Fig. S1

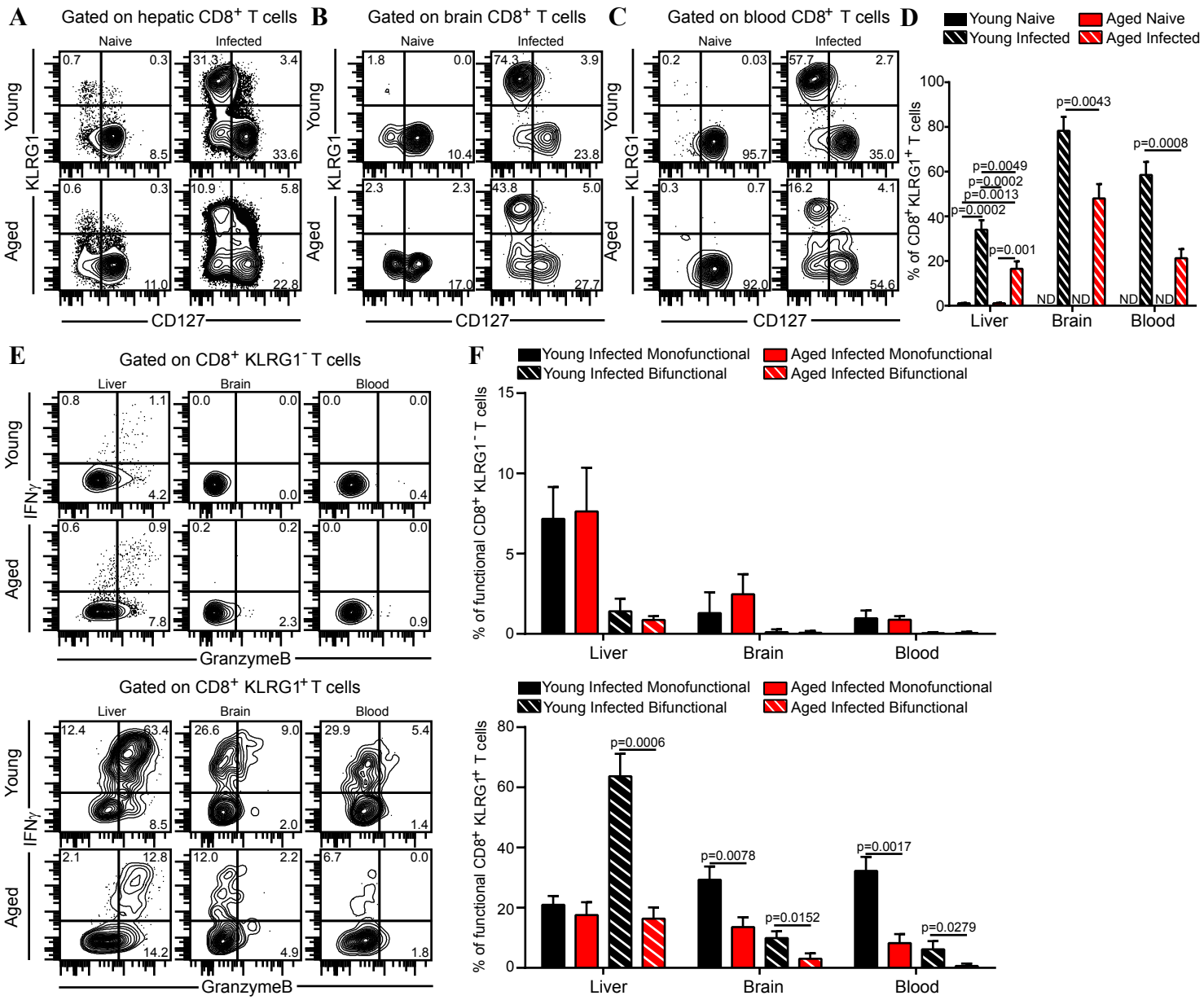

Figure S1. *E. cuniculi* infection downregulates CD8 effector response in non-lympoid tissue in aged mice. (A-D) CD127 and KLRG1 expression was assessed on CD8T cells in young (6-8 weeks old) and aged mice (14-15 month old) at day 12 pi in multiple non-lymphoid tissue.Data is presented as countour plots (A-C) or bar graphs (D). (E, F) IFNγ and GranzymeB expression evaluated in KLRG1- (top panel) and KLRG1+ (bottom panel) CD8 T cells in the aforementioned tissue (parasite challenged mice) is presented as contour plot or bar graphs. ND denotes “not detectable”. Data represent 3 experiments with 4 mice per group. Numbers in dot plots represent percentage.

