Supplementary Information

Supplementary Table 1

Gene name	Forward Primer	Reverse Primer	Comments
beta-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACGCCATG	
IFN-γ	ACTGGCAAAAGGATGGTG	GTTGCTGATGGCCTGATT	
IL-4	AGATCATCGGCATTTTGAACG	TTTGGCACATCCATCTCCG	
IL-17A	CTCCAGAAGGCCCTCAGACTA	AGCTTTCCCTCCGCATTGACA	
IL-17F	CCCATGGGATTACAACATCAC	CACTGGGCCTCAGCGATC	
IL-22	GTGGGATCCCTGATGGCTGTC	AGCGAATTCTCGCTCAGACTG	
IL-23R	ACACTGGGAAGCCTACCTACA	AGCTTGGACCCATACCAAATA	
T-bet	CAACAACCCCTTTGCCAAAG	TCCCCCAAGCAGTTGACAGT	
RORγ	CACGGCCCTGGTTCTCAT	CAGATGTTCCACTCTCCTCTTCTCT	
RORα	TCTCCCTGCGCTCTCCGCAC	TCCACAGATCTTGCATGGA	
Foxp3	GGCCCTTCTCCAGGACAGA	GCTGATCATGGCTGGGTTGT	
CCR6	CCTCACATTCTTAGGACTGGAGC	GGCAATCAGAGCTCTCGGA	
LFA-1	CCAGACTTTTGCTACTGGGAC	GCTTGTTCGGCAGTGATAGAG	
c-MAF	AGCAGTTGGTGACCATGTCG	TGGAGATCTCCTGCTTGAGG	
Runx1	TACCTGGGATCCATCACCTC	GACGGCAGAGTAGGGAACTG	
AHR	AGCAGCTGTGTCAGATGGTG	CTGAGCAGTCCCCTGTAAGC	
IRF-4	GCAGCTCACTTTGGATGACA	CCAAACGTCACAGGACATTG	
SOCS3	AGCTCCAAAAGCGAGTACCA	TGACGCTCAACGTGAAGAAG	
IκBζ	TCCAGAATGTCCCAGTCTCC	GAGTCTCAGTTTGGGGTGGA	

BATF	CCAGAAGAGCCGACAGAGAC	GAGCTGCGTTCTGTTTCTCC	
HIF1	AGCTTCTGTTATGAGGCTCACC	TGACTTGATGTTCATCGTCCTC	
GATA-3	CTCGGCCATTCGTACATGGAA	GGATACCTCTGCACCGTAGC	
hOrai1Δloop2 Fragment 1	GCG CTC GAG ATG CAT CCG GAG CCC GCC CCG	CGC CGC GGC CGC GAG GGG CAA GAA CTT GAC	
hOrai1ΔECloop2 Fragment 2	CGC GCG GCC GCG CCG GGC CAG GCA GCT GCC	CGC GAA TTC GGC ATA GTG GCT GCC GGG CGT	Digest fragment 1 and 2 and ligate with the vector
hOrai1Q108-110AAANotI-fragment 1	GCG CTC GAG ATG CAT CCG GAG CCC GCC CCG	CGC CGC GGC CGC CAC CTC CAC CAT TGC CAC CAT	
hOrai1Q108-110AAANotI-fragment 2	CGC GCG GCC GCG GCT GAC CAC GAC TAC CCA	CGC GAA TTC GGC ATA GTG GCT GCC GGG CGT	Digest fragment 1 and 2 and ligate with the vector
hOrai1D110/112A	GTG GAG GTG CAG CTG GCC GCT GCC CAC GAC TAC CCA CCG	CGG TGG GTA GTC GTG GGC AGC GGC CAG CTG CAC CTC CAC	
hOrai1E106D	ATG GTG GCA ATG GTG GAC GTG CAG CTG GAC GCT	AGC GTC CAG CTG CAC GTC CAC CAT TGC CAC CAT	
hOrai1L95A	AGC CGG ACC TCG GCT GCG CTC TCC GGC TTC GCC	GGC GAA GCC GGA GAG CGC AGC CGA GGT CCG GCT	
hOrai1V102C	TCC GGC TTC GCC ATG TGT GCA ATG GTG GAG GTG	CAC CTC CAC CAT TGC ACA CAT GGC GAA GCC GGA	
hOrai1V102A	TCC GGC TTC GCC ATG GCG GCA ATG GTG GAG GTG	CAC CTC CAC CAT TGC CGC CAT GGC GAA GCC GGA	
RORαp	ATCCTCCCTCTC CTCTTTAACC	AACGCGGATAACCGGATTTGT	ChIP PCR
RORγtp	AGACACCACCCAAGACAGATT	AAACCACAGCTACAGCCGCGG	ChIP PCR

