Thiostrepton induces cell death of acute myeloid leukemia blasts and the associated macrophage population

Isabel Weinhäuser,[1,2,3*] Diego A. Pereira-Martins,[1,2,3*] Jacobien R. Hilberink,[1*] Annet Brouwers-Vos,[1] Eduardo M. Rego,[3] Gerwin Huls[1] and Jan Jacob Schuringa[1]

[1]Department of Experimental Hematology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; [2]Department of Internal Medicine, Medical School of Ribeirao Preto, University of São Paulo, Ribeirao Preto, Brazil and [3]Center for Cell Based Therapy, University of São Paulo, Ribeirao Preto, Brazil

*IW, DAP-M and JRH contributed equally as first authors.

Correspondence:
J.J. SCHURINGA - j.j.schuringa@umcg.nl

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Supplemental data

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Isabel Weinhäuser[1,2,3,4], Diego A. Pereira-Martins[1,2,3,4], Jacobien R. Hilberink[1,4], Annet Brouwers-Vos,[1] Eduardo M. Rego[3], Gerwin Huls[1,5], Jan Jacob Schuringa[1,5,#]

[1]Department of Experimental Hematology, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands

[2]Department of Internal Medicine, Medical School of Ribeirao Preto, University of São Paulo, Ribeirao Preto, Brazil

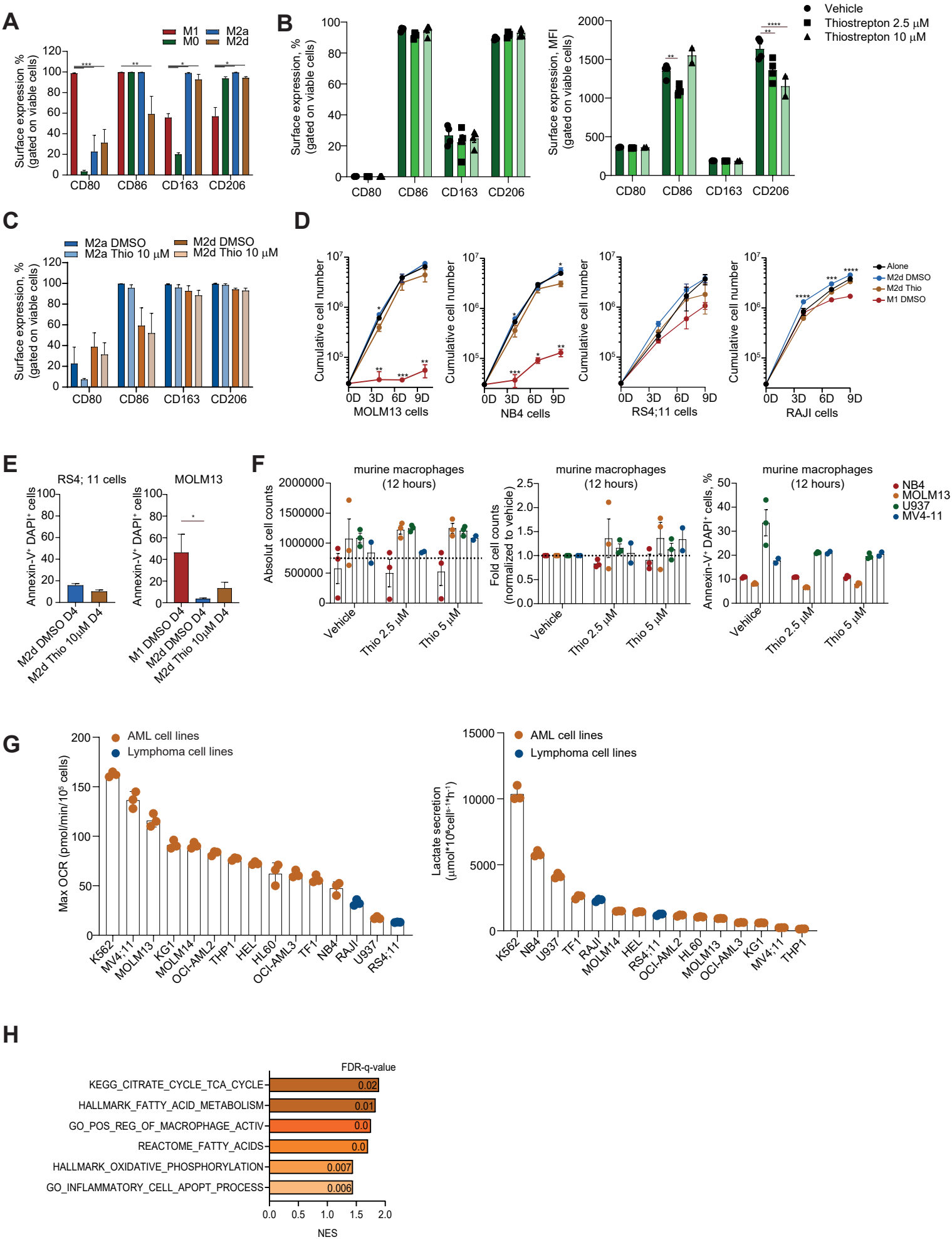
[3]Center for Cell Based Therapy, University of São Paulo, Ribeirao Preto, Brazil