Fig. S2

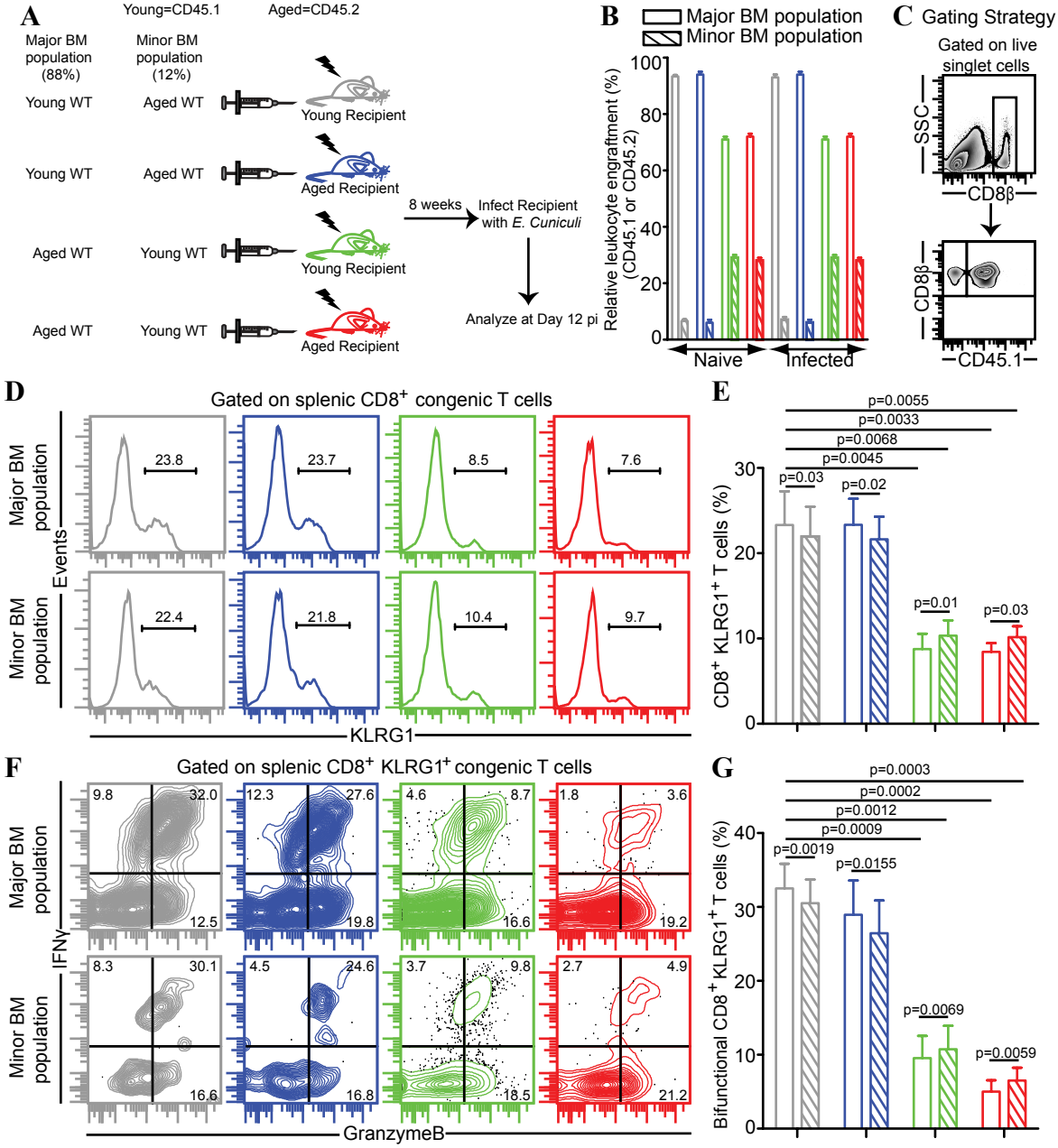

Figure S2. Defective CD8 response in aged mice is primarily CD8 extrinsic and hematopoietic in nature. (A) Schematic used for generating mixed bone marrow chimera in young or aged recipients. (B) Relative leukocyte engraftment was assessed by measuring the proportion of CD45.1+ and CD45.2+ splenic leukocytes in these chimeras. (C) shows the gating strategy. (D, E) Frequency of KLRG1 expressing splenic CD8 T cells was evaluated in the congenic CD8 population, in *E. cuniculi* challenged chimeric mice. (F,G) IFNγ and Gzb production by KLRG1+ effector CD8 T cells is presented as contour plots. Data are representative of 2 experiments with 4 mice per group. Numbers in histograms or dot plots represent percentage.

Fig. S3

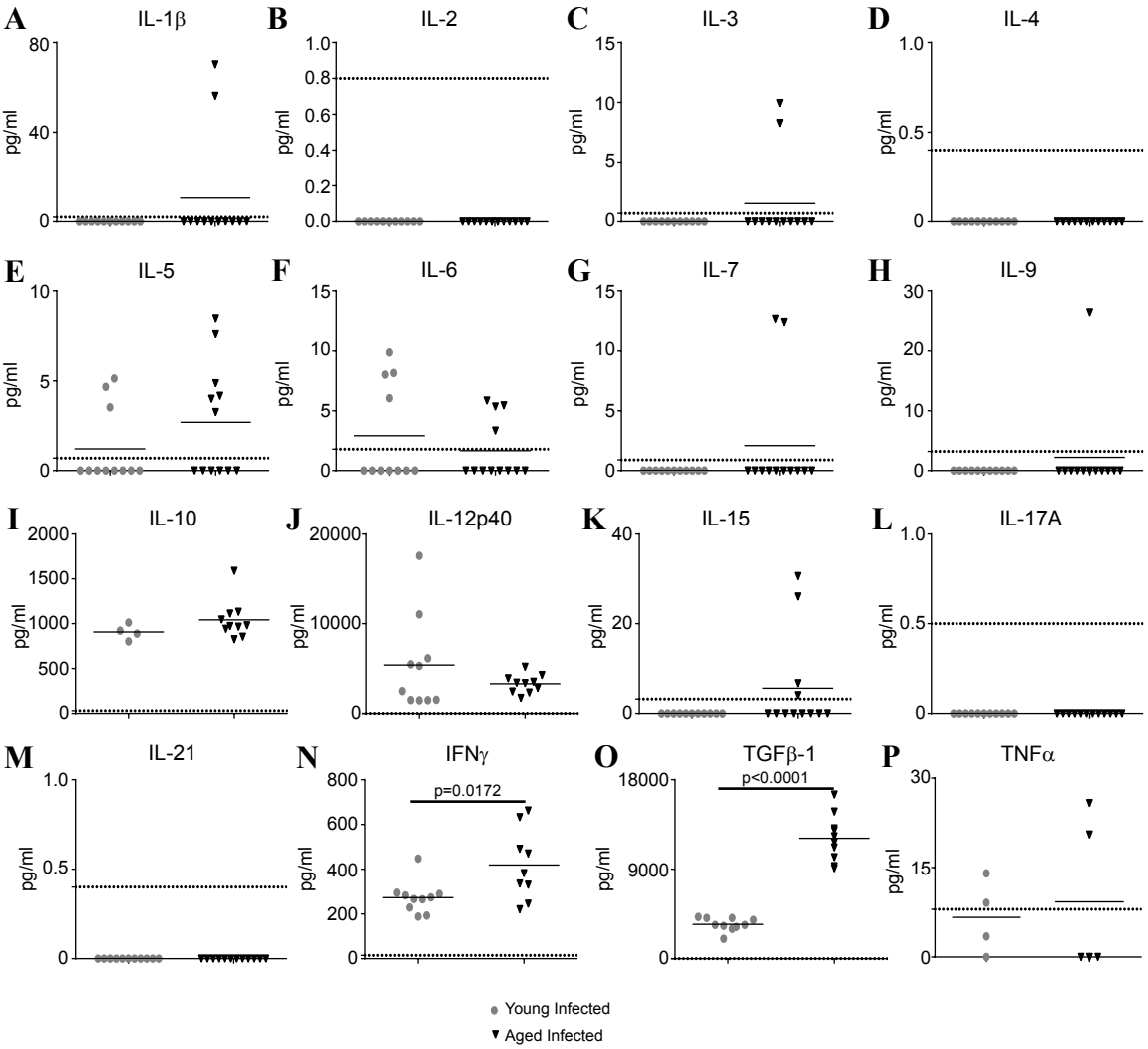

Figure S3. Serum cytokine profile of *E. cuniculi* infected young and aged mice. (A-P) Serum cytokine levels were evaluated in parasited challenged young (6-8 weeks old) and aged mice (14-15 months old) by Luminex or ELISA. Solid line represents mean. Dotted line denotes assay sensitivity. Data represent 2 experiments with 4-12 mice per group.

