



(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION
(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2018/04/05
(87) Date publication PCT/PCT Publication Date: 2018/10/11
(85) Entrée phase nationale/National Entry: 2019/04/15
(86) N° demande PCT/PCT Application No.: IB 2018/052378
(87) N° publication PCT/PCT Publication No.: 2018/185709
(30) Priorités/Priorities: 2017/04/05 (PT110012);
2017/05/15 (EP17171166.6); 2017/08/24 (PT110263);
2017/08/25 (PT110267)

(51) Cl.Int./Int.Cl. *C12N 5/0784* (2010.01),
A61K 35/15 (2015.01)
(71) Demandeur/Applicant:
CENTRO DE NEUROCIENCIAS E BIOLOGIA
CELULAR, PT
(72) Inventeurs/Inventors:
RIBEIRO LEMOS PEREIRA, CARLOS FILIPE, PT;
FERREIRA PIRES, CRISTIANA, PT;
FIUZA ROSA, FABIO ALEXANDRE, PT
(74) Agent: BCF LLP

(54) Titre : COMPOSITIONS DESTINEES A LA REPROGRAMMATION DE CELLULES EN CELLULES DENDRITIQUES
OU EN CELLULES PRESENTATRICES D'ANTIGENE, PROCEDES ET UTILISATIONS ASSOCIES
(54) Title: COMPOSITIONS FOR REPROGRAMMING CELLS INTO DENDRITIC CELLS OR ANTIGEN PRESENTING
CELLS, METHODS AND USES THEREOF

(57) **Abrégé/Abstract:**

The present disclosure relates to compositions, nucleic acid constructs, methods and kits thereof for cell induction or reprogramming cells to the dendritic cell state or antigen presenting cell state, based, in part, on the surprisingly effect described herein of novel use and combinations of transcription factors that permit induction or reprogramming of differentiated or undifferentiated cells into dendritic cells or antigen presenting cells. Such compositions, nucleic acid constructs, methods and kits can be used for inducing dendritic cells in vitro, ex vivo, or in vivo, and these induced dendritic cells or antigen presenting cells can be used for immunotherapy applications.

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
11 October 2018 (11.10.2018)



(10) International Publication Number
WO 2018/185709 A1

(51) International Patent Classification:

C12N 5/0784 (2010.01) A61K 35/15 (2015.01)

(21) International Application Number:

PCT/IB2018/052378

(22) International Filing Date:

05 April 2018 (05.04.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

110012	05 April 2017 (05.04.2017)	PT
17171166.6	15 May 2017 (15.05.2017)	EP
110263	24 August 2017 (24.08.2017)	PT
110267	25 August 2017 (25.08.2017)	PT

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))
- in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE

(71) Applicant: CENTRO DE NEUROCIENCIAS E BIOLOGIA CELULAR [PT/PT]; Universidade de Coimbra, Center for Neuroscience and Cell Biology (CNC), Largo Marquês do Pombal, 3004-517 Coimbra (PT).

(72) Inventors: RIBEIRO LEMOS PEREIRA, Carlos Filipe; Rua João Baptista da Silva 390, Moledo, 4910-264 Caminha (PT). FERREIRA PIRES, Cristiana; Rua Fonte do Areeiro n29, 3770-352 Palhaça (PT). FIÚZA ROSA, Fábio Alexandre; Rua do Brejo, N°53 Touria Pousos, 2410-205 Leiria (PT).

(74) Agent: TEIXEIRA DE CARVALHO, Anabela; Paten-tree, Edifício Net, Rua de Salazares 842, 4149-002 Porto (PT).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

(54) Title: COMPOSITIONS FOR REPROGRAMMING CELLS INTO DENDRITIC CELLS OR ANTIGEN PRESENTING CELLS, METHODS AND USES THEREOF

(57) Abstract: The present disclosure relates to compositions, nucleic acid constructs, methods and kits thereof for cell induction or reprogramming cells to the dendritic cell state or antigen presenting cell state, based, in part, on the surprisingly effect described herein of novel use and combinations of transcription factors that permit induction or reprogramming of differentiated or undifferentiated cells into dendritic cells or antigen presenting cells. Such compositions, nucleic acid constructs, methods and kits can be used for inducing dendritic cells in vitro, ex vivo, or in vivo, and these induced dendritic cells or antigen presenting cells can be used for immunotherapy applications.

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D E S C R I P T I O N**COMPOSITIONS FOR REPROGRAMMING CELLS INTO DENDRITIC CELLS OR ANTIGEN
PRESENTING CELLS, METHODS AND USES THEREOF****TECHNICAL FIELD**

[0001] The present disclosure relates to the development of methods for making dendritic cells or antigen presenting cells with antigen presenting capacity from differentiated, multipotent or pluripotent stem cells by introducing and expressing isolated transcription factors. More particularly, the disclosure provides methods for redirecting differentiated, multipotent or pluripotent stem cells to a dendritic cell or antigen presenting cell state by direct cellular reprogramming with a surprisingly use of combinations of transcription factors.

BACKGROUND

[0002] Cellular reprogramming relies on rewiring the epigenetic and transcriptional network of one cell state to that of a different cell type. Transcription factor (TF)-transduction experiments have highlighted the plasticity of adult somatic or differentiated cells, providing new technologies to generate any desired cell type. Through forced expression of TFs, it is possible to reprogram somatic or differentiated cells into induced pluripotent stem cells (iPSCs) that are remarkably similar to embryonic stem cells (1, 2). Alternatively, a somatic cell can also be converted into another specialized cell type (3). Direct lineage conversion has proven successful to reprogram mouse and human fibroblasts into several cell types, such as neurons, cardiomyocytes and hepatocytes, using TFs specifying the target-cell identity (4). Lineage conversions were also demonstrated in the hematopoietic system, where forced expression of TFs induced a macrophage fate in B cells and fibroblasts (5) and the direct reprogramming of mouse fibroblasts into clonogenic hematopoietic progenitors is achieved with Gata2, Gfi1b, cFos and Etv6 (6). These four TFs induce a dynamic, multi-stage hemogenic process that progresses through an endothelial-like intermediate, recapitulating developmental hematopoiesis *in vitro* (7).

[0003] Reprogrammed cells are very promising therapeutic tools for regenerative medicine, and cells obtained by differentiation of iPSCs are already being tested in clinical studies. For

hematopoietic regeneration, however, approaches to generate mature blood cells from iPSCs are still lacking. Therefore, alternative strategies are needed to generate patient-specific definitive hematopoietic cells that can be used as blood products. Given the opportunity of direct cell reprogramming mediated by TFs, one can envision the generation of antigen-presenting cells (APCs) of the immune system such as Dendritic Cells (DCs).

[0004] DCs are professional APCs capable of activating T cell responses by displaying peptide antigens complexed with the major histocompatibility complex (MHC) on the surface, together with all of the necessary soluble and membrane associated co-stimulatory molecules. DCs induce primary immune responses, potentiate the effector functions of previously primed T-lymphocytes, and orchestrate communication between innate and adaptive immunity. DCs are found in most tissues, where they continuously sample the antigenic environment and use several types of receptors to monitor for invading pathogens. In steady state, and at an increased rate upon detection of pathogens, sentinel DC in non-lymphoid tissues migrate to the lymphoid organs where they present to T cells the antigens they have collected and processed. The phenotype acquired by the T cell depends on the context in which the DC presents its antigen. If the antigen is derived from a pathogen, or damaged self, DC receive danger signals, become activated and the T cells are then stimulated to become effectors, necessary to provide protective immunity.

[0005] The ability of DCs to induce adaptive immunity has boosted research on DC-vaccine strategies for bacterial, viral and parasitic pathogens and cancer immunotherapy. In fact, clinical trials are ongoing utilizing DC-mediated immunotherapy for several tumor types, including solid and hematological tumors (8). However, the clinical outcome has been inconsistent, probably associated with variable efficiency in generating DCs *in vitro*: autologous monocytes give origin to less-efficient DCs, and hematopoietic progenitors are isolated in very low numbers. In addition, these precursor cells are commonly compromised in cancer-bearing patients, resulting in the generation of dysfunctional DCs (8, 9). Cancer evasion mechanisms also may be underlying the lack of consistent therapeutic advantages in DC-based immunotherapies. During tumor progression cancer cells exploit several immunological processes to escape immune surveillance. These adaptations, together with cancer antigen heterogeneity, prevent the recognition of tumor antigens by the immune system and are consequently responsible for the reduced immunogenicity of tumor cells and current immunotherapies.

[0006] The generation of APCs by direct reprogramming opens new opportunities to a better understanding of DC specification and cellular identity, contributing to a more efficient control of immune responses using autologous-engineered cells.

[0007] These facts are disclosed in order to illustrate the technical problem addressed by the present disclosure.

GENERAL DESCRIPTION

[0008] The present subject matter identifies several isolated transcription factors that surprisingly reprogram or induce differentiated cell, multipotent or pluripotent stem cell into dendritic cell, in vitro, ex vivo or in vivo.

[0009] Surprisingly the induced Dendritic Cells generated by reprogramming as described in the present disclosure, are intrinsically more mature than splenic DCs (natural DCs) and are less dependent on exogenous activation stimuli for antigen presentation.

[0010] DCs are professional APCs located throughout the body functioning at the interface of the innate and adaptive immune system. DCs are able to provide a crucial link between the external environment and the adaptive immune system through their ability to capture, process and present antigens to T cells, targeting them to different types of immune responses or to tolerance. Firstly, DCs have to capture antigens and process them through major histocompatibility complex (MHC) class I and MHC class II. Following their activation, DCs are able to migrate towards the local draining lymph nodes priming multiple B cell and T cell responses, a key feature of adaptive immunity. The early protective efficacy is primarily conferred by the induction of antigen-specific antibodies produced by B lymphocytes. The long-term protection against specific antigens requires the persistence of specific antibodies and the generation of immunological memory that could provide a rapid and efficient response after subsequent antigen exposure. DCs, as professional APCs, have the ability to cross-present antigens, meaning that, in addition to its classical ability to present exogenous antigens on MHC class II and endogenous antigens on MHC class I, they are also able to present exogenous antigens on MHC class I, a critical step for the generation of Cytotoxic T Lymphocyte responses (CTL).

[0011] The ontogeny and/or microenvironment in which DC are positioned may result in the expression of distinct combinations of surface receptors by DCs. For example, phenotypic criteria alone allow the classification of mouse DCs into different subpopulations.

Of these, conventional DC (cDC) in lymphoid tissues are traditionally sub-divided into cDC1 and cDC2 subpopulations. It has been argued that different DC subsets may be involved in specific recognition of certain pathogens and/or regulate different immune responses, e.g. Th1 or Th2 (immunity) or regulatory T cells (tolerance). However, the phenotype and functional behavior of DCs is also significantly conditioned by external activating stimuli, denoting significant plasticity. cDC1 and cDC2 subsets differentially prime Th1 and Th2 responses in vivo. Immune therapy for cancer relies on using DCs to prime Th1 or cytotoxic T lymphocyte responses to promote tumor clearance.

[0012] Currently, DC-based immunotherapies rely on autologous DC precursors: either monocytes, which are associated with the production of less-efficient DCs, or hematopoietic progenitors, which are isolated in very low numbers. In addition, these precursor cells are commonly compromised in cancer-bearing patients, resulting in the generation of dysfunctional DCs. In contrast, non-hematopoietic cell-types such as fibroblasts are usually not affected. Human Dermal Fibroblasts (HDFs) also exhibit other competitive advantages, namely are easily obtained from a small skin punch biopsy, are easily expanded in vitro for several passages (15-20 million cells after 4 weeks) and can be conserved frozen and used on-demand. Given the fundamental role of DCs as APCs functioning at the interface of the innate and adaptive immune system, there remains a clinical need to find alternative strategies to generate functional DCs to prime antigen-specific immune responses.

[0013] An aspect of the present disclosure relates to compositions comprising the combination of at least two isolated transcription factors

encoded by a sequence 90% identical to a sequence from a list consisting of: BATF3 (SEQ. ID. 1 or SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14);

as a reprogramming or inducing factor of a cell selected from a list consisting of: stem cell or a differentiated cell, or mixtures thereof,

into dendritic cell or antigen presenting cell in vitro, ex vivo or in vivo.

[0014] In some embodiments, polypeptide variants or family members having the same or a similar activity as the reference polypeptide encoded by the sequences provided in the sequence listing can be used in the compositions, methods, and kits described herein. Generally, variants of a particular polypeptide encoding a DC inducing factor for use in the compositions, methods, and kits described herein will have at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or more

sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art.

[0015] Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch ((1970) J Mol Biol 48: 443-453) to find the global (over the whole the sequence) alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. The BLAST algorithm (Altschul et al. (1990) J Mol Biol 215: 403-10) calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (NCBI). Global percentages of similarity and identity may also be determined using one of the methods available in the MatGAT software package (Campanella et al., BMC Bioinformatics. 2003 Jul 10; 4:29. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences). Minor manual editing may be performed to optimise alignment between conserved motifs, as would be apparent to a person skilled in the art. The sequence identity values, which are indicated in the present subject-matter as a percentage were determined over the entire amino acid sequence, using BLAST with default parameters.

[0016] In an embodiment for better results, the combination of isolated transcription factor may be:

BATF3 (SEQ. ID. 1 or SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or BATF3 (SEQ. ID. 1, SEQ. ID. 2) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and BATF3 (SEQ. ID. 1, SEQ. ID. 2); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) BATF3 (SEQ. ID. 1, SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or TCF4 (SEQ. ID. 13, SEQ. ID. 14), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).

[0017] In an embodiment, the isolated transcription factor of the present disclosure may be used in veterinary or human medicine, in particular in immunotherapy, or in neurodegenerative diseases, or in cancer or in infectious diseases.

[0018] In an embodiment for better results the cell may be selected from a list consisting of: pluripotent stem cell, multipotent stem cell, differentiated cell, tumor cell, cancer cell, and mixtures thereof. In particular mammalian cell, more in particular a mouse or a human cell.

[0019] In an embodiment for better results, the isolated transcription factor of the present disclosure may be use as a reprogramming or inducing factor of a cell selected from a list consisting of: pluripotent stem cell, or multipotent stem cell, or differentiated cell, and mixtures thereof into dendritic cell.

[0020] In an embodiment for better results, the isolated transcription factor of the present disclosure may be use a reprogramming or inducing factor of a cell selected from a list consisting of: tumor cell, cancer cell, and mixtures thereof, into antigen presenting cell.

[0021] Another aspect of the present disclosure is the use of a combination of at least two sequences at least 90% identical, preferably at least 91%, 92%, 93%, 94%, 95%, 96 %, 97%, 98%, 99%, or identical, to a sequence from a list consisting of: BATF3 (SEQ. ID. 1 or SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14), and mixtures thereof;

as a reprogramming or inducing factor of a cell selected from a list consisting of:
stem cell or a differentiated cell, or mixtures thereof,
into dendritic cell or antigen presenting cell in vitro, ex vivo or in vivo.

Preferably the combination may be selected from a list consisting of: BATF3 (SEQ. ID. 1 or SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14), and mixtures thereof. More preferably, the isolated transcription may include the following combination: BATF3 (SEQ. ID. 1 or SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or BATF3 (SEQ. ID. 1, SEQ. ID. 2) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and BATF3 (SEQ. ID. 1, SEQ. ID. 2); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or TCF4 (SEQ. ID. 13, SEQ. ID. 14), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).

[0022] Another aspect of the present disclosure relates to a construct or a vector encoding at least one isolated transcription factor described in the present subject-matter.

[0023] In an embodiment for better results, the construct or the vector may be the combination of three isolated transcription factors is in the following sequential order from 5' to 3': PU.1 (SEQ. ID. 7, SEQ. ID. 8), IRF8 (SEQ. ID. 5, SEQ. ID. 6), BATF3 (SEQ. ID. 1, SEQ. ID. 2); or IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), BATF3 (SEQ. ID. 1, SEQ. ID. 2).

[0024] In an embodiment, the vector is a viral vector; in particular a retrovirus, a adenovirus, a lentivirus, a herpes virus, a pox virus, or adeno-associated virus vectors.

[0025] In an embodiment for better results, the transducing step further comprises at least one vector selected from a list consisting of: a nucleic acid sequence encoding IL12; nucleic acid sequence encoding GM-CSF; nucleic acid sequence encoding IL-7; nucleic acid sequence encoding siRNA targeting IL-10 RNA, and mixtures thereof.

[0026] In an embodiment for better results the transducing of step further comprises at least one vector comprising nucleic acids encoding immunostimulatory cytokines.

[0027] Another aspect of the present disclosure relates to a method for programming or inducing a stem cell or a differentiated cell into a dendritic cell or antigen presenting cell, comprising the following step:

transducing a cell selected from a list consisting of: a stem cell or a differentiated cell, and mixtures thereof,

with one or more vectors comprising at least two nucleic acid sequence encoding a sequence at least 90% identical to a sequence from a list consisting of: BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14), and mixtures thereof; preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or identical;

culturing the transduced somatic cell in a cell media that supports growth of dendritic cells or antigen presenting cells.

[0028] Preferably for better results, wherein the combination of isolated transcription factors is selected from the following encoded combinations: BATF3 (SEQ. ID. 1 or SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or BATF3 (SEQ. ID. 1, SEQ. ID. 2) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and BATF3 (SEQ. ID. 1, SEQ. ID. 2); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or

BATF3 (SEQ. ID. 1, SEQ. ID.2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14) BATF3 (SEQ. ID. 1, SEQ. ID.2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6); or TCF4 (SEQ. ID. 13, SEQ. ID. 14), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8); or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID.2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).

[0029] In an embodiment for better results, the construct or the vector may be the combination of at least three isolated transcription factors in the following sequential order from 5' to 3': PU.1 (SEQ. ID. 7, SEQ. ID. 8), IRF8 (SEQ. ID. 5, SEQ. ID. 6), BATF3 (SEQ. ID. 1, SEQ. ID. 2); or IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), BATF3 (SEQ. ID. 1, SEQ. ID. 2).

[0030] In an embodiment for better results, cells may be transduced with a plurality of isolated transcription factors and cultured during at least 2 days, preferably at least 5 days, more preferably at least 8 days, even more preferably 9 days.

[0031] In an embodiment for better results, the transducing step may further comprise at least one vector selected from a list consisting of: a nucleic acid sequence encoding IL-12; nucleic acid sequence encoding GM-CSF; nucleic acid sequence encoding IL-7; nucleic acid sequence encoding siRNA targeting IL-10 RNA, and mixtures thereof.

[0032] In an embodiment for better results, the cell may be selected from the group consisting of pluripotent stem cell, or multipotent stem cell, differentiated cell, and mixtures thereof. In particular an endoderm derived cell, a mesoderm derived cell, or an ectoderm derived cell, a multipotent stem cell including mesenchymal stem cell, a hematopoietic stem cell, intestinal stem cell, a pluripotent stem cell, a tumor or cancer cell and cell lines.

[0033] In an embodiment for better results, the cell may be a non-human cell, preferably a mouse or a human cell, more preferably cell is a human or mouse fibroblast, or a mammal umbilical cord blood stem cell.

[0034] Another aspect of the present disclosure relates to induced dendritic cell or antigen presenting cell obtained by the method described in the present disclosure.

[0035] Another aspect of the present disclosure relates to induced antigen presenting cell obtained by the method described in the present disclosure. In particular, an induced antigen presenting cell capable to present a cancer antigen, a self-antigen, an allergen, an antigen from a pathogenic and/or infectious organism.

[0036] Another aspect of the present disclosure relates to composition comprising at least one isolated transcription factor as described in the present disclosure, or an induced dendritic cell as described in the present disclosure, or an induced antigen presenting cell as described in the present disclosure, or mixtures thereof, in a therapeutically effective amount and a pharmaceutically acceptable excipient.

[0037] In a preferably embodiment, the composition may be use in veterinary or human medicine, in particular in immunotherapy, or in neurodegenerative diseases, or in cancer or in infectious diseases.

[0038] In a preferably embodiment, the composition may further comprise an anti-viral, an analgesic, an anti-inflammatory agent, a chemotherapy agent, a radiotherapy agent, an antibiotic, a diuretic, or mixtures thereof.

[0039] In a preferably embodiment, the composition may further comprise a filler, a binder, a disintegrant, or a lubricant, or mixtures thereof.

[0040] In a preferably embodiment, the composition may be use in intradermal and transdermal therapies.

[0041] In a preferably embodiment, the composition may be use as an injectable formulation, in particular an in-situ injection.

[0042] In a preferably embodiment, the composition may be use in veterinary or human medicine, in particular in immunotherapy, or in the treatment or therapy neurodegenerative diseases, or in the treatment or therapy of cancer or in the treatment or therapy of an infectious diseases.

[0043] In a preferably embodiment, the composition may be use in the treatment, therapy or diagnostic of a central and peripheral nervous system disorder.

[0044] In a preferably embodiment, the composition may be use in the treatment therapy or diagnostic of neoplasia in particular cancer, namely solid or hematological tumors.

[0045] In a preferably embodiment, the composition may be use in the treatment, diagnostic or therapy of benign tumor, malignant tumor, early cancer, basal cell carcinoma, cervical dysplasia, soft tissue sarcoma, germ cell tumor, retinoblastoma, age-related macular degeneration, Hodgkin's lymphoma, blood cancer, prostate cancer, ovarian cancer, cervix cancer, uterus cancer, vaginal cancer, breast cancer, naso-pharynx cancer, trachea cancer, larynx cancer, bronchi cancer, bronchioles cancer, lung cancer, hollow organs cancer, esophagus cancer, stomach cancer, bile duct cancer, intestine cancer, colon cancer,

colorectum cancer, rectum cancer, bladder cancer, ureter cancer, kidney cancer, liver cancer, gall bladder cancer, spleen cancer, brain cancer, lymphatic system cancer, bone cancer, pancreatic cancer, leukemia, skin cancer, or myeloma.

[0046] In a preferably embodiment, the composition may be use in the treatment, therapy or diagnostic of a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease.

[0047] In a preferably embodiment, the composition may be use in therapy or diagnostic of HIV, infection with SARS coronavirus, Asian flu virus, herpes simplex, herpes zoster, hepatitis, or viral hepatitis.

[0048] In a preferably embodiment, the composition may be use in the treatment, therapy or diagnostic of an amyloid disease in particular Amyloid A (AA) amyloidosis, Alzheimer's disease, Light-Chain (AL) amyloidosis, Type-2 Diabetes, Medullary Carcinoma of the Thyroid, Parkinson's disease, Polyneuropathy, or Spongiform Encephalopathy - Creutzfeldt Jakob disease.

[0049] Another aspect of the present disclosure relates to a vaccine for cancer comprising to compositions comprising at least one isolated transcription factor as described in the present disclosure, or an induced dendritic cell as described in the present disclosure, or an induced antigen presenting cell as described in the present disclosure, or mixtures thereof.

[0050] A kit comprising at least one of the following components: a composition comprising at least one isolated transcription factor as described in the present disclosure, or an induced dendritic cell as described in the present disclosure, or a induced antigen presenting cell as described in the present disclosure, a composition as described in the present disclosure, or a vector as described in the present disclosure, or a construct as described in the present disclosure or mixtures thereof.

[0051] An aspect of the present disclosure relates to compositions, methods, and kits for dendritic cell induction or for reprogramming cells to antigen-presenting dendritic cells (DC). In some embodiments, the compositions comprise at least one DC inducing factor. Such compositions, methods and kits can be used for inducing dendritic cells in vitro, ex vivo, or in vivo, as described herein, and these induced dendritic cells (iDCs) can be used in immunotherapies.

[0052] The compositions, methods, and kits for dendritic cell induction or for reprogramming cells to dendritic cells of the present disclosure are based, in part, in the use

of a novel combination of transcription factors that permit direct reprogramming of differentiated cells to the dendritic cell state. Such compositions, nucleic acid constructs, methods and kits can be used for inducing dendritic cells in vitro, ex vivo, or in vivo, as described herein, and these induced dendritic cells can be used in immunotherapies.

[0053] In an embodiment, the present disclosure relates to the regulation of the immune system, and in particular to the use of reprogrammed dendritic cells to prime immune responses to target antigens.

[0054] In an embodiment, the resulting dendritic cell is an antigen presenting cell which activates T cells against MHC class I-antigen targets. Cancer, viral, bacterial and parasitic infections are all ameliorated by the reprogrammed dendritic cells. As reprogrammed dendritic cells are capable of cross-presenting extracellular antigens via the MHC class I pathway, they are particularly suitable for generation of cytotoxic T lymphocyte responses.

[0055] In an embodiment isolated transcription factor (or exogenous transcription factor) selected from a list consisting of: BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14), and mixtures thereof, upon forced expression in fibroblasts induce activation of the Clec9a DC-specific reporter, DC morphology and a conventional DC type 1 (cDC1) transcriptional program. Induced Dendritic Cells (iDCs) express cDC1 markers, major histocompatibility complex (MHC)-I and II at the cell surface and the co-stimulatory molecules CD80, CD86 and CD40. iDCs are able to engulf particles and upon challenge with LPS or poly I:C secrete inflammatory cytokines. iDCs present antigens to CD4⁺ T cells and cross-present antigens to CD8⁺ T cells.

In an embodiment isolated transcription factor (or exogenous transcription factor) selected from a list consisting of: BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), upon forced expression in fibroblasts induce expression of CLEC9A and HLA-DR, typical DC markers, and DC morphology. Induced Dendritic Cells (iDCs) are able to engulf particles and soluble protein. This disclosure provides powerful new treatments for cancers and cellular infections, as well as a variety of diagnostic and cell screening assays.

In an embodiment, isolated transcription factor (or exogenous transcription factor) selected from a list consisting of: BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), upon forced expression in cancer cell lines induce expression of CLEC9A and MHC-II at the cell surface.

[0056] In an embodiment, CLEC9A is preferentially expressed on the subset cDC1 of dendritic cells. This is an important cell type because it is capable of processing antigens

derived from outside the cell and presenting them to T cells via MHC class I molecules. This is in contrast to most antigen presenting cells, which present extracellularly-derived antigens via MHC class II molecules. Consequently, this mechanism of antigen presentation is sometimes referred to as “cross-presentation”. These cells therefore play an important role in the generation and stimulation of CTL responses, which are an essential part of the immune response against intracellular pathogens, e.g. viruses and cancers.

[0057] In an embodiment, immune responses stimulated via iDCs involve proliferation of T cells, which may be CTL or helper T cells. Antigen presenting cells (and in particular iDCs) can induce proliferation of both CD8+ T cells and CD4+ T cells, and may stimulate proliferation of both types of T cells in any given immune response.

[0058] In an embodiment, iDCs may be implicated in at least Th1, Th2, and Th17-type immune responses. Thus, the methods of the invention may be applied to stimulation of various types of immune response against any antigen. However, these cells are believed to be particularly important in the generation of CTL responses, so the immune response to be stimulated is preferably a CTL response. The method may comprise determining production and/or proliferation of CTLs, which are typically T cells expressing CD8 and are capable of cytotoxic activity against cells displaying their cognate antigen in the context of MHC class I molecules.

[0059] It will therefore be further understood that iDCs may be used for the prophylaxis and/or treatment of any condition in which it is desirable to induce a CTL response, such as cancer, or infection by an intracellular parasite or pathogen, such as a viral infection.

[0060] Nevertheless, if modified, iDCs can result in proliferation of helper T cells as well as, or instead of, CTLs. Thus, the method may additionally or alternatively comprise determining production and/or proliferation of helper T cells. The helper T cells may be CD4+ T cells, and may be of Th1, Th2, Th17 or Treg type.

[0061] Under certain conditions, it is believed that iDCs may be capable of stimulating regulatory T cell (Treg) proliferation. Treg cells are characterised by the expression of the Foxp3 (Forkhead box p3) transcription factor. Most Treg cells are CD4+ and CD25+, and can be regarded as a subset of helper T cells, although a small population may be CD8+. Thus, the immune response, which is to be stimulated by a method of the present disclosure, may comprise inducing proliferation of Treg cells in response to an antigen. Given that Treg cells may be capable of modulating the response of other cells of the immune system against an antigen in other ways, e.g. inhibiting or suppressing their activity, the effect on the immune

system as a whole may be to modulate (e.g. suppress or inhibit) the response against that antigen. Thus, the methods of this aspect of the invention can equally be referred to as methods of modulating (e.g. inhibiting or suppressing) an immune response against an antigen. This may be particularly useful (for example) in the treatment of autoimmune disease.

[0062] iDCs will promote antigen-specific responses. The antigen may be any protein or fragment thereof against which it is desirable to raise an immune response, in particular a CTL response, but also a Th17 response or a Treg response. These may include antigens associated with, expressed by, displayed on, or secreted by cells against which it is desirable to stimulate a CTL response, including cancer cells and cells containing intracellular pathogens or parasites. For example, the antigen may be, or may comprise, an epitope peptide from a protein expressed by an intracellular pathogen or parasite (such as a viral protein) or from a protein expressed by a cancer or tumor cell. Thus, the antigen may be a tumor-specific antigen. The term "tumor-specific" antigen should not be interpreted as being restricted to antigens from solid tumors, but to encompass antigens expressed specifically by any cancerous, transformed or malignant cell.

[0063] The invention therefore provides a primed antigen presenting cell or population thereof. By "primed" is meant that the cell has been contacted with an antigen, is presenting that antigen or an epitope thereof in the context of MHC molecules, preferably MHC I molecules, and is capable of activating or stimulating T cells to proliferate and differentiate into effector cells in response thereof.

[0064] The term "antigen" is well understood in the art and includes immunogenic substances as well as antigenic epitopes. It will be appreciated that the use of any antigen is envisioned for use in the present invention and thus includes, but is not limited to, a self-antigen (whether normal or disease-related), an infectious antigen (e.g., a microbial antigen, viral antigen, etc.), or some other foreign antigen (e.g., a food component, pollen, etc.). Loading the antigen-presenting cells with an antigen can be accomplished utilizing standard methods, for example, pulsing, transducing, transfecting, and/or electrofusing. It is envisioned that the antigen can be nucleic acids (DNA or RNA), proteins, protein lysate, whole cell lysate, or antigen proteins linked to other proteins, i.e., heat shock proteins. The antigens can be derived or isolated from a pathogenic microorganism such as viruses including HIV, influenza, Herpes simplex, human papilloma virus, Hepatitis B, Hepatitis C, EBV, Cytomegalovirus (CMV) and the like. The antigen may be derived or isolated from pathogenic bacteria such as from *Chlamydia*, *Mycobacteria*, *Legionella*, *Meningioccus*,

Group A *Streptococcus*, *Salmonella*, *Listeria*, *Hemophilus influenzae*, and the like. Still further, the antigen may be derived or isolated from pathogenic yeast including *Aspergillus*, invasive *Candida*, *Nocardia*, Histoplasmosis, *Cryptosporidia* and the like. The antigen may be derived or isolated from a pathogenic protozoan and pathogenic parasites including, but not limited to *Pneumocystis carinii*, *Trypanosoma*, *Leishmania*, *Plasmodium* and *Toxoplasma gondii*. In certain embodiments, the antigen includes an antigen associated with a preneoplastic or hyperplastic state. Antigens may also be associated with, or causative of cancer. Such antigens are tumor specific antigen, tumor associated antigen (TAA) or tissue specific antigen, epitope thereof, and epitope agonist thereof. Such antigens include but are not limited to carcinoembryonic antigen (CEA) and epitopes thereof such as CAP-1, CAP-1-6D, MART-1, MAGE-1, MAGE-3, GAGE, GP-100, MUC-1, MUC-2, point mutated ras oncogene, normal and point mutated p53 oncogenes, PSMA, tyrosinase, TRP-1 (gp75), NY-ESO-1, TRP-2, TAG72, KSA, CA-125, PSA, HER-2/neu/c-erb/B2, BRC-I, BRC-II, bcr-abl, pax3-fkhr, ewe-fli-1, modifications of TAAs and tissue specific antigen, splice variants of TAAs, epitope agonists, and the like.

[0065] The term “agent” as used herein means any compound or substance such as, but not limited to, a small molecule, nucleic acid, polypeptide, peptide, drug, ion, etc. An “agent” can be any chemical, entity or moiety, including without limitation synthetic and naturally-occurring proteinaceous and non-proteinaceous entities. In some embodiments, an agent is nucleic acid, nucleic acid analogues, proteins, antibodies, peptides, aptamers, oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNAzymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof etc. In some embodiments, the nucleic acid is DNA or RNA, and nucleic acid analogues, for example can be PNA, pcPNA and LNA. A nucleic acid may be single or double stranded, and can be selected from a group comprising; nucleic acid encoding a protein of interest, oligonucleotides, PNA, etc. Such nucleic acid sequences include, for example, but not limited to, nucleic acid sequence encoding proteins that act as transcriptional repressors, antisense molecules, ribozymes, small inhibitory nucleic acid sequences, for example but not limited to RNAi, shRNAi, siRNA, micro RNAi (mRNAi), antisense oligonucleotides etc. A protein and/or peptide agent or fragment thereof, can be any protein of interest, for example, but not limited to; mutated proteins; therapeutic proteins; truncated proteins, wherein the protein is normally absent or expressed at lower levels in the cell. Proteins of interest can be selected from a group comprising; mutated proteins, genetically engineered proteins, peptides, synthetic peptides,

recombinant proteins, chimeric proteins, antibodies, humanized proteins, humanized antibodies, chimeric antibodies, modified proteins and fragments thereof.

[0066] As used herein, the term “transcription factor” or “TF” refers to a protein that binds to specific parts of DNA using DNA binding domains and is part of the system that controls the transcription of genetic information from DNA to RNA.

[0067] The term “DC inducing factor,” as used herein, refers to a developmental potential altering factor, as that term is defined herein, such as a protein, RNA, or small molecule, the expression of which contributes to the reprogramming of a cell, e.g. a somatic cell, to the DC state. A DC inducing factor can be, for example, transcription factors that can reprogram cells to the DC state, such as PU.1, IRF8, BATF3 and TCF4, and the like, including any gene, protein, RNA or small molecule that can substitute for one or more of these factors in a method of making iDCs in vitro. In some embodiments, exogenous expression of a DC inducing factor induces endogenous expression of one or more DC inducing factors, such that exogenous expression of the one or more DC inducing factor is no longer required for stable maintenance of the cell in the iDC state.

[0068] The term “an antigen-presenting cell” (APC) as used herein, refers to a cell that displays antigen complexed with major histocompatibility complexes (MHCs) on their surfaces; this process is known as antigen presentation. T cells may recognize these complexes using their T cell receptors (TCRs). These cells process antigens and present them to T-cells.

[0069] The term “a somatic cell” used herein, refers to any biological cell forming the body of an organism; that is, in a multicellular organism, any cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell.

[0070] The expression of endogenous DC inducing factors can be induced by the use of DNA targeting systems able to modulate mammalian gene expression in a cell with or without the use of chromatin modifying drugs. In some embodiments, the DNA targeting system may comprise a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) 9-based system (as described in WO2014197748 A2) that may include any modified protein, isolated polynucleotide or vector contacted with the cell and at least one guide RNA targeting a promoter region of at least one gene selected from the group consisting of PU.1, IRF8, BATF3 and TCF4. The DNA targeting system may comprise dCas9-VP64. In some embodiments, the DNA targeting system may comprise two or more transcription activator-like effector transcription factors (as described in US20140309177

A1) that bind to different target regions of at least one gene selected from the group consisting of PU.1, IRF8, BATF3 and TCF4.

[0071] In an embodiment, iDCs can be used as immunotherapy to induce specific immune responses in patients with cancer, such as melanoma, prostate cancer, glioblastoma, acute myeloid leukemia, among others.

[0072] In an embodiment, iDCs can also be used to treat infections caused by viral, bacterial and parasitic pathogens.

[0073] In an embodiment, iDCs can also be used as *in vitro* tools for vaccine immunogenicity testing.

[0074] The pluripotent stem cells used in the present disclosure are obtained without having to recur to a method necessarily involving the destruction of human embryos, namely with the use of induced pluripotent stem cells. Induced pluripotent stem cells (also known as iPS cells or iPSCs) are a type of pluripotent stem cell that can be generated directly from adult cells by cellular reprogramming.

[0075] The induced Dendritic Cells (iDCs) obtainable by this method express MHC-I and II at the cell surface and the co-stimulatory molecules CD80, CD86 and CD40.

[0076] In some embodiments, the composition may comprises the isolated transcription factor discloses in the present subject-matter, in an amount effective to improve the immunotherapy by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 95.7%, at least 98%, or at least 99% in the subject.

[0077] In some embodiments, the composition may comprise the induced dendritic cells disclosure in the present subject-matter, in an amount effective to improve the immunotherapy by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 95.7%, at least 98%, or at least 99% in the subject.

[0078] DC inducing factors of the present disclosure can be delivered to induce reprogramming *in vitro*, *ex vivo* or *in vivo*.

[0079] Differentiated cells of the present disclosure can be isolated from a subject in need, DC inducing factors can be introduced to induce reprogramming into iDCs. Generated iDCs can be infused back to the patient.

[0080] Alternatively, DC inducing factors can be delivered to induce reprogramming in vivo of, for example, cancer cells into iDC with ability to present cancer antigens.

[0081] Preferred routes of administration include but are not limited to oral, parenteral, intramuscular, intravenous, in situ injection, intranasal, sublingual, intratracheal, and inhalation.

[0082] In some embodiments, the dose or dosage form is administered to the subject once a day, twice a day, or three times a day. In other embodiments, the dose is administered to the subject once a week, once a month, once every two months, four times a year, three times a year, twice a year, or once a year.

[0083] The embodiments of the invention provide multiple applications, including kits for research use and methods for generation of cells useful for conducting small molecule screens for immune disorders. In addition, the invention provides commercially and medically useful methods to produce autologous dendritic cells and give them back to a patient in need.

[0084] For example, the methods described herein can be used to produce dendritic cells to treat diseases including hyperproliferative diseases, which can also be further defined as cancer. In still further embodiments, the cancer is melanoma, non-small cell lung, small-cell lung, lung, hepatocarcinoma, leukemia, retinoblastoma, astrocytoma, glioblastoma, gum, tongue, neuroblastoma, head, neck, breast, pancreatic, prostate, colorectal, Esophageal, Non-Hodgkin lymphoma, uterine, liver, thyroid, renal, skin, bone, testicular, ovarian, mesothelioma, cervical, gastrointestinal, lymphoma, brain, colon, sarcoma or bladder. The cancer may include a tumor comprised of tumor cells. For example, tumor cells may include, but are not limited to melanoma cell, a bladder cancer cell, a breast cancer cell, a lung cancer cell, a colon cancer cell, a prostate cancer cell, a liver cancer cell, a pancreatic cancer cell, a stomach cancer cell, a testicular cancer cell, a brain cancer cell, an ovarian cancer cell, a lymphatic cancer cell, a skin cancer cell, a brain cancer cell, a bone cancer cell, or a soft tissue cancer cell. In other embodiments, the hyperproliferative disease is rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, leiomyomas, adenomas, lipomas, hemangiomas, fibromas, vascular occlusion, restenosis, atherosclerosis, pre-neoplastic lesions (such as adenomatous hyperplasia and prostatic intraepithelial neoplasia), carcinoma in situ, oral hairy leukoplakia, or psoriasis.

[0085] Accordingly, provided herein, in an embodiment are dendritic cell (DC) inducing composition comprising one or more expression vectors encoding at least two, three, four,

or more DC inducing factors selected from: BATF3 (SEQ. ID. 1, SEQ. ID. 2), , IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14), or mixtures thereof. In a particular embodiment, the addition of increases the efficiency in at least 8%.

[0086] In some embodiments of these aspects and all such aspects described herein, the one or more expression vectors are retroviral vectors.

[0087] In some embodiments of these aspects and all such aspects described herein, the one or more expression vectors are lentiviral vectors. In some embodiments, the lentiviral vectors are inducible lentiviral vectors.

[0088] Also provided herein, in some aspects, are dendritic cell (DC) inducing compositions comprising modified mRNA sequences encoding at least two, three, four, DC inducing factors selected from BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14) , or mixtures thereof, wherein each cytosine of each said modified mRNA sequence is a modified cytosine, each uracil of each said modified mRNA sequence is a modified uracil, or a combination thereof.

[0089] Provided herein, in some aspects, are dendritic cell (DC) inducing compositions comprising at least two sequences selected from TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), or mixtures thereof, wherein each cytosine of each said modified mRNA sequence is a modified cytosine, each uracil of each said modified mRNA sequence is a modified uracil, or a combination thereof.

[0090] In some embodiments of these aspects and all such aspects described herein, the modified cytosine is 5-methylcytosine and the modified uracil is pseudouracil.

[0091] Also provided herein in some aspects, are methods for preparing an induced dendritic cell (iDC) from a somatic cell comprising:

transducing the somatic cell with one or more vectors comprising a nucleic acid sequence encoding PU.1 (SEQ. ID. 7, SEQ. ID. 8), a nucleic acid sequence encoding IRF8 (SEQ. ID. 5, SEQ. ID. 6); a nucleic acid sequence encoding BATF3 (SEQ. ID. 1, SEQ. ID. 2); wherein each said nucleic acid sequence is operably linked to a promoter; and

culturing the transduced somatic cell in a cell media that supports growth of dendritic cells, thereby preparing an iDC.

[0092] In some embodiments of these aspects and all such aspects described herein, the transducing step further comprises one or more vectors comprising one or more of: a

nucleic acid sequence encoding TCF4 (SEQ. ID. 13, SEQ. ID. 14); a nucleic acid sequence encoding IL12; nucleic acid sequence encoding GM-CSF; nucleic acid sequence encoding IL-7; nucleic acid sequence encoding siRNA targeting IL-10 RNA.

[0093] In some embodiments of these aspects and all such aspects described herein, the transducing step further comprises one or more vectors comprising nucleic acids encoding immunostimulatory cytokines. Preferably, the cytokine is one of the interleukins (e.g., IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-18, IL-19, IL-20), interferons (e.g., IFN- α , IFN- β , IFN- γ), tumor necrosis factor (TNF), transforming growth factor- β (TGF- β), granulocyte colony stimulating factors (G-CSF), macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), Flt-3 ligand or kit ligand. The amino acid sequences of these cytokines are well known in the art. In the case of heterodimeric immunostimulatory cytokines (e.g., IL-12), induced dendritic cells (iDCs) shall be genetically modified to express both subunits of the cytokine molecule.

[0094] The additional vectors may also comprise nucleic acids encoding variants of these cytokines. For example, for those cytokines having both pro-forms and mature forms (e.g., before and after cleavage of a signal peptide, or before and after limited proteolysis to yield an active fragment), the APCs of the invention may be genetically modified to express either the pro- or mature form. Other variants, such as fusion proteins between an active fragment of a cytokine and a heterologous sequence (e.g., a heterologous signal peptide), may also be employed. Species variants may also be employed to the extent that they retain activity in a human subject. Thus, for example, human APCs may be genetically modified to express a murine, bovine, equine, ovine, feline, canine, non-human primate or other mammalian variant of a human cytokine if these species variants retain activity substantially similar to their human homologues.

[0095] It may be desirable also to administer further immunostimulatory agents in order to achieve maximal CTL stimulation and proliferation, and/or stimulation and proliferation of other T cell types. These may include agents capable of activating dendritic cells and stimulating their ability to promote T cell activation. Such an agent may be referred to as an adjuvant. The adjuvant may comprise an agonist for CD40 (such as soluble CD40 ligand, or an agonist antibody specific for CD40), an agonist of CD28, CD27 or OX40 (e.g. an agonist antibody specific for one of those molecules), a CTLA-4 antagonist (e.g. a blocking antibody specific for CTLA-4), and/or a Toll-like receptor (TLR) agonist, and/or any other agent capable of inducing dendritic cell activation. A TLR agonist is a substance that activates a Toll-like receptor. Preferably, the TLR agonist is an activator of TLR3, TLR4, TLR5, TLR7 or TLR9. A

suitable TLR agonist is MPL (monophosphoryl lipid A), which binds TLR4. Other TLR agonists which may be used are LTA (lipoteichoic acid, which binds TLR2; Poly I:C (polyinosine-polycytidylic acid), which binds TLR3; flagellin, which binds TLR5; imiquimod or polyU RNA (1-(2-methylpropyl)-1H-imidazo(4,5-c)quinolin-4-amine), which binds TLR7 and CpG (DNA CpG motifs), which binds TLR9; or any other component which binds to and activates a TLR. For more details, see Reis e Sousa, Toll-like receptors and dendritic cells. Seminars in Immunology 16:27, 2004. Adjuvants which may not work via TLRs include 5' triphosphate RNA, poly I:C, and β -glucans such as curdlan (β -1,3-glucan).

[0096] In some embodiments of these aspects and all such aspects described herein, the culturing step further comprises the use of cell media that supports growth of dendritic cells or antigen presenting cells supplemented with at least one immunostimulatory recombinant cytokine selected from the group consisting of interleukins (e.g., IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-18, IL-19, IL-20), interferons (e.g., IFN- α , IFN- β , IFN- γ), tumor necrosis factor (TNF), transforming growth factor- β (TGF- β), granulocyte colony stimulating factors (G-CSF), macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), Flt-3 ligand or kit ligand. In the case of heterodimeric immunostimulatory cytokines (e.g., IL-12), induced dendritic cells (iDCs) shall be cultured with both subunits of the cytokine molecule. Other pro-inflammatory cytokines may also be used as adjuvants.

[0097] In some embodiments of these aspects and all such aspects described herein, the somatic cell is a fibroblast cell.

[0098] In some embodiments of these aspects and all such aspects described herein, the somatic cell is a hematopoietic lineage cell.

[0099] In some embodiments of these aspects and all such aspects described herein, the hematopoietic lineage cell is selected from promyelocytes, neutrophils, eosinophils, basophils, reticulocytes, erythrocytes, mast cells, osteoclasts, megakaryoblasts, platelet producing megakaryocytes, platelets, monocytes, macrophages, lymphocytes, NK cells, NKT cells, innate lymphocytes, multipotent hematopoietic stem and progenitor cells, oligopotent hematopoietic progenitor cells, lineage restricted hematopoietic progenitors.

[00100] In some embodiments of these aspects and all such aspects described herein, the hematopoietic lineage cell is selected from a multi-potent progenitor cell (MPP), common myeloid progenitor cell (CMP), granulocyte-monocyte progenitor cells (GMP), common lymphoid progenitor cell (CLP), and pre-megakaryocyte-erythrocyte progenitor cell.

[00101] In some embodiments of these aspects and all such aspects described herein, the hematopoietic lineage cell is selected from a megakaryocyte-erythrocyte progenitor cell (MEP), a ProB cell, a PreB cell, a PreProB cell, a ProT cell, a double-negative T cell, a pro-NK cell, a pre-dendritic cell (pre-DC), pre-granulocyte/macrophage cell, a granulocyte/macrophage progenitor (GMP) cell, and a pro-mast cell (ProMC).

[00102] Also provided herein, in some aspects, are kits for making induced dendritic cells (iDC), the kits comprising any of the DC inducing compositions comprising one or more expression vector components described herein.

[00103] Provided herein, in some aspects, are kits for making induced dendritic cells (iDC), the kits comprising any of the DC inducing compositions comprising modified mRNA sequence components described herein.

[00104] In some embodiments of these aspects and all such aspects described herein, the one or more expression vectors are lentiviral vectors. In some embodiments, the lentiviral vectors are inducible lentiviral vectors. In some embodiments, the lentiviral vectors are polycistronic inducible lentiviral vectors. In some embodiments, the polycistronic inducible lentiviral vectors express three or more nucleic acid sequences. In some embodiments, each of the nucleic acid sequences of the polycistronic inducible lentiviral vectors are separated by 2A peptide sequences.

[00105] The use of polycistronic viral expression systems can increase the in vivo reprogramming efficiency of somatic cells to iDCs. Accordingly, in some embodiments of the aspects described herein, a polycistronic lentiviral vector is used. In such embodiments, sequences encoding two or more of the DC inducing factors described herein, are expressed from a single promoter, as a polycistronic transcript. 2A peptide strategy can be used to make polycistronic vectors (see, e.g., Expert Opin Biol Ther. 2005 May; 5(5):627-38). Polycistronic expression vector systems can also use internal ribosome entry sites (IRES) elements to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5'-methylated Cap dependent translation and begin translation at internal sites (Pelletier and Sonenberg, 1988). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, thus creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message. See, for example, U.S. Pat. Nos. 4,980,285; 5,925,565; 5,631,150; 5,707,828;

5,759,828; 5,888,783; 5,919,670; and 5,935,819; and Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press (1989).

BRIEF DESCRIPTION OF THE DRAWINGS

[00106] The following figures provide preferred embodiments for illustrating the description and should not be seen as limiting the scope of invention.

[00107] **Figure 1.** Schematic representation of hematopoietic differentiation. Whereas hematopoietic differentiation normally proceeds from hematopoietic stem cells (HSCs) through progressively more-restricted progenitors into differentiated blood effector cells, such as dendritic cells (DCs) (highlighted with dashed-line box), the results described herein aim to utilize DC-enriched transcription factors to reprogram somatic differentiated cells from other lineages into the dendritic cell fate with antigen-presenting capacity. Multipotent progenitor cells (MPPs), common myeloid progenitor cells (CMPs), common lymphoid progenitor cells (CLPs), granulocyte-monocyte progenitor cells (GMPs), pre-megakaryocyte-erythrocyte (pre-MEG/E) progenitor cell, megakaryocyte-erythrocyte progenitor cells (MEP), colony forming unit-erythroid (CFU-E), megakaryocytic progenitor MkP, pro-mast cells (ProMCs), Monocyte DC progenitors (MDP), Common Dendritic Cell Precursors (CDP), pre-dendritic cell (pre-DC), double negative T lineage precursors (DN1, DN2).

[00108] **Figure 2.** Generating Antigen Presenting Cells by Direct Cellular Reprogramming. Observation of the effect of the TF combination disclosure in the present subject-matter for the induction of dendritic cells (iDCs) from mouse and human fibroblasts. Induced DCs can be applied to generate a personalized immunotherapy after loading with cell extracts or defined antigens (Primed-iDC). DCs are specialized in antigen presentation to Macrophages (M ϕ), T, B and NK cells. Induced DCs stimulate antigen-specific immune responses against cancer, viral, parasitic or bacterial infections.

