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(54) Title: METHODS AND COMPOSITIONS TO POTENTIATE THE IMMUNE RESPONSE WITH LYSINE DEACETYLASE INHIBITORS

(57) Abstract: The invention relates to methods and compositions to boost immune responses to both infection and vaccination against and infection. In particular, the present invention is aimed at providing a method to boost immune responses to both infection with an infectious agent and vaccination against an infection with an infectious agent. It is also aimed at providing a treatment of infectious disease and vaccine adjuvant for the prevention of infection with an infectious agent.

METHODS AND COMPOSITIONS TO POTENTIATE THE IMMUNE RESPONSE WITH LYSINE DEACETYLASE INHIBITORS

Field of Invention

The invention relates to methods and compositions to boost immune responses to both infection
5 and vaccination against and infection.

Introduction

Lasting protection after vaccination or infection depends on the induction of durable memory
responses. Identifying means of expanding immune memory could increase vaccine efficacy,
10 with enhanced cellular responses associated with reduced disease severity and maintenance of
protective antibody levels in infectious diseases such as SARS-CoV-2 and influenza.

Lysine acetylation is a reversible post-translational modification controlled by the opposing
activity of acetyltransferases and deacetylase enzymes (Drummond, D. C. et al. Clinical
15 development of histone deacetylase inhibitors as anticancer agents. *Annu Rev Pharmacol
Toxicol* 45, 495-528, 574 (2005)). The known human KDAC enzymes fall into 4 main classes
(I-IV), and effects of KDACi differ widely by target KDAC specificity, functional impact and target
cell type (Choudhary, C. et al. Lysine acetylation targets protein complexes and co- regulates
major cellular functions. *Science* 325, 834-840, (2009); Scholz, C. et al. Acetylation site
20 specificities of lysine deacetylase inhibitors in human cells. *Nat Biotechnol* 33, 415-423, (2015);
Bantscheff, M. et al. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting
of HDAC complexes. *Nat Biotechnol* 29, 255- 265, (2011)). Promotion of growth arrest or death
of transformed cells has resulted in the development of some KDACi as cancer therapeutics,
and some have also been reported to modulate immune cell differentiation and function but not
25 immune memory (Dinarello et al Histone Deacetylase Inhibitors for Treating a Spectrum of
Diseases Not Related to Cancer. *Mol Med* 17(5-6)333-352, 2011; Li, Y. & Seto, E. HDACs and
HDAC Inhibitors in Cancer Development and Therapy. *Cold Spring Harb Perspect Med* 6,
(2016); Moreira, J. M., Scheipers, P. & Sorensen, P. The histone deacetylase inhibitor
Trichostatin A modulates CD4+ T cell responses. *BMC Cancer* 3, 30, (2003); Tao, R. et al.
30 Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nat Med* 13,
1299-1307, (2007); Licciardi, P. V. & Karagiannis, T. C. Regulation of immune responses by
histone deacetylase inhibitors. *ISRN Hematol* 2012, 690901, (2012)).

The present invention is aimed at providing a method to boost immune responses to both
35 infection with an infectious agent and vaccination against an infection with an infectious agent.
It is also aimed at providing a treatment of infectious disease and vaccine adjuvant for the
prevention of infection with an infectious agent.

Summary of the Invention

The present inventors have surprisingly identified that lysine deacetylase inhibitors (KDACi) have the ability to modulate T-cell memory responses by expanding immune memory cells. In particular, KDACi have ability to promote both an early memory precursor effector cell (MEC) phenotype and expanded stable memory populations and an enhanced secondary recall
5 response. This is critical for promoting a high-affinity antibody response to infectious disease antigens. Thus, KDACi induce durable memory responses to infectious disease antigens. Surprisingly KDACi have been shown herein to enhance the immune response when used at a low dose of up to 50% of the clinically approved or standard (published) dose. Therefore, KDACi may be used to enhance the immune response when administering a vaccine, in particular a
10 vaccine for the prevention of an infection with an infectious disease agent. Furthermore, KDACi may be used to boost immune responses to infection with an infectious disease agent and provide a treatment of infectious disease.

Thus, administration of KDACi has surprisingly been shown herein to promote the memory
15 precursor effector cell phenotype in CD8+ T cells, and as such can be used to enhance the immune response by promoting memory cell differentiation which leads to a strong and durable immune response.

As such, an aspect of the invention relates to a method for enhancing an immune response in a
20 subject comprising administering a lysine deacetylase inhibitor (KDACi) simultaneously, sequentially, or separately with a vaccine, wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

Another aspect relates to a method for the prevention of a disease comprising administering a
25 KDACi simultaneously, sequentially, or separately with a vaccine, and wherein the KDACi is wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

An aspect of the invention relates to a KDACi for use in the prevention of a disease, wherein the
30 KDACi is administered simultaneously, sequentially, or separately with a vaccine, and wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

An aspect of the invention relates to an *in vitro* or *in vivo* method of promoting a T cell memory
35 response comprising exposing cells to a KDACi capable of enhancing glutaminolysis.

An aspect of the invention relates to an *in vitro* or *in vivo* method of promoting differentiation of CD8+ T cells into memory precursor effector cells (MPEC), comprising exposing CD8+ T cells to a KDACi capable of enhancing glutaminolysis.

An aspect of the invention also relates to an *in vitro* or *in vivo* method of increasing IL7R expression in CD8+ T cells, comprising exposing CD8+ T cells to a KDACi capable of enhancing glutaminolysis.

5

A further aspect of the invention relates to an *in vitro* or *in vivo* method of enhancing glutaminolysis, comprising exposing CD8+ T cells to a KDACi.

10 An aspect of the invention also relates to a method of immunising a subject against a disease, comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine, and wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

15 Another aspect relates to a combination therapy comprising administration of administering a KDACi simultaneously, sequentially, or separately with a vaccine, and wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

20 An aspect of the invention relates to the use of a KDACi as an adjuvant for enhancing the efficacy of a vaccine wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

25 An aspect of the invention relates to a composition comprising a KDACi and a vaccine wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

30 An aspect of the invention relates to a kit comprising a KDACi and a vaccine and optionally instructions for use, wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

In yet another aspect, the invention relates to a method for treating a disease comprising administering a KDACi wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

35 In a further aspect, the invention relates to a method for expanding cells for use in adoptive cell therapy comprising:

- providing a population of CD8+ T cells,
- contacting the population of CD8+ T cells to a KDACi capable of enhancing glutaminolysis to produce an expanded population of cells.

Figures

Figure 1. Transcriptomic screening identifies a KDACi subclass promoting memory differentiation in primary human CD8 T cells.

- 5 (A) Schematic illustration of transcriptomic approach to small molecule repurposing. (B) Bubble plots showing enrichment score (x-axis) against significance (y-axis) for matching of three CD8⁺ T cell memory signatures against drug response signatures collated from GEO. Red symbols indicate compounds with KDACi activity, n=700 drug signatures screened against each memory signature. Plots illustrate those with overlap of at least n=5. (C) Schematic illustration of in vitro phenotypic screen. (D-F). Scatter plots showing maximum change in IL7R^{hi} MPEC% (y-axis, fold change v vehicle) against % cell division (x-axis, fc v vehicle) for (D) each of 42 selected treatments (n=844, mean +/-sem), (E) 13 selected treatments given 4 days after stimulation or (F) 6 KDACi given to sorted T cell memory subsets on d0. (G, H) Representative (G) and summary (H) scatterplots illustrating dose-dependent induction of an MPEC phenotype (IL7R^{hi}PD1^{lo}) on valproate treatment (mean +/-sem). Inset numbers (G) indicate % population indicated, inset histograms indicate proliferation (cfse dilution). (I, J) Line and scatterplots showing MPEC phenotype on treatment with low dose valproate (I, n=8) and apicidin (J, n=5). y-axes, ratio v vehicle treatment: red, IL7R:PD1 ratio, green, cell division, blue, activation (CD25⁺) and purple, cell survival by low dose of sodium valproate (H) and apicidin (J). *<0.05, 10 **<0.01, ***<0.001. MPEC = memory precursor effector cell, SLEC = short lived effector cell, fc = fold change.

Figure 2. KDACi-induced glutaminolysis promotes CD8 memory differentiation and a switch towards oxidative metabolism.

- 25 (A) Unrooted tree plots illustrating unsupervised clustering of KDACi compounds by site-specific acetylomic changes induced on treatment of HEK cells. Leaf diameter indicates peak MPEC expansion (A, IL7R fold change v vehicle, red up/blue down) and induced total acetylation (B, panAcK fold change v vehicle, green) on treatment of stimulated primary CD8⁺ T cells. Internal pie charts (A) indicate the target KDAC enzyme class(es) of each compound. Class I= target enzyme 1, 2, 3, 8, class II= target enzyme 4, 5, 7, 9, 6, 8, class IV= target enzyme 11, class III= sirts 1, 2, 3, 4, 5, 6, 7). (C) Bubble plot showing KEGG pathway enrichment of 1115 unique proteins differentially acetylated by KDACi.mem and KDACi.eff compounds (A). Metabolic pathways (blue) and other pathways (orange). (D) Volcano plot showing significance (y-axis, -log₁₀FDR) and magnitude (logFC, x-axis) of differential metabolites (red/blue) identified in metabolomic analysis of CD8⁺T cells, d6 post after stimulation with KDACi (valproate/apicidin, 30 n=16) or vehicle (n=8). (E) Bubble plot showing metabolic pathway enrichment analysis (pathway impact, x-axis v significance, y-axis) for differential metabolites (n=68, FDR<0.05) identified in (D). Pathways coloured blue were significantly (FDR<0.05) KDACi-induced in both metabolomic and acetylomic analyses. (F) KDACi (valproate/apicidin) induced metabolites (green, from D) and acetylated proteins (red, from A) mapped onto the glutaminolysis pathway

(HMDB SMP02298) (G) Scatter/line plots and (H) summary scatterplot showing baseline-normalised oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) against time for CD8⁺ T cells stimulated with valproate (red), vehicle (black) or valproate + R162 (green), n=5, mean $\pm$ sem. (I, J) Representative scatterplots (I) and summary bar plot (J, mean $\pm$ sem, n=4) showing fluoroglucose uptake (NBDG, inset histograms) by MPEC phenotype (IL7R^{hi}PD1^{lo}) and valproate treatment. (K, L) Line and scatter plots (mean $\pm$ sem) showing MPEC differentiation (IL7R^{hi}PD1^{lo}, fold change (fc) vs vehicle control, y-axis) on treatment (d0) with valproate (K, x-axis, n=8) or with 125 μ M sodium valproate + increasing dose of R162 (L, x-axis, n=5). P = 2 way ANOVA.

Figure 3. KDACi treatment induces transcriptomic and epigenetic modification in CD8⁺ T cells consistent with memory differentiation.

(A, B) Volcano plots showing differentially expressed genes (DEG, red/blue, FDR<0.05) induced by treatment of stimulated primary CD8⁺ T cells (n=8) for 6d with 175 μ M valproate (A) or valproate+25 μ M R162 (B). DEG in (B) are colored as in (A) to illustrate impact of R162 inhibition. (C) Circular barplot showing association of each of 9 latent factors describing maximal variance in the transcriptome of valproate and vehicle treated CD8⁺ T cells with treatment group (valproate v valp+R162). (D) Circular barplot showing fgsea enrichment (-log₁₀FDR, radial axis) of LF7 gene weights for selected T cell activation and memory signatures. (E) Circular barplot showing fgsea enrichment of 50 consensus 'hallmark' gene signatures (radial axis) in ranked feature weights for LF7. Circular barplots radial axis = -log₁₀FDR. (F) Annotated line plot showing weighted contribution (y-axis) of all genes (x-axis, ranked by weight) to LF7 with annotation for genes upregulated by memory (red) and exhausted (blue) CD8 T cells (from the lcmv chronic infection model, gse9650). (G) Annotated line plot showing weighted contribution (y-axis) of all genes (x-axis, ranked by weight) to LF7 (correlated with valp/R162 treatment, Fig3C) with annotation for selected genes (red) from the hallmark oxidative phosphorylation pathway. fgsea enrichment performed on whole pathway (n=200, M5936) with top 50 genes illustrated. (H) Waterfall plot showing the top enriched pathways (y-axis heatmap, -log₁₀FDR) amongst 3320 ranked genes (x-axis) with differential ATACseq peaks identified on valproate treatment of primary, stimulated CD8⁺ T cells (n=6 per group). Peak distribution (middle bar, % by region) and frequency of peaks/gene (lower bar) also indicated where multiple peaks were identified. Pathway names in red (left) indicate those known to influence T cell memory differentiation. (I) Representative track plots illustrating increased open chromatin peaks in Wnt pathway genes beta catenin (CTNNB1), Lymphoid enhancer binding factor 1 (LEF1) and Transcription factor 7 (TCF7). (J) Line and scatterplot showing beta-catenin induction in stimulated CD8⁺ T cells (n=6) with increasing dose of sodium valproate (x-axis). *<0.05, **<0.01, ***<0.001.

Figure 4. KDACi treatment in vivo expands immune memory on vaccination and infection.

(A) Schematic illustration of in vivo testing of KDACi-induced MPEC differentiation. X-axis= time (days), y-axis= T cell response. (B) Scatterplot showing MPEC ratio (IL7R^{hi}PD1^{lo}) in CD44⁺CD8⁺ splenocytes d9 post γ MHV infection in KDACi (valproate/apicidin) or vehicle treated mice

(treatment d0 to d9, n=5 per group). (C) Scatterplot (mean $\pm$ -sem) of MPEC (IL7R^{hi}KLRG1^{lo}) and SLEC (IL7R^{lo}KLRG1^{hi}), CD44^{hi} and IL7R/PD1 expression (MFI) on CD3⁺CD8⁺ splenocytes d7 post Nitrophenylacetyl[NP]-OVA immunisation. (D) Schematic illustration of heterotypic influenza rechallenge model. (E-H) Scatterplots (log₂ ratio vs vehicle, mean $\pm$ -sem, n=7-9 per group) illustrating immune cell populations following valproate (V) or combined valproate/R162 (V/R) treatment in the heterotypic 'flu rechallenge model (D). (E) Lung resident T memory (d30pi) pre-rechallenge. (F) Mediastinal lymph node T cell populations, memory markers (G) and cell populations (H) post rechallenge. V = valproate treated, V/R = valproate+R162 treated, NP = influenza A nucleoprotein. Red dashed line = vehicle control level. (I-L) Representative immunofluorescence microscopy images (I, J, K) and summary scatterplot (L, mean $\pm$ sem, n=3) showing peribronchial distribution of proliferating cells (I, Ki67⁺), T cells (J, K CD3⁺8⁺), Treg cells (K, CD3⁺Foxp3⁺) and B cells (K, CD19⁺) on valproate treatment compared to combined valproate/R162 treatment. br = bronchiole, 2-way ANOVA P = 0.01, mean $\pm$ -sem of Ki67 fluorescence assessed in n=16 100 μ m segments in each of n=3 biological replicates. (M) Schematic illustration of SARS-CoV-2 receptor binding domain (RBD) immunisation regimen with valproate treatment or vehicle d0-7. (N) Scatterplot (mean $\pm$ -sem, n=8-10 per group) showing splenic B cell populations d5 post RBD challenge. (O) Scatterplot showing RBD-specific B cell and T cell ELISpot titres (y-axis, spot-forming units (SFU) per 10⁵ splenocytes) d5 post RBD rechallenge by treatment regimen (valproate or vehicle). (P) Scatterplot (mean $\pm$ -sem) showing SARS-CoV-2 neutralising antibody titre (NT50) by treatment group (valproate/vehicle). Red = below detection threshold, plotted at NT=10 for illustrative purposes. PB = plasmablasts, GL7 = germinal centre marker. * = P<0.05, **<0.01, ***<0.001.

Figure 5. Modulating Emerging Memory after Immunisation (the MEMRI study)

(A, B) Schematic illustration of the MEMRI study design (A) and experimental protocol (B). (C, D) Line and scatterplots showing baseline-normalized influenza-specific (Dextramer⁺) CD8⁺ T cell expansion after seasonal influenza vaccination. C line plot valproate is shown in red (top line) and control in blue (bottom line). (E-G) Baseline-normalized seasonal influenza specific IgG (E), IgA (F) valproate shown in red (top line and control shown in blue (bottom line). IgG and IgA titre using the Influenza A Hong Kong H3N2 vaccine strain. Significance assessed using Imm with cubic spline. (G) Representative BCR network plots illustrating global BCR repertoire clonality and diversity, confirmed influenza-specific clones indicated in yellow. (H) Scatter and boxplot showing somatic hypermutation rates (CDR-replacement per unique 'flu-specific BCR sequence) in class-switched (right) and naïve (IgM⁺IgD⁺, left) BCR clusters with confirmed 'flu specificity. (I-J) Representative examples (I) and summary scatterplots (J) of cluster breadth/expansion in naïve (J, left: IgM⁺IgD⁺) and class-switched (J, right) influenza-specific BCR clusters. (K-L) Representative examples (K) and summary scatterplots (L) of cluster breadth in naïve (L, left: IgM⁺IgD⁺) and class-switched (L, right) influenza-specific BCR clusters. BCR cluster = sequences with 85% amino acid homology, identical CDR3 length and identical V/J gene.

Figure 6. (A) Line and scatterplots showing dose dependent impact of KDACi treatment (x-axis) on CD8+ T cell phenotype (y axis). Red = KDACi.mem (apicidin, pano, CI994, vorino, mocet, belino, MS275, abexino, butyrate), Blue = KDACi.eff (sirtinol, bufex, nicotin, tenovin, PCI34051, tubacin). Open circle = reported IC50 KDACi effect, closed circle = clinically approved dose where available.

Figure 7. Line and scatterplots showing effect (fold change vs vehicle control) of (A) the cell-permeable aKG donor dimethyl-ketoglutarate (DMKG) and (B-D) the glutaminolysis pathway inhibitors R162 (B), BPTES (C) and C968 (D). CD8+ T cell phenotype was measured d6 after polyclonal stimulation with anti-CD3/28 bead. Green = cell division, red = IL7R:PD1 ratio, blue = CD25, purple = live CD8+ T cell % of total viable events.

Figure 8. (A, B) Line plot (A, mean +/- sem) and scatterplot (B, mean+/-sem) showing baseline-normalized influenza-specific CD8+ T cell number (Dextramer+) up to 30 days post vaccination with the 2020/21 seasonal influenza vaccine and treatment with either 100mg, 200mg valproate or no drug (control). (C-E) Line plots (mean +/- sem) for influenza-specific IgG (C), IgA (D) and RSV (E) by treatment group. C-E: X-axis=days post vaccine, y-axis= ratio v baseline, E: X-axis=days post vaccine, y-axis= ratio v baseline tire. P values = one tailed Mann Whitney test.

Detailed Description

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology, chemistry, biochemistry and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature, see, e.g., Green and Sambrook et al., Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012).

The present invention is based on the surprising finding that various KDAC inhibitors can enhance glutaminolysis - an anaplerotic pathway, feeding the TCA cycle through generation of α -ketoglutarate. It has been shown herein that the KDACi listed in Table 1 are capable of enhancing glutaminolysis. Through this mechanism KDACi promote the formation of memory precursor effector cells, as such this enhances the memory response and leads to a strong and durable immune response. The effects of KDACi expansion of both cellular and neutralising antibody memory responses in response to infection with an infectious agent can be used to boost immune responses to both infection and vaccination. Therefore, the invention provides a method for treating for an infectious disease. The invention also provides a method for preventing infection with an infectious disease agent. The promotion of memory precursor cells

elicited by KDACi can also be used to prime cells for use in therapies such as adoptive cell transfer. Therefore, the invention also provides methods of preparing memory precursor effector cells and methods of expanding or culturing cells for use in adoptive cell therapy.

5 **Methods for Enhancing an Immune Response**

The present inventors have shown that KDACi can be used to enhance the immune response elicited by a vaccine. As such the invention relates to a method for enhancing an immune response in a subject comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine.

In an aspect the invention relates to a method for enhancing an immune response in a subject comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine, wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

Table 1

KDACi	Clinically approved dose or Published dose	Reference
Belinostat	1 μ M	Center for drug evaluation and research approval package for: application number: 206256orig1s000
NaB	800 μ M	A Phase I Clinical and Pharmacological Evaluation of Sodium Phenylbutyrate on an 120-h Infusion Schedule Carducci et al., Clin Cancer Res, October 1 2001 (7) (10) 3047-3055
Abexinostat	0.1 μ M	Phase 1 study of oral abexinostat, a histone deacetylase inhibitor, in combination with doxorubicin in patients with metastatic sarcoma. Choy et al., (2015), Cancer, 121: 1223-1230. https://doi.org/10.1002/cncr.29175
Mocetinostat	100nM	MGCD0103, a novel isotype-selective histone deacetylase

		inhibitor, has broad spectrum antitumor activity in vitro and in vivo Fournel et al., Mol Cancer Ther April 1 2008 (7) (4) 759-768; DOI: 10.1158/1535-7163.MCT-07-2026
Apicidin	160nM	Prediction of human pharmacokinetics and tissue distribution of apicidin, a potent histone deacetylase inhibitor, by physiologically based pharmacokinetic modeling. Shin et al., Cancer Chemother Pharmacol. 2011 Aug;68(2):465-75. doi: 10.1007/s00280-010-1502-y.
Panobinostat	500nM	Population pharmacokinetics of intravenous and oral panobinostat in patients with hematologic and solid tumors. Savelieva et al., Eur J Clin Pharmacol. 2015;71(6):663-672. doi:10.1007/s00228-015-1846-7
Vorinostat	500nM	Phase I and pharmacokinetic study of vorinostat (suberoylanilide hydroxamic acid) in Japanese patients with solid tumors. Fujiwara et al., Cancer Sci. 2009 Sep;100(9):1728-34. doi: 10.1111/j.1349-7006.2009.01237.x. Epub 2009 May 31
Valproate	1mM (50-125mg/ml, 610mM)	Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. Göttlicher et al., EMBO J. 2001 Dec 17;20(24):6969-78. doi: 10.1093/emboj/20.24.6969.

CI994	2 μ M	A phase I study of the oral combination of CI-994, a putative histone deacetylase inhibitor, and capecitabine. Undevia et al., Phase I and pharmacokinetics Volume 15, Issue 11, P1705-1711, November 01, 2004
MS275	13nM	Phase I and Pharmacokinetic Study of MS-275, a Histone Deacetylase Inhibitor, in Patients With Advanced and Refractory Solid Tumors or Lymphoma. DOI: 10.1200/JCO.2005.02.188 Journal of Clinical Oncology 23, no. 17 (June 10, 2005) 3912-3922.

In another aspect, the invention relates to a method for preventing a disease comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine, wherein the KDACi is capable of enhancing glutaminolysis. In an embodiment the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

In yet another aspect, the invention relates to a method for treating a disease comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine, wherein the KDACi is capable of enhancing glutaminolysis. In an embodiment the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

Lysine deacetylase (KDAC), also known as histone deacetylase (HDAC), are a class of enzymes that catalyse the removal of acetyl groups from the NH₂ terminal tail of histones. This removal of acetyl groups results in a closed chromatin structure and repression of gene expression. The term KDAC and HDAC are used interchangeably herein. There are 18 known KDACs which are split into five classifications based on their sequence homology to the originally identified yeast enzymes. The classifications are as follows, class I, class IIA, class IIB, class III and class IV. Class I comprises KDAC1, KDAC2, KDAC3 and KDAC8. Class IIA comprises KDAC4, KDAC5, KDAC7, and KDAC9. Class IIB comprises KDAC6 and KDAC10. Class 3 comprises sirtuins SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7. Class IV comprises KDAC11.

Lysine deacetylase inhibitors (KDACi) are compounds that inhibit the action of KDAC. As such KDACi can selectively alter gene transcription, in part, by chromatin remodelling and by changes

in the structure of proteins in transcription factor complexes. KDACi may be specific for certain KDAC classes. For example, a KDACi may target class I KDACs and inhibit KDAC1, KDAC2, KDAC3 and KDAC8. A class I KDACi refers to a compound which inhibits any of KDAC1, KDAC2, KDAC3 and KDAC8. A class I KDACi may also demonstrate inhibitory activity against
5 other KDACi provided that it also targets one or more of KDAC1, KDAC2, KDAC3 and KDAC8. Alternatively, the class I KDACi only inhibits one or more of KDAC1, KDAC2, KDAC3 and KDAC8.

It has been shown herein that certain KDACi are capable of enhancing glutaminolysis. Glutaminolysis is an anaplerotic pathway, feeding the TCA cycle through generation of α -ketoglutarate (α KG) while also creating energy and biosynthetic precursors such as nucleic acids and modulating cellular redox balance. It makes an important energetic contribution to T cell activation and can replenish TCA cycle intermediates as they are used for biosynthesis. Glutaminolysis can fuel stem cell-like oxidative metabolism in cancer cells, sustaining chronic proliferation akin to what is required of immune memory. Specific KDACi have demonstrated an effect in enhancing glutaminolysis in particular KDACi capable of inhibiting one or more of KDAC1, KDAC2, KDAC3, KDAC4, KDAC5, KDAC7, KDAC9 or KDAC10 have been shown to enhance glutaminolysis. As shown herein the KDACi listed in Table 1 have been shown to enhance glutaminolysis, however it will be apparent to the skilled person that other KDACi
15 compounds may demonstrate the ability to enhance glutaminolysis. Glutaminolysis is the process by which cells convert glutamine into TCA cycle metabolites through the activity of multiple enzymes including glutaminase, glutamate dehydrogenase, glutamate-oxaloacetate transaminase, glutamate pyruvate transaminase, phosphoserine transaminase. As such, enhanced glutaminolysis may be determined by upregulation of one or more of the enzymes
20 involved in the glutaminolysis pathway or by identifying upregulated metabolites and protein acetylation patterns indicative of glutaminolysis. Metabolites and acetylation patterns may be identified via metabolomic analysis or via functional assessment of glutamine breakdown.

In an embodiment the KDACi capable of enhancing glutaminolysis is selected from abexinostat, belinostat, panobinostat, sodium butyrate, apicidin, mocetinostat, vorinostat, MS275, CI994 and/or valproate. The valproate may be sodium valproate and/or valproic acid. In one embodiment the KDACi is valproate and/or apicidin.
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It has been shown herein that is it advantageous to use the KDACi at a low dose, for example a dose that is up to 50% of the dose listed in Table 1. In an embodiment the KDACi is selected from one of the KDACi listed in Table 1 and the low dose is up to 45%, up to 40%, up to 35%, up to 30%, up to 25%, up to 20%, up to 15%, up to 10%, up to 5% of the dose listed in Table 1 for the selected KDACi. In an embodiment the KDACi is selected from one of the KDACi listed in Table 1 and the low dose is from 1 to 50%, from 1 to 45%, from 1 to 40%, from 1 to 35%, from
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1 to 30%, from 1 to 25%, from 1 to 20%, from 1 to 15%, from 1 to 10%, from 1 to 5% of the dose listed in Table 1 for the selected KDACi. In an embodiment the the KDACi is selected from one of the KDACi listed in Table 1 and the low dose is 1, 2,3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 ,
 5 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% of the dose listed in Table 1 for the selected KDACi. Preferably the KDACi is used at a dose of 1 to 25%, 1 to 20%, 1 to 15%, 1 to 10%, 1 to 5%, 5 to 25%, 5 to 20%, 5 to 15%, or 5 to 10% of the dose listed in Table 1 for the selected KDACi.

As used herein, a vaccine is a substance used to stimulate an immune response and provide
 10 immunity against one or several diseases. A vaccine is generally prepared from the causative agent of a disease, e.g. an infectious disease agent, its products, or a synthetic substitute, treated to act as an antigen without inducing the disease. The term "vaccine" encompasses any vaccine which provides immunity against one or several diseases is used herein to refer to a biological preparation that induces an immunogenic response to a target antigen. Examples of
 15 vaccines include viral, bacterial, protein and nucleic acid vaccines. The term "viral vaccine" refers to a virus that induces an immunogenic response to a target antigen. Suitable antigens include tumour antigens, viral antigens, and in particular, antigens derived from viral pathogenic organisms such as HIV, HepC, FIV, LCMV, Ebola virus, as well as bacterial pathogens such as mycobacterium tuberculosis.

20 In an embodiment the vaccine is an infectious disease vaccine. The term "infectious disease vaccine" as used herein refers to a vaccine that provides a level of immunity against an infectious disease cause by an infectious disease agent. Infectious diseases are generally caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. Thus, the term
 25 infectious disease agent as used herein refers to a pathogenic microorganism, such as bacteria, viruses, parasites or fungi. Also known as "communicable diseases", infectious diseases can be spread directly or indirectly from one person to another. As used herein the terms "infectious disease vaccine" or "viral respiratory disease vaccine" does not extend to cancer vaccines. Thus, the infectious disease vaccine is not a cancer vaccine.

30 In one embodiment, the infectious disease agent (or infectious agent product) is a virus, for example and without limitation, a pox virus (e.g., vaccinia virus), zika virus, smallpox virus, marburg virus, flaviviruses (e.g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus), influenza virus (or antigens, such as F and G proteins or derivatives thereof), e.g., influenza A; or purified or recombinant proteins thereof, such as HA,
 35 NP, NA, or M proteins, or combinations thereof), parainfluenza virus (e.g., sendai virus), respiratory syncytial virus, rubeola virus, human immunodeficiency virus (or antigens, e.g., such as tat, nef, gpl20 or gpl60), human papillomavirus (or antigens, such as HPV6, 11, 16, 18), varicella-zoster virus (or antigens such as gpl, II and IE63), herpes simplex virus (e.g., herpes

simplex virus I, herpes simplex virus II; or antigens, e.g., such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2), cytomegalovirus (or antigens such as gB or derivatives thereof), Epstein-Barr virus (or antigens, such as gp350 or derivatives thereof), JC virus, rhabdovirus, rotavirus, rhinovirus, adenovirus, papillomavirus, parvovirus, picomavirus, poliovirus, virus that causes mumps, virus that causes rabies, reovirus, rubella virus, togavirus, orthomyxovirus, retrovirus, hepadnavirus, hantavirus, junin virion, filovirus (e.g., ebola virus), coxsackievirus, equine encephalitis virus, Rift Valley fever virus, alphavirus (e.g., Chikungunyavirus, sindbis virus), hepatitis A virus, hepatitis B virus (or antigens thereof, for example Hepatitis B Surface antigen or a derivative thereof), hepatitis C virus, hepatitis D virus, or hepatitis E virus.

