

Mice. B6.C RAG1^{-/-} mice were generated by crossing B6 RAG1^{-/-} mice to B6.C mice. B6.C TS1 RAG1^{-/-} mice were generated by crossing B10.D2 TS1 mice to B6.C RAG1^{-/-} mice. BALB/c TS1 RAG2^{-/-} mice were generated by crossing BALB/c TS1 mice to BALB/c RAG2^{-/-} mice. BALB/c TS1 Thy1.1 RAG2^{-/-} mice were generated by crossing BALB/c TS1 RAG2^{-/-} mice to BALB/c Thy1.1 mice.

Antibodies. CD4 Alexa 488 or Alexa 405 (GK1.5), TS1 Alexa 647 (6.5), Thy1.1 Alexa 680 (1A14), Thy1.2 Alexa 405 (30H12), and F4/80 Alexa 488 (HB-198) were lab prepared. CD25 PE (7D4) was from Southern Biotech. CD62L PE-Cy7 (Mel-14), PD-1 PE (29F.1A12), IFN- γ PE and FITC (XMG1.2), IL-2 PE-Cy7 (JES6-1A12), CD8 α PE (53-6.7) and CD44 APC-Cy7 (1M7) were from BioLegend. IL-17A PE (TC11-18H10), Ly76 PE (Ter119), α 4 β 7/LPAM-1 PE (DATK32), and CD69 PE (H1.2F3) were from BD Pharmingen. Gr-1 PE (Mac-1), CD357/GITR PE (DTA-1) and CD11b PE (MI/70) were from eBioscience.

BrdU and apoptosis assays . For BrdU, colon and spleen cells were incubated with ethidium monoazide (EMA) and then stained for surface expression of CD4 and TS1. Cells were resuspended in 0.5mL of 0.15M NaCl and vortexed while 1.2mL of 95% ethanol were added dropwise. Cells were incubated 30 minutes on ice, washed, and then incubated in 1mL of 1% paraformaldehyde with 0.05% Tween 20 (Sigma) for 30 minutes at room temperature followed by 30 minutes on ice. To denature DNA, cells were incubated in 1mL of 0.15M NaCl, 4.2mM MgCl₂, 10pM HCl and 100U/ml DNase

(Sigma) for 30 minutes at 37°C. After washing cells were blocked with 10% rat serum and then stained with FITC-anti-BrdU (Phoenix Flow Systems). For detection of activated caspase-3, cells were incubated in 10uM FITC-VAD (OMe)-FMK (SM Biochemicals) in Clicks media at 37°C for 30 minutes. Cells were washed twice in PBS, incubated with EMA and stained for surface expression of CD4 and TS1.

Colon Isolation. Colons were excised, cut into sections and incubated in 5mM EDTA (American BioAnalytical) at 37°C for 30 minutes. Colons were minced and digested with 200 Mandl U/mL collagenase D (Roche) and 10ug/mL DNase at 37°C for 45 minutes. The resulting digest was filtered through a cell strainer. Lymphocytes were isolated by centrifugation over a Percoll gradient (GE Healthcare Bio-Sciences AB).

Liver Isolation. Livers were perfused with PBS, excised, minced, passed through a cell strainer, and incubated in 0.02% collagenase IV (Sigma) at 37°C for 40 minutes. Lymphocytes were separated on 25% Optiprep gradient (Accurate Chemical & Scientific).

