SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. B cells are a minor population in the donor T cell allograft after magnetic separation, and do not significantly influence the course of thymic GVHD

(A) B6 spleens were first stained with B220-PE or isotype-PE antibody (control), stained with anti-PE antibody, and then negatively selected with anti-PE magnetic to remove B cells. These negatively selected cells were then stained with anti-CD5 magnetic beads and positively selected for T cells. Flow cytometric analyses of remaining B cells (as defined by $CD19^+$ cells or $B220^+$ cells) after B cell depletion or sham depletion with isotype-PE are depicted. Representative plots from one of two independent experiments.

(B) Irradiated BALB/c mice (8.5 Gy) were transplanted with 5×10^6 B6 $CD45.1^+$ TCD-BM ± 0.25×10^6 B-cell replete or B-cell depleted B6 splenic T cells, as prepared in A. Numbers of donor BM-derived $CD45.1^+$ $CD4^+CD8^+$ (DP) thymocytes in all groups were analyzed on day 28 post-transplant. TCD-BM only group, N=9. BM+T cells group, N=12. BM+ B-cell-depleted T cells group, N=16. Combined data from two independent experiments.

Supplemental Figure 2. A low dose of donor T cells in the allograft does not cause significant GVHD mortality

5×10^6 B6 CD45.1 TCD-BM ± $CD5^+$ B6-background T cells → BALB/c (8.5 Gy). The overall survival was assessed until harvest for analysis on day 28. Combined data from 7 experiments. A total of 100 recipients are shown.

Supplemental Figure 3. Thymic GVHD occurs in a clinically-relevant MHC-matched minor antigen-disparate GVHD model system

5×10^6 B6 $CD45.1^+$ TCD-BM ± 1×10^5, 5×10^5, or 1×10^6 B6 $Thy1.1^+$ T cells → LP (12 Gy). N=5 in TCD-BM group, N=7-8 in all T cell-replete groups.

(**A**) Donor CD45.1$^+$ CD4$^+$CD8$^+$ (DP) thymocytes were enumerated on day 28 post-transplant. *p<0.05 versus TCD-BM control.

(**B**) Weight change and (**C**) clinical GVHD score of transplanted mice from A, to four weeks.

5×10^6 B6 CD45.1$^+$ TCD-BM ± 0.5×10^5, 1×10^5, or 2.5×10^5 B6 Thy1.1$^+$ T cells → LP (12 Gy). N=5 in TCD-BM group, N=7-8 in all T cell-replete groups.

(**D**) Donor CD45.1$^+$ CD4$^+$CD8$^+$ (DP) thymocytes were enumerated on day 42 post-transplant. *p<0.05 versus TCD-BM control.

(**E**) Weight change and (**F**) clinical GVHD score of transplanted mice from D, to six weeks.

Supplemental Figure 4. Low doses of alloreactive donor T cells mediate a partially reversible form of thymic GVHD, which is associated with sustained mild systemic GVHD at late time-points post-transplant

5×10^6 B6 CD45.1$^+$ TCD-BM ± 0.5×10^5, 1×10^5, or 2.5×10^5 B6 CD45.2$^+$ T cells → BALB/c (8.5 Gy).

(**A**) Total thymocytes were enumerated on day 60 post-transplant. N=4-8/group. *p<0.05 versus TCD-BM control.

(**B**) Donor bone marrow (BM)-derived CD4$^+$CD8$^+$ thymocytes were subsetted into DN, DP, CD4, and CD8+ thymocytes, and were enumerated on day 60 (mean ± SEM). *p<0.05 versus TCD-BM control for each population. N=4-8/group. Donor bone marrow derived cells are gated as CD45.1$^+$.

(**C**) Weight change and (**D**) clinical GVHD score of transplanted mice from A-B to nine weeks post-transplant are shown.

