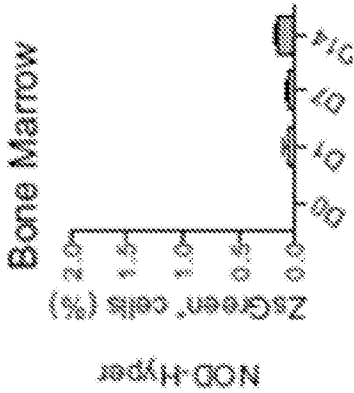


Fig. 4L

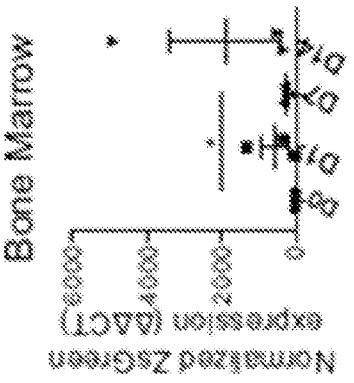


Fig. 4M

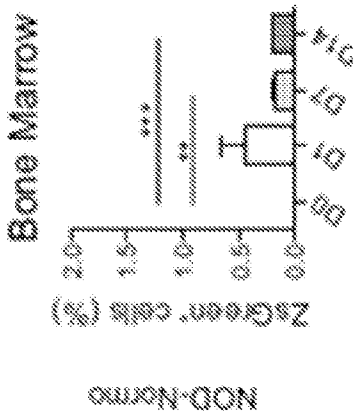


Fig. 4N

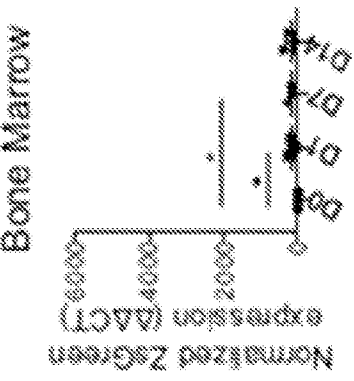


Fig. 4O

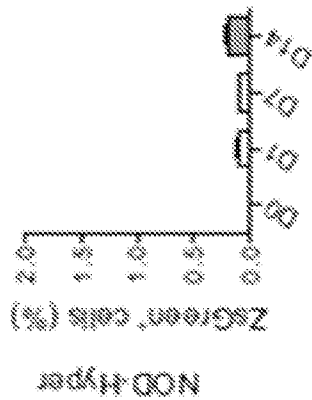


Fig. 4P

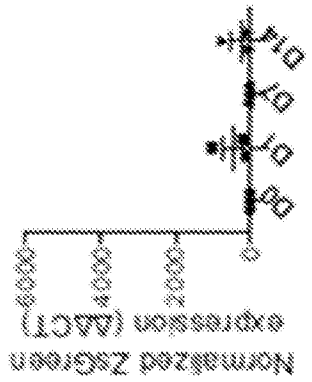


Fig. 4Q

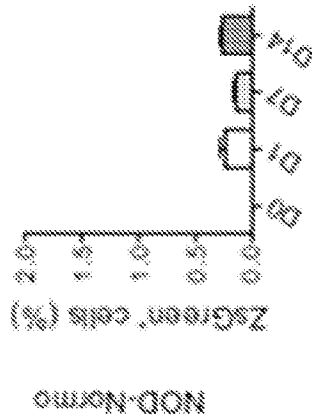


Fig. 4R

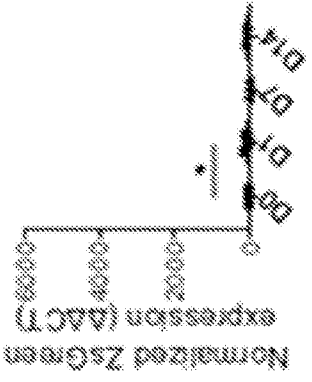


Fig. 4S1-4S4

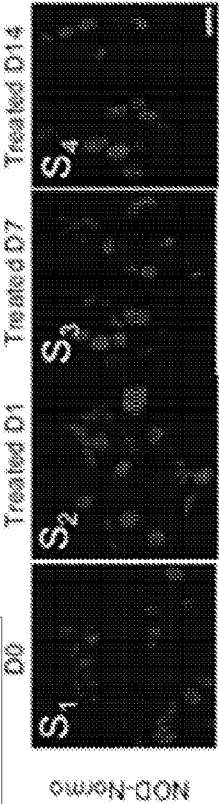


Fig. 4T1-4T4

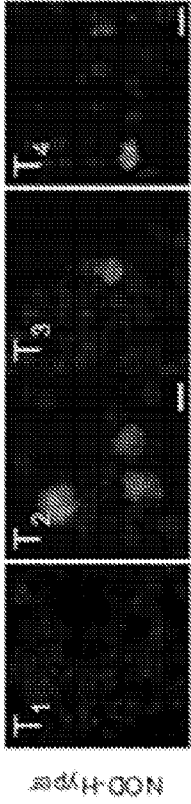


Fig. 4U

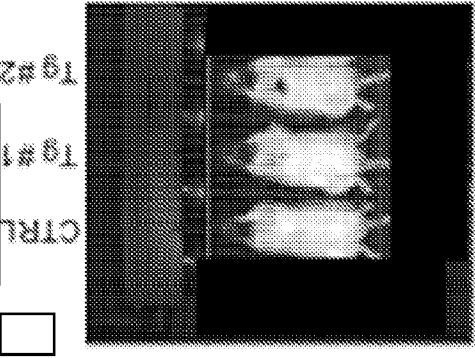


Fig. 4V

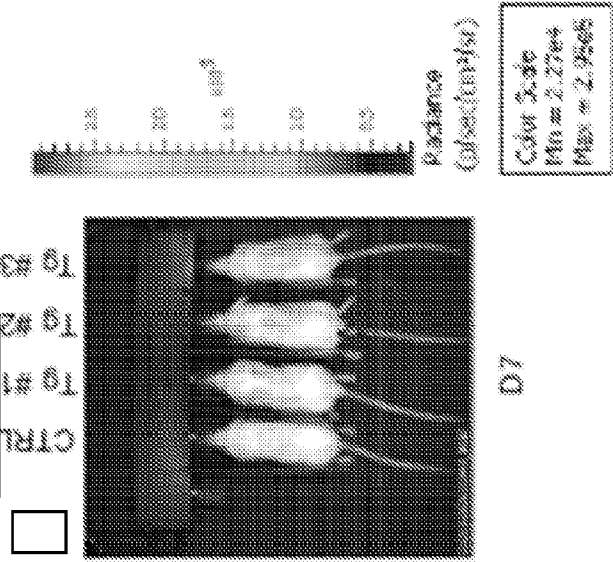


Fig. 5A

Receptor	TLR2/6	TLR1/2	TLR2	TLR3	TLR4	TLR5	TLR7	TLR7/8	TLR8	TLR9	NOD1	NOD2
Ligand	Pam2CSK4	Pam3CSK4	ANK2	Poly(I:C)	LPS-DB	FLAG-ET	Imiquimod	Resiquimod	CDN	CDN	CD14/2	NOP
Conc	ng/ml	ng/ml	cells/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	10 ³	10 ⁴	10 ³	100	10 ⁴	10 ⁴	10 ⁴	10 ⁴	10 ⁶	10 ⁴
B	10	10	10 ¹	10 ³	10 ³	10	10 ³	10 ³	10 ³	10 ³	10 ³	10 ³
C	0	1	10 ⁴	10 ²	10	0	10 ²	10 ²	10 ²	10 ²	10 ²	10 ²
D	0.1	0.1	10 ³	10	1	0.1	10	10	10	10	10	10
E	0.01	0.01	10 ⁴	1	0.1	0.01	1	1	1	1	1	1
F	0.001	0.001	10 ³	0.1	0.01	0.001	0.1	0.1	0.1	0.1	0.1	0.1
G	0.0001	0.0001	10 ²	0.01	0.001	0.0001	0.01	0.01	0.01	0.01	0.01	0.01
H	0	0	0	0	0	0	0	0	0	0	0	0

Fig. 5B

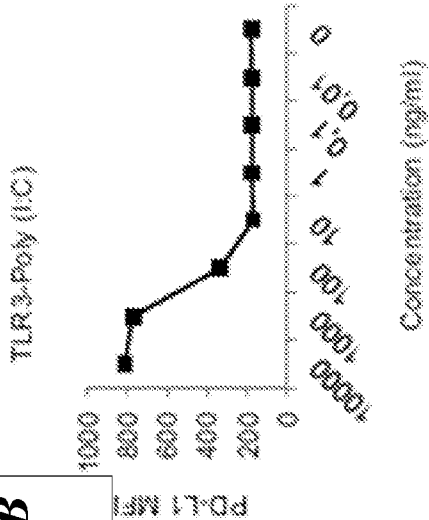


Fig. 5C

TLR screen (96wp) + mobilized CD34+ PD-L1 Expression (MFI)													
	TLR226	TLR112	TLR2	TLR3	TLR4	TLR5	TLR7	TLR7/8	TLR9	TLR9	MD1	MD2	
A	258	170	181	353	158	187	225	215	170	187	185	184	
B	188	185	184	274	156	188	224	213	182	188	172	172	
C	177	187	170	305	187	172	216	201	181	188	188	188	
D	174	175	168	189	168	170	211	200	171	185	188	188	
E	172	173	171	173	170	173	218	197	187	187	188	176	
F	176	170	175	174	177	174	220	203	177	188	188	172	
G	177	184	175	174	174	173	222	213	171	173	172	176	
H	181	178	178	179	177	174	225	211	188	175	173	173	

