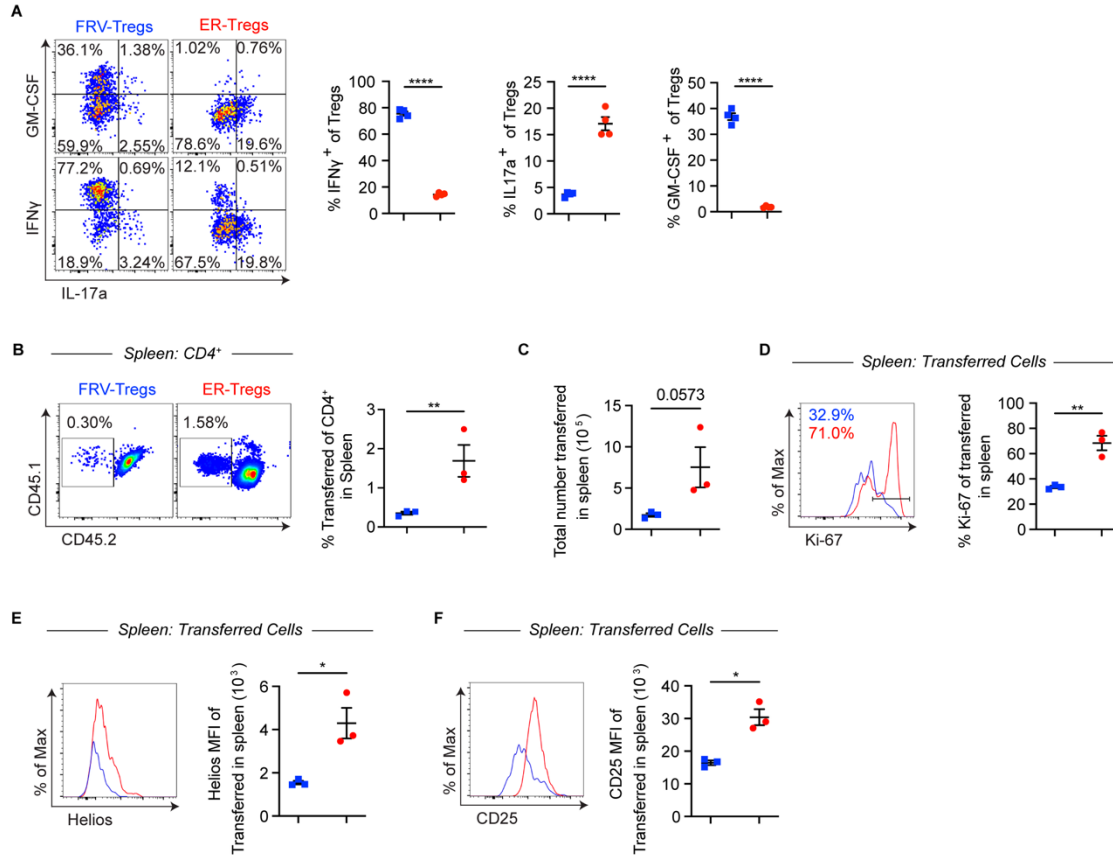


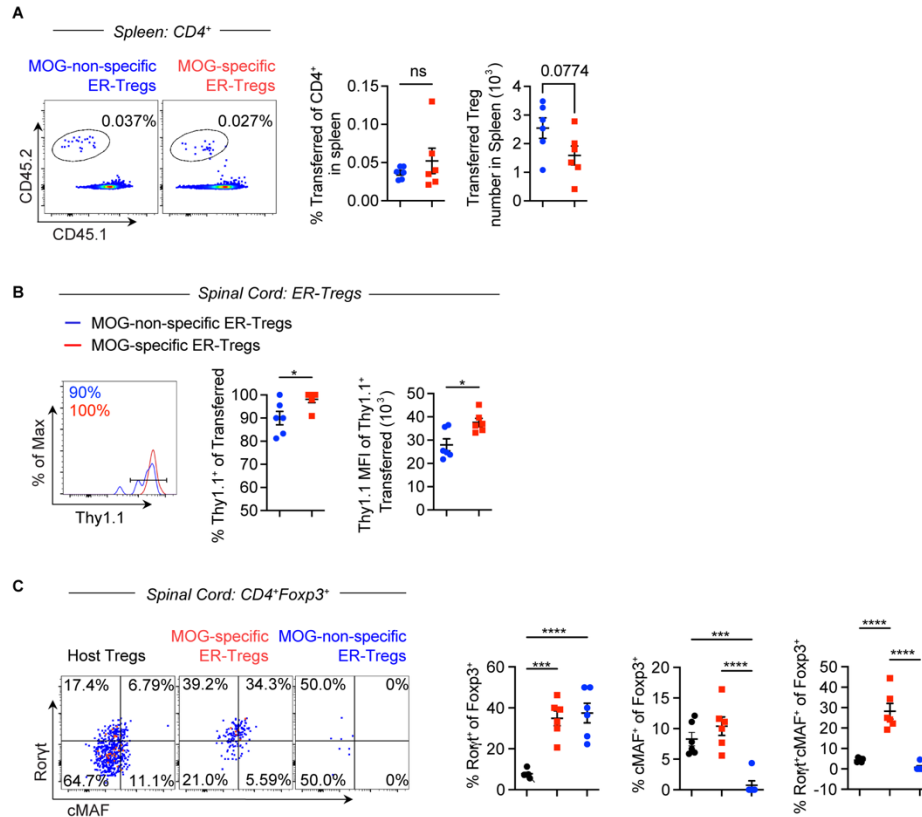
### Supplemental Figure 1. Cellularity and phenotypes of transferred ER-T<sub>regs</sub> in mice with EAE

(A-B) EAE was induced via MOG/CFA immunization in CD45.2<sup>+</sup> mice with or without adoptive transfer of ER-T<sub>regs</sub> reprogrammed from MOG/CFA-primed CD45.1<sup>+</sup>Foxp3<sup>Thy1.1</sup> CD4<sup>+</sup> T<sub>eff</sub> cells, administered one day prior to immunization. Flow cytometry analyses were conducted at 21 days post-immunization (dpi). n = 6 per group. Data are representative of two independent experiments. (A) Flow cytometry analysis of the frequency and total number of host T<sub>regs</sub> and transferred ER-T<sub>regs</sub> in the draining lymph nodes. (B) Flow cytometry analysis of Rorγt and c-MAF expression in both host T<sub>regs</sub> and ER-T<sub>regs</sub> in the draining lymph nodes. (C-E) EAE was induced via MOG/CFA immunization in CD45.2<sup>+</sup> mice with or without adoptive transfer of ER-T<sub>regs</sub> reprogrammed from MOG/CFA-primed CD45.1<sup>+</sup>Foxp3<sup>Thy1.1</sup> T<sub>eff</sub> cells, administered at 11 dpi. Flow cytometry analyses were conducted at 29 dpi. n = 6 per group. Data are representative of two independent experiments. (C) Flow cytometry analysis of the frequency of ER-T<sub>regs</sub> and the total number of host T<sub>regs</sub> and ER-T<sub>regs</sub> in the spinal cords. (D) Flow cytometry analysis of Rorγt and c-MAF expression in both host T<sub>regs</sub> and ER-T<sub>regs</sub> in the spinal cords. (E) Flow cytometry analysis of cytokine expression in spinal cord ER-T<sub>regs</sub>. Mean ± SEM. \*p < 0.05. Unpaired two-sided t-test in (B).



