Supplemental Figure Legends

Supplemental Figure 1. NK cells reside in close proximity to CD4+ T cells in the parafollicular T cell areas of swollen lymph nodes.

(A) A hematoxylin-eosin-stained biopsy of a swollen lymph node (×10). (B, C) The same specimen was immunohistochemically stained using anti-CD4 (brown) and anti-CD56 (red) antibodies. Representative images of parafollicular T cells areas at low (×10) (B) and high (×40) (C) magnification are shown.

Supplemental Figure 2. T cell growth inhibition is not restored by the addition of excess DCs or exogenous IL-2, or by isolation from DCs.

(A) PKH26-labeled, naive CD4 T cells were co-cultured with allo-TNF-DCs or allo-LPS-DCs. Activated NK cells were added the following day at an NK:T ratio of 0:10 or 1:10, together with different concentrations of IL-2 (0, 1, 10, or 100 ng/ml). At day 4 of DC-stimulation, the proliferating T cell numbers were evaluated. The data shown are representative of two independent experiments. (B) PKH26-labeled, naive CD4 T cells were applied at 1 x 10^5 cells/well, which is similar to other proliferation assays, and co-cultured with allo-TNF-DCs at a DC:T ratio of 1:5, 2:5, or 5:5. The

following day, activated NK cells were added to the culture at an NK:DC:T ratio of 1:1:5, 1:2:5, or 1:5:5. At day 4 of DC-stimulation, the numbers of proliferating (open bars) and non-proliferating (filled bars) CD4 T cells were assessed. (C) PKH26-labeled, naive CD4 T cells were co-cultured with allo-TNF-DCs or allo-LPS-DCs for one day. The whole population of CD4 T cells was then isolated from DCs using a cell sorter, and incubated with activated NK cells at an NK:T ratio of 0:10 or 1:10 for three additional days. The numbers of proliferating (open bars) and non-proliferating (filled bars) CD4 T cells were then assessed. The data shown are representative of two independent experiments.

Supplemental Figure 3. Kinetics of CD25 expression and analysis of the dilution of PKH26 signals in allo-DC-stimulated CD4 T cells.

PKH26-labeled, naive $CD4^+$ T cells were co-cultured with allo-TNF-DCs. On days 1, 2, and 3 of DC-stimulation, the cells were harvested and stained with anti-CD4 and anti-CD25 mAbs. The CD4 T cell population was identified by gating on $CD4^+$ $PKH26^+$ cells and the CD25 expression levels and dilution of PKH26 signals were sequentially measured by flow cytometry.

Supplemental Figure 4. Kinetics of MICA/B, HLA class I, and HLA-E expression on non-activated CD4 T cells.

PKH26-labeled, naive CD4 T cells were stimulated with either allo-TNF-DCs or allo-LPS-DCs for three days. To examine the expression kinetics of MICA/B, HLA-E, and HLA class I molecules on non-activated T cells, cells were stained with anti-CD4 mAb, anti-CD25 mAb and antibodies specific to the molecules for 4-color flow cytometric analysis. The non-activated T cell subpopulation was identified by gating on $CD25^{-}$ CD4 T cells at day 1 or on PKH^{high} CD4 T cells at day 3. The ΔMFI of the indicated molecules on non-activated CD4 T cells at days 1 and 3 as well as on resting CD4 T cells on day 0 was then evaluated. Each unique symbol represents an identical donor. (A) The kinetics of MICA/B expression on non-activated T cells in five independent experiments. (B) The kinetics of HLA class I molecule~~s~~ expression on non-activated T cells in three independent experiments. (C) The kinetics of HLA-E expression on non-activated T cells in five independent experiments.

Supplemental Figure 5. TCR signaling and IFN-β are involved in the upregulation of HLA-E on antigen-activated T cells.