Fig. S4

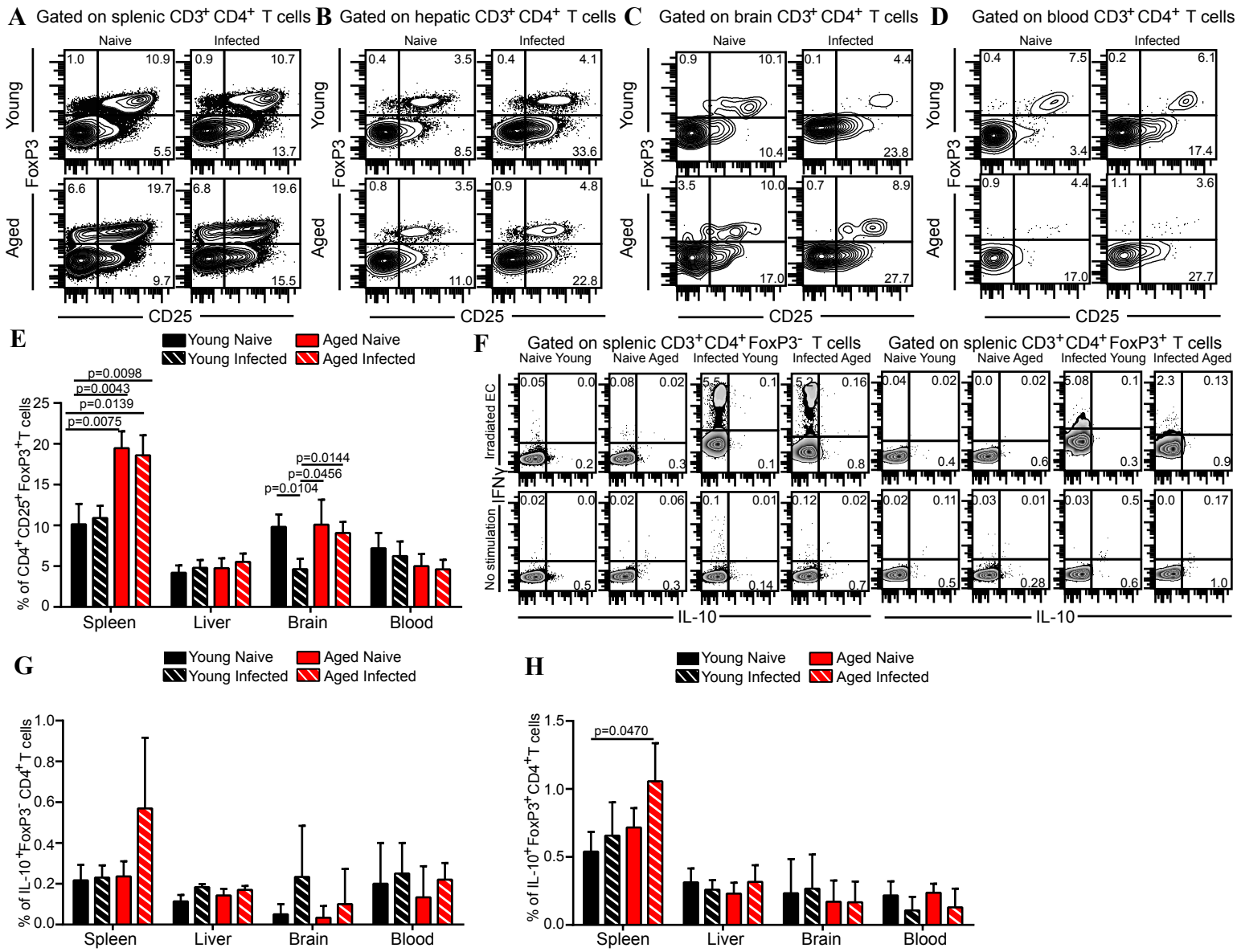

Figure S4. *E. cuniculi* infection does not elicit a robust Treg response in young or aged mice. (A-E) FoxP3 and CD25 expression was assessed on CD4 T cells in young (6-8 weeks old) and aged mice (14-15 month old) at day 12 pi in various tissues. (F-H) IL-10 expression was evaluated in FoxP3+ and FoxP3- CD4 T cells in these animals. Data represent 3 experiments with 4 mice per group. Numbers in dot plots represent percentage.

