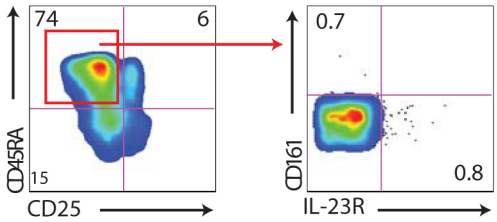
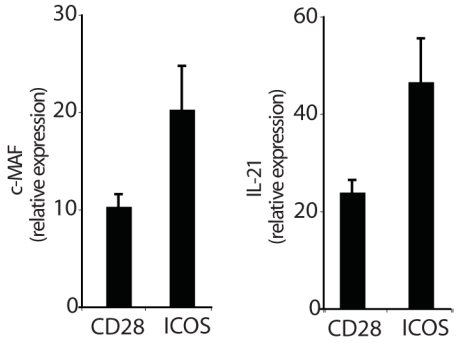
Supplemental Figure 1



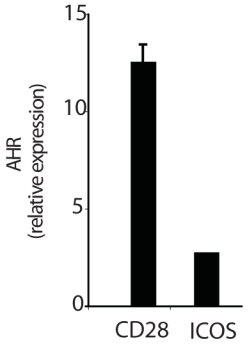
Supplemental Figure 1. UCB CD45RA+CD25-CD4+ T cells contain few CD161+IL-23R+ cells. The expression of CD161 and IL-23R surface markers on CD45RA+CD25-CD4+ T cells was assessed on human umbilical cord blood cells using flow cytometry. Data representative of 2 experiments.

Supplemental Figure 2



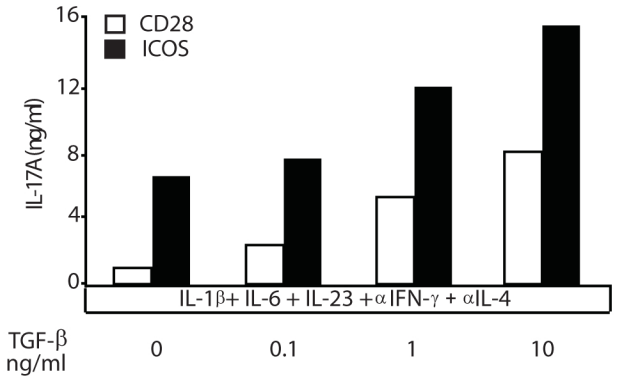
Supplemental Figure 2. ICOS induces c-MAF and IL-21. PB CD4+ T cells were cultured in TH17 polarizing conditions (IL-1β, IL-6, IL-23, plus neutralizing anti-IFN-γ and anti-IL-4) and activated with anti-CD3 beads bearing either anti-CD28 or anti-ICOS antibodies. After their primary expansion, their c-MAF and IL-21 expression mRNA levels was assessed by RT-PCR. Data representative of 2 experiments.

Supplemental Figure 3



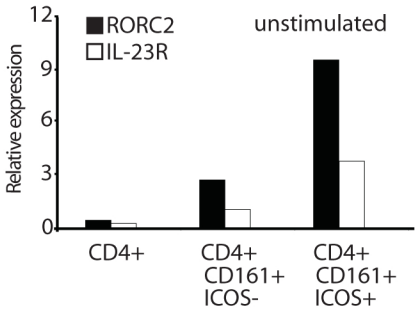
Supplemental Figure 3. CD28 induces expression of the aryl hydrocarbon receptor. PB CD4+ T cells were programmed toward a TH17 phenotype and activated with anti-CD3 beads bearing either anti-CD28 or anti-ICOS antibodies. After their primary expansion, their mRNA expression level of AHR relative to β-actin was assessed by RT-PCR. Data representative of 2 experiments.

