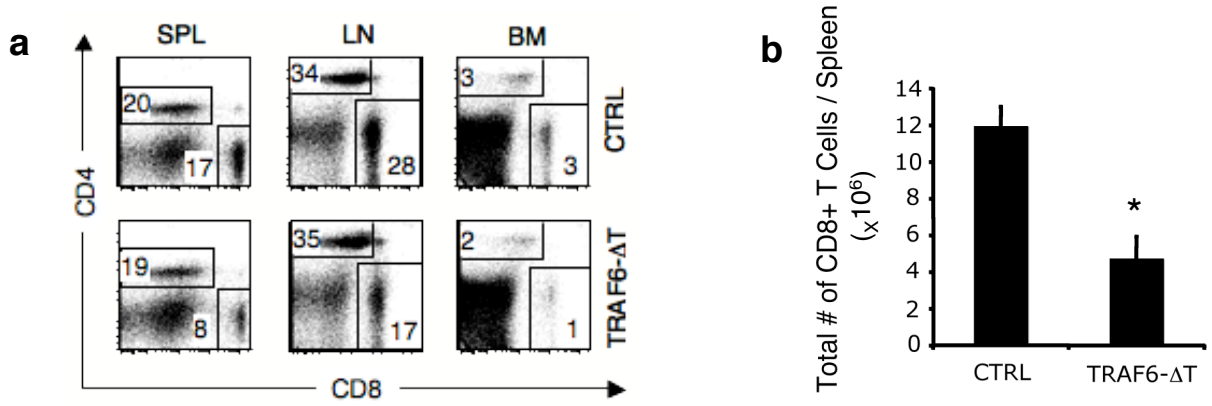
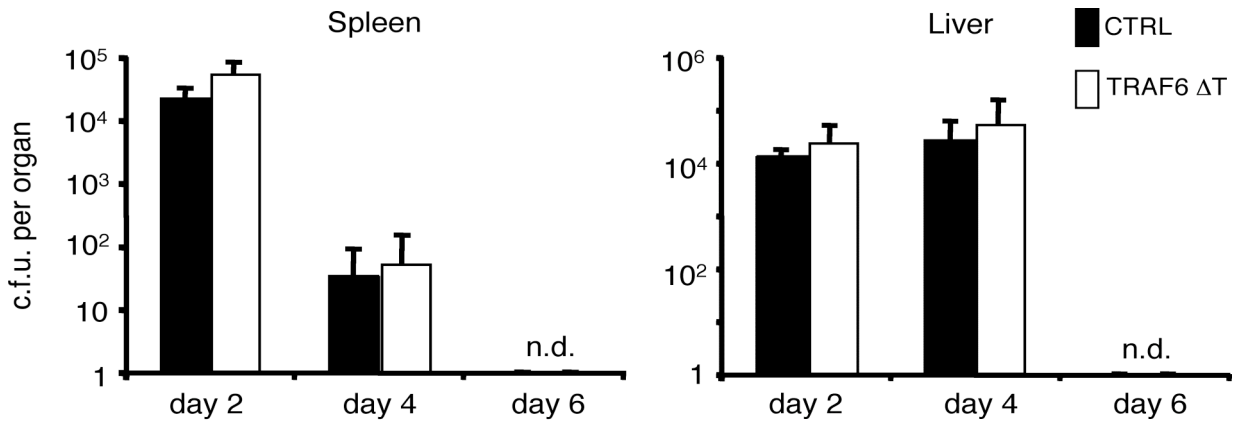
Supplementary Figure 1



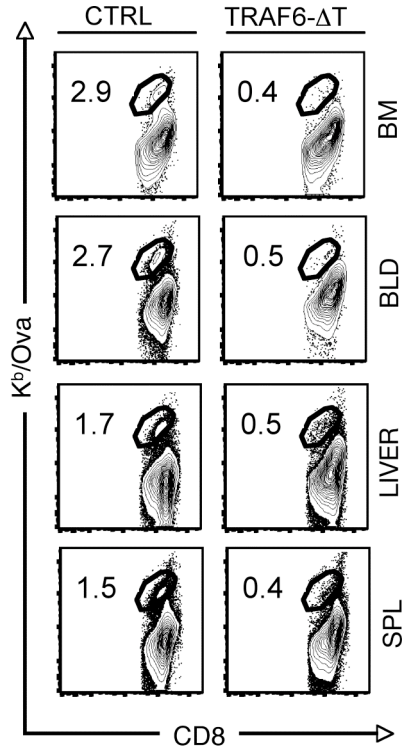
Supplementary Figure 1. TRAF6-ΔT mice have fewer peripheral CD8 T cells compared to CTRL mice. (a) Spleen (SPL), lymph nodes (LN), and bone marrow (BM) cells were isolated from naïve 8-week old CTRL and TRAF6-ΔT mice and stained for surface marker expression. Numbers in the dot plots show the percentages of CD4 and CD8 T cells. (b) Bar graph shows the absolute number of total CD8 T cells per spleen in CTRL and TRAF6-ΔT mice 7 days following infection with LmOva (*n*=3 per group). *p=0.00032

Supplementary Figure 2



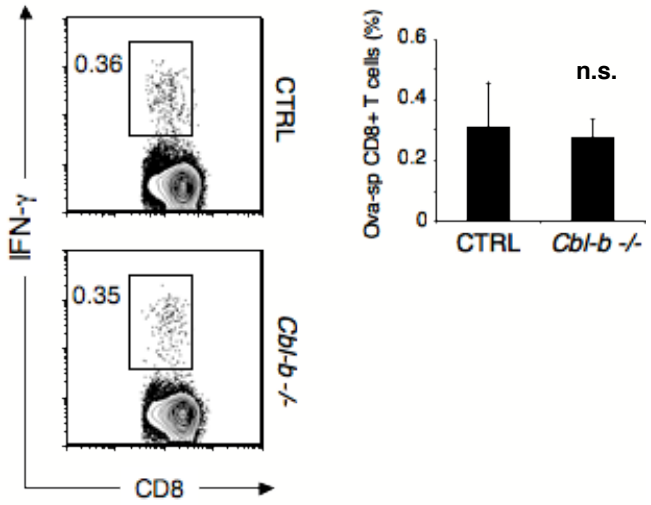
Supplementary Figure 2. Both control and TRAF6-ΔT mice efficiently clear a highly attenuated strain of *L. monocytogenes*. CTRL and TRAF6-ΔT were immunized with attenuated LmOva and bacterial clearance was measured in the spleen and liver 2 (*n*=3-4 per group), 4 (*n*=3-4 per group), and 6 (*n*=2-4 per group) days post-infection. Bar graphs show c.f.u. per organ (means ± standard deviation).

