IL-15 Generates IFN-γ-producing Cells Reciprocally Expressing Lymphoid-Myeloid Markers during Dendritic Cell Differentiation

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Figure S1

A

B
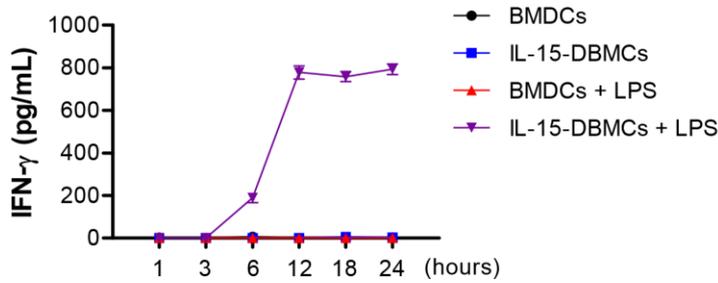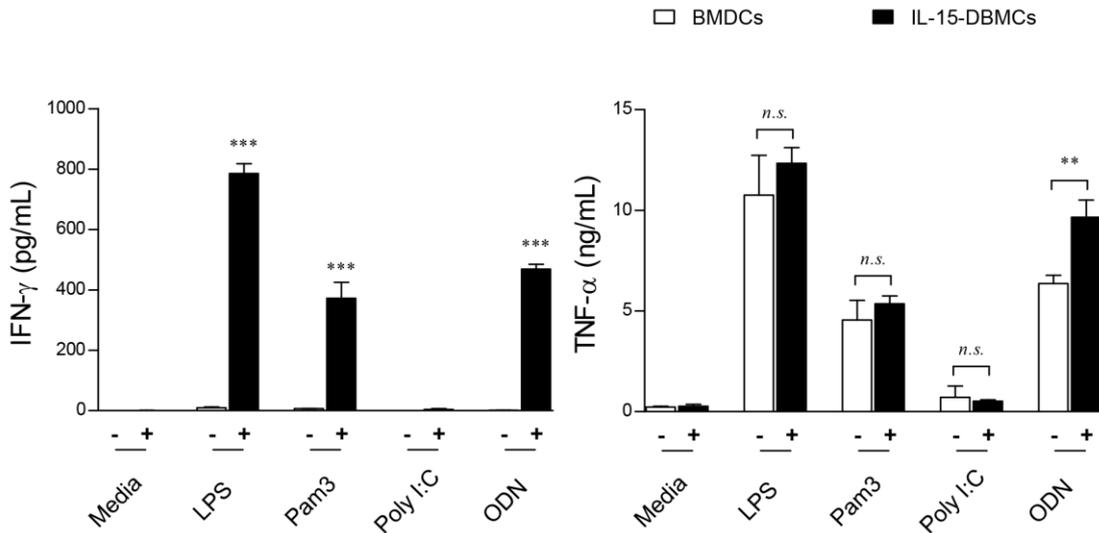
Figure S1. Distinct pattern of IFN-γ secretion upon various types of TLR stimulation between BMDCs and IL-15-DBMCs. BMDCs and IL-15-DBMCs (5×10^5 cells) were stimulated with LPS (100 ng/ml), Pam3 (0.2 μg/ml), Poly(I:C) (1 μg/ml), and ODN (1 μg/ml) for 24 h. (A) BMDCs and IL-15-DBMCs were stimulated with LPS for the same amount of time. (B) BMDCs and IL-15-DBMCs were stimulated with various TLR agonists. Then, cytokines were measured by ELISA using culture media. The results are expressed as the mean ± SD (n = 3) of representative results from three experiments. Significant differences were

1   determined using an unpaired *t*-test. *n.s.*; not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p <$