Supplemental Figure 4



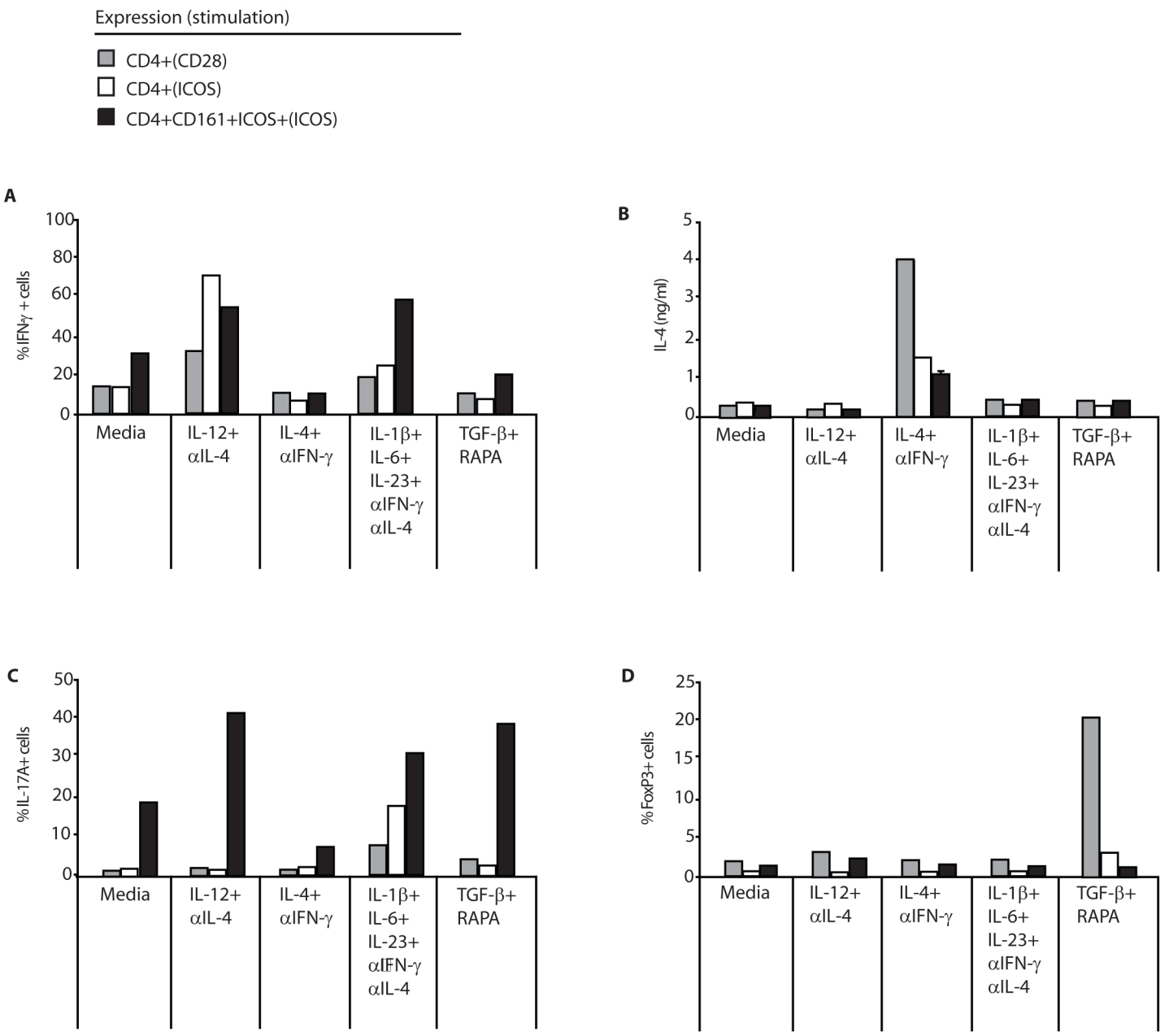
Supplemental Figure 4. Exogenous TGF-β augments the inflammatory potential of human TH17 cells. PB CD4+ T cells were programmed toward a TH17 phenotype and activated with anti-CD3 beads bearing either anti-CD28 or 2 anti-ICOS antibodies in media containing serum and the indicated supplemental TGF-β (from 0.1-10 ng/ml) was added to the culture on day 1. IL-17A secretion by cells was measured on day 5 post-activation by ELISA. Data representative of 2 experiments.

Supplemental Figure 5



Supplemental Figure 5. ICOS+CD161+CD4+ T cells from UCB constitutively express RORC2 and IL23R. CD4+, ICOS+CD161+CD4+ and ICOS-CD161+CD4+ T cells were sorted and their mRNA expression level of RORC2 and IL-23R relative to β-actin was measured by RT-PCR. Data representative of 2 experiments.

Supplemental Figure 6



Supplemental Figure 6. ICOS+CD161+CD4+ T cells are imprinted as TH17 cells. CD4+ and ICOS+CD161+CD4+ T cells from UCB were sorted and cultured in various polarizing conditions as indicated. The frequency of IFN-γ+ (A), IL-4+ (B), IL-17A+ (C) or FoxP3+ (D) cells was measured after their primary expansion with anti-CD3 beads bearing anti-CD28 or anti-ICOS antibodies. As a control, companion control cultures of bulk UCB CD4 T cells were stimulated with anti-CD3/CD28 beads. Cytokines and FoxP3 were measured by flow cytometry or ELISA on day 7 of culture post-stimulation with PMA/ionomycin. Data representative of 2 experiments.