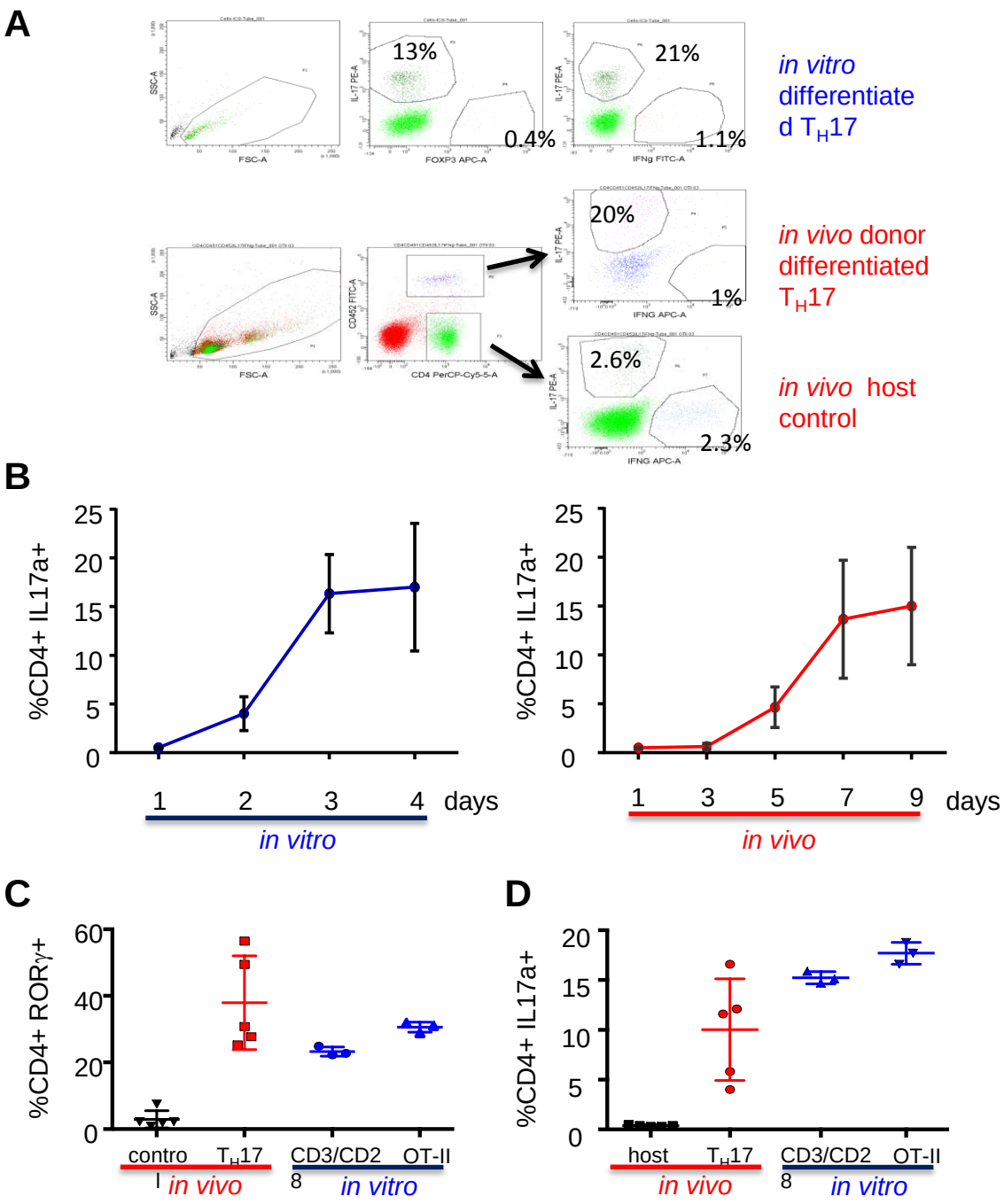
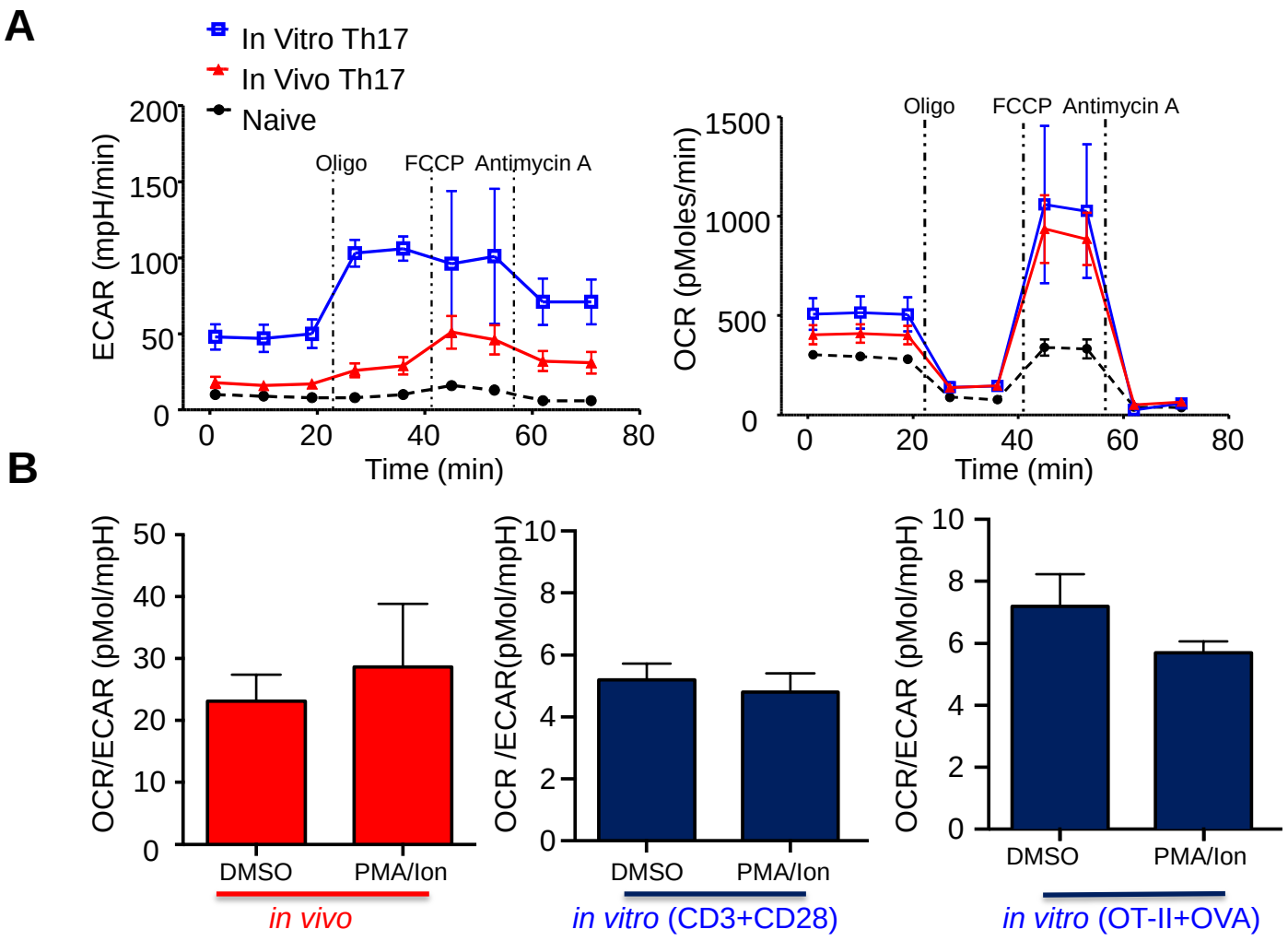


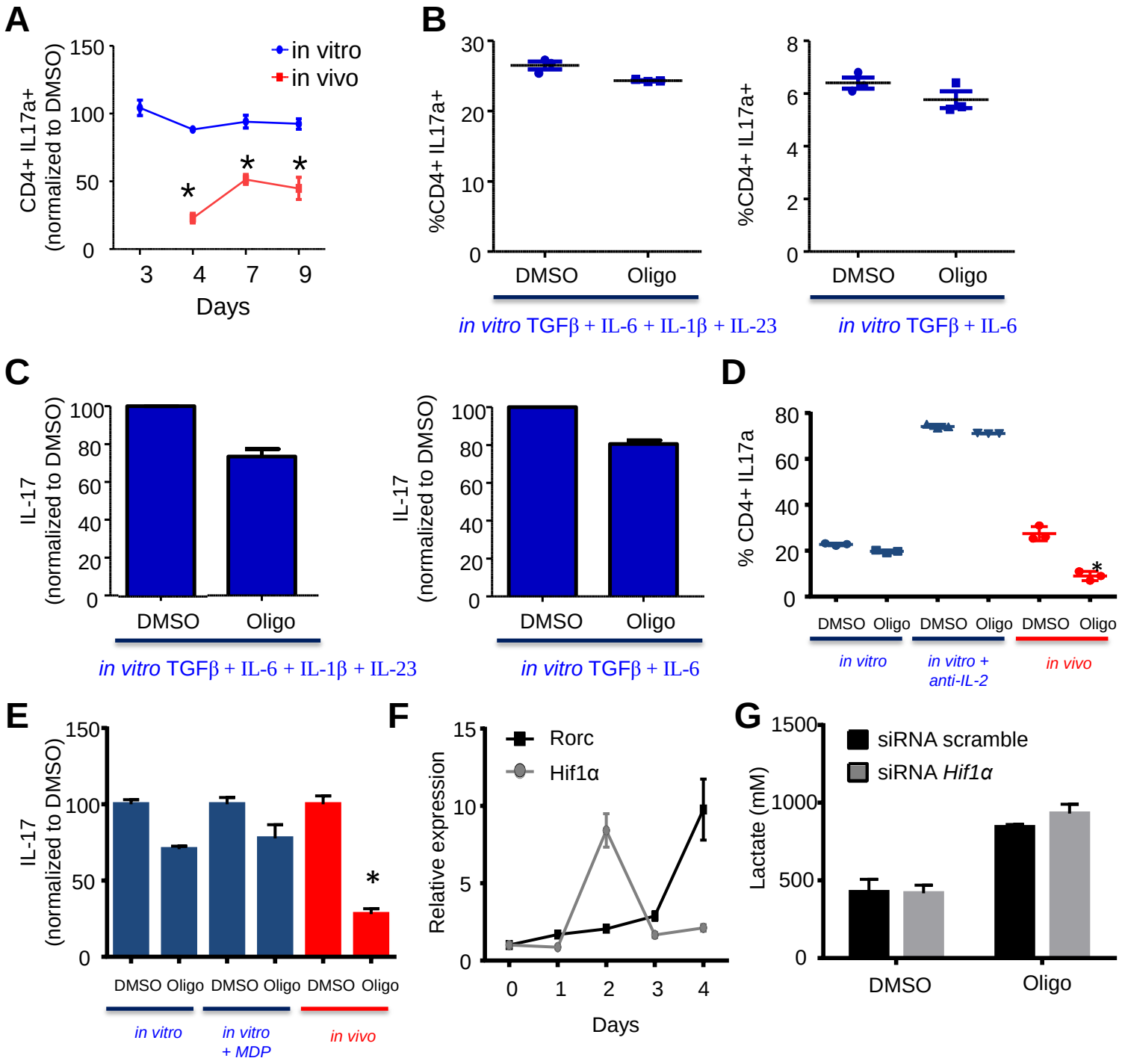
Supplemental Figure 1. Comparable expression of RORγ, IL-17a and IFNγ in in vivo and in vitro differentiated T_H17

(A) Gating strategy for FACS profile of in vitro and in vivo T_H17 cells. In vitro cells were first gated on live cells then the cytokines of interest. In vivo cells were first gated on live cells, than on CD45.2+CD4+ population and then on the cytokines of interest. Representative plot analyzing the expression of IL-17a and IFNγ in cells stimulated with PMA/Ionomycin. (B) In vivo (red) and in vitro (blue) differentiated T_H17 cells were stimulated with PMA/Ionomycin at the indicated time. The percentage of CD4+IL17a+ cells was determined by FACS. (C and D) In vivo-differentiated T_H17 (CD45.2+ CD4+) and in vitro cells differentiated with CD3 and CD28 or OT-II + OVA were stimulated with PMA/Ionomycin. The expression of RORγ and IL-17 was analyzed by FACS. Each dot represent a separate mouse (B,C) Results are representative of three independent experiments. *$p < 0.05$ (unpaired Student's t-test) (error bars, mean ± SEM).



