Supplemental Data

An Activated Th17-Prone T-cell subset involved in Chronic Graft-*versus*-Host Disease Sensitive to Pharmacological Inhibition

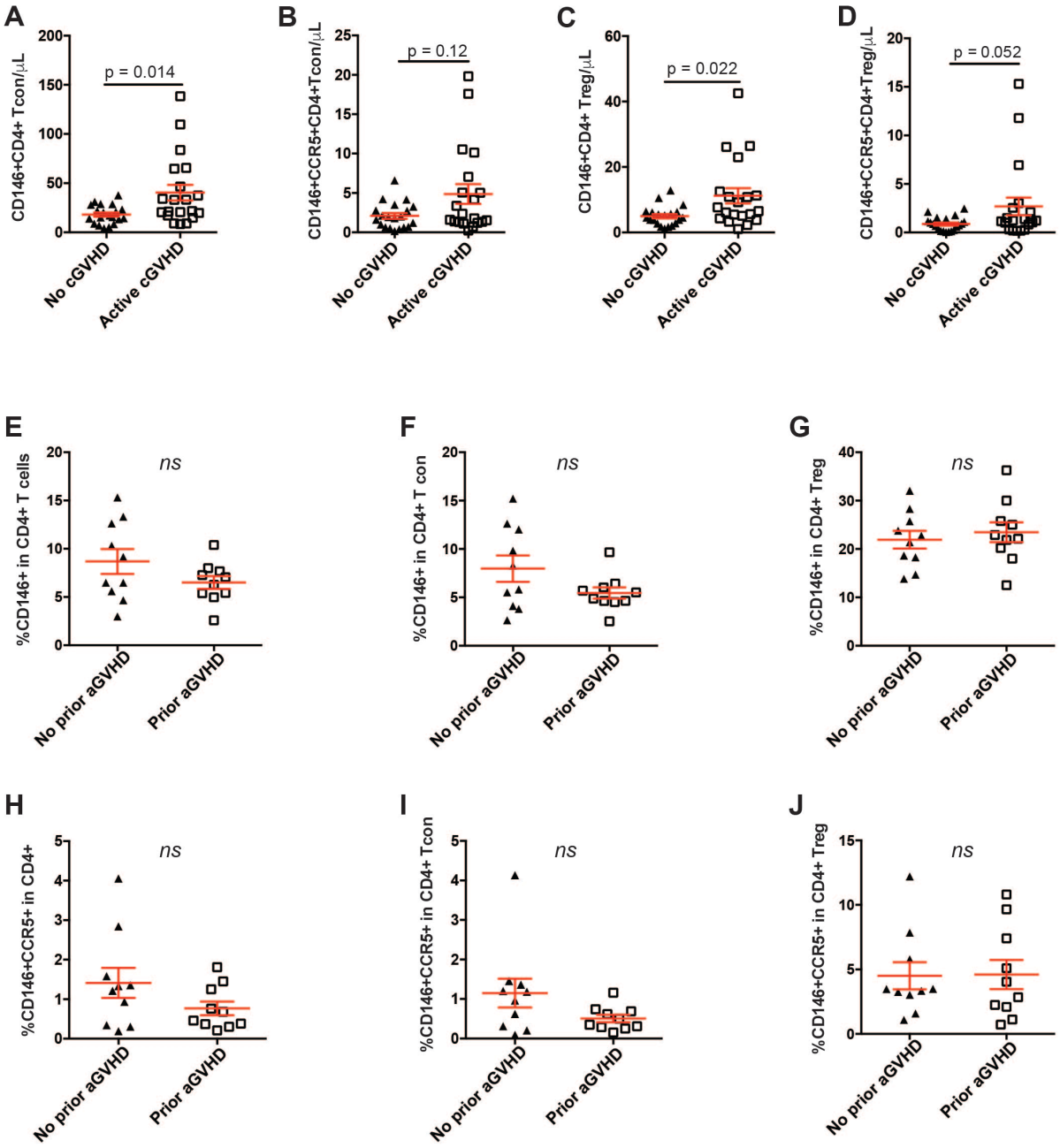
Edouard Forcade, Katelyn Paz, Ryan Flynn, Brad Griesenauer, Tohti Amet, Wei Li, Liangyi Liu, Giorgos Bakoyannis, Di Jiang, Hong Wei Chu, Mercedes Lobera, Jianfei Yang, David S. Wilkes, Jing Du, Kate Gartlan, Geoffrey R. Hill, Kelli P.A. MacDonald, Eduardo L. Espada, Patrick Blanco, Corey S. Cutler, Joseph H. Antin, Robert J. Soiffer, Jerome Ritz, Sophie Paczesny, and Bruce R. Blazar.