[00109] **Figure 3.** *In situ* or *ex vivo* direct reprogramming of cancer cells to stimulate antigen-specific immune responses. Effect of TF combination (PIB) for the induction of DC fate and antigen presenting capacity, when this cocktail is introduced directly into cancer cells *in vivo* or *in situ* or, *ex vivo* or *in vitro*. This strategy enables tumor cells to present their specific antigens (Tumor-APC) to CD4⁺ and CD8⁺ T-cells, triggering a targeted immune response against the tumor.

[00110] **Figure 4. 18 TF candidates for the direct reprogramming of DCs.** (A) Heat map showing gene expression of the 18 candidate factors across multiple mouse tissues (GeneAtlas MOE430). The majority of the 18 factors are specifically enriched in DCs (black box) but not in other tissues (right). (B) Heat maps showing increased gene expression of the 18 factors in mouse DCs when compared with macrophages (M ϕ) derived from bone marrow cultures (left panel, GSE62361). Heat maps displaying gene expression of the 18 TFs in common dendritic cell precursors (CDP), Pre-conventional DCs (Pre-cDC1 and Pre-cDC2) and conventional DCs (cDC1 and cDC2) (right panel, GSE66565). Gene expression data were analyzed by Cluster 3.0 and displayed by Treeview. Red indicates increased expression, whereas blue indicates decreased expression over the mean. (C) Gene ontology biological process (left) and mouse loss-of-function mutant phenotype (right) enrichment analysis was performed for the candidate 18 TFs using Enrichr (<http://amp.pharm.mssm.edu/Enrichr/>). Lists show the most enriched terms (top 19) and left columns show respective p-values. Top enriched biological processes enriched are leucocyte differentiation ($p=2.51E-12$), leucocyte activation ($p=1.02E-11$), DC differentiation ($p=9.58E-12$) and DC activation ($p=6.31E-11$), whilst mutant phenotypes include abnormal adaptive immunity ($p=1.19E-04$) and abnormal antigen presentation ($p=1.25E-03$).

[00111] **Figure 5.** Expression of *Clec9a* is specifically restricted to the conventional DC-lineage. (A) *Clec9a*-Cre X R26-stop-Tomato double transgenic mouse enables identification of conventional DCs and their committed precursors (CDP, Common Dendritic Cell Precursors), but not other leukocytes, due to restricted tdTomato expression. (B) Expression profile of *Clec9a* in DCs and several hematopoietic cell lineages obtained from data available in Immunological Genome Project (www.immgen.org). (C) Gene expression of the *Clec9a* gene in Monocyte DC progenitors (MDPs) and DC-committed precursors (CDPs and pre-DCs) at the single cell level (GSE60783).

[00112] **Figure 6.** *Clec9a* is highly expressed in mature cDC1s. (A) Gene expression of *Clec9a* in DC precursors (CDPs, pre-DC1 and pre-DC2) and mature cells (cDC1 and cDC2) (GSE60782). (B) Confirmation of *Clec9a*-tdTomato on splenic cDC1 (MHC-II⁺ CD11c⁺ CD8⁺ cells) isolated from double transgenic C9a-tdT animals. CD8⁺ T-cells that do not express *Clec9a* were included as control.

[00113] **Figure 7.** Isolation and purification of *Clec9a* reporter MEFs to screen candidate TFs. (A) Double transgenic (*Clec9a*-Cre X R26-stop-Tomato) pregnant females were used to isolate MEFs at embryonic day E13.5. After removal of the head, fetal liver and internal organs, MEFs were cultured until confluency. MEFs were sorted to remove residual CD45⁺

and tdTomato+ cells that could represent cells with hematopoietic potential. (B) Gating strategy to remove residual CD45+ and tdTomato+ cells. Double negative MEFs, approximately 97% of the population, were sorted. (C) Purity confirmation of the sorted population.

[00114] **Figure 8.** Experimental design to screen candidate TFs' ability to activate Clec9a reporter MEFs. Purified MEFs were transduced with different pools of inducible lentiviral vectors encoding DC-specific TFs. MEFs were cultured in the presence of Dox to induce expression of the TFs and monitored from day 1 to 15 for tdTomato expression. Activation of Clec9a promoter induces expression of Cre recombinase, which mediates excision of the Stop codon and consequent expression of tdTomato.

[00115] **Figure 9.** Combinations of candidate DC-inducing TFs induce activation of the Clec9a-tdTomato reporter. (A) MEFs were transduced with M2rtTA (as control), all 18 candidate TFs and pools of 3-4 TFs and analyzed by fluorescent microscopy and flow cytometry 5 days after addition of Dox. (B) Quantification of tdT+ cells after transduction with M2rtTA, all 18 TFs or smaller pools at day 8. Mean $\pm$ SD, n=2. (C) Quantification of tdT+ cells after removal of individual TFs from the pool of 4 TFs or their individual expression at day 8. Mean $\pm$ SD, n=2.

[00116] **Figure 10.** Minimal transcription factor network activates Clec9a reporter and induce DC morphology in mouse fibroblasts. (A) MEFs were transduced with M2rtTA (as control) or PU.1, IRF8 and BATF3 (PIB – mixture of the 3 TF) and analysed by fluorescent microscopy and flow cytometry 5 days after addition of Dox. (B) Kinetics of Clec9a-tdTomato reporter activation analysed by flow cytometry. (C) Quantification of tdTomato+ cells after removal of individual TFs from the pool of PIB or their individual expression at day 5 after addition of Dox. (D) Flow cytometry histograms showing size (FSC) and complexity (SSC) in PIB transduced cells (gated in tdTomato+ or total population) and M2rtTA transduced cells. (E) Morphology of tdTomato+ cells at day 5 after the addition of Dox. (F) Immunofluorescence for F-actin at day 8 after addition of Dox.

[00117] **Figure 11.** Kinetics of Clec9a reporter activation analyzed by time-lapse microscopy from day 0 to day 7. Scale bars represent 200 μ m.

[00118] **Figure 12.** Minimal transcription factor network induces expression of the pan-hematopoietic marker CD45. Flow cytometry analysis at day 8 for expression of CD45 and tdTomato in PIB-transduced MEFs.

[00119] **Figure 13.** TCF4 increases the efficiency of Clec9a reporter activation. (A) MEFs were transduced with PU.1, IRF8 and BATF3, PIB (black bar) or PIB combined with individual TFs

from the 18 candidates (grey bars). tdTomato+ cells were quantified at day 8. M2rtTA transduction was included as control. Mean $\pm$ SD, n=2-6. (B) MEFs were transduced with PIB (black bar) or PIB combined with individual hematopoietic TFs (grey bars). tdTomato+ cells were quantified at day 8. Mean $\pm$ SD, n=2-6.

[00120] **Figure 14.** Optimal culture conditions to induce the activation of the Clec9a reporter. (A) Quantification of tdTomato+ cells in MEFs transduced with PIB (PU.1, IRF8 and BATF3) and cultured in different conditions at day 10 after addition of Dox. (B) Absolute numbers of tdTomato+ cells in MEFs transduced with PIB (black bar) and co-cultured with OP9 and OP9-DL1 cells at day 10 after addition of Dox. OP9 and OP9-DL1 cultures were included as controls.

[00121] **Figure 15.** Expression profiles of PU.1, IRF8, BATF3 and TCF4 at the single cell level. Gene expression of *PU.1*, *IRF8*, *BATF3* and *TCF4* in single monocyte-dendritic cells precursors (MDPs) and restricted DC precursor cells (CDPs, and pre-DCs) (GSE60783). Gene expression level is shown in reads per kilobase of exon model per million mapped reads (RPKM) values.

[00122] **Figure 16.** Analysis of *PU.1*, *IRF8*, *BATF3* factors expression in mouse cells/tissues. (A) Combined gene expression levels of Pu.1+Irf8+Batf3 across 96 tissues and cell-types. Gene expression data from different mouse tissues and cell-types, were obtained from the GeneAtlas MOE430 database. The “GPSforGenes” program was written and used to classify the tissues where combinations of TFs were most enriched (best fit= 1). (B) Gene expression of *Pu.1*, *Irf8* and *Batf3* in DC precursors (CDPs, pre-DC1 and pre-DC2) and mature cells (cDC1 and cDC2) (GSE60782). Gene expression level is shown in relative gene expression. (C) Heat map showing increased expression of *Clec9a*, *PU.1*, *IRF8* and *BATF3* in CD103+ DCs (highlighted in red) belonging to cDC1 subset when compared to other DC subsets and several hematopoietic cell lineages available in the Immunological Genome Project (www.immgen.org). Gene expression data were analyzed by Cluster 3.0 and displayed by Treeview. Red indicates increased expression, whereas blue indicates decreased expression over the mean

[00123] **Figure 17.** Induced DCs express cDC1 markers at cell surface. (A) Flow cytometry analysis of surface phenotype of MEFs transduced with PIB 8 days after the addition of Dox. Quantification of CD103, CD8a, CD4, C11b and B220 expression in tdT+ and tdT- populations. (B) Representative flow cytometry plots.

[00124] **Figure 18.** Induced DCs express antigen-presenting machinery at the cell surface (A) Flow cytometry analysis of MHC-II expression in MEFs transduced with M2rtTA (as control)

and PIB (PU.1, IRF8 and BATF3) 7 days after addition of Dox. TdTomato+ and tdTomato- populations are shown. (B) Kinetics of MHC-II surface expression in M2rtTA and PIB transduced cells. MEFs were analysed by flow cytometry from day 1 to 15 after addition of Dox. (C) Quantification of the percentage of cells expressing MHC-II in high and low levels in bulk cultures after removal of individual TF from the pool of PIB, at day 5 after addition of Dox. (D) Quantification of MHC-II+ cells at day 5 within tdTomato+ population after transduction with 4TFs or upon their individual removal.

[00125] **Figure 19.** Induced DCs express MHC-I at cell surface. Flow cytometry analysis of MHC-I expression in MEFs transduced with M2rtTA and PIB at day 7 after the addition of Dox.

[00126] **Figure 20.** Induced DCs express co-stimulatory molecules at cell surface. Flow cytometry analysis of co-stimulatory molecules (CD80 and CD86) at cell surface of tdTomato+ population in PIB (PU.1, IRF8 and BATF3) transduced MEFs 5 days after addition of Dox. MHC-II+ and MHC-II- populations are shown

[00127] **Figure 21.** Induced DCs up-regulate CD40 expression upon LPS stimuli. (A) Flow cytometry analysis of CD40 expression in tdTomato- and tdTomato+ population in PIB-transduced MEFs at day 8. (B) Histograms show expression of CD40 with or without overnight LPS stimulation at day 13.

[00128] **Figure 22.** PIB factors induce global dendritic cell gene expression program in fibroblasts. (A) Clec9a reporter MEFs were transduced with Pu.1 (P), Irf8 (I) and Batf3 (B) to generate iDCs. Transduced cells were sorted by FACS and sampled using full-transcript single-cell RNA-seq using Fluidigm C1 system, at day 3 (d3, 20 Clec9a-tdTomato+ cells), day 7 (d7, 40 Clec9a-tdTomato+ cells) and day 9 (d9, 36 Clec9a-tdTomato+ MHC-II+ cells). Non-transduced MEFs at day 0 (30 cells) and splenic DCs (sDCs, 66 CD11c+ MHC-II+ CD8a+ cells) isolated from C57Bl/6 animals were used as controls. (B) t-distributed stochastic linear embedding (tSNE) analysis of genome-wide transcriptomes showing clustering of 163 single cells. Each dot represents an individual cell. The number of cells from each sample group is depicted inside brackets. (C) Complete-linkage hierarchical clustering of the consensus matrix obtained by the SC3 clustering algorithm. (D) Heatmap showing expression of the 6525 most variable genes across the 5 different biological sample groups (columns, MEFs, d3, d7, d9 and sDCs). 4 clusters of genes are shown: Cluster I (3014 genes), II (530 genes), III (347 genes) and IV (2634 genes). Color scheme is based on z-score distribution, from -2 (blue) to 2 (red). Examples of genes from each cluster are shown (right panel). (E) Expression

levels of fibroblast genes are shown as Census counts median values $\pm$ 95% confidence interval. (F) Expression levels of genes in Cluster II (*Eea1*, *Aldh1a2*, *Ifit3*), Cluster III (*H2-Pb*, *Ctsc*, *Cd74*) and Cluster IV (*Clec9a*, *Cd45*, *Cd11c*, *Tlr3*, *Ccr2*, *Nlrc5*), presented as violin plots (height, gene expression; width, abundance of cells expressing the gene). Log values of Census counts are shown, horizontal lines corresponding to median values.

[00129] **Figure 23.** PIB factors induce expression of DC transcriptional regulators, including endogenous Pu.1, Irf8 and Batf3. (A) Violin plots showing the expression levels of the DC transcriptional regulators *Zbtb46*, *Bcl11a*, *Stat2*, *Irf7*, *Stat6* and *Stat1*. (B) Expression levels of *Pu.1*, *Irf8* and *Batf3* genes are shown as Log counts presented as box plot with whiskers extending to $\pm 1.5 \times$ interquartile range. Total (left panel) and endogenous transcript (right panel) levels are displayed.

[00130] **Figure 24.** Pathway enrichment for step-wise transitions during iDC reprogramming. (A) Gene set enrichment analysis (GSEA) for step-wise iDC reprogramming was performed using annotated gene sets from NetPath-annotated signaling pathways. Day 3 refers to the pathways upregulated at day 3 versus MEFs, day 7 refers to the pathways upregulated at day 7 versus day 3 and day 9 refers to the upregulated pathways at day 9 versus day 7. Datasets were ordered according to the normalized enrichment score (NES) and the False Discovery Rate (FDR) q-value is shown. The bottom panel shows the enrichment plots for the IL-4 (day 3) and Oncostatin M (day 9) gene sets.

[00131] **Figure 25.** Transcriptional networks for step-wise transitions during iDC reprogramming. (A) Transcription factor covariance networks during iDC reprogramming for each step-wise transition. Shown are transcriptional regulators with more than five edges, with each edge reflecting a correlation > 0.35 between connected transcriptional regulators. The transcription factors PU.1, IRF8 and BATF3 are highlighted in red. (B) Heat maps showing expression of transcriptional regulators shown in panel A in DC precursors (CDPs and pre-DC1) and mature cells (cDC1) from bone marrow (GSE60782). Gene expression data were analyzed by Cluster 3.0 and displayed by Treeview. Red indicates increased expression, whereas blue indicates decreased expression over the mean. (C) PIB-transduced MEFs at day 3, 5, 7, 10 and 25 after addition of Dox were assayed for hematopoietic colony formation (mean $\pm$ SD, n = 2). Sorted sDC1s and unsorted splenocytes and bone marrow cells were included as controls.

[00132] **Figure 26.** PIB factors induce transcriptional reprogramming towards cDC1 expression program. cDC1 and cDC2 gene expression signatures were generated by

analyzing the datasets from Schlitzer et al. (11) (GSE60783). Cumulative median expression levels of cDC1 and cDC2 gene signatures during reprogramming.

[00133] **Figure 27.** Reconstruction of single cell reprogramming trajectory. **(A)** Genome wide transcriptomes of single cells were ordered with TSCAN software (Pseudo-time). Ordering of non-transduced MEFs, induced DCs (iDCs) Clec9a-tdTomato+ at day 3 (d3), day 7 (d7), Clec9a-tdTomato+ MHC-II+ day 9 (d9) and splenic DCs (sDCs, CD11c+ MHC-II+ CD8a+) are shown. Each dot represents an individual cell. **(B)** Cell expression profiles in a two-dimensional independent component space according to predicted trajectory. Solid black line shows pseudo-time ordering constructed by Monocle2. Each dot represents an individual cell, colored according to biological sample groups (left panel) or cell state (right panel). The number of cells from each sample group assigned to each cell state is depicted inside brackets.

[00134] **Figure 28.** Reconstruction of single cell reprogramming trajectory highlights different maturation states of iDCs. **(A)** Five kinetic clusters of branch-dependent genes identified by BEAM. **(B)** Gene set enrichment analysis (GSEA) between cell state 2 and cell state 3 was performed using gene sets present in the Immunologic signatures collection (4,872 gene sets, FDR < 0.02 or maximum of 200 genes per gene set). Gene sets were ordered according to the normalized enrichment score (NES) and the False Discovery Rate (FDR) q-value is shown. Black lines represent DC gene sets. The right panel shows enrichment plots for Mature Stimulatory DC, IFN γ stimulated DC and IFN α stimulated DC gene sets (all enriched in State 3).

[00135] **Figure 29.** PIB factors induce high levels of expression of genes associated with DC maturation. **(A)** GSEA for day 9 iDCs and sDC1s showing the enrichment for 2 MSigDB gene sets (left). Violin plots (right) show expression distribution of day 9 enriched genes.

[00136] **Figure 30.** PIB factors induce high levels of expression of genes associated with DC maturation. **(A)** Expression levels of *Ciita*, *H2-Aa*, *H2-Ab1* and *H2-Eb1* genes in single cells from State 1, 2 and 3 ordered with Monocle2 (Pseudo-time). Each dot represents relative expression values for individual cells. Lines represent branch kinetics curves for State 2 (solid line) and State 3 (dashed line). **(B)** Violin plots showing the expression levels of *Ciita*, *Acp5*, *Tnfrsf1a*, *Tapbp*, *Inpp5d*, *Traf6* and *Itga4* genes. Log values of Census counts are shown, horizontal lines corresponding to median values.

[00137] **Figure 31.** Induced DCs secrete inflammatory cytokines upon TLR stimuli. **(A)** Secretion of cytokines by MEFs transduced with PIB (PU.1, IRF8 and BATF3) or PIBT (PU.1,

IRF8, BATF3 and TCF4) with or without TLR4 (LPS) or TLR3 (PolyI:C) stimuli overnight. Supernatants of MEFs transduced with PIB or PIBT factors were collected at day 10 after addition of Dox and analysed for cytokine concentration using BD Cytometric Bead Array Mouse Inflammation Kit. Cytokine levels for untreated, 100 ng/mL LPS or 25 µg/mL of PolyI:C-treated cells overnight are shown; black or grey bars represent PIB or PIBT-transduced MEFs, respectively.

[00138] **Figure 32.** Induced DCs are able to engulf small particles. MEFs transduced with PIB (PU.1, IRF8 and BATF3) were incubated overnight with FITC-labelled latex beads (1µm) and analysed by fluorescent microscopy at day 7 after addition of Dox.

[00139] **Figure 33.** Induced DCs are able to engulf proteins and dead cells. (A) PIB-transduced MEFs were FACS sorted and tdTomato- and tdTomato+ (iDCs) populations were incubated with AlexaFluor647-labelled Ovalbumin (OVA-Alexa647) at 37°C at day 11 and analysed by flow cytometry. Controls were kept on ice (4°C). (B) Sorted tdTomato- and tdTomato+ (iDCs) populations at day 11 were incubated overnight with dead cells labeled with CellVue Claret Far Red membrane staining and analyzed by flow cytometry. (C, D) iDCs at day 11 were incubated overnight with dead cells labelled with DAPI and analysed by fluorescent or (D) time-lapse microscopy.

[00140] **Figure 34.** Induced DCs express genes involved in TLR signalling and endocytic pathway. Violin plots for genes regulating (A) TLR signalling and (B) incorporation of antigens.

[00141] **Figure 35.** Induced DCs capture and present antigens to CD4+ T cells. (A) Schematic representation of antigen presenting assay. iDCs at day 8 after addition of Dox were co-cultured with OT-II CD4+ T cells isolated from OT-II Rag2KO mice and labelled with CFSE in the presence of Ovalbumin (OVA) or OVA peptide 323-339. After 7 days, activation of CD4+ T cells was evaluated by CFSE dilution and expression of T cell activation marker CD44. (B) Flow cytometry plots of CFSE-labelled CD4+ T cells co-cultured with MEFs transduced with PIB or PIB plus TCF4, in the presence of OVA, stimulated or not with LPS. CD4+ T cells co-cultured with splenic MHC-II+ CD11c+ DCs were included as controls. Grey lines correspond to untouched CD4+ T cells. (C) Flow cytometry plots showing CD44 expression of CFSE-labelled CD4+ T cells co-cultured with MEFs transduced with PIB, in the presence of LPS and OVA or OVA peptide.

[00142] **Figure 36.** Induced DCs capture and present antigens to CD4⁺ T cells. Quantification of the percentage of CFSE^{low} CD4⁺ T-cells co-cultured with MEFs transduced with PIB (iDCs), in the presence of LPS and OVA or OVA peptide. Splenic DCs were included as control.

[00143] **Figure 37.** Induced DCs efficiently export endocytic cargo into the cytoplasm and express cross-presentation genes. (A) iDCs at day 16 were loaded with a FRET-sensitive cytosolic substrate of β -lactamase, CCF4, followed by incubation with β -lactamase. Kinetics of β -lactamase's export to cytosol was measured as CCF4 cleavage by flow cytometry. (B) Violin plots for genes regulating cross-presentation.

[00144] **Figure 38** Induced DCs capture and cross-present exogenous antigens to CD8⁺ T cells. (A) iDCs at day 16 were co-incubated with B3Z T-cell hybridomas for 16 h and increasing concentrations of soluble OVA protein in the absence (left panel) or presence of LPS or poly-I:C (PIC) stimulation (right panel). T-cell activation was measured as up-regulation of β -galactosidase expression in B3Zs (driven by the IL-2 promoter) and quantified using a colorimetric substrate, CPRG. (B) Schematic representation of cross-presentation assay (left panel). iDCs at day 8 after addition of Dox were co-cultured with OT-I CD8⁺ T cells isolated from OT-I Rag2KO mice and labelled with CFSE in the presence of Ovalbumin (OVA) or OVA 257-264 peptide. After 4 days, activation of CD4⁺ T cells was evaluated by CFSE dilution and expression of the T cell activation marker CD44. Flow cytometry plots showing CD44 expression of CFSE-labelled CD8⁺ T cells co-cultured with MEFs transduced with PIB or PIB plus TCF4, in the presence of OVA. CD8⁺ T cells co-cultured with splenic MHC-II⁺ CD11c⁺ DCs were included as controls (middle panel), and respective quantification (right panel).

[00145] **Figure 39.** PU.1, IRF8 and BATF3 induce DC-like morphology in human fibroblasts. (A) Human Dermal Fibroblasts (HDFs) were transduced with PIB (PU.1, IRF8, BATF3) and cultured in the presence of Dox. (B) Bright field images of HDFs transduced with PIB at day 3 after addition of Dox. White arrowheads mark cells with typical DC-like morphology. M2rtTA transduced HDFs are shown as control. (C) Higher magnification of bright field images of PIB-transduced HDFs with DC-like morphology.

[00146] **Figure 40.** PU.1, IRF8 and BATF3 induce expression of HLA-DR and CLEC9A in human fibroblasts. Flow cytometry analysis of HLA-DR and CLEC9A expression in PIB-transduced human fibroblasts at day 9 after addition of Dox.

[00147] **Figure 41.** PU.1, IRF8 and BATF3 induce ability to capture beads and proteins in human fibroblasts. (A) HDFs transduced with PIB were incubated overnight with FITC-labelled latex beads (1 μ m) and analysed by fluorescent microscopy at day 7 after addition of

Dox. CellVue Claret Far Red and DAPI were used to stain cellular membranes and nuclei, respectively. (B) Flow cytometry analysis of PIB-transduced HDFs after incubation with Ovalbumine-AlexaFluor647 for 20 minutes at 37°C at day 7 after addition of Dox. Controls were kept on ice (4°C).

[00148] **Figure 42.** PIB factors induce Clec9a and MHC-II expression in lung cancer cells. Flow cytometry analysis of Clec9a and MHC-II expression in PIB-transduced 3LL cells at day 8 after addition of Dox. M2rtTA-transduced cells are included as control.

[00149] **Figure 43.** PIB factors induce Clec9a and MHC-II expression in melanoma cells. Flow cytometry analysis of Clec9a and MHC-II expression in PIB-transduced B16 cells at day 8 after addition of Dox. M2rtTA-transduced cells are included as control.

[00150] **Figure 44.** Delivery of PIB factors in a polycistronic vector increases reprogramming efficiency. (A) Schematic representation of polycistronic regions encoding either Pu.1, Irf8 and Batf3 (PIB) or Irf8, Pu.1 and Batf3 (IPB) separated by 2A-like sequences. (B) Flow cytometry analysis of Clec9a reporter activation in MEFs transduced with Pu.1, Irf8 and Batf3 in individual vectors (top, right panel) or polycistronic vectors (PIB and IPB) at day 7 after addition of Dox. M2rtTA-transduced cells are included as control. (C) Quantification of tdTomato+ cells after transduction with PIB factors in individual or polycistronic vectors at day 7.

DETAILED DESCRIPTION

[00151] The present disclosure relates to compositions, nucleic acid constructs, methods and kits thereof for cell induction or reprogramming cell to the dendritic cell state or antigen presenting cell state, based, in part, on the surprisingly effect described herein of novel use and combinations of transcription factors that permit induction or reprogramming of differentiated or undifferentiated cells into dendritic cells or antigen presenting cells. Such compositions, nucleic acid constructs, methods and kits can be used for inducing dendritic cells in vitro, ex vivo, or in vivo, and these induced dendritic cells or antigen presenting cells can be used for immunotherapy applications.

[00152] Natural DCs are bone marrow-derived cells that are seeded in all tissues. DCs are poised to sample the environment and to transmit the gathered information to cells of the adaptive immune system (T cells and B cells). Upon antigen engulfment, DCs initiate an immune response by presenting the processed antigen, which is in the form of peptide–major histocompatibility complex (MHC) molecule complexes, to naive (that is, antigen

inexperienced) T cells in lymphoid tissues. After activation, DCs typically overexpress co-stimulatory and MHC molecules in addition to secrete various cytokines responsible for initiating and/or enhancing many T and B lymphocyte responses, i.e. type I interferon, tumor necrosis factor (TNF)- α , IFN- γ , IL-12 and IL-6. Thus, DCs are generally identified by their high expression of major histocompatibility complex class II molecules (MHC-II), co-stimulatory molecules, such as CD80/86 and CD40, and integrin CD11c, as well as their superior capacity to secrete inflammatory cytokines and to migrate from non-lymphoid to lymphoid organs and stimulate naïve T cells. In mice and humans, distinct subsets of DCs can be variably defined by phenotype, ontogeny, and function. They include the conventional DC subset 1 (cDC1, also known as CD8 α + DC subset) found in mouse lymphoid organs and the related CD103+ DC subset in non-lymphoid tissues. Cells bearing a similar phenotype have recently been described in humans, humanized mice, and sheep, indicating cross-species conservation of the cDC1 family. This extended family has distinct functional properties, most notably a remarkable efficiency at capturing material from dead or dying cells, as well as processing exogenous antigens for cross-presentation on MHC class I. These two features allow cDC1 DCs to cross-present cell-associated antigens and trigger CTL responses against infectious agents or tumors. In addition to priming CD8+ T cells, cDC1+ DCs have been implicated in the establishment of cross-tolerance to tissue-specific cell-associated antigens. The ability of cDC1 DCs to either cross-prime or cross-tolerize CD8+ T cells against cell-associated antigens implies that they can decode the context in which they encounter dead cells. DNGR-1, also known as CLEC9A, is a receptor for necrotic cells that favors cross-priming of CTLs to dead cell-associated antigens in mice. DNGR-1 is selectively expressed at high levels by mouse cDC1 DCs, CD103+ DCs and by their human equivalents, being responsible for recognizing an intracellular ligand exposed after cell death. Recently, expression of Clec9a was shown to allow the identification of DC precursors (CDPs) committed to the conventional DC lineage and their progeny in lymphoid tissues (10).

[00153] The successful identification of DC inducing factors capable of reprogramming differentiated cells to induced DCs, as described herein, can advance our basic understanding of DC biology in a number of ways. This work will provide thorough insight into DC minimal transcriptional networks. In addition, the identification of DC inducing factors offer unprecedented opportunities to understand how DC state is established and how key regulatory machinery is put into place.

[00154] Transcription factors play a critical role in the specification of all cell types during development. The success of direct reprogramming strategies using transcription factor-

mediated reprogramming indicates that it is equally plausible to direct the differentiation of pluripotent ES/iPS cells or multipotent stem cells to specific fates using such factors. Accordingly, using the DC inducing factors identified herein, directed differentiation of ES/iPS cells to a definitive DC fate by expression of the DC-enriched transcription factors can be achieved. Additionally, using the DC inducing factors identified herein, directed differentiation of multipotent hematopoietic stem and progenitor cells to a definitive DC fate by expression of the DC-enriched transcription factors can be achieved (forcing differentiation along the hematopoietic tree depicted in **Figure 1**).

[00155] Typically, nucleic acids encoding the DC inducing factors, e.g., DNA or RNA, or constructs thereof, are introduced into a cell, using viral vectors or without viral vectors, via one or repeated transfections, and the expression of the gene products and/or translation of the RNA molecules result in cells that are morphologically, biochemically, and functionally similar to DCs, as described herein. These induced DCs (iDCs) after priming with the adequate antigens have the ability to capture, process and present them to effectors cells of the immune system (macrophages, T-cells, B-cells, NK cells) eliciting antigen-specific immune responses against cancer, viral and parasitic/bacterial infections (**Figure 2**).

[00156] An aspect of the present disclosure is the use of TFs or the use of a combination of TFs in cancer cells (*in situ* or *ex vivo*) to force them to present their own antigens to immune cells (**Figure 3**). This method represents a feasible strategy to increase the clinical outcome of anticancer immunotherapies as it bypasses cancer evasion mechanisms and increases tumor immunogenicity.

[00157] In an embodiment, 18 candidate TFs were selected due to their specifically enriched gene expression in DCs (**Figure 4A**), enriched in DCs when compared to macrophages, which are less efficient APCs (**Figure 4B**, left panel) (20) and during DC ontogeny (**Figure 4B**, right panel). Gene ontology (GO) enrichment analysis for the 18 TFs highlighted their fundamental role on leucocyte and DC differentiation ($p=2.51E-12$ and $p=9.58E-12$, respectively) and activation ($p=1.02E-11$ and $p=6.31E-11$, whilst mouse mutant phenotype enrichment analysis confirmed that genetic perturbations in those genes cause largely hematopoietic phenotypes, in particular abnormal adaptive immunity ($p=1.19E-04$) and abnormal antigen presentation ($p=1.25E-03$) (**Figure 4C**). 18 candidate TFs were cloned individually in a reprogramming proven Doxycycline (Dox)-inducible lentiviral vector (6).

[00158] In an embodiment, for screening the effect of the new dendritic cell-inducing TFs and DC-inducing TF combinations by cellular reprogramming, it has started with Mouse

Embryonic Fibroblasts (MEFs) harboring a DC-specific reporter (Clec9a-Cre X R26-stop-tdTomato) and used the activation of the reporter to shown DC-inducing TFs. In Clec9a-tomato reporter mouse, the tdTomato fluorescent protein is expressed exclusively by CDPs, pre-DCs and in cDCs (10). Macrophages, other immune lineages or monocyte-derived DCs in culture do not express Clec9a and therefore the tdTomato protein (**Figure 5A**). Within the immune system Clec9a gene expression is selectively restricted to CDPs and their progeny (pre-cDCs and cDCs) (**Figure 5B**). Results from gene expression analysis of cDC and precursors also highlighted that Clec9a expression is acquired after commitment to cDC lineage in CDPs and pre-DCs and not before in Monocyte DC progenitors (MDPs) (**Figure 5C**) (11). *Clec9a* is expressed in CDPs, both pre-DCs and cDC subset, reaching high levels in the cDC1 subset (**Figure 6A**) (21). Spleen cells isolated from Clec9a reporter mice were analysed, confirming that 98.8% of cDC1 cells (gated in MHC-II+CD11c+CD8a+) express the tdTomato fluorescent protein (**Figure 6B**).

[00159] Double transgenic Clec9a-tdTomato reporter MEFs were isolated from E13.5 embryos and excluded from any contaminating tdTomato+ or CD45+ cell that could be already committed to the hematopoietic lineage (**Figure 7A and 7B**) by Fluorescent-Activated Cell Sorting (FACS). MEFs used for screening and in the following experiments were tdTomato- CD45- with a purity of 99.8% (**Figure 7C**).

[00160] In an embodiment, PU.1, IRF8 and BATF3 are sufficient for Clec9a activation and to impose dendritic cell morphology.

[00161] In an embodiment, Clec9a reporter MEFs were transduced with combinations of candidate TFs and evaluated for tdTomato expression (**Figure 8**). After transduction with the 18 candidate TFs or one of the pools of 4 TFs, we observed the emergence of tdTomato+ cells 5 days after adding Dox (**Figure 9A, Figure 9B**). The pool comprising of Pu.1, Irf4, Irf8 and Batf3 generated more tdTomato+ cells than 18 TFs (2.36% versus 0.59%, respectively) suggesting that the minimal combination of factors required to induced reporter activation is contained within this pool. TdTomato+ cells were not detected after transduction with control M2rtTA vector. We then removed each of the factors individually (**Figure 9C**). Pu.1, Irf8 and Batf3 (PIB) removal reduced reporter activation while removal of Irf4 did not have an impact. These results suggest that PIB are essential for DC reprogramming.

[00162] In an embodiment when the combination of PU.1, IRF8 or BATF3 (PIB) was expressed in MEFs the Clec9-reporter is activated with an increased efficiency (approx. 3.96%, **Figure 10A**). In an embodiment was then evaluated the kinetics of reporter activation

(**Figure 10B**). TdTomato+ cells start to be detected between day 1 and day 2 and peak between day 5 and day 7 (**Figure 10B**). In an embodiment removal of PU.1, IRF8 or BATF3 completely abolished reporter activation whereas their individual expression was not sufficient to generate tdTomato+ cells (**Figure 10C**). These data suggest that in this embodiment PIB constitute the minimal combination of TFs for Clec9a activation and induced Dendritic Cell (iDC) generation. Importantly, tdTomato+ cells display increased size and complexity (**Figure 10D**), consistent with the observed stellate morphology and the establishment of dendrites characteristic of DCs (**Figure 10E, Figure 10F**). It has been confirmed that reporter activation occurs around 30 hours by time-lapse microscopy and observed that tdTomato+ cells exhibited morphology changes, migration capacity and dendrites gradually being established within 6 days (**Figure 11**). The pan-hematopoietic marker CD45 is expressed in approximately 20% of PIB-transduced MEFs, with approximately 6.6% of tdT+ cells included in this population (**Figure 12**).

[00163] In an embodiment was evaluated the impact of expressing additional factors to PIB. It was assessed the individual impact of each of the TFs from the candidate pool of 18 TFs (**Figure 13A**) as well as other hematopoietic TFs (**Figure 13B**). From the 31 TFs tested it was observed that STAT3, IKZF1, IRF2, NFIL3, BCL6, L-MYC, RUNX1 and KLF4 negatively impact the numbers of tdTomato+ cells generated. The addition of TCF4 and Gfi1b showed a 2.2-fold and 1.9-fold increase in reporter activation, respectively.

[00164] In an effort to optimize the culture conditions for iDC generation it was tested the addition of the cytokines Granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4 and FMS-like tyrosine kinase 3 ligand (Flt3l) during the induction (**Figure 14A**) because of their important role during DC specification (12). It was also tested adding lipopolysaccharides (LPS), different media compositions (RPMI, 2-mercaptoethanol (2-ME) and 4mM L-Glutamine (2xGlut) and co-culture with the stromal cells OP-9 and OP9-DL1 (**Figure 14B**) (13). These culture modifications did not increase the number of induced tdTomato+ cells.

[00165] In an embodiment, the *in vivo* expression patterns of the PIB and TCF4 were analysed. *PU.1*, *IRF8*, *BATF3* and *TCF4* transcripts are expressed in single DC precursor cells (**Figure 15**). While *Pu.1* is equally expressed in MDPs, CDPs and Pre-DCs, *IRF8* expression markedly increases in CDPs and is maintained in pre-DCs. *BATF3* and *TCF4* are only up-regulated at a later stage, in pre-DCs. Moreover, the combined expression of PU.1+IRF8+BATF3 is mostly enriched in CD8a+ DCs among 96 cells and tissues (**Figure 16A**). Importantly, Pu.1 levels are higher in both pre-DC stages, while Irf8 and Batf3 are specifically

enriched in pre-cDC1 and cDC1 subsets (**Figure 16B**). When compared to other DC subsets and several hematopoietic cell lineages, *Clec9a*, *Pu.1*, *Irf8* and *Batf3* display increased expression of in CD103+ DCs belonging to cDC1 subset (**Figure 16C**).

[00166] In an embodiment, it was evaluated whether the activation of the C9a-tdTomato reporter was reflected in the expression of DC markers, such as typical surface markers used to discriminate between conventional cDC and pDC subsets (**Figure 17A**, **Figure 17B**). Interestingly, the cDC1 marker CD103 is expressed in $25 \pm 13.65\%$ of tdTomato+ cells in contrast to undetectable levels in tdTomato- cells suggesting the specification of a non-lymphoid migratory cDC1 program. Moreover, we could detect only residual expression of cDC2 and pDC markers ($0.41 \pm 0.58\%$ CD4+, $2.56 \pm 0.18\%$ CD11b+, $0.77 \pm 1.09\%$ B220+ cells, respectively).

[00167] In an embodiment was evaluated, if the activation of the Clec9a-tdTomato reporter was reflected in the expression of key components of the antigen presentation machinery at the cell surface. Remarkably it was observed that 71.4% of tdTomato+ cells at day 7 expressed MHC-II at the surface (**Figure 18A**), a key molecule for the establishment of APC functionality. The expression of MHC-II is gradually acquired starting between day 1 and day 2 and peak between day 7 and day 11 (**Figure 18B**). The kinetics of MHC-II activation resembles the activation of the Clec9a reporter (**Figure 10B**). At day 7 the tdTomato-compartment contained a lower percentage of MHC-II+ cells (14.2%) (**Figure 18A**). It was then addressed whether expression of MHC-II was controlled directly by PIB factors. By excluding each of the factors individually it was observed that PU.1 expression, but not IRF8 or BATF3 expression, is important for MHC-II activation (**Figure 18C**), consistent with the described Pu.1 role in regulating Class II Transactivator (CIITA) through its promoter I (CIITApl) (14, 15). CIITA is known as the master regulator of MHC Class II genes' expression, determining cell-type-specific, cytokine-induced and developmental-derived modulation of MHC-II expression through the differential usage of CIITA promoters (16). In conventional DCs, CIITApl has been associated with regulation of MHC-II genes.

[00168] In an embodiment, due to the described involvement of IRF4 in inducing MHC-II expression through interaction with CIITA (17), it was evaluated whether IRF4 could compensate for Pu.1 in the generation of MHC-II+ cells within the tdTomato+ population. It was therefore assessed the expression of MHC-II in tdTomato+ cells generated by 4TFs (including IRF4) or their individual exclusion (**Figure 18D**). Inclusion of IRF4 in the pool did not increase MHC-II expression on tdTomato+ cells and IRF4 could not substitute for the loss of Pu.1. Accordingly, IRF4 and PU.1 were found to synergistically promote MHC-II expression

through CIITA promoter III in B cells but not DCs (17). During reprogramming to iDCs, no synergism with PU.1 was observed, which was strictly required for MHC-II expression in tdTomato+ cells.

[00169] In an embodiment, it was evaluated the expression of MHC class I molecules, key molecules for the establishment of APC functionality. 56.7% of tdTomato+ cells at day 7 expressed MHC-I at the surface (**Figure 19**). At day 7 the tdT- compartment contained a lower percentage of MHC-I+ cells (11.2%) (**Figure 19**).

[00170] In an embodiment, it was evaluated the expression the co-stimulatory molecules CD80 and CD86, required for efficient antigen presentation (**Figure 20**). CD80 and CD86 are expressed in 35.2% of tdTomato+MHC-II+ cells in contrast to only 12.9% of tdTomato+ MHC-II- cells. This characterization of the expression of MHC-II, CD80 and CD86 at the cell surface of iDCs suggests that a cohort of tdTomato+ MHC-II+ cells would be competent in antigen presentation. An additional co-stimulatory molecule, CD40, is expressed in 16.1% of tdTomato+ cells, comparing to only 2.8% of tdTomato- cells (**Figure 21A**). Resting cDCs, in particular cDC1 subset, have been described to respond to microbial stimulation up-regulating the expression of co-stimulatory molecules and becoming more effective APCs (25). Accordingly, tdTomato+ cells up-regulate the expression of CD40 (4-fold increase) at cell surface after toll-like receptor TLR4 stimulation (LPS) (**Figure 21B**).

[00171] In an embodiment, in order to define the extent of transcriptional changes during iDC reprogramming, it was measured the full-length single-cell transcriptomes after transduction with PIB (**Figure 22A**). 192 cells were initially profiled from nontransduced MEFs, sorted day 3 Clec9a-tdTomato+, day 7 Clec9a-tdTomato+, day 9 Clec9-tdTomato+MHC-II+ cells and freshly isolated CD11c+ MHC-II+ CD8a+ splenic DCs (sDCs). From these, 163 individual cells passed quality control filters and were used for analysis. After alignment of reads at individual gene *loci*, it has used the Census algorithm to convert relative RNAseq expression levels (transcript per million, TPM) into relative transcript counts (Census counts) without the need for experimental spike-in controls and improving the accuracy of differential expression analysis. It was first employed t-distributed stochastic linear embedding (tSNE) algorithm to perform clustering analysis of the genome-wide transcriptomes (**Figure 22B**) and observed two main clusters of cells. IDCs at day 3 and the majority of day 7 cells map close to MEFs, whilst the remaining day 7 and all day 9 iDCs cluster together with sDCs. It was performed unsupervised clustering using Single-Cell Consensus Clustering (SC3), confirming the global similarity of sDCs with the group of day 9 and some of the day 7 iDCs (**Figure 22C**). Moreover, the timing of global transcriptome

reprogramming correlates well with the peak generation of Clec9a-tdTomato⁺ MHC-II⁺ cells between days 7 and 15 (**Figure 18B**). TdTomato⁺ cells show time-dependent transcriptional changes starting as early as day 3; by day 9 iDCs are remarkably similar to *bona fide* DCs. This analysis suggests that PIB factors induce global transcription reprogramming towards the DC fate. It was extracted the 6525 most variable genes across the dataset, which after clustering could be organized in 4 groups (**Figure 22D**). Cluster I comprises highly expressed genes in MEFs, which are silenced during DC reprogramming. These include typical fibroblast markers, such as *Col5a2*, *Grem1*, *Lox*, *Acta2* and *Thy1* (**Figure 22E**). Cluster II includes transcripts enriched at day 3 and day 7, suggesting activation during the initial stages of reprogramming. This cluster comprises genes such as *Eea1* and *Aldh1a2* that are associated with intracellular trafficking and metabolism as well as type I interferon (IFN) signaling (*Ifit3*) (**Figure 22F**). In contrast, Cluster III encompasses genes enriched at day 9 (**Figure 22D**). Interestingly, MHC-II related genes (such as *H2-Pb*) as well as genes regulating cross-presentation in DCs (such as the cathepsin *Ctsc* and *Cd74*) are enriched in this cluster (**Figure 22F**). Finally, Cluster IV includes genes enriched in sDC1s and reprogrammed iDCs (**Figure 22D**), such as the pan-hematopoietic marker *Cd45* and the general DC marker *Cd11c* (**Figure 22F**). Importantly, cDC1-restricted genes were also upregulated, such as the *Clec9a* gene and *Tlr3* (22), and the key regulator of MHC class I-dependent immune responses (*Nlrp5*) necessary for antigen cross-presentation, a key feature of cDC1s (23). Indeed, we have detected a robust increase in cDC1-signature genes during the reprogramming process when compared to cDC2-signature genes (**Figure 26**) (11). Collectively, these data suggest complete DC reprogramming favoring the cDC1 subset. Accordingly GO analysis showed that categories related to antigen processing and presentation (p-value=3.34E-08, 1.80E-06, 3.53E-06, 4.03E-06) where enriched top biological process and pathways (**Table 1**). Top cellular component GO categories include lysosome (p-value=1.31E-07) and lytic vacuole (p-value=1.44E-07), concordant with the described role of lysosome signaling in coordinating antigen processing and migration of DCs (24). MicroRNA (miRNA) target prediction showed highest enrichment of miR-155 (p-value=1.41E-11) and miR-124 (p-value=1.00E-10) targets (**Table 2**), that have been implicated in DC function and specification, respectively (25,26).

[00172] Table 1. Top 5 gene ontology biological process (left) and cellular component (right) enriched in Cluster I to IV.

	GO Biological Processes	P-value	GO Cellular Processes	P-value
Cluster I	Translation	1.06E-81	Ribonucleoprotein complex	1.46E-96
	Generation of metabolites and energy	1.50E-34	Ribosome	1.75E-90
	Electron transport chain	2.58E-26	Mitochondrion	6.32E-72
	Intracellular transport	1.52E-24	Mitochondrial part	7.38E-50
	Establishment of protein localization	3.61E-19	Ribosomal subunit	6.27E-37
Cluster II	Chromatin modification	2.74E-05	Membrane-enclosed lumen	2.96E-06
	Protein transport	1.07E-04	Intracellular organelle lumen	5.10E-06
	Establishment of protein localization	1.22E-04	Organelle lumen	5.53E-06
	Macromolecule catabolic process	1.54E-04	Endoplasmatic reticulum	9.61E-06
	Protein catabolic process	1.54E-04	Non-membrane bound organelle	9.98E-05
Cluster III	Antigen presentation of exogenous antigen	3.34E-08	Vacuole	1.38E-08
	Antigen presentation of exogenous peptide	1.80E-06	Lysosome	1.31E-07
	Actin cytoskeleton organization	2.71E-06	Lytic vacuole	1.44E-07
	Antigen presentation of peptide antigen	3.53E-06	Cytosol	3.08E-05
	Antigen processing and presentation	4.03E-06	Endoplasmatic reticulum	4.19E-05
Cluster IV	Chromosome organization	3.28E-09	Cytoskeleton	2.51E-11
	Cell cycle	5.10E-09	Non-membrane bound organelle	1.24E-09
	Regulation of GTPase-mediated signaling	9.30E-09	Intracellular organelle	1.24E-09
	DNA metabolic process	2.64E-08	Endomembrane system	3.39E-09
	Regulation Ras protein signal transduction	1.20E-07	Microtubule cytoskeleton	2.31E-08

[00173] Table 2. Gene ontology mouse loss-of-function mutant phenotype (top panel), KEGG pathways (middle panel) and microRNA target interactions (bottom panel) enrichment analysis was performed on the 4 clusters of genes identified using the 6,525 most variable genes across the 5 sample groups (relative to Figure 22). The lists show the most enriched terms and the right columns show respective p-values by fold change in relation to the top enriched term.

	Cluster I	P-value	Cluster II	P-value	Cluster III
Mouse Phenotypes	Abnormal cell death	2.36E-11	Abnormal response to infection	4.25E-06	Abnormal adaptive immunity
	Abnormal cell physiology	3.44E-12	Abnormal embryonic tissue	3.87E-06	Abnormal innate immunity
	Premature death	1.10E-12	Abnormal development patterning	2.68E-06	Abnormal immune physiology
	Abnormal extraembryonic tissue	5.36E-13	Abnormal extraembryonic tissue	1.00E-06	Mammalian phenotype
	Abnormal embryonic tissue	1.86E-13	Abnormal embryo size	5.38E-07	Prenatal lethality
	Abnormal cell proliferation	7.69E-14	Abnormal immune system	1.38E-07	Abnormal bone marrow
	Abnormal embryo size	2.25E-14	Abnormal adaptive immunity	4.15E-08	Abnormal immune cell
	Cellular phenotype	7.15E-19	Abnormal immune cell	3.61E-08	Premature death
	Mammalian phenotype	8.04E-29	Mammalian phenotype	5.99E-09	Abnormal blood cell
	Prenatal Lethality	8.24E-50	Prenatal Lethality	5.05E-10	Abnormal immune system
Pathways	Citrate cycle (TCA cycle)	2.06E-05	Endocytosis	8.69E-02	Endometrial cancer
	Ubiquitin mediated proteolysis	1.51E-05	Apoptosis	8.01E-02	Thyroid cancer
	Nucleotide excision repair	8.73E-06	Vesicular transport	7.84E-02	Glioma
	Spliceosome	9.90E-11	Lysosome	3.79E-02	Prostate cancer
	Proteasome	1.01E-14	Galactose metabolism	3.35E-02	Chronic myeloid leukemia
	Alzheimer's disease	2.76E-20	RIG-I-like receptor signaling	3.35E-02	Chemokine signaling
	Parkinson's disease	3.04E-31	Antigen processing/presentation	3.30E-02	B cell receptor signaling
	Huntington's disease	1.33E-32	Cytosolic DNA-sensing	1.47E-02	Leukocyte migration
	Oxidative phosphorylation	3.72E-34	Toll-like receptor signaling	4.50E-03	Antigen presentation
	Ribosome	2.32E-53	Ubiquitin mediated proteolysis	3.09E-03	Lysosome
MicroRNAs	miR-1-3p	9.67E-17	miR-100-5p	3.85E-05	miR-15b-5p
	let-7b-5p	1.91E-17	miR-7b-5p	2.78E-05	miR-30a-5p
	miR-320a	1.08E-18	miR-425-5p	2.63E-05	miR-19a-3p
	miR-484	1.72E-19	miR-19b-3p	2.07E-05	miR-93-5p
	miR-92a-3p	7.25E-20	miR-98-5p	1.93E-05	miR-34a-5p
	miR-100-5p	1.84E-20	let-106b-5p	4.53E-06	miR-17-5p
	miR-186-5p	1.80E-20	miR-93-5p	4.39E-06	miR-16-5p
	miR-30a-5p	7.02E-21	miR-215-5p	3.24E-06	miR-1-3p
	miR-615-3p	1.11E-26	miR-21-5p	3.01E-06	miR-124-3p
	miR-16-5p	6.33E-40	miR-192-5p	2.43E-06	miR-155-5p

	P-value	Cluster IV	P-value
Mouse Phenotypes	6.36E-11	Abnormal morphology	3.90E-15
	3.22E-11	Cellular phenotype	2.36E-15
	2.73E-11	Premature death	1.73E-16
	6.53E-12	Abnormal brain morphology	1.48E-16
	4.54E-12	Pre-natal lethality	4.28E-19
	2.92E-12	Premeaning lethality	1.80E-20
	5.46E-13	Post-natal lethality	1.64E-20
	4.15E-13	Abnormal survival	1.70E-22
	1.40E-15	Mortality/aging	7.48E-23
	9.50E-17	Mammalian phenotype	8.97E-38
Pathways	6.38E-04	Endometrial cancer	3.56E-03
	6.09E-04	Adherent junction	3.32E-03
	5.83E-04	Pancreatic cancer	1.80E-03
	5.06E-04	Pathways in cancer	1.08E-03
	5.02E-04	Long-term depression	6.48E-04
	4.48E-04	Prostate cancer	6.44E-04
	1.77E-04	Insulin signaling	1.90E-04
	1.33E-04	Inositol metabolism	1.59E-04
	3.02E-05	Acute myeloid leukemia	9.09E-05
	2.08E-09	Phosphatidylinositol signal.	1.16E-05
MicroRNAs	2.92E-07	miR-425-5p	1.64E-06
	2.76E-07	miR-484	9.27E-07
	1.15E-07	miR-21-5p	7.15E-07
	5.32E-08	miR-223-3p	3.97E-07
	4.41E-08	miR-181a-5p	8.57E-08
	1.54E-08	miR-9-5p	1.12E-08
	7.27E-09	miR-149-5p	7.37E-10
	4.60E-09	miR-218-5p	1.85E-10
	1.00E-10	miR-340-5p	1.25E-10
	1.41E-11	miR-324-3p	1.11E-12

[00174] This analysis also revealed activation of DC transcriptional regulators, including *Zbtb46* and *Bcl11a*, which were originally included in our candidate TF list (**Figure 23A**). Several members of the interferon-regulatory factors IFN and signal transducer and activator of transcription (STAT) protein families were also identified. For example, *Stat2* and *Irf7*, key mediators of TLR-Induced DC activation and type I IFN responses, respectively, are detected in high levels at day 3 and 7. *Stat6* also display high median expression values for day 3 and day 7, whilst *Stat1* expression increases at day 9 to levels 128-fold higher than splenic DCs. DC maturation has been reported to be accompanied by a change from STAT6 to STAT1 utilization, which suggests that Pu.1, Irf8 and Batf3 overexpression may also induce DC maturation. It was observed high levels of expression of Pu.1, Irf8 and Batf3 at day 3, day 7 and day 9 when compared to sDCs, consistently with the lentiviral-mediated expression of the 3 TFs (**Figure 23B, top panel**). Since our lentiviral vectors encode the coding sequences without UTRs, it was quantified the expression levels of the endogenous transcripts using the reads at the 3'- and 5'- UTRs. Importantly, it was observed the expression of endogenous *Pu.1*, *Irf8* and *Batf3* starting at day 3 (**Figure 23B, bottom panel**). At day 9 of reprogramming, endogenous expression levels are comparable to splenic DCs.

