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(54) Title: METHODS FOR PREDICTING OR TREATING MYELOPOIESIS-DRIVEN CARDIOMETABOLIC DISEASES AND SEPSIS

(57) Abstract: The present invention relates to a method of treating myelopoiesis-driven cardiometabolic diseases and sepsis in a subject in need thereof comprising the step of administering the subject with a therapeutically effective amount of an agent selected from the group consisting of GLUT1 inhibitors and GOTs inhibitors.

METHODS FOR PREDICTING OR TREATING MYELOPOIESIS-DRIVEN CARDIOMETABOLIC DISEASES AND SEPSIS

FIELD OF THE INVENTION:

The invention is in the field of coronary or vascular disorders, more particularly to myelopoiesis-driven cardiometabolic diseases and sepsis.

The present invention also relates to a pharmaceutical composition for the treatment of myelopoiesis-driven cardiometabolic diseases and sepsis.

BACKGROUND OF THE INVENTION:

Atherosclerosis is a chronic, hypercholesterolemia-driven inflammatory disease that is initiated by the deposition of cholesterol-rich lipoproteins in the artery wall, leading to monocyte-macrophage recruitment. Hypercholesterolemia and/or defective cholesterol efflux have also been documented to induce myelopoiesis, which contributes to atherosclerotic lesion formation by fueling plaques with monocytes and neutrophils.[1,2] The monocyte count, in particular, independently predicts risk for coronary artery disease after adjustment for conventional risk factors.[3,4]

Hematopoietic stem cells (HSCs) are quiescent in the bone marrow (BM) niche and are the source of all hematopoietic stem and multi-potential progenitors (HSPCs) and differentiated cells that are critical for the maintenance and replenishment of peripheral leukocytes in adult life, particularly during emergency hematopoiesis. However, inventors and others have recently shown that chronic cholesterol accumulation in HSPCs due to hypercholesterolemia and/or defective apolipoprotein (Apo)-mediated cholesterol efflux promotes pathogenic HSPC expansion and proliferation leading to uncontrolled myelopoiesis.[5-8] For instance, in the *ApoE*$^{-/-}$ mouse model of atherosclerosis, the progressive HSPC expansion that drives myelopoiesis,[6] contributed to provide the inflamed atherosclerotic lesions with neutrophils and monocytes.[9-11] Although recent research has focused on elucidating the roles of cytokines and the microenvironment in the proliferation, mobilization and commitment of HSPCs in preclinical model of atherosclerosis,[1,2] the cellular metabolic pathways that regulate these processes remain unknown.

Lessons from various mutant mice displaying a wide range of bioenergetic defects *in vivo* have pointed to a central role for the mitochondrial energy metabolism in HSC stemness.[12-17] Mounting evidence also suggests that HSC quiescence requires a hypoxic environment,[18,19] to maintain glycolysis-biased metabolic activity instead of mitochondrial

oxidative phosphorylation (OXPHOS).[20,21] By limiting mitochondrial respiration and ATP production, this could indeed prevent HSCs from producing reactive oxygen species (ROS) to avoid their differentiation and exhaustion.[22-24] In contrast, funneling glucose to the mitochondria for Krebs cycle utilization is required when the HSCs become proliferative or undergo differentiation, most likely due to the high energy demand of these cellular processes.[21,25] More recently, Oburoglu et al., have also reported that glucose utilization can dictate the myeloid lineage commitment in human HSCs.[26] Intriguingly, increased hematopoietic metabolic activity can be visualized by non-invasive PET-CT imaging with ^{18}FDG, a glucose analog, not only in inflamed atherosclerotic plaques,[27-30] but also in the spleen of patients with cardiovascular diseases,[31,32] reflecting most likely an extramedullary hematopoiesis.[33] However, the relevance of these observations as well as the underlying mechanisms are not fully understood.

SUMMARY OF THE INVENTION:

The present invention relates to a method of treating myelopoiesis-driven cardiometabolic diseases and sepsis in a subject in need thereof comprising the step of administrating the subject with a therapeutically effective amount of an agent selected from the group consisting of GLUT1 inhibitors and GOTs inhibitors. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

In an attempt to better understand the relationship between the enhanced hematopoietic glycolytic activity, HSPC proliferation, myelopoiesis and the development of atherosclerotic lesions, inventors first showed that an enhanced hematopoietic glycolytic activity in the aortic arch, the BM and the spleen of *ApoE*$^{-/-}$ BM transplanted mice was associated with an enhanced Glut1 expression in *ApoE*$^{-/-}$ HSPCs. Mechanistic studies showed that the up-regulation of Glut1 in *ApoE*$^{-/-}$ HSPCs was not due to an alteration of the oxygenation status of the BM niche but rather was dependent on Ras signaling downstream of the granulocyte macrophage colony-stimulating factor/interleukin-3 receptor driving glycolytic substrate utilization by mitochondria. Finally, inventors carried out BM transplantation from mice with single or combined deficiencies of ApoE or the glucose transporter Glut1 into *ApoE*$^{-/-}$ mice. Consistent with their hypothesis, *ApoE*$^{-/-}$ mice that had received *ApoE*$^{-/-}$*Glut*$^{+/-}$ BM showed reduced HSPC proliferation and expansion, myelopoiesis and atherogenesis compared to mice that had received *ApoE*$^{-/-}$ BM. Thus, inventors propose a causal relationship between the enhanced hematopoietic glycolytic activity in *ApoE*$^{-/-}$ mice

and their myelopoiesis through regulation of HSPC expansion and fate, offering novel therapeutic perspectives.

Accordingly, the present invention relates to a method of treating myelopoiesis-driven cardiometabolic diseases and sepsis in a subject in need thereof comprising the step of inhibiting Glut1 and GOTs in said subject.

More particularly, the present invention relates to a method of treating myelopoiesis-driven cardiometabolic diseases and sepsis in a subject in need thereof comprising the step of administrating the subject with a therapeutically effective amount of an agent selected from the group consisting of GLUT1 inhibitors and GOTs inhibitors.

As used herein, the terms “treating” or “treatment” refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subject at risk of contracting the disease or suspected to have contracted the disease as well as subject who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., pain, disease manifestation, etc.]).

As used herein, the term "myelopoiesis-driven cardiometabolic diseases" refers to myocardium infarction or chronic atherosclerosis. Myelopoiesis refers to a formation of myeloid cells, including eosinophilic granulocytes, basophilic granulocytes, neutrophilic granulocytes, and monocytes. Hypercholesterolemia and/or defective cholesterol efflux have also been documented to induce myelopoiesis, which contributes to atherosclerotic lesion formation by fueling plaques with monocytes and neutrophils.

Myocardium infarction (MI) is defined by the demonstration of myocardial cell necrosis due to significant and sustained ischaemia. It is usually, but not always, an acute manifestation of atherosclerosis-related coronary heart disease. MI results from either coronary heart disease, which implies obstruction to blood flow due to plaques in the coronary arteries or, much less frequently, to other obstructing mechanisms (e.g. spasm of plaque-free arteries) (definition from World Health Organization, Mendis et al 2010).

Atherosclerosis is a chronic, hypercholesterolemia-driven inflammatory disease that is initiated by the deposition of cholesterol-rich lipoproteins in the artery wall, leading to monocyte-macrophage recruitment. Atherosclerosis contributes to the development of atherosclerotic vascular diseases (AVD) which may affect the coronary arteries (causing ischaemic heart disease), the cerebral circulation (causing cerebrovascular disease), the aorta (producing aneurysms that are prone to thrombosis and rupture) and peripheral blood vessels, typically the legs (causing peripheral vascular disease and intermittent claudication). Ischaemic heart disease (IHD) includes angina (chest pain caused by insufficient blood supply to cardiac muscle) and myocardial infarction (death of cardiac muscle) and cerebrovascular disease includes stroke and transient ischaemic attacks.

Sepsis is a systemic reaction characterized by arterial hypotension, metabolic acidosis, decreased systemic vascular resistance, tachypnea and organ dysfunction. Sepsis (including septic shock) is characterized by a systemic inflammatory response which results from the activation of a number of host defense mechanisms including the release of cytokines, the activation of immune cells, the complement system and the coagulation pathway.

Thus, the method according to the present invention can be supplied to a subject, who has been diagnosed as presenting one of the following myelopoiesis-driven cardiometabolic driven diseases: myocardium infarction or chronic atherosclerosis.

In a particular embodiment, the method according to the present invention wherein the myelopoiesis-driven cardiometabolic disease is atherosclerosis.

The method of the present invention can also be supplied to a subject who has been diagnosed for sepsis.

The term "subject" refers to any mammals, such as a rodent, a feline, a canine, and a primate. Particularly, in the present invention, the subject is a human afflicted with or susceptible to be afflicted with myelopoiesis-driven diseases. In a particular embodiment, the subject is a human afflicted or susceptible to be afflicted with atherosclerosis.

The term "agent selected from the group consisting of GLUT1 inhibitors and GOTs inhibitors" refers to the capacity of any compound to reduce or inhibit Glut1 cell surface expression or GOTs, particularly Glut1 expression or GOTs in HSPCs and myeloid cells. Inhibition includes reduction of function and full blockade.

Glucose transporter 1 (or GLUT1), also known as solute carrier family 2 or facilitated glucose transporter member 1 (SLC2A1), is a uniporter protein that in humans is encoded by the SLC2A1 gene. GLUT1 facilitates the transport of glucose across the plasma membranes of mammalian cells, particularly facilitates the entry of D-glucose across the blood-barrier brain.

Glutamate oxaloacetate transaminases (GOTs) are pyridoxal phosphate-dependent enzymes which exist in cytoplasmic and mitochondrial forms, GOT1 and GOT2, respectively. GOTs play a role in amino acid metabolism and the urea and tricarboxylic acid cycles. The two enzymes are homodimeric and show close homology.

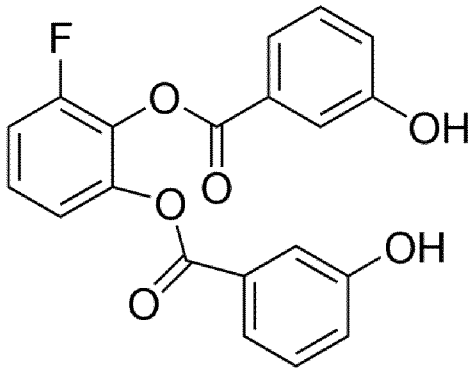
In some embodiments, the inhibitors of GLUT1 could be an antibody, synthetic or native sequence peptides, small molecules or aptamers.

In a particular embodiment, the inhibitor of Glut1 is a small organic molecule.

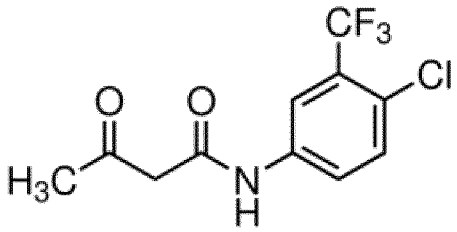
The term "small organic molecule" as used herein, refers to a molecule of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macro molecules (e. g. proteins, nucleic acids, etc.). Typically, small organic molecules range in size up to about 5000 Da, more preferably up to 2000 Da, and most preferably up to about 1000 Da.

In a particular embodiment, the inhibitor of Glut1 is a small organic molecule such as described in WO2011119866, WO2013182612, WO2015112581, Wood et al 2008, Amann et al 2009, Liu et al 2012, Qian et al 2014 and Shibuya et al 2014.

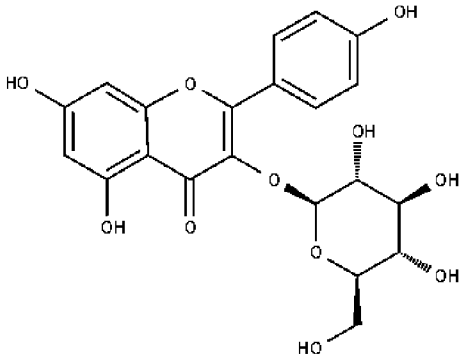
In a particular embodiment, the inhibitor of Glut1 is WZB117, also called 3-Fluoro-1,2-phenylene bis(3-hydroxybenzoate), 3-Hydroxy-benzoic acid 1,1′ -(3-fluoro-1,2-phenylene) ester and has the following formula in the art:



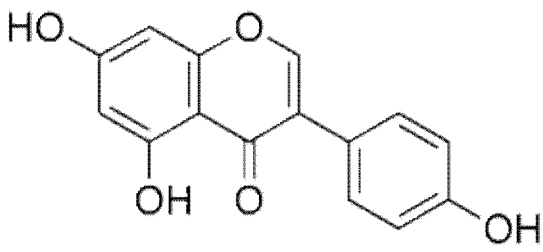
In a particular embodiment, the inhibitor of Glut1 is Fasentin, also called N-[4-Chloro-3-(trifluoromethyl)phenyl]-3-oxobutanamide and has the following formula in the art:



In a particular embodiment, the inhibitor of Glut1 is a plant carbohydrate product such as Astragalin-6-glucoside such as described in Thompson et al 2015.



In a particular embodiment, the inhibitor of Glut1 is genistein, also called 5,7-dihydroxy-3-(4-hydroxyphényl)-4H-chromén-4-one and has the following formula in the art:

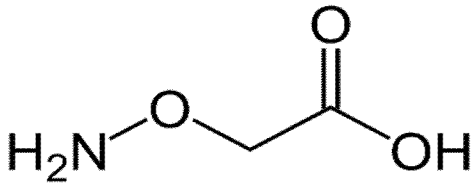


In a particular embodiment, the inhibitor of GLUT1 is an antibody.

As used herein, the term “antibody” is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (l) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The term includes antibody fragments that comprise an antigen binding domain such as Fab', Fab, F(ab')2, single domain antibodies (DABs), TandAbs dimer, Fv, scFv (single chain Fv), dsFv, ds-scFv, Fd, linear antibodies, minibodies, diabodies, bispecific antibody fragments, bibody, tribody (scFv-Fab fusions, bispecific or trispecific, respectively); sc-diabody; kappa(lamda) bodies (scFv-CL fusions); BiTE (Bispecific T-cell Engager, scFv-scFv tandems to attract T cells); DVD-Ig (dual variable domain antibody, bispecific format); SIP (small immunoprotein, a kind of minibody); SMIP ("small modular immunopharmaceutical" scFv-Fc dimer; DART (ds-stabilized diabody "Dual Affinity ReTargeting"); small antibody mimetics comprising one or more CDRs and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art (see Kabat et al., 1991, specifically incorporated herein by reference). Diabodies, in particular, are further described in EP 404, 097 and WO 93/1 1 161; whereas linear antibodies are further described in Zapata et al. (1995). Antibodies can be fragmented using conventional techniques. For example, F(ab')2 fragments can be generated by treating the antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and F(ab')2, scFv, Fv, dsFv, Fd, dAbs, TandAbs, ds-scFv, dimers, minibodies, diabodies, bispecific antibody fragments and other fragments can also be synthesized by recombinant techniques or can be chemically synthesized. Techniques for producing antibody fragments are well known and described in the art. For example, each of

Beckman et al., 2006; Holliger & Hudson, 2005; Le Gall et al., 2004; Reff & Heard, 2001 ; Reiter et al., 1996; and Young et al., 1995 further describe and enable the production of effective antibody fragments. In some embodiments, the antibody is a "chimeric" antibody as described in U.S. Pat. No. 4,816,567. In some embodiments, the antibody is a humanized antibody, such as described U.S. Pat. Nos. 6,982,321 and 7,087,409. In some embodiments, the antibody is a human antibody. A "human antibody" such as described in US 6,075,181 and 6,150,584. In some embodiments, the antibody is a single domain antibody such as described in EP 0 368 684, WO 06/030220 and WO 06/003388. In a particular embodiment, the inhibitor of Glut1 is a monoclonal antibody. Monoclonal antibodies can be prepared and isolated using any technique that provides for the production of antibody molecules by continuous cell lines in culture. Techniques for production and isolation include but are not limited to the hybridoma technique, the human B-cell hybridoma technique and the EBV-hybridoma technique.