Supplemental Figure 5. Donor alloreactive T cells quickly infiltrate the thymus and undergo expansion after allo-BMT

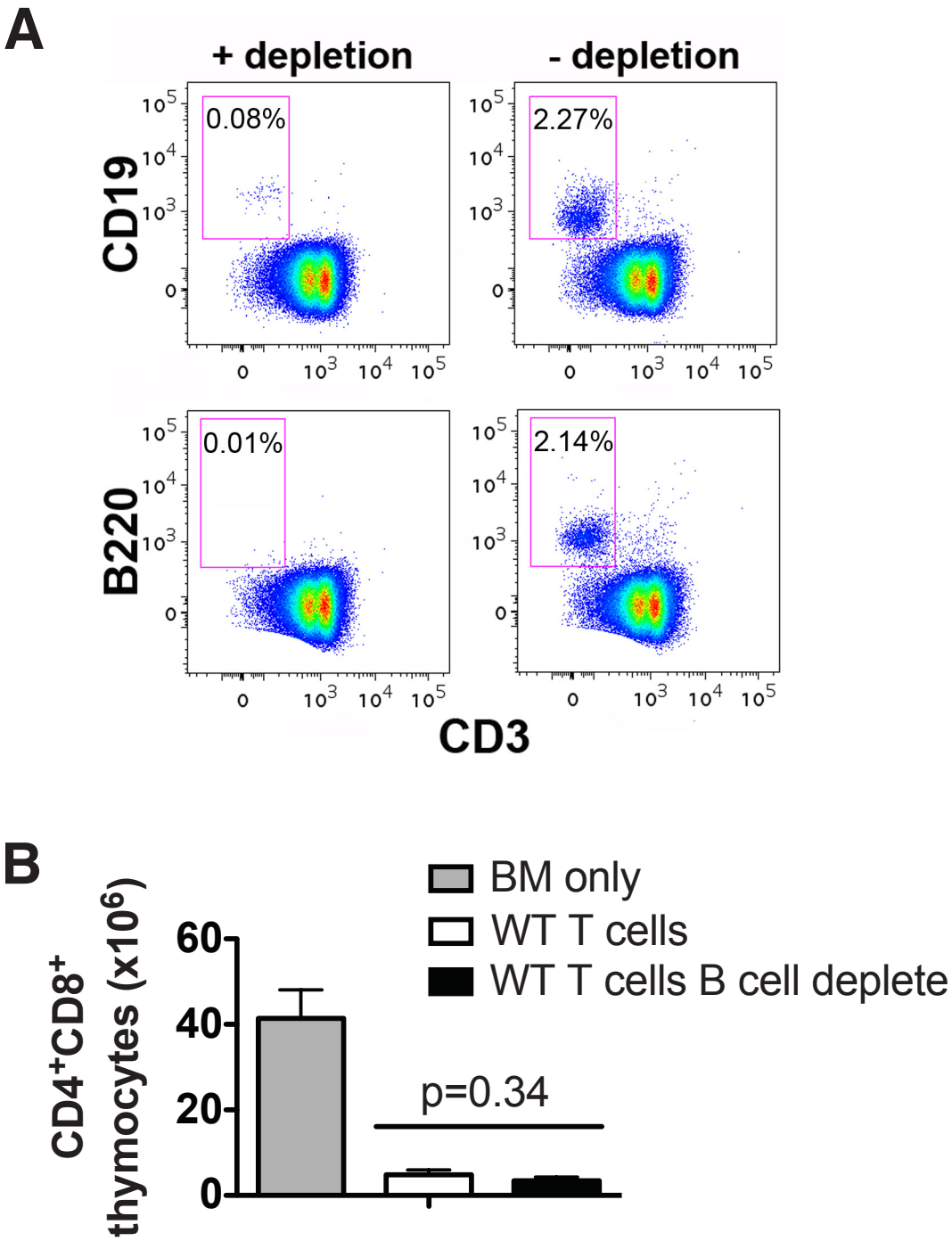
5×10^6 B6 TCD-BM + 0.5 x10^6 (**A**), 1 x10^6 (**B**) and 10 x10^6 (**C**) $CD5^+$ $luciferase^+$ T → BALB/c (8.5 Gy). *In vivo* bioluminescence imaging (BLI) revealed an expansion of alloreactive T cells in lymphoid tissues and gastrointestinal parenchyma by day 2-3. One representative animal is shown. *Ex vivo* imaging of the thymus confirmed thymic infiltration by $luciferase^+$ donor T cells on day 4-5. Representative image from one of two identical experiments shown.

