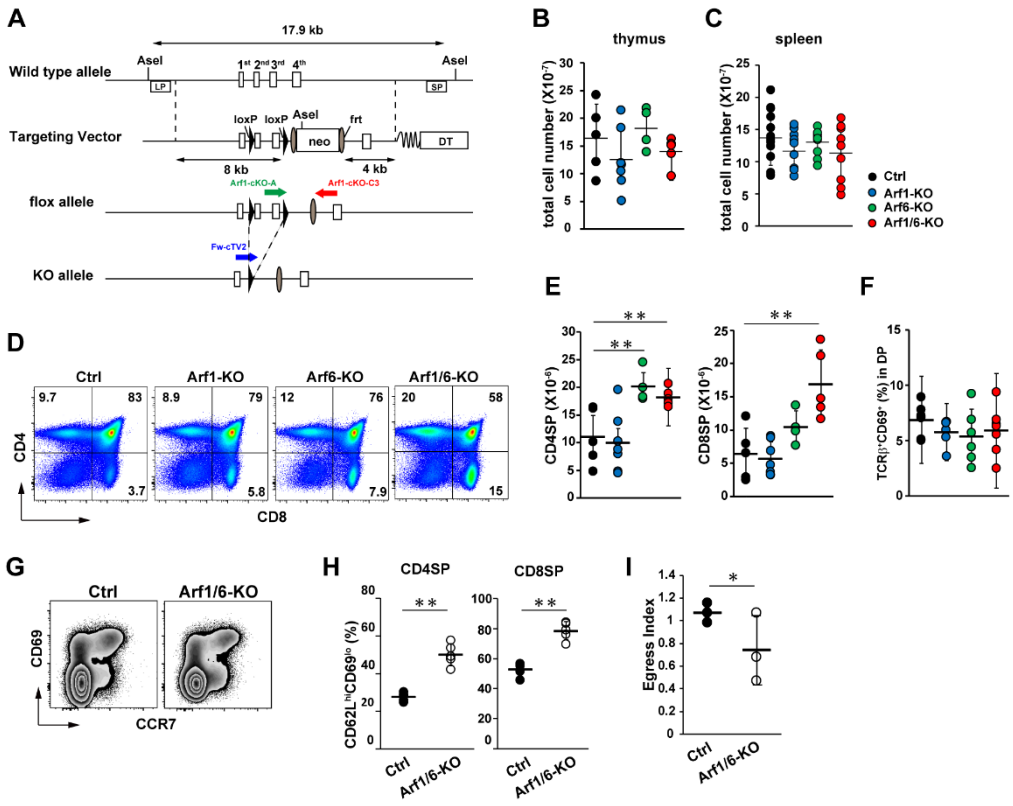
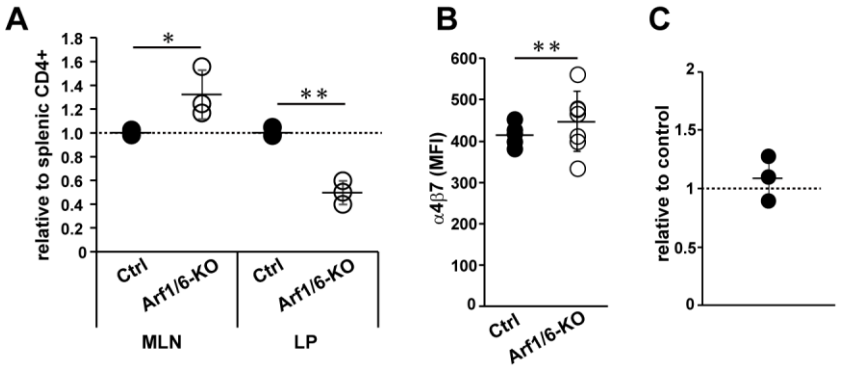