PD-L1 Fold change vs. no treatment

Fig. 5D

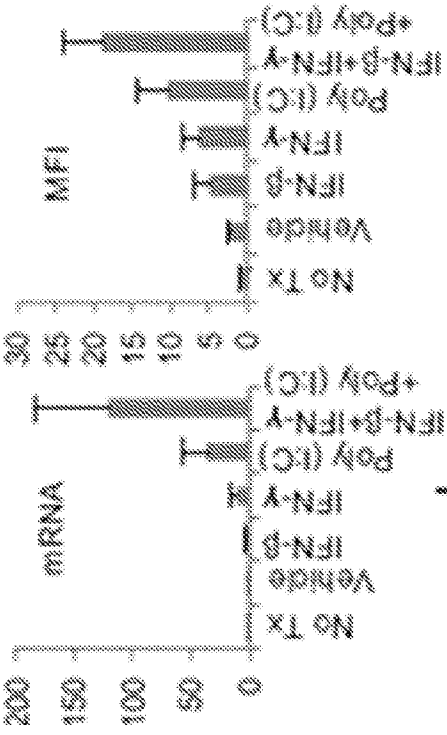


Fig. 5E

Fig. 5F

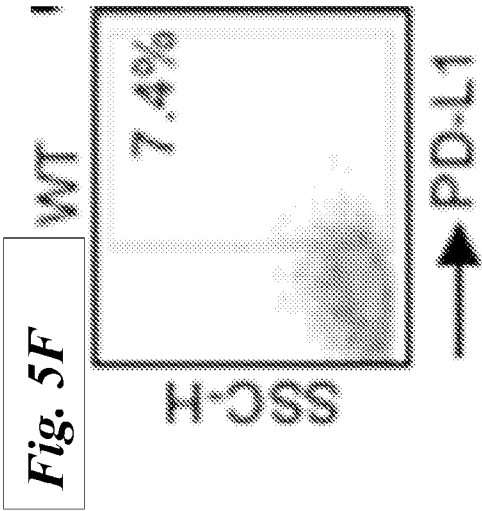


Fig. 5G

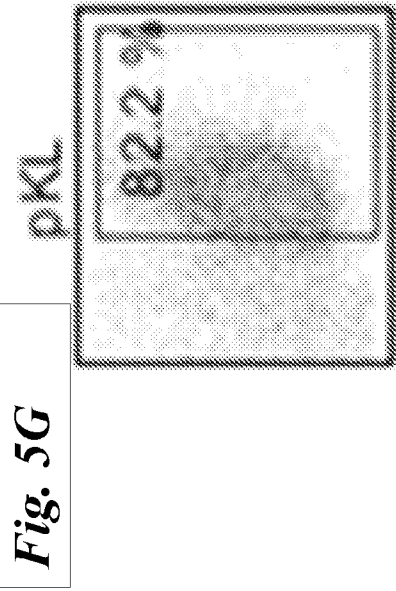


Fig. 5H

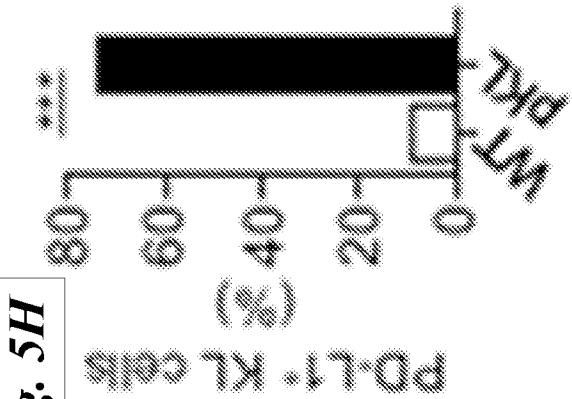


Fig. 5I

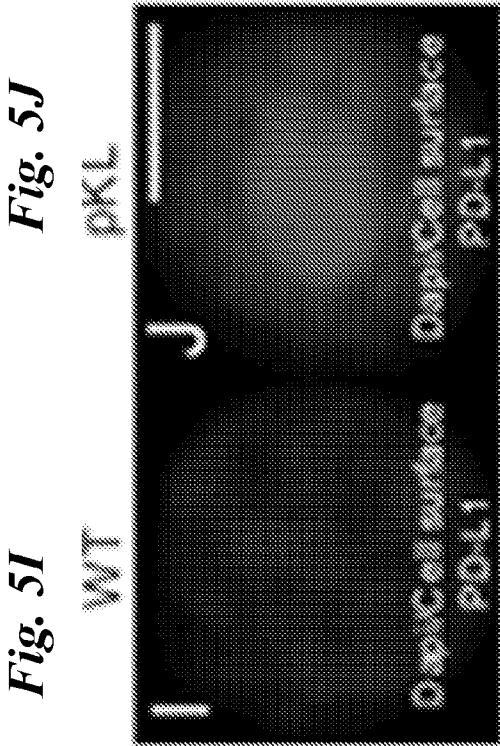


Fig. 5J

Fig. 5K

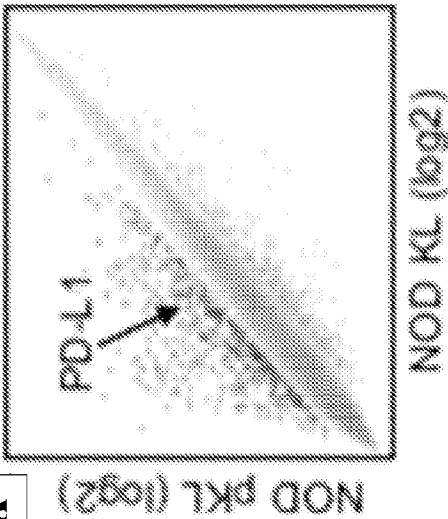


Fig. 5L

Gene Symbol	pKL	WT	Fold change
CD274 (PD-L1)	12.09	8.38	13.06

Fig. 5M

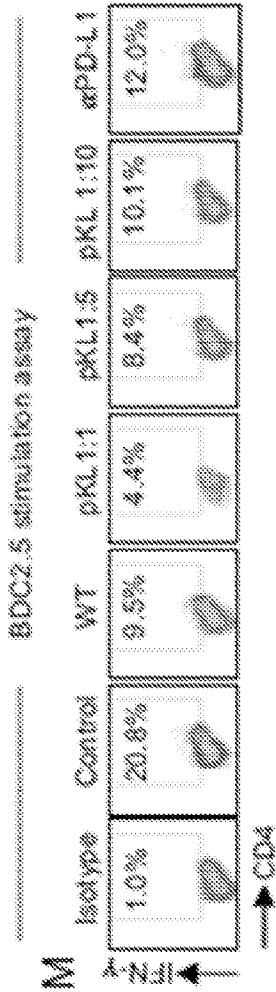


Fig. 5N

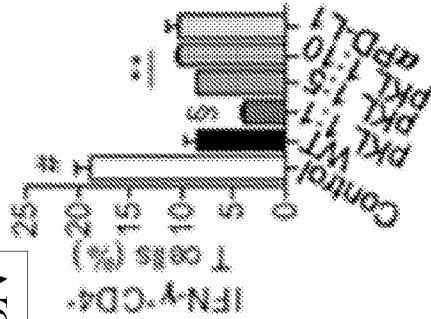


Fig. 5O

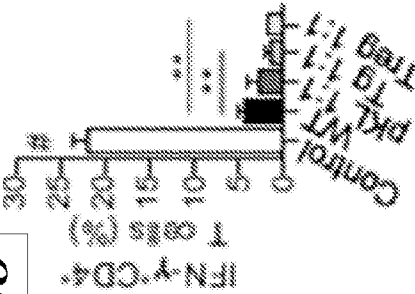


Fig. 5P

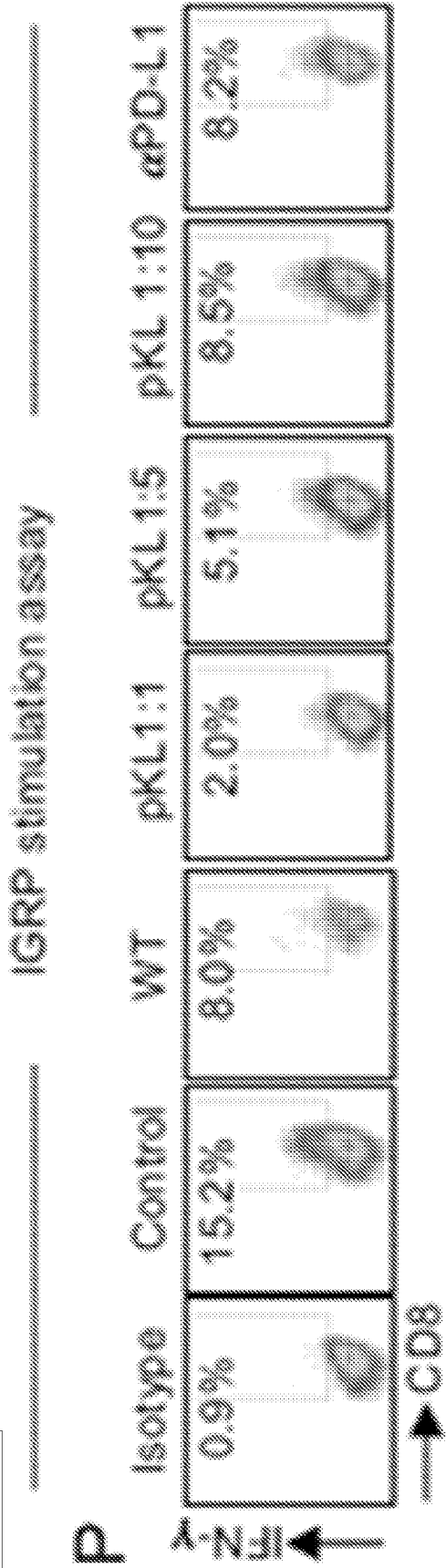


Fig. 5Q

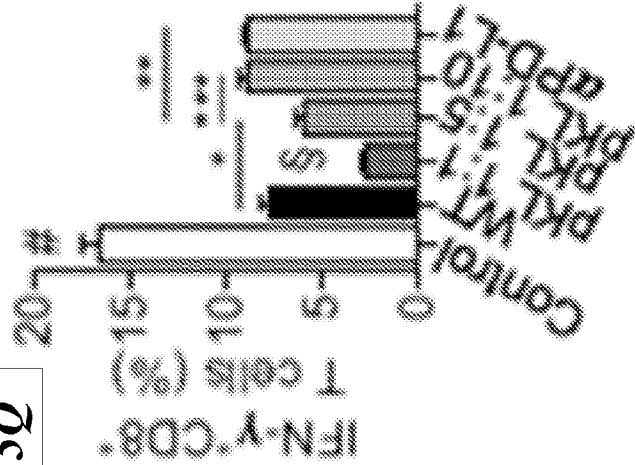


Fig. 5R

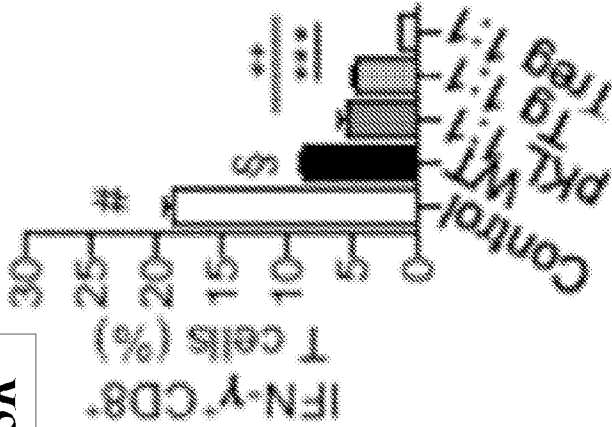


Fig. 5S

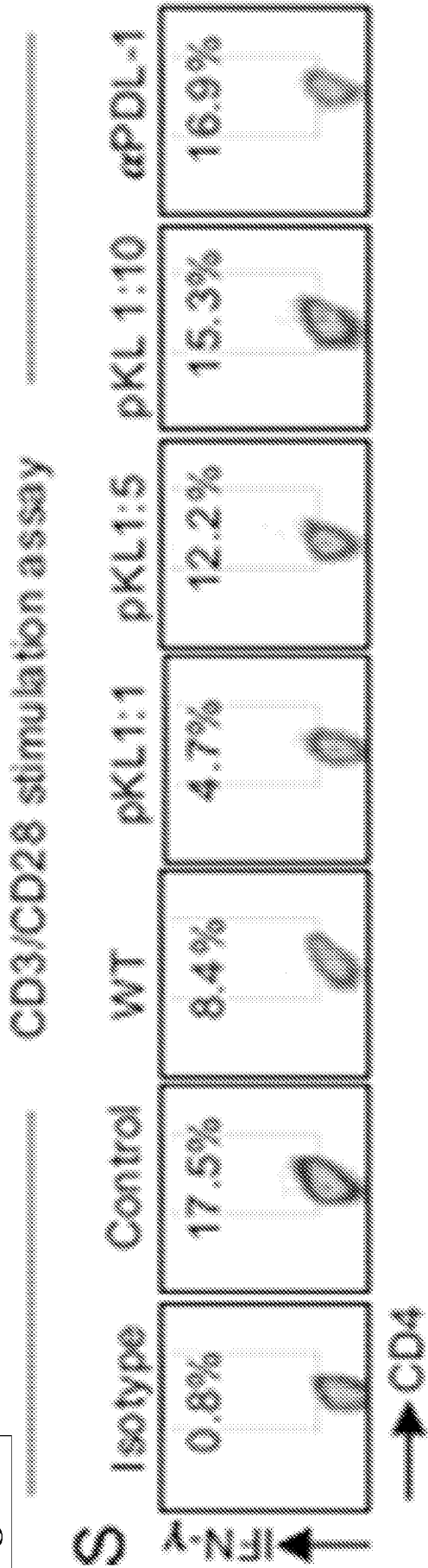


Fig. 5T

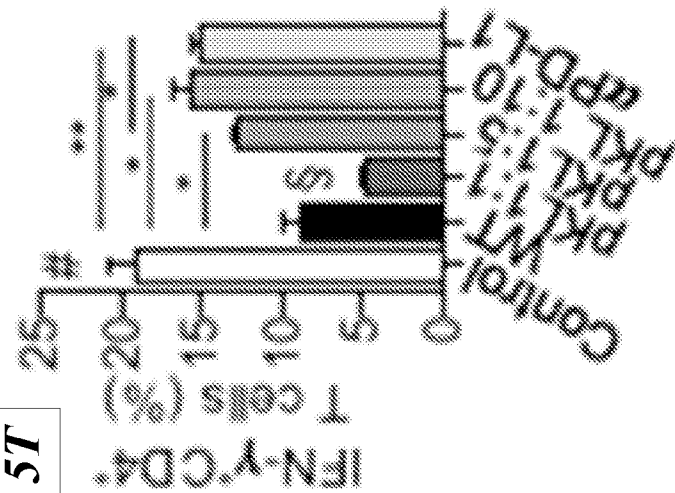


Fig. 5U

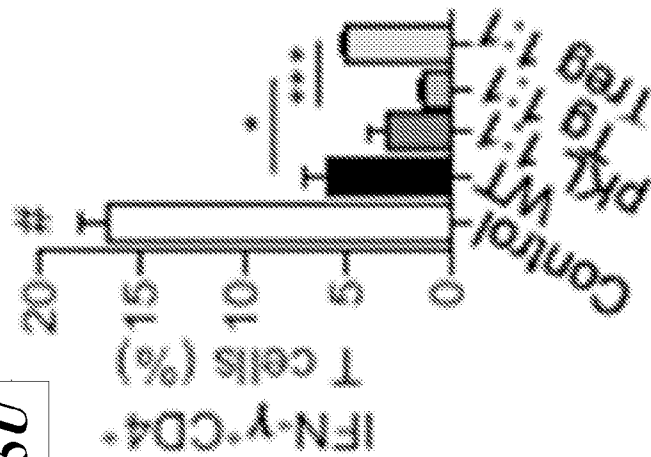


Fig. 6A

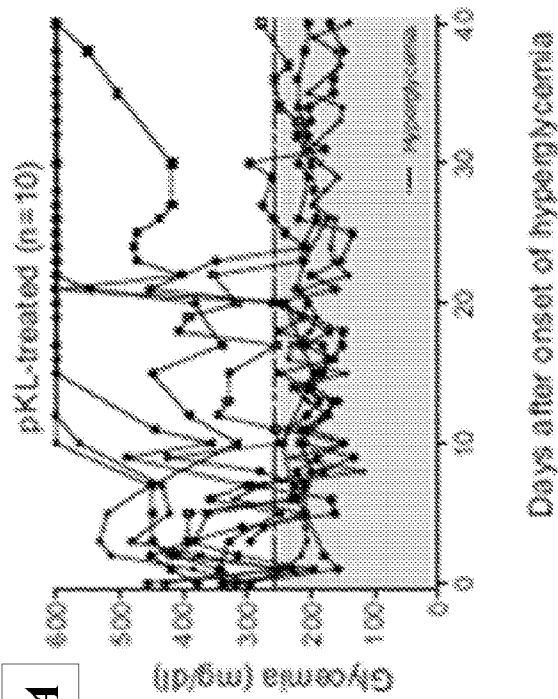


Fig. 6B

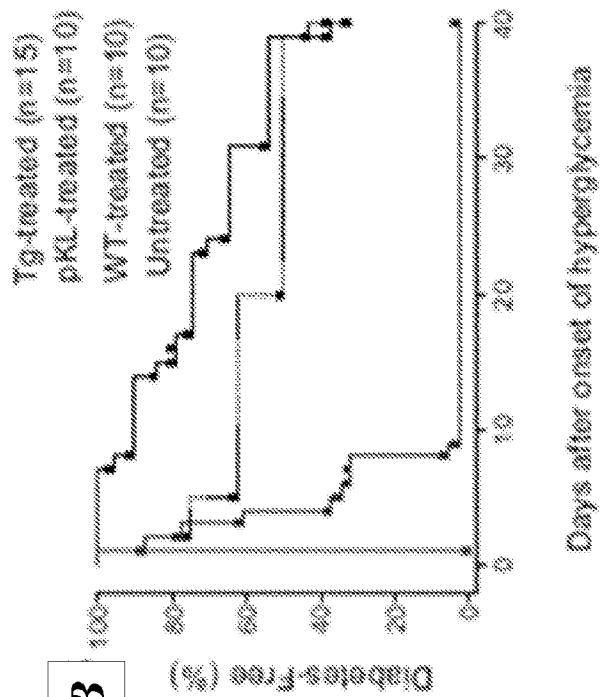


Fig. 6C

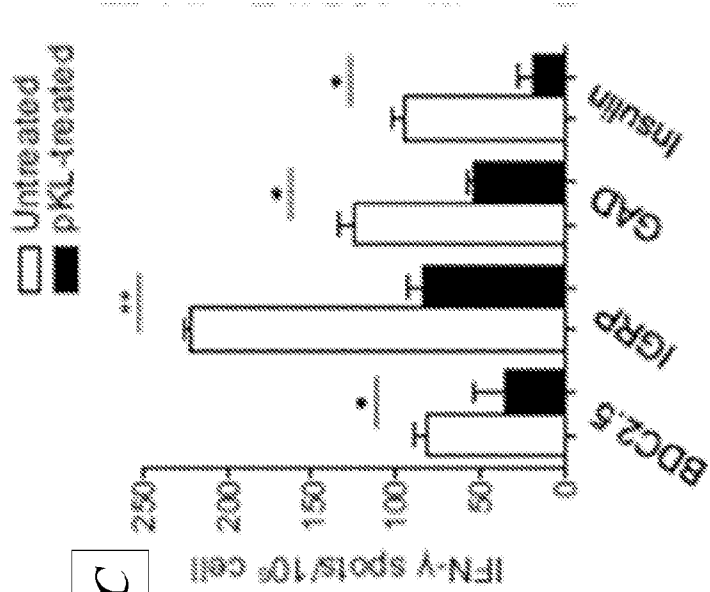


Fig. 6D

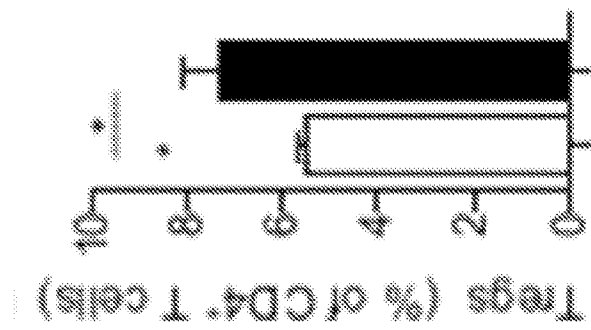


Fig. 6G

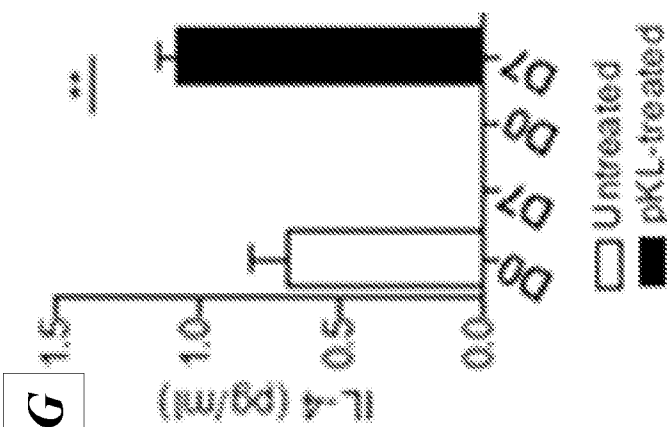


Fig. 6F

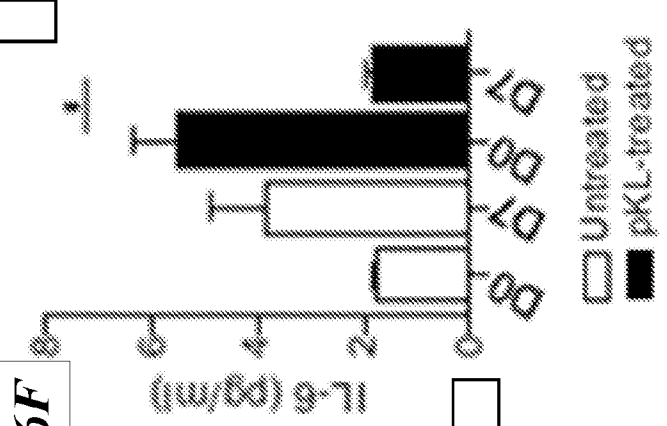


Fig. 6E

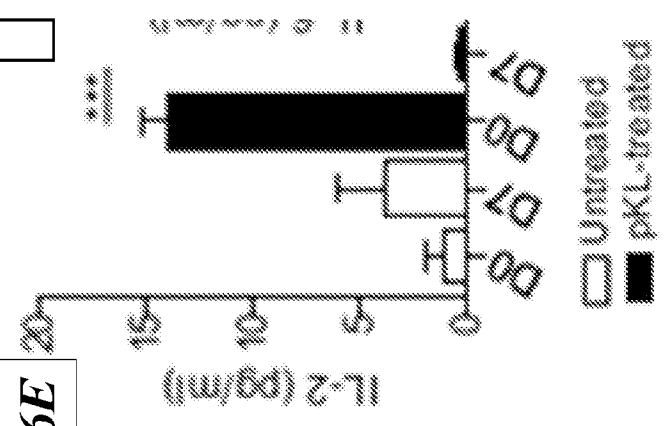


Fig. 6H

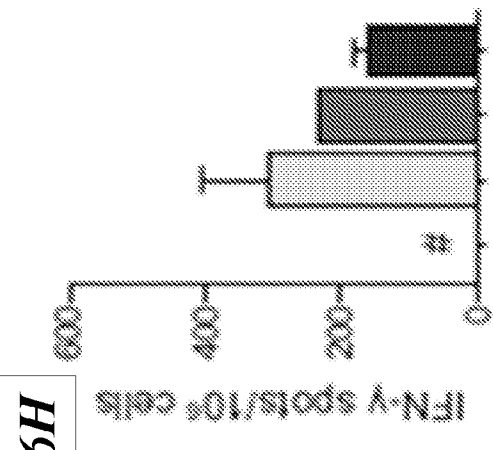


Fig. 6I

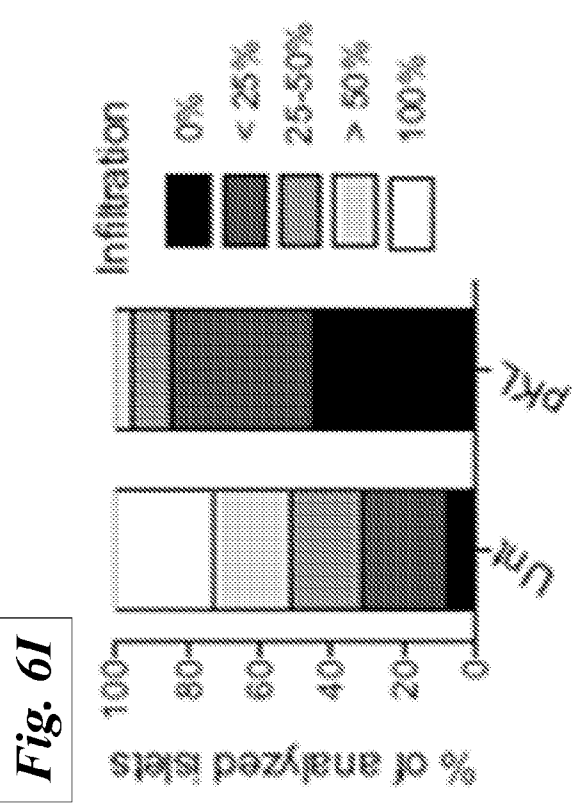


Fig. 6J

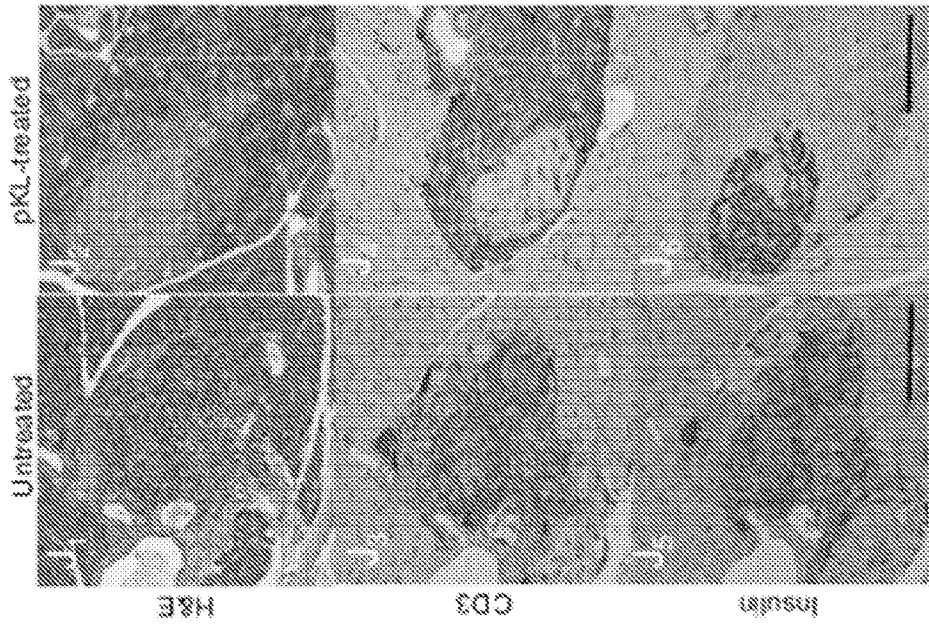


Fig. 6K

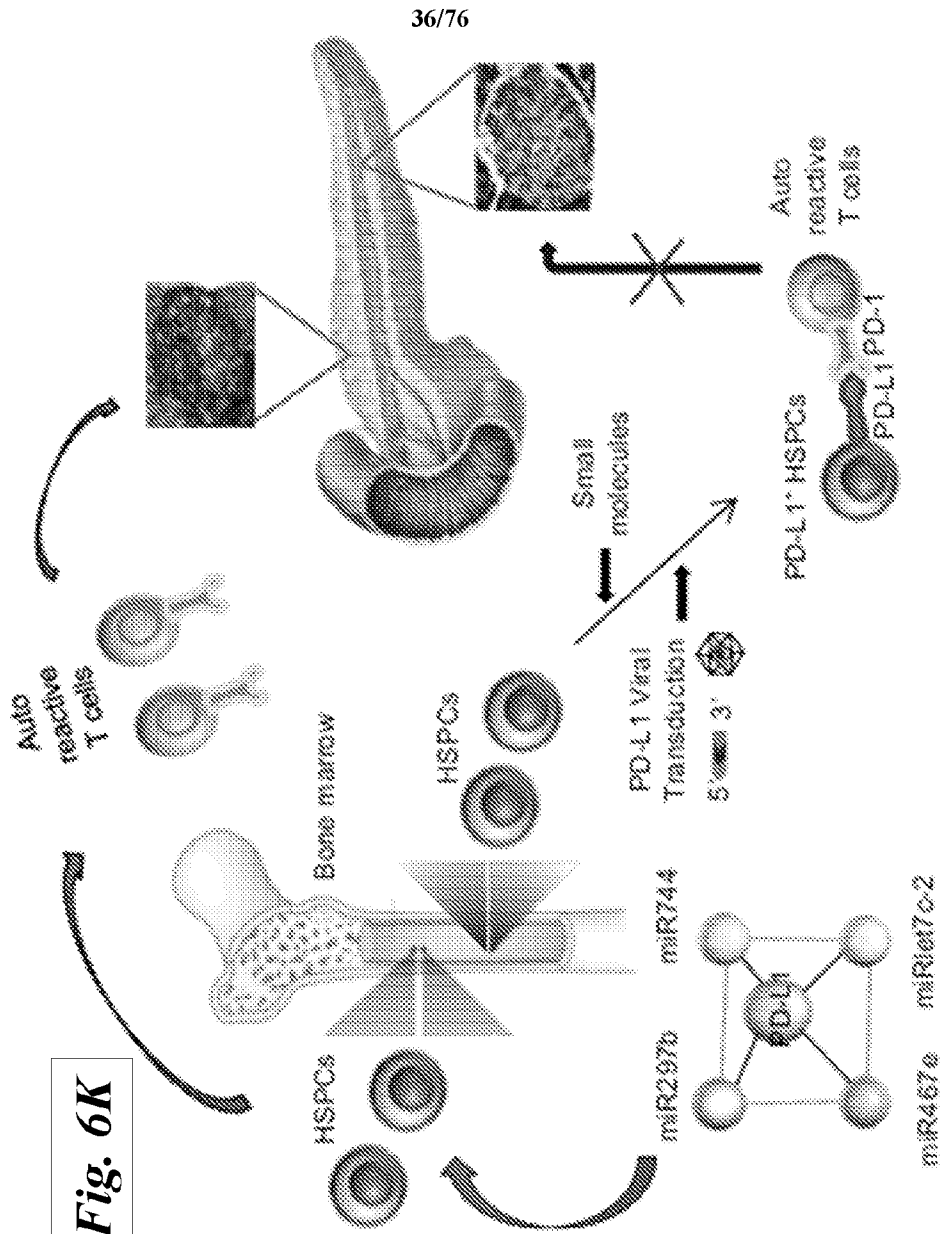


Fig. 7C



Fig. 7B

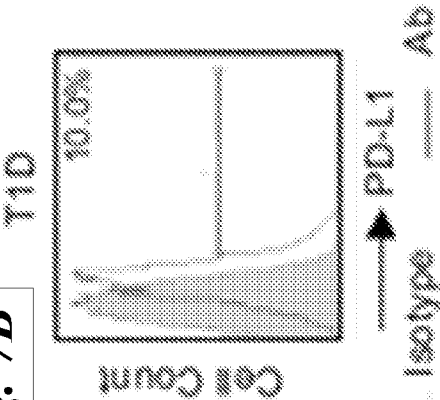


Fig. 7A

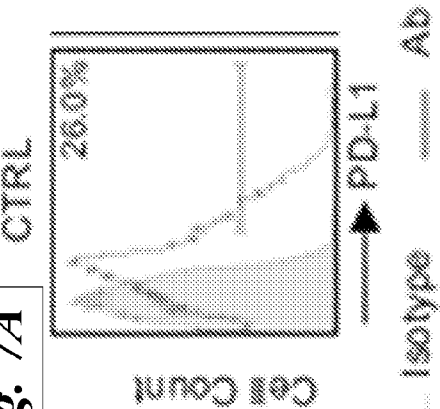


Fig. 7F



Fig. 7E

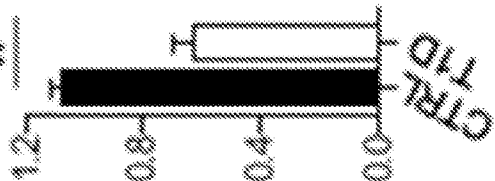
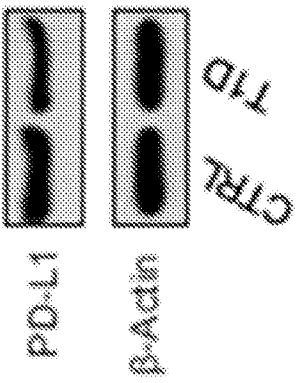


