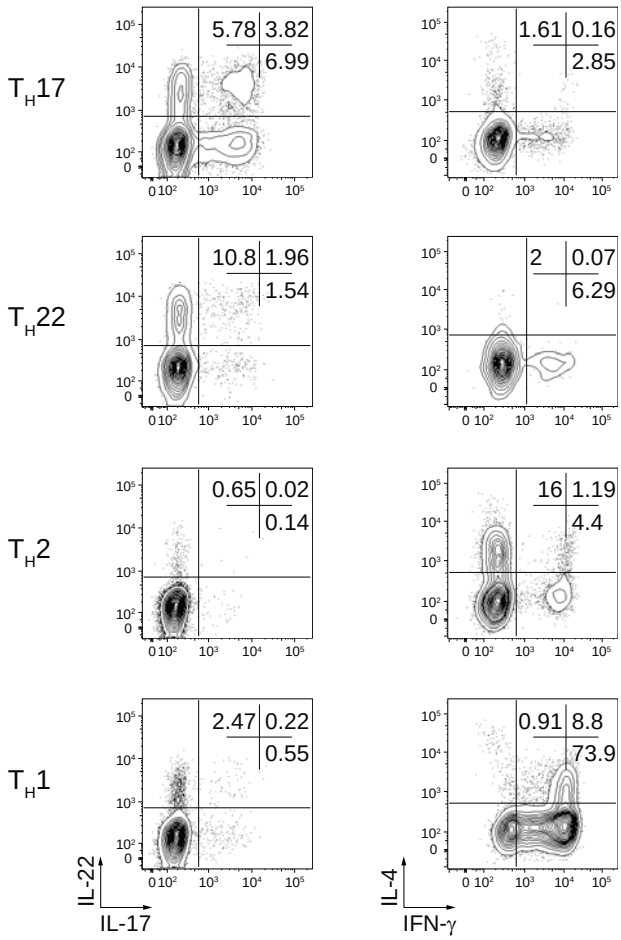
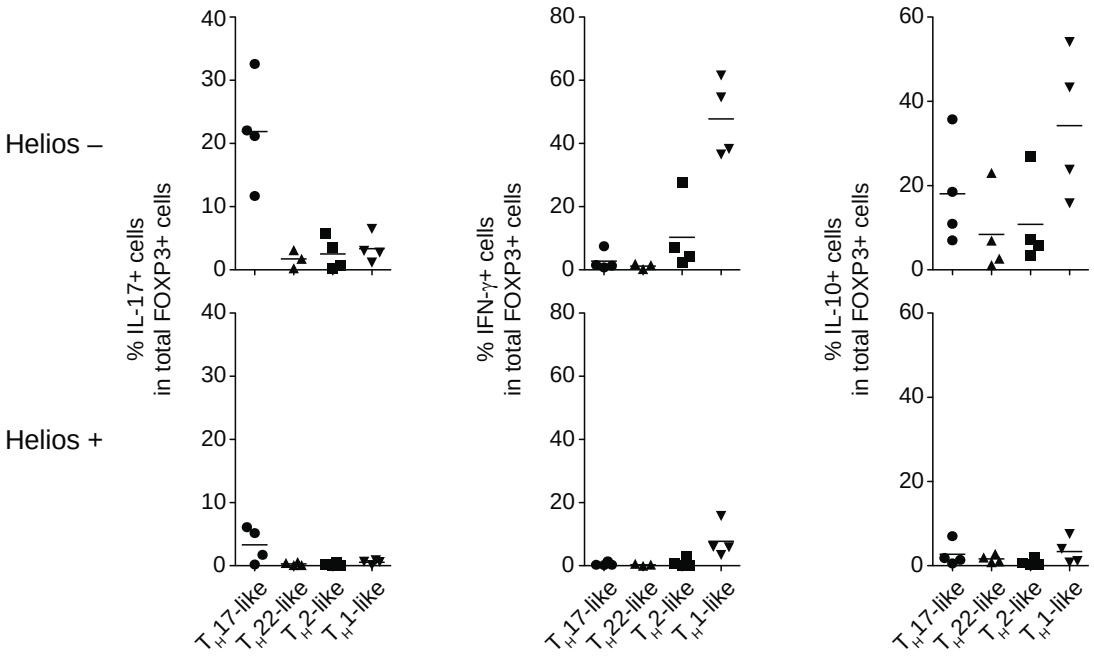


