Type-1 polarized dendritic cells are a potent immunogen against Mycobacterium tuberculosis

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Type-1 polarized dendritic cells are a potent immunogen against *Mycobacterium tuberculosis*

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33 **SUMMARY**
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35 **OBJECTIVE:** Application of immunotherapy using dendritic cells (DCs) is considered an effective treatment
36 strategy against persistent *Mycobacterium tuberculosis* infection. With the goal of developing improved therapeutic
37 vaccination strategies for patients with tuberculosis (TB), we tested the ability of *ex vivo*-generated DCs to induce an
38 effective TB antigen-specific type-1 immune response.
39 **METHODS**: Monocyte-derived DCs from TB patients were induced to mature using a “standard” cytokine cocktail
40 (IL-1β, TNF-α, IL-6, and PGE_2) or a type 1-polarized DC (DC1) cocktail (IL-1β, TNF-α, IFN-α, IFN-γ, and
41 polyinosinic:polycytidylic acid), and were loaded with the established TB antigen 6-kDa early secretory antigenic
42 target protein (ESAT-6).
43 **RESULTS:** Although DC1s from TB patients expressed the same levels of multiple co-stimulatory molecules (CD83,
44 CD86, CD80, CD11c, and CD40) as the standard DCs (sDCs), DC1s secreted substantially higher levels of IL-12p70.
45 Furthermore, when DCs pulsed with or without ESAT-6 were cultured with lymphocytes from the same patients,
46 DC1s induced much higher numbers of ESAT-6-specific IFN-γ-producing T cells than sDCs, as manifested by their
47 superior induction of natural killer cells activation and antigen-independent suppression of regulatory T cells.
48 **CONCLUSION:** TB antigen-loaded DC1s are potent inducers of antigen-specific T cells, which could be used to
49 develop improved immunotherapies of TB.
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51

52 **INTRODUCTION**

53

54 Tuberculosis (TB) remains one of the deadliest human diseases.[1] Although some highly effective anti-TB
55 chemotherapy drugs have been developed, long-term therapy is currently required for patients harbouring a persistent
56 population of slowly replicating or dormant bacilli. In addition to the potential side effects of long-course therapy,
57 such as relapse and drug resistance,[2] the emergence of resistant strains, particularly multidrug-resistant
58 *Mycobacterium tuberculosis* strains, has made disease control even more challenging.[1] Thus, an essential strategy of
59 infection control is to induce the host immune system to efficiently inhibit the growth of the intracellular pathogen
60 such as *M. tuberculosis*. Indeed, immunotherapy has shown good potential to improve the control of TB by enhancing
61 host immune responses to eliminate the bacteria, including persistent bacteria, and to shorten the protracted period of
62 chemotherapy required for TB patients.[3, 4] In TB immunology, there is much evidence to support the critical
63 requirement of the type 1 T helper cell (Th1) immune response for protective immunity against TB.[5-7] Although some
64 recent studies suggest a possible link between the type I immune response and pathogenesis, these opposing roles have
65 not been universally observed.

66 Dendritic cells (DCs) are the most potent antigen-presenting cells. They capture pathogens and then migrate to
67 the regional lymphoid organs, where they present antigens to naïve T cells to initiate an immune response.[8] Since
68 DCs have a distinct ability to prime naïve helper T lymphocytes and cytotoxic T lymphocytes (CTLs),[8-10] there has
69 been much interest in their potential use for immune modulation, and DC-based vaccines have been investigated.
70 However, the standard fully mature human DCs typically used for development of these vaccines show reduced ability
71 to produce interleukin (IL)-12p70, a dominant cytokine for Th1 polarization that is important for the differentiation
72 and maturation of DCs, upon subsequent interaction with antigen-specific T cells,[11,12] thereby limiting their *in vivo*
73 performance for vaccines in cancer treatment. In this context, several methods have been developed to mature DCs or
74 to enhance their ability to secrete high levels of IL-12 during subsequent interactions with T cells.[13-16] These type-1
75 polarized DCs (DC1s) that induce Th1 polarized responses via inflammatory cytokines, memory $CD8^+$ cells, or
76 properly activated natural killer (NK) cells show a strongly elevated ability to activate the Th1 pathway of the
77 differentiation of $CD4^+$ T cells, leading to induction of antigen-specific Th1 and CTLs during *in vitro* sensitization.[13-19]
78 Based on this background, we developed mouse models for preventive cancer vaccines that replicate the phenotype of
79 DC1s,[20] and recently confirmed that DC1s also confer strong protective immunity against an intracellular bacterium,

80 *Listeria monocytogenes*.[21] In the present study, to evaluate the ability of DC1s as a potential immunotherapeutic
81 candidate, we generated DC1s from the blood of patients with active TB infections, which were loaded with the
82 established TB antigen 6-kDa early secretory antigenic target protein (ESAT-6), and tested their feasibility to serve as
83 highly potent inducers of anti-TB immune responses.
84
85

86 **MATERIALS AND METHODS**

87

88 *Patients and blood samples*

89 The characteristics of the patients are summarized in Table 1. All patients were Japanese; 88% of the patients were
90 male, and all cases were pulmonary TB. This study was approved by the Institutional Review Board of all
91 participating hospitals. All patients provided written informed consent.

92 Peripheral blood was collected from patients that were newly diagnosed with TB without infection of human
93 immunodeficiency virus or hepatitis virus, and were not taking steroids or immunosuppressive agents. All TB patients
94 were enrolled in the study within two weeks from the start of TB treatment.

95

96 *Generation of monocyte-derived DCs*

97 Peripheral blood mononuclear cells (PBMCs) were isolated from the blood by density gradients. Monocytes were
98 further isolated using CD14 magnetic beads (Miltenyi Biotech Inc., Auburn, CA, USA) from the PBMCs (purity,
99 >95%, data not shown). The monocytes were cultured for 6 days (Thermo Fischer Scientific, Roskilde, Denmark) at 5
100 $\times 10^5$ cells/well in Iscove's modified Dulbecco's medium (IMDM) with 10% fetal calf serum (Life Technologies,
101 Grand Island, NY, USA) containing *rhu* granulocyte macrophage-colony stimulating factor (GM-CSF) and *rhu* IL-4
102 (both at 1,000 IU/mL; R&D Systems, Minneapolis, MN, USA). On day 6, DCs were induced to mature using either a
103 standard DC (sDC) cytokine cocktail composed of IL-1β (25 ng/mL) (Miltenyi), tumour necrosis factor-alpha (TNF-α,
104 50 ng/mL; Miltenyi), IL-6 (1,000 units/mL; R&D Systems), and prostaglandin E2 (PGE_2, 10^{-6} mol/L; Sigma-Aldrich,
105 St. Louis, MO, USA), or with a DC1 cocktail composed of IL-1β (25 ng/mL), TNF-α (50 ng/mL),
106 polyinosinic:polycytidylic acid (poly-I:C, 20 μg/mL; Sigma-Aldrich), interferon-alpha (IFN-α, 3,000 units/mL; MSD,
107 Tokyo, Japan), and IFN-γ (1,000 units/mL; Miltenyi) for 2 days. For the generation of TB antigen-loaded DCs,
108 ESAT-6 protein (ATGen Co., Ltd., Gyeonggi-do, Korea) at 10 μg/well was pulsed at 20 min after the addition of
109 maturation-inducing cytokines. PBMCs were separately stored frozen and used in the assays described below.