In one embodiment, the infectious agent is a bacterium. Non-limiting examples of suitable bacteria (or bacterially derived products) for use in the vaccines and/or methods of the invention include *Neisseria* species, including *N. gonorrhea* and *N. meningitidis* (or antigens, such as, for example, capsular polysaccharides and conjugates thereof, transferrin-binding proteins, lactoferrin binding proteins, PilC, adhesins); *Haemophilus* species, e.g., *H. influenzae*; *S. pyogenes* (or antigens, such as, for example, M proteins or fragments thereof, C5A protease, lipoteichoic acids), *S. agalactiae*, *S. mutans*; *H. ducreyi*; *Moraxella* spp, including *M. catarrhalis*, also known as *Branhamella catarrhalis* (or antigens, such as, for example, high and low molecular weight adhesins and invasins); *Bordetella* spp, including *B. pertussis* (or antigens, such as, for example, pertactin, pertussis toxin or derivatives thereof, filamentous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis* and *B. bronchiseptica*; *Mycobacterium* species, including *M. tuberculosis* (or antigens, such as, for example, ESAT6, Antigen 85A, -B or -C), *M. bovis*, *M. leprae*, *M. avium*, *M. paratuberculosis*, *M. smegmatis*; *Legionella* spp, including *L. pneumophila*; *Escherichia* spp, including enterotoxigenic *E. coli* (or antigens, such as, for example, colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof), enterohemorrhagic *E. coli*, enteropathogenic *E. coli* (or antigens, such as, for example, shiga toxin-like toxin or derivatives thereof); *Vibrio* spp, including *V. cholera* (or antigens, such as, for example, cholera toxin or derivatives thereof); *Shigella* spp, including *S. sonnei*, *S. dysenteriae*, *S. flexnerii*; *Yersinia* spp, including *Y. enterocolitica* (or antigens, such as, for example, a Yop protein), *Y. pestis*, *Y. pseudotuberculosis*; *Campylobacter* spp, including *C. jejuni* (or antigens, such as, for example, toxins, adhesins and invasins) and *C. coli*; *Salmonella* spp, including *S. typhi*, *S. paratyphi*, *S. choleraesuis*, *S. enteritidis*, *S. typhimurium*, and *S. dysenteriae*; *Listeria* species, including *L. monocytogenes*; *Helicobacter* spp, including *H. pylori* (for example urease, catalase, vacuolating toxin); *Pseudomonas* spp, including *P. aeruginosa*; *Staphylococcus* species, including *S. aureus*, *S. epidermidis*; *Proteus* species, e.g., *P. mirabilis*; *Enterococcus* species, including *E. faecalis*, *E. faecium*; *Clostridium* species, including *C. tetani* (or antigens, such as, for example, tetanus toxin and derivative thereof), *C. botulinum* (or antigens, such as, for example, botulinum toxin and derivative thereof),

C. difficile (or antigens, such as, for example, Clostridium toxins A or B and derivatives thereof), and *C. perfringens*; Bacillus species, including *B. anthracis* (or antigens, such as, for example, botulinum toxin and derivatives thereof), *B. cereus*, *B. circulans* and *B. megaterium*; Corynebacterium species, including *C. diphtheriae* (or antigens, such as, for example, diphtheria toxin and derivatives thereof); Borrelia species, including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (or antigens, such as, for example, OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, OspC, DbpA, DbpB), *B. andersonii* (or antigens, such as, for example, OspA, OspC, DbpA, DbpB), *B. hermsii*; Ehrlichia species, including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis; *Rickettsia spp*, including *R. rickettsii*; Chlamydia species, including *C. trachomatis* (or antigens, such as, for example, MOMP, heparin-binding proteins), *C. pneumoniae* (for example MOMP, heparin-binding proteins), *C. psittaci*; Leptospira species, including *L. interrogans*; Streptococcus species, such as *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*; Treponema species, including *T. pallidum* (or antigens, such as, for example, the rare outer membrane proteins), *T. denticola*, and *T. hyodysenteriae*.

In one embodiment, the infectious agent is a parasite, or a parasite derived product. Non-limiting examples of suitable parasite (or parasite derived products) for use in the vaccines and/or methods of the invention include Plasmodium species, including *P. falciparum*; Toxoplasma species, including *T. gondii* (or antigens, such as, for example SAG2, SAG3, Tg34); Entamoeba species, including *E. histolytica*; Babesia species, including *B. microti*; Trypanosoma species, including *T. cruzi*; Giardia species, including *G. lamblia*; Leshmania species, including *L. major*; Pneumocystis species, including *P. carinii*; Trichomonas species, including *T. vaginalis*; and Schistosoma species, including *S. mansoni*.

In another embodiment, the infectious agent is a fungus, or a fungal derived product. In another embodiment, the infectious agent is a protozoan, or a protozoan derived product. Suitable protozoans (or protozoan derived products) for use in the vaccines and/or methods of the invention include, without limitation, protests (unicellular or multicellular), e.g., Plasmodium falciparum, and helminths, e.g., cestodes, nematodes, and trematodes.

Thus, in one embodiment, the infectious disease vaccine is a respiratory disease vaccine. The respiratory disease vaccine may be selected from a vaccine against Haemophilus influenza, a coronavirus, such as SARS-CoV-2, Haemophilus influenzae type B, measles virus, poliovirus, tetanus, tuberculosis, cholera, typhoid, dengue, diphtheria, hepatitis, Japanese encephalitis, meningococcal meningitis, mumps virus, pertussis, pneumococcal disease, rabies, rotavirus, rubella, coronavirus, such as SARS-CoV-2, SARS-CoV, or MERS-CoV,

In one embodiment, the infectious disease vaccine is a viral respiratory disease vaccine. The viral respiratory disease vaccine may be selected from a vaccine against Haemophilus influenza,

Haemophilus influenzae type B, measles virus, poliovirus, mumps virus, rubella, coronavirus, such as SARS-CoV-2, SARS-CoV, or MERS-CoV.

5 The hemagglutinin of influenza is one of the two main glycoproteins on the viral surface and a major target of neutralizing antibodies. Based on structure and antigenicity, there are eighteen defined subtypes (H1-H18) of IAV HAs belonging to two broad groups. Influenza HA consists of an antigenically variable globular head domain containing the receptor-binding site (RBS) for viral attachment and a more conserved stem domain that mediates fusion of viral and cell membranes in the endosome. The HA head domain is the immunodominant domain of the protein and is the target of most antibody responses induced by IAV vaccine or infection. 10 However, due to the high level of sequence and antigenic diversity occurring in the HA head domain and the incorporation of large number of glycans in this region to evade immune recognition, most head domain specific antibodies exhibit a very narrow breadth of protection. Influenza as used herein may refer to either influenza A, B, C or D. Influenza A includes any of the influenza A subtypes including but not limited to H1N1, H3N2. In one embodiment, the vaccine is directed against influenza A or B. Zoonotic influenza virus with H1, H3, H5, H6, H7, H9 and H10 HAs are also included. 15

20 In an embodiment, the vaccine is directed against a coronavirus. Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses composed of several proteins including the Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) proteins. The S glycoprotein is highly immunogenic with its receptor-binding domain (RBD) being a major target of the humoral response. The 2019 novel coronavirus (2019-nCoV), currently known as SARS-CoV-2, emerged in Wuhan city of the Chinese province of Hubei in December 2019.

25 Infection by SARS-CoV-2 causes a respiratory disease designated CoVID-19, with a median incubation period of 7 days (min: 3 days, max: 14 days). Common symptoms include fever, cough, shortness of breath, muscle pain and fatigue while, in severe cases, the infection can lead to pneumonia, extensive lung damage and death, particularly in aged patients and in those with various underlying conditions. 30

In an embodiment the coronavirus is selected from SARS-CoV-2, SARS-CoV, MERS-CoV, 229E, NL63, OC43, HKU1. In one embodiment, the coronavirus is SARS-CoV-2. SARS-CoV-2 includes any variant of SARS-CoV-2, including but not limited to D614G variant, "Cluster 5" variant, N501Y variant. 35

Various types of vaccine may be used, for example the infectious disease vaccine is selected from a whole pathogen vaccine, live attenuated vaccine, inactivated vaccine, whole killed vaccine, recombinant protein vaccine, toxoid vaccine, conjugate vaccine, virus-like particle

(VLP) vaccine, outer membrane vesicle (OMV) vaccine, DNA vaccine, RNA vaccine, peptide vaccine or viral vectored vaccine. In one embodiment, the vector is an adenoviral vector.

5 A whole pathogen virus uses the whole disease-causing pathogen to produce an immune response similar to that seen during natural infection. A live attenuated vaccine utilises whole bacteria or viruses which have been weakened/attenuated so that they create a protective immune response but do not cause disease in healthy people. Attenuation may be achieved through genetic modification of the pathogen either as a naturally occurring phenomenon or as a modification specifically introduced. Inactivated vaccines comprise whole bacteria or viruses
10 which have been killed or have been altered, so that they cannot replicate. A recombinant protein vaccine comprises one or more specific antigens from the surface of the pathogen and a generally acellular. A toxoid vaccine comprises one or more specific antigens from the surface of a toxin produced by a pathogen. A conjugate vaccine comprises a polysaccharide conjugated to an antigen from the cell surface of a pathogen or from a toxin produced by a pathogen. A
15 VLP comprises molecules that closely resemble viruses, but are non-infectious because they contain no viral genetic material. VLPs may be naturally occurring or synthesized through the individual expression of viral structural proteins, which can then self-assemble into the virus-like structure. In some cases, the antigens in a VLP vaccine are the viral structural proteins themselves. Alternatively, the VLPs can be manufactured to present antigens from another
20 pathogen on the surface, or even multiple pathogens at once. As each VLP has multiple copies of an antigen on its surface it is more effective at stimulating an immune response than a single copy. An OMV are vesicles which are naturally produced from the outer membrane of the bacterial outer cell wall and comprise many of the antigens found on the cell membrane but is a non-infectious particle. An OMV may be modified so that toxic antigens are removed and
25 antigens suitable for stimulating an immune response can be kept. Nucleic acid vaccines comprise genetic material to stimulate a cell to produce the desired antigen. RNA vaccines comprise mRNA inside a lipid membrane, wherein the mRNA encodes the desired antigen. DNA vaccine comprise DNA wherein the DNA encodes the desired vaccine. Viral vectored vaccines utilise viruses to deliver the genetic code of target vaccine antigens to cells of the body, in
30 general the virus is harmless or has been modified to reduce virulence. Viral vectored vaccine may be replicating or non-replicating. Replicating viral vectors retain the ability to make new viral particles alongside delivering the vaccine antigen when used as a vaccine delivery platform. Non-replicating viral vectors do not retain the ability to make new viral particles during the process of delivering the vaccine antigen to the cell. In non-replicating viral vectored vaccines,
35 the virus has been modified to remove key viral genes that enable the virus to replicate.

The KDACi may be administered in combination with any commercially available infectious disease vaccine. In a preferred embodiment, the infectious disease vaccine is a viral respiratory

disease vaccine. In an embodiment the infectious disease vaccine is against SARS-CoV-2 or influenza.

In one example of the various aspects of the invention, the vaccine is against coronavirus and
5 may be selected from the following non-limiting list: Comirnaty (BNT162b2; Pfizer/BioNTech),
mRNA-1273 (Moderna), AZD1222; (Vaxzevria, Covishield; AstraZeneca), Sputnik V (Gamaleya
Research Institute, Acellena Contract Drug Research and Development), Sputnik Light
(Gamaleya Research Institute, Acellena Contract Drug Research and Development), JNJ-
78436735 (Ad26.COV2.S; Johnson & Johnson), CoronaVac (Sinovac), BBIBP-CorV (Beijing
10 Institute of Biological Products; China National Pharmaceutical Group (Sinopharm),
EpiVacCorona (Federal Budgetary Research Institution State Research Center of Virology and
Biotechnology) and Convidicea (PakVac, Ad5-nCoV; CanSino Biologics).

In one example of the various aspects of the invention, the vaccine is against influenza and may
15 be an inactivated influenza vaccines (IIV) and live attenuated influenza vaccines (LAIV). In one
embodiment, the vaccine is a trivalent vaccine. In one embodiment, the vaccine is selected from
Afluria, Fluarix, Flublok, Flulaval, Fluvirin or Fluzone.

The inventors have shown herein that treatment with KDACi is capable of enhancing both the
20 naïve and memory immune responses as such co administration of KDACi with a vaccine has
the ability to increase the breadth of the immune response and thereby improving immune
protection against non-vaccine strains. The term "non-vaccine strains" refers to strains which
the vaccine was not originally developed against. As such the present invention provides
methods of enhancing an immune response in a subject comprising administering a KDACi
25 simultaneously, sequentially, or separately with an infectious disease vaccine, wherein the
immune response enhances protection against non-vaccine strains of the infectious disease.

The inventors have also surprisingly shown that the effects of the KDACi, in particular valproate,
on expanding memory are achieved when a low dose is administered. This dose is lower than
30 the dosages used for KDACi, in particular valproate, for the treatment of cancer. The KDACi,
preferably valproate, may be administered at a dose of 10 to 950 mg/day, 15 to 850 mg/day, 20
to 750 mg/day, 25 to 650 mg/day, 30 to 550 mg/day, 35 to 450 mg/day, 40 to 350 mg/day, 50
to 250 mg/day, 75 to 225 mg/day, 80 to 220 mg/day, 85 to 215 mg/day, 90 to 210 mg/day, 95 to
205 mg/day, preferably at a dose of 100 to 200 mg/day. The KDACi may be administered at a
35 dose of 50 mg/day, 75 mg/day, 100 mg/day, 125 mg/day, 150 mg/day, 175 mg/day, 200 mg/day,
225 mg/day, 250 mg/day. The KDACi may be administered at a dose of approximately 100
mg/day, approximately 100 mg/day may encompass a dose between 95 to 105 mg/day. The
KDACi may be administered at a dose of approximately 200 mg/day, approximately 200 mg/day
may encompass a dose between 195 to 205 mg/day. The dose administered per day is referred

to as the total dose per day, as such the total dose may be split into multiple doses throughout the day, for example where a dose of 200mg/day is administered this may be split into two separate 100mg doses. The total dose per day may be split into multiple administrations, for example the total dose may be split into 2, 3, 4, 5, or 6 administrations per day.

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In an embodiment the KDACi, preferably valproate, is administered at a dose of 100 mg/day. In an embodiment the KDACi, preferably valproate, is administered at a dose of 200 mg/day. In an embodiment the KDACi, preferably valproate, is administered at a dose of 200 mg/day and the dose is split into two 100 mg doses taken at two separate time point during the day. The correct dosage of the KDACi may vary according to the particular formulation, the mode of administration, and its particular site, host. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities may be taken into account.

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Where abexinostat is used in the methods of the invention, abexinostat may be administered at a dose of 2 to 10 nM, 2.5 to 9.5 nM, 3 to 9 nM, 3.5 to 8.5 nM, 4 to 8 nM, 4.5 to 7.5 nM, 2.5 to 7.5 nM, 3.5 to 6.5 nM, 4.5 to 5.5 nM. Abexinostat may be administered at a dose of 2nM, 5nM or 10nM. Abexinostat may be administered at the clinically approved dose range of 0.1µM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering abexinostat simultaneously, sequentially, or separately with a vaccine. In an embodiment the abexinostat is administered at up to 50% of the dose recited in Table 1. In an embodiment the abexinostat is administered in a dose of 2 to 10 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering abexinostat simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the abexinostat is administered in a dose of 2 to 10 nM.

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Where belinostat is used in the methods of the invention, belinostat may be administered at a dose of 5 to 800 nM, 5 to 700 nM, 5 to 600 nM, 5 to 500 nM, 5 to 400 nM, 5 to 300 nM, 5 to 200 nM, 5 to 100 nM, 5 to 50 nM, 5 to 40 nM, 5 to 30 nM, 5 to 20 nM, 5 to 15 nM., 10 to 800 nM, 10 to 700 nM, 10 to 600 nM, 10 to 500 nM, 10 to 400 nM, 10 to 300 nM, 10 to 200 nM, 10 to 100 nM, 10 to 50 nM, 10 to 40 nM, 10 to 30 nM, 10 to 20 nM, 10 to 15 nM. Belinostat may be administered at a dose of 5 nM, 10 nM, or 15 nM. Belinostat may be administered at the clinically approved dose of 1µM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering belinostat simultaneously, sequentially, or separately with a vaccine. In an embodiment the bellinostat is administered at up to 50% of the dose recited in Table 1. In an embodiment the belinostat is administered in a dose of 5 to 800 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering belinostat simultaneously, sequentially, or

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separately with a viral respiratory disease vaccine, wherein the belinostat is administered in a dose of 5 to 800 nM.

- Where panobinostat is used in the methods of the invention, panobinostat may be administered
- 5 at a dose of 0.5 to 500 nM, 0.5 to 400 nM, 0.5 to 300 nM, 0.5 to 200 nM, 0.5 to 100 nM, 0.5 to 50 nM, 0.5 to 40 nM, 0.5 to 30 nM, 0.5 to 20 nM, 0.5 to 10 nM, 0.5 to 9 nM, 0.5 to 8 nM, 0.5 to 7 nM, 0.5 to 6 nM, 0.5 to 5 nM, 0.5 to 4 nM, 0.5 to 3 nM, 0.5 to 2 nM, 1 to 500 nM, 1 to 400 nM, 1 to 300 nM, 1 to 200 nM, 1 to 100 nM, 1 to 50 nM, 1 to 40 nM, 1 to 30 nM, 1 to 20 nM, 1 to 10 nM, 1 to 9 nM, 1 to 8 nM, 1 to 7 nM, 1 to 6 nM, 1 to 5 nM, 1 to 4 nM, 1 to 3 nM, 1 to 2 nM.
- 10 Panobinostat may be administered at a dose of 0.5 nM, 1 nM, or 2 nM. Panobinostat may be administered at the clinically approved dose of 500 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering panobinostat simultaneously, sequentially, or separately with a vaccine. In an embodiment the panobinostat is administered at up to 50% of the dose recited in Table 1. In an embodiment the
- 15 panobinostat is administered in a dose of 0.5 to 500 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering panobinostat simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the panobinostat is administered in a dose of 0.5 to 500 nM.
- 20 Where butyrate is used in the methods of the invention, butyrate may be administered at a dose of 0.1×10^3 to 500×10^3 nM, 0.1×10^3 to 400×10^3 nM, 0.1×10^3 to 300×10^3 nM, 0.1×10^3 to 200×10^3 nM, 0.1×10^3 to 100×10^3 nM, 0.1×10^3 to 50×10^3 nM, 0.1×10^3 to 10×10^3 nM, 0.1×10^3 to 5×10^3 nM, 0.1×10^3 to 1×10^3 nM, 0.1×10^3 to 0.05×10^3 nM, 0.5×10^3 to 500×10^3 nM, 0.5×10^3 to 400×10^3 nM, 0.5×10^3 to 300×10^3 nM, 0.5×10^3 to 200×10^3 nM, 0.5×10^3 to 100×10^3 nM, 0.5×10^3 to 50×10^3
- 25 nM, 0.5×10^3 to 10×10^3 nM, 0.5×10^3 to 5×10^3 nM, 0.5×10^3 to 0.1×10^3 nM, 0.5×10^3 to 0.1×10^3 nM. Butyrate may be administered at a dose of 0.5×10^3 nM, 1×10^3 nM, 2×10^3 nM. Butyrate may be administered at the clinically approved dose of 200 to 800 μ M. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering butyrate simultaneously, sequentially, or separately with a vaccine. In an
- 30 embodiment the butyrate is administered at up to 50% of the dose recited in Table 1. In an embodiment the butyrate is administered in a dose of 0.1 to 500×10^3 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering butyrate simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the butyrate is administered in a dose of 0.1×10^3 to 500×10^3 nM.
- 35 Where apicidin is used in the methods of the invention, apicidin may be administered at a dose of 0.01 to 100 nM, 0.01 to 90 nM, 0.01 to 80 nM, 0.01 to 70 nM, 0.01 to 60 nM, 0.01 to 50 nM, 0.01 to 40 nM, 0.01 to 30 nM, 0.01 to 20 nM, 0.01 to 10 nM, 0.01 to 5 nM, 0.01 to 1 nM, 0.01 to 0.9 nM, 0.01 to 0.8 nM, 0.01 to 0.7 nM, 0.01 to 0.6 nM, 0.01 to 0.5 nM, 0.01 to 0.4 nM, 0.01 to

0.3 nM, 0.01 to 0.2 nM, 0.01 to 0.1 nM, 0.01 to 0.09 nM, 0.01 to 0.08 nM, 0.01 to 0.07 nM, 0.01 to 0.06 nM, 0.01 to 0.05 nM, 0.01 to 0.04 nM, 0.01 to 0.03 nM, 0.01 to 0.02 nM, 0.05 to 100 nM, 0.05 to 90 nM, 0.05 to 80 nM, 0.05 to 70 nM, 0.05 to 60 nM, 0.05 to 50 nM, 0.05 to 40 nM, 0.05 to 30 nM, 0.05 to 20 nM, 0.05 to 10 nM, 0.05 to 5 nM, 0.05 to 1 nM, 0.05 to 0.9 nM, 0.05 to 0.8 nM, 0.05 to 0.7 nM, 0.05 to 0.6 nM, 0.05 to 0.5 nM, 0.05 to 0.4 nM, 0.05 to 0.3 nM, 0.05 to 0.2 nM, 0.05 to 0.1 nM, 0.05 to 0.09 nM, 0.05 to 0.08 nM, 0.05 to 0.07 nM, 0.05 to 0.06 nM. Apicidin may be administered at a dose of 0.01 nM, 0.05 nM, or 0.1 nM. Apicidin may be administered at the clinically approved dose of 160 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering apicidin simultaneously, sequentially, or separately with a vaccine. In an embodiment the apicidin is administered at up to 50% of the dose recited in Table 1. In an embodiment the apicidin is administered in a dose of 0.01 to 100 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering apicidin simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the apicidin is administered in a dose of 0.01 to 100 nM.

Where mocetinostat is used in the methods of the invention, mocetinostat may be administered at a dose of 0.01×10^3 to 1×10^3 nM, 0.01×10^3 to 0.9×10^3 nM, 0.01×10^3 to 0.8×10^3 nM, 0.01×10^3 to 0.7×10^3 nM, 0.01×10^3 to 0.6×10^3 nM, 0.01×10^3 to 0.5×10^3 nM, 0.01×10^3 to 0.4×10^3 nM, 0.01×10^3 to 0.3×10^3 nM, 0.01×10^3 to 0.2×10^3 nM, 0.01×10^3 to 0.1×10^3 nM, 0.1×10^3 to 1×10^3 nM, 0.1×10^3 to 0.9×10^3 nM, 0.1×10^3 to 0.8×10^3 nM, 0.1×10^3 to 0.7×10^3 nM, 0.1×10^3 to 0.6×10^3 nM, 0.1×10^3 to 0.5×10^3 nM, 0.1×10^3 to 0.4×10^3 nM, 0.1×10^3 to 0.3×10^3 nM, 0.1×10^3 to 0.2×10^3 nM. Mocetinostat may be administered at a dose of 0.1×10^3 nM, 0.5×10^3 nM, 1×10^3 nM. Mocetinostat may be administered at the clinically approved dose of 100 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering mocetinostat simultaneously, sequentially, or separately with a vaccine. In an embodiment the mocetinostat is administered at up to 50% of the dose recited in Table 1. In an embodiment the mocetinostat is administered in a dose of 0.01 to 1×10^3 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering mocetinostat simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the mocetinostat is administered in a dose of 0.01×10^3 to 1×10^3 nM.

Where vorinostat is used in the methods of the invention, vorinostat may be administered at a dose of 0.2×10^3 to 5×10^3 nM, 0.2×10^3 to 4×10^3 nM, 0.2×10^3 to 3×10^3 nM, 0.2×10^3 to 2×10^3 nM, 0.2×10^3 to 1×10^3 nM, 0.2×10^3 to 0.5×10^3 nM, 0.2×10^3 to 0.4×10^3 nM, 0.2×10^3 to 0.3×10^3 nM, 1×10^3 to 5×10^3 nM, 1×10^3 to 4.5×10^3 nM, 1×10^3 to 4×10^3 nM, 1×10^3 to 3.5×10^3 nM, 1×10^3 to 3×10^3 nM, 1×10^3 to 2.5×10^3 nM, 1×10^3 to 2×10^3 nM, 1×10^3 to 1.5×10^3 nM. Vorinostat may be administered at a dose of 1.25×10^3 nM, 2.5×10^3 nM, 5×10^3 nM. Vorinostat may be administered at the clinically approved dose of 500 nM. In an embodiment of the invention relates to a method

for enhancing an immune response in a subject comprising administering vorinostat simultaneously, sequentially, or separately with a vaccine. In an embodiment the vorinostat is administered at up to 50% of the dose recited in Table 1. In an embodiment the vorinostat is administered in a dose of 0.2 to 5×10^3 nM. In an embodiment of the invention relates to a method

5 for enhancing an immune response in a subject comprising administering vorinostat simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the vorinostat is administered in a dose of 0.2×10^3 to 5×10^3 nM.

Where MS275 is used in the methods of the invention, MS275 may be administered at a dose

10 of 0.01×10^6 to 1×10^6 nM, 0.01×10^6 to 0.9×10^6 nM, 0.01×10^6 to 0.8×10^6 nM, 0.01×10^6 to 0.7×10^6 nM, 0.01×10^6 to 0.6×10^6 nM, 0.01×10^6 to 0.5×10^6 nM, 0.01×10^6 to 0.4×10^6 nM, 0.01×10^6 to 0.3×10^6 nM, 0.01×10^6 to 0.2×10^6 nM, 0.01×10^6 to 0.1×10^6 nM, 0.01×10^6 to 0.09×10^6 nM, 0.01×10^6 to 0.08×10^6 nM, 0.01×10^6 to 0.07×10^6 nM, 0.01×10^6 to 0.06×10^6 nM, 0.01×10^6 to 0.05×10^6 nM, 0.01×10^6 to 0.04×10^6 nM, 0.01×10^6 to 0.03×10^6 nM, 0.01×10^6 to 0.02×10^6 nM 0.25×10^6 to 1×10^6

15 nM, 0.25×10^6 to 0.9×10^6 nM, 0.25×10^6 to 0.8×10^6 nM, 0.25×10^6 to 0.7×10^6 nM, 0.25×10^6 to 0.6×10^6 nM, 0.25×10^6 to 0.5×10^6 nM, 0.25×10^6 to 0.4×10^6 nM, 0.25×10^6 to 0.3×10^6 nM. MS275 may be administered at a dose of 0.25×10^6 nM, 0.5×10^6 nM, 1×10^6 nM. MS275 may be administered at the clinically approved dose of 13 nM. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering MS275

20 simultaneously, sequentially, or separately with a vaccine. In an embodiment the MS275 is administered at up to 50% of the dose recited in Table 1. In an embodiment the MS275 is administered in a dose of 0.01×10^6 to 1×10^6 nM, In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering MS275 simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the

25 MS275 is administered in a dose of 0.01×10^6 to 1×10^6 nM,

Where CI994 is used in the methods of the invention, CI994 may be administered at a dose of 0.01×10^6 to 2×10^6 nM, 0.01×10^6 to 1×10^6 nM, 0.01×10^6 to 0.9×10^6 nM, 0.01×10^6 to 0.8×10^6 nM, 0.01×10^6 to 0.7×10^6 nM, 0.01×10^6 to 0.6×10^6 nM, 0.01×10^6 to 0.5×10^6 nM, 0.01×10^6 to 0.4×10^6 nM, 0.01×10^6 to 0.3×10^6 nM, 0.01×10^6 to 0.2×10^6 nM, 0.01×10^6 to 0.1×10^6 nM, 0.01×10^6 to 0.09×10^6 nM, 0.01×10^6 to 0.08×10^6 nM, 0.01×10^6 to 0.07×10^6 nM, 0.01×10^6 to 0.06×10^6 nM, 0.01×10^6 to 0.05×10^6 nM, 0.01×10^6 to 0.04×10^6 nM, 0.01×10^6 to 0.03×10^6 nM, 0.01×10^6 to 0.02×10^6 nM 0.25×10^6 to 1×10^6 nM, 0.25×10^6 to 0.9×10^6 nM, 0.25×10^6 to 0.8×10^6 nM, 0.25×10^6 to 0.7×10^6 nM, 0.25×10^6 to 0.6×10^6 nM, 0.25×10^6 to 0.5×10^6 nM, 0.25×10^6 to 0.4×10^6 nM, 0.25×10^6 to 0.3×10^6 nM. CI994 may be administered at a dose of 0.1×10^6 nM, 0.5×10^6 nM, 1×10^6 nM. CI994 may be administered at the clinically approved dose of $2 \mu\text{M}$. In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering CI994 simultaneously, sequentially, or separately with a vaccine. In an embodiment the CI994 is administered at up to 50% of the dose recited in Table 1. In an

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embodiment the CI994 is administered in a dose of 0.01×10^8 to 2×10^8 nM, In an embodiment of the invention relates to a method for enhancing an immune response in a subject comprising administering CI994 simultaneously, sequentially, or separately with a viral respiratory disease vaccine, wherein the CI994 is administered in a dose of 0.01×10^8 to 2×10^8 nM.

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According to the invention the KDACi can be administered simultaneously, sequentially or separately with a vaccine. It is not necessary that the KDACi and vaccine are packed together (but this is one embodiment of the invention). It is also not necessary that they are administered at the same time. As used herein, "separate" administration means that the drugs are administered as part of the same overall dosage regimen (which could comprise a number of days), but preferably on the same day. As used herein "simultaneously" means that the drugs are to be taken together or administered together, or formulated as a single composition. The KDACi and the vaccine may be formulated separately but administered together, via the same or different administration route. For example, the vaccine may be administered subcutaneously or intravenously and the KDACi may be administered orally. As used herein, "sequentially" means that the drugs are administered at about the same time one after another, and preferably within about 1 hour of each other.

The dosage regimen of the KDACi and the vaccine may comprise day 0 for example when the KDACi and vaccine are administered simultaneously. The dosage regimen may comprise additional days wherein further doses of the KDACi and/or the vaccine are administered. The further dose of KDACi may be administered in the absence of the vaccine. For example, the KDACi and the vaccine are administered simultaneously, sequentially or separately on day 0, and a further dose of the KDACi is administered on any or all of days 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or day 1 following the administration of the KDACi and the vaccine. Further doses of the KDACi may be administered on any or all of days 1 to 2, days 1 to 3, days 1 to 4, days 1 to 5, days 1 to 6 or days 1 to 7. In an embodiment the KDACi and the vaccine are administered simultaneously, sequentially or separately on day 0, and a further dose of the KDACi is administered on any or all of days 1 to 7 following the administration of the KDACi and the vaccine.

For example, in embodiments relating to administration with a SARS-CoV-2 vaccine, subjects are administered the SARS-CoV-2 vaccination on day 0 along with a 100mg dose of sodium valproate. The sodium valproate will then be administered for 7 days following the vaccination at a daily dose of 100mg. The same protocol can be used when the SARS-CoV-2 booster vaccination is administered.

For example, in embodiments relating to administration with an influenza, subjects are administered the influenza vaccination on day 0 along with a 100mg or 200mg dose of sodium

valproate. The sodium valproate will then be administered for 7 days following the vaccination at a daily dose of 100mg or 200mg. The same protocol can be used when the booster vaccination is administered.

- 5 As used herein the term "valproate" may refer to valproic acid. Where valproate or valproic acid are is used in the methods of the present invention the compound may be administered as valproic acid or the sodium salt form of valproic acid i.e. sodium valproate, other salt forms may be suitable. Where a salt form is administered the salt form converts to the active form of valproate ion within the blood.