Supplemental Figure 6. β_7 subunit and PSGL-1 are indirectly involved in tGVHD

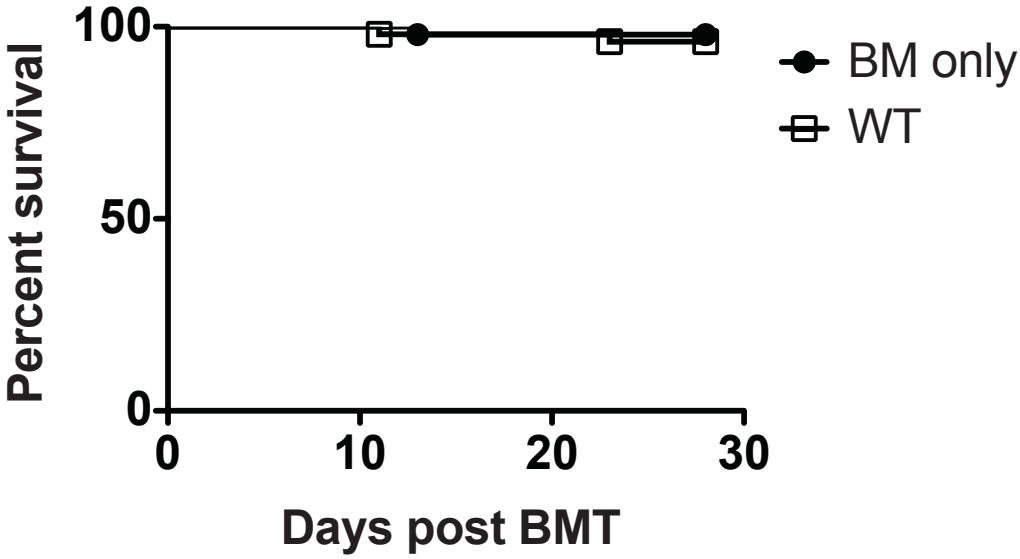
(**A**) $CD45.1^+$CFSE B6 $CD8^+$ T cells were mixed in a 1:1 ratio with either $CFSE^+$ $CD45.2^+$ WT or $CFSE^+$ CD45.2 $\beta_7^{-/-}$ B6 CD8 T cells, and 5×10^6 mixed cells were transferred into irradiated BALB/c mice. Donor thymic and splenic infiltrating CD8 T cells were analyzed at day 6. N=5/group.

(**B**) $CD45.1^+$CFSE B6 T cells were mixed in a 1:1 ratio with either $CFSE^+$ $CD45.2^+$ WT T or $CFSE^+$ CD45.2 PSGL-$1^{-/-}$ B6 T cells and adoptively transferred into irradiated BALB/c mice. Graph depicts relative ratios of donor CD8 T cells that were recovered from the spleen and thymus on day 6. N=10/group. * $p< 0.05$, ** $p< 0.001$ for percentage of $CD45.1^-$ B6.KO vs. B6.WT T cells. One of two identical experiments shown.