2   0.001 for comparisons between BMDCs and IL-15-DBMCs.
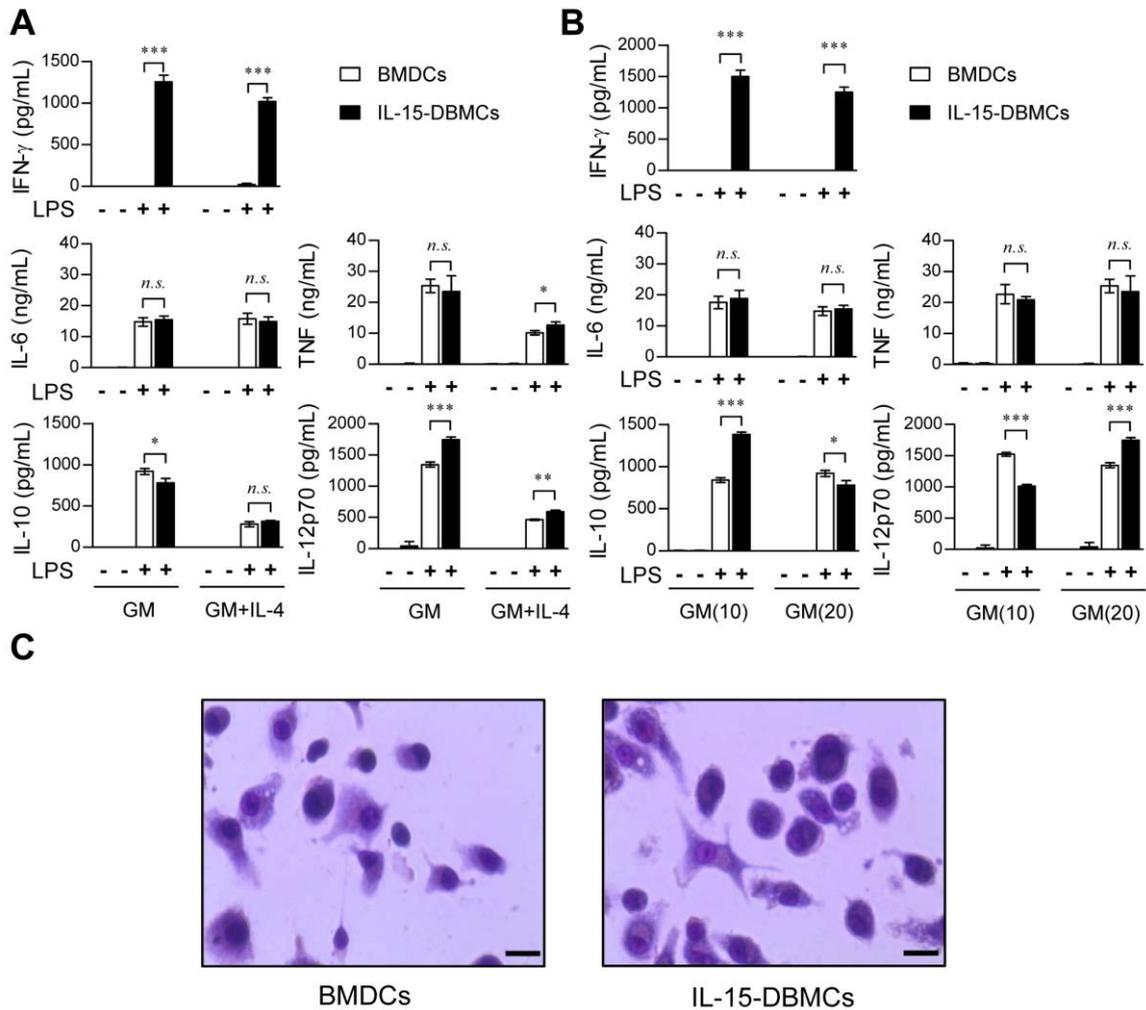
Figure S2

Figure S2. Effects of IL-4 and GM-CSF concentrations on the cytokine-producing properties of BMDCs and IL-15-DBMCs upon LPS stimulation. (A) Responsiveness of both BMDCs and IL-15-DBMCs to LPS (100 ng/ml) stimulation under either IL-4-containing (GM + IL-4) or GM-CSF-alone conditions (GM). (B) Cytokine levels measured at different concentrations of GM-CSF during differentiation of BMCs. (C) Cytospin preparations of each group of cells were stained with May-Grünwald-Giemsa. Cytokines were analyzed at 24 h after stimulation and measured by ELISA (mean ± SD; $n = 3$). Significant differences were determined using an unpaired t-test. $n.s.$; not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p <$

