(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
03 November 2022 (03.11.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/228086 A1

(51) International Patent Classification:
C12N 15/113 (2010.01) *C12N 15/12* (2006.01)
A61K 38/17 (2006.01) *C12N 15/867* (2006.01)
C12N 7/01 (2006.01) *C12N 5/10* (2006.01)

(21) International Application Number:
PCT/CN2022/085852

(22) International Filing Date:
08 April 2022 (08.04.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
202110455720.7 26 April 2021 (26.04.2021) CN
202110761757.2 06 July 2021 (06.07.2021) CN

(71) Applicant: **BEIJING MEIKANG GENO-IMMUNE BIOTECHNOLOGY CO., LTD.** [CN/CN]; Room B104, 1st Floor, No.5 Kaituo Road, Haidian District, Beijing 100085 (CN).

(72) Inventors: **CHANG, Lung-Ji**; Room B104, 1st Floor, No.5 Kaituo Road, Haidian District, Beijing 100085 (CN). **YUAN, Haokun**; Room B104, 1st Floor, No.5 Kaituo Road, Haidian District, Beijing 100085 (CN). **ZHANG, Rui**; Room B104, 1st Floor, No.5 Kaituo Road, Haidian District, Beijing 100085 (CN).

(74) Agent: **BEYOND ATTORNEYS AT LAW**; F6, Xijin Centre, 39 Lianhuachi East Rd., Haidian District, Beijing 100036 (CN).

(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, IT, JM, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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: MYELOID-SPECIFIC PROMOTER AND USE THEREOF

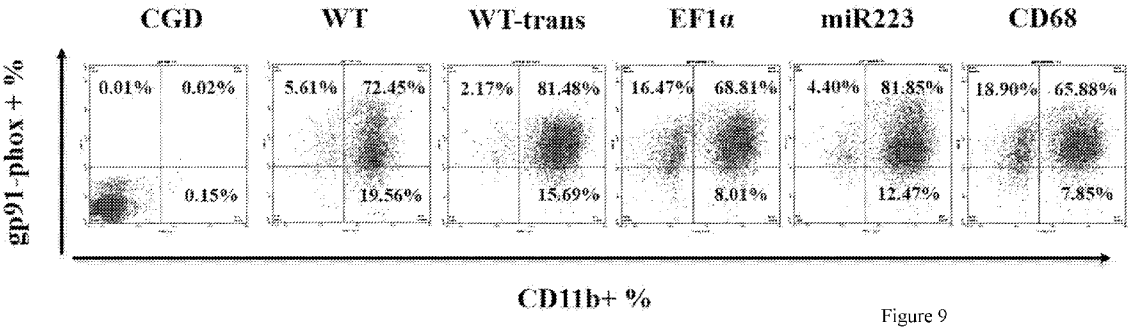

Figure 9

(57) Abstract: Provided are a myeloid-specific promoter and a use thereof. The myeloid-specific promoter includes a nucleic acid sequence as shown in SEQ ID NO: 1 or SEQ ID NO: 2. The myeloid-specific promoter shows specificity to myeloid tissues. It initiates a gene expression with high efficiency in myeloid cells, but with relative low efficiency in non-myeloid cells. As such, the myeloid-specific promoter regulates the specific expression of a gene in myeloid tissues. The myeloid-specific promoter and the CYBB gene are inserted into a lentiviral vector, and the constructed lentiviral expression vector shows specificity to myeloid tissues and can effectively restore the expression of gp91-phox protein and restore the generation function of ROS, which is of great significance for CGD treatment.

WO 2022/228086 A1

MYELOID-SPECIFIC PROMOTER AND USE THEREOF

TECHNICAL FIELD

The present disclosure belongs to the technical field of genetic engineering and relates to a myeloid-specific promoter and a use thereof.

BACKGROUND

Chronic granulomatous disease (CGD) is a hereditary primary immunodeficiency disease affecting neutrophils and monocytes due to defects in functions of a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The CGD is characterized by recurrent severe infections, inflammations and autoimmunity.

The NADPH oxidase consists of a membrane-bound protein and a cytoplasmic protein, which act synergistically, when phagocytes are activated, to help produce reactive oxygen species (ROS) to kill bacteria and fungi. A mutation in any of the five subunits of the NADPH oxidase will result in CGD syndrome. Approximately 67% of CGD cases are caused by defects of cytochrome b-245 beta chain (*CYBB*) gene on the X chromosome, which encodes transmembrane glycoprotein gp91-phox subunit.

At present, hematopoietic stem cell transplantation (HSCT) is the main method for treating CGD. HSCT needs thorough myeloablative preconditioning and needs to find an allogeneic human leukocyte antigen (HLA)-matched donor. However, in most cases, it is difficult for a patient to find a HLA-matched donor. Moreover, HSCT also has a risk of graft-versus-host disease (GVHD). In addition to the problems such as transplantation failure, high mortality and low donor chimerism, HSCT may also lead to immune rejection in the patient, which makes re-transplantation of hematopoietic stem cells very difficult.

Gene therapy refers to that a normal exogenous gene is introduced into target cells to correct or compensate for a defective gene and an abnormal gene for the purpose of treating a disease caused by the defective gene and the abnormal gene. Gene therapy for CGD began in the late 1990s when researchers attempted to use an adenovirus vector for CGD gene therapy. In addition to the inability to express the exogenous gene efficiently and continuously, the method also has the problem that the vector causes an immune response.

γ-retroviral vectors (γ-RVs) are also used for CGD treatment, but only a limited therapeutic effect is achieved. Kang *et al.* performed a gene therapy clinical trial on three X-CGD patients at the age of 19 to 23 by using a γ-RV to mediate gp91-Phox expression. After the cells were transduced with the viral vector, the initial percentage of positive cells was between 25% to 73%. In the seventh month after gene therapy, the percentage of functionally corrected cells in the peripheral

blood of Patient 1 decreased from 24% to 1%. In the eleventh month after gene therapy, the percentage of functionally corrected cells in the peripheral blood of Patient 2 decreased from 4% to 0.03%. Four weeks later, corrected cells could not be detected in the peripheral blood of Patient 3 (see Hyoung, Jin, Kang, *et al.* Retroviral Gene Therapy for X-linked Chronic Granulomatous Disease: Results From Phase I/II Trial[J]. Molecular Therapy, 2011, 19, 2092–2101.).

Ravin *et al.* used CRISPR-Cas9 to repair a mutation in the *CYBB* gene in CGD patients. However, gene editing using the CRISPR-Cas9 system has the problems such as low efficiency and potential safety hazards. In addition, the method requires strict conditions, has a high cost and achieves an unstable result (see De Ravin *et al.* CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease. Science Translational Medicine, 2017, 9, eaah 3480.).

In addition, the function of the NADPH oxidase is to produce ROS, and an overexpression of the ROS in cells may affect the normal functions of cells. Gene therapy can restore the production of the ROS in HCSs. The ROS has a great effect on a balance among processes such as resting, replication, proliferation and differentiation of the HSCs. A heterotopic expression of the NADPH oxidase mediated by a non-tissue-specific promoter will lead to the overexpression of the ROS in the HSCs. Excessive ROS could promote the apoptosis of resting HSCs, induce the HSCs to differentiate and weaken the self-renewal ability of the HSCs, resulting in apparent exhaustion of a HSC pool.

In summary, the adenovirus vector, the gamma-retroviral vector and the CRISPR-Cas9 system have defects in terms of safety, gene transfer efficiency and a long-term expression and the problems such as HSC apoptosis caused by the non-specific overexpression of the NADPH oxidase. Therefore, it is necessary to provide a viral vector having high gene transfer efficiency and suitable for stem cell modification to improve the treatment effect on CGD, which is of great significance in the field of CGD treatment.

SUMMARY

The present disclosure provides a myeloid-specific promoter and a use thereof. The myeloid-specific promoter shows specificity to myeloid tissues. It initiates a gene expression with high efficiency in myeloid cells, but with relative low efficiency in non-myeloid cells.

In a first aspect, the present disclosure provides a myeloid-specific promoter which includes a nucleic acid sequence as shown in SEQ ID NO: 1 or SEQ ID NO: 2.

The myeloid-specific promoter of the present disclosure shows specificity to myeloid tissues. It initiates a gene expression with high efficiency in myeloid cells, but with relative low efficiency in non-myeloid cells. As such, the myeloid-specific promoter regulates the specific expression of a

gene in myeloid tissues, which is of great significance in the field of gene therapy.

