

survival in sublethally irradiated (300cGy TBI) RAG-2^{-/-} BALB/c hosts after adoptive transfer of wild type BALB/c sorted naïve or effector memory phenotype T cells. Details of the sorting the latter cells have been described previously (31).

Supplementary Figure 1. Representative FACS Patterns of T cell -, B cell - and Granulocyte/macrophage Chimerism At 28 days.

Staining was performed for H-2K^b versus TCR, B220, or Mac-1/Gran-1 on gated cell subsets after wild type BALB/c mice were given TLI/ATS conditioning and combined heart and bone marrow transplantation from C57BL/6 donors.

Supplementary Figure 2. Marked T Cell Depletion is Present in NKT Cell or CD4⁺CD25⁺ T Cell Deficient Hosts that Can Reject Grafts

A, mean percentages of CD4⁺ T cells among splenocytes in untreated wild type BALB/c mice (WT-UT), TLI/ATS conditioned wild type hosts given heart transplants (WT-H/T/A), CD25 depleted and conditioned wild type hosts with transplants (WT-H/T/A; CD25 dep) and conditioned CD1d^{-/-} hosts with transplants (CD1d^{-/-} - H/T/A) 24 hours after the completion of conditioning (N=5 per group). **B**, mean absolute number of CD4⁺ cells in groups of mice shown in panel A. **C**, mean percentage of total CD4⁺ cells among splenocytes in wild type mice without heart transplants 24 hours after TLI conditioning (WT-T), or 5 days after ATS conditioning (WT-A) or 24 hours after TLI and ATS conditioning (WT T/A) (N=5). **D**, mean absolute number of CD4⁺ cells among splenocytes in groups of mice shown in panel C.

Supplementary Figure 3. Dominant Influence of ATS on Increase in Proportion of CD4⁺Foxp3⁺ T Cells After Conditioning

A, representative FACS patterns of CD25 versus CD4 among CD4⁺ wild type BALB/c splenocytes in untreated mice (UT), 24 hours after TLI (10 doses) (T), 5 days after ATS (5 doses) (A), and 24 hours after combined TLI/ATS (T/A) conditioning. **B**, mean percentages of CD25⁺CD4⁺ cells among CD4⁺ T cells shown in panel A (N=5). **C**, staining of Foxp3 versus CD4 in groups shown in panel A. **D**, mean percentages of CD4⁺Foxp3⁺ cells among CD4⁺ cells in groups in panel C. **E**, staining of CD25 versus Foxp3 among gated Foxp3⁺ cells in

groups from panel C. **F** and **G**, mean percentages of CD4⁺CD25⁺Foxp3⁺ and CD4⁺CD25⁻Foxp3⁺ T cells respectively among total CD4⁺ T cells in groups from panel E.

Supplementary Figure 4: Conditioning with TLI Increases the Percentage of NKT Cells and Decreases the Percentage of Naive CD8⁺ T cells More Effectively Than ATS.

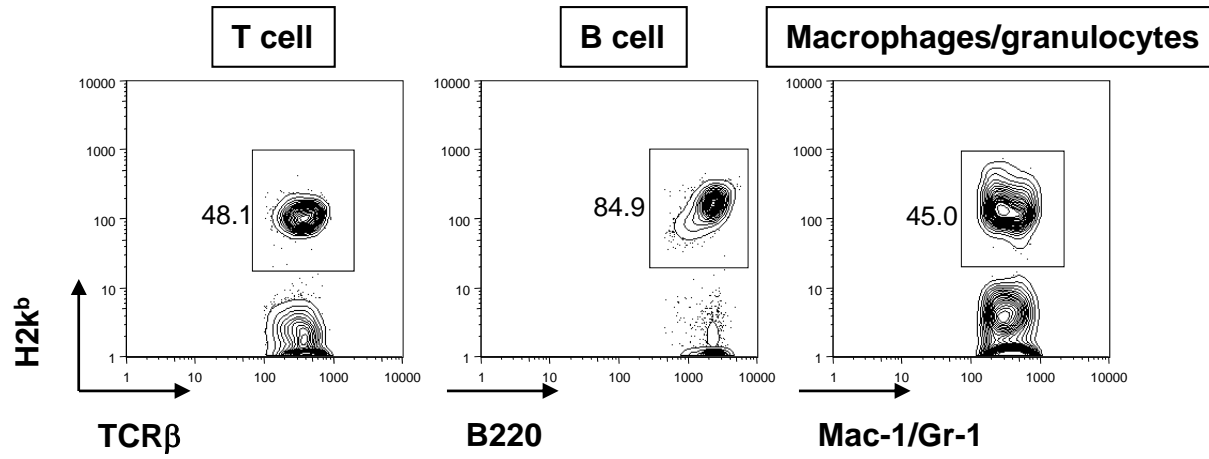
A, BALB/c mice were either untreated or treated with TLI alone, ATS alone, or TLI and ATS. Spleen cells were stained for TCR⁺CD1-tetramer⁺ cells 24 hours after TLI alone, 5 days after ATS alone, or 24 hours after TLI and ATS. Bars show the mean percentages of tetramer⁺ cells among gated TCR⁺ cells. N=8 for untreated mice, and N=5 for treated mice. UT vs TLI, p<0.0001; TLI vs. ATS or TLI/ATS, p<0.0001.