110

111 *Immunophenotyping*

112 Flow cytometric analysis was performed using a Beckman Coulter Gallios system (Beckman Coulter, Inc., Brea CA,
113 USA), after labelling the DCs with CD3, CD4, CD25, CD56, CD69, CD80, CD83, CD86, CD40, CD1a, CCR7,

114 HLA-DR, and Foxp3 (BD Biosciences, Franklin Lakes, NJ, USA) following a staining protocol per the
115 manufacturer's instructions.
116
117 *Chemotaxis*
118 Cell migration was evaluated using a chemotaxis microchamber technique. The lower chamber of 24-well
119 Polycarbonate Membrane Transwell Inserts (Corning Inc., Corning, NY, USA) was filled with 500 μL of IMDM with
120 0.5% bovine serum albumin. Recombinant human CCL19/MIP-3β (PeproTech, Rocky Hill, NJ, USA) and
121 CCL21/6Ckine (Miltenyi) were used separately, at concentrations of 0 ng/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, and
122 1000 ng/mL, respectively. The upper chamber was filled with 2×10^4 cells/100 μL of DCs in triplicate. After 3 h of
123 incubation, the cells that migrated to the underside of the upper chamber were fixed and stained. The membranes were
124 counted manually. The chemotaxis index was calculated by dividing the number of cells observed in the presence of
125 the ligands by the number of cells observed in the absence of the ligand.
126
127 *Measurements of cytokines and chemokines*
128 On day 8, DCs were harvested and washed twice, and then co-cultured (2×10^4 cells/100 μL) with NIH-3T3-hCD40
129 ligand cells (5×10^4 cells/100 μL; kindly provided under the MTA agreement with Dr Gordon Freeman, Dana-Farber
130 Cancer Institute/Brigham and Women's Hospital, USA). The levels of IL-12p70, IL-5, IL-6, IL-10, TNF-α,
131 CCL5/RANTES, CXCL9/MIG, and CXCL10/IP-10 in the supernatants were measured by BD CBA multiplexed
132 bead-based immunoassays (BD Biosciences), and CCL22/MDC in the supernatants was measured with a Quantikine
133 enzyme-linked immunosorbent assay kit (R&D Systems).
134
135 *IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assay*
136 The ELISPOT assay was performed with the antibody pair horseradish peroxidase-conjugated streptavidin and
137 3-amino-9-ethylcarbazole (AEC) chromogen (BD Biosciences) according to the manufacturer instructions. DCs and
138 stored autologous PBMCs (DCs:PBMCs = 1:10, not irradiated) were co-cultured in a MultiScreen-IP Filter 96-well
139 plate (Millipore, Billerica, MA, USA) in duplicate. The plates were incubated for 16 h, and the spots were counted
140 manually.

Assessment of NK cell activation and regulatory T cell (Treg) induction by flow cytometry

To evaluate DC-mediated CD69 expression by NK cells and induction of Tregs by DCs, DCs and stored autologous PBMCs (DCs:PBMCs = 1:10, not irradiated) were co-cultured for 16 h. After co-culture, the cells were harvested and labelled with CD3, CD56, and CD69 (BD Biosciences) for NK cell activation, or were harvested, fixed, permeabilized (Foxp3 Staining Buffer Set; Miltenyi), and labelled with CD4, CD25, and Foxp3 (Miltenyi) for Treg induction.

Statistical methods

The results were compared between different groups with the Mann–Whitney U test or Kruskal-Wallis test; P-values were adjusted using the Holm method. All statistical analyses were performed with EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 3.1.1). More precisely, it is a modified version of R commander (version 2.1-2) designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Characteristics of mature and polarized DCs in patients with TB

sDCs and DC1s showed a typical mature phenotype and DC-like morphology. With the exception of CD1a and CCR7, the expression levels of all other markers tested were significantly elevated in sDCs and DC1s than in immature DCs (iDCs) (Fig. 1A). CD1a and CCR7 also showed relatively high expression in both sDCs and DC1s. Eventually the expression levels of several DC activation markers and co-stimulatory molecules were similar between DC1s and sDCs.

In addition, because of the low expression level of CCR7 on DCs, we tested whether DCs could migrate in response to the CCR7 ligand, CCL19, and CCL21. As shown in Fig. 1B, sDCs and DC1s, but not iDCs, efficiently migrated in response to CCL19 and CCL21, indicating that these combinations of cytokines can provide appropriate signals to induce DCs to migrate to the lymph nodes in patients with TB.

Polarized DCs induce a high level of IL-12p70 production in patients with TB

To determine whether functional DC1s can be induced from TB patients, we measured the level of IL-12p70 production after stimulation of CD40L. The DC1s showed high production of IL-12p70 from the subsequent stimulation of CD40L (Fig. 2A). In contrast to the marked increase in IL-12p70 secretion, the production of IL-5, a Th2 polarizing cytokine, was not increased (Fig. 2B). IL-10 levels were also significantly higher upon CD40 ligation, although these levels seemed relatively low (Fig. 2D). DC1s also induced significantly higher IL-6 production compared to iDCs (Fig. 2C).

Polarized DCs secrete high levels of type-1 lymphocyte- and NK cell-recruiting chemokines in TB patients

We measured the levels of effector (CCL5, CXCL9, CXCL10) and regulatory (CCL22) T cell-attracting chemokines in the DC supernatants. The DC1s secreted significantly more CCL5 compared to sDCs or iDCs (Fig. 2E). The sDCs showed high production of Treg-attracting CCL22 (Fig. 2H). Furthermore, DC1s produced significantly higher amounts of the CXCR3 ligands CXCL9 and CXCL10, as compared with sDCs (Fig. 2F, 2G).

ESAT-6-loaded DC1s show a highly elevated ability to induce antigen-specific IFN-γ-producing cells

The ELISPOT assays confirmed that the DC1s could induce the differentiation of naïve T cells toward Th1 cells,

185 based on the level of IFN-γ production following stimulation with PBMCs. DC1s induced significantly higher
186 frequencies of antigen-specific IFN-γ-producing cells than sDCs or iDCs (Fig. 3A,B). This finding suggests that DC1s
187 strongly promote the Th1 polarization of PBMCs in patients with TB.

188

189 *Type-1 polarized DCs show increased NK cell-activity capacity, but inhibition of Foxp3⁺ Treg cell recruitment,*
190 *compared with sDCs*

191 We next investigated whether NK cells might become activated upon interaction with DC1s or sDCs, and their
192 consequent ability to recruit NK cells. DC1s more successfully recruited NK cells compared to sDCs or iDCs in an
193 antigen-nonspecific manner, as determined by the expression of CD56 and CD69 (Fig. 4A,B). Furthermore, Tregs
194 were suppressed in the presence of highly immunogenic ESAT-6, because of the substantial amounts of IFN-γ, as
195 determined by CD25 and Foxp3 expression levels. However, although DC1s were more effective than sDCs in
196 attracting high numbers of IFN-γ-producing cells, in contrast to sDCs, DC1s did not preferentially recruit Tregs in the
197 absence of ESAT-6 (Fig. 5A,B).

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199

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DISCUSSION

DC1s can be successfully generated from TB patients. The high immunologic activity of such cells suggests their possible use as vaccines or as *ex vivo* inducers of TB-specific type-1 immune cells to overcome the TB-associated immune dysfunction. Although further studies are needed, this study will pave the way to help shorten TB therapy. We consider that DC1s could be used following chemotherapy or in combination with chemotherapy or other immunotherapy. This approach would be promising, especially for improving the treatment of multidrug-resistant/excessive drug-resistant TB. It may also be potentially useful for treating latent TB infection.