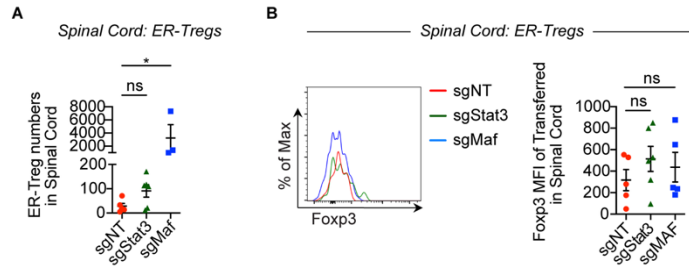
## Supplemental Figure 2. Cellularity, proliferation, and phenotypes of FRV-T<sub>regs</sub> and ER-T<sub>regs</sub>

(A) T<sub>eff</sub> cells retrovirally transduced with a Foxp3 overexpression vector (FRV-T<sub>regs</sub>) or ER-T<sub>regs</sub> were co-cultured with APCs and responder T<sub>eff</sub> in the presence of MOG for 3 days before flow cytometry analysis of IFN $\gamma$ , IL-17a, and GM-CSF expression. (B-F) FRV-T<sub>regs</sub> or ER-T<sub>regs</sub> were transferred into CD45 congenically distinct mice. Recipients were immunized with MOG/CFA and cells were analyzed with flow cytometry 4 days after immunization. (B) Flow cytometry plots (left) and quantification (right) of the frequencies of transferred cells in the spleens of recipient mice. (C) Numbers of transferred cells in the spleens of recipient mice. (D) Flow cytometry histograms (left) and quantification (right) of Ki-67 MFI of transferred cells in the spleens of recipient mice. (E) Flow cytometry histograms (left) and quantification (right) of Helios MFI of transferred cells in the spleens of recipient mice. (F) Flow cytometry histograms (left) and quantification (right) of CD25 MFI of transferred cells in the spleens of recipient mice. Mean  $\pm$  SEM in. \*p < 0.05, \*\*p < 0.01, \*\*\*\*p < 0.0001. Unpaired two-sided t-test in (A-F).



### Supplemental Figure 3. Stability, phenotype, and cellularity of MOG-specific and MOG-nonspecific ER-T<sub>regs</sub> in EAE

(A-C) EAE was induced via MOG/CFA immunization in CD45.1<sup>+</sup> mice with or without adoptive transfer of CD45.2<sup>+</sup>*Foxp3*<sup>Thy1.1</sup> MOG-specific or MOG-nonspecific ER-T<sub>regs</sub>, administered at 11 dpi. Flow cytometry analyses were conducted at 33 dpi. n = 6 per group. (A) Flow cytometry analysis of the frequency and total cell number of MOG specific and MOG non-specific ER-T<sub>regs</sub> in the spleen. (B) Flow cytometry analysis of Thy1.1 expression and Thy1.1 MFI in MOG specific vs MOG non-specific ER-T<sub>regs</sub> in the spinal cord. (C) Flow cytometry analysis of Rorγt and c-MAF expression in host T<sub>regs</sub> and ER-T<sub>regs</sub> in the spinal cord. Mean ± SEM. \*p<0.05, \*\*\*p < 0.001, \*\*\*\*p < 0.0001. Unpaired two-sided t-test in (A) and (B), one-way ANOVA and Holm-Šidák test in (C).



### Supplemental Figure 4. Cellularity and stability of sgNT, sgStat3, and sgMaf transduced ER-T<sub>regs</sub>

EAE was induced via MOG/CFA immunization in *Rag1*<sup>-/-</sup> mice one day after adoptive transfer of MOG/CFA-primed CD45.1<sup>+</sup> CD4<sup>+</sup> T<sub>conv</sub> cells with or without co-transfer of CD45.2<sup>+</sup> *Foxp3*<sup>Thy1.1</sup> *R26*<sup>Cas9</sup> ER-T<sub>regs</sub> transduced with sgRNA-RV targeting *Stat3* (sgStat3), *Maf* (sgMaf), or a non-targeting sgRNA-RV (sgNT). Flow cytometry analyses were conducted at 22 dpi. n = 6-7 per group. (A) Total number of ER-T<sub>regs</sub> in the spinal cords. (B) Flow cytometry histograms (left) and quantification (right) of ER-T<sub>reg</sub> Foxp3 expression in the spinal cord. Mean ± SEM in (A-B). \*p < 0.05. One-way ANOVA and Holm-Šídák test in (A-B).