# SLAMF5 CONTROLS REGULATORY B CELLS IN MULTIPLE SCLEROSIS

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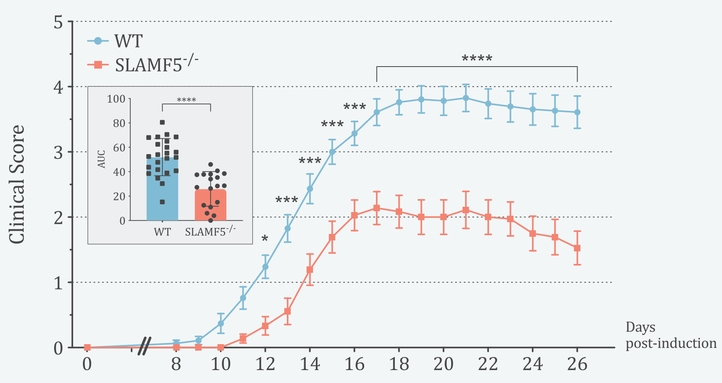
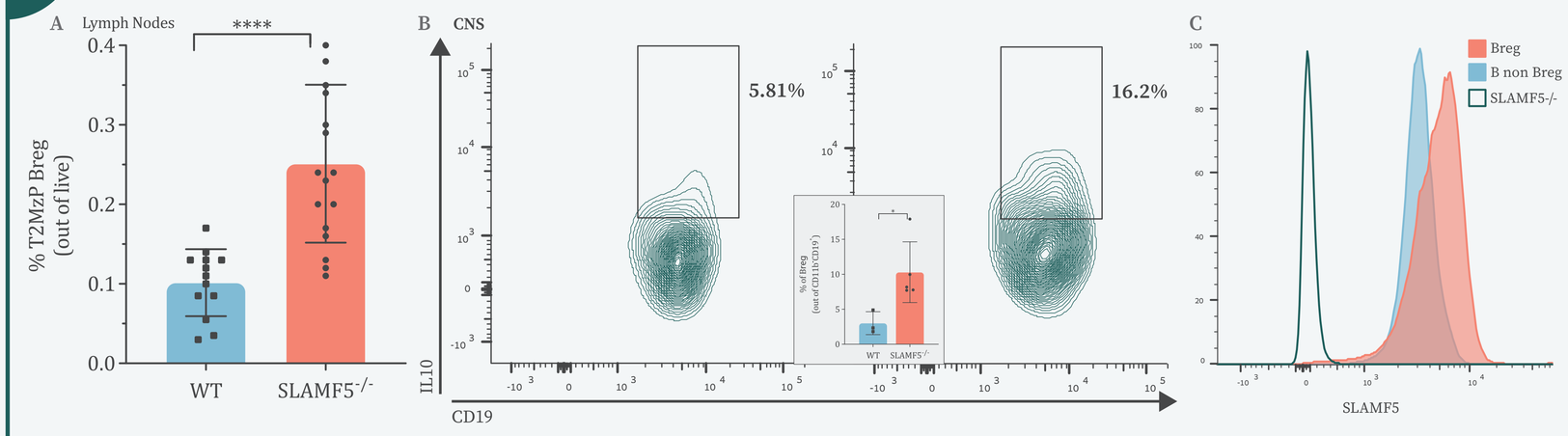
**Background and aim:** B cells are known to contribute to MS by secretion of antibodies, production of pro- inflammatory cytokines and by acting as antigen presenting cells. However, a distinct small subpopulation of B cells was found to negatively regulate inflammation. This subpopulation, known as Regulatory B cells (Bregs), is characterized by the secretion of the anti-inflammatory cytokine IL-10 and their ability to restrain inflammation. In MS patients, Bregs numbers are impaired. In addition, several MS treatments were shown to increase Breg levels. In EAE, the MS mouse model, Bregs were shown to be crucial for overcoming the disease. SLAMF5 is a homophilic receptor belonging to the SLAM (Signaling Lymphocytic Activating Molecules) family. SLAMF5 is expressed mainly hematopoietic cells and facilitates cell-cell interactions and downstream signaling. In this study, we aimed to investigate the role of the receptor SLAMF5 in the fine-tuning of Breg numbers and functionality during EAE.

