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(71) Applicants: **INSTITUT PASTEUR** [FR/FR]; 25 - 28 Rue du Docteur Roux, 75724 PARIS Cédex 15 (FR). **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101, rue de Tolbiac, 75013 PARIS (FR).

(72) Inventors: **DI SANTO, James**; 51 rue Claude Terrasse, 75016 PARIS (FR). **LI, Yan**; 22 rue Emile Dubois, 75014 PARIS (FR).

(74) Agent: **PLASSERAUD IP**; 66 rue de la Chaussée d'Antin, 75440 PARIS CEDEX 09 (FR).

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(54) Title: HUMAN IMMUNE SYSTEM MOUSE MODEL

(57) Abstract: A transgenic mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein a (SIRP α) that is functionally expressed in the mouse, and wherein the mouse genome further comprises a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse or wherein exogenous thymic stromal cell-derived lymphopoietin (TSLP) is administered to the transgenic mouse. A Human Immune System (HIS) mouse made from said transgenic mouse. Use of the HIS mouse as a preclinical mouse model for testing human vaccine candidates, immunotherapeutics or anti-infectious agents. The HIS mouse as a spontaneous model of human atopic dermatitis for testing novel therapeutics.



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HUMAN IMMUNE SYSTEM MOUSE MODEL

FIELD OF THE INVENTION

[0001] The invention relates to a novel transgenic mouse and a Human Immune System (HIS) mouse made from said transgenic mouse which has the capacity to develop functional lymph nodes and enhanced antigen-specific responses. The HIS mouse of the invention is useful in particular as a preclinical mouse model for testing human vaccine candidates and novel immunotherapeutic or anti-infectious agent treatments for humans. The HIS mouse of the invention also provides a spontaneous model of human atopic dermatitis (AD) that is useful for testing novel therapeutics.

BACKGROUND OF THE INVENTION

[0002] Human Immune System (HIS) mice can be used to decipher certain mechanisms of human immunity *in vivo* (reviewed in ¹). Immunodeficient mouse strains have been created by combining mutations affecting antigen receptor rearrangements (*Prkdc*, *Rag1*, *Rag2*) with mutations in γ_c (encoded at *Il2rg*). γ_c is a shared receptor that signals responses to IL-2, -4, -7, -9, -15 and -21 (ref. ²), and its absence affects development of lymphoid precursors and NK cells. The *Il2rg* deficiency allows for substantial increases in human thymopoiesis in HIS mice ^{3, 4, 5}, suggesting a role for NK cell-mediated xenograft rejection and/or competition with mouse thymocyte progenitors.

[0003] Defective IL-7 signaling in *Il2rg*-deficient mice blocks formation of secondary lymphoid tissue (SLT: lymph nodes (LN) and Peyer's patches (PP)) due to an absence of lymphoid tissue inducer (LTi) cells that promote SLT development during fetal life ^{6, 7}. LTi cells belong to group 3 ROR γ t⁺ innate lymphoid cells (ILC3) that inhabit mucosal surfaces and produce IL-17A and IL-22 (reviewed in ^{8, 9}). Accordingly, *Il2rg*^{-/-} mice lack SLT (excepting a residual mesenteric LN). As a consequence, the mature human B, T and NK cells that develop in HIS mice do not interact within normal SLT structures.

[0004] Thymic stromal cell-derived lymphopoietin (TSLP) exhibits structural and functional homology to IL-7, signals through CD127 (IL-7R α chain) but requires a ligand-binding TSLP receptor that is *Il2rg*-independent^{10, 11}. Keratin 14 promoter (K14)-driven over-expression of TSLP can rescue immune developmental defects associated with IL-7 deficiency, enhancing early B and T cell lymphopoiesis¹². Moreover, K14-driven TSLP expression in the embryo rescued fetal LT α i cell function in *Il2rg*^{-/-} mice⁶.

[0005] A shortcoming of HIS mice known in the art is their lack of lymph nodes. Accordingly, there is a need in the art for HIS mice that have lymph nodes. This invention meets these needs and others.

[0006] Atopic dermatitis (AD) is common allergic skin inflammation affecting 15-30% of children and 2-10% of adults in industrialized countries (Bieber, 2008). This disease places not only heavy financial cost on patients and society, but also tremendous negative impact on the quality of life for patients and their family (Carroll et al., 2005). Longitudinal studies often found AD preceding allergic rhinitis and asthma, marking AD as the beginning of a phenomenon known as atopic march (Bantz et al., 2014). The etiology of AD involves the biased type 2 immunity, defective skin barrier, dysbiosis of skin microbiome, and neuropsychological factors (Werfel et al., 2016). This complexity was revealed by a large collection of murine AD models including spontaneous AD models and those induced by epicutaneous sensitization or genetic engineering (Jin et al., 2009). Although individual mouse model recapitulates certain aspects of human AD profile, global transcriptomic profiling from AD mouse models poorly resemble that from human AD patients (Ewald et al., 2017). Hence, cautions should be taken for translating murine data to human cases.

[0007] Treatment options for AD traditionally include allergen avoidance, emollient, and topical/systemic immune suppressants and antibiotics (Weidinger and Novak, 2016). Some treatments associate with significant side effect and transient efficacy. As the knowledge from AD animal models increases, novel immunotherapies against AD have been under development with the hope to bring targeted and safe therapeutics for long term use (Gandhi et al., 2016). Due to differences between human and mouse immunities (Mestas and

Hughes, 2004), human trials are the primarily approach to determine toxicity and efficacy of these biological agents.

[0008] The past efforts model AD in HIS mice through epicutaneous sensitization with oxazolone in NOD-scid *Il2rg*^{-/-} mice reconstituted with human peripheral blood mononuclear cells (PBMC) from AD patients or through mechanical disruption of transplanted artificial human skin on nude mice intradermally injected with in vitro expanded and polarized T helper type 2 (Th2) cells (Carretero et al., 2015; Nolte et al., 2013). Despite advancement made with these models, the requirement for advance technical expertise and limited access to patient blood/tissues makes these models unpractical. Moreover, PBMC engrafted HIS mice often develop graft versus host response (Shultz et al., 2012), which further restrict the usage of these models to study a chronic disease like AD.

[0009] Thymic stromal lymphopoietin (TSLP), expressed by epithelial cells including keratinocytes, is a key cytokine associated with the pathogenesis of AD in human (Takai, 2012). Since TSLP receptor is expressed on both hematopoietic and non-hematopoietic cells, several cell types, such as antigen experienced CD4 T cells, OX40L expressing DCs, basophils, type 2 innate lymphoid cells and even neurons, have been reported to respond to TSLP directly and to mediate type 2 immunity in AD mouse models or in human in in vitro studies (He et al., 2008; Ito et al., 2005; Kim et al., 2013; Siracusa et al., 2011; Wilson et al., 2013). Mouse models with transgenic expression of TSLP develop AD like changes, such as dermal infiltration, dominant type-2 inflammation and skin lesions (Elentner et al., 2009; Yoo et al., 2005).

[0010] Accordingly, there is a need in the art for a HIS mouse model of Atopic dermatitis (AD) that can be used as a preclinical model to test novel therapeutics. This invention meets these needs and others.

25 SUMMARY OF THE INVENTION

[0011] The experiments described in the examples assessed the impact of TSLP over-expression on SLT development in an immunodeficient Balb/c *Rag2*^{-/-} *Il2rg*^{-/-} *Sirpa*^{NOD} (BRGS)-based humanized mouse model¹³. HIS mice created in TSLP transgenic BRGS (BRGST) recipients display a full complement of anatomically distributed LNs, robust

development of follicular helper T cells (T_{FH}) and enhanced antigen-specific T cell and B cell responses after immunization. The examples show that BRGST HIS mice provide the means to visualize lymphocyte behavior and to study host-pathogen interactions in SLT, as illustrated here in the context of HIV-1 infection. The BRGST HIS mouse model with LN offers an opportunity to interrogate the role of LN structures in human immune responses during infection, stress and inflammation.

[0012] The examples also show that BRGST mice alone do not show signs of AD development whereas BRGST HIS mice universally demonstrate clinical, histological, immunological and skin commensal changes resembling human AD. Multiple human hematopoietic lineages associated with human AD were identified in BRGST HIS mice, such as skin infiltrating Th2 and Th22 cells, IgE secreting B cells and hFcεRIα expressing mast cells and basophils. Antibody mediated depletion of CD4, CD8 and CD20 human cells in BRGST HIS mice identified the crucial role of CD4 helper T cell in AD progression. Similar to clinical findings, AD progression and skin homing chemokine receptor expression on CD4 T cells were highly correlated. By targeting skin homing T cells with antibodies against CCR4, AD development was impeded in BRGST HIS mice. Collectively, BRGST HIS mice provide the first spontaneous human AD model with great potential as a preclinical model to test novel therapeutics.

[0013] Accordingly, in an aspect this invention provides a transgenic mice. In certain embodiments the transgenic mice have a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRPα) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse. In certain embodiments the transgenic mice have a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}) and a transgene encoding signal regulatory protein α (SIRPα) that is functionally expressed in the mouse; wherein exogenous thymic stromal cell-derived lymphopoietin (TSLP), such as recombinant TSLP, is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous TSLP, such as recombinant TSLP, was administered. In some embodiments, the exogenous TSLP is a mouse TSLP. In some embodiments the SIRPα is SIRPα^{NOD}. In some embodiments the transgenic mouse has a

genome further comprising a homozygous disruption of a Rag1 gene (Rag1^{-/-}) and/or a homozygous disruption of a Rag2 gene (Rag2^{-/-}). In some embodiments the transgenic mouse has a genome further comprising a homozygous disruption of the Prkdc gene (Prkdc^{-/-}). In some embodiments the transgenic mouse has a genome further comprising a homozygous disruption of the receptor tyrosine kinase Flk2/Flt3 gene (Flt3^{-/-}) and wherein exogenous Flt3 ligand (Flt3L), such as recombinant Flt3L, is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous Flt3L, such as recombinant Flt3L, was administered. In some embodiments, the exogenous Flt3L is a mouse Flt3L. In some embodiments, the exogenous Flt3L is a human Flt3L. In some embodiments the genetic background of the mouse is selected from BALB/c, C57BL/6, 129, such as 129/Ola, DBA/2, C3H, and NOD. In some embodiments the genetic background of the mouse is selected from a BALB/c background and a mixed BALB/c-129 background. In a preferred embodiment of the transgenic mouse, the mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a homozygous disruption of a Rag2 gene (Rag2^{-/-}), a transgene encoding mouse signal regulatory protein α (SIRP α ^{NOD}) that is functionally expressed in the mouse, and a transgene encoding mouse thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and wherein the genetic background of the mouse is BALB/c. In some embodiments the mouse does not express functional mouse MHC I and MHC II proteins. In some embodiments the mouse expresses a functional human MHC I protein and/or a functional human MHC II protein. In some embodiments the mouse is an embryo or a fetus. In some embodiments the mouse develops a full complement of anatomically distributed lymph nodes. In some embodiments the mouse does not develop Peyer's patches.

[0014] In another aspect this invention provides a human immune system mouse. In certain embodiments the human immune system mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse; wherein the mouse has a humanized immune system comprising human B cells, T cells, NK cells, and myeloid cells. In certain embodiments the human immune system mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}) and

a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse; wherein exogenous thymic stromal cell-derived lymphopoietin (TSLP), such as recombinant TSLP, is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous TSLP, such as recombinant TSLP, was administered. In some embodiments, the exogenous TSLP is a mouse TSLP. In some embodiments the SIRP α is SIRP α^{NOD} . In some embodiments the human immune system mouse has a genome further comprising a homozygous disruption of a Rag1 gene (Rag1^{-/-}) and/or a homozygous disruption of a Rag2 gene (Rag2^{-/-}). In some embodiments the human immune system mouse has a homozygous disruption of the Prkdc gene (Prkdc^{-/-}). In some embodiments the human immune system mouse has a genome comprising a homozygous disruption of the receptor tyrosine kinase Flk2/Flt3 gene (Flt3^{-/-}) and wherein exogenous Flt3 ligand (Flt3L), such as recombinant Flt3L, is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous Flt3L such as recombinant Flt3L, was administered. In some embodiments, the exogenous Flt3L is a mouse Flt3L. In some embodiments, the exogenous Flt3L is a human Flt3L. In some embodiments the genetic background of the mouse is selected from BALB/c, C57BL/6, 129, such as 129/Ola, DBA/2, C3H, and NOD. In some embodiments the human immune system mouse the genetic background of the mouse is selected from a BALB/c background and a mixed BALB/c-129 background. In a preferred embodiment, the human immune system mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a homozygous disruption of a Rag2 gene (Rag2^{-/-}), a transgene encoding mouse signal regulatory protein α (SIRP α^{NOD}) that is functionally expressed in the mouse, and a transgene encoding mouse thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and wherein the genetic background of the mouse is BALB/c. In some embodiments the mouse does not express functional mouse MHC I and MHC II proteins. In some embodiments the mouse expresses a functional human MHC I protein and/or a functional human MHC II protein. In some embodiments the mouse is an embryo or a fetus. In some embodiments the mouse develops a full complement of anatomically distributed lymph nodes. In some embodiments the mouse does not develop Peyer's patches. In some embodiments the mouse develops at least two-fold more follicular helper T cells (T_{FH}) than a control mouse having a genome that does not comprise a transgene encoding TSLP that is functionally expressed in the mouse. In

some embodiments the mouse comprises T_{FH} cells comprising an infectious agent. In some embodiments the infectious agent is a virus. In some embodiments the virus is a lentivirus. In some embodiments the lentivirus is a human immunodeficiency virus (HIV).

[0015] The invention also provides a human immune system mouse of this disclosure, wherein the mouse is made by a method comprising providing a transgenic mouse according to this disclosure and engrafting human hematopoietic stem cells (HSCs) into the transgenic mouse.

[0016] Also provided are methods and various uses of the human immune system mouse of the invention. In certain embodiments the methods comprise providing a transgenic mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse; engrafting human hematopoietic stem cells (HSCs) into the transgenic mouse, infecting the transgenic mouse with an infectious agent, and detecting the presence and/or absence of the infectious agent in a lymph node of the transgenic mouse. In certain embodiments the methods comprise providing a transgenic mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}) and a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse; wherein exogenous thymic stromal cell-derived lymphopoietin (TSLP), such as recombinant TSLP, is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous TSLP, such as recombinant TSLP, was administered; engrafting human hematopoietic stem cells (HSCs) into the transgenic mouse, infecting the transgenic mouse with an infectious agent, and detecting the presence and/or absence of the infectious agent in a lymph node of the transgenic mouse. In some embodiments the infectious agent is a virus, bacteria or parasite. In some embodiments, the exogenous TSLP is a mouse TSLP. In some embodiments the SIRP α is SIRP α^{NOD} . In some embodiments the transgenic mouse has a genome further comprising a homozygous disruption of a Rag1 gene (*Rag1*^{-/-}) and/or a homozygous disruption of a Rag2 gene (*Rag2*^{-/-}). In some embodiments the transgenic mouse has a genome further comprising a homozygous disruption of the Prkdc gene (*Prkdc*^{-/-}). In some embodiments the transgenic mouse has a genome comprising a homozygous disruption

of the receptor tyrosine kinase Flk2/Flt3 gene ($Flt3^{-/-}$) and wherein exogenous Flt3 ligand (Flt3L), such as recombinant Flt3L, is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous Flt3L, such as recombinant Flt3L, was administered. In some embodiments, the exogenous Flt3L is a mouse Flt3L. In some
5 embodiments, the exogenous Flt3L is a human Flt3L. In some embodiments the genetic background of the mouse is selected from BALB/c, C57BL/6, 129, ,such as 129/Ola, DBA/2, C3H, and NOD. In some embodiments the genetic background of the mouse is selected from a BALB/c background and a mixed BALB/c-129 background. In a preferred embodiment of the methods, the mouse has a genome comprising a homozygous disruption of a common
10 receptor γ gene ($Il2rg^{-/-}$), a homozygous disruption of a Rag2 gene ($Rag2^{-/-}$), a transgene encoding mouse signal regulatory protein α ($SIRP\alpha^{NOD}$) that is functionally expressed in the mouse, and a transgene encoding mouse thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and wherein the genetic background of the mouse is BALB/c. In some embodiments the mouse does not express functional mouse MHC I and
15 MHC II proteins. In some embodiments the mouse expresses a functional human MHC I protein and/or a functional human MHC II protein. In some embodiments the mouse develops a full complement of anatomically distributed lymph nodes. In some embodiments the mouse does not develop Peyer's patches. In some embodiments the infectious agent is a virus. In some embodiments the virus is a lentivirus. In some embodiments the lentivirus is a human
20 immunodeficiency virus (HIV). In some embodiments the methods further comprise administering an anti-infectious agent therapy to the transgenic mouse. In some embodiments the presence and/or absence of a latent infection of a lymph node of the transgenic mouse by the infectious agent is detected.

[0017] Also provided are methods comprising providing a human immune system
25 mouse according to this disclosure, administering an antigen to the human immune system mouse, and assaying for the presence, absence, and/or level of at least one immune response in the human immune system mouse to the antigen. In some embodiments the at least one immune response is selected from the group consisting of a B-cell response, a T-cell response, and an NK-cell response. In some embodiments the methods further comprise administering
30 an adjuvant to the human immune system mouse. In some embodiments the methods further comprise administering the antigen to a control mouse. In some embodiments the methods

further comprise administering a test molecule to the human immune system mouse and determining whether the test molecule increases or decreases the at least one immune response in the human immune system mouse to the antigen.

[0018] Also provided are methods comprising, providing a human immune system mouse according to this disclosure, administering a vaccine against an infectious agent to the human immune system mouse, administering the infectious agent to the human immune system mouse, and assaying for the presence, absence, and/or level of infection of the human immune system mouse by the infectious agent. In some embodiments the methods further comprise administering the infectious agent to a second human immune system mouse, and assaying for the presence, absence, and/or level of infection of the second human immune system mouse by the infectious agent. In some embodiments the infectious agent is a virus and the vaccine is a viral vaccine. In some embodiments the virus is a lentivirus. In some embodiments the lentivirus is HIV.

[0019] The invention also provides a mouse model of human atopic dermatitis comprising a human immune system (HIS) mouse according to this disclosure, comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and further comprises a humanized immune system. The invention also provides the use of the mouse model of human atopic dermatitis of the present disclosure for testing candidate therapeutic agents or assessing treatments for human allergic and/or inflammatory skin diseases, preferably atopic dermatitis. In some embodiments the candidate therapeutic agent is an antibody.

[0020] The invention also provides a C-C chemokine receptor type 4 (CCR4) antagonist for use in the prevention and/or treatment of atopic dermatitis, in particular in humans. In some embodiments, the CCR4 antagonist is an antibody against CCR4, in particular a monoclonal antibody, more particularly Mogamalizumab. In some embodiments, the CCR4 antagonist is used for the prevention of atopic dermatitis, in particular to slow-down or stop the development of atopic dermatitis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **Figs. 1a-1e. Characterization of human immune cell reconstitution in BRGST HIS mice.** (a) Representative organs (left) and absolute numbers of human cells (right) in the spleens and LNs of BRGS or BRGST HIS mice (n=11 mice/group, bars represent mean, and error bars denote s.e.m., *P* value is shown for Two-tailed Mann-Whitney U test). All visible LNs in BRGS or BRGST HIS mice were pooled. (b) Representative immunohistochemistry images of LN sections from BRGS (left) and BRGST (right) HIS mice. Scale bar represents 500 microns. (c) Percentages of human T and B cells (n=60 mice/BRGS, n=35 mice/BRGST) and CD4⁺ and CD8⁺ T cells (n=23 mice/BRGS, n=22 mice/BRGST) among human hematopoietic cells (hCD45⁺mCD45⁻) in the blood of the indicated mice (bars represent mean, and error bars denote s.e.m., *P* value is shown for Two-tailed Mann-Whitney U test). Each symbol represents an individual mouse. (d) Absolute numbers of human T and B cell (n=11 mice/group) and CD4⁺ and CD8⁺ T cells (n=6 mice/group) in the spleen and LNs of indicated groups of mice (bars represent mean, and error bars denote s.e.m., *P* value is shown for Two-tailed Mann-Whitney U test). All visible LN in BRGS or BRGST HIS mice were pooled. (e) Percentages and absolute numbers of human naïve or memory cell subsets, identified by expression of CCR7 and CD45RA among hCD45⁺CD3⁺CD4⁺ cells or hCD45⁺CD3⁺CD8⁺ cells in the spleen of indicated groups. (n=6 mice/group, bars represent mean, and error bars denote s.e.m., *P* value is shown for Two-tailed Mann-Whitney U test).

[0022] **Figs. 2a-2d. Intravital two photon microscopy of BRGST LN showing human T cell behavior.** 10 million human T cells purified and pooled from 5 HIS mice were fluorescently labeled and transferred into BRGST HIS mouse from the same cohort. After 24h, popliteal lymph nodes from the recipients were subjected to intravital two-photon imaging (a). Representative images of human T cells and corresponding tracks from 8 different movies in 3 different recipients. Scale bar=20 μm. Insets show the migration pattern for human T cells (n=3). Scale bar=10 μm. Graphs show the mean velocity (b) and the straightness index (c) for individual T cells (the measure of centre represents mean). (d) T cells tracks were graphed from a common origin. Data are representative of 8 different movies in 3 different recipients.

[0023] **Figs. 3a-3f. Human thymocyte and T_{FH} cell development in BRGST HIS mice.** (a, b) Representative FACS analysis (gated on indicated populations above each plot) (a) and absolute numbers (b) of human thymocytes in 12 weeks old BRGS and BRGST HIS mice ($n=5$ mice/group, bars represent mean, and error bars denote s.e.m., P value is shown for Two-tailed Mann-Whitney U test). (c) Representative FACS analysis of human T_{FH} cell ($CXCR5^+PD-1^+$) frequencies in blood, spleen and LN of indicated mice (numbers in plots indicate percentages within $mCD45^+hCD45^+CD3^+CD4^+$ cells), and percentages of LN T_{FH} cells ($CXCR5^+PD-1^+$) of indicated mice ($n=11$ mice/group, bars represent mean, and error bars denote s.e.m., P value is shown for Two-tailed Mann-Whitney U test). All visible LN in BRGS or BRGST HIS mice were pooled. (d) Representative FACS analysis of intracellular Bcl-6 expression by subsets of human $CD4^+$ T cells in LN of BRGST HIS mice from 2 independent experiments with similar results. All visible LN in BRGS or BRGST HIS mice were pooled. (e) Analysis of IL-21 and CXCL13 gene expression in sorted spleen/LN $CD45^+CD3^+CD4^+$ human T cells from indicated strains ($n=7$ mice/group, numbers on the y-axis are Nanostring counts of indicated gene normalized to geometric means of housekeeping genes, bars represent mean, and error bars denote s.e.m., P value is shown for Two-tailed Mann-Whitney U test). (f) Representative FACS analysis of intracellular cytokine expression of human T cells from spleens of indicated mice after PMA/Ionomycin stimulation (numbers in plots indicate percentages of $mCD45^+hCD45^+CD3^+$ cells), and percentages of splenic IL-21 $^+$ T cells of indicated mice ($n=10$ mice/group, bars represent mean, and error bars denote s.e.m., P value is shown for Two-tailed Mann-Whitney U test).

[0024] **Figs. 4a-4e. Enhanced humoral responses in BRGST HIS mice.** (a) Frequency (left) or absolute numbers (right) of mature human B cells ($CD24^+CD38^+$ gated on $hCD45^+mCD45^+CD19^+$ cells) in 16 weeks old HIS mice ($n=5$ mice/group, bars represent mean, and error bars denote s.e.m., P value is shown for Two-tailed Mann-Whitney U test). (b) Concentrations of total human IgM, IgG and IgA in sera of 12 to 16 weeks old HIS mice ($n=18-20$ mice/group, bars represent mean, and error bars denote s.e.m., P value is shown for Two-tailed Mann-Whitney U test). Each symbol represents an individual mouse. (c) FACS analysis of IgA and IgG secreting B cell frequencies in pooled BM, spleen and LN of indicated mouse strains after KLH immunization (numbers in plots indicate percentages of gated cells in $mCD45^+hCD45^+CD3^+CD19^+CD10^+$ cells). All visible LN in BRGS or BRGST

HIS mice were pooled. (d) IFN- γ ELISpot from splenocytes (top panel) and KLH specific IgG ELISA results (bottom panel) from sera of immunized HIS mice (n=3 mice/group, bars represent mean, and error bars denote s.e.m., data are representative of 3 independent experiments). (e) Representative immunofluorescence analysis of LN sections of KLH immunized BRGS (top panel) and BRGST (bottom panel) HIS mice co-stained for CD3 (blue), CD20 (green) and AID (red). Scale bar represents 100 micron. Results are representative from 3 independent experiments.

[0025] **Figs. 5a-5e. Immunoglobulin gene repertoire of human class-switched memory B cells in HIS mice.**

(a) Pie charts show the distribution of IgG- and IgA-expressing B cells within clonal expansions (colored) and single clones (white). All unique clones expressed surface IgG except those indicated by an “a” (IgA⁺ cells). (b) Pie charts show shared members of clonal families between analyzed tissues. Each clonal family is represented by the same color, and unique antibodies that are not members of a clonal family are in white. The number of antibody clones analyzed is indicated in the center of each pie chart in A and B. (c) Bar graphs comparing the CDRH3 amino acid length and number of positive charges in the CDRH3 of memory B-cell antibodies between both groups. A total of 105 and 121 IgH sequences were analyzed for BRGS and BRGST, respectively: LN, lymph nodes (n=24 for BRGS, n=29 for BRGST); Sp, spleen (n=61 for BRGS, n=71 for BRGST); BM, bone marrow (n=20 for BRGS, n=21 for BRGST). Groups were compared using two-sided 2x5 Fisher’s Exact test. (d) Violin plots comparing the number of mutations in the variable genes of light (IgL) chains between both groups. The colored bar and the black dot indicate mean and median, respectively. Below each dot plot, pie charts show the distribution of mutation in each group, from 0 (white) to 7 or more (red). The number of sequences analyzed (n) in each group is indicated below the Violon plot. Groups were compared using unpaired two-sided student t-test with Welch’s correction. LN, lymph nodes; Sp, spleen; BM, bone marrow. (e) Graphs show the Bayesian estimation of antigen-driven selection in IgL CDR sequences from both groups as determined with the BASELINE software a total of 238 and 178 IgH sequences for BRGS and BRGST, respectively (following the groups distribution shown in (d)). Values >0 indicate positive selection.

[0026] **Figs. 6a-6e. HIV infection and latency in BRGST HIS mice.** (a) Experimental scheme and timing of HIV-1 infection in BRGS and BRGST HIS mice. 12-week old reconstituted HIS mice were inoculated with 10^5 tissue culture infective dose required for 50% infection (TCID₅₀) HIV_{NLAD8}, and plasma viremia was assayed at indicated days post infection (dpi) in acute phase (7-12dpi) and after HAART (25dpi). (b) Characterization of HIV-1 infected T cell subsets in HIS mice. (left) Representative FACS plots of p24⁺ cells in LNs of BRGS and BRGST HIS mice (numbers in plots indicate percentages of gated cells within hCD45⁺CD3⁺CD8⁻ cells), and (right) percentages and absolute numbers of p24⁺ cells for indicated groups (n=4 mice/group, bars represent mean, and error bars denote s.e.m.). All visible LNs in BRGS or BRGST HIS mice were pooled. (c-e) Viral reservoir quantification in HIV-1-infected HAART-treated BRGST mice. (c) Representative examples of p24 quantification by ultrasensitive digital ELISA after in vitro activation of LN CD4⁺ T cells from in HIV-1-infected HAART-treated BRGST mice (n=4), (d) Representative example of intracellular gag staining of LN CD4⁺ T cells, before and after 14 days of PHA activation from HIV-1-infected HAART-treated BRGST mice (n=4), (e) Infectious unit per millions from LN CD4⁺ T cells in HIV-1-infected HAART-treated BRGST mice (n=4, centre values represent mean, and error bars denote s.e.m.). The dotted line indicates the limit of detection (LOD) of the assay.

[0027] **Figs. 7a-7g. Mouse ILC and LN anlage development in BRGST mice.** (a) Mouse TSLP concentration in plasma from BRGS and BRGST mice was quantified by ELISA (n=12 mice/group, bars represent mean, and error bars denote s.e.m.). (b) Representative FACS plot showing the gating strategy for mouse ILCs in lamina propria of small intestines of BRGST mice from 12 mice with similar results. (c) Absolute numbers of ILC subsets in small intestines of *Rag2*^{-/-}, BRGS and BRGST mice (n=4 mice/group; bars represent mean, and error bars denote s.e.m., *P* value is shown, one-way ANOVA with a Turkey test) (d) Representative images of anlagen of inguinal and iliac LNs in non-engrafted BRGS and BRGST mice 1 week after peritoneal injection of Chicago Sky Blue 6B dye from 2 independent experiments with similar results. LN anlagen are indicated by arrow heads. Scale bar=2 mm. (e) Total number of LN anlagen observed in non-engrafted 8 week-old BRGS and BRGST littermates 1 week after dye injection (n=5 mice/group, bars represent mean, and error bars denote s.e.m., *P* value is shown for Two-tailed Mann-Whitney U test).

(f) Quantification of different LNs present in non-engrafted 8 week-old BRGS and BRGST littermates (n=8 mice/group) 1 week after dye injection. To calculate the percentages, the number of mice observed with indicated LN anlage was divided by total number of mice from indicated strain. (g) Numbers of PPs counted on small intestines of BRGST and C57BL/6 newborn pups after VCAM1 whole mount staining (n=4 mice/group, bars represent mean, and error bars denote s.e.m.).

[0028] **Figs. 8a-8c. Characterization of human immune cell reconstitution in BRGST HIS mice.** (a) Representative pictures indicated LNs in BRGST HIS mice at 10-14 weeks post fetal liver CD34⁺ cell transplantation from 3 independent experiments with similar results. Scale bar=2 mm (b) Kinetics of human cell reconstitution in blood of BRGS and BRGST HIS mice (n=8 mice/group, centre values represent mean, and error bars denote s.e.m.). (c) Representative flow cytometry analysis of human naïve/memory T cell subsets from 12 HIS mice with similar results, identified by expression of CCR7 and CD45RA among hCD45⁺CD3⁺CD4⁺ splenic T cells.

[0029] **Figs. 9a-9d. Two photon microscopy of explanted BRGST LN showing human T cell behavior.** 10 million human T cells purified from health donor PBMC were fluorescently labeled and adoptively transferred into 12 week old BRGST HIS mouse. After 24h, intact inguinal lymph nodes were explanted and subjected to two-photon imaging (a). Representative images of human T cells and corresponding tracks from 8 different movies in 3 different recipients. Scale bar=20 µm. Right side insets show the migration pattern for single human T cells (n=3). Scale bar=10 µm. Graphs show the mean velocity (b) and the straightness index (c) for individual T cells (the measure of centre represents mean). (d) T cells tracks were graphed from a common origin. Data are representative of 8 different movies in 3 different recipients.

[0030] **Figs. 10a-10d. Mouse thymocyte precursor development in BRGST HIS mice.** (a) Percentages and absolute numbers of Ki67⁺ cells among human thymocyte subsets in 12 week old BRGS and BRGST HIS mice (n=5 mice/BRGS, 6 mice/BRGST. bars represent mean, and error bars denote s.e.m., *P* value is shown for Two-tailed Mann-Whitney U test). (b) Representative images of thymi in 8 week old non-reconstituted BRGS and BRGST mice 1 week after peritoneal injection of Chicago Sky Blue 6B dye. Data from 2

independent experiments with similar results. Scale bar=2 mm (c) Representative flow cytometry analysis of murine thymocytes gated on indicated populations above each plot (numbers next to outlined areas indicate percentages of cells) from 11 HIS mice with similar results. (d) Absolute numbers of mouse thymocyte subsets in BRGS and BRGST mice (n = 6 mice/BRGS, 5 mice/BRGST, bars represent mean, and error bars denote s.e.m., *P* value is shown for Two-tailed Mann-Whitney U test).

[0031] **Figs. 11a-11c. B cell phenotype and antigen-specific immune responses after KLH immunization in HIS mice.** (a) FACS analysis of mature human B cells (gated on hCD45⁺mCD45⁻CD19⁺ cells) from HIS mice. Representative analysis from 10 independent mice with similar results. (b) KLH specific IgG ELISA results from sera and (c) IFN- γ ELISpot results from splenocytes of KLH-immunized HIS mice in 2 independent experiments. (n=4 mice/group, centre values represent mean, and error bars denote s.e.m.).

[0032] **Figs. 12a-12d. Immunoglobulin gene repertoire of human class-switched memory B cells in HIS mice.** (a) Circular phylograms of representative clonal expansions as shown in B (green and blue clones for BRGS and BRGST, respectively). GL, germline; LN, lymph nodes; Sp, spleen; BM, bone marrow. (b) Circos plots comparing the frequency of VH(DH)JH rearrangements of IgA⁺/IgG⁺ memory B-cell antibodies between both groups. (c) Violin plots comparing the number of mutations in the variable genes of heavy (IgH) chain between both groups. The colored bar and the black dot indicate mean and median, respectively. Below each dot plot, pie charts show the distribution of mutation in each group, from 0 (white) to 7 or more (red). The number of sequences analyzed (n) in each group is indicated below the Violon plot. Groups were compared using unpaired two-sided student t-test with Welch's correction. LN, lymph nodes; Sp, spleen; BM, bone marrow. (d) Graphs show the Bayesian estimation of antigen-driven selection in IgH CDR sequences from both groups as determined with the BASELINE software using a total of 121 and 115 IgL sequences for BRGS and BRGST, respectively (following the groups distribution shown in (c)). Values >0 indicate positive selection.

[0033] **Figs. 13a-13b. HIV infection in BRGS and BRGST HIS mice.** Longitudinal analysis in the blood of HIV viremia (RNA copies/mL) and of human CD45⁺ cells, CD4⁺ and

CD8⁺ T cells, respectively in BRGS (a) and BRGST (b) HIS mice. (n=6 BRGS mice, n=12 BRGST mice for acute infection; n=3 BRGS, n=4 BRGST mice for HAART treatment).

[0034] **Figs. 14a-14b. Cell sorting report.** (a) Representative flow cytometry analysis for T cell enrichment by lineage depletion of pooled HIS mouse splenocytes. Data from 3 independent experiments with similar results. (b) Representative flow cytometry analysis for CD4⁺ T cell selection from pooled splenocytes and lymph node cells. Data from 3 independent experiments with similar results.

[0035] **Fig. 15a-15e. TSLP injection and immunization.**

[0036] **Fig. 16a-16c. Human cell development in TSLP injected HIS mice.**

10 [0037] **Figure 17A-17D. Overexpression of mouse TSLP does not induce atopic dermatitis in BRGST mice.** (A-B) 20 week old BRGS and BRGST littermates were examined for skin condition (A) and ear thickness (B) (n=10 mice/BRGST, 13 mice/BRGS). (C) absolute numbers of CD11c⁺MHC-II⁺ dendritic cell (DC), Langerin⁺CD103⁻ migratory DC and Langerin⁺ CD103⁺ dermal DC from splenocytes of 8 week-old BRGS and BRGST littermate (n=3 mice/group). (D) Flow cytometry analysis of DC activation (CD80⁺MHC II⁺) after 24 hour culture with indicated conditions (numbers next to outlined areas indicate percentages of human CD45⁺lin⁻CD11c⁺ cells). *P<0.05, ns, not significant, Mann-Whitney U test.

20 [0038] **Figure 18A-18C. BRGST mice develop severe skin lesions after long-term humanization.** (A) 25 week-old BRGS and BRGST HIS littermates were examined for skin condition. Severe dermatitis with hemorrhage, erosion, dryness, crust and alopecia appears throughout the body of BRGST HIS mice. (B) Clinical skin conditions of BRGS and BRGST HIS mice were macroscopically examined and scored weekly by two persons blinded to group allocation. (C) Ear thickness was measured by a thickness gauge micrometer with a ratchet stop weekly

25 [0039] **Figure 19A-19G. Dysbiosis and *Staphylococcus aureus* colonization in the skin of BRGST AD HIS mice.** (A, B) Histological features of skin lesions from BRGS HIS and BRGST AD HIS mice post 25-week human cell engraftment. Hematoxylin& eosin-

stained sections show: 1, dermal inflammation; 2, epidermis hyperplasia; 3, destruction of basal layers of epidermis and hair follicles; 4, Hyperkeratosis (A). A magnified image of stained sections show: 1, Mast cell; 2, Macrophage; 3, Lymphocyte; 4, Neutrophil; 5, Eosinophil (B). (C) Absolute cell numbers of skin infiltrating cells from BRGS and BRGST AD HIS mice (n=5/group). (D) Determination of bacterial and fungi load measured by universal 16S and 18S qPCR in upper-back skins of BRGS HIS mice and BRGST AD HIS mice. (E) Principal coordinate analysis (PCoA) plot comparing microbiota of upper-back skins of BRGS HIS mice and BRGST AD HIS mice along the first two principal coordinate (PC) axes using canberra distances. (F) Absolute abundance of total *Staphylococcus aureus* species on (F) Relatively abundance of *Staphylococcus* genus in upper-back skins of BRGS HIS mice and BRGST AD HIS mice evaluated by quantitative qPCR. (G) Level of human inflammatory mediators in (F) Relatively abundance of *Staphylococcus* genus in upper-back skins of BRGS HIS mice and BRGST AD HIS mice. *P<0.05, **P<0.01, ***P<0.001, n.s., not significant, Mann-Whitney U test.

[0040] **Figure 20A-20E Characterization of human immune responses in BRGST HIS mice.** (A) Serum IgE levels in BRGS and BRGST HIS mice post 16-week human cell engraftment. (B) Cytokine profiles in sera of BRGST and BRGST HIS mice (n=5 mice/BRGS, n=8 mice/BRGST). (C) Flow cytometry analysis of human B cells (Red, hCD45⁺hFcεRIα⁺CD117⁺CD203⁺SSC^{low}FSC^{low}), mast cells (Green, hCD45⁺hFcεRIα⁺CD117⁺CD203⁺SSC^{high}FSC^{high}), and basophils (Blue, hCD45⁺hFcεRIα⁺CD117⁺CD203⁺SSC^{low}FSC^{low}) from skin draining lymph nodes from BRGST HIS mice post 16-week human cell engraftment (numbers next to outlined areas indicate percentages of hIgE⁺ cells). (D-E) Flow cytometry analysis of intracellular cytokine expression of human T cells from spleen, skin draining lymph nodes, and upper-back skin of indicated mice after PMA/Ionomycin stimulation (numbers next to outlined areas indicate percentages of mCD45⁺hCD45⁺CD3⁺ cells). *P<0.05, **P<0.01, ***P<0.001, Mann-Whitney U test.

[0041] **Figure 21A-21D. Human CD4 T cells drive the development of atopic dermatitis in BRGST HIS mice.** (A, B) 12 week-old HIS mice received weekly injection of mAbs against CD4(OKT4, 300μg/injection), CD8(OKT8, 100μg/injection) or

CD20(Rituximab, 200µg/injection) for 10 weeks. (A) Percentages of blood CD19⁺ B cells within mCD45⁻hCD45⁺ cells, CD8⁺ T cells within mCD45⁻hCD45⁺CD3⁺ cells, and CD4⁺ T cells within mCD45⁻hCD45⁺CD3⁺ cells during the period of mAb treatment (n=3 mice/BRGS, 6 mice/BRGST). (B) Ear thickness was measured by a thickness gauge micrometer with a ratchet stop weekly for indicated groups. (n=9 mice/BRGS, 8 mice/BRGST, 6/other groups). (C, D, E) 20 week-old BRGST AD HIS mice received mAbs against CD4 (OKT4, 200µg/injection) twice a week for 6 weeks (n=6 mice/BRGST+CD4, n=5 mice/other groups). (C, D) Clinical skin conditions of indicated groups were macroscopically scored and ear thickness was measured weekly by two persons blinded to group allocation. (E) Comparison of skin lesions between indicated groups at indicated weeks post OKT4 mAb injection.

[0042] **Figure 22A-22F. Skin homing receptors on T cells as therapeutic targets.** (A) Volcano plots of (-Log₁₀ (*P*-value) versus the Log₂ (fold change expression)) of genes listed in Nanostring nCounter GX Human Immunology kit V2. Significant, differentially expressed genes (>1.5-fold comparing CD4 T cells from 20 week-old BRGST AD HIS mice to CD4 T cells from BRGS HIS littermates, moderated *t*-test *P*-value <0.05, Benjamini-Hochberg corrected) were plotted with green dots annotated by gene name. Each dot represents a gene expression assay (n=5) for the cell type indicated. (B) CLA, CCR10, CXCR3, and CCR8 mRNA gene expression is displayed as absolute nCount units (n=5 mice/group). (C) The percentages of CLA, CCR4, CCR10 and CXCR3 expression on blood CD4 T cells from indicated groups as measured by flow cytometry. (D) correlation(Spearman rank test) between ear thickness and percentages of CLA and CCR4 expression on blood CD4 T cells. Each dot represents a BRGST HIS mouse between 12-20 week-old. (E, F) 10 week-old HIS mice received weekly injection of mAbs against CCR4(100µg/injection) for 10 weeks (n=5 mice/group). (E) Percentages of CCR4⁺ expression on blood mCD45⁻hCD45⁺CD3⁺CD4⁺ cells during the period of mAb treatment. (F) Clinical skin conditions of indicated groups were macroscopically scored and ear thickness was measured weekly by two persons blinded to group allocation. **P*<0.05, ***P*<0.01, ****P*<0.001, Mann-Whitney U test.

[0043] **Figure 23A-23B. Skin infiltration and dysbiosis of BRGST AD HIS mice** (A) Histological features of skin lesions from BRGS HIS and BRGST AD HIS mice post 20-

week human cell engraftment as shown by hematoxylin& eosin-stained sections. Arrows indicate destruction and inflammation in basal layers of epidermis and hair follicles (infundibulum and isthmus: superficial parts of hair follicles). **(B)** Relatively abundance of *Staphylococcus* genus in upper-back skins of BRGS HIS mice and BRGST AD HIS mice.

5 [0044] **Figure 24. Depletion efficiency of CD4 T cells by OKT4 antibody treatment.** 20 week-old BRGST AD HIS mice received mAbs against CD4 (OKT4, 200µg/injection) twice a week for 6 weeks (n=6 mice/BRGST+CD4, n=5 mice/other groups). Percentages of CD4 expressing cells of blood mCD45^hCD45⁺CD3⁺ cells during the period of mAb treatment.

10 DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

[0045] As used herein, “homozygous disruption” refers to the situation when both copies of a gene in the genome of a mouse are null alleles of that gene. A “null allele” is an allele that does not rescue the phenotype of a deletion allele of the gene when one copy of the deletion allele and one copy of the null allele are present in the genome of a mouse. For the avoidance of doubt, a deletion allele is one type of null allele. A non-limiting alternative embodiment is an allele in which the open reading frame for the gene is intact but is not expressed. Another non-limiting alternative embodiment is an allele in which a mutant version of the protein encoded by the gene is expressed but is inactive. In some embodiments of a homozygous disruption both allelels in the genome of the mouse are the same. In some embodiments of a homozygous disruption the two allelels in the genome of the mouse are different.

[0046] As used herein, a common receptor γ gene (*Il2rg*) is a gene that encodes interleukin-2 receptor subunit gamma (IL-2RG) protein. IL-2RG is a cytokine receptor subunit that is common to the receptor complexes for at least six different interleukin receptors: IL-2, IL-4, IL-7, IL-9, IL-15 and interleukin-21 receptor. The γ c glycoprotein is a member of the type I cytokine receptor family expressed on most lymphocyte (white blood cell) populations, and its gene is found on the X-chromosome of mammals. Non-limiting examples of mouse *Il2rg* coding sequences are available at NCBI reference sequences NM_013563 and

NM_001308535. Non-limiting examples of mouse IL-2RG sequences are available at NCBI reference sequences NP_001295464 and NP_038591.

[0047] As used herein, a signal regulatory protein α (SIRP α) is a regulatory membrane glycoprotein from SIRP family expressed mainly by myeloid cells and also by stem cells or neurons. SIRP α recognizes CD47, that is an antiphagocytic signal distinguished live cells from dying. CD47 has a single Ig-like extracellular domain and five membrane spanning region. Non-limiting examples of mouse SIRP α coding sequences are available at NCBI reference sequences NM_001177646, NM_001177647, NM_001291019, NM_001291020, NM_001291021. Non-limiting examples of mouse SIRP α sequences are available at NCBI reference sequences NP_001171118, NP_001277948, NP_001277949, NP_001277950, NP_001277951. In some embodiments the SIRP α is a mouse SIRP α . In some embodiments the SIRP α is the SIRP α^{NOD} allele. Non-limiting examples of SIRP α^{NOD} sequences are provided in Katsuto Takenaka, et al., "Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells," Nature Immunology, Vol. 8, No. 12, pp. 1313-1323 (2007). In some embodiments the SIRP α is a human SIRP α .

[0048] As used herein, a *Rag1* gene is a gene that encodes the protein RAG1, which is involved in the initiation of V(D)J recombination during B and T cell development. Non-limiting examples of mouse *Rag1* coding sequences are available at NCBI reference sequence NM_009019. Non-limiting examples of mouse RAG1 sequences are available at NCBI reference sequence NP_033045.

[0049] As used herein, a *Rag2* gene is a gene that encodes the protein RAG2, which is involved in the initiation of V(D)J recombination during B and T cell development. Non-limiting examples of mouse *Rag2* coding sequences are available at NCBI reference sequence NM_009020. Non-limiting examples of mouse RAG2 sequences are available at NCBI reference sequence NP_033046.

[0050] As used herein, a *TSLP* gene is a gene that encodes thymic stromal cell-derived lymphopoietin (TSLP). TSLP is a protein belonging to the cytokine family. It is known to play an important role in the maturation of T cell populations through activation of antigen presenting cells. Non-limiting examples of mouse *TSLP* coding sequences are available at

NCBI reference sequence NM_021367. Non-limiting examples of mouse TSLP sequences are available at NCBI reference sequence NP_067342. In some embodiments the TSLP is a mouse TSLP.

[0051] As used herein, a Flk2/Flt3 gene is a gene that encodes receptor tyrosine kinase
5 FLT3 (or FLK2). FLT3 (or FLK2) is a protein that acts as cell-surface receptor for the cytokine FLT3 ligand (FLT3LG or Flt3L) and regulates differentiation, proliferation and survival of hematopoietic progenitor cells and of dendritic cells. Non-limiting examples of mouse Flk2/Flt3 coding sequences are available at NCBI reference sequence NM_010229.2. Non-limiting examples of mouse FLT3 sequences are available at NCBI reference sequence
10 NP_034359.2. Non-limiting examples of human Flk2/Flt3 coding sequences are available at NCBI reference sequence NM_004119.2. Non-limiting examples of human FLT3 sequences are available at NCBI reference sequence NP_004110.2. Non-limiting examples of mouse Flt3lg coding sequences are available at NCBI reference sequence NM_013520.3. Non-limiting examples of mouse Flt3L sequences are available at NCBI reference sequence
15 NP_038548.3. Non-limiting examples of human Flt3lg coding sequences are available at NCBI reference sequence NM_001204502.1, NM_001204503.1, NM_001278637.1, NM_001278638.1, NM_001459.3, NM_001459.3, and XM_011526682.2. Non-limiting examples of human Flt3L sequences are available at NCBI reference sequence NP_001191431.1, NP_001191432.1, NP_001265566.1, NP_001265567.1, NP_001450.2, and
20 XP_011524984.1.

[0052] As used herein, a “genetic background” of a mouse refers to the genome of a mouse that is a member of a particular mouse strain. The background may be either an inbred strain or an F1 hybrid strain. When mice of different first and second strains are crossed the resulting F1 progeny have a genome that is a mix of the two strains. If this F1 mouse is then
25 backcrossed to one of the parental mouse strains for at least ten generations the genetic background of the resulting mouse is that of the parental mouse strain used for the backcross. For example, a C57BL/6 strain mouse may be bred with a Balb/c strain mouse. The F1 offspring is a hybrid. If the F1 offspring is then backcrossed to Balb/c strain mice for at least ten generations the resulting offspring are said to have a Balb/c genetic background.

Alternatively, if the backcross is to an F1 hybrid mouse for at least ten generations then the resulting offspring are said to have a genetic background of the F1 hybrid.