IL-12p70 derived from DCs has been reported as the key factor of the Th1 polarization of $CD4^+$ T cells, and can act directly on $CD8^+$ T cells to promote effector and memory $CD8^+$ T cell expansion.[22,23] The susceptibility of IL-12p40 gene-deficient mice to *M. tuberculosis* infection and the therapeutic role of IL-12 against TB strongly support an important role for this cytokine in the protective immune response against *M. tuberculosis*.[24] Indeed, administration of IL-12 DNA has been reported to reduce the bacterial load in chronically *M. tuberculosis*-infected mice.[25] However, *M. tuberculosis* impairs the maturation of DCs, reduces the secretion of IL-12 by DCs, and inhibits their ability to stimulate T cell proliferation.[26] In light of the previously reported contribution of the dysfunction of endogenous DCs to the overall immunosuppression observed in TB patients, the current data suggest the possibility of restoring the "immunosuppression" resulting from TB infection in this group of patients using *ex vivo*-generated DCs.

Studies on the functionality of NK cells in cases of human TB are limited; however, there is some evidence that NK cells may be functionally impaired during TB infection. Patients newly diagnosed with pulmonary TB display decreased frequencies of NK cell subsets, coinciding with lowered expression of NKp30, NKp46, and IFN-γ.[27] Anti-TB treatment regimens leading to reductions in mycobacterial load have been shown to partially restore the cytolytic capabilities of NK cells.[27] The findings in the present study are consistent with a previous report[28] showing that mature DC1s from healthy donors efficiently recruited NK cells in a CXCL9-dependent manner. In conjunction with our present data, the evidence collected to date supports the potential clinical use of DCs, combining a fully mature status with high migratory function, the ability to produce IL-12p70 upon cognate interaction with T cells expressing CD40L, and the ability to recruit and activate NK cells in the presence of CD40L-expressing lymphocytes. Collectively, these properties predict that DC1-based vaccines loaded with TB antigens should induce a strong Th1-polarized anti-TB immune response.

228 Tregs participate in the control of effector T cells in chronic diseases, preventing tissue damage and resolution
229 of the inflammatory process; however, in the case of *M. tuberculosis* infection, an increased Treg frequency may be
230 harmful during the first stages of bacterial replication, because they may downregulate antigen-specific T cells,
231 dampening effective macrophage activation, and thereby suppressing *M. tuberculosis* replication control.[29,30]
232 Consistent with previous reports, our data indicate that immature DCs have tolerogenic properties by inducing T cell
233 anergy or Tregs lacking sufficient expression of co-stimulatory molecules. Moreover, sDCs showed weaker induction
234 of Tregs compared to iDCs with sufficient expression of co-stimulatory molecules through the induction of
235 anti-inflammatory cytokines. For an effective vaccine, DCs should optimally mediate effector T cells to the site of
236 antigen-dependent DC–CD4$^+$ T cell interactions by secretion of CCL5 chemokines.[31] To efficiently recruit NK cells
237 within the draining lymph node, immigrating DCs should produce CXCR3 ligands.[32] Our data show that DC1s
238 produced significantly higher amounts of CXCR3 ligands. Although numerous chemokines are known to
239 preferentially attract Tregs to different tissues, the ability of human DCs to attract Foxp3$^+$ Treg cells is strictly
240 CCR4-dependent, implicating a key role of CCL22 in the process, which is the only DC-produced CCR4 ligand. In
241 accordance with the possibility that the PGE_2 used in the process of sDC generation may preferentially promote their
242 interaction with Tregs,[28,33,34] we observed that sDCs were highly effective in attracting CD4$^+$Foxp3$^+$ T cells from the
243 freshly isolated bulk population of CD4$^+$ T cells. In contrast, alternatively matured DC1s showed strongly reduced
244 production of CCL22, indicating that DC1s did not preferentially recruit Foxp3$^+$ T cells from the total population of
245 CD4$^+$ T cells. In the presence of highly immunogenic ESAT-6, a strong IFN-γ milieu may inhibit the differentiation of
246 Treg cells under highly inflammatory conditions.[35] Our results confirm that the presence of IFN-γ creates a highly
247 inflammatory environment that negatively affects Treg generation.

248 In conclusion, we show that DC1s as a potential immunotherapeutic candidate in active TB patients, and further
249 investigation of these effects is warranted.

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251

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263 **References**

264 1. World Health Organization. Global tuberculosis report. 2015.
265 http://www.who.int/tb/publications/global_report/en/

266 2. Yamamoto Y, Hasegawa Y, Ogawa K. Retrospective cohort study of risk factors for adverse effects of
267 antituberculosis therapy. Kekkaku 2011; 86: 499–507. [article in Japanese]

268 3. Stanford J L, Bahr G M, Rook G A, Shaaban M A, Chugh T D, Gabriel M, et al. Immunotherapy with
269 *Mycobacterium vaccae* as an adjunct to chemotherapy in the treatment of pulmonary tuberculosis. Tubercle 1990; 71:
270 87–93.

271 4. Abou-Zeid C, Gares M P, Inwald J, Janssen R, Zhang Y, Young D B, et al. Induction of a type 1 immune response to
272 a recombinant antigen from *Mycobacterium tuberculosis* expressed in *Mycobacterium vaccae*. Infect Immun 1997; 65:
273 1856–1862.

274 5. Desvignes L, Wolf A J, Ernst J D. Dynamic roles of type I and type II IFNs in early infection with *Mycobacterium*
275 *tuberculosis*. J Immunol 2012; 188: 6205–6215.

276 6. Lande R, Giacomini E, Grassi T, Remoli M E, Iona E, Miettinen M et al. IFN-alpha beta released by
277 *Mycobacterium tuberculosis*-infected human dendritic cells induces the expression of CXCL10: selective recruitment
278 of NK and activated T cells. J Immunol 2003; 170: 1174–1182.

279 7. Rai P K, Chodisetti S B, Nadeem S, Maurya S K, Gowthaman U, Zeng W, et al. A novel therapeutic strategy of
280 lipidated promiscuous peptide against *Mycobacterium tuberculosis* by eliciting Th1 and Th17 immunity of host. Sci
281 Rep 2016; 6: 23917.

282 8. Banchereau J, Steinman R M. Dendritic cells and the control of immunity. Nature 1998; 392: 245–252.

283 9. Kalinski P, Hilkens C M, Wierenga E A, Kapsenberg M L. T-cell priming by type-1 and type-2 polarized dendritic
284 cells: the concept of a third signal. Immunol Today 1999; 20: 561–567.

285 10. Nakamura Y, Suda T, Nagata T, Aoshi T, Uchijima M, Yoshida A, et al. Induction of protective immunity to
286 *Listeria monocytogenes* with dendritic cells retrovirally transduced with a cytotoxic T lymphocyte epitope minigene.
287 Infect Immun 2003; 71: 1748–1754.

288 11. Kalinski P, Schuitemaker J H, Hilkens C M, Wierenga E A, Kapsenberg M L. Final maturation of dendritic cells is
289 associated with impaired responsiveness to IFN-gamma and to bacterial IL-12 inducers: decreased ability of mature
290 dendritic cells to produce IL-12 during the interaction with Th cells. J Immunol 1999; 162: 3231–3236.

