Supplementary Figure

Fig. S1 Frequency of Foxp3^{+}CD4^{+} natural occurring Tregs in naive *Arrb2*$^{-/-}$ or *Arrb2*$^{+/+}$ mice.

Mononuclear cells were purified from spleen (SPL, a and d), draining lymph nodes (DLN, b and e) and mesenteric lymph nodes (MLN, c and f) of naive *Arrb2*$^{-/-}$ mice and wild-type littermates. Foxp3^{+}CD4^{+} cells were assessed by surface and intracellular staining of CD4 and Foxp3 followed by Flow cytometry measurement. Both their frequencies in all live leukocytes (a-c) or among CD4^{+} population (d-f) were shown. The data were shown as the means ± SEM (n=3-5).

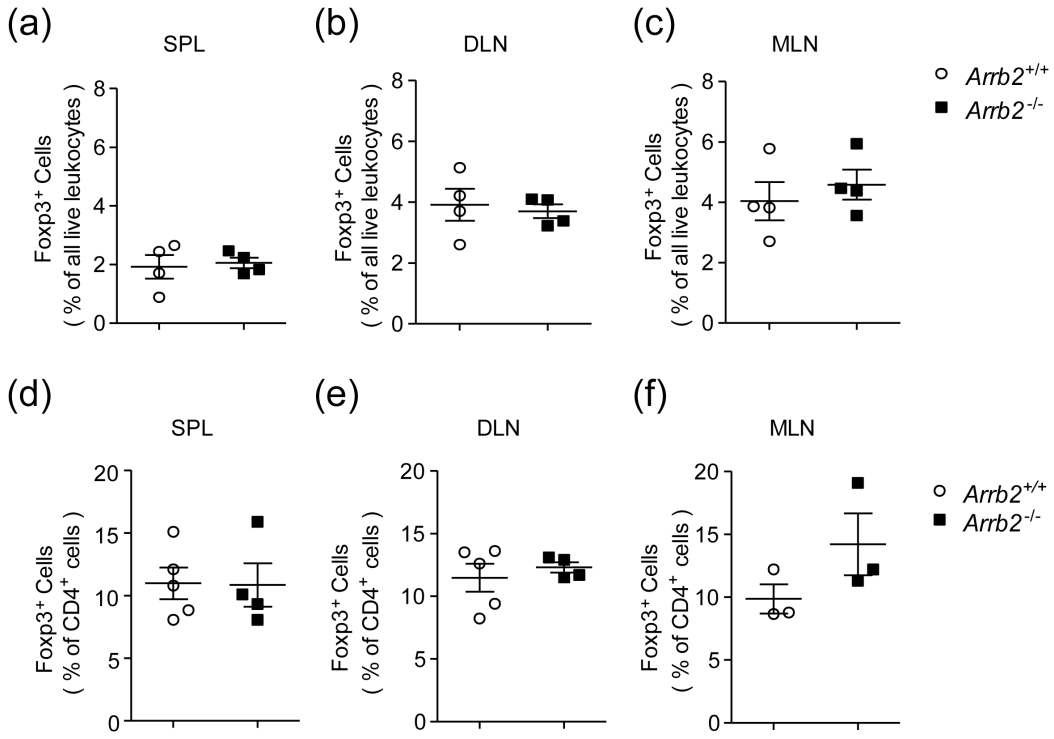

Fig. S2 Immune cell populations from CNS and peripheral immune organs of *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ EAE mice during early stage of EAE (day 12-16).

Mononuclear cells were isolated from CNS (a), SPL (b), DLN (c) and peripheral blood (PBL, d) of *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ EAE mice on day 12-16 after immunization, and the frequencies of CD11b^{+} myeloid cells, B220^{+} B cells, CD4^{+} and CD8^{+} T cells, in these tissues were measured by flow cytometry. The data were shown as mean ± SEM (n=5). ***P* < 0.01, *versus* wild type control (two-tailed Student's *t*-test)