## 1 SLAMF5 enhances disease severity in EAE



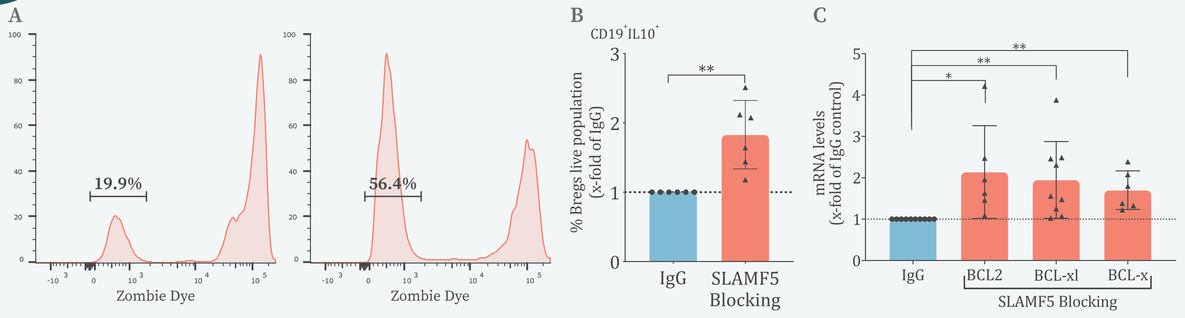

EAE (MOG35-55) was induced in WT and SLAMF5-/- mice. Mice were followed for 26 days. Graph depicts Daily Mean clinical score of the disease . Data represents mean ± SEM. (Inserts depicts the area under curve for days 0–26. WT group n=23, SLAMF5-/- group n=19, two independent experiments) *P < 0.05, **P < 0.01, ***P < 0.001, ****P <0.0001.

## 2 SLAMF5 negatively controls Breg accumulation



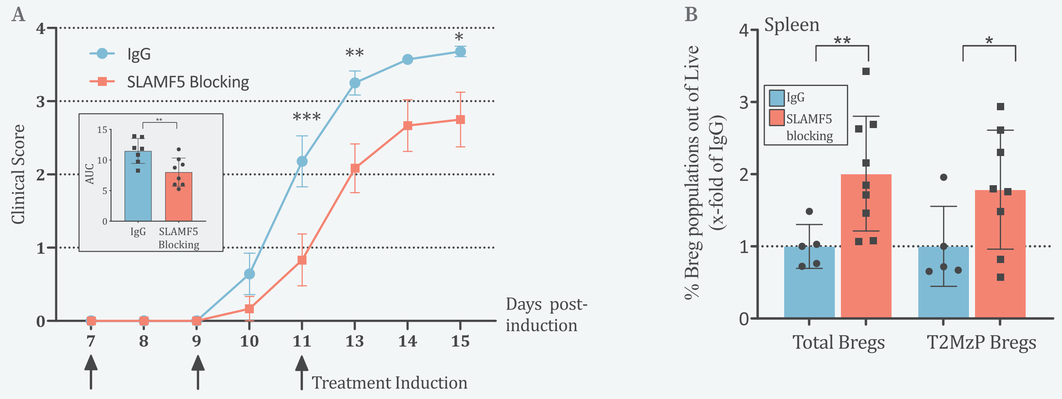

EAE was induced in WT and SLAMF5-/- mice. (A) on day 15 Lymph Nodes were analysed by flow cytometry for T2-MzP Bregs; CD19+IL10+CD24+CD21+CD23+. Each dot represents one mouse. (B) CNS were analysed for Breg population; CD45+ CD11b-CD19+CDIL10+. (insert) Data represent mean ± SD. Each dot represents a combination of 4-5 mice. (C) SLAMF5 expression on T2 B cells and T2MzP Bregs was analysed by flow cytometry.