In a particular embodiment, the inhibitor of GOTs belongs to the aminooxyacetic acid (AOA) class which called also 2-(aminooxy) acetic acid (Troy et al 1976; Risto et al 2003) and has the formula in the art:



In a particular embodiment, the inhibitor of Glut1 or GOTs is an inhibitor of GLUT1 or GOTs expression.

An "inhibitor of GLUT1 or GOTs expression" refers to a natural or synthetic compound that has a biological effect to inhibit or significantly reduce the expression of the gene encoding for GLUT1 or GOTs. Typically, the inhibitor of GLUT1 or GOTs expression has a biological effect on one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

In some embodiments, the inhibitor of GLUT1 or GOTs expression is an antisense oligonucleotide. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of GLUT1 mRNA or GOTs

mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of GLUT1 or GOTs proteins, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding GLUT1 or GOTs can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically alleviating gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

In some embodiments, the inhibitor of GLUT1 or GOTs expression is a small inhibitory RNAs (siRNAs). GLUT1 or GOTs expression can be reduced by contacting the subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that GLUT1 or GOTs expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002); Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

In some embodiments, inhibitor of GLUT1 or GOTs expression is ribozyme. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of GLUT1 or GOTs mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

A “therapeutically effective amount” is intended for a minimal amount of active agent which is necessary to impart therapeutic benefit to a subject. For example, a "therapeutically effective amount" to a subject is such an amount which induces, ameliorates or otherwise causes an improvement in the pathological symptoms, disease progression or physiological conditions associated with or resistance to succumbing to a disorder. It will be understood that the total daily usage of the compounds of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidential with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

The GLUT1 inhibitors and GOTs inhibitors as described above may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can

be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms. Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The polypeptide (or nucleic acid encoding thereof) can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and

by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active polypeptides in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. Enhanced glucose utilization in the aortic arch, splenocytes, BM and HSPCs of *ApoE*$^{-/-}$ BM chimeras. (A) 2-deoxy-[^{14}C]-glucose uptake in aortic arch, bone marrow and spleen of *ApoE*$^{-/-}$ recipients transplanted with WT or *ApoE*$^{-/-}$ BM at 12 weeks after the transplantation procedure. **(B)** 2-deoxy-[^{14}C]-glucose uptake was also determined in colony forming unit assays of multipotential progenitors (CFU-GEMM) and granulocyte macrophage progenitors (CFU-GM) from the BM of WT and *ApoE*$^{-/-}$ mice. **(C)** The citric acid metabolites were determined by LC-MS in BM cells isolated from *ApoE*$^{-/-}$ recipients transplanted with WT or *ApoE*$^{-/-}$ BM at 12 weeks after the transplantation procedure. **(D)** The mitochondrial membrane potential (MMP) was measured by flow cytometry using a fluorescent tetramethylrhodamine ethyl ester (TMRE) dye in Lineage marker (Lin)$^-$, Lin$^+$ and CD34$^-$ or CD34$^+$ HSPCs isolated from the BM of these mice. **(E)** NBD-glucose binding and/or uptake and **(F)** cell surface expression of Glut1 was also quantified in these cells. All results are the means ± SEM and are representative of at least one experiment performed with 6-10 animals per group. **P*<0.05 *vs.* WT. §*P*<0.05 *vs.* the untreated condition.

Figure 2. HIF1α-independent regulation of Glut1 expression and ApoE-/- HSPC expansion and myeloid lineage fate. (A) Experimental overview. Bone marrow from Mx1-Cre (controls), Mx1-cre HIF1□fl/fl, ApoE-/- Mx1-Cre, ApoE-/- Mx1-cre HIF1□fl/fl mice were transplanted into ApoE-/- recipient mice and, after a 5 week recovery period, the mice were injected with Poly:IC and fed a high fat diet for 12 weeks to induce the expansion of HSPCs. (B) Representative Western blots showing HIF1□□□□□□□□□□□levels in BM cells freshly isolated from these mice at the end of the study period. Quantification (normalized to □-actin) is expressed as arbitrary unit and indicated by numbers below (C) mRNA expression of HIF1□ and HIF1□ target genes Ldha and Glut1 in BM cells freshly isolated from these mice at the end of the study period. (D) Histograms showing Glut1 cell surface expression (expressed as the mean fluorescence intensity (MFI)) in CD34- and CD34+ HSPCs. (E) Quantification of the CD34- or CD34+ HSPCs by flow cytometry was expressed as the percentage of total BM. (F) peripheral blood neutrophils, monocytes and eosinophils were also quantified in these mice at the end of the study period. The results are the means ± SEM of 6-10 animals per group. *P<0.05 vs. Mx1-Cre. §P<0.05 vs. ApoE-/- Mx1-Cre.

Figure 3. The *ApoE*$^{-/-}$ HSPC expansion and myeloid lineage fate and Glut1 upregulation are driven by the IL3Rβ signaling pathway. (A) Twenty-week-old WT and *ApoE*$^{-/-}$ mice were injected with IgG control or 100μg of the IL-3Rβ blocking antibody for 24 h and analyzed for peripheral blood myeloid cells by flow cytometry. **(B)** The CD34$^-$ or

$CD34^+$ HSPCs were quantified in the BM of these mice and was expressed as the percentage of total BM. **(C)** The percentage of these cells in S/G2M phase was determined by Hoechst staining, and **(D)** Glut1 cell surface expression was expressed as the mean fluorescence intensity (MFI). The results are the means ± SEM of 5 to 6 animals per group. *P<0.05 *vs.* WT IgG control. §P<0.05 *vs.* *ApoE*$^{-/-}$ IgG control.

Figure 4. Mitochondrial glycolytic substrate utilization is required for ApoE-/- HSPC proliferation and myelomonyctic fate in vitro. Schematic representation of the metabolic pathways analyzed using pharmacological inhibitors with key enzymes indicated in blue, inhibitors in red, metabolites in black and cholesterol in green. Red boxes also indicated key signaling molecules. Bone marrow cells from fluorouracil-treated WT and ApoE-/- mice were grown for 72h in liquid culture containing 10% FBS IMDM in the presence of the indicated chemical compounds and 6ng/mL IL-3 or 2ng/mL GM-CSF. (A) Quantification of HSPCs and (B) CD11b+Gr-1+ myeloid cells after in vitro culture. Arrows on the y-axis indicate the starting percentage of cells per well before culture. The results are the means ± SEM of an experiment performed with 4 animals per group. P<0.05, genotype effect. NS, non-significant.

Figure 5. Glut1 is required *in vitro* for the IL3Rβ-dependent *ApoE*$^{-/-}$ HSPC expansion and myeloid lineage fate. Bone marrow cells from WT, *Glut1*$^{+/-}$, *ApoE*$^{-/-}$, and *ApoE*$^{-/-}$*Glut1*$^{+/-}$ mice were sorted for Lin^- cells (i.e, enriched in HSPCs) and cultured for 72h in liquid culture in presence or absence of 6ng/mL IL-3 or 2ng/mL GM-CSF. **(A)** Representative dot plots and **(B)** quantification of HSPCs after *in vitro* culture. **(C)** Representative dot plots and **(D)** quantification of $CD11b^+Gr\text{-}1^+$ myeloid cells after *in vitro* culture. (E) Quantification of ROS generation and (F) mitochondrial membrane potential (MMP) by flow cytometry using fluorescent carboxy-H_2DCFDA and tetramethylrhodamine ethyl ester (TMRE) dyes, respectively in HSPCs after *in vitro* culture. The results are the means ± SEM of n=4 per group. *P<0.05, genotype effect. §P<0.05, Glut1-dependent effect. #P<0.05, growth hormone effect.

Figure 6. Glut1-dependence of ApoE-/- HSPC expansion and myelopoiesis in vivo. (A) Experimental overview. Bone marrow from WT, Glut1+/-, ApoE-/-, and ApoE-/-Glut1+/- mice were transplanted into ApoE-/- recipient mice and, after a 5 week recovery period, the mice were fed a high fat diet for 12 weeks to induce the expansion of HSPCs. Histograms show (B) the Glut1 cell surface expression and (C) NBD-glucose binding and/or uptake in HSPC subpopulations from the most quiescent (long-term LT-HSCs) to the most cycling multipotential progenitors (CD34-CD150+Flt3->CD34+CD150+Flt3-

>CD34+CD150-Flt3->CD34+CD150-Flt3+) and are expressed as the mean fluorescence intensity (MFI). (D) The percentage of cells in S/G2M phase was determined by DAPI staining and flow cytometry, and (E) quantified as the percentage of total BM. Histograms showing the quantification of granulocyte macrophage progenitor (GMP), common myeloid progenitor (CMP) and megakaryocyte-erythroid progenitor (MEP) populations are expressed as percentage of (F) total BM or (G) spleen. Quantification of (H) the peripheral blood leukocytes, (I) monocytes, (J) neutrophils and (K) eosinophils over the course of a 12-week high fat diet period. The data are the means ± SEM and are representative of an experiment performed with n=6 (WT and Glut1+/- BM transplanted into ApoE-/- recipients) or n=10-12 (ApoE-/- and ApoE-/-Glut1+/- BM transplanted into ApoE-/- recipients) animals per group. *P<0.05 vs. ApoE-/- mice receiving WT BM. §P<0.05 vs. ApoE-/- mice receiving ApoE-/- BM.

Figure 7. Cell autonomous role of Glut1 on *ApoE*$^{-/-}$ HSPC expansion and myeloid lineage commitment. Schematic diagram showing the protocol for the competitive repopulation assay. Equally mixed portions of BM from the respective genotypes were transplanted into WT recipients. Chow-fed recipient mice were analyzed at 10 weeks after reconstitution by flow cytometry for the contribution of the donor (CD45.1^{+}/CD45.2^{+}) to the HSPC subpopulations from the most quiescent (long-term LT-HSCs) to the most cycling multipotential progenitors (CD34^{-}CD150^{+}>CD34^{+}CD150^{+}> CD34^{+}CD150^{-}) in the bone marrow. **(B)** The percentage of CD45.1^{+} and CD45.2^{+} HSPCs in S/G2M phase was also determined by DAPI staining and flow cytometry in the BM of these mice. The contribution of the donor (CD45.1^{+}/CD45.2^{+}) to **(C)** the monocytes and **(D)** neutrophils in the peripheral blood was also analyzed. The results are the means ± SEM of 6 to 8 mice per groups. *P<0.05 *vs.* WT mice receiving CD45.1^{+}*Apoe*$^{-/-}$/CD45.2^{+}*Apoe*$^{-/-}$ mixed BM. §P<0.05 *vs.* CD45.1^{+}*Apoe*$^{-/-}$ cells within the same transplanted mice.

Figure 8. Glut1 deficiency reduces the accelerated atherosclerosis of *ApoE*$^{-/-}$ BM chimeras. Representative hematoxylin and eosin (H&E) staining (magnification, x100) and quantification by morphometric analysis of the development of atherosclerotic lesions in the proximal aorta of *ApoE*$^{-/-}$ recipient mice transplanted with *ApoE*$^{-/-}$ (n=10) or *ApoE*$^{-/-}$*Glut1*$^{+/-}$ BM that were fed a high fat diet (n=12). The values for individual mice are shown as open circles, representing an average of 6 sections per mouse. The horizontal bars represent the group medians. The macrophages were detected by F4/80 immunofluorescent staining in the proximal aorta and quantified as the mean intensity (magnification, X200). Aortic arch and spleen uptake of 2-deoxy-[^{14}C]-glucose in these mice at the end of the study period. All

results are the means ± SEM and are representative of 10 to 12 animals per group. $^{\S}P<0.05$ *vs.* *ApoE*$^{-/-}$ mice receiving *ApoE*$^{-/-}$ BM.

EXAMPLE:

Material & Methods

Results

The enhanced glucose uptake in atheromatous plaques under hypercholesterolemic conditions correlates with higher metabolic activity of hematopoietic cells and is associated with higher Glut1 expression in HSPCs. To monitor the metabolic activity of hematopoietic cells, we first investigated the uptake of the radiolabeled D-glucose analogue 2-deoxy [14C] glucose in organs isolated from irradiated ApoE-/- recipient mice transplanted with either WT or ApoE-/- bone marrow (BM). A more than 2-fold increase in 2-deoxy [14C] glucose uptake was observed not only in the aortic arch of ApoE-/- BM transplanted mice compared to controls but also in their BM and spleen (Fig. 1A). An approximately 3.5-fold increase in 2-deoxy [14C] glucose uptake was also consistently observed in colony forming unit assays with the multipotential progenitors (CFU-GEMM) and granulocyte macrophage progenitors (CFU-GM) from the ApoE-/- mice (Fig. 1B). Quantification of the citric acid metabolites by LC-MS showed higher citrate, fumarate and malate but not succinate in ApoE-/- leukocytes (Fig. 1C). This was associated with a 1.7-fold increase in succinate dehydrogenase activity in ApoE-/- leukocytes (WT, 13.7±1.9. vs. ApoE-/-, 24.2±4.1 ΔOD/min/mg protein, respectively). Consistent with these findings, an approximately 1.3-fold increase in the mitochondrial membrane potential was observed in ApoE-/- Lineage marker-positive (Lin+) and Lin- BM cells by flow cytometry using a fluorescent tetramethylrhodamine ethyl ester (TMRE) dye; these cell types represent mature leukocytes or a mix of HSPCs, respectively (Fig. 1D). An analysis of the different populations within the HSPCs showed that the mitochondrial potential of the CD34- long-term hematopoietic stem cells (LT-HSCs) and CD34+ HSPCs,34,35 was increased to the same extent as the Lin- BM cells in the ApoE-/- mice (Fig. 1D). This was associated with an increase in reactive oxygen species (ROS) staining in these cells (data not shown). To test whether this phenotype was caused by modulation of the glycolytic pathway in HSPCs, we next used the fluorescent D-glucose analog 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) as a tool to examine the glucose uptake in these cells.36 The NBDG staining was increased by approximately 1.5-fold in the ApoE-/- Lin+ and Lin- BM cells and CD34+ HSPCs, but not in the more primitive fractions of the CD34- LT-HSCs (Fig.

1E). We next assessed the cell surface expression of the glucose transporter 1 (Glut1) in these cells by flow cytometry. An approximately 1.25-fold increase in the Glut1 cell surface expression was observed in the ApoE-/- Lin+ and Lin- BM cells and CD34+ HSPCs but not CD34- LT-HSCs (Fig. 1D). This was similar to the NBDG pattern. Together, these observations suggest that the enhanced Glut1 expression and associated glycolytic activity in organs from ApoE-/- BM transplanted mice could reflect the metabolic state not only of leukocytes but also HSPCs.