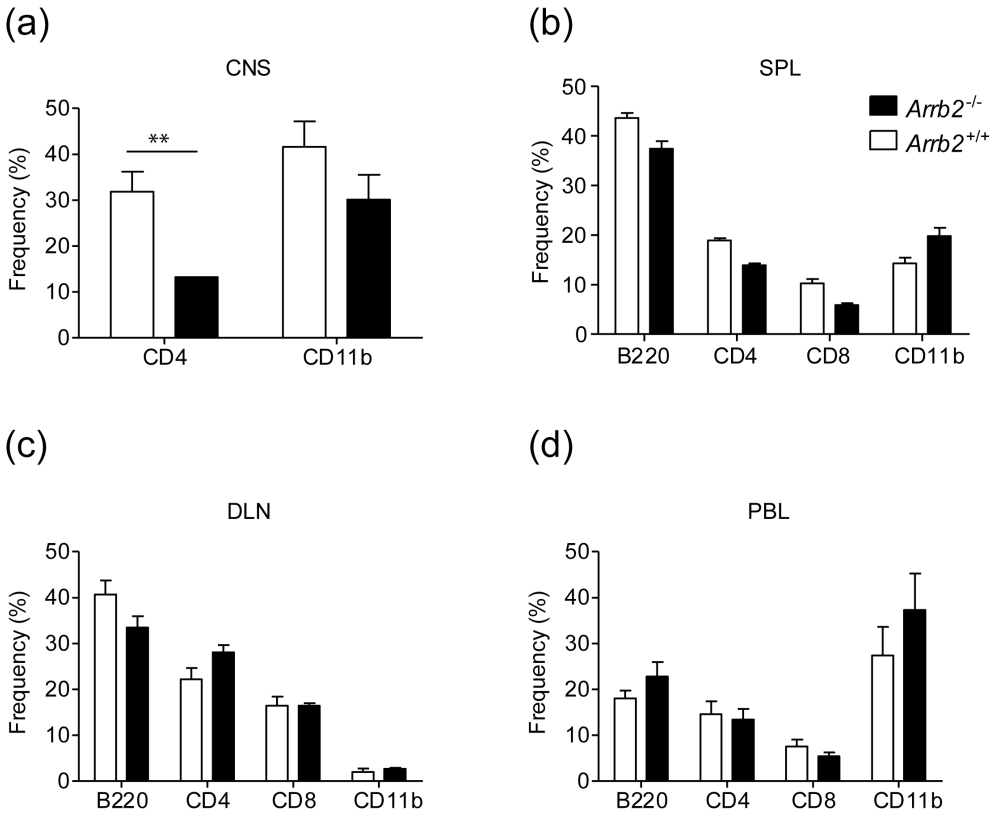

Fig. S3 Immune cell populations from peripheral immune organs of *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ mice during late stage of EAE (day 18-22).
Mononuclear cells were isolated from SPL (a), DLN (b), and PBL (c) of *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ EAE mice on day 18-22 after immunization, and the frequencies of CD11b^{+} myeloid cells, B220^{+} B cells, CD4^{+} and CD8^{+} T cells, in these tissues were measured by flow cytometry. The data were shown as mean ± SEM (n=5).