SEQ ID NO: 1:

acttgtacagcttcacagggctccatgcttagaaggaccccacacttagtttaatgttctgctgtcatcatcttgatattcttaatttttaaataaa
gggcctatcgttttcatttttttactgggccttgcaaattatgtagctggttctgtatgccaggagagaagttggaagtaaaatggtattccaggaccag
gaggcattctggcagagtgaaagaacatgtgatttggagtccatggggatgggtttaaatttcagctttccactaatttgctttgtgatactgagtattt
ccttttatccctcagaggctctgtttctcaattttgactacgggtttttttcattagataatgtctcagttctggtattccaggtttccctcaattattctggga
aaacctccttgacccacaggcagagcctagggcagccaggtgctttctactctctctctctctgcagcttggaaagttagtgtctgttgaaggtcag
ctgggagttggtggaggcagggcagtggcctgctactattgctgcagtagcagacccttcacaacagcattgttttgtcattttgcatccagatttc
cgttggctaacctcagtcttatcttcctcatttctgtttcctgttgaagacaccaagggcccttcaaaacacagaagcttcttgctcacggcagaaag
cccaattccatctggcccctgcaggttggctcagcactggggaatcagagtcccctccatgaccaaggcaccactccactgacag.

SEQ ID NO: 2:

tagccatttgctggaccaaatctggagggagaacccctaaaacccctaagtgaggttgcccaggggttgtccccaggtgggggaag
caggggagagaaaatggtagccatttttacattgttttgtatagtatttattgattcaggaaacaaacacaaaattctgattataatgacttggaaactg
cctgtttgggcttctcatttcttacctccccttccctctcccacctgctactgggtgcatctctgctccccccttccccagcagatggttacctttgggct
gttgctttcttgtcaccatctgagttctcagacgctggaaagccatgttctcggctctgtgaatgacaatgctgactggagtgctgcccctctgtaaa
gggctgggtgtggatggtcacaagcccctcacatgcctcagccaagaggaagtagtacaggggtcagcccagaggtccaggggaaaggagt
ggaaaccgatttccccaccaagggaggggcctgtacctcagctgttcccatagctacttgccacaactgccaagcaagtttcgctgagtttgaca
catggaaccctgtggatcaactgccctaggactccgtttgcacccatgtgacactgttgactttgccctgatgaagcagggccaacagtccccta
acttaattacaaaaactaatgactaagagagaggtggctagagctgaggcccctgagtcaggctgtgggtgggatcatctccagtacaggaagt
gagactttcatttcctcctttccaagagagggctgagggagcagggttgagcaactggtgcagacagcctagctggactttgggtgaggcggtt
cagcc.

In a second aspect, the present disclosure provides a recombinant expression vector which includes the myeloid-specific promoter according to the first aspect.

In some specific embodiments, the recombinant expression vector includes a viral vector or a plasmid vector containing the myeloid-specific promoter according to the first aspect.

Preferably, the viral vector includes a pTYF lentiviral vector.

Preferably, the recombinant expression vector further includes a *CYBB* gene.

Preferably, the *CYBB* gene includes a nucleic acid sequence as shown in SEQ ID NO: 3.

SEQ ID NO: 3:

atggggaactgggctgtgaatgaggggctctccatttttgtcattctggtttggctggggttgaacgtcttcctctttgtctggtattaccgggt
ttatgatattccacctaagttcttttacacaagaaaacttcttgggtcagcactggcactggccagggcccctgcagcctgcctgaatttcaactgca
tgctgattctcttgccagtctgtcgaaatctgctgtccttcctcaggggttccagtgcgtgctgctcaacaagagttcgaagacaactggacaggaa
tctcacctttcataaaatggtggcatggatgattgcacttcactctgcgattcacaccattgcacatctatttaatgtggaatggtgtgtgaatgcccg
agtcaataattctgatccttattcagtagcactctctgaacttggagacaggcaaaatgaaagttatctcaattttgctcgaaagagaataaagaacc

ctgaaggaggcctgtacctggctgtgaccctgttggcaggcatcactggagttgtcatcacgctgtgcctcatattaattatcacttcctccaccaa
aaccatccggaggtcttactttgaagtcttttggtacacacatcatctctttgtgatcttcttcattggccttgccatccatggagctgaacgaattgtac
gtgggcagaccgcagagagtttggctgtgcataatataacagtttgtgaacaaaaaatctcagaatggggaaaaataaaggaatgcccaatccc
tcagtttgctggaaaccctcctatgacttggaaatggatagtgggtcccatgtttctgtatctctgtgagaggttggtgcggttttggcgatctcaaca
gaaggtggtcatcaccaaggtggtcactcacccttcaaaaccatcgagctacagatgaagaagaaggggttcaaaatggaagtgggacaata
cattttgtcaagtgcccaaaggtgtccaagctggagtggcacccttttacactgacatccgcccctgaggaagacttctttagtatccatatccgca
tcgttggggactggacagaggggctgttcaatgcttgtggctgtgataagcaggagtttcaagatgcgtggaaactacctaagatagcggttgat
gggccctttggcactgccagtgaagatgtgttcagctatgaggtggtgatgttagtgggagcagggattggggtcacacccttcgcatccattctc
aagtcagtctggtacaaatattgcaataacgccaccaatctgaagctcaaaaagatctacttctactggctgtgccgggacacacatgcctttgagt
ggtttgcagatctgctgcaactgctggagagccagatgcaggaaaggaacaatgccggcttcctcagctacaacatctacctcactggctggga
tgagtctcaggccaatcactttgctgtgcaccatgatgaggagaaagatgtgatcacaggcctgaaacaaaagactttgtatggacggcccaact
gggataatgaattcaagacaattgcaagtcaacaccctaataccagaataggagttttcctctgtggacctgaagccttggctgaaaccctgagta
aacaaagcatctccaactctgagtctggccctcggggagtgcatttcattttcaacaaggaaaacttctaa.

Preferably, the myeloid-specific promoter initiates the expression of the *CYBB* gene.

In some specific embodiments, a lentiviral vector is used for transduction of blood stem cells or somatic cells with high efficiency, high stability and high safety so that the gene can be transferred efficiently during gene therapy. Meanwhile, the myeloid-specific promoter is used so that the lentiviral vector specifically expresses the *CYBB* gene in myeloid cells, thereby effectively treating the chronic granulomatous disease caused by gene mutation on the X chromosome.

In a third aspect, the present disclosure provides a recombinant lentivirus containing the recombinant expression vector according to the second aspect.

In a fourth aspect, the present disclosure provides a recombinant cell containing the myeloid-specific promoter according to the first aspect.

In some specific embodiments, the recombinant cell contains the recombinant expression vector according to the second aspect.

In some specific embodiments, the recombinant cell contains the recombinant lentivirus according to the third aspect.

In a fifth aspect, the present disclosure provides a method for preparing the recombinant cell according to the fourth aspect, which includes:

introducing the recombinant expression vector according to the second aspect or the recombinant lentivirus according to the third aspect into a host cell to obtain the recombinant cell.

Preferably, the introduction is carried out by a method which includes any one of electrical gene transfer, a viral vector system, a non-viral vector system or gene gun injection.

Preferably, the host cell includes a hematopoietic stem cell.

Preferably, the method includes:

(1) constructing a lentiviral vector;

(2) co-transfecting the lentiviral vector in step (1) and a packaging plasmid or packaging plasmids into a mammalian cell for lentiviral vector packaging; and

(3) introducing the packaged lentiviral vector in step (2) into a host cell to obtain the recombinant cell.

Preferably, the step (1) of constructing a lentiviral vector includes: inserting the myeloid-specific promoter according to the first aspect and a *CYBB* gene into a pTYF lentiviral vector.

Preferably, the packaging plasmids in step (2) include pNHP and pHEF-VSVG.

Preferably, the mammalian cell in step (2) includes a 293T cell.

In a sixth aspect, the present disclosure provides a pharmaceutical composition which includes any one or a combination of at least two of the myeloid-specific promoter sequences according to the first aspect, the recombinant expression vector according to the second aspect, the recombinant lentivirus according to the third aspect or the recombinant cell according to the fourth aspect.

Preferably, the pharmaceutical composition further includes any one or a combination of at least two of a pharmaceutically acceptable carrier, excipient or diluent.

In a seventh aspect, the present disclosure provides a use of the myeloid-specific promoter according to the first aspect, the recombinant expression vector according to the second aspect, the recombinant lentivirus according to the third aspect, the recombinant cell according to the fourth aspect or the pharmaceutical composition according to the sixth aspect in the preparation of a drug for treating a disease.

Preferably, the disease includes CGD.

Compared with the existing art, the present disclosure has the following beneficial effects:

(1) The myeloid-specific promoter of the present disclosure shows specificity to myeloid tissues. It initiates a gene expression with high efficiency in myeloid cells, but with relative low efficiency in non-myeloid cells. As such, the myeloid-specific promoter regulates the specific expression of a gene in myeloid tissues.

(2) In the present disclosure, the myeloid-specific promoter is inserted into a lentiviral vector to obtain a lentiviral vector which has high transduction efficiency, high stability and high safety and can perform specific expression in myeloid cells.