[4]Authors contributed equally to this study

[5]Lead contact authors

*Correspondence: j.j.schuringa@umcg.nl (J.J.S.), Experimental Hematology, UMCG, University of Groningen, Hanzeplein 1, 9700RB Groningen, The Netherlands, phone 0031503619391; fax: 0031503614862

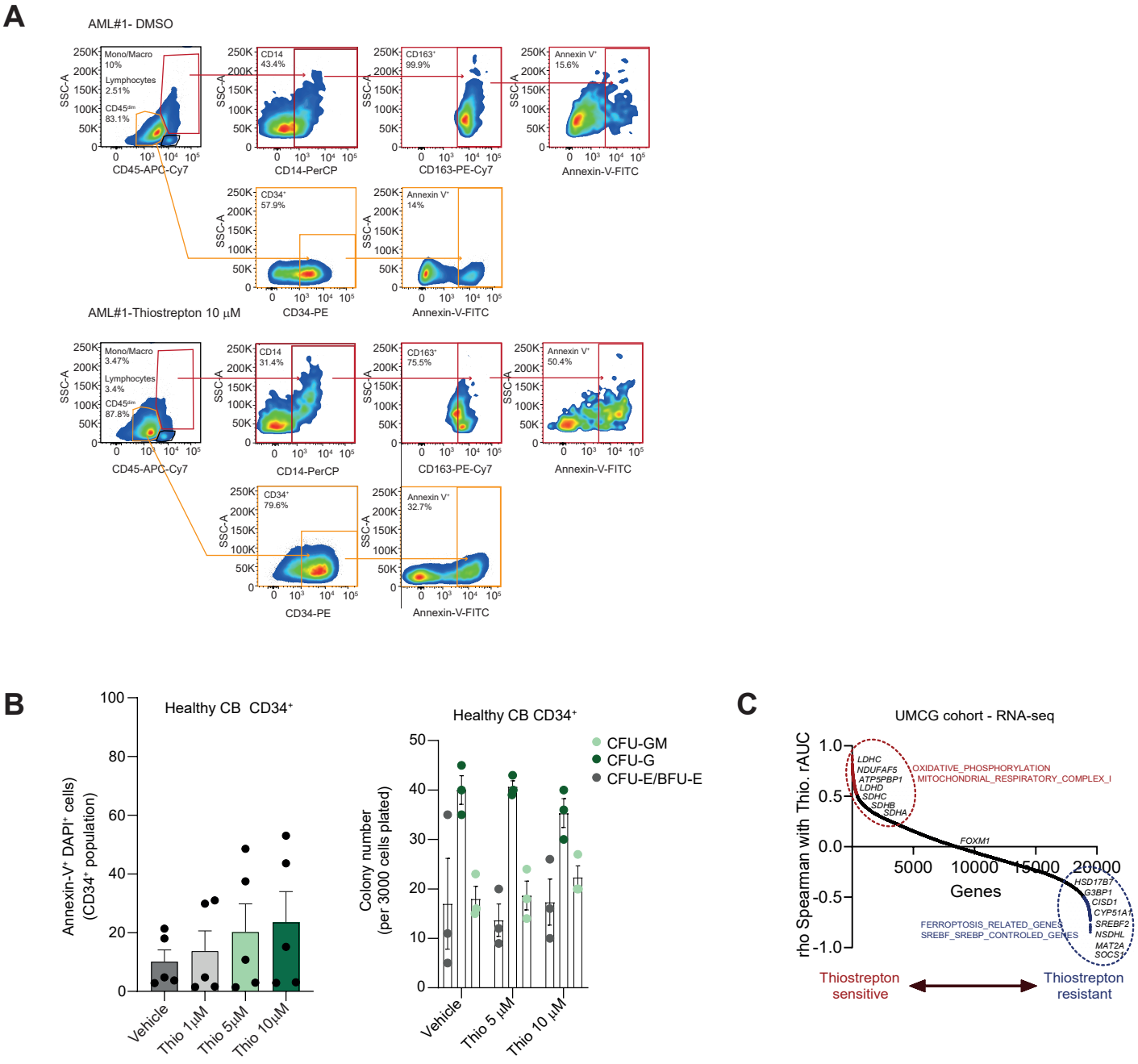
Supplemental figure 1



Supplemental Figure 1. Thiostrepton treatment induces macrophage cytotoxicity but not M2-to-M1 repolarization. (A) Bar plot comparing the levels (percentage, %) of M2 (CD163 and CD206) and M1 (CD80 and CD86) markers measured by Flow cytometer measurements in healthy macrophages activated with respective cytokines for 24 h. M0-macrophages were generated with 50 ng/mL of MCSF (upper panels). (B) Bar plot comparing the levels (left panel: percentage, and right panel: mean fluorescence intensity, MFI) of M2 (CD163 and CD206) and M1 (CD80 and CD86) markers measured by flow cytometry in Peripheral blood isolated healthy macrophages cultured in the presence of 50 ng/mL recombinant human MCSF (M0-macrophages) for 7 days and treated in the absence of cytokines with thiostrepton for 24 h. (C) Bar plot comparing the levels (%) of M2 (CD163 and CD206) and M1 (CD80 and CD86) markers measured by FACS in healthy activated M2a and M2d macrophages treated with DMSO or thiostrepton (10 µM) for 24 h. (D) Cumulative cell count of MOLM13, NB4, RS4;11, and RAJI leukemic cell lines cultured on M1 and M2d macrophages (treated or not with thiostrepton, 10 µM) for 9 days. (E) Percentage of apoptotic RS4;11 and MOLM13 cells after 4 days of culture on M1 and M2d pre-treated with thiostrepton (10 µM) or vehicle. (F) Cumulative cell counts (left panel), fold of proliferation relative to vehicle (middle panel) and apoptosis levels (evaluated by Annexin-V/DAPI