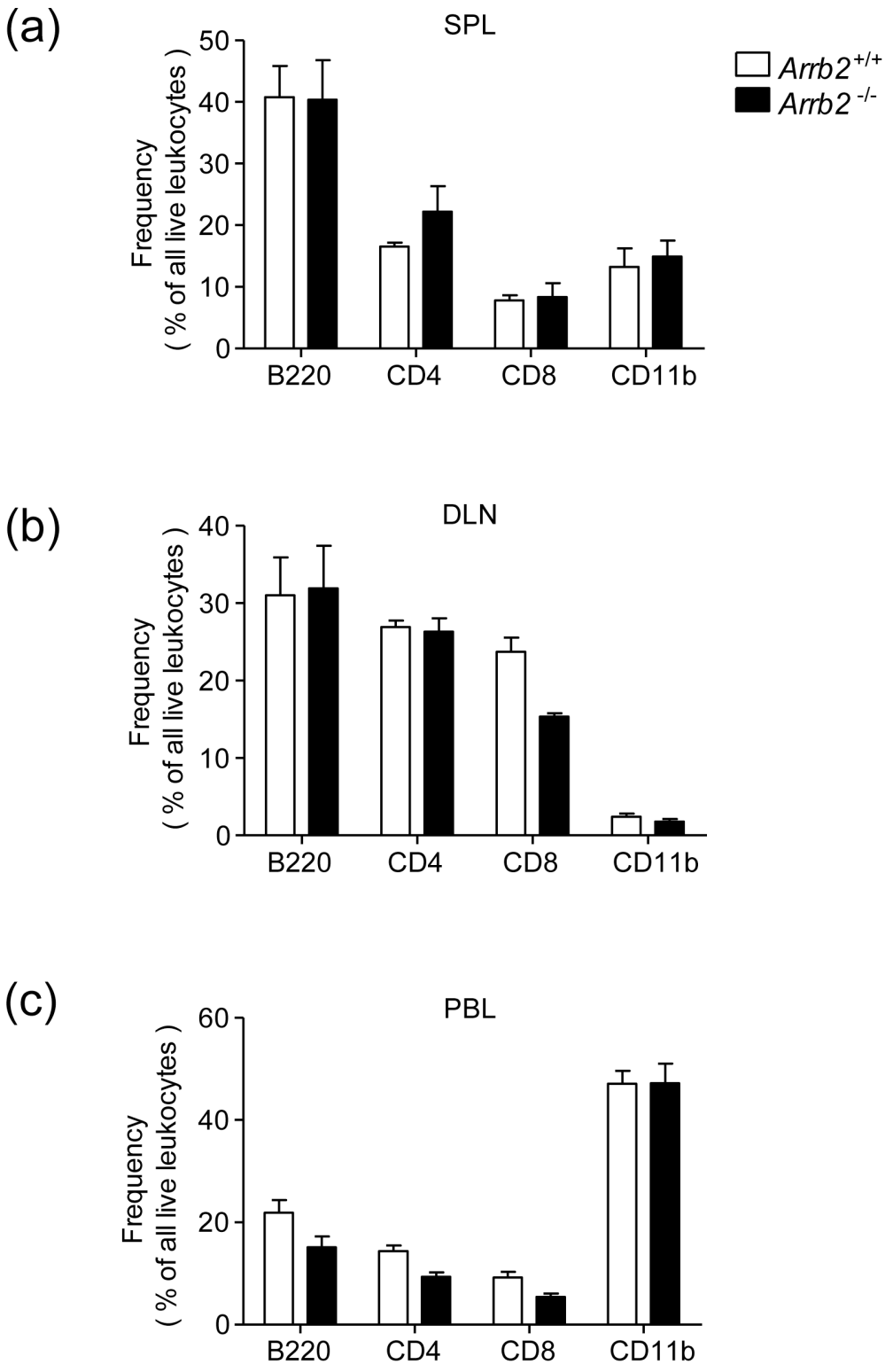

Fig. S4 Frequencies of effector T cells in *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ EAE mice during early stage of EAE (day 12 to 16).

Mononuclear cells were isolated from CNS (a), SPL (b), DLN (c) and PBL (d) of *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ EAE mice on day 12-16 after immunization, and the frequency of Foxp3^{+}, IFN-γ^{+}, and IL-17^{+} cells among CD4^{+} population were measured by flow cytometry. DP represents IFN-γ^{+} IL-17^{+} cells. The data are shown as the means ± SEM (n=5). **P* < 0.05, *versus* wild-type control (two-tailed Student's *t*-test)

