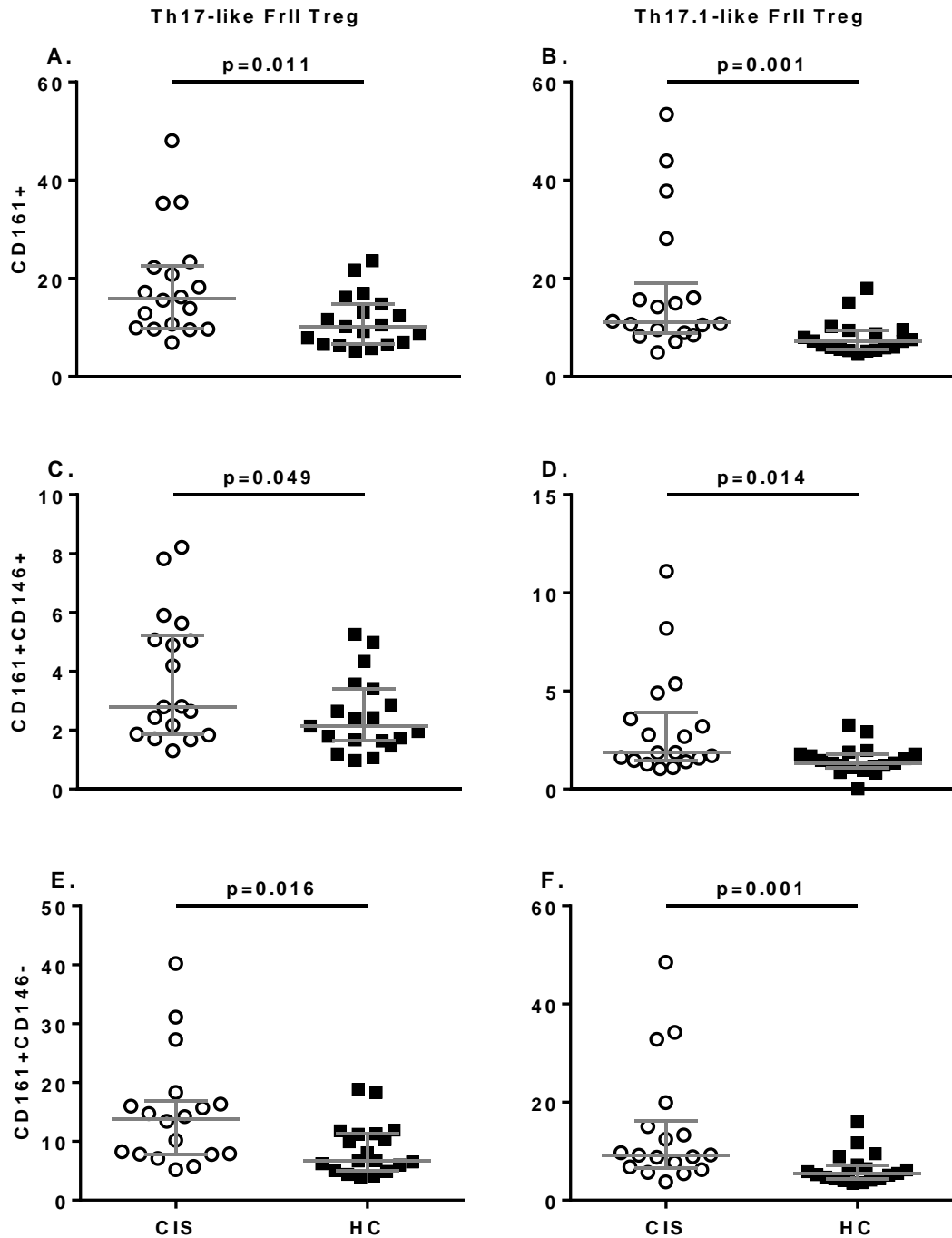
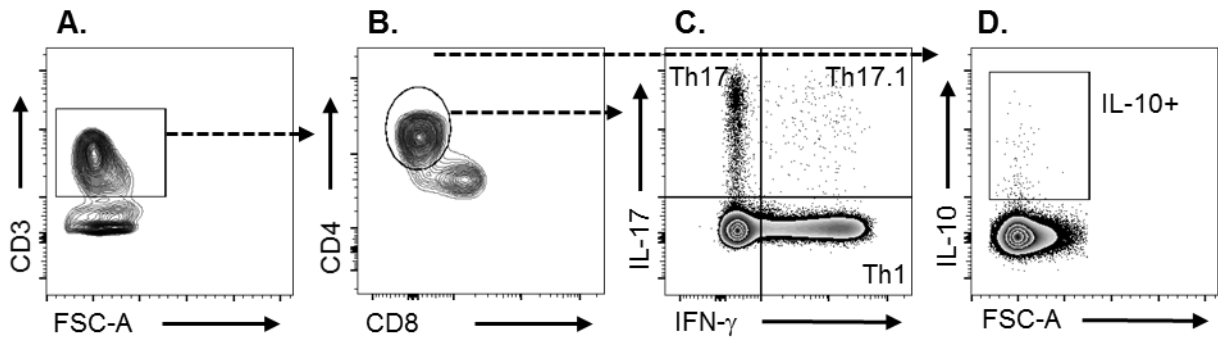


