Supplementary Figure 1. Expression of T-bet and IL-17F in differentiated Th17 cells

Real-time PCR analysis of T-bet expression in differentiating Th17 cells and in committed Th17 cells after the 2^{nd} stimulation (a). Concentrations of IL-17F in 72 h supernatants collected from cell cultures after 1^{st} and 2^{nd} stimulation as described in Figure 2 (b). *p< 0.001. Data are representative of 2 experiments. (error bars, s.e.m).

Supplementary Figure 2. Increased IL-27 concentration in culture does not suppress committed Th17 cells.

Th17 cells differentiated from 2D2 splenocytes were reactivated in the presence of MOG_{35-55} (20 μg/ml), IL-23 and increasing concentrations of IL-27 (0, 10, 50 and 100 ng/ml) during 72 h. Cells were then activated with PMA and ionomycin in the presence of GolgiPlug for 4 h and analysed by flow cytometry for the expression of IL-17A and IFN-γ on gated $CD4^+$ cells (a). IL-17A levels were measured by ELISA in the supernatants of cells activated during 72 h in the presence of IL-23 and IL-27 (b). Changes in IL-17A concentration (%) when IL-27 was added to the culture compared to PBS are indicated above the bars. Data are representative of two experiments. (error bars, s.e.m).

Supplementary Figure 3. Th17 cells retain their phenotype during three rounds of stimulation.

Th17 cells that underwent a 2^{nd} stimulation in the presence of TGF-β+IL-6 were rested 2 days in the presence of IL-2 and then restimulated (3^{rd} stimulation) with anti-CD3 and anti-CD28 antibodies, in the presence of cytokine combinations indicated on each panel. After 72 h cells

were stimulated with PMA and ionomycin in the presence of GolgiPlug for the final 4 h, stained and analyzed by flow cytometry for IL-17A and IFN-γ expression.

Supplementary Figure 4. Th17 cells do not retain their phenotype in the presence of IL-12

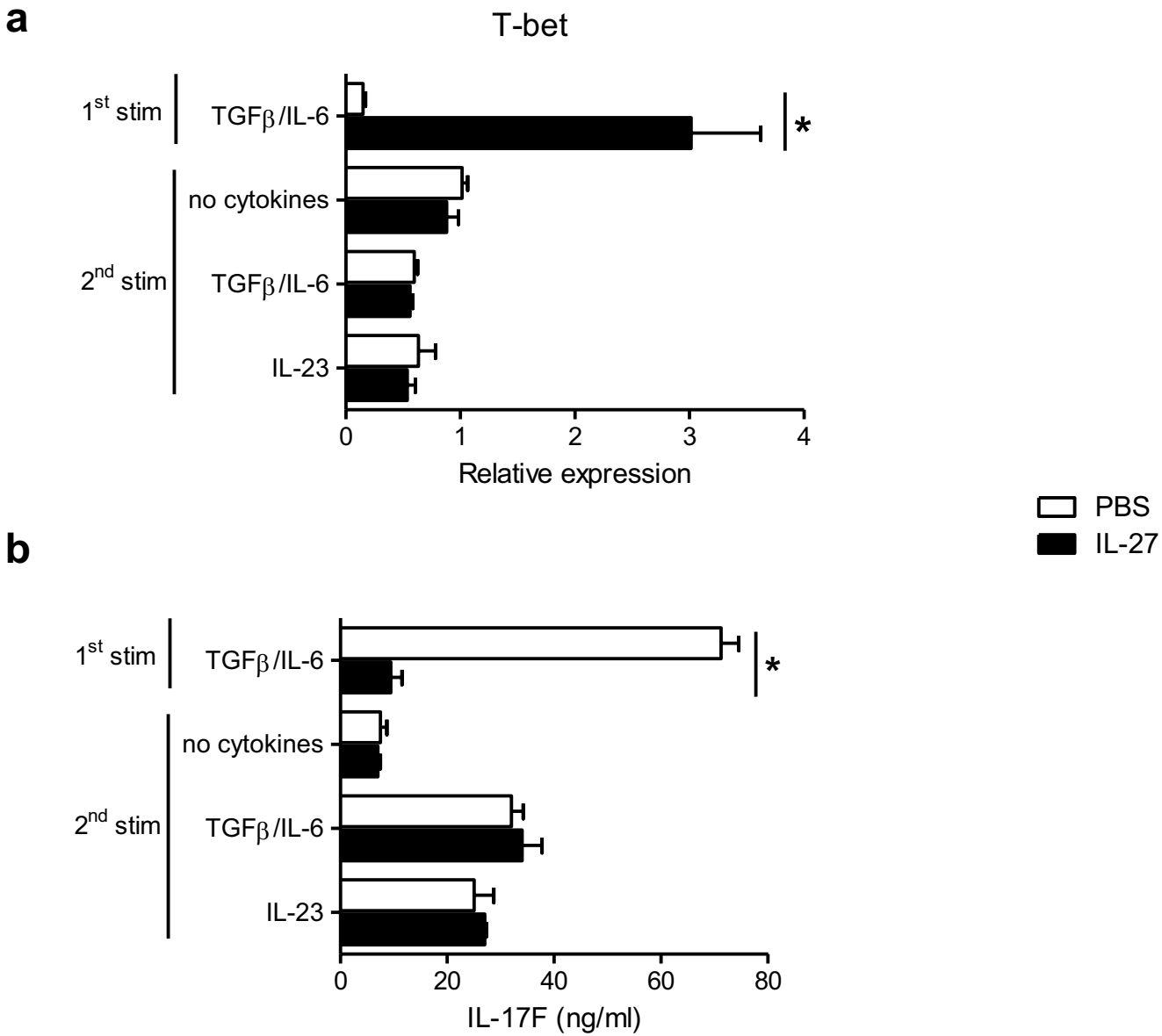
Splenocytes from 2D2 mice were activated with MOG_{35-55} peptide (20 μg/ml) in the presence of TGF-β+IL-6 for 72 h (1^{st} stimulation). Following the 1^{st} stimulation, cells were rested 48 h in the presence of IL-2 and then reactivated with peptide (2^{nd} stimulation) during 3 days in the presence of cytokines indicated on each panel. After each stimulation, cells were activated with PMA and ionomycin in the presence of GolgiPlug for the final 4 h, stained and analyzed by flow cytometry for IL-17A and IFN-γ expression in $CD4^+$ cells.