291 12. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of
292 TH1, TH2 and nonpolarized T cells. Nat Immunol 2000; 1: 311–316.
293 13. Kalinski P, Okada H. Polarized dendritic cells as cancer vaccines: directing effector-type T cells to tumors. Semin
294 Immunol 2010; 22: 173–182.
295 14. Mailliard R B, Wankowicz-Kalinska A, Cai Q, Wesa A, Hilkens C M, Kapsenberg M L, et al. Alpha-type-1
296 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. Cancer Res 2004; 64:
297 5934–5937.
298 15. Ten Brinke A, Karsten M L, Dieker M C, Zwaginga J J, van Ham S M. The clinical grade maturation cocktail
299 monophosphoryl lipid A plus IFNgamma generates monocyte-derived dendritic cells with the capacity to migrate and
300 induce Th1 polarization. Vaccine 2007; 25: 7145–7152.
301 16. Watchmaker P B, Berk E, Muthuswamy R, Mailliard R B, Urban J A, Kirkwood J M, et al. Independent regulation
302 of chemokine responsiveness and cytolytic function versus CD8+ T cell expansion by dendritic cells. J Immunol 2010;
303 184: 591–597.
304 17. Kalinski P, Nakamura Y, Watchmaker P, Giermasz A, Muthuswamy R, Mailliard R B. Helper roles of NK and
305 CD8+ T cells in the induction of tumor immunity. Polarized dendritic cells as cancer vaccines. Immunol Res 2006; 36:
306 137–146.
307 18. Mailliard R B, Egawa S, Cai Q, Kalinska A, Bykovskaya S N, Lotze M T, et al. Complementary dendritic
308 cell-activating function of CD8+ and CD4+ T cells: helper role of CD8+ T cells in the development of T helper type 1
309 responses. J Exp Med 2002; 195: 473–483.
310 19. Mailliard R B, Son Y I, Redlinger R, Coates P T, Giermasz A, Morel P A, et al. Dendritic cells mediate NK cell
311 help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function. J Immunol
312 2003; 171: 2366–2373.
313 20. Giermasz A S, Urban J A, Nakamura Y, Watchmaker P, Cumberland R L, Gooding W, et al. Type-1 polarized
314 dendritic cells primed for high IL-12 production show enhanced activity as cancer vaccines. Cancer Immunol
315 Immunother 2009; 58: 1329–1336.
316 21. Kono M, Nakamura Y, Suda T, Uchijima M, Tsujimura K, Nagata T, et al. Enhancement of protective immunity
317 against intracellular bacteria using type-1 polarized dendritic cell (DC) vaccine. Vaccine 2012; 30: 2633–2639.

318 22. Chang J, Cho J H, Lee S W, Choi S Y, Ha S J, Sung Y C. IL-12 priming during in vitro antigenic stimulation
319 changes properties of CD8 T cells and increases generation of effector and memory cells. J Immunol 2004; 172:
320 2818–2826.
321 23. Lee J B, Lee K A, Chang J. Phenotypic changes induced by IL-12 priming regulate effector and memory CD8 T
322 cell differentiation. Int Immunol 2007; 19: 1039–1048.
323 24. Cooper AM, Kipnis A, Turner J, Magram J, Ferrante J, Orme I M. Mice lacking bioactive IL-12 can generate
324 protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present. J
325 Immunol 2002; 168: 1322–1327.
326 25. Triccas J A, Sun L, Palendira U, Britton W J. Comparative affects of plasmid-encoded interleukin 12 and
327 interleukin 18 on the protective efficacy of DNA vaccination against *Mycobacterium tuberculosis*. Immunol Cell Biol
328 2002; 80: 346–350.
329 26. Rajashree P, Krishnan G, Das S D. Impaired phenotype and function of monocyte derived dendritic cells in
330 pulmonary tuberculosis. Tuberculosis 2009; 89: 77–83.
331 27. Bozzano F, Costa P, Passalacqua G, Dodi F, Ravera S, Pagano G, et al. Functionally relevant decreases in
332 activatory receptor expression on NK cells are associated with pulmonary tuberculosis in vivo and persist after
333 successful treatment. Int Immunol 2009; 21: 779–791.
334 28. Gustafsson K, Ingelsten M, Bergqvist L, Nystrom J, Andersson B, Karlsson-Parra A. Recruitment and activation of
335 natural killer cells in vitro by a human dendritic cell vaccine. Cancer Res 2008; 68: 5965–5971.
336 29. Singh A, Dey A B, Mohan A, Sharma P K, Mitra D K. Foxp3+ regulatory T cells among tuberculosis patients:
337 impact on prognosis and restoration of antigen specific IFN-gamma producing T cells. PloS One 2012; 7: e44728.
338 30. Li L, Wu CY. CD4+ CD25+ Treg cells inhibit human memory gammadelta T cells to produce IFN-gamma in
339 response to M tuberculosis antigen ESAT-6. Blood 2008; 111: 5629–5636.
340 31. Lebre M C, Burwell T, Vieira P L, Lora J, Coyle A J, Kapsenberg M L, et al. Differential expression of
341 inflammatory chemokines by Th1- and Th2-cell promoting dendritic cells: a role for different mature dendritic cell
342 populations in attracting appropriate effector cells to peripheral sites of inflammation. Immunol Cell Biol 2005; 83:
343 525–535.
344 32. Gustafsson K, Junevik K, Werlenius O, Holmgren S, Karlsson-Parra A, Andersson P O. Tumour-loaded alpha-type

345 1-polarized dendritic cells from patients with chronic lymphocytic leukaemia produce a superior NK-, NKT- and
346 CD8+ T cell-attracting chemokine profile. Scand J Immunol 2011; 74: 318–326.
347 33. McIlroy A, Caron G, Blanchard S, Fremaux I, Duluc D, Delneste Y, et al. Histamine and prostaglandin E
348 up-regulate the production of Th2-attracting chemokines (CCL17 and CCL22) and down-regulate
349 IFN-gamma-induced CXCL10 production by immature human dendritic cells. Immunology 2006; 117: 507–516.
350 34. Muthuswamy R, Urban J, Lee J J, Reinhart T A, Bartlett D, Kalinski P. Ability of mature dendritic cells to interact
351 with regulatory T cells is imprinted during maturation. Cancer Res 2008; 68: 5972–5978.
352 35. Gigante M, Mandic M, Wesa A K, Cavalcanti E, Dambrosio M, Mancini V, et al. Interferon-alpha
353 (IFN-alpha)-conditioned DC preferentially stimulate type-1 and limit Treg-type in vitro T-cell responses from RCC
354 patients. J Immunother 2008; 31: 254–262.
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357 Table 1. Clinical characteristics of 17 patients. Data are presented as the median (range) or no. (%).

358

Characteristic	**N = 17**
Sex (M/F)	15/2
Age (years)	68 (23–86)
Ethnic Group	
Japanese	17 (100)
Body mass index	18.8 (14.4–27.1)
System involved	
Pulmonary tuberculosis [no. (%)]	17 (100)
Microbiological findings	
*Sputum smear (0, scanty, 1+, 2+, 3+)	3, 3, 6, 5, 0
Clinical characteristics	
Body temp (°C)	36.8 (35.3–37.9)
Heart rate (beats/min)	81 (68–108)
Laboratory findings	
BUN (mg/dL)	13.8 (4.5–38.8)
Alb (mg/dL)	3.9 (2.1–4.5)
Cre (mg/dL)	0.74 (0.05–1.12)
WBC (/mm^3)	7130 (4640–10240)
ESR (mm/h)	38 (25–72)

359 BUN, blood urea nitrogen; Alb, albumin; Cre, creatinine; WBC, white blood cell count; ESR, erythrocyte sedimentation rate.

360 *Sputum smear was defined according to the Ziehl-Neelson method for acid-fast bacteria. Data given refer to respective scale

361 numbers.