Fig. S5

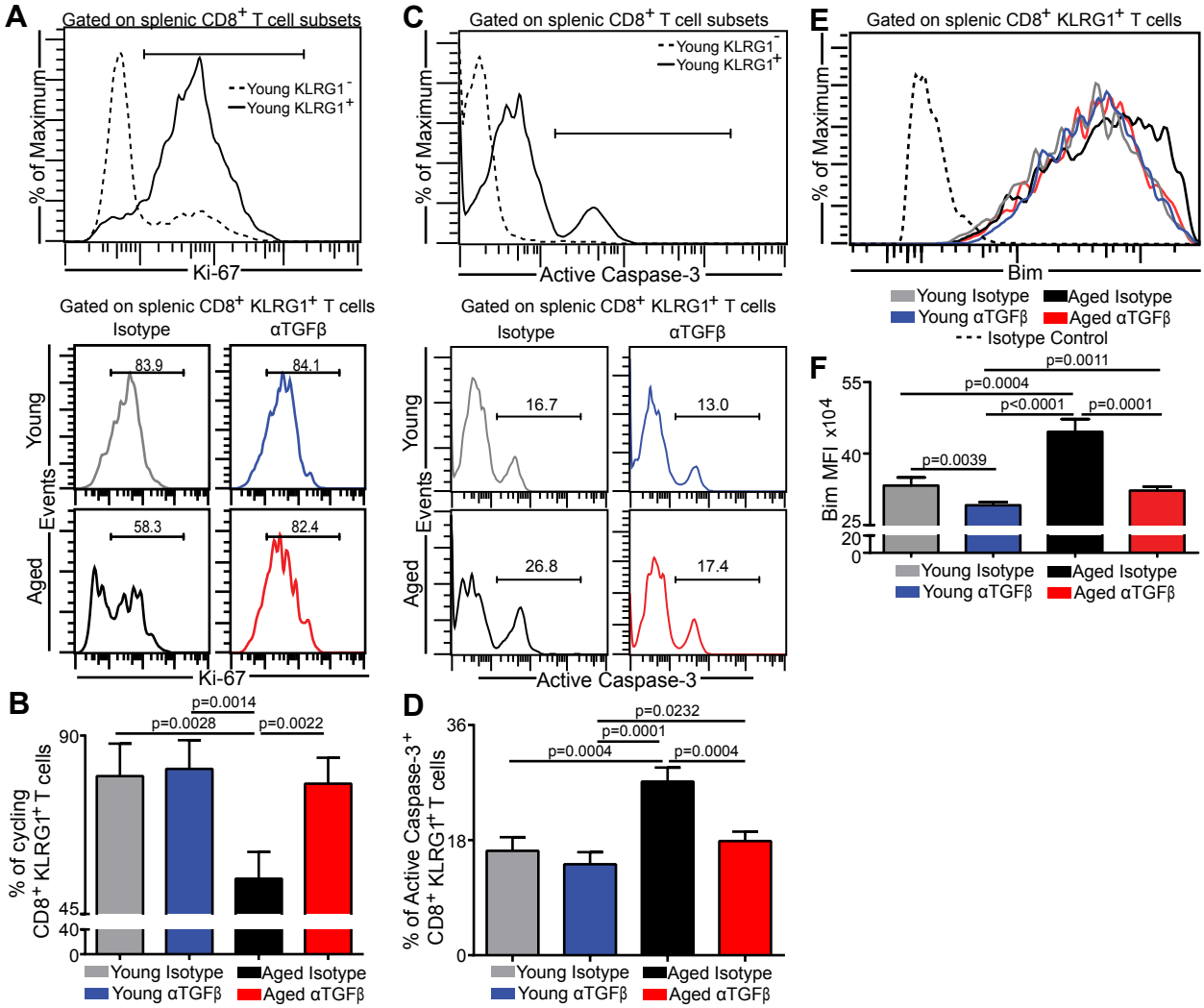

Figure S5. αTGFβ treatment rescues effector CD8 proliferation and survival in *E. cuniculi* infected aged mice. (A,B) Splenocytes from isotype control antibody or αTGFβ treated young (6-8 week old) or aged (14-15 month old) mice were assessed for proliferating KLRG1+ CD8 effectors at day 12 pi by intracellular staining for Ki-67. (C,D) Splenocytes from the above groups were incubated at 37°C for 5h and active caspase 3, was detected on KLRG1+ CD8 T cells by flow cytometry. (E,F) Bim expression was assessed in these cells direct ex vivo by intracellular staining. Data are representative of 3 experiments with 4-6 mice per group. Numbers in histograms represent percentage.