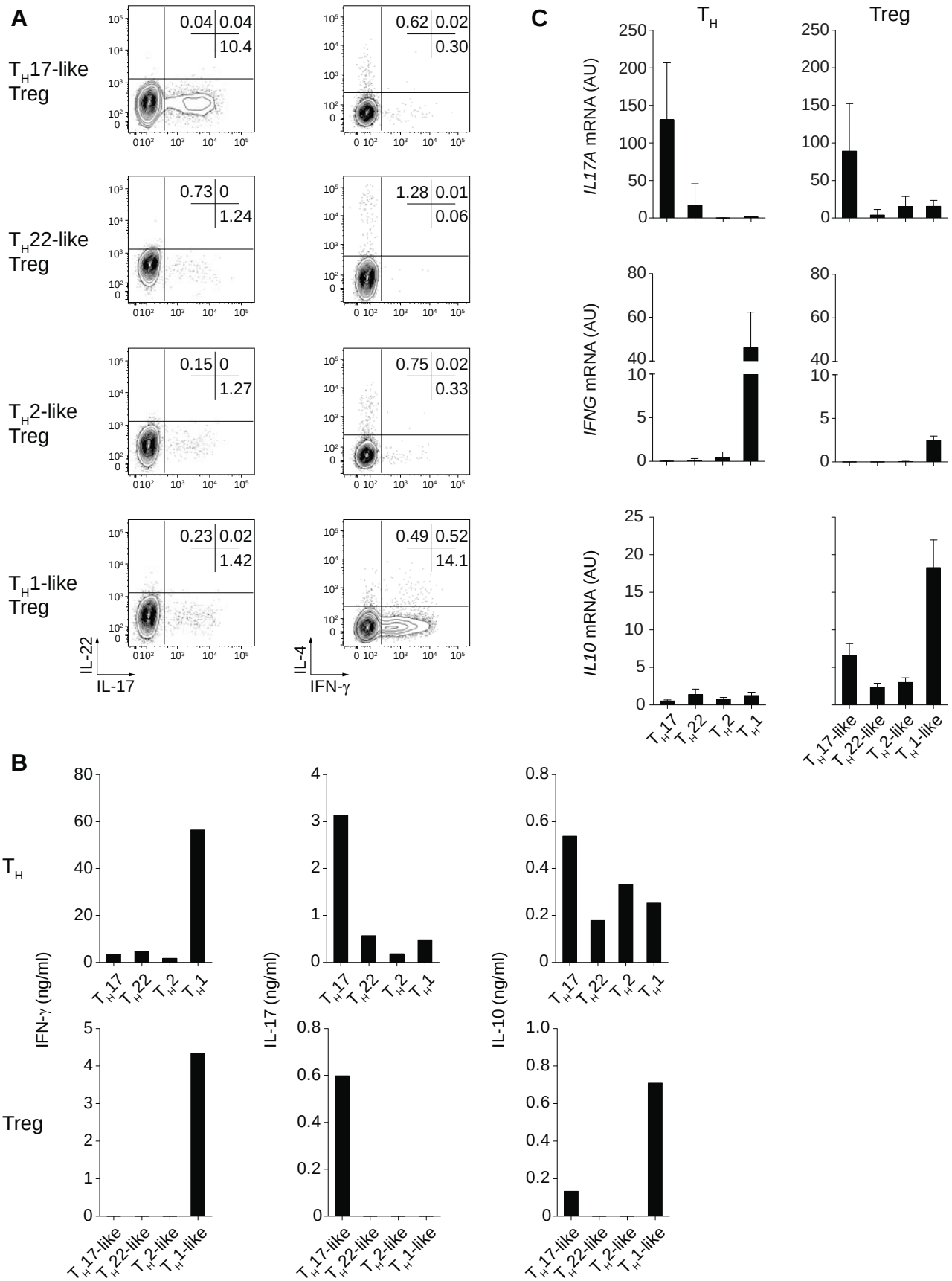
Supplemental Figure 1. Cytokine production by T_H subsets.

