Supporting Information

Selective ORAI1 inhibition ameliorates CNS inflammation in EAE by suppressing effector but not regulatory T cell function.

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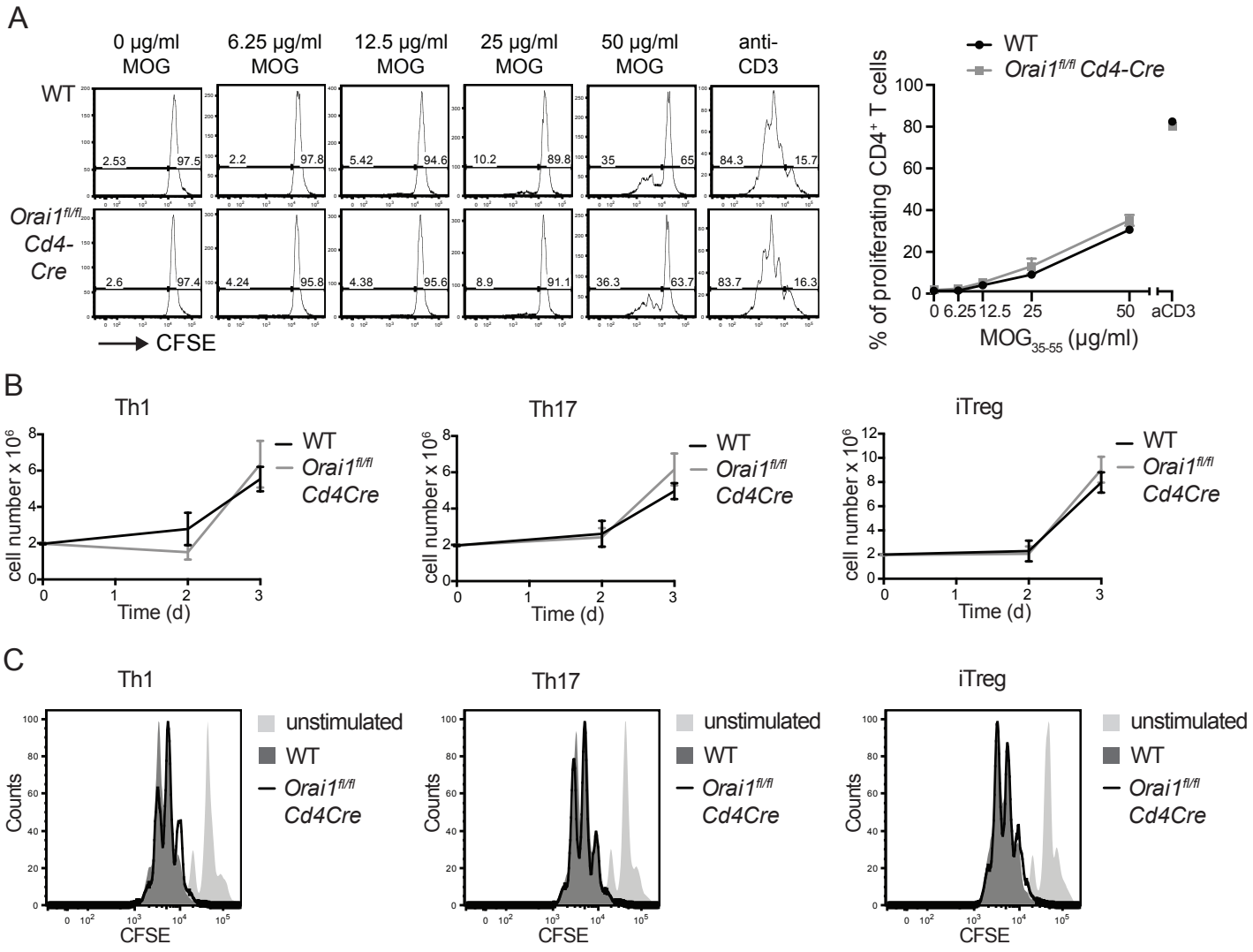
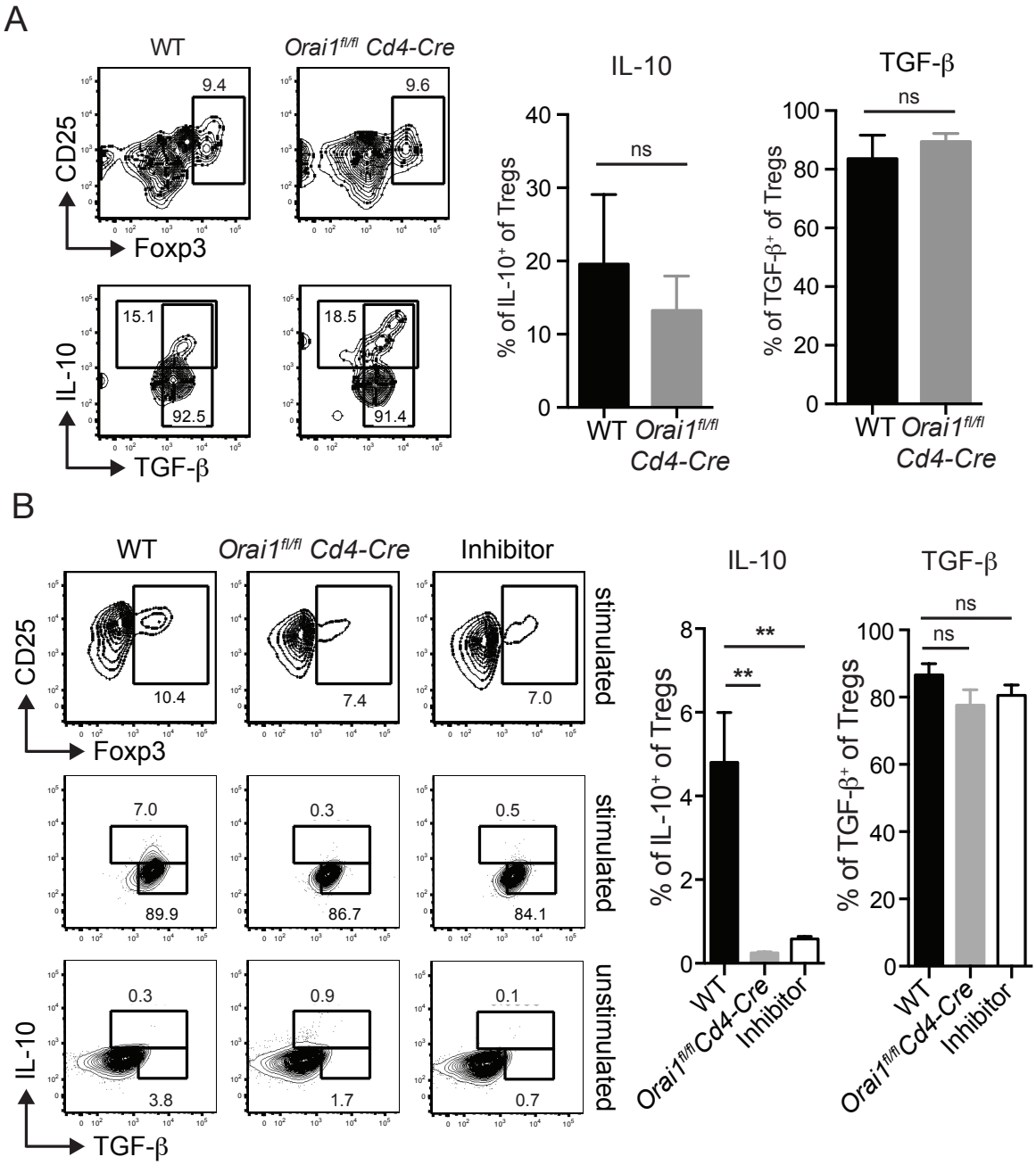
Supporting Figures



