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(54) **Title:** NOVEL INNATE LYMPHOID CELLS (ILCS)

(57) **Abstract:** This invention relates to a novel human cell type, namely a human regulatory innate lymphoid cell (ILCreg). The invention provides a human ILCreg. The invention also provides an in vitro population of human ILCregs, pharmaceutical compositions and uses thereof.

NOVEL INNATE LYMPHOID CELLS (ILCS)

TECHNICAL FIELD

This invention relates to a novel human cell type, named a human regulatory innate lymphoid cell (ILCreg). The invention provides a human ILCreg. The invention also provides an *in vitro* population of human ILCregs, pharmaceutical compositions comprising the human ILCregs and uses thereof.

BACKGROUND

Innate lymphoid cells (ILCs) are mucosal-enriched and tissue-resident cells that mediate mucosal barrier integrity during homeostasis and provide a rapid, antigen non-specific source of cytokines during infection. ILCs currently fall into three groups, comprising five subtypes. The first group encompasses T-bet$^+$ Eomes$^+$ cytotoxic Natural Killer (NK) cells and T-bet$^+$ Eomes$^-$ ILC1. The second group comprises Rorα$^+$ Gata3$^+$ ILC2, and the third group encompasses RORγt$^+$ Lymphoid tissue inducer cells (LTi) as well as Natural Cytotoxicity Receptor (NCR)$^{+/-}$ ILC3. These categories appear to be less rigidly defined in humans than in mice, and plasticity can occur between groups.

The relative frequency and phenotypes of these ILC subsets varies between tissues in both mice and humans. Understanding what drives this heterogeneity is a topic of considerable interest, as relative ILC frequencies are not only altered during acute infection, but can become chronically dysregulated in inflammatory diseases and cancer, making them attractive targets for therapeutic manipulation. This is especially true as ILC engage in bi-directional interactions with epithelial cells: ILC1 drive epithelial cell proliferation through TGF-ß1; ILC2 are activated and proliferate in response to Tuft-cell derived IL-25; and ILC3 drive Lgr5$^+$ intestinal stem cell proliferation through IL-22; finally, foetal Lti mediate development of secondary lymphoid structures, whereas NK cells are circulatory and not specifically enriched in mucosa.

Mature ILC stem from the Common Lymphoid Precursor (CLP), which differentiate toward an ILC restricted Lineage$^-$ CD127$^+$ Id2$^+$ IL-7R$^+$ α4ß7$^+$ ILC precursor (ILCP) in mice. Sources of ILCP have been identified in the murine adult bone marrow, foetal liver, small intestine and lung (Bando *et al.*, 2015). ILC precursors have also been identified in human bone marrow, tonsils foetal, paediatric and adult intestines, but these are less well characterised than their murine counterparts (Elmentaite *et al.*, 2021).

Common to mice and humans is the low frequency of ILCs *in vivo*. Such limited numbers for isolation can hinder attempts to further study ILCs *in vitro* or *in vivo*. The therapeutic potential of ILCs is also yet to be realised, given the difficulties in obtaining large enough numbers for cell therapy.

In vitro attempts to produce ILCs have found that $CD34^+$ hematopoietic cells can produce ILC when cultured with feeder cells modified to express Notch ligands and with/without IL-15 supplementation (Hernández *et al.*, 2021). However, these approaches do not efficiently drive maturation of all subsets in parallel.

5 A regulatory ILC (ILCreg) population has previously been described in mice and humans (Wang et al., 2017), through their capacity to produce IL-10. However, the characteristics of this population are controversial as other ILC subsets such as ILC2 can also produce IL-10 (Bando et al., 2019). In none of those cases, was the population described to express Foxp3, the hallmark transcription factor for regulatory T cells. The present invention seeks 10 to address one or more of the aforementioned issues.

SUMMARY OF THE INVENTION

The inventors have identified a subset of human ILCs called human regulatory ILCs (or ILCregs). These are defined in more detail below and may be used to suppress inflammation, especially in the intestine.

15 In particular, the invention provides a human regulatory innate lymphoid cell (ILCreg) which expresses a detectable level of FOXP3 and/or CTLA4. The invention also provides:

- an *in vitro* population of human ILCregs, wherein the population comprises at least about two human ILCregs of the invention;

- a pharmaceutical composition comprising a human ILCreg of the invention or an *in* 20 *vitro* population of the invention and a pharmaceutically or physiologically acceptable diluent and/or carrier;

- a method of treating or preventing a disease in a subject, wherein the method comprises administering to the subject a human ILCreg of the invention, an *in vitro* population of the invention or a pharmaceutical composition of the invention;

25 - a human ILCreg of the invention, an *in vitro* population of the invention or a pharmaceutical composition of the invention for use in the treatment or prevention of a disease;

- a method for expanding a human ILCreg of the invention or an *in vitro* population of the invention, the method comprising co-culturing the human ILCreg or the *in vitro* 30 population and at least one epithelial organoid, wherein the epithelial organoid comprises more epithelial cells than mesenchymal cells; and

- a method for producing a human ILCreg of the invention or an *in vitro* population of the invention, the method comprising co-culturing human ILC precursors and at least one epithelial organoid, wherein the epithelial organoid comprises more epithelial

cells than mesenchymal cells, to expand the human ILC precursors and differentiate the ILC precursors into the human ILCregs.

DETAILED DESCRIPTION

Human ILCregs

5 The invention also provides a human regulatory ILC (ILCreg) which expresses a detectable level of FOXP3. The human ILCreg preferably further expresses detectable levels of one or more of CD25 (IL2RA), CD127 and CTLA4, such as CD25 (IL2RA), CD127, CTLA4, CD25 and CD127, CD25 and CTLA4, CD127 and CTLA4, or CD25, CD127 and CTLA4. The human ILCreg preferably further expresses detectable levels of one or more, such as 2, 3, 4, 5, 6, 10 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17, of the following genes: CCR5, CTLA4, FGGY, GATA3, GZMB, IL10, IL1R1, IL2RA, IL2RB, KAT2B, LGLS3, PIM1, PRDM1, RUNX1, SOX4, TNFRSF18 and TRAF1. The human ILCreg preferably expresses detectable levels of all of these genes. Gene expression is typically measured by measuring messenger RNA (mRNA) expression, for instance using RNA sequencing or single cell RNAseq (scRNAseq). Gene 15 expression, especially of FOXP3, can also be measured by measuring protein in the cell. Protein expression can be measured using standard methods, such as flow cytometry, Western blotting, immunohistochemistry, or enzyme-linked immunosorbent assay (ELISA). It is worthy of note FOXP3, RUNX1, IL1R1 and CTLA4 are associated with human T-regulatory cells (Tregs). The human ILCreg preferably further secretes a detectable level of 20 IL-10. This can be measured using a standard cytokine release assay and by intracellular flow cytometry. The human ILCreg preferably expresses detectable levels of one or more, such as 2 or 3, of the following cell surface markers: CD25 (IL2RA), CD127 and CTLA4. The human ILCreg preferably expresses detectable levels of CD25 (IL2RA), CD127, CTLA4, CD25 and CD127, CD25 and CTLA4, CD127 and CTLA4, or CD25, CD127 and CTLA4. 25 Surface marker expression can be measured using standard methods, such as flow cytometry.

The invention also provides a human regulatory ILC (ILCreg) which expresses detectable levels of CTLA4. Surface expression of CTLA4 can be measured using standard methods, such as flow cytometry. The human ILCreg preferably further expresses a detectable level 30 of FOXP3. The human ILCreg preferably further expresses detectable levels of CD25 (IL2RA), and/or CD127. The human ILCreg preferably further expresses detectable levels of one or more, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17, of the following genes: CCR5, FGGY, FOXP3, GATA3, GZMB, IL10, IL1R1, IL2RA, IL2RB, KAT2B, LGLS3, PIM1, PRDM1, RUNX1, SOX4, TNFRSF18 and TRAF1. The human ILCreg preferably 35 expresses detectable levels of all of these genes. Gene expression is typically measured by measuring messenger RNA (mRNA) expression, for instance using RNA sequencing or single cell RNAseq (scRNAseq). Gene expression, especially of FOXP3, can also be measured by measuring protein in the cell. Protein expression can be measured using standard methods,

such as flow cytometry, Western blotting, immunohistochemistry, or enzyme-linked immunosorbent assay (ELISA). It is worthy of note FOXP3, RUNX1, IL1R1 and CTLA4 are associated with human T-regulatory cells (Tregs). The human ILCreg preferably further secretes a detectable level of IL-10. This can be measured using a standard cytokine
5 release assay and by intracellular flow cytometry.

The invention also provides a human regulatory ILC (ILCreg) which expresses detectable levels of FOXP3 and CTLA4. Expression of FOXP3 and CTLA4 can be measured as described above. The human ILCreg preferably further expresses detectable levels of CD25 (IL2RA), and/or CD127. The human ILCreg preferably further expresses detectable levels of one or
10 more, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16, of the following genes: CCR5, FGGY, GATA3, GZMB, IL10, IL1R1, IL2RA, IL2RB, KAT2B, LGLS3, PIM1, PRDM1, RUNX1, SOX4, TNFRSF18 and TRAF1. The human ILCreg preferably expresses detectable levels of all of these genes. Gene expression can be measured as described above. It is worthy of note FOXP3, RUNX1, IL1R1 and CTLA4 are associated with human T-regulatory
15 cells (Tregs). The human ILCreg preferably further secretes a detectable level of IL-10. This can be measured using a standard cytokine release assay and by intracellular flow cytometry.

The invention also provides a human regulatory ILC (ILCreg) which expresses detectable levels of one or more, such as 2 or 3, of the following cell surface markers: CD25 (IL2RA),
20 CD127 and CTLA4. The human ILCreg preferably expresses detectable levels of CD25 (IL2RA), CD127, CTLA4, CD25 and CD127, CD25 and CTLA4, CD127 and CTLA4, or CD25, CD127 and CTLA4. Surface marker expression can be measured using standard methods, such as flow cytometry. The human ILCreg preferably further expresses a detectable level of FOXP3. The human ILCreg preferably further expresses detectable levels of one or more,
25 such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15, of the following genes: CCR5, FGGY, GATA3, GZMB, IL10, IL1R1, IL2RB, KAT2B, LGLS3, PIM1, PRDM1, RUNX1, SOX4, TNFRSF18 and TRAF1. The human ILCreg preferably expresses detectable levels of all of these genes. Gene expression is typically measured by measuring messenger RNA (mRNA) expression, for instance using RNA sequencing or single cell RNAseq (scRNAseq). It is worthy of note
30 FOXP3, RUNX1, IL1R1 and CTLA4 are associated with human T-regulatory cells (Tregs). The human ILCreg preferably further secretes a detectable level of IL-10. This can be measured using a standard cytokine release assay and by intracellular flow cytometry.

In any of the embodiments above, the human ILCreg preferably does not express detectable levels of one or more, such as 2, 3, 4, 5 or 6, of CD3, CD4, CD19, CD20, TCRαβ,
35 and TCRγδ. The human ILCreg preferably does not express detectable levels of one or more, such as 2, 3, 4 or 5, of CD3, CD4, CD19, CD20, and TCRαβ. The human ILCreg preferably does not express detectable levels of one or more these markers at the cell surface and/or

at the RNA level. The human ILCreg preferably does not express extracellular detectable levels of CD3 and CD4. The human ILCreg preferably does not express extracellular detectable levels of any of these markers. The human ILCreg preferably comprises a $CD3^-$, $CD4^-$, $CD19^-$, $CD20^-$, $TCR\alpha\beta^-$, $TCR\gamma\delta^-$ expression profile. The human ILCreg preferably comprises a $CD3^-$, $CD4^-$, $CD19^-$, $CD20^-$, $TCR\alpha\beta^-$ expression profile. The human ILCreg preferably expresses a detectable level of CD127.

The ILCregs of the invention are human. It is straightforward for the skilled person to determine whether or not a cell is human, for instance by examining the number and size of the chromosomes present in the nucleus, by sequencing part of the genome or by identifying the presence of specific human markers. The source of the cell also helps the skilled person confirm the ILCreg is human.

Populations

The invention also provides an *in vitro* population of human ILCregs, wherein the population comprises at least about two human ILCregs of the invention. The population preferably comprises at least about 5, at least about 10, at least about 50, at least about 100, at least about 1000 or at least about 5×10^3 human ILCregs of the invention. The population preferably comprises at least about 5, at least about 10, at least about 50, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1000 or at least about 5×10^3 human ILCregs of the invention. The human ILCregs of the invention may be any of those defined above.

In all embodiments of the invention, an *in vitro* population includes a population of cells in a format suitable for administration to a subject. This may include a vial, bag or needles containing the cells. The cells may be in a liquid solution or frozen form.

In some embodiments the population comprises at least about 5×10^3 human ILCregs, at least about 1×10^4 human ILCregs, at least about 5×10^4 human ILCregs, at least about 1×10^5 human ILCregs, at least about 5×10^5 human ILCregs, at least about 1×10^6 human ILCregs, at least about 5×10^6 human ILCregs, at least about 1×10^7 human ILCregs, at least about 5×10^7 human ILCregs, at least about 1×10^8 human ILCregs, at least about 5×10^8 human ILCregs, at least about 1×10^9 human ILCregs, at least about 5×10^9 human ILCregs, at least about 1×10^{10} human ILCregs, at least about 5×10^{10} human ILCregs, at least about 1×10^{11} human ILCregs, at least about 1×10^{12} human ILCregs, at least about 1×10^{13} human ILCregs, at least about 1×10^{14} human ILCregs, at least about 1×10^{15} human ILCregs, at least about 1×10^{16} human ILCregs, at least about 1×10^{17} human ILCregs, at least about 1×10^{18} human ILCregs, at least about 1×10^{19} human ILCregs, at least about 1×10^{20} human ILCregs, at least about 1×10^{25} human ILCregs, at least about 1×10^{30} human ILCregs, at least about

1×10^{35} human ILCregs, at least about 1×10^{40} human ILCregs, at least about 1×10^{45} human ILCregs, at least about 1×10^{50} human ILCregs, at least about 1×10^{60} human ILCregs, at least about 1×10^{70} human ILCregs, at least about 1×10^{80} human ILCregs, at least about 1×10^{90} human ILCregs, at least about 1×10^{100} human ILCregs, at least about 1×10^{150} human ILCregs, at least about 1×10^{200} human ILCregs, at least about 1×10^{250} human ILCregs, at least about 1×10^{300} human ILCregs, at least about 1×10^{350} human ILCregs, at least about 1×10^{400} human ILCregs, at least about 1×10^{450} human ILCregs, at least about 1×10^{500} human ILCregs, at least about 1×10^{550} human ILCregs, at least about 1×10^{600} human ILCregs, at least about 1×10^{650} human ILCregs, at least about 1×10^{700} human ILCregs, at least about 1×10^{750} human ILCregs, at least about 1×10^{850} human ILCregs, at least about 1×10^{950} human ILCregs, at least about 1×10^{1000} human ILCregs, at least about 1×10^{2000} human ILCregs, at least about 1×10^{3000} human ILCregs, at least about 1×10^{4000} human ILCregs, at least about 1×10^{4000} human ILCregs, at least about 1×10^{5000} human ILCregs, at least about 1×10^{6000} human ILCregs, at least about 1×10^{7000} human ILCregs, at least about 1×10^{8000} human ILCregs, at least about 1×10^{9000} human ILCregs or at least about 1×10^{10000} human ILCregs.

In some embodiments the population comprises at least about 1×10^{20} human ILCregs. In some embodiments the population comprises at least about 1×10^{100} human ILCregs. In some embodiments the population comprises at least about 1×10^{500} human ILCregs. In some embodiments the population comprises at least about 1×10^{1000} human ILCregs.

In some embodiments the population comprises no more than about 1×10^{10000} human ILCregs. In some embodiments the population comprises no more than about 1×10^{500} human ILCregs. In some embodiments the population comprises of from about 5×10^{4} human ILCregs to about 1×10^{10000} human ILCregs. In some embodiments the population comprises of from about 1×10^{200} human ILCregs to about 1×10^{10000} human ILCregs.

The *in vitro* population may comprise other ILCs in addition to the human ILCregs. The *in vitro* population of ILCs may comprise a plurality of Group 1 and Group 2 ILCs. In some embodiments the *in vitro* population of ILCs comprise a plurality of Group 1 and Group 3 ILCs. Alternatively, the *in vitro* population of ILCs may comprise a plurality of Group 2 and Group 3 ILCs. In some embodiments, the *in vitro* population of ILCs comprise a plurality of Group 1, Group 2 and Group 3 ILCs. In some embodiments, the *in vitro* population of ILCs comprises a plurality of Group 1 ILCs and human ILCregs. In some embodiments, the *in vitro* population of ILCs comprises a plurality of human ILCregs and Group 2 ILCs. In some embodiments, the *in vitro* population of ILCs comprises a plurality of human ILCregs and Group 3 ILCs. In some embodiments, the *in vitro* population of ILCs comprises a plurality of Group 1 ILCs, Group 2 ILCs and human ILCregs. In some embodiments, the *in vitro* population of ILCs comprises a plurality of Group 1 ILCs, Group 3 ILCs and human ILCregs. In some embodiments, the *in vitro* population of ILCs comprises a plurality of Group 2 ILCs,

Group 3 ILCs and human ILCregs. In some embodiments, the *in vitro* population of ILCs comprises a plurality of Group 1 ILCs, Group 2 ILCs, Group 3 ILCs and human ILCregs.

The *in vitro* population of human ILCregs of the invention may comprise at least about 0.1%, at least about 0.2%, at least about 0.5%, at least about 0.8%, at least about 1%, at least about 2%, at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% human ILCregs of the invention.

In some embodiments, the *in vitro* population of ILCs is a heterogenous population of ILCs. In other embodiments the *in vitro* population of ILCs is a homologous population of ILCs.

In some embodiments at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the human ILCregs express or secrete a detectable level of IL-10.

In some embodiments at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the human ILCregs express a detectable level of FOXP3.

In some embodiments at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the human ILCregs express a detectable level of CD25 (IL2RA).

In some embodiments at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the human ILCregs express a detectable level of CD127.

In some embodiments at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the human ILCregs express a detectable level of CTLA4.

At least 50% of the *in vitro* population of human ILCs may co-express detectable levels of two or more cytokines.

The *in vitro* population of human ILCregs may be a stimulated *in vitro* population of human ILCregs. Various stimulation methods are known to those skilled in the art. For example, the population may comprise a PMA and Ionomycin stimulated *in vitro* population of human ILCregs. Preferably, a stimulated *in vitro* population of human ILCregs expresses detectable levels of one or more cytokines as described above.

The *in vitro* population of human ILCregs may have a tissue-specific imprint. By "tissue-specific imprint", this will be understood to refer to a genetic signature and phenotype specific of *in vivo* immune cells of a particular organ. Thus, in some embodiments the *in vitro* population of human ILCregs comprises or consists of a population of tissue-specific human ILCregs. Such tissue-specific human ILCregs may have utility for the treatment of particular diseases and/or may have improved homing capacity to the specific tissue when administered to a subject. In some embodiments, the *in vitro* population of human ILCregs comprise or consist of skin, intestinal, lung, thymic, thyroid, reproductive, bladder, kidney, pancreas, oral mucosal or liver specific human ILCregs. In some embodiments, the *in vitro* population of human ILCregs comprises or consists of skin, intestinal, lung, thyroid, reproductive, bladder, kidney, pancreas, oral mucosal or liver specific human ILCregs. In some embodiments, the *in vitro* population of human ILCregs comprises or consists of intestinal, lung, reproductive or oral mucosal specific human ILCregs.

Reproductive-specific human ILCregs may comprise fallopian tube specific human ILCregs, ovary specific human ILCregs, prostate specific human ILCregs, endometrium specific human ILCregs, cervix specific human ILCregs, vaginal specific human ILCregs and testes-specific human ILCregs. Reproductive-specific human ILCregs may be selected from fallopian tube specific human ILCregs, ovary specific human ILCregs, prostate specific human ILCregs and endometrium specific human ILCregs.

Oral mucosal specific human ILCregs may comprise salivary gland taste bud specific ILCs, lingual region specific human ILCregs and oesophagus specific human ILCregs. In some embodiments, the oral mucosal specific human ILCregs are oesophageal specific human ILCregs.

In some embodiments, the *in vitro* population of human ILCregs comprises or consists of intestinal-specific human ILCregs and/or lung-specific human ILCregs. The intestinal specific human ILCregs may be small intestinal-specific human ILCregs and/or lung specific human ILCregs.

Alternatively, the *in vitro* population of human ILCregs may comprise or consist of epithelial cancer specific human ILCregs. In some embodiments the *in vitro* population of human ILCregs may comprise or consist of skin cancer specific human ILCregs, intestinal cancer specific human ILCregs, lung cancer specific human ILCregs, thymic cancer specific human ILCregs, thyroid cancer specific human ILCregs, reproductive cancer specific human ILCregs, bladder cancer specific human ILCregs, kidney cancer specific human ILCregs, pancreas cancer specific human ILCregs, oral mucosal cancer specific human ILCregs or liver cancer specific human ILCregs the *in vitro* population of human ILCregs may comprise or consist of skin cancer specific human ILCregs, intestinal cancer specific human ILCregs , lung cancer specific human ILCregs, thyroid cancer specific human ILCregs, reproductive

cancer specific human ILCregs, bladder cancer specific human ILCregs, kidney cancer specific human ILCregs, pancreas cancer specific human ILCregs, oral mucosal cancer specific human ILCregs or liver cancer specific human ILCregs. invention.

Preferably, the human ILCregs are primary human ILCregs. A "primary cell" is a cell that is obtained from or is derived from a subject. Primary cells are not immortalised cells from a cell line. Primary human ILCregs can be produced from primary human ILC precursors, i.e., from human ILC precursors that have been obtained from a subject. The primary human ILCregs may be autologous. Alternatively, the primary human ILCregs may be allogeneic. In some embodiments, the population comprises a mixture of allogeneic and autologous human ILCregs.

Alternatively, the human ILCregs may comprise or consist of immortalised immune cells from a cell line. Alternatively, the human ILCregs may comprise or consist of immortalised human ILCregs or a human ILCreg cell line.

In some embodiments, at least about 10% of the *in vitro* population comprises an exogenous polynucleotide. By "exogenous polynucleotide", this will be understood to refer to a polynucleotide which has been introduced into the human ILCreg, such that the ILCreg is genetically modified. Thus, in some embodiments the *in vitro* population of human ILCreg is a genetically modified *in vitro* population of human ILCreg. Typically, the exogenous polynucleotide is recombinant. The exogenous polynucleotide typically encodes an exogenous polypeptide. It will be appreciated that the exogenous polypeptide may be a polypeptide which is endogenous to the human ILCreg but is expressed in the cell at higher levels following introduction of the exogenous polynucleotide by genetic modification Alternatively, the exogenous polypeptide may be a polypeptide which is not naturally expressed in the human ILCreg.

In some embodiments at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the cells in the *in vitro* population comprise an exogenous polynucleotide.

In some embodiments, at least about 10% of the cells in the *in vitro* population express the exogenous polypeptide encoded by the exogenous polynucleotide at a detectable level. In some embodiments at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the cells in the *in vitro* population express the exogenous polypeptide encoded by the exogenous polynucleotide at a detectable level.

The exogenous polypeptide may comprise a marker protein or an immunotherapeutic molecule. The marker protein may otherwise be referred to as a reporter protein. Suitable

marker proteins include, but are not necessarily limited to GFP, a MYC epitope tag or a FLAG epitope tag.

In some embodiments, the exogenous polypeptide further comprises a purification tag. As the skilled person will appreciate, a purification tag can assist with purification. Examples of purification tags include but are not necessarily limited to a His-tag, Arg-tag, T7-tag, Strep-tag, S-tag, aptamer-tag, V5 tag, or AviTag™. Various other tags are well known in the art.

The immunotherapeutic molecule may be any immunotherapeutic molecule which may further increase the immunotherapeutic use of the in vitro population of human ILCregs. For example, the immunotherapeutic molecule may comprise an enzyme, an antibody, an antigen, a chimeric antigen receptor (CAR), MHC class II cell receptor, chemokine receptor and/or a cytokine. The MHC class II cell receptor may preferably comprise HLA-DR.

In some embodiments the immunotherapeutic molecule comprises or consists of a chimeric antigen receptor (CAR). Chimeric antigen receptors are immune cell receptors which have been genetically engineered to confer the ability to target a specific antigen or antigens. Generally, chimeric antigen receptors are specific for one or more cancer-associated antigens. As such, chimeric antigen receptors are commonly used in the treatment of cancer.

Various CARs are known to those skilled in the art. In particular, the CAR may comprise or consist of a first, second, third, or fourth generation CAR.

First-generation CARs comprise or consist of a binding domain that is capable of specifically binding to an epitope on a target antigen, a transmembrane domain, and one or more intracellular signalling domains. The extracellular binding domain may comprise a single-chain variable fragment (scFv) from a monoclonal antibody. A first-generation CAR typically comprises a CD3ζ chain domain or a variant thereof as the intracellular signalling domain, which is the primary transmitter of signals.

In addition to the components specified for first-generation CARs, second-generation CARs also contain a co-stimulatory domain, such as CD28 and/or 4-1BB. The inclusion of an intracellular co-stimulatory domain improves T-cell proliferation, cytokine secretion, resistance to apoptosis, and *in vivo* persistence. The co-stimulatory domain of a second-generation CAR is typically *in cis* with and upstream of the one or more intracellular signalling domains.

Third-generation CARs combine multiple co-stimulatory domains *in cis* with one or more intracellular signalling domains, to augment T-cell activity. For example, a third-generation CAR may comprise co-stimulatory domains derived from CD28 and 41BB, together with an intracellular signalling domain derived from CD3 zeta. Other third-generation CARs may

comprise co-stimulatory domains derived from CD28 and OX40, together with an intracellular signalling domain derived from CD3 zeta.

Fourth-generation CARs (also known as TRUCKs or armoured CARs), combine the features of a second-generation CAR with further factors to enhance anti-tumour activity (e.g., cytokines, co-stimulatory ligands, chemokines receptors or further chimeric receptors of immune regulatory or cytokine receptors). The factors may be *in trans* or *in cis* with the CAR, typically *in trans* with the CAR.

In some embodiments, the CAR is specific for a cancer antigen. The cancer antigen may be a solid tumour cancer antigen. By "specific", in the context of the CAR, this will be understood to refer to being capable of specifically binding to a target antigen.

Cancer antigens include, but are not necessarily limited to Erbb1, Erbb3, Erbb4, Erbb2, mucins, PSMA, carcinoembryonic antigen (CEA), mesothelin, GD2, MUC1, folate receptor, NKG2D ligands, ligands bound by other NK receptors such as NKp30, NKp44 or NKp46, GPC3, CAIX, FAP, NY-ESO-1, gp100, PSCA, ROR1, PD-L1, PD-L2, EpCAM, EGFRvIII, CD19, CD20, CD22, GD3, CLL-1, ductal epithelial mucin, CA-125, GP36, TAG-72, glycosphingolipids, glioma-associated antigen, beta-hCG, AFP (alpha-fetoprotein) and lectin-reactive AFP, thyroglobulin, receptor for advanced glycation end products (RAGE), TERT, telomerase, carboxylesterase, M-CSF, M-CSF receptor, PSA, tyrosinase, survivin, PCTA-1, melanoma-associated antigen (MAGE), for example MAGE A1, MAGE A2, MAGE A4, MAGE A8, CD22, IGF-1, IGF-2, IGF-1 receptor, MHC-associated tumour peptide, 5T4, tumour stroma-associated antigens, WT1, MLANA, CA 19-9, epithelial tumour antigen (ETA), BCMA, cancer testis antigens such as CTA New York (o)esophageal squamous cell carcinoma (NYESO) and glycoprotein 100 (GP100), preferentially expressed antigen in melanoma (PRAME), collagen type IV alpha 3 chain (COL6A3), MR1, CD1c, human epidermal growth factor receptor 2 (HER2), solute carrier family 3 member 2 (SLC3A2) and avb6 integrin.

In some embodiments, the cancer antigen is selected from NYESO, GP100, PRAME, COL6A3, MR1, CD1c, HER2, SLCA2, CD19, PSMA, AFP, CEA, CA-125, MUC1, ETA, tyrosinase and MAGE. In some embodiments, the CAR is an anti-CD19, anti-SLC3A2 or anti-PSMA CAR.

In some embodiments, the CAR is an anti-CD19 or anti-PSMA CAR.

MAGE may be selected from MAGE A1, MAGE A2, MAGE A4 or MAGE A8.

The CAR may be linked to a reporter protein, for example GFP, MYC epitope flag or a FLAG epitope tag. Other suitable reporter proteins will be known to those skilled in the art.

In some embodiments, the CAR comprises a second-generation CAR.

Suitable CAR intracellular signalling domains may include any suitable signalling domain, including any region comprising an Immune-receptor-Tyrosine-based-Activation-Motif (ITAM), as reviewed for example by Love et al. Cold Spring Harbor Perspect. Biol 2010 2(6)l a002485. In some embodiments, the signalling domain comprises the intracellular domain of human CD3 [zeta] chain as described for example in US Patent No 7,446,190, or a variant thereof.

Various co-stimulatory domains are known to engineer CAR cells. The CAR may comprise one or more of these domains. Suitable co-stimulatory domains include, but are not necessarily limited to members of the B7/CD28 family such as B7-1, B7-2, B7-H1, B7-H2, B7-H3, B7-H4, B7-H6, B7-H7, BTLA, CD28, CTLA-4, Gi24, ICOS, PD-1, PD-L2 or PDCD6; or ILT/CD85 family proteins such as LILRA3, LILRA4, LILRB1, LILRB2, LILRB3 or LILRB4; or tumour necrosis factor (TNF) superfamily members such as 4-1BB, BAFF, BAFF R, CD27, CD30, CD40, DR3, GITR, HVEM, LIGHT, Lymphotoxin-alpha, OX40, RELT, TACI, TL1A, TNF-alpha or TNF RII; or members of the SLAM family such as 2B4, BLAME, CD2, CD2F-10, CD48, CD58, CD84, CD229, CRACC, NTB-A or SLAM; or members of the TIM family such as TIM-1, TIM-3 or TIM-4; or other co-stimulatory molecules such as CD7, CD96, CD160, CD200, CD300a, CRTAM, DAP12, Dectin-1, DPPIV, EphB6, Integrin alpha 4 beta 1, Integrin alpha 4 beta 7/LPAM-1, LAG-3 or TSLP R.

In some embodiments the immunotherapeutic molecule comprises a MHC Class II cell surface receptor. Preferably, the MHC Class II cell surface receptor comprises or consists of HLA-DR. Without wishing to be bound by theory, exogenous expression of HLA-DR by the human ILCregs may aid immunoregulatory activity.

In some embodiments the immunotherapeutic molecule comprises a cytokine. The cytokine may be an immunoregulatory cytokine, for example IL-10 or TGF-β. The expression of an immunoregulatory cytokine or receptor in the *in vitro* population of the human ILCregs may have particular use when the human ILCregs are used for the treatment of an autoimmune disease, for example inflammatory bowel disease (IBD) or multiple sclerosis, or an allergy. Further autoimmune diseases and specific allergies are described below.

In other embodiments, the cytokine is an inflammatory cytokine. Exemplary inflammatory cytokines include, but are not necessarily limited to IL-22, IL-17A, IL-5, IL-4, Amphiregulin, IFN-γ, IL-2, IL-1, IL-18, TNF-α and GM-CSF. Thus, in some embodiments the cytokine comprises one or more of IL-22, IL-17A, IL-5, IL-4, Amphiregulin, IFN-γ, IL-2, IL-1, IL-18, TNF-α and GM-CSF. In some embodiments the cytokine comprises one or more of IL-22, IL-17A, IL-5, IL-4, IFN-γ, TNF-α and GM-CSF. The expression of such an inflammatory cytokine may have particular use when the human ILCregs are used for the treatment of a cancer.

Various chemokine receptors are known in the art. Exemplary chemokine receptors include, but are not necessarily limited to CXC chemokine receptors, CC chemokine receptors, XCR1 and CX3CR1.

In some embodiments the chemokine receptor comprises a CC chemokine receptor. For example, the chemokine receptor may comprise one or more of CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10 and CCR11. In some embodiments, the chemokine receptor comprises or consists of CCR7 or CCR3. In some embodiments the chemokine receptor comprises or consists of CCR7.

Without wishing to be bound by theory, the present inventors believe that the expression of a chemokine receptor in the *in vitro* population of human ILCregs may assist with tissue-specific therapeutic targeting of the human ILCregs. This may further increase the therapeutic efficacy of the in vitro population of human ILCregs.

Preferably, a vector comprises the exogenous polynucleotide. The vector may be viral or non-viral. Various viral and non-viral vectors are known to those skilled in the art. Non-viral vectors include plasmids, episomal vectors, and human artificial chromosomes (see, e.g., Harrington et al., 1997, Nat Genet. 15:345). For example, non-viral vectors useful for expression of the exogenous polypeptide in mammalian (e.g., human) cells include pThioHis A, B and C, pcDNA3.1/His, pEBVHis A, B and C, (Invitrogen, San Diego, Calif.), MPS V vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include vectors based on retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent et al., supra; Smith, 1995, Annu. Rev. Microbiol. 49:807; and Rosenfeld et al., 1992, Cell 68: 143. In particular, retroviral, lentiviral, adenoviral or adeno-associated viral vectors are commonly used for expression in immune cells such as T-cells. Examples of such vectors include the SFG retroviral expression vector (see Riviere et al., 1995, Proc. Natl. Acad. Sci. (USA) 92:6733-6737).

In some embodiments, the vector is a retroviral or lentiviral vector. Optionally, the vector is an SFG retroviral vector. In some embodiments the vector is a lentiviral vector. Lentiviral vectors include self-inactivating lentiviral vectors (so-called SIN vectors).

The choice of vector depends on the intended host cells in which the vector is to be expressed. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, e.g., Queen, et al., 1986, Immunol. Rev. 89:49-68), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-

specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPS V promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, the EF1 alpha promoter, the phosphoglycerate kinase (PGK) promoter and promoter-enhancer combinations known in the art.

The vector may further comprise a polynucleotide encoding a reporter gene. Suitable reporter genes include, but are not necessarily limited to HNIS, hNET and HSVtK.

Cultures of transformed organisms can be expanded under non-inducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the in vitro population of human ILCregs. In addition to promoters, other regulatory elements may also be required or desired for efficient expression. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, e.g., Scharf et al., 1994, Results Probl. Cell Differ. 20:125; and Bittner et al., 1987, Meth. Enzymol., 153:516). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

The genetic engineering of immune cells such as human ILCregs can be carried out according to standard cloning and expression techniques, which are known in the art (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989). The vector may be introduced into the *in vitro* population of human ILCregs using such techniques. Introduction may comprise transfection or transduction into the in vitro population of human ILCregs.

The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

As used herein, the term polynucleotide refers to a polymer comprising two or more nucleotides. Preferably, the polynucleotide comprises at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides or at least 100 nucleotides. The nucleotides can be naturally occurring or artificial.

A nucleotide typically contains a nucleobase, a sugar and at least one linking group, such as a phosphate, 2′O-methyl, 2′ methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate group. The nucleobase is typically heterocyclic. Nucleobases include, but

are not limited to, purines and pyrimidines and more specifically adenine (A), guanine (G), thymine (T), uracil (U) and cytosine (C). The sugar is typically a pentose sugar. Nucleotide sugars include, but are not limited to, ribose and deoxyribose. The sugar and the nucleobase together form a nucleoside. Preferred nucleosides include, but are not limited to, adenosine, guanosine, 5-methyluridine, uridine, cytidine, deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine and deoxycytidine. The nucleosides may be adenosine, guanosine, uridine and cytidine.

The nucleotides are typically ribonucleotides or deoxyribonucleotides. The nucleotides may be deoxyribonucleotides. The nucleotides typically contain a monophosphate, diphosphate or triphosphate. Phosphates may be attached on the 5′ or 3′ side of a nucleotide.

Nucleotides include, but are not limited to, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), guanosine monophosphate (GMP), guanosine diphosphate (GDP), guanosine triphosphate (GTP), thymidine monophosphate (TMP), thymidine diphosphate (TDP), thymidine triphosphate (TTP), uridine monophosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP), cytidine monophosphate (CMP), cytidine diphosphate (CDP), cytidine triphosphate (CTP), 5-methylcytidine monophosphate, 5-methylcytidine diphosphate, 5-methylcytidine triphosphate, 5-hydroxymethylcytidine monophosphate, 5-hydroxymethylcytidine diphosphate, 5-hydroxymethylcytidine triphosphate, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), deoxyadenosine monophosphate (dAMP), deoxyadenosine diphosphate (dADP), deoxyadenosine triphosphate (dATP), deoxyguanosine monophosphate (dGMP), deoxyguanosine diphosphate (dGDP), deoxyguanosine triphosphate (dGTP), deoxythymidine monophosphate (dTMP), deoxythymidine diphosphate (dTDP), deoxythymidine triphosphate (dTTP), deoxyuridine monophosphate (dUMP), deoxyuridine diphosphate (dUDP), deoxyuridine triphosphate (dUTP), deoxycytidine monophosphate (dCMP), deoxycytidine diphosphate (dCDP) and deoxycytidine triphosphate (dCTP), 5-methyl-2′-deoxycytidine monophosphate, 5-methyl-2′-deoxycytidine diphosphate, 5-methyl-2′-deoxycytidine triphosphate, 5-hydroxymethyl-2′-deoxycytidine monophosphate, 5-hydroxymethyl-2′-deoxycytidine diphosphate and 5-hydroxymethyl-2′-deoxycytidine triphosphate. The nucleotides may be selected from AMP, UMP, GMP, CMP, dAMP, dTMP, dGMP or dCMP. In some embodiments, the nucleotides are selected from dAMP, dTMP, dGMP or dCMP.

The nucleotides may contain additional modifications. In particular, suitable modified nucleotides include, but are not limited to, 2′amino pyrimidines (such as 2′-amino cytidine and 2′-amino uridine), 2′-hyrdroxyl purines (such as , 2′-fluoro pyrimidines (such as 2′-fluorocytidine and 2′fluoro uridine), hydroxyl pyrimidines (such as 5′-α-P-borano uridine), 2′-O-methyl nucleotides (such as 2′-O-methyl adenosine, 2′-O-methyl guanosine, 2′-O-

methyl cytidine and 2′-O-methyl uridine), 4′-thio pyrimidines (such as 4′-thio uridine and 4′-thio cytidine) and nucleotides have modifications of the nucleobase (such as 5-pentynyl-2′-deoxy uridine, 5-(3-aminopropyl)-uridine and 1,6-diaminohexyl-N-5-carbamoylmethyl uridine).

5 One or more nucleotides in the polynucleotide may be modified, for instance with a label or a tag. The label may be any suitable label which allows the nucleotides to be detected. Suitable labels include, but are not limited to, fluorescent molecules, radioisotopes, e.g. ^{125}I, ^{35}S, enzymes, antibodies, antigens, other polynucleotides and ligands such as biotin.