(A) Unlabeled, naive CD4 T cells were stimulated with 100 μg/ml of plate-bound

anti-CD3 mAb plus 5 μg/ml of soluble anti-CD28 mAb (referred to as stimulation with anti-CD3/28 mAbs) for three days. To assess the kinetics of HLA-E expression on activated T cells, the cells were stained with PE-conjugated anti-HLA-E mAb together with PC7-conjugated anti-CD4 mAb and APC-conjugated anti-CD25 mAb in flow cytometric analysis, and the activated T cell population was defined by gating on $CD25^+$ CD4 T cells. The ΔMFI of HLA-E on $CD25^+$, activated CD4 T cells at day 1 and day 3 and on resting naive CD4 T cells on day 0 was evaluated in three independent experiments. (B) Unlabeled, naive CD4 T cells were stimulated with anti-CD3/28 mAbs in the presence or absence of 20ng/ml of IFN-β. HLA-E expression on $CD25^+$, activated CD4 T cells was evaluated at day 1 by flow cytometry using PE-conjugated anti-HLA-E mAbs, as described for Supplemental Figure 5A, in three independent experiments. (C) Unlabeled, naive CD4 T cells were co-cultured with allo-TNF-DCs or allo-LPS-DCs in the presence or absence of 20ng/ml of IFN-β. At day 1, the cells were stained with unconjugated anti-HLA-E mAbs followed by staining with APC-conjugated GAM, PC7-conjugated anti-CD4 mAb and FITC-conjugated anti-CD25 mAb. HLA-E expression on alloantigen-activated CD4 T cells (defined as $CD25^+$ CD4 T cells) was evaluated by flow cytometry in four experiments, two each for TNF-DC-activated T cells and LPS-DC-activated T cells. (D) Monocyte-derived immature DCs were seeded

at a concentration of 1 x 10^6 cells/ml and stimulated with 10 ng/ml of TNF-α or 100 ng/ml of LPS for 24 hours. The supernatants were then collected and the concentration of IFN-β was measured by ELISA in three independent experiments. Each unique symbol represents an identical donor. (E) Unlabeled, naive CD4 T cells were co-cultured with allo-LPS-DCs in the presence or absence of 20 μg/ml of anti-IFN-α/β receptor blocking mAbs. HLA-E expression on $CD25^+$, alloantigen-activated CD4 T cells was evaluated at day 1 by flow cytometry using PE-conjugated anti-HLA-E mAbs, as described in Supplemental Figure 5A, in two independent experiments.