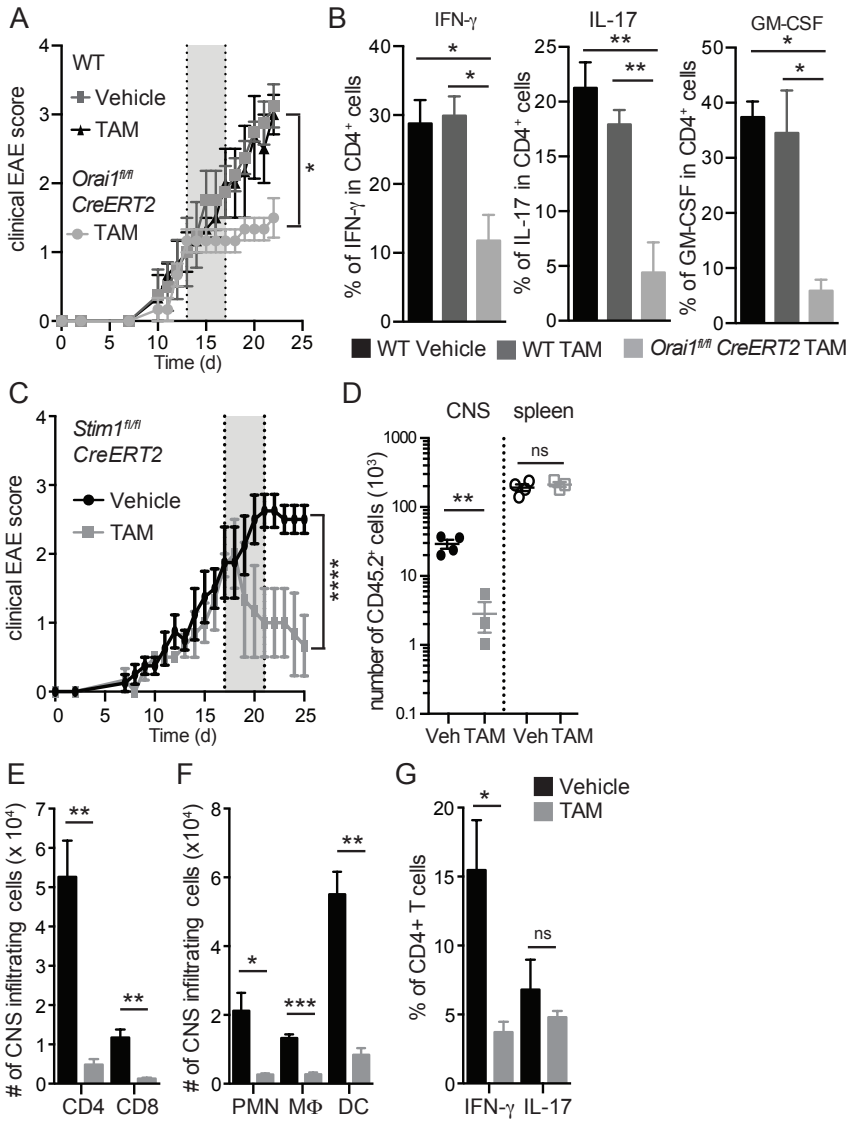
Supporting Figure 1: Early T cell activation as well as proliferation of in vitro differentiated Th1, Th17 and iTreg cells was not impaired in Orai1 deficient T cells.

