



(51) International Patent Classification:

C12N 5/0783 (2010.01) C07H 19/20 (2006.01)
 A61K 31/7076 (2006.01) C07K 14/525 (2006.01)
 A61K 35/17 (2015.01) C07K 14/54 (2006.01)
 A61P 1/00 (2006.01) C07K 16/22 (2006.01)
 A61P 35/00 (2006.01) C07K 16/28 (2006.01)

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, 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).

(21) International Application Number:

PCT/CA2017/000274

Published:

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

(22) International Filing Date:

21 December 2017 (21.12.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/447,491 18 January 2017 (18.01.2017) US

(71) Applicant: UNIVERSITY HEALTH NETWORK

[CA/CA]; 190 Elizabeth Street, R. Fraser Elliott Building - Room 1S-417, Toronto, Ontario M5G 2C4 (CA).

(72) Inventors: St. PAUL, Michael; 239 Strathearn Ave, Richmond Hill, Ontario L4B 3C4 (CA). OHASHI, Pam; 208 La Rose Ave, Toronto, Ontario M9P 1B5 (CA). SAIBIL, Sam; 63 Salem Ave, Toronto, Ontario M6H 3C2 (CA).

(74) Agent: CHIU, Jung-Kay; Norton Rose Fulbright Canada LLP/S.E.N.C.R.L., s.r.l., 1, Place Ville Marie, Suite 2500, Montreal, Quebec, H3B 1R1 (CA).

(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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: ANTI-CANCER T CELLS AND THEIR PREPARATION USING COENZYME A

(57) Abstract: There is described herein, a method for inducing Tc22 lineage T cells from a population of CD8+ T cells, the method comprising: a) providing a population of CD8+ T cells; b) activating the population; and c) culturing or contacting the population of CD8+ T cells with Coenzyme A.

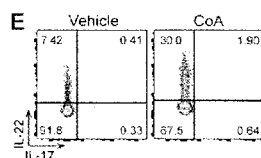


Figure 2

ANTI-CANCER T CELLS
AND THEIR PREPARATION USING COENZYME A

5 RELATED APPLICATION

This application claims priority to U.S. Provisional Patent No. 62/447,491 filed on January 18, 2017, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

- 10 The invention relates to T cells and specifically, to a IL-22⁺ CD8⁺ T cell subset with anti-tumor function.

BACKGROUND OF THE INVENTION

- Metabolic programming is a central regulator of T cell activation, effector
15 function and differentiation. Upon activation, T cells engage in aerobic glycolysis and increase their rate of glycolysis and lactate production disproportionately to the increase in oxidative phosphorylation (OXPHOS)¹⁻⁴. Adoption of this highly glycolytic phenotype supports effector function, as cytokine production is coupled to the flux of glucose down the glycolytic
20 pathway⁵⁻⁷. Metabolic pathways also control the differentiation of CD8⁺ memory cells as well as the commitment of CD4⁺ cells to specific subset lineages⁸⁻¹⁴.

SUMMARY OF THE INVENTION

- 25 In an aspect, there is provided, a method for inducing Tc22 lineage T cells from a population of CD8⁺ T cells, the method comprising: a) providing a population of CD8⁺

T cells; b) activating the population; and c) culturing or contacting the population of CD8+ T cells with Coenzyme A.

In an aspect, there is provided, a population of cells comprising Tc22 lineage T cells that are CD8+/IL-22+/IL17-/IFN γ low.

- 5 In an aspect, there is provided, a population of Tc22 lineage T cells produced by the method described herein.

In an aspect, there is provided, the population described herein, for use in the treatment of cancer, preferably a cancerous tumor.

- 10 In an aspect, there is provided, a method of treating cancer, preferably a cancerous tumor, in a patient, the method comprising administering to the patient the population described herein.

In an aspect, there is provided, a use of the population described herein, in the manufacture of a medicament for the treatment of cancer, preferably a cancerous tumor.

- 15 In an aspect, there is provided a method of treating cancer, preferably a cancerous tumor, or an inflammatory disease, comprising administering to the patient Coenzyme A or Coenzyme A treated T cells.

In an aspect, there is provided a use of Coenzyme A or Coenzyme A treated T cells for the treatment of cancer, preferably a cancerous tumor, or an inflammatory disease.

- 20 In an aspect, there is provided a use of Coenzyme A or Coenzyme A treated T cells in the manufacture of a medicament for the treatment of cancer, preferably a cancerous tumor, or an inflammatory disease.

BRIEF DESCRIPTION OF FIGURES

- 25 These and other features of the preferred embodiments of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings wherein:

Figure 1. Tc22s are metabolically distinct and require OXPHOS for polarization.

(A) Cytokine profile of CD8+ T cells activated for three days in the presence of polarizing cytokines (B-D) Metabolites were extracted and quantified from polarized Tc subsets derived from 5 mice and displayed as a (B) heatmap or (C) PCA plot. (D) Number of significantly different metabolites relative to each subset. (E) Basal extracellular acidification rate (ECAR), oxygen consumption rate (OCR) and OCR:ECAR as determined by Seahorse Bioanalyzer. (F) ATP levels in polarized Tc subsets. (G) Cytokine profile of CD8+ Tc subsets polarized in the presence of oligomycin (Oligo) or vehicle control. (E,F) Bar graphs are means of (E) 6 or (F) 3 replicates $\pm$ SEM. Statistical significance was calculated with one-way ANOVA with a Tukey test. ** $p < 0.01$, *** $p < 0.001$. (A,E-G) Results shown are representative of at least 2 independent experiments.

Figure 2. CoA induces Tc22s in the absence of polarizing cytokines.

(A) Metabolic pathways enriched in CD8+ Tc22s (B) Quantification of pantothenate pathway metabolites in Tc subsets. Results shown are mean scaled intensity of metabolites as determined by mass spectrometry $\pm$ standard error (N=5) (C,D) Cytokine expression in (C) mouse and (D) human CD8+ T cells activated in Tc22 conditions plus vehicle or pantothenate. (E) Cytokine expression in CD8+ T cells activated in Tc22 conditions plus vehicle or CoA for three days. (F,G) Cytokine expression in CD8+ T cells activated in non-polarizing conditions plus vehicle or CoA. (H) CD8+ T cells were activated for three days in the presence of CoA with or without anti IL-6 (10 ug/mL). (I) Cytokines were quantified in supernatants collected after a three day culture with CD8+ T cells activated in the presence of vehicle or CoA. Results shown are mean $\pm$ standard error (N=2) (J) Expression level of top significantly up-regulated genes in Tc22s as determined by RNA seq. Values are expressed as mean fold change from 3 mice relative to vehicle treated control cells on a log2 scale. (B) Statistical significance was calculated using Welch's two tailed t-test adjusted for multiple comparisons * $p \leq 0.05$, ** $p \leq 0.01$. (C-I) Results shown are representative of at least 2 independent experiments.

Figure 3. Oxidative HIF signaling mediates the induction of Tc22s by CoA.

Basal (A) OCR, ECAR and (B) OCR:ECAR ratio quantified by Seahorse of CD8+ T cells activated in the presence of vehicle or CoA. (C) ATP levels in CoA treated CD8+ T cells. (D) CD8+ T cells activated for three days in presence of CoA or Vehicle were incubated for 2.5 hours in media containing ^{13}C glucose and analyzed by mass

spectrometry. (E) CD8⁺ T cells were activated for three days with or without exogenous succinate added. (F) Western blotting for HIF-1 α and β -actin in CD8⁺ T cells activated in the presence of vehicle or CoA. (G) Cytokine expression in CD8⁺ T cells that were activated in the presence of CoA and either a HIF inhibitor or vehicle control. (H) WT or HIF-Transgenic CD8⁺ T cells were activated for three days in non-polarizing conditions using anti-CD3 (5 μ g/mL) and anti-CD28 (1 μ g/mL). Results shown are (A-C) mean replicates (N=3-6) $\pm$ SEM, or (D) means of triplicates $\pm$ SEM of activated T cells pooled from 3 mice. Statistical significance was calculated with two-tailed t test. ** p<0.01, *** p<0.001. (A-C, E-H) are representative of at least 2 independent experiments.

Figure 4. CoA enhances anti-tumor function of T cells and protects against DSS colitis. (A). Mice were inoculated with B16-gp33 melanoma tumors and received an adoptive transfer of 0.5×10^6 CD8⁺ P14-gp33 specific T cells activated in the presence of CoA or vehicle 10 days post tumor-inoculation. Results shown are mean tumor area (LxW) of 4-5 mice/group $\pm$ SEM. (B-D) Mice received 2.5% DSS in water for 5 days $\pm$ CoA or Vehicle. After 5 days, mice were switched to DSS-free water $\pm$ CoA or Vehicle (C) Mean % weight loss relative to day 0 weight of mice (n=5 mice/group $\pm$ SEM). (D) Cumulative mean % weight loss of DSS treated mice on day 8 pooled from 2 independent experiments (n=9 mice/group $\pm$ SEM). Results shown are (A,C) representative of at least 2 independent experiments or (D) pooled data from multiple independent experiments. (A,C) Repeated measures ANOVA with a Sidak test and (D) two-tailed t-test were used to calculate statistical significance relative to vehicle treated controls. *p<0.05, ** p<0.01, *** p<0.001

Figure 5. Evaluation of amino acid metabolism in Tc subsets. Metabolites were quantified in the metabolic pathways of (A) Alanine and Aspartate (B) Leucine, Isoleucine and Valine (C) Tryptothan (D) Lysine (E) Glutamate (F) Phenylalanine and Tyrosine (G) Glycine, Serine and Threonine (H) Histidine (I) Arginine and Proline and (J) Methionine, Cysteine and Taurine metabolic pathways. Results shown are mean scaled intensity of polarized T cells derived from 5 mice as determined by mass spectrometry.

Figure 6. Tc subsets differentially utilize oxidative and glycolytic pathways. (A) Levels of metabolites involved in glycolysis, nucleotide metabolism and TCA cycle from CD8⁺ T cells activated for three days in Tc1, Tc17 and Tc22 conditions as

determined by mass spectrometry. Each column represents an individual mouse. (B-F) Transcript levels in different CD8⁺ Tc subsets of genes involved in (B) glycolysis (C) pentose phosphate pathway (D) TCA cycle and (E) glutamine metabolism. Results shown are mean log₂ FPKM+1 values from day 3 polarized CD8⁺ T cells derived from three mice as determined by RNA sequencing.

Figure 7. Tc22 polarization is not dependent on fatty acid metabolism. (A) Transcript levels of genes involved in fatty acid metabolism in Tc subsets. Levels of (B) Long chain and (C) Polyunsaturated fatty acids in Tc1 (blue), Tc17 (black) and Tc22 (red) subsets. (D) Expression levels of genes involved in fatty acid transport. (E) Cytokine expression in CD8⁺ T cells that were polarized in the presence of etomoxir (40 μ m) or vehicle control. (A,D) Results shown are mean log₂ FPKM+1 values from 3 mice as determined by RNA sequencing or (B,C) Mean metabolite levels as determined by mass spectrometry from polarized T cells derived from 5 mice $\pm$ standard error .

Figure 8. CoA acts directly on CD8⁺ T cells to induce or enhance Tc22 polarization. Cytokine expression in CD8⁺ T cells that were activated using platebound anti-CD3 (5 ug/mL) and soluble anti CD28 (1 ug/mL) for three days in (a) non-polarizing conditions or (b) Tc22 conditions. CoA or vehicle was added 24 hours after the start of activation.