The nucleotides in the exogenous polynucleotide may be attached to each other in any 10 manner. The nucleotides may be linked by phosphate, 2′O-methyl, 2′ methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate linkages. The nucleotides are typically attached by their sugar and phosphate groups. The nucleotides may be connected via their nucleobases as in pyrimidine dimers.

The exogenous polynucleotide may comprise a deoxyribonucleic acid (DNA) or a ribonucleic 15 acid (RNA). Preferably, the exogenous polynucleotide comprises DNA. The exogenous polynucleotide may be any synthetic nucleic acid known in the art, such as peptide nucleic acid (PNA), glycerol nucleic acid (GNA), threose nucleic acid (TNA), locked nucleic acid (LNA), morpholino nucleic acid or other synthetic polymers with nucleotide side chains.

Substitutions may be used for the practices of codon optimisation and codon wobble, both 20 of which are known to those skilled in the art. Thus, it will be appreciated that codon-optimised and codon-wobbled exogenous polynucleotide are also envisaged. In some embodiments, the exogenous polynucleotide is codon-optimised for human expression.

The exogenous polynucleotide can be produced by de novo solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence. Direct chemical synthesis of polynucleotides can 25 be accomplished by methods known in the art, such as the phosphotriester method of Narang et al., 1979, Meth. Enzymol. 68:90; the phosphodiester method of Brown et al., 1979, Meth. Enzymol. 68:109; the diethylphosphoramidite method of Beaucage et al., 1981, Tetra. Lett., 22:1859; and the solid support method of U.S. Pat. No. 4,458,066. Introducing mutations to a polynucleotide sequence by PCR can be performed as described 30 in, e.g., PCR Technology: Principles and Applications for DNA Amplification, H. A. Erlich (Ed.), Freeman Press, NY, N.Y., 1992; PCR Protocols: A Guide to Methods and Applications, Innis et al. (Ed.), Academic Press, San Diego, Calif, 1990; Mattila et al., 1991, Nucleic Acids Res. 19:967; and Eckert et al., 1991, PCR Methods and Applications 1:17.

The invention also provides a pharmaceutical composition comprising a human ILCreg of the 35 invention or an *in vitro* population of human ILCregs of the invention and a pharmaceutically or physiologically acceptable diluent and/or carrier.

The carrier and/or diluent is generally selected to be suitable for the intended mode of administration and can include agents for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, colour, isotonicity, odour, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Typically, these carriers and/or diluents include aqueous or alcoholic/aqueous solutions, emulsions, or suspensions, including saline and/or buffered media.

Suitable further agents for inclusion in the pharmaceutical compositions include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulphite, or sodium hydrogen-sulphite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as free serum albumin, gelatin, or immunoglobulins), colouring, flavouring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as Polysorbate 20 or Polysorbate 80; Triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides, such as sodium or potassium chloride, or mannitol sorbitol), delivery vehicles, excipients and/or pharmaceutical adjuvants.

The carrier and/or diluent may be a parenteral, optionally intravenous vehicle. Suitable parenteral vehicles include sodium chloride solution, Ringer’s dextrose, dextrose and sodium chloride and lactated Ringer’s. Suitable physiologically-acceptable thickeners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin and alginates may be included. Intravenous vehicles include fluid and nutrient replenishers and electrolyte replenishers, such as those based on Ringer’s dextrose. In some cases, one might include agents to adjust tonicity of the composition, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in a pharmaceutical composition. For example, in many cases it is desirable that the composition is substantially isotonic. Preservatives and other additives, such as antimicrobials, antioxidants, chelating agents, and inert gases, may also be present. The precise formulation will depend on the route of administration. Additional relevant

principle, methods and components for pharmaceutical formulations are well known (see, e.g., Allen, Loyd V. Ed, (2012) Remington's Pharmaceutical Sciences, 22nd Edition).

A pharmaceutical composition of the present invention can be administered by one or more routes of administration using one or more of a variety of methods known in the art. As will
5 be appreciated by the skilled person, the route and/or mode of administration will vary depending upon the desired results. Routes of administration for pharmaceutical compositions of the invention include intravenous, intramuscular, intradermal, intraperitoneal, intrapleural, subcutaneous, intratumoural, spinal, or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral
10 administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intratumoural, intrapleural and intra-sternal injection
15 and infusion. In some embodiments, the pharmaceutical composition is administered intratumourally. In other embodiments, administration is intrapleural or intraperitoneal. When parenteral administration is contemplated, the pharmaceutical compositions are usually in the form of a sterile, pyrogen-free, parenterally acceptable composition. A particularly suitable vehicle for parenteral injection is a sterile, isotonic solution, properly
20 preserved. The pharmaceutical composition can be in the form of a lyophilizate, such as a lyophilized cake.

Alternatively, the pharmaceutical composition of the invention can be administered by a non-parenteral route, such as a topical, epidermal, or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually, or topically.

25 In some embodiments, the pharmaceutical composition is for subcutaneous administration. Typically, the pharmaceutical compositions for subcutaneous administration contain suitable stabilizers (e.g., amino acids, such as methionine, and or saccharides such as sucrose), buffering agents and tonicifying agents. Alternatively, the pharmaceutical composition may be for intravenous administration.

30 The invention also provides a kit comprising the human ILCreg of the invention, the *in vitro* population and/or the pharmaceutical composition as defined above. The kit may further comprise instructions for use. In some embodiments, the human ILCreg, the *in vitro* population and/or the pharmaceutical composition is provided in an aqueous solution, optionally buffered solution and/or at a temperature of at least -20°C.

Also provided is a method of treating or preventing a disease in a subject, wherein the method comprises administering to the subject the human ILCreg, the *in vitro* population and/or the pharmaceutical composition of the invention.

The method typically comprises administering a therapeutically effective amount or a prophylactically effective amount of human ILCregs, the *in vitro* population and/or the pharmaceutical composition of the invention. A therapeutically effective amount is an amount which ameliorates one or more symptoms, such as all the symptoms, of the disease and/or abolishes one or more symptoms, such as all the symptoms, of the disease. The therapeutically effective amount preferably cures the disease. A prophylactically effective amount is an amount which prevents the onset of the disease and/or prevents the onset of one or more symptoms, such as all the symptoms, of the disease. The prophylactically effective amount preferably prevents the subject from developing the disease. Suitable amounts are discussed in more detail below.

The human ILCreg, the *in vitro* population and/or the pharmaceutical composition of the invention may be administered to a subject that displays symptoms of disease. The human ILCreg, the *in vitro* population and/or the pharmaceutical composition of the invention may be administered to a subject that is asymptomatic, i.e. does not display symptoms of disease. The human ILCreg, the *in vitro* population and/or the pharmaceutical composition of the invention may be administered when the subject's disease status is unknown or the patient is expected not to have a disease. The human ILCreg, the *in vitro* population and/or the pharmaceutical composition of the invention may be administered to a subject that is predisposed, such as genetically predisposed, to developing the disease.

The subject is typically human.

Various diseases are suitable for treatment or prophylaxis by administration of the human ILCreg, the *in vitro* population and/or the pharmaceutical composition of the invention. Any disease which can be treated or prevented using immunotherapy is envisaged. The ILCregs of the invention are capable of reducing inflammation or an immune response, especially in the lung, intestine or any of the other specific tissue discussed above. The ILCregs of the invention are capable of reducing inflammation or an immune response associated with any of the diseases described below.

For example, the disease may be cancer, an infection, an autoimmune disease or an allergy. In some embodiments the disease is cancer, an autoimmune disease or an allergy. In some embodiments the disease is a cancer or an autoimmune disease. In other embodiments the disease is an autoimmune disease or an allergy. Alternatively, the disease is an allergy or cancer. In some embodiments the disease is a cancer. In some embodiments the disease is an autoimmune disease. In other embodiments the disease is an allergy.

In some embodiments the disease is an inflammatory disease. Such diseases are discussed in more detail below. In some embodiments the disease comprises a chronic or acute inflammatory disease. The chronic or acute inflammatory disease may comprise a chronic or acute infection.

5 The autoimmune disease may include, but not necessarily be limited to inflammatory bowel disease, eczema, rheumatoid arthritis, psoriasis, multiple sclerosis (MS), myasthenia gravis, type 1 diabetes mellitus, systemic lupus erythematosus (SLE or Lupus), Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, Graves' disease, Hashimoto's thyroiditis and vasculitis.

10 In some embodiments, the autoimmune disease is selected from inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis (MS), type 1 diabetes mellitus and systemic lupus erythematosus (SLE or Lupus). Additional autoimmune diseases are discussed below. In some embodiments the autoimmune disease comprises inflammatory bowel disease. Exemplary inflammatory bowel diseases include Crohn's disease and 15 ulcerative colitis.

The cancer may include, but not necessarily be limited to, a solid tumour cancer, a soft tissue tumour, a metastatic lesion, and a haematological cancer. For example, the cancer can be liver cancer, lung cancer, breast cancer, prostate cancer, lymphoid cancer, colon cancer, renal cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or 20 neck, such as squamous cell carcinoma of the head and neck (SCCHN), cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the 25 oesophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukaemias including acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, solid tumours of childhood, lymphocytic lymphoma, cancer 30 of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumour angiogenesis, spinal axis tumour, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, myelodysplastic syndrome (MDS), chronic myelogenous leukaemia-chronic phase (CMLCP), diffuse large B-cell lymphoma (DLBCL), 35 cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL), hepatocellular carcinoma (HCC), gastrointestinal stromal tumours (GIST), non-small cell lung carcinoma (NSCLC), cutaneous melanoma, mucosal melanoma, cutaneous squamous cell carcinoma

(CSCC), small-cell lung cancer, squamous cell carcinoma of the lung, Merkle cell carcinoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. In some embodiments, the cancer is selected from the above group.

The cancer may be a solid tumour cancer.

5 In some embodiments, the cancer is selected from the group consisting of cancer of the head and/or neck, ovarian cancer, malignant mesothelioma, breast cancer, pancreatic cancer, colorectal cancer, lung cancer, gastric cancer, bladder cancer, prostate cancer, oesophageal cancer, endometrial cancer, hepatobiliary cancer, chronic or acute leukaemia including acute myeloid leukaemia, duodenal carcinoma, thyroid carcinoma, cancer of the 10 central nervous system or renal cell carcinoma.

In some embodiments, the cancer is selected from ovarian cancer, breast cancer, optionally triple-negative breast cancer, pancreatic cancer, chronic or acute leukaemia including acute myeloid leukaemia, malignant mesothelioma, and combinations of said cancers.

The subject may have been pre-treated with a chemotherapeutic agent. In some 15 embodiments, the disease is cancer or autoimmune disease and the subject has been pre-treated with a chemotherapeutic agent.

When the disease is cancer, the administration of the human ILCreg, the *in vitro* population and/or the pharmaceutical composition of the invention to the subject may result in a decrease in tumour size of about 10%, about 20%, about 30%, about 40%, about 50%, 20 about 60%, about 70%, about 80%, about 90%, or even about 100%, when compared to an untreated tumour.

The allergy may include, but not necessarily be limited to allergic rhinitis (which may otherwise be referred to as hayfever), dust mite allergy, animal allergy, food allergy, insect bite/sting allergy, medicinal allergy, latex allergy, mould allergy, allogeneic rejection and/or 25 graft versus host disease.

Common food allergies include, but are not necessarily limited to nut allergy, fruit allergy, shellfish allergy, cow's milk protein allergy, egg allergy and a lactose allergy. The nut allergy may be a peanut allergy. The fruit allergy may be a strawberry, rhubarb, pineapple, apple or pear allergy.

30 In some embodiments the allergy is selected from allergic rhinitis, food allergy, allogeneic rejection and graft versus host disease.

Also provided is a method of treating or preventing a disease in a subject, wherein the method comprises administering to the subject a human ILCreg of the invention, an *in vitro* population of human ILCregs of the invention and/or a pharmaceutical composition of the

invention comprising human ILCregs of the invention. The discussion above relating to the amounts of cells and subjects equally applies to this method. The disease is preferably an inflammatory disease, such as an autoimmune disease, an infection or cancer. The inflammatory disease may be chronic or acute as discussed above. The inflammatory disease may be present in the cells of any of the tissues discussed above, including skin, gastro-intestinal, lung, thymic, thyroid, reproductive, bladder, kidney, pancreas, or liver tissue. An inflammatory disease is a disease or infection which comprises the damage or destruction of healthy viable cells. Examples of inflammatory diseases include, but not limited to, an autoimmune disease, an allergy, asthma, coeliac disease, nephritis, hepatitis, reperfusion injury, graft versus host disease (GvHD), transplant rejection and an infection. By "infection", this will be understood to infection with a pathogen. In some embodiments, the inflammatory disease comprises an autoimmune disease, an infection, or cancer.

An autoimmune disease may comprise rheumatoid arthritis, psoriasis, system lupus erythematosus (lupus), inflammatory bowel disease, multiple sclerosis, diabetes, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, Graves' disease, Hashimoto's thyroiditis, Myasthenia gravis, Aplastic Anaemia (AA), Vasculitis or combinations thereof. The autoimmune disease preferably comprises inflammatory bowel disease. Exemplary inflammatory bowel diseases include Crohn's disease and ulcerative colitis.

The infection may be an infection with any pathogen. The pathogen may be a bacterium, an archaeon, a single cell eukaryote, such as an amoeba or a paramecium, a fungus, or a virus.

The bacterium may be Gram negative or Gram positive. The Gram-positive bacterium is preferably from the genus *Bacillus, Clostridium, Enterococcus, Mycobacterium, Staphylococcus* or *Streptococcus.* The Gram-positive bacterium may be from the genus *Pasteurella* or *Nocardia*.

The Gram negative bacterium is preferably from the genus *Aggregatibacter, Bacteroides, Bartonella, Brucella, Campylobacter, Chylamidia, Enterbacter, Francisella, Haemophilus, Heliobacter, Klebsiella, Legionella, Moraxella, Neisseria, Porphyromonas, Pseudomonas, Salmonella, Serratia, Stenotrophomonas, Vibrio* or *Yersinia*. The Gram negative bacterium may be from the genus *Escherichia* or *Pseudomonas*.

The bacterium may be from the genus *Borrelia, Chlamydophila, Listeria, Mycoplasma, Proteus* or *Treponema.* The bacterium is preferably *Aggregatibacter actinomycetemcomitans, Bacillus anthracis, Bacillus licheniformis, Bacteroides fragilis, Bartonella henselae, Bordetella pertussis, Borrelia burgdorferi, Brucella abortus, Campylobacter jejuni, Chlamydia trachomatis, Chlamydophila pneumoniae, Clostridium*

difficile, Clostridium perfringens, Enterobacter aerogenes, Enterococcus faecalis, Enterococcus faecium, Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Klebsiella oxytoca, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Porphyromonas gingivalis, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enter ica, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Stenotrophomonas maltophilia, Streptococcus mutans, Streptococcus pyogenes, Streptococcus salivarius, Streptococcus sanguinis, Treponema pallidum, Vibrio cholera, Vibrio parahaemolyticus or *Yersinia enter ocolitica*.

Other specific examples of bacteria include, but are not limited, to *Mycobacterium tuberculosis, Mycobacterium intracellilare, Mycobacterium kansaii, Mycobacterium gordonae, Streptococcus agalactiae, Streptococcus viridans group, Streptococcus faecalis, Streptococcus bovis, Streptococcus pneumoniae, Corynebacterium diptheriae, Erysipelothrix rhusiopathie, Clostridium tetani, Klebsiella pneumoniae, Pasteurella multocida, Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pertenue* and *Actinomyces israelii*.

The microbe is preferably a Mycobacterium species that are capable of causing tuberculosis. The microbe is preferably *Mycobacterium tuberculosis (M. tuberculosis), Mycobacterium africanum (M. africanum), Mycobacterium orygis (M. orygis*, which may otherwise be referred to as the oryx bacilli), *Mycobacterium bovis (M. bovis), Mycobacterium microti (M. microti), Mycobacterium canetti (M. canetti), Mycobacterium caprae (M. caprae), Mycobacterium pinnipedii (M. pinnipedii), Mycobacterium suricattae (M. suricattae)* or *Mycobacterium mungi (M. mungi)*

The fungus is preferably from the genus *Absidia, Acremonium, Aspergillus, Aureobasidium, Basidiobolus, Blastomyces, Blastoschizomyces, Candida, Cladosporium, Coccidioides, Cryptococcus, Cunninghamella, Curvularia, Debaryomyces, Exophiala, Exserohilum, Fonsecea, Fusarium, Geotrichum, Histoplasma, Issatchenkia, Kluyveromyces, Malezzesia, Mucor, Paracoccidioides, Paecilomyces, Penicillium, Pichia, Pneumocystis, Rhizomucor, Rhizopus, Rhodotorula, Saccharomyces, Scedosporium, Schizophyllum, Scopulariopsis, Sporothrix, Trichoderma, Trichophyton* or *Trichosporon*.

The fungus is preferably *Aspergillus fumigatus, Aspergillus flavus, Aspergillus lentulus, Aspergillus terreus, Aspergillus nidulans, Aspergillus oryzae, Aspergillus niger, Candida albicans, Candida caribbica (Candida fermentati), Candida dubliniensis, Candida famata (Debaryomyces hansenii), Candida fukuyamaensis (Candida xestobii or Candida carpophila), Candida guilliermondii, Candida kefyr (Kluyveromyces marxianus), Candida krusei (Issatchenkia orientalis), Candida metapsilosis, Candida orthopsilosis, Candida parapsilosis,*

Candida parapsilosis, Candida pelliculosa, Candida psychrophila, Candida rugosa, Candida smithsonii, Candida tropicalis, Candida utilis, Coccidioides immitis , Cryptococcus bacillisporus, Cryptococcus gattii, Cryptococcus grubii, Cryptococcus neoformans, Debaryomyces coudertii, Debaryomyces maramus, Debaryomyces nepalensis, Debaryomyces prosopidis, Debaryomyces robertsiae, Debaryomyces udenii, Histoplasma capsulatum, Kluyveromyces lactis, Pichia cecembensis, Rhodotorula araucariae, Rhodotorula babjevae, Rhodotorula dairensis, Rhodotorula diobovatum, Rhodotorula glutinis, Rhodotorula kratochvilovae, Rhodotorula paludigenum, Rhodotorula sphaerocarpum, Rhodotorula toruloides, Rhodotorula mucliaginosa, Saccharomyces 'sensu stricto', Saccharomyces bayanus, Saccharomyces boulardii, Saccharomyces cariocanus, Saccharomyces kudiavzevii, Saccharomyces mikatae, Saccharomyces paradioxus, Saccharomyces pastorianus, Saccharomyces uvarum, Saccharomyces cerevisiae or *Tsuchiyaea wingfieldii*.

The virus may belong to the family Retroviridae, such as human deficiency viruses, such as HIV-I (also referred to as HTLV- III), HIV-II, LAC, IDLV-III/LAV, HIV-III or other isolates such as HIV-LP, the family Picornaviridae, such as poliovirus, hepatitis A, enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses, the family Calciviridae, such as viruses that cause gastroenteritis, the family Togaviridae, such as equine encephalitis viruses and rubella viruses, the family Flaviviridae, such as dengue viruses, encephalitis viruses and yellow fever viruses, the family Coronaviridae, such as coronaviruses (e.g., SARS-CoV or SARS-CoV-2/COVID-19), the family Rhabdoviridae, such as vesicular stomata viruses and rabies viruses, the family Filoviridae, such as Ebola viruses, the family Paramyxoviridae, such as parainfluenza viruses, mumps viruses, measles virus and respiratory syncytial virus, the family Orthomyxoviridae, such as influenza viruses, the family Bungaviridae, such as Hataan viruses, bunga viruses, phleoboviruses and Nairo viruses, the family Arena viridae, such as hemorrhagic fever viruses, the family Reoviridae, such as reoviruses, orbiviruses and rotaviruses, the family Bimaviridae, the family Hepadnaviridae, such as hepatitis B virus, the family Parvoviridae, such as parvoviruses, the Papovaviridae, such as papilloma viruses and polyoma viruses, the family Adenoviridae, such as adenoviruses, the family Herpesviridae, such as herpes simplex virus (HSV) I and II, varicella zoster virus and pox viruses, or the family Iridoviridae, such as African swine fever virus). The virus may be an unclassified virus, such as the etiologic agents of Spongiform encephalopathies, the agent of delta hepatitis, the agents of non-A, non-B hepatitis (class 1 enterally transmitted; class 2 parenterally transmitted such as Hepatitis C); Norwalk and related viruses and astroviruses.

The cancer may be any of those discussed above.

Administration of the human ILCregs of the invention, including in the population or pharmaceutical composition of the invention, to the subject may reduce symptoms by at

least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or even about 100%, when compared to an untreated subject. Administration of the human ILCregs of the invention, including in the population or pharmaceutical composition of the invention, to the subject may reduce inflammation or an immune response associated with the disease by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or even about 100%, when compared to an untreated subject.

In embodiments administering human ILCreg or the *in vitro* population, the number of cells administered to the subject should take into account the route of administration, the disease being treated, the weight of the subject and/or the age of the subject. In general, from about 1×10^6 to about 1×10^{11} human ILCregs are administered to the subject. In some embodiments, from about 1×10^7 to about 1×10^{10} human ILCregs, or from about 1×10^8 to about 1×10^9 human ILCregs are administered to the subject.

The invention also provides a human ILCreg of the invention, an *in vitro* population of human ILCregs of the invention and/or a pharmaceutical composition of the invention comprising human ILCregs of the invention for use in any of the therapeutic methods described above. Thus, also provided is a human ILCreg of the invention, an *in vitro* population of human ILCregs of the invention and/or a pharmaceutical composition of the invention comprising human ILCregs of the invention for use in the treatment or prevention of a disease. This may otherwise be referred to for use in therapy. In particular, the invention provides a human ILCreg of the invention, an *in vitro* population of human ILCregs of the invention and/or a pharmaceutical composition of the invention comprising human ILCregs of the invention for use in the treatment or prevention of an inflammatory disease, such as such as an autoimmune disease, an infection or cancer.

Also provided is the use of a human ILCreg of the invention, an *in vitro* population of human ILCregs of the invention and/or a pharmaceutical composition of the invention comprising human ILCregs of the invention for the manufacture of a medicament for the treatment or prevention of a disease. Also provided is use of a human ILCreg of the invention, an *in vitro* population of human ILCregs of the invention and/or a pharmaceutical composition of the invention comprising human ILCregs of the invention for the treatment or prevention of a disease. In both instances, the disease is preferably an inflammatory disease, such as such as an autoimmune disease, an infection or cancer. Further provided is use of a human ILCreg of the invention, an *in vitro* population of human ILCregs of the invention and/or a pharmaceutical composition of the invention comprising human ILCregs of the invention for therapy.

Methods of producing the ILCregs

Human ILCregs of the invention can be produced by co-culturing human ILC precursors with at least one epithelial organoid, wherein the epithelial organoid comprises more epithelial cells than mesenchymal cells, to expand the human ILC precursors and differentiate the ILC precursors into the human ILCregs. The ILCregs of the invention can be expanded by co-culturing them with at least one epithelial organoid, wherein the epithelial organoid comprises more epithelial cells than mesenchymal cells. These methods are described in detail in International Application No. PCT/EP2023/066317 (which is incorporated herein by reference in its entirety).

The term "organoid" is a known term of the art, which refers to a plurality of cells which self-assemble *in vitro* to form a complex structure. The organoid is 3D and resembles an *in vitro* miniaturized version of an organ or section of an organ thereof. Generally, organoids are 3D. Further generally, co-culture is with at least one whole epithelial organoid. However, in some embodiments culture is with a portion of at least one epithelial organoid. A portion may comprise a layer of cells, which will comprise all cell types comprised in the full organoid. In such embodiments, it will be appreciated that the portion may be obtained from mechanically or chemically breaking up the organoid. In the context of the present invention, the term "epithelial organoid" refers to an organoid comprising epithelial cells.

Advantageously, organoids can remain viable and stable *in vitro* for extended periods of time. For example, organoids may remain viable and stable *in vitro* for at least two weeks, at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least three months, at least six months, at least nine months, at least 12 months, at least 18 months or at least 24 months. The long-term stability of the organoid, together with its complex structure, provides an *in vitro* culture environment which closely correlates to the *in vivo* environment. Such long-term stability also enables research of the organoid over an extended period of time which also more closely correlates to *in vivo* time periods.

The present inventors have found that the co-culture of at least one epithelial organoid comprising more epithelial cells than mesenchymal cells and human ILCregs leads to significant expansion of the human ILCregs. Prior to this finding, it was believed that mesenchymal cells were essential to ensure maintenance and expansion of the immune cells. Therefore, the significant expansion achieved by the present method is entirely unexpected. The inventors have also surprisingly found that the co-culture of at least one epithelial organoid comprising more epithelial cells than mesenchymal cells and ILC precursors differentiates the ILC precursors into substantial numbers of human ILCregs. The generation of such substantial numbers of immune cells may have particular utility in cell therapy, where it currently can be difficult to obtain sufficient numbers of mature immune cells for therapeutic purposes or even for *in vitro* study.

As the skilled person will appreciate, a mesenchymal cell is a stromal cell. In the context of the present invention, a mesenchymal cell is a cell having plastic adherent properties under normal culture conditions and has a fibroblast-like morphology. Cultured mesenchymal cells may be CD90 positive. Cultured mesenchymal cells may be CD90 and CD105 positive. Cultured mesenchymal cells may be CD90, CD105 and CD73 positive. Cultured mesenchymal cells may be CD73, CD90, CD105, CD44, CD106 and CD166 positive. Cultured mesenchymal cells may be CD11b, CD14, CD19, CD34, CD45, CD79a and HLA-DR negative and CD73, CD90 and CD105 positive. Cultured mesenchymal cells may be CD11b, CD14, CD19, CD34, CD45, CD79a and HLA-DR negative and CD73, CD90, CD105, CD44, CD106 and CD166 positive.

In the context of the present invention, if a cell is defined as positive for a particular marker, for example CD45, it will be appreciated that the cell comprises a detectable level of the marker. Conversely, if a cell is defined as negative for a particular marker, it will be appreciated that the cell comprises an undetectable level of the marker. Methods for measuring the presence of markers/proteins/mRNA are known in the art and discussed in more detail below. In the context of the present invention, the terms "amount" and "level" are interchangeable.

Mesenchymal cells may comprise multipotent mesenchymal cells. Multipotent mesenchymal cells are capable of differentiating into a plurality of different cell types.

Preferably, the mesenchymal cells comprise or consist of fibroblasts.

In the context of the present invention, the term "expansion" refers to the generation or production of human ILCregs. Thus, in the context of the present invention, the term "expansion" comprises proliferation of the human ILCregs and/or differentiation of the ILC precursors into differentiated human ILCregs. The term "expansion" does not relate to activation of a specific sub-clone, such as a specific T-cell clone.

In some embodiments, the epithelial organoid comprises less than about 45% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than about 44% mesenchymal cells, less than about 43% mesenchymal cells, less than about 42% mesenchymal cells, less than about 41% mesenchymal cells, less than about 40% mesenchymal cells, less than about 39% mesenchymal cells, less than about 38% mesenchymal cells, less than about 37% mesenchymal cells, less than about 36 % mesenchymal cells, less than about 35% mesenchymal cells, less than about 34% mesenchymal cells, less than about 33% mesenchymal cells, less than about 32% mesenchymal cells, less than about 31% mesenchymal cells, less than about 30% mesenchymal cells, less than about 29% mesenchymal cells, less than about 28% mesenchymal cells, less than about 27% mesenchymal cells, less than about 26%

mesenchymal cells, less than about 25% mesenchymal cells, less than about 24% mesenchymal cells, less than about 23% mesenchymal cells, less than about 22% mesenchymal cells, less than about 21% mesenchymal cells, less than about 20 mesenchymal cells, less than about 19% mesenchymal cells, less than about 18% mesenchymal cells, less than about 17% mesenchymal cells, less than about 16% mesenchymal cells, less than about 15% mesenchymal cells, less than about 14 mesenchymal cells, less than about 13% mesenchymal cells, less than about 12% mesenchymal cells, less than about 11% mesenchymal cells, less than about 10% mesenchymal cells, less than about 9% mesenchymal cells, less than about 8% mesenchymal cells, less than about 7% mesenchymal cells, less than about 6% mesenchymal cells, less than about 5% mesenchymal cells, less than about 4% mesenchymal cells, less than about 3% mesenchymal cells, less than about 2% mesenchymal cells or less than about 1% mesenchymal cells.

In some embodiments, the epithelial organoid comprises less than about 40% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than about 35% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 30% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 25% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 24% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 23% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 22% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 21% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 20% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 15% mesenchymal cells. In some embodiments, the epithelial organoid comprises less than 10% mesenchymal cells.

In some embodiments, the epithelial organoid comprises of from about 0.01% mesenchymal cells to about 45% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 0.01% mesenchymal cells to about 30% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 0.01% mesenchymal cells to about 25% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 0.01% mesenchymal cells to about 20% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 0.01% mesenchymal cells to about 15% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 0.01% mesenchymal cells to about 10% mesenchymal cells.

In some embodiments, the epithelial organoid comprises of from about 0.1% mesenchymal cells to about 45% mesenchymal cells. In some embodiments, the epithelial organoid

comprises of from about 0.1% mesenchymal cells to about 30% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 0.1% mesenchymal cells to about 25% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 0.1% mesenchymal cells to about 20% mesenchymal cells.

5 In some embodiments, the epithelial organoid comprises of from about 1% mesenchymal cells to about 45% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 1% mesenchymal cells to about 30% mesenchymal cells. In some embodiments, the epithelial organoid comprises of from about 1% mesenchymal cells to about 25% mesenchymal cells. In some embodiments, the epithelial organoid comprises of
10 from about 1% mesenchymal cells to about 20% mesenchymal cells.

The epithelial organoid may not comprise a detectable level of mesenchymal cells. Thus, in some embodiments, the epithelial organoid comprises an undetectable level of mesenchymal cells.

Various methods for detecting a percentage or level are known to those skilled in the art.
15 For example, the percentage or level of mesenchymal cells in the organoid may be detected by flow cytometry. Another suitable method of detection may comprise fluorescence microscopy, for example using a confocal microscope. Other suitable methods will be known to the skilled person. Detection of the percentage or level of mesenchymal cells may be prior to step (a). Alternatively, detection of the percentage or level of mesenchymal cells
20 may be during or after step (a).

Before step (a), the method may comprise a step of depleting mesenchymal cells from the epithelial organoid. Alternatively, the epithelial organoid of the invention may have undergone depletion of mesenchymal cells. By "depleting" mesenchymal cells, this will be understood to refer to removal of mesenchymal cells from the epithelial organoid. Thus,
25 "undergone depletion" will refer to an epithelial organoid which has already undergone removal of mesenchymal cells.

Before step (a), the method may comprise a step of depleting mesenchymal cells from the epithelial organoid immediately before step (a) and/or after full maturation of the epithelium.

30 An epithelial organoid which has undergone depletion of mesenchymal cells may comprise less than about 45% mesenchymal cells, less than about 44% mesenchymal cells, less than about 43% mesenchymal cells, less than about 42% mesenchymal cells, less than about 41% mesenchymal cells, less than about 40% mesenchymal cells, less than about 39% mesenchymal cells, less than about 38% mesenchymal cells, less than about 37%
35 mesenchymal cells, less than about 36 % mesenchymal cells, less than about 35% mesenchymal cells, less than about 34% mesenchymal cells, less than about 33%

mesenchymal cells, less than about 32% mesenchymal cells, less than about 31% mesenchymal cells, less than about 30% mesenchymal cells, less than about 29% mesenchymal cells, less than about 28% mesenchymal cells, less than about 27% mesenchymal cells, less than about 26% mesenchymal cells, less than about 25% mesenchymal cells, less than about 24% mesenchymal cells, less than about 23% mesenchymal cells, less than about 22% mesenchymal cells, less than about 21% mesenchymal cells, less than about 20 mesenchymal cells, less than about 19% mesenchymal cells, less than about 18% mesenchymal cells, less than about 17% mesenchymal cells, less than about 16% mesenchymal cells, less than about 15% mesenchymal cells, less than about 14 mesenchymal cells, less than about 13% mesenchymal cells, less than about 12% mesenchymal cells, less than about 11% mesenchymal cells, less than about 10% mesenchymal cells, less than about 9% mesenchymal cells, less than about 8% mesenchymal cells, less than about 7% mesenchymal cells, less than about 6% mesenchymal cells, less than about 5% mesenchymal cells, less than about 4% mesenchymal cells, less than about 3% mesenchymal cells, less than about 2% mesenchymal cells or less than about 1% mesenchymal cells, as defined above.

Depletion may comprise mechanical disruption of the epithelial organoid. Advantageously, mechanical disruption separates a mesenchymal fraction (if present) from the epithelial structure of the epithelial organoid. The mesenchymal fraction can then be removed, leaving the epithelial structure of the organoid. In some embodiments, depletion comprises digestion of the epithelial organoid, for example digestion using collagenase.

Depletion may be repeated two or more times. For example, depletion may be repeated three, four, five or six times.

In some embodiments, depletion comprises mechanical disruption of the epithelial organoid which is repeated three, four or five times. In other embodiments, depletion comprises i) mechanical disruption of the epithelial organoid and ii) digestion of the epithelial organoid using collagenase. Preferably, digestion of the epithelial organoid using collagenase is after mechanical disruption.

The epithelial organoid may be a primary organoid or derived from stem cells. The stem cells may comprise or consist of induced pluripotent stem cells (iPSCs) or adult stem cells. As the skilled person will appreciate, iPSCs are a pluripotent stem cell obtained by genetic reprogramming of adult somatic cells into an embryonic state. By primary organoid, this will be understood to refer to an organoid obtained from a subject biopsy sample. The subject biopsy sample may have been obtained during an endoscopy. The subject biopsy sample is preferably a human biopsy sample. The subject biopsy sample is more preferably a human small intestine biopsy sample or a human colon biopsy sample. The subject biopsy sample is

most preferably a human small intestine biopsy sample. The organoid is preferably a human small intestine biopsy derived organoid or a human colon biopsy derived organoid. The organoid is most preferably a human small intestine biopsy derived organoid. The effectiveness of such organoids is shown in Example 10.

5 The subject biopsy sample is preferably from a surgical resection. The subject biopsy sample is preferably from a human surgical resection. The subject biopsy sample is more preferably from a human small intestine surgical resection or a human colon surgical resection. The subject biopsy sample is more preferably from a human small intestine surgical resection. The organoid is preferably a human small intestine surgical resection 10 derived organoid or a human colon surgical resection derived organoid. The organoid is most preferably a human small intestine surgical resection derived organoid.

In embodiments comprising an organoid derived from iPSCs, the iPSCs may be obtained from the Human Induced Pluripotent Stem Cells Initiative (HipSci, https://www.hipsci.org).

Alternatively, in embodiments comprising an organoid derived from iPSCs, the method may 15 comprise a step before step (a) of producing the iPSCs.

Producing the iPSCs may comprise introducing a polynucleotide sequence encoding one or more of OCT3/4, SOX2, KLF4 and MYC into isolated primary cells. Preferably, the polynucleotide sequence encodes OCT3/4, SOX2, KLF4 and MYC. More preferably, the polynucleotide sequence encodes human OCT3/4, human SOX2, human KLF4 and human 20 MYC. Introduction may comprise transduction or transfection, typically transduction. In some embodiments a vector comprises the polynucleotide sequence. The vector may be a Sendai vector. After introduction, the cells may be cultured in an iPS cell medium for at least five, at least ten, at least 20, at least 30 or at least 40 days. Culture may be on a feeder layer. The iPS cell medium may comprise advanced DMEM.

25 The iPS cell medium may further comprise Knockout Serum Replacement (KSR), which is commercially available from Life Technologies. The iPS cell medium may comprise about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15% or about 20% KSR. In some embodiments the iPS cell medium comprises about 10% KSR.

30 In some embodiments, the iPS cell medium further comprises L-glutamine and/or Fibroblast Growth Factor-2. The iPS cell medium may comprise L-glutamine at a concentration of at least about 0.5 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM or about 5 mM. The iPS cell medium may comprise less than about 10 mM, less than about 7 mM, less than about 6 mM or less than about 5 mM L-glutamine. In some embodiments the iPS cell 35 medium comprises of from about 1 mM to about 3 mM L-glutamine. In some embodiments the iPS cell medium comprises about 2 mM L-glutamine.

The Fibroblast Growth Factor-2 may be Zebrafish Fibroblast Growth Factor-2. In some embodiments the Fibroblast Growth Factor-2 is recombinant. The iPS cell medium may comprise Fibroblast Growth Factor-2 at a concentration of at least about 1ng/ml, about 2ng/ml, about 3ng/ml, about 4ng/ml, about 5ng/ml or at least about 10ng/ml. In some embodiments the iPS cell medium comprises less than about 20ng/ml, less than about 10ng/ml or less than about 5ng/ml Fibroblast Growth Factor-2. In some embodiments the iPS cell medium comprises about 4ng/ml Fibroblast Growth Factor-2.

The iPS cell medium may further comprise an antibiotic, for example pen/strep. The iPS cell medium may comprise about 0.1%, about 0.2%, about 0.5%, about 1%, about 2%, about 3%, about 4% or about 5% pen/strep. In some embodiments the iPS cell medium comprises about 1% pen/strep.

The iPS cell medium may further comprise 2-mercaptoethanol. The iPS cell medium may comprise about 0.001%, about 0.002%, about 0.005%, about 0.007%, about 0.01%, about 0.02%, about 0.03%, about 0.04% or about 0.05% 2-mercaptoethanol. In some embodiments the iPS cell medium comprises about 0.007% 2-mercaptoethanol.

Methods for obtaining primary epithelial organoids from subject biopsy samples are well known in the art. In embodiments comprising primary epithelial organoids, the method may comprise a step before step (a) of obtaining the primary epithelial organoid from a subject biopsy sample. In embodiments comprising primary epithelial organoids, the method may comprise a step before step (a) of obtaining the primary epithelial organoid from a human biopsy sample. Obtaining the primary epithelial organoid from a subject biopsy sample may comprise culturing a subject biopsy sample (or portions of the subject biopsy sample thereof) suspension in a matrix and a basal medium. In the context of the present invention, matrix will be understood to refer to a cell culture matrix. Various cell culture matrices are commercially available. Suitable matrices may include, but not necessarily be limited to Geltrex, Cultrex, Matrigel, Collagen I hydrogels, IV hydrogels and other synthetic hydrogels derived from crosslinking of functionalised polypeptides and/or polymers like Polyethylene Glycol (PEG) as described, for example, in Jowett *et al.* 2021. In some embodiments the matrix comprises or consists of Matrigel.

Alternatively, obtaining the primary epithelial organoid from a subject biopsy sample may comprise culturing a subject biopsy sample (or portions of the subject biopsy sample thereof) suspension in a hanging drop suspension. By "hanging drop suspension", this will be understood to refer to suspension in a basal medium from a surface. Various hanging drop suspension culture modules are available to those skilled in the art.