362

363 **Figure legends**

364 Figure 1. Immunophenotyping of immature DCs (iDCs), standard DCs (sDCs), and type-1 polarized DCs (DC1s), and
365 migration assay. (A) Flow cytometric analysis of DCs. DCs were labelled with CD1a, CD40, CD80, CD83, CD86,
366 CCR7, and HLA-DR antibodies. The results are expressed as median and interquartile range of data from 8 patients
367 (*; compared with iDC, $P < 0.05$). (B) The data of the migration assay; the results are expressed as median and
368 interquartile range of data from 5 patients (*; compared with iDC, $P < 0.05$). The results are reported from five fields
369 counted under a light microscope at 400× magnification in triplicate for a total of 15 fields per ligand. The chemotaxis
370 index was calculated by dividing the cell numbers observed in the presence of the ligands by those observed in the
371 absence of ligands.

372

373 Figure 2. Production of cytokines and chemokines by DCs. DCs were harvested and co-cultured with
374 NIH-3T3-hCD40 ligand cells for 16 h. Cytokines and chemokines in the supernatant were measured by multiplexed
375 bead-based immunoassays or enzyme-linked immunosorbent assay (CCL22 only). (A) IL-12p70, (B) IL-5, (C) IL-6,
376 (D) IL-10, (E) CCL5, (F) CXCL9, (G) CXCL10, and (H) CCL22. The results are expressed as median and
377 interquartile range of data from 9 patients (*; $P < 0.05$).

378

379 Figure 3. IFN-γ enzyme-linked immunosorbent spot assay. DCs with/without ESAT-6 (10 μg/mL) were harvested and
380 co-cultured with autologous PBMCs for 16 h (DC:PMBC = 1:10). (A) The results are expressed as median and
381 interquartile range of data from 6 patients (*: $P < 0.05$). (B) Representative image of an ELISPOT plate.

382

383 Figure 4. Assessment of DC-induced activation of NK cells by flow cytometric analysis. (A) DCs with/without
384 ESAT-6 co-cultured with autologous PBMCs for 16 h (DC:PBMC = 1:10). Cells were gated by $CD3^{-}CD56^{+}$ NK cells,
385 and then NK cell activity was assessed according to CD69 expression. The results are representative data. (B) The
386 results show $CD3^{-}CD56^{+}CD69^{+}$-positive cells, as median and interquartile range of data from 6 patients (*: $P < 0.05$).

387

388 Figure 5. Assessment of the ability of DCs to induce regulatory T cells (Tregs) by flow cytometric analysis. (A) DCs
389 with/without ESAT-6 co-cultured with autologous PBMCs for 16 h (DC:PBMC = 1:10). Cells were gated by

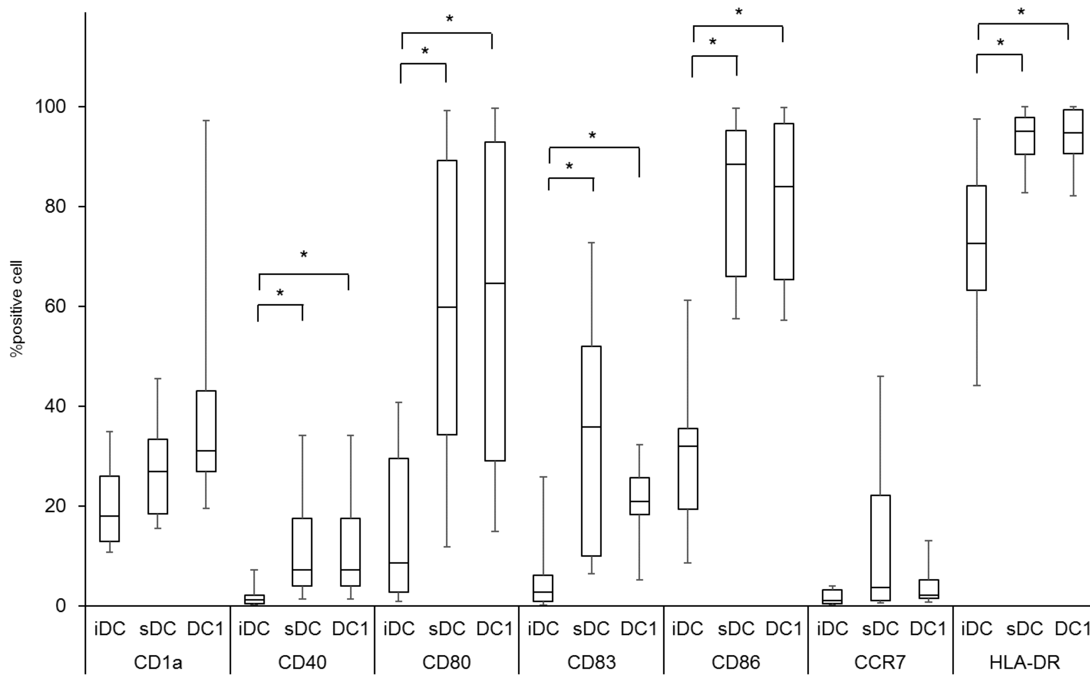
390 $CD4^+CD25^+$ T cells and then $Foxp3^+$-positive cells were analysed. The results are representative data. (B) The results

391 show $CD4^+CD25^+Foxp3^+$-positive Treg cells as median and interquartile range of data from 7 patients (*: $P < 0.05$).

392

393 Figure 1.

394 A



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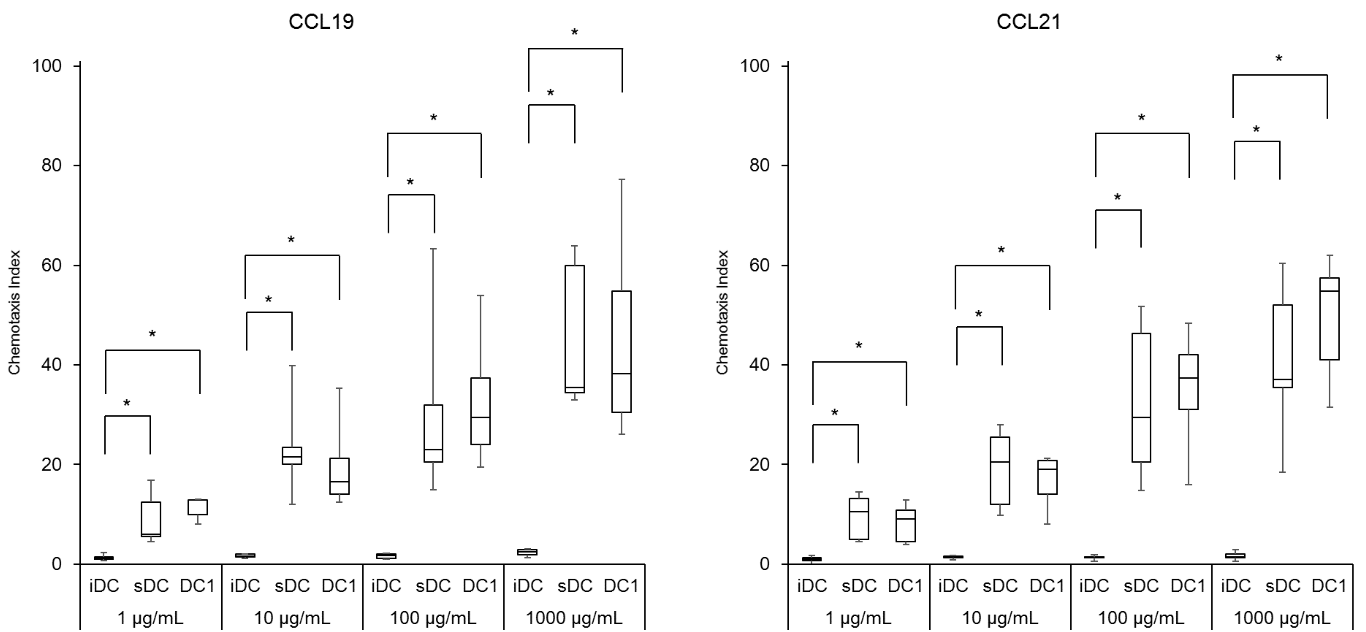
397 B



