mTORC1 and mTORC2 selectively regulate CD8$^+$ T cell differentiation

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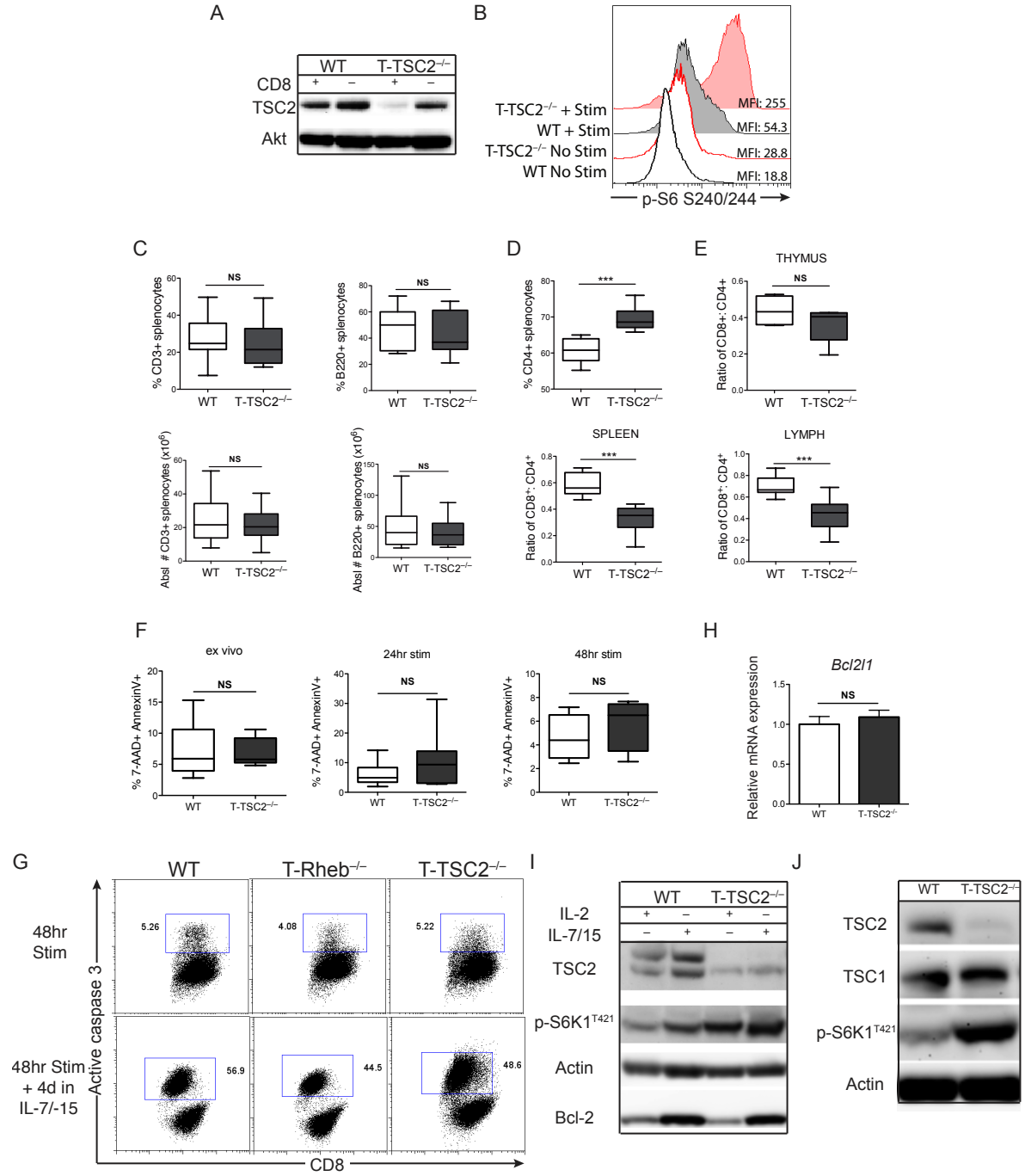
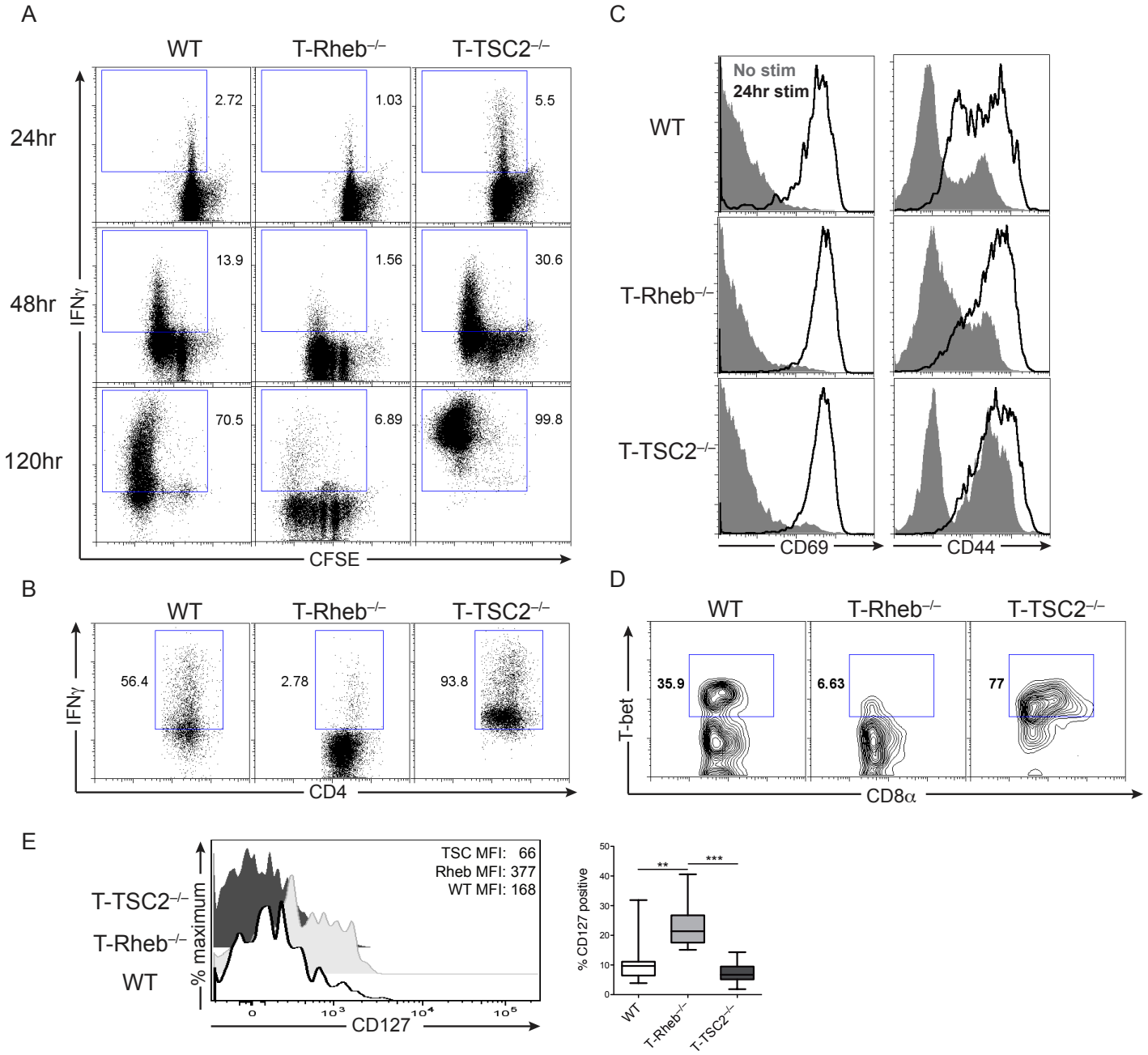
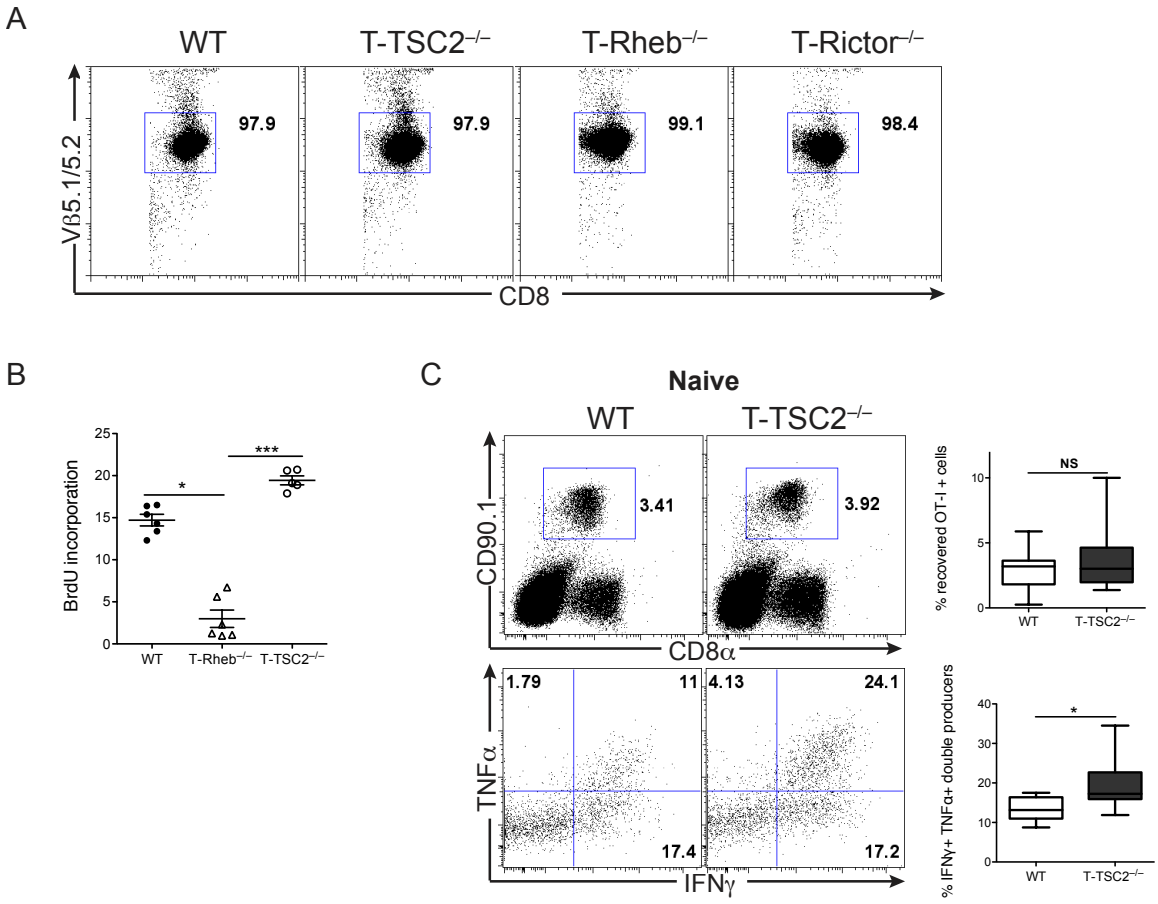
Supplemental Figures



Supplemental Figure 1: TSC2 deficiency does not promote enhanced cellular death. (A-G) WT and T-TSC2$^{-/-}$ splenocytes were harvested from 6 wk old mice. (A) CD8$^+$ and non-CD8$^+$ cells were analyzed by immunoblot analysis for TSC2 level. Total Akt is shown as a loading control. (B) T-TSC2$^{-/-}$ cells have enhanced