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The KDACi is administered to enhance an immune response to an infectious agent. In particular, administration of the KDACi either alone or with a vaccine can increase the intensity, rate, and duration of immune response, and/or shorten onset time of antibody responses. When administered with a vaccine, the immune response to the vaccine is enhanced compared to the
15 immune response generated by administration of the vaccine alone to the subject.

- The immune system encompasses cellular immunity and humoral immunity. Cellular immunity includes a network of cells and events. Memory T cells are key components of the immune response, are antigen-specific, are developed after exposure and recognition of a particular
20 antigen. Humoral immunity involves B cells and antibodies. When B cells become transformed to plasma cells, the plasma cells express and secrete antibodies. The secreted antibodies can subsequently bind to antigens. Expansion of germinal centre (GCB) and expansion of T cell follicular helper cells (TFH) are critical for promoting a high affinity antibody response.

- 25 Thus, the term "inducing and/or enhancing an immune response" means that the method evokes and/or enhances any response of the subject's immune system. "Immune response" is defined as any response of the immune system, for example, of either a cell-mediated or humoral (i.e. antibody mediated) nature. In particular, the response is humoral. These immune responses can be assessed by a number of *in vivo* or *in vitro* assays well known to one skilled in the art
30 including, but not limited to, antibody assays (for example ELISA assays) antigen specific cytotoxicity assays, production of cytokines (for example ELISPOT assays), etc. Suitable assays are also shown in the examples.

- In particular, as used herein, the enhanced immune response includes one or more of the
35 following: expansion of germinal centre (GCB), expansion of T cell follicular helper cells (TFH), and/or differentiation of stimulated primary human CD8+ T cells into MPEC and expansion of MPEC.

Thus, in one embodiment, the production of memory T cells produced during infectious disease challenge simulated by administration of the vaccine. In one embodiment, differentiation of stimulated primary human CD8+ T cells into MPEC is promoted.

5 Therefore, using a vaccine together with a KDACi at the dose described herein can stimulate greater protection against the infectious disease immunogenic targets expressing the antigen in the vaccine. In particular, as discussed above, using a vaccine together with a KDACi results in a immune response with a greater breadth thereby providing protection against both the vaccine strain and non-vaccine strain of the infectious disease. In addition, using a vaccine together
10 with a KDACi could lead to enhanced clonal expansion and memory and a quicker/faster, more intense, and more prolonged humoral response upon re-challenge to the antigen in question. The combination of vaccine and a KDACi can produce an additive or synergistic effect and achieve greater benefit than using vaccine alone; and the combination also allows for possible use of smaller doses of the vaccine to achieve protective antibody titers.

15 Where a vaccine administration comprises a prime boost dosage regimen, the KDACi may be administered, as described herein, with both the priming dose of the vaccine and the boost dose of the vaccine or only one of these doses. As used herein the "prime boost dosage regimen" refers to a regimen of immunization with the same immunogen during the prime and booster
20 doses or a regimen of priming the immune system with an immunogen and then boosting with a different immunogen. During a prime boost dosage regimen, the prime and boost doses may be administered one or more weeks apart, one or more months apart or one or more years apart. When the prime dose of the vaccine is administered on day 0 the KDACi is also administered separately, sequentially or simultaneously. On subsequent days further doses of the KDACi may
25 be administered, for example on any or all of days 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or day 1 following the administration of the KDACi and the vaccine. A prime boost dosage regimen is common with vaccines directed against SARS-CoV-2 and so this administration regimen, wherein the KDACi is administered with both the priming and boost doses of the SARS-CoV-2 vaccine, may be adopted.
30 Alternatively, the KDACi may be administered either with the priming dose of the vaccine or with the boost dose of the vaccine.

Administration of the KDACi and/or the vaccine may be via any reasonable route, for example any parenteral or enteral route. For example, any convenient route, includes but is not limited to
35 oral, topical, parenteral, sublingual, rectal, vaginal, ocular, intranasal, pulmonary, intradermal, intravitreal, intramuscular, intraperitoneal, intravenous, subcutaneous, intracerebral, transdermal, transmucosal, by inhalation. Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intranasal, rectal, intravesical, intradermal, topical or subcutaneous administration. As discussed above, the KDACi or the

infectious disease vaccine can take the form of one or more dosage units. Administration of the KDACi may be performed by the oral, sublingual, buccal, intravenous, intramuscular, or subcutaneous route. Where more than one administration of the KDACi is used, the same or different routes of administration may be used. Various oral dosage forms can be used, including such solid forms as tablets, capsules, liquids, granules and bulk powders. Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed, containing suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules, and effervescent preparations reconstituted from effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, coloring agents and flavoring agents.

In one embodiment, administration of the vaccine may be performed by intravenous, intramuscular, intradermal, intranasal, or subcutaneous route.

The vaccine and/or the KDACi can be in the form of a liquid, e.g., a solution, emulsion or suspension. The liquid compositions, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides, polyethylene glycols, glycerin, or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; and agents for the adjustment of tonicity such as sodium chloride or dextrose. The composition can be enclosed in an ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material.

An intravenous formulation of the vaccine or the KDACi may be in the form of a sterile injectable aqueous or non-aqueous (e.g. oleaginous) solution or suspension. The sterile injectable preparation may also be in a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, phosphate buffer solution, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils may be employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may be used in the preparation of the intravenous formulation of the invention.

The vaccine or the KDACi can be prepared using methodology well known in the pharmaceutical art. For example, a composition intended to be administered by injection can be prepared by combining with water so as to form a solution. A surfactant can be added to facilitate the formation of a homogeneous solution or suspension.

In an embodiment the subject is a mammal, preferably a human. It has been shown herein that the administration of the KDACi valproate in conjunction with a vaccine was able to enhance both the naïve T cell response and the memory response. As such, the methods of the invention are particularly suitable for use in the elderly, children or immunocompromised subjects. In an embodiment the subject is over the age of 65. In an embodiment the subject is between the age of 1 year and 18 years old. In an embodiment the subject is immunocompromised or immunosuppressed. Immunocompromised or immunosuppressed subject may include; transplant patients, subjects with weakened immune system, subjects taking immunosuppressive drugs, subjects with HIV/AIDS, cancer, diabetes or genetic disorders.

The invention relates to a KDACi for use in a method of enhancing an immune response in a subject comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine.

The invention also relates to a KDACi for use in the prevention or treatment of a disease, wherein the KDACi is administered simultaneously, sequentially, or separately with a vaccine, and wherein the KDACi is capable of enhancing glutaminolysis. In an embodiment the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

The invention also relates to a KDACi for use in the treatment of a disease, wherein KDACi is capable of enhancing glutaminolysis. In an embodiment the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.

The invention also relates to a KDACi for use in the prevention of SARS-CoV-2, wherein the KDACi is administered simultaneously, sequentially, or separately with a SARS-CoV-2 vaccine. Preferably wherein the KDACi is administered at a dose of 10 to 950 mg/day. In an embodiment the KDACi is valproate.

The invention also relates to a KDACi for use in the treatment of SARS-CoV-2, preferably wherein the KDACi is administered at a dose of 10 to 950 mg/day. In an embodiment the KDACi is valproate.

The invention also relates to a KDACi for use in the prevention of influenza, wherein the KDACi is administered simultaneously, sequentially, or separately with an influenza vaccine, preferably wherein the KDACi is administered at a dose of 10 to 950 mg/day. In an embodiment the KDACi is valproate.

The invention also relates to a KDACi for use in the treatment of influenza, preferably wherein the KDACi is administered at a dose of 10 to 950 mg/day. In an embodiment the KDACi is valproate.

- 5 The invention also relates the use of a KDACi in the manufacture of a medicament for the prevention and/or treatment of a disease wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1. In one embodiment, the KDACi is administered together with an infectious disease vaccine.
- 10 The terms "treat", "treatment" or "treating," as used herein refer to administering a compound to a subject for prophylactic and/or therapeutic purposes. Where the KDACi is for use as part of an immunization regimen, the KDACi is for use in the prevention of an infectious disease by enhancing the production of memory precursor effector cells. As explained herein, the KDACi can also be used to treat disease, in particular at the early onset of the disease. Thus in one
- 15 embodiment, the KDACi is being used to prevent the onset of an infectious disease and is administered prophylactically together with a vaccine. As such the term "prevention" refers to preventing the onset of an infectious disease or preventing an infection. The term "prevention" can refer to a reduction in the risk of contracting a disease. The term "prophylactic or preventative treatment" refers to treating a subject who does not yet exhibit symptoms of a
- 20 disease or condition, but who is susceptible to, or otherwise at risk of, a particular disease or condition, whereby the treatment reduces the likelihood that the patient will develop the disease or condition. The term "therapeutic treatment" refers to administering treatment to a subject already suffering from a disease or condition.

25 **Methods of Enhancing Memory T cell Response**

- It has been shown herein that exposure to KDACi results in expanded memory cell populations and improved recall responses. As such the invention also relates to an *in vivo*, *in vitro* or *ex vivo* method of promoting a T cell memory response, comprising exposing cells to a KDACi. According to the method, for administration to a human subject, the KDACi is administered at a
- 30 dose as set out herein. In particular the KDACi may enhance the T cell memory response. The enhanced T cell memory response may be characterised by an expanded population of memory precursor effector cells. Memory precursor effector cells can be characterized by the presences of specific markers and cell surface markers. Methods to identify and quantify these markers are well known in the art. Examples of suitable methods include but are not limited to affinity-based
- 35 separation methods, magnetic cell sorting techniques, fluorescence-based cell sorting techniques such as FACS (fluorescence activated cell sorting). Memory precursor effector cells may be characterised by a number of markers, examples include but are not limited to; ILR7^{hi}PD1^{lo}. The term "hi" and "lo" refer to relative expression of these markers on the cells. "lo" may refer to cells wherein there is no expression of the markers, it may also refer to cells wherein

there is low expression of the markers relative to other cells in the sample. "hi" may refer to cells wherein there is high expression of the markers, for example where there is high expression of the markers relative to other cells in the sample.

5 The invention also relates to an *in vivo*, *in vitro* or *ex vivo* method of promoting differentiation of CD8⁺ T cells into memory precursor effector cells, comprising exposing CD8⁺ T cells to a class I KDACi. Unless otherwise specified, the term CD8⁺ T cells as used herein relates to human CD8⁺ T cells. As used herein, the term "differentiation" refers to cell differentiation and has its usual meaning in the art and refers to the process wherein a cell transitions from one cell type
10 to another. Cell differentiation can be characterised by the presence of cell surface markers. CD8⁺ T cells may be identified by the following cell surface markers CD3⁺CD8⁺. The transition of CD8⁺ T cells into memory precursor effector cells may be characterised by an increase in cell population expressing ILR7^{hi}PD1^{lo}. CD8⁺ T cells may be obtained from a subject and then exposed *in vitro/ex vivo* in order to stimulate differentiation of the CD8⁺ T cells into memory
15 precursor effector cells. The expanded population of memory precursor effector cells may be reintroduced to a subject. According to the method, for administration to a human subject, the KDACi is administered at a dose as set out herein.

It has been shown herein that KDACi stimulates expression of IL7R (interleukin-7 receptor). An
20 aspect of the invention related to an *in vivo*, *in vitro* or *ex vivo* method of enhancing IL7R expression in CD8⁺ T cells, comprising exposing CD8⁺ T cells to a KDACi. IL7R plays a key role in the development of lymphocytes and VDJ recombination. Enhanced expression of IL7R may be detected using standard techniques including but not limited to include but are not limited to affinity-based separation methods, magnetic cell sorting techniques, fluorescence-based cell
25 sorting techniques such as FACS (fluorescence activated cell sorting). According to the method, for administration to a human subject, the KDACi is administered at a dose as set out herein.

A shown herein exposing cells to KDACi results in a cell population with strong and durable immune responses. Therefore these cell populations are particularly suitable for use in therapies
30 such as adoptive cell therapy. Adoptive cell therapy is a type of immunotherapy wherein T cells are provided to a subject in order to enhance the subjects immune response to diseases such as cancer. Types of adoptive cell therapy include chimeric antigen receptor T-cell (CAR T-cell) therapy and tumor-infiltrating lymphocyte (TIL) therapy. An aspect of the invention relates to a method for expanding cells for use in adoptive cell therapy comprising:

35 providing a population of CD8⁺ T cells,
contacting the population of CD8⁺ T cells to a KDACi capable of enhancing glutaminolysis to produce an expanded population of cells.

The term “expanding cells” as used herein refers to culturing cells under conditions such that the number of cells increases. In an embodiment the resultant expanded population of cells comprises memory precursor effector cells. The expanded population of cells may be characterised by specific cell surface markers for example the memory precursor effector cells are characterised by the markers IL7Rhi, PD1lo. In an embodiment the expanded population of memory precursor effector cells are further characterised by the markers IL7Rhi, PD1lo, CX3CR1, CCR7, CD27 and CD62L.

The invention also provides a method of providing a population of cells enriched for memory precursor effector cells comprising:

- providing a population of CD8+ T cells,
- contacting the population of CD8+ T cells to a KDACi capable of enhancing glutaminolysis to produce an expanded population of cells.

In an aspect the invention relates to a method of treatment comprising administering a population of cells expanded by the methods described herein. An embodiment relates to an expanded population of cells for use in therapy wherein the expanded population of cells are obtained by the following method:

- providing a population of CD8+ T cells,
- contacting the population of CD8+ T cells to a KDACi capable of enhancing glutaminolysis to produce an expanded population of cells.

The cells for use in therapy or methods of treatment may be characterised as memory precursor effector cells as identified by one or more of the cell surface markers IL7Rhi, PD1lo, CX3CR1, CCR7, CD27 and CD62L, in particular IL7Rhi, PD1lo. The cells may be for use in adoptive cell therapy

Further it has been shown herein that KDACi enhance glutaminolysis in cells. As used herein “glutaminolysis” refers to the process which feeds the TCA cycle through the generation of α -ketoglutarate. The process of glutaminolysis comprises a series of biochemical reactions by which the amino acid glutamine is lysed to glutamate, aspartate, CO₂, pyruvate, lactate, alanine and citrate. As such, an aspect of the invention relates to an *in vivo*, *in vitro* or *ex vivo* method of enhancing glutaminolysis, comprising exposing CD8+ T cells to a KDACi. According to the method, for administration to a human subject, the KDACi is administered at one of the doses as set out above. In an embodiment where valproate is used as the KDACi, valproate is administered at a dose of 10 to 950 mg/day.

In the methods described herein cells are exposed to the KDACi *in vivo*, *in vitro* or *ex vivo*. As such the cells may be exposed to the KDACi by administering the KDACi directly to the subject.

Alternatively, cells may be obtained from a subject and exposed to the KDACi. Once the cells have been exposed to the KDACi the cells may be returned to the subject.

5 In the methods described herein the cells may also be exposed to a viral respiratory disease vaccine. The viral respiratory disease vaccine may be any vaccine as described herein. The cells may be exposed to the viral respiratory disease vaccine *in vivo*, *in vitro* or *ex vivo*. As such the cells may be exposed to the viral respiratory disease vaccine by administering the viral respiratory disease vaccine directly to the subject. Alternatively, cells may be obtained from a subject and exposed to the viral respiratory disease vaccine. Once the cells have been exposed to the viral respiratory disease vaccine, the cells may be returned to the subject.

Methods of Immunising

The invention relates to a method of immunising a subject against a disease, comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine, and wherein the KDACi is capable of enhancing glutaminolysis.

20 The invention relates to the use of a KDACi as an adjuvant for enhancing the efficacy of a vaccine. As used herein the term "adjuvant" refers to a substance that enhances the immune system's response to the presence of an antigen. An adjuvant is commonly used to improve the effectiveness of a vaccine. The use of the KDACi as an adjuvant may be performed by administering the KDACi in combination with a vaccine, preferably an infectious disease vaccine, the KDACi and the vaccine may be administered separately, sequentially or simultaneously. Where the KDACi is used as an adjuvant for administration to a subject, it is provided in a dose as set out herein. In an embodiment the KDACi is one of the listed in Table 1 and used at a dose of up to 50% of the dose set out in Table 1 for said KDACi. In an embodiment where valproate is used as the KDACi, valproate is administered at a dose of 10 to 950 mg/day.

30 The invention also relates to a composition comprising a KDACi and a vaccine wherein the KDACi is capable of enhancing glutaminolysis. In an embodiment the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1. The composition may comprise the KDACi a vaccine formulated together, preferably an infectious disease vaccine. The composition may comprise one or more additional active ingredients, pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The compositions may optionally include other drug actives.

35 The invention also relates to a kit comprising a KDACi and a vaccine and optionally instructions for use, wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1. The kit may comprise the KDACi and a vaccine formulated together, preferably an infectious disease vaccine. The kit may comprise the KDACi and i

vaccine formulated separately. The kit may also comprise one or more additional active ingredients, pharmaceutically acceptable carrier, diluent, excipient or adjuvant, and optionally instructions for use.

- 5 The term “pharmaceutically acceptable carrier” or “pharmaceutically acceptable excipient” includes any and all solvents, dispersion media, coatings, antiviral, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Some examples of substances, which can serve as pharmaceutically-acceptable carriers or components thereof, are sugars, 10 such as lactose, dextrose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and methyl cellulose; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, 15 sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the TWEENS; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions.
- 20 Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. While the foregoing disclosure provides a general description of the subject matter encompassed within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are provided to further 25 enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of the present invention will be apparent to those skilled in the 30 art in view of the present disclosure.

All documents mentioned in this specification are incorporated herein by reference in their entirety.

The invention is further described in the non-limiting examples.

Examples

- 35 **Example 1 – Genomic drug repurposing expands immune memory following immunisation and infection**

Drug repurposing allows the rapid use of existing therapeutics for new disease indications and can be directed by matching disease-associated transcriptional changes to those induced by candidate small molecules. Here we screen multiple transcriptional signatures of T cell memory against libraries of drug response signatures, identifying a subgroup of compounds with lysine deacetylase inhibitory (KDACi) activity with the ability to promote, at low doses, a memory precursor effector cell (MPEC) phenotype in primary human CD8⁺ T cells. Using combined proteomics, metabolomics, transcriptomics and epigenomics on *in vitro* treated cells, we identify enhanced glutaminolysis and oxidative metabolism during T cell activation as the immunometabolic mechanism promoting memory cell differentiation. KDACi treatment reproduced - and glutaminolysis inhibition reversed - the transcriptional memory signatures used for initial screening with concurrent epigenetic modification of Wnt pathway genes controlling glutamine breakdown. We demonstrate *in vivo* efficacy of a repurposed, clinically-approved KDACi (sodium valproate) in four murine models of immunisation and infection, including SARS-CoV-2 spike protein immunisation, showing expanded memory populations and improved recall responses. Memory expansion *in vivo* is also reversed by inhibition of glutaminolysis, highlighting its contribution to T cell memory formation. The observed expansion of both cellular and neutralising antibody memory responses to SARS-CoV-2 immunisation suggest that rapid translation of selected KDACi may enhance the efficacy of vaccination strategies.

Results

To identify existing small molecules with the potential to modulate T cell memory responses we undertook an *in silico* repurposing screen. We matched three independent transcriptional signatures of CD8⁺ T cell memory (Fig1A, B) against three libraries of small molecule transcriptional response signatures comprising over 30,000 compounds (Methods). Amongst predicted matches we observed multiple instances of compounds with lysine (K) deacetylase inhibitory (KDACi) activity (Fig1B), with strongest enrichment from drug libraries containing signatures from human immune and primary cells. We therefore included 16 KDACi compounds amongst selected treatments in an *in vitro* phenotypic screen comprising 844 assays across 3 doses per compound. The doses of each compound are set out in the table below. The high-throughput assay tested each compound's ability to modulate differentiation of stimulated primary human CD8⁺ T cells into memory precursor effector cells (MPEC), characterised by increased expression of IL7R on dividing cells (Fig1C). We observed significant expansion of MPEC following treatment with multiple KDACi with the effect diminishing if treatment was delayed following stimulation (Fig1E) and arising predominantly from differentiation of naïve T cells, rather than from restimulation of those in a pre-existing memory pool (Fig1F).

Table 2. KDACi dose range selection

KDACi.name	Clinical dose	HDACi target class	IC50 <i>in vitro</i>	clinical dose range	dose range used <i>in vitro</i> , nM
Belinostat	94mM	1-10	27nM	1mM	5/10/15

NaB	800mM	1-3,8	1mM	200-800mM	0.5/1/2e6
Abexinostat	0.1mM	1-10	7nM	0.1mM	2/5/10
Nicotinamide	82mM	Sirt1-7	2mM	80mM	0.1/0.5/1e6
Bufexamac	N/A	6,10	9mM	30-100mM	1/2.5/5
Sirtinol	100mM	Sirt1,2	40mM		4.5/9/18e3
Mocetinostat	5-10mM	1-3,11	0.15mM	100nM	0.1/0.5/1e3
PCI34051	10mM	8	10nM		0.1/0.5/1e3
Apicidin	160nM ~ 100ng/ml	1-3	16nM	160nM	0.01/0.05/0.1
Tubacin	5mM	6	4nM		2/4/8
Panobinostat	1mM	1-10	5nM	500nM	0.5/1/2
Tenovin-6	10mM	Sirt1,2	10mM		2.5/5/10e3
vorinostat	2.5mM	1-10	10nM	500nM	1.25/2.5/5e3
Valproate	1mM	1-3	0.4mM	1mM (50-125mg/ml, 610mM)	0.125/0.25/0.5e6
CI994	2mM	1-3	0.9mM	2mM	0.1/0.5/1e6
MS275	13nM	1-3	0.5mM	13nM	0.25/0.5/1e6

Following KDACi treatment of primary human CD8⁺ T cells we observed a biphasic dose response, with MPEC expansion occurring only at low doses at which no impact on cell survival, proliferation or activation was seen (Fig1G, H). Higher doses limited cell proliferation and survival consistent with their clinical use as anti-proliferative agents in the context of cancer (Fig 6).

Dose-dependent MPEC differentiation was characterised by expansion of a population of IL7RhiPD1lo cells (Fig1I-J) and was apparent with only a subset (9/16) of KDACi (Fig1H-J, Fig6). We therefore sought to investigate the mechanism of enhanced memory differentiation, systematically comparing genomic analyses of compounds promoting MPEC differentiation (KDACi.mem) with compounds favouring short lived effector differentiation (KDACi.eff, Fig. 2A 84 and Fig 6). We reasoned that KDACi-induced differences in site-specific protein acetylation may be responsible and compared acetylated protein data quantified by mass spectrometry in KDACi-treated HEK cells. Consistent with this, unsupervised clustering of proteomic acetylation signatures derived from 88 segregated compounds by their effect on MPEC differentiation (Fig2A), but not by target KDAC enzyme class (Fig2A) or by the magnitude of total induced acetylation (quantified in primary stimulated CD8⁺ T cells, Fig2B, C). This indicated that protein-specific acetylation – rather than simply increased total acetylation – was important so we further investigated protein specific acetylation differences following KDACi.mem or KDACi.eff exposure. Analysis of peptide acetylomic data identified 1560 acetylation sites from 1116 proteins differentially induced by KDACi.mem and KDACi.eff compounds, with pathway enrichment analysis indicating over-representation of multiple metabolic pathways amongst them. Given this result, we undertook targeted mass spectrometric metabolomic analysis of

primary human CD8⁺ T cells stimulated in the presence of selected KDACi.mem, sodium valproate and apicidin (compounds selected for largest effect sizes at lowest concentrations, Fig 6Fig1H-K). Compared to vehicle-treated stimulated control cells, 68 metabolites were found to be differentially expressed (Fig2D, 103) amongst which the 'alanine, aspartate and glutamate' pathway demonstrated strongest enrichment, greatest predicted pathway impact while showing similar enrichment amongst differentially-acetylated proteins also (Fig2E, Fig 6). Mapping differential metabolites onto this pathway revealed a specific and significant (FDR<5%.) increase in both metabolites and acetylation of enzymes regulating glutamine breakdown and the TCA cycle (Fig2F).

Glutaminolysis feeds the TCA cycle through generation of α -ketoglutarate (aKG), creating energy and biosynthetic precursors such as nucleic acids and modulating cellular redox balance. It makes an important energetic contribution to T cell activation and can replenish TCA cycle intermediates as they are used for biosynthesis. Glutaminolysis is known to promote B cell memory formation and can fuel stem cell-like oxidative metabolism in cancer cells, sustaining chronic proliferation. This process is controlled by Wnt/B-catenin signalling, a pathway also known to arrest effector T cell differentiation and promote memory formation. However, the specific contribution of glutaminolysis to T cell memory formation remains unclear. We therefore sought to determine whether KDACi.mem-induced glutaminolysis had a functional impact on T cell 'metabolic switching' – a relative increase in oxidative over glycolytic metabolism on activation that is a critical determinant of memory differentiation. Low-dose valproate treatment enhanced oxidative metabolism and reduced the glycolytic rate (Fig2G-I) without changing cell activation, proliferation or glucose uptake (Fig2J, K). Both valproate-induced metabolic switching (Fig2G-I) and MPEC differentiation (Fig2L-M) could be reversed by administration of the specific glutaminolysis inhibitor R162 (which targets GLUD1: Fig2F Fig 7). MPEC differentiation could also be induced by bypassing glutaminolysis through direct provision of the cell-permeable aKG analogue dimethyl ketoglutarate (DMKG: Fig 7). Together, these data indicate that increased glutaminolysis induced by a subgroup of KDACi in activated primary human CD8⁺ T cells promotes a switch to oxidative metabolism, promoting their differentiation towards a memory precursor phenotype.

We next sought to test whether valproate treatment of stimulated CD8⁺ T cells would induce global transcriptional changes consistent with T cell memory differentiation. RNAseq analysis showed marked changes in stimulated CD8⁺ T cell transcriptomes induced by valproate treatment (Fig3A, compared to vehicle-treated, stimulated controls) that could be reversed by concurrent inhibition of glutaminolysis (Fig3B). To better understand these changes we performed a matrix factorisation analysis (methods), identifying 9 latent factors of which one (LF7) showed strong, specific association with valproate treatment 144 (Fig3C, FDR<1%). Systematic enrichment analysis of T cell differentiation signatures amongst LF7 gene weights

identified specific polarisation of T cell memory and exhaustion signatures used for initial compound screening (Fig3D, E). Enrichment analysis using consensus 'hallmark' signalling pathways (Fig3F) identified specific induction of two signatures, reflecting oxidative phosphorylation and also Myc signalling. Myc is a known upstream regulator of Wnt signaling and memory formation and also glutaminolysis-driven functional metabolic switching. Other latent factors reflected inter-individual differences in the extent of T cell activation and proliferation but were not associated with response to valproate treatment.

The generation of durable cellular memory requires epigenetic modification as cells undergo functional differentiation. We therefore used ATACseq analysis of valproate or vehicle-treated and stimulated CD8⁺ T cells to identify differentially open chromatin regions (Fig3G, H). Amongst differential peaks in 3220 genes associated with 92 pathways we observed strongest enrichment for the Wnt/B-catenin pathway (Fig3G), known to regulate glutaminolysis and a stem-like T cell memory phenotype. In addition, 6 of the top 9 valproate-induced pathways have established roles in T cell memory formation including HIF1a-33, Hippo and glutamate signalling (Fig3G). We observed significant expansion of peaks in both promoter and regulatory 3'UTR regions in key Wnt pathway genes including CTNNB1, LEF1 and TCF726 165 (Fig3H) and confirmed dose dependent beta catenin (CTNNB1) induction by valproate treatment of CD8⁺ T cells in vitro (Fig3I). We next sought to investigate whether the memory phenotype induced on primary stimulation was preserved on subsequent restimulation in vitro. We sorted IL7Rhi/lo MPEC/SLECs after either KDACi (valproate/apicidin) or vehicle treatment and, after resting and normalisation of cell numbers, restimulated them in vitro (using polyclonal anti-CD2/3/28 microbeads). In both cases, but especially after KDACi pre-treatment, the initial MPEC phenotype observed on primary stimulation was preserved on restimulation (Fig3J-L).

Epigenetic modification and preservation of MPEC phenotype indicated that durable expansion of memory may occur following KDACi.mem treatment. We therefore next tested whether low dose valproate treatment during a primary challenge could enhance immune memory in vivo. This was undertaken in four different murine models of infection or immunisation, testing early MPEC differentiation (d7post-immunisation/infection (pi)), the stable T cell memory pool (>d30pi) and recall responses on rechallenge (d5 post-rechallenge (pr)). Valproate treatment during primary infection (gMHV, Fig4A) or immunisation (Nitrophenylacetyl(NP)-OVA, Fig4B) promoted MPEC differentiation as with treatment of stimulated CD8⁺ T cells in vitro. Consequently, to allow assessment of functional in vivo memory responses, we used a heterosubtypic influenza rechallenge model. In this model (Fig4C) primary intranasal infection with H3N2 influenza (A/HKx31) is followed by intranasal rechallenge with a recombinant H1N1 strain (A/PR8/34). The PR8 strain shares all internal proteins with HKx31 but is serologically distinct, allowing detection of secondary T cell responses without confounding effects of antibody mediated viral clearance. Secondary influenza responses are characterised by expansion of antigen-specific T cells in mediastinal lymph nodes (MLN) followed by migration to lung with

severe infection characterised by diffuse lymphocytic infiltration and virus-induced lung immunopathology. Following low dose valproate treatment for 7 days during the primary infection (~10% clinically approved dose), we observed significant expansion of lung-resident T cell memory by d30pi (Fig4D). This was followed by expanded secondary lymphoid responses on rechallenge (Fig4E, F) characterised by enhanced expression of a memory phenotype (IL7RhiPD1lo, Fig 4G) and reduced pulmonary lymphocytic infiltration (Fig4H). In each case, concurrent administration of the glutaminolysis inhibitor (R162) reversed the valproate-induced expansion of T cell memory (Fig4D-H, VR). Although valproate treatment resulted in an overall reduction in lung T cell infiltration on rechallenge, we observed marked clustering of proliferating cells around the peribronchiolar site of infection (Fig 4I, J). This is consistent with the enhanced antigen-specific memory seen at d30pi compared to a more diffuse parenchymal T cell infiltrate characteristic of immunopathology seen in the controls (Fig 4I-J). Epigenetic (ATACseq) analysis of CD44+CD8+ T cells from spleens of valproate-treated animals again showed enrichment of Wnt, Hippo and HIF pathways amongst differentially open chromatin regions, consistent with in vitro observations on similarly treated primary human CD8+ T cells (Fig 3G). Although the influenza model was specifically designed to test secondary T cell responses (with no sharing of B cell epitopes between primary infection and rechallenge), we also observed an expansion of germinal centre B (GCB) and T follicular helper cells (TFH), critical for promoting a high-affinity antibody response (Fig4H). We therefore tested the impact of valproate treatment on both cellular and humoral responses in a murine model of SARS-CoV2 immunisation. Mice were immunised with SARS-CoV2 receptor binding domain protein (RBD) 221 alongside low dose oral valproate treatment (Fig 4K). Treated animals again showed expansion of germinal centre and plasmablast responses (Fig 4L) alongside a >2-fold expansion in the magnitude of RBD-specific B and T cell memory responses following peptide rechallenge (Fig 4L, M) along with a similar expansion in SARS-CoV-2 neutralising antibody titre (Fig 4N).

