



(51) International Patent Classification:

G01N 33/50 (2006.01)

(21) International Application Number:

PCT/EP2018/065364

(22) International Filing Date:

11 June 2018 (11.06.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/518,382 12 June 2017 (12.06.2017) US

(71) Applicant: MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.

[DE/DE]; Hofgartenstr. 8, 80539 München (DE).

(72) Inventors: PEARCE, Erika; Am Rebberg 68, 79194

Gundelfingen (DE). CORRADO, Mauro; Eschholzstr. 1,

79106 Freiburg (DE).

(74) Agent: VOSSIUS & PARTNER (NO 31); Siebertstraße 3,

81675 München (DE).

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

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: IMMUNE CELL MODULATION THROUGH CARDIOLIPIN SYNTHESIS

(57) Abstract: The present invention relates to a method for determining whether a compound has the capability of inhibiting cardiolipin synthesis, comprising (a) contacting a sample comprising T cells and/or M2 macrophages with the compound; (b) culturing the sample in the presence of the compound for at least 48h, preferably at least 60h and most preferably at least 72h; and (c) assaying in the sample the mitochondrial function of the T cells and/or M2 macrophages, wherein a reduction of the mitochondrial function as compared to a control sample not contacted with the compound indicates that the compound has the capability of inhibiting cardiolipin synthesis.



IMMUNE CELL MODULATION THROUGH CARDIOLIPIN SYNTHESIS

FIELD OF THE INVENTION

[0001] The present invention relates to a method for determining whether a compound has the capability of inhibiting cardiolipin synthesis, comprising (a) contacting a sample comprising T cells and/or M2 macrophages with the compound; (b) culturing the sample in the presence of the compound for at least 48h, preferably at least 60h and most preferably at least 72h; and (c) assaying in the sample the mitochondrial function of the T cells and/or M2 macrophages, wherein a reduction of the mitochondrial function as compared to a control sample not contacted with the compound indicates that the compound has the capability of inhibiting cardiolipin synthesis.

BACKGROUND OF THE INVENTION

[0002] In this specification, a number of documents including patent applications and manufacturer's manuals are cited. The disclosure of these documents, while not considered relevant for the patentability of this invention, is herewith incorporated by reference in its entirety. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

[0003] Immune cell plasticity and function are highly regulated by mitochondria. In particular, memory T (TMEM) cells have higher mitochondrial mass and spare respiratory capacity (SRC) due to tight organization of cristae structure and, thus, respiratory chain supercomplexes (RCS). Mitochondria respiration is an important feature also in other immune cells, such as M2 macrophages that are characterized by high SRC. Although the role of OPA1 (or dynamin-like 120 kDa protein) – being the protein master regulator of cristae structure - has been characterized in TMEM development, not much has been done to characterize the role of mitochondrial lipid composition in determining metabolism and function of immune cells. Among different lipids, cardiolipin, the most abundant phospholipid of the Inner Mitochondrial Membrane (IMM) accounting for 20% of total lipids, contributes to membrane curvature and respiratory chain complexes (RCC) organization. Cardiolipin is exclusively synthesized in the IMM in a pathway where dephosphorylation of phosphatidylglycerolphosphate (PGP) by phosphatidylglycerophosphatase1 (PTPMT1) is a limiting step reaction (Figure 1).

[0004] Cardiolipin-dependent IMM curvature increases surface area for the RCC to organize in supercomplexes making oxidative phosphorylation more efficient. Indeed, genetic ablation of PTPMT1 or CLS1 in fibroblasts or hepatocytes negatively affect mitochondrial fusion, ultrastructure and oxidative phosphorylation.

[0005] Although some knowledge on the cardiolipin synthesis pathway has been collected in the prior art, a full picture of all downstream cellular processes being governed by the cardiolipin synthesis pathway is not available. It is thus also not fully known which cellular processes can be controlled by interfering with the the cardiolipin pathway, in particular the dephosphorylation of PGP by PTPMT1.

SUMMARY OF THE INVENTION

[0006] The present invention aims at providing novel screening methods and therapeutic uses encompassing interference with the cardiolipin synthesis pathway.

BRIEF DESCRIPTION OF THE FIGURES

[0007] **Figure 1** is a depiction of the cardiolipin synthesis pathway. Cardiolipin synthesis starts form the generation of phosphatidylglycerol (PG) from phosphatidylglycerolphosphate (PGP) mediated by the phosphatase PTPMT1. Cardiolipin synthase 1 (CLS1) catalyzes cardiolipin (CL) in a reaction between PG and a molecule of cytidine diphosphate diacylglycerol (CDP-DAG).

[0008] **Figure 2A-D** are graphs that show inhibition of PTPMT1 by AD affects TMEM cell function and viability. **(A)** T cells from WT animals were CD3/CD28 activated and cultured for 3 days in IL2. At day 3 post activation cells were differentiated in TMEM by culturing them in IL15 for 3 additional days. AD was added at the indicated concentrations starting from day 3 post activation. At day 6, oxygen consumption rate (OCR - an indicator of oxidative phosphorylation) was measured using a Seahorse Analyser. **(B)** Cells were differentiated as in A, except that control vehicle or AD were added directly during Seahorse analysis. **(C)** Cells were treated as described and viability was measured every day as 7-AAD exclusion by flow cytometry. **(D)** Cells were differentiated as in A. At day 6 post activation mitochondrial mass was measured by MitoTracker staining.

[0009] **Figure 3** is a graph showing that AD disrupts oxidative phosphorylation in M2 macrophages. Bone marrow-derived macrophages (BMDMs) on day 7 of culture were polarised into M2 cells with IL-4 in the presence or absence of AD at the indicated concentrations. After 24 hours, oxygen consumption rate (OCR - an indicator of oxidative phosphorylation) was measured using a Seahorse Analyser.

[0010] **Figure 4** is a graph showing that Alexidine Dihydrochloride (AD) is able to reduce cardiolipin species in T cells.

[0011] **Figure 5** TMEM cells have higher levels of cardiolipin due to higher expression of PTPMT1 and CRLS1.

[0012] **Figure 6** Reduction of cardiolipin levels mediated by AD impair TMEM function and viability.

[0013] **Figure 7** RNAseq data comparing overall gene expression of TEFF, TMEM and TMEM treated with AD.

[0014] **Figure 8** Effect of AD treatment on TEFF cells.

[0015] **Figure 9** AD treatment during priming does not alter TMEM differentiation and function.

[0016] **Figure 10** Acute treatment of TEFF or TMEM cells during seahorse experiment does not alter mitochondrial respiration.

[0017] **Figure 11** Opa1 over-expression, NAC or pyruvate do not rescue the effect of AD in TMEM cells.

[0018] **Figure 12** AD reduce OXPHOS in M2 macrophages and inhibits their differentiation.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention therefore relates in a first aspect to a method for determining whether a compound has the capability of inhibiting cardiolipin synthesis, comprising (a) contacting a sample comprising T cells and/or M2 macrophages with the compound; (b) culturing the sample in the presence of the compound for at least 48h, preferably at least 60h and most preferably at least 72h; and (c) assaying in the sample the mitochondrial function of the T cells and/or M2 macrophages, wherein a reduction of the mitochondrial function as compared to a control sample not contacted with the compound indicates that the compound has the capability of inhibiting cardiolipin synthesis.

[0020] The nature of the compound to be tested for its capability of inhibiting cardiolipin (CL) synthesis is not particularly limited and preferred examples thereof will be described herein below. As used herein "cardiolipin" refers to a chemical compound having the (IUPAC name "1,3-bis(*sn*-3'-phosphatidyl)-*sn*-glycerol". Cardiolipin is an important component of the inner mitochondrial membrane (IMM), where it constitutes about 20% of the total lipid composition. The name cardiolipin is derived from the fact that it was first found in animal hearts. In mammalian and plant cells cardiolipin is found almost exclusively in the IMM, where it is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism. The compound may interfere with any step in the

cardiolipin synthesis pathway as shown in Figure 1. As shown in Figure 1, cardiolipin synthesis starts from the generation of phosphatidylglycerol (PG) from phosphatidylglycerolphosphate (PGP) mediated by the phosphatase PTP localized to mitochondrion 1 (PTPMT1). Cardiolipin synthase 1 (CLS1) then catalyzes cardiolipin (CL) in a reaction between PG and a molecule of cytidine diphosphate diacylglycerol (CDP-DAG). The compound having the capability of inhibiting cardiolipin synthesis may interfere with any one of the precursors of CL, e.g. PGP, PG or CDP-DAG, so that CL cannot be generated as one or more of the required precursors are absent or not processible. The compound having the capability of inhibiting cardiolipin synthesis may also interfere with one or more of the enzymes in the pathway, e.g. PTPMT1 or CLS1, so that the precursors no longer can be processed into CL as the required enzymes are absent or inhibited.

[0021] The method of the invention may be performed in high through-put format for testing several compounds at the same time. High-throughput methods are capable of screening up to several thousand compounds in parallel. High-throughput assays, independently of being biochemical, cellular or other assays, generally may be performed in wells of microtiter plates, wherein each plate may contain 96, 384 or 1536 wells. Handling of the plates, including incubation at temperatures other than ambient temperature, and bringing into contact of test compounds with the assay mixture is preferably effected by one or more computer-controlled robotic systems including pipetting devices. In case large libraries of test compounds are to be screened and/or screening is to be effected within short time, mixtures of, for example 10, 20, 30, 40, 50 or 100 test compounds may be added to each well. In case a well exhibits inhibition of cardiolipin synthesis, said mixture of test compounds may be de-convoluted to identify the one or more test compounds in said mixture giving rise to the inhibition of cardiolipin synthesis.

[0022] The compound having the capability of inhibiting cardiolipin synthesis may act (i) on the DNA level (e.g. a siRNA against the mRNA encoding PTPMT1 or CLS1), or (ii) on the protein level (e.g. an antibody against the PTPMT1 or CLS1 enzyme), or (iii) on the precursor level (e.g. an antibody against PGP, PH or CDP-DAG). It follows that the compound having the capability of inhibiting cardiolipin synthesis may be (i) a compound inhibiting the expression of an mRNA encoding an enzyme being required for the cardiolipin synthesis, or (ii) a compound inhibiting an enzyme being required for the cardiolipin synthesis, or (iii) or a compound inhibiting a precursor being required for the cardiolipin synthesis.

[0023] Compounds under option (i) include compounds interfering with the transcriptional machinery and/or its interaction with the promoter of said gene and/or with expression control elements remote from the promoter such as enhancers. Compounds under option (i) also include compounds interfering with the translational machinery. The

compound inhibiting the expression of a mRNA encoding an enzyme being required for the cardiolipin synthesis inhibits the expression and/or translation of said mRNA, for example, by specifically interfering with the promoter region controlling the expression of the mRNA or leading to the silencing of the mRNA. Preferably, the transcription and/or translation of the mRNA is reduced by at least 50%, more preferred at least 75% such as at least 90% or 95%, even more preferred at least 98% and most preferred by about 100% (e.g., as compared to the same experimental set up in the absence of the compound).

[0024] Compounds under options (ii) and (iii) cause said protein or precursor to perform its function with lowered efficiency. The compound preferably specifically inhibits the activity of the enzyme or precursor. The activity of the enzyme is its capability of catalyzing a chemical reaction being involved in the pathway leading to cardiolipin. The activity of the precursor is that it can be processed into another precursor further upstream in the pathway of cardiolipin synthesis or cardiolipin itself. Preferably, the activity of the enzyme or precursor is reduced by at least 50%, more preferred at least 75% such as at least 90% or 95%, even more preferred at least 98%, and most preferably about 100% (e.g., as compared to the same experimental set up in the absence of the compound).

[0025] The claimed method requires assaying in the sample the mitochondrial function. A mitochondrion is a membrane bound cellular structure and is generally found in eukaryotic cells. The size of mitochondria ranges from 0.5 to 1.0 micrometer in diameter. Mitochondria are sometimes described as power plants of the cells. These organelles generate most of the energy of the cell in the form of adenosine triphosphate (ATP) and it is used as a source of chemical energy.

[0026] Hence, in accordance with the method of the invention the capability of the compound of inhibiting cardiolipin synthesis is determined by comparing the mitochondrial function in the presence of the compound to that in the absence of the compound. In case the mitochondrial function is reduced in the presence of the compound the compound is capable of inhibiting cardiolipin synthesis. The term "mitochondrial function" designates any one of the functions that mitochondria exert as a cell organelle. The most important function of mitochondria is to produce energy. Simpler molecules of nutrition are sent to the mitochondria to be processed and to produce charged molecules. These charged molecules combine with oxygen and produce ATP molecules. This process is known as oxidative phosphorylation. Mitochondria also function to help the cells to maintain proper concentration of calcium ions within the compartments of the cell. Mitochondria aid in building certain parts of blood and hormones, like testosterone and estrogen. The mitochondria of liver cells comprise enzymes that detoxify ammonia. Mitochondria play an important role in the process of apoptosis or programmed cell death. Abnormal death of cells due to the dysfunction of mitochondria can affect the function of organs. A compound is preferably qualified as being

capable of inhibiting cardiolipin synthesis in case the mitochondrial function is reduced by at least 50%, more preferred at least 75% such as at least 90% or 95%, even more preferred at least 98% and most preferred by about 100% (e.g., as compared to the same experimental set up in the absence of the compound). Means and methods for determining and quantifying the mitochondrial function are known in the art and will be further detailed herein below.

[0027] In this connection a sample comprising T cells and/or M2 macrophages is contacted with the test compound. The sample can be a body sample or *in vitro* cultured cells. Preferred body samples are a tissue sample (e.g. bone marrow derived macrophages or organ biopsy, such as skin biopsy comprising T cells), including a tumor sample derived from a biopsy or resection, a blood sample (e.g. whole blood, plasma or serum), spinal fluid, and fluid from bronchoalveolar lavage.

[0028] As used herein and in the state of the art the term "T cell" (or "T lymphocyte") designates a type of lymphocytes (i.e. a subtype of white blood cells) that plays a central role in cell-mediated immunity. T cells can be distinguished from other lymphocytes, such as B cells and natural killer cells, by the presence of a T-cell receptor (TCR) on the cell surface. The TCR is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules. The major component of the TCR is CD3. Several subsets of T cells are known, each having a distinct function. Non-limiting examples of these subsets are effector T cells, T-helper cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cell, mucosal associated invariant T cells, and gamma delta T cells. It is believed that any subset of T cells can be employed in the method of the invention.

[0029] Since memory T cells (also designated herein TMEM or T_{MEM}) are employed in the examples herein below the T cells herein are preferably memory T cells. Antigen-naïve T cells expand and may differentiate into memory T cells after they encounter their cognate antigen within the context of an MHC molecule on the surface of a professional antigen presenting cell (e.g. a dendritic cell). The single unifying theme for all memory T cell subtypes is that they are long-lived and can quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen. By this mechanism they provide the immune system with "memory" against previously encountered pathogens. Memory T cells may be either CD4⁺ or CD8⁺ and usually express CD45RO. *Inter alia* the following memory T cell subtypes are known: (i) Central memory T cells (T_{CM} cells) express CD45RO, C-C chemokine receptor type 7 (CCR7), and L-selectin (CD62L). Central memory T cells also have intermediate to high expression of CD44. This memory subpopulation is commonly found in the lymph nodes and in the peripheral circulation. (ii) Effector memory T cells (T_{EM} cells and T_{EMRA} cells) express CD45RO but lack expression of CCR7 and L-selectin. They also have intermediate to high expression of CD44. These memory T cells lack lymph node-

homing receptors and are thus found in the peripheral circulation and tissues. T_{EMRA} stands for terminally differentiated effector memory cells re-expressing CD45RA, which is a marker usually found on naive T cells. (iii) Tissue resident memory T cells (T_{RM}) occupy tissues (skin, lung, etc.) without recirculating. One cell surface marker that has been associated with T_{RM} is the integrin $\alpha\beta 7$. (iv) Virtual memory T cells differ from the other memory subsets in that they do not proliferate following a strong clonal expansion event. Thus, although this population as a whole is abundant within the peripheral circulation, individual virtual memory T cell clones reside at relatively low frequencies. One theory is that homeostatic proliferation gives rise to this T cell population. Although CD8 virtual memory T cells were the first to be described, it is now known that CD4 virtual memory cells also exist. Among the mentioned memory T cell subtypes (i) to (iv), subtypes (i) to (iii) are preferred.

[0030] As used herein the term “macrophage” designates a cell of myeloid origin. Macrophages are large white blood cells, occurring principally in connective tissue and in the bloodstream. They ingest foreign particles and infectious microorganisms by phagocytosis and have the capacity for antigen presentation. Macrophages are classified in the art into M0, M1 and M2 macrophages. As will be further detailed herein below, M1 and M2 macrophage activation represent two possible, distinct fates that unactivated macrophages can differentiate into.

[0031] Unactivated macrophages derived from precursors undergo specific differentiation depending on the local tissue environment. They respond to environmental cues within tissues such as damaged cells, activated lymphocytes, or microbial products, to differentiate into distinct functional phenotypes. For instance, monocytes in the blood can enter the tissue during inflammation or insult and are, depending on the local microenvironment, polarised towards an M1 or M2 phenotype. The M1 macrophage phenotype is characterized by the production of high levels of pro-inflammatory cytokines, an ability to mediate resistance to pathogens, strong microbicidal properties, high production of reactive nitrogen and oxygen intermediates, and promotion of Th1 responses. In contrast, M2 macrophages are characterized by their involvement in parasite control, tissue remodeling, immune regulation, tumor promotion and efficient phagocytic activity.

[0032] In accordance with the method of the invention the sample may comprise M2 macrophages. Conditions that can be used in order to differentiate monocytes and/or macrophages into M2 macrophages *in vitro* are known in the art. Preferred examples are the differentiation of monocytes and/or unactivated macrophages (M0 cells) into M2 macrophages with (i) IL-4, (ii) IL-10, (iii) glucocorticoids (GC), or (iv) GC plus TGF-beta plus. A more preferred example is the differentiation of monocytes and/or unactivated macrophages (M0 cells) into M2 macrophages with IL-4, preferably in conjunction with M-CSF. In this respect it is further preferred to use IL-4 at a concentration of about 10ng/mL

and, if present, M-CSF at a concentration of about 20ng/ml. The sample comprising M2 macrophages may also be obtained from a subject. As will be further discussed herein below macrophages, including M2 macrophages are known to be distributed in tissues throughout the body.

[0033] A M2 macrophage is also designated as alternatively activated macrophage in the art and hence as a macrophage that was activated in the absence of a bacterium, a virus, or a bacterial or viral compound (such as and preferably LPS) and IFN-gamma. Preferably, a M2 macrophage as used herein refers to a macrophage cell that was activated *in vitro* or *in vivo* in the presence of a compound selected from IL-4, IL-10, glucocorticoids (GC), and GC plus TGF-beta plus, or any combination of these compounds. Based on the mode of activation M2 cells are also designated M(IL-4), M(IL-10), M(GC) or M(GC+TGF-beta) cells. The M2 cells herein are preferably M(IL-4), M(IL-10), M(GC) or M(GC+TGF-beta) cells and are most preferably M(IL-4) cells.

[0034] M(IL-4) cells express CD68, GATA3, IRF4, SOCS1, CCL4, CCL13, CCL17, CCL18, MRC1, STAB1, F13A1, TGFB1, MMP12, TGM2, ALOX15, CD200R, but do not express MARCO and CD163. M(IL-10) cells express CD68, SOCS3 and IL-4Ra. M(GC) cells express CD68, CD163, STAB1, MARCO, TGFB2, ADORA3. Finally, M(GC+TGF-beta) cells express CD68, ID3, RGS1, pSMAD2, TGFB2, ALOX5AP, IL17RB. An overview of the expressed markers being characteristic for M2 cells that were activated by IL-4, IL-10, (GC), and GC plus TGF-beta, respectively, is provided in Table 1.

Table 1

Alternatively activated macrophage (M2 cell)	Expressed Markers
IL-4-stimulated M2 cells (M(IL-4))	CD68, GATA3, IRF4, SOCS1, CCL4, CCL13, CCL17, CCL18, MRC1, STAB1, F13A1, TGFB1, MMP12, TGM2, ALOX15, CD200R Not expressed: CD163 and MARCO
IL-10-stimulated M2 cells (M(IL-10))	CD68, SOCS3, IL-4Ra
Glucocorticoid-stimulated M2 cells (M(GC))	CD68, CD163, STAB1, MARCO, TGFB2, ADORA3
Glucocorticoid plus TGF-beta-stimulated M2 cells (M(GC+TGF))	CD68, ID3, RGS1, pSMAD2, TGFB2, ALOX5AP, IL17RB

[0035] Herein M(IL-4), M(IL-10), M(GC) and M(GC+TGF-beta) cells are preferably identified by the presence or absence of at least three of the respective markers listed in Table 1. For instance, M(IL-10) cells are preferably identified by the presence of CD68, SOCS3 and IL-4Ra. (M(IL-4)) cells are, for example, preferably identified by the presence of CCL17 and CCL18, and the absence of MARCO. M(IL-4), M(GC) and M(GC+TGF-beta) cells are more preferably identified by the presence or absence of at least six of the markers listed in Table 1. For example, (M(GC)) cells are more preferably identified by the presence of CD68, CD163, STAB1, MARCO, TGFBR2, and ADORA3. (M(IL-4)) cells are, for example, preferably identified by the presence of MRC1, STAB1, F13A1, TGFB1 and MMP12, and the absence of CD163. (M(GC+TGF)) cells are most preferably identified by the presence of CD68, ID3, RGS1, pSMAD2, TGFBR2, ALOX5AP, and IL17RB. (M(GC+TGF)) cells are most preferably identified by the presence of CD68, GATA3, IRF4, SOCS1, CCL4, CCL13, CCL17, CCL18, MRC1, STAB1, F13A1, TGFB1, MMP12, TGM2, ALOX15 and CD200R, and the absence of CD163 and MARCO. In addition, CD206 is a general marker of M2 cells *in vivo*.

[0036] In connection with the claimed method the sample comprising T cells and/or M2 macrophages is cultured in the presence of the compound for at least 48h, preferably at least 60h and most preferably at least 72h. A minimum time of at least 48h is required because cardiolipin synthesis is a slow process, so that any phenotypic consequence of the reduction of the mitochondria function by the compound cannot be observed directly upon the contact of the compound with the sample comprising T cells and/or M2 macrophages. After at least 60h and in particular after at least 72h the phenotypic consequences of the reduction of the mitochondria function might be more profound. Conditions that can be used in order to culture a sample comprising T cells and/or M2 macrophages are known in the art. Non-limiting examples of culture conditions for macrophages are described in Davis and Gordon (2005), *Methods Mol Biol.*;290:105-16. Non-limiting examples of culture conditions for T cells are described in Raulf-Heimsoth (2008), *Methods Mol Med.*; 138:17-30.

[0037] As can be taken from the examples herein below it was surprisingly found that the inhibition of cardiolipin synthesis by Alexidine Dihydrochloride (AD) impairs the mitochondrial function of T cells and M2 macrophages. In memory T cells the mitochondrial mass and spare respiratory capacity (SRC) are both reduced upon contact with AD in a dose dependent manner. Moreover AD decreases oxidative phosphorylation in M2 cells, while M1 metabolism is not affected. Hence, it was unexpectedly revealed that an inhibitor of cardiolipin synthesis leads to the impairment of mitochondrial function of T cells and M2 macrophages while other immune cells, in particular M1 do not show such impairment of mitochondrial function. As again exemplified for AD, it was also found that an inhibitor of

cardiolipin has a more profound effect on T memory cells as compared to effector T-cells, pointing to a particularly important role of cardiolipin for mitochondrial function in memory T cells. Moreover, it was found that the impairment of mitochondrial function by cardiolipin inhibition can only be detected after an incubation time of at least two days, preferably at least three days but not directly after contacting the inhibitor with the T cells. These findings translate into the claimed method, which employs mitochondrial function of T cells, in particular T memory cells, and M2 macrophages for determining after an incubation time of at least 48h whether a compound is an inhibitor of cardiolipin synthesis or not.

[0038] In accordance with a preferred embodiment of the first aspect of the invention, in the sample the mitochondrial function is assayed by the quantification of mitochondrial respiratory complexes and supercomplexes, determination of the ultrastructure of mitochondrial cristae, determination of the mitochondrial morphology, massspectrometric analysis of cardiolipin species, quantifying oxidative phosphorylation and/or quantification of mitochondrial mass.

[0039] Within this list of assays the massspectrometric analysis of cardiolipin species and/or quantifying mitochondrial oxidative phosphorylation and mass are preferred, because these methods were employed in the examples herein below.

[0040] The mitochondrial respiratory chain consists of 5 enzyme complexes that are responsible for ATP generation. In the solid state supercomplexes the respiratory complexes associate with each other to form supramolecular complexes. Means and methods for the quantification of mitochondrial respiratory complexes and supercomplexes are, for example, described in Jha et al. (2016), Curr Protoc Mouse Biol; 6(1):1-14 and Sabar et al. (2005), Plant J.; 44(5):893-901. A reduction of mitochondrial respiratory complexes and supercomplexes indicates a reduction of the mitochondrial function.

[0041] A crista (plural cristae) is a fold in the inner membrane of a mitochondrion. Cristae give the inner membrane its characteristic wrinkled shape, and provide a large amount of surface area for chemical reactions to occur. This aids aerobic cellular respiration, because the mitochondrion requires oxygen. Cristae are studded with proteins, including ATP synthase and a variety of cytochromes. Means and methods for the determination of the ultrastructure of mitochondrial cristae are, for example, described in Zick et al. (2009), Biochim Biophys Acta.; 1793(1):5-19 and Cogliati et al. (2013), Cell; 155(1):160–171. An impaired morphology of the cristae and/or a reduction of the number of cristae indicate a reduction of the mitochondrial function.

[0042] Mitochondrial morphology is an actively regulated and dynamic feature altered via mitochondrial dynamics (MD) - the combination of mitochondrial fission, fusion, biogenesis, and mitochondrial autophagy (mitophagy). Changes in MD have been *inter alia*

associated with regulation of oxidative metabolism, calcium homeostasis, and apoptotic or necrotic cell death. Means and methods for determination of the mitochondrial morphology are, for example, described in Leonard et al. (2015), *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1853(2):348–360. An impaired mitochondrial morphology indicates a reduction of the mitochondrial function.

[0043] Generally three regions within a mass spectrum for cardiolipin can be used to deduce the structure of the cardiolipin species: the diacylglycerol phosphate region, the monoacylglycerol phosphate region, and the fatty acid region. Structurally, the diversity of cardiolipin species is found in both the identity and position of its four fatty acyl moieties. Means and methods for the massspectrometric analysis of cardiolipin species are, for example, described in Minkler and Hoppel (2010), *J Lipid Res*; 51(4): 856–865. A reduction of the cardiolipin species indicates a reduction of the mitochondrial function.

[0044] Oxidative phosphorylation is the metabolic pathway in which cells use enzymes to oxidize nutrients, thereby releasing energy which is used to reform ATP. In most eukaryotes, this takes place inside mitochondria. Means and methods for quantifying oxidative phosphorylation are, for example, described in Lark et al. (2016), *Am J Physiol Cell Physiol.*; 311(2):C239-45 and Zerbetto et al. (1997), *Electrophoresis*; 18(11):2059-64. A reduction of oxidative phosphorylation indicates a reduction of the mitochondrial function.

[0045] For example, MitoTracker™ Green FM is a useful tool for *in vivo* determining the mass of mitochondria. MitoTracker™ Green FM is a non-fluorescent in aqueous solutions and becomes fluorescent only when it accumulates in the mitochondrial lipid environment, regardless of membrane potential. MitoTracker™. Means and methods for quantifying mitochondrial mass are, for example, described in Puleston (2015), *Cold Spring Harb Protoc.*; 2015(9):pdb.prot086298 and Agnello et al. (2008); *Cytotechnology*, 56(3): 145–149. A reduction of the mitochondrial mass indicates a reduction of the mitochondrial function.

[0046] In accordance with a preferred embodiment of the first aspect of the invention, quantifying oxidative phosphorylation comprises quantifying the oxygen consumption rate.

[0047] As mentioned, oxidative phosphorylation leads to the formation of ATP. This chemical process involves the reduction of oxygen. For this reason measuring the mitochondrial oxygen consumption rate is a means for determining mitochondrial function; see, for example, Brand and Nicholls (2011), *Biochem J*; 435(Pt 2): 297–312. A reduced oxygen consumption rate indicates a reduction of the mitochondrial function.

[0048] In accordance with a preferred embodiment of the first aspect of the invention, the compound is an antisense molecule, siRNA, shRNA, antibody, ribozyme, aptamer, protein drug or small molecule.

[0049] The aptamer, ribozyme, antibody, small molecule, protein drug, siRNA, a shRNA or an antisense oligonucleotide of this embodiment specifically binds to / interacts with a compound being involved in the synthesis of cardiolipin thereby inhibiting cardiolipin synthesis. The compounds are the precursors of cardiolipin and enzymes as shown in Figures 1 and the respective nucleic acid molecules encoding the enzymes

[0050] As shown in Figure 1, the enzymes PTPMT1 (Ensembl ID ENSG00000110536) and CLS1 (Ensembl ID ENSG00000088766) are involved in cardiolipin synthesis. The mRNAs of human PTPMT1 transcripts variants are shown in SEQ ID NOs 1, 2, 3, and 4 (together with the corresponding Ensembl transcripts ID numbers) and of human CLS1 in SEQ ID NOs 5, 6, and 7 (together with the corresponding Ensembl transcripts ID numbers). The amino acid sequence encoded by the PTPMT1 and CLS1 transcripts variants are shown in SEQ ID NOs 8, 9, 10, and 11 (together with Ensembl peptide ID numbers) and in SEQ ID NOs 12, 13, and 14 (together with the corresponding Ensembl peptide ID numbers), respectively. Within the mRNAs of SEQ ID NOs 1, 2, 3, and 4 and within the amino acid sequences of SEQ ID NOs 8, 9, 10, and 11 are preferred. This is because in the cardiolipin synthesis pathway CLS1 catalyzes the last chemical reaction being required for the formation of cardiolipin. As the enzyme CLS1 is only involved in cardiolipin synthesis, a compound targeting the mRNA of SEQ ID NOs 1, 2, 3, and 4 or the amino acid sequence of SEQ ID NOs 8, 9, 10, and 11 is expected to prevent unwanted off target effects.