Supplementary Figure 1. Confirmation of Treg fraction functional characteristics based on surface and intracellular marker expression in PBMC samples from 19 healthy controls. The predominantly CD25+CD127lo (A) and Helios+ (B) phenotype of FcRI, together with a low percentage of CD161+ Th17-like Treg (C), is supportive of the “resting” designation given to these cells. FcRII contained the greatest proportion of CD25+CD127lo and Helios+ Treg, consistent with “activated” Treg possessing suppressive functionality. FcRIII, previously described as “cytokine-producing” non-Treg, had the lowest proportion of cells of a suppressive phenotype (CD25+CD127lo and Helios+), and the greatest frequency of CD161+ Th17-like Treg. Box-and-whisker plots are mean, IQR, and minimum and maximum values.



Supplementary Figure 2. Proinflammatory Th17- and Th17.1-like FrII Treg are present at greater frequency in CIS. The CIS group had a greater proportion of CD161+ Th17-like (A) and Th17.1-like (B) FrII Treg, including CD161+CD146- single-positive cells (E and F), indicating IL-17 production potential. The proportion of cells with a CD161+CD146+ double positive phenotype, associated with IL-17 and

GM-CSF production, was also greater in the CIS group (**C** and **D**). Data are median (IQR) for n=18 individuals with CIS (circles) and n=19 HC (squares).



Supplementary Figure 3. Representative intracellular cytokine staining gating plots. Following dead cell and doublet exclusion, PBMCs were gated to identify CD3+CD4+CD8- T cells (**A** and **B**), which were subsequently analysed to obtain the percentage of Th1 (IFN- γ +IL-17-), Th17 (IFN- γ -IL-17+), and Th17.1 (IFN- γ +IL-17+) (**C**) or IL-10+ cells (**D**).

Supplementary Table 1. Regulatory cell phenotype and Helios expression correlates with the frequency of Tfh EM cells and CD27-IgD- double-negative (DNB) cells

	Tfh1 EM	Tfh2 EM	Tfh17 EM	Tfh17.1 EM	DNB
% Treg					
Frl	-0.421*	-0.467*	-0.388†	-0.395†	-0.112
% Tfr					
Frl	-0.582**	-0.632**	-0.539**	-0.560**	-0.137
FrlII	0.599**	0.644**	0.576**	0.556**	0.256
Helios MFI - Treg					
Frl	-0.594**	-0.578**	-0.562**	-0.594**	-0.393†
FrlII	-0.621**	-0.628**	-0.626**	-0.645**	-0.349†
FrlIII	-0.629**	-0.637**	-0.619**	-0.638**	-0.426*
Helios MFI - Tfr					
Frl	-0.547**	-0.522**	-0.514**	-0.540**	-0.364†
FrlII	-0.540**	-0.538**	-0.570**	-0.589**	-0.290
FrlIII	-0.575**	-0.570**	-0.568**	-0.588**	-0.411†
Data are Pearson's correlation coefficient for n=37 (Tfh cells: n=18 CIS and n=19 HC) or n=36 individuals (DNB cells: n=18 CIS and n=18 HC). **p≤0.001, *(p<0.01), † (p<0.05)					