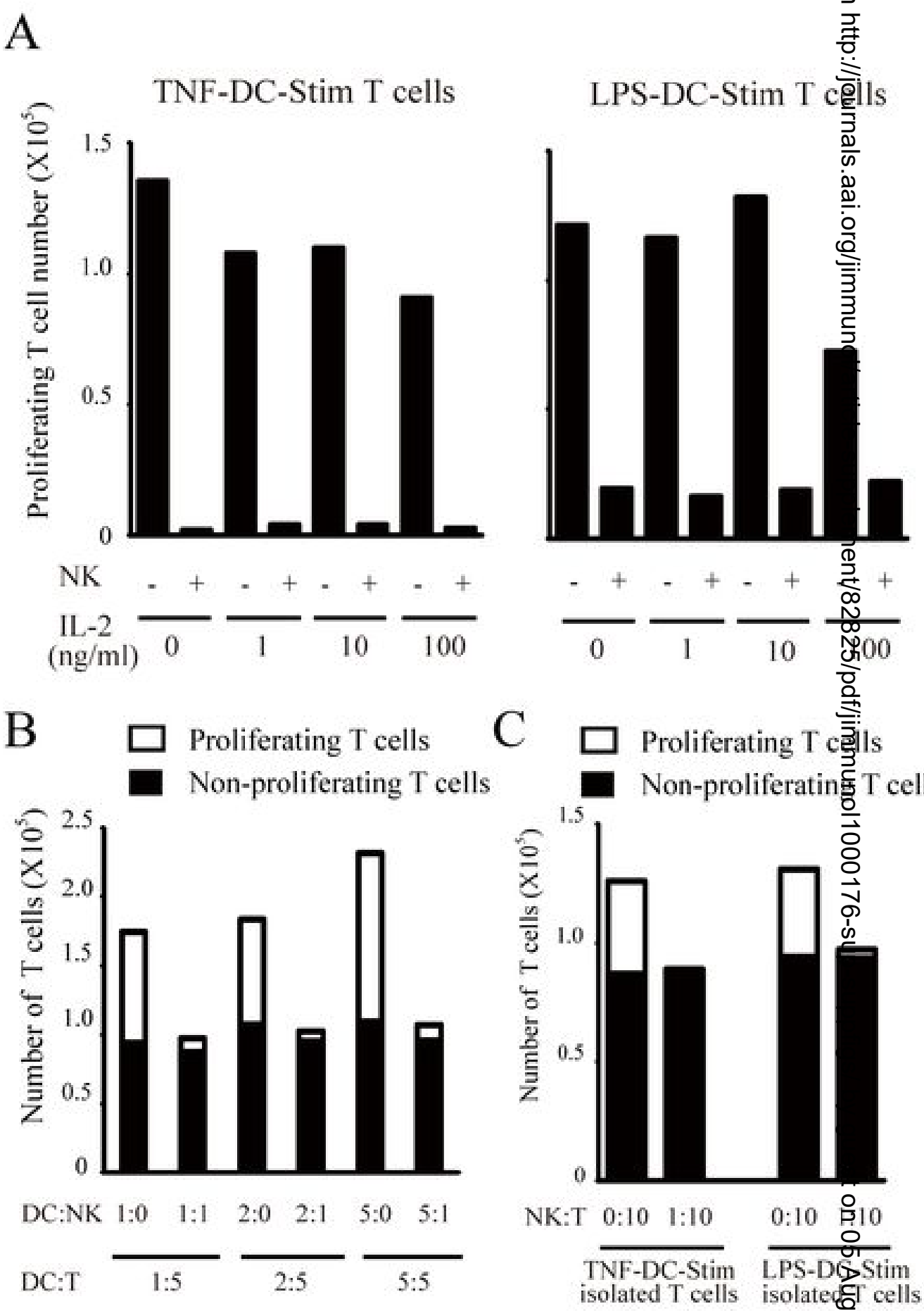
Supplemental Figure 1

A

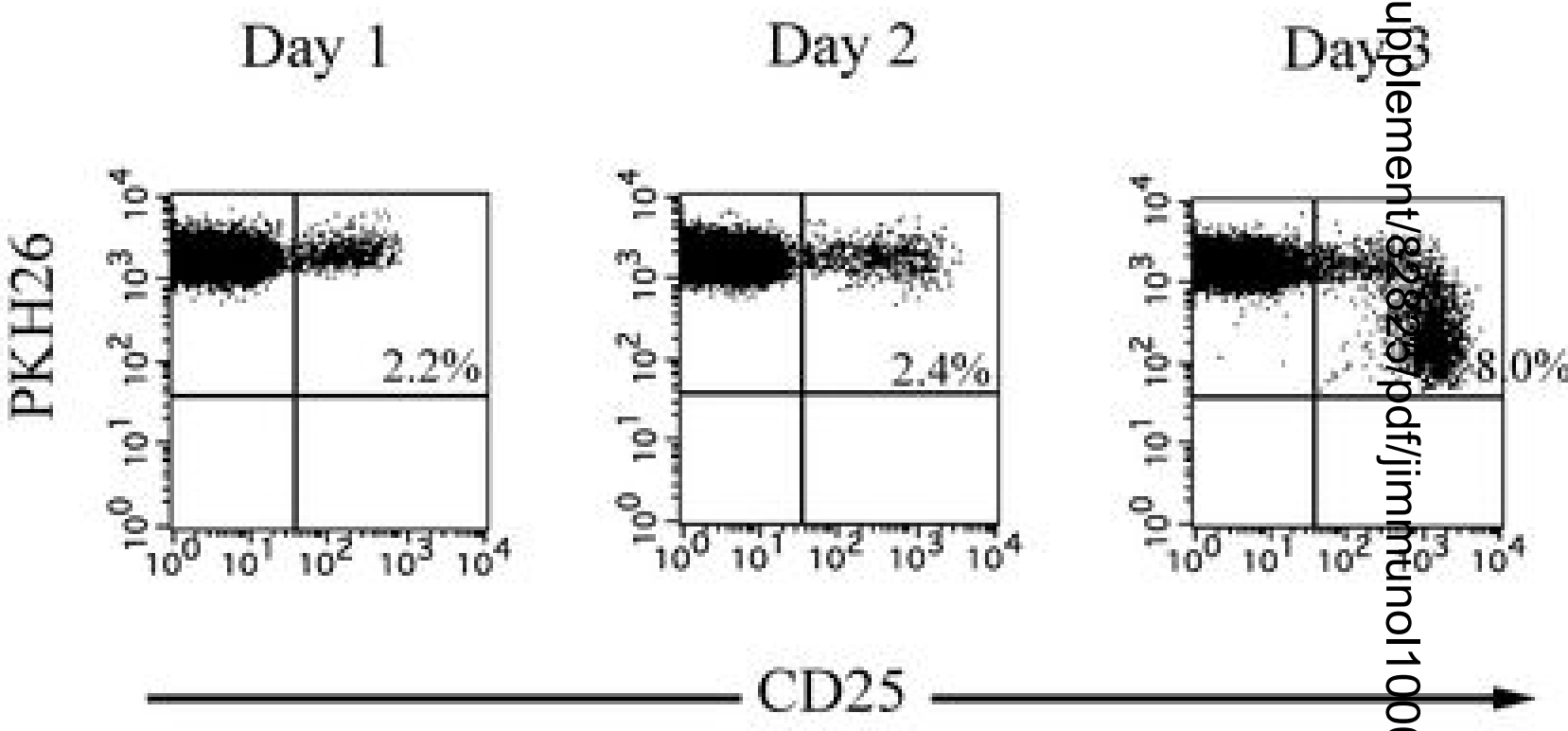
B

C

