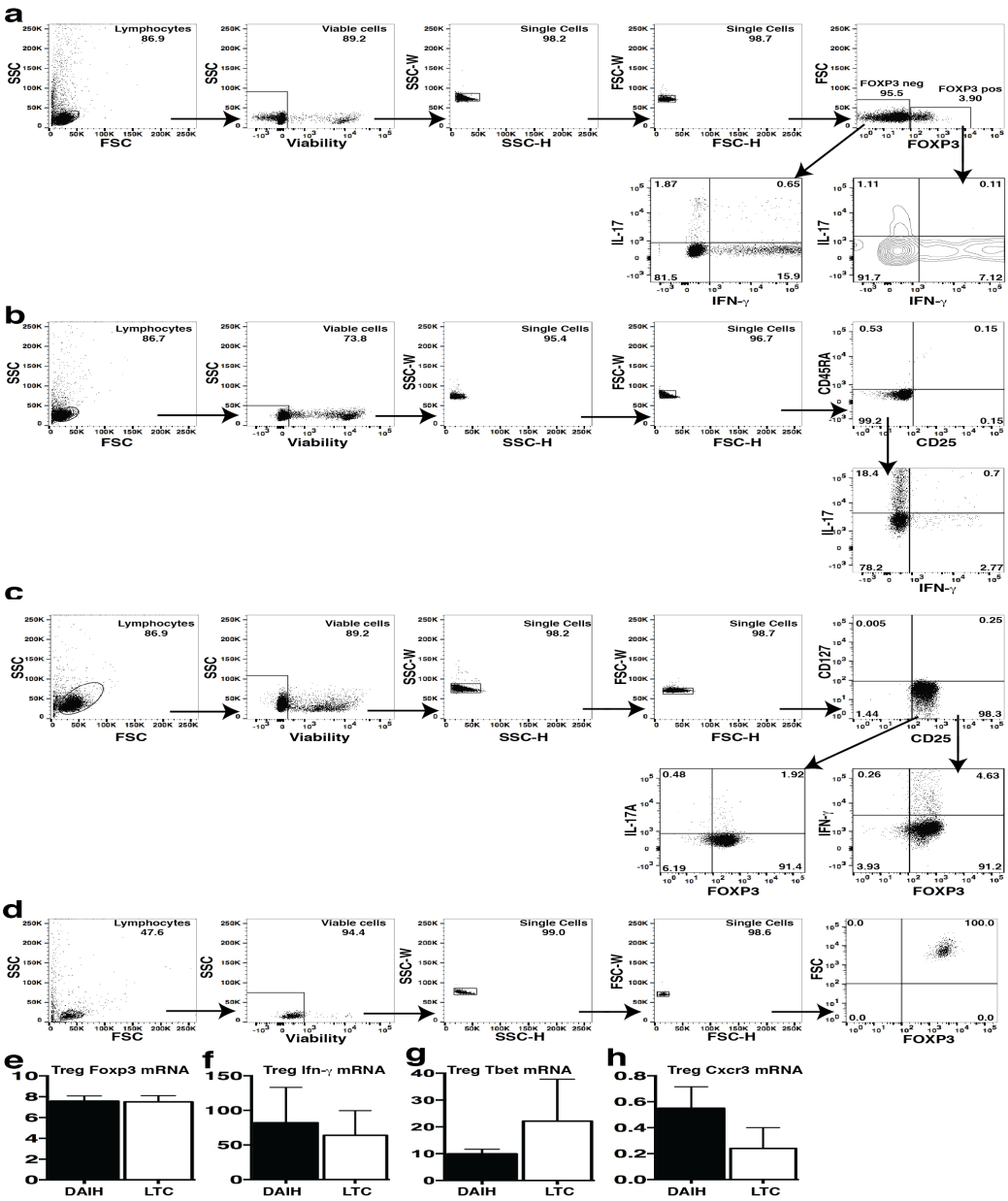
Probe	Catalog Number
IL17A	Hs00936345_m1
FOXP3	Hs01085834_m1
Tbet	Hs00203436_m1
Rorc	Hs01076112_m1
Gata3	Hs00231122_m1
Rorα	Hs00536545_m1
Tgfβ	Hs00998133_m1
PD1	Hs00169472_m1
PDL1	Hs00204257_m1
Tim3	Hs00958618_m1
Cccr5	Hs00152917_m1
Cxcr3	Hs00171041_m1
IL4	Hs00929862_m1
IL5	Hs99999031_m1
IL10	Hs00961622_m1
IL13	Hs00174379_m1
Lag3	Hs00158563_m1
Klf5	Hs00156145_m1
IL23R	Hs00332759_m1
β2m	Hs00152917_m1