[00175] In order to further characterize the dynamic nature of the transcriptional reprogramming, gene set enrichment analysis (GSEA) was performed using NetPath gene sets to compare the transitions between day 0, 3, 7 and 9 (**Figure 24, top panel**). It was observed that several immune-related gene sets were highly enriched in day 3 compared with MEFs. Interestingly, IL-4 used for *in vitro* differentiation of DCs ranked on top (NES: 1.97, FDR q-value: 0.02) (**Figure 24, bottom left panel**). Some gene sets were also enriched on day 7, although with smaller NES values, suggesting that more subtle transitions might occur during this phase. In contrast, several gene sets were highly enriched in day 9 compared with day 7, including interleukin pathways and Oncostatin M, previously associated with DC maturation (**Figure 24, bottom right panel**).

[00176] In an embodiment, the transcriptional networks for step-wise transitions during iDC reprogramming were evaluated (**Figure 25A**). It was observed that the transition of MEFs to day 3 was associated with the expression of a dense TF network that highly connected to the PIB reprogramming factors; the transition of day 3 to day 7 was softer, characterized by a less dense TF network, which do not include the PIB factors; and the transition of day 7 to day 9, characterized by a dense TF network which can be divided in 2 clusters of TFs, one denser that includes the cDC marker *Zbtb46*, and one composed by fewer TFs including the PIB factors. This reinforces the idea that a subtler transition might occur between day 3 and day 7, and suggests that day 9 iDCs might have acquired a stable cell fate. Importantly, all these transcriptional regulators are enriched in mature DCs and not in DC progenitors, irrespective of the day that are activated, suggesting that the reprogramming process do not pass through an intermediate progenitor state (**Figure 25B**). Moreover, the absence of intermediate progenitor cells in the iDC reprogramming process was further validated by performing hematopoietic colony formation assays with PIB-transduced MEFs at day 3, 5, 7, 10 and 25 after addition of Dox, including sorted sDC1s and unsorted splenocytes and bone marrow cells as controls (**Figure 25C**). No colonies were observed in iDCs or sDC1 cultures whereas, as expected, unsorted splenocytes and bone marrow cells in culture gave origin to colonies. This supports the idea that the reprogramming process is direct and do not transit through intermediate progenitor cells.

[00177] In an embodiment, it was set out to reconstruct the DC reprogramming path by establishing a pseudo-temporal order based on the gradual transition of cell transcriptomes (**Figure 27A**). Using pseudo-Time reconstruction in Single-Cell RNA-seq Analysis (TSCAN) software, it was observed that the order is consistent with the temporal reprogramming events, with MEFs being followed by day 3 iDCs and subsequently by the majority of day 7

iDCs. Interestingly, 4 individual day 7 and some day 9 iDCs are positioned in line with sDCs in the pseudotime ordering, suggesting that the transcriptome reprogramming was complete. However, the remaining day 9 iDCs seem to be further away from the initial timepoint than the splenic DCs. In order to confirm the robustness of these results, it was performed pseudo-time ordering using Monocle2, an alternative algorithm for delineating differentiation paths. This reconstruction positioned biological sample groups along 3 branches of pseudotime ordering (**Figure 27B, left panel**). MEFs, d3 iDCs and 26 day 7 iDCs were placed along the first branch, considered cell state 1 (**Figure 27B, right panel**), which then reaches a branching point and divides into State 2 and State 3. State 2 includes 82% of sDCs as well as 3 day 7 and 13 day 9 iDCs. However, 55% of day 9 iDCs as well as 11 sDCs are placed within State 3, which is consistent with TSCAN results. To understand the transcriptional differences between these 2 states, GO enrichment analysis was performed using the differentially expressed genes (**Table 3**), which showed that top biological processes and pathways in State 3 include type I and type II (IFN- γ) IFN signaling, known inflammatory mediators of DC activation and maturation (38, 39). Genetic perturbations for State 3 enriched genes highlighted corresponding immune phenotypes, such as abnormal APC and abnormal immune system. Consistently, BEAM analysis revealed 2 kinetic clusters of branch-dependent genes upregulated in State 3 (cluster 2 and 4) functionally enriched for antigen presentation and other immune-related processes (**Figure 28A and Table 4**).

[00178]Table 3. Top 5 gene ontology biological process, mouse phenotypes and wiki pathways enrichment analysis of genes differentially expressed between State 2 and State 3. Relative to Figure 27.

	State 2	P-Value	State 3	P-Value
GO Biological processes	Phosphatidylinositol dephosphorylation	5.90E-04	IFN γ signaling	2.58E-06
	Regulation of error-prone translesion synthesis	6.50E-04	Response to IFN γ	6.98E-06
	Actin filament capping	1.05E-03	Cellular response to IFN γ	7.39E-06
	Actin filament reorganization	1.19E-03	Neutrophil degranulation	2.21E-05
	Peptidyl-serine autophosphorylation	2.13E-03	Type I interferon signaling	2.24E-05
Phenotypes	Lethality at weaning	2.56E-06	Abnormal antigen presenting cell	1.00E-09
	Premature death	9.47E-05	Abnormal immune system	8.88E-08
	Abnormal mineral homeostasis	1.25E-03	Abnormal blood cell	1.34E-07
	Mammalian phenotype	9.44E-04	Abnormal response to infection	1.40E-07
	Abnormal startle reflex	3.40E-03	Abnormal bone marrow	1.79E-07

	State 2	P-Value	State 3	P-Value
Pathways	Estrogen signaling pathway	7.03E-03	IFN γ signaling	9.80E-07
	Regulation of actin cytoskeleton	9.07E-03	G13 Signaling	3.09E-06
	Breast cancer	9.50E-03	Alzheimers Disease	5.68E-06
	MAPK signaling	1.06E-02	Heart Hypertrophy	1.52E-05
	Gastric cancer	1.06E-02	IL-3 Signaling Pathway	1.94E-05

[00179] Table 4. Top 5 gene ontology biological process and mouse loss-of-function mutant phenotype enrichment analysis of genes in Cluster 1 to 5. Relative to Figure 28.

	GO Biological processes	P-value	Mouse Phenotypes	P-value
Cluster 1	Translation	6.97E-05	Abnormal cell death	8.32E-04
	DNA packaging	5.26E-03	Abnormal lacrimal gland	1.25E-03
	Nucleosome assembly	1.41E-02	Abnormal sex gland	2.11E-03
	Chromatin assembly	1.51E-02	Abnormal hormone levels	3.18E-03
	Protein-DNA assembly	1.57E-02	Abnormal muscle contractility	4.46E-03
Cluster 2	Negative regulation signal transduction	7.46E-03	Abnormal blood cell	2.33E-06
	Negative regulation cell communication	1.20E-02	Abnormal immune system	2.52E-05
	Regulation of cell killing	1.29E-02	Abnormal immune cell	7.16E-04
	Regulation leukocyte cytotoxicity	1.29E-02	Perinatal lethality	9.04E-04
	Regulation lymphocyte immunity	2.23E-02	Abnormal adaptive immunity	6.72E-04
Cluster 3	Sensory perception chemical stimulus	9.34E-23	Mammalian phenotype	3.04E-14
	Neurological system process	1.04E-22	Abnormal blood homeostasis	9.59E-09
	Sensory perception	1.67E-22	Abnormal hormone levels	9.62E-09
	Sensory perception smell	6.48E-22	Abnormal nervous system	7.57E-08
	Cognition	2.96E-21	Abnormal neuron morphology	6.91E-08
Cluster 4	Antigen presentation of exogenous peptide	1.94E-05	Abnormal blood cell	1.81E-09
	Antigen presentation via MHC-II	1.94E-05	Abnormal Immune system	5.66E-08
	Polysaccharide antigen presentation	4.03E-05	Abnormal antigen presenting cell	1.77E-07
	Positive regulation leukocyte activation	8.20E-05	Abnormal immune cell	8.54E-07
	Exogenous antigen presentation	8.91E-05	Abnormal bone marrow	1.87E-06
Cluster 5	Protein-DNA complex assembly	4.71E-03	Mammalian phenotype	4.93E-05
	Chromosome organization	7.89E-03	No abnormal phenotype	4.81E-04
	Chromatin	1.01E-02	Normal phenotype	5.16E-04
	Lens development in eye	1.34E-02	Metabolism phenotype	1.54E-03
	Positive regulation of secretion	1.47E-02	Abnormal Social interaction	5.30E-03

[00180] In an embodiment, GSEA also showed that 4705 vs 167 gene sets for immunological signatures were upregulated on State 3 when compared with State 2, such as Mature Stimulatory DC, IFN γ and IFN α stimulated DC gene sets (**Figure 28B**). As State 3 contained the majority of day 9 iDCs, it was sought to confirm that similar maturation trait was observed when comparing sDC1s (naïve) with day 9 iDCs. GSEA showed that antigen processing and presentation and DC maturation gene sets are enriched at day 9 iDCs (**Figure 29A**). Interestingly, Stat6, which is associated with immature DCs, was up regulated in sDC1s, whilst Stat1, described to increase with maturation, was up regulated in day 9 iDCs (**Figure 23A**).

[00181] In an embodiment, given that it was previously observed that iDCs express high levels of MHC-II molecules, which is reported to be associated with maturation of DCs (**Figure 18**), it was sought to investigate if the observed pseudotime trajectories were indicative of different maturation states. It was observed that the branch kinetic curves reflect a continuous upregulation of *Ciita*, the known master regulator of MHC-II genes' expression, and several MHC-II genes (*H2-Aa*, *H2-Ab1* and *H2-Eb1*) towards State 3 as compared with State 2 (**Figure 30A**). Consistently, expression of *Ciita* and genes associated with mouse (*Tnfrs1a*, *Tapbp*, *Inpp5d* and *Traf6*) and human (*Acp5* and *Itag4*) DC maturation were enriched at day 9 iDCs (**Figure 30B**). These data suggest that iDCs are intrinsically more mature than sDCs and may be less dependent on exogenous activation stimuli for antigen presentation.

[00182] In an embodiment, the induced dendritic cells in some aspects of all the embodiments of disclosure, while similar in functional characteristics, differ in their gene expression from the naturally occurring endogenous dendritic cells (**Table 5**).

[00183] Table 5. Top 500 differentially expressed genes between day 9 iDCs and sDC1 cells ordered by fold change.

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Cd74	8.378400519	AY036118	3.630967107
Ucp2	7.999114666	Sfi1	3.546674749
Gri	7.650480054	Gprc5c	3.440084658
Cdkn1a	7.079169574	Olfr648	3.00881167
S100a11	7.048233116	Rsph9	2.959047864
Gapdh	6.979316778	Tmsb4x	2.890522248
Cct8	6.923788452	Mtmt1	2.654214297
Ly6e	6.336631107	Il15	2.574244187
Ubb	6.19368097	Fanci	2.24583185
B2m	6.151779369	Ptprk	2.113646962

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Mir6240	6.053040315	Cnot6l	2.002604494
Irf8	5.725731065	Bdkrb1	1.987001179
Mir6236	5.674808976	Pdgfb	1.930540437
Spi1	5.640038385	Letm1	1.915941208
Cd81	5.563653083	Abca3	1.880254741
Ctsa	5.501860486	Plpp5	1.867877278
Rnf13	5.457727173	1700095J03Rik	1.847936727
Ifitm3	5.400810698	Dcun1d4	1.788829125
Samhd1	5.263523757	Lrif1	1.769132531
Pgam1-ps2	5.203763065	Fus	1.758030571
Prkar1a	5.192511183	Ltbp1	1.719160475
Gns	5.151976827	Prr15l	1.702342332
Gdi2	5.020765205	Rps20	1.686771039
mt-Nd5	4.991318969	Fam92a	1.682146576
Usp14	4.895884787	Rpl32	1.661123211
Eif2ak3	4.883252352	Clstn3	1.658201639
mt-Tm	4.874702287	Usp10	1.651957116
Med21	4.82652063	2900009J06Rik	1.642513164
Shisa5	4.762756365	4930553I04Rik	1.639560609
Stat1	4.717469627	Nceh1	1.633238968
Scpep1	4.654917481	Kdelr1	1.623624988
Tmem59	4.630781554	Amdhd2	1.620291498
Drg1	4.622644784	Snhg14	1.60518724
Pttglip	4.613419142	Ppcdc	1.593210807
Batf3	4.567419599	Cit	1.571609014
Grina	4.563608382	Lef1	1.571116453
Ctsc	4.562081251	Cinp	1.555760027
Calm2	4.5319627	Cep290	1.542877805
Ifi44	4.526513658	Eya4	1.542329932
Arhgdib	4.448903084	Ssbp2	1.541337751
Ifitm2	4.43505433	Stard3nl	1.53918705
Itm2b	4.41388282	Ppp2r5a	1.529811517
Sbds	4.328188964	Rps27	1.514118829
Bst1	4.322914109	Rpain	1.512378029
Nnat	4.253027937	Rps15a	1.503115843
Sulf2	4.233589183	Ushbp1	1.489434725
Lgals3bp	4.212602771	Caprin2	1.48766573
Dazap2	4.138256756	Glis1	1.468306086
Slc30a9	4.045135818	Rpl24	1.455561714
Rbms1	4.026259385	Rgs1	1.452134922
Ftl1	4.022138158	Lsp1	1.445858249
Eif3a	4.002306157	Malat1	1.441188932
Axl	3.997197448	Kctd19	1.439866506
Cited2	3.993185485	Sfxn5	1.438464054
Lars2	3.932022979	Brca2	1.429734511
Cfl1	3.902855666	Fgd4	1.429251043

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Pfn1	3.862256545	Mir762	1.42527755
Ate1	3.846471162	Rabgap1	1.418468643
Myadm	3.828349737	Notch1	1.417303263
Bgn	3.827554891	Anapc5	1.415567561
Ywhab	3.794470188	Slc6a17	1.412700849
mt-Rnr1	3.783136169	Ncapd2	1.407349715
Tnfrsf1a	3.708506639	Ccdc40	1.382403998
Tmbim6	3.677054824	4930519L02Rik	1.382401843
Ppp3r1	3.659734805	Aacs	1.378156309
Cap1	3.650896891	Arhgef40	1.377963701
Sparc	3.634708576	Olfr986	1.376630166
Tgtp2	3.628901251	Dnm1	1.369144578
Chordc1	3.627931997	Adgrv1	1.36678555
Mir8114	3.606630393	Reg2	1.36266606
Tma7	3.600667604	Kif24	1.360757058
Slc25a3	3.59535779	Khk	1.354650399
Unc93b1	3.541471457	Camk2d	1.353347692
Mapk1	3.539792266	Disp1	1.337935937
Spp1	3.53813155	Msh3	1.330172804
Trim25	3.537049716	Pmf1	1.328658766
Ywhaz	3.533742941	Mrpl48	1.320851411
Pla2g7	3.518104367	Fry	1.318521603
Cyfip1	3.485665307	Adgra3	1.315125368
Ncoa4	3.473391046	Tssc1	1.313253438
Tgtp1	3.454518062	Fbf1	1.312311242
Pros1	3.417372505	Hsd3b2	1.311309901
Dda1	3.40525625	Snord57	1.309355259
Cmtr1	3.387409585	Adsl	1.308071098
Stt3a	3.385900731	Banp	1.307751088
mt-Cytb	3.381159175	Diexf	1.303869281
Edem1	3.367122678	Ctage5	1.296409102
Lgmn	3.355164538	Olfr1089	1.295835162
Serinc3	3.35283067	Arhgef19	1.293919043
Plbd2	3.337813415	Trpm4	1.291888876
Slfn5	3.330993072	Olfr980	1.290974033
Rab1b	3.324732402	Ap3b2	1.290262191
Ap3d1	3.315238282	Ndufs1	1.282010137
Icam1	3.293730801	Prob1	1.279252518
Mdh1	3.270873537	Tox	1.277128111
Hsp90aa1	3.26235373	Tnk2	1.275196071
Zmpste24	3.256672868	Pcdh15	1.274945531
mt-Rnr2	3.227837673	Use1	1.273360442
Sp100	3.20878081	Znrf1	1.273285759
Surf4	3.208326066	Il16	1.265024831
Pkm	3.190910497	Gsn	1.264820606
Ciita	3.181654915	Tyrobp	1.254979854

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Glul	3.165849559	Zfp57	1.25469807
Cmc4	3.145763373	Dnm3	1.249092237
Ifit2	3.140223266	Btbd19	1.246939299
AA474408	3.12954966	Tmeff2	1.244721598
Pigt	3.127377903	Pde1c	1.241625438
Serinc1	3.116484011	Slc16a14	1.241236737
Lyn	3.111346193	Herc4	1.240940968
Tnfaip1	3.108541206	Pdzd2	1.240932703
Rnf145	3.108426516	Cenpu	1.239620462
Ubl5	3.09022469	Ccdc9	1.237773411
Map2k4	3.089586057	Cd63	1.235857882
Hmga1	3.069250123	Scrib	1.229623359
Cox6a1	3.069034679	Lats2	1.222817428
Laptm4a	3.060750646	Plbd1	1.220326423
Psmc5	3.047865774	1700030K09Rik	1.212538771
Plekho2	3.039215546	Gpsm3	1.212322462
Lgals9	3.035253178	Rasa4	1.212195177
Mlxip	3.031480133	Jade1	1.211805352
Fos	2.995472377	Astn1	1.206888241
Fkbp10	2.995150234	Abca2	1.205733273
Gps2	2.993152938	Ptpn5	1.2051801
Tecr	2.987574318	Tpk1	1.201688574
Mbnl1	2.96248689	Kantr	1.201432145
Sez6l	2.951436702	Slc15a2	1.199012945
Sh3bp2	2.949222732	Fgf1	1.196064338
Mov10	2.935927814	4930511M06Rik	1.193287597
Fam167a	2.91423349	A430010J10Rik	1.193177327
Asns	2.907465523	Pde4c	1.186996538
C530025M09Rik	2.889428766	2610203C20Rik	1.183842191
Zdhhc5	2.875076576	Cep85	1.182775307
Rpl38	2.871058843	Auts2	1.181857217
Tmod3	2.869947135	Pld1	1.179419755
Tspan9	2.868408159	Lsamp	1.179262613
Arhgap1	2.861787688	Ercc5	1.174619478
Rn7s1	2.857560775	B230216N24Rik	1.172531942
Rn7s2	2.857560775	Cdkal1	1.172233483
St3gal5	2.850664779	Plb1	1.170618218
Lamp2	2.838622934	Ttc3	1.169286246
Zfp36l1	2.828619356	Smarcad1	1.1661693
Qsox1	2.816348613	B130055M24Rik	1.164407791
Nubp2	2.801535041	Glrbl	1.162769632
Dap	2.798447222	Fam71e1	1.161503582
H2-Eb1	2.790275212	Actr1b	1.160650079
Trpc4ap	2.790223774	Fam120c	1.159839707
Ptk2	2.774986716	Lamb3	1.1544153
Litaf	2.76910688	Rpl18	1.147822978

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Samd12	2.756415888	B430219N15Rik	1.147593372
Hspa8	2.738492847	Pnpla7	1.146410398
Psap	2.725482169	Myo1b	1.145796458
Rap1b	2.708283782	Tmcc1	1.145088425
Rab11b	2.707195739	Mxd1	1.142557058
Kdm5c	2.659888994	Gtpbp4	1.140348186
Atp6v0c	2.652904455	Mbtd1	1.139896426
Pnkp	2.627212937	Mir101c	1.139676828
Atp1b3	2.57433971	Srcin1	1.138957445
Plin2	2.569589842	C130026I21Rik	1.13879651
Dusp1	2.564304737	Qrich1	1.135006953
Dennd6a	2.55766128	Snora7a	1.129825352
Syt9	2.54591498	P2ry2	1.12826657
Park7	2.538892352	Tle6	1.127997617
Thbs1	2.526658864	Gstt2	1.127976061
Ndel1	2.520436416	Rnf214	1.125647634
Eif2s2	2.517791626	Mier2	1.125490293
Degs1	2.516228756	Rad52	1.123514249
Pcsk6	2.508442547	Tsfm	1.121124616
Washc4	2.501207547	Rpl34	1.118504821
H2-Pb	2.494182352	Ampd2	1.118010861
Gins4	2.480528831	Col6a6	1.11565181
Ap2m1	2.479969069	6820408C15Rik	1.11415417
Stam	2.460346325	Plekha7	1.11413288
Calm1	2.459547652	Kifc1	1.113349023
Cd47	2.452322667	Entpd8	1.11138608
Arhgap5	2.436336219	Trmu	1.111070484
Msn	2.427359	Dhx30	1.110688069
Arhgef2	2.424471636	Becn1	1.108510602
Rnps1	2.421957675	Gtf2h1	1.107621797
Agpat3	2.420651749	Dennd2d	1.107444842
Hexb	2.406950442	St14	1.10685259
Jmjd1c	2.403960577	Sema6c	1.105449952
Uba7	2.393024725	Olfr1321	1.105249776
Stat3	2.392854797	Nin	1.104494546
Aqr	2.390209543	2900076A07Rik	1.103729098
Rasgrp3	2.389626162	Alg13	1.102785341
Ifi207	2.378045514	Rapgef3	1.101622786
Tcn2	2.366636661	Trps1	1.101516996
Jkamp	2.362648719	Morc2a	1.100797248
Xpnpep2	2.36068485	Myo15b	1.097398552
Pld4	2.345478622	Dlg1	1.096480818
Csnk1a1	2.333344842	Pus7	1.092393911
Cmklr1	2.331529539	H2afz	1.091796066
mt-Co1	2.330383946	Cacna1f	1.091419504
Commd7	2.308633577	Rps7	1.091078271

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Gabarap	2.303502443	Kctd15	1.087832781
Aes	2.298975567	Slc22a15	1.083405985
Nfe2l1	2.296002657	Nbr1	1.082686573
Sgpl1	2.2917436	Cd27	1.081589891
Gbf1	2.287585646	Itga2b	1.080996825
Gstm1	2.280980951	Eci2	1.080130803
Mtcl1	2.275467469	Cd6	1.080089799
Vcl	2.249503311	Mical1	1.080032007
Slc25a5	2.249170226	Serpina6	1.079365549
2610507B11Rik	2.249033444	Cadm2	1.079157707
Tmem248	2.243289507	Kmt5b	1.075999202
Chd9	2.24028321	Scn8a	1.075722849
B4galt5	2.23488477	Zfp239	1.075383073
Rictor	2.234163721	Ap1s1	1.075103048
Srrm2	2.233556776	Erdr1	1.071138918
Sh3bgrl	2.232075277	Cacna1a	1.069720493
Cdc42se1	2.209710506	Ivns1abp	1.067892703
Lrp1	2.208956871	Dhodh	1.067584269
Ipo9	2.195716865	Ttn	1.067147365
Tcp1	2.194202192	Ddx19a	1.066321446
Ppp1cb	2.188862064	Stx4a	1.064577603
Pgam1	2.187328026	Safb2	1.063265347
Atp6v0e	2.185473454	Aloxe3	1.063249994
Pik3r1	2.177807689	Stx18	1.062644106
mt-Nd4l	2.170033442	Notch4	1.062408256
Ddx5	2.1526024	Vars2	1.06236208
n-R5-8s1	2.137222743	Ces5a	1.06224942
Ddost	2.137080797	Fxyd2	1.061290421
Btf3	2.136870648	Olfr539	1.061124921
Pitpna	2.130897293	Ubl4a	1.06102377
Zfp451	2.121640696	Plxnc1	1.059510561
Msrbb3	2.108023782	Tars2	1.059081003
Tram1	2.103942132	A430073D23Rik	1.058179534
Gpx1	2.092125019	Brpf1	1.058170111
Rab3il1	2.091780481	E2f6	1.05716641
Anxa3	2.082860042	Gle1	1.055917907
Prkaa1	2.07920508	Clca3a2	1.053662602
Rab8b	2.076422781	Mir99ahg	1.052669091
Srpr	2.060721132	Grk2	1.052345211
Ncstn	2.048033241	Firre	1.052005979
mt-Nd2	2.046373118	Cp	1.049988888
Sf3b1	2.04486528	Cacna2d4	1.04830389
H2-Ab1	2.037298163	Isy1	1.046348457
Plp2	2.021912335	4930402H24Rik	1.046335717
Dnm1l	2.011874745	D430042O09Rik	1.045493524
Nptx1	2.007694218	Pax6	1.043231341

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Clec16a	1.998844774	Rcbtb2	1.042445583
Uggt1	1.98850241	Wapl	1.042368448
Pxk	1.982701164	Rab14	1.040736807
Tiparp	1.982534756	Magi3	1.038947888
Impad1	1.982393464	Ctbp2	1.037976024
Gpcpd1	1.979316308	Prpf4b	1.037971623
Tmem214	1.973030883	Csmd2	1.037185914
Coro1b	1.970528885	Btbd11	1.036685875
Naaa	1.968987323	Vwf	1.036683252
Snx12	1.960818344	Cdh4	1.036640143
Anpep	1.959292302	Apbb1	1.036638533
Ptk2b	1.94897573	Ccdc162	1.0359892
Gusb	1.944307923	Sipa1l3	1.035923762
Ccnd3	1.940195368	Slc35a3	1.035192913
Syf2	1.939540412	Ahrr	1.034311688
Tubb5	1.927300046	Opcml	1.034020412
Ap2b1	1.927092864	Sirt5	1.032682041
Col4a1	1.92298865	Nox4	1.032270813
Myl12b	1.918466916	Spint2	1.031953157
Ccnd1	1.912610788	Aebp2	1.031681705
Sfxn3	1.906741793	Dtnbp1	1.030548993
Timp2	1.906668629	lqca	1.030543359
B230219D22Rik	1.902284133	Lbp	1.029703558
Rhog	1.887582293	Kcnj16	1.029523684
Scap	1.88592784	Gtf2ird1	1.028843965
Qk	1.88561085	Aldoa	1.026887502
Bfar	1.882090302	Pfdn5	1.026732581
Slfn5os	1.880082558	Mtx3	1.026090598
Lipa	1.879981666	Zfp950	1.025199024
Plekha1	1.876160326	Rasgrp4	1.024647963
Errfi1	1.86913871	Lmf1	1.024604555
Ccnd2	1.866791989	Smc3	1.024558747
Snhg4	1.85926484	Fam118b	1.024505164
Zmat3	1.858777231	Kif15	1.023953631
Ptpn9	1.856452888	Cpeb3	1.023923019
Egr1	1.853897687	Adgra1	1.023026376
Dnajc10	1.852044296	Safb	1.022908183
A630033H20Rik	1.851287962	Psmb3	1.022863235
Ctsb	1.832770858	Dhtkd1	1.022813101
Sgsh	1.831682811	Bmpr1b	1.02276876
Ctnna1	1.825509641	Cdk14	1.022075321
Gng12	1.823669353	Abcd3	1.020692022
Tmem176b	1.82285949	A530040E14Rik	1.019535471
Atp6v0a2	1.820322631	Phf24	1.018368684
Dmd	1.814015876	Frmd5	1.017848005
Ssbp4	1.808520665	Cldn34c1	1.017608358

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Dck	1.807120995	Mfap4	1.017464779
Tmed10	1.804630829	Lgi1	1.016790218
Plekha2	1.788241201	Fgfr2	1.016403211
Ywhae	1.787342486	Espn	1.015617966
Prdx6	1.782181966	Olfr90	1.015595827
Cpne8	1.780683612	Ahcyl2	1.015502202
Pan3	1.76632335	Zbtb46	1.015224258
Tsn	1.765727062	Ghrhr	1.013691524
Postn	1.760918581	Slu7	1.013465906
5031439G07Rik	1.754798139	Rgs6	1.011093407
Tcf25	1.751773197	Hacl1	1.010778823
Capza2	1.748806651	Myo1g	1.01023387
Ssr3	1.745487096	Tsen54	1.00985654
Pafah1b1	1.741737246	Tdo2	1.006294884
Sbf2	1.740917993	Mrgprc2-ps	1.006158745
Ubc	1.738762972	Sez6	1.005579581
Rnpep	1.733612821	Fmr1	1.004972266
Tnpo1	1.73276259	Olfr295	1.004922065
1110037F02Rik	1.731726851	Stard10	1.004561273
Ogt	1.722820519	Ikzf3	1.003969279
Nras	1.722695811	Mad1l1	1.003331901
Ddx39b	1.722321035	Sun2	1.002634026
Elovl5	1.716649083	Zfp532	1.001725437
Inpp5d	1.708655113	C2cd3	1.001540813
Stx7	1.705989329	Gpr89	1.001332809
Klf3	1.704318719	C920009B18Rik	1.001138066
Sdc3	1.691630397	Itsn1	1.001091655
Pltp	1.689657257	BC034090	1.000446333
Gnai2	1.686128128	Gripap1	0.999489556
Nfib	1.677548572	Lmo7	0.998665132
Eef1a1	1.666817084	Cep250	0.99700681
Sval2	1.654461632	Mkln1	0.996736898
Cxcl16	1.653930195	9030624J02Rik	0.996673208
Gpr108	1.649897733	C2cd5	0.994435489
Atp5h	1.648363833	Racgap1	0.994262554
Ppp1ca	1.648036693	Epb41	0.993574572
Amfr	1.646430431	Rgs3	0.9935682
2310014F06Rik	1.642609288	Map2k2	0.991526748
mt-Tl1	1.638589779	Zfp369	0.990602507
Twsg1	1.636545598	Zcchc4	0.990232518
Magt1	1.631891466	Celf3	0.989871179
Gria3	1.614777482	Nfrkb	0.988761687
Gna12	1.611782252	1500012K07Rik	0.987475187
Ppp4r1l-ps	1.611024994	Csnk1g1	0.987302343
Mfge8	1.606938172	Tbk1	0.987242185
Lasp1	1.606641944	Ube2e2	0.986976054

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Gstp1	1.60150536	C2cd2l	0.986252848
Sh3pxd2b	1.598640187	Nlgn1	0.985395138
Coq10b	1.597748785	Atad3aos	0.98523759
Cdk1	1.590738694	Lair1	0.98518503
Wnk1	1.589077278	Lamtor3	0.983751037
Calm3	1.575849963	Man2c1	0.98191137
Rad23b	1.575037653	Phc2	0.981299938
Naa20	1.570197421	Rnf123	0.980776141
Nkx2-2	1.566605188	Rgs11	0.980226854
Nfix	1.555019024	Fbxo18	0.980008734
Nans	1.547167513	Plxna3	0.979857188
Sidt2	1.545962364	Adam23	0.979186928
Oasl2	1.532383232	Thsd7a	0.979160545
Cyb561a3	1.53078711	Pde4d	0.979053978
Rasal2	1.530399343	Smim1	0.977702838
Flt4	1.527570552	Pum2	0.977669452
2810474O19Rik	1.526141876	Dlec1	0.977609037
3222401L13Rik	1.520827618	Arhgef4	0.977585828
Fyttd1	1.520231944	Zbtb49	0.977502295
Ilgp1	1.515968737	Senp3	0.977451995
Atp6v1a	1.514613918	Trpm2	0.976880999
Lrrc42	1.513457922	1810032O08Rik	0.976754675
Trim16	1.513078049	Gramd1c	0.976420001
Tmub2	1.511718079	Zfp13	0.975991964
Slc25a12	1.511137106	Ppp4r1	0.975773873
Oasl1	1.509642511	Proser2	0.975505959
Rpph1	1.507834495	Nek10	0.975435395
Crtc3	1.506271977	Mcf2l	0.974595726
Rnf44	1.502558854	Cald1	0.974287067
Rab43	1.4997319	Homez	0.973060396
Lrch4	1.494344133	Plcg1	0.972958606
Trim35	1.493644106	Pkp4	0.972945011
Slit2	1.489830377	Hnrnpk	0.972631359
Cyp2f2	1.488373517	Ppp2r1a	0.97246017
Snx3	1.481595088	Trmt1	0.972303044
Etv5	1.479921021	Rab3gap1	0.972187837
Oas2	1.473261403	4930431F12Rik	0.972082764
Psme1	1.470561263	Tcte2	0.97206615
Lsm12	1.468223249	Aoah	0.9714557
Impact	1.466855064	1700110I01Rik	0.970367518
Dcakd	1.465419814	4933427J07Rik	0.970106045
Tbp	1.463219259	Polrmt	0.969835731
Alg8	1.463180722	Plekhg3	0.969499396
Csrp1	1.460388067	Chrna9	0.969212179
Znfx1	1.459793233	Fgfr1op2	0.969013346
Ctps	1.457382832	Olfr889	0.968607339

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Zc3h14	1.456860742	Gnas	0.968283741
Nisch	1.454173681	Egflam	0.967634053
Polr2a	1.45089857	Clk4	0.966015603
Hectd1	1.443602365	Metap1d	0.965868178
Mir195b	1.442373355	Rap1gap	0.965285567
Rnf139	1.439425895	Inpp5f	0.965246376
Hist1h4m	1.436213948	Olfir509	0.964350171
Yap1	1.436000261	Trpm1	0.964078371
Cse1l	1.432919664	Palm	0.963865503
Hist1h4n	1.420524579	Capn10	0.96369017
Lhx9	1.412383525	Acad10	0.962450168
Plekhn1	1.410987625	Xndc1	0.962213076
Arpc4	1.404917958	Tesk2	0.961846014
Vamp3	1.404006857	Acox2	0.961694735
Phkb	1.402306049	Ptpn3	0.960945148
Atp1a1	1.398979263	Slf1	0.960893662
Scamp2	1.393517046	Rpl23	0.95970086
Rnf213	1.392440751	Hdac7	0.959267451
Grb10	1.389174949	Prkcb	0.958836304
Znrf2	1.388534731	Bcas3	0.958813276
Hspa5	1.385032431	Rpl19-ps10	0.958779312
Dnase2a	1.37456459	Efcab7	0.958287456
Cyr61	1.371301936	Pabpc1	0.95824206
Cystm1	1.370152464	Rassf8	0.95818526
Hnrnp1	1.35916454	Lrmp	0.957724619
Ppia	1.357022789	1700034P13Rik	0.957400444
Pptc7	1.353998906	Rspry1	0.95567678
Fxr1	1.347297931	Sorbs2	0.955664718
Kif1c	1.345550329	Rtel1	0.955325539
Ctsd	1.339661798	Snph	0.955256444
Tgoln1	1.338617781	Clk1	0.952095381
Fam65a	1.336440467	Tdh	0.951361116
Synpo	1.335052229	4930571N24Rik	0.95129822
Fbrs	1.332797189	Frmd4b	0.951044409
Abcc1	1.330281279	Txnrd2	0.950976492
Ranbp2	1.330010856	D10Wsu102e	0.950757437
Ubr4	1.327808199	Stxbp2	0.950655783
Sel1l	1.325945409	Mum1	0.950334319
Tsg101	1.322854408	Adam12	0.949845685
Bag1	1.32115605	Gramd1b	0.949409727
Cmtm3	1.319131098	Duxbl1	0.94925517
Rsu1	1.318682847	Pmaip1	0.949000209
Il6st	1.318295734	Fance	0.948894855
Gng2	1.315481592	Prosc	0.948788392
Tmem184b	1.31525017	Lima1	0.948520832
Gatm	1.314698511	Aen	0.94849098

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Mir1193	1.31441673	Prdm16	0.948418105
Pias1	1.314346226	Pcca	0.948384277
Elk3	1.309805838	4933411E06Rik	0.948034366
Rnf130	1.301482051	Slc26a4	0.947701486
Rpl13	1.300399066	Dgkd	0.946864762
Lpp	1.296602519	Csnk1e	0.945641621
Mrpl45	1.296477005	Katnal2	0.945160424
Cyb5r3	1.296253036	Vcam1	0.944460807
Shprh	1.289669952	Tmem200a	0.944191277
Cpt1c	1.289477514	Chek2	0.943761214
Ptpn1	1.282425471	Sgk2	0.943639303
Fam160a2	1.279579655	Nsun5	0.943217275
Cfh	1.26988982	Tcf7l1	0.941235147
Hnrnpul1	1.266397252	Uckl1	0.941137918
Txndc12	1.266168771	Rasgrp2	0.941009198
Eri3	1.264671312	Smarcd2	0.940938344
Gsk3b	1.256436011	Epha7	0.939472345
Rnh1	1.255232223	Armc6	0.938877158
Man1b1	1.250578892	Ptpn22	0.938849214
Fkbp1a	1.245105748	Fev	0.93824194
Mia3	1.244304037	Serpinb6a	0.938234426
Ruvbl2	1.239887848	Mier1	0.938199749
Adam10	1.233094367	4930567H12Rik	0.938007376
Mfap5	1.232219563	Sgsm1	0.936969063
Trim56	1.226423401	Csn1s1	0.936473012
Aaed1	1.225534965	Herpud1	0.936370431
Mapre1	1.223804151	Braf	0.936045549
Laptm5	1.223212463	Npsr1	0.935924917
Glpr2	1.21815041	Cox6b1	0.935454199
Dock10	1.213163554	Dpm1	0.935059488
Tmx2	1.211625711	Rhoa	0.935000601
Tor1aip2	1.210578968	Chrn3	0.934196093
Etv6	1.208482154	Cobl	0.934107222
Vmn1r70	1.208124474	AI838599	0.933705762
Anxa5	1.205547419	Vars	0.932030709
Cuta	1.203534407	Clspn	0.931082728
Larp1	1.202436659	Dvl2	0.930782278
Tapbp	1.19794257	Dync1h1	0.930369494
Ddx6	1.193876061	Luc7l2	0.930153333
Mbnl2	1.186460159	Rel2	0.929377802
Ncoa3	1.179909627	Zfp260	0.929183972
Tpm3	1.179006377	Dock3	0.929117216
Dok1	1.178638573	Bace1	0.928792603
Per1	1.177725503	Sh3gl2	0.927925558
Prrc2b	1.177576351	Pde1a	0.927007191
Memo1	1.173534294	Zfyve1	0.925770343

Day 9 up (vs sDC1)	Fold change	Day9 Down (vs sDC1)	Fold Change
Pcbp1	1.173138043	Tacc2	0.925726245
Ccs	1.172429114	Col16a1	0.925278858
F11r	1.168823701	Urod	0.924496139
Mmp23	1.167621462	Kntc1	0.924441575
Ssr2	1.167292126	Tprn	0.92401978
Pmpcb	1.16244984	lpmk	0.923602568
Mtmr2	1.161744403	Tns4	0.923451301
Atxn10	1.15851223	Zfp512b	0.923242144
Glg1	1.156216287	Rnf10	0.922947177
Fndc3a	1.156036991	Pus10	0.922597079
Zdhhc13	1.153699404	Slc39a9	0.922555226
Mef2c	1.150436919	Arhgap22	0.921849391
Mir8116	1.144949195	Mknk1	0.921395425
Slc6a6	1.143929269	1810059H22Rik	0.921384279
Dmpk	1.143512387	Ttc29	0.921368016
Prr32	1.137396445	Grip1	0.921036891
Zcchc6	1.136983879	Nudt16	0.920946667
Pfkip	1.136528919	Sf3b5	0.920695962
1600014C10Rik	1.134651281	Sbno1	0.920236532
Pdhub	1.13293917	4933407L21Rik	0.92002246
Elk4	1.130558075	Bend5	0.919256559
Casp3	1.129817521	Pard3	0.918710037
Gskip	1.129732393	Fam81a	0.918653108
Dnase1l3	1.127323549	Abcg2	0.918618185
Pde4b	1.124393895	Flt3	0.917955107
B4galt1	1.124344501	Nebi	0.917936388
Ube2v1	1.120565671	Ddc	0.917229801
Ifi213	1.120530139	Lrrc8d	0.916139547
Cops8	1.119841447	Focad	0.915711203
Sf3a2	1.118870719	Tlk2	0.915663628

[00184] In an embodiment, in addition to the membrane associated co-stimulatory molecules, mature DCs express cytokines with a pro-inflammatory function that are important for the development of T-cell responses. These responses can be initiated by the triggering of at least 11 different Toll-like receptors (TLRs), allowing the specific recognition of distinct conserved microbial or viral structures. It was asked whether iDCs secrete cytokines to the media when challenged with TLR3 (using Polyinosinic-polycytidylic acid (poly-I:C)) or TLR4 (Lipopolysaccharides (LPS)) stimulation (**Figure 31**). Upon LPS or polyI:C challenge of iDCs it was observed an increase in the secretion of IL-6 (14- or 10-fold, respectively). An increase in the secretion of tumor necrosis factor TNF (7-fold) and

interferon IFN- γ (2-fold) was also observed after LPS stimulation or polyI:C, respectively. Cells transduced with PIB plus TCF4 (PIBT) respond equivalently to stimulation displaying increased secretion of IL-6, TNF and IFN- γ . Importantly, upon stimulation of iDCs it was not observed increase in the secretion of the anti-inflammatory cytokine IL-10. These results suggest that iDCs underwent maturation towards a proinflammatory profile.

[00185] In an embodiment, it was evaluated the capacity of iDCs to mount an antigen-specific immune response. First it was evaluated whether iDCs would be able to engulf particles by incubation with 1 μ m FITC-labeled latex beads. After incubation tdTomato+ cells contained numerous fluorescent beads in the cytoplasm (**Figure 32**), suggesting that iDCs have established the competence for phagocytosis.

[00186] Then, we evaluated the ability of iDCs to capture soluble proteins. Remarkably, 13,8% of tdTomato+ cells were able to actively uptake soluble protein after incubation at 37°C for 20 minutes in contrast to only 5.6% when incubated at 4°C (**Figure 33A**), further suggesting that iDCs have established the competence for phagocytosis/endocytosis. In contrast, only 4.6% of tdTomato- cells showed similar ability. Next, we evaluated whether iDCs were able to internalize dead cell material *in vitro* (**Figure 33B-D**). This unique ability has been shown to be associated with cDC1 DC subtypes to cross-present cell-associated antigens on MHC-I (27). After overnight incubation with labeled dead cells, 65.7% of purified tdTomato+ cells have incorporated dead cell material in contrast to only 10.5% of tdTomato- (**Figure 33B**). Uptake of dead cells was further analysed by live imaging and it was observed that tdTomato+ cells avidly accumulated dead cell material in the cytoplasm (**Figure 33C**). TdTomato+ cells move actively and, upon encountering a dead cell, projected cellular protrusions to incorporate and engulf it (**Figure 33D**).

[00187] Moreover, we have confirmed that iDCs express genes encoding TLR (*Tlr3* and *Tlr4*) and other mediators of TLR signaling, including MyD88-dependent (TRAM (encoded by *Ticam2*), and *Traf6*) and independent (*IKK ϵ*) pathways (**Figure 34A**). Also, we have confirmed that iDCs express key mediators of receptor-mediated endocytosis (*Fcgr2b*, *Tfr2* and *Mrc1*) and macropinocytosis of dead cells (*Axl*, *Lrp1* and *Scarf1*), further suggesting that iDCs have acquired the ability for sense and incorporate antigens (**Figure 34B**).

[00188] In an embodiment, it was evaluated the functional capacity of iDCs to promote antigen-specific proliferation of CD4 T-cells (**Figure 35A**). For this it was employed MHC class II-restricted ovalbumin-specific T cells (OT-II cells) isolated from lymph nodes and spleen of OT-II Rag2 KO mice (18). In this model T cells respond to the processed antigenic peptide

(OVA 323-339) when shown in the context of MHC-II of DCs. Therefore OT-II CD4 T-cells were co-cultured with iDCs when given the Ovalbumin protein (OVA) or pre-processed antigenic peptide (OVA 323-339). Functional DCs are able to capture the protein, process and present the processed antigenic peptide in the context of MHC-II. Induced CD4+ T cell proliferation was measured by CFSE dilution and the activation of the T-cell activation marker CD44 after 7 days of co-culture. Remarkably, 56% of CD4+ T cells diluted CFSE when co-cultured with PIB-generated iDCs in the presence of OVA protein (**Figure 35B**). When co-cultured with MEFs transduced with PIB+TCF4, 38.2% of CD4+ T cells diluted CFSE content, which suggests that inclusion of TCF4 in the reprogramming pool does not increase the stimulatory ability of iDCs. Splenic MHC-II+CD11c+ DCs were used as controls and generated 24.1% of proliferative T-cells. Importantly, addition of LPS stimuli, which is commonly employed to induce DC “maturation”, increased the antigen-specific stimulatory ability of MEFs transduced with PIB (1.5-fold) or PIB+TCF4 (2-fold) and also splenic DCs (3-fold) (**Figure 35B and Figure 36**). As expected, T-cells that were not co-cultured did not proliferate with or without LPS stimuli. To further assess the stimulatory ability of iDCs, it was evaluated if they were able to induce expression of T-cell activation markers, such as CD44. When given the pre-processed antigen, OT-II CD4 T cells diluted CFSE and upregulated the expression of CD44 when co-cultured with both PIB-generated iDCs and splenic MHC-II+CD11c+ DCs (**Figure 35C**). Importantly, when given OVA protein, iDCs display comparable ability to induce CD44 expression in OT-II T cells when compared with splenic DCs (52.2% versus 63.8%). This data supports iDCs’ functional ability to incorporate and process OVA protein followed by presentation of processed Ovalbumin peptides in MHC-II complexes at cell surface. Collectively, these results highlight the functional capacity of iDCs and support the feasibility of using directly reprogrammed fibroblast to present antigens and inducing antigen-specific adaptive immune responses.

[00189] In an embodiment, it was evaluated if iDCs acquire ability to export antigens to cytosol and express key genes essential for cross-presentation ability. Cross-presentation via the cytosolic pathway involves antigen export from endocytic compartments to the cytosol. Thus, the ability of iDCs to perform antigen export was evaluated using a cytofluorimetry-based assay (**Figure 37A**). Remarkably, after 90-minute incubation with b-lactamase, approximately 80% of CCF4-loaded iDCs expressed cleaved CCF4. Thus, iDCs were able to uptake b-lactamase and efficiently export it into the cytoplasm, leading to the generation of cleaved CCF4. It was also confirmed that iDCs express genes involved in cross-presentation pathway, such as *Cybb*, *Atg7*, *Tap1* and *Tap2* (**Figure 37B**).

[00190] In an embodiment, it was evaluated if iDCs were able to cross-present antigens to CD8⁺ T-cells. For this, cross-presentation of OVA at MHC-I molecules was evaluated by co-culturing iDCs with B3Z T-cell hybridoma cells that express β -galactosidase under the control of IL-2 promoter (**Figure 38A, left panel**). It was observed that iDCs were able to induce antigen-specific T-cell activation in a concentration-dependent manner. Moreover, it was observed an increase of activation of B3Z T-cells after TLR3 stimulation with polyI:C and not with LPS (**Figure 38A, right panel**). Accordingly, it has been described that maturation of cDC1s with polyI:C enhances the MHC-I cross-presentation process (28). Moreover, cross-presentation was also evaluated employing MHC class I-restricted ovalbumin-specific T cells (OT-I cells) isolated from lymph nodes and spleen of OT-I Rag2 KO mice (19). In this model T-cells respond to the processed antigenic peptide (OVA 257-264) when shown in the context of MHC-I of DCs. Therefore OT-I CD8 T-cells were co-cultured with iDCs in the presence of the Ovalbumin protein (OVA) and polyI:C stimulation (**Figure 38B**). Functional DCs are able to capture the exogenous protein, process and perform cross-presentation of the processed antigenic peptide in the context of MHC-I, inducing activation of CD8⁺ T cells. Induced CD8⁺ T cell proliferation was measured by CFSE dilution and the activation of the T-cell activation marker CD44 after 4 days of co-culture. Remarkably, in the presence of OVA protein, $11.3 \pm 1.13\%$ of CD8⁺ T cells diluted CFSE and up-regulated CD44 expression when co-cultured with iDCs, respectively (**Figure 38B**). Splenic MHC-II⁺CD11c⁺ DCs were used as controls and generated $18.05 \pm 0.78\%$ of proliferative and CD44⁺ T-cells.