In some embodiments, obtaining the primary epithelial organoid from a subject biopsy sample comprises culturing a subject biopsy sample (or portions of the subject biopsy sample thereof) suspension in Matrigel and a basal medium.

The basal medium may comprise Advanced DMEM/F12. Other suitable basal mediums will be known to those skilled in the art.

The basal medium may further comprise L-glutamine, antibiotic, N2 supplement, B27 supplement, HEPES and/or N-acetylcysteine.

The basal medium may comprise L-glutamine at a concentration of at least about 0.5 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM or about 5 mM. The basal medium may comprise less than about 10 mM, less than about 7 mM, less than about 6 mM or less than about 5 mM L-glutamine. In some embodiments the basal medium comprises of from about 1 mM to about 3 mM L-glutamine. In some embodiments the basal medium comprises about 2 mM L-glutamine.

The basal medium may comprise HEPES at a concentration of at least about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 12 mM, about 15 mM or at least about 20 mM. The basal medium may comprise less than about 50 mM, about 40 mM, about 30 mM, about 20 mM or about 10 mM HEPES. In some embodiments the basal medium comprises HEPES at a concentration of from about 5 mM to about 15 mM. In some embodiment the basal medium comprises about 10 mM HEPES.

The basal medium may comprise N-acetylcysteine at a concentration of at least about 0.5 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM or about 5 mM. The basal medium may comprise less than about 10 mM, less than about 7 mM, less than about 6 mM or less than about 5 mM N-acetylcysteine. In some embodiments the basal medium comprises of from about 0.5 mM to about 3 mM N-acetylcysteine. In some embodiments the basal medium comprises about 1 mM N-acetylcysteine.

Culturing the subject biopsy sample (or portions of the subject biopsy sample thereof) suspension may be for a period of at least 120 hours, at least 168 hours, at least two weeks, at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks or at least eight weeks. In some embodiments, culturing a subject biopsy sample (or portions of the subject biopsy sample thereof) suspension is for a period of from 120 hours to eight weeks.

Preferably, obtaining the primary epithelial organoid from a subject biopsy sample comprises culturing a subject biopsy sample (or portions of the subject biopsy sample thereof) suspension in a matrix and a basal medium for a period of at least 120 hours, at

least 168 hours, at least two weeks, at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks or at least eight weeks. In some embodiments, obtaining the primary epithelial organoid from a subject biopsy sample comprises culturing a subject biopsy sample (or portions of the subject biopsy sample thereof) suspension in a matrix and a basal medium for a period of from 120 hours to eight weeks.

Preferably, the basal medium comprises Noggin. In some embodiments, the basal medium comprises R-Spondin1. In some embodiments, the basal medium comprises EGF. In some embodiments the basal medium comprises Noggin, R-Spondin1 and EGF. The basal medium may further comprise one or more of FGF10, CHIR and RhoK-inhibitor.

More preferably, the epithelial organoid is derived from iPSCs or adult stem cells. Most preferably, the epithelial organoid is derived from iPSCs. Advantageously, the derivation of an organoid from a stem cell allows the *in vitro* differentiation of the stem cells into a plurality of different cell types. This plurality of different cell types can self-assemble *in vitro* into the complex 3D structure of the organoid.

Methods for generating epithelial organoids from iPSCs or adult stem cells are known in the art. Thus, in some embodiments, before step (a) the method comprises an initial step of generating the organoid *in vitro* from iPSCs or adult stem cells. Preferably, before step (a) the method comprises an initial step of generating the organoid *in vitro* from iPSCs. Generating the organoid *in vitro* from iPSCs may comprise culture of the iPSCs in an organoid differentiation medium. In some embodiments, the method comprises:

i) culturing the iPSCs in a plurality of different organoid differentiation media for a total time period of at least about 48 hours to form organoid colonies;

ii) selecting organoid colonies; and optionally

iii) culturing the organoid colonies.

In some embodiments, the iPSCs are human iPSCs.

The organoid differentiation media may comprise RPMI medium or E8 medium supplemented with the appropriate supplements for differentiation.

For example, for the generation of an intestinal organoid, suitable mediums may comprise endoderm differentiation and mid/hindgut differentiation media. An exemplary endoderm differentiation medium may comprise RPMI medium, B27 and Activin A. An exemplary mid/hindgut differentiation medium may comprise RPMI medium, B27, FGF4 and CHIR.

The endoderm or mid/hindgut differentiation medium may comprise at least about 0.1% B27, at least about 0.2% B27, at least about 0.3% B27, at least about 0.4% B27, at least about 0.5% B27, at least about 0.6% B27, at least about 0.7% B27, at least about 0.8% B27, at least about 0.9% B27, at least about 1% B27, at least about 1.5% B27, at least about 2% B27 or at least about 3% B27. In some embodiments the endoderm differentiation medium comprises less than about 10% B27, less than about 5% B27 or less than about 3% B27. In some embodiments the endoderm differentiation medium comprises about 0.2% B27. In other embodiments the endoderm differentiation medium comprises about 1% B27. In some embodiments the mid/hindgut differentiation medium comprises about 2% B27.

The endoderm differentiation medium may comprise at least about 0.5μl/ml Activin A, at least about 1μl/ml Activin A, at least about 2μl/ml Activin A, at least about 3μl/ml Activin A or at least about 4μl/ml Activin A. In some embodiments the endoderm differentiation medium comprises less than about 5μl/ml Activin A. In some embodiments the endoderm differentiation medium comprises about 1 μl/ml Activin A.

The mid/hindgut differentiation medium may comprise at least about 0.5μl/ml Fibroblast Growth Factor 4 (FGF4), at least about 1μl/ml FGF4, at least about 2μl/ml FGF4, at least about 3μl/ml FGF4 or at least about 4μl/ml FGF4. In some embodiments the mid/hindgut differentiation medium comprises less than about 5μl/ml FGF4. In some embodiments the mid/hindgut differentiation medium comprises about 1 μl/ml FGF4.

An exemplary organoid generation method for the generation of a human intestinal organoid, is as follows:

1. Culture the human iPSCs in E8 medium comprising B27 and Activin A, for about 24 hours;

2. Replace the E8 medium with endoderm differentiation medium and culture the human iPSCs in the endoderm differentiation medium for at least about 72 hours;

3. Replace the endoderm differentiation medium with mid/hindgut differentiation medium and culture the human iPSCs in the mid/hindgut differentiation medium for at least about 72 hours.

Alternatively, the method comprises an initial step of generating the organoid *in vitro* from adult stem cells, preferably murine adult stem cells. Generating the organoid *in vitro* from adult stem cells may comprise culture of the adult stem cells in an organoid differentiation medium for a period of at least about 4 hours. In some embodiments, the method comprises:

i) culturing the adult stem cells in a organoid differentiation medium for at least about 4 hours to form organoid colonies;

ii) selecting organoid colonies; and optionally

iii) culturing the organoid colonies.

The organoid differentiation media may comprise RPMI medium or E8 medium supplemented with the appropriate supplements for differentiation.

For the generation of an intestinal organoid from adult murine stem cells, the organoid differentiation medium may comprise a basal medium as defined above. For example, the organoid differentiation medium may comprise Advanced DMEM/F12, L-glutamine, antibiotic, N2 supplement, B27 supplement, R-spondin, EGF, Noggin, HEPES and/or N-acetylcysteine. The basal medium may further comprise one or more of FGF10, CHIR and RhoK-inhibitor.

Other suitable organoid generation methods will be known and available to those skilled in the art.

The at least one epithelial organoid may be a plurality of epithelial organoids. For example, the at least one epithelial organoid may be at least two epithelial organoids, at least three epithelial organoids, at least four epithelial organoids, at least five epithelial organoids, at least six epithelial organoids, at least seven epithelial organoids, at least eight epithelial organoids, at least nine epithelial organoids, at least ten epithelial organoids, at least 20 epithelial organoids, at least 30 epithelial organoids, at least 40 epithelial organoids, at least 50 epithelial organoids, at least 60 epithelial organoids, at least 70 epithelial organoids, at least 80 epithelial organoids, at least 90 epithelial organoids, at least 100 epithelial organoids, at least 150 epithelial organoids, at least 200 epithelial organoids or at least 500 epithelial organoids.

In some embodiments, the at least one epithelial organoid is less than 150 epithelial organoids, less than 100 epithelial organoids, less than 90 epithelial organoids, less than 80 epithelial organoids, less than 70 epithelial organoids, less than 60 epithelial organoids, less than 50 epithelial organoids, less than 40 epithelial organoids or less than 30 epithelial organoids.

In some embodiments, the at least one epithelial organoid is of from two organoids to 100 organoids. In some embodiments, the at least one epithelial organoid is of from 25 to 100 epithelial organoids, preferably, of from 25 to 50 epithelial organoids.

It will be appreciated that the numbers of organoids described above may be in relation to one co-culture, for example one co-culture well of a tissue culture plate. Thus, the total

numbers of organoids may be much greater when multiple co-culture wells are used and/or when the method is a high throughput method. For example, the total number of organoids may be at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 500, at least 1000, at least 10000, at least 50,000, at least 100,000, at least 200,000, at least 300,000, at least 400,000, at least 500,000, at least 1000000 or at least 5000000.

In some embodiments, the total number of organoids is less than 100000000 organoids. In some embodiments the total number of organoids is of from 10 to 100000000 organoids. The total number of organoids may be of from 20 to 500,000 organoids. In some embodiments, the total number of organoids is of from 20 to 10000 organoids, optionally of from 20 to 1000 organoids. In some embodiments, the total number of organoids is of from 20 to 500 organoids. The total number of organoids may be of from 20 to 250 organoids.

Preferably, the epithelial organoid is a human or murine epithelial organoid. More preferably, the epithelial organoid is a human epithelial organoid. In the context of the present invention, the term "murine" will be understood to encompass rat and mouse. Thus, in some embodiments, the epithelial organoid is a murine epithelial organoid, preferably a mouse epithelial organoid.

The epithelial organoid may be a primary human epithelial organoid or derived from human iPSCs or human adult stem cells. Alternatively, the epithelial organoid may be a primary mouse epithelial organoid or derived from mouse iPSCs, mouse embryonic stem cells or mouse adult stem cells. When the epithelial organoid is a primary mouse epithelial organoid, the mouse from which the organoid is isolated may be germ-free or specific pathogen free. The present inventors have found that organoids from such germ-free or specific pathogen free mice are suitable for the present invention. This is surprising given the known role of microorganisms in organ differentiation and maintenance, particularly intestinal differentiation and maintenance.

In embodiments comprising a plurality of epithelial organoids, the plurality of epithelial organoids preferably comprise or consist of epithelial organoids from the same species. For example, the plurality of epithelial organoids may comprise or consist of a plurality of epithelial human organoids. Alternatively, the plurality of epithelial organoids may comprise or consist of a plurality of epithelial murine organoids, for example a plurality of epithelial mouse organoids. Alternatively, the plurality of epithelial organoids may consist of epithelial organoids from at least two different species, for example human and mouse. Optionally, the plurality of epithelial organoids comprise or consist of a plurality of murine and human epithelial organoids.

Since the epithelial organoid is an *in vitro* miniaturized version of an organ or section of an organ thereof, it will be appreciated that any epithelial organoid can be used in the present invention. In particular, the epithelial organoid can be an *in vitro* miniaturized version of any organ (or section of an organ thereof) which comprises epithelial cells. For example, the epithelial organoid may be a skin, gastro-intestinal, lung, thymic, thyroid, reproductive, bladder, kidney, pancreas or liver epithelial organoid. In some embodiments, the epithelial organoid is a skin, gastro-intestinal, lung, thyroid, reproductive, bladder, kidney, pancreas, or liver epithelial organoid. In some embodiments, the epithelial organoid is a lung, reproductive or gastro-intestinal epithelial organoid. In some embodiments the epithelial organoid is a lung or gastro-intestinal epithelial organoid.

Reproductive epithelial organoids may comprise fallopian tube epithelial organoids, ovary epithelial organoids, prostate epithelial organoids, endometrium epithelial organoids, cervix epithelial organoids, vaginal epithelial organoids and testes epithelial organoids (which may otherwise be referred to as gonadal epithelial organoids). Cervix epithelial organoids may comprise endocervical canal epithelial organoids and/or ectocervix epithelial organoids. Reproductive epithelial organoids may be selected from fallopian tube epithelial organoids, ovary epithelial organoids, prostate epithelial organoids and endometrium epithelial organoids.

In the context of the present invention, the term "gastro-intestinal" will be understood to refer to oral mucosal organs, stomach, intestine and anus. The term "oral mucosal" will be understood to refer to mucosal organs of the oral tract, such as the salivary gland, pharynx, taste buds, lingual region, gingival epithelial and oesophagus. Gastro-intestinal epithelial organoids may thus comprise stomach epithelial organoids, salivary gland epithelial organoids, taste bud epithelial organoids, lingual region epithelial organoids, ginigival, epithelial organoids, oesophagus epithelial organoids, pharynx epithelial organoids, intestinal epithelial organoids and anal epithelial organoids. In some embodiments the epithelial organoid is an oral mucosal epithelial organoid or an intestinal epithelial organoid. In some embodiments, the epithelial organoid is an oral mucosal epithelial organoid. The oral mucosal epithelial organoid may be selected from a stomach epithelial organoid and oesophageal epithelial organoid.

In some embodiments the epithelial organoid is an intestinal epithelial organoid. It will be appreciated that intestinal organoids comprise small intestinal organoids, large intestinal organoids and rectum organoids. In some embodiments, the intestinal epithelial organoids are small intestinal epithelial organoids. In other embodiments, the intestinal epithelial organoids are large intestinal epithelial organoids. Large intestinal epithelial organoids may comprise or consist of colon epithelial organoids. In some embodiments, the intestinal epithelial organoids are rectum organoids.

Preferably, the epithelial organoid is an intestinal epithelial organoid or lung epithelial organoid. In some embodiments the epithelial organoid is an intestinal epithelial organoid. In some embodiments the epithelial organoid is a small intestinal epithelial organoid or a lung epithelial organoid. Alternatively, the epithelial organoid may be a lung epithelial organoid.

The epithelial organoid may be an epithelial cancer organoid. By epithelial cancer organoid, this will be understood to refer to an organoid obtained from a cancerous tumour biopsy sample. For example, the epithelial cancer organoid may be an epithelial skin cancer, intestinal cancer, lung cancer, thymic cancer, thyroid cancer, reproductive cancer, bladder cancer, kidney cancer, pancreas cancer, oral mucosal cancer or liver cancer organoid. In some embodiments, the epithelial cancer organoid is an epithelial skin cancer, intestinal cancer, lung cancer, thyroid cancer, reproductive cancer, bladder cancer, kidney cancer, pancreas cancer, oral mucosal cancer or liver cancer organoid. The epithelial cancer organoid may be an epithelial head and neck cancer organoid. It will be appreciated that an epithelial head and neck cancer organoid originates from a head and neck cancerous tumour biopsy sample.

In some embodiments, the epithelial organoid is a primary skin, intestinal, lung, thymic, thyroid, reproductive, bladder, kidney, pancreas, oral mucosal or liver epithelial organoid. In some embodiments, the epithelial organoid is a primary skin, intestinal, lung, thyroid, reproductive, bladder, kidney, pancreas, oral mucosal or liver epithelial organoid. In some embodiments, the epithelial organoid is a primary intestinal, lung, reproductive or oral mucosal epithelial organoid. Primary epithelial organoids, as defined above, are organoids comprising epithelial cells obtained from a subject biopsy sample. A primary epithelial intestinal organoid may otherwise be referred to as an enteroid. A primary colon epithelial organoid may otherwise be referred to as a colonoid.

The present inventors have surprisingly found that human ILCregs obtained by the method of the present invention may be imprinted with a genetic signature and phenotype specific of *in vivo* human ILCregs in the primary organ. This advantageously allows the generation of tissue-specific human ILCregs which may have utility for the treatment of particular diseases and/or may have improved homing capacity to the specific tissue when administered to a subject. When the epithelial organoid is an intestinal organoid, the human ILCregs obtained may thus have an intestinal-specific genetic signature and phenotype. When the epithelial organoid is a lung organoid, the human ILCregs obtained may have a lung-specific genetic signature and phenotype. The tissue-specific genetic signatures and phenotypes are discussed in more detail herein.

Preferably, the human ILCregs or human ILC precursors and at least one epithelial organoid are co-cultured in a medium which supports the culture of both the human ILCregs or

human ILC precursors and the epithelial organoid(s). By "supports the culture" this will be understood to enable growth, maintenance and differentiation, if necessary. The skilled person can design such a medium. The medium preferably comprises a basal medium comprising one or more of R-Spondin, Noggin, EGF, 2-mercaptoethanol, IL-2, and IL-7.
5 Suitable basal mediums include, but are not necessarily limited to Advanced DMEM/F12 and Essential 8™ medium. Further suitable basal media are known to those skilled in the art. In some embodiments the medium comprises a basal medium comprising R-Spondin, Noggin, EGF, IL-2, and IL-7. In some embodiments the medium comprises a basal medium comprising R-Spondin, Noggin, EGF, 2-mercaptoethanol, IL-2, and IL-7. In some
10 embodiments the medium comprises a basal medium comprising IL-2 and IL-7. In some embodiments the medium comprises a basal medium comprising IL-2, IL-7 and 2-mercaptoethanol. In some embodiments the medium comprises Advanced DMEM/F12 medium comprising R-Spondin, Noggin, EGF, IL-2, and IL-7. In other embodiments the medium comprises Essential 8™ medium comprising R-Spondin, Noggin, EGF, IL-2, and IL-
15 7. In some embodiments the basal medium further comprises IL-23. In some embodiments the basal medium further comprises IL-15. In some embodiments the basal medium further comprises IL-22. In some embodiments the basal medium further comprises TGF-β.

In some embodiments the basal medium comprises IL-2, IL-7, IL-15 and 2-mercaptoethanol. In some embodiments the basal medium comprises R-Spondin, Noggin,
20 EGF, IL-2, IL-7, IL-15 and β-mercaptoethanol. In some embodiments the basal medium comprises Advanced DMEM/F12 medium comprising R-Spondin, Noggin, EGF, IL-2, IL-7, IL-15 and β-mercaptoethanol.

The basal medium may comprise EGF at a concentration of at least about 1ng/ml, at least about 10ng/ml, at least about 20ng/ml, at least about 25ng/ml, at least about 30ng/ml, at
25 least about 35ng/ml, at least about 40ng/ml, at least about 45ng/ml, at least about 50ng/ml, at least about 55ng/ml, at least about 60ng/ml, at least about 65ng/ml, at least about 70ng/ml, at least about 75ng/ml, at least about 80 ng/ml, at least about 85ng/ml, at least about 90ng/ml, at least about 95ng/ml or at least about 100ng/ml. In some embodiments the basal medium comprises EGF at a concentration of at least about
30 10ng/ml. In some embodiments the basal medium comprises EGF at a concentration of at least about 25ng/ml, optionally at least about 50ng/ml.

The basal medium may comprise EGF at a concentration of less than about 1000ng/ml, less than about 900ng/ml, less than about 800ng/ml, less than about 700ng/ml, less than about 600ng/ml, less than about 500ng/ml, less than about 400ng/ml, less than about 300ng/ml,
35 less than about 200ng/ml, less than about 100ng/ml or less than about 50ng/ml. In some embodiments the basal medium comprises EGF at a concentration of less than about 50 ng/ml.

In some embodiments the basal medium comprises EGF at a concentration of from about 1ng/ml to about 1000ng/ml. The basal medium may comprise EGF at a concentration of from about 10ng/ml to about 500ng/ml, optionally at a concentration of from about 10ng/ml to about 100ng/ml. In some embodiments the basal medium comprises EGF at a concentration of about 50ng/ml.

The EGF may be recombinant. In some embodiments, the EGF is murine or human.

The basal medium may comprise R-spondin at a concentration of at least about 100ng/ml, at least about 200ng/ml, at least about 300ng/ml, at least about 400ng/ml, at least about 500ng/ml, at least about 600ng/ml, at least about 700ng/ml, at least about 800ng/ml, at least about 900ng/ml, at least about 1µg/ml, at least about 2µg/ml, at least about 3µg/ml, at least about 4µg/ml, at least about 5µg/ml, at least about 6µg/ml, at least about 7µg/ml, at least about 8µg/ml, at least about 9µg/ml, at least about 10µg/ml, at least about 15µg/ml or at least about 20µg/ml. In some embodiments the basal medium comprises R-spondin at a concentration of at least about 500ng/ml. In some embodiments the basal medium comprises R-spondin at a concentration of at least about 700ng/ml, optionally at least about 900ng/ml.

The basal medium may comprise R-spondin at a concentration of less than about 25µg/ml, less than about 24µg/ml, less than about 23µg/ml, less than about 22µg/ml or less than about 21µg/ml. In some embodiments the basal medium comprises R-spondin at a concentration of less than about 10µg/ml.

In some embodiments the basal medium comprises R-spondin at a concentration of from about 100ng/ml to about 20µg/ml. The basal medium may comprise R-spondin at a concentration of from about 200ng/ml to about 10µg/ml, optionally at a concentration of from about 500ng/ml to about 2µg/ml. In some embodiments the basal medium comprises R-spondin at a concentration of about 1µg/ml.

The R-spondin may be recombinant. In some embodiments, the R-spondin is murine or human. In some embodiments, the R-spondin is an R-spondin-comprising supernatant. The supernatant may have been isolated from an R-spondin-producing cell line, of which various cell lines are available.

The basal medium may comprise Noggin at a concentration of at least about 1ng/ml, at least about 2 ng/ml, at least about 3ng/ml, at least about 4ng/ml, at least about 5ng/ml, at least about 6ng/ml, at least about 7ng/ml, at least about 8ng/ml, at least about 9ng/ml, at least about 10ng/ml, at least about 11ng/ml, at least about 12ng/ml, at least about 13ng/ml, at least about 14ng/ml, at least about 15ng/ml, at least about 16ng/ml, at least about 17ng/ml, at least about 18ng/ml, at least about 19ng/ml, at least about 20ng/ml, at least about 21ng/ml, at least about 22ng/ml, at least about 23ng/ml, at least about

24ng/ml, at least about 25ng/ml, at least about 26ng/ml, at least about 27ng/ml, at least about 28ng/ml, at least about 29ng/ml, at least about 30ng/ml, at least about 31ng/ml, at least about 32ng/ml, at least about 33ng/ml, at least about 34ng/ml, at least about 35ng/ml, at least about 36ng/ml, at least about 37ng/ml, at least about 38ng/ml, at least about 39ng/ml, at least about 40ng/ml, at least about 41ng/ml, at least about 42ng/ml, at least about 43ng/ml, at least about 44ng/ml, at least about 45ng/ml, at least about 46ng/ml, at least about 47ng/ml, at least about 48ng/ml, at least about 49ng/ml or at least about 50ng/ml. In some embodiments the basal medium comprises Noggin at a concentration of at least about 20ng/ml. In some embodiments the basal medium comprises Noggin at a concentration of at least about 50ng/ml, optionally at least about 70ng/ml.

The basal medium may comprise Noggin at a concentration of less than about 1000ng/ml, less than about 900ng/ml, less than about 800ng/ml, less than about 700ng/ml, less than about 600ng/ml, less than about 500ng/ml, less than about 400ng/ml, less than about 300ng/ml, less than about 200ng/ml, less than about 100ng/ml or less than about 50ng/ml. In some embodiments the basal medium comprises Noggin at a concentration of less than about 200ng/ml.

In some embodiments the basal medium comprises Noggin at a concentration of from about 1ng/ml to about 1000ng/ml. The basal medium may comprise Noggin at a concentration of from about 10ng/ml to about 500ng/ml, optionally at a concentration of from about 10ng/ml to about 200ng/ml. In some embodiments the basal medium comprises Noggin at a concentration of from about 50ng/ml to about 200ng/ml. In some embodiments the basal medium comprises Noggin at a concentration of about 100ng/ml.

The Noggin may be recombinant. In some embodiments, the Noggin is murine or human. In some embodiments, the Noggin is a Noggin-comprising supernatant. The supernatant may have been isolated from a Noggin-producing cell line, of which various cell lines are available.

The basal medium may comprise IL-2 at a concentration of at least about 1ng/ml, at least about 2 ng/ml, at least about 3ng/ml, at least about 4ng/ml, at least about 5ng/ml, at least about 6ng/ml, at least about 7ng/ml, at least about 8ng/ml, at least about 9ng/ml, at least about 10ng/ml, at least about 11ng/ml, at least about 12ng/ml, at least about 13ng/ml, at least about 14ng/ml, at least about 15ng/ml, at least about 16ng/ml, at least about 17ng/ml, at least about 18ng/ml, at least about 19ng/ml, at least about 20ng/ml, at least about 21ng/ml, at least about 22ng/ml, at least about 23ng/ml, at least about 24ng/ml, at least about 25ng/ml, at least about 26ng/ml, at least about 27ng/ml, at least about 28ng/ml, at least about 29ng/ml, at least about 30ng/ml, at least about 31ng/ml, at least about 32ng/ml, at least about 33ng/ml, at least about 34ng/ml, at least about 35ng/ml, at

least about 36ng/ml, at least about 37ng/ml, at least about 38ng/ml, at least about 39ng/ml, at least about 40ng/ml, at least about 41ng/ml, at least about 42ng/ml, at least about 43ng/ml, at least about 44ng/ml, at least about 45ng/ml, at least about 46ng/ml, at least about 47ng/ml, at least about 48ng/ml, at least about 49ng/ml or at least about 50ng/ml. In some embodiments the basal medium comprises IL-2 at a concentration of at least about 10ng/ml. In some embodiments the basal medium comprises IL-2 at a concentration of at least about 15ng/ml, optionally at least about 20ng/ml.

The basal medium may comprise IL-2 at a concentration of less than about 1000ng/ml, less than about 900ng/ml, less than about 800ng/ml, less than about 700ng/ml, less than about 600ng/ml, less than about 500ng/ml, less than about 400ng/ml, less than about 300ng/ml, less than about 200ng/ml, less than about 100ng/ml or less than about 50ng/ml. In some embodiments the basal medium comprises IL-2 at a concentration of less than about 50 ng/ml.

In some embodiments the basal medium comprises IL-2 at a concentration of from about 1ng/ml to about 1000ng/ml. The basal medium may comprise IL-2 at a concentration of from about 10ng/ml to about 500ng/ml, optionally at a concentration of from about 10ng/ml to about 100ng/ml. In some embodiments the basal medium comprises IL-2 at a concentration of from about 10ng/ml to about 50ng/ml. In some embodiments the basal medium comprises IL-2 at a concentration of about 20ng/ml.

Preferably, the IL-2 is recombinant. In some embodiments, the IL-2 is murine or human. More preferably, the IL-2 is recombinant human IL-2.

The basal medium may comprise IL-7 at a concentration of at least about 1ng/ml, at least about 2 ng/ml, at least about 3ng/ml, at least about 4ng/ml, at least about 5ng/ml, at least about 6ng/ml, at least about 7ng/ml, at least about 8ng/ml, at least about 9ng/ml, at least about 10ng/ml, at least about 11ng/ml, at least about 12ng/ml, at least about 13ng/ml, at least about 14ng/ml, at least about 15ng/ml, at least about 16ng/ml, at least about 17ng/ml, at least about 18ng/ml, at least about 19ng/ml, at least about 20ng/ml, at least about 21ng/ml, at least about 22ng/ml, at least about 23ng/ml, at least about 24ng/ml, at least about 25ng/ml, at least about 26ng/ml, at least about 27ng/ml, at least about 28ng/ml, at least about 29ng/ml, at least about 30ng/ml, at least about 31ng/ml, at least about 32ng/ml, at least about 33ng/ml, at least about 34ng/ml, at least about 35ng/ml, at least about 36ng/ml, at least about 37ng/ml, at least about 38ng/ml, at least about 39ng/ml, at least about 40ng/ml, at least about 41ng/ml, at least about 42ng/ml, at least about 43ng/ml, at least about 44ng/ml, at least about 45ng/ml, at least about 46ng/ml, at least about 47ng/ml, at least about 48ng/ml, at least about 49ng/ml or at least about 50ng/ml. In some embodiments the basal medium comprises IL-7 at a concentration of at

least about 10ng/ml. In some embodiments the basal medium comprises IL-7 at a concentration of at least about 15ng/ml, optionally at least about 20ng/ml.

The basal medium may comprise IL-7 at a concentration of less than about 1000ng/ml, less than about 900ng/ml, less than about 800ng/ml, less than about 700ng/ml, less than about 600ng/ml, less than about 500ng/ml, less than about 400ng/ml, less than about 300ng/ml, less than about 200ng/ml, less than about 100ng/ml or less than about 50ng/ml. In some embodiments the basal medium comprises IL-7 at a concentration of less than about 50 ng/ml.

In some embodiments the basal medium comprises IL-7 at a concentration of from about 1ng/ml to about 1000ng/ml. The basal medium may comprise IL-7 at a concentration of from about 10ng/ml to about 500ng/ml, optionally at a concentration of from about 10ng/ml to about 100ng/ml. In some embodiments the basal medium comprises IL-7 at a concentration of from about 10ng/ml to about 50ng/ml. In some embodiments the basal medium comprises IL-7 at a concentration of about 20ng/ml.

Preferably, the IL-7 is recombinant. In some embodiments, the IL-7 is murine or human. More preferably, the IL-7 is recombinant murine IL-7.

Preferably, the basal medium comprises R-Spondin at a concentration of about 1µg/ml, Noggin at a concentration of about 100ng/ml, EGF at a concentration of about 50ng/ml, IL-2 at a concentration of about 20ng/ml and IL-7 at a concentration of about 20ng/ml.

The R-spondin may be recombinant. In some embodiments, the R-spondin is murine or human. In some embodiments, the R-spondin is a R-spondin-comprising supernatant. The supernatant may have been isolated from a R-spondin-producing cell line, of which various cell lines are available.

In some embodiments the basal medium comprises IL-15 at a concentration of at least about 0.1ng/ml, at least about 0.2 ng/ml, at least about 0.3ng/ml, at least about 0.4ng/ml, at least about 0.5ng/ml, at least about 0.6ng/ml, at least about 0.7ng/ml, at least about 0.8ng/ml, at least about 0.9ng/ml, at least about 1ng/ml, at least about 1.1ng/ml, at least about 1.2ng/ml, at least about 1.3ng/ml, at least about 1.4ng/ml, at least about 1.5ng/ml, at least about 1.6ng/ml, at least about 1.7ng/ml, at least about 1.8ng/ml, at least about 1.9ng/ml, at least about 2ng/ml, at least about 3ng/ml, at least about 4ng/ml, at least about 5ng/ml, at least about 6ng/ml, at least about 7ng/ml, at least about 8ng/ml, at least about 9ng/ml or at least about 10ng/ml. The basal medium may comprise IL-15 at a concentration of less than about 20ng/ml, less than about 15ng/ml, less than about 12ng/ml, less than about 10ng/ml or less than about 5ng/ml. In some embodiments the basal medium comprises IL-15 at a concentration of from about 0.1ng/ml to about 5ng/ml. In some embodiments the basal medium comprises IL-15 at a concentration of from about

0.5ng/ml to about 2ng/ml. In some embodiments the basal medium comprises IL-15 at a concentration of about 1ng/ml. The IL-15 may be recombinant.

In some embodiments the basal medium comprises 2-mercaptoethanol. The basal medium may comprise at least 1mM, at least 2mM, at least 3mM, at least 4mM, at least 5mM, at least 6mM, at least 7mM, at least 8mM, at least 9mM, at least 10mM, at least 11mM, at least 12mM, at least 13mM, at least 14mM, at least 15mM, at least 16mM, at least 17mM, at least 18mM, at least 19mM, at least 20mM, at least 21mM, at least 22mM, at least 23mM, at least 24mM, at least 25mM, at least 26mM, at least 27mM, at least 28mM, at least 29mM, at least 30mM, at least 31mM, at least 32mM, at least 33mM, at least 34mM, at least 35mM, at least 36mM, at least 37mM, at least 38mM, at least 39mM, at least 40mM, at least 41mM, at least 42mM, at least 43mM, at least 44mM, at least 45mM, at least 46mM, at least 47mM, at least 48mM, at least 49mM, at least 50mM, at least 51mM, at least 52mM, at least 53mM, at least 54mM, at least 55mM, at least 56mM, at least 57mM, at least 58mM, at least 59mM, at least 60mM, at least 70mM, at least 80mM, at least 90mM or at least 100mM 2-mercaptoethanol.

In some embodiments the basal medium comprises no more than 500mM, no more than 400mM, no more than 300mM, no more than 200mM, no more than 100mM, no more than 90mM, no more than 80mM, no more than 70mM or no more than 60mM 2-mercaptoethanol.

In some embodiments the basal medium comprises of from 1mM to 500mM 2-mercaptoethanol. In some embodiments the basal medium comprises of from 10mM to 100mM 2-mercaptoethanol. Optionally the basal medium comprises about 50mM 2-mercaptoethanol.

Preferably, the basal medium comprises R-Spondin at a concentration of about 1μg/ml, Noggin at a concentration of about 100ng/ml, EGF at a concentration of about 50ng/ml, IL-2 at a concentration of about 20ng/ml, IL-7 at a concentration of about 20ng/ml, IL-15 at a concentration of about 1ng/ml and 20 μm 2-mercaptoethanol.

In some embodiments the medium/basal medium does not comprise a detectable level of one or more of IL-23, IL-1ß, IL-15, IL-12, IL-18, IL-25, and IL-33. In the context of the present invention, the medium/basal medium not comprising a detectable level of a particular marker refers to the marker not being exogenously added or included in the medium. Thus, the medium/basal medium not comprising a detectable level of the marker is in relation to the medium at the start of the method, and does not exclude detectable levels of the marker produced by the immune cells and/or epithelial organoid into the medium during the method. In some embodiments the medium/basal medium does not comprise a detectable level of IL-15, IL-12 and IL-18. In some embodiments the

medium/basal medium does not comprise a detectable level of IL-1ß, IL-15, IL-12, IL-18 and IL-25. In some embodiments the medium/basal medium does not comprise a detectable level of IL-1ß, IL-15, IL-12, IL-18, IL-25 and IL-33. In some embodiments the medium/basal medium does not comprise a detectable level of IL-23, IL-1ß, IL-15, IL-12, IL-18 and IL-25. In some embodiments the medium/basal medium does not comprise a detectable level of IL-23, IL-1ß, IL-15, IL-12, IL-18, IL-25, and IL-33.

Preferably, the medium is germ-free or specific pathogen free. Most preferably, the medium is medium A as disclosed in the Examples.

Preferably, the human ILCregs or human ILC precursors and the at least one epithelial organoid are co-cultured in a matrix. The matrix may comprise or consist of Geltrex, Cultrex or Matrigel. Other commercially available matrices, particularly synthetic hydrogels, are known to the skilled person. In some embodiments the matrix comprises or consists of Matrigel. More preferably, the human ILCregs or human ILC precursors and the at least one epithelial organoid are co-cultured in Matrigel in a medium as defined above, preferably a basal medium.

In some embodiments, the human ILCregs or human ILC precursors and the at least one epithelial organoid are co-cultured in a transwell. Typically, in such embodiments, a permeable insert separates cell populations. Thus, in such embodiments, the human ILCregs or human ILC precursors may be separated from the at least one epithelial organoid, preferably by a permeable insert.

In some embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 99% of the medium is replaced of from about every 24 hours to about every 72 hours.

In some embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 99% of the medium is replaced about every 24 hours. In some embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 99% of the medium is replaced about every 48 hours. In some embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 99% of the medium is replaced about every 72 hours.

Preferably, of from about 10% to about 70% of the medium is replaced of from about every 24 hours to about every 72 hours. More preferably, about 50% of the medium is replaced of from about every 24 hours to about every 72 hours. This allows conditioned medium to remain with the cells while supplementing with fresh growth factors.

Preferably, the human ILCregs or human ILC precursors and the at least one epithelial organoid are co-cultured at a temperature of at least about 20°C, at least about 25°C, at least about 30°C or at least about 35°C. More preferably, the human ILCregs or human ILC precursors and the at least one epithelial organoid are co-cultured at a temperature of about 37°C.

In some embodiments the human ILCregs or human ILC precursors and at least one epithelial organoid are co-cultured in at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10% or at least about 20% CO_2. Preferably, the human ILCregs or human ILC precursors and at least one epithelial organoid are co-cultured in of from about 1% to about 10% CO_2. More preferably, the human ILCregs or human ILC precursors and at least one epithelial organoid are co-cultured in about 5% CO_2.

In some embodiments, the epithelial organoid comprises a metabolite, for example a bacterial metabolite such as succinate or butyrate. For example, the epithelial organoid may be injected with a metabolite, preferably succinate.

Preferably, the human ILCregs or human ILC precursors are primary human ILCregs or human ILC precursors. More preferably, the human ILCregs or human ILC precursors are primary human cells. By "primary cell" this will be understood to refer to a cell that has been obtained from or is derived from a subject. Primary cells are not immortalised cells from a cell line.

The primary human ILCregs or human ILC precursors may be autologous. Alternatively, the primary human ILCregs or human ILC precursors may be allogeneic. In some embodiments, the primary human ILCregs or human ILC precursors comprise a mixture of allogeneic and autologous human ILCregs or human ILC precursors.

As the skilled person will appreciate, autologous cells are cells from the same subject, i.e. cells which have been obtained from a subject which will be administered back to the same subject. Allogeneic cells are cells obtained from a different subject to the subject to which the cells will be administered. The different subjects are typically from the same species. Allogenic cells are thus genetically different to the subject to which they are administered.

Alternatively, the human ILCregs or human ILC precursors may comprise or consist of immortalised human ILCregs or human ILC precursors from a cell line.

In some embodiments the human ILCregs or human ILC precursors are primary human ILCregs or human ILC precursors and the epithelial organoid is a primary epithelial organoid. In some embodiments, the human ILCregs or human ILC precursors are primary human ILCregs or human ILC precursors and the epithelial organoid is derived from iPSCs

or adult stem cells. In some embodiments, the human ILCregs or human ILC precursors are primary human human ILCregs or human ILC precursors and the epithelial organoid is a primary human epithelial organoid. In some embodiments, the human ILCregs or human ILC precursors are primary human human ILCregs or human ILC precursors and the epithelial organoid is derived from human iPSCs or human adult stem cells.

In some embodiments the human ILCregs or human ILC precursors are primary human human ILCregs or human ILC precursors and the epithelial organoid is a primary mouse epithelial organoid. Alternatively, the human ILCregs or human ILC precursors may be primary human human ILCregs or human ILC precursors and the epithelial organoid may be derived from mouse iPSCs or mouse adult stem cells.

As the skilled person will appreciate, ILCs are innate counterparts of T cells and are capable of expressing cytokines at a detectable level. ILCs are typically found at mucosal barriers *in vivo* and can be identified by the skilled person.