[0053] As used herein, an “infectious agent” is any biological organism that infects a mammal, including without limitation a virus, a bacterium, and other parasites. In certain
5 embodiments the infectious agent is a virus. In certain embodiments the virus is a lentivirus. In certain embodiments the lentivirus is a human immunodeficiency virus (HIV) or a simian immunodeficiency virus (SIV). In some embodiments the infectious agent is HIV1. In some
embodiments the infectious agent is HIV2.

[0054] As used herein, “anatomically distributed lymph nodes” are a set of lymph
10 nodes occurring at the expected anatomical locations that are visible to the eye following die injection.

[0055] As used herein, “exogenous thymic stromal cell-derived lymphopoietin (TSLP)”
or an “exogenous Flt3 ligand (Flt3L)” is TSLP or Flt3L that is provided to the mouse in the form
of : (i) a TSLP or Flt3L protein that was made outside of a mouse, (ii) a polynucleotide encoding
15 a TSLP or Flt3L protein, preferably inserted in an expression vector. For example, the exogenous
TSLP or Flt3L may be a recombinant TSLP or Flt3L that may be made using a recombinant cell
culture system and then injected into the mouse.

B. Transgenic Mice

[0056] Provided herein are transgenic mice having a genome comprising a
20 homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal
regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene
encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in
the mouse. In some embodiments the SIRP α is SIRP α ^{NOD}. This combination of genetic
features may be combined in the same mouse using any suitable method known in the art.
25 Additional genetic features may also be present in the genome of the mouse. For example, the
transgenic mouse may have a genome further comprising a homozygous disruption of a Rag
gene (Rag1 or Rag2).

[0057] In some embodiments the genetic background of the mouse is selected from BALB/c, C57BL/6, 129, such as 129/Ola, DBA/2, C3H, and NOD. In some embodiments the genetic background of the mouse is selected from an F1 hybrid of any two strains selected from BALB/c, C57BL/6, 129, such as 129/Ola, DBA/2, C3H, and NOD. In some
5 embodiments the genetic background of the mouse is selected from a BALB/c background and a mixed BALB/c-129 background (i.e., a BALB/c-129 F1 hybrid background). In some embodiments the transgenic mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a homozygous disruption of a Rag2 gene (*Rag2*^{-/-}), a transgene encoding mouse signal regulatory protein α (SIRP α), especially SIRP α ^{NOD}, that is
10 functionally expressed in the mouse, and a transgene encoding mouse thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and wherein the genetic background of the mouse is BALB/c.

[0058] For example, a first mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}) may be created. This first mouse may be
15 bred with a second mouse having a genome comprising a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse to create a third mouse having a genome that comprises *Il2rg*^{-/-} and SIRP α . This third mouse may then be bred with a fourth mouse having a genome comprising a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse to create a fifth mouse that is
20 *Il2rg*^{-/-}, SIRP α , TSLP.

[0059] A transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse comprises expression regulatory elements functionally linked to a coding sequence sufficient to drive expression of the SIRP α protein in the mouse. In some embodiments a human SIRP α coding sequence is used and the endogenous regulatory elements that drive
25 expression of human SIRP α in the human genome are present in the transgene and drive expression of SIRP α in the transgenic mouse. In some embodiments a mouse SIRP α coding sequence is used and the endogenous regulatory elements that drive expression of mouse SIRP α in the mouse genome are present in the transgene and drive expression of SIRP α in the transgenic mouse. Alternatively, a known constitutive or inducible promoter may be functionally
30 linked to the SIRP α coding sequence. The transgene may be introduced into the genome of the

mouse using pronuclear injection methods, homologous recombination in ES cells, or any suitable method known in the art.

[0060] A transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse comprises expression regulatory elements functionally linked to a coding sequence sufficient to drive expression of the TSLP protein in the mouse. In some embodiments a mouse TSLP coding sequence is used and the endogenous regulatory elements that drive expression of mouse TSLP in the mouse genome are present in the transgene and drive expression of TSLP in the transgenic mouse. Alternatively, a known constitutive or inducible promoter may be functionally linked to the TSLP coding sequence. A non-limiting example of a suitable promoter is the keratin 14 (K14) promoter. The transgene may be introduced into the genome of the mouse using pronuclear injection methods, homologous recombination in ES cells, or any suitable method known in the art.

[0061] In alternative embodiments an exogenous TSLP, such as recombinant TSLP protein, is administered to a mouse, a mouse embryo, or a mouse fetus. In some embodiments exogenous TSLP, such as recombinant TSLP protein, is administered to a mouse embryo or mouse fetus by administering the protein to a pregnant female mouse. In some embodiments, the TSLP is a mouse TSLP.

[0062] The transgenic mice may be genetically modified so they have a genome comprising a homozygous disruption of the receptor tyrosine kinase Flk2/Flt3 gene ($Flt3^{-/-}$) and wherein exogenous Flt3 ligand (Flt3L), such as recombinant Flt3L, is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous Flt3L, such as recombinant Flt3L, was administered. In some embodiments the Flt3L is human Flt3L. In some embodiments the Flt3L is a mouse Flt3L. $Flt3^{-/-}$ transgenic mice and treatment of $Flt3^{-/-}$ transgenic mice with exogenous Flt3L are disclosed for example in Li et al., Eur. J. Immunol., 2013, 46, 1291-1299 and WO 2010/115115.

[0063] The transgenic mice may optionally be genetically modified so that they do not express mouse MHC class I proteins and/or do not express mouse MHC class II proteins. For example, the transgenic mice may comprise a homozygous disruption of a $\beta 2$ -microglobulin ($\beta 2$ -m) gene, such that the mouse does not express functional major histocompatibility

complex (MHC) I proteins. For example, the transgenic mice may also or alternatively comprise a homozygous disruption of a $I-A\beta^b$ gene, such that the mouse does not express functional major histocompatibility complex (MHC) II proteins.

[0064] The transgenic mice may optionally be genetically modified to have a genome comprising a transgene encoding a functional human MHC I protein. In some embodiments the MHC I protein is human leukocyte antigen (HLA) A2 (HLA-A2).

[0065] The transgenic mice may optionally be genetically modified to have a genome comprising a transgene encoding a functional human MHC II protein. In some embodiments the MHC II protein is human leukocyte antigen HLA-DR1.

[0066] The results presented in the examples create and compare transgenic mice having a genome that is $Il2rg^{-/-}$, $Rag2^{-/-}$, $SIRP\alpha$, and TSLP in a BALB/c genetic background (abbreviated BRGST) with transgenic mice having a genome that is $Il2rg^{-/-}$, $Rag2^{-/-}$, and $SIRP\alpha$ in a BALB/c genetic background (abbreviated BRGS). Accordingly, provided herein are BRGST mice. As shown in the examples, the BRGST mice display several useful attributes, including development of anatomically distributed lymph nodes.

C. Human Immune System (HIS) Mice

[0067] Also provided are human immune system (HIS) mice made from a transgenic mouse of the invention. The HIS mice may be made by engrafting the transgenic mouse of the invention with human primary haematopoietic cells. The human primary haematopoietic cells may be any suitable human primary haematopoietic cells. For example, the cells may be human CD34⁺ cells. For example, the cells may be human fetal liver CD34⁺ cells (available, for example, from Advanced Bioscience Resources Inc., USA). The transgenic mice may be engrafted at from about 3 to about 5 days old. For example, newborn (3 to 5 days old) pups may receive a sub-lethal irradiation (3 Gy) and may then be injected intrahepatically with 5×10^4 CD34⁺CD38⁻ human fetal liver cells. Alternatively, the cells may be peripheral blood mononuclear hematopoietic progenitors.

[0068] The results presented in the examples compare HIS mice made from transgenic BRGST mice with HIS mice made from transgenic BRGS mice. As shown in the examples,

HIS mice made from BRGST mice have several unexpected useful attributes compared to HIS mice made from BRGS mice, including development of anatomically distributed lymph nodes.

[0069] In certain embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-}, SIRPα, and TSLP, and a HIS mouse made from the transgenic mouse supports development of an absolute number of human cells within LNs of at least 5-fold higher than a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-} and SIRPα, but not TSLP. In some embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-}, SIRPα, and TSLP, and a HIS mouse made from the transgenic mouse supports development of an absolute number of human cells within LNs of at least 10-fold higher than a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-} and SIRPα, but not TSLP. The transgenic mouse and control transgenic mouse may have a BALB/c genetic background.

[0070] In certain embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-}, SIRPα, and TSLP, and a HIS mouse made from the transgenic mouse supports development of at least 50% more follicular helper T cells (T_{FH}) than a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-} and SIRPα, but not TSLP. In some embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-}, SIRPα, and TSLP, and a HIS mouse made from the transgenic mouse supports development of at least 75% more follicular helper T cells (T_{FH}) than a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-} and SIRPα, but not TSLP. In some embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-}, SIRPα, and TSLP, and a HIS mouse made from the transgenic mouse supports development of at least 100% more (i.e., two-fold more) follicular helper T cells (T_{FH}) than a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-} and SIRPα, but not TSLP. The transgenic mouse and control transgenic mouse may have a BALB/c genetic background.

[0071] In certain embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-}, SIRPα, and TSLP, and an HIV-1 infected HIS mouse made from the transgenic mouse supports maintenance of at least 5-fold more T_{FH} cells than a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-} and SIRPα, but not TSLP. In some embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, Rag2^{-/-}, SIRPα, and

TSLP, and an HIV-1 infected HIS mouse made from the transgenic mouse supports maintenance of at least 10-fold more T_{FH} cells than a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, *Rag2*^{-/-} and SIRPα, but not TSLP. The transgenic mouse and control transgenic mouse may have a BALB/c genetic backbround.

- 5 [0072] In certain embodiments the transgenic mouse has a genome comprising *Il2rg*^{-/-}, *Rag2*^{-/-}, SIRPα, and TSLP, and an HIV-1 infected HIS mouse made from the transgenic mouse that is treated with highly active anti-retroviral therapy (HAART) maintains an HIV-1 reservoir in infected T_{FH} cells, but a control transgenic mouse that has a genome comprising *Il2rg*^{-/-}, *Rag2*^{-/-} and SIRPα, but not TSLP does not maintain an HIV-1 reservoir. The
 10 transgenic mouse and control transgenic mouse may have a BALB/c genetic backbround. The maintenance of the HIV-1 viral reservoir may be detected using an *ex vivo* quantitative viral outgrowth assay. The maintenance of the HIV-1 viral reservoir may be detected using a method comprising treating the T_{FH} cells cells with PHA.

D. Methods and uses

- 15 [0073] The transgenic mice of the invention, and HIS mice made from the transgenic mice of the invention, are useful for assessing lymphoid clearance in the context of a HIS mouse. In some embodiments the infectious agent is a virus, bacteria or parasite. For example, the HIS mice disclosed herein may be used to characterize viral clearance from T_{FH} cells. Accordingly, also provided are methods comprising providing a transgenic mouse of this
 20 disclosure, infecting the transgenic mouse with an infectious agent, and detecting the presence and/or absence of the infectious agent in a lymph node of the transgenic mouse. In some embodiments the infectious agent is a virus, bacteria or parasite. In some embodiments the virus is a lentivirus. In some embodiments the virus is a virus that infects T_{FH} cells. In some
 25 embodiments the virus is a virus that forms a latent infection stage in T_{FH} cells. In some embodiments the virus is HIV. In some embodiments the virus is HIV-1. In some embodiments the methods further comprise administering an anti-infectious agent therapy to the transgenic mouse. In some embodiments the methods further comprise administering an antiviral therapy to the transgenic mouse. In some embodiments the methods comprise
 30 detecting the presence and/or absence of a latent infection of a lymph node of the transgenic mouse by the infectious agent. In some embodiments the methods comprise detecting the

presence and/or absence of the virus in T_{FH} cells of the transgenic mouse. In some embodiments the methods comprise detecting the presence and/or absence of a latent viral infection in T_{FH} cells of the transgenic mouse.

[0074] Also provided are methods comprising, providing a first transgenic mouse of
5 this disclosure, infecting the first transgenic mouse with a virus and administering an antiviral
therapy to the first transgenic mouse, providing a second transgenic mouse of this disclosure,
infecting the second transgenic mouse with the virus and not administering the antiviral
therapy to the second transgenic mouse, and measuring the level of the virus in a lymph node
10 of the first transgenic mouse and measuring the level of the virus in a lymph node of the
second transgenic mouse. In some embodiments the level of the virus in the lymph node of
the first transgenic mouse is lower than the level of the virus in the lymph node of the second
transgenic mouse, and wherein the antiviral therapy is identified as effective to reduce lymph
node viral load. In some embodiments the level of the virus in the lymph node of the first
15 transgenic mouse is not lower than the level of the virus in the lymph node of the second
transgenic mouse, and wherein the antiviral therapy is identified as not effective to reduce
lymph node viral load. In some embodiments the virus is a lentivirus. In some embodiments
the virus is a virus that infects T_{FH} cells. In some embodiments the virus is a virus that forms a
latent infection stage in T_{FH} cells. In some embodiments the virus is HIV. In some
20 embodiments the virus is HIV-1. In some embodiments the methods comprise detecting the
presence and/or absence of the virus in T_{FH} cells of the transgenic mouse. In some
embodiments the methods comprise detecting the presence and/or absence of a latent viral
infection in T_{FH} cells of the transgenic mouse. In some embodiments the transgenic mice used
in the methods are HIS mice.

[0075] Also provided are methods comprising, providing a human immune system
25 mouse according to this disclosure, administering an antigen to the human immune system
mouse, and assaying for the presence, absence, and/or level of at least one immune response
in the human immune system mouse to the antigen. In some embodiments the at least one
immune response is selected from the group consisting of a B-cell response, a T-cell response,
and an NK-cell response. In some embodiments the methods further comprise administering
30 an adjuvant to the human immune system mouse. In some embodiments the methods further

comprise administering the antigen to a control mouse. In some embodiments the methods further comprise administering a test molecule to the human immune system mouse and determining whether the test molecule increases or decreases the at least one immune response in the human immune system mouse to the antigen.

5 [0076] Also provided are methods comprising, providing a human immune system mouse according to this disclosure, administering a vaccine against an infectious agent to the human immune system mouse, administering the infectious agent to the human immune system mouse, and assaying for the presence, absence, and/or level of infection of the human immune system mouse by the infectious agent. In some embodiments the methods further
10 comprise administering the infectious agent to a second human immune system mouse, and assaying for the presence, absence, and/or level of infection of the second human immune system mouse by the infectious agent. In some embodiments the infectious agent is a virus, bacteria or parasite. In some embodiments the infectious agent is a virus and the vaccine is a viral vaccine. In some embodiments the virus is a lentivirus. In some embodiments the
15 lentivirus is HIV.

[0077] Also provided are methods comprising, providing a human immune system mouse according to this disclosure, administering an infectious agent to the human immune system mouse, administering an anti-infectious agent to the human immune system mouse, and assaying for the presence, absence, and/or level of infection of the human immune system
20 mouse by the infectious agent. In some embodiments the methods further comprise administering the infectious agent to a second human immune system mouse, and assaying for the presence, absence, and/or level of infection of the second human immune system mouse by the infectious agent. In some embodiments the infectious agent is a virus, bacteria or parasite. In some embodiments the infectious agent is a virus and the anti-infectious agent is
25 an antiviral agent. In some embodiments the virus is a lentivirus. In some embodiments the lentivirus is HIV.

E. HIS mouse model of human atopic dermatitis and use thereof

[0078] The results presented in the examples also show that HIS mice made from transgenic BRGST mice spontaneously develop clinical signs closely resembling human

atopic dermatitis. In addition to clinical findings, histological, immunological and skin changes resembling human atopic dermatitis are also found in BRGST HIS mice. Therefore, the BRGST HIS mice of the invention provides a spontaneous human atopic dermatitis model that is very useful as a preclinical model to test candidate therapeutic agents and assess novel treatments for human allergic and/or inflammatory skin diseases, in particular atopic dermatitis.

[0079] Accordingly, the invention also provides a mouse model of human atopic dermatitis comprising a human immune system (HIS) mouse according to this disclosure. The HIS mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and further comprises a humanized immune system. The human immune system mouse comprises in particular human B cells, T cells, NK cells, and myeloid cells. The HIS mice may be made by engrafting the transgenic mouse of the invention with human primary haematopoietic cells as disclosed above.

[0080] The invention also provides the use of the mouse model of human atopic dermatitis of the present disclosure for testing candidate therapeutic agents or assessing treatments for human allergic and/or inflammatory skin diseases, preferably atopic dermatitis. The candidate therapeutic agent may be any agent of interest, for example an antibody or others.

[0081] Also provided are methods comprising, providing a human immune system mouse model of human atopic dermatitis according to this disclosure, administering a candidate therapeutic agent for treating atopic dermatitis to the human immune system mouse model, and evaluating the therapeutic effect of the candidate agent.

[0082] The therapeutic effect of the candidate agent is determined using objective parameters or criteria of the therapeutic response such as clinical signs or predictive biomarkers. The objective criteria for evaluating the therapeutic response to the candidate agent or treatment can be determined easily by one skilled in the art, for example by using clinical scores, as shown in the examples of the present application. The therapeutic effect of

the candidate agent or treatment in the mouse model may be evaluated by measuring the parameters before and after administration of the candidate agent or treatment to a mouse or by comparing the parameters in a treated mouse and an untreated mouse (control).

F. Treatment of human atopic dermatitis

5 [0083] The results presented in the examples also show that C-C chemokine receptor type 4 (CCR4) is an immunotherapeutic target for atopic dermatitis, in particular human atopic dermatitis.

[0084] Therefore, the invention also provides a C-C chemokine receptor type 4 (CCR4) antagonist for use in the prevention and/or treatment of atopic dermatitis, in particular
10 human atopic dermatitis. The invention provides a method of treating atopic dermatitis, comprising administering a CCR4 antagonist to a subject in need thereof, in particular a human subject. In some embodiments, the CCR4 antagonist is an antibody against CCR4, in particular a monoclonal antibody, more particularly Mogamolizumab. In some embodiments, the CCR4 antagonist is used for the prevention of atopic dermatitis, in particular to slow-
15 down or stop the development of atopic dermatitis.

EXAMPLES

Example 1: Materials and Methods

[0085] The following materials and methods were used to perform the examples.

[0086] *Mice*

20 [0087] Animals were housed in isolators under pathogen-free conditions with human care and anesthesia was performed using Isoflurane to minimize suffering. All experiments generating and characterizing humanized mice were approved by an ethical committee at the Institut Pasteur (CETEA-2013-0131) and validated by the French Ministry of Education and Research (Reference # 02162.01).

25 [0088] Mice transgenic for mouse TSLP (mTSLP) under the control of the Keratin 14 promoter on the C57BL/6 background have been previously described ¹². TSLP transgenic

mice were extensively backcrossed (>10 generations) to the Balb/c *Rag2*^{-/-} *Il2rg*^{-/-} *Sirpa*^{NOD} strain (BRGS)¹³ to create TSLP transgenic BRSG (BRGST) hosts.

[0089] *Human blood and tissue samples*

[0090] Blood samples from healthy donors were obtained from Etablissement
5 Français du Sang (EFS, Paris) in an agreement signed with Institut Pasteur. Fetal liver was obtained from Advanced Bioscience Resources Inc. with gestational age ranging from 14 to 20 weeks. Experiment with human fetal liver were approved by Medical and Ethical Committees at Institut Pasteur and the French Ministry of Education and Research (IF-20080451) and performed in full compliance with French Law.

10 [0091] *Enumeration of LN anlagen and PPs*

[0092] LN enumeration was performed a week after peritoneal injection of 100 µl of 1% Chicago Sky Blue 6B (Sigma-Aldrich) ink in PBS. Images of LNs were captured with a Canon PowerShot S90 camera (Canon). Whole mount VCAM-1 immunostaining of mouse intestine was performed to enumerate PPs from newborn pups. Briefly, entire intestine
15 removed of mesentery was fixed in 4% paraformaldehyde, dehydrated with methanol, and stained with rat anti-VCAM antibody (CD106, clone 429, Thermofisher, 1:50) overnight. Results were revealed with horseradish peroxidase-labeled goat anti-rat IgG (ab7097, Abcam) followed by DAB substrate kit (Abcam). Images of PPs were captured with a stereoscopic Leica M80 microscope.

20 [0093] *Generation of HIS mice*

[0094] BRGS and BRGST mice were used as recipients to create HIS mice as previously described¹³⁻¹⁵. Briefly, fetal liver CD34⁺ cells (Advanced Bioscience Resources Inc., USA) were isolated using affinity matrices according to manufacturer's instructions (Miltenyi Biotec) and subsequently phenotyped for CD38 expression. Newborn (3 to 5 days
25 old) pups received sub-lethal irradiation (3 Gy) and were injected intrahepatically with 5x10⁴ CD34⁺CD38⁻ human fetal liver cells. All manipulations of HIS mice were performed under laminar flow conditions.

[0095] *Cell isolation*

[0096] HIS mice were perfused with PBS prior to tissue preparation. Thymus, LNs (all visible LN in BRGS or BRGST HIS mice as detailed in Fig. 7f were pooled) and spleen were minced and filtered through 100µm cell strainer (Falcon). Femur and tibia bones were crushed with mortar and pestle to extract cells. Erythrocytes in spleen and BM were lysed using red blood cell lysing buffer Hybri-Max (Sigma). Percoll density gradient centrifugation (GE Healthcare Life Sciences) was used for liver lymphocyte preparation. Intestines were perfused with cold RPMI 1640 medium to remove feces, cut open longitudinally and washed. Tissue was cut into 1-cm pieces and agitated in pre-warmed medium containing 10 mM EDTA (Sigma-Aldrich) at 37°C for 30 min to remove epithelial cells. Remaining tissue was collected, minced and digested for 60 min in medium containing 5% FCS and Liberase (1 mg/ml; Roche). Lamina propria lymphocytes were enriched by Percoll gradient centrifugation, filtered and washed. All cell preparation steps were performed using RPMI 1640 Glutamax (Life Technologies) 100U/mL penicillin, 100g/mL streptomycin (Invitrogen) unless otherwise stated. For isolation of skin leukocyte, shaved upper back skins (2cm x 2cm) were cut into small pieces in cold RPMI 1640 Glutamax (Life Technologies) medium plus 100U/mL penicillin, 100g/mL streptomycin (Invitrogen), and digested twice at 37°C for 1 hour with 10ml RPMI 1640 Glutamax medium containing 100U/mL penicillin, 100g/mL streptomycin (Invitrogen), 50µg/ml liberase (Roche) and 50U/ml DNase I (Roche). After digestion, remaining tissues and cells were filtered with 100µm cell strainers to receive single-cell suspension. Skin leucocytes were then enriched by Percoll (GE Healthcare Life Sciences) gradient centrifugation, filtered and washed.

[0097] *Flow cytometric analysis and cell sorting*

[0098] Fixable viability dye eFlour506 (Thermofisher) were used to exclude dead cells before Ab staining. Fluorochrome-conjugated Abs from BD Biosciences, Biolegend, Thermofisher and Miltenyi were used in this study (Table 1 and 2). For surface marker staining, cells were incubated with Abs on ice for 30 min. For Foxp3 staining, cells were stained with surface first and then permeabilized and fixed with Foxp3/Transcription Factor Staining Buffer set (Thermofisher). For intracellular cytokine detection, freshly isolated cells were stimulated with 50 ng/ml PMA (Sigma-Aldrich) and 1 µg/ml ionomycin (Sigma-

Aldrich) for 4 hours in the presence of Golgi-plug (BD biosciences) prior to intracellular cytokine staining using the BD cytofix/cytoperm kit (BD biosciences). Fortessa (BD Biosciences) were used to acquire the data and Flowjo software (TreeStar) were used for data analysis. Cell sorting was performed using a FACS Aria II (BD biosciences).

5 [00099] *Cell enrichment*

[00100] Human CD3⁺ T cells from HIS mice were purified by staining cells with biotin conjugated anti-human CD19, CD14, CD11b, CD56, CD123, NKp46 and biotin conjugated anti-mouse Ter119 and CD45 (**Table 1**). Human CD3⁺ T cells from human peripheral blood were isolated by staining cells with biotin conjugated anti-human CD19, CD14, CD11b, 10 CD56, CD123 and NKp46 (**Table 1**). Stained cells were then incubated with anti-biotin microbeads and depleted by immunomagnetic selection (Miltenyl Biotec) and untouched human T cells (Fig. 14a) were used for 2-photon microscopy (see below).

[00101] *Immunohistochemistry*

[00102] Tissue samples were fixed in 10% neutral buffered formalin, embedded in 15 paraffin and 4 µm sections were cut. To assess lymphocyte phenotypes, histochemistry analysis was performed using primary mouse anti-human CD3 (clone F7.2.38, M7254, Dako, Glostrup, Denmark; dilution 1:50) and mouse anti-human CD20cy (clone L26, M0755, Dako, Glostrup, Denmark; dilution 1:1000) antibodies, revealed using a Venatana Benchmark XT automated IHC slide staining system (Roche).

20 [00103] *Immunofluorescence analysis*

[00104] Cryostat sections of LN fixed in 4% paraformaldehyde were immunostained with the following antibodies diluted in PBS with 0,1% Triton X-100 and 1% FCS: anti-human CD3 (A0452, Dako), anti-human CD20 (clone L26 M0755, Dako), anti-AID (clone mAID-2, Thermofisher). Secondary antibodies were coupled to Alexa Fluor 488, Alexa Fluor 25 555, Alexa Fluor 647 (Molecular Probes). Photomicrographs were taken with an Axioimager microscope (Zeiss).

[00105] *Immunization*

[00106] HIS mice were immunized *i.p.* using a 29-gauge needle, three times, on weeks 12, 15, and 18 with 100µg mariculture keyhole limpet hemacyanin (mcKLH, Prod# 77600, Thermo Scientific) and 60µg aluminum hydroxide in 100µl PBS. Negative controls received
5 the same volume of PBS buffer with 60µg aluminum hydroxide. Two weeks after the last immunization, HIS mice were analyzed for antigen-specific T and B cell responses.

[00107] *Single B-cell FACS sorting, immunoglobulin gene amplification and analysis*

[00108] Single IgA⁺ and IgG⁺ memory B cells were identified and sorted into 96-well PCR plates using a FACS Aria III sorter (Becton Dickinson) as previously described ³⁵.

10 Single-cell cDNA synthesis using SuperScript IV reverse transcriptase (Fisher Scientific) followed by nested-PCR amplifications of IgH, Igκ and Igλ genes were performed as previously described ³⁵. All immunoglobulin gene characteristics were determined by analyzing IgH and IgL sequences of single IgG⁺ and IgA⁺ B cells using IgBLAST; (<http://www.ncbi.nlm.nih.gov/igblast>) and IMGT® (<http://www.imgt.org>) online tools. Circos
15 plots were generated from the immunoglobulin gene analysis using *Circlize* R package (v0.3.1). Phylogenetic trees were generated following sequences alignment using CLC Main Workbench 7 software (v7.0) with default parameters. The relationship between sequences was generated using the Neighbor-Joining method. The bootstrap consensus tree inferred from 100 replicates was taken to represent the relationship. IgH and IgL mutations selection
20 strengths were determined using the BASELINE program (<http://selection.med.yale.edu/baseline/>). P values for Ig gene repertoire analyses, analysis of lengths, and positive charges of IgH CDR3 were calculated by two-sided 2x2 or 2x5 Fisher's Exact test. The numbers of VH, Vκ and Vλ mutations were compared across groups of antibodies using unpaired student t-test with Welch's correction. Statistical analyses were
25 performed using *GraphPad Prism* software (v6.0a), and SISA online tools for 2x5 Fisher test (<http://www.quantitativeskills.com/sisa>).

[00109] *ELISA assay*

[00110] The plasma harvested from BRGS and BRGST mice were screened by ELISA for mouse TSLP (88-7490-22, ThermoFisher). The sera harvested from HIS mice were screened by ELISA for the presence of antigen-specific and total antibodies. Species specific
5 ELISA assays for human anti-KLH IgG (700-140-KLG, Alpha Diagnostic), human IgM (109-035-043, Jackson ImmunoResearch), human IgG (109-035-008, Jackson ImmunoResearch), and human IgA (88-50600-22, Thermofisher) were done according to the manufacturer's instructions.

[00111] *ELISPOT assay*

10 [00112] 96-well plates with a nitrocellulose filter base (MultiScreen, Millipore) were coated with purified anti-IFN- γ capture antibodies (human IFN- γ ELISPOT pair, BD biosciences). After overnight incubation at 4°C, plates were washed twice in IMDM with L-Glutamine (Lonza), 10% FCS and penicillin/streptomycin (Gibco) and incubated in complete
15 IMDM for 2 hours at room temperature for the blocking step. Splenocytes from immunized mice were added in doubling dilutions starting from 5×10^5 cells per well with 10 μ g/ml KLH and 1 μ g/ml purified anti-CD28 (clone 28.2, eBioscience) or anti-human CD3/CD28 activating beads (Dynabeads, Gibco) for restimulation, with IL-2 (10 IU/mL) and IL-7 (100ng/mL) (Miltenyi Biotech). Doubling dilutions were also applied to non-stimulated
20 splenocytes (only maintained in IL-2 and IL7) to determine the background. After 36h in a 37°C 5%CO₂ incubator, plates were washed following manufacturer's protocol (human IFN- γ ELISPOT pair, BD biosciences) and incubated with biotinylated anti-IFN- γ detection antibody (BD Biosciences), followed by streptavidin-horseradish peroxidase conjugate (BD Biosciences), and revealed with an AEC substrate (BD Biosciences). Plates were dried and red spots were enumerated using an ELISPOT counter (Bioreader 5000-E β cytometer,
25 Biosys).

[00113] *Two photon microscopy*

[00114] Isolated human T cells (from HIS mice or from human peripheral blood) were labeled with 4 μ M CFSE (Vybrant CFDA-SE Cell Tracer Kit, Fisher) before adoptive transfer.

10 million human T cells were injected retro-orbitally into 12 to 14 week old BRGST HIS mice. Two-photon imaging (intravital, explanted LN) was performed as previously described^{19, 20} using an upright microscope (FVMPE-RS, Olympus) with a 25X/1.05 NA dipping objective (Olympus). Excitation was provided by an Insight DS + Dual laser (Spectra-
5 Physics). Movies were processed and analyzed with Imaris software (Bitplane). Straightness index was calculated as the ratio of the distance from origin to the total distance travelled.

[00115] *Gene expression analysis*

[00116] CD4⁺ T cells were isolated by immunomagnetic selection (Miltenyl Biotec) and RNA extracted using RNeasy plus minikit (Qiagen, CA). To avoid unnecessary freeze
10 and thaw of the RNA, distinct aliquots for quantification and gene expression analysis were prepared, and all aliquots were frozen at -80°C. RNA concentration was estimated using Qubit RNA HS Assay Kit (Life Technologies) according to the manufacturer's protocol. Gene expression including *Il21* and *Cxcl13* expression were analyzed on minimum 50 ng of
15 total RNA from each sample using the preassembled nCounter GX Human Immunology kit v2 and nCounter system (Nanostring Technologies, Seattle, WA) according to manufacturer's instructions. Each sample was analyzed in a separate multiplexed reaction including in each, eight negative probes and six serial concentrations of positive control probes. Negative control analysis was performed to determine the background for each sample. Data was imported into nSolver analysis software (version 3.0) for quality check and normalization of
20 data. For each gene in a sample, the geometric means of the eight negative probe counts were subtracted to remove the background from nonspecific probe binding. To normalize for differences in RNA input, samples were then normalized based on the geometric means of both the supplied positive controls and the 15 housekeeping genes, as recommended by the manufacturer. Gene expression is presented as normalized nCounter Units and visualized by
25 *GraphPad Prism* software (v6.0a). The differential gene expression between groups was analyzed and visualized as volcano-plot by nCounter Advanced Analysis Software (version 1.1.5).

[00117] *HIV-1 infection in HIS mice*

[00118] *HIV-1_{NLAD8}* molecular clone was obtained from the AIDS Research and Reference Reagent Program, NIAID, NIH. NLAD8 viruses were produced using 293T cell transfection with plasmid pNLAD8 (Trans IT, Mirus). Virus was titrated using the Reed-Muench method on activated PBMCs and p24 was titrated with Alliance HIV-1 p24 ELISA kit (Perkin-Elmer). BRGS and BRGST HIS mice were inoculated by intra-peritoneal injection of 10⁵ tissue culture infective dose required for 50% infection (TCID₅₀) HIV_{NLAD8} and housed in isolators. Viral RNA was extracted from 25µL of plasma (EDTA-harvested blood, Microvette CB300, Sarstedt) using QIAamp Viral RNA kit (Qiagen) using retrotranscription (SuperScript III Reverse Transcriptase, Invitrogen) and a linear 15-cycle pre-amplification PCR, followed by a probe-based qPCR (JumpStart, Sigma-Aldrich) using the following primers and probes defined on HIV_{NLAD8} gag gene (Sigma-Aldrich). (PCR1: For 5'-GCCTCAATAAAGCTTGCCTTGA-3' (SEQ ID NO: 1) Rev 5'-CCTGGCCTTAACCGAATTT3' (SEQ ID NO: 2), qPCR For 5'-GCCTCAATAAAGCTTGCCTTGA-3' (SEQ ID NO: 3), Rev 5'-GGCGCCACTGCTAGAGATTTT-3 (SEQ ID NO: 4), Probe 5'-FAM-AAGTAGTGTGTGCCCCGTCTGTTGTGTGACT-3' BHQ1 (SEQ ID NO: 5)). Quantification was reached using serum HIV standards (Biocentric kit, LOD= 300 RNA copies/mL). Oral administration of highly active antiretroviral therapy (HAART) was initiated at 8 dpi during 20 days with a 3 drug regimen composed of Emtricitabine (FTC, Emtriva, Gilead), Tenofovir disoproxil fumarate (TDF, Viread, Gilead), Raltegravir (RAL, Isentress, MSD). The tablets were powdered and resuspended in solution (MediDrop Sucralose, ClearH20). The doses were scaled for mouse metabolism using animal weights and Km studies (mouse Km=3, human Km=37). The final dose mg/kg/day without excipients were: FTC (72.9) TDF (72.9) RAL (729.2).

[00119] FACS analysis was performed on 30µl total blood stained with antibodies (EDTA-harvested blood, Microvette CB300, Sarstedt) and counting beads to quantify absolute numbers of cells/mL blood (CountBright absolute counting beads, Invitrogen). After incubation with antibodies, red blood cells were lysed using FACS Lysing solution (BD) before FACS analysis.

[00120] *Quantitative viral outgrowth assay for HIV*

[00121] CD4⁺ positive cells isolated from lymph nodes were plated in duplicate in limiting dilution in 48 wells plates (starting with 1×10^6 or 7.5×10^5 , and a dilution factor of 5). Cells were then stimulated with PHA (1 µg/mL) and IL-2 (100 UI/mL). Levels of p24 in supernatants were quantified at the indicated time points with the ultrasensitive digital ELISA Simoa assay (Quanterix) and the fraction of Gag⁺ cells was determined after 14 days of culture as previously described³⁸. Infectious unit per millions (IUPM) were calculated using IUPM calculator v1.0 (<http://silicianolab.johnshopkins.edu/>) and cytometry data were analyzed using Flowjo X (Tristar). Conservative thresholds were set for p24 positivity (ranging from 0.05 to 0.16 pg/mL), and only wells with at least 2 consecutive detectable p24 values were considered as positive for IUPM calculation.

[00122] *Measuring the AD scores and ear thickness*

[00123] The dermatitis score is measured based on 5 criteria: hair density, hair loss, the presence of dryness/scales, hemorrhage/erosion and infection/crusts. 4 regions of the mouse body were checked for each criterion: Head/neck, dorsal skin, ventral skin and 4 limbs. For the hair density, only one region with most severe hair loss is scored on the scale of 0 to 4; for the rest criteria, the score is based on the number of regions (0-4). The score system is detailed in (Table 3). For measuring the ear thickness, a thickness gauge micrometer with a ratchet stop was used for a constant measuring force (293-831-30, mitutoyo).

[00124] *Mice skin swab collection and processing*

[00125] Specimens were obtained with sterile dry swabs (COPAN LQ Stuart Transport Swab; COPAN Italia S.p.A, Brescia, Italy), which are rotated five times around the mice skin while applying constant pressure. All swabs are immediately frozen at -80°C until use. To the processing the TECAN Freedom EVOware robot was used which allows the establishment of a broad range of high-throughput protocols using Matrix barcoded tubes. First, the samples are thawed, vortex for 30 seconds at 2500 rpm, to insure complete recuperation of the cells and microbes fixed to the flocked swabs. Next, the samples were pipetted to a 96 well deep

well plate and was directly centrifuged at 16 000g for 10 minutes at 4°C to pellet the cells and microbes. The swab supernatant was aliquoted to the exploration of the proteome.

[00126] *DNA extraction, library preparation, quantification and sequencing*

[00127] In the present study, the extraction of total genomic DNA from swabs samples
5 was performed using the TECAN Freedom EVOware workstation, and the NucleoSpin® 96
Genomic DNA kit (Macherey-Nagel ®). Briefly, the pellets that remain in the deep well plate
were incubated with Ready-Lyse™ Lysozyme Solution (250 U/μl) (Epicentre, Hessisch
Oldendorf, Germany) for 30 minutes at 37°C. Next, the suspension were incubated with
Proteinase K/buffer T1 working solution at 55°C overnight until the samples are completely
10 lysed. 20 μg of glycogen (DNA carrier) was added and the DNA extraction kit protocol was
followed. Finally the DNA was eluted in 30 μl and the DNA box is immediately frozen at -
80°C at local center until use. The concentration of extracted DNA is determined using
TECAN (QuantiFluor® ONE dsDNA System, Promega), and DNA integrity and size were
also confirmed with the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). In general,
15 the amplification of V1-V4 region of 16S rRNA resulted in the identification of more bacteria
(Castelino et al., 2015; Chakravorty et al., 2007; Conlan et al., 2012). Therefore, the V3-V4
region of 16S rRNA were PCR amplified from each sample using a composite forward primer
(340F; 5'-AATGATACGGCGACCACCGAGATCTACAC-3', (SEQ ID NO : 6)) and a
reverse primer (806R: 5'-CAAGCAGAAGACGGCATACGAGA-3' (SEQ ID NO : 7))
20 containing a primer linker, primer pad, unique 8-mer Golay barcode which was used to tag
PCR products from respective samples, and the Illumina adaptor (Kozich et al., 2013). PCR
reactions consisted of 18 μl of AccuPrime Pfx SuperMix (12344-040; Invitrogen), 0.5 μl of
each primers and 1 μl of DNA (10 ng). PCR was carried out as follows: 95 °C for 2 min, 30
cycles of 95 °C for 20 s, 55 °C for 15 s and 72 °C for 5 min, and a final extension step at 72
25 °C for 10 min on a Biorad thermocycler. Next, each PCR reaction was cleaned individually
with NucleoMag magnetic purification beads (MACHEREY-NAGEL Kit) following the
protocol for DNA double size selection (fragment libraries with a size range of 400-600 bp)
and resuspended in 20 μL of TE buffer. PCR products were then quantified with the
QuantiFluor® ONE dsDNA kit (Promega). Equal amounts of each PCR product (50 ng per
30 sample) were pooled and thoroughly mixed. Library pools were diluted followed by NaOH

denaturation as per manufacturer's instructions (Illumina Inc., USA). Denatured libraries were loaded at 12pM with a 15% PhiX spike for diversity and sequencing control, onto a v2 300-bp paired end reads cartridge for sequencing on the Illumina MiSeq. The 16S rDNA amplicon library was sequenced at the Institut Pasteur Biomix NGS platform.

5 [00128] *Sequence processing and statistical analysis*

[00129] Bioinformatic analysis was performed as previously described (Quereda et al., 2016). After removed reads containing incorrect primer or barcode sequences and sequences with more than one ambiguous base, a total of 470.947 mapped reads from the 8 mice with a mean length of 416-base-long paired-end through Illumina MiSeq analysis. Each individual
10 was covered by an average of 58.868 reads. Briefly, amplicons were clustered into operational taxonomic units (OTU) with VSEARCH (v1.4) and aligned against the greengene and SILVA database. The clustering was performed at 98% sequence identity threshold, producing 283 OTUs. The normalization, statistical analyses and multiple visualization were performed with SHAMAN (SHiny application for Metagenomic Analysis (shaman.c3bi.pasteur.fr) based on R
15 software. All the individual rarefaction curves tended to approach the saturation plateau (Figure 24). Principal coordinates analysis (PCOA) based on Canberra distance matrix was computed at phylum and genus taxonomic level to describe the similarity between microbiota.

[00130] *Quantification of total fungi (18S rDNA), bacteria (16S rDNA) and S. aureus in skin measured by qPCR.*

20 [00131] To gain further insight into microbiota counts, a qPCR was applied in the extracted DNA, using universal 16S rRNA primers to measure total bacteria (16S_F: 5'-ATTACCGCGGCTGCTGG-3' (SEQ ID NO: 8) and 16S_R: 5'-ATTACCGCGGCTGCTGG-3, (SEQ ID NO: 9)) and 18S rRNA primers to measure total fungi (18S_F: 5'-ATTGGAGGGCAAGTCTGGTG-3 (SEQ ID NO: 10) and 18S_R: 5'-CCGATCCCTAGTCGGCATAG-3, (SEQ ID NO: 11)) (Qiu et al., 2015). The absolute
25 abundance of *S. aureus* was also evaluated by quantitative qPCR using primers specific to sa442 gene (sa442_F:5'-GTCGGGTACACGATATTCTTCACG-3' (SEQ ID NO: 12) and sa442_R: 5'-CTCTCGTATGACCAGCTTCGGTAC-3'(SEQ ID NO: 13)) (Cattoir et al., 2011; Martineau et al., 1998; Reischl et al., 2000; Shrestha et al., 2002). PCR reactions

consisting of 10 μ L SYBR Green PCR master mix (Roche), 1 μ L (10 nM) each primer, 200 ng template DNA in 20 μ L of reaction carried out on an ABI StepOne Plus Sequence Detection System (Applied Biosystems). Thermocycling reactions consisted of 1 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 15 s at 56 °C, and 45 s at 72 °C.

5 [00132] *ELISA and Cytokine multiplex assay*

[00133] Blood from HIS mice was collected by facial vein into an EDTA coated tubes (Microvette CB 300, Sarstedt) and plasma was collected by centrifuge at 2000g for 10min. Species specific ELISA assays for human IgE (88-50610-22, eBioscience) were done according to the manufacturer's instructions. Cytokines were analyzed using human cytokine
 10 magnetic 25-plex panel (Invitrogen) on a MAGPIX machine (Luminex). Secreted human cytokines from mouse skin swabs was detected by a digital enzyme-linked immunosorbent assay (ELISA). The Single Molecule Array (Simoa) Analyzer (Quanterix®, Lexington, MA 02421, USA) is automated equipment and enables detection of lower concentrations, compared to conventional ELISA and radioimmunoassay technology, by providing the
 15 capability of detection of proteins by a so-called digital ELISA principle based on counting individual enzyme-labeled immunocomplexes of proteins captured on paramagnetic beads in single-molecule arrays (Rissin et al., 2010). The instrument has recently been described in detail (Wilson et al., 2016). The Simoa HD-1 Analyzer consumables were purchased from Quanterix Corporation (Quanterix®). IL-1 β , IL-6 and TNF, IL-17A and IL-22 were
 20 quantified by ultrasensitive assay kits according to the manufacturer's instructions (ref. 101605, 101319 and 101953; Simoa; Quanterix).

[00134] *Cell culture and stimulation*

[00135] For human DC culture, peripheral blood mononuclear cells (PBMC) from healthy PB were isolated by Ficoll-Paque (GE Healthcare) density gradient centrifugation.
 25 PBMC were first depleted of T cell, B cell, monocytes, NK cells and erythrocyte by labeling with biotin-conjugated anti-CD3, anti-CD19, anti-CD14, anti-CD56, or anti-CD235a followed by anti-biotin microbeads (Miltenyi) according to manufacturer's instructions. Depleted cells were further stained with PE-Cy7 conjugated anti-CD11c, FITC conjugated anti-CD4, and PE-CF594 conjugated anti-CD3/19/14 (Table 2). Lin⁻CD11c⁺CD4^{low} were sorted by a

FACS Aria II (BD biosciences) to reach >95% purity. CD11c⁺ DCs were cultured immediately after sorting in RPMI 1640 Glutamax medium containing 100U/mL penicillin, 100g/mL streptomycin (Invitrogen), 10% fetal calf serum (FCS), 1% sodium pyruvate (Life Technologies), and 10mM HEPES (Dutscher). Cells were seeded at 3×10^5 /ml in flat bottomed 96-well plates in the presence of mouse TSLP (50ng/ml, R&D), human TSLP (50ng/ml, R&D), poly I:C (25µg/ml, Invivogen), or culture media alone. After 24 hours of culture, DCs were collected and resuspended by incubating with ice chilled DPBS containing 2mM EDTA for 5 minutes under room temperature. For human T cell culture, PBMCs were stained with FITC-CD4, PerCP-Cy5.5-CD3 and AF700-CD45. Sorted CD4⁺ T cells (>95%) were seeded at 2.5×10^5 /ml in U-bottomed 96-well plates with RPMI 1640 Glutamax medium containing 100U/mL penicillin, 100g/mL streptomycin (Invitrogen), and 10% FCS. Cells were cultured in the presence of mouse IL-12 (50ng/ml, R&D), human IL-12 (50ng/ml, R&D), mouse TSLP (50ng/ml, R&D) or media alone and supplemented with or without dynabeads (1×10^4 /well, Life Technologies). Half of media were replaced at day 5 and day 7 with corresponding cytokines. At day 8, cells were washed and resuspended in staining buffers for FACS staining. The duplicated wells were stimulated with PMA and Ionomycin for 4 hours to check for cytokine production.

[00136] *In vivo antibody depletion*

[00137] Anti-hCD4 (OKT4), anti-hCD8 (OKT8), anti-hCD20 (Rituximab), anti-hIL-12 p70 (20C2), and anti-mIL12 p75 (R2-9A5) mAbs were purchased from bioXcell (UK). Anti-hCCR4 (KM2160) was provided by Shimon Sakaguchi and Atsushi Tanaka (Osaka University, Japan). 300µg anti-hCD4, 100µg anti-hCD8 and 200µg anti-hCD20 antibodies were injected intraperitoneally (i.p.) every week to check for the AD progression and the depletion of corresponding cell types in HIS mice. 200µg anti-hCD4 antibodies were given twice a week as a therapeutic agent by i.p. injections to BRGST HIS mice with AD. 100µg hIL-12 and 100µg mIL-12 blocking antibodies were mixed and injected weekly i.p. to HIS mice to check for the AD progression and the prevention of CLA upregulation on CD4 T cells. 100µg anti-hCCR4 antibodies were injected weekly i.p. to HIS mice to check for the depletion of CCR4⁺CD4⁺ T cells and AD progression.

30 [00138] *Statistics*

[00139] Statistical significance of the data was calculated using GraphPad Prism version 6 and data were depicted as mean $\pm$ s.e.m. if not stated otherwise. To compare two groups, an unpaired two-tailed Mann-Whitney U test was applied. When more than two groups of samples were compared, one-way ANOVA (with Tukey's multiple-comparison post-tests) was used. All P values less than or equal to 0.05 were considered significant. No statistical method was used to pre-determine sample sizes.

Example 2: TSLP expression in *Il2rg*-deficient mice promotes formation of SLT anlagen

[00140] Mouse TSLP levels were elevated in the serum of adult BRGS mice expressing K14 promoter-driven mTSLP (BRGST) (Fig. 7a). While gut NK cell and ILC subsets were severely depleted in *Il2rg*^{-/-} BRGS mice compared to *Rag2*^{-/-} mice (Fig. 7b, c), BRGST mice showed improved ILC development with no effect on NK cells (Supplementary Fig. 7c), suggesting that TSLP complements IL-7 signaling pathways *in vivo*. Previous studies showed that TSLP over-expression can restore LT α i function in *Il2rg*^{-/-} hosts⁶. This finding was confirmed in BRGST mice where LN anlagen robustly developed throughout the body (Fig. 7d-f). The few LN anlagen in BRGS mice were smaller than those in BRGST mice and only visible through dye staining. In contrast, the defective PP development secondary to *Il2rg*-deficiency was not rescued by TSLP over-expression (Fig. 7g).