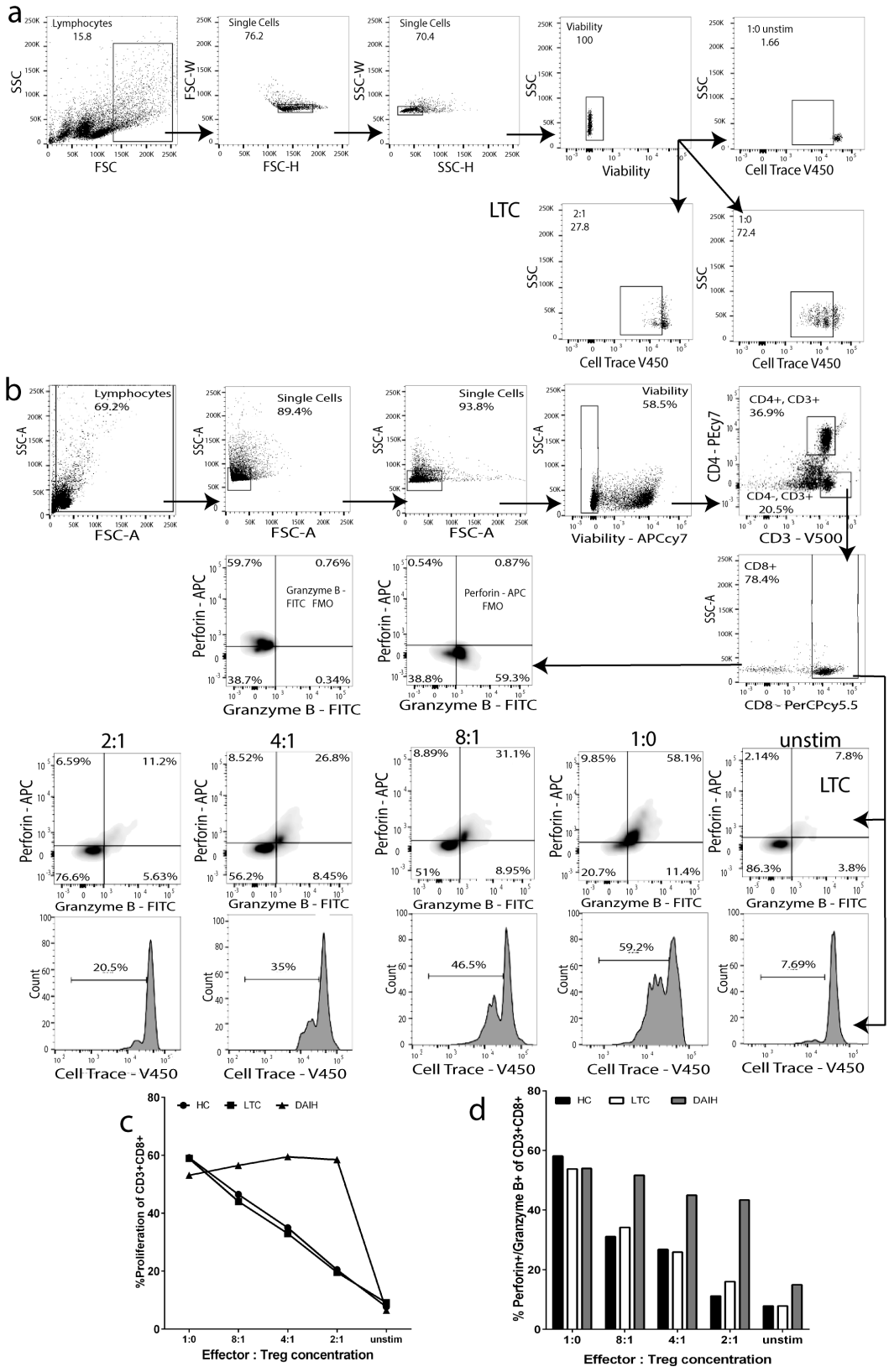
SUPPLEMENTAL FIGURE 1: TaqMan Probes used for qRT-PCR.