B, BALB/c mice were either untreated or treated with TLI and/or ATS, and the percentage of CD622^{hi} CD44^{lo} (naive) cells among gated CD8⁺ T cells in the spleen was determined after conditioning. Bars show mean percentages of naive cells. UT vs ATS, p<0.0001; UT vs TLI, p<0.0001; UT vs TLI/ATS, p<0.0001. N=5 for UT, N=7 for ATS and TLI alone, N=5 for TLI/ATS.

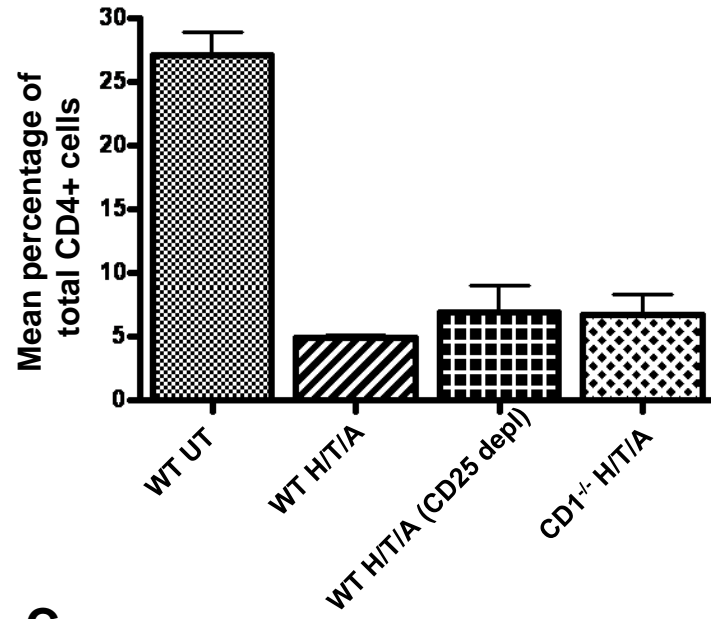
Supplementary Figure 5: CD4⁺CD25⁺ T Cells from Untreated and Chimeric BALB/c Mice Suppress the MLR to C57BL/6 and C3H Third Party Stimulator Cells

Stimulation index was calculated from ³H-thymidine incorporation with or without stimulator cells. BALB/c sorted TCR⁺ splenocytes were used as responders, irradiated (20Gy) C57B/6 (donor type) splenocytes or C3H (third party) splenocytes as stimulators with and without addition of sorted untreated (UT BALB/c) wild type BALB/c CD4⁺CD25⁺ Treg cells (Panel A) or H-2K^{d+} Treg cells (CHIM BALB/c) from BALB/c hosts given combined C57BL/6 heart and bone marrow transplants 100 days earlier (Panel B). Panel C shows the percentage suppression of ³H-thymidine incorporation in Panels A and B. Bars show means of triplicate wells. One of 2 replicate experiments is shown. There were 1x10⁵ responder, stimulator, and/ or Treg cells in each well.