Example 3: A novel humanized mouse model with robust lymph node development

[00141] Engraftment of BRGS hosts with human CD34⁺ hematopoietic stem cell (HSC) progenitors generates diverse human lymphocytes (B, T, NK cells) and myeloid cells¹³⁻¹⁵. Nevertheless, reconstituted HIS mice in BRGS^{3, 5, 13-15} or other backgrounds^{4, 16} show poor and variable LN reconstitution. In contrast, reconstituted BRGST HIS mice harbored a diverse set of systemic LNs (Fig. 1a; Fig. 8a). Total numbers of human CD45⁺ cells were similar in the spleen of BRGS and BRGST HIS mice, while absolute numbers of human cells within LNs were at least 10-fold higher in BRGST HIS mice (Fig. 1a). LNs in these mice harbored distinct human B zones within follicle-like structures and more diffuse human T cell distribution, in contrast with the disorganized tissues observed in BRGS HIS mice (Fig. 1b).

[00142] An increase in T cells and a corresponding decrease in B cells in the blood of BRGST HIS mice was observed (Fig. 1c). This was not due to preferential expansion of CD4⁺ or CD8⁺ T cells (Fig. 1c), but rather reflected a redistribution of B cells between the spleen and LN and a generalized enhanced T cell development (Fig. 1d). Stability of immune reconstitution was similar in the two models (Fig. 8b). Moreover, notable effects on human memory T cell homeostasis in BRGST HIS mice with increased numbers of both effector (CCR7⁻CD45RA⁺) and central memory (CCR7⁺CD45RA⁺) T cell subsets were observed (Fig. 1e; Fig. 8c). Although percentages of naïve T cells decreased in BRGST HIS mice, total numbers of naïve T cells were similar in both models (Fig. 1e).

10 Example 4: Human T cell dynamics in LN of BRGST HIS mice

[00143] Murine T cells exhibit robust motility and scanning behavior in LNs, allowing encounters with dendritic cells and B cells during the initiation of the adaptive immune response^{17, 18}. To test whether our BRGST HIS model supports the motility of human T cells within LNs, CFSE-labeled human T cells were adoptively transferred from BRGST HIS mice and their behavior within intact popliteal LNs of BRGST HIS mice were analyzed by intravital two-photon microscopy^{19, 20}. Human T cells were readily detected, indicating efficient *in vivo* trafficking to LN (Fig. 2a, additional data not shown). Human T cells were highly motile and exhibited an apparent random walk with mean velocity (8 μm/min) and mean straightness index (0.5) (Fig. 2b, c) resembling the behavior already described for mouse T cells²¹. Finally, tracks of human T cells migrating in LN of BRGST HIS mice resembled those seen with mouse T cells, with no apparent directional bias (Fig. 2d). This analysis of explanted LNs showed similar results using adoptively transferred human peripheral blood T cells to BRGST HIS mice (Fig. 9a-d; additional data not shown). These results suggest that the LN microenvironment in BRGST HIS mice allows for efficient human T cell migration.

Example 5: Enhanced T cell development in BRGST HIS mice

[00144] This example analyzed whether the improved human T cell engraftment in BRGST mice was due to enhanced thymopoiesis. In HIS mice, human T cells develop within the murine thymus, and total thymocyte numbers rarely exceed 3 million cells (reviewed in 5 ²²). In addition, the distribution of major CD4⁺ and CD8⁺ thymocyte subsets is frequently altered with a loss of CD4⁺CD8⁺ ‘double positive’ (DP) cells and a higher frequency of NK cells (refs. ^{5, 23}; Fig. 3a, b). In contrast, thymi from BRGST HIS mice were substantially larger, harbored more hCD45⁺ cells and contained a higher proportion of DP cells (Fig. 3a, b). Notable increases in numbers of ‘immature single positive’ (ISP4; CD3⁻CD4⁺) and DP cells 10 (Fig. 3b) account for the increase in thymic cellularity. Moreover, increased proliferation of human thymocytes and the high proportions of Ki67⁺ ISP4 cells in BRGST HIS mice may explain the increased numbers of ISP4 and DP cells (Fig. 10a) through more efficient β -selection.

[00145] It was hypothesized that the improved human thymopoiesis in BRGST HIS 15 mice might result from improved ‘crosstalk’ between the developing thymocytes and mouse epithelial cells. Thymus size and cellularity of non-reconstituted BRGST mice were increased compared to BRGS mice (Fig. 10b, c). Mouse TSLP is active on ‘double negative’ (DN) 1 and DN2 pro-T progenitors and can expand these early murine thymocyte progenitors *in vitro* ²⁴. Thymocytes from non-reconstituted BRGST mice showed a increase in DN subsets, 20 especially DN2, DN3 and ISP cells (Fig. 10d) that can deliver signals to thymic epithelial cells (reviewed in ^{25, 26}). The inventors speculate that this creates a ‘feed-forward’ system facilitating human thymopoiesis in BRGST recipients.

Example 6: BRSGT HIS mice demonstrate robust TFH cell development

[00146] Specialized T follicular helper (T_{FH}) cells provide cognate support to B cells 25 and are indispensable for germinal center formation, IgG affinity maturation, and plasma cell and memory B cell homeostasis ²⁷. T_{FH} differentiation primarily occurs in SLT ²⁸. As such, it is not surprising that few T_{FH} cells (defined as PD-1 and CXCR5 expressing CD4⁺ T cells) are found in the blood, spleen and mesenteric LN of BRGS HIS mice (Fig. 3c). In contrast, a CXCR5⁺PD-1⁺ T_{FH} population was detected in LNs of BRGST HIS mice, that expressed 30 BCL6 (Fig. 3c, d). T_{FH} secrete signature cytokines and chemokines (including IL-21 and

CXCL13) that attract and activate CXCR5⁺ follicular B cells during the germinal center reaction^{27, 28}. CD4⁺ T cells from BRGST HIS mice expressed substantially higher levels of IL-21 and CXCL13 transcripts compared to BRGS mice (Fig. 3e). Moreover, stimulation of T cells from BRGST, but not BRGS, HIS mice revealed abundant IL-21-producing T cells (Fig. 3f) indicating robust T_{FH} development in this model.

Example 7: Enhanced B cell development and antibody responses in BRGST HIS mice

[00147] Peripheral human B cells in HIS mice retain an immature, transitional phenotype with elevated expression of CD24 and CD38 (refs.^{29, 30}). In BRGS and BRGST HIS mice, this predominant population of CD24^{hi}CD38^{hi} immature B cells was observed in the bone marrow, liver and spleen (Fig. 4a; Fig. 11a). In contrast, mature CD24^{lo}CD38^{lo} cells were the dominant human B cell subset in LNs of BRGST mice (Fig. 4a; Fig. 11a). Although notable differences in these different B cell subsets were not observed between the two models, the total numbers of mature CD24^{lo}CD38^{lo} B cells in LN were significantly increased (p=0.02) in BRGST HIS mice (Fig. 4a).

[00148] Immunoglobulin isotype analysis revealed comparable human IgM levels in the two models, but substantially higher levels of serum human IgG and IgA in BRGST HIS mice (Fig. 4b). Global IgM and IgG levels are similar to those reported in other HIS mouse models^{13, 29, 30}. BRGS and BRGST HIS mice were immunized with Keyhole Limpet Hemocyanin (KLH) that primes both T and B cell responses³¹. IgG⁺ and IgA⁺ B cells were detected in both models (Fig. 4c), although their frequencies were higher in BRGST HIS mice. KLH-specific T cell responses were also increased in immunized BRGST HIS mice (Fig. 4d; Fig. 11b). Moreover, in immunized BRGST HIS mice, elevated levels of KLH-specific IgG antibodies were detected (Fig. 4d; Fig. 11b) that were associated with AID⁺ B cell follicles within LNs (Fig. 4e). Taken together, these results demonstrate the enhanced capacity of BRGST HIS mice to generate antigen-specific cellular and humoral immune responses following immunization.

Example 8: Genetic features of human IgG and IgA antibodies in BRGS-based HIS mice

[00149] Global splenic B cell repertoires of BRG-based HIS mice comprised a large diversity of IgH chains³². Therefore the antibody repertoire of class-switched memory B cells was characterized in lymphoid organs of KLH-immunized HIS mice. In both models, clonal expansions of various sizes (Fig. 5a, b; Fig. 12a) were found, some of which were shared amongst class-switched B-cell compartments and lymphoid organs (Fig. 5a, b). Comparative analyses of IgH and IgL variable (V) and joining (J) gene usage did not reveal any notable differences (Fig. 12b). Nevertheless, BGRST-derived memory B-cell antibodies showed an increased frequency of positively charged IgH complementary determining region (CDRH) 3 as well as a trend towards longer CDRH3 lengths (Fig. 5c). These characteristics reflect the distribution of CDRH3 length and charges found in human circulating IgG⁺ and IgA⁺ memory B cells and bone marrow plasma cells³³⁻³⁵.

[00150] A decrease of somatic hypermutation levels in IgH variable genes of BGRST memory B-cells isolated from LNs was observed (in total for all tissues, 1.19 vs 1.73 for BGRS) (Fig. 5d; Fig. 12c). However, this difference originated mainly from the major B-cell expansion detected in the LNs of BGRS mice, which represented about 60% of all clones (Fig. 5b, yellow clone). Importantly, for the IgL repertoire in which no light chains of this specific clone could be amplified, memory B cells from LN and spleen of BGRST HIS mice displayed more somatic mutations than their BGRS counterparts (In total, 3.55 vs 2.62; p=0.0012) (Fig. 5d), which was also evident in CDRL loops (Fig. 5e). Collectively, our data show that BGRST HIS mice develop a diverse antibody B-cell repertoire resembling the one found in humans and contain circulating class-switched memory B cells exhibiting clear signs of antigen-driven antibody maturation.

Example 9: Analysis of acute and latent HIV-1 infection in BRGST HIS mice

[00151] HIV-1 replication in BRGS and BRGST HIS mice was assessed, using a CCR5-tropic viral strain (NLAD8, ref.³⁶) (Fig. 6a). The acute phase of infection was assessed by measuring viral loads and the levels of T cell subsets, at days 7 and 12 post infection (pi). Both models supported a robust HIV-1 replication with viremia reaching 10⁸ copies/ml at day 12 pi and a loss of human CD45⁺ cells (including CD4⁺ T cells; Fig. 13a, b) as previously

observed³⁷. Active viral replication within different CD3⁺CD8⁻ T cells subsets was measured by HIV-1 p24 staining and flow cytometry (Fig. 6b). No HIV-specific antibodies were measurable during this period, likely due to the strong virus-induced depletion of immune cells. BRGST HIS mice showed elevated frequencies of HIV1-infected T_{FH} cells (IL-21⁺p24⁺ cells) resulting in a 10-fold increase in their absolute numbers within LNs compared to BRGS HIS mice (Fig. 6b).

[00152] Whether a viral reservoir could be established in the LNs of BGRST mice was assessed. Highly active anti-retroviral therapy (HAART) decreased viremia to undetectable levels, resulting in the maintenance of human CD45⁺ cells as well as CD4⁺ T cells in both models (Fig. 13a, b). The level of the viral reservoir was measured in LN of HAART-treated HIV-1-infected mice by *ex vivo* quantitative viral outgrowth assays³⁸. As expected, LN could not be retrieved from BRGS HIS mice, whereas LN from all BRGST HIS mice could be studied. Immediately after sorting, the CD4⁺ T cells did not show evidence of active viral production (Fig. 6c). However, stimulation of these cells by PHA led to productive viral infection, demonstrating the presence of a viral reservoir in LNs of HAART-treated BRGST HIS mice (Fig. 6c-e; Table 4).

Example 10: TSLP injection as an alternative method to induce LN in HIS mice

[00153] As LN formation is the result of interaction between LT_i and stromal organizer cells during embryo development, the inventors reasoned that persistent expression of mouse TSLP after birth might not be necessary for the maintenance of LNs in HIS mice. To test this hypothesis and provide an alternative approach to induce LNs in HIS mice, 2μg mouse TSLP was injected retro-orbitally into pregnant BRGS mice every other day starting from E12.5 for total 4 injections. Newborn pups (3-5 days old) were injected intra-hepatically with a mixture of human CD34⁺ cells and 0.5ug mouse TSLP. Since internal littermate controls were not available in this context, non-TSLP treated BRGS pups injected with same batch of human CD34⁺ cells were used as controls. 12-14 weeks post-graft, mice were sacrificed and analyzed for the presence of LNs. The number of LNs increased considerably in TSLP treated HIS mice with recovery of gastric, renal, brachial, iliac and inguinal LNs (Figure 15A-C). Similar to BRGST HIS mice, more human cells were present in the LNs of TSLP treated HIS mice compared with BRGS HIS mice (Figure 16). Next KLH immunization was compared in

TSLP treated and control HIS mice. Consistent with results obtained using BRGST HIS mice, an increase in KLH specific IFN- γ producing T cells and an increase in KLH specific IgGs were detected in TSLP treated HIS mice. Thus, TSLP treatment of BRGS HIS mice in utero and in the early post-natal period provides an alternative way to boost LN development, human immune cell reconstitution and induced antigen-specific immune responses in humanized mice.

Example 11: Discussion

[00154] Secondary lymphoid tissues are critical to initiate and coordinate immune responses (reviewed in ³⁹). Using TSLP over-expression in BRGS (BRGST), a HIS mouse model with a full complement of LNs was generated, that develop robust T_{FH} differentiation and improved antigen-specific cellular and humoral immune responses. These results suggest that SLT can positively influence human immune responses. As such, the BRGST HIS model should be useful to dissect the mechanisms by which SLT impact on human cellular and humoral immunity.

[00155] Two photon microscopy allows visualization of ongoing immune reactions *in situ* or in tissue explants *ex vivo* (reviewed in ⁴⁰). To date, most of these experiments have focused on mouse lymphocytes. Thus the ability to visualize human T cells dynamics in the lymph nodes of BRGST HIS mice should provide new opportunities to study migration and interactions established by human T and B cells in the context of infection with human pathogens or in response to immunomodulatory therapy targeting human molecules.

[00156] T_{FH} cells play critical roles in germinal center formation through activation of antigen-specific B cells ²⁷. A previous study reported T_{FH} cells in the gut mucosa and female reproductive tract but not in the spleen of HIS mice ⁴¹, and robust human T_{FH} differentiation was observed in lymphoid tissues of BRGST HIS mice. These results suggest that T_{FH} generation requires particular environmental signals. How enhanced LN generation in BRGST HIS mice facilitates T_{FH} differentiation is not known. One possibility is that LN structures provide the necessary spatial requirements for efficient interactions of naïve T cells with DC that drive T_{FH} differentiation. Alternatively, mature B cells within LN of BRGST

HIS mice may help promote T_{FH} development and maintenance, for example via ICOS-L signaling⁴².

[00157] Antigen-specific immune responses involve clonal expansion of lymphocytes recognizing MHC:peptide complexes. The generation of antigen-specific immune responses in BRGS-based HIS mice occurs despite the absence of HLA expression by host tissues, suggesting that mouse H-2 molecules are sufficient to promote intrathymic human T cell selection and that this process may be improved in BRGST HIS mice. Whether expression of HLA transgenes will impact on the generation of antigen-specific T and B cell responses within SLT remains to be determined.

[00158] Although increased LT_i cells promoted robust LN development in BRGST HIS mice (including gut-associated mesenteric LNs), recovery of intestinal PP was not observed. While both PP and LN formation require LT_i function, CD11c⁺ cells are additionally required for normal PP development and in particular, signaling through the RET tyrosine kinase receptor expressed by CD11c⁺ cells appear critical in this process⁴³. A potential lack of CD11c⁺RET⁺ cells in BRGS mice may explain the selective defect of PP restoration in BRGST hosts.

[00159] Secondary lymphoid organs and mucosal tissues are thought to be actively involved in the maintenance of pro-viral DNA and to contribute to HIV persistence in HAART treated, viremia-suppressed patients. In particular, lymphoid organ specific CD4⁺ T cell subsets, such as T_{FH} and T_{reg} are important compartments of HIV persistence⁴⁴⁻⁴⁶. BRGST HIS mice offer the possibility to dissect the immunological features of HIV latency and replication in secondary lymphoid tissues and to test strategies to eliminate them.

[00160] The TSLP transgene can be used to create lymph nodes in mice lacking the Il2rg gene that do not develop secondary lymphoid structures (lymph nodes and Peyer patches) since TSLP can restore lymphoid tissue inducer cell (LT_i cell) function that is absent in Il2rg^{-/-} mice and is needed for development of secondary lymphoid structures.

[00161] Humanized immune system mice that are created in Il2rg^{-/-} hosts also utilize mutations that affect endogenous mouse T and B cell development, including Rag1, Rag2 or

SCID (Prkdc). The TSLP transgene can restore lymph nodes in Rag2/Il2rg deficient hosts (and should also work in Rag1/Il2rg deficient hosts). Interestingly, the TSLP transgene causes thymic tumors (thymomas) in SCID/Il2rg deficient hosts that results in death of animals by 8-12 weeks of age. As such, TSLP transgenes can not be used to restore lymph nodes in certain SCID-based mouse strains that are currently used to make humanized mice (such as ‘NSG’ mice from JAX, USA or ‘NOG’ mice from CIEA, Japan). Moreover, TSLP transgenic mice bearing heterozygous mutations in Prkdc (Prkdc+/- mice) were found also to generate thymic tumors.

[00162] In order to eventually use TSLP to restore LT α i cell function in SCID-based mouse strains (such as ‘NSG’ mice from JAX, USA or ‘NOG’ mice from CIEA, Japan), TSLP injection in utero is an alternative approach that will not provoke thymic tumors.

Example 12: Overexpression of mouse TSLP does not induce AD in immunodeficient mice

[00163] The BRGST mice were generated by backcrossing BRGS mice with mice expressing mouse TSLP under the control of the Keratin 14 promoter (Chappaz and Finke, 2010). Examples 2-11 show that mouse lymphoid node development is recovered in BRGST mice due to the presence of innate lymphoid cells. Since skin specific expression of TSLP has also been associated with the spontaneous development of AD in both immune-competent and –deficient mice (Yoo et al., 2005), the skin conditions of BRGST mice were examined and were indistinguishable compared to BRGS littermates (**Figure 17A**), suggesting that the presence of mouse lymphocytes were indispensable for the initiation of AD. In addition, ear thickness is often used as a quantitative measurement for skin inflammation associated with AD (Elentner et al., 2009; Kim et al., 2013) and there was no evident difference between BRGST and BRGS mice at 20 week-old (**Figure 17B**). Although TSLP has a broad effect on cells of both hematopoietic and non-hematopoietic origin, its unique ability to activate dermal DCs to prime T-helper type 2 (Th2) response is considered one primary mechanism of AD development (Elentner et al., 2009; Ito et al., 2005; Soumelis et al., 2002). Hence, the DC subsets were analyzed in spleen of 8 week-old mice, and significantly more dermal DCs but not conventional DCs and migratory Langerhans cells were found (**Figure 17C**), supporting the established role of TSLP in promoting the activation and mobilization of dermal DCs.

Mouse TSLP protein shares low degree of identity with human TSLP (43%) and does not bind to human cell lines expressing human TSLP receptors (Park et al., 2000; Quentmeier et al., 2001). To investigate the cross-species reactivity of mouse TSLP on primary human cells, human DCs (CD45⁺lin⁻CD11c⁺CD4^{low}) were sorted from PBMC and simulated them *in vitro* with mouse or human TSLP. After 24h culture, a strong induction of CD80 is observed on human TSLP or poly I:C stimulated DCs (**Figure 17D**). Mouse TSLP stimulated DCs, however, showed similar levels of CD80 expression as DCs cultured in media only. These data suggest that human cells would not be directly affected by mouse TSLP in BRGST mice.

Example 13: Severe AD develops in BRGST HIS mice

[00164] Examples 2-11 demonstrates that engraftment of BRGST host with human CD34⁺ hematopoietic stem cells (HSC) progenitors mounts efficient adaptive immunity with the presence of lymph nodes. HIS mice used in that experiment setting were sacrificed before 20 weeks post human cell engraftment. Surprisingly, visible skin lesions started to appear on BRGST HIS mice after 20 weeks post human cell engraftment, while their littermate BRGS HIS mice show no signs of skin lesions (**Figure 18A**, 25 week post human cell engraftment). Their lesions generally began with loss of hair and erythema on the snout and postauricular region, and followed with moderate loss of hair and erosions on the neck and upper back, and progressed to severe loss of hair throughout the body, crust on the neck and upper back and scales on the lower back and hind legs. To quantify these lesions, a system scoring the hair density and coverage, the presence of erosions, crusts and scales from 4 regions of the body on a scale of 0 to 4 was created (**Table 3**). AD scores were tracked based on this system from week 12 post human cell engraftment and it was found that BRGST HIS mice developed skin disease beginning at ~16 weeks post human cell engraftment (**Figure 18B**). AD scores based on this system matched the increase of ear thickness, which is another indicator for skin inflammation. (**Figure 18A, B**). Together, these observations closely resemble clinical features of human AD.

[00165] AD took 4-5 months to develop in BRGST HIS mice. Hence, dynamics of human immune subsets were recorded over a 2-month period to investigate the potential cellular target that might drive the AD development. The overall human cell engraftment (hCD45⁺mCD45.2⁻ cells) in the blood was similar at 14-week post reconstitution when AD

was not initiated. However, human cells in blood of BRGST HIS mice increased with the ear thickness and AD score after 16-week. More human cells were likely the results of CD3 T cell activation and expansion as the percentages of CD3 T cells within human cells increased while the percentages of CD19 B cells decreased over time. These data indicate the activation and expansion of human T cells might link with the progression of AD in BRGST HIS mice.

Example 14: AD skin is associated with immune cell infiltration and dysbiosis

[00166] Facial, back and abdomen skin samples from BRGST HIS mice were subjected to histological analysis and compared with skin from BRGS HIS mice at 20-week post humanization (**Figure 19A** and **Figure 23**). Major characteristic changes of skin lesions observed in human AD were recapitulated in BRGST HIS mice (Eckert, 1991), including dermal inflammation of mononuclear cells, epidermis hyperplasia, destruction of basal layers of epidermis and hair follicles and hyperkeratosis, whereas no skin lesions were present in skins from BRGS HIS mice (**Figure 19A**). A closer examination of the skin lesion revealed that the infiltrated cells composed of a predominance of lymphocytes and a mixture of mast cell, neutrophil, macrophage and eosinophil (**Figure 19B**). Consistent with the histological analysis, significantly higher number of mononucleated cells per cm² was extracted from upper back skin of BRGST HIS mice than the skin of BRGS HIS mice (**Figure 19C**).

[00167] Swabs collect microbiota from the superficial layer of the skin allows collection of superficial skin cells and associated microbes (Grice et al., 2008). Therefore, skin swab samples were obtained from shaved upper back skin of BRGS mice and AD BRGST HIS mice after 25 weeks of human cell engraftment. Adequate quantities of DNA were obtained (all samples > 10 ng/μl) from BRGS HIS mice and AD BRGST HIS. To gain further insight into microbiota counts, a quantitative polymerase chain reaction (qPCR) was applied in the extracted DNA, using universal 16S rRNA primers to measure total bacteria and 18S rRNA primers to measure total fungi (Qiu et al 2015). The inventors observed that the total amount of bacteria and fungi is not different between normal skin of BRGS HIS mice (n=10) and AD affected skin of BRGST HIS mice (n=13) (**Figure 19E**). To study microbiota diversity an Illumina sequencing of the V3-V4 region of bacterial 16S rRNA genes (Castelino et al., 2015) was performed. The principal component analysis (PCoA) of samples based on 16S rRNA sequences demonstrate a significant clustering by group, showing that mice with

AD lesions have a prominent skin microbiota dysbiosis (**Figure 19F**). Particularly, with 16S rDNA sequencing relatively higher abundance of *Staphylococcus* genus was detected in mice with AD than those of BRGS HIS mice (**Figure 23B**). Next, the absolute abundance of total *Staphylococcus aureus* (*S. aureus*) specie on skin was evaluated by quantitative qPCR using primers specific to sa442 gene (Martineau et al., 1998). The inventors observed that only 50% of BRGS HIS mice are *S. aureus* carriers (**Figure 19F**). On the other hand, 85% of AD mice are carriers and showed an increase in *S. aureus* colonization abundance (**Figure 19F**).

[00168] *S. aureus* can interfere in many ways with the immune system (Hepburn et al., 2017) and can drive dermatitis inflammation (Kobayashi et al., 2015). For example, *S. aureus* superantigens can induce a massive CD4⁺ T cell hyperactivation (Marrack and Kappler, 1990) and induce directly the production of IL-1 β , Th2 (e.g. IL-6 and TNF) and Th17 (e.g. IL-17A and IL-22) cytokines (Kobayashi et al., 2015; Nakatsuji et al., 2016; Niebuhr et al., 2011). Therefore, the secretion of specific human cytokines (i.e. IL-1 β , IL-6, IL-17A, and TNF) was studied in normal BRGS HIS skin and AD affected skin of BRGST HIS mice, using the digital ELISA Simoa assay (see methods). A higher concentration of human IL-1 β , IL-6 and IL-17A was detected in healthy skin of AD BRGST HIS mice (**Figure 19G**). The inventors believe that the results of secreted human cytokines presented here give further insight into the complex interactions between human immune cells and skin dysbiosis during the AD development of BRGST HIS mice.

Example 15: Multiple hematopoietic lineages contribute to AD development in BRGST mice

[00169] As clinical diagnosis criteria for AD include characteristic skin lesions and increased blood IgE levels (Weidinger and Novak, 2016), the inventors therefore checked the IgE in plasma of BRGST HIS mice and found it elevated to the levels found in human AD patients (**Figure 20A**) (Stone et al., 1973). In allergic inflammation, IgE binds to and mediate the activation of mast cells, basophils and eosinophils (Liu et al., 2011). As lymphocytes and granulocytes presented in the inflamed skin of BRGST HIS mice (**Figure 19B**), the inventors further characterized the IgE⁺ human cells from the skin draining LNs by flow cytometry (**Figure 20C**). Beside human B cells which express membrane bound IgE or low-affinity IgE receptor (Fc ϵ RII; CD23), the majority of IgE⁺ cells detected with surface bound IgE were

human basophils and mast cells, which express high affinity IgE receptor (FcεRI) (**Figure 20C**).

[00170] Although a dominant Th2 response has been well documented in acute AD models and in AD patients, cytokines detected from serum or skin evolve with time and manifest a mixed Th1/2 profile in chronic AD (Bieber, 2008). To characterize the cytokine profile, sera from AD BRGST HIS mice were collected at 20 weeks post humanization and performed Luminex human cytokine 25-plex assay, which quantifies key cytokines and chemokines. The inventors observed cytokine/chemokine signatures of both Th1 and Th2 (**Figure 20B**), which resemble the features of chronic AD in human (Bieber, 2008). In addition, 4 cytokines measured from skin swab (IL-1β, IL-6, IL-17A, and TNF) were verified in plasma samples (**Figure 20B**). Plasma IL-6 and TNF showed similar trend as in skin swabs, but IL-1β and IL-17A were below the detection threshold of Luminex cytokine assay, likely reflecting the technical advance of Simoa digital ELISA.

[00171] The elevated cytokines from skin and serum suggested that AD is both a local and systemic inflammatory disease. However, whether these cytokines were produced locally or systemically still remains to be explored. With the advantages of HIS mouse model, the inventors investigated the cellular source of two cytokines IL-13 and IL-22, which are progressively elevated in AD patients (Gittler et al., 2012). Cells from spleen, skin draining LNs and upper-back skin of BRGS and BRGST HIS mice were stimulated with PMA and ionomycin. The production of these two cytokines was elevated in BRGST HIS mice (**Figure 20D-E**). Of note, both IL-13 and IL-22 showed a gradient increase of production from spleen to skin T cells, suggesting that elevated serum cytokines in AD HIS mice were mainly produced by T cells from skin or skin draining LNs.

Example 16: Human CD4 T cells are the master driver for the development of AD

[00172] Multiple mouse immune subsets, such as Th2 cells, myeloid cells, and type 2 innate lymphoid cells, contributed to AD progression (Werfel et al., 2016). However, considerable differences exist between murine and human immune system (Mestas and Hughes, 2004). Hence, it is necessary to examine the knowledge from mouse AD models with HIS mice. The inventors first attempted to deplete human CD4, CD8 T cells and B cells in

HIS mice with weekly intraperitoneal (i.p.) injection of antibodies for 10 weeks (**Figure 21A**). CD4 and CD8 T cells were depleted by OKT4 and OKT8 clones, respectively, and checked in blood by flow cytometry with different clones. B cells were depleted by anti-CD20 antibody (Rituximab) and checked in blood by anti-CD19 antibodies. CD8 T cells could be efficiently depleted after the 1st injection of antibody. However, it took nearly 10 weeks to deplete the B cells and longer than 10 weeks to deplete CD4 T cells in both BRGS and BRGST HIS mice. Nevertheless, the progression of AD measured by ear thickness was not correlated to the efficiency of the depletion, rather to the cell types that was targeted (**Figure 21B**). Depletion of CD8 T cells delayed the onset of the AD in HIS mice but did not change the final outcome, suggesting CD8 T cell might play an important role in the acute phase of AD. On the contrary, depletion of CD20 B cells did not delay the onset of the AD, rather slowed down the increase of ear thickness in the chronic phase. The reduction of CD4 T cells, despite an incomplete depletion, not only delayed the onset of AD but also had a long-term effect. These data pointed to the central role of CD4 T cells in the development of acute and chronic AD.

[00173] To further test the therapeutic potential of T cell depletion in AD, the inventors waited for the AD to develop in HIS mice and then treated the mice with CD4 depleting antibody twice a week for 6 weeks (**Figure 21C and D**). The increased frequency of injections of CD4 depleting antibody resulted in a reduction of CD4 at first 2 weeks, but the percentages of CD4 in blood rebounded at week 4 and 6 (**Figure 24**). Despite the incomplete depletion of CD4 T cells, both AD scores and ear thickness were reduced in treated AD mice, while the AD in untreated BRGST HIS mice continued to progress (**Figure 21C**). The impact of CD4 T cell reduction could also be directly visualized by the picture comparison between treated and untreated groups (**Figure 21D**). These data confirmed that CD4 T cells is required for the maintenance and progression of AD in HIS mice, and AD was a reversible process by targeted depletion of CD4 T cells.

Example 17: Targeting the skin-homing T cells in AD HIS mice

[00174] Although CD4 T cell depletion delayed the onset and progression of AD, it had limited clinical impact. Therefore, specific subsets within CD4 T cells need to be identified as biomarkers for AD or as novel therapeutic targets. The inventors sorted mCD45⁻ CD45⁺CD3⁺CD8⁻CD4⁺ T cells from splenocytes of BRGST AD HIS mice and BRGS HIS mice, and performed Nanostring nCounter assay with GX Human Immunology kit V2 (**Figure 22A**). Differential expressed genes in CD4 T cells of AD HIS mice included genes for T cell activation, follicular helper T cells, and Th2 cytokines. Of note, a set of genes involved in T cell migration and adhesion, such as CCR10, CLA, CXCR3, and CCR8 were revealed and had the potential to be biomarkers or therapeutic targets (**Figure 22B**). CCR4, a chemokine receptor reported to be expressed on Th2 cells and associated with AD progression in patients (Wakugawa et al., 2001), is not in the probe list of GX Human Immunology kit V2. To verify CCR4 expression and chemokine receptors identified, surface expression of these skin homing receptors were evaluated on blood CD4 T cells (**Figure 22C**). Indeed, the inventors observed considerably increased percentages of CLA⁺, CCR4⁺, CCR10⁺, CXCR3⁺ and CCR8⁺ CD4 T cells in the AD BRGST HIS mice compared to BRGS littermates. Since clinical studies correlate the expression of skin homing receptors with the severity of the AD (Wakugawa et al., 2001), the inventors plotted the percentages of CLA and CCR4 with ear thickness and they are highly correlated (**Figure 22D**).

[00175] For decades, systemic corticosteroid is the mainstay of treatments for moderate and severe AD while other treatments such as emollients, topical glucocorticoids and calcineurin inhibitors have limited efficacy (Gandhi et al., 2016). Nevertheless, patients suffer from the long-term side effects from systemic corticosteroid if given for prolonged period. Recently, a human monoclonal antibody targeting the shared alpha subunit of the IL-4 receptors and blocking signaling from Th2 cytokines IL-4 and IL-13 achieved rapid improvement on AD patients in clinical trials and therefore approved by FDA as a new immunotherapy for AD (Beck et al., 2014). To evaluate the potential of skin homing receptors as a novel category of immunotherapeutic targets, the inventors obtained and tested a CCR4 deleting antibody, which were initially developed to deplete a subset of regulatory T cells in cancer patients (Sugiyama et al., 2013), in BRGST HIS mice. 100µg anti-CCR4 antibody

were injected weekly i.p. into BRGST HIS mice for 6 weeks. A reduction of CCR4⁺ CD4 T cells was achieved at 3 weeks post injection of antibodies and the difference was maintained for the following period of experiment (**Figure 22E**). Despite incomplete depletion of CCR4⁺ CD4 T cells, the progression of AD was significantly impeded in antibody treated group as measured by the AD score and the ear thickness (**Figure 22F**). Together, these data supported that skin homing receptors identified in HIS AD mice can be targeted not only as biomarker but also as novel immunotherapy candidates.

Example 18: Discussion

[00176] It is a challenge to model a complex disease such as AD that involves clinical presentations of skin lesions, interactions among multiple cell types, and connections to environmental hues and neuropsychological factors (Werfel et al., 2016). Knowledge of AD has been expanded by the use of animal models developed, especially genetically modified mouse models, which allow to investigate in-depth the pathogenesis and experiment on novel therapeutics (Jin et al., 2009). However, animal models of AD often share limited features with human AD and they are of little value to evaluate immunotherapeutic candidates from pipelines of pharmaceutical industry (Ewald et al., 2017; Mestas and Hughes, 2004). With the development of BRGST AD HIS mice, the inventors were able to track macroscopic change of skin lesions and link it with the dynamic of human cells in both the acute phase and chronic phase of AD. Moreover, skin histology and FACS analysis of skin and draining lymph nodes from BRGST HIS mice provide deeper understanding of this complex involvement and activation of multiple immune subsets in the progression of human AD. Previous efforts to model human AD directly in humanized mouse models more likely reflects the acute phase of intrinsic human AD as characterized by a predominant Th2 response and lack of IgE response (Carretero et al., 2015), whereas analysis of BRGST HIS mice with high AD scores showed a mixed Th1/2 cytokine profile, high IgE levels in the sera, and the presence of both Th2 and Th22 in the skin, similar to the chronic phase of AD in patients. Hence, BRGST HIS mice represent the first AD animal model developed in human context and cover different stages of the clinical manifestations of the disease. This model may help the field to understand the sequential activation of human immune system during AD development from acute phase to chronic phase.

[00177] The skin of specific pathogen free (SPF) mice home a diverse microbiota that might trigger keratinocyte proliferation and antimicrobial peptides (AMPs) production, and promote local production of inflammatory mediators (Ichinohe et al., 2011). Under homeostatic condition, skin microbiota play an important role in local tissue immunity, e.g. induces IL-17A⁺ CD8⁺ T cells that home to the epidermis, enhance innate barrier immunity and limit pathogen invasion (Naik et al., 2015). Conversely, the imbalance of the microbiome (dysbiosis) may leads to or accelerates the development and pathophysiology of some skin diseases, such as atopic dermatitis (Naik et al., 2012). Despite that *S. aureus* colonizes the skin of most healthy individuals (Li et al., 2015), atopic dermatitis patients are well known to have increased colonization by this bacteria species (Gong et al., 2006; Nakatsuji et al., 2017) and a loss in bacterial diversity on the skin (Kong et al., 2012). Interestingly enough, BRGST HIS mice develop atopic dermatitis-like lesions with dysbiosis towards *S. aureus*. It has been reported that *S. aureus* produces extracellular proteases that disrupts the skin epidermal barrier, and induces immune cell activation with toxins (Hirasawa et al., 2010). In this study the inventors observed that the skin microbiota composition changes correlated with enhanced cytokine production in AD HIS mice. As there is no known genetic deficiency reported in BRGS strain for skin barrier integrity, the dysbiosis of *S. aureus* is likely secondary to the AD induced by human cells. In the future, it will be interesting to generate germ-free BRGST HIS mice to evaluate the contribution of skin dysbiosis to the progression of AD.

[00178] The elevated expression of TSLP in human AD skin and TSLP polymorphism in AD patients has supported a role of this cytokine in the pathogenesis of AD (Gao et al., 2010; Takai, 2012). Subsequent in vitro studies and mouse model studies identified direct cellular targets of TSLP, including B cell precursors, DCs, Th2 cells, basophils, ILC2, and neurons (He et al., 2008; Ito et al., 2005; Kim et al., 2013; Siracusa et al., 2011; Vosshehrich et al., 2003; Wilson et al., 2013). In addition, skin-specific (keratin 5 or 14 promoter) overexpression of TSLP in mice leads to an AD-like phenotype (Elentner et al., 2009; Yoo et al., 2005), but T cell deficiency in K5-TSLPtg mice still demonstrates many features of AD (Yoo et al., 2005), suggesting T cells are not necessary for AD development in this scenario. However, K14-TSLPtg in BRGST mice did not drive the development of AD-like phenotype in the absence of mouse T cells, possibly due to further reduction of skin ILC2 by *Il2rg*^{-/-}. Nevertheless, reconstitution with human immune cells, especially CD4 T cells, initiated the

AD progression in BRGST mice, despite that mouse TSLP does not cross-react on human cells. Since mouse dermal DCs responded to TSLP overexpression by migrating to secondary lymphoid organs in BRGST mice, activated dermal DCs might cross-activate naïve human T cells into allergic Th2 cells in skin and secondary lymphoid organs to initiate the AD cascades.

[00179] Reducing inflammation with nonspecific immunosuppressant has been proven to be effective immunotherapies for moderate and severe AD (Weidinger and Novak, 2016). However, systemic immunosuppression has broad mechanism of activation and often leads to toxicities (Paller et al., 2017). With increased understanding the pathogenesis of AD through animal models and clinical trials, antibodies targeting key components of the type-2 immunity were developed to achieve more specific immune suppression and potentially less side effect (Gandhi et al., 2016). Unexpectedly, an IgE (Omalizumab) neutralizing antibody targeting the degranulation of IgE from mast cells and basophils was not sufficient to achieve a good clinical response for AD patients, despite that serum IgE was reduced (Heil et al., 2010). Subsequently, pharmaceutical companies invested heavily into antibodies targeting 3 key cytokines driving the Th2 response, including IL-4, IL-5 and IL-13 (Gandhi et al., 2016). Although many candidates delivered promising results from animal studies and in vitro works, most of them failed to bring clinical improvement during clinical trials. To this day, only 1 antibody (Dupilumab) targeting IL-4 and IL-13 shared receptor subunit IL-4R α passed Phase II trails and got approved by FDA to treat moderate and severe AD (Beck et al., 2014; Gandhi et al., 2016; Paller et al., 2017). The high rate of failures of these antibodies can be attributed to the suboptimal activity of antibodies in vivo, route of administration, and/or incorrect dosing schedule (Gandhi et al., 2016). In BRGST AD HIS mice, the inventors could observe high levels of IgE and 3 key Th2 cytokines in serum. Also, similar to human AD, the inventors proved that CD4 T cells are the key driver in the pathogenesis. Hence, this model could be ideal to serve as a preclinical platform to test the efficacy and toxicity of future antibody candidates in vivo.

[00180] Chemokines and their receptors play critical roles in initiation and exacerbation of AD. Th2 cytokines and bacteria infection with *S. aureus* can stimulate the production of chemokines (e.g. CCL1, CCL17, CCL18, CCL22, and CCL27) from DCs,

keratinocytes and endothelial cells, which attract circulating CLA⁺, CCR4⁺, CCR8, and/or CCR10⁺ T cells to the skin (Nedoszytko et al., 2014). These chemokine receptors were also enriched in the list of genes differentially expressed by CD4 T cells from AD mice. The inventors validated the expression of these chemokine receptors on CD4 T cells from AD mice by FACS and established a correlation between the severity of AD and the expression of CCR4/CLA. To establish a causal relationship and evaluate the therapeutic potential, the inventors treated AD HIS mice with anti-CCR4 depleting antibodies and found an impeded disease progression. In conclusion, BRGST AD HIS mouse will not only provide a valuable tool to save the investment on money-draining clinical trials, but also advance our knowledge on human AD and accelerate the drug discovery process.

[00181] **Table 1: Antibodies used for FACS analysis and sorting**

Antigen	Reactivity	Clone	Supplier	Catalogue Number	Fluorochrome
CD45	Human	HI30	BD Biosciences	564105	PerCP-Cy5.5
				560566	Alexa Fluor 700
				563792	BUV395
CD3 ϵ	Human	UCHT1	Biolegend	300430	PerCP-Cy5.5
			BD Biosciences	564307	BUV737
CD4	Human	RPA-T4	BD Biosciences	555346	FITC
				557871	APC-Cy7
				562658	BV605
CD8 α	Human	RPA-T8	BD Biosciences	562282	PE-CF594
				301046	BV786
CD10	Human	HI10a	BD Biosciences	563734	BV650
CD14	Human	MOP9	BD Biosciences	562335	PE-CF594
CD19	Human	HIB19	BD Biosciences	555413	PE
CD24	Human	ML5	Biolegend	311104	FITC
CD38	Human	HIT2	BD Biosciences	555460	PE
CD45RA	Human	T6D11	Miltenyi Biotec	130-113-360	VioBlue
CCR7	Human	3D12	BD	561008	PE
CXCR5	Human	RF8B2	BD Biosciences	558112	Alexa Fluor 488
PD-1	Human	EH12.1	BD Biosciences	329924	BV605
NKp46	Human	9E2/NKp46	BD Biosciences	558051	APC
IL-2	Human	5344.111	BD Biosciences	340448	FITC

[00182] **Table 1 (continuation): Antibodies used for FACS analysis and sorting**

IL-17A	Human	BL168	Biolegend	512312	Pacific Blue
IL-21	Human	3A3-N2	Miltenyi Biotec	130-096-868	PE
IgA	Human	IS11-8E10	Miltenyi Biotec	130-113-475	FITC
IgG	Human	G18-145	BD Biosciences	564230	BV786
Ki67	Human	B56	BD Biosciences	556026	FITC
Bcl-6	Human	K112-91	BD Biosciences	561525	Alexa Fluor 647
Foxp3	Human	PCH101	eBioscience	56-4776-41	Alexa Fluor 700
p24	HIV antigen	Kc57	Beckman Coulter	6604665	FITC
CD49b	Mouse	DX5	eBioscience	11-5971-82	FITC
CCR6	Mouse	29-2L17	Biolegend	129819	BV605
NKp46	Mouse	29A1.4	eBioscience	50-3351-82	eFluor 660
ROR γ t	Mouse	Q31-378	BD Biosciences	562684	PE-CF594
GATA3	Mouse	TWAI	eBioscience	46-9966-42	PerCP-Cy5.5
CD127	Mouse	A7R34	eBioscience	25-1271-82	PE-Cy7
CD5	Mouse	53-7.3	BD Biosciences	100608	PE
CD45.2	Mouse	104	Biolegend	109824	APC-Cy7
CD3 ϵ	Mouse	145-2C11	eBioscience	11-0031-82	FITC
CD4	Mouse	RM4-5	eBioscience	48-0042-82	Pacific Blue
CD8 β	Mouse	eBioH35-17.2	eBioscience	47-0083-82	APC
CD44	Mouse	IM7	eBioscience	12-0441-82	PE
CD25	Mouse	PC61.5	eBioscience	25-0251-82	PE-Cy7
Biotin conjugated antibody for untouched human T cell enrichment					
CD19	Human	HIB19	eBioscience	13-0199-82	

[00183] **Table 2: Antibodies used for FACS analysis and sorting**

Antigen	Reactivity	Clone	Cat ID	Supplier
CD80	human	2D10	305206	Biolegend
HLA-DR	human	L243	307618	Biolegend
CD45	human	HI30	563792	BD
CD11c	human	3.9	25-0116-42	ThermoFisher
CD3	human	UCHT1	562280	BD
CD19	human	HIB19	562294	BD
CD56	human	B159	562289	BD
CD14	human	MφP9	562335	BD
hFcεRIα	human	AER-37	334608	Biolegend
CD117	human	104D2	313218	Biolegend
CD203c	human	NP4D6	324606	Biolegend
IgE	human	MHE-18	325510	Biolegend
CD4	human	RPA-T4	555349	BD
CD8α	human	BW135/80	130-097-911	Miltenyi
IL-13	human	JES10-5A2	563580	BD
IL-22	human	IL22JOP	46-7222-82	ThermoFisher
CLA	human	HECA-452	130-104-194	Miltenyi
CCR4	human	1G1	557864	BD
CCR10	human	1B5	564770	BD
CCR8	human	L263G8	360604	Biolegend
CD45RA	human	T6D11	130-095-464	Miltenyi
CXCR3	human	1C6	550967	BD
Langerin	mouse	4C7	144206	Biolegend
CD103	mouse	2E7	121420	Biolegend
CD11c	mouse	N418	56-0114-80	ThermoFisher
CD45.2	mouse	104	109824	Biolegend
IA-E	mouse	M5/114.15.2	107620	Biolegend

[00184] **Table 3: Atopic dermatitis score system**

Score system	Hair density	Hair loss	Dryness/scales	Hemorrhage/erosion	Infection/crust
4 regions:	0-100%	0-no	0-no	0-no	0-no
Head and neck	1-75%	1-1 region	1-1 region	1-1 region	1-1 region
Dorsal skin	2-50%	2-2 region	2-2 region	2-2 region	2-2 region
Limbs	3-25%	3-3 region	3-3 region	3-3 region	3-3 region
Ventral skin	4-nude	4-4 region	4-4 region	4-4 region	4-4 region

[00185] **Table 4: p24 quantification by ultrasensitive digital ELISA and validation by FACS after in vitro activation of BRGST LN CD4+ T cells**

p24 + wells digital ELISA			gag+ cells Flow Cytometry		
580	#1	#2	580	#1	#2
0,75M	+	+	0,75M	+	+
0,15M	+	+	0,15M	+	
0,03M			0,03M		
0,006M	+		0,006M		
581	#1	#2	581	#1	#2
1M	+	+	1M	+	+
0,2M	+	+	0,2M	+	+
0,04M		+	0,04M		
0,008M			0,008M		
582	#1	#2	582	#1	#2
1M		+	1M		+
0,2M			0,2M		
0,04M			0,04M		
0,008M			0,008M		
584	#1	#2	584	#1	#2
1M	+	+	1M	+	+
0,2M	+	+	0,2M	+	+
0,04M			0,04M		
0,008M			0,008M		

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CLAIMS

1. A transgenic mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse.
2. A transgenic mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}) and a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse; wherein exogenous thymic stromal cell-derived lymphopoietin (TSLP) is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous TSLP was administered.
3. The transgenic mouse of claim 1 or claim 2, wherein the SIRP α is SIRP α ^{NOD}.
4. The transgenic mouse of any one of claims 1 to 3, wherein the TSLP is a mouse TSLP.
5. The transgenic mouse of any one of claims 1 to 4, having a genome further comprising a homozygous disruption of a Rag1 gene (*Rag1*^{-/-}) and/or a homozygous disruption of a Rag2 gene (*Rag2*^{-/-}).
6. The transgenic mouse of any one of claims 2 to 4, having a genome comprising a homozygous disruption of the Prkdc gene (*Prkdc*^{-/-}).
7. The transgenic mouse of any one of claims 1 to 6, having a genome comprising a homozygous disruption of the receptor tyrosine kinase Flk2/Flt3 gene (*Flt3*^{-/-}) and wherein exogenous Flt3 ligand (Flt3L) is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous Flt3L was administered.
8. The transgenic mouse of any one of claims 1 to 7, wherein the genetic background of the mouse is selected from BALB/c, C57BL/6, 129, DBA/2, C3H, and NOD.

9. The transgenic mouse of any one of claims 1 to 7, wherein the genetic background of the mouse is selected from a BALB/c background and a mixed BALB/c-129 background.

5

10. The transgenic mouse of claim 1, wherein the mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a homozygous disruption of a Rag2 gene (*Rag2*^{-/-}), a transgene encoding mouse signal regulatory protein α (SIRP α ^{NOD}) that is functionally expressed in the mouse, and a transgene encoding mouse thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and wherein the genetic background of the mouse is BALB/c.