Supplemental methods section

Murine CD146 WT vs. CD146 KO Th17 differentiation

T cells were purified from CD146 WT vs. CD146 KO fresh splenocytes. Naïve total T cells were negatively selected using a Pan T cell isolation kit followed by positive selection using CD62L microbeads (both Miltenyi Biotec). Purified naïve total T cells were activated with anti-CD3/CD28 or anti-CD3/ICOS antibody-coated Dynabeads M-450 Tosylactivated (Invitrogen) at a bead/cell ratio of 1:5. Naïve total T cells were then differentiated under Th17 conditions using IL-1β (20 ng/ml), IL-6 (30 ng/ml), IL-23 (30 ng/ml), and TGF-β (2 ng/ml). Exposure to differentiating cytokines (all from R&D Systems) was maintained throughout the 5-day culture period.

Supplemental Figure 1.



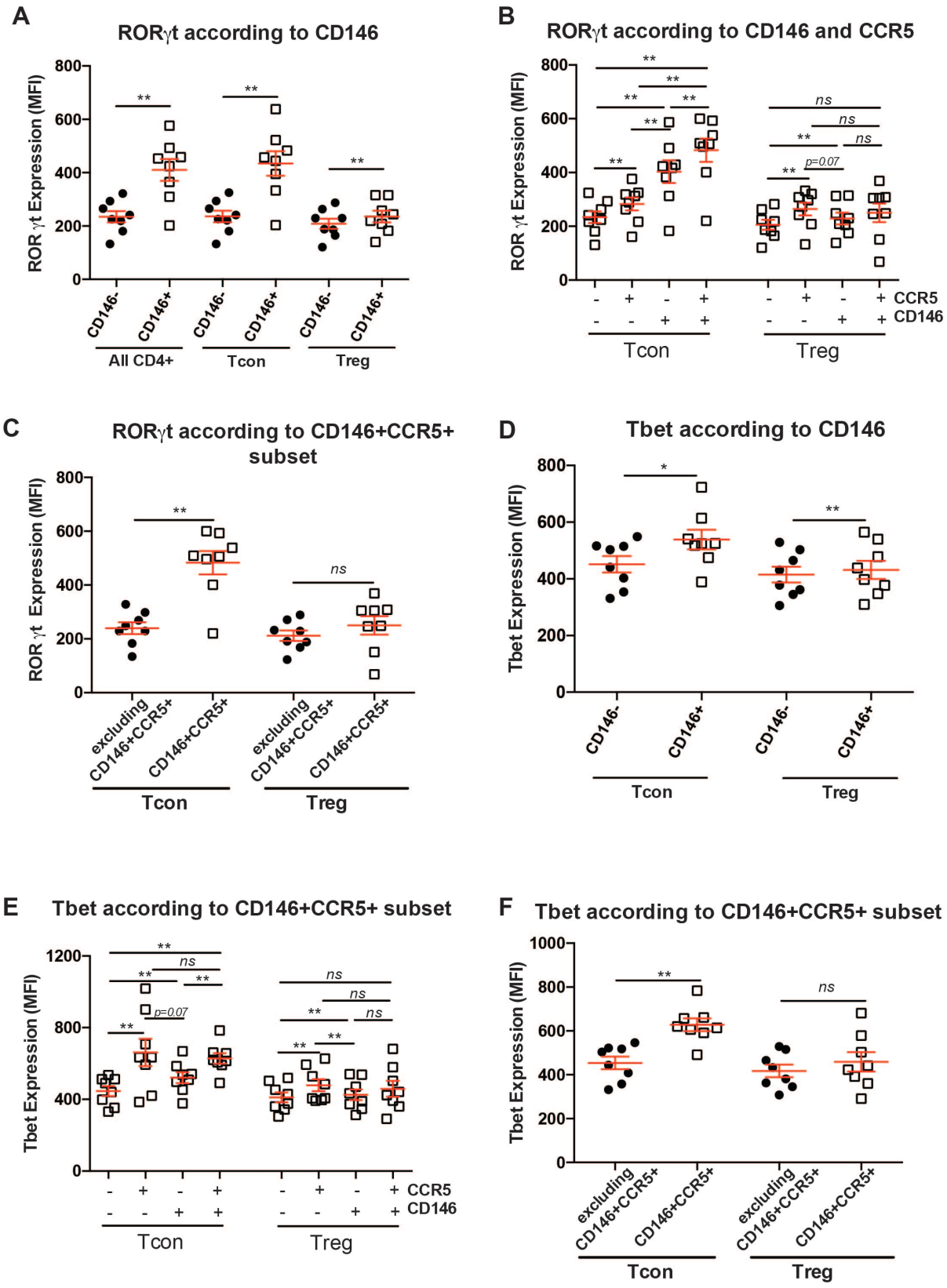
Supplemental Figure 1.