Figure 9. CoA primarily alters immune and not metabolic genes. GO term analysis of top 100 genes in CD8⁺ T cells significantly (A) up-regulated and (B) downregulated by CoA treatment as determined by RNA seq. (C-G) Transcript levels in CD8⁺ T cells activated in the presence of vehicle, CoA or Tc22 conditions genes involved in (C) glycolysis (D) pentose phosphate pathway (E) TCA cycle (F) glutamine and (G) fatty acid metabolism. Results shown are mean log₂ FPKM+1 values from three mice as determined by RNA sequencing.

Figure 10. CoA induces IL-22 and OXPHOS in previously activated effectors. (A) Cytokine profile (B) OCR (C) ECAR, (D) OCR:ECAR ratio and (E) ATP Production was evaluated in previously activated CD8⁺ T cells that were re-stimulated for 48 hours with anti-CD3 in the presence of vehicle or CoA. (B-E) Results shown are mean values of 3-6 replicates $\pm$ standard error.

Figure 11. CD4⁺ Th22s require OXPHOS and can be induced with CoA. (A) Cytokine profile in CD4⁺ T cells activated in Th1 or Th22 conditions with vehicle control or Oligomycin (B,C) Cytokine expression in SMARTA CD4⁺ T cells activated in the presence of vehicle or CoA in (B) non-polarizing or (C) Th22 conditions. (D) OCR, (E) OCR:ECAR ratio and (F) ATP production was evaluated in CD4⁺ T cells activated in the presence of vehicle or CoA. (D-F) Results shown are mean values of 3-4 replicates $\pm$ standard error.

DETAILED DESCRIPTION

In the following description, numerous specific details are set forth to provide a thorough understanding of the invention. However, it is understood that the invention may be practiced without these specific details.

Generally, in one aspect, the present invention is directed to stimulation, activation, or expansion of T cells, including but not limited to CD4⁺ and CD8⁺ T cells.

Akin to CD4⁺ T cells, CD8⁺ T cells also differentiate into multiple different T cytotoxic (Tc) effector subsets (St. Paul et al., submitted). The metabolic phenotype of the Tc subsets and the impact of metabolism upon their differentiation and function are undefined. Here, we utilize a metabolomics approach to interrogate the metabolic profile of Tc1, Tc17 and Tc22 differentiated CD8⁺ effector cells and found each subset to possess a unique metabolic signature with Tc22 cells being distinct in that they displayed increased utilization of OXPHOS. This increased OXPHOS was required for Tc22 differentiation, as pharmacological inhibition of OXPHOS abrogated Tc22 differentiation but not Tc1 or Tc17 development. Moreover, we identified coenzyme A (CoA) as a reagent that enhances OXPHOS in CD8⁺ T cells and promotes Tc22 differentiation. Surprisingly, we found that CoA alone, in the absence of polarizing cytokines, was sufficient to generate CD8⁺ IL-22⁺ Tc22 cells as well as CD4⁺ Th22 cells. To our knowledge, this is the first example of an effector T cell population whose differentiation can be induced solely via altering T cell metabolism in the absence of polarizing cytokines.

In an aspect, there is provided, a method for inducing Tc22 lineage T cells from a population of CD8⁺ T cells, the method comprising: a) providing a population of CD8⁺

T cells; b) activating the population; and c) culturing or contacting the population of CD8⁺ T cells with Coenzyme A.

Methods of activating T cells are known in the art, for example, as described in T Cell Activation, *Annu. Rev. Immunol.* 2009. 27:591–619.

5 The term “activation”, as used herein, refers to the state of a cell following sufficient cell surface moiety ligation to induce a noticeable biochemical or morphological change. Within the context of T cells, such activation refers to the state of a T cell that has been sufficiently stimulated to induce cellular proliferation. Activation of a T cell may also induce cytokine production and performance of regulatory or cytolytic effector
10 functions. Within the context of other cells, this term infers either up or down regulation of a particular physico-chemical process. The term “activated T cells” indicates T cells that are currently undergoing cell division, cytokine production, performance of regulatory or cytolytic effector functions, and/or has recently undergone the process of “activation.”

15 In some embodiments, the activation comprises culturing or contacting the population with at least one of (i) anti-CD3 antibody and (ii) gp33 peptide from LCMV (KAVYNFATM).

In some embodiments, the population of CD8⁺ T cells is additionally cultured or contacted with TNF- α or IL-6.

20 In some embodiments, the population of CD8⁺ T cells is additionally cultured or contacted with aryl hydrogen receptor (AhR) agonist, preferably 6-Formylindolo(3,2-b)carbazole (FICZ).

In some embodiments, the population of CD8⁺ T cells is additionally cultured or contacted with anti-IFN γ antibody.

25 In some embodiments, the population of CD8⁺ T cells is additionally cultured or contacted with anti-TGF- β antibody.

In some embodiments, the population of CD8⁺ T cells is additionally cultured or contacted with succinate.

In some embodiments, the population of CD8+ T cells is additionally cultured or contacted with pantothenate.

In some embodiments, the Tc22 lineage T cells are CD8+/IL-22+/IL17-/IFN γ low.

In some embodiments, the Tc22 lineage T cells are additionally IL-4-, IL-5-, IL-9-, IL-10- and/or IL-13-.

In some embodiments, the Tc22 lineage T cells are additionally TNF- α hi and/or IL-2hi.

In some embodiments, step c) is performed before or after step b). In other embodiments, step c) is performed simultaneously with step b).

In an aspect, there is provided, a population of cells comprising Tc22 lineage T cells that are CD8+/IL-22+/IL17-/IFN γ low.

In an aspect, there is provided, a population of Tc22 lineage T cells produced by the method described herein.

The T cell populations generated herein would be understood to be beneficial in treating cancer, and may include adrenal cancer, anal cancer, bile duct cancer, bladder cancer, bone cancer, brain/cns tumors, breast cancer, castleman disease, cervical cancer, colon/rectum cancer, endometrial cancer, esophagus cancer, ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumor (gist), gestational trophoblastic disease, hodgkin disease, kaposi sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, leukemia (acute lymphocytic, acute myeloid, chronic lymphocytic, chronic myeloid, chronic myelomonocytic), liver cancer, lung cancer (non-small cell, small cell, lung carcinoid tumor), lymphoma, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma - adult soft tissue cancer, skin cancer (basal and squamous cell, melanoma, merkel cell), small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, waldenstrom macroglobulinemia, and wilms tumor.

In an aspect, there is provided, the population described herein, for use in the treatment of cancer, preferably a cancerous tumor.

In an aspect, there is provided, a method of treating cancer, preferably a cancerous tumor, in a patient, the method comprising administering to the patient the population
5 described herein.

In an aspect, there is provided, a use of the population described herein, in the manufacture of a medicament for the treatment of cancer, preferably a cancerous tumor.

In an aspect, there is provided a method of treating cancer, preferably a cancerous
10 tumor, or an inflammatory disease, comprising administering to the patient Coenzyme A or Coenzyme A treated Tcells. In some embodiments, the inflammatory disease is a gastro-intestinal inflammatory disease, preferably colitis or inflammatory bowel disease.

In an aspect, there is provided a use of Coenzyme A or Coenzyme A treated Tcells for
15 the treatment of cancer, preferably a cancerous tumor, or an inflammatory disease.

In an aspect, there is provided a use of Coenzyme A or Coenzyme A treated Tcells in the manufacture of a medicament for the treatment of cancer, preferably a cancerous tumor, or an inflammatory disease.

As used herein, "*pharmaceutically acceptable carrier*" means any and all solvents,
20 dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for
25 example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the pharmacological agent.

As used herein, "*therapeutically effective amount*" refers to an amount effective, at
30 dosages and for a particular period of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the pharmacological agent

may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the pharmacological agent to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the pharmacological agent are outweighed by the therapeutically beneficial effects.

The advantages of the present invention are further illustrated by the following examples. The examples and their particular details set forth herein are presented for illustration only and should not be construed as a limitation on the claims of the present invention.

10

EXAMPLES

Materials and Methods:

Mice

C57BL/6 were purchased from The Jackson Laboratory and Taconic. LSL-HIF-1 α -dPA mice (JAX strain 009673) were obtained from The Jackson Laboratory and bred to Vav-Cre mice to generate HIF-1 α -Transgenic mice. The HIF-1 α in these mice has a proline to alanine substitution rendering HIF-1 α resistant to degradation by von Hippel Lindau only in cells that express Cre recombinase (22). Generation of P14 mice, which express a transgenic TCR specific for the H2-Db gp33 peptide of the lymphocytic choriomeningitis virus (LCMV) was described previously (27). SMARTA mice have been described previously (28). All mice were maintained at the Ontario Cancer Institute animal facility according to institutional guidelines and with approval of the Ontario Cancer Institute Animal Ethics Committee.

Tc Subset Polarization

P14 CD8⁺ T cells were magnetically purified (Miltenyi Biotec) and co-cultured with mature bone marrow dendritic cells (BMDCs) pulsed with gp33 peptide from LCMV (KAVYNFATM) (29) for three days in IMDM (Gibco) supplemented with 10% FCS, L-glutamine, β -mercaptoethanol, penicillin and streptomycin. For CD4⁺ T cells, SMARTA CD4⁺ cells were co-cultured for three to four days with BMDCs pulsed with gp61 from LCMV (GLNGPDIYKGVYQFKSVEFD). To generate Tc or Th subsets, polarizing

cocktails were added at the start of the co-culture as follows –Tc1: IL-12 (5 ng/mL), Tc17: IL-6 (20 ng/mL) + TGF- β 1 (3 ng/mL) +IL-23 (10 ng/mL) + α -IFN- γ (XMG1.2 - 10 μ g/mL), and Tc22: IL-6 (20 ng/mL) +TNF- α (40 ng/mL) + 6-Formylindolo(3,2-b)carbazole (FICZ at 2 ng/mL) + α -IFN- γ (10 μ g/mL) + α -TGF- β (1D11.16.8 - 10 μ g/mL). Cytokines and neutralizing antibodies were purchased from Biolegend, eBioscience and R&D. FICZ was purchased from Enzo Life Sciences.

Flow Cytometry, Antibodies and Cytokine Assays

Antibodies used for flow cytometry were purchased from eBioscience, Biolegend and BD Pharmingen. Antibody clones used were: CD4 (RM-45), CD8 (53-6.7), IL-22 (IL22JOP and Poly5164), IL-17 (eBio17B7), IFN- γ (XMG1.2) and TNF- α (MP6-XT22). Antibodies used for western blotting were HIF-1 α (Caymen Chem -10006421) and β -actin (Sigma - A2066). For intracellular cytokine staining, cells were re-stimulated for 5-6 hours with Cell-Stimulation Cocktail (eBioscience) in the presence of Brefeldin A (eBioscience), followed by staining using Cytofix/Cytoperm (BD Pharmingen). Flow cytometry data was acquired on a FACSCanto II (BD) and analyzed using FlowJo software (Tree Star). Cytokines were quantified in the supernatant using LEGENDPLEX cytometric bead array (Biolegend) or by ELISA (eBioscience)

Metabolomic Profiling

Metabolomic and statistical analyses were conducted at Metabolon (Durham, NC) as described previously (17). Briefly, cell pellets (n=5 biological replicates per group) were disrupted using a GenoGrinder (675 strokes/minute, 2 minutes) and subjected to methanol extraction. Extracts were split into four aliquots and processed for analysis by ultra-high performance liquid chromatography/mass spectrometry (UHPLC/MS) in the positive (two methods), negative or polar ion mode. Metabolites were identified by automated comparison of ion features to a reference library of chemical standards followed by visual inspection for quality control. For statistical analyses and data display, any missing values were assumed to be below the limits of detection; these values were imputed with the compound minimum (minimum value imputation). To determine statistical significance, Welch's two-factor t-test was performed on protein-normalized data in ArrayStudio (Omicsoft) or "R" to compare data between experimental groups; $p < 0.05$ was considered significant. An estimate of the false discovery rate (Q-value) was calculated to take into account the multiple comparisons that normally occur in metabolomic-based studies, with $Q < 0.05$ used as an indication

of high confidence in a result. ArrayStudio was also used to generate high level overview display items (PCA, heatmaps). Pathway enrichment scores for Tc22 were calculated relative to control effector CD8+ T cells using the formula: (# of significant metabolites in pathway(k) / total # of detected metabolites in pathway(m)) / (total # of significant metabolites(n) / total # of detected metabolites(N)) (k/m)/(n/N). A P-value cut off of $p < 0.01$ was used. Only pathways where at least 3 metabolites were examined were included.

