Supplemental Information

Arp2/3 complex controls T cell homeostasis by maintaining surface TCR levels via regulating TCR^{+} endosome trafficking

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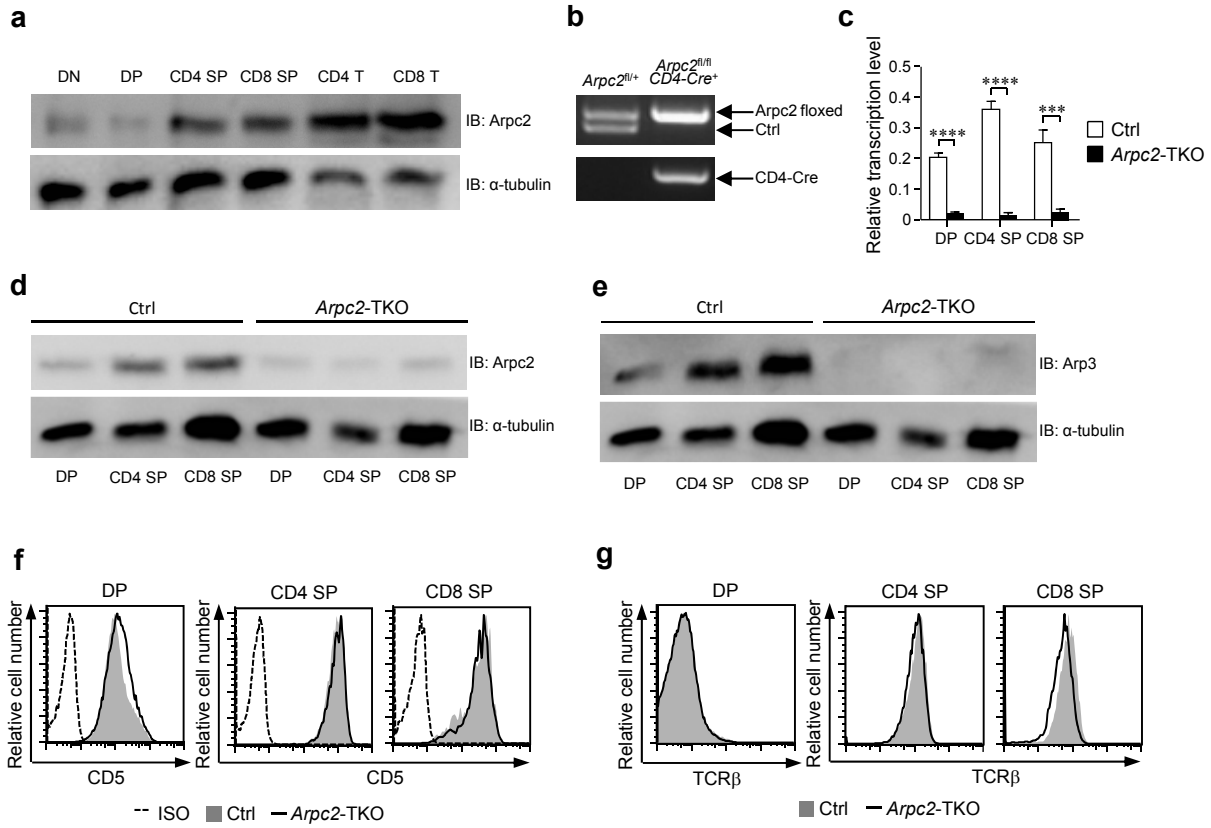
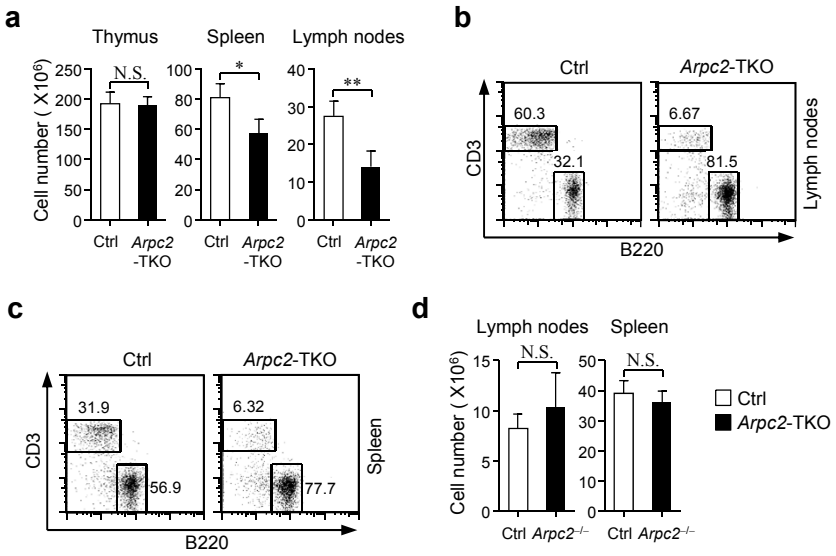
Supplementary Figure 1