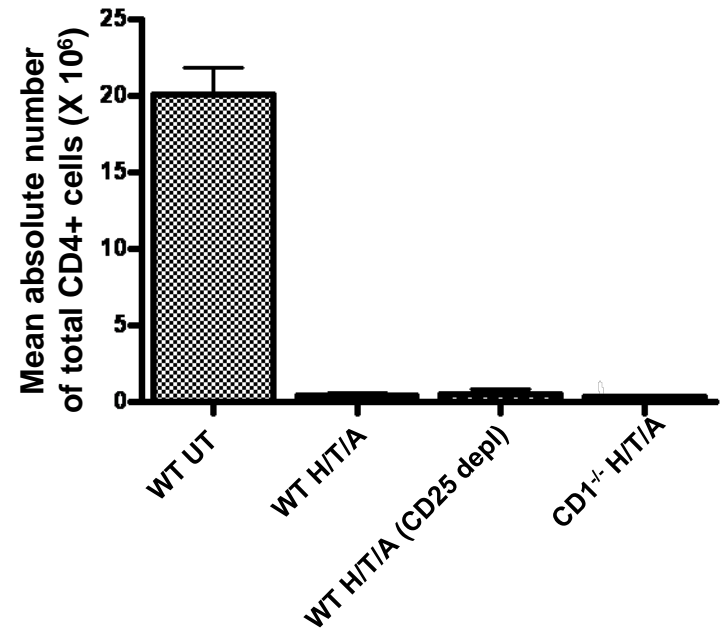
Supplementary Figure 1.