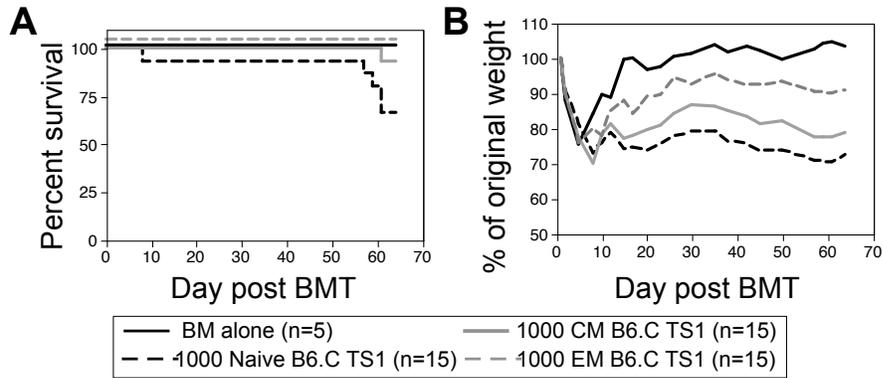


Figure S1. B6.C TS1 T_N and T_{CM} cause severe GVHD whereas B6.C TS1

T_{EM} cause only mild GVHD. HA104 mice received 750cGy and 8×10^6 B6.C RAG^{-/-} BM cells alone or in combination with 1000 sorted B6.C TS1 T_N , T_{CM} or T_{EM} . Survival is shown in (A). $P=0.016$ comparing survival of recipients of T_N vs. recipients of T_{EM} . Weight loss is shown in (B). $P<0.0001$ comparing weight loss in recipients of T_N or T_{CM} vs. recipients of BM alone for days 15-64. $P<0.0001$ comparing weight loss in recipients of T_{EM} vs. recipients of T_N for days 15-64. $P<0.05$ comparing weight loss in recipients of T_{EM} vs. recipients of BM alone for days 15-20 and 30-64.

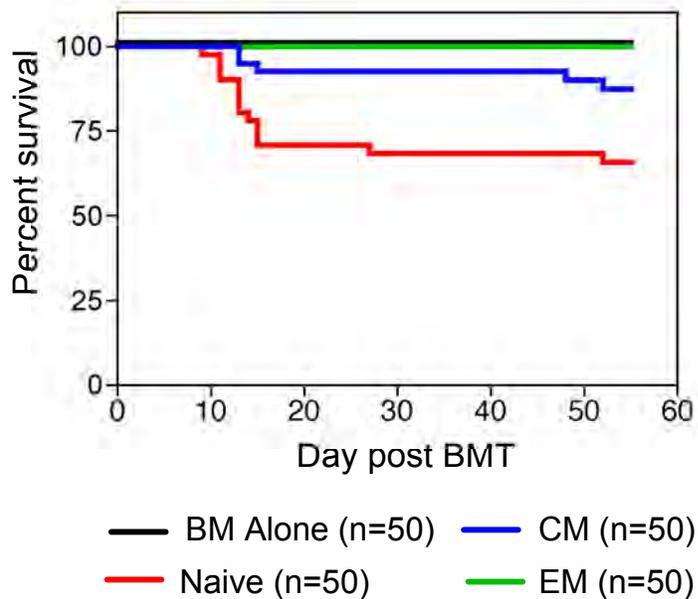


Figure S2. Survival in HA104 recipients of TS1 T_N , T_{EM} and T_{CM} . HA104

mice were irradiated and reconstituted with 8×10^6 RAG^{-/-} or WT BALB/c BM cells alone or in combination with TS1 T_N , T_{EM} or T_{CM} , in doses ranging from 1000 to 3000 cells per mouse. Data are combined from 5 independent experiments in which 10 mice per group received the same number of TS1 T_N , T_{EM} or T_{CM} . $P < 0.0001$ for T_{EM} vs. T_N , $P = 0.0217$ for T_{EM} vs. T_{CM} , $P = 0.0175$ for T_N vs. T_{CM} .