Together these data indicate that short duration, low dose sodium valproate treatment following primary immunisation or infection results in a significant expansion of both early MPEC and expanded stable memory populations and an enhanced secondary recall response. Combined genomic analyses indicate that this expansion is directed by increased glutaminolysis, oxidative metabolism and Wnt/Myc signalling accompanied by epigenetic modification of Wnt and other memory associated pathway genes. These effects of valproate treatment may be reversed both in vitro and in vivo by specifically blocking glutaminolysis, indicating an immunometabolic mechanism underpinning treatment-induced memory differentiation. With demonstrated in vivo efficacy at low dose, selected KDACi could be rapidly translated to boost immune memory responses to both infection and vaccination, including to influenza and SARS-CoV2 vaccines. Such enhanced cellular memory may be of particular benefit in the elderly, where vaccine responses may be suboptimal, or to limit the ability of new viral variants to evade vaccine-induced serological protection.

Example 2 – Experimental medicine study in healthy volunteers receiving immune challenge by seasonal influenza vaccination with/without concurrent administration of low-dose sodium valproate

This is an experimental medicine study into the development of immunological memory following seasonal influenza vaccine aiming to investigate the extent and mechanism by which a repurposed drug alters that process.

The population we aimed to study were healthy adults in two broad age groups (18-65 years and >65 years) who are positive for specific HLA alleles that will facilitate identification of antigen specific CD4 (HLA-DRB*07:01, HLA-DRB*11:01, HLA-DRB*04:01) and CD8 (A*02:01) T cells. Individuals will be identified and invited to participate through the NIHR Cambridge BioResource, to facilitate identification by HLA haplotype.

We aimed to recruit up to 180 volunteers, to achieve full datasets from 50 volunteers per treatment group (1.vaccine,+drug.dose#1 (100mg/day), 2.vaccine+drug.dose#2 (200mg/day) of the study (allowing for 20% dropout). Group 3 received seasonal influenza immunization alone, groups 1 and 2 received oral low dose sodium valproate from day 0 to day 7 inclusive. Each group was further stratified by age into those 18-65 years old and those >65 years old. Peripheral blood samples will be collected before and after vaccination (day0 and day7) and will be processed to retain serum and peripheral blood mononuclear cells (PBMC) for storage as viable aliquots of 107 cells. Samples will be processed in batches to minimize batch artefact structure once all have been collected. Serum samples will be used to quantitate influenza specific humoral responses (ELISA, HAI titre) at each timepoint while frozen, viable cell aliquots will be used to identify and sort influenza specific CD8 and CD4 T cells for quantitation and sequencing. The immune traits being measured as outcomes in this study design have been developed taking into account previous evidence of immunological correlates of protection following influenza vaccination:

- The cellular response following influenza vaccine shows a significant correlation with protection from severe infection.
- Increased titre of influenza specific antibody is a correlate of protection from influenza infection following seasonal vaccination
- Influenza responses to seasonal vaccination are demonstrably lower in aged individuals compared to younger vaccines.
- There is a dose-dependent effect of sodium valproate on memory differentiation during polyclonal stimulation of T lymphocytes in vitro culture.
- There is a differential effect of sodium valproate on memory and naïve populations during polyclonal stimulation of T lymphocytes in vitro culture.

Results - Modulating Emerging Memory Responses to Immunisation (The MEMRI study)

Neutralising antibody responses have been established as robust correlates of protection with multiple vaccines, while enhanced cellular responses associate with reduced disease severity

and maintenance of protective antibody levels in both SARS-CoV-2 and influenza. In human influenza infection the antibody response has been established as a robust correlate of protection from infection while the magnitude of the heterotypic T cell response was associated with reduced severity of influenza infection during the 2009-2001 H1N1 pandemic. We therefore

5 sought to test whether repurposed low dose sodium valproate treatment could act as an immunometabolic adjuvant, promoting expanded cellular or humoral correlates of protection after seasonal influenza vaccination. We designed an interventional experimental medicine study (the MEMRI study, Fig 5A, + Protocol, methods) with stratified randomisation of HLA - genotyped (A*-0201) healthy volunteers to receive either sodium valproate (at one of two low

10 doses (100 or 200mg/day) equivalent to 5-10% of its approved dose as an anti-convulsant,) or no additional treatment daily for seven days starting on the day of seasonal influenza (2020/21) vaccination (Fig 5B). Blood samples taken both before (day 0) and at days 7 and 30 following vaccination (Fig 5B) were used to quantify both the CD8+ T cell response to the immunodominant HLA-A*0201 restricted influenza protein MP1 and the humoral response using

15 both vaccine-specific influenza antibody titres and functional haemagglutination inhibition (HAI) assays. We observed treatment-induced expansion of baseline-normalized influenza-specific cellular and humoral responses post vaccination with low dose sodium valproate treatment resulting in both a >10-fold expansion and maintenance of 'flu-specific CD8+ T cells (Fig 5C, D) and a >2-fold expansion and maintenance of vaccine strain-specific influenza IgG titre (Fig 5E).

20 Mean influenza-specific IgA titre in plasma was expanded >10-fold in the treatment group on day 7 post-vaccination although levels had fallen in both groups by day 30 (Influenza A/Hong Kong H3, Fig 5F). Overall, influenza-specific HAI titre was increased >1.5 fold in the treatment group (Fig 5G) with much of the observed humoral response due to the lowest dose treatment group . Conversely, the impact on the CD8+ T cell response was observed to be greater with

25 the higher dose treatment.

We also observed post-vaccination expansion of influenza-specific IgG and IgA titres to viral serotypes that were not included in the seasonal vaccine used, including some which were increased by sodium valproate treatment. This implied that treatment may promote a broader response, as well as expansion and maintenance. Such a hypothesis is also supported by our

30 observations of increased TFH and germinal centre B cells (Fig4B, M) in the murine influenza (Fig 4B) and SARS-CoV-2 immunisation models (Fig 4M) as these changes would be expected to result in increased affinity maturation and somatic hypermutation leading to a broader, more effective vaccine-induced antibody response. To test this hypothesis further, we undertook targeted B cell receptor sequencing and repertoire analysis of whole blood samples from the

35 MEMRI study. In the global BCR repertoire we observed longitudinal changes in isotype usage, somatic hypermutation and clonal diversity peaking at day 7 post-vaccination. However, no global repertoire differences were observed between treatment groups (Fig 5H). To investigate changes in the influenza-specific BCR repertoire, we matched the observed BCR sequences to those of over 27,000 known influenza-specific clones (methods), identifying 5274 BCR clusters

with confirmed influenza specificity. Amongst these sequences, valproate treatment was associated with significant increases in somatic hypermutation (Fig 5I), clonal expansion (Fig 5J, K) and diversity (Fig 5L, M). Measures of expansion and diversity were increased by treatment in both class-switched and naïve (IgM+IgD+) sequences (Fig5K, M) suggesting a treatment effect on both de novo primary and also secondary influenza-specific responses following vaccination.

Discussion

Prior to this study, it was known that metabolic changes could be causal drivers of immune cell differentiation as well as important consequences of that process (McKinney and Smith, 2018). It was also known that metabolic intermediates – such as acetate (Qiu et al., 2019) – could serve as signalling events to modify critical cellular processes (Choudhary et al., 2009) including chromatin accessibility, such as are necessary to mediate long-lived changes in cellular phenotype (Choudhary et al., 2014). Consequently, what were initially known as ‘histone deacetylases’ have expanded to become lysine (K) deacetylases, capable of modulating many protein groups and with many targets in the mitochondria (Scholz et al., 2015). Our data are consistent with a model in which a subclass of KDACi compounds are capable of altering protein acetylation resulting in increased glutaminolysis during initial antigen encounter and cellular activation. The resulting switch towards oxidative phosphorylation promotes immune cell differentiation towards a memory phenotype and enhanced secondary responses. T cell exposure to certain KDACi at the time of activation results in acquisition of a memory phenotype along with functional, transcriptional and epigenetic features of memory. However, it is less clear whether other immune cell types (such as CD4+ T cells and B cells) undergo similar cell-intrinsic epigenetic modulation, or whether altering the phenotype of one cell type may have secondary consequences on the entire response. In addition, KDACi can have direct effects on histone acetylation and, while the reversal of treatment effects by inhibition of glutaminolysis (both in vitro and in vivo) suggests that epigenetic modulation is secondary to immunometabolic modulation, our experiments do not definitively differentiate these two possibilities.

It was also previously known that KDACi can have immunomodulatory effects, and in particular that cell survival, proliferation and differentiation can be altered by certain compounds. Our data are consistent with this – we see a biphasic dose response relationship with limitations in cell cycle and proliferation at higher doses and promotion of cellular memory only at lower doses. Only through screening multiple KDACi compounds was the subclass effect apparent.

Our experimental approach demonstrates that, by combining multiple genomic analyses with both in silico and in vitro phenotypic screening, it is possible to identify repurposable small molecules capable of rapid clinical translation. Similar approaches to the identification of repurposable small molecules have been tried before, although with only rare examples of validation in human studies (Pushpakom et al., 2019). However, the inclusion of multi-omic analysis also allows us to also consider the drugs screened to be molecular probes, using

genomic data to interpret their mechanism of action and inform our biological understanding of immune memory in the process. A stepwise progression from in silico screen, through in vitro mechanistic evaluation into preclinical and ultimately human experimental medicine studies offers the potential to understand why as well as which compounds should be repurposed.

- 5 The MEMRI study described here indicates that low dose KDACi (valproate) treatment can significantly expand the size, breadth and durability of vaccine-induced cellular and humoral immunity (Fig 5). The study does not confirm clinical efficacy of the observed expansion but uses validated correlates of vaccine-induced protection from infection as key endpoints. Data from both murine and human studies are consistent with a model in which enhanced cellular and
- 10 humoral responses are secondary to expanded germinal centre and TFH cell differentiation, rather than direct expansion of T cell memory alone. Our observation that both antigen naïve (IgM+IgD+) and secondary (d7 IgG+) humoral responses show increased expansion, breadth and SHM on treatment suggests that susceptible groups – such as the elderly – who are more reliant on heterologous secondary responses for vaccine-induced protection could gain
- 15 particular benefit from KDACi adjuvanted vaccination.

Together these data indicate that short duration, low dose sodium valproate treatment following primary immunisation or infection results in a significant expansion of immune memory in mice and humans. Multi-omic analyses indicate that this expansion is directed by increased glutaminolysis, oxidative metabolism and Wnt/Myc signalling accompanied by epigenetic

20 modification of Wnt and other memory associated pathway genes. These effects of valproate treatment may be reversed both in vitro and in vivo by specifically blocking glutaminolysis, indicating an immunometabolic mechanism underpinning treatment-induced memory differentiation. With demonstrated in vivo efficacy at low dose, selected KDACi could be rapidly translated to boost immune memory responses to both infection and vaccination, including to

25 influenza and SARS-CoV2 vaccines. Such enhanced cellular memory may be of particular benefit in the elderly, where vaccine responses may be suboptimal, or to limit the ability of new viral variants to evade vaccine-induced serological protection.

30 **Example 3 - Experimental medicine study in healthy volunteers receiving immune challenge by SARS-CoV-2 vaccination with/without concurrent administration of low-dose sodium valproate**

This is an experimental medicine study into the development of immunological memory following seasonal influenza vaccine aiming to investigate the extent and mechanism by which a repurposed drug alters that process.

- 35 Subjects will be administered the SARS-CoV-2 vaccination on day 0 along with a 100mg dose of sodium valproate. The sodium valproate will then be administered for 7 days following the vaccination. The same protocol will be used when the SARS-CoV-2 booster vaccination is administered.

Control subjects will also be included in the study who will not receive sodium valproate.

Blood samples will be taken on the day of the first and second vaccinations as well as 6 months and 12 months after the first vaccination.

Methods

5 **In silico screen**

Target CD8⁺ T cell memory signatures defining CD8 SLEC v MPEC (ImmuneSigDB M3027, M3028), CD8⁺ exhaustion v memory (GSE41867, ImmuneSigDB, M9480,9482), CD2 memory (E-MTAB-3470) were obtained from MSigDB or GEO. Signatures were screened against characteristic direction drug response signature libraries curated from GEO
10 (Drug_perturbations_from_GEO_2014), LINCS (L100 chem perturbation) and the connectivity map (CMAP) using the EnrichR platform. Compounds with significant overlap (overlap ≥ 5 and adjusted P95%) were considered for inclusion for in vitro phenotypic screening. Combined enrichment scores are 11 255 computed as modified Fisher's exact test multiplied by deviation from an expected rank value.

15 **In vitro phenotypic screen**

Primary human CD8⁺ T cells were separated from leucocyte cones obtained from NHS Blood and Transplant (Addenbrooke's Hospital, Cambridge, UK) by centrifugation over ficoll and positive selection using magnetic beads as previously described. The purity of separated cell subsets was determined by three-colour flow cytometry. Purified T cells (>95%) werelabelled
20 with 2.5 μ M CFSE (Invitrogen) and resuspended in complete RPMI 1640 (Sigma Aldrich) in the presence of 10% FCS and then stimulated in sterile, U-bottomed 96 well culture plates (Greiner) using MACS iBead particles (1:2 bead:cell ratio, Miltenyi) conjugated to anti-CD2/CD3/CD28 in the presence of non-limiting IL2 (10ng/ml, Gibco Life technologies) for 6 days. Selected compounds were added at the time of stimulation (d0), or 2 (d2) or 4 (d4) days after as indicated.
25 T cell memory subsets were isolated using flow cytometric sorting (FACS Aria III cell sorter, BD Biosciences) following MACS enrichment of CD8⁺ T cells as above, into naïve (Tn, CD45RA⁺CD62L⁺), effector memory (Tem, CD45RA⁺CD62L⁻), central memory (CD45RA⁺CD62L⁺) and Temra (CD45RA⁺CD62L⁺) populations before co-culture with drugs as indicated. T cells were stimulated in the presence of treatments as indicated for 6 days before undergoing
30 high-throughput immunophenotypic screening (BD LSR Fortessa HTS) with quantification of IL7R. Reactions were standardised with multicolour calibration particles (BD Biosciences) with saturating concentrations of the following antibodies: IL7R (BD Biosciences, Clone HIL-7R-M21), CD25 (Clone M-A251), live/dead discrimination (AquaFluorescent amine-reactive dye, Invitrogen). Data was analysed using FlowJo software (Tree Star), extracting immunophenotypic
35 traits (IL7Rmfi, division (CFSEdii), CD25mfi, % live cells) with ratios calculated against pooled vehicle treated controls in triplicate.

Compound selection and titration

Compounds were selected from the *in silico* screen on the basis of consistent enrichment against multiple signatures taking into consideration likely impact on cellular viability, toxicity and

availability. Additional ligands and cytokines with a demonstrated association with T cell memory formation were also included with Fc-Chimaeric ligands hybridised to individual wells of a 96-well U-bottomed culture plate (Greiner) for 2h at 37C before washing in sterile complete RPMI-1640 and addition of cell suspensions for stimulation. Dose ranges for inclusion were selected on the basis of demonstrated in vitro IC50 for target effects with 3 doses included per treatment. Effects were considered by dose and also by peak effect per treatment with each value compared to the median of triplicate repeat vehicle stimulation. In vitro KDACi efficacy was confirmed by measuring impact on total cellular protein acetylation by flow cytometry (acetylated lysine multiMab mix, CST: Fig2B) and for selected compounds (apicidin and sodium valproate) taken into genomic, functional and in vivo assays standard concentrations were generated using targeted LC-MS/MS quantitation (PK/B Core facility, CRUK, Cambridge).

Lysine Acetylation proteomic data

Stable isotope labelling with amino acids in cell culture (SILAC) mass spectroscopic (MS) quantitation of enriched acetylated peptides was obtained from. We extracted SILAC H/L ratios from KDACi-treated HeLa cells, verifying comparability between and then combining data across technical replicates. Median H/L ratios were converted to a pairwise distance matrix using the ape and phylobase packages in R before visualisation as an annotated, unrooted tree using iTOL (<https://itol.embl.de/>). KDACi compounds were classified on the basis of MPEC phenotype induction (KDACi.mem/eff) and differentially acetylated peptides were determined by comparison between these groups (threshold fdr 5%). Peptides were summarised to protein level and pathway enrichment analysis of differentially acetylated proteins was undertaken against the KEGG pathway database using EnrichR.

Metabolomic analysis

Primary human CD8+ T cells were snap frozen in liquid nitrogen following 20 isolation stimulation and culture as described above in the presence of either apicidin (1.4nM), valproate (87.5µM) or vehicle control (<0.001% DMSO n=8 per group). Cell pellets were subjected to LC-MS/MS analysis (Metabolon Ltd) with identified compounds normalised to dsDNA concentration, log transformed and missing values imputed as the lowest value identified per compound. A Mixed model ANOVA with biological replicate as a random effect was used to identify differential compounds between experimental conditions (FDRq) Pathway enrichment of differential metabolites was undertaken using MetaboAnalyst42 and metabolite set enrichment analysis, taking pathways from HMDB43 as indicated.

Seahorse metabolic flux analysis

Measurement of oxygen consumption rate (OCR, XF MitoStress test, Agilent) and extracellular acidification rate (ECAR, XF Glycolysis Stress test, Agilent) was undertaken using the Seahorse XFp energy phenotype system (Agilent) according to the manufacturer's instructions. Primary human CD8+ T cells were isolated, stimulated and cultured as above for 2 days in the presence of either 87.5µM sodium valproate before being transferred to Seahorse-XF base medium (Agilent, Cat No. 103335-100), immobilised onto a Seahorse XF96 cell culture microplate

(Agilent) using Cell-Tak (Corning) with concurrent aliquots taken for immunophenotypic assessment including live/dead (AquaFluorescent amine-reactive dye, Invitrogen) and cell proliferation (CFSE dilution) quantification. Raw data was extracted using Wave software (Agilent) and normalised to baseline values with comparative flux estimates obtained from summarized peak values across biological replicates.

Glucose uptake assay

Primary human CD8⁺ T cells were isolated, stimulated and cultured as above for 6 days after which cells were incubated in glucose-free media for 60min at 37C in the presence of 50μM NBDG (ThermoFisher N13195) before staining with live/dead (AquaFluorescent amine-reactive dye, Invitrogen) and IL7R (BD Biosciences, Clone HIL-7R-M21) and acquisition of NBDG fluorescence on a BD LSR Fortessa instrument.

Glutaminolysis inhibition

Following isolation as above, CD8⁺ T cells were stimulated and co-cultured in the presence of either a dose range of sodium valproate (Sigma) or of the specific glutaminolysis inhibitors GDH1 (Focus Biomolecules), C968 (Sigma) or BPTES (Sigma), or with the cell-permeable α-ketoglutarate analogue dimethyl-2-ketoglutarate (DMKG, Sigma) as indicated.

RNA sequencing

Primary CD8⁺ T cells were cultured and stimulated as above for 6 days in the presence of either sodium valproate (175μM, Sigma) or sodium valproate plus R162 (175μM/25μM, Focus Biomolecules) before total RNA was extracted using an RNeasy mini kit (Qiagen) with quality assessed using an Agilent BioAnalyser 2100 and RNA quantification performed using a NanoDrop ND-1000 367 spectrophotometer. Sequencing library preparation was performed using the SMARTer stranded Total RNA-Seq kit v2 pico input (Takara Bio) and 75bp paired end sequencing performed on a NextSeq500 instrument with titrated 1% PhiX. Raw sequence read QC was undertaken using fastqc followed by adapter removal (TrimGalore), extraction of ribosomal reads (BBsplit) and sequence mapping (HISAT2) to the reference genome (GRCh38). Merged bam file QC was undertaken using the QoRTs package in R with assimilation using multiQC. Merged bam files were read into R using featureCounts (Rsubread) with feature filtration to >cpm1 in >6 samples and annotation using BioMart followed by pairwise differential expression using DGEList (edgeR). Matrix factorisation analysis was undertaken using the MOFA package in R with feature set enrichment performed using the Bioconductor package fgsea in R with gene sets extracted from MSigDB using the msigdb package as indicated.

ATAC sequencing

ATACseq was undertaken following the protocol established by Buenrostro et al 201545 with modifications. Following CD8⁺ T cell culture as above, 50,000 cells were lysed (1M Tris-HCl) and the nuclear pellet transposed using the Tn5 transposase (TDE1, Illumina) at 37C for 30min before DNA isolation (Qiagen 386 minElute) and PCR amplification (NEBnext) guided by qPCR (KAPA-SYBR) estimation of reporter signal v cycle number and PCR cycle length tailored to 1/3 maximal amplification. Libraries were purified (AMPure XP) with quality assessed using an

Agilent BioAnalyser 2100 and RNA quantification performed using a NanoDrop ND-1000 spectrophotometer. 50bp paired-end sequencing was performed on a NextSeq500 instrument with downstream QC matching against ENCODE standards and peak calling and differential peak identification undertaken using the esATAC BioConductor package in R. KEGG pathway enrichment and visualisation of treatment-specific differential peaks was undertaken using the BioConductor packages clusterProfiler, GenVisR and rtracklayer in R.

***in vitro* restimulation**

Primary CD8⁺ T cells were isolated, stimulated and cultured as above for 6 days in the presence of either sodium valproate (175µM, Sigma) or vehicle control (<0.001%DMSO) followed by flow cytometric sorting (FACSARIAIII cell sorter, BD Biosciences) into divided CFSE^{lo}IL7R^{hi} and CFSE^{lo}IL7R^{lo} subpopulations. These were rested for 5 days in complete RPMI-1640 (Sigma), with normalization of live cell numbers (1x10⁶/ml) and CFSE-relabelling before polyclonal restimulation repeated as for the primary stimulation (anti- CD2/3/28, Miltenyi). After 5 days restimulation cells were analysed by flow cytometry (BD LSR Fortessa) with staining for live/dead (AquaFluorescent amine-reactive dye, Invitrogen) and IL7R (BD Biosciences, Clone HIL-7R-M21)

Infection and immunisation models

C57BL/6 mice were infected i/n with gMHV virus or immunised with 200µl NP-412 OVA in alum with spleens harvested d7 (NP-OVA) or d9pi (yMHV). For the heterotypic influenza model C57BL/6 mice were given a primary i/n challenge with 500pfu H3N2 influenza (A/HKx31) and a secondary challenge with 105pfu 415 i/n H1N1 (A/PR8/34) influenza d31 post-primary challenge with tissues (lung, spleen, MLN, blood) harvested on d30pi and d38pi for stable memory and 417 rechallenge assessment respectively (Fig4C). For each model mice were administered sodium valproate 0.1% w/v in drinking water, sodium valproate plus R162 (90% corn oil, 10% DMSO) 20mg/kg/day intraperitoneally or matched vehicle control as indicated (Fig4). For SARS-CoV-2 immunisation, mice were administered receptor binding domain (RBD) peptide (gift, YM) 50µg s/c in QuilA adjuvant either once (R1) or three times (R2) as indicated (Fig4K). Tail bleeds were taken (d58pi) prior to RBD re-immunisation (d60) with tissues harvested on d65pi.

Expression and purification of SARS-CoV-2 RBD

Recombinant SARS-CoV-2 Spike RBD glycoprotein carrying a C-terminal hexahistidine purification (His6) tag was expressed in the Pichia pastoris X-33 yeast strain using the pPICZα A expression vector (ThermoFisher). Cultures were grown on the 10-liter scale in an Eppendorf BioFlo 510 fermenter with active control of temperature, pH, dissolved oxygen concentration and methanol feed (as the carbon source) to maximize protein yield based on a protocol developed previously to purify SARS-CoV-1 RBD^{46,47}. RBD glycoprotein was secreted into the cell culture supernatant and purified by nickel-affinity and size-exclusion chromatography. To produce untagged RBD, the His6 tag was removed using Carboxypeptidase A (Sigma), followed by nickel-affinity chromatography to remove uncleaved protein and size-exclusion chromatography.

Tissue processing

Splenocytes and draining lymph nodes were weighed and then homogenised through a 70µm cell strainer (BD bioscience) using complete tissue culture media [IMDM (Thermo), 10% Fetal Bovine Serum (Sigma), 1mM Sodium Pyruvate (Thermo), 1 X Non-essential Amino Acid Solution (Sigma), 2mM 444 Glutamax (Thermo), 100U/ml Penicillin-Streptomycin (Thermo) and 50µM b-
 5 445 Mercaptoethanol (Sigma)]. Splenocyte solutions were red blood cell lysed, resuspended in culture media and counted before use.

ELISPOTS

For B cell ELISPOTS, MultiScreen-IP Filter PDVF, 0.45 µm ELISPOT plates (Merck Millipore) were first activated in 70% ethanol for 2 minutes before being washed twice with sterile PBS.
 10 Plates were then coated overnight at 4°C with 50µl of purified RBP protein in PBS (5µg/ml). The next day, plates were washed twice with sterile PBS and blocked with 200µl complete tissue culture media for 2 hours at 37°C. After this time, blocking media was aspirated and 100µl of splenocyte suspensions were added in two concentrations (10⁶/mL and 10⁵ /mL) to appropriate triplicate wells. Plates were then incubated at 37°C overnight. After 18hrs cells were aspirated
 15 from the wells and plates were washed 3 times with PBS/0.05% Tween-20 (Sigma) [PBST] followed by 3 washes in PBS. 50µl of goat anti-mouse IgG-HRP conjugate (Southern Biotech) in PBS at 1:2000 dilution was then added to the plates and incubated at room temperature for 1 hour. Plates were then washed as previous, before being developed with 3-Amino-9-ethylcarbazole tablets (Sigma) using the manufacturer's instructions. After stopping
 20 development, plates were left to dry for 48 hours before being read and analysed using an ELISPOT plate scanner (ELISPOT Reader System, AID) and spots enumerated using ImageJ (National Institutes of Health).

For IFNγ ELISPOTS, plates were prepared with ethanol and washed as above before being coated with 50µl of anti-mouse IFNγ (AN18, Thermo) at 2ug/ml in PBS. Plates were incubated
 25 overnight at 4°C before being washed and blocked as described above. First, 50µl of culture media containing 60U/ml IL-2 (Peprotech) and 20µg/ml purified RBP protein were added to the appropriate wells, followed by 50µl of cell suspension corresponding to 104 or 105 cells per well. Plates were then incubated at 37°C for 48 hours before cells were aspirated and washed as above. 50µl of biotinylated anti-mouse IFNγ (R4-6A2, Thermo) in PBS/0.5% BSA (Fischer) was
 30 then added to each well and plates were incubated for 2 hours at room temperature before being washed. 50µl of Streptavidin-horseradish peroxidase (Thermo) diluted 1:5000 in PBS/0.5% BSA was then added to each 20 well and plates were incubated for 1 hour at room temperature, washed and then developed as above.

Immunophenotyping

35 After harvesting cell suspensions were normalised to 1x10⁶ /ml and stained with non-limiting concentrations of IL7R (CloneA7R34, Biolegend), PD1 (Clone RMP1- 30, Biolegend), KLRG1 (), CD44 (CloneIM7, Biolegend), NP-dextramer (Immudex), GL7 (Clone GL7, Biolegend), CD8 (Clone 53-6.7, Biolegend), BCL6 4(CloneIG191E/A8, Biolegend), FOXP3 (Clone FJK-16s,

ThermoFisher), B220 (Clone RA3-6B2, Biolegend) before data acquisition by flow cytometry (BD LSR Fortessa) and analysis in FlowJo (BD Biosciences) and prism 6.0 (Graphpad).

Multiplex IHC

- Lung and spleen samples were fixed and processed into FFPE blocks. 4.5um paraffin sections
- 5 were collected on Super Frost Plus slides and dried overnight. For immunostaining slides were deparaffinized in xylene and rehydrated with series of graded ethanol. Antigen Retrieval was performed by gentle simmering in 700ml of Tris-EDTA, pH9 buffer, following by endogenous peroxidases blocking and incubation with 2.5% Horse Serum. Ki67 antibody (1:500) were incubated in a humidified chamber overnight at +4C, detected using ImmPRESS anti-Rabbit-
- 10 HRP Polymer detection system (VectorLab, MP-7401) and visualized using TSA-Plus Opal 620 (Akoya, FP1495001KT). Slides were then placed in Tris-EDTA, pH9 buffer and simmered for 15 min. anti-CD3 antibodies (1:50) were applied and incubated in a humidified chamber overnight at +4C. As before primary anti-CD3 antibodies were detected with ImmPRESS anti-Rabbit-HRP
- 15 21 Polymer detection system and visualized with TSA-Plus Opal 520 (Akoya, FP1495001KT). Slides were mounted with Dapi-supplemented ProlongGold and imaged using Zeiss Apotome 2 microscope and Hamamatsu monochrome camera.