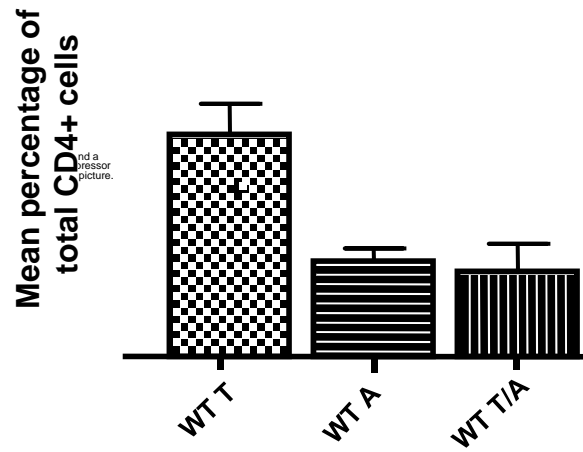
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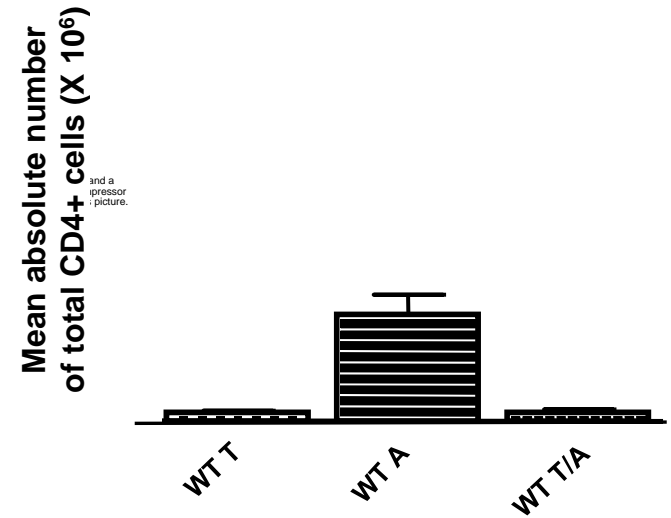
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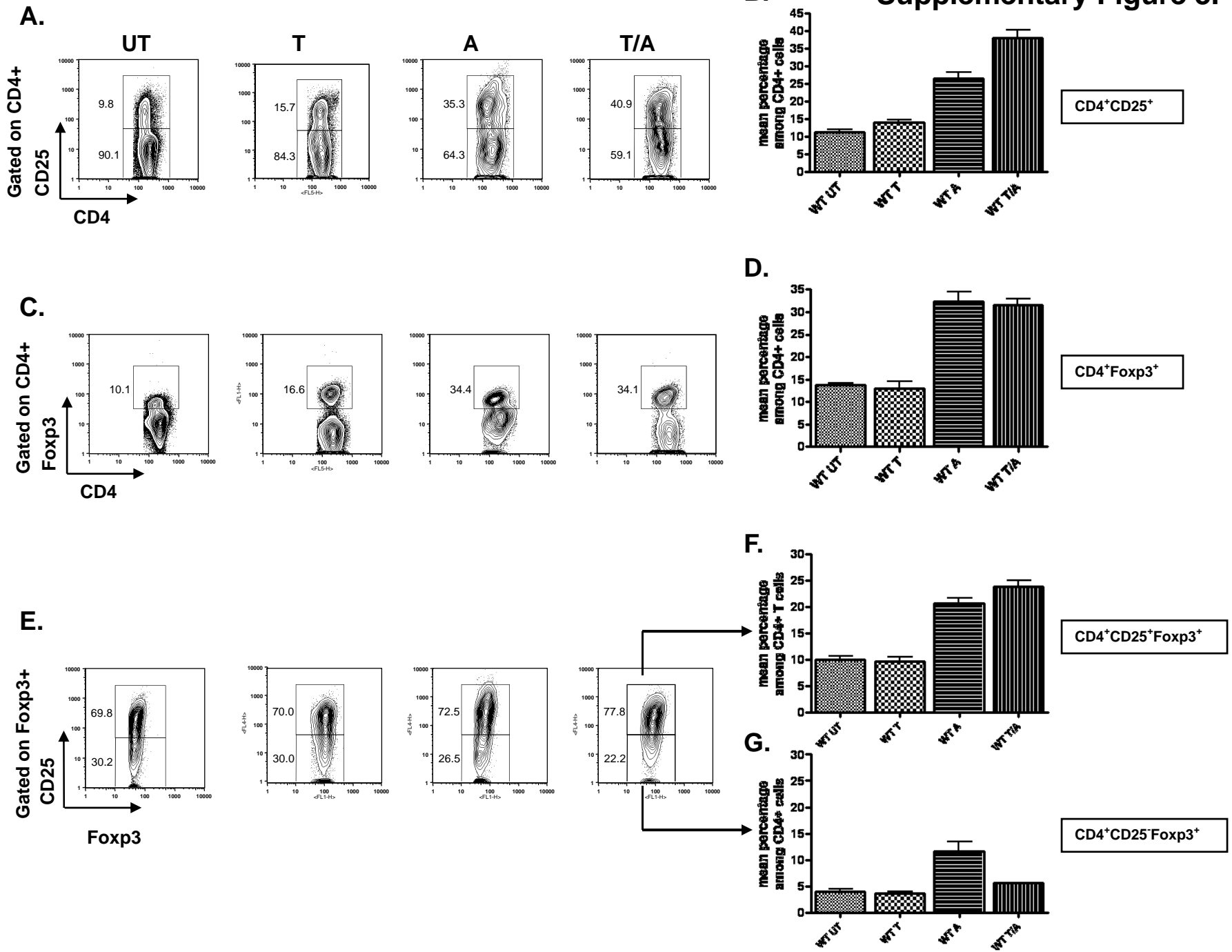
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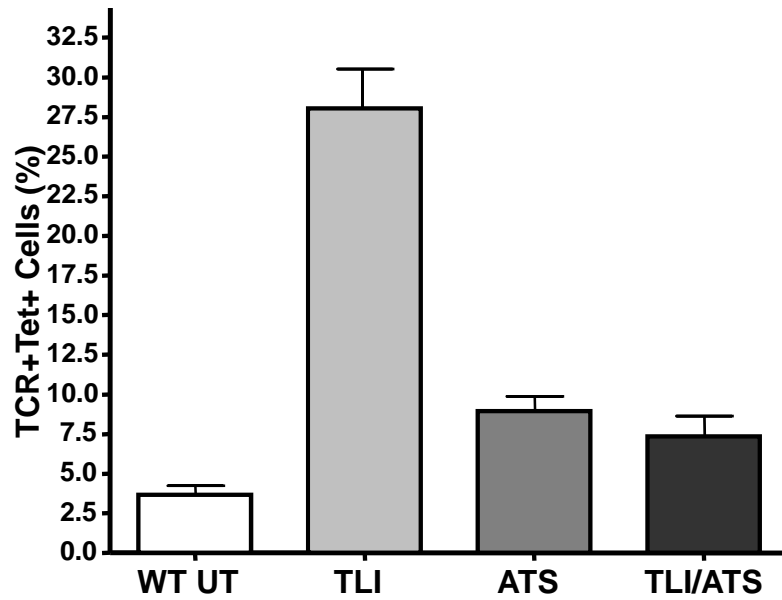
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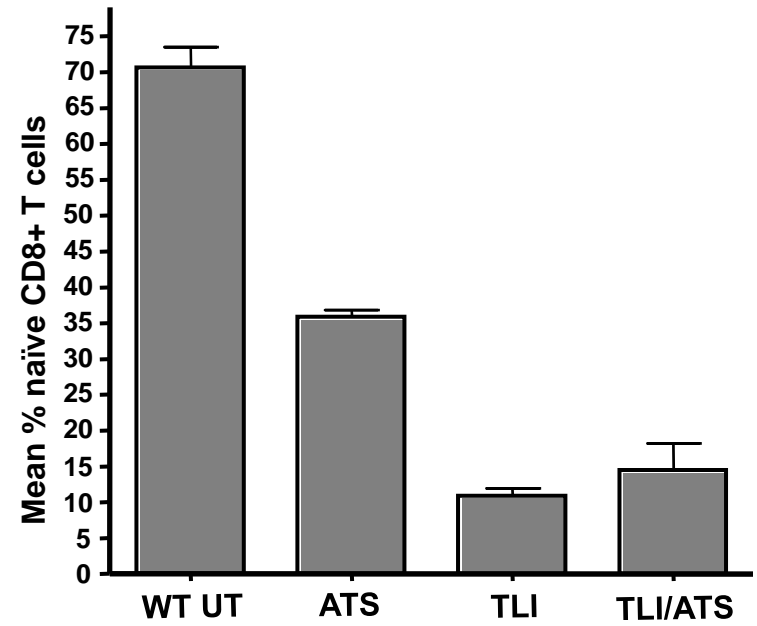
B. Supplementary Figure 3.



