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Supplemental Material to:

Maria Sole Chimenti, Paola Tucci, Eleonora Candi, Roberto Perricone, Gerry Melino, and Anne E Willis

Metabolic profiling of human CD4+ cells following treatment with methotrexate and anti-TNF-α infliximab

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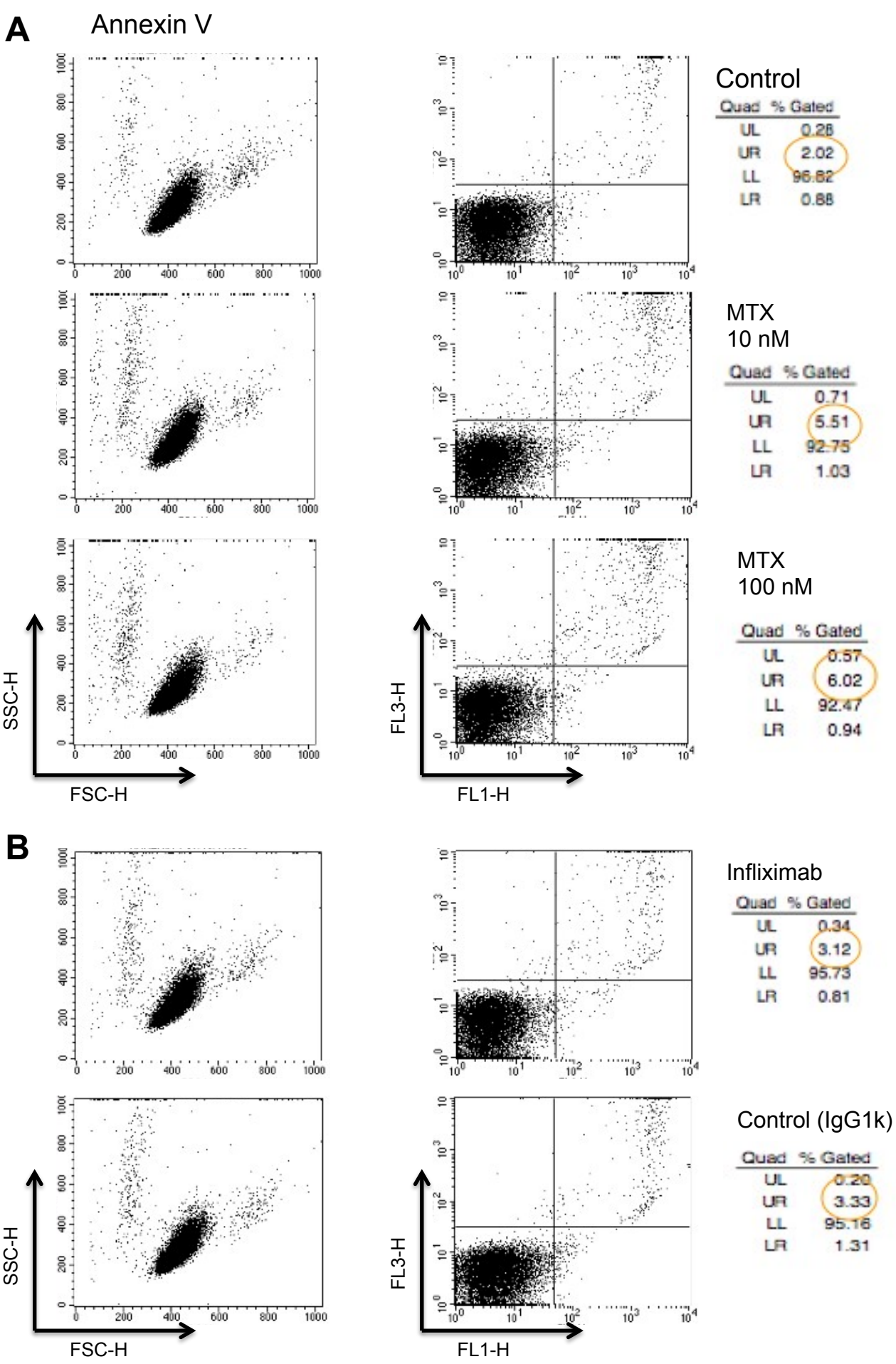
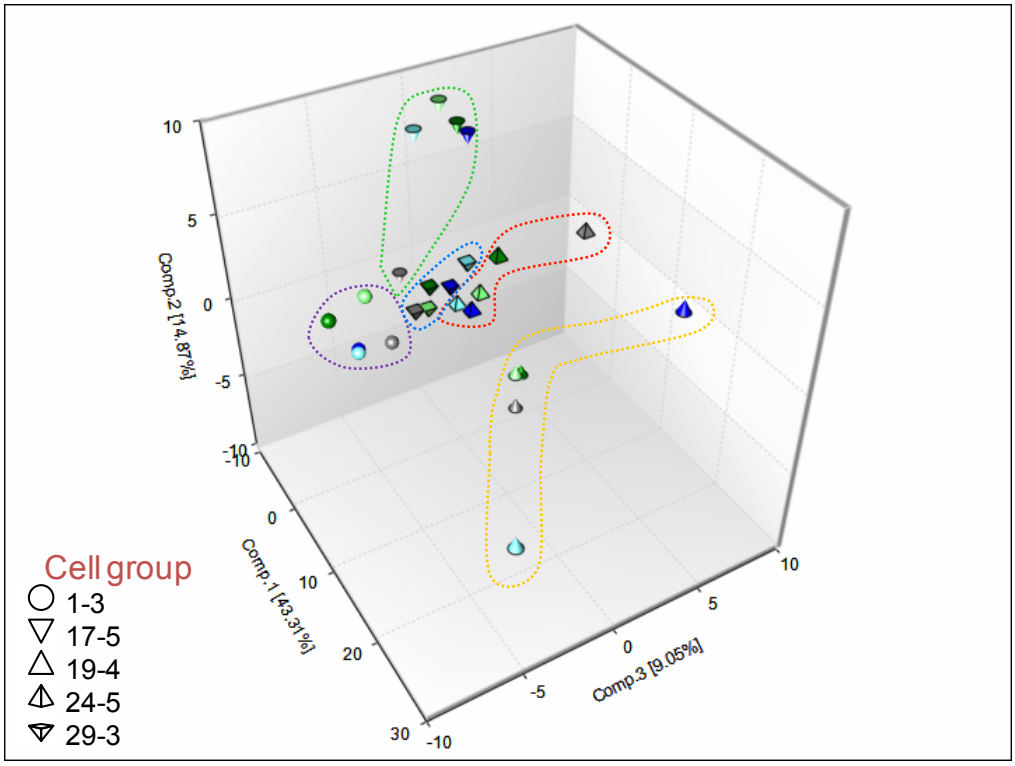