Fig. 7D



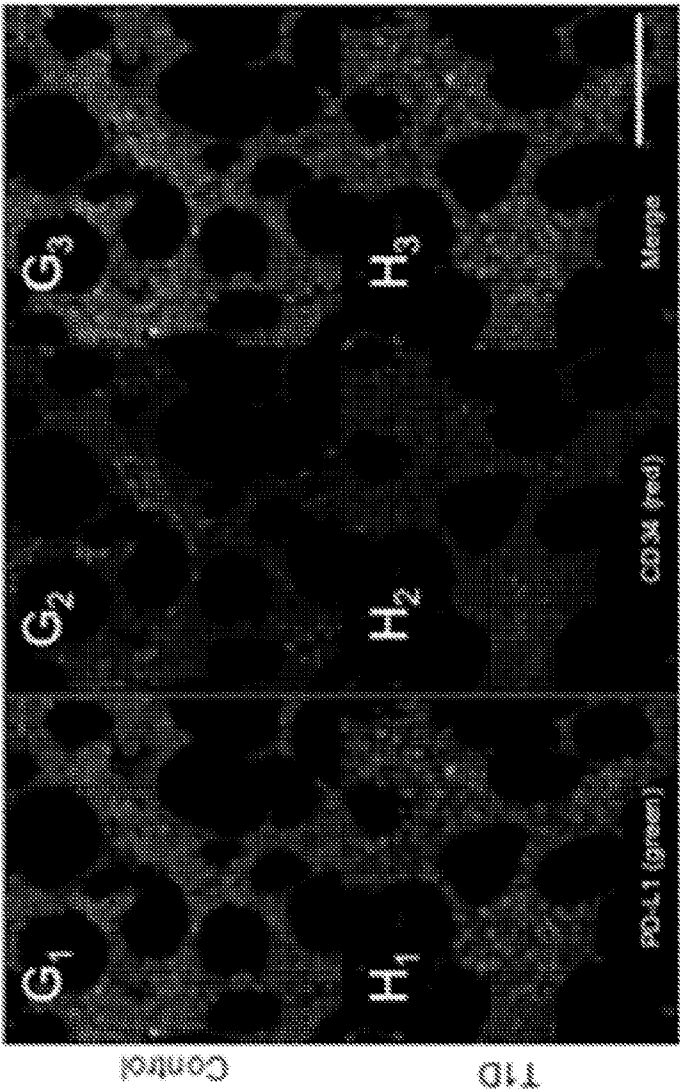


Fig. 7G

Fig. 7H

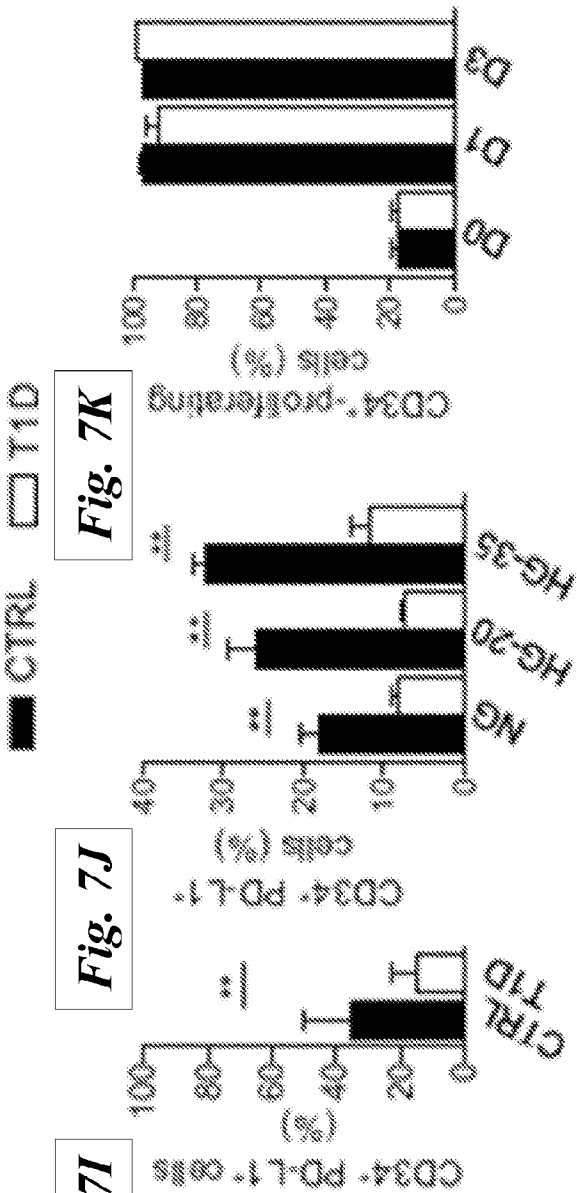


Fig. 7I

Fig. 7J

Fig. 7K

Fig. 7L

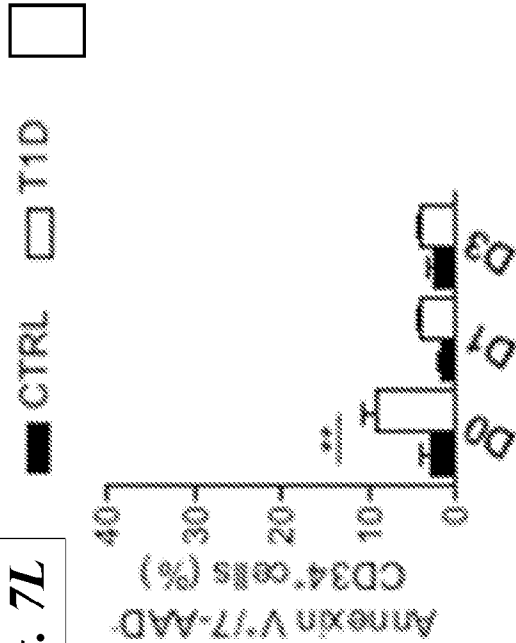


Fig. 7M

Gene Symbol	Target rank	Target score
hsa-miR-4282	1	91
hsa-miR-7853-5p	2	88
hsa-miR-105-5p	3	88
hsa-miR-224-3p	4	86
hsa-miR-4279	5	86
hsa-miR-522-3p	6	86
hsa-miR374c-5p	.	.

Fig. 7N

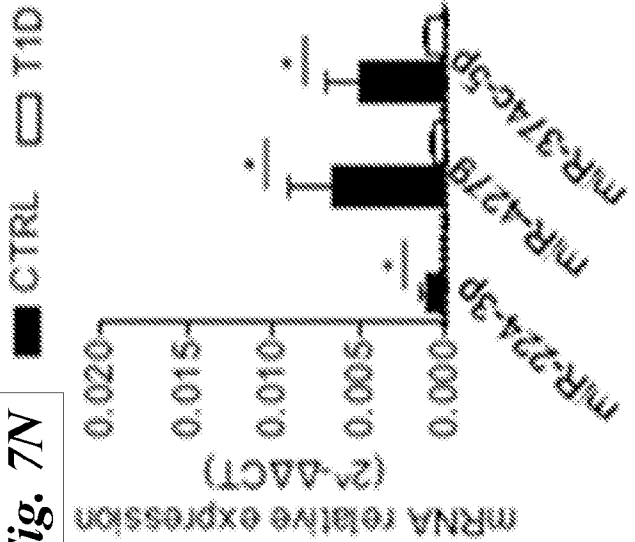
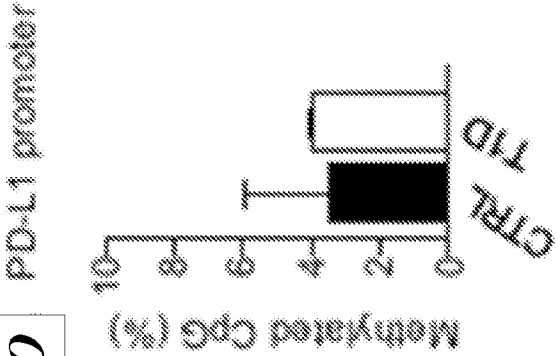


Fig. 7O



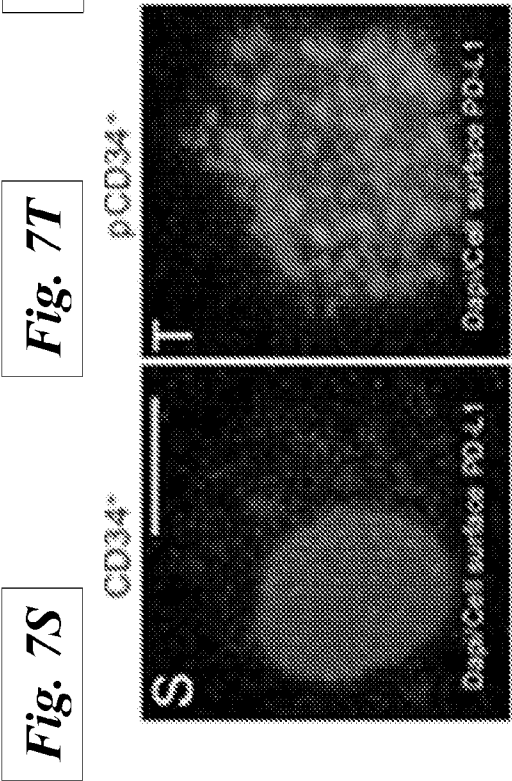
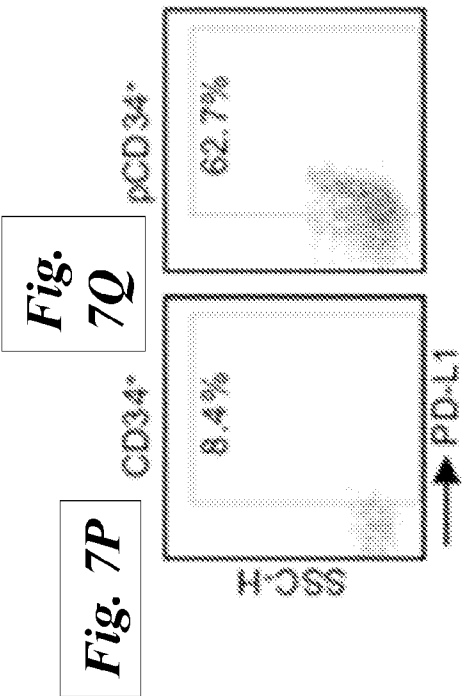
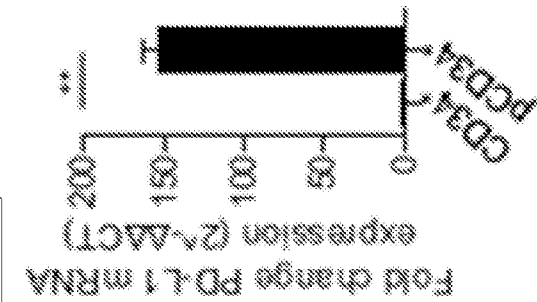
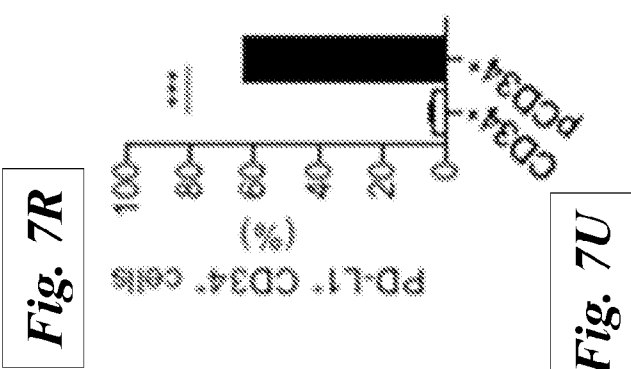


Fig. 7V

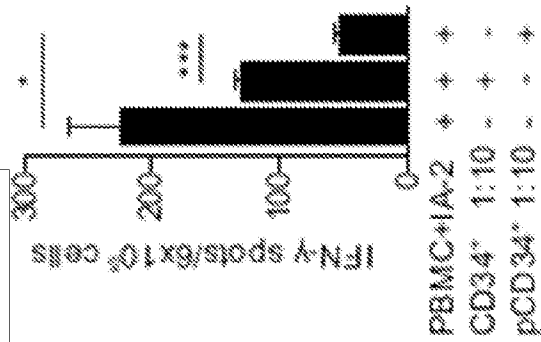


Fig. 7W

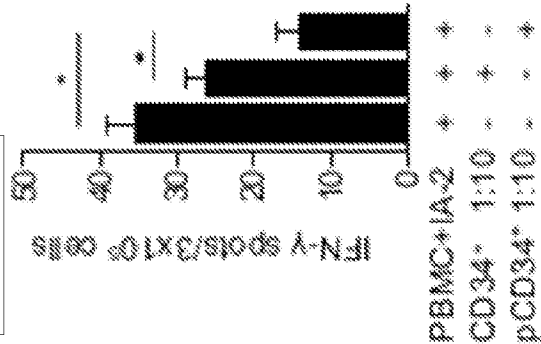


Fig. 7X

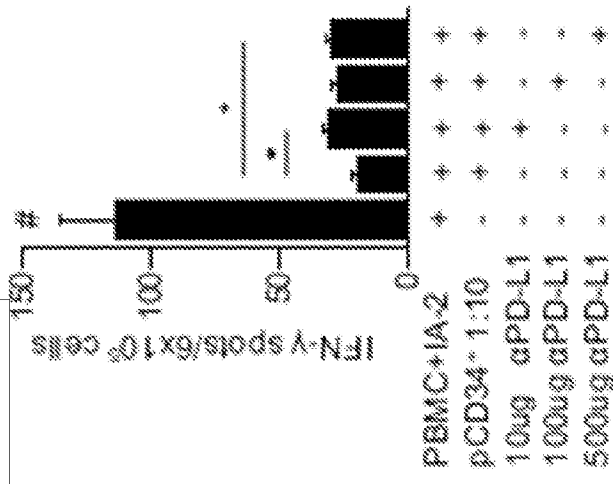


Fig. 7Y

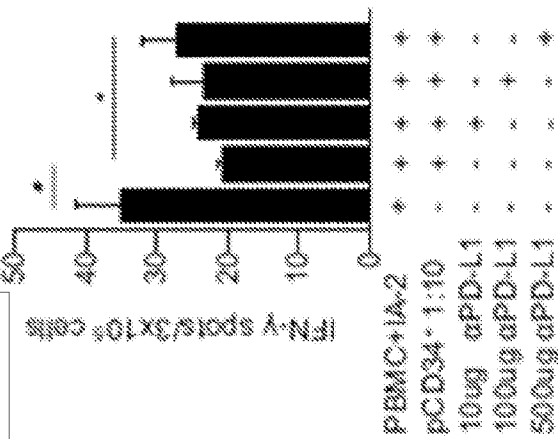


Fig. 7Z

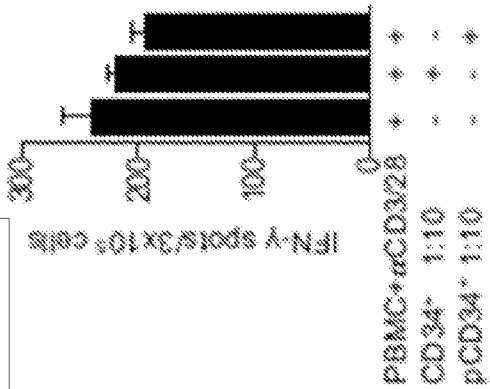


Fig. 8A

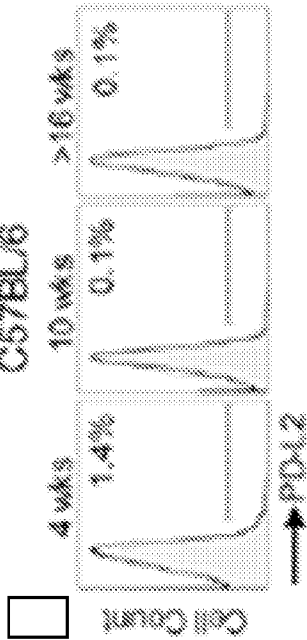


Fig. 8B

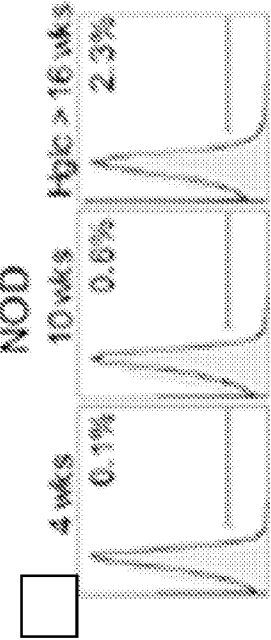


Fig. 8D

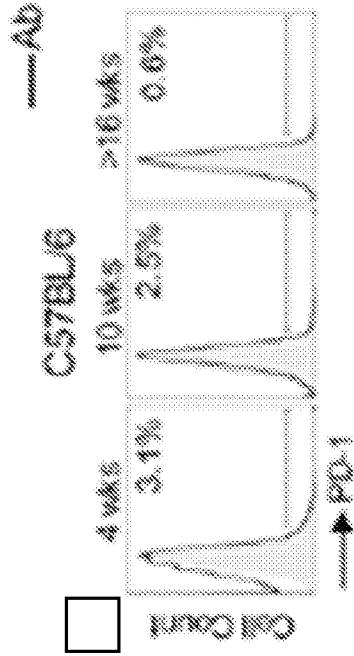


Fig. 8E

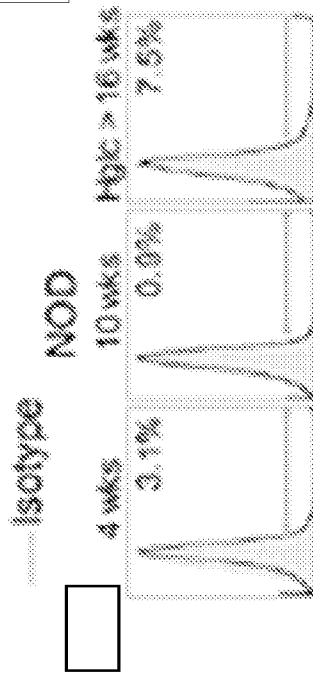


Fig. 8C

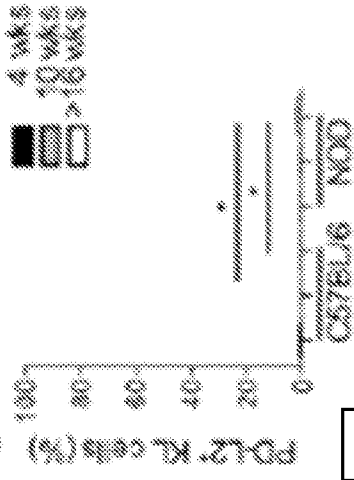
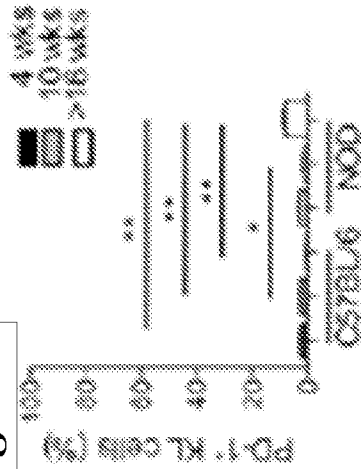


Fig. 8F



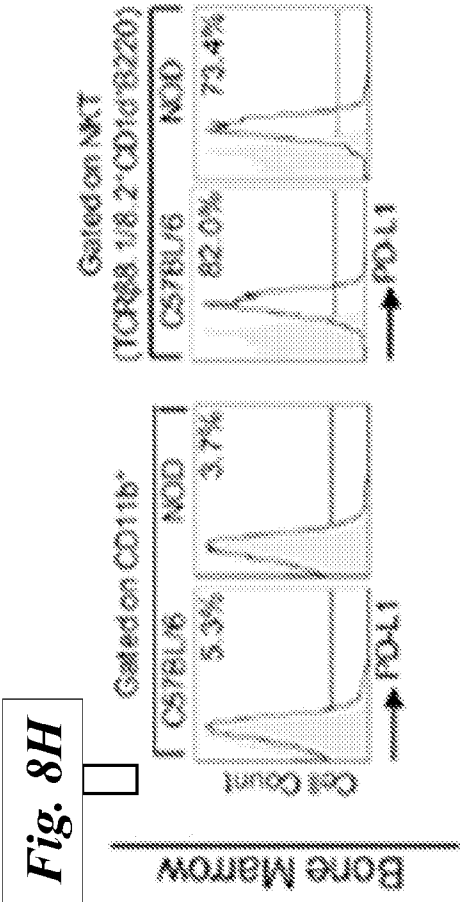
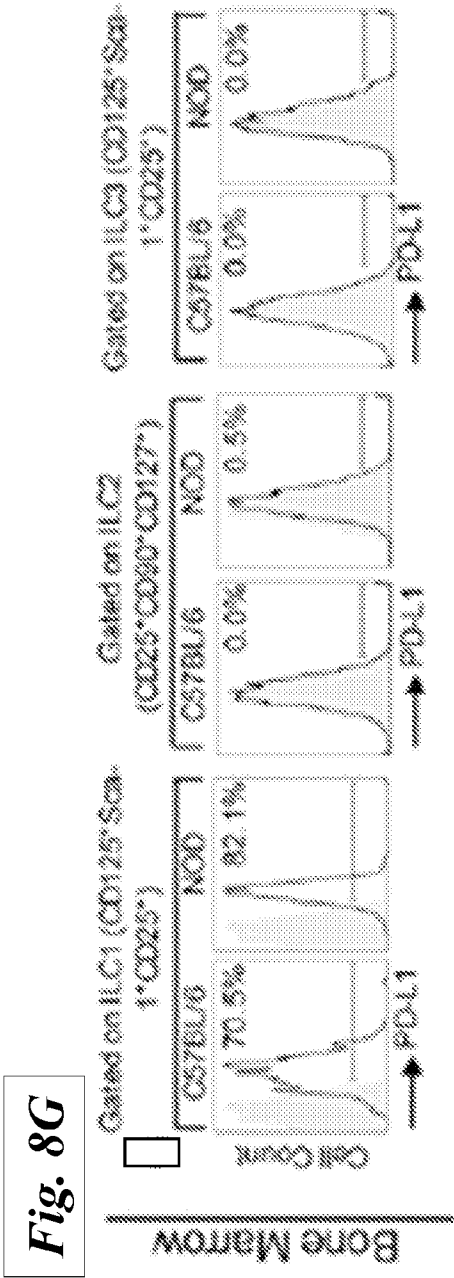


