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 marrowderived 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 \rightarrow 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 BMderived 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 gutassociated lymphoid tissues after BMT in athymic recipients. Analysis of mature T cells at day 42 after congenic BMT (CD45.1⁺CD45.2⁺ B6 \rightarrow 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 $(CD45.2^+ BALB/c \rightarrow CD45.2^+ BALB/c) BMT$ 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

Small intestine В A Bone marrow Lamina propria Intraepithelial Peyer's patches 10⁵ 10⁵ · 10⁵ -10⁵ 1.18 0.25 6.07 44.4 7.46 7.25 34.6 10⁴ 82 10⁴ 10⁴ · 10' 10³ I 10³ 10³ 10³ CD4 CD4 10² 16.5 10² -2.24 7.62 10² 4.53 10² 0 10⁵ 10⁵ 10⁵ ^{0 10²} 10⁴ 105 0 10² 1 10⁴ 10³ ' . 10³ 10³ 0 10² 10⁴ 0 10² 10³ 10⁴

Supplemental figure 2 В WT B6 BMT WT B6 BMT A CD8+ BM bost preT CD45+ CD4+ % of BM-derived cells % of preT-derived cells 6-2.0 **BM-derived** preT-derived 40 Splenocytes (10⁶) 100₁ CD4 3-CD4 CD8 30 1.5 4 2-20 1.0 50 2 1. 10 0.5 0.0 0 0 0 0 + preT + DN2 preT CD8+ С B6 nude BMT D B6 nude BMT % of preT-derived cells preT-derived CD4 CD45+_ CD4+ % of BM-derived cells 0.0 **BM-derived** 60 Splenocytes (10⁶) 0.3 0.6 40-CD4 CD8 40 0.2 0.4 20 20 0.1 0.2 + preT + DN2 preT 0.0 0 0.0 0 + preT + DN2 preT WT B6 BMT Е B220+ NK1.1+ CD11c+ CD11b+ Gr-1+ 1.5 r 0.9 6т 8-20 -Splenocytes (10⁶) 🗖 BM 15 6 1.0 0.6 4 □ host 10 · 4 preT 0.5 0.3 2 2 5 0. 0.0 0 0.0 0 + preT + DN2 preT B6 nude BMT F B220+ NK1.1+ CD11c+ CD11b+ Gr-1+ 3-60 20 -10 -1.5 Splenocytes (10⁶) 8. 15 🗖 BM 2 40 1.0 6 □ host 10 4 preT 1 20 0.5 5 2 0 0 0 0. 0.0

+ preT + DN2 preT

+ preT + DN2 preT

+ preT + DN2 preT

+ DN2 preT

+ preT

+ preT + DN2 preT

Supplemental figure 3





0

10

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10⁴ 10⁵

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10⁴

9 mo

Thymus

MLN

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10⁵

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