



(51) International Patent Classification:

A61K 38/20 (2006.01) A61K 39/00 (2006.01)

A61K 31/404 (2006.01) A61P 19/02 (2006.01)

A61K 31/519 (2006.01) A61P 29/00 (2006.01)

A61K 35/17 (2015.01) C12N 5/10 (2006.01)

(21) International Application Number:

PCT/US2024/031858

(22) International Filing Date:

31 May 2024 (31.05.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/471,677 07 June 2023 (07.06.2023) US

63/530,409 02 August 2023 (02.08.2023) US

(71) Applicants: NORTHWESTERN UNIVERSITY

[US/US]; 633 Clark Street, Evanston, Illinois 60208 (US).

THE BRIGHAM AND WOMEN'S HOSPITAL, INC.

[US/US]; 75 Francis Street, Boston, Massachusetts 02115

(US).

(72) Inventors: CHOI, Jaehyuk; c/o NORTHWESTERN

UNIVERSITY, 633 Clark Street, Evanston, Illinois 60208

(US). RAO, Deepak; 11 Halifax Street #2, Jamaica Plain,

Massachusetts 02130 (US). LAW, Calvin; c/o NORTH-

WESTERN UNIVERSITY, 633 Clark Street, Evanston,

Illinois 60208 (US).

(74) Agent: MAEBIUS, Stephen B. et al.; FOLEY & LARD-

NER LLP, 3000 K Street NW, Suite 600, Washington, Dis-

trict of Columbia 20007-5109 (US).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM,

DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG,
KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY,
MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA,
NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO,
RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS,
ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, CV,

GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST,

SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ,

RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ,

DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT,

LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,

GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))

— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii))

Published:

— with international search report (Art. 21(3))

— before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))

(54) Title: TARGETING AUTOIMMUNITY-ASSOCIATED T CELLS THROUGH REGULATION OF TRANSCRIPTION FAC-
TORS

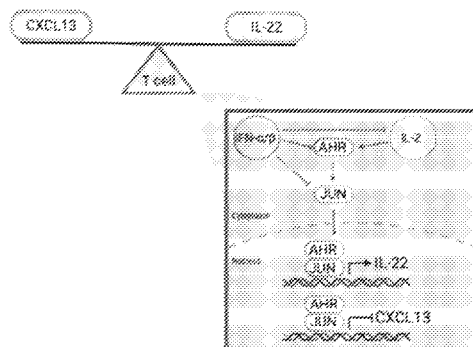


Figure 6k

(57) Abstract: Provided is an aryl hydrocarbon receptor (AHR) agonist, wherein the AHR agonist negatively regulates T cell differentiation as well as methods of using such AHR agonist. Also provided herein are methods of use of transcription factors and gene knockout to negatively regulate T cell differentiation.



TARGETING AUTOIMMUNITY-ASSOCIATED T CELLS THROUGH REGULATION OF TRANSCRIPTION FACTORS

CROSS-REFERENCE TO RELATED PATENT APPLICATION

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Nos. 63/530409, filed August 2, 2023, and 63/471677, filed June 7, 2023, the entire contents of which are incorporated herein by reference.

STATEMENT OF GOVERNMENT SUPPORT

[0001] This invention was made with government support under grant numbers AR078769, and CA268839 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0002] Multiple human autoimmune diseases involve the activation of both autoreactive T cells and B cells and the production of autoantibodies. Systemic lupus erythematosus (SLE) provides a prototype example of an autoimmune disease with central features of pathologic T cell-B cell interactions^{1,2}. Both activated B cell populations and B cell-helper T cells are expanded in patients with SLE and correlate with disease activity³⁻⁹. B cell activation and autoantibody production in autoimmune diseases generally require help from T cells, which stimulate B cell activation and differentiation via CD40L and IL-21^{2,10}. T follicular helper (Tfh) cells are the principal T cell population that helps B cells within follicles of secondary lymphoid organs; however, chronic autoimmune diseases often involve varied B cell-helper T cells with distinct phenotypes, including T peripheral helper (Tph) cells, which couple B cell helper functions to a migratory program targeting inflamed peripheral tissues and accumulate prominently in the joints of patients with seropositive rheumatoid arthritis (RA) and in the circulation of patients with SLE^{9,11}.

[0003] In addition to IL-21 and CD40L, a hallmark function of human B cell-helper T cells is the production of CXCL13, a B cell chemoattractant that uniquely binds CXCR5. Chemokine

(C-X-C motif) ligand 13 (CXCL13) is a chemokine encoded by the *CXCL13* gene. CXCL13 production is critical for the accumulation and organization of B cell follicles in secondary lymphoid organs, and overexpression of CXCL13 is sufficient to induce the formation of ectopic lymphoid structures in murine models^{12,13}. Circulating levels of CXCL13 have been proposed as a biomarker of global germinal center activity and correlate with disease activity in both SLE and RA^{14,15}. In rodents, CXCL13 is primarily produced by stromal cells and not by T cells; yet, in humans and primates, Tfh and Tph cells appear to be the primary source of this chemokine^{14,16,17}.

[0004] Despite its functional significance in autoimmune disease, the extrinsic signals and transcriptional networks that control CXCL13 production by T cells remain largely unknown, with roles for TGF- β , IL-2, and SOX4 previously reported^{18,19}. More broadly, the mechanisms that control CXCL13⁺ Tph cell expansion in autoimmune diseases remain unclear. CXCL13 is also highly expressed by T cells in the tumor microenvironment^{20,21}. In particular, tumor antigen-reactive CD8⁺ T cells have been found to express CXCL13, raising the possibility that some of these regulatory circuits may be shared with CD8⁺ T cells²²⁻²⁴.

[0005] T cell functions are strongly influenced by signals derived from the environment, including cytokines, metabolites, and other small molecules, which can push T cells towards specific phenotypes and away from others. These divergent T cell fates establish axes of opposing functional outcomes, which can regulate pathogenic inflammation.

[0006] The disclosure herein identifies regulators that control the differentiation of CXCL13⁺ Tph and Tfh cells, which may place this phenotype in the context of other differentiated T cell states.

SUMMARY

[0007] Expansion of B cell-helper T cells including T follicular helper (Tfh) and T peripheral helper (Tph) cells is a prominent feature of systemic lupus erythematosus (SLE), a prototypical autoimmune disease with broad autoantibody production. Human Tfh and Tph cells are marked by high production of the B cell chemoattractant CXCL13, yet regulation of T cell CXCL13 production and the relationship between a CXCL13⁺ state and other

differentiated T cell states remain largely undefined. As disclosed herein, Applicants identified a dramatic imbalance in CD4 T cell phenotypes in SLE patients, with expansion of PD-1⁺/ICOS⁺ CXCL13⁺ T cells and reduction of CD96^{hi} IL-22⁺ T cells. Using CRISPR screens, the aryl hydrocarbon receptor (AHR) was identified as a potent negative regulator of CXCL13 production by human CD4 T cells. Transcriptomic, epigenetic, and functional studies demonstrate that AHR coordinates with AP-1 (Activator Protein 1) family member JUN to prevent CXCL13⁺ Tph/Tfh cell differentiation and promote an IL-22⁺ phenotype. Type I interferon (IFN), a pathogenic driver of SLE, opposes AHR and JUN to promote T cell production of CXCL13. The data presented herein places CXCL13⁺ Tph/Tfh cells on a polarization axis opposite from Th22 cells and reveals AHR, JUN, and IFN as key regulators of these divergent T cell states.

[0008] According to one aspect, provided herein are compositions and methods for negatively regulating pathological T cell differentiation in autoantibody driven autoimmune disease. The negative regulation of pathological T cell differentiation can play a role in treating or mitigating symptoms of an autoimmune disease. According to another aspect, provided herein are compositions and methods for preventing B cell-helper T cell function, thereby inhibiting differentiation of B cells.

[0009] One embodiment is an aryl hydrocarbon receptor (AHR) agonist, wherein the AHR agonist negatively regulates pathological T cell differentiation in autoantibody driven autoimmune disease. In some embodiments, the agonist negatively regulates T follicular helper (Tfh) cell and/or T peripheral helper (Tph) cell differentiation. In some embodiments, the agonist is conjugated to a T cell targeting moiety, such as anti-T cell antibody. Another embodiment is a vector comprising the AHR agonist. In one aspect, the vector is a lentivirus. Another embodiment is a composition comprising the AHR agonist.

[0010] Another embodiment is preventing CXCL13⁺ Tph/Tfh cell generation, comprising contacting a cell population with one or more AHR agonists, or a vector comprising an AHR agonist, or a composition comprising an AHR agonist and a pharmaceutically acceptable carrier, optionally where the contacting is *in vivo*, *in vitro*, or *ex vivo*. In other embodiments the method can be used *in situ*.

[0011] Yet another embodiment is a method of inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, comprising administering one or more AHR agonist, or a vector comprising an AHR agonist, or a composition comprising an AHR agonist and a pharmaceutically acceptable carrier, to a subject in need. In one aspect, the subject in need has an autoimmune disease. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0012] Yet another embodiment is a method of treating an autoimmune disease in a subject in need thereof, the method comprising administering an effective amount of one or more AHR agonists, or a vector comprising an AHR agonist, or a composition comprising an AHR agonist and a pharmaceutically acceptable carrier. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0013] Yet another embodiment is a method of inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, comprising administering an effective amount of one or more AHR agonists, a vector comprising an AHR agonist, or a composition comprising an AHR agonist to a subject in need thereof or to a cell population. In some embodiments, the contacting is *in vivo*, *in vitro*, or *ex vivo*. In other embodiments the method may be used *in situ*. In one embodiment the subject in need has an autoimmune disease. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0014] In another aspect, the present disclosure provides a method of using one or more AHR agonists, or vectors or compositions including an AHR agonist, to prevent B cell differentiation in a cell population or subject in need. In one aspect, the method comprises contacting an effective amount of the AHR agonist with a cell population *in vivo*, *in vitro*, or *ex vivo*, or *in situ*. In another aspect, the method comprises administering an effective amount of the AHR agonist to a subject in need. In some embodiments, AHR agonist may be administered intravenously, subcutaneously, orally, or topically to a subject in need.

[0015] And yet another embodiment is a method of treating an autoimmune disease in a subject in need thereof, comprising obtaining a population of T cells from the subject, treating the population of T cells with an AHR agonist, and administering an effective amount of the treated population of T cells to the subject. In some embodiments, the

treatment with an AHR agonist reduces the frequency of PD-1+CXCR5- Tph cells in the treated T cell population. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0016] Yet another embodiment comprises treatment with an AHR agonist to stabilize JUN function in a subject in need thereof.

[0017] According to another embodiment is a transcription factor selected from AHR, JUN, FOS, ATF3, FOSL1, and FOSL2, wherein the transcription factor negatively regulates Tfh and Tph cell function in autoantibody driven autoimmune disease, and methods of use for this purpose. Another embodiment is a vector, such as a lentivirus, comprising the transcription factor. Another embodiment is a composition comprising the transcription factor.

[0018] One embodiment is preventing CXCL13+ Tph/Tfh cell generation, comprising contacting a cell population with one or more transcription factors selected from AHR, JUN, FOS, ATF3, FOSL1, and/or FOSL2, or a vector comprising the transcription factor, or a composition comprising the transcription factor and a pharmaceutically acceptable carrier. In some embodiments, the contacting is *in vivo*, *in vitro*, or *ex vivo*. In other embodiments the method may be used *in situ*.

[0019] Yet another embodiment is a method of inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, comprising administering an effective amount of one or more transcription factors selected from AHR, JUN, FOS, ATF3, FOSL1, and/or FOSL2, or a vector comprising the transcription factor, or a composition comprising the transcription factor and a pharmaceutically acceptable carrier to a subject in need thereof or to a cell population. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0020] Another embodiment is treating an autoimmune disease in a subject in need thereof, comprising administering an effective amount of one or more transcription factors selected from AHR, JUN, FOS, ATF3, FOSL1, and/or FOSL2, or a vector comprising the transcription factor, or a composition comprising the transcription factor and a

pharmaceutically acceptable carrier, to the subject in need thereof, wherein the transcription factor causes a target gene in the subject to be overexpressed. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0021] According to yet another embodiment is a method of treating an autoimmune disease in a subject in need thereof, comprising administering an effective amount of an AHR agonist and a transcription factor selected from AHR, JUN, FOS, ATF3, FOSL1, and/or FOSL2. In one aspect, the AHR agonist and transcription factor are administered consecutively or sequentially. The AHR agonist may be in a vector or composition. The transcription factor may be in a vector or composition. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0022] Yet another embodiment is a gRNA targeting a gene selected from *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, or *STAT2*, wherein the gRNA has a sequence according to one of the sequences in Table 5. These genes were selected from a larger selection of genes upregulated in Tph/Tfh cells in the current RNA-Seq dataset or in previously published RNA-Seq datasets of RA synovial T cells¹⁶, as well as from genes previously correlated with CXCL13 expression, or genes in the AP-1 family.^{19,36} When *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, or *STAT2*, were knocked out, CXCL13 in a cell or cell population decreased.

[0023] Another embodiment is a CRISPR-Cas9-gRNA ribonucleoprotein (crRNP) complex comprising at least one gRNA as described herein. Another aspect is a vector or a composition comprising the crRNP complex or vector, and a pharmaceutically acceptable carrier.

[0024] Yet another embodiment is a method of decreasing CXCL13 production in a cell, comprising contacting the cell with the crRNP complex, vector, or composition as described herein, optionally where the contacting is *in vivo*, *in vitro*, or *ex vivo*. The crRNP complex knocks out a target gene. In other embodiments the method may be used *in situ*.

[0025] Yet another embodiment is a method of treating an autoimmune disease in a subject in need thereof, comprising administering the crRNP complex described herein to a subject in

need thereof. The crRNP complex knocks down a target gene. In some embodiments, administering is intravenous, subcutaneous, oral, or topical.

[0026] According to another aspect of the methods described herein, an additional therapeutic agent is administered to the subject in need, optionally wherein the additional therapeutic agent is anifrolumab.

[0027] In some embodiments, methods of treatment described herein are used to treat an autoimmune disease selected from rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, and/or systemic sclerosis.

[0028] In some embodiments the autoimmune disease is RA or SLE.

FIGURES

[0029] Figures 1a-j: Imbalanced CXCL13⁺ Tph and Tfh cells versus IL-22⁺ CD96^{hi} cells in SLE patients. **Fig. 1a**, Differentially expressed proteins on gated memory CD4⁺ T cells from systemic lupus erythematosus patients compared to controls. p-values from t-test with Bonferroni correction. **Fig. 1b**, UMAP feature plots showing expression of indicated proteins on memory CD4⁺ T cells. **Fig. 1c**, UMAP showing Cell Neighborhood Analysis (CNA) results (red, enriched in SLE; blue, enriched in controls). **Fig. 1d**, UMAP showing CD4⁺ T cell assigned to clusters. **Fig. 1e**, Quantification of indicated clusters in systemic lupus erythematosus patients (n=19) and controls (n=19), $P=6.32\text{e-}6$ for PD-1/ICOS⁺, $p=2.9\text{e-}9$ for CD96^{hi}. **Fig. 1f**, Correlation plot of PD-1/ICOS⁺ cluster and CD96^{hi} cluster abundances in systemic lupus erythematosus patients and controls. Spearman statistics shown. **Fig. 1g**, Heatmap of upregulated genes in Treg, Tph/Tfh cells, and CD96^{hi} cells in bulk RNA-seq data from cell subsets sorted from blood (n=11; 6 systemic lupus erythematosus patients, 5 controls). **Fig. 1h**, Th22 (left) and Tph (right) gene signature scores in bulk RNA-seq data of sorted T cell subsets from healthy controls (n=5) and systemic lupus erythematosus patients (n=6). Th22 score compared to CD96^{hi} T cells, p-values from left to right: $6.24\text{e-}8$, $9.53\text{e-}3$, $1.11\text{e-}7$, $4.75\text{e-}3$. Tph score compared to Tph cells, from left to right $p=3.42\text{e-}7$, $4.73\text{e-}4$,

2.04e-4 **Fig. 1i**, Expression of *IL22* and *CXCL13* by qPCR in sorted T cell populations from systemic lupus erythematosus patients (n=6), plotted relative to expression in CD96^{hi} cells. *IL22* relative expression p-values from left to right: 0.0063, 0.0075. *CXCL13* relative expression p-values from left to right: 0.0128, 0.0012, 0.0283. **Fig. 1j** Example of flow cytometry detection of IL-22 and IL-17A in PMA/ionomycin-stimulated CD96^{hi} CD4 T cells (left) and quantification of IL-22 and IL-17A T cells single or double positive populations (right) in sorted cell subsets from healthy individuals (n=6). Only comparing between CD96^{hi} and Th17 subsets, IL-17a-IL-22⁺ $P=0.0312$, IL-17a⁺IL-22⁻ $P=0.0312$. Data for **Figs. 1e, 1h, and 1i** are shown as mean $\pm$ DS, and min/max/median for **Fig. 1j**. p-values (NS ≥ 0.05 , * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$) were obtained by Mann-Whitney test in **e**, by Friedman test with Dunn multiple comparisons test in **Fig. 1h**, by ratio paired T-test in **Fig. 1i**, or by Wilcoxon test in **j**.

[0030] Figures 2a-2n: AHR controls a CXCL13-IL-22 differentiation axis in human T cells. **Fig. 2a**, Schematic of arrayed CRISPR-deletion screen to detect regulators of CXCL13 production. **Fig. 2b**, CXCL13 levels by ELISA in supernatants of cells from CRISPR screen, with screen results of 2 independent experiments using different donors on x- and y-axes. **Fig. 2c**, ELISA data for indicated cytokines in supernatants of memory CD4⁺ T cells nucleofected with sgAHR or sgCD8 control. All cells were cultured with TGF- β , and each line represents a separate donor (n=12). For CXCL13 $p=4.88e-4$ and IL-22 $p=4.88e-4$. **Fig. 2d**, ELISA data for indicated cytokine, normalized to control condition (DMSO), in supernatant from total CD4⁺ T cells stimulated with indicated AHR modulators in the presence of TGF- β (n=10). For AHRinh and TCDD, respectively, $p=1.44e-6$ and $1.55e-4$ for CXCL13, and $p=1.33e-4$ and $2.76e-4$ for IL-22. **Fig. 2e**, Expression of ICOS (left) and CD96 (right) by flow cytometry on memory CD4⁺ T cells, normalized to DMSO condition per donor (n=8). For AHRinh and TCDD compared to DMSO, respectively, $p=0.0052$ and 0.0004 for ICOS (left) and $p=0.0020$ and 0.018 for CD96 (right). **Fig. 2f**, UMAPs of scRNA-seq data of memory CD4⁺ T cells stimulated under indicated conditions for 2 weeks. **Fig. 2g**, UMAP of scRNA-seq data with cells coloured by enrichment of Tph gene signature. **Fig. 2h** UMAP of rheumatoid arthritis (RA) synovial CD4⁺ T cell clusters (left) and feature plot of expression of CXCL13 (right). **Fig. 2i**, UMAP of *in vitro* cultured memory CD4⁺ T cells from **Fig. 2f** mapped to RA synovial CD4⁺ T cell dataset. **Fig. 2j**, Bar plot of cluster

abundance of *in vitro* cultured memory CD4⁺ T cells from **Fig. 2f** mapped to RA synovial CD4⁺ T cell clusters by treatment conditions (n=3). Comparing against DMSO condition, from top to bottom, for TGF- β +DMSO p=0.0092, 0.0023, 0.0275, 0.0323, and for TGF- β +AHRinh P=0.0188, 0.0023, 0.0323. **Fig. 2k**, GSEA plot of Tph gene signature enrichment (red) and CD96^{hi} gene signature enrichment (blue) in cells treated with TGF- β +AHRinh for 2 weeks versus TCDD without TGF- β for 1 week. **Fig. 2l**, Normalized luciferase activity from AHR reporter cell line treated with DMSO or TCDD in the presence of serum from patients with systemic lupus erythematosus (n=11) or anti-nuclear antibody (ANA)+ control patients without systemic lupus erythematosus (n=12), p=2.80e-3. **Fig. 2m**, Normalized (to DMSO condition) flow cytometry quantification of CD4⁺, PD-1⁺ CXCR5⁻ (left, n=9) or CD96^{hi} T cells (right, n=9) T cells. Statistical testing was performed comparing AHR agonist or inhibitor to DMSO treated PBMCs from either systemic lupus erythematosus patient samples or healthy controls. For PD-1+CXCR5- p=0.0022. For CD96^{hi}, from left to right, p=0.0041, 2.2e-5, 7.9e-5. **Fig. 2n**, Percent of B cells with a CD38^{hi} CD27⁺ plasmablast phenotype after 5-day co-culture with Tfh cells that were pre-treated with vehicle control (DMSO), AHR inhibitor (AHRinh) or AHR agonist (TCDD). Percentage was normalized to total B cells in culture. B cells and Tfh cells were donor matched, sorted from healthy donor PBMCs (n=4), p=0.0212. Data for **Figs. 2d, 2e, 2l-n** are shown as mean $\pm$ SD. P-values (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) were obtained by Wilcoxon test in **Fig. 2c**, by ratio paired t-test in **Figs. 2d, 2e, 2m** and **2n**, by ANOVA with Holm-Sidak test in **Fig. 2j**, and by Mann-Whitney test for **Fig. 2l**. TCDD = AHR agonist; AHRinh = AHR inhibitor CH-223191.

[0031] Figures 3a-3l. JUN coordinates with AHR to divert T cells away from CXCL13 and towards IL-22. Fig. 3a, Time course RNA-Seq analysis with maSigPro cluster 6 identified as AHR response gene set by pathway enrichment. **Fig. 3b**, Transcription factor motif and common co-occurrence enrichment analysis. **Fig. 3c**, AHR CUT&RUN peak heatmap, signal profile and motif analysis. **Fig. 3d**, Representative AHR binding regions. **Fig. 3e**, Comparison of AHR binding in cells treated with AHR agonist or AHR antagonist. **Fig. 3f**, Pathway enrichment analysis of AHR-bound peak associated genes. **Figs. 3g, 3h**, CXCL13(**Fig. 3g**) and IL-22(**Fig. 3h**) expression levels measured by ELISA in supernatants of cells from AP-1 transcription factor family CRISPR arrayed screen, with screen results of 2 independent experiments using different donors on x- and y-axes. **Fig. 3i**, Illustration of

JUN-targeting sgRNAs used in CRISPR screens and validation experiments (top), and Western blot detection of JUN protein expression in T cells nucleofected with control (sgCTRL) or JUN-targeting guide (*JUN*-sg5, bottom). **Fig. 3j**, Representative flow cytometry plots of CXCL13 detection by intracellular flow cytometry in memory CD4⁺ T cells nucleofected with control (upper left) or *JUN*-sg5 (lower left). Flow cytometry measurements are plotted as the percentage of cells that are CXCL13⁺ after stimulation with TGF- β and either DMSO, AHRinh or TCDD (n=4, top right), p-value from left to right: 3.81e-4, 0.0372. ELISA measurements of CXCL13 levels in supernatants was also done in corresponding stimulation conditions (n=3, lower right), p-value from left to right: 0.00979, 0.0528, 0.0108. **Fig. 3k**, Representative flow cytometry plots of IL-22 detection by intracellular flow cytometry and quantification of IL-22 production by intracellular flow cytometry assay or ELISA in memory CD4⁺ nucleofected with control or *JUN*-sg5, as done for CXCL13 T cells in **Fig. 3j**. For flow cytometry data (top right, n=4, $P=3.6e-5$), and for ELISA data (n=3, bottom right), p-values from left to right: 0.0517, 0.0226, 0.045. **Fig. 3l**, GSEA of Th22 (red) and Tph (teal) gene signature enrichment in cells nucleofected with *JUN*-sg5 or control. p-values (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$) were obtained by ratio paired t-test for **Figs. 3j** and **3k**. TCDD = AHR agonist; AHRinh = AHR inhibitor CH-223191.

Figures 4a-4l. JUN shares binding regions with AHR as coregulator of AHR

transcription program. Fig. 4a, Top 10 HOMER motifs (left) from called JUN CUT&RUN peaks in TCDD-treated memory CD4⁺ T cells with pathway enrichment analysis (middle and right). **Fig. 4b**, Venn diagram of CUT&RUN peaks bound by AHR (left) and JUN (right). **Fig. 4c**, Venn diagram of JUN and AHR co-bound CUT&RUN peak-associated genes that overlap with AHR-induced genes identified by Time-course RNA-Seq in **Fig. 4a**. **Fig. 4d**, Representative JUN binding peaks in relation to AHR peaks in memory CD4⁺ T cells treated with TCDD. **Fig. 4e**, Overall JUN binding signal at AHR peaks in cells treated with AHR agonist (TCDD, green) or inhibitor (AHRinh, purple) and representative JUN binding peaks at *CXCL13* (middle) and *IL22* (right) in memory CD4⁺ T cells treated with AHRinh. **Fig. 4f**, Analysis of JUN binding peaks lost with AHR inhibitor. Venn diagram of JUN bound CUT&RUN peaks in memory CD4⁺ T cells treated with AHRinh and TCDD (top) and heatmap of total JUN-bound regions in the same conditions (bottom). **Fig. 4g**, Example Western blot of JUN and phospho-JUN (JUN-pS73) in memory CD4⁺ T cells stimulated as

indicated. **Fig. 4h**, Densitometry quantification of Western blot results as in **Fig. 4g** with results normalized to DMSO condition (n=13), p-value from left to right: 0.0010, 3.1e-5, 0.0265, 6.6e-5. **Fig. 4i**, ELISA measurements of CXCL13 (left) and IL-22 (right) in JUN-overexpressing (JUN OE) or vector control transduced memory CD4⁺ T cells, stimulated in TGF- β with either DMSO, AHRinh or TCDD (n=4). p-value from left to right: 0.00343, 0.0153, 0.0312, 0.069, 0.091, 0.0391. **Fig. 4j**, Representative JUN binding peaks from CUT&RUN of AHRinh treated memory CD4⁺ T cells either expressing the control vector (Empty) or JUN overexpression vector (JUN OE) at the *CXCL13* and *IL22* gene loci. **Fig. 4k**, KEGG pathway (top) and Elsevier Pathway (bottom) enrichment analysis of genes associated with upregulated peaks (by Diffbind) in JUN OE versus empty vector control. **Fig. 4l**, GSEA analysis of Th22 (red) and Tph (teal) gene signature enrichment in JUN-overexpressing T cells and empty vector control when treated with TCDD (top) or AHRinh (bottom). Data in **Fig. 4h** are shown as mean $\pm$ S.D. p-value (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) was obtained by ratio paired t-test for **Figs. 4h** and **4i**. TCDD = AHR agonist; AHRinh = AHR inhibitor, CH-223191.

[0032] Figure 5a-5m: Increased IFN in systemic lupus erythematosus patients promotes Tph cell differentiation and inhibits AHR. Fig. 5a, *CXCL13* expression in transcripts per million (TPM) in RNA-Seq data of CD4⁺ T cells from systemic lupus erythematosus patients stratified as IFN-high or IFN-low (n=12, p=0.0013). **Fig. 5b**, Serum CXCL13 levels in systemic lupus erythematosus patients treated longitudinally with anti-IFNAR antibody (anifrolumab) or placebo, stratified by IFN signature level, IFN High (left) and IFN Low (right). Sample size for each cohort indicated in figure. For IFN High p=2.67e-07. **Fig. 5c**, Schematic of longitudinal Lupus patient derived scRNA-seq data. **Fig. 5d**, UMAP clustering of memory CD4⁺ T cells from Lupus patients pre-anifrolumab treatment (left), and feature plot highlighting clusters enriched in indicated gene signatures (right). **Fig. 5e**, Violin plots comparing Tph (left) and CD96^{hi} (right) signature enrichments in respective clusters found in **d**, gene signatures were taken from gene sets used in **Fig. 1g**. p<2.2e-16 for Tph and p<2.2e-16 for CD96^{hi}. **Fig. 5f**, Violin plot comparing ISG signature enrichment between Tph and Th22 cell clusters from **Fig. 5d**, p=4.57e-8. **Fig. 5g**, Violin plots comparing JUN transcriptional signature (left) and JUN mRNA expression levels (right) between Tph and Th22 cell clusters from **Fig. 5d**. p<2.2e-16 for JUN signatures and p=0.81 for *JUN* mRNA

expression. **Fig. 5h**, Comparison of Tph and Th22 (as indicated) cell population as a percentage of memory CD4⁺ T cells, before (blue circle) and after (orange upside down triangle) anifrolumab treatment. $p=0.03$ for Tph and 0.016 for Th22. **Fig. 5i**, ELISA for CXCL13 (left) and IL-22 (right) from memory CD4⁺ T cells stimulated with or without IFN- α for 24 hours prior to addition of AHR agonist/inhibitor or DMSO control as indicated ($n=6$). From left to right, p -value for CXCL13: $8.5e-5$, $4.05e-4$, $2.9e-5$, and for IL-22: $2.35e-3$, $8.76e-4$, 0.0338 . **Fig. 5j**, ELISA for CXCL13 in total CD4⁺ T cells nucleofected with sgAHR or control CRISPR guides, treated with IFN- α (when indicated) in the absence or presence of TGF- β ($n=4$). p -value from left to right: $5.17e-4$, $2.8e-4$, $7.1e-5$, $1.35e-3$. **Fig. 5k**, Venn diagram of overlapped DAR regions in IFN- β treated CD4⁺ T cells (left) or Control (right) with SF PD-1^{hi} and AHRinh treated CD4⁺ T cells. **Fig. 5l**, Accessible regions near the *CXCL13* gene loci in CD4⁺ T cells treated with IFN- β or control for 72h. **Fig. 5m**, Luciferase activity from AHR reporter cell line treated with TCDD with or without IFN- β pre-treatment (3 experiments with 2-4 replicates each, total $n=9$, left to right $p=0.0117$, 0.00391). Data in **Figs. 5a**, **5i**, and **5j** are shown as mean $\pm$ S.D. p -value (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) was obtained by Mann-Whitney in **a**, by linear mixed model with random effect for patients applied in **e**, **f**, **g** and **m**, by paired t-test in **h**, or by ratio paired t-test in **Fig. 5i** and **Fig. 5j**. TCDD = AHR agonist; AHRinh = AHR inhibitor, CH-223191.

[0033] Figures 6a-6k. IFN opposes IL-2 and JUN to promote CXCL13⁺ Tph cells. Fig. 6a, CXCL13 (left) and IL-22 (right) expression levels measured by ELISA in supernatants of cells from regulators of IFN induced CXCL13 CRISPR arrayed screen, with screen results of 2 independent experiments using different donors on x- and y-axes. **Fig. 6b**, Violin plot comparing IL-2 induced gene signature between Tph and Th22 cell clusters. Signature genes were taken from Hallmark IL2 STAT5 signaling human gene set found in the GSEA molecular signatures database, $p=0.0175$. **Fig. 6c**, ELISA for CXCL13 (left, $n=9$) and IL-22 (right, $n=6$) from memory CD4⁺ T cells stimulated with plate-bound CD3 antibody and IL-2 at indicated concentrations for 6 days. From left to right, p -values for CXCL13: $9.45e-4$, $4.34e-5$, $1.34e-5$, $3.05e-6$, $4.30e-6$, $4.24e-6$ and for IL-22: $2.21e-3$, $4.51e-4$. **Fig. 6d**, Western blot verification of STAT5 deletion by respective guides (left), ELISA for CXCL13 (left) and IL-22 (right) in memory CD4⁺ T cells nucleofected with sgSTAT5A, sgSTAT5B, both (combined) or control CRISPR guides, stimulated in the absence or presence of IL-2 ($n=3$).

Statistical significance was calculated based on comparing cell stimulated in the absence or presence of IL-2 within each respective CRISPR condition. p-values from left to right for CXCL13: 4.16e-5, 4.12e-4, 1.73e-3 and for IL-22: 1.94e-2, 2.53e-2, 6.06e-4. **Fig. 6e**, ELISA for CXCL13 production in memory CD4⁺ T cells stimulated with CD3/CD28 dynabeads (left, n=6) or anti-CD3 plate bound antibodies (right, n=5) and cultured with addition of indicated cytokines or nothing (media) for 6 days. p-value from left to right for cells stimulated with CD3/CD28 dyanbeads: 5.52e-3, 1.70e-2, 1.08e-2, 2.40e-2 and with anti-CD3: 2.54e-2, 7.2e-3, 1.57e-2. **f**, ELISA for IL-2 production in memory CD4⁺ T cells stimulated with CD3/CD28 dynabeads in the absence or presence of IFN- β (n=8), p=8e-4. **g**, Representative Western blot of phospho-JUN (pS73) and JUN expression in memory CD4⁺ T cells under indicated conditions (left) and quantification of expression normalized to Control DMSO condition (n=5, right). JUN p=0.0044, and JUN-pS73 p=0.0016. **Fig. 6h**, Overall JUN binding signal at JUN peaks in memory CD4⁺ T cells stimulated in the presence (purple) or absence (green) of IFN- α (left) and representative JUN binding peaks at IL22 (right) in memory CD4⁺ T cells of respective conditions or IgG control (bottom). **Fig. 6i**, Elsevier pathway enrichment for annotated differentially bound peaks by JUN, upregulated in control over IFN- α treated conditions from the same data set generated in **Fig. 6h**. **Fig. 6j**, ELISA of CXCL13 (top) or IL-22 (bottom) comparing memory CD4⁺ T cells either overexpressing of JUN (JUN OE) or control vector in the presence or absence of IFN- α (n=4). p-values from left to right for CXCL13: 0.011, 0.041 and for IL-22: 0.013, 0.033. **Fig. 6k**, Graphical representation of proposed model in type I IFN regulation of IL-2, AHR and JUN to promote Tph cells in systemic lupus erythematosus. Data in **Figs. 6c, 6d, 6e** and **6g** shown was mean $\pm$ S.E.M. p-value (*p<0.05, **p<0.01, ***p<0.001) was obtained by linear mixed model with random effect for patients applied in **Fig. 6b**, by 2-way ANOVA in **Fig. 6c**, and by ratio paired T-test in **Figs. 6d, 6e, 6f, 6g** and **6j**. TCDD = AHR agonist; AHRinh = AHR inhibitor, CH-223191.

[0034] Figures 7a-7e. Clinical associations of PD-1⁺/ICOS⁺ and CD96^{hi} cell clusters. Fig. 7a, Heatmap of marker expression on mass cytometry cell clusters (left) and MASC association statistics for each cluster comparing systemic lupus erythematosus vs controls (right). SLE OR = odds ratio of representation in systemic lupus erythematosus vs control. CI = confidence interval. **Fig. 7b**, Association of indicated cluster proportions with serum anti-dsDNA antibody level in systemic lupus erythematosus patients (n=19). **Fig. 7c**, Association

of indicated cluster proportions with systemic lupus erythematosus disease activity by SLEDAI-2K (n=19). **Fig. 7d**, Association of indicated cluster proportions with prednisone dose or equivalent at time of sample collection. **Fig. 7e**, Cluster proportions of PD-1⁺/ICOS⁺ (left) and CD96^{hi} (right) clusters in SLE patients stratified by immunosuppressant drug use at time of sample collection. Spearman correlation statistics shown in **Figs. 7b-7d**. Data in **Fig. 7e** shown as median $\pm$ interquartile, and statistically tested with Mann-Whitney test.

[0035] Figures 8a-f. CD96^{hi} cells are a Th22 cell population. **Fig. 8a**, Example of flow cytometry sorting of CD4⁺ T cell subsets for bulk RNA-seq analysis. **Fig. 8b**, PCA plot of bulk RNA-seq profiles of CD4⁺ T cell subsets sorted from SLE (n=6) or healthy control (n=5) donors. Colors indicate cell subsets and shapes indicate clinical group. **Fig. 8c**, Multi-set Venn diagram of the number of differentially expressed genes between CD96^{hi} cells and indicated CD4⁺ T cell subsets. **Fig. 8d**, UMAP of scRNA-seq data of blood CD4⁺ T cells from pediatric SLE patients and controls, colored by intensity of expression of genes differentially expressed in bulk sorted Tph cells or CD96^{hi} cells from **Figs. 8a-8c**. **Figs. 8e, 8f**, Flow cytometry quantification of cytokines from PMA/ionomycin stimulated CD4⁺ T cell subsets sorted from healthy donors (e, n=5, $P=0.0012$ for Th17 versus Tph) and base chemokine receptor expression (f, n=6-7). P -value for f from left to right, all comparing to CD96^{hi} subset, for CCR6: 0.0156, 0.0156, 0.0156, for CXCR5: 0.0313, 0.0313, 0.0313, for CXCR3: 0.0156, 0.0156, 0.0156. Data for **Figs. 8e** and **8f** are shown as mean $\pm$ S.D. P -values (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$) were obtained by ratio paired t-test in **8e** or by Wilcoxon test in **8f**.

[0036] Figures 9a-9l: AHR controls T cell production of CXCL13. **Fig. 9a**, Western blot detection of CBLB in memory CD4⁺ T cells treated with control or sgCBLB CRISPR guide (left) and ELISA quantification of CXCL13 from indicated cells (n=4, 2 biological donors each with 2 technical replicates, $p=0.031$). **Fig. 9b**, Representative Western blot for AHR protein expression in cells nucleofected with sgAHR and sgCD8a control. **Fig. 9c**, ELISA data of CXCL13 in supernatants of memory CD4⁺ T cells nucleofected with sgAHR or sgCD8a in the presence or absence of TGF- β (n=8). From left to right, $p=0.0078$, 0.0078, 0.0078, 0.0156. **Figs. 9d, 9e**, Normalized (to DMSO control) ELISA quantification of indicated cytokines in supernatants of stimulated memory (**Fig. 9d**) or naive CD4⁺ T cells

(**Fig. 9e**) treated with AHR agonist TCDD, AHR inhibitor (AHRinh) CH-223191, or DMSO control (n=5-7). For AHRinh and TCDD in **Fig. 9d**, respectively, $p=7.31\text{e-}4$ and 0.00304 for CXCL13, and $p=0.00159$ and 0.0124 for IL-22. For AHRinh and TCDD in **Fig. 9e**, respectively, $p=0.0679$ and 0.00108 for CXCL13, and $p=0.0192$ and 0.0157 for IL-22. f, Normalized (to DMSO control) ELISA quantification of indicated cytokines in supernatants of memory CD4^+ T cells stimulated with AHR agonist FICZ, AHR inhibitor GNF-351, or DMSO control (n=3-4). For FICZ and GNF-351, respectively, $p=0.0109$ (GNF-351 only) for CXCL13, and $p=0.0084$ and 0.0393 for IL-22. **Fig. 9g**, Effects of AHR CRISPR deletion (left, n=10) and pharmacological modulation (middle[n=9] and right[n=3]) on $\text{IFN}\gamma$ production as measured by ELISA. 2 sets of AHR modulators (used in **Figs. 9e** and **9f**) were tested. Results for AHR modulation are shown normalized to DMSO control. **Fig. 9h**, $\text{IFN}\gamma$, IL-4 and IL-17 production by memory CD4^+ T cells cultured in indicated T helper subset polarizing conditions with the addition of DMSO, AHRinh or TCDD, measured by Flow cytometry (n=6). $p=0.0316$ for IL-17. **Fig. 9i**, ELISA data for CXCL13 (left) and IL-22 (right), normalized to control (DMSO) condition, in supernatants of CD4^+ T cells stimulated and cultured with indicated factors. Each dot represents a donor (n=4-5). j, ELISA data for CD8^+ T cells stimulated in the presence of $\text{TGF-}\beta$ with indicated AHR modulators, normalized to DMSO condition per donor (n=6). For AHRinh and TCDD compared to DMSO, respectively, $p=0.0021$ and 0.0038 for CXCL13, and $p=0.0103$ and 0.0032 for IL-22. **Fig. 9k**, ELISA measurement for CXCL13 in supernatants of memory CD8^+ T cells nucleofected with sgAHR or control CRISPR guide (n=6, $P=0.0312$). **Fig. 9l**, Expression of ICOS (left) and CD96 (right) by flow cytometry in memory CD8^+ T cells treated with DMSO or AHR agonist/inhibitor. Respective quantifications are normalized to DMSO condition (n=8). For AHRinh and TCDD, respectively, $p=0.0158$ (AHRinh only) for ICOS, and $p=7.09\text{e-}3$ and 0.0371 for CD96. Data for **Figs. 9d, 9e, 9f, 9g, 9j, and 9l** are shown as mean $\pm$ S.D. p-values ($\text{NS}\geq 0.05$, $*p<0.05$, $**p<0.01$, $***p<0.001$) are obtained by ratio paired t-test for **Figs. 9a, 9d, 9e, 9f, 9g, 9h, 9j, 9l** or Wilcoxon test in **Figs. 9c** and **9k**. TCDD = AHR agonist; AHRinh = AHR inhibitor, CH-223191.

[0037] Figures 10a-10d: Effects of chronic AHR modulation in CD4^+ T cells. **Fig. 10a**, ELISA data for indicated cytokines in supernatants of memory (top) and naïve (bottom) CD4^+ T cells re-stimulated each week for 3 weeks, normalized to DMSO 1 week result for

each donor (n=3-4 donors). **Fig. 10b**, CXCL13 expression (by fragments per kilobase of transcript per million mapped reads, FPKM) in bulk RNA-seq samples of cell stimulated under indicated conditions. **Fig. 10c**, GSEA enrichment plots of Tph gene signature in naïve or memory CD4⁺ T cells stimulated with TGF- β plus either AHR agonist TCDD or inhibitor (AHRinh) CH-223191. **Fig. 10d**, GSEA enrichment plots for Tph gene signature in T cells stimulated with or without TGF- β , under indicated conditions of AHR agonist TCDD, AHR inhibitor (AHRinh) CH223191, or DMSO control.

[0038] Figures 11a-11e: Effects of AHR and TGF- β on CD4⁺ T-cell subsets. Fig. 11a, ELISA measurement of CXCL13 in supernatants of sorted CD4⁺ T cell subsets from healthy donors (n=10), stimulated with or without TGF- β and indicated AHR modulators or DMSO control. Statistical comparisons shown compare AHR agonist/inhibitor to DMSO within presence or absence of TGF- β , and TGF- β versus no TGF- β within each treatment. p-values from left to right for Naïve: 0.0188, 0.0188, Tph: 0.0032, 4.7e-5, Tfh: 0.0123, 0.0050, CD96hi: 0.0063, 0.0003, 0.0079, Th17: 0.0231, 0.0032, 0.0188, Th1: 0.0050, 0.0050, 0.0421. **Fig. 11b**, ELISA measurement of CXCL13 production in sorted CD4⁺ T cell subsets nucleofected with either sgAHR or sgCD8a CRISPR guides (n=4). From left to right, p=0.0331, 0.0507, 0.0539, 0.0154, 0.0127. **Fig. 11c**, TGF- β gene signature score in bulk RNA-seq data of sorted CD4⁺ T cell subsets as in **Fig. 1h**. Comparisons made against Tph subset, from left to right p=1.14e-4, 1.85e-3, 0.0197, 1.85e-3. **Fig. 11d**, ELISA measurement for IL-22 in supernatants of indicated CD4⁺ T cell subsets stimulated under indicated conditions in the presence (orange) or absence (teal) of TGF- β (n=10). For each subset, statistical comparisons shown compare AHR agonist/inhibitor to DMSO within presence or absence of TGF- β , and TGF- β versus no TGF- β within each treatment. p-values from left to right in each subset is as follows, Naïve: 0.0123, CD96hi: 0.0188, 7.24e-4, 1.96e-3, Th17; 4.31e-4, 0.028, 3.17e-3, 0.0188, Th1: 0.002. **Fig. 11e**, Surface expression of indicated markers in CD4⁺ T cell subsets by flow cytometry, plotted as normalized (to DMSO w/o TGF- β) mean fluorescence intensity (MFI), after 5 days of stimulation in the presence (orange) or absences (teal) of TGF- β (n=4-5). For each subset of each marker, statistical comparisons shown compare AHR agonist/inhibitor to DMSO within presence or absence of TGF- β , and TGF- β versus no TGF- β within each treatment. For ICOS, p-value from left to right in each subset is as follows, Naïve: 0.0188, 8.98e-3, 0.0264, 3.57e-3, Tph: 2.73e-3,

5.21e-3, 5.57e-3, 0.0104, 0.0109, 0.0293, Tfh: 2.20e-4, 1.54e-3, 3.34e-4, 8.89e-4, 5.49e-5, 1.31e-3, CD96^{hi}: 5.17e-3, 6.66e-3, 4.52e-4, 0.0154, 2.10e-3, 3.14e-7, 4.39e-3. For CD96, p-value from left to right in each subset is as follows, Naïve: 0.0112, Tph: 0.0312, 0.0205, 6.75e-3, 5.57e-3, Tfh: 0.0315, CD96^{hi}: 0.0463, 5.93e-3, 0.0296, 0.0224. For TIGIT, p-value from left to right in each subset is as follows, Naïve: 0.0160, 0.0203, Tph: 2.41e-3, 0.0115, 0.0165, 1.69e-3, Tfh: 3.26e-3, 0.0238, 0.0321, 4.35e-3, 8.51e-3, 6.84e-3, CD96^{hi}: 0.0105, 0.0157. For PD-1, p-value from left to right in each subset is as follows, Naïve: 9.17e-3, 4.63e-3, 1.04e-3, 2.33e-3, 8.55e-4, 0.0331, Tph: 0.0133, 0.0119, 3.49e-3, 0.0104, Tfh: 8.49e-4, 5.38e-3, 3.73e-4, 1.03e-3, 1.75e-3, 0.0292, 6.66e-3, CD96^{hi}: 9.75e-3, 0.0485, 0.0129, 0.0197, 2.94e-3, 2.07e-3, 0.0328. Data shown as min/max/median for **Figs. 11a** and **11d**, and as mean $\pm$ S.D for **Fig. 11c** and **11e**. p-values (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) were obtained by Friedman's test with post-test by Dunn's test for **Figs. 11a**, **11c** and **11d**, and by ratio paired t-test for **Figs. 11b** and **11e**. TCDD = AHR agonist; AHRinh = AHR inhibitor, CH-223191.