HIF1α is neither involved in the up-regulation of Glut1 in ApoE-/- HSPCs nor the enhanced myelopoiesis of ApoE-/- mice. We next set out to better understand the mechanism leading to Glut1 regulation in the ApoE-/- HSPCs. Studies have proposed that the hypoxia inducible factor 1□ (HIF1□) up-regulates Glut1,37 and HIF1□ contributes to HSPC homeostasis.18,20 We first assessed the hypoxic state of the BM cells isolated from irradiated ApoE-/- recipient mice transplanted with either WT or ApoE-/- BM by flow cytometry with a fluorescein (FITC)-conjugated anti-Pimonidazole (Pimo) antibody at 90 min after intravenous Pimo administration. We did not observe significant changes in Pimo staining in either the ApoE-/- Lin+ and Lin- BM cells or CD34+ HSPCs and CD34- LT-HSCs (Supplemental Fig. 1A). HIF1□ protein was also barely detectable in WT and ApoE-/- BM cell lysates under normoxic culture conditions and cell lysates from ApoE-/- BM cells showed amounts of HIF1□ protein similar to those of WT cells under hypoxia (Supplemental Fig. 1A). Even though, the increase in lactate dehydrogenase A (Ldha) mRNA expression, a HIF1□ target gene, upon hypoxia was similar between WT and ApoE-/- BM cultures, Glut1 gene expression was higher under both normoxic and hypoxic culture conditions in ApoE-/- BM cultures (Supplemental Fig. 1C). Thus, to directly test the contribution of HIF1□ in the regulation of Glut1-dependent glucose metabolism in ApoE-/- HSPC in vivo, we next generated an inducible, hematopoietic-specific HIF1□□knockout (Mx1-cre HIF1□fl/fl) on a WT or ApoE-/- background. The BM from these mice was transplanted into irradiated ApoE-/- mice and after a recovery period, the recipients were then placed on a high fat diet (HFD) for 12 weeks to exacerbate their HSPC expansion (Fig. 2A).6 HIF1□□was deleted from hematopoietic cells before the start of the diet by sequential polyI:polyC (PIpC) injections, which efficiently excised the HIF1□□gene from the BM cells (Fig. 2B and 2C). Ldha mRNA expression was also significantly reduced in the BM of these mice, but Glut1 was only marginally regulated (Fig. 2C). HIF1□□deficiency also did not alter the cell surface expression of Glut1 in CD34+ HSPCs and CD34- LT-HSCs (Fig. 2D) or the frequency of these cells (Fig. 2E). Furthermore, quantification of the blood myeloid cells in these mice

revealed that HIF1□□deficiency further increased the neutrophil, monocyte and eosinophil counts in these mice (Fig. 2F). Together, these findings suggest that HIF1□ does not mediate the upregulation of Glut1 in ApoE-/- HSPC or their expansion and minimally contributed to myelopoiesis under hypercholesterolemic conditions.

The IL-3Rβ signaling pathway concomitantly controls the cycling and the up-regulation of Glut1 in ApoE-/- HSPCs. Glut1 can be up-regulated by growth hormone-dependent activation of oncogenes, such as Ras or Src.38,39 Therefore, we investigated the expression of Glut1 in WT and ApoE-/- BM cultures in response to various growth hormones. The Glut1 mRNA levels in WT BM cells were increased upon stimulation with GM-CSF and IL-3, but not Flt3L or TPO, and this response was further increased in the ApoE-/- BM cells and blunted by a farnesyl transferase inhibitor that blocks Ras activation (Supplemental Fig. 2A). These responses were not observed for the HIF1□ or Ldha mRNAs (Supplemental Fig. 2A). Flow cytometry analysis confirmed an increase in Glut1 cell surface expression in ApoE-/- HSPCs upon IL-3 and GM-CSF stimulation compared to WT HSPCs (Supplemental Fig. 2B). These effects were abrogated by blocking the IL3R□ signaling pathway and downstream Ras activation with a farnesyl transferase inhibitor, but not by the Jak2 inhibitor, AG490 or the AMPK activator, metformin (Supplemental Fig. 2B). Removing plasma membrane cholesterol with cyclodextrin also prevented the enhanced Glut1 expression in ApoE-/- HSPCs confirming the central role of cholesterol in this regulation (Supplemental Fig. 2B), To directly test the relevance of these observations in vivo, an IL3R□ blocking antibody was next injected into the WT and ApoE-/- mice. Consistent with earlier work,40 we first showed that this antibody efficiently reduced myelopoiesis in the ApoE-/- mice and had no effect in WT mice over a 24 h period (Fig. 3A). An analysis of the genes in the glycolytic pathway in the BM cells at the end of the study period revealed no significant changes in the HIF1□ or Ldha mRNAs, but the Glut1 mRNA was down-regulated after treatment with the IL3R□ blocking antibody in the ApoE-/- BM (Supplemental Fig. 2C). Quantification of the HSPCs in the BM of these mice by flow cytometry also revealed reduced numbers of CD34+ but not CD34- HSPCs in the ApoE-/- mice but not the WT mice (Fig. 3B), which correlated with reduced cycling of these cells (Fig. 3C). This was associated with the reduced cell surface expression of Glut1 in the ApoE-/- CD34+ HSPCs (Fig. 3D). These results revealed that the metabolic requirements for proliferation and expansion of the ApoE-/- HSPCs is associated with the IL-3/Glut1 axis and not the HIF1□/Glut1 axis in vivo.

Inhibition of mitochondrial glycolytic substrate utilization prevents ApoE-/- HSPC proliferation and myelomonyctic fate in vitro. To determine the contribution of

mitochondrial oxidative phosphorylation (OXPHOS) on ApoE-/- HSPC proliferation and lineage specification upon IL-3 and GM-CSF treatment, we next artificially suppressed various enzymes that are intricately involved in the regulation of the TCA cycle using pharmacological inhibitors (Fig. 4A). We first validated our in vitro BM culture assay by showing that inhibition of the IL-3R signaling pathway (IL3R blocking antibody), inhibition of Ras signaling (farnesyl transferase inhibitor FTI-277) and plasma membrane cholesterol depletion (cyclodextrin CD) prevented ApoE-/- HSPC expansion (Fig. 4B) and the generation of CD11b+Gr-1+ myeloid cells upon IL-3 and GM-CSF treatment (Fig. 4C). In contrast, inhibition of lactate dehydrogenase (LDH) and acetyl-CoA carboxylase (ACC) using oxamate and Tofa, respectively or activation of AMP-activated protein kinase (AMPK) with metformin did not alter ApoE-/- HSPC expansion (Fig. 4B) or their myeloid fate (Fig. 4C). Surprisingly, inhibition of mitochondrial respiratory chain complex I with rotenone was also not sufficient to dampen ApoE-/- HSPC expansion and myeloid commitment (Fig. 4B and 4C). Given the enhanced SDH activity observed in ApoE-/- BM cells, we next evaluated the contribution of the mitochondrial complex II. Inhibition of SDH with 3-nitropropionic acid (3-NPA) specifically prevented the myeloid fate of ApoE-/- HSPCs but not their expansion (Fig. 4B and 4C). We next assessed whether inhibition of the conversion of pyruvate for entry to the TCA cycle with a pyruvate dehydrogenase (PDH) inhibitor (CPI-613) and/or a pyruvate carboxylase (PC) inhibitor (chlorothricin) could alter the expansion and myeloid fate of ApoE-/- HSPCs. Although ApoE-/- HSPC expansion required both inhibition of PDH and PC (Fig. 4B), their myeloid fate was actually suppressed by inhibiting either PDH or PC (Fig. 4C). This revealed that the conversion of both succinate and pyruvate into the TCA cycle are central metabolic checkpoints for ApoE-/- HSPC myeloid lineage specification and to some extent ApoE-/- HSPC expansion. Interestingly, the conversion of succinate to fumarate and pyruvate to oxaloacetate (OAA) converge to the malate-aspartate shuttle, known to maximize the number of ATP molecules produced in glycolysis.41 To address the contribution of this pathway, we suppressed transaminases including glutamate oxaloacetate transaminases (GOTs) using aminooxyacetic acid (AOA). This molecule has been shown to prevent the mitochondria from utilizing glycolytic substrates by inhibiting the malate-aspartate shuttle.42 Remarkably, treatment with AOA not only prevented ApoE-/- HSPC expansion (Fig. 4B) but also their myeloid fate upon IL-3 and GM-CSF treatment (Fig. 4C). These data collectively suggest that mitochondrial metabolic reprogramming of ApoE-/- HSPCs is required for their expansion and commitment to the myeloid lineage.

Glut1 mediates the IL-3Rβ-dependent ApoE-/- HSPC proliferation and myelomonyctic fate in vitro. To bolster our observations, we next generated single or combined knockout of ApoE (ApoE-/-) and Glut1 (Glut1+/-) and isolated Lin- bone marrow cells (containing predominantly HSPCs) that were placed in vitro in medium either alone or supplemented with IL-3 or GM-CSF. We found that Glut1 deficiency led to significantly decreased HSPC expansion either in WT Lin- cultures after IL-3 and GM-CSF stimulation or in ApoE-/- Lin- cultures under both unstimulated and stimulated conditions (Fig. 5A and 5B). Similarly, Glut1 deficiency blunted the generation of CD11b+Gr-1+ myeloid cells both in response to IL-3 and GM-CSF or in ApoE-/- Lin- cultures (Fig. 5C and 5D). This mirrored the ROS production and mitochondrial membrane potential assessed by flow cytometry in HSPCs at the end of the culture period (Fig. 5E and 5F). Mechanistically, we next tested whether Glut1 may mediate the effect of IL-3 on autophagy,43 since autophagy has recently emerged to regulate HSPC maintenance and a bias toward myelopoiesis.44,45 Western blot analysis of LC3-II protein levels, an hallmark of autophagy, revealed that Glut1 deficiency prevented the decrease of LC3-II expression in WT and ApoE-/- BM cells under basal and IL-3 stimulated conditions (Supplemental Fig. 2D). To analyze the autophagic flux of HSPCs, we next used the Cyto-ID probe allowing analysis by multicolor flow cytometry. Remarkably, Glut1 deficiency prevented the reduced Cyto-ID staining induced by IL-3 in HSPCs isolated from WT Lin- cultures and restored the autophagic flux of ApoE-/- HSPCs to the level of control cells (Supplemental Fig. 2E and 2F). These data identify that Glut1 is a key metabolic sensor mediating the growth-regulatory effects of IL-3 through autophagy-dependent modulation of HSPC expansion and myeloid commitment in vitro.

Reduced glycolytic activity in mice with hematopoietic Glut1 deficiency prevents ApoE-/- HSPC expansion and proliferation. To directly test the in vivo physiological relevance of Glut1 on ApoE-/- HSPCs, we transplanted the BM of single or combined knockout of ApoE (ApoE-/-) and Glut1 (Glut1+/-) into irradiated ApoE-/- mice. After a recovery period, the recipients were placed on a high fat diet (HFD) for 12 weeks using the similar experimental design described earlier (Fig. 6A). At the end of the study period, an analysis of the different populations within the HSPCs of these mice was performed (Supplemental Fig. 3). As shown in Figure 1F, low Glut1 cell surface expression was observed in the CD34- LT-HSCs (Fig. 6B and Supplemental Fig. 3D), which were also characterized by CD150+ and Flt3- markers (Supplemental Fig. 3A-C).34,35 Further analysis of the different populations within the HSPCs revealed higher Glut1 expression in the CD34+CD150+Flt3- multi-potent progenitors (MMP2) compared to the CD34+CD150-Flt3-

and CD34+CD150-Flt3+ multi-potent progenitors (MMP3 and MMP4, respectively)(Fig. 6B). Consistent with these findings, the Glut1 cell surface expression was decreased by an approximately 1.4-fold in the MMP2 of mice receiving either Glut1+/- or ApoE-/-Glut1+/- BM (Fig. 6B and Supplemental Fig. 3D). This confirmed the efficiency of the transplantation procedure. Interestingly, the 2-NBDG staining quantified by flow cytometry suggested Glut1-independent glucose utilization in different populations within the HSPCs, but confirmed an approximately 1.35-fold decrease in 2-NBDG staining in the CD34+CD150+Flt3- MMP2 of mice receiving either Glut1+/- or ApoE-/-Glut1+/- BM compared to their respective controls (Fig. 6C and Supplemental Fig. 3E). We next investigated the relationship between the proliferation capacity of the MMP2 and the Glut1-dependent glucose utilization. Although there was no significant decrease in the S/G2M fraction in the CD34+CD150+Flt3- MMP2 and other populations within the HSPCs of mice receiving Glut1+/- BM, a significant 1.3-fold decrease in the S/G2M fraction was observed in the CD34+CD150+Flt3- MMP2 and downstream CD34+CD150-Flt3- MMP3 of mice receiving ApoE-/-Glut1+/- BM compared to mice receiving ApoE-/- BM (Fig. 5D and Supplemental Fig. 3F). Quantification of the bone marrow HSPCs confirmed an approximately 1.4-fold decrease in the frequency of the CD34+CD150+Flt3- MMP2 and downstream MMPs in mice receiving ApoE-/-Glut1+/- BM compared to the controls (Fig. 6E). Similar findings were also observed in chow-fed ApoE-/-Glut1+/- BM-transplanted mice compared to the ApoE-/- BM-transplanted mice (data not shown). Together, these findings reveal that Glut1-dependent glucose utilization was required for ApoE-/- MMP2 proliferation and downstream MMP expansion.

Reduced myeloid commitment in ApoE-/- BM with Glut1 deficiency. While working on this manuscript, Pietras et al., elegantly showed that the CD34+CD150+Flt3- MMP2 and downstream CD34+CD150-Flt3- MMP3 exhibited a myeloid-biased multipotential progenitor phenotype.35 This prompted us to test whether the decreased MMP2 and MMP3 expansion observed in mice receiving ApoE-/-Glut1+/- BM could be associated with a defective myeloid fate specification. The common myeloid progenitor (CMP), granulocyte macrophage progenitor (GMP) and megakaryocyte-erythroid progenitor (MEP) populations were analyzed by flow cytometry (Supplemental Fig. 3G) and were not significantly reduced in the mice receiving Glut1+/- BM despite a trend towards CMPs (Fig. 6F). Nevertheless, the CMP numbers were significantly decreased by more than 1.2-fold in mice receiving ApoE-/-Glut1+/- BM compared to controls receiving ApoE-/- BM (Fig. 6F). We noticed that the splenomegaly in the mice receiving the ApoE-/- BM was rescued by Glut1 deficiency (Table S1), and the spleen represents an important reservoir of myeloid cells

through extramedullary hematopoiesis in ApoE-/- mice.6,33 Therefore, the hematopoietic progenitors were next quantified in this organ. Similar to the BM, we observed a 1.6-fold decrease in the frequency of splenic CMPs in mice receiving ApoE-/-Glut1+/- BM and, to some extent, a 1.3-fold decrease in the percentage of GMPs, but no changes in the MEP population (Fig. 6G). Consistent with these findings, the platelet and red blood cell counts, mean platelet volume (MPV) and hematocrit were unchanged in these mice (Table S1). Peripheral T- and B-cell numbers were also not affected in these mice (Supplemental Fig. 4A and 4B). In contrast, the blood counts indicated that the leukocytosis, monocytosis, neutrophilia and eosinophilia of mice receiving ApoE-/- BM in response to feeding a high fat diet were rescued by Glut1 deficiency (Fig. 6H to 6K). These data indicate that Glut1-dependent glucose utilization is required at the early stage of ApoE-/- HSPC commitment to the myeloid lineage.