[00191] In an embodiment, Human Dermal Fibroblasts (HDFs) were transduced with PIB (**Figure 39A**). Importantly, it was observed morphologic alterations when PIB TFs were introduced in HDFs. Three days after transduction it was observed that HDFs lost the characteristic bipolar and elongated shapes and acquired a stellate DC-like morphology (**Figure 39B and 39C**). Moreover, it was evaluated the expression of typical DC surface markers. Human foreskin fibroblasts BJ5 transduced with PIB express HLA-DR molecules and CLEC9A, known specific human DC markers (**Figure 40**), suggesting that PIB combination of DC-inducing factors is conserved between the mouse and human and is sufficient to generate human iDCs.

[00192] In an embodiment, it was evaluated whether human iDCs would be able to engulf particles by incubation with 1 μ m FITC-labeled latex beads. After incubation PIB-transduced HDFs contained numerous fluorescent beads in the cytoplasm (**Figure 41A**), suggesting that iDCs have established the competence for phagocytosis. Additionally, the ability to incorporate proteins was evaluated by incubating iDCs with AlexaFluor647-labelled

ovalbumine (**Figure 41B**). After incubation at 37°C, 1.2% of PIB-transduced HDFs contained labelled protein, suggesting that iDCs are able to actively engulf proteins.

[00193] In an embodiment, it was evaluated whether cancer cells would acquire DC phenotypic traits after transduction with PU.1, IRF8 and BATF3. Remarkably, 2.13% of PIB-transduced lung cancer cells (3LL cell line) expressed MHC-I molecules and 2.33% co-expressed MHC-II and CLEC9A at cell surface 8 days after addition of Dox (**Figure 42**). Similarly, 26% of PIB-transduced B16 melanoma cancer cells expressed CLEC9A, whilst 1.04 % of cells co-expressed it with MHC-II molecules (**Figure 43**). These results suggest that it is possible to induce DC phenotype in cancer cells using PIB factors.

[00194] In an embodiment, coding regions of PU.1, IRF8 and BATF3 were cloned into polycistronic inducible lentiviral vectors that express the three nucleic acid sequences, each of them separated by 2A peptide sequences (**Figure 44A**). The 3 TFs were included in different orders, PU.1, IRF8 and BATF3 (PIB) or IRF8, PU.1 and BATF3 (IPB). Impressively, Clec9a reporter activation was observed in 10.8% and 6.6% of MEFs transduced with the polycistronic vectors (PIB and IPB, respectively). In contrast, only 1.9% of tdTomato+ cells were observed in MEFs transduced with lentiviral vectors encoding individual factors (**Figure 44B** and **44C**). As expected, delivery of PIB factors in polycistronic vectors increased reprogramming efficiency up to 6-fold.

[00195] In an embodiment, coding regions of each candidate TF were individually cloned into an inducible lentiviral pFUW-TetO vector (6) in which the expression of the TFs is under the control of the tetracycline operator and a minimal CMV promoter. A previously described lentiviral vector containing the reverse tetracycline transactivator M2rtTA under the control of a constitutively active human ubiquitin C promoter (FUW-M2rtTA) was used in combination. Human Embryonic Kidney (HEK) 293T cells were transfected with a mixture of TF-encoding plasmids, packaging constructs and the VSV-G envelope protein. Viral supernatants were harvested after 36, 48 and 60 hours, filtered (0.45 µm, Corning) and used fresh or concentrated 40-fold with Amicon ultra centrifugal filters (Millipore).

[00196] In some embodiments, polypeptide variants or family members having the same or a similar activity as the reference polypeptide encoded by the sequences provided in the sequence list can be used in the compositions, methods, and kits described herein. Generally, variants of a particular polypeptide encoding a DC inducing factor for use in the compositions, methods, and kits described herein will have at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or more sequence

identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art.

[00197] *Homo sapiens* Basic Leucine Zipper ATF-Like Transcription Factor (BATF3), mRNA (SEQ. ID. 1) and a codon optimized, or different codons encoding the same amino acids, are naturally also contemplated to be covered by the reference to the nucleic acid as set forth herein.

[00198] *Homo sapiens* Spi-1 proto-oncogene (PU.1), mRNA (SEQ. ID. 7) and a codon optimized, or different codons encoding the same amino acids, are naturally also contemplated to be covered by the reference to the nucleic acid as set forth herein.

[00199] *Homo sapiens* Interferon Regulatory Factor 8 (IRF8), mRNA (SEQ. ID. 5) and a codon optimized, or different codons encoding the same amino acids, are naturally also contemplated to be covered by the reference to the nucleic acid as set forth herein.

[00200] *Homo sapiens* Transcription factor 4 (TCF4), mRNA (SEQ. ID. 13) and a codon optimized, or different codons encoding the same amino acids, are naturally also contemplated to be covered by the reference to the nucleic acid as set forth herein.

[00201] In some embodiments of the compositions, methods, and kits provided herein, the number of DC inducing factors used or selected to generate iDCs from a starting somatic cell, such as a fibroblast cell or hematopoietic lineage cell, a multipotent stem cell, an induced pluripotent stem cell, a cancer or tumor cell is at least three. In some embodiments, the number of DC inducing factors used or selected is at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least thirty, at least thirty three, at least thirty five, at least forty, or more.

[00202] Also provided herein, in various aspects of the compositions, methods, and kits, are isolated amino acid sequences, and isolated DNA or RNA nucleic acid sequences encoding one or more DC inducing factors for use in making iDCs.

[00203] In some embodiments of the compositions, methods, and kits described herein, the nucleic acid sequence or construct encoding the DC inducing factor(s), such as PU.1, IRF8, BATF3 and TCF4, is inserted or operably linked into a suitable expression vector for transfection of cells using standard molecular biology techniques. As used herein, a “vector”

refers to a nucleic acid molecule, such as a dsDNA molecule that provides a useful biological or biochemical property to an inserted nucleotide sequence, such as the nucleic acid constructs or replacement cassettes described herein. Examples include plasmids, phages, autonomously replicating sequences (ARS), centromeres, and other sequences that are able to replicate or be replicated in vitro or in a host cell, or to convey a desired nucleic acid segment to a desired location within a host cell. A vector can have one or more restriction endonuclease recognition sites (whether type I, II or IIs) at which the sequences can be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a nucleic acid fragment can be spliced or inserted in order to bring about its replication and cloning. Vectors can also comprise one or more recombination sites that permit exchange of nucleic acid sequences between two nucleic acid molecules. Vectors can further provide primer sites, e.g., for PCR, transcriptional and/or translational initiation and/or regulation sites, recombination signals, replicons, additional selectable markers, etc. A vector can further comprise one or more selectable markers suitable for use in the identification of cells transformed with the vector.

[00204] In some embodiments of the compositions, methods, and kits described herein, the expression vector is a viral vector. Some viral-mediated expression methods employ retrovirus, adenovirus, lentivirus, herpes virus, pox virus, and adeno-associated virus (AAV) vectors, and such expression methods have been used in gene delivery and are well known in the art.

[00205] In some embodiments of the compositions, methods, and kits described herein, the viral vector is a retrovirus. Retroviruses provide a convenient platform for gene delivery. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to target cells of the subject either in vivo or ex vivo. A number of retroviral systems have been described. See, e.g., U.S. Pat. No. 5,219,740; Miller and Rosman (1989) *BioTechniques* 7:980-90; Miller, A. D. (1990) *Human Gene Therapy* 1:5-14; Scarpa et al. (1991) *Virology* 180:849-52; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033-37; Boris-Lawrie and Temin (1993) *Curr. Opin. Genet. Develop.* 3:102-09. In some embodiments of the compositions, methods, and kits described herein, the retrovirus is replication deficient. Retroviral vector systems exploit the fact that a minimal vector containing the 5' and 3' LTRs and the packaging signal are sufficient to allow vector packaging, infection and integration into target cells, provided that the viral structural proteins are supplied in trans in the packaging cell line. Fundamental advantages of retroviral vectors for gene transfer include efficient infection and gene

expression in most cell types, precise single copy vector integration into target cell chromosomal DNA and ease of manipulation of the retroviral genome.

[00206] In some embodiments of the compositions, methods, and kits described herein, the viral vector is an adenovirus-based expression vector. Unlike retroviruses, which integrate into the host genome, adenoviruses persist extrachromosomally, thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham (1986) *J. Virol.* 57:267-74; Bett et al. (1993) *J. Virol.* 67:5911-21; Mittereder et al. (1994) *Human Gene Therapy* 5:717-29; Seth et al. (1994) *J. Virol.* 68:933-40; Barr et al. (1994) *Gene Therapy* 1:51-58; Berkner, K. L. (1988) *BioTechniques* 6:616-29; and Rich et al. (1993) *Human Gene Therapy* 4:461-76). Adenoviral vectors infect a wide variety of cells, have a broad host-range, exhibit high efficiencies of infectivity, direct expression of heterologous genes at high levels, and achieve long-term expression of those genes in vivo. The virus is fully infective as a cell-free virion so injection of producer cell lines is not necessary. With regard to safety, adenovirus is not associated with severe human pathology, and the recombinant vectors derived from the virus can be rendered replication defective by deletions in the early-region 1 ("E1") of the viral genome. Adenovirus can also be produced in large quantities with relative ease. Adenoviral vectors for use in the compositions, methods, and kits described herein can be derived from any of the various adenoviral serotypes, including, without limitation, any of the over 40 serotype strains of adenovirus, such as serotypes 2, 5, 12, 40, and 41. The adenoviral vectors used herein are preferably replication-deficient and contain the DC inducing factor of interest operably linked to a suitable promoter.

[00207] In some embodiments of the compositions, methods, and kits described herein, the nucleic acid sequences encoding the DC inducing factor(s), such as, PU.1, IRF8, BATF3 and TCF4 are introduced or delivered using one or more inducible lentiviral vectors. Control of expression of DC inducing factors delivered using one or more inducible lentiviral vectors can be achieved, in some embodiments, by contacting a cell having at least one DC inducing factor in an expression vector under the control of or operably linked to an inducible promoter, with a regulatory agent (e.g., doxycycline) or other inducing agent. When using some types of inducible lentiviral vectors, contacting such a cell with an inducing agent induces expression of the DC inducing factors, while withdrawal of the regulatory agent inhibits expression. When using other types of inducible lentiviral vectors, the presence of the regulatory agent inhibits expression, while removal of the regulatory agent permits expression. As used herein, the term "induction of expression" refers to the expression of a gene, such as a DC inducing factor encoded by an inducible viral vector, in the presence of

an inducing agent, for example, or in the presence of one or more agents or factors that cause endogenous expression of the gene in a cell.

[00208] In some embodiments of the aspects described herein, a doxycycline (Dox) inducible lentiviral system is used. Unlike retroviruses, lentiviruses are able to transduce quiescent cells making them amenable for transducing a wider variety of hematopoietic cell types. For example, the pFUW-tetO lentivirus system has been shown to transduce primary hematopoietic progenitor cells with high efficiency.

[00209] In some embodiments of the methods described herein, the nucleic acid sequences encoding the DC inducing factor(s), such as PU.1 (SEQ. ID. 7, SEQ. ID. 8), IRF8 (SEQ. ID. 5, SEQ. ID. 6), BATF3 (SEQ. ID. 1, SEQ. ID. 2) and/or TCF4 (SEQ. ID. 13, SEQ. ID. 14), are introduced or delivered using a non-integrating vector (e.g., adenovirus). While integrating vectors, such as retroviral vectors, incorporate into the host cell genome and can potentially disrupt normal gene function, non-integrating vectors control expression of a gene product by extra-chromosomal transcription. Since non-integrating vectors do not become part of the host genome, non-integrating vectors tend to express a nucleic acid transiently in a cell population. This is due in part to the fact that the non-integrating vectors are often rendered replication deficient. Thus, non-integrating vectors have several advantages over retroviral vectors including, but not limited to: (1) no disruption of the host genome, and (2) transient expression, and (3) no remaining viral integration products. Some non-limiting examples of non-integrating vectors for use with the methods described herein include adenovirus, baculovirus, alphavirus, picornavirus, and vaccinia virus. In some embodiments of the methods described herein, the non-integrating viral vector is an adenovirus. Other advantages of non-integrating viral vectors include the ability to produce them in high titers, their stability in vivo, and their efficient infection of host cells.

[00210] Nucleic acid constructs and vectors for use in generating iDCs in the compositions, methods, and kits described herein can further comprise, in some embodiments, one or more sequences encoding selection markers for positive and negative selection of cells. Such selection marker sequences can typically provide properties of resistance or sensitivity to antibiotics that are not normally found in the cells in the absence of introduction of the nucleic acid construct. A selectable marker can be used in conjunction with a selection agent, such as an antibiotic, to select in culture for cells expressing the inserted nucleic acid construct. Sequences encoding positive selection markers typically provide antibiotic resistance, i.e., when the positive selection marker sequence is present in the genome of a cell, the cell is sensitive to the antibiotic or agent. Sequences encoding negative selection

markers typically provide sensitivity to an antibiotic or agent, i.e., when the negative selection marker is present in the genome of a cell, the cell is sensitive to the antibiotic or agent.

[00211] Nucleic acid constructs and vectors for use in making iDCs in the compositions, methods, and kits thereof described herein can further comprise, in some embodiments, other nucleic acid elements for the regulation, expression, stabilization of the construct or of other vector genetic elements, for example, promoters, enhancers, TATA-box, ribosome binding sites, IRES, as known to one of ordinary skill in the art.

[00212] In some embodiments of the compositions, methods, and kits described herein, the DC inducing factor(s), such as PU.1 (SEQ. ID. 7, SEQ. ID. 8), IRF8 (SEQ. ID. 5, SEQ. ID. 6), BATF3 (SEQ. ID. 1, SEQ. ID. 2) and/or TCF4 (SEQ. ID. 13, SEQ. ID. 14), are provided as synthetic, modified RNAs, or introduced or delivered into a cell as a synthetic, modified RNA, as described in US Patent Publication 2012-0046346-A1, the contents of which are herein incorporated by reference in their entireties. In those embodiments where synthetic, modified RNAs are used to reprogram cells to iDCs according to the methods described herein, the methods can involve repeated contacting of the cells or involve repeated transfections of the synthetic, modified RNAs encoding DC inducing factors, such as for example, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 25, at least 30, or more transfections.

[00213] In addition to one or more modified nucleosides, the modified mRNAs for use in the compositions, methods, and kits described herein can comprise any additional modifications known to one of skill in the art and as described in US Patent Publications 2012-0046346-A1 and 20120251618A1, and PCT Publication WO 2012/019168. Such other components include, for example, a 5' cap (e.g., the Anti-Reverse Cap Analog (ARCA) cap, which contains a 5'-5'-triphosphate guanine-guanine linkage where one guanine contains an N7 methyl group as well as a 3'-O-methyl group; caps created using recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-O-methyltransferase enzyme, which can create a canonical 5'-5'-triphosphate linkage between the 5'-most nucleotide of an mRNA and a guanine nucleotide where the guanine contains an N7 methylation and the ultimate 5'-nucleotide contains a 2'-O-methyl generating the Cap1 structure); a poly(A) tail (e.g., a poly-A tail greater than 30 nucleotides in length, greater than 35 nucleotides in length, at least 40 nucleotides, at least 45 nucleotides, at least 55 nucleotides, at least 60 nucleotide, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at

least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 600 nucleotides, at least 700 nucleotides, at least 800 nucleotides, at least 900 nucleotides, at least 1000 nucleotides, or more); a Kozak sequence; a 3' untranslated region (3' UTR); a 5' untranslated region (5' UTR); one or more intronic nucleotide sequences capable of being excised from the nucleic acid, or any combination thereof.

[00214] The modified mRNAs for use in the compositions, methods, and kits described herein can further comprise an internal ribosome entry site (IRES). An IRES can act as the sole ribosome binding site, or can serve as one of multiple ribosome binding sites of an mRNA. An mRNA containing more than one functional ribosome binding site can encode several peptides or polypeptides, such as the DC inducing factors described herein, that are translated independently by the ribosomes ("multicistronic mRNA"). When nucleic acids are provided with an IRES, further optionally provided is a second translatable region. Examples of IRES sequences that can be used according to the invention include without limitation, those from picornaviruses (e.g. FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SW) or cricket paralysis viruses (CrPV).

[00215] In some embodiments of the compositions, methods, and kits described herein, the synthetic, modified RNA molecule comprises at least one modified nucleoside. In some embodiments of the compositions, methods, and kits described herein, the synthetic, modified RNA molecule comprises at least two modified nucleosides.

[00216] In some embodiments of the compositions, methods, and kits described herein, the modified nucleosides are selected from the group consisting of 5-methylcytosine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'-deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2,N2,7-trimethylguanosine (m2,2,7G), and inosine (I). In some embodiments, the modified nucleosides are 5-methylcytosine (5mC), pseudouracil, or a combination thereof.

[00217] Modified mRNAs need not be uniformly modified along the entire length of the molecule. Different nucleotide modifications and/or backbone structures can exist at various

positions in the nucleic acid. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) can be located at any position(s) of a nucleic acid such that the function of the nucleic acid is not substantially decreased. A modification can also be a 5' or 3' terminal modification. The nucleic acids can contain at a minimum one and at maximum 100% modified nucleotides, or any intervening percentage, such as at least 50% modified nucleotides, at least 80% modified nucleotides, or at least 90% modified nucleotides.

[00218] In some embodiments, it is preferred, but not absolutely necessary, that each occurrence of a given nucleoside in a molecule is modified (e.g., each cytosine is a modified cytosine e.g., 5-methylcytosine, each uracil is a modified uracil, e.g., pseudouracil, etc.). For example, the modified mRNAs can comprise a modified pyrimidine such as uracil or cytosine. In some embodiments, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the uracil in the nucleic acid are replaced with a modified uracil. It is also contemplated that different occurrences of the same nucleoside can be modified in a different way in a given synthetic, modified RNA molecule. The modified uracil can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures). In some embodiments, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the cytosine in the nucleic acid may be replaced with a modified cytosine. The modified cytosine can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures) (e.g., some cytosines modified as 5mC, others modified as 2'-O-methylcytosine or other cytosine analog). Such multi-modified synthetic RNA molecules can be produced by using a ribonucleoside blend or mixture comprising all the desired modified nucleosides, such that when the RNA molecules are being synthesized, only the desired modified nucleosides are incorporated into the resulting RNA molecule encoding the DC inducing factor.

[00219] In certain embodiments it is desirable to intracellularly degrade a modified nucleic acid introduced into the cell, for example if precise timing of protein production is desired. Thus, in some embodiments of the compositions, methods, and kits described herein, provided herein are modified nucleic acids comprising a degradation domain, which is capable of being acted on in a directed manner within a cell.

[00220] While it is understood that iDCs can be generated by delivery of DC inducing factors in the form of nucleic acid (DNA or RNA) or amino acid sequences, in some embodiments of the compositions, methods, and kits described herein, iDC induction can be induced using

other methods, such as, for example, by treatment of cells with an agent, such as a small molecule or cocktail of small molecules, that induce expression one or more of the DC inducing factors.

[00221] Detection of expression of DC inducing factors introduced into cells or induced in a cell population using the compositions, methods, and kits described herein, can be achieved by any of several techniques known to those of skill in the art including, for example, Western blot analysis, immunocytochemistry, and fluorescence-mediated detection.

[00222] In order to distinguish whether a given combination of DC inducing factors has generated iDCs, one or more DC activities or parameters can be measured, such as, in some embodiments, differential expression of surface antigens. The generation of induced DCs using the compositions, methods, and kits described herein preferably causes the appearance of the cell surface phenotype characteristic of endogenous DCs, such as CLEC9A, MHC-I, MHC-II, CD40, CD80, CD86, CD103, for example.

[00223] DCs are most reliably distinguished from other immune cells by their functional behavior. Functional aspects of DC phenotypes, or dendritic cell activities, such as the ability of a dendritic cell to induce antigen specific T cell responses, can be easily determined by one of skill in the art using routine methods known in the art, and as described herein, for example, in the Drawings, i.e., FIGS. 1-44. In some embodiments of the aspects described herein, functional assays to identify reprogramming factors can be used. For example, in some embodiments, antigen presentation and antigen cross-presentation assays can be used to confirm antigen-specific induction of T cell responses (antigen presentation potential) of iDCs generated using the compositions, methods, and kits thereof. Cytokine secretion can be used to confirm immune-modulatory properties of iDCs generated using the compositions, methods, and kits described herein. Ability to engulf particles, proteins and dead cells of iDCs generated using the compositions, methods, and kits described herein can be evaluated by culturing transduced cells in the presence of labelled beads, ovalbumine or dead cells, followed by flow cytometry analysis, respectively.

[00224] As used herein, “cellular parameter,” “DC parameter,” or “antigen presentation activity” refer to measureable components or qualities of endogenous or natural DCs, particularly components that can be accurately measured. A cellular parameter can be any measurable parameter related to a phenotype, function, or behavior of a cell. Such cellular parameters include, changes in characteristics and markers of a DC or DC population, including but not limited to changes in viability, cell growth, expression of one or more or a

combination of markers, such as cell surface determinants, such as receptors, proteins, including conformational or posttranslational modification thereof, lipids, carbohydrates, organic or inorganic molecules, nucleic acids, e.g. mRNA, DNA, global gene expression patterns, etc. Such cellular parameters can be measured using any of a variety of assays known to one of skill in the art. For example, viability and cell growth can be measured by assays such as Trypan blue exclusion, CFSE dilution, and 3H-thymidine incorporation. Expression of protein or polypeptide markers can be measured, for example, using flow cytometric assays, Western blot techniques, or microscopy methods. Gene expression profiles can be assayed, for example, using RNA-sequencing methodologies and quantitative or semi-quantitative real-time PCR assays. A cellular parameter can also refer to a functional parameter or functional activity. While most cellular parameters will provide a quantitative readout, in some instances a semi-quantitative or qualitative result can be acceptable. Readouts can include a single determined value, or can include mean, median value or the variance, etc. Characteristically a range of parameter readout values can be obtained for each parameter from a multiplicity of the same assays. Variability is expected and a range of values for each of the set of test parameters will be obtained using standard statistical methods with a common statistical method used to provide single values.

[00225] In some embodiments of the compositions, methods, and kits described herein, additional factors and agents can be used to enhance iDC reprogramming. For example, factors and agents that modify epigenetic pathways can be used to facilitate reprogramming into iDCs.

[00226] Essentially any primary somatic cell type can be used for producing iDCs or reprogramming somatic cells to iDCs according to the presently described compositions, methods, and kits. Such primary somatic cell types also include other stem cell types, including pluripotent stem cells, such as induced pluripotent stem cells (iPS cells); other multipotent stem cells; oligopotent stem cells; and (5) unipotent stem cells. Some non-limiting examples of primary somatic cells useful in the various aspects and embodiments of the methods described herein include, but are not limited to, fibroblast, epithelial, endothelial, neuronal, adipose, cardiac, skeletal muscle, hematopoietic or immune cells, hepatic, splenic, lung, circulating blood cells, gastrointestinal, renal, bone marrow, and pancreatic cells, as well as stem cells from which those cells are derived. The cell can be a primary cell isolated from any somatic tissue including, but not limited to, spleen, bone marrow, blood, brain, liver, lung, gut, stomach, intestine, fat, muscle, uterus, skin, spleen, endocrine organ, bone, etc. The term "somatic cell" further encompasses, in some

embodiments, primary cells grown in culture, provided that the somatic cells are not immortalized. Where the cell is maintained under in vitro conditions, conventional tissue culture conditions and methods can be used, and are known to those of skill in the art. Isolation and culture methods for various primary somatic cells are well within the abilities of one skilled in the art.

[00227] In some embodiments of these aspects and all such aspects described herein, the somatic cell is a fibroblast cell.

[00228] In some embodiments of these aspects and all such aspects described herein, the somatic cell can be a hematopoietic lineage cell.

[00229] In some embodiments of these aspects and all such aspects described herein, the somatic cell can be a cancer cell or a tumor cell.

[00230] In some embodiments of the compositions, methods, and kits described herein, a somatic cell to be reprogrammed or made into an iDC cell is a cell of hematopoietic origin. As used herein, the terms “hematopoietic-derived cell,” “hematopoietic-derived differentiated cell,” “hematopoietic lineage cell,” and “cell of hematopoietic origin” refer to cells derived or differentiated from a multipotent hematopoietic stem cell (HSC). Accordingly, hematopoietic lineage cells for use with the compositions, methods, and kits described herein include multipotent, oligopotent, and lineage-restricted hematopoietic progenitor cells, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, and lymphocytes (e.g., T-lymphocytes, which carry T-cell receptors (TCRs), B-lymphocytes or B cells, which express immunoglobulin and produce antibodies, NK cells, NKT cells, and innate lymphocytes). As used herein, the term “hematopoietic progenitor cells” refer to multipotent, oligopotent, and lineage-restricted hematopoietic cells capable of differentiating into two or more cell types of the hematopoietic system, including, but not limited to, granulocytes, monocytes, erythrocytes, megakaryocytes, and lymphocytes B-cells and T-cells. Hematopoietic progenitor cells encompass multi-potent progenitor cells (MPPs), common myeloid progenitor cells (CMPs), common lymphoid progenitor cells (CLPs), granulocyte-monocyte progenitor cells (GMPs), and pre-megakaryocyte-erythrocyte progenitor cell. Lineage-restricted hematopoietic progenitor cells include megakaryocyte-erythrocyte progenitor cells (MEP), ProB cells, PreB cells, PreProB cells, ProT cells, double-negative T cells, pro-NK cells, pre-granulocyte/macrophage cells, granulocyte/macrophage

progenitor (GMP) cells, and pro-mast cells (ProMCs). A differentiation chart of the hematopoietic lineage is provided at Figure 1.

[00231] Cells of hematopoietic origin for use in the compositions, methods, and kits described herein can be obtained from any source known to comprise these cells, such as fetal tissues, umbilical cord blood, bone marrow, peripheral blood, mobilized peripheral blood, spleen, liver, thymus, lymph, etc. Cells obtained from these sources can be expanded ex vivo using any method acceptable to those skilled in the art prior to use in with the compositions, methods, and kits for making iDCs described herein. For example, cells can be sorted, fractionated, treated to remove specific cell types, or otherwise manipulated to obtain a population of cells for use in the methods described herein using any procedure acceptable to those skilled in the art. Mononuclear lymphocytes may be collected, for example, by repeated lymphocytophereses using a continuous flow cell separator as described in U.S. Pat. No. 4,690,915, or isolated using an affinity purification step of CLP method, such as flow-cytometry using a cytometer, magnetic separation, using antibody or protein coated beads, affinity chromatography, or solid-support affinity separation where cells are retained on a substrate according to their expression or lack of expression of a specific protein or type of protein, or batch purification using one or more antibodies against one or more surface antigens specifically expressed by the cell type of interest. Cells of hematopoietic origin can also be obtained from peripheral blood. Prior to harvest of the cells from peripheral blood, the subject can be treated with a cytokine, such as e.g., granulocyte-colony stimulating factor, to promote cell migration from the bone marrow to the blood compartment and/or promote activation and/or proliferation of the population of interest. Any method suitable for identifying surface proteins, for example, can be employed to isolate cells of hematopoietic origin from a heterogeneous population. In some embodiments, a clonal population of cells of hematopoietic origin, such as lymphocytes, is obtained. In some embodiments, the cells of hematopoietic origin are not a clonal population.

[00232] Further, in regard to the various aspects and embodiments of the compositions, methods, and kits described herein, a somatic cell can be obtained from any mammalian species, with non-limiting examples including a murine, bovine, simian, porcine, equine, ovine, or human cell. In some embodiments, the somatic cell is a human cell. In some embodiments, the cell is from a non-human organism, such as a non-human mammal.

[00233] In general, the methods for making iDCs described herein involve culturing or expanding somatic cells, such as cells of hematopoietic origin, in any culture medium that is

available and well-known to one of ordinary skill in the art. Such media include, but are not limited to, Dulbecco's Modified Eagle's Medium® (DMEM), DMEM F12 Medium®, Eagle's Minimum Essential Medium®, F-12K Medium®, Iscove's Modified Dulbecco's Medium®, RPMI-1640 Medium®, and serum-free medium for culture and expansion of DCs. Many media are also available as low-glucose formulations, with or without sodium. The medium used with the methods described herein can, in some embodiments, be supplemented with one or more immunostimulatory cytokine. Commonly used growth factors include, but are not limited to, G-CSF, GM-CSF, TNF- α , IL-4, the Flt-3 ligand and the kit ligand. In addition, in preferred embodiments, the immunostimulatory cytokine is selected from the group consisting of the interleukins (e.g., IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-6, IL-8, IL-9, IL-10, IL-12, IL-18, IL-19, IL-20), the interferons (e.g., IFN- α , IFN- β , IFN- γ), tumor necrosis factor (TNF), transforming growth factor- β (TGF- β), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), the Flt-3 ligand and the kit ligand.

[00234] Cells in culture can be maintained either in suspension or attached to a solid support, such as extracellular matrix components or plating on feeder cells, for example. Cells being used in the methods described herein can require additional factors that encourage their attachment to a solid support, in some embodiments, such as type I and type II collagen, chondroitin sulfate, fibronectin, "superfibronectin" and fibronectin-like polymers, gelatin, poly-D and poly-L-lysine, thrombospondin and vitronectin. In some embodiments, the cells are suitable for growth in suspension cultures. Suspension-competent host cells are generally monodisperse or grow in loose aggregates without substantial aggregation. Suspension-competent host cells include cells that are suitable for suspension culture without adaptation or manipulation (e.g., cells of hematopoietic origin, such as lymphoid cells) and cells that have been made suspension-competent by modification or adaptation of attachment-dependent cells (e.g., epithelial cells, fibroblasts).

[00235] In some embodiments of these aspects and all such aspects described herein, the isolated induced dendritic cells (iDCs) further comprise a pharmaceutically acceptable carrier for administration to a subject in need.

[00236] Also provided herein, in some aspects, are methods of treating a subject in need of treatment to induce antigen-specific immune responses to eliminate cancer cells or infectious agents using the DC inducing compositions and methods of preparing iDCs described herein, or using the isolated induced dendritic cells (iDCs) and cell clones thereof produced using any of the combinations of DC inducing factors, DC inducing compositions,

or methods of preparing iDCs described herein. In such methods of treatment, somatic cells, such as fibroblast cells or hematopoietic lineage cells, can first be isolated from the subject, and the isolated cells transduced or transfected, as described herein with a DC inducing composition comprising expression vectors or synthetic mRNAs, respectively. The isolated induced dendritic cells (iDCs) produced using any of the combinations of DC inducing factors, DC inducing compositions, or methods of preparing iDCs described herein, can then be administered to the subject, such as via systemic injection of the iDCs to the subject.

[00237] Also provided herein, in some aspects, are methods of treating a subject in need of treatment to induce antigen-specific immune responses to eliminate cancer cells or infectious agents using the DC inducing compositions and any of the combinations of DC inducing factors described herein. In such methods of treatment, cancer cells are transduced, as described herein with a DC inducing composition comprising expression vectors. Cancer cells can be first isolated from the subject, transduced with a DC inducing composition comprising expression vectors and then administered to the subject, such as via systemic injection. Alternatively, cancer cells can be transduced in situ or in vivo with DC inducing composition comprising viral expression vectors. The modified cancer cell acquires antigen presentation ability, presenting their tumor antigens to T cells and eliciting cytotoxic responses against themselves.

[00238] The reprogrammed iDCs generated using the compositions, methods, and kits described herein can, in some embodiments of the methods of treatment described herein, be used directly or administered to subjects in need of immunotherapies. Accordingly, various embodiments of the methods described herein involve administration of an effective amount of a iDC or a population of iDCs, generated using any of the compositions, methods, and kits described herein, to an individual or subject in need of a cellular therapy. The cell or population of cells being administered can be an autologous population, or be derived from one or more heterologous sources. Further, such iDCs can be administered in a manner that permits them to migrate to lymph node and activate effector T cells.

[00239] A variety of means for administering cells to subjects are known to those of skill in the art. Such methods can include systemic injection, for example, i.v. injection, or implantation of cells into a target site in a subject. Cells may be inserted into a delivery device which facilitates introduction by injection or implantation into the subject. Such delivery devices can include tubes, e.g., catheters, for injecting cells and fluids into the body of a recipient subject. In one preferred embodiment, the tubes additionally have a needle, e.g., through which the cells can be introduced into the subject at a desired location. The

cells can be prepared for delivery in a variety of different forms. For example, the cells can be suspended in a solution or gel or embedded in a support matrix when contained in such a delivery device. Cells can be mixed with a pharmaceutically acceptable carrier or diluent in which the cells remain viable.

[00240] Accordingly, the cells produced by the methods described herein can be used to prepare cells to treat or alleviate several cancers and tumors including, but not limited to, breast cancer, prostate cancer, lymphoma, skin cancer, pancreatic cancer, colon cancer, melanoma, malignant melanoma, ovarian cancer, brain cancer, primary brain carcinoma, head-neck cancer, glioma, glioblastoma, liver cancer, bladder cancer, non- small cell lung cancer, head or neck carcinoma, breast carcinoma, ovarian carcinoma, lung carcinoma, small-cell lung carcinoma, Wilms' tumor, cervical carcinoma, testicular carcinoma, bladder carcinoma, pancreatic carcinoma, stomach carcinoma, colon carcinoma, prostatic carcinoma, genitourinary carcinoma, thyroid carcinoma, esophageal carcinoma, myeloma, multiple myeloma, adrenal carcinoma, renal cell carcinoma, endometrial carcinoma, adrenal cortex carcinoma, malignant pancreatic insulinoma, malignant carcinoid carcinoma, choriocarcinoma, mycosis fungoides, malignant hypercalcemia, cervical hyperplasia, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, chronic granulocytic leukemia, acute granulocytic leukemia, hairy cell leukemia, neuroblastoma, rhabdomyosarcoma, Kaposi's sarcoma, polycythemia vera, essential thrombocytosis, Hodgkin's disease, non- Hodgkin's lymphoma, soft-tissue sarcoma, osteogenic sarcoma, primary macroglobulinemia, and retinoblastoma, and the like.

[00241] In addition to the above, the methods of the invention can be used to prevent or eliminate infection by pathogens known to predispose to certain cancers. Pathogens of particular interest for use in the cancer vaccines provided herein include the hepatitis B virus (hepatocellular carcinoma), hepatitis C virus (heptomas), Epstein Barr virus (EBV) (Burkitt lymphoma, nasopharynx cancer, PTLD in immunosuppressed individuals), HTLV (adult T cell leukemia), oncogenic human papilloma viruses types 16, 18, 33, 45 (adult cervical cancer), and the bacterium *Helicobacter pylori* (B cell gastric lymphoma). Other medically relevant microorganisms that may serve as antigens in mammals and more particularly humans are described extensively in the literature, e.g., C. G. A Thomas, Medical Microbiology, Bailliere Tindall, (1983).

[00242] In addition to the above, the methods of the invention can be used for viral infections. Exemplary viral pathogens include, but are not limited to, infectious virus that

infect mammals, and more particularly humans. Examples of infectious virus include, but are not limited to: Retroviridae (e.g., human immunodeficiency viruses, such as HIV-I (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g. strains that cause gastroenteritis); Togaviridae (e.g. equine encephalitis viruses, rubella viruses); Flaviridae (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g. coronaviruses such as the SARS coronavirus); Rhabdoviridae (e.g. vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g. ebola viruses); Paramyxoviridae (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g. influenza viruses); Bunyaviridae (e.g. Hantaan viruses, bunya viruses, phleboviruses and Nairo viruses); Arenaviridae (hemorrhagic fever viruses); Reoviridae (e.g. reoviruses, orbiviruses and rotaviruses); Birnaviridae; Hepadnaviridae (Hepatitis B virus); Parvoviridae (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g. African swine fever virus); and unclassified viruses (e.g. the etiological agents of Spongiform encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class I=enterally transmitted; class 2=parenterally transmitted (i.e. Hepatitis C); Norwalk and related viruses, and astro viruses).

[00243] In addition to the above, the methods of the invention can be used to target gram negative and gram positive bacteria in vertebrate animals. Such gram positive bacteria include, but are not limited to *Pasteurella* sp., *Staphylococci* sp., and *Streptococcus* sp. Gram negative bacteria include, but are not limited to, *Escherichia coli*, *Pseudomonas* sp., and *Salmonella* sp. Specific examples of infectious bacteria include but are not limited to: *Helicobacter pylori*, *Borrelia burgdorferi*, *Legionella pneumophila*, *Mycobacteria* sp. (e.g. *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. goodii*), *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A *Streptococcus*), *Streptococcus agalactiae* (Group B *Streptococcus*), *Streptococcus* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic sps.), *Streptococcus pneumoniae*, pathogenic *Campylobacter* sp., *Enterococcus* sp., *Haemophilus influenzae*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Corynebacterium* sp., *Erysipelothrix rhusiopathiae*, *Clostridium perfringens*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Bacteroides*

sp., *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidum*, *Treponema pertenue*, *Leptospira*, *Rickettsia*, and *Actinomyces israelii*.

[00244] In addition to the above, the methods of the invention can be used to target pathogens that include, but are not limited to, infectious fungi and parasites that infect mammals, and more particularly humans. Examples of infectious fungi include, but are not limited to: *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Chlamydia trachomatis*, and *Candida albicans*.

[00245] In addition to the above, the methods of the invention can be used to target parasites such as intracellular parasites and obligate intracellular parasites. Examples of parasites include but are not limited to *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Babesia microti*, *Babesia divergens*, *Trypanosoma cruzi*, *Toxoplasma gondii*, *Trichinella spiralis*, *Leishmania major*, *Leishmania donovani*, *Leishmania braziliensis*, *Leishmania tropica*, *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, *Ascaris lumbricoides*, *Onchocerca volvulus* and *Schistosoma mansoni*.

[00246] If modified induced dendritic cells can be used to induce a tolerogenic response including the suppression of a future or existing immune response, to one or more target antigens. Thus, induce DCs are useful for treating or preventing an undesirable immune response including, for example, transplant rejection, graft versus host disease, allergies, parasitic diseases, inflammatory diseases and autoimmune diseases. Examples of transplant rejection, which can be treated or prevented in accordance with the present invention, include rejections associated with transplantation of bone marrow and of organs such as heart, liver, pancreas, kidney, lung, eye, skin etc. Examples of allergies include seasonal respiratory allergies; allergy to aeroallergens such as hayfever; allergy treatable by reducing serum IgE and eosinophilia; asthma; eczema; animal allergies, food allergies; latex allergies; dermatitis; or allergies treatable by allergic desensitisation. Autoimmune diseases that can be treated or prevented by the present invention include, for example, psoriasis, systemic lupus erythematosus, myasthenia gravis, stiff-man syndrome, thyroiditis, Sydenham chorea, rheumatoid arthritis, diabetes and multiple sclerosis. Examples of inflammatory disease include Crohn's disease, chronic inflammatory eye diseases, chronic inflammatory lung diseases and chronic inflammatory liver diseases, autoimmune haemolytic anaemia, idiopathic leucopenia, ulcerative colitis, dermatomyositis, scleroderma, mixed connective tissue disease, irritable bowel syndrome, systemic lupus erythematosus (SLE), multiple sclerosis, myasthenia gravis, Guillan-Barre syndrome (antiphospholipid syndrome), primary

myxoedema, thyrotoxicosis, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, insulin-dependent diabetes mellitus (IDDM), Goodpasture's syndrome, Behcet's syndrome, Sjogren's syndrome, rheumatoid arthritis, sympathetic ophthalmia, Hashimoto's disease/hypothyroiditis, celiac disease/dermatitis herpetiformis, and demyelinating disease primary biliary cirrhosis, mixed connective tissue disease, chronic active hepatitis, Graves' disease/hyperthyroiditis, scleroderma, chronic idiopathic thrombocytopenic purpura, diabetic neuropathy and septic shock.

[00247] Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid. Preferably, prior to the introduction of cells, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like.

[00248] It is preferred that the mode of cell administration is relatively non-invasive, for example by intravenous injection, pulmonary delivery through inhalation, topical, or intranasal administration. However, the route of cell administration will depend on the tissue to be treated and may include implantation. Methods for cell delivery are known to those of skill in the art and can be extrapolated by one skilled in the art of medicine for use with the methods and compositions described herein.

[00249] Also provided herein, in some aspects, are kits for making induced dendritic cells (iDCs), the kits comprising any of the DC inducing compositions comprising one or more expression vector components described herein.

[00250] Also provided herein, in some aspects, are kits comprising one or more of the DC inducing factors described herein as components for the methods of making the induced dendritic cells described herein.

[00251] Accordingly, in some aspects, provided herein, are kits for preparing induced dendritic cells comprising the following components: (a) one or more expression vectors encoding at least one, two, three, four, five, six, seven, eight, or more DC inducing factors selected from: BATF3 (SEQ. ID. 1, SEQ. ID. 2), SPIB (SEQ. ID. 3, SEQ. ID. 4), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), STAT3 (SEQ. ID. 11, SEQ. ID. 12), TCF4 (SEQ. ID. 13, SEQ. ID. 14), IKZF1 (SEQ. ID. 15, SEQ. ID. 16), ID2 (SEQ. ID. 17, SEQ. ID. 18), BCL11A (SEQ. ID. 19, SEQ. ID. 20), RELB (SEQ. ID. 21, SEQ. ID. 22), ZBTB46 (SEQ. ID. 23, SEQ. ID. 24), RUNX3

(SEQ. ID. 25, SEQ. ID. 26), GFI1 (SEQ. ID. 27, SEQ. ID. 28), IRF2 (SEQ. ID. 29, SEQ. ID. 30), NFIL3 (SEQ. ID. 31, SEQ. ID. 32), BCL6 (SEQ. ID. 33, SEQ. ID. 34), L-MYC (SEQ. ID. 35, SEQ. ID. 36), NR4A3 (SEQ. ID. 37, SEQ. ID. 38), and (b) packaging and instructions therefor.

[00252] The kits described herein, in some embodiments, can further provide the synthetic mRNAs or the one or more expression vectors encoding DC inducing factors in an admixture or as separate aliquots.

[00253] In some embodiments, the kits can further comprise an agent to enhance efficiency of reprogramming. In some embodiments, the kits can further comprise one or more antibodies or primer reagents to detect a cell-type specific marker to identify cells induced to the dendritic cell state.

[00254] In some embodiments, the kits can further comprise a buffer. In some such embodiments, the buffer is RNase-free TE buffer at pH 7.0. In some embodiments, the kit further comprises a container with cell culture medium.

[00255] All kits described herein can further comprise a buffer, a cell culture medium, a transduction or transfection medium and/or a media supplement. In preferred embodiments, the buffers, cell culture mediums, transfection mediums, and/or media supplements are DNase and RNase-free. In some embodiments, the synthetic, modified RNAs provided in the kits can be in a non-solution form of specific quantity or mass, e.g., 20 µg, such as a lyophilized powder form, such that the end-user adds a suitable amount of buffer or medium to bring the components to a desired concentration, e.g., 100 ng/µl.

[00256] All kits described herein can further comprise devices to facilitate single-administration or repeated or frequent infusions of the cells generated using the kits components described herein, such as a non-implantable delivery device, e.g., needle, syringe, pen device, or an implantable delivery device, e.g., a pump, a semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular), or a reservoir. In some such embodiments, the delivery device can include a mechanism to dispense a unit dose of a pharmaceutical composition comprising the iDCs. In some embodiments, the device releases the composition continuously, e.g., by diffusion. In some embodiments, the device can include a sensor that monitors a parameter within a subject. For example, the device can include pump, e.g., and, optionally, associated electronics.

[00257] In an embodiment, induced dendritic cells are made by the hand of man by, e.g., modifying the gene expression of at least one of the factors disclosed herein of a somatic cell, a pluripotent cell, a progenitor cell or a stem cell, or by exposing any one of these cell

types to at least one protein or RNA that produces at least one protein as disclosed herein. The cells can further be made by exposing them to small molecules that turn on at least one of the factors disclosed herein. In some aspects at least two, three, four, five, six, seven, or eight factors are used to make the induced dendritic cells.

[00258] In an embodiment, the induced dendritic cells in some aspects of all the embodiments of disclosure, while similar in functional characteristics, differ significantly in their gene expression from the naturally occurring endogenous dendritic cells.

[00259] In an embodiment, the induced dendritic cells as described herein differ from naturally occurring dendritic cells by both their posttranslational modification signatures and their gene expression signatures.

[00260] In an embodiment, the induced dendritic cells as described herein differ from naturally occurring dendritic cells by their ability to growth in vitro as adherent cultures and to survive in culture for more than one month.

[00261] In an embodiment, induced dendritic cell is also defined as comprising a gene expression signature that differs from naturally occurring dendritic cells. One can experimentally show the difference by comparing the gene expression pattern of a naturally occurring dendritic cell to that of the induced dendritic cells. Therefore, in some aspects of all the embodiments of the invention, the induced dendritic cells comprise an expression signature that is about 1-5%, 5-10%, 5-15%, or 5-20% different from the expression signature of about 1-5%, 2-5%, 3-5%, up to 50%, up to 40%, up to 30%, up to 25%, up to 20%, up to 15%, or up to 10% of specific genes. For example, expression levels of DC inducing factor(s), such as PU.1, IRF8, BATF3 and TCF4, in iDCs are higher than in naturally occurring DCs as the DC inducing factors are being overexpressed.

[00262] In an embodiment, mouse Embryonic Fibroblasts (MEFs) were isolated and purified in the following way: Clec9aCre/Cre animals (10) were crossed with Rosa26-stopflox-tdTomato reporter mice (The Jackson Laboratory) to generate double homozygous Clec9a^{Cre/Cre} Rosa^{tdTomato/tdTomato} (C9A-tdTomato) mice. C57BL/6 mice, Rag2 constitutive knock-out (KO)/OT-II random transgenic (Rag2KO/OT-II) mice and Rag2KO/OT-I random transgenic mice were acquired from Charles River and Taconic, respectively (17-19). All animals were housed under controlled temperature (23 ± 2 °C), subject to a fixed 12-h light/dark cycle, with free access to food and water.

[00263] In an embodiment, primary cultures of MEFs were isolated from E13.5 embryos of C9A-tdTomato or C57BL/6 mice (6, 10). Head, fetal liver and all internal organs were

removed and the remaining tissue was mechanically dissociated. Dissected tissue was enzymatic digested using 0.12% trypsin/0.1 mM Ethylenediaminetetraacetic acid (EDTA) solution (3 mL per embryo), and incubation at 37°C for 15 min. Additional 3 mL of same solution per embryo were added, followed by another 15 min incubation period. A single cell suspension was obtained and plated in 0.1% gelatin-coated 10-cm tissue culture dishes in growth media. Cells were grown for 2-3 days until confluence, dissociated with Tryple Express and frozen in Fetal Bovine Serum (FBS) 10% dimethyl sulfoxide (DMSO). Before plating for lentiviral transduction, MEFs were sorted to remove residual CD45+ and tdTomato+ cells that could represent cells with hematopoietic potential. MEFs used for screening and in the following experiments were tdTomato- CD45- with a purity of 99.8% and expanded up to 4 passages.

[00264] In an embodiment, HEK293T cells, MEFs and Human Dermal Fibroblasts (HDFs, ScienCell) were maintained in growth medium [Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) FBS, 2mM L-Glutamine and antibiotics (10 µg/ml Penicillin and Streptomycin)], OP-9 and OP-9-DL1 cell lines were cultured in Minimum Essential Medium (MEM) Alpha containing 20% FBS, 1mM L-Glutamine and penicillin/streptomycin (10 µg/ml). OP-9 and OP-9-DL1 were routinely passaged at 80% confluency. All cells were maintained at 37°C and 5% (v/v) CO₂. All tissue culture reagents were from Thermo Fisher Scientific unless stated otherwise.

[00265] In an embodiment, viral transduction and reprogramming experiments were performed in the following way: C9A-tdTomato MEFs were seeded at a density of 40,000 cells per well on 0.1% gelatin coated 6-well plates. Cells were incubated overnight with a ratio of 1:1 FUW-TetO-TFs and FUW-M2rtTA lentiviral particles in growth media supplemented with 8 µg/mL polybrene. When testing combinations of TFs, equal MOIs of each individual viral particles were applied. Cells were transduced twice in consecutive days and after overnight incubation, media was replaced with fresh growth media. After the second transduction, growth media was supplemented with Doxycycline (1 µg/mL) – day 0. Media was changed every 2-3 days for the duration of the cultures. Emerging tdTomato+ cells were analyzed 1-15 days post-transduction. When stated, variations of culture conditions were applied, namely RPMI-1640, Lipopolysaccharide (LPS, 100 ng/ml, Sigma), 2-Mercaptoethanol (1x10⁻⁴ µM; 2-ME), L-glutamine (2 µmol/ml), GM-CSF (10 ng/ml, STEMCELL Technologies), IL-4 (20 ng/ml, STEMCELL Technologies) and Flt3l (100 ng/ml, STEMCELL Technologies).

[00266] In an embodiment, fluorescent microscopy and immunofluorescence was evaluated in the following way: C9A-driven tdTomato in MEFs and transduced HDFs were visualized directly on 6-well plates under an inverted microscope (Zeiss AxioVert 200M) and images processed with AxioVision and Adobe Photoshop software. DAPI (4',6-diamidino-2-phenylindole, 1 µg/mL, Sigma) and Phalloidin (50 µg/ml, Sigma) were used to stain nuclei and F-actin, respectively. For time-lapse microscopy fluorescent pictures were acquired after adding Dox every 1 hour for 6 days and 4 hours using an INCELL Analyzer 2200 (GE Healthcare). Movies were generated with ImageJ software (NIH).