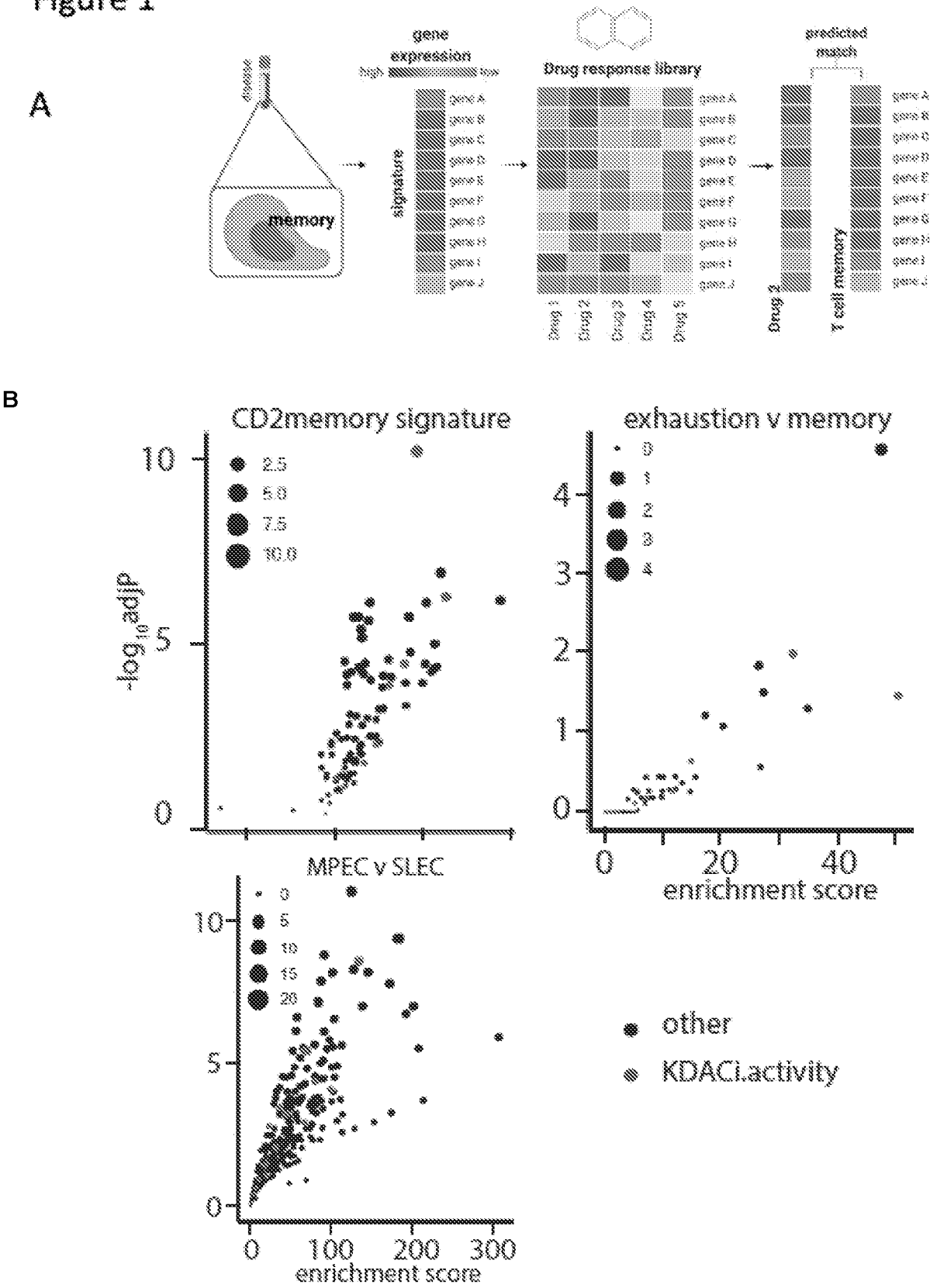
CLAIMS

1. A method for enhancing an immune response in a subject comprising administering a lysine deacetylase inhibitor (KDACi) simultaneously, sequentially, or separately with a vaccine, wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1 for the selected KDACi.
2. The method according to claim 1, wherein the KDACi is provided at a dose of up to 25% of the dose listed in Table 1.
3. The method according to claim 1 or 2, wherein the vaccine is an infectious disease vaccine against a viral, bacterial, fungal or parasitic infectious agent.
4. The method according to a preceding claim, wherein the vaccine is a respiratory disease vaccine selected from a vaccine against *Haemophilus influenza*, a coronavirus, such as SARS-CoV-2, *Haemophilus influenzae* type B, measles virus, poliovirus, tetanus, tuberculosis, cholera, typhoid, dengue, diphtheria, hepatitis, Japanese encephalitis, meningococcal meningitis, mumps virus, pertussis, pneumococcal disease, rabies, rotavirus, rubella, coronavirus, such as SARS-CoV-2, SARS-CoV, or MERS-CoV,
5. The method according to a preceding claim, wherein the vaccine is selected from a whole pathogen vaccine, live attenuated vaccine, inactivated vaccine, whole killed vaccine, recombinant protein vaccine, toxoid vaccine, conjugate vaccine, virus-like particle (VLP) vaccine, outer membrane vesicle (OMV) vaccine, DNA vaccine, RNA vaccine, or viral vectored vaccine.
6. The method according to a preceding claim, wherein the KDACi is administered at a dose of 10 to 950 mg/day, or 10 to 500 mg/day, or 50 to 250 mg/day, or 75 to 225 mg/day, preferably at a dose of 100 to 200 mg/day.
7. The method according to a claim 5, wherein the KDACi is valproate and administered at a dose of 100 mg/day.
8. The method according to claim 5, wherein the KDACi valproate and is administered at a dose of 200 mg/day.
9. The method according to a preceding claim, wherein the KDACi and the vaccine are administered on day 0, and wherein a further dose of the KDACi is administered on days 1 to 2, days 1 to 3, days 1 to 4, days 1 to 5, days 1 to 6 or days 1 to 7.
10. The method according to a preceding claim, wherein the KDACi is administered by the oral, sublingual, buccal, intravenous, intramuscular, or subcutaneous route.
11. The method according to a preceding claim, wherein the KDACi is provided as a tablet, capsule or liquid.
12. The method according to a preceding claim, wherein the vaccine is administered by the intravenous, intramuscular, or subcutaneous route.

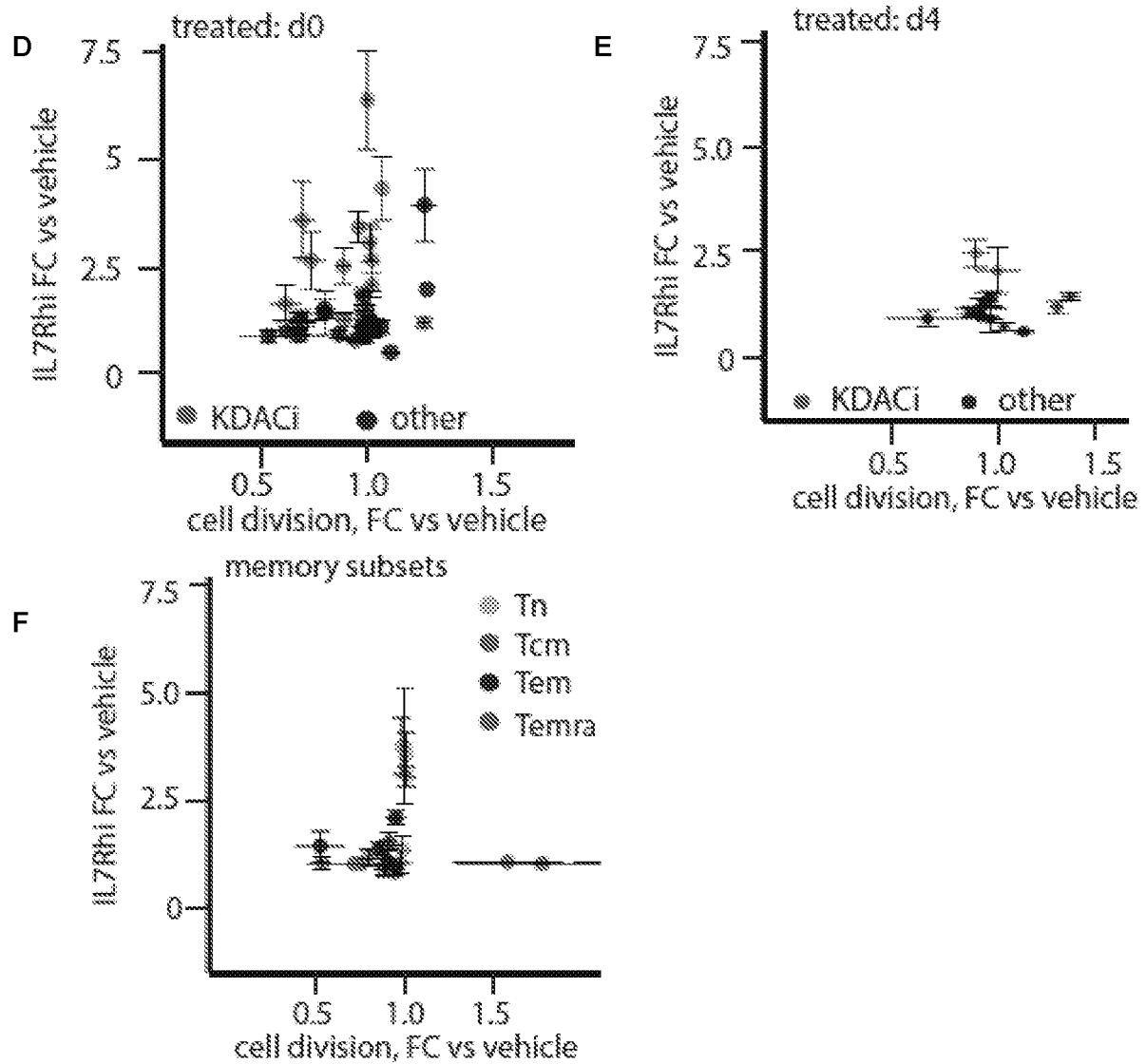
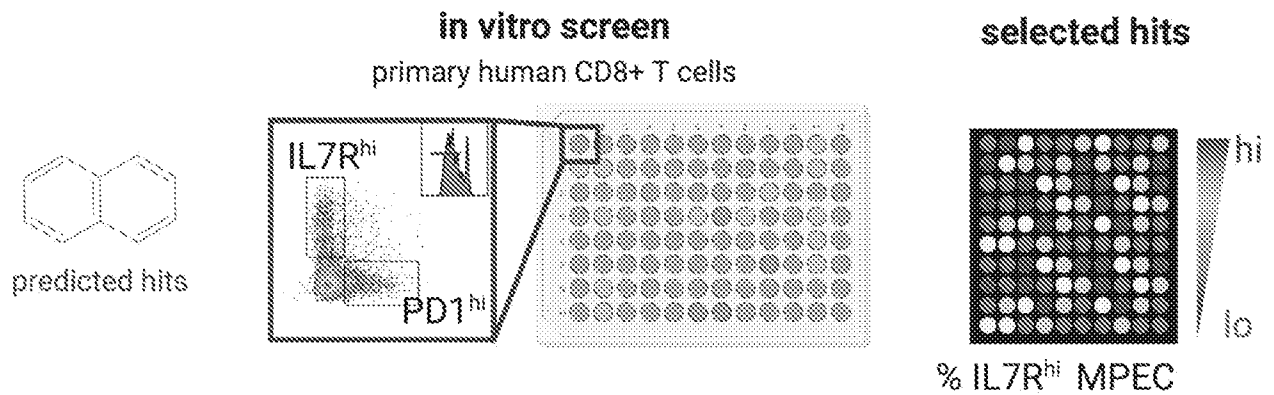
13. A method for the treatment of a disease comprising administering a lysine deacetylase inhibitor (KDACi) wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1 for the selected KDACi.
- 5 14. A KDACi for use in the prevention of a disease, wherein the KDACi is administered simultaneously, sequentially, or separately with a vaccine, wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1 for the selected KDACi.
- 15 15. A KDACi for use in the prevention of SARS-CoV-2, wherein the KDACi is administered simultaneously, sequentially, or separately with a SARS-CoV-2 vaccine.
- 10 16. A KDACi for use in the prevention of influenza, wherein the KDACi is administered simultaneously, sequentially, or separately with an influenza vaccine.
17. A method of promoting a T cell memory response comprising exposing cells to a KDACi capable of enhancing glutaminolysis.
- 15 18. A method of promoting differentiation of CD8+ T cells into memory precursor effector cells, comprising exposing CD8+ T cells to a KDACi capable of enhancing glutaminolysis.
19. A method of increasing IL7R expression in CD8+ T cells, comprising exposing CD8+ T cells to a KDACi capable of enhancing glutaminolysis.
- 20 20. A method of enhancing glutaminolysis comprising exposing CD8+ T cells to a KDACi capable of inhibiting one or more of KDAC1, KDAC2, KDAC3, KDAC4, KDAC5, KDAC7, KDAC9 or KDAC10.
21. The method of any one of claims 17 to 20, wherein the cells are exposed to the KDACi *in vivo*, *in vitro* or *ex vivo*.
- 25 22. The method of any one of claims 17 to 21, wherein the cells are also exposed to a vaccine.
23. A method of immunising a subject against a disease, comprising administering a KDACi simultaneously, sequentially, or separately with a vaccine, and wherein the KDACi is capable of enhancing glutaminolysis.
- 30 24. Use of a class I KDACi as an adjuvant for enhancing the efficacy of a vaccine wherein the KDACi is capable of enhancing glutaminolysis.
25. A composition comprising a KDACi and a vaccine wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.
- 35 26. A kit comprising a KDACi and an infectious disease vaccine and optionally instructions for use, wherein the KDACi is selected from the KDACi listed in Table 1 and provided at up to 50% of the dose listed in Table 1.
27. A method for enhancing an immune response in a subject comprising administering valproate simultaneously, sequentially, or separately with a vaccine, wherein the valproate is administered in a dose of 10 to 950 mg/day.

28. A method for enhancing an immune response in a subject comprising administering abexinostat simultaneously, sequentially, or separately with a vaccine, wherein the abexinostat is administered in a dose of 2 to 10 nM.
- 5 29. A method for enhancing an immune response in a subject comprising administering bellinostat simultaneously, sequentially, or separately with a vaccine, wherein the bellinostat is administered in a dose of 5 to 800 nM.
30. A method for enhancing an immune response in a subject comprising administering panobinostat simultaneously, sequentially, or separately with a disease vaccine, wherein the panobinostat is administered in a dose of 0.5 to 500 nM,
- 10 31. A method for enhancing an immune response in a subject comprising administering butyrate simultaneously, sequentially, or separately with a vaccine, wherein the sodium butyrate is administered in a dose of 0.1×10^3 to 500×10^3 nM.
32. A method for enhancing an immune response in a subject comprising administering apicidin simultaneously, sequentially, or separately with a vaccine, wherein the apicidin is administered in a dose of 0.01 to 100 nM.
- 15 33. A method for enhancing an immune response in a subject comprising administering mocetinostat simultaneously, sequentially, or separately with a vaccine, wherein the mocetinostat is administered in a dose of 0.01×10^3 to 1×10^3 nM.
34. A method for enhancing an immune response in a subject comprising administering vorinostat simultaneously, sequentially, or separately with a vaccine, wherein the vorinostat is administered in a dose of 0.2×10^3 to 5×10^3 nM.
- 20 35. A method for enhancing an immune response in a subject comprising administering MS275 simultaneously, sequentially, or separately with a vaccine, wherein the MS275 is administered in a dose of 0.01×10^6 to 1×10^6 nM,
- 25 36. A method for enhancing an immune response in a subject comprising administering CI994 simultaneously, sequentially, or separately with a vaccine, wherein the CI994 is administered in a dose of 0.01×10^6 to 2×10^6 nM.
37. A method for expanding cells for use in adoptive cell therapy comprising:
providing a population of CD8⁺ T cells,
30 contacting the population of CD8⁺ T cells to a KDACi capable of enhancing glutaminolysis to produce an expanded population of cells.
38. The method of claim 37, wherein the expanded population of cells comprises memory precursor effector cells
39. The method of claims 37 to 38, wherein the expanded population of cells are characterised by the markers IL7R^{hi}, PD1^{lo}.
- 35 40. The method of claims 37 to 39, wherein the expanded population of cells are further characterised by the markers IL7R^{hi}, PD1^{lo}, CX3CR1⁺, CCR7⁺, CD27⁺ and CD62L⁺.

Figure 1



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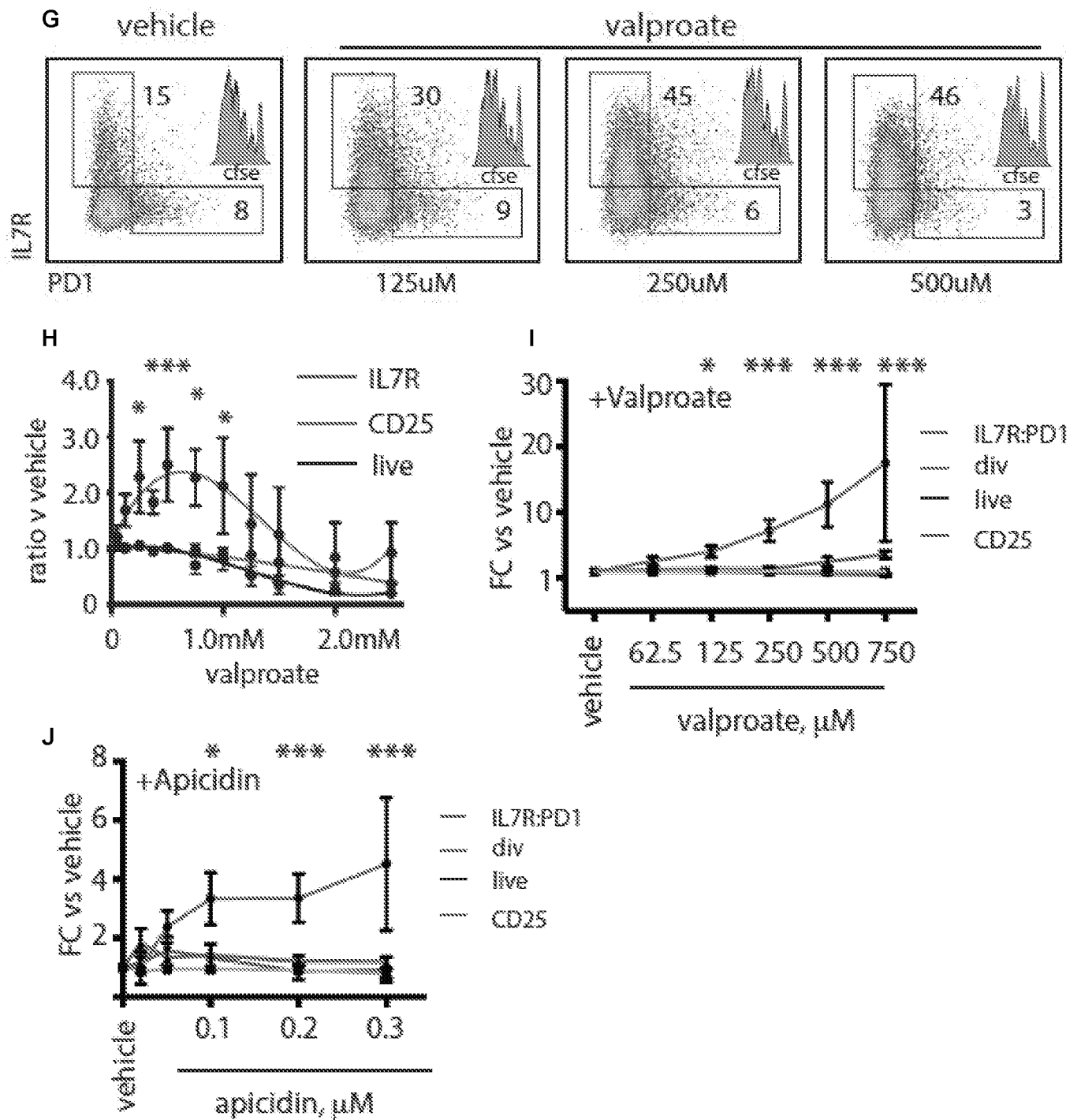
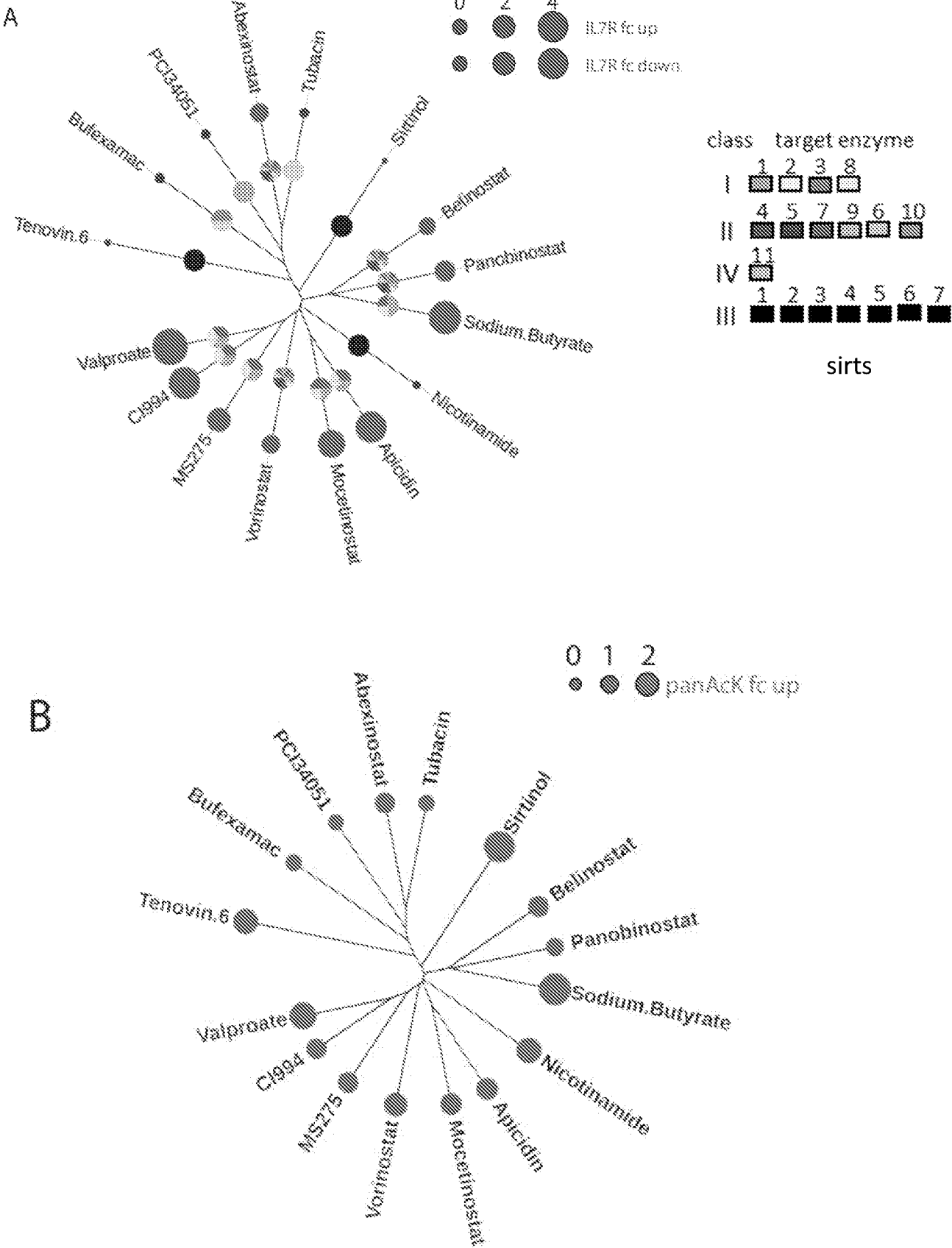
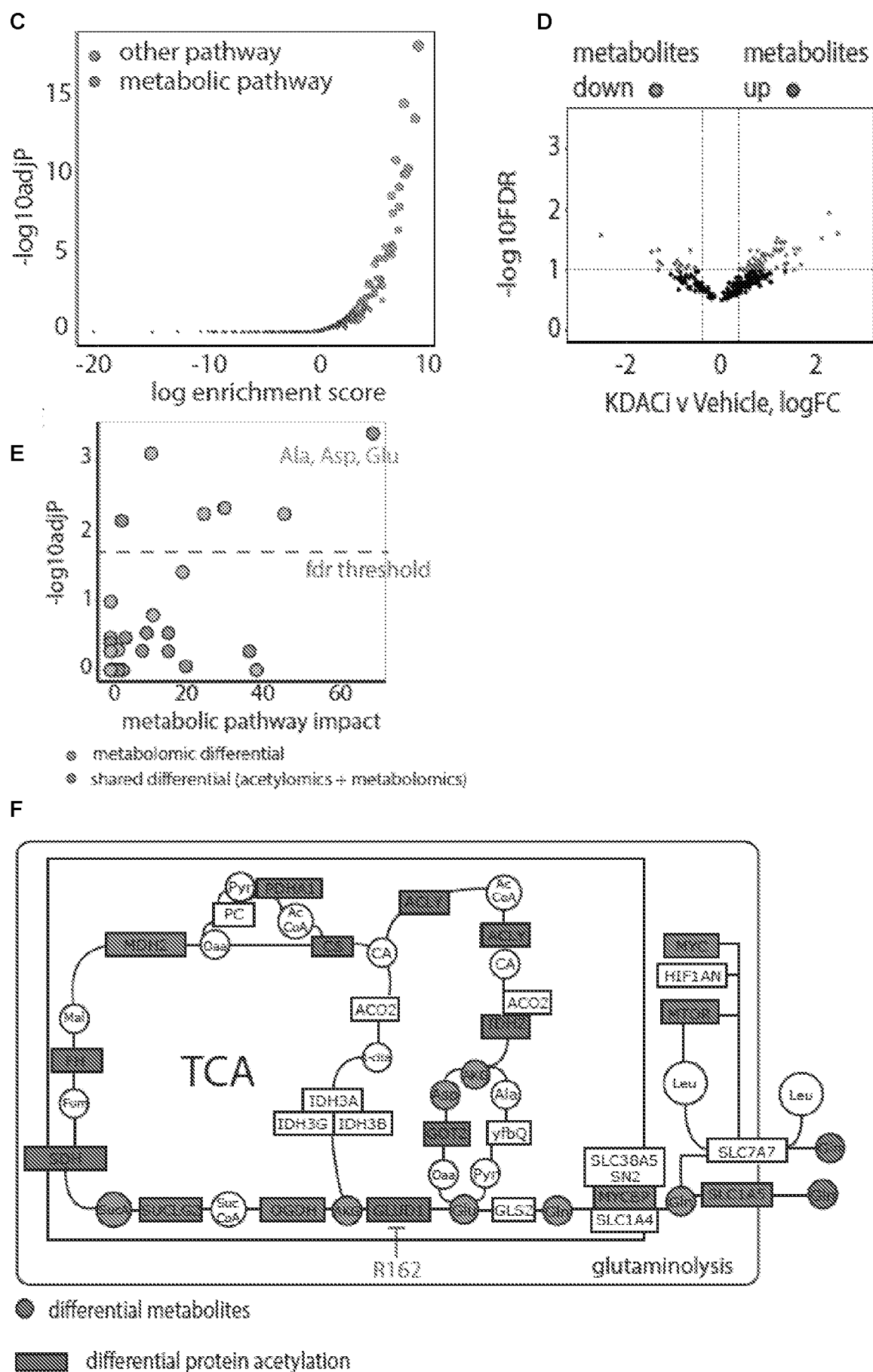


Figure 2

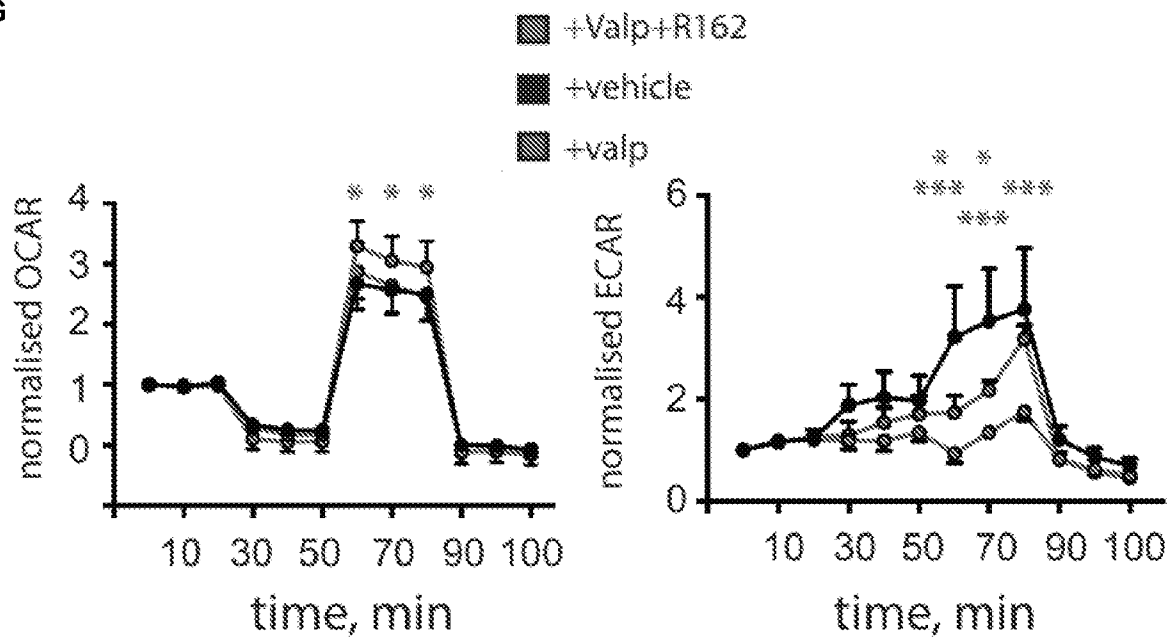


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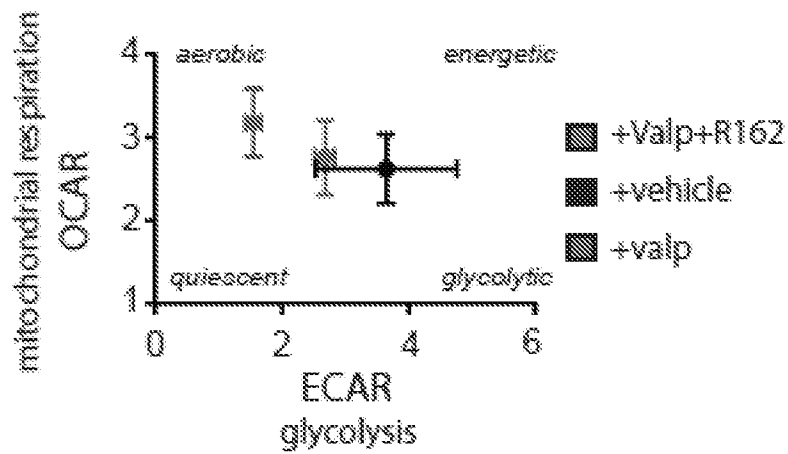


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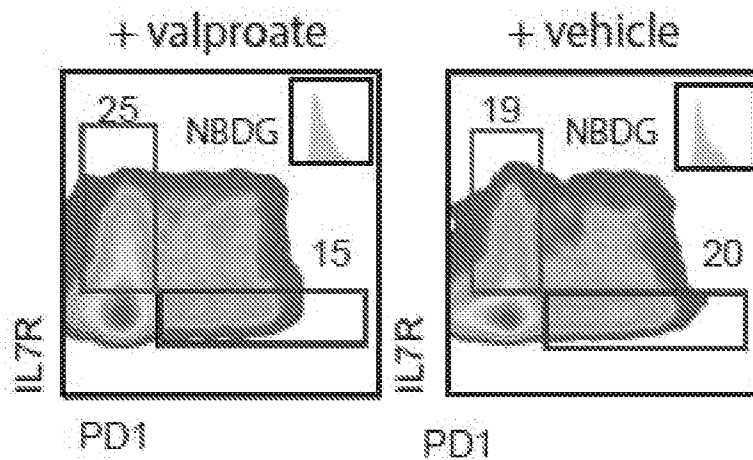
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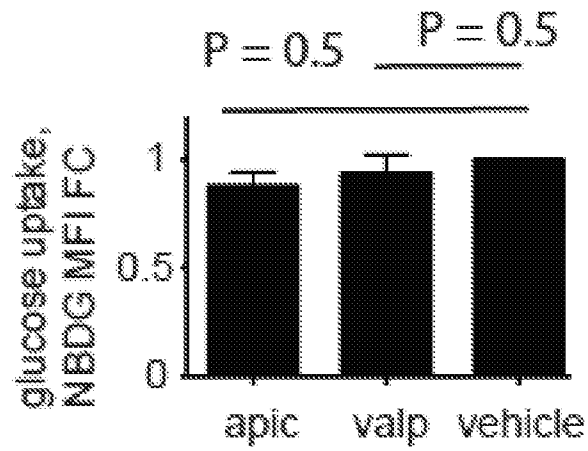