Metabolic Assays and Stable Isotope Tracing Analysis

Seahorse was performed as previously described (11). ATP quantification was performed on day 3 polarized T cells using a commercial kit (Sigma) according to the recommended protocol. For SITA, CD8+ T cells activated for three days in the presence of vehicle or CoA were harvested, washed and incubated for 2.5 hours with U-13C6-glucose (Cambridge Isotopes). Cells were then washed and pellet was frozen in methanol. The cells were thawed on ice and lysed by sonication at 4°C using a Diagenode Bioruptor (30s. on, 30s off, 5mins). The resulting mixtures were spun down (15mins 13000rpm 4°C) to remove the cellular debris, the resulting supernatants were transferred to clean microfuge tubes and the solvent was removed using a speed-vac at room temperature. The resulting pellets were stored at -70°C until derivitization. The pellets were solubilized in 30 µL of pyridine containing methoxyamine(10mg/mL; Sigma) and each sample was spiked with 1µL of myristic acid-D27 (750ng/mL in pyridine; Cambridge Isotopes). The samples were vortexed, sonicated, transferred to autoinjection vials and incubated at room temperature for 30mins. Subsequent to this, 70uL of N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA; Sigma) was added and the samples were incubated at 70°C for 30mins. GC/MS analysis was performed using an Agilent 5975C GC/MS equipped with a DB-5MS+DG capillary column. 1uL of sample was injected onto the GC column in splitless mode with an inlet temperature of 280°C and the data was collected by electron impact set at 70 eV. Helium was used as the carrier gas with a flow rate of 1.55 mL/min. The quadrupole was set at 150°C and the GC/MS interface at 285°C. The oven program for all metabolite analyses started at 60°C held for 1 min, then increasing at a rate of 10°C/min until 320°C. Bake-out was at 320°C for 10 mins and the sample data were acquired in scan mode (1-600 m/z).

The data was analyzed using Agilent MSD-Chemstation interfaced to the NIST11 library. Metabolite identities were further cross-validated to standards (Sigma) run in-house on the same instrument. Mass isotopomer analysis was used to derive the flux data employing in-house algorithms to take into account ¹³C natural abundances.

- 5 Integrated ion intensities are reported relative to the myristic acid-D27 internal standard.

Pharmacologic Compounds

- Oligomycin, Etomoxir, Diethyl succinate and Calcium-D Pantothenate were purchased from Sigma, and Coenzyme A Trilithium Salt (CoA) and HIF-inhibitor (CAS 934593-90-5) were purchased from EMD Millipore. Oligomycin (100 nm) was added to CD8+ T cells 24 hours after the start of activation, while Etomoxir (40 μM), Pantothenate (8 mM), Diethyl Succinate (14 mM) and HIF-Inhibitor (40 μM) were added at the start of activation. CoA was added at the start of activation unless otherwise indicated at a concentration of 2.5-3.5 mM depending on the lot.
- 10

15 RNA Extraction and Sequencing

- RNA was extracted from CD8+ T cells activated for three days in the presence of CoA or Vehicle using Qiagen RNEasy kit. Libraries were prepared using TruSeq Stranded Total RNA kit. Two hundred nanograms from RNA samples were ribosomal RNA depleted using Ribo-zero Gold rRNA beads, following purification the RNA was fragmented. The cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers. This is followed by second strand cDNA synthesis using RNase H and DNA Polymerase I. A single "A" based were added and adapter ligated followed by purification and enrichment with PCR to create cDNA libraries. Final cDNA libraries were size validated using Agilent Bioanalyzer and concentration validated by qPCR. All libraries were normalized to 10nM and pooled together. 10pM of pooled libraries were loaded onto Illumina cBot for cluster generation. Clustered flow cell was then sequenced Pair-end 100 cycles V3 using Illumina HighSeq 2000 to achieve ~30 million reads per sample.
- 20
- 25

RNA Sequencing Data Analysis

- 30 The Tophat (2.0.8b) software suite with Bowtie (2.0.5) was used to align reads to the Mus musculus mm10 mouse genome (igenome). RNA_seqc (1.1.7) was used to

assess the quality of the aligned data and depletion based on median coverage across transcript length and identities of top expressed transcripts. Samtools (0.1.18) was used to merge aligned technical replicates and sort alignment files. The cufflinks (2.2.1) software suite was used to quantify alignments. Cuffquant was used to quantify
5 individual sample alignments and Cuffnorm was used to normalize quantified data for each group of biological replicates. Cuffdiff was used in conjunction with the R (3.2.2) library CummeRbund (2.10.0) to explore the data at replicate and grouping levels. A custom python script was used to subset data based on an FPKM threshold of 1, and to apply additional thresholds as indicated. GO term analysis was performed using the
10 database for annotation, visualization and integrated discovery (DAVID) bioinformatics resources (30, 31).

The RNA sequencing analysis performed for the figures comparing Tc1, Tc17 and Tc22 was done as previously described (St. Paul et al., submitted).

Tumors and DSS Colitis

15 For anti-tumor experiments, 8-12 week old female C57BL/6 mice were randomized into different cohorts and inoculated subcutaneously with 4×10^5 B16F10-gp33 cells and received 0.5×10^6 polarized CD8⁺ P14 T cells by tail vein infusion 10 days later. Tumor size was continually assessed using calipers until mice reached experimental endpoint (diameter ≥ 1.5 cm or severe ulceration/necrosis). Upon death, mice were
20 given a tumor size of 225 mm² representing the maximum endpoint value of 1.5 cm x 1.5 cm.

For DSS-colitis experiments, 8-12 week old female C57BL/6 mice were randomized into different cohorts and colitis was induced using 2.5% DSS (MP Bio) added to the drinking water of mice for 5 consecutive days. CoA (0.5 mg/mL) or vehicle control was
25 added to the drinking water on day 0. On day 5, mice were switched to fresh water without any DSS added, supplemented with either CoA (0.5 mg/mL) or vehicle control.

Human T cell experiments

Peripheral blood mononuclear cells were obtained from healthy donors following institutional review board approval. Written informed consent was obtained from all
30 donors who provided the samples. PBMCs were magnetically sorted for naïve T cells (Miltenyi Biotec) and seeded into a 96-well plate previously coated with 5 µg/mL anti-

CD3 (eBioscience, clone OKT3). To induce Tc22 polarization, the following antibodies and cytokines were added to culture: 1 µg/mL anti-CD28 (eBioscience, clone CD28.2), 5 µg/mL anti-IFN γ (BioLegend, clone B27), 5 µg/mL anti-IL-4 (BioLegend, clone 8D4-8), 5 µg/mL anti-TGF β (eBioscience, clone 1D11.16.8), 20 ng/mL IL-6, 10 ng/mL IL-21, 10 ng/mL IL-23, 40 ng/mL TNF- α (All from BioLegend) and 2 ng/mL FICZ. Pantothenate (8 mM) or vehicle control was also added on day 0. Five days later, cells were stimulated with PMA/ionomycin + Brefeldin for 5-6 hours. Cells were then stained and analyzed for intracellular cytokines by flow cytometry.

Statistical Analysis

Statistical significance was calculated using Graphpad Prism as indicated in the figure legends. $p < 0.05$ was considered statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

RESULTS AND DISCUSSION

To start, we differentiated Tc1, Tc17 and Tc22 cells *in vitro* (**Figure 1A**) and employed an untargeted global metabolomics approach to interrogate their metabolic profiles (**Figure 1B**). The intracellular abundance of over 400 metabolites in each Tc subset was quantified using established liquid chromatography-mass spectrometry protocols (17). Using principal component analysis (PCA) and unsupervised hierarchical cluster analysis, we found that each Tc subset formed a unique cluster with Tc17s being the most metabolically distinct, while Tc1s and Tc22s appeared more similar to each other (**Figure 1B,C**). Each Tc subset, however, was clearly separate as there were at least 100 metabolites whose levels were significantly altered between each individual subset (**Figure 1D**). Assessing the major macromolecular components of metabolism, there were fewer differences in amino acid metabolism between the Tc subsets (**Figure 5**) relative to the differences in nucleotide and carbohydrate metabolism (**Figure 6**). Some of the most notable differences in metabolites were in the levels of glycolytic and tricarboxylic acid (TCA) cycle intermediates, despite having minimal alterations of these pathways at the transcript levels (**Figure 6**). Thus, these data confirmed a unique metabolic profile for Tc1, Tc17 and Tc22 cells and suggested that differences in glycolytic and oxidative metabolism may underpin the Tc1, Tc17 and Tc22 phenotypes.

To further characterize these observed differences in each Tc subset, we analyzed their respective utilization of glycolysis versus OXPHOS using the Seahorse Bioanalyzer. This method allows the concurrent measurement of the oxygen consumption rate (OCR – indicative of OXPHOS) and the extracellular acidification rate (ECAR – indicative of glycolysis) in real-time. Interestingly, we found that the Tc22 subset had the highest OCR rates and the highest ratio of OCR to ECAR amongst the three subsets (**Figure 1E**), similar to what has previously been reported for memory CD8⁺ T cells relative to activated effector cells (11). Consistent with the increased ATP yield of oxidative metabolism compared to glycolysis, Tc22s had the highest amounts of intracellular ATP (**Figure 1F**). We also observed metabolites involved in the TCA cycle and OXPHOS, particularly succinate, to be increased in Tc22s (**Figure 6**).

Given the increased utilization of OXPHOS by Tc22s, we tested whether Tc22 polarization was dependent on this metabolic shift. After activating cells in Tc22 conditions in the presence of oligomycin, an inhibitor of oxidative metabolism, we noted a decrease in Tc22 polarization (**Figure 1G**). In contrast, oligomycin did not affect Tc17 polarization but did enhance Tc1 polarization, which is consistent with published reports linking glycolytic metabolism to the production of IFN- γ (5) (**Figure 1G**). Interestingly, treatment with the carnitine palmitoyltransferase 1a (CPT1a) inhibitor etomoxir had no effect on Tc22 polarization, indicating that Tc22 polarized cells do not depend on fatty acid oxidation (FAO) to fuel OXPHOS (**Figure 7**). This distinguishes the metabolic program of Tc22s from other oxidative T cell lineages, such as CD8⁺ memory cells and CD4⁺ T-regs, which have been reported to rely on FAO for their differentiation (8–10). Collectively, these data demonstrate that enhanced utilization of oxidative metabolism is a distinct metabolic phenotype of Tc22 cells as compared to Tc1 and Tc17 effector cells.