Arterbery et al., Supplemental Figure 2a-h



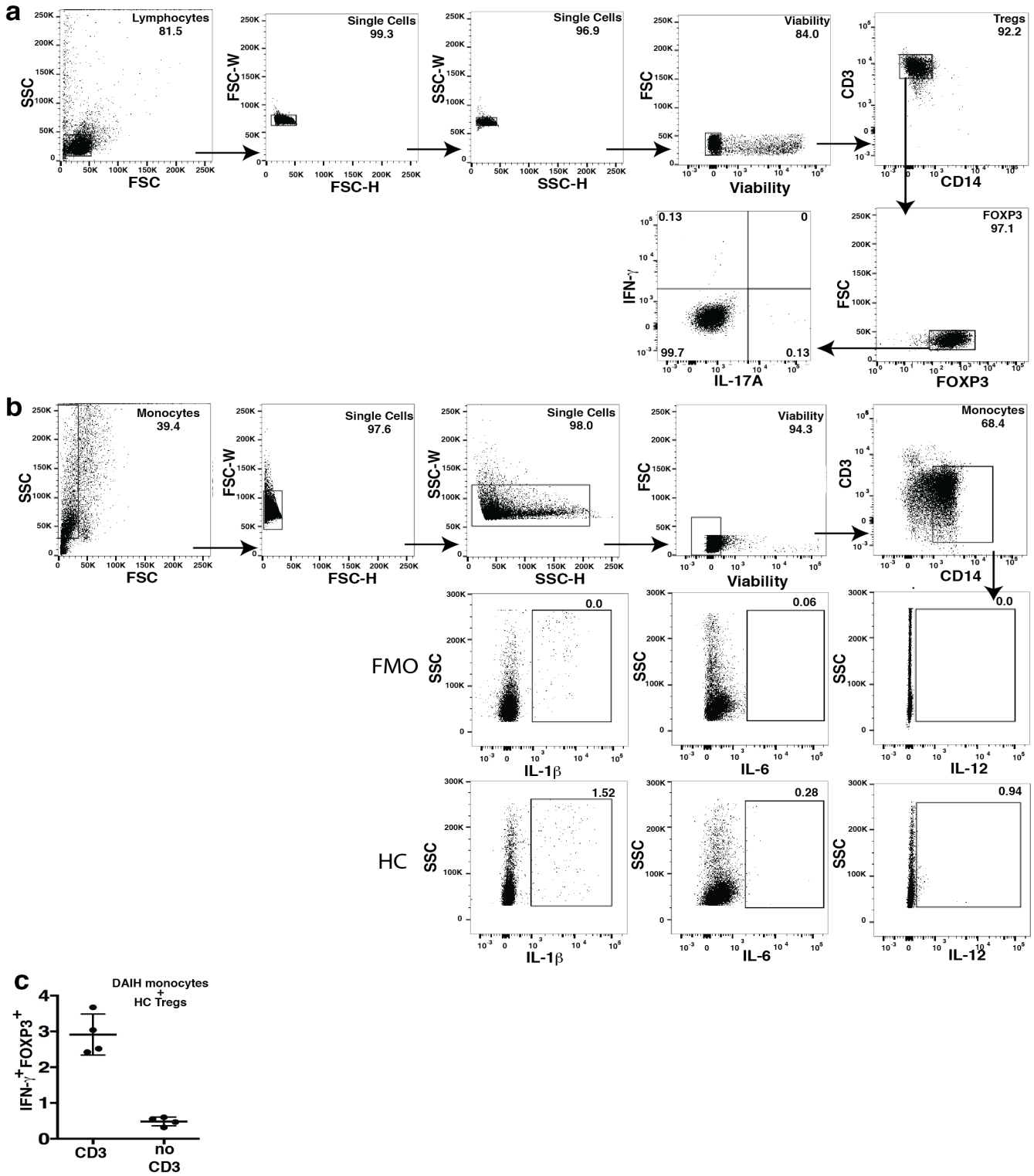
SUPPLEMENTARY FIGURE 2A-H: Gating strategy for sorted T cell subsets. FOXP3 purity. mRNA expression of FOXP3 of Tregs. Total CD4+ T cells were isolated by negative selection and stained for fluorescence-activated cell sorting (FACS) with the following antibodies: anti-CD45RA, CD25, and CD127. The Treg (CD4+CD25hiCD127low/neg), Tmemory (CD4+CD25low/negCD45RA-) and Tnaive (CD4+CD25low/negCD45RA+) populations were sorted on a FACS Aria and stimulated with phobol-12-myristate-13-acetate (PMA) and ionomycin for 4-hours and intracellular staining of IFN-γ, IL-17A and FOXP3 was performed. A small fraction of the sorted Tregs was used for purity analysis and intracellular staining for FOXP3 was performed. RNA was isolated from the stimulated T cell populations and subjected to quantification of mRNA expression levels by RT-PCR. A) Forward and side scatter identified lymphocyte population; viable cells were gated on, single cells were next gated on using FSC-W vs. FSC-H and SSC-W vs. SSC-H, next FOXP3+ and FOXP3- cells were gated on and of these, IFN-γ vs. IL-17 secretion identified. Gate placement determined using unstimulated sample. B) Forward and side scatter identified lymphocyte population; viable cells were gated on, single cells were next gated on using FSC-W vs. FSC-H and SSC-W vs. SSC-H, next CD45RA- and CD25- cells were gated on and of these, IFN-γ vs. IL-17 secretion identified. Gate placement determined using unstimulated sample. C) Forward and side scatter identified lymphocyte population; viable cells were gated on, single cells were next gated on using FSC-W vs. FSC-H and SSC-W vs. SSC-H, next CD25 hi and CD127 lo cells were gated on and of these, FOXP3+ cells secreting IFN-γ/IL-17 were identified. Gate placement determined using unstimulated sample. D) Forward and side scatter identified lymphocyte population; viable cells were gated on, single cells were next gated on using FSC-W vs. FSC-H and SSC-W vs. SSC-H, and of these, CD25 hi and FOXP3+ cells were identified. E) Similar FOXP3 mRNA expression in Tregs isolated from subjects with de novo autoimmune hepatitis (dAIH) (n=4) compared to liver transplanted subjects without de novo autoimmune hepatitis (LTC) (n=4). F) IFN-γ mRNA expression not significantly different in Tregs isolated from subjects with dAIH (n=9) vs. LTC (n=9). G) TBET mRNA expression not significantly different in Tregs isolated from subjects with dAIH (n=9) vs. LTC (n=9). H) CXCR3 mRNA expression not significantly different in Tregs isolated from subjects with dAIH (n=4) vs. LTC (n=5).