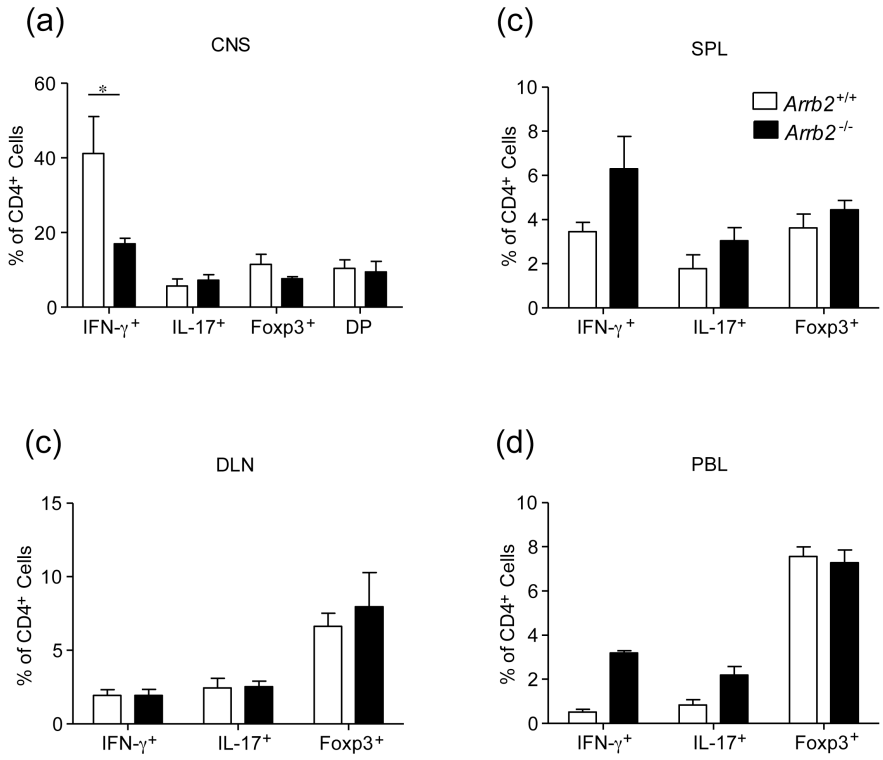

Fig. S5 Frequencies of peripheral blood effector and regulatory T cells in *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ EAE mice during early disease stage.
Mononuclear cells were isolated from PBL of *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ EAE mice on day 18-22 after immunization, and the frequency of IFN-γ^+, IL-17$^+$, and Foxp3$^+$ cells among CD4$^+$ population were measured by flow cytometry. The data were shown as the means ± SEM (n=5).

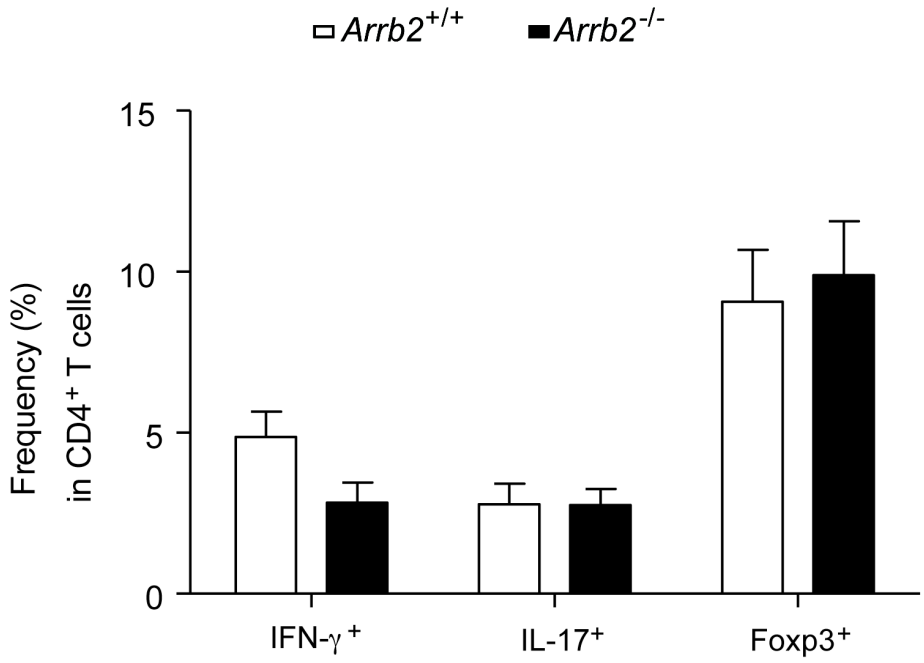

Fig. S6 Comparable cell viabilities in TGF-β induced *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ iTreg cultures.