T-betp	GCGTAACAGCTAGCGAAAGA	GGGACTTTCAGGCAAAGGAA	ChIP PCR
IL-17p	CATGTGAATGGCACGATAGG	TGAGGTCAGCACAGAACCAC	ChIP PCR
IL-17 CNS2	CAGCGTGTGGTTTGGTTTAC	CTAGGTGGGTTCCTCACTGG	ChIP PCR
IFN-γp	ATCCCACAAGAATGGCACAG	TATACCTGATCGAAGGCTCCTC	ChIP PCR

Supplementary Figures

Suppl. Figure 1 Characterization of the inhibitory activity of compound 5D on CRAC channels

(**A**) Comparison of the inhibitory effects of the lead compound 5 (10 μM) and its analogue 5D (1 μM) on CRAC channel-mediated SOCE in HeLa-OSN cells.

(**B**) TIRF microscopy analysis of HEK293 cells expressing STIM1-mCherry and Orai1-GFP in the presence of DMSO (left three panels) or compound 5D (10 μM, right three panels). 1 μM thapsigargin was added to deplete the intracellular stores (t = 0) and epifluorescence (top panels) and TIRF (lower two panels) images of STIM1 and Orai1 clustering into ER-PM junctions are shown before and after store depletion. The graphs represent an average of normalized fluorescence intensity ± s.e.m. of STIM1-mCherry (left panel) and Orai1-GFP (right panel) from measurements of 5 cells treated with (blue traces) and without (black traces) compound 5D (10 μM).

(**C**) Compound 5D blocks currents generated by $Orai1^{W176C}$. Measurement of currents from HEK293 cells expressing $Orai1^{W176C}$ (in the absence of STIM1). I-Vs were plotted in the absence (black trace), presence (red trace) or after washing out (green trace) of 10 μM compound 5D. Each trace is representative of data obtained from six different cells.

(**D**) Compound 5D partially blocks currents generated by $Orai1^{V102C}$. I-V relationship of currents from HEK293 cells expressing $Orai1^{V102C}$ (without STIM1) in the absence (black trace) and presence (blue trace) of 20 μM compound 5D. Each trace is representative of data obtained from 8-10 different cells.

(**E**) Block of currents generated by $Orai1^{V102}$ mutants by compound 5D. Inhibition of currents generated by $Orai1^{V102C}$ (n = 8) and $Orai1^{V102A}$ (n = 4) mutants by 20 μM compound 5D. Data represent average ± s.e.m and are normalized to block of wild-type Orai1.

(**F**) Compound 5D does not block TrpC1 mediated SOCE. HEK293 cells co-expressing TrpC1 and STIM1 were treated with thapsigargin in Ca^{2+}-free medium to deplete intracellular stores. Subsequently 2mM Ca^{2+}-containing solution with and without compound 5D (20 μM) was added to measure SOCE. Data represent average ± s.e.m. of peak SOCE from 20-30 cells.

(G) Compound 5D blocks SOCE mediated by all three Orai proteins. Orai1$^{-/-}$ naïve CD4^{+} T cells were transduced with viral vectors encoding Orai1, Orai2, and Orai3, cultured under non-polarizing conditions for 5 days, and examined for reconstitution of SOCE and inhibition by compound 5D (10 μM). Compound 5D suppressed SOCE induced by Orai proteins (blue trace), as well as the residual SOCE observed in Orai1$^{-/-}$ T cells (black trace). The control trace of SOCE from Orai1$^{-/-}$ T cells is repeated in all the panels. Data represent average ± s.e.m. of peak SOCE from 30-40 cells.

Suppl. Figure 2 Compound 5D inhibits functions of effector T cells

(**A**) Effects of compound 5D treatment in differentiation of regulatory T cells. Naïve T cells differentiated under Treg-polarizing conditions with compound 5D were examined for Foxp3 expression. A representative of three independent experiments is shown.

(**B**) Expression levels of IL-23R, CCR6, and LFA-1 (Integrin αL) from naïve T cells differentiated under T_H17-skewing conditions in the presence or absence of compound 5D. The transcript levels were normalized to those of DMSO-treated cells. Data represent average ± standard deviation from triplicates.