10

11. The transgenic mouse of any one of claims 1 to 10, wherein the mouse does not express functional mouse MHC I and MHC II proteins.

15

12. The transgenic mouse of any one of claims 1 to 10, wherein the mouse expresses a functional human MHC I protein and/or a functional human MHC II protein.

13. The transgenic mouse of any one of claims 1 to 12, wherein the mouse is an embryo or a fetus.

20

14. The transgenic mouse of any one of claims 1 to 13, wherein the mouse develops a full complement of anatomically distributed lymph nodes.

15. The transgenic mouse of claim 14, wherein the mouse does not develop Peyer's patches.

25

16. A human immune system mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse;

30

wherein the mouse has a humanized immune system comprising human B cells, T cells, NK cells, and myeloid cells.

17. A human immune system mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}) and a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse; wherein exogenous thymic stromal cell-derived lymphopoietin (TSLP) is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous TSLP was administered

18. The human immune system mouse of claim 16 or claim 17, wherein the SIRP α is SIRP α ^{NOD}.

19. The human immune system mouse of any one of claims 16 to 18, wherein the TSLP is a mouse TSLP.

20. The human immune system mouse of any one of claims 16 to 19, having a genome further comprising a homozygous disruption of a Rag1 gene (*Rag1*^{-/-}) and/or a homozygous disruption of a Rag2 gene (*Rag2*^{-/-}).

21. The transgenic mouse of claim 18 or claim 19, having a homozygous disruption of the Prkdc gene (*Prkdc*^{-/-}).

22. The transgenic mouse of any one of claims 16 to 21, having a genome comprising a homozygous disruption of the receptor tyrosine kinase Flk2/Flt3 gene (*Flt3*^{-/-}) and wherein exogenous Flt3 ligand (Flt3L) is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous Flt3L was administered.

23. The human immune system mouse of any one of claims 16 to 22, wherein the genetic background of the mouse is selected from BALB/c, C57BL/6, 129, DBA/2, C3H, and NOD.

24. The human immune system mouse of any one of claims 16 to 22, wherein the genetic background of the mouse is selected from a BALB/c background and a mixed BALB/c-129 background.

5 25. The human immune system mouse of claim 16, wherein the mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a homozygous disruption of a Rag2 gene (*Rag2*^{-/-}), a transgene encoding mouse signal regulatory protein α ((SIRP α ^{NOD}) that is functionally expressed in the mouse, and a transgene encoding mouse thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the
10 mouse, and wherein the genetic background of the mouse is BALB/c.

26. The human immune system mouse of any one of claims 16 to 25, wherein the mouse does not express functional mouse MHC I and MHC II proteins.

15 27. The human immune system mouse of any one of claims 16 to 25, wherein the mouse expresses a functional human MHC I protein and/or a functional human MHC II protein.

20 28. The human immune system mouse of any one of claims 16 to 27, wherein the mouse is an embryo or a fetus.

29. The human immune system mouse of any one of claims 16 to 28, wherein the mouse develops a full complement of anatomically distributed lymph nodes.

25 30. The human immune system mouse of claim 29, wherein the mouse does not develop Peyer's patches

30 31. The human immune system mouse of claim 29 or 30, wherein the mouse develops at least two-fold more follicular helper T cells (T_{FH}) than a control mouse having a genome that does not comprise a transgene encoding TSLP that is functionally expressed in the mouse.

32. The human immune system mouse of any one of claims 29 to 31, wherein the mouse comprises T_{FH} cells comprising an infectious agent.

33. The human immune system mouse of claim 32, wherein the infectious agent is a virus.

34. The human immune system mouse of claim 33, wherein the virus is a lentivirus.

35. The human immune system mouse of claim 34, wherein the lentivirus is a human immunodeficiency virus (HIV).

36. The human immune system mouse of any one of claims 16 to 35, wherein the mouse is made by a method comprising providing a transgenic mouse according to any one of claims 1 to 14 and engrafting human hematopoietic stem cells (HSCs) into the transgenic mouse.

37. A method, comprising:
providing a transgenic mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a transgene encoding signal regulatory protein α (SIRP α) that is functionally expressed in the mouse, and a transgene encoding thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse;
engrafting human hematopoietic stem cells (HSCs) into the transgenic mouse,
infecting the transgenic mouse with an infectious agent, and
detecting the presence and/or absence of the infectious agent in a lymph node of the transgenic mouse.

38. A method, comprising:
providing a transgenic mouse having a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}) and a transgene encoding signal regulatory protein α

(SIRP α) that is functionally expressed in the mouse; wherein exogenous thymic stromal cell-derived lymphopoietin (TSLP) is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous TSLP was administered;

engrafting human hematopoietic stem cells (HSCs) into the transgenic mouse,

5 infecting the transgenic mouse with an infectious agent, and

detecting the presence and/or absence of the infectious agent in a lymph node of the transgenic mouse.

39. The method of claim 37 or claim 38, wherein the SIRP α is SIRP α^{NOD} .

40. The method of any one of claims 37 to 39, wherein the transgenic mouse has a genome further comprising a homozygous disruption of a Rag1 gene (Rag1^{-/-}) and/or a homozygous disruption of a Rag2 gene (Rag2^{-/-}).

41. The method of any claim 38 or claim 39, wherein the transgenic mouse has a genome further comprising a homozygous disruption of the Prkdc gene (Prkdc^{-/-}).

42. The method of any one of claims 37 to 41, wherein the transgenic mouse has a genome comprising a homozygous disruption of the receptor tyrosine kinase Flt2/Flt3 gene (Flt3^{-/-}) and wherein exogenous Flt3 ligand (Flt3L) is administered to the transgenic mouse or the transgenic mouse developed from a mouse to which exogenous Flt3L was administered.

43. The method of any one of claims 37 to 42, wherein the genetic background of the mouse is selected from BALB/c, C57BL/6, 129, DBA/2, C3H, and NOD.

44. The method of any one of claims 37 to 42, wherein the genetic background of the mouse is selected from a BALB/c background and a mixed BALB/c-129 background.

45. The method of claim 37, wherein the mouse has a genome comprising a homozygous disruption of a common receptor γ gene (*Il2rg*^{-/-}), a homozygous disruption of a Rag2 gene (Rag2^{-/-}), a transgene encoding mouse signal regulatory protein α ((SIRP α^{NOD}) that

is functionally expressed in the mouse, and a transgene encoding mouse thymic stromal cell-derived lymphopoietin (TSLP) that is functionally expressed in the mouse, and wherein the genetic background of the mouse is BALB/c.

5 46. The method of any one of claims 37 to 45, wherein the mouse does not express functional mouse MHC I and MHC II proteins.

 47. The method of any one of claims 37 to 45, wherein the mouse expresses a functional human MHC I protein and/or a functional human MHC II protein.

10

 48. The method of any one of claims 37 to 47, wherein the mouse develops a full complement of anatomically distributed lymph nodes.

 49. The method of claim 48, wherein the mouse does not develop Peyer's patches.

15

 50. The method of any one of claims 37 to 49, wherein the infectious agent is a virus, bacteria or parasite.

 51. The method of claim 50, wherein the infectious agent is a virus.

20

 52. The method of claim 51, wherein the virus is a lentivirus.

 53. The method of claim 52, wherein the lentivirus is a human immunodeficiency virus (HIV).

25

 54. The method of any one of claims 37 to 53, further comprising administering an anti-infectious agent therapy to the transgenic mouse.

30 55. The method of any one of claims 37 to 54, wherein the presence and/or absence of a latent infection of a lymph node of the transgenic mouse by the infectious agent is detected.

56. A method comprising, providing a human immune system mouse according to any one of claims 16 to 36, administering an antigen to the human immune system mouse, and assaying for the presence, absence, and/or level of at least one immune response in the human immune system mouse to the antigen.

57. The method of claim 56, wherein the at least one immune response is selected from the group consisting of a B-cell response, a T-cell response, and an NK-cell response.

58. The method of claim 56 or claim 57, further comprising administering an adjuvant to the human immune system mouse.

59. The method of any one of claims 56 to 58, further comprising administering the antigen to a control mouse.

60. The method of any one of claims 56 to 59, further comprising administering a test molecule to the human immune system mouse and determining whether the test molecule increases or decreases the at least one immune response in the human immune system mouse to the antigen.

61. A method comprising, providing a human immune system mouse according to any one of claims 16 to 36, administering a vaccine against an infectious agent to the human immune system mouse, administering the infectious agent to the human immune system mouse, and assaying for the presence, absence, and/or level of infection of the human immune system mouse by the infectious agent.

62. The method of claim 61, further comprising administering the infectious agent to a second human immune system mouse, and assaying for the presence, absence, and/or level of infection of the second human immune system mouse by the infectious agent.

63. The method of claim 61 or claim 62, wherein the infectious agent is a virus and the vaccine is a viral vaccine.

64. The method of claim 63, wherein the virus is a lentivirus.

5

65. The method of claim 64, wherein the lentivirus is HIV.

66. The transgenic mouse of claim 2, the human immune system mouse of claim 17 or the method of claim 38, wherein said exogenous TSLP is recombinant TSLP.

10

67. The transgenic mouse of claim 7, the human immune system mouse of claim 22 or the method of claim 42, wherein said exogenous Flt3L is recombinant Flt3L.

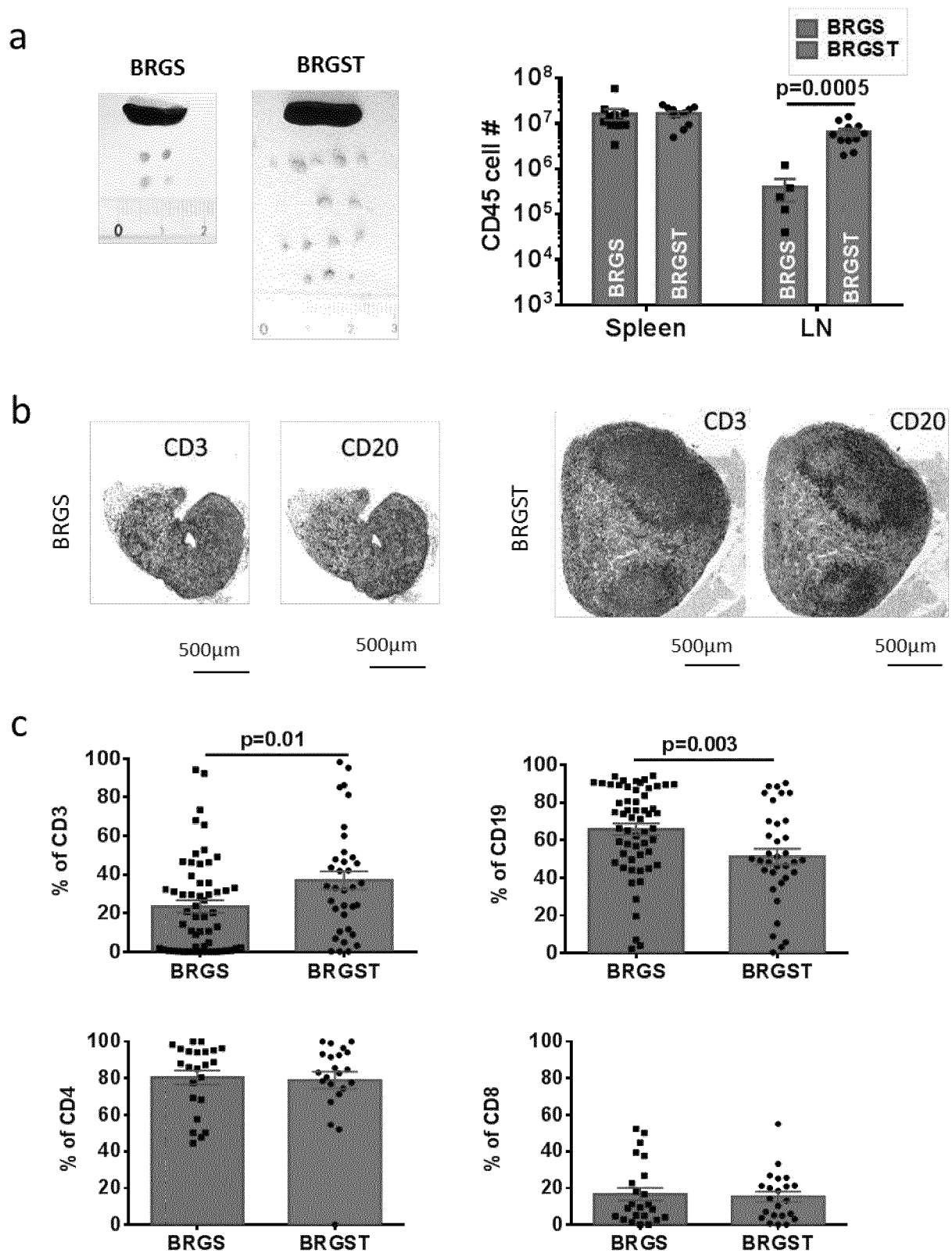
68. A mouse model of human atopic dermatitis comprising the human immune system mouse of any one of claims 16, 18 to 31 and 36.

15

69. Use of the mouse model of claim 68 for testing candidate therapeutic agents to treat human atopic dermatitis.

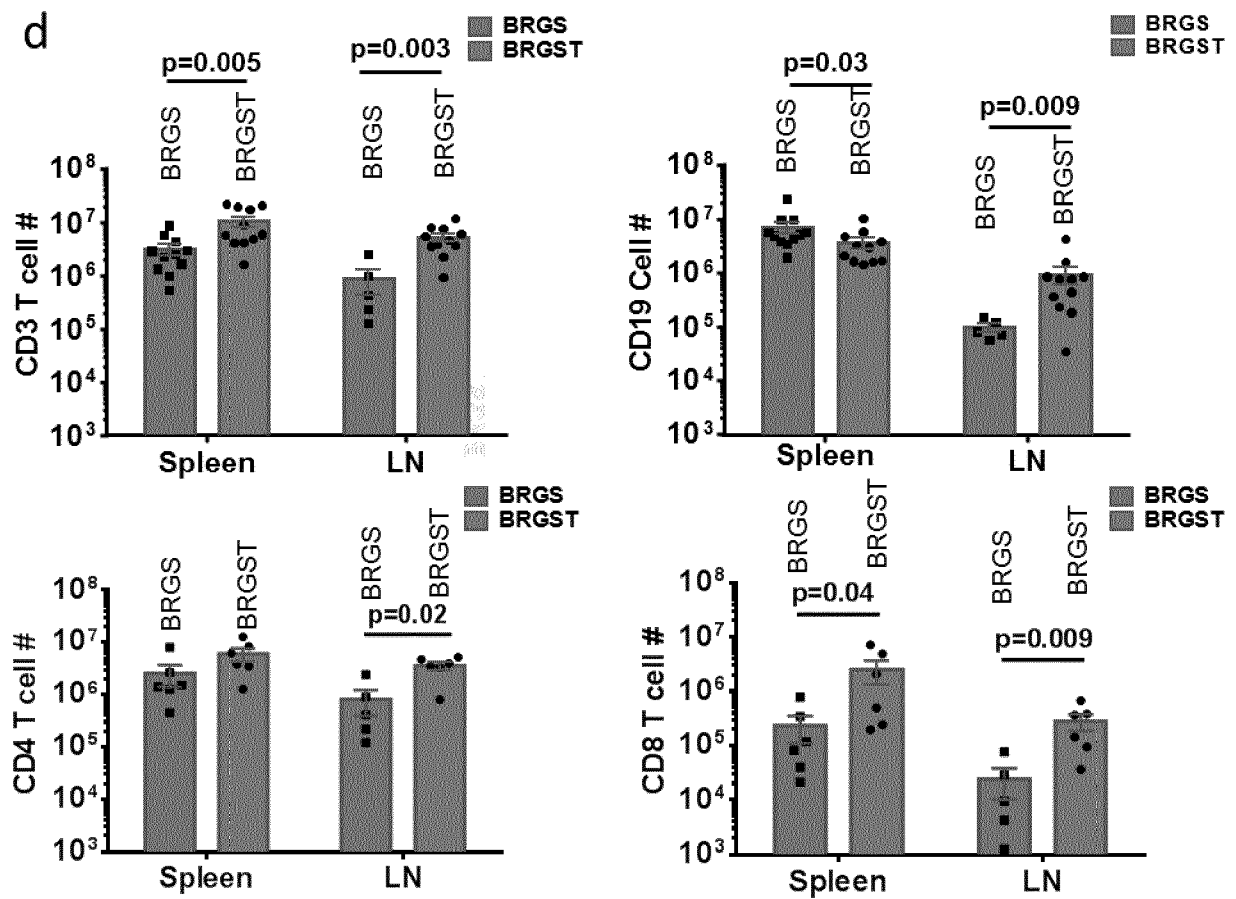
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Figure 1



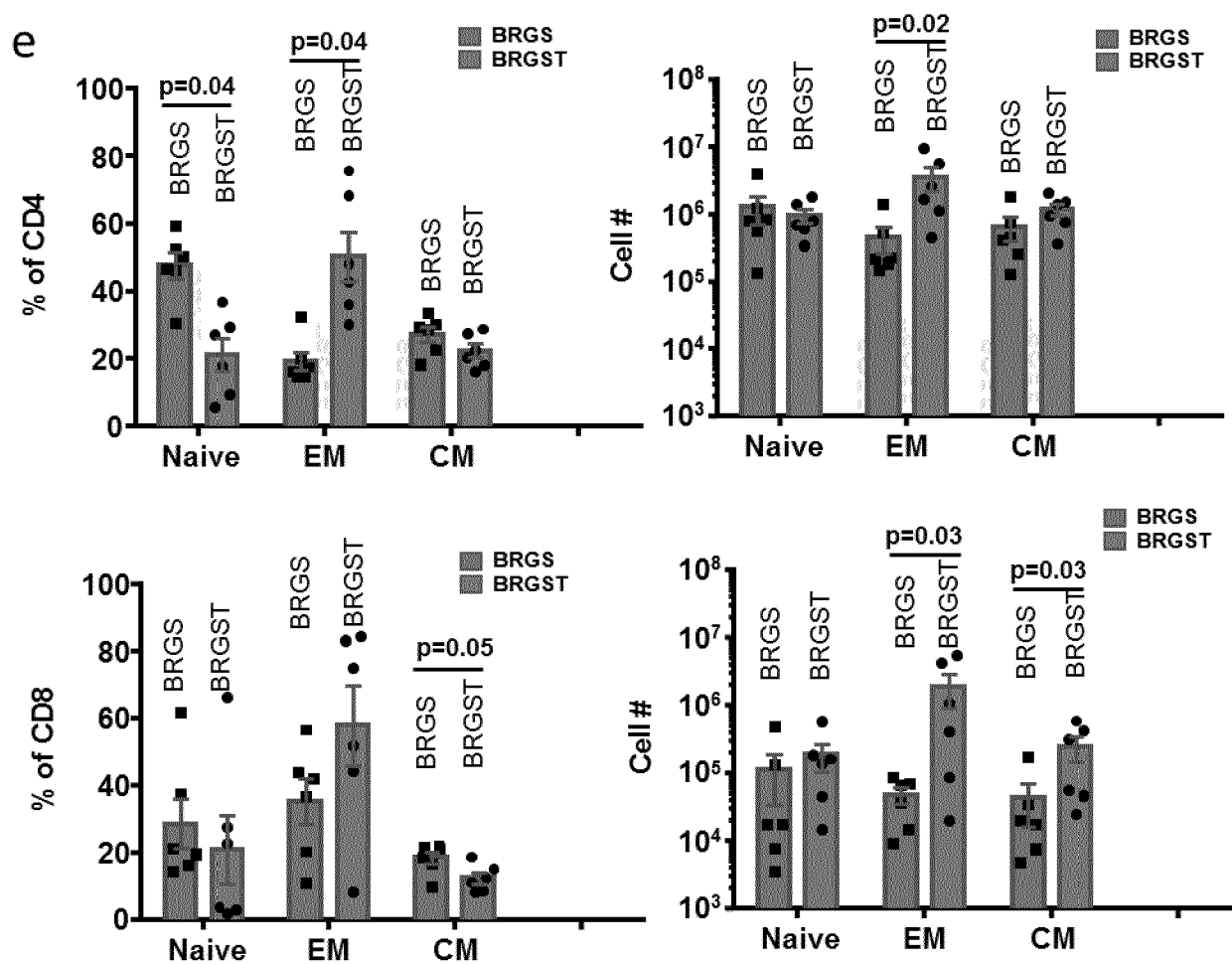
2/70

Figure 1 (continuation)



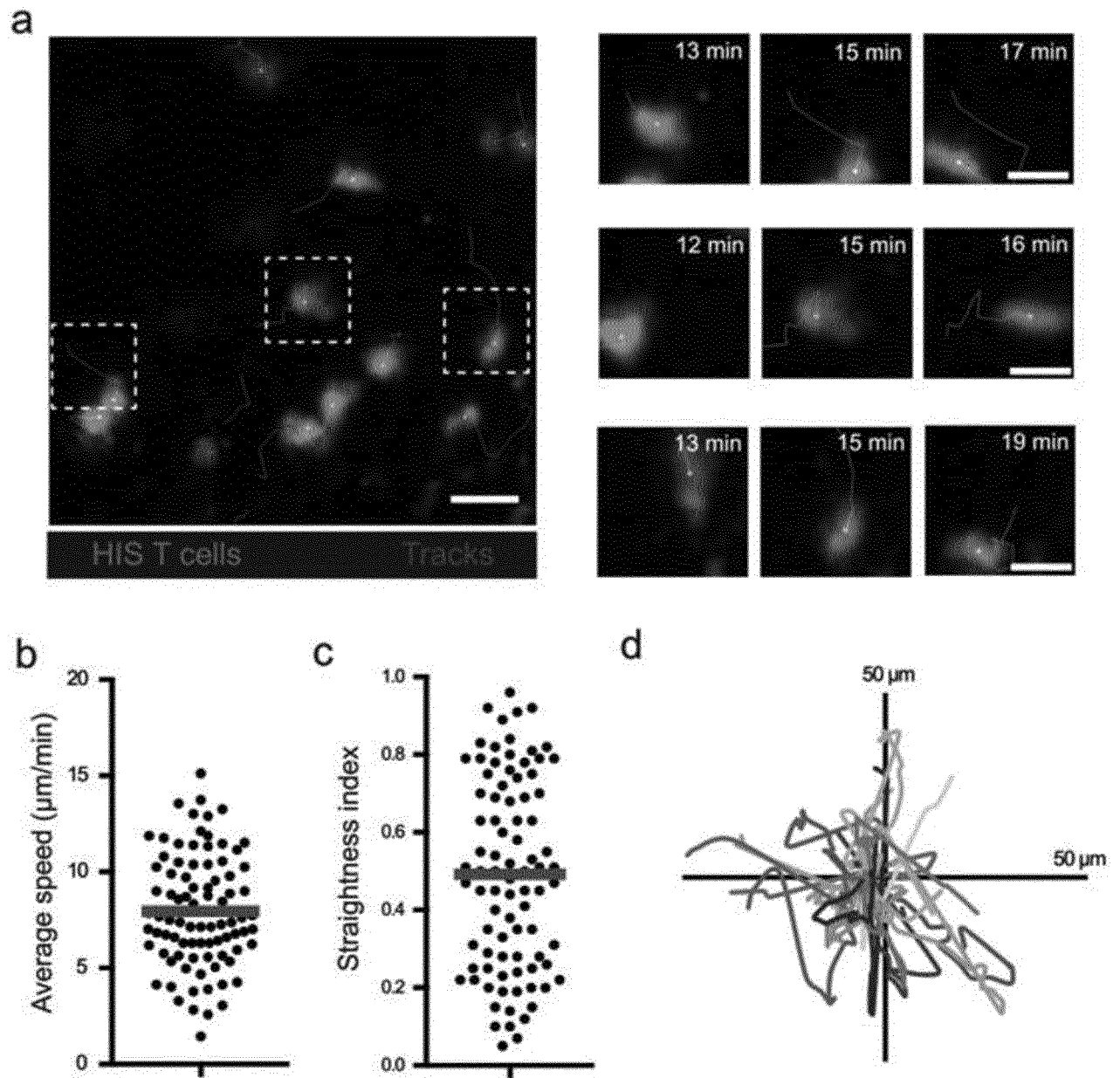
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Figure 1 (continuation)



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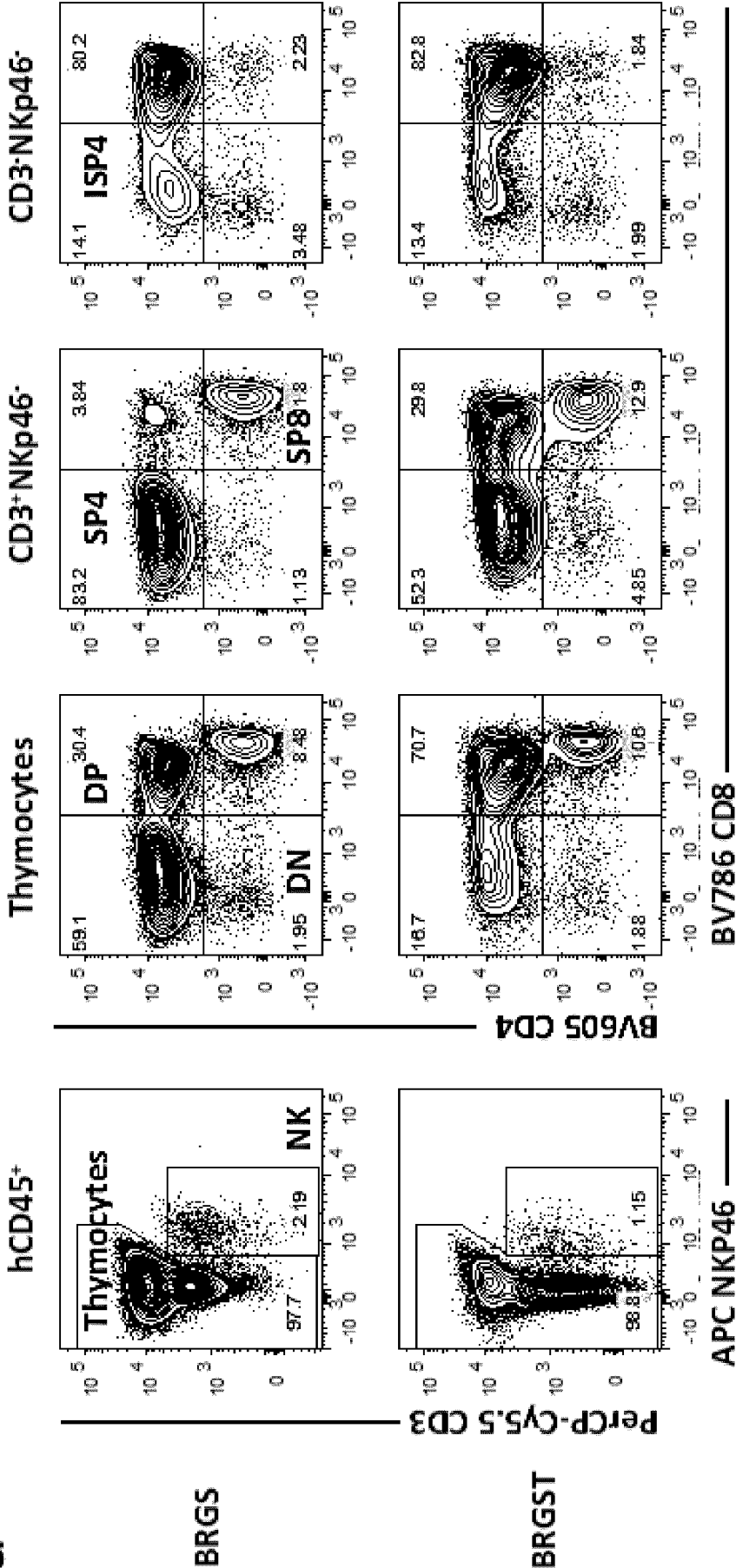
Figure 2



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Figure 3

a



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Figure 3 (continuation)

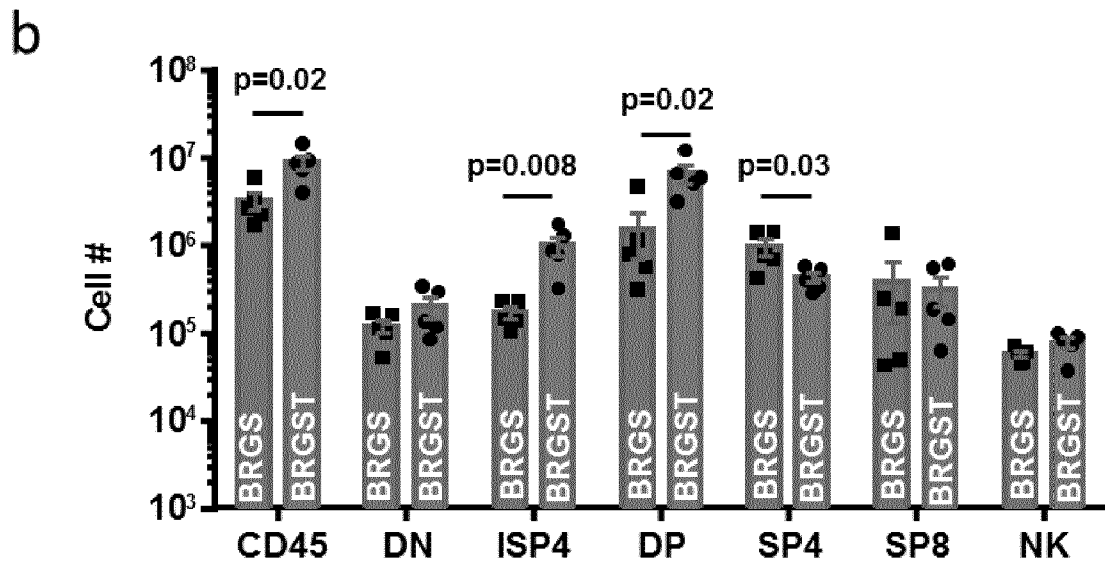
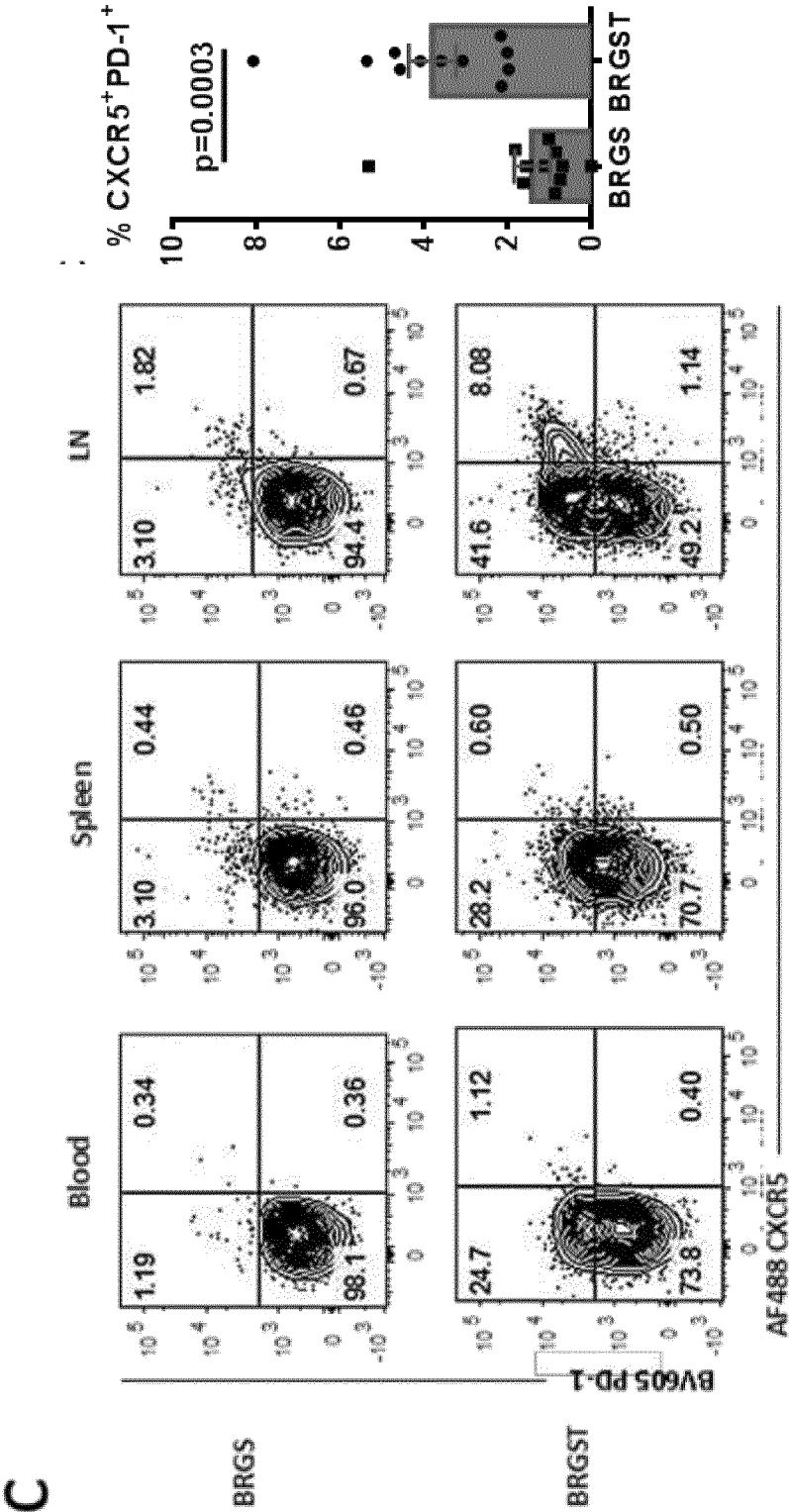


Figure 3 (continuation)



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Figure 3 (continuation)

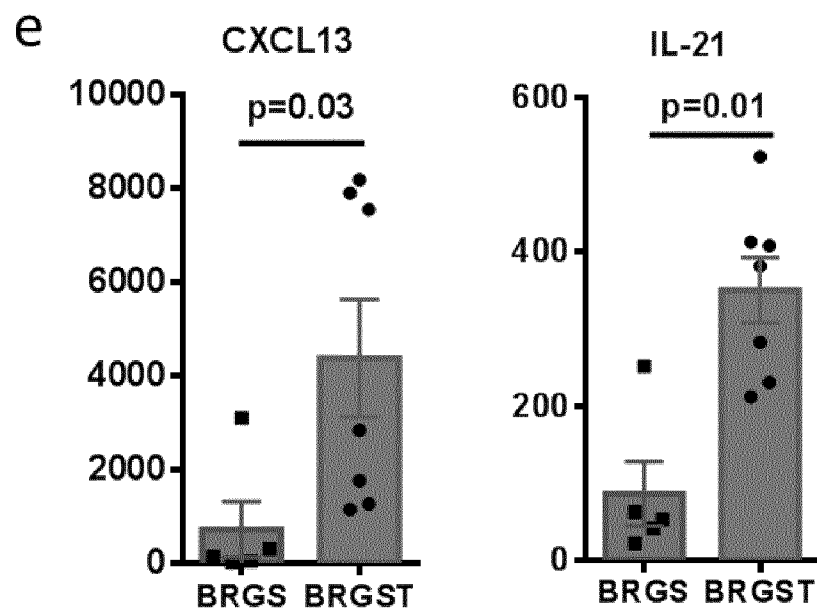
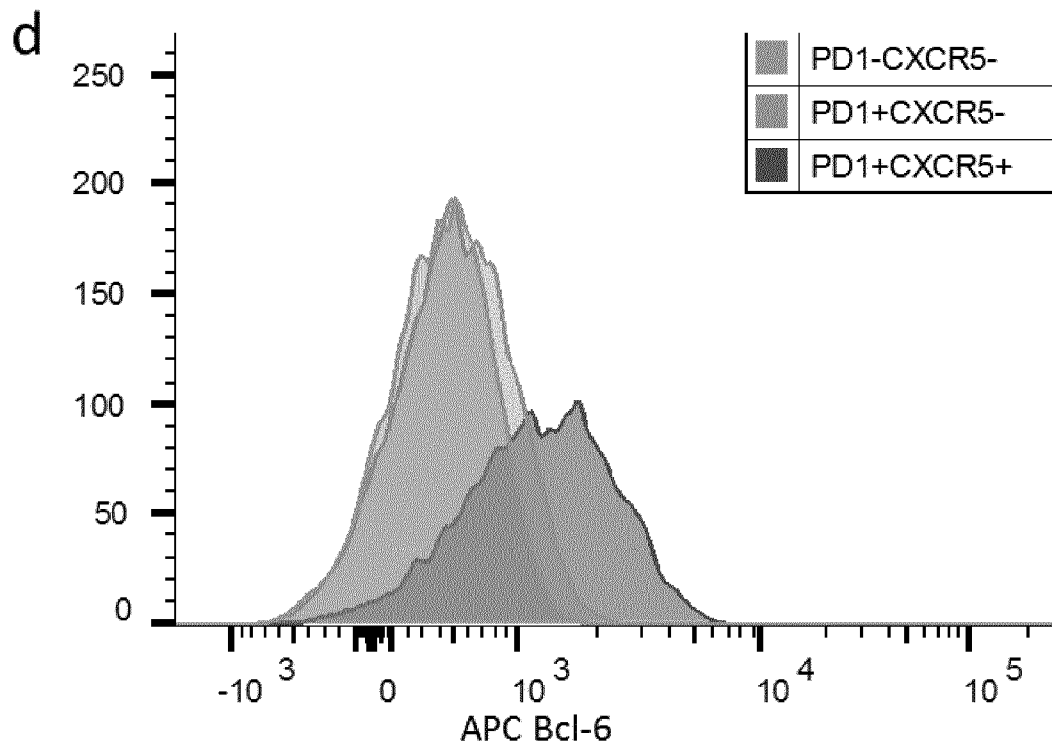
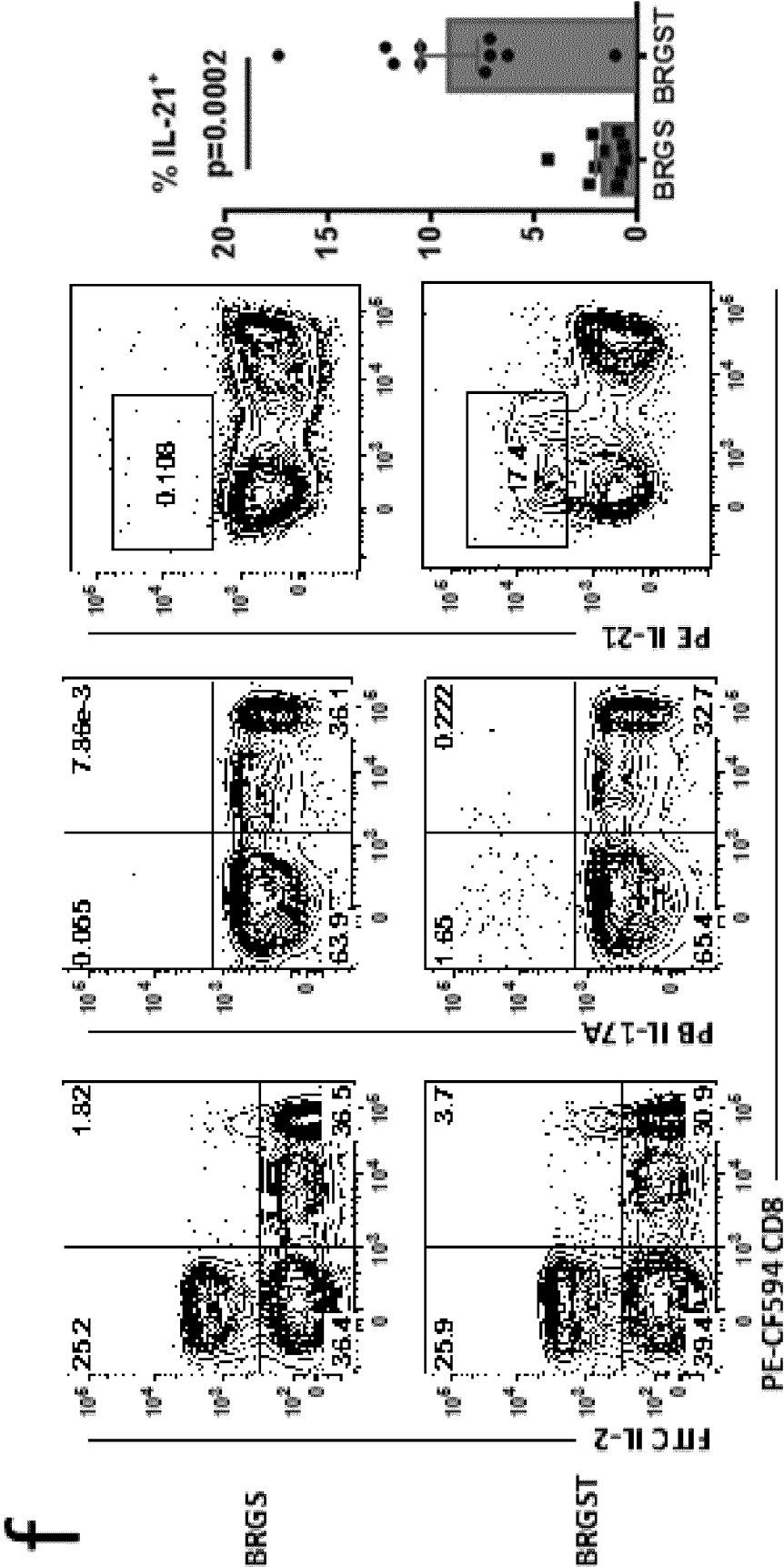
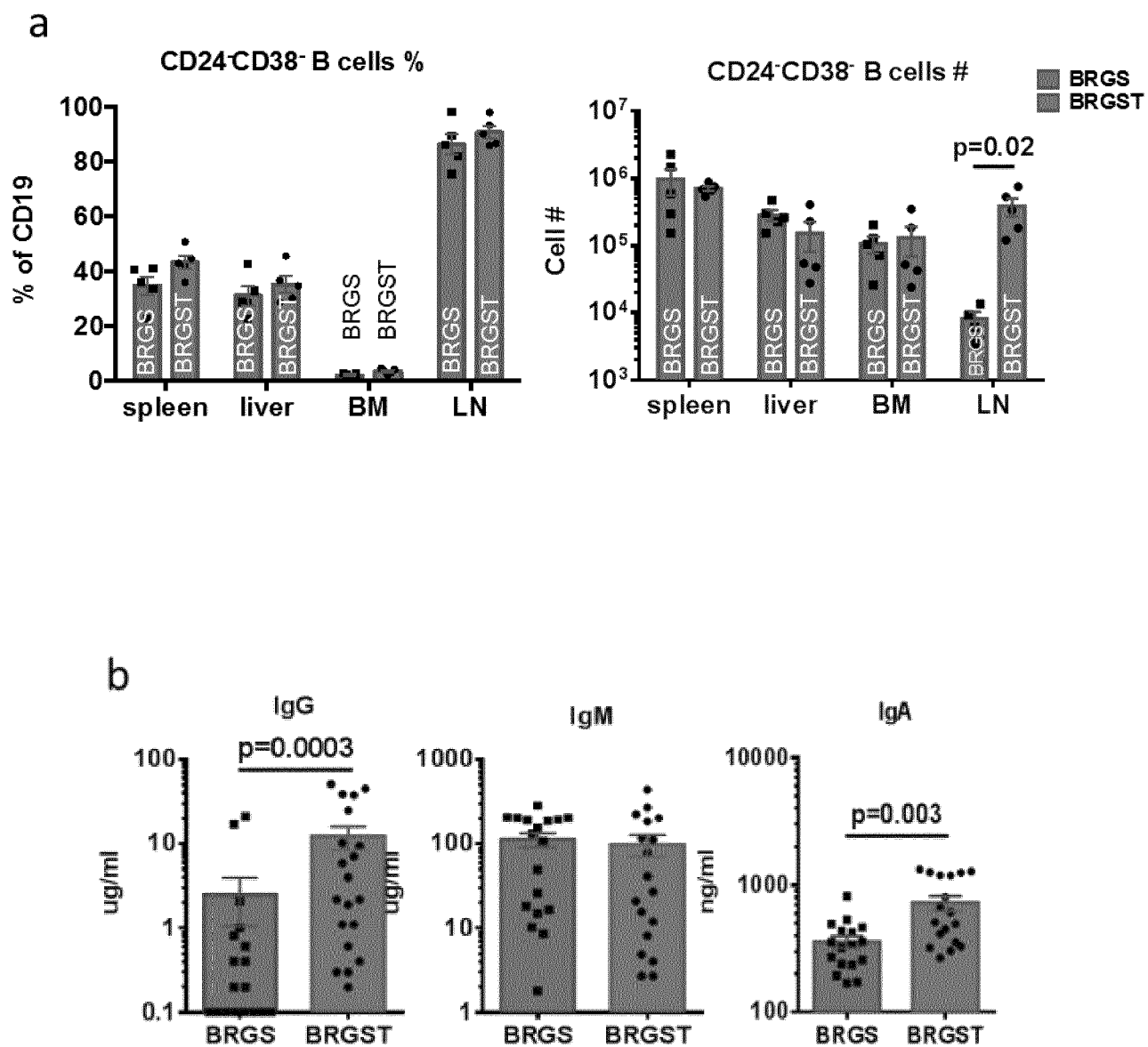


Figure 3 (continuation)



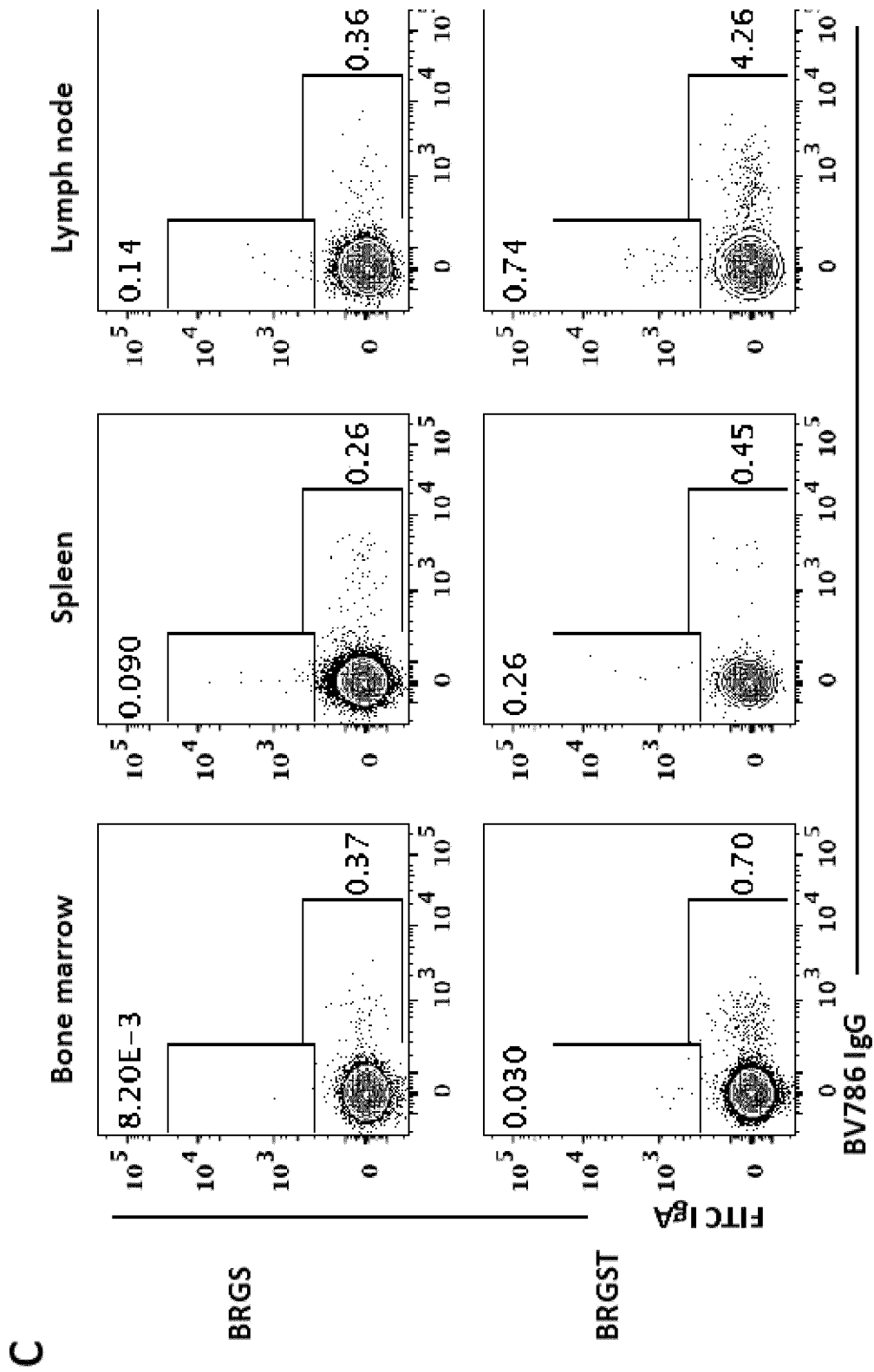
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Figure 4