Naive CD4^{+}CD25^{-} T cells purified from *Arrb2*$^{-/-}$*Foxp3*^{egfp} or *Arrb2*$^{+/+}$*Foxp3*^{egfp} mice were cultured under iTreg condition and harvested on day 4. A fixable viability dye (Cat. No. 65-0865, eBioscience) was used to identify the dead cells. Representative flow cytometry graphs show the frequencies of viable cell among all Foxp3^{+}GFP^{+} iTreg population (a), and Foxp3^{+}GFP^{+} iTreg cells among all viable CD4^{+} cells (b), respectively.

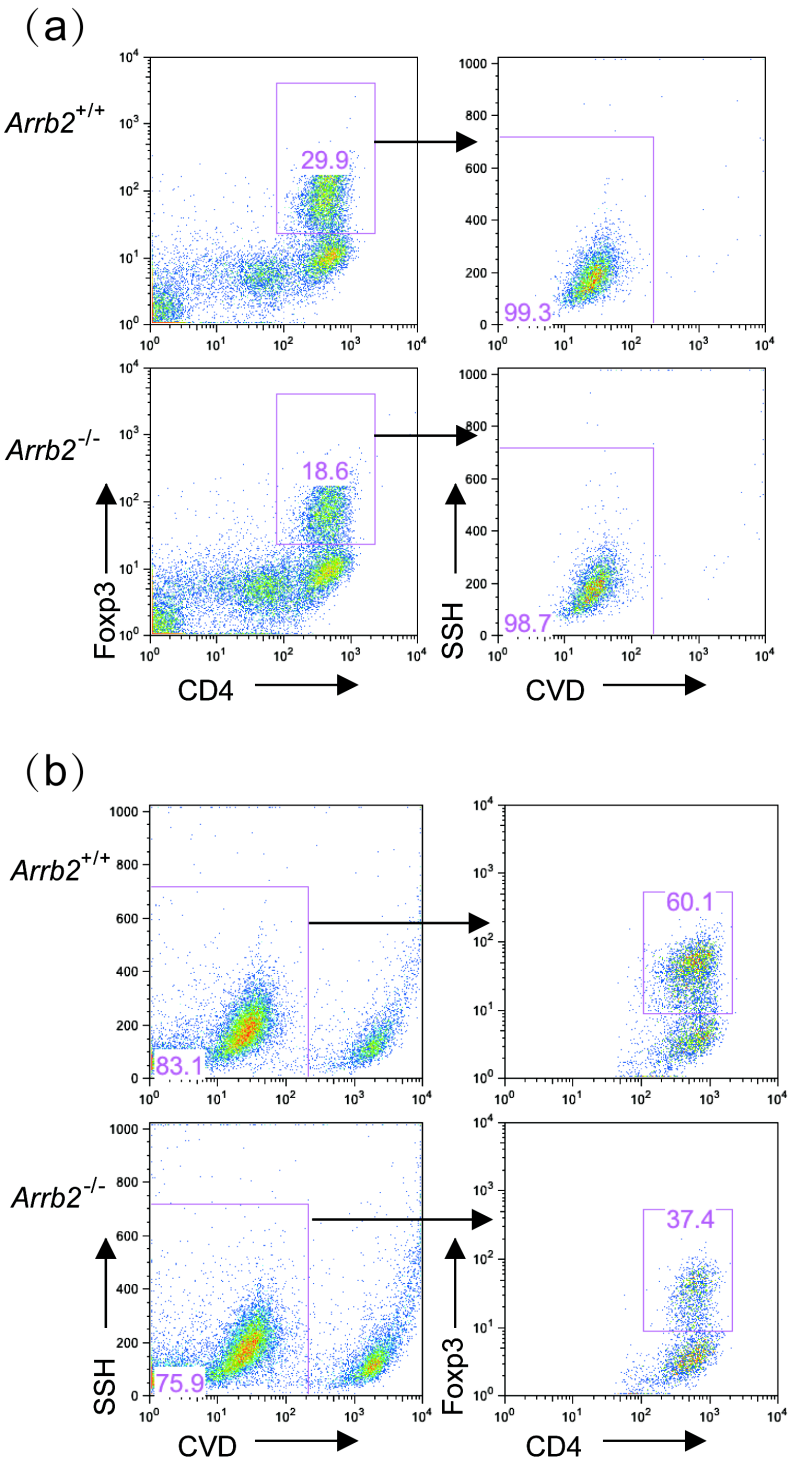

Fig. S7 Mixed lymph nodes reaction.
Magnetic sorted $CD4^+CD25^+$ T cells from *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ mice were cultured with wild type $CD4^+CD25^-$ T responder cells (Tresp) at different ratios indicated together with irradiated wild-type APCs (T-cell depleted splenocytes) and anti-CD3 for 96 h. Proliferation of cells was measured by incorporation of [^{3}H] thymidine. Data are means ± SEM of triplicates and representative of three independent experiments. **P* < 0.05, *versus* control (two-tailed Student's *t* test).