Glut1 acts in a cell-autonomous fashion to regulate ApoE-/- HSPC proliferation and myelopoiesis. To test whether this phenotype was caused by cell autonomous effects of Glut1 within the myeloid-biased HSPCs or involved a cell extrinsic effect, we performed a competitive BM transplantation experiment with equally mixed BM cells from CD45.1 ApoE-/- mice and either CD45.2 ApoE-/- BM or CD45.2 ApoE-/-Glut1+/ BM into irradiated WT recipients. After BM reconstitution, we found that the frequency of CD45.1 ApoE-/- HSPCs, particularly the CD34+ HSPCs, were not affected by the presence of CD45.2 ApoE-/- Glut1+/ BM cells, despite the reduced frequency of the CD45.2 ApoE-/-Glut1+/ HSPCs (Fig. 7A). These findings mirrored the reduced S/G2M fraction in the CD45.2 ApoE-/-Glut1+/ HSPCs without altering the S/G2M fraction in the mixed CD45.1 ApoE-/- HSPCs (Fig. 7B). Consistent with these findings on BM HSPCs, there was a preferential accumulation of CD45.1 ApoE-/- vs. CD45.2 ApoE-/-Glut1+/- blood monocytes and neutrophils (Fig. 7C and 7D), indicative of a cell autonomous proliferative disadvantage of Glut1 deficiency.

Glut1 deficiency prevents the progression of atherosclerosis in ApoE-/- BM-transplanted mice. We next explored the in vivo relevance of reducing ApoE-/- HSPC proliferation and myelopoiesis through Glut1 deficiency on the development of atherosclerosis. This was tested in ApoE-/- recipient mice that received ApoE-/- or ApoE-/- Glut1+/- BM fed a high fat diet for 12 weeks (Fig. 6A). As shown in Table S1, the body weight, plasma LDL and HDL cholesterol or plasma glucose were not significantly different with regard to Glut1 deficiency. However, ApoE-/- mice receiving ApoE-/-Glut1+/- BM showed an approximately 1.4-fold decrease in the development of atherosclerosis in their proximal aortas (Fig. 8A). Immunohistochemical staining of the aortic root plaques revealed

that this phenotype was associated with a massive decrease in the F4/80+ macrophages in the ApoE-/-Glut1+/-BM-transplanted mice (Fig. 8B). We also examined the uptake of the radiolabeled D-glucose analog (2-[14C]-DG) after ex vivo incubation of the aortic arch and spleen from the ApoE-/- mice that received ApoE-/- or ApoE-/-Glut1+/- BM. ApoE-/-Glut1+/- BM-transplanted mice showed a significant 1.4-fold and 1.3-fold decrease in total uptake of 2-[14C]-DG in the aortic arch (Fig. 8C) and the spleen (Fig. 8D), respectively compared to controls. Thus, we showed that Glut1 connects the enhanced glucose uptake in atheromatous plaques of ApoE-/- mice,27-30 with their myelopoiesis through regulation of HSPC maintenance and myelomonocytic fate.

Discussion

Previous studies have shown that inflamed atherosclerotic plaques can be visualized by non-invasive PET-CT imaging with ^{18}FDG, a glucose analog, which correlates with macrophage accumulation and inflammation.[27-30] However, a recent study has called into question the relevance of these observations, as macrophage-specific overexpression of Glut1 did not aggravate atherosclerosis in mice compared to Glut1 sufficient controls,[46] reflecting the need for a better understanding of the underlying mechanisms. Our study provides direct evidence that Glut1 connects the enhanced glucose uptake in atheromatous plaques of *ApoE*$^{-/-}$ mice with their myelopoiesis through Glut1-dependent regulation of HSPC maintenance and myelomonocytic fate.

Recent studies have suggested that HSPC expansion and the associated myelopoiesis could underlie the hypercholesterolemia-induced atherosclerosis in mice.[1,2] However, the rate of ATP generation required for cell proliferation and differentiation cannot be directly explained by cholesterol and requires alternative sources of energy.[47] Our observations indicate that the leukocytes and HSPCs from hypercholesterolemic *ApoE*$^{-/-}$ mice exhibited an increased Glut1-dependent glucose uptake that was associated with increased mitochondrial potential suggesting that the influx of glycolytic metabolites in these cells fuel the mitochondria for oxidative phosphorylation and ATP generation.[21] The low expression of Glut1 in CD34$^-$ LT-HSCs compared to other CD34$^+$ HSPCs was first counterintuitive, as we initially speculated that the presence of the LT-HSCs in the 'hypoxic BM niche' could favor the expression of Glut1 by HIF1α.[37] However, increasing glucose metabolism through translocation of Glut1 to the cell surface is thought to be crucial for the active cells and cell cycle entry rather than quiescence.[48,49] During aerobic respiration, the ATP yield is linked to NAD$^+$-dependent oxidative steps, including oxidative decarboxylation of pyruvate, that

requires metabolic shuttle systems to convey reducing equivalents from cytosol to mitochondria.[41] Our findings indicate that both the oxidative decarboxylation of pyruvate and the transamination reactions of the malate-aspartate shuttle were essential for HSPC expansion and commitment to the myeloid lineage. Thus, it is probably not surprising that we did not observe an accumulation of succinate in *ApoE*$^{-/-}$ BM cultures due to higher succinate dehydrogenase activity favoring fumarate and malate production. The absence of succinate accumulation in *ApoE*$^{-/-}$ BM cells could also contribute to the lack of HIF1α activation in these cells.[50] Consistent with these observations, we did not observe modulation of the oxygenation status of *ApoE*$^{-/-}$ LT-HSCs in chronic hypercholesterolemia, and HIF1α deficiency in WT or *ApoE*$^{-/-}$ hematopoietic cells did not alter Glut1 expression or the HSPC frequency. In fact, HIF1α deficiency in hematopoietic cells rather led to increased myeloid expansion, which could contribute to the role of hypoxia in the development of atherosclerosis.[51] In contrast, and consistent with the alternative regulation of Glut1 by growth hormone-dependent activation of Ras or Src,[38,39] the enhanced Glut1 expression in proliferating *ApoE*$^{-/-}$ HSPCs was prevented by IL3Rβ blockade. This metabolic regulation was critical for the expansion of *ApoE*$^{-/-}$ HSPCs and the associated IL-3-dependent downregulation of autophagy,[43] which is most likely required to limit intracellular lysosomal degradation and fulfill the high-energy demand of these cells for proliferation.

Very recently, increased splenic activity in patients with cardiovascular diseases has been demonstrated by non-invasive PET-CT imaging with ^{18}FDG,[31,32] which could reflect the metabolic activity of extramedullary hematopoiesis required to generates monocytes that infiltrate atherosclerotic plaques.[33] However, these observations do not prove causality. Intriguingly, inhibition of glucose uptake with a 2-deoxyglucose (2-DG) analog has recently ben shown to inhibit myelopoiesis in human HSCs,[26] but the relevance to atherosclerosis has not been tested. The present study clearly establishes that the increased Glut1-dependent glucose utilization in the *ApoE*$^{-/-}$ HSPCs could divert these cells to a myelomonocytic fate leading to extramedullary myelopoiesis and subsequent macrophage deposition-dependent atherosclerotic plaque formation. Indeed, we now provide direct *in vivo* evidence that Glut1 deficiency can significantly reduce the number of CMPs in the *ApoE*$^{-/-}$ BM as well as the number of CMPs and GMPs in the *ApoE*$^{-/-}$ spleen and this was associated with reduced splenic glucose uptake. This led to inhibition of the monocytosis, neutrophilia and eosinophilia in the *ApoE*$^{-/-}$ mice transplanted with the *ApoE*$^{-/-}$ BM. Consistent with the lack of effect of Glut1 deficiency on resting T-cells,[51] we also did not observe variations in the

number of lymphocytes in our models. While working on this manuscript, Pietras et al., elegantly showed that the $CD34^{+}CD150^{+}Flt3^{-}$ MMP2, which is now showed to express the most Glut1, and downstream $CD34^{+}CD150^{-}Flt3^{-}$ MMP3 exhibited a myeloid-biased multipotential progenitor phenotype,[35] offering an alternative explanation to the role of Glut1 in favoring myelopoiesis that is independent of a change in other lineage commitments. Together, these findings reveal that the mechanism by which defective ApoE-dependent cholesterol efflux pathways skew hematopoietic stem cells towards myelopoiesis,[5,6] relies on the regulation of Glut1-dependent glucose uptake by the IL-3Rβ signaling pathway.

The metabolic phenotype of *ApoE*$^{-/-}$ HSPCs outlined here could be relevant to the adaptability of HSPCs to cholesterol overload and may indicate that the glycolytic phenotype of HSPCs is not merely a product of their hypoxic environment. Thus, the existence of different molecular mechanisms underlying the different glycolytic phenotypes in HSPCs may suggest strategies for specifically modulating the pool of HSPCs that are committed to the myeloid lineage under stressed conditions, such as in myeloproliferative disorders,[36] sepsis,[53] myocardium infarction,[54] or chronic atherosclerosis, as shown in the present study. Inhibition of glucose uptake by a Glut1 inhibitor that does not cross the blood-brain barrier could ultimately provide a novel therapeutic approach to prevent myelopoiesis-driven diseases such as atherosclerosis.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A method of treating myelopoiesis-driven cardiometabolic diseases and sepsis in a subject in need thereof comprising the step of administering the subject with a therapeutically effective amount of an agent selected from the group consisting of GLUT1 inhibitors and GOTs inhibitors.

2. The method according to claim 1, wherein the myelopoiesis-driven cardiometabolic disease is atherosclerosis.

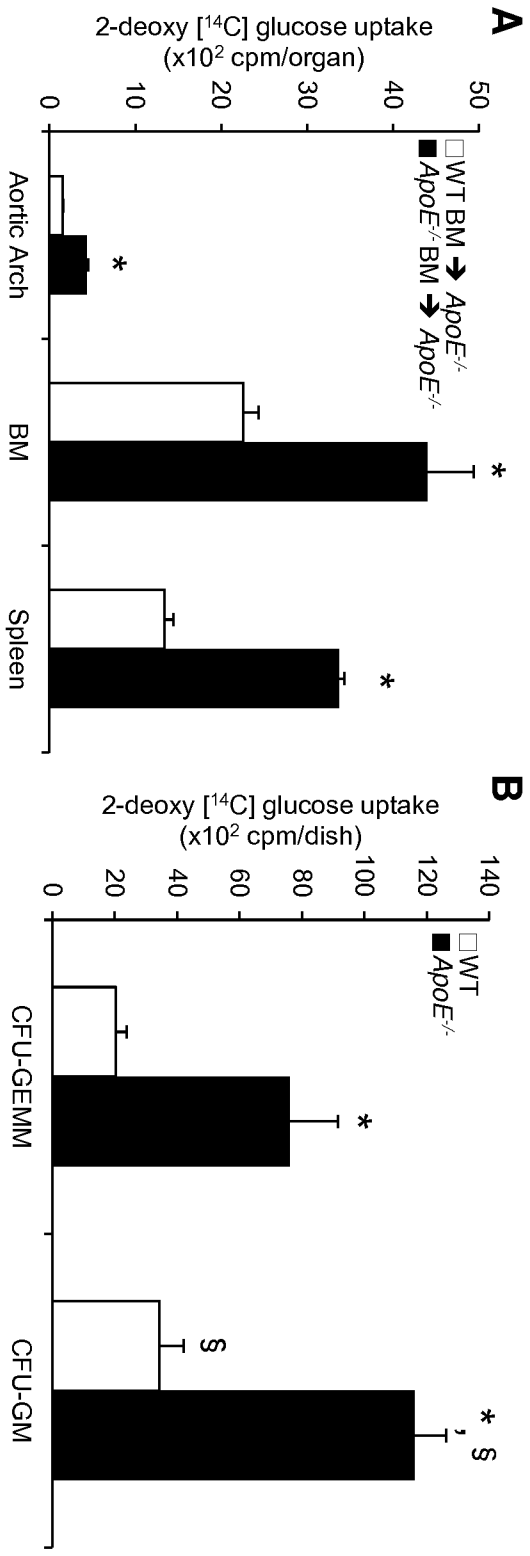
3. The method according to claim 1, wherein the inhibitor of Glut1 is a small organic molecule.

4. The method according to claim 1, wherein the inhibitor of Glut1 is WZB117.

5. The method according to claim 1, wherein the inhibitor of Glut1 is Fasentin.

6. . The method according to claim 1, wherein the inhibitor of GLUT1 is an antibody.

7. The method according to claim 1, wherein the inhibitor of GOTs belongs to the aminooxyacetic acid (AOA) class.


A
2-deoxy [14C] glucose uptake
(x10² cpm/organ)
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10
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□WT BM → ApoE-/-
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Aortic Arch
BM
Spleen
*
*
*
B
2-deoxy [14C] glucose uptake
(x10² cpm/dish)
0
20
40
60
80
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140
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■ApoE-/-
CFU-GEMM
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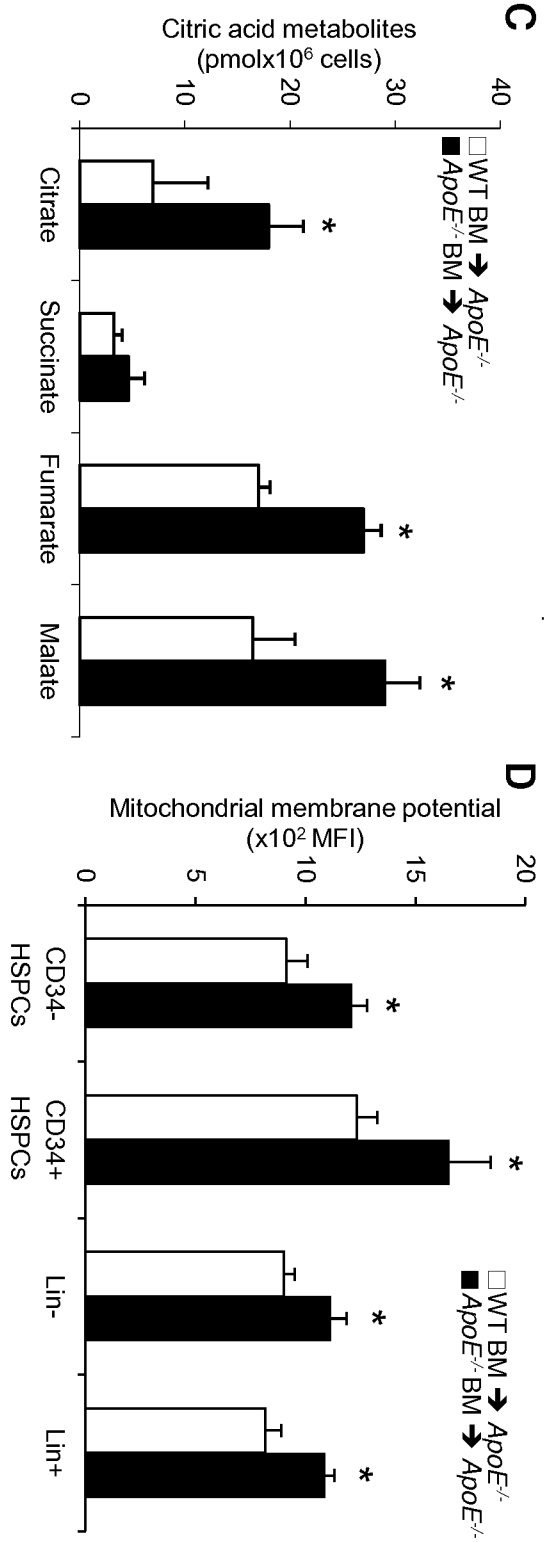
Figure 1A&B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Citric acid metabolites
(pmolx10^6 cells)
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30
40
Citrate
Succinate
Fumarate
Malate
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WT BM → ApoE-/-
ApoE-/- BM → ApoE-/-
D
Mitochondrial membrane potential
(x10^2 MFI)
0
5
10
15
20
CD34- HSPCs
CD34+ HSPCs
Lin-
Lin+
*
WT BM → ApoE-/-
ApoE-/- BM → ApoE-/-