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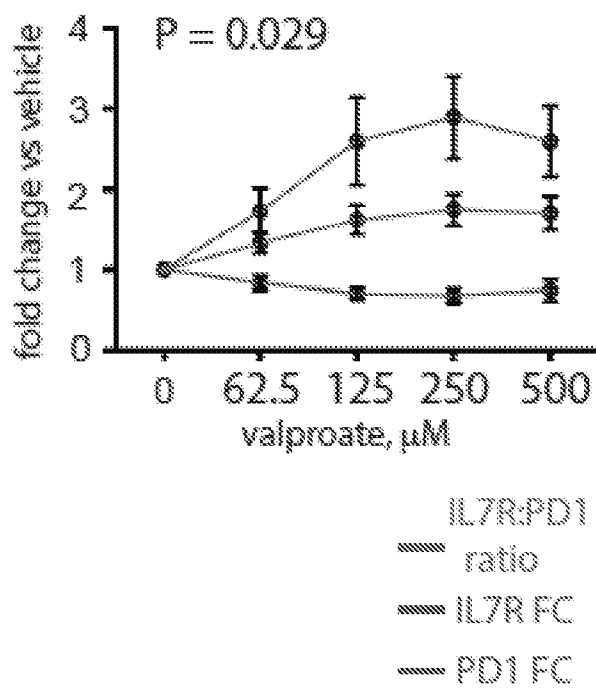


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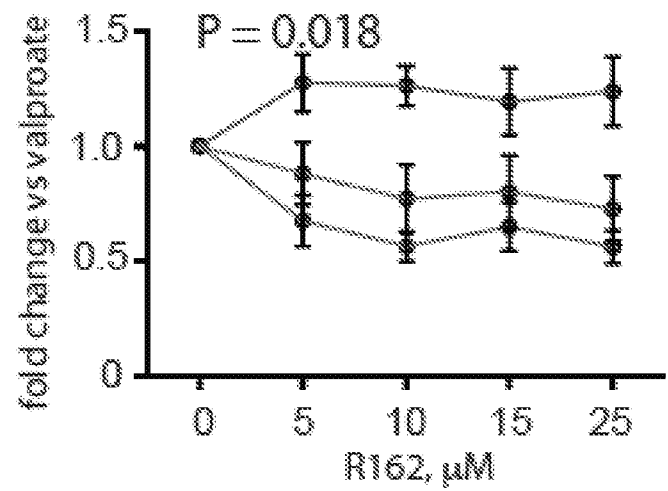
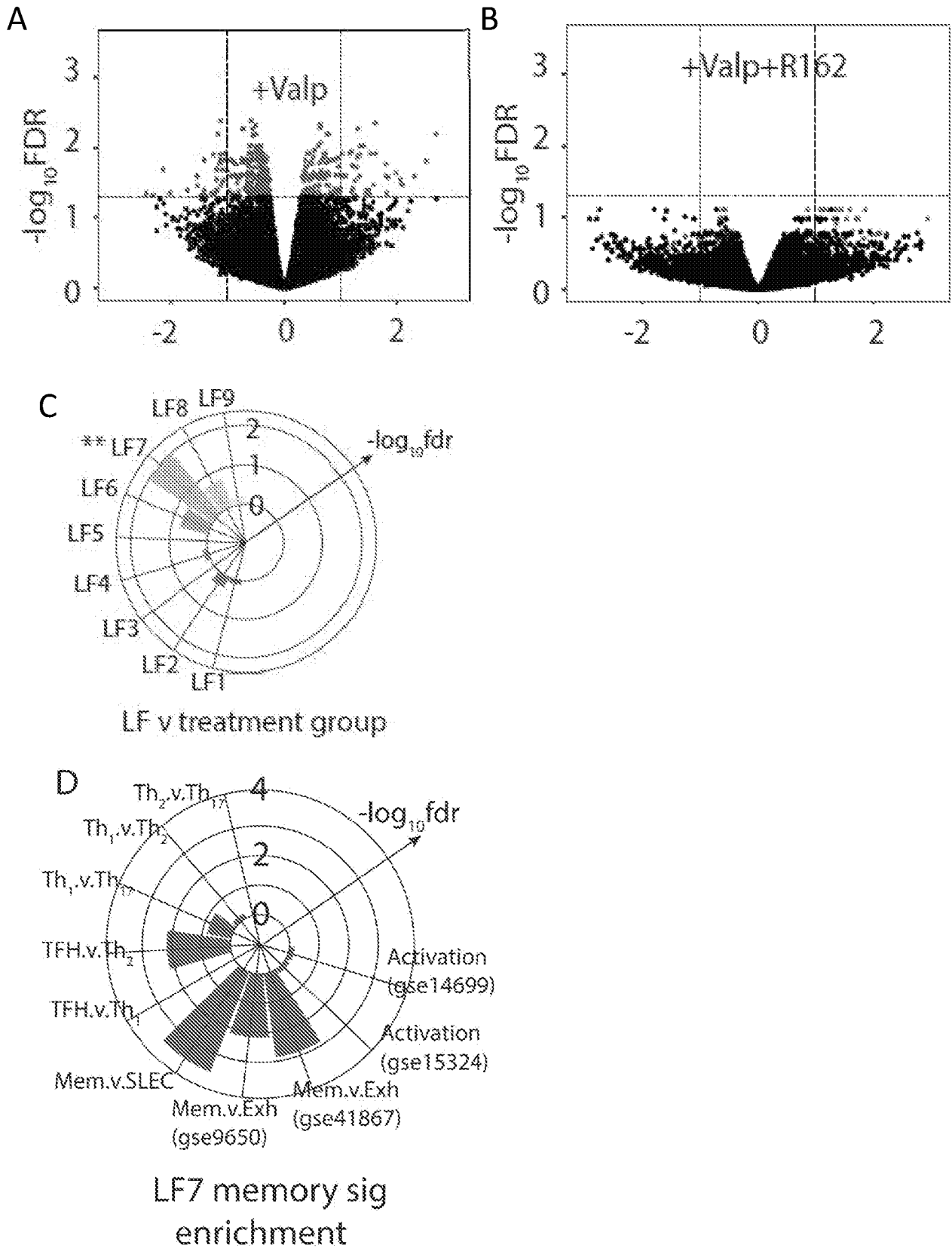
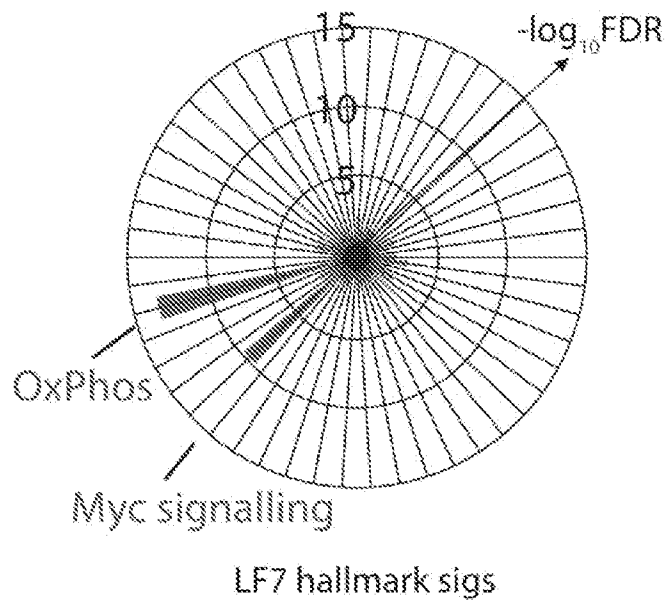


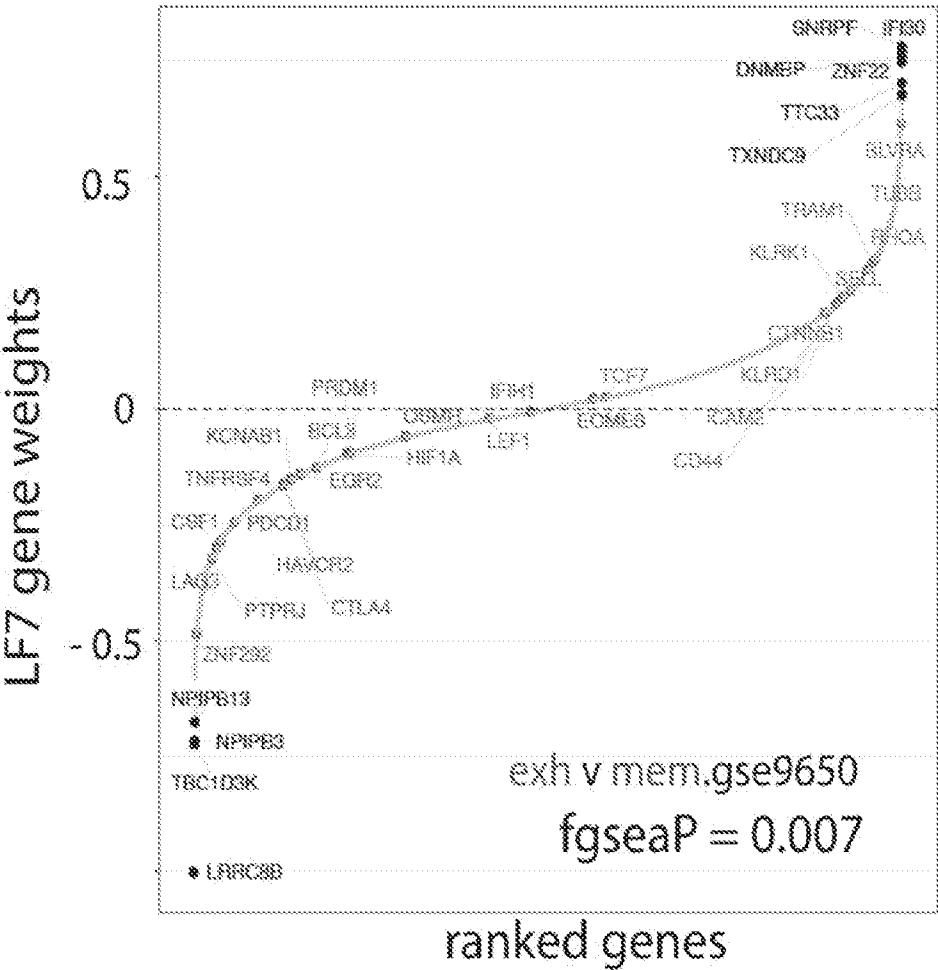
Figure 3



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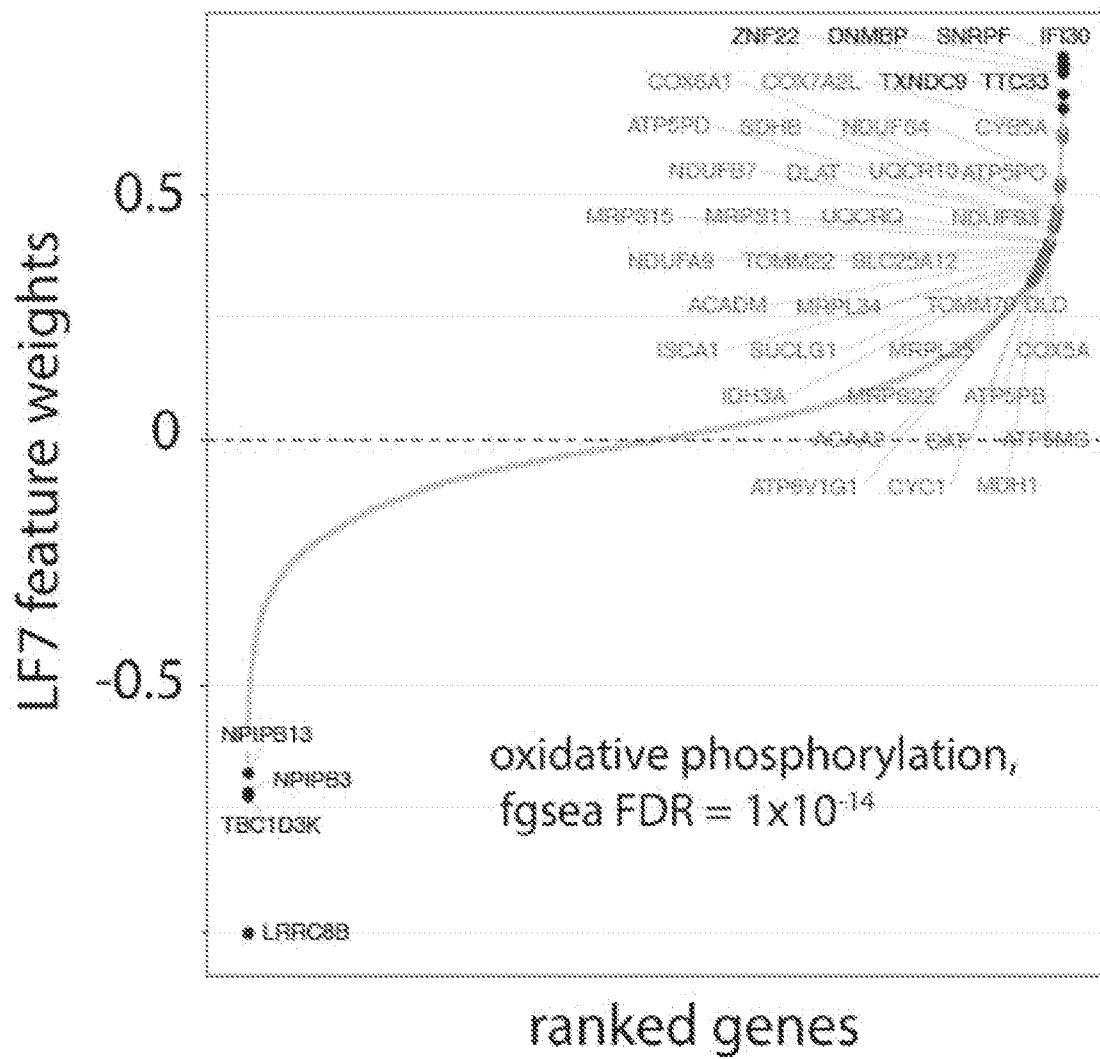


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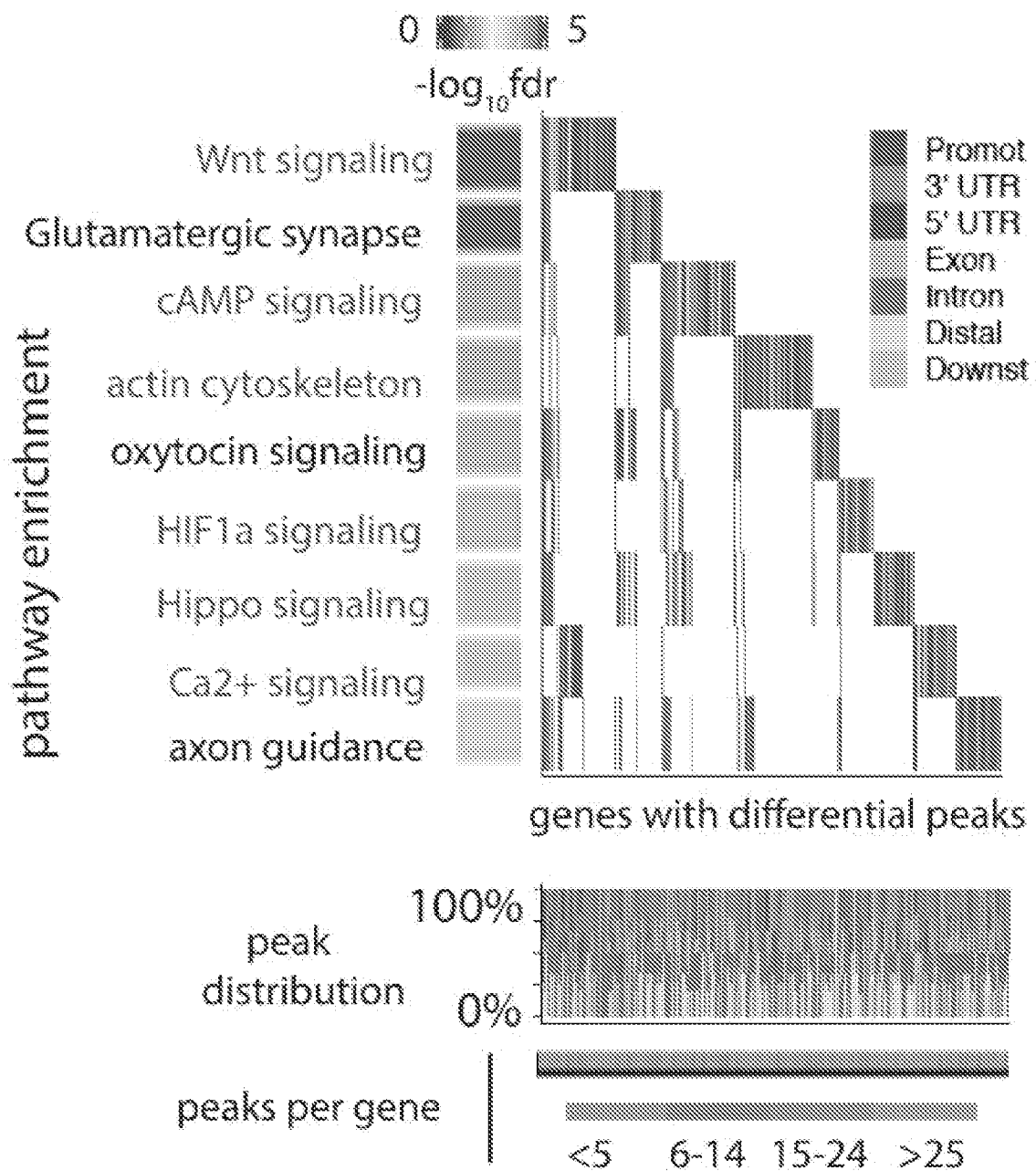


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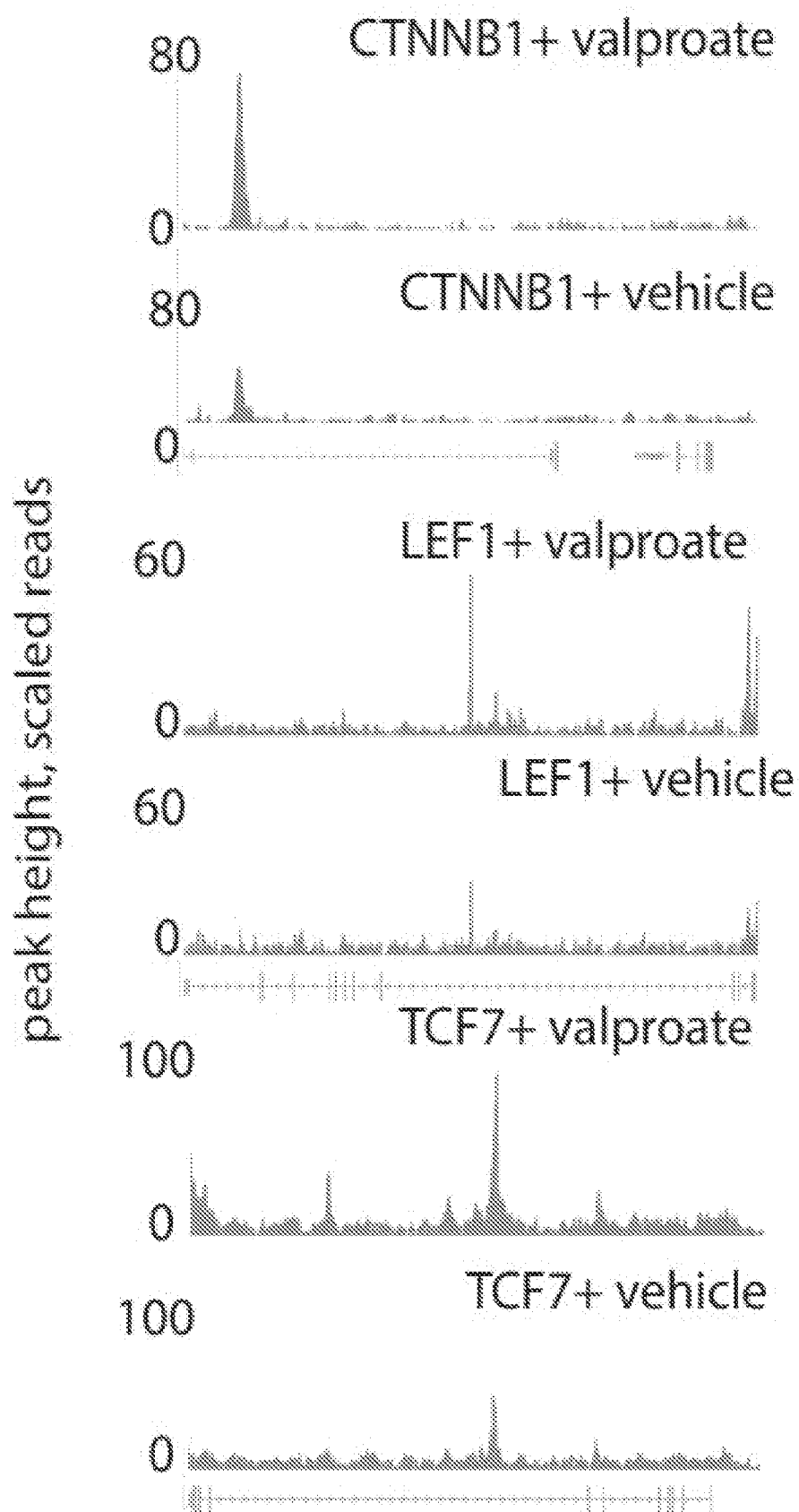
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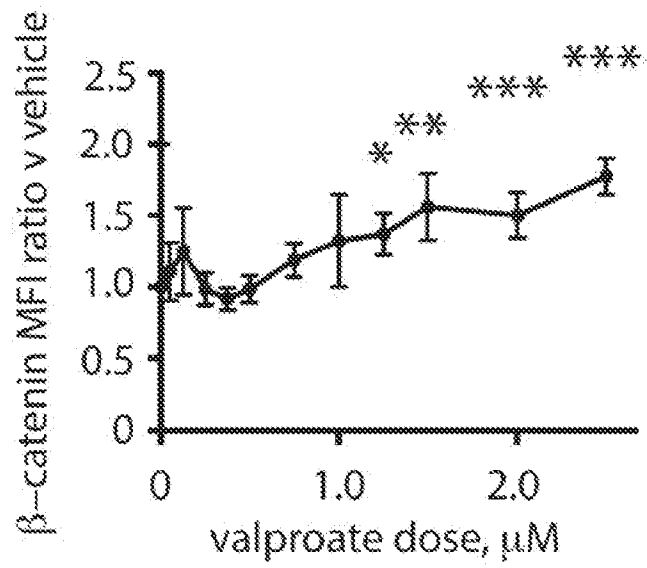
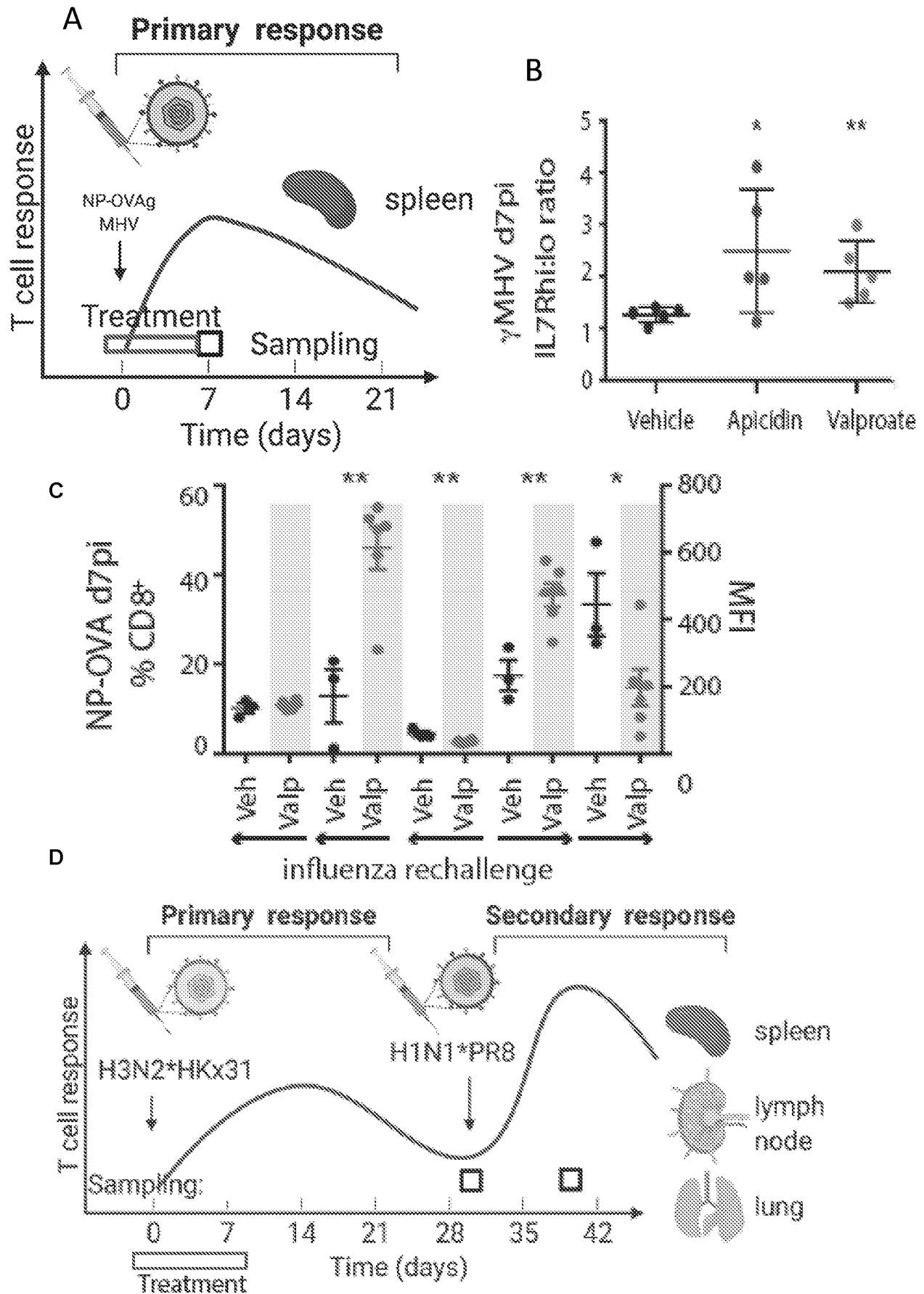
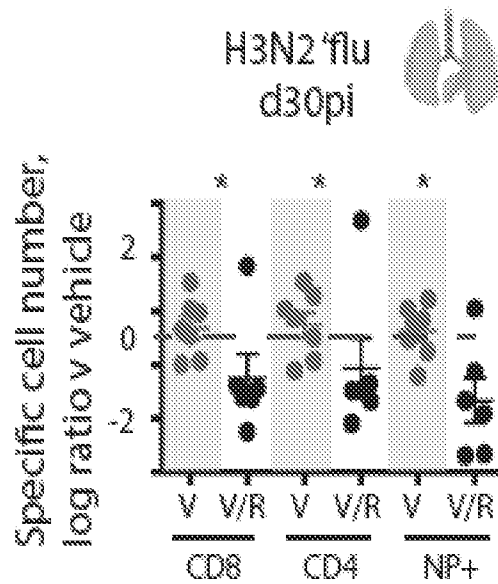