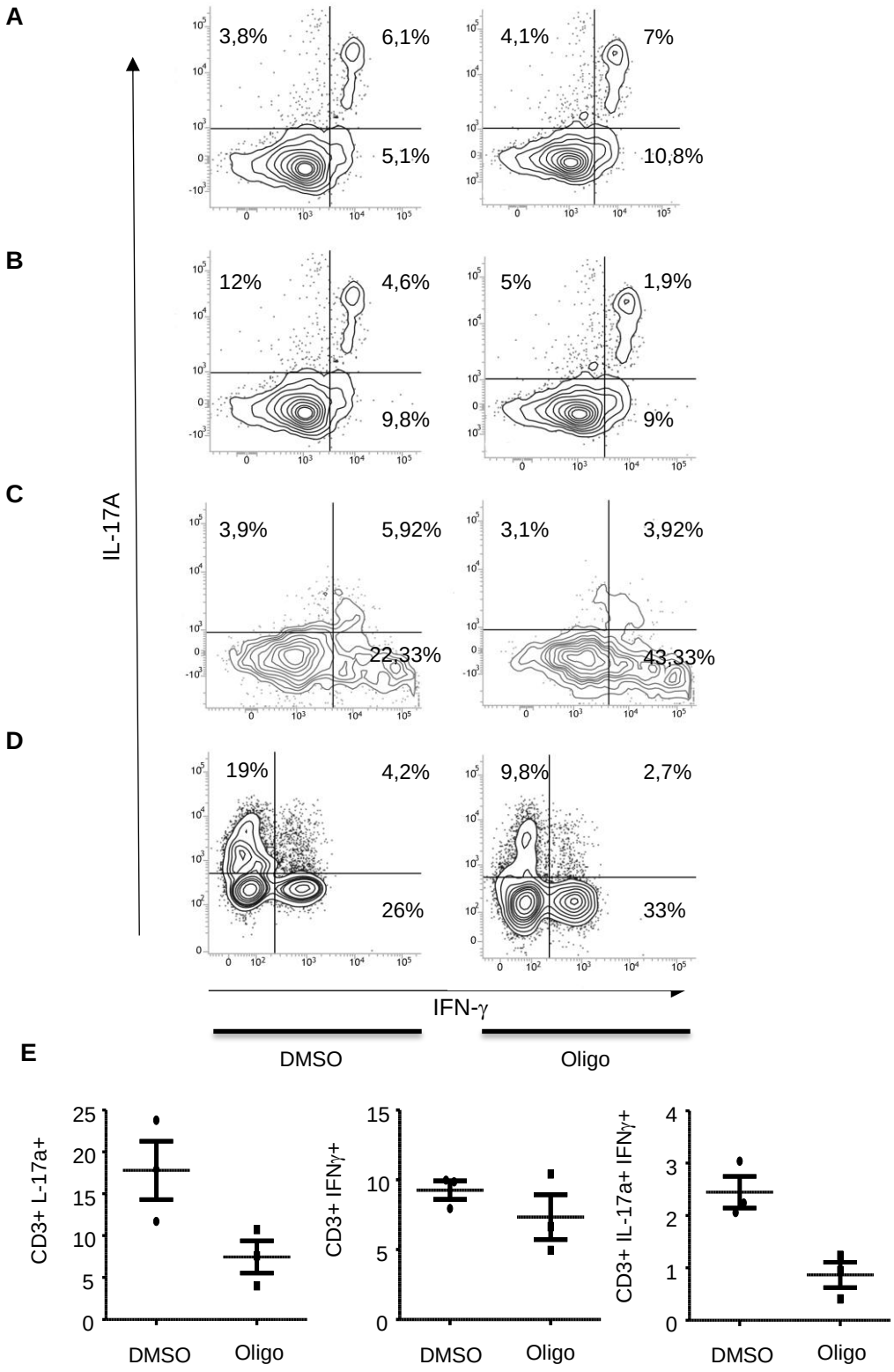
Supplemental Figure 2. Energetic profile of in vitro and in vivo differentiated T_H17

(A) Metabolic profile of naïve CD4 controls (CD4+CD62L-, oval), in vivo TH17 cells (CD45.2+CD4+, triangles), in vitro T_H17 cells (CD45.1+CD4+, square) was analyzed to measure OCR and ECAR. Where indicated cells were treated with oligomycin (1 mM), FCCP (1.5 mM) and antimycin A (1 mM). (B) OCR/ECAR profile of in vivo differentiated T_H17 (CD45.2+ CD4+), or naïve CD4 differentiated in vitro into T_H17 by anti-CD3/anti-CD28, or OT-II splenocytes differentiated in vitro into T_H17 by OT-II peptide stimulation, with or without PMA/Ionomycin stimulation. (A,B) Results are representative of three independent experiments. *$p < 0.05$ (unpaired Student's t-test) (error bars, mean ± SEM).