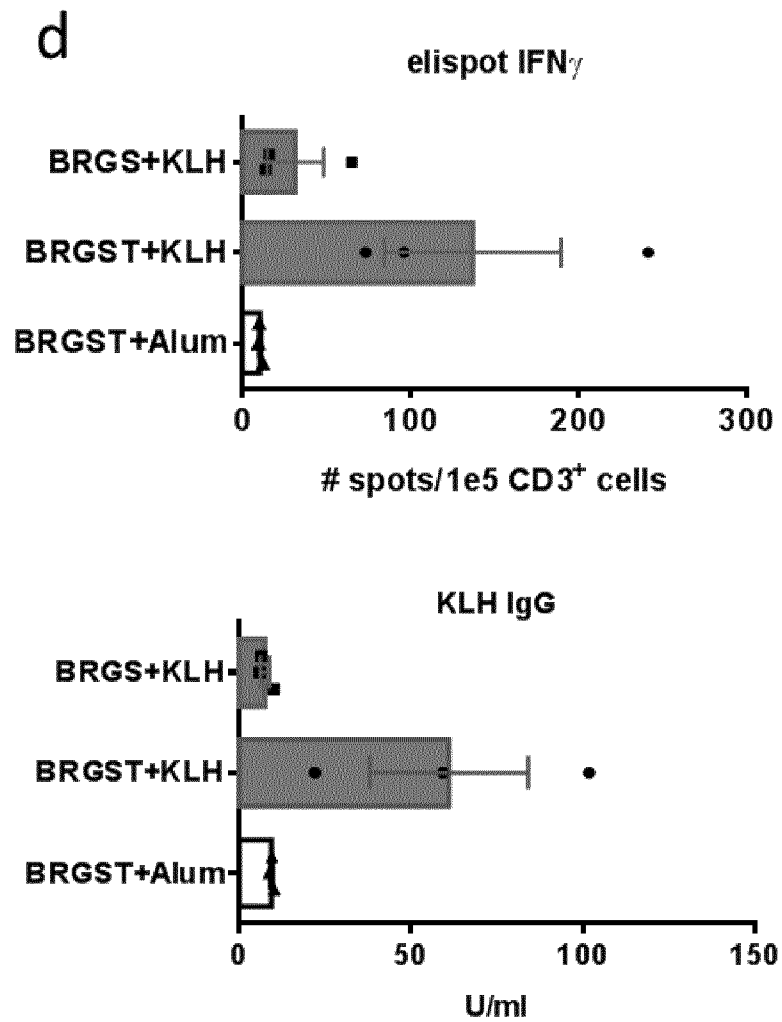
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Figure 4 (continuation)



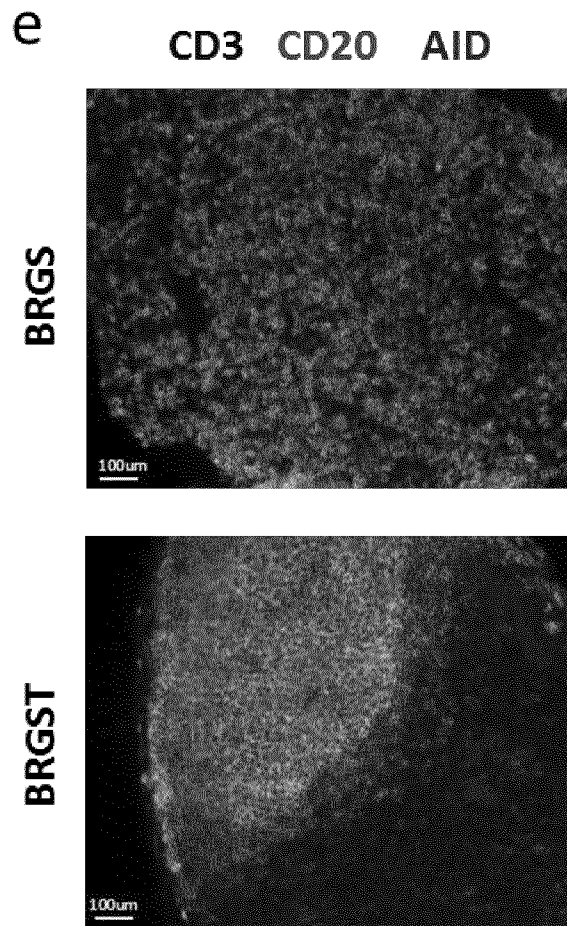
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Figure 4 (continuation)



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Figure 4 (continuation)



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Figure 5

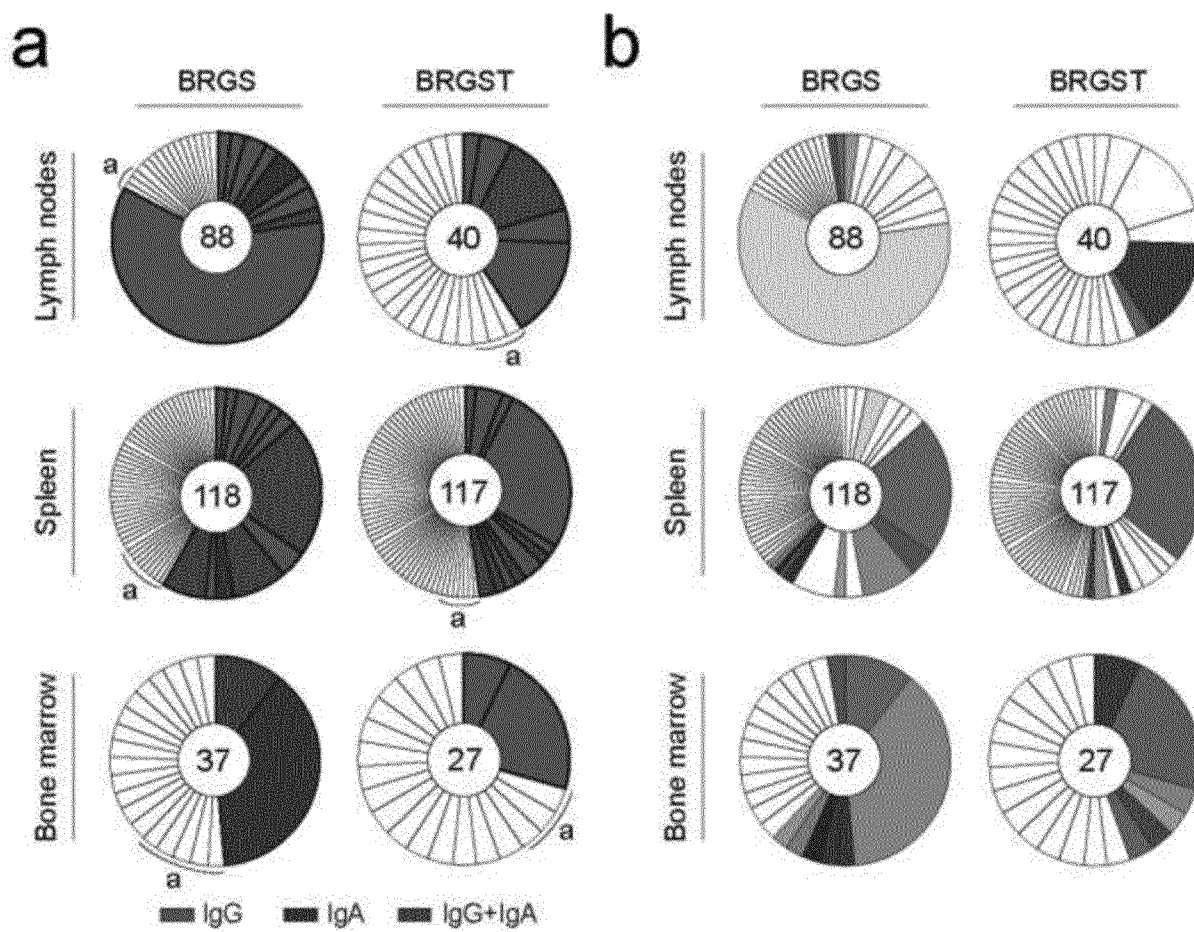


Figure 5 (continuation)

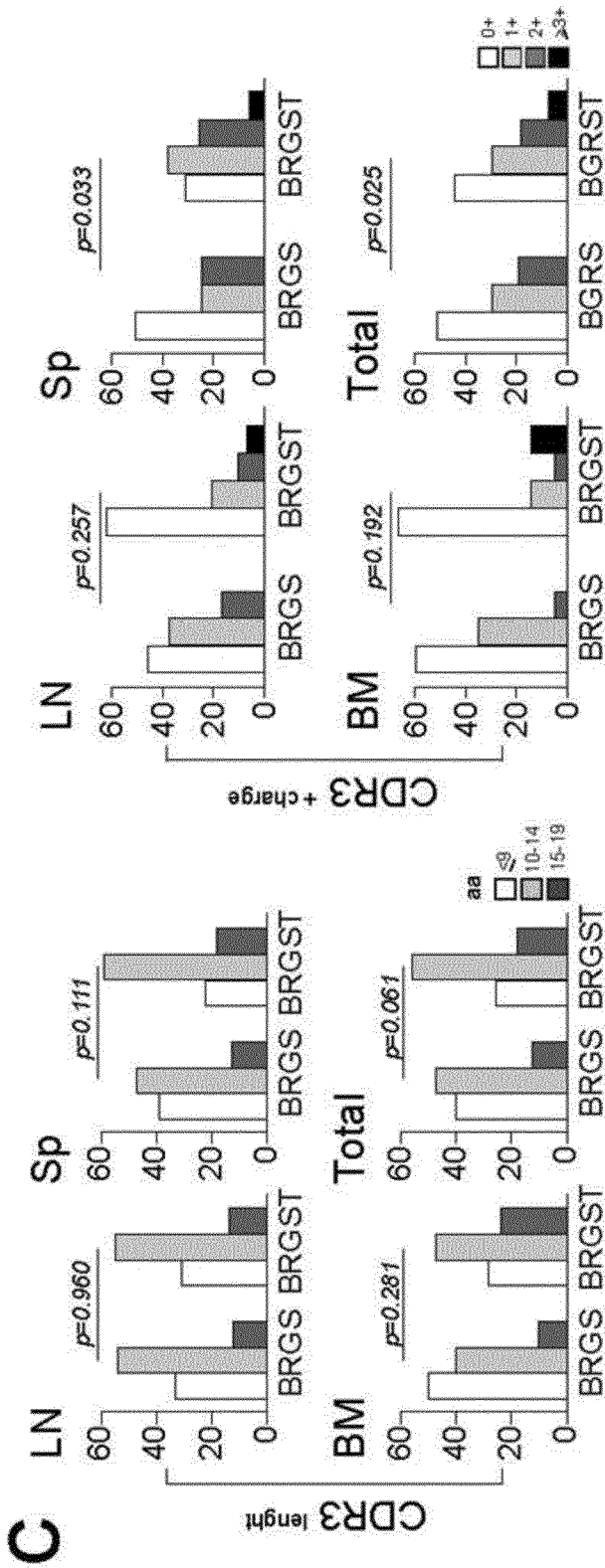
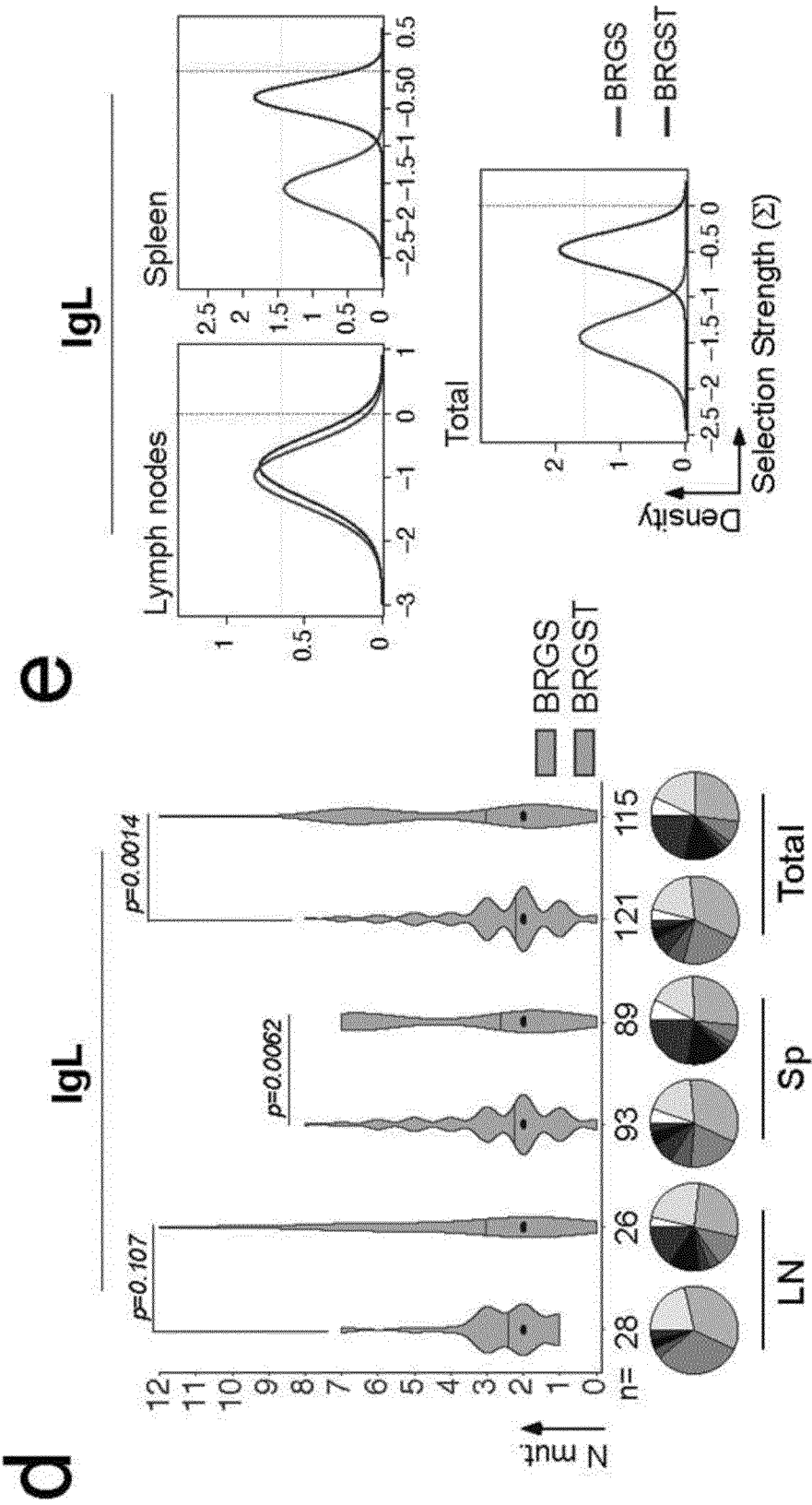
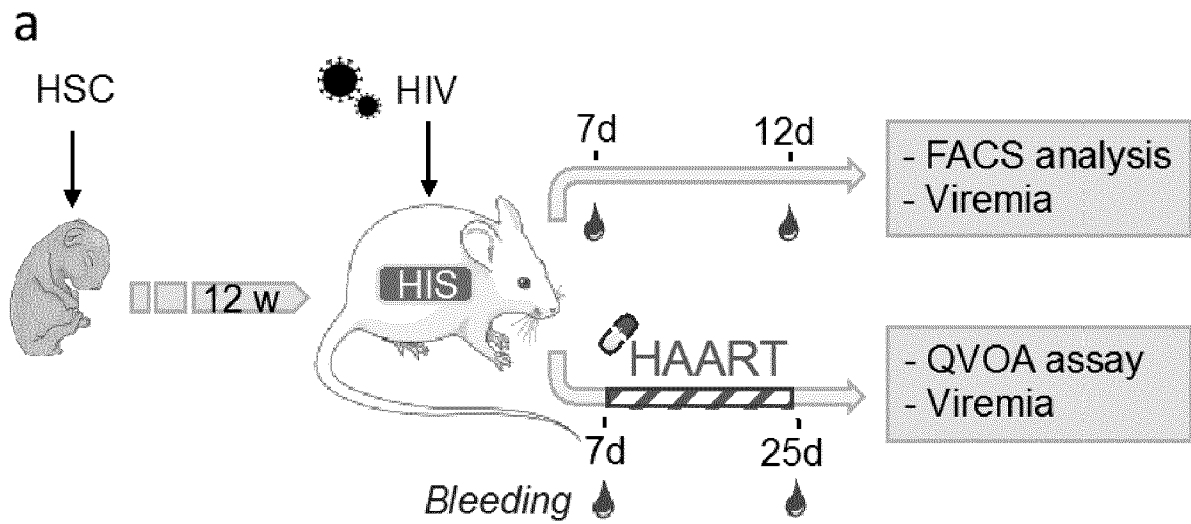


Figure 5 (continuation)



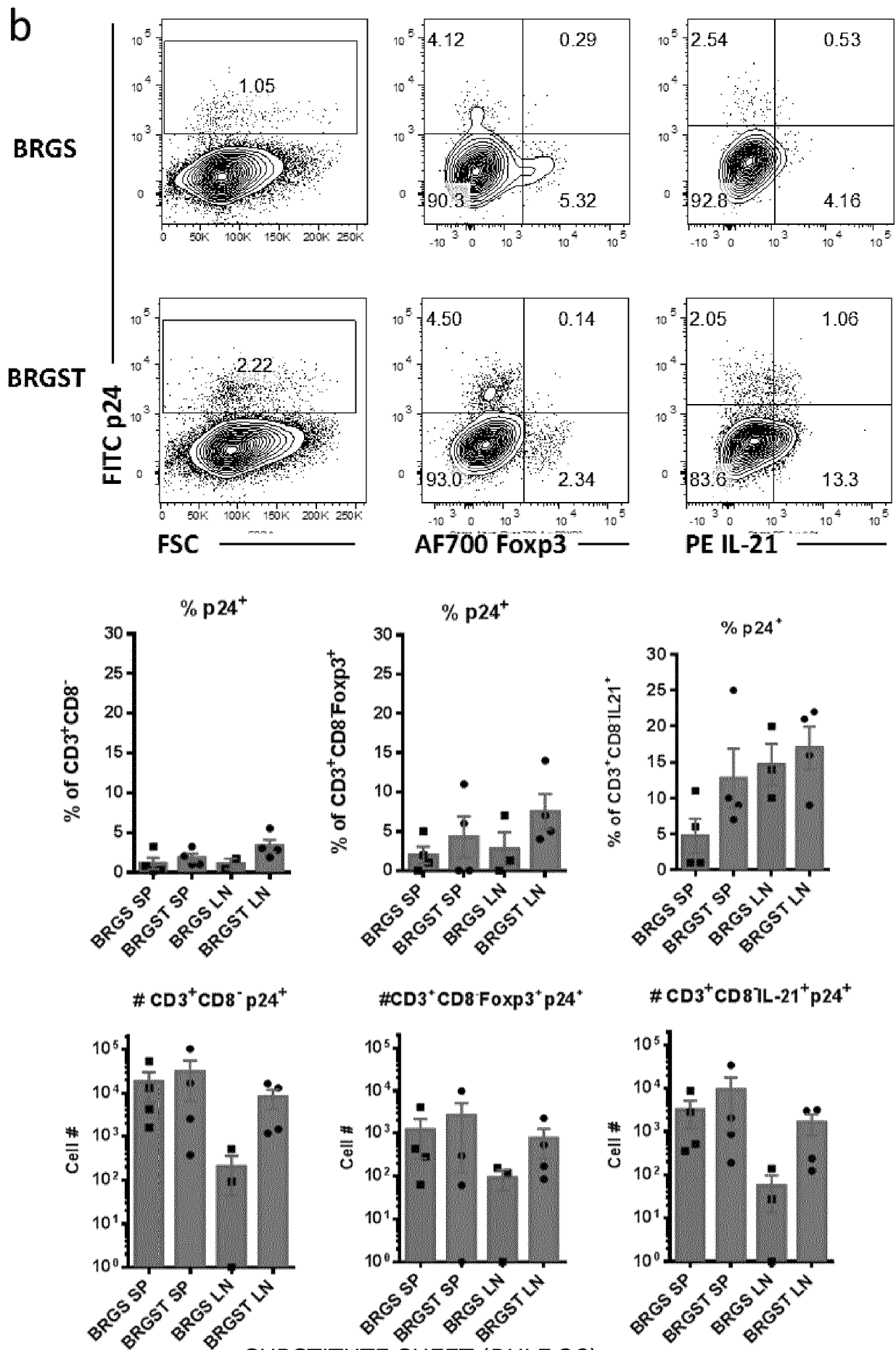
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Figure 6



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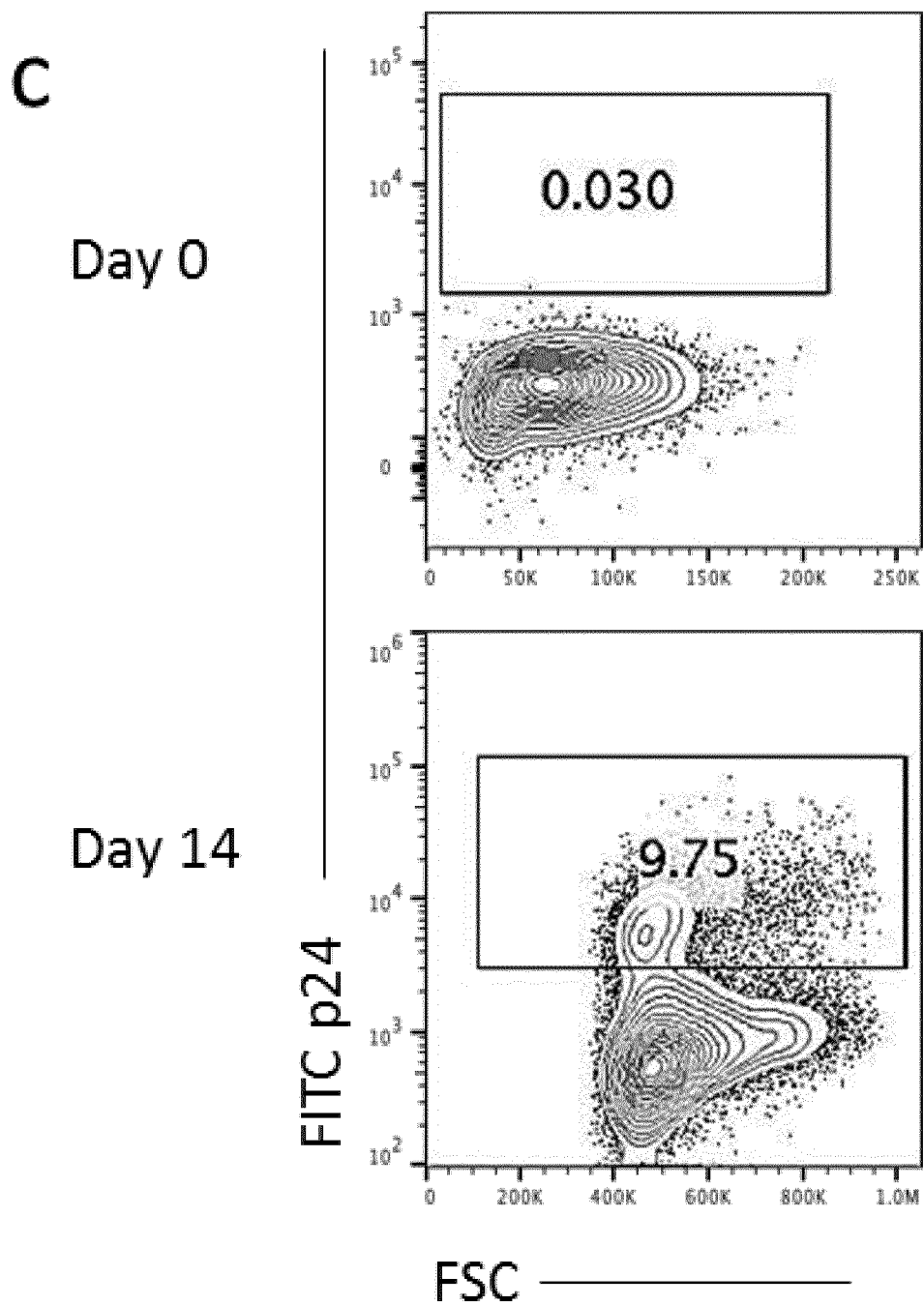
Figure 6 (continuation)



SUBSTITUTE SHEET (RULE 26)

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Figure 6 (continuation)



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Figure 6 (continuation)

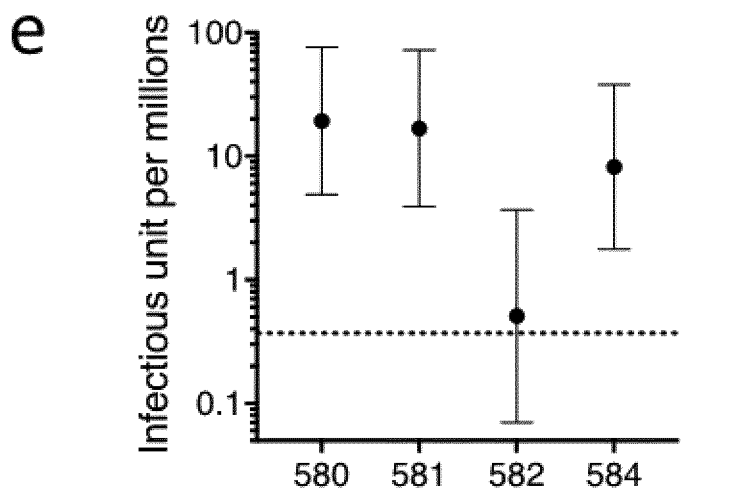
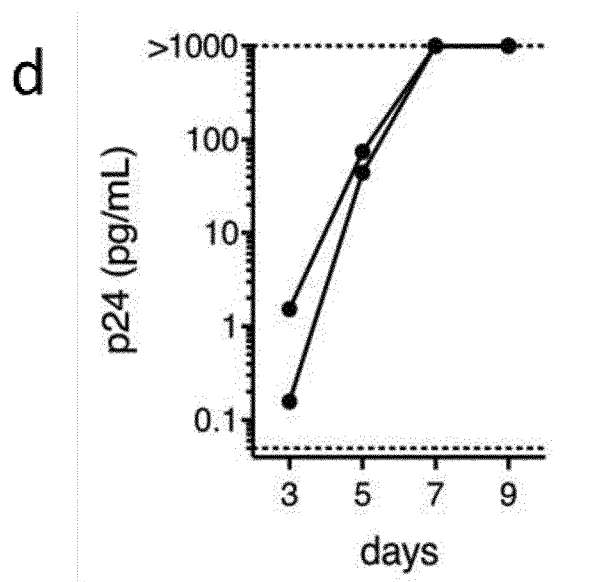
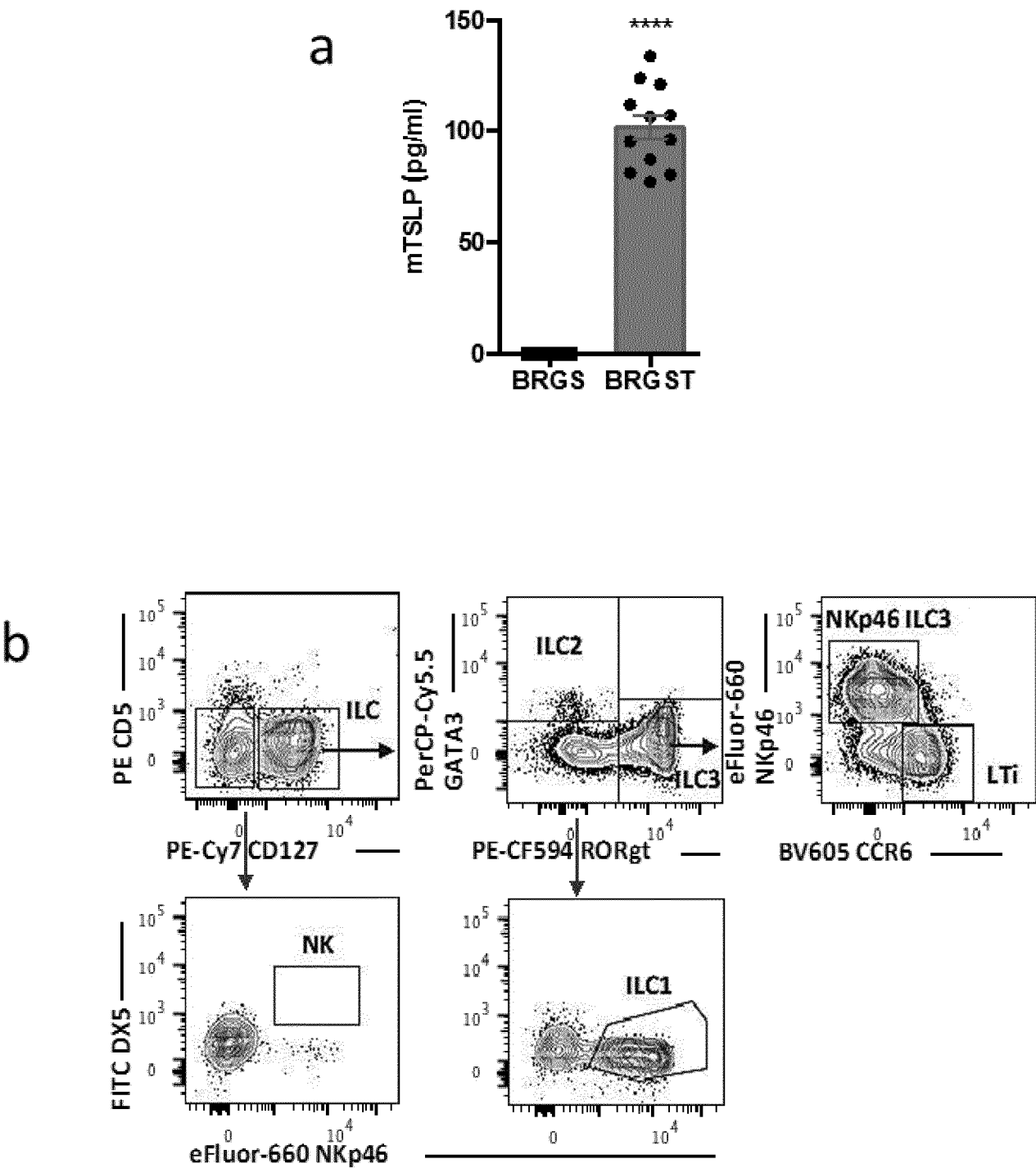
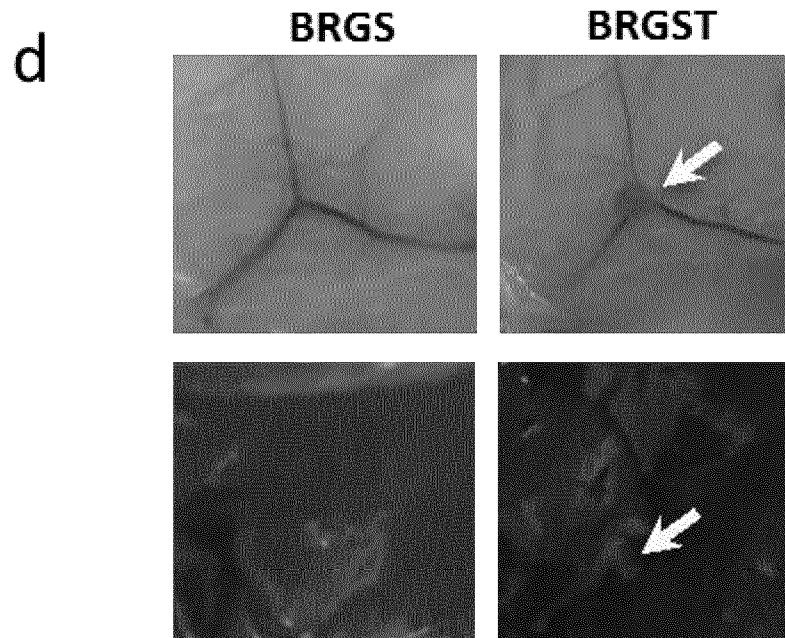
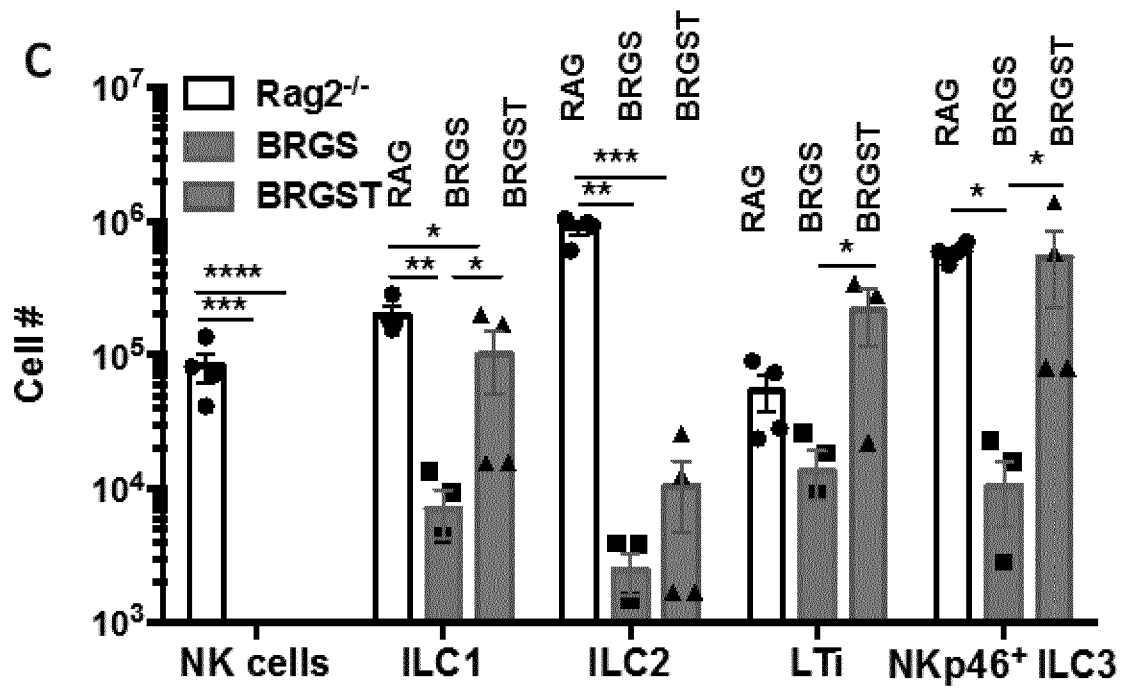


Figure 7



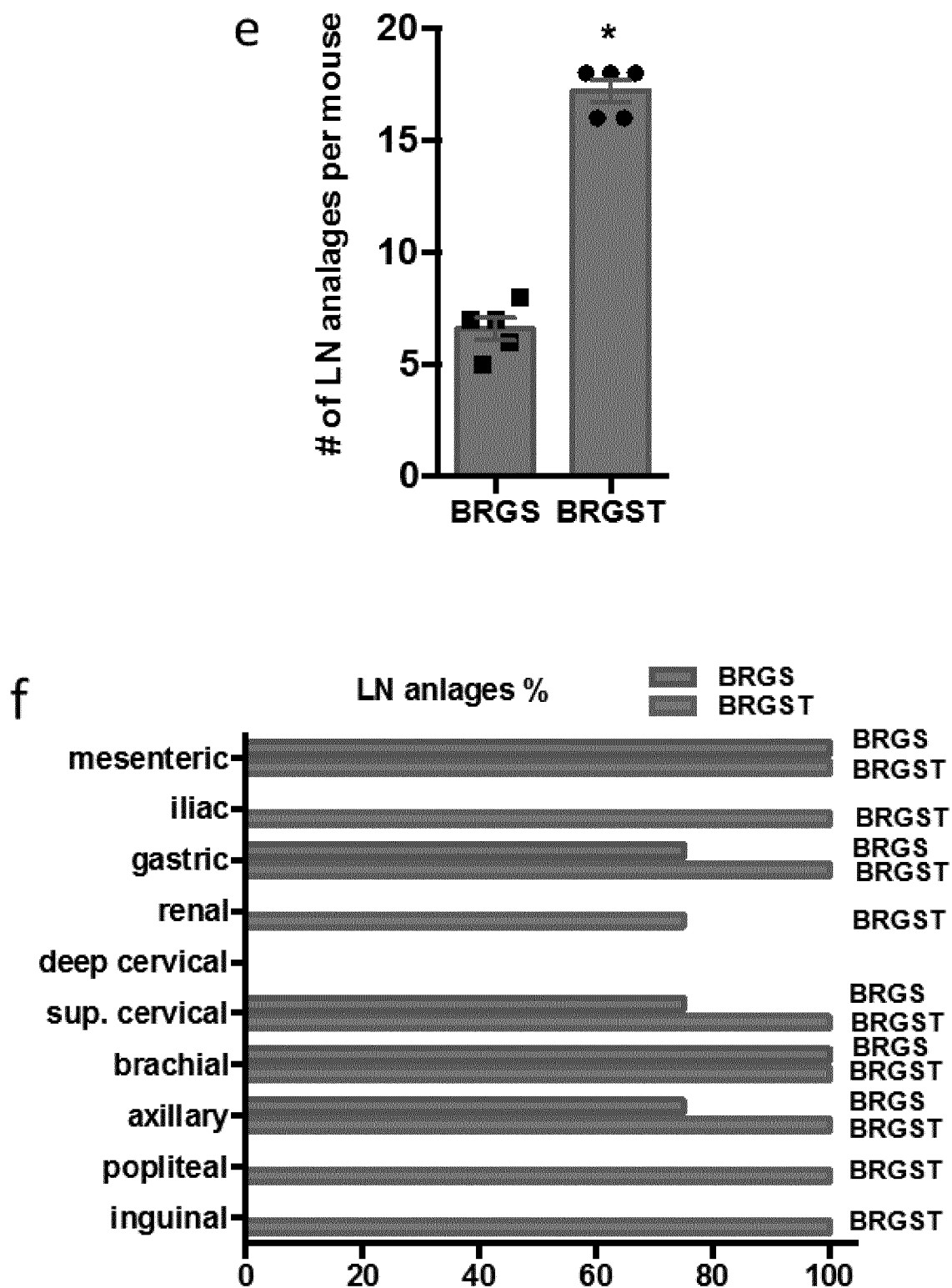
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Figure 7 (continuation)



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Figure 7 (continuation)



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Figure 7 (continuation)

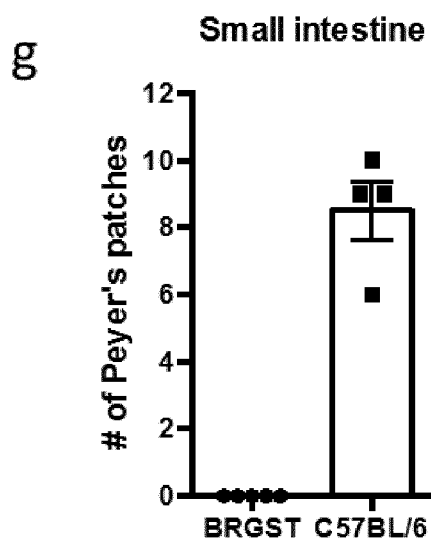
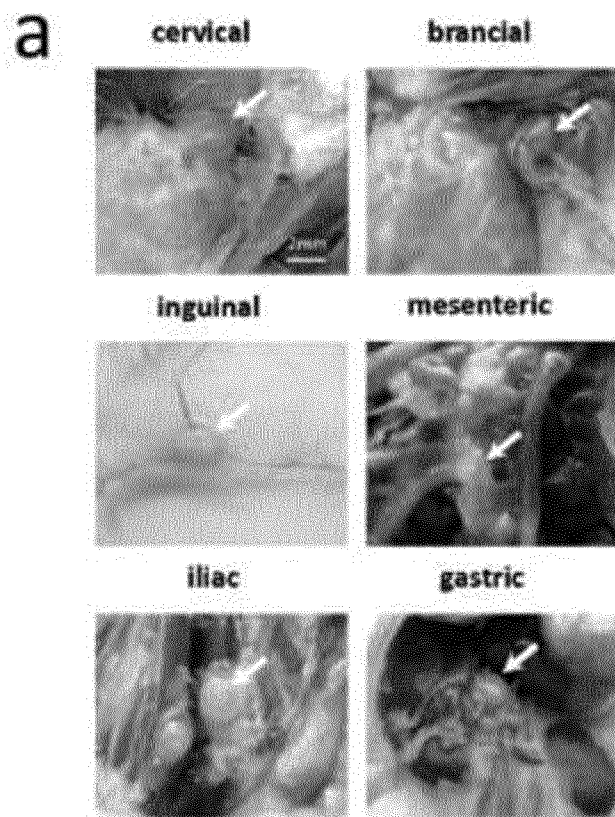
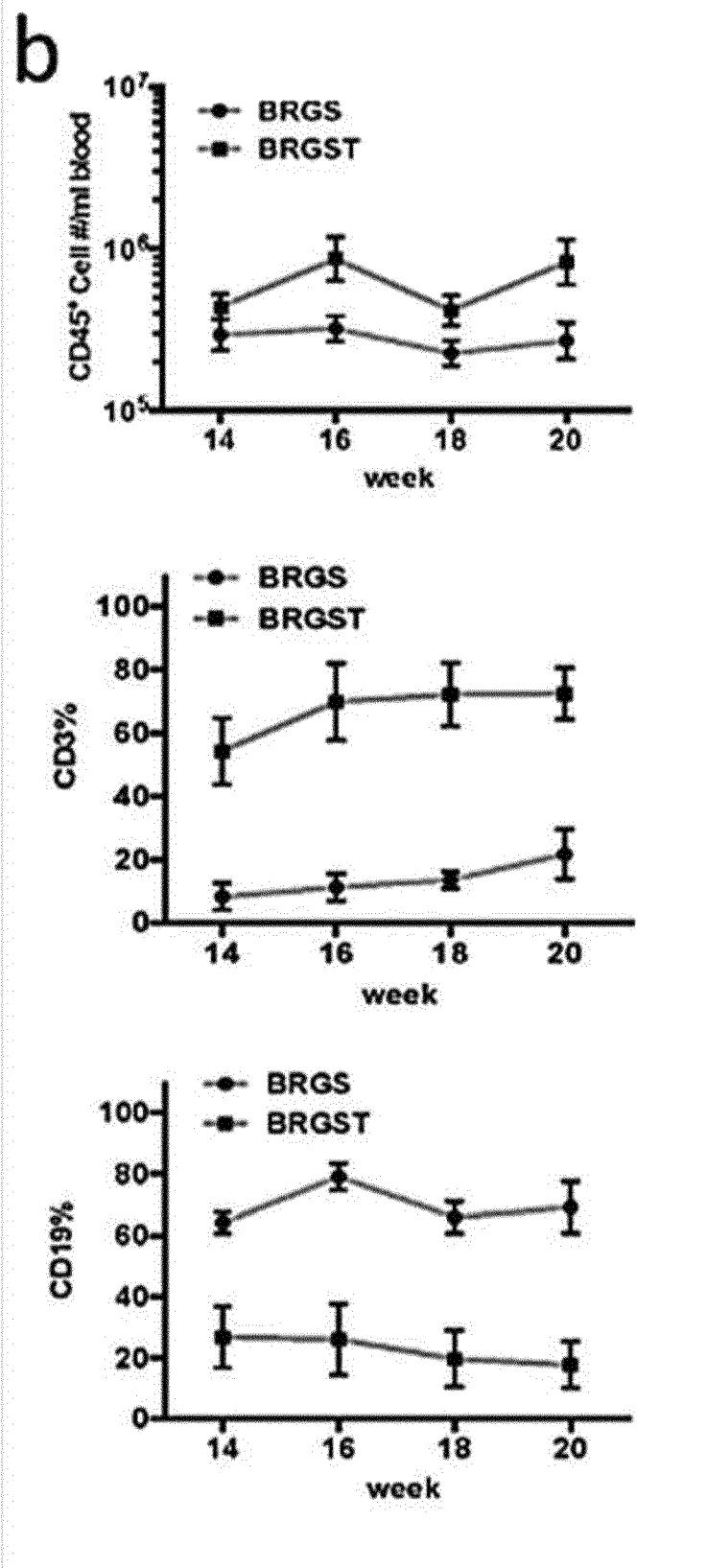


Figure 8



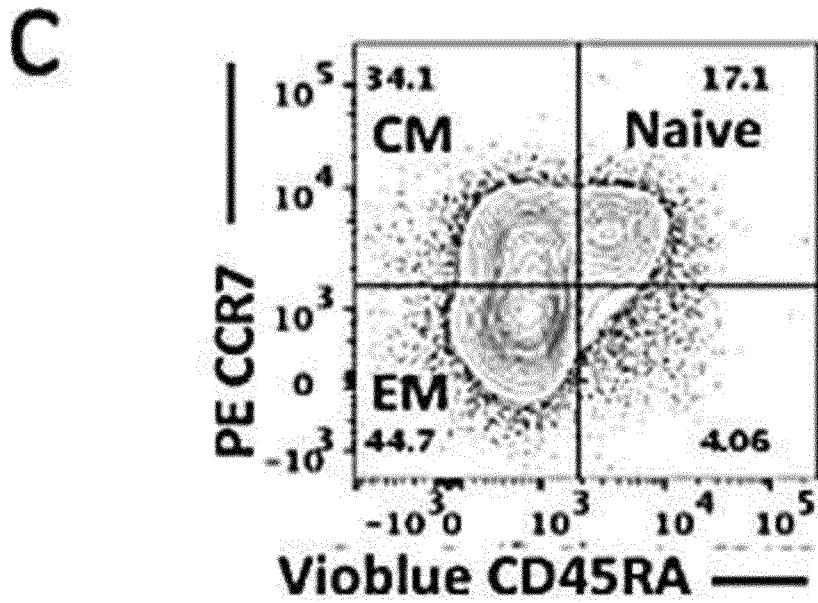
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Figure 8 (continuation)



