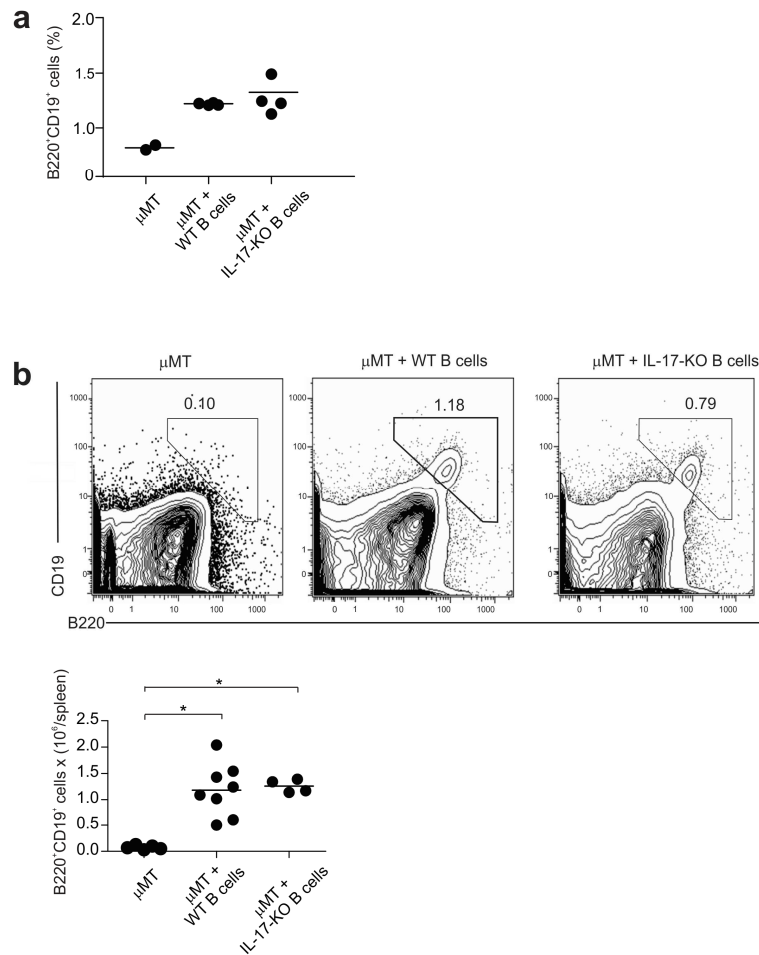
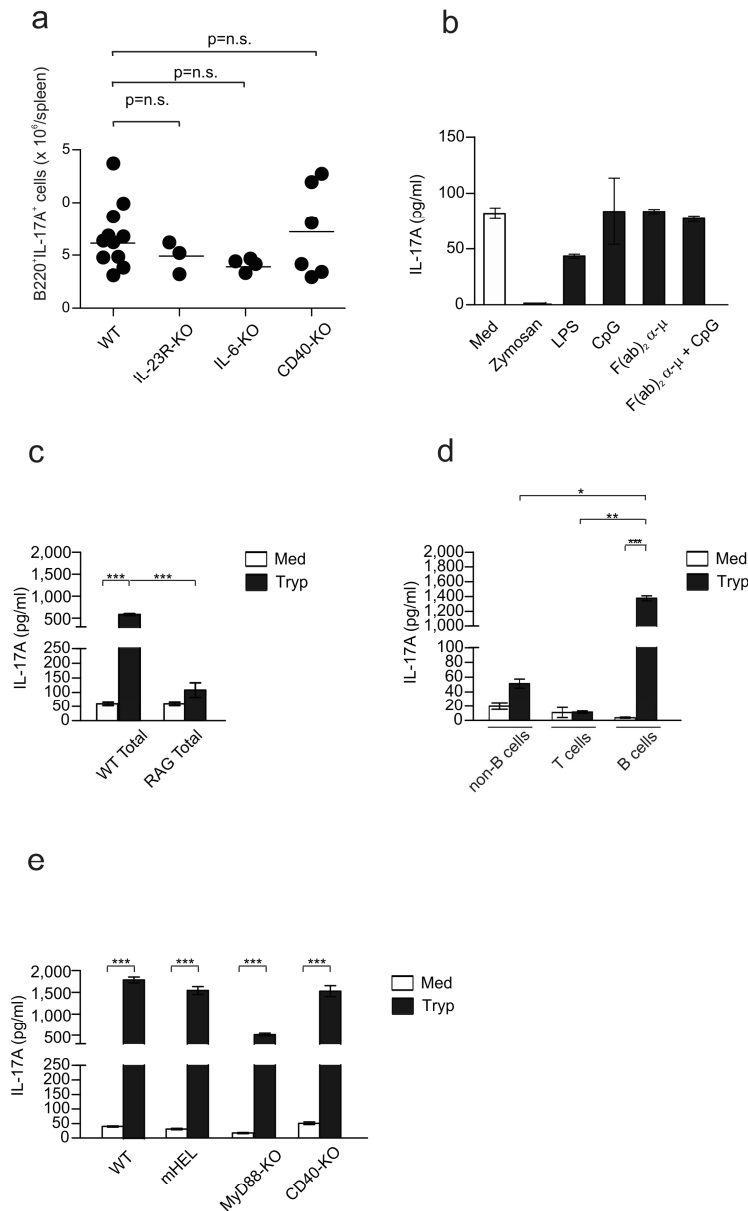