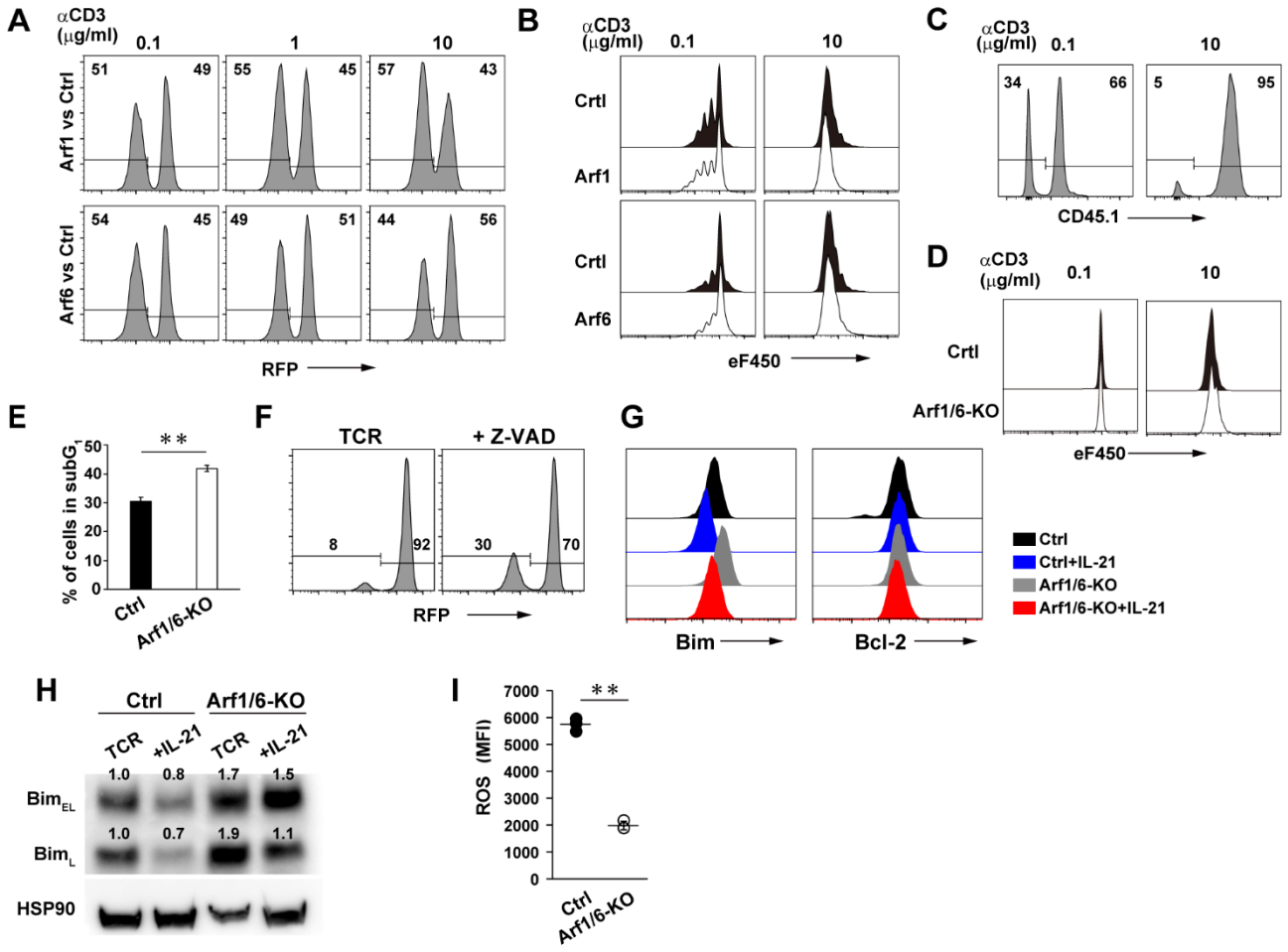
Supplemental Figure 1. Characterization of T-lineage specific Arf-deficient mice.

