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(54) Title: INHIBITION OF ACETYL-COA METABOLISM FOR TREATMENT AND PREVENTION OF IMMUNE SYSTEM DISEASES AND DISORDERS

(57) Abstract: Provided are methods for treating or preventing T cell-mediated diseases or disorders by administering an inhibitor of acetyl-CoA production, such as an inhibitor of lactate dehydrogenase A or an inhibitor of ATP-citrate lyase.



**INHIBITION OF ACETYL-CoA METABOLISM FOR TREATMENT AND
PREVENTION OF IMMUNE SYSTEM DISEASES AND DISORDERS**

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/394,859, filed on September 15, 2016, the entire contents of which are incorporated by reference.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under grant number CA008748, awarded by the National Institutes of Health. The government has certain rights in the invention.

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BACKGROUND

[0005] T cell activation and differentiation are associated with metabolic rewiring (1-4). A metabolic hallmark of activated T cells is aerobic glycolysis (the Warburg effect) (5), the conversion of glucose to lactate in the presence of oxygen. However, its physiopathological functions remain incompletely understood (6-8). As the major carbon source, glucose plays

important roles in T cell development, proliferation, and function (9-15). Aerobic glycolysis has been implicated in augmenting effector T cell responses, including expression of the pro-inflammatory cytokine interferon (IFN)- γ , via 3' untranslated region (3'UTR)-mediated mechanisms. However, the specific contribution of aerobic glycolysis to T cell responses has not been well defined. Using galactose as a sugar source, aerobic glycolysis was proposed to support IFN- γ expression through 3'UTR-mediated mechanisms (12). Although galactose is metabolized at a slower rate than glucose via the Leloir pathway, both sugars are converted to lactate (16), rendering the galactose system unable to model aerobic glycolysis deficiency in a definitive manner.

[0006] By converting pyruvate to lactate with regeneration of nicotinamide adenine dinucleotide (NAD⁺) (17), lactate dehydrogenase (LDH) defines the biochemical reaction of aerobic glycolysis. LDHA and LDHB form five tetrameric LDH isoenzymes (A₄B₀, A₃B₁, A₂B₂, A₁B₃, and A₀B₄) with distinct kinetic properties (17). ATP-citrate lyase (ACL) is an enzyme converting citrate to acetyl-CoA in the cytosol.

[0007] A better understanding of LDH and ACL activity upon T cell activation could have therapeutic implications for diseases and disorders of the immune system, including autoimmunity and transplant rejection.

SUMMARY OF THE INVENTION

[0008] Some of the main aspects of the present invention are summarized below. Additional aspects are described in the Detailed Description of the Invention, Examples, Drawings, and Claims sections of this disclosure. The description in each section of this disclosure is intended to be read in conjunction with the other sections. Furthermore, the various embodiments described in each section of this disclosure can be combined in various different ways, and all such combinations are intended to fall within the scope of the present invention.

[0009] The inventors show herein that LDHA is induced in activated T cells to support aerobic glycolysis, but promotes IFN- γ expression independently of its 3'UTR. Instead, LDHA maintains high levels of acetyl-CoA to enhance histone acetylation and transcription of *Ifng*. Ablation of LDHA in T cells protects mice from immunopathology triggered by excessive IFN- γ expression or deficiency of regulatory T cells. These findings reveal an epigenetic mechanism by which aerobic glycolysis promotes effector T cell differentiation, and indicate that LDHA and downstream enzymes such as ACL can be targeted

therapeutically in diseases or disorders associated with T cell activation and/or differentiation and/or proliferation, such as autoinflammatory and autoimmune diseases, graft rejection, and transplant rejection.

[0010] Accordingly, the invention provides a method of treating or preventing a T cell-mediated disease or disorder, the method comprising reducing acetyl-CoA production in a subject having or susceptible to developing a T cell-mediated disease or disorder.

[0011] In one aspect, the invention provides a method of treating or preventing a T cell-mediated disease or disorder, the method comprising administering to a subject having or susceptible to developing a T cell-mediated disease or disorder a composition comprising an effective amount of a lactate dehydrogenase A (LDHA) inhibitor. In another aspect, the invention provides a composition comprising an LDHA inhibitor for use in the treatment of a T cell-mediated disease or disorder. The invention also provides a composition comprising an LDHA inhibitor for the manufacture of a medicament for use in the treatment of a T cell-mediated disease or disorder. In some embodiments, the LDHA inhibitor is selected from the group consisting of FX11, Galloflavin, GNE-140, GSK 2837808A, and NHI 2. In a preferred embodiment, the LDHA inhibitor is FX11.

[0012] In a further aspect, the invention provides a method of treating or preventing a T cell-mediated disease or disorder, the method comprising administering to a subject having or susceptible to developing a T cell-mediated disease or disorder a composition comprising an effective amount of an ATP-citrate lyase (ACL) inhibitor. In yet another aspect, the invention provides a composition comprising an ACL inhibitor for use in the treatment of a T cell-mediated disease or disorder. The invention also provides a composition comprising an ACL inhibitor for the manufacture of a medicament for use in the treatment of a T cell-mediated disease or disorder. In some embodiments, the ACL inhibitor is selected from the group consisting of BMS 303141, ETC-1002, and SB 204990. In a preferred embodiment, the ACL inhibitor is BMS 303141.

[0013] In some embodiments, the composition is administered orally. In some embodiments, the composition is administered intravenously.

[0014] In a preferred embodiment, the subject is a human.

[0015] T cell-mediated diseases or disorders that can be treated or prevented by the methods of the invention include, but are not limited to, autoimmune disorders, graft rejection, inflammation, and organ rejection. In certain embodiments, the autoimmune disorder is selected from the group consisting of graft-versus-host disease (GVHD),

inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, rheumatoid arthritis, systemic lupus erythematosus (SLE), and type-1 diabetes. In one embodiment, the composition is administered prophylactically within about 24 hours of an organ transplant or tissue graft.

BRIEF DESCRIPTION OF THE FIGURES

- [0016]** **Fig. 1A-1B** show that LDHA is upregulated in CD4⁺ T cells upon TCR stimulation. **Fig. 1A** shows zymography of LDH isoenzymes in muscle, heart, and activated CD4⁺ T cells (T cell), 4 LDHA and 0 LDHB (A₄B₀), A₃B₁, A₂B₂, A₁B₃, and A₀B₄. **Fig. 1B** shows naïve CD4⁺ T cells isolated from wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) mice, left untreated or stimulated with anti-CD3 and anti-CD28 in the presence of IL-2 for 3 days. The expression of LDHA, LDHB, and GAPDH was determined by immunoblotting with tissues from muscle and heart as controls. Asterisk indicates the LDHB band recognized by the cross-reactive anti-LDHA.
- [0017]** **Fig. 2A-2I** show that LDHA dictates aerobic glycolysis in activated CD4⁺ T cells. Naïve CD4⁺ T cells isolated from wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) mice were stimulated with anti-CD3 and anti-CD28 in the presence of IL-2 for 2 days. Cells were replenished with fresh medium, which was harvested 24 h later. Lactate production (**Fig. 2A**) and glucose consumption (**Fig. 2B**) were determined with triplicates. Naïve WT or KO CD4⁺ T cells were stimulated as in Fig. 2A. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured with a glycolysis stress test kit (**Fig. 2C, 2E, and 2F**) or a Mito stress test kit (**Fig. 2D, 2G-2I**). Baseline ECAR (**Fig. 2E**) and stressed ECAR (**Fig. 2F**) of activated CD4⁺ T cells were calculated according to Fig. 2C. Baseline OCR (**Fig. 2G**), stressed OCR (**Fig. 2H**), and baseline OCR/ECAR (**Fig. 2I**) were calculated according to Fig. 2D. Statistics (**Fig. 2E-2I**) were from one of two independent experiments, each with 8 biological replicates (n = 8); data represent mean ± SD, two-tailed unpaired *t*-test, ***P ≤ 0.001.
- [0018]** **Fig. 3** shows metabolic flux analysis of wild-type (WT) and LDHA-deficient CD4⁺ T cells. Naïve CD4⁺ T cells isolated from WT or CD4^{Cre}*Ldha*^{fl/fl} (KO) mice were differentiated under Th1 conditions for 3 days. Cells were labeled with ¹³C₆-glucose (10 mM) in glucose-deficient RPMI1640 supplemented with 10% dialyzed FBS and 100 U/mL IL-2 for 2 h. Metabolites were extracted by methanol and analyzed by CE-MS. Data

represent mean $\pm$ SD, $n = 3$. Two-tailed unpaired t -test, $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; $****P \leq 0.0001$.

[0019] **Fig. 4A-4C** show that LDHA deficiency does not affect T cell development in mice. **Fig. 4A** shows flow cytometry analysis of CD4 and CD8 expression in thymocytes (left panels), and Foxp3 expression in CD4⁺CD8⁻ T cells (right panels) of 8-week-old wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) mice. **Fig. 4B** and **4C** show frequencies of CD4⁻CD8⁻ (DN), CD4⁺CD8⁺ (DP), CD4⁺CD8⁻ (CD4SP), and CD4⁻CD8⁺ (CD8SP) thymocytes (**Fig. 4B**, $n = 7$) and Foxp3⁺ regulatory T cells among CD4SP cells (**Fig. 4C**, $n = 5$). Data represent mean $\pm$ SD. Two-tailed unpaired t -test; ns, not significant.

[0020] **Fig. 5A-5G** show that LDHA deficiency does not substantially affect T cell homeostasis in mice. **Fig. 5A** shows total peripheral lymph node (pLN) and spleen (SPL) cell numbers from wild-type (WT) and CD4^{Cre}*Ldha*^{fl/fl} (KO) mice. Data represent mean $\pm$ SD, $n = 7$ per genotype. Two-tailed unpaired t -test, ns, not significant. **Fig. 5B-5E** show flow cytometry analysis of pLN and SPL T cell populations from WT and KO mice. **Fig. 5B** and **5C** show representative plots of TCR β ⁺, CD4⁺TCR β ⁺, CD8⁺TCR β ⁺, and CD4⁺TCR β ⁺Foxp3⁺ T cell populations. **Fig. 5D** and **5E** show frequencies and numbers of TCR β ⁺, CD4⁺TCR β ⁺, CD8⁺TCR β ⁺, and CD4⁺TCR β ⁺Foxp3⁺ T cell populations. Data represent mean $\pm$ SD, $n = 7$ per genotype. Two-tailed unpaired t -test, ns, not significant. **Fig. 5F** and **5G** show flow cytometry analysis of CD44 and CD62L expression in pLN and SPL CD4⁺TCR β ⁺ or CD8⁺TCR β ⁺ T cells. **Fig. 5F** shows representative plots of CD44 and CD62L expression. **Fig. 5G** shows frequencies of CD4⁺ (upper panel) and CD8⁺ (lower panel) T cell populations with the indicated activation phenotypes. Data represent mean $\pm$ SD, $n = 8$ per genotype. Two-tailed unpaired t -test, $*P \leq 0.05$; ns, not significant.

[0021] **Fig. 6A-6E** show that LDHA is non-essential for CD4⁺ T cell activation, growth, proliferation, and survival *in vitro*. **Fig. 6A-6D** show naïve CD4⁺ T cells isolated from wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) mice, and stimulated with anti-CD3 and anti-CD28 in the presence of IL-2. **Fig. 6A** shows flow cytometry analysis of CD44, CD69, and CD25 expression one day post-activation, and CD40L expression 3 days post-activation. **Fig. 6B** shows cell size of WT and KO T cells, measured 3 days post-activation. **Fig. 6C** and **6D** show flow cytometry analysis of DAPI staining of WT and KO T cells 3 days post-activation. **Fig. 6C** shows representative plots of DAPI staining. **Fig. 6D** shows frequencies of DAPI⁻ T cells, $n = 4$. Two-tailed paired t -test, ns, not significant. **Fig. 6E** shows naïve CD4⁺ WT and KO T cells labeled with CFSE and stimulated as in Fig. 6A. CFSE dilution

was examined 1, 2, and 3 days post-stimulation. Data are representative of three independent experiments.

[0022] **Fig. 7A-7H** show that LDHA regulates IFN- γ expression independent of its 3' UTR. **Fig. 7A** and **7B** show naïve wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) CD4⁺ T cells differentiated under Th1 conditions for 3 days, and re-stimulated with phorbol myristate acetate (PMA) and ionomycin for 4 h. IFN- γ expression was determined by intracellular staining. Representative plots (**Fig. 7A**) and mean fluorescence intensity (MFI) (**Fig. 7B**) are shown. **Fig. 7C-7E** show naïve WT or KO CD4⁺ T cells activated for 2 days and transduced with green fluorescent protein (GFP) constructs fused with the 3' untranslated region (3'UTR) of the *Ifng* or *Gapdh* gene. GFP expression was measured 48 h after transduction. Representative plots (**Fig. 7C**) and MFI of GFP in GFP⁺ cells (**Fig. 7D**) are shown. **Fig. 7E** shows MFI of GFP from IFN- γ 3'UTR, normalized to that of GAPDH 3'UTR control. **Fig. 7F** shows a diagram of IFN- γ mRNA expressed from the WT *Ifng* or *Yeti* allele. **Fig. 7G** and **7H** show naïve CD4⁺ T cells from *Yeti*/*Yeti* or KO*Yeti*/*Yeti* mice, differentiated as in Fig. 7A. The expression of IFN- γ and eYFP were determined by flow cytometry. Representative plots and respective statistical analysis are shown in **Fig. 7G** and **7H**. Statistics (**Fig. 7B, 7E, and 7H**) were from one of two independent experiments, each with 3 biological replicates (n = 3); data represent mean $\pm$ SD, two-tailed unpaired *t*-test, *P $\leq$ 0.05; ***P $\leq$ 0.001; ns, not significant.

[0023] **Fig. 8A-8C** show genome-wide analysis of LDHA-regulated transcripts in Th1 cells. **Fig. 8A** shows a volcano plot of transcripts differentially expressed between wild-type (WT) and CD4^{Cre}*Ldha*^{fl/fl} (KO) T cells, differentiated under Th1 conditions. Arrow indicates the differentially expressed *Ifng* gene. FDR corrected p value < 0.01. **Fig. 8B** shows a scatter plot of relative histone acetylation (H3K9Ac) levels of the differentially expressed transcripts between WT and KO Th1 cells. Arrow indicates the differential H3K9Ac at the promoter region of the *Ifng* gene. FDR corrected p value < 0.01. **Fig. 8C** shows a selected list of genes with either decreased or increased level of mRNA (heat map) and H3K9Ac (black box) in KO Th1 cells compared to those of WT Th1 cells.

[0024] **Fig. 9A-9C** show that IFN- γ but not T-bet expression is diminished in LDHA-deficient Th1 cells. Naïve wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) CD4⁺ T cells were differentiated under Th1 conditions for 3 days. **Fig. 9A** shows IFN- γ mRNA measured by qPCR and normalized to β -Actin. **Fig. 9B** and **9C** show T-bet expression measured by flow cytometry. Representative plots (**Fig. 9B**) and statistics (**Fig. 9C**) are shown. In **Fig. 9A** and

Fig. 9C, data represent mean $\pm$ SD, $n = 3$. Two-tailed unpaired t -test, *** $P \leq 0.001$; ns, not significant.

[0025] **Fig. 10A-10F** show that LDHA promotes IFN- γ expression through an epigenetic mechanism. **Fig. 10A** shows representative H3K9 acetylation (H3K9Ac) peaks at the *Cd3e* or *Ifng* promoter and enhancer (CNS22) regions from one of two ChIP-Seq experiments. **Fig. 10B** shows H3K9Ac at the *Ifng* promoter and CNS22 enhancer regions in WT or KO Th1 cells, assessed by ChIP-qPCR. Enrichment was normalized to H3K9Ac at the *Cd3e* promoter region. **Fig. 10C** shows naïve WT or KO CD4⁺ T cells differentiated under Th1 conditions, and acetyl-CoA levels, measured by an acetyl-CoA assay kit. **Fig. 10D and 10E** show naïve WT or KO CD4⁺ T cells differentiated under Th1 conditions for 3 days, and either left untreated or supplemented with 20 mM sodium acetate for another 24 h. Cells were subsequently re-stimulated with phorbol myristate acetate (PMA) and ionomycin for 4 h. The expression of IFN- γ protein (**Fig. 10D**) and mRNA (**Fig. 10E**) were determined by flow cytometry and qPCR, respectively. mRNA level of IFN- γ was normalized to that of β -Actin. MFIs of IFN- γ are shown in **Fig. 10D**. **Fig. 10F** shows T cells cultured as in Fig. 10D. H3K9Ac at the *Ifng* promoter and CNS22 enhancer regions were assessed by ChIP-qPCR. Enrichment was normalized to H3K9Ac at the *Cd3e* promoter region. Statistics (**Fig. 10B, 10C, 10E, and 10F**) were from one of three independent experiments, each with 3 biological replicates ($n = 3$); data represent mean $\pm$ SD, two-tailed unpaired t -test, * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, not significant.