Supplementary Figure 3



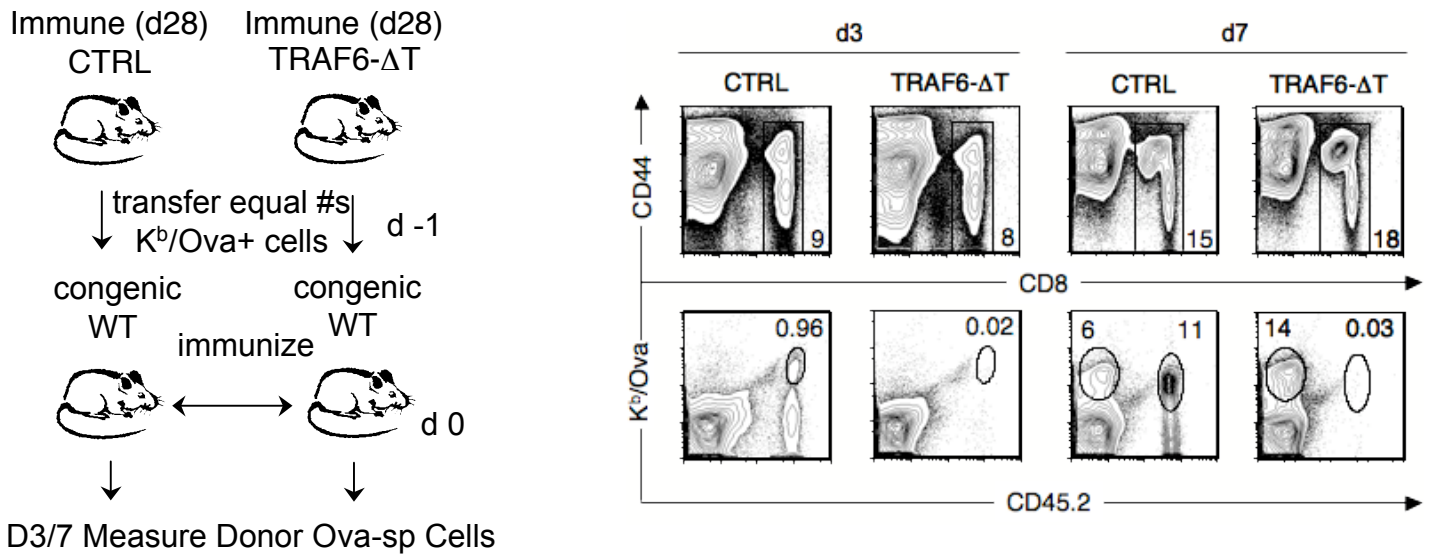
Supplementary Figure 3. CD8 memory T cell development is impaired in TRAF6-ΔT mice early following infection with LmOva. Control (CTRL) and TRAF6-ΔT mice were immunized with LmOva. Spleen (SPL), liver, blood (BLD), and bone marrow (BM) cells were isolated 28 days post-infection and stained with CD8 and K^b/Ova tetramer to measure Ova-specific cells. Numbers reflect percentage of CD8 T cells that are Ova-specific (*n*=3 per group).

Supplementary Figure 4



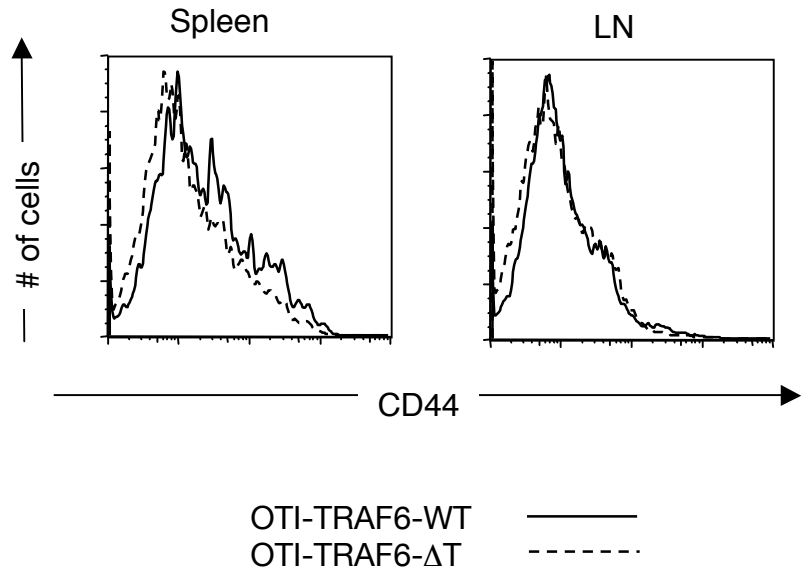
Supplementary Figure 4. *Cbl-b-/-* mice generate normal CD8 T_M in response to *L. monocytogenes*. Control (CTRL) and *Cbl-b-/-* mice (*n*=5 per group) were immunized with LmOva and splenocytes were restimulated with the Ova peptide and analyzed 60 days post-infection by intracellular IFN-γ. Dot plots show IFN-γ-producing CD8 T cells (numbers indicate the percentage of CD8 T cells that produce IFN-γ). Bar graphs show the percentage of total CD8 T cells per spleen that are Ova-specific (means ± standard deviation). n.s.= not significant.

Supplementary Figure 5



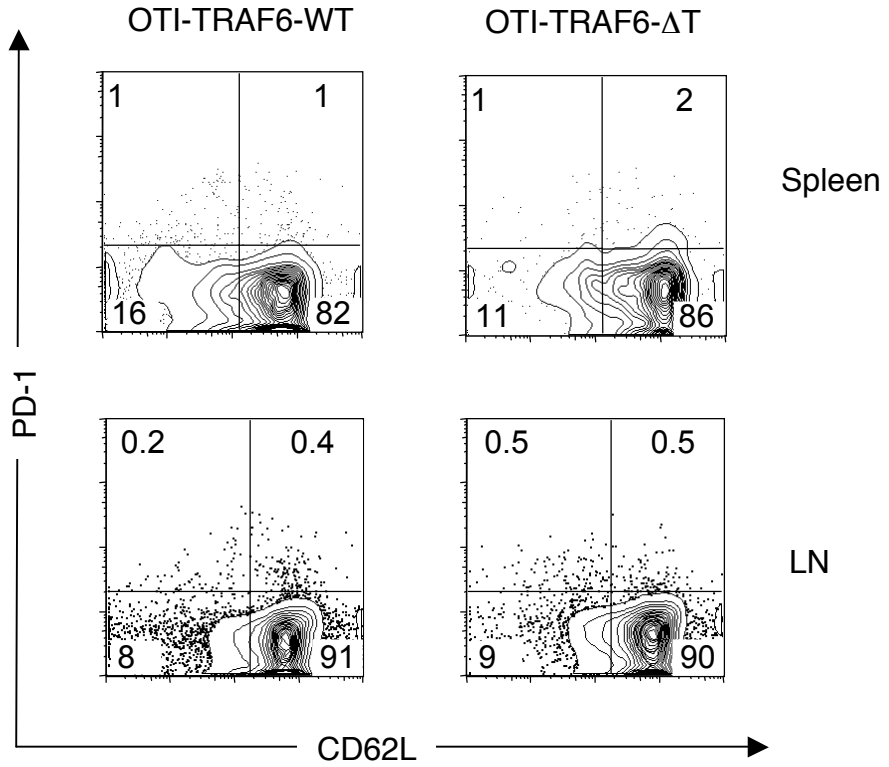
Supplementary Figure 5. CD8 memory T cell development is impaired in TRAF6-ΔT mice even when compared on a per cell basis. Equal numbers of K^b/Ova tetramer+ CD8 T cells from immunized CTRL and TRAF6-ΔT mice 28 days post-infection were adoptively transferred into congenic (CD45.1) recipients (*n*=3-4 per group) and then challenged with LmOva. Splenocytes were analyzed 3 and 7 days post-challenge for surface marker expression. Numbers indicate the percentage of CD8 T cells (live gated, top panel) or the percent of donor-derived (CD45.2) Ova-specific CD8 T cells (CD8 gated, bottom panel).

