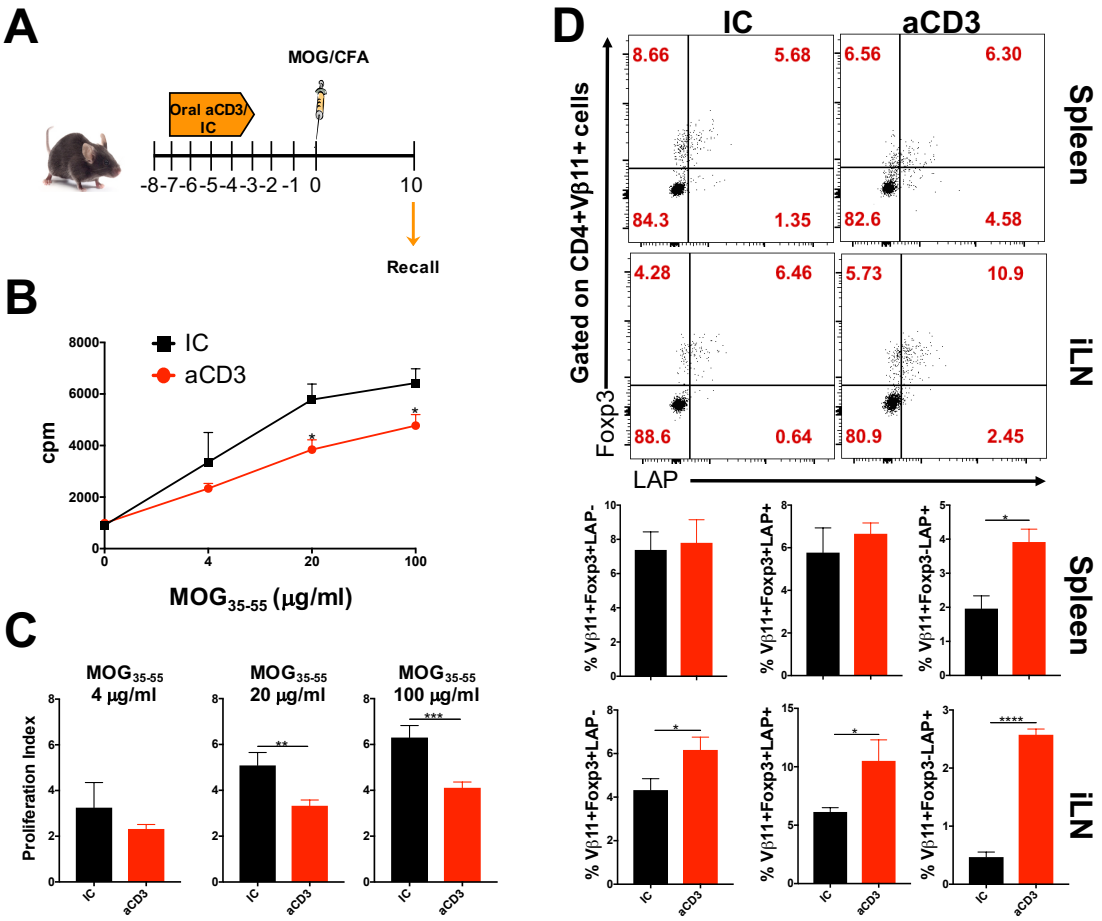
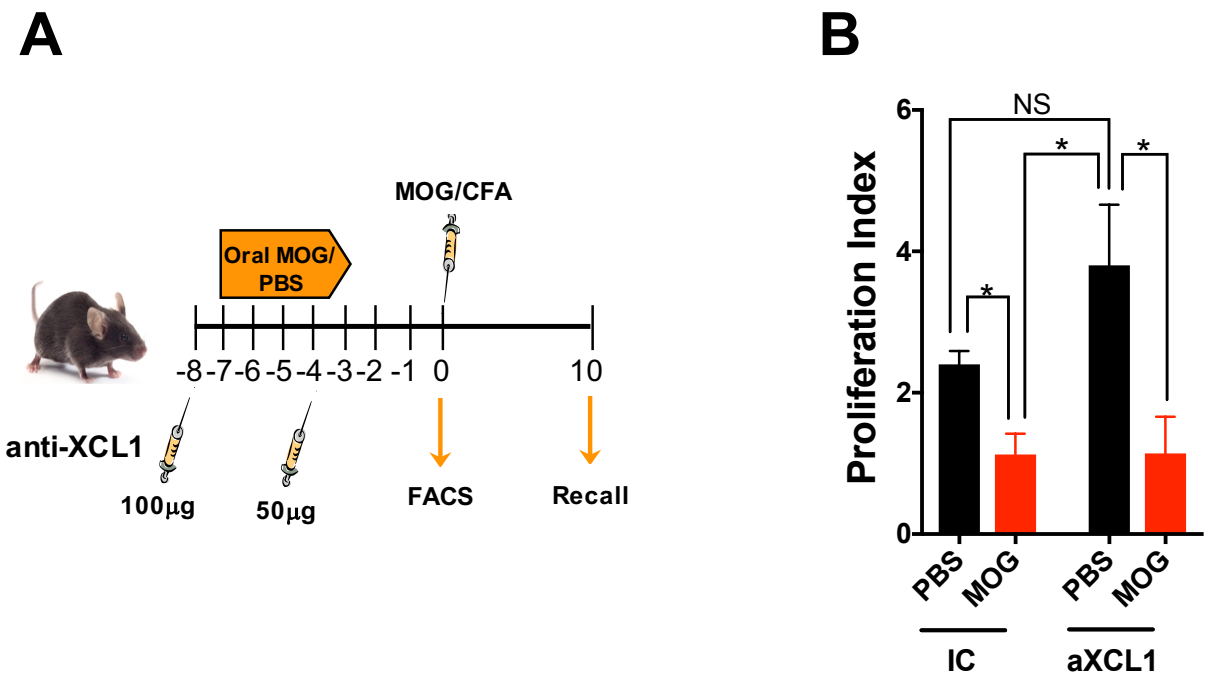
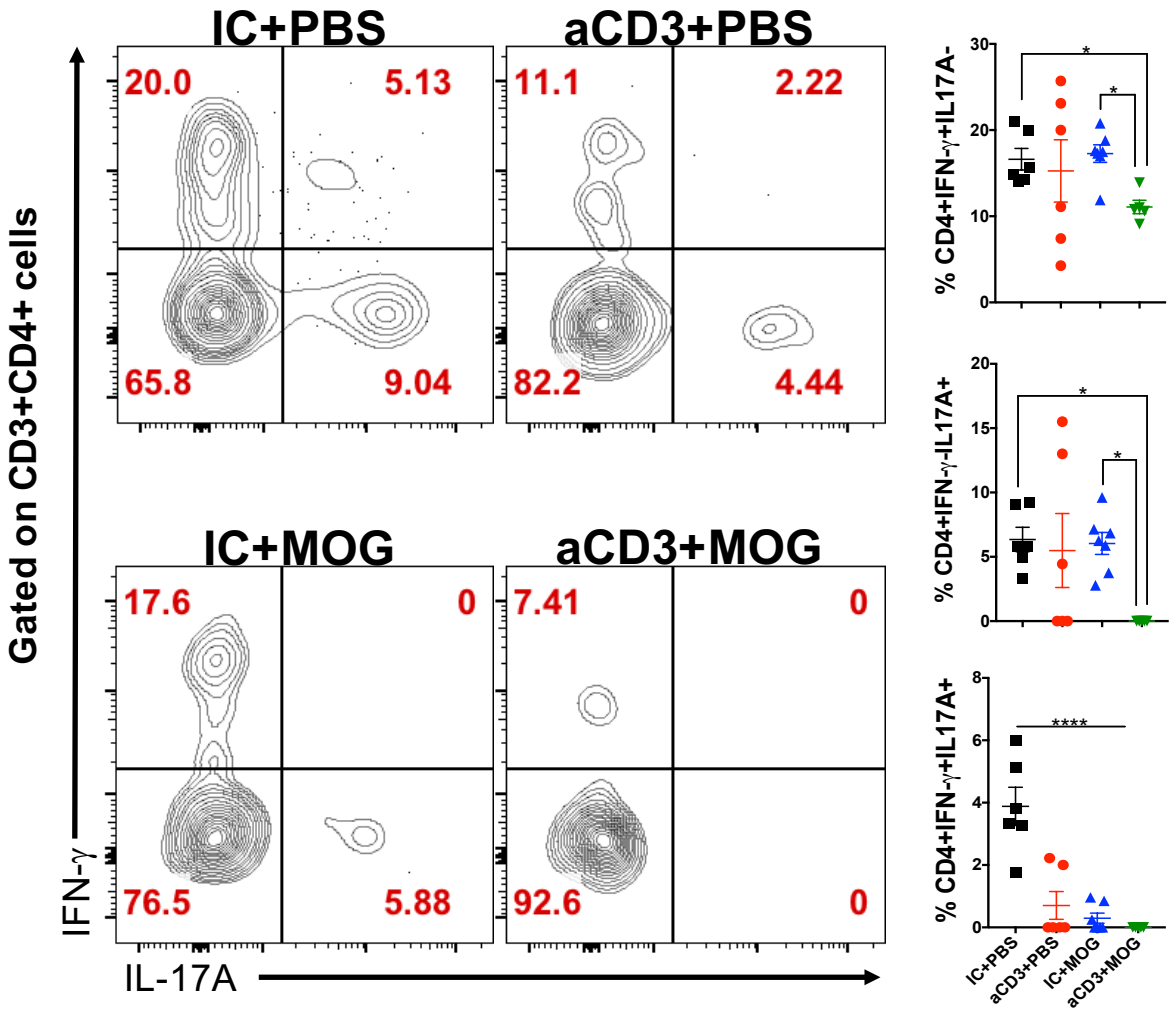
SUPPLEMENTAL DATA



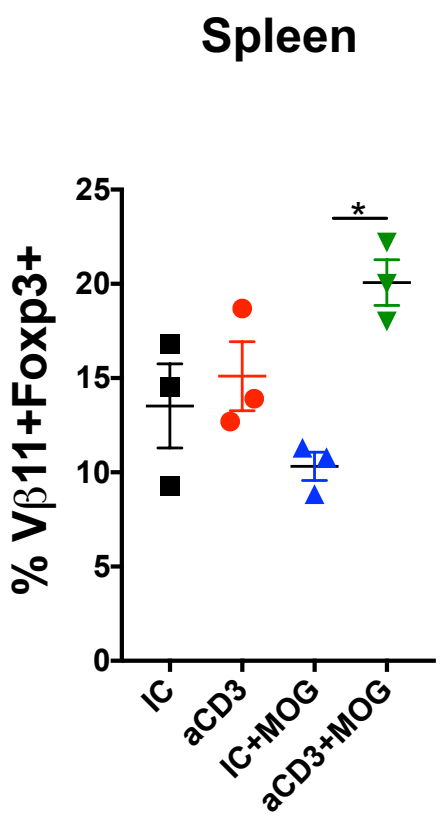
Supplementary Figure 1. Increased MOG_{35-55}-specific Treg cells after anti-CD3-induced oral tolerance. (A) Scheme for oral administration of anti-CD3 (aCD3) or isotype control (IC), both given at 10 µg/mouse; MOG_{35-55}/CFA immunization and recall assay. **(B, C)** Ten days after MOG_{35-55}/CFA immunization, mice were sacrificed and spleens removed for recall assay. Splenocytes were stimulated in the presence of 4, 20 or 100 µg/ml of MOG_{35-55} for three days and proliferation analyzed by H^3-thymidine incorporation in counts per minute (cpm; **B**) or index (**C**; see methods for details); n=8-20 mice/group. **(D)** FACS plots showing Foxp3 and LAP expression in CD4+Vβ11+ cells from the spleen and inguinal lymph node (iLN); n=5 mice/group. Data are shown as mean ± SEM and are representative of three independent experiments. One-way ANOVA **(B)** or Student's t test **(C, D)** were used. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.