To further investigate the metabolic profile of Tc22s, we performed metabolic pathway analysis and found the most enriched pathway to be that of pantothenate/ coenzyme A (CoA) (**Figure 2A**). This pathway produces the metabolic cofactor CoA from pantothenate through a series of metabolic intermediates. Many of these intermediates were downregulated in Tc22s, while CoA itself was elevated, thereby suggestive of an increased flux of pantothenate to generate CoA (**Figure 2B**). Hypothesizing that pantothenate may therefore facilitate Tc22 differentiation, we added exogenous pantothenate during Tc22 polarization and noted enhanced polarization in

both mouse (**Figure 2C**) and human (**Figure 2D**) CD8⁺ cells. This indicated that the pantothenate pathway can be targeted to enhance Tc22s and IL-22 production.

As several steps are required to generate CoA from pantothenate, we wondered whether targeting the pathway downstream of pantothenate may prove to be more effective, as this may bypass any rate-limiting steps. CoA itself provides an attractive target in this context, given that recent evidence indicates that cells can uptake extracellular CoA (18). Indeed, we found that the addition of exogenous CoA during Tc22 polarization greatly enhanced polarization, more so than what we observed for pantothenate (**Figure 2E**). Surprisingly, we found that CD8⁺ T cells activated in the presence of CoA alone skewed primarily towards an IL-22⁺ IL-17⁻ IFN γ ⁻ population in the absence of polarizing cytokines (**Figure 2F,G**). This was dependent on the direct interaction of CoA with the T cells, and not the antigen presenting cells (APC) used to activate the T cells, as CoA induced and enhanced Tc22 polarization in an APC-free system (**Figure 8**). CoA also functions independent of IL-6, the cytokine that drives the Tc22 lineage, as CoA retained the ability to induce Tc22s despite the presence of an IL-6 neutralizing antibody (**Figure 2H**).

To further confirm that CoA was inducing the full Tc22 phenotype, we evaluated the cytokine profile of CoA treated cells and found them to primarily up-regulate IL-22 and none of the other Tc subset lineage-defining cytokines (**Figure 2I**). We also performed RNA sequencing on CoA treated cells to determine if they resembled cytokine-induced Tc22s at the level of the transcriptome. Indeed, vast majority of genes up-regulated by Tc22 polarizing cytokines, the "Tc22 signature", were also strongly up-regulated by CoA alone (**Figure 2J**). These genes include granzyme C and D, which we previously observed to be expressed highly by Tc22s relative to other Tc subsets (St. Paul et al., submitted). Together, these findings establish that CoA acts on CD8⁺ T cells to induce the Tc22 phenotype both at a functional and transcriptional level. To our knowledge, we have discovered the first instance in which adding a pharmacologic compound can induce a T cell subset without the need for any polarizing cytokines.

We found that the enhanced utilization of OXPHOS was a defining metabolic requirement for cytokine-induced Tc22 cells (**Figure 1**). Similar to polarizing cytokines (**Figure 6**), however, CoA treatment primarily altered the expression of immune system genes and not metabolic genes (**Figure 9**). Therefore, by transcriptional analysis, it was unclear if CoA treatment resulted in the same metabolic shift to

OXPHOS required by polarizing cytokines to induce the Tc22 lineage. Metabolic analysis of CoA treated cells revealed that they also had a higher basal OCR and a lower basal ECAR compared to vehicle controls (**Figure 3A**). This resulted in a high OCR:ECAR ratio for the CoA-treated cells (**Figure 3B**). Treatment with CoA also increased intracellular levels of ATP relative to vehicle treated controls (**Figure 3C**). These data indicated that CoA promoted an oxidative phenotype similar to what we had described for cytokine-induced Tc22s.

To further characterize this oxidative phenotype of the CoA treated cells, we employed stable isotope tracer analysis (SITA) with ^{13}C -glucose to track glucose utilization by the cells. We found that CoA treatment resulted in enhanced incorporation of the ^{13}C label into TCA metabolites, consistent with an increased flux of glucose into the TCA cycle (**Figure 3D**). Interestingly, CoA treatment also increased total intracellular pools of many of the TCA intermediates, including succinate. It has previously been demonstrated that succinate can stabilize and activate the transcription factor hypoxia inducible factor (HIF)-1 α under normal oxygen tensions (19, 20), and that activation of HIF-1 α can promote IL-22 production in CD4 $^{+}$ T cells (21). Thus, we hypothesized that the mechanistic link between OXPHOS and the induction of Tc22s was the succinate-induced activation of HIF-1 α signaling in an oxidative environment downstream of CoA or polarizing cytokines. Consistent with this hypothesis, we noted enhanced IL-22 production in CD8 $^{+}$ T cells activated in the presence of succinate (**Figure 3E**). Moreover, CoA treated CD8 $^{+}$ T cells had substantially increased levels of HIF-1 α (**Figure 3F**), and blocking HIF-1 α activity inhibited the ability of CoA to induce Tc22s (**Figure 3G**). Furthermore, we demonstrated that the activation of CD8 $^{+}$ T cells that constitutively express HIF-1 α (22) induced IL-22 production in the absence of polarizing cytokines (**Figure 3H**). Together, these findings indicated that the oxidative metabolic phenotype induced by CoA is required to generate succinate and activate HIF-1 α signaling to facilitate the polarization of Tc22s.

Having found that the addition of CoA could polarize naïve cells to Tc22s during activation, we investigated the effects of CoA on previously activated CD8 $^{+}$ T cells. The addition of CoA during a secondary re-stimulation of CD8 $^{+}$ T cells previously activated in the absence of polarizing cytokines was able to induce IL-22 production and promote an oxidative phenotype (**Figure 10**). The effects of CoA were not only limited to CD8 $^{+}$ T cells, as we also demonstrated that CD4 $^{+}$ Th22s are dependent on OXPHOS for polarization (**Figure 11A**) and that CoA similarly induced and enhanced

Th22 polarization by providing a metabolic shift towards OXPHOS and enhanced ATP production (**Figure 11B-F**). Together, these findings demonstrate the versatility of CoA and its ability to induce OXPHOS and IL-22 producing T cell subsets in different contexts.

5 Recently, it has been reported that CD8⁺ T cells with an increased capacity for OXPHOS are superior at mediating anti-tumor responses (23–25). Thus, we evaluated the role of CoA-treated cells in an *in vivo* mouse tumor model. Tumor-specific P14 CD8⁺ T cells, activated for three days in the presence of vehicle or CoA, were transferred into mice inoculated with B16-gp33 melanoma tumors. Mice that received
10 CoA treated P14s demonstrated a significantly greater reduction in tumor growth compared to animals that received activated T cells treated with vehicle (**Figure 4A**). We also tested CoA in a dextran sulfate sodium (DSS) colitis model (**Figure 4B**) in which IL-22 has been shown to be protective (26). Indeed, mice administered oral CoA demonstrated a decrease in disease severity, suggesting that CoA has the potential
15 for *in vivo* therapeutic applications (**Figure 4C,D**).

Overall, we have demonstrated that Tc1, Tc17 and Tc22 CD8⁺ T cells subsets have distinct metabolic signatures. By further interrogating these metabolic differences, we found that a key distinction of Tc22 polarization is the requirement for OXPHOS. Importantly, we identified CoA as a molecule capable of promoting and enhancing
20 Tc22 and Th22 polarization as a stand alone agent, mediated in part by oxidative HIF-1 α signaling. Although previous studies have modified T cell lineage commitment by altering metabolism, these observations were still dependent on the presence of polarizing cytokines (13, 14). Here, we show that CoA alone is sufficient to induce Tc22 or Th22 generation. These data represent a paradigm shift in the generation of
25 specific T cell subsets, as we suggest that the selective manipulation of cellular metabolism is sufficient to drive the induction of the Tc22 subset with potential therapeutic applications to treat diseases.

Although preferred embodiments of the invention have been described herein, it will be understood by those skilled in the art that variations may be made thereto without
30 departing from the spirit of the invention or the scope of the appended claims. All documents disclosed herein, including those in the following reference list, are incorporated by reference.

Reference List

1. N. J. MacIver, R. D. Michalek, J. C. Rathmell, Metabolic regulation of T lymphocytes. *Annu. Rev. Immunol.* 31, 259–83 (2013).
2. E. L. Pearce, M. C. Poffenberger, C.-H. Chang, R. G. Jones, Fueling immunity: insights into metabolism and lymphocyte function. *Science.* 342, 1242454 (2013).
3. Y. Cao, J. C. Rathmell, A. N. Macintyre, Metabolic reprogramming towards aerobic glycolysis correlates with greater proliferative ability and resistance to metabolic inhibition in CD8 versus CD4 T cells. *PLoS One.* 9, 1–15 (2014).
4. R. Wang et al., The Transcription Factor Myc Controls Metabolic Reprogramming upon T Lymphocyte Activation. *Immunity.* 35, 871–882 (2011).
5. C. H. Chang et al., Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell.* 153, 1239–1251 (2013).
6. P.-C. Ho et al., Phosphoenolpyruvate Is a Metabolic Checkpoint of Anti-tumor T Cell Responses. *Cell.* 162, 1217–1228 (2015).
7. J. Blagih et al., The Energy Sensor AMPK Regulates T Cell Metabolic Adaptation and Effector Responses In Vivo. *Immunity.* 42, 41–54 (2015).
8. R. D. Michalek et al., Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J. Immunol.* 186, 3299–303 (2011).
9. D. O'Sullivan et al., Memory CD8(+) T Cells Use Cell-Intrinsic Lipolysis to Support the Metabolic Programming Necessary for Development. *Immunity.* 41, 75–88 (2014).
10. E. L. Pearce et al., Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature.* 460, 103–107 (2009).
11. G. J. W. van der Windt et al., Mitochondrial Respiratory Capacity Is a Critical Regulator of CD8+ T Cell Memory Development. *Immunity.* 36, 68–78 (2012).

12. L. Z. Shi et al., HIF1 α -dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J. Exp. Med.* 208, 1367–76 (2011).
13. V. A. Gerriets et al., Metabolic programming and PDHK1 control CD4⁺ T cell subsets and inflammation. *J. Clin. Invest.* 125, 194–207 (2015).
14. L. Berod et al., De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat. Med.* 20, 1327–1333 (2014).
15. C. S. Hinrichs et al., Type 17 CD8⁺ T cells display enhanced antitumor immunity. *Blood*. 114, 596–9 (2009).
- 10 16. H.-W. Mittrücker, A. Visekruna, M. Huber, Heterogeneity in the differentiation and function of CD8⁺ T cells. *Arch. Immunol. Ther. Exp. (Warsz)*. 62, 449–58 (2014).
17. S.-Y. Shin et al., An atlas of genetic influences on human blood metabolites. *Nat. Genet.* 46, 543–50 (2014).
18. B. Srinivasan et al., Extracellular 4'-Phosphopantetheine is a source for intracellular Coenzyme A synthesis. *Nat. Chem. Biol.* 11, 1–26 (2015).
- 15 19. G. M. Tannahill et al., Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature*. 496, 238–242 (2013).
20. M. A. Selak et al., Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- α prolyl hydroxylase. *Cancer Cell*. 7, 77–85 (2005).
- 20 21. S. A. Budda, A. Girton, J. G. Henderson, L. A. Zenewicz, Transcription Factor HIF-1A Controls Expression of the Cytokine IL-22 in CD4 T Cells. *J. Immunol.* 197, 2646–2652 (2016).
22. W. Y. Kim et al., Failure to prolyl hydroxylate hypoxia-inducible factor α phenocopies VHL inactivation in vivo. *EMBO J.* 25, 4650–62 (2006).
- 25 23. N. E. Scharping et al., The Tumor Microenvironment Represses T Cell Mitochondrial Biogenesis to Drive Intratumoral T Cell Metabolic Insufficiency and Dysfunction. *Immunity*. 45, 374–388 (2016).