[0039] Figures 12a-12f: ATAC-seq analysis of Tph cells, Tfh cells, and AHR inhibitor-treated cells. **Figs. 12a, 12b**, Example flow cytometry cell sorting of CD4⁺ T cell populations from RA synovial fluid (**Fig. 12a**) or tonsil (**Fig. 12b**) mononuclear cells. **Fig. 12c**, PCA plot of ATAC-seq data from CD4 T cell populations sorted from RA synovial fluid or from tonsil based on PD-1 expression level. **Fig. 12d**, PCA plot of ATAC-seq data from blood CD4⁺ T cells of healthy donors cultured with DMSO, AHR agonist TCDD or AHR inhibitor (AHRinh) CH-223191 in the presence of TGF- β . **Fig. 12e**, GSEA plots of annotated genes of DARs from synovial fluid Tph cells (top) and tonsil Tfh cells (bottom) in CD4⁺ T cells treated with AHRinh versus TCDD in presence of TGF- β for 1 week. **Fig. 12f**, Differentially accessible regions (red square) near the CXCL13 gene locus from ATAC-seq of each indicated cell type/culture condition. TCDD = AHR agonist; AHRinh = AHR inhibitor CH-223191.

[0040] Figure 13: Detection of PD-1⁺ Tph cells in systemic lupus erythematosus peripheral blood mononuclear cells (PBMC). Gating strategy for flow cytometry detection of PD-1⁺ CXCR5⁻ Tph cells and CD96^{hi} cells in PBMC from SLE patient after treatment with AHR inhibitor (AHRinh) CH-223191.

[0041] Figures 14a-14h: Transcriptomic and epigenetic evaluation of AHR activation in T cells and association with AP-1 family members. **Fig. 14a**, Schematic of RNA-Seq time course experiment to identify early transcriptomic events of AHR modulation. **Fig. 14b**, PCA plots of RNA-seq samples after 12 hours (left) and 48 hours (right) of stimulation with TGF- β and either AHR agonist (TCDD) or AHR inhibitor (AHRinh) CH-223191. **Figs. 14c, 14d**, Volcano plots of DESeq2 results from RNA-Seq analysis of memory CD4⁺ T cells cultured for 12 hours (**Fig. 14c**) and 48 hours (**Fig. 14d**) in TGF- β and either TCDD or AHRinh. The samples used for DESeq2 analysis correspond with the PCA plots in **Figs. 14b, 14e**, Pathway enrichment analysis of genes upregulated in TCDD-treated CD4⁺ T cells at 48 and 72 hours of culture, based on Elsevier pathway collection. **Fig. 14f**, Transcription factor enrichment analysis using EnrichR databases TRRUST Transcription factors 2019 (left) and EnrichR Transcription factor Co-occurrence (right). **Fig. 14g**, Volcano plot of AHR CUT&RUN Diffbind analysis comparing samples with and without AHR CRISPR knockout (left) and HOMER motif analysis of all upregulated peaks found in AHR WT samples (right). **Fig. 14h**, Venn diagram of overlapped genes bound by AHR with Th22 signature genes as shown in **Fig. 1g**, hypergeometric P-value is shown. TCDD = AHR agonist; AHRinh = AHR inhibitor, CH-223191.

[0042] Figures 15a-15f: Overexpression of JUN in human CD4⁺ T cells. **Fig. 15a, 15b**, Verification of AHR and JUN as interactors. Immunoblot (WB) analysis of HA (**Fig. 15a**) or Flag (**Fig. 15b**) immunoprecipitates from the indicated cell lysates probed with the indicated antibodies. **Fig. 15c**, Cytoplasmic or nuclear extracts (as indicated on bottom) from HEK293T cells stably expressing HA-AHR or vector control treated with AHRinh, TCDD or vehicle control (DMSO) were immunoblotted for AHR, JUN and respective controls (β -tubulin for cytoplasmic extract, Histone H3 for nuclear extract). **Fig. 15d**, JUN expression by Western blot in T cells transduced with JUN overexpression construct or control vector. **Fig. 15e**, Example of flow cytometry sorting to obtain JUN-overexpressing cells based on GFP positivity. **Fig. 15f**, JUN overexpression (JUN OE) CUT&RUN assessment by peak density on total JUN bound peaks compared to vector control as in **Fig. 4f** and top HOMER motifs for each respective condition.

[0043] Figs. 16a-16c: Increased IFN in systemic lupus erythematosus patients inhibit AHR signaling. **Fig. 16a**, IFN signature score in RNA-seq data of CD4⁺ T cell subsets from systemic lupus erythematosus and control patients as in **Fig. 1g**. **Fig. 16b**, Elsevier pathway enrichment from annotated genes of DAR in No IFN- β control treated CD4⁺ T cells. **Fig. 16c**, Normalized relative expression of CYP1A1 measured by qPCR in CD4⁺ T cells cultured in TCDD with the addition of vehicle control or IFN- α , $p=0.0016$ ($n=8$) by ratio paired t-test. Dotted line represents average expression of cells cultured without TCDD for both IFN- α and control. TCDD = AHR agonist.

DETAILED DESCRIPTION

[0044] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. While not explicitly defined below, such terms should be interpreted according to their common meaning.

[0045] The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0046] Unless explicitly indicated otherwise, all specified embodiments, features, and terms intend to include both the recited embodiment, feature, or term and biological equivalents thereof.

[0047] Definitions

[0048] Unless otherwise specified “a” or “an” means one or more.

[0049] All numeric values should be treated as having the term “about” placed before a specific numeric value.

[0050] As used herein, the term “about” placed before a specific numeric value may mean $\pm 20\%$ of the numeric value; $\pm 18\%$ of the numeric value, $\pm 15\%$ of the numeric value; $\pm 12\%$ of the numeric value; $\pm 8\%$ of the numeric value; $\pm 5\%$ of the numeric value; $\pm 3\%$ of the numeric value; $\pm 2\%$ of the numeric value; $\pm 1\%$ of the numeric value or $\pm 0.5\%$ of the numeric value.

[0051] “Optional” or “optionally” means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

[0052] As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0053] As used herein, the term “comprising” is intended to mean that the compounds, compositions and methods include the recited elements, but not exclude others. “Consisting essentially of” when used to define compounds, compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants, e.g., from the isolation and purification method and pharmaceutically acceptable carriers, preservatives, and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients. Embodiments defined by each of these transition terms are within the scope of this technology.

[0054] A “gene” refers to a polynucleotide containing at least one open reading frame (ORF) that is capable of encoding a particular polypeptide or protein after being transcribed and translated.

[0055] The term “express” refers to the production of a gene product, such as mRNA, peptides, polypeptides or proteins. As used herein, “expression” refers to the process by which polynucleotides are transcribed into mRNA or the process by which the transcribed

mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0056] As used herein, the term “overexpress” intends a level of expression of the mRNA, the protein or the polypeptide” that is greater than or exceeds the level of expression of the mRNA, the protein or the polypeptide in a native, wild-type or cell that has not been engineered to increase expression.

[0057] A “gene product” or alternatively a “gene expression product” refers to the amino acid (e.g., peptide or polypeptide) generated when a gene is transcribed and translated. In some embodiments, the gene product may refer to an mRNA or other RNA, such as an interfering RNA, generated when a gene is transcribed.

[0058] The term “encode” as it is applied to polynucleotides refers to a polynucleotide which is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed to produce the mRNA for the polypeptide or a fragment thereof, and optionally translated to produce the polypeptide or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom. Further, as used herein an amino acid sequence coding sequence refers to a nucleotide sequence encoding the amino acid sequence.

[0059] The term “a regulatory sequence”, “an expression control element” or “promoter” as used herein, intends a polynucleotide that is operatively linked to a target polynucleotide to be transcribed or replicated, and facilitates the expression or replication of the target polynucleotide. A promoter is an example of an expression control element or a regulatory sequence. Promoters can be located 5' or upstream of a gene or other polynucleotide, that provides a control point for regulated gene transcription. Polymerase II and III are examples of promoters. In some embodiments, a regulatory sequence is bidirectional, i.e., acting as a regulatory sequence for the coding sequences on both sides of the regulatory sequence. Such bidirectional regulatory sequence may comprise, or consists essentially of, or consists of a bidirectional promoter (see for example Trinklein ND, et al. (2004) An abundance of bidirectional promoters in the human genome. *Genome Res.* Jan;14(1):62-6).

[0060] The term “protein,” “peptide” and “polypeptide” are used interchangeably and in their broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs or peptidomimetics. The subunits (which are also referred to as residues) may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. A protein or peptide must contain at least two amino acids and no limitation is placed on the maximum number of amino acids which may comprise a protein's or peptide's sequence. As used herein the term “amino acid” refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics.

[0061] As used herein, the term “antibody” collectively refers to immunoglobulins or immunoglobulin-like molecules including by way of example and without limitation, IgA, IgD, IgE, IgG and IgM, combinations thereof, and similar molecules produced during an immune response in any vertebrate, for example, in mammals such as humans, goats, rabbits and mice, as well as non-mammalian species, such as shark immunoglobulins. Unless specifically noted otherwise, the term “antibody” includes intact immunoglobulins and “antibody fragments” or “antigen binding fragments” that specifically bind to a molecule of interest (or a group of highly similar molecules of interest) to the substantial exclusion of binding to other molecules (for example, antibodies and antibody fragments that have a binding constant for the molecule of interest that is at least 10^3 M^{-1} greater, at least 10^4 M^{-1} greater or at least 10^5 M^{-1} greater than a binding constant for other molecules in a biological sample). The term “antibody” also includes genetically engineered forms such as chimeric antibodies (for example, murine or humanized non-primate antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Owen et al., Kuby Immunology, 7th Ed., W.H. Freeman & Co., 2013; Murphy, Janeway's Immunobiology, 8th Ed., Garland Science, 2014; Male et al., Immunology (Roitt), 8th Ed., Saunders, 2012; Parham, The Immune System, 4th Ed., Garland Science, 2014.

[0062] As used herein, the term “T cell,” refers to a type of lymphocyte that matures in the thymus. T cells play an important role in cell-mediated immunity and are distinguished from other lymphocytes, such as B cells, by the presence of a T-cell receptor on the cell surface. T-

cells may either be isolated or obtained from a commercially available source. “T cell” includes all types of immune cells expressing CD3 including T-helper cells (CD4⁺ cells), cytotoxic T-cells (CD8⁺ cells), natural killer T-cells, T-regulatory cells (Treg) and gamma-delta T cells.

[0063] As used herein, the term “sample” refers to clinical samples obtained from a subject. In certain embodiments, a sample is obtained from a biological source (i.e., a “biological sample”), such as tissue, bodily fluid, or microorganisms collected from a subject. Sample sources include, but are not limited to, mucus, sputum, bronchial alveolar lavage (BAL), bronchial wash (BW), whole blood, bodily fluids, cerebrospinal fluid (CSF), urine, plasma, serum, or tissue.

[0064] As used herein, the term “stimulation” refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules, such as downregulation of TGF β , and/or reorganization of cytoskeletal structures, and the like.

[0065] Transforming growth factor beta (TGF- β) is a cytokine. It can act as an inducer of CXCL13 (Kobayashi et al. (2016) *European Journal of Immunology* 46(2): 360-71).

[0066] A “stimulatory molecule,” as the term is used herein, means a molecule on a T cell that specifically binds with a cognate stimulatory ligand present on an antigen presenting cell.

[0067] The term “therapeutically effective amount” refers to the amount of the subject compound that will elicit the biological or medical response of a tissue, system, or subject that is being sought by the researcher, veterinarian, medical doctor or other clinician. The term “therapeutically effective amount” includes that amount of a compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the signs or symptoms of the disorder or disease being treated. The therapeutically effective amount will vary depending on the compound, the disease and its severity and the age, weight, etc., of the subject to be treated. As used in the methods provided herein, the terms “therapeutically effective amount” and “effective amount” are used interchangeably.

[0068] “Treating” or “treatment” as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, i.e., arresting its development; (ii) relieving a disease or disorder, i.e., causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. Therapeutic effects of treatment include, without limitation, inhibiting recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

[0069] Treatment of cancer or an infection, immune disorder, or autoimmune response, disorder or disease can be at any time during the cancer or an infection, immune disorder, or autoimmune response, disorder or disease. Certain embodiments of the present disclosure can be administered as a combination (e.g., with a second active), or separately concurrently or in sequence (sequentially) in accordance with the methods described herein as a single or multiple dose e.g., one or more times hourly, daily, weekly, monthly or annually or between about 1 to 10 weeks, or for as long as appropriate, for example, to achieve a reduction in the onset, progression, severity, frequency, duration of one or more symptoms or complications associated with or caused by cancer or an infection, immune disorder, or autoimmune response, disorder or disease, or an adverse symptom, condition or complication associated with or caused by cancer or an infection, immune disorder, or autoimmune response, disorder or disease. Thus, a method can be practiced one or more times (e.g., 1-10, 1-5 or 1-3 times) an hour, day, week, month, or year. The skilled artisan will know when it is appropriate to delay or discontinue administration. A non-limiting dosage schedule is 1-7 times per week, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more weeks, and any numerical value or range or value within such ranges.

[0070] The term “contacting” means direct or indirect binding or interaction between two or more. A particular example of direct interaction is binding. A particular example of an indirect interaction is where one entity acts upon an intermediary molecule, which in turn acts upon the second referenced entity. Contacting as used herein includes in solution, in solid

phase, *in vitro*, *ex vivo*, in a cell and *in vivo*. Contacting *in vivo* can be referred to as administering, or administration.

[0071] CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an acronym for DNA loci that contain multiple, short, direct repetitions of base sequences. The prokaryotic CRISPR/Cas system has been adapted for use as gene editing (silencing, enhancing or changing specific genes) for use in eukaryotes (see, for example, Cong, Science, 15:339(6121):819-823 (2013) and Jinek, et al., Science, 337(6096):816-21 (2012)). By transfecting a cell with elements including a Cas gene and specifically designed CRISPRs, nucleic acid sequences can be cut and modified at any desired location. Methods of preparing compositions for use in genome editing using the CRISPR/Cas systems are described in detail in US Pub. No. 2016/0340661, US Pub. No. 20160340662, US Pub. No. 2016/0354487, US Pub. No. 2016/0355796, US Pub. No. 20160355797, and WO 2014/018423, which are specifically incorporated by reference herein in their entireties.

[0072] Thus, as used herein, “CRISPR system” refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated (“Cas”) genes, including sequences encoding a Cas gene, a *tracr* (trans-activating CRISPR) sequence (*e.g.*, *tracr*RNA or an active partial *tracr*RNA), a *tracr*-mate sequence (encompassing a “direct repeat” and a *tracr*RNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a “spacer”, “guide RNA” or “gRNA” in the context of an endogenous CRISPR system), or other sequences and transcripts from a CRISPR locus. One or more *tracr* mate sequences operably linked to a guide sequence (*e.g.*, direct repeat-spacer-direct repeat) can also be referred to as “pre-crRNA” (pre-CRISPR RNA) before processing or crRNA after processing by a nuclease.

[0073] In some embodiments, one or more vectors driving expression of one or more elements of a CRISPR system are introduced into a target cell such that expression of the elements of the CRISPR system direct formation of a CRISPR complex at one or more target sites. While the specifics can be varied in different engineered CRISPR systems, the overall methodology is similar. A practitioner interested in using CRISPR technology to target a DNA sequence can insert a short DNA fragment containing the target sequence into a guide RNA expression plasmid. The sgRNA expression plasmid contains the target sequence (about

20 nucleotides), a form of the tracrRNA sequence (the scaffold) as well as a suitable promoter and necessary elements for proper processing in eukaryotic cells. Such vectors are commercially available (see, for example, Addgene). Many of the systems rely on custom, complementary oligos that are annealed to form a double stranded DNA and then cloned into the sgRNA expression plasmid. Co-expression of the sgRNA and the appropriate Cas enzyme from the same or separate plasmids in transfected cells results in a single or double strand break (depending of the activity of the Cas enzyme) at the desired target site.

[0074] C-X-C motif chemokine 13 (CXCL13) is a cytokine that can act as a B cell chemoattractant. The human CXCL13 has an amino acid sequence according to Uniprot O43927. The *CXCL13* gene has an mRNA sequence according to NCBI NM_001371558.1.

[0075] Transcription Factors

[0076] Described herein are a number of transcription factors which can play a role in T cell differentiation, such as through enhancement or suppression of CXCL13.

- Aryl hydrocarbon receptor (AHR) is a transcription factor encoded by the *AHR* gene. The human AHR protein has an amino acid sequence according to Uniprot P35869. The *AHR* gene has an mRNA sequence according to NCBI NM_001621.5.
- Transcription factor JUN (JUN) is a transcription factor that is a part of the AP-1 transcription factor family. The human JUN protein has an amino acid sequence according to Uniprot P05412. The *JUN* gene has an mRNA sequence according to NCBI NM_002228.4.
- Protein c-FOS (FOS) is a nuclear phosphoprotein that forms a complex with the JUN/AP-1 transcription factor. FOS is a transcription factor part of the AP-1 transcription factor family. The human FOS protein has an amino acid sequence according to Uniprot P01100. The *FOS* gene has an mRNA sequence according to NCBI NM_005252.4.
- Cyclic AMP-dependent transcription factor ATF3 (ATF3) is a transcription factor that binds the cAMP response element. It is a part of the AP-1 transcription factor family.

The human ATF3 protein has an amino acid sequence according to Uniprot P18847. The *ATF3* gene has an mRNA sequence according to NCBI NM_001030287.4.

- Fos-related antigen 1 (FOSL1) is a transcription factor that is a part of the AP-1 transcription factor family. The human FOSL1 protein has an amino acid sequence according to Uniprot P15407. The *FOSL1* gene has an mRNA sequence according to NCBI NM_001300844.2.
- Fos-related antigen 2 (FOSL2) is a transcription factor that is a part of the AP-1 transcription factor family. The human FOSL2 protein has an amino acid sequence according to Uniprot P15408. The *FOSL2* gene has an mRNA sequence according to NCBI NM_005253.4.
- Basic leucine zipper transcriptional factor ATF-like (BATF) is a transcription factor that is a part of the AP-1 transcription factor family. The human BATF protein has an amino acid sequence according to Uniprot Q16520. The *BATF* gene has an mRNA sequence according to NCBI NM_006399.5.
- E3 ubiquitin-protein ligase (CBLB) is a transcription factor that has an amino acid sequence according to Uniprot Q13191. The *CBLB* gene has an mRNA sequence according to NCBI NM_001321786.1.
- Signal transducer and activator of transcription 5B (STAT5B) is a transcription factor that has an amino acid sequence according to Uniprot P51692. The *STAT5B* gene has an mRNA sequence according to NCBI NM_012448.4.
- Signal transducer and activator of transcription 5A (STAT5A) is a transcription factor that has an amino acid sequence according to Uniprot P42229. The *STAT5A* gene has an mRNA sequence according to NCBI NM_001288718.2.
- Signal transducer and activator of transcription 1-alpha/beta (STAT1) is a transcription factor that has an amino acid sequence according to Uniprot P42224. The *STAT1* gene has an mRNA sequence according to NCBI NM_001384880.1.

- Tyrosine-protein kinase (JAK1) is a transcription factor that has an amino acid sequence according to Uniprot P23458. The *JAK1* gene has an mRNA sequence according to NCBI NM_001320923.2.
- Transcription factor JunB (JUNB) has an amino acid sequence according to Uniprot P17275. The *JUNB* gene has an mRNA sequence according to NCBI NM_002229.3.
- Mineralocorticoid receptor (NR3C2) is a transcription factor that can have an amino acid sequence according to Uniprot P08235. The *NR3C2* gene has an mRNA sequence according to NCBI NM_000901.5.
- T-cell surface glycoprotein CD3 delta chain (CD3D) is a transcription factor that has an amino acid sequence according to Uniprot P04234. The *CD3D* gene has an mRNA sequence according to NCBI NM_000732.6.
- Lymphocyte cytosolic protein 2 (LCP2) is a transcription factor that has an amino acid sequence according to Uniprot Q13094. The *LCP2* gene has an mRNA sequence according to NCBI NM_005565.5.
- Cyclic AMP-dependent transcription factor ATF-4 (ATF4) is a transcription factor that has an amino acid sequence according to Uniprot P18848. The *ATF4* gene has an mRNA sequence according to NCBI NM_001675.4.
- Interferon alpha/beta receptor 2 (IFNAR2) is a transcription factor that has an amino acid sequence according to Uniprot P48551. The *IFNAR2* gene has an mRNA sequence according to NCBI NM_000874.5.
- Signal transducer and activator of transcription 2 (STAT2) is a transcription factor that has an amino acid sequence according to Uniprot P52630. The *STAT2* gene has an mRNA sequence according to NCBI NM_001385110.1.

Modes for Carrying Out the Disclosure

[0077] In one aspect, the present disclosure provides regulators that control the differentiation of CXCL13⁺ Tph (T peripheral helper) and Tfh (T follicular helper) cells, which may place this phenotype in the context of other differentiated T cell states. In another aspect, the present disclosure provides the ligand gated receptor aryl hydrocarbon receptor (AHR) as a central regulator of an unexpected axis (“T cell differentiation axis”) of T cell differentiation with CXCL13⁺ and IL-22⁺ states at opposing ends of this polarization (see **Fig. 6k**) Manipulation of AHR can regulate the CXCL13⁺ and IL-22⁺ T cell states. This T cell differentiation axis can be markedly skewed in patients with systemic lupus erythematosus (see e.g. **Fig. 1i**), in part due to actions of type I interferon, which opposes AHR and JUN to drive T cells towards autoimmunity-associated CXCL13⁺ Tph and Tfh cell states. AHR can reduce the B cell-helper function of Tfh cells by regulating B cell chemoattractant CXCL13.

[0078] The present disclosure demonstrates that activating AHR (aryl hydrocarbon receptor) with AHR agonists, ideally directed towards T cells, can suppress the generation of Tph/Tfh cells and may inhibit disease activity in autoantibody-associated autoimmune diseases. AHR agonists can include FICZ, TCDD, and chemokines including IL-2. The present disclosure identifies the aryl hydrocarbon receptor as a potent negative regulator of Tfh/Tph cell differentiation, and demonstrates that at least two AHR agonists (FICZ, TCDD) inhibit T cell ability to acquire Tph/Tfh cell features including inhibiting production of the B cell chemoattractant CXCL13 (chemokine (C-X-C) motif ligand 13) (see e.g. **Fig. 2d, Figs. 9f and 9i**).

[0079] FICZ (6-formylindolo(3,2-b)carbazole) is commercially available, for example from InvivoGen.com. TCDD (2,3,7,8-tetrachlorodibenzodioxin, also referred to as 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin) is also commercially available, for example at caymanchem.com.

[0080] AHR Agonist—compositions and methods

[0081] In certain aspects, the present disclosure provides an AHR agonist for treatment of autoimmune disease. In certain aspects, the present disclosure provides a T cell-directed AHR agonist, optionally capable of more specific targeting of AHR to T cells (e.g. anti-CD5 or

other anti-T cell antibody conjugated to AHR agonist). According to some embodiments, the AHR agonist is FICZ, TCDD, and/or chemokines including IL-2.

[0082] According to other embodiments the AHR agonist is selected from TCDD, FICZ, ITE, tapinarof, TEACOP270, kynurenic acid, indole[3,2-b]carbazole, 1,3-di(1H-indol-3-yl)propan-2-one, and 1-(1H-indol-3-yl)-3-(3H-indol-3-ylidene)propan-2-one.

[0083] According to some embodiments, the present disclosure provides a vector including the AHR agonist. The vector may be comprised of, or derived from, a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector. In other aspects, the AHR agonist or vector are part of a composition. The composition also includes a carrier, which may be a pharmaceutically acceptable carrier.

[0084] AHR activation strongly drives T cells away from a CXCL13⁺ phenotype and towards an IL-22⁺ phenotype (see e.g. **Fig. 2b**, which shows deletion of the AHR gene upregulated CXCL13 production in Memory CD4⁺ T cells). In certain aspects, the present disclosure provides a method of preventing CXCL13⁺ Tph/Tfh cell generation or differentiation. The method comprises, consists essentially of, or consists of contacting a cell or cell population (i.e. a T cell population) with one or more AHR agonists, or vectors or compositions including the AHR agonist, thereby preventing CXCL13⁺ Tph/Tfh cell generation or differentiation. In some embodiments, the T cells are CD4⁺ or CD8⁺ cells. The T cells may be a CD4⁺ subset selected from Th1, Th17, Tfh, and/or Tph cells. The T cells may be a CD8⁺ subset selected from T_{CM}, T_{EM}, and/or T_{EMRA} cells. The T cell populations may be antigen specific. In these methods, an AHR agonist, or vector or composition including the AHR agonist, is contacted with the T-cell populations. Contacting may occur *in vitro*, *in vivo*, or *ex vivo*. In other embodiments these methods may be used *in situ*, for example via delivery through lipid nanoparticles, retroviruses, lentiviruses, or other agents to specifically target T cell subsets. *In situ* routes of administration or contacting may be systemic or targeted. In some embodiments, the *in situ* administration or contacting is intravenous, subcutaneous, or topical. In these methods of preventing CXCL13⁺ Tph/Tfh cell generation or differentiation

with the addition of the AHR agonist, the T-cell populations are reprogrammed towards an IL-22⁺ phenotype and/or away from a B-cell helper phenotype.

[0085] In some aspects, the AHR agonist may be used in combination with inhibition of proteins critical for T-cell receptor (TCR) signaling such as the CD3 protein complex or LCP2. Knockdown of the genes encoding CD3 (*CD3D*) and LCP2 (*LCP2*) inhibited CXCL13 production (**Fig. 2b**), indicating the genes play a role in increased CXCL13 production in T-cells.

[0086] In certain aspects, the present disclosure also provides methods of using AHR agonists to inhibit functions of autoimmunity-associated T cell populations—including T follicular helper (Tfh) cells and T peripheral helper (Tph) cells—in a subject in need or a cell population. In some aspects, the method comprises, consists essentially of, or consists of contacting a cell or cell population (i.e. a T cell population) with one or more AHR agonists or vectors or compositions including the AHR agonist, thereby inhibiting functions of autoimmunity-associated T cell populations. In some aspects, the method comprises, consists essentially of, or consists of administering one or more AHR agonists or vectors or compositions including an AHR agonist to a subject in need, thereby inhibiting functions of autoimmunity-associated T cell populations. In some embodiments, the contacting is *in vivo*, *in vitro*, or *ex vivo*. In other embodiments the method may be used *in situ*. In one embodiment the subject in need has an autoimmune disease. In some embodiments, administering is intravenous, subcutaneous, oral, or topical. In some aspects, the function is Tph/Tfh differentiation or B cell recruitment (see e.g. **Figs. 2d and 2n, Figs. 9f and 9i**).

[0087] In yet other aspects, the present disclosure provides a method directed to preventing the expansion of pathological T cells found in autoimmune diseases using AHR agonists. The method comprises, consists essentially of, or consists of administering one or more AHR agonists or vectors or compositions including an AHR agonist to a subject in need, thereby preventing the expansion of pathological T cells. In some embodiments, administering is intravenous, subcutaneous, oral, or topical. T cells with the capacity to help B cells, Tfh cells and Tph cells, are highly expanded in patients with autoantibody-associated autoimmune diseases such as lupus and rheumatoid arthritis. The AHR agonist can target key biological

markers specific to the pathological T cells, and inhibiting either function or fit of these cells (see e.g. **Fig. 8**).

[0088] In another aspect, the present disclosure provides a method of using one or more AHR agonists, or vectors or compositions including an AHR agonist, to prevent B cell differentiation in a cell or cell population or subject in need. In some aspects, the method comprises, consists essentially of, or consists of contacting the cell or cell population with an AHR agonist, thereby preventing B cell differentiation. Contacting may occur *in vivo*, *in vitro*, or *ex vivo*, or *in situ*. In some aspects, the method comprises, consists essentially of, or consists of administering the AHR agonist to a subject in need, thereby preventing B cell differentiation. The AHR agonist may be administered intravenously, subcutaneously, orally, or topically. The B cell-helper function of T cells (Tfh cells) treated with an AHR agonist is reduced, preventing the induction of B cell differentiation into plasmablasts (**Fig. 2n**).

[0089] In another aspect, the present disclosure provides a method of treating an autoimmune disease using AHR agonists. The method comprises, consists essentially of, or consists of obtaining a population of T cells from the subject, treating the population of T cells with an AHR agonist, or vector or composition including an AHR agonist, and administering the treated population of T cells to the subject, thereby treating the autoimmune disease. In some embodiments, the T cells populations are CD4⁺ or CD8⁺ cells. The T cells may be a CD4⁺ subset selected from Th1, Th17, Tfh, and/or Tph cells. The T cells may be a CD8⁺ subset selected from T_{CM}, T_{EM}, and/or T_{EMRA} cells. The T cell populations may be antigen specific. With the treatment of the T-cell populations with an AHR agonist, the T-cell populations may be reprogrammed towards an IL-22⁺ phenotype and/or away from a B-cell helper phenotype. In one aspect, treatment of a cell population with an AHR agonist reduces the frequency of PD-1⁺ CXCR5⁻ Tph cells in the treated T cell population (**Fig. 2m**.) In another aspect, treatment of a cell population with an AHR agonist can stabilize the transcription factor JUN function.

[0090] AHR supports expression of AP-1 family member JUN, which directly suppresses CXCL13 and supports IL-22 expression. In some embodiments, AHR supports expression of

other AP-1 family members including FOSL2, FOSL1, ARID5B, and HIF1A (**Fig. 3b, Fig. 14f**).

[0091] In some aspects, the AHR agonist is administered to the T cells or T cell populations (i.e. contacted with the cells) at a rate of about 1-500nM, or about 1-250nM, or about 1-100nM, or about 1-50nM, or about 1-10nM, or about 1-5nM, or about 0.1-5nM. In one embodiment the AHR agonist is applied at a rate of about 10μM. In yet another embodiment the AHR agonist is applied at a rate of about 3-5nM.

[0092] The autoimmune disease may be selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis. The subject in need may be a human.

[0093] Transcription Factors—compositions and methods

[0094] The present disclosure also identifies transcription factors including— AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and BATF—which can regulate Tfh and Tph cell function in autoantibody driven autoimmune disease.

[0095] In particular, JUN is identified as a key regulator of Tfh and Tph function, including but not limited to the production of CXCL13 (see e.g. **Fig. 3g**, which shows expression in a CRISPR screen). In certain aspects, the present disclosure demonstrates AHR antagonists and/or inhibitors reduces JUN functions. In certain aspects, the present disclosure provides JUN as a targetable biomolecule for treatment of autoantibody driven autoimmune disease. JUN activation drives T cells away from a CXCL13⁺ phenotype and towards an IL-22⁺ phenotype (see e.g. **Fig. 3i and Fig. 3k**, which shows deletion of the JUN gene upregulates CXCL13 production and downregulates IL-22 production).

[0096] In certain aspects, the present disclosure provides a transcription factor— AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and/or BATF—for treatment of autoimmune disease. The

transcription factor can suppress the generation of Tph/Tfh cells and may inhibit disease activity in autoantibody-associated autoimmune diseases.

[0097] According to some embodiments, the present disclosure provides a vector including the transcription factor. The vector may be comprised of, or derived from, a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector. In some aspects the vector is a VSV-G pseudotyped lentivirus. In other aspects, the transcription factor or vector are part of a composition. The composition may also include a carrier, which may be a pharmaceutically acceptable carrier.

[0098] In certain aspects, the present disclosure provides a method of preventing CXCL13+ Tph/Tfh cell generation or differentiation. The method comprises, consists essentially of, or consists of contacting a cell or cell population (i.e. a T cell population) with one or more transcription factor, vectors, or compositions including the transcription factor, thereby preventing CXCL13+ Tph/Tfh cell generation or differentiation. The transcription factors may be selected from AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and BATF. In these methods, the transcription factor, or vector or composition including the transcription factor, is contacted with the T cell populations. Application of the transcription factors results in the transcription factor to be overexpressed in the cell population. Contacting may occur *ex vivo*, *in situ*, *in vitro* or *in vivo*. In some embodiments, the T cells populations are CD4+ or CD8+ cells. The T cells may be a CD4+ subset selected from Th1, Th17, Tfh, and/or Tph cells. The T cells may be a CD8+ subset selected from T_{CM}, T_{EM}, and/or T_{EMRA} cells. The T cell populations may be antigen specific. With the treatment of the T-cell populations with a transcription factor described herein, the T-cell populations may be reprogrammed towards an IL-22+ phenotype and/or away from a B-cell helper phenotype.

[0099] In certain aspects, overexpression of select transcription factors—naturally occurring or mutants—decreases CXCL13 when overexpressed in a cell or population of cells. Transcription factors which decrease CXCL13 when overexpressed are found below in Table 1, which includes DNA sequences for *AHR* and *JUN*, and mutants of each. As shown in Table 1, the mutants can be mutants with constitutive activity, or mutants with loss of a

transactivation domain (TAD). In still other embodiments, the transcription factors may have other mutants which decrease CXCL13 activity.

[0100] Table 1. Transcription factors that decrease CXCL13 when overexpressed.

Name and Description (SEQ ID NO:)	DNA Sequences
AHR (SEQ ID NO:)	ATGAACAGCAGCAGCGCCAACATCACCTACGCCAGTCGCAA GCGGCGGAAGCCGGTGCAGAAAACAGTAAAGCCAAATCCCAG CTGAAGGAATCAAGTCAAATCCTTCCAAGCGGCATAGAGAC CGACTTAATACAGAGTTGGACCGTTTGGCTAGCCTGCTGCCT TTCCCACAAGATGTTATTAATAAGTTGGACAACTTTCAGTT CTTAGGCTCAGCGTCAGTTACCTGAGAGCCAAGAGCTTCTTT GATGTTGCATTAAAATCCTCCCCTACTGAAAGAAACGGAGG CCAGGATAACTGTAGAGCAGCAAATTTTCAGAGAAGGCCTGA ACTTACAAGAAGGAGAATTCTTATTACAGGCTCTGAATGGCT TTGTATTAGTTGTCACTACAGATGCTTTGGTCTTTTATGCTTC TTCTACTATACAAGATTATCTAGGGTTTCAGCAGTCTGATGT CATACATCAGAGTGTATATGAACTTATCCATACCGAAGACCG AGCTGAATTTTCAGCGTCAGCTACACTGGGCATTAAATCCTTC TCAGTGTACAGAGTCTGGACAAGGAATTGAAGAAGCCACTG GTCTCCCCCAGACAGTAGTCTGTTATAACCCAGACCAGATTC CTCCAGAAAACCTCTCCTTTAATGGAGAGGGTGCTTCATATGTC GTCTAAGGTGTCTGCTGGATAATTCATCTGGTTTTCTGGCAA TGAATTTCCAAGGGAAGTTAAAGTATCTTCATGGACAGAAA AAGAAAGGGAAAGATGGATCAATACTTCCACCTCAGTTGGC TTTGTTTTCGATAGCTACTCCACTTCAGCCACCATCCATACTT GAAATCCGGACCAAAAATTTTATCTTTAGAACCAAAACACAA ACTAGACTTCACACCTATTGGTTGTGATGCCAAAGGAAGAAT TGTTTTAGGATATACTGAAGCAGAGCTGTGCACGAGAGGCTC AGGTTATCAGTTTATTCATGCAGCTGATATGCTTTATTGTGCC GAGTCCCATATCCGAATGATTAAGACTGGAGAAAGTGGCAT GATAGTTTTCCGGCTTCTTACAAAAACAACCGATGGACTTG GGTCCAGTCTAATGCACGCCTGCTTTATAAAAATGGAAGACC AGATTATATCATTGTAACCTCAGAGACCACTAACAGATGAGG AAGGAACAGAGCATTTACGAAAACGAAATACGAAGTTGCCT TTTATGTTTACCACTGGAGAAGCTGTGTTGTATGAGGCAACC AACCCTTTTCTGCCATAATGGATCCCTTACCACTAAGGACT AAAAATGGCACTAGTGGAAGAAAGACTCTGCTACCACATCCAC TCTAAGCAAGGACTCTCTCAATCCTAGTTCCCTCCTGGCTGC CATGATGCAACAAGATGAGTCTATTTATCTCTATCCTGCTTC

	AAGTACTTCAAGTACTGCACCTTTTGAAAACAACCTTTTTTCAA CGAATCTATGAATGAATGCAGAAATTGGCAAGATAATACTG CACCGATGGGAAATGATACTATCCTGAAACATGAGCAAATT GACCAGCCTCAGGATGTGAACTCATTGCTGGAGGTCACCCA GGGCTCTTTCAAGATAGTAAAAACAGTGACTTGTACAGCATA ATGAAAAACCTAGGCATTGATTTTGAAGACATCAGACACAT GCAGAATGAAAAATTTTTCAGAAATGATTTTCTGGTGAGGT TGACTTCAGAGACATTGACTTAACGGATGAAATCCTGACGTA TGTC CAAGATTCTTTAAGTAAGTCTCCCTTCATACCTTCAGAT TATCAACAGCAACAGTCCTTGGCTCTGAACTCAAGCTGTATG GTACAGGAACACCTACATCTAGAACAGCAACAGCAACATCA CCAAAAGCAAGTAGTAGTGGAGCCACAGCAACAGCTGTGTC AGAAGATGAAGCACATGCAAGTTAATGGCATGTTTGAAAAT TGGAACCTAACCAATTCGTGCCTTTCAATTGTCCACAGCAA GACCCACAACAATATAATGTCTTTACAGACTTACATGGGATC AGTCAAGAGTTCCCCTACAAATCTGAAATGGATTCTATGCCT TATACACAGAACTTTATTTCTGTAAATCAGCCTGTATTACCA CAACATTCCAAATGTACAGAGCTGGACTACCCTATGGGGAG TTTTGAACCATCCCCATACCCCACTACTTCTAGTTTAGAAGA TTTTGTCACTTGTTTACAACCTTCCTGAAAACCAAAAGCATGG ATTAAATCCACAGTCAGCCATAATAACTCCTCAGACATGTTA TGCTGGGGCCGTGTCGATGTATCAGTGCCAGCCAGAACCTCA GCACACCCACGTGGGTCAGATGCAGTACAATCCAGTACTGC CAGGCCAACAGGCATTTTAAACAAGTTTCAGAATGGAGTTT TAAATGAAACATATCCAGCTGAATTAAATAACATAAATAAC ACTCAGACTACCACACATCTTCAGCCACTTCATCATCCGTCA GAAGCCAGACCTTTTCTGTATTTGACATCCAGTGTGA
CA-AHR (Constitutively Active AHR) (SEQ ID NO:)	ATGAACTCTTCATCCGCTAATATCACCTACGCATCACGGAAG CGGAGGAAACCGGTCCAAAAGACCGTTAAACCCATTCTGC AGAGGGTATCAAAAGCAACCCTAGTAAACGGCACCGCGACC GCTTGAACACGGAACCTGACCGACTTGCGTCTCTTCTCCCAT TCCCTCAAGATGTAATCAACAACTGGATAAGTTGAGCGTAT TGCGGCTGTCTGTTTCTTACTTGAGGGCAAAGTCATTCTTTGA CGTGCGCACTTAAAAGTAGTCCAACGGAACGGAATGGCGGTC AGGATAATTGCCGCGCCGCTAACTTTAGAGAGGGTCTGAATC TTCAGGAGGGCGAATTTCTCCTGCAGGCCCTTAACGGTTTTG TTCTCGTAGTGACCACAGACGCGTTGGTGTTTTACGCTAGTT CCACTATACAAGATTACTTGGGGTTTCAGCAATCAGATGTGA TTCATCAGTCTGTCTACGAACTCATCCATACTGAAGATAGGG CAGAATTCCAAAGACAGCTTCATTGGGCCTTGAATCCCAGTC AATGTACAGAAAGTGGCCAGGGTATCGAGGAAGCCACCGGT CTCCCGCAGACGGTTGTCTGCTATAATCCCGATCAGATCCCA CCTGAAAATTCACCCCTTATGGAACGGTGCTTTATTTGTGCG CTTCGATGCCTGCTGGACAACCTCTAGCGGTTTCTTGCGGATG AATTTCCAAGGGAAATTGAAGTACTTGACGGTCAGAAAAA

	AAAGGGTAAAGACGGGTCCATACTGCCCCACAGCTGGCTC TTTTTGCCATTGCTACCCCCCTGCAGAACGGAAGTAGCGGAA AAGACAGTGCCACGACCAGTACCCTGAGTAAGGACTCTCTG AATCCTTCCTCTTTGCTCGCTGCCATGATGCAACAGGATGAG TCCATATATTTGTATCCCGCCTCTAGCACTAGCAGCACAGCG CCATTCGAGAACAATTTTTTTAATGAAAGTATGAATGAATGT AGAAACTGGCAAGACAACACGGCTCCTATGGGAAACGATAC GATTCTTAAGCATGAACAAATTGACCAGCCCCAAGATGTAA ATAGTTTTGCAGGTGGCCACCCAGGGCTTTTCCAGGACAGTA AGAATAGTGATCTGTACTCAATCATGAAGAACCTTGGAATA GATTCGAGGACATCCGCCACATGCAAAATGAAAAGTTTTTC CGCAATGACTTTAGTGGGGAGGTCTGACTTCCGCGATATCGAC CTTACCGACGAGATACTCACGTACGTTTCCAGGATTCTCTTTCA AAGAGCCCTTTTATTCCTTCAGACTACCAACAACAACATCC CTGGCCCTCAACTCTTCATGTATGGTTCAGGAACACTTGCAC CTGGAACAACAGCAACAACACCACCAAAAACAGGTAGTAGT AGAACCACAGCAGCAACTCTGCCAAAAGATGAAGCACATGC AAGTTAACGGCATGTTTGAGAATTGGAAGTCTAATCAATTCG TACCTTTCAATTGTCCGCAACAAGATCCCCAGCAGTACAATG TTTTTACGGACTTGCATGGAATATCACAAGAATTCCTTACA AGTCCGAAATGGATTCCATGCCCTATACCCAAAACCTTCATCT CTTGTAATCAGCCAGTCCTGCCCCAGCACAGCAAGTGTACCG AATTGGATTACCCTATGGGGTCATTTGAACCTCACCTTACC CCACGACGTCTAGCCTCGAGGATTTTGTACATGCCTTCAGC TGCCTGAGAATCAAAAACATGGCCTGAACCCACAATCAGCG ATCATCACCCCGCAGACTTGCTACGCTGGTGTCTGTCAGTATG TATCAGTGTCAGCCAGAACCACAGCATACTCACGTTGGTCAA ATGCAATATAACCCAGTCTTGCCGGGACAACAAGCTTTTTTG AATAAGTTCCAGAATGGTGTCTTAACGAGACATACCCTGCG GAGTTGAATAACATAAACAATACGCAGACCACTACTCACCTT CAACCTTTGCACCATCCGTCTGAGGCTCGACCCTTTCCAGAT TTGACATCATCTGGCTTTCTGTAA
JUN (SEQ ID NO:)	ATGACTGCAAAGATGGAAACGACCTTCTATGACGATGCCCTC AACGCCTCGTTTCTCCCGTCCGAGAGCGGACCTTATGGCTAC AGTAACCCCAAGATCCTGAAACAGAGCATGACCCTGAACCT GGCCGACCCAGTGGGGAGCCTGAAGCCGCACCTCCGCGCCA AGAACTCGGACCTCCTCACCTCGCCCGACGTGGGGCTGCTCA AGCTGGCGTCGCCCCGAGCTGGAGCGCCTGATAATCCAGTCC AGCAACGGGCACATCACCACCACGCCGACCCCCACCCAGTT CCTGTGCCCCAAGAACGTGACAGATGAGCAGGAGGGCTTCG CCGAGGGCTTCGTGCGCGCCCTGGCCGAACCTGCACAGCCAG AACACGCTGCCCAGCGTCACGTCGGCGGCGCAGCCGGTCAA CGGGGCAGGCATGGTGGCTCCCGCGGTAGCCTCGGTGGCAG GGGGCAGCGGCAGCGGGCGGCTTCAGCGCCAGCCTGCACAGC GAGCCGCCGGTCTACGCAAACCTCAGCAACTTCAACCCAGG

	CGCGCTGAGCAGCGGCGGCGGGGCGCCCTCCTACGGCGCGG CCGGCCTGGCCTTTCCCGCGCAACCCAGCAGCAGCAGCAG CCGCCGACACCTGCCCCAGCAGATGCCCCGTGCAGCACCC GCGGCTGCAGGCCCTGAAGGAGGAGCCTCAGACAGTGCCCG AGATGCCCCGGCGAGACACCGCCCCCTGTCCCCATCGACATG GAGTCCCAGGAGCGGATCAAGGCGGAGAGGAAGCGCATGA GGAACCGCATCGCTGCCTCCAAGTGCCGAAAAAGGAAGCTG GAGAGAATCGCCCGGCTGGAGGAAAAAGTGAAAACCTTGAA AGCTCAGAACTCGGAGCTGGCGTCCACGGCCAACATGCTCA GGGAACAGGTGGCACAGCTTAAACAGAAAGTCATGAACCAC GTTAACAGTGGGTGCCAACTCATGCTAACGCAGCAGTTGCA AACATTTTGA
JUNdTAD (JUN mutant with loss of transactivation domain (TAD) (SEQ ID NO:)	ATGGTGACAGACGAGCAGGAGGGCTTTGCAGAGGGCTTCGT CCGCGCCTTGGCCGAGCTCCACTCACAGAACACCTTGCCTTC AGTGACTAGCGCGGCGCAGCCCGTGAACGGCGCAGGTATGG TGGCCCCCGCTGTGGCATCCGTTGCAGGGGGGTCCGGTAGTG GAGGCTTTTCTGCTTCTCTGCACTCTGAACCCCCAGTCTACGC CAATCTGTCCAACTTTAATCCTGGTGCCTTAGCTCAGGCGG GGGAGCGCCAGCTACGGAGCGGCTGGCCTGGCTTTCCCCG CTCAGCCTCAGCAACAGCAGCAGCCTCCCCACCATTTGCCTC AGCAGATGCCGGTACAGCACCCCCGGTTACAGGCCCTCAAA GAGGAACCACAGACAGTTCCCGAGATGCCCGGAGAAACACC CCCCTTATCACCTATCGACATGGAATCCCAGGAGAGAATCAA GGCCGAACGCAAACGCATGAGGAACCGTATCGCAGCGAGCA AATGTAGAAAGCGGAAGTTGGAGCGGATTGCAAGACTCGAG GAAAAAGTTAAAACACTTAAAGCACAGAATAGCGAACTCGC TTCTACCGCTAACATGCTGCGCGAGCAGGTAGCCCAGCTGAA GCAGAAAGTTATGAATCATGTGAACTCAGGTTGCCAGCTCAT GTTGACGCAGCAACTTCAGACATTCTGA

[0101] In certain aspects, the present disclosure also provides a method of using transcription factors to inhibit functions of autoimmunity-associated T cell populations—T follicular helper (Tfh) cells and T peripheral helper (Tph) cells—in a subject in need. In some aspects, the transcription factors are selected from AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and BATF. In another aspect, the present disclosure also provides a method of using the transcription factor mutants found in Table 1 to inhibit functions of autoimmunity-associated T cell populations in a subject in need. In some other aspects, other mutants of transcription factors AHR, JUN, FOS, AFT3, FOSL1, FOSL2, and/or BATF are used. In yet other aspects, the present disclosure provides a method directed to preventing the expansion of pathological

T cells found in autoimmune diseases using the transcription factors. These methods comprise, consist essentially of, or consist of administering a transcription factor, vectors, or compositions including a transcription factor mutant to a subject in need, thereby inhibiting functions of autoimmunity-associated T cell populations or preventing the expansion of pathological T cells. Application of the transcription factors results in the transcription factor to be overexpressed in the subject. Administration may be systemic or targeted. In some embodiments, administration is intravenous, subcutaneous, oral, or topical. T cells with the capacity to help B cells, Tfh cells and Tph cells, are highly expanded in patients with autoantibody-associated autoimmune diseases such as lupus and rheumatoid arthritis. The transcription factor can target key biological markers specific to the pathological T cells, and inhibiting either function or fit of these cells.