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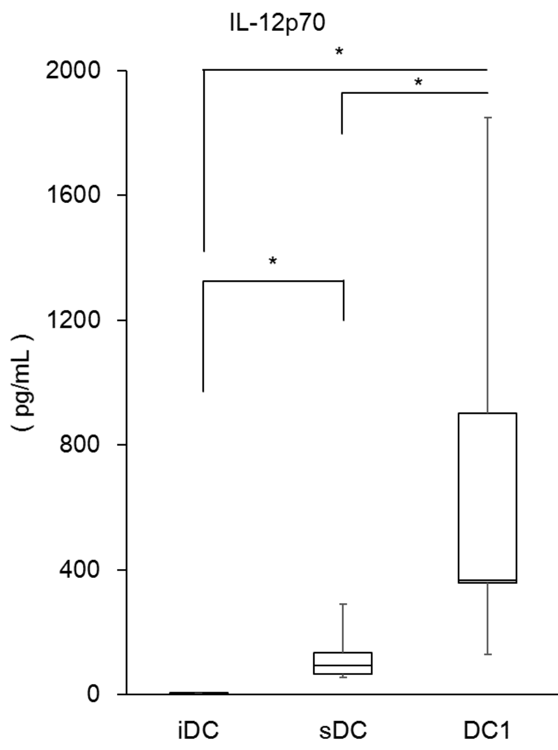
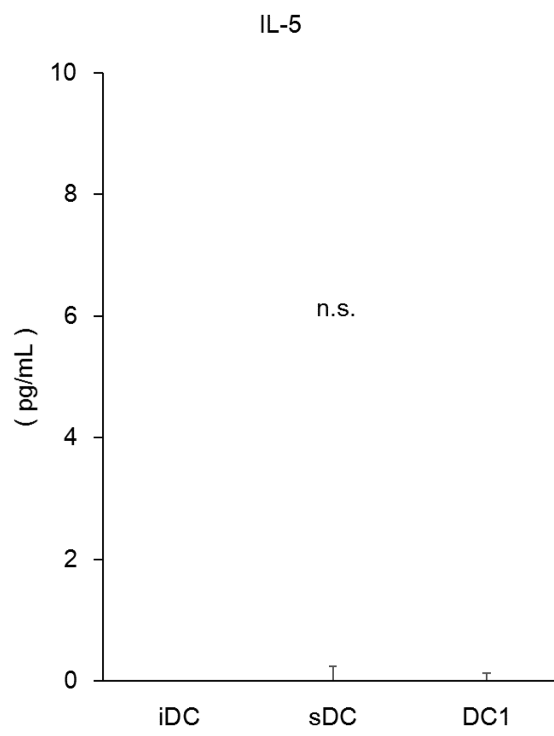
401

402 Figure 2.

403 A B





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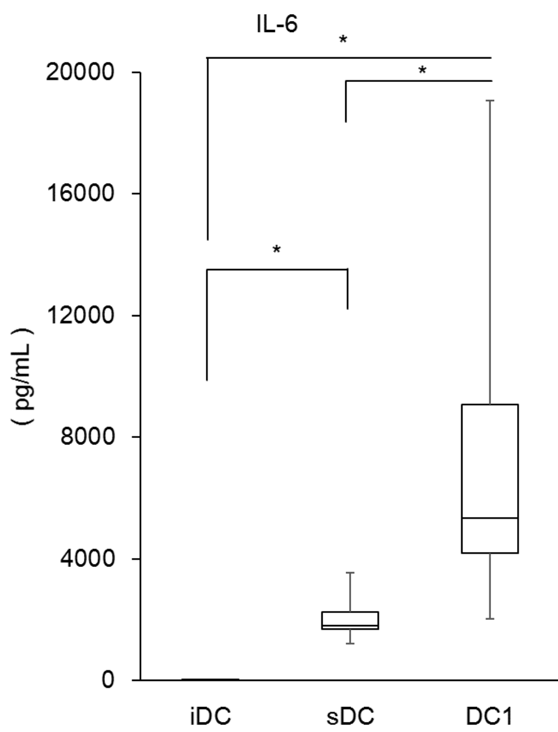
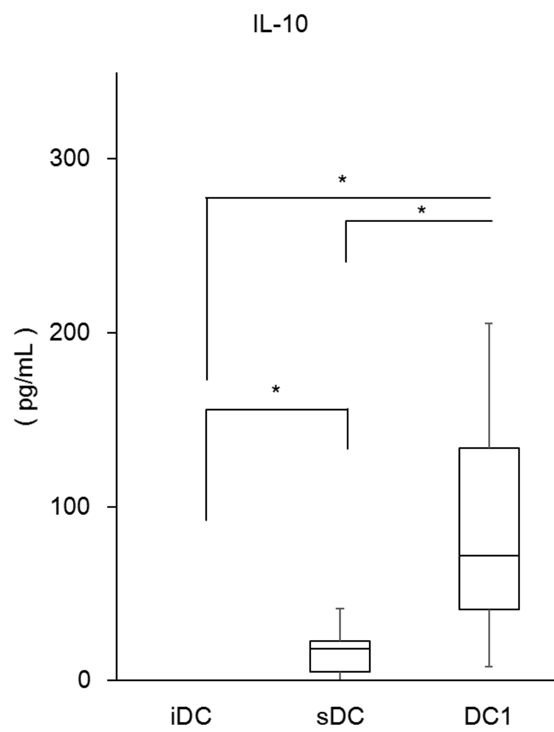
406 C D





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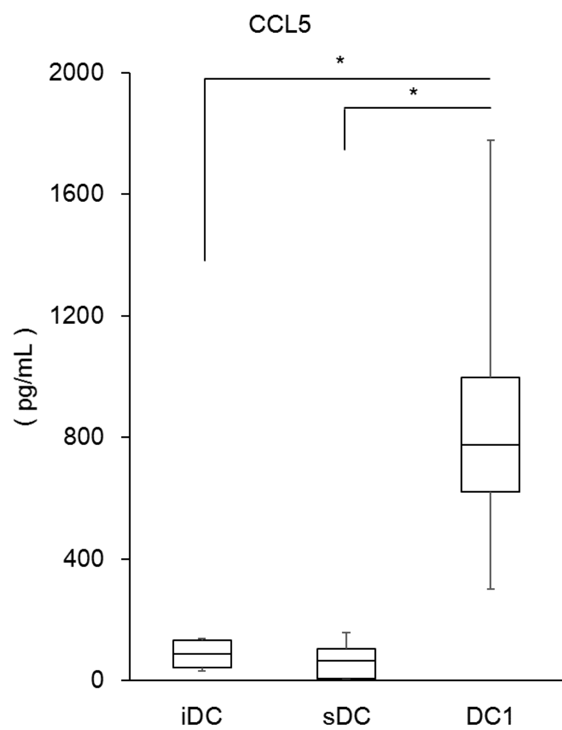