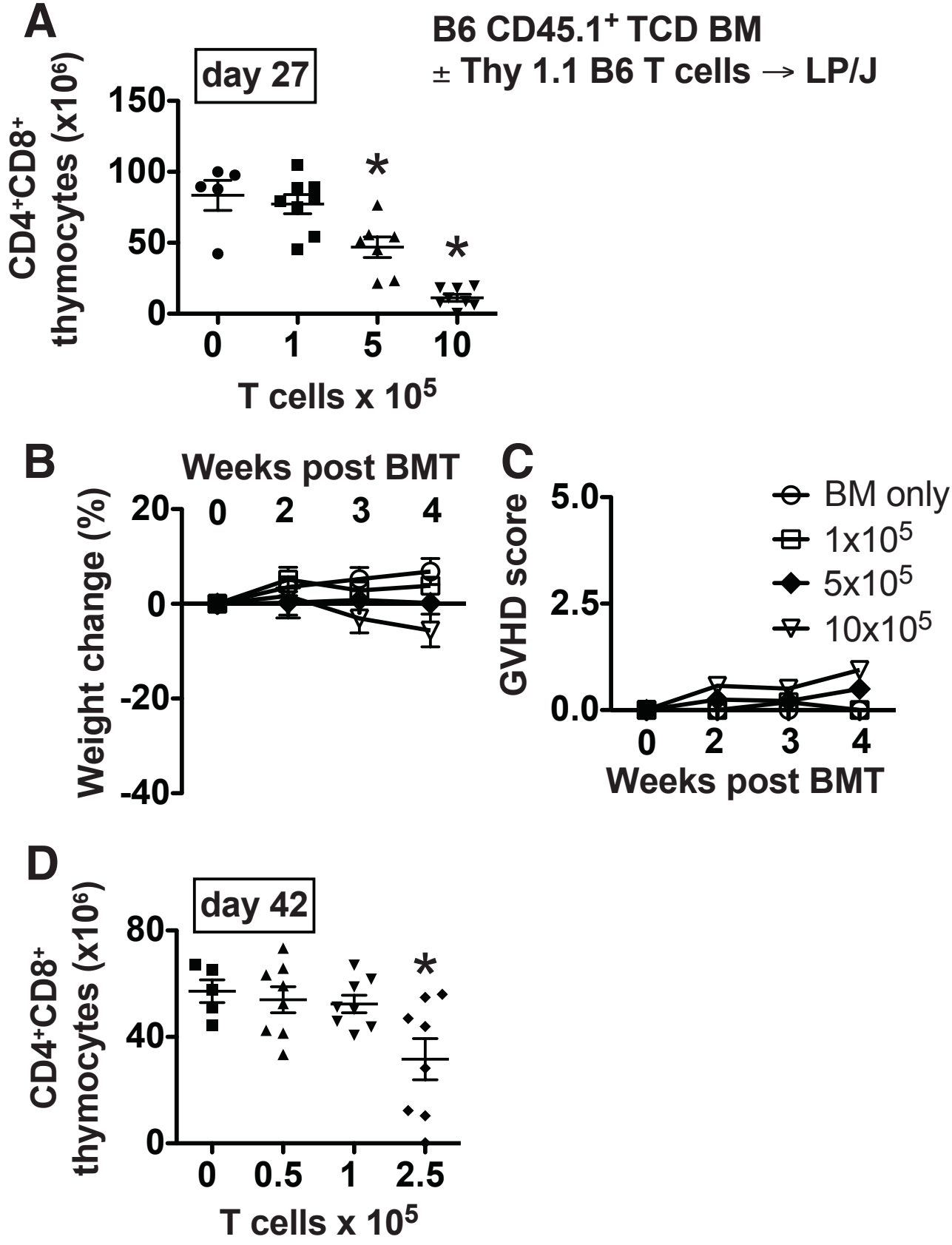
Suppl. FIGURE 1



Suppl. FIGURE 2



Suppl. FIGURE 3