[0102] In another aspect, the present disclosure provides a method to treating an autoimmune disease using transcription factors (AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and/or BATF). The method comprises, consists essentially of, or consists of obtaining a population of T cells from the subject, treating the population of T cells with a transcription factor selected from AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and/or BATF, or vector or composition including the transcription factor, and administering the treated population of T cells to the subject, thereby treating the autoimmune disease. In another aspect, the present disclosure also provides a method to treating an autoimmune disease using the transcription factor mutants found in Table 1, which includes obtaining a population of T cells from the subject, treating the population of T cells with a transcription factor mutant, or vector or composition including the transcription factor mutant, and administering the treated population of T cells to the subject, thereby treating the autoimmune disease. In some aspects, other mutants of the transcription factors are used.

[0103] In certain aspects, the present disclosure also provides a method of using both an AHR agonist and transcription factor (AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and/or BATF and/or transcription factor mutant) to treat an autoimmune disease in a subject in need. The method comprises, consists essentially of, or consists of administering both an AHR agonist and transcription factor to the subject, thereby treating the autoimmune disease. In some embodiments transcription factor is JUN. The AHR agonist and the transcription factor

may be administered consecutively, or they may be administered sequentially. In some embodiments, the AHR and/or transcription factor may be administered intravenously, subcutaneously, orally, or topically.

[0104] The autoimmune disease may be selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis. The subject in need may be a human.

[0105] crRNP complexes—compositions and methods

[0106] Finally, in some aspects provided herein are methods and compositions using gRNA targeting a gene selected from *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, or *STAT2*, wherein the gRNA has a sequence according to one of the sequences in **Table 5**. The gRNAs are designed for use in a CRISPR system. In one aspect, the CRISPR system is a CRISPR-Cas9-gRNA ribonucleoprotein (crRNP) complex comprising the gRNA for targeting at least one of *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, and/or *STAT2*. In another aspect, the CRISPR system includes other RNAs, for example crRNAs for targeting at least one of *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, and/or *STAT2*. As provided herein, when knocked out, the genes in **Table 5** can decrease CXCL13 production in T cells

[0107] Another embodiment is a vector comprising a crRNP complex as described herein, optionally wherein the vector is, comprises, or is derived from a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector. In one aspect, the vector is a VSV-G pseudotyped lentivirus. In another aspect, the vector is a lipid nanoparticle. In yet another aspect, the vector is a gamma retrovirus.

[0108] Another embodiment is a composition comprising a crRNP complex as described herein, or the vector as described herein, and a carrier. Optionally the carrier is pharmaceutically acceptable.

[0109] In one aspect herein is described a method of decreasing CXCL13 production in a cell. The method comprises, consists essentially of, or consists of contacting a T-cell or cell population with the crRNP complex, vector, or composition as described herein, thereby decreasing CXCL13 production. Contacting may occur *ex vivo*, *in situ*, *in vitro* or *in vivo*. In some embodiments the crRNP complex comprises a gRNA for targeting at least one of *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, and/or *STAT2* and the target gene in the cell is knocked out upon introduction of the crRNP complex. In some embodiments, the T cells populations are CD4⁺ or CD8⁺ cells. The T cells may be a CD4⁺ subset selected from Th1, Th2, TH17, Tfh, Tph, iTreg, Th9, Tr1, and/or Th22 cells. The T cells may be a CD8⁺ subset selected from T_{CM}, T_{EM}, and/or T_{EMRA} cells. The T cell populations may be antigen specific. With the treatment of the T-cell populations with a crRNP complex as described herein, the T-cell populations may be reprogrammed towards an IL-22⁺ phenotype.

[0110] In one aspect herein is described a method of treating an autoimmune disease in a patient in need. The method comprises, consists essentially of, or consists of administering an effective amount of the crRNP complex, vector, or composition as described herein to a subject in need, thereby treating the autoimmune disease. The target gene is knocked out. In one embodiment, the administering is done intravenously, subcutaneously, orally, or topically. In one embodiment the patient in need is human.

[0111] The autoimmune disease may be selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis. The subject in need may be a human.

[0112] Compositions, Subject Population and Methods of Administration

[0113] As used herein “patient” and “subject” are used interchangeably.

[0114] In the methods described herein, the term “subject” refers includes but is not limited to a subject at risk of an immune disorder, or autoimmune response, disorder or disease, as well as a subject that has already developed an immune disorder, or autoimmune response,

disorder or disease. Such subjects, include mammalian animals (mammals), such as a non-human primate (apes, gibbons, gorillas, chimpanzees, orangutans, macaques), a domestic animal (dogs and cats), a farm animal (poultry such as chickens and ducks, horses, cows, goats, sheep, pigs), experimental animal (mouse, rat, rabbit, guinea pig) and humans. Subjects include animal disease models, for example, mouse and other animal models of immune disorders, or autoimmune response, disorder or disease known in the art.

[0115] In some embodiments in the methods described herein, the subject is an adult human. In other embodiments the subject is a juvenile human.

[0116] In the methods described herein, administering an AHR agonist, transcription factor, crRNP complex, vector, and/or composition can be accomplished by any method known in the art suitable for the particular type of agonist and formulation selected. Suitable routes of administration include without limitation oral, parenteral (including intramuscular, subcutaneous, intradermal, intravascular, intravenous, intraarterial, intraarticular, intramedullary and intrathecal), intraperitoneal, and topical (including dermal/epicutaneous, transdermal, mucosal, transmucosal, intranasal (e.g., by nasal spray or drop), intraocular (e.g., by eye drop), pulmonary (e.g., by inhalation), buccal, sublingual, rectal and vaginal). Furthermore, AHR agonists can be formulated for administration by oral inhalation.

[0117] In some aspects, in the methods described herein, the administering to a subject in need can be done intravenously or subcutaneously. In other aspects, in the methods described herein, the administering to a subject in need can be topical.

[0118] In some aspects of the methods described herein, an additional therapeutic agent is administered to the subject in need. In one embodiment, the additional therapeutic agent is anifrolumab.

[0119] Doses in the methods described herein can be based upon current existing protocols, empirically determined, using animal disease models or optionally in human clinical trials. Initial study doses can be based upon animal studies, e.g. a mouse, and the amount treatment or agent disclosed herein administered in an amount that is determined to be effective. Exemplary non-limiting amounts (doses) are in a range of about 0.1 mg/kg to about 100

mg/kg, and any numerical value or range or value within such ranges. Greater or lesser amounts (doses) can be administered, for example, 0.01-500 mg/kg, and any numerical value or range or value within such ranges. The dose can be adjusted according to the mass of a subject, and will generally be in a range from about 1-10 ug/kg, 10-25 ug/kg, 25-50 ug/kg, 50-100 ug/kg, 100-500 ug/kg, 500-1,000 ug/kg, 1-5 mg/kg, 5-10 mg/kg, 10-20 mg/kg, 20-50 mg/kg, 50-100 mg/kg, 100-250 mg/kg, 250-500 mg/kg, or more, two, three, four, or more times per hour, day, week, month or annually. A typical range will be from about 0.3 mg/kg to about 50 mg/kg, 0-25 mg/kg, or 1.0-10 mg/kg, or any numerical value or range or value within such ranges.

[0120] The maximum tolerable dose of the methods described herein can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a patient in practicing the present disclosure.

[0121] Doses can vary and depend upon whether the treatment is prophylactic or therapeutic, whether a subject has previously had cancer or an infection, immune disorder, or autoimmune response, disorder or disease, the onset, progression, severity, frequency, duration probability of or susceptibility of the symptom, condition, pathology or complication, the treatment protocol and compositions, the clinical endpoint desired, the occurrence of previous or simultaneous treatments, the general health, age, gender, race or immunological competency of the subject and other factors that will be appreciated by the skilled artisan. The skilled artisan will appreciate the factors that may influence the dosage and timing required to provide an amount sufficient for providing a therapeutic or prophylactic benefit.

[0122] In the methods described herein, the route, dose, number and frequency of administrations, treatments, and timing/intervals between treatment and disease development can be modified. In certain embodiments, a desirable treatment of the present disclosure will elicit robust, long-lasting immunity against cancer or an infection, immune disorder, or autoimmune response, disorder or disease. Thus, in certain embodiments, disclosure methods,

uses and compositions provide long-lasting immunity to cancer or an infection, immune disorder, or autoimmune response, disorder or disease.

[0123] As used herein the term “pharmaceutically acceptable” and “physiologically acceptable” mean a biologically acceptable formulation, gaseous, liquid or solid, or mixture thereof, which is suitable for one or more routes of administration, *in vivo* delivery or contact. Such formulations include solvents (aqueous or non-aqueous), solutions (aqueous or non-aqueous), emulsions (e.g., oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or *in vivo* contact or delivery. Aqueous and non-aqueous solvents, solutions and suspensions may include suspending agents and thickening agents. Such pharmaceutically acceptable carriers include tablets (coated or uncoated), capsules (hard or soft), microbeads, powder, granules and crystals. Supplementary active compounds (e.g., preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions.

[0124] Compositions (i.e. pharmaceutical compositions) described herein can be formulated to be compatible with a particular route of administration. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes. Exemplary routes of administration for contact or *in vivo* delivery which a composition can optionally be formulated include inhalation, respiration, intranasal, intubation, intrapulmonary instillation, oral, buccal, intrapulmonary, intradermal, topical, dermal, parenteral, sublingual, subcutaneous, intravascular, intrathecal, intraarticular, intracavity, transdermal, iontophoretic, intraocular, ophthalmic, optical, intravenous (i.v.), intramuscular, intraglandular, intraorgan, or intralymphatic.

[0125] Formulations suitable for parenteral administration comprise aqueous and non-aqueous solutions, suspensions or emulsions of the active compound, which preparations are typically sterile and can be isotonic with the blood of the intended recipient. Non-limiting illustrative examples include water, saline, dextrose, fructose, ethanol, animal, vegetable or synthetic oils.

EXAMPLE

[0126] The following Example is provided to illustrate and not limit this disclosure.

[0127] Expansion of B cell-helper T cells including T follicular helper (**Tfh**) and T peripheral helper (**Tph**) cells is a prominent feature of systemic lupus erythematosus (**SLE**), a prototypical autoimmune disease with broad autoantibody production. Human Tfh and Tph cells are marked by high production of the B cell chemoattractant CXCL13, yet regulation of T cell CXCL13 production and the relationship between a CXCL13⁺ state and other differentiated T cell states remain largely undefined. Applicants identify a dramatic imbalance in CD4 T cell phenotypes in systemic lupus erythematosus patients, with expansion of PD-1⁺/ICOS⁺ CXCL13⁺ T cells and reduction of CD96^{hi} IL-22⁺ T cells. Using CRISPR screens, the aryl hydrocarbon receptor (**AHR**) was identified as a potent negative regulator of CXCL13 production by human CD4 T cells. Transcriptomic, epigenetic, and functional studies demonstrate that AHR coordinates with AP-1 family member JUN to prevent CXCL13⁺ Tph/Tfh cell differentiation and promote an IL-22⁺ phenotype. Type I interferon (**IFN**), a pathogenic driver of SLE, opposes AHR and JUN to promote T cell production of CXCL13. The data presented herein places CXCL13⁺ Tph/Tfh cells on a polarization axis opposite from Th22 cells and reveals AHR, JUN, and IFN as key regulators of these divergent T cell states.

[0128] As demonstrated herein, mass cytometry immunophenotyping revealed a marked imbalance in CD4 T cell phenotypes in systemic lupus erythematosus patients, with expansion of CXCL13⁺ Tfh/Tph cells and specific reduction of CD96^{hi} IL-22⁺ T cells. Without wishing to be bound by any particular theory, Inventors hypothesized that a common regulator might drive this skewed balance. Using CRISPR screens of human CD4 T cells, the present disclosure identifies the transcription factor aryl hydrocarbon receptor (**AHR**) as a central regulator of an axis of T cell polarization with CXCL13⁺ and IL-22⁺ states at opposing ends. Transcriptomic, epigenetic, CUT&RUN, and functional studies provided herein demonstrated that 1) AHR activation drives T cells away from a CXCL13⁺ phenotype and towards an IL-22⁺ phenotype, and 2) AHR engages the AP-1 family member JUN to regulate CXCL13⁺ and IL-22-associated phenotypes. Treatment of T cells from SLE patients with an AHR agonist reduced the frequency of PD-1⁺ Tph cells. In contrast, type I interferon

(IFN-I), a central mediator in SLE, represses AHR activation in T cells and synergizes with AHR inhibition to boost CXCL13 production and promote a Tph cell phenotype. These results reveal AHR, JUN, and IFN-I as regulators of a previously unrecognized CXCL13 <— > IL-22 polarization axis and highlight an imbalance of this axis in SLE.

Results

[0129] Immunophenotyping reveals an altered balance of Tph/Tfh cells versus CD96^{hi} Th22 cells in systemic lupus erythematosus patients

[0130] Expansion of B cell-helper CD4⁺ T cells is one characteristic feature of systemic lupus erythematosus, yet the scope of abnormalities in circulating CD4⁺ T cell phenotypes in SLE patients is incompletely defined. Mass cytometry was used to broadly evaluate the CD4⁺ T cell immunophenotypes of 19 patients with SLE and 19 non-autoimmune controls. Patients with SLE ranged in age and gender, and anti-rheumatic drugs taken. All but one patient took at least one anti-rheumatic drug. Differential expression analysis comparing memory CD4⁺ T cells from SLE patients and controls identified increased expression of PD-1, KI67, and TBET in cells from SLE patients, and increased expression of CD96 in cells from controls (t-test with Bonferroni-corrected p-value<0.05) (**Fig. 1a**). Visualization of memory CD4⁺ T cells by uniform manifold approximation and projection (UMAP) showed differing patterns of expression of immunomodulatory receptors, with overlapping expression of immune checkpoint receptors PD-1 and TIGIT, as well as co-stimulatory molecule ICOS, yet distinct expression of inhibitory molecule CD96 in a separate region (**Fig. 1b**). Co-varying neighborhood analysis was used to identify regions of the UMAP that are enriched with cells from SLE patients compared to controls³¹. Consistent with differential expression results, CNA analysis indicated an enrichment in neighborhoods containing PD-1/ICOS/TIGIT⁺ cells in SLE patients, while neighborhoods containing CD96^{hi} cells were enriched in controls (global p-value <0.001, neighborhood false discovery rate (FDR)<0.05, **Fig. 1c**).

[0131] As a complementary approach, the cells were clustered by Louvain-based clustering. Two clusters were identified as significantly enriched in SLE patients and 1 cluster was identified as significantly depleted in SLE patients (odds ratio (OR) >2 or <0.5). This approach confirmed enrichment of PD1/ICOS⁺ cells (cluster 8, OR=2.8, p<0.0002) in SLE

patients, which also expressed CTLA4, KI67, and TBET as well as an increase in TIGIT⁺ FOXP3⁺ T regulatory cells (**Tregs**) (cluster 2, OR 2.1, $p < 0.0001$) (**Figs. 1c-1e; Fig. 7a**). In contrast, a cluster containing CD96^{hi} cells was significantly depleted in SLE patients compared to controls (cluster 7, OR=0.3, $p < 0.0001$). Across all patients, the abundance of the PD-1⁺/ICOS⁺ cluster was inversely correlated with the CD96^{hi} cluster (**Fig. 1f**). Moreover, the abundance of PD-1⁺/ICOS⁺ cluster was positively associated with SLE disease activity as measured by SLE disease activity index 2000 (**SLEDAI-2K**) ($p = 0.007$, $r = 0.6$) and with anti-double stranded DNA (**dsDNA**) antibody level ($p = 0.033$, $r = 0.49$) in this SLE cohort (**Figs. 7b, 7c**). In contrast, the abundance of CD96^{hi} cells (cluster 7) did not correlate with disease activity or anti-dsDNA antibody level. Neither population was clearly associated with prednisone dose or immunosuppressive drug treatment (**Figs. 7d, 7e**).

[0132] To define potential functions of the cell populations altered in SLE patients, bulk RNA-Seq was performed of sorted subsets of CD4⁺ T cells based on the markers highlighted by immunophenotyping. These subsets included PD-1^{hi} cells (divided into CXCR5⁺ Tfh cells and CXCR5⁻ Tph cells), CD96^{hi} cells, and CD25⁺ CD127⁻ Tregs. Cell subsets were sorted from peripheral blood mononuclear cells (**PBMC**) of patients with SLE ($n = 6$) and control patients ($n = 5$) (**Fig. 8a**). By principal component analysis (PCA), cell subsets generally clustered together, with a secondary separation of cells from SLE patients vs controls (**Fig. 8b**). Differential expression analyses identified gene sets upregulated in each cell population. Both PD-1^{hi} CXCR5⁻ and PD-1^{hi} CXCR5⁺ populations expressed genes characteristic of Tph/Tfh cells including *MAF*, *CXCL13*, and *PDCD1* (**Figs. 1g, 1h**), confirming their identities as Tph cells and Tfh cells respectively, as previously described⁹. CD96^{hi} cells showed a distinct pattern of gene expression, with selective upregulation of *CCR6*, *RORC*, *IL22*, and *AHR* but not *IL17A* or *IL17F*, suggesting that CD96^{hi} cells represent IL-22-producing Th22 cells (**Fig. 1g; Fig. 8d**). Consistent with this idea, CD96^{hi} cells showed the highest enrichment score for a Th22-associated gene set³² (**Fig. 1h**). This pattern was validated in PBMC from additional SLE patients, showing that CD96^{hi} cells expressed higher transcript levels of *IL22* compared to PD-1^{hi} Tfh and Tph cells, while Tfh and Tph cells expressed significantly higher levels of *CXCL13* (**Fig. 1i**). Similarly, there was also a pattern of distinct localization of cells with signatures of CD96^{hi} cells or Tph/Tfh cells in a published scRNA-Seq dataset of PBMC from pediatric SLE patients and controls⁹¹ (**Fig. 8d**).

Moreover, *in vitro* stimulation confirmed that CD96^{hi} cells sorted from PBMC of healthy donors frequently produced IL-22 protein. While total IL-22 production was comparable in CD96^{hi} cells and CD96^{low} CCR6⁺ CXCR3⁻ Th17 cells, CD96^{hi} cells were more often single producers of IL-22 (IL-22⁺ IL-17A⁻) and less often single producers of IL-17A (IL-22⁻ IL-17A⁺) compared to traditional CD96^{low} Th17 cells (**Fig. 1j**). IFN- γ and TNF production were comparable in CD96^{hi} cells and Tfh/Tph cells (**Fig. 8e**). Flow cytometry also demonstrated higher expression of CCR6, a receptor expressed on Th17 and Th22 cells, on CD96^{hi} T cells compared to Tph and Tfh cells, while CXCR3 was similarly expressed by all 3 populations (**Fig. 8f**). CXCR5 was minimally expressed on CD96^{hi} cells. Together, these results indicate that SLE patients show a marked expansion of CXCL13⁺ Tfh/Tph cells and a concurrent reduction in an IL-22-producing CD96^{hi} T cell population.

[0133] AHR and other transcription factors are negative regulators of CXCL13 production and Tph differentiation

[0134] The imbalance in CXCL13⁺ versus IL-22⁺ T cells in SLE patients suggested that CXCL13 and IL-22 may exist on opposite sides of an axis of T cell differentiation. Without wishing to be bound by any particular theory, Inventors hypothesized that specific transcriptional regulators may control such a differentiation axis. To identify molecular drivers of these distinct phenotypes, a CRISPR depletion screening assay in memory CD4⁺ T cells was conducted to identify regulators of CXCL13 and IL22 production. Pools of four guide RNA (gRNA) targeting 86 genes and 10 controls were synthesized in arrayed format in a 96-well plate and complexed with purified Cas9 protein *in vitro* to form CRISPR-Cas9 ribonucleoproteins (**crRNPs**). Multiplexed pools of 4 gRNAs per gene were chosen to improve depletion efficiency and minimizes off-target effects³³⁻³⁵. These 86 target genes were selected from genes upregulated in Tph/Tfh cells in the current RNA-Seq dataset (**Fig. 1g**) or in previously published RNA-Seq datasets of RA synovial T cells¹⁶, as well as from genes previously correlated with CXCL13 expression, however only the target genes of particular interest are included in **Table 2** below^{19,36}. **Table 2** further includes gRNAs for AP-1 family member which was also a CRISPR array screen target. In **Table 2** the genes of particular interest are those that resulted in increased CXCL13 levels when knocked out. This indicates genes that could reduce CXCL13 levels when overexpressed in a cell.

[0135] Memory CD4⁺ T cells from 2 healthy donors were nucleofected with the arrayed crRNP libraries and cultured for 4 days in the presence or absence of TGF- β , a known inducer of CXCL13 production³⁶ (**Fig. 2a**). CXCL13 in supernatants from the two replicate screens were quantified. Deletion of *CD3* or *LCP2* (also called *SLP-76*), two proteins critical for TCR (T-Cell receptor) signaling included as positive controls, inhibited CXCL13 production (**Fig. 2b**), reflecting the need for TCR stimulation and validating the screen performance. Cbl Proto-Oncogene B (*CBLB*), an E3 ubiquitin ligase, and the aryl hydrocarbon receptor (*AHR*) were the top regulators of CXCL13 production; deletion of either of these genes strongly upregulated CXCL13 production, with similar results in the absence or presence of TGF- β (**Fig. 2b; Fig. 9a**).

[0136] AHR has a recognized role in regulating IL-22 production and Th22 differentiation^{25,37,38}. Utilizing novel guides (that were not part of the original screen), CRISPR deletion of AHR in multiple donors confirmed that AHR deletion increases memory T cell CXCL13 production (**Fig. 9b; Fig. 2c**). This increase was further enhanced by the presence of TGF- β (**Fig. 9c**). In parallel, deletion of AHR decreased production of IL-22, consistent with prior studies³⁸ (**Fig. 2c**). As with deleting AHR, the AHR inhibitor CH-223191 (**AHRinh**) also increased CXCL13 and decreased IL-22 production from total CD4 T cells, with similar effects in purified naïve and memory CD4⁺ cells (**Figs. 2d; Figs. 9d, 9e**). In contrast, AHR agonist 2,3,7,8-tetrachlorodibenzodioxin (**TCDD**) decreased CXCL13 production and increased IL-22 production. Similar results were obtained with a second AHR agonist, 6-formylindolo(3,2-b)carbazole (**FICZ**), and a second AHR inhibitor, GNF-351 (**Fig. 9f**). Neither CRISPR deletion nor pharmacological modulation of AHR substantially affected IFN- γ production by CD4 T cells (**Fig. 9g**). Further, AHR agonism did not suppress *in vitro* polarization of CD4 T cells into IFN- γ ⁺ Th1 cells, IL-4⁺ Th2 cells, or IL-17A⁺ Th17 cells, indicating that AHR selectively suppresses CXCL13 production (**Fig. 9h**).

[0137] T cell differentiation into Tfh cells or Th17 cells can be influenced by multiple cytokines or extrinsic factors^{10,39,40}. Therefore, Inventors evaluated whether other cytokines or signals associated with Tfh or Th17/Th22 differentiation similarly influenced CXCL13 production by CD4⁺ T cells. Among these factors, only TGF- β and modulation of AHR activity altered CXCL13 and IL-22 production (**Fig. 9i**). TGF- β increased CXCL13

production and decreased IL-22 and IFN γ production. In addition to cytokine production, AHR and TGF- β also controlled key features of the Tfh/Tph cell surface phenotype. AHR inhibition with CH-223191 increased ICOS expression and decreased CD96 expression in CD4⁺ T cells from control donors, while AHR activation with TCDD decreased ICOS expression and increased CD96 (**Fig. 2e**). AHR modulation altered similar functional and phenotypic features in human CD8⁺ T cells. Both CRISPR deletion and pharmacologic inhibition of AHR increased CXCL13 production and decreased IL-22 production by CD8⁺ T cells, while AHR activation decreased CXCL13 production and increased IL-22 production, with no effect on IFN- γ production (**Figs. 9j, 9k**). AHR inhibition also inhibited CD96 expression and upregulated ICOS expression in CD8⁺ T cells (**Fig. 9l**). Together, these observations suggest that CXCL13 production and IL-22 production lie on opposites ends of a T cell differentiation axis that is regulated by AHR and TGF- β .

[0138] The effects of AHR activity on CXCL13 and IL-22 in the setting of persistent or repeated TCR activation were also investigated. Memory and naïve CD4⁺ T cells were stimulated weekly for 3 weeks in the presence or absence of AHR modulators. Under neutral conditions, repeated stimulation of memory or naïve CD4⁺ T cells induced progressively higher production of CXCL13 over time, while production of IL-22 and IFN- γ remained stable over 3 weeks (**Fig. 10a**). CXCL13 production was amplified in the presence of AHR inhibitor, while AHR agonism blunted the rise in CXCL13 production over time, with similar results obtained using either memory or naïve CD4⁺ T cells.

[0139] TGF- β and AHR control CXCL13-associated transcriptomic features

[0140] To determine broader transcriptomic changes associated with the CXCL13⁺ phenotype induced by TGF- β and AHR inhibition, a single cell RNA-Seq (**scRNA-Seq**) analysis was performed on memory CD4⁺ T cells from 3 donors after 2 weeks of culture with DMSO, TGF- β +DMSO, TGF- β +AHR agonist (TCDD), or TGF- β +AHR inhibitor (CH-223191). By UMAP visualization, cells treated with TGF- β or TGF- β +AHR inhibitor were spatially separated from cells not treated with TGF- β (**Fig. 2f**). Strikingly, cells treated with TGF- β +AHR agonist TCDD clustered together with cells that were not treated with TGF- β , suggesting that AHR agonism inhibited the transcriptomic program associated with chronic

TGF- β treatment in these cultures. A gene signature of Tph cells defined from RA synovial tissue, the site where Tph cells were originally defined was used to assess whether cells in the TGF- β +AHR inhibitor or TGF- β +DMSO conditions upregulated a gene signature of prototypical Tph cells¹⁶. Results indicate T cells cultured with TGF- β +AHR inhibitor or TGF- β +DMSO co-localized in a specific region of the UMAP demonstrating the highest Tph signature (**Fig. 2g**). To compare these *in vitro* differentiated cells to *ex vivo* Tph and Tfh cells obtained from rheumatoid arthritis synovial tissue, the cells from these treatment conditions were mapped onto a reference map of T cells obtained from 70 RA synovial tissue biopsies¹⁷ using Symphony⁴¹. The RA synovial T cell dataset contained 2 clusters marked by high expression of *CXCL13* that represented synovial Tph cells (cluster 9) and Tfh+Tph cells (cluster 5)⁴², with high expression of genes including *CXCL13*, *PDCD1*, *ICOS*, *CTLA4*, *IL21*, and *MAF* (**Figs. 2h–2j**). T cells treated with TGF- β +AHR inhibitor or TGF- β +DMSO preferentially mapped to the synovial Tph cell cluster (cluster 9), as well as one proliferating cluster (cluster 12) and a cluster with high IFN signature (cluster 14), while DMSO-treated cells and TGF- β +AHR agonist-treated cells preferentially mapped to a different T cell cluster (cluster 3) separate from the *CXCL13*⁺ region (**Figs. 2i, 2j**).

[0141] To further characterize the transcriptional programs bulk RNA-Seq data at different timepoints was generated. In T cell cultures stimulated for 1 or 2 weeks, *CXCL13* expression was strongly induced by TGF- β +AHR inhibitor, especially after 2 weeks (**Fig. 10b**). Consistent with scRNA-Seq data, bulk RNA-Seq analyses of naïve CD4⁺ T cells stimulated in the presence of TGF- β +AHR inhibitor were enriched for a Tph signature compared to cells stimulated with TGF- β +TCDD (**Fig. 10c**). Memory T cells showed a similar direction of enrichment though a less robust change, consistent with the greater plasticity of naïve T cells. Comparing transcriptomes of memory CD4⁺ T cells from the strongest *CXCL13*-inducing condition (TGF- β +AHR inhibitors for 2 weeks) versus the weakest *CXCL13*-inducing condition (TCDD only for 1 week) showed a significant enrichment of Tph-associated genes in the TGF- β +AHR inhibitor condition and an enrichment for CD96^{hi}-associated genes in the AHR agonist condition (**Fig. 2k**). The ability of TGF- β to promote a Tph cell signature was strongly influenced by AHR activity in that TGF- β treatment strongly induced a Tph

signature when AHR was inhibited yet could not induce a Tph signature when AHR was activated, consistent with scRNA-Seq results (**Fig. 10d**).

[0142] A strong interaction between TGF- β and AHR at the protein level across diverse T cell subsets was confirmed. In the presence of TGF- β , both CRISPR deletion of AHR and AHR inhibition induced CXCL13 production from multiple sorted T cell subsets, including Tph cells, Tfh cells, Th17 cells, Th1 cells, and naïve CD4⁺ T cells (**Figs. 11a, 11b**). The ability of AHR inhibition to induce CXCL13 production depended on the presence of TGF- β for most cell subsets; however, Tph cells showed a unique ability to produce CXCL13 with AHR inhibition alone without co-treatment with TGF- β . Without wishing to be bound by any particular theory, Inventors hypothesized that Tph cells may have been exposed to TGF- β *in vivo* before isolation, obviating the need for exogenous TGF- β . In support of this hypothesis, Tph cells isolated from the blood of systemic lupus erythematosus patients showed upregulation of a TGF- β gene signature compared to other T cell subsets, suggesting that these cells may have recently responded to TGF- β *in vivo* (**Fig. 11c**). Both TGF- β and AHR inhibition reduced IL-22 production from CD96^{hi} and Th17 cells, yet AHR activation did not induce IL-22 production from Tph or Tfh cells (**Fig. 11d**). AHR inhibition also increased ICOS and PD-1 expression in stimulated Tph, Tfh, CD96^{hi}, and naïve cell subsets while suppressing CD96 and TIGIT expression, generally with stronger effects in the absence of TGF- β (**Fig. 11e**).

[0143] Next, to determine whether *in vitro* stimulated T cells acquire epigenetic features of Tph or Tfh cells, ATAC-Seq was used to evaluate areas of open chromatin in CD4⁺ T cells stimulated with TGF- β plus either AHR agonist or inhibitor. This ATAC-Seq was compared data to ATAC-Seq data from sorted PD-1^{hi} CXCR5⁻ Tph cells from RA synovial fluid (SF) and PD-1^{hi} CXCR5⁺ Tfh cells from tonsil (**Figs. 12a, 12b**). PD-1^{mid} and PD-1^{lo} CD4 T cells from each respective tissue were sorted as comparator cell populations. By PCA, SF Tph cells and tonsil Tfh cells clustered together, separated from PD-1^{mid} and PD-1^{lo} CD4⁺ T cells (**Fig. 12c**). Blood CD4⁺ T cells stimulated *in vitro* with the AHR inhibitor CH-223191 clustered separately from cells treated with TCDD and DMSO control, consistent with scRNA-Seq results (**Fig. 12d**).

[0144] Further, DESeq2⁴³ was used to identify differentially accessible regions (**DAR**) in Tph and Tfh cells from SF and tonsil tissues, respectively, by comparing PD-1^{hi} versus PD-1^{lo} cells from each tissue. This DESeq2 data was compared to DAR found in CD4 T cells stimulated *in vitro* with TGF- β plus TCDD or AHR inhibitor. Results shows DAR annotated genes of CD4⁺ T cells treated with TGF- β +AHR inhibitor were significantly enriched for both Tph- and Tfh-associated DAR annotated genes ($p=0.001$ for both) compared to cells treated with TGF- β +TCDD (**Fig. 12e**). Furthermore, peaks at the *CXCL13* gene locus were preferentially open in Tph cells, Tfh cells, and AHR inhibitor-treated cells, consistent with their capacity to produce CXCL13 (**Fig. 12f**). Taken together, the data presented here indicates that TGF- β and AHR activity control the development of CXCL13⁺ Tph and Tfh cells.

[0145] Without wishing to being bound by any particular theory, Inventors hypothesized that aberrant AHR activation may be a systemic feature in systemic lupus erythematosus patients that allows for expansion of Tph and Tfh cells. The effects of serum from SLE patients on AHR activation were tested using a HepG2 cell line with a luciferase reporter driven by AHR response elements⁴⁴. Serum from either new-onset SLE patients or controls showed comparably low intrinsic AHR agonist activity; however, treatment of HepG2 reporter cells with serum from SLE patients significantly inhibited TCDD-induced activation of the AHR reporter, as compared to serum samples from anti-nuclear antibody⁺ (ANA⁺) control patients without SLE (**Fig. 2l**).

[0146] To assess the therapeutic potential of modulating this pathway, Inventors tested whether strong AHR activation *in vitro* is sufficient to reduce the frequency of PD-1⁺ Tph or Tfh cell populations within PBMC purified from SLE patients. PBMC of SLE or healthy donor controls were treated with AHR agonists and/or inhibitors for 4 days without further TCR stimulation, and the frequency of T cell subsets within memory CD4 T cells was evaluated (**Fig. 13**). The AHR agonist TCDD significantly reduced the frequency of PD-1⁺ CXCR5⁻ Tph cells among memory CD4⁺ T cells from SLE patients (**Fig. 2m**). In contrast, the AHR inhibitor CH-223191 reduced the frequency of CD96^{hi} cells in both control and SLE patients. Generation of new PD-1⁺CXCR5⁻ Tph cells and CD96^{hi} cells appears to require TCR stimulation, as AHR inhibition or agonism alone was not sufficient to increase the

respective cell populations in PBMC from SLE patients. To evaluate the ability of AHR to influence the B cell helper function of T cells *ex vivo*, circulating PD-1^{hi} CXCR5⁺ Tfh cells were separated from blood of control donors, and Tfh cells were pretreated with an AHR agonist, AHR inhibitor, or control prior to co-culture with memory B cells. Tfh cells treated with an AHR agonist were less able to induce B cell differentiation into plasmablasts *in vitro* (**Fig. 2n**), which indicates that AHR activation can reduce the B cell-helper function of circulating Tfh cells.

[0147] AHR controls CXCL13-IL22 polarization through AP-1 family member JUN

[0148] To elucidate the mechanisms by which AHR controls CXCL13-IL22 polarization, a high-resolution time-course RNA-Seq of CD4⁺ memory T cells treated with TGF- β and either an AHR agonist or inhibitor for 12, 24, 48 or 72 hours was conducted (**Fig. 14a**). These early time points were chosen to capture proximal transcriptional events induced by AHR agonism. Expression of canonical AHR target genes (*CYP1A1*, *CYP1B1*, *TIPARP*, *AHRR*)⁴⁵⁻⁴⁷ increased within 12 hours of culture with TCDD, and PCA analysis revealed clear separation of samples based on AHR activation status by 48 hours (**Figs. 14b, 14c; Table 3**). AHR agonism significantly downregulated expression of *CXCL13* and other Tph genes such as *ICOS*, *MAF* and *IL21* at 48 hours and significantly upregulated *CD96* expression (**Fig. 14d; Table 3**).

[0149] Pathway analysis showed AHR signaling pathways first enriched at 48 hours in CD4⁺ memory T cells cultured with TCDD (AHR agonist), followed by co-enrichment of T cell activation and differentiation pathways at 72 hours (Elsevier Pathway collection database, $p_{adj} < 0.05$, **Fig. 14e**). Analysis of transcription factor signatures for each time points revealed additional transcription factors (TF) altered by AHR agonism, including upregulation of genes enriched for putative cis-binding sites for AP-1 transcription factors such as JUN, FOS, ATF3, FOSL1, and FOSL2 in cells treated with TCDD after 12 hours (**Fig. 14f**).

[0150] This analysis was confirmed by maSigPro⁴⁸, which identifies clusters of genes that are upregulated coordinately across time. Nine clusters of differentially expressed gene transcripts were identified, including one gene set (cluster 6) that was progressively

upregulated by AHR activation and highly enriched in AHR binding sites near the promoters (adjusted $p=0.00009$) (**Table 4, Fig. 3a**).

[0151] EnrichR TF co-occurrence analysis highlighted a co-enrichment of AP-1 family transcription factor motifs (adjusted $p=1.03e-8$) in genes within this AHR-upregulated gene set—FOSL2, JUN, FOSL1, ARID5B, and HIF1A (**Fig. 3b**)—which suggests that AP-1 family members may co-regulate a set of target genes with AHR.

[0152] To corroborate and extend these findings, CUT&RUN was used to identify the sites directly bound by AHR using an anti-AHR antibody or anti-IgG control⁴⁹. AHR ChIP-Seq and/or CUT&RUN has not been previously reported in primary human T cells. To confirm specificity, CUT&RUN was done for AHR on cells in which AHR had been targeted by CRISPR and on control cells (**Fig. 3c**). CUT&RUN yielded 2736 AHR-bound peaks in AHR-intact cells and 1379 peaks in AHR-depleted cells. Motif analysis of differentially bound peaks (DiffBind)⁵⁰ between AHR-intact and AHR-depleted cells confirmed that the peaks dependent on AHR expression were significantly enriched for AHR motifs (**Fig. 14g**). AHR binding was observed at gene loci of canonical AHR target genes such as *CYP1B1* and *AHR*, as well as enhancers/promoters in *CD96* and *IL22* with AHR agonist. These peaks were absent in cells treated with AHR antagonist or subjected to AHR CRISPR deletion (**Figs. 3d, 3e**). Notably, AHR also bound to *CXCL13*, *ICOS* and *MAF* gene loci, suggesting direct repression of genes associated with Tph/Tfh cells.

[0153] Conversely, a gene set of 210 Th22 signature genes³² was highly enriched among AHR-bound peaks (**Fig. 14h**, hypergeometric score $p=0.0019$). Broadly, Pathway analysis utilizing the Kyoto Encyclopedia of Genes and genomes (KEGG) database for T cell specific pathways showed that AHR peak-associated genes were enriched in a Th17 signature (**Fig. 3f**); however, there were no AHR peaks in the *IL17A* or *IL17F* gene loci, consistent with a Th22 phenotype. Unbiased analysis for transcription factor binding sites in AHR peaks using HOMER revealed enrichment the expected AHR binding motif ($p=1e-37$, third most significant motif), as well as multiple motifs shared by members of the AP-1 transcription factor family (TGA[C/G]TCA) (**Figs. 3c, Fig. 14g**). Thus, both RNA-Seq and AHR

CUT&RUN transcription factor motif analyses strongly suggested that AP-1 transcription factors and AHR co-regulate gene expression.

[0154] AP-1 transcription factors act as hetero- or homodimers with highly similar DNA binding motifs, such that it is not possible to identify the specific AP-1 family member(s) responsible for this effect based on motif analysis alone^{51,52}. To determine which AP-1 family members regulate CXCL13 versus IL-22 associated phenotypes, a targeted, arrayed CRISPR screen in human memory CD4⁺ T cells targeting 22 AP-1 family members was conducted using an approach similar to **Fig. 2a**, but in the presence of TGF- β plus AHR agonist TCDD or AHR inhibitor CH-223191.

[0155] Results of the CRISPR screen with AP-1 family members indicate deletion of *JUN* strongly upregulated T cell CXCL13 (**Fig. 3g**) and downregulated IL-22 production, showing the largest effect of any AP-1 family member (**Fig. 3h**). Validation experiments using an independent gRNA confirmed that JUN deletion upregulated CXCL13 production and downregulated IL-22 production in CD4⁺ memory T cells from multiple healthy donors (**Figs. 3i–3k**).

[0156] To determine the effects of JUN on gene expression, RNA-Seq on JUN-deleted cells and JUN-intact cells treated with AHR agonist or inhibitor was conducted. GSEA analysis showed significant enrichment of Tph signatures in JUN-deleted cells stimulated with an TCDD (FDR<0.001), and also a trend towards depletion of Th22 gene signatures (**Fig. 3l**). These results identify JUN as a key regulator of a CXCL13-IL22 functional polarization in human T cells and a repressor of the CXCL13⁺ phenotype.

