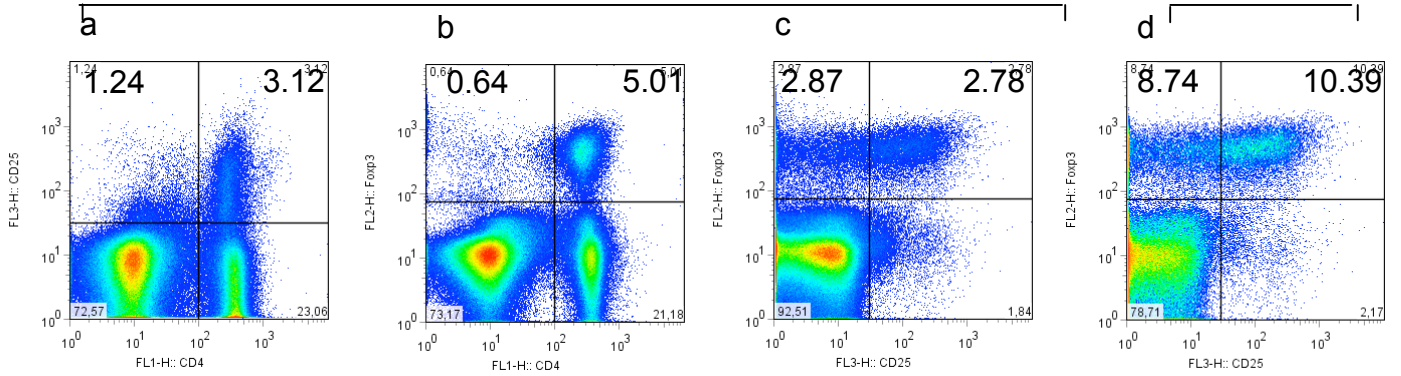


## Supplementary Fig 1

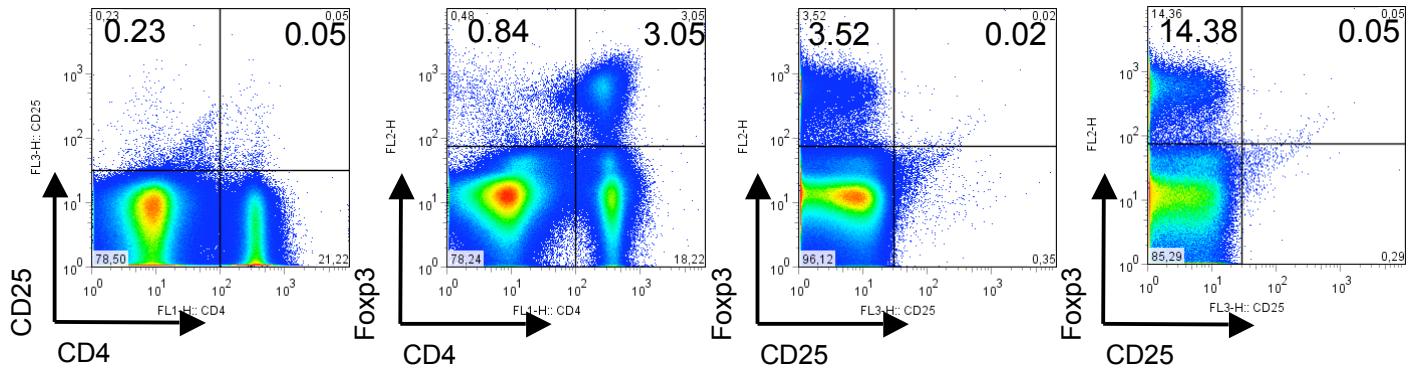
Without treatment

Total cells

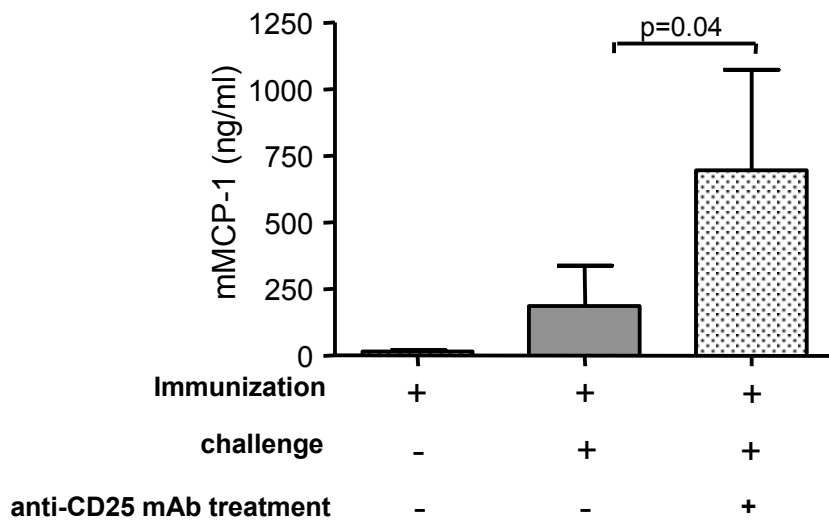
CD4+ cells



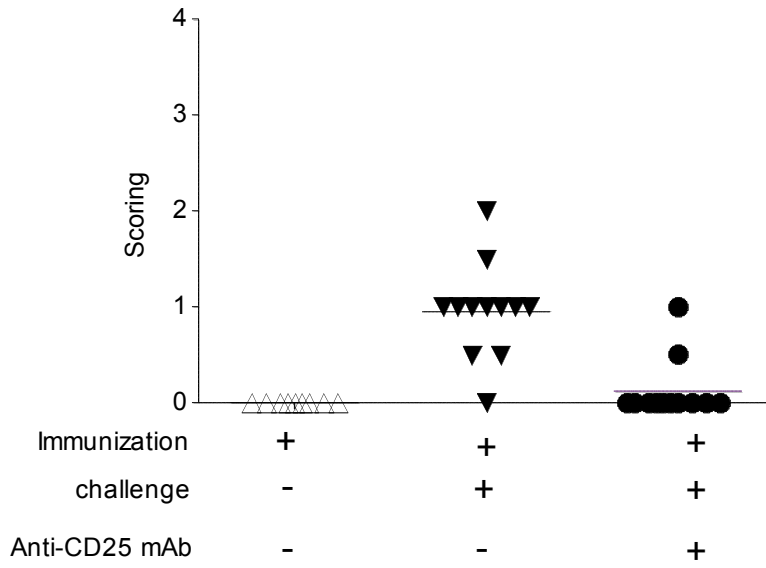
Anti-CD25 mAb treatment



Supplementary Fig 2



Supplementary Fig 3



## SUPPLEMENTARY MATERIAL

### **Materials and Methods**

#### **Flow cytometry staining:**

Axillary and inguinal lymph nodes were removed from mice one day after the last treatment with anti-CD25 mAb. Lymphocytes were prepared by enzymatic digestion as described before and incubated with FACS medium (PBS, 2% SVF, 0,05% mM EDTA, and 0,01% NaN<sub>3</sub>) for at least 15 minutes. Cells were distributed in 96-wells plates and Fc- $\gamma$  receptors were blocked using 50  $\mu$ l of 2.4G2 mAb (supernatant of hybridoma culture) and incubated for 15 minutes at +4°C. After washing with FACS medium, cells were incubated with FITC anti-CD4 and PerCPcy5.5 anti-CD25 mAbs (both from BD Biosciences). After washing with FACS medium, cells were resuspended in fix/permeabilisation medium (eBioscience, France) and incubated for 1.50 h at +4°C. After washing with permeabilisation medium, cells were incubated with rat anti-mouse Fc $\gamma$ -receptor antibody (supernatant from the hybridoma 2.4G2) for 15 minutes at +4°C. Anti-Foxp3-PE was added and incubated for 30 minutes at +4°C. FACS analysis was performed using a FACS Canto (Becton Dickinson) and the data were analysed with the FlowJo software .