## 3 Blocking SLAMF5 in vitro increases Breg levels and their survival



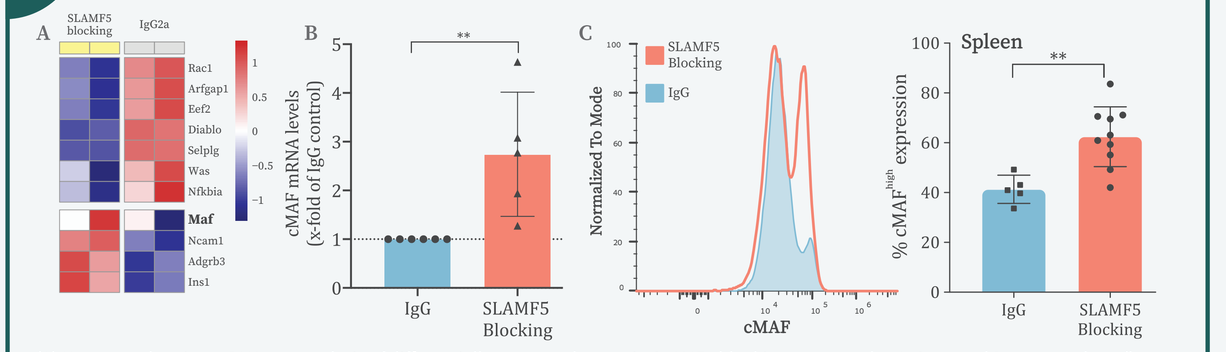

Splenic B cells of EAE induced WT mice were cultured and treated with LPS in the presence of SLAMF5 blocking or control antibodies for 48h. Analysis or Bregs and non-Bregs survival was done by Zombie staining. (A) Representative histograms of live Bregs. (B) Graph summarizes the percentage of live (Zombie-) Bregs shown as x-fold of IgG. (C) B cells of EAE induced Vert-x mice were sorted for Bregs (CD19+GFP+) and treated with SLAMF5 blocking or control antibodies for 24h. mRNA levels of BCL-2, BCL-x and BCL-xl were analysed by qPCR. Data represent mean ± SD and is shown as x-fold of treatment compared to control.; Each dot represents combination of 2-3 mice.

## 4 Blocking SLAMF5 in vivo alleviates EAE and increases Breg levels



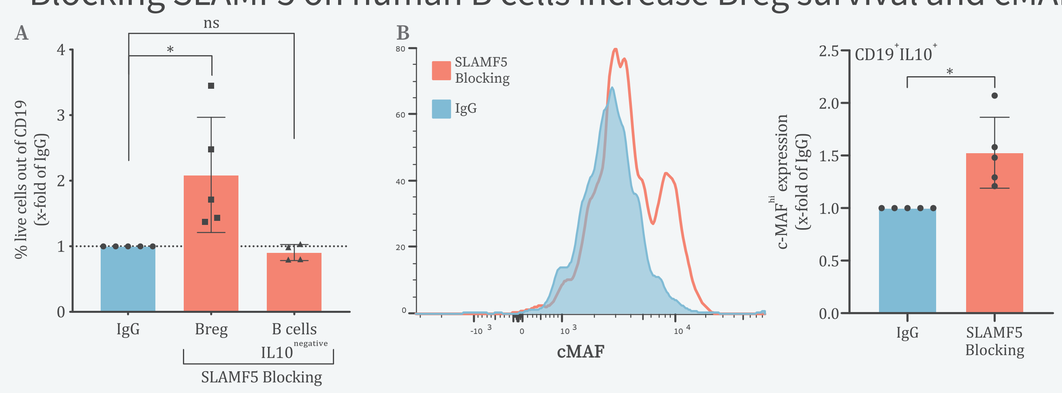

EAE was induced in WT mice. On days 7,9,11 mice were injected i.v. with 30ug SLAMF5 blocking or IgG ab. (A) clinical score. (B) On day 15 spleens were collected. And analyzed for Breg levels; Total Bregs (CD19+IL10+), T2MzP and (CD19+ IL10+ CD24+ CD23+CD21+).