(3) In the present disclosure, the myeloid-specific promoter and the *CYBB* gene are inserted into a lentiviral vector, and the constructed lentiviral expression vector shows specificity to myeloid tissues and can effectively restore the expression of gp91-phox protein and restore the generation

function of ROS, which is of great significance for CGD treatment.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a diagram illustrating the viral vector copy number (VCN) in C57 mouse bone marrow HSCs.

FIG. 2 is a diagram illustrating the expression of GFPs in C57 mouse HSCs on Day 5 and Day 14 after transfected with lentiviruses.

FIG. 3 is a diagram illustrating expression percentages of GFPs in C57 mouse HSCs on Day 5 and Day 14 after transduced with lentiviruses.

FIG. 4 is a diagram illustrating results of the expression of the *CYBB* gene in X-CGD mouse HSCs.

FIG. 5 is a diagram illustrating generation levels of ROS in X-CGD mouse HSCs.

FIG. 6 is a diagram illustrating percentages of mouse HSCs that differentiated into myeloid cells on Day 14 of differentiation induction.

FIG. 7 is a diagram illustrating results of an *Escherichia coli*-phagocytizing experiment.

FIG. 8 is a diagram illustrating results of VCN in X-CGD mouse HSCs transduced with lentiviruses.

FIG. 9 is a diagram illustrating results of the expression of the *CYBB* gene in mouse cells *in vivo*.

FIG. 10 is a diagram illustrating results of the generation level of ROS in mouse cells *in vivo*.

DETAILED DESCRIPTION

To further elaborate on the technical means adopted and effects achieved in the present disclosure, the present disclosure is further described below in conjunction with examples and drawings. It is to be understood that the specific examples set forth below are intended to explain the present disclosure and not to limit the present disclosure.

Experiments without specific techniques or conditions noted in the examples are conducted according to techniques or conditions described in the literature in the art or a product specification. The reagents or instruments used herein without manufacturers specified are conventional products commercially available from proper channels.

Example 1

A recombinant lentivirus was prepared. The method for preparing the recombinant lentivirus includes steps described below.

(1) Construction of a lentiviral vector

1) A pTYF lentiviral vector was modified by mutating wild-type 5' splice donor site GT into CA, and deleting the enhancer in the U3 region. For a specific modification method, see "Cui Y,

Iwakuma T, Chang L J. Contributions of Viral Splice Sites and cis-Regulatory Elements to Lentivirus Vector Function[J]. Journal of Virology, 1999, 73 (7): 6171."

Wild-type 5' splice donor site SEQ ID NO: 4:

GGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTA;

Mutant 5' splice donor site SEQ ID NO: 5:

GGCAAGAGGCGAGGGGCGGCGACTGCAGAGTACGCCAAAAATTTTGACTAGCGGAGGCTA.

2) A cDNA sequence of *CYBB* gene (SEQ ID NO: 3), an miR223 promoter sequence (SEQ ID NO: 1) and a CD68 promoter sequence (SEQ ID NO: 2) were synthesized, and these sequences were correspondingly ligated into lentiviral vector TYF through restriction enzyme sites to obtain an miR223+CYBB lentiviral vector and a CD68+CYBB lentiviral vector.

(2) Lentivirus packaging and concentration

1) 293T cells were inoculated in a fresh Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and incubated for 17 h.

2) The two lentiviral vectors prepared in step (1), DMEM, pNHP and pHEF-VSV-G were added to a sterile centrifuge tube in sequence, vortexed and mixed, and then a Superfect transfection reagent (QIAGEN) was added to the centrifuge tube. The system was allowed to stand at room temperature for 8 min.

3) The mixture prepared in the centrifuge tube was added dropwise to 293T cells and incubated for 5 h at 37 °C under 5% CO_2.

4) The cell culture medium was discarded, and the cells were rinsed and added with a fresh medium to continue the culture.

5) The cell culture medium was collected, the cells were rinsed, and the culture medium was replaced with a fresh culture medium. The fresh medium was incubated in a 5% CO_2 incubator overnight. Then, the cell culture medium was collected and stored at –80 ℃.

6) The packaged lentivirus was centrifuged for 5 min at 1000×g, cell fragments were removed and the remaining lentivirus was stored at –80 °C.

7) The supernatant of the lentivirus was added to a centrifuge filter tube and centrifuged at 2500×g for 30 min. The concentrated virus was collected into a centrifuge tube and stored at –80 °C to obtain lentiviruses LV-miR223 and LV-CD68 expressing *CYBB*.

Example 2

Gene transfer efficiency and promoter specificity were verified in C57 mouse HSCs.

C57 mouse bone marrow HSCs were separately transduced with *CYBB*-expressing lentiviruses

LV-EF1α, LV-miR223, LV-CD68 and LV-VEC, where LV-EF1α was a lentivirus carrying a widely expressed strong mammalian EF1α promoter, LV-VEC was a lentivirus carrying an endothelial cell-specific promoter, and cells transduced with no lentiviruses were used as a negative control (NC).

C57 mouse HSCs were transduced by the method described below.

(1) Bone marrow was taken from the tibia of a C57 mouse, and HSCs were isolated and extracted from the bone marrow using EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit available from STEMCELL Technologies.

(2) 1×10^6 mouse HSCs were resuspended in 100 μL medium (StemSpan SFEM Medium available from STEMCELL Technologies) containing cytokines (including 50 ng/mL stem cell growth factor (SCF), 50 ng/mL FMS-like tyrosine kinase 3 ligand (FLT3-L), 10 ng/mL interleukin 6 (IL6) and 50 ng/mL thrombopoietin (TPO) available from Biotech Company) and stimulated and incubated for 17 h.

(3) 50 μL medium was discarded, and 50 μL fresh medium containing cytokines was added to resuspend the cells. 8 μg/mL polybrene was added, and the lentivirus was added and mixed. The multiplicity of infection (MOI) of the transfection was 200. The cells were transfected once a day, twice in total. Centrifuged at 100×g at room temperature for 100 min.

(4) After the transduction was completed, the cells were collected and induced by 20 ng/mL murine granulocyte colony-stimulating factor (an mG-CSF cytokine available from PeproTech, Inc.) to differentiate into myeloid cells. On Day 5 and Day 14, cells were collected and measured for the expression of green fluorescent proteins (GFPs) through flow cytometry.

After the virus transduction, q-PCR was used to determine the VCN in the cells. The results are shown in FIG. 1. The viral VCNs of the lentiviruses LV-miR223 and LV-CD68 after the transduction were 206.33% and 196.87%, respectively, indicating that the lentiviral vector containing a myeloid-specific promoter constructed in the present disclosure can be effectively transfected into cells and meet the requirements of gene therapy.

The lentiviral vector carried a GFP fluorescent gene. Photos were taken and the expression of the lentiviral vector was analyzed by measuring the expression percentage of GFPs. The expression of GFPs on Day 5 (the cells were not differentiated into myeloid cells (undiffs)) and the expression of GFPs on Day 14 (the cells were differentiated into myeloid cells (diffs)) were compared, and the myeloid specificity of two promoters was analyzed.

The results are shown in FIGS. 2 and 3. FIG. 2 is a diagram illustrating the expression of GFPs in cells on Day 5 and Day 14 after induced differentiation, where the first column is a fluorescent photograph, and the second column is a white light photograph. FIG. 3 is a diagram illustrating

expression percentages of GFPs in C57 mouse HSCs on Day 5 and Day 14 after transduced with lentiviruses. The expression percentages of GFPs in the undiff cells and the diff cells in the EF1α group were 84.72% and 85.35%, respectively, which are similar. The expression percentages of GFPs in the undiff cells and the diff cells in the VEC group were 28.28% and 32.22%, respectively, which are similar. The expression percentages of GFPs mediated by miR223 in the undiff cells and the diff cells were 26.42% and 89.16%, respectively, which have a significant difference. The expression percentages of GFPs mediated by CD86 in the undiff cells and the diff cells were 58.01% and 77.49%, respectively, which have a significant difference. It can be seen that the miR223 promoter and the CD86 promoter initiate gene expression in the myeloid cells with higher efficiency than in non-myeloid cells, that is, the miR223 promoter and the CD86 promoter have myeloid specificity. Moreover, the miR223 promoter has a greater difference in expression, that is, the miR223 promoter has higher specificity.

Example 3

Gene transfer efficiency was verified and the abilities of promoters to initiate the expression of *CYBB* gene and restore functions of NADPH oxidase and the specificity of the promoters were compared in HSCs of CGD mice (X-CGD mice, B6.129S-Cyb btm1Din/J).

X-CGD mouse HSCs were transduced by the method described below.

(1) Bone marrow was taken from the tibia of a X-CGD mouse, and HSCs were isolated and extracted from the bone marrow using EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit available from STEMCELL Technologies.