[0157] Together, the above data suggested co-regulation of a set of genes by both AHR and the AP-1 family member, JUN. To confirm this biochemically, performed CUT&RUN was performed utilizing antibodies targeting JUN on AHR agonist TCDD treated memory CD4 T cells. This analysis identified 6901 peaks. Motif analysis by HOMER confirmed specificity, as AP-1 sites were significantly enriched in these CUT&RUN peaks (**Fig. 4a**). JUN binding sites were significantly enriched for Th17-like genes in both KEGG and Elsevier pathway databases by EnrichR analysis (**Fig. 4a**). However, like AHR, JUN bound to IL-22 but not IL-17A or IL17F, consistent with a specific role in Th22 cells. Other Elsevier pathways that

were enriched among JUN-bound genes included AHR signaling in Th17 and regulatory T (Tr1) cells, which further suggests that JUN and AHR co-regulate T cell-relevant genes. JUN also bound to the CXCL13 gene locus, which suggests that it inhibits CXCL13 expression directly (**Fig. 4d**).

[0158] Genome-wide, JUN bound 66% of AHR binding sites, a proportion significantly higher than expected by chance alone (hypergeometric $p=1.22e-254$, **Fig. 4b**). Genes associated with peaks bound by both JUN and AHR (989 genes from 1170 peaks, data not shown) included 23 genes from the AHR-agonized gene cluster identified by time course RNA-Seq (cluster 6, **Fig. 3a**, hypergeometric $p=2.36e-13$, **Fig. 4c**). Of relevance, both AHR and JUN bound to the same enhancer region of the *IL22* gene locus, while JUN also bound to the promoter (**Fig. 4d**).

[0159] Given the overlapping effects of AHR and JUN in human T cells, the effects of AHR activation or inhibition on JUN binding to target loci was evaluated (**Fig. 4e**). AHR inhibition reduced the peak sizes at genes co-bound by AHR and JUN, including both *CXCL13* and *IL22* gene loci (**Fig. 4e**). Diffbind analysis confirmed significant reduction of JUN binding to *IL22* in AHR inhibited condition ($FDR=5.402e-4$).

[0160] AHR inhibition also had broader effects on JUN binding peaks, with 60% of the total 7181 JUN peaks lost in AHR inhibitor-treated cells, including 87% of peaks that are not co-bound by AHR (**Fig. 4f**), which suggests that AHR inhibitors do not modulate JUN-dependent genes merely by abrogating recruitment of JUN to AHR-bound sites.

[0161] Without being bound to any theory, Inventors hypothesized that AHR may modulate JUN expression. There were no significant changes in *JUN* RNA expression in the time course RNA-Seq data of AHR activation ($\text{Log2FC} = 0.01$ at 12h and -0.06 at 48h). However, regulation of JUN activity and protein stability occurs mainly through post-translational modifications, including phosphorylation by c-JUN N-terminal kinases (JNK) at Ser63/73 residues, which modulates JUN transcriptional activity, binding with other AP-1 transcription factors, and protein stability⁵³⁻⁵⁵.

[0162] AHR inhibition (with or without TGF- β) moderately but significantly reduced levels of native JUN protein and its Ser73 phosphorylated proteoform in 13 healthy donors, which suggests that AHR inhibition may reduce JUN protein stability (**Figs. 4g, 4h**). This effect was not dependent on TGF- β . Taken together, these results suggest that AHR inhibition may moderately inhibit JUN protein stability, leading to reduced DNA binding to enforce an AHR-induced transcriptional program.

[0163] Because AHR and JUN frequently co-bound genomic loci, Inventors evaluated whether AHR and JUN proteins interact biochemically. Co-immunoprecipitation (coIP) using either HA-tagged AHR (HA-AHR) or Flag-tagged JUN (JUN-Flag) confirmed protein-protein interactions (**Figs. 15a, 15b**). Surprisingly, the presence of AHR agonists or inhibitors had no effect on this protein-protein binding; however, subcellular fractionation experiments demonstrated that an AHR agonist increased JUN abundance in the nucleus (**Fig. 15c**), an effect that parallels the known ability of AHR agonists to induce AHR translocation from the cytoplasm to the nucleus⁴⁶.

[0164] Without wishing to be being bound to any theory, Inventors hypothesized that restoring JUN levels would rescue IL-22 expression and CXCL13 inhibition. To test this, memory CD4⁺ T cells were transduced with a lentiviral construct encoding JUN (**Fig. 15d**) in the presence or absence of the AHR agonist or antagonist. JUN overexpression (JUN OE) significantly decreased CXCL13 production and increased IL-22 expression, even in cells treated with AHR inhibitor (**Fig. 4i**). CUT&RUN analysis showed that JUN overexpression restored DNA binding in *CXCL13* and *IL22* gene loci at regions suppressed by AHR inhibition (**Fig. 4j; Figs. 15e, 15f**). Furthermore, JUN overexpression restored JUN binding to other peaks in the Th17/Th22 differentiation pathways that are otherwise lost in AHR inhibitor-treated cells (**Fig. 4k**). RNA-Seq analysis of JUN-overexpressing CD4⁺ T cells demonstrated enrichment of Th22 signatures and suppression of Tph signatures compared to control cells (**Fig. 4l**). Collectively, these results suggest that JUN upregulates Th22 genes (including IL-22) and inhibits CXCL13 directly.

[0165] Type I IFN promotes CXCL13⁺ Tph cells at the expense of Th22 cells in a JUN-dependent manner

[0166] Lastly, extrinsic factors that may skew the CXCL13⁺ Tph/Tfh versus IL-22⁺ Th22 axis polarization in systemic lupus erythematosus patients were identified. Differential expression analyses comparing T cell populations from SLE patients and controls highlighted a prominent upregulation of IFN-inducible genes in cells from SLE patients, consistent with a well-recognized IFN signature in SLE⁵⁶ (**Fig. 16a**). Using published bulk RNA-Seq data on T cells from a larger cohort of SLE patients stratified in to IFN-high and IFN-low subgroups, Inventors found that CXCL13 was significantly higher in T cells from IFN-high SLE patients compared to IFN-low patients⁵⁷ (**Fig. 5a**). The effect of blockade of the IFN- α/β receptor (IFNAR) on circulating CXCL13 levels was then evaluated in SLE patients treated with either anifrolumab (anti-IFNAR) or placebo in the TULIP-1 randomized controlled trial⁵⁸.

[0167] Patients with a high IFN signature had significantly higher levels of circulating CXCL13 than patients with a low IFN signature (**Fig. 5b**). In IFN-high SLE patients, IFNAR blockade with anifrolumab significantly reduced serum CXCL13 levels compared to placebo ($p=2.67e-07$), while CXCL13 levels remained low and unchanged in IFN-low patients ($p=0.18$). These data provide clear *in vivo* evidence that type I IFN promotes CXCL13 production in SLE patients.

[0168] To evaluate whether IFN promotes Tph cells in SLE patients, scRNA-Seq was performed on CD3⁺ T cells sorted from blood of patients with lupus ($n=7$ total; 5 SLE, 2 cutaneous lupus) obtained before and 1-2 months after treatment with anifrolumab (**Figs. 5c, 5d**). Clustering of memory CD4⁺ T cells yielded clusters with distinct T helper subset gene signatures, including a Tph cluster (cluster 0), which was enriched for a Tph signature ($p<2.2e-16$, Linear Mixed Model [LMM]), and a Th22 cluster (cluster 11), which was enriched for both Th22 and CD96^{hi} gene signatures ($p<2.2e-16$, **Fig. 5e**). Consistent with the functional data, an IFN stimulated gene (ISG) signature was higher in Tph cells compared to Th22 ($p=4.57e-8$, **Fig. 5f**). Furthermore, the Tph cluster had higher ISG signature and significantly lower signature of JUN target genes compared to Th22 cells ($p<2.2e-16$, **Fig. 5g**) despite similar mRNA expression of *JUN* ($p=0.81$). Treatment with anifrolumab significantly reduced the proportion of cells in the Tph cluster ($\log_2FC$ -0.33, $p=0.03$, **Fig. 5h**) and increased the proportion of cells in the Th22 cluster in lupus patients ($\log_2FC$ 0.7,

p=0.016). Taken together, these data indicate that IFN promotes Tph accumulation and CXCL13 production in SLE patients.

[0169] To evaluate whether IFNs act directly on T cells to promote CXCL13, memory CD4⁺ T cells from healthy donors in the presence or absence of IFN- α were stimulated under varied AHR activation conditions (**Fig. 5i**). IFN- α increased T cell CXCL13 production in all cases and significantly decreased IL-22 production in T cells treated with AHR agonist. In addition, the combination of IFN- α and AHR inhibition or deletion further amplified CXCL13 production, particularly in the presence of TGF- β (**Fig. 5j**).

[0170] To examine its effects on the broader Tph phenotype, a previously published RNAseq data from naïve CD4⁺ T cells treated with IFN- β was analyzed⁵⁹. In this dataset, IFN was sufficient to induce Tph genes such as *CXCL13*, *ICOS* and *MAF* (data not shown), similar to AHR inhibition in memory CD4⁺ T cells.

[0171] To examine whether type I IFN induces a Tph epigenetic profile, ATAC-Seq data from CD4⁺ T cells treated with IFN- β ⁵⁹ were compared with ATAC-Seq profiles of rheumatoid arthritis SF PD-1^{hi} Tph cells and of CD4⁺ T cells treated with an AHR inhibitor. This analysis revealed a significant overlap in chromatin accessibility among IFN-treated CD4⁺ T cells with SF Tph cells (hypergeometric p=1.111e-06), AHR inhibitor-treated CD4⁺ T cells (hypergeometric p=2.891e-08), and between all three groups (hypergeometric p=6.041e-14) (**Fig. 5k**). These overlaps were de-enriched in DAR upregulated in the T cells not treated with IFN- β . Notably, the CXCL13 gene locus was more accessible in IFN- β treated CD4⁺ T cells, much as it is for SF PD-1^{hi} and AHR inhibitor-treated T cells (**Fig. 5l**). Moreover, IFN- β downregulated ATAC-Seq peaks in genes associated with AHR signaling (**Fig. 16b**).

[0172] These results suggested that type I IFN can function as a physiological AHR inhibitor. Supporting this idea, pretreatment of HepG2 cells expressing an AHR reporter⁴⁴ with IFN- β inhibited TCDD-induced luciferase activation (**Fig. 5m**). In addition, pretreatment of human CD4 T cells with IFN- α inhibited TCDD-induced upregulation of *CYP1A1* (**Fig. 16c**).

[0173] To identify potential mechanisms linking IFN signaling to Tph/Th22 cell differentiation, Inventors performed a CRISPR arrayed screen targeting a set of IFN regulatory module genes in CD4⁺ T cells⁵⁹ (**Fig. 6a**). As expected, CRISPR deletion of IFN alpha/beta receptor 2 (IFNAR2) obviated IFN induced CXCL13. Interestingly, Applicant found that CRISPR deletion of *STAT5A*, *STAT1*, and *JAK1* increased CXCL13 and decreased IL-22 production, much like AHR deletion (see gRNAs in **Table 2**). While these genes were implicated in a study of IFN signaling⁵⁹, all three are also mediators of signaling by IL-2^{60,61}, a potential inducer of AHR ligands⁶² and a factor that is known to suppress CXCL13 production^{19,36,63}. Consistent with this observation, scRNA-Seq data from SLE patients showed a low IL-2 response gene signature in Tph cells compared to Th22 cells (p=0.0175, LMM, **Fig. 6b**).

[0174] *In vitro*, addition of exogenous IL-2 to memory CD4⁺ T cells stimulated with anti-CD3 alone, a condition that induces little endogenous IL-2 production, significantly decreased CXCL13 production and increased IL-22 production (**Fig. 6c**). This effect required STAT5, as CRISPR depletion of *STAT5A* and *STAT5B* blocked the effect of IL-2 on CXCL13 and IL-22 production (**Fig. 6d**).

[0175] Addition of type I IFN to IL-2-treated CD4⁺ T cells restored CXCL13 production, indicating that IFN can block the ability of an IL-2-STAT5 axis to suppress CXCL13 production (**Fig. 6e**). In addition to altering the response to IL-2, IFN- β also reduced production of IL-2 from *in vitro* stimulated CD4⁺ T cells (**Fig. 6f**), consistent with prior studies^{64,65}.

[0176] Finally, Inventors investigated whether IFN influences JUN expression or function. Treatment of CD4⁺ T cells with IFN- α moderately but significantly reduced expression of both phospho-JUN and total JUN (**Fig. 6g**), similar to AHR inhibition. As with AHR inhibition, CUT&RUN analyses of JUN performed in CD4⁺ T cells cultured with and without IFN- α demonstrated significantly reduced JUN binding across the genome, including at the *IL22* locus (**Fig. 6h**). Consistent with the model, pathway analysis of genes annotated to the 89 JUN-bound peaks lost with IFN- α treatment (Diffbind, FDR<0.1, data not shown)

suggested that IFN reduces JUN binding to genes associated with Th17 differentiation and AHR signaling (**Fig. 6i**).

[0177] To demonstrate a functional significance, testing was performed to determine whether overexpression of JUN would be sufficient to reverse the effects of IFN. Lentiviral overexpression of JUN was sufficient to reverse the effects of IFN, as JUN overexpression blunted the ability of IFN- α to augment CXCL13 and repress IL-22 production (**Fig. 6j**). Together, these results highlight a potent effect of IFN in controlling accumulation of a CXCL13⁺ T cell population in SLE patients, with effects at multiple levels to oppose AHR-, IL-2-, and JUN-mediated repression of a CXCL13⁺ phenotype (**Fig. 6k**).

[0178] Select transcription factors are positive regulators of CXCL13 production and Tph differentiation

[0179] In **Table 5** the genes of particular interest are those that resulted in decreased CXCL13 levels when knocked out. CXCL13 levels decrease when select transcription factors were knocked out in the CRISPR screen, including *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, and *STAT2*. This is indicative of genes that could increase CXCL13 levels when overexpressed in a cell or genes that play a role in increasing levels of CXCL13 production in a cell.

Discussion

[0180] Described herein is an imbalance in T cell differentiation in patients with systemic lupus erythematosus, which manifests not as an isolated expansion of activated population, but as a dysregulated balance of distinct effector T cell states. Expansion of Tph and Tfh cells has been consistently observed across multiple SLE cohorts^{9,66-68}, yet the deficiency in a counterbalanced CD96^{hi} Th22 T cell subset has not been previously recognized. Perhaps analogous to a disrupted Th17/Treg balance in certain autoimmune diseases, herein it is shown through multiple, orthogonal assays that Tph/Tfh and Th22 developmental pathways are reciprocally interconnected.

[0181] Also described herein are AHR+JUN and type I IFN as critical, opposing regulators of a T cell differentiation axis, which were identified using CRISPR screening,

pharmacologic interventions, and cytokine assays. Herein is defined a driver role for AHR in restricting production of CXCL13, an essential B cell chemoattractant. Further, AHR blunts T cell acquisition of several phenotypic, transcriptomic, and epigenetic marks of B cell-helper function. These results suggest that augmenting AHR activation may prevent Tph/Tfh differentiation and the subsequent recruitment of B cells, providing a potential strategy to dampen formation of lymphoid aggregates in inflamed targeted tissues^{13,69}. Boosting Th22 cell generation may also have benefits in mucosal barrier integrity in systemic lupus erythematosus given reports of compromised gut barrier integrity in both SLE patients and murine lupus models⁷⁰⁻⁷², though potential inflammatory consequences are possible⁷³. Collectively, these data support the therapeutic potential of AHR agonism for autoimmune diseases, either systemically or directed towards T cells⁷⁴⁻⁷⁷.

[0182] Mechanistically, AHR acts in concert with JUN to limit CXCL13⁺ Tph/Tfh cell generation and usher T cells towards IL-22 production. AHR activation increases JUN protein levels and promotes JUN nuclear localization, which may occur through direct protein-protein interaction. CUT&RUN analyses indicated that AHR and JUN coregulate a shared gene transcriptional program. While the roles of AP-1 in T cell differentiation and cytokine production have been investigated for decades, these results add a previously unappreciated layer to the regulation and function of JUN in controlling T cell phenotypes. Although AP-1 factors appear to bind to similar DNA-binding motifs, there are repeated examples of functional specificity^{78,79}. Applicants have shown here that JUN is shown to be necessary and sufficient to induce the Th22 phenotype and antagonize CXCL13 expression.

[0183] Insufficient or altered AHR activation has been suggested in multiple autoimmune contexts^{25,80}, including SLE⁸¹, yet mechanisms underlying this remain unclear.

[0184] Applicant's results identify IFN as an endogenous inhibitor of AHR activation that may contribute to inadequate AHR activation and function in systemic lupus erythematosus through multiple mechanisms. IFN inhibits AHR-induced gene upregulation and induces epigenetic changes that resemble those caused by AHR inhibition, pushing T cells towards CXCL13 production and away from IL-22 production. The synergistic effects of IFN plus AHR inhibition indicates that IFN has additional roles in promoting a CXCL13⁺ phenotype

beyond inhibiting AHR. For one, IFN inhibited JUN expression and disrupted JUN binding to multiple sites across the genome, indicating that IFN substantially alters AP-1 activation in T cells. Inhibition of AP-1 activity is a key function of BCL6 in Tfh cells, enabling full Tfh cell differentiation and function^{82,83}; it is possible that IFN can similarly disrupt or alter AP-1 activity in BCL6^{low} Tph cells, helping to enable their B cell-helper function in the absence of high BCL6 expression. In addition, IFN blocked IL-2-STAT5-mediated suppression of CXCL13 production. IL-2 has been shown sustain AHR activation in tumor infiltrating lymphocytes⁶², highlighting the connected interactions between these regulators. The ability of IFNAR blockade to reduce levels of Tph cells and CXCL13 in lupus patients strongly implicates IFN in this pathway *in vivo*.

[0185] Further, Inventors comprehensively identified microenvironmental changes that can mediate epigenetic changes associated with CXCL13 production, many of which appear to be shared with CXCL13⁺ CD8⁺ T cells in the tumor microenvironment²²⁻²⁴. Like CD4⁺ Tph cells in systemic lupus erythematosus, CXCL13⁺ CD8⁺ T cells in tumors appear chronically TCR-activated, exposed to TGF- β and/or type I IFN, and deprived of IL-2, a factor that can promote intrinsic AHR signaling. The need for persistent TCR stimulation for CXCL13 production was highlighted by CRISPR deletion of *CBLB* and repeated TCR stimulation assays. Intriguingly, as for Tph cells, CXCL13-associated dysfunctional states in CD8⁺ T cells may critically rely on downregulation of JUN⁷⁸.

[0186] In conclusion, a comprehensive approach combining cellular, molecular and computational biology revealed an unexpected, fundamental axis of T cell differentiation with CXCL13 and IL-22 production at opposite ends. In one aspect, the identified critical controlling factors, including AHR, IFN, and JUN, may be manipulated therapeutically to alter this axis and dampen the production of pathologically expanded CXCL13⁺ Tph and Tfh cells in systemic autoimmune diseases.

Methods

[0187] Human subjects research

[0188] Human subjects research was performed according to the Institutional Review Board at Mass General Brigham (IRB protocol 2014P002558, 2018P001961, 2021P002267) via approved protocols with informed consent as required. Synovial fluid samples were collected from patients with rheumatoid arthritis as discarded fluid from clinically indicated arthrocentesis. Seropositive (RF+ and/or anti-CCP+) RA patients fulfilled 2010 ACR/EULAR classification criteria. SLE patients fulfilled the 2019 ACR/EULAR criteria⁸⁵. Blood samples were obtained from individuals with SLE, RA, as well as individuals without inflammatory diseases. Mononuclear cells from synovial fluid and peripheral blood were isolated by density centrifugation using Ficoll-Paque Plus (GE healthcare) and cryopreserved in FBS + 10% DMSO by slow freeze, followed by storage in liquid nitrogen for batched analyses. For experimental analyses, cryopreserved samples were thawed into warm RPMI medium + 10% FBS.

[0189] Mass cytometry staining

[0190] Samples were processed in 3 batches, including balanced numbers of SLE and control samples per batch. Cryopreserved cells were thawed and trypan blue negative viable cells were counted by hemocytometry. Approximately 1 million live cells per sample were used for mass cytometry staining. All antibodies were obtained from the Longwood Medical Area CyTOF Core. Buffers were from Fluidigm. Cells were stained with rhodium (Fluidigm) for viability then washed. Cells were washed and stained with primary antibody cocktails using custom metal-conjugated antibodies obtained from the Longwood Medical Area CyTOF Antibody Resource Core (Boston, MA). Cells were then washed, fixed and permeabilized using the Ebioscience Transcription Factor Fix/Perm Buffer for 45 min, washed in PBS/1% BSA/0.3% saponin, then stained for intracellular markers. Cells were re-fixed in formalin (Sigma), washed with Milli-Q water, and analysed on a CyTOF2 (Fluidigm). Mass cytometry data were normalized using EQ Four Element Calibration Beads (Fluidigm).

[0191] Mass cytometry data analysis

[0192] Normalized FCS files were uploaded in FlowJo v.10.4.2. Live singlet cells were determined by manual gating and normalization beads were excluded. FCS files including all manually gated T CD4 memory cells (CD3+CD8-CD4+CD45RO+) were uploaded and read

in R (v.4.0.3) using the flowCore package. Marker expressions were arcsinh transformed using a co-factor of 5. The transformed matrix of expression was transposed and implemented into a Seurat object with the corresponding metadata. To check data quality, the distribution of the markers across the three batches and confirmed minimal batch effect was verified. From the 30 initial markers, 18 of them that were non-redundant in each sample and variable across samples were selected, as determined by the PCA-based non-redundancy score⁸⁶. To ensure equal representation of samples and conditions (SLE versus controls) for further unsupervised analysis, the data was downsampled randomly to 2000 cells/sample. The data was then processed and analyzed using the Seurat pipeline (Seurat package v.4.1.1). ScaleData and RunPCA were used with the default settings. The nearest-neighbor graph was built using the function FindNeighbors on the 10 first principal components followed by a Louvain-based clustering analysis (function FindClusters) with a resolution of 0.5. To visualize the data, UMAP was used as a dimensionality reduction tool (runUMAP) based on the 10 first principal components.

[0193] To compare cell abundances within T CD4 memory between SLE and control samples co-varying neighborhood analysis (CNA)³¹ was applied using its R implementation for Seurat (rcna package v.0.0.99; Korsunsky). CNA defines the abundance of cells from each sample within defined neighborhoods (small groups of cells based on the nearest-neighbor graph) and applies PCA to the neighborhood abundance matrix for dimensionality reduction. Association testing is then performed with a specified clinical feature using a linear model. CNA was used here to capture small groups of cells that co-vary with SLE disease (control = 1, SLE = 2, while controlling for age, sex and batch **Fig. 1C**). In addition to CNA, Applicant used Mixed-effects Association testing for Single Cells (MASC)⁸⁷, an approach previously validated for mass cytometry analysis testing cluster attribution and association with a specific clinical variable. Here MASC was used to test for the contribution of SLE (versus control) to cluster membership for each single cell. SLE versus control was defined as the contrast variable, age and sex as fixed factors, and patient and batch as random factors (**Fig. 1d**, **Fig. 7a** right panel). MASC determines an odds ratio (and 95% confidence intervals) for SLE disease for each clusters. P values were then adjusted for multiple testing using Bonferroni's correction. Manual biaxial gating was performed using FlowJo (v.10.4.2)

for quality control and independent examination of the expression of markers and frequencies of populations.

[0194] *In vitro* culture of human T cells

[0195] Total or memory CD4⁺ T cells were isolated from PBMC by negative selection using magnetic beads (Miltenyi Biotec). Alternatively, CD4⁺ T cells were isolated by magnetic positive selection using Dynabeads (Invitrogen #11331D). CD45RO⁺ memory CD4 T cells were further isolated from bulk CD4⁺ T cells by negative selection, depleting CD45RA⁺ naïve CD4 T cells using CD45RA mouse IgG antibodies (Invitrogen #14-0458-82) and Pan IgG Dynabeads (Invitrogen #11531D). Cells were cultured in complete RPMI consisting of RPMI-1640 medium (Gibco #21875034), 10% FBS, 1% penicillin/streptomycin, 10mM HEPES, 1% L-glutamine. In some cases, the 1% L-glutamine was replaced with 1mM sodium pyruvate. For *in vitro* culturing with AHR modulation, T cells were cultured in complete RPMI medium with Dyna anti-CD3/CD28 T activator beads (Invitrogen #11131D) at 1:5 bead:cell ratio with 2ng/mL human TGF- β 1 (Peprotech #100-21C R&D 7754-BH-025), and either 10 μ M of CH-223191 (Sigma C8124) or 3-5nM of TCDD as indicated (AccuStandard, #1746-01-6). For experiments with IFN- α or IFN- β , 1000U/mL of IFN- α (R&D PHC4814) or IFN- β (Peprotech #300-02BC) were added. For experiments with IL-2, unless indicated, 10ng/mL of IL-2 (Peprotech #200-02) was added to complete RPMI medium and cells were either stimulated with CD3/CD28 T activator beads or plate-bound anti-CD3 antibodies (Thermo #16-0036-81, clone SK7).

CRISPR-Cas9 Delivery

[0196] Electroporation of human CD4⁺ T cells with CRISPR-Cas9-gRNA ribonucleoprotein (**crRNP**) complexes was performed as previously described³⁴ with minor modifications. Briefly, guide RNAs (gRNAs) were designed using CRISPick online design tool by Broad Institute or ITDNA⁸⁸ and purchased from Integrated DNA technologies (IDT, Alt-R CRISPR Cas9 crRNA). crRNAs were duplexed with tracrRNA (IDT #1072534) for 2 minutes at 95°C or 40minutes at 37°C in 5% CO₂ incubator and complexed with Cas9 protein (Macrolab, Berkeley, 40 μ M stock) at 1:2 or 1:1 molar ratio for up to 60 minutes at 37°C. After 48-72 hours of stimulation with Dyna CD3/CD28 T activator beads, cells were collected, stripped

from beads, pelleted, and resuspended in Lonza electroporation buffer P3 (Lonza #V4XP-3032) at $0.2\text{--}2 \times 10^6$ cells / 20 μ L. Cells were electroporated in 16-well cuvettes using pulse code EH115 (Lonza #AAF-1002X). 100 μ L of pre-warmed complete RPMI culture media was added and cuvettes were incubated at 37°C and 5% CO₂ for 15 minutes. Cells were then transferred to 48 or 96-well plates containing complete RPMI media and CD3/CD28 T activator beads.

[0197] CRISPR Arrayed Screen

Custom 96-well CRISPR array library plates were purchased from Horizon Discovery Ltd. Each well consisted of 4 individual gRNA guides targeting the same gene.

[0198] Ribonucleoprotein (crRNP) complexes were made from gRNA from each well as previously described³⁴, and aliquoted and stored at -80°C in lo-bind plates (Eppendorf). Electroporation of pre-stimulated T cells was done as described above. Non-targeting guides *CD8a*, *CD19* and *OR1A1* sgRNAs were included as controls in the CRISPR-Cas9 screen. Roughly 60 million memory CD4⁺ T cells were stimulated with anti-CD3/CD28 Dyna activator beads for 72 hours prior to electroporation. On day of electroporation, stimulator beads were removed, and cells were resuspended in electroporation buffer P3 (Lonza) at $0.5\text{--}0.7$ million cells / 20 μ L, pipette mixed with crRNP complexes in lo-bind plate, transferred into 96-well Nucleofector™ plate (Lonza #V4SP-3096), and electroporated using 96-well Shuttle™ (Lonza). Immediately after electroporation, 80 μ L of pre-warmed complete RPMI culture media was added to each well of the Nucleofector™ plate and rested in incubator at 37°C and 5% CO₂ for 15 minutes. Cells were then transferred to 96-well plates containing complete RPMI media and anti-CD3/CD28 T activator beads and incubated at 37°C and 5% CO₂ for 4 days, supplementing 100 μ L of fresh complete RPMI on day 3. After 4 days, cells were resuspended with gentle pipetting, and 20 μ L was removed for cell count using CountBright™ beads (Invitrogen #C36950). Roughly 0.3 million cells were removed from each well to make protein lysates for CRISPR knockout verification as previously described³⁴. Remaining cells were split evenly between two new 96-well plates for culture conditions of anti-CD3/CD28 bead stimulation with or without TGF- β 1 (2ng/mL). Cells were cultured for 8 days after splitting into new conditions, and 100 μ L of supernatants collected on

days 4, 6 and 8 of culture. 100 μ L of fresh complete RPMI with or without TGF- β 1 was re-added to respective culture plate after each supernatant collection. For AP-1 family transcription factor screen, cells were cultured in 4 different conditions of anti-CD3/CD28 bead stimulation with either DMSO, DMSO with TGF- β 1 (2ng/mL), TGF- β 1 with AHR inhibitor (CH-223191, 10 μ M), or TGF- β 1 with AHR agonist (TCDD, 3nM). For IFN- α screen, cells were cultured with anti-CD3/CD28 bead stimulation with either PBS or IFN- α (1000U/mL).

[0199] Cleavage Under Targets & Release Using Nuclease (CUT&RUN)

[0200] CUT&RUN was performed using the CUTANA ChIC / CUT&RUN Kit (EpiCypher #14-1048) and following manufacture protocol with minor modifications. Briefly, human CD4 memory T cells were isolated and stimulated for 72 hours with TGF- β 1 and either AHR agonist or AHR antagonist, or in a separate experiment with either PBS or IFN- α . T cells were then collected and washed in wash buffer provided by kit. Cells were then incubated with activated ConA beads for 10 minutes at room temperature. Bead bound cells were resuspended in kit-provided antibody buffer supplemented with 0.1% bovine serum albumin, 100nM trichostatin A, 0.1 U citrate synthase, and 1mM oxaloacetic acid. Cells in antibody buffer were incubated with antibodies targeting either AHR (CST #83200), JUN (CST #9165), or IgG control (Epiccypher #13-0042) overnight on nutator at 4°C. The following day, cells were washed with cell permeabilization buffer twice and incubated with pAG-Mnase for 1 hour on nutator at 4°C. After incubation, cells were washed and while on ice, 100mM calcium chloride was added to each sample and incubated for 1 hour on nutator at 4°C. Finally, cells were incubated at 37°C for 30 minutes for DNA release, and DNA was cleaned and concentrated using the kit-provided DNA purification buffers and columns. DNA quantity was measured using Qubit dsDNA HS assay kit (Invitrogen #Q32851) and Qubit 2.0 fluorometer (Invitrogen). CUT&RUN library prep was performed using KAPA HyperPrep Kit (Roche), and paired-end DNA sequencing was performed on a HiSeq 2500 platform (Illumina) at Admera Health Inc.

[0201] CUT&RUN Data analysis

[0202] Raw CUT&RUN Sequenced reads (FASTQ files) were processed using script adapted and modified from the following repository: https://github.com/wherrylab/jogiles_ATAC. Briefly, samples were aligned to human genome hg38 (GRCh38) using Bowtie 2 (v.2.2.6). Samtools was used to remove unmapped, unpaired and mitochondrial reads. ENCODE blacklist regions were also removed (<https://sites.google.com/site/anshulkundaje/projects/blacklists>). PCR duplicates were removed using Picard. Peak calling was performed using SEACR (v.1.3) in relaxed setting normalized to IgG control. The number of reads in each peak was determined using BedTools coverage. Peak annotation was performed using ChIPseeker⁸⁹ package (v.1.30.3) in R (v.4.1.1). Differentially bound peaks were identified using Diffbind package (v.3.4.11) in R, following DESeq2 (v.1.34.0) normalization using an FDR cut-off <0.05 unless otherwise indicated. Tracks were visualized using Integrative Genomics Viewer (v.2.13.0, Broad Institute). Motif enrichment analysis and gene-to-peak association was performed using HOMER (v.4.10) with default settings. Significance in overlapped genes or bound regions was calculated with hypergeometric tests. P values and q values <0.05 were considered to indicate a significant difference. Tracks shown in figures were generated on IGV using bigwig files.

[0203] Time-course RNA-Seq experiment and module analysis

[0204] RNA from human memory CD4 T cells was isolated from cells (200,000 cells) stimulated with anti-CD3/CD28 Dyna beads and cultured in TGF- β 1 with either AHR antagonist or agonist for 12, 24, 48 and 72 hours. RNA was isolated with Rneasy Plus Micro Kit (Qiagen #74034) following manufacture protocol. RNA libraries were prepared using the QuantSeq FWD Kit for Illumina Sequencing (Lexogen) for 3'RNA-Seq. Sequencing was performed on Illumina NextSeq platform. Three biological replicates of each sample were sequenced. Sequence quality was assessed with FastQC (v.0.11.5), FASTQ files were trimmed using Bbduk from BBMap (v.38.90) according to Lexogen QuantSeq manufacturer's parameters, and mapped to hg38 using STAR (v.2.6.0). HTSeq was used to count uniquely mapped reads and significantly differentially expressed genes at each time point were determined using DESeq2. Time-course analyses were performed using

maSigPro⁴⁸ using default settings. Volcano plots and PCA plots were generated using R (v.4.1.1).

Lentiviral Transduction of Human T cells for JUN overexpression

[0205] cDNA encoding c-JUN (*JUN*) was reverse transcribed from total mRNA isolated from activated primary CD4 T cells and cloned into lentiviral expression vector (System Bioscience #CD511b-1) to create JUN overexpressing vector. *JUN* open reading frame (ORF) was subcloned into the EcoRI site of lentiviral vector using Gibson Assembly (NEB). JUN overexpressing vector was transformed into NEB Stable (NEB #C3040H) chemically competent cells and purified ZymoPURE plasmid Midiprep kit (Zymo Research #D4201-A). Pantropic. VSV-G pseudotyped lentivirus was produced via transfection of 293T cells with JUN overexpression vector and the viral packaging plasmids pCMVdr8.91 and pCMV-VSV-G using FuGENE (Promega #E2311). Primary CD4 memory T cells were isolated as described above, on the same day of 293T cell transfection. After 24 hours in culture, memory T cells were stimulated with CD3/CD28 T-activator Dynabeads (Life Technologies #11131D) at a 1:2 bead:cell ratio. At 48 hours, viral supernatant was harvested, filtered, concentrated, and added to primary T cell culture for 24 hours. At day 5 post T cell stimulation, Dynabeads were removed and T cells were re-cultured in AHR modulating conditions with or without TGF- β for 8 days while supernatants were collected on days 4, and 8 for ELISA assays.

[0206] For CUT&RUN and RNA-Seq of JUN overexpressing cells, cells were sorted at day 5 post T cell stimulation based on GFP positivity. Sorted cells were cultured for 72 hours before being processed for CUT&RUN and RNA-Seq.

[0207] Flow cytometry staining

[0208] Cryopreserved cells were thawed, washed and counted. Cells ranging from 0.1-2 million cells were stained in PBS with Aqua fixable live/dead dye (Invitrogen) for 20 minutes at 4 °C.

[0209] For surface staining, cells were stained in PBS with 1% BSA with the following antibodies for 20-30 minutes at 4°C: anti-CD3 BV711 (OKT3), anti-CD3 A700 (OKT3), anti-CD4 Pe-Cy7(RPA-T4), anti-CD8 BV510 (RPA-T8), anti-CD56 BV510 (HCD56), anti-CD25 FITC (M-A251), anti- anti-CD25 PerCPy5.5 (M-A251), anti-CD127 Alexa Fluor 700 (A019D5), anti-CD127 APC (A019D5), anti-CXCR5 BV421 (J252D4), anti-PD-1 BV711 (EH12.2H2), anti-ICOS FITC (C398.4A), anti-IL-17A PB (BL168), anti-IFN- γ (B27), anti-TNF FITC (Mab11), anti-CD14 APC (M5E2), anti-CD3 Alexa700 (HIT3a), anti-CD19 PE (HIB19), anti-CD27 BV421 (O323), and anti-IgD FITC (IA6-2), anti-CD4 FITC (RPA-T4), anti-CD19 APC-Cy7 (HIB19), anti-CD27 PE-Cy7 (M-T271), anti-CD38 BV785 (HIT2) (all from BioLegend), anti-CD45RA APC-eFluor788 (HI100), LIVE/DEAD Fixable Aqua, anti-AHR (FF3399), anti-IL-22, PeCy7 (22URTI), anti-IL-4 APC (8D4-8) (all from Invitrogen), anti-CD4 BUV395 (RPA-T4) and anti-CD8 BUV395 (RPA-T8) (BD Biosciences). Cells were washed in cold PBS 1% BSA. For intracellular or intranuclear staining, the eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set (ThermoFisher) was used. For detection of intracellular cytokines, depending on the experiment, cells were stimulated for 4 days with anti-CD3/CD28 Dyna beads (1:5 bead: cell ratio) or 10 days with Th1, Th2, Th17 polarization cocktails and then re-stimulated with 1x PMA/ionomycin (Invitrogen) for 6 hours. Brefeldin A (Invitrogen) was added 2 hours later and incubated with the cells for 4 hours. Cells were stained for viability and indicated surface markers as above. Following surface staining, cells were washed and incubated with 1xFixation/Permeabilization Buffer at room temperature for 40 minutes at 4°C. Cells were then washed in 1x eBioscience Permeabilization Buffer. For intracellular cytoplasmic staining, cells were incubated with indicated intracellular antibodies for 30min at 4°C. For intranuclear staining, cells were incubated with indicated intracellular antibodies for 1 hour at room temperature. Cells were then washed twice in 1x eBioscience Permeabilization Buffer and washed once more with PBS 1% BSA. Data acquired on a BD Fortessa analyzer using FACSDiva software and were analyzed using FlowJo (v.10.4.2).

[0210] Flow cytometric cell sorting

[0211] Two flow cytometry panels of 9 and 11 colors were used to identify memory CD4 T cell populations. Panel #1 included anti-CD3 BV510, anti-CD4 Pe-Cy7, anti-CD45RA

BV605, anti-CD25 FITC, anti-CD127 BV711, anti-PD-1 APC-Cy7, anti-CXCR5 BV421, anti-CD96 APC, anti-TIGIT PE. Panel #2 included anti-CD8 BV510, anti-CD19 BV510, anti-CD56 BV510, anti-CD25 FITC, anti-CD127 Alexa Fluor 700, anti-CD96 APC, anti-CXCR5 BV421, anti-TIGIT PE, anti-CCR6 BV605, anti-PD-1 BV711, propidium iodide (all from BioLegend), and anti-CD45RA APC-eFluor788, anti-CXCR3 PE-Cy7 (Invitrogen). Memory B cells were identified with this panel: propidium iodide, anti-CD14 APC, anti-CD3 Alexa700, anti-CD19 PE, anti-CD27 BV421 and anti-IgD FITC. Cells were incubated at 4°C with antibodies in PBS /1% BSA for 15-30 minutes. Cells were washed once in PBS/1% BSA, centrifuged and passed through a 70µM filter, and propidium iodide was added immediately prior to sorting. Cells were sorted on a 4-laser BD FACSAria Fusion cell sorter. Intact cells were gated according to forward scatter and side scatter area (FSC-A and SSC-A). Doublets were excluded by serial FSC-H/FSC-W and SSC-H/SSC-W gates (H, height; W, width). Non-viable cells were excluded based on propidium iodide uptake. Cells were sorted through a 70µM nozzle at 70 psi. Cell purity was routinely >98%. For functional analyses, 0.2-1 million cells were sorted from each population into cold RPMI/10% FBS. For RNA-Seq, up to 2000 cells were collected from each cell subset directly into buffer TCL (Qiagen) with 1% β-mercaptoethanol (Sigma). Flow cytometric quantification of cell populations was performed using FlowJo (v.10.0.7).

[0212] T:B co-culture assay

[0213] Tfh cells were flow sorted with panel #2 and activated with anti-CD3/CD28 Dynabeads in the presence of either DMSO, TCDD, or CH-223191 for 48 hours. Tfh cells were then collected and stained with LIVE/DEAD, and live Tfh cells were flow sorted again. In parallel, B cells were isolated from PBMCs from the same donor, and CD27⁺ IgD⁻ memory B cells were flow sorted. Tfh and memory B cells were co-cultured in the presence of SEB (1µg/ml) for 5 days. Cells were then analyzed by flow cytometry to quantify CD38^{hi} CD27⁺ plasmablasts among B cells.

[0214] Th1/Th2/Th17 polarization

[0215] Naïve and memory CD4⁺ T cells were stimulated with anti-CD3/CD28 Dynabeads for 4 days in Th1-polarizing conditions (IL-12 (10 ng/ml), IL-2 (20ng/ml) and anti-IL-4 Abs

(1 µg/ml MAB204)) (R&D Systems), Th2-polarizing conditions (IL-4 (20 ng/ml), anti-IFN γ (1µg/ml MAB285) (R&D Systems), or Th17-polarizing conditions (IL-6 (50ng/ml), TGF- β (2ng/mL), IL-23 (40ng/mL), IL-1 β (10ng/mL), anti-IFN γ (1µg/ml), anti-IL-4 Abs (1 µg/ml) (R&D Systems) in the presence of DMSO, TCDD (5nM) or CH-223191 (10µM). Cells were split every 2-3 days and replenished with media containing the Th1- or Th2-polarization cocktails together with AHR modulators for 6 additional days. Intracellular staining was performed as indicated.

[0216] ELISA quantification

[0217] Cytokine levels from supernatant of T-cell culture or from patient serum were quantified by ELISA using Human DuoSet ELISA kits for CXCL13, IL-22, IFN- γ .

[0218] AHR Luciferase Assay

[0219] HEPG2 cells were obtained from Dr. Gary Perdew, Penn State University. Cells were first cultured in DMEM, 15% FBS, 1% penicillin/streptomycin at 37°C until reaching 70% confluence. Cells were then washed, counted, and cultured at a density of 70,000 cells in 96-well plate wells overnight. Cells were then incubated with 10% serum from healthy or SLE patients in RPMI 1640 overnight. The following day, cells were treated with trypsin (Gibco), rinsed, and washed at 800 rpm for 8 minutes. Cells were resuspended in DMEM and lysed with Dual-Glo Luciferase Assay System (Promega#, E2920). The luciferase activity was read with GloMax® Explorer Multimode Microplate Reader, Promega).

[0220] Western Blotting

[0221] Rabbit anti-JUN, anti-phospho-JUN (Ser73), anti-AHR, anti- β -actin, anti-tubullin, anti-cyclophilin B, anti-vincullin antibodies were purchased from Cell Signaling Technology. Cells were pelleted and lysed with Laemmli buffer 1X (Biorad #1610747) or RIPA buffer (Thermofisher #89901) for 1h at 4 degrees using a micro-tube shaker. Lysed cells were then centrifuged at >14000rpm for 10 min, and lysates stored at -80 degrees. Protein was measured using Pierce BCA assay (ThermoScientific #23225), according to manufacturer's instructions. Protein lysates were loaded in 10x Tris 10% or 12% Criterion™ TGX™ Precast

Midi protein gels, transferred to Immun-Blot® PVDF membrane at 4°C for 2 hours with 0.2 amps. Membranes were blocked either with 5% milk or 5% BSA for 1 hour, or EveryBlot Blocking Buffer (Bio-Rad) for 5 min and then incubated with primary antibody overnight (1:1000 for anti-AHR, 1:50,000 for anti-tubulin, anti-cyclophilin B, anti-vinculin or anti- β -actin). Membranes were then incubated with the horseradish peroxidase conjugate-labeled secondary antibody (goat anti-rabbit IgG H+L, Invitrogen) for 1-2 hours and then washed with TBS-T. Protein bands were detected by SuperSignal™ West Femto Maximum Sensitivity substrate (#34096; Thermo Fisher Scientific). Images were obtained and quantified via Chemi Doc and Image Lab Software (Bio-Rad).

[0222] Subcellular fractionation

[0223] Human embryonic kidney 293T (HEK293T) cells were separately transduced with GFP containing lentiviral construct either native or encoding HA-tagged AHR as described above. Transduced HEK293Ts were sorted based on GFP positivity. Cells were then cultured for 72 hours with either AHR agonist, AHR antagonist or vehicle control as above and then harvested for protein lysates. The Standard Cell Fractionation Kit (Abcam #ab109719) was used to collect the cytoplasmic and nuclear fractions, as per the manufacturer's instructions. The Pierce BCA Protein Assay (ThermoFisher, #23225) was used to quantify the protein concentration within the cytoplasmic and nuclear fractions, as per the manufacturer's instructions. Western blot was performed as described above.

[0224] Co-immunoprecipitation

[0225] Human embryonic kidney 293T (HEK293T) cells were transduced with GFP containing lentiviral constructs encoding either HA-tagged AHR or 3xFLAG-tagged c-Jun as described above. Transduced HEK293Ts were sorted based on GFP positivity. Cells were cultured for 72 hours with either AHR agonist, AHR antagonist or vehicle control as above and then harvested for protein lysates using Pierce IP Lysis Buffer (ThermoFisher, #87787) containing Halt Protease Inhibitor Cocktail (ThermoFisher, #78429). The Pierce BCA Protein Assay (ThermoFisher, #23225) was used to quantify the protein concentration within the cytoplasmic and nuclear fractions, as per the manufacturer's instructions.