[00267] In an embodiment, flow cytometry analysis was performed in the following way: Transduced C9A-tdTomato MEFs or transduced human fibroblasts were dissociated with TrypLE Express, resuspended in 200 µL PBS 5% FBS and kept at 4°C prior analysis in BD Accuri C6 Flow Cytometer (BD Biosciences). Sample acquisition was performed with the configuration 3-blue-1-red (533/30 filter in FL1; 585/40 in FL2, 670 LP in FL3 and 675/25 in FL4). tdTomato fluorescence was analyzed in the FL2 channel. For the analysis of CD45 or MHC-II cell surface marker expression, dissociated cells were incubated with APC-Cy7 rat anti-mouse CD45 antibody or Alexa Fluor 647 rat anti-mouse I-A/I-E diluted in PBS 5% FBS at 4°C for 30 minutes in the presence of rat serum (1/100, GeneTex) to block unspecific binding. Cells were washed with PBS 5% FBS, resuspended in PBS 5% FBS and analyzed in a BD Accuri C6 Flow cytometer. CD45 APC-Cy7 and I-A/I-E Alexa Fluor 647 fluorescence were analyzed in FL4 channel. For the combined analysis of MHCII, CD80 and CD86 cell surface expression, dissociated cells were stained with Alexa Fluor 647 rat anti-mouse I-A/I-E, BV650 rat anti-mouse CD80 and PE-CY7 rat anti-mouse CD86 and analyzed in BD FACS Aria III (BD Biosciences). For the analysis of transduced HDFs, dissociated cells were stained with APC mouse anti-human CLEC9A and FITC mouse anti-human HLA-DR. To assess CD4+ and CD8+ T cell proliferation and activation after 7 days of co-culture with APCs, carboxyfluorescein succinimidyl ester (CFSE)-labeled T cells were incubated with PE rat anti-mouse CD44 and analyzed in BD Accuri C6. Flow cytometry data were analyzed using FlowJo software (FLOWJO, LLC, version 7.6).

[00268] In an embodiment, fluorescence activated cell sorting (FACS) was performed in the following way: To purify C9A-tdTomato MEFs, cells were incubated at 4°C for 30 minutes with APC-Cy7 anti-CD45 antibody diluted in PBS 5% FBS. Subsequently, MEFs were washed with PBS 5% FBS, resuspended in PBS 5% FBS and tdTomato- CD45- MEFs were purified in BD FACS Aria III. When described tdTomato+ cells were purified using BD FACS Aria III and cultured in the absence or presence of doxycycline. For the isolation of splenic DCs, splenic

cells were incubated with Alexa Fluor 647 rat anti-mouse I-A/I-E, FITC rat anti-mouse CD11c and APC-Cy7 rat anti-mouse CD8a. CD11c+MHCII+CD8a+ splenic DCs were purified in BD FACS Aria III (BD Biosciences). FACS data was processed in FlowJo software.

[00269] In an embodiment, GPSforGenes software was used to calculate the specificity of Pu.1, Irf8 and Batf3 combination for the DC lineage. Gene expression data was downloaded from BioGPS database (GeneAtlas MOE430), transformed to log-space and normalized to bring the expression values to 0-1 range for each gene across different samples. The resulting data was then searched for samples with the highest averaged expression for Pu.1+Irf8+Batf3.

[00270] In an embodiment, Single cell mRNAseq analysis was performed in the following way: Single-end reads were mapped to the mm10 mouse genome (Ensembl annotation, release 89) using Salmon v0.8.1 with k = 21. The resulting TPM were imported into R using tximport library and converted into mRNA counts using the Census algorithm implemented in monocle library. Scatter library was used to discard cells and genes that didn't pass quality control threshold. The following QC criteria were used: 1) library size per cell; 2) number of genes detected in each single cell; 3) percentage of counts in mitochondrial genes. From the 192 cells initially profiled, 163 individual cells passed quality control filters and were used for analysis. Custom R scripts were used to perform t-distributed stochastic neighbor embedding (tSNE) (Monocle and scatter package), principal component analysis (PCA) (Monocle and scatter package), hierarchical clustering (SC3 package), variance analysis and to construct heat maps, box plots, scatter plots, violin plots, dendrograms, bar graphs, and histograms. Generally, ggplot2, gplots, graphics and pheatmap packages were used to generate data graphs.

[00271] In an embodiment, differential expression analysis was performed using Monocle package, and selecting genes with BH-corrected p-value less than 0.05. The resulting genes were next filtered by variance (genes with variance ≥ 1 across all conditions were selected). Finally, the resulting 6,525 genes were grouped into 4 distinct clusters based on hierarchical clustering.

[00272] In an embodiment, endogenous expression of genes was determined using STAR v2.5.3a with default settings. A window was defined based on -10kb, start of the gene and end of the gene, +10kb, which correspond to 5' and 3' untranslated regions (UTRs) and used to calculate the number of reads in the UTRs using multicov from bedtools v2.27.0.

[00273] In an embodiment, DC lineage of iDCs was determined by using cDC1 and cDC2 gene signatures from Schlitzer (11). The majority of genes were highly expressed in MEF, and across all our condition. These genes were discarded. Moreover, as sDC cells were purified for the cDC1 markers CD11c, MHC-II and CD8 α , genes that were expressed in sDC but at the same time were found in cDC2 signature list were discarded. cDC1/cDC2 gene lists were then used to performed hierarchical clustering. Next, only clusters in which median expression of genes in MEF cells were significantly lower compared to day3, day7 and day9 were selected. Besides that, for cDC2 gene list, in addition to procedure described above, gene clusters with median expression of genes in sDC cells significantly higher compared to day3, day7 and day9 were also discarded. Next, the median of gene expression across each selected gene was calculated from each particular condition. Finally, the median of gene expression across all pre-sorted cDC1 and cDC2 gene signatures defined by Schlitzer and colleagues, was calculated.

[00274] In an embodiment, the Monocle package, an algorithm that uses independent component analysis with minimal spanning tree to connect cells along a pseudotemporally ordered path, was used to order cells on a pseudo-time course during MEF to iDC cell reprogramming. Monocle analysis was performed based on cDC1 and cDC2 genes from Schlitzer, 2015 (11) as genes, which define a cell's progress, as this was an alternative approach to prove that day9 are cDC1-like cells. The resulting trajectories were visualized using Monocle functions. Since single-cell trajectories included branches, branched expression analysis modeling (BEAM) was used, a special statistical test implemented in Monocle package in order to find differentially expressed genes between the branches. As an alternative approach to Monocle branching algorithm, TSCAN was used, which combines clustering with pseudotime analysis, by building a minimum-spanning tree to connect the clusters. TSCAN, in contrast to Monocle, can use all genes to order the cells.

[00275] In an embodiment, gene ontology (biological process, cellular component and KEGG pathway) was performed using using Enrichr (<http://amp.pharm.mssm.edu/Enrichr/>) and Database for Annotation, Visualization and Integrated Discovery (DAVID) clustered functional analysis (david.ncifcrf.gov/).

[00276] In an embodiment, microRNA target interaction analysis was performed using miRTarBase 2017, Enrichr website (<http://amp.pharm.mssm.edu/Enrichr/>).

[00277] In an embodiment, Mouse phenotype analysis was performed using Network2canvas (<http://www.maayanlab.net/N2C/#.WmRvOILc8yk>).

[00278] In an embodiment, gene set enrichment analysis (GSEA) between all possible conditions and states were performed against C7: immunologic signatures from Molecular Signatures Database (MSigDB) and NetPath.

[00279] In an embodiment, TF network analysis was computed by pairwise correlation matrix using Pearson correlation. TFs were selected based on DBD: Transcription factor prediction database (<http://www.transcriptionfactor.org/>) in mouse. As the objective was to investigate the switch between condition from mef to day9, 3 lists were created corresponding to switch from mef to day3; day3 to day7; day7 to day9 and included only those TF which have logFC = 0.5 for pair of conditions. Next, out of TFs defined based on logFC for 3 pair of conditions TFs were selected with a Pearson correlation of greater than 0.35 with at least five other TFs. Taking into consideration the fact that those results could be obtained by chance, permutations were used in order to determine the probability of TFs passing this threshold by chance. 100 permutations were performed and all of them resulted in 0 TFs that pass this threshold. The function `graph.adjacency()` of igraph R package was used, which took Pearson pairwise correlation matrix for the selected TFs for 3 pair of conditions.

[00280] In an embodiment, Methylcellulose clonogenic assays were performed in the following way: PIB-transduced MEFs at day 3, 5, 7, 10 and 25 after addition of Dox were assayed in 1% methylcellulose media (Methocult M3434, Stem Cell Technologies). Sorted sDC1 (MHC-II+CD11c+CD8a+) as well as unsorted splenocytes and bone marrow cells were used as control. Hematopoietic colonies were scored and counted after 7-10 days of culture in 5% CO₂ at 37°C.

[00281] In an embodiment, bead incorporation assay was evaluated in the following way: transduced C9A-tdTomato MEF or transduced HDF cultures were incubated with 2.5% yellow-green fluorescent-coupled solid latex beads (carboxylate-modified polystyrene, Sigma) at 1:1000 ratio in growth medium. Sixteen hours later, cells were washed twice in PBS 5% FBS and analyzed under an inverted microscope. DAPI (1 µg/mL, Sigma) was used for nuclear staining.

[00282] In an embodiment, incorporation of labelled ovalbumin was evaluated in the following way: transduced MEFs and human fibroblast cultures were incubated with Alexa647-labelled ovalbumin (Life Technologies) for 20 minutes at 37°C or 4°C. After washing with PBS 5%FBS, cells were analysed in BD Accuri C6.

[00283] In an embodiment, incorporation of dead cells was evaluated in the following way: HEK293T cells were exposed to ultra-violet (UV) irradiation to induce cell death and labelled with CellVue® Claret Far Red Fluorescent Cell Linker Kit (Sigma), according to manufacturer's instructions. Transduced MEFs were incubated with Far red-labelled dead cells overnight, and analysed in BD Accuri C6.

[00284] In an embodiment, inflammatory cytokine assay was performed in the following way: Levels of the cytokines interleukin- 6 (IL-6), interleukin-10 (IL-10), interleukin-12p70 (IL-12p70), interferon- γ (IFN- γ) and tumor necrosis factor (TNF) were assessed in supernatants of iDCs cultures 10 days after Dox supplementation. At day 9, 100 ng/mL LPS or 25 μ g/mL of Polyinosinic-polycytidylic acid (PolyI:C) (Invivogen) were added for overnight stimulation. 50 μ L of culture supernatants from a 6-well plate well were collected and analyzed by CBA Mouse Inflammation Kit (BD Biosciences), according to manufacturer's instructions. Acquisition was performed with a BD Accuri C6 and data were analyzed using FCAP array software, version 3.0 (BD Biosciences). The limit of detection in CBA was: IL-6, 20.91 pg/ml; IL-10, 10.55 pg/ml; IFN- γ , 18.2 pg/ml; TNF, 18.13 pg/ml; IL-12p70, 20.05 pg/ml.

[00285] In an embodiment, splenic DC isolation was evaluated in the following way: Freshly isolated spleens were homogenized using the frosted ends of 2 sterile slides. Cells were harvested in PBS supplemented with 2% FBS and filtered through a 70 μ m cell strainers (BD Biosciences). Red blood cells were lysed with BD Pharm Lyse (BD Biosciences) for 8 min at room temperature. MHC-II+CD11c+ DCs were purified by FACS (BD FACSAria III, BD Biosciences) and immediately used for antigen presenting assays.

[00286] In an embodiment, CD4+ T cell isolation and antigen presenting assays was evaluated in the following way: CD4+ T cells from spleen of Rag2KO/OT-II mice were enriched using Dynabeads Untouched Mouse CD4 Cells Kit (BD Biosciences), according to manufacturer's instructions. Enriched CD4+ T cells were labeled with CFSE 5 μ M at room temperature for 10 min, washed, and counted before cultured with APCs. iDCs cultures at day 8 after the addition of Dox or splenic CD11c+ MHC-II+ DCs cells were incubated with OVA protein (10 μ g/mL) or OVA323-339 peptide (10 μ g/mL) in the presence or absence of 100 ng/mL of LPS and co-cultured with untouched CFSE-labeled OT-II CD4+ T cells. iDC cultures (20000 cells) or 20000 splenic CD11c+ MHC-II+ DCs were incubated with 20000 CFSE-labeled CD4+ T cells in 96-well round-bottom tissue culture plates. T cell proliferation (dilution of CFSE staining) and activation (CD44 expression) were assessed by flow cytometry after 7 days of co-culture.

[00287] In an embodiment, CD8⁺ T cell isolation and antigen cross-presentation was evaluated in the following way: CD8⁺ T cells from spleen of Rag2KO/OT-I mice were enriched using Dynabeads Untouched Mouse CD8 Cells Kit (BD Biosciences), according to manufacturer's instructions. Enriched CD8⁺ T cells were labelled with CFSE 5 μ M at room temperature for 10 min, washed, and counted before cultured with APCs. iDCs cultures at day 8 after the addition of Dox or splenic CD11c⁺ MHC-II⁺ DCs cells were incubated with OVA protein (10 μ g/mL) in the presence of 25 μ g/mL of polyI:C and co-cultured with untouched CFSE-labelled OT-I CD8⁺ T cells. iDC cultures (20000 cells) or 20000 splenic CD11c⁺ MHC-II⁺ DCs were incubated with 20000 CFSE-labelled CD8⁺ T cells in 96-well round-bottom tissue culture plates. T cell proliferation (dilution of CFSE staining) and activation (CD44 expression) were assessed by flow cytometry after 4 days of co-culture.

[00288] In an embodiment, hybridoma cross-presentation assays were performed in the following way: PIB-transduced Clec9a-tdTomato MEFs at day 16 after addition of Dox were dissociated with TrypLE Express, resuspended in growth media and incubated for 4 hours with different concentrations of OVA protein. After being extensively washed, PIB-transduced MEFs (100,000 cells) were co-cultured with 100,000 B3Z cells in 96-well round-bottom tissue culture plates in the presence or absence of 100 ng/mL LPS or 25 μ g/mL PIC. After 18 h, cells were lysed in a buffer containing 0.125% Nonidet P-40 (substitute), 9 mM MgCl₂, and a colorimetric CPRG β -galactosidase substrate. β -galactosidase activity was measured on MicroPlate Reader as optical density at 590 nm.

[00289] In an embodiment, the efficiency of antigen export to the cytosol by Clec9a-tdTomato⁺ cells were analyzed by cytofluorimetry-based assay. Briefly, PIB-transduced MEFs at day 16 after addition of Dox were dissociated with TrypLE Express, resuspended in loading buffer and loaded with 1 μ M CCF4-AM for 30 min at room temperature. Cells were then washed and incubated with 2 mg/mL β -lactamase at 37°C for 30, 60 and 90 minutes. To stop the reaction, cells were transferred to ice cold PBS. Immediately before flow cytometry analysis in a BD FACSAriaIII, the cells were stained with Fixable Viability Dye eFluor 780 (eBioscience). The percentage of live Clec9a-tdTomato⁺ cells with a high blue-to-green (V450/V500) fluorescence ratio was used as a measure of the efficiency of antigen export into the cytosol.

[00290] In an embodiment, comparisons among groups were performed by one-way ANOVA followed by Bonferroni's multiple comparison test with GraphPad Prism 5 software. P-values are shown when relevant (*p<0.05; **p<0.01, ***p<0.001, ****p<0.0001).

Table 6 - Primary Antibodies Used in the analysis

Antibody/Antigen	Specie	Clone	Conjugate	Source
CD45	Mouse	30-F11	PE	BD Pharmingen
CD4	Mouse	GK1.5	PE-CY7	eBioscience
CD8 α	Mouse	53-6.7	APC-Cy7	Biolegend
CD44	Mouse	IM7	PE	BD Pharmingen
CD103	Mouse	2E7	APC-Cy7	Biolegend
MHC Class II (I-A/I-E)	Mouse	M5/114.15.2	Alexa Fluor 647	BD Pharmingen
MHC Class I (H-2Kb)	Mouse	AF6-88.5.5.3	FITC	eBioscience
CD80 (B7 1)	Mouse	16-101A	BV650	BD Horizon
CD86 (B7 2)	Mouse	GL1	PE-CY7	eBioscience
CD40	Mouse	HM40-3	eFluor450	eBioscience
B220	Mouse	RA3-6B2	FITC	eBioscience
CD11b	Mouse	M1/70	AlexaFluor700	eBioscience
CD11c	Mouse	N418	FITC	Biolegend
HLA-DR	Human	L243	FITC	Biolegend
CLEC9A	Human	8F9	APC	Biolegend

[00291] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above description, but rather is as set forth in the appended claims.

[00292] Where singular forms of elements or features are used in the specification of the claims, the plural form is also included, and vice versa, if not specifically excluded. For example, the term “a cell” or “the cell” also includes the plural forms “cells” or “the cells,” and vice versa. In the claims articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or

descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[00293] Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the claims or from relevant portions of the description is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[00294] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

[00295] In addition, it is to be understood that any particular embodiment of the present invention may be explicitly excluded from any one or more of the claims. Where ranges are given, any value within the range may explicitly be excluded from any one or more of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the invention, can be excluded from any one or more claims. For

purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

[00296] The above described embodiments are combinable.

[00297] The following claims further set out particular embodiments of the present disclosure.

[00298] All references recited in this document are incorporated herein in their entirety by reference, as if each and every reference had been incorporated by reference individually.

References

1. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131(5):861-72. Epub 2007/11/24. doi: 10.1016/j.cell.2007.11.019. PubMed PMID: 18035408.
2. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663-76. Epub 2006/08/15. doi: 10.1016/j.cell.2006.07.024. PubMed PMID: 16904174.
3. Pereira CF, Lemischka IR, Moore K. Reprogramming cell fates: insights from combinatorial approaches. *Ann N Y Acad Sci*. 2012;1266:7-17. Epub 2012/08/21. doi: 10.1111/j.1749-6632.2012.06508.x. PubMed PMID: 22901251.
4. Xu J, Du Y, Deng H. Direct lineage reprogramming: strategies, mechanisms, and applications. *Cell Stem Cell*. 2015;16(2):119-34. Epub 2015/02/07. doi: 10.1016/j.stem.2015.01.013. PubMed PMID: 25658369.
5. Xie H, Ye M, Feng R, Graf T. Stepwise reprogramming of B cells into macrophages. *Cell*. 2004;117(5):663-76. Epub 2004/05/28. PubMed PMID: 15163413.
6. Pereira CF, Chang B, Qiu J, Niu X, Papatsenko D, Hendry CE, et al. Induction of a hemogenic program in mouse fibroblasts. *Cell Stem Cell*. 2013;13(2):205-18. Epub 2013/06/19. doi: 10.1016/j.stem.2013.05.024. PubMed PMID: 23770078; PubMed Central PMCID: PMC3735774.
7. Pereira CF, Chang B, Gomes A, Bernitz J, Papatsenko D, Niu X, et al. Hematopoietic Reprogramming In Vitro Informs In Vivo Identification of Hemogenic Precursors to Definitive Hematopoietic Stem Cells. *Dev Cell*. 2016;36(5):525-39. Epub 2016/03/10. doi: 10.1016/j.devcel.2016.02.011. PubMed PMID: 26954547; PubMed Central PMCID: PMC4785845.
8. Datta J, Terhune JH, Lowenfeld L, Cintolo JA, Xu S, Roses RE, et al. Optimizing dendritic cell-based approaches for cancer immunotherapy. *Yale J Biol Med*. 2014;87(4):491-518. Epub 2014/12/17. PubMed PMID: 25506283; PubMed Central PMCID: PMC4257036.

9. Subklewe M, Geiger C, Lichtenegger FS, Javorovic M, Kvalheim G, Schendel DJ, et al. New generation dendritic cell vaccine for immunotherapy of acute myeloid leukemia. *Cancer Immunol Immunother.* 2014;63(10):1093-103. Epub 2014/09/05. doi: 10.1007/s00262-014-1600-5. PubMed PMID: 25186611.
10. Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell.* 2013;154(4):843-58. Epub 2013/08/21. doi: 10.1016/j.cell.2013.07.014. PubMed PMID: 23953115.
11. Schlitzer A, Sivakamasundari V, Chen J, Sumatoh HR, Schreuder J, Lum J, et al. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. *Nat Immunol.* 2015;16(7):718-28. Epub 2015/06/10. doi: 10.1038/ni.3200. PubMed PMID: 26054720.
12. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol.* 2013;31:563-604. Epub 2013/03/23. doi: 10.1146/annurev-immunol-020711-074950. PubMed PMID: 23516985; PubMed Central PMCID: PMC3853342.
13. Senju S, Hirata S, Matsuyoshi H, Masuda M, Uemura Y, Araki K, et al. Generation and genetic modification of dendritic cells derived from mouse embryonic stem cells. *Blood.* 2003;101(9):3501-8. Epub 2002/10/31. doi: 10.1182/blood-2002-07-2254. PubMed PMID: 12406878.
14. Kitamura N, Yokoyama H, Yashiro T, Nakano N, Nishiyama M, Kanada S, et al. Role of PU.1 in MHC class II expression through transcriptional regulation of class II transactivator pl in dendritic cells. *J Allergy Clin Immunol.* 2012;129(3):814-24 e6. Epub 2011/11/25. doi: 10.1016/j.jaci.2011.10.019. PubMed PMID: 22112519.
15. Smith MA, Wright G, Wu J, Tailor P, Ozato K, Chen X, et al. Positive regulatory domain I (PRDM1) and IRF8/PU.1 counter-regulate MHC class II transactivator (CIITA) expression during dendritic cell maturation. *J Biol Chem.* 2011;286(10):7893-904. Epub 2011/01/11. doi: 10.1074/jbc.M110.165431. PubMed PMID: 21216962; PubMed Central PMCID: PMC3048676.
16. Reith W, LeibundGut-Landmann S, Waldburger JM. Regulation of MHC class II gene expression by the class II transactivator. *Nat Rev Immunol.* 2005;5(10):793-806. Epub 2005/10/04. doi: 10.1038/nri1708. PubMed PMID: 16200082.
17. van der Stoep N, Quinten E, Marcondes Rezende M, van den Elsen PJ. E47, IRF-4, and PU.1 synergize to induce B-cell-specific activation of the class II transactivator promoter III (CIITA-PIII). *Blood.* 2004;104(9):2849-57. Epub 2004/07/10. doi: 10.1182/blood-2004-03-0790. PubMed PMID: 15242870.
18. Shinkai Y, Rathbun G, Lam KP, Oltz EM, Stewart V, Mendelsohn M, et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell.* 1992;68(5):855-67. Epub 1992/03/06. PubMed PMID: 1547487.
19. Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR. T cell receptor antagonist peptides induce positive selection. *Cell.* 1994;76(1):17-27. Epub 1994/01/14. PubMed PMID: 8287475.
20. J. Helft et al., GM-CSF Mouse Bone Marrow Cultures Comprise a Heterogeneous Population of CD11c(+)MHCII(+) Macrophages and Dendritic Cells. *Immunity* 42, 1197 (Jun 16, 2015).

21. G. E. Grajales-Reyes et al., Batf3 maintains autoactivation of Irf8 for commitment of a CD8alpha(+) conventional DC clonogenic progenitor. *Nat Immunol* 16, 708 (Jul, 2015).
22. B. T. Edelson et al., Peripheral CD103⁺ dendritic cells form a unified subset developmentally related to CD8α⁺ conventional dendritic cells. *The Journal of Experimental Medicine* 207, 823 (2010).
23. K. S. Kobayashi, P. J. van den Elsen, NLRC5: a key regulator of MHC class I-dependent immune responses. *Nat Rev Immunol* 12, 813 (12//print, 2012).
24. M. Bretou et al., Lysosome signaling controls the migration of dendritic cells. *Science Immunology*, (2017).
25. S. M. Han et al., TCF4-Targeting miR-124 is Differentially Expressed amongst Dendritic Cell Subsets. *Immune Network* 16, 61 (2016).
26. I. Dunand-Sauthier et al., Silencing of c-Fos expression by microRNA-155 is critical for dendritic cell maturation and function. *Blood* 117, 4490 (2011).
27. O. Schulz, C. Reis e Sousa, Cross-presentation of cell-associated antigens by CD8alpha+ dendritic cells is attributable to their ability to internalize dead cells. *Immunology* 107, 183 (2002).
28. L. Delamarre, H. Holcombe, I. Mellman, Presentation of Exogenous Antigens on Major Histocompatibility Complex (MHC) Class I and MHC Class II Molecules Is Differentially Regulated during Dendritic Cell Maturation. *The Journal of Experimental Medicine* 198, 111 (2003).

C L A I M S

1. A composition comprising a combination of at least two isolated transcription factors encoded by a sequence at least 90% identical to a sequence from a list consisting of selected from a list consisting of: BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14), and mixtures thereof,
as a reprogramming or inducing factor of a cell selected from a list consisting of: stem cell or a differentiated cell, or mixtures thereof,
into dendritic cell or antigen presenting cell.
2. The composition according to the previous claim comprising a combination of at least two isolated transcription factors
encoded by a sequence at least 95% identical to a sequence from a list consisting of selected from a list consisting of: BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), TCF4 (SEQ. ID. 13, SEQ. ID. 14), and mixtures thereof.
3. The composition according to any of the previous claim wherein the combination of isolated transcription factors is selected from the following encoded combinations:
BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);
or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6);
or TCF4 (SEQ. ID. 13, SEQ. ID. 14), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);
or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).
4. The composition according to any of the previous claims wherein the combination of isolated transcription factors is the encoded combination of BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).

5. The composition according to any of the previous claims wherein the encoded combination of isolated transcription factors is a combination of BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), and TCF4 (SEQ. ID. 13, SEQ. ID. 14).
6. The composition according to any of the previous claims, wherein the cell is selected from a list consisting of: pluripotent stem cell, multipotent stem cell, differentiated cell, tumour cell, cancer cell, and mixtures thereof.
7. The composition according to any of the previous claims, as a reprogramming or inducing factor of a cell selected from a list consisting of: pluripotent stem cell, or multipotent stem cell, differentiated cell, and mixtures thereof into dendritic cell.
8. The composition according to any the previous claims, as a reprogramming or inducing factor of a cell selected from a list consisting of: tumour cell, cancer cell, and mixtures thereof, into antigen presenting cell.
9. The composition according to any the previous claims, as reprogramming or inducing factors of antigen presenting cells, wherein the antigen is: a cancer antigen, a self-antigen, an allergen, an antigen from a pathogenic and/or infectious organism.
10. The composition according to any the previous claims, for use in veterinary or human medicine, in particular in immunotherapy, or in neurodegenerative or ageing diseases, or in cancer or in infectious diseases, or as a drug screening.
11. The composition according to any of the previous claims, wherein the pluripotent stem cell, multipotent stem cell or differentiated cell is a mammalian pluripotent stem cell, multipotent stem cell or differentiated cell, in particular a mouse or a human cell.
12. A construct or a vector encoding at least the combination of two isolated transcription factors described in any one of the claims 1-11, preferably the encoded combination of three transcription factors, more preferably four transcription factors.

13. The construct or the vector according to the previous claim wherein the combination of three isolated transcription factors is in the following sequential order from 5' to 3':

PU.1 (SEQ. ID. 7, SEQ. ID. 8), IRF8 (SEQ. ID. 5, SEQ. ID. 6), BATF3 (SEQ. ID. 1, SEQ. ID. 2); or
 IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), BATF3 (SEQ. ID. 1, SEQ. ID. 2).

14. The vector according to any of the claims 12-13 wherein the vector is a viral vector; in particular a retrovirus, a adenovirus, a lentivirus, a herpes virus, a pox virus, or adeno-associated virus vectors.

15. A method for reprogramming or inducing a stem cell or a differentiated cell into a dendritic cell or antigen presenting cell, comprising the following steps:

transducing a cell selected from a list consisting of: stem cell or a differentiated cell, and mixtures thereof,

with one or more vectors comprising at least two nucleic acid sequences encoding a sequence at least 90% identical, preferably at least 95% identical, to a sequence from a list consisting of SEQ. ID. 1, SEQ. ID.2, SEQ. ID. 5, SEQ. ID. 6, SEQ. ID. 7, SEQ. ID. 8, SEQ. ID. 13, SEQ. ID. 14, and mixtures thereof;

culturing the transduced cell in a cell media that supports growth of dendritic cells or antigen presenting cells.

16. The method according to the previous claim, wherein the sequence is selected from the group consisting of:

BATF3 (SEQ. ID. 1, SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6);
 or BATF3 (SEQ. ID. 1, SEQ. ID. 2) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);
 or IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);
 or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and BATF3 (SEQ. ID. 1, SEQ. ID. 2);
 or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and IRF8 (SEQ. ID. 5, SEQ. ID. 6);
 or TCF4 (SEQ. ID. 13, SEQ. ID. 14) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);

or BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);

or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6);

or TCF4 (SEQ. ID. 13, SEQ. ID. 14), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);

or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).

17. The method according to the previous claim, wherein the sequence is selected from the group from the following combinations:

BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);

or TCF4 (SEQ. ID. 13, SEQ. ID. 14) BATF3 (SEQ. ID. 1, SEQ. ID. 2) and IRF8 (SEQ. ID. 5, SEQ. ID. 6);

or TCF4 (SEQ. ID. 13, SEQ. ID. 14), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8);

or TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).

18. The method according to the previous claim, wherein the sequence is selected from the group is a combination of BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8), preferably TCF4 (SEQ. ID. 13, SEQ. ID. 14), BATF3 (SEQ. ID. 1, SEQ. ID. 2), IRF8 (SEQ. ID. 5, SEQ. ID. 6) and PU.1 (SEQ. ID. 7, SEQ. ID. 8).

19. The method according to the previous claim, wherein the combination of three isolated transcription factors is in the following sequential order from 5' to 3':

PU.1 (SEQ. ID. 7, SEQ. ID. 8), IRF8 (SEQ. ID. 5, SEQ. ID. 6), BATF3 (SEQ. ID. 1, SEQ. ID. 2); or

IRF8 (SEQ. ID. 5, SEQ. ID. 6), PU.1 (SEQ. ID. 7, SEQ. ID. 8), BATF3 (SEQ. ID. 1, SEQ. ID. 2).

20. The method according to the previous claims 15-19, culturing the cell transduced with a plurality of isolated transcription factors during at least 2 days, preferably at least 5 days, more preferably at least 8 days, even more preferably at least 9 days.

21. The method according to the previous claims 15-20, wherein transducing step further comprises at least one vector selected from a list consisting of: a nucleic acid sequence encoding a nucleic acid sequence encoding IL12; nucleic acid sequence encoding GM-CSF; nucleic acid sequence encoding IL-7; nucleic acid sequence encoding siRNA targeting IL-10 RNA, and mixtures thereof.
22. The method according to the previous claims 15-21, wherein transducing step further comprises at least one vector comprising nucleic acids encoding immunostimulatory cytokines.
23. The method according to the previous claims 15-22 wherein the cell is selected from a list consisting of: of pluripotent stem cell, or multipotent stem cell, differentiated cell, and mixtures thereof.
24. The method according to the previous claims 15-23, wherein the cell is a mammalian cell.
25. The method according to the previous claims 15-24, wherein the pluripotent stem cell, multipotent stem cell, or differentiated cell, is selected from the group consisting of: an endoderm derived cell, a mesoderm derived cell, or an ectoderm derived cell, a multipotent stem cell including mesenchymal stem cell, a hematopoietic stem cell, an intestinal stem cell, a pluripotent stem cell and a cell line.
26. The method according to any of the previous claims 15-25, wherein the cell is a non-human cell.
27. The method according to any of the previous claims 15-26, wherein the cell is a mouse cell.
28. The method according to any of the previous claims 15-27, wherein the cell is a human cell.
29. The method according to any of the previous claims 15-28, wherein the cell is a human or mouse fibroblast, or a mammalian umbilical cord blood stem cell.
30. An induced dendritic cell obtained by the method described in any of the claims 15-29.

31. The induced dendritic according to the previous claim, wherein said cell is intrinsically more mature than splenic DCs - natural DCs - and/or is less dependent on exogenous activation stimuli for antigen presentation.
32. An induced antigen presenting cell obtained by the method described in any of the claims 15-29.
33. The induced antigen presenting cell of the previous claim wherein the antigen is: a cancer antigen, a self-antigen, an allergen, an antigen from a pathogenic and/or infectious organism.
34. A composition comprising an induced dendritic cell as described in claims 30-31, or an induce antigen presenting cell as described in claim 32-33, or mixtures thereof, in a therapeutically effective amount and a pharmaceutically acceptable excipient.
35. The composition according to any of the previous claims 1-11, 34, for use in veterinary or human medicine.
36. The composition according to any of the claims 34-35, for use in immunotherapy, or in the treatment or therapy of neurodegenerative diseases, or in the treatment or therapy of cancer or in the treatment or therapy of an infectious diseases.
37. The composition according to any of the claims 34-36 further comprising an anti-viral, an analgesic, an anti-inflammatory agent, a chemotherapy agent, a radiotherapy agent, an antibiotic, a diuretic, or mixtures thereof.
38. The composition according to any of the previous claims 34-37 further comprising a filler, a binder, a disintegrant, or a lubricant, or mixtures thereof.
39. The composition according to any of the previous claims 34-38 for use in intradermal and transdermal therapies.
40. The composition according to any of the previous claims 34-39 wherein the composition is an injectable formulation, in particular an in situ injection.
41. The composition according to any of the previous claims 34-40 for use in veterinary or human medicine, in particular in immunotherapy, or in neurodegenerative or ageing diseases, or in cancer or in infectious diseases, as a drug screening.

42. The composition according to any of the previous claims 34-41, for use in the treatment, therapy or diagnostic of a central and peripheral nervous system disorder.
43. The composition according to any of the previous claims 34-42, for use in the treatment therapy or diagnostic of neoplasia in particular cancer, namely solid or hematological tumours.
44. The composition according to any of the previous claims 34-43 for use in the treatment, diagnostic or therapy of benign tumour, malignant tumour, early cancer, basal cell carcinoma, cervical dysplasia, soft tissue sarcoma, germ cell tumour, retinoblastoma, age-related macular degeneration, Hodgkin's lymphoma, blood cancer, prostate cancer, ovarian cancer, cervix cancer, uterus cancer, vaginal cancer, breast cancer, naso-pharynx cancer, trachea cancer, larynx cancer, bronchi cancer, bronchioles cancer, lung cancer, hollow organs cancer, esophagus cancer, stomach cancer, bile duct cancer, intestine cancer, colon cancer, colorectum cancer, rectum cancer, bladder cancer, ureter cancer, kidney cancer, liver cancer, gall bladder cancer, spleen cancer, brain cancer, lymphatic system cancer, bone cancer, pancreatic cancer, leukaemia, skin cancer, or myeloma.
45. The composition according to any of the previous claims 34-44, for use in the treatment, therapy or diagnostic of a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease.
46. The composition according to any of the previous claims 34-45, for use in the treatment, therapy or diagnostic of HIV, infection with SARS coronavirus, Asian flu virus, herpes simplex, herpes zoster, hepatitis, or viral hepatitis.
47. The composition according to any of the previous claims 34-46, for use in the treatment, therapy or diagnostic of an amyloid disease in particular Amyloid A amyloidosis, Alzheimer's disease, Light-Chain (AL) amyloidosis, Type-2 Diabetes, Medullary Carcinoma of the Thyroid, Parkinson's disease, Polyneuropathy, or Spongiform Encephalopathy - Creutzfeldt Jakob disease.
48. A vaccine for cancer comprising the composition as described in any one of the claims 1-11, 34-47, or an induced dendritic cell as described in claims 30-31, or an induce antigen presenting cell as described in claim 32-33, or mixtures thereof.

49. A kit comprising at least one of the following components:

- an induced dendritic cell as described in claims 30-31;
- an induce antigen presenting cell as described in claim 32-33,
- a composition as described in any of the previous claims 1-11, 34-47;
- a vector or a construct as described in claims 12-14; or mixtures thereof.