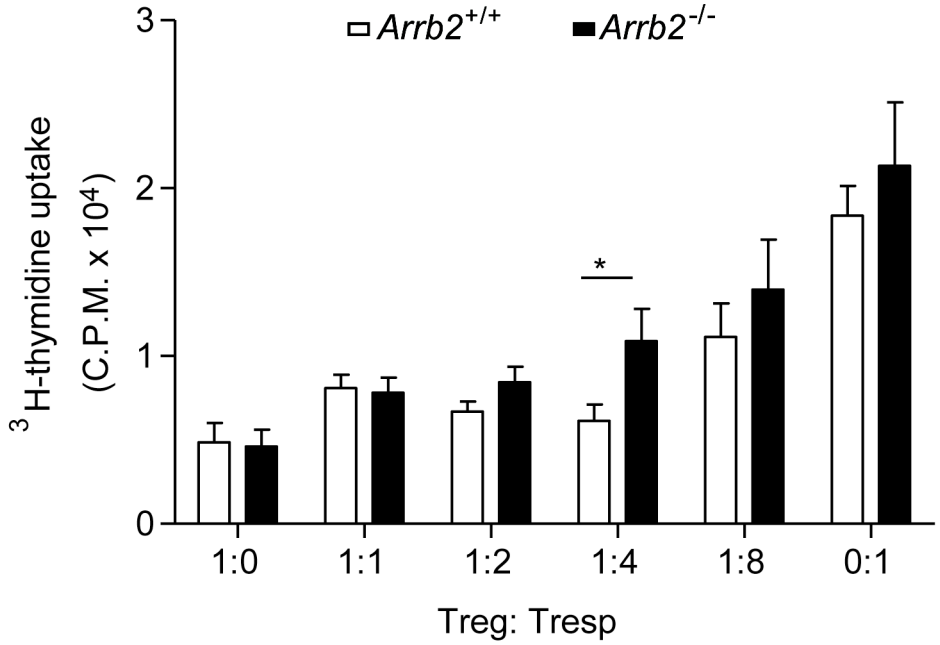

Fig. S8 Primer list

	Sense (5'—3')	Anti-sense (5'—3')
Arrb1	CCTGGATGTCTTGGGTCTG	TGATGGGTTCTCCGTGGTA
Arrb2	CAGCCAGGACCAGAGGACA	TGATAAGCCGCACAGAGTT
Foxp3	GGTACACCCAGGAAAGACAG	ATCCAGGAGATGATCTGCTTG
Ctla4	CATGGTGTCGCCAGCTTTC	AGTCACCCGGACCTCATCA
Gitr	GAGCAATACGGCCATTTGACT	GAGCTGGACTGTGGTTAGGAA
Lag3	CTACAACTCACCGCGTCATTT	GCTCCAGACCCAGAACCTT
Ifng	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
Il17a	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
Il2	TCTGCGGCATGTTCTGGATTT	ATGTGTTGTCAGAGCCCTTTAG
Il12a	TGGCTACTAGAGAGACTTCTTCCACAA	GCACAGGGTCATCATCAAAGAC
hprt	CCTGCTGGATTACATTAAAGCACTG	TTCAACACTTCGAGAGGTCCT

Fig. S9 Enhanced pS6 (mTOR1) activation in *Arrb2*$^{-/-}$ iTreg cell cultures. Magnetic sorted CD4^{+} T cells from *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ mice were activated under iTreg condition and harvested at indicated time points. Expressions of mTOR downstream protein were measured by Western Blot, respectively. Data are representative of three independent experiments.