Fig. 8I

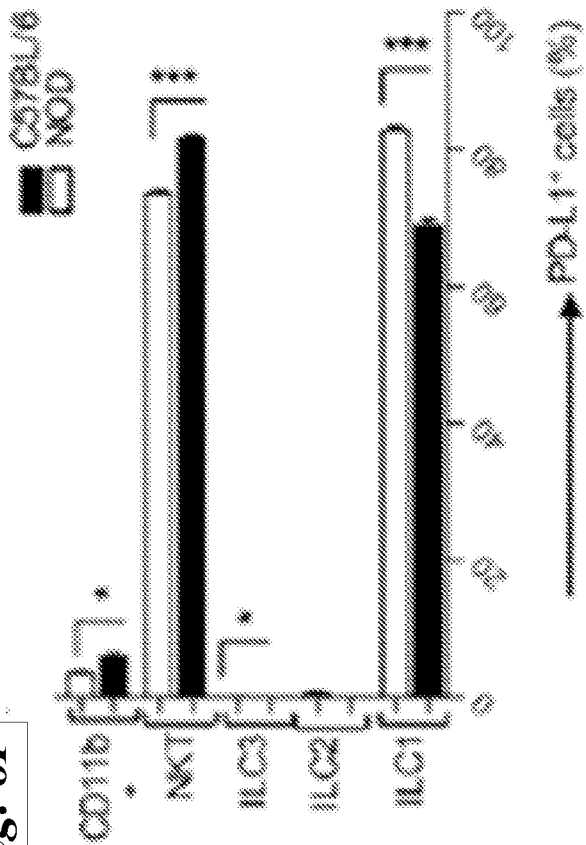


Fig. 8J

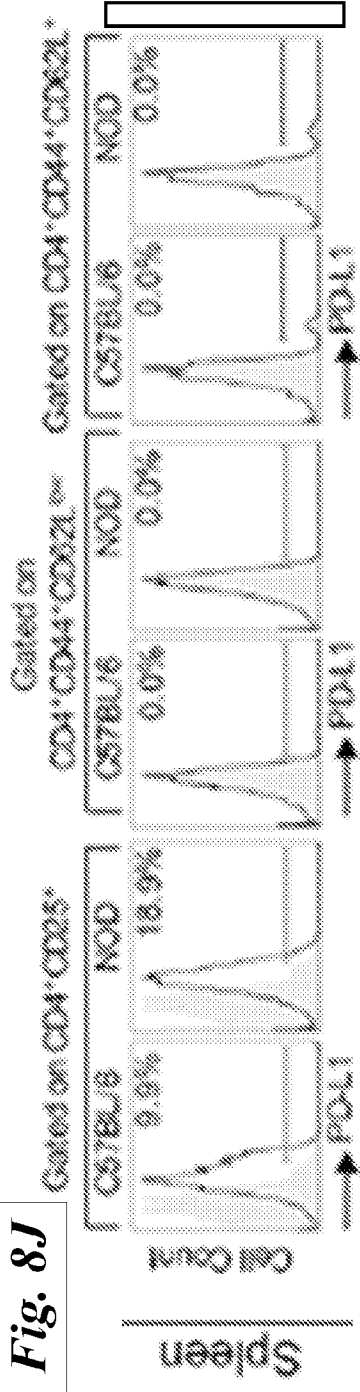


Fig. 8K

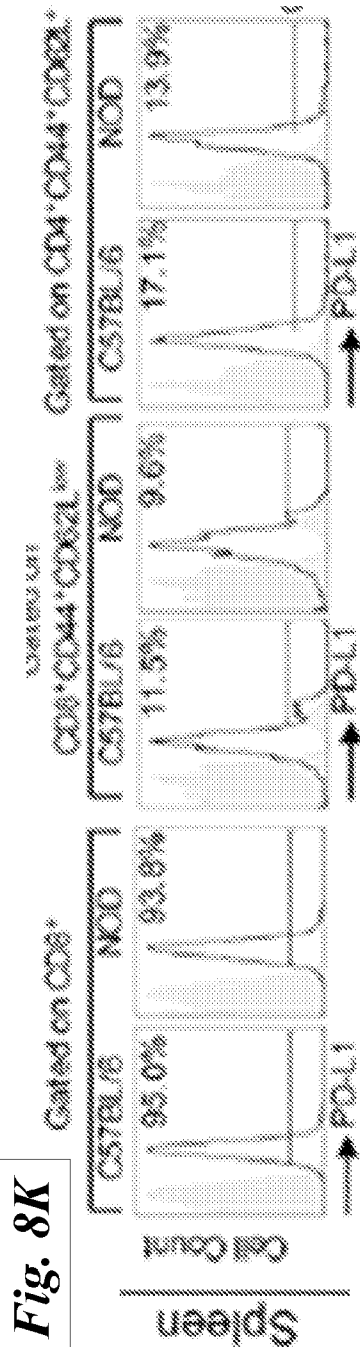


Fig. 8L

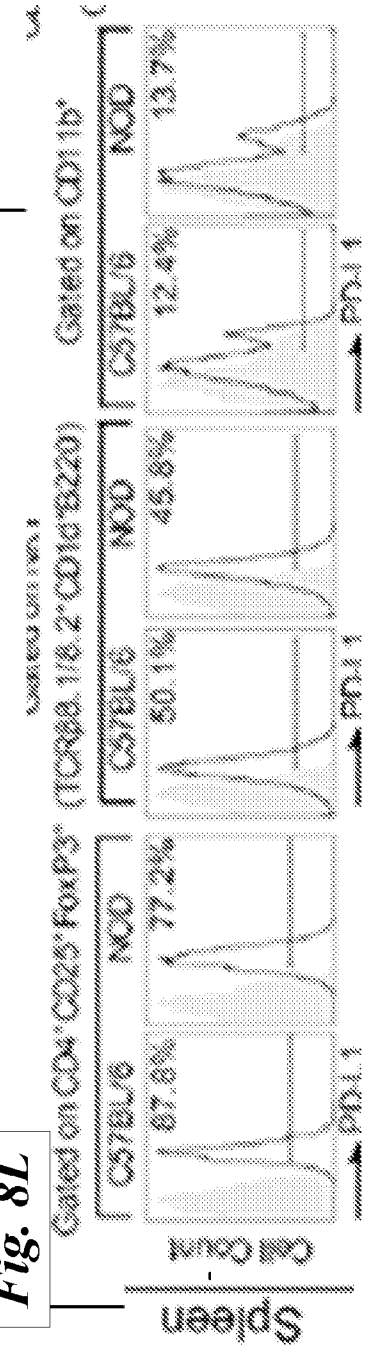


Fig. 8M

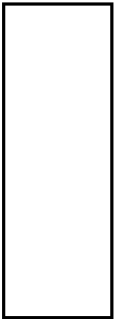
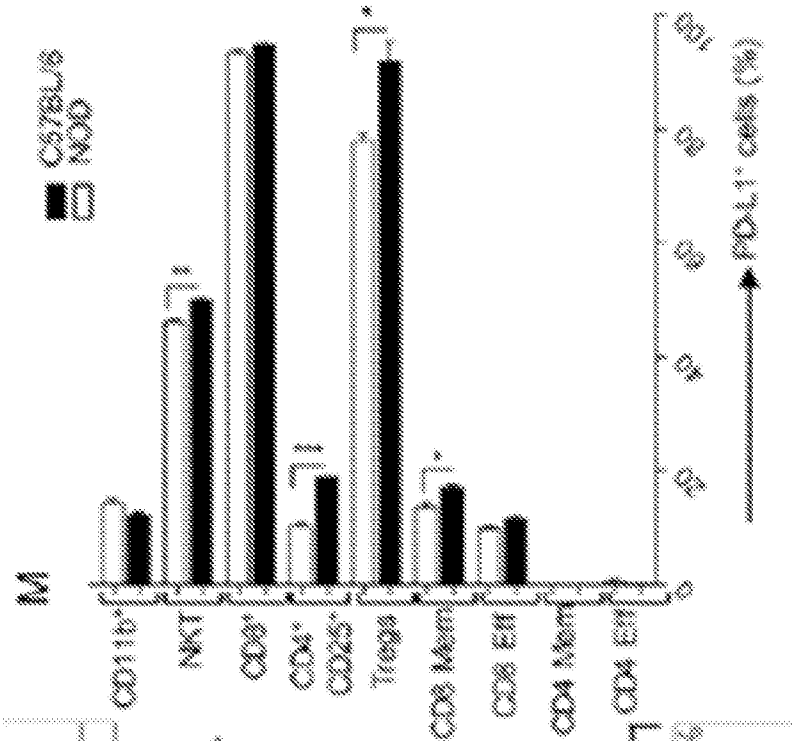


Fig. 9A

Generation of PD-L1⁺ HSPCs by lentiviral transduction (genetic approach)

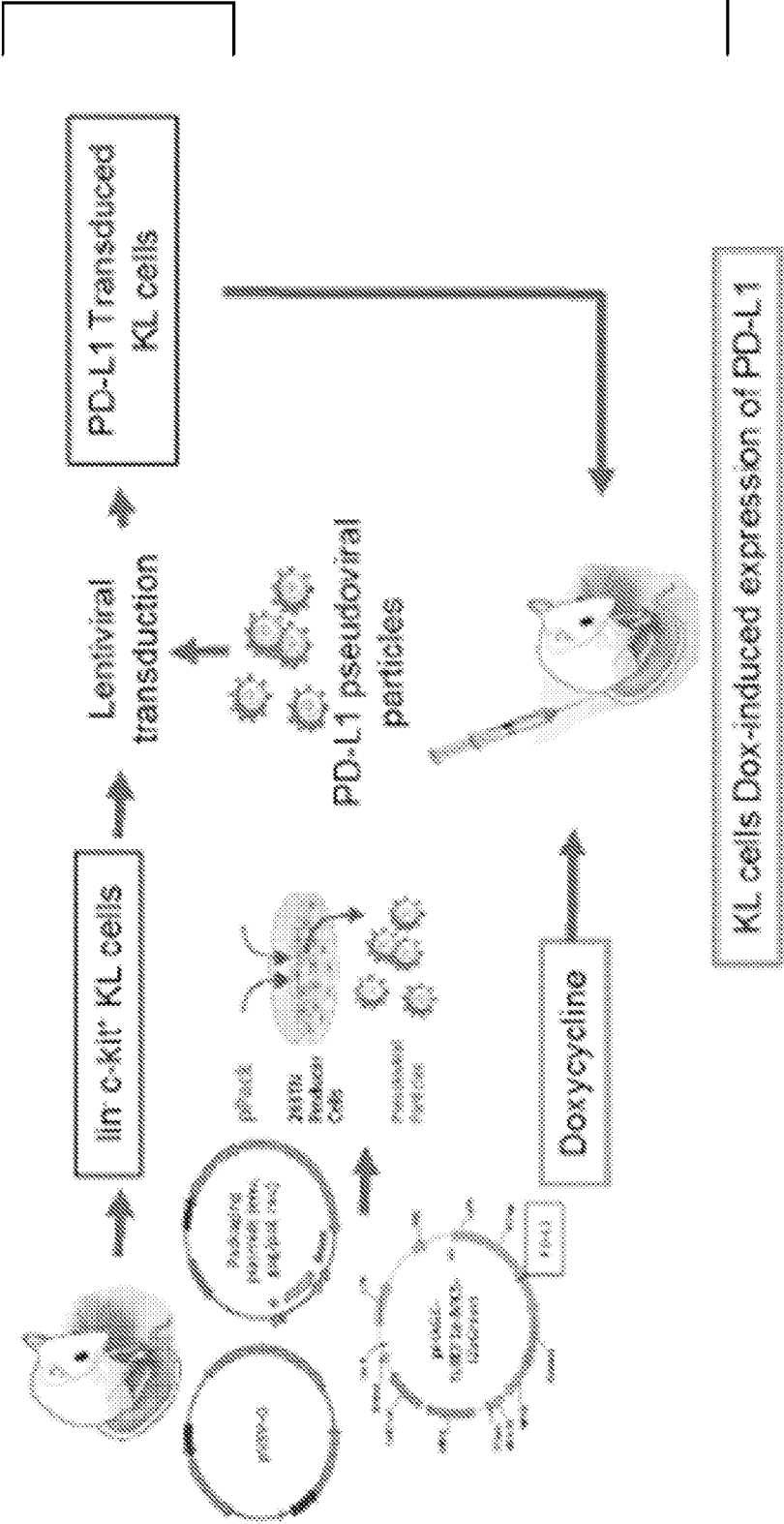
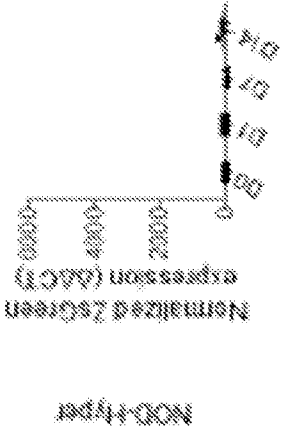
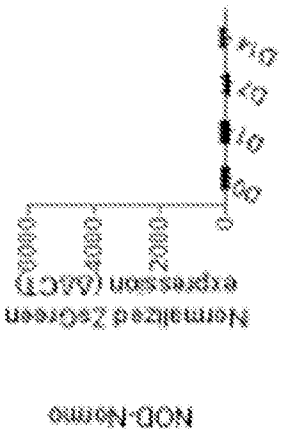


Fig. 9B



PLN

Fig. 9C



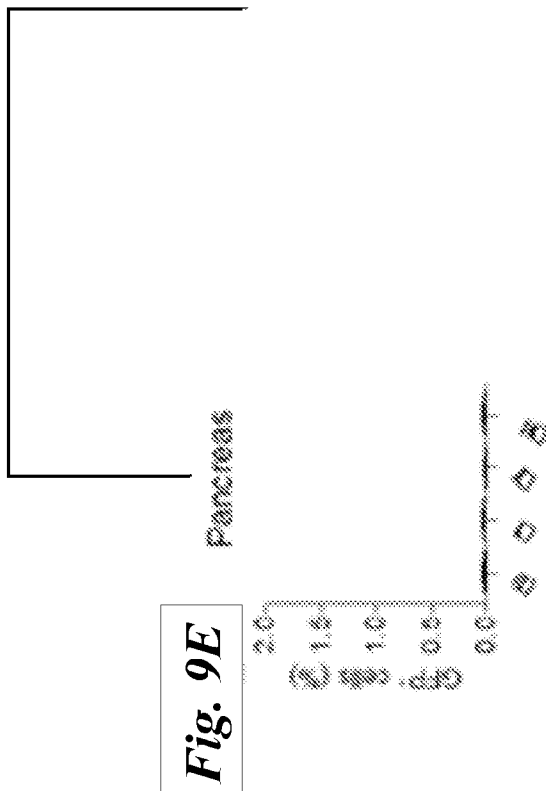


Fig. 9E

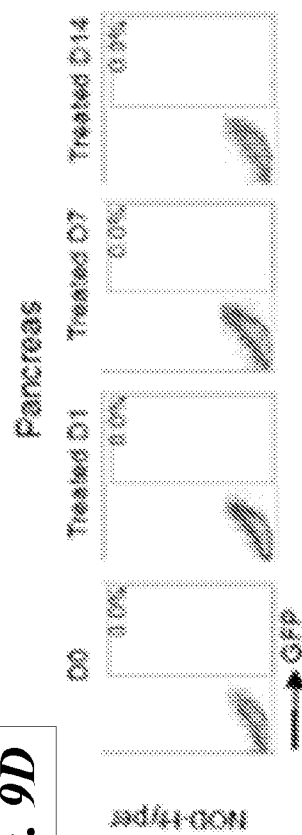


Fig. 9D

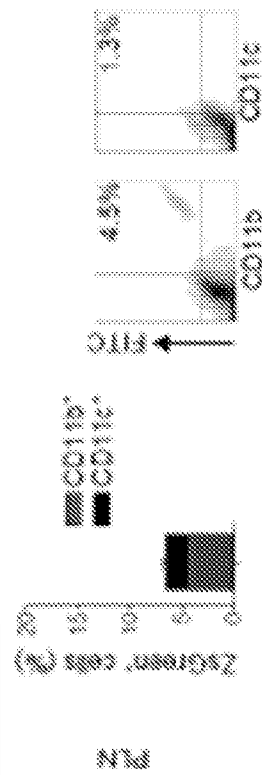


Fig. 9H

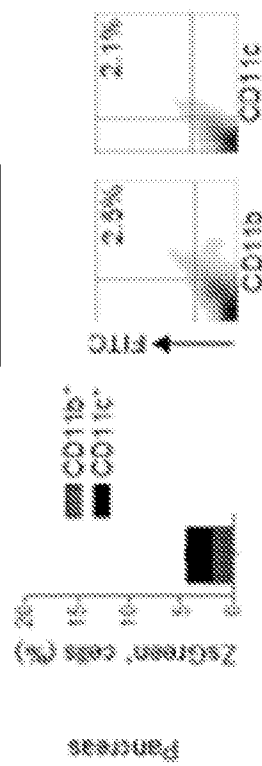


Fig. 9F

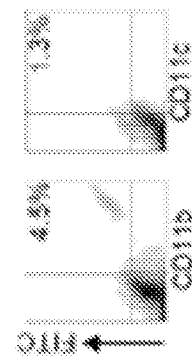


Fig. 9I

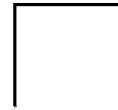


Fig. 10A

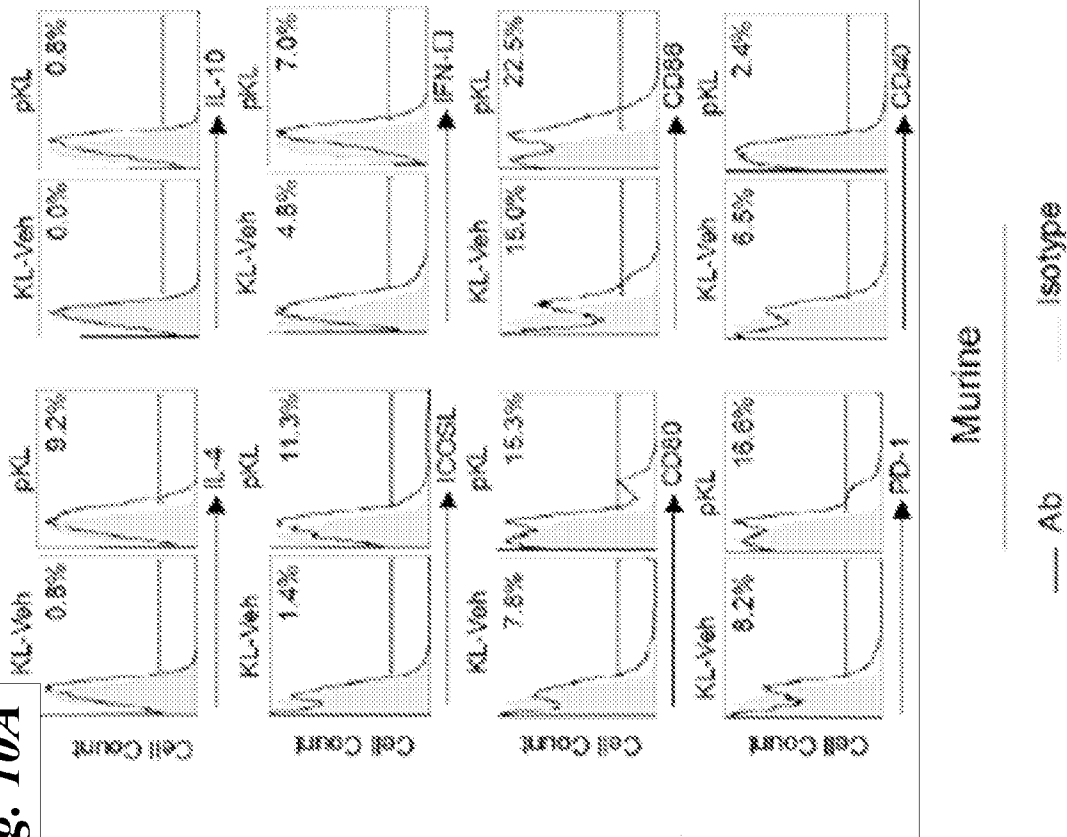
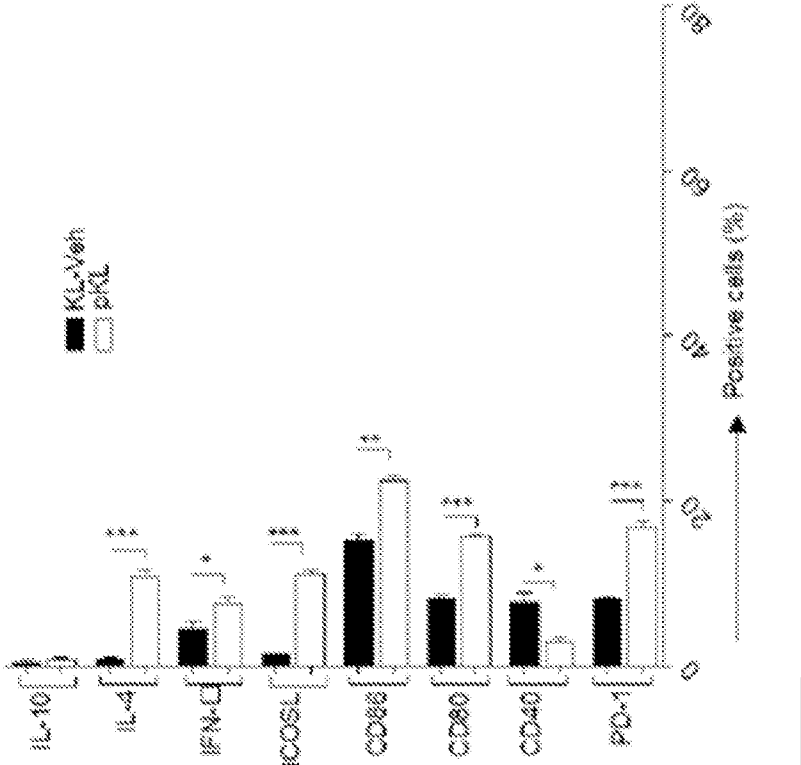
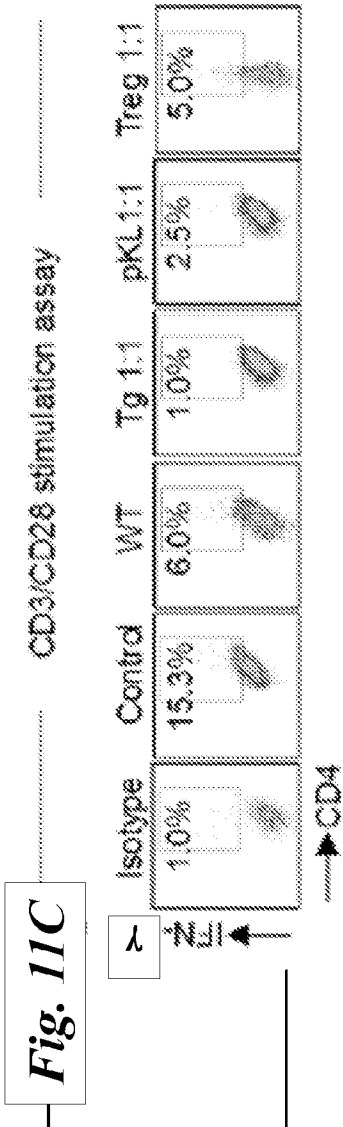
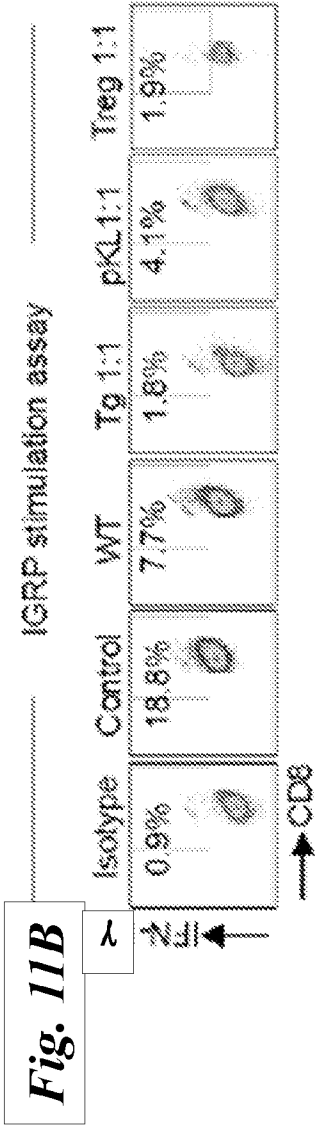
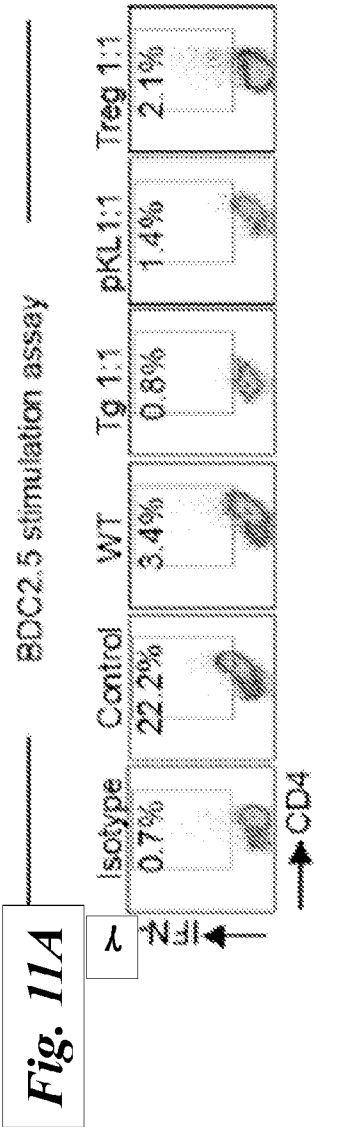


