

Supplemental Figure legends

Supplemental Figure 1 Gut-associated sites, but not bone marrow, support extrathymic CD4⁺CD8⁺ cells after transplant. Analysis of CD4⁺CD8⁺ bone marrow-derived cells after BMT alone by flow cytometry. **(A)** Representative dot plot showing CD4 and CD8 on cells from preT origin at day 14 after BMT in BM. Data representative of at least five mice. **(B)** Representative dot plots showing CD4⁺CD8⁺ cells from preT origin at day 14 after BMT in Peyer's patches, small bowel epithelium, and small bowel lamina propria. Data representative of at least five mice.

Supplemental Figure 2 PreT possess greater T cell potential than BM cells in athymic recipients. Splenocytes in CD45.1⁺CD45.2⁺ B6 → CD45.2⁺ B6 **(A-B)** or B6 nude **(C-D)** recipients receiving preT or DN2 preT (CD45.1⁺) 28 days post-BMT. White bars: host; gray bars: BM; black bars: preT. PreT → WT, n=3; DN2 preT → WT, n=7; PreT → nude, n=5; DN2 → preT nude, n=9. Combined from 2-3 experiments. **(E-F)** Splenocytes in CD45.1⁺CD45.2⁺ B6 → CD45.2⁺ B6 **(E)** or B6 nude **(F)** recipients receiving preT or DN2 preT (CD45.1⁺) 28 days post-BMT. White bars: host; gray bars: BM; black bars: preT. PreT → WT, n=3; DN2 preT → WT, n=7; PreT → nude, n=5; DN2 → preT nude, n=9. Combined from 2-3 experiments.

Supplemental Figure 3 Ability to support DP in MLN is not associated with changes in architecture or adhesion molecule expression. Lymph node stromal cells were analyzed by flow cytometry after enzymatic dissociation. **(A)** Analysis of CD45⁻ lymph node cells from nontransplanted BALB/c or BALB/c → BALB/c + preT recipients for fibroblastic reticular cells (FRC, gp38⁺CD31⁻), lymphatic endothelial cells (LEC, gp38⁺CD31⁺), blood endothelial cells (BEC, gp38⁻CD31⁺), and double negative (DN, gp38⁻CD31⁻) populations. n=3, representing 15 pooled mice from

three (of five) representative experiments. **(B)** Analysis of CD45- lymph node cells from nonirradiated B6 nude mice or B6 nude mice on day 7 post-lethal irradiation for FRC, LEC, BEC, and DN populations. n=6 each, combined from two experiments. **(C-F)** Representative histograms of FRC and BEC from **B**: **(C and E)** P-selectin on BEC in MLN and PLN, and **(D and F)** ICAM and VCAM on FRC in MLN and PLN. Black lines represent nonirradiated controls and gray shaded histograms represent cells on day 7 post-irradiation.

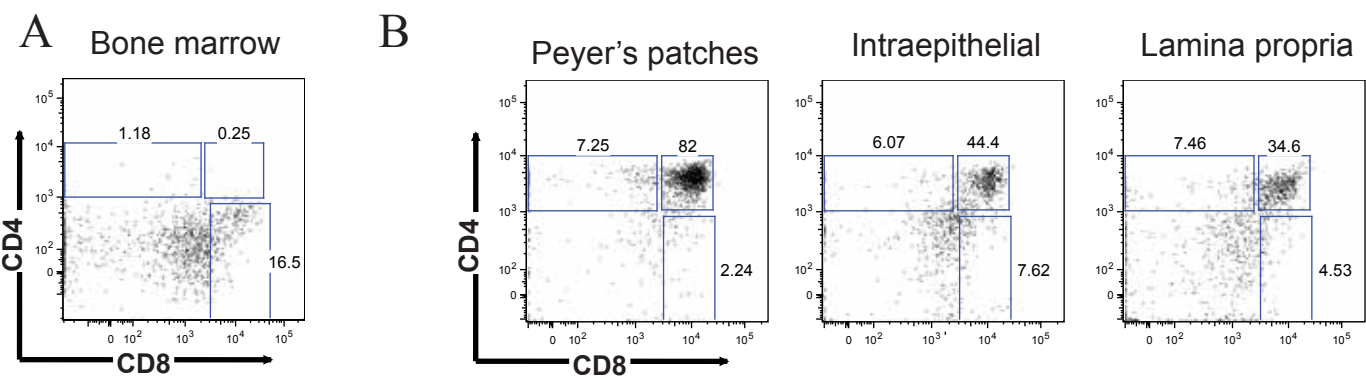
Supplemental Figure 4 Extrathymic T cell development is less susceptible to aging than the thymic counterpart. **(A)** CD4⁺CD8⁺ cells in the thymus and MLN of 3 month old (n=10) and 9 month old (n=9) recipients at day 21 post-BMT (CD45.2⁺ CBF1 → CD45.2⁺ CBF1). Data combined from two experiments. **(B)** Percentage of CD4⁺CD8⁺ cells in thymus and MLN in **A**. **(C)** Representative plot showing BM-derived cells at day 21 in thymus and MLN after BMT in **A**. **(D)** Representative histograms of CD4⁺CD8⁺ cells from BM origin in thymus (gray shaded histogram) and MLN (black line) of 24 month old recipients. **(E)** Representative dot plot of CD4 versus CD8 in MLN of 12 month old nontransplanted B6 mouse (n=5, one experiment).