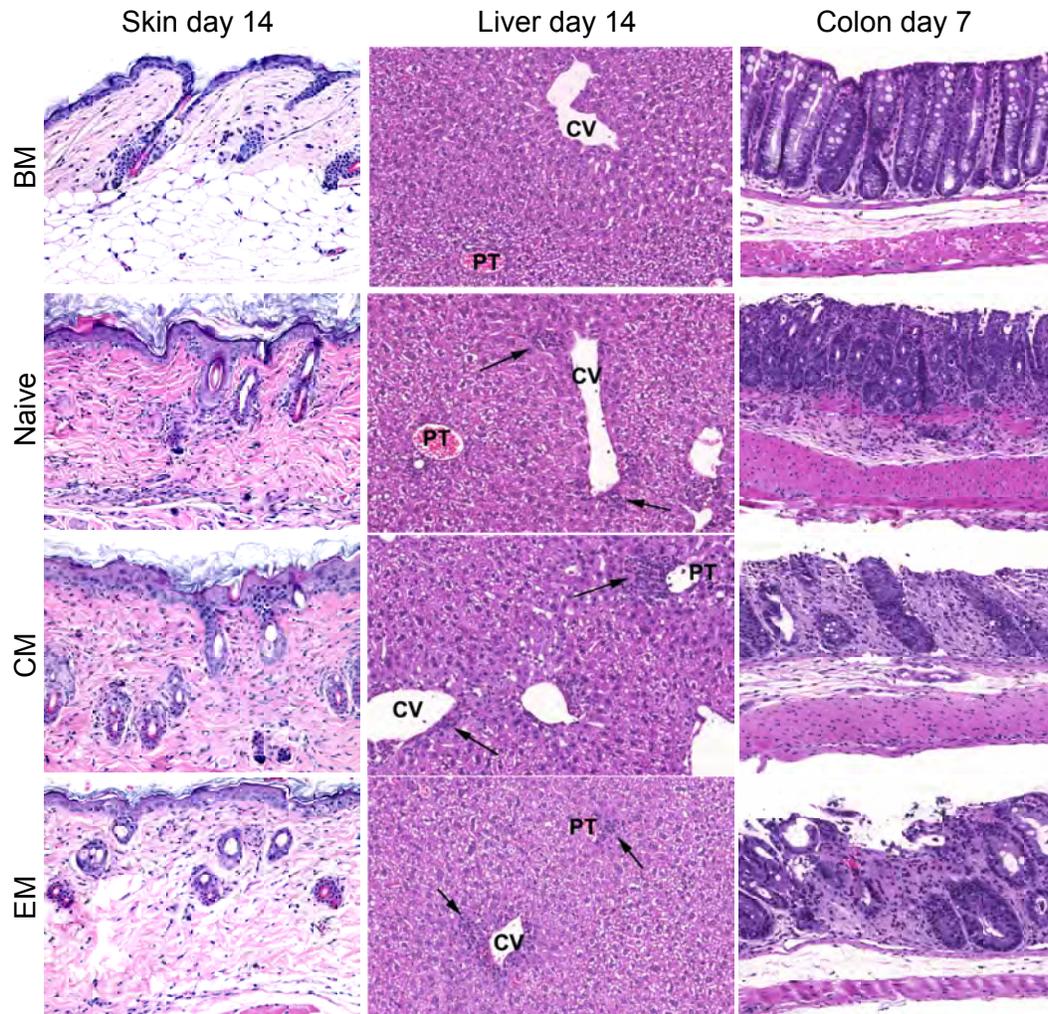


Figure S3. Representative histopathology for data shown in Fig. 4

Representative sections are shown for skin (day 14), liver (day 14) and colon (day 7).