Arterbery et al., Supplemental Figure 3a-d



SUPPLEMENTARY FIGURE 3: Gating strategy for suppressor assay. Sorted Tregs were co-cultured with CFSE-labeled effector (responder) CD4+CD25-CD127+ T cells at 1:1, 2:1, 4:1, 8:1 Tresponder:Treg ratio and proliferation of viable responder T cells was analyzed on a LSR II flow cytometer. For the CD8 T cell cytolytic function assay, Tregs were co-cultured with Cell Trace V450-labeled total PBMCs at a ratio of 1:2, 1:4, and 1:8. A-B) Forward and side scatter identified lymphocyte population; single cells were next gated on using FSC-W vs. FSC-H and SSC-W vs. SSC-H, viable cells were gated on, and of these, CFSE vs. SSC-A identified. Gate placement determined using unstimulated sample. For perforin/granzyme B, gate placement determined using FMO. C-D) Tregs from a subject with de novo autoimmune hepatitis suppress CD8 T cell proliferation less efficiently compared to sorted Tregs from a healthy non-transplanted subject and a liver transplanted subject without de novo autoimmune hepatitis (LTC). They similarly suppress perforin and granzyme B secretion from CD8 T cells less efficiently compared to sorted Tregs from a healthy non-transplanted subject and liver transplanted subject without de novo autoimmune hepatitis (LTC).

Arterbery et al., Supplemental Figure 4a-c



SUPPLEMENTARY FIGURE 4: Gating strategy for monocyte/Treg co-culture experiments. CD14+ monocytes from healthy non-transplanted subjects (n=6), liver transplanted subjects without de novo autoimmune hepatitis (n=5) and subjects with de novo autoimmune hepatitis (n=5) were co-cultured with sorted Tregs from healthy non-transplanted subjects in the presence of plate bound anti-CD3 for 5-days and IL-17A and IFN-γ secretion from FOXP3+ Tregs was assessed using flow cytometry. Gating strategy for stimulated monocyte experiment. Monocytes from the above three groups were also stimulated with LPS for 24-hours and stained with anti-CD3, anti-CD14, intracellular cytokines IL-12, IL-6 and IL-1β. Cytokine secretion analyzed using flow cytometry. A) Forward and side scatter identified lymphocyte population; single cells were next gated on using FSC-W vs. FSC-H and SSC-W vs. SSC-H, viable cells were gated on, and of these, CD3 vs. CD14, CD14-CD3+ cells were gated on, and of these, FOXP3+ cells were gated on, and of these, IFN-γ vs. IL-17. Gate placement determined using stimulated healthy control Tregs alone sample. B) Forward and side scatter identified monocyte population; single cells were next gated on using FSC-W vs. FSC-H and SSC-W vs. SSC-H, viable cells were gated on, and of these, CD14+ and CD3- cells were gated upon; of these, SSC-A vs IL-12, IL-6 and IL-1β identified. Gate placement for cytokines determined by FMO. C) Absence of cytokine production in the absence of plate bound anti-CD3 confirming that cytokine production from FOXP3+ Tregs in the co-culture experiments with monocytes is not a result of allogeneity.