Supplementary Figure 5. Resistance of committed Th17 cells to IL-27 is not modified by accessory cells or antigen specific stimulation.

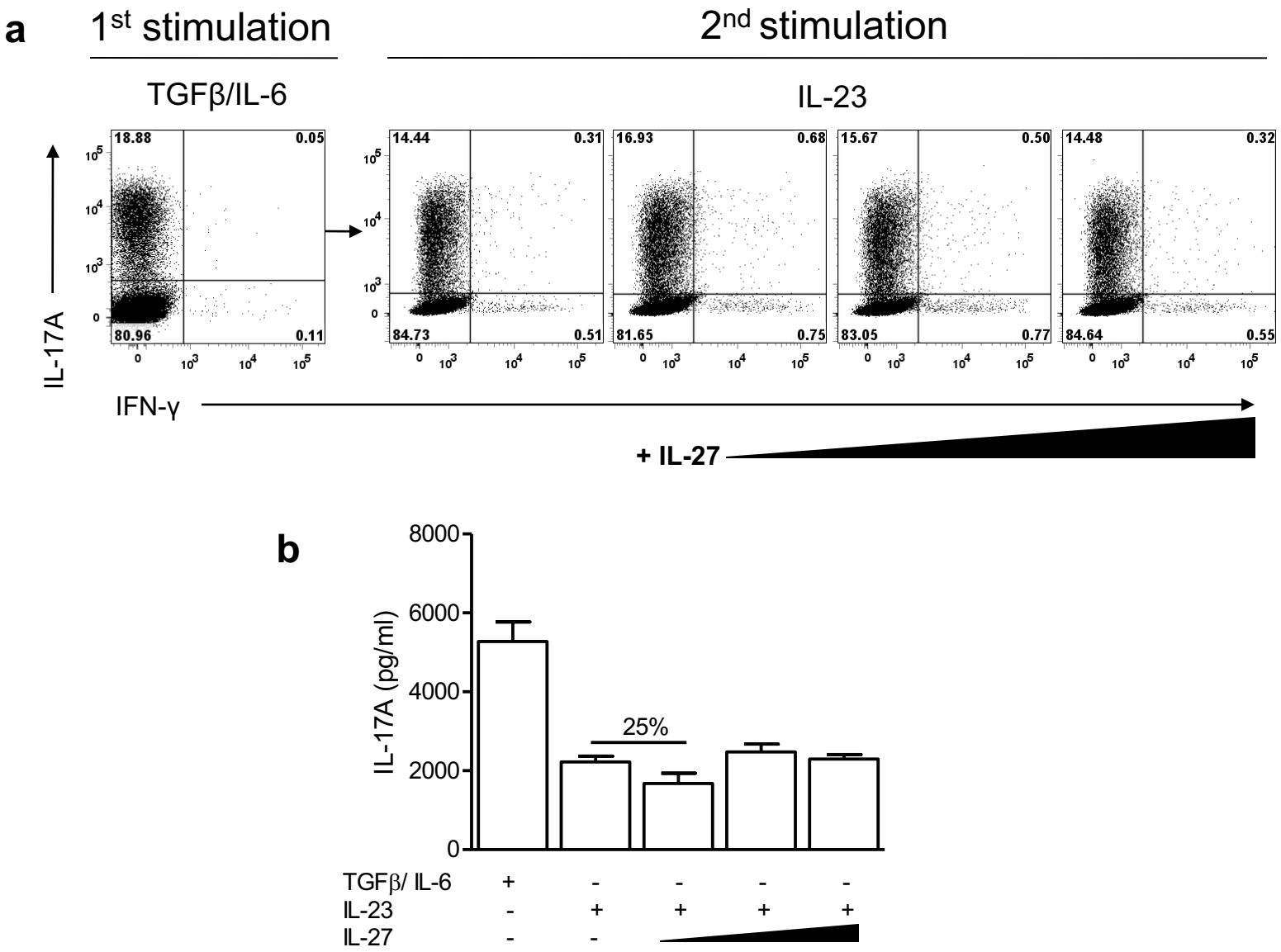
Splenocytes from C57BL/6 (a) or 2D2 mice (c) were activated with anti-CD3 and anti-CD28 antibodies (B6 mice) or MOG_{35-55} peptide (20 μg/ml; 2D2 mice) in the presence of TGF-β+IL-6 (± IL-27) for 72 h (1^{st} stimulation). Following the 1^{st} stimulation, cells were rested 48 h in the presence of IL-2 and then reactivated with antibodies or peptide (2^{nd} stimulation) for 3 days in the presence of cytokine combinations indicated on each panel. After 72 h cells were stimulated with PMA and ionomycin in the presence of GolgiPlug for the final 4 h, stained and analyzed by flow cytometry for IL-17A and IFN-γ expression in $CD4^+$ cells. IL-17A levels in B6 cultures (b) and 2D2 cultures (d) were measured by ELISA in the supernatants of cells activated during 72 h as described above. Changes in IL-17A concentration (%) when

IL-27 was added to the culture compared to PBS are indicated above the bars. Data are representative of two experiments. (error bars, s.e.m).