1   0.001 for comparisons between BMDCs and IL-15-DBMCs.
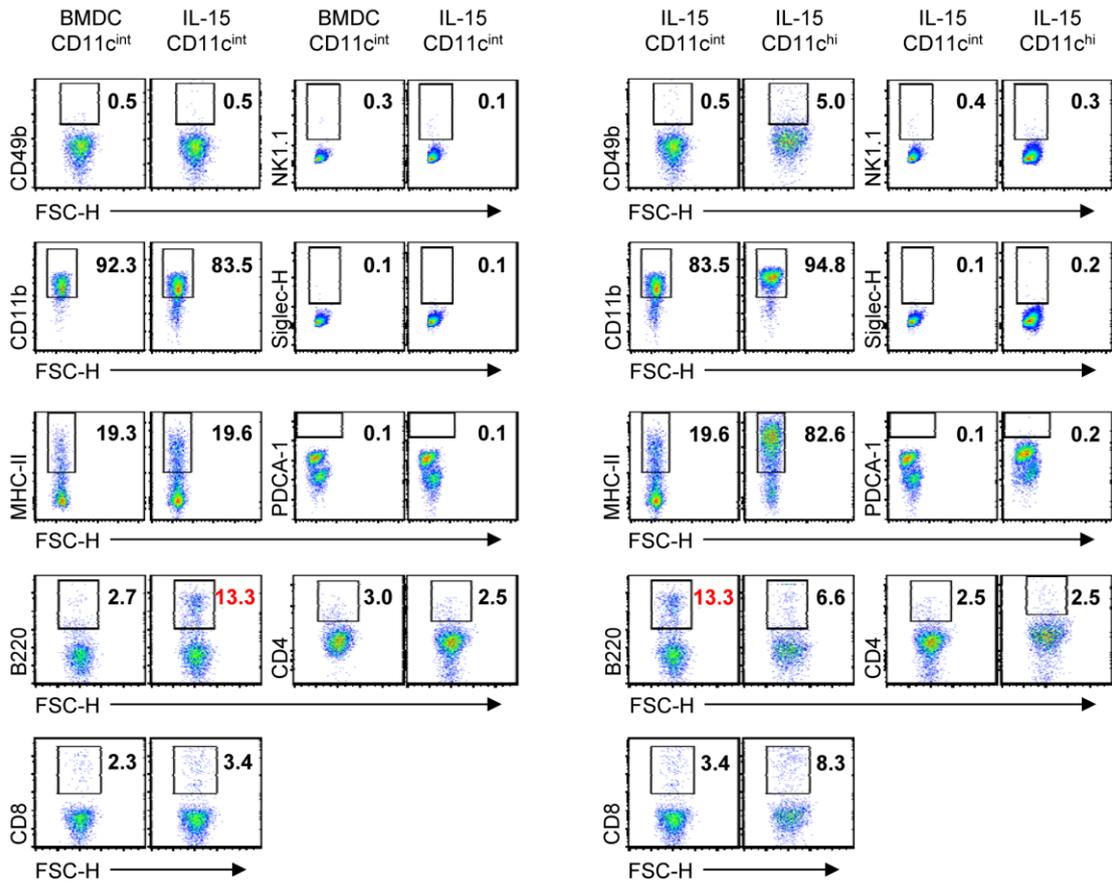
Figure S3

Figure S3. Differential surface expression profiles of IL-15-DBMC-derived CD11c^{int} cells compared to those of both IL-15-DBMC-derived CD11c^{hi} and BMDC-derived CD11c^{hi} cells. The IL-15-DBMC-derived CD11c^{int}CD11c^{hi} population and BMDC-derived CD11c^{int} population were stained for the indicated markers as described in Figure 2C. One representative plot from three independent experiments is described. The number of squares indicates the percentage of the corresponding population.