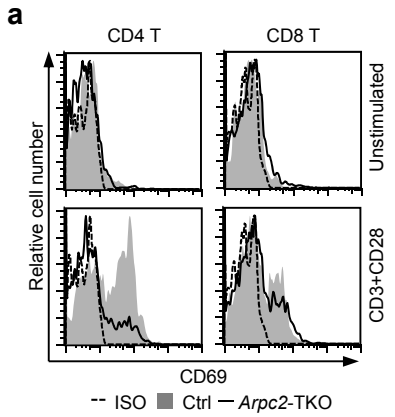
Supplementary Figure 1. Specific deletion of Arpc2 in T cells.
(**a**) Protein level of Arpc2 in DN, DP, CD4 SP, CD8 SP, CD4 T and CD8 T cells in control mice. The expression of total a-tubulin served as a loading control. (**b**) Genomic DNA was extracted from tails of control mice (*Arpc2*$^{fl/+}$) and Arpc2-deficient (*CD4-cre*$^{+}$*Arpc2*$^{fl/fl}$) mice and then were analyzed by PCR. (**c**) The relative expression level of Arpc2 in DP, CD4 SP and CD8 SP thymocytes obtained from Ctrl or *Arpc2*-TKO mice. (n=3; ***P < 0.001 and ****P < 0.001 by two-tailed unpaired *t*-test). (**d**) Analysis of Arpc2 protein levels in DP, CD4 SP and CD8 SP thymocytes. The expression of total a-tubulin served as a loading control. (**e**) Analysis of Arp3 protein levels in DP, CD4 SP and CD8 SP thymocytes. The expression of total a-tubulin served as a loading control. (**f**) Flow cytometry analysis of the surface markers CD5 in DP and SP thymocytes obtained from Ctrl and *Arpc2*-TKO mice, IgG2a isotype control were used. (**g**) Flow cytometry analysis of the surface markers TCRβ in DP and SP thymocytes obtained from Ctrl and *Arpc2*-TKO mice.

Supplementary Figure 2



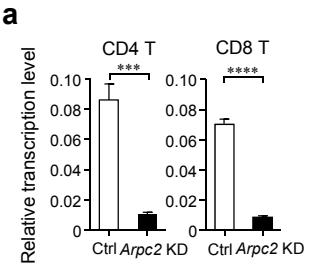
Supplementary Figure 2. Cellularity of thymus, spleen and lymph nodes.
(**a**) Cellularity of thymus, spleen and lymph nodes (n=4; *P < 0.05 and **P < 0.01 by two-tailed unpaired *t*-test; N.S.: no significance). (**b-c**) Flow cytometry analysis of CD3 and B220 expression in (**b**) lymph nodes and (**c**) spleen. (**d**) Total number of $B220^+$ B cells in lymph nodes and spleen (n=4; two-tailed unpaired *t*-test; N.S.: no significance).

Supplementary Figure 3



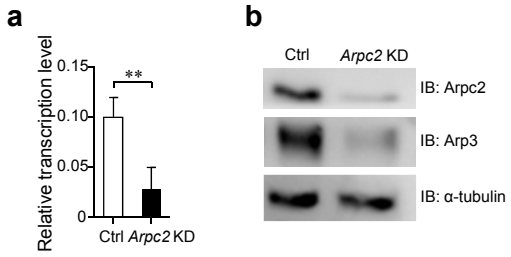
Supplementary Figure 3. Decreased T cell activation in *Arpc2*-TKO mice T cells.
(**a**) Histogram show Flow cytometry analysis of CD69 expression in T cells that were unstimulated or stimulated with anti-CD3 and anti-CD28 activator beads for 4h. IgG1, κ isotype control were used.

Supplementary Figure 4



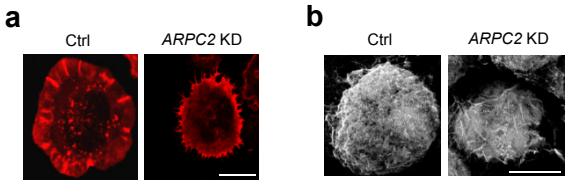
Supplementary Figure 4. Knockdown of Arpc2 in primary T cells.
(**a**) Analysis of Arpc2 mRNA level in control and Arpc2 knockdown peripheral T cells (n=3; ***P < 0.001 by two-tailed unpaired *t*-test). Representative images are shown. Error bars indicate s.d.. All results are representative from at least three independent experiments.