24. J. G. Crompton et al., Akt inhibition enhances expansion of potent tumor-specific lymphocytes with memory cell characteristics. *Cancer Res.* 75, 296–305 (2015).
25. M. D. Buck et al., Mitochondrial Dynamics Controls T Cell Fate through
5 Metabolic Programming. *Cell.* 166, 63–76 (2016).
26. L. A. Zenewicz et al., Innate and Adaptive Interleukin-22 Protects Mice from Inflammatory Bowel Disease. *Immunity.* 29, 947–957 (2008).
27. H. Pircher, K. Bürki, R. Lang, H. Hengartner, R. M. Zinkernagel, Tolerance
10 induction in double specific T-cell receptor transgenic mice varies with antigen. *Nature.* 342, 559–561 (1989).
28. A. Oxenius, M. F. Bachmann, R. M. Zinkernagel, H. Hengartner, Virus-specific MHC-class II-restricted TCR-transgenic mice: effects on humoral and cellular immune responses after viral infection. *Eur. J. Immunol.* 28, 390–400 (1998).
29. D. Dissanayake et al., Nuclear factor- κ B1 controls the functional maturation of
15 dendritic cells and prevents the activation of autoreactive T cells. *Nat. Med.* 17, 1663–7 (2011).
30. D. W. Huang, B. T. Sherman, R. A. Lempicki, Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* 37, 1–13 (2009).
- 20 31. D. W. Huang, B. T. Sherman, R. A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4, 44–57 (2009).

CLAIMS:

1. A method for inducing Tc22 lineage T cells from a population of CD8⁺ T cells, the method comprising:
 - a) providing a population of CD8⁺ T cells;
 - 5 b) activating the population; and
 - c) culturing or contacting the population of CD8⁺ T cells with Coenzyme A.
2. The method of claim 1, wherein the activation comprises culturing or contacting the population with at least one of (i) anti-CD3 antibody and (ii) gp33 peptide from LCMV (KAVYNFATM).
- 10 3. The method of claim 1 or 2, wherein the population of CD8⁺ T cells is additionally cultured or contacted with TNF- α or IL-6.
4. The method of any one of claims 1 to 3, wherein the population of CD8⁺ T cells is additionally cultured or contacted with aryl hydrogen receptor (AhR) agonist, preferably 6-Formylindolo(3,2-b)carbazole (FICZ).
- 15 5. The method of any one of claims 1 to 4, wherein the population of CD8⁺ T cells is additionally cultured or contacted with anti-IFN γ antibody.
6. The method of any one of claims 1 to 5, wherein the population of CD8⁺ T cells is additionally cultured or contacted with anti-TGF- β antibody.
7. The method of any one of claims 1 to 5, wherein the population of CD8⁺ T cells is additionally cultured or contacted with succinate.
- 20 8. The method of any one of claims 1 to 5, wherein the population of CD8⁺ T cells is additionally cultured or contacted with pantothenate.
9. The method of any one of claims 1 to 8, wherein the Tc22 lineage T cells are CD8⁺/IL-22⁺/IL17⁻/IFN γ ^{low}.
- 25 10. The method of claim 9, wherein the Tc22 lineage T cells are additionally IL-4⁻, IL-5⁻, IL-9⁻, IL-10⁻ and/or or IL-13⁻.

11. The method of claim 9 or 10, wherein the Tc22 lineage T cells are additionally TNF- α^{hi} and/or IL-2 ^{hi} .
12. The method of any one of claims 1-11, wherein step c) is performed before or after step b).
- 5 13. The method of any one of claims 1-11, wherein step c) is performed simultaneously with step b).
14. A population of cells comprising Tc22 lineage T cells that are CD8 $^{+}$ /IL-22 $^{+}$ /IL17 $^{-}$ /IFN γ^{low} .
- 10 15. A population of Tc22 lineage T cells produced by the method of any one of claims 1-13.
16. The population of claim 13 or 14, for use in the treatment of cancer, preferably a cancerous tumor.
17. A method of treating cancer, preferably a cancerous tumor, in a patient, the method comprising administering to the patient the population of claim 13 or 15 14.
18. Use of the population of claim 13 or 14, in the manufacture of a medicament for the treatment of cancer, preferably a cancerous tumor.
19. A method of treating cancer, preferably a cancerous tumor, or an inflammatory disease, comprising administering to the patient Coenzyme A or Coenzyme A 20 treated Tcells.
20. Use of Coenzyme A or Coenzyme A treated Tcells for the treatment of cancer, preferably a cancerous tumor, or an inflammatory disease.
21. Use of Coenzyme A or Coenzyme A treated Tcells in the manufacture of a medicament for the treatment of cancer, preferably a cancerous tumor, or an 25 inflammatory disease.
22. The method of claim 19, or the use of claims 20 or 21, wherein the inflammatory disease is a gastro-intestinal inflammatory disease, preferably colitis or inflammatory bowel disease.