Supplementary Figure 1

A



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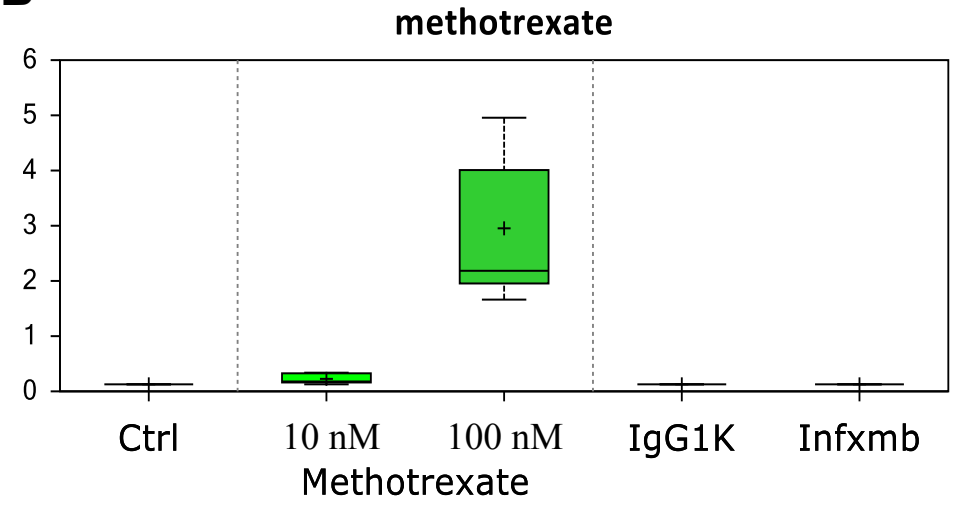

Supplementary Figure 2

Metabolic profiling of human CD4+ cells following treatment with Methotrexate and anti-TNF-α Infliximab

*Maria Sole Chimenti[1], Paola Tucci[2,3], Eleonora Candi[4], Roberto Perricone[1], Gerry Melino[3,4 *], Anne E. Willis[3,*],*

[1] Rheumatology, Allergology and clinical immunology, Department of Internal Medicine, University of Rome Tor Vergata, 00133, Rome, Italy.
[2] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende (CS), Italy.
[3] Medical Research Council, Toxicology Unit, Leicester LE1 9HN, UK.
[4] Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, 00133 Rome, Italy.

[*]***Correspondence***: Anne E. Willis, MRC Toxicology Unit, Hodgkin Building, Leicester University, Lancaster Road, P.O. Box 138, Leicester LE1 9HN, UK. Email: aew5@le.ac.uk AND Gerry Melino, MRC Toxicology Unit, Hodgkin Building, Leicester University, Lancaster Road, P.O. Box 138, Leicester LE1 9HN, UK and IDI-IRCCS laboratory c/o Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, via Montpellier 1, 00133 Rome, Italy. Email: gm89@le.ac.uk

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Table 1. Metabolomic data. Left panel. Effect of MTX and infliximab on CD4+ T cells at 24 h. Heat map of statistical significant biochemical profiled. Shaded cells indicate p≤0.05 (RED indicates that the mean values are significantly higher for that comparison; GREEN values significantly lower). BLUE-bolded text indicates 0.05<p<0.10. All data are normalized to Bradford protein assay values. **Central panel.** Fold changes, Welch's two sample t-tests. **Right panel.** Absolute values and mean values.

Supplementary Figure 1. Effect of MTX and infliximab on apoptosis evaluated by measuring phosphatidylserine externalization by flow cytometry using annexin V^{FITC} as a probe. Cells were treated with 10 nM and 100 nM MTX (A) and with infliximab (1mg/ml), for 24 h. Cells were collected and analysed (BD, FACSCalibur) for each time point. The data shown are one representative experiment of three.

Supplementary Figure 2. (A) PCA showed no clear separation among treatment groups. However, PCA demonstrated moderate separation among different CD4+ T cell preparations suggesting a confounding effect of T cell sources. Treatment group: GREY, control; LIGHT GREEN, MTX 10 nM; GREEN, MTX 100 nM; LIGHT BLUE, IgG1K 1μg/ml; BLUE, infliximab 1μg/ml. (B) Confirmation of Methotrexate (MTX). MTX was detected in the treatment groups and was absent in the other groups. The relative concentrations of MTX reflected the treatment doses used in the two MTX groups.