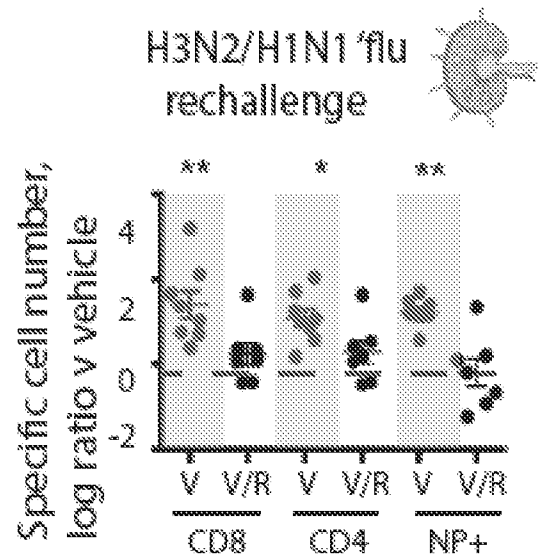
Figure 4



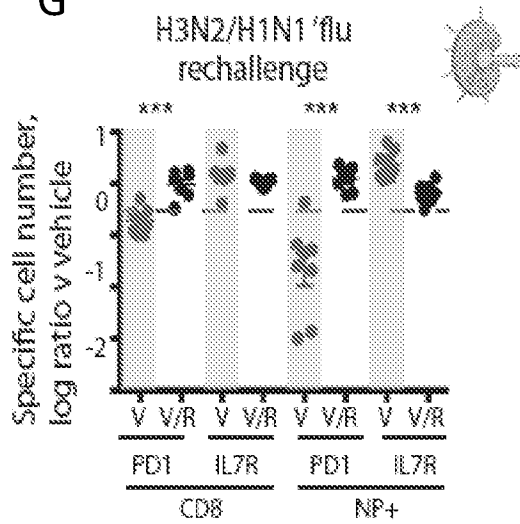
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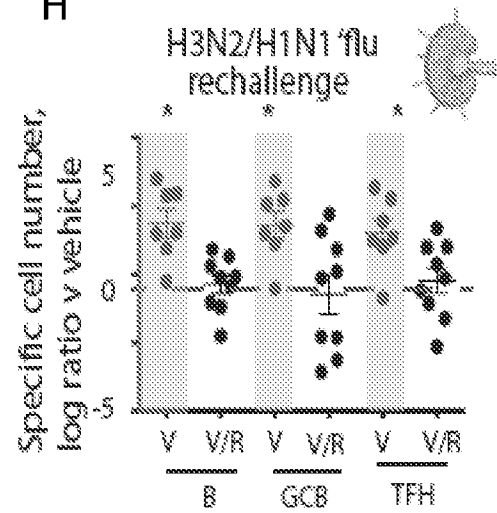
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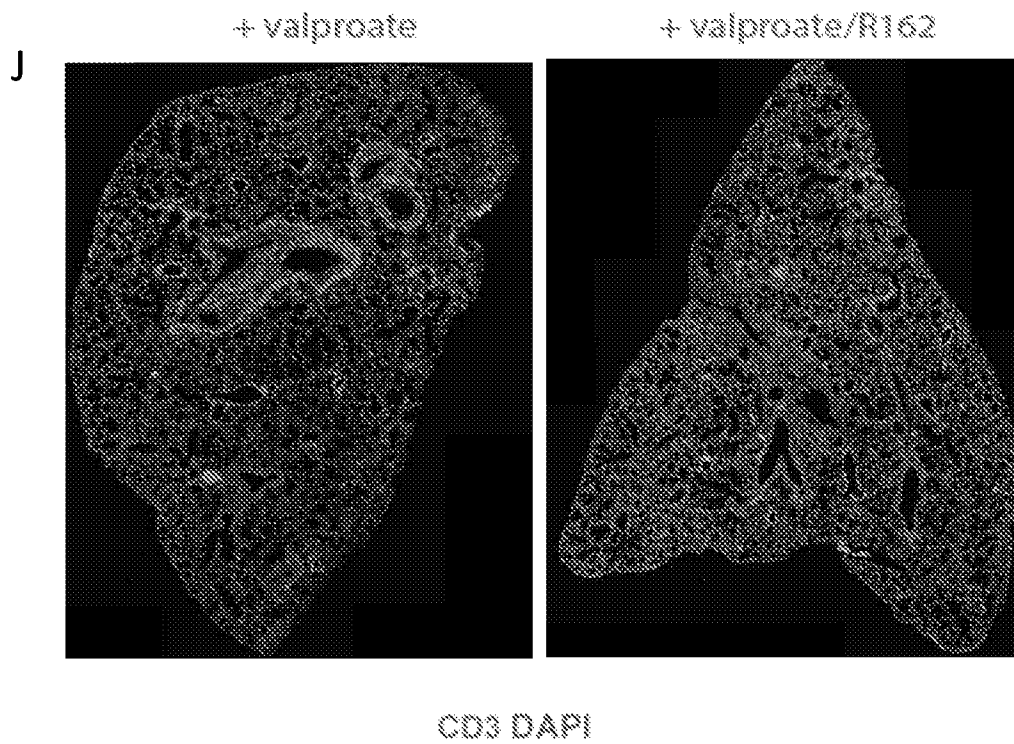
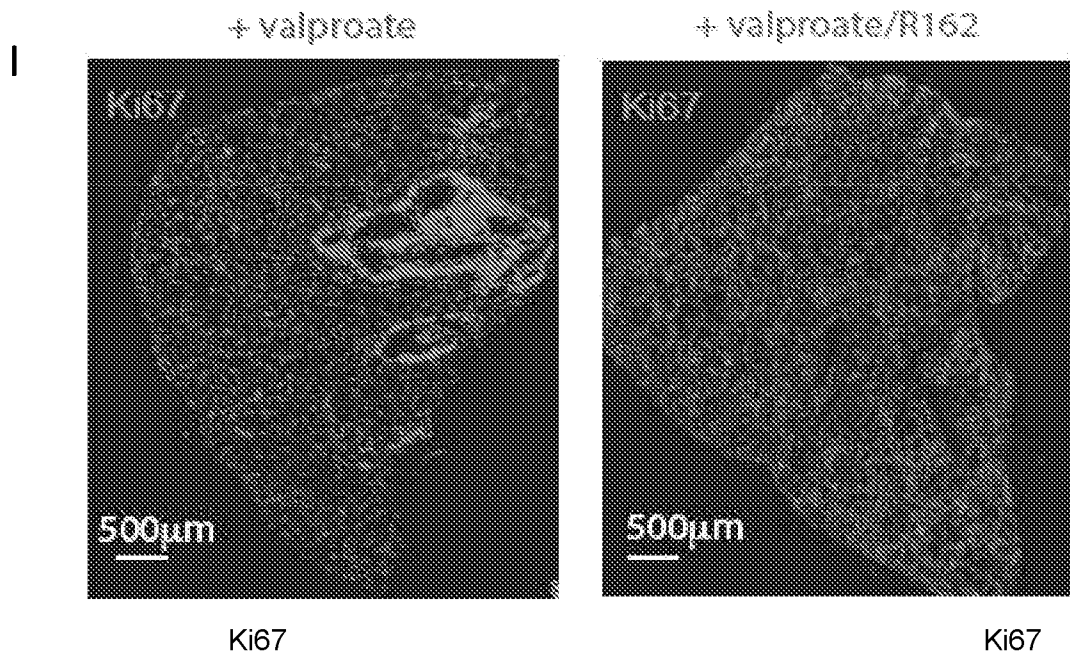


G

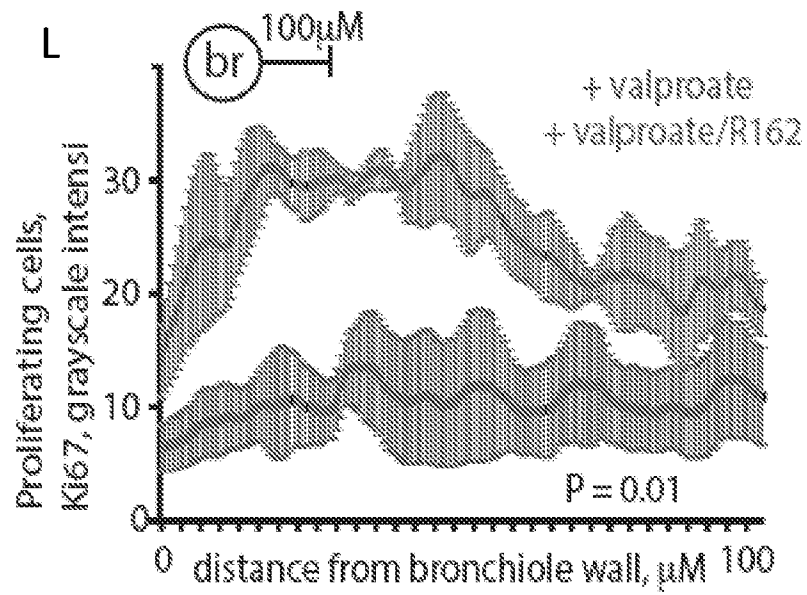
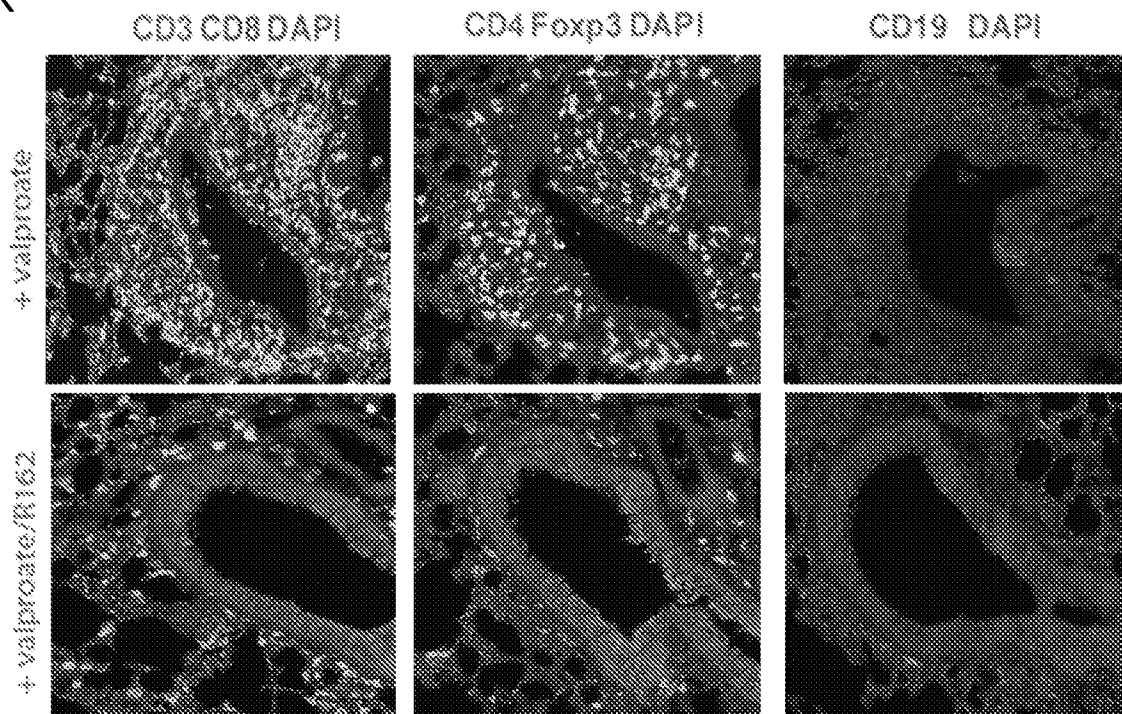


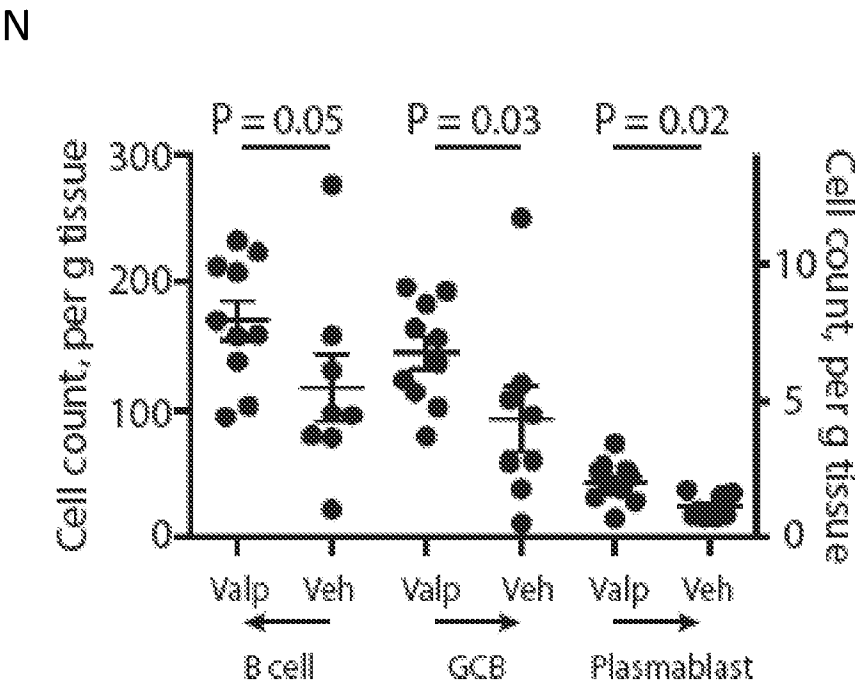
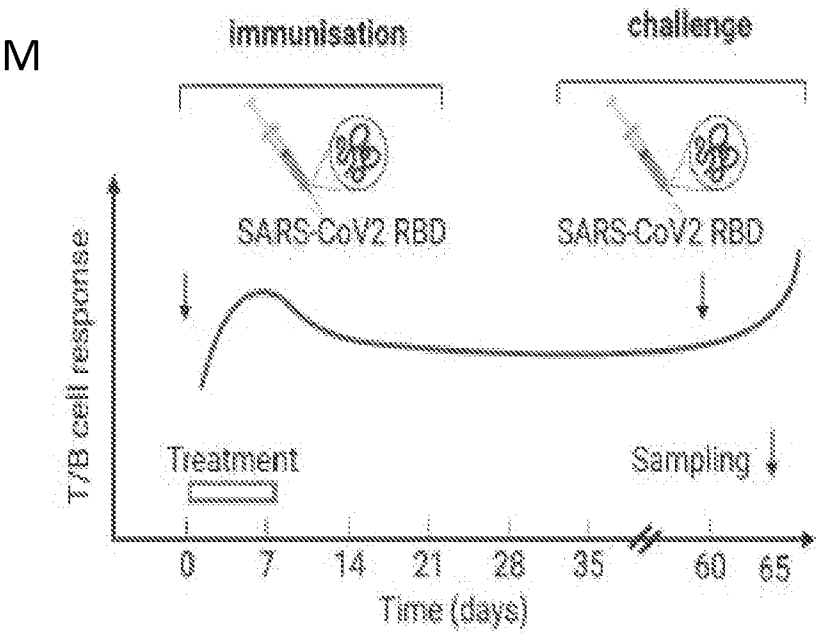
H



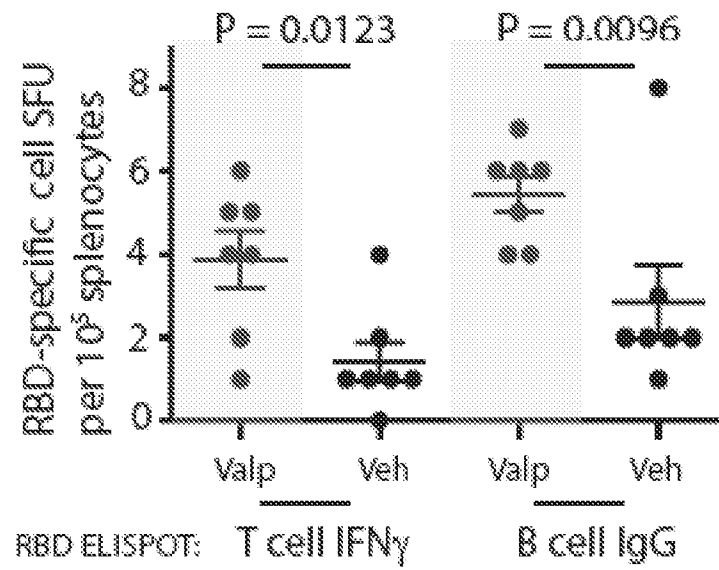


K





O



P

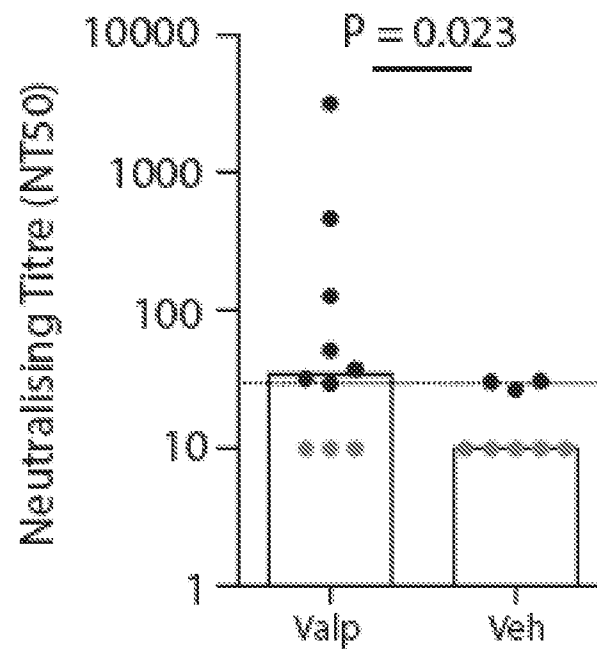
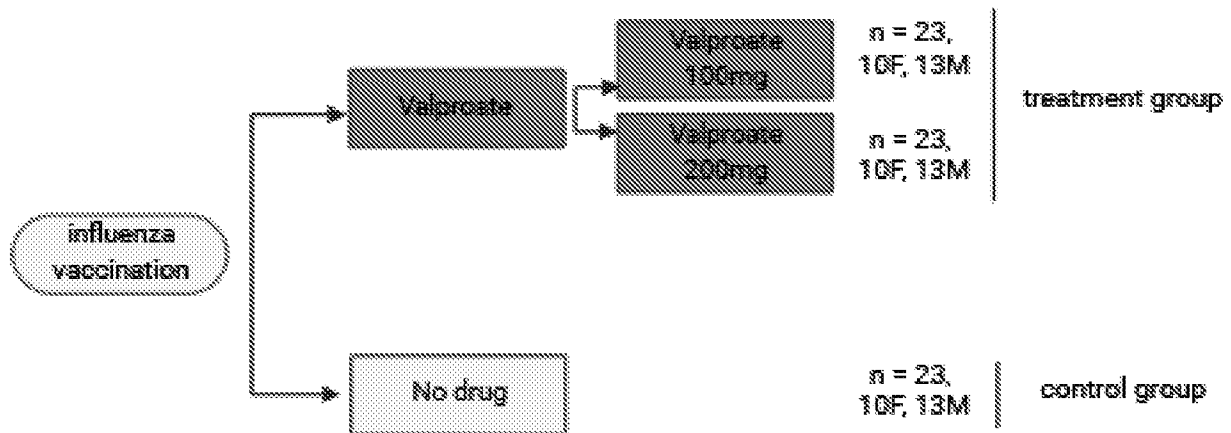
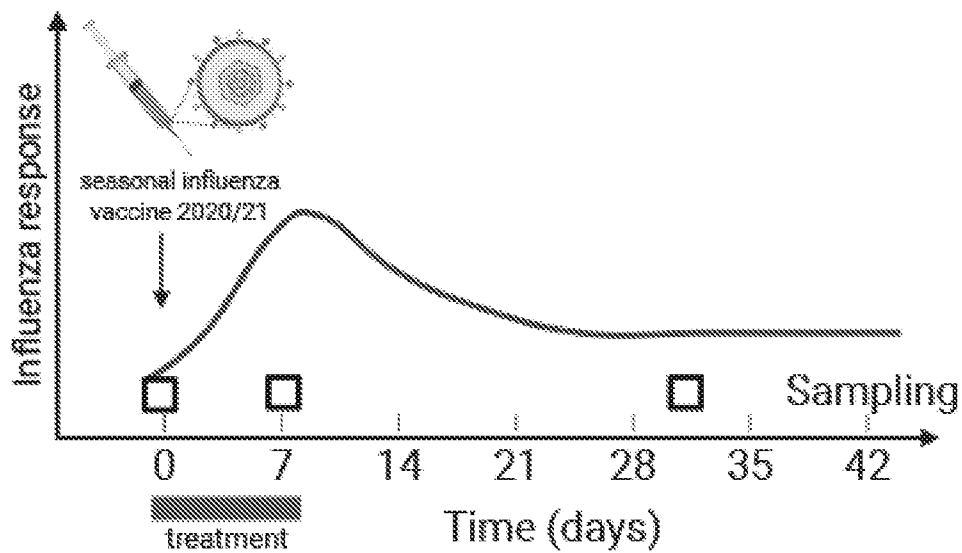


Figure 5

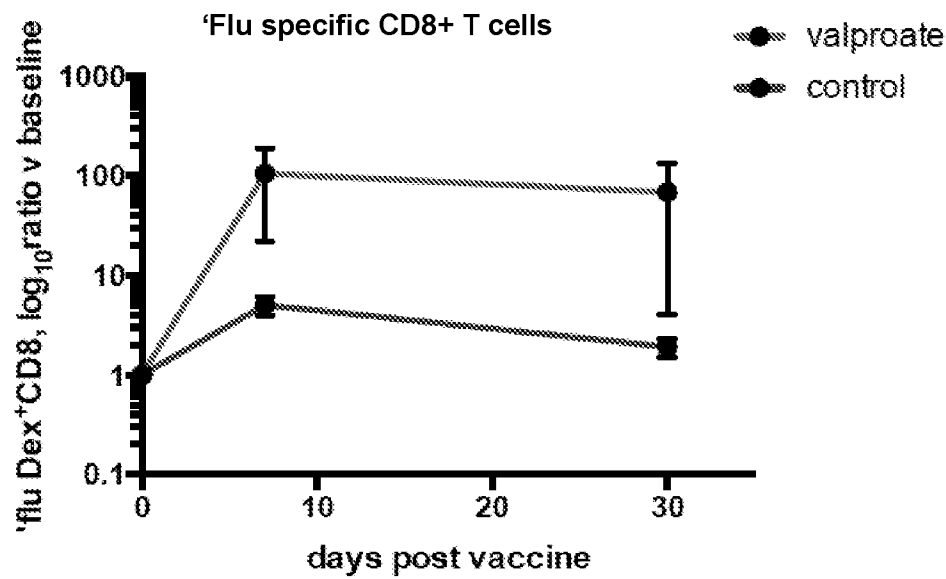
A



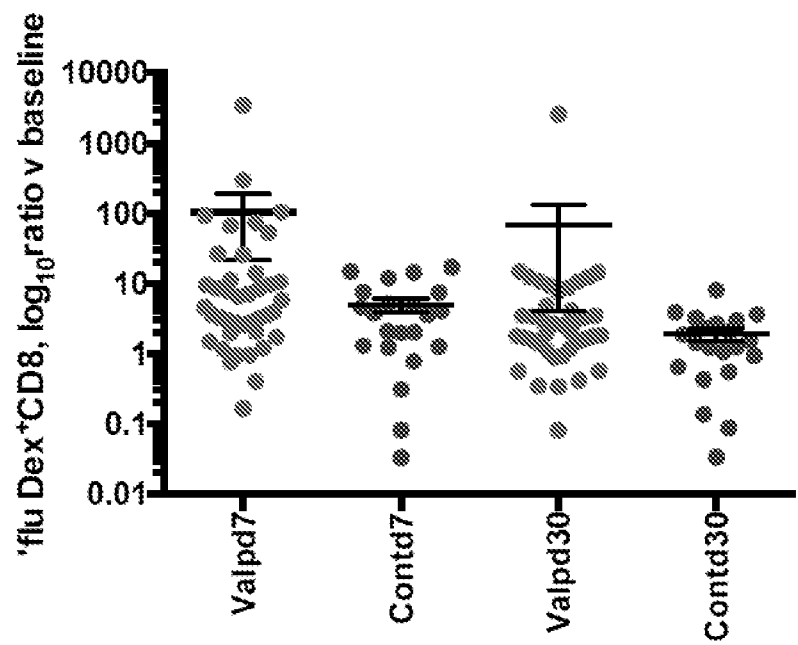
B



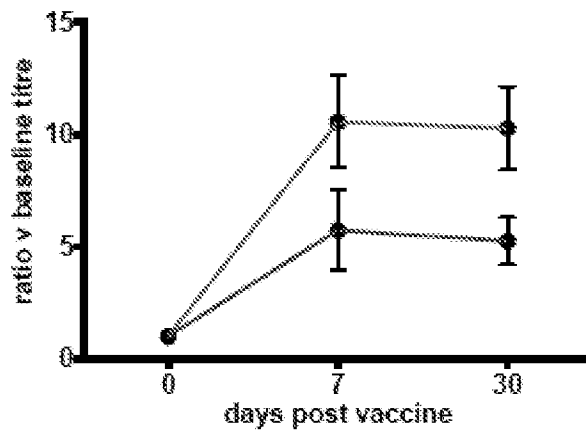
C



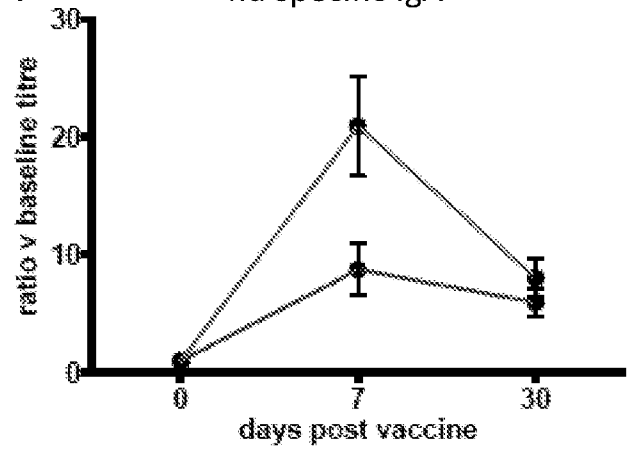
D

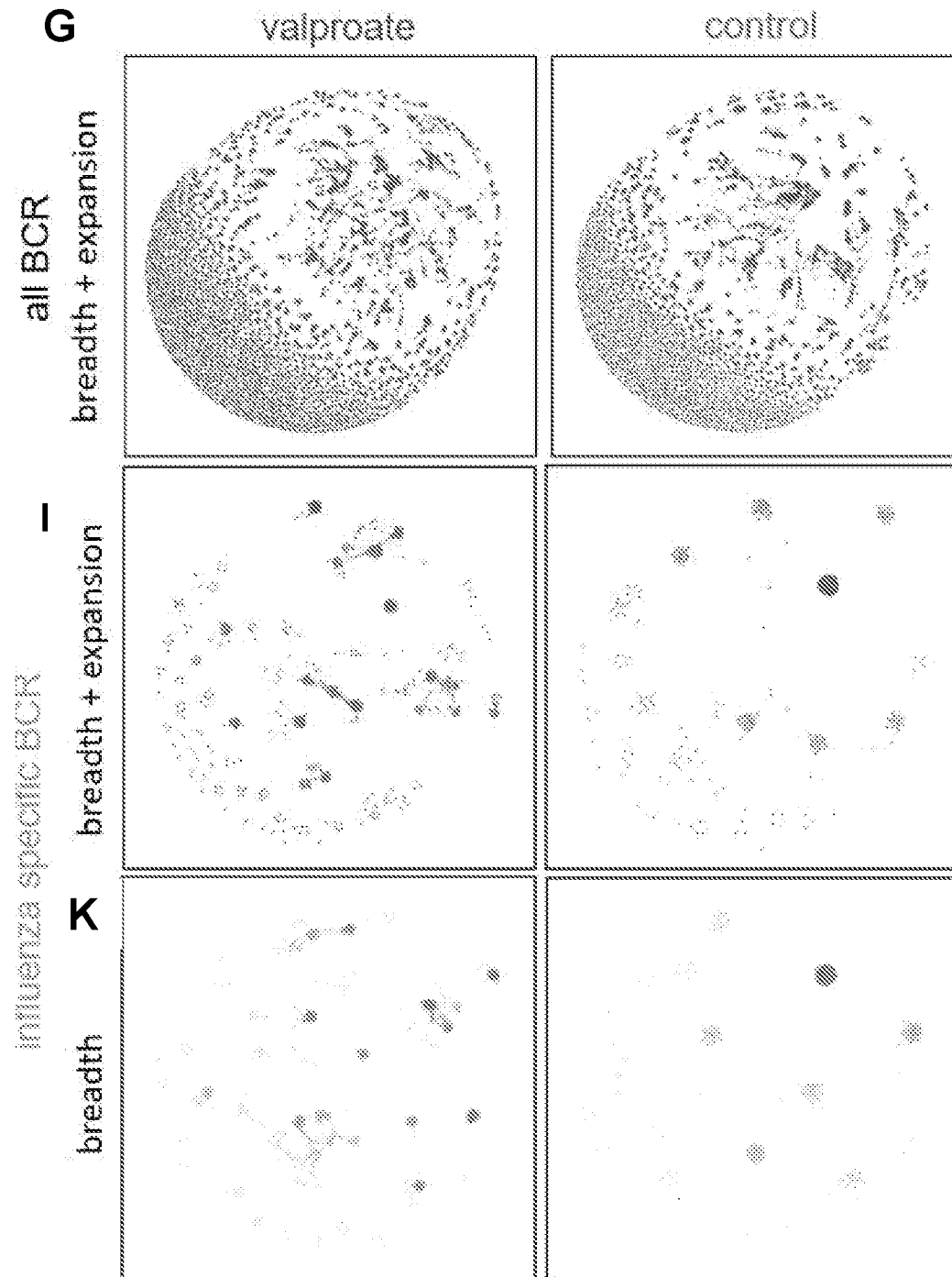


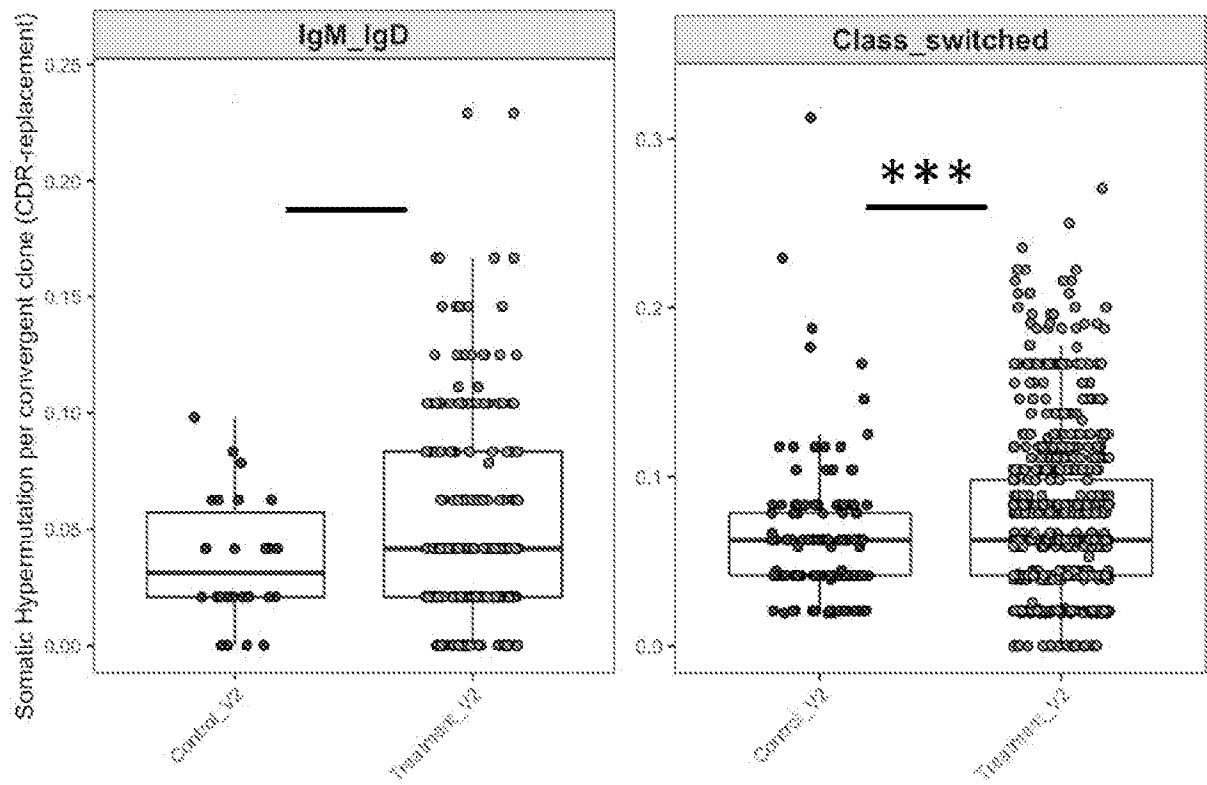
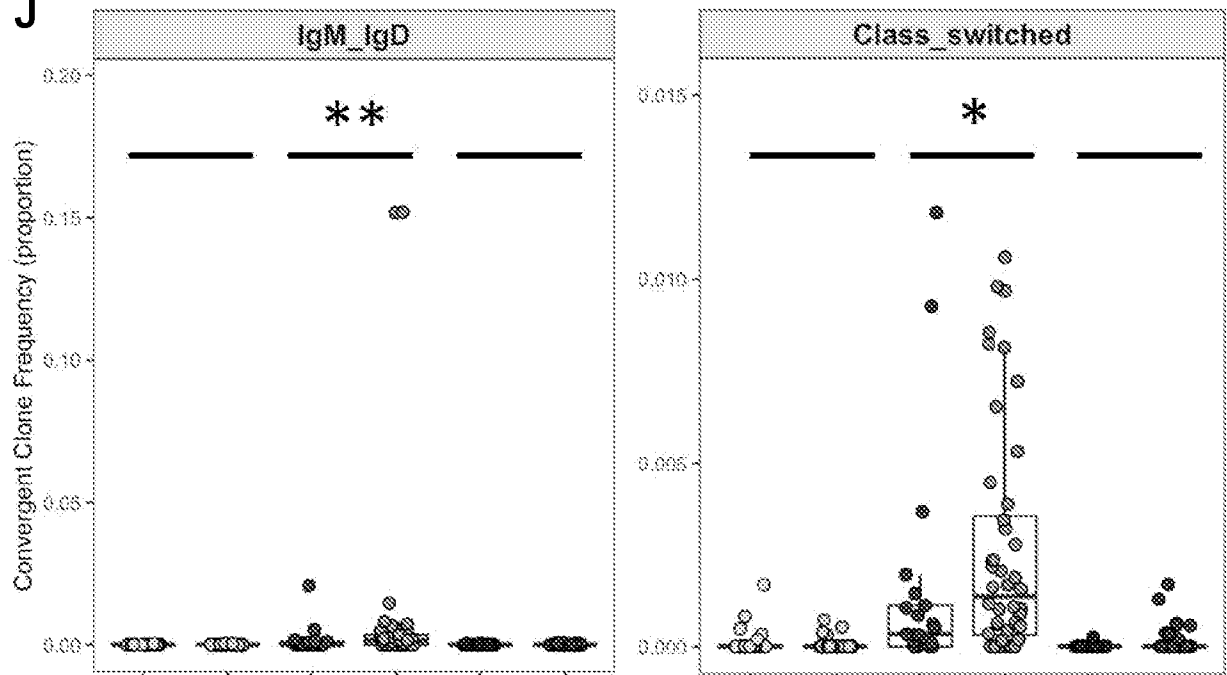
E 'flu specific IgG