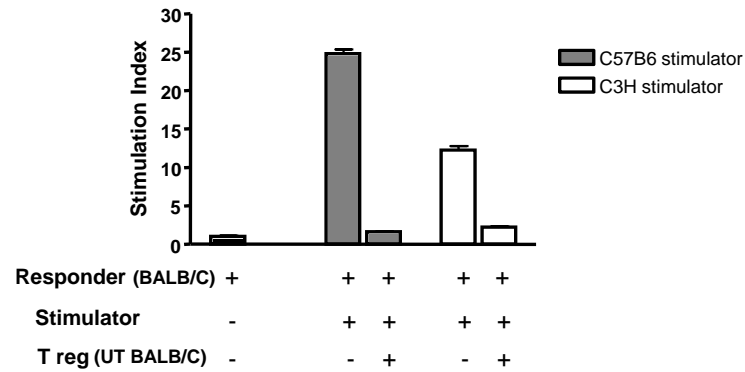
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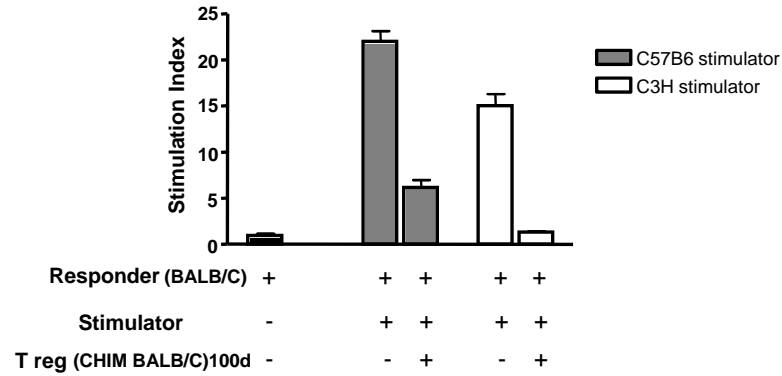
B.



A.



B.



C.