Supplemental Figure 3. In vitro differentiated T_H17 are insensitive to oligomycin treatment

(A) At the indicated time after the induction of the differentiation in vivo or in vitro, effector T_H17 cells were treated for one hour with DMSO or oligomycin (1 μM) and then stimulated with PMA/ionomycin. IL-17A expression in CD4+ cells was analyzed by FACS. Results are representative of two independent experiments; *p < 0.05 (unpaired Student's t-test; error bars, mean ± SEM. (B and C) Naïve CD4 cells were differentiated into T_H17 cells with TGFβ and IL6, with or without the addition of IL-1β and IL23, treated with DMSO or oligomycin (1 μM) for three hours and then stimulated with PMA/ionomycin. IL-17A expression in CD4+ cells was analyzed by FACS (B) and IL-17 production in cell-free supernatant by ELISA (B and C) Representative of two independent experiments. (D) Naïve CD4 cells were differentiated into T_H17 cells with or without anti-IL-2 blocking antibodies. In vitro and in vivo effector T_H17 cells were treated for one hour with DMSO or oligomycin (1 μM) and then stimulated with PMA/ionomycin. IL-17A expression in CD4+ cells was analyzed by FACS. (E) Splenocytes from OT-2 mice were differentiated into T_H17 cells by OVA peptide and polarizing cytokines in the presence, or absence, of MDP (muramildipepdide) In vitro and in vivo effector T_H17 cells were treated for one hour with DMSO or oligomycin (1 μM) and then stimulated with PMA/ionomycin. IL-17 production in cell-free supernatant from triplicate cultures was analyzed by ELISA (D and E) Results are representative of three independent experiments; *p < 0.05 (unpaired Student's t-test; error bars, mean ± SEM. (F) Gene expression in vivo-differentiated T_H17 cells was analyzed by RT-PCR at the indicated time points. Representative of three independent experiments (G) In vitro-differentiated T_H17 cells nucleofected with siRNA scramble or siRNA targeting HIF1α were treated for one hour with DMSO or oligomycin (1 μM) and then stimulated with PMA/ionomycin. Lactate concentration in cell-free supernatant from triplicate cultures was determined by a colorimetric assay. Representative of two independent experiments.



Supplemental Figure 4. OXPHOS controls IL-17 production in IBD.

LPMC isolated from mice treated with ethanol (A), TNBS (B and C), DSS (E), or from human IBD patients (D) were exposed for 1 h to DMSO or oligomycin (1 mM) and then stimulated with PMA/Ionomycin. (a-e) IL-17 and IFNγ production in CD45+CD3+ (A,B,D) and CD45+CD3+CD8+ (C) was evaluated by FACS analysis. (A-D) Representative dot plot. (E) Each dot represents an individual mouse, target cell populations expressed as percentage of total CD3+ cells (E) Representative of two independent experiments *p<0.05 (paired Student's t-test) (error bars, mean ± SEM).