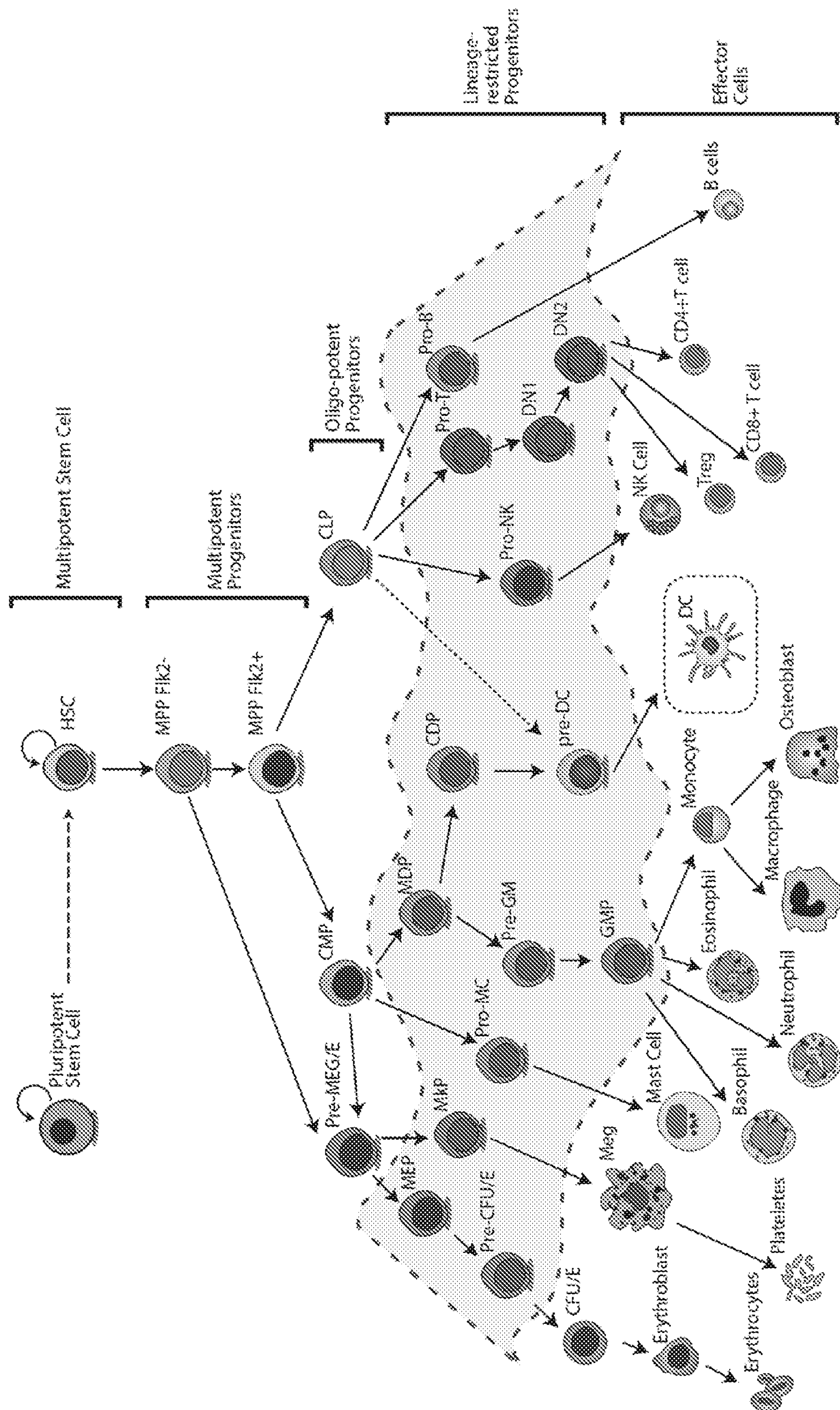


Fig. 1

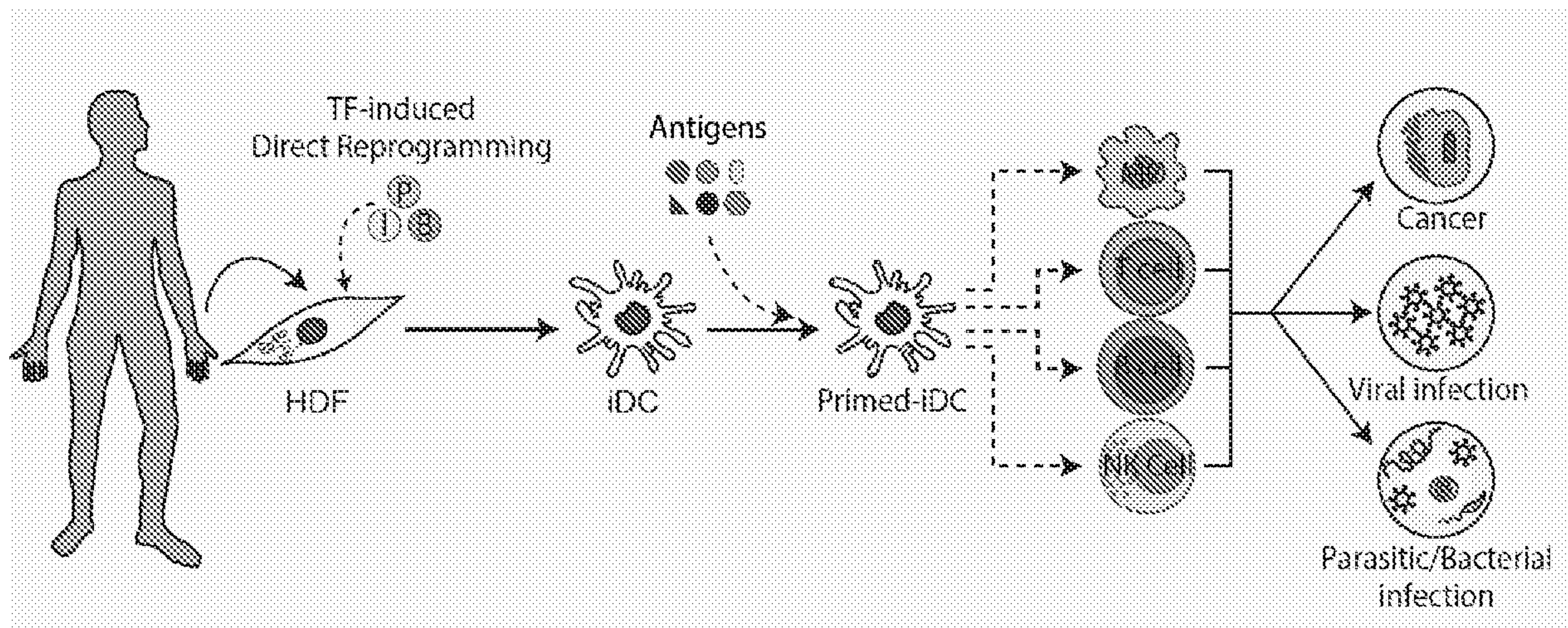


Fig. 2

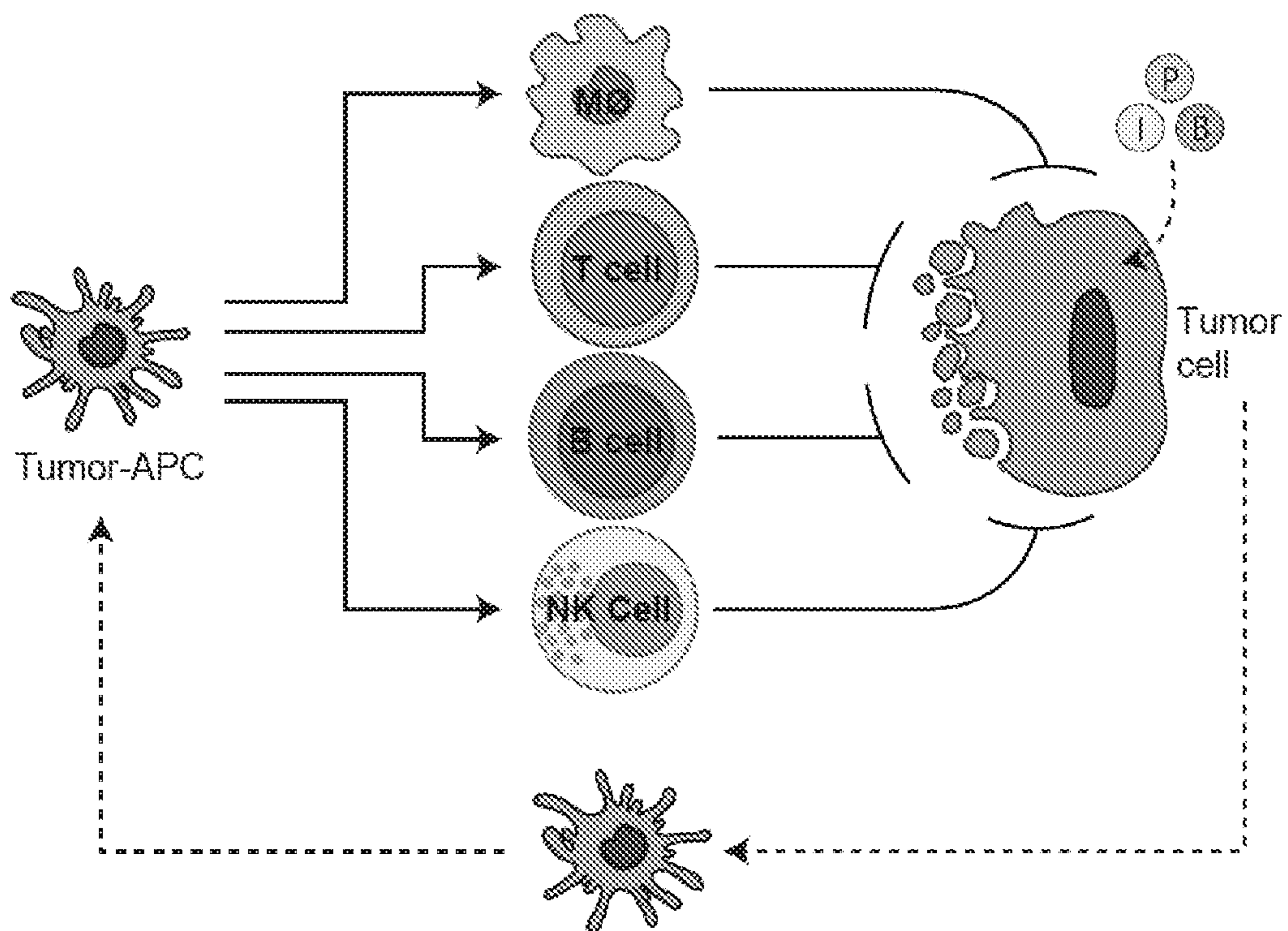


Fig. 3

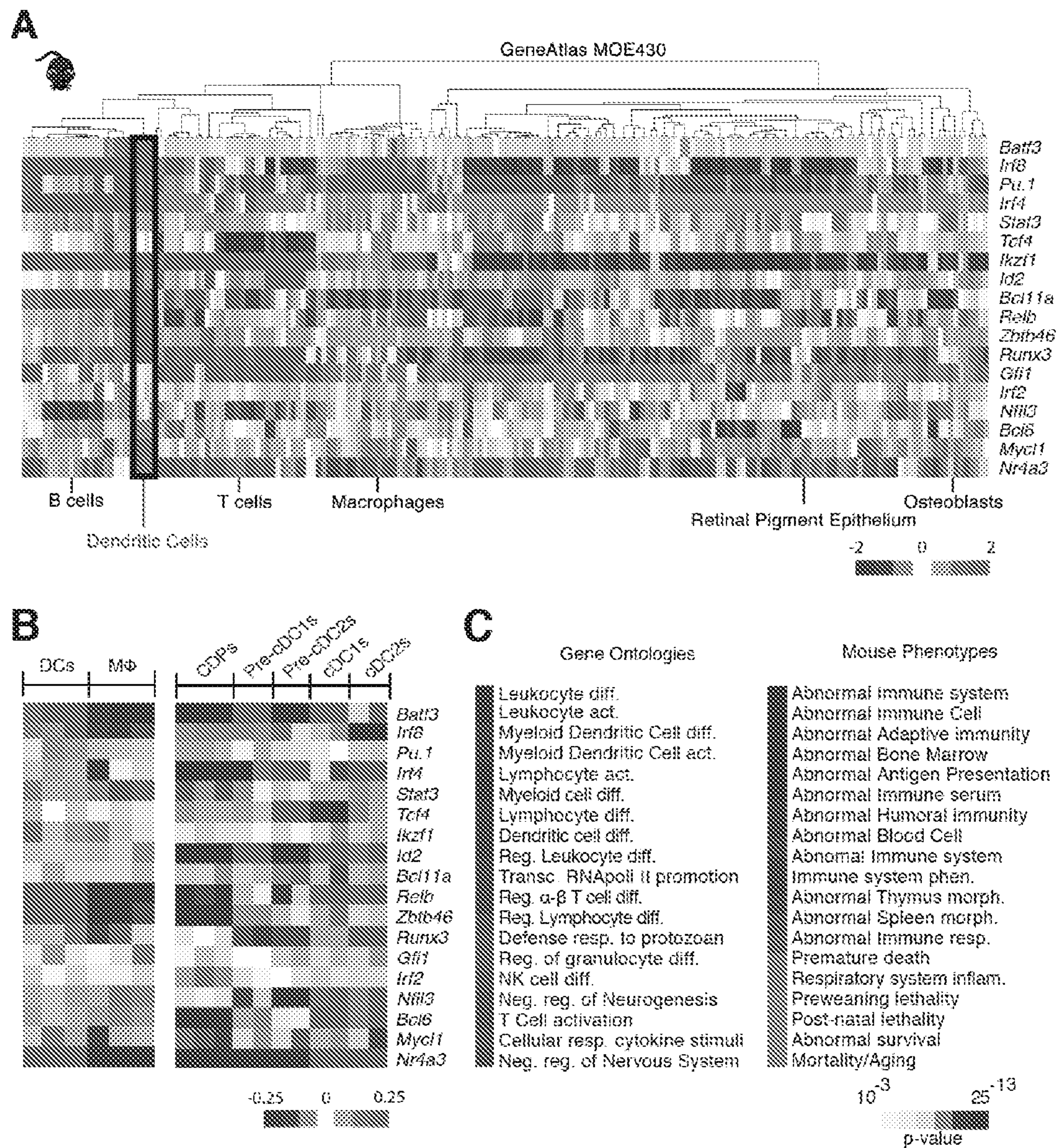


Fig. 4

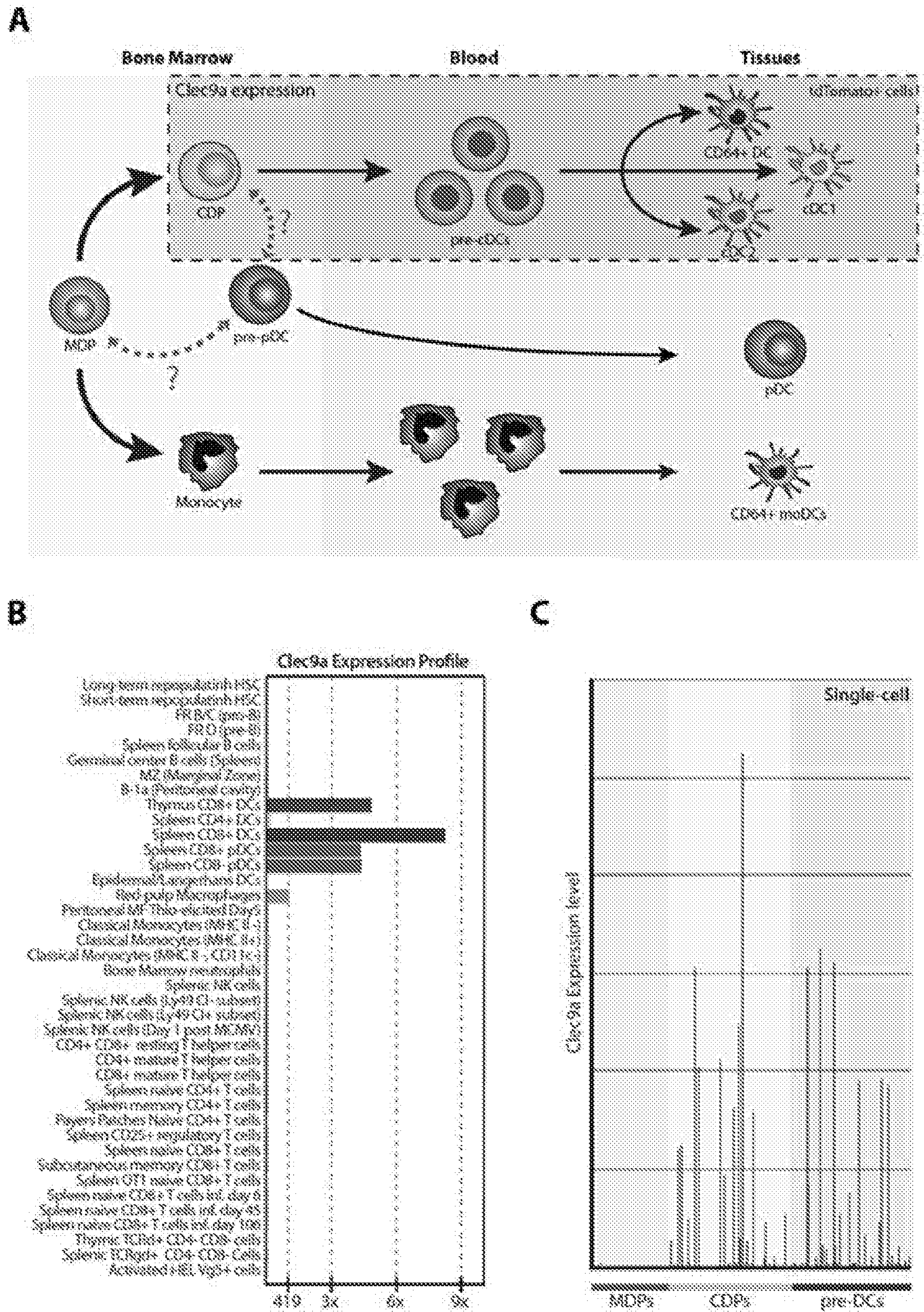


Fig. 5

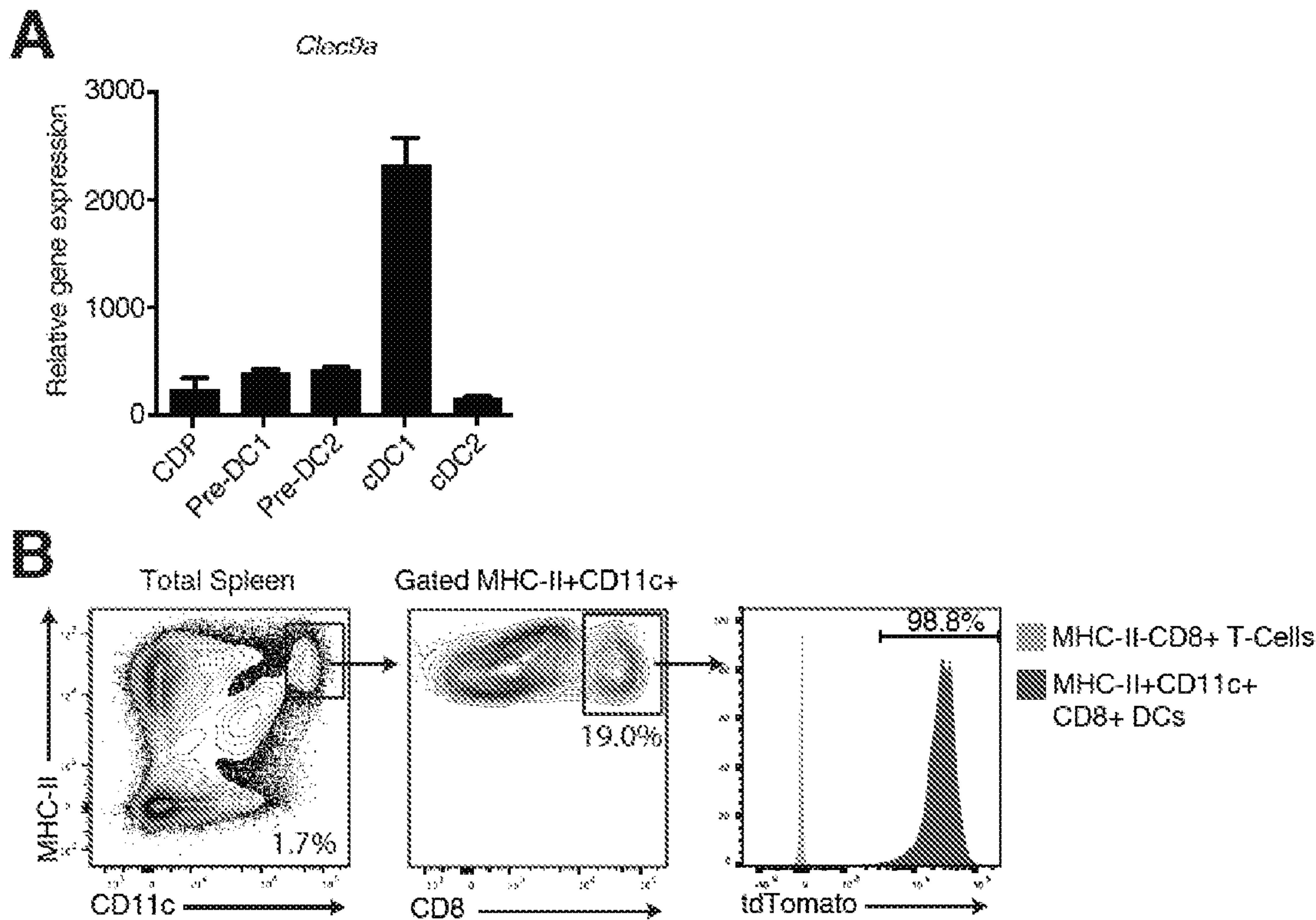


Fig. 6

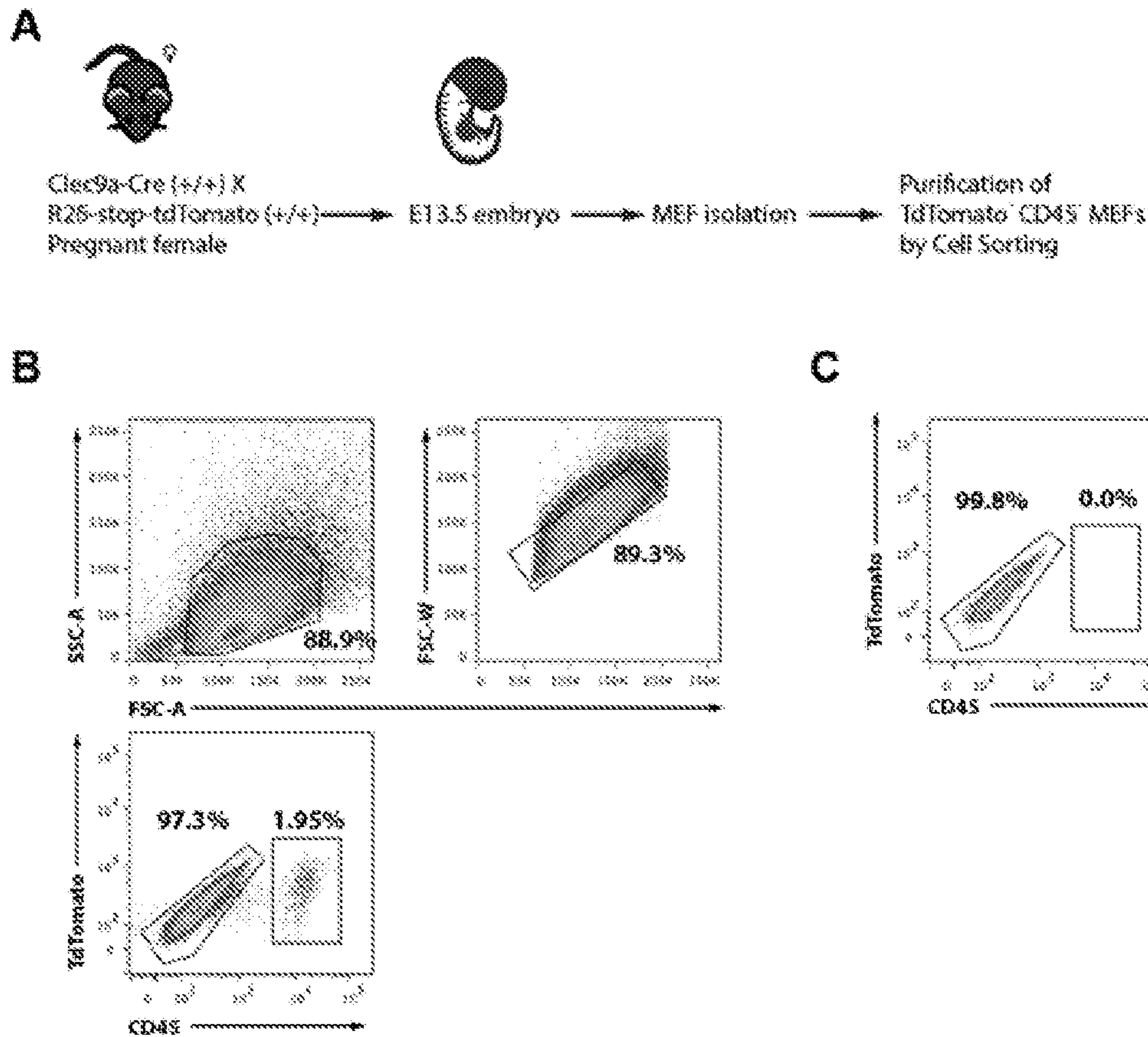


Fig. 7

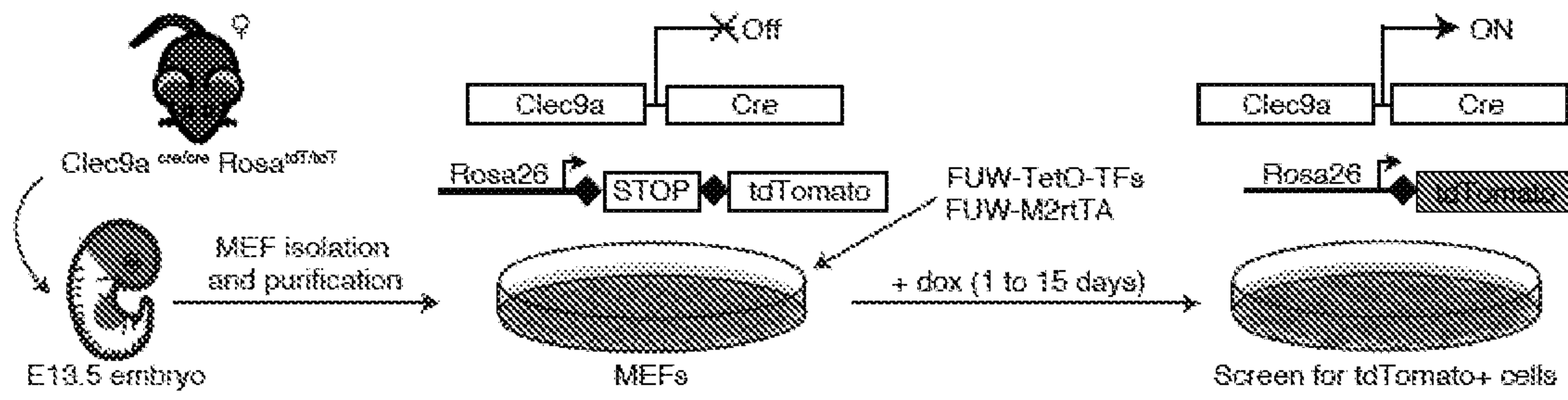


Fig. 8

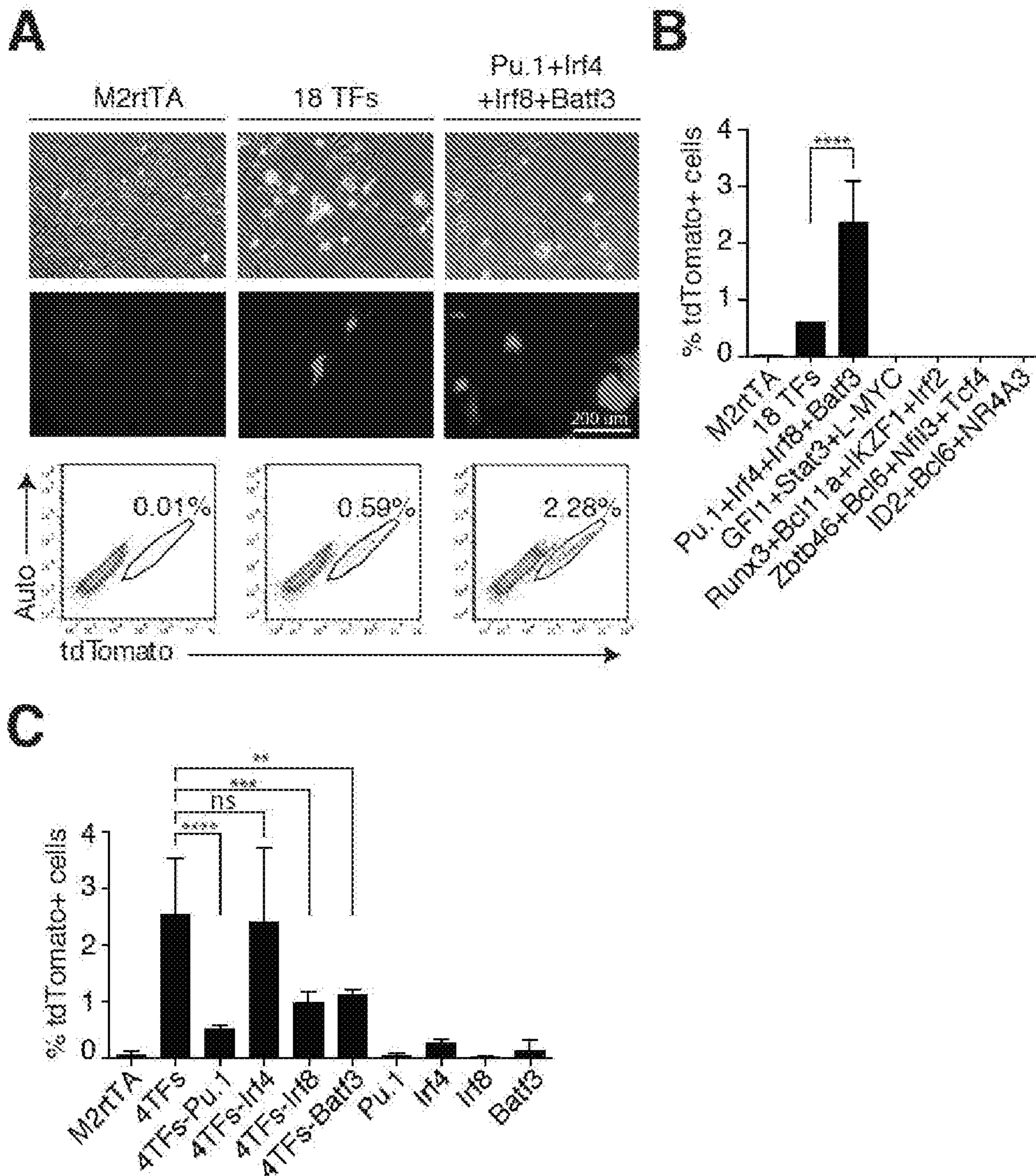


Fig. 9

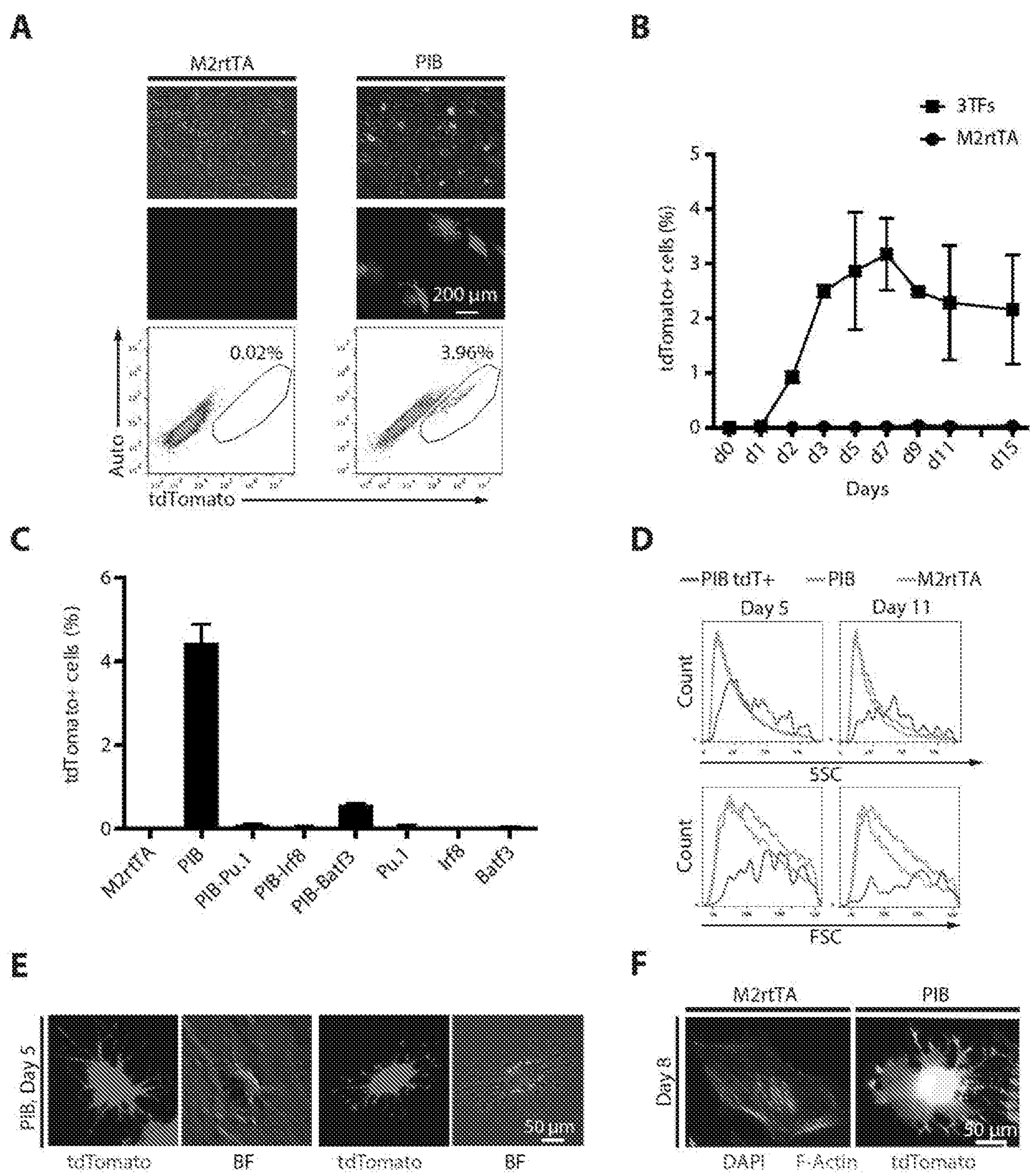


Fig. 10

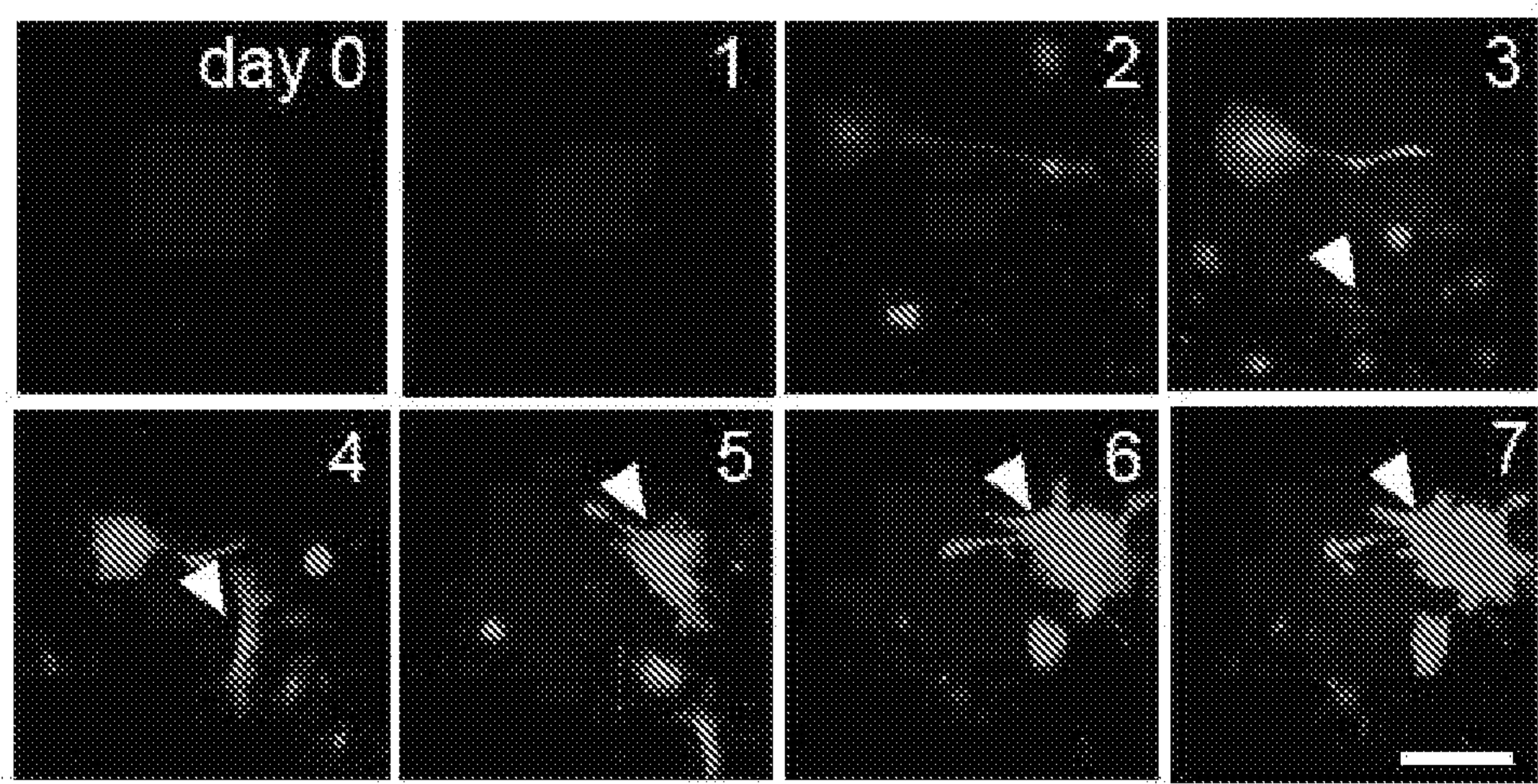


Fig. 11

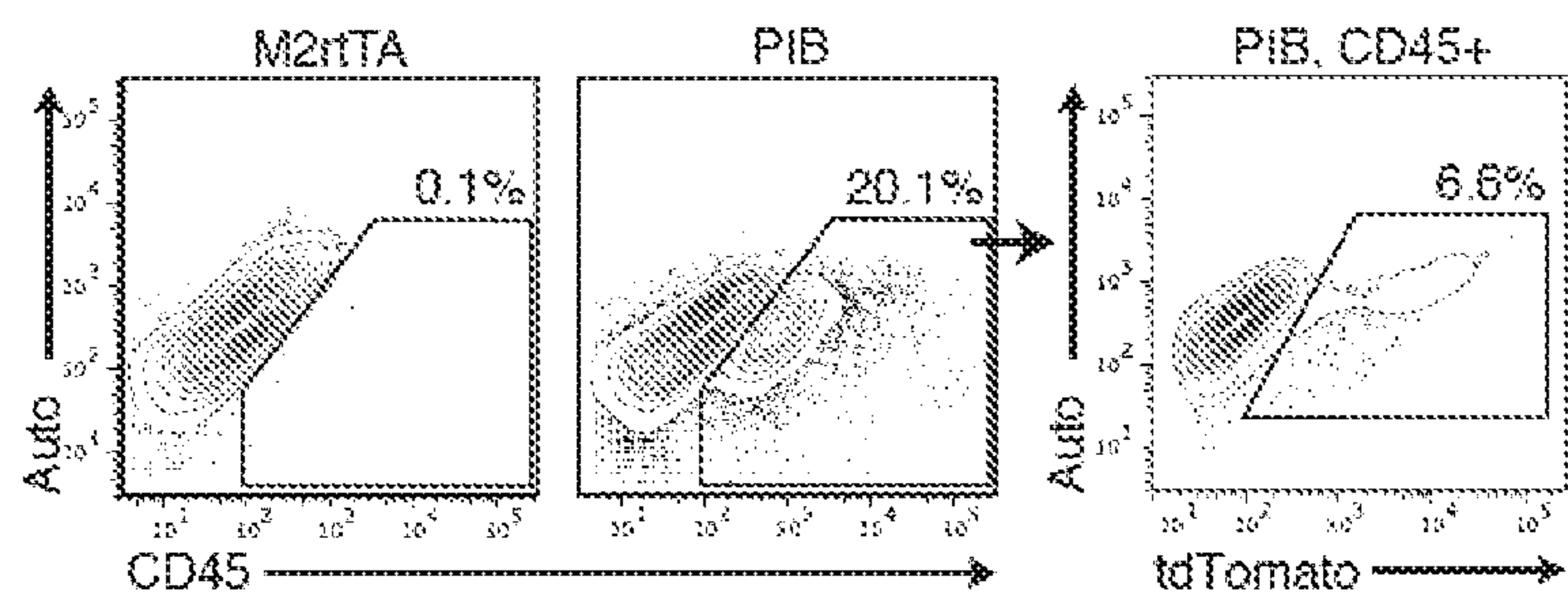


Fig. 12

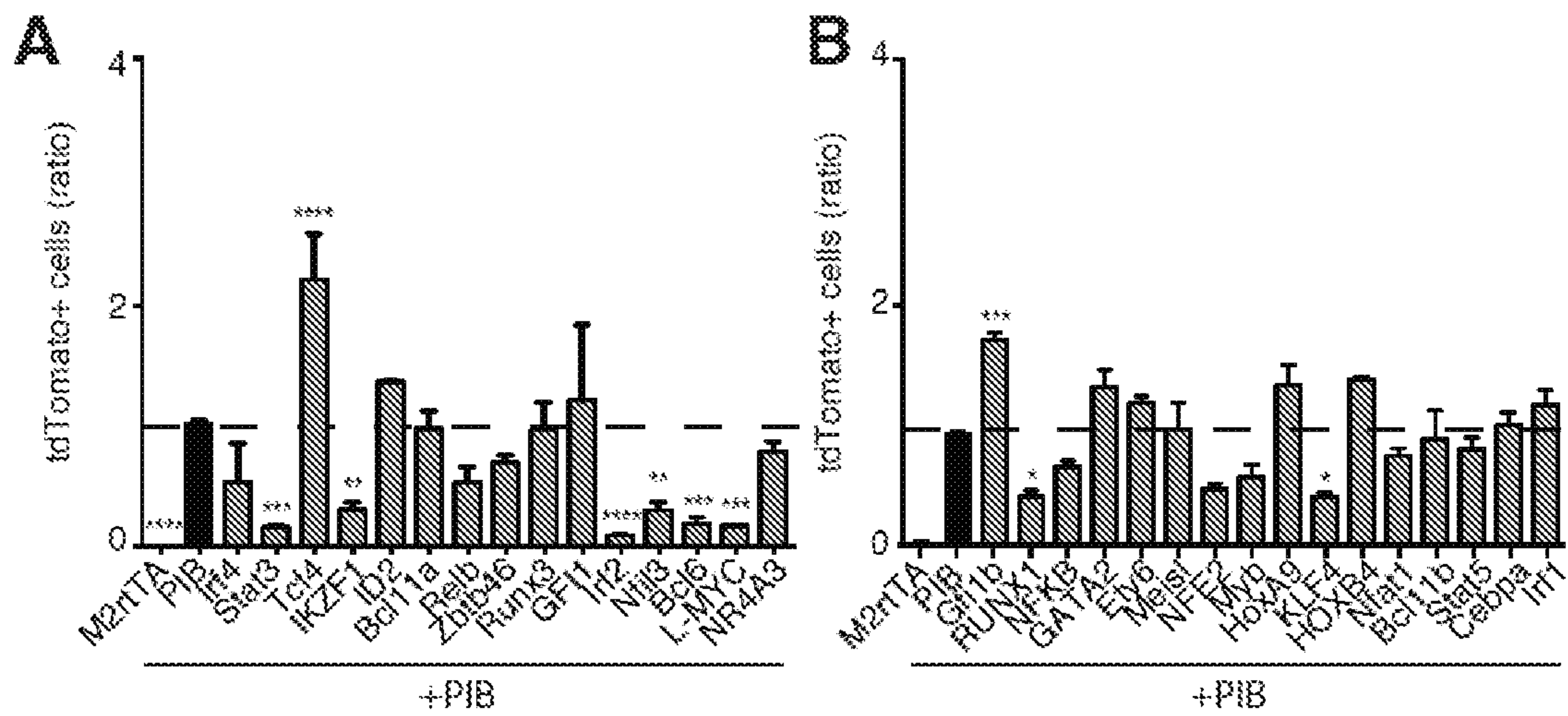


Fig. 13

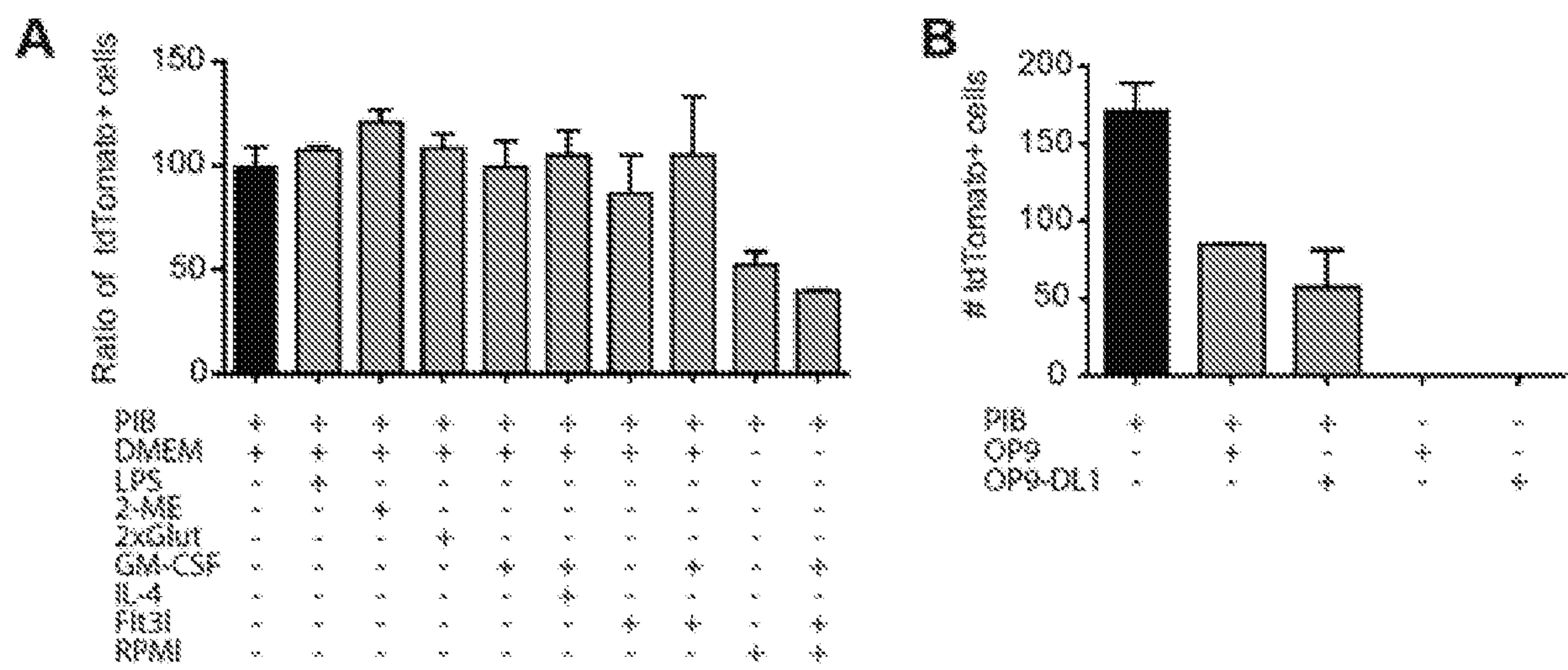


Fig. 14

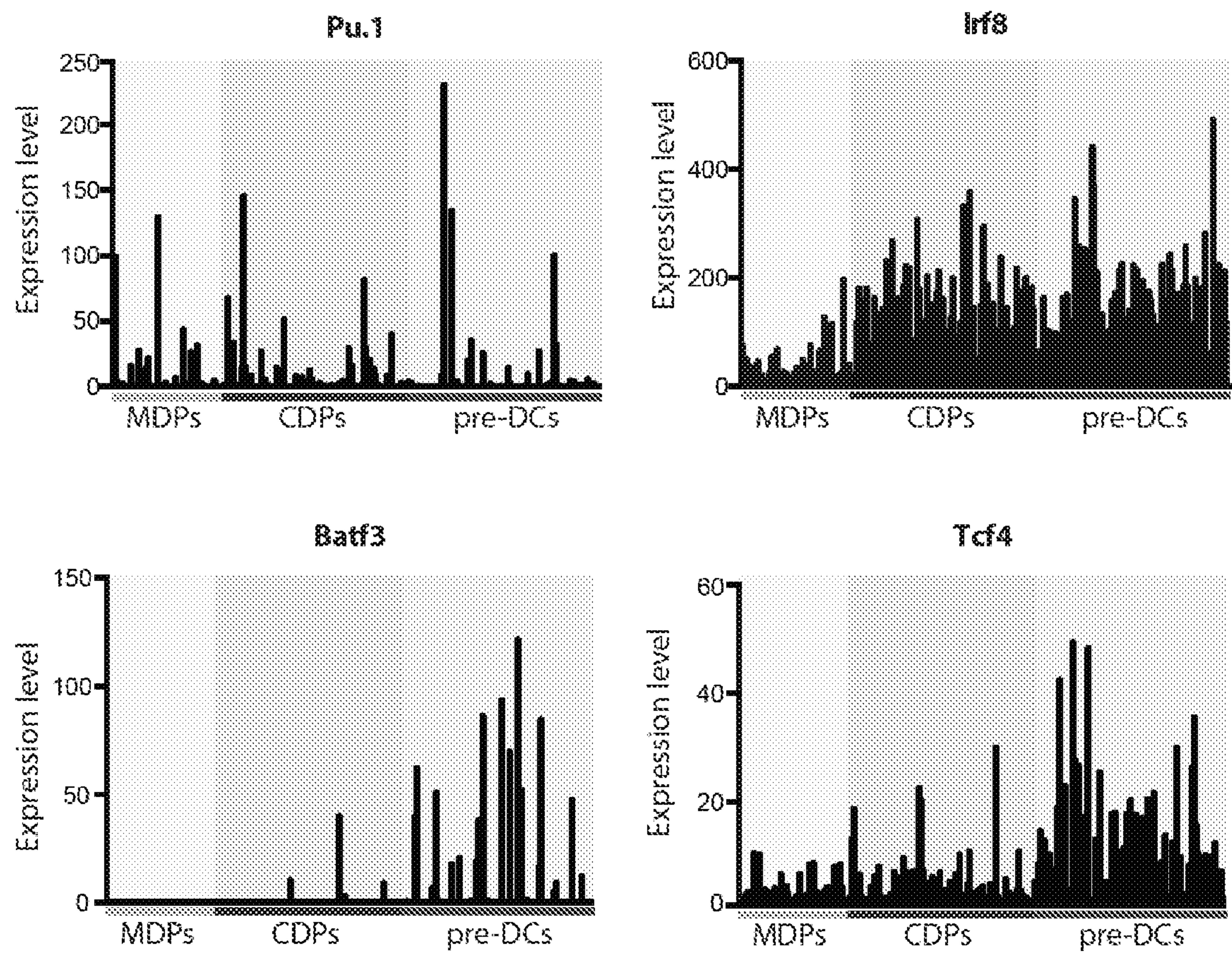


Fig. 15

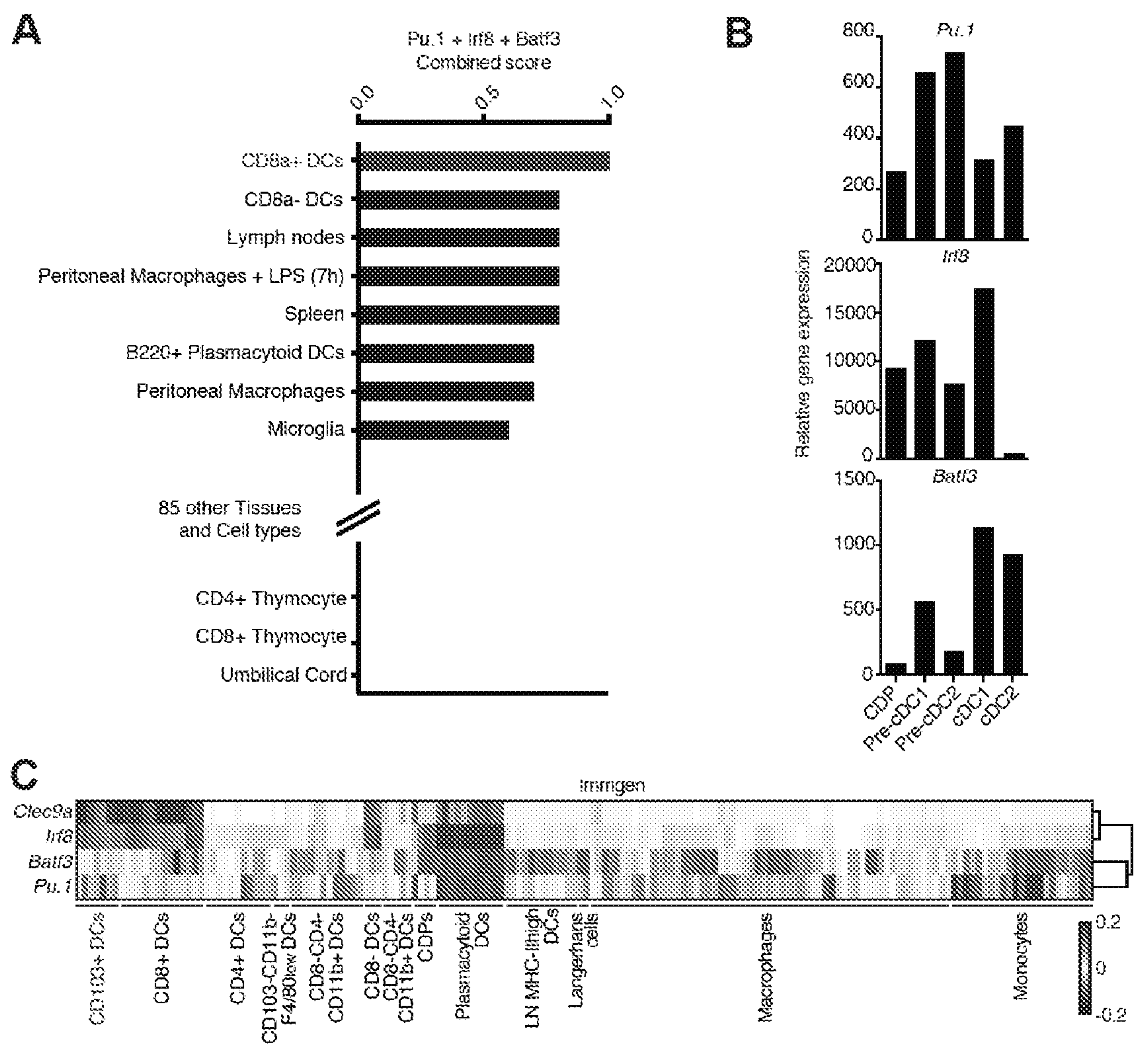


Fig. 16

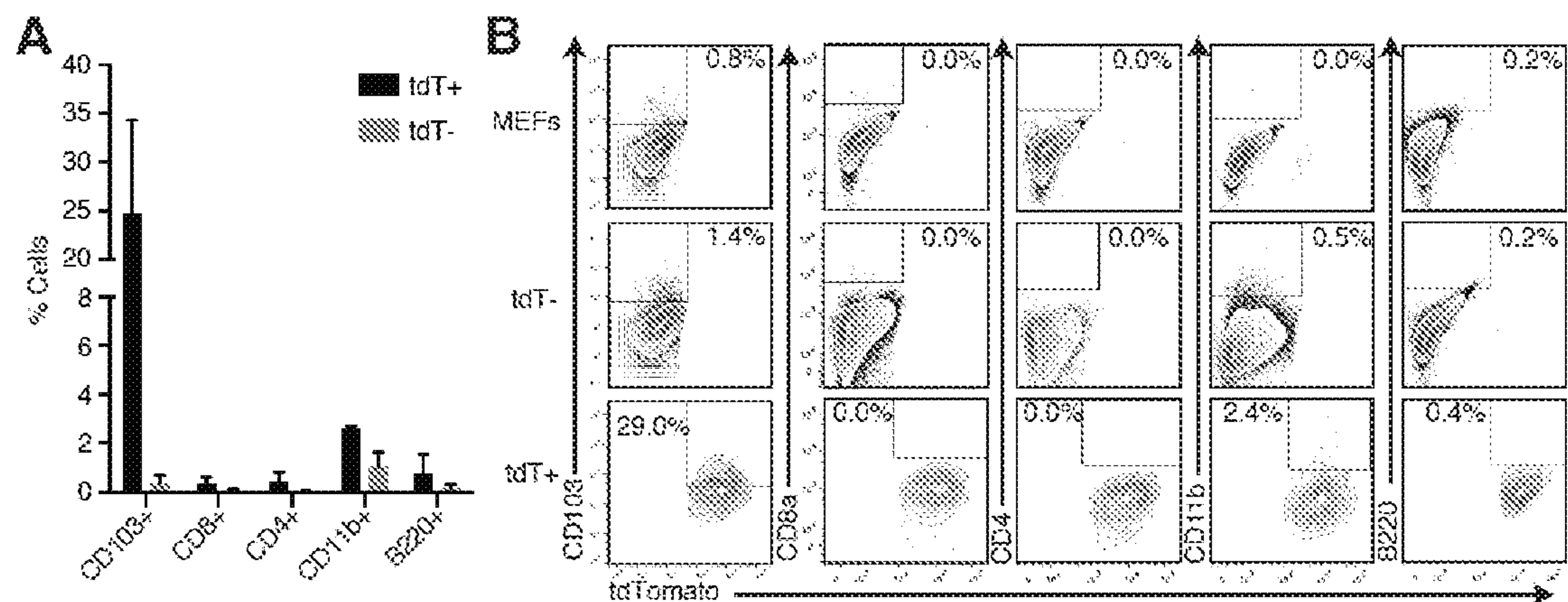


Fig. 17

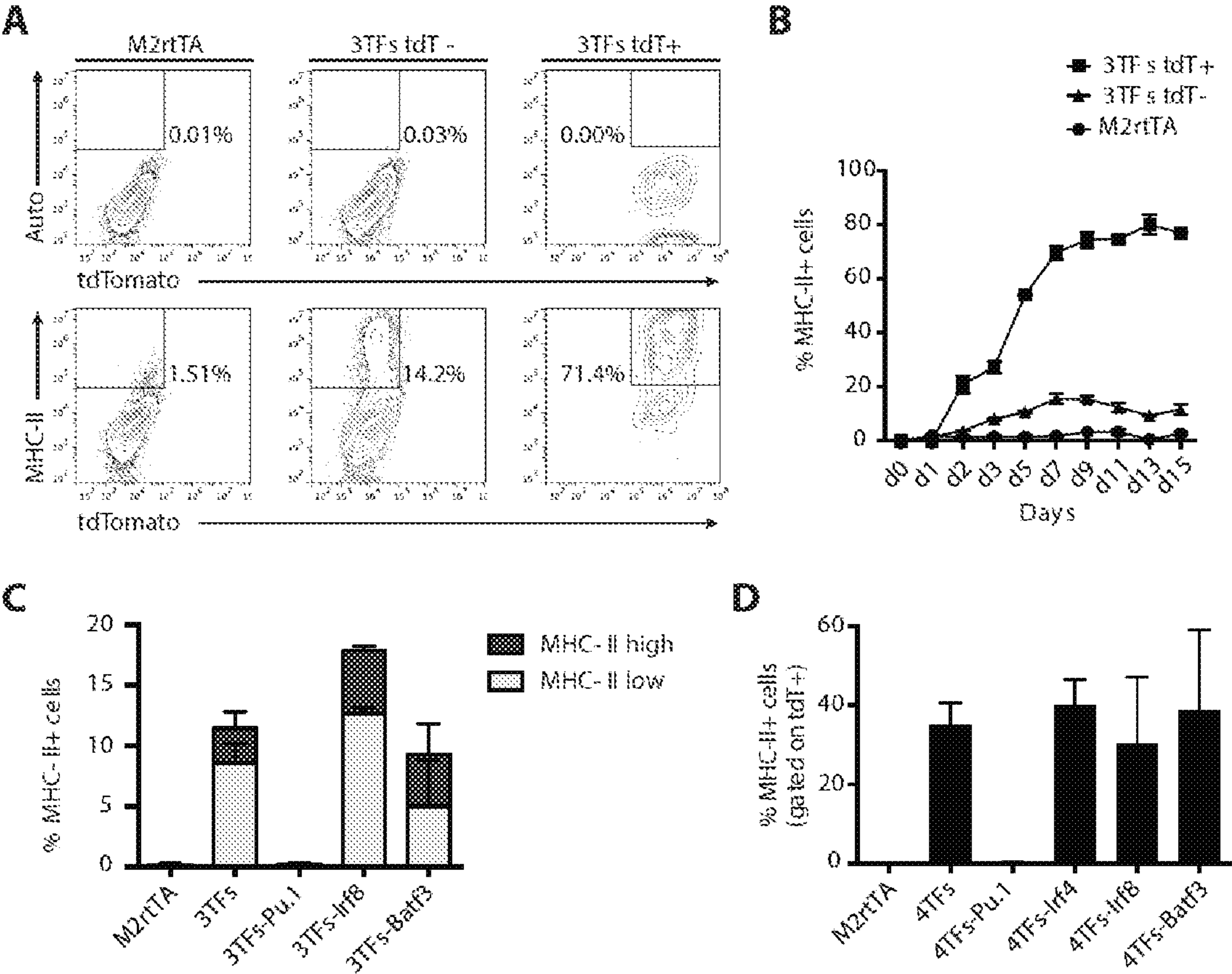
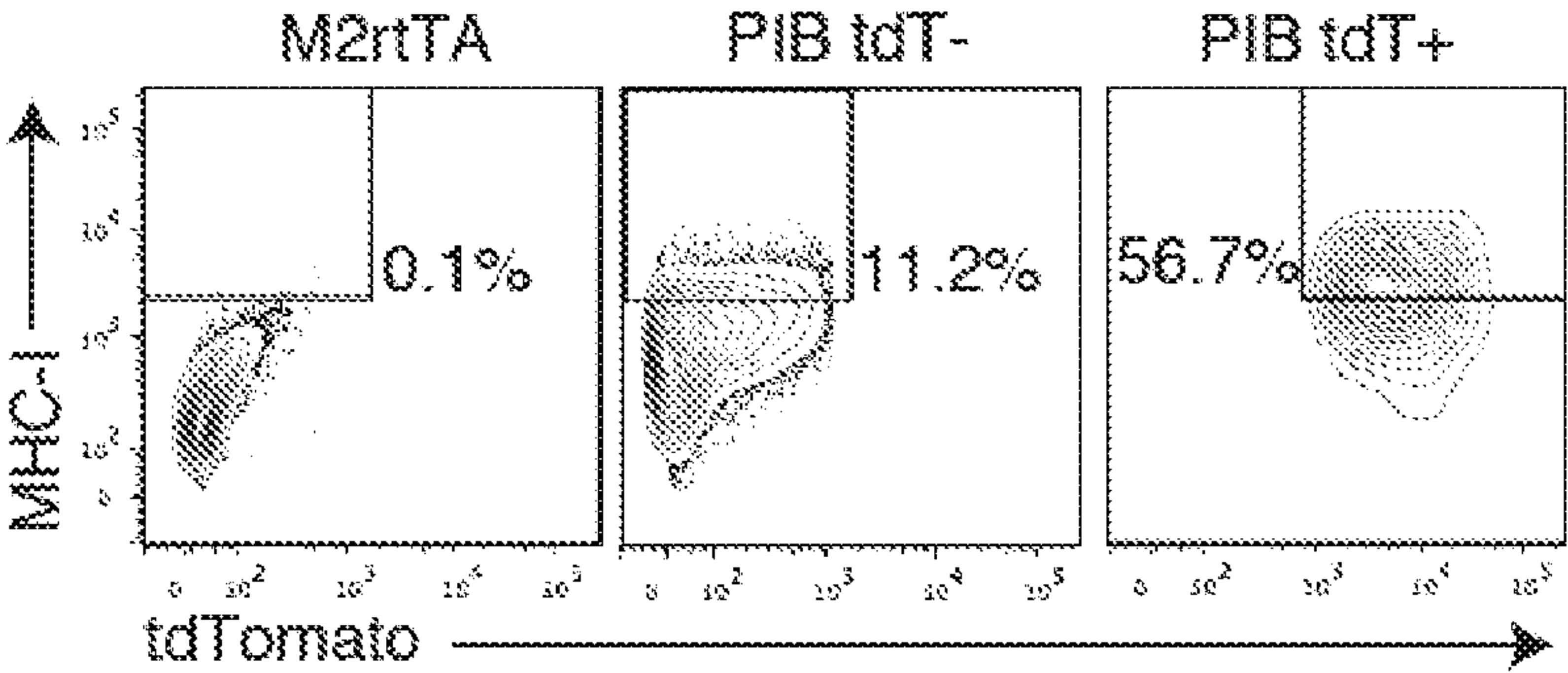