activation of mTORC1 compared to WT levels even in the absence of TCR stimulation. Cells were left unstimulated or stimulated in vitro for 24hr. (C) The percentage and absolute number of T cells and B cells, (D) CD4$^+$ splenocytes, and the ratio of CD8$^+$ to CD4$^+$ splenocytes, (E) thymocytes, or lymph node derived T cells were determined from WT and T-TSC2$^{-/-}$ mice, n=9. (F) Cellular death of WT and T-TSC2$^{-/-}$ CD8+ T cells was assessed by Annexin V and 7-AAD staining directly ex vivo, or 24 and 48hrs after in vitro stimulation. (G) Active caspase 3 levels were detected by flow cytometry 48hr after in vitro stimulation or after expansion in IL-7 and IL-15 for 4 days. (H) Purified CD8$^+$ T cells from WT and T-TSC2$^{-/-}$ mice were stimulated in vitro for 48hr and then expanded in media supplemented with IL-7 and IL-15 for 3 days. On day 3, relative transcript expression of *Bcl2l1* (Bcl-xL) was determined by qPCR. Data are mean ± SEM of 3 measurements. (I) Bcl-2 protein expression and mTORC1 activation was assessed after expansion in IL-2 or IL-7 and IL-15 for 3 days. (J) TSC1, TSC2 and p-S6K levels were detected after culture in IL-7 and IL-15 for 3 days.