Supplemental Figure 5 Extrathymic-derived T cells repopulate peripheral and gut-associated lymphoid tissues after BMT in athymic recipients. Analysis of mature T cells at day 42 after congenic BMT (CD45.1⁺CD45.2⁺ B6 → CD45.2⁺ B6 WT or nude). **(A-C)** Quantification of mature T cells in nude mice (n=3), nude BMT alone (n=16), or nude BMT+preT recipients (n=9) (mean±sem) in **(A)** MLN, **(B)** Peyer's patches, and **(C)** PLN. Data combined from one to three experiments. Analyzed by nonparametric Mann-Whitney test; * signifies $p<0.05$; ** signifies $p<0.01$. White bars: host; gray bars: BM; black bars: preT. **(D)** Quantification of small bowel IEL in

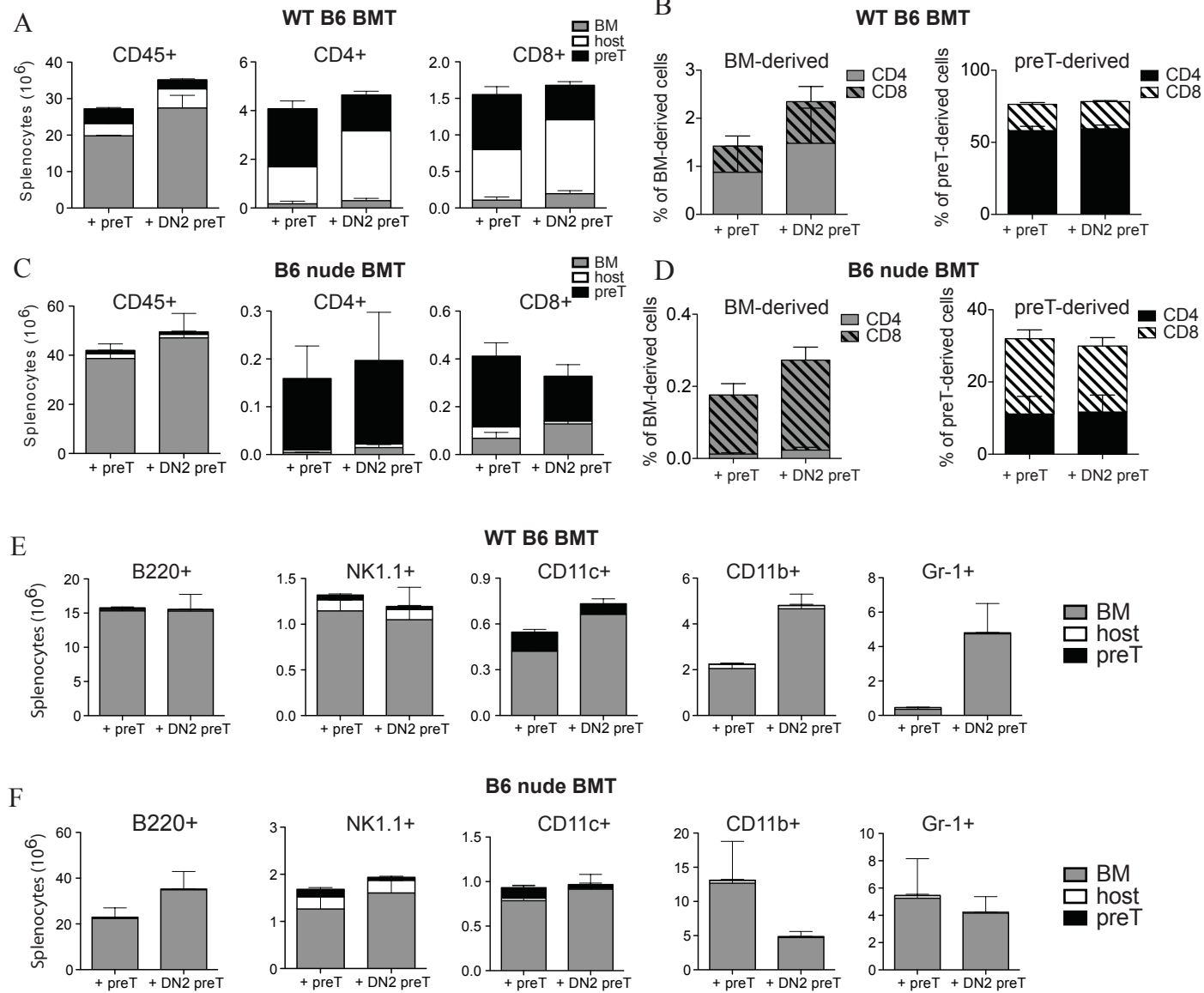
euthymic (n=5) and athymic (n=7) BMT recipients at day 42 after transplant. Data combined from one to two experiments.