(A-D) Data shown are absolute count (median and range) according to different clinical groups: No (n=20) or Active cGvHD (n=20).

(E-I) Analysis of prior acute GVHD development on CD146 expressing subsets. Within our 20 patients that presented with active cGVHD, we compared 10 patients that

previously developed acute GVHD and 10 patients that did not. Graphs represent frequencies (mean±SEM) of the different subsets according to the prior development of acute GVHD. Unpaired *t test*. *ns* = not significant.

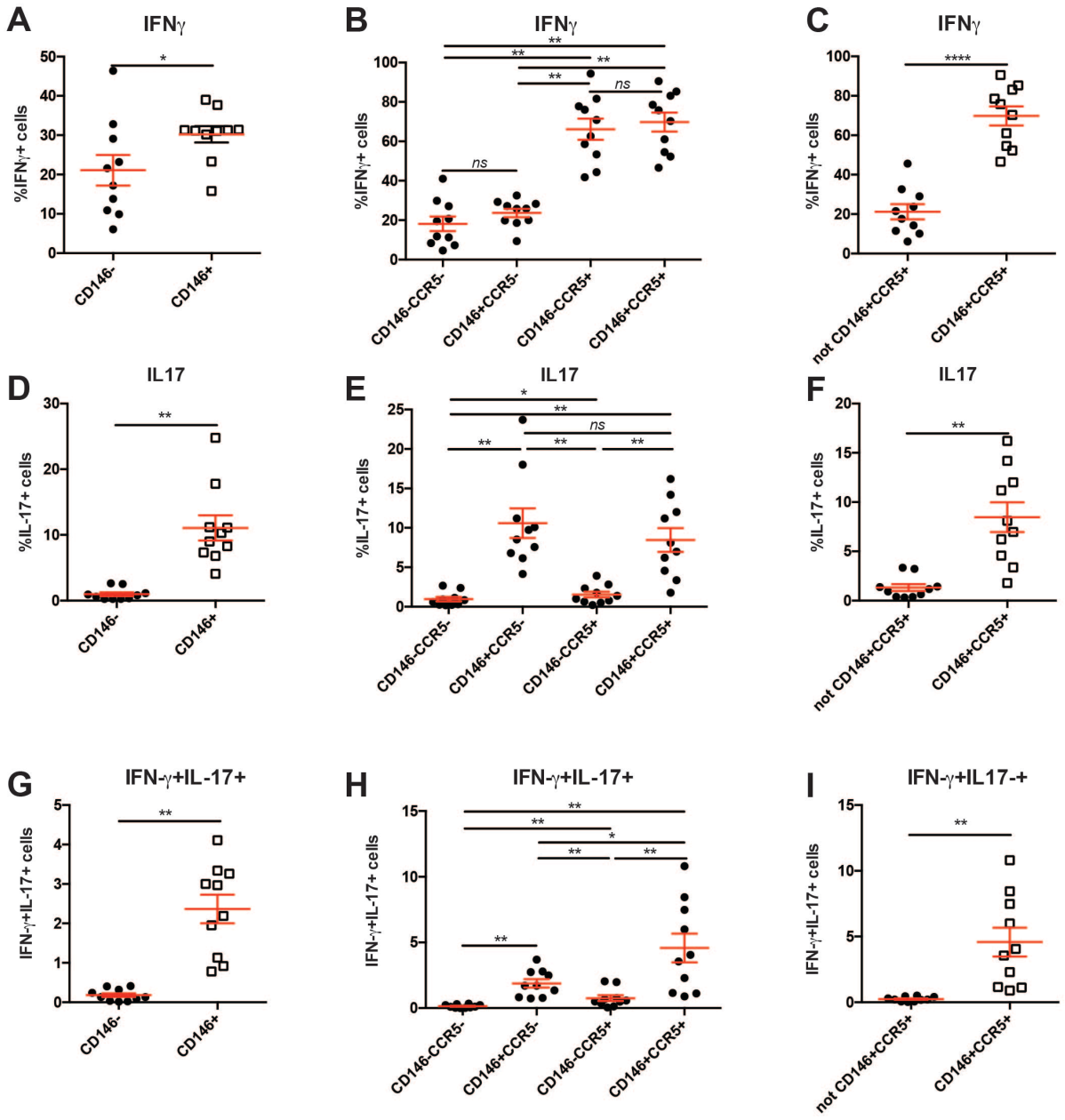
Supplemental Figure 2



Supplemental Figure 2. RORγt and T-bet expression is associated with CD146