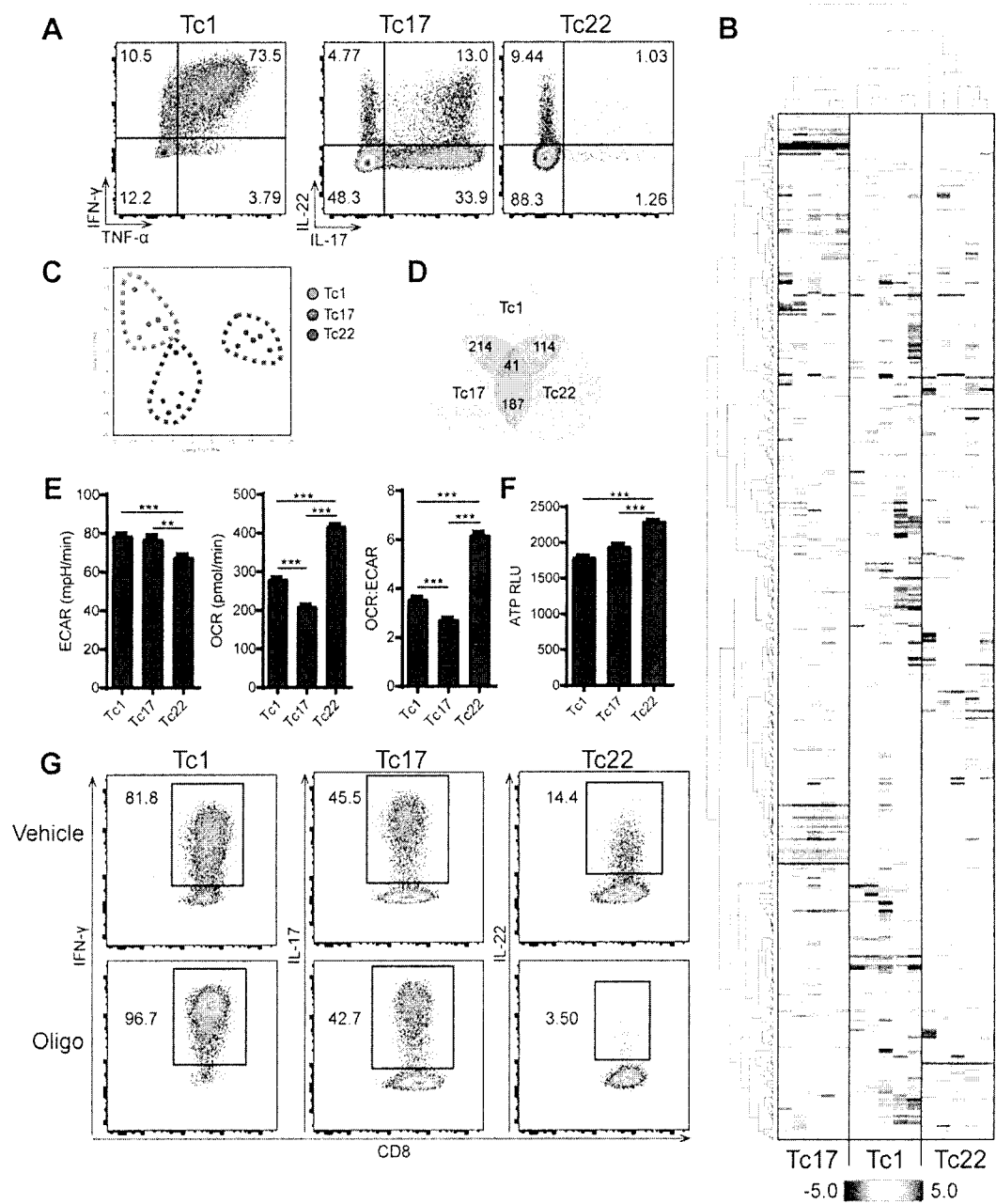


Figure 1

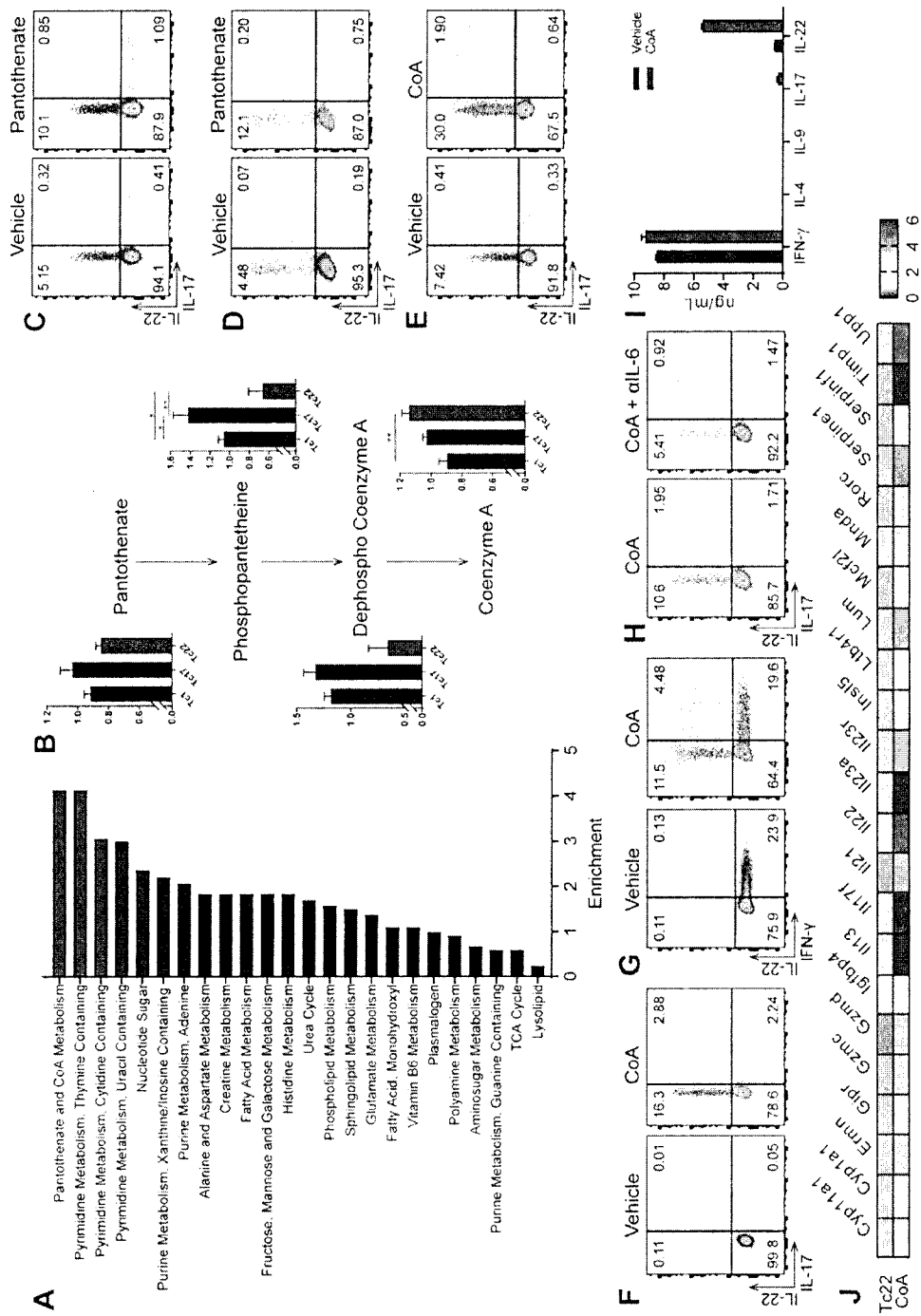


Figure 2

Figure 3

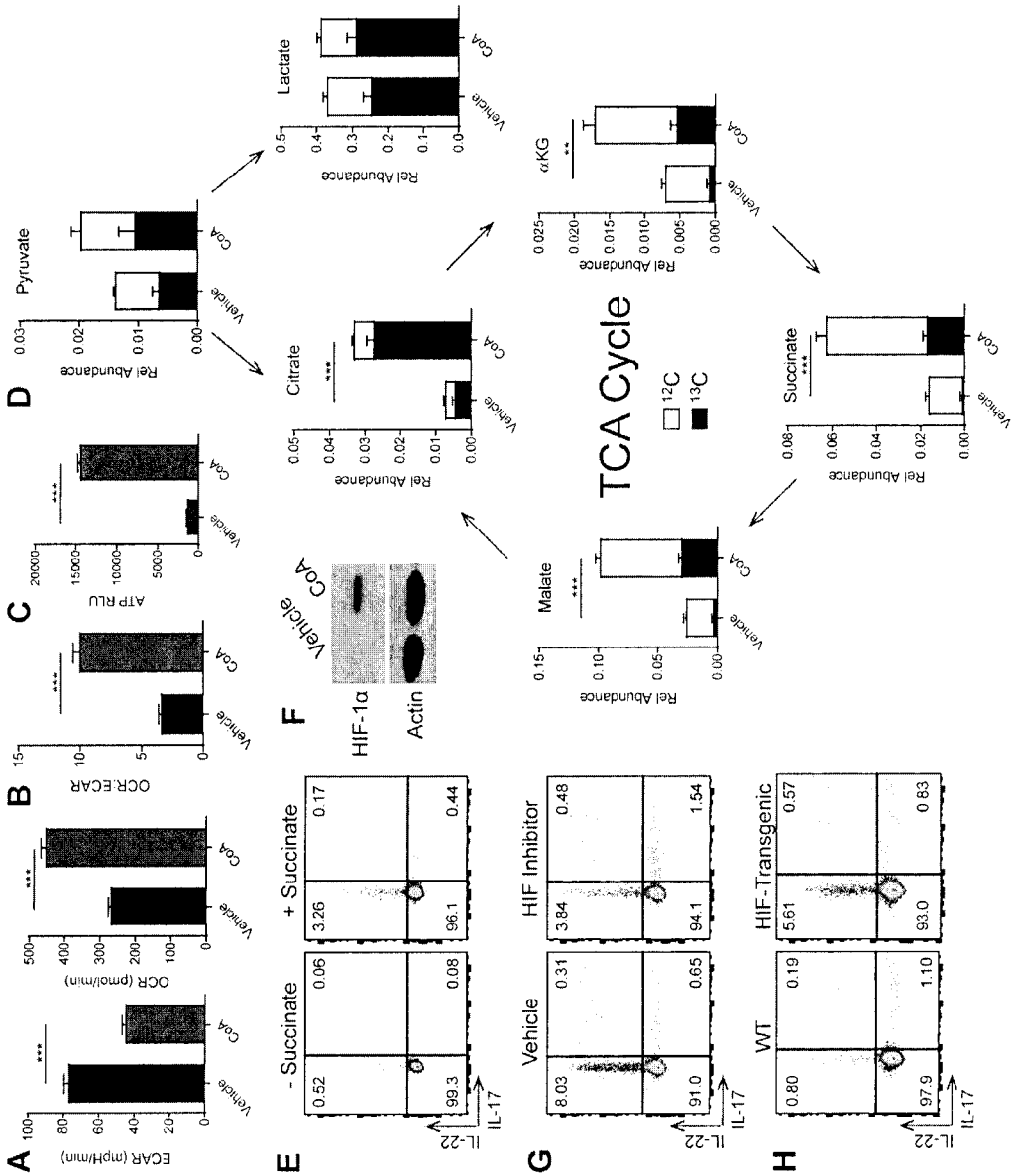


Figure 4

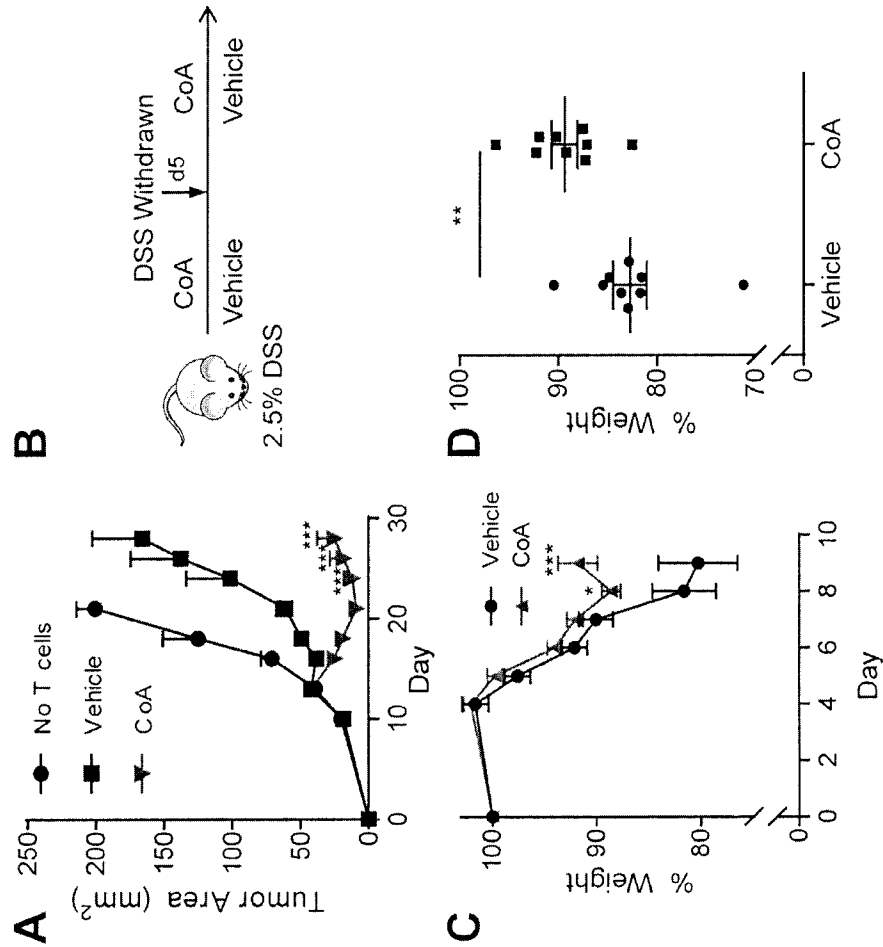


Figure 5

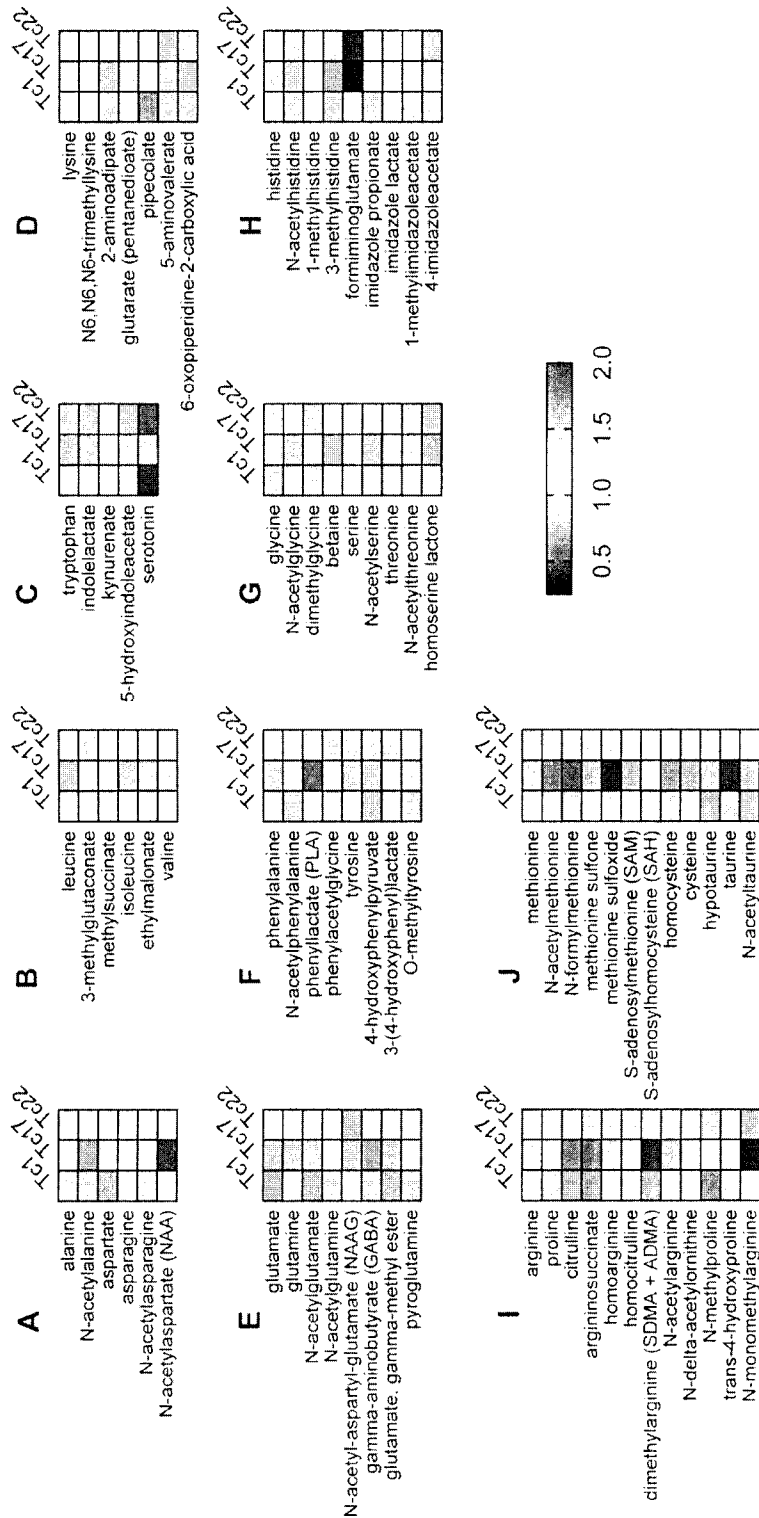


Figure 6

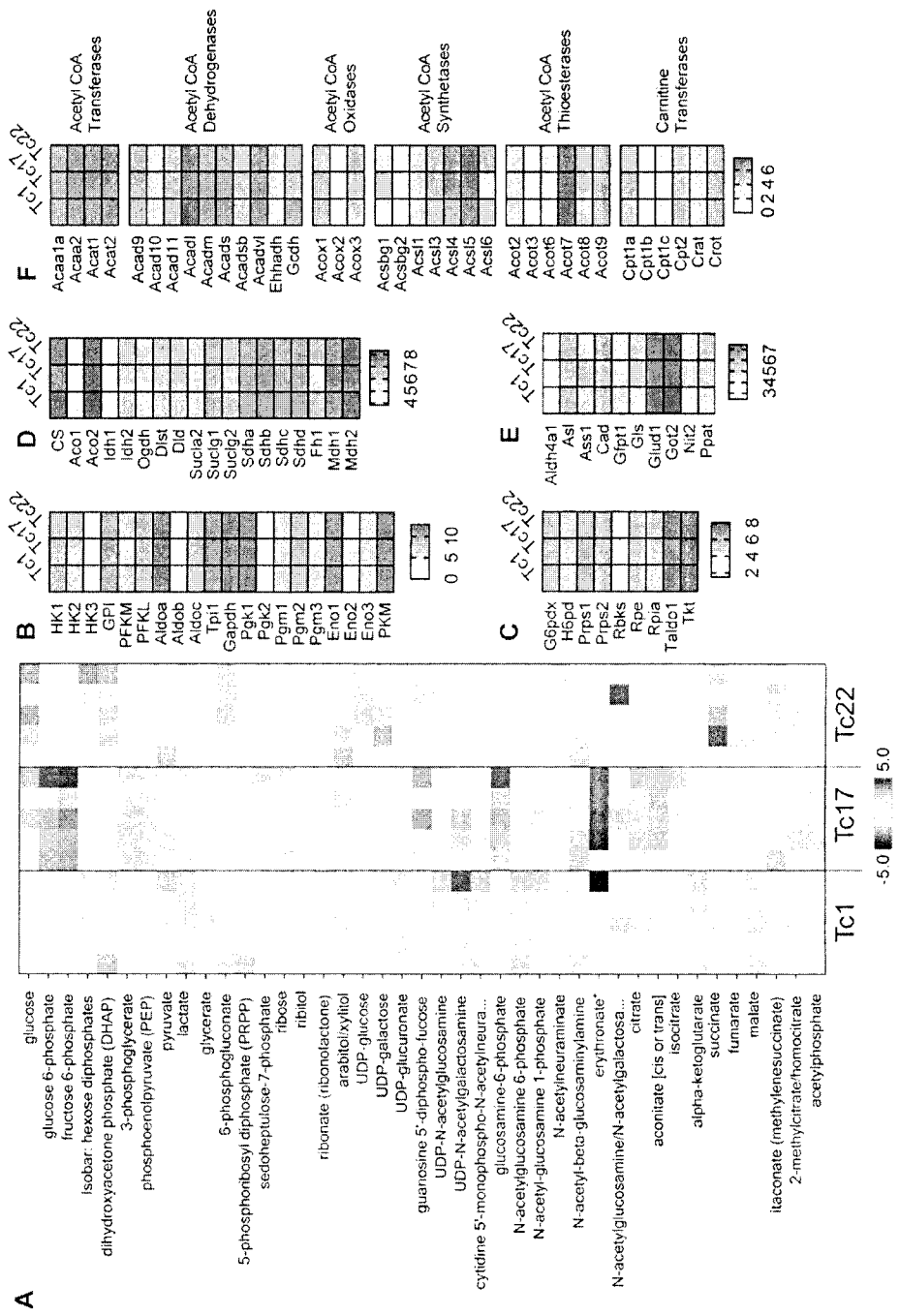


Figure 7

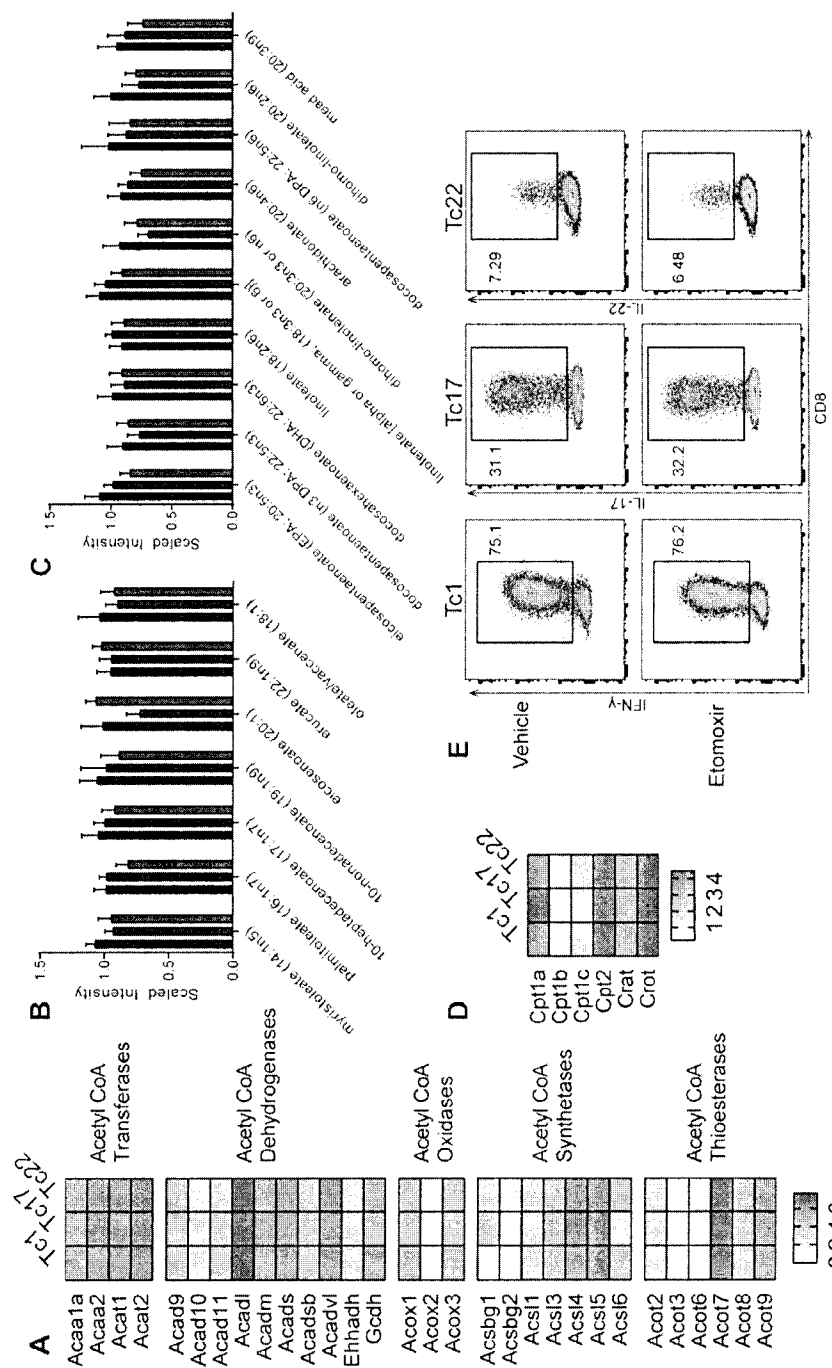


Figure 8

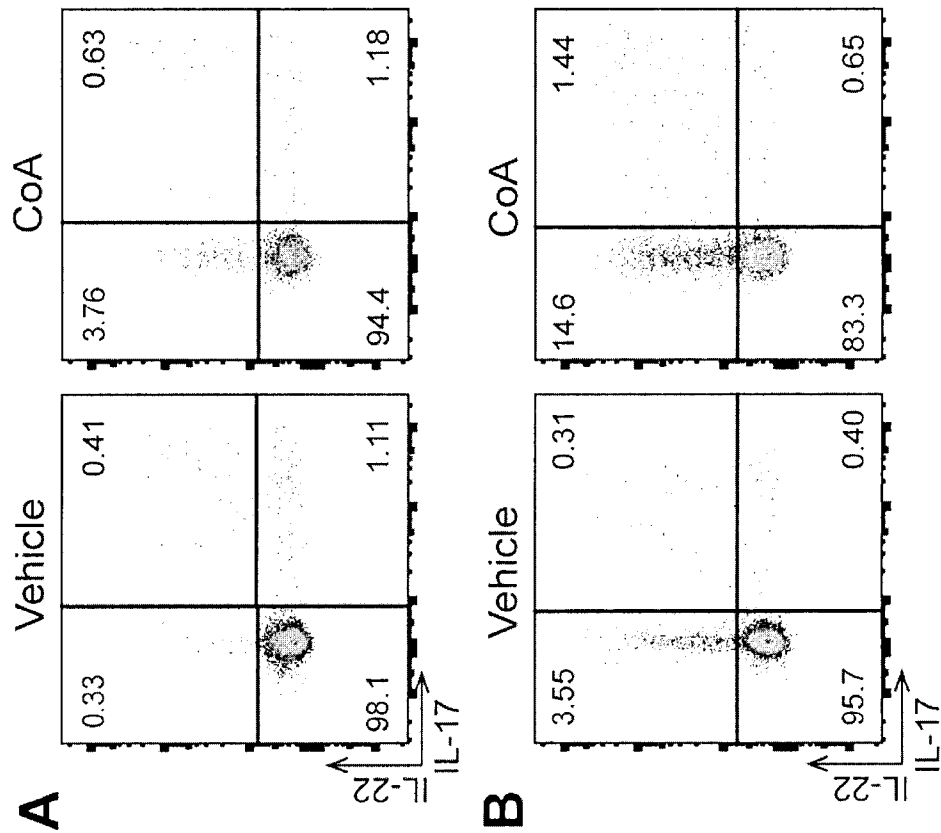


Figure 9

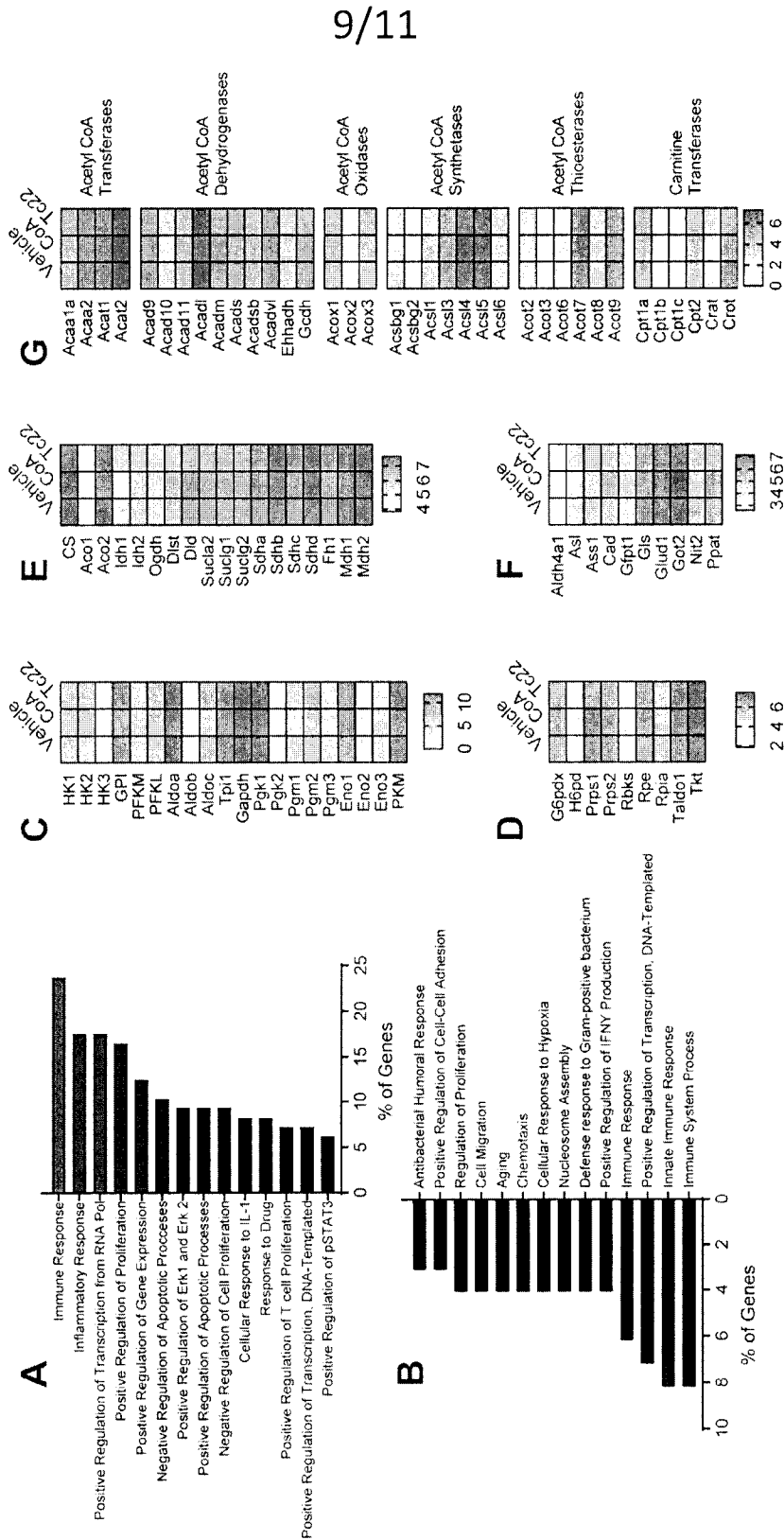


Figure 10

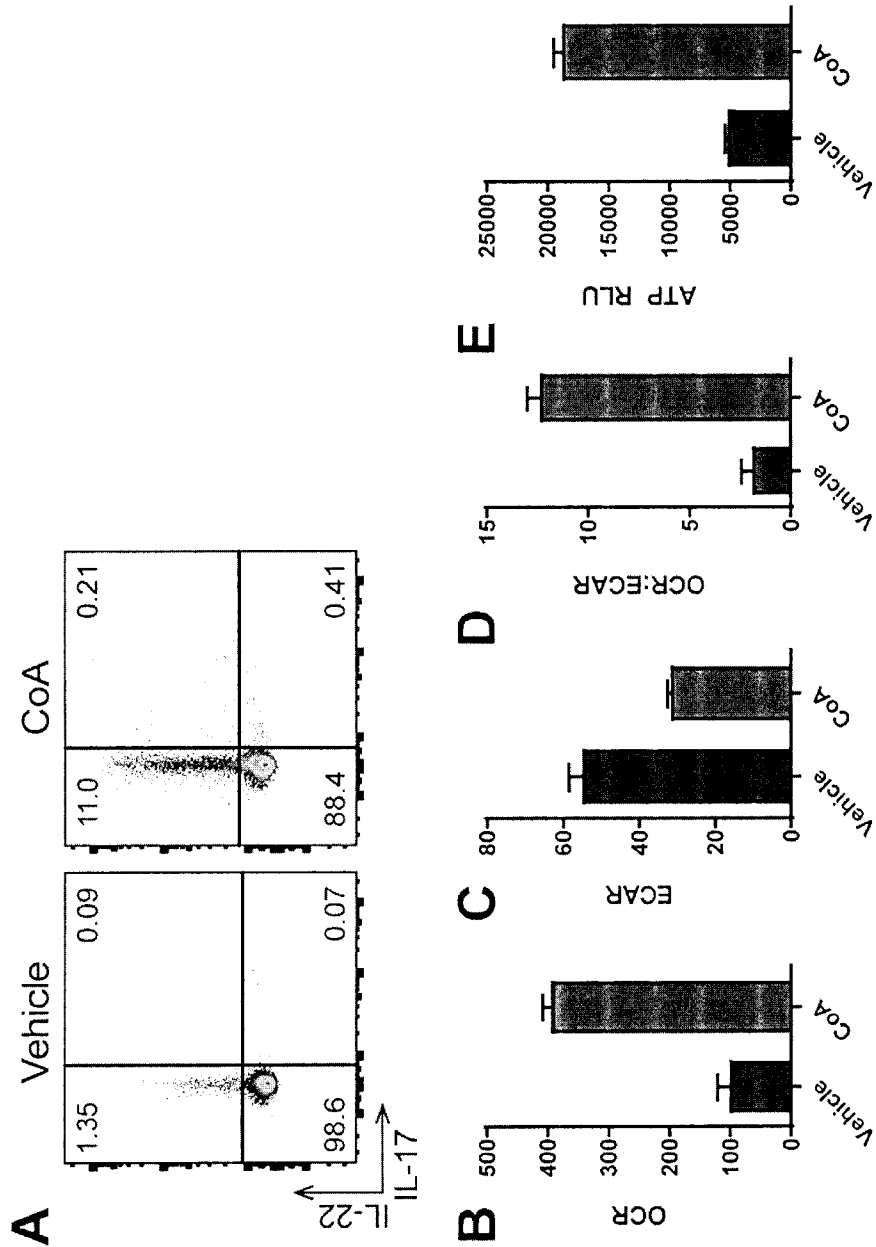
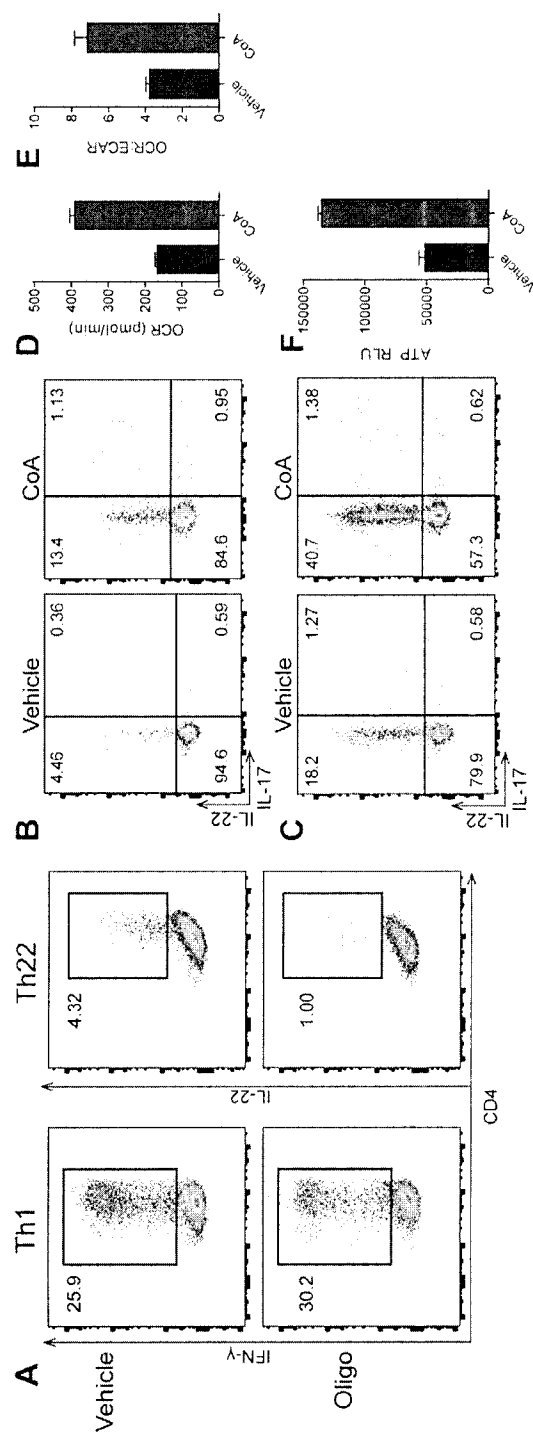


Figure 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/000274

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C12N 5/0783** (2010.01), **A61K 31/7076** (2006.01), **A61K 35/17** (2015.01), **A61P 1/00** (2006.01), **A61P 35/00** (2006.01), **C07H 19/20** (2006.01) (more IPCs on the last page)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 5/0783 (2010.01), **A61K 31/7076** (2006.01), **A61K 35/17** (2015.01), **A61P 1/00** (2006.01), **A61P 35/00** (2006.01), **C07K 14/525** (2006.01), **C07K 14/54** (2006.01), **C07K 16/22** (2006.01), **C07K 16/28** (2006.01), **C07H 19/20** (2006.01)

