

Tolerogenic dendritic cells are efficiently generated using minocycline and dexamethasone

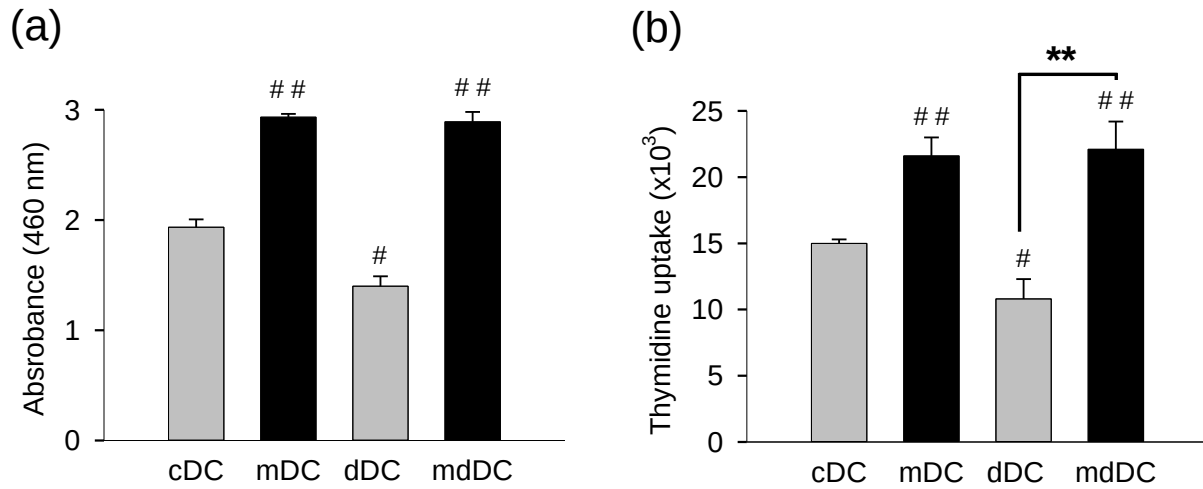
Jae-Hee Lee^{1,+}, Chan-Su Park^{1,+}, Sundong Jang^{1,+}, Ji-Wan Kim¹, Sang-Hyeon Kim¹, Sukgil Song¹, Kyungjae Kim², and Chong-Kil Lee^{1,*}

¹College of Pharmacy, Chungbuk National University, Cheongju 28644, South Korea

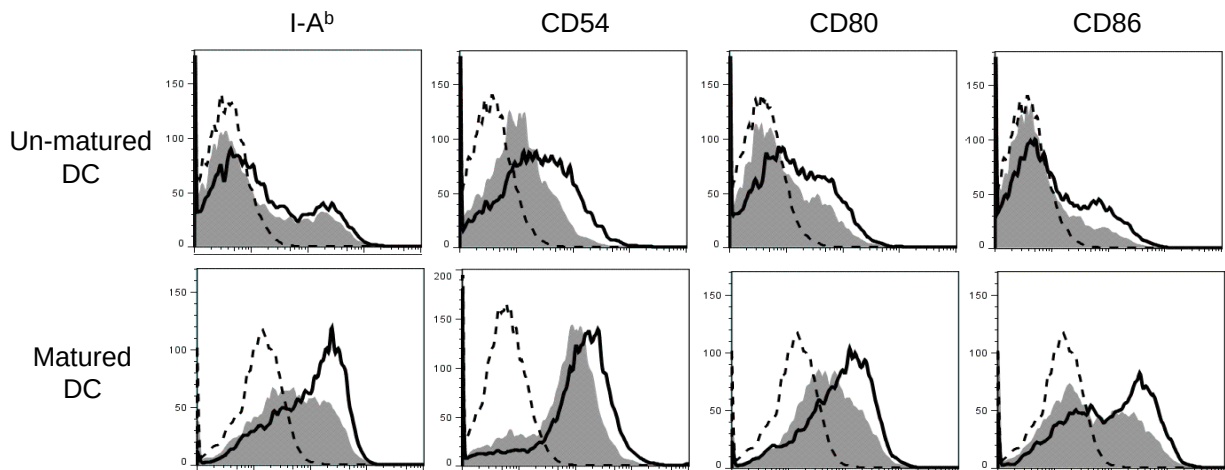
²College of Pharmacy, Sahmyook University, Seoul 01795, South Korea

* Corresponding author. cklee@chungbuk.ac.kr

⁺ These authors contributed equally in this work



Supplementary Figure S1. Growth-promoting activity of minocycline. **(a)** Immature cDCs, mDCs, dDCs, or mdDCs generated from BM cells of C57BL/6 mice were harvested on day 4 from the initiation of culture, washed, and added wells of 96-well plate (2×10^5 cells/well) in a culture containing minocycline (5 μ M, for mDCs), dexamethasone (1 μ M, for dDCs), or both (for mdDCs) together with 40 ng/ml GM-CSF and 40 ng/ml IL-4. After culturing for 24 h, 50 μ l of a solution containing 1 mg/ml XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) (Sigma-Aldrich) and 0.383 mg/ml phenazine methosulfate (PMS, Fluka, Buchs, Switzerland) diluted in PBS were added to each well and incubated for 4 h at 37 °C. Absorbance was measured at 460 nm. **(b)** Immature cDCs, mDCs, dDCs, or mdDCs generated from BM cells of C57BL/6 mice were harvested on day 4 from the initiation of culture, washed, and added wells of 96-well plate (2×10^5 cells/well) in a culture containing minocycline (5 μ M, for mDCs), dexamethasone (1 μ M, for dDCs), or both (for mdDCs) together with 40 ng/ml GM-CSF and 40 ng/ml IL-4. After incubating for 18 h, cells were pulsed with 1 μ Ci/ml [3 H]thymidine (Du Pont, Boston, MA, USA) for an additional 6 h. After harvesting the cells, [3 H]thymidine uptake was measured using a scintillation counter. The data are presented as the mean $\pm$ SD of three independent experiments performed in triplicate cultures. One way ANOVA tests were performed in order to evaluate significance. # $P < 0.05$, ## $P < 0.01$ compared with cDC group. ** $P < 0.01$ compared with matched group.



Supplementary Figure S2. mdDCs are refractory to maturation. cDCs and mdDCs were harvested on day 6 from the initiation of the culture (un-matured DC), or exposed to 50 ng/mL IFN- γ plus 50 ng/mL TNF- α for 24 h to induce maturation (matured DC), and analyzed by flow cytometry for the expression of I-A^b, CD54, CD80 and CD86. cDCs, black line; mdDCs, shaded area; isotype-matched control, dotted line. A representative set of histograms from 2 separate experiments was shown.