Flow cytometric analysis of cytokine production by the indicated T_H cell subsets stimulated for 5h with PMA/ionomycin. Data are representative of six independent experiments.



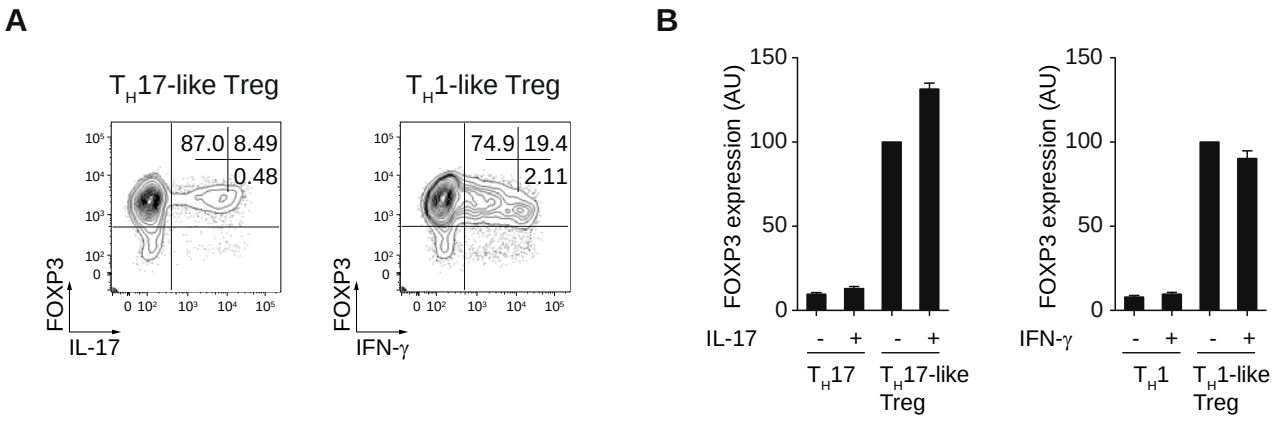
Supplemental Figure 2. IL-17-, IFN-γ- and IL-10-producing Treg cells are predominantly Helios^{-}.

Frequency of IL-17-, IFN-γ- and IL-10-producing cells among gated FOXP3^{+}Helios$^{+/-}$ cells from the indicated Treg cell subsets stimulated for 5h with PMA/ionomycin. Each symbol represents one donor; horizontal bars indicate the mean. Data are from four donors.


A
TH17-like Treg
TH22-like Treg
TH2-like Treg
TH1-like Treg
0.04 0.04 10.4
0.62 0.02 0.30
0.73 0 1.24
1.28 0.01 0.06
0.15 0 1.27
0.75 0.02 0.33
0.23 0.02 1.42
0.49 0.52 14.1
IL-22
IL-17
IL-4
IFN-γ
C
TH
Treg
IL17A mRNA (AU)
IFNG mRNA (AU)
IL10 mRNA (AU)
TH17
TH22
TH2
TH1
TH17-like
TH22-like
TH2-like
TH1-like
B
TH
Treg
IFN-γ (ng/ml)
IL-17 (ng/ml)
IL-10 (ng/ml)