[0051] The term "aptamer" in accordance with the present invention refers to DNA or RNA molecules being either in the natural D-conformation or in the L-conformation ("spiegelmer") that usually have been selected from random pools based on their ability to bind other molecules. Aptamers have been selected which bind nucleic acid, proteins, small organic compounds, and even entire organisms. Hence, aptamer can be designed against the precursors, enzymes and the mRNA encoding the enzymes. A database of aptamers is maintained at <http://aptamer.icmb.utexas.edu/>. More specifically, aptamers can be classified as DNA or RNA aptamers or peptide aptamers. Whereas the former consist of (usually short) strands of oligonucleotides, the latter consist of a short variable peptide domain, attached at both ends to a protein scaffold. Nucleic acid aptamers are nucleic acid species that have been engineered through repeated rounds of *in vitro* selection or equivalently, SELEX (systematic evolution of ligands by exponential enrichment) to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers offer the utility for biotechnological and therapeutic applications as they offer molecular recognition properties that rival those of the commonly used biomolecules, in

particular antibodies. In addition to their discriminate recognition, aptamers offer advantages over antibodies as they can be engineered completely in a test tube, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications. Non-modified aptamers are cleared rapidly from the bloodstream, with a half-life of minutes to hours, mainly due to nuclease degradation and clearance from the body by the kidneys, a result of the aptamer's inherently low molecular weight. The rapid clearance of aptamers can be an advantage in applications such as *in vivo* diagnostic imaging. Several modifications, such as 2'-fluorine-substituted pyrimidines, polyethylene glycol (PEG) linkage, etc. are available to scientists with which the half-life of aptamers easily can be increased to the day or even week time scale.

[0052] The term "ribozymes" refers to RNA molecules that act as enzymes in the absence of proteins. These RNA molecules act catalytically or autocatalytically and are capable of cleaving e.g. other RNAs at specific target sites but they have also been found to catalyze the aminotransferase activity of the ribosome. Hence, the ribozymes may act on the mRNA encoding the enzymes being involved in the cardiolipin synthesis. Selection of appropriate target sites and corresponding ribozymes can be done as described for example in Zaher and Unrau (2007), RNA, 13 (7): 1017–1026. Examples of well-characterized small self-cleaving RNAs are the hammerhead, hairpin, hepatitis delta virus, and *in vitro*-selected lead-dependent ribozymes. The principle of catalytic self-cleavage has become well established in the last 10 years. The hammerhead ribozymes are characterized best among the RNA molecules with ribozyme activity. Since it was shown that hammerhead structures can be integrated into heterologous RNA sequences and that ribozyme activity can thereby be transferred to these molecules, it appears that catalytic sequences for almost any target sequence can be created, provided the target sequence contains a potential matching cleavage site. The basic principle of constructing hammerhead ribozymes is as follows: An interesting region of the RNA, which contains the GUC (or CUC) triplet, is selected. Two oligonucleotide strands, each with 6 to 8 nucleotides, are taken and the catalytic hammerhead sequence is inserted between them. Molecules of this type were synthesized for numerous target sequences. They showed catalytic activity *in vitro* and in some cases also *in vivo*. The best results are usually obtained with short ribozymes and target sequences.

[0053] The aptamers and ribozymes may comprise modified nucleotides, such as locked nucleic acids (LNAs).

[0054] The term "antibody" as used in accordance with the present invention comprises, for example, polyclonal or monoclonal antibodies. Furthermore, also derivatives or fragments thereof, which still retain the binding specificity, are comprised in the term "antibody". Antibody fragments or derivatives comprise, *inter alia*, Fab or Fab' fragments, Fd,

F(ab')₂, Fv or scFv fragments, single domain V_H or V-like domains, such as VhH or V-NAR-domains, as well as multimeric formats such as minibodies, diabodies, tribodies, tetrabodies or chemically conjugated Fab'-multimers (see, for example, Altshuler et al., Biochemistry (Mosc). 2010 Dec; 75(13):1584-605, Holliger and Hudson, Nat Biotechnol., 2005; 23(9):1126-36). The term "antibody" also includes embodiments such as chimeric (human constant domain, non-human variable domain), single chain and humanized (human antibody with the exception of non-human CDRs) antibodies. Various techniques for the production of antibodies and fragments thereof are well known in the art and described, e.g. in Altshuler et al., Biochemistry (Mosc). 2010 Dec; 75(13):1584-605. Thus, polyclonal antibodies can be obtained from the blood of an animal following immunisation with an antigen in mixture with additives and adjuvans and monoclonal antibodies can be produced by any technique which provides antibodies produced by continuous cell line cultures. Examples for such techniques are described, e.g. Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988 and Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999 and include the hybridoma technique originally described by Köhler and Milstein, Nature 256 (1975), 495-497, the trioma technique, the human B-cell hybridoma technique (see e.g. Kozbor, Immunology Today 4 (1983), 72; Milstein, C (1999), BioEssays 21 (11): 966-73.) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985), 77-96). Furthermore, recombinant antibodies may be obtained from monoclonal antibodies or can be prepared *de novo* using various display methods such as phage, ribosomal, mRNA, or cell display. A suitable system for the expression of the recombinant (humanized) antibodies or fragments thereof may be selected from, for example, bacteria, yeast, insects, mammalian cell lines or transgenic animals or plants (see, e.g., US patent 6,080,560; Holliger and Hudson, Nat Biotechnol., 2005; 23(9):1126-36). Further, techniques described for the production of single chain antibodies (see, inter alia, US Patent 4,946,778) can be adapted to produce single chain antibodies specific for the target of this invention. Surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies. Antibodies may be directed to proteins or small organic molecules. Hence, the antibody used by the present invention may act on one of the enzymes or precursors being in cardiolipin synthesis. The antibody thus preferably specifically binds to one or more of the amino acid sequences of SEQ ID NOs 8 to 11 (i.e. proteins encoding the splice variants of PTPMT1) or one or more of the amino acid sequences of SEQ ID NOs 12 to 14 (i.e. proteins encoding the splice variants of CLS1), or a precursor being selected from PGP and PG. As regards antibodies being directed to proteins encoding splice variants it is to be understood that the antibody may specifically bind to more than one protein being encoded by the splice variants

of PTPMT1 or CLS1 in case the epitope of the antibody can be found in more than one of these proteins, or the antibody may specifically bind to only protein being encoded by a splice variant of PTPMT1 or CLS1 in case the epitope of the antibody can be found in only one of these proteins. It is preferred in this respect that the antibody specifically binds an epitope that can be found in all proteins being encoded by the splice variants of PTPMT1 or CLS1, respectively, so that the activity of all variants of PTPMT1 or CLS1, respectively can be inhibited by a single antibody. Means and methods for determining to which proteins being encoded by the discussed splice variants an antibody can bind are known in the art and may comprise, for example, a Western Blot, immunohistochemistry, or an ELISA assay. In these cases the antibody inhibits the synthesis of cardiolipin by inhibiting an enzyme or a precursor being required for the synthesis of cardiolipin.

[0055] The term “protein drug” designates designer drugs that are derivatives of proteins, preferably human proteins. These proteins are used as scaffold to create a protein drug by well-established screening procedures (see Tomlinson et al (2004), *Nature Biotechnology*, 22(5): 521-522). Non-limiting examples of human proteins which serve as a scaffold for designing protein drugs are transferrin, C-type lectins, trinectins, domain antibodies, kunitz domains, lipocalins and the Fyn SH3 domain. Also the protein drug used by the present invention may act on one of the enzymes or precursors being involved in the cardiolipin synthesis.

[0056] The term “small molecule” as used herein designates a low molecular weight organic compound that may have or has the capability to inhibit cardiolipin synthesis. The upper molecular weight limit for a small molecule is preferably about 900 Daltons. Small molecule of this molecular weight can in general rapidly diffuse across cell membranes, so that they can reach intracellular sites of action. As lower molecular weight a cut-off of 500 Daltons is preferred. This is because clinical attrition rates may be significantly reduced if the molecular weight is kept below 500 Daltons.

[0057] The antisense technology for the downregulation of RNA is well-established and widely used in the art to treat various diseases. The basic idea of the antisense technology is the use of oligonucleotides for silencing a selected target mRNA through the exquisite specificity of complementary-based pairing (Re, Ochsner J. 2000 Oct; 2(4): 233–236). Herein below details on the antisense construct compound classes of siRNAs, shRNAs and antisense oligonucleotides will be provided. As will be further detailed herein below, antisense oligonucleotides are single stranded antisense constructs while siRNAs and shRNAs are double stranded antisense constructs with one strand comprising an antisense oligonucleotide sequence (i.e. the so-called antisense strand). All these compound classes may be used to achieve downregulation or inhibition of a target RNA.

[0058] In accordance with the present invention the target of antisense constructs in general as well as all the specific classes of antisense constructs being described herein is preferably an mRNA of an enzyme being involved in the cardiolipin synthesis. Accordingly, the target is preferably one or more nucleic acids selected from the sequences of SEQ ID NOs 1 to 4 (i.e. the splice variants of PTPMT1), or one or more nucleic acids selected from the sequences of SEQ ID NOs 5 to 7 (i.e. the splice variants of CLS1). It is a matter of routine in the field of antisense technology to design an antisense construct with a sufficient number of nucleotide mismatches to any off-target in order to ensure that no off-targets become down-regulated. As regards antisense constructs in general as well as all the specific classes of antisense constructs being described herein it is to be understood that the antisense construct may specifically bind to more than one of the splice variants of PTPMT1 or CLS1 in case the target sequence of the construct can be found in more than one of these splice variants, or the antisense construct may specifically bind to only one splice variant of PTPMT1 or CLS1 in case the target sequence of the construct can be found in only one of the splice variants. It is preferred in this respect that the antisense construct specifically binds to a target sequence that can be found in all the splice variants of PTPMT1 or CLS1, respectively, so that the activity of all variants of PTPMT1 or CLS1, respectively can be inhibited by a single antisense construct.

[0059] The term "siRNA" in accordance with the present invention refers to small interfering RNA, also known as short interfering RNA or silencing RNA. siRNAs are a class of 12 to 30, preferably 18 to 30, more preferably 20 to 25, and most preferred 21 to 23 or 21 nucleotide-long double-stranded RNA molecules that play a variety of roles in biology. Most notably, siRNA is involved in the RNA interference (RNAi) pathway where the siRNA interferes with the expression of a specific gene. In addition to their role in the RNAi pathway, siRNAs also act in RNAi-related pathways, e.g. as an antiviral mechanism or in shaping the chromatin structure of a genome. siRNAs have a well defined structure: a short double-strand of RNA (dsRNA), advantageously with at least one RNA strand having a 5' or 3' overhang. Each strand typically has a 5' phosphate group and a 3' hydroxyl (-OH) group. This structure is the result of processing by dicer, an enzyme that converts either long dsRNAs or small hairpin RNAs into siRNAs. siRNAs can also be exogenously (artificially) introduced into cells to bring about the specific knockdown of a gene of interest. Thus, any gene of which the sequence is known can in principle be targeted based on sequence complementarity with an appropriately tailored siRNA. The double-stranded RNA molecule or a metabolic processing product thereof is capable of mediating target-specific nucleic acid modifications, particularly RNA interference and/or DNA methylation. Preferably, one or both ends of the double-strand have a 3'-overhang from 1-5 nucleotides, more preferably from 1-3 nucleotides and most preferably 2 nucleotides. The most efficient silencing was so far

obtained with siRNA duplexes composed of 21-nt sense and 21-nt antisense strands, paired in a manner to have 2-nt 3'- overhangs. The sequence of the 2-nt 3' overhang makes a small contribution to the specificity of target recognition restricted to the unpaired nucleotide adjacent to the first base pair (Elbashir et al. Nature. 2001 May 24; 411(6836):494-8). 2'-deoxynucleotides in the 3' overhangs are as efficient as ribonucleotides, but are often cheaper to synthesize and probably more nuclease resistant. The siRNA used in the invention preferably comprises an antisense strand which comprises or consists of a sequence which is with increasing preference complementary to at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, or at least 21 nucleotides of one or more sequences of SEQ ID NOs 1 to 7.

[0060] A preferred example of a siRNA is an endoribonuclease-prepared siRNA (esiRNA). An esiRNA is a mixture of siRNA oligos resulting from cleavage of a long double-stranded RNA (dsRNA) with an endoribonuclease such as *Escherichia coli* RNase III or dicer. esiRNAs are an alternative concept to the usage of chemically synthesized siRNA for RNA interference (RNAi). For the generation of esiRNAs a cDNA of an mRNA template may be amplified by PCR and tagged with two bacteriophage-promotor sequences. RNA polymerase is then used to generate long double-stranded RNA that is complementary to the target-gene cDNA. This complementary RNA may be subsequently digested with RNase III from *Escherichia coli* to generate short overlapping fragments of siRNAs with a length between 18-25 base pairs. This complex mixture of short double-stranded RNAs is similar to the mixture generated by dicer cleavage in vivo and is therefore called endoribonuclease-prepared siRNA or short esiRNA. Hence, esiRNA are a heterogeneous mixture of siRNAs that all target the same mRNA sequence. esiRNAs lead to highly specific and effective gene silencing.

[0061] A "shRNA" in accordance with the present invention is a short hairpin RNA, which is a sequence of RNA that makes a (tight) hairpin turn that can also be used to silence gene expression via RNA interference. shRNA preferably utilizes the U6 promoter for its expression. The shRNA hairpin structure is cleaved by the cellular machinery into siRNA, which is then bound to the RNA-induced silencing complex (RISC). This complex binds to and cleaves mRNAs which match the shRNA that is bound to it. The shRNA used in the invention preferably comprises an antisense strand which comprises or consists of a sequence which is with increasing preference complementary to at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides of one or more sequences of SEQ ID NOs 1 to 7.

[0062] The term "antisense oligonucleotide" in accordance with the present invention preferably refers to a single-stranded nucleotide sequence being complementary by virtue of Watson-Crick base pair hybridization to one or more mRNAs selected from SEQ ID NOs 1 to 7 whereby the respective mRNA is blocked and cannot be translated into protein. The antisense oligonucleotides may be unmodified or chemically modified. In general, they are relatively short (preferably between 13 and 25 nucleotides). Moreover, they are specific for one or more mRNAs of SEQ ID NOs selected from SEQ ID NOs 1 to 7, i.e. they hybridize to a unique sequence in the total pool of targets present in the target cells/organism. The antisense oligonucleotide according to the invention comprises or consists a sequence which is with increasing preference complementary to at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides of one or more sequences of SEQ ID NOs 1 to 7. These at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides are preferably complementary to a contiguous part of one or more sequences of SEQ ID NOs 1 to 7, i.e. the nucleotides are consecutive in the respective SEQ ID NO(s).

[0063] The antisense oligonucleotide is preferably a LNA-GapmeR, an Antagomir, or an antimiR.

[0064] LNA-GapmeRs or simply GapmeRs are potent antisense oligonucleotides used for highly efficient inhibition of mRNA function. GapmeRs function by RNase H dependent degradation of complementary RNA targets. They are an excellent alternative to siRNAs for knockdown of mRNA. They are advantageously taken up by cell without transfection reagents. GapmeRs contain a central stretch of DNA monomers flanked by blocks of LNAs. The GapmeRs are preferably 14-16 nucleotides in length and are optionally fully phosphorothioated. The DNA gap activates the RNase H-mediated degradation of targeted RNAs and is also suitable to target transcripts directly in the nucleus. The LNA-GapmeR used in the invention preferably comprises a sequence which is with increasing preference complementary to at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides of one or more sequences of SEQ ID NOs 1 to 7. These at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides are preferably complementary to a contiguous part of one or more sequences of SEQ ID NOs 1 to 7, i.e. the nucleotides are consecutive in the respective SEQ ID NO(s). LNA-GapmeRs are routinely designed using

established algorithms. LNA-GapmeRs to a selected target are commercially available including positive and negative controls, for example, from Exiqon.

[0065] As mentioned, AntimiRs are oligonucleotide inhibitors that were initially designed to be complementary to a miRNA. AntimiRs against miRNAs have been used extensively as tools to gain understanding of specific miRNA functions and as potential therapeutics. As used herein, the AntimiRs are preferably designed to be complementary to one or more of the sequences of SEQ ID NOs 1 to 7. AntimiRs are preferably 14 to 23 nucleotides in length. An AntimiR according to the invention more preferably comprises or consists a sequence which is with increasing preference complementary to at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, or at least 23 nucleotides of one or more sequences of SEQ ID NOs 1 to 7. These at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, or at least 23 nucleotides are preferably complementary to a contiguous part of one or more sequences of SEQ ID NOs 1 to 7, i.e. the nucleotides are consecutive in the respective SEQ ID NO(s).

[0066] AntimiRs are preferably AntagomiRs. AntagomiRs are synthetic 2-O-methyl RNA oligonucleotides, preferably of 21 to 23 nucleotides which are preferably fully complementary to the selected target RNA. While AntagomiRs were initially designed against miRNAs they may also be designed against mRNAs. The AntagomiRs used according to the invention therefore preferably comprise a sequence being complementary to 21 to 23 nucleotides of one or more sequences of SEQ ID NOs 1 to 7. These 21 to 23 nucleotides are preferably complementary to a contiguous part of one or more sequences of SEQ ID NOs 1 to 7, i.e. the nucleotides are consecutive in the respective SEQ ID NO(s). AntagomiRs are preferably synthesized with 2'-OMe modified bases (2'-hydroxyl of the ribose is replaced with a methoxy group), phosphorothioate (phosphodiester linkages are changed to phosphorothioates) on the first two and last four bases, and an addition of cholesterol motif at 3' end through a hydroxyprolinol modified linkage. The addition of 2'-OMe and phosphorothioate modifications improves the bio-stability whereas cholesterol conjugation enhances distribution and cell permeation of the AntagomiRs.

[0067] Antisense molecules (including antisense oligonucleotides, such as LNA-GapmeR, an Antagomir, an antimiR), siRNAs and shRNAs of the present invention are preferably chemically synthesized using a conventional nucleic acid synthesizer. Suppliers of nucleic acid sequence synthesis reagents include Proligo (Hamburg, Germany), Dharmacon Research (Lafayette, CO, USA), Pierce Chemical (part of Perbio Science, Rockford, IL,

USA), Glen Research (Sterling, VA, USA), ChemGenes (Ashland, MA, USA), and Cruachem (Glasgow, UK).

[0068] The ability of antisense molecules (including antisense oligonucleotides, such as LNA-GapmeR, an Antagomir, an antimiR), siRNA, and shRNA to potently, but reversibly, silence or inhibit a target mRNA *in vivo* makes these molecules particularly well suited for use in the medical applications of the invention being further described herein below. Ways of administering siRNA to humans are described in De Fougerolles et al., Current Opinion in Pharmacology, 2008, 8:280-285. Such ways are also suitable for administering other small RNA molecules like antisense oligonucleotides or shRNAs. Accordingly, such pharmaceutical compositions may be administered directly formulated as a saline, via liposome based and polymer-based nanoparticle approaches, as conjugated or complexation pharmaceutical compositions, or via viral delivery systems. Direct administration comprises injection into tissue, intranasal and intratracheal administration. Liposome based and polymer-based nanoparticle approaches comprise the cationic lipid Genzyme Lipid (GL) 67, cationic liposomes, chitosan nanoparticles and cationic cell penetrating peptides (CPPs). Conjugated or complexation pharmaceutical compositions comprise PEI-complexed antisense molecules (including antisense oligonucleotides), siRNA, or shRNA. Further, viral delivery systems comprise influenza virus envelopes and virosomes.

[0069] The antisense molecules (including antisense oligonucleotides, such as LNA-GapmeR, an Antagomir, an antimiR), siRNAs, shRNAs may comprise modified nucleotides such as locked nucleic acids (LNAs). The ribose moiety of an LNA nucleotide is modified with an extra bridge connecting the 2' oxygen and 4' carbon. The bridge "locks" the ribose in the 3'-endo (North) conformation, which is often found in the A-form duplexes. LNA nucleotides can be mixed with DNA or RNA residues in the oligonucleotide whenever desired. Such oligomers are synthesized chemically and are commercially available. The locked ribose conformation enhances base stacking and backbone pre-organization. This significantly increases the hybridization properties (melting temperature) of oligonucleotides.

[0070] In accordance with a more preferred embodiment of the first aspect of the invention the compound comprises (a) a nucleic acid sequence which comprises or consists of a nucleic acid sequence being complementary to at least 12 continuous nucleotides of the nucleic acid sequence of one or more sequences of SEQ ID NOs 1 to 7, (b) a nucleic acid sequence which comprises or consists of a nucleic acid sequence which is at least 70% identical to the complementary strand of one or more sequences of SEQ ID NOs 1 to 7, (c) a nucleic acid sequence which comprises or consists of a nucleic acid sequence according to (a) or (b), wherein the nucleic acid sequence is DNA or RNA, (d) an expression vector expressing the nucleic acid sequence as defined in any one of (a) to (c), preferably under the

control of a macrophage-specific promoter, or (e) a host comprising the expression vector of (d).

[0071] The nucleic acid sequences as defined in items (a) to (c) of this preferred embodiment comprise or consist of sequences being complementary to nucleotides of the amino acids encoding the enzymes required for the synthesis of cardiolipin as defined by SEQ ID NOs 1 to 7. Hence, the nucleic acid sequences as defined in items (a) to (c) comprise or are antisense nucleic acid sequences.

[0072] The nucleic acid sequence according to item (a) of this further preferred embodiment of the invention comprises or consists of a sequence which is with increasing preference complementary to at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides of one or more sequences of SEQ ID NOs 1 to 7. These at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, or at least 21 nucleotides are preferably complementary to a contiguous part of one or more sequences of SEQ ID NOs 1 to 7. The format of the nucleic acid sequence according to item (a) is not particularly limited as long as it comprises or consists of at least 12 continuous nucleotides being complementary to a nucleic acid sequence of one or more sequences of SEQ ID NOs 1 to 7. Hence, the nucleic acid sequence according to item (a) reflects the above-mentioned basic principle of the antisense technology which is the use of an oligonucleotide for silencing a selected target RNA through the exquisite specificity of complementary-based pairing. Therefore, it is to be understood that the nucleic acid sequence according to item (a) is preferably in the format of a siRNA, shRNA or an antisense oligonucleotide as defined herein above. The antisense oligonucleotides are preferably LNA-GapmeRs, AntagomiRs, or antimiRs as defined herein above.

[0073] A nucleic acid sequence according to item (b) of the above preferred embodiment of the invention is capable of interacting with, more specifically hybridizing with one or more target mRNAs being selected from SEQ ID NOs 1 to 7. By formation of the hybrid the function of one or more mRNAs being selected from SEQ ID NOs 1 to 7 is reduced or blocked.

[0074] The sequence identity of the molecule according to item (b) in connection with one or more sequences of SEQ ID NOs 1 to 7 is with increasing preference at least 75%, at least 80%, at least 85%, at least 90%, at least 92.5%, at least 95%, at least 98%, at least 99% and 100%. The sequence identity in connection with each of SEQ ID NOs 1 to 7 can be individually selected. For instance, a non-limiting example is at least 85% in connection with one or more of SEQ ID NOs 1 to 4 and at least 90% in connection with one

or more of SEQ ID NOs 5 to 7. Means and methods for determining sequence identity are known in the art. Preferably, the BLAST (Basic Local Alignment Search Tool) program is used for determining the sequence identity with regard to one or more of SEQ ID NOs 1 to 7. Most preferred examples of nucleic acid sequences which comprise a nucleotide sequence which is at least 70% identical to one or more of SEQ ID NOs 1 to 4 or 5 to 7 are the complementary strands of SEQ ID NOs 1 to 4 and 5 to 7.

[0075] In the nucleic acid sequence according to item (c) the nucleotide sequences may be RNA or DNA. RNA or DNA encompasses chemically modified RNA nucleotides or DNA nucleotides. As commonly known RNA comprises the nucleotide U while DNA comprises the nucleotide T.

[0076] In accordance with items (d) and (e) of the above preferred embodiment the inhibitor may also be an expression vector or host, respectively being capable of producing an nucleic acid sequence as defined in any one of items (a) to (c).

[0077] An expression vector may be a plasmid that is used to introduce a specific transcript into a target cell. Once the expression vector is inside the cell, the inhibitor of the cardiolipin synthesis being encoded by the expression vector is produced by the cellular-transcription. The plasmid is in general engineered to contain regulatory sequences that act as enhancer and/or promoter regions and lead to efficient transcription of the transcript. In accordance with the present invention the expression vector preferably contains a T-cell-specific promoter or a macrophage-specific promoter. T-cell-specific promoters are known in the art, for example, from Ji et al (2002), J Biol Chem.; 277(49):47898-906. Also macrophage-specific promoters are known in the art, for example, from Levin et al., Gene Ther., 2012; 19(11):1041-7. Using a T-cell-specific promoter ensures that the nucleic acid sequence is only expressed in T cells and may avoid potential undesired side effects by expression in other cell types. Likewise, using a macrophage-specific promoter ensures that the nucleic acid sequence is only expressed in macrophages and may avoid potential undesired side effects by expression in other cell types.

[0078] Non-limiting examples of expression vectors include prokaryotic plasmid vectors, such as the pUC-series, pBluescript (Stratagene), the pET-series of expression vectors (Novagen) or pCARTOPO (Invitrogen) and vectors compatible with an expression in mammalian cells like pREP (Invitrogen), pcDNA3 (Invitrogen), pCEP4 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2neo, pBPV-1, pDBPVMNTneo, pRSVgpt, pRSVneo, pSV2-dhfr, pIZD35, pLXIN, pSIR (Clontech), pIRES-EGFP (Clontech), pEAK-10 (Edge Biosystems) pTriEx-Hygro (Novagen) and pCINeo (Promega). Examples for plasmid vectors suitable for *Pichia pastoris* comprise e.g. the plasmids pAO815, pPIC9K and pPIC3.5K (all Invitrogen). For the formulation of a pharmaceutical composition a suitable vector is selected in accordance with good

manufacturing practice. Such vectors are known in the art, for example, from Ausubel et al, Hum Gene Ther. 2011 Apr; 22(4):489-97 or Allay et al., Hum Gene Ther. May 2011; 22(5): 595–604.

[0079] A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Moreover, elements such as origin of replication, drug resistance gene, regulators (as part of an inducible promoter) may also be included. The *lac* promoter is a typical inducible promoter, useful for prokaryotic cells, which can be induced using the lactose analogue *isopropylthiol-b-D-galactoside* ("IPTG"). For recombinant expression and secretion, the polynucleotide of interest may be ligated between e.g. the PelB leader signal, which directs the recombinant protein in the periplasm and the gene III in a phagemid called pHEN4 (described in Ghahroudi et al, 1997, FEBS Letters 414:521-526). Additional elements might include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from retroviruses, e.g., RSV, HTLVI, HIVI, and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Alternatively, the inhibitor can be expressed in stable cell lines that contain the gene construct integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells. The transfected nucleic acid can also be amplified to express large amounts of the encoded (poly)peptide. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the nucleic acid molecule encoding the inhibitor. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al.1991, *Biochem J.* 227:277-279; Bebbington et al. 1992, *Bio/Technology* 10:169-175). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. As indicated above, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. For vector modification techniques, see Sambrook and Russel (2001), *Molecular Cloning: A Laboratory Manual*, 3 Vol. Generally, vectors can contain one or more origins of replication (ori) and inheritance systems for cloning or expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more

expression cassettes. Suitable origins of replication (ori) include, for example, the Col E1, the SV40 viral and the M 13 origins of replication.

[0080] The sequences to be inserted into the vector can e.g. be synthesized by standard methods, or isolated from natural sources. Ligation of the coding sequences to transcriptional regulatory elements and/or to other amino acid encoding sequences can be carried out using established methods. Transcriptional regulatory elements (parts of an expression cassette) ensuring expression in prokaryotes or eukaryotic cells are well known to those skilled in the art. These elements comprise regulatory sequences ensuring the initiation of the transcription (e.g., translation initiation codon, promoters, enhancers, and/or insulators), internal ribosomal entry sites (IRES) (Owens, Proc. Natl. Acad. Sci. USA 98 (2001), 1471-1476) and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Preferably, the nucleotide sequence as defined in item (a) of the above preferred embodiment of the invention is operatively linked to such expression control sequences allowing expression in prokaryotic or eukaryotic cells.

[0081] The host may be a prokaryotic or eukaryotic cell. A suitable eukaryotic host may be a mammalian cell, an amphibian cell, a fish cell, an insect cell, a fungal cell or a plant cell. Representative examples of bacterial cells are *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; of fungal cells are yeast cells; and of insect cells are *Drosophila* S2 and *Spodoptera* Sf9 cells. It is preferred that the cell is a mammalian cell such as a human cell. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells. The cell may be a part of a cell line, preferably a human cell line. Appropriate culture mediums and conditions for the above-described host cells are known in the art. The host is preferably a host cell and more preferably an isolated host cell. The host is also preferably a non-human host.

[0082] In accordance with another preferred embodiment of the method of the invention, the compound is directed against the protein tyrosine phosphatase localized to the mitochondrion 1 (PTPMT1) or cardiolipin synthase 1 (CLS1).

[0083] As discussed above and as shown in Figure 1, PTPMT1 and CLS1 are enzymes being required for the synthesis of cardiolipin. Depending on the nature of the compound the compound is directed against the mRNA encoding one of the enzymes or one of the enzymes in their protein form. For instance and as detailed herein above, in case the compound is an antisense molecule, siRNA or shRNA it may be directed against the mRNA encoding one of the enzymes. On the other hand, in case the compound is an antibody it may be directed against one of the enzymes in their protein form. The compounds are

preferably directed against the human mRNAs (SEQ ID NOs 1 to 7) and enzymes (SEQ ID NOs 8 to 14).

[0084] In accordance with a further preferred embodiment of the method of the invention, the efficacy of the compound for inhibiting cardiolipin synthesis in a patient is determined, wherein the sample of step (a) and the control sample are samples that have been obtained from the patient.

[0085] The compound to be tested for its capability of inhibiting cardiolipin synthesis may also be a compound for which it is already known that it has the capability of cardiolipin synthesis and in this case the method can determine how much inhibition is achieved by the compound. This option of performing the claimed method is particularly suitable for determining the efficacy of a compound for cardiolipin synthesis in a patient.

[0086] Hence, the first aspect of the present invention also encompasses a method for determining the efficacy of a compound for inhibiting cardiolipin synthesis in a patient, comprising (a) obtaining a sample comprising T cells and/or M2 macrophages from a patient, (b) contacting the obtained sample with the compound; and (c) culturing the sample in the presence of the compound for at least 48h, preferably at least 60h and most preferably at least 72h; and (d) assaying in the sample the mitochondrial function, wherein a reduction of the mitochondrial function as compared to a control sample not contacted with the compound indicates that the compound has the capability of inhibiting cardiolipin synthesis.