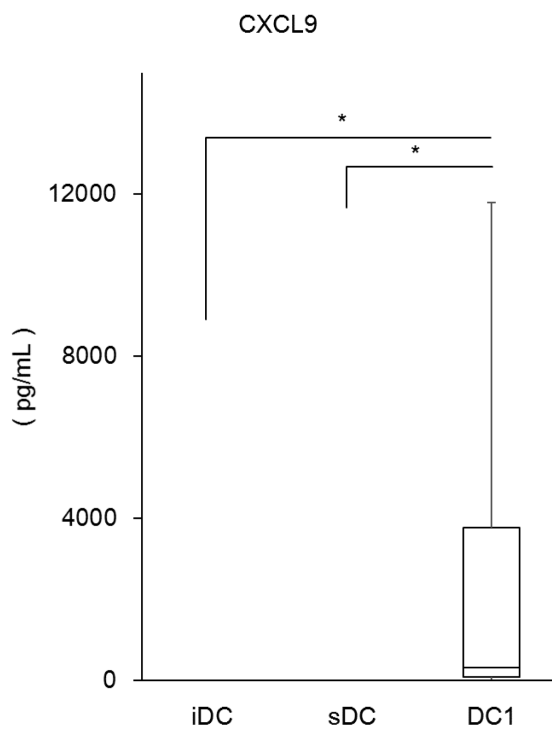

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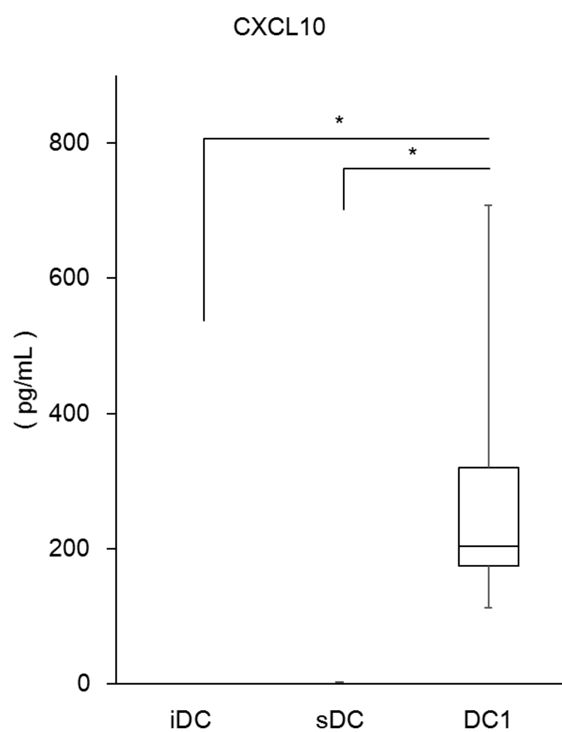
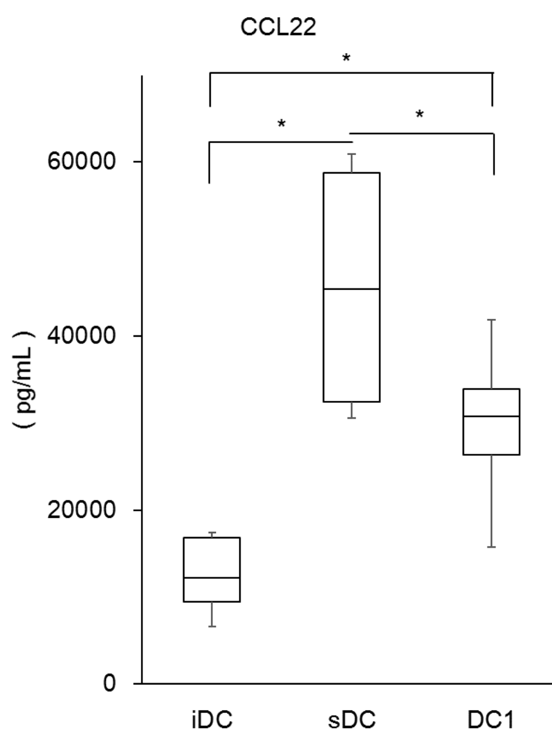
412 G H





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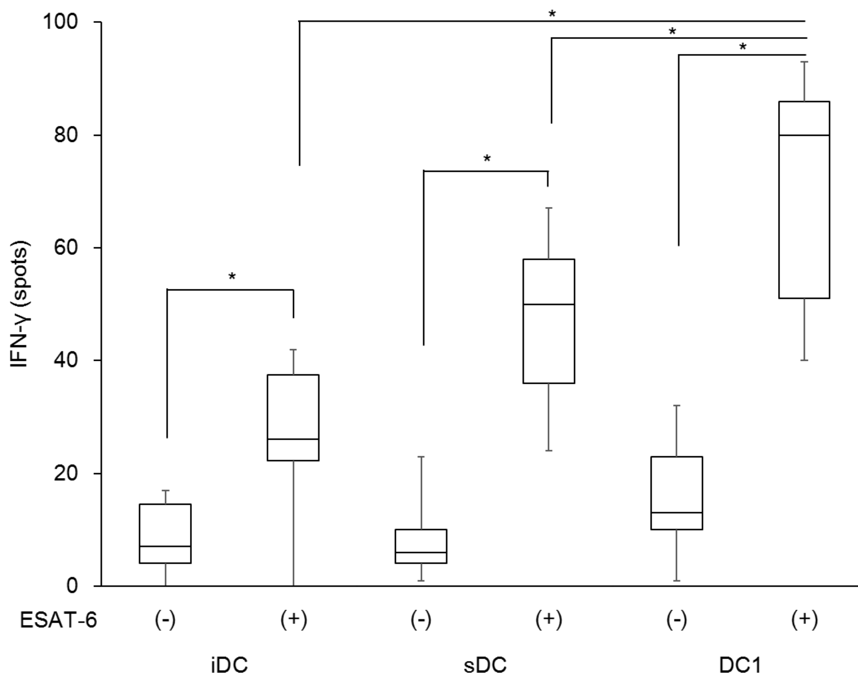
415 Figure 3.

416 A



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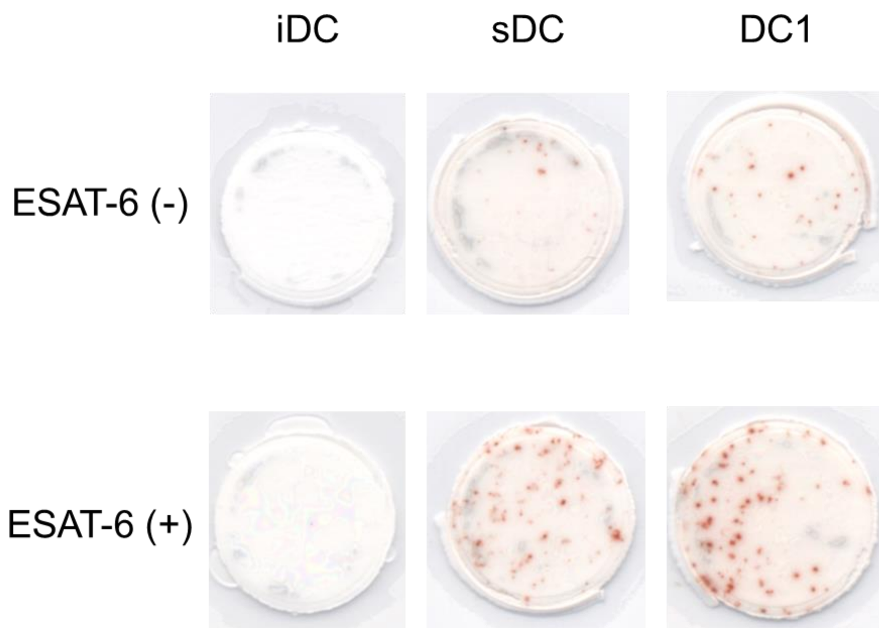