Fig. 10B





Diabetes Late Prevention

Fig. 11D

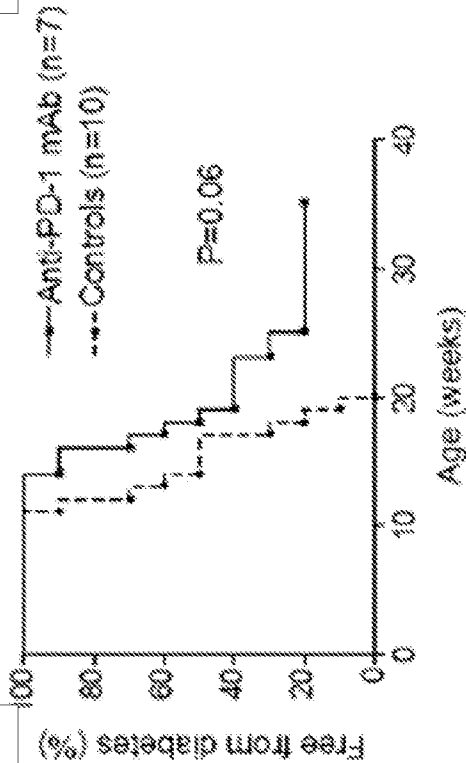
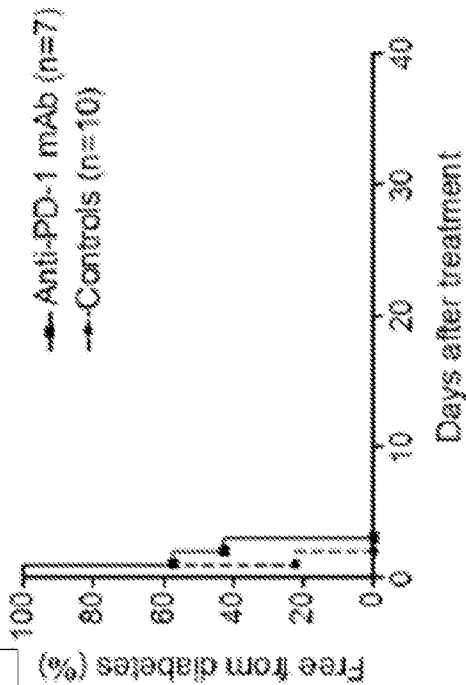


Fig. 11E



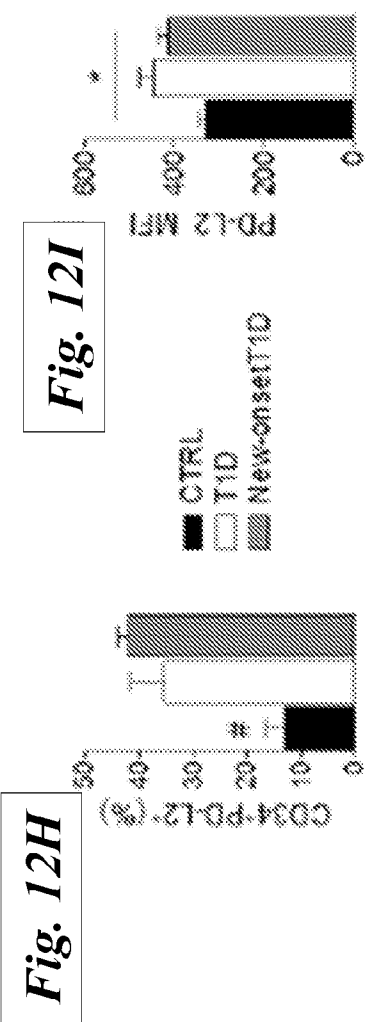
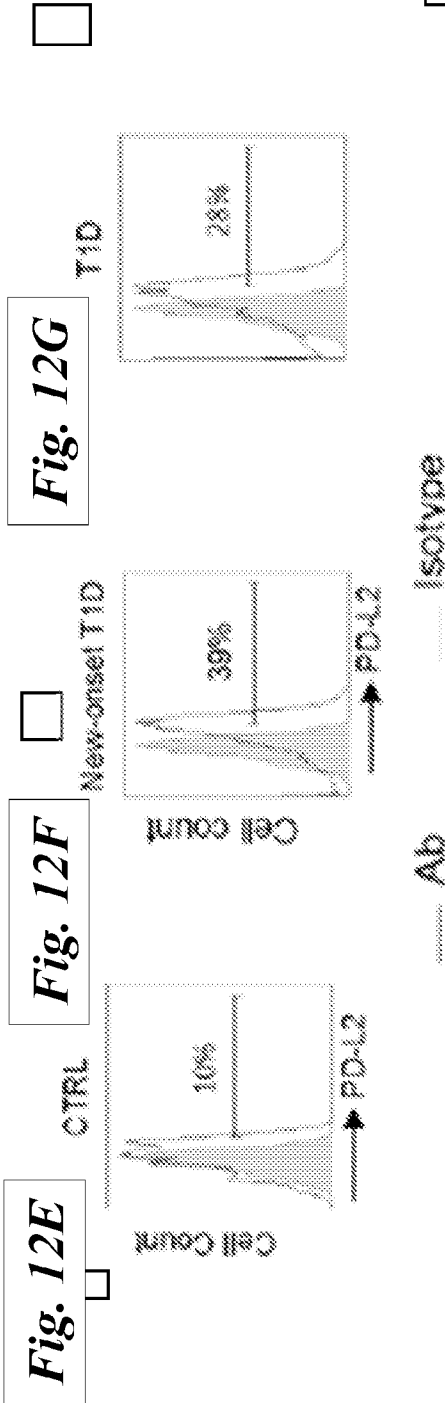
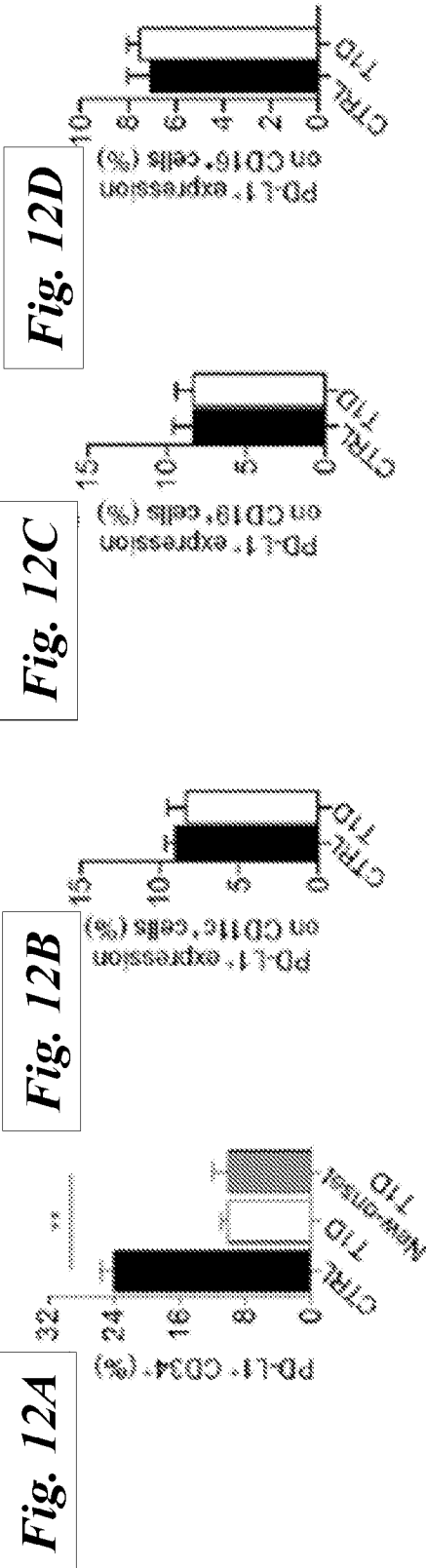


Fig. 12J

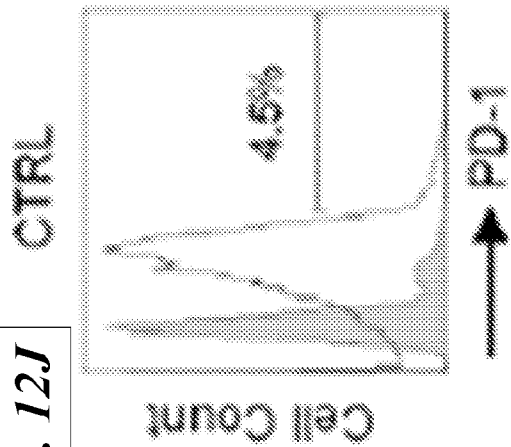


Fig. 12K

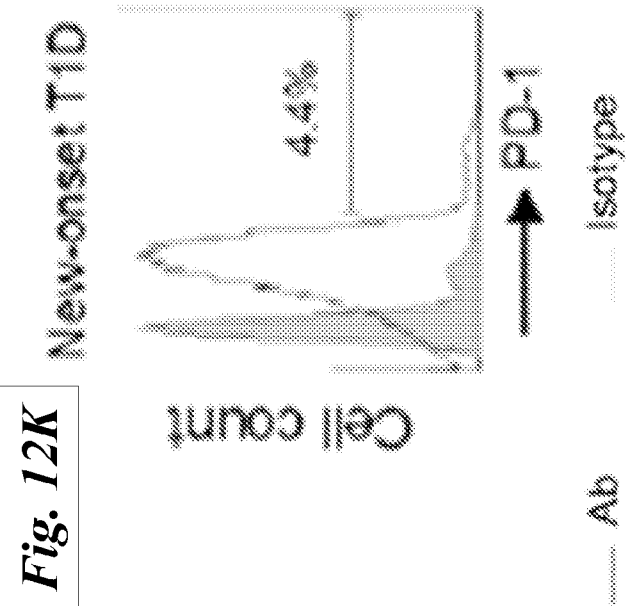


Fig. 12L

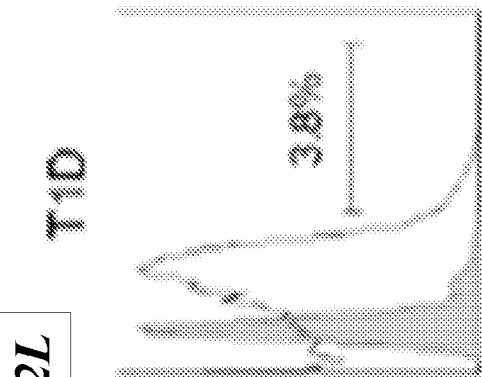


Fig. 12M

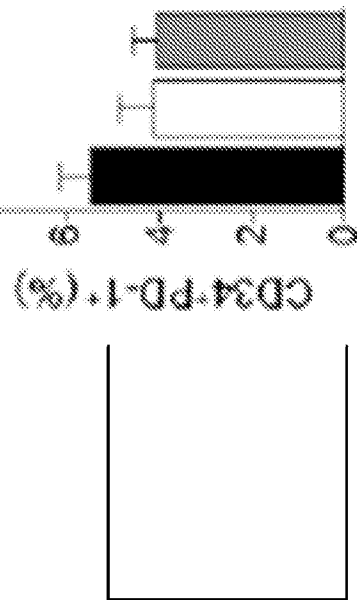


Fig. 12N

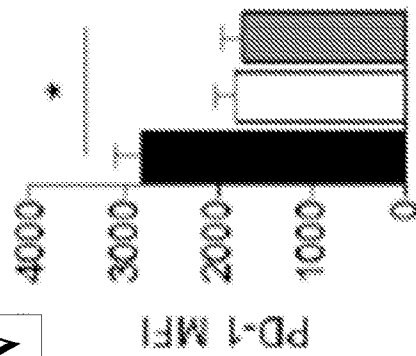


Fig. 13A

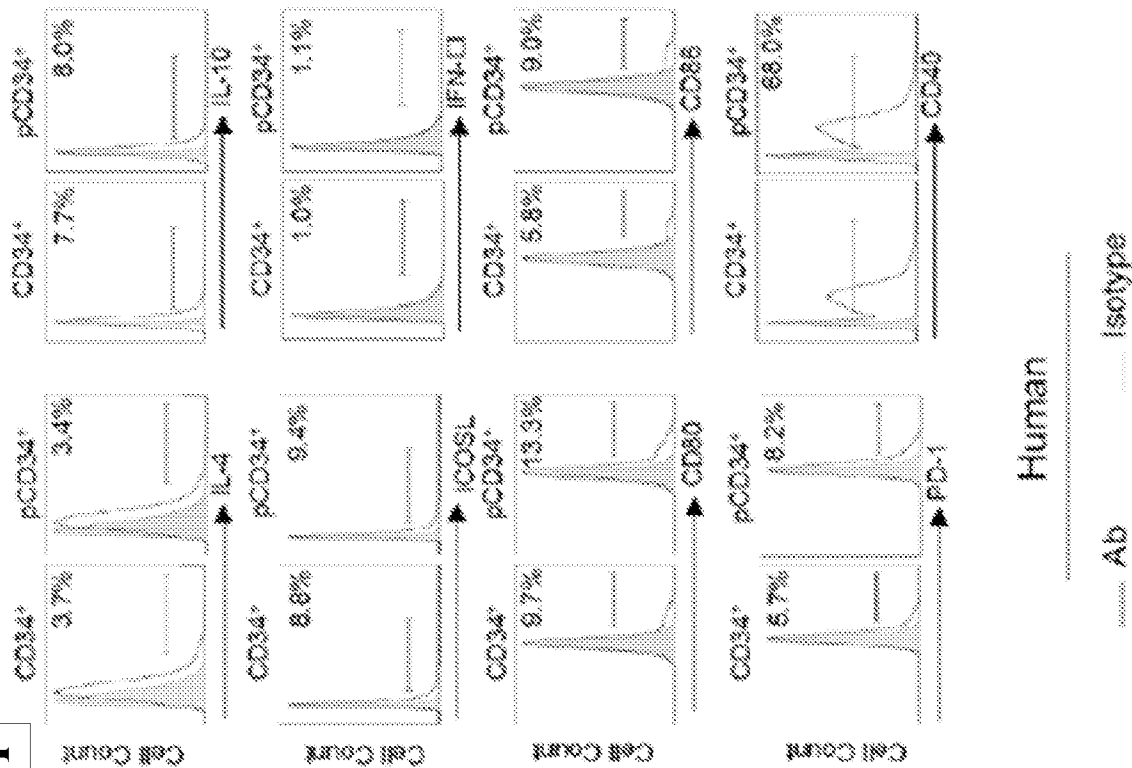


Fig. 13B

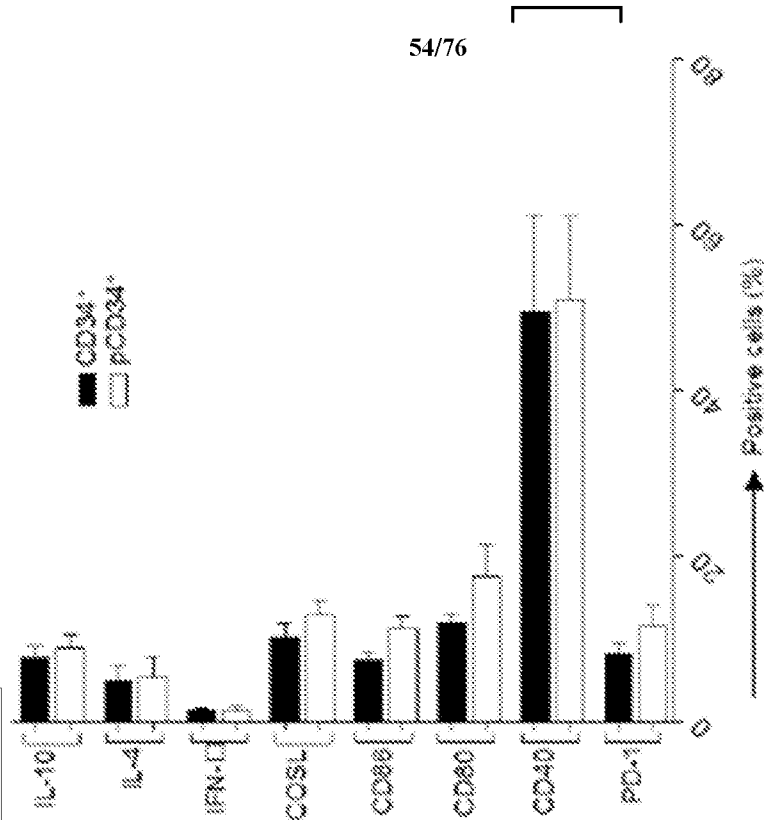


Fig. 14A

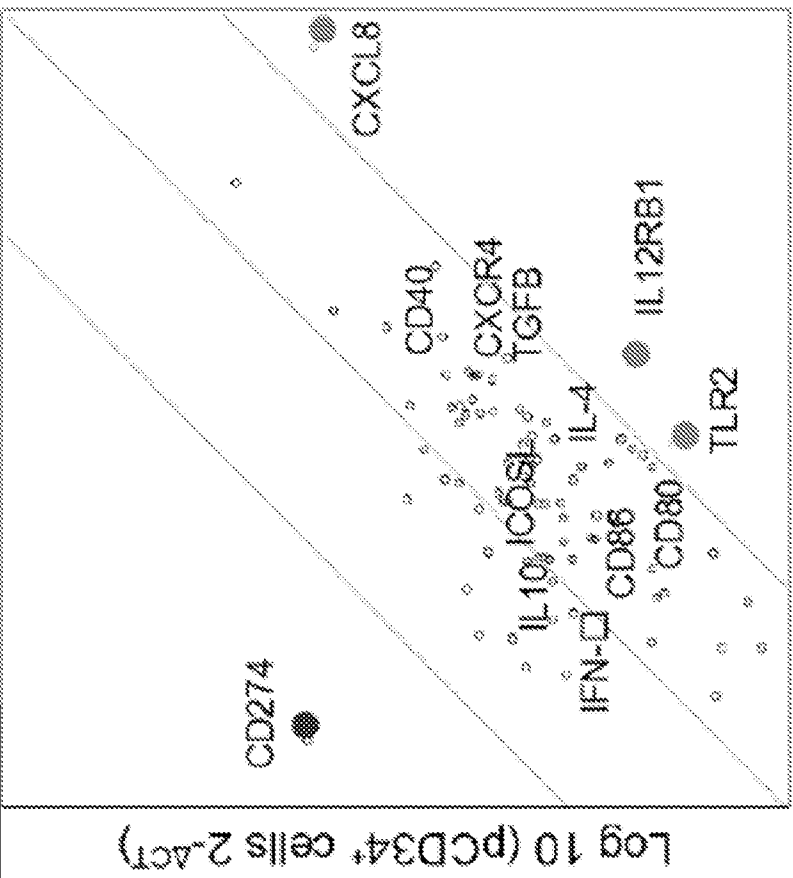


Fig. 14B

Gene Symbol	P-value	Fold change
CD274 (PD-L1)	0.028999	13.9304

Fig. 15A

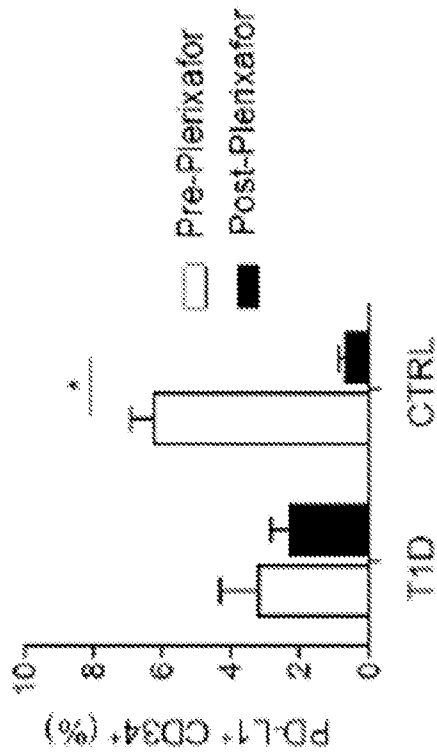


Fig. 15B

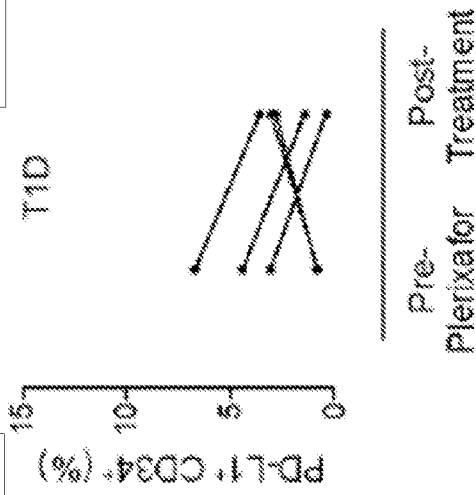
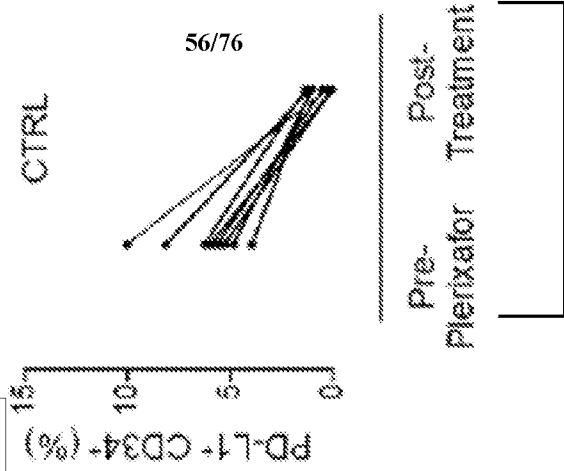