[0026] **Fig. 11A-11C** show that acetylation of histone H3 and recruitment of RNA polymerase II to the *Ifng* locus are reduced in LDHA-deficient Th1 cells. Naïve wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) CD4⁺ T cells were differentiated under Th1 conditions for 3 days, and subjected to ChIP with a control antibody (IgG) or antibodies against total histone H3 (**Fig. 11A**), acetylated lysine 27 of histone H3 (H3K27Ac) (**Fig. 11B**), or RNA polymerase II (PolII) (**Fig. 11C**). Enrichment was normalized to the *Cd3e* promoter region. Data represent mean $\pm$ SD, $n = 3$. Two-tailed unpaired t -test, * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, not significant.

[0027] **Fig. 12A-12I** show that an ACL inhibitor suppresses IFN- γ expression, while acetate and an HDAC inhibitor promote IFN- γ expression. In **Fig. 12A-12D**, naïve CD4⁺ T cells isolated from wild-type (WT) mice were differentiated under Th1 conditions for 2 days. DMSO or the ATP citrate lyase inhibitor (ACLi) BMS-303141 was added to the culture for another 24 h. Subsequently, cells were re-stimulated with phorbol myristate acetate (PMA)

and ionomycin for 4 h. IFN- γ expression was determined by intracellular staining. A representative plot (**Fig. 12A**) and statistical analysis (**Fig. 12B**) are shown. Cell viability was determined by LIVE/DEAD staining. A representative plot (**Fig. 12C**) and statistical analysis (**Fig. 12D**) are shown. Data represent mean $\pm$ SD, $n = 3$. Two-tailed unpaired t -test, * $P \leq 0.05$; ** $P \leq 0.01$; ns, not significant. **Fig. 12E** shows naïve CD4^{Cre}*Ldha*^{fl/fl} (KO) CD4⁺ T cells differentiated under Th1 conditions for 2 days. Cells were replated in fresh medium with or without 20 mM sodium acetate for another 24 h. Acetyl-CoA levels were measured by an acetyl-CoA assay kit. Data represent mean $\pm$ SD, $n = 3$. Two-tailed unpaired t -test, ** $P \leq 0.01$. **Fig. 12F** shows naïve CD4⁺ T cells isolated from WT or KO mice, differentiated under Th1 conditions for 3 days. Cells were further cultured in the absence or presence of 20 mM sodium acetate for another 24 h, followed by re-stimulation with PMA and ionomycin for 4 h. The expression of T-bet was determined by flow cytometry. **Fig. 12G** and **12H** show naïve CD4⁺ T cells isolated from WT or KO mice, differentiated under Th1 conditions for 3 days. Cells were further cultured in the absence or presence of 30 nM TSA for another 24 h, followed by re-stimulation with PMA and ionomycin for 4 h. The expression of IFN- γ (**Fig. 12G**) and T-bet (**Fig. 12H**) were determined by flow cytometry. The mean fluorescence intensity (MFI) of IFN- γ is shown in **Fig. 12G**. **Fig. 12I** shows cells cultured as in Fig. 12G. H3K9Ac at the *Ifng* promoter region was assessed by ChIP-qPCR. Enrichment was normalized to H3K9Ac at the *Cd3e* promoter region. Data represent mean $\pm$ SD, $n = 4$. Two-tailed unpaired t -test, ** $P \leq 0.01$; *** $P \leq 0.001$; ns, not significant.

[0028] **Fig. 13A-13B** show that the LDHA inhibitor FX11 suppresses IFN- γ expression in T cells. Naïve CD4⁺ T cells isolated from wild-type mice were differentiated under Th1 conditions for 3 days. DMSO or indicated concentration of Fx11 was added to the cell culture for another 24 h, with or without 20 mM sodium acetate. Subsequently, cells were re-stimulated with phorbol myristate acetate (PMA) and ionomycin for 4 h. IFN- γ expression was determined by intracellular staining. Representative plots (**Fig. 13A**) and quantification (**Fig. 13B**) are shown.

[0029] **Fig. 14A-14D** show that LDHA deficiency in T cells protects *Yeti/Yeti* mice from a lethal autoinflammatory disease. **Fig. 14A** shows the survival curve of *Yeti/Yeti* ($n = 5$) and CD4^{Cre}*Ldha*^{fl/fl}*Yeti/Yeti* (KO *Yeti/Yeti*) mice ($n = 6$). **Fig. 14B** shows representative haematoxylin and eosin staining of liver sections from wild-type (WT), CD4^{Cre}*Ldha*^{fl/fl} (KO), *Yeti/Yeti*, and KO *Yeti/Yeti* mice. Arrows indicate the necrotic regions. In **Fig. 14C**

and **14D**, splenocytes from mice of the indicated genotypes were stimulated with phorbol myristate acetate (PMA) and ionomycin for 4 h. IFN- γ and YFP expression in CD4⁺ T cells and in NK1.1⁺TCR⁻ NK cells were determined by flow cytometry. Representative plots (**Fig. 14C**) and statistics (**Fig. 14D**) are shown; data represent mean $\pm$ SD, n = 4 - 5 mice per genotype, two-tailed paired *t*-test, ***P $\leq$ 0.001; ns, not significant.

[0030] **Fig. 15A-15E** show that LDHA promotes IFN- γ expression in the absence of its 3' UTR *in vivo*. **Fig. 15A** shows representative images of livers from wild-type (WT), CD4^{Cre}*Ldha*^{fl/fl} (KO), *Yeti/Yeti*, and CD4^{Cre}*Ldha*^{fl/fl}*Yeti/Yeti* (KO *Yeti/Yeti*) mice. Arrows indicate the damaged liver in *Yeti/Yeti* mice. **Fig. 15B** and **15C** show total cell numbers (**Fig. 15B**) and CD4⁺ T cell numbers (**Fig. 15C**) from spleens of WT, KO, *Yeti/Yeti*, and KO *Yeti/Yeti* mice. Data represent mean $\pm$ SD, n = 4-5 per genotype. Two-tailed unpaired *t*-test, ns, not significant. **Fig. 15D** and **15E** show flow cytometry analysis of CD44 and CD62L expression in CD4⁺ T cells from WT, KO, *Yeti/Yeti*, and KO *Yeti/Yeti* mice. **Fig. 15D** shows representative plots of CD44 and CD62L expression. **Fig. 15E** shows frequencies of CD4⁺ T cell populations with the indicated activation phenotypes; data represent mean $\pm$ SD, n = 4-5 per genotype. Two-tailed unpaired *t*-test, *P $\leq$ 0.05; ns, not significant.

[0031] **Fig. 16A-16J** show that LDHA deficiency in T cells attenuates the autoimmune phenotype of Scurfy mice. **Fig. 16A** shows a representative image of 21-day-old wild-type (WT), CD4^{Cre}*Ldha*^{fl/fl} (KO), *Foxp3*^{sf}, and CD4^{Cre}*Ldha*^{fl/fl}*Foxp3*^{sf} (KO *Foxp3*^{sf}) mice. Arrows indicate the scurfy tails and ears of *Foxp3*^{sf} mice, which were rescued in KO *Foxp3*^{sf} mice. **Fig. 16B** shows the survival curve of *Foxp3*^{sf} (n = 25) and KO *Foxp3*^{sf} (n = 16) mice. **Fig. 16C** shows representative haematoxylin and eosin (H&E) staining of sections from the lung, liver, and ear of WT, KO, *Foxp3*^{sf}, and KO *Foxp3*^{sf} mice. **Fig. 16D** shows a representative image of peripheral lymph nodes and spleens of 21-day-old WT, KO, *Foxp3*^{sf}, and KO *Foxp3*^{sf} mice. **Fig. 16E** and **16F** show total cell numbers (**Fig. 16E**) and CD4⁺ T cell numbers (**Fig. 16F**) from peripheral lymph nodes (pLN) and spleens (SPL) of WT, KO, *Foxp3*^{sf}, and KO *Foxp3*^{sf} mice; data represent mean $\pm$ SD, n = 4-9 per genotype. Two-tailed unpaired *t*-test, *P $\leq$ 0.05; ns, not significant. **Fig. 16G** and **Fig. 16H** show flow cytometry analysis of CD44 and CD62L expression in CD4⁺ T cells from WT, KO, *Foxp3*^{sf}, and KO *Foxp3*^{sf} mice. **Fig. 16G** shows representative plots of CD44 and CD62L expression. **Fig. 16H** shows frequencies of CD4⁺ T cell populations with the indicated activation phenotypes; data represent mean $\pm$ SD, n = 4-9 per genotype. Two-tailed unpaired *t*-test,

* $P \leq 0.05$; *** $P \leq 0.001$; ns, not significant. **Fig. 16I** and **16J** show $CD4^+$ T cells from peripheral pLN or spleens SPL of WT, KO, *Foxp3^{fl}*, and KO *Foxp3^{fl}* mice, stimulated with PMA and ionomycin for 4 h. IFN- γ expression was determined by flow cytometry. Representative plots (**Fig. 16I**) and paired analyses (**Fig. 16J**) are shown. Data represent mean $\pm$ SD, $n = 2-7$ per genotype. Two-tailed paired t -test, * $P \leq 0.05$; *** $P \leq 0.001$; ns, not significant.

[0032] **Fig. 17** shows that LDHA deficiency results in compromised Th17 cell differentiation, which can be recapitulated by pharmacologic inhibition of LDHA with an LDHA inhibitor (GNE-140). Naïve WT or LDHA-deficient (KO) $CD4^+$ T cells were differentiated under Th17 conditions for 4 days. Increasing doses of GNE-140 were also included in the WT T cell culture. Cell viability was determined by a live/dead cell dye, and the percentage of live cell population is shown. In addition, cells were re-stimulated with PMA/ionomycin for 4 h. IL-17a and Foxp3 protein expression was examined by flow cytometry, and used as readouts for Th17 and Treg cell differentiation, respectively.

[0033] **Fig. 18A-18H** show that compromised Th17 cell differentiation in the absence of LDHA is associated with reduced H3K9Ac, and that inhibition of acetyl-CoA generation by an ACL inhibitor (BMS303141) protects mice from experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. In **Fig. 18A-18C**, naïve WT or LDHA-deficient (KO) $CD4^+$ T cells were differentiated under Th17 conditions for 3 days. Cells were re-stimulated with PMA/ionomycin for 4 h. **Fig. 18A** shows IL-17a protein expression examined by flow cytometry. **Fig. 18B** shows IL-17a mRNA expression measured by qPCR, and normalized to that of β -actin. Data represent mean $\pm$ SD, $n = 2$. Two-tailed unpaired t -test, ** $P \leq 0.01$. **Fig. 18C** shows representative H3K9Ac peaks at the *Cd3e* and *Il17a* loci from one of two ChIP-Seq experiments. In **Fig. 18D-18F**, WT or KO mice were subjected to EAE induction by immunization with MOG peptide with CFA. Clinical scoring in **Fig. 18D**: 0, no overt signs of disease; 1, limp tail; 2, partial hind-limb paralysis; 3, total hind-limb paralysis; 4, hind-limb paralysis and 75% body paralysis; 5, complete body paralysis, or moribund state, or death by EAE, or sacrifice for humane reasons. **Fig. 18E** shows immune cells from spinal cord and cerebellum, re-stimulated with PMA/ionomycin for 4 h. IL-17a and IFN- γ protein expression were examined by flow cytometry. **Fig. 18F** is quantification of **Fig. 18E**; data represent mean $\pm$ SD, $n = 4$. Two-tailed unpaired t -test, *** $P \leq 0.001$. **Fig. 18G** shows that an ACL inhibitor (ACLi, BMS 303141) inhibits Th17 cell differentiation *in vitro*. Naïve WT or KO $CD4^+$ T cells were

differentiated under Th17 conditions for 3 days, and BMS 303141 was included in the culture for another 24 hours. Cells were re-stimulated with PMA/ionomycin for 4 h. IL-17a protein expression was examined by flow cytometry. **Fig. 18H** shows that BMS 303141 treatment protects mice from EAE. WT mice were subjected to EAE induction by immunization with MOG peptide with CFA. BMS 303141 (35 mg/kg/day) was intraperitoneally injected daily, starting from day 7. Clinical scoring was the same as in Fig. 18D.

[0034] **Fig. 19** shows an epigenetic model of aerobic glycolysis in the control of IFN- γ expression. A major role of LDHA-mediated aerobic glycolysis in activated CD4⁺ T cells is to maintain glycolytic production of ATP, which relieves the burden of mitochondria as an energy house to burn acetyl-CoA to generate ATP. As a result, acetyl-CoA can be readily used as a substrate for histone acetyltransferases (HATs) for epigenetic regulation of target genes including *Ifng*. In the absence of LDHA, glucose consumption is reduced and more acetyl-CoA is oxidized in the mitochondria to generate ATP, leading to reduced nucleocytoplasmic acetyl-CoA for histone acetylation and attenuated transcription of target genes.

DETAILED DESCRIPTION OF THE INVENTION

[0035] We demonstrate that genes involved in aerobic glycolysis, such as lactate dehydrogenase A (LDHA), also play a role in T cell differentiation by an epigenetic mechanism, which is distinct from how they were thought to act. Deficiency of this enzyme (or inhibition of the enzyme) reverses the symptoms of immune disorders seen in the Scurfy mouse model. Scurfy mice have defective T cell tolerance leading to an X-linked lymphoproliferative disease that parallels the X-linked autoimmunity-allergic dysregulation syndrome (XLAAD) in humans. This work has identified a critical role for LDHA-mediated aerobic glycolysis in promoting autoreactive Th1 and Th17 cell responses. LDHA inhibitors are being developed to target tumor cell metabolism. Our data indicate that LDHA inhibitors are immunosuppressive, and thus may complicate their applications in cancer. Instead, LDHA inhibitors, as well as inhibitors targeting other enzymes involved in acetyl-CoA metabolism, including ACL, can be useful in treatment of diseases and disorders associated with T cell activation, differentiation, or expansion.

[0036] Unless defined otherwise, 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

invention is related. For example, *The Dictionary of Cell and Molecular Biology* (5th ed. J.M. Lackie ed., 2013), the *Oxford Dictionary of Biochemistry and Molecular Biology* (2d ed. R. Cammack et al. eds., 2008), and *The Concise Dictionary of Biomedicine and Molecular Biology* (2d ed. P-S. Juo, 2002) can provide one of skill with general definitions of some terms used herein.

[0037] As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents, unless the context clearly dictates otherwise. The terms “a” (or “an”) as well as the terms “one or more” and “at least one” can be used interchangeably.

[0038] Furthermore, “and/or” is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” is intended to include A and B, A or B, A (alone), and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to include A, B, and C; A, B, or C; A or B; A or C; B or C; A and B; A and C; B and C; A (alone); B (alone); and C (alone).

[0039] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range, and any individual value provided herein can serve as an endpoint for a range that includes other individual values provided herein. For example, a set of values such as 1, 2, 3, 8, 9, and 10 is also a disclosure of a range of numbers from 1-10. Where a numeric term is preceded by “about,” the term includes the stated number and values $\pm 10\%$ of the stated number. The headings provided herein are not limitations of the various aspects or embodiments of the invention, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

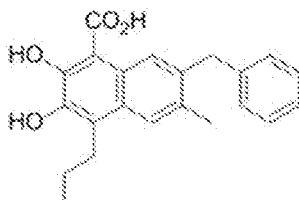
[0040] Amino acids are referred to herein by their commonly known three-letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, are referred to by their commonly accepted single-letter codes. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation, and nucleic acid sequences are written left to right in 5' to 3' orientation.

[0041] Wherever embodiments are described with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are included.

[0042] The term “T cell-mediated disease or disorder,” as used herein, includes diseases and disorders characterized by T cell activation, T cell differentiation, and/or T cell proliferation. T cell-mediated inflammation, graft or organ rejection, and autoimmune diseases, such as graft-versus-host disease (GVHD), inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, rheumatoid arthritis, systemic lupus erythematosus (SLE), and type-1 diabetes, are examples of T cell-mediated diseases or disorders.

[0043] An “active agent” is an agent which itself has biological activity, or which is a precursor or prodrug that is converted in the body to an agent having biological activity. Active agents useful in the methods of the invention include inhibitors of acetyl-CoA production, such as LDHA inhibitors and ACL inhibitors.

[0044] An “LDHA inhibitor” is an active agent that agonizes or antagonizes the activity of lactate dehydrogenase A or reduces its production in a cell. Examples of LDHA inhibitors that are suitable for use in the present invention include FX11, Galloflavin, GNE-140, quinoline 3-sulfonamides (38), including GSK 2837808A, and NHI 2. “FX11” refers to a compound having the structure:



“Galloflavin” refers to 3,8,9,10-tetrahydroxy-pyrano[3,2-c][2]benzopyran-2,6-dione. “GNE-140” refers to (2R)-5-(2-chlorophenyl)sulfanyl-4-hydroxy-2-(4-morpholin-4-ylphenyl)-2-thiophen-3-yl-1,3-dihydropyridin-6-one. “GSK 2837808A” refers to 3-[[3-(cyclopropylsulfamoyl)-7-(2,4-dimethoxypyrimidin-5-yl)quinolin-4-yl]amino]-5-(3,5-difluorophenoxy)benzoic acid. “NHI 2” refers to methyl 1-hydroxy-6-phenyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate. Derivatives of these compounds that act as LDHA inhibitors, and their pharmaceutically acceptable salts, are also suitable for use in the invention.