Figure 1C&D

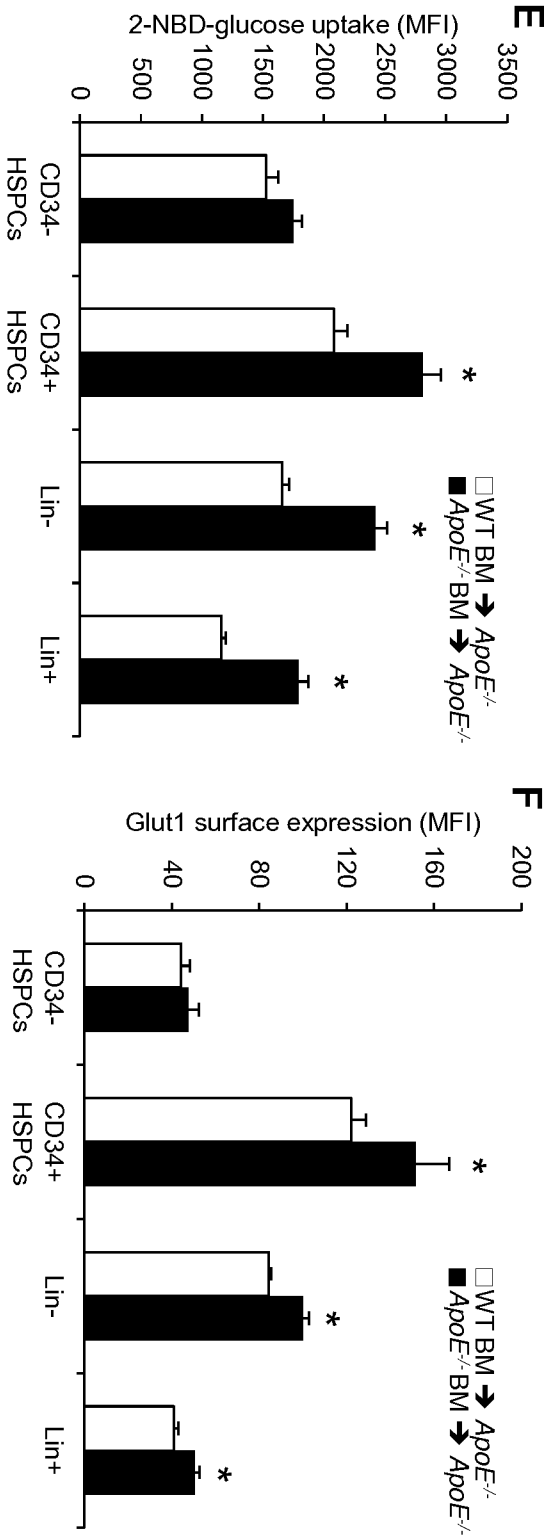
E
2-NBD-glucose uptake (MFI)
0
500
1000
1500
2000
2500
3000
3500
CD34- HSPCs
CD34+ HSPCs
Lin-
Lin+
*
WT BM → ApoE-/-
ApoE-/- BM → ApoE-/-
F
Glut1 surface expression (MFI)
0
40
80
120
160
200
CD34- HSPCs
CD34+ HSPCs
Lin-
Lin+
*
WT BM → ApoE-/-
ApoE-/- BM → ApoE-/-

Figure 1E&F

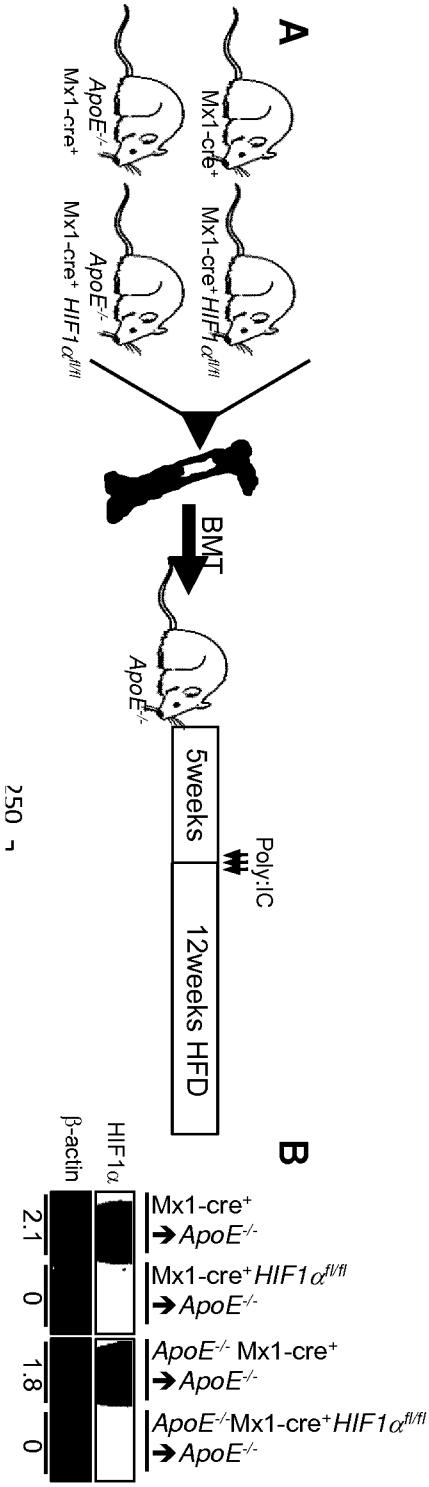
A
Mx1-cre+
ApoE-/-
Mx1-cre+
Mx1-cre+ HIF1αfl/fl
ApoE-/-
Mx1-cre+ HIF1αfl/fl
BMT
ApoE-/-
5weeks
Poly:IC
12weeks HFD
B
β-actin
HIF1α
2.1
0
1.8
0
Mx1-cre+
→ApoE-/-
Mx1-cre+HIF1αfl/fl
→ApoE-/-
ApoE-/- Mx1-cre+
→ApoE-/-
ApoE-/-Mx1-cre+HIF1αfl/fl
→ApoE-/-

Figure 2A&B

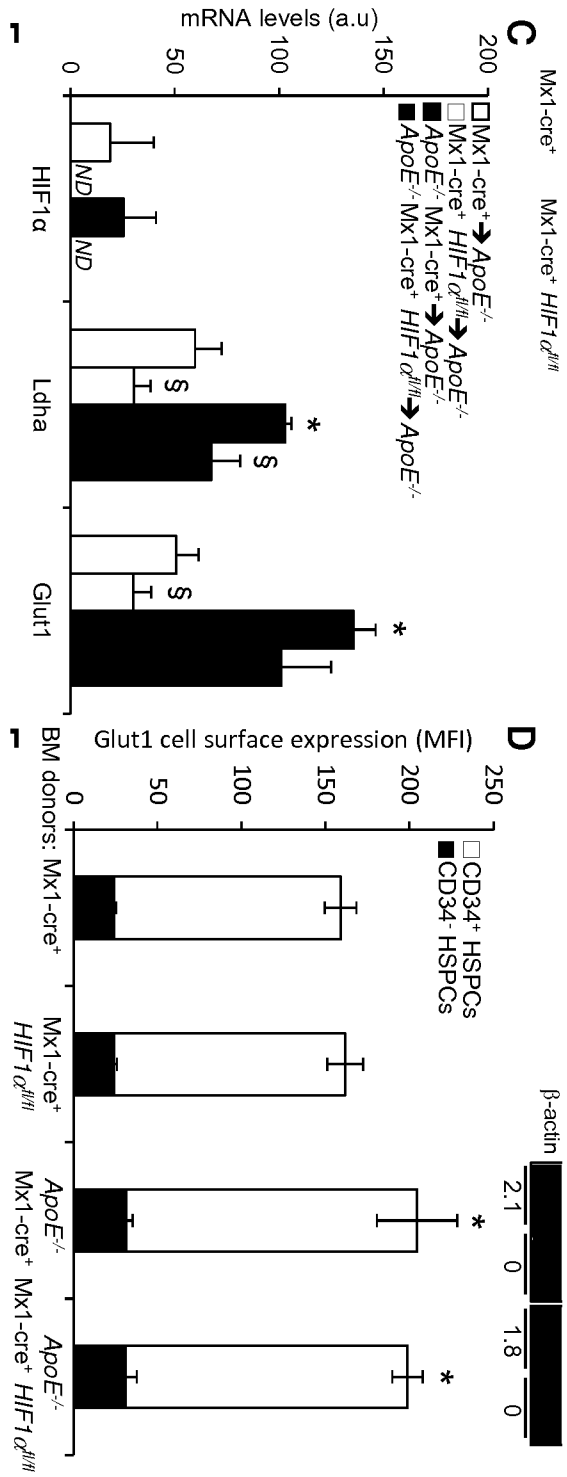
C
mRNA levels (a.u)
200
150
100
50
0
Mx1-cre+
Mx1-cre+ HIF1α fl/fl
Mx1-cre+ → ApoE-/-
Mx1-cre+ HIF1α fl/fl → ApoE-/-
ApoE-/- Mx1-cre+ → ApoE-/-
ApoE-/- Mx1-cre+ HIF1α fl/fl → ApoE-/-
HIF1α
Ldha
Glut1
ND
ND
S
S
S
S
*
*
D
Glut1 cell surface expression (MFI)
250
200
150
100
50
0
CD34+ HSPCs
CD34- HSPCs
BM donors: Mx1-cre+
Mx1-cre+ HIF1α fl/fl
ApoE-/- Mx1-cre+
ApoE-/- Mx1-cre+ HIF1α fl/fl
*
*
β-actin
2.1
0
1.8
0

Figure 2C&D

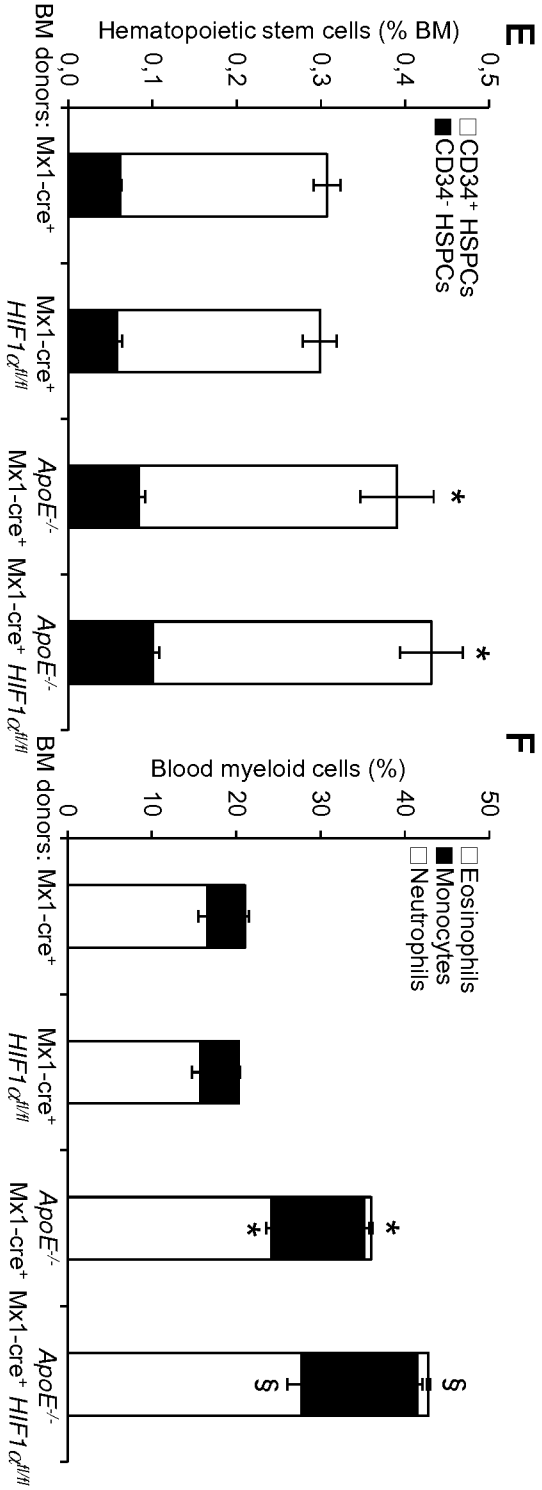
E
Hematopoietic stem cells (% BM)
0,0
0,1
0,2
0,3
0,4
0,5
CD34+ HSPCs
CD34- HSPCs
BM donors: Mx1-cre+
Mx1-cre+ HIF1α fl/fl
ApoE-/- Mx1-cre+
ApoE-/- Mx1-cre+ HIF1α fl/fl
*
*
F
Blood myeloid cells (%)
0
10
20
30
40
50
Eosinophils
Monocytes
Neutrophils
BM donors: Mx1-cre+
Mx1-cre+ HIF1α fl/fl
ApoE-/- Mx1-cre+
ApoE-/- Mx1-cre+ HIF1α fl/fl
*
*
§
§