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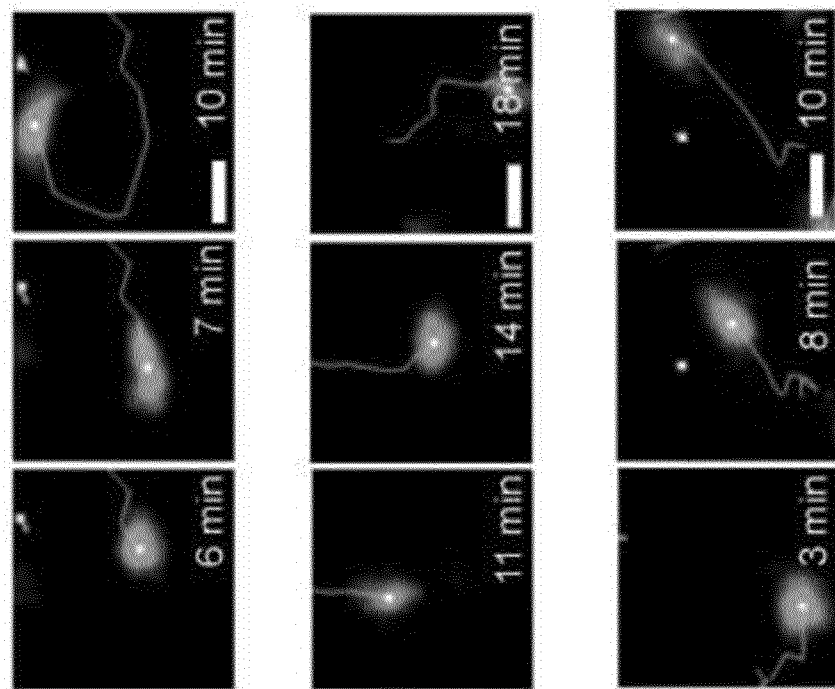
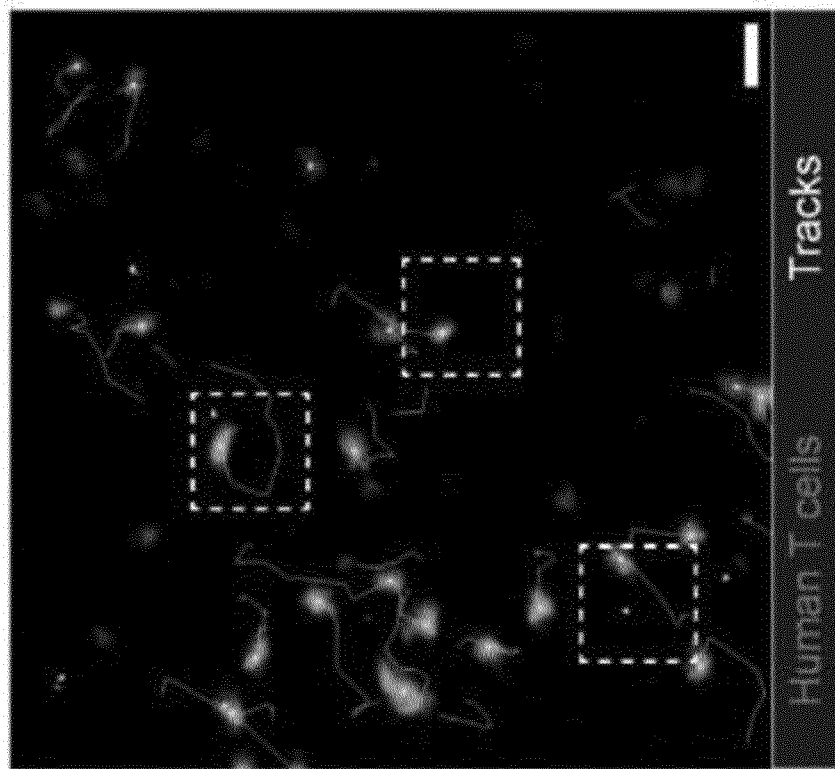
Figure 8 (continuation)



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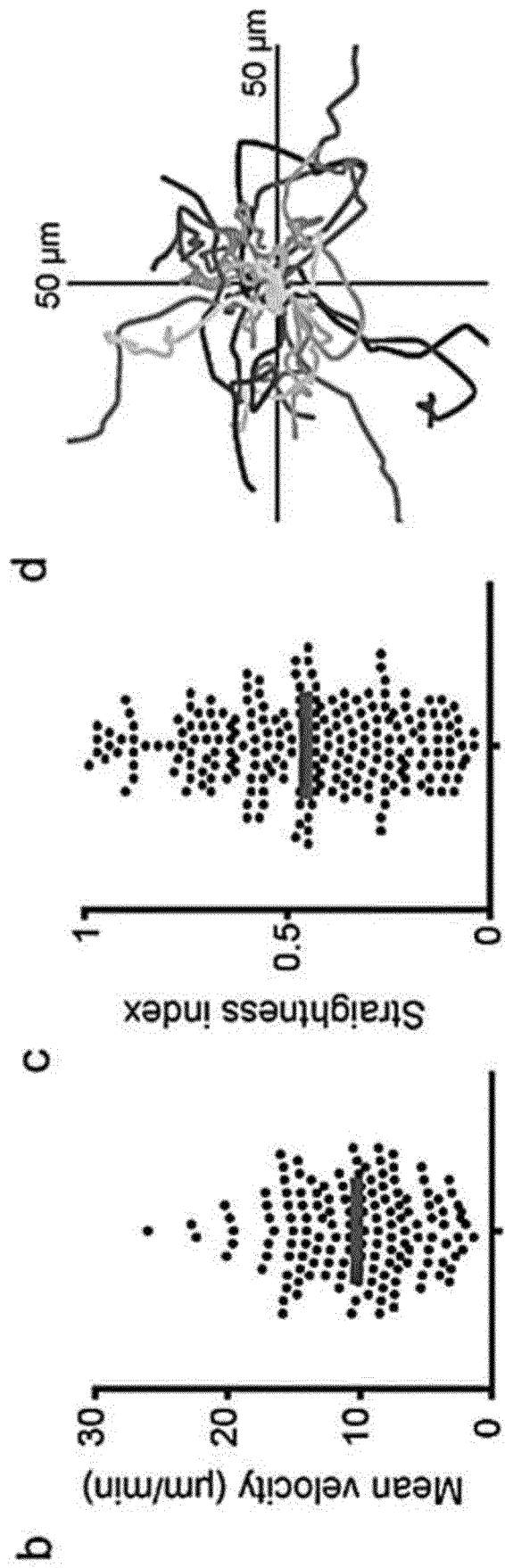
Figure 9

a



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Figure 9 (continuation)



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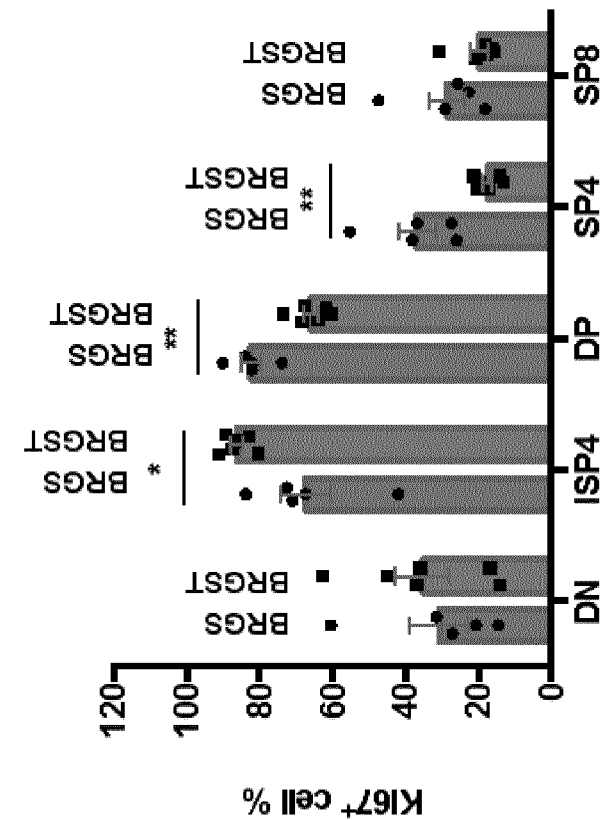
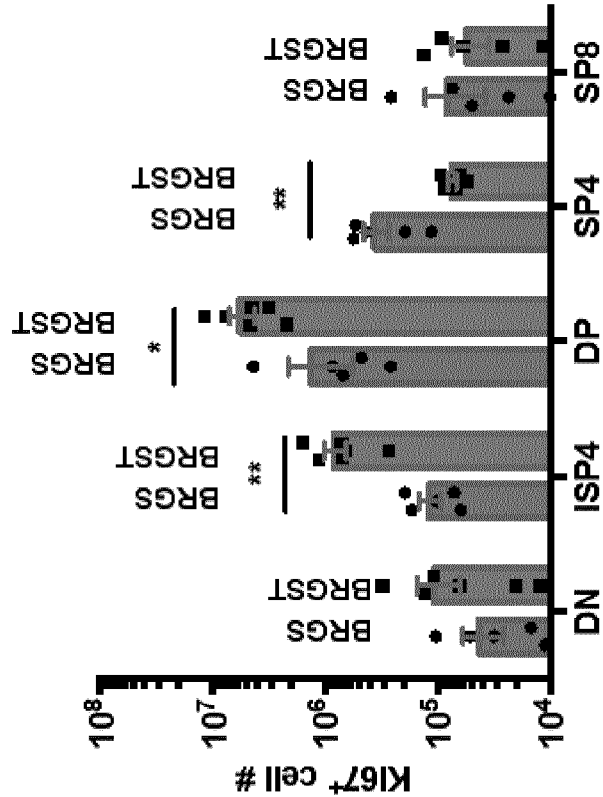


Figure 10

a

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Figure 10 (continuation)

b

BRGS



BRGST

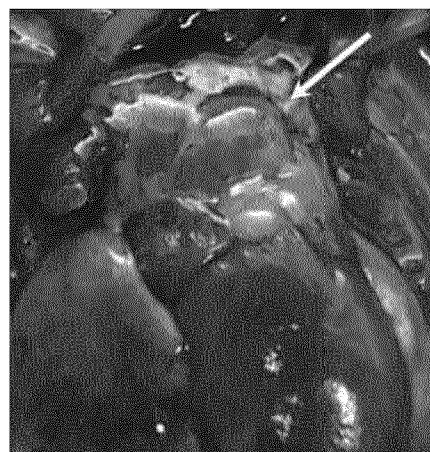
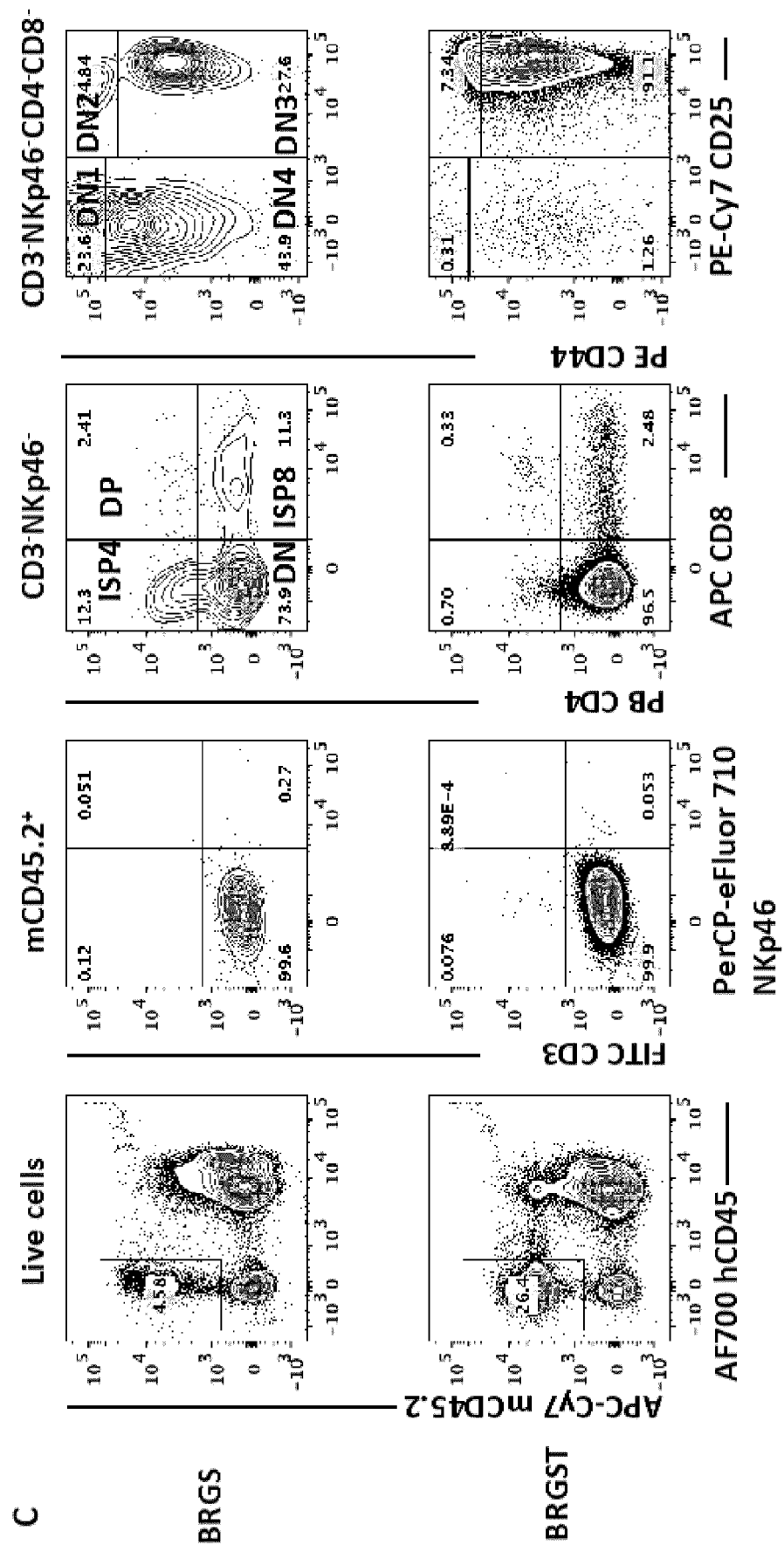


Figure 10 (continuation)



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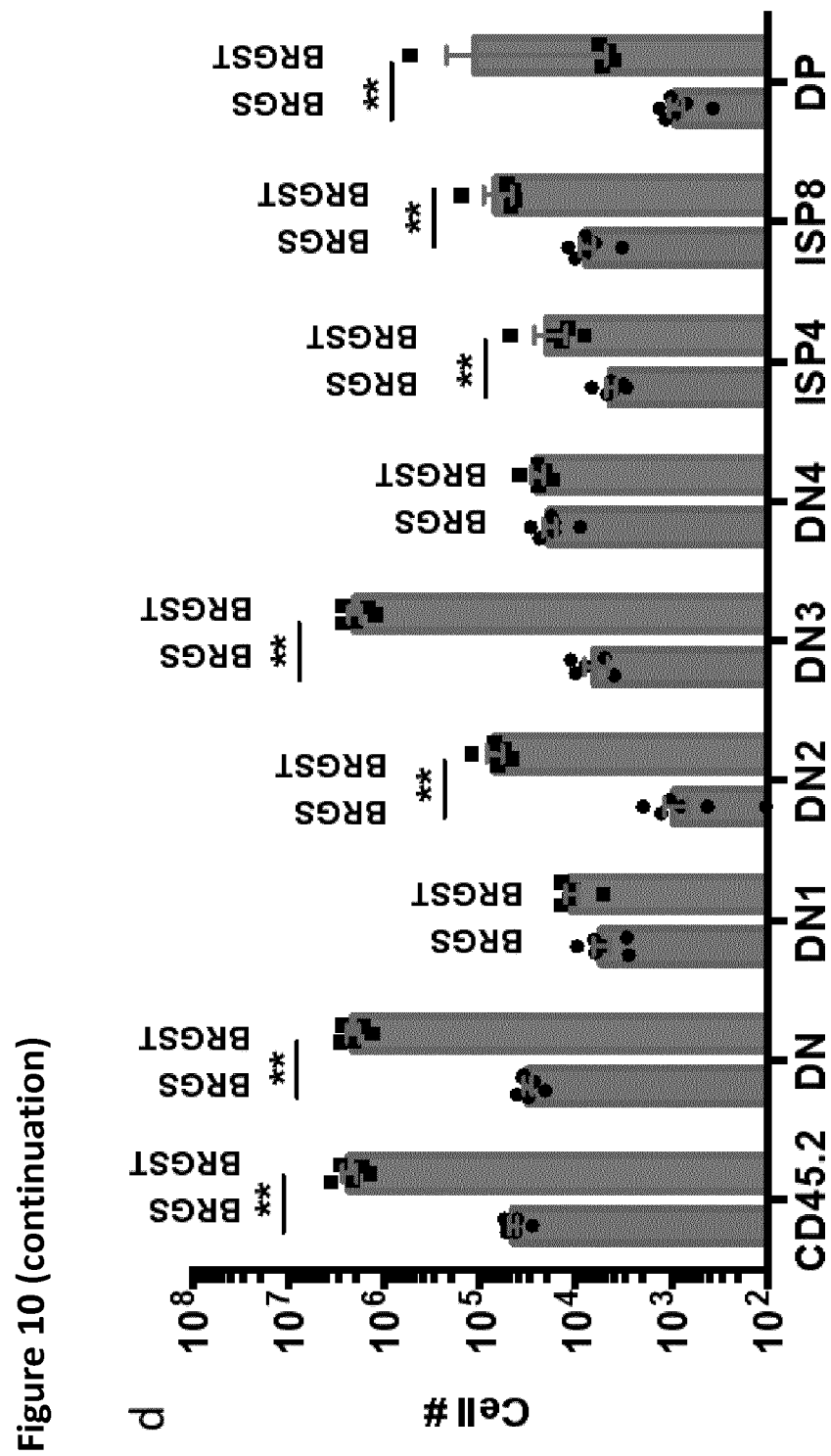
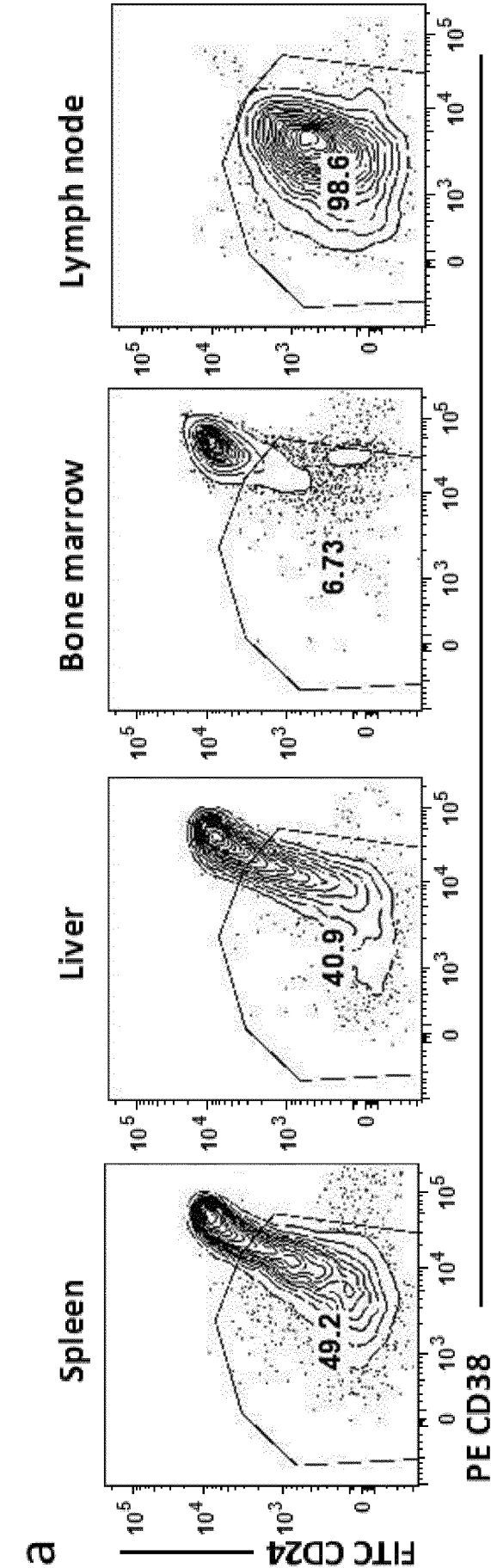
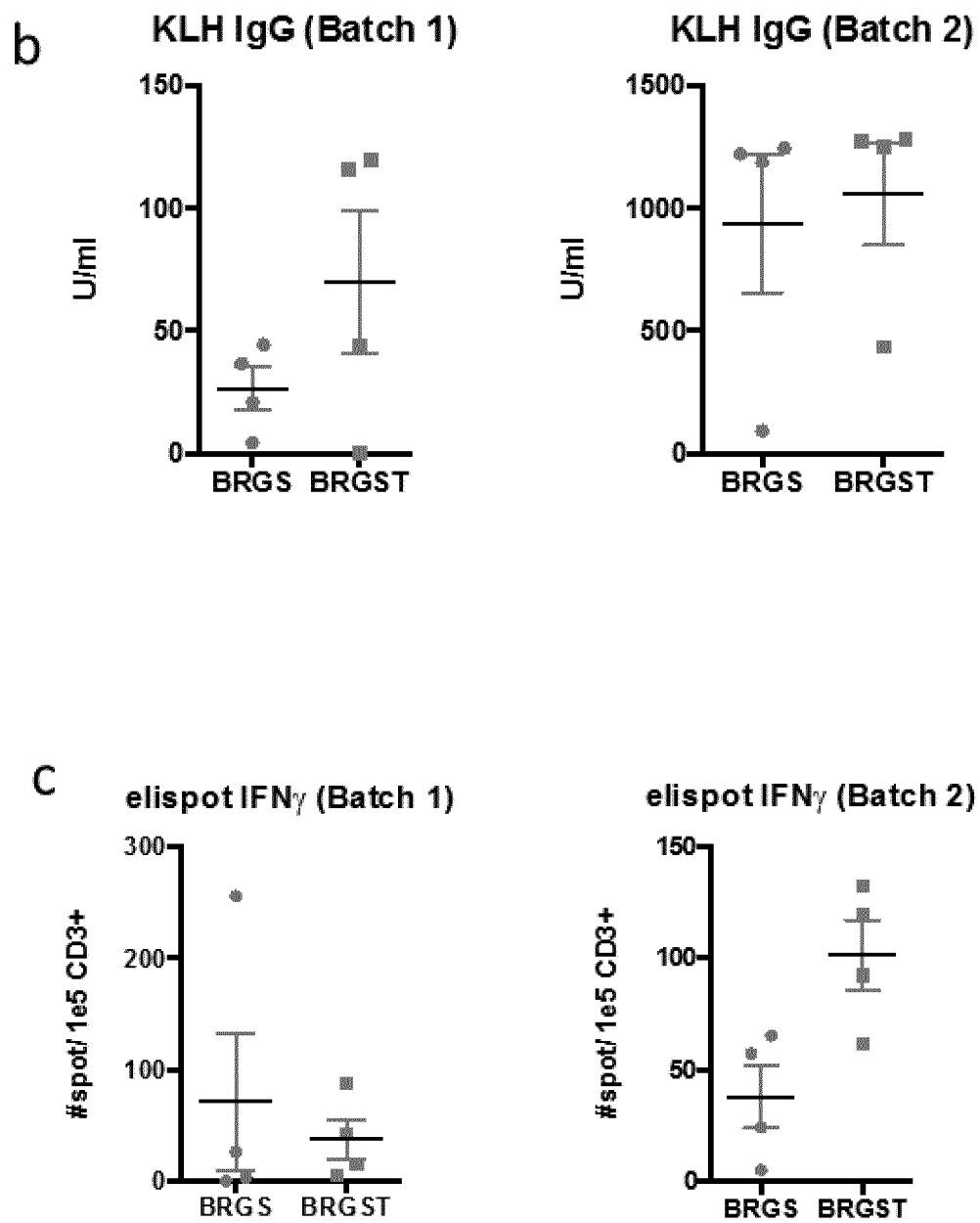


Figure 11



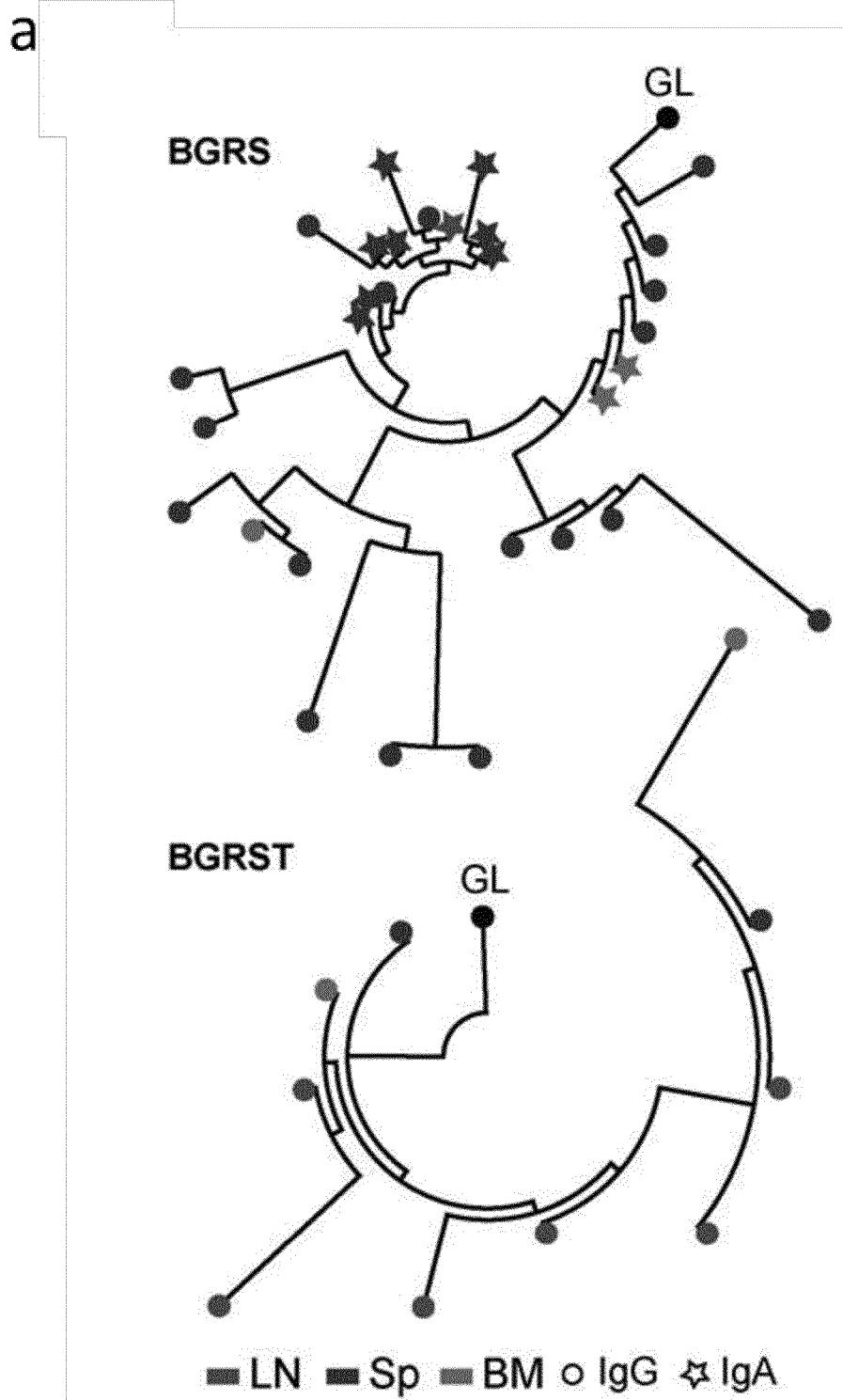
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Figure 11 (continuation)



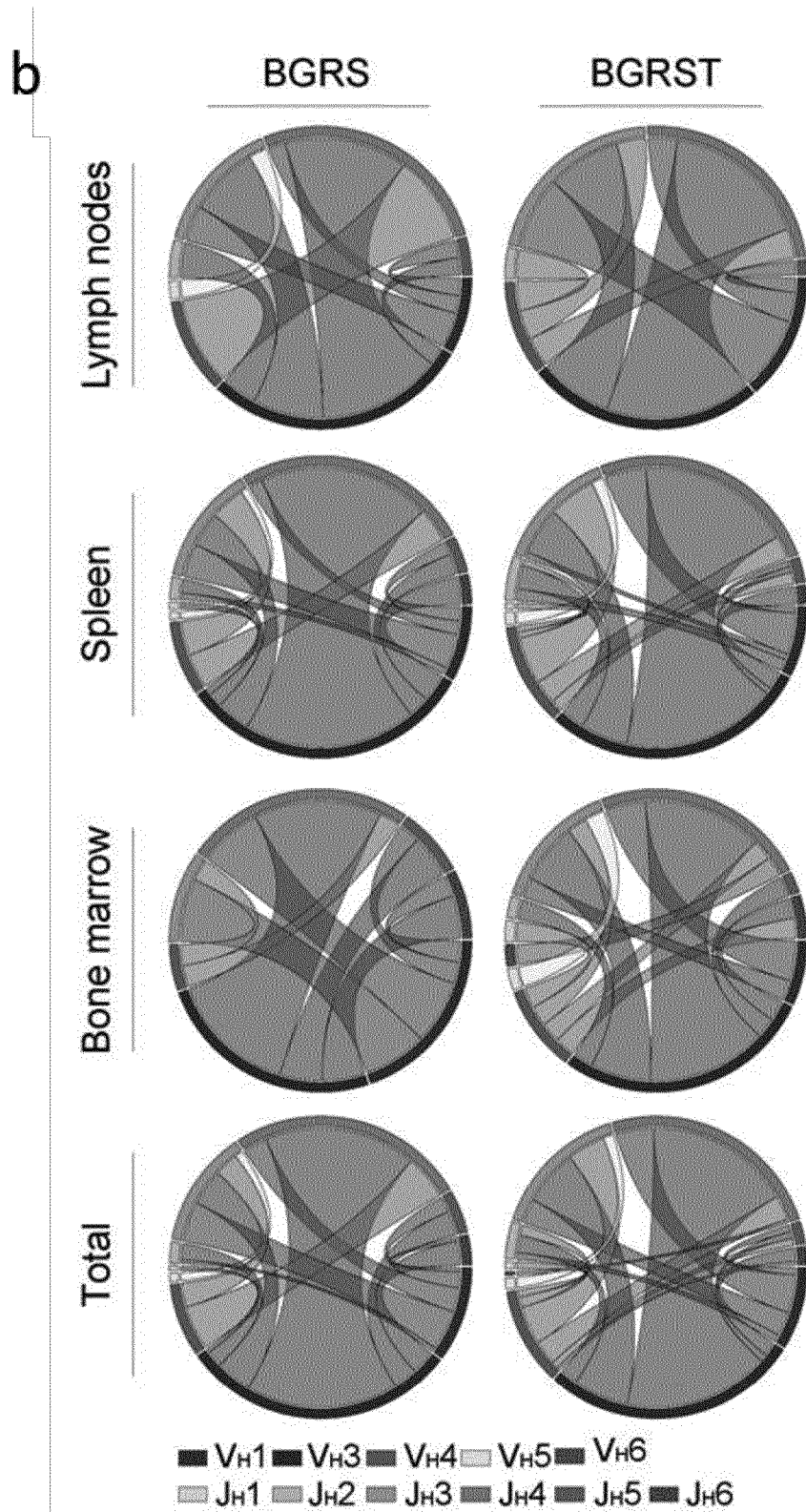
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Figure 12



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Figure 12 (continuation)



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Figure 12 (continuation)

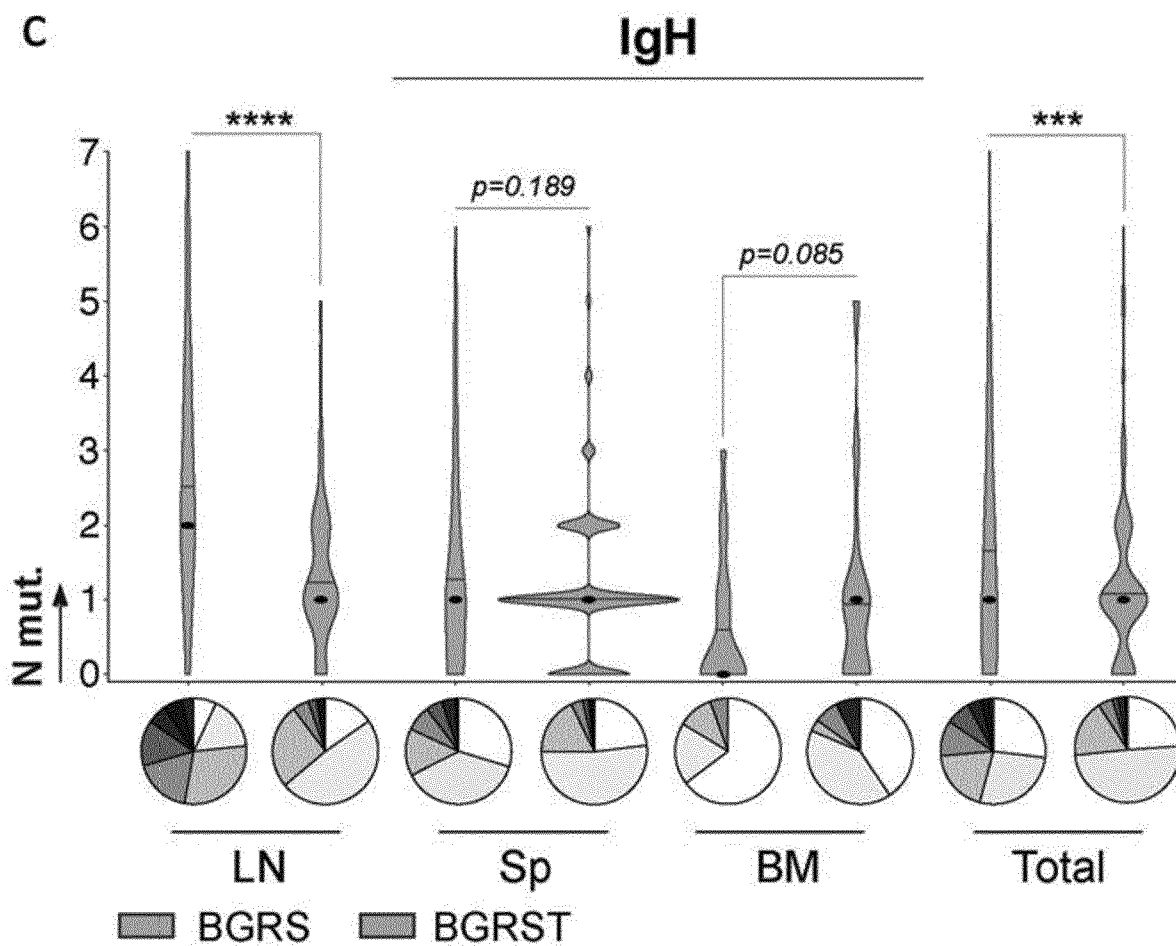
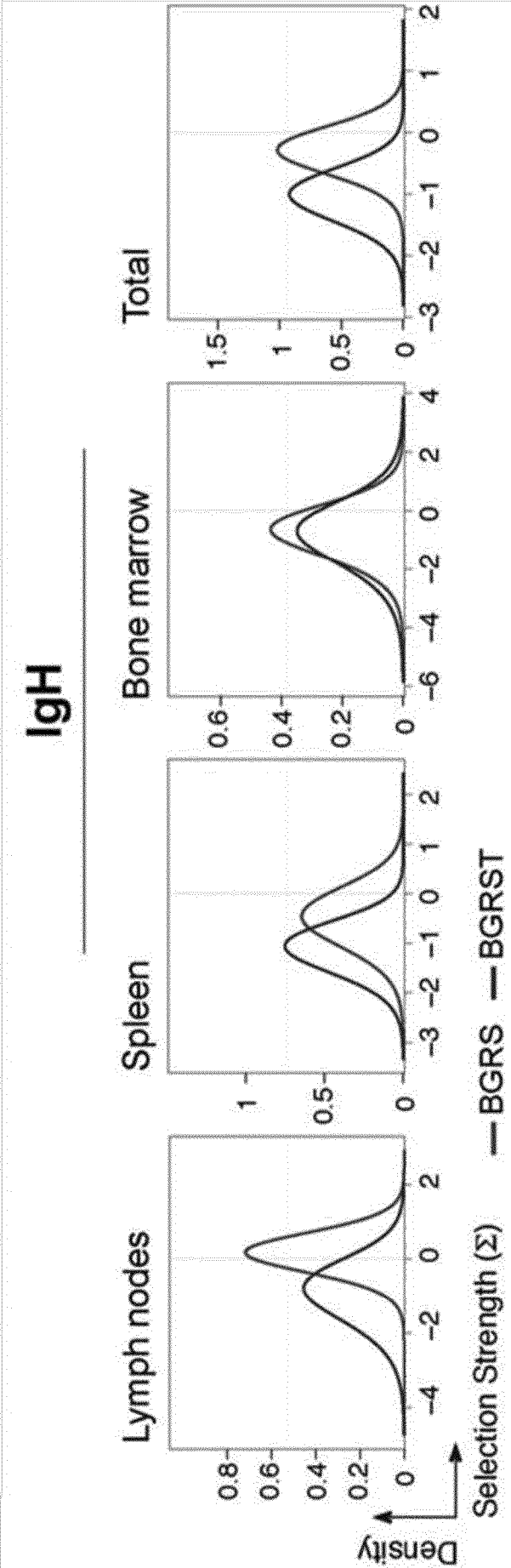


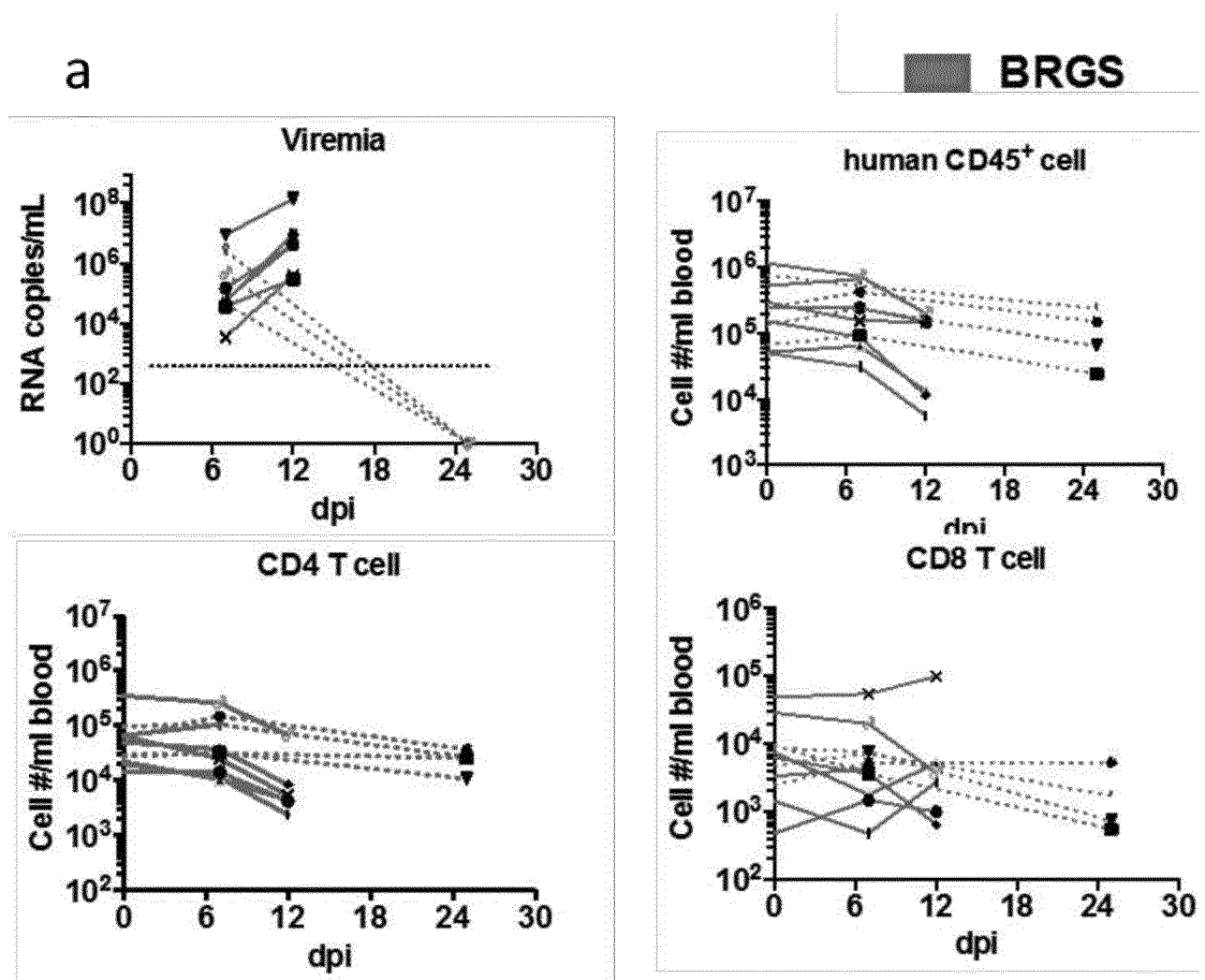
Figure 12 (continuation)

d



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Figure 13



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Figure 13 (continuation)

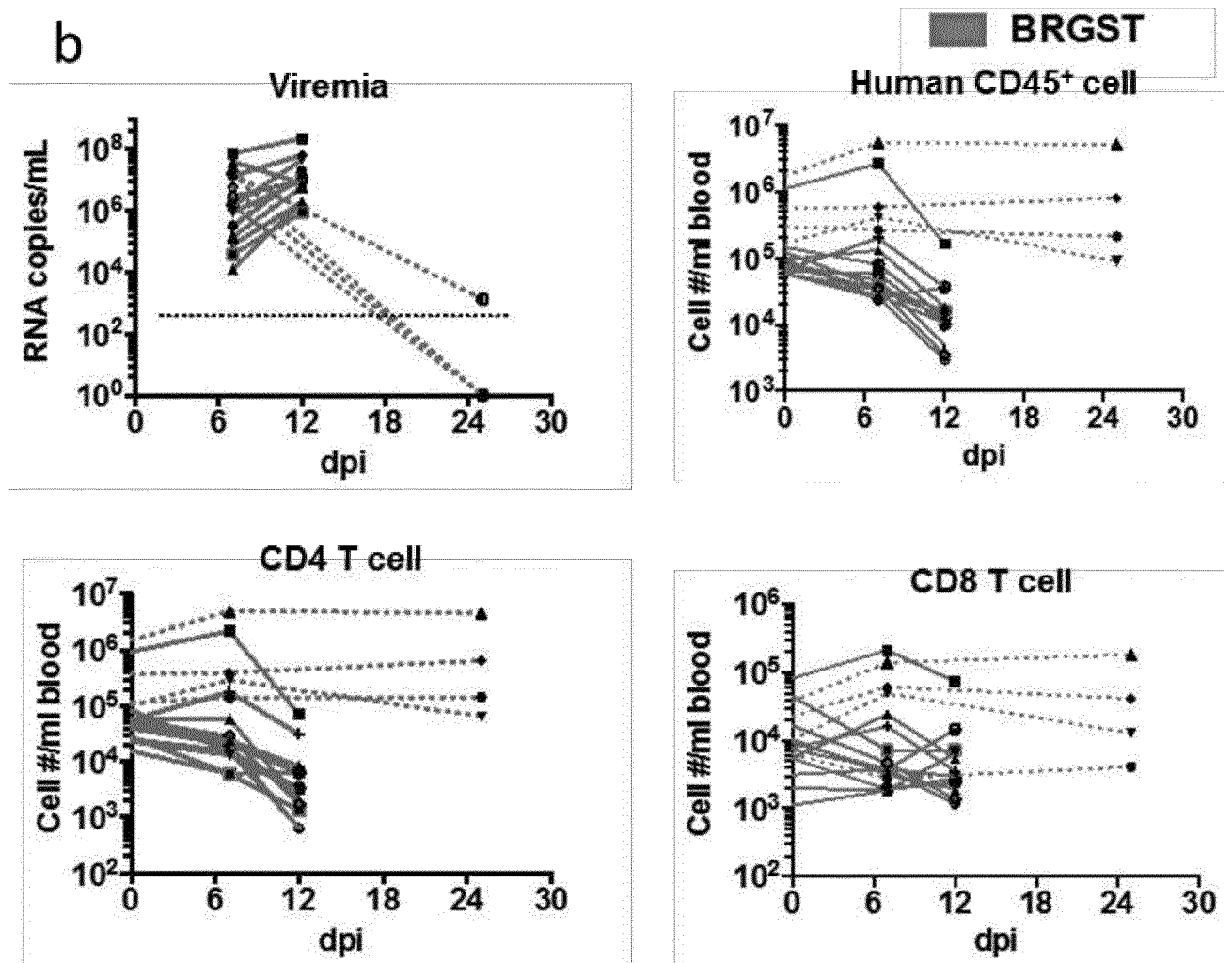
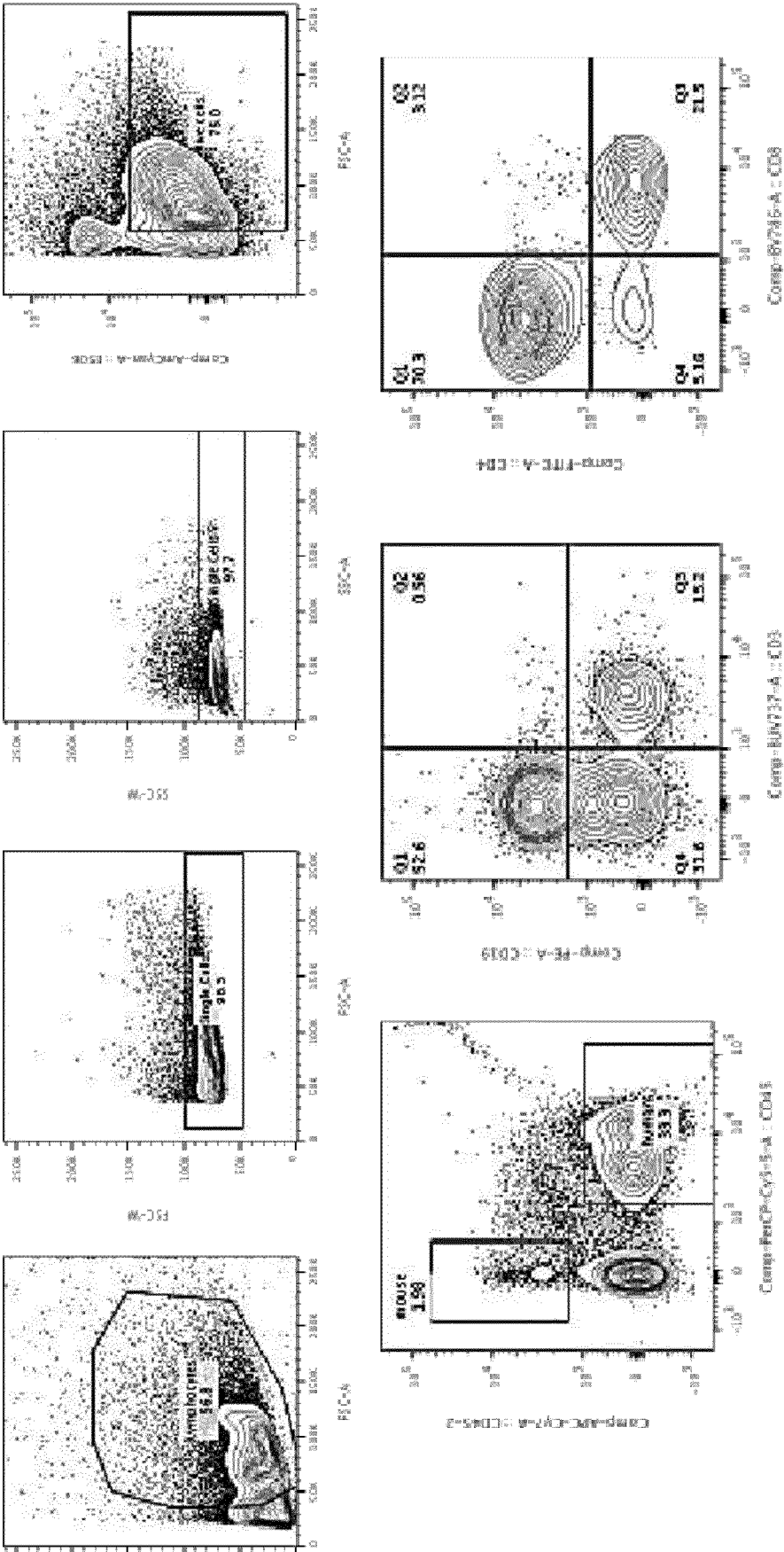