## 5 Blocking SLAMF5 increases cMAF, which regulates IL10



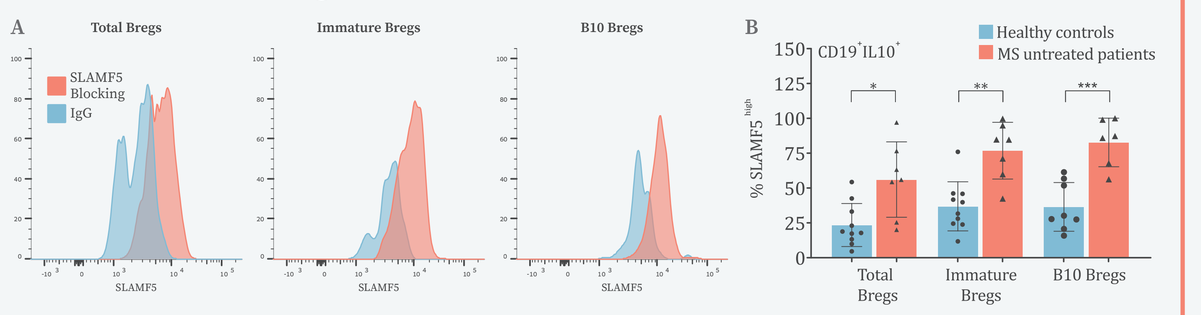

(A) Heatmap showing mRNA-seq analysis of differentially expressed genes in SLAMF5 blocking compared to IgG treated Bregs was done (log2 normalized counts standardized for each gene to a mean of zero). (B) qPCR validation of c-MAF mRNA in SLAMF5 blocked Vert-x sorted Bregs (CD19+GFP+), Each dot represents a combination of 2-3 mice. (C) EAE was induced in WT mice. On days 7,9,11 mice were injected i.v. with 30ug SLAMF5 blocking or IgG ab. cMAF levels in splenic Bregs were analysed on day 15 by flow cytometry. (left) representing histogram and (right) bar charts showing cMAF high expression. Data represent mean ± SD and is shown as x-fold of treatment compared to IgG control.; **P < 0.01.

## 6 Blocking SLAMF5 on human B cells increase Breg survival and cMAF




Purified healthy human B cells were activated with B4 SLAMF5 blocking ab or control IgG for 48 hours. (A) Bregs (CD19+IL10+) and non Breg B cells (CD19+IL10-) were then analysed for (A) cell survival by Zombie staining, data shown as shown as x-fold of treatment compared to IgG control. (B) Cells were also analysed for cMAF levels (right) representing histogram and (left) high cMAF expression on Bregs. Data represent mean ± SD. Each dot represents one donor. *P < 0.05, **P < 0.01

## 7 Multiple Sclerosis patients express abnormal high levels of SLAMF5 on Bregs




Blood samples derived from new untreated Multiple Sclerosis patients and matched healthy controls were processed and PBMC were activated for 5 hours with PMA, Ionomycin, Monensin and Brefeldin A and stained for SLAMF5 and IL-10 on Breg populations. Total Bregs: CD19+IL10+, Immature Bregs: CD19+IL10+CD24+CD38+, B10 Bregs: CD19+IL10+, CD24+ CD27+. (A) representative histograms and (B) bar charts of SLAMF5 expression Breg populations. Data represent mean ± SD. **P < 0.01, ***P < 0.001.