Fig. 15C



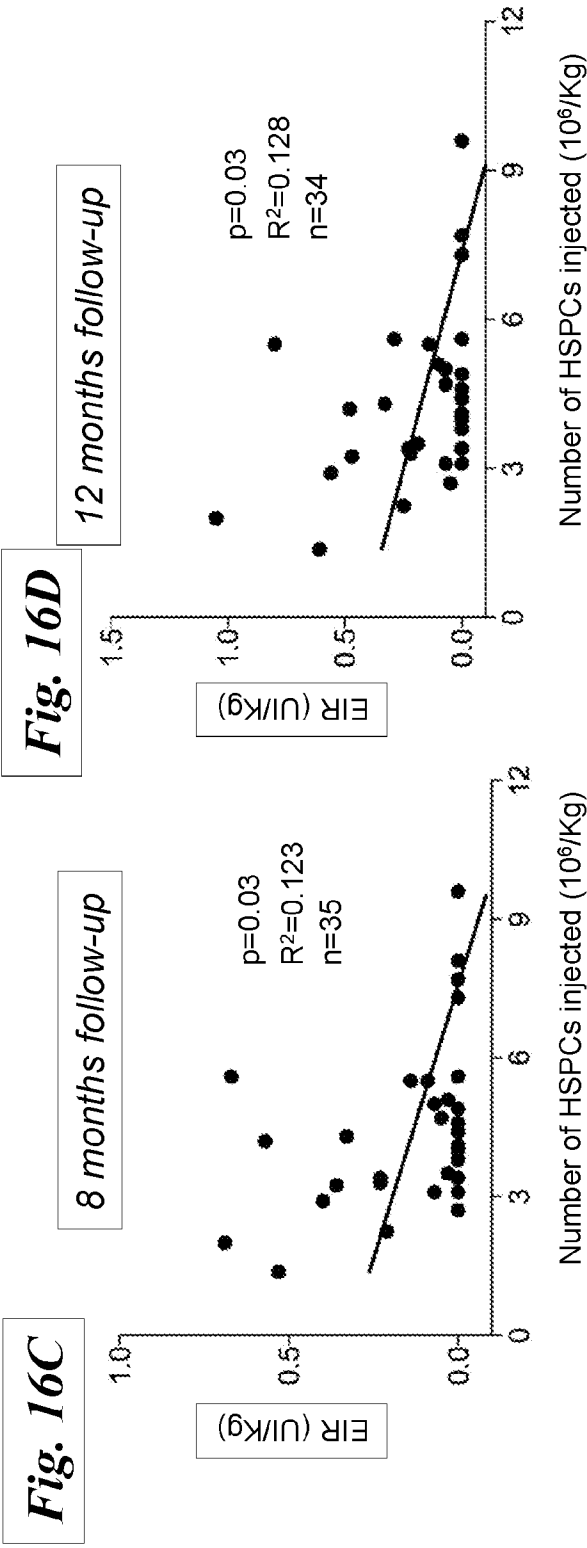
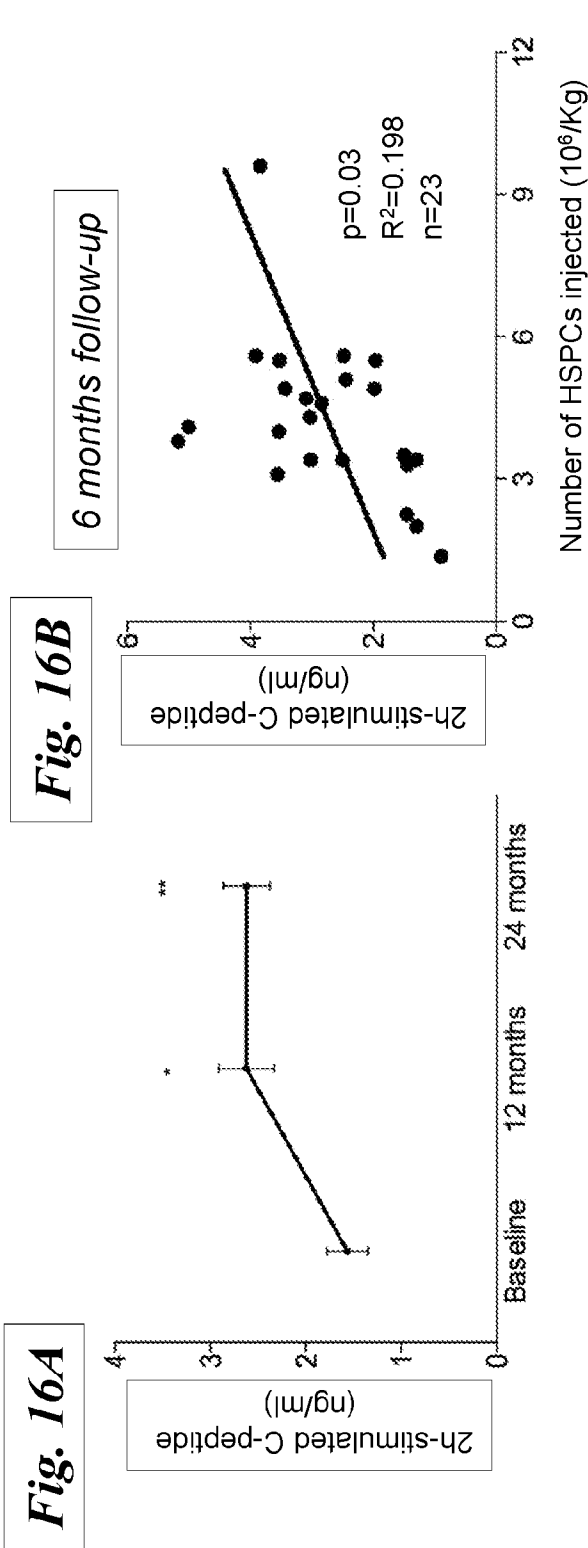
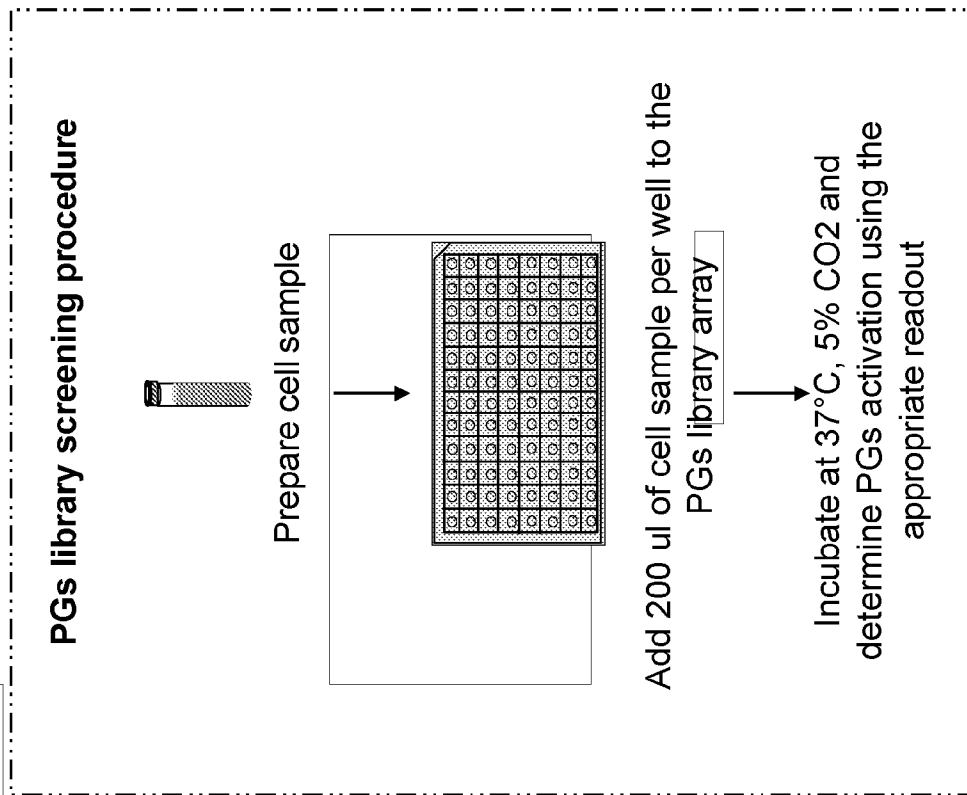
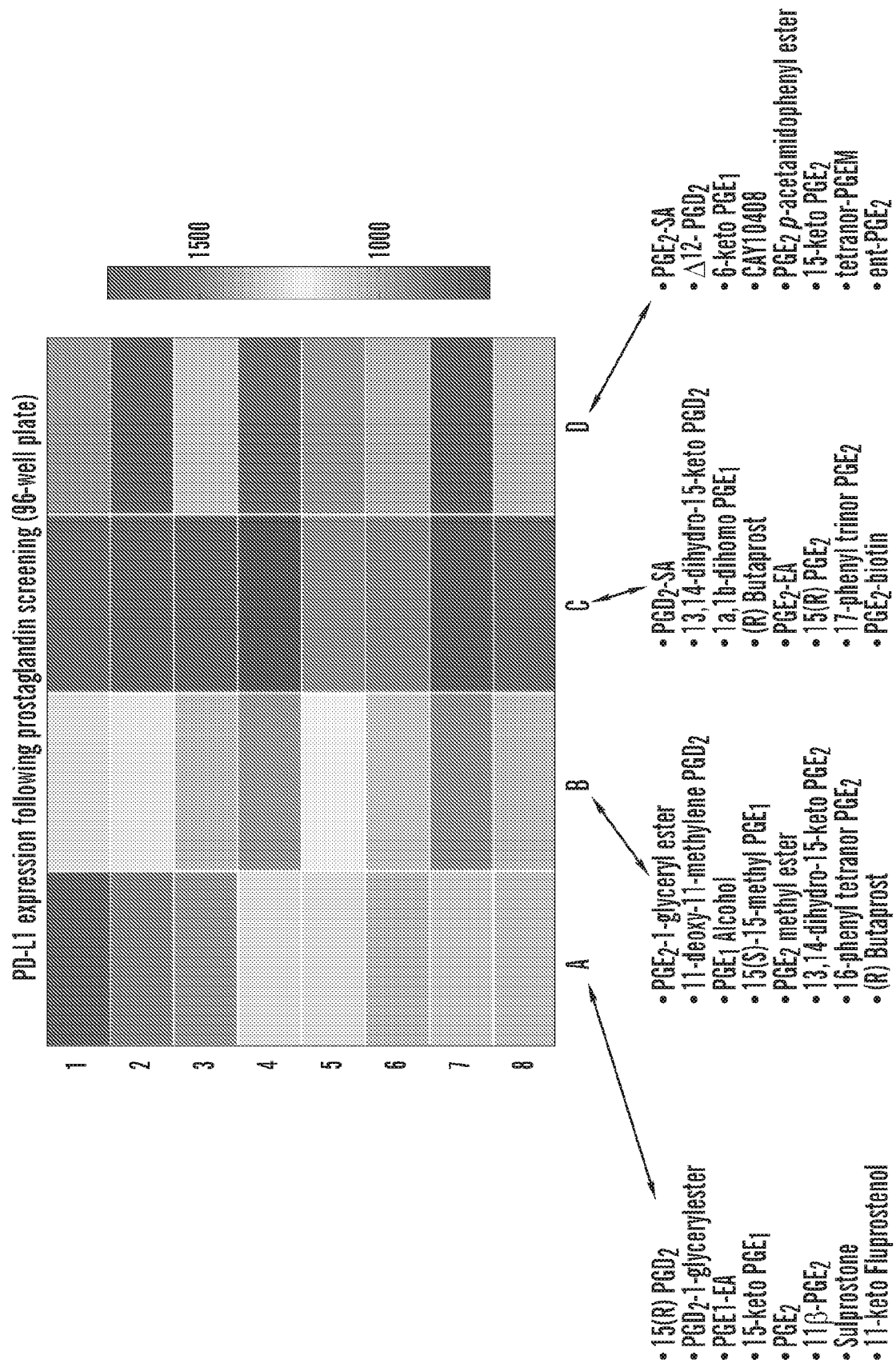
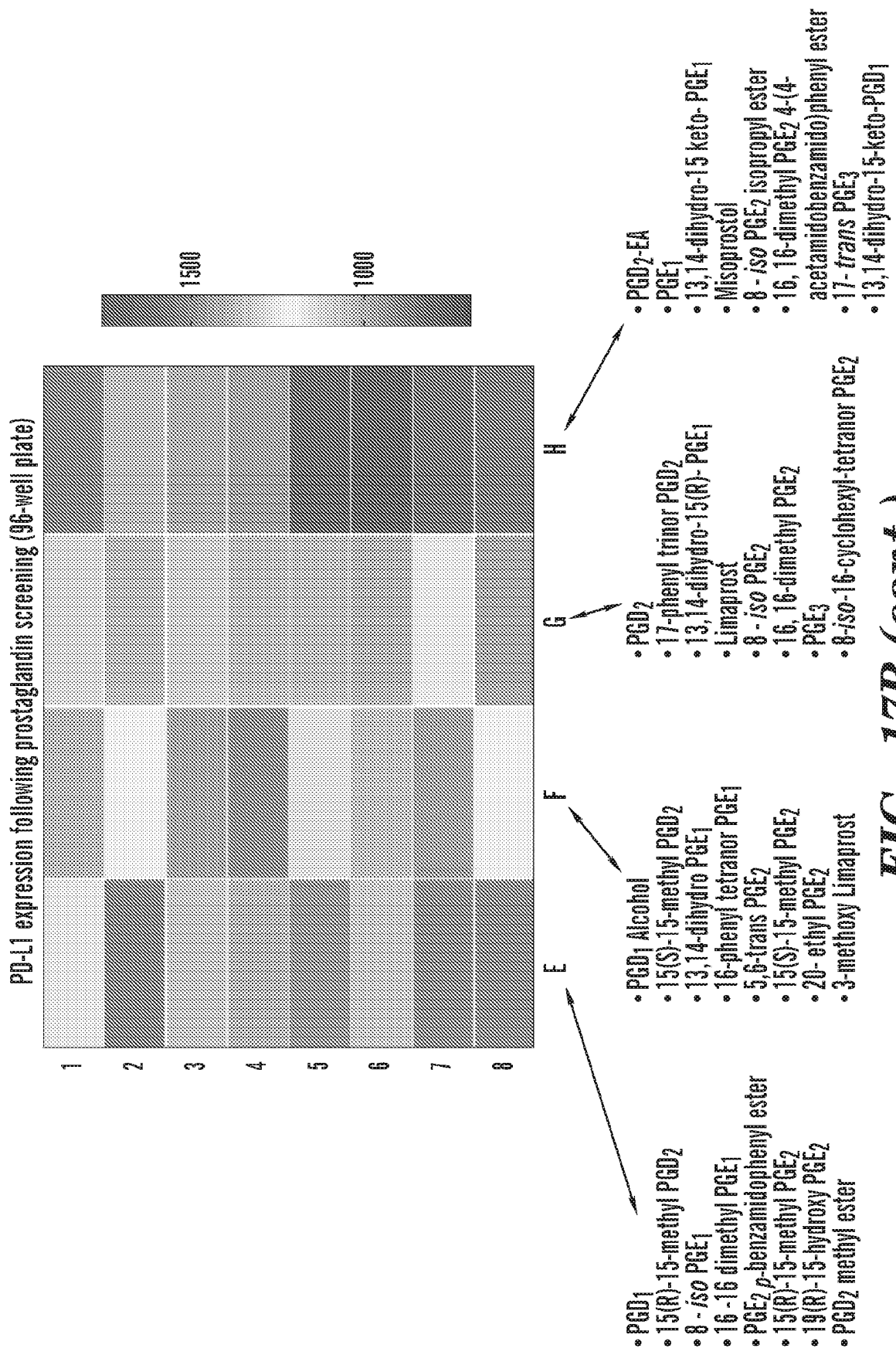


Fig. 17A

**FIG. 17B**

**FIG. 17B (cont.)**

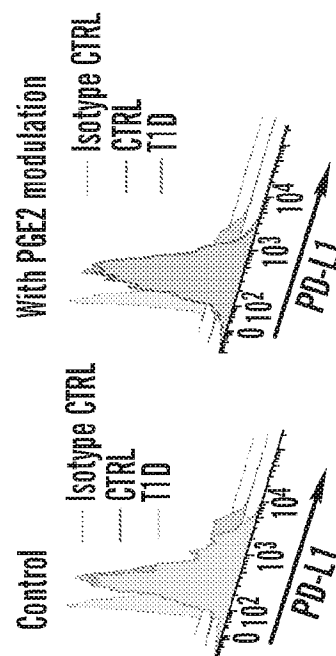
**FIG. 17C**

Fig. 17D

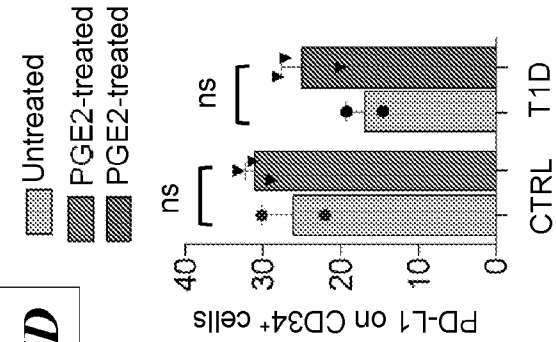


Fig. 17E

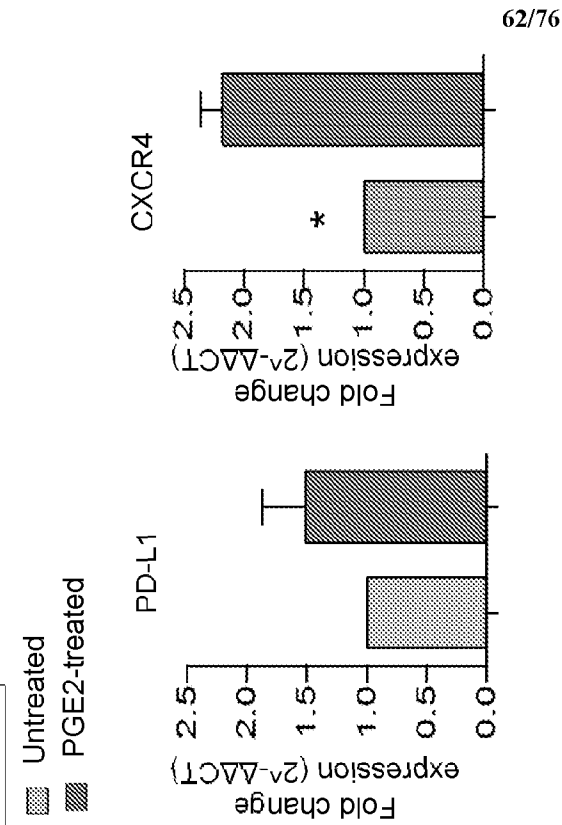


Fig. 17F

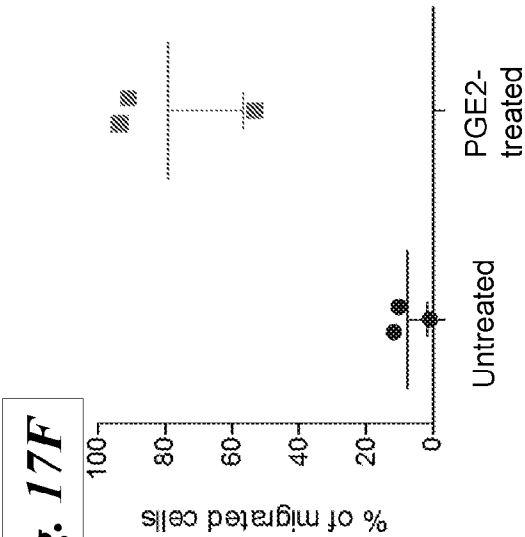
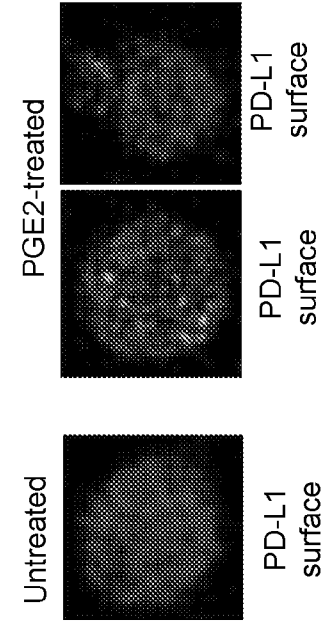
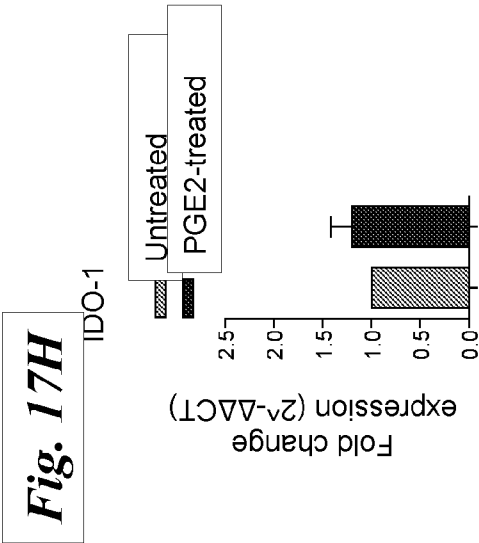
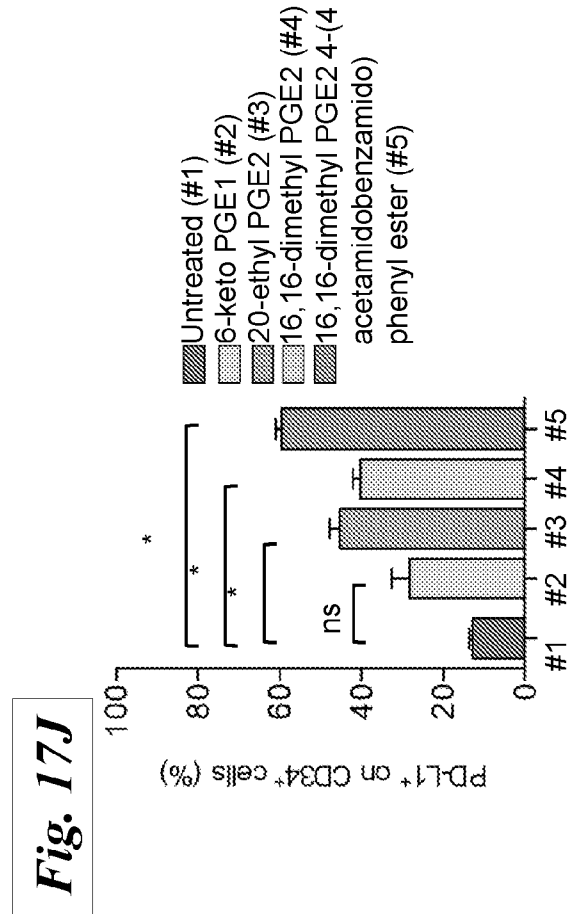