Supplementary Figure 6



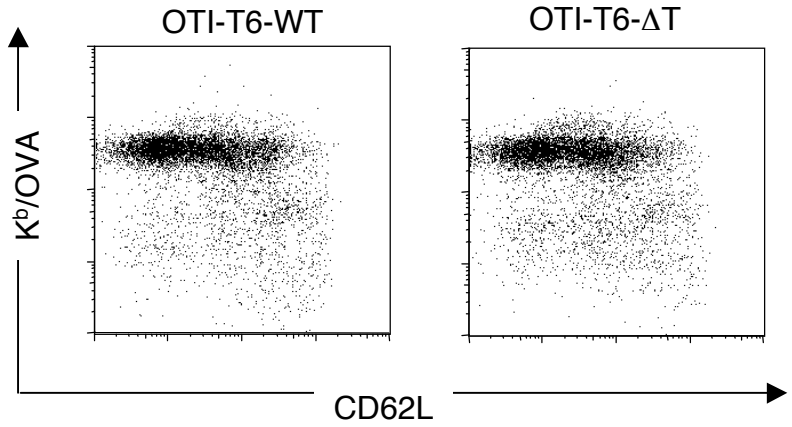
Supplementary Figure 6. CD44 expression on CD8 T cells from OTI-TRAF6-WT and OTI-TRAF6-ΔT mice. Histograms depict the levels CD44 on CD8 T cells from the spleen and lymph nodes (LN) of naïve mice.

Supplementary Figure 7



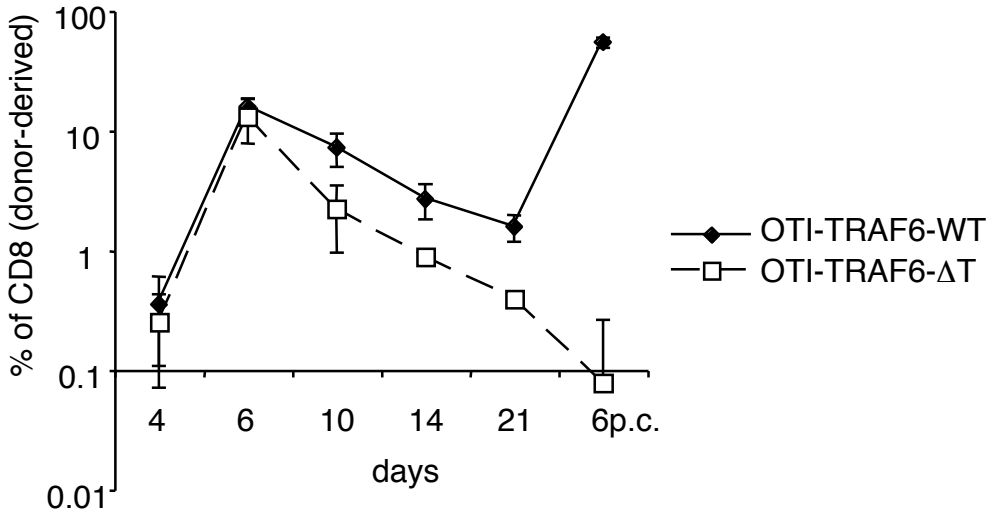
Supplementary Figure 7. Surface marker expression on CD8 T cells from OTI-TRAF6-WT and OTI-TRAF6-ΔT mice. Dot plots depict the levels of PD-1 and CD62L from the spleen and lymph nodes (LN) of naïve mice (K^b/Ova gate).

Supplementary Figure 8



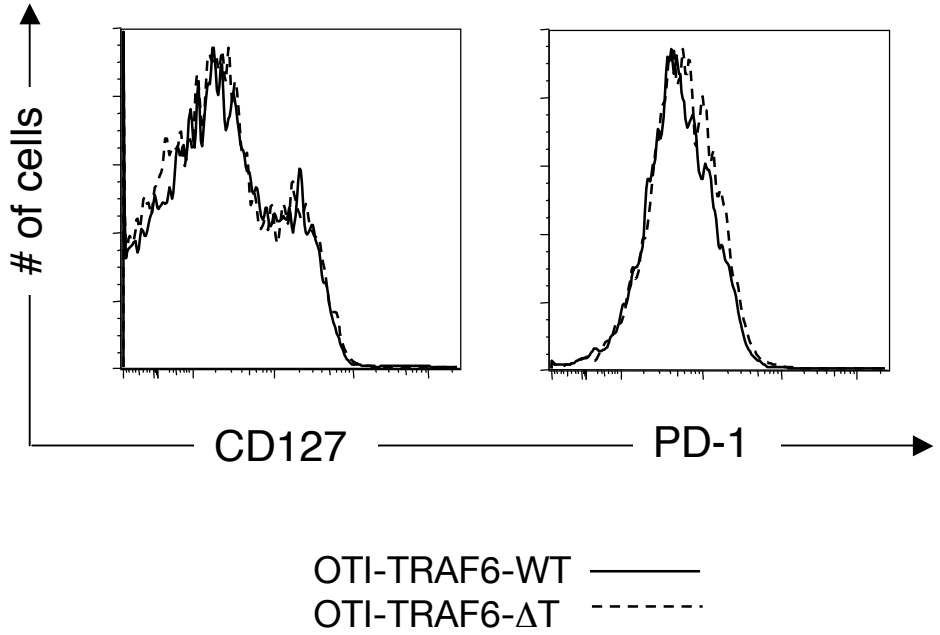
Supplementary Figure 8. Surface marker and TCR expression on CD8 T cells from OTI-TRAF6-WT and OTI-TRAF6-ΔT mice. Dot plots depict the levels of K^b/Ova-specific TCR and CD62L from the spleen of naïve mice (CD8 gate).