(2) 1×10^6 mouse HSCs were resuspended in a 100 μL medium (StemSpan SFEM Medium available from STEMCELL Technologies) containing cytokines (including 50 ng/mL SCF, 50 ng/mL FLT3-L, 10 ng/mL IL6 and 50 ng/mL TPO available from Biotech Company) and stimulated and incubated for 17 h.

(3) 50 μL medium was discarded, and 50 μL fresh medium containing cytokines was added to resuspend the cells. 8 μg/mL polybrene was added, and the viral vector was added and mixed. The MOI of the transduction was 200. The cells were transduced once a day, twice in total. Centrifuged at 100×g at room temperature for 100 min.

(4) After the transduction was completed, the cells were collected and induced by 20 ng/mL murine granulocyte colony-stimulating factor (an mG-CSF cytokine available from PeproTech, Inc.) to differentiate into myeloid cells.

The expression of the *CYBB* gene (expressing gp91-phox protein) was detected on Day 5 and Day 14, respectively, that is, percentages of gp91-phox-positive cells on Day 5 (undiff) and Day 14 (diff) were measured through flow cytometry. The results are shown in FIG. 4, where NC represents

X-CGD mouse HSCs transduced with no lentivirus, CGD represents X-CGD mouse HSCs transduced with no lentivirus but stained with an anti-gp91-phox antibody, and WT represents wild-type mouse cells.

As can be seen from FIG. 4, the expression percentages of gp91-phox protein in diff cells and undiff cells in the WT group were72.58% and 64.38%, respectively; the expression percentages of gp91-phox protein in diff cells and undiff cells in the EF1α group were 80.28% and 81.7%, respectively; the expression percentages of gp91-phox protein in diff cells and undiff cells in the miR223 group were 71.17% and 54.17%, respectively; and the expression percentages of gp91-phox protein in diff cells and undiff cells in the CD68 group were 70.8% and 65.9%, respectively. In summary, the miR223 promoter and the CD86 promoter initiate gene expression in the myeloid cells with higher efficiency than in non-myeloid cells, that is, the miR223 promoter and the CD86 promoter have myeloid specificity. Moreover, the miR223 promoter has higher specificity.

The cells were stimulated by phorbol ester (PMA) and stained with dihydrorhodamine (DHR123), and the generation level of ROS in the cells was measured through flow cytometry on Day 14 to further verify the expression of *CYBB* gene. The results are shown in FIG. 5. The DHR123+% in the WT group was 72.97%, the DHR123+% in the EF1α group was 62.99%, the DHR123+% in the MiR223 group was 62.76%, and the DHR123+% in the CD68 group was 53.58%. It can be seen that the lentiviral vector constructed in the present disclosure can effectively express the *CYBB* gene, that is, the lentiviral vector can effectively restore the generation level of ROS in CGD cells to a level close to that of ROS in normal wild-type cells.

Example 4

The effect of the viral vector on the differentiation ability of X-CGD mouse HSCs was determined.

X-CGD mouse HSCs were transduced by the method described below.

(1) Bone marrow was taken from the tibia of a X-CGD mouse, and HSCs were isolated and extracted from the bone marrow using EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit available from STEMCELL Technologies.

(2) 1×10^6 mouse HSCs were resuspended in a 100 μL medium (StemSpan SFEM Medium available from STEMCELL Technologies) containing cytokines (including 50 ng/mL SCF, 50 ng/mL FLT3-L, 10 ng/mL IL6 and 50 ng/mL TPO available from Biotech Company) and stimulated and incubated for 17 h.

(3) 50 μL medium was discarded, and 50 μL fresh medium containing cytokines was added to resuspend the cells. 8 μg/mL polybrene was added, and the viral vector was added and mixed. The

MOI of the transduction was 200. The cells were transduced once a day, twice in total. Centrifuged at 100×g at room temperature for 100 min.

(4) After the transfection was completed, the cells were collected, inoculated in a fresh RPMI1640 medium containing 20% FBS and induced to differentiate by 20 μg/mL murine granulocyte colony-stimulating factor (an mG-CSF cytokine available from PeproTech, Inc.). The medium was replaced every two days, and the cells were cultured for 14 days.

Mouse HSCs can be induced by the murine granulocyte colony-stimulating factor to differentiate into myeloid cells (phagocytes and neutrophils). Since CD11b is an important marker of the myeloid cells, the percentage of CD11b-positive cells was measured through flow cytometry in order to determine cell differentiation. The results are shown in FIG. 6, where cells transduced with no lentivirus and treated with an isotype antibody were used as a negative control (ISO).

As can be seen from FIG. 6, the CD11b+% in the WT group was 85.8%, the CD11b+% in the miR223 group was 97.26%, and the CD11b+% in the CD68 group was 83.86%, indicating that the lentiviral vector constructed in the present disclosure will not affect the differentiation ability of cells transduced with the lentiviral vector, that is, the lentiviral vector is safe.

Example 5

The effect of the lentiviral vector on the phagocytic function of X-CGD mouse HSCs after differentiation was determined.

The lentiviral transduction and induced differentiation experiments were the same as that described in Example 4. Cells that had been completely induced to differentiate were taken, washed using PBS and counted, and an experiment was carried out according to 1:100 of cell/E. coli-GFP+. The medium was a fresh RPMI1640 medium containing 20% FBS, and the cells were cultured for 2.5 h in total and washed using PBS. The fluorescence of fluorescein isothiocyanate (FITC) was tested through flow cytometry. The results are shown in FIG. 7.

As can be seen from FIG. 7, in the wild-type cells (WT group), the CD11b+% was 83.27% and the E. coli-GFP+% was 87.07%; in the cells transduced with the lentivirus LV-miR223 (miR223 group), the CD11b+% was 89.76% and the E. coli-GFP+% was 84.59%; and in the cells transduced with the lentivirus LV-CD68 (CD68 group), the CD11b+% was 83.99% and the E. coli-GFP+% was 82.77%. It can be seen from the comparison that after the lentiviruses designed in the present disclosure are transfected into the HSCs, the lentiviruses have no effect on the differentiation of HSCs into myeloid cells and the phagocytic function of the differentiated cells. Therefore, the lentiviral vectors designed in the present disclosure are proved to be safe.

Example 6

The ability of the lentiviral vector to correct the functions of phagocytes and neutrophils was

evaluated in X-CGD mice.

1.5×10^6 X-CGD mouse HSCs were taken and separately transduced with lentiviruses LV-miR223, LV-CD86 and LV-EF1α *in vitro* with an MOI of 200. The X-CGD mouse HSCs were transduced by the same method as those in Example 4.

Myeloablative preconditioning was conducted on X-CGD mice through irradiation at a radiation dose of 4.5 Gy. On Day 4 after the treatment, the above cells transduced with the lentiviruses were transplanted via tail veins. Four weeks later, the peripheral blood was taken for detection, including detecting the VCN through qPCR, detecting the expression of the *CYBB* gene through flow cytometry and measuring the generation level of ROS in the cells stained with DHR123.

FIG. 8 is a diagram illustrating VCN results. FIG. 9 is a diagram illustrating results of the expression of the *CYBB* gene. FIG. 10 is a diagram illustrating results of the generation level of ROS in cells. FIG. 8 shows that lentiviruses can be efficiently transfected. It can be seen from FIGS. 9 and 10 that in the isotype wild-type C57 mice (WT group), the gp91-phox+% was 59.37% and the Rhodamine123+% was 68.59%; in the CGD mice transplanted with wild-type C57 mouse HSCs (WT-trans group), the gp91-phox+% was 57.14% and the Rhodamine123+% was 61.26%; in the CGD mice transplanted with HSCs transduced with LV-miR223 (miR223 group), the gp91-phox+% was 58.98% and the Rhodamine123+% was 58.29%; and in the CGD mice transplanted with HSCs transduced with LV-CD68 (CD68 group), the gp91-phox+% was 58.29% and the Rhodamine123+% was 61.35%. It can be seen from the comparison that after the HSCs transduced with the lentiviral vectors designed in the present disclosure are transplanted back into the X-CGD mice, the lentiviral vectors can effectively restore the expression of gp91-phox proteins and the generation function of ROS. Therefore, the lentiviral vectors designed in the present disclosure are proved to be effective.

In summary, in the present disclosure, the myeloid-specific promoter and the *CYBB* gene are inserted into the lentiviral expression vector. The constructed lentiviral expression vector has high transduction efficiency, stable expression ability, safety and myeloid specificity. The lentiviral expression vector is effectively expressed in the myeloid cells and can effectively restore the expression of gp91-phox proteins and restore the generation function of ROS, which is of great significance for CGD treatment.

The applicant has stated that although the detailed method of the present disclosure is described through the examples described above, the present disclosure is not limited to the detailed method described above, which means that implementation of the present disclosure does not necessarily depend on the detailed method described above. It should be apparent to those skilled in the art that any improvements made to the present disclosure, equivalent replacements of raw

materials of the product of the present disclosure, additions of adjuvant ingredients to the product of the present disclosure, and selections of specific manners, etc., all fall within the protection scope and the disclosure scope of the present disclosure.