In the context of the present invention, ILCs can be categorised into three groups: Group 1, Group 2 and Group 3 ILCs. Group 1 ILCs may comprise NK cells and ILC1 cells. The NK cells are preferably cytotoxic NK cells. Group 2 ILCs may comprise ILC2 cells, while group 3 may comprise ILC3 and Lymphoid tissue inducer (LTi) cells. Group 2 and Group 3 ILCs typically express CD127. Human regulatory ILCs (ILCregs) are a subset of ILCs identified by the inventors and these are defined in more detail above.

Each subset of ILCs can be identified using various methods. Each subset of ILCs may be identified using flow cytometry and/or RNA sequencing to determine the expression profile.

Human group 1 ILCs may comprise a Lin$^-$, RORγt$^-$, CD127$^{+/-}$, CD56$^{+/-}$, CD161$^{+/-}$ expression profile.

Human group 1 ILCs preferably express detectable levels of one or more, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28, of the following genes: AOAH, CCL3, CCL4, CCL5, CD244, CD247, CST7, EOMES, FCGR3A, FGR, GNLY, GZMB, GZMK, IFNG, IKZF3, IL12RB2, ITGAX, ITGB2, KLRC1, KLRD1, NCAM1, NCR1, NKG7, PRF1, SAMD3, TBX21, TIGIT and ZNF683. Human group 1 ILCs preferably express detectable levels of all these genes. Gene expression is typically measured by measuring messenger RNA (mRNA) expression, for instance using RNA sequencing or single cell RNAseq (scRNAseq) as shown in Example 8. As explained above, human group 1 ILCs preferably comprise human NK cells and/or human ILC1 cells.

Human group 2 ILCs may comprise a Lineage$^-$, RORγt$^-$, CD127$^+$, GATA-3$^+$, CRTH2$^+$, c-Kit$^{+/-}$ expression profile. Alternatively, human group 2 ILCs may comprise a Lineage$^-$, GATA-3$^+$, CRTH2$^+$, c-Kit$^{+/-}$, ST2$^+$ expression profile.

Human group 2 ILCs preferably express detectable levels of one or more, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, of the following genes: ANXA1, BCL11B, CCR2, GATA3, HPGD, HPGDS, IL10RA, IL13, IL17RB, IL32, IL5, IL9R, KLRG1, LGALS1, MAF, MBOAT2, PPARG, PTGDR2, PTGER2 and TNFSF10. Human group 2 ILCs preferably express detectable levels of all of these genes. Gene expression is typically measured by measuring messenger RNA (mRNA) expression, for instance using RNA sequencing or single cell RNAseq (scRNAseq) as shown in Example 8.Murine group 3 ILCs may comprise a Lineage$^-$, CD127$^+$, RORγt$^+$, NK1.1$^{+/-}$, NKp46$^{+/-}$, CCR6$^{+/}$, CD4$^{+/-}$ expression profile.

Human group 3 ILCs may comprise a Lineage$^-$, CD127$^+$, RORγt$^+$, NKp44$^{+/-}$, c-Kit$^{+/-}$, CCR6$^{+/-}$ HLA-DR$^{+/-}$ expression profile.

Human group 3 ILCs preferably express detectable levels of one or more, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, of the following genes: AHR, BCL2A1, CCL20, CD81, CSF2, CXCL8, ID2, IKZF2, IL1R1, IL23R, IL4I1, IRF4, KIT, LIF, LTA4H, NCR1, PECAM1, RBPJ, RORC, TNFRSF25, TNFSF4 and TOX2. Human group 3 ILCs preferably express detectable levels of all of these genes. Gene expression is typically measured by measuring messenger RNA (mRNA) expression, for instance using RNA sequencing or single cell RNAseq (scRNAseq) as shown in Example 8.

"Lineage$^-$", as used herein in relation to human ILCs will be understood to refer to a CD3$^-$ CD20$^-$ CD14$^-$ CD19$^-$ expression profile.

ILC1 cells may comprise the expression profile RORγt$^-$ Klrg1$^-$ NK1.1$^{+/-}$ NKp46$^+$ Eomes$^-$ T-bet$^+$. Human ILC1 cells may express detectable levels of one or more of the genes discussed above.

NK cells may comprise the expression profile T-bet$^+$ Eomes$^+$. Alternatively, NK cells may comprise the expression profile T-bet$^-$ Eomes$^+$. The NK cells are preferably cytotoxic NK cells. Human NK cells preferably express detectable levels of one or more of the genes discussed above.

The inventors have unexpectedly found that numerous different human ILCs can be expanded in the method of the present invention. In other words, numerous different human ILCs can unexpectedly be expanded under the same conditions. Thus, in some embodiments, the immune cells comprise a plurality of different human ILCs. In some embodiments, the immune cells comprise a plurality of human Group 1 ILCs and human ILCregs. In some embodiments, the immune cells comprise a plurality of human ILCregs and human Group 2 ILCs. In some embodiments, the immune cells comprise a plurality of human ILCregs and human Group 3 ILCs. In some embodiments, the immune cells

comprise a plurality of human Group 1 ILCs, human Group 2 ILCs and human ILCregs. In some embodiments, the immune cells comprise a plurality of human Group 1 ILCs, human Group 3 ILCs and human ILCregs. In some embodiments, the immune cells comprise a plurality of human Group 2 ILCs, human Group 3 ILCs and human ILCregs. In some embodiments, the immune cells comprise a plurality of human Group 1 ILCs, human Group 2 ILCs, human Group 3 ILCs and human ILCregs.

Without wishing to be bound by theory, the present inventors believe that the ability to generate a plurality of different cell types more accurately represents the *in vivo* environment and so enables the generation of immune cells more suitable for cell therapy. In some embodiments, the method generates a heterogenous population of human ILCs. The heterogenous population of human ILCs may comprise or consist of human ILCregs, but have varying expression profiles. For example, the heterogenous population of ILCs may comprise a heterogenous population of ILCs. The inventors believe that such a heterogenous population may have increased utility for in vitro research since it more accurately represents an *in vivo* population. Without wishing to be bound by theory, the inventors also believe that such a heterogenous population may have improved viability and function given its more accurate representation of an *in vivo* population. This may have particular utility in therapeutic applications.

Alternatively, in other embodiments the method generates a homologous population of human ILCregs.

In some embodiments, one or more of the different immune cell subsets in the plurality are enriched over time. In embodiments comprising a plurality of different human ILC groups, the human ILCregs may be enriched over time.

In some embodiments, tissue-specific human ILCregs may be enriched over time. Tissue-specific human ILCregs are described herein. For example, when the epithelial organoid is an intestinal organoid, intestinal-specific human ILCregs may be enriched over time. When the epithelial organoid is a lung organoid, lung-specific human ILCregs may be enriched over time.

As the skilled person will appreciate, human ILC precursors are progenitor cells of the human ILCregs. Thus, expansion and differentiation of the ILC precursors leads to the generation of the human ILCregs. In some embodiments, the method comprises a precursor step of expanding an ILC precursor to generate a plurality of the ILC precursors. Thus, in some embodiments the method is for the production and expansion of human ILCregs and wherein the method comprises before step (a):

culturing an human ILC precursor to generate human ILC precursors; and

co-culturing the human ILC precursors and the epithelial organoid to expand the human ILC precursors and differentiate them into the human ILCregs.

In the context of the present invention, human ILC precursors may comprise a Lineage$^-$, CD127$^+$ expression profile. In some embodiments, human ILC precursors comprise a Lineage$^-$, CD127$^+$, CD7$^{+/-}$, c-Kit$^+$, CRTh2$^-$, KLRG1$^-$, CD56$^-$, NKp46$^-$ expression profile. In some embodiments, human ILC precursors comprise a Lineage$^-$, CD127$^+$, c-Kit$^+$ expression profile. Human ILC precursors may comprise a Lineage$^-$, CD34$^+$ expression profile. Alternatively, human ILC precursors may comprise a Lineage$^-$, CD127$^+$, CD45RA$^+$, CD62L$^-$ expression profile. Various methods can be used to determine the expression profile, for example flow cytometry, fluorescence microscopy, RT-PCR and RNA-sequencing.

"Lineage$^-$", as used herein in relation to human ILCs precursors will be understood to refer to a CD3$^-$, CD4$^-$, CD19$^-$, CD20$^-$, TCR$\alpha\beta^-$, TCR$\gamma\delta^-$ expression profile.

Preferably, the human ILC precursors are primary human ILC precursors. More preferably, the human ILC precursors are primary human human ILC precursors. By "primary cell" this will be understood to refer to a cell that has been obtained from a subject. Primary cells are not immortalised cells from a cell line.

The primary human ILC precursors may be autologous. Alternatively, the primary human ILC precursors may be allogeneic. In some embodiments, the primary human ILC precursors comprise a mixture of allogeneic and autologous human ILC precursors.

Alternatively, the human ILC precursors may comprise or consist of immortalised human ILC precursors from a cell line.

In some embodiments the human ILC precursors are primary human ILC precursors and the epithelial organoid is a primary epithelial organoid. In some embodiments, the human ILC precursors are primary human ILC precursors and the epithelial organoid is derived from stem cells, preferably iPSCs or adult stem cells. In some embodiments, the human ILC precursors are primary human ILC precursors and the epithelial organoid is a primary human epithelial organoid. In some embodiments, the human ILC precursors are primary human ILC precursors and the epithelial organoid is derived from human iPSCs or human adult stem cells.

In some embodiments the ILC precursors are primary mouse ILC precursors and the epithelial organoid is a primary human epithelial organoid. In some embodiments the ILC precursors are primary mouse ILC precursors and the epithelial organoid is derived from human iPSCs or human adult stem cells.

In some embodiments the human ILC precursors are primary human ILC precursors and the epithelial organoid is a primary mouse epithelial organoid. Alternatively, the human ILC precursors may be primary human ILC precursors and the epithelial organoid may be derived from mouse embryonic stem cells (ESC), mouse adult stem cells or mouse iPSCs,
5 preferably derived from mouse ESCs or mouse adult stem cells.

In the context of the present invention, it will be appreciated that primary I human LCs or primary human ILC precursors are isolated primary human ILCs or primary human ILC precursors, given the *in vitro* nature of the method of the present invention. The primary human ILCs or primary human ILC precursors may have been isolated from blood, bone
10 marrow, foetal liver, tonsils or intestine. In some embodiments, the primary human ILCs or human ILC precursors are isolated blood or bone marrow primary human ILCs or primary human ILC precursors. In some embodiments the primary human ILCs or human ILC precursors are isolated blood primary human ILCs or primary human ILC precursors.

Advantageously, the present method can be maintained for prolonged periods of time,
15 which enables the ongoing and reliable production of a large number of human ILCregs. In some embodiments, the immune cells and the epithelial organoid are co-cultured for at least about 72 hours. In some embodiments, the human ILCregs or human ILC precursors and the epithelial organoid are co-cultured for at least about 96 hours, at least about 120 hours, at least about 144 hours, at least about 168 hours, at least about 192 hours, at least
20 about 216 hours, at least about 240 hours, at least about 264 hours, at least about 288 hours, at least about 312 hours, at least about 336 hours, at least about 504 hours, at least about 672 hours or at least about 1008 hours.

In some embodiments, the human ILCregs or human ILC precursors and the epithelial organoid are co-cultured for no more than about 1440 hours, about 1008 hours, about 720
25 hours, about 672 hours or about 504 hours.

In some embodiments, the human ILCregs or human ILC precursors and the epithelial organoid are co-cultured for of from about 96 hours to about 504 hours. Preferably, the human ILCregs or human ILC precursors and the epithelial organoid are co-cultured for of from about 120 hours to about 504 hours. More preferably, the human ILCregs or human
30 ILC precursors and the epithelial organoid are co-cultured for of from about 168 hours to 336 hours.

During the method of the present invention, the human ILCregs or human ILC precursors preferably expand at a rate of at least 2-fold every about 24 hours. In some embodiments, the human ILCregs or human ILC precursors expand at a rate of at least 3-fold every about
35 24 hours.

The human ILCregs or human ILC precursors may expand at a rate of at least 4-fold every about 48 hours, optionally at a rate of at least about 6 fold every about 48 hours.

Optionally, the human ILCregs or human ILC precursors expand at a rate of at least 256-fold every about 168 hours.

5 In some embodiments, the human ILCregs or human ILC precursors expand at a rate of at least 200-fold, at least 250-fold, at least 300-fold, at least 350-fold, at least 400-fold, at least 450-fold, at least 500-fold, at least 550-fold, at least 600-fold, at least 650-fold, at least 700-fold, at least 750-fold, at least 800-fold, at least 850-fold or at least 900-fold about every 336 hours of co-culture.

10 In some embodiments, the human ILCregs or human ILC precursors expand at a rate of no more than 5000-fold, no more than 2000-fold, no more than 1000-fold, or no more than 950-fold about every 336 hours of co-culture. For example, the human ILCregs or human ILC precursors may expand at a rate of from 400-fold to 900-fold about every 336 hours of co-culture. In some embodiments, the human ILCregs or human ILC precursors expand at a 15 rate of from 500-fold to 1000-fold about every 336 hours of co-culture. The human ILCregs or human ILC precursors may expand at a rate of from 500-fold to 800-fold about every 336 hours of co-culture. In some embodiments, the human ILCregs or human ILC precursors expand at a rate of about 750-fold about every 336 hours of co-culture.

20 Throughout the description and claims of this specification, the words “comprise” and “contain” and variations of the words, for example “comprising” and “comprises”, mean “including but not limited to”, and do not exclude other components, integers or steps. Moreover the singular encompasses the plural unless the context otherwise requires: in particular, where the indefinite article is used, the specification is to be understood as 25 contemplating plurality as well as singularity, unless the context requires otherwise.

Preferred features of each aspect of the invention may be as described in connection with any of the other aspects. Within the scope of this application it is expressly intended that the various aspects, embodiments, examples and alternatives set out in the preceding paragraphs, in the claims and/or in the following description and drawings, and in particular 30 the individual features thereof, may be taken independently or in any combination. That is, all embodiments and/or features of any embodiment can be combined in any way and/or combination, unless such features are incompatible.

BRIEF DESCRIPTION OF THE DRAWINGS

One or more embodiments of the invention will now be described, by way of example only, 35 with reference to the accompanying drawings, in which:

Figure 1: HIO promote proliferation and maturation of systemic human ILCP. a) Gating strategy for healthy PBMC-derived ILCP, pregated on live, single, CD45^{+} cells, with apprropriate FMOs overlayed in blue and magenta. b) Schematic of HIO ILCP co-cultures, indicating presence of mesenchymal cells and CD45^{+} ILCP c) Representative flow cytometry plot overlays of EpCAM^{+} intestinal epithelial cells (E, green), double negative mesenchymal cells (M, magenta), and CD45^{+} ILC (blue) after 14 day co-cultures, highlighting Matrigel debris in grey, and quantified in (d) as count of EpCAM^{-} CD45^{+} Lineage^{-} ILC and (e) fold change expansion of ILC after 14 day co-culture relative to the number of ILCP seeded on day 1 (N=3-15 across ~7experiments). f) Count of Live, EpCAM^{-}, CD45^{+}, Lineage^{-}, RORγt^{+} ILC after 14 day co-culture with mesenchyme depleted HIO or epithelial depleted mesenchyme/fibroblasts expressing markers CCR6, NKp44, and/or T-bet (N=3). g) Pie charts of relative group 1 (Live, CD45^{+} Lin^{-} CRTH2^{-} c-kit^{-}, and [CD127^{+}, CD161^{+} in primary gut ILC only] and [EpCAM^{-}, RORγt^{-}, GATA3^{-} in MD-HIO and ED-HIO only) group 2 (Live, CD45^{+} Lin^{-} CRTH2^{+} c-kit$^{+/-}$, and [CD127^{+}, CD161^{+} in primary gut ILC only] and [EpCAM^{-}, RORγt^{-}, GATA3^{+} in MD-HIO and ED-HIO only), group 3 (Live, CD45^{+} Lin^{-} CRTH2^{-} c-kit^{+} NKp44$^{+/-}$, and [CD127^{+}, CD161^{+} in primary gut ILC only] and [EpCAM^{-}, RORγt^{+}, GATA3^{-} in MD-HIO and ED-HIO only) and other Lineage^{-} ILC (e.g. undifferentiated precursors, (Live, CD45^{+} Lin^{-} CRTH2^{-} c-kit^{-}, and [CD127^{+}, CD161^{+} in primary gut ILC only] and [EpCAM^{-}, RORγt^{-}, GATA3^{-} in MD-HIO and ED-HIO only) in unstimulated, primary human intestine (N=13, adapted from Kraemer et al., 2017), MD-HIO (N=13), and ED-FB (N=7). h) Representative image of CD45^{+} ILCP co-cultured with mesenchyme-depleted, E-cadherin^{+} HIO (scale bar 25µm). Error bars S.E.M, p-values unpaired student t-tests.

Figure 2. a) Representative confocal image of a human intestinal organoid (HIO, apical actin ring magenta, hindgut expression of transcription factor CDX2 white, Nuclei stained with DAPI, cyan) in co-culture with ILCP (CD45, yellow arrows). Mesenchyme surrounds complete epithelial-mesenchymal HIO structures Comp. HIO) as CD45- CDX2- nuclei (blue arrows). Scale bar 50µm. b) Count of seeded ILCP (day 1), CD45 immune cells after 14 day co-culture with or without complete epithelial-mesenchymal HIO. c) Representative flow plot of GATA3 and IL-13 expression in CD45^{+} Lineage^{-} ROR-γt^{-} CRTh2^{+} ILC2, stimulated with PMA/Ionomcyin for 4h, with corresponding quantification of expression in day 1 ILCP pre-co-culture and 14 day co-culture with complete HIO (ILCP from N=3 donors). d) Overlays of target gene expression in putative group 1 (magenta^{-} Live, EpCAM^{-} CD45^{+} LIN^{-} RORγt^{low} T-bet^{+}), and group 3 (Live, EpCAM^{-} CD45^{+} LIN^{-}, ROR-γt^{+}) cells expressing CCR6 (orange), NKp44 (dark blue), and no NKp44 (light blue) after 4h stimulation with PMA/Ionomycin after 14 day co-culture with mesenchyme depleted HIO (MD-HIO). e) Overlay of putative group 3, T-bet^{+} and Eomes^{+} group 1 ILC, showing expression of IFN-γ in these PMA/Ionomycin stimulated co-cultures with complete HIO with or without IL-18 stimulation, with corresponding quantifications. f) Representative flow plots CD161 and RORγt expression in Live EpCAM^{-} CD45^{+} LIN^{-} HIO-derived ILC, with corresponding quantification of CD161^{+}

RORγt^{+} cells and g) the frequency of NKp44^{+} ILC within the CD161+ RORγt+ population. h) Representative flow plots of IL-22 and IL-17A expression with corresponding quantification in complete HIO-derived ILCP after 4h stimulation with PMA/Ionomycin with or without additional IL-23 stimulation. Error bars S.E.M., unpaired student t-tests, ILCP from N=3 donors.

Figure 3: Human epithelial cells, not mesenchymal cells, drive proliferation and maturation of functional human ILC. a) Frequency of Live, EpCAM^{-}, CD45^{+}, Lineage^{-}, RORγt^{+} ILC after 14 day co-culture expressing markers CCR6, NKp44, and/or T-bet. b) Frequency of IL-22^{+} and IL-17A^{+} MD-HIO and ED-FB derived ILC after 4h stimulation with PMA/Ionomycin. c) Representative flow plots corresponding to (b) (FMOs overlayed in blue and magenta). d) Frequency of CD45^{+} LIN^{-} RORγt^{-} expressing T-bet and/or Eomes e) Representative flow plots of CD56 and IFN-γ expression in T-bet^{+} and Eomes^{+} populations after 4h stimulation with PMA/Ionomycin (IFN-γ FMO overlayed magenta), with corresponding quantification. f) Total count of putative ILC2 after 14 day co-culture. g) Expression of IL-5 and IL-13 in putative ILC2 after 4h stimulation with PMA/Ionomycin (FMOs overlayed magenta and blue). With corresponding quantification. Error bars S.E.M, p-values unpaired student t-tests; ILCP from N=3 donors.

Figure 4. Representative flow plots of group 1 associated genes visualised by overlaying ROR-γt^{-} T-bet^{+} (red), Eomes^{+} (purple), or "other" T-bet- (grey) after 14 day co-culture with MD-HIO or ED-FB after 4h stimulation with PMA/Ionomycin, with relevant quantification of geometric mean fluorescence intensities. Error bars S.E.M., unpaired student t-tests, ILCP from N=3 donors.

Figure 5. a) Relative frequency among CD45^{+} Lineage^{-} of putative ILC2 (gating strategy in (b)), after 14 day co-culture with MD-HIO or ED-FB. b) Representative flow plots overlaying putative ILC2 (green) and other Klrg1^{-} non-ILC2 (magenta) showing expression of group 2 associated genes after 4h stimulation with PMA/Ionomycin (ILCP from N=3 donors). c) Representative flow plots of putative ILC2 and IL-5/IL-13 expression after 4h stimulation with PMA-Ionomycin after ILCP co-culture with MD-HIO, MD-HIO separated from ILCP by a transwell separator, or ILCP cultured in Matrigel without organoids (N=1 donor, n=2 technical replicates). d) Count of EpCAM^{-}CD45^{+} immune cells and EpCAM-CD45- fibroblasts/mesenchyme in co-cultures derived from 14-day MD-HIO co-cultures, which were then FACS purified and reseeded either with fresh MD-HIO or with ED-FB. e) Frequency of IL-13^{+} and IL-5^{+} ILC within putative ILC2 pre-matured with MD-HIO and reseeded with MD-HIO or ED-FB for an additional 7 days. ILCP from N=3 donors. All error bars S.E.M., unpaired student t-tests.

Figure 6. Transfer of gut-matured ILC to HLO recapitulate tissue-specific human ILC2 phenotypes. a) Representative image of HIO and human lung organoids (HLO) showing E-

cadherin^{+} epithelium (magenta), CD45^{+} ILC (yellow), and nuclei (Hoechst, cyan) after 14-day co-culture (scale bars 50μm). b) Count of EpCAM^{-}, CD45^{+}, LIN^{-} ILC after 14-day co-culture with MD-HIO or MD-HLO, with corresponding count of EpCAM^{-} CD45^{-} fibroblasts/mesenchyme. c) Representative flow plots of RORγt, CCR6, IL-22, IL-17A, IFN-γ, and NKp44 after 14 day co-culture with MD-HIO or MD-HLO after 4h PMA/Ionomycin stimulation (pregated population indicated in grey, representative of N=3). d) IL-5 and IL-13 expression in putative GATA3^{+} ILC2 after 14 day co-culture and 4h PMA/Ionomcyin stimulation (FMOs overlayed magenta and cyan) with corresponding quantification. e) Expression of CD25 and ST2 in putative GATA3^{+} ILC2 after 14 day co-culture with HIO or HLO with corresponding quantification. f) Quantification of the relative frequency of CD25^{+} and ST2^{+} putative ILC2 and (g) MFI of ST2 in the same population with corresponding histogram overlay of ST2-PE after 14 day co-culture with HIO or HLO, followed by a reseeding from HIO back to HIO, a swap from HIO to HLO, or a swap from HIO to HLO with 50ng/ml hIL-33 neutralising antibody. All experiments performed with ILCP from N=3 donors, unpaired two-tailed student t-tests, error bars S.E.M.

Figure 7. Expression of HLA-DR on ILC3 generated from 13 days co-culture of ILC precursors (ILCP) on mesenchymal depleted human intestinal organoids (MD-HIO). Showing percentage of HLA-DR+ from the ILC3 population pre-gated on Live, CD45+, Lin- (CD3- CD20- CD14- CD19-), ckit^{+} ILC3. Red: ILC3, blue: fluorescence minus one (FMO) control.

Figure 8. Antigen processing by ILC via MHC-II shown by hydrolysis of DQ-BSA each leads to fluorescent signal. a) Expression of Dye Quenched-Bovine Serum Albumin (DQ-BSA) fluorescence in expanded ILCs followed by a 4 hour incubation with DQ-BSA at 37°C, on Ice or without DQ-BSA. ILCs were expanded from ILC precursors derived from healthy adult PBMCs. (CD45+, Lin- then ILC1s are cKit- CRTH2-, CD56- and CD161+, ILC2s are CRTH2+ and ILC3s are cKit+, CRTH2-). b) Expression of HLA-DR in expanded ILC populations following 4 hour incubation with Dye Quenched-Bovine Serum Albumin (*CD45+, Lin- then ILC1s are cKit- CRTH2-, CD56- and CD161+, ILC2s are CRTH2+ and ILC3s are cKit+, CRTH2-)*. c) Expression of HLA-DR in expanded ILC populations following 4 hour incubation with Dye Quenched-Bovine Serum Albumin where grey dots are DQ-BSA- ILCs (*CD45+, Lin- then ILC1s are cKit- CRTH2-, CD56- and CD161+, ILC2s are CRTH2+ and ILC3s are cKit+, CRTH2-)*.

Figure 9. Single cell RNAseq (scRNAseq) on the organoid-generated ILCs. **A)** UMAP visualisation of all cells which passed quality controls ($n = 7{,}446$) color-coded based on annotated ILC subsets. **B)** Dot plot showing the relative enrichment of the selected and previously described human ILC subset-specific genes used to annotate the clusters. Whilst the gene expression of ILCregs has yet to be characterised within the human intestine, key genes used to differentiate these cells from the other ILC subsets in mice (Wang *et al.,*

2017) were found to be highly expressed in this cluster, including *IL10, SOX4* and *ID3*, alongside other genes characteristic of Tregs (e.g., *FOXP3, CTLA4* and *IL2RA*). **C)** UMAP visualisation of the enrichment scores of a human ILCP gene signature (Liu *et al.,* 2021). Despite these cells not forming their own distinct cluster, their clear clustering observed here within the ILC3 population indicates that a small proportion of ILCPs were captured and sequenced. **D)** UMAP visualisation of the density of *TBX21* and *EOMES* expression. Density mapping was employed here due to the weak detected expression of these two transcription factors. At the resolution used here, ILC1 and NK cells do not form distinct clusters; however, the patterning of *EOMES* expression suggests that both cell types were successfully generated within this co-culture. **E-F)** Density plot and UMAP visualisations of the enrichment scores of gene modules associated with human tissue specific ILC populations (Mazzurana *et al.,* 2021) in ILC3s. The three modules examined here correspond to a circulatory ILC module (mod11), a colonic ILC module (mod3) and a lung ILC module (mod34). Only ILC3s were examined as Mazzurana *et al.,* did not identify any distinct clusters of ILC1s or ILC2s in the colon. Despite ILC3s exhibiting greatest enrichment of a circulatory phenotype, this was not uniform across the ILC3 cluster (F) with the cluster dividing into those cells showing a greater enrichment for a circulatory ILC signature, which includes the putative ILCP cells, and those displaying a more colonic (mod3) enrichment. Notably, these ILC3s exhibited the weakest enrichment for the lung ILC gene module. This indicates that within the intestinal co-cultures, ILC3s may mature and differentiate towards a more intestinal tissue-specific phenotype.

Figure 10. NK cells generated by co-culture of ILC precursors (ILCP) with intestinal organoids are cytotoxic. Percentage (%) of dead CFSE stained K562 target cells. CFSE cells (target cells, T) were labelled carboxyfluorescein succinimidyl ester (CFSE) prior to co-culture with $CD56^{+}$ NK cells (effector cells, E) expanded from ILCP in human intestinal organoid co-cultures. They were co-cultured at different effector-to-target (E:T) ratios from 5:1, 2.5:1 to 1.25:1 with IL-2 supplementation (20ng/ml). Three control samples were prepared, target cells alone, effector cells and a positive control for cell dead (targets cells treated with Tween 20). To determine cellular viability, all experimental groups were stained with LIVE/DEAD fixable UV blue stain.

Figure 11. Generation of ILCs in human small intestine biopsy derived organoids. Generation of differentiated ILCs (including ILC1, ILC2, ILC3 and NK cells) from ILC precursors (ILCP) after 15 days in culture with human small intestine biopsy derived organoids in which 50% Intesticult^{TM} (commercially available from StemCell Technologies) and 50% homemade organoid medium were used. A. Cell counts at the start and end of the culture. B. Visual representation (brightfield microscope) of the expanded ILCs in contact with human small intestinal organoids (dark areas).

Figure 12. Intestinal organoid generated Foxp3+ Innate Lymphoid Cells (ILC). A) Flow cytometry plots showing intracellular staining for Foxp3+ in cells generated from 13 days co-culture of blood ILC precursors (ILCP) with mesenchymal depleted hIPSC-derived intestinal organoid. Cells pre-gated on single cells, live, CD45+, EpCAM-, Lin- (CD3, CD14, 5 CD19, CD20), CD56-. Blood obtained from 3 donors. B) Graphical representation of the percentage of Foxp3 expressing cells within the LiveCD45+Lin+CD56- population.

Figure 13: CTLA-4 expression on intestinal organoid generated Foxp3+ Innate Lymphoid Cells (ILC). A) Flow cytometry plots showing intracellular staining for CTLA-4 in Foxp3+ cells generated from 13 days co-culture of blood ILC precursors (ILCP) with mesenchymal 10 depleted hIPSC-derived intestinal organoid. Cells pre-gated on single cells, live, CD45+, EpCAM-, Lin- (CD3, CD14, CD19, CD20), CD56-Foxp3+. Blood obtained from 3 donors. B) Graphical representation of the percentage of CTLA-4 expressing cells within the LiveCD45+Lin+CD56-Foxp3+ population.

EXAMPLES

15 Materials and Methods

Human organoid differentiations

KUTE-4 (Leha *et al.*, 2016) and FS13B human iPSCs previously generated using established protocols (Kilpinen *et al.*, 2017; Yusa *et al.*, 2011, both incorporated by reference). Human iPSCs were maintained on Vitronectin (StemCell Technologies) in E8 media and passaged as 20 disrupted clusters every 4-6 days using Versene (GIBCO).

HIO were derived following previously established protocols (McCracken *et al.*, 2011, incorporated by reference), with substitution of CHIR99021 for recombinant Wnt3a. HIO were further matured through addition of 20ng/ml IL-2 to the expansion media. HIO were passaged and reseeded in Matrigel as whole structures every 10-14 days. Organoids were 25 matured for 4-8 weeks before use in experiments.

Human ILCP isolation

Systemic ILCP were isolated from leukocyte cones (NHS-BT) following established protocols (Lim *et al.*, 2017a, herein incorporated by reference). Briefly, lymphocytes were purified from cones using FICOLL density gradient separation. A small aliquot from each cone was 30 phenotyped using the hILCP panel to assess ILCP frequency and titre antibodies, and the remaining cone was distributed between 20-30 cryo-preserved vials of approximate equal hILCP frequency (10% DMSO in foetal calf serum, added dropwise and stored in liquid nitrogen after being frozen in a Mr Frosty Isopranol container), allowing for experiments to be performed from the same three donors to reduce variability and ensure that sufficient 35 Lineage antibody was added to exclude non ILC subtypes. On day-1 of experiments, cryovials were thawed and rested in media containing 10%FCS and Fc block for 1h, rinsed

with PBS for fixable Live/Dead staining, stained and sorted on a FACSARIA-III (BD biosciences).

ILCP co-cultures with organoids

On day 1, HIO/HLO were digested with 0.1 mg/ml Collagenase for 15 min at 37 ^{o}C, then mechanically disrupted until a cloudy mesenchyme-enriched fraction appeared. Epithelial structures were allowed to settle to the base of a 15 ml falcon tube, and the mesenchyme-rich fraction was removed, repeating this step 3-5 times or until the epithelial-enriched fraction became clear. Approximately 25-50 human organoid structures were transferred to 1.5 ml eppendorfs, then approximately 500-1000 ILCP were added to these tubes, and centrifuged at 300 G for 5min. After carefully removing the supernatant, the resulting cell mix was rigorously resuspended in 30 µl ice cold Matrigel, avoiding bubble formation, and the combined cell mixes were pipetting in the centre of a pre-warmed tissue-culture treated well, and allowed to solidify for at least 15 min at 37 ^{o}C. Pre-warmed basal media supplemented with R-Spondin, Noggin, EGF, and with 50 mM 2-mercaptoethanol (R&D), 20 ng/ml rhIL-2 (Sigma), and 20 ng/ml rmIL-7 (R&D) was then carefully added to each well. This medium may otherwise be referred to herein as Medium A.

Half media changes (50% media out, replenished with 60% of the remaining volume) were performed every 1-3 days, allowing for conditioned media to remain in the wells while supplementing with fresh 2X growth factors to ensure viability of organoids and ILC without disrupting ILC-epithelial interactions. No small molecules, FGF7, or FGF10 were supplemented to lung organoid cultures to maintain consistency between conditions.

For transwell experiments, permeable inserts separated the organoid fraction (top) and the ILCP fraction (bottom), with both resuspended in 25 µl Matrigel (Falcon 24 well (Corning), 1.6 x 10^6 pores per cm^2).

In inter-organ swapping experiments, ILCP+organoid co-cultures were dissociated with TryPLE on day 7, and live EpCAM-, CD45+, Lineage- whole populations were isolated from half of the culture by FACS, with the other half being used for flow cytometry analysis. This prevented epitopes from being blocked for secondary analysis on day 14. The organoid-matured ILC populations yielded by FACS were then re-seeded with the same or the opposite organoid cultures in fresh Matrigel, and the protocol was restarted as on day 1.

For regular analysis, day 7 or day 14 (swapped) co-cultures were either fixed in 4% PFA for immunocytochemistry or rinsed with PBS and dissociated with TrypLE (Gibco) and DNAse (due to potential dead epithelial cells) for 20 min to obtain single-cell suspension to be analysed by flow, or to FACS purify individual populations for RT-qPCR. Any suspension containing single epithelial cells were maintained in 2%FCS with 0.1 mM EDTA and 1mM HEPES to reduce cell clumping.

Co-culture analysis

Flow cytometry

Flow cytometry data was acquired on a Fortessa II (BD Biosciences) using DIVA software and analysed using FlowJo 10.4.1.

5 *RT-qPCR*

RNA was extracted using the RNAeasy micro kit (Qiagen), with 10 µl/ml ß-ME supplemented to the RLT lysis buffer to mitigate degradation in RNAse-rich epithelial tissues. cDNA reverse transcription was performed following manufacturer's protocols with the RevertAid synthesis kit (ThermoFischer), using 0.5 µl random primer and 0.5 µl Oligo dTTT primer per 10µl
10 reaction. RTqPCR were with primers ordered from Invitrogen with SYBR (Applied Bioscences) or with TAQ-FAM probes (ThermoFisher) with TAQ enzyme (Applied Biosciences) and run on a BioRad Real Time CFX384 Touch with CFX Maestro software, and resulting data were processed and normalised in Microsoft excel.

Confocal and live imaging

15 Co-cultures were fixed in 4% PFA for 5-15 min at room temperature within 3D Matrigel bubbles to retain relative ILC-organoid localisation. Samples were either stained as whole organoids, or were cryo-preserved in 30% glucose, then embedded in OCT overnight at 4 ^{o}C to desiccate the Matrigel, then frozen and cryosectioned. Samples were permeabilised using 0.05% Triton-X, stained in primary antibody overnight at 4 ^{o}C, and stained in
20 secondary antibody and Hoechst for 1h at room temperature (RT). Images were acquired on a Leica SP8 confocal microscope using LAX software, and resulting images were processed using FIJI (ImageJ).

Quantification and Statistical Analysis

Data was analysed using Microsoft Office 365 Excel 16.16.20 and GraphPad Prism 8.2.1.
25 Meta-analysis of public deposited RNA-sequencing was performed by normalising raw FPKM values to the Geometric mean of housekeeping genes *Actb/ACTB, Hprt1/HPRT1*, and *Gapdh/GAPDH,* and the (logX+1, 2) of these values was analysed with multiple row t-tests. Log-q values were visualised as Volcano plots in GraphPad Prism 8, heatmaps were produced using heatmapper.ca/expression/, applying clustering to rows and columns
30 (applying clustering dendrograms to columns) using centroid linkage and Eucledian distance measurement.