### **Figure legends**

#### **Supplementary figure 1: Efficacy of anti-CD25 mAb treatment**

Mice were injected with anti-CD25 mAb twice 2 days apart as described in the legend of figure 2 and the frequency of Treg in lymph nodes 1 day later was compared to that of normal untreated mice. Cells were prepared from inguinal and axillary lymph nodes and stained for CD4, CD25 and Foxp3 as described in the supplementary materials and methods. Expression of CD4, CD25 and Foxp3 on total lymph node cells is shown (a-c), as well as the expression of CD25 versus Foxp3 on gates CD4<sup>+</sup> cells (d).

**Supplementary figure 2: Anti-CD25 mAb injection before challenge enhances serum mMCP1.**

Mice were orally immunized with 10 mg BLG + CT and challenged with either PBS (*white bar*) or BLG (*grey bar and black bar*). One group of BLG-sensitized mice received 100 µg of anti-CD25 mAb one day before BLG challenge (*black bars*). mMCP-1 was titrated by ELISA in serum 1 hr after BLG challenge. Data are representative of 1 out 2 experiments using 4 mice per group.

**Supplementary figure 3: Anti-CD25 mAb injection before BLG challenge abrogates clinical symptoms of ITH**

Mice were orally immunized with 10 mg BLG/ CT and challenged with either PBS (*open triangles*) or 50 mg BLG (*black triangles*), as described in the Figure 1's legend. One group of BLG-immunized and -challenged mice received 100 µg of anti-CD25 mAb on day 25 and 27 , e.g. just before the BLG challenge (*black circles*). The cumulative clinical score was determined as described in Material and Methods. Data represent pooled values from 2 independent experiments using 4-7 mice/group.