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## Supporting Information
## for
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**Lack of adiponectin leads to increased lymphocyte activation and increased disease severity in a mouse model of multiple sclerosis**

# SUPPORTING INFORMATION

## FIGURE S1
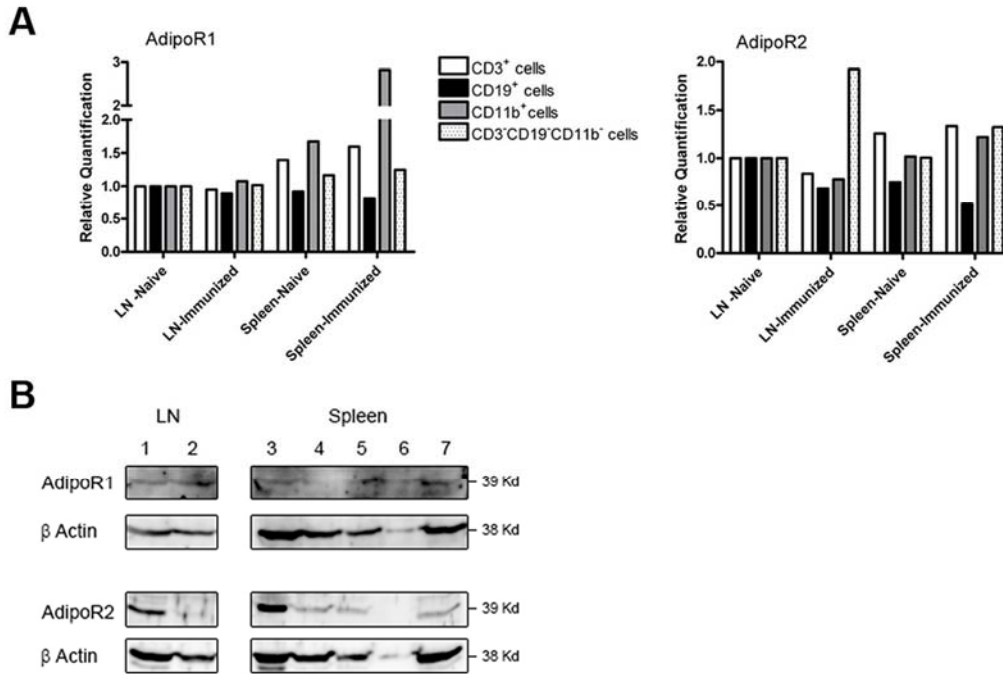


**Figure S1. AdipoR1 and R2 are expressed by murine lymph node cells and splenocytes.** Spleen and lymph node cells where isolated from naïve and $MOG_{35-55}$ immunized C57BL/6 WT mice and the $CD3^+$ T lymphocytes, $CD19^+$ B lymphocytes and the $CD11b^+$ cell populations were separated by cell sorting. To obtain sufficient cells for each subpopulation, splenocytes and lymph node cells from five mice per treatment group were pooled before sorting. **(A)** Messenger RNA expression for AdipoR1 and AdipoR2 was investigated in the different cell subpopulations by quantitative RT-PCR. Target gene expression was normalized to a housekeeping gene expression, GAPDH. Data are given as quantification for each gene relative to expression levels in spinal cords from naïve C57BL/6 mice (calibrator). **(B)** AdipoR1 and R2 protein expression was