1 **Figure S4**
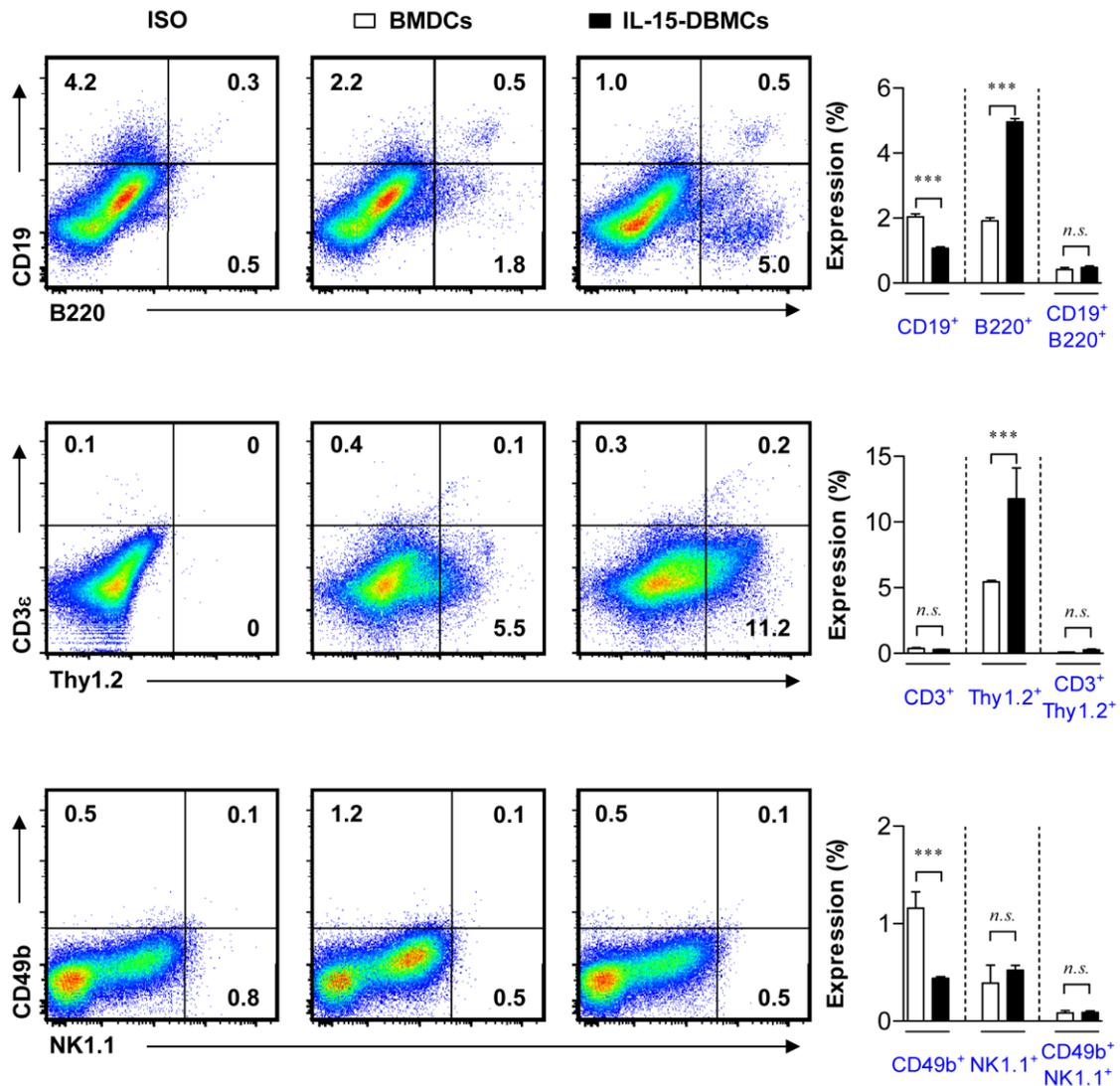
2

3 **Figure S4**. **Expression assessment of specific surface markers for B, T, and NK cells in**

4 **BMDMs and IL-15-DBMCs.** BMDCs and IL-15-DBMCs were collected on day six of culture

5 and were stained with the indicated markers. PE-conjugated anti-CD19 and APC-conjugated

6 anti-B220 antibodies were used to detect B cells (top); PE-conjugated anti-CD49b (DX5) and

7 APC-eFluor® 780-conjugated anti-NK1.1 antibodies were used to detect NK cells (middle);

8 and PE-conjugated anti-Thy1.2 and Alexa 647-conjugated anti-CD3 antibodies were used to

9 detect T cells (bottom). The numbers in the plots indicate the percentages of the corresponding

1   populations, and the percentage of expression of each surface markers is displayed in the bar

2   graphs. The data are presented as the mean ± SD ($n = 3$). Significant differences were

3   determined using an unpaired *t*-test. *n.s.*; not significant, *** $p < 0.001$ for comparisons