Figure 2E&F

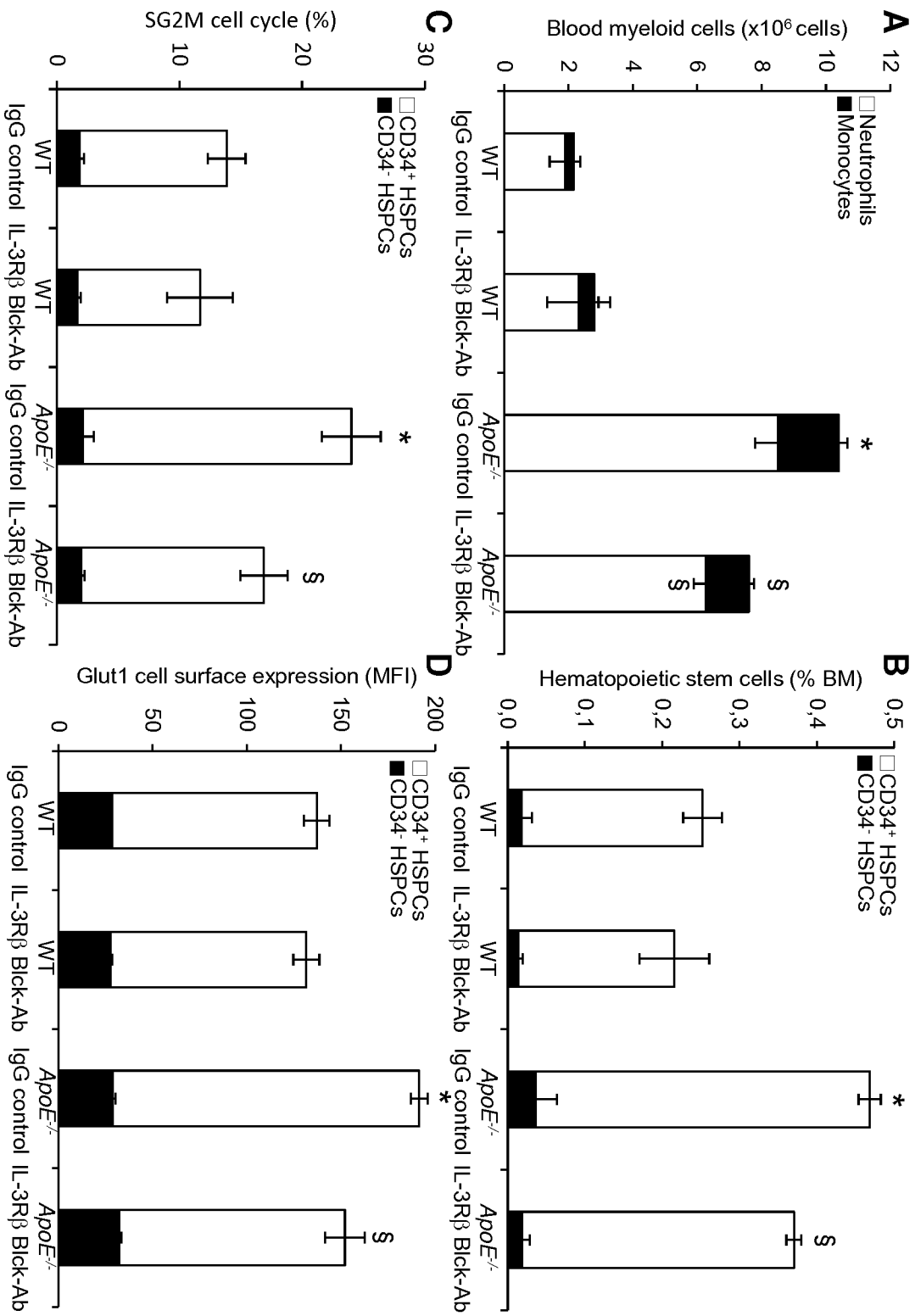
A
Blood myeloid cells (x10⁶ cells)
Neutrophils
Monocytes
B
Hematopoietic stem cells (% BM)
CD34+ HSPCs
CD34- HSPCs
C
SG2M cell cycle (%)
D
Glut1 cell surface expression (MFI)
WT
ApoE-/-
IgG control
IL-3Rβ Blck-Ab

Figure 3

A

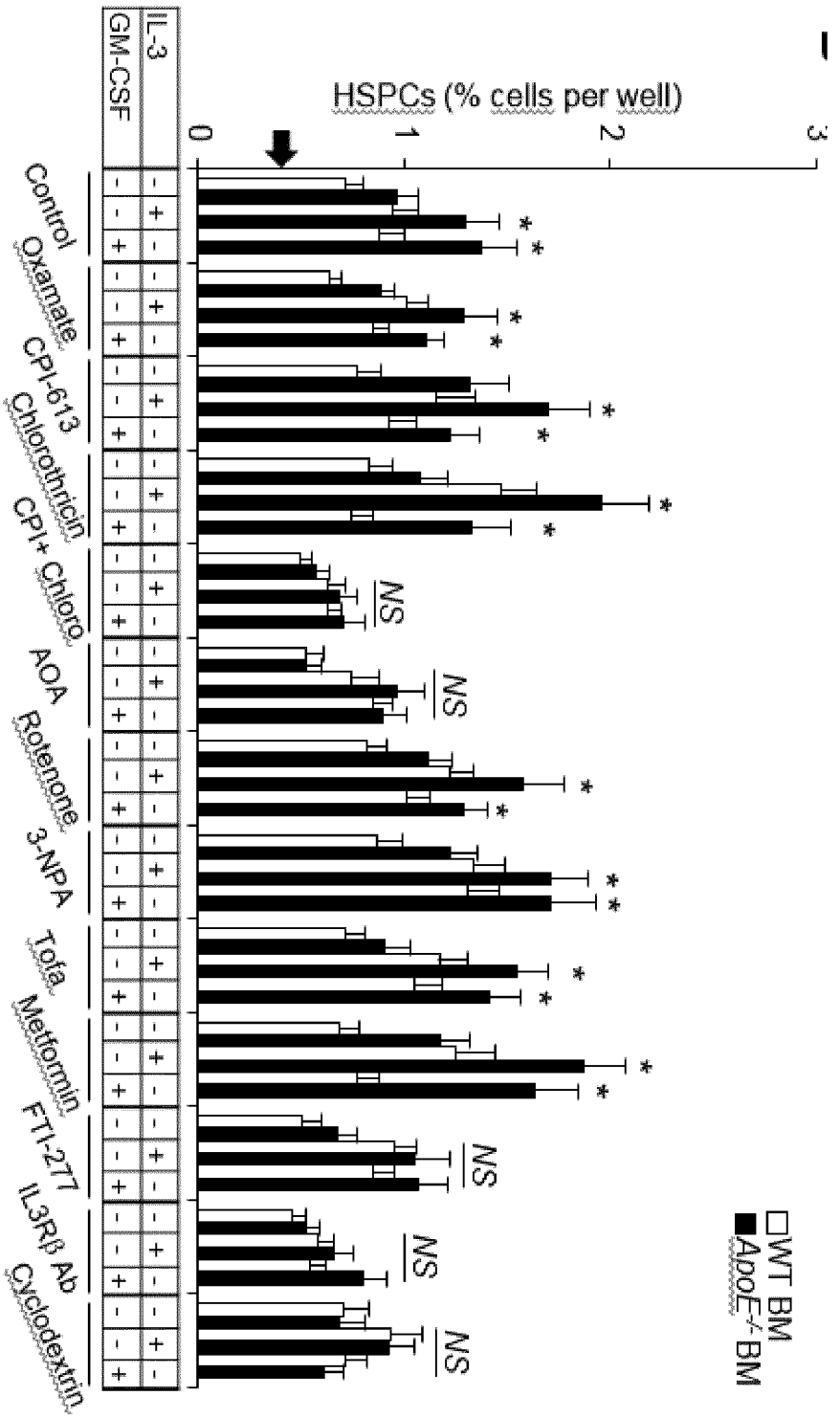
HSPCs (% cells per well)
0
1
2
3
GM-CSF
IL-3
Control
Oxamate
CPI-613
Chlorothricin
CPI+ Chloro
AOA
Rotenone
3-NPA
Tofa
Metformin
FTI-277
IL3Rβ Ab
Cyclodextrin
NS
WT BM
ApoE-/- BM

Figure 4A

B

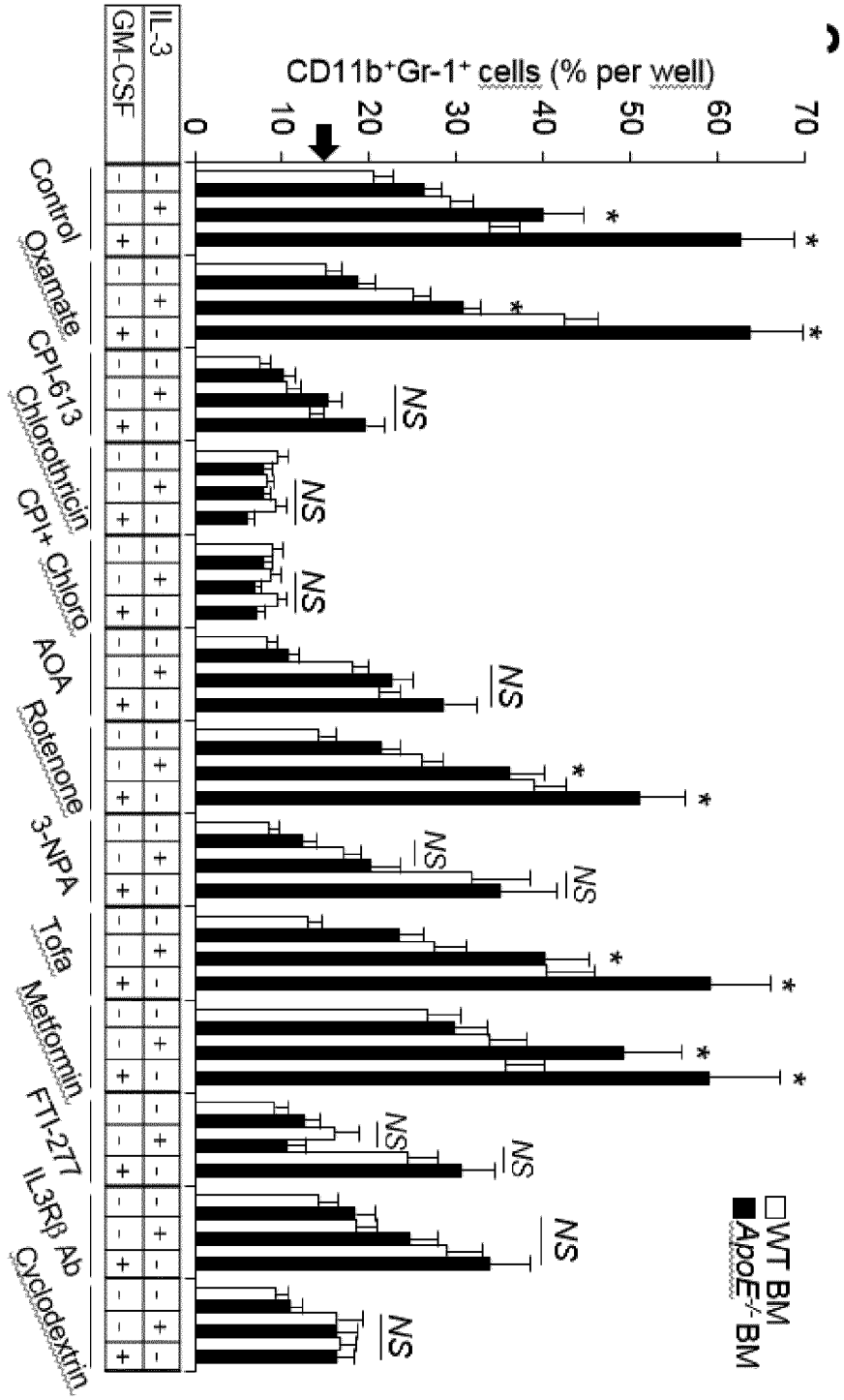
CD11b+Gr-1+ cells (% per well)
0
10
20
30
40
50
60
70
GM-CSF
IL-3
Control
Oxamate
CPI-613
Chlorothricin
CPI+ Chloro
AOA
Rotenone
3-NPA
Tofa
Metformin
FTI-277
IL3Rβ Ab
Cyclodextrin
NS
*
WT BM
ApoE-/- BM

Figure 4B

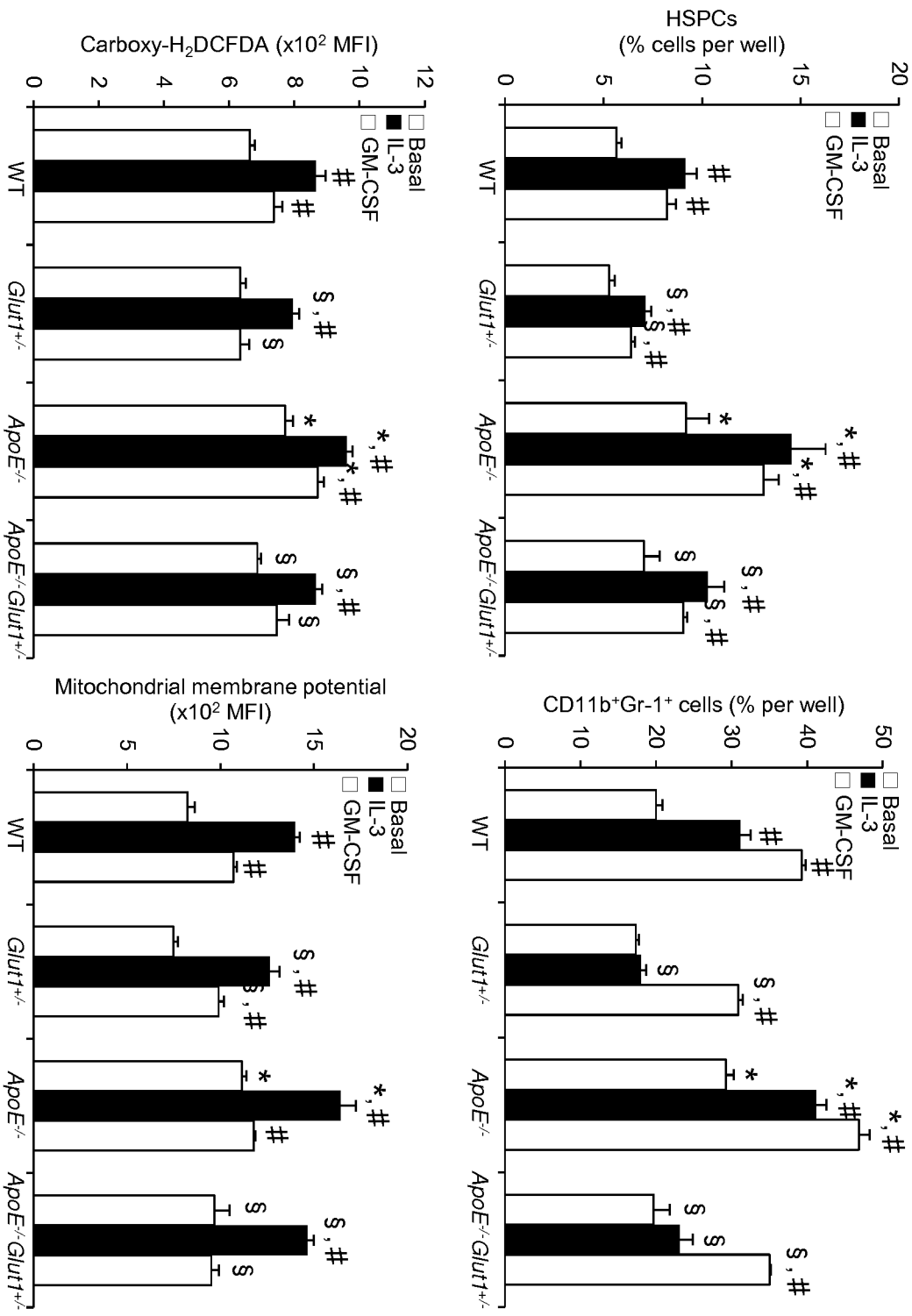
HSPCs
(% cells per well)
Carboxy-H2DCFDA (x10² MFI)
CD11b⁺Gr-1⁺ cells (% per well)
Mitochondrial membrane potential
(x10² MFI)
Basal
IL-3
GM-CSF
WT
Glut1+/-
ApoE-/-
ApoE-/-Glut1+/-