(**C**) Expression levels of various transcription factors involved in T_H17 differentiation. Transcripts were analyzed from naïve T cells differentiated under T_H17-polarizing conditions in the presence or absence of 10 μM of compound 5D. mRNA levels were normalized to that of β-actin. Data represent average ± standard deviation from triplicates.

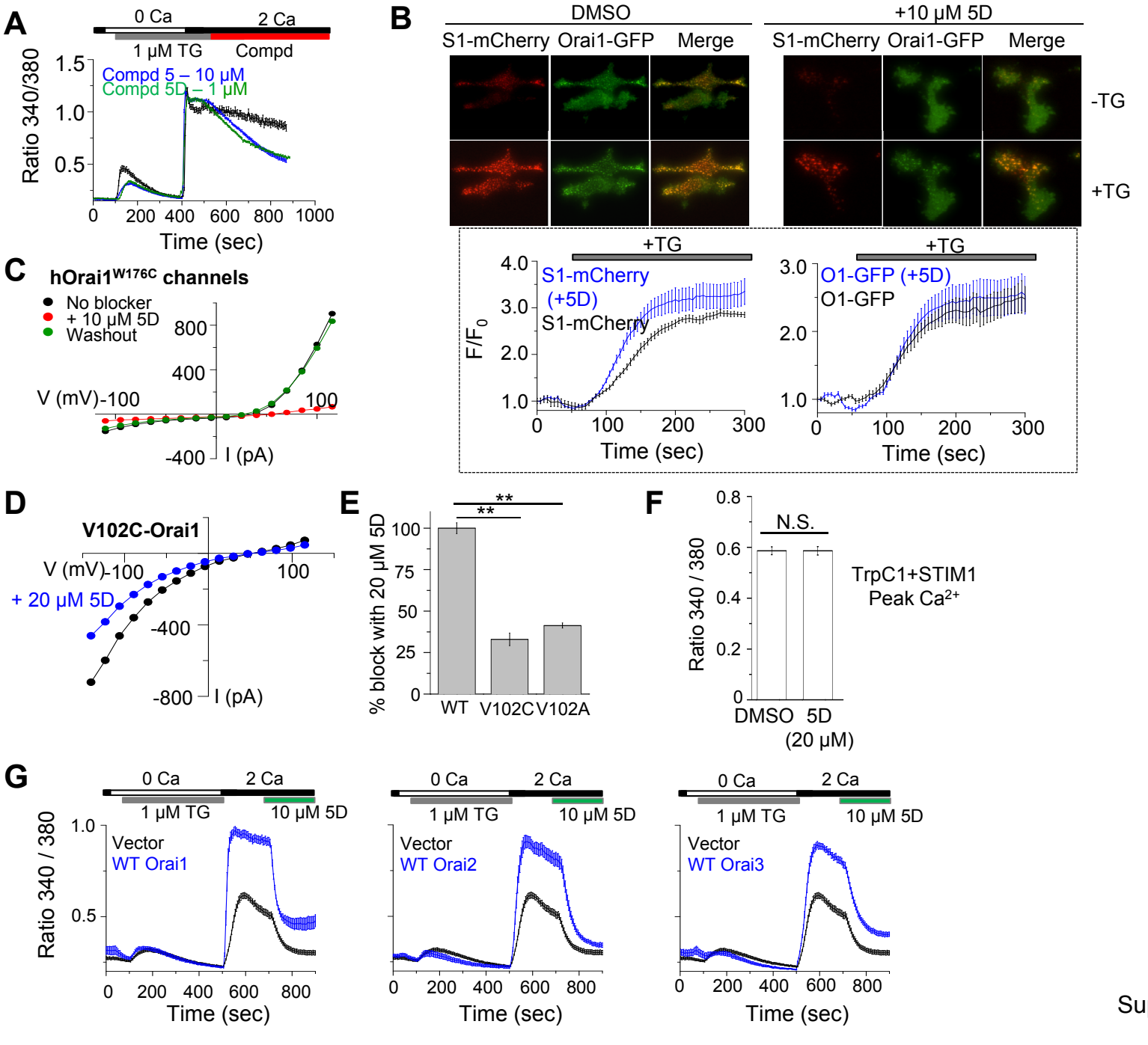
Suppl. Figure 3 Compound 5J-4 reduces EAE symptoms without affecting the survival of mice

(**A**) Survival curve of mice after alternate day intraperitoneal injection with compound 5J-4 (2 mg/kg) or carrier (DMSO).