Supplementary Figure 9



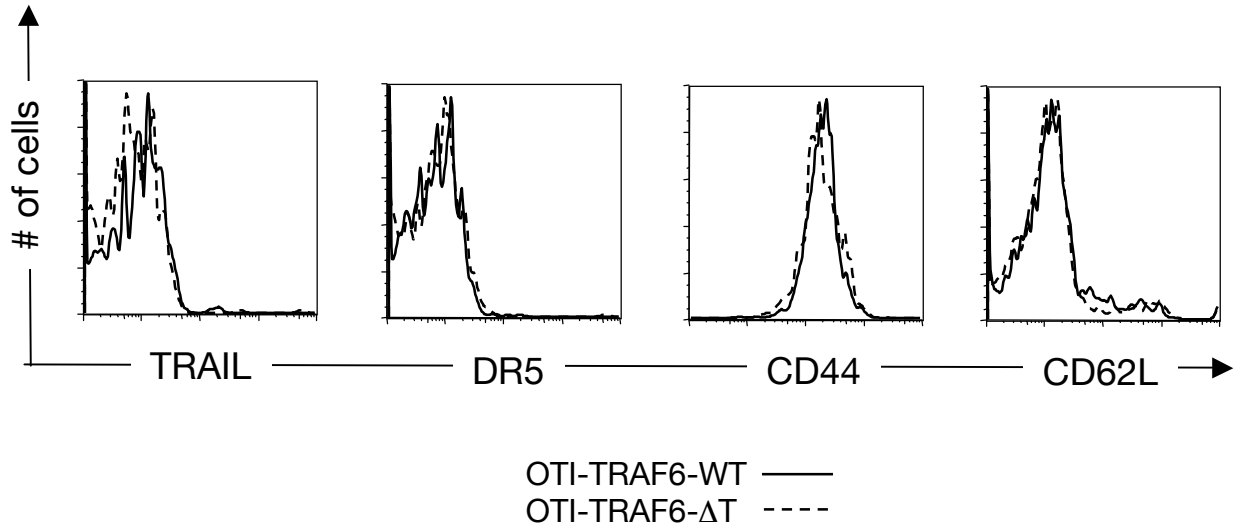
Supplementary Figure 9. OTI-TRAF6-ΔT donor cells are not maintained during contraction following primary immunization with LmOva. This line graph is on log scale and is generated from the data in Figure 2c.

Supplementary Figure 10



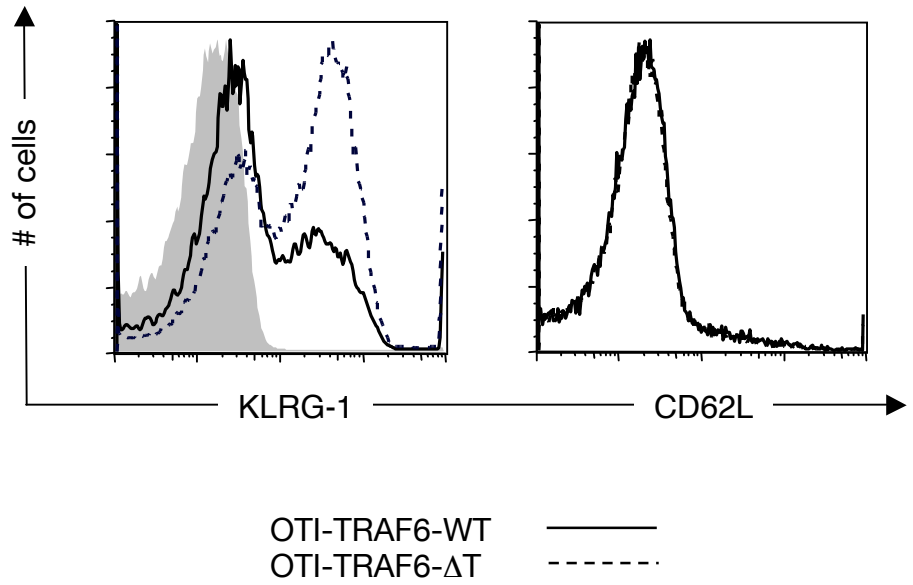
Supplementary Figure 10. Surface marker expression on donor CD8 T cells from OTI-TRAF6-WT and OTI-TRAF6-ΔT mice. <5000 OTI-TRAF6-WT and OTI-TRAF6-ΔT CD8 T cells were transferred into congenic recipients. Recipient mice were then infected with LmOva and surface marker expression assessed on donor cells 7 days following infection. Histograms depict the levels of CD127 and PD-1 on donor K^b/Ova-specific splenocytes.

Supplementary Figure 11



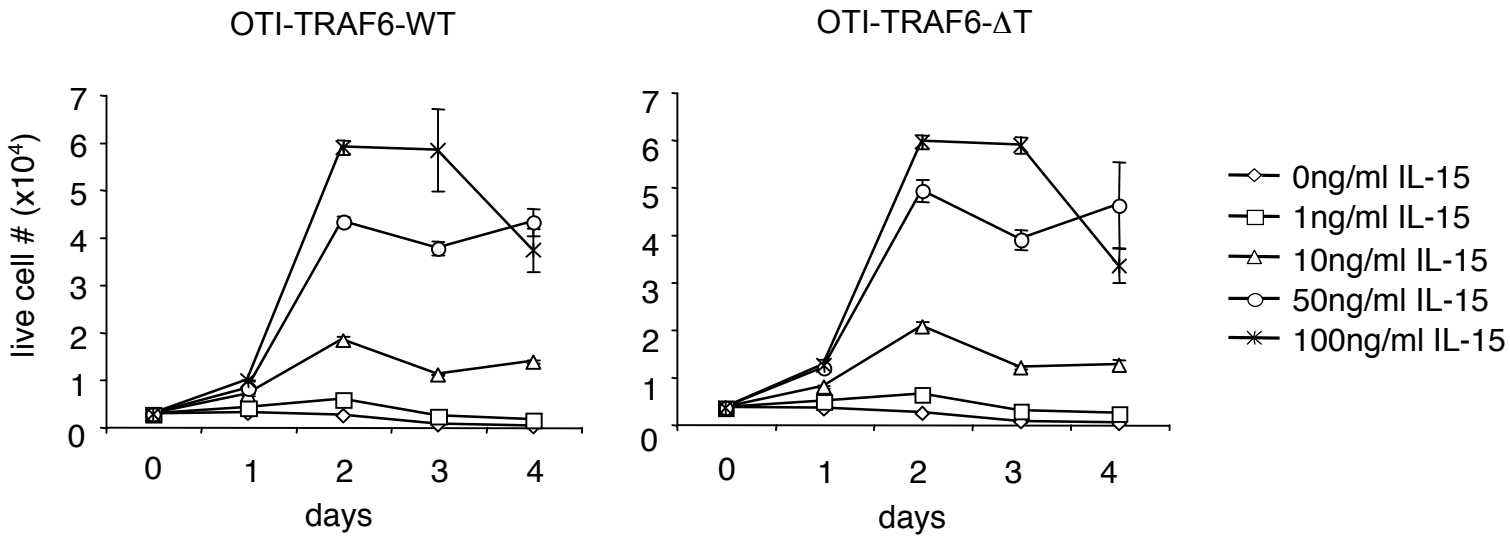
Supplementary Figure 11. Surface marker expression on donor CD8 T cells from OTI-TRAF6-WT, and OTI-TRAF6-ΔT mice. <5000 OTI CD8 T cells were transferred into congenic recipients. Recipient mice were then infected with LmOva and surface marker expression assessed on donor cells 7 days following infection. Histograms depict the levels of TRAIL, DR5 (TRAIL receptor), CD44, and CD62L donor K^b/Ova-specific splenocytes.