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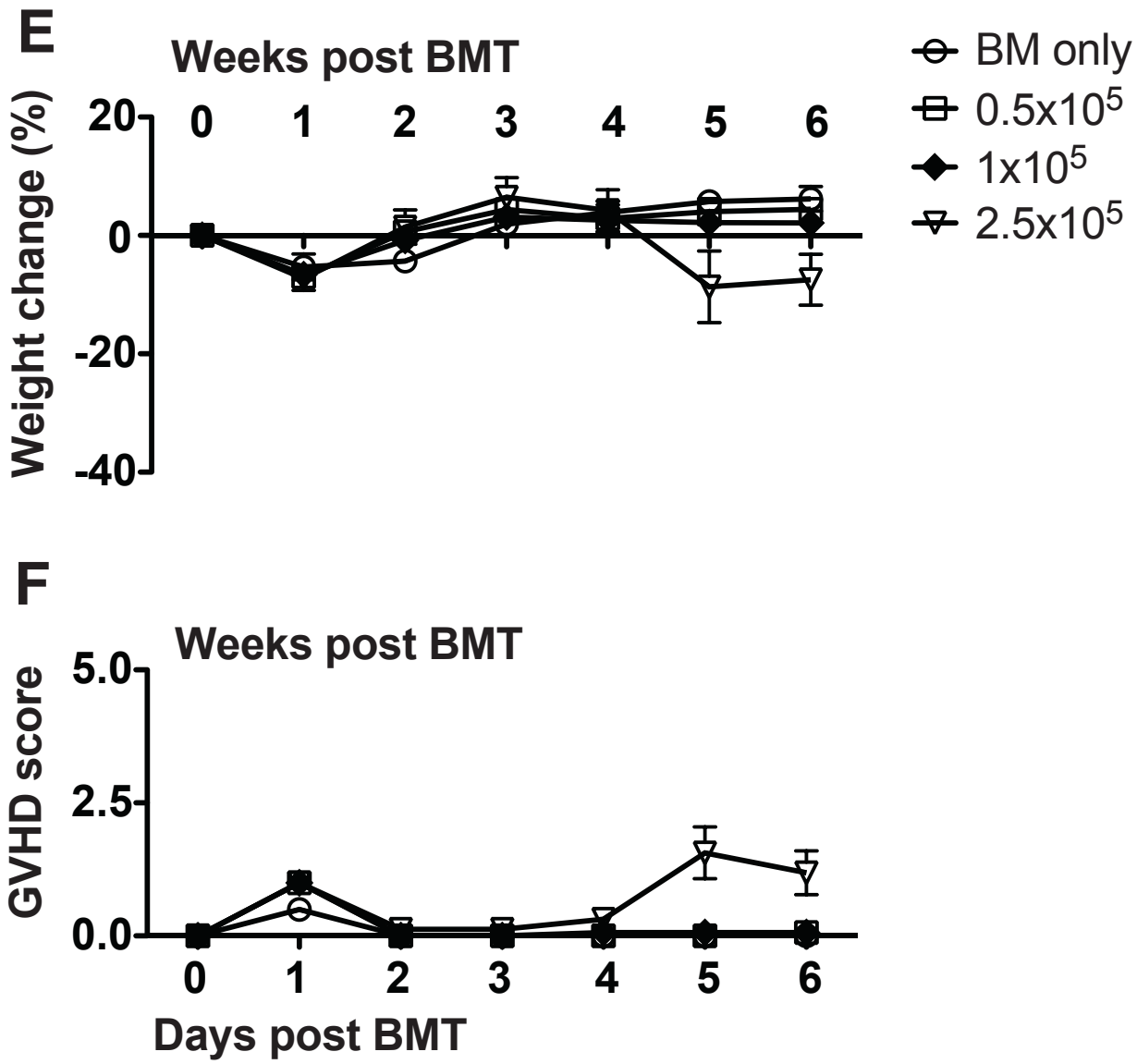