Figure 5

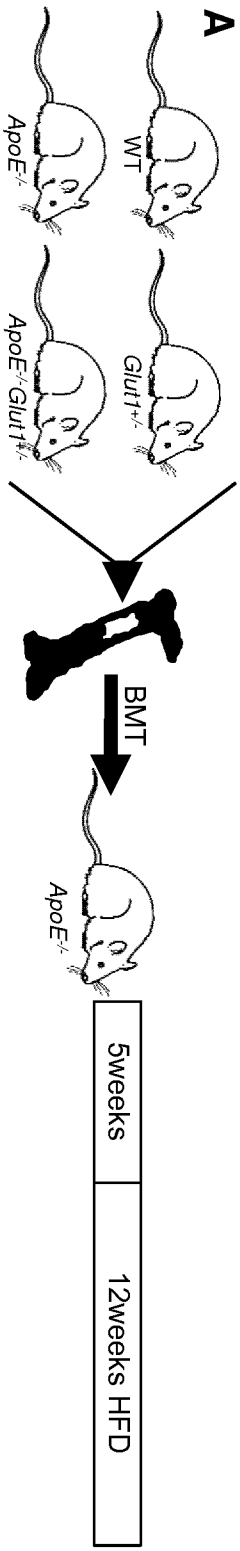

Figure 6A

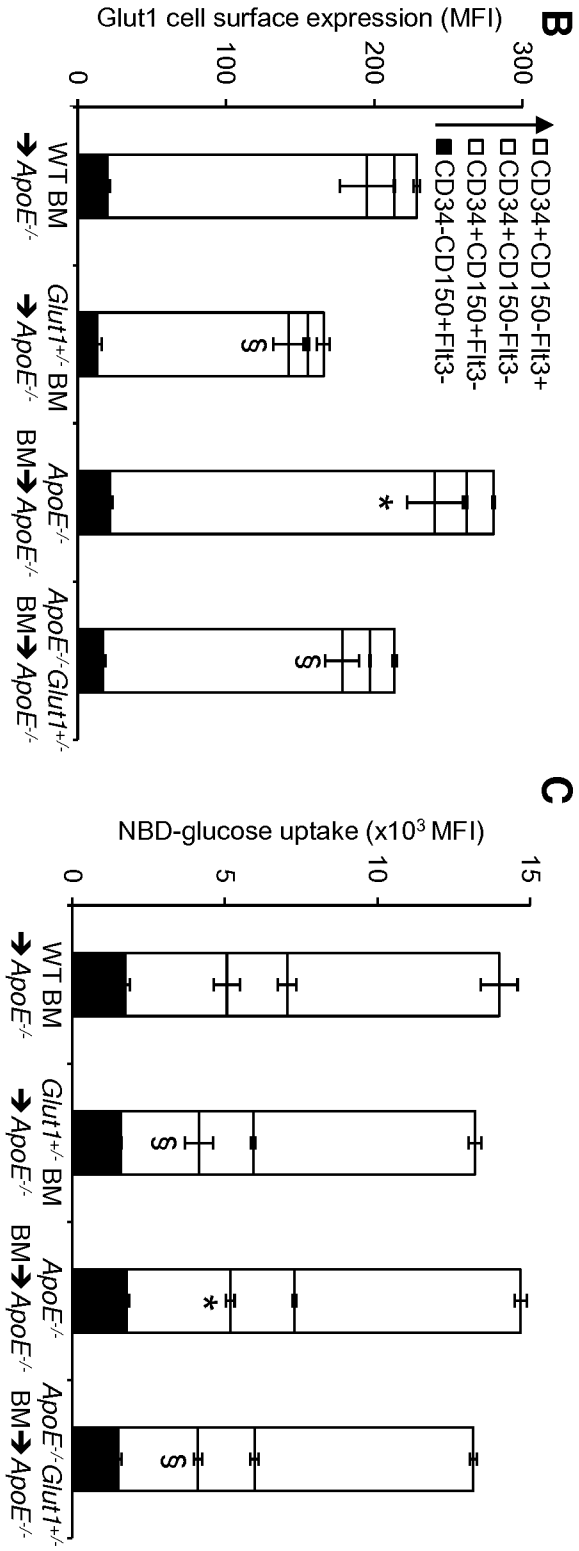
B
Glut1 cell surface expression (MFI)
0
100
200
300
CD34+CD150-Flt3+
CD34+CD150-Flt3-
CD34+CD150+Flt3-
CD34-CD150+Flt3-
WT BM → ApoE-/-
Glut1+/- BM → ApoE-/-
ApoE-/- BM → ApoE-/-
ApoE-/-Glut1+/- BM → ApoE-/-
ns
*
ns
C
NBD-glucose uptake (x10³ MFI)
0
5
10
15
WT BM → ApoE-/-
Glut1+/- BM → ApoE-/-
ApoE-/- BM → ApoE-/-
ApoE-/-Glut1+/- BM → ApoE-/-
ns
*
ns

Figure 6B&C

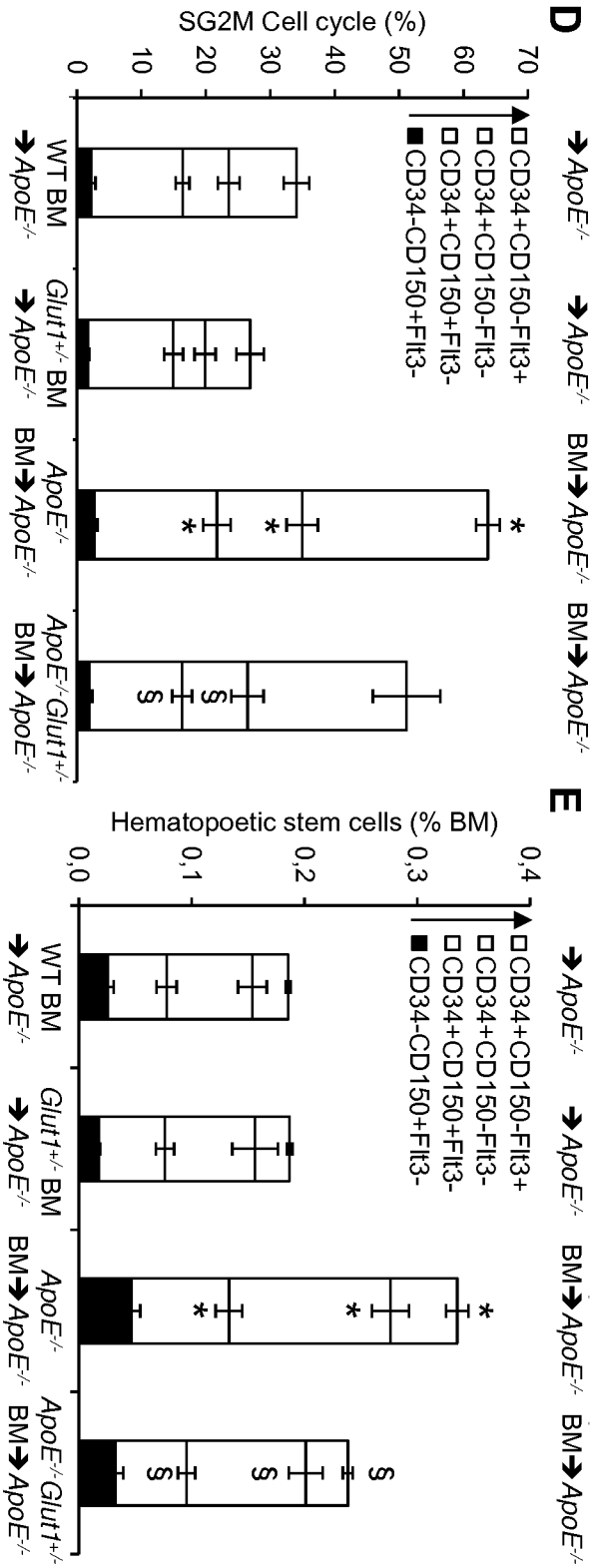
D
SG2M Cell cycle (%)
0
10
20
30
40
50
60
70
CD34+CD150-Flt3+
CD34+CD150-Flt3-
CD34+CD150+Flt3-
CD34-CD150+Flt3-
ApoE-/- → ApoE-/-
BM → ApoE-/-
BM → ApoE-/-
WT BM → ApoE-/-
Glut1+/- BM → ApoE-/-
ApoE-/- BM → ApoE-/-
ApoE-/-Glut1+/- BM → ApoE-/-
E
Hematopoetic stem cells (% BM)
0,0
0,1
0,2
0,3
0,4

Figure 6D&E

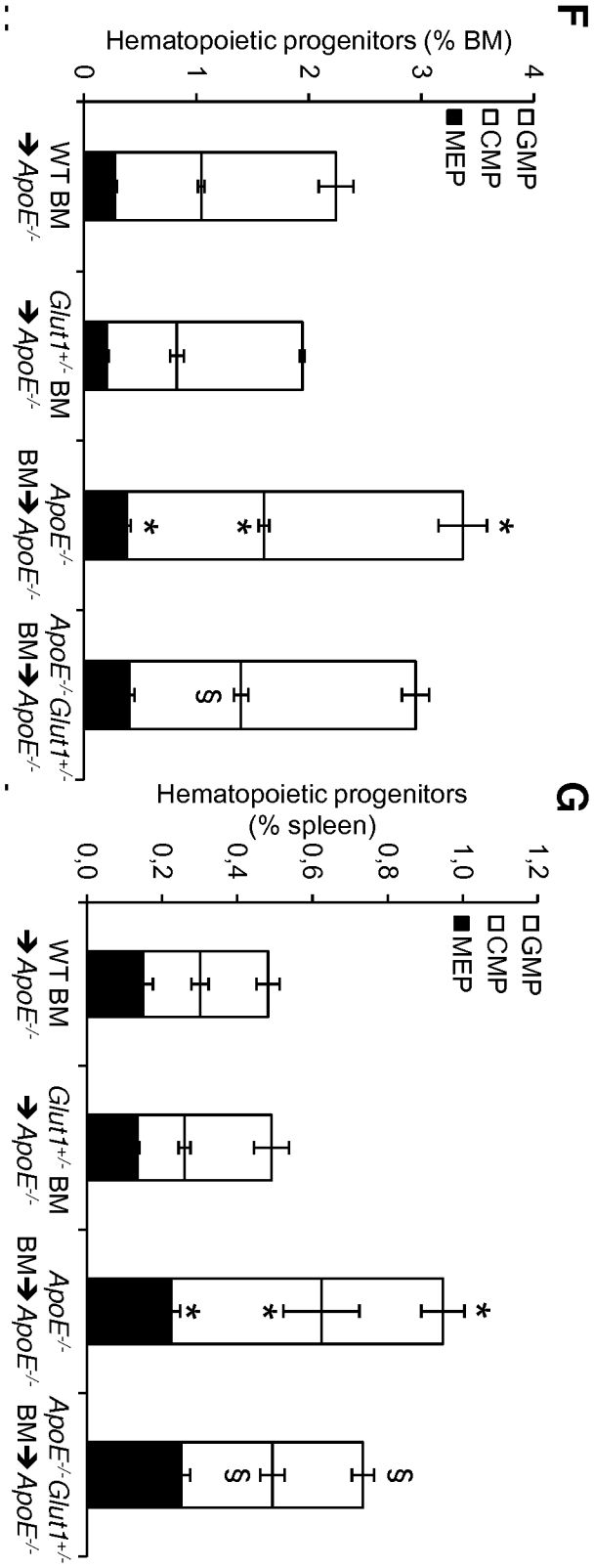
F
Hematopoietic progenitors (% BM)
0
1
2
3
4
GMP
CMP
MEP
WT BM → ApoE-/-
Glut1+/- BM → ApoE-/-
BM → ApoE-/-
BM → ApoE-/-
ApoE-/-Glut1+/-
*
*
*
§§
G
Hematopoietic progenitors (% spleen)
0,0
0,2
0,4
0,6
0,8
1,0
1,2
GMP
CMP
MEP
WT BM → ApoE-/-
Glut1+/- BM → ApoE-/-
BM → ApoE-/-
BM → ApoE-/-
ApoE-/-Glut1+/-
*
*
*
§§