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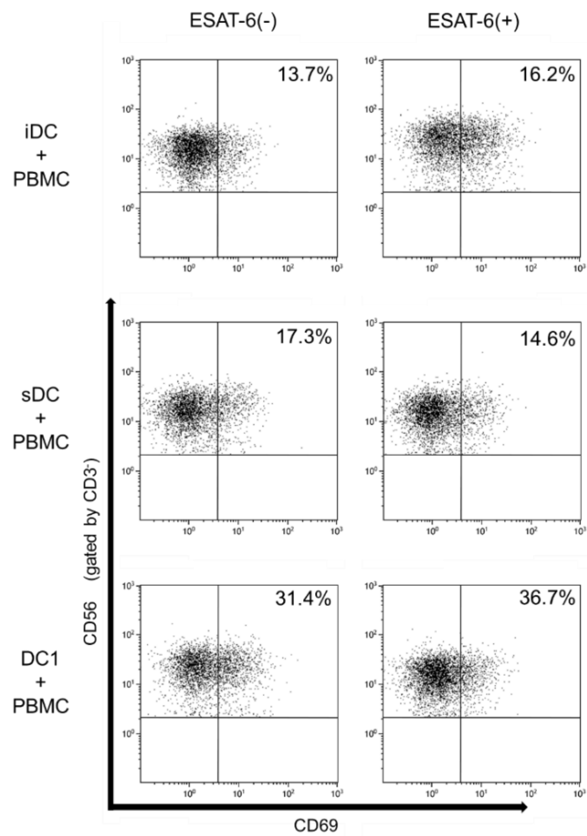
422

423 Figure 4.

424 A



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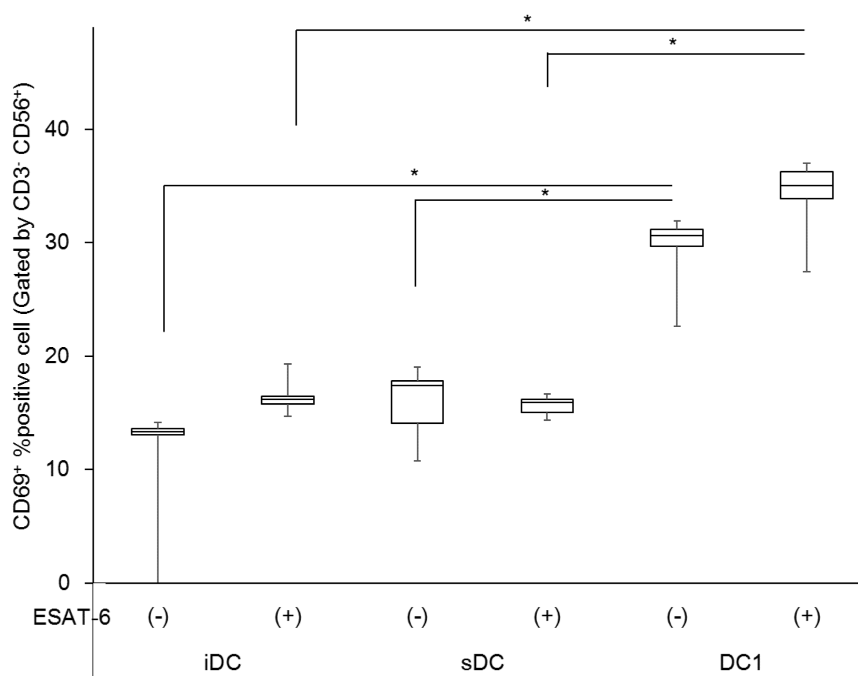
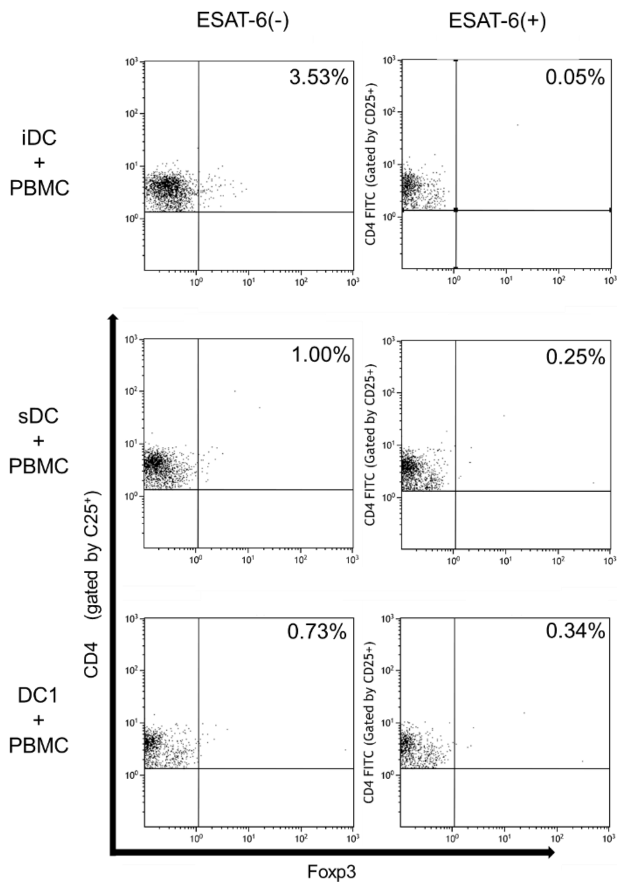


427

428

429 Figure 5.

430 A



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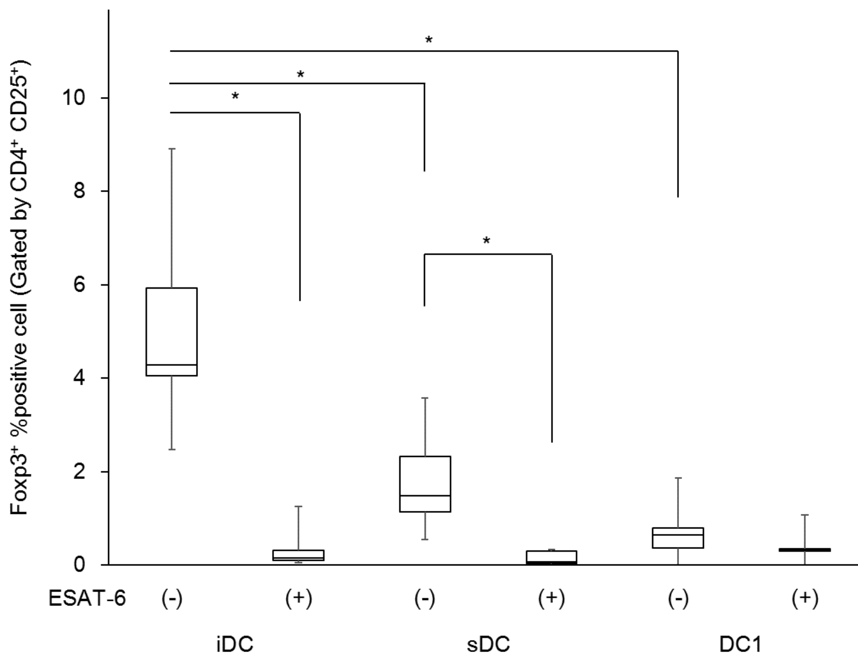

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