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

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

Databases: Canadian Patent Database, PubMed, Orbit Intelligence-Questel

Keywords: CD8+ T cells, Coenzyme A, anti-CD3 antibody, gp33 peptide, Tc22, cancer, inflammatory disease

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PIETER et al.: 'Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis', PLoS ONE, November 2010, Volume 5, Issue 11, pages 1-11, ISSN 1932-6203 the whole document	14, 15
X	WO200112200 A1 (REN AND FEI) 22 February 2001 (22-02-2001) the whole document	19-22
X	CN102397259 A (YONGJIE ET AL.) 4 April 2012 (04-04-2012) the whole document	19-21
A	LIU et al.: 'Interleukin-21 induces the differentiation of human Tc22 cells via phosphorylation of signal transducers and activators of transcription', IMMUNOLOGY, April 2011, Volume 132, pages 540-548, ISSN 1365-2567 the whole document	1-13, 16-18

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date 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) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	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 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 document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
--------------------------------------	--	--------------------------	--

Date of the actual completion of the international search
08 March 2018 (09-03-2018)

Date of mailing of the international search report
28 March 2018 (28-03-2018)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Keely Ingrey (819) 639-7697

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/CA2017/000274

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO200112200 A1	22 February 2001 (22-02-2001)	JP2001064184 A AU6553500 A CN1092986 C JP4484338 B2 US6509035 B1 WO200112200 A1	13 March 2001 (13-03-2001) 13 March 2001 (13-03-2001) 23 October 2002 (23-10-2002) 16 June 2010 (16-06-2010) 21 January 2003 (21-01-2003) 22 February 2011 (22-02-2001)
CN102397259 A	4 April 2012 (04-04-2012)	CN102397259 A	4 April 2012 (04-04-2012)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/000274

C07K 14/525 (2006.01), *C07K 14/54 (2006.01)*, *C07K 16/22 (2006.01)*, *C07K 16/28 (2006.01)*