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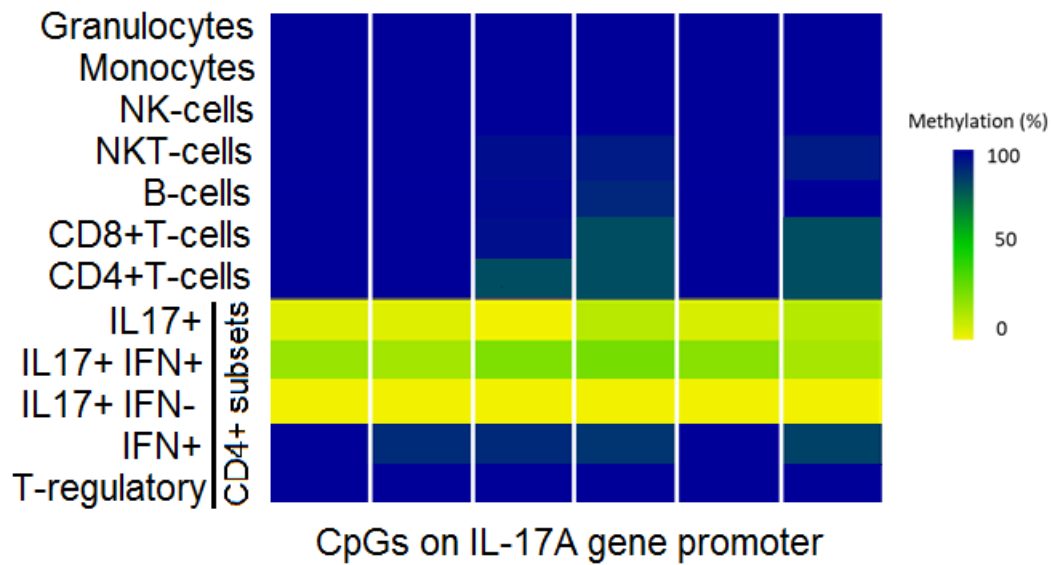
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SUPPLEMENTARY MATERIAL

Details of the epigenetic qPCR assay

Bisulfite sequencing analysis of the IL-17A promotor region in various purified cell types indicated that selected CpG dinucleotides in the analysed region were overwhelmingly methylated in myeloid derived blood cells (monocytes and granulocytes in Figure S1). In lymphocyte subsets, data were more varied. In the bulk populations of CD8+T-cells, B-cells, NK and NKT cells, bisulfite sequencing did not show significant levels of unmethylated CpGs in the IL-17A promotor region. Equally, purified CD4⁺ Treg showed fully methylated CpGs in the analysed region. In total CD4+T-cells however, a small % of cells presented with demethylated CpGs at discrete positions in the region (showed by lighter blue/green colour on the head map). More specific purification of CD4+T-cells subsets using cell sorting based on IFN- γ and IL-17 expression, indicated that all IL-17 expressing cells presented with almost fully unmethylated CpGs at the analysed CpG positions in the promotor region of the IL17A gene, with IL-17A+/IFN- γ - cells showing the highest percentage of unmethylation over the overall region. In contrast, IFN γ ⁺ cells were almost fully methylated at all positions.

Based on the differentially methylation observed in this region, a qPCR assay was designed [55], using primers aligning to unmethylated DNA at specific CpGs position in the promotor of IL-17A gene, that would not generate a product when the DNA is methylated.



Supplementary Figure 1: Heatmap presenting CpG-methylation in the IL-17A gene promoter in different cell subsets

Immune cell subsets were sorted by flow cytometry to high purity. DNA was extracted, converted with bisulfite and a region of the IL-17A gene promoter containing 6 CpGs was sequenced. Results are presented as % of methylated DNA at each of the 6 CpG positions.