4   between BMDCs and IL-15-DBMCs.
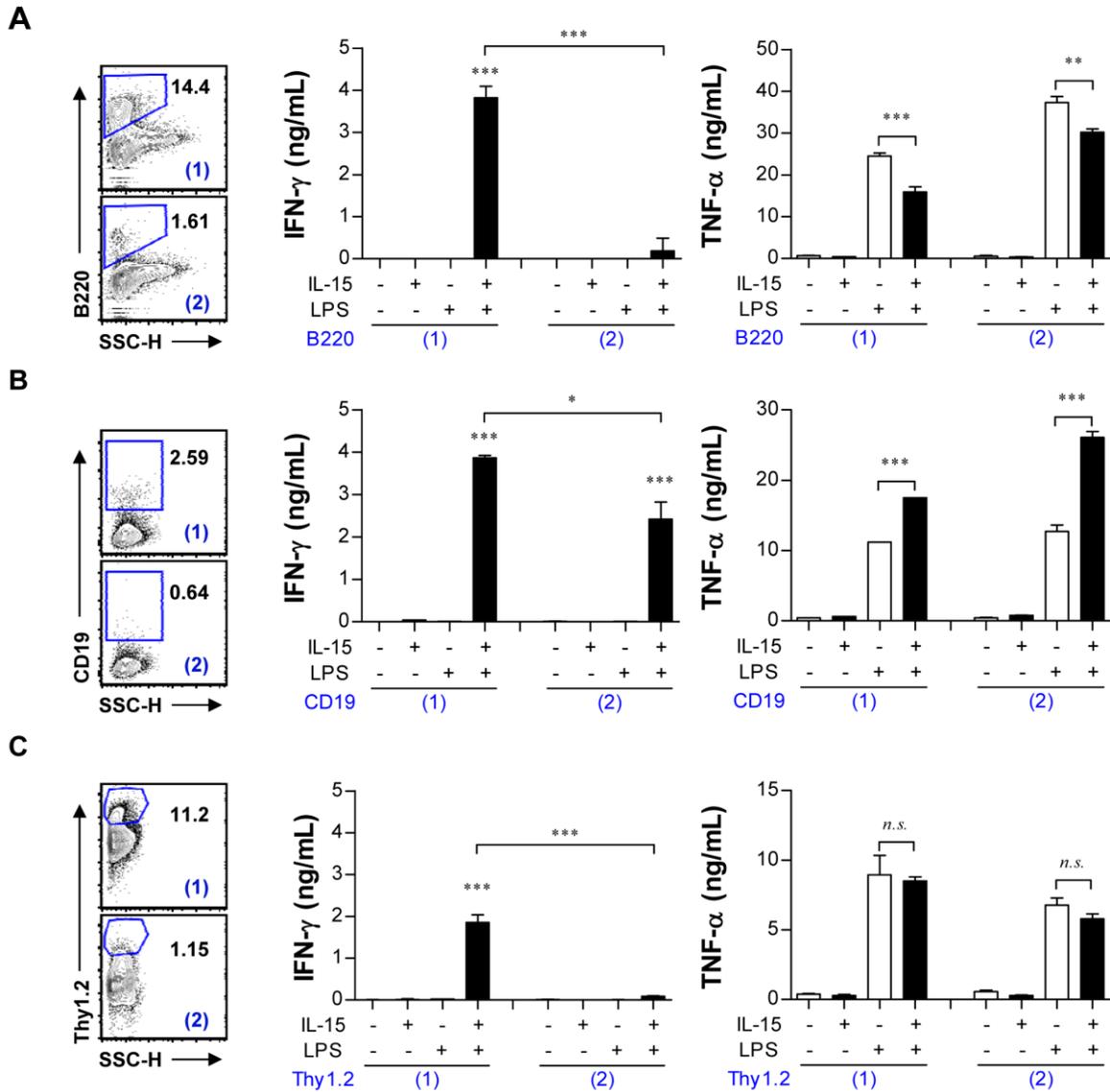
Figure S5

Figure S5. Depletion of B220- or Thy1.2-positive populations from IL-15-DBMCs

abolishes IFN-γ secretion. Each targeted cell population from BMDCs and IL-15-DBMCs

was selectively depleted using B220 MicroBeads (A), CD19 MicroBeads (B), and Thy1.2

MicroBeads (C). The cells represented in (1) were not subjected to MACS sorting, and the cells

represented in (2) were deficient in the molecule corresponding to each Microbead. The

number of squares indicates the percentage of the corresponding populations of Groups (1) and

(2). Groups (1) and (2) from BMDCs and IL-15-DBMCs were stimulated with LPS, and the

1   cytokines were measured. The data are presented as the mean $\pm$ SD ($n = 3$). Significant

2   differences were determined using an unpaired *t*-test. *n.s.*; not significant, * $p < 0.05$, ** $p <$

3   0.01, and *** $p < 0.001$.