F 'flu specific IgA





H**J**

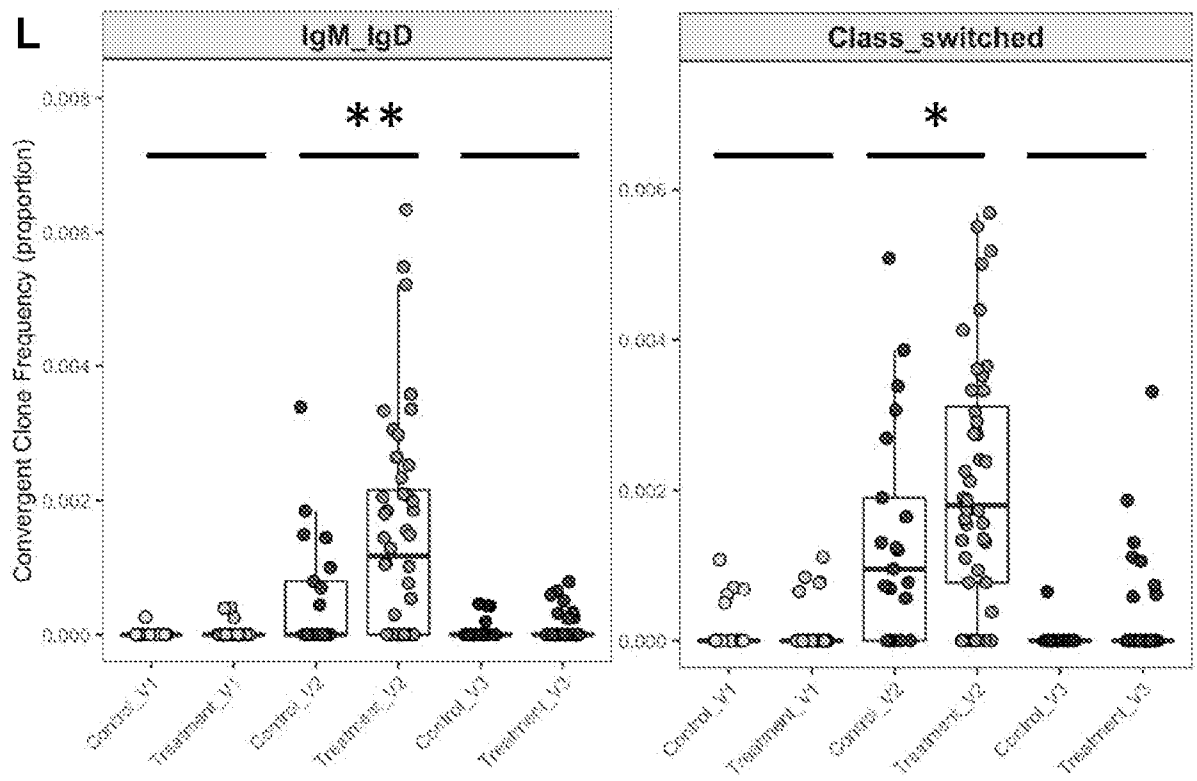


Figure 6

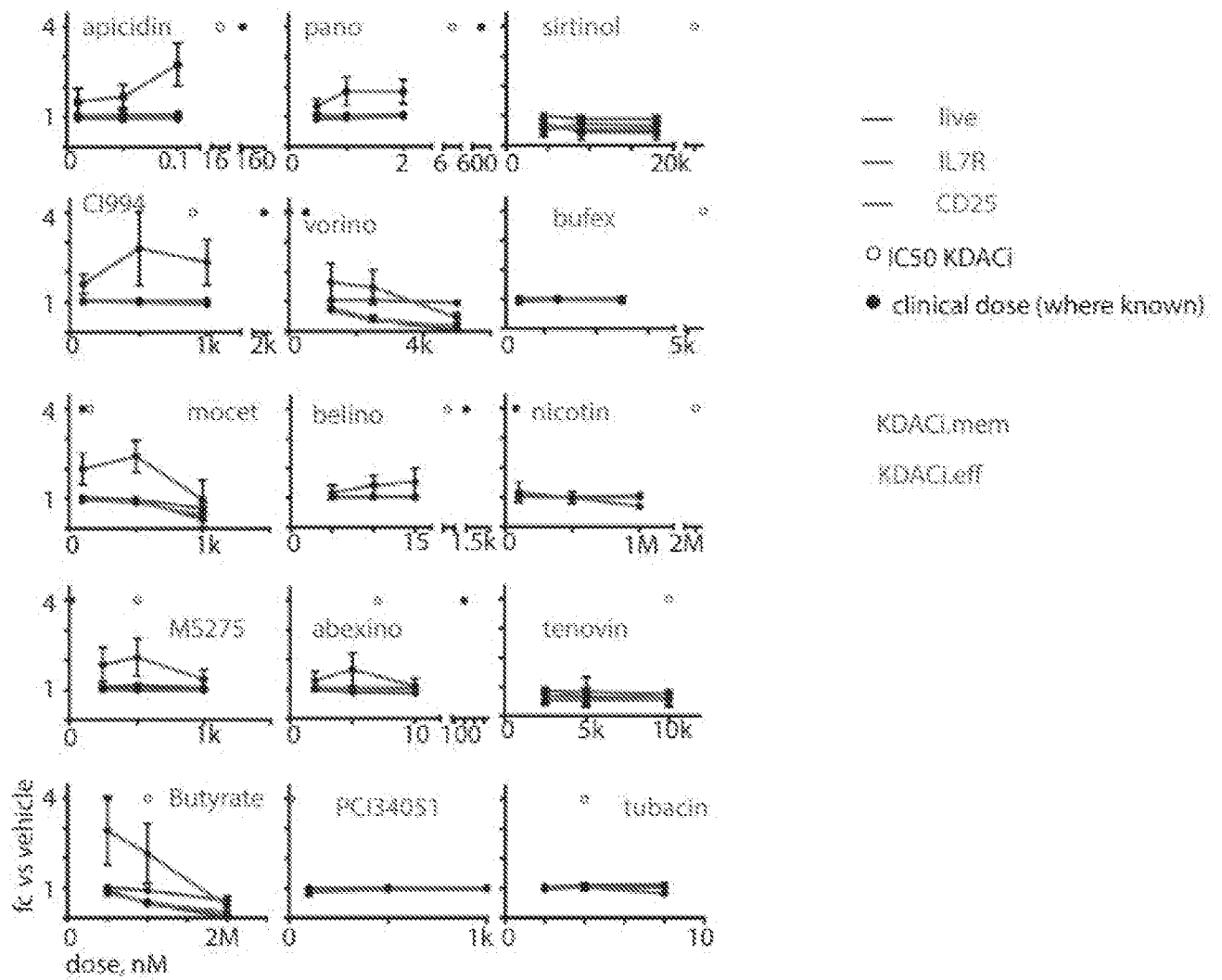
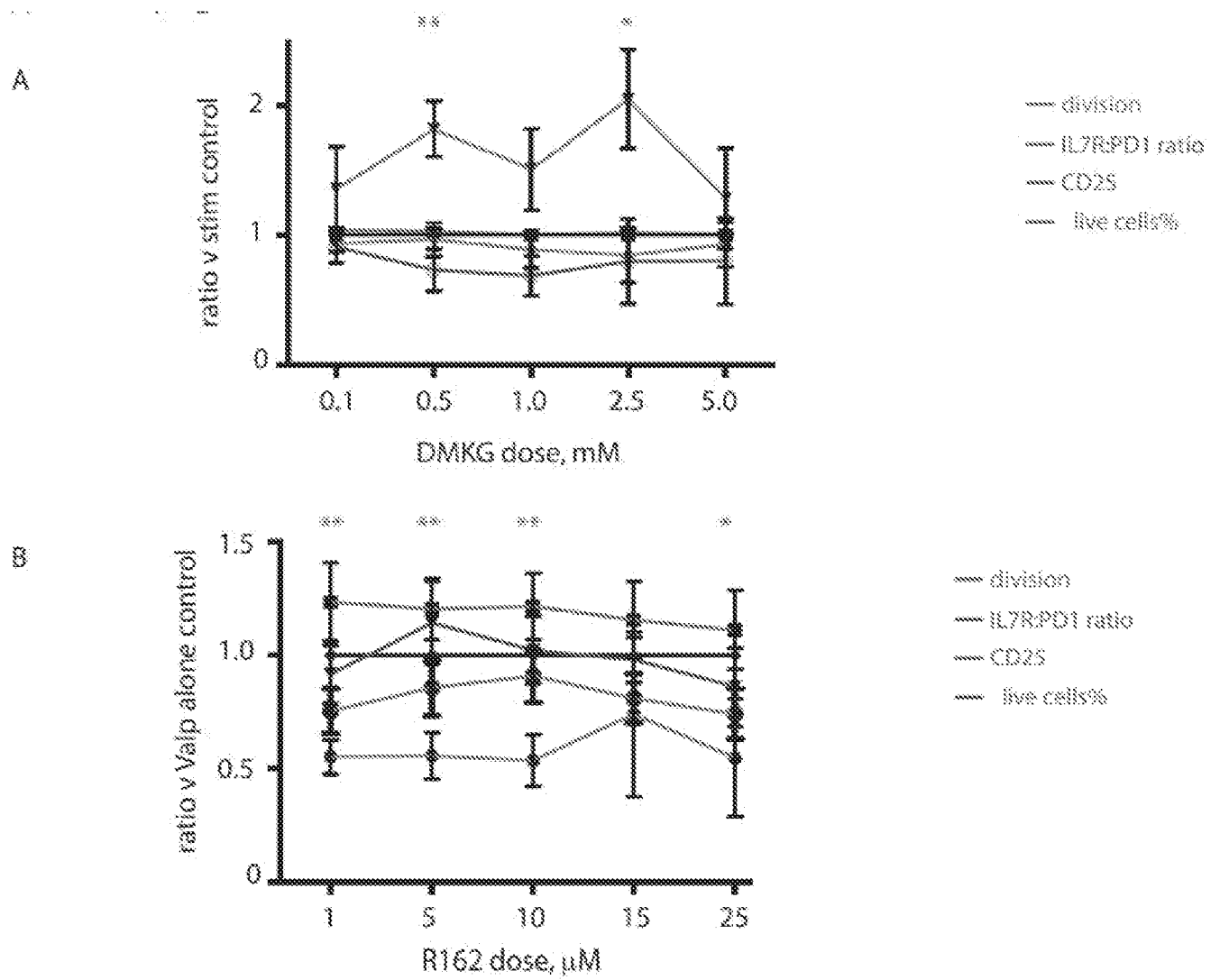
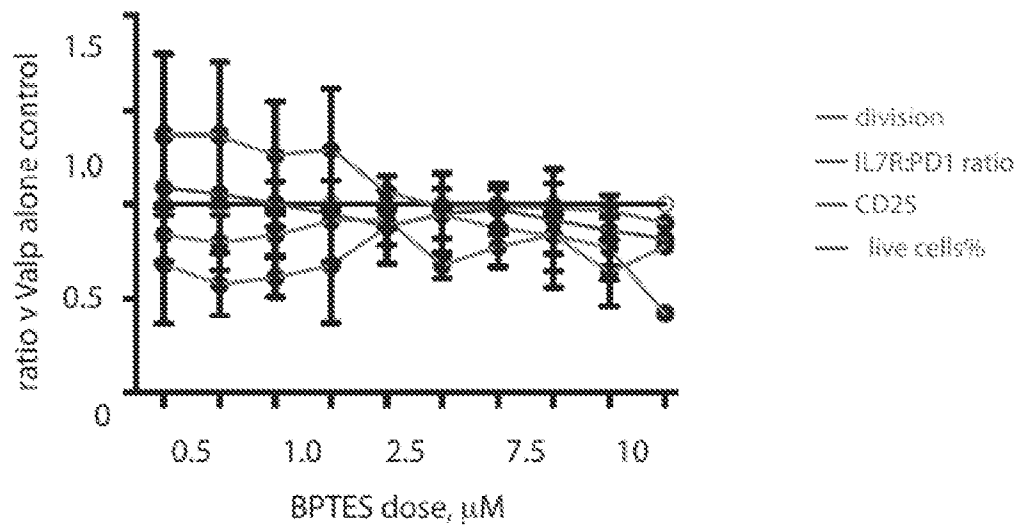


Figure 7



29 / 33

C



D

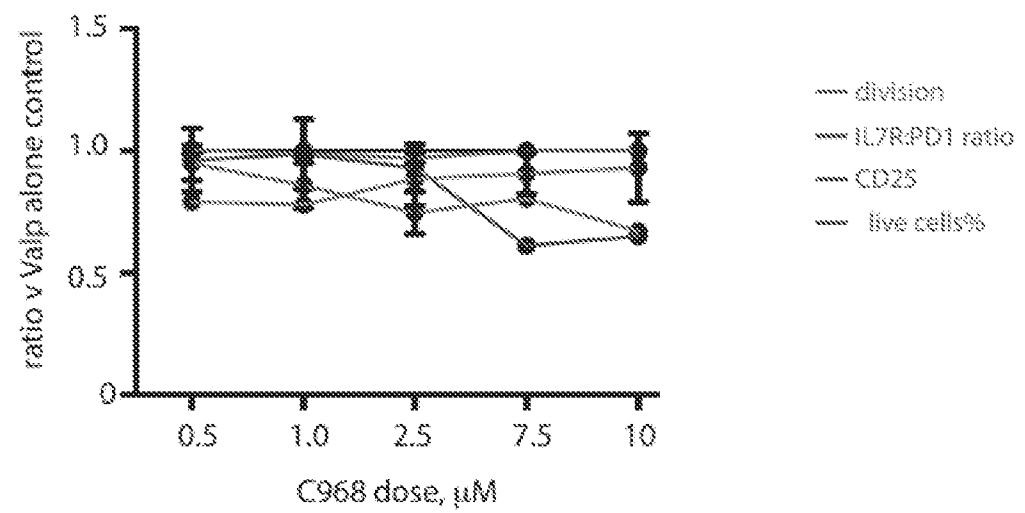
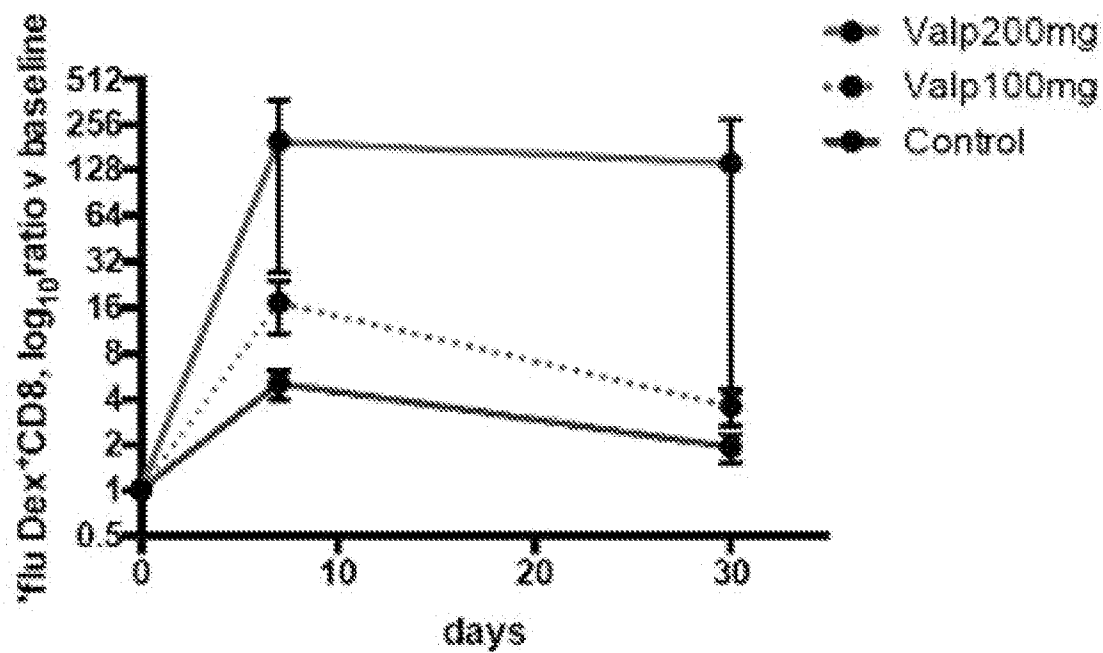
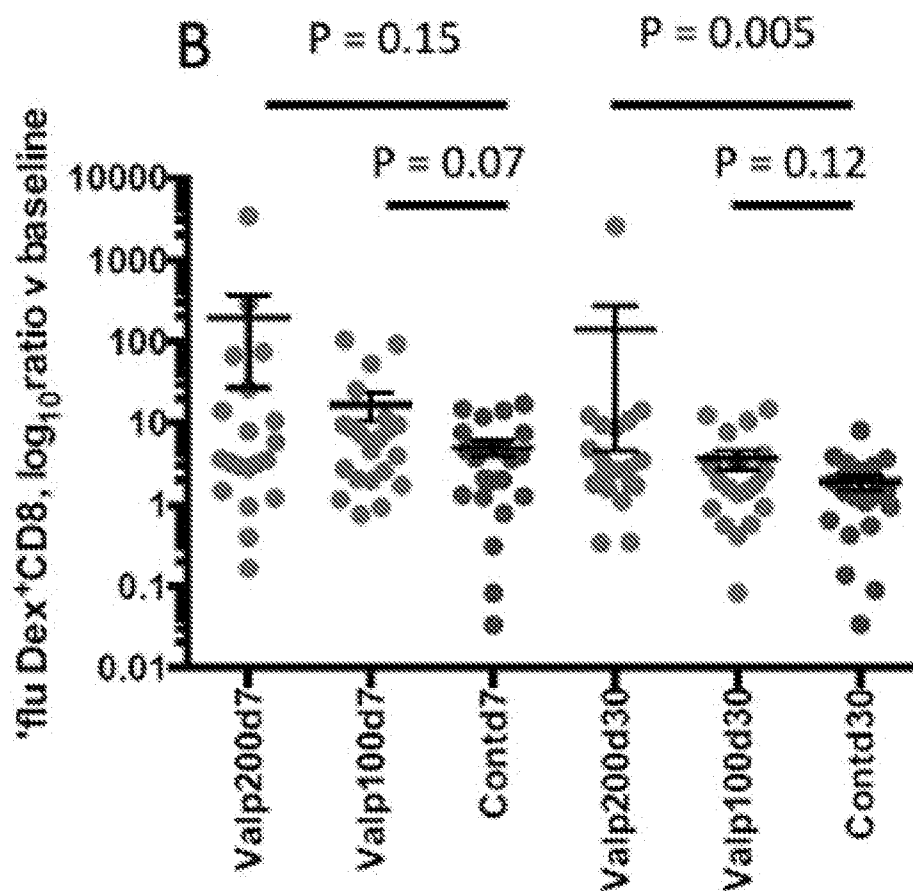


Figure 8

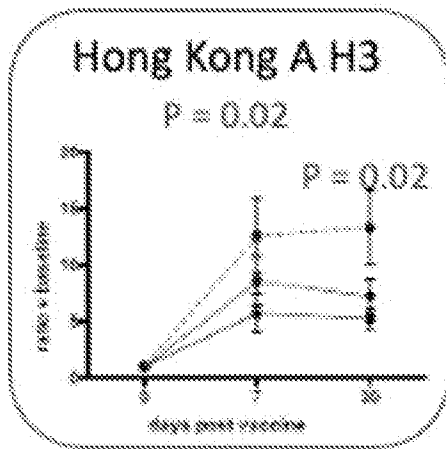
A



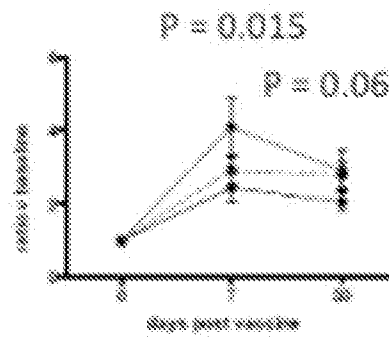
B



C IgG ratio

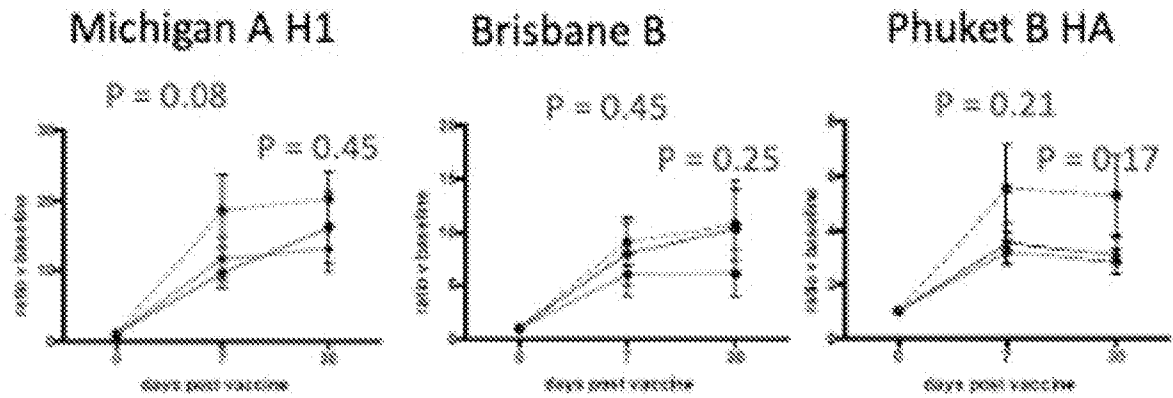


Shanghai A H7



- Valp200mg
- Valp100mg
- Control

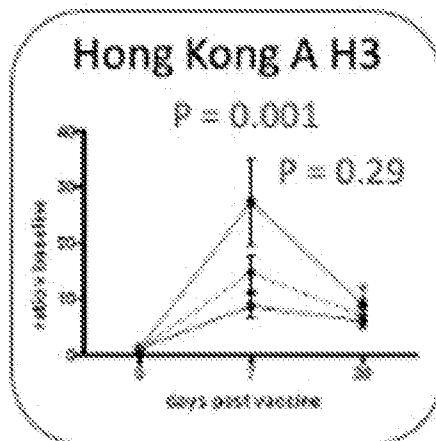
included in seasonal vaccine 2020/21



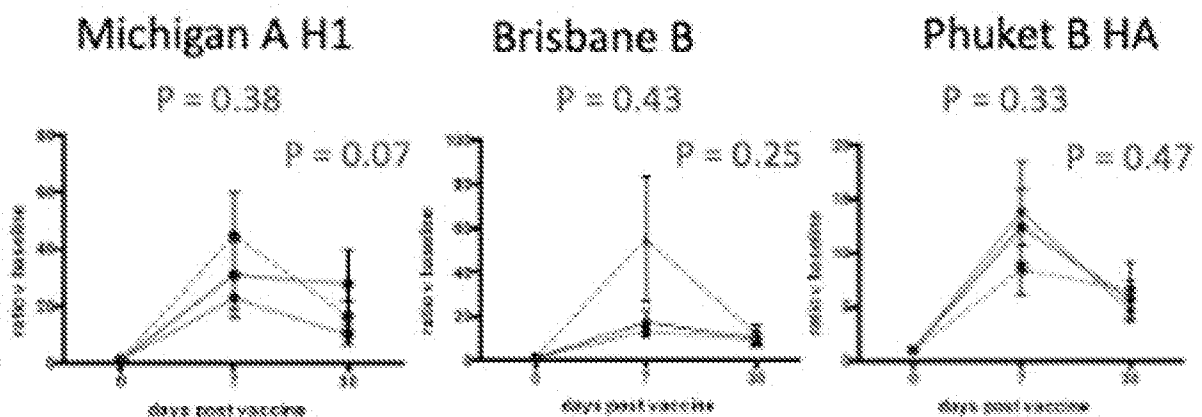
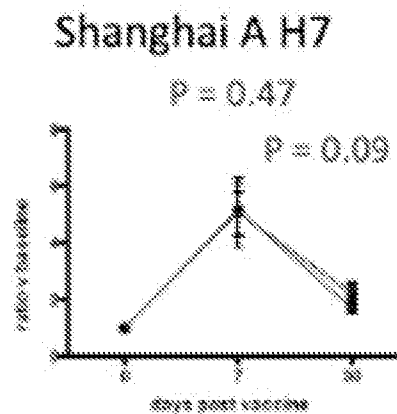
non-vaccine influenza strains

- Valp200mg
- Valp100mg
- Control

D IgA ratio

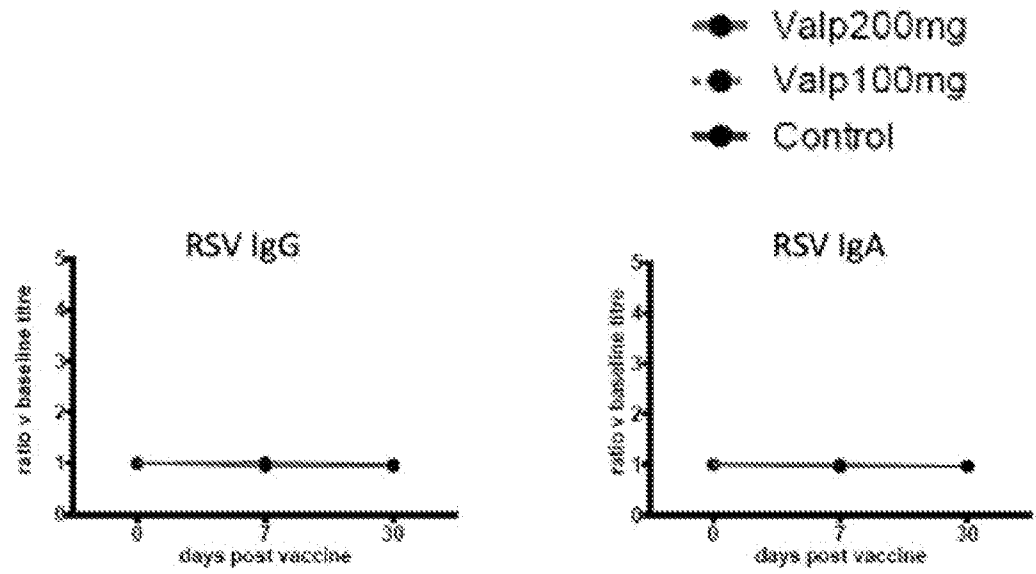


included in seasonal vaccine 2020/21.



non-vaccine influenza strains

E



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2022/053173

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61K31/167	A61K31/18
	A61K31/4406	A61K31/506
	A61P35/00	A61P31/14
		A61K31/19
		A61K38/12
		A61P31/16
		A61K31/343
		A61K39/00
		A61K31/4045
		A61P37/04
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/122629 A1 (UNIV MCMASTER [CA]; BRIDLE BRYAM [CA] ET AL.) 20 September 2012 (2012-09-20) paragraph [0008]; claims 1-2, 6, 14-20 paragraph [0032] paragraph [0053] - paragraph [0054] -----	1-12, 14, 23-36
X	WO 2020/030634 A1 (INST NAT SANTE RECH MED [FR]; UNIV PARIS SUD [FR] ET AL.) 13 February 2020 (2020-02-13) claim 1 page 4, line 6 - line 8 page 42, line 32 - line 34 page 49, lines 32-34 page 52, line 28 - line 30 page 53, line 13 - line 15 ----- --/--	1-12, 14, 23-36
☒ 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 1 March 2023		Date of mailing of the international search report 04/05/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Matos de Brito, P

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2022/053173

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MCCAW TYLER R ET AL: "Histone deacetylase inhibition promotes intratumoral CD8T-cell responses, sensitizing murine breast tumors to anti-PD1", CANCER IMMUNOLOGY IMMUNOTHERAPY, SPRINGER, BERLIN/HEIDELBERG, vol. 68, no. 12, 12 November 2019 (2019-11-12), pages 2081-2094, XP036952137, ISSN: 0340-7004, DOI: 10.1007/S00262-019-02430-9 [retrieved on 2019-11-12] the whole document -----	1-12, 14-16, 23-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2022/053173

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

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

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

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-12, 14-16, 23-36

Remark on Protest

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-12, 14-16, 23-36

A method for enhancing an immune response in a subject (i.e. in vivo) comprising administering a lysine deacetylase inhibitor with a vaccine, wherein the KDACi is listed in table 1.

2. claims: 13, 17-22, 37-40

A method, in vivo, in vitro or ex-vivo wherein cells are exposed to a KDACi, of promoting a T-cell memory response, of promoting a differentiation of CD8+ T cells into memory precursor effector cells, of increasing IL7R expression, of enhancing glutaminolysis; for the treatment of a disease comprising administration of a KDACi; for expanding cells for use in the treatment of a disease, and more particularly to be used, in adoptive cell therapy comprising: providing a population of CD8+ T cells; contacting the population of CD8+ T cells to a KDACi capable of enhancing glutaminolysis to produce an expanded population of cells.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2022/053173

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012122629 A1	20-09-2012	CA 2829607 A1 CN 103619350 A CN 108126204 A EP 2683402 A1 JP 6309274 B2 JP 2014510073 A US 2014193458 A1 US 2018064805 A1 WO 2012122629 A1	20-09-2012 05-03-2014 08-06-2018 15-01-2014 11-04-2018 24-04-2014 10-07-2014 08-03-2018 20-09-2012
WO 2020030634 A1	13-02-2020	CN 112703011 A EP 3833383 A1 US 2021236633 A1 WO 2020030634 A1	23-04-2021 16-06-2021 05-08-2021 13-02-2020