Fig. S6

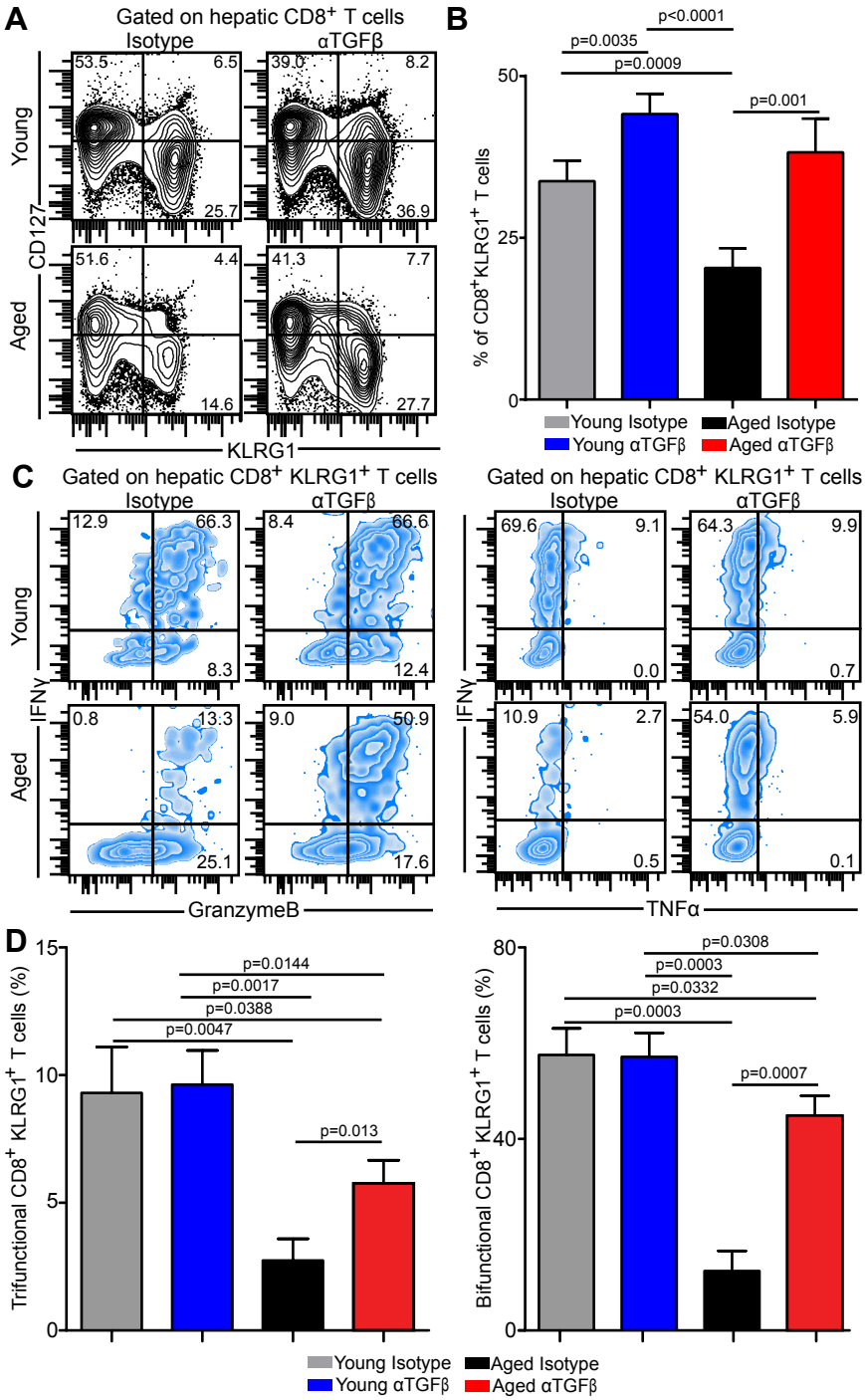

Figure S6. αTGFβ treatment reinvigorates effector CD8 response in a non-lymphoid tissue in *E. cuniculi* infected aged mice. (A,B) KLRG1 expression was evaluated on hepatic CD8 T cells in *E. cuniculi* challenged young (6-8 weeks old) or aged (14-15 month old) mice treated with αTGFβ or isotype control antibody. (C,D) IFNγ, TNFα and Gzb levels were assayed in hepatic KLRG1+ effector CD8 T cells in these animals. Data represent 2 experiments with at least 4 mice per group. Numbers in dot plots represent percentage.

Fig. S7

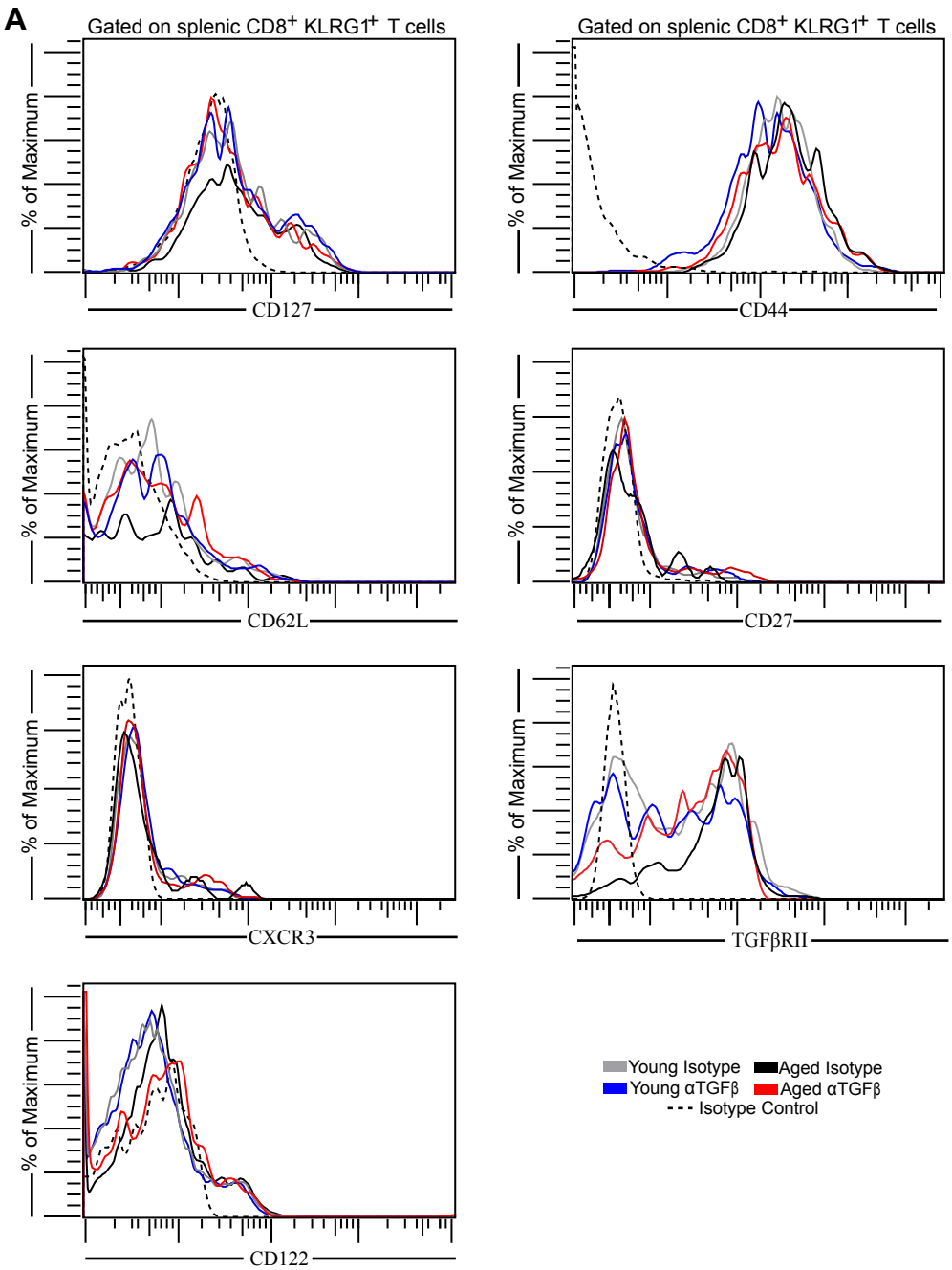

Fig S7. αTGFβ treatment does not dramatically alter KLRG1+ effector CD8 activation profile. (A) Expression of a panel of activation and maturation markers was evaluated on KLRG1+ splenic effector CD8 T cells in *E. cuniculi* challenged young (6-8 week old) or aged mice (14-15 month old) treated with αTGFβ or isotype control antibody. Data are representative of 2 experiments with 4 mice per group.