2
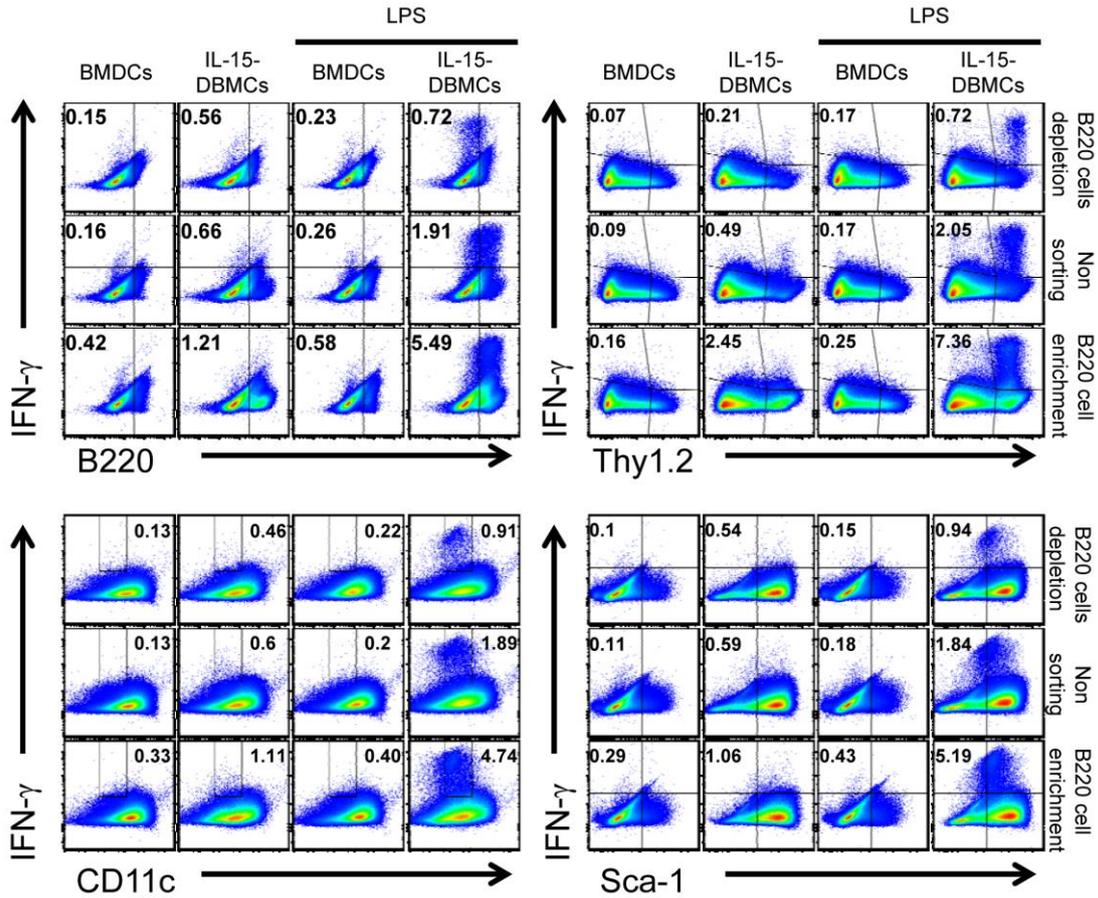
Figure S6. IL-15-derived cells expressing B220$^+$, Thy1.2$^+$, CD11c^{int}, and Sca-1$^+$ are the source of IFN-γ. Related to Figure 5B, B220-enriched, B220-depleted, and non-sorted populations from BMDCs and IL-15-DBMCs simultaneously expressed IFN-γ with B220, Thy1.2, CD11c, and Sca-1. The co-expression of IFN-γ and other surface markers is plotted for B220-enriched IL-15-DBMCs. One representative plot from three independent experiments is described. The numbers in the plots indicate the percentages of the corresponding populations.