Supplementary material to article by S-H. Shin et al. "Inhibiting Sphingosine Kinase 2 Derived-sphingosine-1-phosphate Ameliorates Psoriasis-like Skin Disease via Blocking Th17 Differentiation of Naïve CD4+ T Lymphocytes in Mice"

Fig. S3. T-helper 17 (Th17) differentiation of naïve CD4+ T cells was blocked by sphingosine kinase 2 inhibition. For Th17 differentiation, naïve CD4+ T cells isolated from the mouse spleen were cultured in the presence of soluble anti-CD28 (1 μg/ml), rmIL-2 (20 ng/ml), rmTGF-β (5 ng/ml), rmIL-6 (25 ng/ml), and anti-IL-4 and anti-IFN-γ neutralizing antibodies (10 μg/ml) for 5 days. The cells were transferred to a new plate to incubate for 2 days and then were re-stimulated with Dynabeads Mouse T-Activator CD3/CD2 for 40 h. IL-17A levels in the supernatant were measured using an ELISA Kit (BioLegend), according to the manufacturer’s instructions. IL-17A levels secreted from differentiated CD4+ T cells with chemical treatment of (A) Ceranib-1 (30 μM), Ceranib-2 (30 μM), or MP-A08 (15 μM); (B) PF-543 (30 nM) or ABC294690 (26 μM). (C) mRNA expression levels of sphingosine-1-phosphate receptors in murine naïve CD4+ T cells were examined using real-time PCR. IL-17A levels secreted from differentiated CD4+ T cells with chemical treatment of (D) JTE013 (5 μM), (E) CAY10444 (5 μM), and (F) CYM50358 (5 μM). mRNA expression levels of (G) SOCS1 and (H) SOCS3 in differentiated Th17 murine CD4+ T cells. Results are mean ± SEM values (n = 6). **p < 0.01, ***p < 0.001.