(**A**) *Arf1* conditional deficient mice were generated as described previously (http://www2.clst.riken.jp/arg/methods.html). Schematic representation of wild-type genomic locus of *Arf1* with 4 exons (boxes with 1st, 2nd, 3rd, 4th), introns (lines) and restriction enzyme sites of AseI are shown. LP and SP probes for Southern blot analysis of AseI digested genomic DNA are also shown (boxes with LP, SP). Targeting vector containing 2 loxP sites (black arrowheads) and 2 frt sites (shaded ellipses), a PGK-Neo-polyA cassette (box with neo) for positive selection, and a MC1-DT-A-polyA cassette (box with DT) for negative selection is represented. AseI site in the PGK-Neo-polyA cassette is also shown. The conditional allele after deleting the PGK-Neo-polyA cassette by using FLP deleter strain B6-Tg(CAG-FLPe)36, provided by RIKEN (RBRC01834), is shown as flox allele. The targeted allele following Cre recombination is shown as KO allele. Genotyping of offspring was performed by PCR with the following primers: Arf1-cKO-A (green), 5'-GCTTGATCTTCGTAGTGGACAGCAATGAC-3'; Arf1 cKO-C3 (red), 5'-TGAGGAAAAGGAAGAATTAGTGGCAGGGAC-3'; Fw-cTV2 (blue), 5'-CGTCTAAGAAACCATTATTATCATGAC-3'. Primer pair of Arf1-cKO-A and Arf1 cKO-C3 yields products of wild-type (249 bp) and floxed (377 bp) alleles, whereas primer pair of Fw-cTV2 and Arf1 cKO-C3 yields products of floxed (729 bp) and deleted (190 bp) alleles. (**B**) Number of total cells in the thymus from 5-7 weeks old control (n=5, black), Arf1-KO (n=7, blue), Arf6-KO (n=4, green), and Arf1/6-KO (n=5, red) mice. Each symbol represents an individual mouse (mean ± S.D.). (**C**) Number of total cells in the spleen from the indicated mice as in Fig. 1B. (**D**) FACS analysis for CD8 and CD4 in the thymocytes from the indicated mice. Data are representative of four independent experiments. (**E**) CD4SP (left) and CD8SP (right) thymocyte numbers in the indicated mice as in (B). Mean ± S.D. **p< 0.01. (**F**) Proportions of TCRβ$^+$CD69$^+$ cells in DP cells in the indicated mice as in (B) (mean ± S.D.). (**G**) Shown are CD69 and CCR7 expression in thymocytes of the indicated mice. Data are representative of three independent experiments. (**H**) Proportions of the most mature cells (CD62L^{hi}CD69^{lo}) in CD4SP and CD8SP in 5-7 weeks old control (n=5) and Arf1/6-KO (n=5) mice. Each symbol represents an individual mouse. Mean ± S.D. **p< 0.01. (**I**) Control (CD45.1$^+$) and Arf1/6-KO (CD45.1$^-$) bone marrow cells were mixed at an equal ratio and transferred into sub-lethally irradiated recipient (RFP$^+$) mice (n=3). Two months after transfer, "Egress Index" was evaluated as the proportion of splenic CD4$^+$ T cells relative to the proportion of thymic CD4SP cells. Mean ± S.D. *p< 0.05, Mann-Whitney U test.



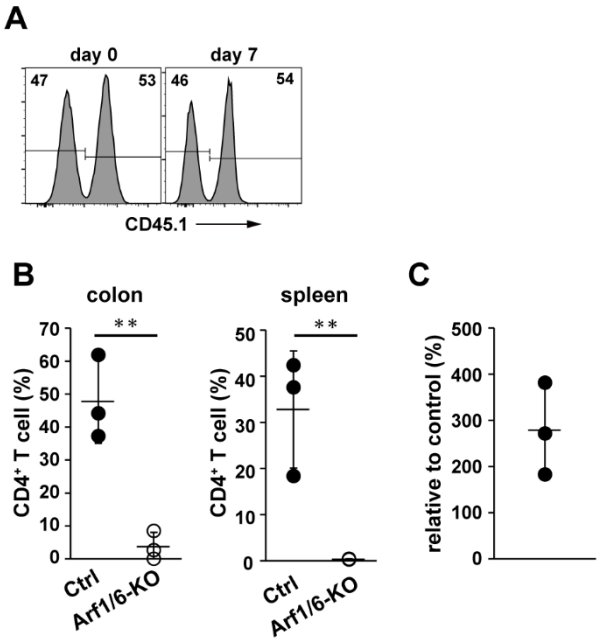
Supplemental Figure 2. Arf-deficient CD4$^+$ T cells in the colonic LP are significantly decreased, but normally migrate into the colon.

(**A**) Control (CD45.1$^+$) and Arf1/6-KO (CD45.1$^-$) bone marrow cells were mixed at an equal ratio and transferred into sub-lethally irradiated recipient (RFP$^+$) mice as in Supplemental Figure 1I. Two months after transfer, the ratios of MLN or colonic LP CD4$^+$ T cells to splenic CD4$^+$ T cells gated in RFP$^-$CD45.1$^+$ (control) or RFP$^-$CD45.1$^-$ (Arf1/6-KO) were evaluated by FACS analysis. Indicated are the values normalized to control. (**B**) Expression levels of α4β7 in the colonic LP CD4$^+$ T cells from control (Ctrl, n=5) and Arf1/6-KO (n=7) mice. Each symbol represents an individual mouse. Mean ± S.D. **p< 0.01. (**C**) Mixture of CD4$^+$ T cells from control (CD45.1$^+$) and Arf1/6-KO mice (CD45.1$^-$) at an equal ratio were transferred into *Rag2*$^{-/-}$ mice (n=3). After 24 hours, the ratios of colonic LP CD4$^+$ T cells between control and Arf-deficient cells were evaluated by FACS analysis.