expressing subsets. RORγt and T-bet expression (MFI) were evaluated by flow cytometry according to T cell subsets expressing CD146 and/or CCR5 or not, in a set of 8 healthy donors. Tcon: non-$CD25^{hi}CD127^{low}$ within CD4+ T cells, and Treg: $CD25^{hi}FoxP3^{+}$ within CD4+ T cells. Dot plots represent mean±SEM of MFI. Paired *t test*. * *p<0.05*, ** *p<0.01*, *** *p<0.001*, *ns* = not significant.

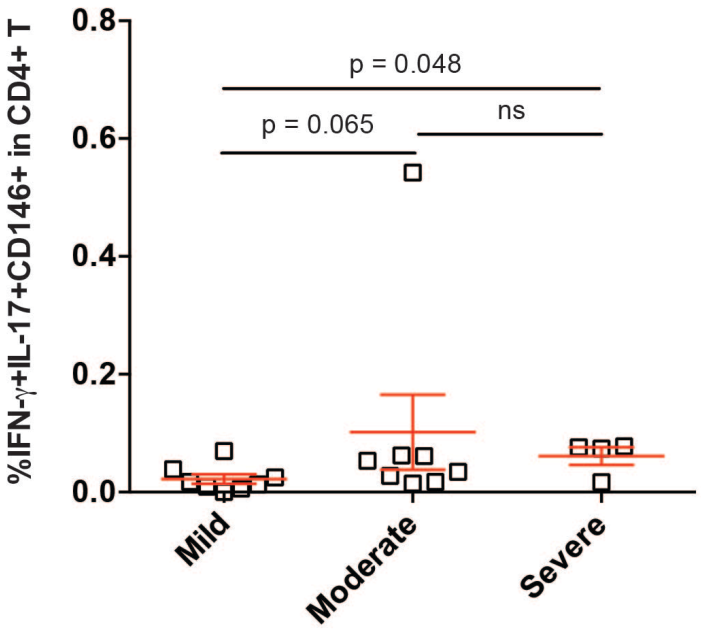
Supplemental Figure 3



Supplemental Figure 3. IFN-γ and IL-17 production is associated with CD146 and CCR5 expression. IFN-γ and IL-17 production (% of positive cells) were evaluated by flow cytometry upon stimulation by PMA ionomycine for 5 hours in presence of Brefeldin A (n=8 healthy donors). Graphs represent cytokine production according to