[0087] As will be discussed in more detail herein below, a compound inhibiting cardiolipin synthesis can be used to treat several diseases in a patient. Hence, determining the efficacy of a compound for inhibiting cardiolipin synthesis in a patient allows to predict whether this compound will be effective to treat or prevent a disease in the patient, in particular a disease being mediated by cardiolipin synthesis of T cells and/or M2 macrophages, or that can be treated by inhibiting synthesis of T cells and/or M2 macrophages.

[0088] In accordance with a more preferred embodiment of the method of the invention, the patient is afflicted with an autoimmune disorder, a hyperproliferative disorder or a fibrotic disorder.

[0089] An autoimmune disorder is any disease arising from an abnormal immune response to a normal body part. There are at least 80 types of autoimmune diseases. Nearly any body part can be involved. Common symptoms include low grade fever and feeling tired. Non-limiting examples are celiac disease, diabetes mellitus type 1, Graves disease, inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

[0090] A hyperproliferative disorder is any disease comprising an abnormally high rate of proliferation of cells by rapid cell division. Non-limiting examples are psoriasis, psoriatic arthritis, rheumatoid arthritis, cutaneous hyperkeratoses, inflammatory bowel disease tumors and cancers.

[0091] A fibrotic disorder is any disorder comprising the formation of excess fibrous connective tissue, e.g. in an organ or tissue in a reparative or reactive process. Non-limiting examples of fibrotic disorders will be provided herein below.

[0092] Autoreactive T cells are key players in autoimmune diseases. They can act both as regulatory and effector cells. Various animal models have been used to show that the transfer of autoreactive T cells is sufficient to induce a model of an autoimmune disease (Dornmair et al. (2003), *Am J Pathol*; 163(4): 1215–1226 or Yang et al. (2015), *Arthritis Res Ther*; 17(1): 29.). Hyperproliferative disorders, such as T cell lymphomas may be mediated by T cells. T cells may also play a role in fibrotic diseases (Bank (2016), *Rambam Maimonides Med J*; 7(4):e0029). It follows that T cells play an important role in promoting the formation of autoimmune, hyperproliferative or fibrotic disorders and interfering with the formation of T cells is a suitable means for treating or preventing these disorders. M2 macrophages are known to secrete large amounts of pro-fibrotic factors, such as TGF- β and Galactin-3 (Braga et al. (2015), *Front Immunol*; 6:602 and Wermuth and Jimenez (2015), *Clin Transl Med*; 4: 2.). M2 macrophages are also known to promote cell proliferation (Mills (2012), *Crit Rev Immunol.*; 32(6):463-88.). It follows that M2 macrophages play an important role in promoting the formation of hyperproliferative or fibrotic disorders and interfering with the formation of M2 macrophages is a suitable means for treating or preventing these disorders.

[0093] In connection with the method of the invention it is therefore particularly useful to determine the efficacy of a compound for inhibiting cardiolipin synthesis in a patient being afflicted with an autoimmune, a hyperproliferative or a fibrotic disorder.

[0094] In accordance with another more preferred embodiment of the method of the invention, the sample is a tissue sample or a blood sample.

[0095] In order to be capable of determining the efficacy of a compound for inhibiting cardiolipin synthesis in a patient the sample has to be a sample that has been obtained from the body of the patient and has to comprise T cells and/or M2 macrophages. Such a sample is preferably a tissue sample or a blood sample. T cells and macrophages are known to be distributed in tissues throughout the body, e.g. in the brain, liver, lung, spleen, intestine, skin, heart, kidney and peritoneum (Epelman et al. (2014), *Immunity*; 41(1):21–35 and Mueller and Mackey (2016), *Nature Reviews Immunology*, 16:79–89). As the patient is preferably afflicted with an autoimmune, hyperproliferative or fibrotic disorder

also the tissue sample is preferably obtained from such a patient. T cells and macrophages are likewise found in the blood, including the peripheral blood. The blood sample may be a whole blood sample, serum or plasma.

[0096] In accordance with a further more preferred embodiment of the method of the invention, the compound is alexidine dihydrochloride (AD).

[0097] Alexidine dihydrochloride (AD) is a potent and selective PTPMT1 inhibitor ($IC_{50} = 1.08 \mu M$, *in vitro*) (Doughty-Shenton et al. (2010), Journal of Pharmacology and Experimental Therapeutics, 333(2):584-592). Alexidine is an alkyl bis(biguanide) having the CAS No. 1715-30-6.

[0098] The present invention relates in a second aspect to a compound inhibiting cardiolipin synthesis for use in the treatment or prevention of a disease being mediated by cardiolipin synthesis in T cells and/or M2 macrophages, wherein the disease is preferably an autoimmune disorder, a hyperproliferative disorder or a fibrotic disorder.

[0099] The present invention likewise relates to a method for treating or preventing a disease being mediated by cardiolipin synthesis in T cells and/or M2 macrophages, wherein the disease is preferably an autoimmune disorder, a hyperproliferative disorder or a fibrotic disorder, comprising administering a therapeutically effective amount of a compound inhibiting cardiolipin synthesis to a subject in need thereof.

[00100] The present invention relates in a third aspect to a compound inhibiting cardiolipin synthesis for use in the treatment or prevention of an autoimmune disorder, a hyperproliferative disorder or a fibrotic disorder in a patient by inhibiting cardiolipin synthesis in T cells and/or M2 macrophages of the patient.

[00101] The present invention also relates to a method for treating or preventing an autoimmune, hyperproliferative or fibrotic disorder by inhibiting cardiolipin synthesis comprising administering a therapeutically effective amount of a compound inhibiting cardiolipin synthesis to a subject in need thereof, thereby inhibiting cardiolipin synthesis in T cells and/or M2 macrophages of the patient.

[00102] The preferred embodiments, definitions and explanations described herein above in connection with the first aspect of the invention as far as being applicable to the second and third aspect of the invention apply *mutatis mutandis* to the second and third aspect of the invention.

[00103] The subject to be treated in accordance with the second and third aspect of the invention is preferably a mammal, more preferably a primate and most preferably a human.

[00104] The compounds inhibiting cardiolipin synthesis are preferably admixed with a pharmaceutically acceptable carrier or excipient to form a pharmaceutical composition.

By “pharmaceutically acceptable carrier or excipient” is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type (see also Handbook of Pharmaceutical Excipients 6ed. 2010, Published by the Pharmaceutical Press). The compounds inhibiting cardiolipin synthesis or the pharmaceutical composition may be administered, for example, orally, parenterally, such as subcutaneously, intravenously, intramuscularly, intraperitoneally, intrathecally, transdermally, transmucosally, subdurally, locally or topically via iontophoresis, sublingually, by inhalation spray, aerosol or rectally and the like in dosage unit formulations optionally comprising conventional pharmaceutically acceptable carriers or excipients.

[00105] The compounds inhibiting cardiolipin synthesis may be formulated as vesicles, such as liposomes. Liposomes have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. Liposomal delivery systems have been used to effectively deliver nucleic acids, such as siRNA *in vivo* into cells (Zimmermann et al. (2006) Nature, 441:111-114). Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are phagocytosed by macrophages and other cells *in vivo*.

[00106] The compounds inhibiting cardiolipin synthesis can be administered to the subject at a suitable dose. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. The therapeutically effective amount for a given situation will readily be determined by routine experimentation and is within the skills and judgement of the ordinary clinician or physician. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 µg to 5 g units per day. However, a more preferred dosage is in the range of 0.01 mg to 100 mg, even more preferably 0.01 mg to 50 mg and most preferably 0.01 mg to 10 mg per day. Furthermore, if for example said compound comprises or is a nucleic acid molecule, such as an siRNA, the total pharmaceutically effective amount of pharmaceutical composition administered will typically be less than about 75 mg per kg of body weight, such as for example less than about 70, 60, 50, 40, 30, 20, 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, or 0.0005 mg per kg of body weight. More preferably, the amount will be less than 2000 nmol of nucleic acid molecule per kg of body weight, such as for example less than 1500, 750,

300, 150, 75, 15, 7.5, 1.5, 0.75, 0.15, 0.075, 0.015, 0.0075, 0.0015, 0.00075 or 0.00015 nmol per kg of body weight.

[00107] The length of treatment needed to observe changes and the interval following treatment for responses to occur vary depending on the desired effect. The particular amounts may be determined by conventional tests which are well known to the person skilled in the art. Suitable tests are, for example, described in Tamhane and Logan (2002), Journal of the American statistical association, 97(457):1-9.

[00108] As detailed above, T cells and/or M2 macrophages play an important role in promoting the formation of autoimmune, hyperproliferative or fibrotic disorders and therefore these diseases can be treated or prevented by interfering with the cardiolipin synthesis, noting that interfering with the cardiolipin synthesis leads to a reduced function of the mitochondria in the T cells and/or M2 macrophages. By the surprising finding of the inventors that the cardiolipin synthesis is important for the function of the mitochondria in T cells and/or M2 macrophages, it has also been revealed that a compound inhibiting cardiolipin synthesis can be used to treat or prevent a disease being mediated by T cells and/or M2 macrophages, or a autoimmune, hyperproliferative or fibrotic disorder by inhibiting cardiolipin synthesis. In other words, the finding that the T cells and/or M2 macrophages can be inhibited by inhibiting cardiolipin synthesis also revealed that diseases that are mediated by T cells and/or M2 macrophages can be treated or prevented by inhibiting the cardiolipin synthesis.

[00109] In accordance with a preferred embodiment of the second and third aspect of the invention, the compound targets protein tyrosine phosphatase localized to the mitochondrion 1 (PTPMT1) or cardiolipin synthase 1 (CLS1).

[00110] As can be taken from Figure 1, PTPMT1 and CLS1 are enzymes being involved in the cardiolipin synthesis. Further details on how the compounds of the invention target these enzymes are provided herein above in connection with the first aspect of the invention. These details apply *mutatis mutandis* to the second and third aspect of the invention.

[00111] In accordance with a further preferred embodiment of the second and third aspect of the invention, the compound is an antisense molecule, siRNA, shRNA, antibody, ribozyme, aptamer, protein drug or small molecule.

[00112] In connection with the second aspect and third of the invention the compound inhibiting cardiolipin synthesis may be any one of an antisense molecule, siRNA, shRNA, antibody, ribozyme, aptamer, protein drug and small molecule as defined herein above in connection with the first aspect of the invention. The details on the compounds of the invention as provided herein above in connection with the corresponding embodiment of

the first aspect of the invention apply *mutatis mutandis* to the second and third aspect of the invention.

[00113] In accordance with another preferred embodiment of the second and third aspect of the invention, the compound is alexidine dihydrochloride (AD).

[00114] This compound is known to inhibit PTPMT1. Further details on this inhibitor are provided herein above in connection with the first aspect of the invention.

[00115] In accordance with a still further preferred embodiment of the second and third aspect of the invention, the (i) the autoimmune disorder is multiple sclerosis, type 1 diabetes, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, Behçet's disease; antiphospholipid antibody syndrome (APLS), opr Sjögren's syndrome (SjS, SS); (ii) the hyperproliferative disorder is a neoplasm, tumor or cancer and is preferably selected from cancer of the breast, lung, prostate, kidney, skin, neural, ovary, uterus, liver, pancreas, epithelial, gastric, intestinal, exocrine, endocrine, lymphatic, hematopoietic system or a head and neck tissue; and/or (iii) the fibrotic disorder is selected from sarcoidosis, renal fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, liver fibrosis, cardiac fibrosis, endomyocardial fibrosis, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, chronic kidney disease, nephrogenic systemic fibrosis, Crohn's disease, hypertrophic scarring, keloid, scleroderma, organ transplant-associated fibrosis and ischemia-associated fibrosis.

[00116] The above-listed examples of autoimmune disorders, hyperproliferative disorders and fibrotic disorders are non-limiting examples or defined diseases that can be treated in accordance with the second and third aspect of the invention. A neoplasm or tumor is an abnormal benign or malignant new growth of tissue that possesses no physiological function and arises from uncontrolled usually rapid cellular proliferation. The WHO classifies neoplasms into four main groups: benign neoplasms, in situ neoplasms, malignant neoplasms, and neoplasms of uncertain or unknown behavior. A malignant neoplasm is also referred to as cancer.

[00117] As regards the embodiments characterized in this specification, in particular in the claims, it is intended that each embodiment mentioned in a dependent claim is combined with each embodiment of each claim (independent or dependent) said dependent claim depends from. For example, in case of an independent claim 1 reciting 3 alternatives A, B and C, a dependent claim 2 reciting 3 alternatives D, E and F and a claim 3 depending from claims 1 and 2 and reciting 3 alternatives G, H and I, it is to be understood that the specification unambiguously discloses embodiments corresponding to combinations A, D, G; A, D, H; A, D, I; A, E, G; A, E, H; A, E, I; A, F, G; A, F, H; A, F, I; B, D, G; B, D, H; B,

D, I; B, E, G; B, E, H; B, E, I; B, F, G; B, F, H; B, F, I; C, D, G; C, D, H; C, D, I; C, E, G; C, E, H; C, E, I; C, F, G; C, F, H; C, F, I, unless specifically mentioned otherwise.

[00118] Similarly, and also in those cases where independent and/or dependent claims do not recite alternatives, it is understood that if dependent claims refer back to a plurality of preceding claims, any combination of subject-matter covered thereby is considered to be explicitly disclosed. For example, in case of an independent claim 1, a dependent claim 2 referring back to claim 1, and a dependent claim 3 referring back to both claims 2 and 1, it follows that the combination of the subject-matter of claims 3 and 1 is clearly and unambiguously disclosed as is the combination of the subject-matter of claims 3, 2 and 1. In case a further dependent claim 4 is present which refers to any one of claims 1 to 3, it follows that the combination of the subject-matter of claims 4 and 1, of claims 4, 2 and 1, of claims 4, 3 and 1, as well as of claims 4, 3, 2 and 1 is clearly and unambiguously disclosed.

EXAMPLES

[00119] The following examples illustrate the invention.

Example 1 –Material and Methods

Cell culture and drug treatments

[00120] OT-I splenocytes were activated with OVA-peptide (SIINFEKL, New England Peptide) and IL-2 (100 U/mL) for 3 days. WT cells were activated with plate bound CD3 (5ug/ml)/soluble CD28 (0,5ug/ml) and IL-2 (100 U/mL) for 3 days. Subsequently OT-I and WT cells were cultured in the presence of either IL-2 or IL-15 (10ng/mL) for an additional 3 days in TCM (RPMI 1640 media supplemented with 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin/streptomycin, and 55 μ M β -mercaptoethanol). AD was added at the indicated concentrations either starting from day 3 post activation or acutely during Seahorse analysis.

[00121] For in vitro survival assays, cells were activated for 3 days as described, then cultured in either IL-2 at 5x10⁴ cells/mL or IL-15 at 1x10⁵ cells/mL in 96 well round bottom plates. Survival was analyzed by 7AAD exclusion using flow cytometry.

[00122] Bone marrow cells were differentiated for 7 days into BMDMs by culturing in complete medium (RPMI 1640 supplemented with 10% FBS, 100 U/mL penicillin/streptomycin, 2 mM L-glutamine) with 20 ng/mL mouse macrophage colony-stimulating factor (M-CSF; PeproTech). Bone marrow-derived macrophages (BMDMs) on day 7 of culture were polarised into M2 cells with IL-4 in the presence or absence of AD at the indicated concentrations.

Mass spectrometry

[00123] Cell samples were suspended in water (to give 1.0×10^6 cells/mL suspension) and ultra-sonication was used to homogenize the cells. Protein precipitation was performed to extract CL from 100 μ L of cell suspension. CL (14:0) was used as internal standard and was added to the samples before extraction. CL analysis was performed with a Shimadzu 20AD HPLC system, coupled to a triple quadrupole mass spectrometer (API 4000 QTrap) operated in MRM mode. The negative ion ESI mode was used for detection. Data processing was conducted with Analyst 1.5.1 (Applied Biosystems).

Metabolism assays

[00124] Oxygen consumption rates (OCR) was measured in XF media (non-buffered RPMI 1640 containing 25 mM glucose, 2mM L-glutamine, and 1 mM sodium pyruvate) under basal conditions and in response to 1 μ M oligomycin, 1.5 μ M fluoro-carbonyl cyanide phenylhydrazone (FCCP) and 100 nM rotenone + 1 μ M antimycin A, using a 96 well XF Extracellular Flux Analyzer (EFA) (Seahorse Bioscience)

Example 2 – Results

[00125] Given the observation that in TMEM cristae shape is tighter and RCC are less susceptible to digitonin treatment - which suggests a role for membrane composition and organization in this cell type – it was hypothesized that cardiolipin could govern metabolic capacity and cell fate of immune cells, in particular TMEM cells and M2 macrophages, by tightening cristae structure and organizing RCS thus allowing higher SRC.

[00126] Indeed, using Alexidine Dihydrochloride (AD), a drug that specifically inhibits the function of PTPMT1 and thus reduces cardiolipin synthesis, it was shown that SRC, metabolic hallmark of TMEM cells, is reduced in a dose dependent manner (Figure 2A). Moreover, it was shown that AD treatment is effective when cells were treated for 3 days and not after acute injection (Figure 2A-B). The action of AD has also a profound effect on viability of *in vitro* differentiated TMEM cells whilst no increase in apoptosis is observed in effector T (TEFF) cells, pointing to a specific role of cardiolipin in memory T cell differentiation (Figure 2C). Memory T cells also show lower mitochondrial mass upon AD treatment (Figure 2D).

[00127] To understand whether cardiolipin synthesis is a conserved strategy exploited by different immune cells anytime oxidative phosphorylation is increased, the effect of AD in M2 macrophages was checked, which engage oxidative phosphorylation to help drive their differentiation. It was observed that AD decreases oxidative phosphorylation in M2 cells, while M1 metabolism is not affected (Figure 3).

[00128] Alexidine Dihydrochloride (AD) is able to reduce cardiolipin species in T cells (Figure 4). The analysis of the amount of Cardiolipin was performed by mass spectrometry (HPLC-triple quadrupole mass spectrometer). This assay can be used as a screening method to check the effect of AD on patients. To do this, T cells would be isolated from patients before and after treatment with AD, followed by mass spectrometry analysis of cardiolipin species.

[00129] Moreover, it has been shown that autoimmune diseases are often associated with an increase in anti-cardiolipin antibodies in the plasma. As an additional screening method, an ELISA-based screening assay against anticardiolipin antibodies is proposed, as described in Harris et al. J Infect Dis1988;157:23–32.

Example 3 – Further Results

[00130] In order to test our hypothesis that cardiolipin plays a role in T_{MEM} differentiation and function, in vitro memory and effector cells were generated as shown in Fig.5A. The higher SRC, which represents a hallmark of memory T cells over effector T cells (Fig.5B), is indeed paralleled by an increase in cardiolipin species (Fig.5C). This increase is mediated by higher expression of both PTPMT1 and CRLS1 mRNA (Fig.5D) and protein levels (Fig.5E,F).

[00131] AD was used to selectively inhibit PTPMT1 in the synthesis of Cardiolipin (Fig.6A) in activated CD8⁺ T cell during T_{MEM} cell development (Fig.6B). This treatment effectively inhibits the total amount of cardiolipin (Fig.6C) and, in particular, the most abundant forms of cardiolipin species in T cells as the mass spec analysis revealed (Fig.6D). High spare respiratory capacity (SRC) is a metabolic hallmark of T_{MEM} cells, which is completely abrogated by the use of AD (Fig.6E). The reduced OCR/ECAR ratio (Fig.6F) together with higher uptake of glucose from the media and lactate production (Fig.6G,H) suggests that AD is able to shift T_{MEM} cells from oxidative phosphorylation to aerobic glycolysis. Moreover, when an inhibitor of fatty acid oxidation (FAO) is added to the culture (ETO, Etomoxir), SRC is additionally reduced meaning that residual FAO-dependent respiration can occur even in low content of cardiolipin in mitochondria (Fig.6I). From a functional point of view, the inhibition of cardiolipin synthesis by AD reduces the expression of CD62L which is usually highly expressed in T_{MEM} cells, without affecting the expression of the activation marker CD25 (Fig.6J). Additionally, AD reduces specifically the survival of T_{MEM} cells while T_{EFF} cells survival is not affected (Fig.6K). T_{MEM} cells show an elongated mitochondrial network with tight cristae structure (Buck et al, Cell, 2016, Cell, 166:63–76). AD treatment alters mitochondrial morphology by inducing mitochondrial fragmentation (Fig.6L). This is accompanied by a slight reduction in mitochondrial mass dependent on reduced levels of specific mitochondrial proteins (Fig.6M-O). At ultrastructural level, AD

induces cristae remodeling characterized by enlarged cristae (Fig.6P,Q). Tight organization of cristae structure is linked to efficient mitochondrial respiration obtained by strong embedding of mitochondrial respiratory chain in the inner mitochondrial membrane (IMM) to form respiratory chain supercomplexes. An extraction assay based on the use of increasing concentration of the detergent digitonin followed by separation of extracted (S) and non-extracted proteins (P) was used to determine whether inhibition of cardiolipin synthesis is linked to differential embedding of respiratory chain complexes into the IMM. The experiment shows that in AD treated T_{MEM} cells, respiratory chain complexes are poorly embedded into the IMM compared to untreated T_{MEM} cells being extracted at higher extent also at low concentration of digitonin (Fig.6R). Functionally, T_{MEM} cells are characterized by the ability to respond more quickly and more strongly to a second challenge by the same antigen. This is obtained due to their metabolic advantage represented by higher SRC and it results in production of higher amount of cytokines. AD treatment reduces SRC in memory T cell upon in vitro reactivation in a seahorse experiment (Fig.6S,T) and results in lower cytokines production (Fig.6U) indicating impaired function of T_{MEM} cells when cardiolipin levels are pharmacologically reduced.

[00132] To understand the overall effect of cardiolipin synthesis inhibition by AD in T_{MEM} cells an RNAseq was performed while comparing effector and memory T cells, the latter treated or not with AD. As expected, the results show a wide change in gene expression between T_{EFF} and T_{MEM} cells. Interestingly, analyzing a subset of genes whose expression is significantly altered between effector and memory T cells, the clustering analysis shows that AD reprograms gene expression of a sub-group of genes toward a more effector/short-lived effector T cell-like pattern (Fig.7A).

[00133] Effector T cells are highly metabolically active with predominance of glycolysis over mitochondrial metabolism. It was tested whether AD was able to induce a metabolic effect also in this T cell population according to the scheme in Fig.8A. Mass-spec analysis of cardiolipin species amount shows that AD has a milder effect compared to the one observed in T_{MEM} cells with the reduction of only one of the analyzed forms of cardiolipin (Fig.8B). Nevertheless, this reduction results in lower basal and maximal respiration and to a reduced OCR/ECAR ratio suggesting that after AD treatment effector T cells rely even more on glycolysis to support their energy production (Fig.8C,D). Residual oxidative phosphorylation is ablated by inhibition of fatty acid oxidation by etomoxir (Fig.8E). Differently from T_{MEM} cells, viability is not affected by inhibition of cardiolipin synthesis suggesting that given the different metabolic requirements, effector cells are less sensitive to reduced cardiolipin-mediated toxicity (Fig.8F). No major differences are observed in mitochondrial morphology given the already fragmented mitochondrial network in T_{EFF} cells (Fig.8G). Functionally, inhibition of cardiolipin synthesis results in cells expressing higher

levels of cytokines (especially interferon gamma) when restimulated with PMA/Ionomycin (Fig.8H,I).

[00134] To understand whether new synthesis of cardiolipin might be important during T cell priming, naïve T cells were activated in the presence of AD. After 48h, AD was washed out and cells were differentiated towards memory T cells (Fig.9A). Metabolism, in particular in regards to basal and maximal respiration and OCR/ECAR ratio were not altered (Fig.9B,C). Similarly, cytokine production upon T cell reactivation was not affected suggesting T cells rely on the existing pool of cardiolipin during the early phases of activation (Fig.9D,E).

[00135] Acute treatment with AD during Seahorse analysis does not alter the metabolic profile of memory T cells suggesting that cardiolipin synthesis is not a quick process but it takes time to occur (Fig.10A,B).

[00136] Opa1 is the protein master regulator of mitochondrial ultrastructure and inhibition of cardiolipin synthesis alters the ratio between long and short forms of the protein, with the accumulation of short forms, which have been linked to cristae remodeling and loss of respiratory chain supercomplexes organization. AD treatment exerts a similar effect, by inducing accumulation of short forms of Opa1, which is consistent with structural data showing alteration of cristae morphology (Fig.11A). Given the role of Opa1, it was tested whether the over-expression of Opa1 was able to rescue the mitochondrial metabolic defect in T_{MEM} cells treated with AD. The experiment shows that even in the presence of higher levels of Opa1, the reduction of cardiolipin is able to impair oxidative phosphorylation (Fig.11B,C). Moreover, it was tested whether antioxidant treatment with N-Acetyl-Cysteine (NAC) or supplementation with pyruvate could rescue the effect of AD but our experiments show no rescue in this particular setting (Fig.11D,E).

[00137] To understand whether cardiolipin synthesis is a conserved strategy exploited by different immune cells any time OXPHOS is increased, the effect of AD in M2 macrophages was checked, which engage OXPHOS to help driving their differentiation. Bone marrow-derived macrophages were differentiated to M0, M1 and M2 cells (Fig.12A) and it was confirmed that AD treatment during M2 differentiation results in decrease of cardiolipin species (Fig.12B). This reduction results in turn in decreased OXPHOS and higher basal glycolysis rates in M2 cells (Fig.12C,D), while M1 metabolism is not affected (data not shown). M2 macrophages are characterized by elongated mitochondria, whose morphology shifts to a more fragmented phenotype when cardiolipin synthesis is inhibited (Fig.12E). The reduction of mitochondrial respiration and alteration of mitochondrial morphology when M2 macrophages are incubated with AD is functionally paralleled by reduced expression of specific activation markers of M2 macrophages like RELMa, CD301 and CD206 (Fig.12F).

Example 4 – Discussion and future applications

[0010] Altered cardiolipin content and species were observed in patients affected by the genetic inherited disease Barth's Syndrome and the autoimmune disease Multiple Sclerosis. Multiple Sclerosis, Type 1 diabetes and Systemic Lupus Erythematosus are autoimmune disease whose incidence is 5%-10% of the total world population and it is on the surge. In autoimmune diseases, when memory cells are formed against the "self", they help mount a highly efficient pathogenic response against the body's own tissues. Thus inhibiting T memory cell function is a credible therapeutic strategy in these pathological conditions.

[0011] Alternatively activated macrophages have a pivotal role in cancer and fibrosis. In particular, in cancer M2-like macrophages establish an immunosuppressive environment, which contributes to the immune-evasion of cancer cells. In regards to fibrosis, M2 cells contributes to fibrosis in lung and liver causing the progressive loss of function of these organs. In these settings, M2 cells could be a potential target to inhibit in order to reduce the severity of the pathology.

CLAIMS

1. A method for determining whether a compound has the capability of inhibiting cardiolipin synthesis, comprising
 - (a) contacting a sample comprising T cells and/or M2 macrophages with the compound;
 - (b) culturing the sample in the presence of the compound for at least 48h, preferably at least 60h and most preferably at least 72h; and
 - (c) assaying in the sample the mitochondrial function of the T cells and/or M2 macrophages,wherein a reduction of the mitochondrial function as compared to a control sample not contacted with the compound indicates that the compound has the capability of inhibiting cardiolipin synthesis.
2. The method of claim 1, wherein in the sample the mitochondrial function is assayed by the quantification of mitochondrial respiratory complexes and supercomplexes, determination of the ultrastructure of mitochondrial cristae, determination of the mitochondrial morphology, massspectrometric analysis of cardiolipin species, quantifying oxidative phosphorylation and/or quantification of mitochondrial mass.
3. The method of claim 2, wherein quantifying oxidative phosphorylation comprises quantifying the oxygen consumption rate.
4. The method of any one of claims 1 to 3, wherein the compound is an antisense molecule, siRNA, shRNA, antibody, ribozyme, aptamer, protein drug or small molecule.
5. The method of any one of claims 1 to 4, wherein the compound is directed against the protein tyrosine phosphatase localized to the mitochondrion 1 (PTPMT1) or cardiolipin synthase 1 (CLS1).
6. The method of any one of claims 1 to 5, wherein the efficacy of the compound for inhibiting cardiolipin synthesis in a patient is determined, wherein the sample of step (a) and the control sample are samples that have been obtained from the patient.
7. The method of claim 6, wherein the patient is afflicted with an autoimmune disorder, a hyperproliferative disorder or a fibrotic disorder.

8. The method of claim 6 or 7, wherein the sample is a tissue sample or a blood sample.
9. The method of any one of claims 6 to 8, wherein the compound is alexidine dihydrochloride (AD).
10. A compound inhibiting cardiolipin synthesis for use in the treatment or prevention of a disease being mediated by cardiolipin synthesis in T cells and/or M2 macrophages, wherein the disease is preferably an autoimmune disorder, a hyperproliferative disorder or a fibrotic disorder.
11. A compound inhibiting cardiolipin synthesis for use in the treatment or prevention of an autoimmune disorder, a hyperproliferative disorder or a fibrotic disorder in a patient by inhibiting cardiolipin synthesis in T cells and/or M2 macrophages of the patient.
12. The compound for use of claim 10 or 11, wherein the compound targets protein tyrosine phosphatase localized to the mitochondrion 1 (PTPMT1) or cardiolipin synthase 1 (CLS1).
13. The compound for use of any one of claims 10 to 12, wherein the compound is an antisense molecule, siRNA, shRNA, antibody, ribozyme, aptamer, protein drug or small molecule.
14. The compound for use of claim 13, wherein the compound is alexidine dihydrochloride (AD).
15. The compound for use of any one of claims 10 to 14 or the method of any one of claims 7 to 9, wherein
 - (i) the autoimmune disorder is multiple sclerosis, type 1 diabetes, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, Behçet's disease; antiphospholipid antibody syndrome (APLS), or Sjögren's syndrome (SjS, SS);
 - (ii) the hyperproliferative disorder is a neoplasm, tumor or cancer and is preferably selected from cancer of the breast, lung, prostate, kidney, skin, neural, ovary, uterus, liver, pancreas, epithelial, gastric, intestinal, exocrine, endocrine, lymphatic, hematopoietic system or a head and neck tissue; and/or
 - (iii) the fibrotic disorder is selected from sarcoidosis, renal fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, liver fibrosis, cardiac

fibrosis, endomyocardial fibrosis, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, chronic kidney disease, nephrogenic systemic fibrosis, Crohn's disease, hypertrophic scarring, keloid, scleroderma, organ transplant-associated fibrosis and ischemia-associated fibrosis.