Fig. 17G





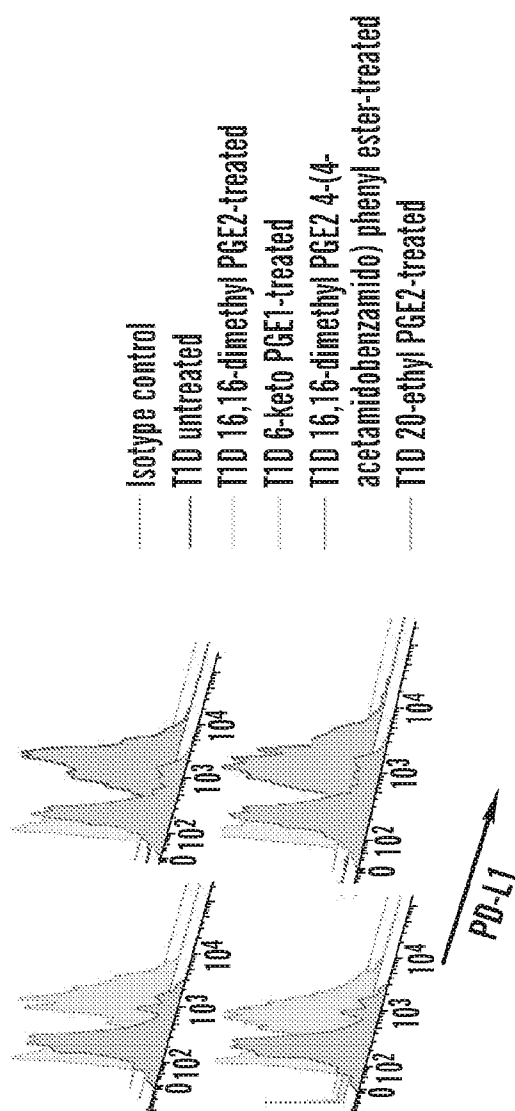


FIG. 17I

Fig. 18A

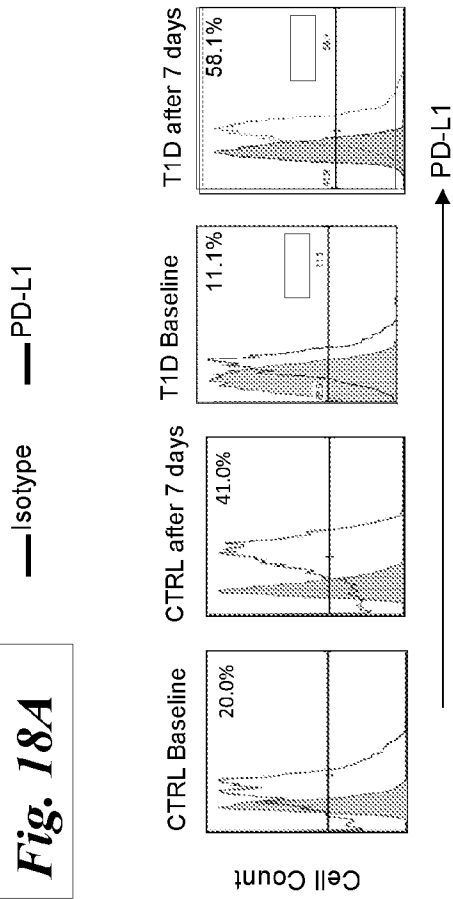


Fig. 18B

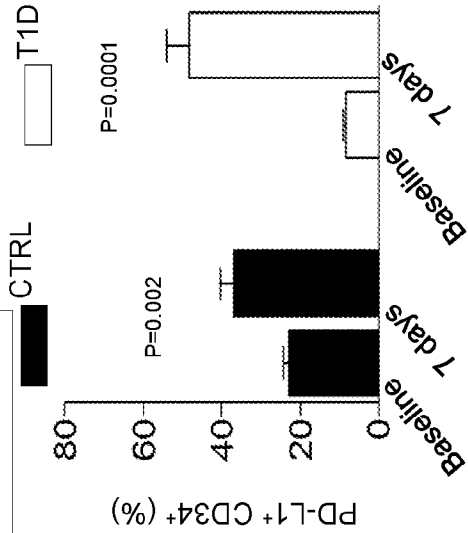
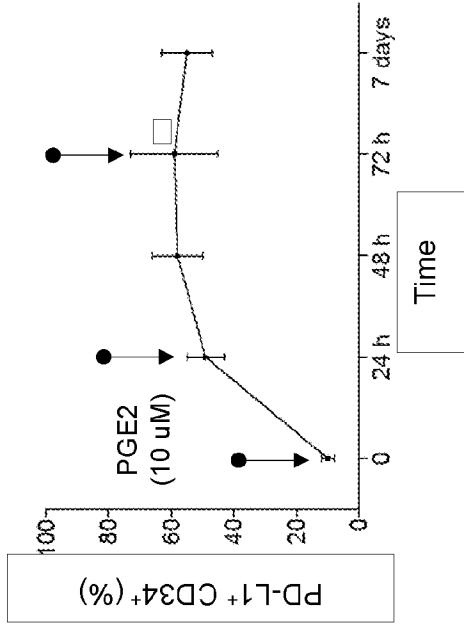
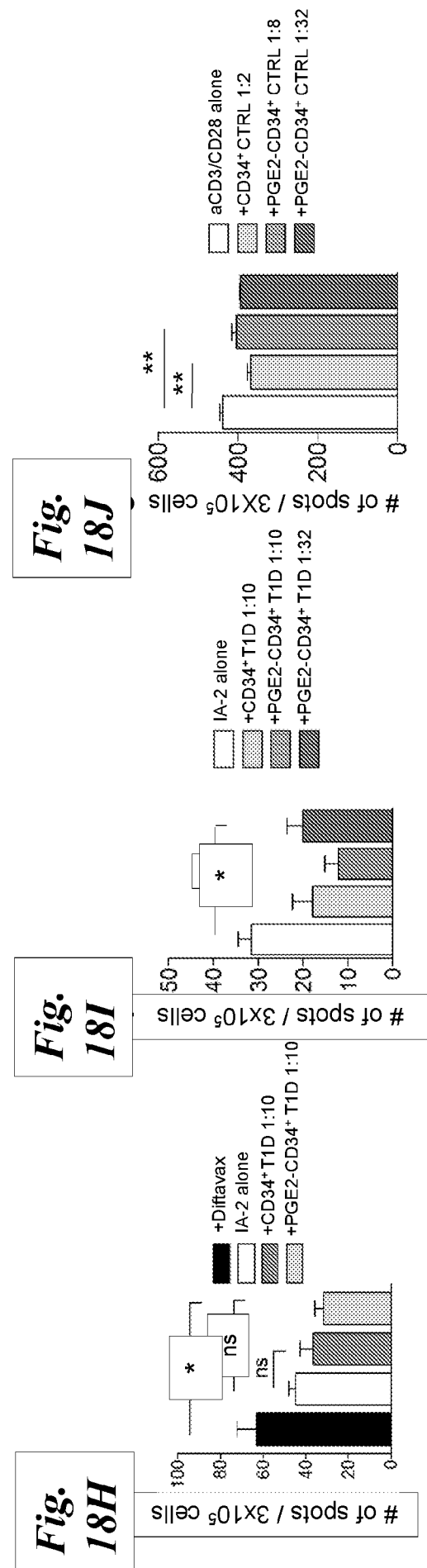
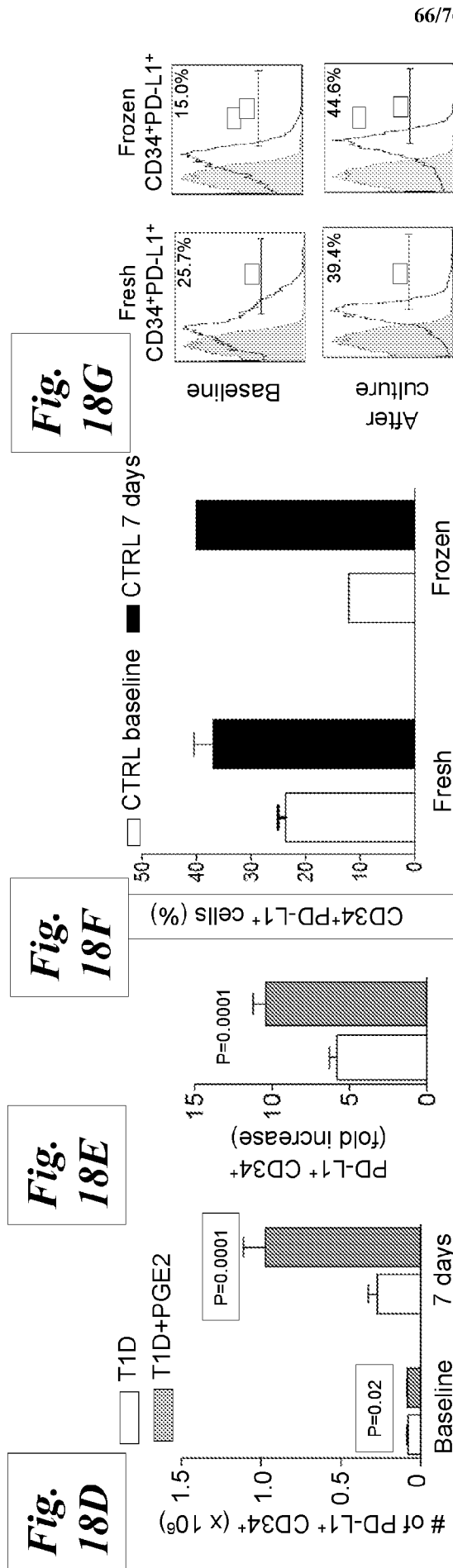
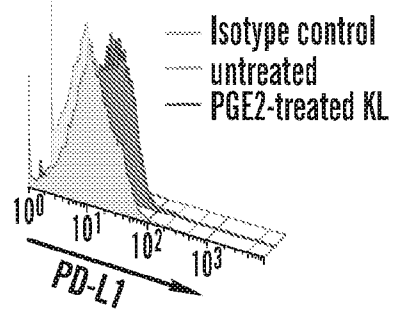
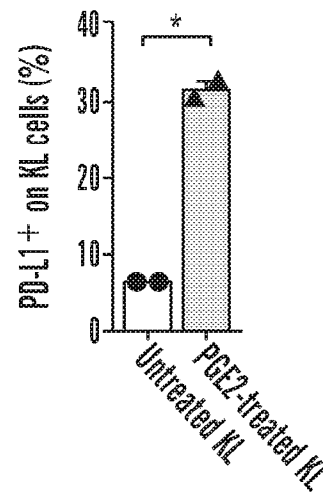
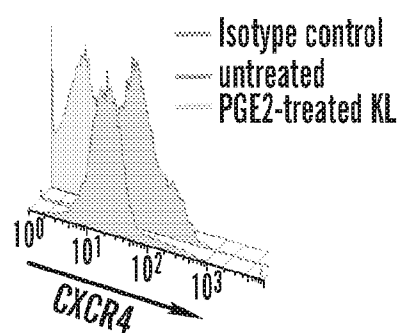
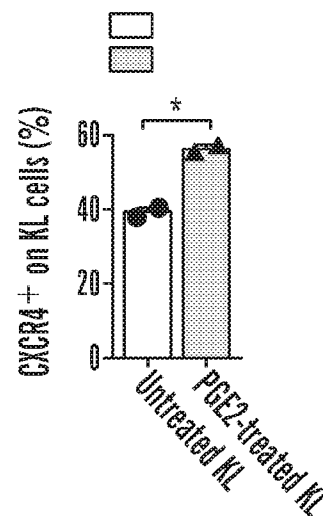