evaluated by Western blot on each subpopulation of immune cells, isolated from lymph nodes and spleens of $MOG_{35-55}$ immunized mice. Lanes 1 and 3= $CD3^+$ cells; lanes 2 and 4= $CD19^+$ cells; lane 5= $CD11^+$ cells; lane 6= $CD3^-CD19^-CD11b^-$ cells; and from total splenocytes from naïve mice (lane 7). LN= lymph nodes.
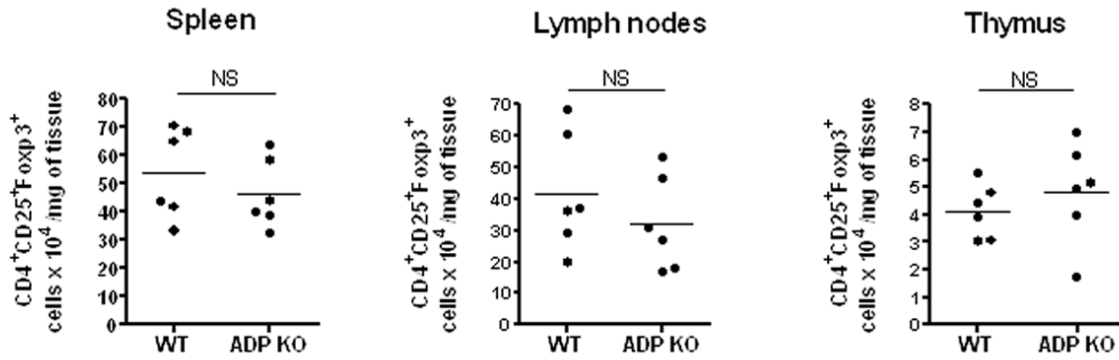
**FIGURE S2**
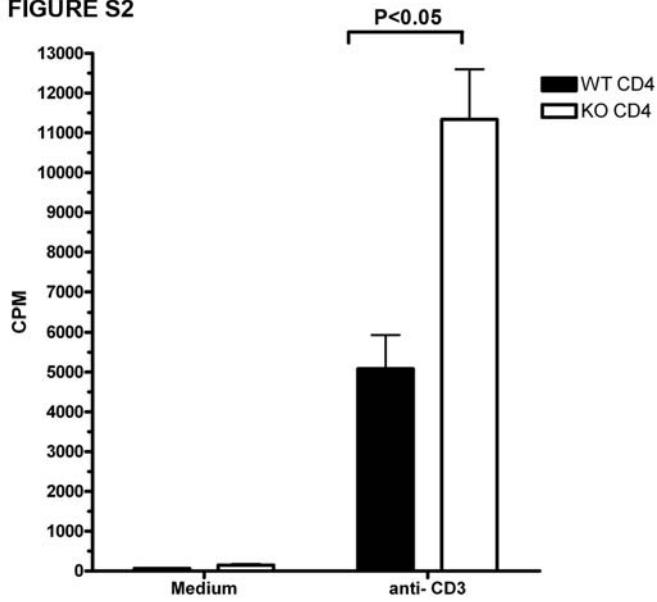
**Figure S2. Higher proliferation of ADPKO CD4⁺ T cell compared to WT CD4⁺ T cells in response to anti-CD3 stimulation in the absence of APCs.** Lymph node cells were isolated from immunized ADPKO and WT mice on day 11 pi (n=3/group), CD4⁺ T cells were purified by magnetic sorting and stimulated *in vitro* with anti-CD3 antibody coated on the plate without APCs or cultured with medium alone. For each individual mouse, mean CPM ± SD was calculated from triplicate wells. Results are shown as means ± SD. Data representative of three different experiments with similar results. Black bars: WT; Open bars: ADPKO. CPM=counts per minute. Statistical significance determined by *t*- test.
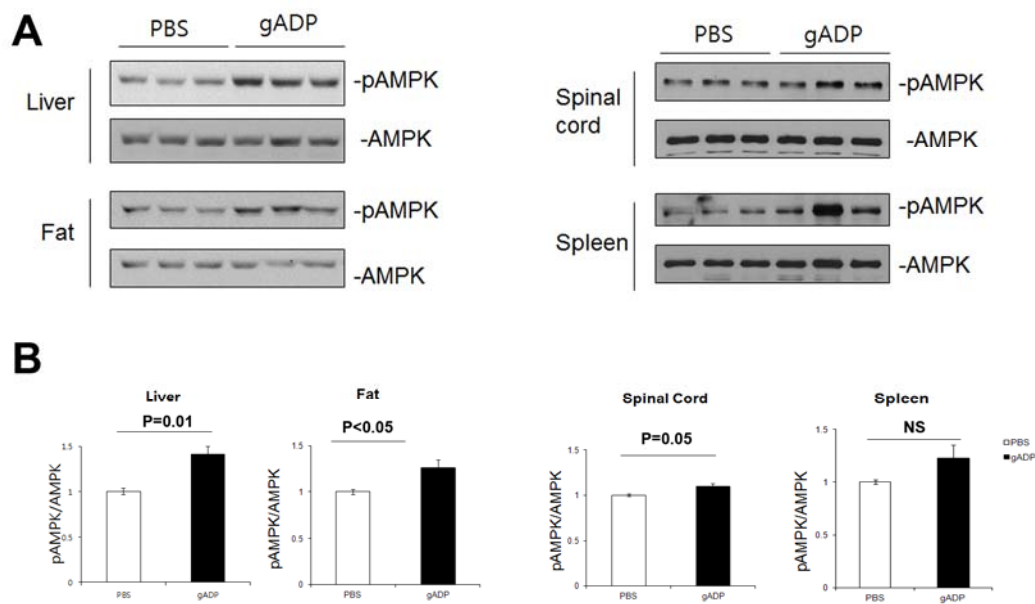
**FIGURE S3**
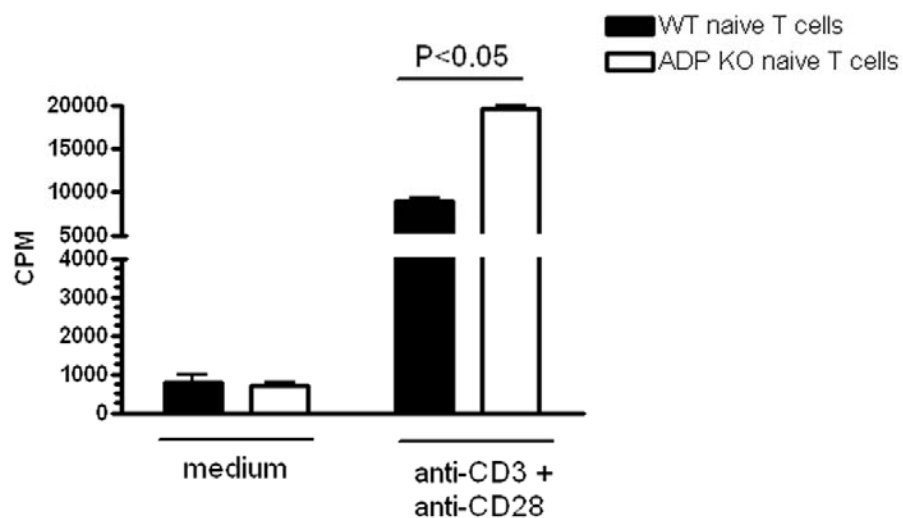