[0045] An “ACL inhibitor” is an active agent that agonizes or antagonizes the activity of ATP-citrate lyase or reduces its production in a cell. Examples of ACL inhibitors that are suitable for use in the present invention include BMS 303141, ETC-1002, MEDICA 16, and

SB 204990. “BMS 303141” refers to 3,5-dichloro-2-hydroxy-*N*-(4-methoxy[1,1'-biphenyl]-3-yl)-benzenesulfonamide. “ETC-1002” refers to 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid. “MEDICA 16” refers to 3,3,14,14-tetramethylhexadecanedioic acid. “SB 204990” refers to (3*R*,5*S*)-rel-5-[6-(2,4-dichlorophenyl)hexyl]tetrahydro-3-hydroxy-2-oxo-3-furanacetic acid.

[0046] The terms “inhibit,” “block,” and “suppress” are used interchangeably and refer to any statistically significant decrease in biological activity, including full blocking of the activity.

[0047] By “subject” or “individual” or “patient” is meant any subject, preferably a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, sports animals, and zoo animals including, *e.g.*, humans, non-human primates, dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, and so on.

[0048] Terms such as “treating” or “treatment” or “to treat” or “alleviating” or “to alleviate” refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder. In certain embodiments, a subject is successfully “treated” for a disease or disorder according to the methods provided herein if the patient shows, *e.g.*, total, partial, or transient alleviation or elimination of symptoms associated with the disease or disorder.

[0049] “Prevent” or “prevention” refers to prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus, those in need of prevention include those at risk of or susceptible to developing the disorder. Subjects that are at risk of or susceptible to developing a T cell-mediated disease or disorder include, but are not limited to, patients receiving a tissue graft or an organ transplant, patients having a genetic predisposition to a T cell-mediated disease or disorder, and patients having another disease or disorder of the immune system, including another T cell-mediated disease or disorder. In certain embodiments, a disease or disorder is successfully prevented according to the methods provided herein if the patient develops, transiently or permanently, *e.g.*, fewer or less severe symptoms associated with the disease or disorder, or a later onset of symptoms associated with the disease or disorder, than a patient who has not been subject to the methods of the invention.

[0050] In a prophylactic context, a composition for inhibiting acetyl-CoA production, such as a composition comprising an LDHA inhibitor or an ACL inhibitor, can be administered at any time before or after an event, for example, a tissue graft or an organ transplant, which places a subject at risk of or susceptible to developing a T cell-mediated disease or disorder. In some aspects, the composition for inhibiting acetyl-CoA production is administered prophylactically up to about one week before the event, such as 1, 2, 3, 4, 5, 6, or 7 days before the event. In some instances, the composition for inhibiting acetyl-CoA production is administered prophylactically on the same day as the event. In some embodiments, the pharmaceutical composition is administered prophylactically within 7 days of the event, for example, within about 1, 2, 3, 4, 5, 6, or 7 days. In a preferred embodiment, the composition for inhibiting acetyl-CoA production is administered within 24 hours of the event, *i.e.*, about 24 hours before the beginning of the event to about 24 hours after the completion of the event.

[0051] The term “pharmaceutical composition” refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components that are unacceptably toxic to a subject to which the composition would be administered. Pharmaceutical compositions can be in numerous dosage forms, for example, tablet, capsule, liquid, solution, softgel, suspension, emulsion, syrup, elixir, tincture, film, powder, hydrogel, ointment, paste, cream, lotion, gel, mousse, foam, lacquer, spray, aerosol, inhaler, nebulizer, ophthalmic drops, patch, suppository, and/or enema. Pharmaceutical compositions typically comprise a pharmaceutically acceptable carrier, and can comprise one or more of a buffer (*e.g.* acetate, phosphate or citrate buffer), a surfactant (*e.g.* polysorbate), a stabilizing agent (*e.g.* human albumin), a preservative (*e.g.* benzyl alcohol), a penetration enhancer, an absorption promoter to enhance bioavailability and/or other conventional solubilizing or dispersing agents. Choice of dosage form and excipients depends upon the active agent to be delivered and the disease or disorder to be treated or prevented, and is routine to one of ordinary skill in the art.

[0052] “Systemic administration” means that a pharmaceutical composition is administered such that the active agent enters the circulatory system, for example, via enteral, parenteral, inhalational, or transdermal routes. Enteral routes of administration involve the gastrointestinal tract and include, without limitation, oral, sublingual, buccal, and rectal delivery. Parenteral routes of administration involve routes other than the gastrointestinal tract and include, without limitation, intravenous, intramuscular,

intraperitoneal, intrathecal, and subcutaneous. “Local administration” means that a pharmaceutical composition is administered directly to where its action is desired (*e.g.*, at or near the site of the injury or symptoms). Local routes of administration include, without limitation, topical, inhalational, subcutaneous, ophthalmic, and otic. It is within the purview of one of ordinary skill in the art to formulate pharmaceutical compositions that are suitable for their intended route of administration.

[0053] An “effective amount” of a composition as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An “effective amount” can be determined empirically and in a routine manner, in relation to the stated purpose, route of administration, and dosage form.

[0054] In some embodiments, administration of the LDHA inhibitor or the ACL inhibitor can comprise systemic administration, at any suitable dose and/or according to any suitable dosing regimen, as determined by one of skill in the art. The LDHA inhibitor or the ACL inhibitor can be administered according to any suitable dosing regimen, for example, where the daily dose is divided into two or more separate doses. It is within the skill of the ordinary artisan to determine a dosing schedule and duration for administration. In some embodiments, the pharmaceutical composition is administered orally at least once a day or at least twice a day. In some embodiments, the pharmaceutical composition is administered intravenously at least once a day or at least twice a day. In some embodiments, the pharmaceutical composition is administered subcutaneously at least once a day or at least twice a day.

[0055] Embodiments of the present disclosure can be further defined by reference to the following non-limiting examples. It will be apparent to those skilled in the art that many modifications, both to materials and methods, can be practiced without departing from the scope of the present disclosure.

EXAMPLES

Example 1. Absence of LDHA Alters Glucose Metabolism in Activated CD4⁺ Cells

[0056] To characterize LDH activity in activated CD4⁺ cells, we performed a zymography assay. Activated CD4⁺ T cells manifested LDH activity predominantly in the form of A₄B₀, similar to that of muscle tissues (**Fig. 1A**). Consistently, LDHA, but not LDHB, was induced upon T cell activation (**Fig. 1B**), likely due to HIF-1 α - and c-Myc-induced transcription of *Ldha* (10, 11).

[0057] To study the definitive function of aerobic glycolysis, we deleted LDHA specifically in T cells ($CD4^{Cre}Ldha^{fl/fl}$, designated as knock-out, KO) (**Fig. 1B**) (18). Compared to activated wild-type (WT) $CD4^{+}$ T cells, KO T cells barely produced lactate (**Fig. 2A**). Furthermore, glucose consumption in KO T cells was reduced to $\sim 30\%$ WT levels (**Fig. 2B**), in line with a critical role for LDHA in sustaining aerobic glycolysis through regeneration of NAD^{+} consumed at the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) step of glycolysis (17). Consistent with low Glut1 (13) and LDHA expression (**Fig. 1B**), naïve $CD4^{+}$ T cells showed little activity of glucose metabolism with negligible glucose-induced extracellular acidification rate (ECAR) or oxygen consumption rate (OCR) (**Fig. 2C and 2D**). Upon activation, ECAR was increased in WT $CD4^{+}$ T cells, which was largely diminished in KO T cells (**Fig. 2C, 2E, and 2F**). In contrast, both basal and maximal OCR were elevated in KO T cells (**Fig. 2D, 2G, and 2H**).

[0058] ^{13}C -isotope labeled glucose ($^{13}C_6$ -glucose) tracing experiments showed that glycolysis was slowed down at the GAPDH step in KO T cells (**Fig. 3**). However, ^{13}C -labelled citrate was increased in the absence of LDHA (**Fig. 3**). In particular, using doubly ($m+2$) and quadruply ($m+4$) ^{13}C -labeled citrate, readouts of tricarboxylic acid (TCA) cycle activity through the first and second turns, respectively, were much increased in KO T cells (**Fig. 3**), in line with enhanced OCR (**Fig. 2D, 2G, and 2H**). Together, these findings reveal that glucose metabolism in activated $CD4^{+}$ T cells is shifted from aerobic glycolysis towards oxidative phosphorylation in the absence of LDHA (**Fig. 2I**).

[0059] LDHA deficiency did not affect thymic development of conventional or regulatory T (Treg) cells (**Fig. 4A-4C**), or T cell homeostasis in peripheral lymph node and spleen (**Fig. 5A-5G**). LDHA deficiency also did not alter expression of activation markers, size or survival of activated $CD4^{+}$ T cells (**Fig. 6A-6D**), while proliferation was slightly delayed (**Fig. 6E**). These phenotypes were distinct from those of Glut1-deficient T cells (13), suggesting that general glucose metabolism, but not aerobic glycolysis, is required for T cell development and homeostasis.

Example 2. LDHA Deficiency Results in Decreased IFN- γ Expression in T Cells

[0060] Glycolysis promotes expression of effector molecules including the type 1 cytokine IFN- γ (9, 12-15). Indeed, LDHA deficiency led to diminished IFN- γ expression in T cells differentiated under T helper 1 (Th1) conditions (**Fig. 7A and 7B**). A recent study proposed that aerobic glycolysis enhanced IFN- γ production by sequestering GAPDH away from binding to the *Ifng* 3'UTR thereby enhancing IFN- γ translation (12). To determine

whether such regulation accounted for reduced IFN- γ expression in KO T cells, we used a green fluorescent protein (GFP) reporter in which the GFP open reading frame was fused to the 3'UTR of *Ifng* or *Gapdh*, with the latter not known to repress mRNA translation (19). As previously reported (19), GFP expression controlled by the *Ifng* 3'UTR showed decreased mean fluorescence intensity compared to that regulated by the *Gapdh* 3'UTR in WT T cells (**Fig. 7C-7E**). Surprisingly, GFP expression under the control of *Ifng* 3'UTR was diminished to a similar extent in KO T cells (**Fig. 7C-7E**), suggesting that *Ifng* 3'UTR is insufficient to mediate aerobic glycolysis regulation of IFN- γ expression.

[0061] To explore the definitive function of 3'UTR in LDHA control of IFN- γ expression, we used an *Ifng* reporter allele *Yeti* (Yellow-enhanced transcript for IFN- γ) (20), in which the *Ifng* 3'UTR was replaced by 3'UTR of the bovine growth hormone (*BGH*) gene (**Fig. 7F**). We found that expression of both IFN- γ and yellow fluorescent protein (YFP) driven by the internal ribosome entry site (IRES) element in the reporter allele were proportionally reduced in the absence of LDHA (**Fig. 7G and 7H**), demonstrating that *Ifng* 3'UTR is not required for LDHA control of IFN- γ expression.

Example 3. Histone Acetylation and Cytosolic Acetyl CoA Are Reduced in LDHA-Deficient T Cells

[0062] To determine whether reduced IFN- γ production in KO T cells was caused by diminished transcription, we performed RNA sequencing experiments. 363 transcripts were differentially expressed between WT and KO Th1 cells, among which 220 transcripts, including *Ifng*, were downregulated in KO T cells (**Fig. 8A and 9A**). IFN- γ transcription in Th1 cells is induced by T-bet (21). However, T-bet expression was not affected in KO T cells (**Fig. 9B and 9C**), suggesting that LDHA promotes IFN- γ expression via T-bet-independent mechanisms.

[0063] Glucose metabolism is implicated in the control of gene expression through epigenetic mechanisms including histone acetylation (7, 22). We performed chromatin immunoprecipitation-sequencing (ChIP-seq) analysis of histone H3 acetylation at the lysine 9 residue (H3K9Ac), a histone mark associated with active transcription. The ChIP-seq analysis showed that the differentially expressed genes between WT and KO Th1 cells had varying levels of H3K9Ac (**Fig. 8B**), which encoded proteins involved in signal transduction, transcription, metabolism, and effector functions (**Fig. 8C**). Notably, 86%

downregulated transcripts including *Ifng* had decreased H3K9Ac in KO T cells (**Fig. 8B**), suggesting that LDHA may promote IFN- γ expression by modulating histone acetylation.

[0064] Compared to the constitutively active *Cd3e* locus, diminished H3K9Ac was observed in *Ifng* promoter, gene body and the conserved noncoding sequence 22 kilobase pairs upstream of *Ifng* (CNS-22) in KO T cells (**Fig. 10A and 10B**). Acetylation of histone H3 at lysine 27 (H3K27Ac) was also reduced, while total histone H3 was comparable (**Fig. 11A and 11B**). Importantly, diminished histone acetylation was associated with reduced RNA polymerase II (PolII) recruitment to the *Ifng* locus in KO T cells (**Fig. 11C**). Histone acetylation requires acetyl coenzyme A (acetyl-CoA) as a substrate, with glucose being a critical source. Considering enhanced TCA cycle activity in KO T cells (**Fig. 2A-2I and Fig. 3**), we hypothesized that in the absence of LDHA, less citrate would be exported from the mitochondria for acetyl-CoA regeneration. Indeed, cytosolic acetyl-CoA was decreased in LDHA-deficient Th1 cells (**Fig. 10C**).

[0065] To determine whether reduced cytosolic acetyl-CoA was sufficient to repress IFN- γ expression, we inhibited ATP-citrate lyase (ACL), the enzyme converting citrate to acetyl-CoA, and found that IFN- γ expression was diminished in WT T cells (**Fig. 12A-12D**). Acetyl-CoA can be generated from acetate by acetyl-CoA synthetase, independent of citrate (23). Acetate supplementation augmented acetyl-CoA production (**Fig. 12E**), and corrected IFN- γ expression in KO T cells (**Fig. 10D and 10E**) without affecting T-bet expression (**Fig. 12F**). Instead, enhanced IFN- γ production was associated with normalization of H3K9Ac in *Ifng* promoter and enhancer regions (**Fig. 10F**). Histone acetylation is a dynamic process controlled by competing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) (24). TSA, a HDAC inhibitor, rectified IFN- γ expression in KO T cells without affecting T-bet expression (**Fig. 12G and 12H**), which was associated with increased H3K9Ac in the *Ifng* locus (**Fig. 12I**). Collectively, these findings demonstrate that LDHA promotes IFN- γ expression through an epigenetic mechanism of histone acetylation.

Example 4. LDHA Inhibition or Deficiency Inhibits IFN- γ Expression and Reduces T-Cell Mediated Autoinflammation

[0066] To test whether LDHA activity is required for its regulation of cytokine production, a LDHA inhibitor FX11 was used. We found that FX11 dose-dependently inhibited IFN- γ expression in T cells (**Fig. 13A-13B**). Importantly, the inhibitory effect of FX11 on IFN- γ expression was fully nullified by supplementing cells with acetate,

supporting the conclusion that FX11 inhibits IFN- γ expression through modulation of acetyl-CoA levels. These data indicate that LDHA and ACL inhibitors can be useful in treatment of autoimmune/autoinflammatory diseases and rejection of transplant. Also, these inhibitors may be useful in treatment of any other diseases caused by excessive production of pro-inflammatory cytokines, such as sepsis shock in various infections (both bacterial and viral).

[0067] In the absence of endogenous 3'UTR, the *Ifng* transcript in *Yeti/Yeti* mice is stable, resulting in sustained IFN- γ production and a lethal autoinflammatory phenotype (25). Indeed, all *Yeti/Yeti* mice succumbed to death by 3 weeks of age (**Fig. 14A**), which was associated with severe liver damage (**Fig. 14B** and **Fig. 15A**). Deletion of *Ldha* in T cells corrected the liver immunopathology and conferred long-term survival of *Yeti/Yeti* mice (**Fig. 14A**, **14B**, and **Fig. 15A**). LDHA deficiency did not substantially affect the cellularity or activation of *Yeti/Yeti* CD4⁺ T cells (**Fig. 15B-15E**). However, IFN- γ expression in *Yeti/Yeti* CD4⁺ T cells was diminished in the absence of LDHA, while its expression in LDHA-sufficient *Yeti/Yeti* NK cells was not affected (**Fig. 14C** and **14D**). The correction of a lethal autoinflammatory disease in *Yeti/Yeti* mice by T cell-specific deletion of *Ldha* supports that aerobic glycolysis promotes IFN- γ expression in T cells through an epigenetic mechanism of *Ifng* transcription but not via a 3'UTR-dependent mechanism of translation *in vivo*.