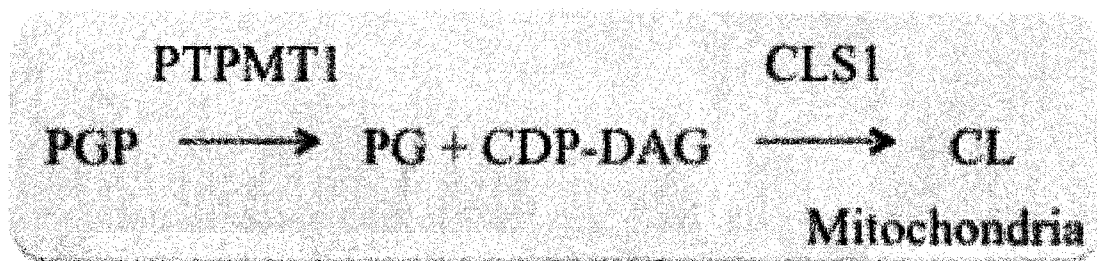


FIG. 1

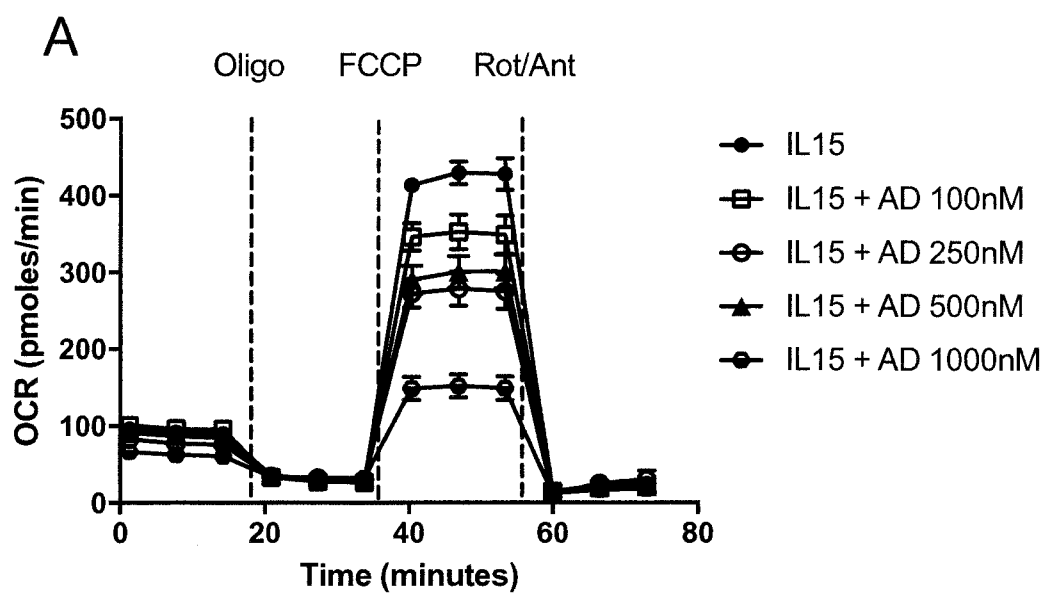


FIG. 2A

2/52

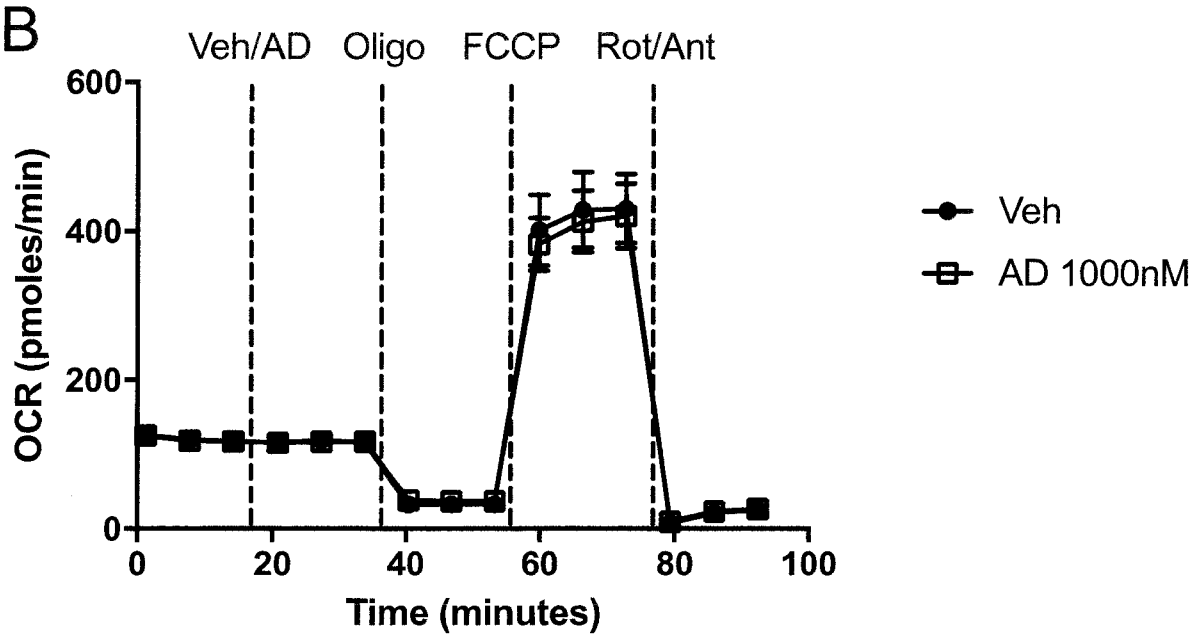


FIG. 2B

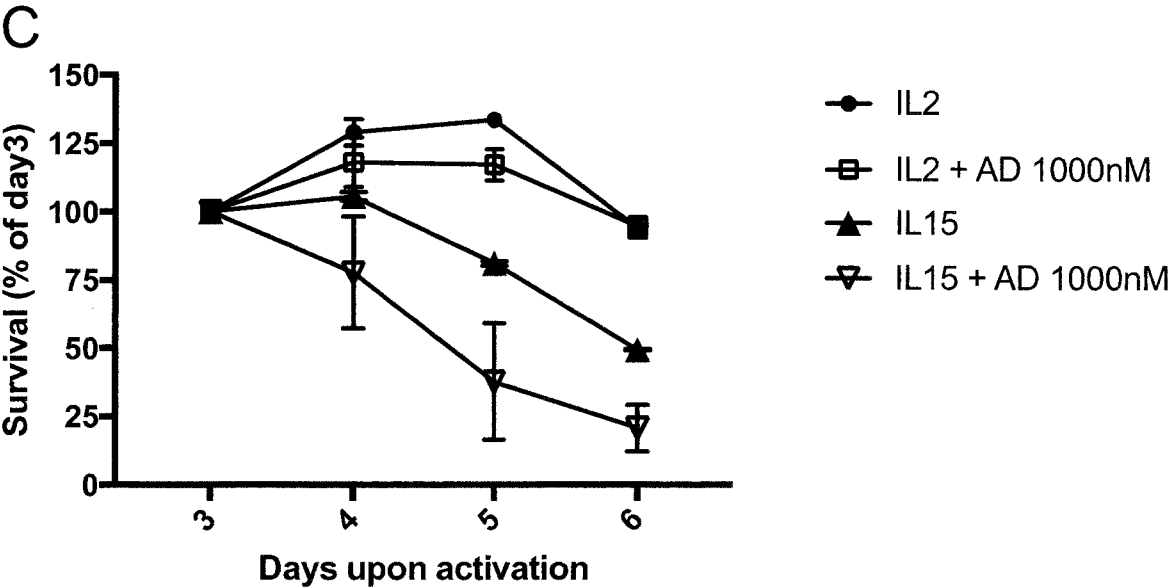


FIG. 2C

3/52

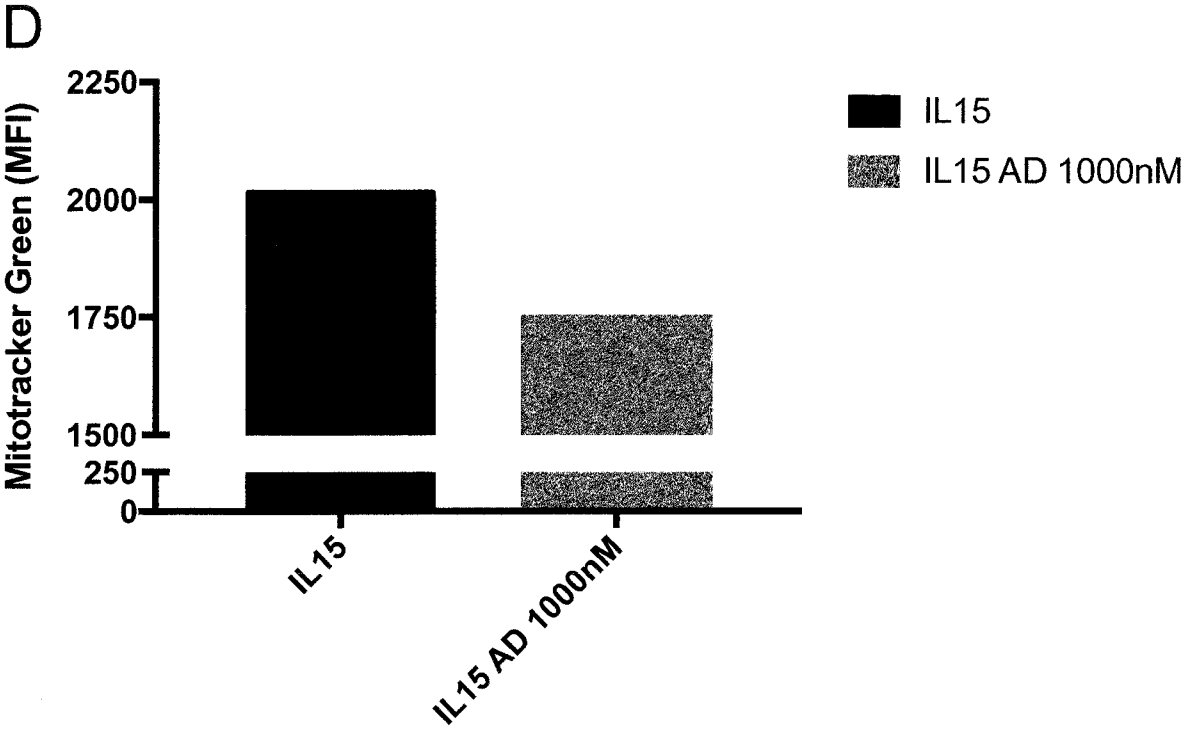


FIG. 2D

M2 OCR b/w

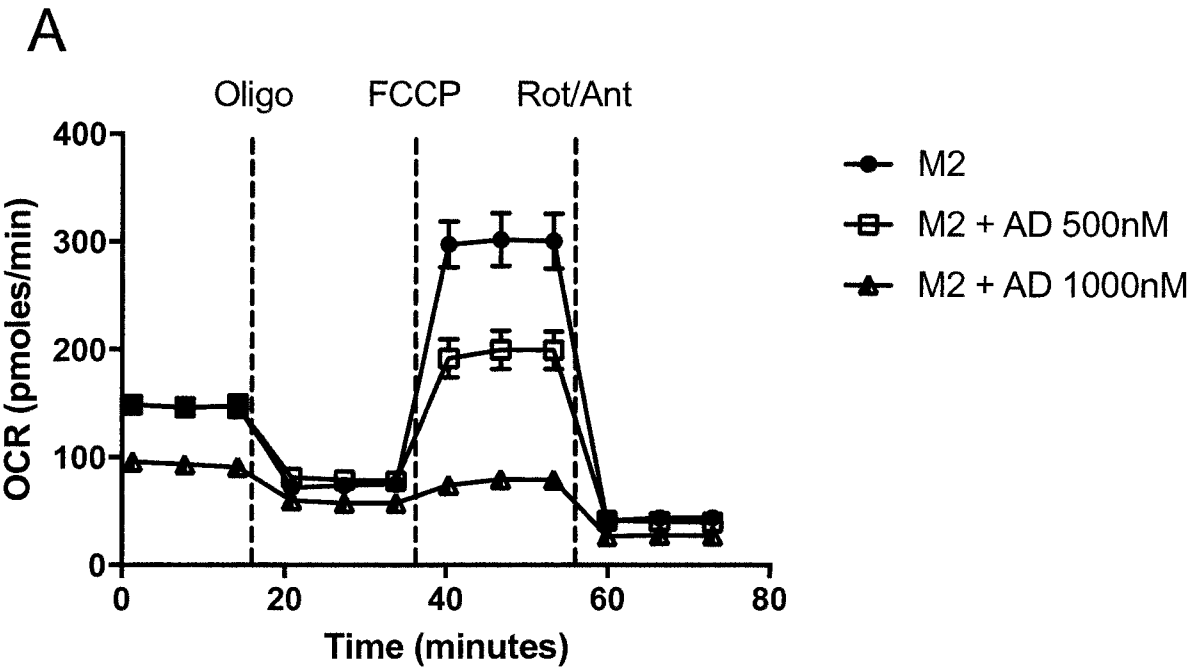


FIG. 3

5/52

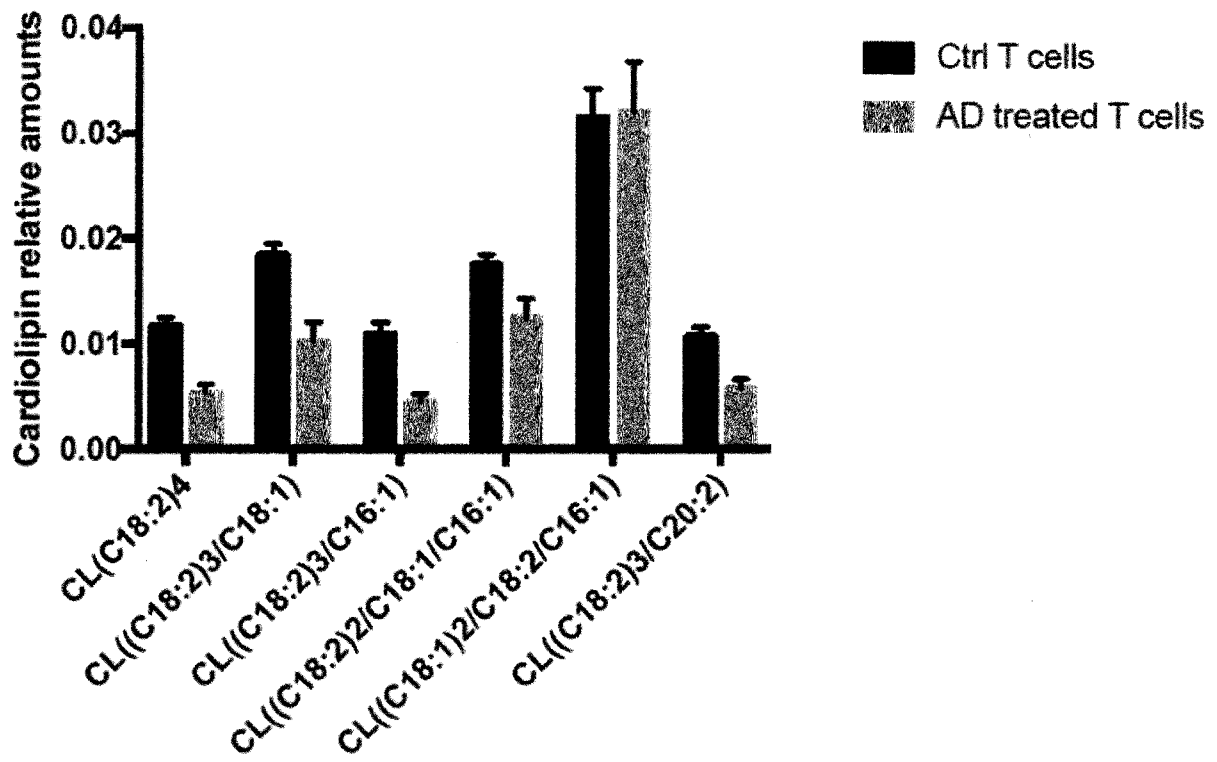


FIG. 4

A

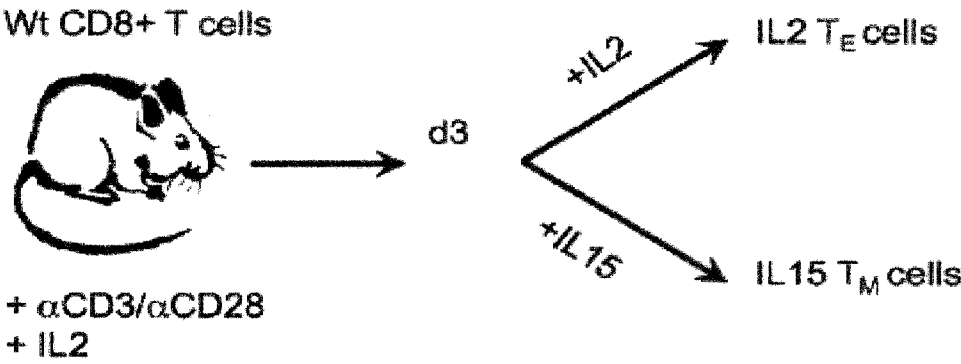


FIG. 5A

B

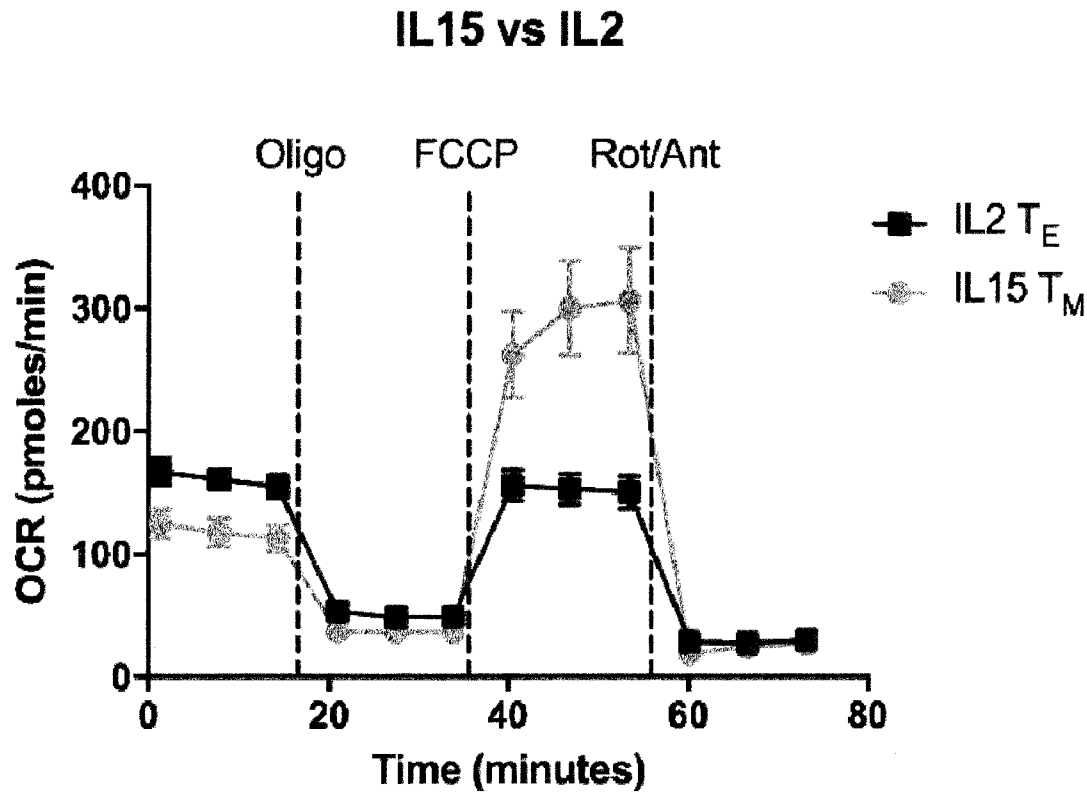


FIG. 5B

C

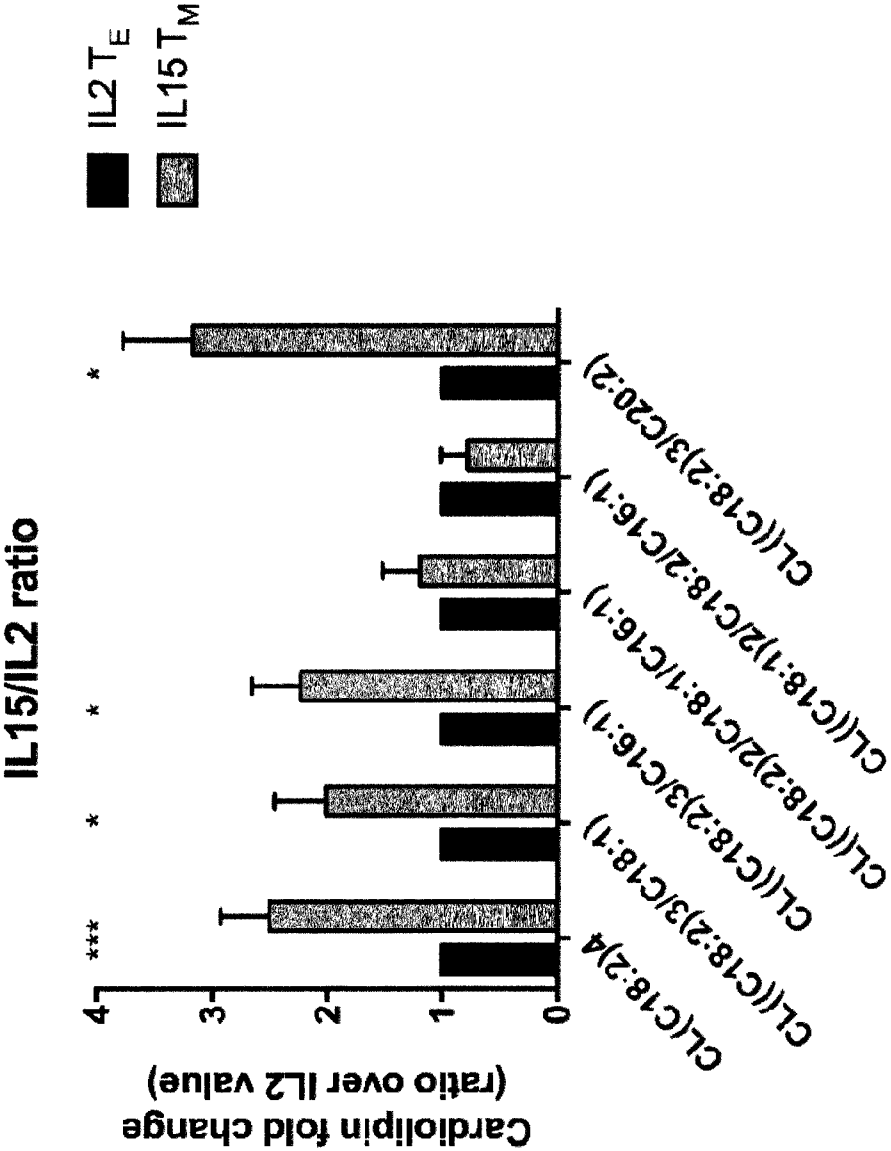


FIG. 5C

8/52

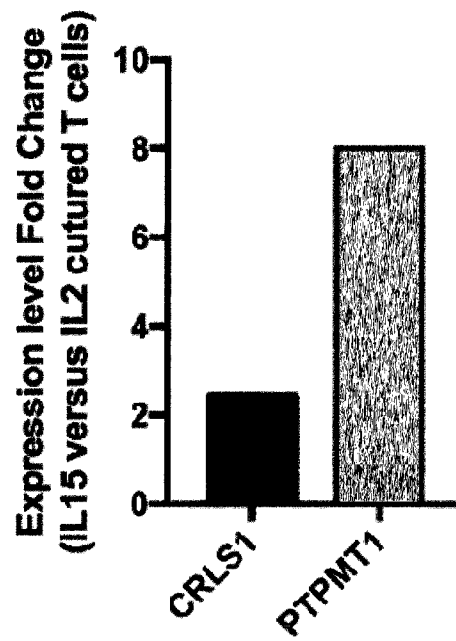
D

FIG. 5D

9/52

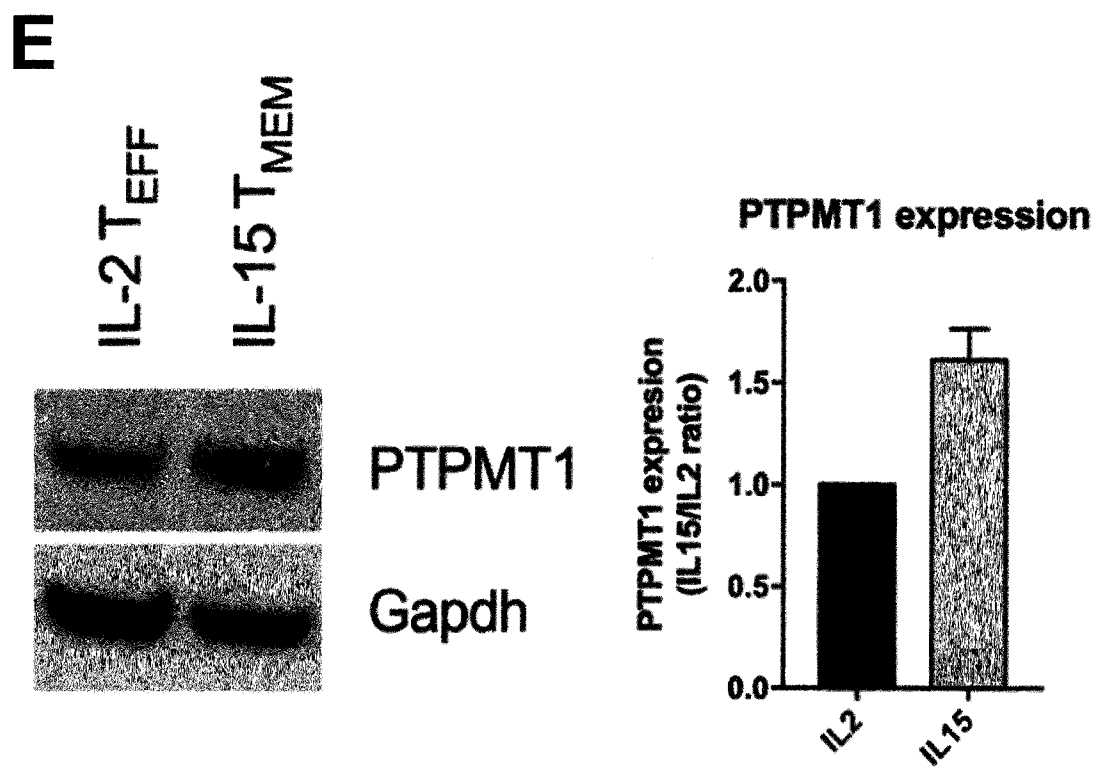


FIG. 5E

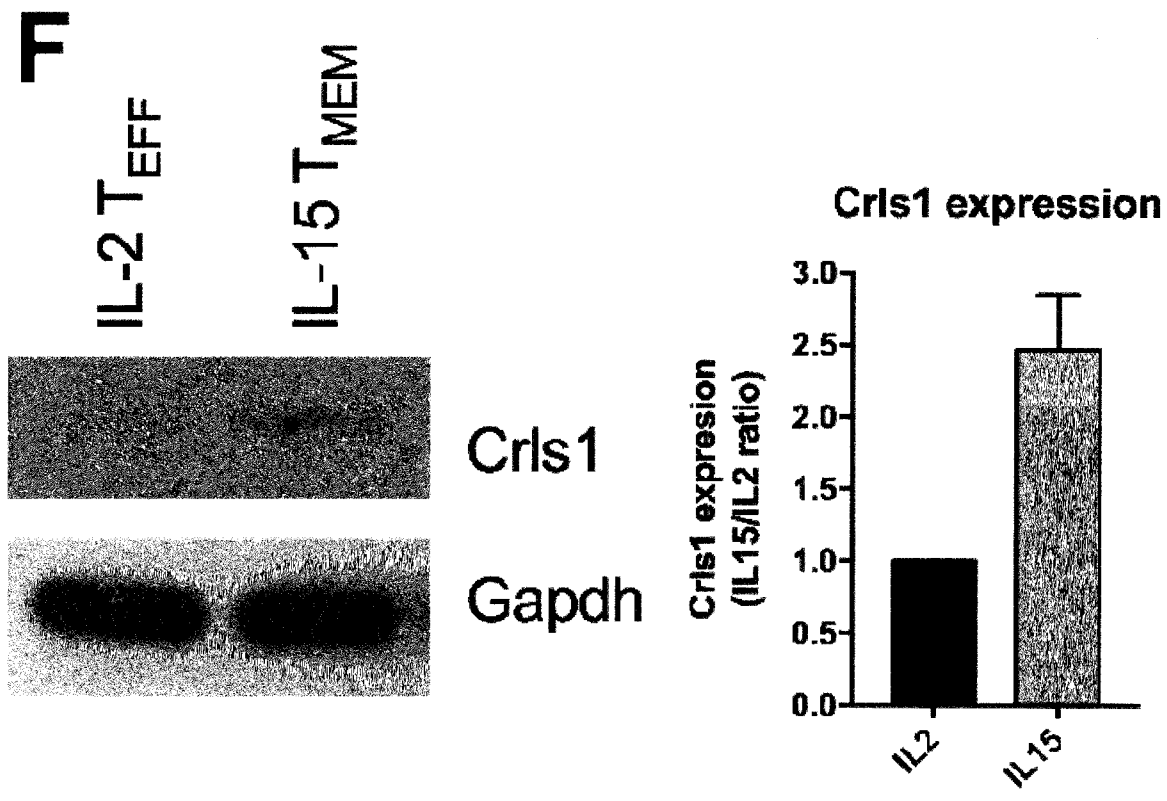
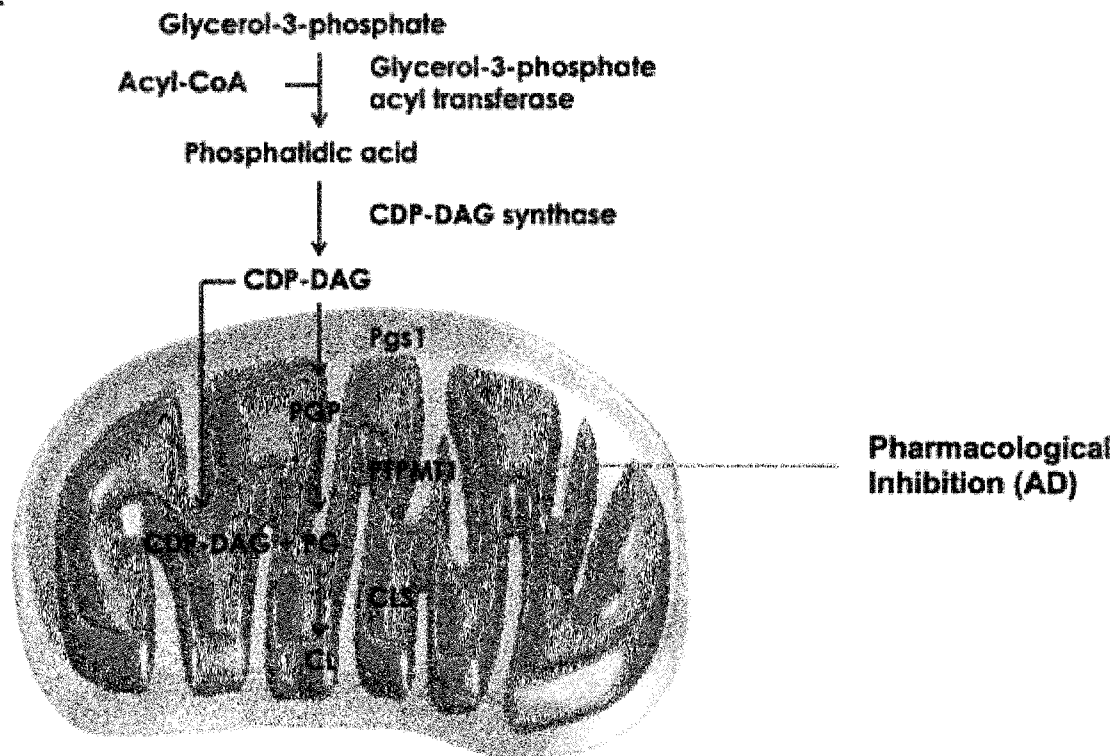
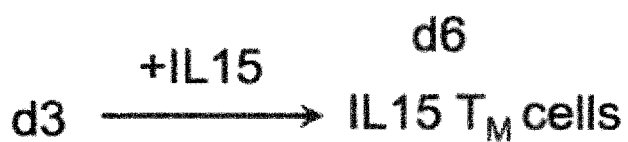