Fig. S8

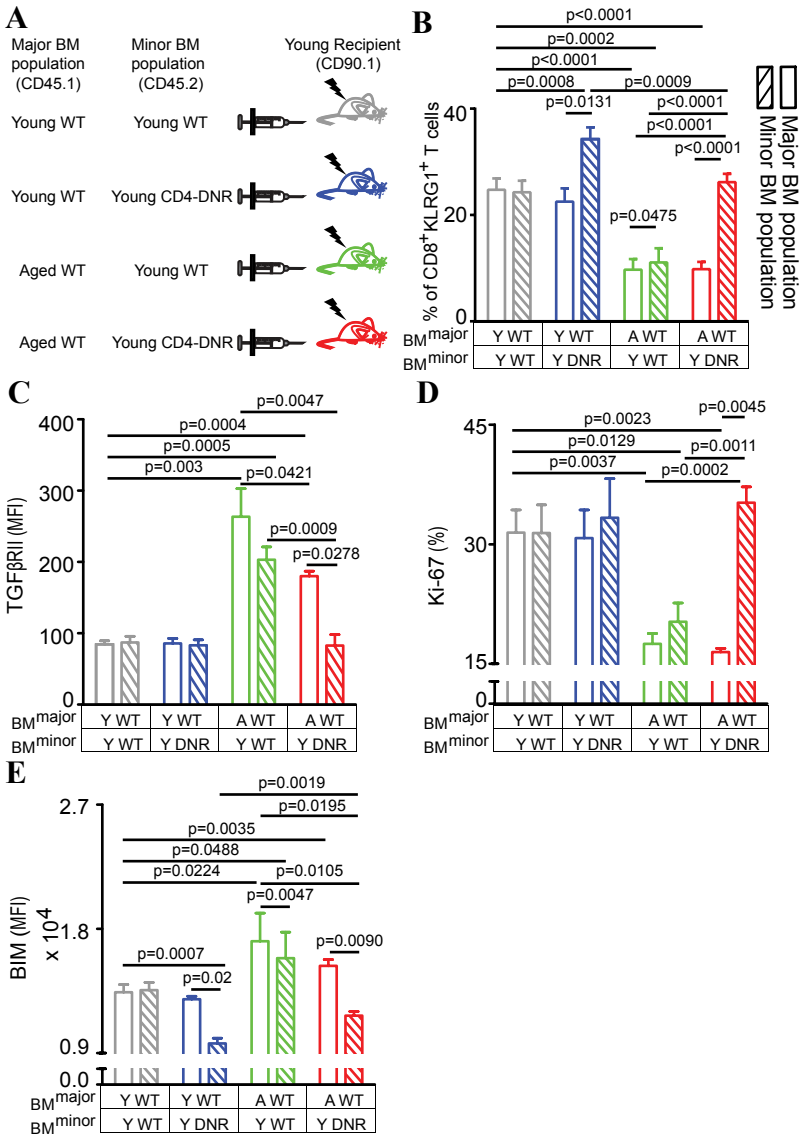

Figure S8. CD8 intrinsic TGFβ signaling is primarily responsible for poor *E. cuniculi* specific effector CD8 proliferation and survival in aged mice. (A) Schematic of mixed bone marrow chimera generation using a combination of young or aged WT and young CD4-DNR donors. (B) Bar graph represents the percentage of splenic KLRG1+ CD8 T cells in these chimeras at day 12 pi. (C) TGFβRII expression levels on splenic KLRG1+ effector CD8 T cells. (D) Proliferation was assessed in these cells by assaying for Ki-67. (E) Bim expression levels were evaluated by intracellular staining.The data represent 2 experiments with 3-4 chimeras per group.