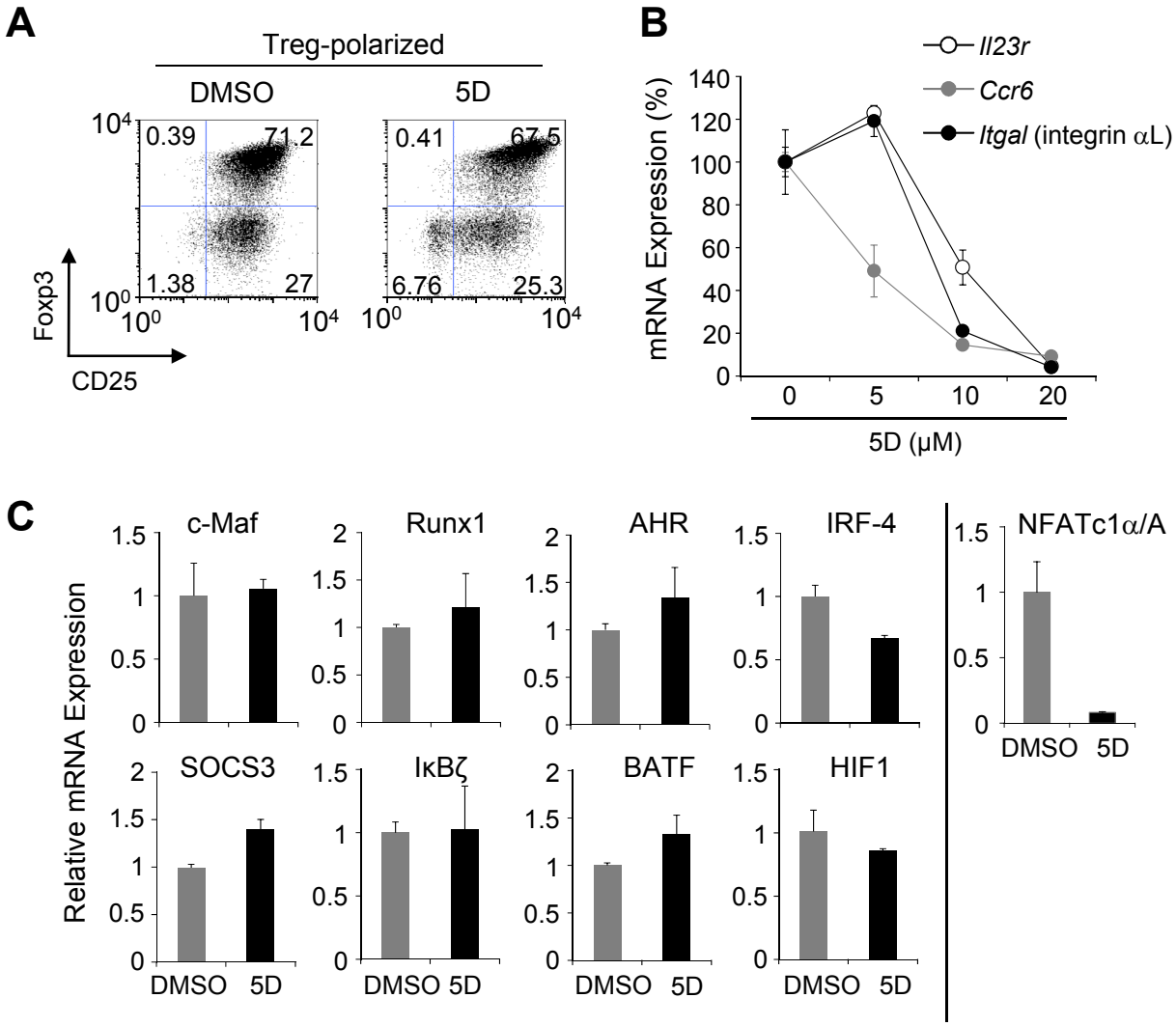
(**B**) Incidence of EAE induction by MOG_{35-55} immunization in animals injected with vehicle or compound 5J-4 (2 mg/kg).

(**C**) Histology of transverse sections of spinal cords from DMSO and compound 5J-4-injected mice. Luxol Fast Blue and H&E staining of spinal cords. Left images are depicted at 4x magnification while those on right show 10x magnification of boxed areas.