Trypanosoma cruzi trans-sialidase initiates an ROR- γ t-AHR-independent program leading to IL-17 production by activated B cells

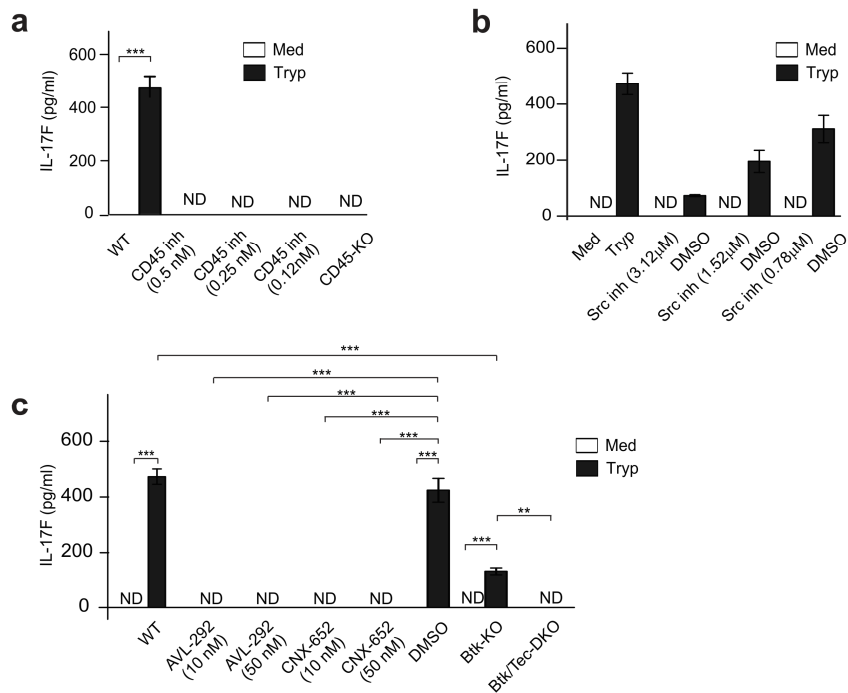
Daniela A Bermejo, Shaun W Jackson, Melisa Gorosito-Serran, Eva V Acosta-Rodriguez, Maria C Amezcua-Vesely, Blythe D Sather, Akhilesh K. Singh, Socheath Khim, Juan Mucci, Denny Liggitt, Oscar Campetella, Mohamed Oukka, Adriana Gruppi & David J Rawlings