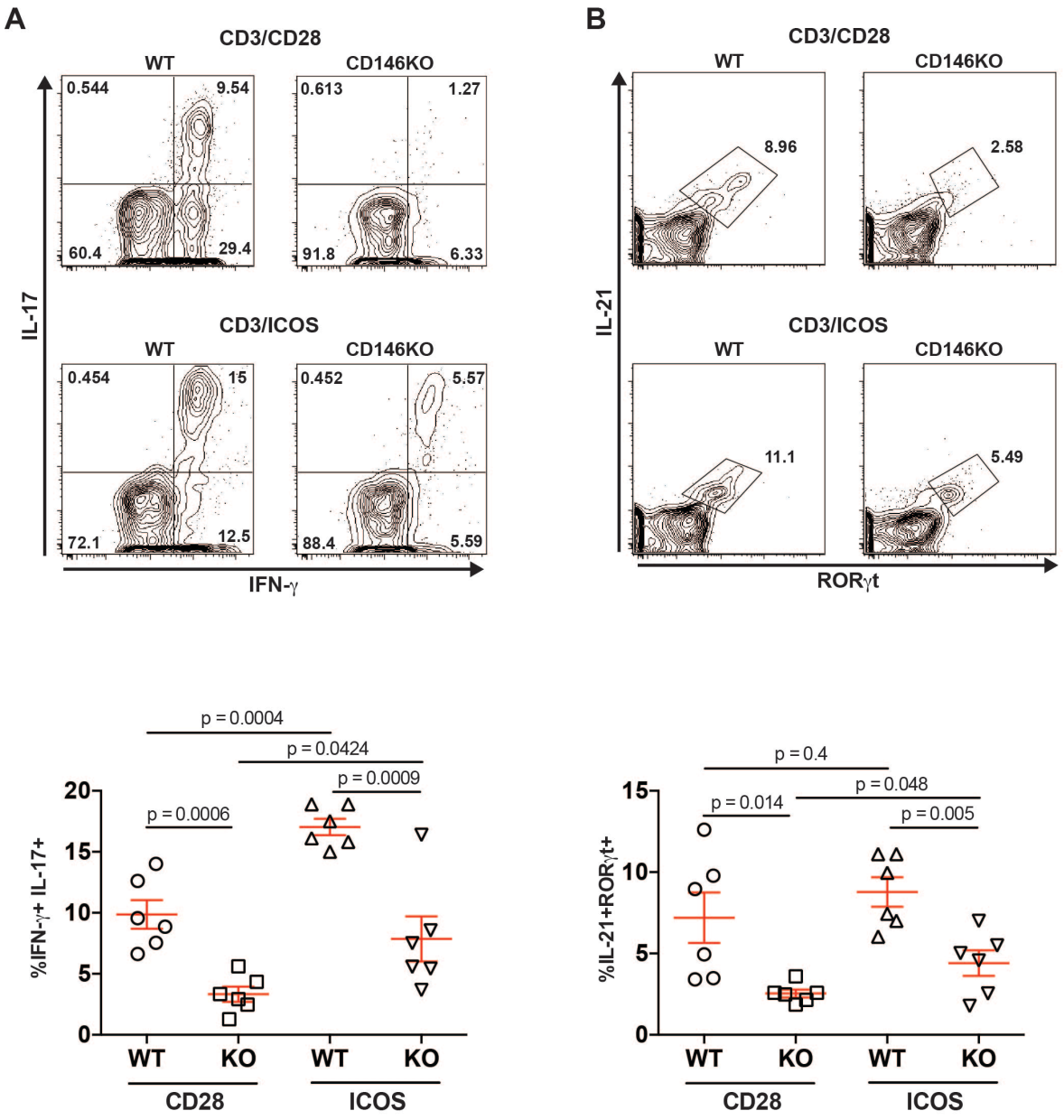
T cell subsets expressing CD146 and/or CCR5 or not, in a set of 8 healthy donors. Data represent mean±SEM. Paired *t-test*. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, *ns* = not significant.