Fig. 18C

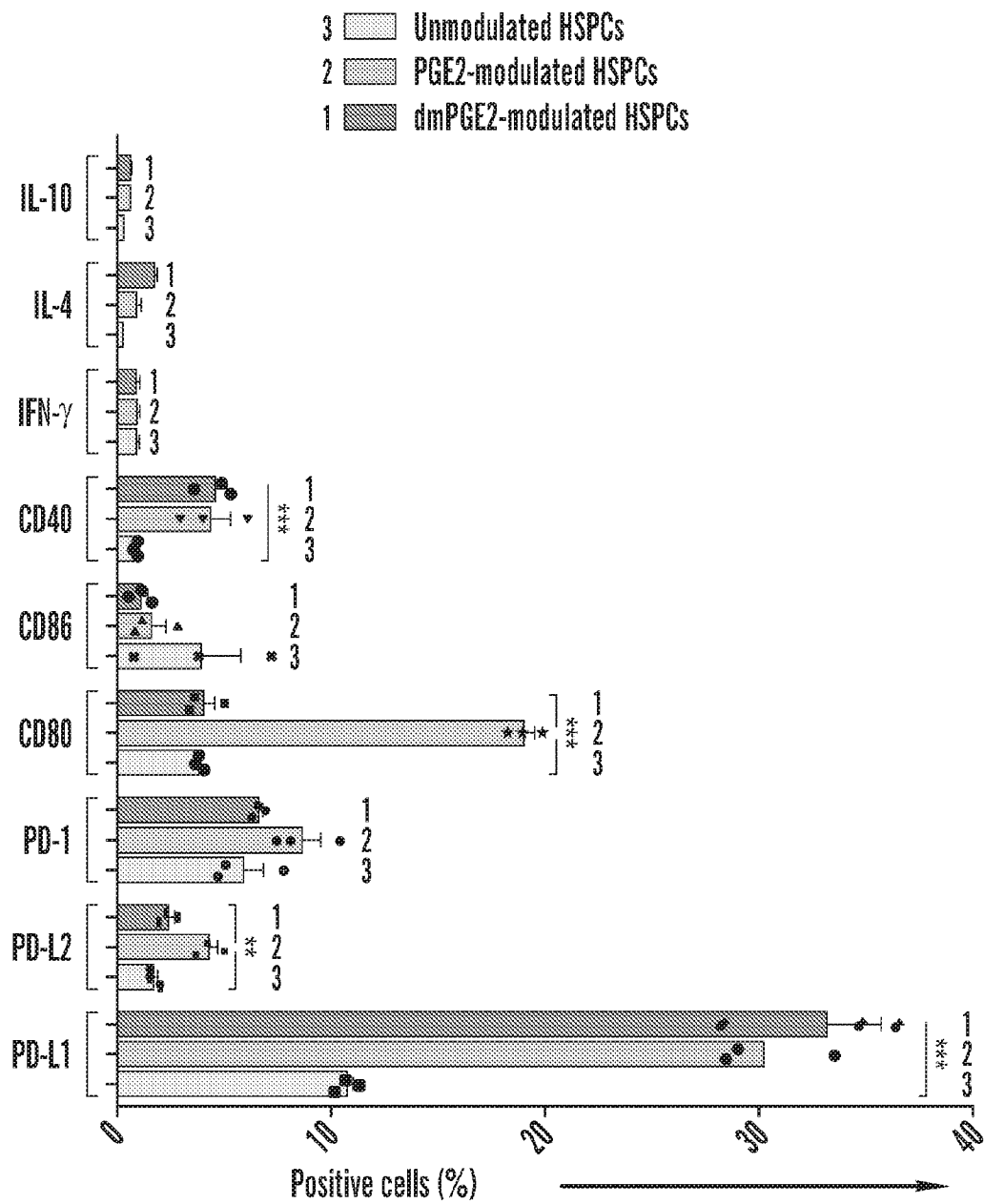




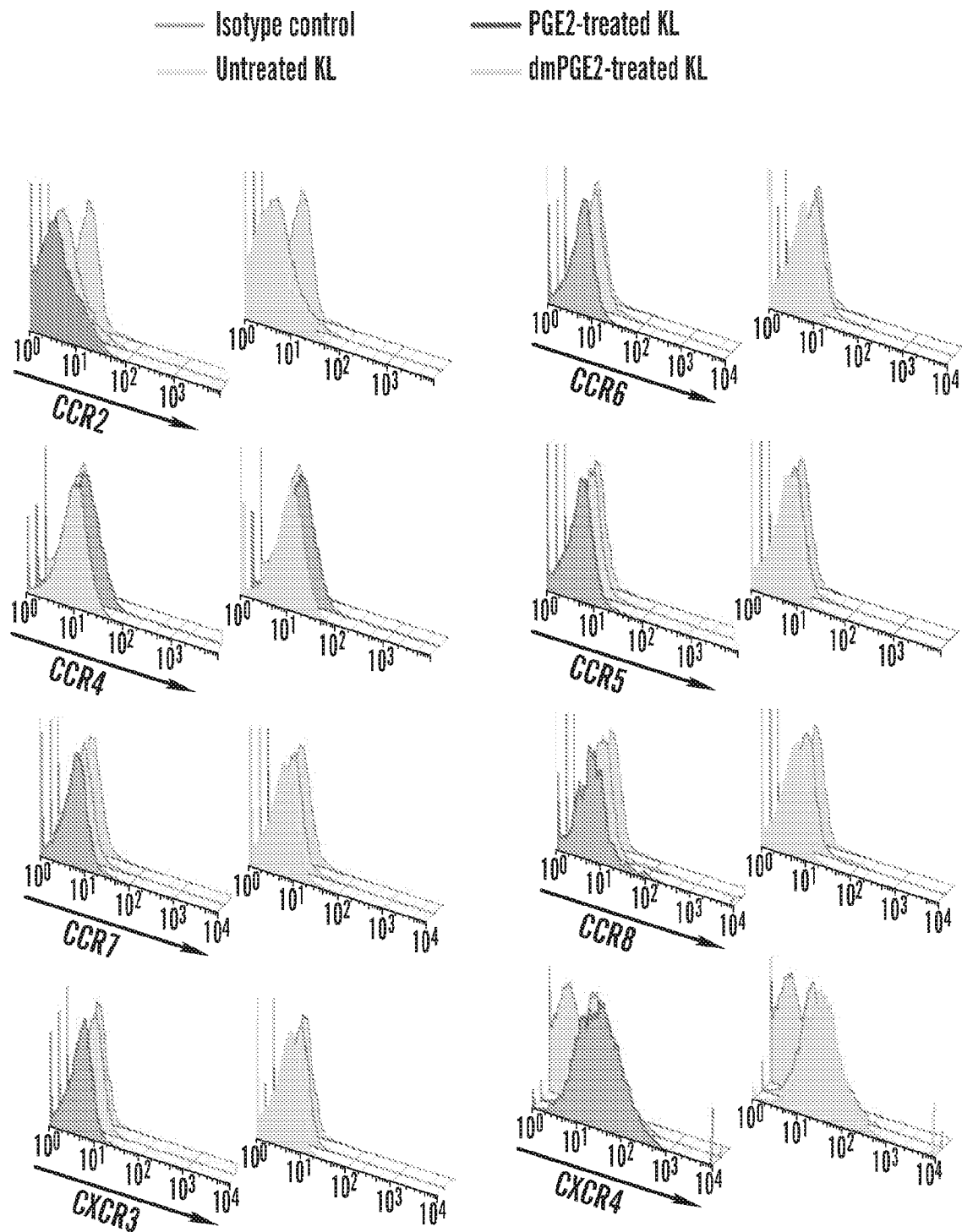
67/76

**FIG. 19A****FIG. 19B****FIG. 19C****FIG. 19D**

68/76

**FIG. 19E**

69/76

**FIG. 19F**

70/76

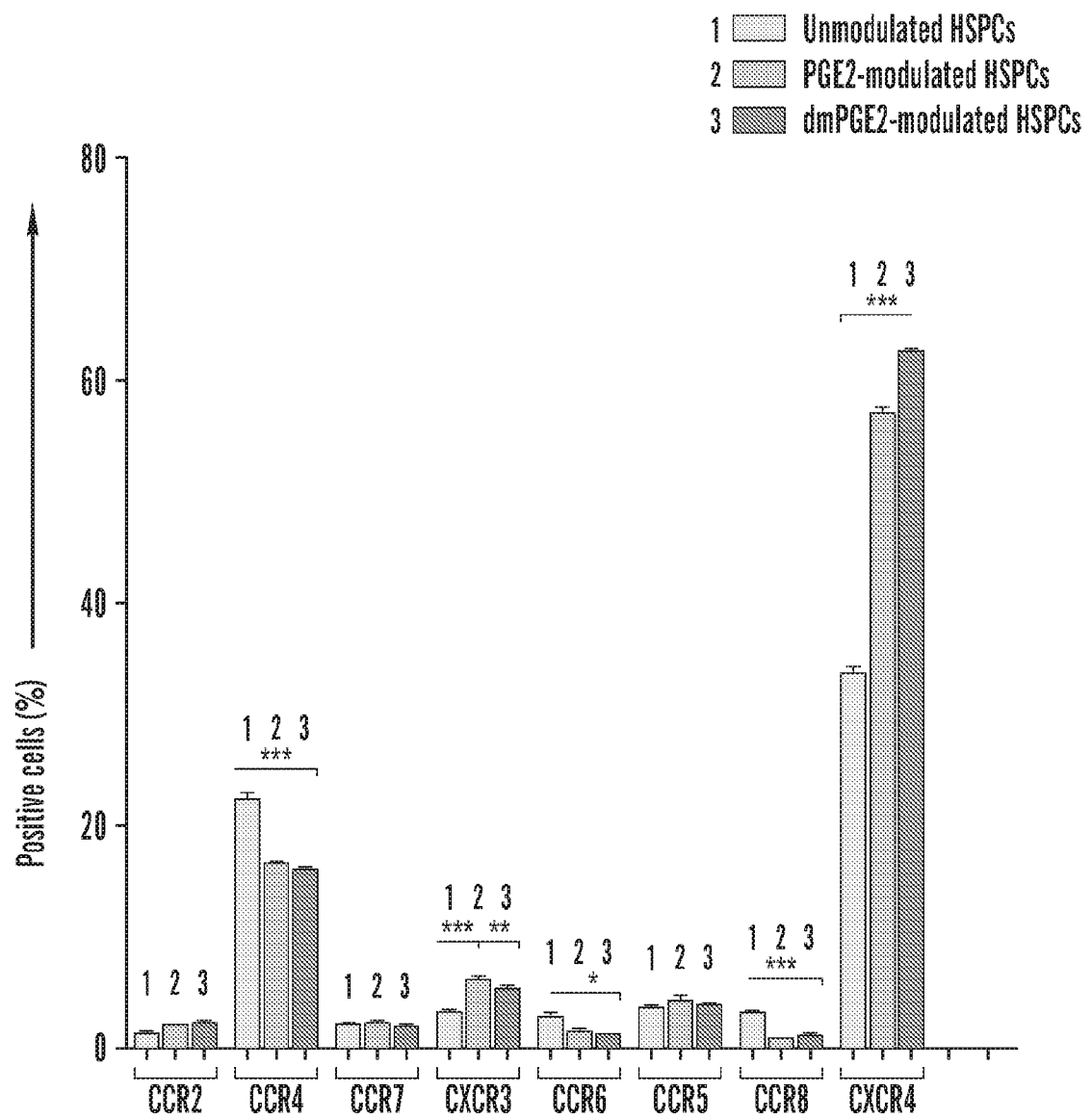
**FIG. 19G**

Fig. 20A

BDC2.5 stimulation assay

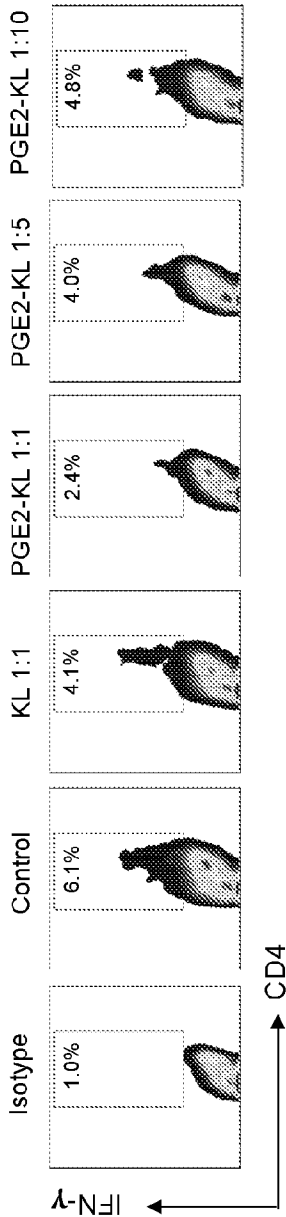


Fig. 20B

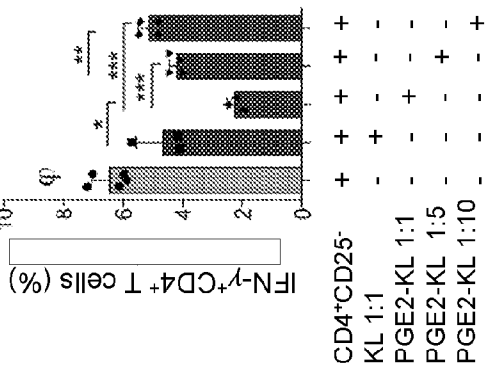


Fig. 20C

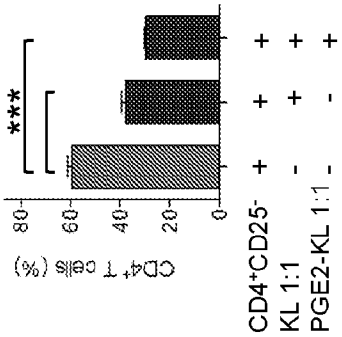


Fig. 20D

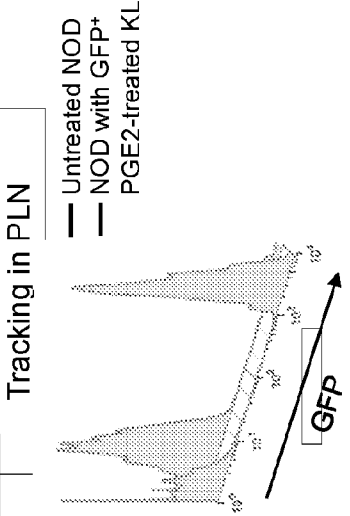
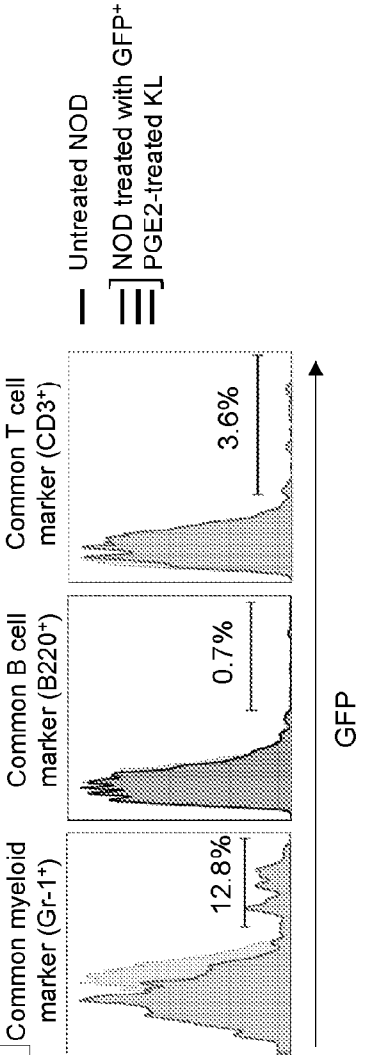


Fig. 20E

Tracking in Pancreas



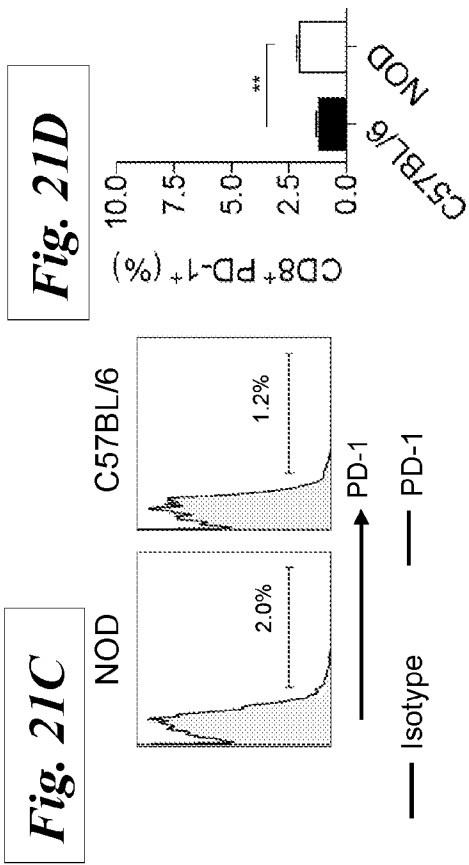
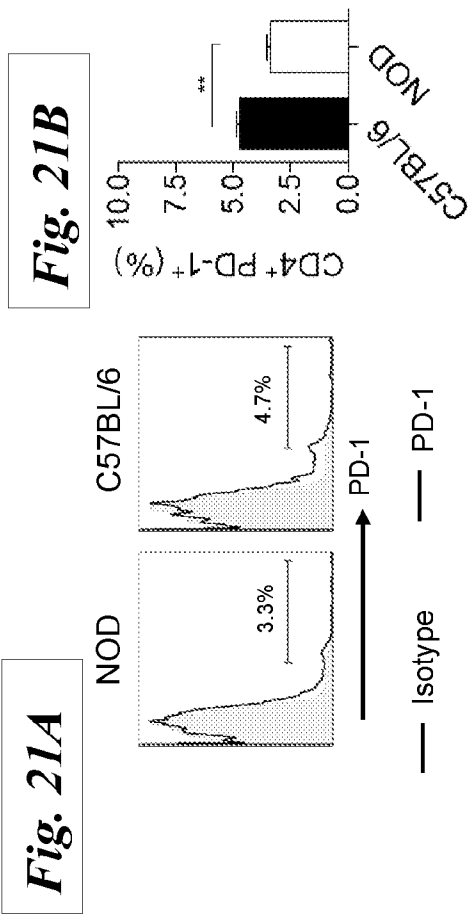
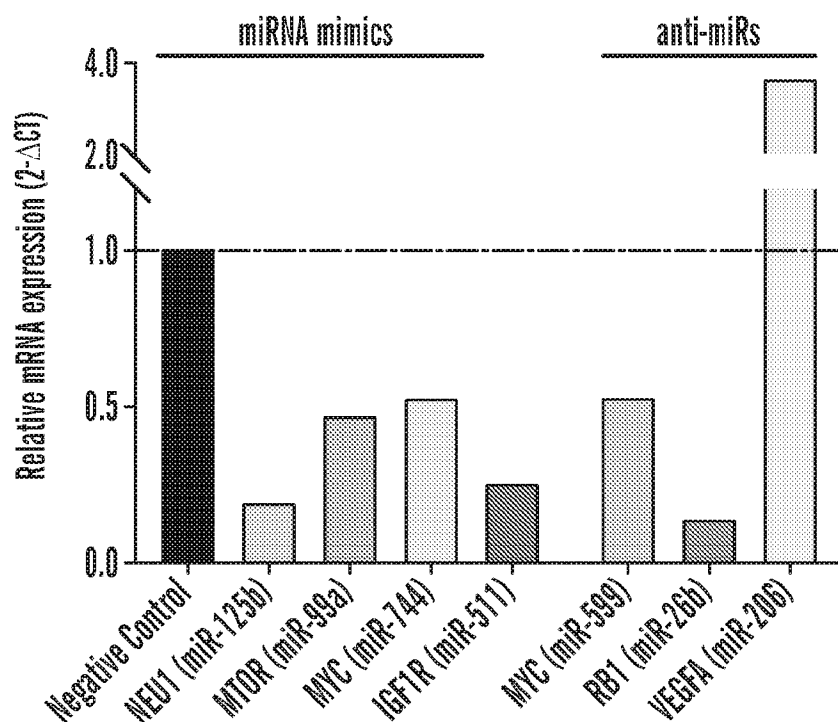
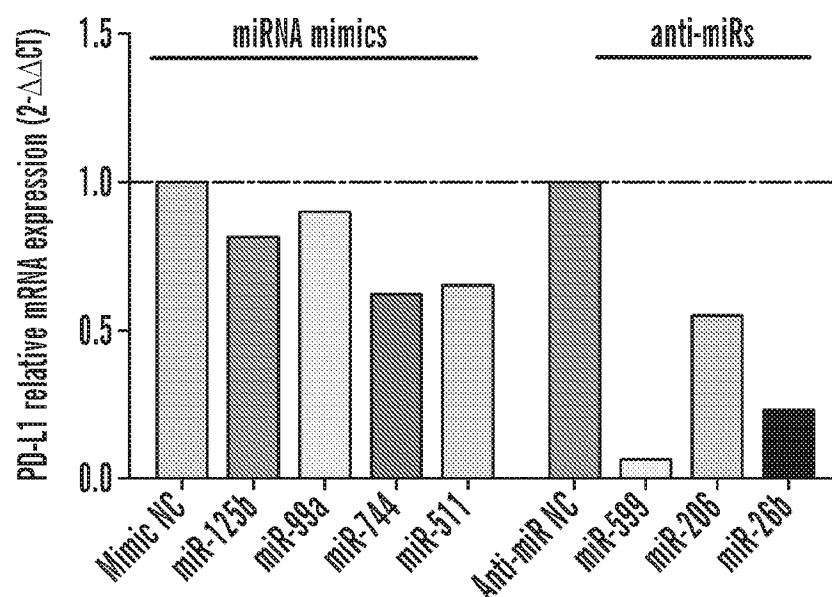


Fig. 21D

73/76

**FIG. 22A****FIG. 22B**

74/76

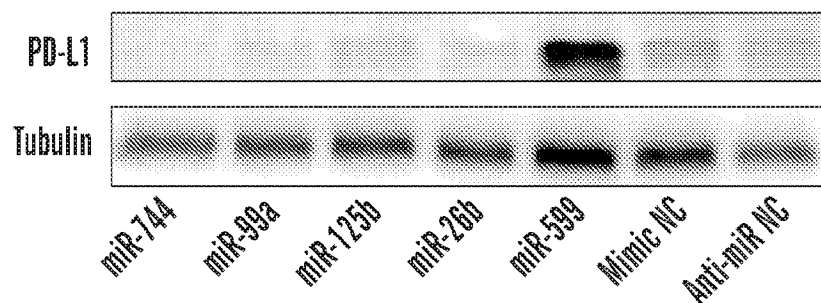
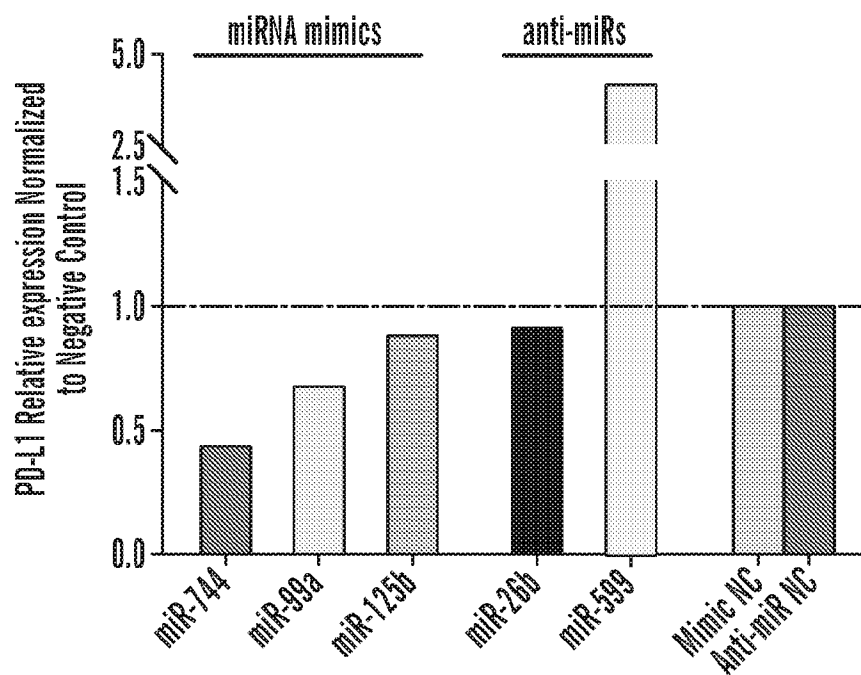
**FIG. 22C****FIG. 22D**

Fig. 23A

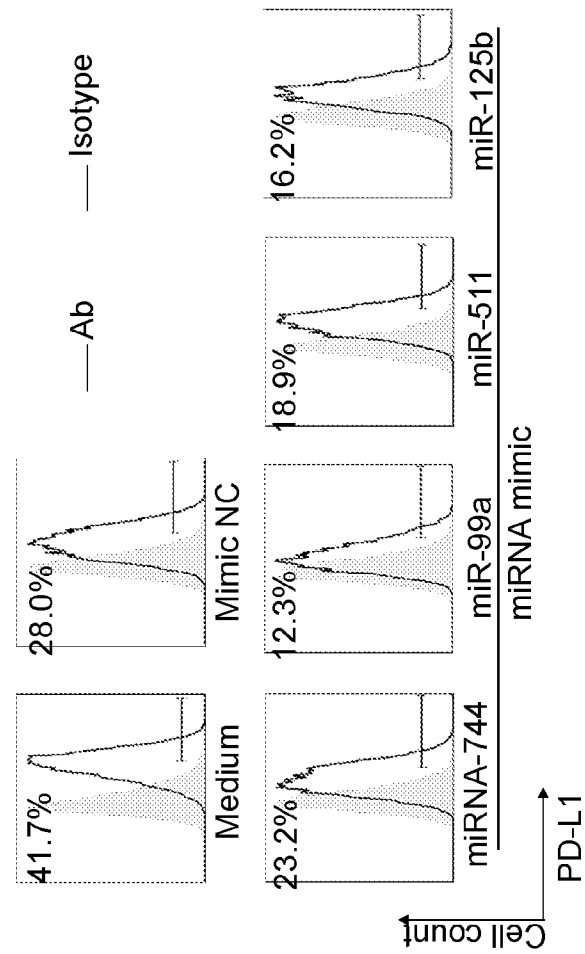


Fig. 23B

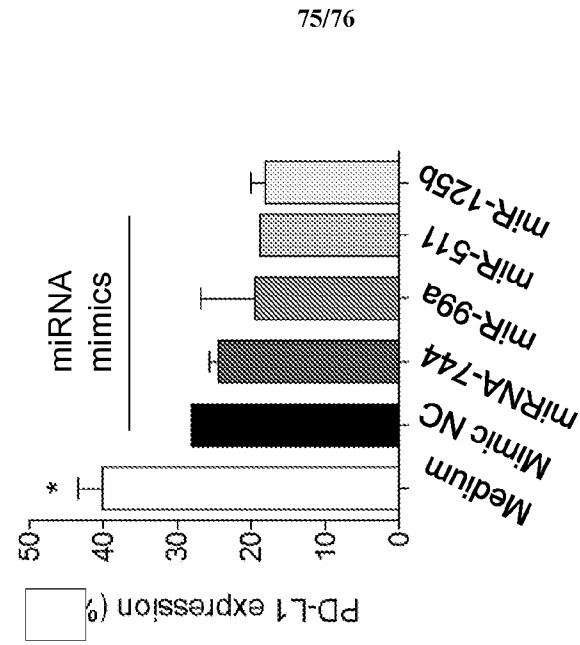


Fig. 23D

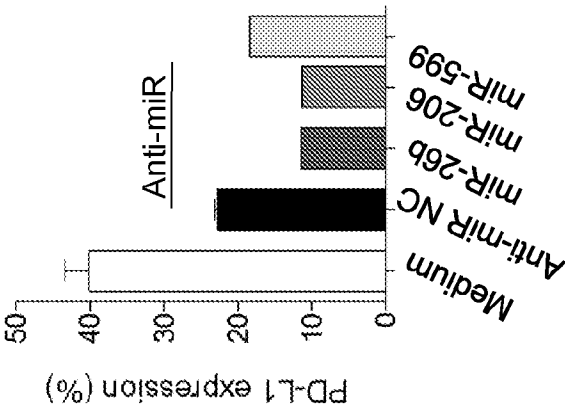
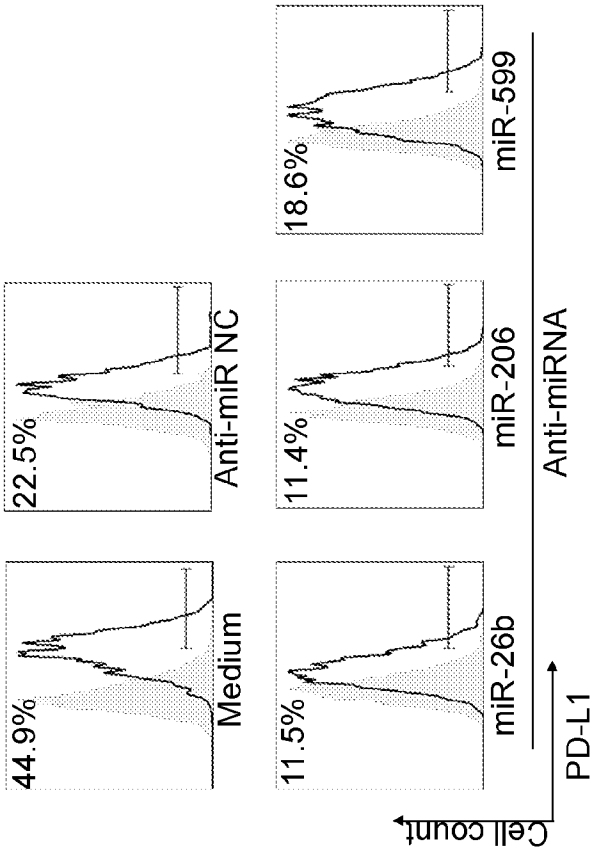


Fig. 23C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/052198

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7105; A61K 31/713; A61K 35/12; A61K 35/17; A61K 48/00; A61P 35/00 (2019.01)
 CPC - A61K 31/711; A61K 2035/124; A61P 35/00; A61P 37/00; C12N 15/111; C12N 15/113; C12N 15/1138; C12N 2310/14; C12N 2310/141; C12N 2320/11; C12Q 2600/178 (2019.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 514/44R; 514/44A; 424/93.71; 435/372; 435/375; 435/6.16; 435/6.12; 435/6.14 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0341933 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA et al) 20 November 2014 (20.11.2014) entire document	1-6, 10-15, 27-32, 39, 46
Y	WO 2016/161196 A1 (MIRNA THERAPEUTICS, INC. et al) 06 October 2016 (06.10.2016) entire document	1-6, 10-15, 27-32, 39, 46
Y	US 2009/0209621 A1 (MENDELL et al) 20 August 2009 (20.08.2009) entire document	4, 6, 11, 46
Y	US 2015/0366914 A1 (OHIO STATE INNOVATION FOUNDATION) 24 December 2015 (24.12.2015) entire document	15
Y	WO 2016/077574 A1 (THE GENERAL HOSPITAL CORPORATION) 19 May 2016 (19.05.2016) entire document	30, 31
P, X	BEN NASR et al. "PD-L1 Genetic Overexpression or Pharmacological Restoration in Hematopoietic Stem and Progenitor Cells Reverses Autoimmune Diabetes," Science Translational Medicine, 15 November 2017 (15.11.2017), Vol. 9, Iss. 416, Pgs. 1-14. entire document	1-6, 10-15, 27-32, 39, 46
A	ANSARI et al. "The Programmed Death-1 (PD-1) Pathway Regulates Autoimmune Diabetes in Nonobese Diabetic (NOD) Mice," Journal of Experimental Medicine, 07 July 2003 (07.07.2003), Vol. 198, No. 1, Pgs. 63-69. entire document	1-6, 10-15, 27-32, 39, 46
A	US 2010/0203056 A1 (IRVING et al) 12 August 2010 (12.08.2010) entire document	1-6, 10-15, 27-32, 39, 46



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 January 2019

Date of mailing of the international search report

07 FEB 2019

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/052198

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CORTEZ et al. "PDL1 Regulation by p53 via miR-34," Journal of the National Cancer Institute, 17 November 2015 (17.11.2015), Vol. 108, Pgs. 1-9. entire document	1-6, 10-15, 27-32, 39, 46
A	GRENDAL et al. "New Dancing Couple: PD-L1 and MicroRNA," Scandinavian Journal of Immunology, 22 June 2017 (22.06.2017), Vol. 86, No. 3, Pgs. 130-134. entire document	1-6, 10-15, 27-32, 39, 46

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/052198

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 7-9, 16-26, 33-38, 40-45, 47-51
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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