FIG. 5F

10/52

A.**FIG. 6A****B**Wt CD8⁺ T cells+ α CD3/ α CD28

+ IL2



AD treatment
Cardiolipin synthesis
inhibition

FIG. 6B

11/52

C

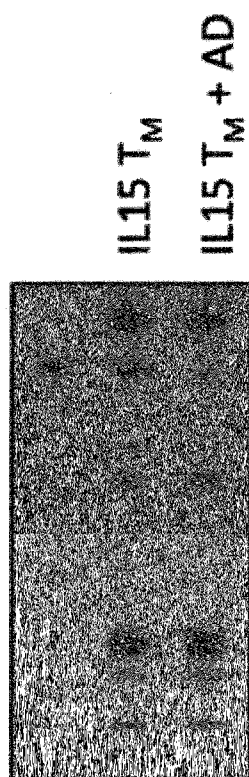


FIG. 6C

D

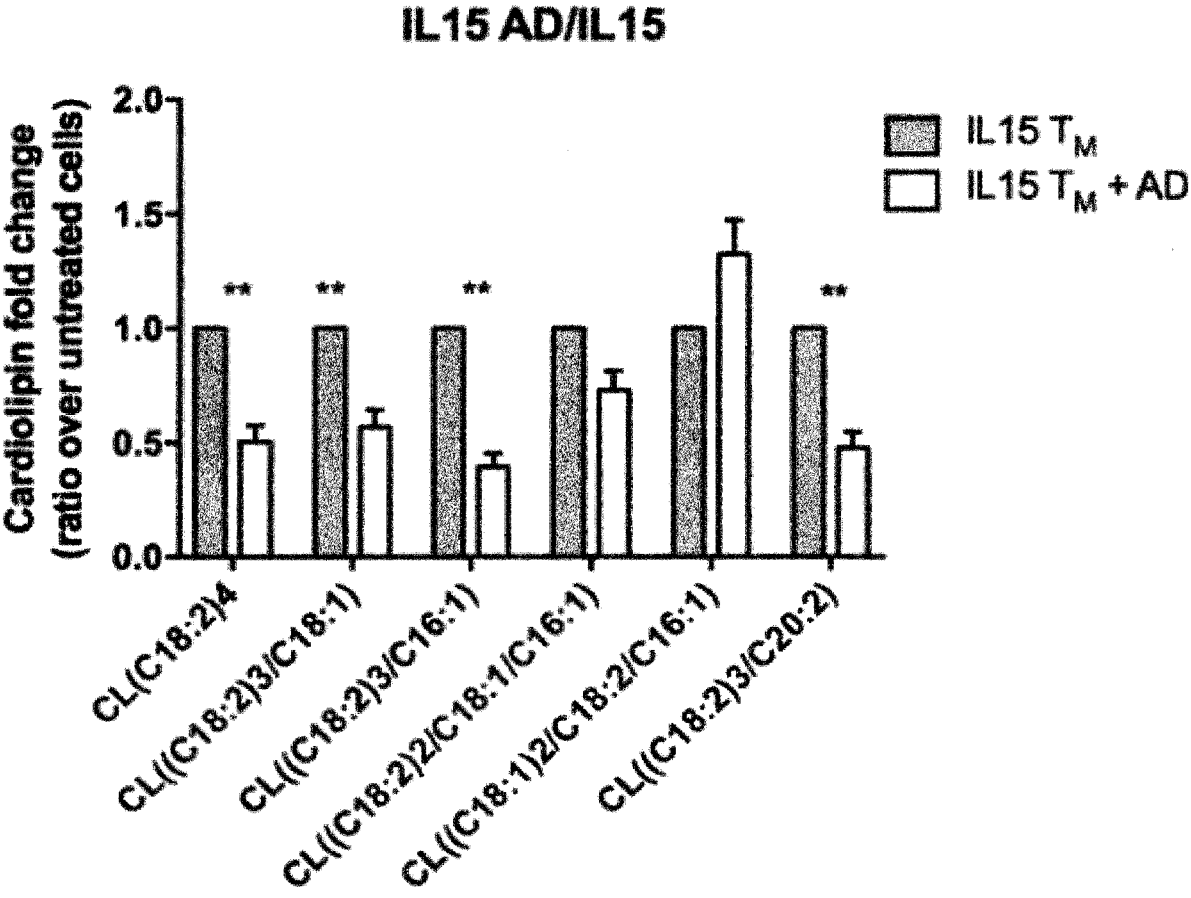
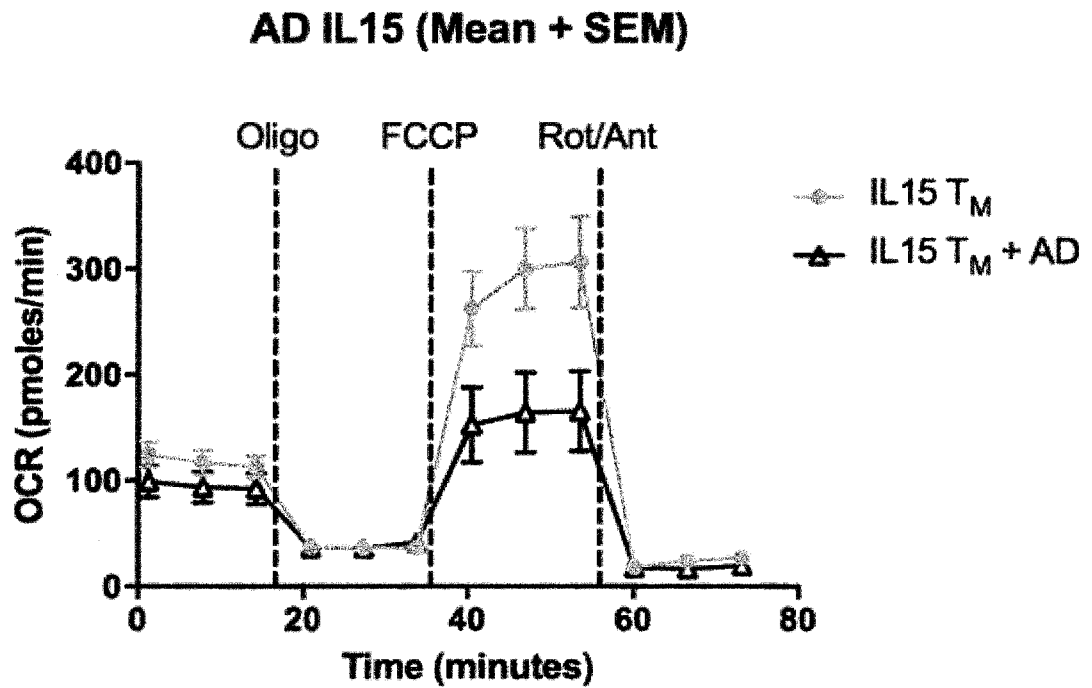
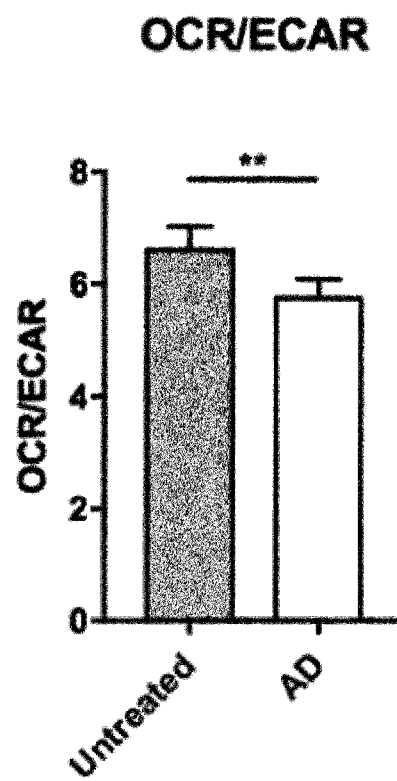


FIG. 6D

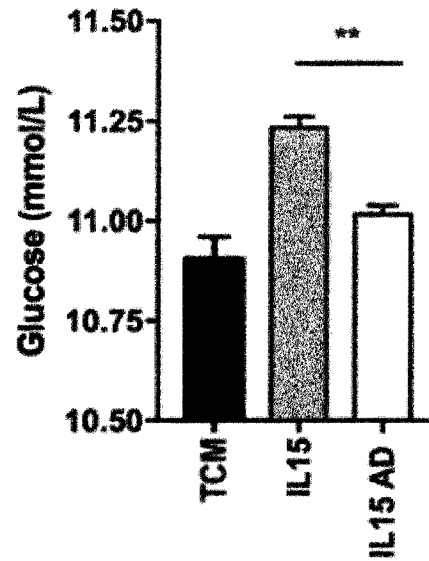
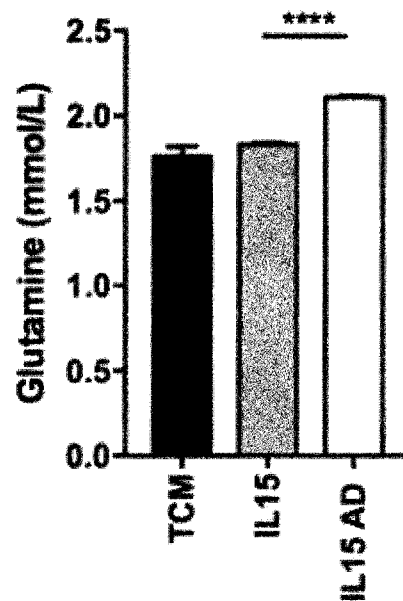
13/52

E**FIG. 6E**

14/52

F**FIG. 6F**

15/52

G**CD8 Day6 IL15 Glucose in the media****FIG. 6G****H****CD8 Day6 IL15 Lactate in the media****FIG. 6H**

16/52

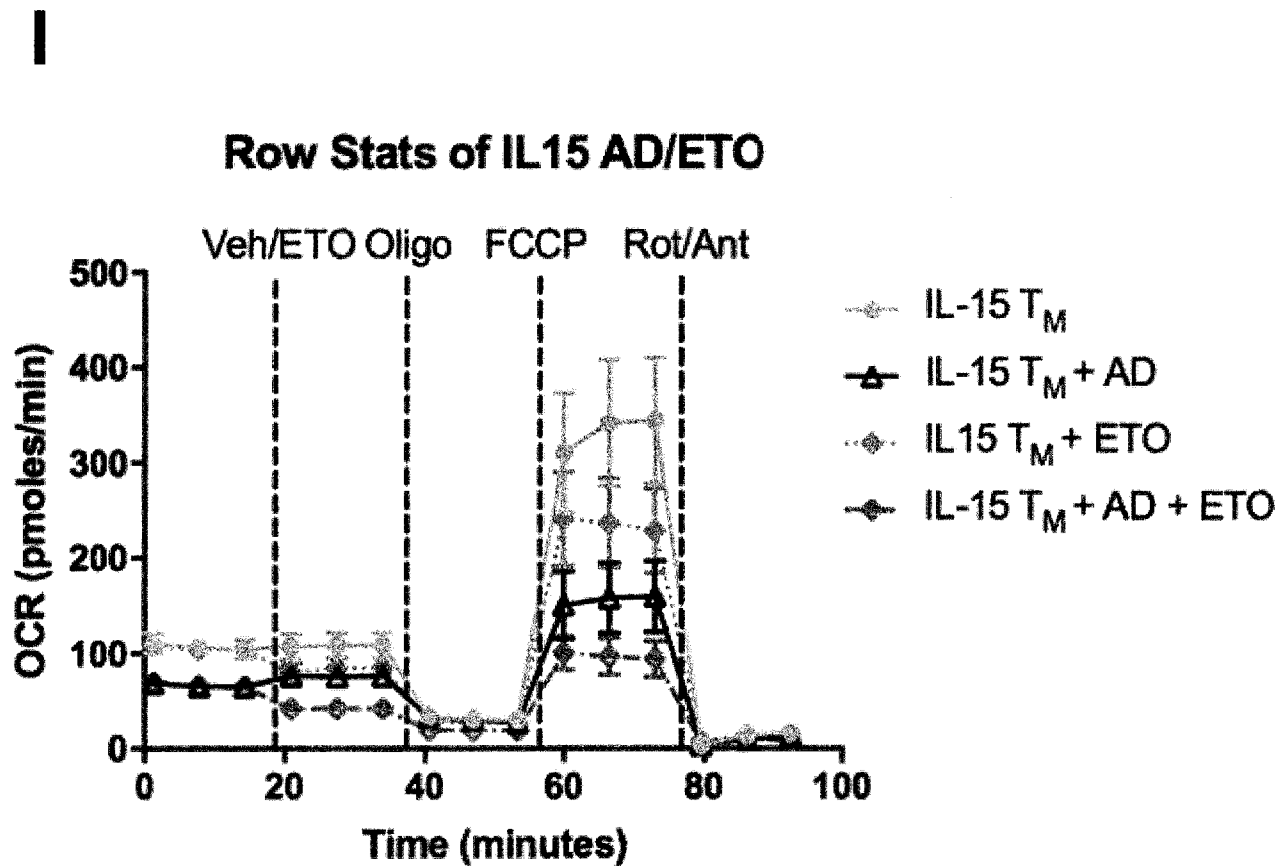


FIG. 6I

17/52

J

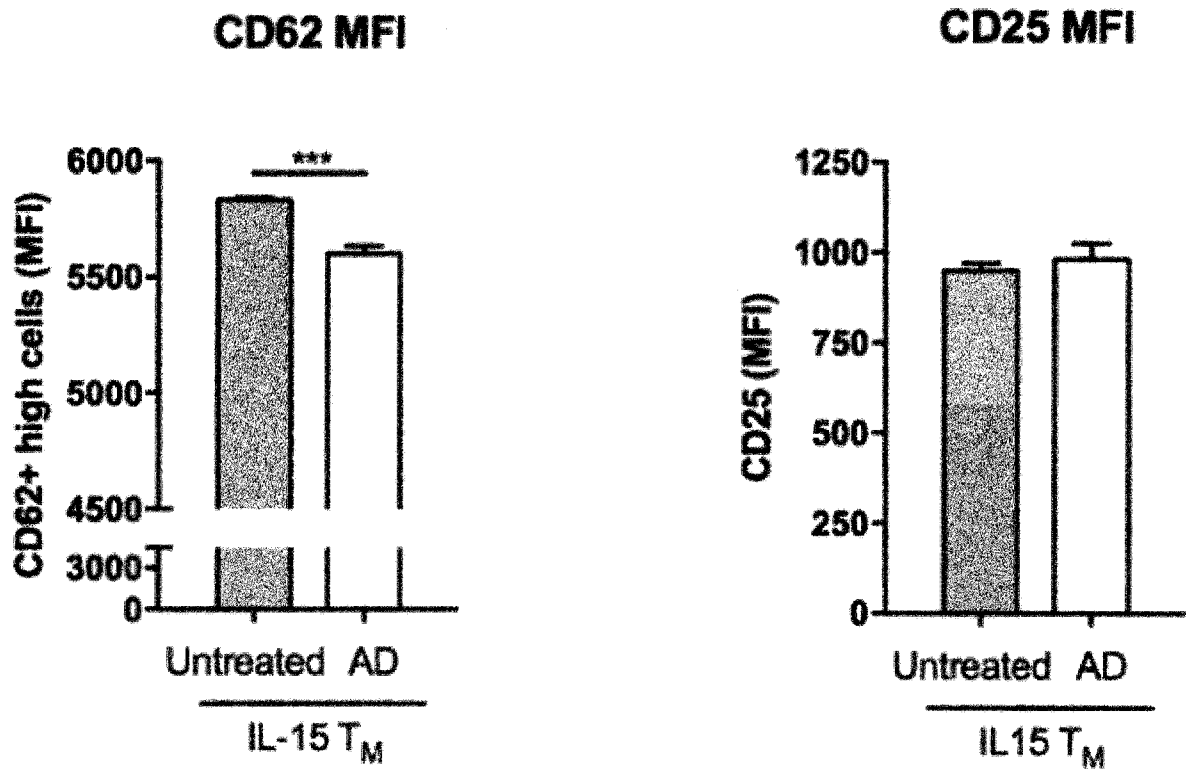
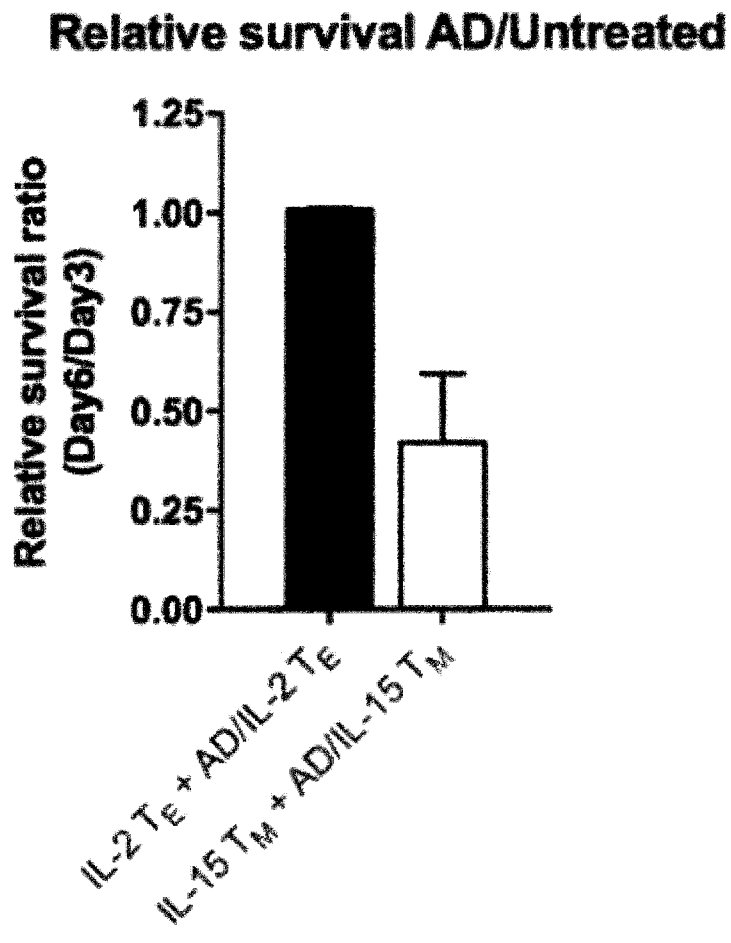


FIG. 6J

18/52

K**FIG. 6K**

19/52

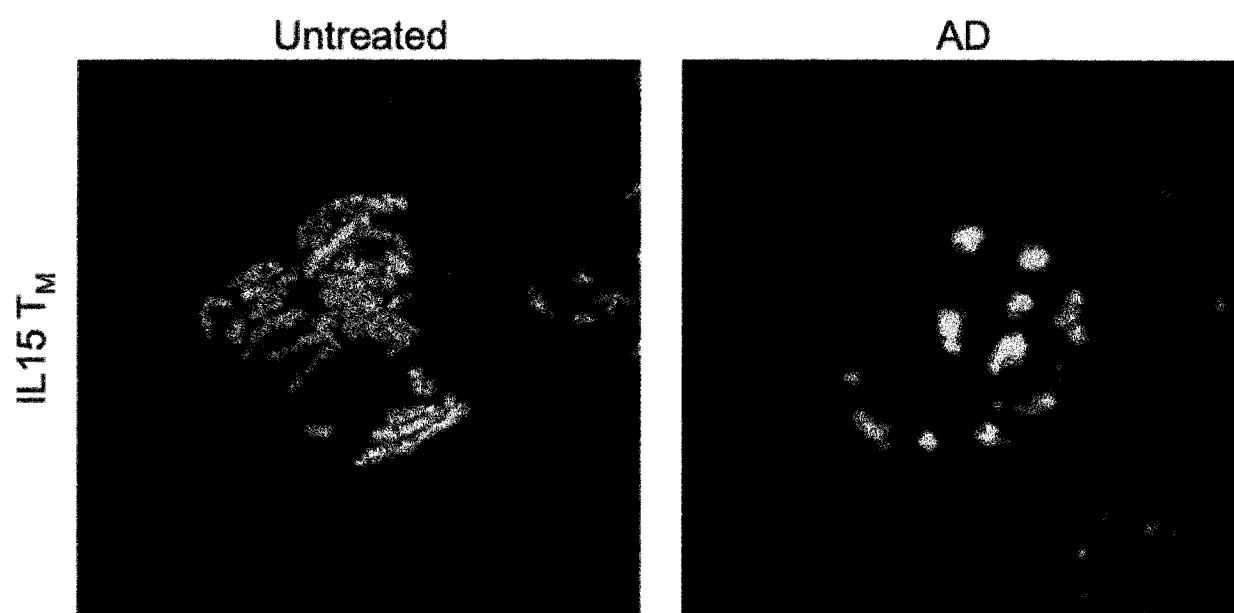
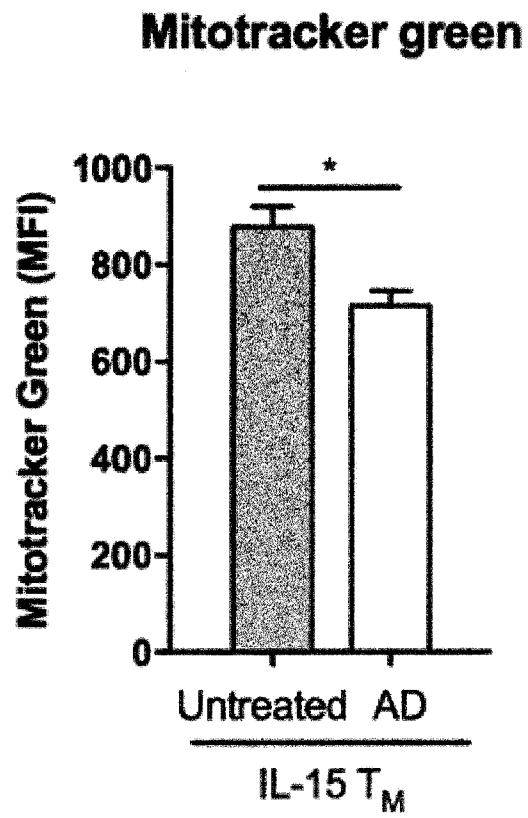
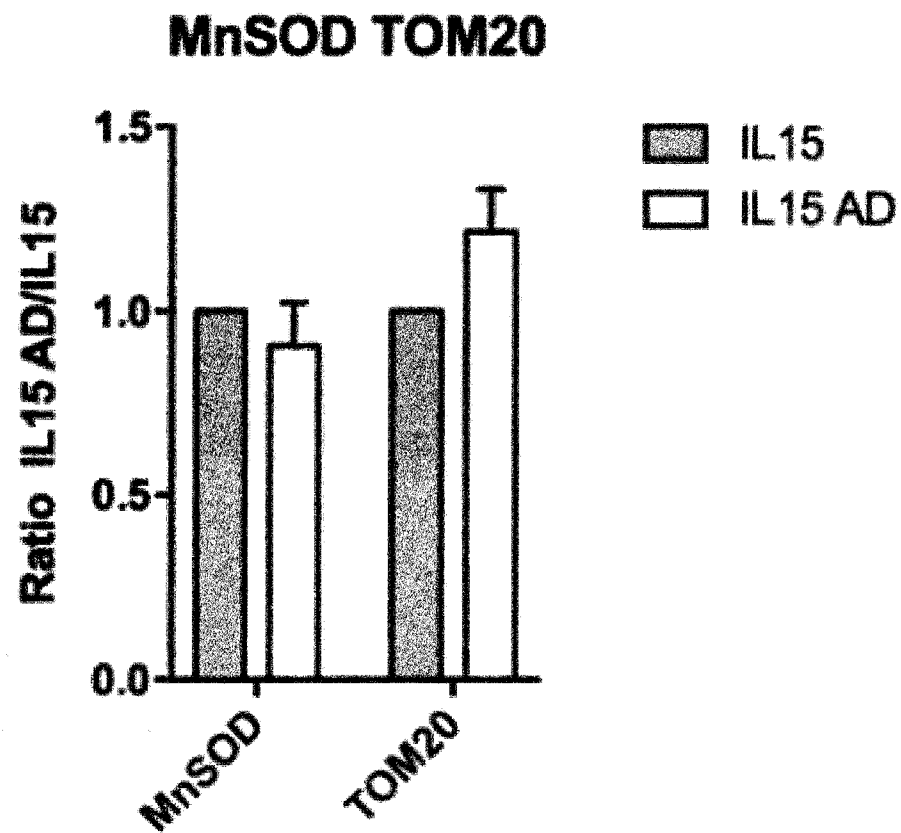
L

FIG. 6L

20/52

M**FIG. 6M**

21/52

N**FIG. 6N**

22/52

O

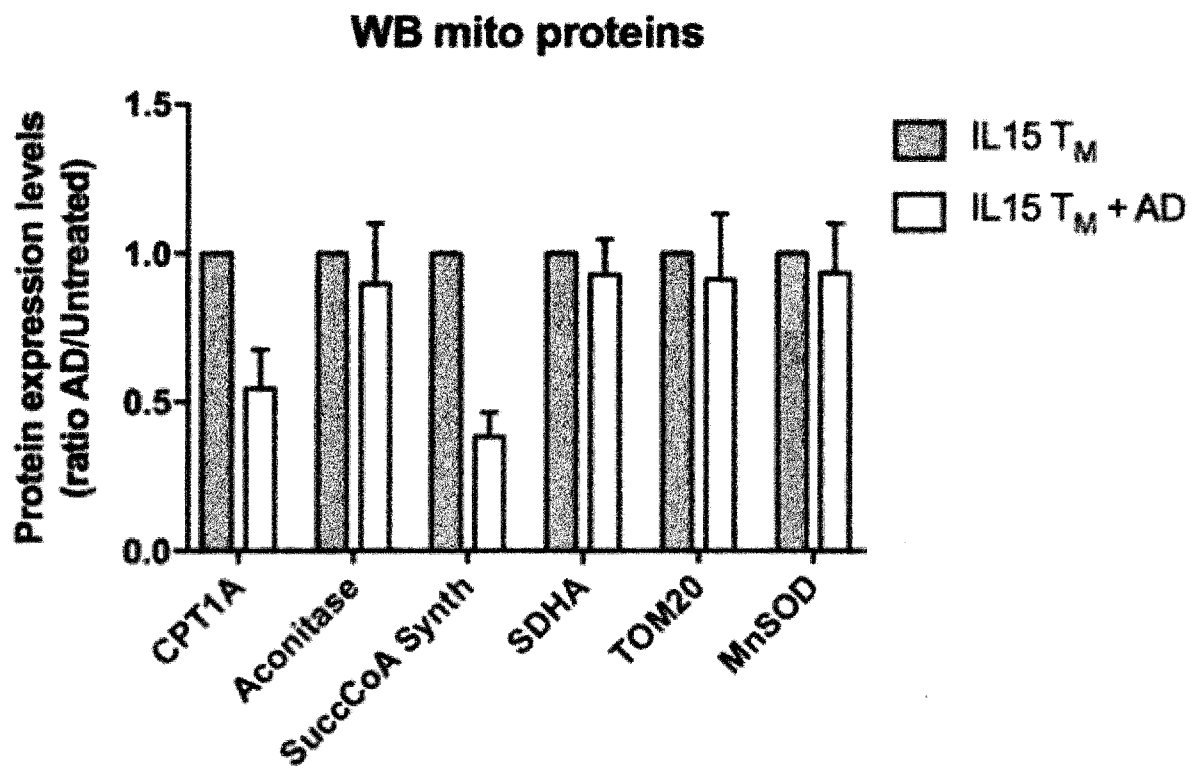


FIG. 6O

23/52

P

IL15 T_M

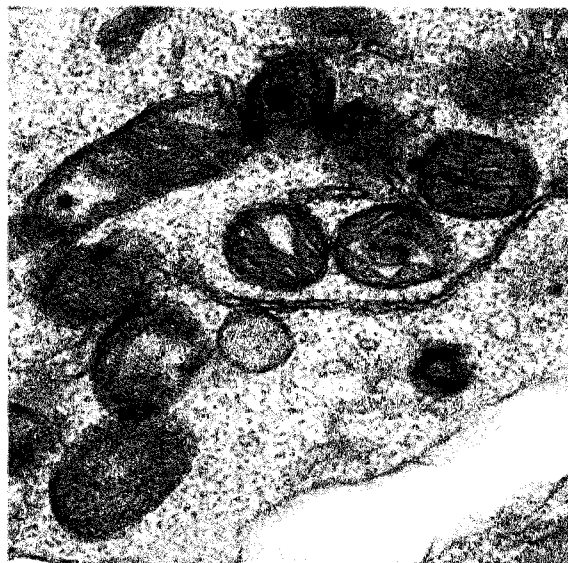
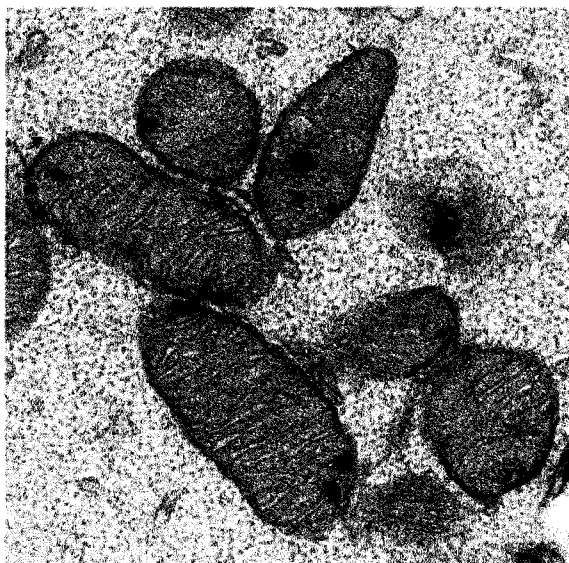