Supplementary Figure 5



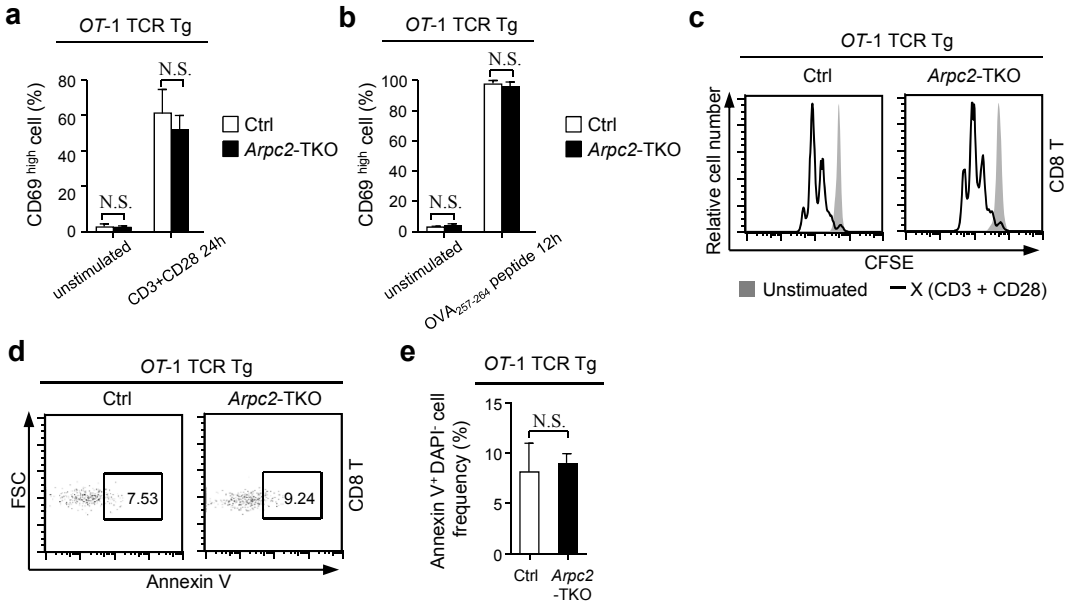
Supplementary Figure 5. Arpc2 was efficiently deleted in Jurkat T cells.
(**a**) Analysis of Arpc2 mRNA (n=4; **P < 0.01 by two-tailed unpaired *t*-test) and (**b**) protein levels in Control and *Arpc2* KD Jurkat T cells. Representative images are shown. Error bars indicate s.d.. All results are representative from at least three independent experiments.

Supplementary Figure 6



Supplementary Figure 6. Loss of Arpc2 leads to defective branched actin polymerization in T cells.
(**a**) β-actin-mCherry Jurkat T cells (red) were transfected with control or sh*Arpc2* and plated on coverslips coated with anti-CD3mAb for 15 min. They were then imaged using microscopy. Bar is 10 μm. At least 30 cells are imaged. Representative images are shown. (**b**) Control and Arpc2 KD Jurkat T cells were allowed to spread for 15 min at 20 μg/ml OKT3 on coated coverslips. The cells were then unroofed and photographed to observe the organization of F-actin under SEM. Bar is 4 μm. At least 30 cells were imaged. Representative images are shown. All results are representative from at least three independent experiments.

Supplementary Figure 7



Supplementary Figure 7. The signaling, proliferation and apoptosis was normal in *OT*-1 TCR Tg *Arpc2*-TKO T cells.

(**a**) Flow cytometry analysis of CD69 expression in *OT*-1 TCR Tg T cells obtained from Ctrl and *Arpc2*-TKO mice that were unstimulated or stimulated with anti-CD3 and anti-CD28 activator beads for 24 h (n=3; two-tailed unpaired *t*-test; N.S.: no significance) or (**b**) with antigen presenting cells pulsed SIINFEKL ($OVA_{257-264}$) peptide for 12h (n=4; two-tailed unpaired *t*-test; N.S.: no significance). (**c**) Lymphocytic T cells of *OT*-1 TCR Tg T cells obtained from Ctrl and *Arpc2*-TKO mice were labeled with CFSE and they were unstimulated or stimulated with anti-CD3 and anti-CD28 activator beads for 48 h. (**d**) Survival was assessed of *OT*-1 TCR Tg naïve T cells using flow cytometry analysis with Annexin V and DAPI staining. (**e**) The percentage statistics for Annexin V are illustrated. (n=3; two-tailed unpaired *t*-test; N.S.: no significance).