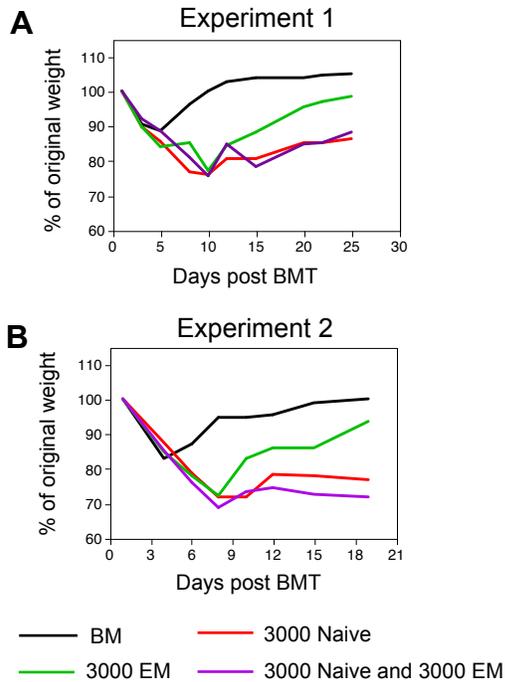


Figure S4. TS1 T_{EM} do not suppress weight loss induced by TS1 T_N . HA104

mice were transplanted with TS1 T_N , T_{EM} or a mix of both as described in Figure 5. Weight curves are shown for each of the two independent experiments that were combined in Figure 5. In (A), there were initially 8 mice per group, 4 of which were sacrificed on day 14. $P < 0.05$ comparing weight loss in recipients of T_N vs. T_{EM} or $T_N + T_{EM}$ vs. T_{EM} from day 15 onward. $P > 0.05$ for recipients of T_N vs. $T_N + T_{EM}$ from day 10 onward. In (B), there were a 11 mice per group; 4 were sacrificed on days 14 and 21. $P < 0.01$ comparing weight loss in recipients of T_N vs. T_{EM} or $T_N + T_{EM}$ vs. T_{EM} from day 10 onward. $P > 0.3$ for recipients of T_N vs. $T_N + T_{EM}$ from day 10 onward.

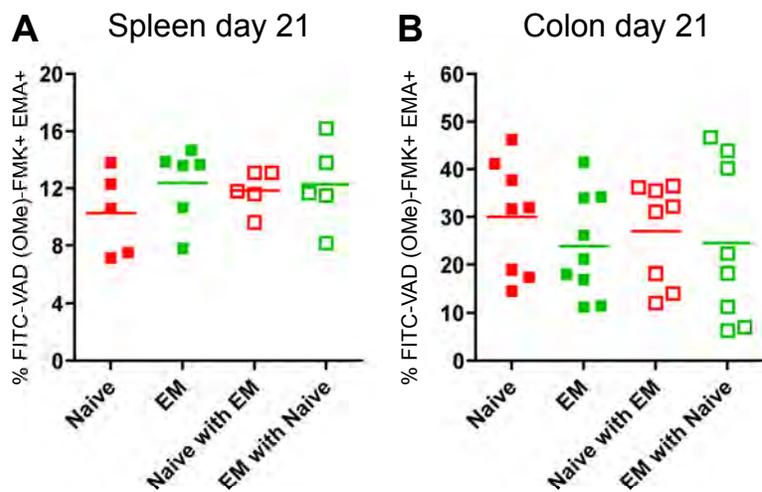


Figure S5. Apoptosis is not increased in progeny of T_{EM} compared to progeny of T_N . Mice were transplanted as in Figure 5. On day 21, spleen and colon cells were isolated and TS1 cells were assayed for activated caspase-3 by incubation with FITC-VAD (OME)-FMK and by staining with EMA. The percentages of $CD4^+TS1^+$ cells that were FITC⁺EMA⁺ in spleen and colon are shown in (A) and (B), respectively. Spleen data are representative of two independent experiments; colon data are combined from two independent experiments with similar results. For spleen data, $P > 0.24$ for any one group compared to any other group. For colon data, $P > 0.28$ for any one group compared to any other group.

