



### Supplementary Data S1. Gating strategy for defining T cell subpopulations and ROS expression

PBMC from patients with RA (n = 40) and healthy controls (n = 10) were isolated from whole blood samples. Single cells were gated in isolated PBMCs using FSC-A and FSC-H, and lymphocyte populations were determined with Live/Dead Aqua staining. CD3-expressing T cells were gated from live singlet lymphocytes. Following CD4 and CD8 expression, CD3<sup>+</sup> T cells were defined as THs (CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>-</sup>) and TCs (CD3<sup>+</sup>CD8<sup>+</sup>CD4<sup>-</sup>). Following fixation and permeabilization using FOXP3/transcription factor staining buffer, cells were stained with specific antibodies for FOXP3 and IL-17A. THs were further classified as TH17s (CD3<sup>+</sup>CD4<sup>+</sup>IL-17<sup>+</sup>) and Tregs (CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup>). After washing, each T cell subset was incubated with two types of ROS dye, CellROX or MitoSOX. Plots and histograms are representative examples with PBMCs from patient with Moderate RA.