Supplemental Figure 3. Survival and proliferation of Arf1- or Arf6-single deficient CD4$^+$ T cells.

(**A** and **B**) Control (RFP$^+$) CD4$^+$ T cells were mixed with either Arf1-KO (RFP$^-$) or Arf6-KO (RFP$^-$) CD4$^+$ T cells and labeled with eF450, followed by stimulation with 0.1-10 μg/ml plate-bound anti-CD3ε mAb along with 1 μg/ml soluble anti-CD28 mAb for 4 days. Shown are representative FACS plots indicating proportions of control (Ctrl) and either Arf1-KO (*upper*) or Arf6-KO cells (*lower*) (A), or eF450 dilution plots of control (Ctrl) vs Arf1-KO (*upper*) and control (Ctrl) vs Arf6-KO (*lower*) (B). Data are representative of at least two independent experiments. **(C** and **D)** Control (CD45.1$^+$) CD8$^+$ T cells were mixed with Arf1/6-KO (CD45.1$^-$) CD8$^+$ T cells and labeled with eF450, followed by stimulation with 0.1 or 10 μg/ml plate-bound anti-CD3ε mAb along with 1 μg/ml soluble anti-CD28 mAb for 3 days. Shown are representative FACS plots indicating proportions of control and Arf1/6-KO (C), or eF450 dilution plots of control (Ctrl) vs Arf1/6-KO (D). Data are representative of at least three independent experiments. (**E**) CD4$^+$ T cells from the indicated mice (n=3, each) were activated with anti-CD3ε mAb for 4 days, and proportions of subG1 cells were evaluated by FACS. Mean ± S.D. **p< 0.01. (**F**) Mixture of CD4$^+$ T cells from control (RFP$^+$) and Arf1/6-TKO mice (RFP$^-$) was stimulated with anti-CD3ε/anti-CD28 mAbs (TCR) along with or without 50 μM Z-VAD-FMK (+Z-VAD) for 4 days, followed by FACS analysis. Shown are representative of three. (**G** and **H**) Naïve CD4$^+$ T cells from the indicated mice were stimulated with anti-CD3ε/anti-CD28 mAbs along with or without IL-21, and analyzed at 48 h by FACS against Bcl-2 (G) or at 96 h by immunoblot against Bim and HSP90 as a loading control (H). The values indicated are relative density of the band normalized to HSP90. Data are representative of two independent experiments. **(I)** CD4$^+$ T cells from the indicated mice (n=3, each) were activated with anti- CD3ε/anti-CD28 mAbs for 3 days, followed by evaluation of ROS levels by FACS. Mean ± S.D. **p< 0.01.



Supplemental Figure 4. Survival and differentiation of Arf-deficient CD4$^+$ T cells *in vivo.*

(**A**) *In vitro*-differentiated control (CD45.1$^+$) and Arf1/6-KO (CD45.1$^-$) pathogenic Th17 cells were mixed at an equal ratio (day 0), and transferred into colitis-induced recipient mice. The ratios of control to Arf1/6-KO cells in the colonic LP of recipient mice were evaluated by FACS on day 7. Shown are representative of four independent experiments. (**B**) FACS analysis for CD4$^+$ T cells found in the colon or spleen of recipient *Rag2*$^{-/-}$ mice (n=3) three weeks after transfer with the indicated naïve CD4$^+$ T cells. Mean ± S.D. **p< 0.01. **(C)** Proportions of IL-17A producing cells in the splenic CD4$^+$ T cells of recipient *Rag2*$^{-/-}$ mice (n=3) as in (B) were evaluated by FACS. Shown are proportions of IL-17A$^+$ cells in *Rag2*$^{-/-}$ mice transferred with Arf1/6-deficient naïve CD4$^+$ T cells normalized to those found in *Rag2*$^{-/-}$ mice with control. Mean ± S.D.