Supplemental Figure 6 Recipients of preT have greater numbers of extrathymic T cell development even after preT are no longer present. **(A-B)** T cell subsets in (CD45.2⁺ BALB/c→ CD45.2⁺ BALB/c) BMT only (WT, n=7; nude, n=7) or BMT + preT (CD45.1⁺ B6; WT, n= 3; nude, n=7) recipients 4.5 months after transplant. Data combined from one to two experiments. Gray bars: BM + host; black bars: preT. **(A)** WT recipients. **(B)** nude recipients.

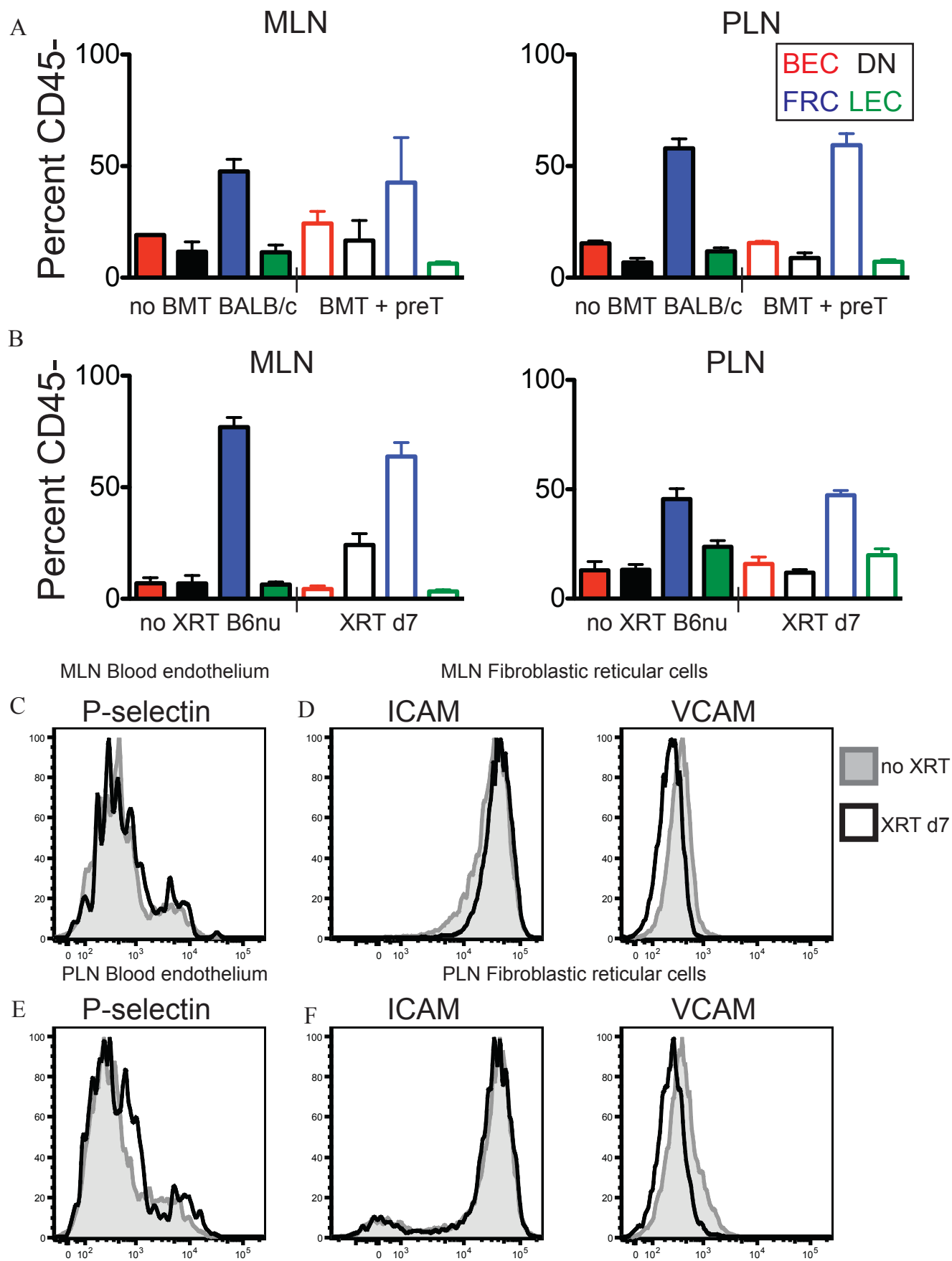