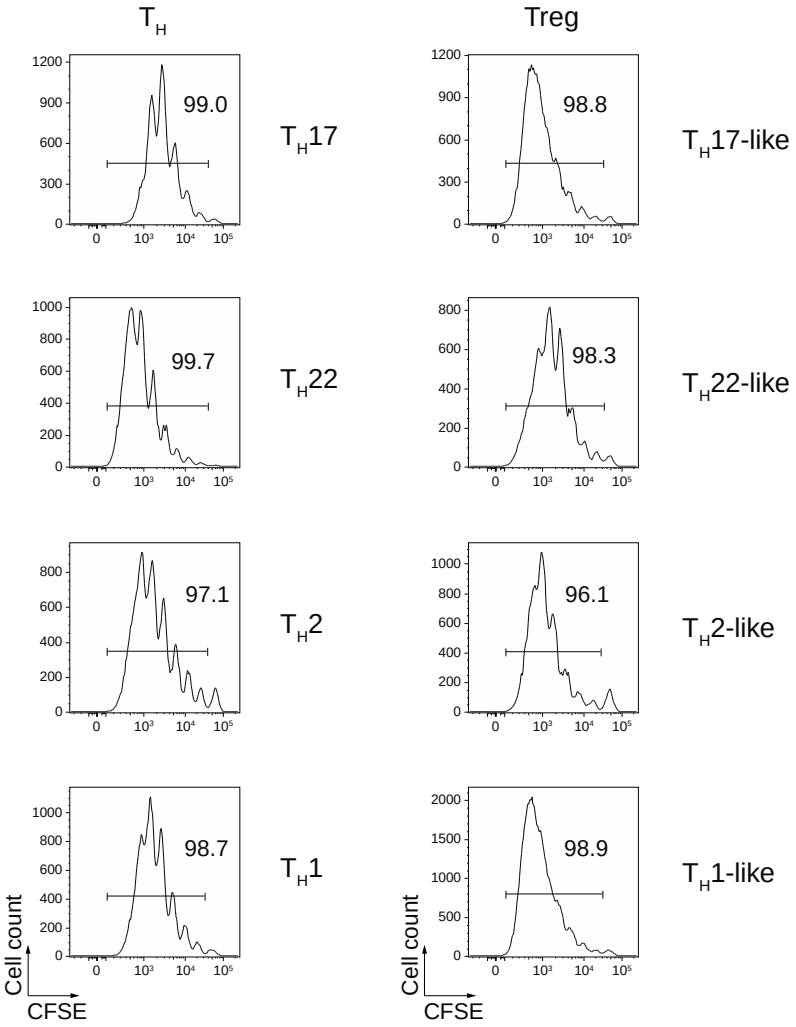
Supplemental Figure 3. Treg cell subsets express *IL17A*, *IFNG* and *IL10* mRNA directly *ex vivo* but do not produce IL-22 and IL-4.

(**A**) Flow cytometric analysis of cytokine production by the indicated Treg cell subsets stimulated for 5h with PMA/ionomycin. Data are representative of six independent experiments. (**B**) Cytokine production by the indicated T_H and Treg cells subsets stimulated for 24h with PMA/ionomycin and measured by ELISA. Data are representative of four independent experiments. (**C**) Real-time RT-PCR analysis of IL-17A (*IL17A*), IFN-γ (*IFNG*) and IL-10 (*IL10*) mRNA expression by the indicated T_H and Treg cell subsets. AU, arbitrary units. Data are mean ± SEM of five donors.



Supplemental Figure 4. IL-17 and IFN-γ production by T_H17-like and T_H1-like Treg cells is not due to decreased FOXP3 expression.

(**A**) Flow cytometric analysis of cytokine production by the indicated Treg cell subsets stimulated for 5h with PMA/ionomycin. Data are representative of six independent experiments. (**B**) Analysis of FOXP3 expression levels in cytokine $^+$ or cytokine$^-$ T_H17-like and T_H1-like Treg cells. For each donor, the MFI of FOXP3 staining in cytokine$^-$ T_H17-like or T_H1-like Treg cells was set to 100. AU, arbitrary units. Data are mean ± SEM of eight donors.



Supplemental Figure 5. All T_H and Treg cell subsets proliferate in response to CD3/CD28 stimulation.

Proliferation of CFSE-labeled T_H and Treg cell subsets isolated by cell sorting and stimulated with autologous monocytes in the presence of anti-CD3 and anti-CD28 antibodies. For Treg cells, IL-2 (20 U/ml) was added to the cultures at day 0 and day 2. CFSE dilution was analyzed after 6 days.