Figure 14

a

Lineage positive fraction



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Figure 14 (continuation)

a

Lineage negative fraction for untouched T cell selection

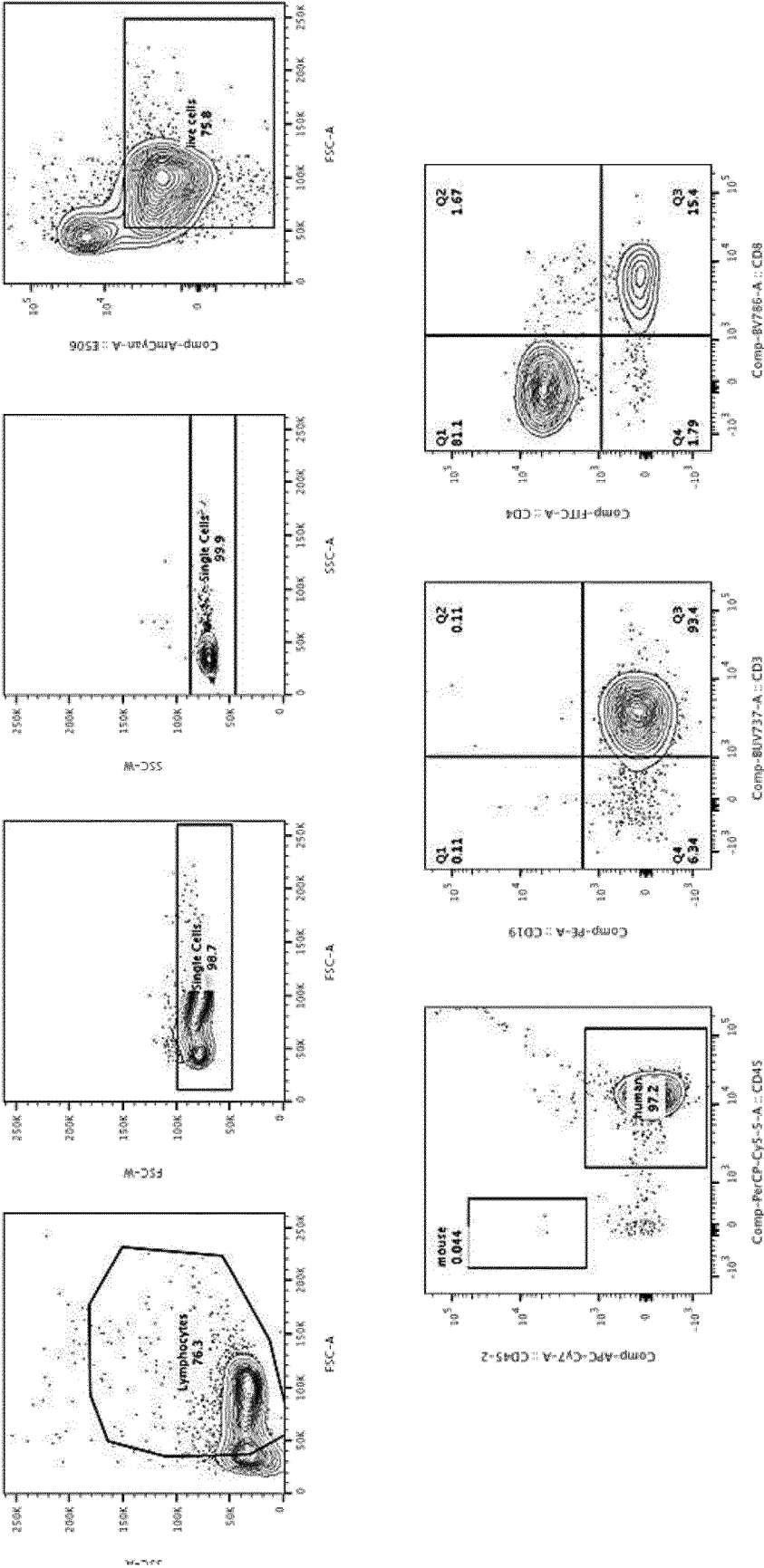
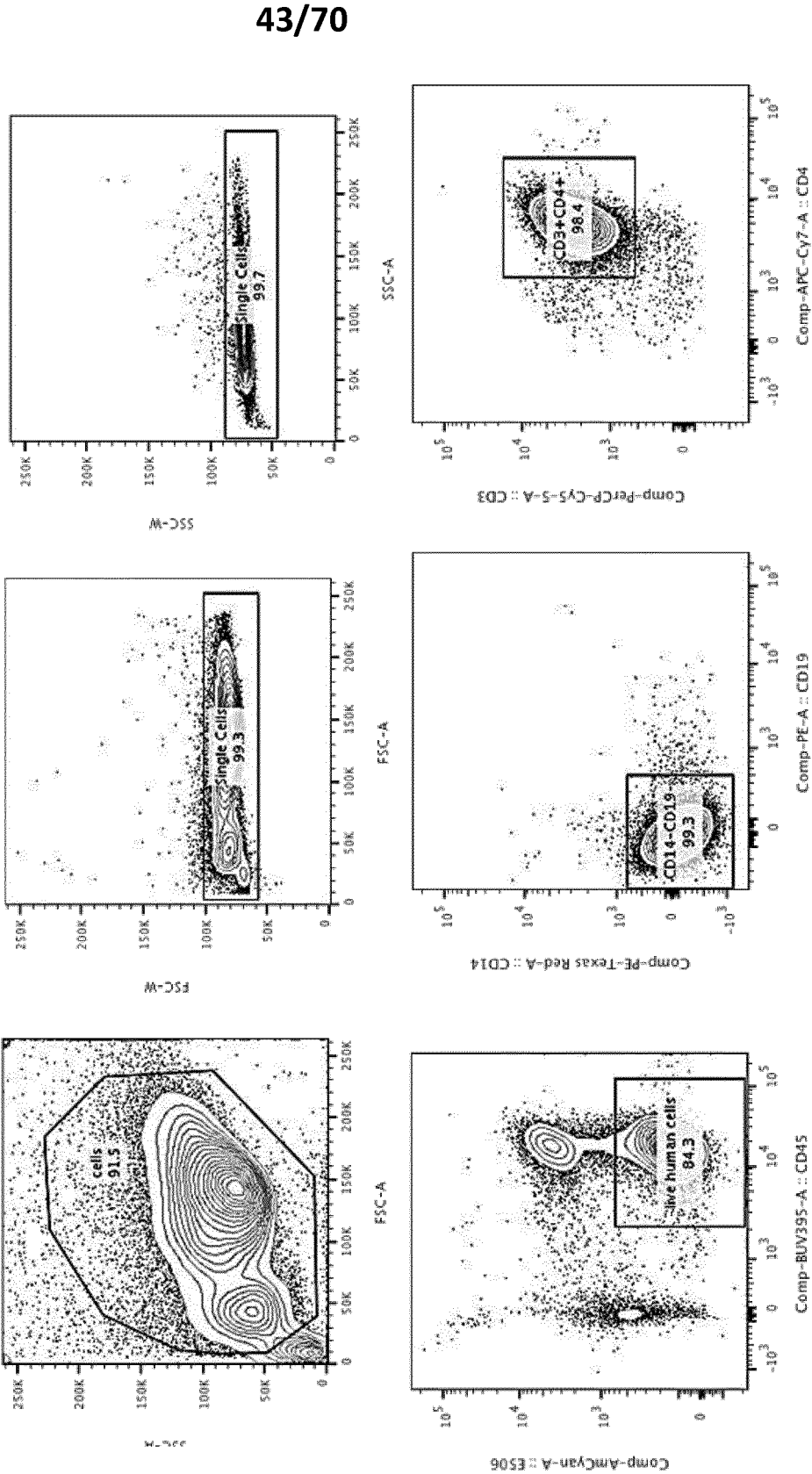


Figure 14 (continuation)

b

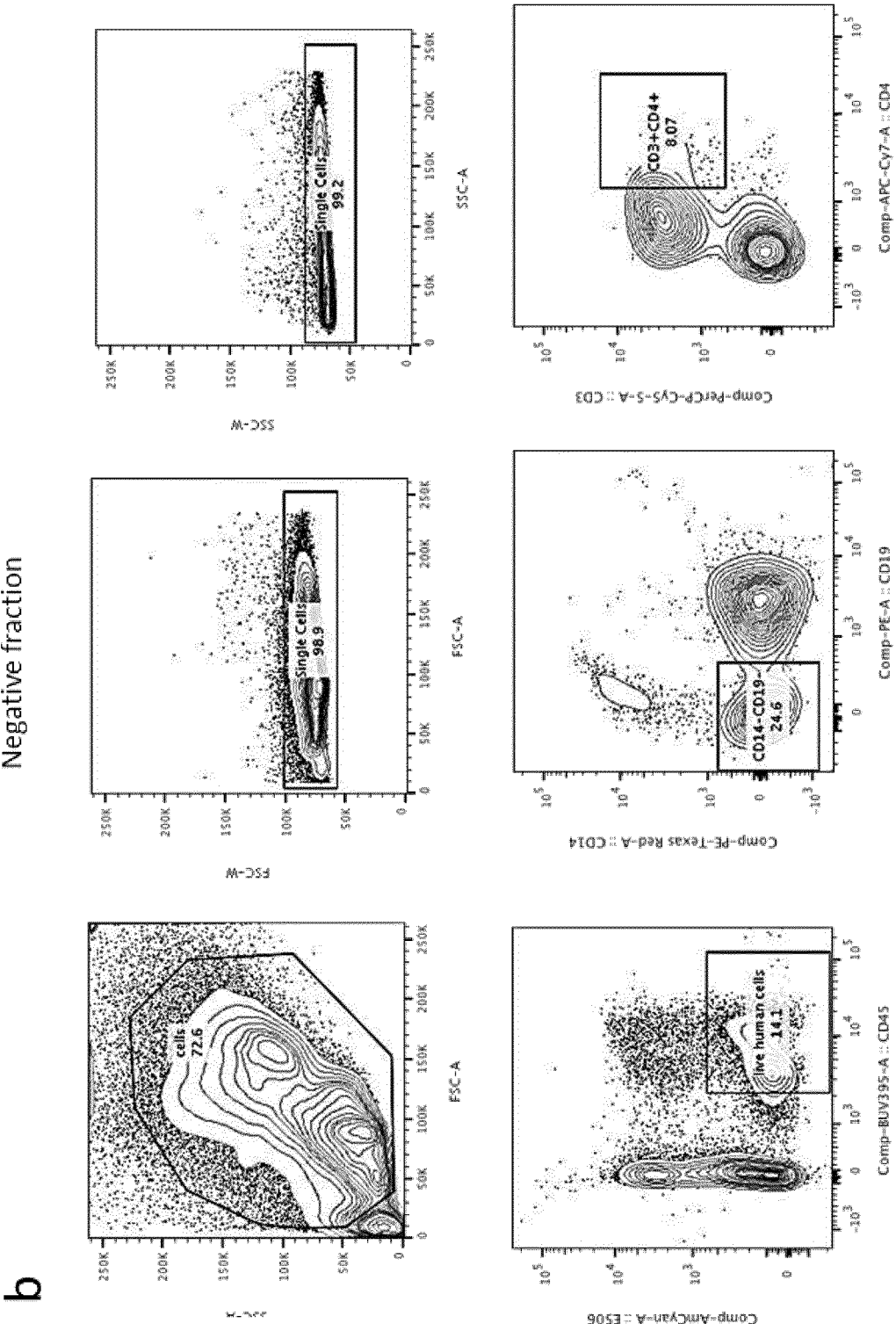
Positive fraction for CD4 selection



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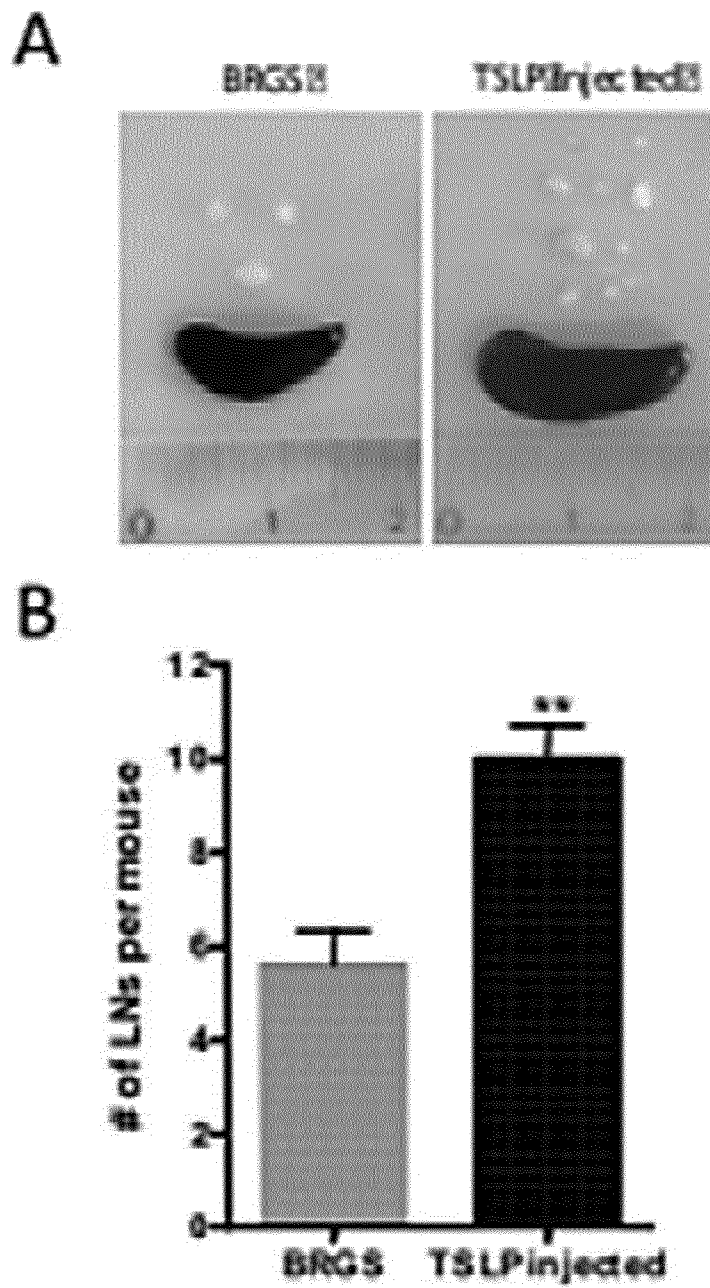
Figure 14 (continuation)

b



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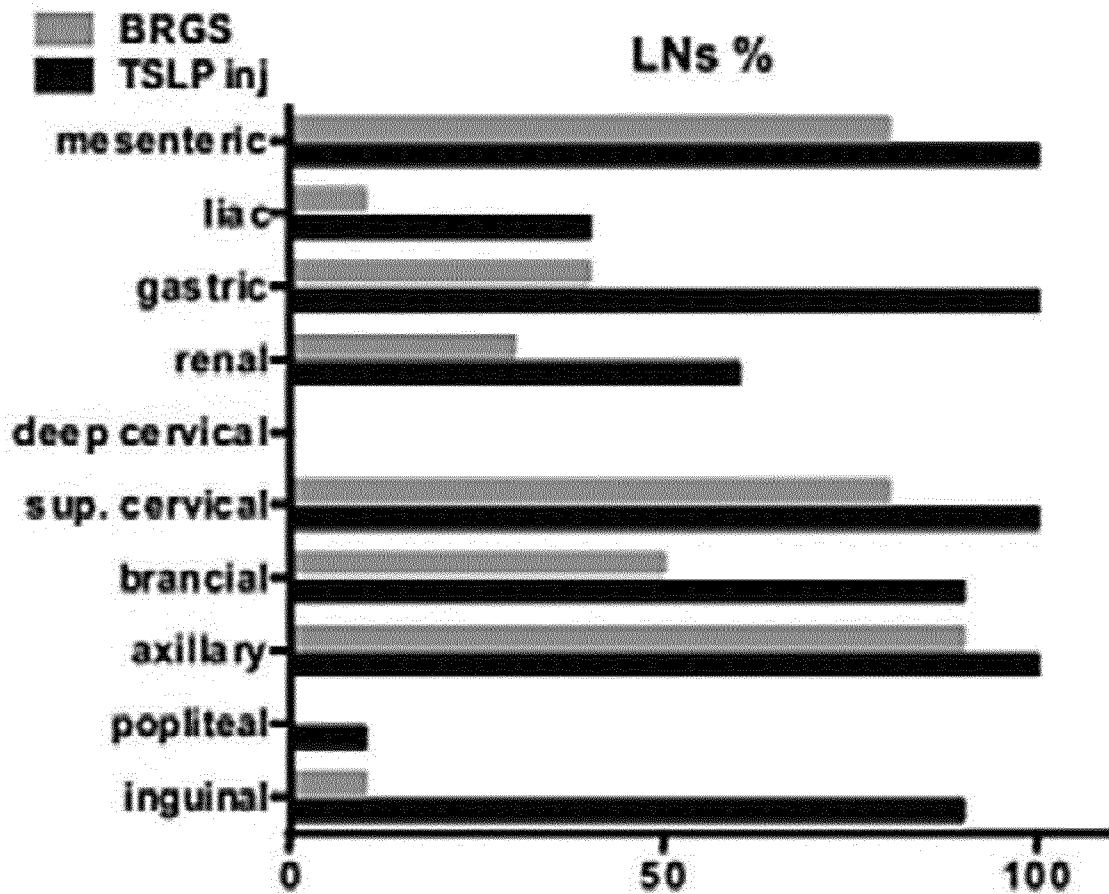
Figure 15



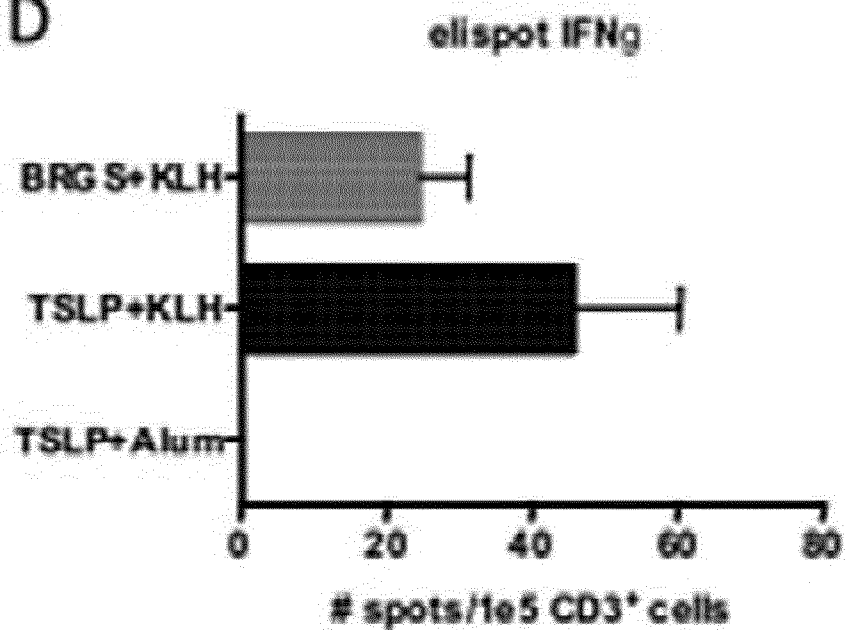
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Figure 15 (continuation)

C

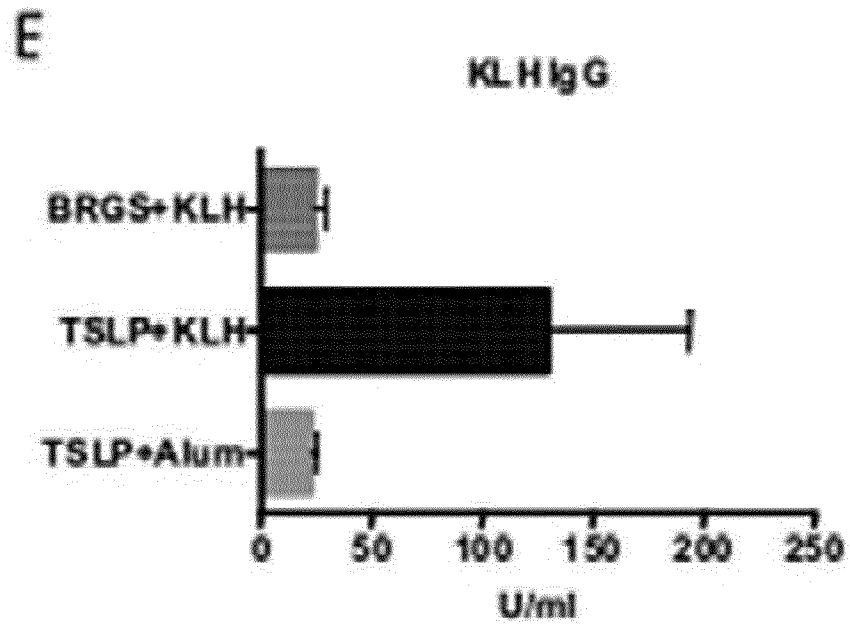


D



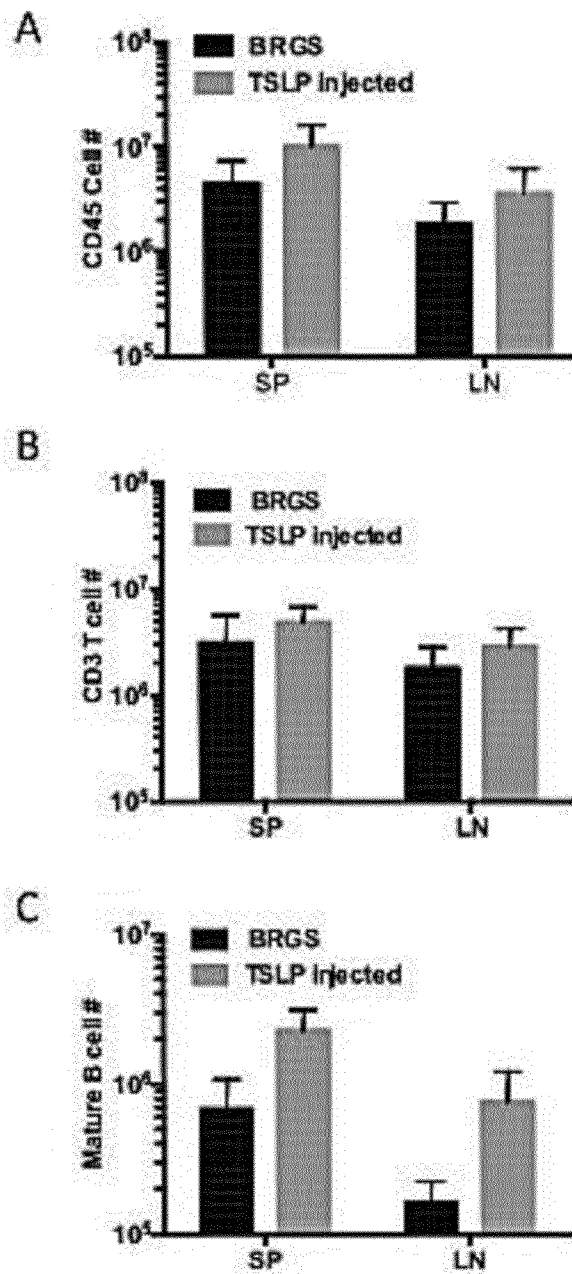
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Figure 15 (continuation)



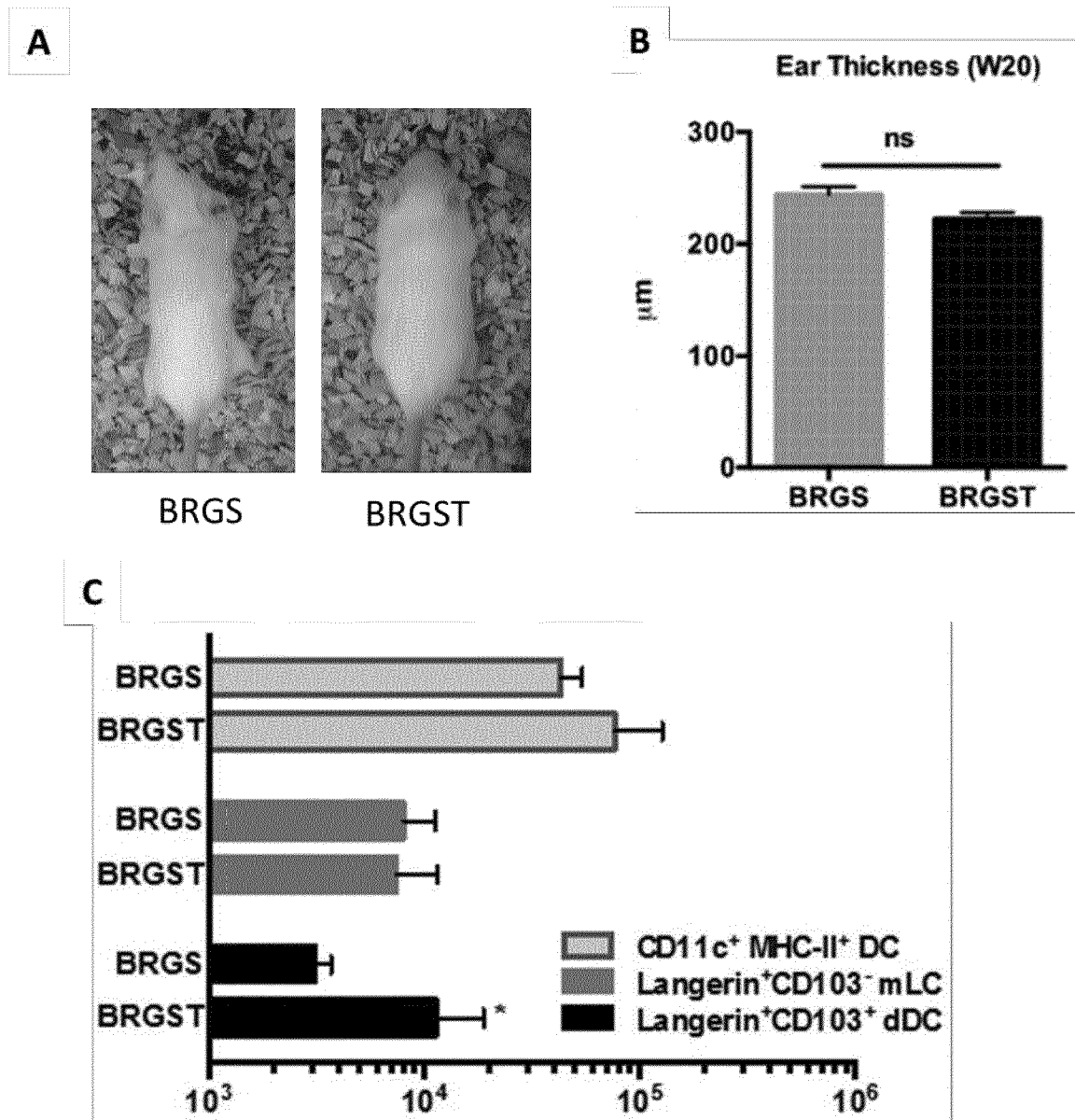
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Figure 16



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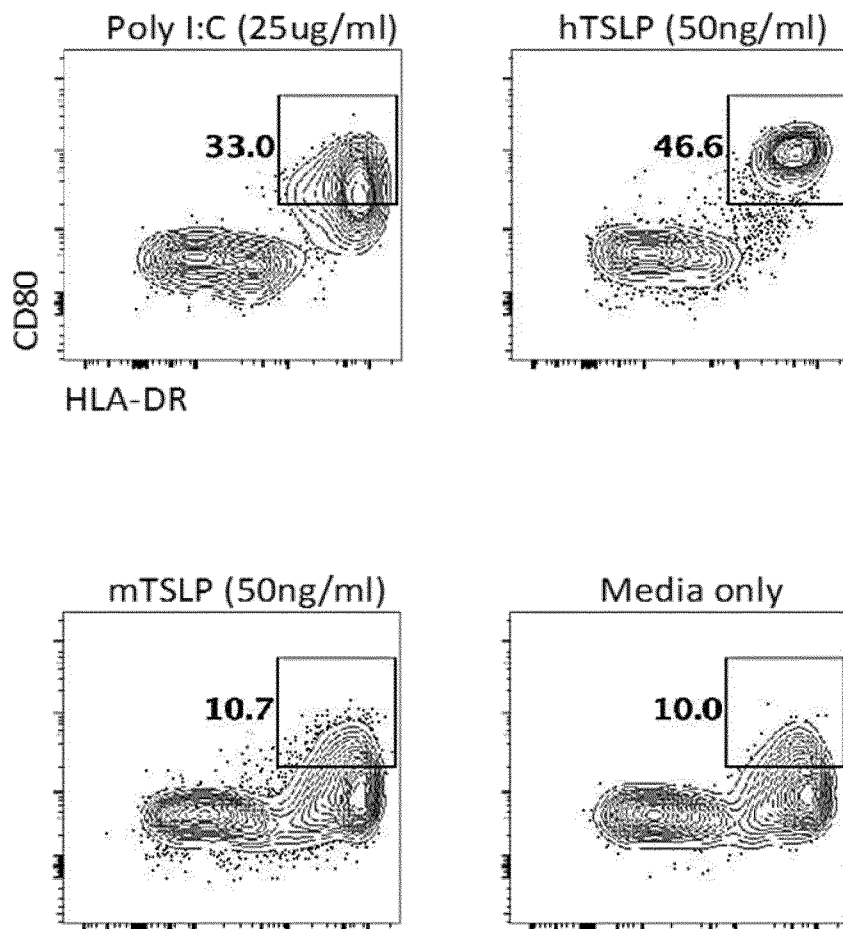
Figure 17



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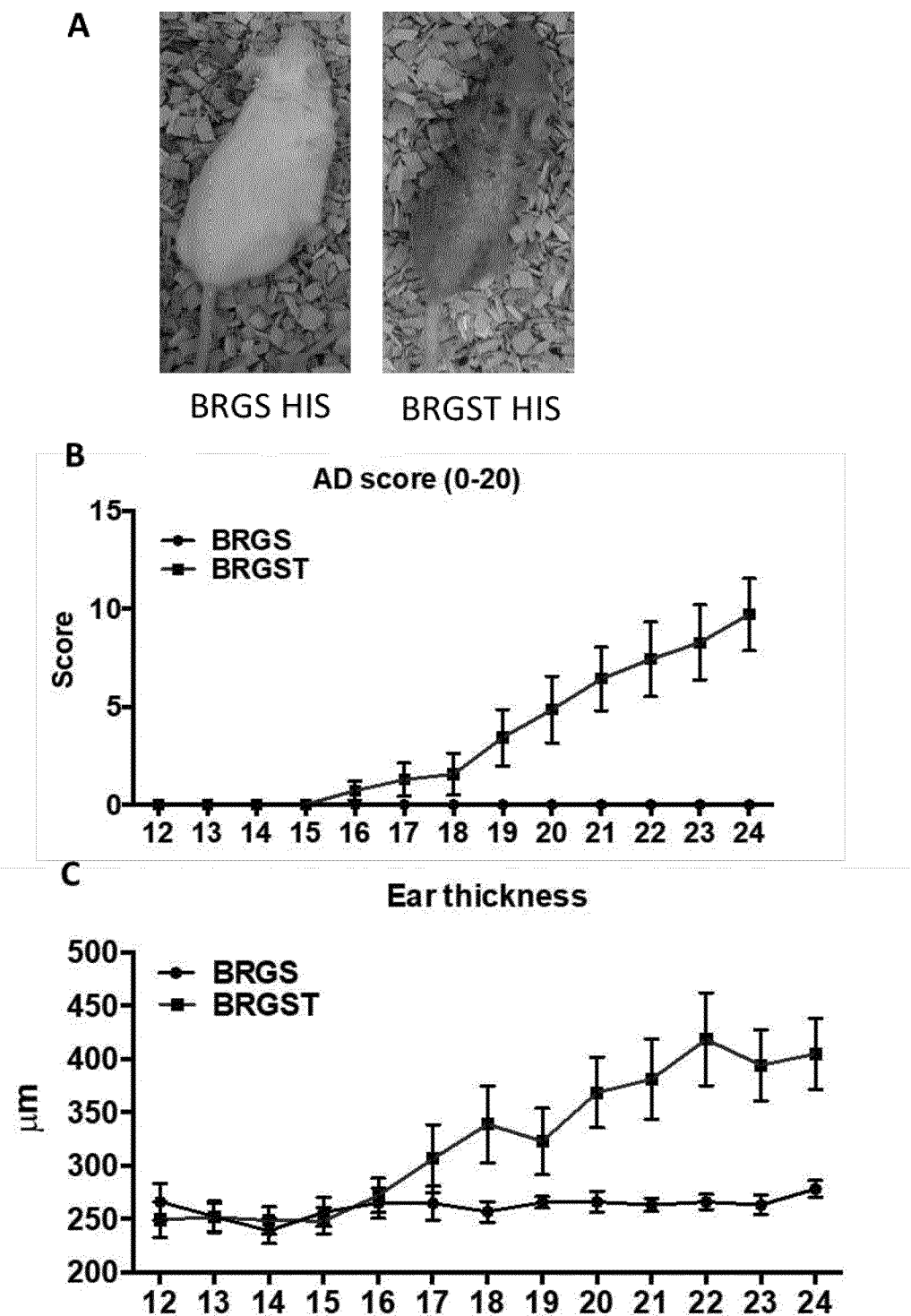
Figure 17 (continuation)

D



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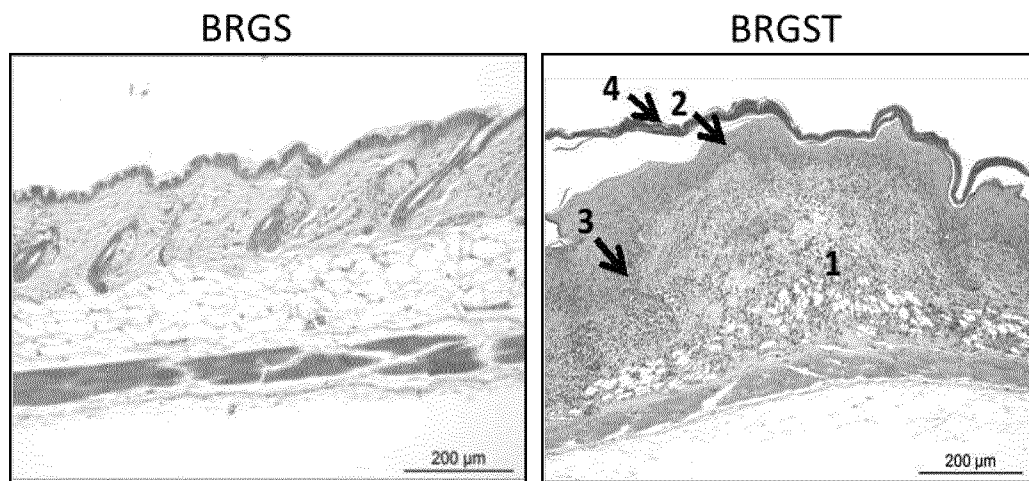
Figure 18



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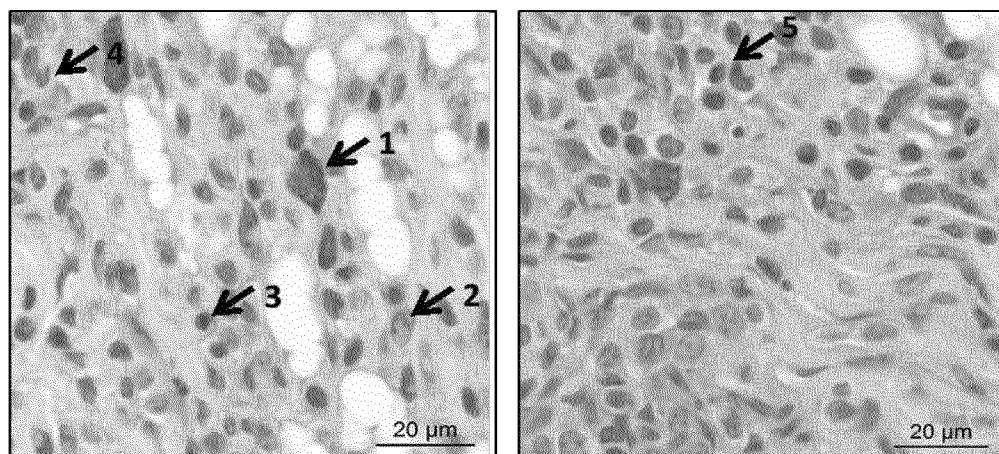
Figure 19

A



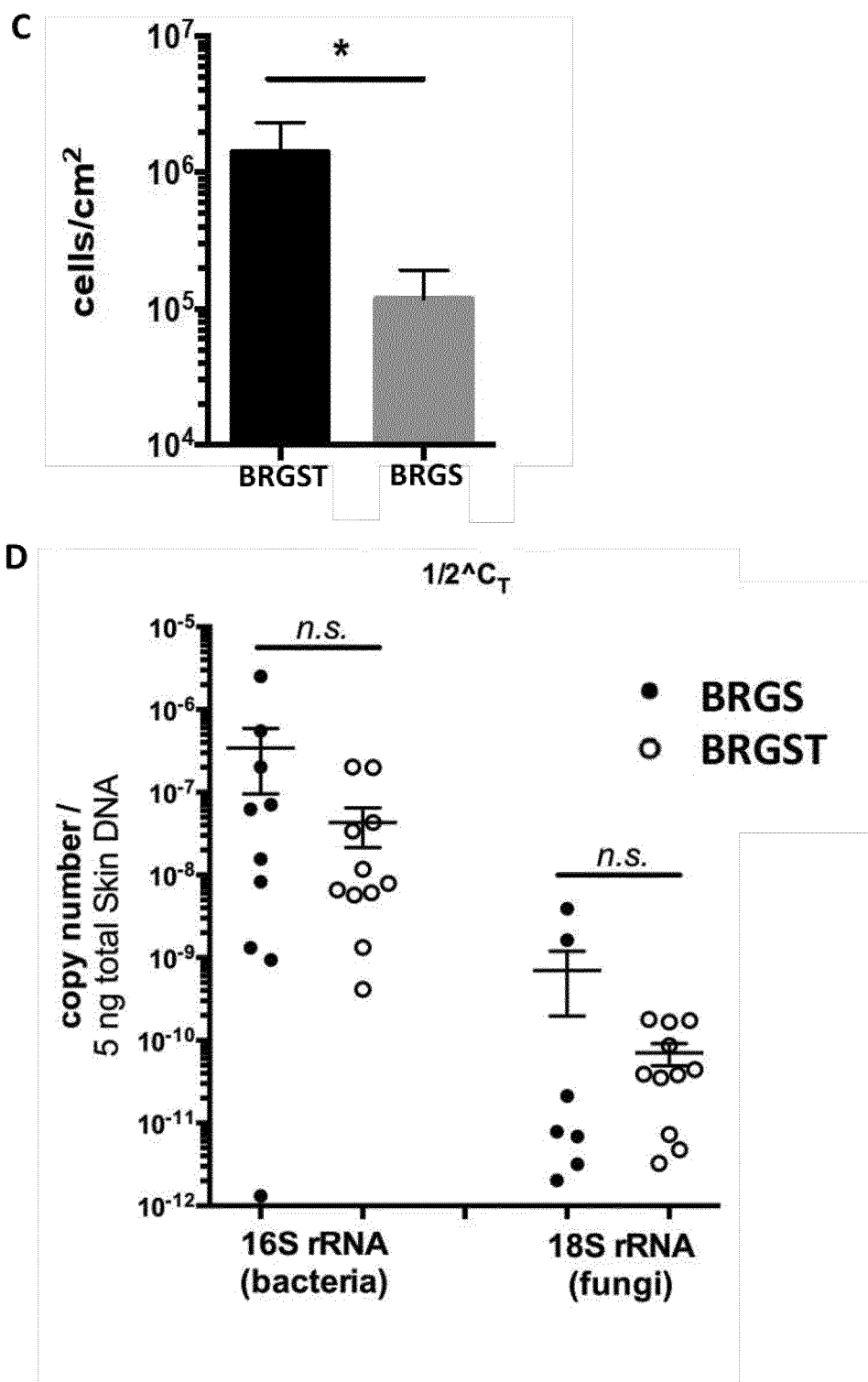
B

BRGST



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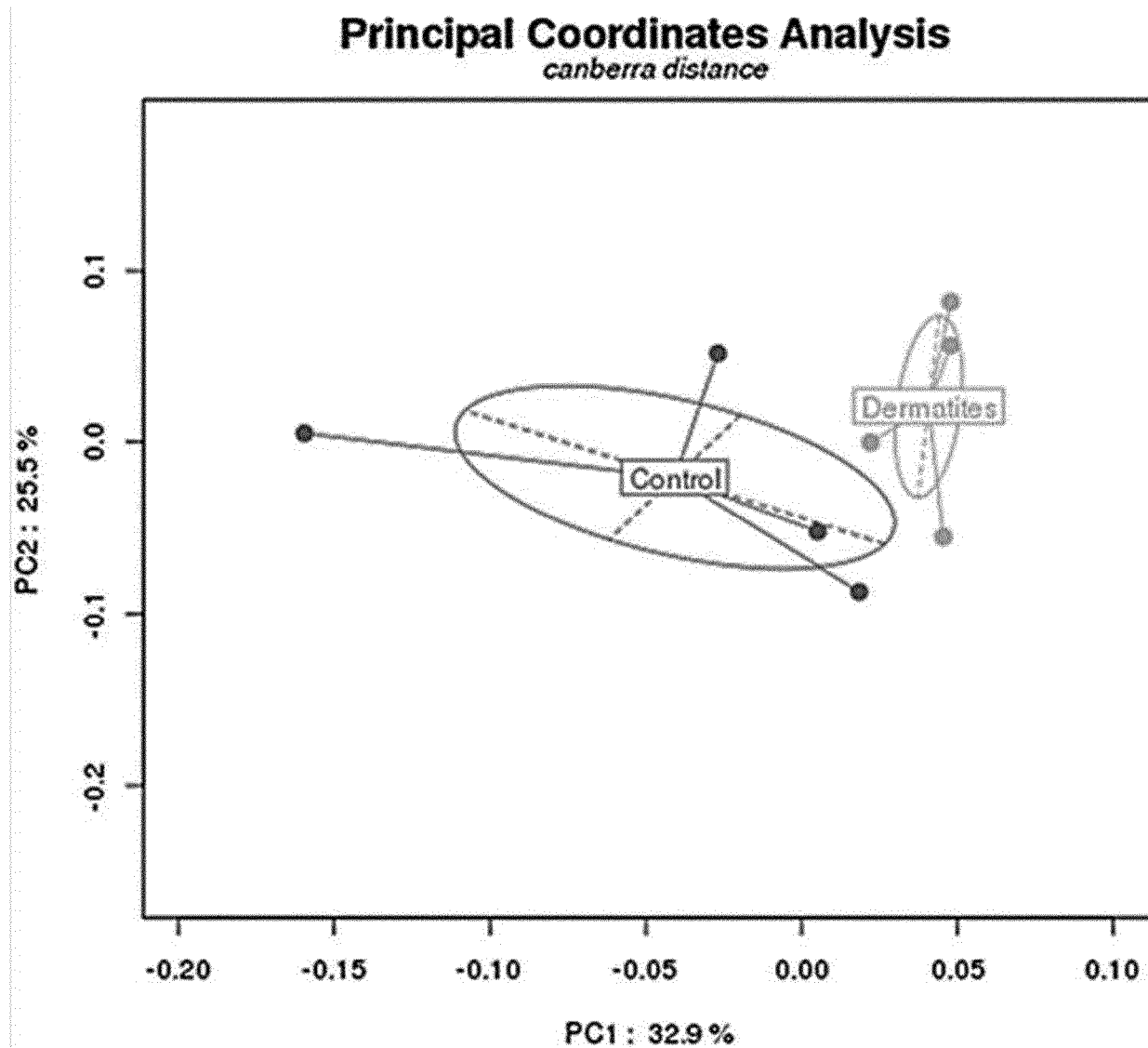
Figure 19 (continuation)



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Figure 19 (continuation)

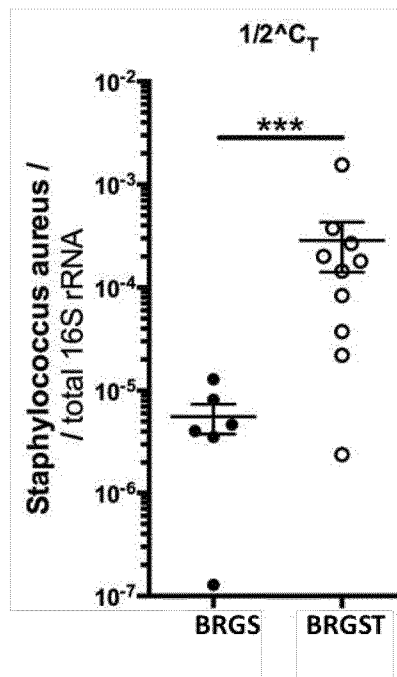
E



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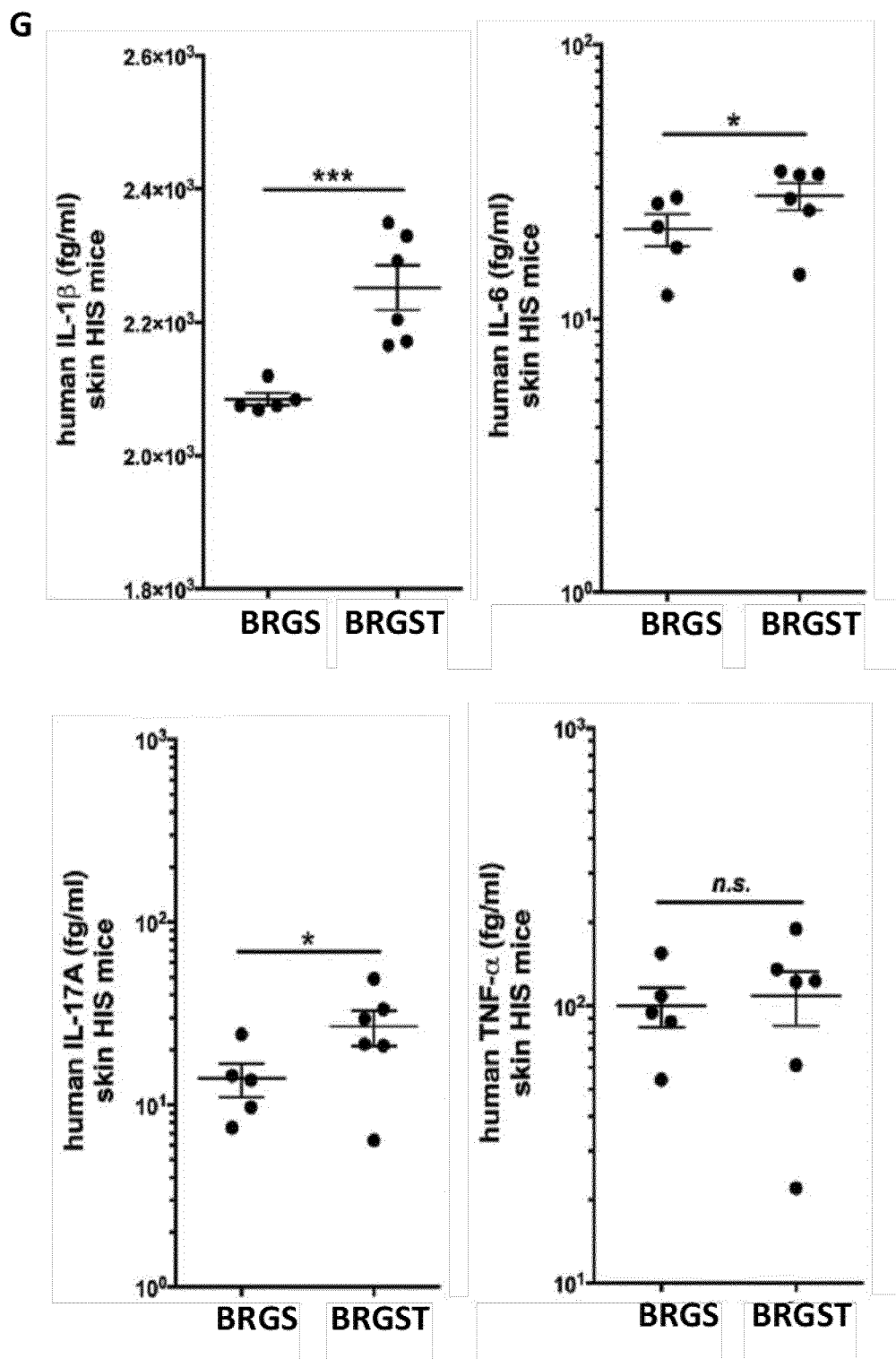
Figure 19 (continuation)