Supplemental Figure 2: mTORC1 deficiency does not impair antigen-induced activation, but does reduce effector function. (A) CFSE labeled splenocytes from WT, T-Rheb$^{-/-}$, and T-TSC2$^{-/-}$ mice were stimulated with αCD3. At 20 hr and 44 hr after stimulation, golgi stop was added to a sampling of each culture for 4hr and IFNγ production was determined compared to CFSE dilution. Plots were gated on CD8^{+} T cell populations. At 48hr, cells were expanded in media supplemented with IL-2, and 116 hr post initial stimulation, live cells were stimulated with αCD3/αCD28 for 4hr to assess IFNγ production. Gates were determined from unstimulated controls. (B) WT, T-Rheb$^{-/-}$, and T-TSC2$^{-/-}$ splenocytes were stimulated with αCD3. 44hr after stimulation, golgi stop was added to cultures for 4hr and IFNγ production was determined from the CD4^{+} population. (C) Splenocytes from WT, T-Rheb$^{-/-}$, and T-TSC2$^{-/-}$ mice were harvested and expression of CD69 and CD44 was determined from the CD8^{+} T cell population directly ex vivo or after 24hr in vitro stimulation. (D-E) As in Figure 3A, (D) T-bet protein and (E) CD127 expression was assessed from OVA specific CD8+ T cells after in vivo infection with Vaccinia-OVA 6 days prior. Statistics shown to the right, n=16.



Supplemental Figure 3: Phenotypic analysis of OT-I$^+$ CD8$^+$ T cells from conditional KO mice. (A) Splenocytes from WT OT-I$^+$, T-Rheb$^{-/-}$ OT-I$^+$, T-TSC2$^{-/-}$ OT-I$^+$, and T-Rictor$^{-/-}$ OT-I$^+$ mice were harvested and stained ex vivo for Vβ5.1/Vβ5.2 and CD8 to determine SIINFEKL specificity. Plots gated on CD8$^+$ T cell population. (B) WT, T-Rheb$^{-/-}$, and T-TSC2$^{-/-}$ CD8$^+$ OT-I$^+$ CD90.1$^+$ cells were adoptively transferred into WT CD90.2$^+$ recipients infected with vaccinia-OVA, 5 days later mice were injected with 100ug brdU and after 16hrs mice were harvested and brdU incorporation of OT-I$^+$ splenocytes was determined. (C) 7.5x10^4 naïve sorted WT and T-TSC2$^{-/-}$ CD8$^+$ OT-I$^+$ CD90.1$^+$ cells were adoptively transferred into WT CD90.2$^+$ recipients infected with vaccinia-OVA. Six days post transfer and infection, top panel: percentage of CD8$^+$ CD90.1$^+$ splenocytes was determined. Right graph depicts cumulative percentages of CD8$^+$CD90.1$^+$ splenocytes, n=25. Bottom panel: splenocytes were stimulated with SIINFEKL and IFNγ and TNFα production was determined by flow cytometry. Right plot depicts percentage of CD90.1$^+$ cells producing IFNγ and TNFα, n=10.