Supplementary Figure 12



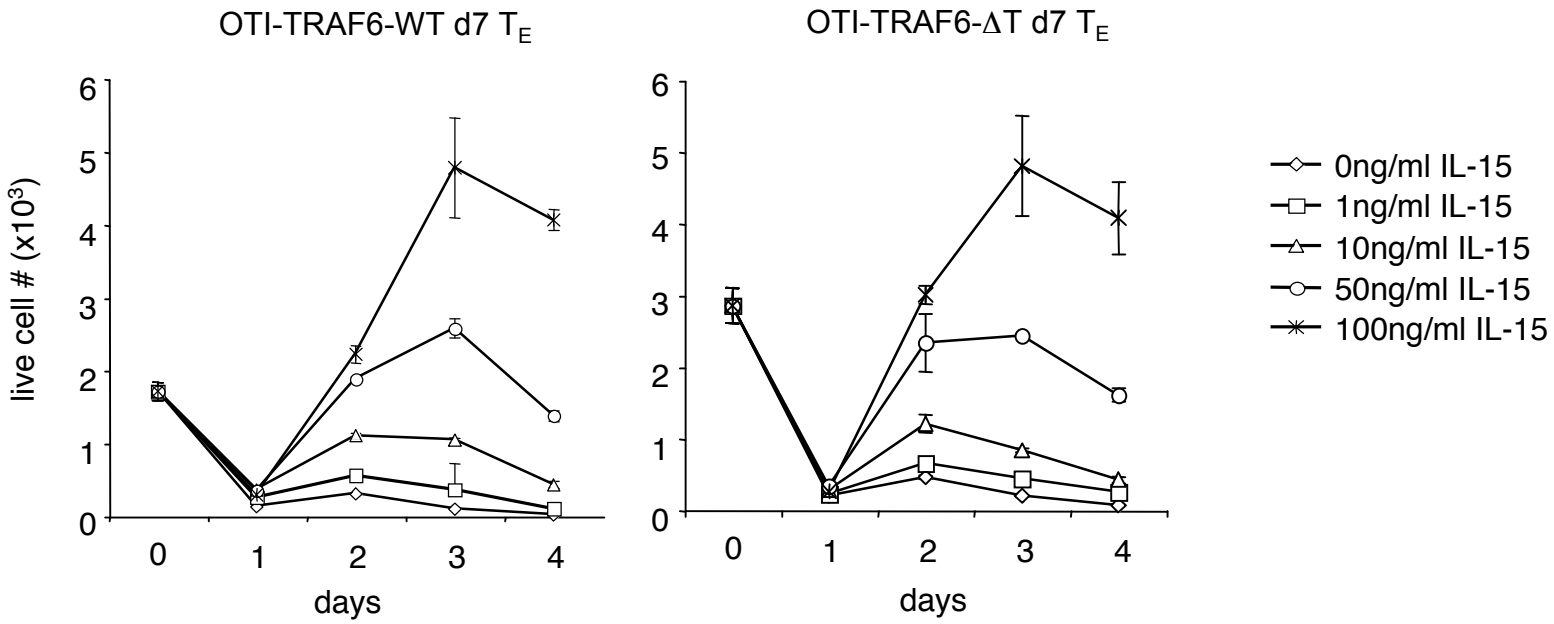
Supplementary Figure 12. Surface marker expression on donor CD8 T cells from OTI-TRAF6-WT and OTI-TRAF6-ΔT mice. <5000 OTI-TRAF6-WT and OTI-TRAF6-ΔT CD8 T cells were transferred into CD45.1+ congenic recipients. Recipient mice were then infected with LmOva and surface marker expression assessed on donor cells 7 days following infection. Histograms depict the levels of KLRG1 (shaded area, isotype control) and CD62L on donor CD45.2+ CD8 T cells.