F



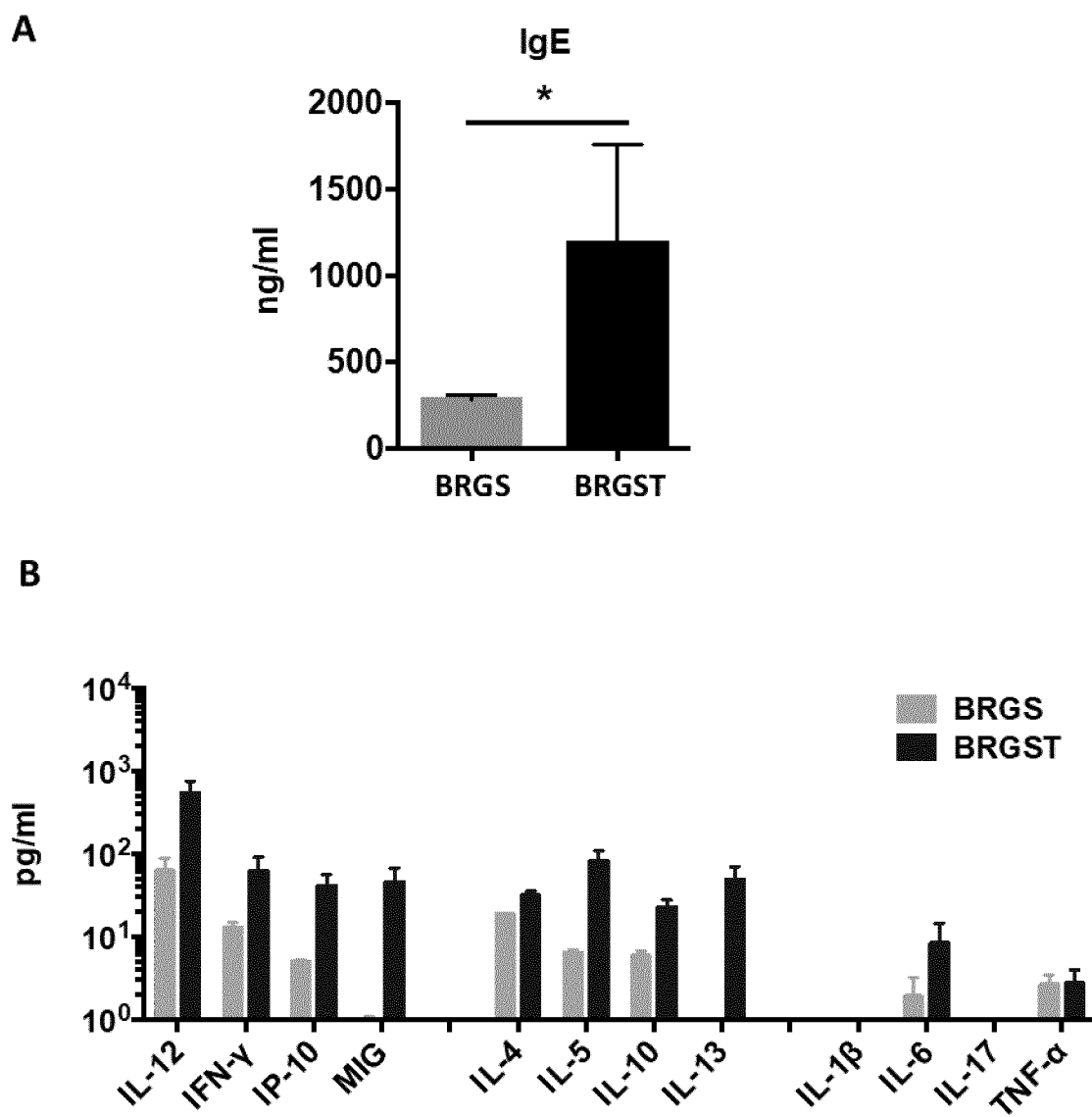
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Figure 19 (continuation)



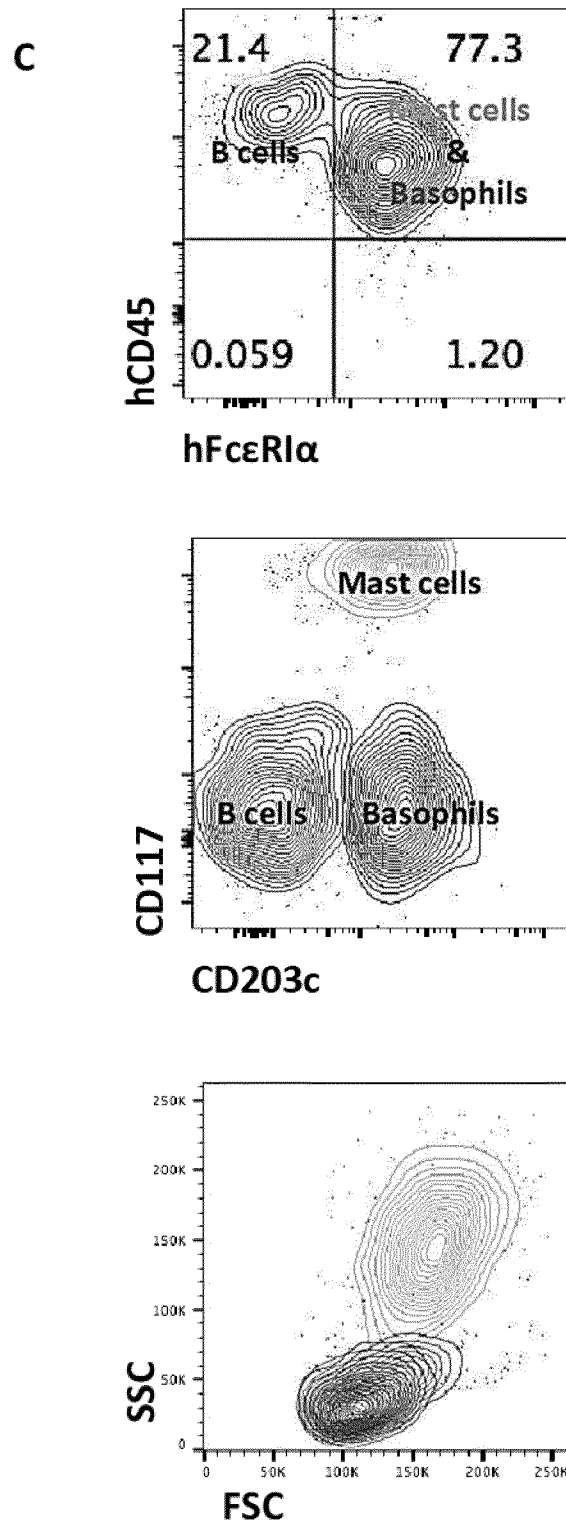
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Figure 20



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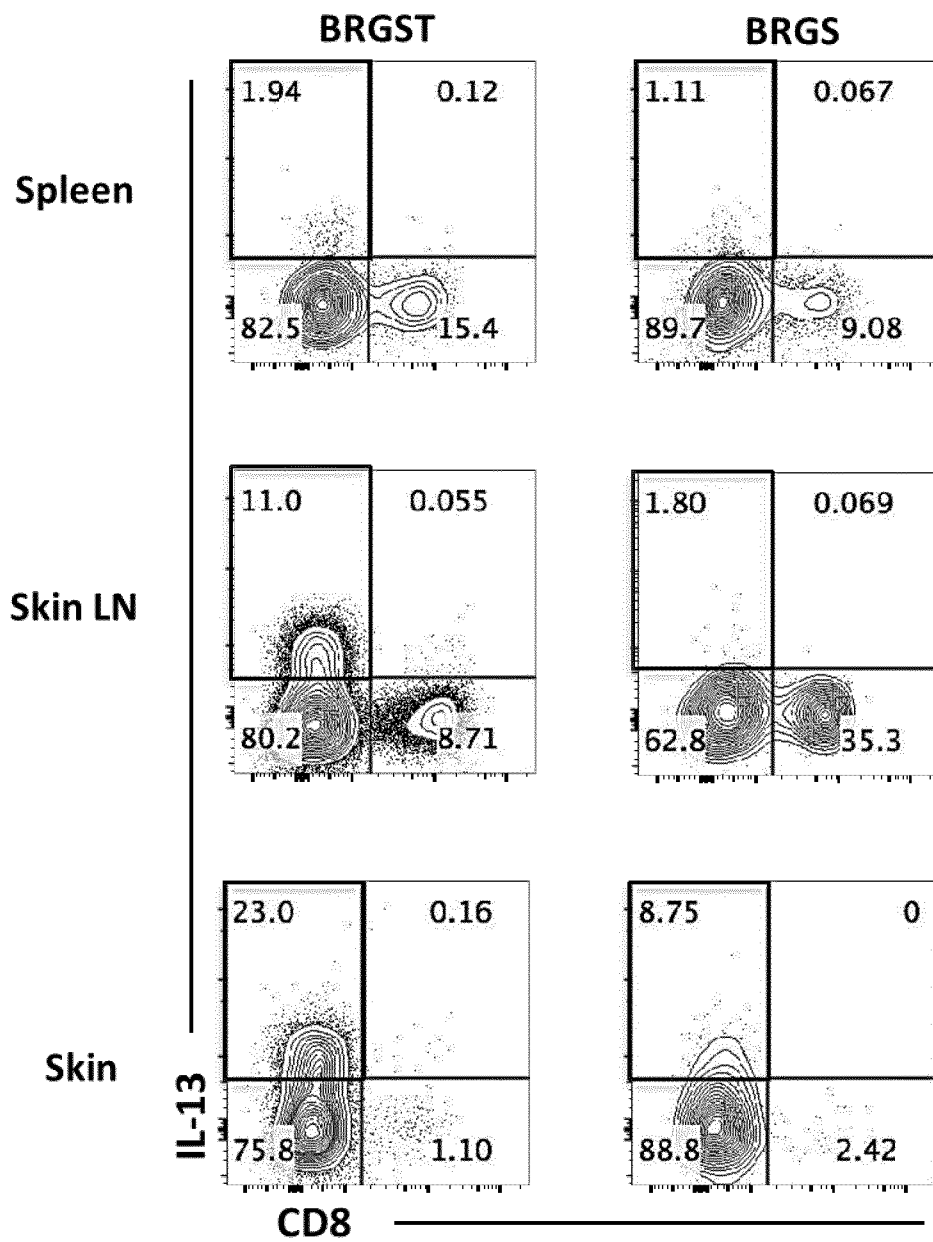
Figure 20 (continuation)



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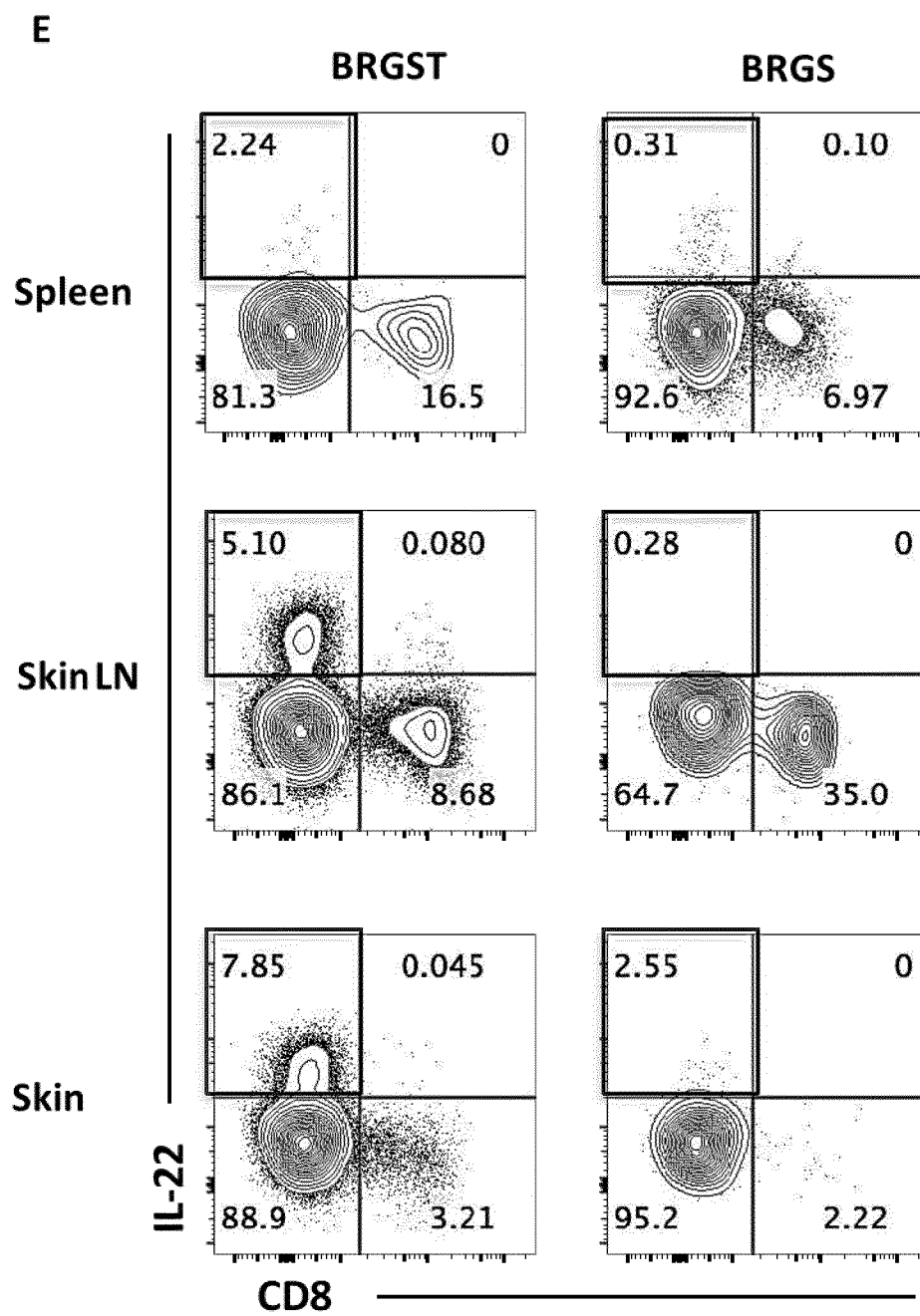
Figure 20 (continuation)

D



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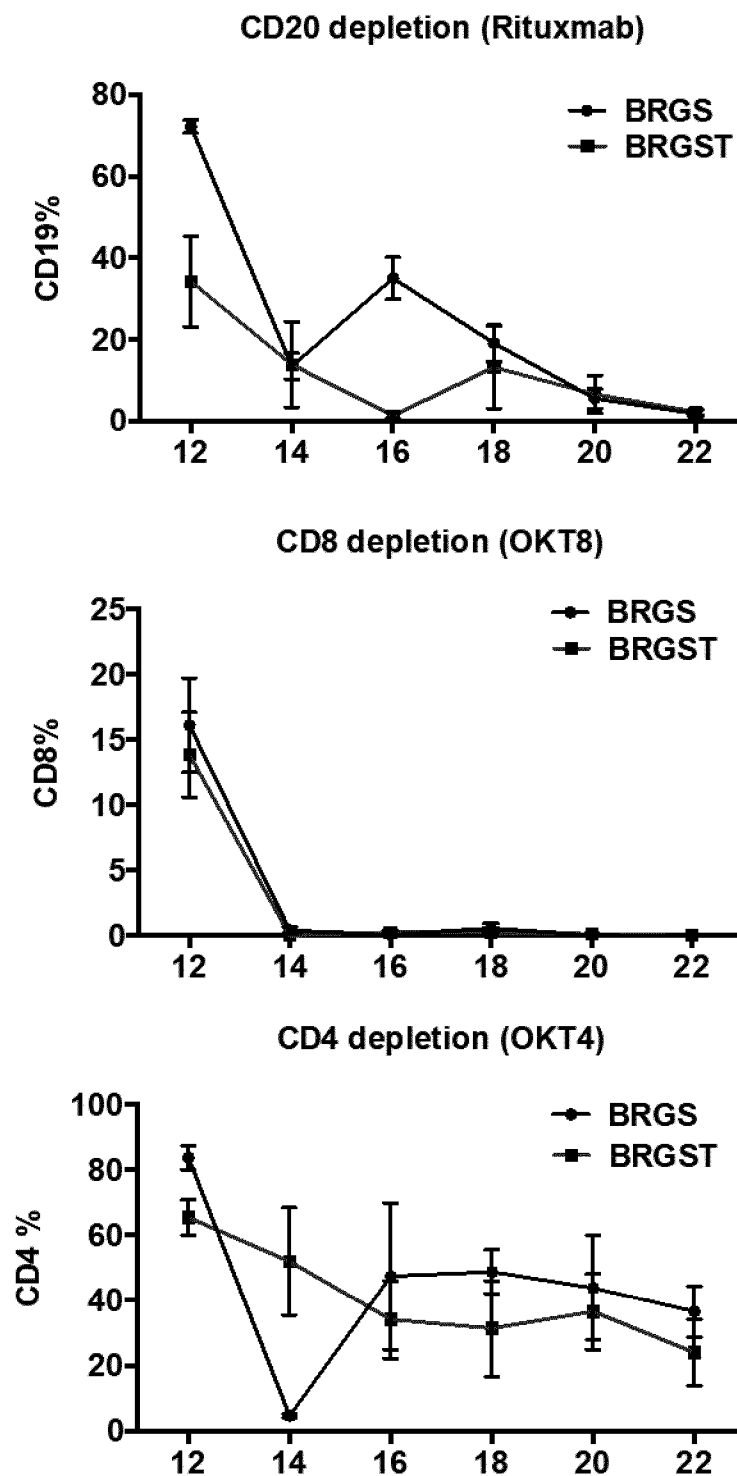
Figure 20 (continuation)



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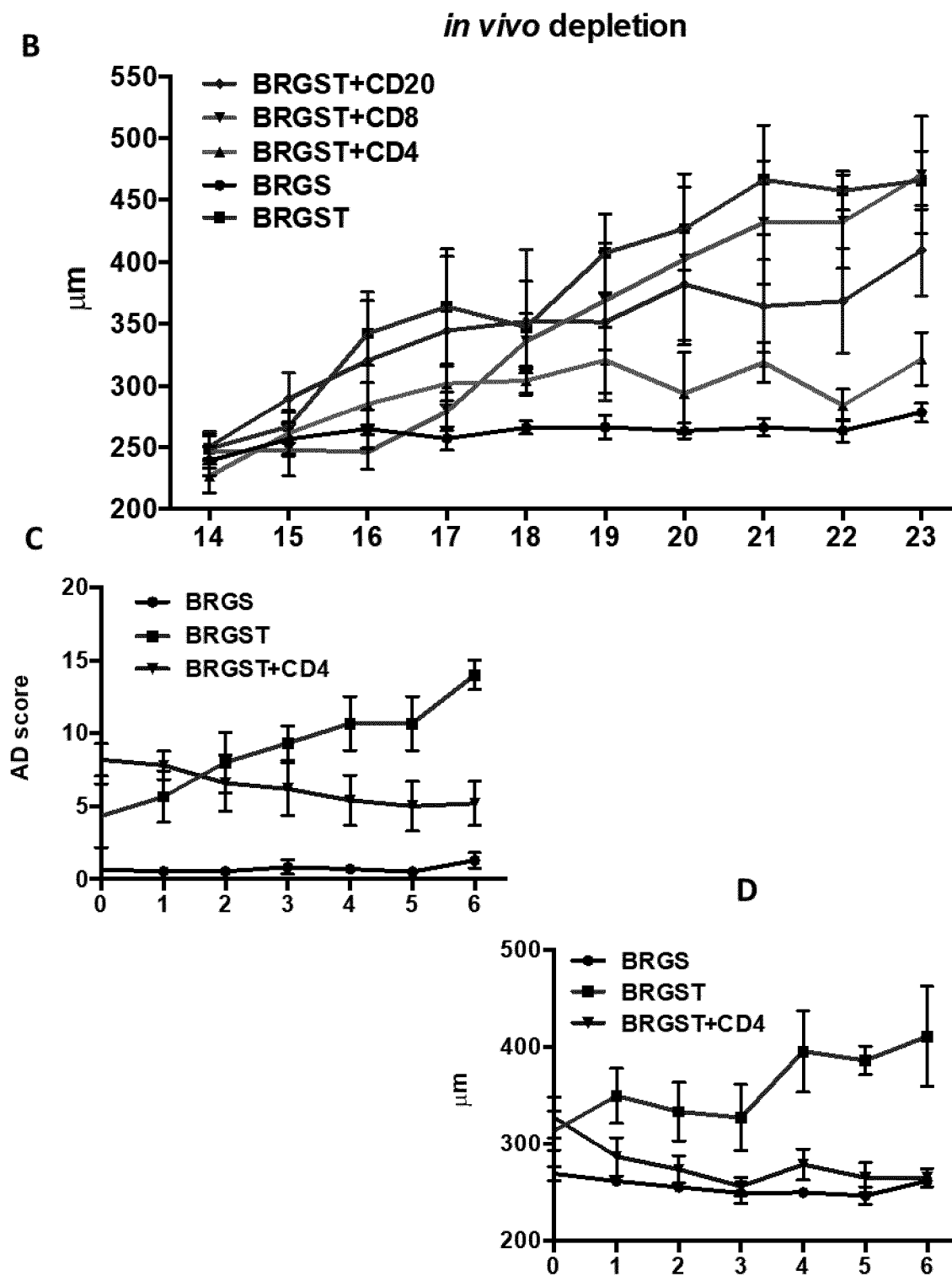
Figure 21

A



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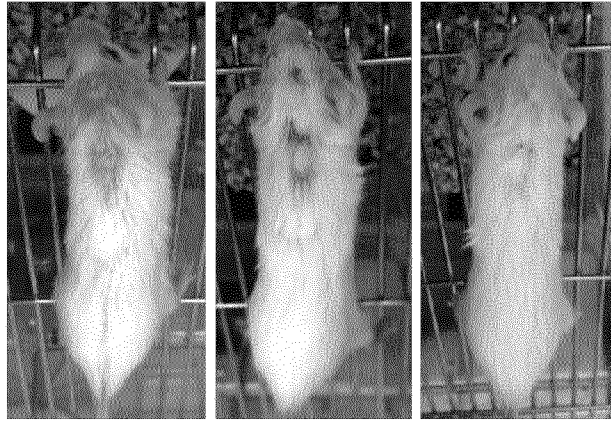
Figure 21 (continuation)



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Figure 21 (continuation)

E

**BRGST
+CD4****BRGST**

W0

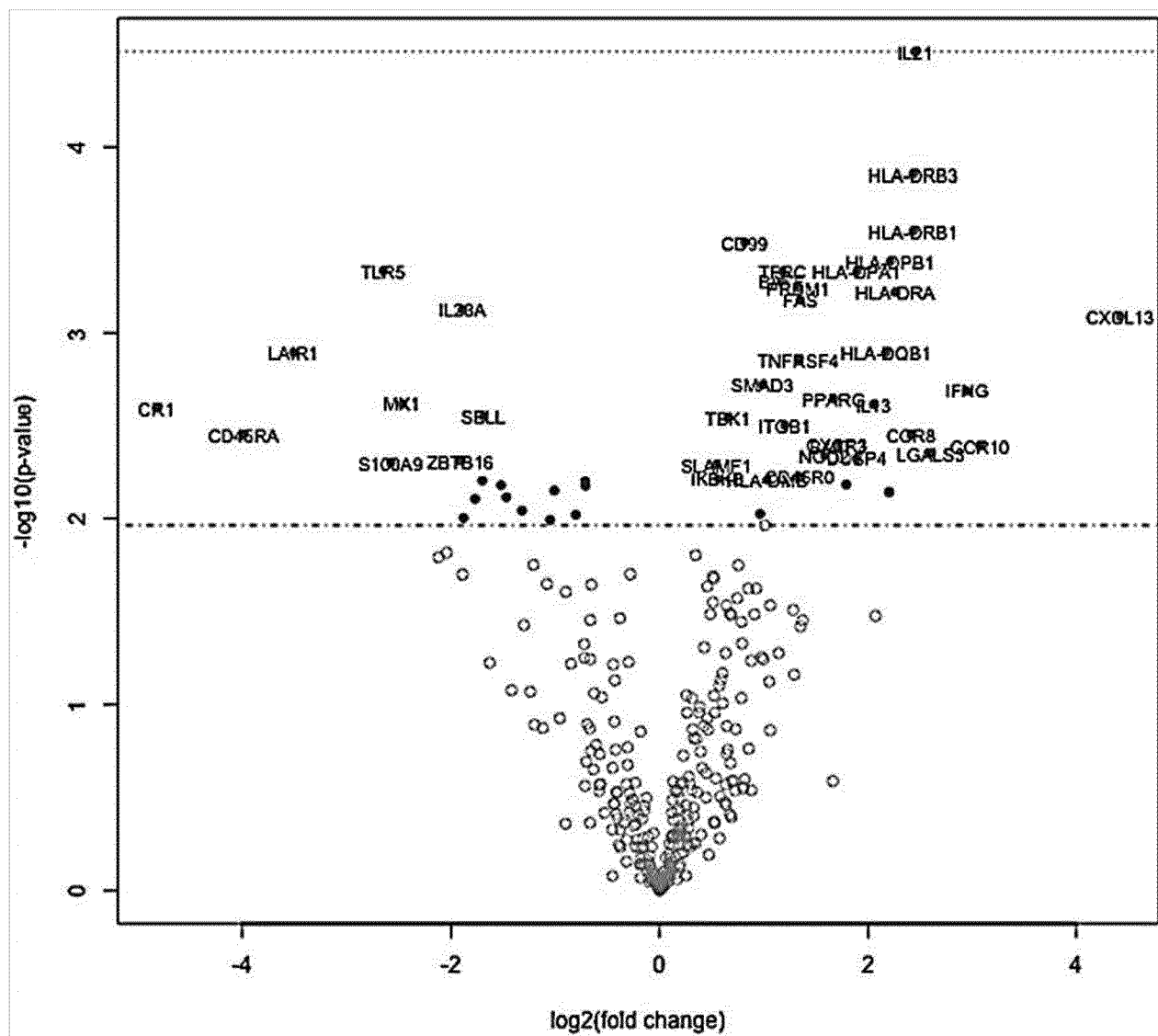
W3

W6

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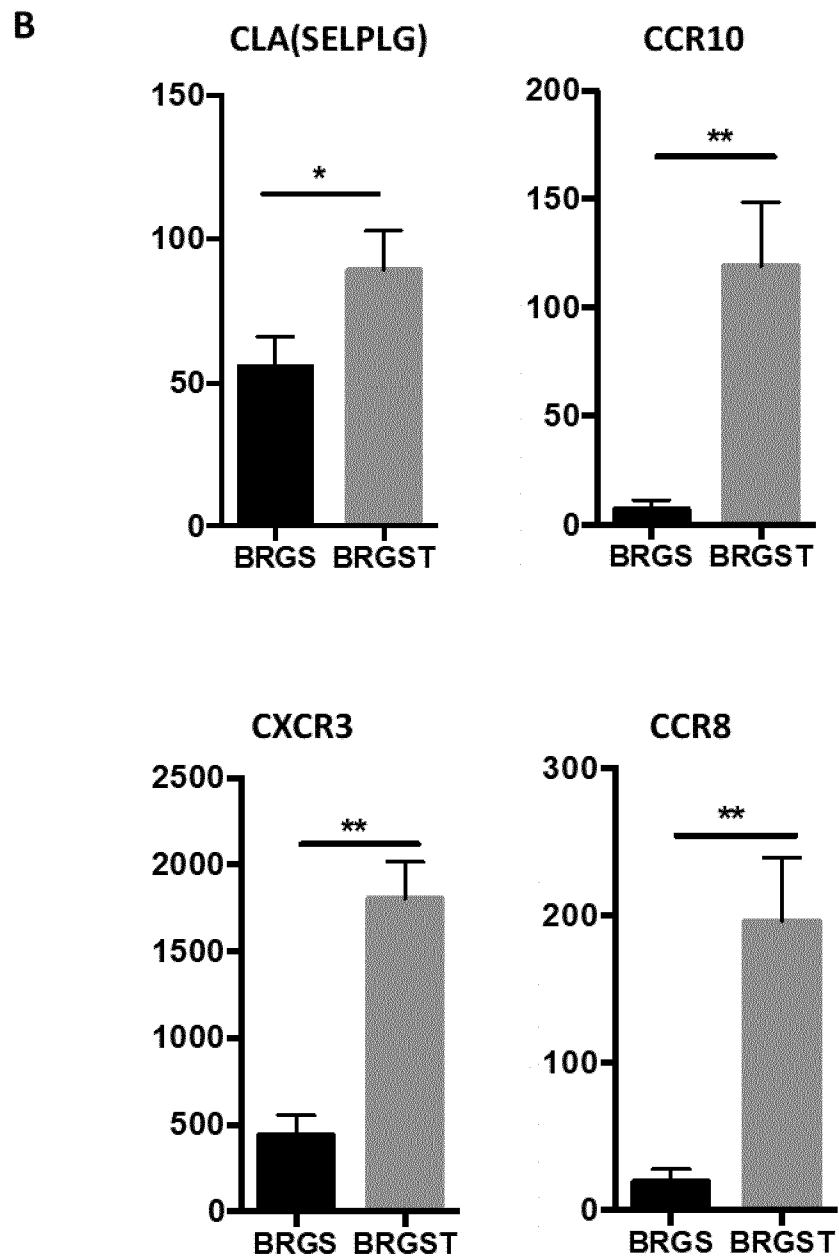
Figure 22

A



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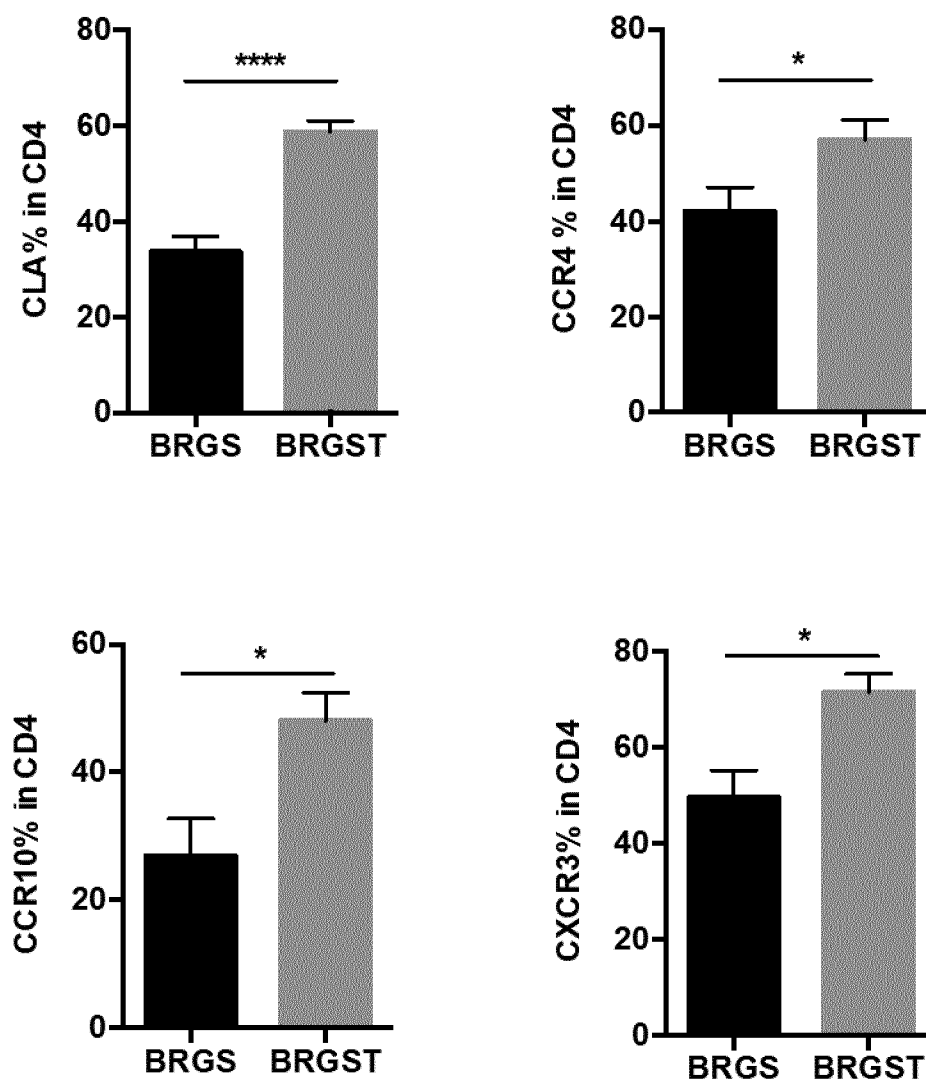
Figure 22 (continuation)



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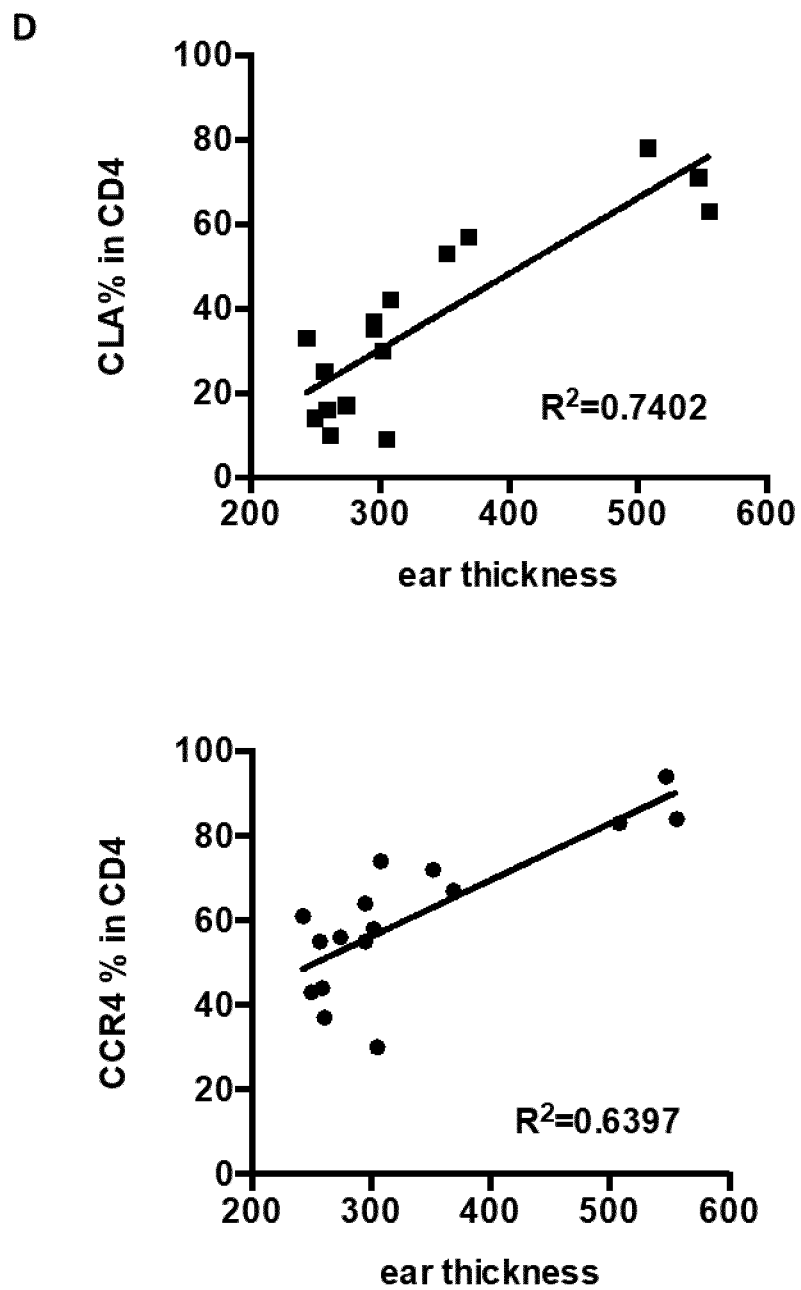
Figure 22 (continuation)

C



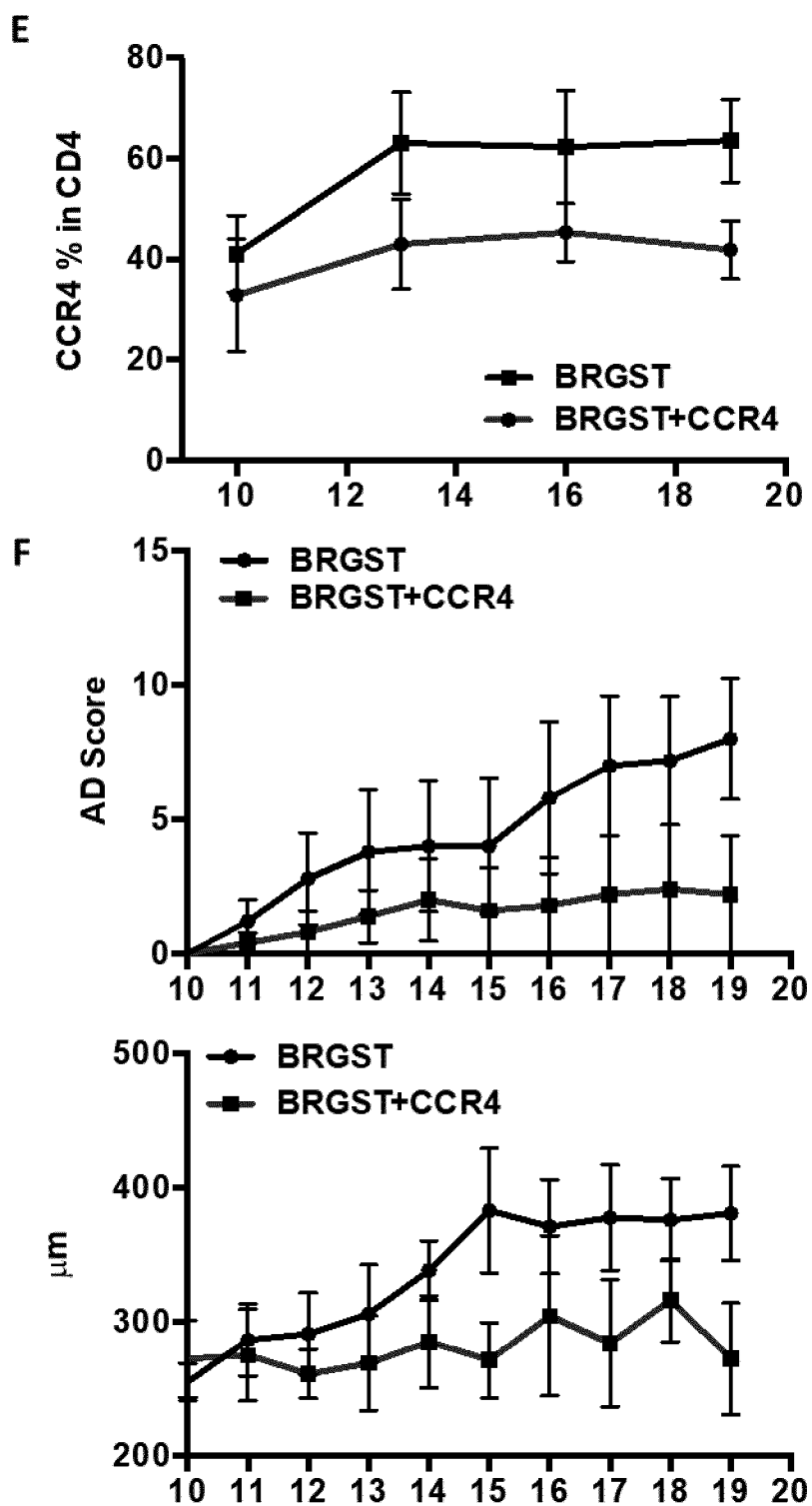
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Figure 22 (continuation)



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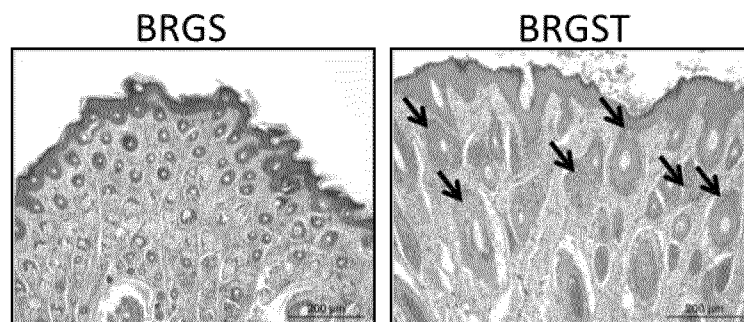
Figure 22 (continuation)



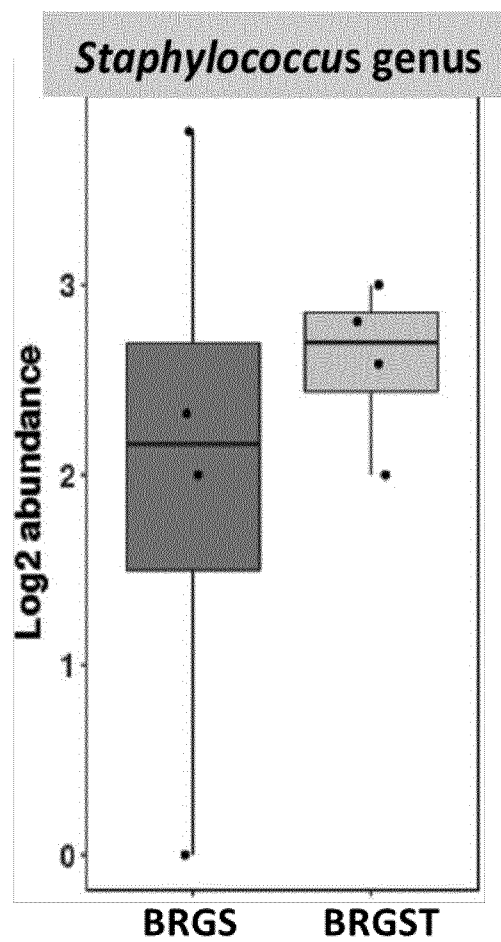
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Figure 23

A

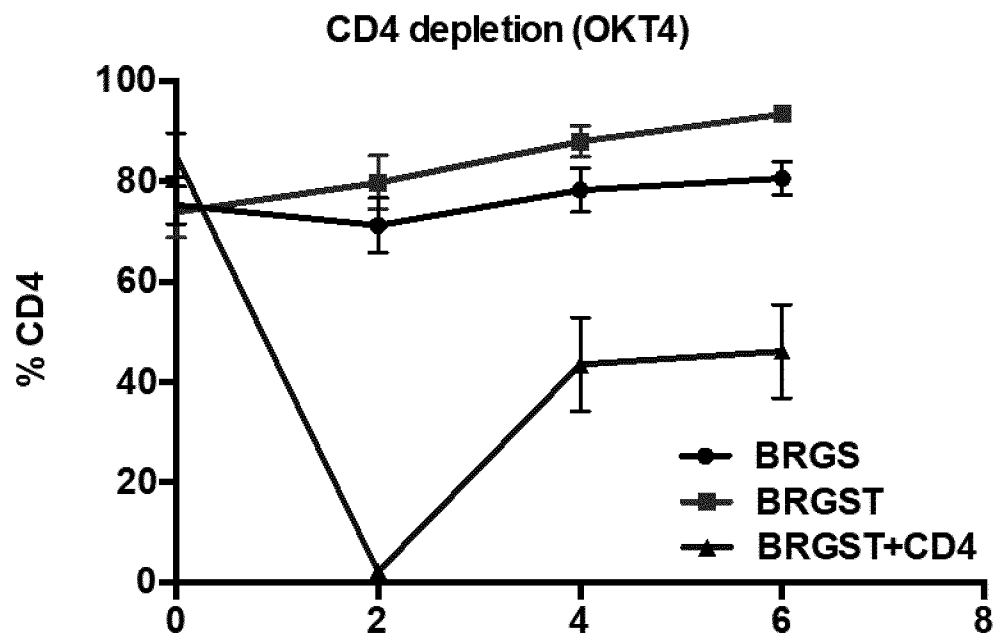


B



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Figure 24



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/068172

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01K67/027 C07K14/54
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)
A01K C07K

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MEGAN M MACBRIDE: "Meeting report: International Workshop on Humanized Mice 5", INTERNET CITATION, 8 March 2016 (2016-03-08), pages 1-5, XP002758867, Retrieved from the Internet: URL: http://www.taconic.com/taconic-insights/precision-research-models/meeting-report-international-workshop-on-humanized-mice-5.html [retrieved on 2016-06-15]	1-3,5, 8-10, 13-18, 20, 23-25, 28-31,36
Y	page 5 ----- -/-	4,6,7, 11,12, 19,22, 26,27, 32-35, 37-67



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

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"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

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"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

4 October 2019

Date of mailing of the international search report

14/10/2019

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Deleu, Laurent

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/068172

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TAKESHI TAKAHASHI ET AL: "Enhanced Antibody Responses in a Novel NOG Transgenic Mouse with Restored Lymph Node Organogenesis", FRONTIERS IN IMMUNOLOGY, vol. 8, 17 January 2018 (2018-01-17), XP055627796, DOI: 10.3389/fimmu.2017.02017 page 5 -----	6,21,41
Y	BREHM MICHAEL A ET AL: "Generation of improved humanized mouse models for human infectious diseases", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 410, 4 March 2014 (2014-03-04), pages 3-17, XP029053086, ISSN: 0022-1759, DOI: 10.1016/J.JIM.2014.02.011 page 7 - page 8; figure 1 -----	6,11,12, 21,26, 27,41, 46,47, 56-65
Y	YAN LI ET AL: "A novel Flt3-deficient HIS mouse model with selective enhancement of human DC development", EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 46, no. 5, May 2016 (2016-05), pages 1291-1299, XP55627989, Weinheim ISSN: 0014-2980, DOI: 10.1002/eji.201546132 page 1292 - page 1298 -----	7,22,42, 66,67
Y	ATEF ALLAM ET AL: "TFH cells accumulate in mucosal tissues of humanized-DRA mice and are highly permissive to HIV-1", SCIENTIFIC REPORTS, vol. 5, no. 1, September 2015 (2015-09), XP055627938, DOI: 10.1038/srep10443 page 9 - page 11 -----	32-35, 37-55
Y	STÉPHANE CHAPPAZ ET AL: "The IL-7 Signaling Pathway Regulates Lymph Node Development Independent of Peripheral Lymphocytes", THE JOURNAL OF IMMUNOLOGY, vol. 184, no. 7, 5 March 2010 (2010-03-05), pages 3562-3569, XP055628031, US ISSN: 0022-1767, DOI: 10.4049/jimmunol.0901647 abstract ----- -/--	4,19

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/068172

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JANE YOO ET AL: "Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin", THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 202, no. 4, 15 August 2005 (2005-08-15), pages 541-549, XP055628051, US ISSN: 0022-1007, DOI: 10.1084/jem.20041503 abstract	68,69
A	----- YRINA ROCHMAN ET AL: "New insights into the regulation of T cells by [gamma]c family cytokines", NATURE REVIEWS IMMUNOLOGY, vol. 9, no. 7, July 2009 (2009-07), pages 480-490, XP55627800, GB ISSN: 1474-1733, DOI: 10.1038/nri2580 page 9 - page 10	68,69
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A	----- MARSHALL E KARPEL ET AL: "BLT humanized mice as a small animal model of HIV infection", CURRENT OPINION IN VIROLOGY, vol. 13, August 2015 (2015-08), pages 75-80, XP055627935, United Kingdom ISSN: 1879-6257, DOI: 10.1016/j.coviro.2015.05.002 the whole document	33-35, 51-53, 63-65
A	----- WO 2013/062134 A1 (ISHIKAWA FUMIHIKO [JP]; KOSEKI HARUHIKO [JP] ET AL.) 2 May 2013 (2013-05-02) page 14 - page 16 ----- -/--	1-31

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/068172

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	IURII KOBOZIEV ET AL: "Use of Humanized Mice to Study the Pathogenesis of Autoimmune and Inflammatory Diseases :", HHS PUBLIC ACCESS AUTHOR MANUSCRIPT, vol. 21, no. 7, July 2015 (2015-07), pages 1652-1673, XP055628037, US ISSN: 1078-0998, DOI: 10.1097/MIB.00000000000000446 page 7 - page 15 -----	1-67

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/068172

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013062134	A1	02-05-2013	NONE