Supplemental Figure 4



Supplemental Figure 4. Frequency of IFNγ+IL-17+CD146+ within CD4+ T cells evaluated by flow cytometry upon stimulation by PMA ionomycine in presence of Brefeldin A. Data represent mean and SEM according to clinical groups. Unpaired *t-test*.

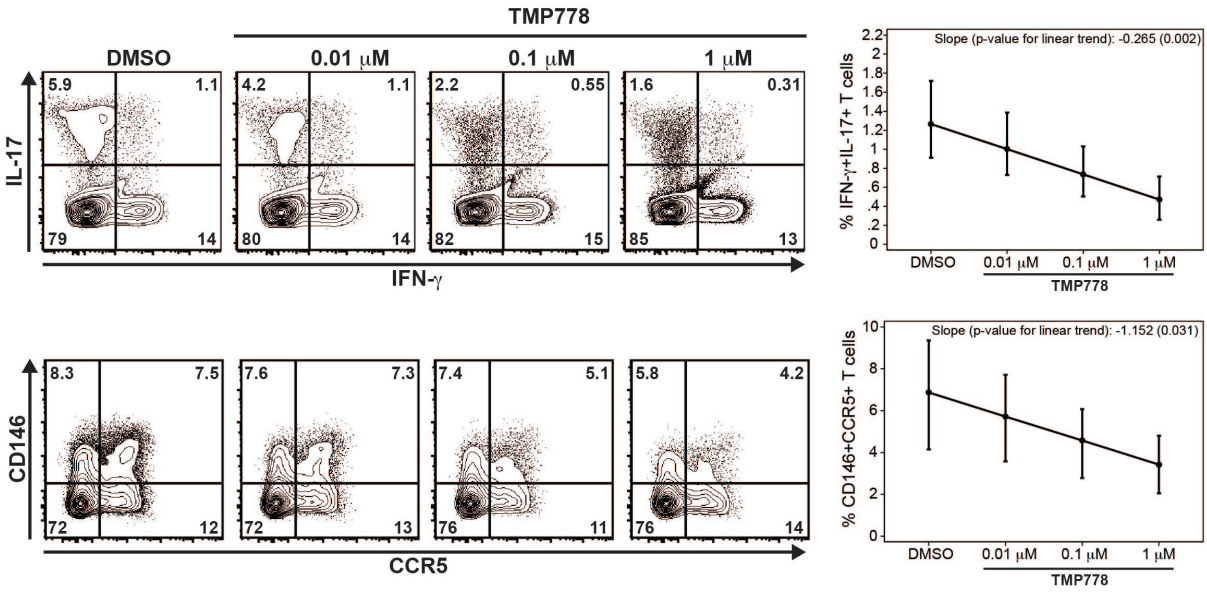
Supplemental Figure 5



Supplemental Figure 5. Th17 differentiation of naïve T cells from murine CD146 WT or CD146 KO after anti-CD3/CD28 or anti-CD3/ICOS stimulation as described in Materials and Methods. Representative plots showing IL-17 and IFN-γ coexpression or IL-21 and RORγt coexpression in live CD4 T cells and dot plots depicting mean±SEM values for frequency of IFN-γ+ IL-17+ T cells or IL-21+ RORγt+ T cells, n

= 6. Unpaired *t-test*.

Supplemental Figure 6



Supplemental Figure 6. Effect of RORγt inhibitor TMP778 on generation of Th1/Th17 cells and CD146+CCR5+CD4 T cells from human mixed lymphocyte reactions (MLRs). MLRs were carried out in the presence of indicated concentrations of TMP778 and vehicle control DMSO for 8-10 days. (**A**) (Top panels) Representative plots of IL-17 and IFN-γ expression depicting mean±SEM values for frequency of IFN-γ+IL-17+ T cells (n=7). (**B**) (Bottom panels) Representative plots of CD146 and CCR5 expression depicting mean±SEM values for frequency of CD146+CCR5+ T cells (n=5). Paired *t-test*.