[0226] The 3xFLAG co-immunoprecipitation was completed with the ANTI-FLAG M2 Affinity Gel (Millipore Sigma, #A2220) and the HA co-immunoprecipitation was completed using Pierce Anti-HA magnetic beads (Fisher, #PI88836). Briefly, 250µg of protein was incubated with washed agarose slurry or magnetic beads overnight at 4°C as per manufacturer instructions. Incubated anti-Flag M2 agarose slurry was washed with 50mM Tris-HCl buffer supplemented with 150mM NaCl, pH 7.6 (Millipore Sigma, #524750) before eluting with 1x Laemmli Sample Buffer (Biorad, #161-0747) at 70°C for 10min. 1µL β-mercaptoethanol was added to eluted product before running on SDS-PAGE for Western blot. Separately, incubated anti-HA magnetic beads were washed with 0.05% Tween-20 in 150mM NaCl, 50mM Tris-HCl buffer, pH 7.6 (Millipore Sigma, #524750) before eluting with acidic 0.1M glycine buffer solution, pH 2.0-2.8 and neutralized with UltraPure 1M Tris-HCl Buffer, pH 9.5. 4x Laemmli Sample Buffer (Biorad, #161-0747) and β-mercaptoethanol were added to elution product before running on SDS-PAGE for Western blot.

[0227] Single Cell RNA-Seq of *in vitro* stimulated cells

[0228] *Sample preparation*

[0229] Isolated memory CD4 T cells were cultured at 1×10^5 cell per well in a 96 well plate with 200ul of RPMI/10% FBS and stimulated with Dynabeads (ThermoFisher). As indicated, cells were cultured with DMSO alone, TGF-β (2ng/mL) and DMSO, CH-223191 (10µM), or TCDD (3nM). Cells were collected at day 6 cells, restimulated, and collected at day 13. Cell counts were normalized across conditions and stained with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen) and cell hashing antibodies specific for each condition and donor, pooled, and stained with TotalSeq-C Human Universal Cocktail (BioLegend). Viable cells were then flow sorted and subjected to encapsulation and library preparation at the BWH Center for Cellular Profiling via the 10X Genomics pipeline, with 24-30K cells loaded per run with 82-95% viability.

[0230] *Single cell RNA-seq data processing and QC*

[0231] Libraries were prepared according to the 10x Genomics User Guide and processed by Cell Ranger (v.6.1.1) workflow. FASTQ files containing gene expression and feature

barcodes were aligned to the human genome hg38 (GRCh38). The filtered features, barcodes, and matrix files were imported into R and used to generate a Seurat object with Seurat package (v.4.3.0). Quality control was first performed filtering out cells with more than 10% mitochondrial reads and with <200 or >5,500 reads. Cells that passed the QC were log normalized and scaled. The Seurat object was then demultiplexed using the hashtag oligos. HTO reads were normalized and cells assigned a HTO ID using the HTODemux function from Seurat. Doublets were removed and the filtered cells were used to run PCA using the Seurat Package. To account for donor variation, the Seurat object for each timepoint was individually integrated utilizing Harmony (v.0.1.1), where each Seurat object was corrected by donor. The top 10 harmony embeddings were then used to generate a UMP and clustering was performed with a resolution of 0.7. Differentially expressed genes between the four culture conditions were identified using the FindMarkers function in the Seurat Package.

[0232] *Symphony Mapping*

[0233] The Rheumatoid Arthritis reference dataset was generated through the Accelerating Medicines Partnership (AMP)¹⁷. T cells were identified as described¹⁷, and filtered for cells with mitochondrial read <20% and with reads >200 and <5000. Cells passing filtering were normalized, scaled, and run through Harmony (v.0.1.1) integration correcting for donor. The top 15 harmony embeddings were used to generate a UMAP and clustered with a resolution of 0.8. The harmonized object was then used to generate a reference object with the buildReferenceFromSeurat Function in the Symphony package (v.0.1.1). Data from day 13 *in vitro* single cell data was then query mapped to the AMP reference. The frequency at which cells mapped to a given cluster was extracted and compared between culture conditions by donor.

[0234] **Single Cell RNA-Seq of T cells from patients with lupus**

[0235] *Sample collection*

[0236] Blood samples were also obtained from patients with SLE (n=5) or with a diagnosis of refractory cutaneous lupus (n=2) before starting treatment with anifrolumab (first day of infusion) and 1-3 months after the starting date. Cryopreserved PBMC were thawed,

resuspended in Fc Receptor block (BioLegend) and then stained with hashing antibodies specific for each donor and timepoint, CD3-APC (BioLegend), and propidium iodide. Viable CD3⁺ T cells were flow-sorted, pooled, and stained with TotalSeq-C Human Universal Cocktail (BioLegend). Cells were then subjected to encapsulation and library preparation at the BWH Center for Cellular Profiling via the Chromium X system from 10X Genomics, with 2x100k cells loaded with 89% viability.

[0237] *Anifrolumab single cell data analysis*

[0238] Quality control was first performed filtering out cells with more than 15% mitochondrial reads and with <1,000 or >4,000 reads. Samples were demultiplexed and clustered as above, and memory CD4 T cells were selected for further analysis. Cells passing filtering were normalized, scaled, and run through Harmony (v.0.1.1) integration correcting for donor and 10x sequencing batch. The top 20 harmony embeddings were then used to generate a UMAP and clustering was performed with a resolution of 1.2. The signature scores are calculated using addmoduleScore function from Seurat package.

[0239] **Low-input bulk RNA-Seq analysis**

[0240] Low input bulk RNA-Seq libraries were prepared at Broad Technology Labs at the Broad Institute of Harvard and MIT using the Illumina SmartSeq2 platform. Libraries were sequenced to generate 38 base paired-end reads. FASTQ files from sequencing were examined with FastQC for quality control and trimmed with trimmomatic. Reads were aligned to human genome hg38 (GRCh38) using hisat2 alignment program. Lowly expressed genes (log2 FPKM<10 in 10 samples) were filtered out for downstream analysis. Differentially expressed genes (DEGs) were identified using DESeq2 with an adjusted p-value threshold of < 0.05 . Principal component analysis (PCA) was performed using the prcomp function in R. The top 20% most variable genes were selected for this analysis. Heatmap is generated using the pheatmap package (v.1.0.12) in R using FPKM values that have been scaled by each gene. The signature genes for Tregs, CD96hi cells, or Tph/Tfh cells are the common DEGs in individual comparisons with each of the other cell groups. The ssGSEA score is calculated by gsva package (GSVA v.1.38.2) in R. The Th22 cell gene signature list and Tph cell gene signature list were derived from previous reports^{16,32}.

[0241] ATAC-Seq data analysis

[0242] Raw sequencing data were trimmed using cutadapt (v.1.18) with Python (v.2.7.15) to remove the adapter. Trimmed reads were aligned to the GRCh38 human reference genome with Bowtie2; Aligned reads were filtered to remove mitochondrial reads and PCR duplicates, then peaks were called using Genrich in ATAC-Seq mode on individual samples. The fragment size distribution is checked using deepTools (v.3.1.2). The intervals were set to the default length of 100bp, and the peak-calling significance threshold was set to $-\log(p) > 2$. A union peak list for each data set was created by combining all peaks in all samples, merging overlapping peaks using bedtools (v.2.26.0), and retaining only peaks that were called in more than one sample. Normalized read counts for consensus peaks were computed for each sample using Diffbind, and differential accessibility between different groups was determined using a matched pairs t-test with the edgeR package (v.3.30). Peaks were annotated using ChIPseeker (v.1.26.2). Enrichment analysis of peaks and Gene Sets Enrichment Analysis was conducted using the clusterProfiler (v.3.16) package with a threshold of $FDR < 0.05$ to define enriched pathways. For ATAC-Seq GSEA, peaks were declared differentially accessible at the genome-wide level with a false discovery rate adjusted p-value < 0.05 , and those exhibiting a log₂ fold change of ± 2 or greater were defined as PD1^{hi} signature in tonsil and SF. The PD-1^{hi} signatures were used for GSEA analysis using the clusterProfiler(v3.16) package. Peak signal tracks were generated using the rtracklayer package (v.1.48) or IGV software.

[0243] Systemic lupus erythematosus patient plasma CXCL13 comparison from TULIP-1 randomized clinical trial

[0244] Circulating plasma samples taken at baseline, Week 12, and Week 52 (end of trial) from 302 patients in the TULIP-1 trial⁵⁸ were assessed for protein biomarkers by targeted high-multiplex immunoassay panels on the Olink platform; Olink Target 96 Immuno-Oncology (v.3112). Data for CXCL13 protein is presented as relative Normalized Protein eXpression (NPX), which is on a Log₂ scale. Statistical analysis and data visualization conducted using standard packages in R version 4.0.1.

[0245] A longitudinal mixed effect model was used to test whether 1-year trajectory of CXCL13 protein were statistically different between anifrolumab and placebo arms. Model was adjusted for the trial stratification factors; baseline oral corticosteroid dose (<10 mg/day, ≥10 mg/day), Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI 2K) score at screening (<10 points, ≥10 points), and IFN 4-gene status⁹⁰. Multiple testing was accounted for using the Benjamini-Hochberg (FDR) procedure with < 0.05 threshold.

[0246] Statistics

[0247] Statistical analysis was performed as described in each section and figure legends using Graphpad Prism 8 software and R (v.4.0.3). Unless otherwise indicated, multi-group analyses were performed using non-parametric Friedman test with Dunn's multiple comparisons test, and two group comparisons were performed using either paired t-test, Wilcoxon test or paired ratio t-test as indicated in figure legends. P<0.05 was considered significant.

[0248] Tables (Examples Section)

[0249] **Table 2. Tph cell CRISPR Array Screen Targets. Each gene included four gRNA sequences.** The CRISPR screens initially screened 86 target genes were selected from genes upregulated in Tph/Tfh cells in a current RNA-Seq dataset, or in previously published RNA-Seq datasets of rheumatoid arthritis synovial T cells¹⁶, or from genes previously correlated with CXCL13 expression, or AP-1 family members. Included in Table 2 are the genes that increased CXCL13 in cells when knocked out and the corresponding CRISPR gRNA sequences for the gene.

SEQ ID NO:	Gene Symbol	Sequence
5	<i>AHR</i>	TAGTGGTAAGGGATCCATTA
6	<i>AHR</i>	TAATGGATCCCTTACCACTA
7	<i>AHR</i>	TCATGGTGCCCAGCCGACGG
8	<i>AHR</i>	GGCCTCCGTTTCTTTCAGTA
9	<i>BATF</i>	ATGAGGTTGGTGAATGCGT
10	<i>BATF</i>	CCGCCCAGAAGAGCCGACAG
11	<i>BATF</i>	GCTGTCGGAGCTGTGAGGCA
12	<i>BATF</i>	CACATCATCAGATGAGTCCT

13	<i>CBLB</i>	TGTGACACATCCAGGTTACA
14	<i>CBLB</i>	CCAATCGAGTGCAACTTAAC
15	<i>CBLB</i>	GCTGCTATAGATTTAGACGT
16	<i>CBLB</i>	GATTTCCTCCTCGACCACCA
17	<i>JUN</i>	CGTTGCTGGACTGGATTATC
18	<i>JUN</i>	CTCCTCACCTCGCCCGACGT
19	<i>JUN</i>	CGTGTTCTGGCTGTGCAGTT
20	<i>JUN</i>	GTTGAGGGCATCGTCATAGA
21	<i>STAT5B</i>	TATGCCACACAGCTCCAGGT
22	<i>STAT5B</i>	GATTGAAAGCCAAGCATGGT
23	<i>STAT5B</i>	CATCAGATGCAAGCGTTATA
24	<i>STAT5B</i>	TATTCCTGAAGTGGGCACTA
25	<i>STAT5A</i>	GTTGATCTGAAGGTGCTTCT
26	<i>STAT5A</i>	TACGCCACGCAGCTCCAGGT
27	<i>STAT5A</i>	CATCGAGGTCCGGCACTACT
28	<i>STAT5A</i>	CACTGAACTGAGACTCAAAC
29	<i>STAT1</i>	GATCACCACAACGGGCAGAG
30	<i>STAT1</i>	TGTGATAGGGTCATGTTCGT
31	<i>STAT1</i>	GCTGAATTTTCGGCACCTGGT
32	<i>STAT1</i>	CTGAATTTTCGGCACCTGGTA
33	<i>JAK1</i>	TATGCCTACAGATATCATGG
34	<i>JAK1</i>	CTTAGTAGCCACCAGCAGGT
35	<i>JAK1</i>	CTTGGTGTTCTCGTCATACA
36	<i>JAK1</i>	TGCCTAGACAGCACCGTAAT
37	<i>JUNB</i>	GGTAGCGCCCAGGGACACGT
38	<i>JUNB</i>	TATGAGTCGTCGTGGTAGAA
39	<i>JUNB</i>	CTGACAGCCGTTGCTGACGT
40	<i>JUNB</i>	TCTGGTCAGGGCTCGGACAC
41	<i>NR3C2</i>	GACCGATGAGAATAACTACA
42	<i>NR3C2</i>	ACACAGAGTTGATTCCAGCA
43	<i>NR3C2</i>	TCAGAGGGCTGCAAACAGAC
44	<i>NR3C2</i>	CTTGAGCACCAATCCTGTAG

[0250] Table 3. Differential transcript expression at 12 and 24 hours after stimulation with an AHR agonist or inhibitor. Log2 fold changes is a comparison of T cells treated with an AHR inhibitor+ TGF- β compared to an AHR agonist+ TGF- β . A positive log2 fold change indicated higher expression in T cells treated with the AHR agonist.

12 hours

	log2 Fold Change	<i>P</i> value	Adjusted <i>P</i> value
<i>CYP1B1</i>	4.6858963	4.69E-17	5.37E-13
<i>TIPARP</i>	2.3464807	3.73E-13	2.13E-09
<i>LMNA</i>	1.3048018	7.22E-09	2.75E-05
<i>IKZF3</i>	0.9203728	5.77E-08	0.0001649
<i>ITGA1</i>	2.4543184	8.02E-08	0.0001794
<i>ITGAL</i>	1.4553643	9.41E-08	0.0001794
<i>DUSP4</i>	1.0703486	5.42E-07	0.0008857
<i>TRAF4</i>	1.9322586	6.48E-07	0.0009265
<i>LPXN</i>	1.4090928	8.14E-07	0.0010344
<i>RNF144A</i>	2.027682	9.68E-07	0.0011074
<i>CXCL8</i>	2.2081591	3.06E-06	0.0031778
<i>CLDND1</i>	1.2358017	4.70E-06	0.0044815
<i>SCD</i>	-1.961364	7.04E-06	0.0061902
<i>PPP2R5C</i>	0.8277342	9.74E-06	0.0079588
<i>IL1B</i>	1.6476733	3.03E-05	0.0216735
<i>SOS1</i>	1.0374021	3.03E-05	0.0216735
<i>CTSL</i>	1.9427539	3.49E-05	0.0234795
<i>GALNT2</i>	1.8651553	7.72E-05	0.0490662
48 hours			
	log2 Fold Change	<i>P</i> value	Adjusted <i>P</i> value
<i>RHOA</i>	4.2114591	2.17E-51	2.43E-47
<i>GPR157</i>	2.7793111	2.18E-34	1.22E-30
<i>TIPARP</i>	2.705468	3.77E-22	1.41E-18
<i>CD226</i>	2.2271063	2.71E-21	7.59E-18
<i>AIM2</i>	-1.899453	1.88E-20	4.21E-17
<i>CD40LG</i>	-1.818956	3.32E-20	6.19E-17
<i>GJB2</i>	4.5913992	2.86E-19	4.58E-16
<i>RGS16</i>	-2.157395	2.87E-18	4.02E-15
<i>LMNA</i>	1.5273662	1.08E-16	1.34E-13
<i>TNFRSF11A</i>	2.650977	2.27E-16	2.55E-13
<i>RNF19A</i>	-1.389284	1.41E-15	1.44E-12
<i>STING1</i>	-1.765282	1.86E-15	1.74E-12
<i>GPR68</i>	4.2396159	1.71E-14	1.48E-11
<i>CYP1B1</i>	10.590944	3.47E-14	2.78E-11
<i>ITGA1</i>	2.4322158	2.11E-13	1.57E-10
<i>IL3</i>	-2.928894	3.18E-13	2.23E-10
<i>IFNG</i>	-1.609179	3.94E-13	2.59E-10
<i>SRGN</i>	-0.881356	1.16E-12	7.22E-10

<i>CD44</i>	-1.045856	1.51E-12	8.90E-10
<i>SLA</i>	-1.200464	2.72E-12	1.52E-09
<i>AHRR</i>	6.4005966	4.63E-12	2.47E-09
<i>SOS1</i>	1.3069934	1.08E-11	5.50E-09
<i>LOC100288175</i>	3.2572938	2.62E-11	1.22E-08
<i>SEPTIN9</i>	1.0137024	2.53E-11	1.22E-08
<i>CCR1</i>	-2.34175	4.67E-11	2.02E-08
<i>LBH</i>	-1.595998	4.69E-11	2.02E-08
<i>MYB</i>	1.6489335	7.56E-11	3.14E-08
<i>CYP1A1</i>	8.0840055	1.43E-10	5.74E-08
<i>CLDND1</i>	1.5751164	2.92E-10	1.13E-07
<i>TIGIT</i>	2.0754118	4.44E-10	1.66E-07
<i>CRYBG1</i>	-1.173035	4.91E-10	1.77E-07
<i>NFKBIA</i>	-0.799773	6.49E-10	2.27E-07
<i>STMND1</i>	7.8890608	6.89E-10	2.34E-07
<i>RALB</i>	1.0989987	7.90E-10	2.60E-07
<i>UCP2</i>	-1.161888	1.15E-09	3.68E-07
<i>CPOX</i>	1.9664371	1.79E-09	5.56E-07
<i>PFKFB3</i>	1.037257	3.45E-09	1.05E-06
<i>CXCL13</i>	-3.100432	4.51E-09	1.33E-06
<i>GBP2</i>	-0.79737	4.65E-09	1.34E-06
<i>GJB6</i>	6.4636801	5.88E-09	1.65E-06
<i>ITM2C</i>	2.7402481	7.11E-09	1.90E-06
<i>PIGX</i>	0.911614	6.95E-09	1.90E-06
<i>LPXN</i>	1.0773775	9.43E-09	2.46E-06
<i>PALM2AKAP2</i>	1.3401117	2.52E-08	6.41E-06
<i>ALOX5AP</i>	-1.265716	2.59E-08	6.45E-06
<i>MSL3</i>	0.9488386	5.63E-08	1.37E-05
<i>NPTX1</i>	6.9169124	5.85E-08	1.39E-05
<i>RAB11FIP1</i>	-0.917751	8.79E-08	2.05E-05
<i>DUSP5</i>	-1.00158	1.20E-07	2.74E-05
<i>COL6A3</i>	-1.323102	1.36E-07	3.05E-05
<i>LAG3</i>	-1.619184	1.83E-07	4.03E-05
<i>ADGRG5</i>	5.1493502	2.16E-07	4.66E-05
<i>ADD2</i>	2.4149603	2.25E-07	4.76E-05
<i>ABI3</i>	1.4418068	2.56E-07	5.31E-05
<i>STAT1</i>	0.7966264	2.87E-07	5.85E-05
<i>TIMP1</i>	-1.435576	4.71E-07	9.43E-05
<i>ZMIZ1</i>	1.2389496	5.10E-07	0.0001002
<i>PIM2</i>	-0.717567	5.29E-07	0.0001021

<i>TRAF1</i>	-0.851936	5.73E-07	0.0001088
<i>DUSP2</i>	-1.121852	5.86E-07	0.0001094
<i>IL21</i>	-2.020773	7.62E-07	0.0001399
<i>CD82</i>	-0.755025	1.89E-06	0.0003407
<i>ID2</i>	-0.771332	2.21E-06	0.0003936
<i>IL9</i>	-0.885727	2.34E-06	0.0004089
<i>IL2</i>	-3.554747	2.41E-06	0.0004101
<i>MALT1</i>	0.7021634	2.42E-06	0.0004101
<i>USP12</i>	0.7760647	2.81E-06	0.0004693
<i>IL1R1</i>	0.9698061	2.97E-06	0.0004848
<i>RGL4</i>	2.4280405	2.99E-06	0.0004848
<i>SCD</i>	-1.219751	3.31E-06	0.0005301
<i>SLC1A4</i>	1.0235069	3.49E-06	0.0005513
<i>CENPF</i>	0.6400842	3.57E-06	0.000556
<i>ANK3</i>	1.2196186	3.89E-06	0.0005973
<i>BTG1</i>	-0.606402	4.34E-06	0.0006574
<i>IL7R</i>	-0.859727	4.90E-06	0.0007316
<i>DUSP4</i>	0.7895353	5.01E-06	0.0007383
<i>RDX</i>	0.6802702	5.15E-06	0.000749
<i>EED</i>	-0.718892	5.27E-06	0.0007565
<i>HIPK2</i>	0.7642931	5.52E-06	0.000782
<i>IL6R</i>	-0.788	5.89E-06	0.0008253
<i>NAPSB</i>	2.2375787	6.42E-06	0.0008882
<i>TBL1X</i>	-0.897727	6.92E-06	0.0009446
<i>TERC</i>	1.1908771	7.94E-06	0.001071
<i>RCBTB2</i>	3.001567	8.77E-06	0.0011688
<i>TAGAP</i>	-1.559033	9.36E-06	0.0012051
<i>TRAF4</i>	1.3139171	9.33E-06	0.0012051
<i>ZFP36L2</i>	-0.829742	9.19E-06	0.0012051
<i>SLAMF7</i>	1.4015347	9.53E-06	0.001213
<i>ICOS</i>	-0.729976	9.74E-06	0.001226
<i>ZBED2</i>	0.7167055	1.13E-05	0.0014122
<i>ZBTB10</i>	-1.754928	1.23E-05	0.0015084
<i>MGAT5</i>	-0.977231	1.31E-05	0.0015966
<i>CD96</i>	0.9667371	1.41E-05	0.0016989
<i>RMRP</i>	-2.301186	1.59E-05	0.0018972
<i>FOSL2</i>	-0.9464	1.66E-05	0.0019344
<i>GPR55</i>	2.8160629	1.65E-05	0.0019344
<i>LINC00892</i>	-1.9592	1.79E-05	0.0020622
<i>RNF144A</i>	1.7083418	1.95E-05	0.0022305

<i>MAPKAPK2</i>	-0.667926	2.14E-05	0.0024238
<i>CCR2</i>	-1.556592	2.24E-05	0.0025084
<i>MBD2</i>	0.6004829	2.32E-05	0.0025762
<i>CSF1</i>	-1.015454	2.63E-05	0.0028284
<i>MAF</i>	-0.880552	2.60E-05	0.0028284
<i>MYOF</i>	5.8213016	2.61E-05	0.0028284
<i>CYTOR</i>	-0.898155	2.85E-05	0.0030133
<i>SIPRI</i>	1.1731538	2.84E-05	0.0030133
<i>LFNG</i>	-0.983513	3.00E-05	0.0031404
<i>RNU4-2</i>	-1.443442	3.22E-05	0.0033358
<i>UBALD2</i>	-0.816424	3.29E-05	0.003386
<i>CFLAR</i>	-0.732902	3.38E-05	0.0033863
<i>PIM3</i>	-0.583934	3.37E-05	0.0033863
<i>TIAMI</i>	-1.053014	3.39E-05	0.0033863
<i>CYLD</i>	-0.849662	3.42E-05	0.0033897
<i>SEMA7A</i>	-0.942905	4.05E-05	0.0039763
<i>CTSB</i>	-0.683141	4.24E-05	0.0041285
<i>PPIF</i>	0.6866764	4.31E-05	0.004163
<i>XBP1</i>	-0.875443	4.46E-05	0.0042675
<i>ATP6V0A2</i>	0.7945725	4.59E-05	0.0043572
<i>KDM5B</i>	-0.834906	4.64E-05	0.0043697
<i>KIAA0586</i>	0.9561603	4.74E-05	0.0044243
<i>LGALS8</i>	0.8208088	4.86E-05	0.0044967
<i>APOBEC3C</i>	-0.760839	5.08E-05	0.0046609
<i>IL12RB1</i>	-1.432531	6.07E-05	0.0055259
<i>GLO1</i>	0.6524317	6.19E-05	0.0055909
<i>IKZF3</i>	0.6034999	6.41E-05	0.0057403
<i>IL26</i>	1.4965406	6.78E-05	0.0060295
<i>RPTOR</i>	1.6583509	6.93E-05	0.0061098
<i>NDUFA13</i>	-0.753424	7.60E-05	0.0066513
<i>ETS2</i>	0.8095858	7.75E-05	0.0067308
<i>CHAC1</i>	-2.03616	8.04E-05	0.0069235
<i>ADAM9</i>	-3.182392	8.49E-05	0.0072634
<i>SREBF1</i>	-2.27838	8.87E-05	0.0075259
<i>ETHE1</i>	0.7422263	9.13E-05	0.0076897
<i>THBS1</i>	5.545079	9.24E-05	0.0077213
<i>GALR2</i>	-1.620879	9.92E-05	0.0082287
<i>GNA15</i>	-1.054425	0.0001025	0.0083814
<i>WARS1</i>	-0.574056	0.0001018	0.0083814
<i>SERF2</i>	-0.91204	0.0001069	0.0086731

<i>PCLAF</i>	0.5812841	0.0001102	0.0088774
<i>ASCL2</i>	-2.333575	0.0001197	0.009455
<i>PRR5L</i>	-1.282766	0.0001199	0.009455
<i>RN7SL1</i>	-1.208419	0.0001189	0.009455
<i>IFITM2</i>	-0.663718	0.000122	0.0095576
<i>HMGCS1</i>	-0.898063	0.0001248	0.0097044
<i>BNIP3</i>	-0.990061	0.0001386	0.0107053
<i>ATP6V0D1</i>	-1.356756	0.0001461	0.0112059
<i>FADS1</i>	-1.188536	0.0001567	0.0119435
<i>NTRK3</i>	3.8247249	0.0001579	0.01195
<i>CDKN2A</i>	-1.354643	0.0001655	0.0124429
<i>LYZ</i>	5.7774082	0.0001696	0.0126678
<i>B3GNT5</i>	-5.389034	0.0001746	0.0129543
<i>DDB2</i>	0.800694	0.0001759	0.0129651
<i>CDI09</i>	1.7819806	0.0001773	0.0129831
<i>POU2F2</i>	-0.639821	0.0001814	0.0131926
<i>BCL2L11</i>	-1.069603	0.0001858	0.0134268
<i>IL24</i>	-2.934094	0.0001909	0.0137087
<i>SMAD3</i>	1.2615893	0.0001955	0.0139465
<i>FUT8</i>	1.0220321	0.0001992	0.0141234
<i>MT2A</i>	1.521686	0.0002029	0.0142901
<i>IL1RL2</i>	4.6691982	0.0002058	0.0144041
<i>SP100</i>	0.5574788	0.0002127	0.0147982
<i>CAMTA2</i>	-0.980303	0.0002148	0.0148519
<i>VSIR</i>	-1.953011	0.0002219	0.0152461
<i>SSR4</i>	-0.691494	0.0002354	0.0160798
<i>HDAC7</i>	-0.579445	0.0002397	0.0162691
<i>SAT1</i>	-0.788619	0.0002533	0.0170904
<i>HIC1</i>	4.1980807	0.0002735	0.0183435
<i>HEATR5A</i>	-1.179758	0.000282	0.0188005
<i>MAPK13</i>	-0.892596	0.0002861	0.0188476
<i>TUBA8</i>	-0.858722	0.0002859	0.0188476
<i>IL13</i>	-2.008043	0.0002878	0.0188526
<i>FHL3</i>	-1.602218	0.0003044	0.019826
<i>FLVCR2</i>	1.6634604	0.0003142	0.0203409
<i>MALAT1</i>	-0.471766	0.0003189	0.0205261
<i>SAMSN1</i>	0.7854464	0.0003242	0.0207534
<i>SNORA16A</i>	-1.555477	0.0003468	0.0220735
<i>MYO5B</i>	-4.523484	0.0003546	0.0224395
<i>RRM2B</i>	1.141136	0.000368	0.0231571

<i>SNX9</i>	-0.883571	0.0003741	0.0234104
<i>PCSK7</i>	1.1647584	0.0003764	0.0234205
<i>HBA2</i>	-4.086547	0.0004021	0.0248846
<i>ASPM</i>	0.5350211	0.0004283	0.0262179
<i>EGR3</i>	-1.522642	0.0004269	0.0262179
<i>PMM2</i>	0.6906288	0.0004523	0.0275315
<i>DOK1</i>	0.7589581	0.0004558	0.0275958
<i>VOPPI</i>	-0.57456	0.0004702	0.0283131
<i>RASGRP1</i>	-0.553681	0.0004915	0.0294372
<i>NEK6</i>	1.5751275	0.0004976	0.0296455
<i>ABCA1</i>	-2.095053	0.0005182	0.0307114
<i>PPDPF</i>	-0.535099	0.0005239	0.030883
<i>FAM102A</i>	-0.819956	0.0005289	0.0310168
<i>ORAI2</i>	0.5917956	0.0005323	0.0310521
<i>APOBEC3G</i>	-0.660007	0.0005518	0.0320266
<i>SNX10</i>	-0.602515	0.0005561	0.0321091
<i>TENT5C</i>	-1.125821	0.0005662	0.0325224
<i>ARAP2</i>	-0.779799	0.0005826	0.0332931
<i>ANXA6</i>	-0.607296	0.0005888	0.0334783
<i>ZNF365</i>	-2.619862	0.0006321	0.0357585
<i>ARHGAP25</i>	-0.693915	0.0006439	0.0362401
<i>TNFAIP3</i>	-0.629438	0.000657	0.0367967
<i>MDFIC</i>	-0.617716	0.0006753	0.0376337
<i>SSH1</i>	0.9544877	0.0006902	0.0382699
<i>RGS3</i>	-1.456071	0.0007105	0.0392025
<i>KIF21B</i>	-0.806572	0.0007345	0.0399352
<i>PPP6R3</i>	0.6166736	0.0007305	0.0399352
<i>RUNX3</i>	0.5870499	0.0007325	0.0399352
<i>NDFIP1</i>	-0.644877	0.000765	0.0413962
<i>HSP90AA1</i>	0.4041844	0.0008064	0.0432327
<i>TSPAN3</i>	0.9593337	0.0008067	0.0432327
<i>SELPLG</i>	-0.886503	0.0008149	0.0434643
<i>GATA3</i>	-0.645222	0.0008634	0.0458048
<i>TNIP2</i>	-0.641858	0.0008669	0.0458048
<i>C2CD4A</i>	-3.412064	0.0008739	0.045811
<i>REL</i>	-0.646797	0.0008752	0.045811
<i>TMSB10</i>	-0.545785	0.0008886	0.0462935
<i>DGAT1</i>	-1.561375	0.0009003	0.0466874
<i>DAPP1</i>	0.652567	0.0009055	0.0467418
<i>CBX6</i>	-0.428362	0.0009112	0.0468178

<i>FCRL4</i>	5.4803674	0.0009267	0.0473965
<i>ACTN1</i>	-1.003466	0.0009368	0.0476972
<i>FAM83D</i>	1.17483	0.0009688	0.0491026

[0251] Table 4. AHR modulated gene expression. Shown here are transcripts identified as part of cluster 6, which is progressively upregulated over time by AHR activation, as seen in **Fig. 3a**. The default setting of maSigPro breaks down profiles into 9 clusters, however only the cluster shown below was observed by Applicant to be progressively upregulated.

CLUSTER 6			
Genes			
<i>ABI3</i>	<i>ADD2</i>	<i>ADGRG5</i>	<i>ADGRG6</i>
<i>AHRR</i>	<i>ARMC9</i>	<i>BCOR</i>	<i>CBFA2T3</i>
<i>CD226</i>	<i>CEMP2</i>	<i>CGAS</i>	<i>CLDND1</i>
<i>CPOX</i>	<i>CTSL</i>	<i>CYP1A1</i>	<i>CYP1B1</i>
<i>DENND3</i>	<i>DPF1</i>	<i>DUSP4</i>	<i>ETS2</i>
<i>FAM167A</i>	<i>FCRL4</i>	<i>FZD1</i>	<i>GIMAP6</i>
<i>GJB2</i>	<i>GJB6</i>	<i>GPR157</i>	<i>GPR55</i>
<i>GPR68</i>	<i>HIC1</i>	<i>HIPK2</i>	<i>ID1</i>
<i>IKZF3</i>	<i>ITGA1</i>	<i>ITM2C</i>	<i>KIAA1671</i>
<i>LMNA</i>	<i>LOC100288175</i>	<i>LOXL3</i>	<i>LPXN</i>
<i>MAP3K20</i>	<i>MT1A</i>	<i>MT1E</i>	<i>MT2A</i>
<i>MYL9</i>	<i>MYOF</i>	<i>NEK6</i>	<i>NPTX1</i>
<i>NTRK3</i>	<i>OLIG3</i>	<i>PAX2</i>	<i>PFKFB3</i>
<i>PIK3R1</i>	<i>PLAT</i>	<i>PLEKHO1</i>	<i>PPP2R5C</i>
<i>RALB</i>	<i>RCBTB2</i>	<i>RHOA</i>	<i>RNF144A</i>
<i>RNF144A-AS1</i>	<i>RPTOR</i>	<i>RRM2B</i>	<i>SLC8A1-AS1</i>
<i>SMAD3</i>	<i>SMG6</i>	<i>SOS1</i>	<i>STAT1</i>
<i>TIGIT</i>	<i>TIPARP</i>	<i>TNFRSF11A</i>	<i>TPRG1</i>
<i>TRAF4</i>			

[0252] Table 5. Tph cell CRISPR Array Screen Targets. Each gene included four gRNA sequences. The CRISPR screens initially screened 86 target genes were selected from genes upregulated in Tph/Tfh cells in a current RNA-Seq dataset, or in previously published RNA-Seq datasets of rheumatoid arthritis synovial T cells¹⁶, or from genes previously correlated with CXCL13 expression, or AP-1 family members. Included in Table 5 are the genes that

decreased CXCL13 in cells when knocked out and the corresponding CRISPR gRNA sequences for the gene.

SEQ ID NO:	Gene Symbol	Sequence
45	<i>CD3D</i>	TTGCAATACCAGCATCACAT
46	<i>CD3D</i>	CTGTCCTCAAGTTCCTCTAT
47	<i>CD3D</i>	ACATGGGTAGAGGGAACGGT
48	<i>CD3D</i>	TACACCTATATATTCCTCGT
49	<i>LCP2</i>	TACCTTCCCGCTGGAATCGA
50	<i>LCP2</i>	AGAGCTTCCTCGTCATTGGA
51	<i>LCP2</i>	CTGCCTTCGGCTCTGATAGG
52	<i>LCP2</i>	CGGAGCTAATGAACGATCTA
53	<i>ATF4</i>	AGATGACCTTCTGACCACGT
54	<i>ATF4</i>	ATCCACAGCCAGCCATTTCGG
55	<i>ATF4</i>	AGGTCTCTTAGATGATTACC
56	<i>ATF4</i>	TCACCCTTGCTGTTGTTGGA
57	<i>IFNAR2</i>	GCCTATGTCACCGTCCTAGA
58	<i>IFNAR2</i>	TTTCCGGTCCATCTTATCAT
59	<i>IFNAR2</i>	GTGTATATCAGCCTCGTGTT
60	<i>IFNAR2</i>	GCTGTATACAATCATGAGGT
61	<i>STAT2</i>	CTCTGTGCAACCGTACACGA
62	<i>STAT2</i>	GGCTCATTGTGGTCTCTAAT
63	<i>STAT2</i>	TGTGATACCATGCATAGTGT
64	<i>STAT2</i>	TCTGTGCTCCAGACGCATCA

[0253] **Numbered Embodiments**

[0254] Additional embodiments include the following numbered embodiments:

1. An aryl hydrocarbon receptor (AHR) agonist, wherein the AHR agonist negatively regulates pathological T cell differentiation in autoantibody driven autoimmune diseases.
2. The agonist of embodiment 1, wherein the agonist negatively regulates T follicular helper (Tfh) cell and/or T peripheral helper (Tph) cell differentiation.

3. The agonist of embodiment 1 or 2, wherein the agonist is conjugated to a T cell targeting moiety.
4. The agonist of embodiment 3, wherein the targeting moiety is an anti-T cell antibody.
5. The agonist of any of embodiments 1-4, wherein the agonist is the cytokine IL-2, 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and/or 6-formylindolo(3,2-b)carbazole (FICZ).
6. A vector comprising the agonist of any of embodiments 1-5, optionally wherein the vector is, comprises, or is derived from a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector.
7. The vector of embodiment 6, wherein the vector comprises a lentivirus.
8. A composition comprising the agonist of any of embodiments 1-5 or the vector of embodiment 6 or 7, and a carrier, optionally wherein the carrier is a pharmaceutically acceptable carrier.
9. A method of preventing CXCL13+ Tph/Tfh cell generation, comprising contacting a cell population with one or more agonists of any of embodiments 1-5, the vector of embodiment 6 or 7, or the composition of embodiment 8, thereby preventing CXCL13+ Tph/Tfh cell generation, wherein the contacting is *in vivo*, *in vitro*, *in situ*, or *ex vivo*.
10. A method of treating an autoimmune disease in a subject in need thereof, the method comprising administering an effective amount to the subject in need of one or more agonists of any one of embodiments 1-5, the vector of embodiment 6 or 7, or the composition of embodiment 8, thereby treating the autoimmune disease, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, orally, and/or intravenously.
11. A method of inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, comprising administering an effective amount of one or more agonists of any one of embodiments 1-5, the vector of embodiment 6 or 7, or the composition of

embodiment 8 to a subject in need thereof, thereby inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, optionally wherein the function is Tph/Tfh differentiation or B cell recruitment, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, orally, and/or intravenously.

12. The method of embodiment 11, wherein the subject has an autoimmune disease.

13. The method of embodiment 11 or 12, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

14. The method of embodiment 13, wherein the autoimmune disease is rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE).

15. The method of any of embodiments 11-14 where the subject is a human.

16. A method of treating an autoimmune disease in a subject in need thereof, comprising obtaining a population of T cells from the subject, treating the population of T cells with an AHR agonist, and administering an effective amount of the treated population of T cells to the subject, thereby treating the autoimmune disease, optionally wherein the subject in need thereof is a human, optionally wherein the administering is done subcutaneously, orally, and/or intravenously.

17. The method of embodiment 16, wherein the treatment with an AHR agonist reduces the frequency of PD-1+CXCR5- Tph cells in the treated T cell population.

18. The method of embodiment 16, wherein the treatment with an AHR agonist stabilizes JUN function.

19. A transcription factor selected from AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and BATF, wherein the transcription factor negatively regulates Tfh and Tph cell function in autoantibody driven autoimmune disease.
20. The transcription factor JUN, wherein JUN negatively regulates Tfh and Tph cell function in autoantibody driven autoimmune disease.
21. A vector comprising the transcription factor of embodiment 19 or 20, optionally wherein the vector is, comprises, or is derived from a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector.
22. The vector of embodiment 21, wherein the vector comprises a lentivirus.
23. A composition comprising the transcription factor of embodiment 19 or 20 or the vector of embodiment 21 or 22, and a carrier, optionally wherein the carrier is a pharmaceutically acceptable carrier.
24. A method of preventing CXCL13+ Tph/Tfh cell generation, comprising contacting a cell population with one or more transcription factors of embodiment 19 or 20, vectors of embodiment 21 or 22, or composition of embodiment 23, thereby preventing CXCL13+ Tph/Tfh cell generation, optionally wherein the contacting is *in vivo*, *in vitro*, *in situ*, or *ex vivo*.
25. A method of inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, comprising administering an effective amount of one or more transcription factors of embodiment 19 or 20, vectors of embodiment 21 or 22, or composition of embodiment 23 to a subject in need thereof, thereby inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, optionally wherein the function is Tph/Tfh differentiation or B cell recruitment, and optionally wherein the subject in need thereof is a human.
26. A method of treating an autoimmune disease in a subject in need thereof, comprising administering an effective amount of one or more transcription factors of embodiment 19 or

20, vectors of embodiment 21 or 22, or composition of embodiment 23 to the subject in need thereof, thereby treating the autoimmune disease, wherein the transcription factor causes a target gene in the subject to be overexpressed, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, topically, orally, and/or intravenously.

27. The method of embodiment 26, further administering an additional therapeutic agent to the subject in need thereof, optionally wherein the additional therapeutic agent is anifrolumab.

28. The method of embodiment 26 or 27, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

29. A method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject in need thereof:

a) one or more transcription factor of embodiment 19 or 20, vector of embodiment 21 or 22, or composition of embodiment 23, wherein the transcription factor causes a target gene in the subject to be overexpressed; and

b) one or more AHR agonist of any of embodiment 1-5, the vector of embodiment 6 or 7, or the composition of embodiment 8,

thereby treating the autoimmune disease, optionally wherein the transcription factor is JUN, optionally wherein the one or more transcription factor and one or more AHR agonist are administered consecutively or sequentially, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, topically, orally, and/or intravenously.

30. The method of embodiment 29, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren

disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

31. A method of inhibiting B cell differentiation in a population of cells, comprising contacting the population of cells with at least one of the AHR agonists of any of embodiments 1-5, the vector of embodiment 6 or 7, the composition of embodiment 8, transcription factors of embodiment 19 or 20, vectors of embodiment 21 or 22, or compositions of embodiment 23, thereby inhibiting B cell differentiation, optionally wherein the contacting takes place *in vivo*, *in vitro*, *in situ* or *ex vivo*.

32. A method of inhibiting B cell differentiation in a subject in need thereof, comprising administering to the subject in need thereof an effective amount of at least one of the AHR agonists of any of embodiments 1-5, the vector of embodiment 6 or 7, the composition of embodiment 8, transcription factor of embodiment 19 or 20, vector of embodiment 21 or 22, or compositions of embodiment 23, thereby inhibiting B cell differentiation, optionally wherein the administering is done subcutaneously, topically orally, and/or intravenously, , optionally wherein the subject in need thereof is a human.

33. A gRNA targeting a gene selected from *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, or *STAT2*, wherein the gRNA has a sequence according to one of the sequences in Table 5.

34. A CRISPR-Cas9-gRNA ribonucleoprotein (crRNP) complex comprising at least one gRNA of embodiment 33.

35. A vector comprising the crRNP complex of embodiment 34, optionally wherein the vector is, comprises, or is derived from a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector.

36. A composition comprising the crRNP complex of embodiment 34, or the vector of embodiment 35, and a carrier, optionally wherein the carrier is a pharmaceutically acceptable carrier.

37. A method of decreasing CXCL13 production in a cell or cell population, comprising contacting the cell or cell population with the crRNP complex of embodiment 34, the vector of embodiment 35, or the composition of embodiment 36, thereby decreasing CXCL13 production, optionally where the contacting is *in vivo*, *in vitro*, *in situ*, or *ex vivo*.

38. A method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject in need thereof an effective amount of the crRNP complex of embodiment 34, the vector of embodiment 35, or the composition of embodiment 36, thereby treating the autoimmune disease, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, orally, and/or intravenously.