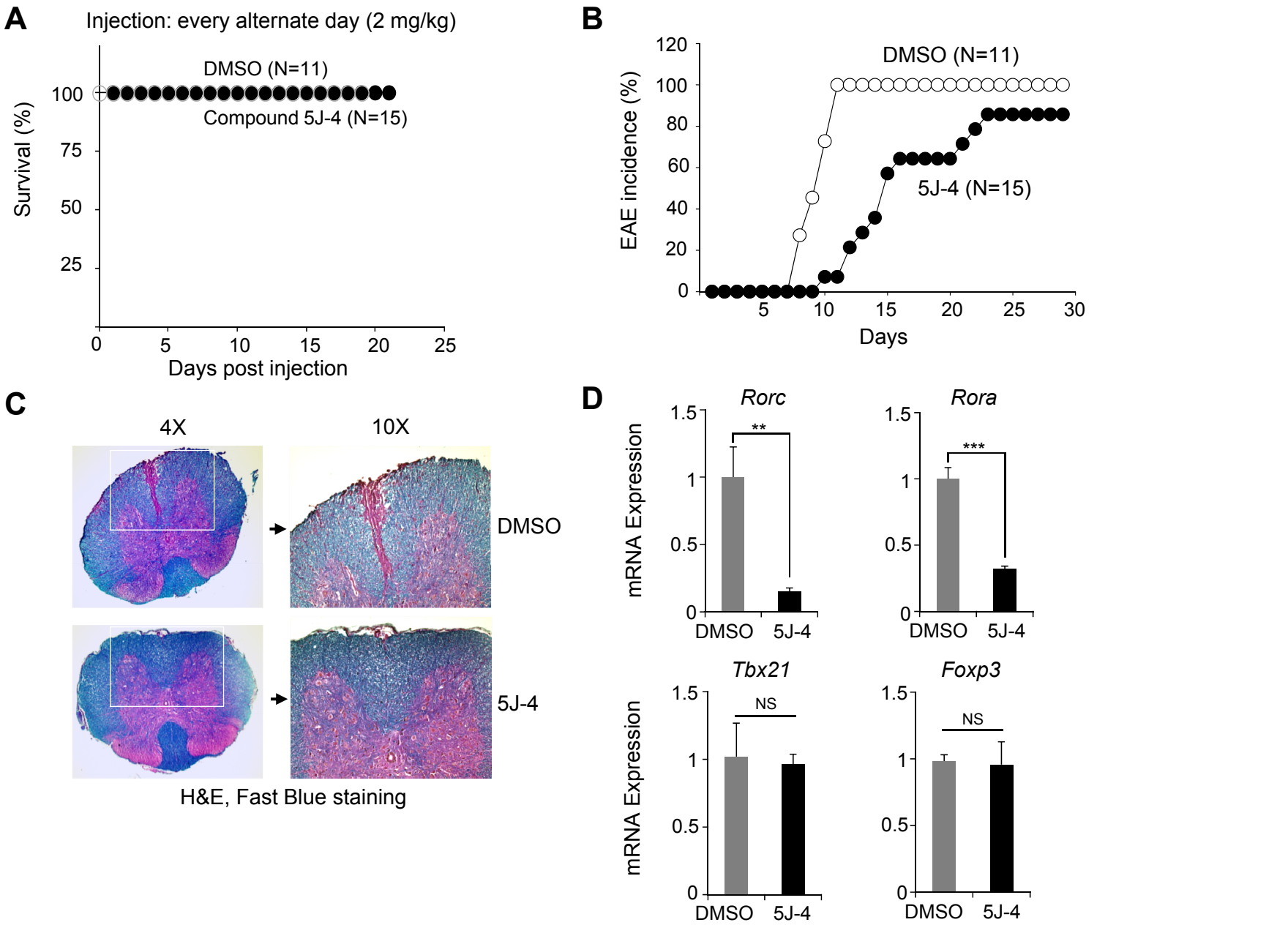
(**D**) Compound 5J-4 treatment decreases inflammatory T cell differentiation in vivo. The transcript levels of transcription factors were measured in cells isolated from the draining lymph nodes of DMSO and compound 5J-4-treated mice after 14 days of immunization with MOG_{35-55} peptide/CFA. The graph shows average ± standard deviation from triplicates.



Suppl Figure 1



Suppl Figure 2



Suppl Figure 3