Supplemental Figure 2. Anti-XCL1 monoclonal antibody administration does not affect oral tolerance induced by a fed antigen. (A) Scheme for anti-XCL1 monoclonal antibody (mAb) administration, injected subcutaneously twice (100 and 50 µg/mouse) on oral administration of MOG_{35-55} (250 µg/mouse) or PBS; MOG_{35-55}/CFA immunization and recall assay. (**B)** Ten days after MOG_{35-55}/CFA immunization, mice were sacrificed, and spleens removed for recall assay. Splenocytes were stimulated in the presence of 100 µg/ml of MOG_{35-55} for three days and proliferation index analyzed by H^3-thymidine incorporation; n=5 mice/group. Data are shown as mean ± SEM and are representative of two independent experiments. One-way ANOVA was used. NS=non-significant, * $p<0.05$.



Supplemental Figure 3. Decreased inflammation in the spinal cord of mice treated orally with both anti-CD3 and MOG_{35-55}. FACS plots showing IFN-γ and IL-17A expression in infiltrating CD3+CD4+ T cells in the spinal cord of EAE-induced mice 18 days after immunization with MOG_{35-55}/CFA. Mice were treated orally with either 10 μg of anti-CD3 (aCD3) or 250 μg of MOG_{35-55} (MOG) alone or both together (or their respective vehicle controls alone or together) for five consecutive days and EAE induced three days after the last dose of aCD3 and MOG; n=6-7 mice/group. Data are shown as mean ± SEM and are representative of two independent experiments. One-way ANOVA was used. * $p<0.05$, **** $p<0.0001$.



Supplemental Figure 4. Increased Treg cell frequency following administration of both anti-CD3 and MOG_{35-55}. Scatter plot showing Foxp3 expression in CD3+CD4+Vβ11+ T cells in the spleen of EAE-induced mice 10 days after immunization with MOG_{35-55}/CFA. Mice were treated orally with either 10 μg of anti-CD3 (aCD3) or 250 μg of MOG_{35-55} (MOG) alone or both together (or their respective vehicle controls alone or together) for five consecutive days and EAE induced three days after the last dose of aCD3 and MOG; n=3 mice/group. Data are shown as mean ± SEM and are representative of two independent experiments. One-way ANOVA was used. * $p<0.05$, **** $p<0.0001$.