Fig. 18



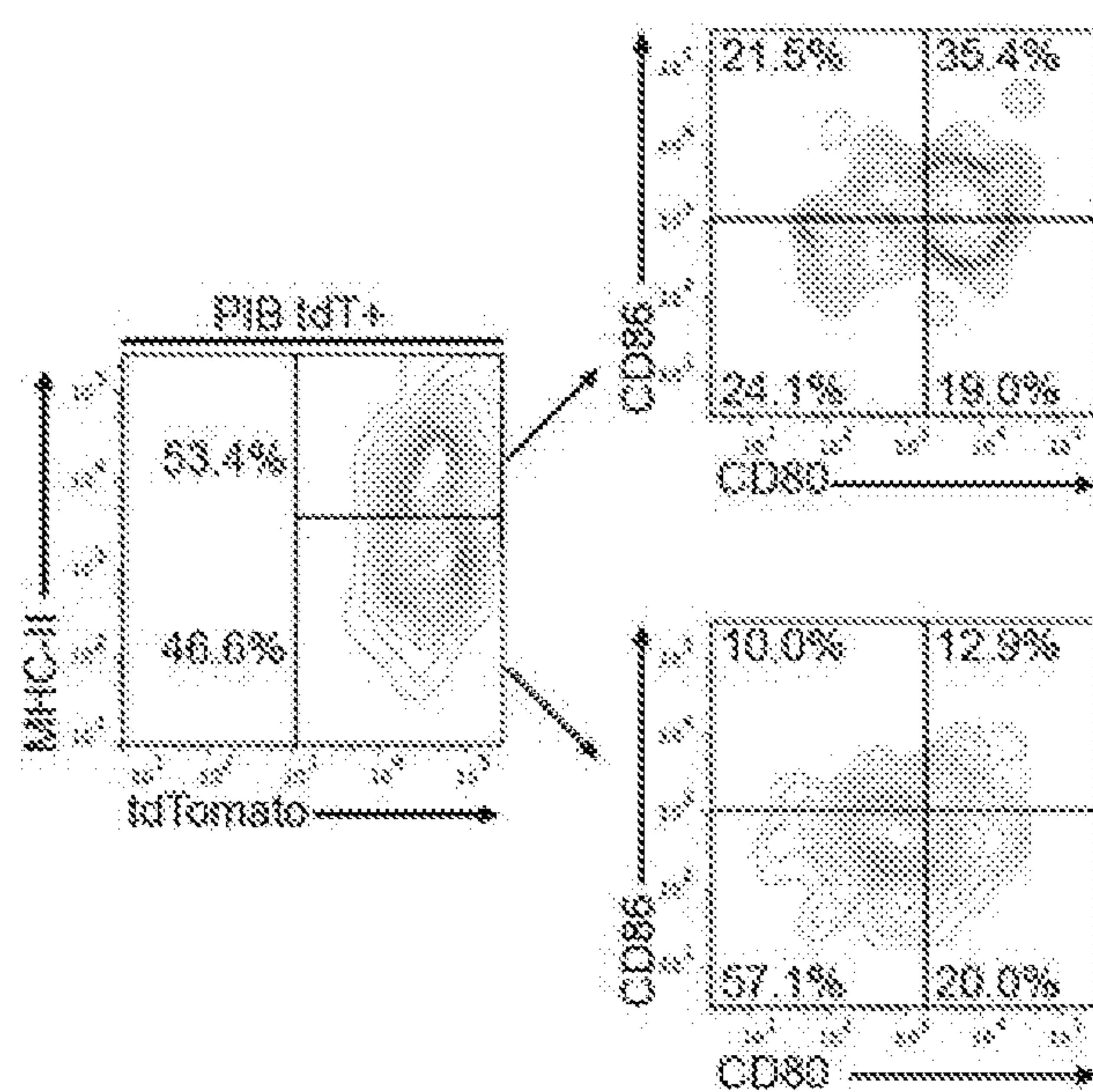


Fig. 20

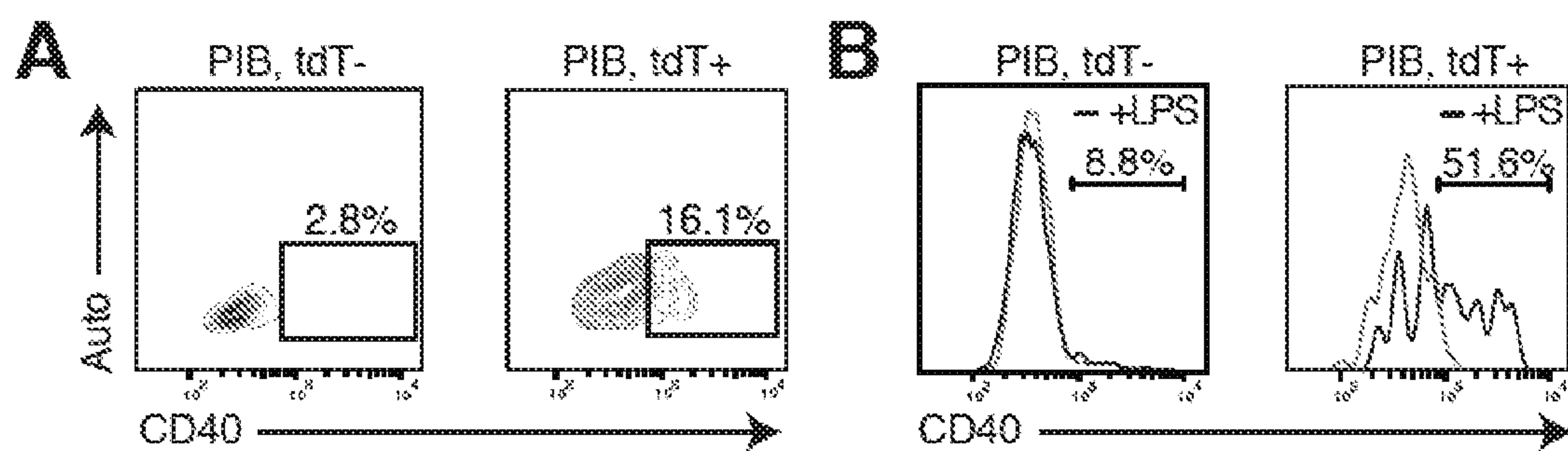


Fig. 21

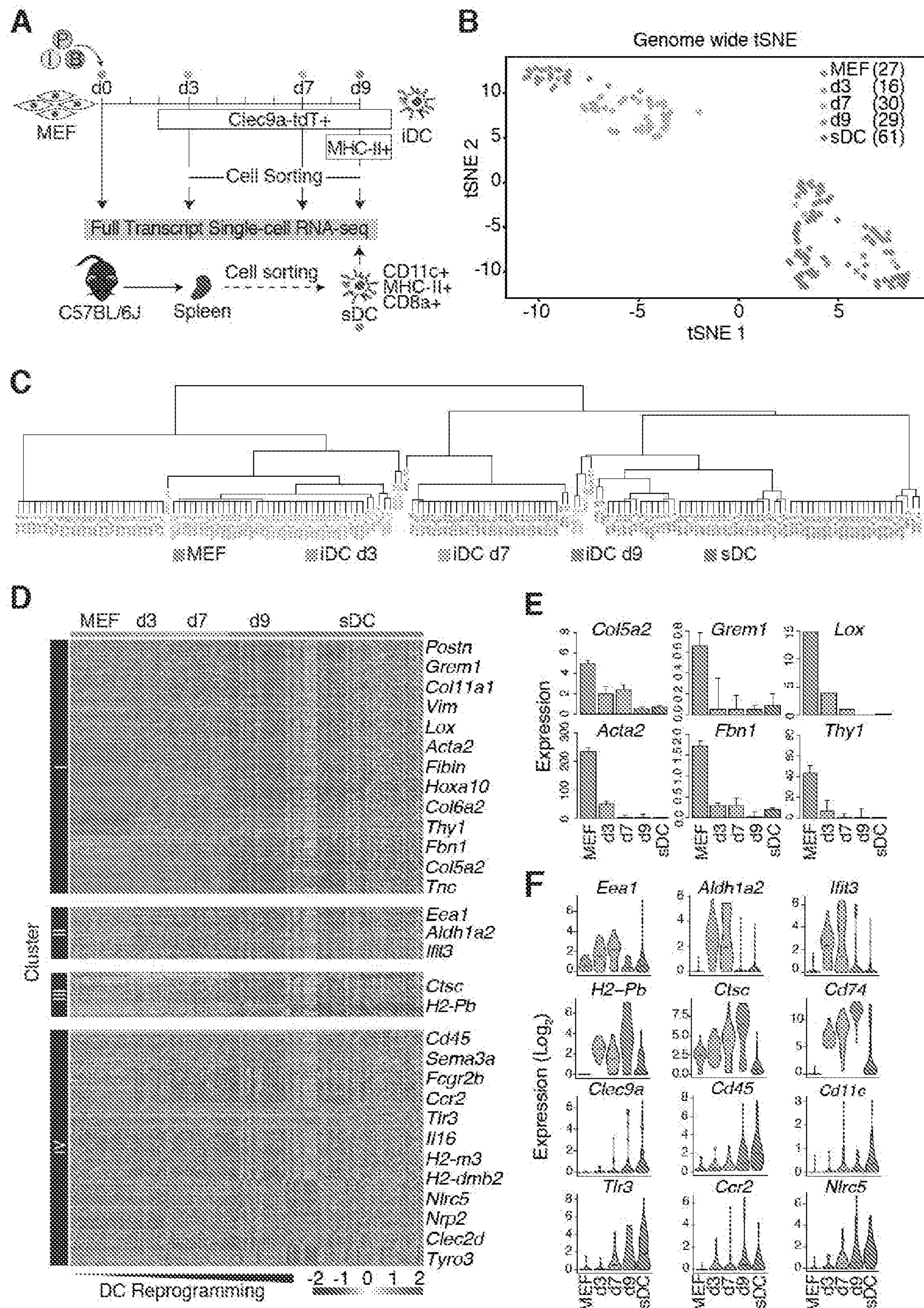


Fig. 22

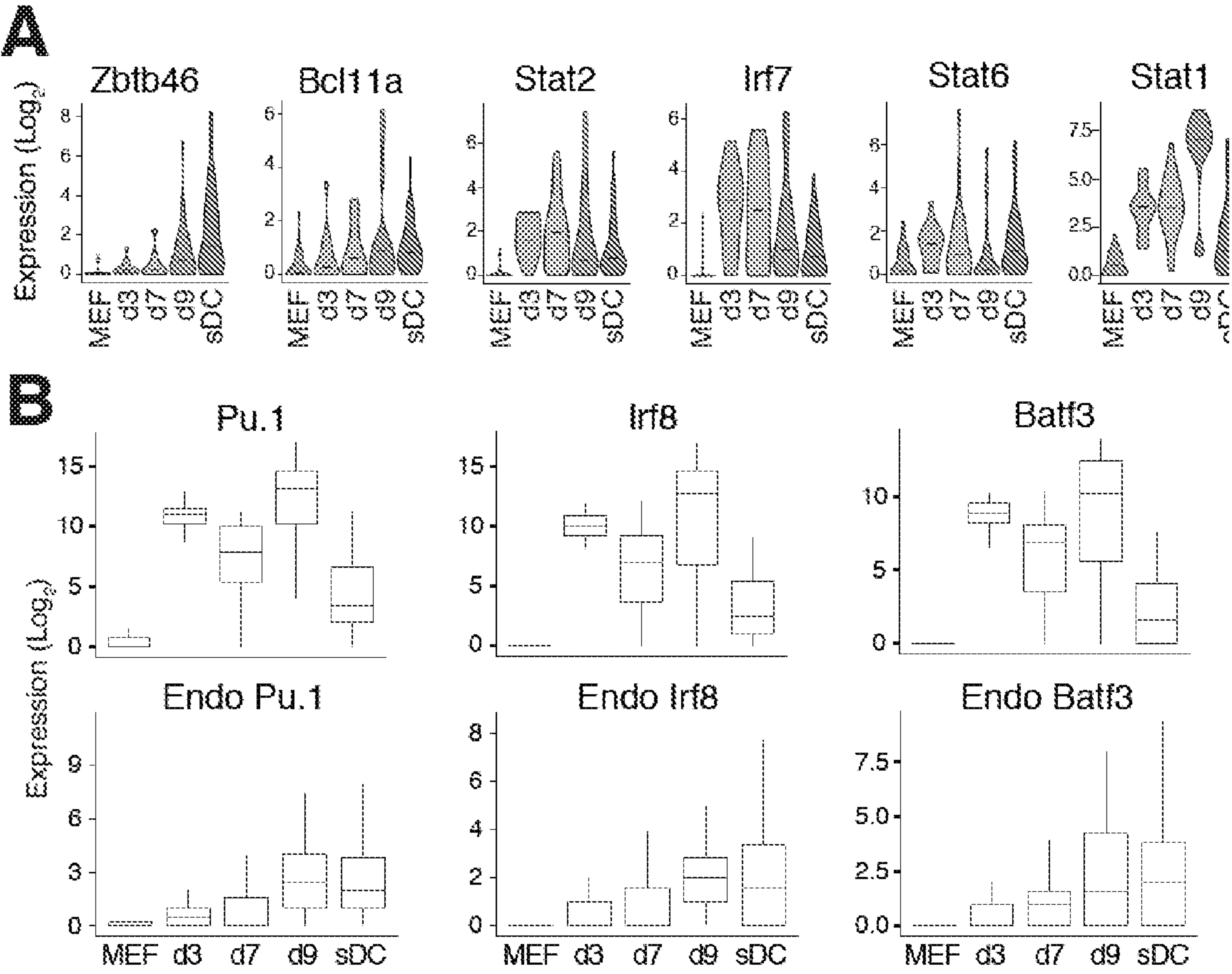


Fig. 23

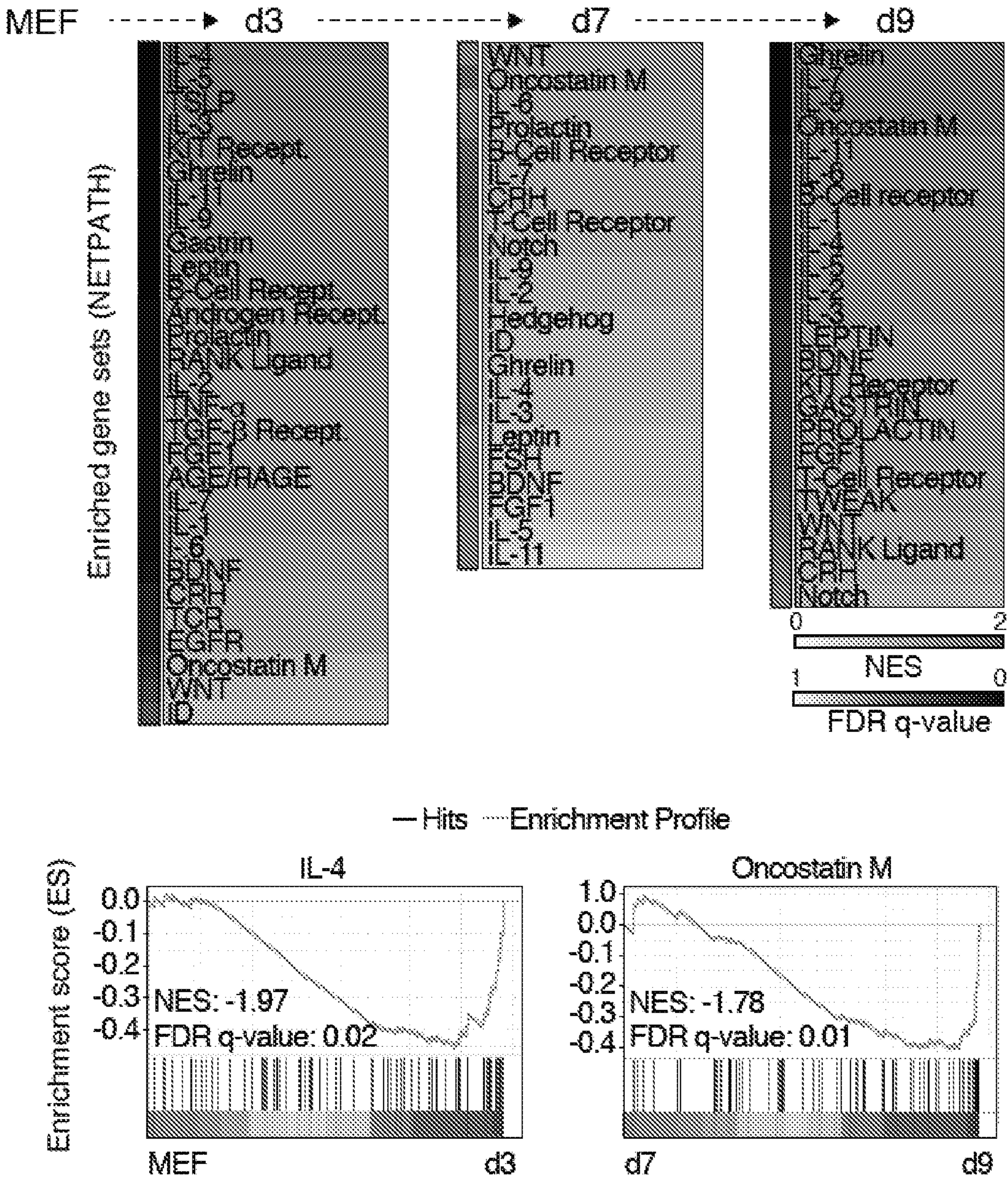


Fig. 24

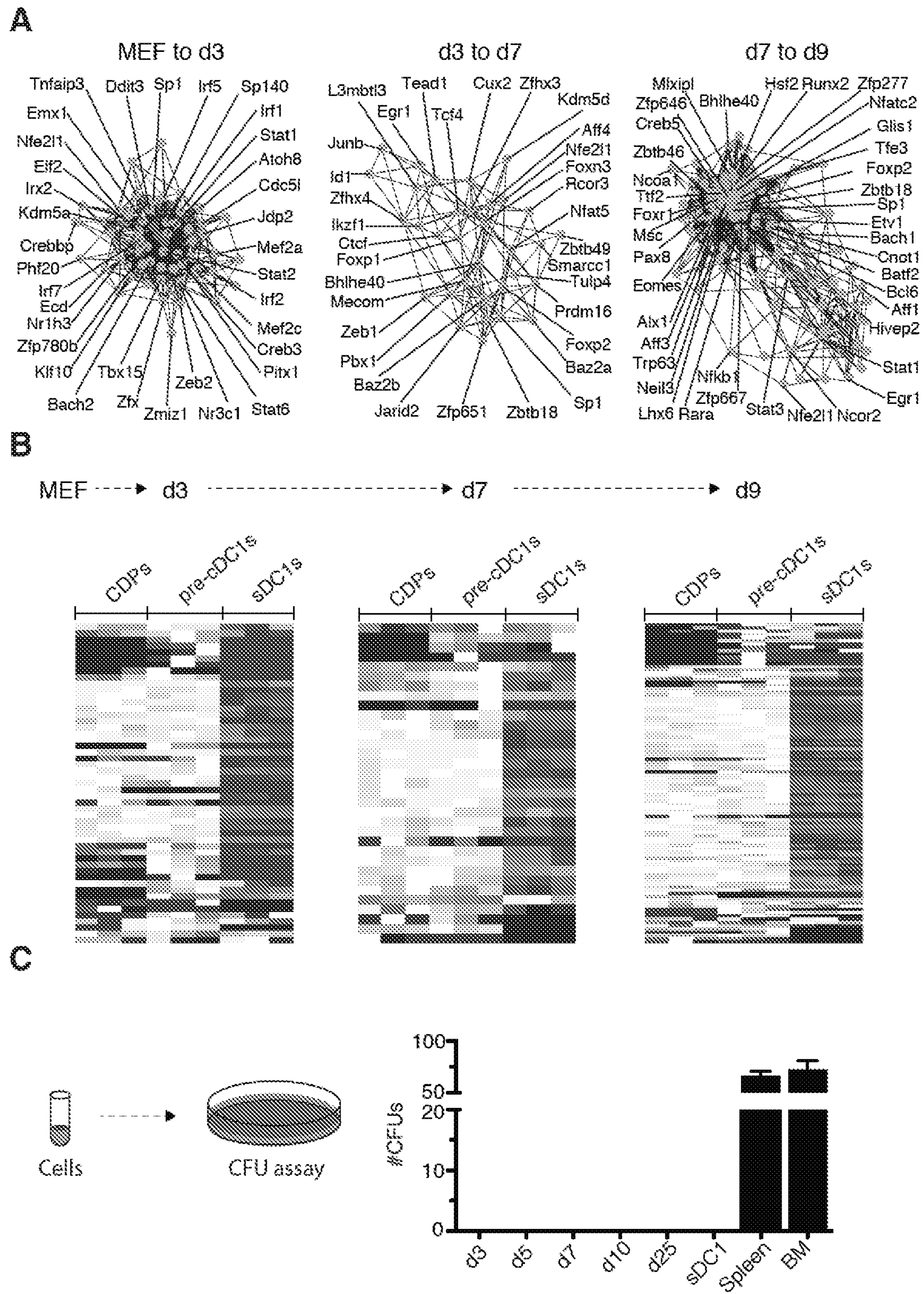


Fig. 25

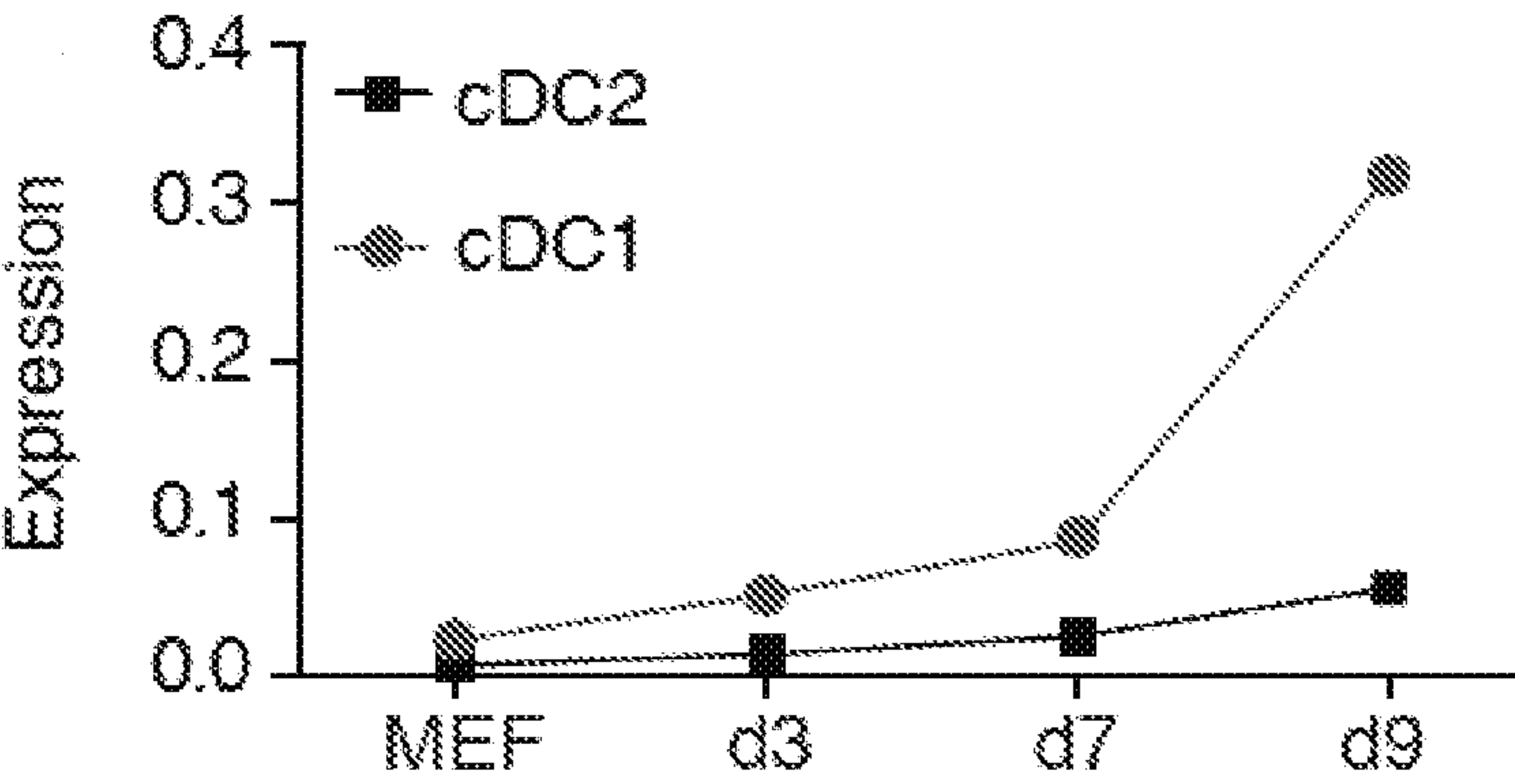


Fig. 26

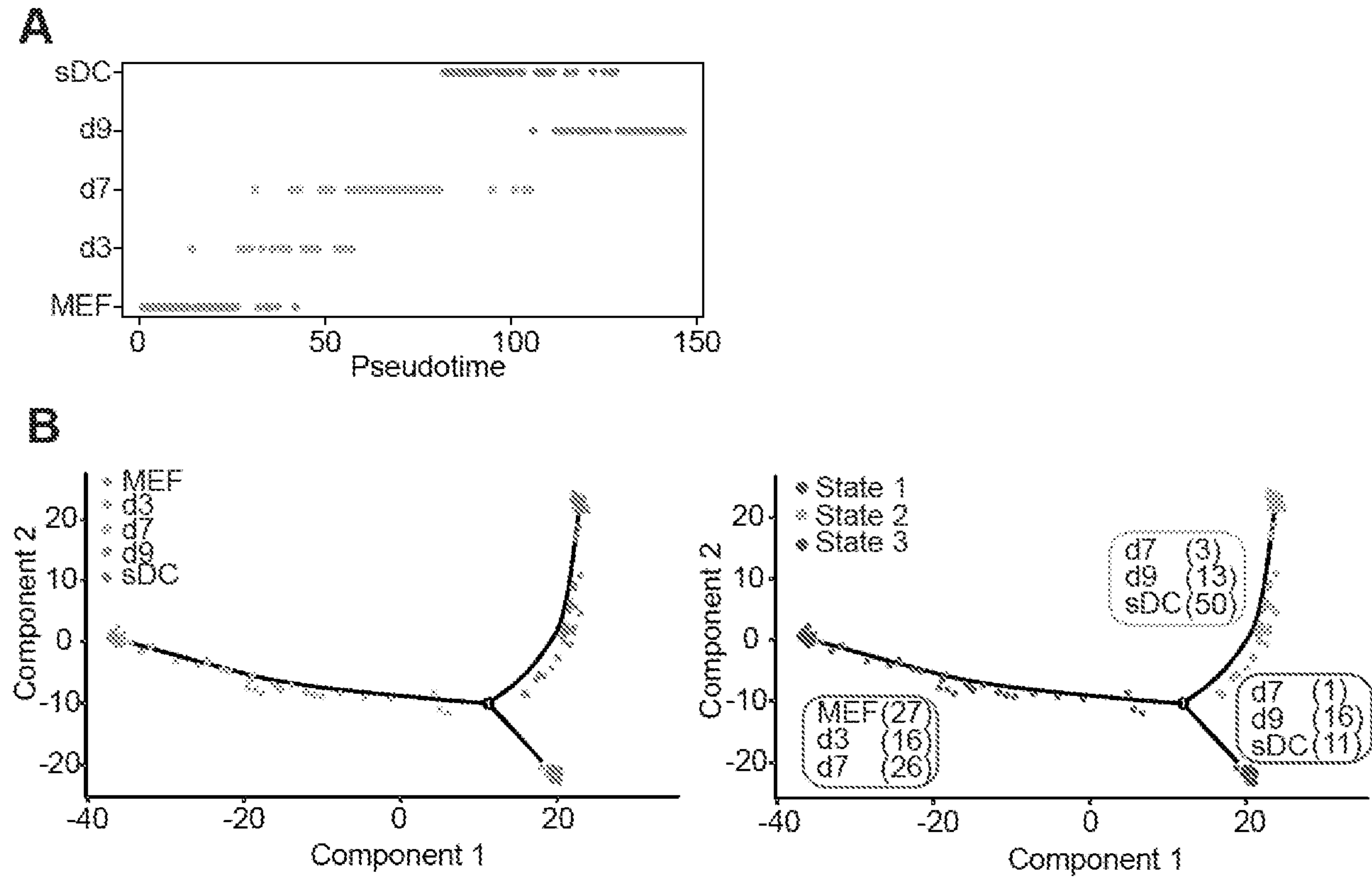


Fig. 27

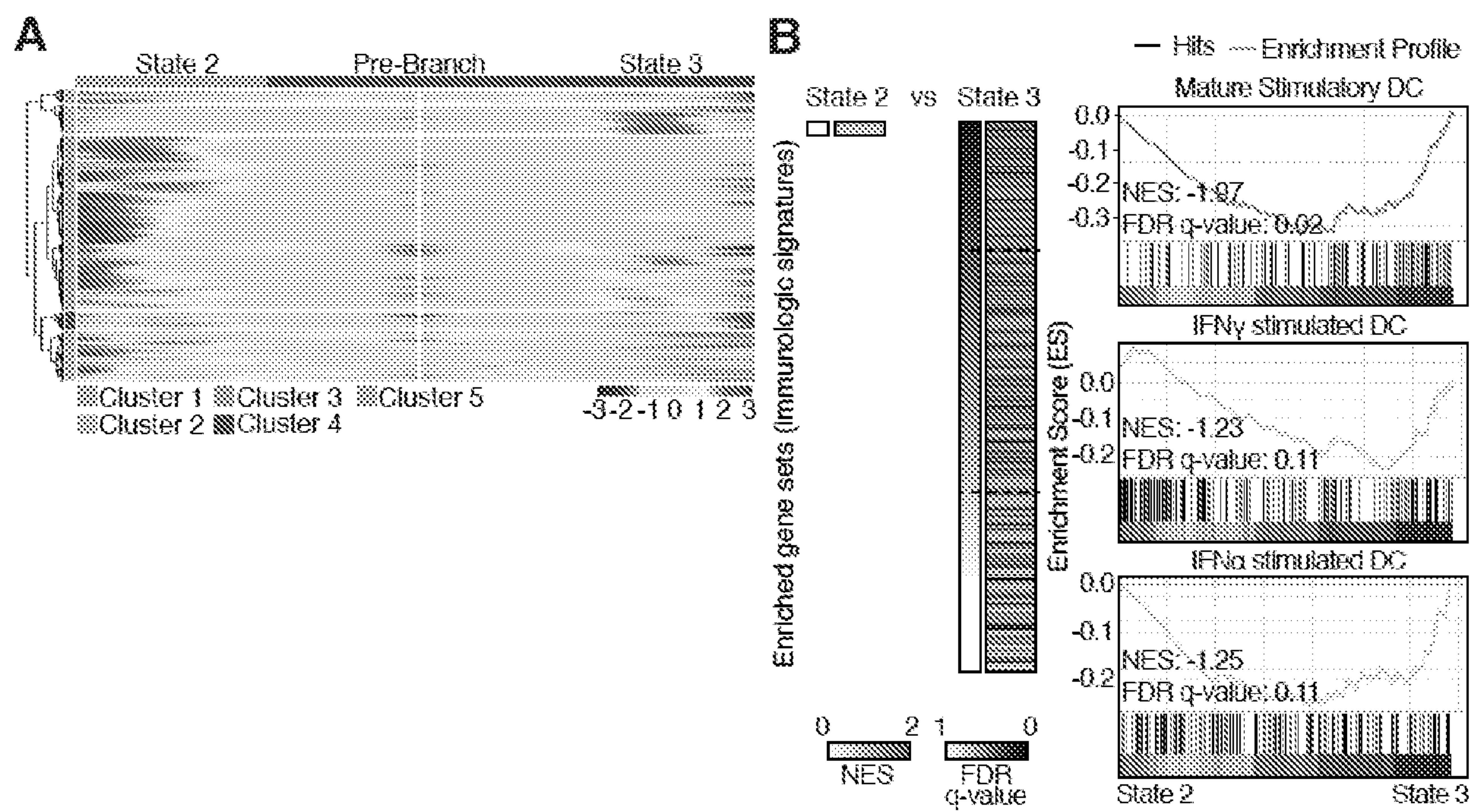


Fig. 28

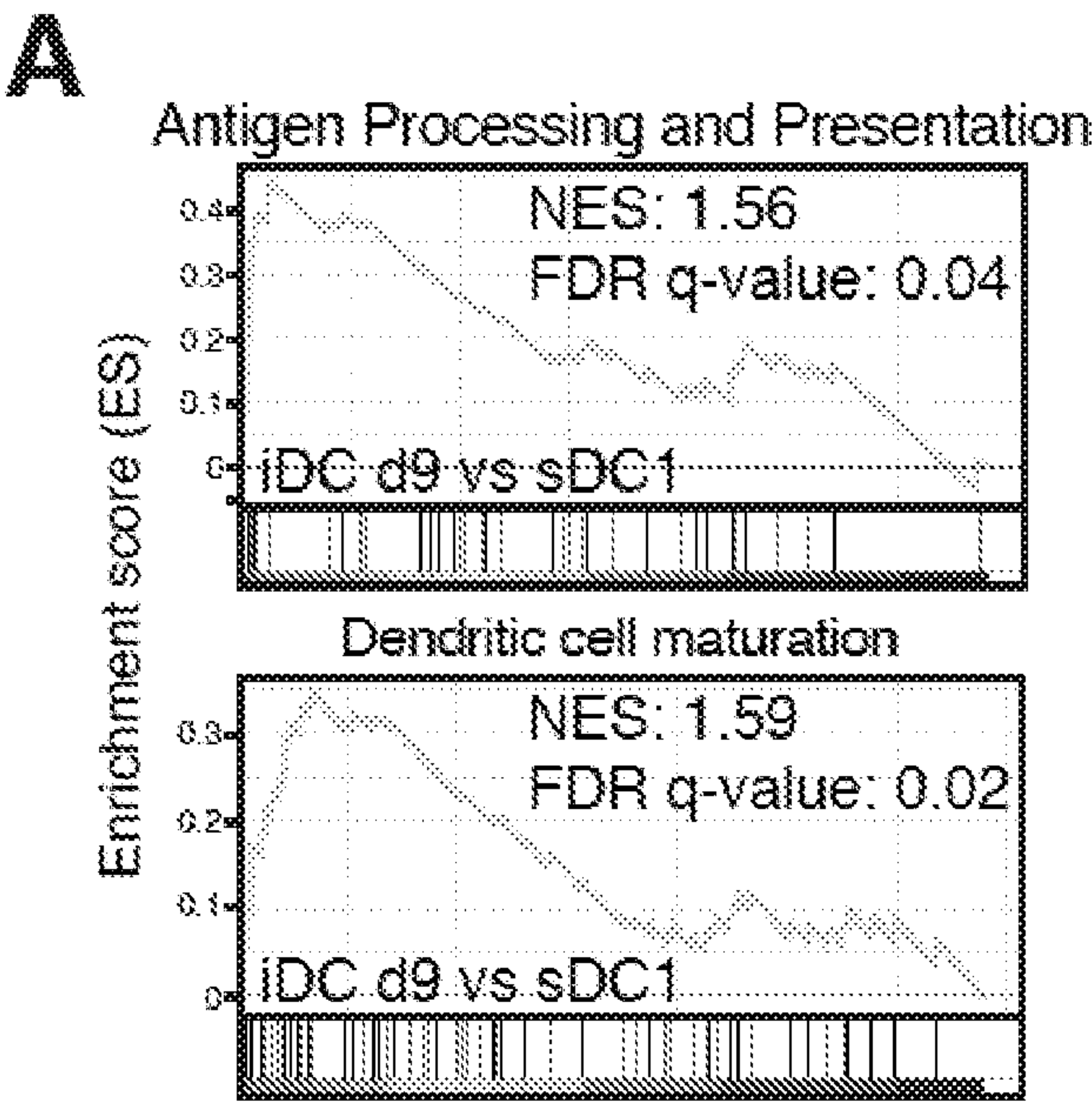


Fig. 29

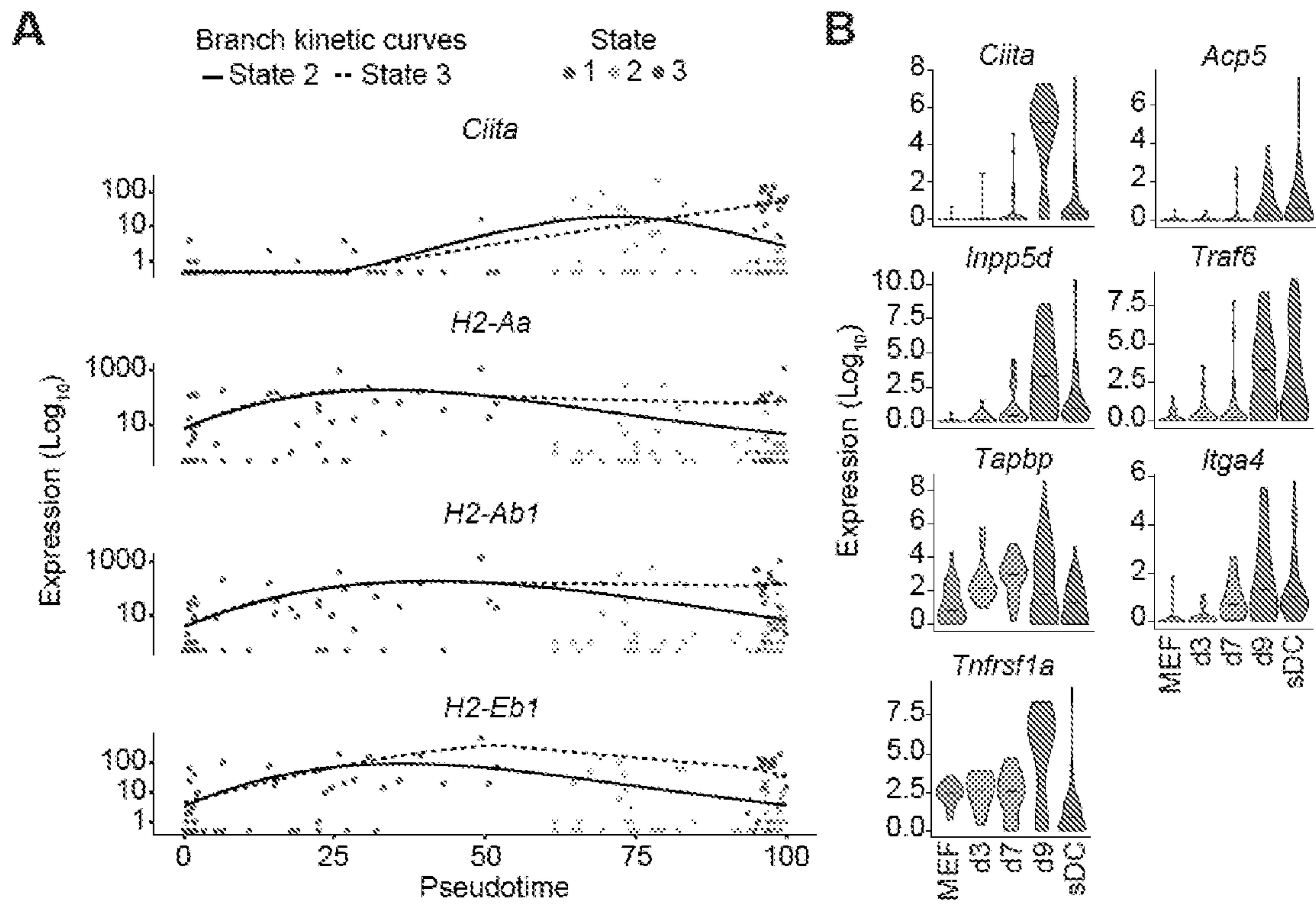


Fig. 30

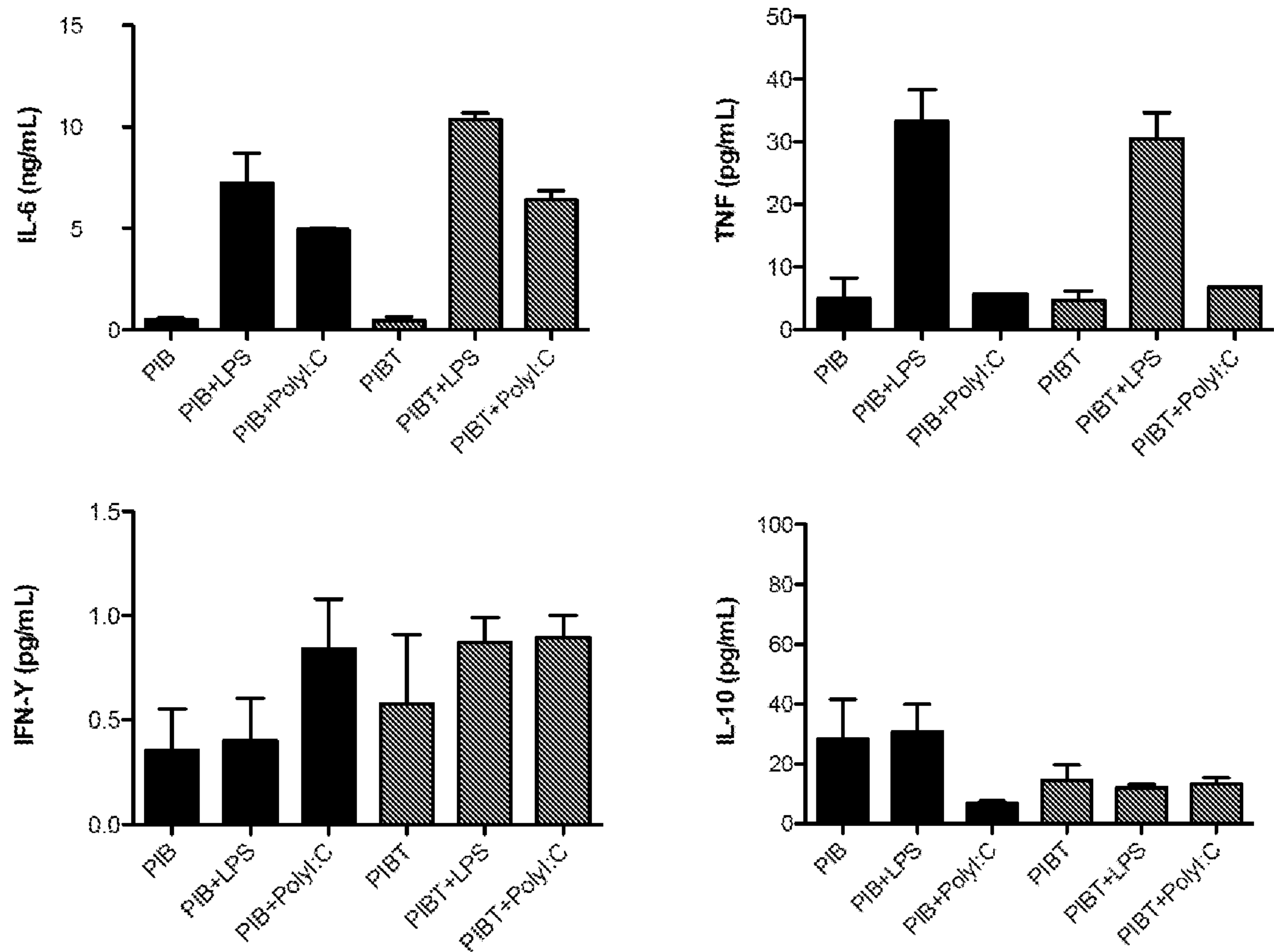


Fig. 31

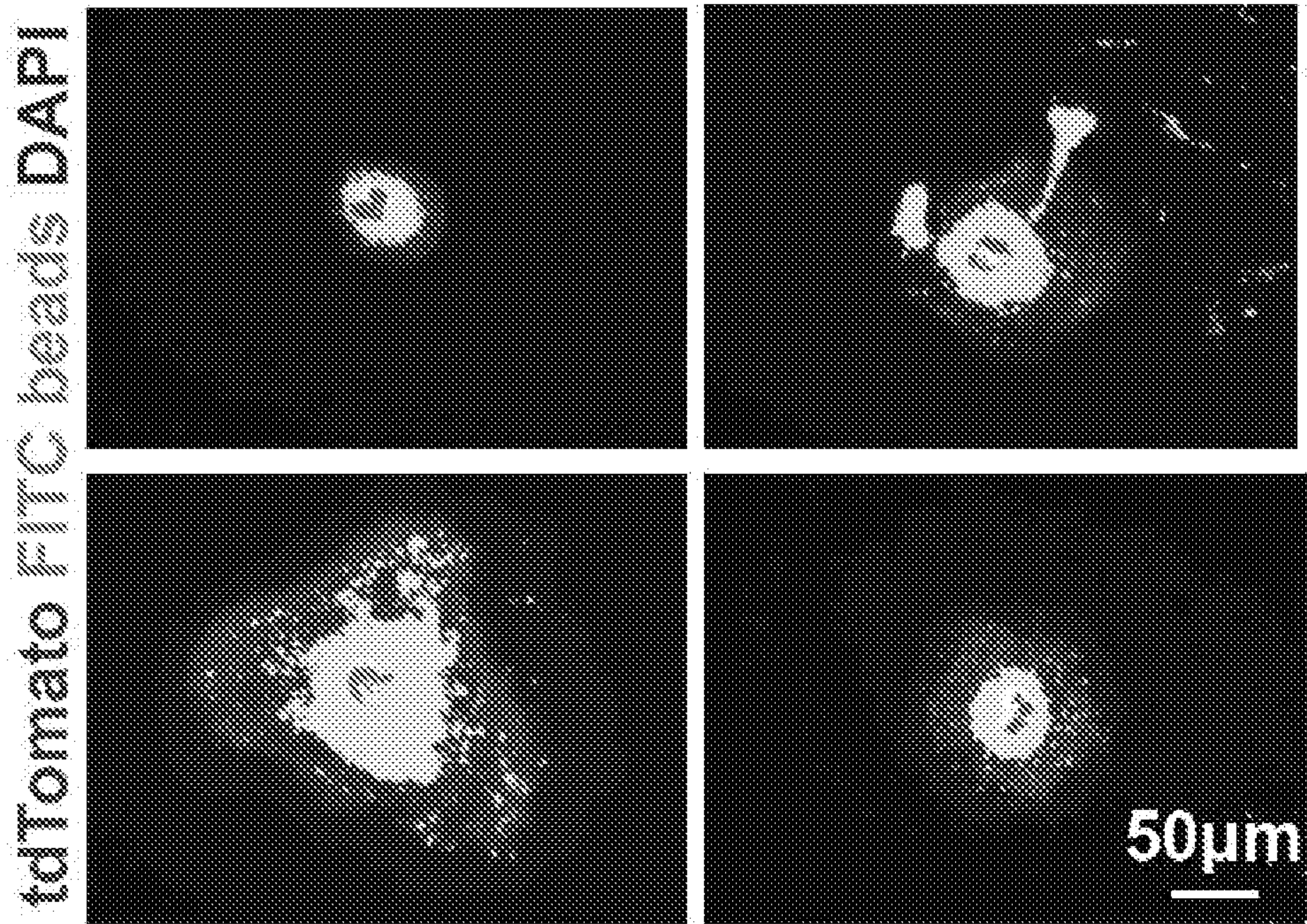


Fig. 32