(**A**) WT and *Orai1*$^{fl/fl}$ *Cd4-Cre* mice were immunized with MOG_{35-55} peptide but without PTX. At day 7 after immunization cells were isolated from spleen and draining LNs and stimulated with different concentrations of MOG_{35-55} peptide (0-50 µg/ml) or anti-CD3 for 3 days in vitro. Cells were labeled with CFSE and 3 days later $CD4^+$ T cells were analyzed for CFSE dilution by flow cytometry. Histograms are representative of 5 mice per group and line graphs on the right represent the average ± SEM of 5 mice per group. Statistical analysis was performed using an unpaired Student's t test. (**B, C**) Naïve $CD4^+$ T cells from spleen and LNs of WT and *Orai1*$^{fl/fl}$ *Cd4-Cre* mice were cultured under Th1, Th17 and iTreg polarizing conditions for 3 days. (**B**) Cells were counted on day 0, 2 and 3. Line graphs represent the average cell number ± SEM for 3 mice per group. (**C**) Cells were labeled with CFSE and 3 days later analyzed for CFSE dilution by flow cytometry. Shown are representative flow cytometry plots.



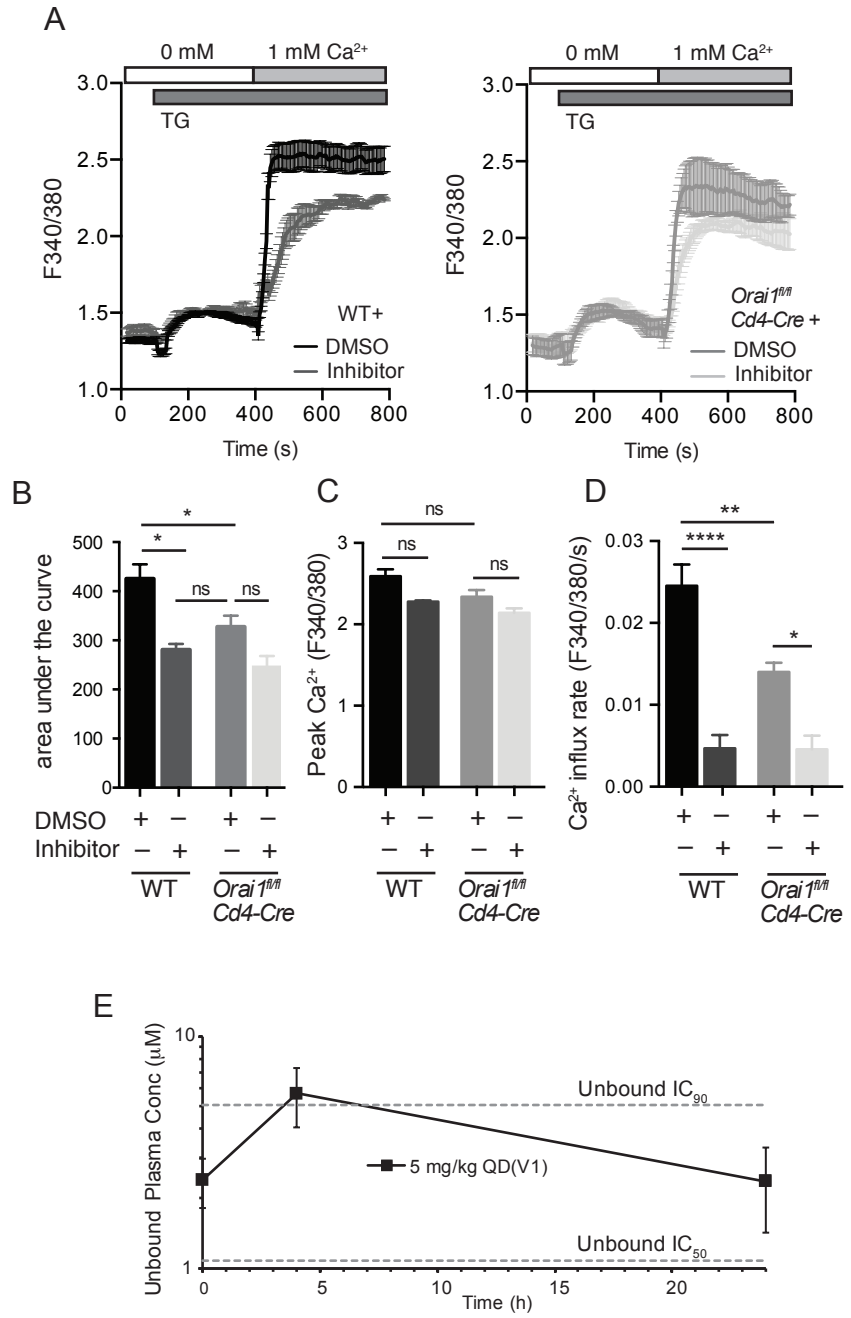
Supporting Figure 2: IL-10 and TGF-β expression in Treg cells isolated from the CNS of mice with EAE and iTreg cells differentiated in vitro.

(**A**) EAE was induced in WT and *Orai1*$^{fl/fl}$ *Cd4-Cre* mice by immunization with MOG_{35-55} peptide in CFA. Lymphocytes were isolated from the CNS on day 27 after immunization. Treg cells were identified by gating on $CD4^+$ $CD25^+$ $Foxp3^+$ cells. For cytokine staining, cells were stimulated with PMA and ionomycin in the presence of Brefeldin A for 6 h in vitro. IL-10 and TGF-β expression was analyzed in $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells. Bar graphs represent the average of 4-6 mice per group. Statistical analysis was performed using an unpaired Student's t test. (**B**) $CD4^+$ T cells isolated from the spleen and LNs of WT and *Orai1*$^{fl/fl}$ *Cd4-Cre* mice were stimulated with anti-CD3/CD28 under iTreg polarizing conditions in vitro. The CRAC channel inhibitor AMG1 (100 nM) was added to some WT T cells (right column) on day 0 and was present throughout the iTreg cell differentiation (day 0-3). iTreg cells were left unstimulated or stimulated with PMA plus ionomycin for 6h in the presence of Brefeldin A. The frequency of IL-10 and TGF-β expressing cells of all $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells was measured by flow cytometry. Data represent the average ± SEM of 3 mice per group. Statistical analysis was performed using an unpaired Student's t test. ** $p < 0.01$.