Reagents and models used

CELL LINE	**SOURCE**
KUTE-4, BOBC, and FS13B human induced	Feeder-free hiPSC derived from skin tissue via CtyoTune 2 in 2015 as part of the HipSci consortium phenotyping project (Kilpinen, Goncalves, Lega *et al.* Nature (2017). The cell lines

pluripotent stem cell lines	are banked at ECACC and have catalogue number 77650426, disease status of the female anonymous donors was normal, and all details and pluripotency scores can be found at http://www.hipsci.org/lines/#/lines/HPSI0714i-kute_4. The hiPSC are titled HPSI0714i-Kute_4. The Wellcome Trust Sanger Institute and the Wellcome Trust and MRC Cambridge Stem Cell Institute provided human iPS cell lines. Mycoplasma testing was performed by the King's College London Centre for Stem Cells and Regenerative Medicine on a monthly basis.
PBMC Leukocyte cones	NHS-BT

Table 1: Experimental Model and Subject Details

REAGENT	SOURCE	IDENTIFIER	CLONE
Human CD45 – e450, Alexa700	Invitrogen	48-0459-42	HI30
Human EpCAM – FITC	BioLegend	324203	9C4
Human LIN cocktail 3 – FITC, PacBlue	BD biosciences	643510	CD3 SK7, CD20 L27, CD19 SJ25C1, CD14 MφP9
Human CD4 – APC	eBioscience	17-0048-42	OKT4
Human KLRG1 – APC	BioLegend	138411	2F1-AG
Human CD335 NKp46 – APC	BioLegend	331917	9E2
Human TCR α/β - FITC	BioLegend	306706	IP26
Human TCR γ/δ - FITC	BioLegend	331208	B1
Human CD127 – PE Cy7	eBioscience	25-1278-42	eBioRDR5
Human CD56 – Alexa 700	BioLegend	318316	HCD56
Human c-KIT/CD117 bv605	BioLegend	313218	104D2
Human CRTh2 – PE	Miltenyi Biotec	130-113-600	BM16
Human CRTh2– BV711	BioLegend	350124	BM16
Human CRTh2 – BV421	BioLegend	350112	BM16
Human CD161 APC, Alexa700	BioLegend	302012	V NK80
Human ST2 – APC	R&D Sys	FAB5231A	N/A
Human ST2 – PE	R&D Sys	FAB5231P	N/A
Human NKp44 – PE Cy7	BioLegend	325116	P44-8
Human NKp44 – PerCP Cy5.5	BioLegend	325114	P44-8
Tbet – PE-Cy7	BioLegend	644824	4B10
Human RORγt – APC	eBioscience	17-6988-82	AFKJS-9
Human RORγt – PE	BDBiosciences	563081	Q21-559
Human GATA3 – APC Cy7	Santa Cruz	sc-268, APCC7	HG3-31
Human IL22 – PerCP Cy5.5	BioLegend	366709	2G12A41
Human IL17A – PE-Dazzle	BioLegend	512335	BL168

Human IL17A – e450, BV786	BD Horizon/ bioscience	560610 (e450), 563745 (BV786)	N49-653
Human IFNg –APCe780	Invitrogen	47-7319-41	4S.B3
Human IL-5 – APC	BioLegend	504305	TRFK5
Human IL-13 – FITC	eBioscience	11-7139-41	PVM13-1
Human IL-13 – bv711	BDBiosciences	564288	JES10-5A2
Human CD25 – PerCP-Cy 5.5	BDBiosciences	560503	M-A251
Human Klrg1 – APC	eBioscience	25-5893-80	2F1
Human CCR6 – APC	BioLegend	353416	G034E3
Human CCR6 – BV605	BioLegend	353419	G034E3
Human NKp46 APC	BioLegend	331918	9E2
Human Foxp3 AF647	BioLegend	320214	206D
Human CTLA4-PE/ Dazzle	Biolegend	349922	L3D10
BLOCKING ANTIBODIES			
FcR CD16/32 blocking mouse	BioCell	BE0307	2.4G2
FcR blocking human	Miltenyi Biotec	130-059-901	N/A
Anti-rhIL33 (neutralising, ICC, goat polyclonal)	R&D	AF3625	Ser112-Thr270
Anti-rmIL33 (neutralising, ICC, goat polyclonal)	R&D	AF3626	Ser109-Ile266
ICC ANTIBODIES			
E-Cadherin – human (rat)	eBioscience	51-3249-82	DECMA-1
CDX2 – human (rabbit)	abcam	Ab76541	
EpCAM – mouse (rabbit)	abcam	Ab71916	
Purified anti-human CD45 – (mouse)	BioLegend	304001	HI30
ZO-1	Abcam	Ab96587	Aa 1-266
Dclk1	Abcam	Ab31704	AL: 82.2 KDa and BL: 47.6 KDa
CD44	eBioscience	14-0551-82	IM7

Table 2: Antibodies

EGF	R&D	236-EG-01M
rhIL-2 (CF)	BioLegend	589104
rmIL-7	BioLegend	577806
rmIL-33	BioLegend	580502
FGF10	PeproTech	100-26-50ug
FGF7	R&D	251-KG-010/CF
rmFlt3 ligand	R&D	427_FL
IL-22	PeproTech	200-22-2uG
IL-18	BioLegend	592102
rhIL-33	BioLegend	581802
Advanced DMEM/F12	Gibco	12634-010
Glutamax	Gibco	1932118
Antibiotic-Antimycotic	Gibco	15240096
HEPES 1M	Gibco	2259252

2-Mercaptoethanol 50mM	Gibco	31350-010
PBS	Gibco	10010-015
PFA 16%	Pierce	28906
heat inactivated qualified FBS/FCS	Gibco	26140087
N-Acetylcysteine	Sigma-Aldrich	A9165
Matrigel	Corning	356231
Vitronectin XF	Stem Cell Technologies	07180
Essential 8™ Medium	Gibco	A1517001
Versene	Gibco	15040033
Y-27632 dihydrochloride (Rho-K inhibitor)	Tocris	1254
Recombinant Human/Mouse/Rat Activin A Protein, 50 ug	Bio-Techne	338-AC-050
Recombinant Human FGF-4 (aa 71-206)	Bio-Techne	7460-F4-025
CHIR-99021 (CHIR)	TOCRIS	4423
B27	Gibco	17504-044
PerColl	cytiva	17089101
TaqMan™ Fast Advanced Master Mix	ThermoFischer	4444556
SYBR	Applied Biosystems	
TrypLE-phenol free	Gibco	12604-021
Ethylenediaminetetraacetic acid (EDTA)	Fischer	BP2481-100, CAS 60-00-4
Succinate	Sigma	S3674-100G
FITC – 4kDa	Sigma	FD4-100MG
DAPI	ThermoFischer	62248
Hoechst	ThermoFischer	62249
Texas Red™-X Phalloidin	ThermoFischer	T7471
Brefeldin A	Invitrogen	00-4506-51
Monensin	Sigma	M5273
PMA	Sigma-Aldrich	P1585
Ionomycin	Sigma-Aldrich	I0634

Table 3: Cell culture reagents

REAGENT	SOURCE	IDENTIFIER
Foxp3 / Transcription Factor Staining Buffer Set	Invitrogen eBioscience	00-5523-00
RNeasy	Qiagen	74106
RevertAid First Strand cDNA Synthesis Kit	ThermoFisher	K1622
Live Dead fixable blue/UV	ThermoFischer	L34961

UltraComp eBeads	Invitrogen	01-2222-42

Table 4: Kits used

EXAMPLES

Example 1: Human intestinal organoids promote maturation of human systemic ILCP

The translation of experimental approaches used in mice to distal human tissues, especially
5 when studying rare populations like ILC, can be challenging. This makes the potential of *in vitro* human mucosal organoid systems particularly appealing. However, human bone marrow ILC precursors are poorly accessible. Thus, to translate the previously described murine ILCP-organoid system, readily available systemic ILCP were isolated from peripheral blood mononuclear cells (PBMC) instead of bone marrow, while human intestinal organoids
10 (HIO) were derived from healthy KUTE-4 human induced pluripotent stem cells (McCracken *et al.*, 2011). These GF structures are well-established and can be matured through addition of IL-2.

Heterogeneous systemic ILCPs (Figure 1a) were co-cultured with these hiPSC-derived epithelial-mesenchymal HIO (Figure 1b). Co-culture with HIO significantly expanded (~20
15 fold) ILCPs relative to the number of cells seeded without HIO, and when compared to the number of ILCP seeded on day 1 (d1) (Figure 2a, b). As in murine co-cultures, human ILCP developed as putative group 2 (Figure 2c), group 3 (Figure 2d), and group 1 ILC (Figure 2e) when cultured with whole HIO supplemented with IL-2 and IL-7. This fold expansion and development occurred without requiring the addition of IL-1β or subset-specific cytokines,
20 whereas previous *in vitro* systems required supplementation with IL-1β+IL-23 for ILC3s, IL-25+IL-33 for ILC2s, and IL-12+IL-18 for robust ILC1 development (Lim *et al.*, 2017b), or of IL-15 and differential Notch ligands when matured from $CD34^{+}$ hematopoietic stem cells (Hernández *et al.*, 2021). Instead, HIO alone significantly supported maturation of $ROR\gamma t^{+}$ ILC Figure 2f) which significantly upregulated NKp44 (Figure 2g), recapitulating the
25 difference in $c\text{-}KIT^{+}$ ILC observed between periphery and gut by Lim and colleagues. These $NKp44^{+/-}$ $CCR6^{+/-}$ group 3 $ROR\gamma t^{+}$ ILC produced low levels of IL-22 and IL-17A upon non-specific PMA/Ionomycin stimulation, and significantly responded to additional 4h stimulation with IL-23 (Figure 2h), while putative group 1 ILC significantly upregulated expression of T-bet and IFN-γ secretion in response to 4h stimulation with IL-18 (Figure 2e).

30 Example 2: Epithelial cells, not mesenchyme, drive robust ILC expansion and maturation

These data suggest the human intestinal organoid microenvironment provides stimuli for baseline maturation of ILC subsets from ILCP. However, unlike the murine primary epithelial-only organoids, hiPSC-derived hindgut organoids co-develop with rich and complex native mesenchyme. These cells not only contribute to the maturation of epithelial
35 cells (Stallmach *et al.*, 1989), but are a known source of ILC-survival factor and CD127-ligand IL-7 (Xu *et al.*, 2015). To assess whether epithelial cells or mesenchymal cells were

predominantly driving this ILC maturation, the matrix of HIO structures was digested with Collagenase, and a single cell suspension of mesenchyme was separated from 3D epithelial structures through serial gravity-gradient separation, resulting in a mesenchyme-depleted HIO fraction (MD-HIO) and an epithelial-depleted fibroblast-rich fraction (ED-FB) (Figure 1c). These fractions were cultured with ILCP for 14 days, and both fractions were able to maintain ILC viability and expansion (Figure 1c). However, the mesenchyme-depleted epithelial fraction unexpectedly yielded a dramatic increase in $EpCAM^-$, $CD45^+$, $Lineage^-$ cells after culture (Figure 1d), significantly increasing the fold-change expansion of seeded ILCP relative to those seeded with epithelial-depleted mesenchyme or without organoids in Matrigel only (Figure 1e). Moreover, while complete epithelial-mesenchymal HIO induced NKp44 expression in ~2-6% of group 3 ILC (Figure 2g), this upregulation was significantly greater in mesenchyme-depleted co-cultures (15-22%) (Figure 1f, 3a). Indeed, the epithelial-enriched MD-HIO fraction promoted patterns of ILC-subset maturation that more closely resembled the distribution of mature ILC in the healthy human intestine (Krämer *et al.*, 2017) than the epithelial-depleted mesenchyme fraction (Figure 1g). The relative proportion of $NKp44^+$ to $NKp44^-$ putative ILC3 biased maturation towards an NCR^- population in GF, environmentally controlled MD-HIO (Figure 1f, g), in which ILCP could freely interact with the epithelium (Figure 1h).

The current gold standard for investigating murine and human ILC maturation *in vitro* relies on modified murine bone marrow stromal cells (Nakano, 1996). Therefore, the relatively poor ILCP expansion rates induced by hiPSC-derived intestinal mesenchyme were unexpected. We hypothesized that the increased cell number in mesenchyme-depleted HIO could represent proliferation of poorly differentiated, immature ILC, while stromal cells may promote a smaller but more mature ILC yield. To test this, unbiased stimulation with PMA/Ionomycin was performed in ILC derived from MD-HIO and ED-FB co-cultures. First, the maturation of putative Group 3 ILCs was assessed. Here, only the frequency of $NKp44^+$ cells of $ROR\gamma t^+$ ILC significantly expanded in MD-HIO, with the relative frequency of $CCR6^+$, $NKp44^-$, and $T\text{-}bet^+$ ILC remaining constant in both conditions (Figure 3a). However, $CCR6^+$ and $NKp44^{+/-}$ ILC expressed significantly greater amounts of IL-22, and $CCR6^+$ and $NKp44^-$ ILC expressed significantly more IL-17A than did the respective corresponding populations derived from the epithelial-depleted mesenchymal fraction (Figure 3b). In fact, the frequency of $IL\text{-}22^+$ ILC was either comparable or greater in the mesenchyme-depleted fractions with unbiased PMA/Ionomycin stimulation than in complete HIO co-cultures stimulated with IL-22-inducing IL-23 (Figure 2h). The proportion of putative group 1 $CD56^{+/-}$, $T\text{-}bet^+$ and $Eomes^+$ ILC (Figure 4) was not only significantly decreased in the epithelial-depleted fraction (Figure 3d), but no expression of IFN-γ was observed in either population in the absence of epithelial cells when stimulated with PMA/Ionomycin (Figure 3e). Comparable to group 3 ILC, the proportion of $T\text{-}bet^+$ ILC and the number of $IFN\text{-}\gamma^+$ cells was greater in the absence of mesenchyme than in the absence of epithelium (Figure 2e).

Unlike group 1 and 3 ILC, the overall count (Figure 3f) and relative frequency (Figure 5a) of putative group 2 ILC (Figure 5b) was not significantly affected by the depletion of mesenchyme. Nevertheless, expression of IL-5 and IL-13 were both significantly and greatly increased in mesenchyme-depleted HIO fractions upon unbiased stimulation with PMA/Ionomycin (Figure 3g). The lack of difference in ILC2 numbers induced by epithelial depletion stands in contrast to findings in murine ILC2P transwell cultures, where the ILC2 number was significantly decreased upon physical separation from epithelial cells (Figure 1d-f). To assess whether contact-dependent mechanisms were conserved in maturing human ILCP-derived ILC2, ILCP were also separated from mesenchyme depleted HIO (Figure 5c). Though the yield of ILC2 was still greater in transwell-separated co-cultures than in ILCP cultured without organoids, both the overall cell count and relative ILC2 frequency appeared to decrease in transwell culture, and these ILC2 failed to express IL-5 or IL-13 (Figure 5c). Finally, we aimed to assess whether the lack of cytokine expression in epithelial-depleted co-cultures was because ILC2 failed to mature without epithelial cells, or if this was the result of active ILC2 cytokine repression by mesenchyme either through secretion of repressive factors or via competitive depletion of necessary ligands. To test this, ILCP were first successfully matured with MD-HIO, then resulting EpCAM$^-$ CD45$^+$ Lineage$^-$ ILC were FACS-purified and reseeded either with MD-HIO or swapped to ED-FB (Figure 5d). ILC reseeded without epithelial cells appeared to decrease their rate of proliferation, and while there was no difference in the expression of IL-13 (Figure 5e), the greater presence of mesenchyme resulted in a significant decrease in IL-5 expression by ILC2 (Figure 5e). This suggests that stable development of IL-13$^+$ ILC2 may be promoted by intestinal epithelial contact, but ILC2 proliferation and expression of IL-5 may either be actively repressed by intestinal mesenchyme or require constant epithelial exposure.

Example 3: Human mucosal epithelial identity contributes to tissue-specific ILC maturation

To assess if any tissue-characteristic ILC phenotypes could be captured in these human organoid systems (Figure 6a), ILCP were cultured either with hiPSC-derived small intestine or hiPSC-derived lung organoids. ILCP expanded in both co-cultures (Figure 6b), in which the absolute number of fibroblasts post mesenchyme-depletion remaining sufficiently consistent between conditions. HLO demonstrated comparable capacity to yield mature group 1 and group 3 cells that expressed IL-22, IL-17A, and IFN-γ (Figure 6c). Both HIO and HLO yielded GATA3$^+$ ILC that expressed IL-5 and IL-13 (Figure 6d). Much like ED-FB, HLO did not support maturation of IL-5$^+$ as significantly as HLO. Similarly, a recently described c-KIT$^+$, CRTh2^{low}, IL-17A$^+$ ILC2 population was additionally present in HIO, but not in HLO cultures, suggesting that much like the murine system, the human intestinal microenvironment may favour group 3 maturation, even in inducing ex-ILC2 to ILC3 plasticity in mature MD-HIO-derived ILC2. However, unlike in the murine system, co-culture with HIO or HLO alone was not sufficient to induce statistically significant differences in the frequency of CD25 and ST2 ILC within this GATA3+ putative ILC2 population (Figure 6e).

Finally, we assessed the capacity of the human organoids to recapitulate the gut-lung translocation approach. ILCP were matured with MD-HIO for two weeks, then FACS-purified and reseeded with MD-HIO, MD-HLO, or MD-HLO with human IL-33 neutralisation, as performed in the murine system. HiPSC-derived lung organoids did not significantly impact the frequency of $CD25^{+}$ (Figure 6f). However, the anticipated increase in pulmonary ILC2 ST2 expression that was not captured by HIO and HLO co-culture alone was significantly observed upon transfer of MD-HIO-derived ILC2 to lung culture (Figure 6g). This effect was dampened by addition of neutralising IL-33.

Example 4: Antigen processing of the ILCs of the invention

The ILCs of the invention express HLA-DR, an MHC-II surface receptor (Figure 7). The ability of the ILCs of the invention to process antigens via MHC-II was tested by hydrolysis of DQ-BSA (which leads to fluorescent signal). The positive results are shown in Figure 8.

Example 5: Single cell RNAseq (scRNAseq) on the organoid-generated ILCs

From the co-cultures, CD45+ Lin- cells were isolated via fluorescence activated cell sorting (FACS). We identified 5 'super-clusters' of cells corresponding to a cytotoxic ILC1 population, two distinct ILC2 clusters (ILC2a and ILC2b), an ILC3 cluster, and a small cluster of cells which exhibited significantly upregulated expression of genes characteristic of regulatory T cells (Tregs) and a regulatory ILC (ILCreg) population previously described in mice (Wang et al., 2017) (Fig. 9A).

The helper-like ILC cell types (ILC1-3) were identified via determining expression patterns of key genes previously described to distinguish the different ILC family members in single cell RNA sequencing studies using human samples (Björklund et al., 2016; Cella et al., 2019; Liu et al., 2021; Mazzurana et al., 2021). The significant enrichment of these genes in each cluster was determined via unbiased differential gene expression analysis comparing expression of the genes within each cluster to that of all other clusters (Figure 9B). Accordingly, the ILC1 population was identified via its enriched expression of key cytokines and chemokines including *IFNG* and *CCL3* alongside their relatively high expression of genes such *KLRC1*, *PRF1*, and *GNLY*, previously shown to characterise cytotoxic populations of ILC1. The increased expression of the transcription factor GATA-3 (encoded via *GATA3*) and the cytokine signalling genes *IL33* and *IL17RB* were used to identify the two ILC2 populations, alongside their upregulation of additional ILC2-associated genes such as *HPGDS* and *KLRG1*. Finally, ILC3s showed significantly greater enrichment of genes encoding their characteristic transcription factors (e.g., *RORC*), cell surface markers (e.g., *KIT*), and cytokine signalling pathways (e.g., *IL23RA*). These genes were found coupled to expression of markers previously shown to be highly expressed in both ILC3s and ILC1s, including *NCR1* and *PECAM1*, further confirming the identity of both clusters.

The annotation of the ILCreg cluster, however, was primarily performed using markers identified in mouse research on ILCregs as this population has yet to be extensively characterised in human patients, although an IL-10 expressing ILC population was identified in the human intestine (Wang et al., 2017). This included genes encoding their key cytokine, IL-10 (*IL10*), the cell surface marker CD25 (*IL2RA*) and the transcription factors *ID3* and *SOX4*. Interestingly, the ILCreg cluster additionally expressed genes associated with human T-regulatory (Tregs) cells including *FOXP3, RUNX1, IL1R1* and *CTLA4*. Importantly, the T-cell markers CD4 and CD3 were included within the lineage gating panel when sorting the ILCs from the co-cultures, and the lack of their expression was further confirmed at the RNA level. Accordingly, these cells were deemed to be the human ILC equivalent of Tregs.

Using cell-specific enrichment scores for a human ILCP gene signature (Liu et al., 2021) we identified a small population of these precursor cells within the larger ILC3 super-cluster (Fig. 9C). Moreover, the cytotoxic ILC1 super-cluster showed distinct expression patterns of the transcription factors *TBX21* and *EOMES*, suggesting this contained both ILC1 and NK-like cells (Fig. 9D). Overall, we found cells corresponding to the major ILC cell types expected to be observed in our co-culture model, alongside a putative population of human ILCregs.

To determine whether the ILCs exhibited an intestinal tissue specific phenotype, we generated cell-specific enrichment scores for three gene modules identified by Mazzurana *et al.*, (2021) associated with blood/tonsillar circulatory ILCs (mod11), colonic ILCs (mod3) or lung ILCs (mod34) (Mazzurana et al., 2021). Only ILC3s were examined here as Mazzurana *et al.,* did not identify any distinct clusters of ILC1s or ILC2s in the colon. The ILC3 super-cluster was found to exhibit the greatest enrichment for the circulatory gene module (Fig. 9E), followed by the colonic gene module, with minimal cells showing enriched expression of lung ILC associated genes. Considering we observed the putative ILCP population within our ILC3s, we subsequently overlaid the enrichment scores onto the UMAP to determine any heterogeneity in the pattern of expression of these gene modules (Fig. 9F). This revealed that the ILC super-cluster could be clearly differentiated into those cells which exhibit a more circulatory phenotype compared to those which express a gene signature more closely resembling that of colonic ILC3s. As expected, there were minimal numbers of cells enriched for the lung module. Taken together, these data suggest that a substantial proportion of the ILC3s in this co-culture model differentiate away from a circulatory phenotype to acquire a colonic gene expression signature.

Experiment outline:

Circulatory innate lymphoid cell precursors (ILCPs; CD45+ Lin- CD56- CD127+ CRTh2- cKIT+ NKp46- KLRG1-) were isolated from the blood of age-matched male and female

donors (n = 1), placed into co-culture with iPSC-derived human intestinal organoids and allowed to expand for 14 days. After this 14-day period, four populations of ILCs were isolated from the co-cultures using FACS: ILC2 (CD45+ Lin- CRTh2+), ILC3 (CD45+ Lin- CRTh2+ cKIT+), ILC1/NK cells (CD45+ Lin- CRTh2- cKIT- CD56+/- CD161+) and 'other'
5 ILC (CD45+ Lin- CRTh2- cKIT- CD56+/- CD161-). Each population was subsequently pooled in a 1:1 ratio of male to female cells to give a final total proportion of each ILC population at 25, 15, 35 and 15% respectively. These proportions were decided upon based on prior knowledge of the heterogeneity of each ILC subset, with those ILCs displaying greater heterogeneity contributing a larger percentage of the final cell counts. This pool of cells was
10 then sequenced as single sample via 10X sequencing.

scRNA-seq data processing and analysis:

Barcoding, cDNA synthesis, and library preparation were performed using Chromium™ Next GEM Single Cell 3', Library & Gel Bead Kit v3.1 according to the manufacturer's instructions. Libraries were sequenced by Novogene using NovaSeq with a target of 20,000 reads per
15 cell. Pre-processing of single cell RNA-sequencing fastq files was performed using Cell Ranger v7.0.1 (10X Genomics), with reads aligned to the GRCh38 reference genome. Further data pre-processing was performed in R (v4.2.2) using the package Seurat (v4.3.0). Cells with less than 1,000 genes and greater than 10 % of transcripts derived from mitochondrial genes were removed as they were considered lysed/apoptotic. Moreover, cells
20 with more than 7,500 genes or 40,000 UMIs were excluded as these were presumed to contain doublets. The filtered dataset was subsequently normalised using the Seurat SCTransform function (v2) in which the effects of the cell cycle were regressed out. The top 30 principal components were then used as input for Louvain clustering via the FindClusters() function in Seurat at resolution 0.2. UMAP was used for cluster visualisation.
25 Differentially expressed genes were determined using the Wilcox test in the FindAllMarkers() function in Seurat, and genes with log fold change >= 2.5 and a false discovery rate $P < 0.05$ were considered significantly differentially expressed. To visualise lowly expressed genes (*TBX21* and *EOMES*) the plot_density function was used from the R package Nebulosa (v1.8.0). To determine the enrichment of different ILC gene signatures, the R package
30 AUCell (v1.6.1) was used and the resultant scores were plotted for visualisation.

The results are shown in Figure 9 and the Table 5 below.

Table 5: Results of scRNAseq on the organoid-generated ILCs

ILC1 / NK cell genes	ILC2 genes	ILC3 genes	ILCreg genes
AOAH	ANXA1	AHR	CCR5
CCL3	BCL11B	BCL2A1	CTLA4
CCL4	CCR2	CCL20	FGGY
CCL5	GATA3	CD81	FOXP3
CD244	HPGD	CSF2	GATA3
CD247	HPGDS	CXCL8	GZMB
CST7	IL10RA	ID2	IL10
EOMES	IL13	IKZF2	IL1R1
FCGR3A	IL17RB	IL1R1	IL2RA
FGR	IL32	IL23R	IL2RB
GNLY	IL5	IL4I1	KAT2B
GZMB	IL9R	IRF4	LGLS3
GZMK	KLRG1	KIT	PIM1
IFNG	LGALS1	LIF	PRDM1
IKZF3	MAF	LTA4H	RUNX1
IL12RB2	MBOAT2	NCR1	SOX4
ITGAX	PPARG	PECAM1	TNFRSF18
ITGB2	PTGDR2	RBPJ	TRAF1
KLRC1	PTGER2	RORC	
KLRD1	TNFSF10	TNFRSF25	
NCAM1		TNFSF4	

NCR1		TOX2	
NKG7			
PRF1			
SAMD3			
TBX21			
TIGIT			
ZNF683			

Example 6: NK cells generated by co-culture of ILC precursors (ILCP) with intestinal organoids are cytotoxic

The cytotoxicity of NK cells generated by co-culture of ILC precursors (ILCP) with intestinal
5 organoids was tested using standard methods. The positive results are shown in Figure 10.

Example 7: Generation of ILCs in human small intestine biopsy derived organoids

Differentiated ILCs (including ILC1, ILC2, ILC3 and NK cells) were generated from ILC precursors (ILCP) after 15 days in culture with human small intestine biopsy derived organoids. The results are shown in Figure 11.

10 Example 8: Producing and testing ILCregs

Circulatory innate lymphoid cell precursors (ILCPs; CD45+ Lin- (CD3, CD14, CD19, CD20) CD56- CD127+ CRTh2- cKIT+ NKp46- KLRG1-) were isolated from the blood cones (*n* = 3), placed into co-culture with iPSC-derived human intestinal organoids, which were depleted of mesenchymal cells, and allowed to expand for 13 days. After this 13-day period, cells were
15 analysed by flow cytometry for extracellular (CD45, EpCAM, Lin) and intracellular (Foxp3, CTLA-4) markers. The results are shown in Figures 12 and 13 and demonstrate that the ILCs express at the protein level markers associated with regulatory lymphocyte populations supporting the generation of a human population of ILCregs with this invention.

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CLAIMS

1. A human regulatory innate lymphoid cell (ILCreg) which expresses a detectable level of FOXP3 and/or CTLA4.

2. A human ILCreg according to claim 1, wherein the human ILCreg further expresses a detectable level of CD25 (IL2RA) and/or CD127.

3. A human ILCreg according to claim 1 or 2, wherein the human ILCreg further expresses detectable levels of one or more of CCR5, FGGY, GATA3, GZMB, IL10, IL1R1, IL2RA, IL2RB, KAT2B, LGLS3, PIM1, PRDM1, RUNX1, SOX4, TNFRSF18 and TRAF1.

4. A human ILCreg according to any one of the preceding claims, wherein the human ILCreg further expresses detectable levels of CCR5, FGGY, GATA3, GZMB, IL10, IL1R1, IL2RA, IL2RB, KAT2B, LGLS3, PIM1, PRDM1, RUNX1, SOX4, TNFRSF18 and TRAF1.

5. A human ILCreg according to any one of the preceding claims, wherein the human ILCreg does not express detectable levels of one or more of CD3, CD4, CD19, CD20, TCR$\alpha\beta$.

6. A human ILCreg according to any one of the preceding claims, wherein the human ILCreg does not express detectable levels of one or more of CD3, CD4, CD19, CD20, TCR$\alpha\beta$, and TCR$\gamma\delta$.

7. A human ILCreg according to any one of the preceding claims, wherein the human ILCreg does not express detectable levels of CD3, CD4, CD19, CD20, TCR$\alpha\beta$, and TCR$\gamma\delta$.

8. An *in vitro* population of human ILCregs, wherein the population comprises at least about two human ILCregs according to any one of claims 1-7.

9. An *in vitro* population according to claim 8, wherein the population comprises at least about 100 human ILCregs.

10. An *in vitro* population according to claim 8 or 9, wherein at least 10% of the human ILCregs in the *in vitro* population comprise an exogenous polynucleotide.

11. A pharmaceutical composition comprising a human ILCreg according to any one claims 1-7 or an *in vitro* population according to any one of claims 8-10 and a pharmaceutically or physiologically acceptable diluent and/or carrier.

12. A method of treating or preventing a disease in a subject, wherein the method comprises administering to the subject a human ILCreg according to any one claims 1-7, an *in vitro* population according to any one of claims 8-10 or a pharmaceutical composition according to claim 11.

13. A method according to claim 12, wherein the disease is an inflammatory disease.

14. A method according to claim 12, wherein the inflammatory disease is an allergy, an autoimmune disease, an infection or cancer.

15. A method according to any one of claims 12-14, wherein the subject is human.

16. A human ILCreg according to any one claims 1-7, an *in vitro* population according to any one of claims 8-10 or a pharmaceutical composition according to claim 11 for use in the treatment or prevention of a disease.

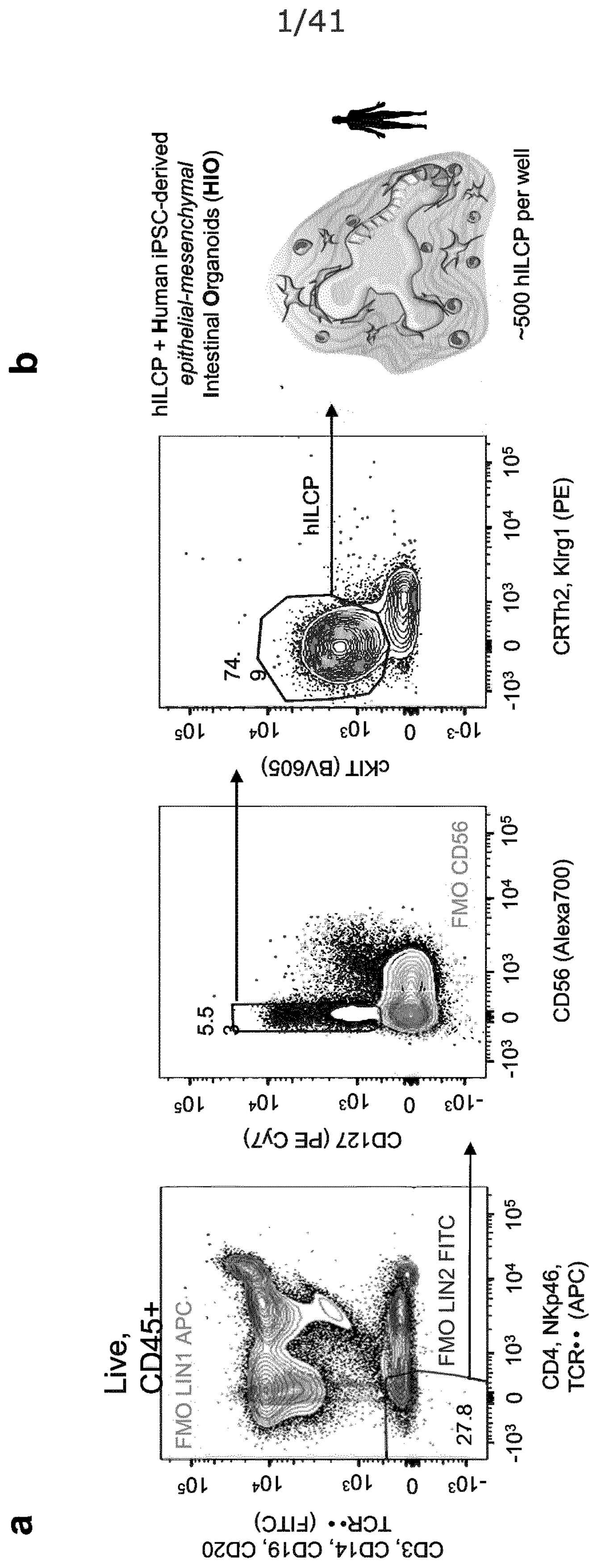
17. A method for expanding a human ILCreg according to any one claims 1-7 or an *in vitro* population according to any one of claims 8-10, the method comprising:

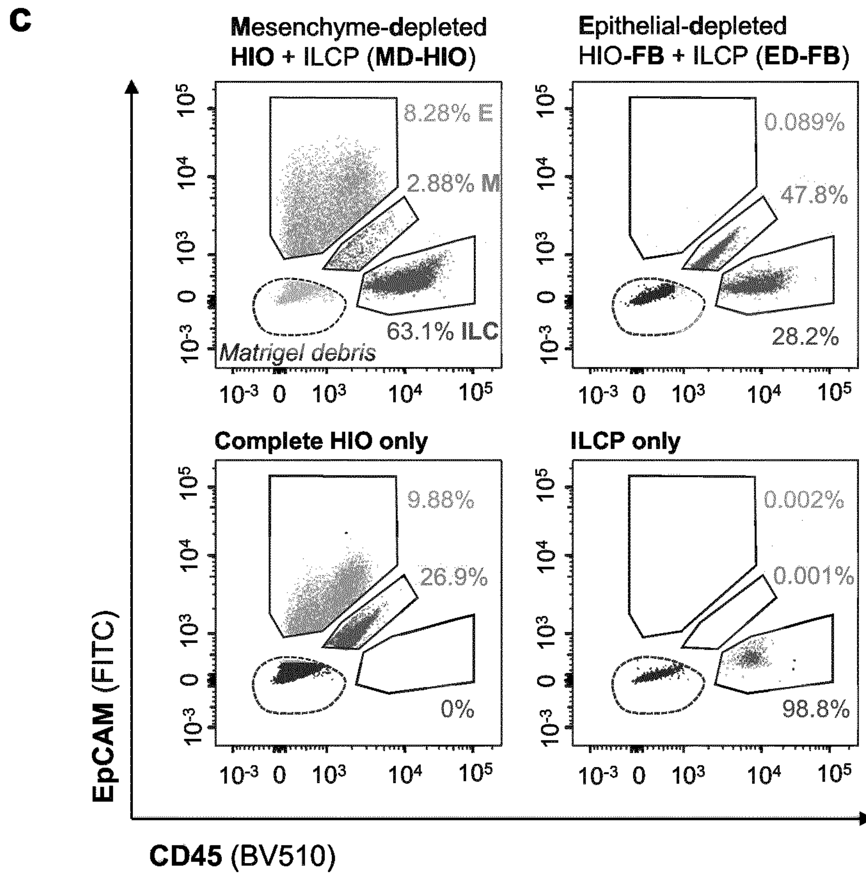
(a) co-culturing the human ILCreg or the *in vitro* population and at least one epithelial organoid, wherein the epithelial organoid comprises more epithelial cells than mesenchymal cells.

18. A method for producing a human ILCreg according to any one claims 1-7 or an *in vitro* population according to any one of claims 8-10, the method comprising:

(a) co-culturing human ILC precursors and at least one epithelial organoid, wherein the epithelial organoid comprises more epithelial cells than mesenchymal cells, to expand the human ILC precursors and differentiate the ILC precursors into the human ILCregs.