39. The method of embodiment 38, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

References

- 1 Tsokos, G. C. Autoimmunity and organ damage in systemic lupus erythematosus. *Nat Immunol* **21**, 605-614 (2020). doi.org:10.1038/s41590-020-0677-6
- 2 Jenks, S. A., Cashman, K. S., Woodruff, M. C., Lee, F. E. & Sanz, I. Extrafollicular responses in humans and SLE. *Immunol Rev* **288**, 136-148 (2019). Doi.org:10.1111/imr.12741
- 3 Banchereau, R. *et al.* Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell* **165**, 551-565 (2016). Doi.org:10.1016/j.cell.2016.03.008
- 4 Choi, J. Y. *et al.* Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. *Arthritis & rheumatology* **67**, 988-999 (2015). Doi.org:10.1002/art.39020
- 5 He, J. *et al.* Circulating precursor CCR7(lo)PD-1(hi) CXCR5(+) CD4(+) T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity* **39**, 770-781 (2013). Doi.org:10.1016/j.immuni.2013.09.007
- 6 Jacquemin, C. *et al.* OX40 Ligand Contributes to Human Lupus Pathogenesis by Promoting T Follicular Helper Response. *Immunity* **42**, 1159-1170 (2015). Doi.org:10.1016/j.immuni.2015.05.012
- 7 Jenks, S. A. *et al.* Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. *Immunity* **49**, 725-739 e726 (2018). Doi.org:10.1016/j.immuni.2018.08.015
- 8 Wang, S. *et al.* IL-21 drives expansion and plasma cell differentiation of autoreactive CD11c(hi)T-bet(+) B cells in SLE. *Nature communications* **9**, 1758 (2018). Doi.org:10.1038/s41467-018-03750-7
- 9 Bocharnikov, A. V. *et al.* PD-1hiCXCR5- T peripheral helper cells promote B cell responses in lupus via MAF and IL-21. *JCI Insight* **4** (2019). Doi.org:10.1172/jci.insight.130062
- 10 Crotty, S. Follicular helper CD4 T cells (TFH). *Annual review of immunology* **29**, 621-663 (2011). Doi.org:10.1146/annurev-immunol-031210-101400
- 11 Rao, D. A. *et al.* Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* **542**, 110-114 (2017). Doi.org:10.1038/nature20810
- 12 Ansel, K. M. *et al.* A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* **406**, 309-314 (2000). Doi.org:10.1038/35018581

- 13 Luther, S. A., Lopez, T., Bai, W., Hanahan, D. & Cyster, J. G. BLC expression in pancreatic islets causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis. *Immunity* **12**, 471-481 (2000). Doi.org:10.1016/s1074-7613(00)80199-5
- 14 Havenar-Daughton, C. *et al.* CXCL13 is a plasma biomarker of germinal center activity. *Proc Natl Acad Sci U S A* **113**, 2702-2707 (2016). Doi.org:10.1073/pnas.1520112113
- 15 Bao, Y. Q. *et al.* Increased circulating CXCL13 levels in systemic lupus erythematosus and rheumatoid arthritis: a meta-analysis. *Clin Rheumatol* **39**, 281-290 (2020). Doi.org:10.1007/s10067-019-04775-z
- 16 Zhang, F. *et al.* Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat Immunol* **20**, 928-942 (2019). Doi.org:10.1038/s41590-019-0378-1
- 17 Zhang, F. *et al.* Cellular deconstruction of inflamed synovium defines diverse inflammatory phenotypes in rheumatoid arthritis. *bioRxiv*, 2022.2002.2025.481990 (2022). Doi.org:10.1101/2022.02.25.481990
- 18 Kobayashi, S. *et al.* TGF- β induces the differentiation of human CXCL13-producing CD4⁺ T cells. *European Journal of Immunology* **46**, 360-371 (2016). Doi.org:10.1002/eji.201546043
- 19 Yoshitomi, H. *et al.* Human Sox4 facilitates the development of CXCL13-producing helper T cells in inflammatory environments. *Nat Commun* **9**, 3762 (2018). Doi.org:10.1038/s41467-018-06187-0
- 20 Park, J. *et al.* Integrated genomic analyses of cutaneous T-cell lymphomas reveal the molecular bases for disease heterogeneity. *Blood* **138**, 1225-1236 (2021). Doi.org:10.1182/blood.2020009655
- 21 Liu, B. *et al.* Temporal single-cell tracing reveals clonal revival and expansion of precursor exhausted T cells during anti-PD-1 therapy in lung cancer. *Nat Cancer* **3**, 108-121 (2022). Doi.org:10.1038/s43018-021-00292-8
- 22 Litchfield, K. *et al.* Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. *Cell* **184**, 596-614 e514 (2021). Doi.org:10.1016/j.cell.2021.01.002
- 23 Liu, B., Zhang, Y., Wang, D., Hu, X. & Zhang, Z. Single-cell meta-analyses reveal responses of tumor-reactive CXCL13(+) T cells to immune-checkpoint blockade. *Nat Cancer* **3**, 1123-1136 (2022). Doi.org:10.1038/s43018-022-00433-7

- 24 Lowery, F. J. *et al.* Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. *Science* **375**, 877-884 (2022).
Doi.org:10.1126/science.abl5447
- 25 Veldhoen, M. *et al.* The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* **453**, 106-109 (2008).
Doi.org:10.1038/nature06881
- 26 Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A. & Sallusto, F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol* **10**, 857-863 (2009). Doi.org:10.1038/ni.1767
- 27 Holloman, B. L., Cannon, A., Wilson, K., Nagarkatti, P. & Nagarkatti, M. Aryl Hydrocarbon Receptor Activation Ameliorates Acute Respiratory Distress Syndrome through Regulation of Th17 and Th22 Cells in the Lungs. *mbio* **14**, e0313722 (2023).
Doi.org:10.1128/mbio.03137-22
- 28 Eyerich, K., Dimartino, V. & Cavani, A. IL-17 and IL-22 in immunity: Driving protection and pathology. *Eur J Immunol* **47**, 607-614 (2017).
Doi.org:10.1002/eji.201646723
- 29 Plank, M. W. *et al.* Th22 Cells Form a Distinct Th Lineage from Th17 Cells In Vitro with Unique Transcriptional Properties and Tbet-Dependent Th1 Plasticity. *J Immunol* **198**, 2182-2190 (2017). Doi.org:10.4049/jimmunol.1601480
- 30 Eyerich, S. *et al.* Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest* **119**, 3573-3585 (2009).
Doi.org:10.1172/JCI40202
- 31 Reshef, Y. A. *et al.* Co-varying neighborhood analysis identifies cell populations associated with phenotypes of interest from single-cell transcriptomics. *Nature biotechnology* **40**, 355-363 (2022). Doi.org:10.1038/s41587-021-01066-4
- 32 Hollbacher, B. *et al.* Transcriptomic Profiling of Human Effector and Regulatory T Cell Subsets Identifies Predictive Population Signatures. *Immunohorizons* **4**, 585-596 (2020). Doi.org:10.4049/immunohorizons.2000037
- 33 Doench, J. G. *et al.* Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol* **34**, 184-191 (2016).
Doi.org:10.1038/nbt.3437
- 34 Hultquist, J. F. *et al.* CRISPR-Cas9 genome engineering of primary CD4(+) T cells for the interrogation of HIV-host factor interactions. *Nat Protoc* **14**, 1-27 (2019).
Doi.org:10.1038/s41596-018-0069-7

- 35 Hultquist, J. F. *et al.* A Cas9 Ribonucleoprotein Platform for Functional Genetic Studies of HIV-Host Interactions in Primary Human T Cells. *Cell Rep* **17**, 1438-1452 (2016). Doi.org:10.1016/j.celrep.2016.09.080
- 36 Kobayashi, S. *et al.* TGF-beta induces the differentiation of human CXCL13-producing CD4(+) T cells. *Eur J Immunol* **46**, 360-371 (2016). Doi.org:10.1002/eji.201546043
- 37 Ramirez, J. M. *et al.* Activation of the aryl hydrocarbon receptor reveals distinct requirements for IL-22 and IL-17 production by human T helper cells. *Eur J Immunol* **40**, 2450-2459 (2010). Doi.org:10.1002/eji.201040461
- 38 Trifari, S., Kaplan, C. D., Tran, E. H., Crellin, N. K. & Spits, H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat Immunol* **10**, 864-871 (2009). Doi.org:10.1038/ni.1770
- 39 Korn, T., Bettelli, E., Oukka, M. & Kuchroo, V. K. IL-17 and Th17 Cells. *Annu Rev Immunol* **27**, 485-517 (2009). Doi.org:10.1146/annurev.immunol.021908.132710
- 40 Schmitt, N. *et al.* The cytokine TGF-beta co-opts signaling via STAT3-STAT4 to promote the differentiation of human TFH cells. *Nature immunology* **15**, 856-865 (2014). Doi.org:10.1038/ni.2947
- 41 Kang, J. B. *et al.* Efficient and precise single-cell reference atlas mapping with Symphony. *Nat Commun* **12**, 5890 (2021). Doi.org:10.1038/s41467-021-25957-x
- 42 Zhang, F. *et al.* Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. *Nature* **623**, 616-624 (2023). Doi.org:10.1038/s41586-023-06708-y
- 43 Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**, 550 (2014). Doi.org:10.1186/s13059-014-0550-8
- 44 Long, W. P., Pray-Grant, M., Tsai, J. C. & Perdew, G. H. Protein kinase C activity is required for aryl hydrocarbon receptor pathway-mediated signal transduction. *Mol Pharmacol* **53**, 691-700 (1998). Doi.org:10.1124/mol.53.4.691
- 45 MacPherson, L. *et al.* Aryl hydrocarbon receptor repressor and TiPARP (ARTD14) use similar, but also distinct mechanisms to repress aryl hydrocarbon receptor signaling. *Int J Mol Sci* **15**, 7939-7957 (2014). Doi.org:10.3390/ijms15057939
- 46 Gutierrez-Vazquez, C. & Quintana, F. J. Regulation of the Immune Response by the Aryl Hydrocarbon Receptor. *Immunity* **48**, 19-33 (2018). Doi.org:10.1016/j.immuni.2017.12.012

- 47 Rothhammer, V. & Quintana, F. J. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat Rev Immunol* **19**, 184-197 (2019). Doi.org:10.1038/s41577-019-0125-8
- 48 Conesa, A., Nueda, M. J., Ferrer, A. & Talon, M. maSigPro: a method to identify significantly differential expression profiles in time-course microarray experiments. *Bioinformatics* **22**, 1096-1102 (2006). Doi.org:10.1093/bioinformatics/btl056
- 49 Meers, M. P., Bryson, T. D., Henikoff, J. G. & Henikoff, S. Improved CUT&RUN chromatin profiling tools. *Elife* **8** (2019). Doi.org:10.7554/eLife.46314
- 50 Ross-Innes, C., Stark, R., Teschendorff, A. *et al.* Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature* **481**, 389–393 (2012). doi.org/10.1038/nature10730
- 51 Fonseca, G. J. *et al.* Diverse motif ensembles specify non-redundant DNA binding activities of AP-1 family members in macrophages. *Nat Commun* **10**, 414 (2019). Doi.org:10.1038/s41467-018-08236-0
- 52 Hess, J., Angel, P. & Schorpp-Kistner, M. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci* **117**, 5965-5973 (2004). Doi.org:10.1242/jcs.01589
- 53 Behrens, A. *et al.* Jun N-terminal kinase 2 modulates thymocyte apoptosis and T cell activation through c-Jun and nuclear factor of activated T cell (NF-AT). *Proc Natl Acad Sci U S A* **98**, 1769-1774 (2001). Doi.org:10.1073/pnas.98.4.1769
- 54 Derijard, B. *et al.* JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76**, 1025-1037 (1994). Doi.org:10.1016/0092-8674(94)90380-8
- 55 Waudby, C. A. *et al.* An intrinsic temporal order of c-JUN N-terminal phosphorylation regulates its activity by orchestrating co-factor recruitment. *Nat Commun* **13**, 6133 (2022). Doi.org:10.1038/s41467-022-33866-w
- 56 Bennett, L. *et al.* Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* **197**, 711-723 (2003). Doi.org:10.1084/jem.20021553
- 57 Panwar, B. *et al.* Multi-cell type gene coexpression network analysis reveals coordinated interferon response and cross-cell type correlations in systemic lupus erythematosus. *Genome Res* **31**, 659-676 (2021). Doi.org:10.1101/gr.265249.120
- 58 Furie, R. A. *et al.* Type I interferon inhibitor anifrolumab in active systemic lupus erythematosus (TULIP-1): a randomised, controlled, phase 3 trial. *The Lancet Rheumatology* **1**, e208-e219 (2019). Doi.org/10.1016/S2665-9913(19)30076-1

- 59 Sumida, T. S. *et al.* Type I interferon transcriptional network regulates expression of coinhibitory receptors in human T cells. *Nat Immunol* **23**, 632-642 (2022).
Doi.org:10.1038/s41590-022-01152-y
- 60 Frank, D. A., Robertson, M. J., Bonni, A., Ritz, J. & Greenberg, M. E. Interleukin 2 signaling involves the phosphorylation of Stat proteins. *Proc Natl Acad Sci U S A* **92**, 7779-7783 (1995). Doi.org:10.1073/pnas.92.17.7779
- 61 Moriggl, R. *et al.* Stat5 Is Required for IL-2-Induced Cell Cycle Progression of Peripheral T Cells. *Immunity* **10**, 249-259 (1999). Doi.org/10.1016/S1074-7613(00)80025-4
- 62 Liu, Y. *et al.* IL-2 regulates tumor-reactive CD8(+) T cell exhaustion by activating the aryl hydrocarbon receptor. *Nat Immunol* **22**, 358-369 (2021).
Doi.org:10.1038/s41590-020-00850-9
- 63 Gu-Trantien, C. *et al.* CXCL13-producing TFH cells link immune suppression and adaptive memory in human breast cancer. *JCI Insight* **2** (2017).
Doi.org:10.1172/jci.insight.91487
- 64 Otero, D. C., Fares-Frederickson, N. J., Xiao, M., Baker, D. P. & David, M. IFN-beta Selectively Inhibits IL-2 Production through CREM-Mediated Chromatin Remodeling. *J Immunol* **194**, 5120-5128 (2015). Doi.org:10.4049/jimmunol.1403181
- 65 Zella, D. *et al.* IFN-alpha 2b reduces IL-2 production and IL-2 receptor function in primary CD4+ T cells. *J Immunol* **164**, 2296-2302 (2000).
Doi.org:10.4049/jimmunol.164.5.2296
- 66 Blanco, P., Ueno, H. & Schmitt, N. T follicular helper (Tfh) cells in lupus: Activation and involvement in SLE pathogenesis. *Eur J Immunol* **46**, 281-290 (2016).
Doi.org:10.1002/eji.201545760
- 67 Lin, J., Yu, Y., Ma, J., Ren, C. & Chen, W. PD-1+CXCR5-CD4+T cells are correlated with the severity of systemic lupus erythematosus. *Rheumatology (Oxford)* **58**, 2188-2192 (2019). Doi.org:10.1093/rheumatology/kez228
- 68 Makiyama, A. *et al.* Expanded circulating peripheral helper T cells in systemic lupus erythematosus: association with disease activity and B cell differentiation. *Rheumatology (Oxford)* **58**, 1861-1869 (2019).
Doi.org:10.1093/rheumatology/kez077
- 69 Rao, D. A. T Cells That Help B Cells in Chronically Inflamed Tissues. *Front Immunol* **9**, 1924 (2018). Doi.org:10.3389/fimmu.2018.01924

- 70 Azzouz, D. *et al.* Lupus nephritis is linked to disease-activity associated expansions and immunity to a gut commensal. *Ann Rheum Dis* **78**, 947-956 (2019). Doi.org:10.1136/annrheumdis-2018-214856
- 71 Botia-Sanchez, M. *et al.* Gut epithelial barrier dysfunction in lupus triggers a differential humoral response against gut commensals. *Front Immunol* **14**, 1200769 (2023). Doi.org:10.3389/fimmu.2023.1200769
- 72 Manfredo Vieira, S. *et al.* Translocation of a gut pathobiont drives autoimmunity in mice and humans. *Science* **359**, 1156-1161 (2018). Doi.org:10.1126/science.aar7201
- 73 Arshad, T., Mansur, F., Palek, R., Manzoor, S. & Liska, V. A Double Edged Sword Role of Interleukin-22 in Wound Healing and Tissue Regeneration. *Front Immunol* **11**, 2148 (2020). Doi.org:10.3389/fimmu.2020.02148
- 74 Barroso, A., Mahler, J. V., Fonseca-Castro, P. H. & Quintana, F. J. The aryl hydrocarbon receptor and the gut-brain axis. *Cell Mol Immunol* **18**, 259-268 (2021). Doi.org:10.1038/s41423-020-00585-5
- 75 Cannon, A. S., Nagarkatti, P. S. & Nagarkatti, M. Targeting AhR as a Novel Therapeutic Modality against Inflammatory Diseases. *Int J Mol Sci* **23** (2021). Doi.org:10.3390/ijms23010288
- 76 Rikken, G. *et al.* Lead optimization of aryl hydrocarbon receptor ligands for treatment of inflammatory skin disorders. *Biochem Pharmacol* **208**, 115400 (2023). Doi.org:10.1016/j.bcp.2022.115400
- 77 Yoshimatsu, Y. *et al.* Aryl hydrocarbon receptor signals in epithelial cells govern the recruitment and location of Helios(+) Tregs in the gut. *Cell Rep* **39**, 110773 (2022). Doi.org:10.1016/j.celrep.2022.110773
- 78 Lynn, R. C. *et al.* c-Jun overexpression in CAR T cells induces exhaustion resistance. *Nature* **576**, 293-300 (2019). Doi.org:10.1038/s41586-019-1805-z
- 79 Yamazaki, S. *et al.* The AP-1 transcription factor JunB is required for Th17 cell differentiation. *Sci Rep* **7**, 17402 (2017). Doi.org:10.1038/s41598-017-17597-3
- 80 Rothhammer, V. *et al.* Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat Med* **22**, 586-597 (2016). Doi.org:10.1038/nm.4106
- 81 Shinde, R. *et al.* Apoptotic cell-induced AhR activity is required for immunological tolerance and suppression of systemic lupus erythematosus in mice and humans. *Nat Immunol* **19**, 571-582 (2018). Doi.org:10.1038/s41590-018-0107-1

- 82 Hatzi, K. *et al.* BCL6 orchestrates Tfh cell differentiation via multiple distinct mechanisms. *J Exp Med* **212**, 539-553 (2015). Doi.org:10.1084/jem.20141380
- 83 Seth, A. *et al.* AP-1-independent NFAT signaling maintains follicular T cell function in infection and autoimmunity. *J Exp Med* **220** (2023). Doi.org:10.1084/jem.20211110
- 84 Crotty, S. Follicular Helper CD4 T Cells (TFH). *Annual Review of Immunology* **29**, 621-663 (2011). Doi.org:10.1146/annurev-immunol-031210-101400
- 85 Aringer, M. *et al.* 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. *Arthritis Rheumatol* **71**, 1400-1412 (2019). Doi.org:10.1002/art.40930
- 86 Nowicka, M. *et al.* CyTOF workflow: differential discovery in high-throughput high-dimensional cytometry datasets. *F1000Res* **6**, 748 (2017). Doi.org:10.12688/f1000research.11622.3
- 87 Fonseka, C. Y. *et al.* Mixed-effects association of single cells identifies an expanded effector CD4(+) T cell subset in rheumatoid arthritis. *Sci Transl Med* **10** (2018). Doi.org:10.1126/scitranslmed.aag0305
- 88 Doench, J. G. *et al.* Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature Biotechnology* **34**, 184-191 (2016). Doi.org:10.1038/nbt.3437
- 89 Yu, G., Wang, L. G. & He, Q. Y. ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. *Bioinformatics* **31**, 2382-2383 (2015). Doi.org:10.1093/bioinformatics/btv145
- 90 Morand, E. F. *et al.* Trial of Anifrolumab in Active Systemic Lupus Erythematosus. *N Engl J Med* **382**, 211-221 (2020). doi.org:10.1056/NEJMoa1912196
- 91 Nehar-Belaid, D. *et al.* Mapping systemic lupus erythematosus heterogeneity at the single-cell level. *Nat Immunol* **21**, 1094-1106 (2020).

* * *

[0255] Equivalents

[0256] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs.

[0257] Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention.

[0258] The present technology illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” “containing,” etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present technology claimed.

[0259] Thus, it should be understood that the materials, methods, and examples provided here are representative of preferred aspects, are exemplary, and are not intended as limitations on the scope of the present technology.

[0260] The present technology has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the present technology. This includes the generic description of the present technology with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0261] In addition, where features or aspects of the present technology are described in terms of Markush groups, those skilled in the art will recognize that the present technology is also

thereby described in terms of any individual member or subgroup of members of the Markush group.

[0262] All publications, patent applications, patents, GenBank citations, ATCC citations, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the specification, including definitions, will control.

WHAT IS CLAIMED IS:

1. An aryl hydrocarbon receptor (AHR) agonist, wherein the AHR agonist negatively regulates pathological T cell differentiation in autoantibody driven autoimmune diseases.
2. The agonist of claim 1, wherein the agonist negatively regulates T follicular helper (Tfh) cell and/or T peripheral helper (Tph) cell differentiation.
3. The agonist of claim 1 or 2, wherein the agonist is conjugated to a T cell targeting moiety.
4. The agonist of claim 3, wherein the targeting moiety is an anti-T cell antibody.
5. The agonist of any of claims 1-4, wherein the agonist is the cytokine IL-2, 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and/or 6-formylindolo(3,2-b)carbazole (FICZ).
6. A vector comprising the agonist of any of claims 1-5, optionally wherein the vector is, comprises, or is derived from a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector.
7. The vector of claim 6, wherein the vector comprises a lentivirus.
8. A composition comprising the agonist of any of claims 1-5 or the vector of claim 6 or 7, and a carrier, optionally wherein the carrier is a pharmaceutically acceptable carrier.
9. A method of preventing CXCL13+ Tph/Tfh cell generation, comprising contacting a cell population with one or more agonists of any of claims 1-5, the vector of claim 6 or 7, or the composition of claim 8, thereby preventing CXCL13+ Tph/Tfh cell generation, wherein the contacting is *in vivo*, *in vitro*, *in situ*, or *ex vivo*.
10. A method of treating an autoimmune disease in a subject in need thereof, the method comprising administering an effective amount to the subject in need of one or more agonists of any one of claims 1-5, the vector of claim 6 or 7, or the composition of claim 8, thereby

treating the autoimmune disease, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, orally, and/or intravenously.

11. A method of inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, comprising administering an effective amount of one or more agonists of any one of claims 1-5, the vector of claim 6 or 7, or the composition of claim 8 to a subject in need thereof, thereby inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, optionally wherein the function is Tph/Tfh differentiation or B cell recruitment, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, orally, and/or intravenously.

12. The method of claim 11, wherein the subject has an autoimmune disease.

13. The method of claim 11 or 12, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

14. The method of claim 13, wherein the autoimmune disease is rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE).

15. The method of any of claims 11-14 where the subject is a human.

16. A method of treating an autoimmune disease in a subject in need thereof, comprising obtaining a population of T cells from the subject, treating the population of T cells with an AHR agonist, and administering an effective amount of the treated population of T cells to the subject, thereby treating the autoimmune disease, optionally wherein the subject in need thereof is a human, optionally wherein the administering is done subcutaneously, orally, and/or intravenously.

17. The method of claim 16, wherein the treatment with an AHR agonist reduces the frequency of PD-1+CXCR5- Tph cells in the treated T cell population.

18. The method of claim 16, wherein the treatment with an AHR agonist stabilizes JUN function.
19. A transcription factor selected from AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and BATF, wherein the transcription factor negatively regulates Tfh and Tph cell function in autoantibody driven autoimmune disease.
20. The transcription factor JUN, wherein JUN negatively regulates Tfh and Tph cell function in autoantibody driven autoimmune disease.
21. A vector comprising the transcription factor of claim 19-20, optionally wherein the vector is, comprises, or is derived from a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector.
22. The vector of claim 21, wherein the vector comprises a lentivirus.
23. A composition comprising the transcription factor of claim 19 or 20 or the vector of claim 21 or 22, and a carrier, optionally wherein the carrier is a pharmaceutically acceptable carrier.
24. A method of preventing CXCL13+ Tph/Tfh cell generation, comprising contacting a cell population with one or more transcription factor of claim 19 or 20, vector of claim 21 or 22, or composition of claim 23, thereby preventing CXCL13+ Tph/Tfh cell generation, optionally wherein the contacting is *in vivo*, *in vitro*, *in situ*, or *ex vivo*.
25. A method of inhibiting the function of one or more autoimmunity-associated T cells or T cell populations, comprising administering an effective amount of one or more transcription factor of claim 19 or 20, vector of claims 21 or 22, or composition of claim 23 to a subject in need thereof, thereby inhibiting the function of one or more autoimmunity-associated T cells or T cell populations optionally wherein the function is Tph/Tfh differentiation or B cell recruitment, and optionally wherein the subject in need thereof is a human.

26. A method of treating an autoimmune disease in a subject in need thereof, comprising administering one or more transcription factor of claim 19 or 20, vector of claim 21 or 22, or composition of claim 23 to the subject in need thereof, thereby treating the autoimmune disease, wherein the transcription factor causes a target gene in the subject to be overexpressed, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, topically, orally, and/or intravenously.

27. The method of claim 26, further administering an additional therapeutic agent to the subject in need thereof, optionally wherein the additional therapeutic agent is anifrolumab.

28. The method of claim 26, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

29. A method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject in need thereof:

a) one or more transcription factor of claim 19 or 20, vector of claim 21 or 22, or composition of claim 23, wherein the transcription factor causes a target gene in the subject to be overexpressed; and

b) one or more AHR agonist of any of claims 1-5, the vector of claim 6 or 7, or the composition of claim 8,

thereby treating the autoimmune disease, optionally wherein the transcription factor is JUN, optionally wherein the one or more transcription factor and one or more AHR agonist are administered consecutively or sequentially, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, topically, orally, and/or intravenously.

30. The method of claim 29, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-

antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

31. A method of inhibiting B cell differentiation in a population of cells, comprising contacting the population of cells with at least one of the AHR agonist of any of claims 1-5, the vector of claim 6 or 7, the composition of claim 8, transcription factor of claim 19 or 20, vector of claims 21 or 22, or compositions of claim 23, thereby inhibiting B cell differentiation, optionally wherein the contacting takes place *in vivo*, *in vitro*, *in situ* or *ex vivo*.

32. A method of inhibiting B cell differentiation in a subject in need thereof, comprising administering to the patient in need thereof with at least one of the AHR agonists of any of claims 1-5, the vector of claim 6 or 7, the composition of claim 8, transcription factor of claim 19 or 20, vector of claim 20 or 22, or compositions of claim 23, thereby inhibiting B cell differentiation, optionally wherein the administering is done subcutaneously, topically orally, and/or intravenously, optionally wherein the subject in need thereof is a human.

33. A gRNA targeting a gene selected from *CD3D*, *LCP2*, *ATF4*, *IFNAR2*, or *STAT2*, wherein the gRNA has a sequence according to one of the sequences in Table 5.

34. A CRISPR-Cas9-gRNA ribonucleoprotein (crRNP) complex comprising at least one gRNA of claim 33.

35. A vector comprising the crRNP complex of claim 34, optionally wherein the vector is, comprises, or is derived from a plasmid, an adenovirus, and adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), a lentivirus, or a synthetic vector.

36. A composition comprising the crRNP complex of claim 34, or the vector of claim 35, and a carrier, optionally wherein the carrier is a pharmaceutically acceptable carrier.

37. A method of decreasing CXCL13 production in a cell or cell population, comprising contacting the cell or cell population with the crRNP complex of claim 34, the vector of

claim 35, or the composition of claim 37, thereby decreasing CXCL13 production in the cell, optionally where the contacting is *in vivo*, *in vitro*, *in situ*, or *ex vivo*.

38. A method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject in need thereof an effective amount of the crRNP complex of claim 34, the vector of claim 35, or the composition of claim 37, thereby treating the autoimmune disease, optionally wherein the subject in need thereof is a human, and optionally wherein the administering is done subcutaneously, topically, orally, and/or intravenously.

39. The method of claim 38, wherein the autoimmune disease is selected from rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), cutaneous lupus, Sjogren disease, auto-antibody driven autoimmune disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, juvenile idiopathic arthritis, ulcerative colitis, or systemic sclerosis.

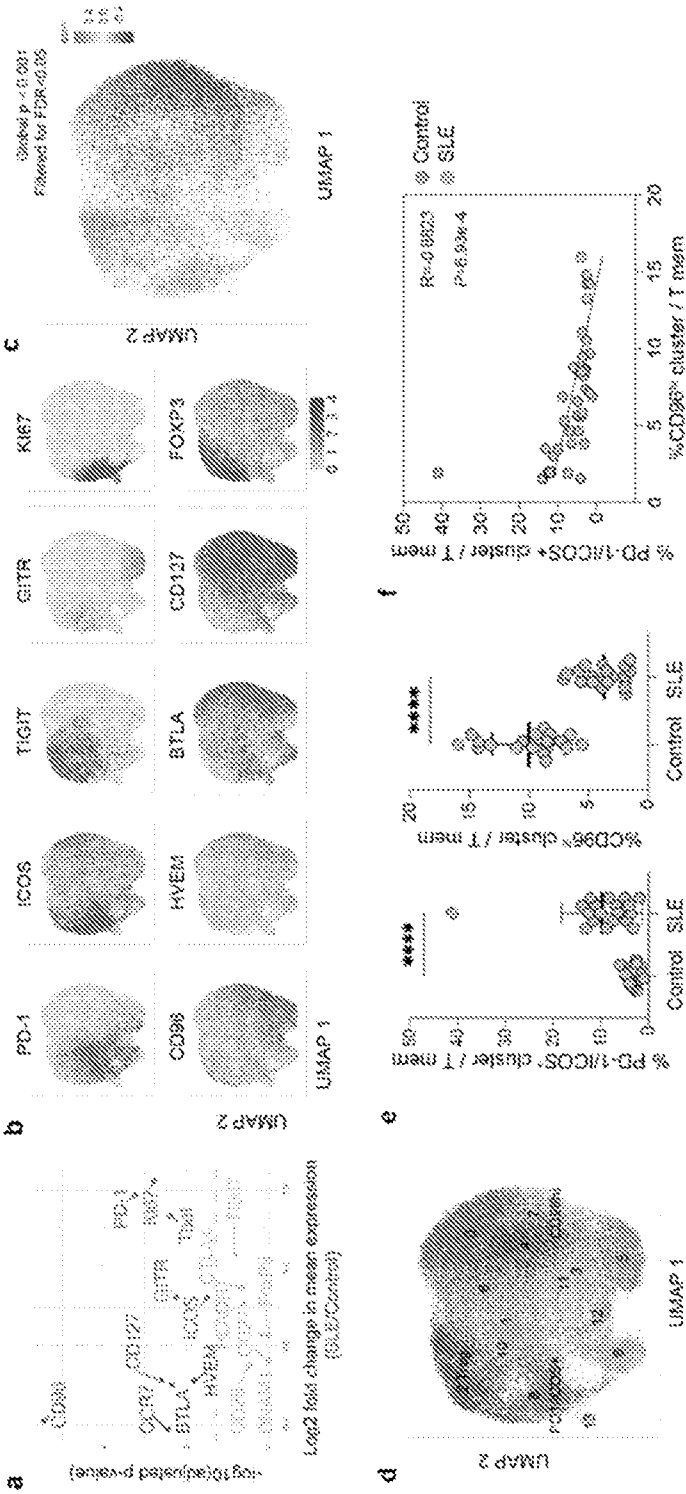


Figure 1a-1f

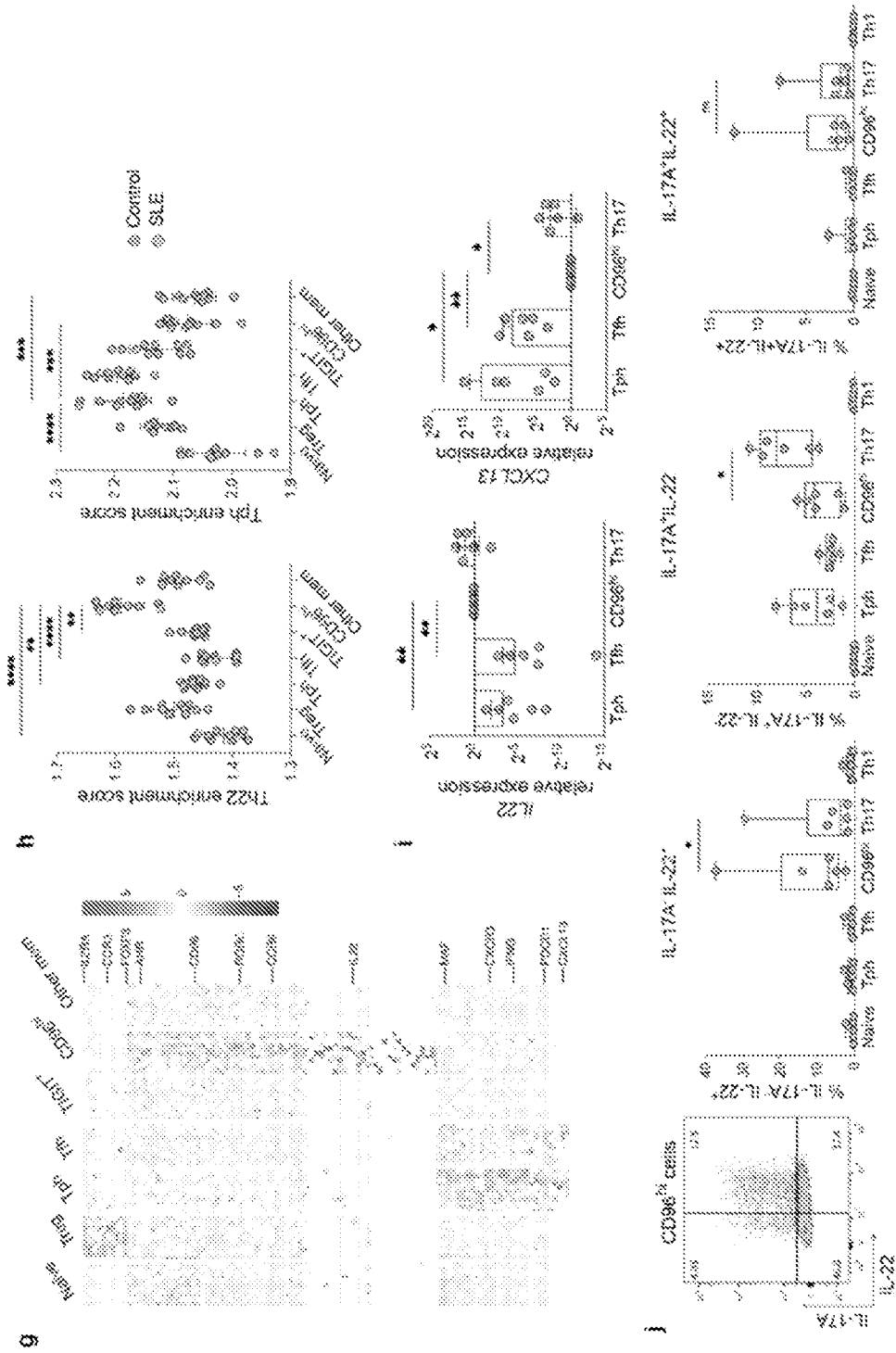
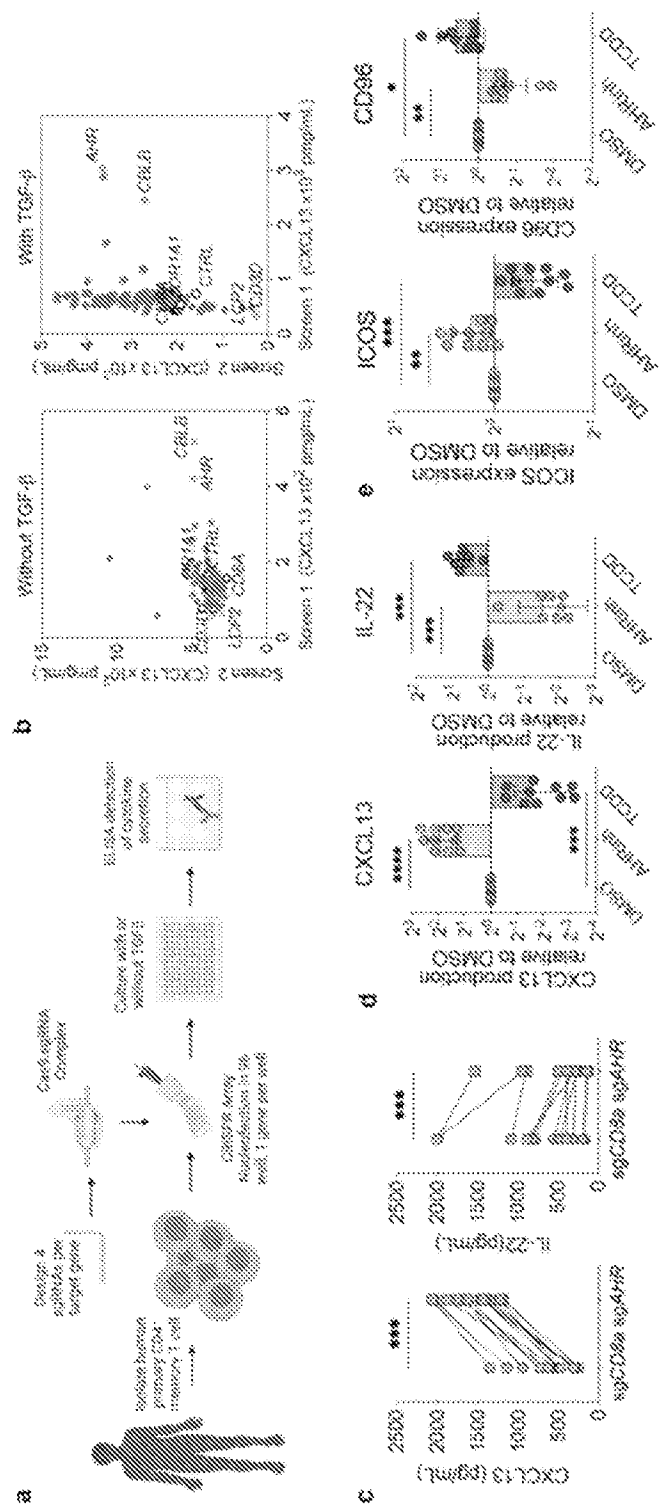
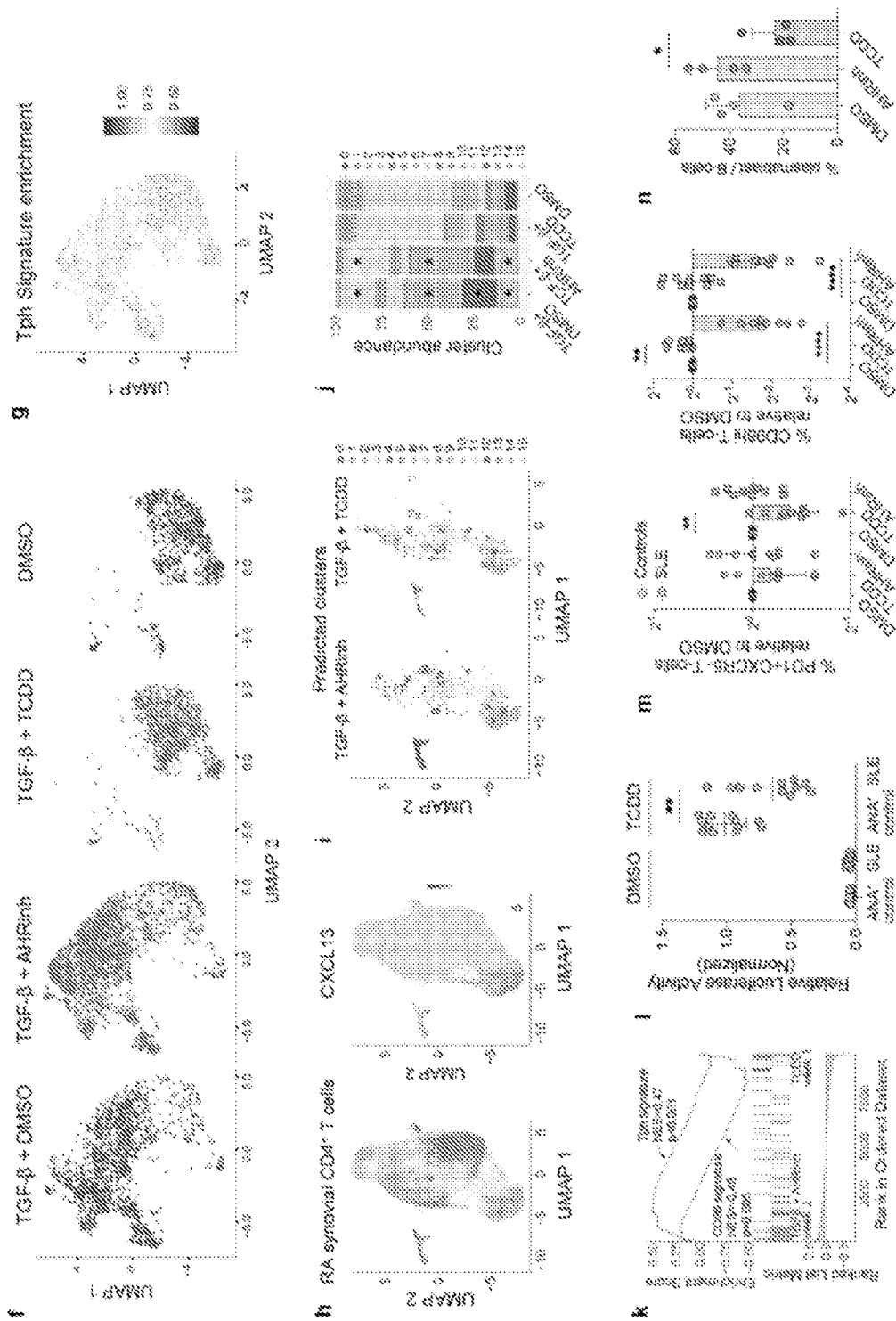