Supplementary Figure 13



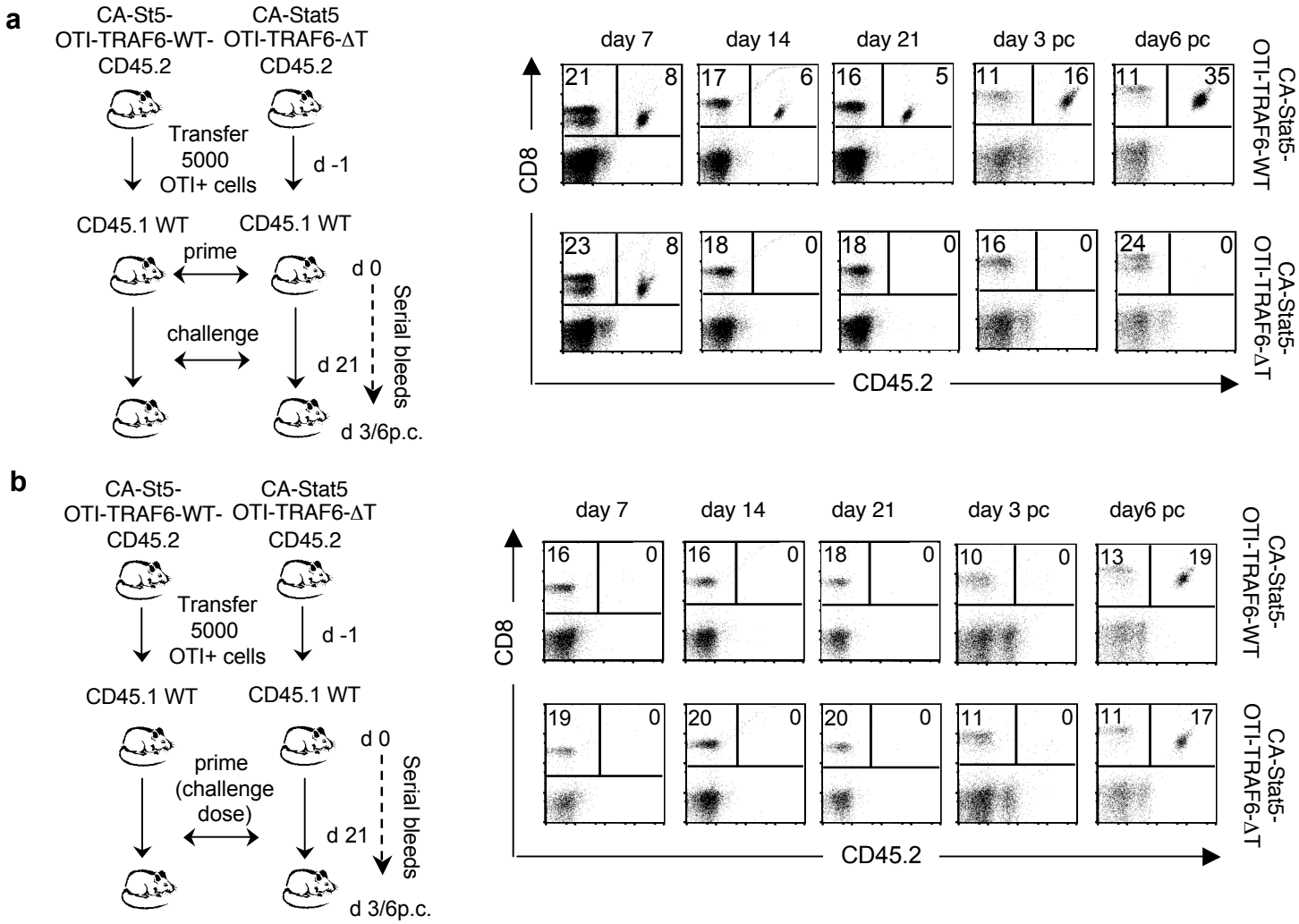
Supplementary Figure 13. *In vitro* activated OTI-TRAF6-WT and OTI-TRAF6-ΔT T_E cells exhibit similar responsiveness to IL-15 in culture. Purified CD8 T cells from OTI-TRAF6-WT and OTI-TRAF6-ΔT mice were activated with αCD3/28 and cultured with 100 U/ml of IL-2 for 3 days. Cells were then counted and re-plated in triplicate with decreasing concentrations of IL-15 and cultured for 4 days. Live cells were quantified by FACS (7-AAD exclusion) for each timepoint indicated (means ± standard deviation).

Supplementary Figure 14



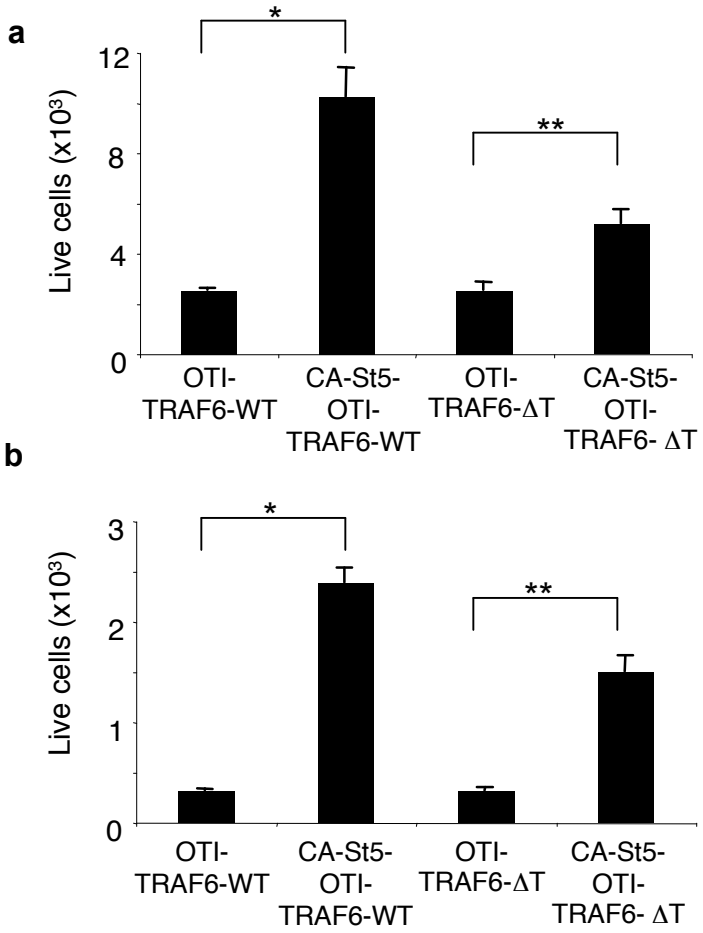
Supplementary Figure 14. *In vivo* activated OTI-TRAF6-WT and OTI-TRAF6-ΔT T_E cells exhibit similar responsiveness to IL-15 in *ex vivo* culture. <5000 OTI-TRAF6-WT and OTI-TRAF6-ΔT CD8 T cells were transferred into congenic recipients (*n*=3 per group). Recipient mice were then infected with LmOva and 7 days later donor cells were purified and cultured with decreasing concentrations of IL-15 for 4 days (wells in triplicate). Live cells were quantified by FACS (7-AAD exclusion) for each timepoint indicated (means ± standard deviation).