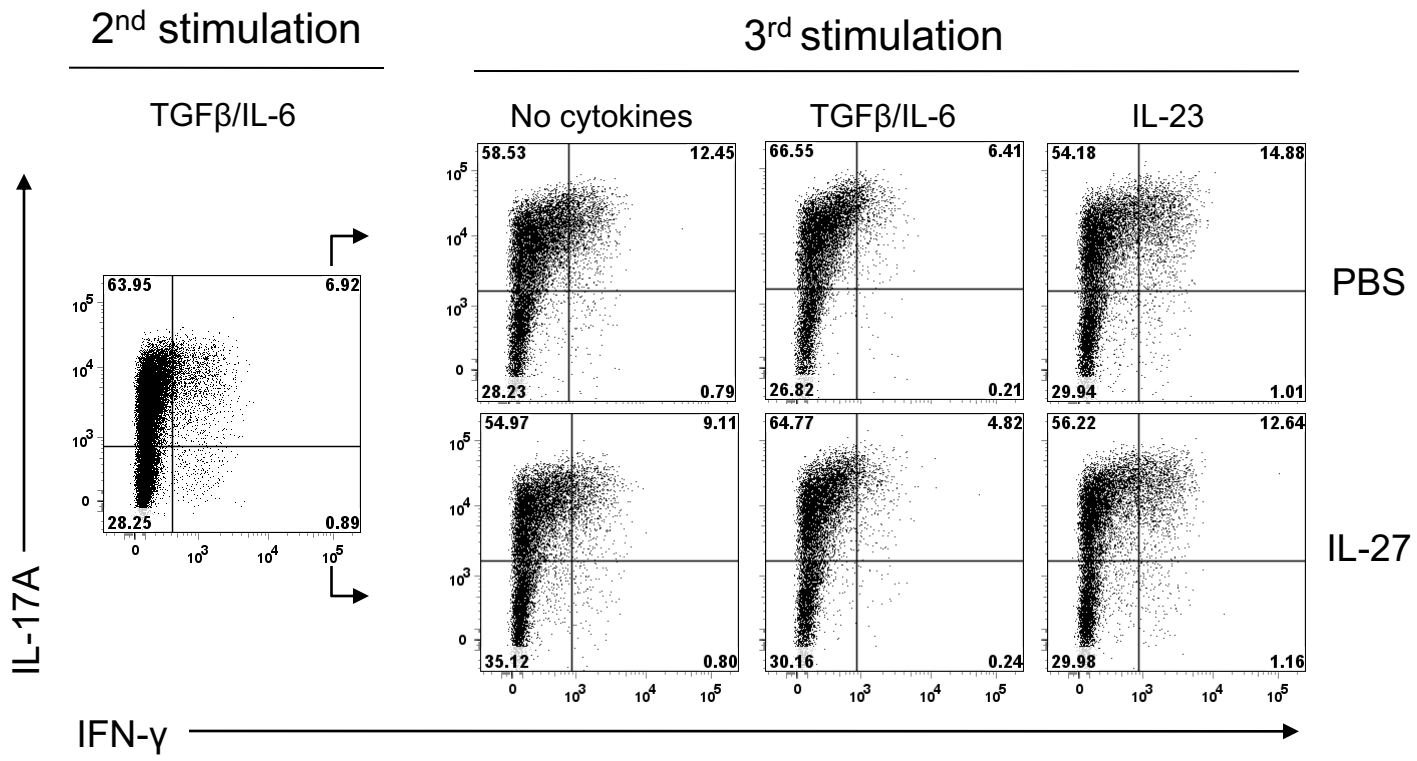
Supplementary Figure 1



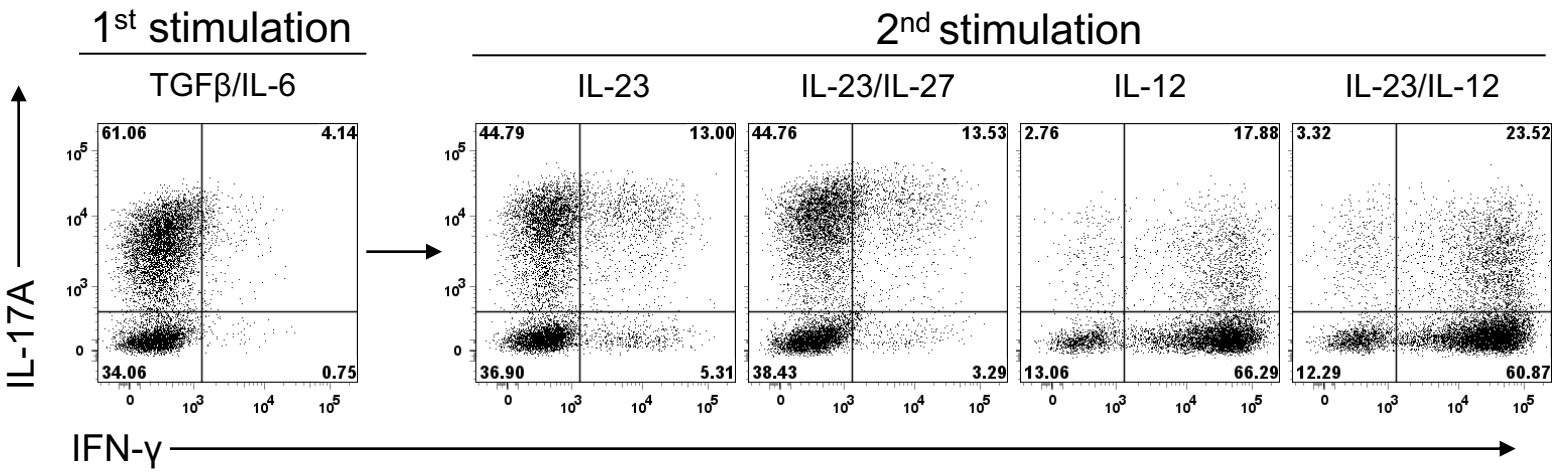
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplemental Figure 5