

## SUPPLEMENTAL DATA

**FIGURE S1.** Kinetics of MOG-specific T cell proliferation in lymphoid organs.

*A and B*, Groups of four WT and KO mice were immunized with MOG/CFA. Pooled splenocytes (*A*) and dLN cells (*B*) isolated 15 or 24 days post-immunization were stimulated for 72 h with increasing MOG<sub>35-55</sub> peptide concentrations, and proliferation was analyzed by [<sup>3</sup>H]TdR uptake. Statistical differences were determined as in Fig. 1.

**FIGURE S2.** Kinetics of IFN- $\gamma$  and IL-17 production in lymphoid organs upon MOG immunization.

*A and B*, dLN cells (*A*) or splenocytes (*B*) from WT and KO mice isolated 15 days post-immunization were challenged *in vitro* with the indicated concentrations of MOG<sub>35-55</sub> peptide, and culture supernatants collected after 72 h were analyzed for IFN- $\gamma$  (*left panel*) and IL-17 (*right panel*) concentration by ELISA. Statistical differences were determined as in Fig. 1.

**FIGURE S3.** Normal development and function of regulatory T cell in *Def6*<sup>-/-</sup> mice.

*A and B*, Thymocytes (*A*) and splenocytes (*B*) from WT and KO mice were analyzed for CD4 and intracellular FoxP3 expression by flow cytometry. Numbers in the plots represent the % of CD4<sup>+</sup>FoxP3<sup>+</sup> cells in the thymus (*A*) and among the CD4-gated cells (*B*). *C*, Naïve CD4<sup>+</sup> T cells were purified from spleens of WT and KO mice and then stimulated with 1  $\mu$ g/ml of coated anti-CD3 mAb in the absence or presence of 2.5 ng/ml TGF- $\beta$ . Three days later, cells were harvested and resuspended in fresh TGF- $\beta$ - and IL-

2-containing medium for an additional day, and then analyzed for CD25 and FoxP3 expression. The numbers represent the percentage of CD4<sup>+</sup>FoxP3<sup>+</sup> cells. *D*, Induced Tregs cells prepared as in (C) were stimulated for 5 h *in vitro* with PMA plus ionomycin in the presence of Golgi Stop, and analyzed for IL-10 expression by ICS.

Data shown are representative of five (*A-B*) and three (*C*) independent experiments.

Figure S1

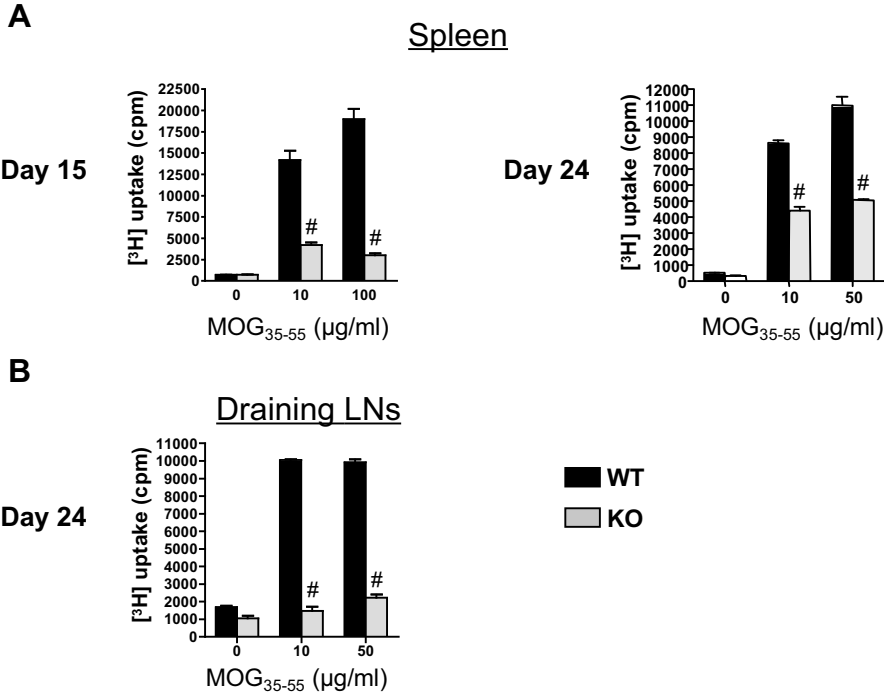


Figure S2

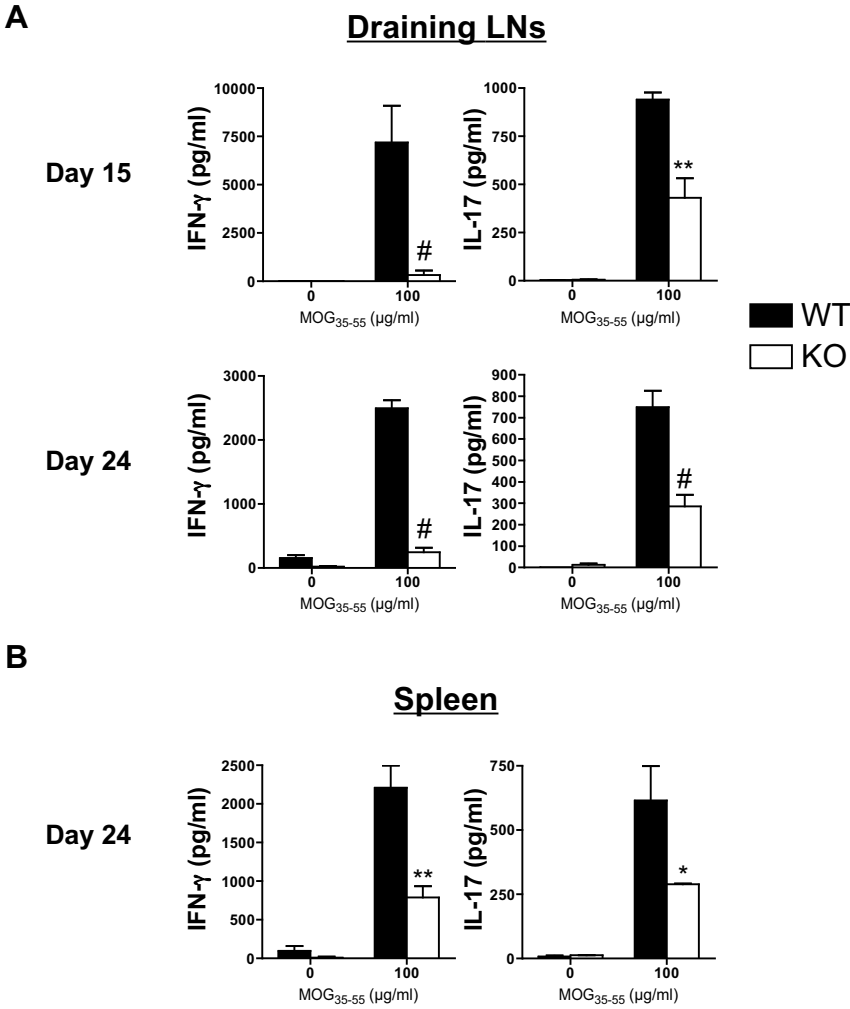


Figure S3