1. A myeloid-specific promoter, comprising a nucleic acid sequence as shown in SEQ ID NO: 1 or SEQ ID NO: 2.

2. A recombinant expression vector, comprising the myeloid-specific promoter according to claim 1;

preferably, the recombinant expression vector comprises a viral vector or a plasmid vector comprising the myeloid-specific promoter according to claim 1; and

preferably, the viral vector comprises a pTYF lentiviral vector.

3. The recombinant expression vector according to claim 2, wherein the recombinant expression vector further comprises a cytochrome b-245 beta chain (*CYBB*) gene;

preferably, the *CYBB* gene comprises a nucleic acid sequence as shown in SEQ ID NO: 3; and

preferably, the myeloid-specific promoter initiates the expression of the *CYBB* gene.

4. A recombinant lentivirus containing the recombinant expression vector according to claim 2 or 3.

5. A recombinant cell containing the myeloid-specific promoter according to claim 1;

preferably, the recombinant cell contains the recombinant expression vector according to claim 2 or 3; and

preferably, the recombinant cell contains the recombinant lentivirus according to claim 4.

6. A method for preparing the recombinant cell according to claim 5, comprising:

introducing the recombinant expression vector according to claim 2 or 3 or the recombinant lentivirus according to claim 4 into a host cell to obtain the recombinant cell;

preferably, the introduction is carried out by a method which comprises any one of electrical gene transfer, a viral vector system, a non-viral vector system or gene gun injection; and

preferably, the host cell comprises a hematopoietic stem cell.

7. The method according to claim 6, comprising the following steps:

(1) constructing a lentiviral vector;

(2) co-transfecting the lentiviral vector in step (1) and a packaging plasmid or packaging plasmids into a mammalian cell for lentiviral vector packaging; and

(3) introducing the packaged lentiviral vector in step (2) into a host cell to obtain the recombinant cell.

8. The method according to claim 7, wherein step (1) of constructing the lentiviral vector comprises: inserting the myeloid-specific promoter according to claim 1 and a *CYBB* gene into a pTYF lentiviral vector;

preferably, the packaging plasmids in step (2) comprise pNHP and pHEF-VSVG; and

preferably, the mammalian cell in step (2) comprises a 293T cell.

9. A pharmaceutical composition, comprising any one or a combination of at least two of the myeloid-specific promoter according to claim 1, the recombinant expression vector according to claim 2 or 3, the recombinant lentivirus according to claim 4 or the recombinant cell according to claim 5;

preferably, the pharmaceutical composition further comprises any one or a combination of at least two of a pharmaceutically acceptable carrier, excipient or diluent.

10. Use of the myeloid-specific promoter according to claim 1, the recombinant expression vector according to claim 2 or 3, the recombinant lentivirus according to claim 4, the recombinant cell according to claim 5 or the pharmaceutical composition according to claim 9 in the preparation of a drug for treating a disease;

preferably, the disease comprises chronic granulomatous disease.

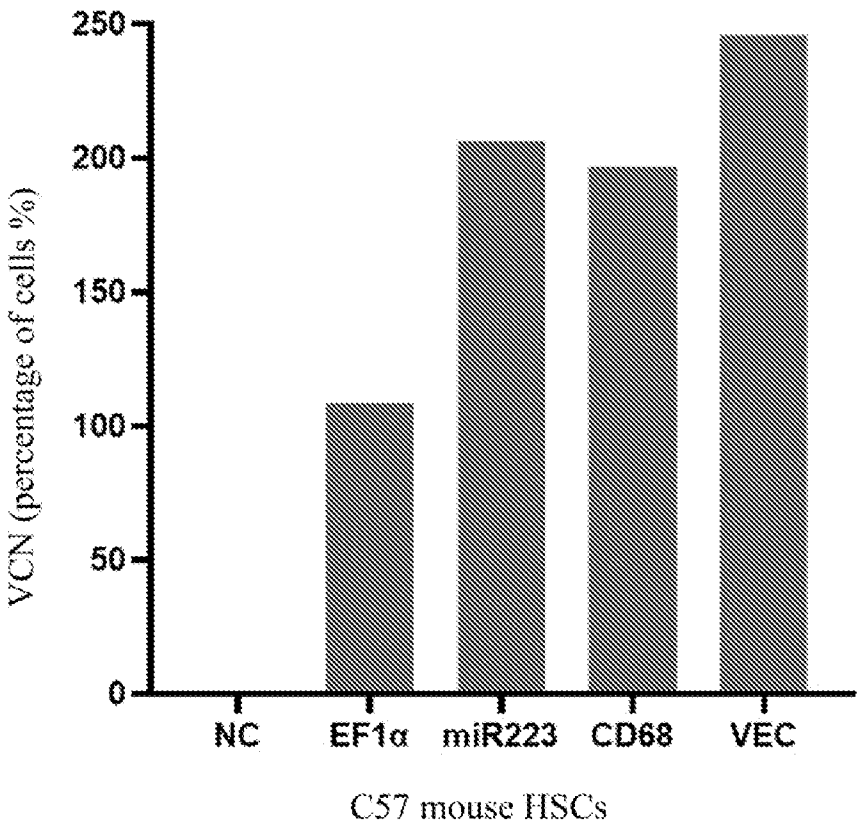
250
200
150
100
50
0
VCN (percentage of cells %)
NC
EF1α
miR223
CD68
VEC
C57 mouse HSCs

Figure 1

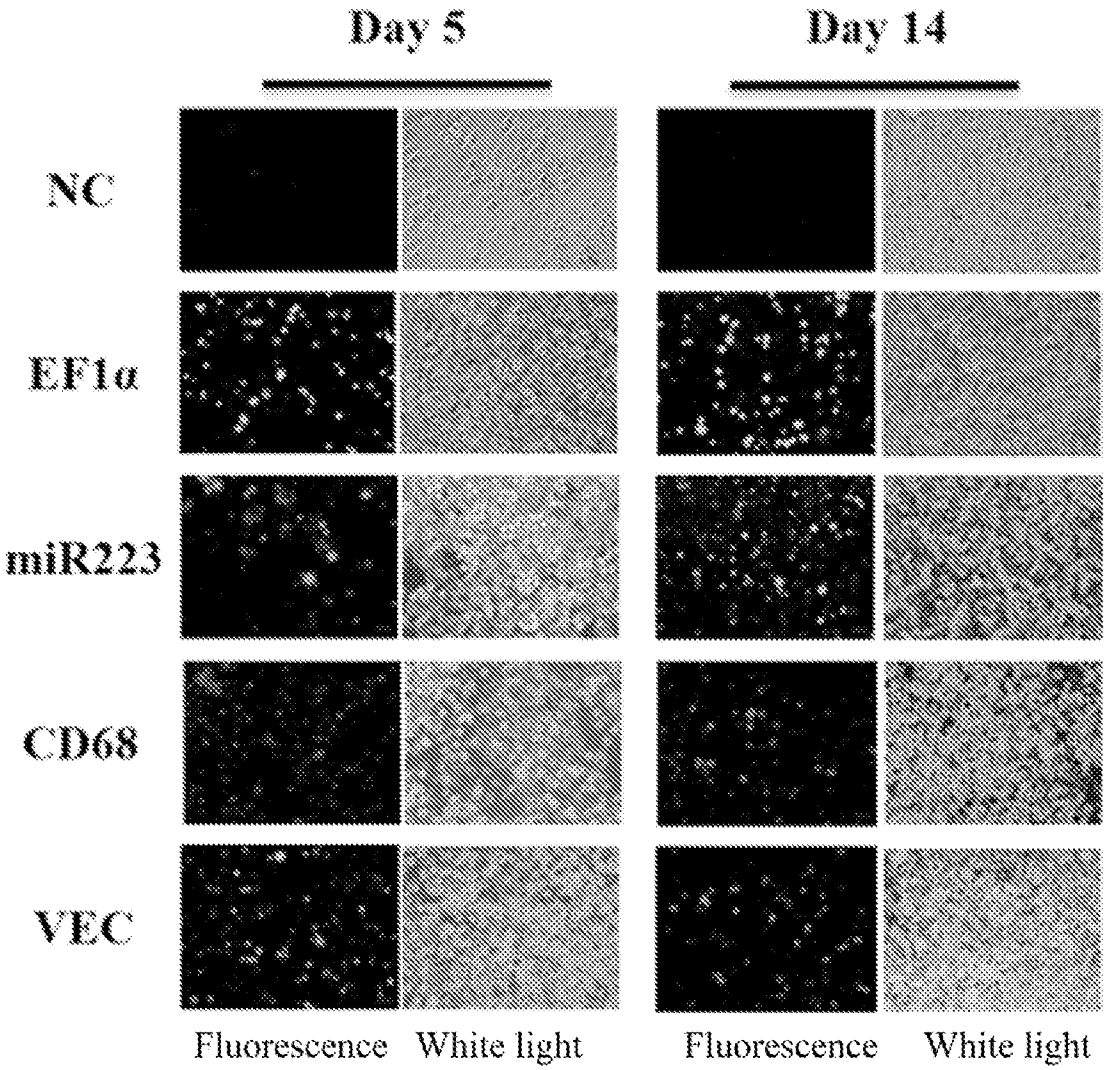
Day 5
Day 14
NC
EF1α
miR223
CD68
VEC
Fluorescence
White light
Fluorescence
White light