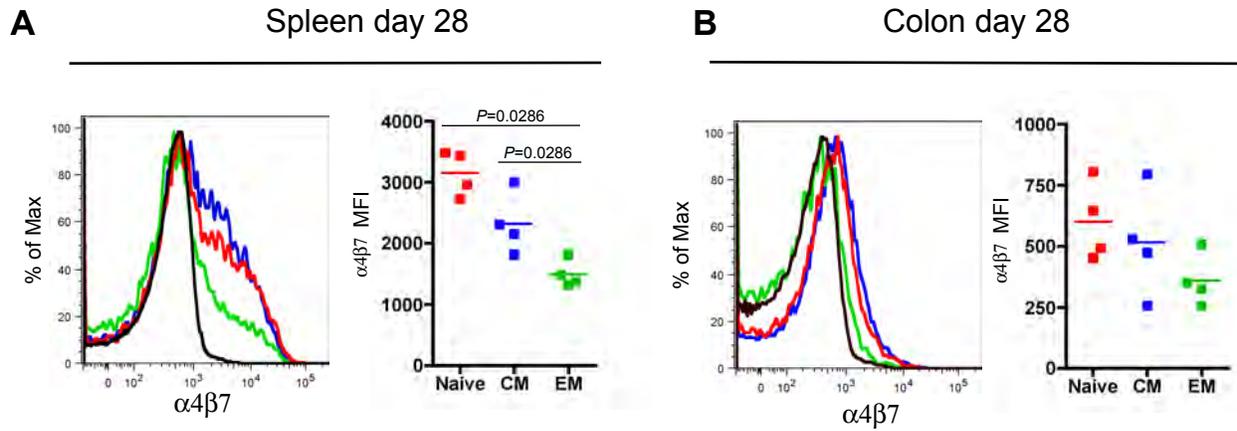


Figure S6. TS1 T_{EM} progeny express lower levels of $\alpha 4\beta 7$ than do TS1 progeny in T_N and T_{CM} recipients. HA104 mice received 750cGy and 8×10^6 RAG^{-/-} BM cells in combination with 3000 TS1 T_N , T_{CM} or T_{EM} . Shown is expression of $\alpha 4\beta 7$ on CD4⁺TS1⁺ cells on day 28 in the spleen (A) and colon (B). Data from recipients of T_N , T_{CM} and T_{EM} are shown in red, blue and green, respectively. $\alpha 4\beta 7$ staining of TS1 T_N from unmanipulated mice are shown in black. Splenic TS1 T_N serve as a baseline for colon analyses because sufficient colon TS1 cells cannot be extracted from unmanipulated TS1 mice.