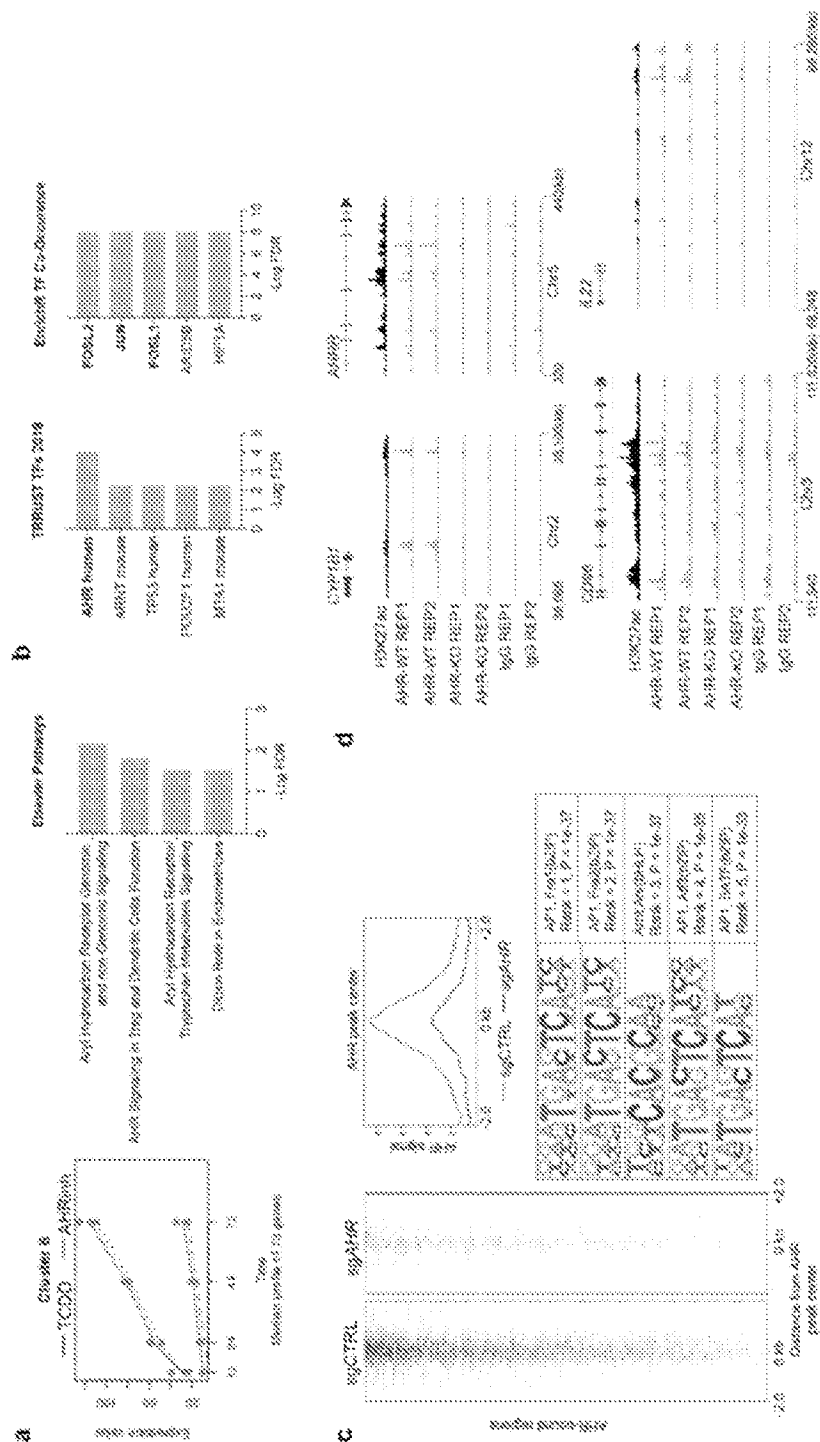
Figure 1g-1j



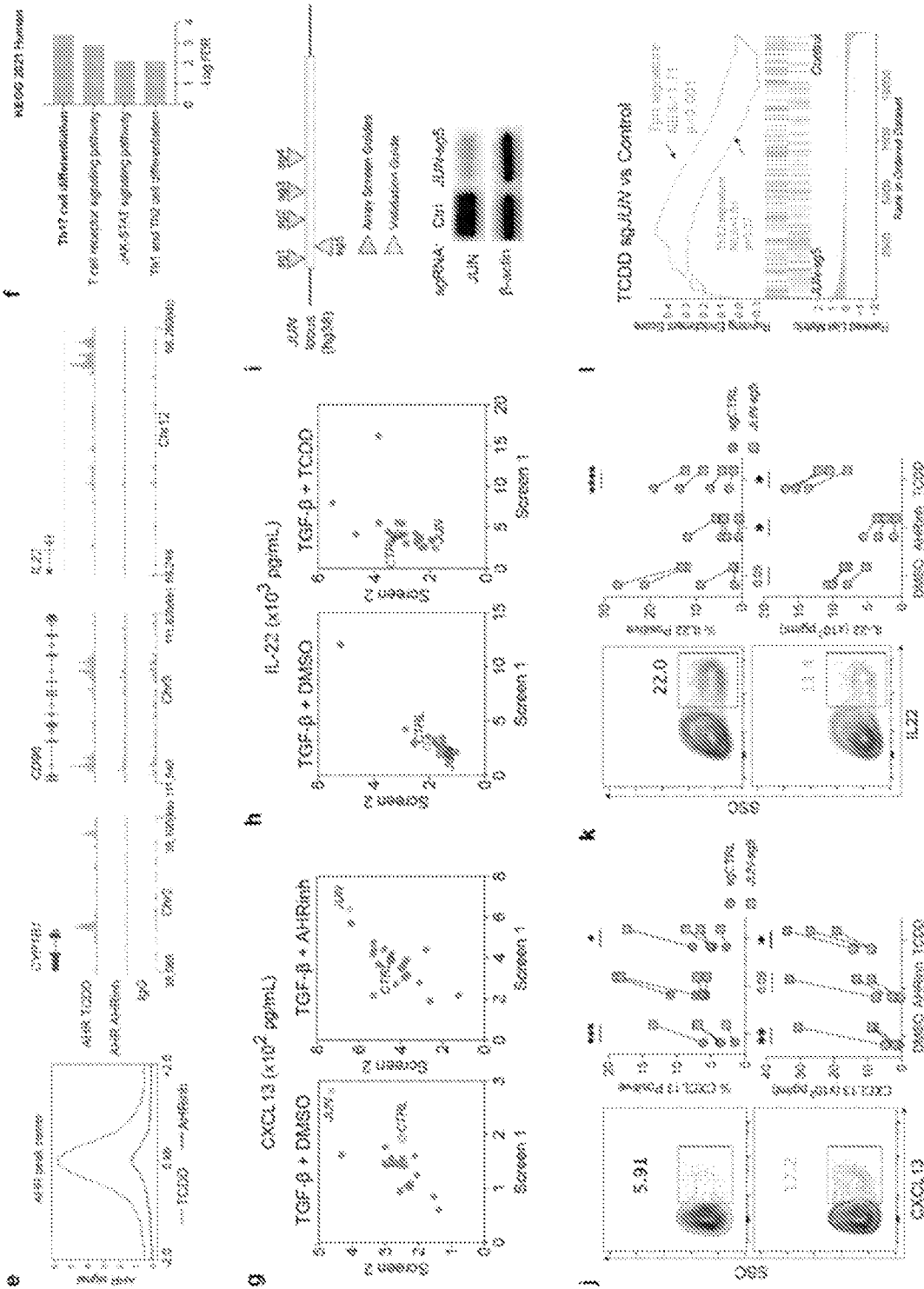
Figures 2a-2e

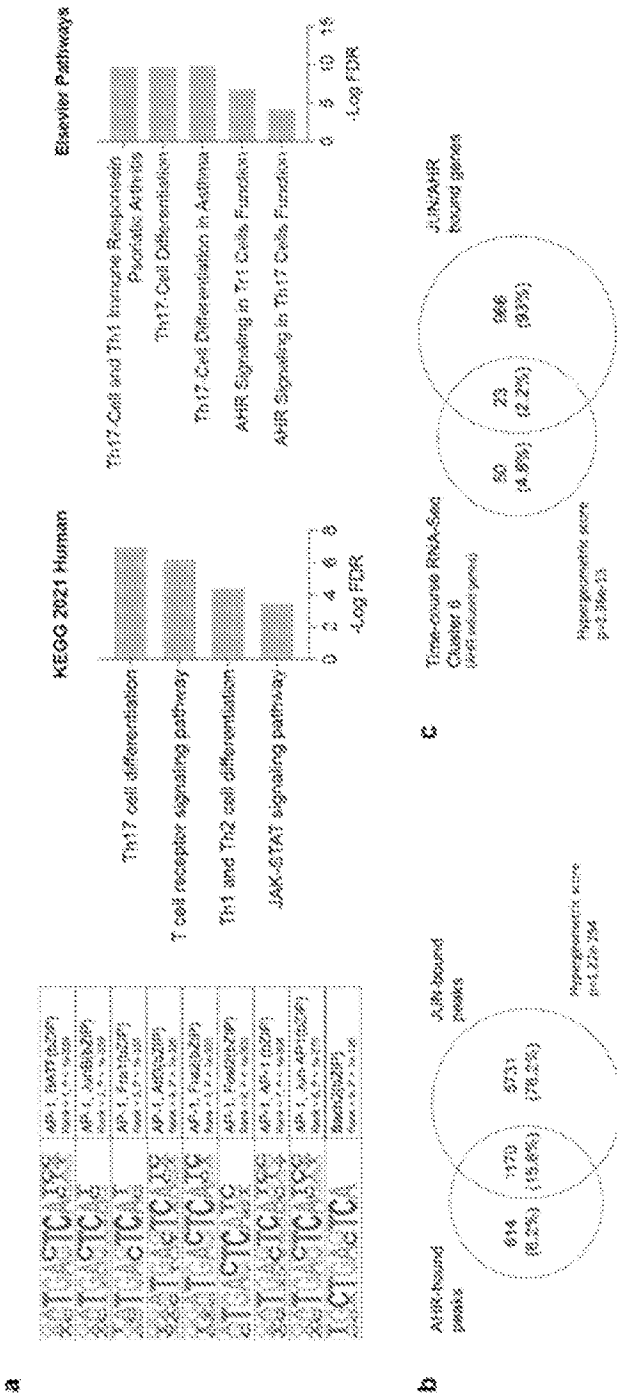


Figures 2f-2n

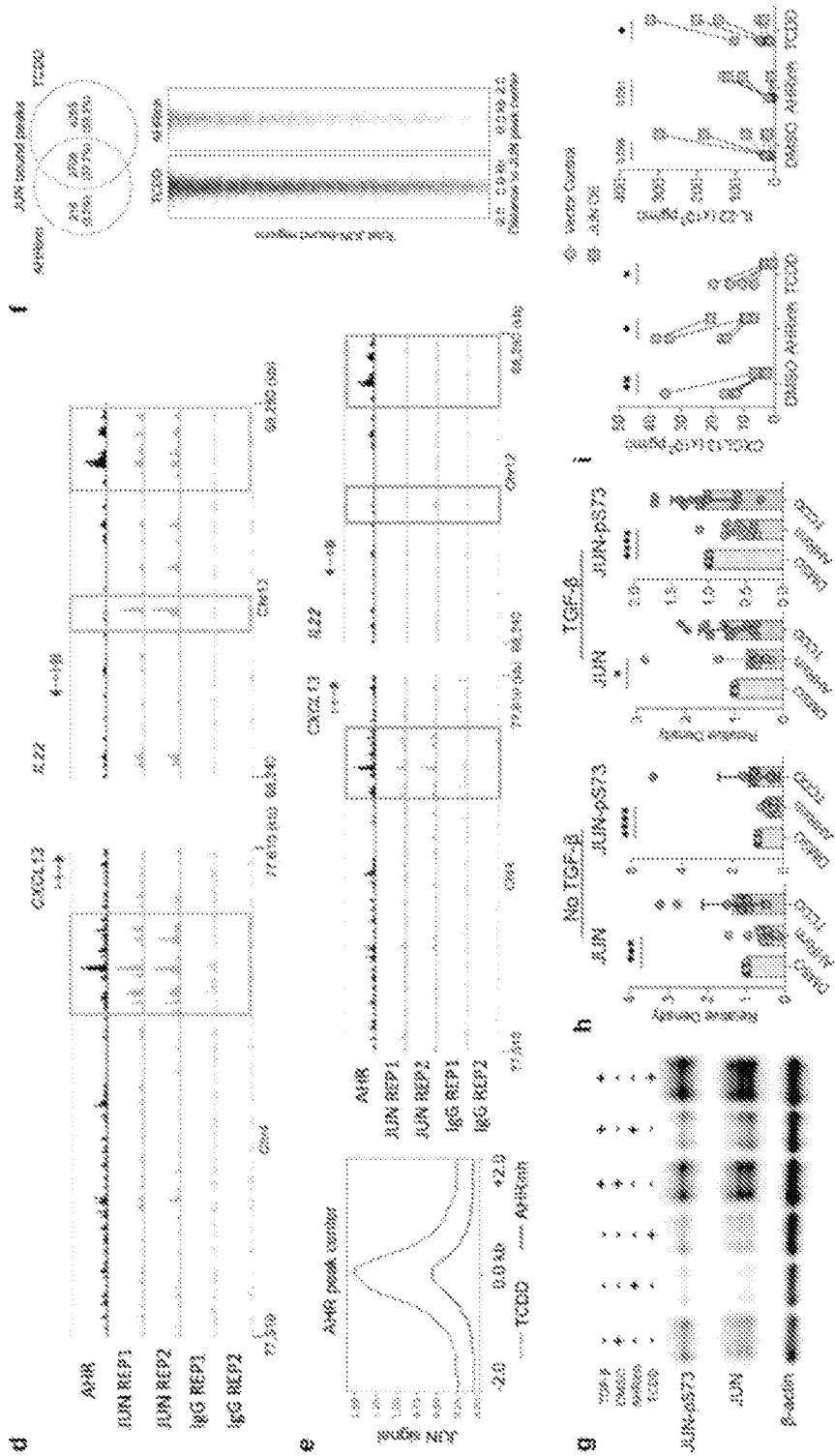


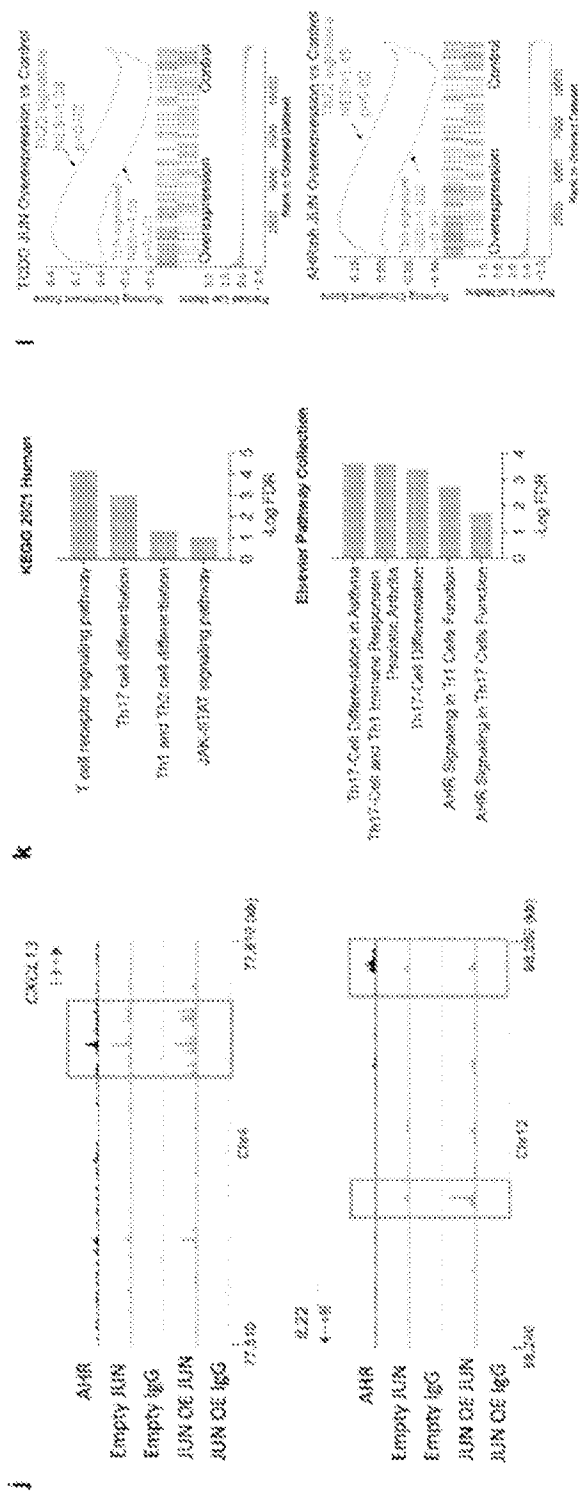
Figures 3a-3d



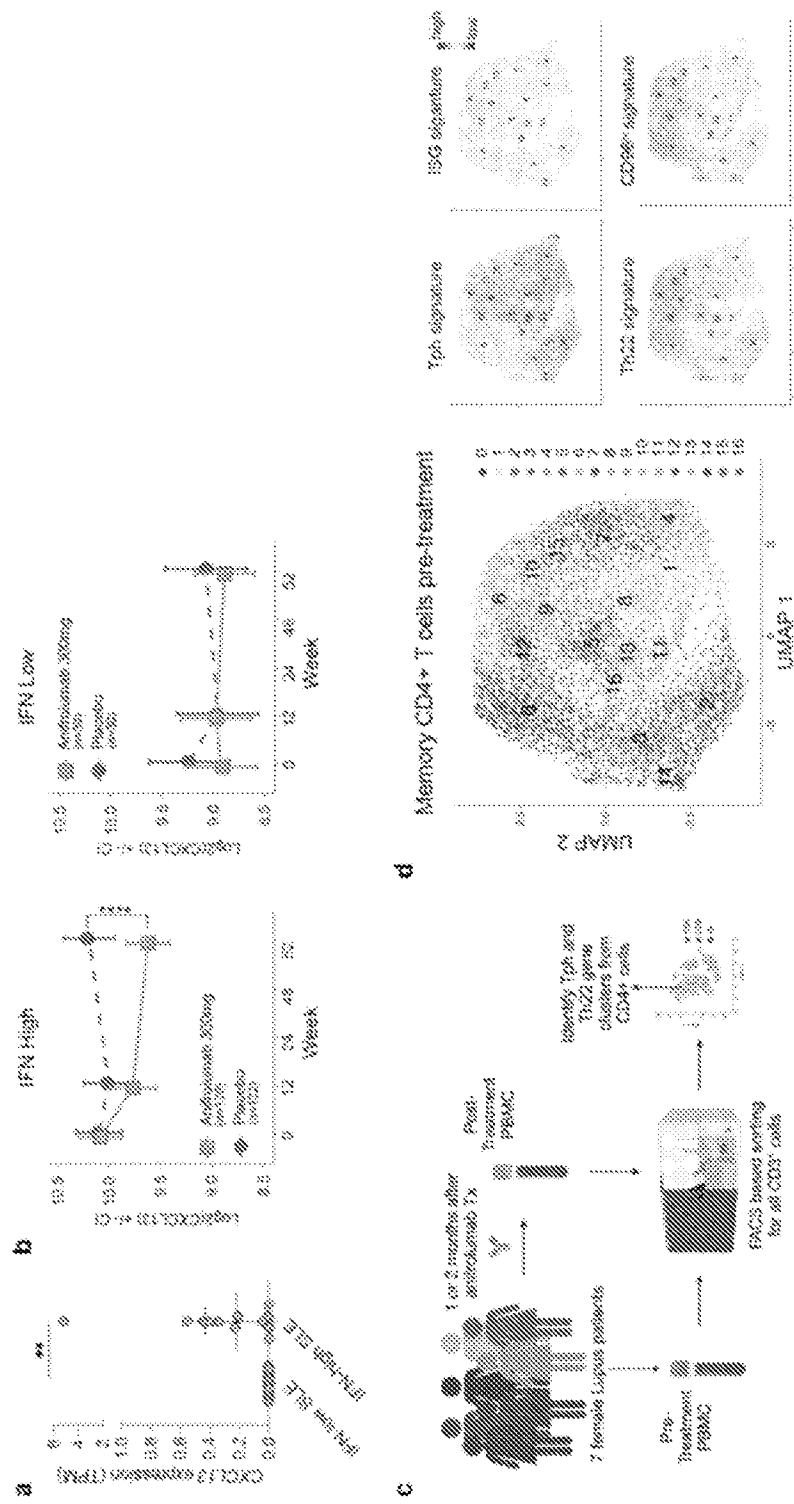


Figures 4a-4c

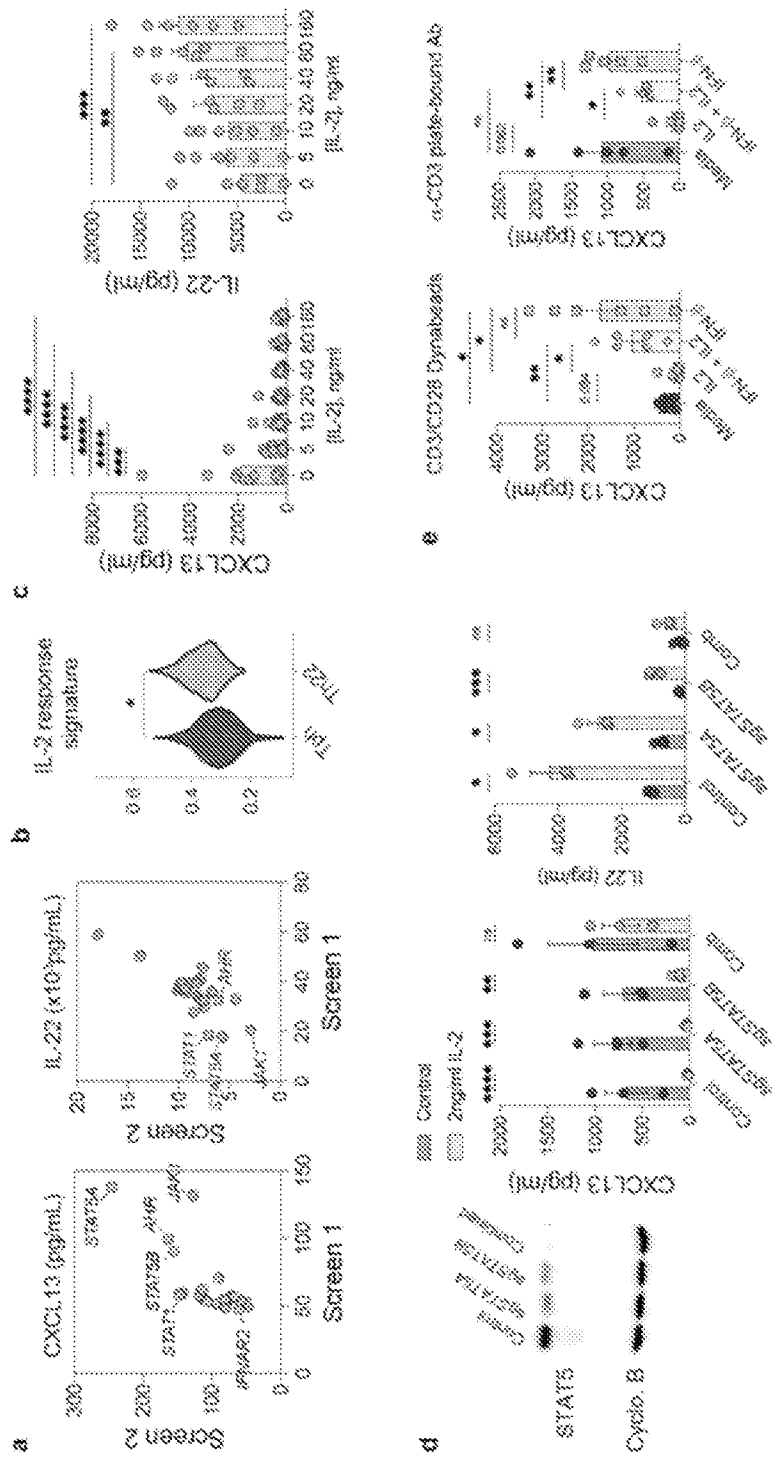




Figures 4j-4l



Figures 5a-5d



Figures 6a-6e

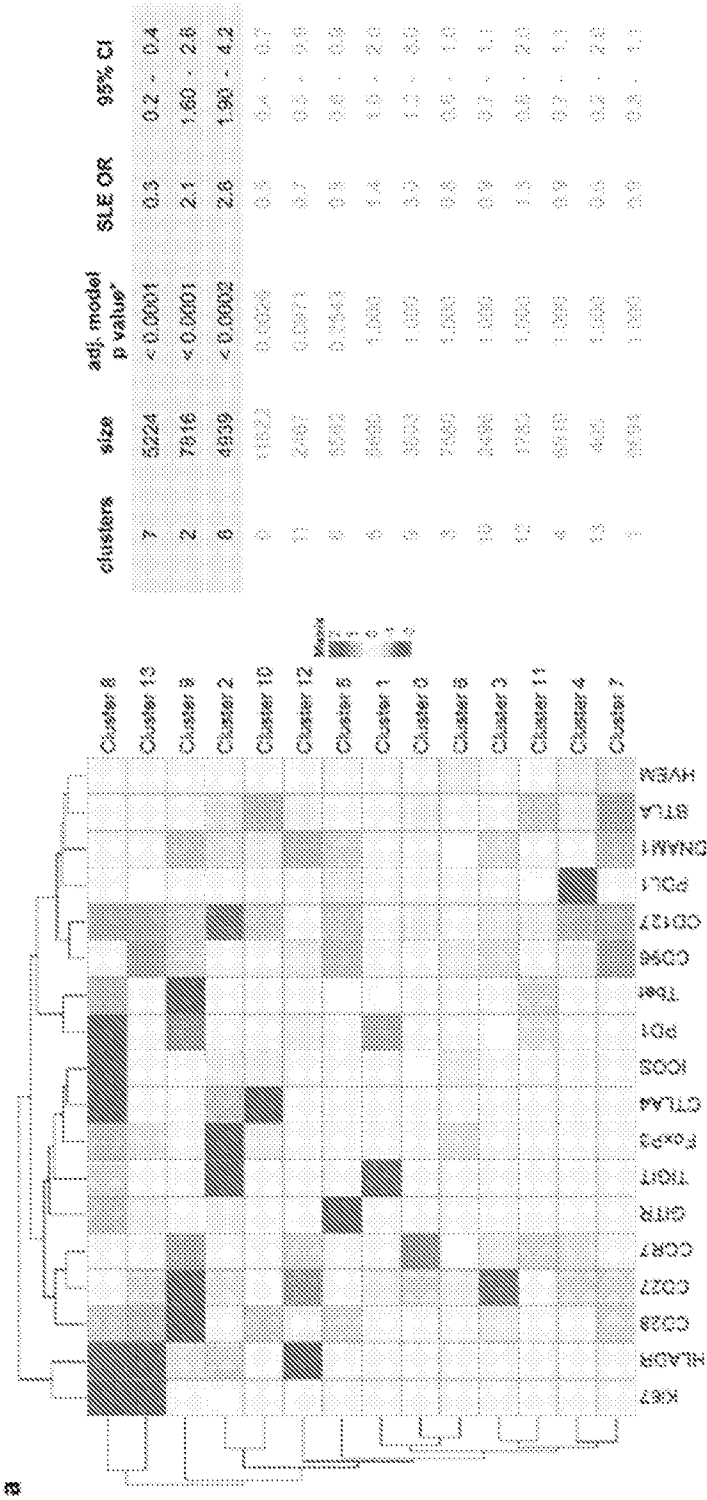
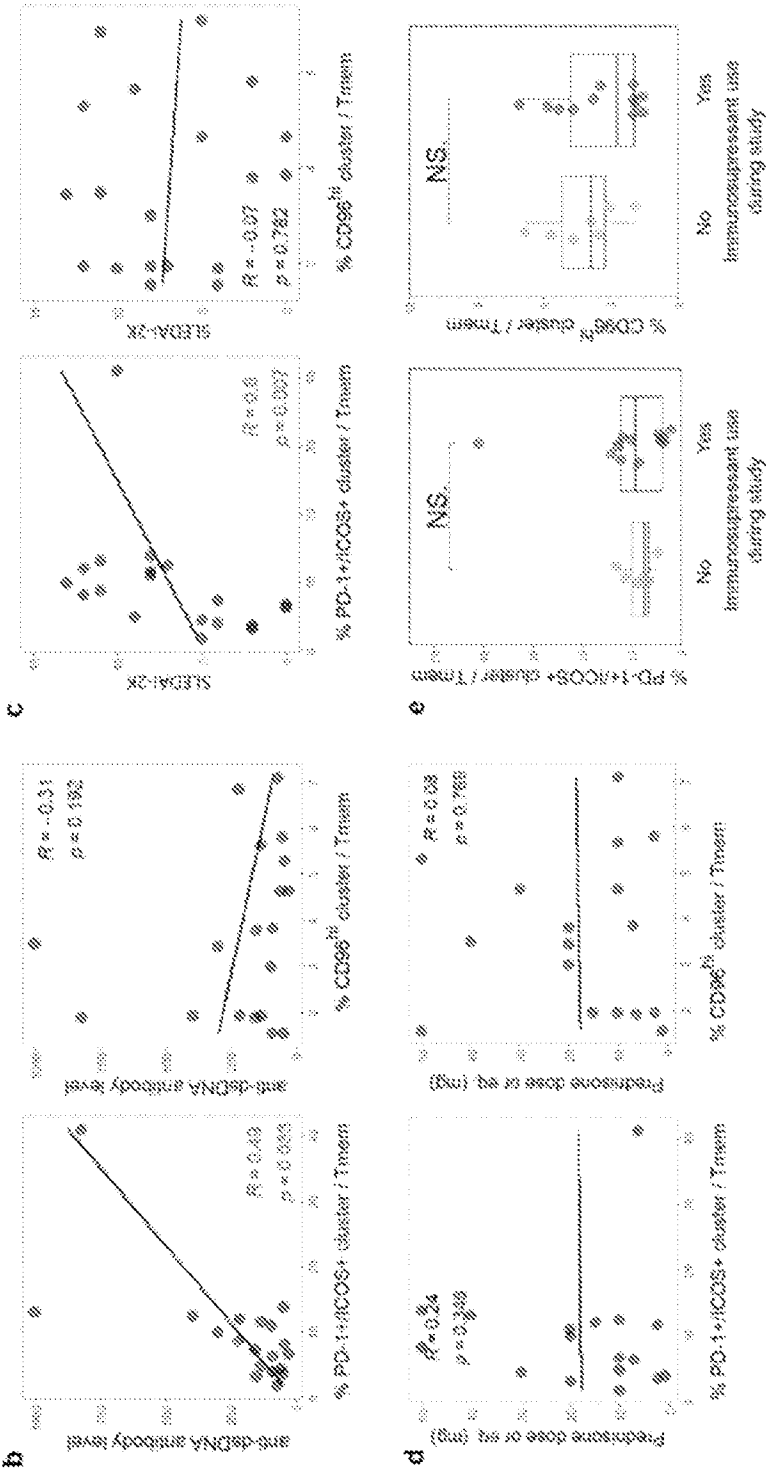
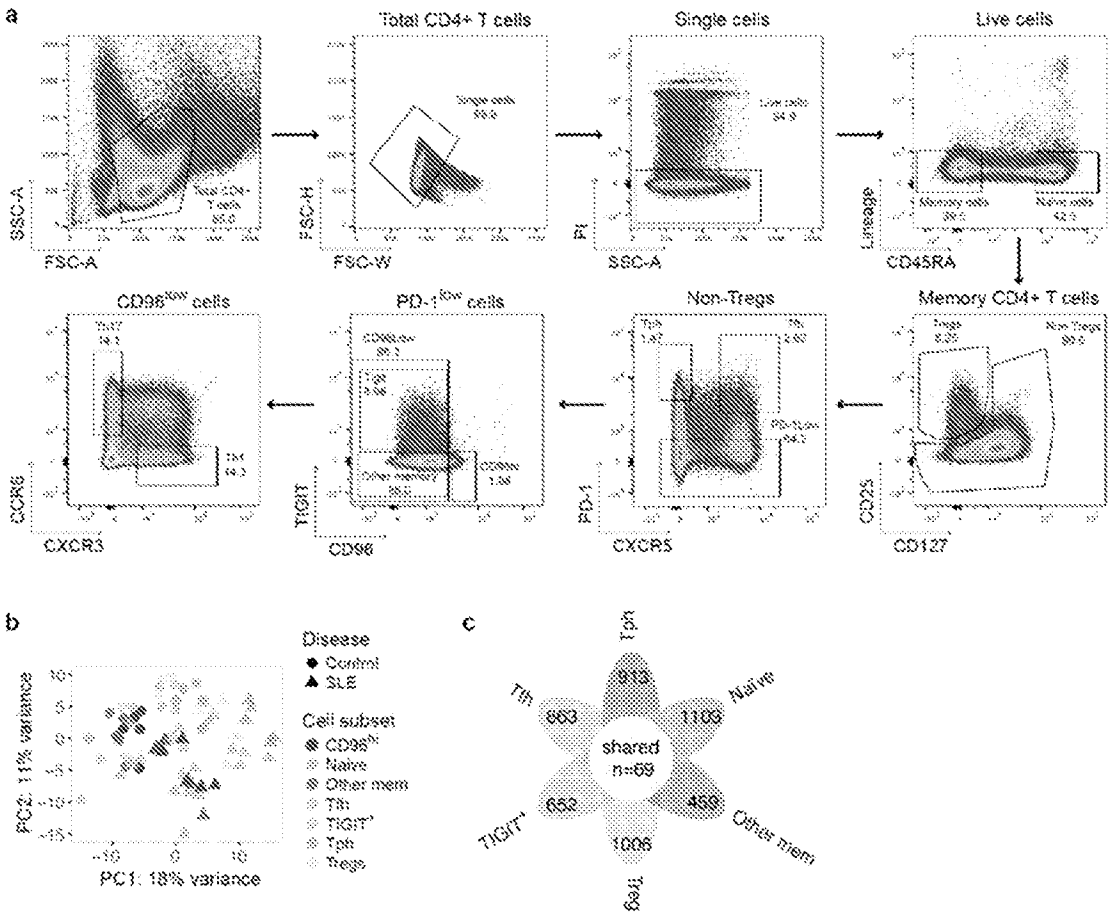


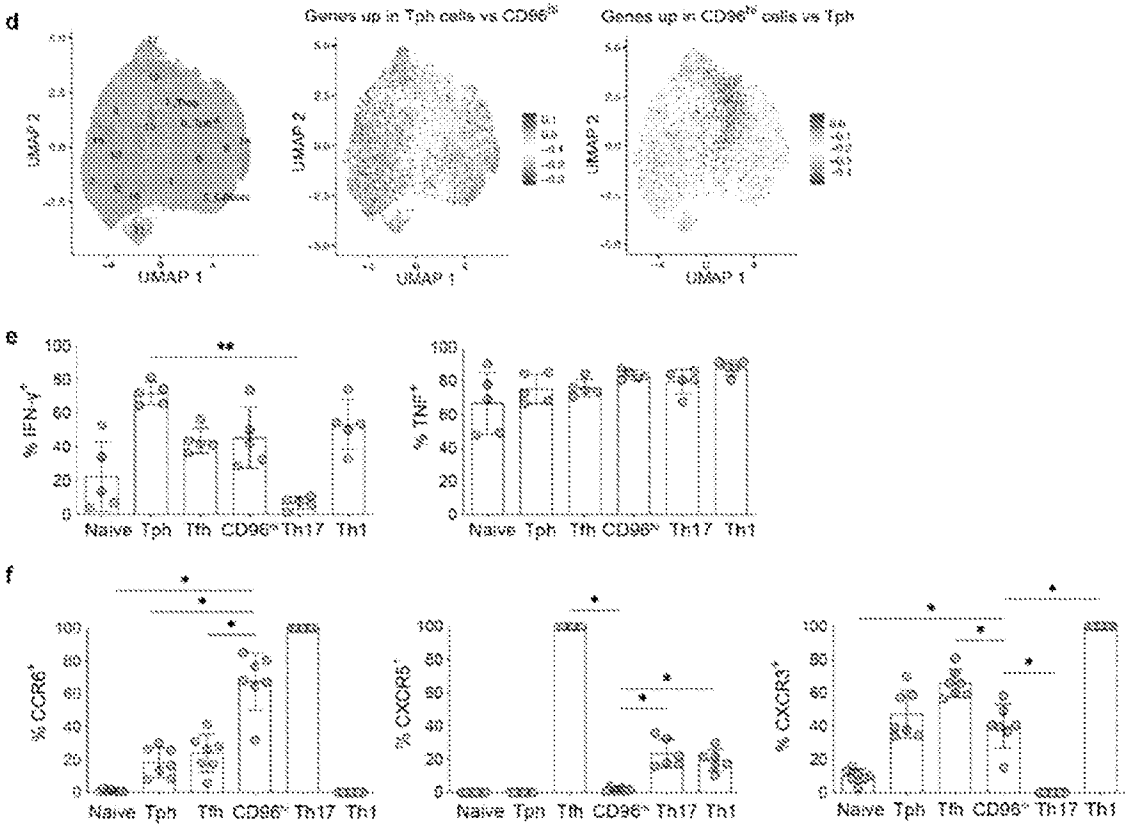
Figure 7a



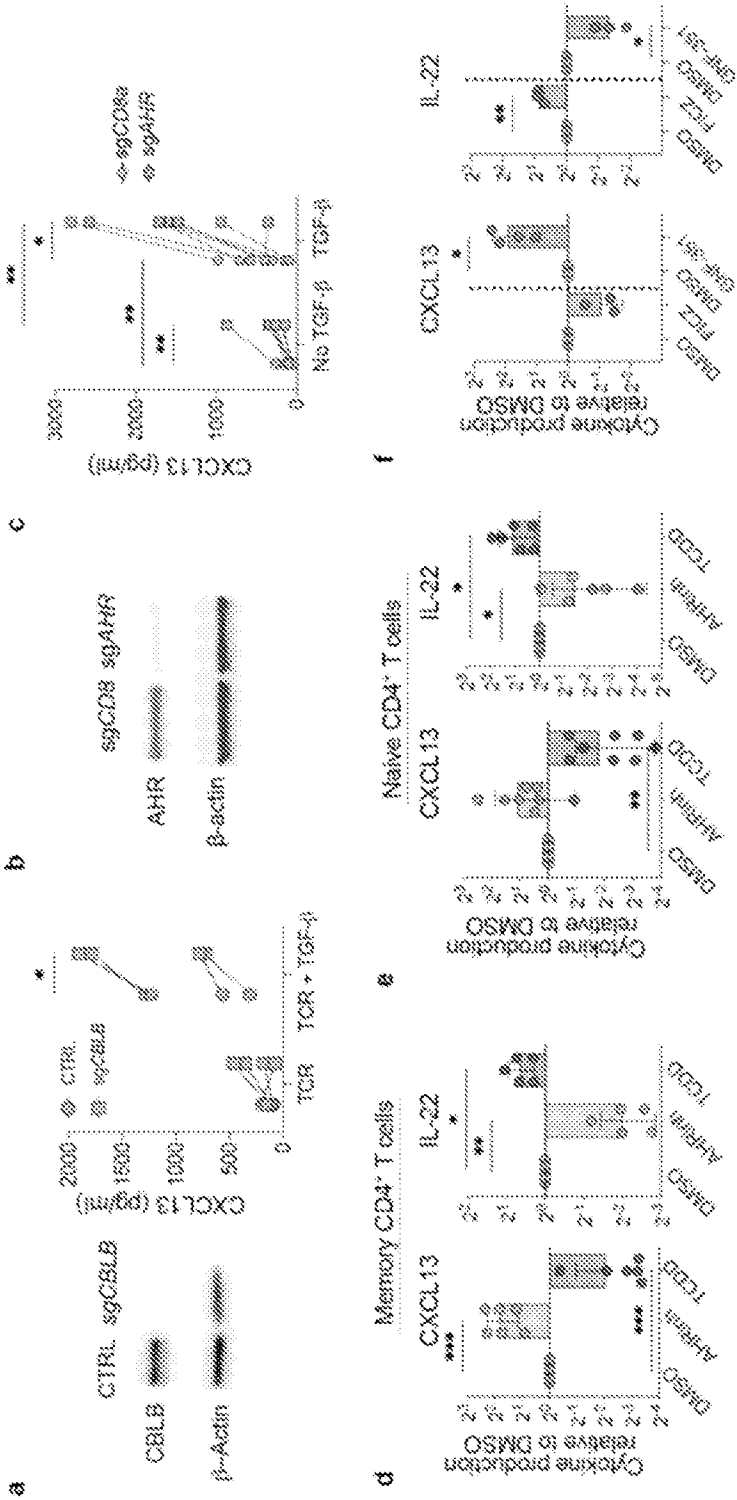
Figures 7b-7e



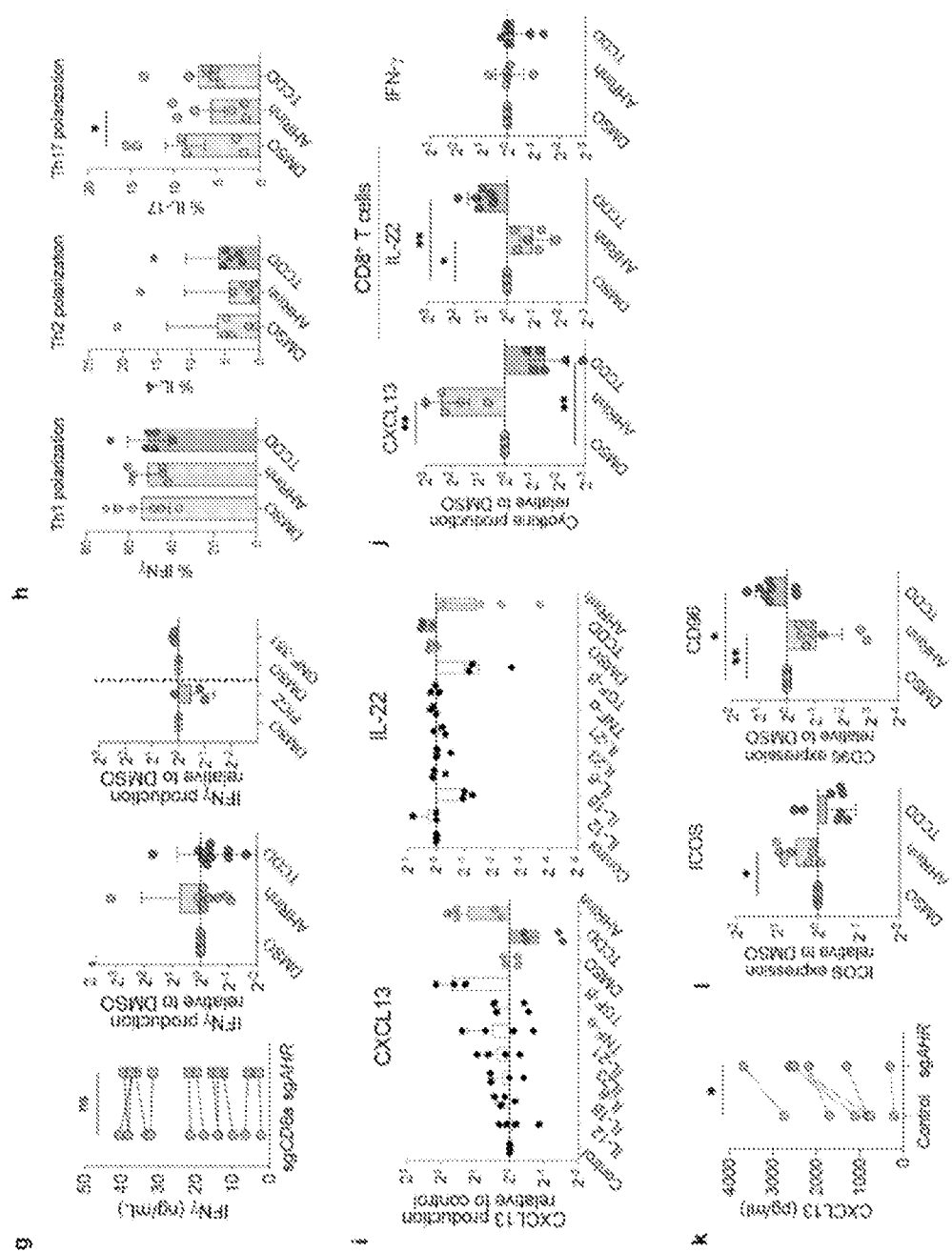
Figures 8a-8c



Figures 8d-8f



Figures 9a-9f



Figures 9g-9l

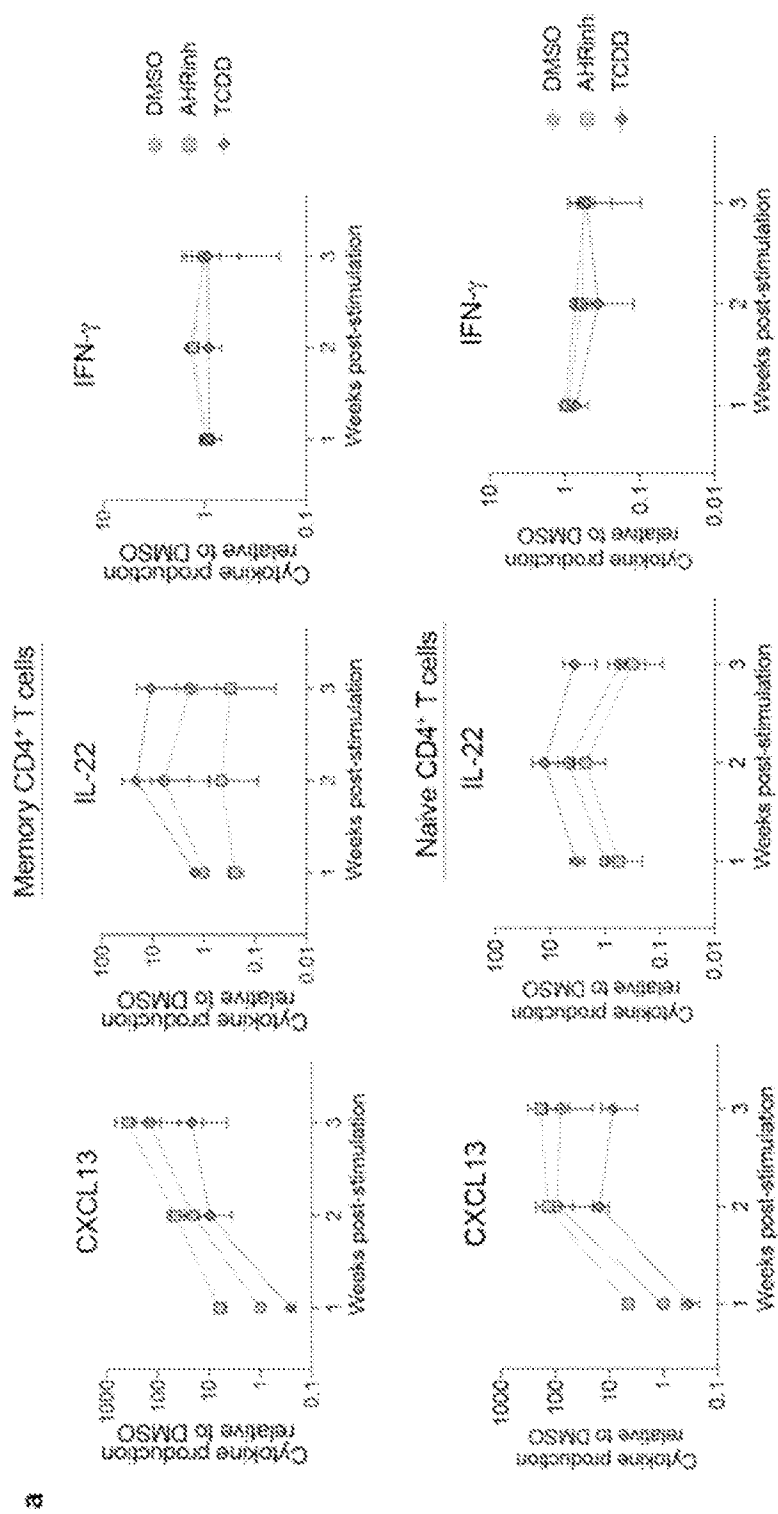
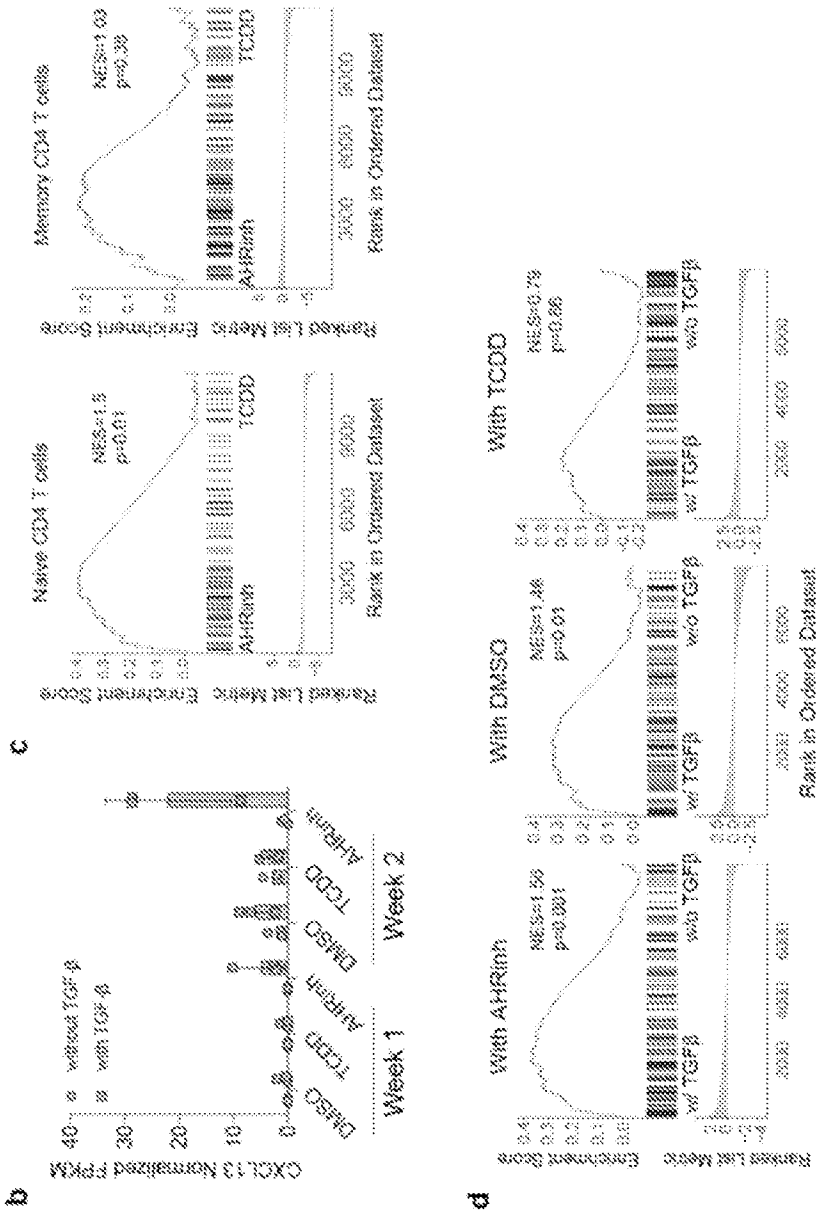
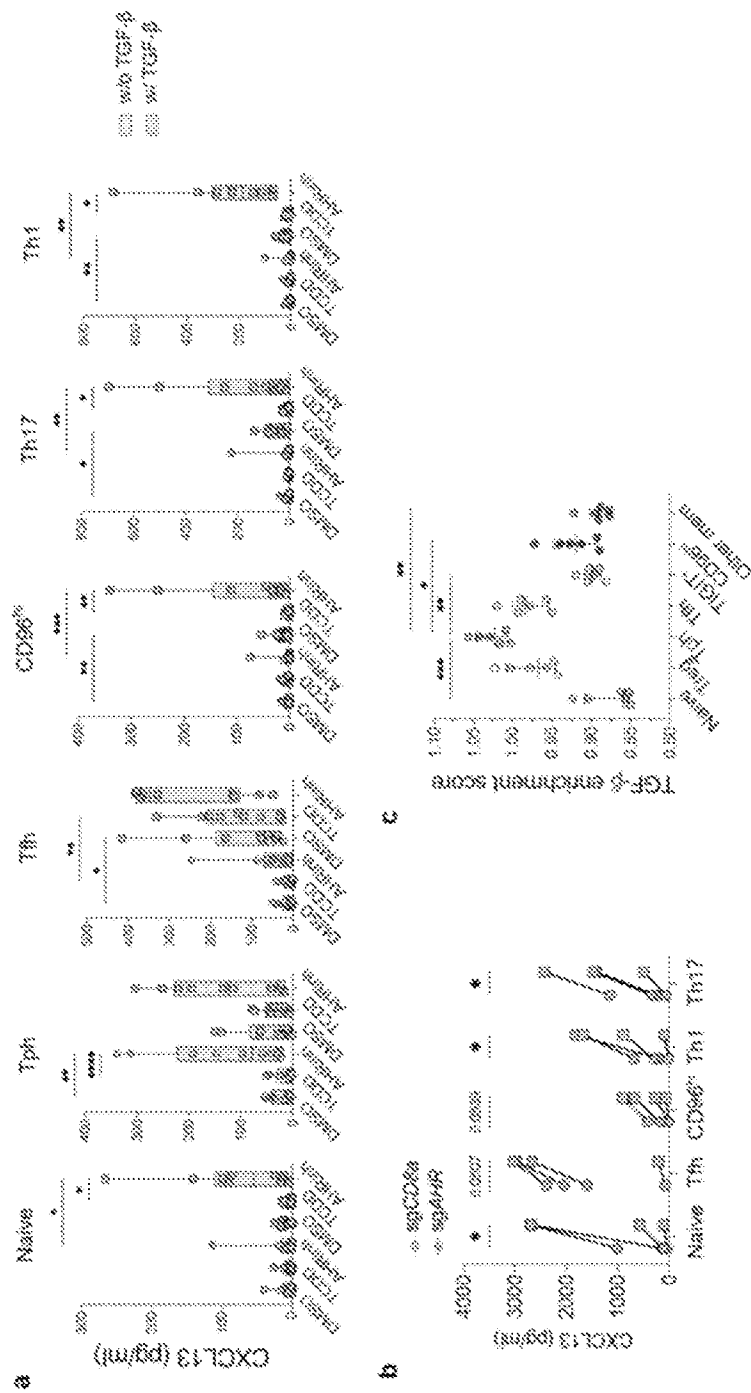