Figure 1





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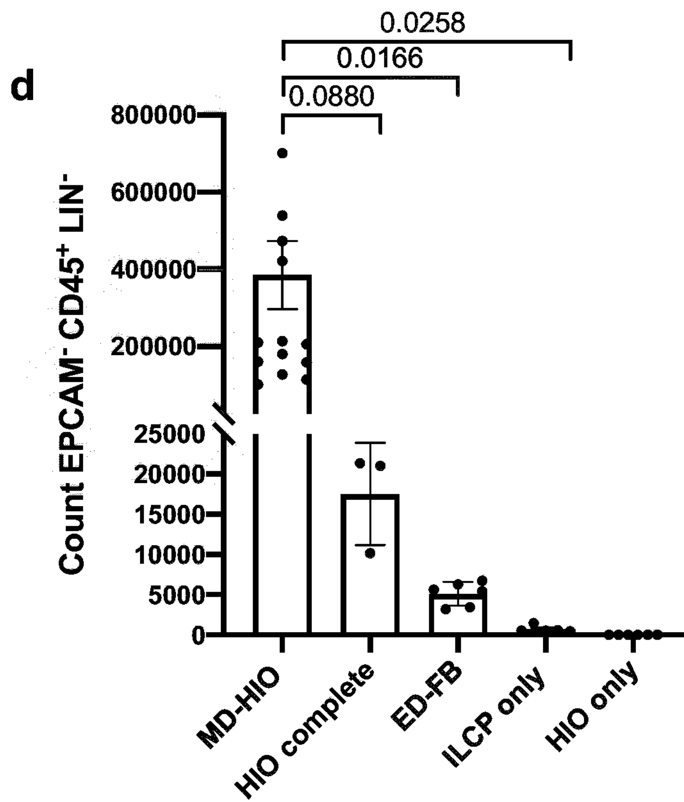

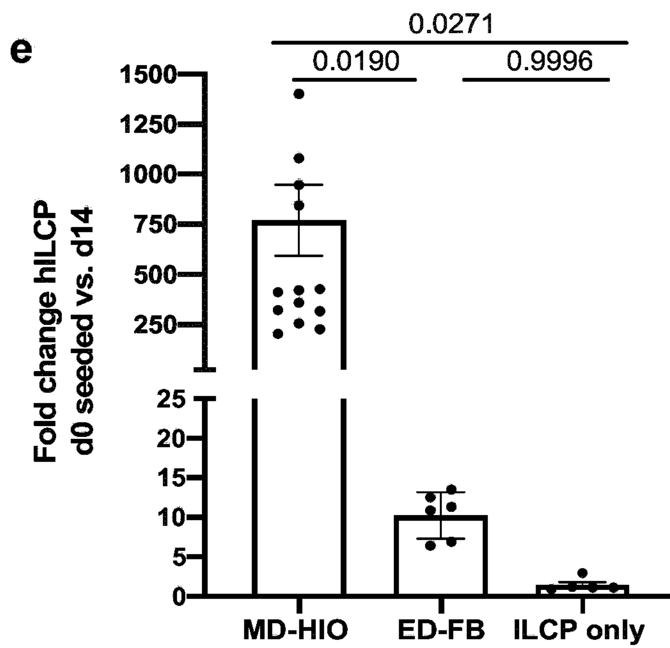

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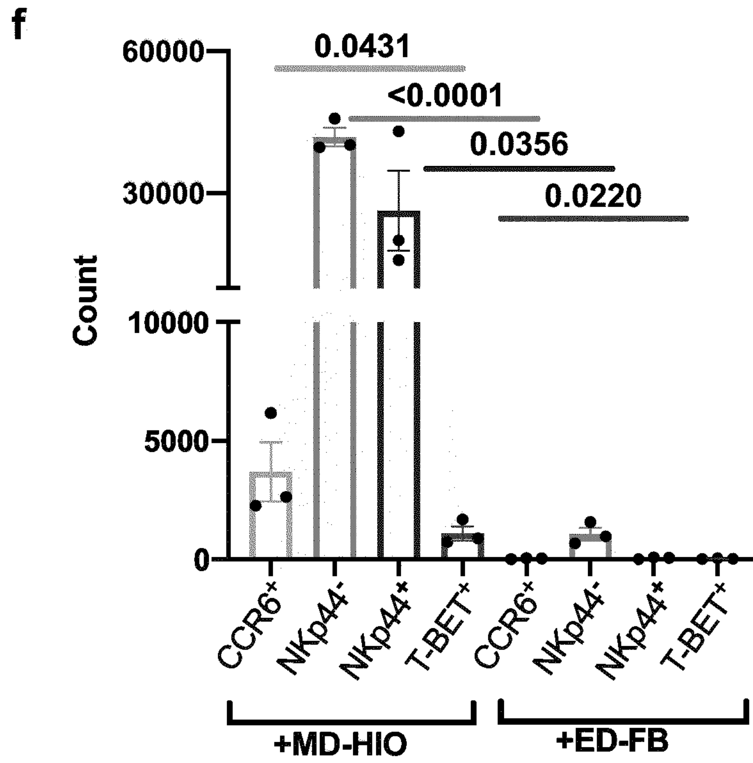

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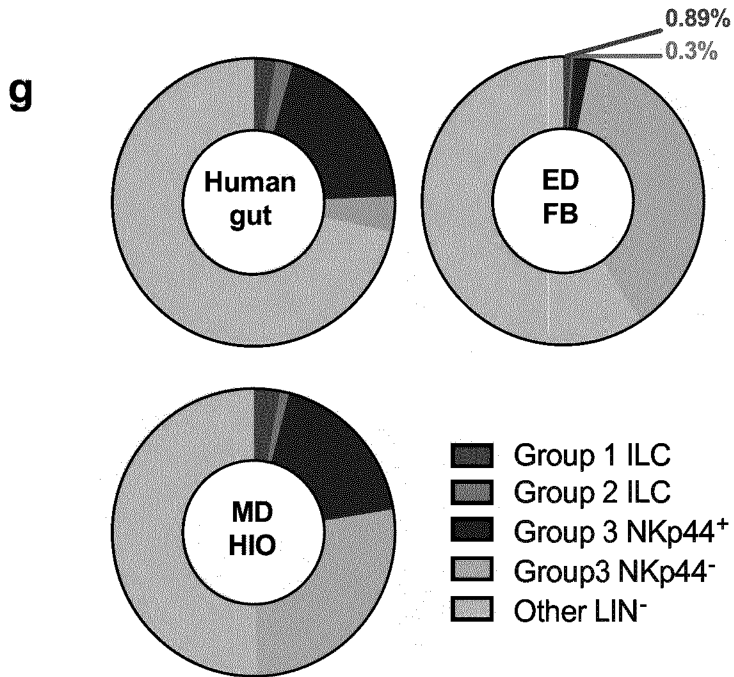


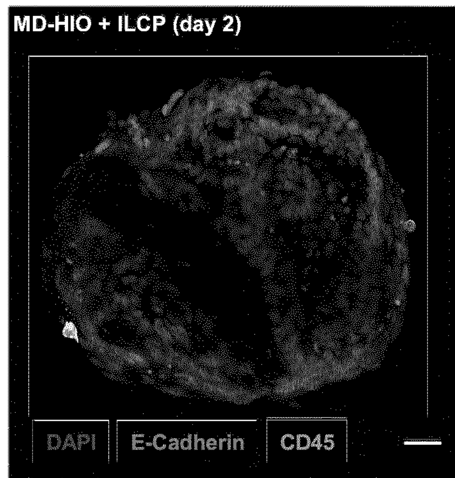
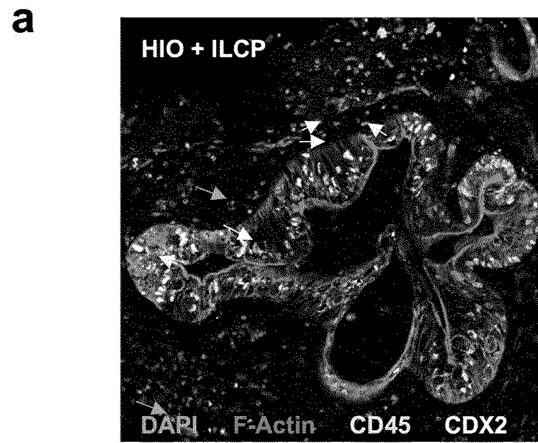


Figure 1 cont.

a



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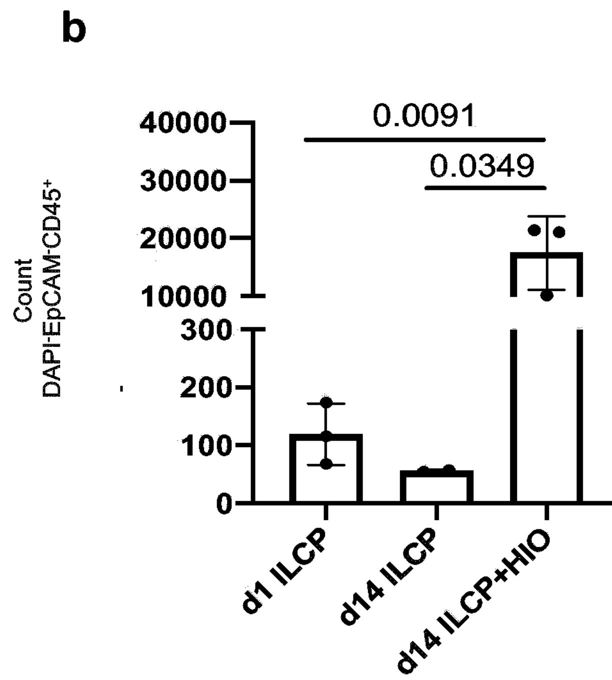
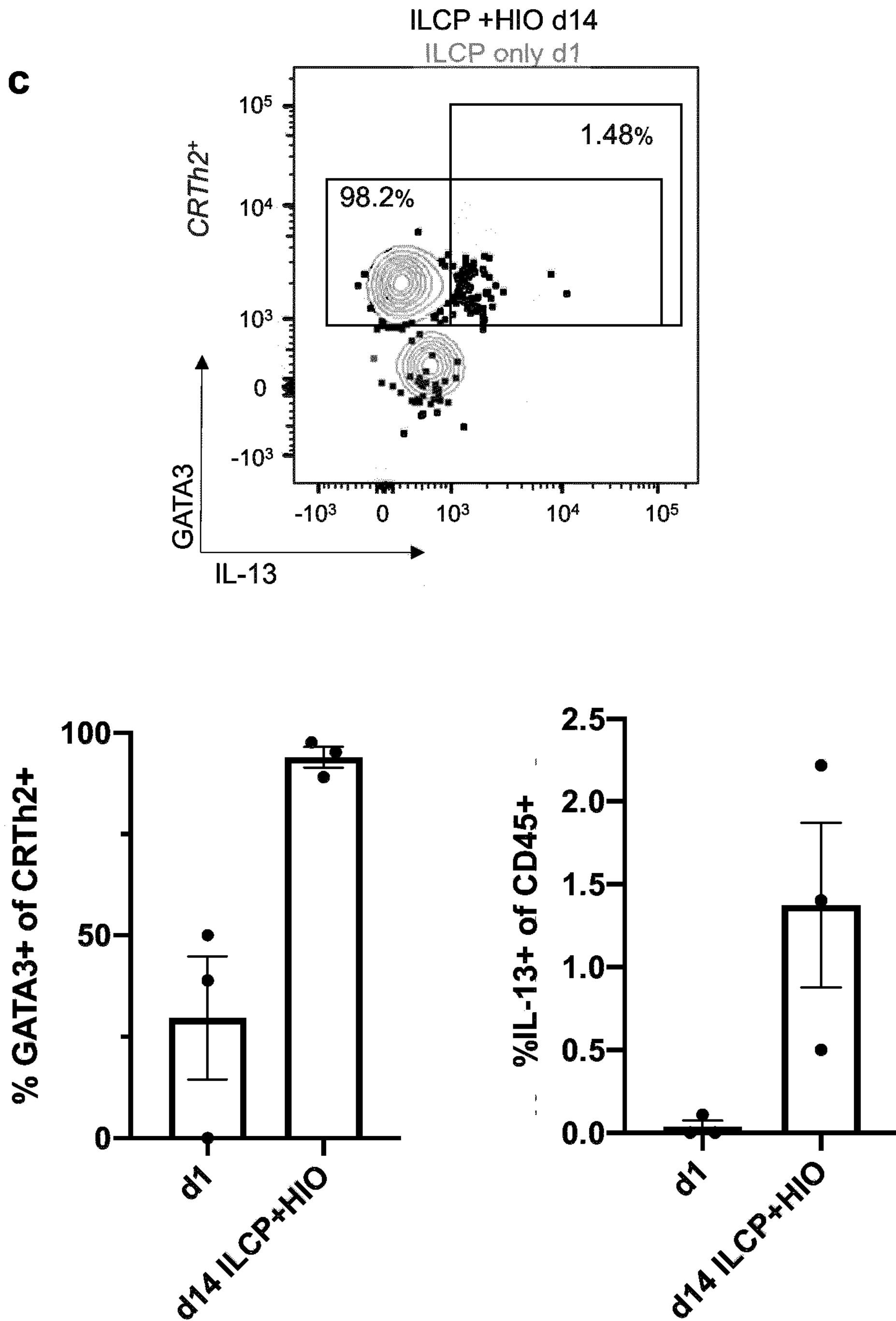


Figure 2



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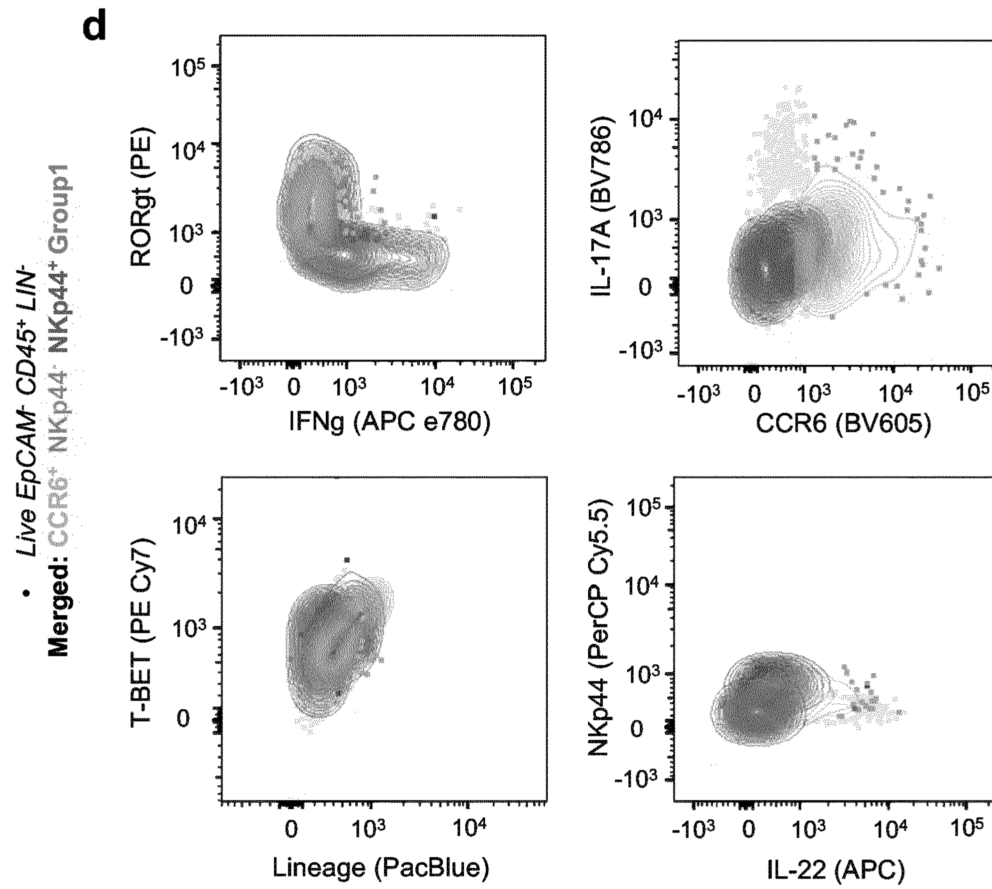

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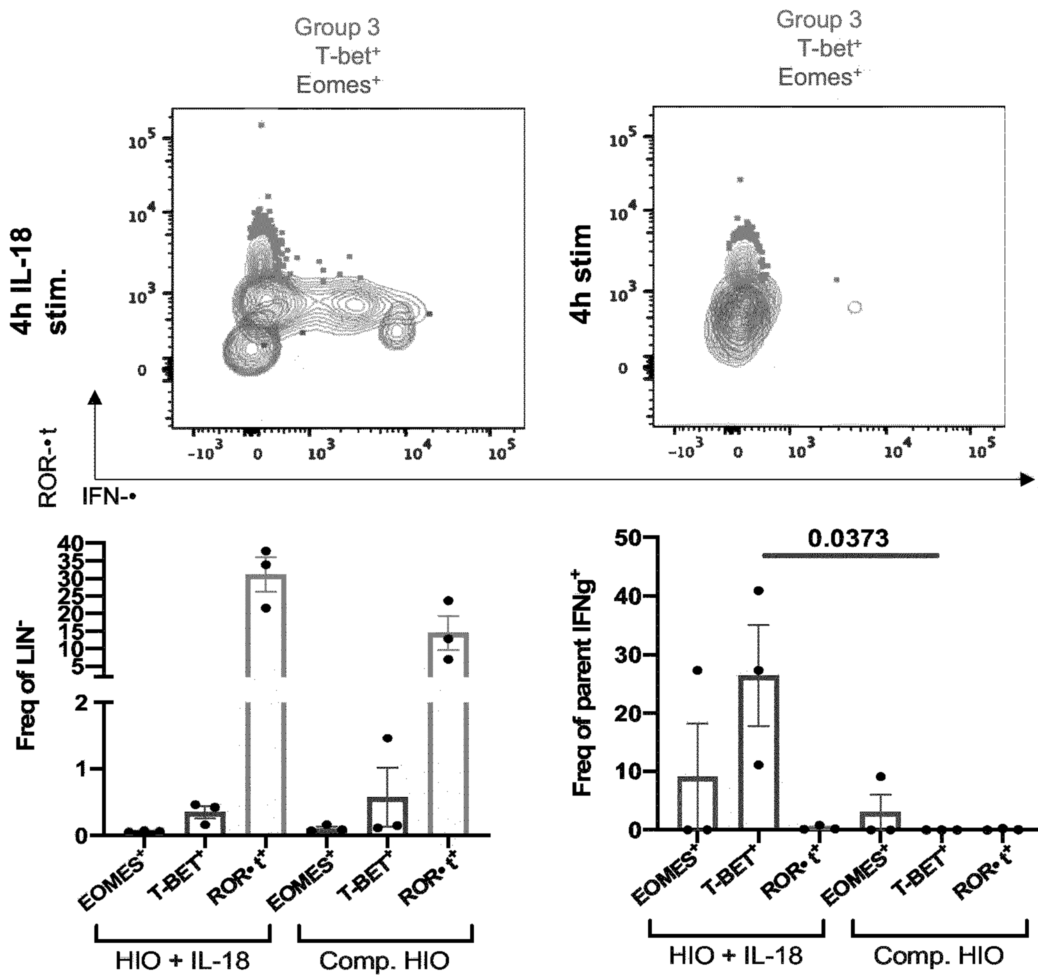

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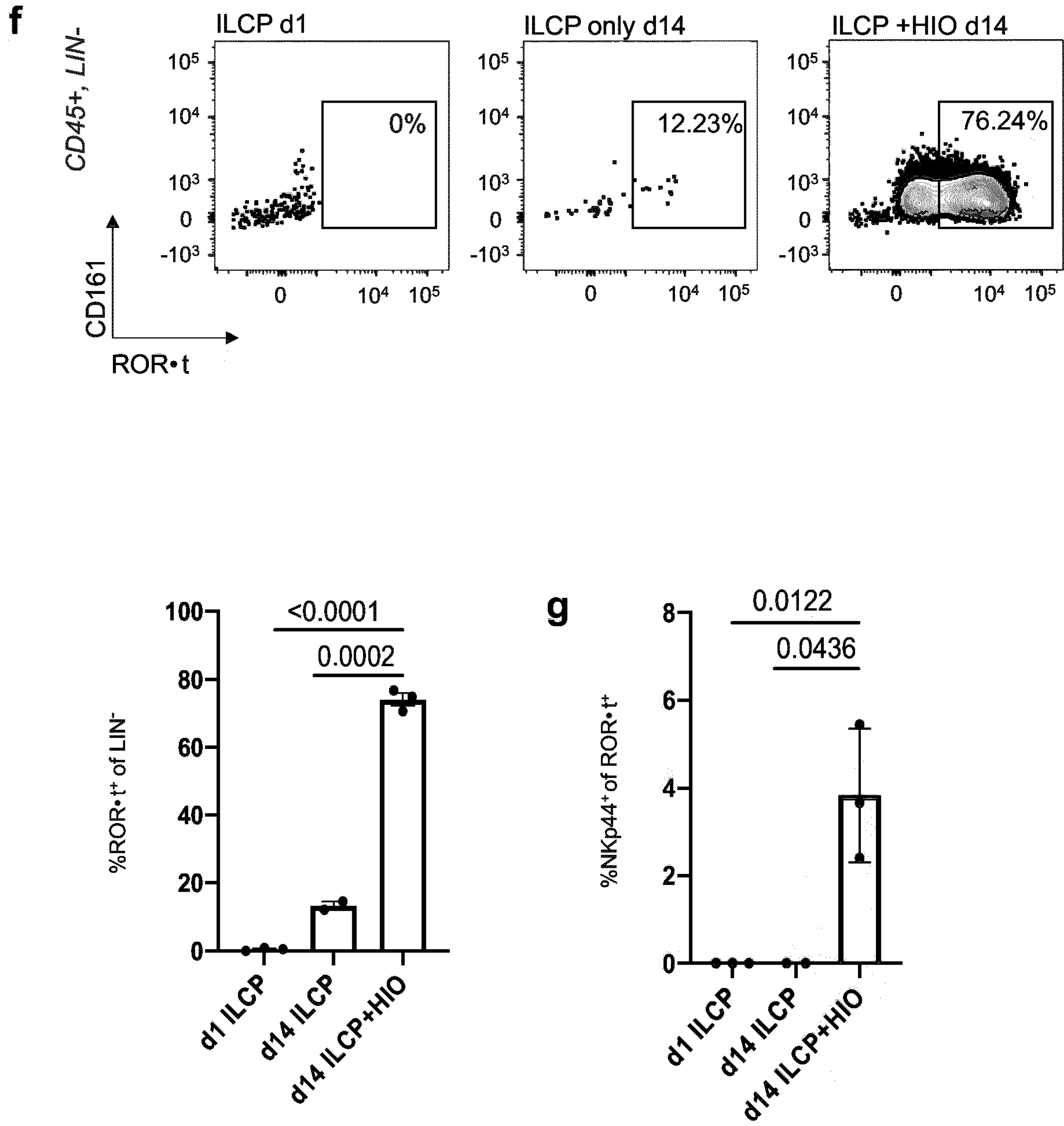

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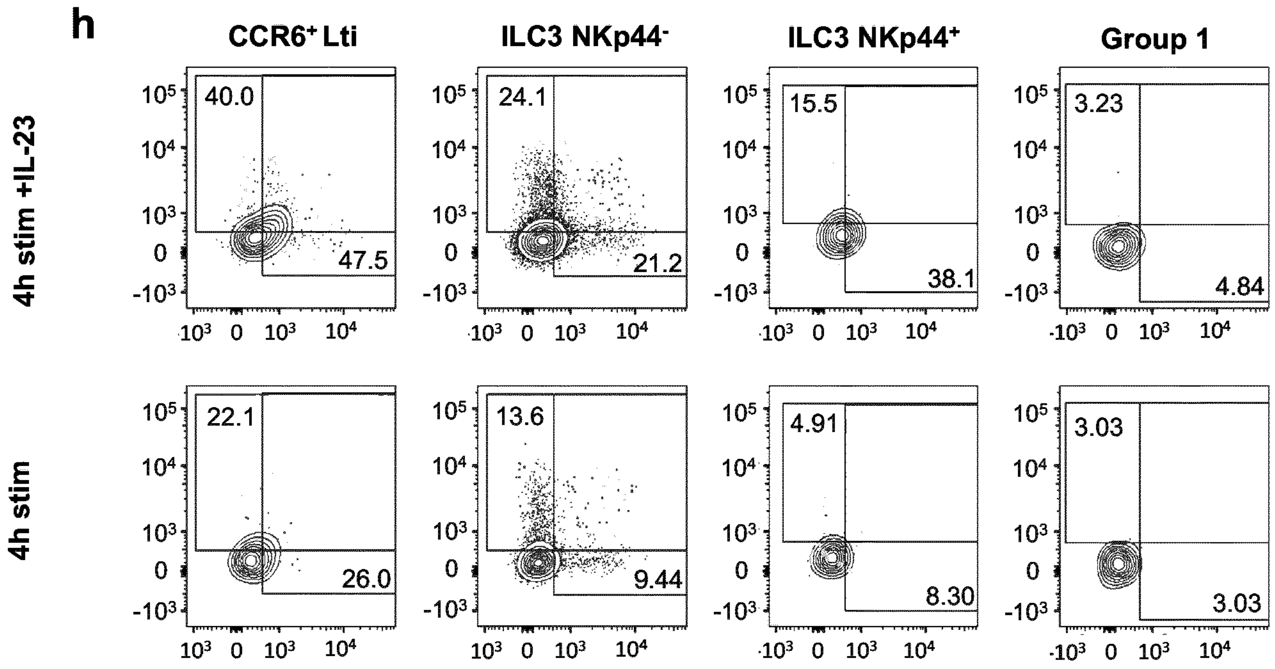

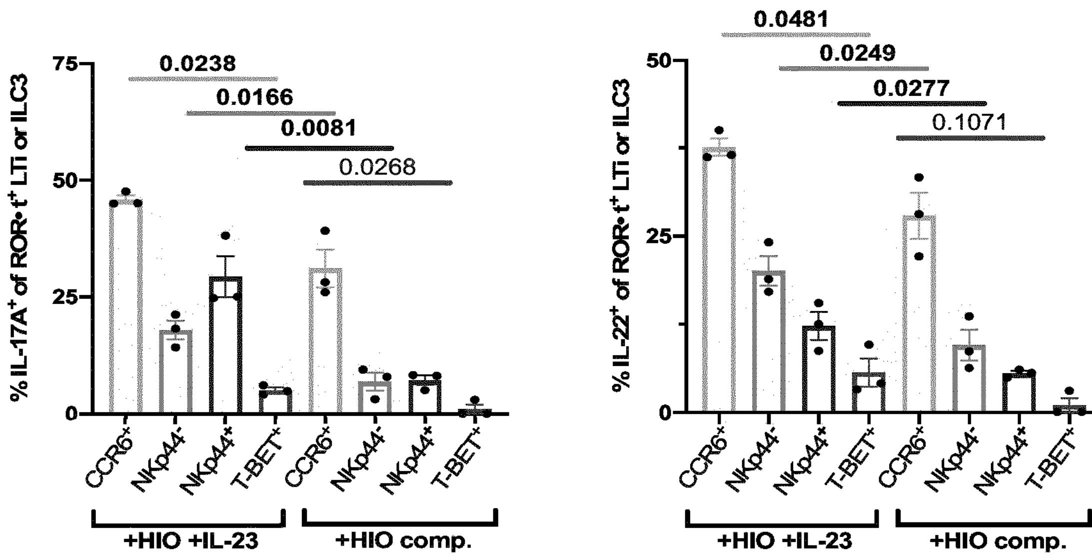

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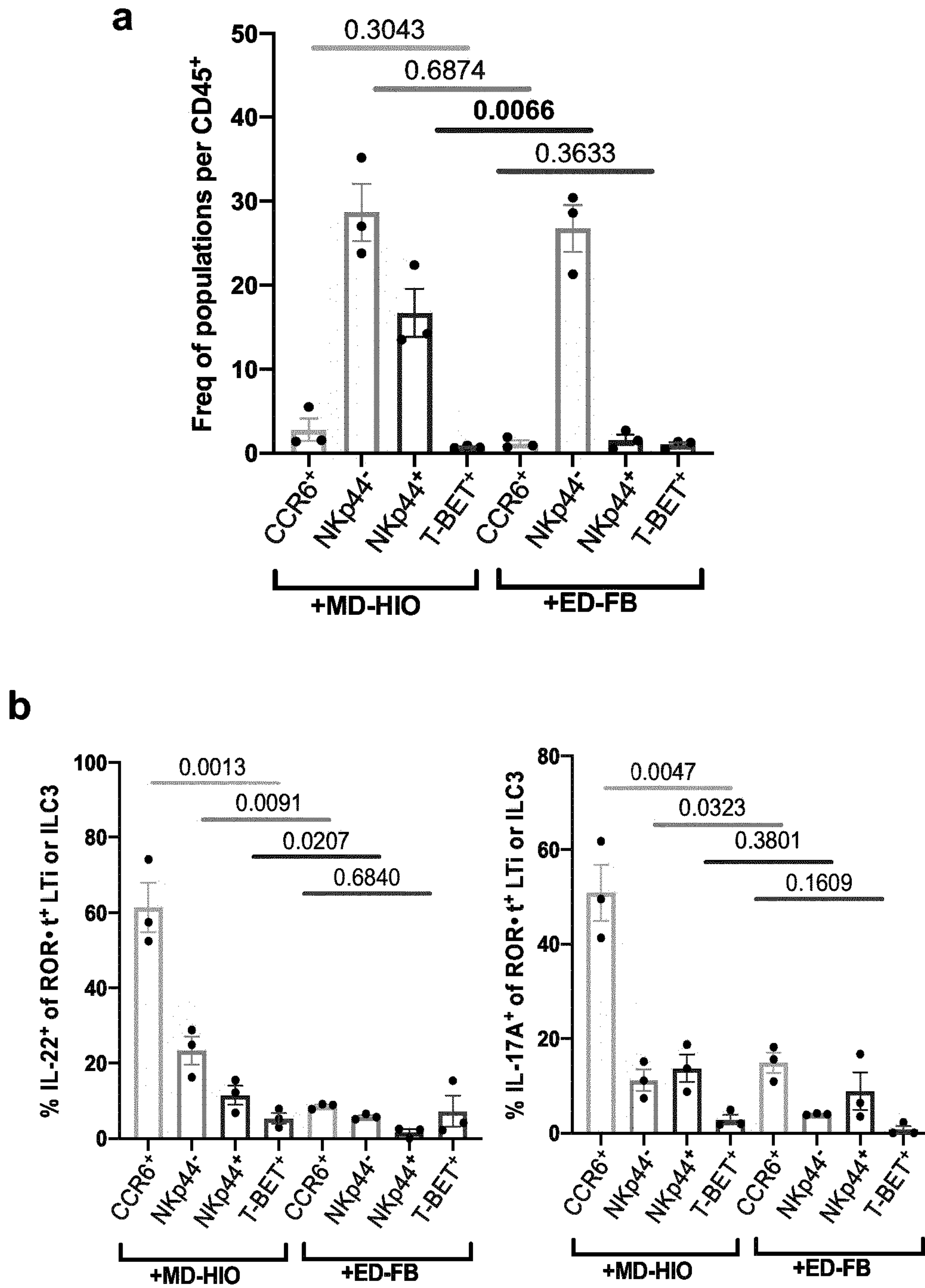
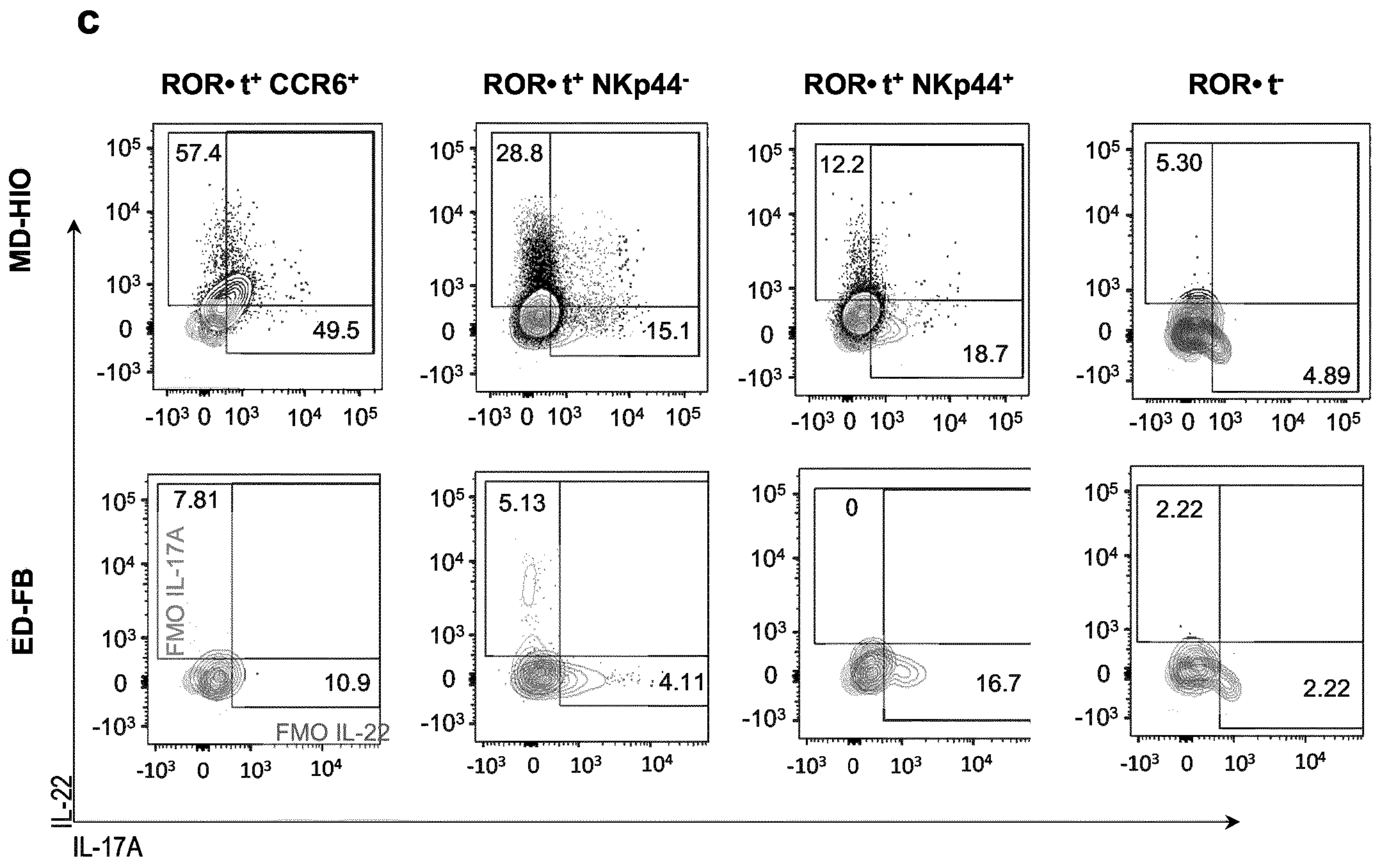


Figure 3



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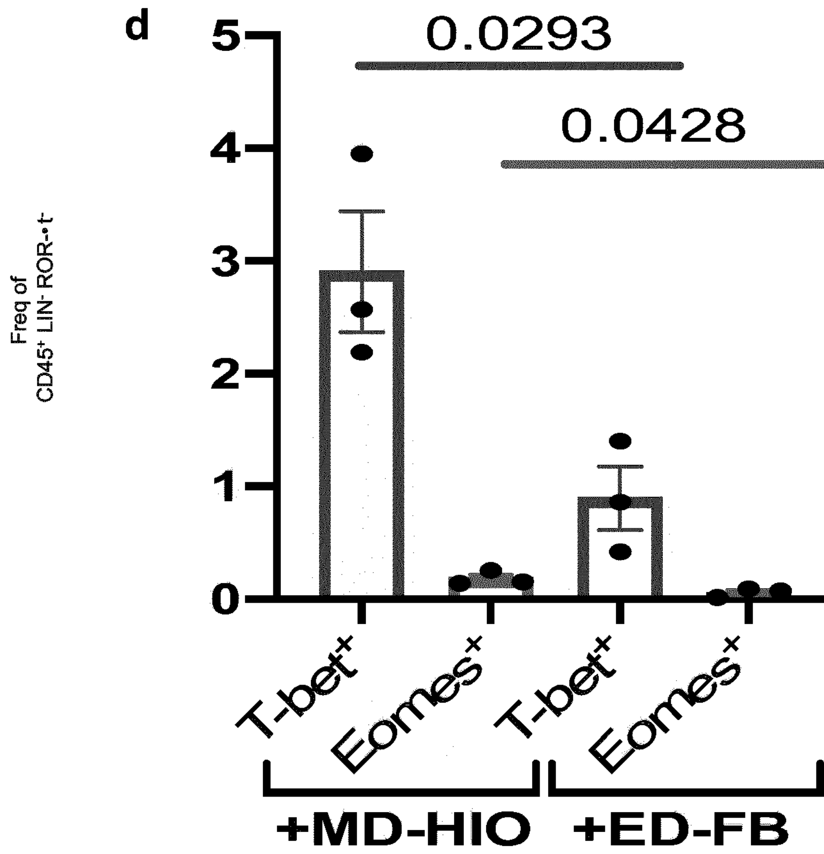

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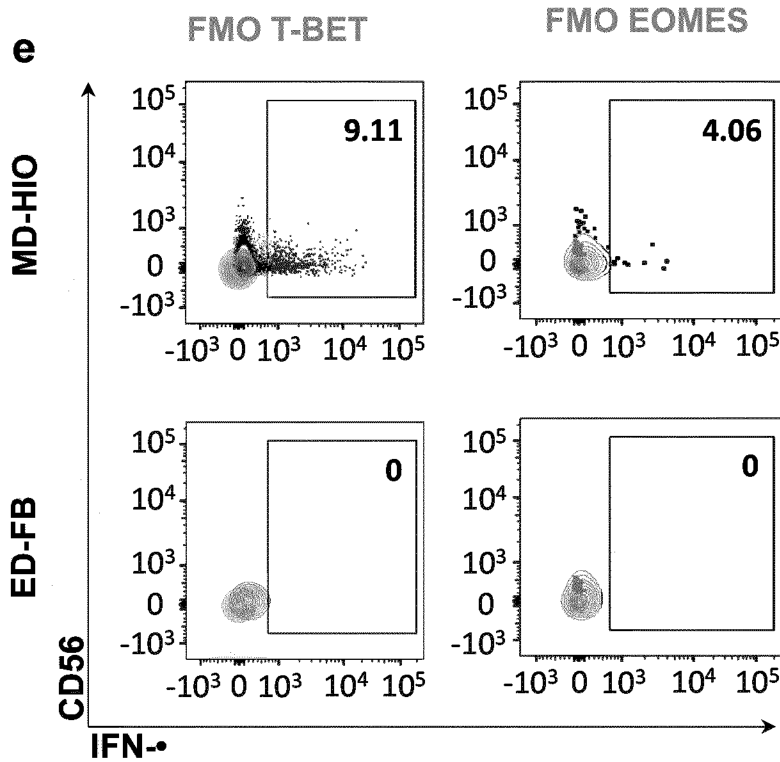

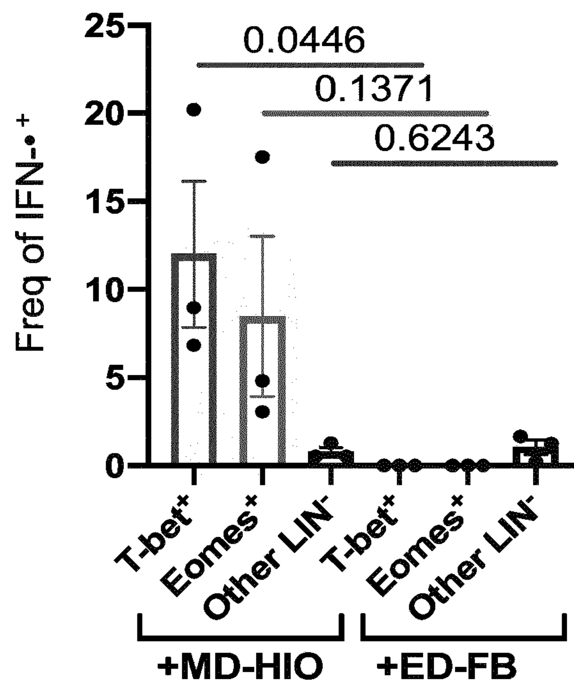

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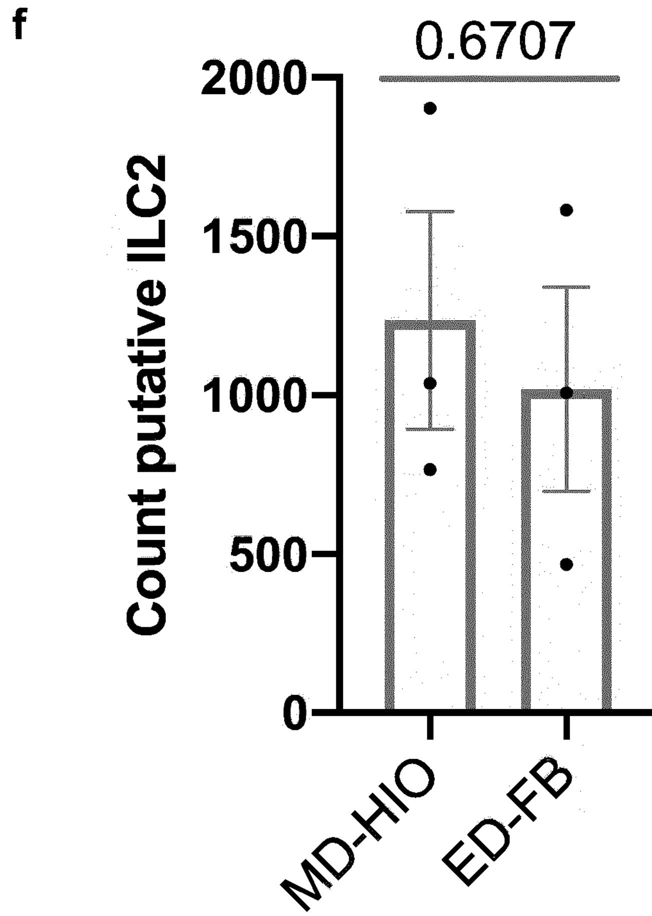