Fig. S9

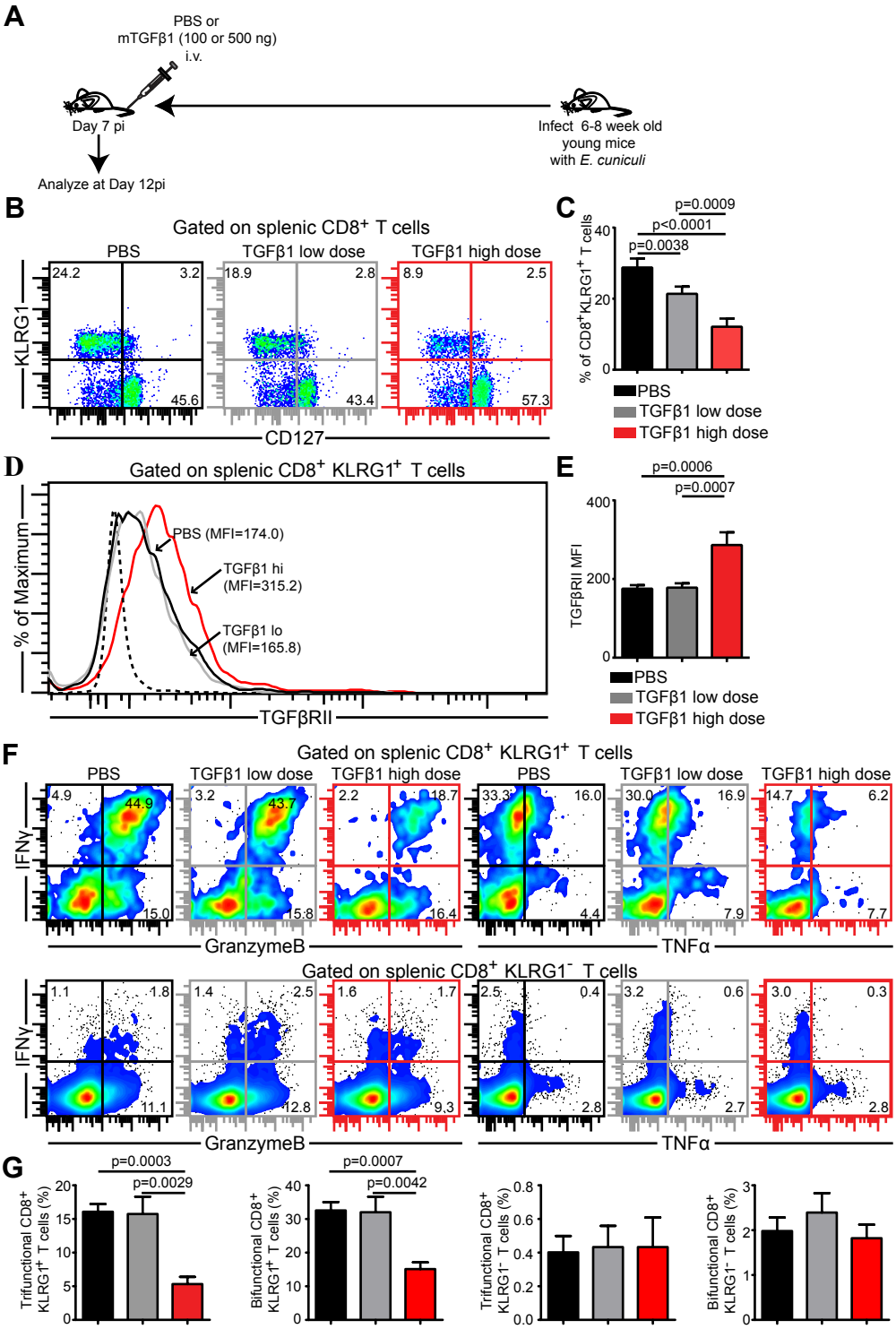

Figure S9. Administration of high but not low TGFβ1 dose results in downregulation of effector CD8 functionality. (A) shows the schematic of TGFβ1 administration. (B, C) Percentage of KLRG1+ CD8 effectors is presented as pseudocolor plots (B) or bar graph (C). (D, E) TGFβRII expression on KLRG1+ effector CD8 T cells is presented as histogram (D) or bar graph (E). (F, G) CD8 polyfunctionality was evaluated in KLRG1+ and KLRG1- CD8 T cells. Data are presented as pseudocolor plots (F) or bar graphs (G). Data are representative of 2 experiments with 4 mice per group. Data in dot plots and histograms represent percentage and MFI respectively.

Fig. S10

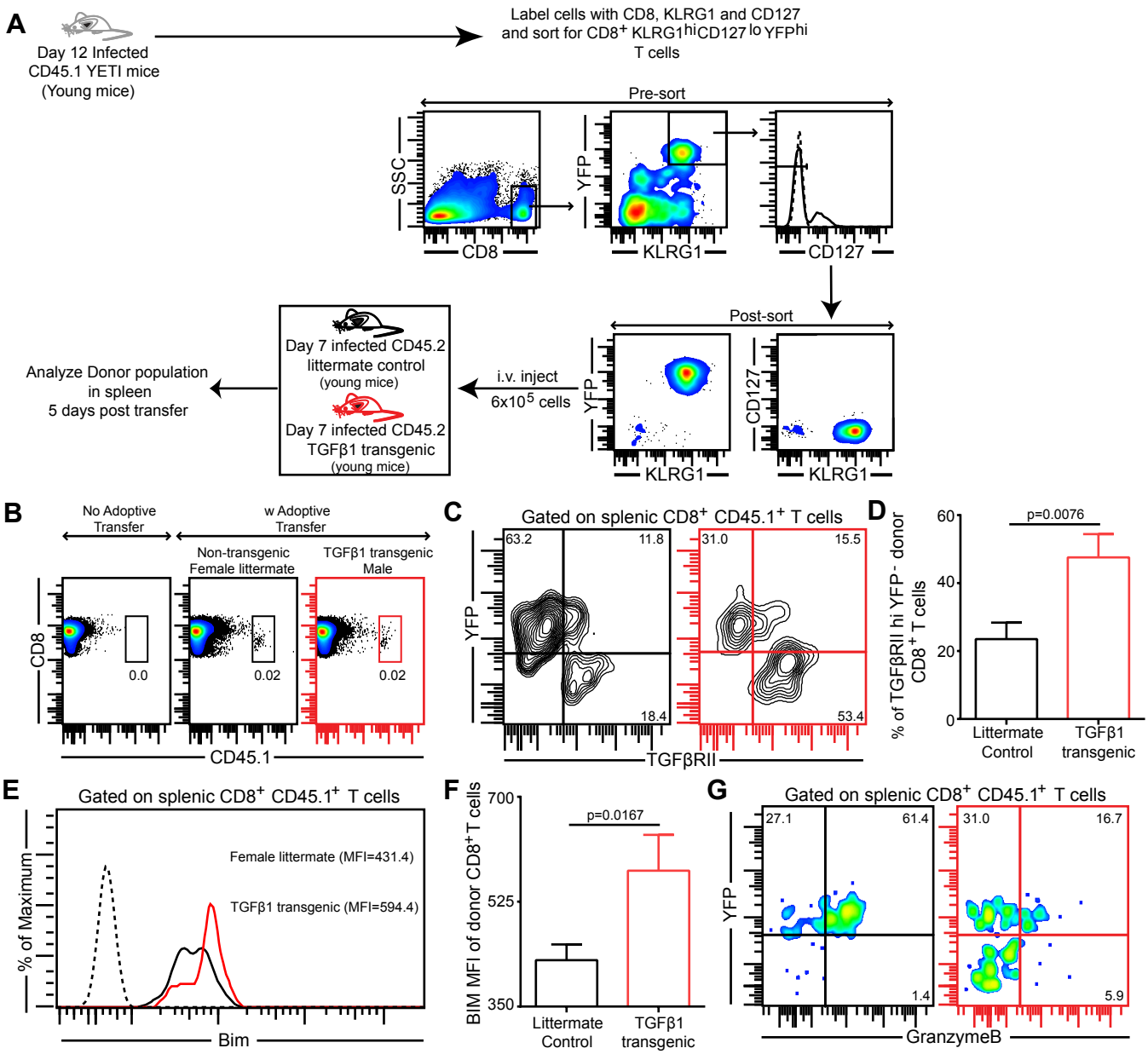

Figure S10. TGFβ mediated loss of CD8 polyfunctionality occurs post effector commitment. (A) Schematic illustrating CD8 sorting. (B) Percentage evaluation of donor cells in the recipient spleens. (C, D) TGFβRII expression on donor cells is presented as contour plots (C) or bar graph (D). (E, F) Bim expression was assayed in these cells by intracellular staining. (G, H) YFP and GranzymeB expression on donor CD8 T cells was evaluated direct ex vivo. Data represent 2 experiments with 3 mice per group. Data in dot plots and histograms represent percentage and MFI respectively.