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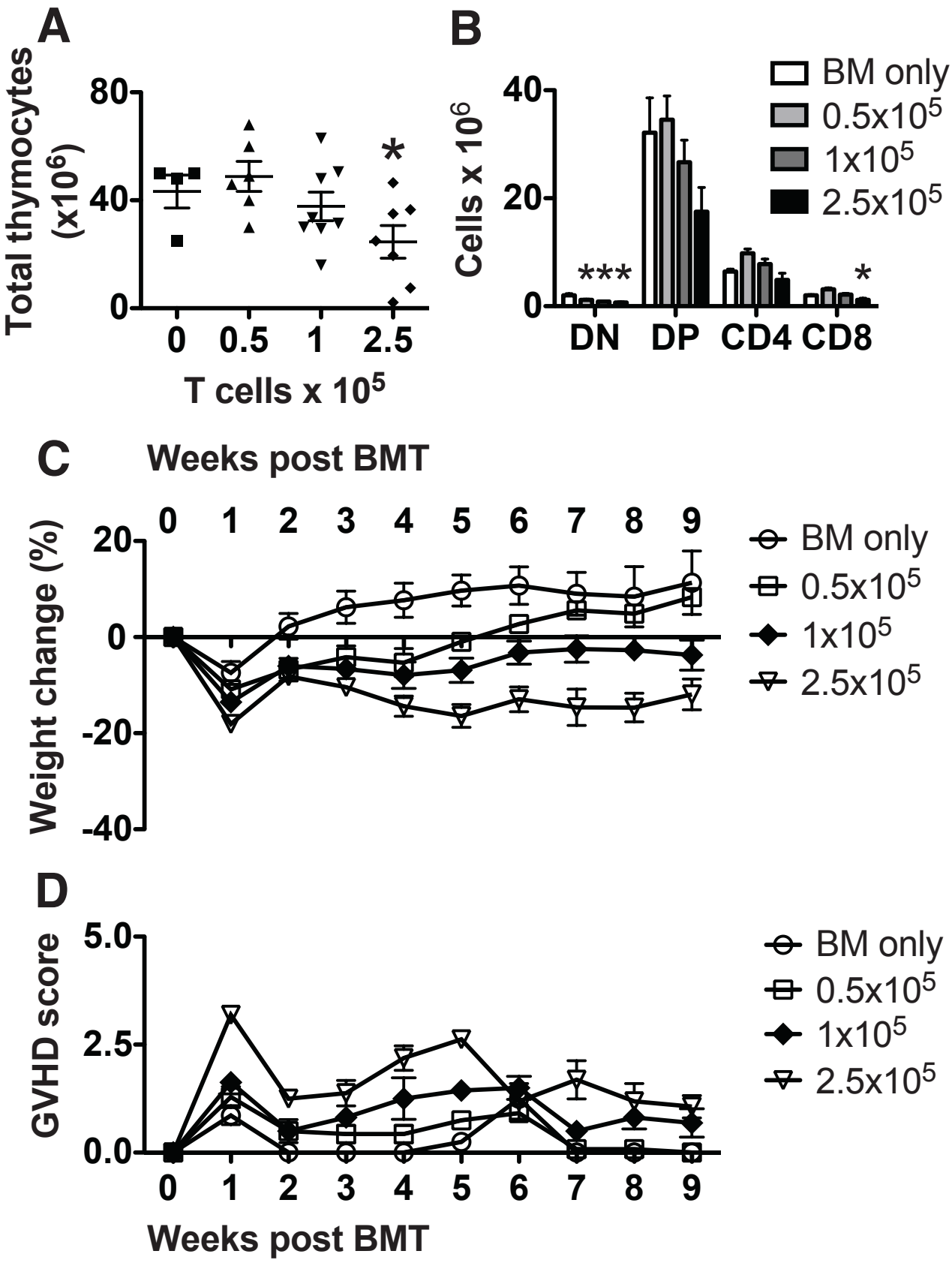