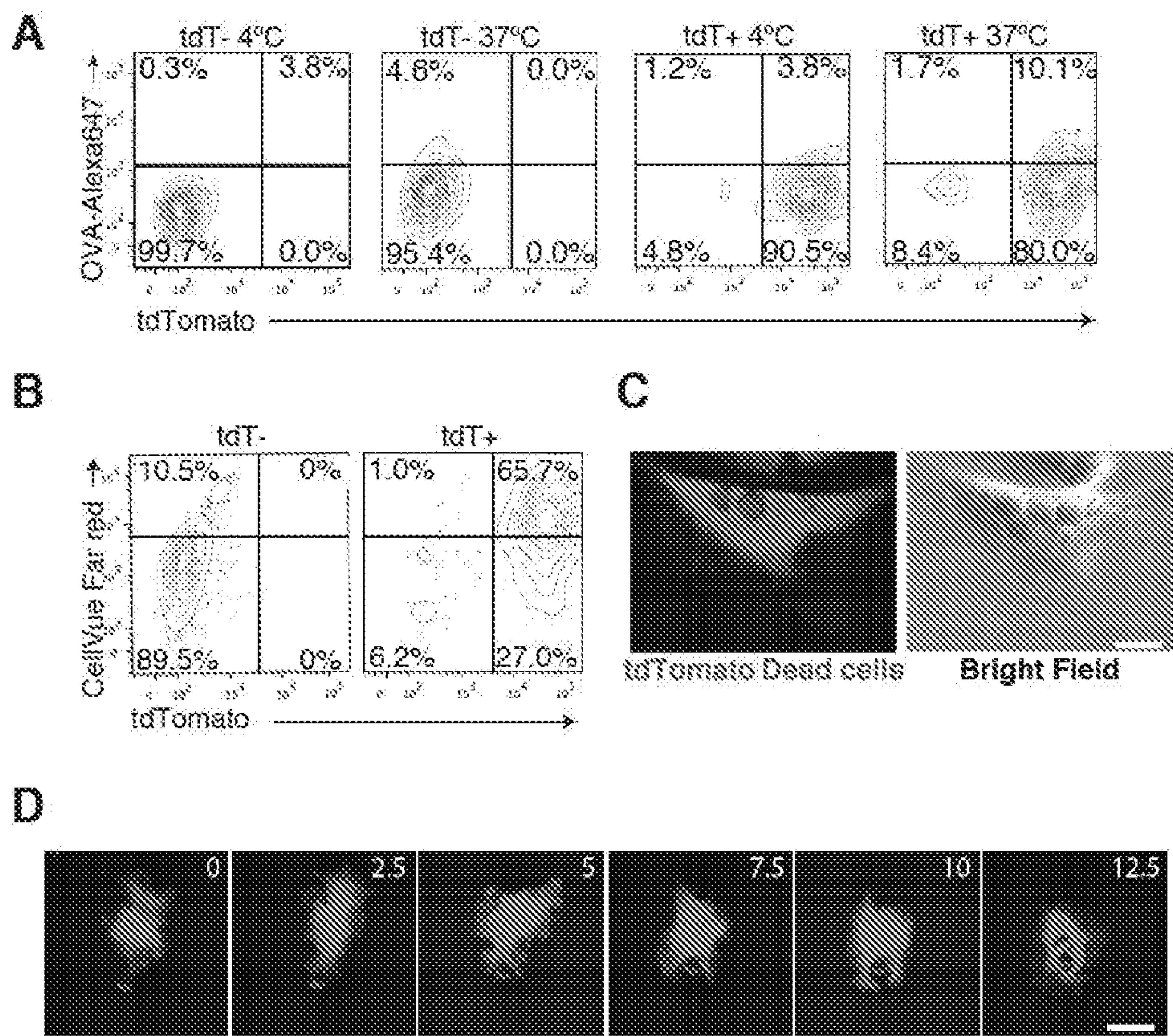


Fig. 33

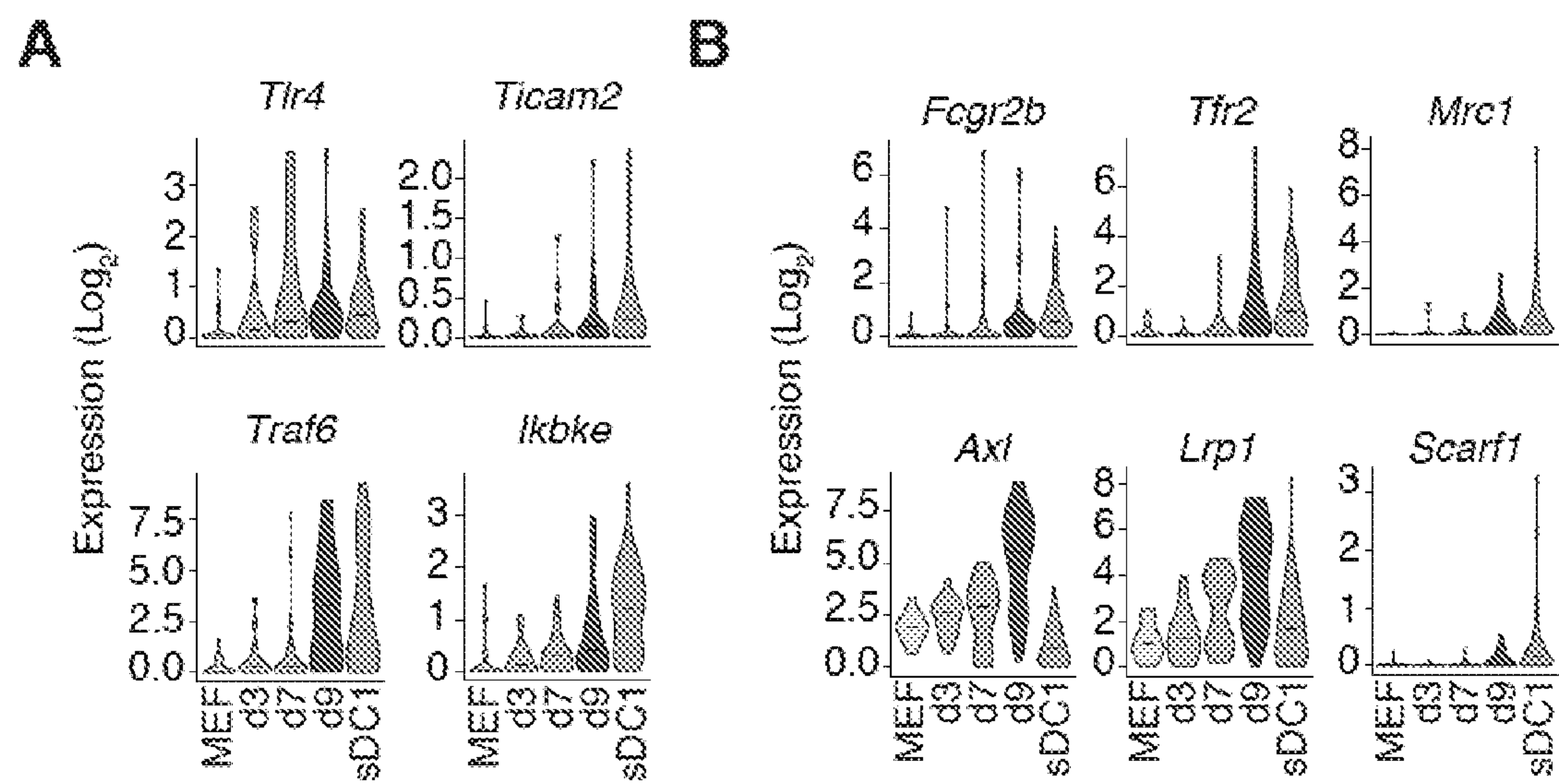


Fig. 34

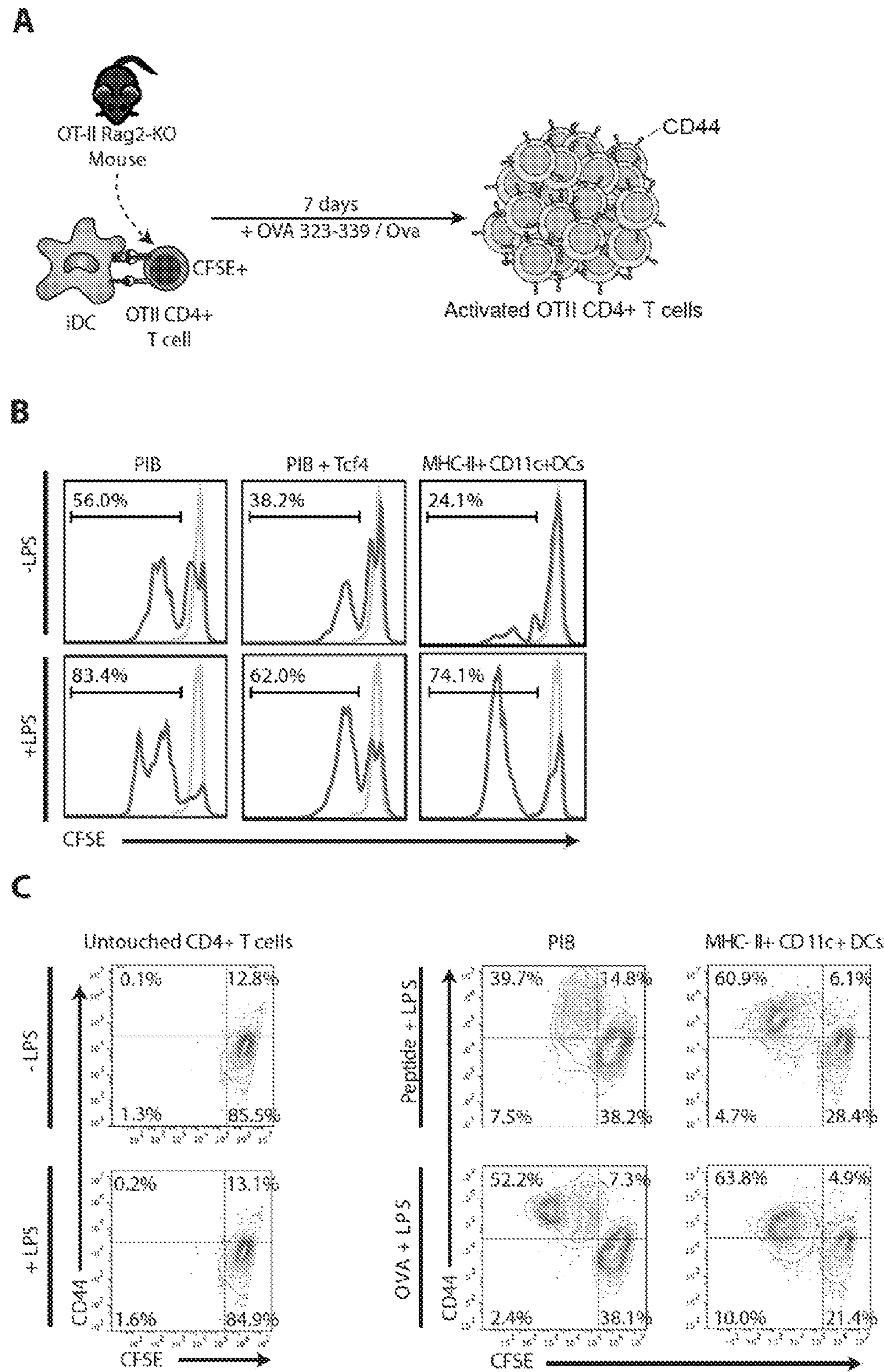


Fig. 35

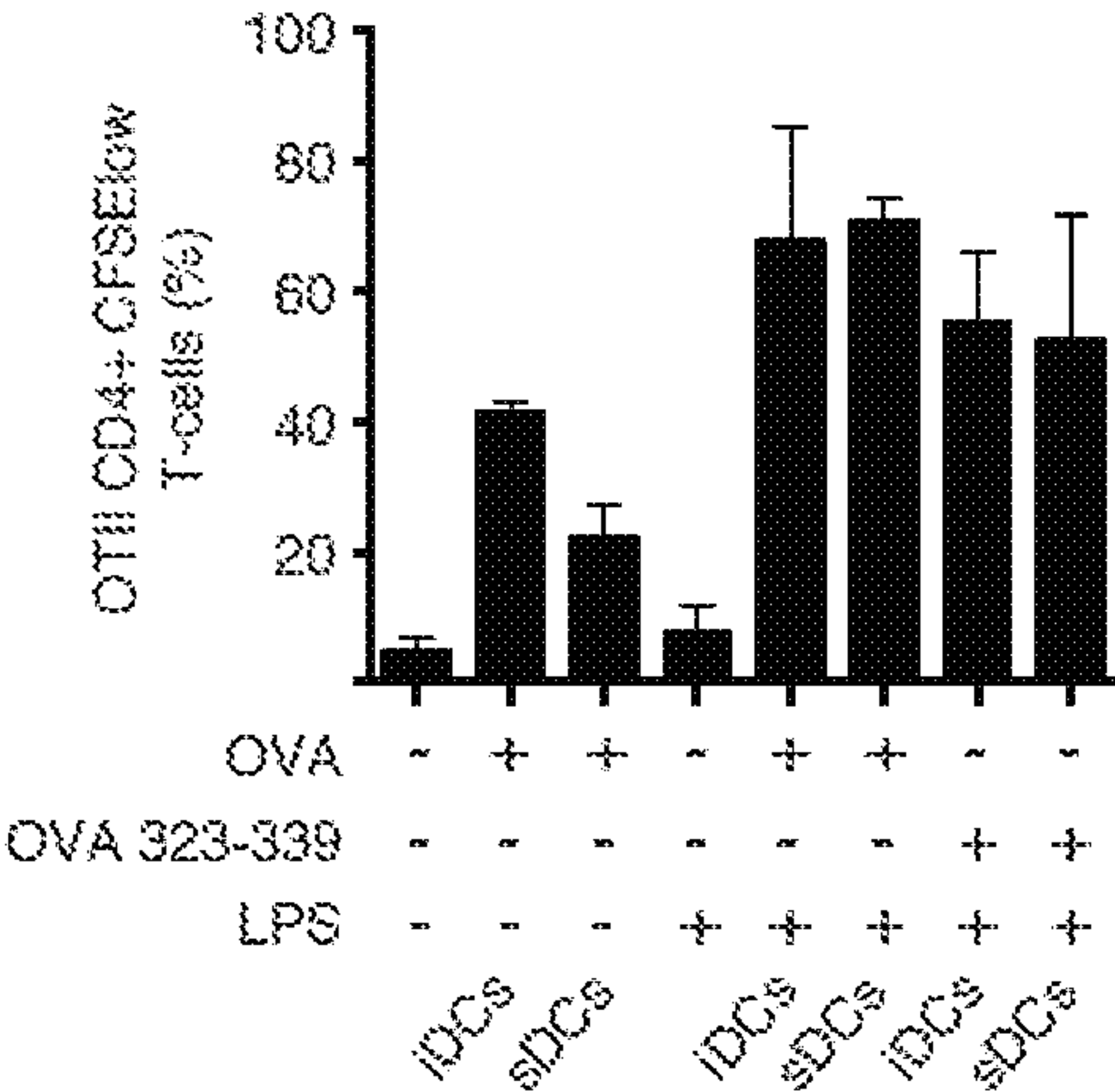


Fig. 36

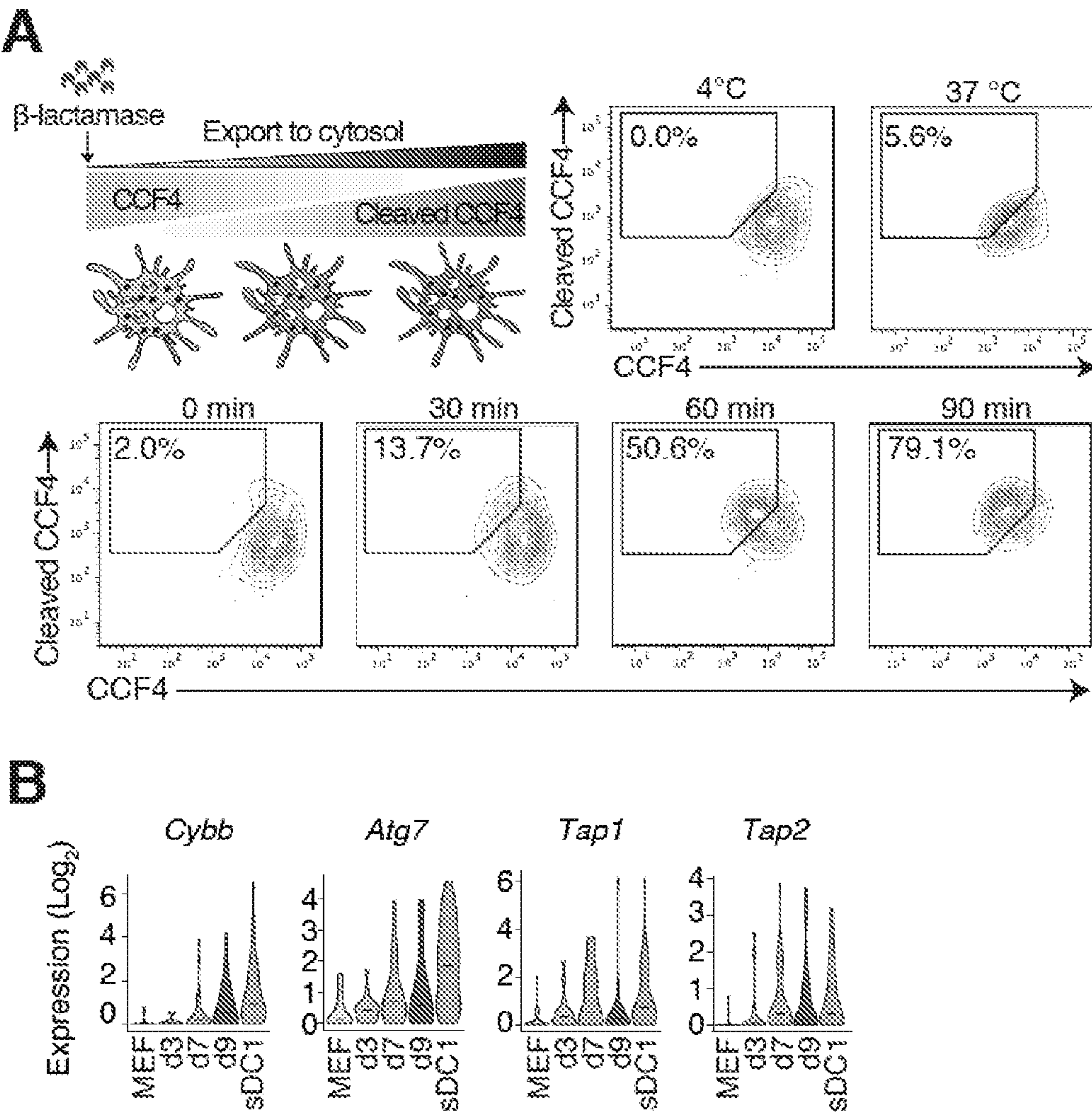


Fig. 37

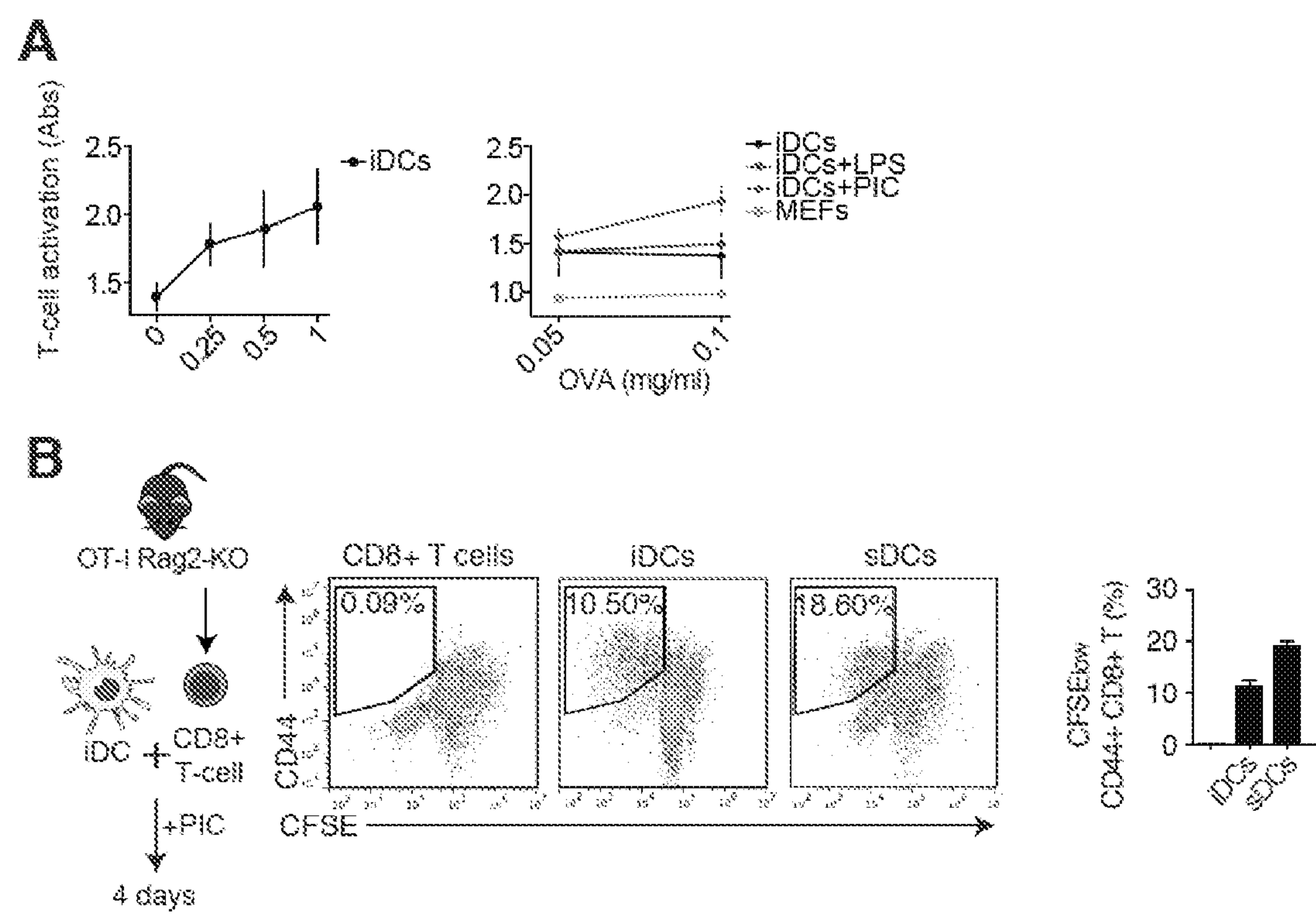


Fig. 38

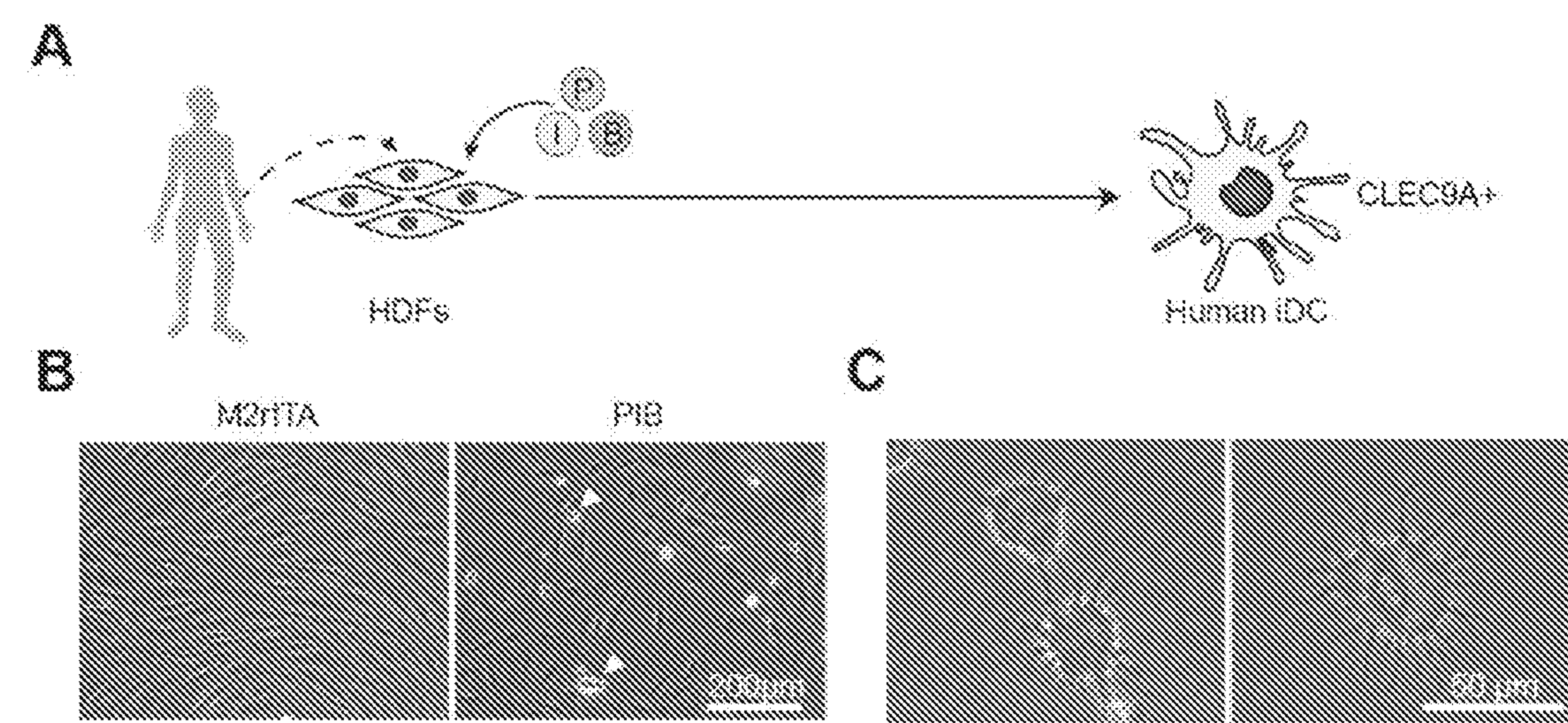


Fig. 39

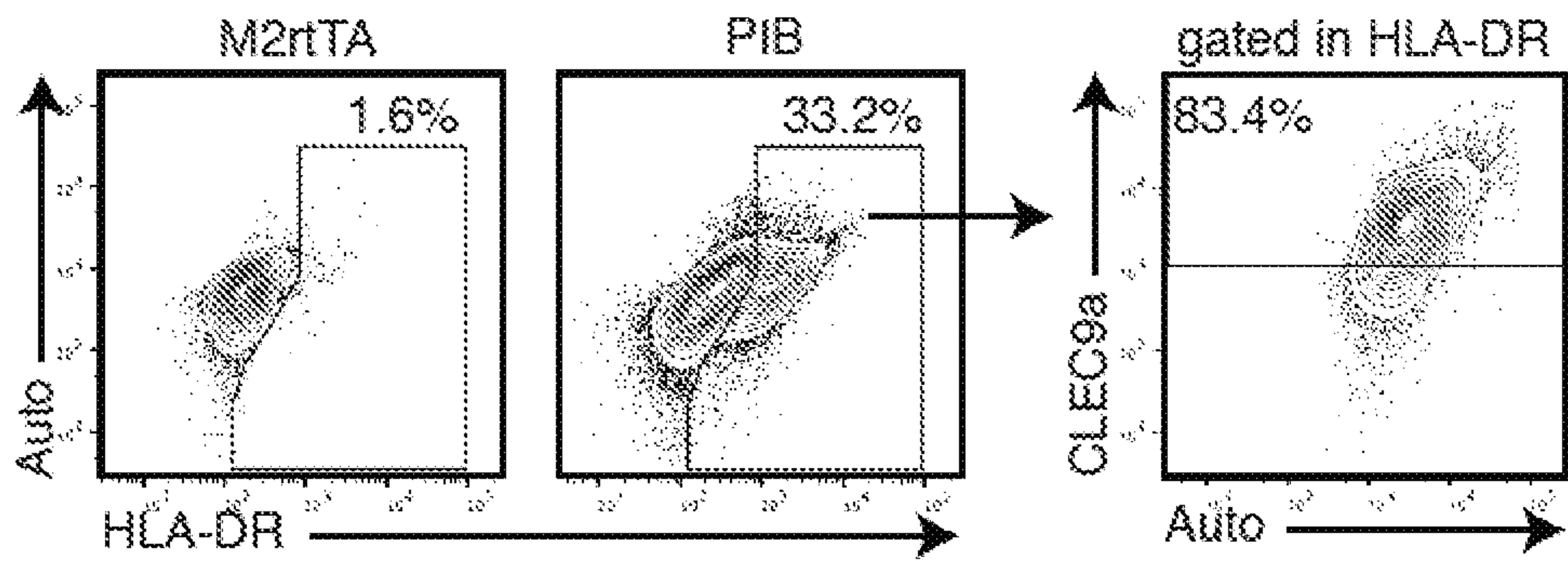


Fig. 40

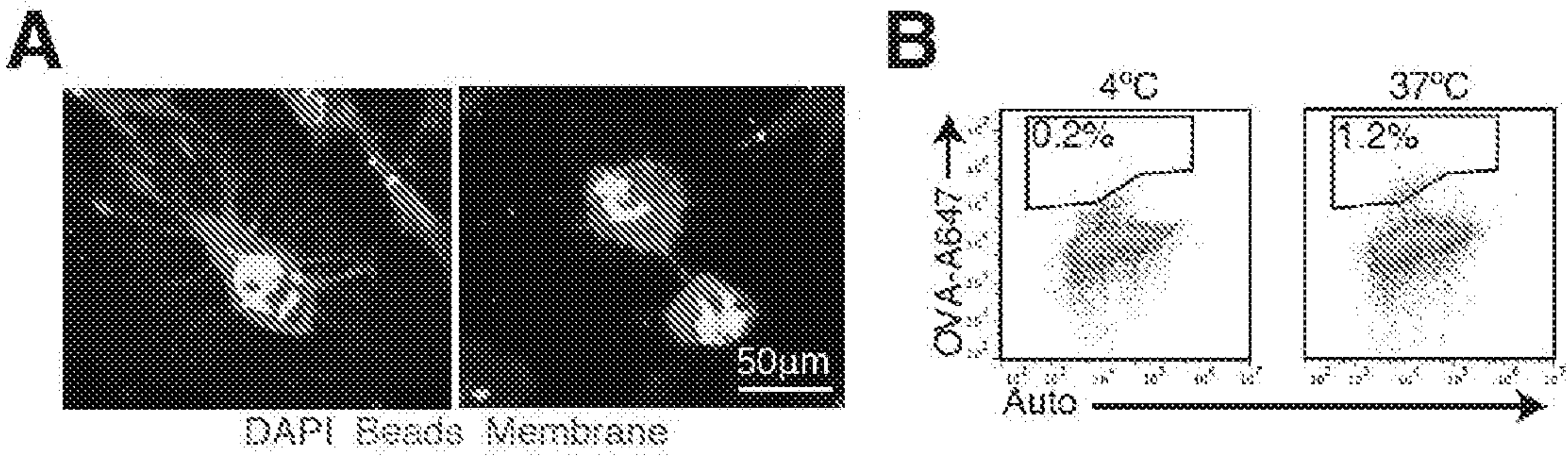


Fig. 41

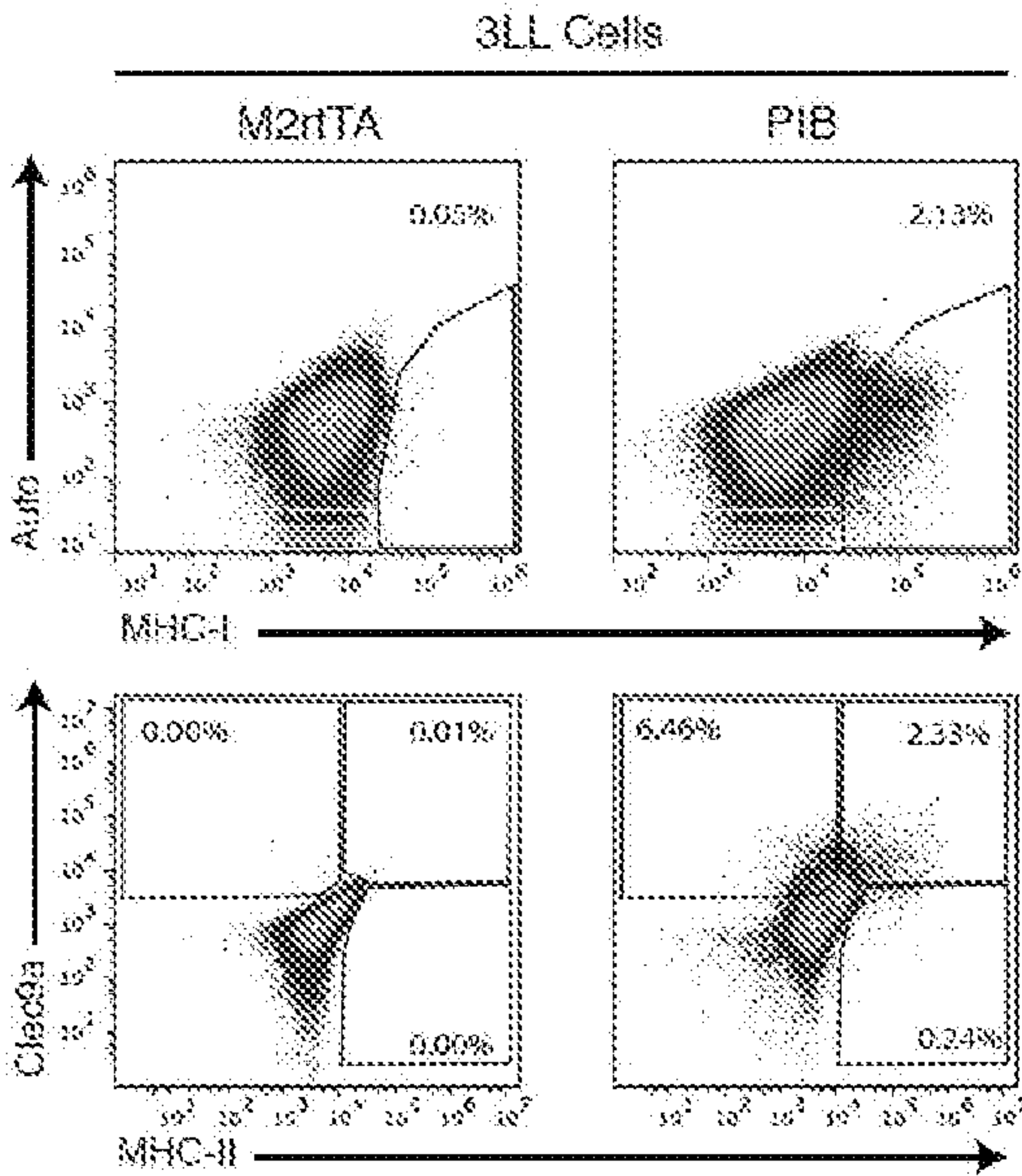


Fig. 42

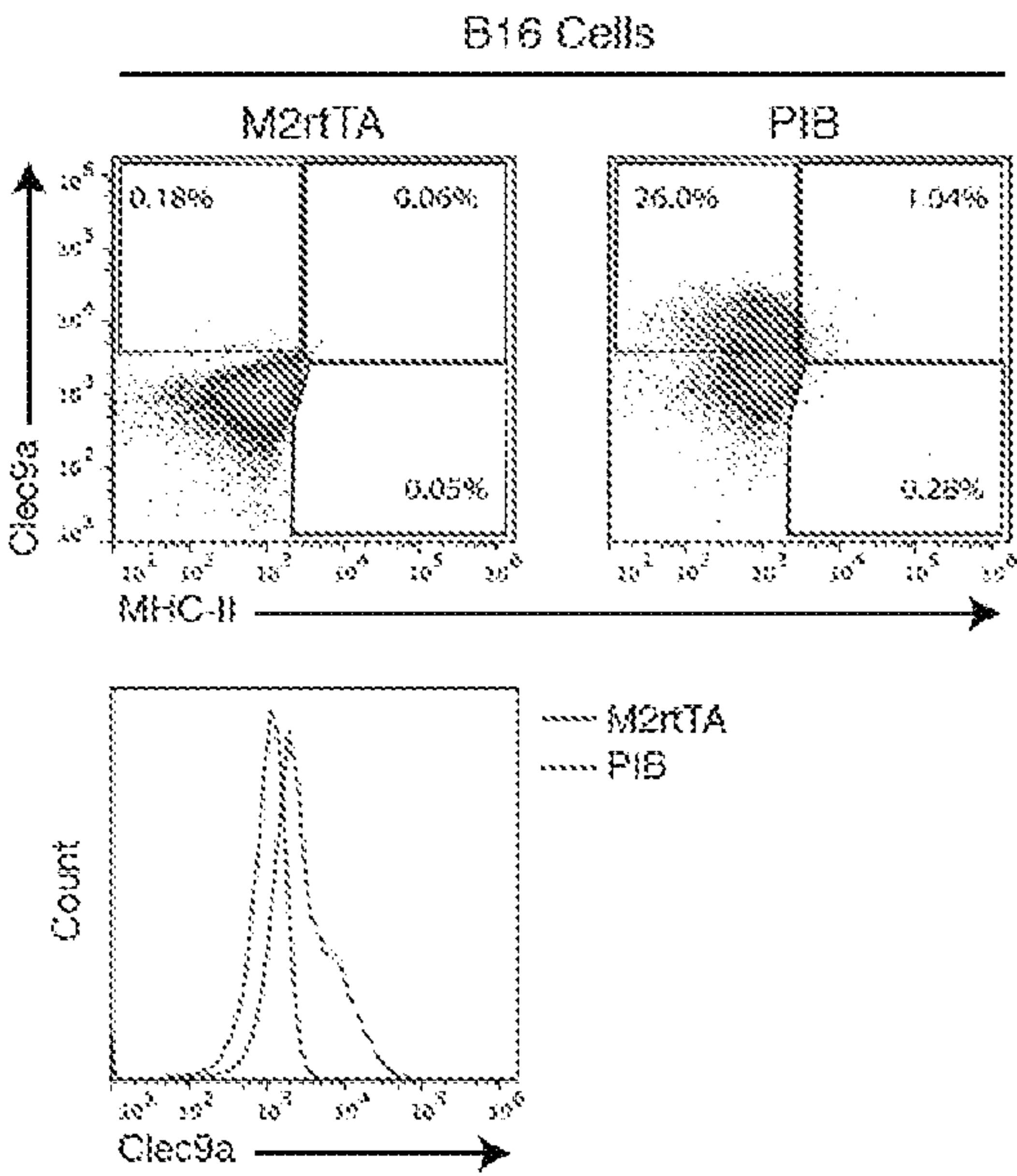
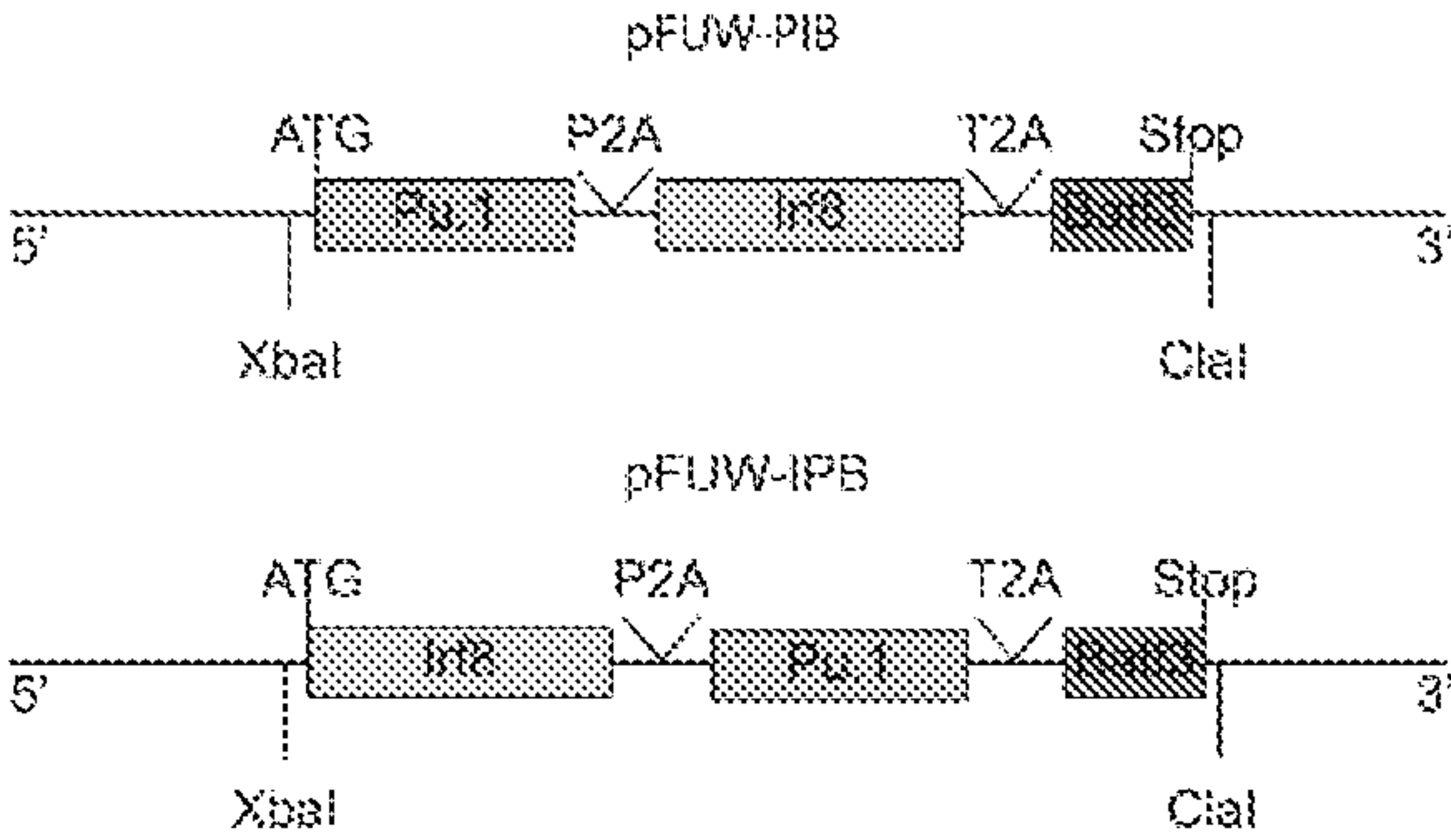
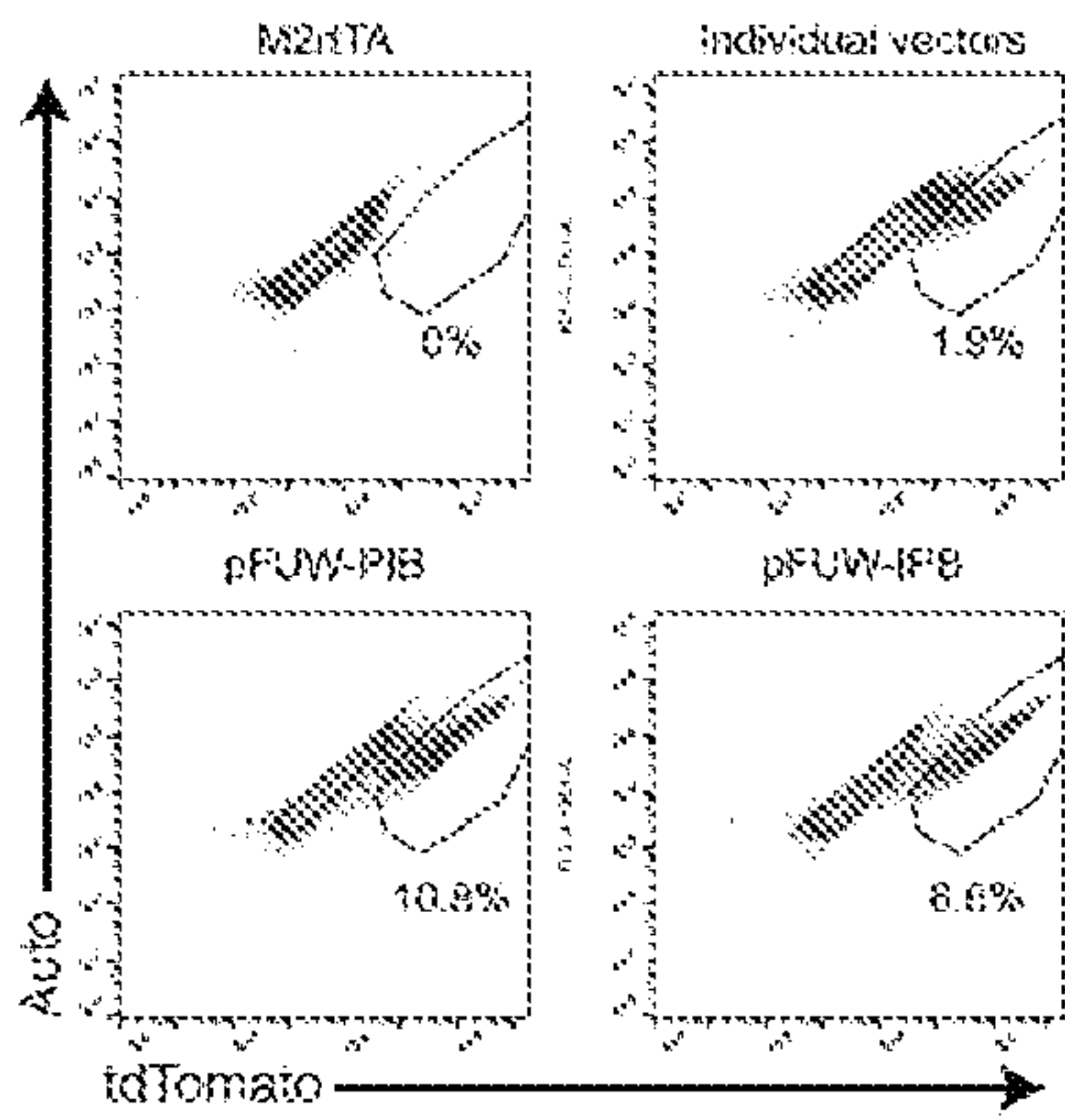


Fig. 43

A



B



C

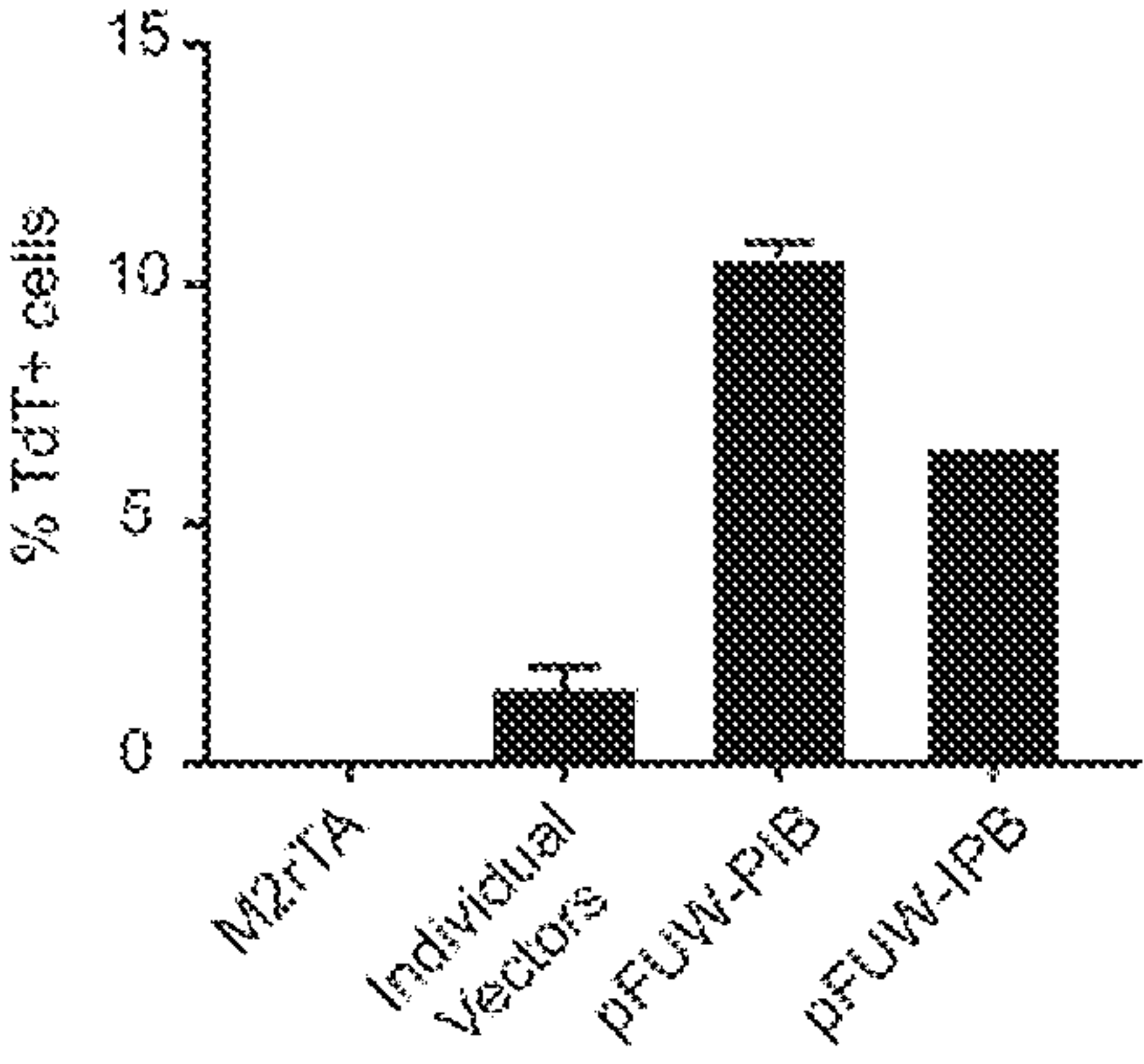


Fig. 44