Figure 2

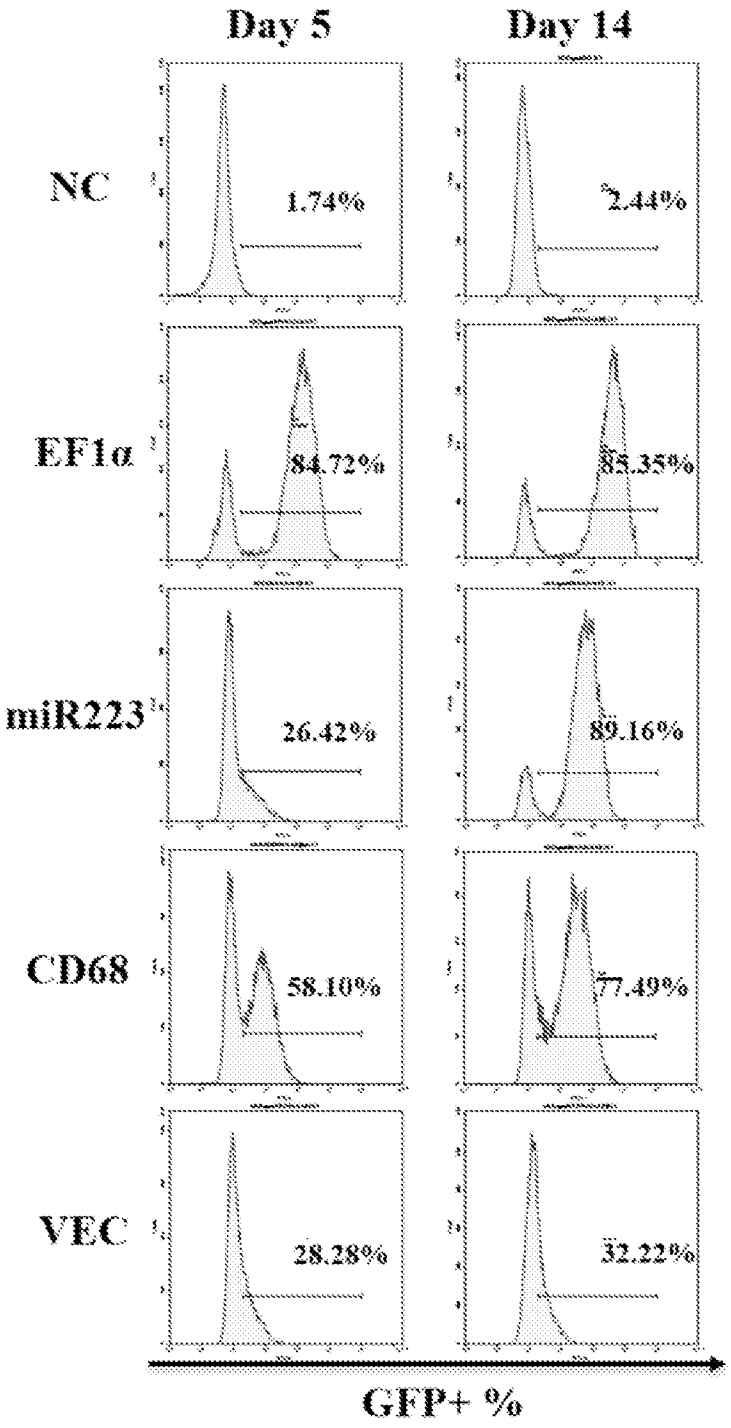
Day 5
Day 14
NC
1.74%
2.44%
EF1α
84.72%
85.35%
miR223
26.42%
89.16%
CD68
58.10%
77.49%
VEC
28.28%
32.22%
GFP+ %

Figure 3

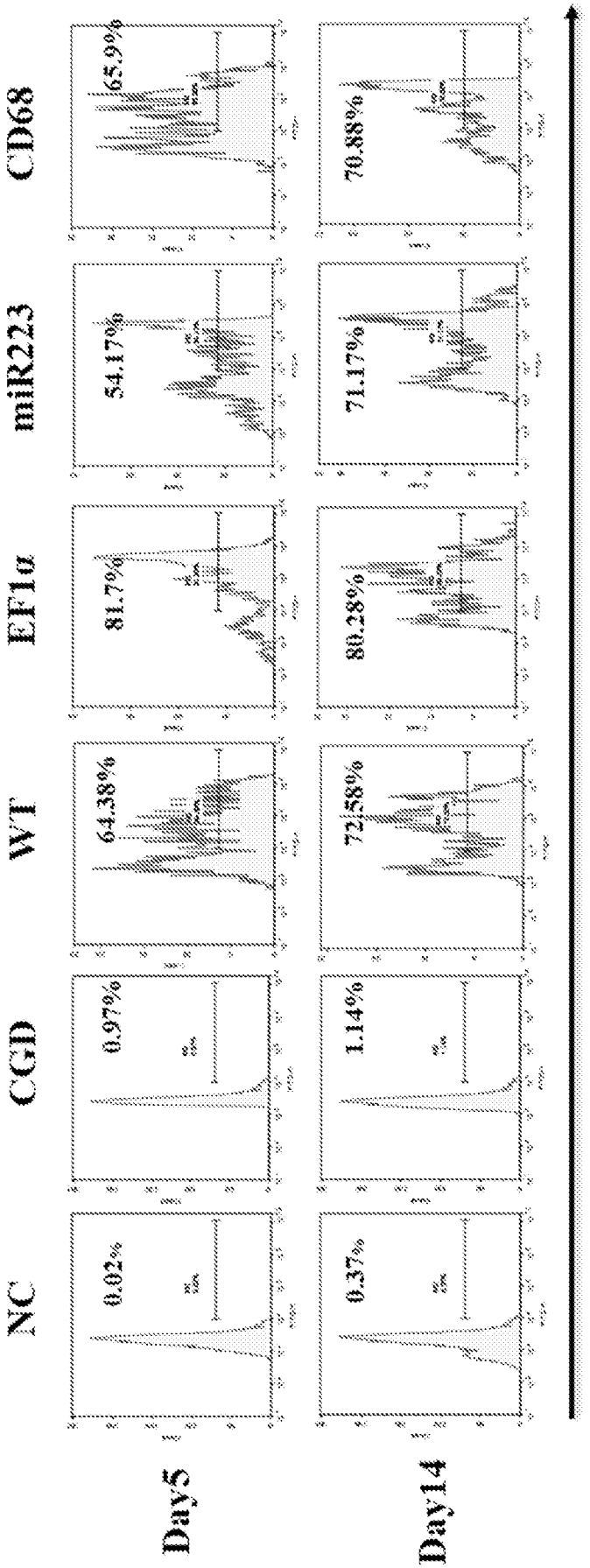
NC
CGD
WT
EF1α
miR223
CD68
Day5
Day14
0.02%
0.97%
64.38%
81.7%
54.17%
65.9%
0.37%
1.14%
72.58%
80.28%
71.17%
70.88%
gp91-phox+ %