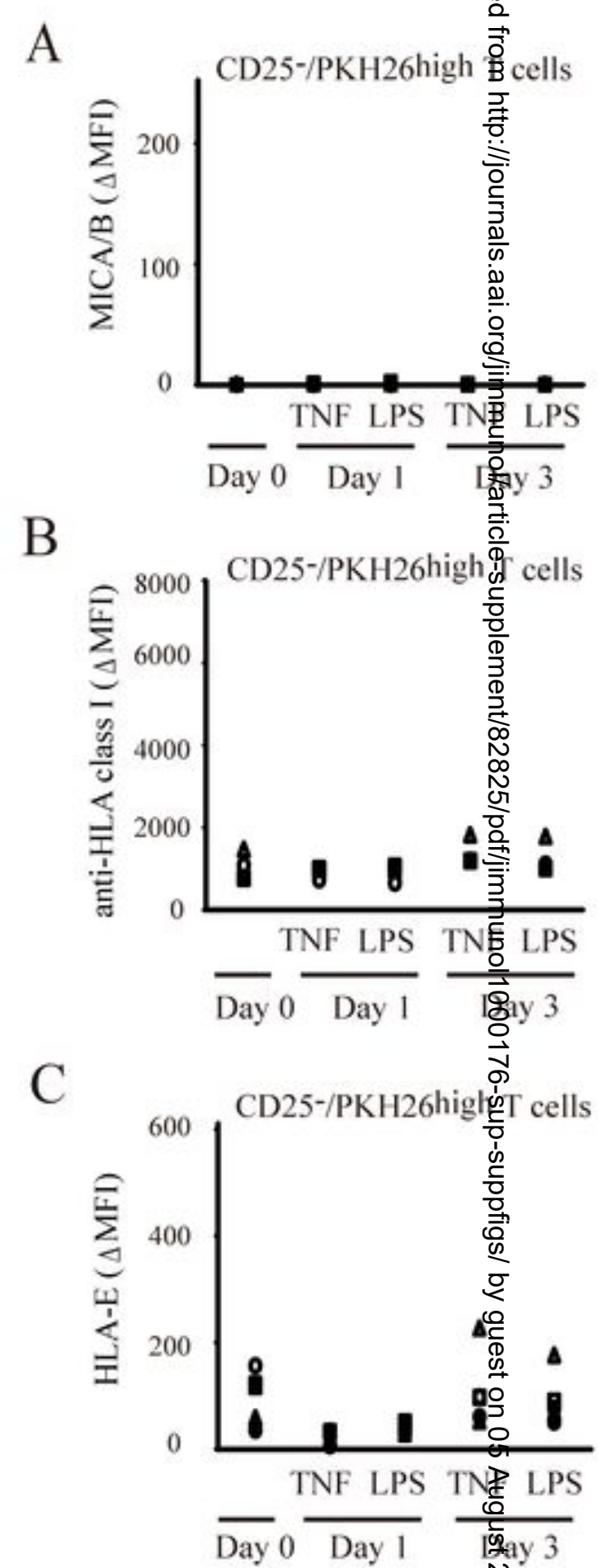
Supplemental Figure 2



Suppelmental Figure 3



Supplemental Figure 4



Supplemental Figure 5