Supplementary Figure 15



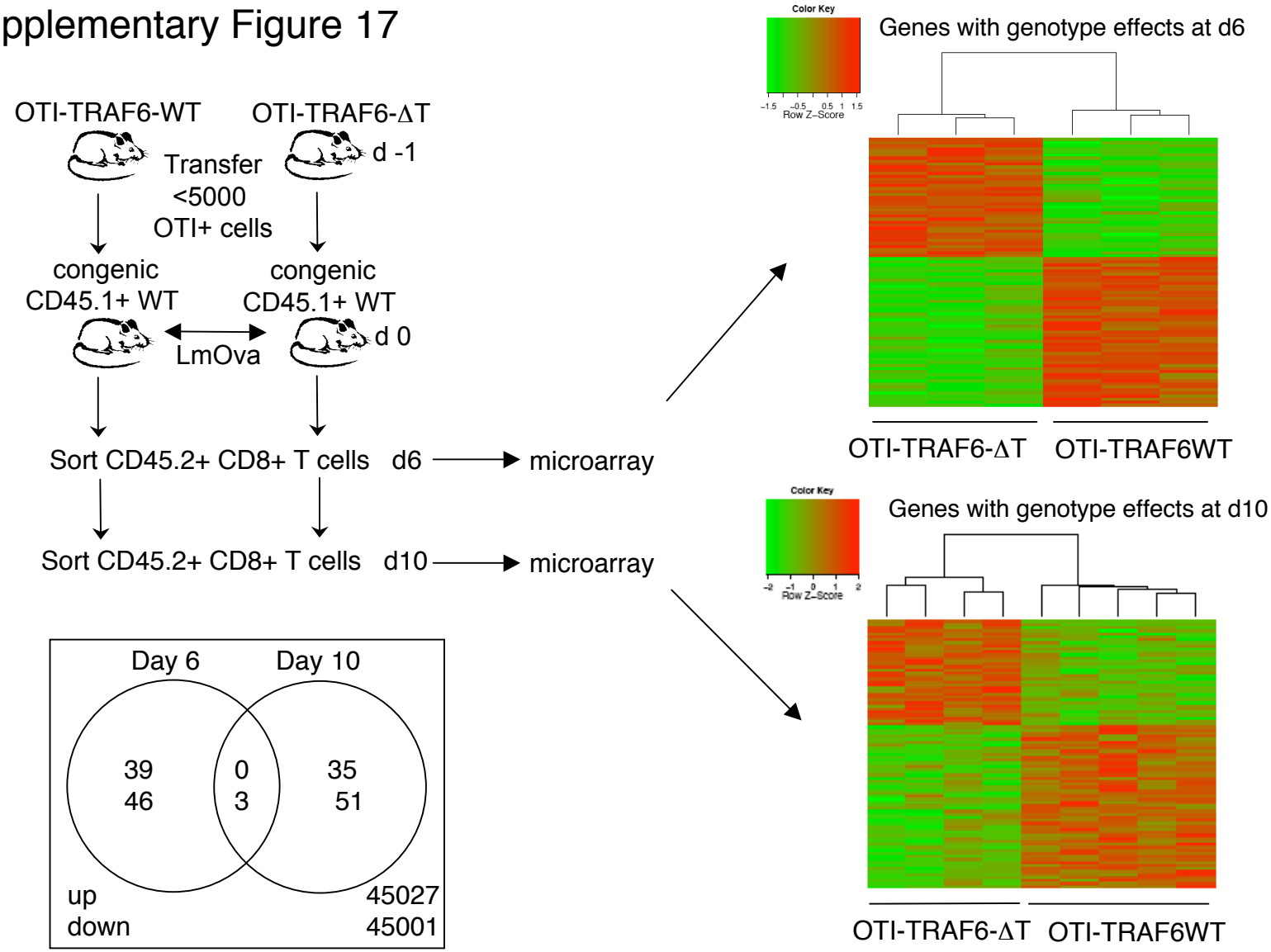
Supplementary Figure 15. Defect in TRAF6-deficient CD8 T_M generation by is not rescued by CD8 T cell overexpression of constitutively active Stat5 (CA-St5). These schematics and dot plots were generated from the same experiment outlined in Fig. 2d (a) and Fig. 2e (b). Dot plots show donor OT-I cells by CD45.2 (numbers indicate the percentage of CD8 T cells that are host or donor-derived, live gated).

Supplementary Figure 16



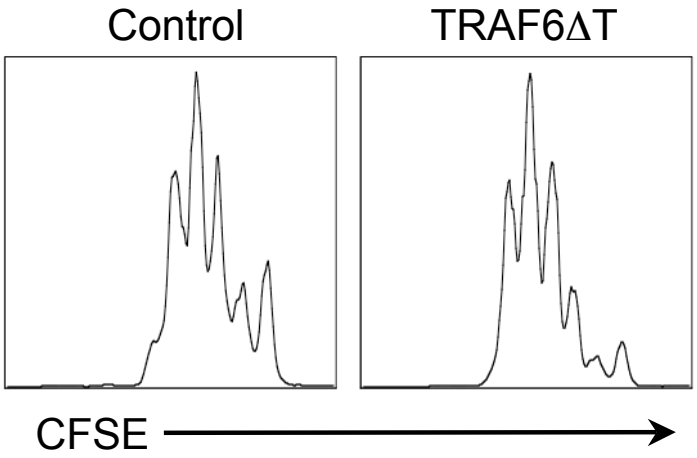
Supplementary Figure 16. Overexpression of constitutively active Stat5 has a positive effect on CD8 T cell survival. <5000 OTI CD8 T cells from OTI-TRAF6-WT,OTI-TRAF6-ΔT, CA-St5-OTI-TRAF6-WT, and CA-St5-OTI-TRAF6-ΔT mice were transferred into congenic WT recipients (*n*=4 per group) and recipient mice were then infected with LmOva. 7 days following infection donor cells were purified, counted, and equal numbers re-plated in triplicate and cultured with low doses of IL-2 (10U/ml), (a) or IL-15 (10ng/ml), (b). Line graphs show numbers of live cells following 96 hours of culture (means ± standard error). Live cell numbers were quantified by FACS (7-AAD exclusion). *p value= 0.007,**p value=0.003, (a). *p value= 0.001,**p value=0.004, (b).

Supplementary Figure 17



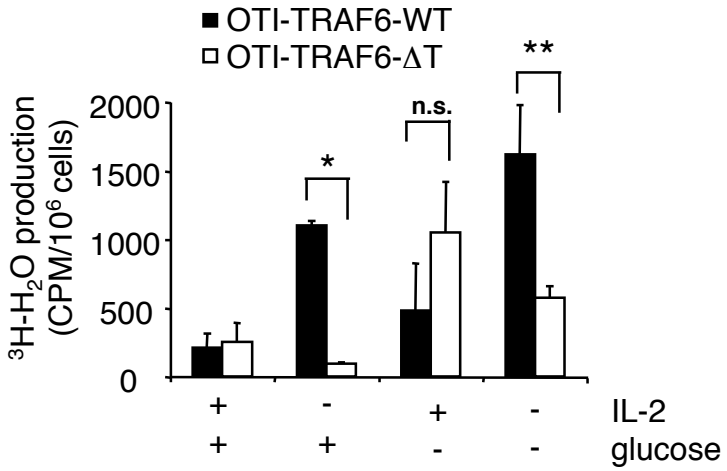
Supplementary Figure 17. Microarray analysis of OTI-TRAF6-WT and OTI-TRAF6-ΔT early and late-stage T_E cells following LmOva infection. Schematic of experimental setup (a) heat maps (b,c) and Venn diagram (d) from the microarray analysis.