[0068] To further explore aerobic glycolysis in control of effector T cell responses, we used Scurfy mice with a mutation in the Treg cell lineage *Foxp3* gene (*Foxp3^{sf}*) (26). LDHA deficiency in T cells corrected the Scurfy phenotype and extended life span of *Foxp3^{sf}* mice (**Fig. 16A** and **16B**), which was associated with reduced inflammation in multiple organs without affecting lymphadenopathy or splenomegaly (**Fig. 16C-16F**). T cell activation and expansion were comparable between *Foxp3^{sf}* and KO *Foxp3^{sf}* mice (**Fig. 16G** and **16H**), which was in line with our *in vitro* findings (**Fig. 6A-6E**). Nevertheless, CD4⁺ T cells produced less IFN- γ in the absence of LDHA (**Fig. 16I** and **16J**), demonstrating a critical role for LDHA-mediated aerobic glycolysis in promoting autoreactive Th1 cell responses.

Example 5. LDHA Inhibition or Deficiency Inhibits IL-17A Expression

[0069] Naïve CD4 T cells (2.5 x 10⁴) were isolated from spleen and peripheral lymph nodes of *Ldha^{fl/fl}* (WT) and CD4^{Cre}*Ldha^{fl/fl}* (KO) mice. T cells were cultured with antigen-presenting cells (APCs) at a ratio of 10:1 (APC:T) in T-cell medium (RPMI-1640, 10% fetal bovine serum, 2 mM L-glutamine, 55 μ M 2-mercaptoethanol, 100 U/mL penicillin, and 100

mg/mL streptomycin) under Th17 differentiation conditions (1 μ g/mL anti-CD3, 1 μ g/mL anti-CD28, 2 ng/mL TGF- β , 10 ng/mL IL-6, and 10 μ g /L α -IL2). WT cells were treated with 0.3 μ M, 10 μ M, or 30 μ M GNE-140, or were untreated. Cultured cells were harvested on day 4, and were stimulated with ionomycin and PMA for 4 hours. Cytokines and transcription factors were analyzed by flow cytometry.

[0070] Under untreated conditions, KO T cells produced a significantly lower amount of IL-17A than WT T cells (**Fig. 17**). GNE-140 treatment inhibited IL-17A expression in WT T cells in a dose-dependent manner. At a concentration of 30 μ M, WT T cells produced a similar level of IL-17A as KO T cells.

Example 6. Inhibition of ACL Is Protective in a Mouse Model of Multiple Sclerosis

[0071] The same mechanism that we proposed for LDHA regulation of Th1 cell differentiation also operates in Th17 cells. We found that LDHA-deficiency resulted in diminished IL-17a production under Th17 culture conditions (**Fig. 18A and 18B**), which was associated with reduced histone acetylation at *Il17a* locus (**Fig. 18C**). Th17 cells play a critical role in experimental autoimmune encephalomyelitis (EAE), a mouse model of the human multiple sclerosis disease. Remarkably, LDHA deficiency in T cells completely protected mice from EAE (**Fig. 18D**), which was associated with reduced production of both IL-17a and IFN- γ from CD4⁺ T cells isolated from spinal cord and cerebellum (**Fig. 18E and 18F**).

[0072] To test whether reduced acetyl-CoA production was sufficient to blunt Th17 response, we added an ACL inhibitor (BMS 303141) to the Th17 culture, and found that it repressed IL-17a production (**Fig. 18G**). Remarkably, *in vivo* treatment of mice with BMS 303141 completely protected mice from EAE (**Fig. 18H**), indicating that ACL inhibition can be used to treat multiple sclerosis.

[0073] Overall, our findings in Examples 1-6 do not support a translational mechanism of aerobic glycolysis in IFN- γ production (12), as LDHA promotes IFN- γ expression independent of its 3'UTR. Previous studies have shown that utilization of glycolytic intermediates for biosynthesis accounts for a small fraction (~ 7%) of the glucose consumed in activated T cells (27), while aerobic glycolysis produces the majority (~ 60%) of ATP (28). Hence, LDHA-mediated aerobic glycolysis may primarily relieve the burden of mitochondria as an energy house to 'burn' carbons to generate ATP. As a result, more citrate can be exported out of mitochondria to generate acetyl-CoA and promote histone

acetylation in selected gene loci (**Fig. 19**). LDHA inhibitors are being developed to target tumor cell metabolism (29). Our data suggest that LDHA inhibitors could be immunosuppressive, and thus may complicate their applications in cancer. Instead, LDHA inhibitors, as well as inhibitors targeting other enzymes involved in acetyl-CoA metabolism, including ACL, may be useful in treatment of T-cell mediated immune diseases, such as autoimmunity and transplant rejection.

Example 7. Materials and Methods

Mice

[0074] Mice with one targeted allele of *Ldha* (*Ldha*^{tm1a(EUCOMM)Wtsi}) were obtained from EUCOMM, in which the third exon was flanked by two loxp sites. The mice were first crossed with a transgenic Flipase strain (Jackson Laboratory) to remove the Neo cassette, and then crossed with CD4^{Cre} transgenic mice to specifically delete *Ldha* in T cells. *Yeti* mice were kindly provided by Dr. Richard Locksley (UCSF). C57BL/6, CD4^{Cre} and *Foxp3*^{sf} mice were purchased from the Jackson Laboratory. All mice were maintained in the MSKCC animal facility under SPF conditions, and animal experimentation was conducted in accordance with institutional guidelines.

Antibodies

[0075] Anti-LDHA (2012S), anti-GAPDH (D16H11), anti-Histone 3 (D2B12), and normal rabbit IgG were purchased from Cell Signaling. Anti-LDHB (60H11), anti-Histone H3 (acetyl K9) (ab4441), anti-Histone H3 (acetyl K27) (ab4729), and anti-RNA polymerase II CTD repeat YSPTSPS (ab5131) were obtained from Abcam. Fluorescent-dye-labeled antibodies against CD4, CD8, TCR-β, CD44, CD62L, CD69, CD25, CD40L, IFN-γ, NK1.1, Foxp3, and T-bet were purchased from eBiosciences, BioLegend, or Tonbo. Blocking antibodies for FcγR (2.4G2) and IL-4 (11B11), as well as anti-CD3 (145-2C11) and anti-CD28 (37.51) were obtained from Bio X Cell.

Flow Cytometry

[0076] Cells from thymi, spleens, and lymph nodes were depleted of erythrocytes by hypotonic lysis. Cells were incubated with specific antibodies for 15 min on ice in the presence of anti-FcγR to block FcγR binding. Dead cells were excluded by DAPI (Invitrogen) staining. To determine cytokine expression, cells were stimulated with 50 ng/mL phorbol 12-myristate 13-acetate (Sigma) and 1 μM ionomycin (Sigma) in the presence of GolgiStop (BD Biosciences) for 4 h. After stimulation, cells were stained with

cell-surface marker antibodies and LIVE/DEAD Fixable dye (Invitrogen) to exclude dead cells, fixed, and permeabilized with a transcription factor staining kit (eBioscience), followed by staining with cytokine antibodies. All samples were acquired with an LSR II flow cytometer (Becton, Dickinson) and analyzed with FlowJo software (TreeStar).

In Vitro Cell Culture

[0077] CD62L⁺CD44⁺CD25⁻CD4⁺ T cells were isolated from spleen and peripheral lymph nodes of wild-type (WT) or CD4^{Cre}*Ldha*^{fl/fl} (KO) mice with naïve CD4⁺ T cells isolation kits (Miltenyi or Stem Cell). Purity was checked by flow cytometry, and was over 98%. For *in vitro* T cell activation, $0.2 - 0.5 \times 10^6$ naïve CD4⁺ T cells were cultured on a 24-well plate pre-coated with 5 µg/mL anti-CD3 in T cell medium [RPMI/1640, 10% fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 1x non-essential amino acid (Invitrogen), 10 mM HEPES, 55 µM 2-mercaptoethanol, 100 U/ml penicillin, 100 mg/ml streptomycin] supplemented with 2 µg/mL anti-CD28 and 100 U/ml IL-2. For T helper 1 (Th1) cell differentiation, T cells were cultured with the addition of 10 µg/mL anti-IL-4 and 10 ng/mL IL-12. For T helper 17 (Th17) cell differentiation, T cells were cultured with the addition of 10 µg/mL anti-IL-4, 10 µg/mL anti-IFN-γ, 10 µg/mL anti-IL-2, 2 ng/ml TGF-β, 10 ng/ml IL-6, 10 ng/ml IL-23, and 10 ng/mL IL-1β. For cell proliferation experiments, naïve CD4⁺ T cells were labeled with CFSE (Invitrogen), and activated on a 24-well plate, pre-coated with 5 µg/mL anti-CD3, in T cell culture medium supplemented with 2 µg/mL anti-CD28 and 100 U/mL IL-2. CFSE dilution was assessed by flow cytometry on days 1, 2, and 3 post-activation. For sodium acetate and TSA treatment experiment, cells were differentiated under Th1 conditions for 3 days, and cultured in T cell medium supplemented with 100 U/mL IL-2, 10 µg/mL anti-IL-4, 10 ng/mL IL-12, and the indicated concentrations of sodium acetate (Sigma), TSA (Promega), or vesicle for 24 h. For ATP citrate lyase (ACL) inhibitor experiments, naïve CD4⁺ T cells isolated from WT mice were cultured under Th1 conditions for 2 days or under Th17 conditions for 3 days. DMSO and indicated concentrations of ATP citrate lyase inhibitor (BMS-303141, Sigma) were added into the culture for another 24 hours. For LDHA inhibitor experiments, naïve CD4⁺ T cells isolated from WT mice were cultured under Th1 or Th17 conditions for 3-4 days. DMSO and indicated concentrations of FX11 or GNE-140 were added into the culture for another 24 hours.

3' UTR GFP Reporter Assay

[0078] 3'UTR vectors were co-transfected with pCL-Eco helper plasmid into Phoenix cells, and the culture supernatant was used to infect activated T cells. Naïve CD4⁺ T cells isolated from WT and KO mice were activated with plated-bound anti-CD3 and soluble anti-CD28 for 2 days, and transduced with retroviral supernatants via spin-infection (2600 rpm for 2 h at 35 °C) and cultured for another 2 days. The GFP signal was examined by flow cytometry.

Zymography for LDH Isoenzymes

[0079] Heart and muscle tissues were harvested from mice perfused with ice-cold PBS, and homogenized to single cells with non-denaturing cell lysis buffer (Cell Signaling). Activated CD4⁺ T cells and tissues were lysed with non-denaturing cell lysis buffer on ice for 20 min, and the cell lysate was centrifuged at 20,000 g for 10 min at 4 °C. The supernatants were diluted with 2X native loading buffer (Biorad), and subjected to electrophoresis with precast 4 - 20% native gels (Biorad). The gels were subsequently incubated in a development solution containing 100 mM Tris-HCl (pH 8.0), 0.3 mM NAD (Sigma), 0.1 M sodium lactate (Sigma), 0.5 mM phenazin-methosulphate (Sigma) and 0.4 mM tetrazolium-blue (Sigma) at 37 °C for 30 min.

Immunoblotting

[0080] Cells were lysed with a cell lysis buffer (Cell Signaling), and protein concentrations were determined with a BCA kit (Thermo Scientific). Protein extracts were separated by SDS-PAGE gels and transferred to nitrocellulose blotting membranes (GE Healthcare). The membranes were probed with antibodies and visualized with the Immobilon Western Chemiluminescent HRP Substrate (Millipore).

Metabolic Assays

[0081] To determine glucose consumption and lactate production of activated CD4⁺ T cells, culture medium was replaced with fresh medium 48 h after T cell activation, and collected 24 h later. Medium alone in wells of the same plate was used as control. Glucose and lactate levels in culture media were measured with a glucose assay kit II (BioVison) and lactate assay kit II (Sigma).

[0082] For measurement of cytosolic acetyl-CoA, naïve CD4⁺ T cells were differentiated under Th1 conditions for 2 days and then cultured in fresh T cell medium for another 24 h. Cells were lysed with lysis buffer (1% Triton X-100, 20 mM Tris-HCl, pH=7.4, 150 mM

NaCl) on ice for 10 min. The lysates were spun at 20,000 g for 10 min at 4 °C, the pellets (nuclei and heavy membrane) were discarded, and the supernatants were used for acetyl-CoA measurement with an Acetyl-CoA Assay Kit (Sigma). Freshly isolated naive WT and KO CD4⁺ T cells were rested in T cell medium with 10 ng/mL IL-7 for 4 h at 37 °C in the incubator, and the level of acetyl-CoA was measured.

[0083] OCR and ECAR were measured with an XF96 extracellular flux analyzer (Seahorse Bioscience), following protocols recommended by the manufacturer. Briefly, freshly isolated CD4⁺ naïve or activated cells were seeded on XF96 microplates (150,000 cells/well) that had been pre-coated with Cell-Tak adhesive (BD Biosciences). The plates were quickly centrifuged to immobilize cells. Cells were maintained in a non-buffered assay medium (Seahorse Biosciences) in a non-CO₂ incubator for 30 min before the assay. Glycolysis was measured with XF glycolysis stress test kit (Seahorse Biosciences). Initially, cells were incubated in the glycolysis stress test medium without glucose, and ECAR was assessed. Three baseline recordings were made, followed by sequential injection of 10 mM glucose and 1 μM oligomycin, which inhibited mitochondrial ATP production and shifted the energy production to glycolysis. The increased ECAR revealed the maximum glycolytic capacity of T cells. The final injection was 100 mM 2-DG, a glucose analog that inhibited glycolysis. The resulting decrease of ECAR confirmed that the ECAR observed in experiments was due to glycolysis. The Mito stress test kit (Seahorse Biosciences) was used to test OCR under different conditions. Three baseline recordings were made, followed by sequential injection of 1 μM oligomycin, 0.25 μM FCCP, which uncoupled oxygen consumption from ATP production to obtain maximal OCR, and 0.5 μM rotenone/antimycin A, which inhibited complex I and III.

[0084] Metabolic flux analysis of ¹³C₆-glucose in Th1 cells was determined by CE-MS. Briefly, ~10 million WT or KO Th1 cells were labeled with ¹³C₆-glucose (10 mM) in glucose-deficient RPMI1640 supplemented with 10% dialyzed FBS and 100 U/mL IL-2 for 2 h. Metabolites were extracted according to the Floating Cells Protocol E-150782, and shipped to Human Metabolome Technologies Inc. (Boston) for CE-MS analysis (F-SCOPE). Data were from three biological replicates.

RNA-Seq and Quantitative RT-PCR

[0085] Total RNA was extracted with RNeasy kit (QIAGEN), and cDNA libraries were generated and sequenced using a HiSeq 2000 platform (Illumina) at the Integrated Genomics Operation of MSKCC. For quantitative RT-PCR, RNA was reverse transcribed with

Superscript III (Invitrogen). mRNA levels were normalized to β -Actin. The primers used were: *Actin*-F: 5'-GGCACCACACCTTCTACAATG-3' (SEQ ID NO: 1); *Actin*-R: 5'-GTGGTGGTGAAGCTGTAGCC-3' (SEQ ID NO: 2). *Ifng*-F: 5'-CTTTGGACCCTCTGACTTGAG-3' (SEQ ID NO: 3), *Ifng*-R: 5'-TTCCACATCTATGCCACTTGAG-3' (SEQ ID NO: 4).

RNA-Seq Analysis

[0086] Quality Control of raw reads was done using FastQC (v0.11.2) (10) to ensure that there were no major flaws in sequencing. They were then mapped to mm10 genome using STAR (v2.3.0e_r291) (30) and default parameters. The mapped reads were counted using htseq-count (v0.6.0, parameters -t exon) (31) and gene models from Ensembl (Mus_musculus.GRCm38.75.gtf). Differential expression was performed using DESeq2 (v1.2.10, default parameters) (32). Human orthologs were retrieved from Ensembl v75. DESeq2 normalized counts with human orthologs were provided to GSEA (v2-2.0.14) (33) for gene set enrichment analysis.

ChIP-Seq and ChIP-qPCR

[0087] Chromatin immunoprecipitations were performed with SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling) according to manufacturer's instructions. Briefly, naïve CD4⁺ T cells were differentiated to Th1 cells for 3 days, and were fixed for 10 min at 25 °C with 1% formaldehyde. After incubation, glycine was added to a final concentration of 0.125 M to quench formaldehyde. Subsequently, cells were lysed, and chromatin was harvested and fragmented using enzymatic digestion, followed by sonication. The chromatin was then subjected to immunoprecipitation with anti-H3K9Ac at 4 °C overnight, and was incubated with protein G magnetic beads at 4 °C for 2 h. The immune complexes were washed and eluted in 150 μ L elution buffer. Elute DNA and input DNA were incubated at 65 °C to reverse the crosslinking. After digestion with proteinase K, DNA was purified with spin columns. The library was prepared with KAPA library preparation kit (Kapa Biosystems) and sequenced using a HiSeq 2000 platform (Illumina) at the Integrated Genomics Operation of MSKCC. The relative abundance of precipitated DNA fragments was analyzed by qPCR using Power SYBR Green PCR Master Mix (Qiagen) and the enrichments were normalized to *Cd3e* promoter. The following primers were used for qPCR: *Cd3e* promoter-F: 5'-TCAGTGTGGAGGTGCTTTG-3' (SEQ ID NO: 5), *Cd3e* promoter-R: 5'-CAGCCTTCCCATAAGGATGAA-3' (SEQ ID NO: 6); *Ifng* promoter-F: 5'-GGAGCCTTCGATCAGGTATAAA-3' (SEQ ID NO: 7), *Ifng* promoter-R: 5'-

CTCAAGTCAGAGGGTCCAAAG-3' (SEQ ID NO: 8); CNS22-F: 5'-
GAGGCCAAATTTCTGCTCATTG-3' (SEQ ID NO: 9), CNS22-R: 5'-
GTTCTTTCAGGAAGCCCGTTA-3' (SEQ ID NO: 10).