Figure 4

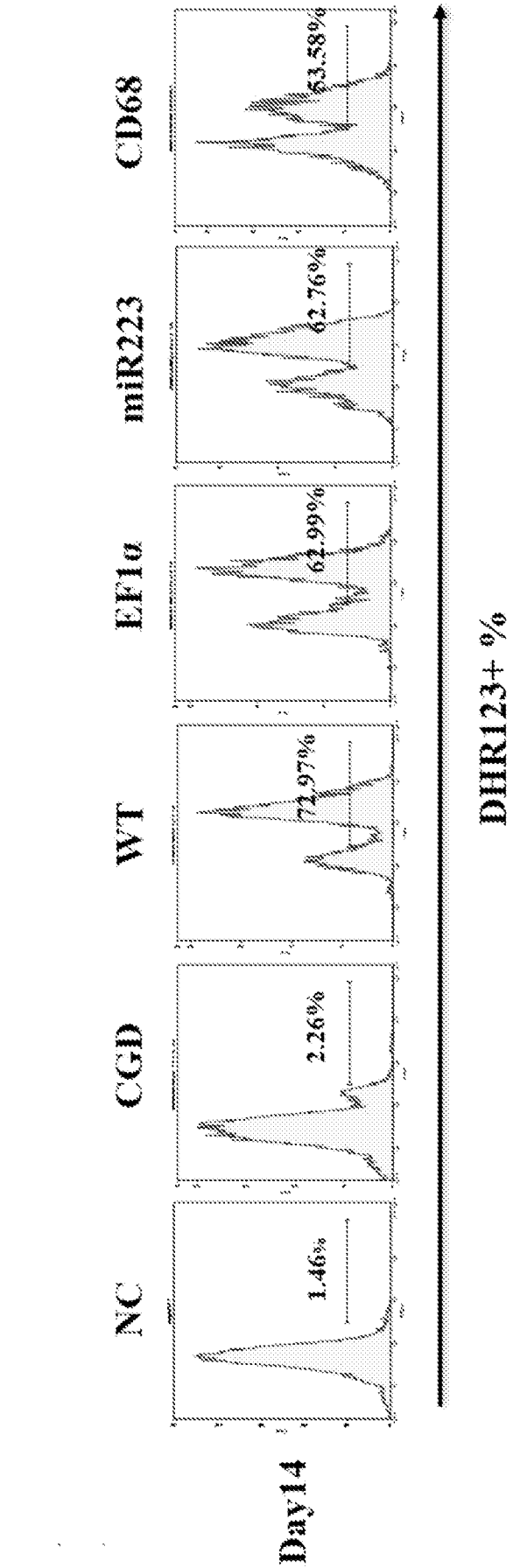
NC
CGD
WT
EF1α
miR223
CD68
Day14
1.46%
2.26%
72.97%
62.99%
62.76%
63.58%
DHR123+ %

Figure 5

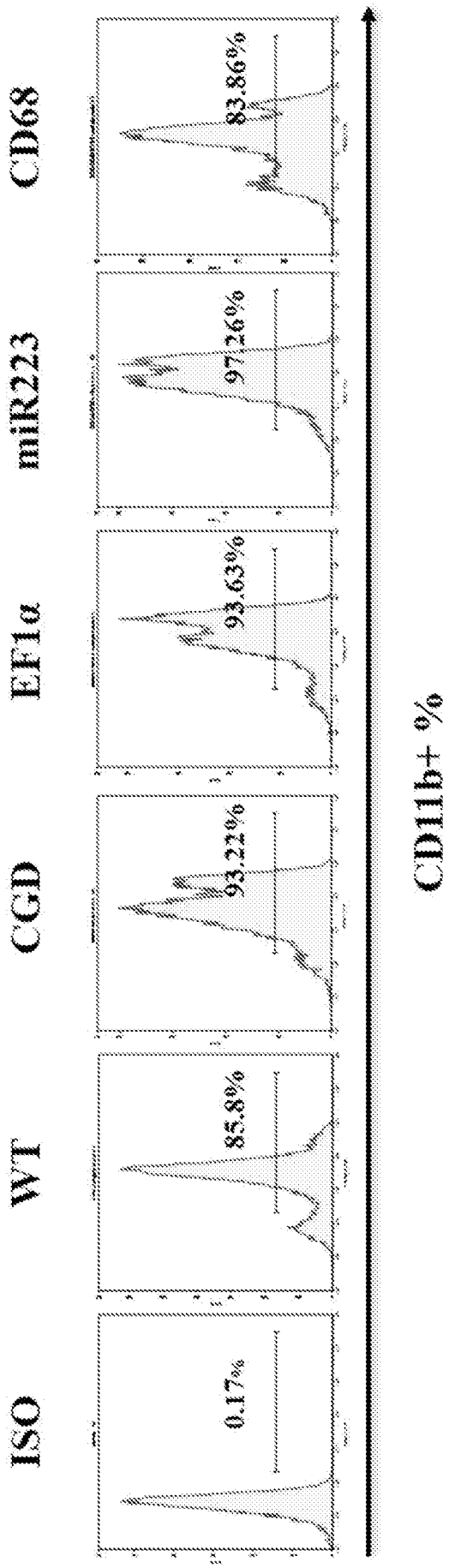

Figure 6

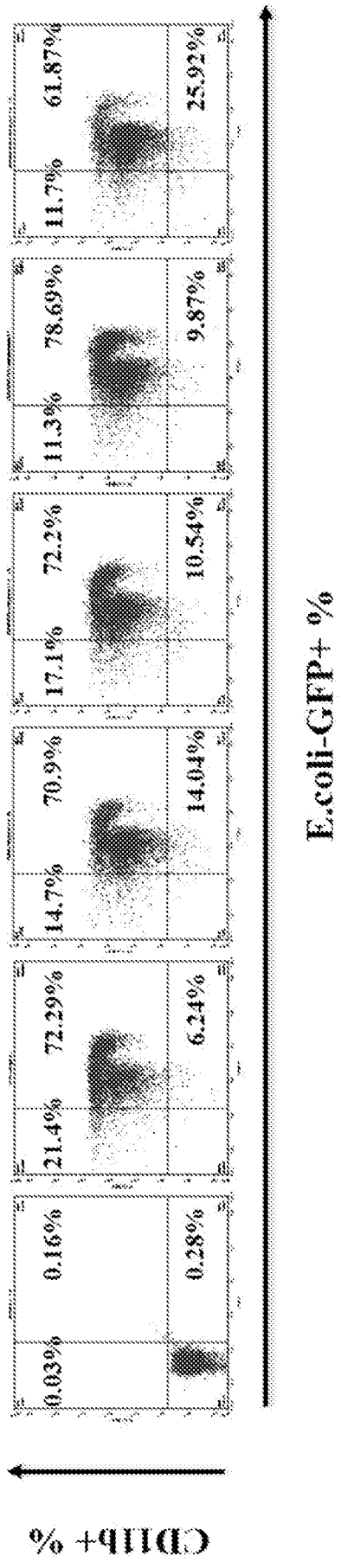
ISO
WT
CGD
EF1α
miR223
CD68
0.03%
0.16%
0.28%
21.4%
72.29%
6.24%
14.7%
70.9%
14.04%
17.1%
72.2%
10.54%
11.3%
78.69%
9.87%
11.7%
61.87%
25.92%
CD11b+ %
E.coli-GFP+ %

Figure 7

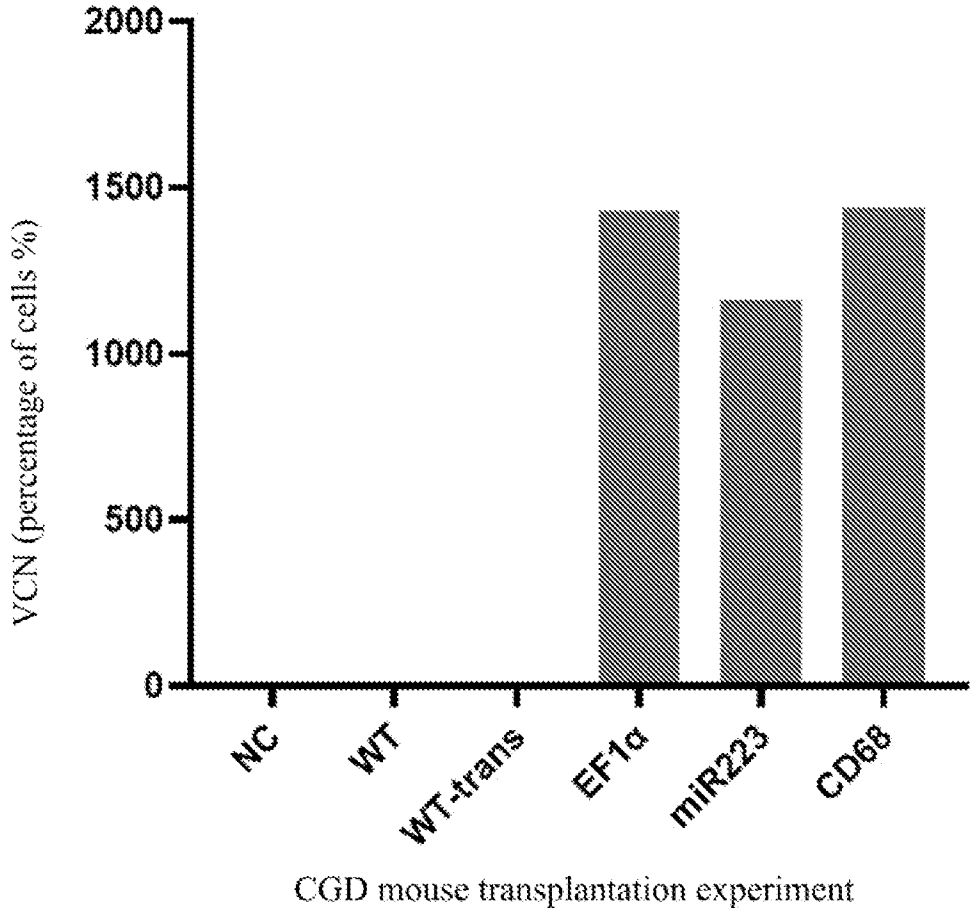
2000
1500
1000
500
0
VCN (percentage of cells %)
NC
WT
WT-trans
EF1α
miR223
CD68
CGD mouse transplantation experiment