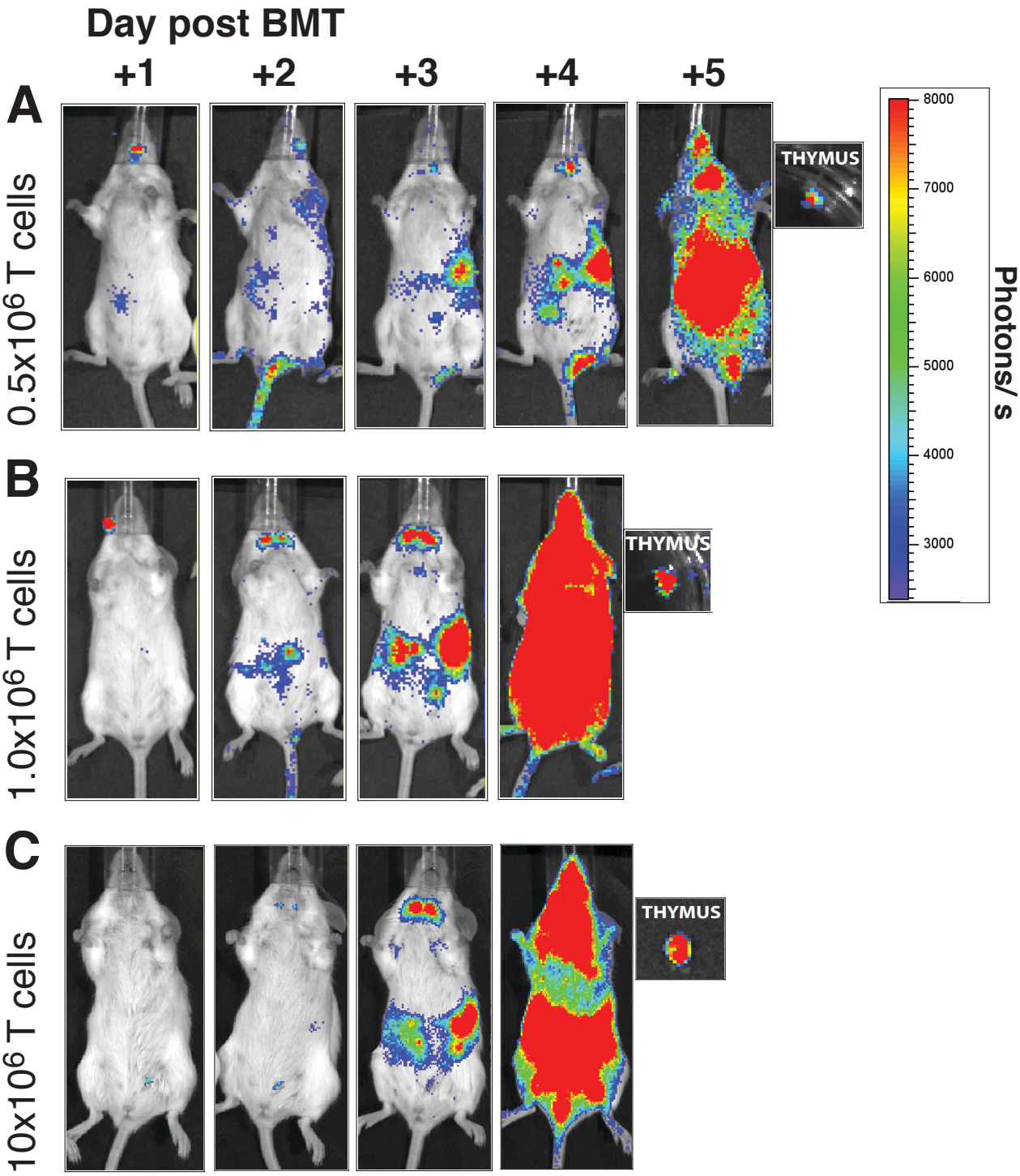


Suppl. FIGURE 5



Suppl. FIGURE 6