ChIP-Seq Analysis

[0088] Reads were first trimmed using Trimmomatic (v0.33) (34). Reads were trimmed if first/last 3 nucleotides had phred quality score of < 15, or at the point where a sliding window of 4 nucleotides averaged a phred quality score < 15. Illumina adapters were also removed. The reads were then aligned using bowtie2 (v 2.2.6, options -fr --no-discordant) (35). Multimapping reads were removed after alignment. Peak calling was done using MACS2 (v2.1.0) (36) on pooled replicates and individual samples using p-value cutoff of 0.01. The peaks were then filtered further using IDR (37) to make sure the peaks were consistent among replicates. The promoter was annotated as region within 2,000 bp from TSS, intron was annotated as region within the gene body but not in the promoter region, and peaks at regions outside but close to gene body were annotated as intergenic.

[0089] RNA-seq and ChIP-Seq data are deposited in the Genome Expression Omnibus under accession number GSE86188, the content of which is incorporated herein by reference.

Histopathology

[0090] Tissues from euthanized animals were fixed in Safefix II (Fisher) and embedded in paraffin. 5-µm sections were stained with haematoxylin and eosin.

Statistical Analysis

[0091] Statistical tests were performed with Prism (GraphPad). A value of $p < 0.05$ were considered statistically significant. All error bars represent standard error (SD).

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[0092] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance. The present invention is further described by the following claims.

CLAIMS

1. A method of treating or preventing a T cell-mediated disease or disorder, the method comprising administering to a subject having or susceptible to developing a T cell-mediated disease or disorder a composition comprising an effective amount of a lactate dehydrogenase A (LDHA) inhibitor.
2. The method of claim 1, wherein the LDHA inhibitor is selected from the group consisting of FX11, Galloflavin, GNE-140, GSK 2837808A, and NHI 2.
3. The method of claim 2, wherein the LDHA inhibitor is FX11.
4. A method of treating or preventing a T cell-mediated disease or disorder, the method comprising administering to a subject having or susceptible to developing a T cell-mediated disease or disorder a composition comprising an effective amount of an ATP-citrate lyase (ACL) inhibitor.
5. The method of claim 4, wherein the ACL inhibitor is selected from the group consisting of BMS 303141, ETC-1002, and SB 204990.
6. The method of claim 5, wherein the ACL inhibitor is BMS 303141.
7. The method of claim 1 or claim 4, wherein the composition is administered orally.
8. The method of claim 1 or claim 4, wherein the composition is administered intravenously.
9. The method of claim 1 or claim 4, wherein the subject is a human.
10. The method of claim 1 or claim 4, wherein the T cell-mediated disease or disorder is an autoimmune disorder, graft rejection, inflammation, or organ rejection.
11. The method of claim 10, wherein the autoimmune disorder is selected from the group consisting of graft-versus-host disease (GVHD), inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, rheumatoid arthritis, systemic lupus erythematosus (SLE), and type-1 diabetes.
12. The method of claim 10, wherein the composition is administered prophylactically within about 24 hours of an organ transplant or tissue graft.

13. A composition comprising a lactate dehydrogenase A (LDHA) inhibitor for use in the treatment of a T cell-mediated disease or disorder.
14. A composition comprising an ATP-citrate lyase (ACL) inhibitor for use in the treatment of a T cell-mediated disease or disorder.
15. The composition of claim 13 or claim 14, wherein the LDHA inhibitor is selected from the group consisting of FX11, Galloflavin, GNE-140, GSK 2837808A, and NHI 2.
16. The composition of claim 13 or claim 14, wherein the ACL inhibitor is selected from the group consisting of BMS 303141, ETC-1002, and SB 204990.
17. The composition of claim 13 or claim 14, wherein the T cell-mediated disease or disorder is an autoimmune disorder, graft rejection, inflammation, or organ rejection.
18. The composition of claim 13 or claim 14, wherein the autoimmune disorder is selected from the group consisting of graft-versus-host disease (GVHD), inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, rheumatoid arthritis, systemic lupus erythematosus (SLE), and type-1 diabetes.

Figure 1

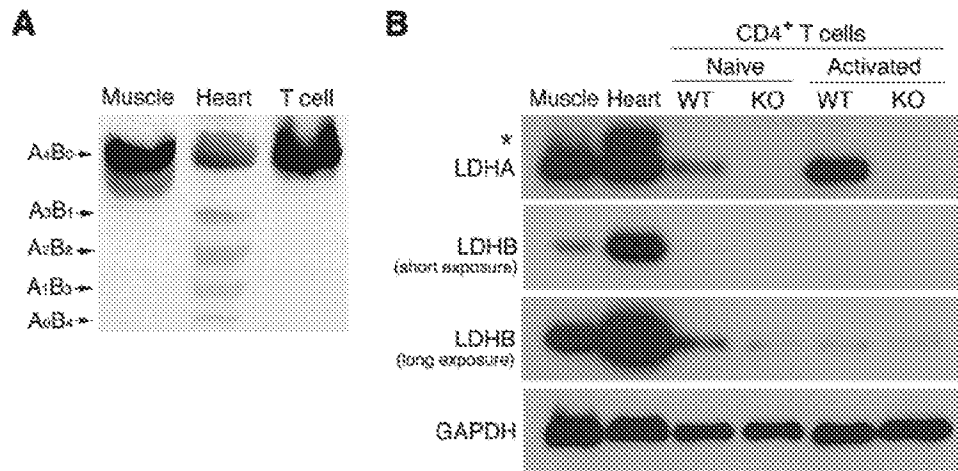
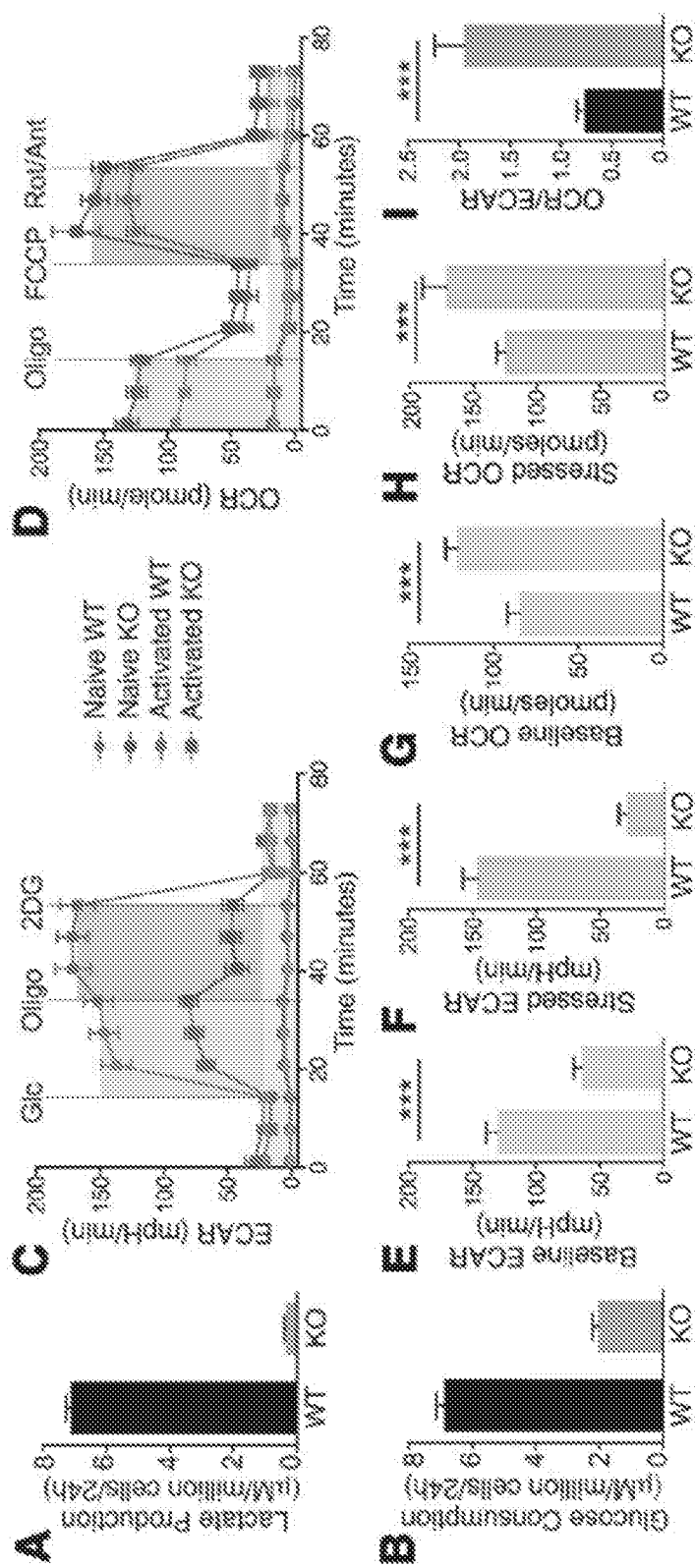