Fig. S11

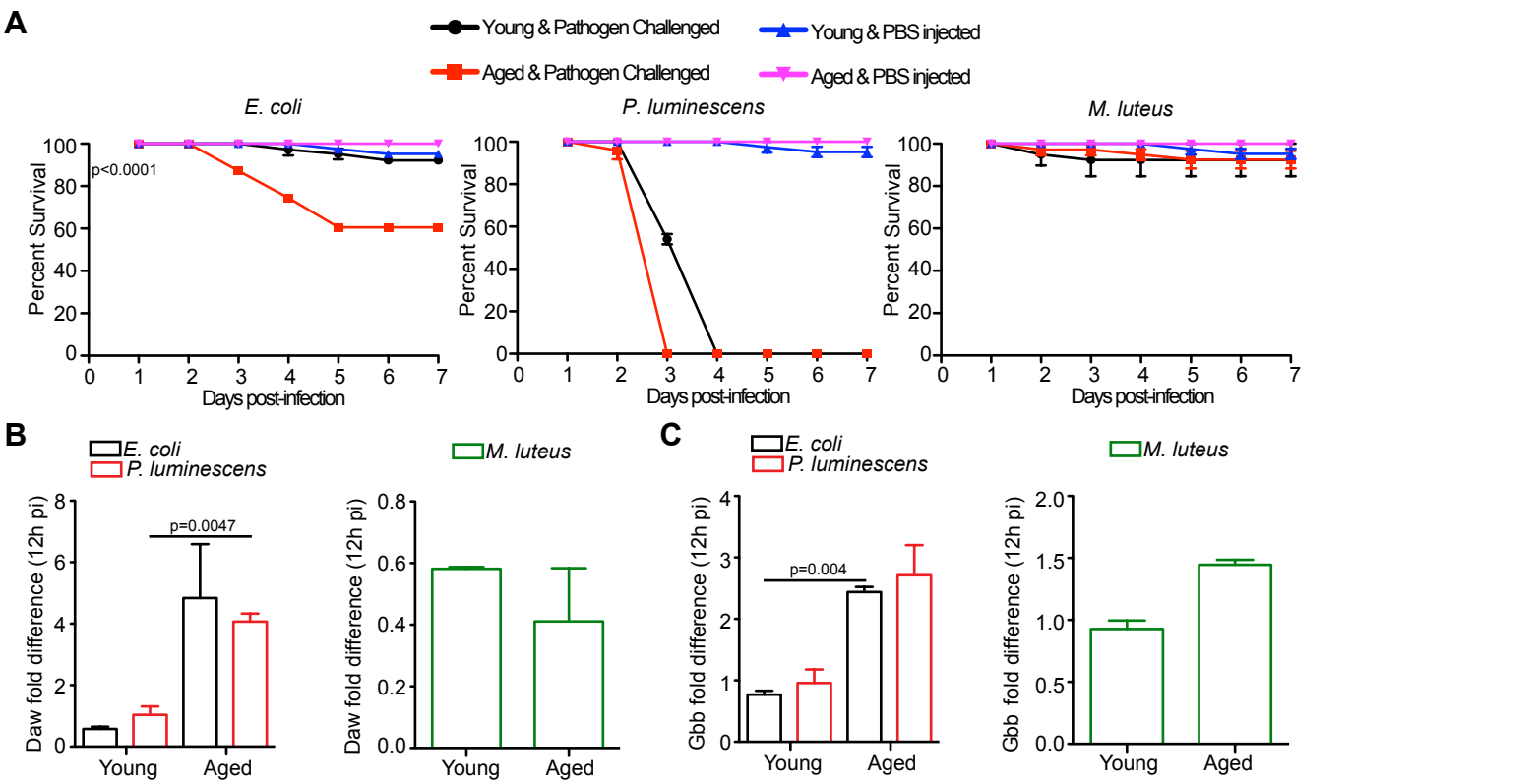

Figure S11. Infection with gram-negative bacteria elicits similar levels of TGF-β homologues in aged Drosophila flies but not in young flies. (A) Survival of flies infected with *E. coli*, *P. luminescens* and *M. luteus*. PBS-injected flies were used as controls. Data represent 2 experiments (n=20). (B) Transcription levels of the TGF-β homologues Dawdle (Daw) and Glass bottom boat (Gbb) in young and aged Drosophila adult flies infected with *E. coli*, *P. luminescens* and *M. luteus* compared to PBS-injected controls. Data represent 3 replicates (5 pooled flies per replicate). Survival data were analyzed using Kaplan-Meier curves and Log-rank analysis. Gene transcription data were analyzed using unpaired two-tailed Student's-t test. Bar graph represents Mean ± SEM from 3 biological replicates.

Fig. S12

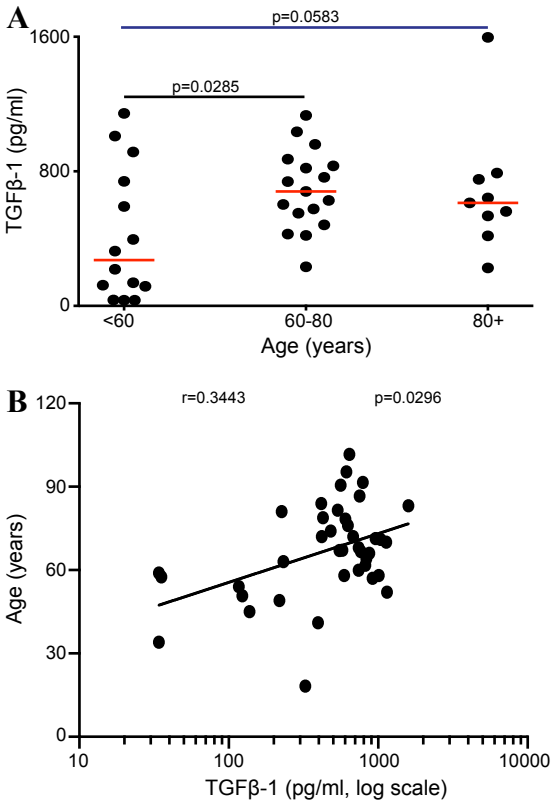

Figure S12. Aged humans exhibit elevated plasma TGFβ-1 levels. (A) Plasma TGF-β1 levels (pg/mL) among the different age groups: Younger than 60 years old (<60), between 60 and 80 years old (60-80) and older than 80 years old (+80). P values for Mann-Whitney U (<60 vs 60-80) and Kruskal-Wallis tests are shown. Red line in graph denotes mean TGFβ-1 level. (B) Direct relationship between the plasma levels of TGFβ-1 (pg/mL) and age (years). Pearson's analysis: r = 0.3443; p = 0.0296.