Supporting Figure 3: Inducible deletion of *Orai1* and *Stim1* during ongoing EAE ameliorates disease severity.

(**A**) EAE was induced in WT and *Orai1*$^{fl/fl}$ *Cre-ERT2* mice by immunization with MOG_{35-55} peptide in CFA. Mice were treated with 1 mg/kg tamoxifen (TAM) or vehicle (corn oil) control for 5 days starting from days 13-17 after immunization. Clinical disease scores were monitored daily. Data represent the average ± SEM of 3-4 mice per group. (**B**) $CD4^+$ T cells were isolated from the CNS at day 22 after EAE induction and restimulated in vitro with PMA and ionomycin in the presence of Brefeldin A for intracellular cytokine analysis by flow cytometry. (**C-G**) For passive induction of EAE, *Stim1*$^{fl/fl}$ *Cre-ERT2* and WT mice ($CD45.2^+$) were immunized with MOG peptide. T cells were isolated from spleen and LNs 12 days later and restimulated in vitro with MOG peptide in the presence of IL-23 for 3 days. 4 x 10^6 $CD4^+$ T cells were transferred i.v. into sublethally irradiated CD45.1 recipient mice. After EAE symptoms developed, recipient mice were injected with 1 mg/kg tamoxifen (TAM) or vehicle (corn oil) for 5 days (days 17-21 after T cell transfer). (**C**) Clinical EAE scores. (**D**) Absolute numbers of $CD45.2^+$ cells in CNS and spleen. (**E**) Absolute numbers of $CD4^+$ and $CD8^+$ T cells as well as (**F**) $CD11b^+Gr\text{-}1^+$ polymorphonuclear cells (PMN), $CD11b^+CD11c^-Gr\text{-}1^-$ macrophages and $CD11c^+Gr\text{-}1^-$ DC in the CNS 25 days after adoptive transfer. (**G**) Frequencies of $CD4^+IFN\text{-}\gamma^+$ and $CD4^+IL\text{-}17^+$ T cells isolated from the CNS and restimulated in vitro with PMA and ionomycin for 6h. Data in C-G represent the average ± SEM of 3-4 mice per group. Statistical analysis of EAE scores in A (days 13-22) and C (days 17-25) was performed using a Mann-Whitney test; data in panels B, D-G were analyzed using an unpaired Student's t test. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, **** $p < 0.0001$.



Supporting Figure 4: Specificity and pharmacokinetics of CRAC channel inhibitor AMG1.

(**A**) $CD4^+$ T cells were isolated from the spleen and LNs of WT and *Orai1$^{fl/fl}$Cd4-Cre* mice, loaded with Fura-2 and incubated with 1000 nM AMG1 or DMSO in 0 mM Ca^{2+} Ringer solution for 15 min prior to Ca^{2+} measurements by FlexStation. Cells were stimulated with 1 μM Thapsigargin in Ca^{2+} free buffer, followed by addition of 1 mM Ca^{2+} Ringer solution. SOCE in experiments shown in A was quantified as the area under the curve (AUC) after Ca^{2+} re-addition (400-800s) (**B**), peak Ca^{2+} influx (**C**) and Ca^{2+} influx rate (400-440s) (**D**). Data represent the average ± SEM from 3 repeat experiments. (**E**) Pharmacokinetics of the CRAC inhibitor AMG1 in BALB/c mice. Shown are the mean ± SD of AMG1 concentrations (nM) in the plasma of 5 female BALB/c mice after 10 days of QD dosing. Mice were administered 5 mg/kg inhibitor AMG1 for 10 days by oral gavage, and plasma concentrations measured at trough (0 h) prior to the final dose as well as 4 h and 24 h after final gavage of compound. Dotted lines represent the IC_{50} and IC_{90} concentrations for inhibition of IL-2 production by mouse splenocytes in vitro. Data in panels A-C were analyzed using a one-way ANOVA test. * $p < 0.05$, ** $p < 0.005$, **** $p < 0.0001$.