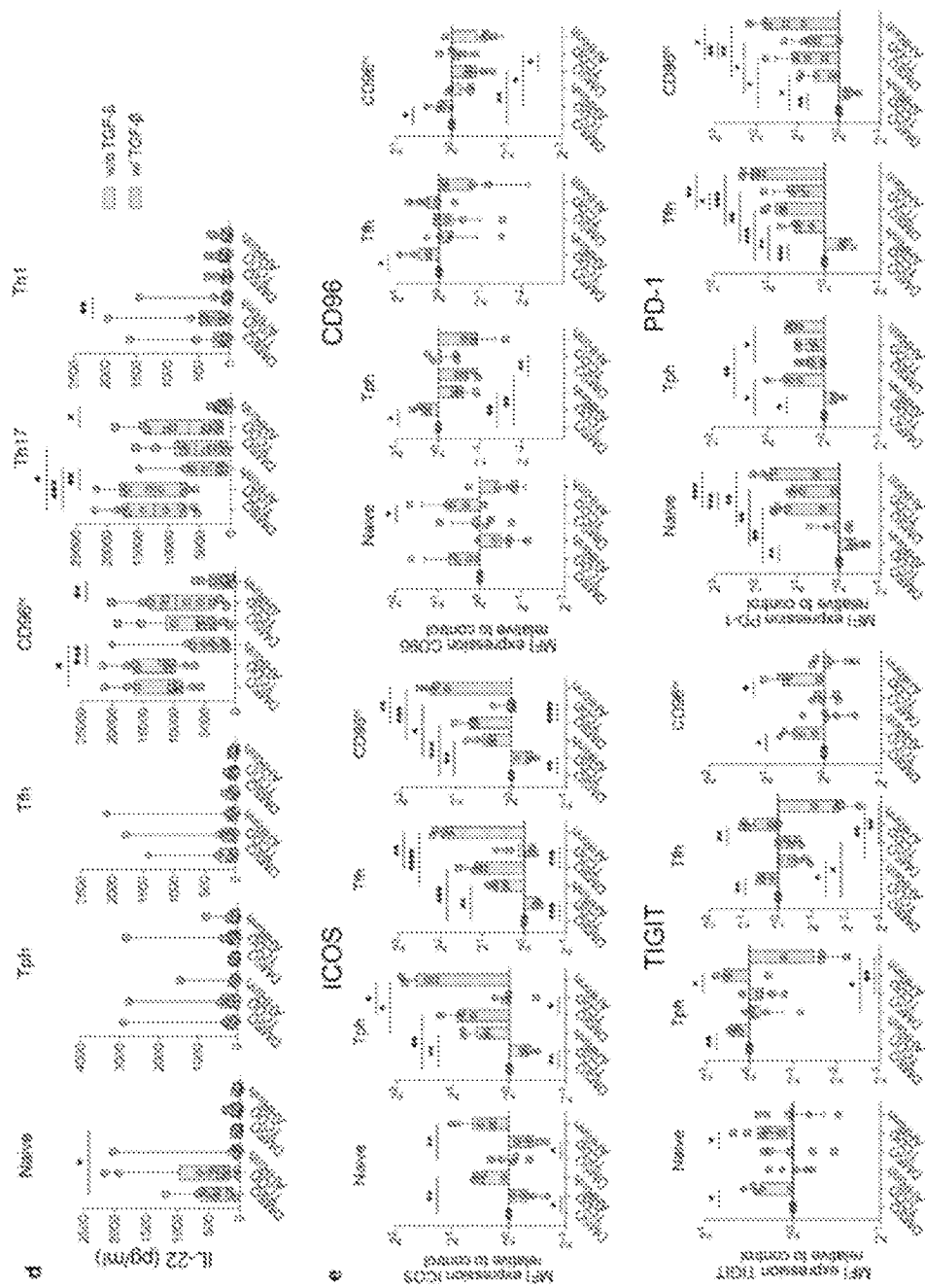
Figure 10a



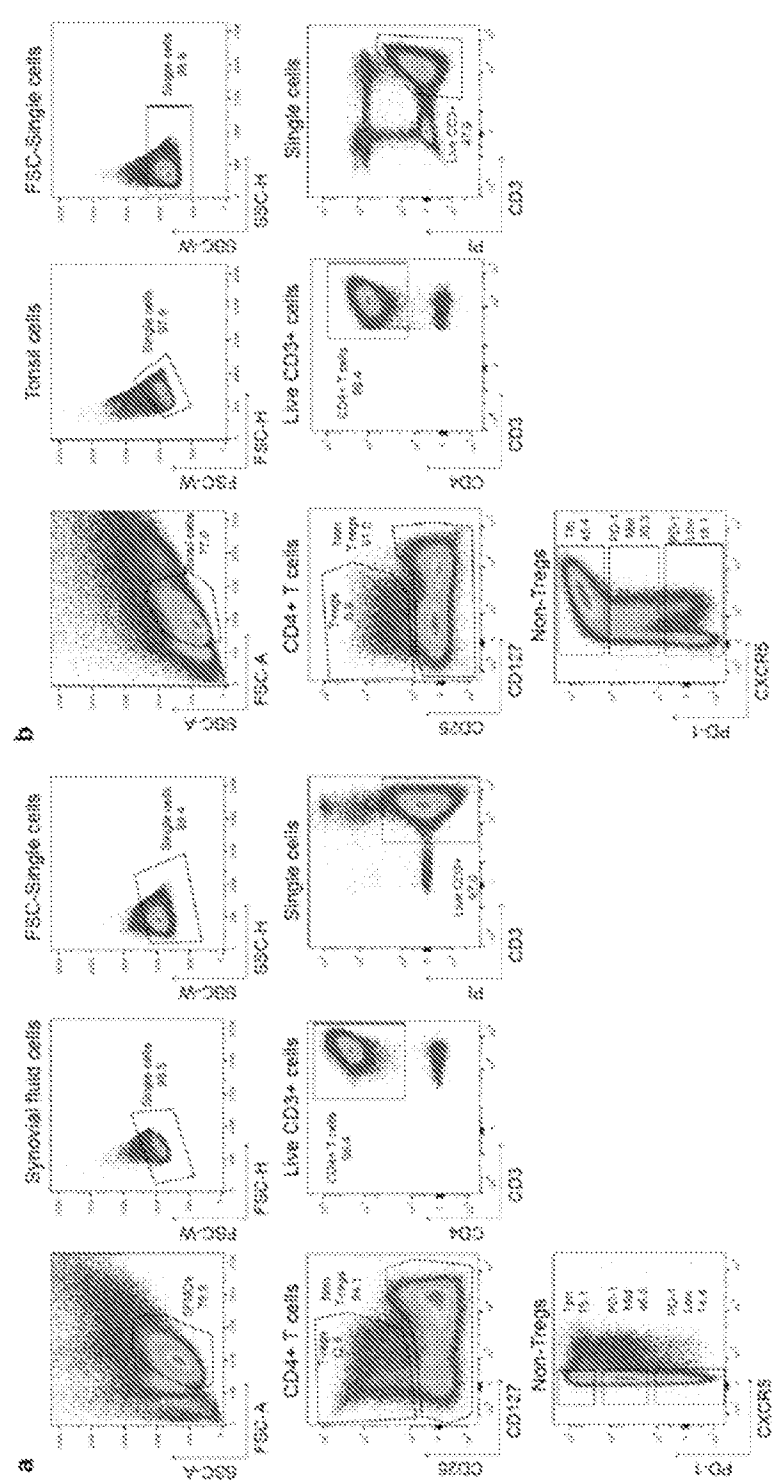
Figures 10b-10d



Figures 11a-11c



Figures 11d-11e



Figures 12a-12b

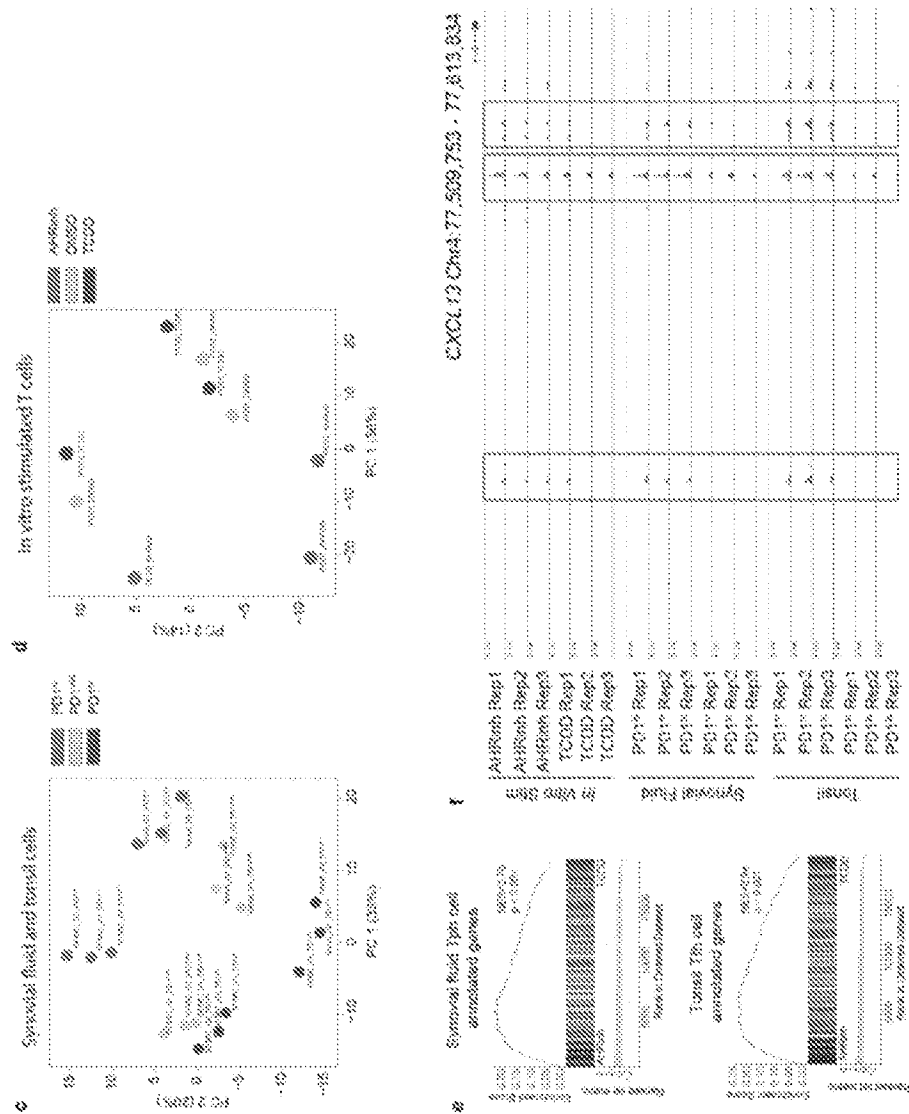


Figure 12c-12f

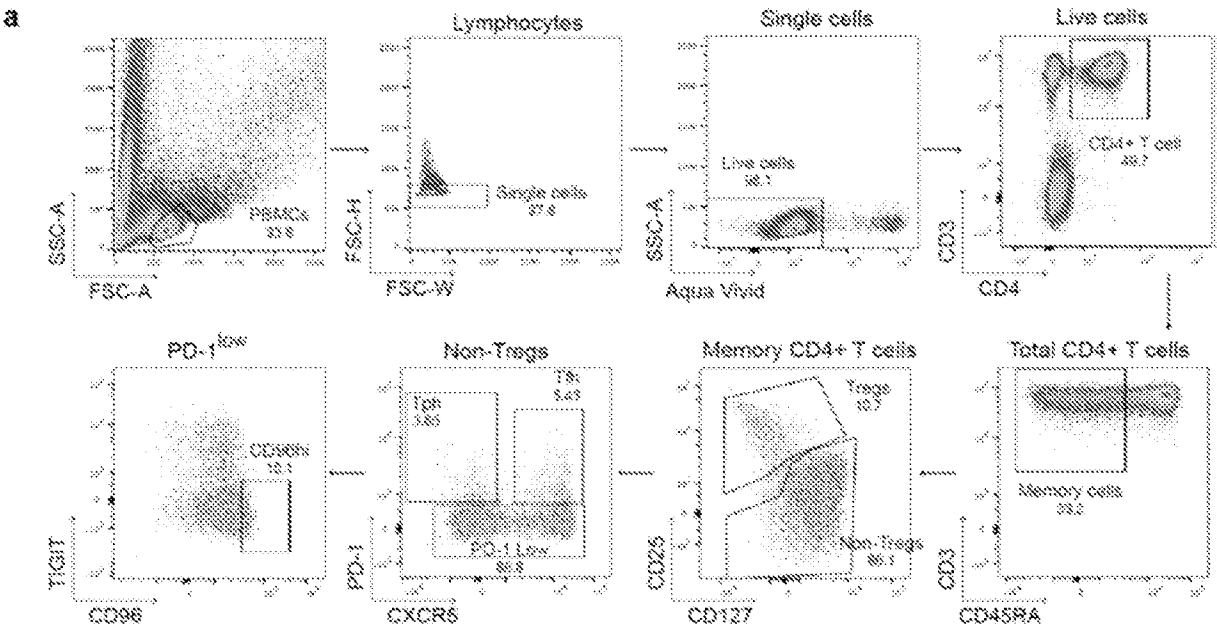
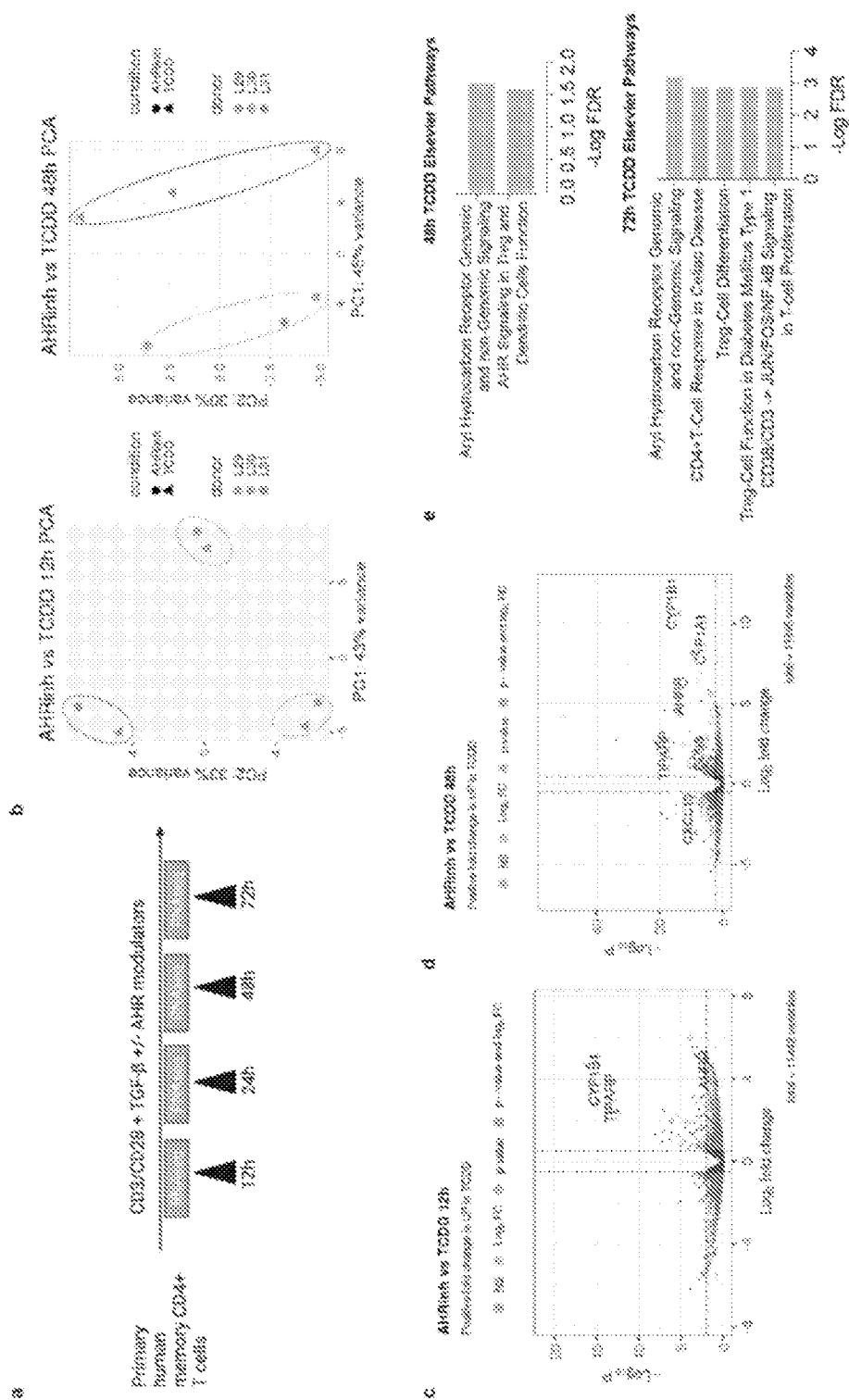
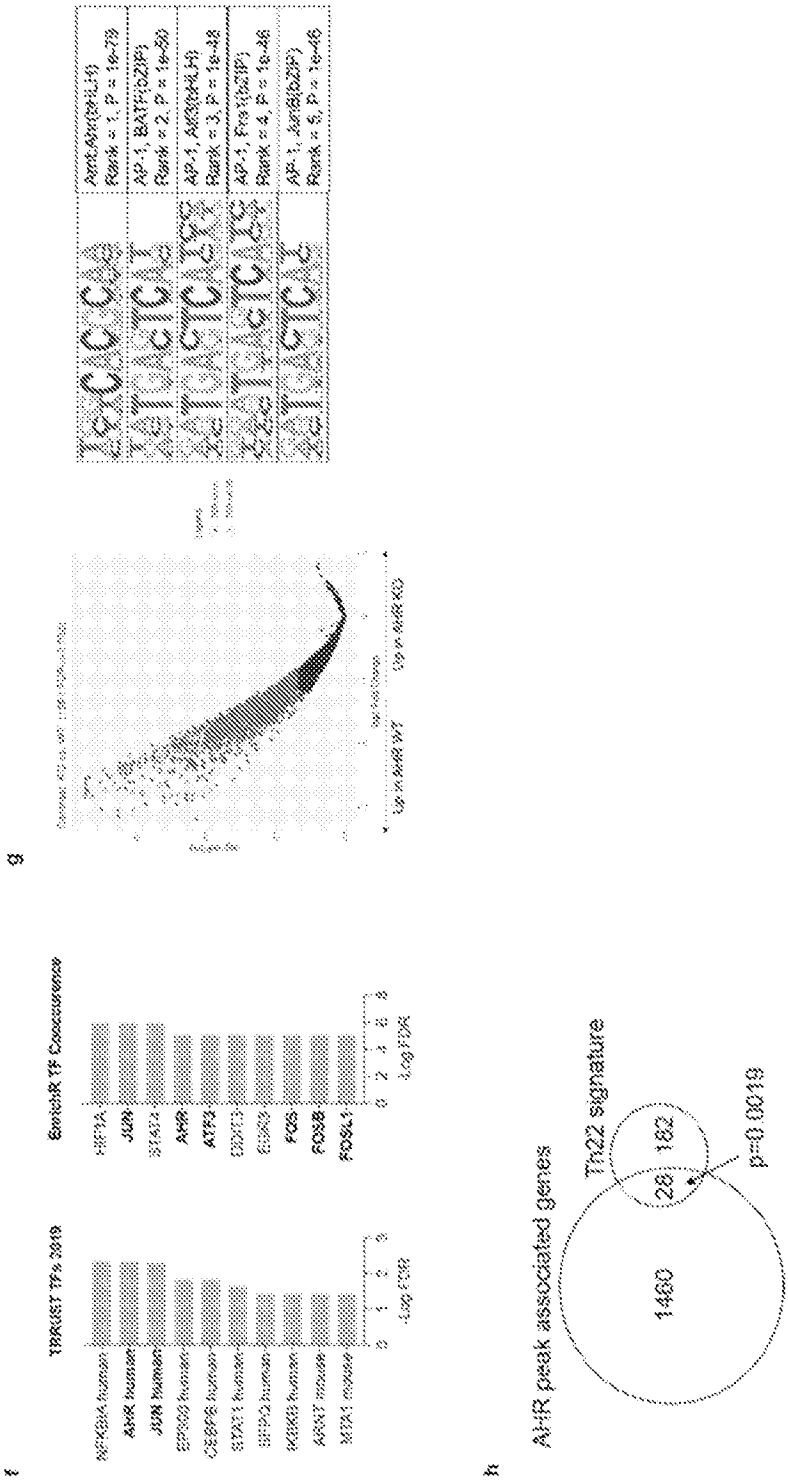


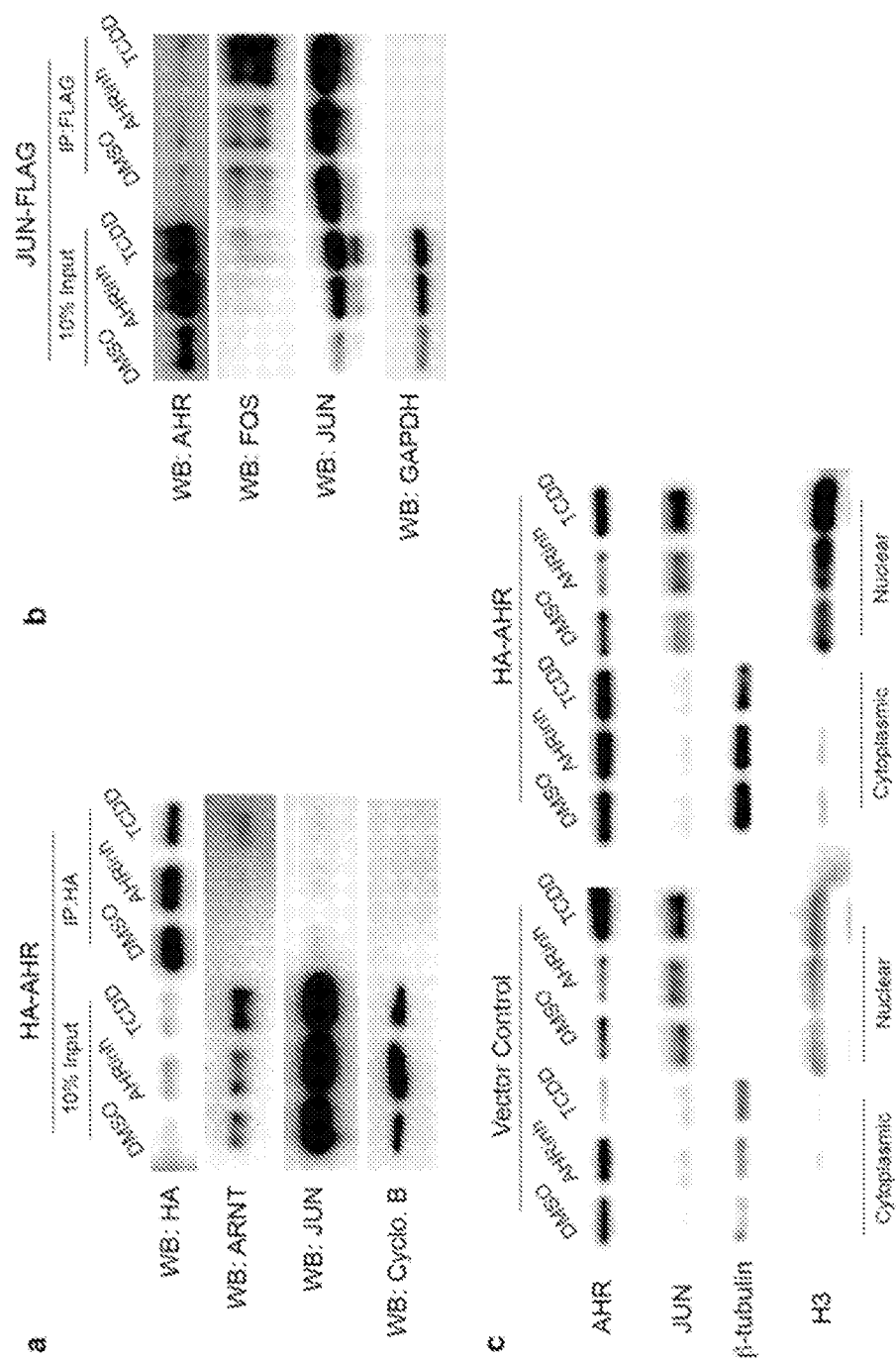
Figure 13



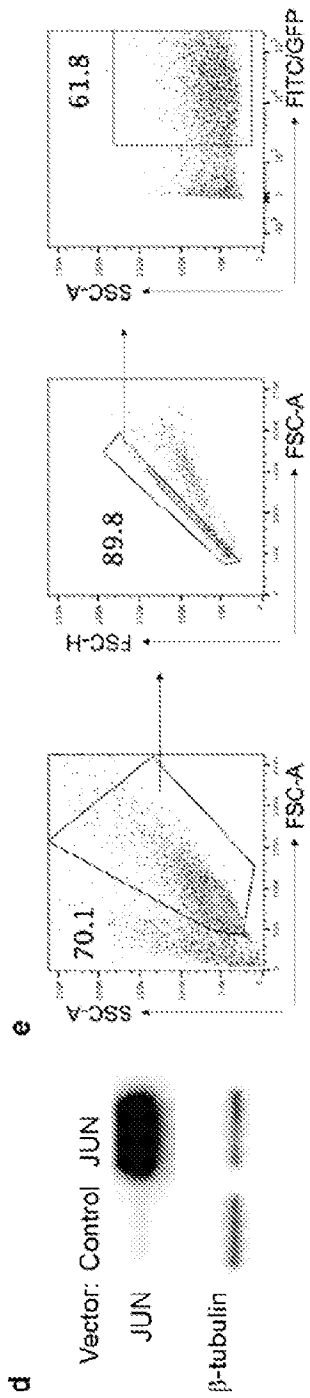
Figures 14a-14e



Figures 14f-14h



Figures 15a-15c



Figures 15d-15e

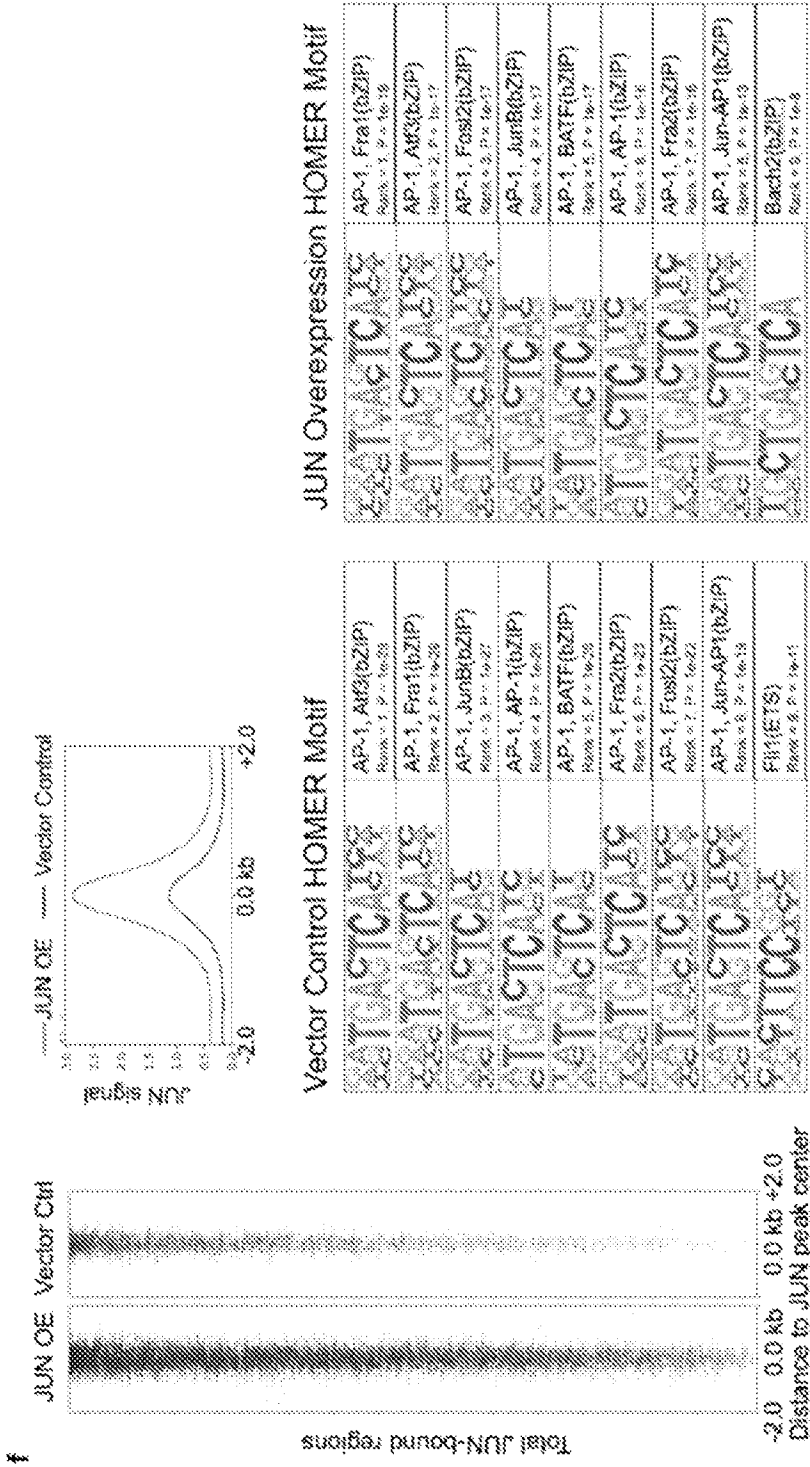
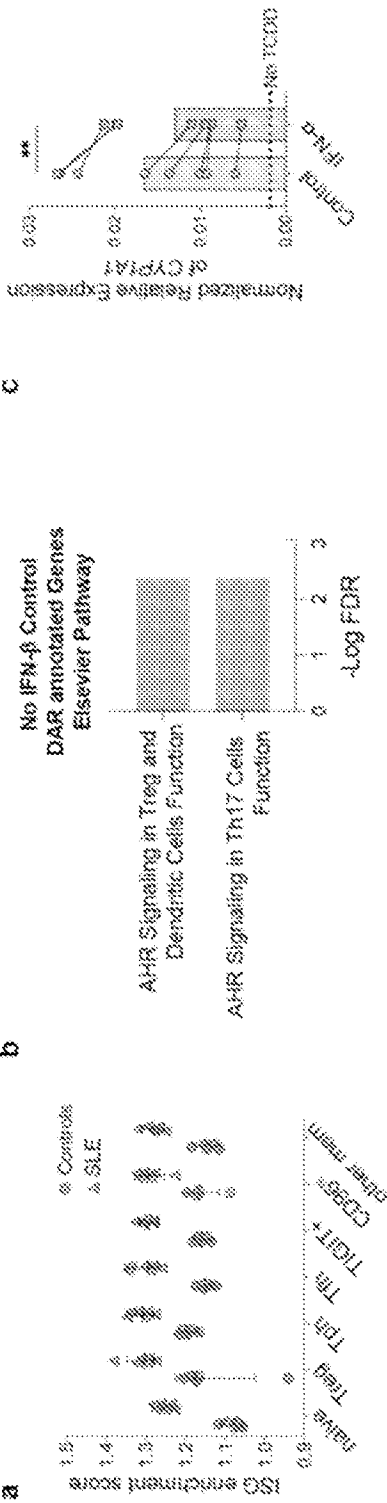


Figure 15f



Figures 16a-16c

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/031858

A. CLASSIFICATION OF SUBJECT MATTER					
INV.	A61K38/20	A61K31/404	A61K31/519	A61K35/17	A61K39/00
	A61P19/02	A61P29/00	C12N5/10		
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)					
A61K C07K A61P C12N					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)					
EPO-Internal, BIOSIS, EMBASE, 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	US 2018/042899 A1 (MEZRICH JOSHUA DAVID				1 - 15,
	[US] ET AL) 15 February 2018 (2018-02-15)				29 - 32
Y	abstract;				1 - 15,
	p. 1, left-hand column, paragraph [0003] -				29 - 32
	p. 20, left-hand column, paragraph [0170];				
	claims 1-32				

	- / - -				
☒ 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			"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention		
"E" earlier application or patent but published on or after the international filing date			"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		
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)			"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		
"O" document referring to an oral disclosure, use, exhibition or other means			"&" document member of the same patent family		
"P" document published prior to the international filing date but later than the priority date claimed					
Date of the actual completion of the international search			Date of mailing of the international search report		
16 August 2024			13/11/2024		
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 Camilleri, Alain		

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/031858

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEI LIAO ET AL: "Interleukin-2 at the Crossroads of Effector Responses, Tolerance, and Immunotherapy", IMMUNITY, vol. 38, no. 1, 1 January 2013 (2013-01-01), pages 13-25, XP055225914, AMSTERDAM, NL ISSN: 1074-7613, DOI: 10.1016/j.immuni.2013.01.004	1-15, 29-32
Y	abstract; p. 13, left-hand column, paragraph 1 - p. 21, left-hand column, paragraph 1 -----	1-15, 29-32
X	KARAKUS UFUK ET AL: "Receptor-gated IL-2 delivery by an anti-human IL-2 antibody activates regulatory T cells in three different species", SCIENCE TRANSLATIONAL MEDICINE, vol. 12, no. 574, 16 December 2020 (2020-12-16), XP093130080, ISSN: 1946-6234, DOI: 10.1126/scitranslmed.abb9283	1-15, 29-32
Y	abstract; p. 1, left-hand column, paragraph 1 - p. 14, left-hand column, paragraph 4 -----	1-15, 29-32
X	XU LE ET AL: "New therapeutic strategies based on IL-2 to modulate Treg cells for autoimmune diseases", INTERNATIONAL IMMUNOPHARMACOLOGY, vol. 72, 18 April 2019 (2019-04-18), pages 322-329, XP093000063, NL ISSN: 1567-5769, DOI: 10.1016/j.intimp.2019.03.064	1-15, 29-32
Y	abstract; p. 322, left-hand column, paragraph 1 - p. 328, left-hand column, paragraph 2 -----	1-15, 29-32
X	HE JING ET AL: "Low-dose interleukin-2 treatment selectively modulates CD4+ T cell subsets in patients with systemic lupus erythematosus", NATURE MEDICINE, NATURE PUBLISHING GROUP US, NEW YORK, vol. 22, no. 9, 8 August 2016 (2016-08-08), pages 991-993, XP037922194, ISSN: 1078-8956, DOI: 10.1038/NM.4148 [retrieved on 2016-08-08]	1-15, 29-32
Y	abstract; p. 991, left-hand column, paragraph 1 - p. 5, right-hand column, paragraph 1 ----- -/-	1-15, 29-32

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/031858

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAVID KLATZMANN ET AL: "The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases", NATURE REVIEWS IMMUNOLOGY, vol. 15, no. 5, 17 April 2015 (2015-04-17), pages 283-294, XP055408855, London ISSN: 1474-1733, DOI: 10.1038/nri3823	1-15, 29-32
Y	abstract; p. 283, left-hand column, paragraph 1 - p. 293, right-hand column, paragraph 1 -----	1-15, 29-32
Y	ZHU CONGHUI ET AL: "The role of AhR in autoimmune regulation and its potential as a therapeutic target against CD4 T cell mediated inflammatory disorder", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, MOLECULAR DIVERSITY PRESERVATION INTERNATIONAL (MDPI), BASEL, CH, vol. 15, no. 6, 5 June 2014 (2014-06-05), pages 10116-10135, XP002790335, ISSN: 1422-0067, DOI: 10.3390/IJMS150610116 abstract; p. 10117, paragraph 1 - p. 10130, paragraph 4 -----	1-15, 29-32
Y	FRANCISCO J QUINTANA: "The aryl hydrocarbon receptor: a molecular pathway for the environmental control of the immune response", CANCER RESEARCH, WILEY-BLACKWELL PUBLISHING LTD, GB, vol. 138, no. 3, 5 February 2013 (2013-02-05), pages 183-189, XP071276353, ISSN: 0019-2805, DOI: 10.1111/IMM.12046 abstract; p. 183, left-hand column, paragraph 1 - p. 187, left-hand column, paragraph 2 -----	1,2, 5-15, 29-32
Y	WANG XIAO-SONG ET AL: "Therapeutic potential of aryl hydrocarbon receptor in autoimmunity", INFLAMMOPHARMACOLOGY, KLUWER ACADEMIC PUBLISHERS, DORDRECHT, NL, vol. 28, no. 1, 15 October 2019 (2019-10-15), pages 63-81, XP037001330, ISSN: 0925-4692, DOI: 10.1007/S10787-019-00651-Z [retrieved on 2019-10-15] abstract; p. 63, left-hand column, paragraph 1 - p. 75, right-hand column, paragraph 2 -----	1,2, 5-15, 29-32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/031858

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed.
 - b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/031858

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

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

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

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-15, 29-32 (all partially)

Remark on Protest

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15, 29-32 (all partially)

An aryl hydrocarbon receptor (AHR) agonist and its use in the treatment of an autoimmune disease, wherein the AHR agonist is interleukin-2. The AHR agonist IL-2 may also be conjugated to a T-cell targeting moiety such as an anti-T-cell antibody.

2. claims: 1-15, 29-32 (all partially)

An aryl hydrocarbon receptor (AHR) agonist and its use in the treatment of an autoimmune disease, wherein the AHR agonist is TCDD. The AHR agonist TCDD may also be conjugated to a T-cell targeting moiety such as an anti-T-cell antibody.

3. claims: 1-15, 29-32 (all partially)

An aryl hydrocarbon receptor (AHR) agonist and its use in the treatment of an autoimmune disease, wherein the AHR agonist is FICZ. The AHR agonist FICZ may also be conjugated to a T-cell targeting moiety such as an anti-T-cell antibody.

4. claims: 16-18

A population of T cells which have been treated with an AHR agonist and the use of the resulting T cells in the treatment of an autoimmune disease.

5. claims: 19-28 (completely); 29-32 (partially)

A transcription factor selected from AHR, JUN, FOS, ATF3, FOSL1, FOSL2, and BATF, and use of said transcription factor in the treatment of an autoimmune disease.

6. claims: 33-39

A guide RNA targeting CD3D, LCP2, ATF4, IFNAR2, or STAT2, and wherein the gRNA has a sequence according to one of the sequences in Table 5, or a Caspr-Cas9-gRNA (crRNP) complex comprising at least one such gRNA, and used of said crRNP complex in the treatment of an autoimmune disease.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2024/031858

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2018042899 A1	15-02-2018	US 2014050694 A1	20-02-2014
		US 2018042899 A1	15-02-2018