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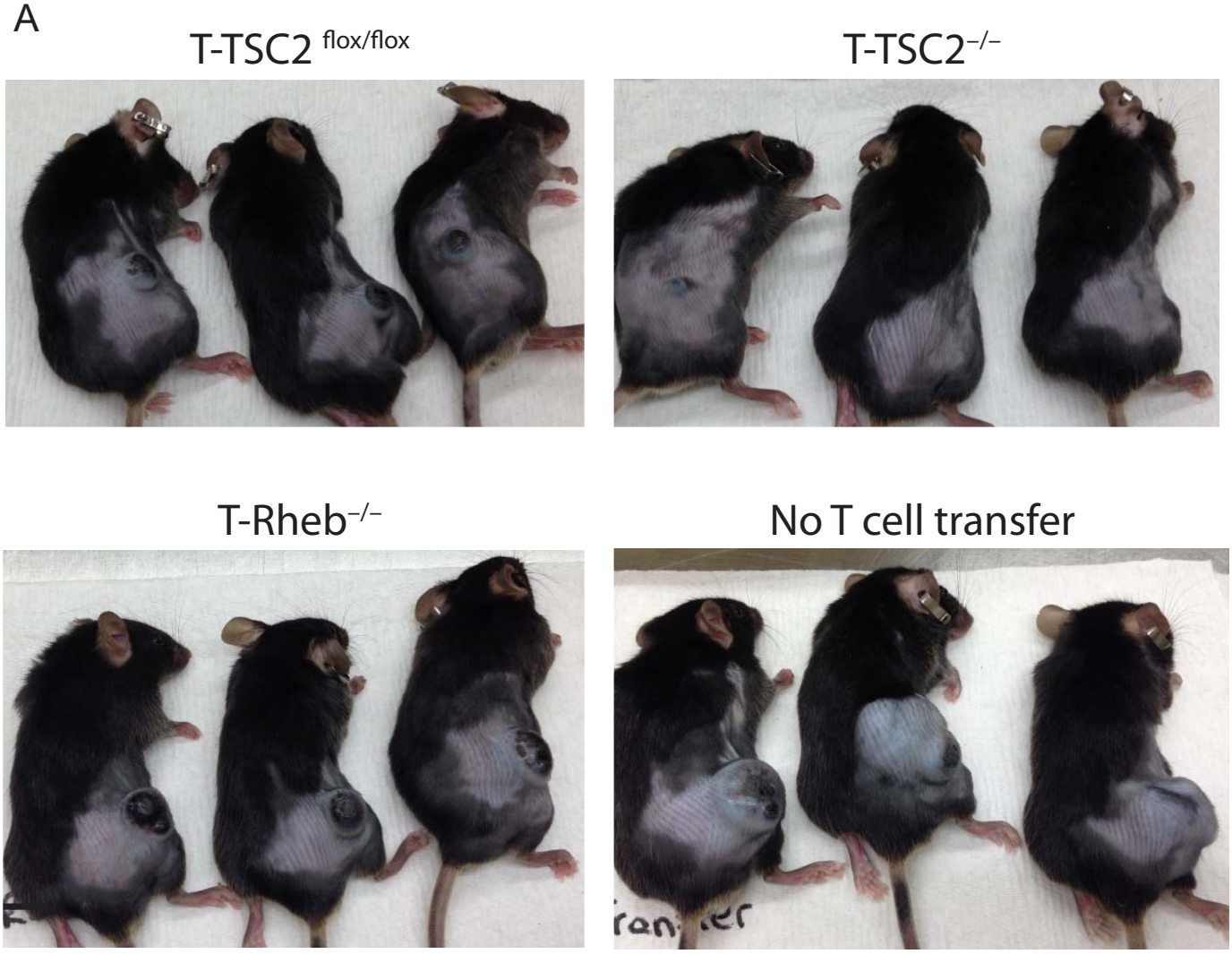

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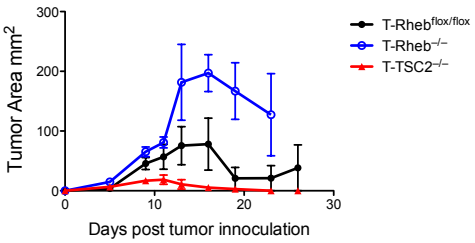
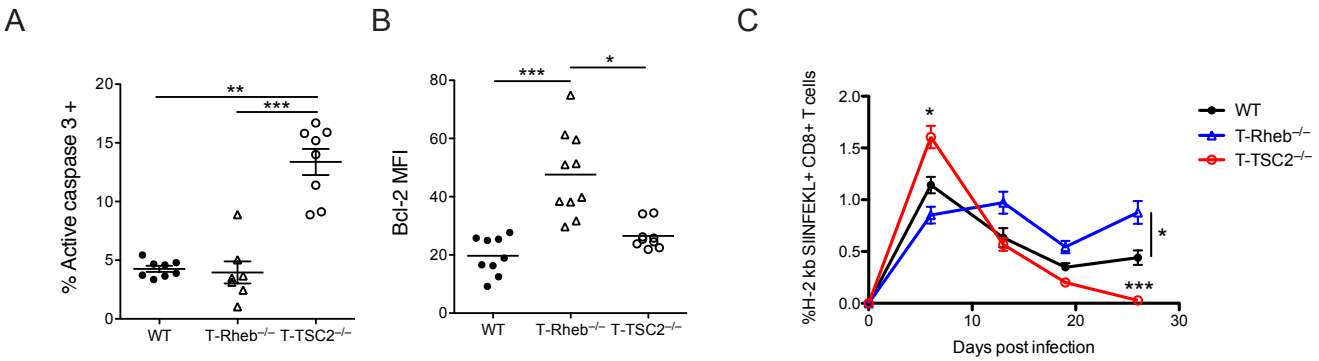


Supplemental Figure 4: mTORC1 deficiency impairs tumor clearance. (A) As in Figure 4C, in vitro activated T-Rheb$^{-/-}$ and T-TSC2$^{-/-}$ and Cre negative littermate control OT-I^{+} CD8^{+} T cells were injected r.o. into WT recipients that had received B16-OVA cells s.q. 6 days prior. Pictures of replicate mice per group were taken on Day 18 after tumor inoculation. 'No T cell transfer' indicates that mice did not receive OT-I^{+} cells. (B) T-Rheb$^{flox/flox}$,T-Rheb$^{-/-}$, and T-TSC2$^{-/-}$ mice received 1x10^{6} EL4 tumor cells s.q., and tumor burden was assessed every 2-3 days, n=10.



Supplemental Figure 5: mTORC1 hyper activation results in enhanced antigen specific cellular death at the contraction phase. WT, T-Rheb$^{-/-}$ and T-TSC2$^{-/-}$ CD90.1^{+}, OT-I^{+} cells were adoptively transferred into congenically distinct WT recipients infected with vaccinia-OVA. (A) 6 days later, percentage of OT-I^{+} cells with active caspase 3 protein expression was determined from blood. (B) 9 days after infection, splenocytes were harvested and Bcl-2 expression was determined from the OT-I^{+} splenocytes. (C) WT, T-Rheb$^{-/-}$ and T-TSC2$^{-/-}$ mice were infected with vaccinia-OVA and the percentage of OVA tetramer^{+} CD44^{+}CD8^{+} T cells was assessed from the blood over time, n=16.