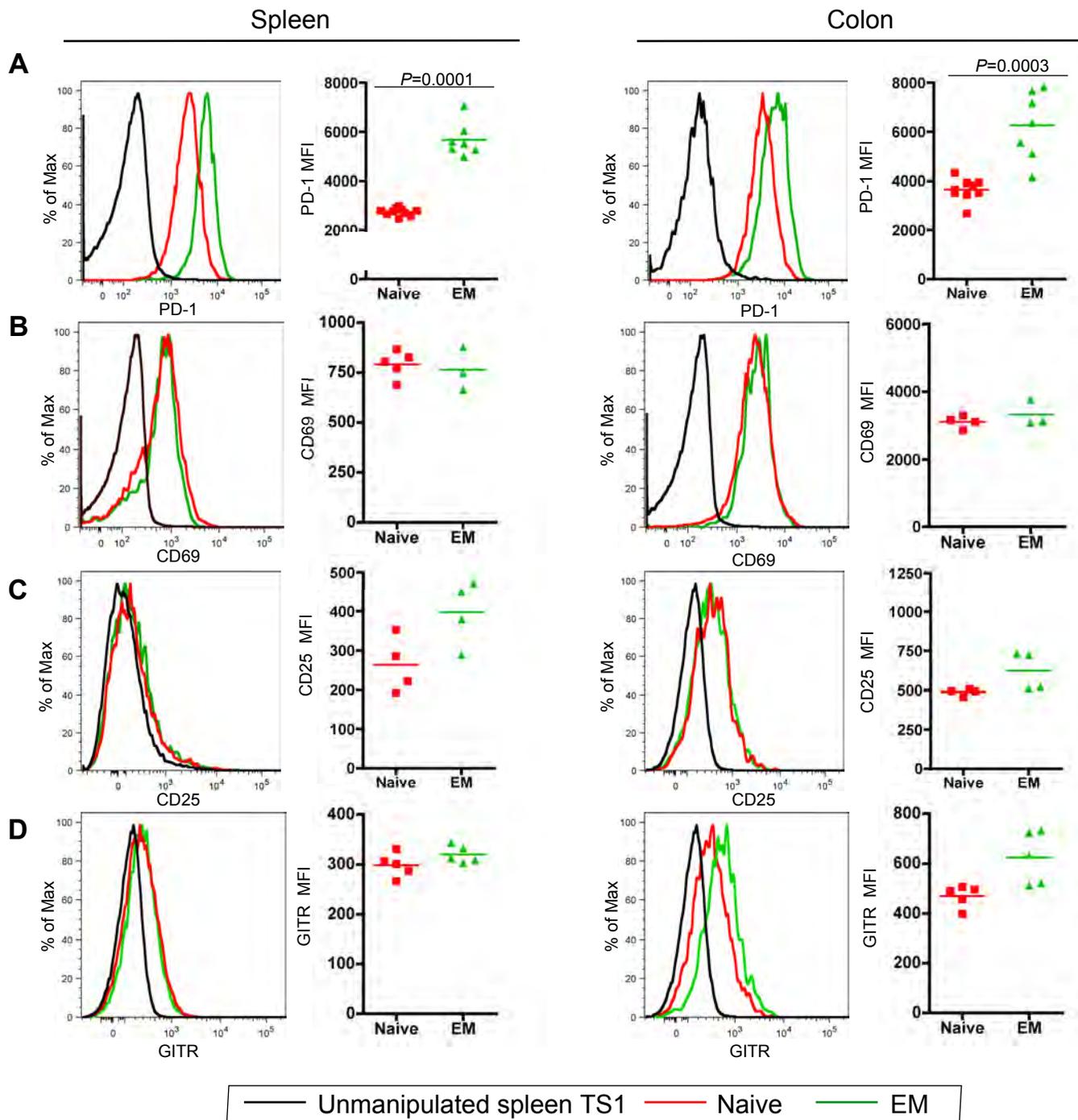


Figure S7. T_N and T_{EM} progeny express similar levels of CD25, CD69 and GITR but T_{EM} progeny express more PD-1. HA104 mice received 750cGy and 8×10^6 RAG^{-/-} BM cells in combination with 3000 TS1 T_N or 3000 TS1 T_{EM} . On days 28 and 32, spleen and colon cells were isolated and phenotypic analysis was performed by flow cytometry. For all markers analyzed, TS1 were identified with antibodies against CD4 (GK1.5, Alexa 405), TS1 (6.5, Alexa 647), with the marker analyzed stained with PE-conjugated antibodies. Representative histograms and plots of MFIs of CD4⁺TS1 from spleen and colon are shown. For all panels, data from T_N and T_{EM} TS1 recipients are shown in red and green, respectively. Staining of splenic TS1 T_N from unmanipulated mice are in black. Splenic TS1 T_N serve as a baseline for colon analyses because sufficient colon TS1 cells cannot be extracted from unmanipulated TS1 mice. PD-1 data (A) are

combined from mice analyzed on days 28 and 32; CD69 data (B) are from day 32; CD25 (C) are and GITR data (D) are from day 28. All data are representative of at least two experiments, with the exception of GITR data, which are from one experiment.

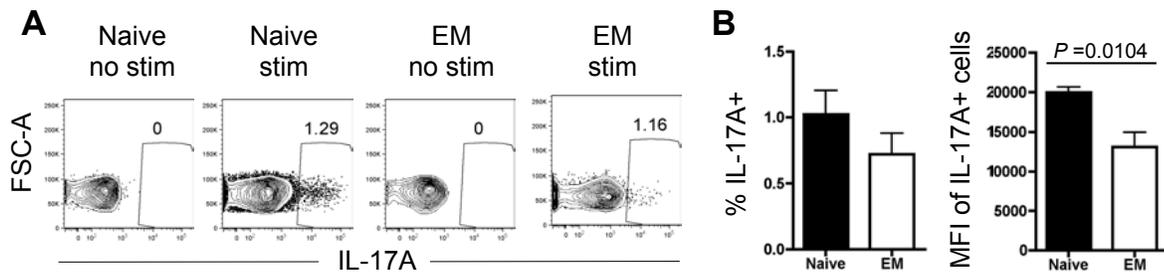


Figure S8. In the colon, TS1 cells produce little IL-17A. HA104 mice received 750cGy and 8×10^6 RAG^{-/-} BM cells in combination with 3000 TS1 T_N or 3000 TS1 T_{EM}. On day 21, colon cells were isolated, stimulated with PMA and ionomycin and stained for IL-17A. Representative FACS staining with and without PMA/ionomycin stimulation are shown in (A). The percentages of TS1 cells that are IL-17A⁺ and the MFI of the IL-17A⁺ cells are shown in (B). Data are combined from two independent experiments (total 8 mice per group).