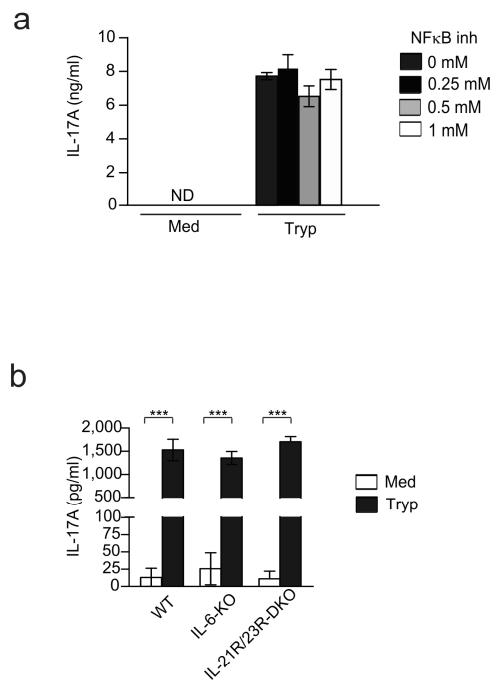
Supplementary Figure 1. Splenic B cells in μ MT mice following adoptive transfer of WT or *Il17a*^{-/-} B cells. Control μ MT mice, or μ MT mice reconstituted with WT or *Il17a*^{-/-} B cells, were infected with *T. cruzi*. **(a)** Percentage of B220⁺CD19⁺ cells in peripheral blood of control, or reconstituted, μ MT mice at 10 d post-infection. Animals were sacrificed at 20 d and splenic B cell populations were evaluated. **(b)** Representative FACS plots showing splenic B220⁺CD19⁺ B cells; and **(c)** Total number of splenic B220⁺CD19⁺ B cells. Each symbol denotes a single animal and the line represents the mean value in each group. The reduced total number of control μ MT and μ MT mice reconstituted with *Il17a*^{-/-} B cells reflects increased mortality in these cohorts. Data are shown as means \pm s.e.m. *, P = 0.05 (calculated by one-way ANOVA with Bonferroni correction). Data (a-b) are representative of 2 experiments with results in (c) showing combined data from 3-4 animals per group in each experiment.



Supplementary Figure 2. Deletion of IL-23R, IL-6, MyD88 or CD40, or restriction of the BCR repertoire, has no impact on *T. cruzi* triggered IL-17 production. (a) Number of IL-17A-expressing B220⁺ cells in the spleen of WT, *IL23r*^{-/-}, *Il6*^{-/-} and *Cd40*^{-/-} mice infected with *T. cruzi* (10 d). Each symbol denotes a mouse and the line represents the media value with 3-6 animals per group. Results are representative of 2 independent experiments. (b) IL-17A production by purified splenic B220⁺CD19⁺ WT B cells stimulated with F(ab)₂ anti-IgM, TLR agonists or both (3 replicates per condition). Representative of 3 experiments. (c) IL-17A production by total splenic cells derived from UI WT and *Rag2*^{-/-} mice (3 replicates per condition). Representative of 3 experiments. (d) IL-17A production by Tryp stimulated non-B cells (B220⁻ cells) or sort-purified CD3⁺ T cells derived from UI WT mice (3 replicates per condition). Representative of 2 experiments. (e) IL-17A production by WT, anti-HEL-Tg, *Myd88*^{-/-} or *Cd40*^{-/-} B incubated with *T. cruzi* Tryp (3 replicates per condition). Representative of 2 experiments. All analyses performed at 72 h and data are shown as means \pm s.e.m. *, P = 0.05 **, P = 0.005 (calculated by Mann-Whitney *U* test).



Supplementary Figure 3. CD45 and Src and Btk family kinases are required for B cell IL-17F production. (a) IL-17F production by WT B cells treated with or without various doses of CD45 inhibitor and by *Cd45*^{-/-} B cells. (b) IL-17F production by WT FM B cells stimulated with Tryp and treated with different concentration of Src inhibitor (PP2) vs. DMSO control. (c) IL-17F production by WT FM B cells stimulated with Tryp and increasing doses of Btk-specific inhibitors (AVL-292 or CNX-652) vs. DMSO control. All analyses performed at 72 h and results are representative of three (a) or two (b-c) independent experiments for each strain or condition. Data are shown as means ± s.e.m. ***, P = 0.0005 (calculated by one-way ANOVA with Bonferroni correction).



Supplementary Figure 4. NFκB, IL-6 and IL-21R and -23R signaling are not required for IL-17 production by B cells stimulated with *T. cruzi* Tryps. (a) IL-17A secretion in B220⁺CD19⁺ B cells from WT mice in response to *in vitro* exposure to Tryp and concurrent treatment with different doses of an NF-κB inhibitor (BAY). (b) IL-17A production by B220⁺CD19⁺ B cells from WT, *Il6*^{-/-} or *Il21*^{-/-}*Il23r*^{-/-} mice stimulated with Tryp. Data are shown as means ± s.e.m. *, P = 0.05 **, P = 0.005 (calculated by Mann-Whitney *U* test). All analyses performed at 72 h (3 replicates per condition) and results are representative of two independent experiments.