**Figure S3. Naïve ADPKO T cells are more activated than naïve WT T cells.** CD4$^+$ CD62L$^{high}$ CD44$^{low}$ naïve T cells sorted from total lymph node cells and splenocytes of ADPKO and WT mice were stimulated *in vitro* with anti-CD3 coated on plates and anti-CD28 for 72h. A significant higher T cell proliferation was observed in the ADPKO group compared to WT (P<0.05 by *t*-test). Black bars: WT; Open bars: ADPKO. CPM=counts per minute.
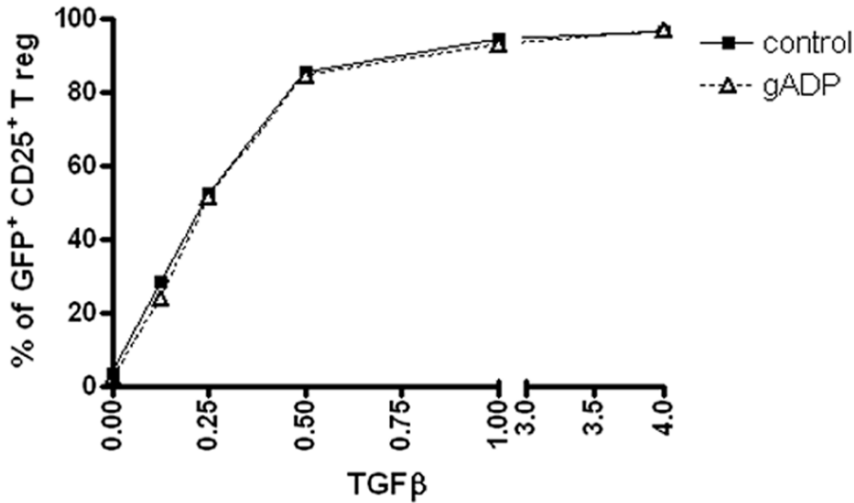
**FIGURE S4**



**Figure S4. Numbers of Treg cells in naïve WT and ADPKO mice**. Numbers of CD25$^+$ Foxp3$^+$ cells within the CD4$^+$ cell population were calculated. Absolute numbers of CD4$^+$CD25$^+$Foxp3$^+$ Treg cells were calculated based on the total number of splenocytes, lymph node cells or thymocytes/mouse. Results are means ± SD.

## FIGURE S5



**Figure S5. Treatment with gADP led to adenosine monophosphate (AMP)-activated protein kinase (AMPK) activation**

***in vivo*. (A)** Expression of AMPK and phosphorylated (p) AMPK was detected by western blot using specific antibodies in

the liver , fat tissue, spinal cord and spleen of C57BL/6 mice treated with gADP or PBS (n=3/group). (B) The ratio of

intensities for pAMPK and AMPK detections were calculated and they were higher in tissues obtained from gADP treated

mice compared to PBS treated controls with differences reaching significance in all the tissues, but the spleens. Error

bars represent  mean ± SD. NS: not significant. Statistical significance determined by Mann Whitney test.

**FIGURE S6**



**Figure S6. gADP does not affect the conversion of naïve T cell into Treg _in vitro._**

$CD25^{neg}Foxp3(RFP)^{neg}CD4^{+}CD62^{high}CD44^{low}$ naïve T cells were isolated from spleen and lymph nodes of Foxp3-IRES-RFP reporter mice. Cells were plated in the presence of plate-bound anti-CD3 antibody, soluble antiCD28, TGFβ, IL-2 and with or without gADP. On day 5 in culture cells were analyzed by flow cytometry for expression of CD25 and RFP which would be up-regulated in those cells that differentiated into Treg cells.