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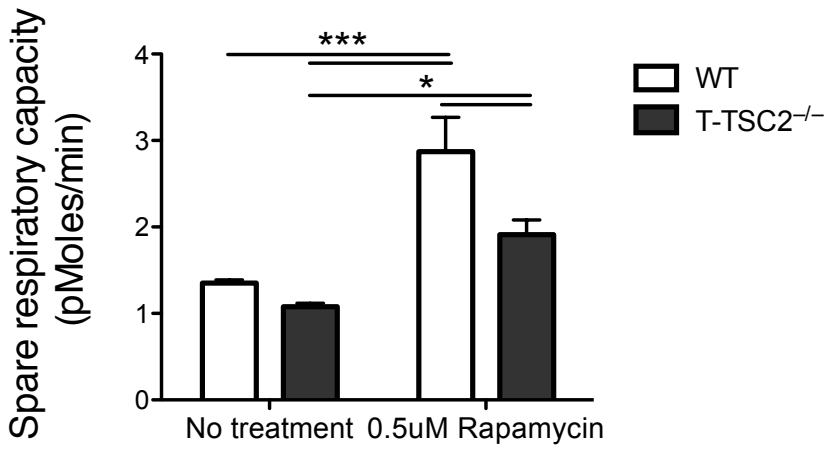

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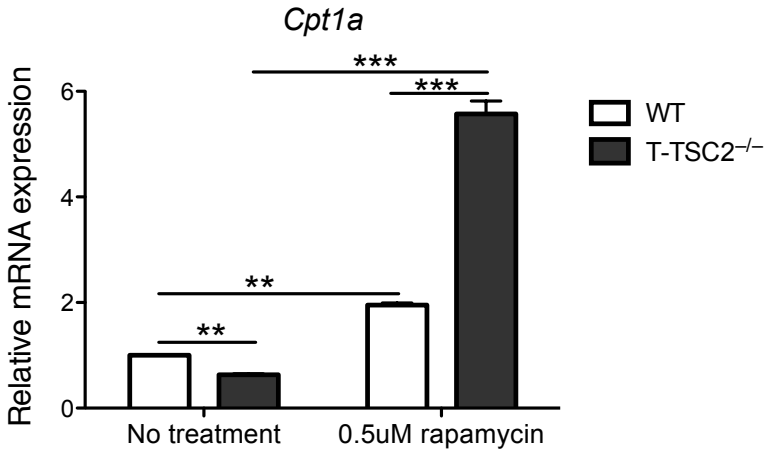
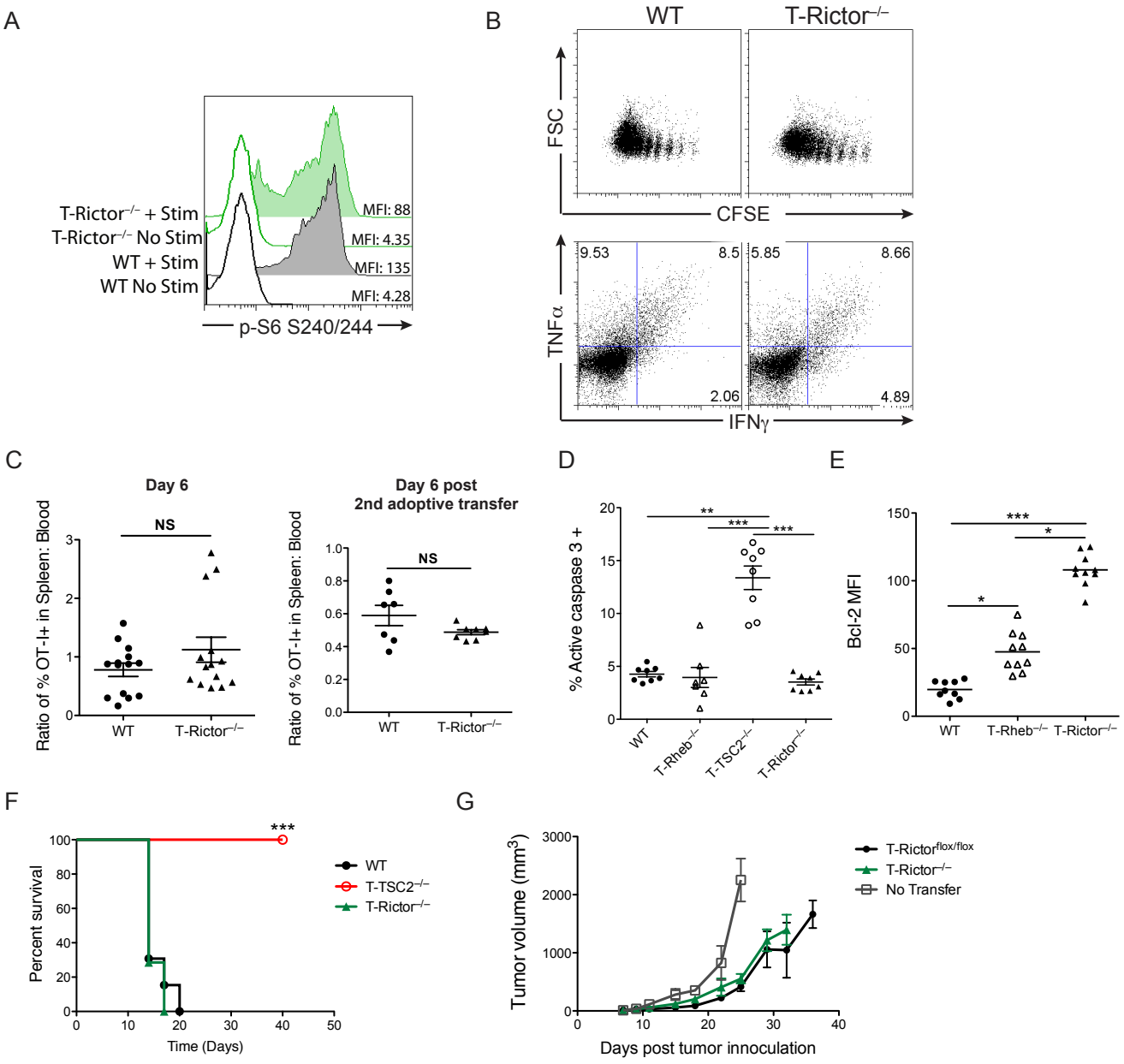


Supplemental Figure 6: Rapamycin treatment enhances the mitochondrial fitness of $CD8^+$ T cells. Purified $CD8^+$ T cells from WT, and T-$TSC2^{-/-}$ mice were stimulated in vitro for 48hr and expanded in media supplemented with IL-7 and IL-15 for 3 days. (A) Spare respiratory capacity was determined after cells were run on a extracellular flux analyzer. Data are mean ± SEM of 9 measurements. (B) RNA was extracted and relative expression of *Cpt1a* mRNA transcript was determined by qPCR. Data are mean ± SEM of 3 measurements.



Supplemental Figure 7: mTORC2 deficiency does not hinder effector function of activated CD8$^+$ T cells. (A) mTORC1activity was assessed from WT and T-Rictor$^{-/-}$ splenocytes left untreated or stimulated 24hr with αCD3. Histograms gated from CD8+ population, p-S6 MFI is shown per condition. (B) CFSE labeled splenocytes from WT and T-Rictor$^{-/-}$ mice were stimulated with αCD3. After 48hr, cells were expanded in media supplemented with IL-2, and 116hr post initial stimulation, live cells were restimulated to assess cytokine production. Top panel shows CFSE dilution and bottom panel shows cytokine production gated from the CD8$^+$ T cell population. (C) Ratios of OT-I$^+$ cells found in the spleen versus the blood are shown at different time points after infection, each dot represents 1 mouse. (D-E) Replicate data from Supplemental Figure 5 including T-Rictor$^{-/-}$ cells. (F) WT, T-Rictor$^{-/-}$, and T-TSC2$^{-/-}$ mice received 1x10^6 EL4 tumor cells s.q., and survival was determined, n=8. (G) As in Figure 4C, in vitro activated T-Rictor$^{flox/flox}$ and T-Rictor$^{-/-}$ OT-I$^+$ CD8$^+$ T cells were injected r.o. into WT recipients that had received B16-OVA cells s.q. 6 days earlier. 'No transfer' indicates that mice did not receive OT-I$^+$ cells. Tumor volume was assessed every 2-3 days. Each point represents an average per genotype, n=10.