FIG. 6P

24/52

Q

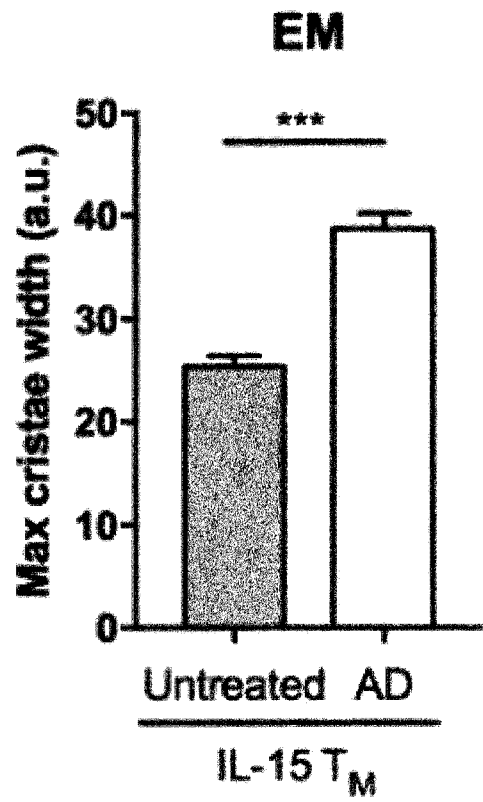


FIG. 6Q

R

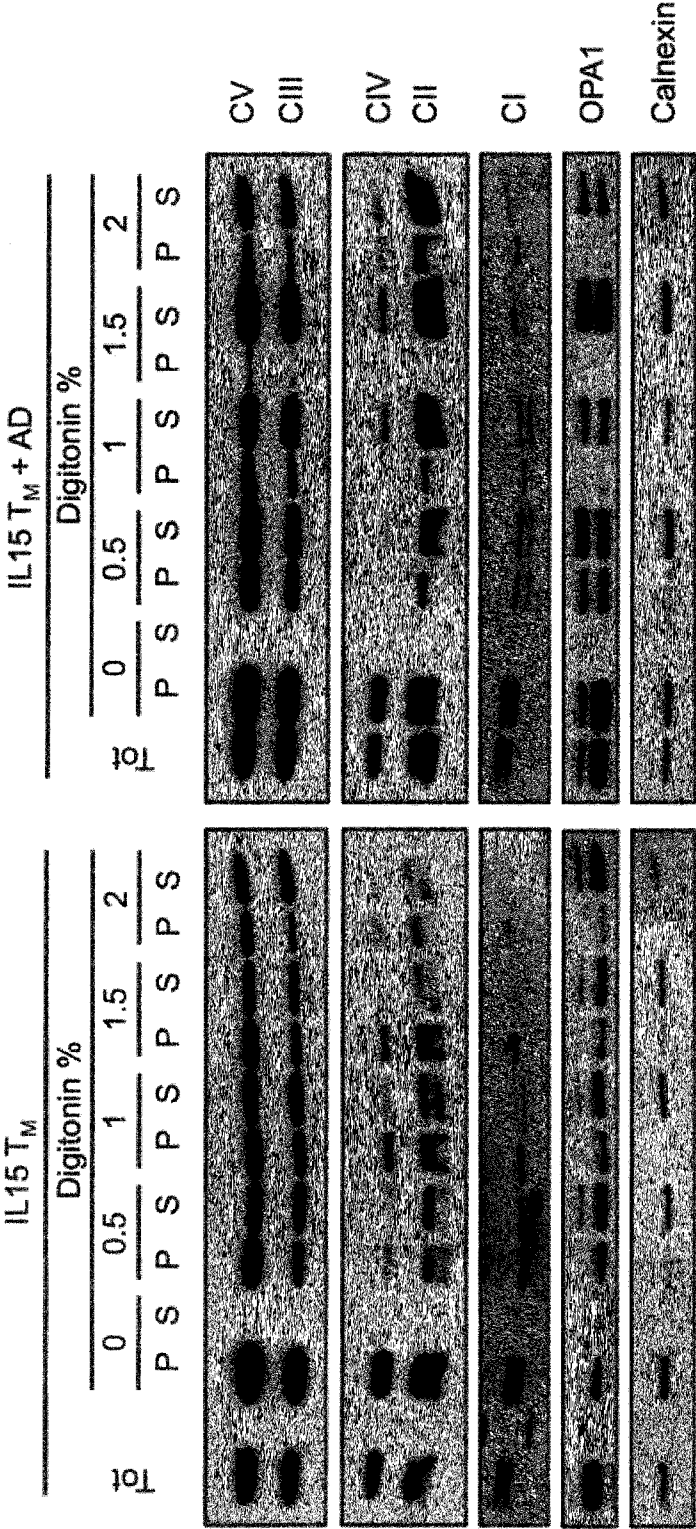


FIG. 6R

26/52

S

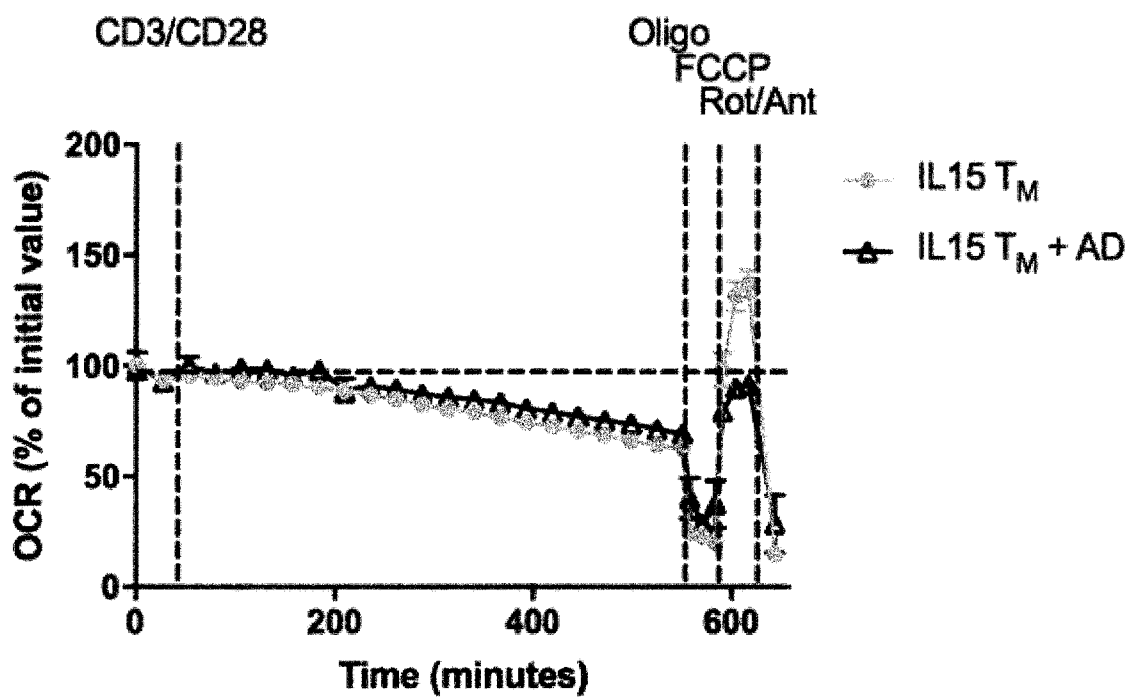
Copy of Baseline-corrected of OCR after react

FIG. 6S

27/52

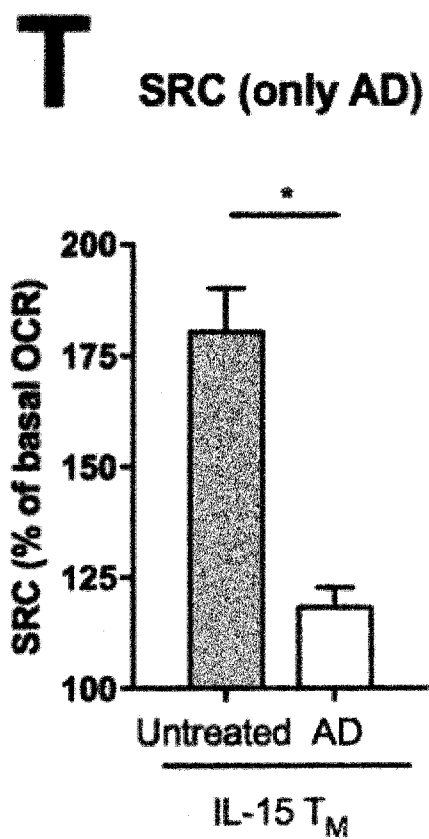


FIG. 6T

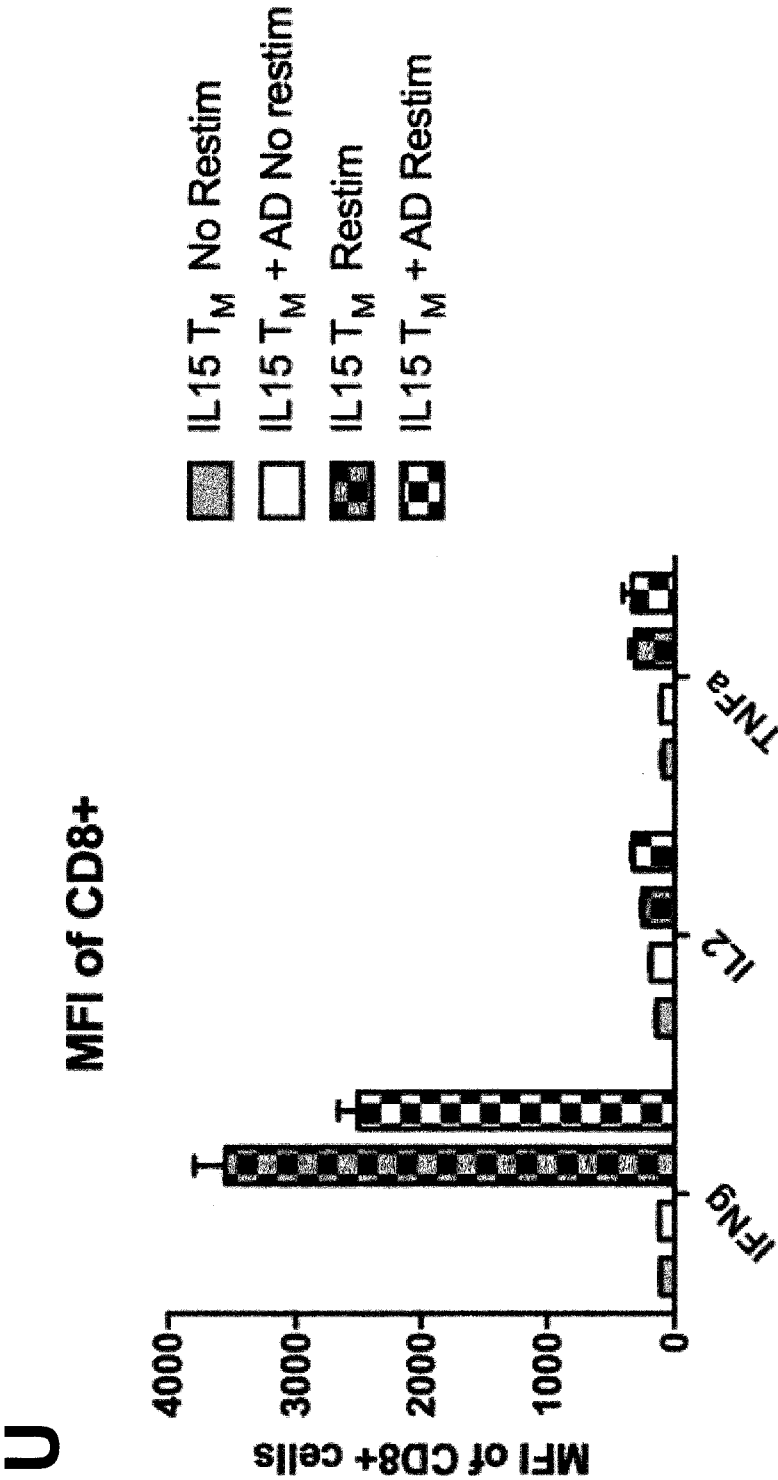


FIG. 6U

29/52

A

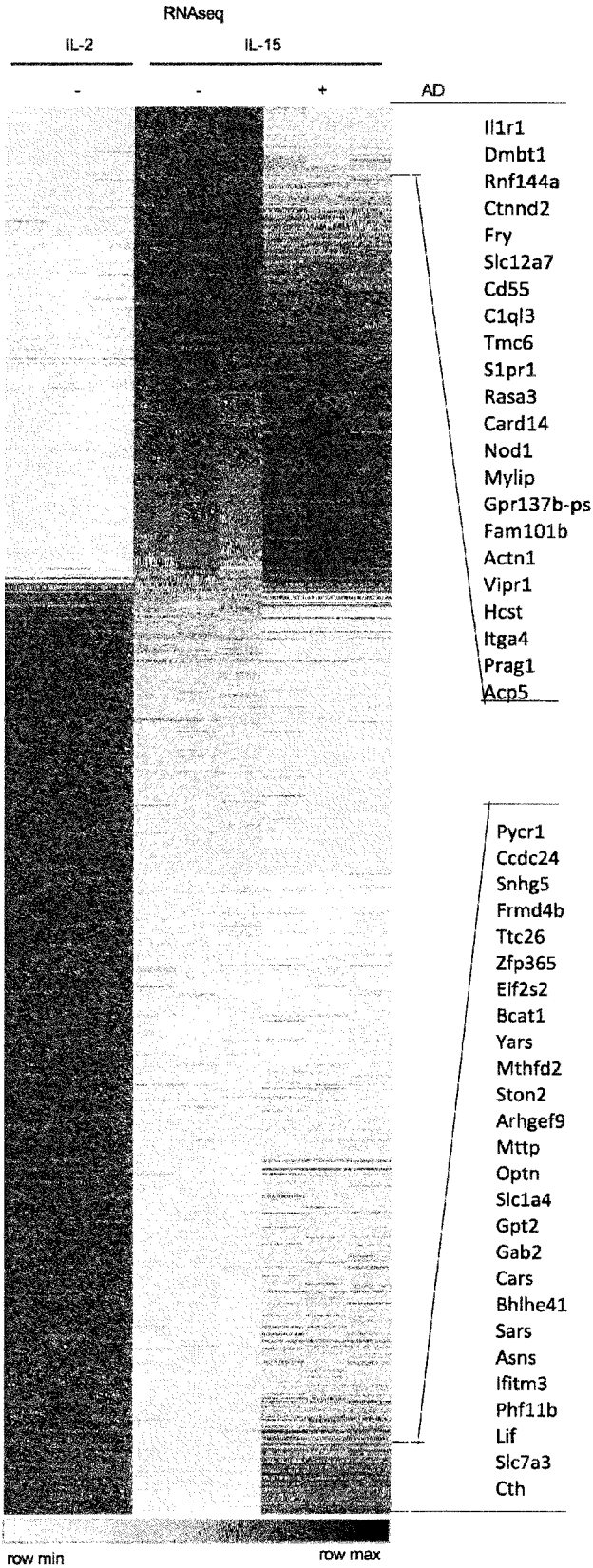


FIG. 7

30/52

AWt CD8⁺ T cells

+ α CD3/ α CD28
+ IL2

d3

+IL2

d6

IL2 T_E cells

AD treatment
Cardiolipin synthesis
inhibition

FIG. 8A

B

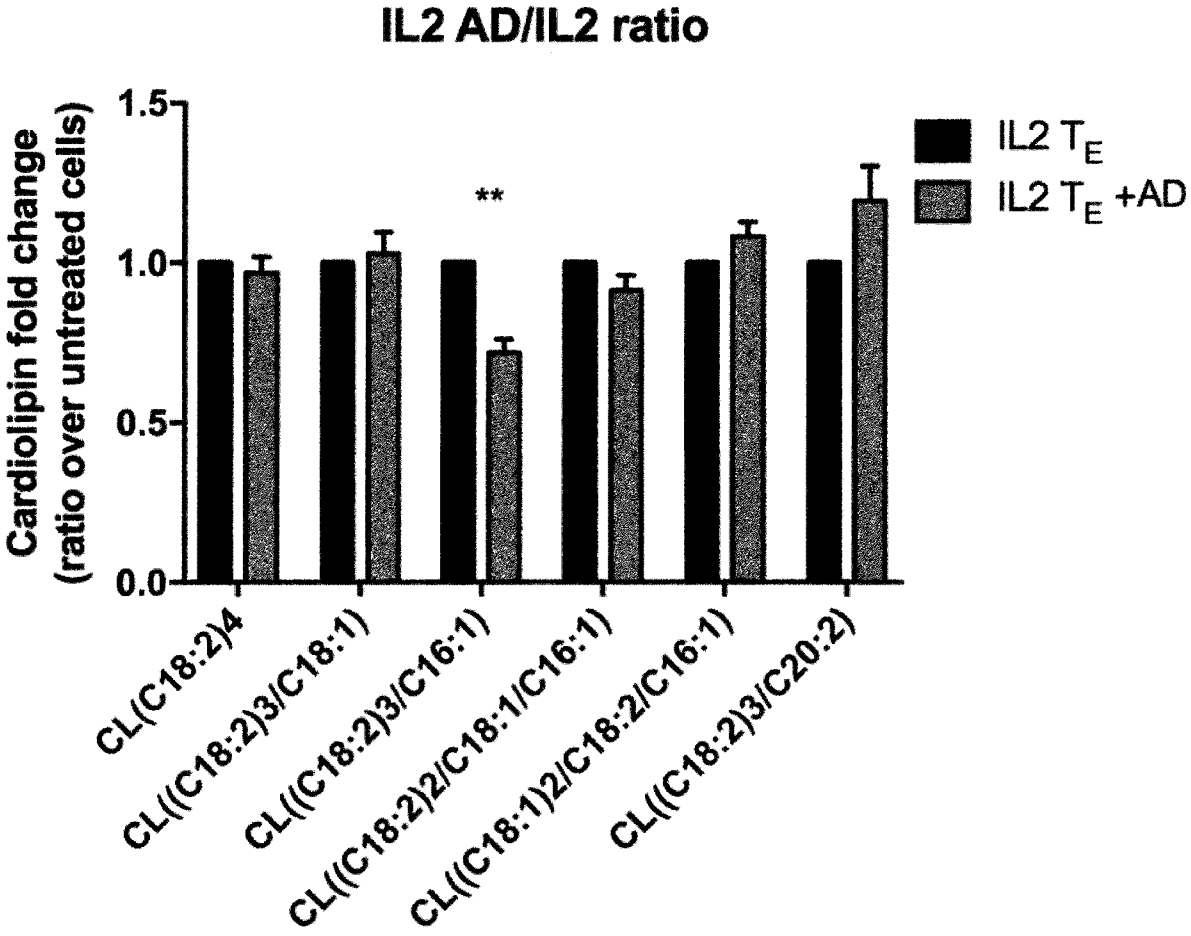


FIG. 8B

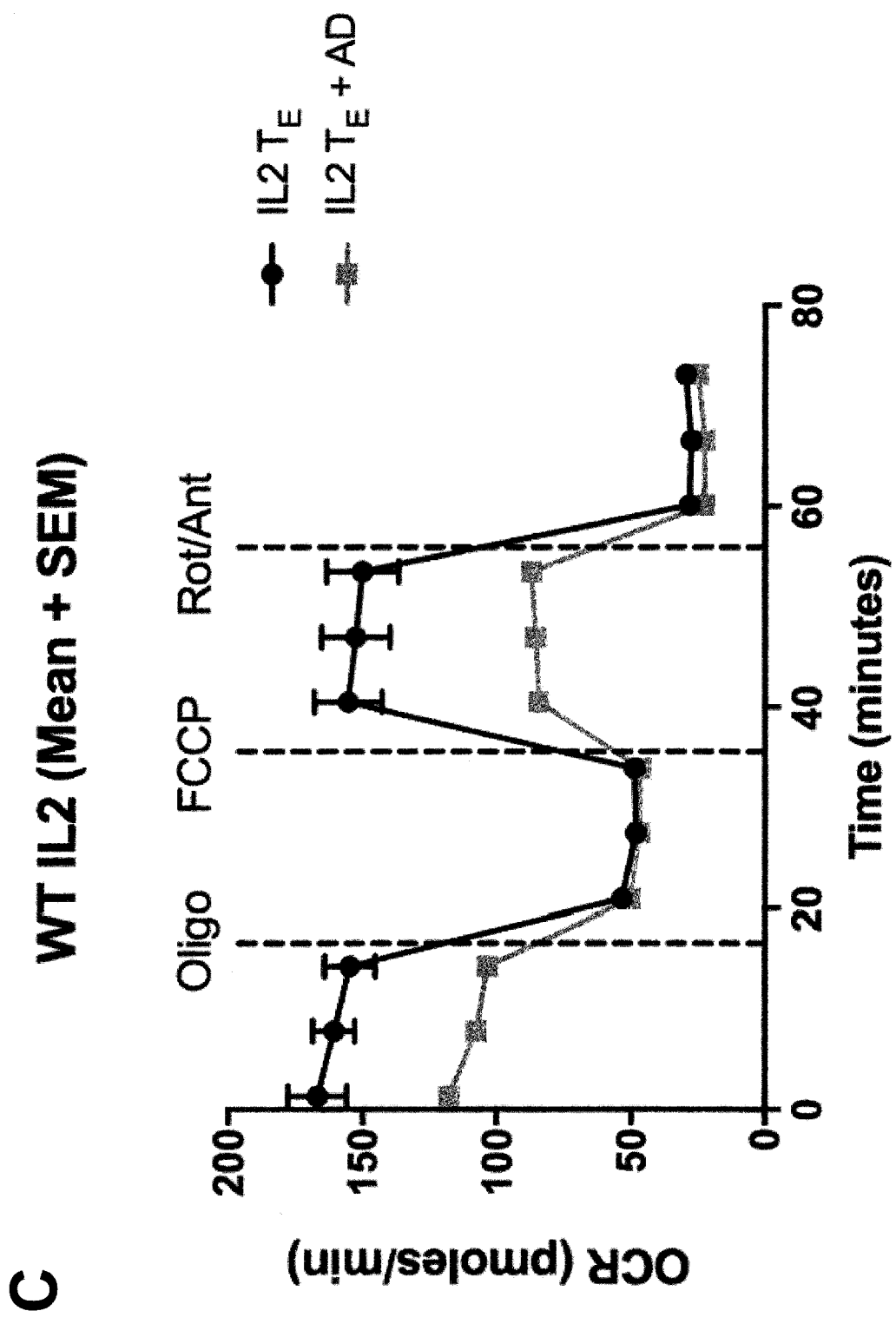
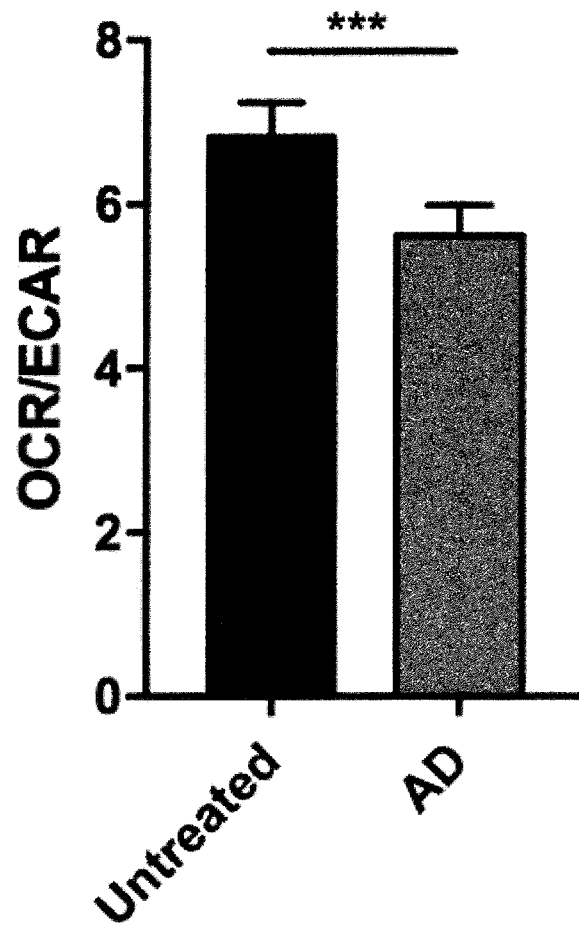


FIG. 8C

33/52

D**OCR/ECAR IL2****FIG. 8D**

E

Row Stats of IL2 AD/ETO

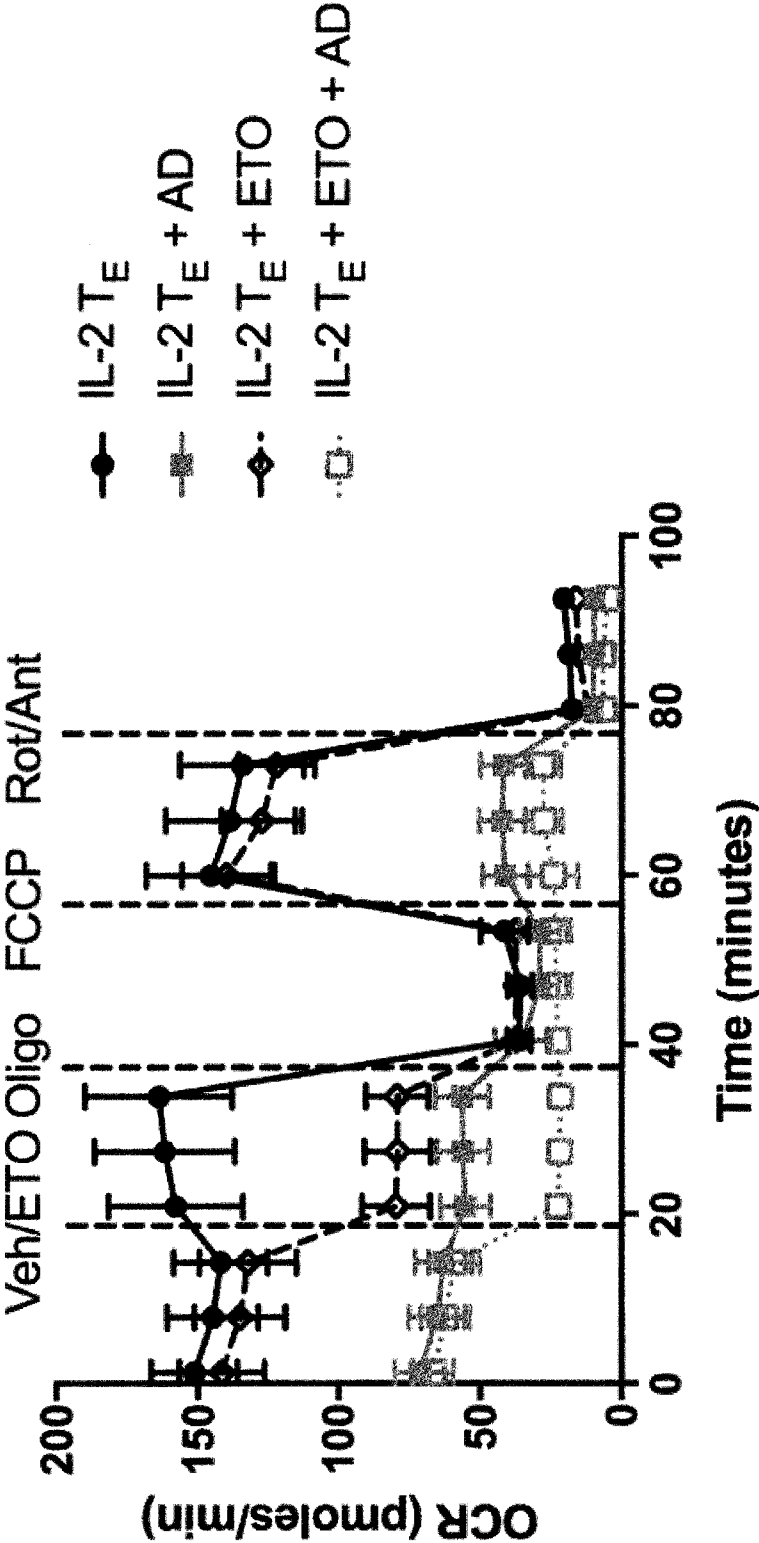
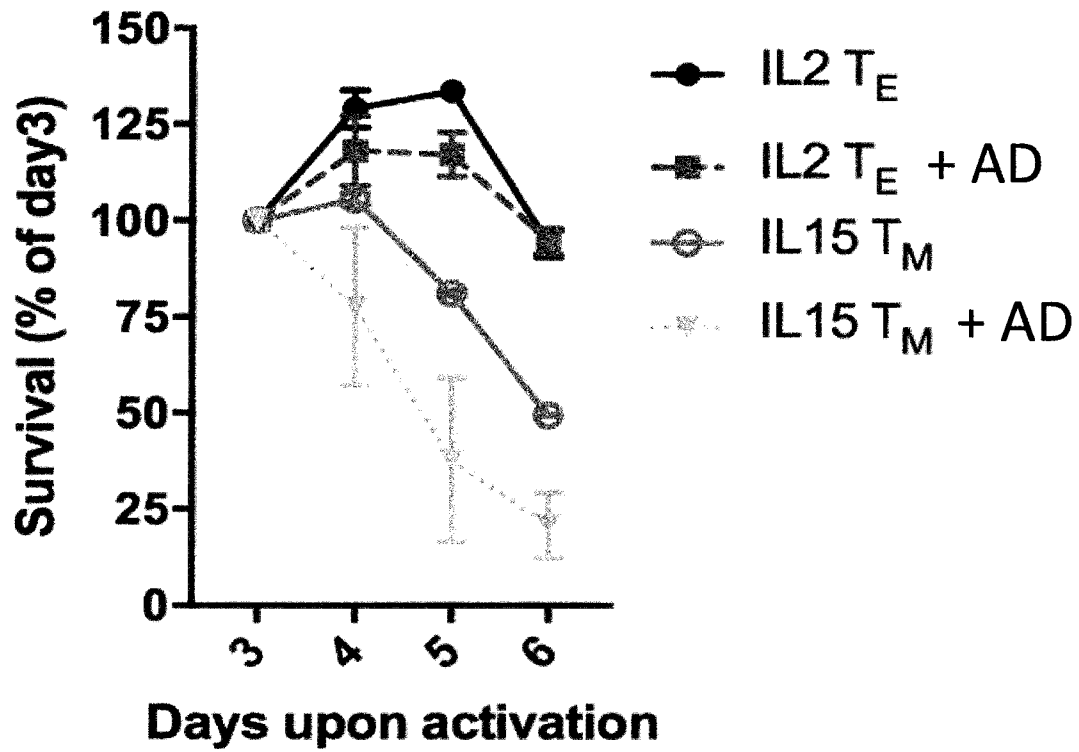