Figure 8

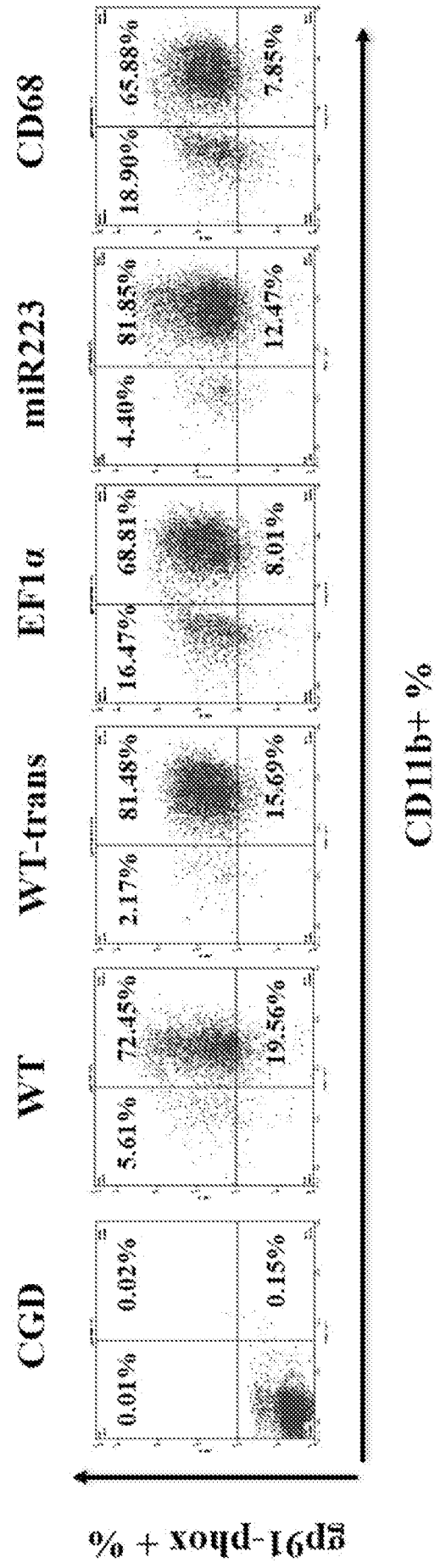
CGD
WT
WT-trans
EF1α
miR223
CD68
0.01%
0.02%
5.61%
72.45%
2.17%
81.48%
16.47%
68.81%
4.40%
81.85%
18.90%
65.88%
0.15%
19.56%
15.69%
8.01%
12.47%
7.85%
gp91-phox + %
CD11b+ %

Figure 9

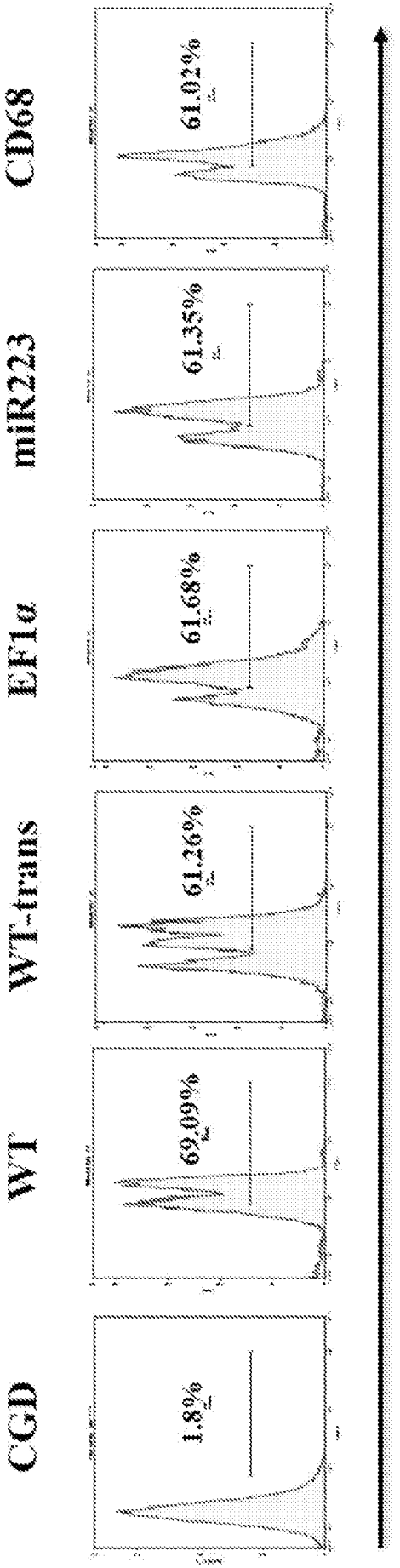
CGD
WT
WT-trans
EF1α
miR223
CD68
1.8%
69.09%
61.26%
61.68%
61.35%
61.02%
Rhodamine123+ %

Figure 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/085852

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/113(2010.01)i; A61K 38/17(2006.01)i; C12N 7/01(2006.01)i; C12N 15/12(2006.01)i; C12N 15/867(2006.01)i; C12N 5/10(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N; A61K

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)

CNABS,DWPI,SIPOABS,CNTXT,WOTXT,EPTXT,USTXT,Baidu,CNKI,Wanfang Database, GenBank,EBI-EMBL, STN, ISI Web of Knowledge,PubMed,SpringerLink, Chinese Patent Biological Sequence Search System: Applicant/Inventor, SEQ ID NOs:1-5, myeloid, specific, promoter, mir223, cd68, cd86, CYBB, gp91-phox, CGD, lentiviral vector, transduc+, pTYF, chronic granulomatous disease.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	CN 113621611 A (BEIJING MEIKANG GENO-IMMUNE BIOTECHNOLOGY CO., LTD.) 09 November 2021 (2021-11-09) claims 1-10	1-10
X	WO 2021064164 A1 (UNIV ZUERICH) 08 April 2021 (2021-04-08) claims 1-6, examples	1-10
X	CN 108713059 A (BLUEBIRD BIO. INC.) 26 October 2018 (2018-10-26) claims 1-326, description paragraphs 340-753	1-9
Y	CN 108713059 A (BLUEBIRD BIO. INC.) 26 October 2018 (2018-10-26) claims 1-326, description paragraphs 340-753	10
Y	O'REILLY, D.et al. "Cell-type-specific expression of the human CD68 gene is associated with changes in Pol II phosphorylation and short-range intrachromosomal gene looping" *GENOMICS*, Vol. 90, 20 June 2007 (2007-06-20), page 408, left column, the 2nd paragraph	10

☑ 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	Date of mailing of the international search report
15 June 2022	**06 July 2022**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing** **100088, China** Facsimile No. **(86-10)62019451**	Authorized officer **SHEN,Jingjing** Telephone No. **86-(10)-53961944**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/085852

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 109971787 A (BEIJING MEIKANG GENO-IMMUNE BIOTECHNOLOGY CO., LTD.) 05 July 2019 (2019-07-05) the whole document	1-10
A	CN 112575034 A (JINAN CELL BIOLOGY TECHNOLOGY CO., LTD.) 30 March 2021 (2021-03-30) the whole document	1-10
A	WO 2021064162 A1 (HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUR GESUNDHEIT UND UMWELT GMBH) 08 April 2021 (2021-04-08) the whole document	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/085852

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/CN2022/085852

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	113621611	A	09 November 2021	None			
WO	2021064164	A1	08 April 2021	AU	2020360965	A1	17 March 2022
				CA	3148895	A1	08 April 2021
				IL	291372	D0	01 May 2022
CN	108713059	A	26 October 2018	JP	2019504635	A	21 February 2019
				EP	3414321	A1	19 December 2018
				KR	20180110112	A	08 October 2018
				BR	112018016450	A2	26 December 2018
				WO	2017139576	A1	17 August 2017
				SG	11201806758V	A	27 September 2018
				MX	2018009750	A	07 February 2019
				CA	3014078	A1	17 August 2017
				AU	2017217813	A1	20 September 2018
				RU	2021103425	A	25 February 2021
				RU	2018132210	A	12 March 2020
CN	109971787	A	05 July 2019	EP	3956458	A1	23 February 2022
				WO	2020211828	A1	22 October 2020
				GB	202115560	D0	15 December 2021
CN	112575034	A	30 March 2021	None			
WO	2021064162	A1	08 April 2021	AU	2020357668	A1	10 March 2022
				CA	3149259	A1	08 April 2021