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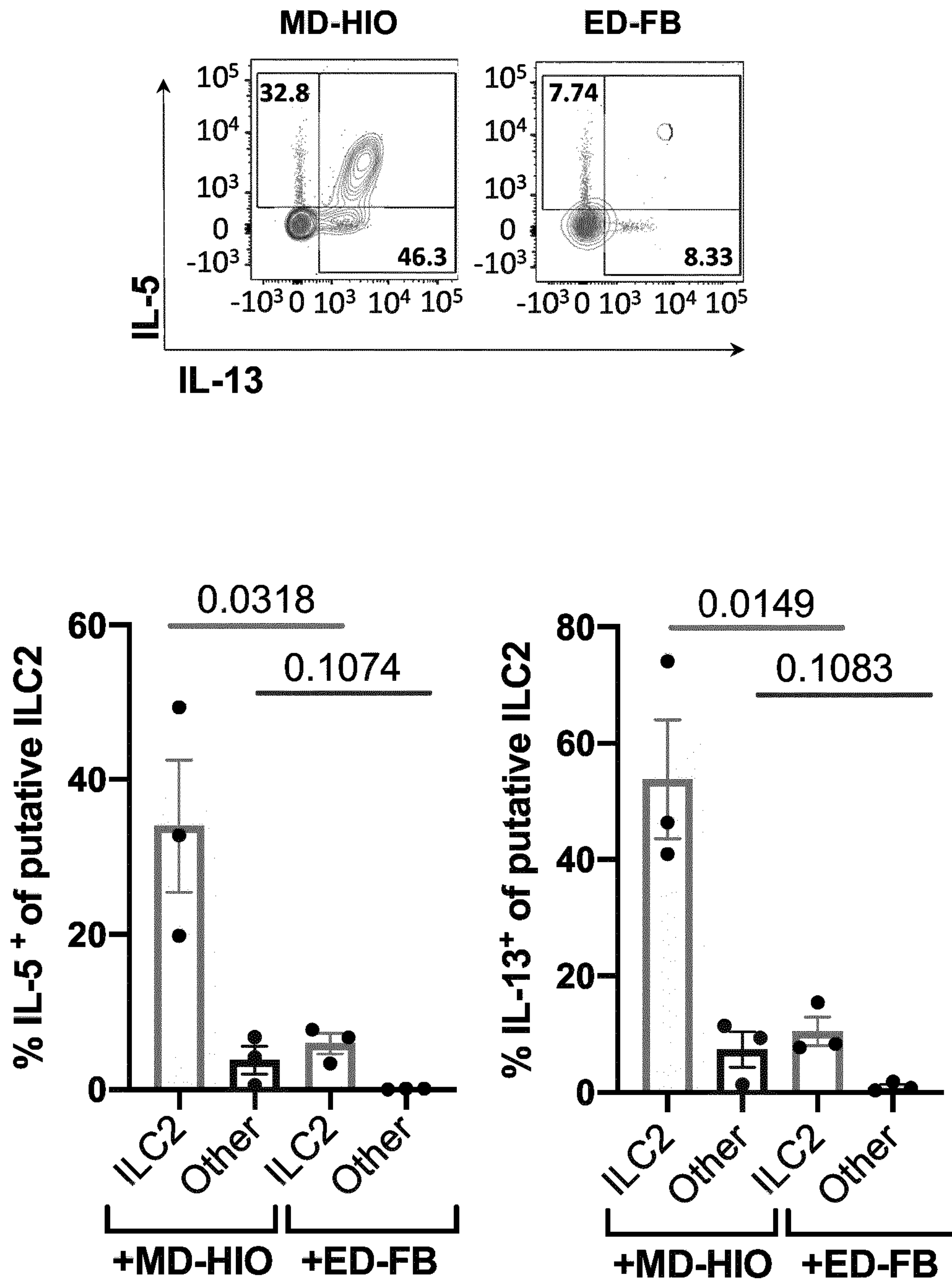

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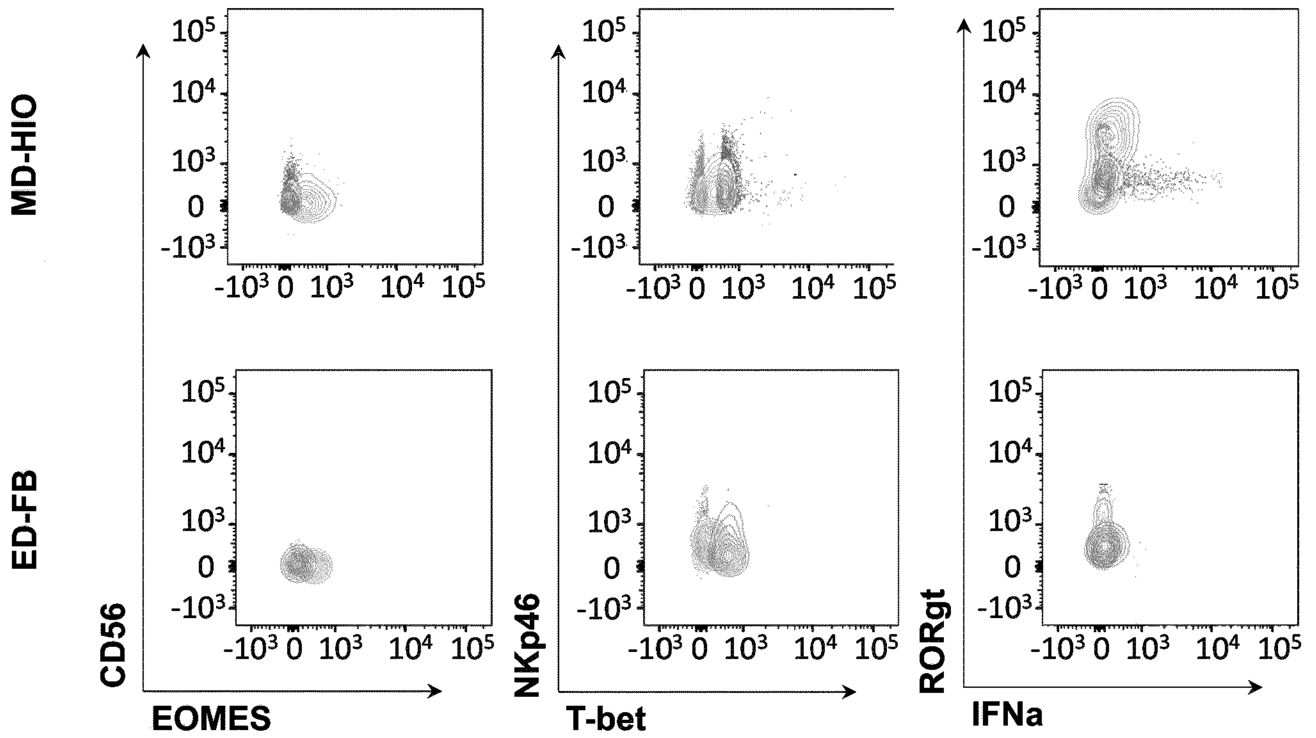

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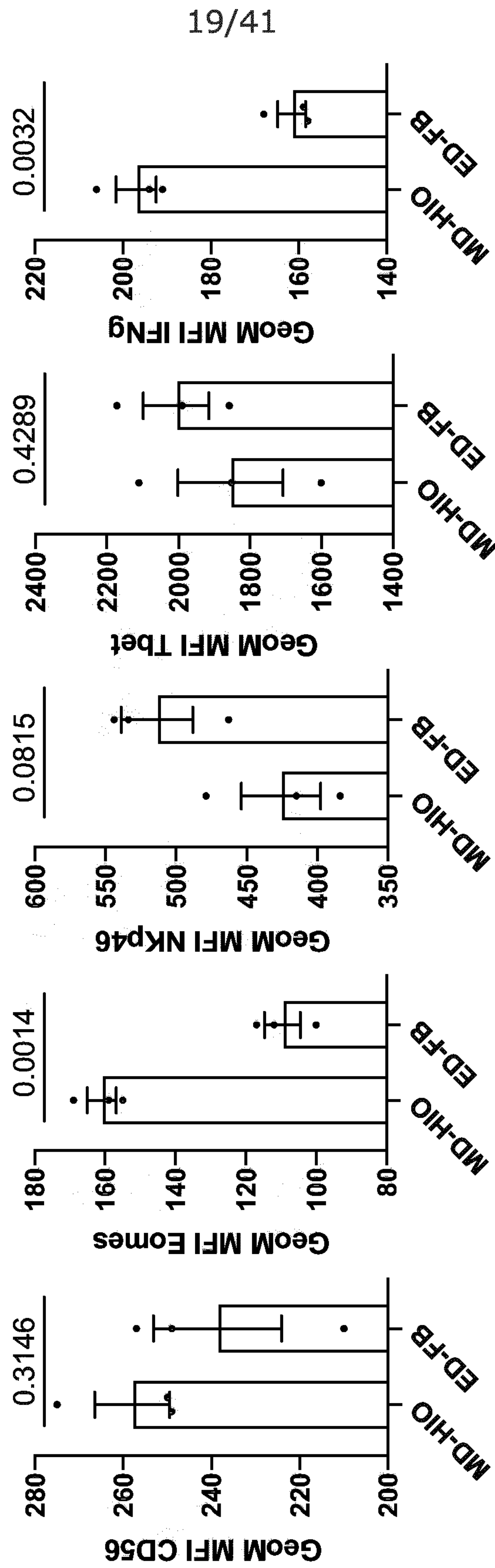

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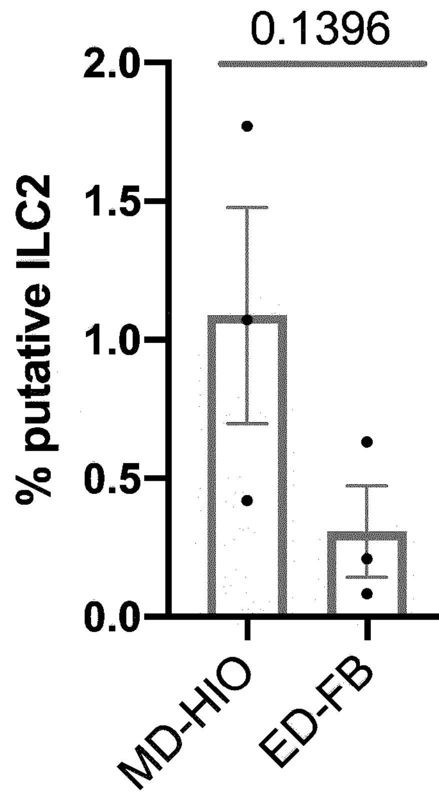
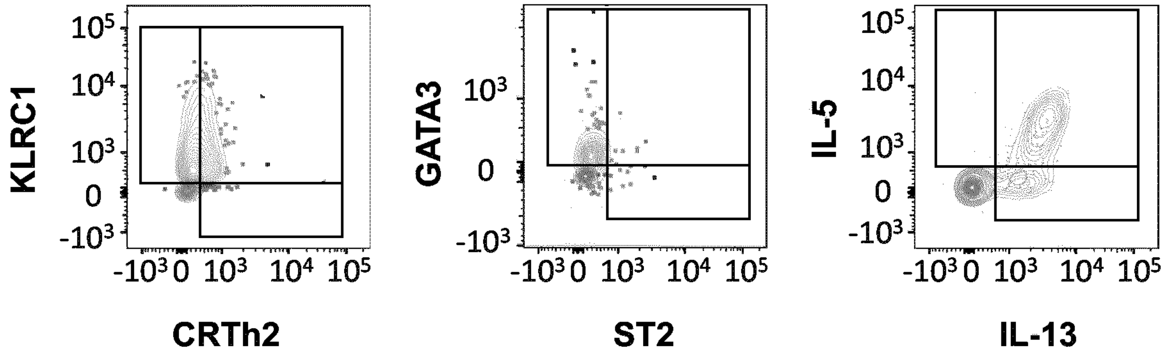
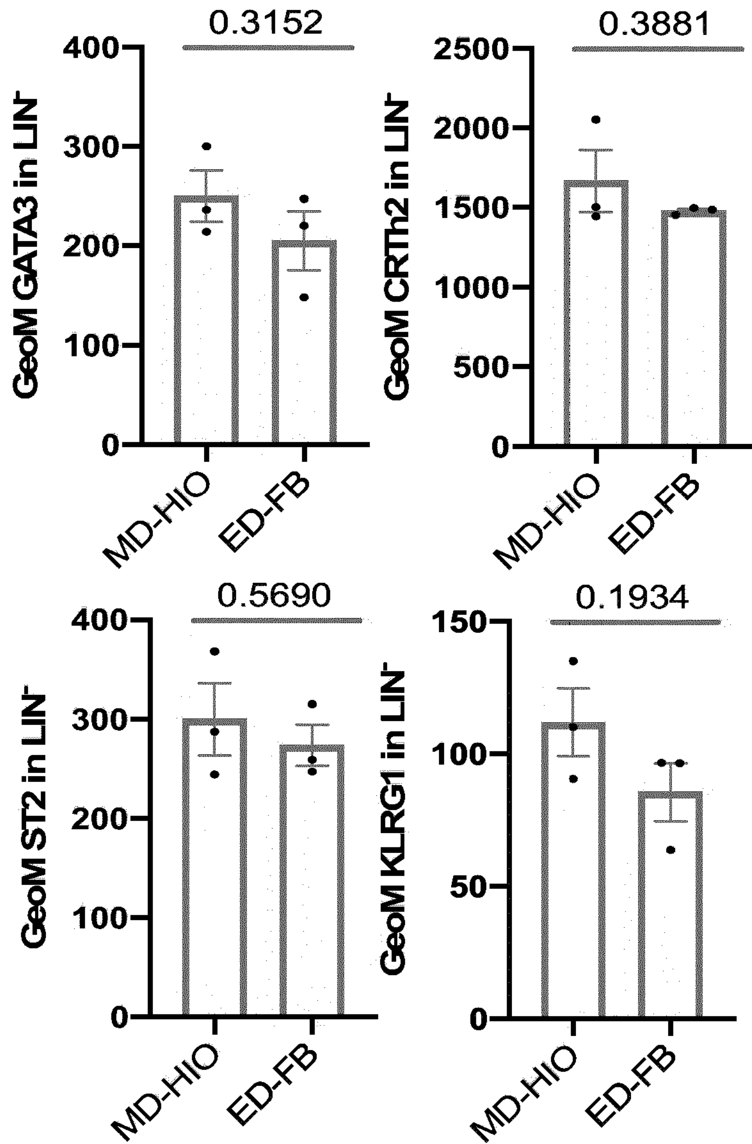


Figure 5

b





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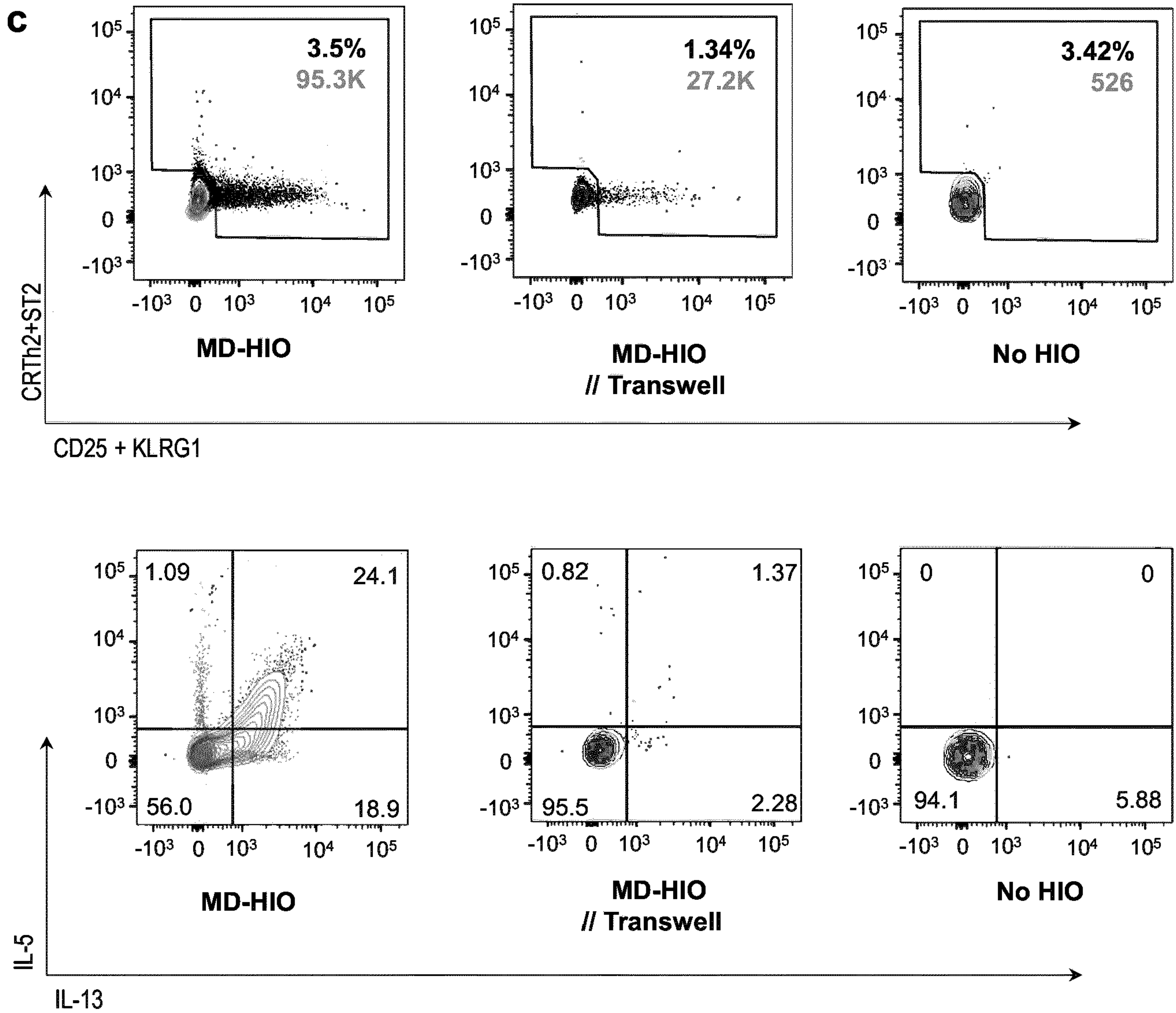

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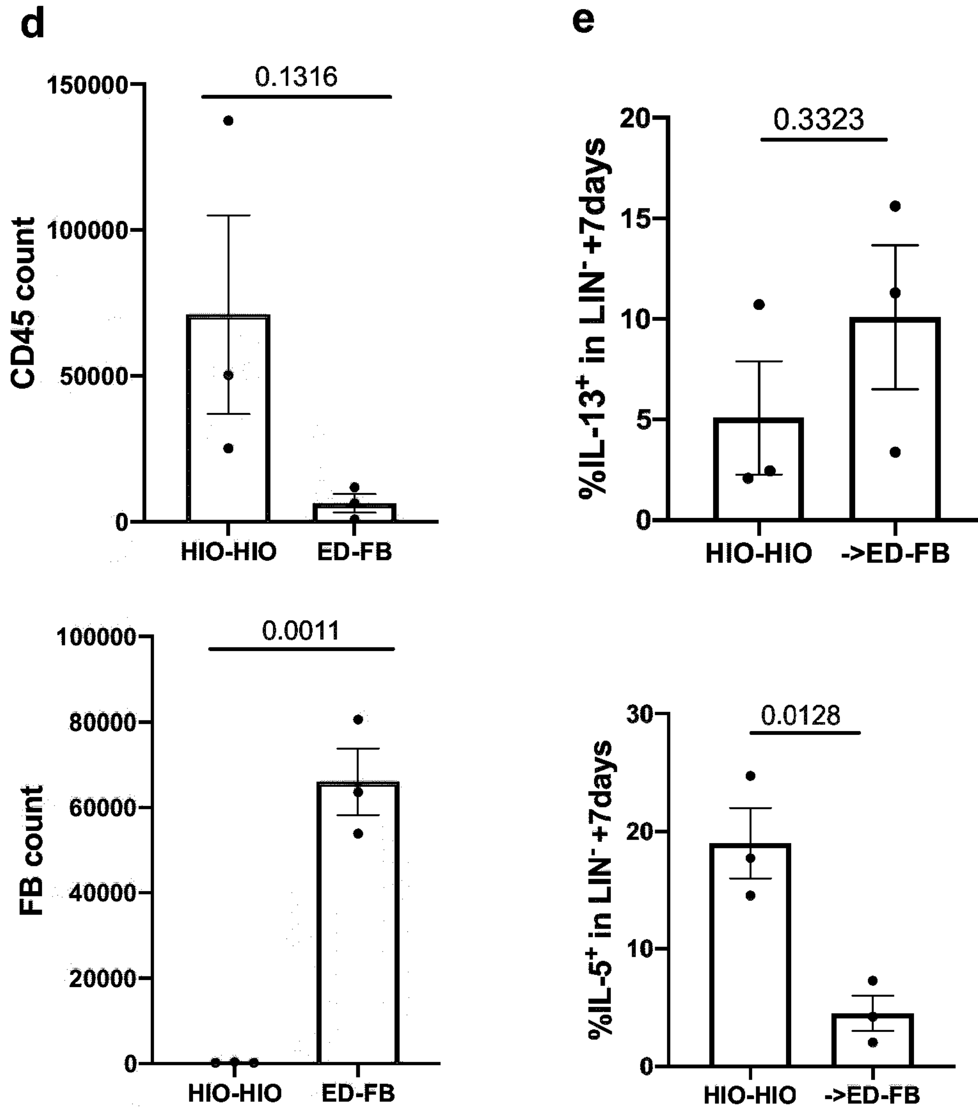

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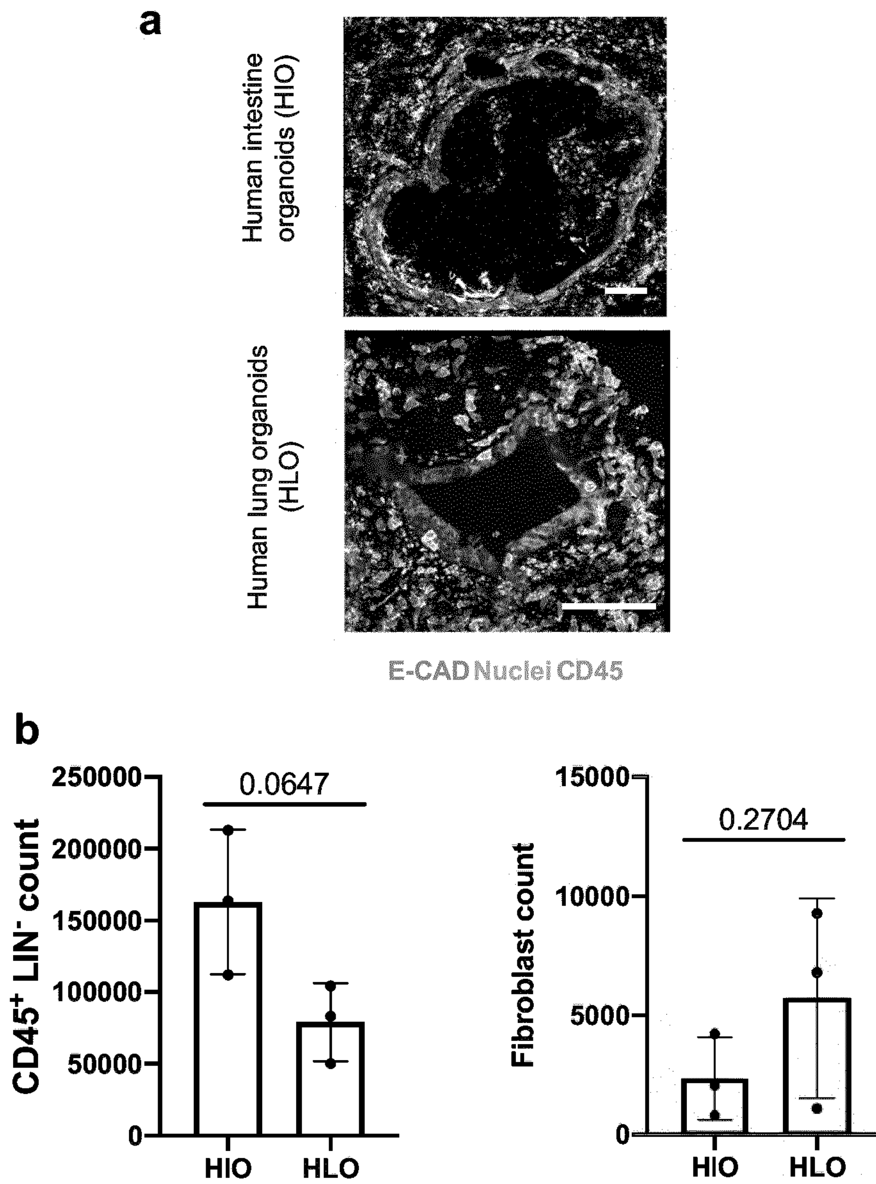

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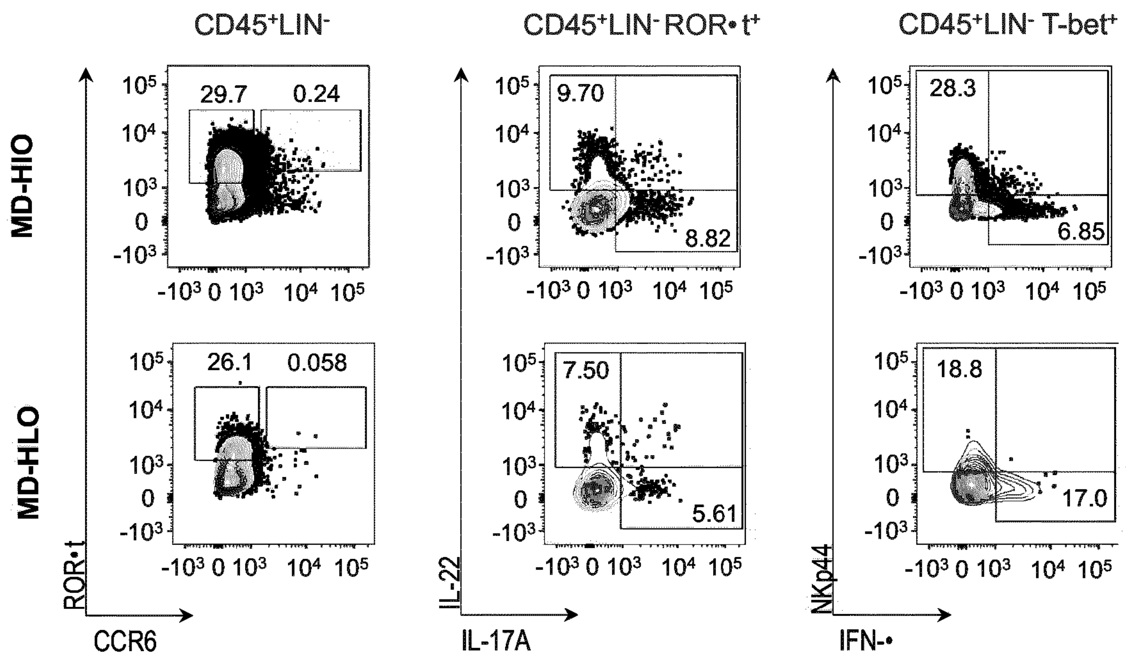

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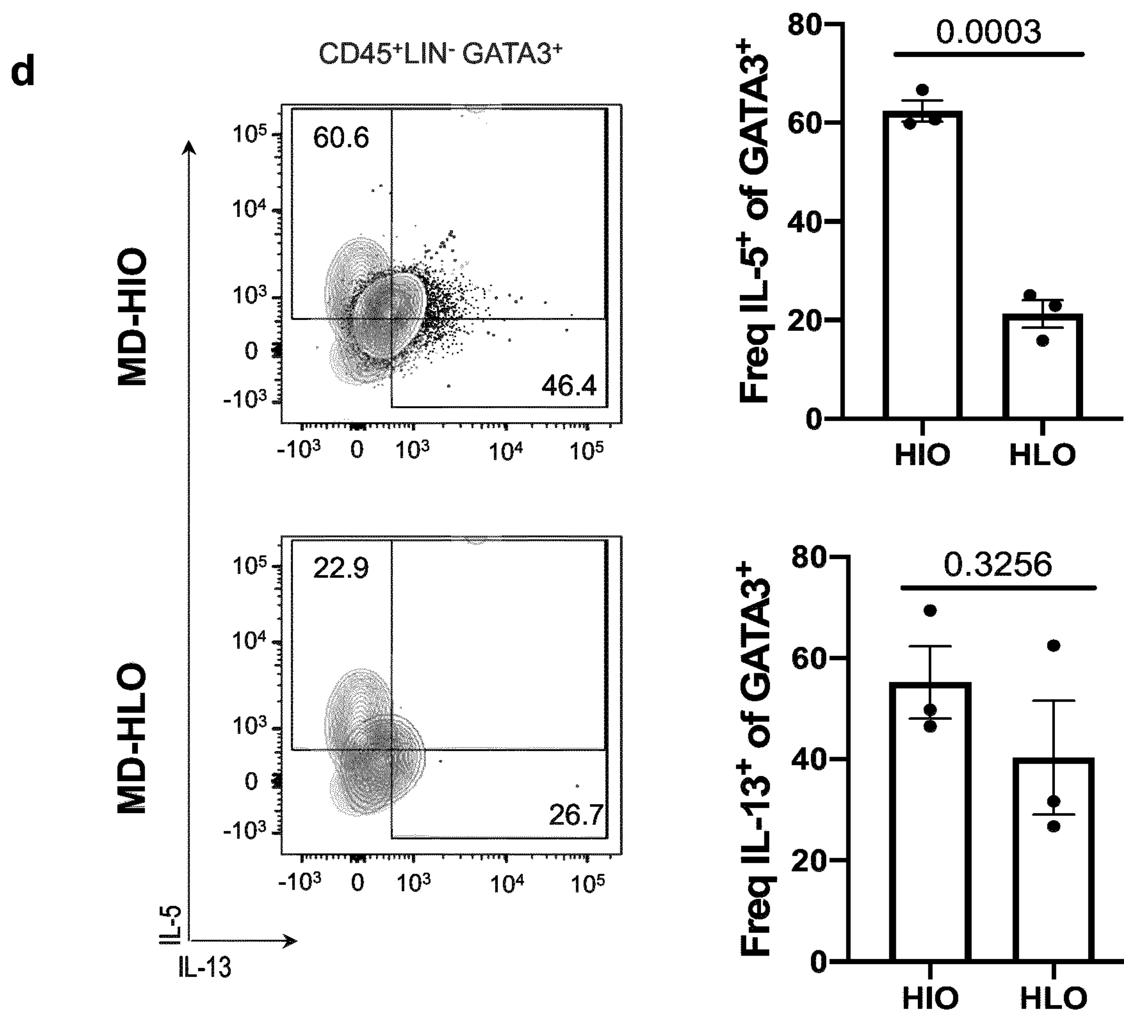

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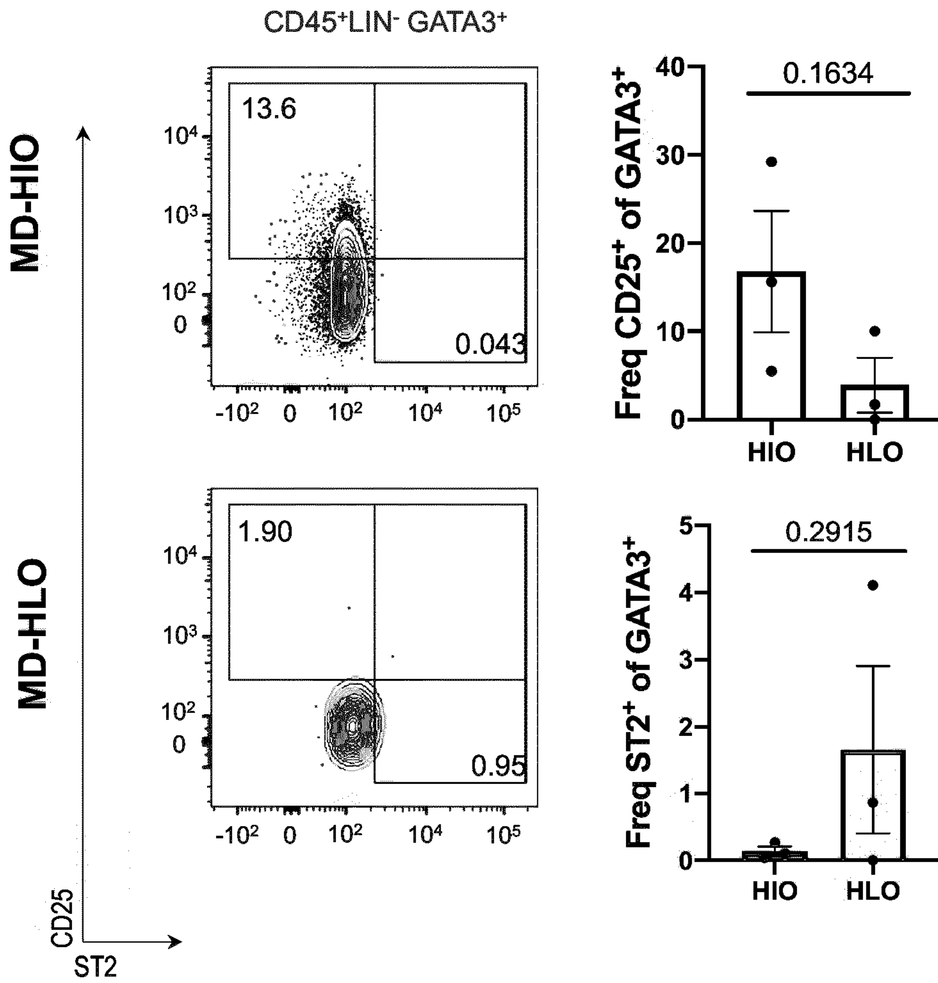

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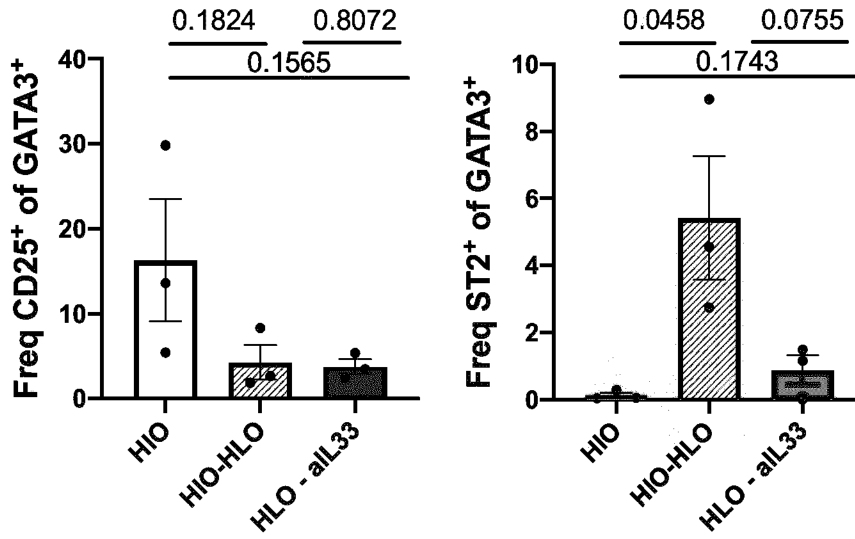

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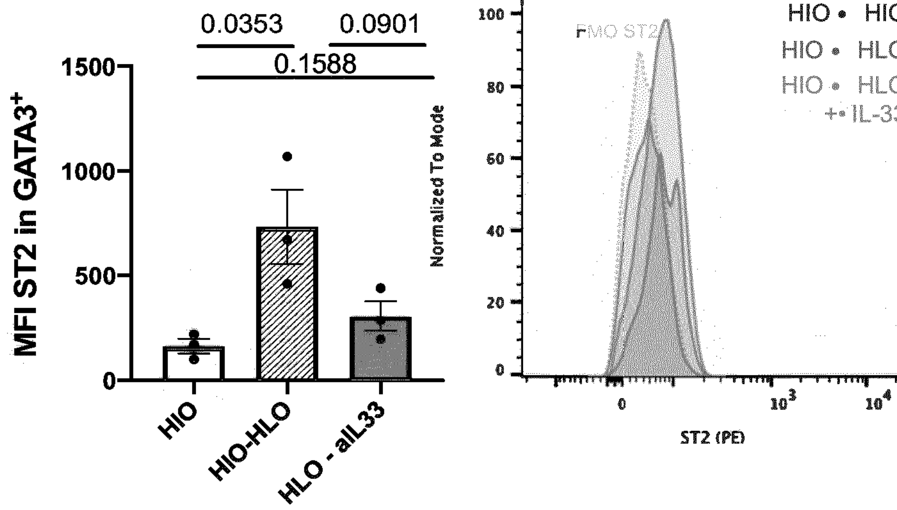

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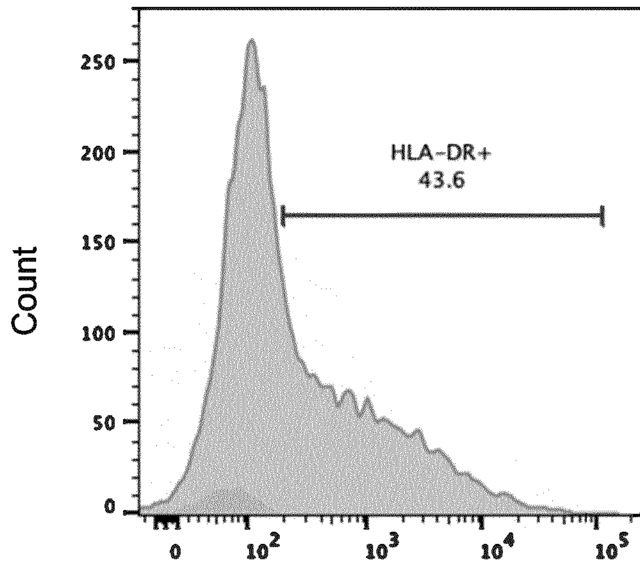

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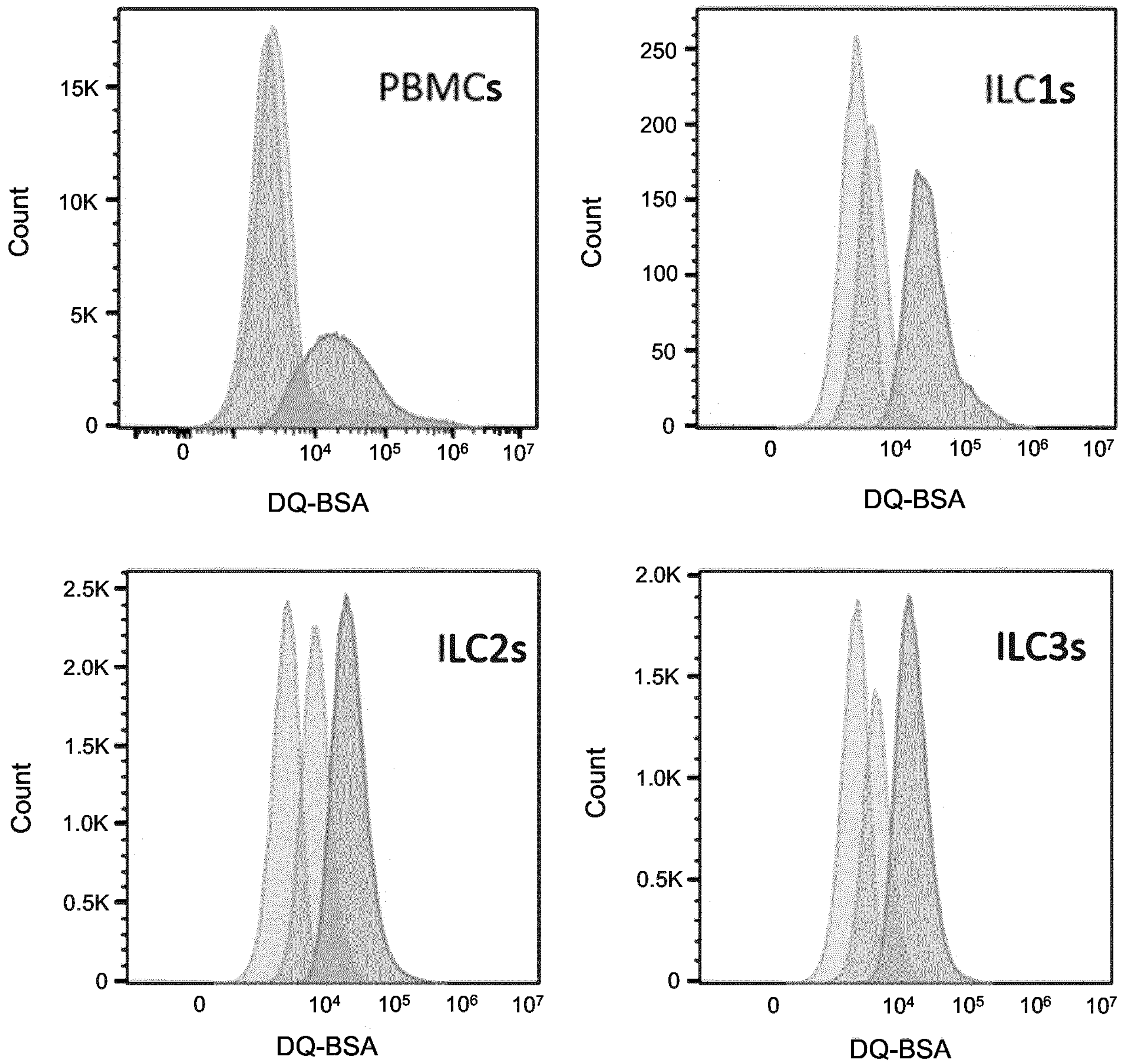