staining) of human leukemic cells co-cultured for 12 h on murine bone-marrow isolated macrophages. Macrophages were pre-treated with vehicle (DMSO) or thiostrepton (2.5 and 5 µM) prior to the co-culture (24 hours). For flow-cytometry evaluations, cells were stained with human CD45-FITC to exclude cross-contamination with floating murine macrophages. (G) Lactate secretion per 1×10^6 cells per hour (upper panel) and maximum oxygen consumption rate (Max OCR, lower

panel) in leukemic cells (n = 4 measured in quadruplicates). (H) Gene Ontology (GO) analysis using the Pearson correlations depicted in Figure 2B.

Supplemental figure 2



Supplemental Figure 2. Flow cytometry gating schemes and effects of thiostrepton on healthy CD34$^+$ cells

(A) Representative Flow cytometer plots depicting the gating strategy used for evaluating the thiostrepton-induced apoptosis in AML CD45$^+$ subpopulations treated *ex vivo*. (B) Ex vivo drug induced apoptosis of thiostrepton in a set of 5 independent healthy CD34$^+$ cells isolated from cord blood samples (left panel) and colony formation capacity (right panel). Cells were treated for 72 h, and apoptosis was evaluated by Annexin-V/DAPI staining by flow cytometry. Clonogenic assay was performed in methylcellulose medium supplemented with human cytokines. Colonies were scored after 14 days of plating. One-way or two-way analysis of variance (ANOVA) were used for statistical analysis. *p<0.05, **p<0.01, ***p<0.001. Source data are provided as a Source Data file. (C) Hockey stick plot displaying the correlation between RNA-seq data from *ex vivo*-treated primary AML samples (UMCG cohort) and thiostrepton sensitivity. Expression of Ferroptosis related genes and SREBP target genes was correlated with Thiostrepton insensitivity.