Supplemental figure 1



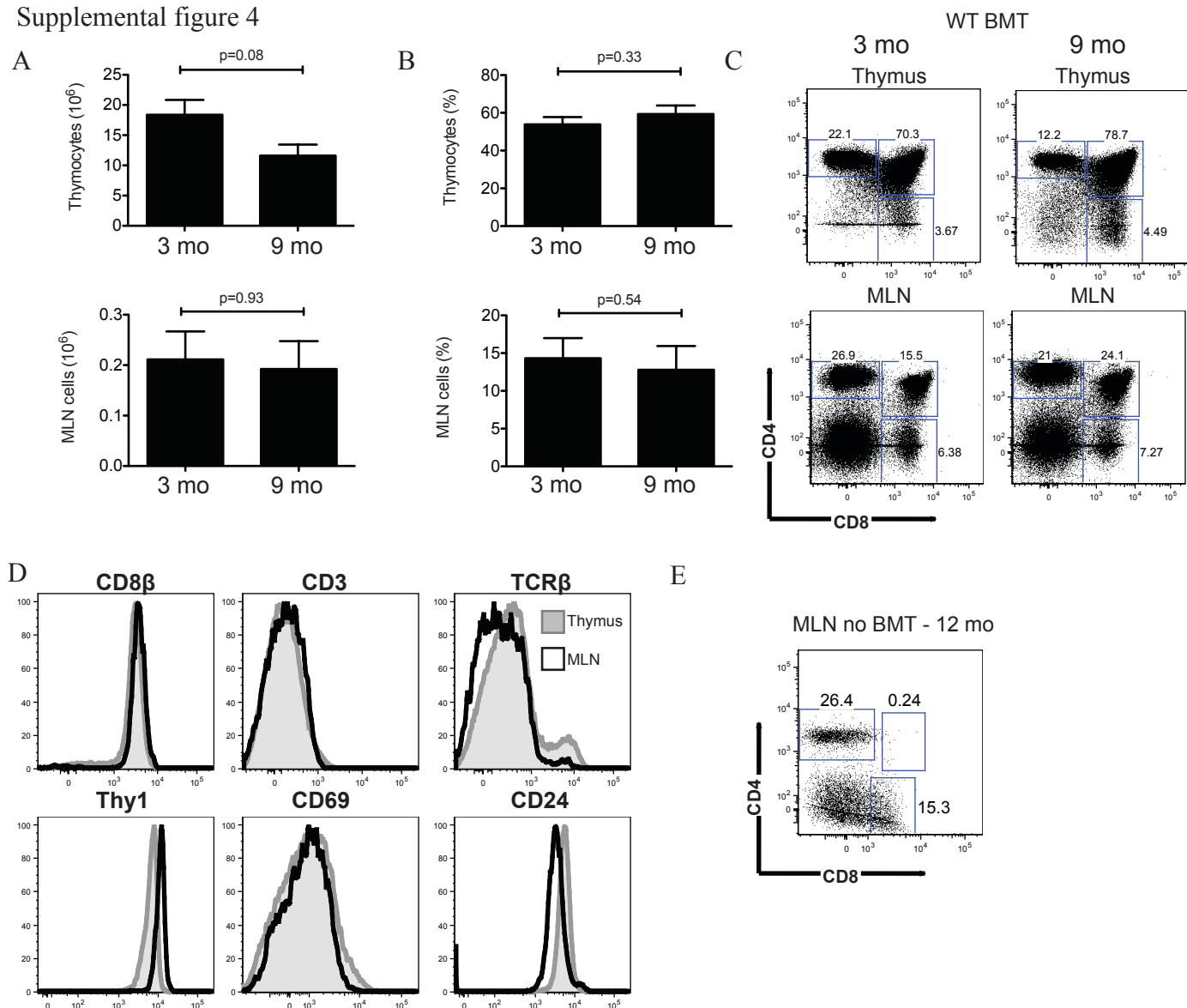
Supplemental figure 2



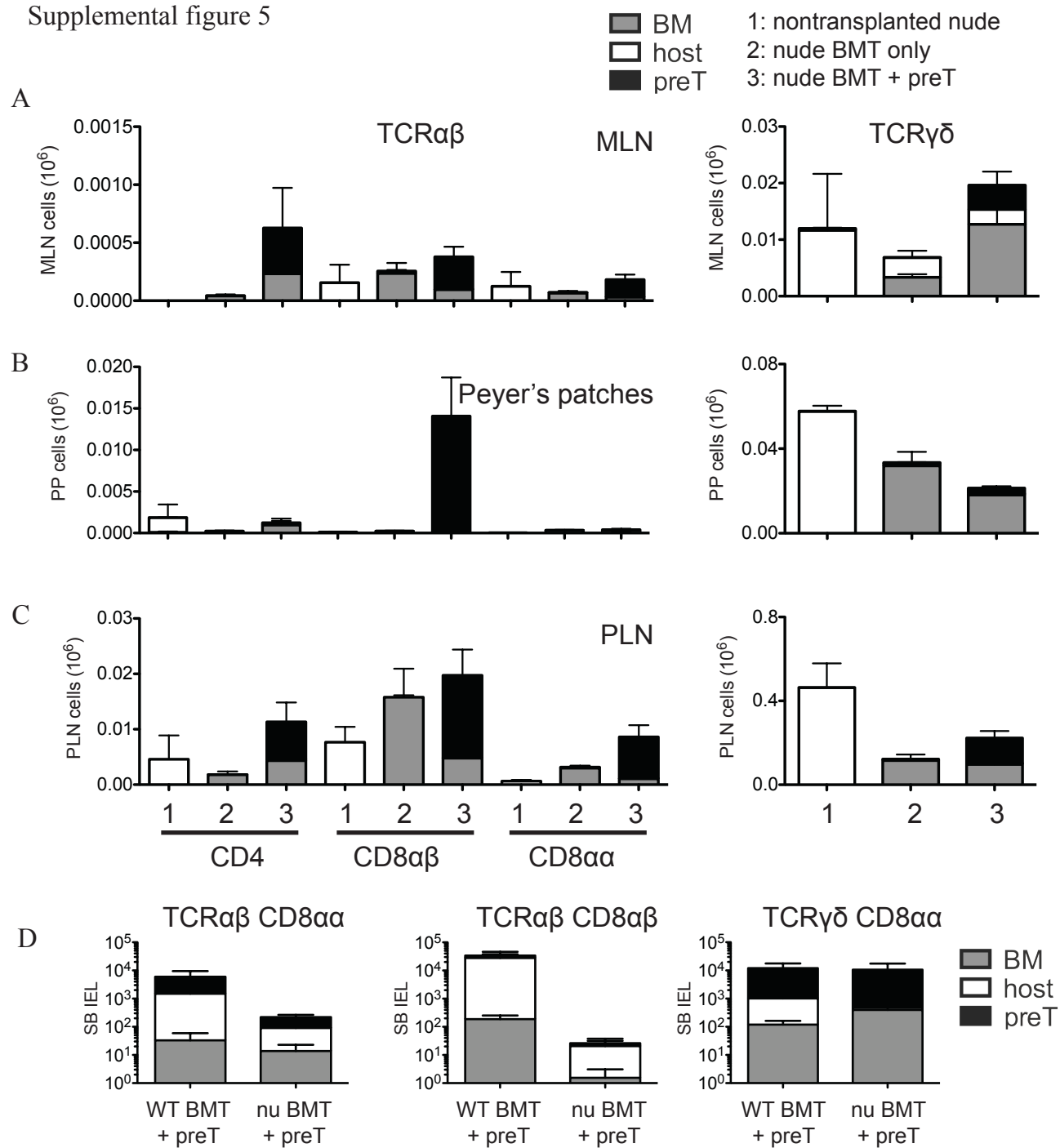
Supplemental figure 3



Supplemental figure 4



Supplemental figure 5



Supplemental figure 6