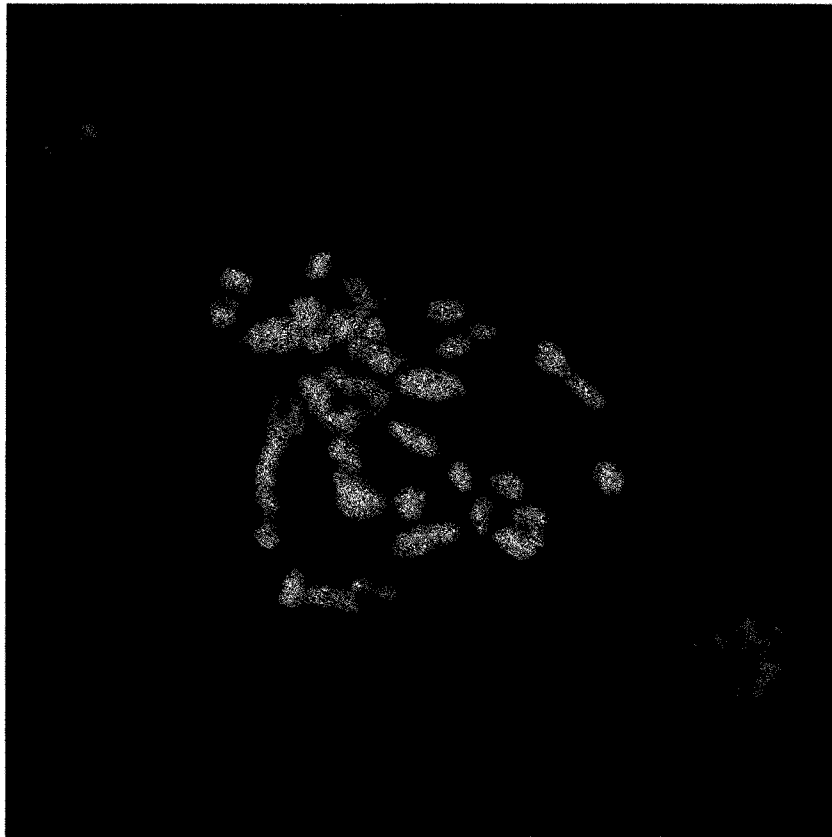
FIG. 8E

35/52

F**Survival upon AD treatment****FIG. 8F**

G

IL2



IL2 AD

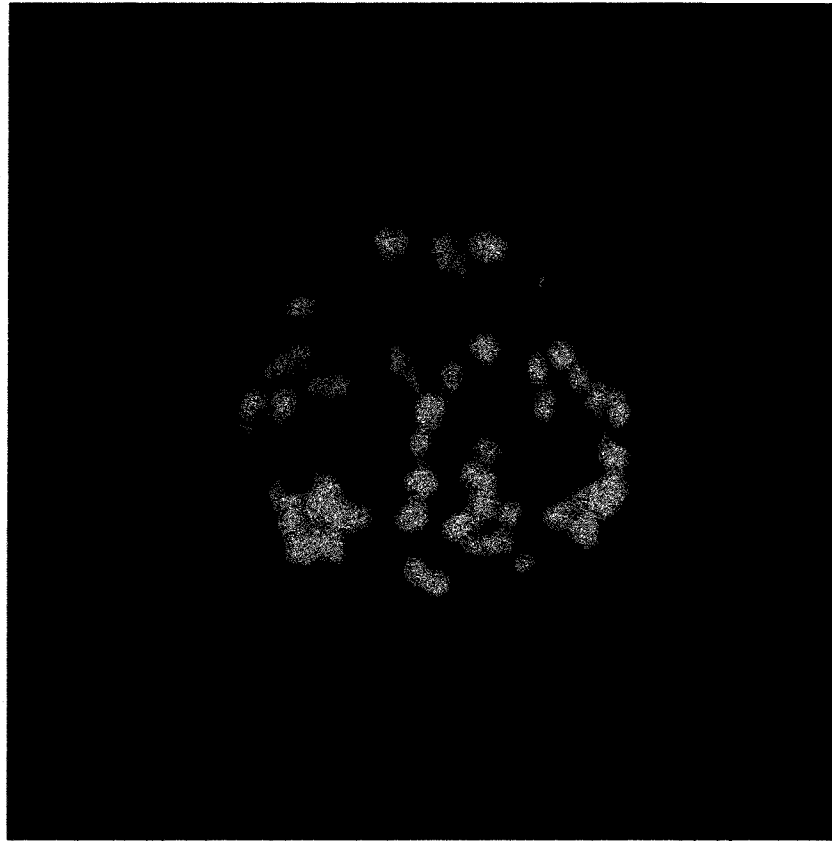


FIG. 8G

H

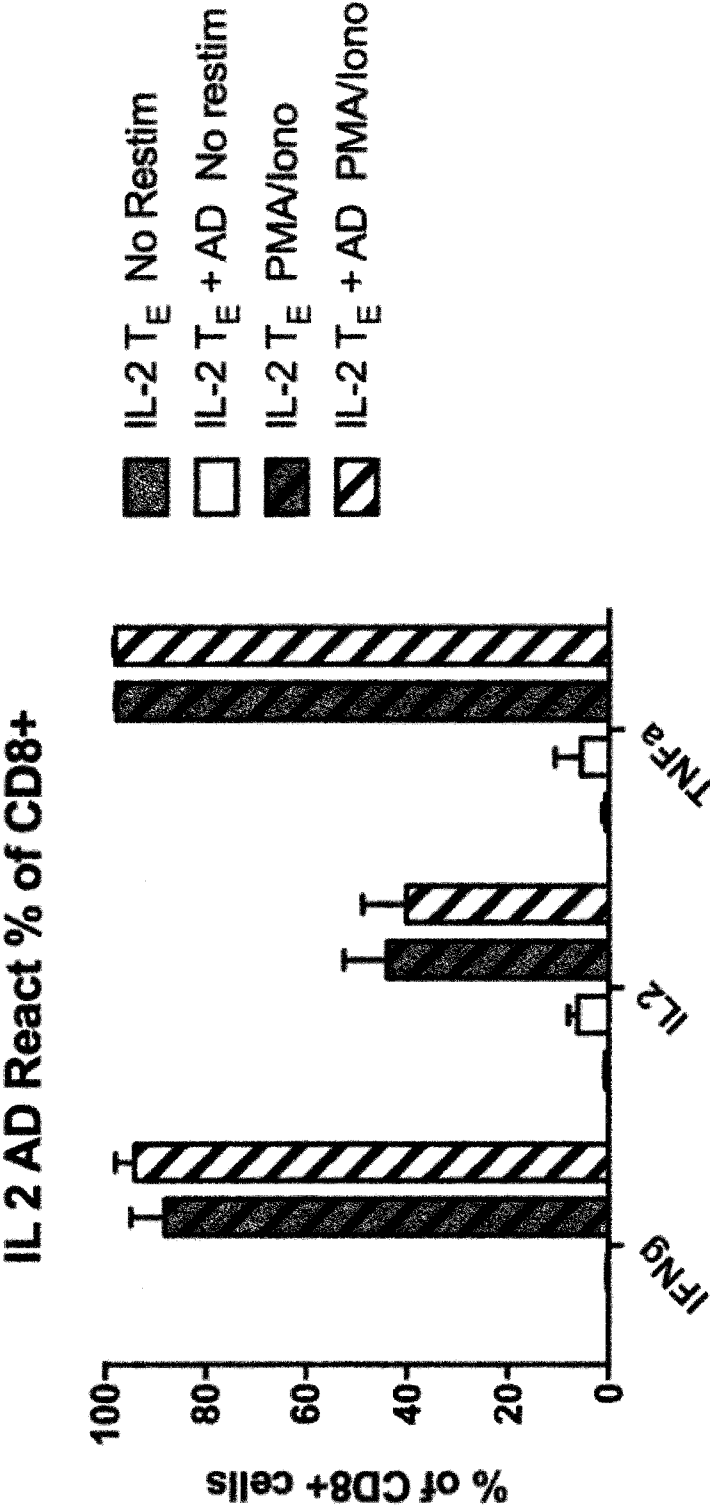


FIG. 8H

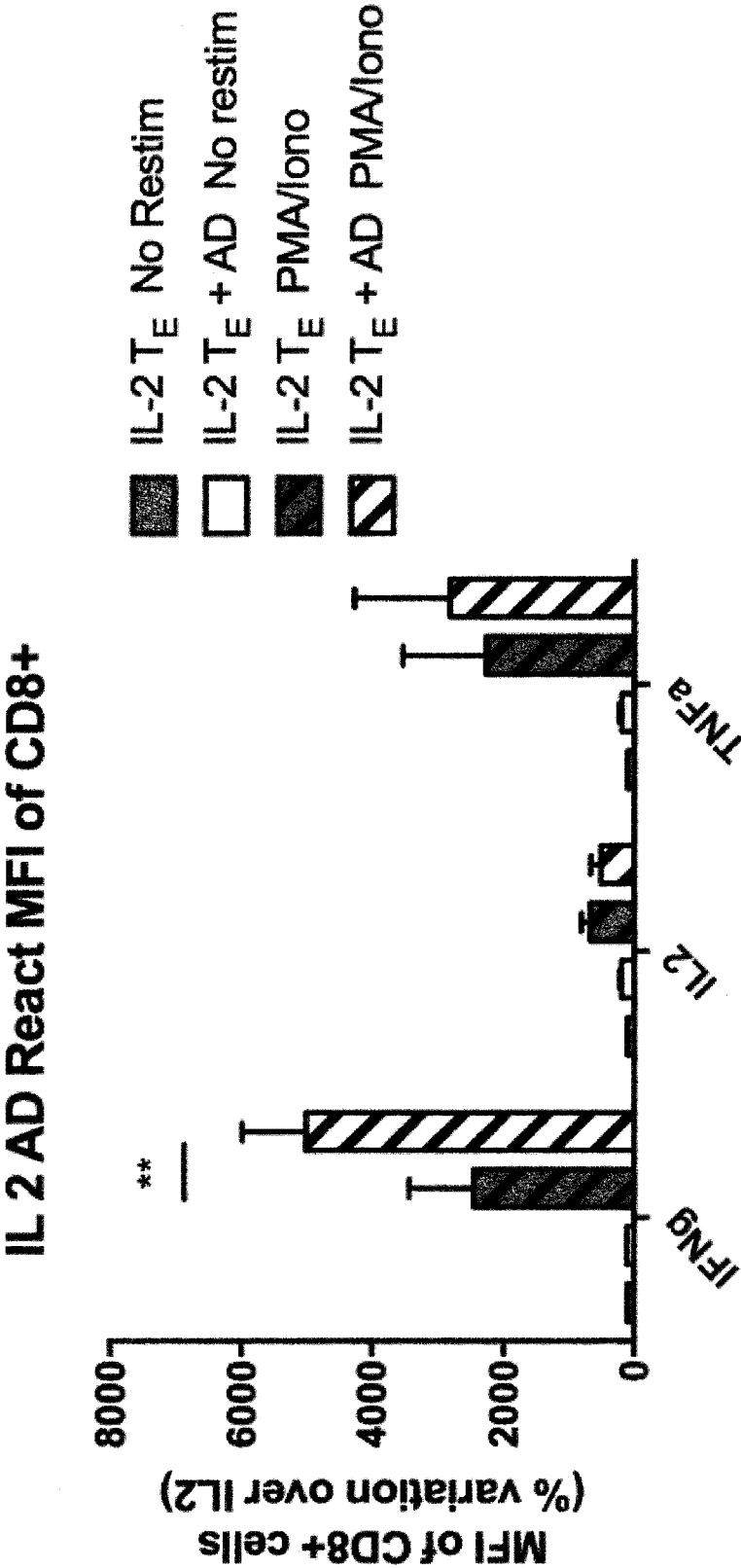


FIG. 8I

A

39/52

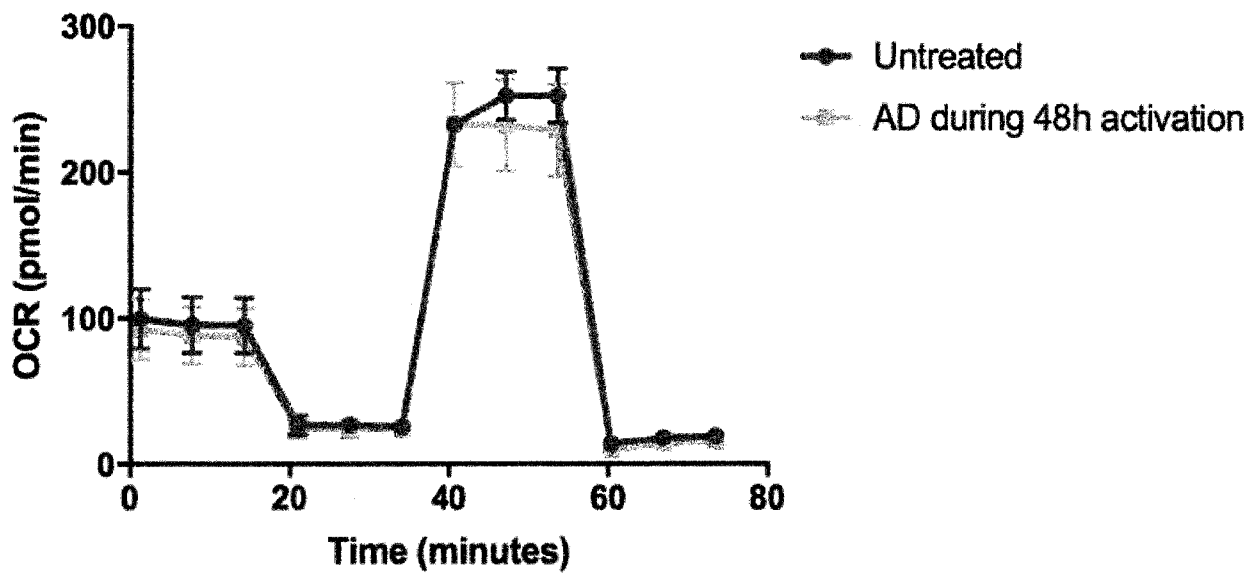
Wt CD8⁺ T cells

+ α CD3/ α CD28
+ IL2

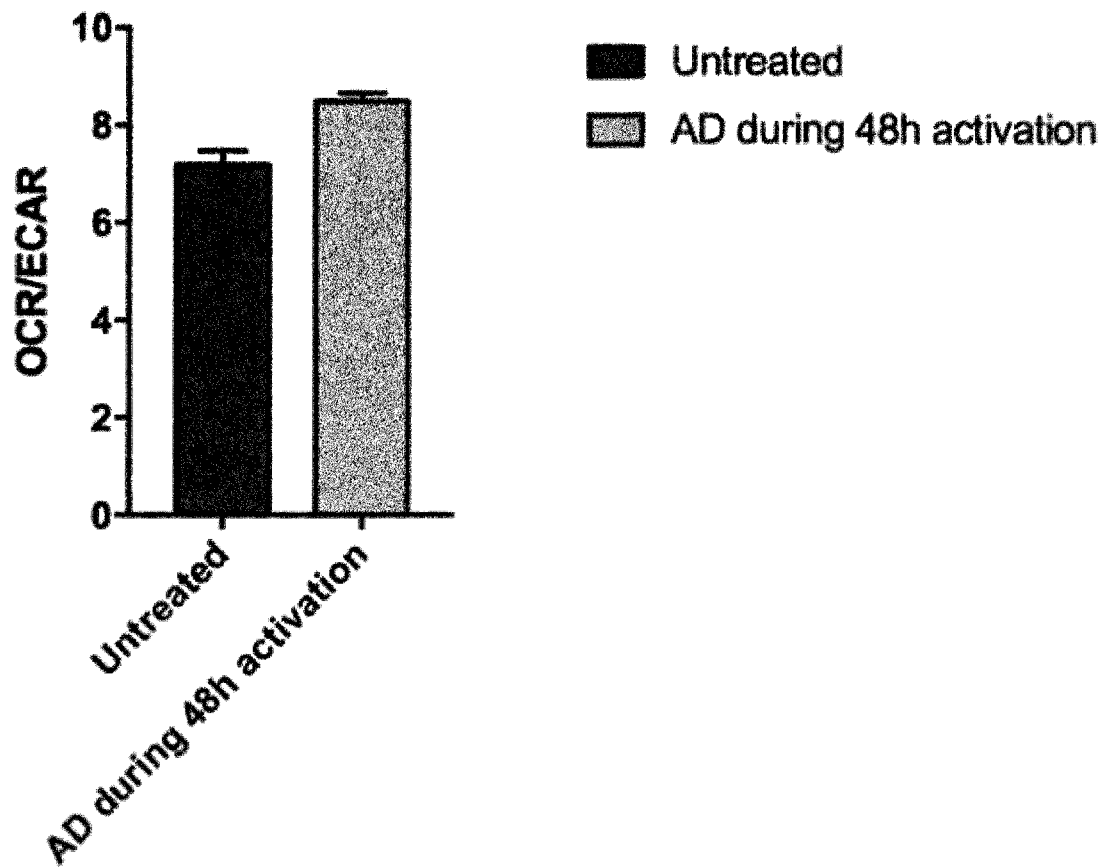
d2 $\xrightarrow{+IL2}$ d3 $\xrightarrow{+IL15}$ d6

IL15 T_M cells

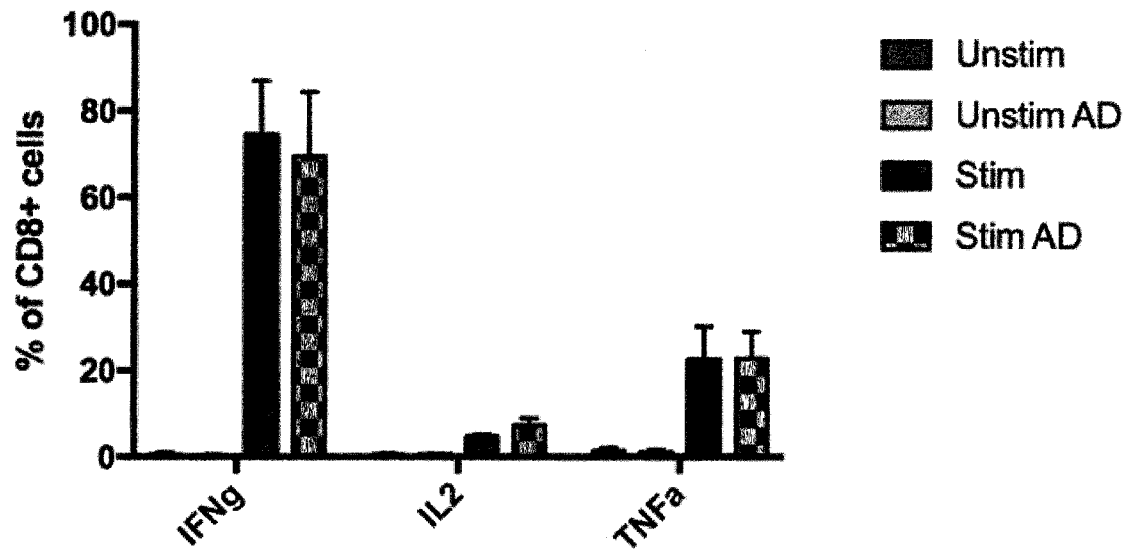
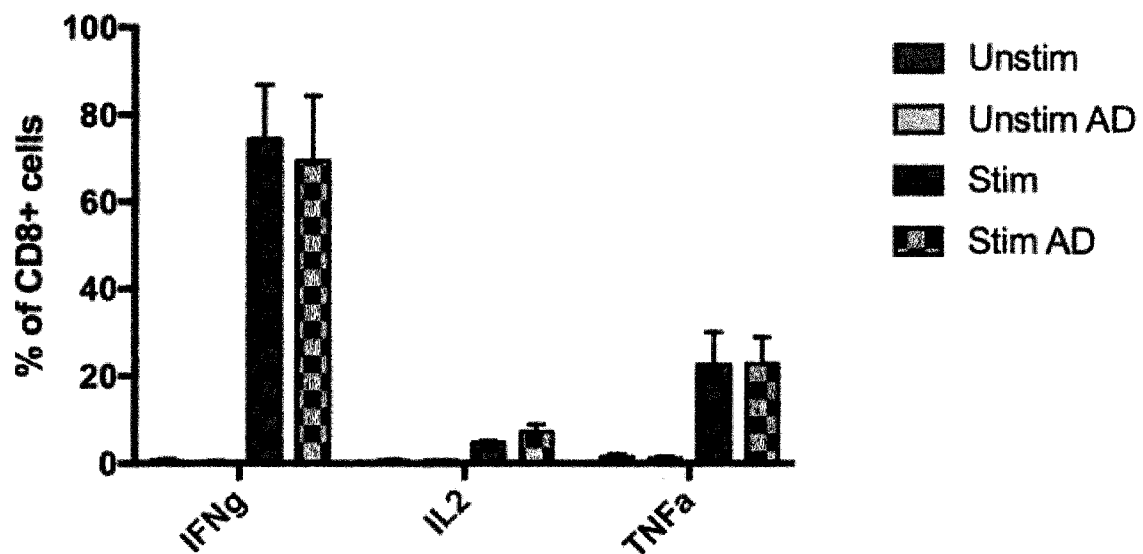
AD treatment
Cardiolipin synthesis
inhibition

FIG. 9A**B****OCR IL-15 (AD 48h act)****FIG. 9B**

40/52

C**OCR/ECAR IL-15 (AD 48h act)****FIG. 9C**

41/52

D**OCR IL-15 (AD 48h act) React % of CD8+ cells****FIG. 9D****E****OCR IL-15 (AD 48h act) React % of CD8+ cells****FIG. 9E**

A

WT Day6 IL2 AD acute injection (Mean + SEM)

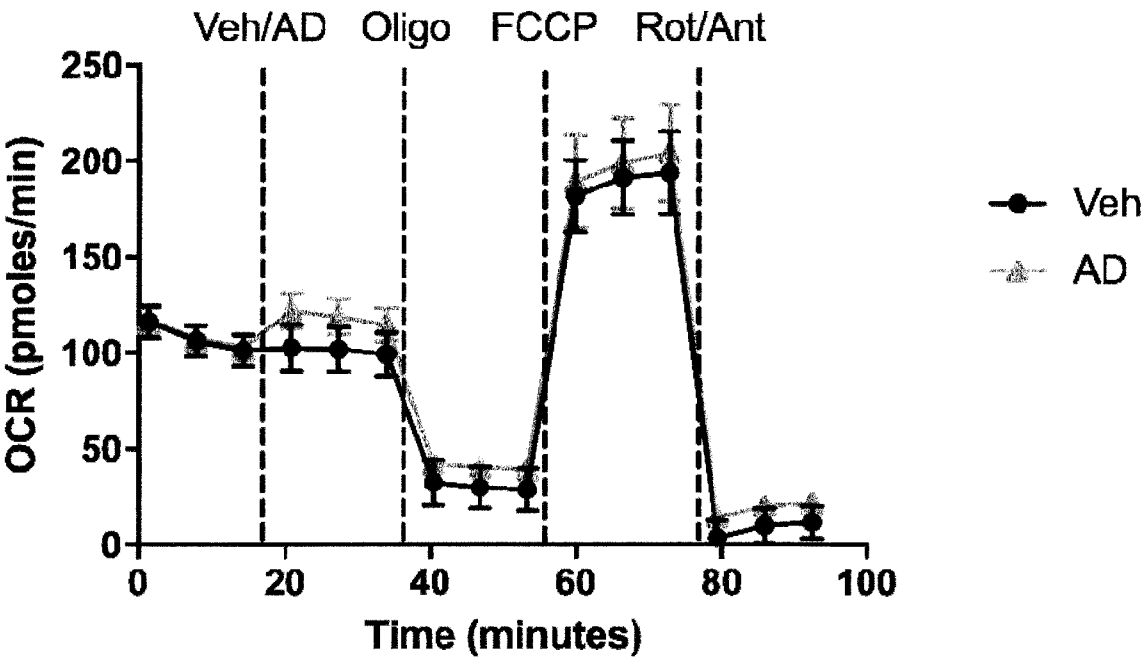


FIG. 10A

B

WT Day6 IL15 AD acute injection (Mean + SEM)