Figure 6F&G

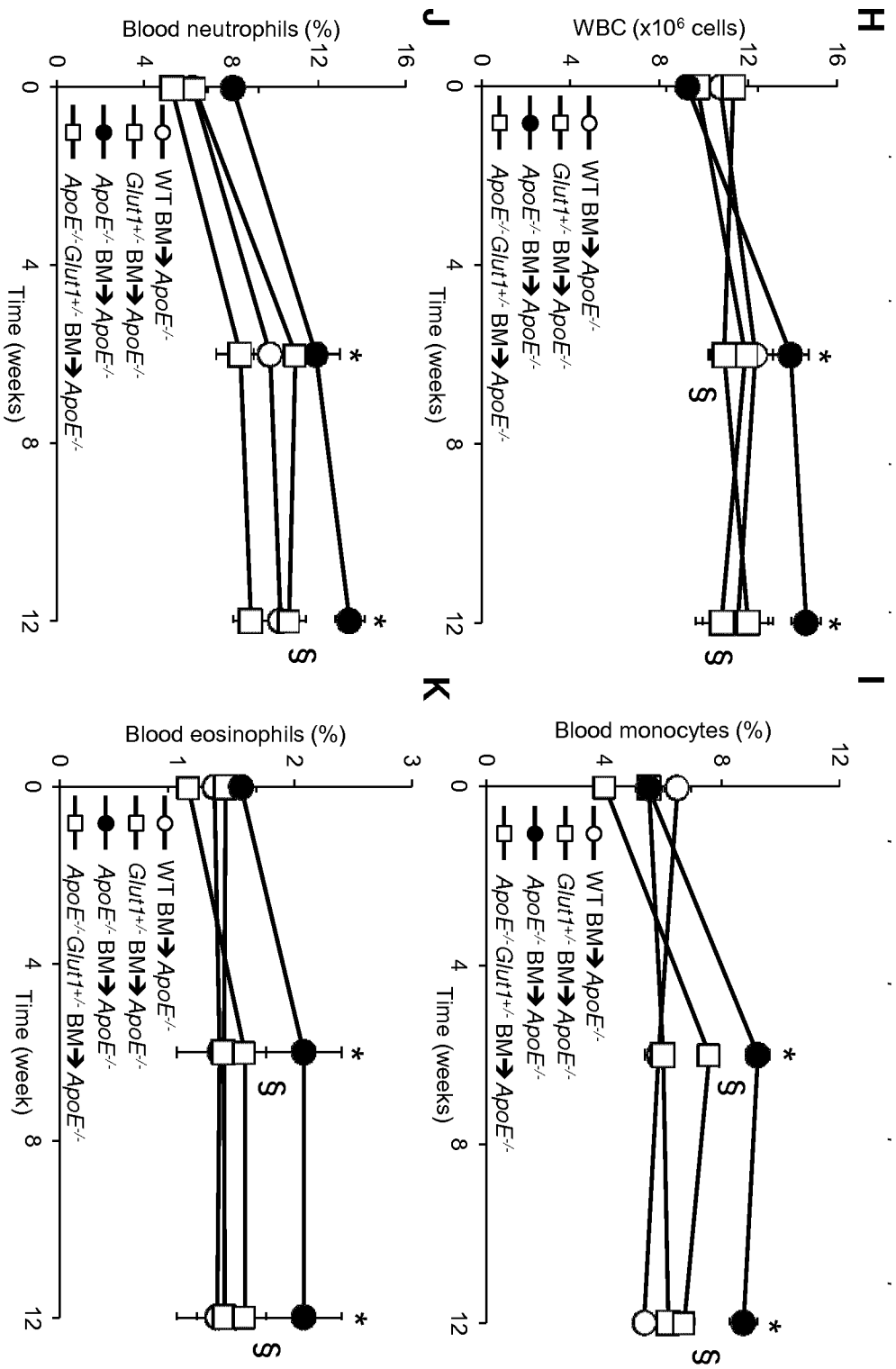

Figure 6 H, I, J & K

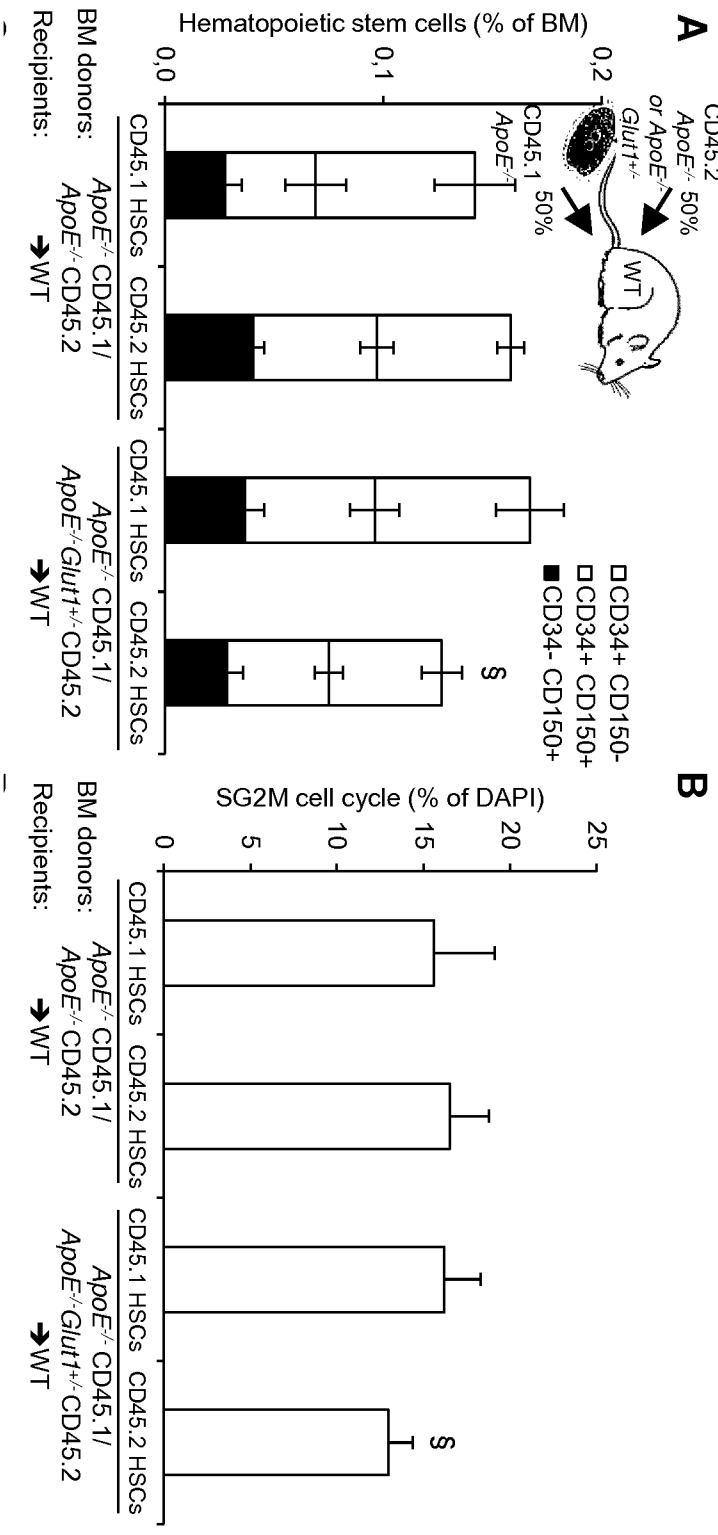
A
CD45.2 ApoE-/- 50%
or ApoE-/-Glut1+/-
CD45.1 ApoE-/- 50%
WT
Hematopoietic stem cells (% of BM)
0,0
0,1
0,2
CD34+ CD150-
CD34+ CD150+
CD34- CD150+
§
CD45.1 HSCs CD45.2 HSCs CD45.1 HSCs CD45.2 HSCs
BM donors: ApoE-/- CD45.1/ ApoE-/- CD45.2 ApoE-/- CD45.1/ ApoE-/-Glut1+/- CD45.2
Recipients: WT WT
B
SG2M cell cycle (% of DAPI)
0
5
10
15
20
25
§
CD45.1 HSCs CD45.2 HSCs CD45.1 HSCs CD45.2 HSCs
BM donors: ApoE-/- CD45.1/ ApoE-/- CD45.2 ApoE-/- CD45.1/ ApoE-/-Glut1+/- CD45.2
Recipients: WT WT

Figure 7A&B

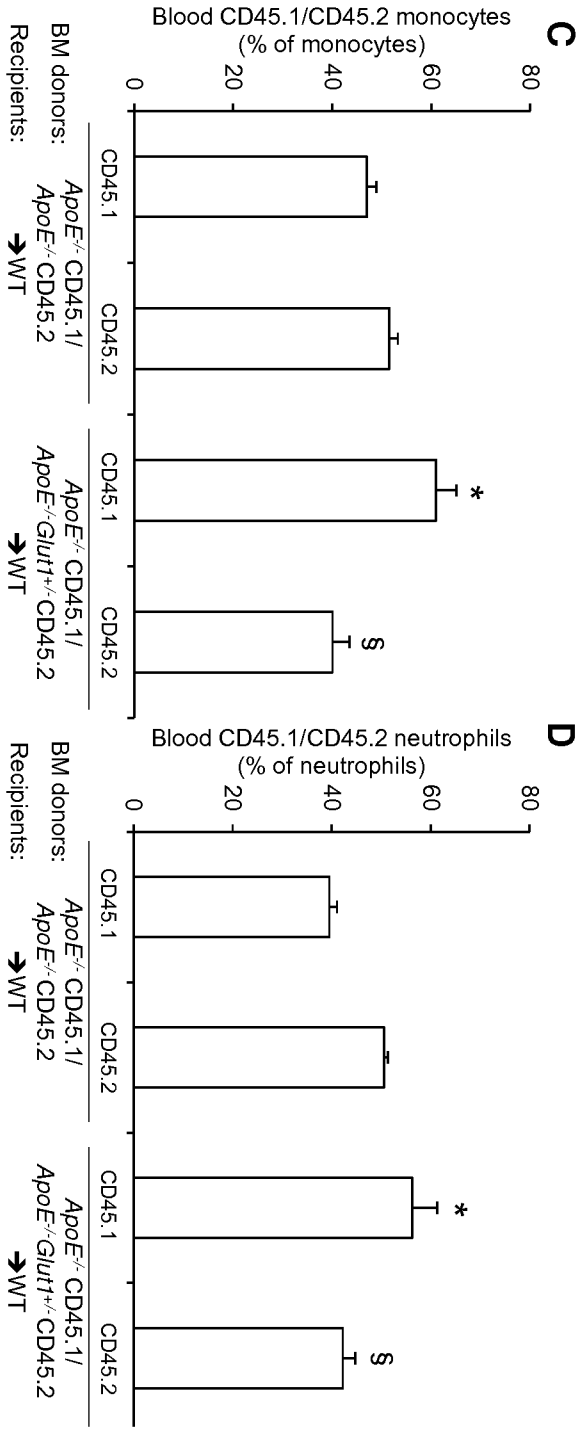
C
Blood CD45.1/CD45.2 monocytes
(% of monocytes)
0
20
40
60
80
CD45.1
CD45.2
CD45.1
CD45.2
*
§
BM donors:
Recipients:
ApoE-/- CD45.1/
ApoE-/- CD45.2
➔WT
ApoE-/- CD45.1/
ApoE-/-Glut1+/- CD45.2
➔WT
D
Blood CD45.1/CD45.2 neutrophils
(% of neutrophils)
0
20
40
60
80
CD45.1
CD45.2
CD45.1
CD45.2
*
§
BM donors:
Recipients:
ApoE-/- CD45.1/
ApoE-/- CD45.2
➔WT
ApoE-/- CD45.1/
ApoE-/-Glut1+/- CD45.2
➔WT

Figure 7 C&D

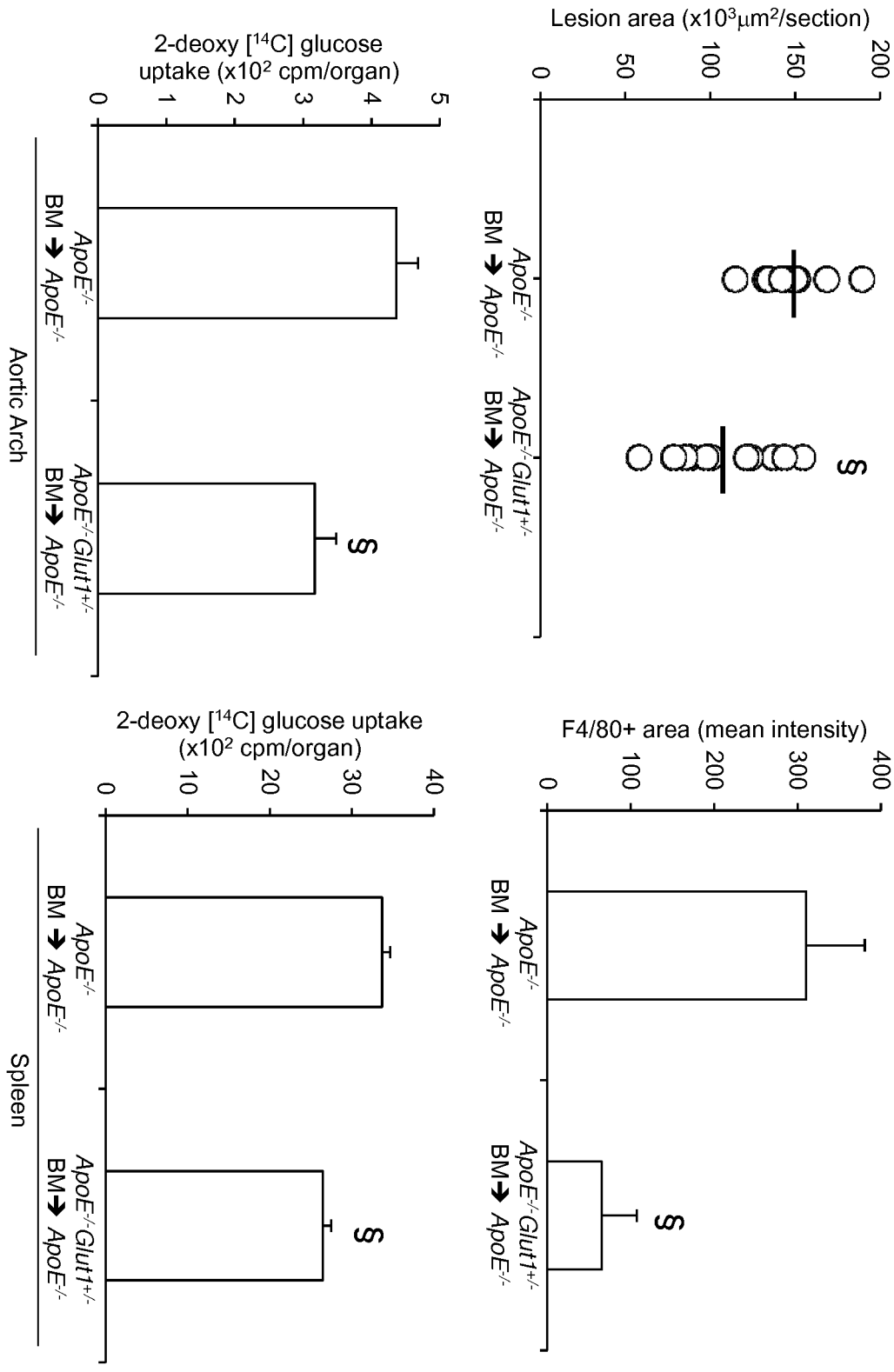
Lesion area (x10³μm²/section)
0
50
100
150
200
ApoE-/- BM → ApoE-/-
ApoE-/-Glut1+/- BM→ ApoE-/-
$
F4/80+ area (mean intensity)
0
100
200
300
400
ApoE-/- BM → ApoE-/-
ApoE-/-Glut1+/- BM→ ApoE-/-
$
2-deoxy [14C] glucose uptake (x10² cpm/organ)
Aortic Arch
0
1
2
3
4
5
ApoE-/- BM → ApoE-/-
ApoE-/-Glut1+/- BM→ ApoE-/-
$
2-deoxy [14C] glucose uptake (x10² cpm/organ)
Spleen
0
10
20
30
40
ApoE-/- BM → ApoE-/-
ApoE-/-Glut1+/- BM→ ApoE-/-
$

Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/051418

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/197 A61K39/00 A61K45/00 A61K31/235 A61K31/166
A61P9/10 A61P31/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/314740 A1 (CAMPOCHIARO PETER A [US] ET AL) 23 October 2014 (2014-10-23) claims -----	1-6
X	ANUP SRIVASTAVA ET AL: "PKM2 regulates the Warburg effect and promotes HMGB1 release in sepsis", FRONTIERS IN PHYSIOLOGY, vol. 5, 9 March 2015 (2015-03-09), page 4436, XP055276187, CH ISSN: 1664-042X, DOI: 10.1038/ncomms5436 page 2 -----	1-7
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
16 March 2017	02/06/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Büttner, Ulf

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. J. LEE ET AL: "Oxidized Low-Density Lipoprotein Stimulates Macrophage 18F-FDG Uptake via Hypoxia-Inducible Factor-1 Activation Through Nox2-Dependent Reactive Oxygen Species Generation", THE JOURNAL OF NUCLEAR MEDICINE, vol. 55, no. 10, 1 October 2014 (2014-10-01), pages 1699-1705, XP055276190, US ISSN: 0161-5505, DOI: 10.2967/jnumed.114.139428 figure 2	1-7
X	TOMOHIRO NISHIZAWA ET AL: "Testing the Role of Myeloid Cell Glucose Flux in Inflammation and Atherosclerosis", CELL REPORTS, vol. 7, no. 2, 1 April 2014 (2014-04-01), pages 356-365, XP055276493, US ISSN: 2211-1247, DOI: 10.1016/j.celrep.2014.03.028 page 364	1-7
X	CHIONG MARIO ET AL: "Influence of glucose metabolism on vascular smooth muscle cell proliferation", VASA, BERN, CH, vol. 42, no. 1, 1 January 2013 (2013-01-01), pages 8-16, XP009190260, ISSN: 0301-1526 page 10	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2017/051418

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

2(completely); 1, 3-7(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 2(completely); 1, 3-7(partially)

   A method of treating myelopoiesis-driven cardiometabolic diseases in a subject in need thereof comprising the step of administrating the subject with a therapeutically effective amount of an agent selected from the group consisting of GLUT 1 inhibitors and GOTs inhibitors.

   ---

2. claims: 1, 3-7(all partially)

   A method of treating sepsis in a subject in need thereof comprising the step of administrating the subject with a therapeutically effective amount of an agent selected from the group consisting of GLUT 1 inhibitors and GOTs inhibitors.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/051418

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014314740 A1	23-10-2014	US 2014314740 A1	23-10-2014
		WO 2013086515 A1	13-06-2013