Figure 8a

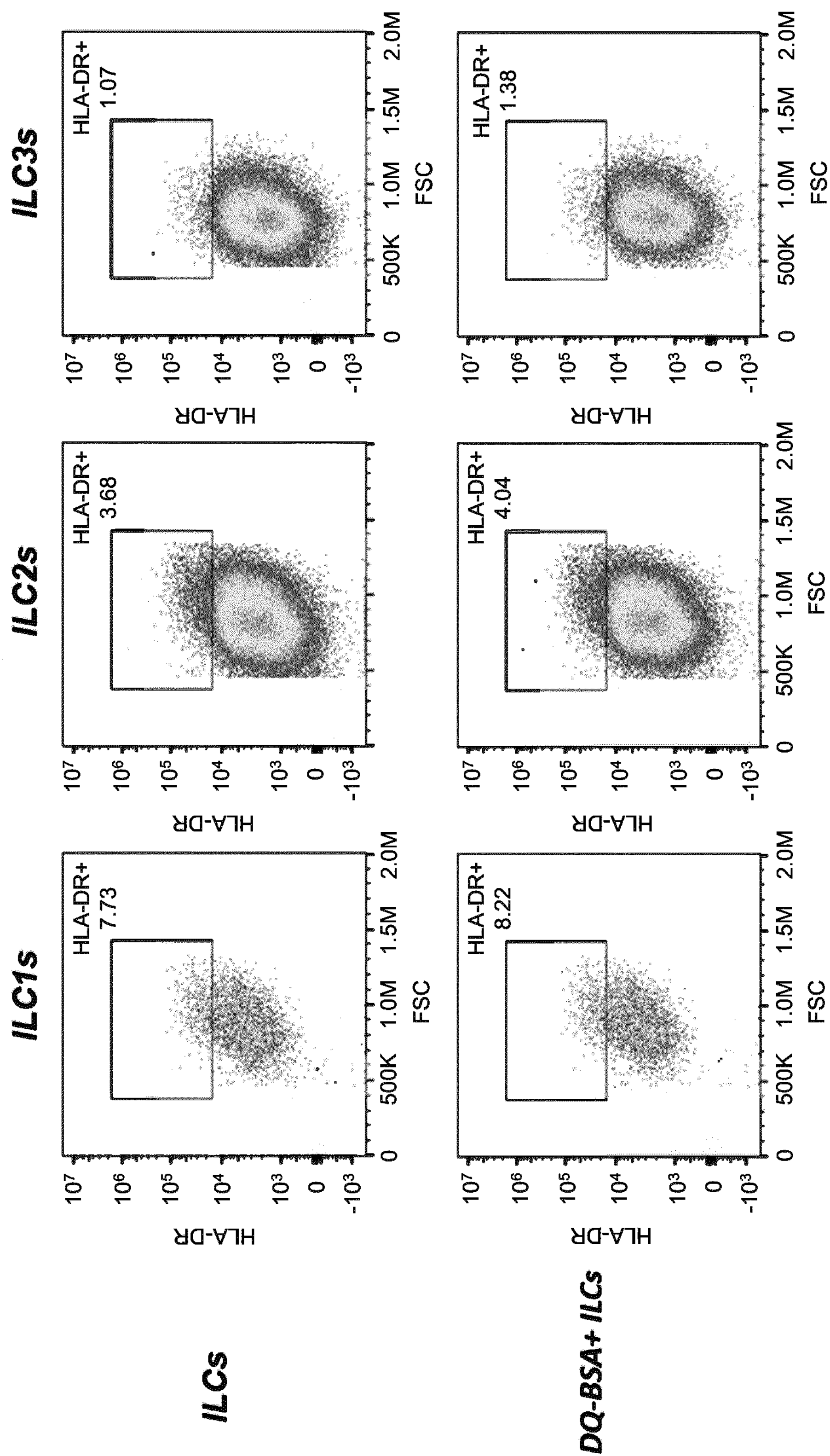
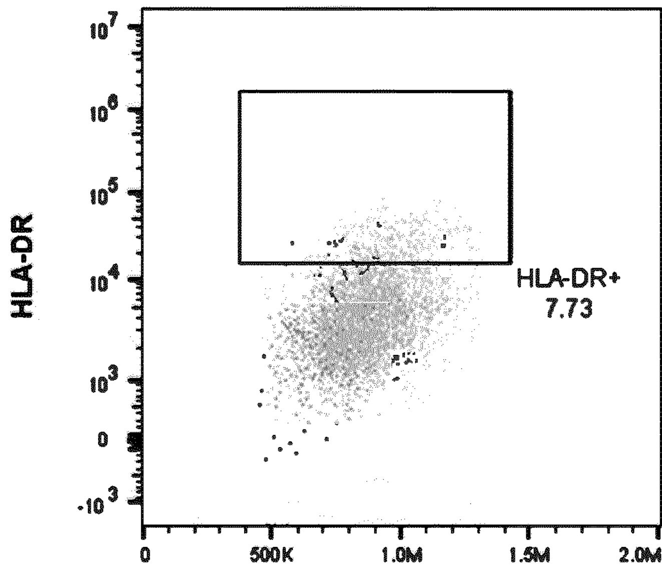
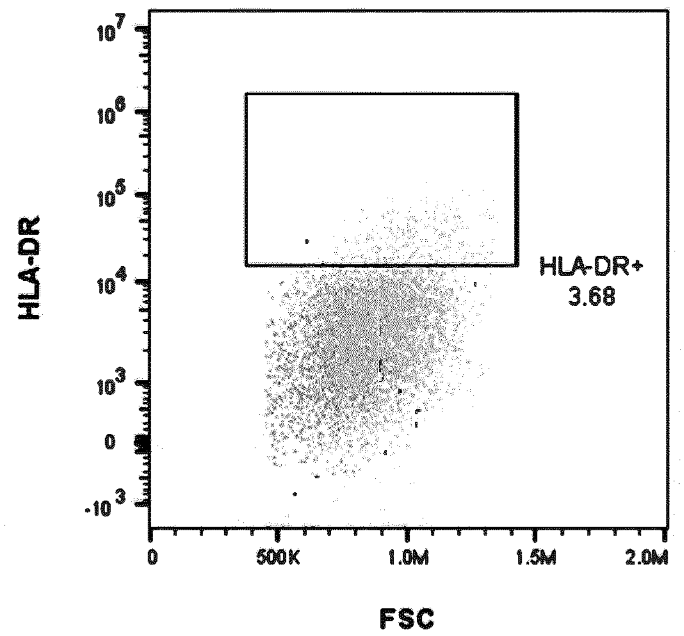


Figure 8b

	DQ-BSA+ ILCs
	DQ-BSA- ILCs





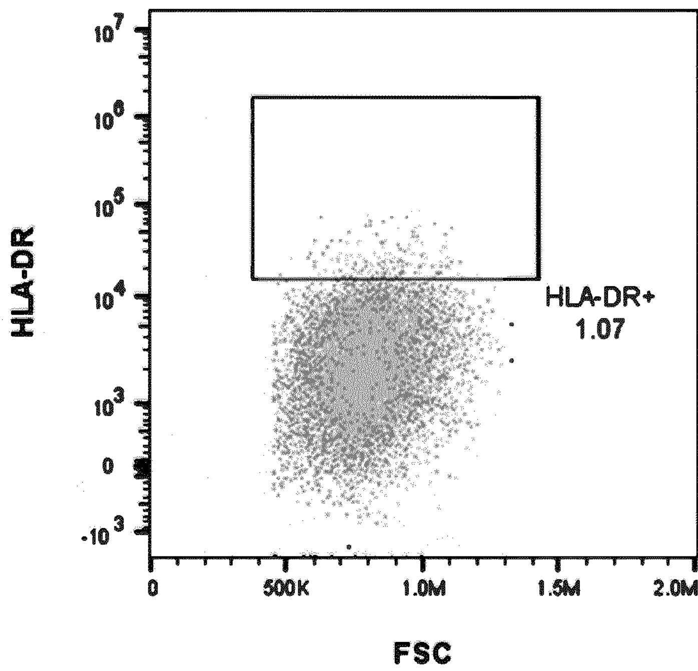


Figure 8c

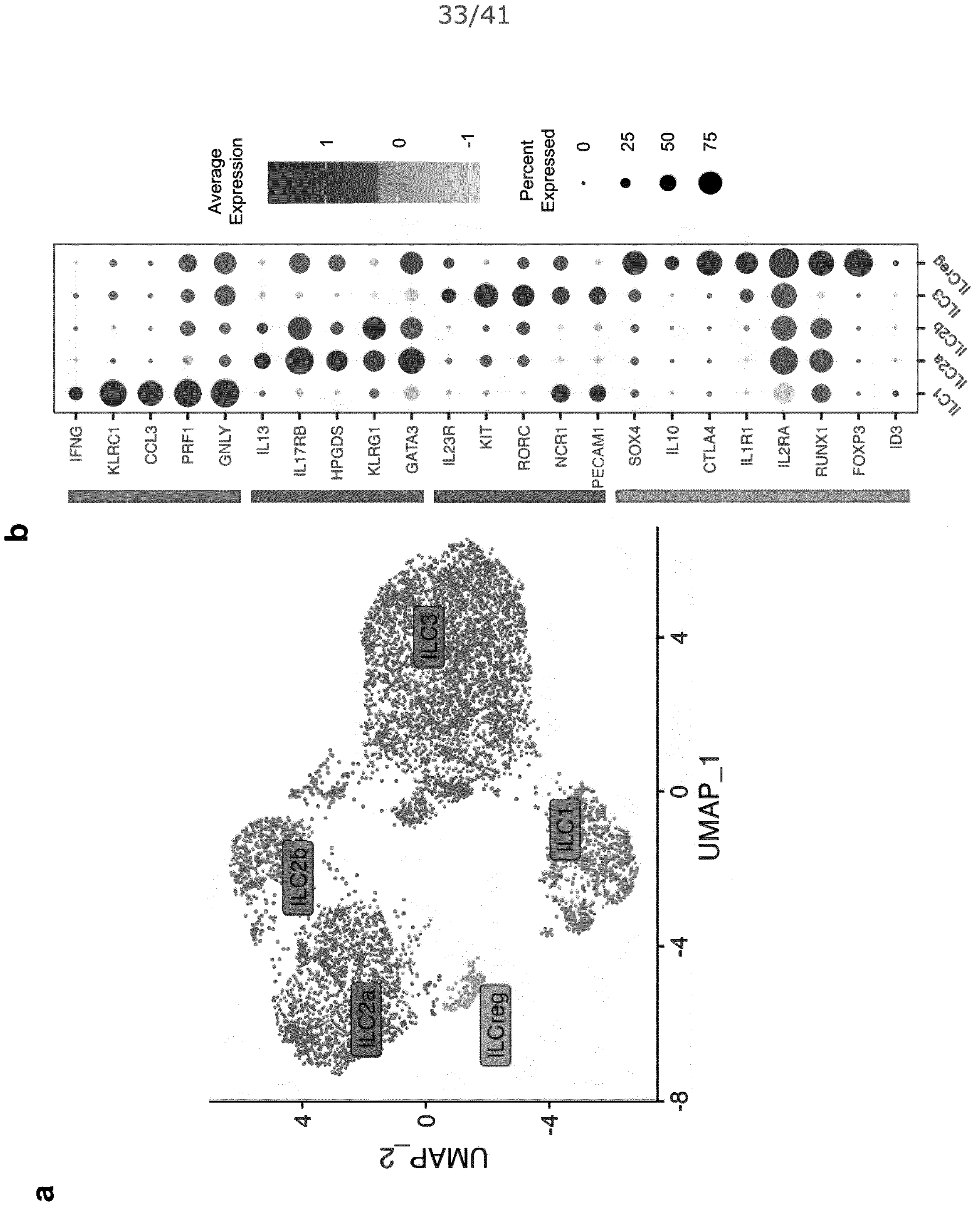
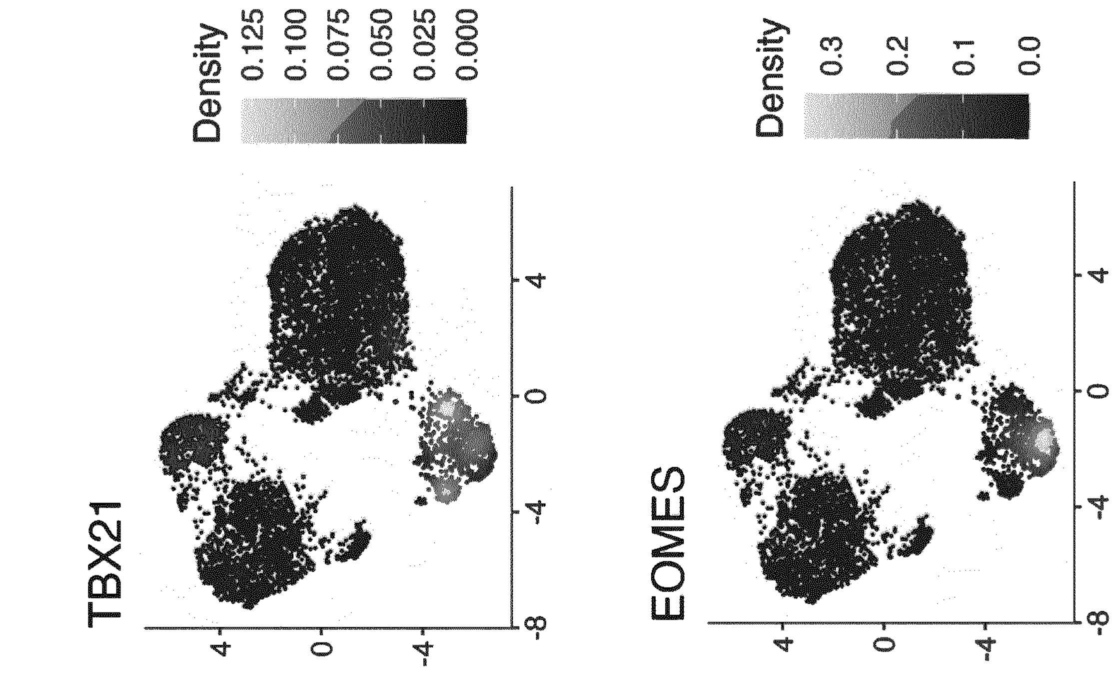


Figure 9

d



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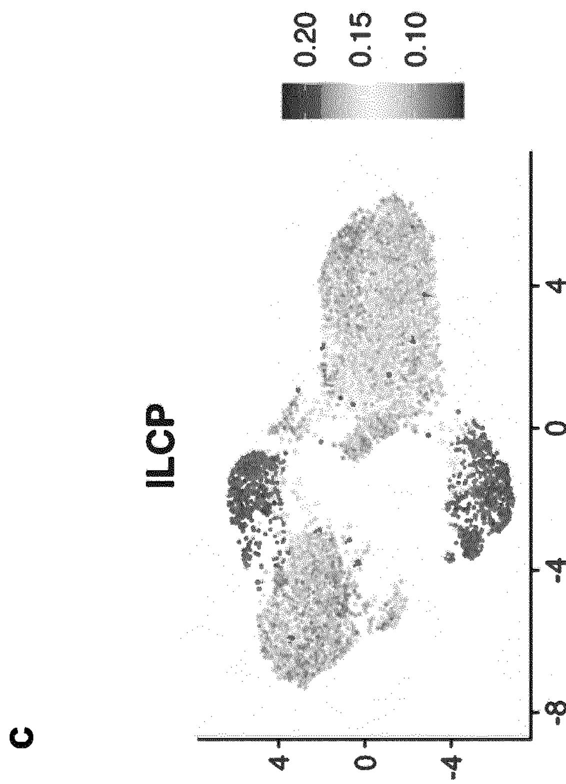
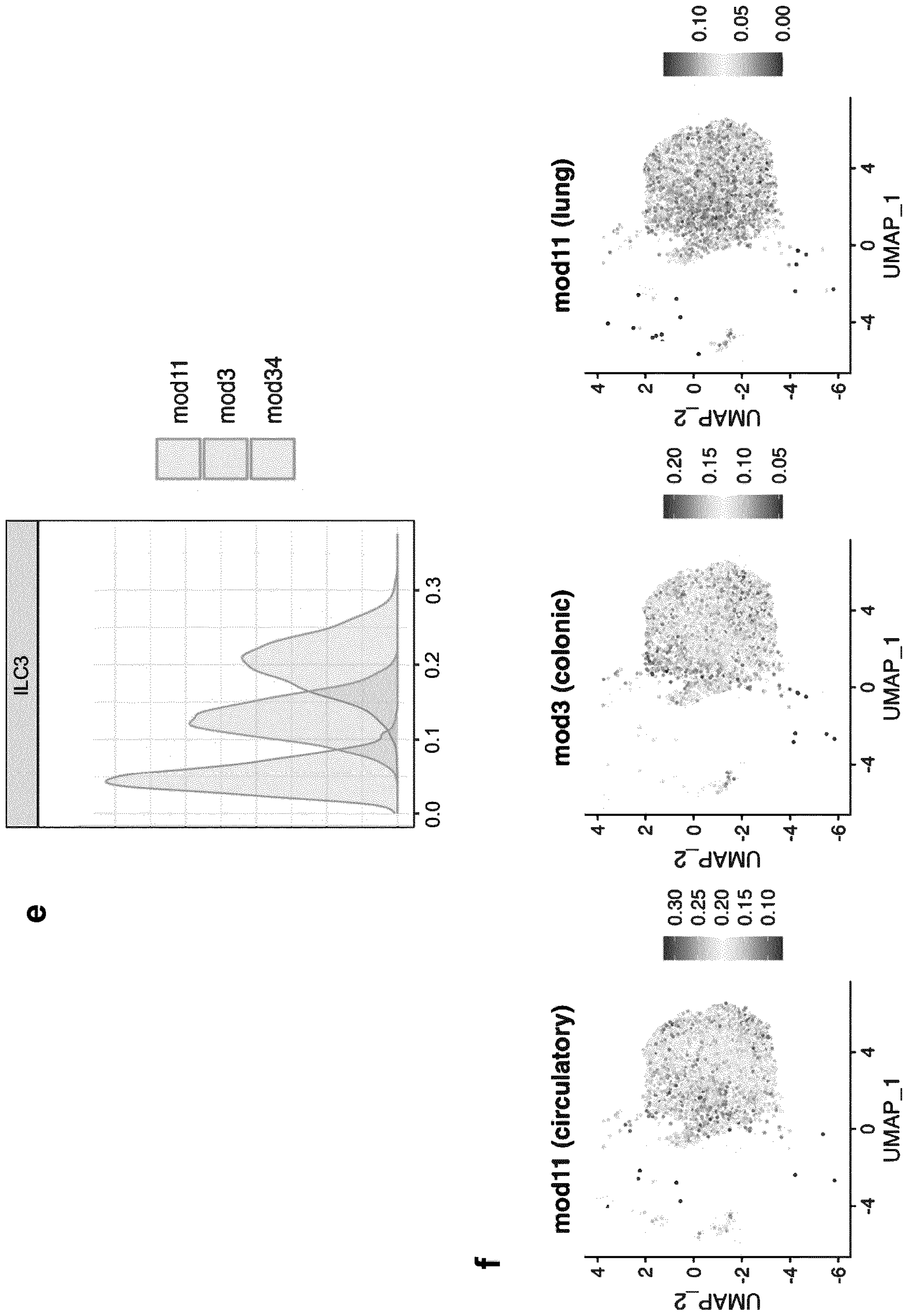


Figure 9 cont



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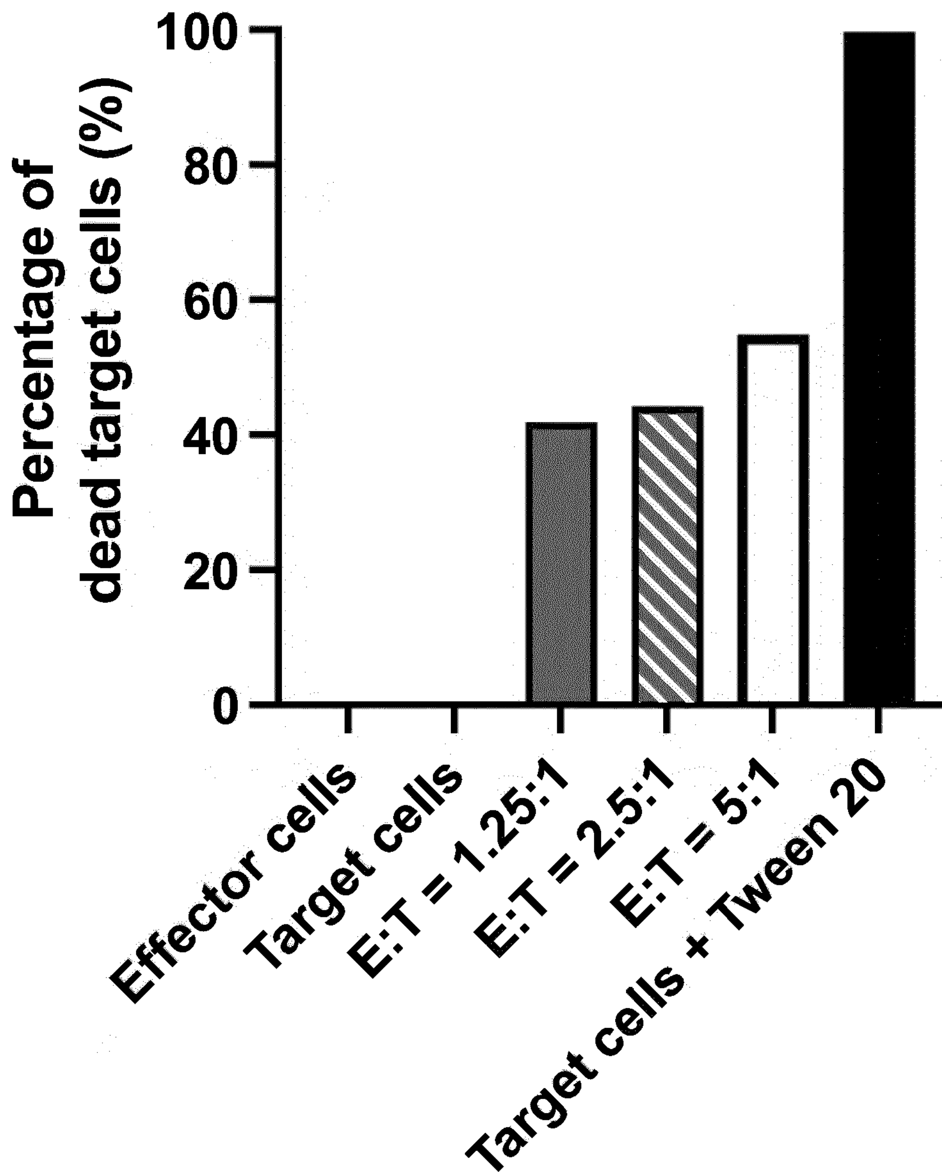
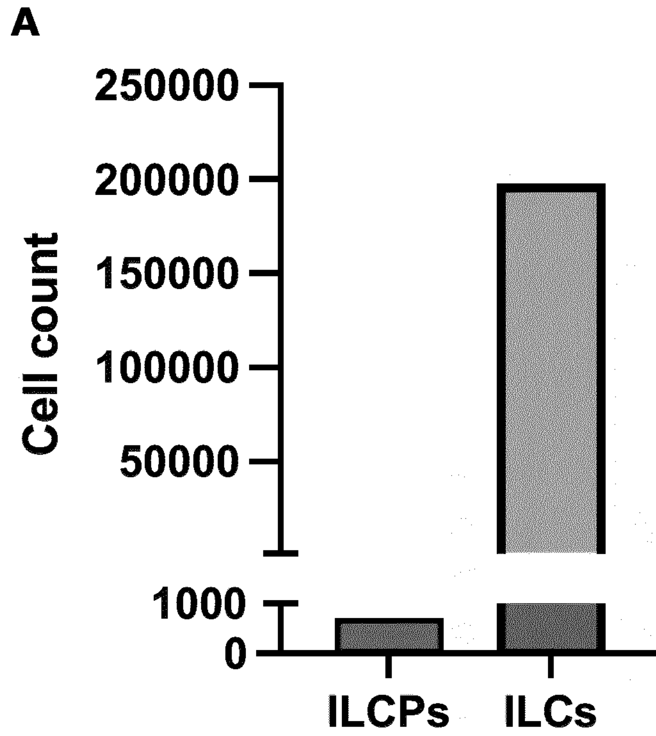


Figure 10

A



B

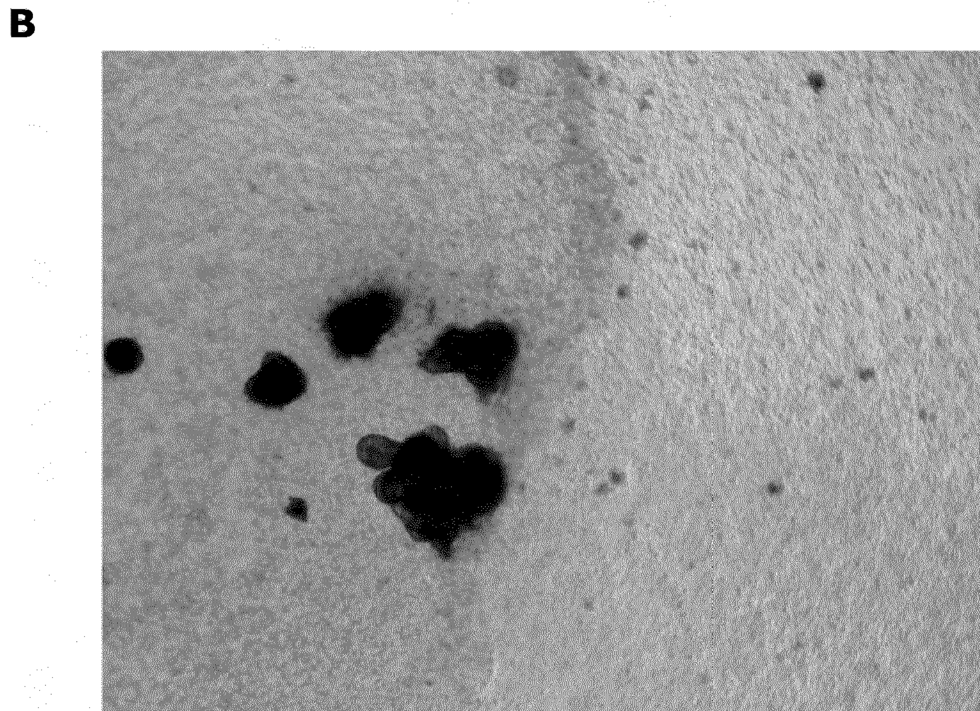


Figure 11

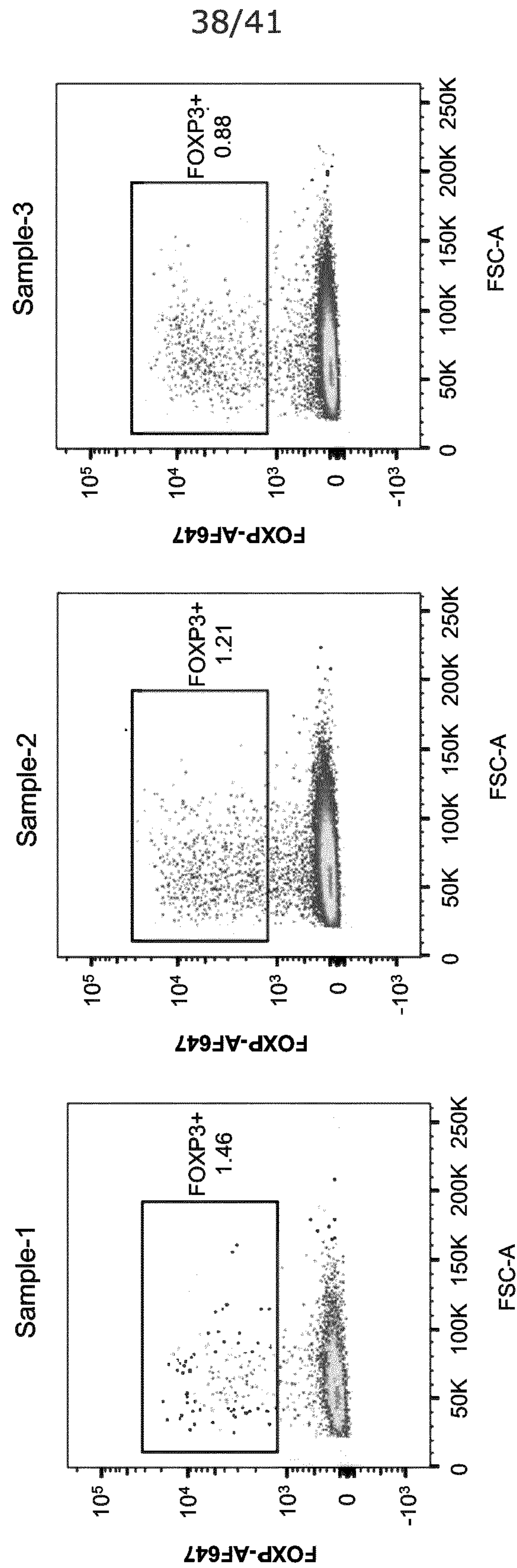


Figure 12A

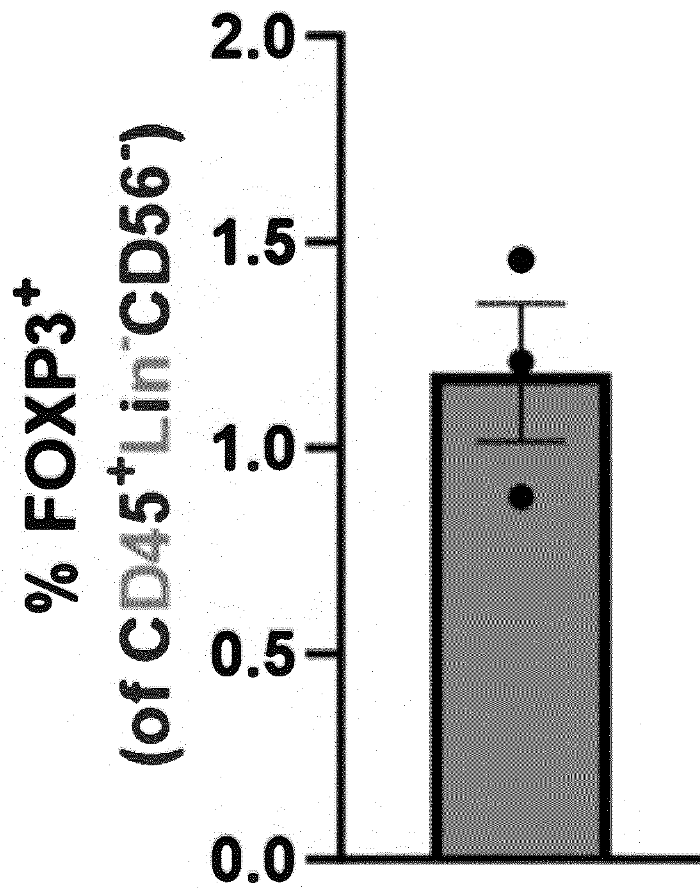


Figure 12B

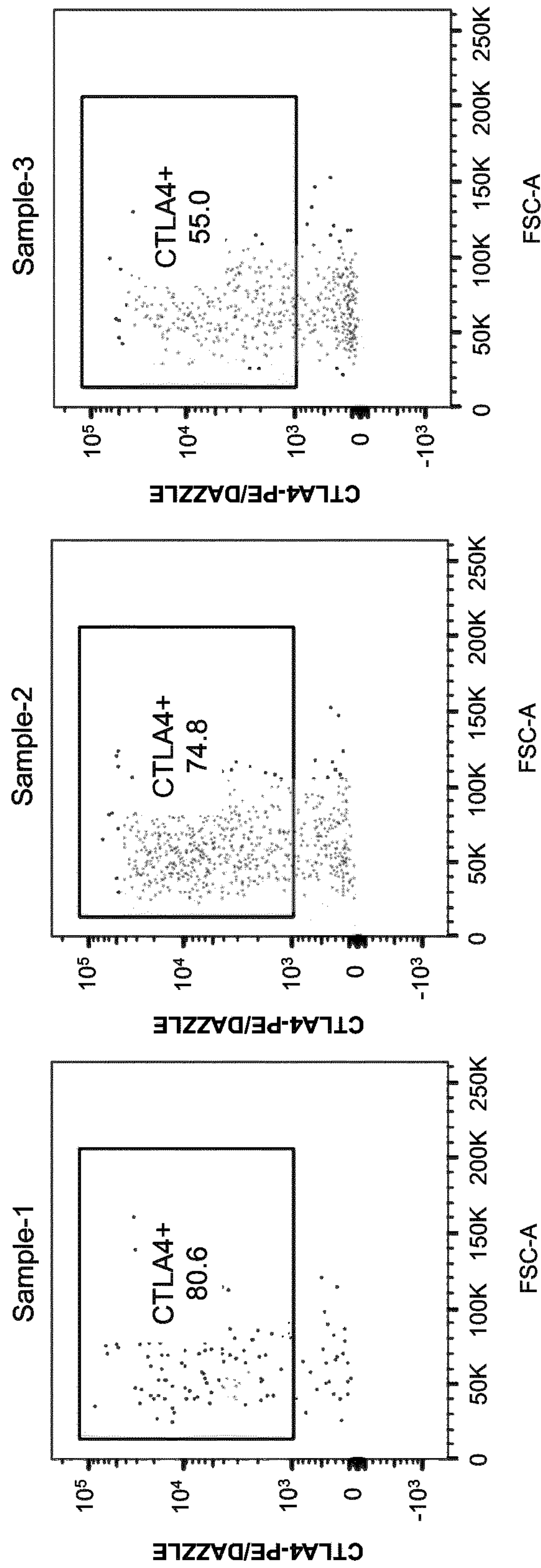


Figure 13A

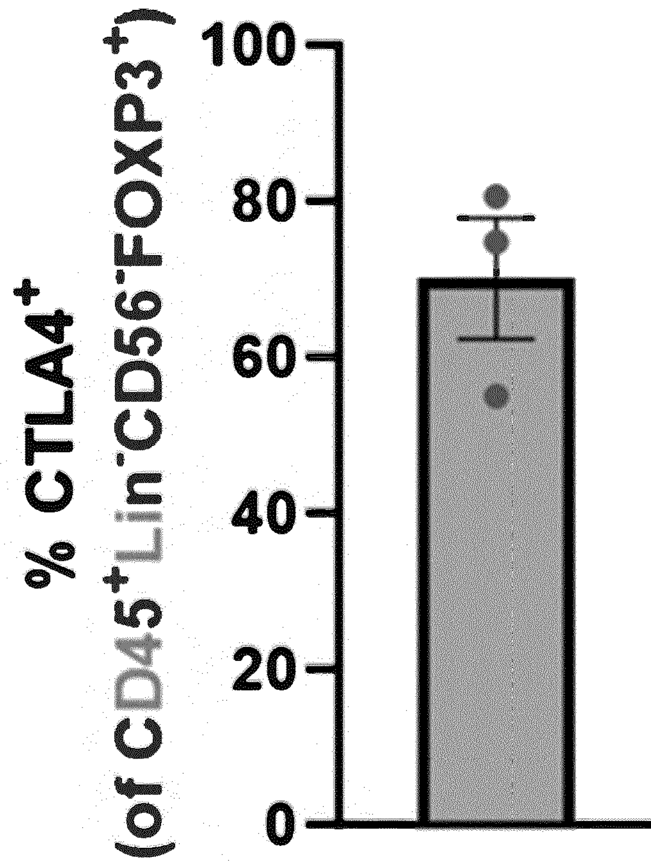


Figure 13B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/066529

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0783
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORITA HIDEAKI ET AL: "Induction of human regulatory innate lymphoid cells from group 2 innate lymphoid cells by retinoic acid", JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 143, no. 6, 22 January 2019 (2019-01-22), page 2190, XP085705064, ISSN: 0091-6749, DOI: 10.1016/J.JACI.2018.12.1018	1-16
Y	the whole document figures 1, 2 -----	18
	-/--	

[X] 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	Date of mailing of the international search report
1 October 2024	15/10/2024

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

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/066529

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WANG SHUO ET AL: "Regulatory Innate Lymphoid Cells Control Innate Intestinal Inflammation", CELL, vol. 171, no. 1, 1 September 2017 (2017-09-01), pages 201-216.e18, XP093123830, Amsterdam NL ISSN: 0092-8674, DOI: 10.1016/j.cell.2017.07.027 Retrieved from the Internet: URL:https://www.sciencedirect.com/science/article/pii/S0092867417308632?via%3Dihub> the whole document figure S1 -----	1-16
X	READ EMILY ET AL: "Co-Culture of Murine Small Intestine Epithelial Organoids with Innate Lymphoid Cells", JOURNAL OF VISUALIZED EXPERIMENTS, no. 181, 23 March 2022 (2022-03-23), pages 1-21, XP093072805, US ISSN: 1940-087X, DOI: 10.3791/63554 Retrieved from the Internet: URL:http://dx.doi.org/10.3791/63554> the whole document -----	17
Y	JOWETT GERALDINE M. ET AL: "Organoids capture tissue-specific innate lymphoid cell development in mice and humans", CELL REPORTS, vol. 40, no. 9, 1 August 2022 (2022-08-01), page 111281, XP093074149, US ISSN: 2211-1247, DOI: 10.1016/j.celrep.2022.111281 the whole document -----	18

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