Supplementary Figure 18



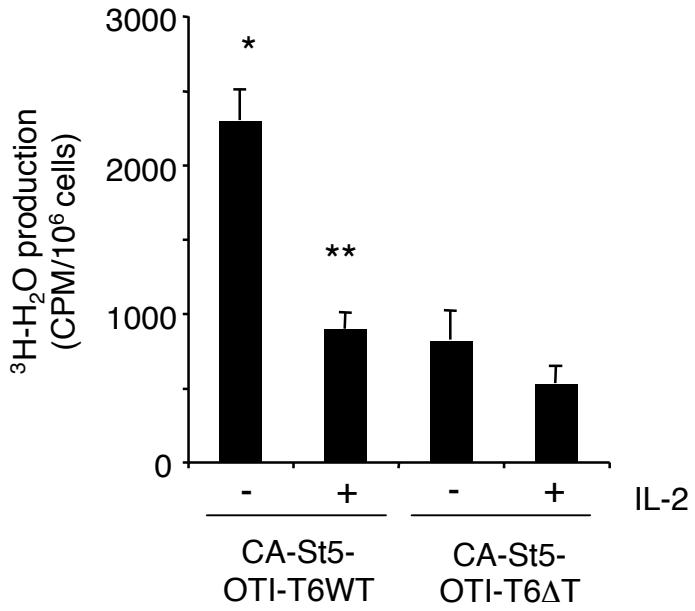
Supplementary Figure 18. TRAF6-ΔT CD8 T cells are not defective in their activation following *in vitro* stimulation. CFSE profile of sorted naïve CD8 T cells on plate-bound αCD3 (2.5 μg/ml) and αCD28(1 μg/ml) cultured for 3 days.

Supplementary Figure 19



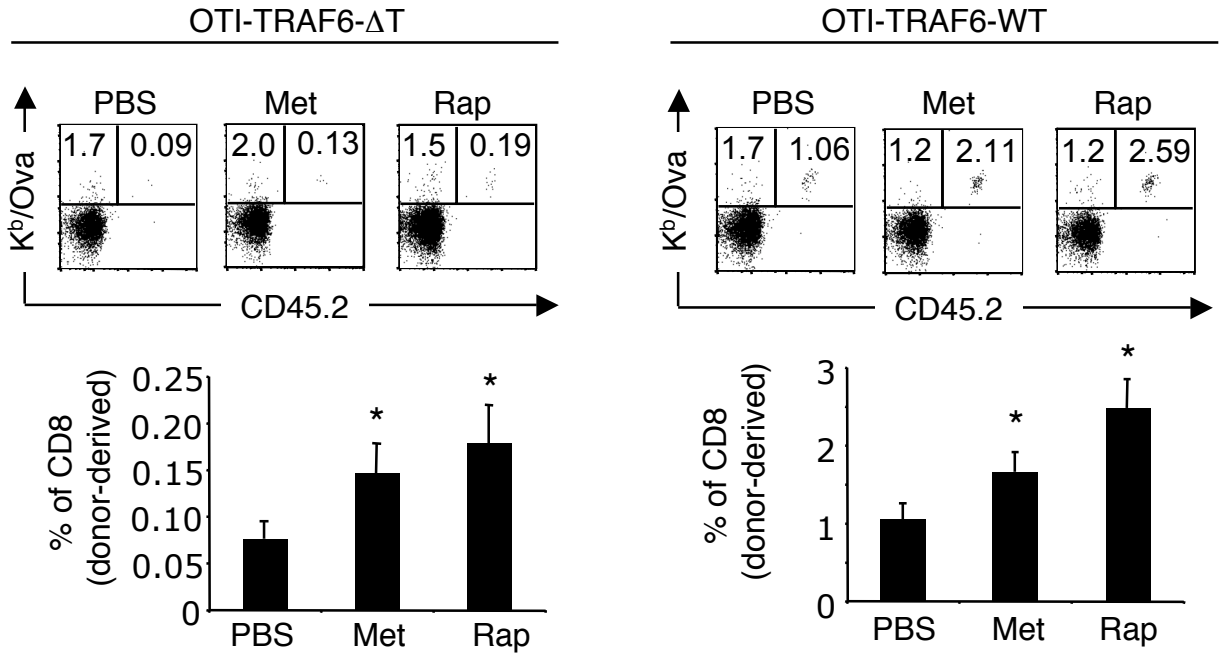
Supplementary Figure 19. Mitochondrial β-oxidation of activated OTI-TRAF6-WT and OTI-TRAF6-ΔT cells following IL-2 and/or glucose withdrawal (data generated from experiment in Fig. 3b). p =* 0.012, ** 0.027, n.s., not significant.

Supplementary Figure 20



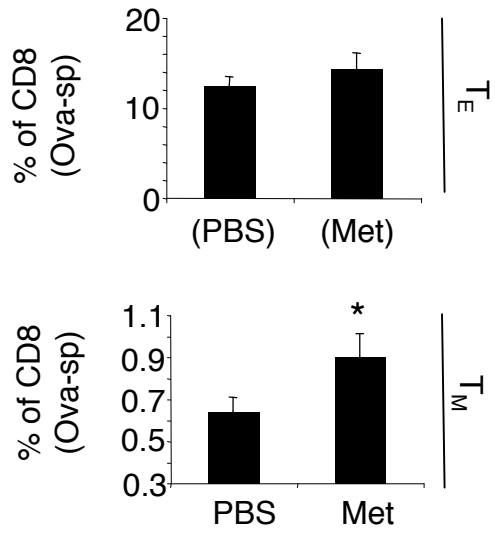
Supplementary Figure 20. Transgenic expression of constitutively active Stat5 (CA-St5) does not rescue the defect in β-oxidation in TRAF6-deficient CD8 T cells following IL-2 withdrawal. Bar graph shows mitochondrial β-oxidation of activated CA-St5-OTI-TRAF6-WT and CA-St5-OTI-TRAF6-ΔT +/- IL-2. Comparing between genotypes, *p value =0.001 (-)IL-2 and **p value =0.027 (+) IL-2.

Supplementary Figure 21



Supplementary Figure 21. Metformin and rapamycin treatment promote T_M generation. OT-I cells (<5000) isolated from OTI-TRAF6-WT and OTI-TRAF6-ΔT mice (CD45.2) were adoptively transferred into CD45.1 congenic recipients followed by primary immunization with LmOva. On day 8 postinfection mice were treated with daily injections of PBS (*n*=7-9 per group), metformin (*n*=7-9 per group), or rapamycin (*n*=5 per group) for three weeks. Numbers in the dot plots reflect the percentages of total CD8 T_M cells in the blood that are host or donor donor-derived (Ova-specific) at 28 days post-infection (when treatments ceased) and bar graphs represent the percent of CD8 T cells in the blood that are donor-derived (means ± standard error). *p value (compared to PBS controls) = 0.0307 (Met) and 0.0108 (Rap)(a), 0.036 (Met) and 0.0013 (Rap)(b).

Supplementary Figure 22



Supplementary Figure 22. Daily metformin injections increase Ova-specific T_M following LmOva immunization in C57Bl/6 mice. C57BL/6 mice were immunized with LmOva and 7 days post-infection daily injections of metformin or PBS were given for 3 weeks. Bar graphs represent the percentage of Ova-specific cells in the blood 7 days (upper panel, mice destined for PBS (*n*=8 per group) or metformin (*n*=9 per group) treatment) and 28 days post-infection (lower panel, mice after receiving PBS or metformin treatments) (means ± standard error) *p value=0.028.