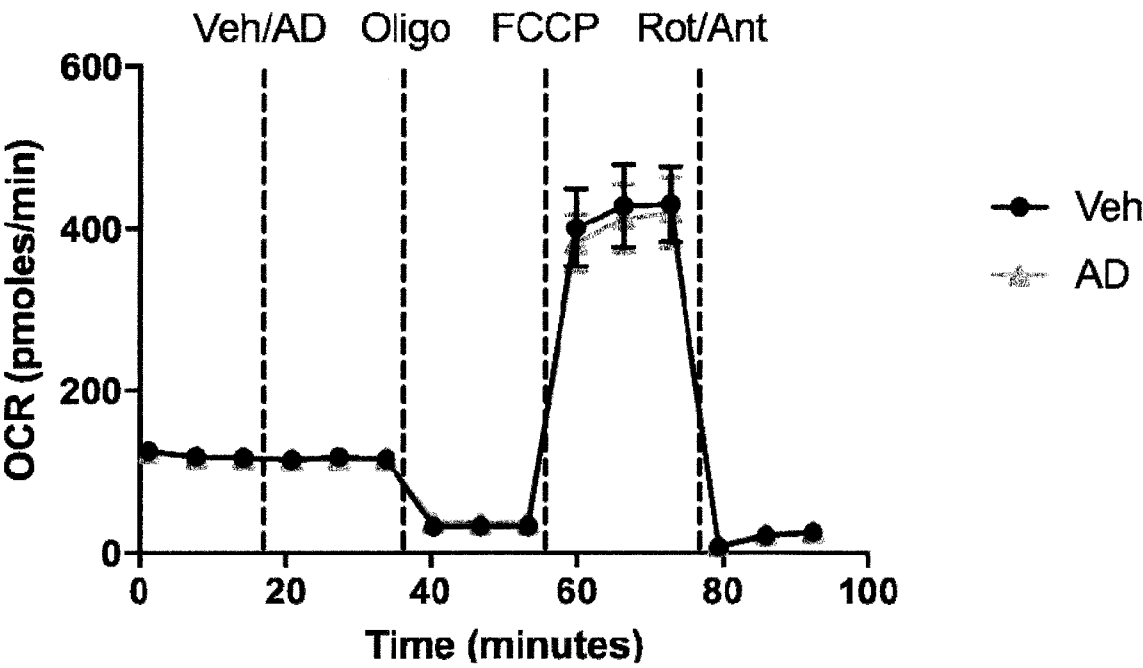


FIG. 10B

A

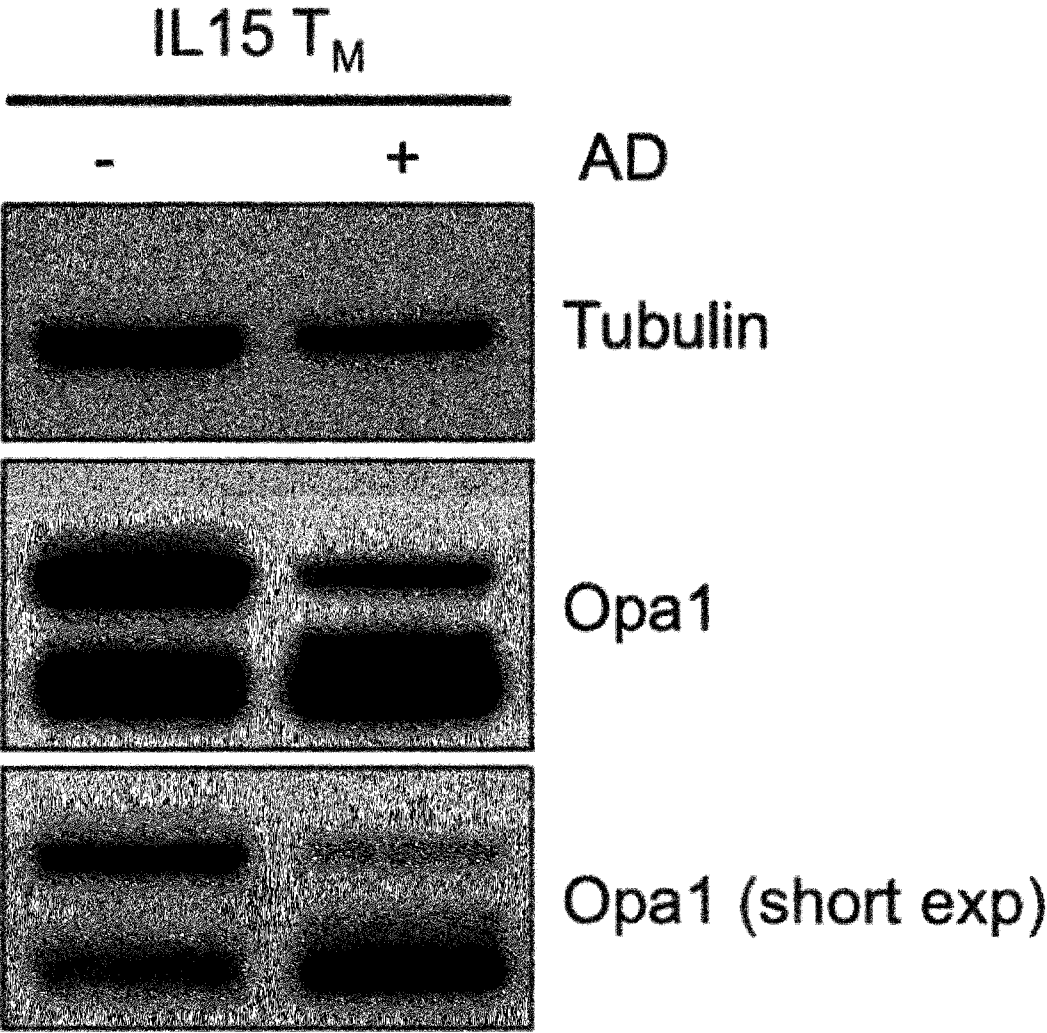


FIG. 11A

B

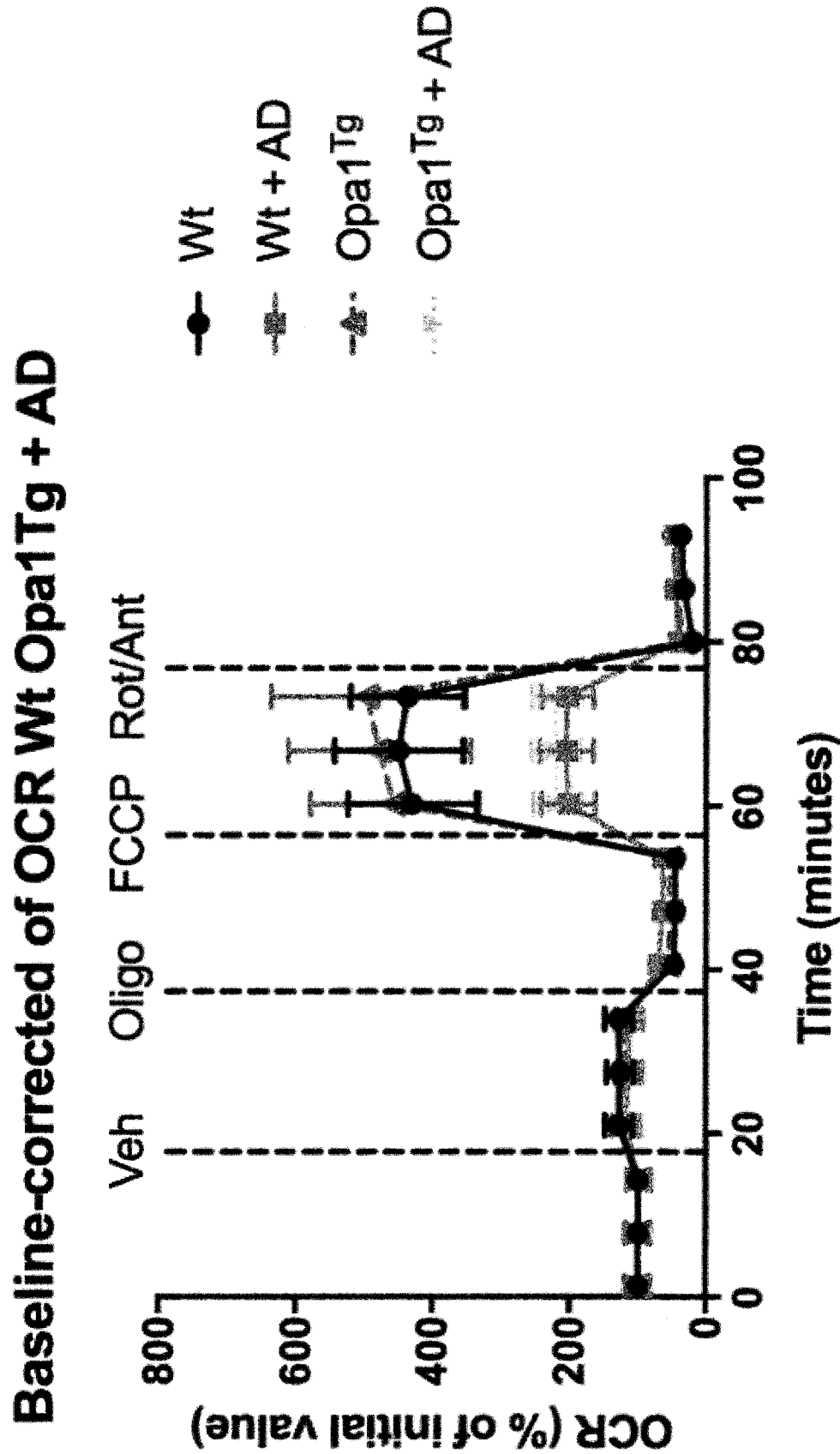


FIG. 11B

C

Proton Leak

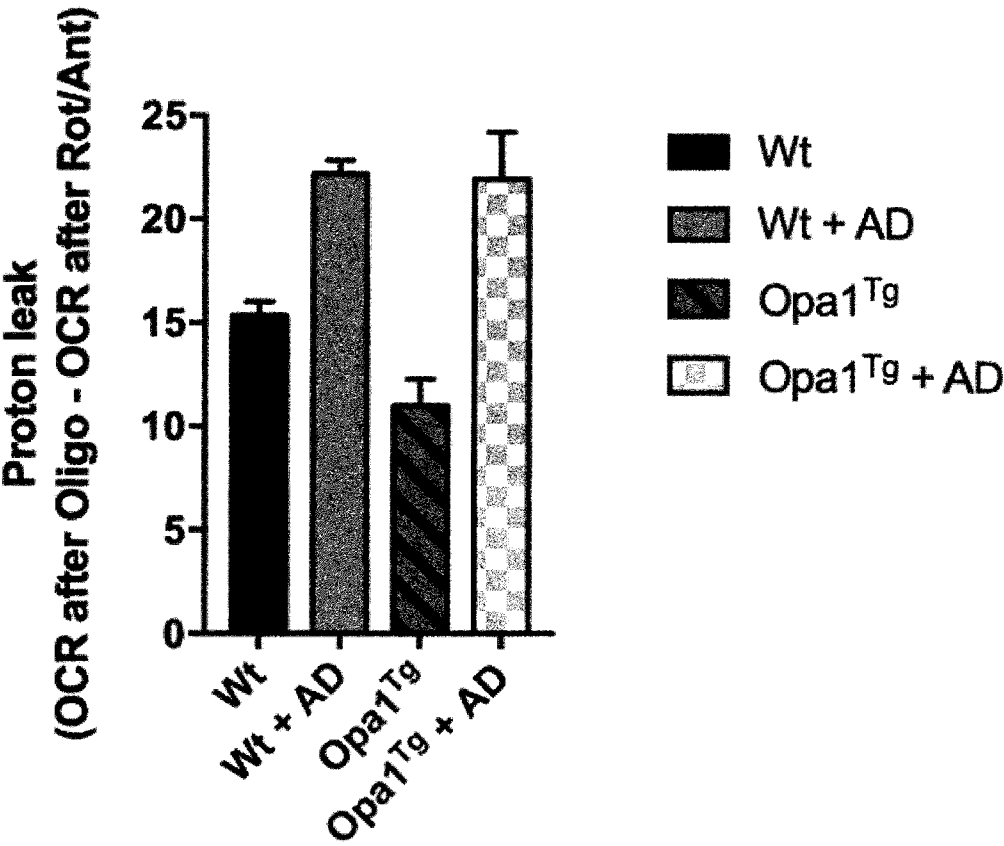
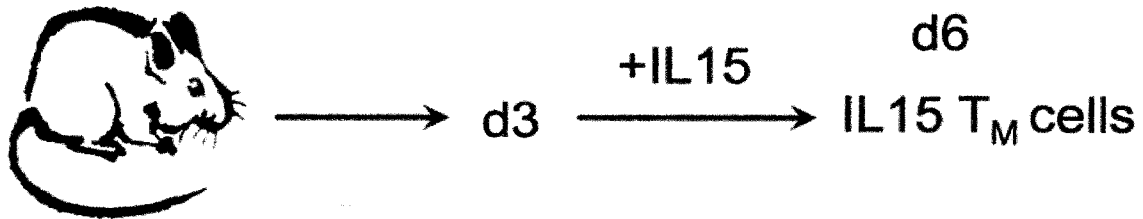


FIG. 11C

47/52

DWt CD8⁺ T cells

+ α CD3/ α CD28
+ IL2

AD treatment
Cardiolipin synthesis
inhibition
+ rescue treatment

FIG. 11D

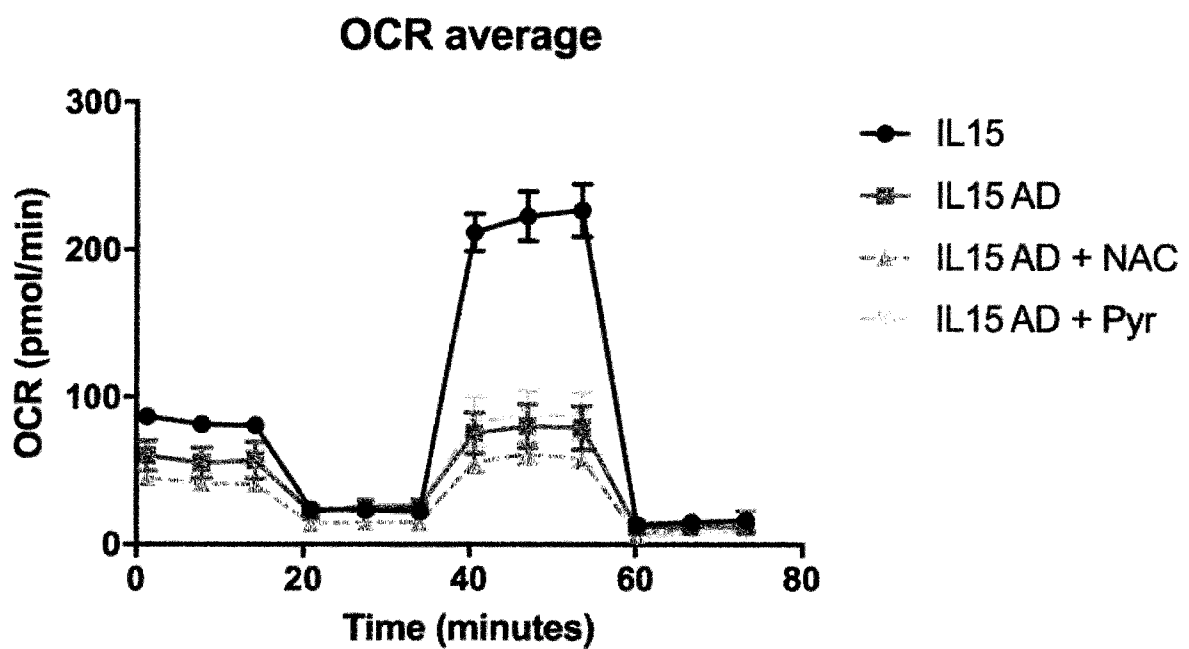
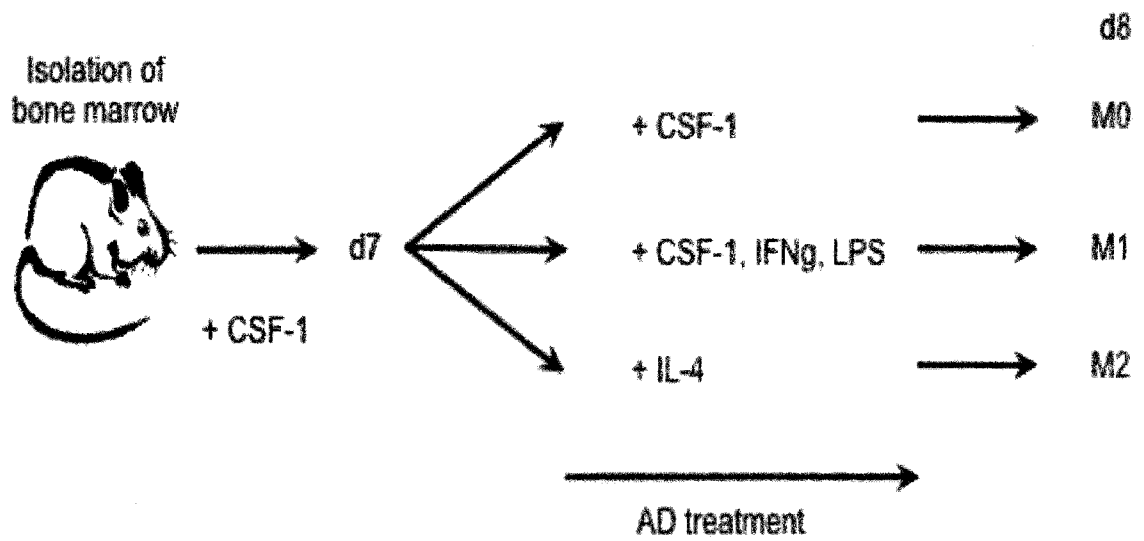
E

FIG. 11E

48/52

A**FIG. 12A**

49/52

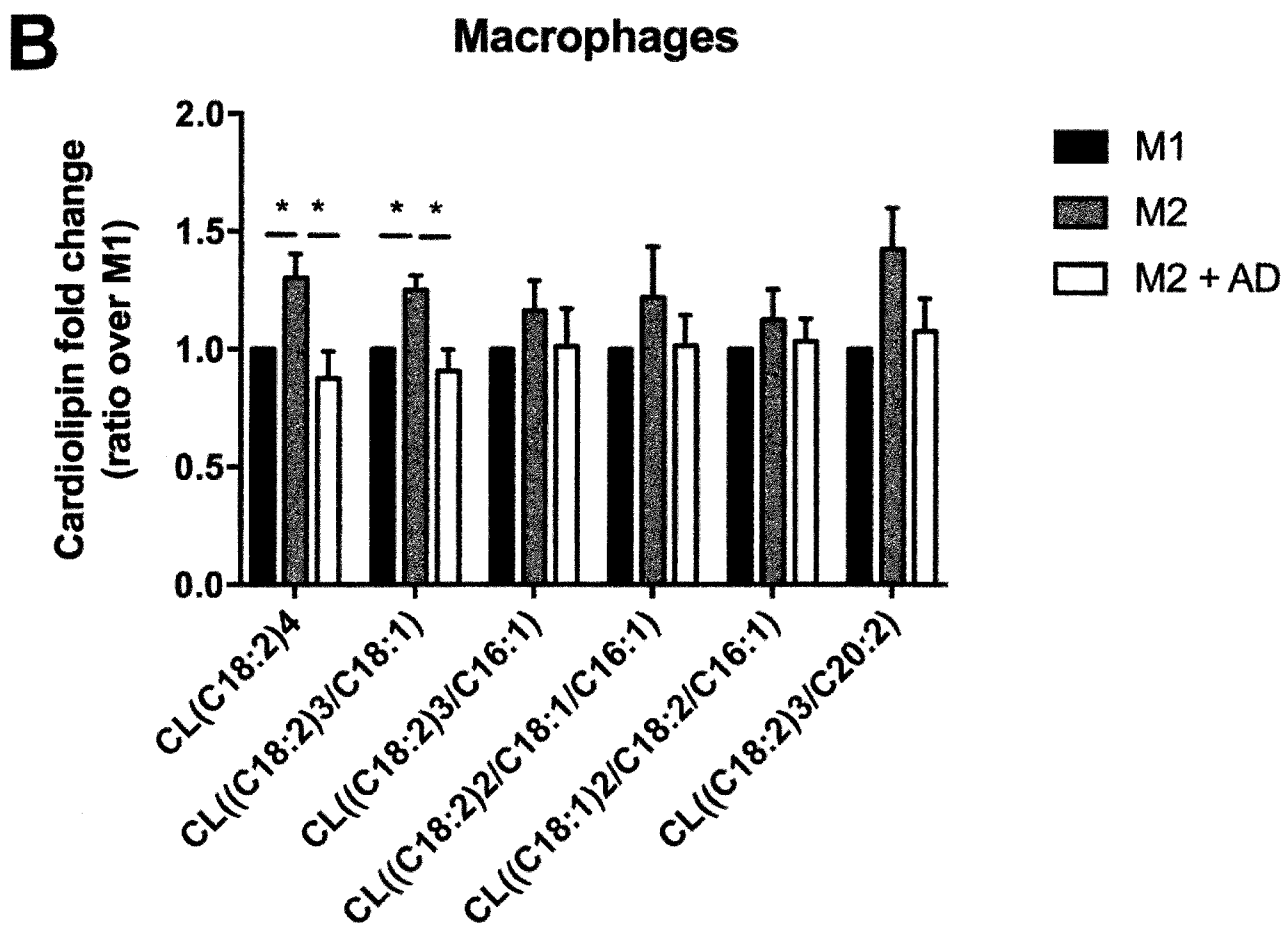


FIG. 12B

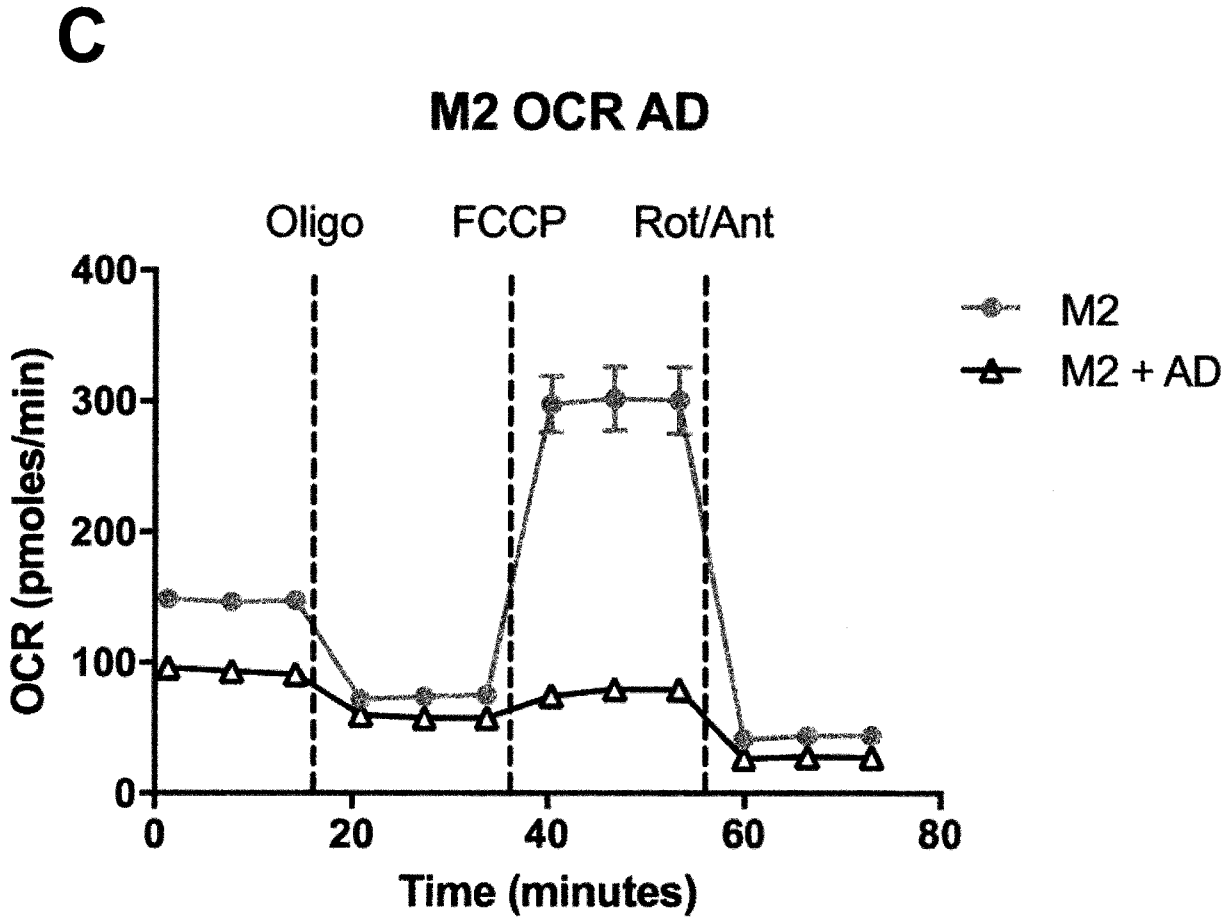


FIG. 12C

51/52

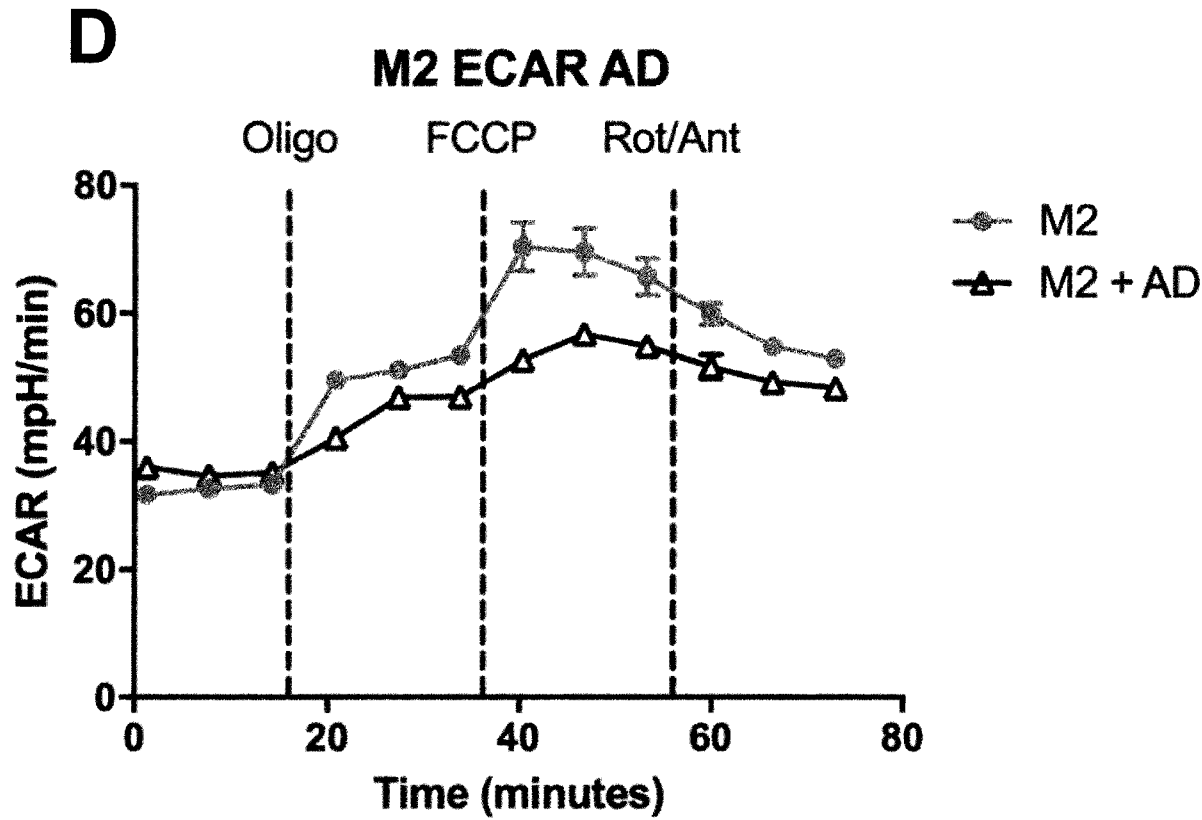


FIG. 12D

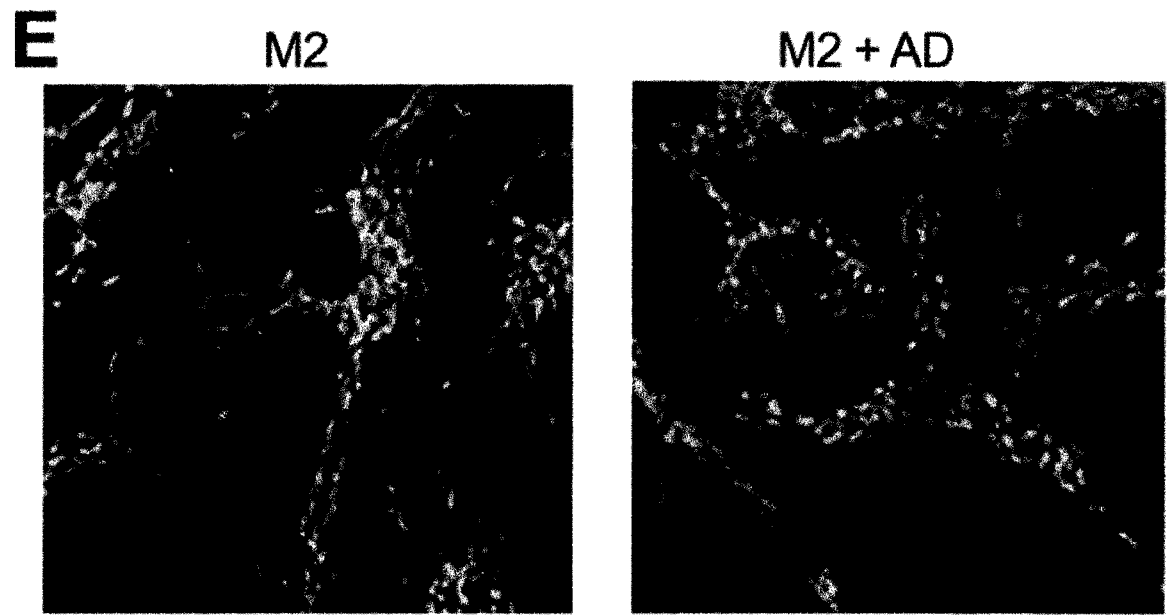


FIG. 12E

F

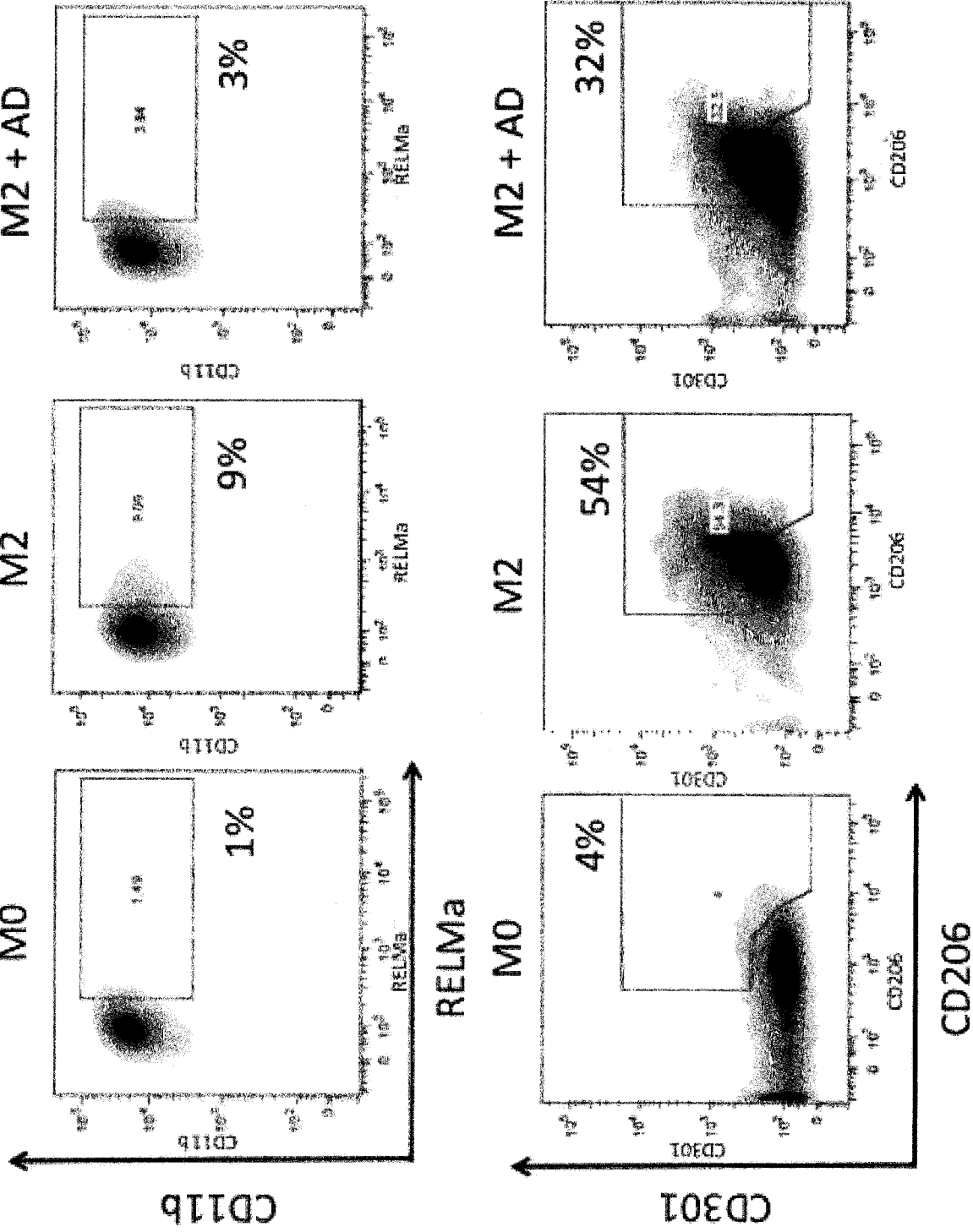


FIG. 12F

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/065364

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/50

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/078560 A1 (JAMIL HARIS [US] ET AL) 13 April 2006 (2006-04-13)	10-15
A	claims 1-14	1-9
X	US 2005/250709 A1 (KHODADOUST MEHRAN [US]) 10 November 2005 (2005-11-10)	10-15
A	0007, 0046, 0047	1-9
E	WO 2018/104172 A1 (CENTRE NAT RECH SCIENT [FR]; UNIV DE BORDEAUX [FR]; ASS FRANCAISE CONT) 14 June 2018 (2018-06-14) claims 1-15	1-15
	----- -/-	



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

12 July 2018

Date of mailing of the international search report

02/08/2018

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

Hoesel, Heidi

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/065364

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MCMILLIN J B ET AL: "Cardiolipin and apoptosis", BIOCHIMICA ET BIOPHYSICA ACTA (BBA) - MOLECULAR AND CELL BIOLOGY OF LI, ELSEVIER, AMSTERDAM, NL, vol. 1585, no. 2-3, 30 December 2002 (2002-12-30), pages 97-107, XP004402365, ISSN: 1388-1981, DOI: 10.1016/S1388-1981(02)00329-3 abstract -----	1-15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2018/065364

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2018/065364

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006078560	A1	13-04-2006	NONE

US 2005250709	A1	10-11-2005	US 2005250709 A1 10-11-2005
		WO 2005060951 A2	07-07-2005

WO 2018104172	A1	14-06-2018	NONE