Figure 2



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Figure 3

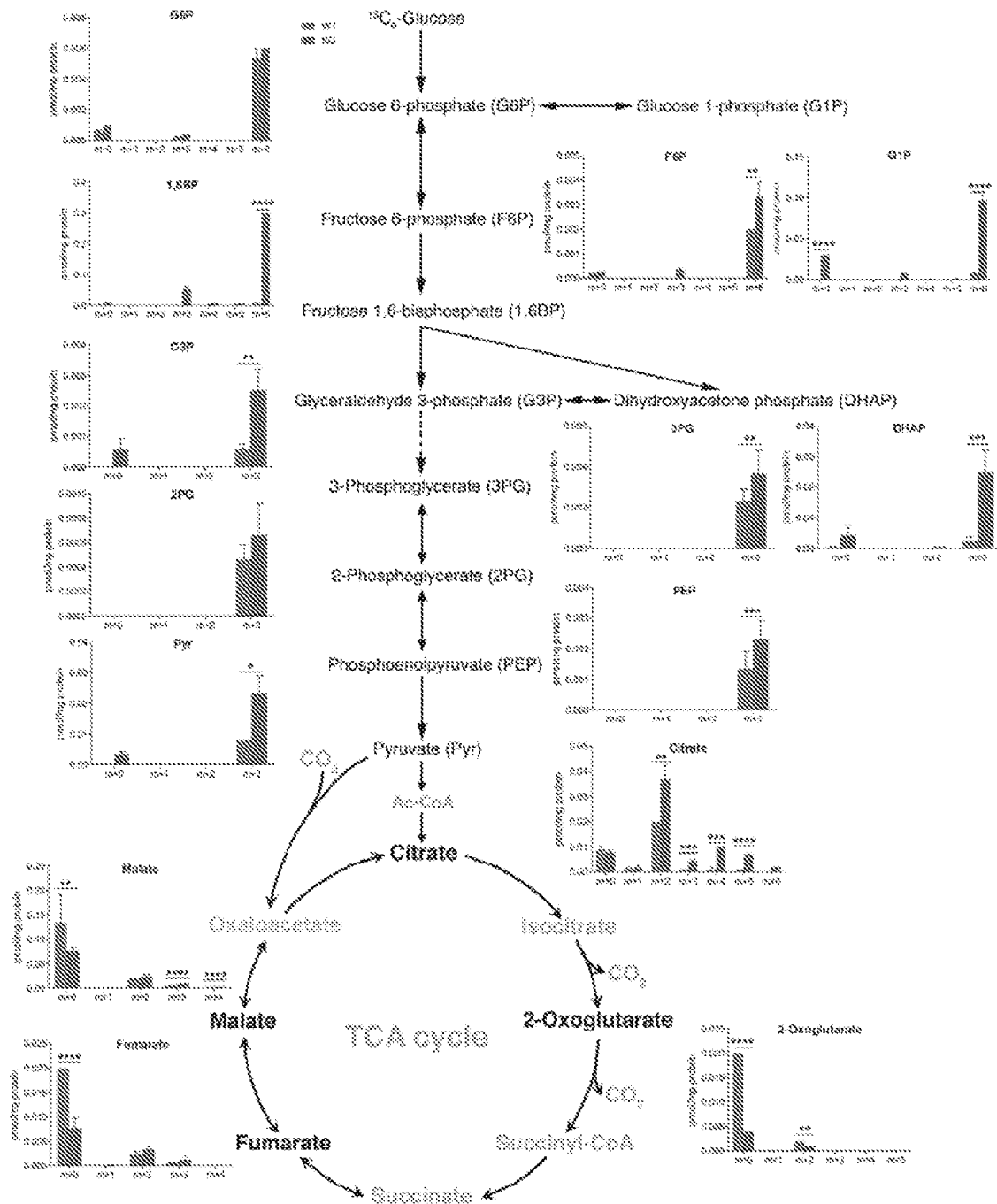


Figure 4

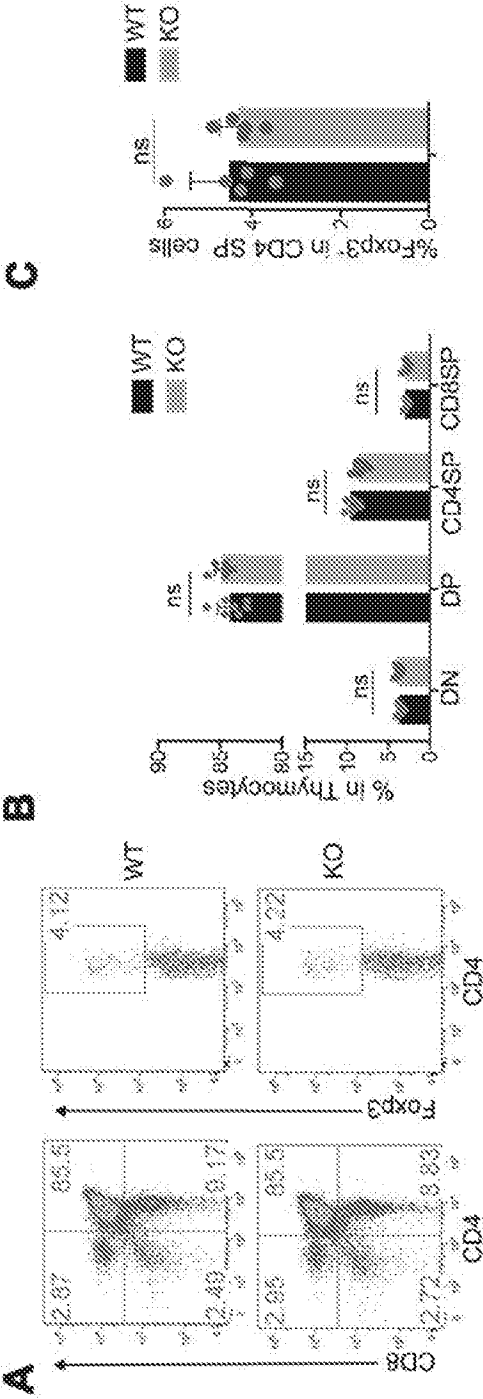


Figure 5

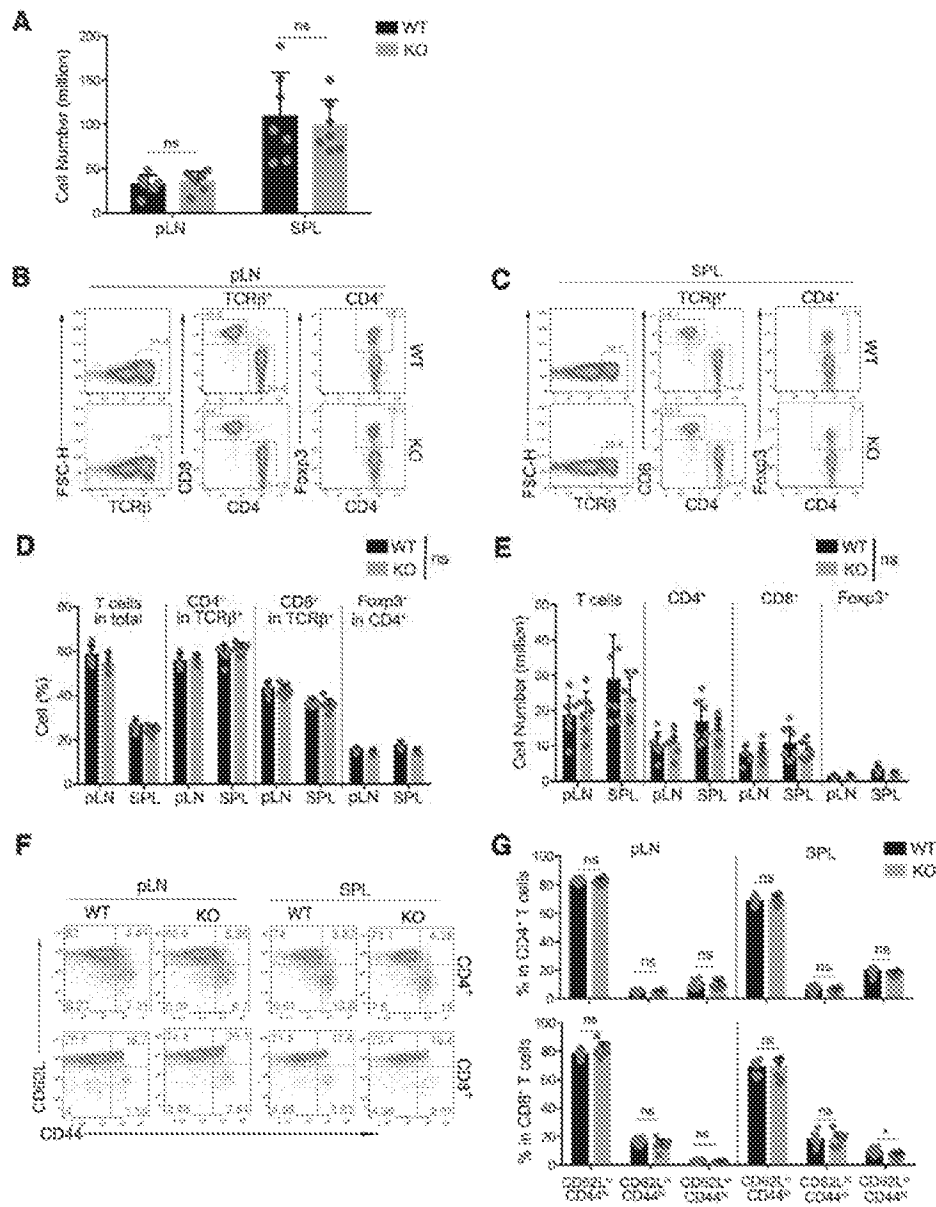


Figure 6

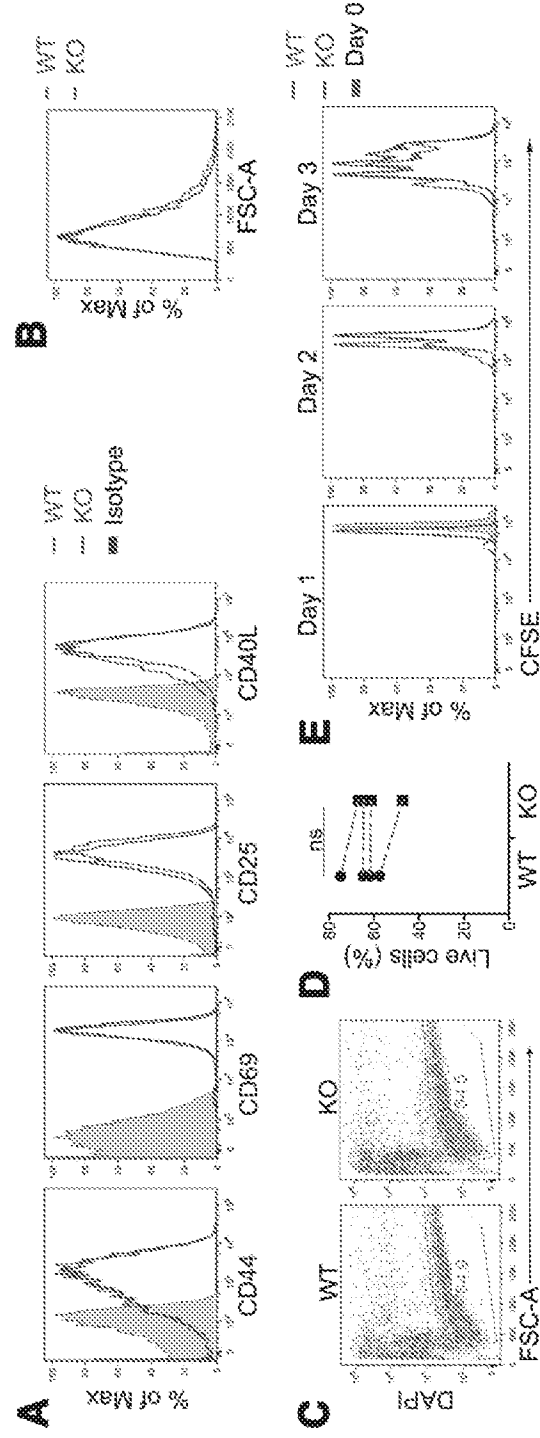


Figure 7

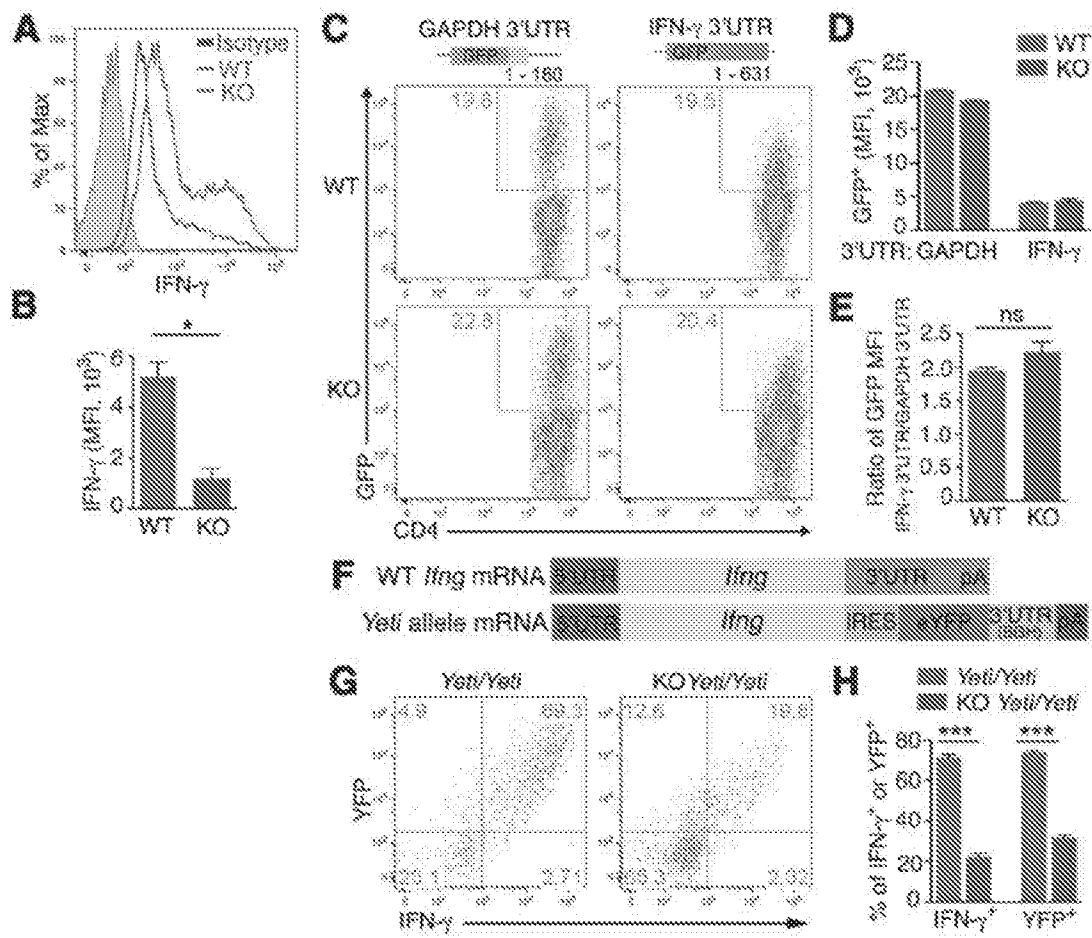


Figure 8

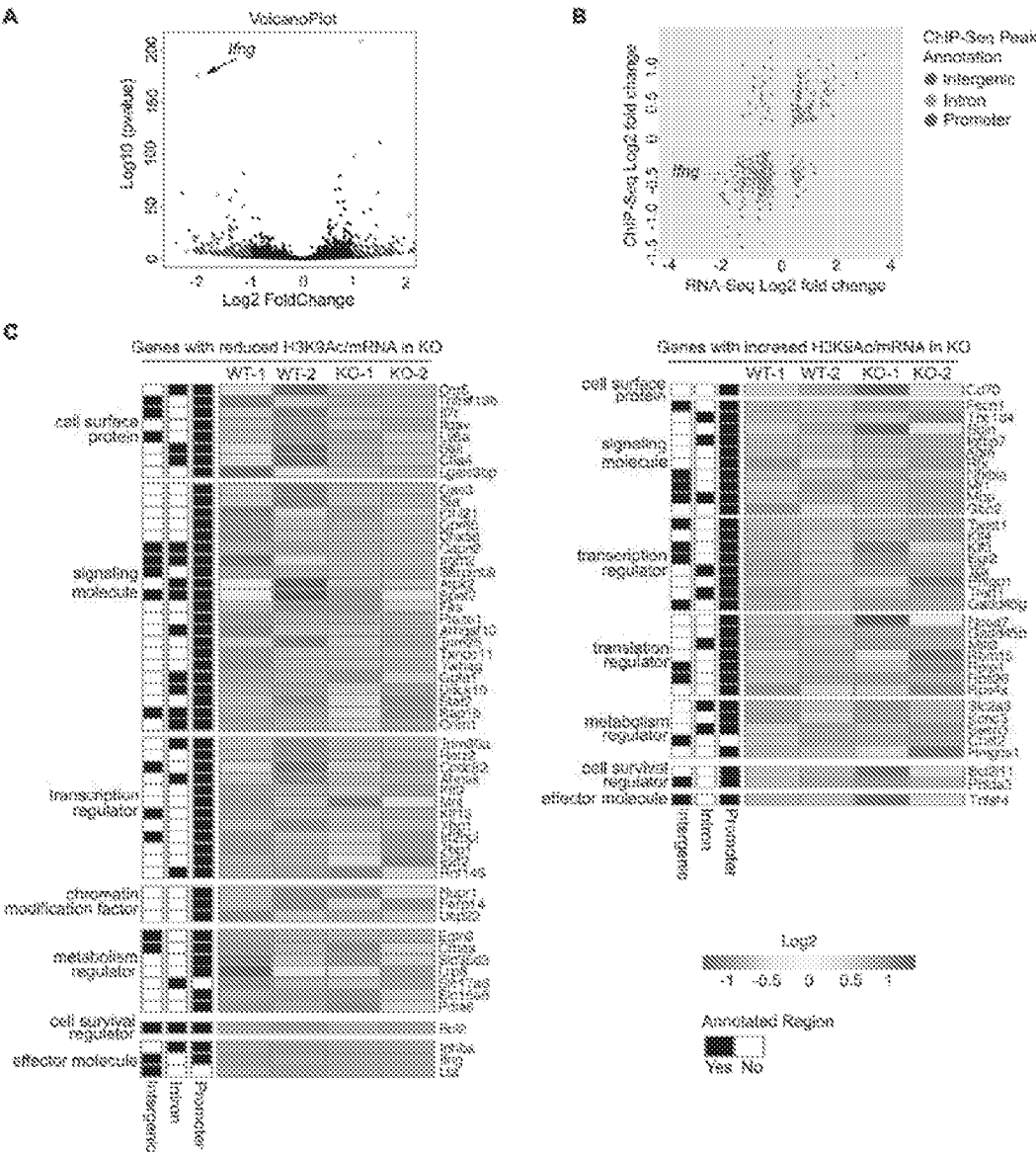


Figure 9

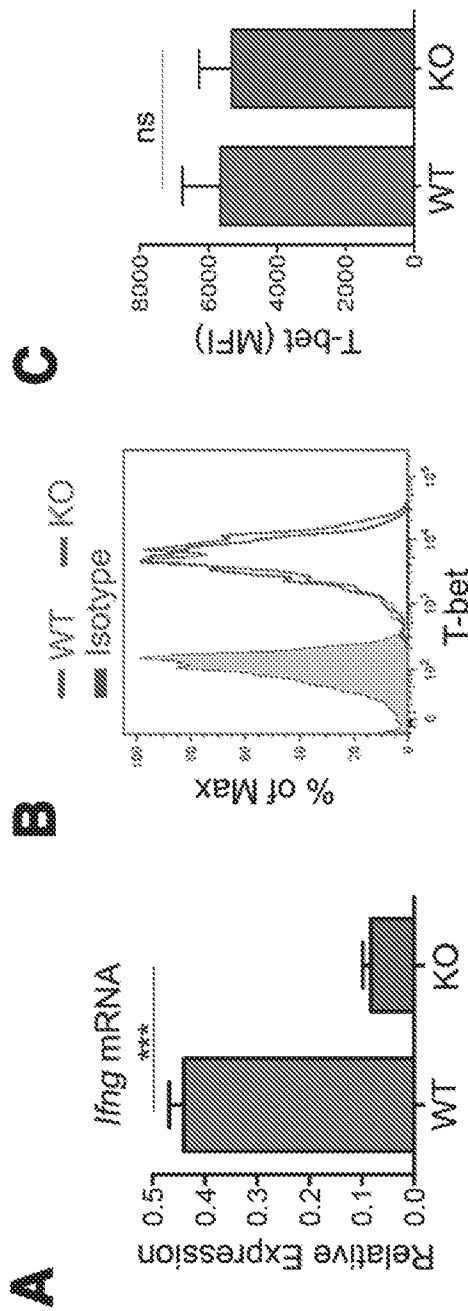


Figure 10

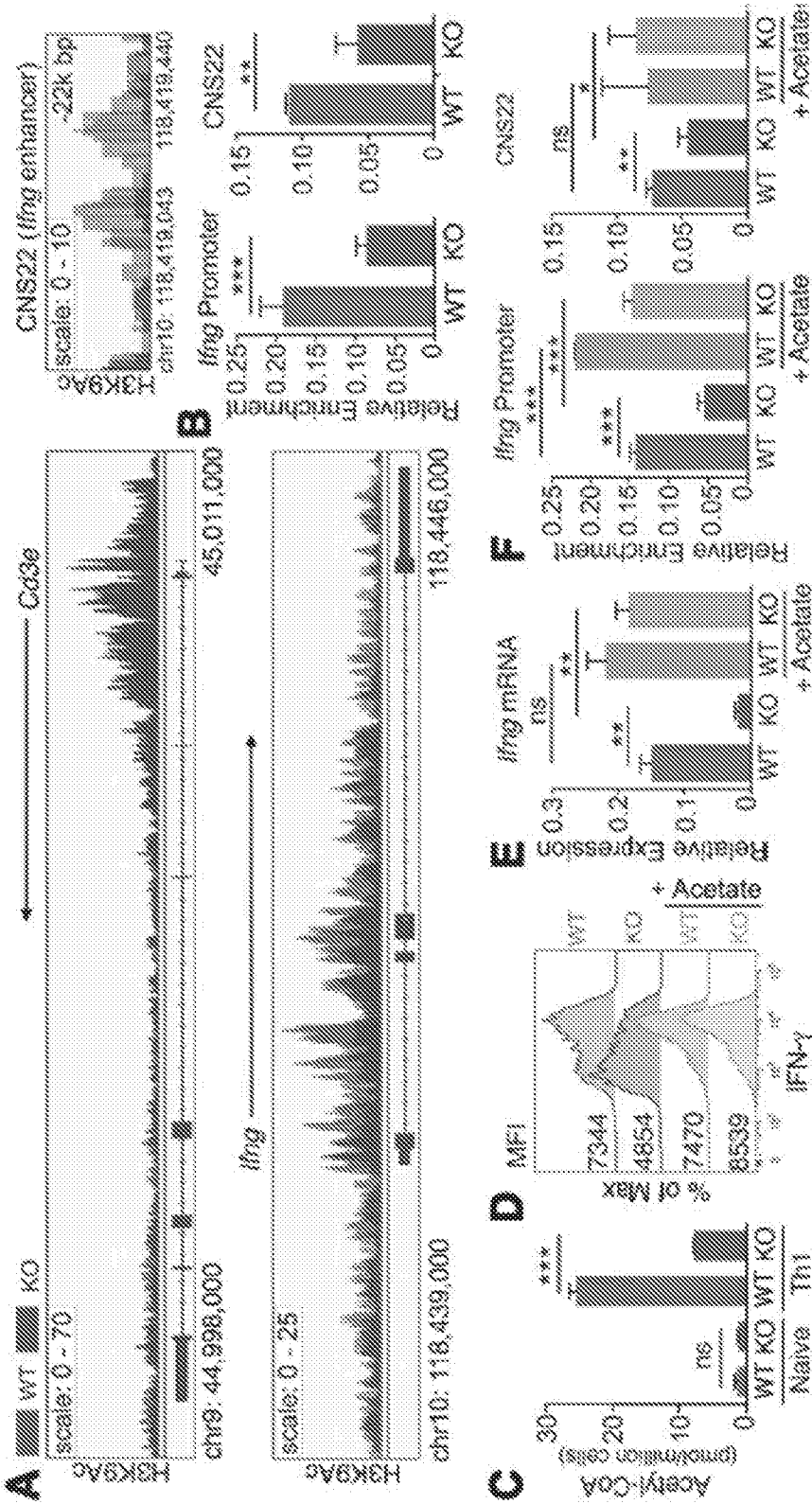


Figure 11

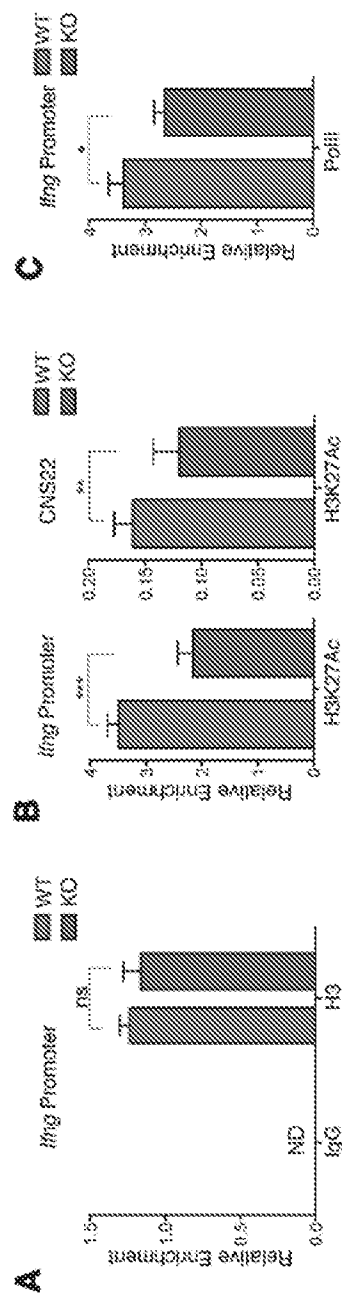


Figure 12

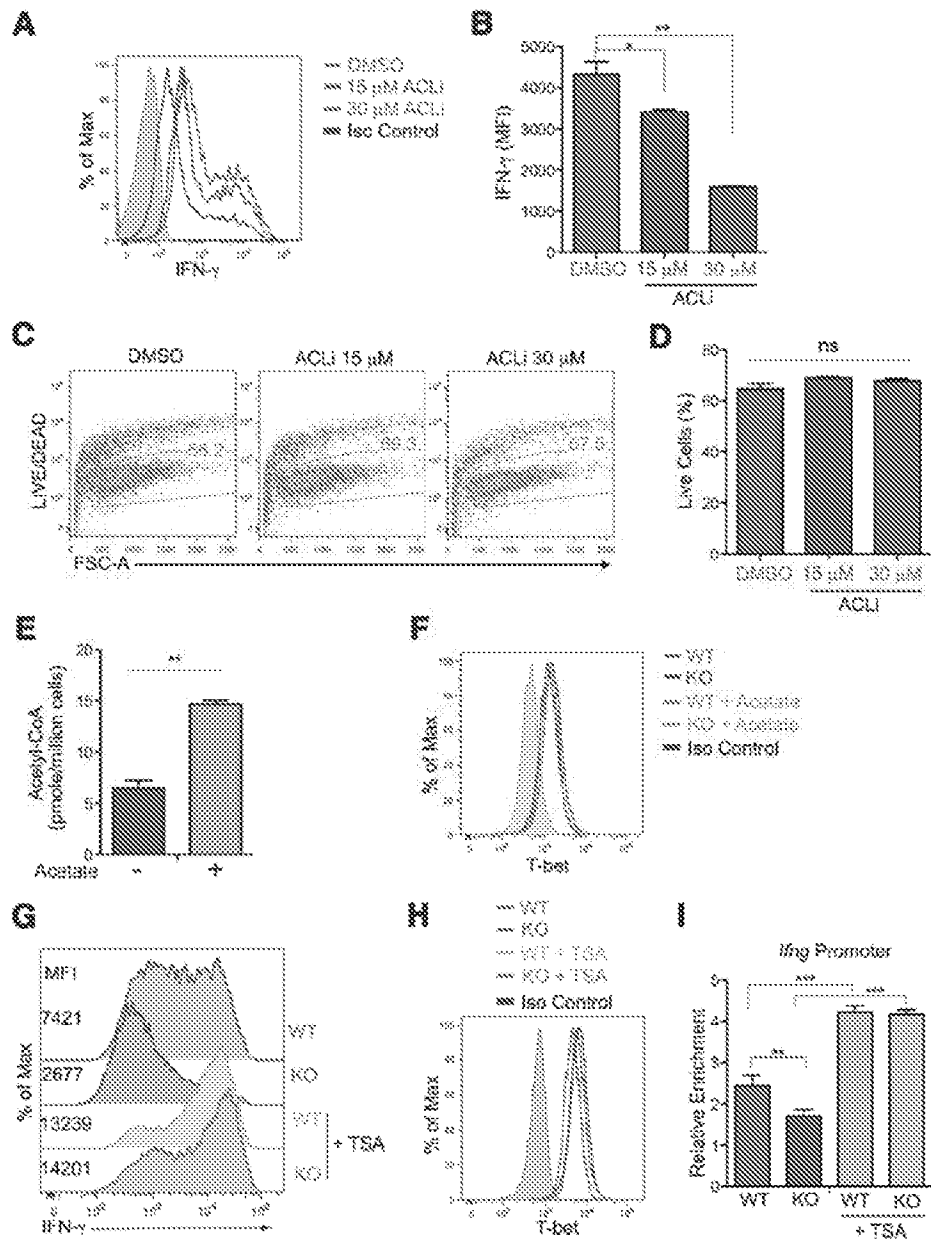


Figure 13

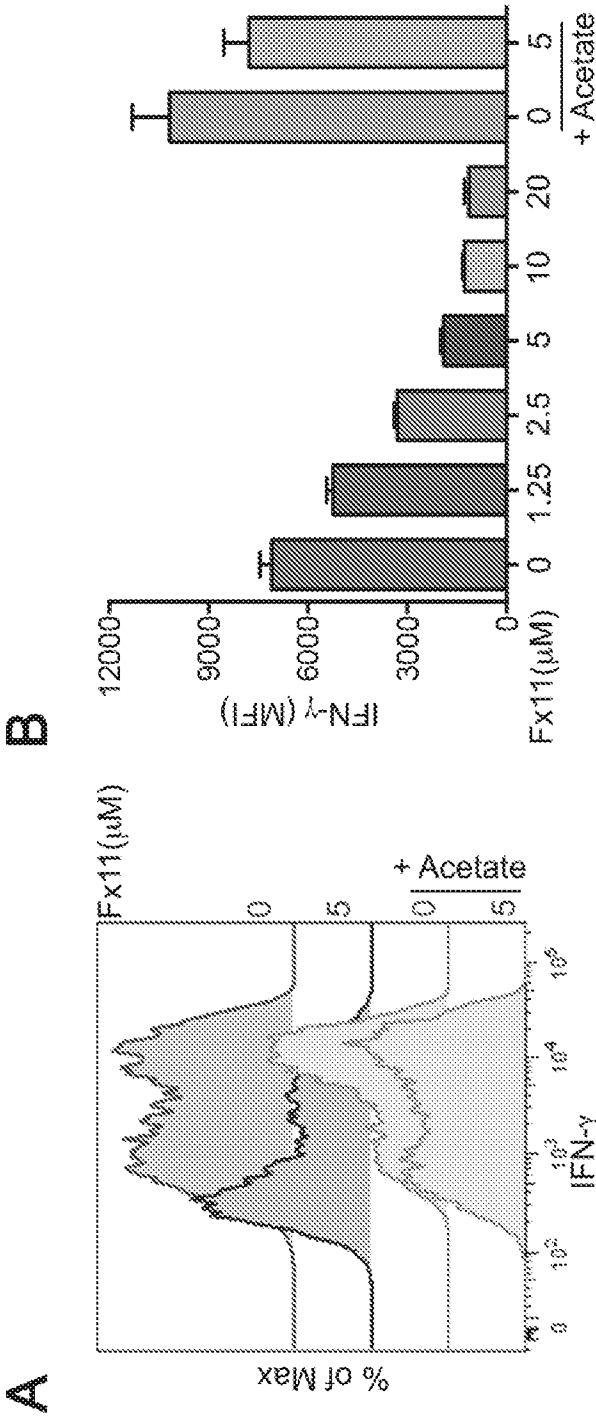


Figure 14

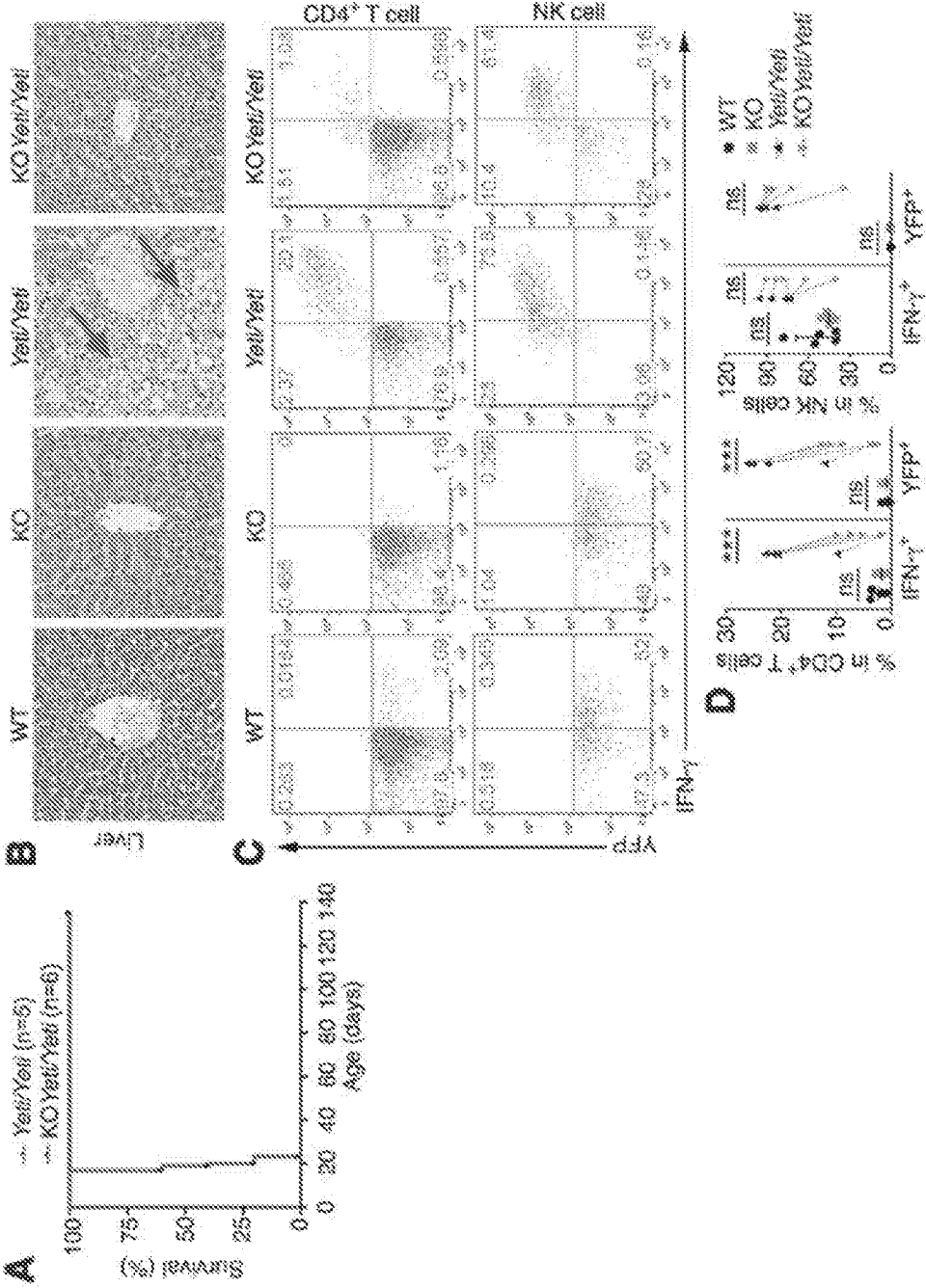


Figure 15

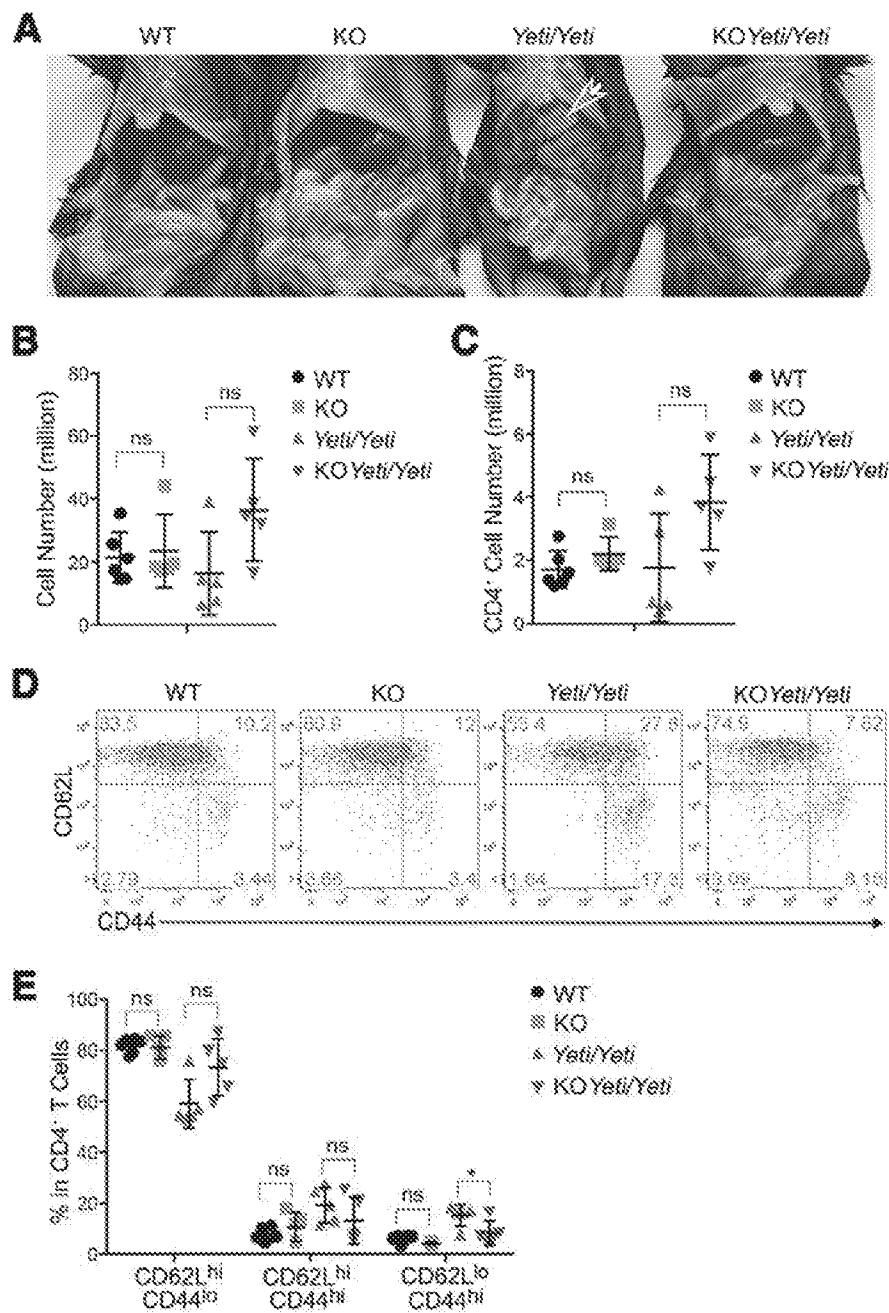


Figure 16

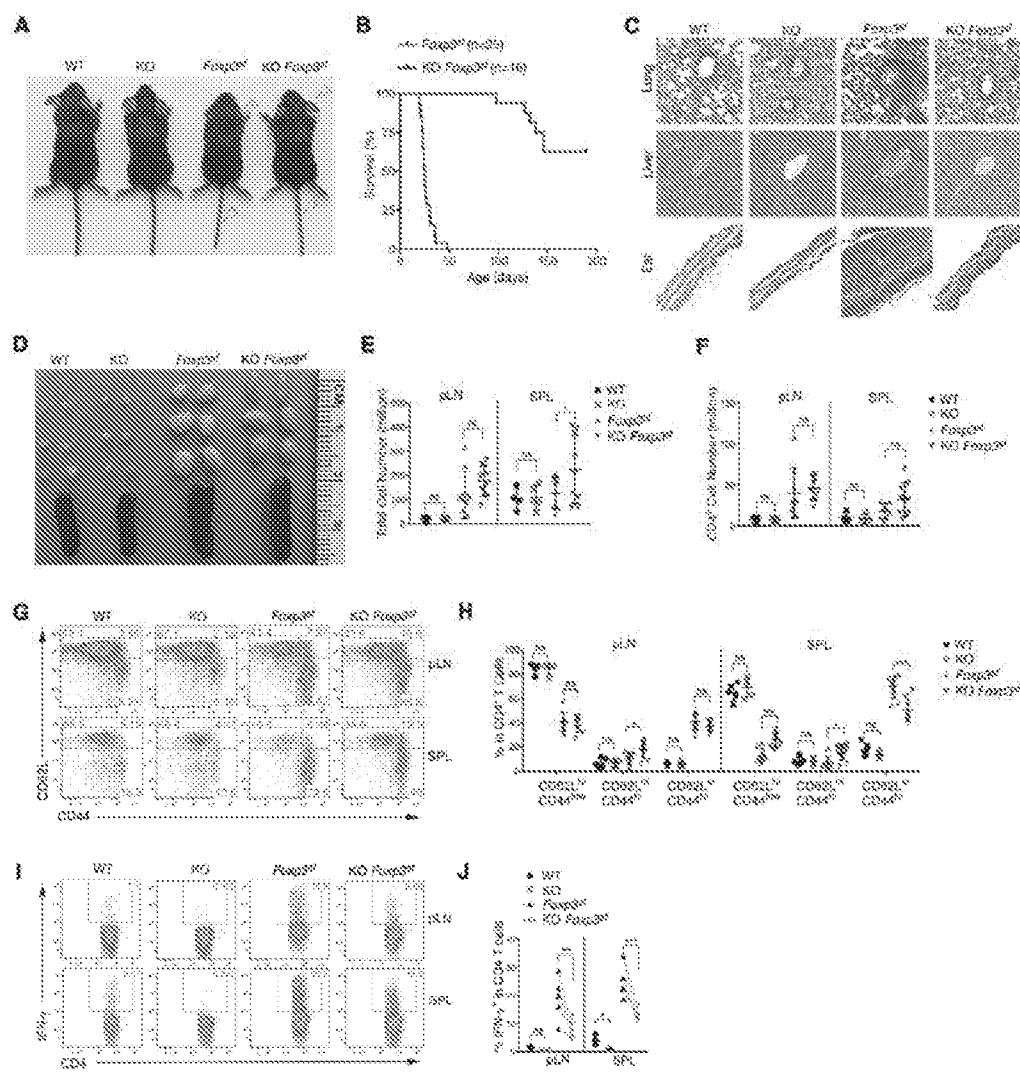


Figure 17

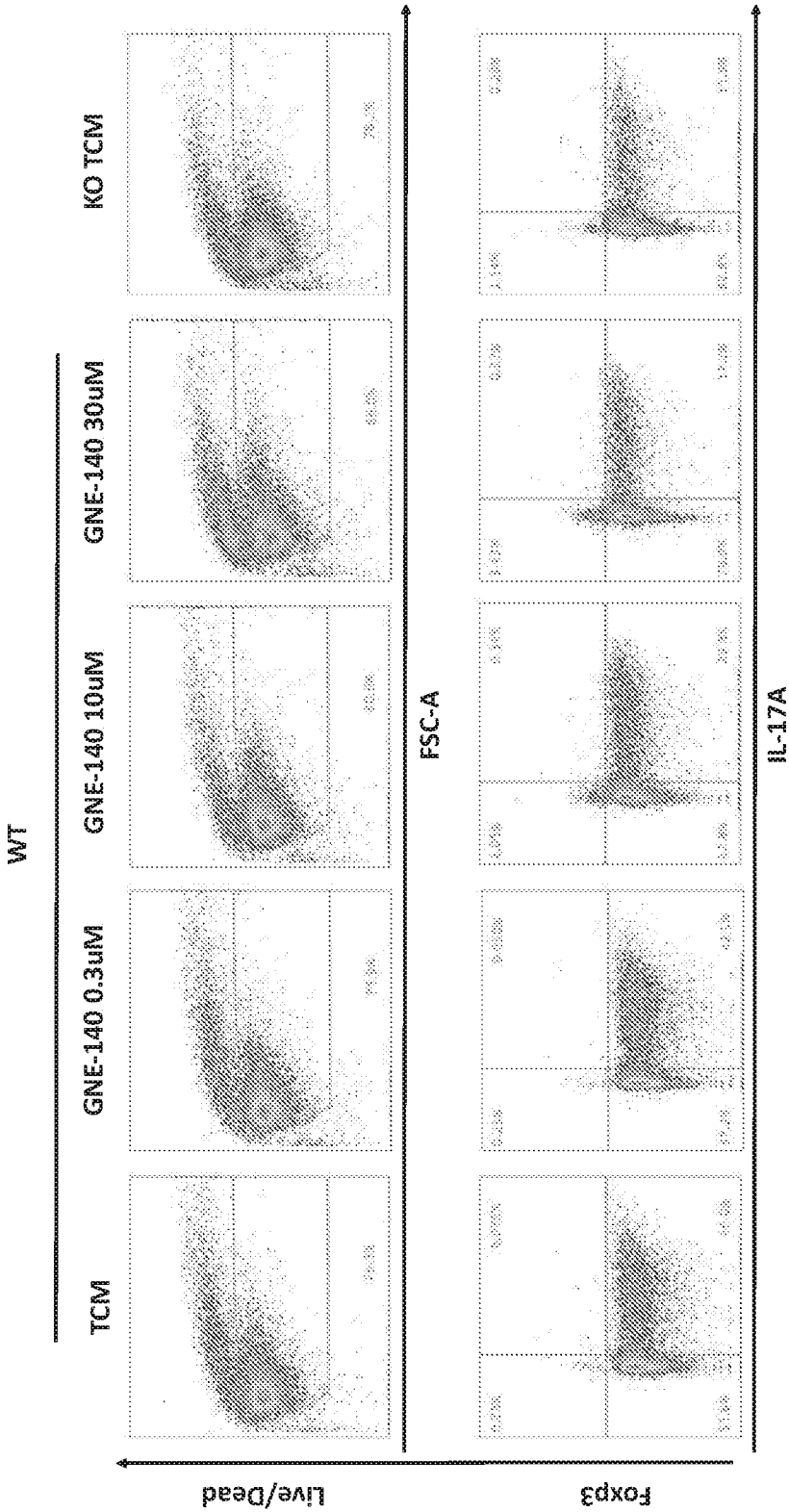
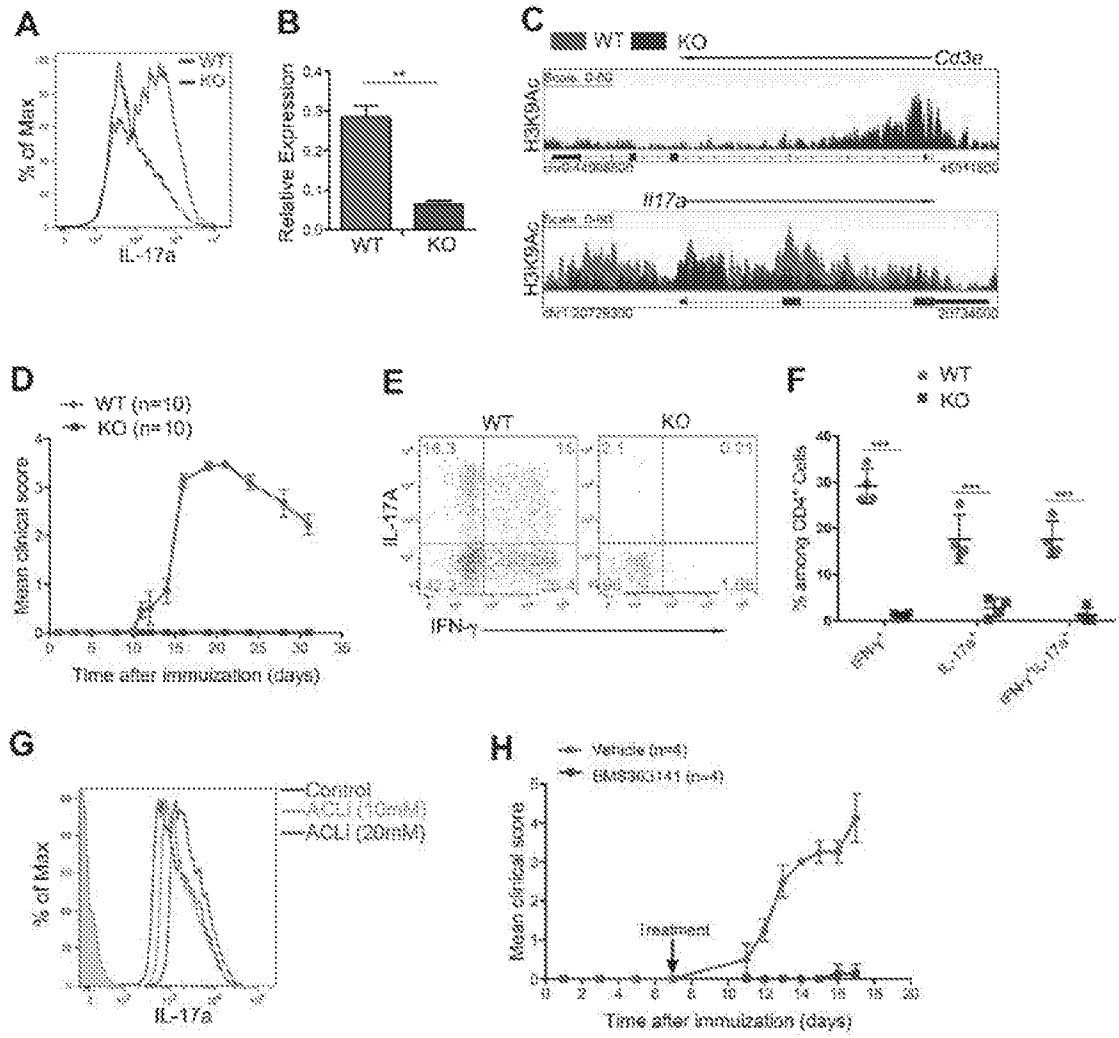
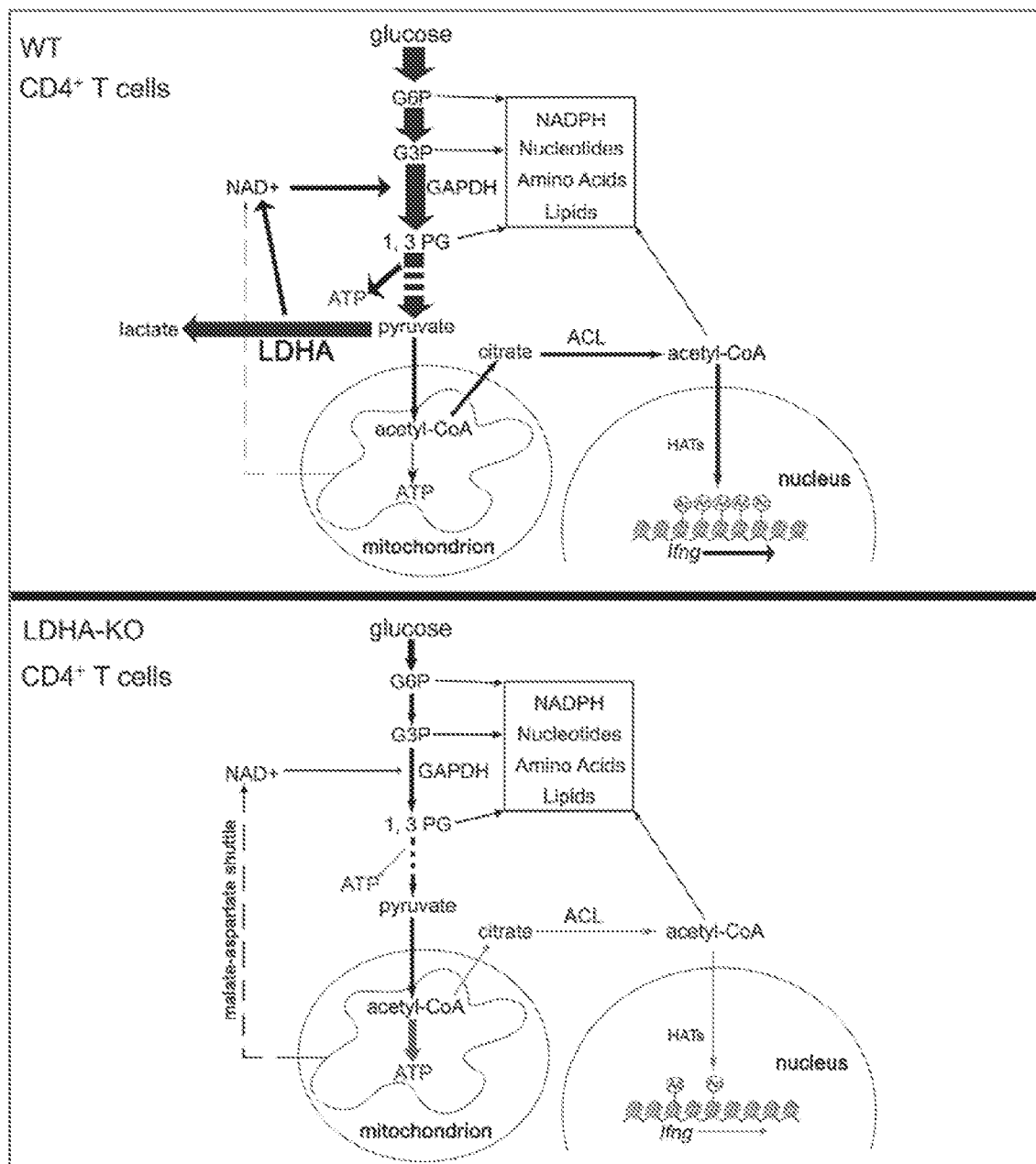


Figure 18



19/19

Figure 19



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/51724

A. CLASSIFICATION OF SUBJECT MATTER
 IPC - A61K 31/4178; C12N 15/113 (2017.01)
 CPC - A61K 31/4178; C12N 15/1137

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0135385 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 15 May 2014; abstract; paragraphs [0010], [0013], [0060]	1-3, 4/1, 8/1, 9/1, 10/1, 11/10/1, 12/10/1, 13, 15/13, 17/13, 18/13
Y	US 2015/0335674 A1 (KOTTMANN, RM et al.) 26 November 2015; abstract; paragraphs [0033], [0053], [0283]-[0285]	1-3, 4/1, 8/1, 9/1, 10/1, 11/10/1, 12/10/1, 13, 15/13, 17/13, 18/13
Y	US 2015/0190433 A1 (ANTHROGENESIS CORPORATION) 09 July 2015; claims 19, 29	12/10/1

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

☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

04 December 2017 (04.12.2017)

Date of mailing of the international search report

01 FEB 2018

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
 P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/51724

Box No. 1 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:
☐ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*. 1(a)).
☐ on paper or in the form of an image file (Rule 13*ter*. 1(b) and Administrative Instructions, Section 713).
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/51724

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:

---Please See Within the Next Supplemental Box---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 7/1, 8/1, 9/1, 10/1, 11/10/1, 12/10/1, 13, 15/13, 17/13, 18/13

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/51724

-Continued from Box No. III Observations where unity of invention is lacking-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-3, 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 11 (in-part), 12 (in-part), 13, 15 (in-part), 17 (in-part), 18 (in-part) are directed toward compositions and methods for treating or preventing T cell-mediated diseases or disorders by administering an inhibitor of lactate dehydrogenase A.

Group II, Claims 4-6, 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 11 (in-part), 12 (in-part), 14, 16 (in-part), 17 (in-part), 18 (in-part) are directed toward compositions and methods for treating or preventing T cell-mediated diseases or disorders by administering an inhibitor of ATP-citrate lyase.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include administering an inhibitor of lactate dehydrogenase A, not present in Group II; the special technical features of Group II include administering an inhibitor of ATP-citrate lyase, not present in Group I.

Groups I and II share the technical features including: a method of treating or preventing a T cell-mediated disease or disorder, the method comprising administering to a subject having or susceptible to developing a T cell-mediated disease or disorder a composition comprising an effective amount of an inhibitor; a composition comprising an inhibitor for use in the treatment of a T cell-mediated disease or disorder.

However, these shared technical features are previously disclosed by US 9,301,947 B2 to The Regents of the University of California (hereinafter 'California').

California discloses a method of treating or preventing a T cell-mediated disease or disorder (method of treating a T cell-mediated disease or disorder; abstract), the method comprising administering to a subject having or susceptible to developing a T cell-mediated disease or disorder a composition comprising an effective amount of an inhibitor (the method comprising administering to a subject having or susceptible to developing a T cell-mediated disease or disorder a composition comprising an effective amount of an inhibitor; abstract; column 3, lines 26-30); a composition comprising an inhibitor for use in the treatment of a T cell-mediated disease or disorder (a composition comprising an inhibitor for use in the treatment of a T cell-mediated disease or disorder; abstract; column 3, lines 26-30).

Since none of the special technical features of the Groups I and II inventions are found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the California reference, unity of invention is lacking.