**SUPPORTING INFORMATION**

# Hypoxia triggers major metabolic changes in AML cells without altering indomethacin-induced TCA cycle deregulation

Alessia Lodi[1,2†], Stefano Tiziani[1,3†], Farhat L. Khanim[4], Mark T. Drayson[5], Ulrich L. Günther[1§], Christopher M. Bunce[4§], and Mark R. Viant[4§*]

[1]The Henry Wellcome Building for Biomolecular NMR Spectroscopy, School of Cancer Sciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.; [2] Department of Radiology and Biomedical Imaging, University California San Francisco, San Francisco, CA 94158 USA; [3]Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA; [4]School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.; [5]School of Immunity and Infection, Medical School, University of Birmingham, B15 2TH, UK.

[†]Contributed equally to this work

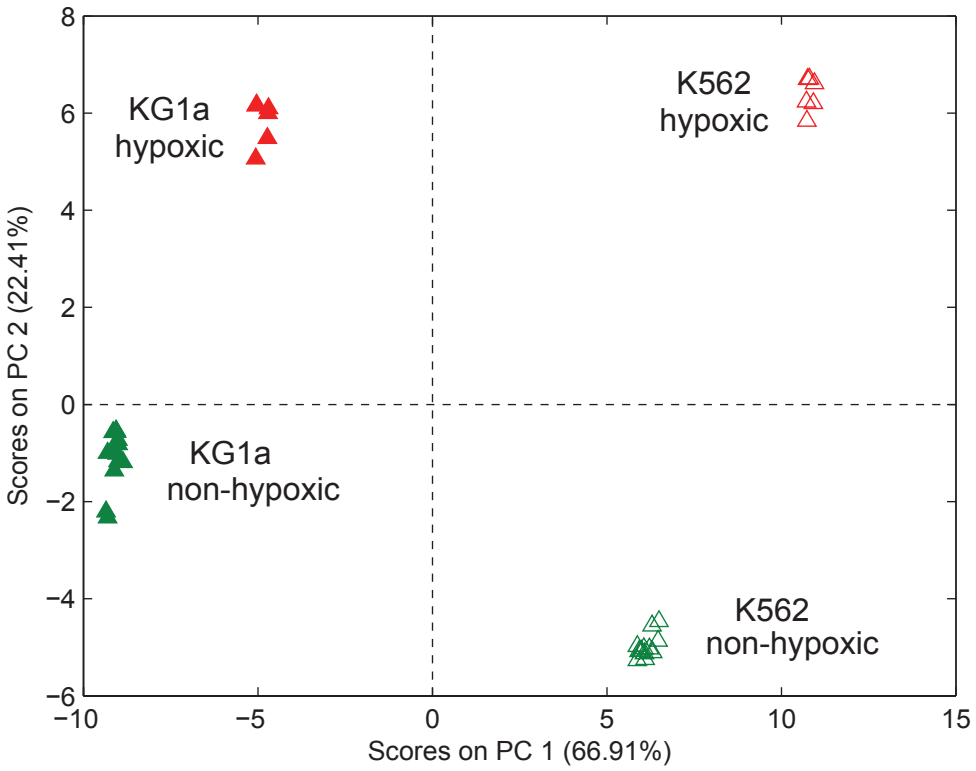
[§]Contributed equally as senior authors

*To whom correspondence should be addressed:

Phone: +44 (0)121 4142219

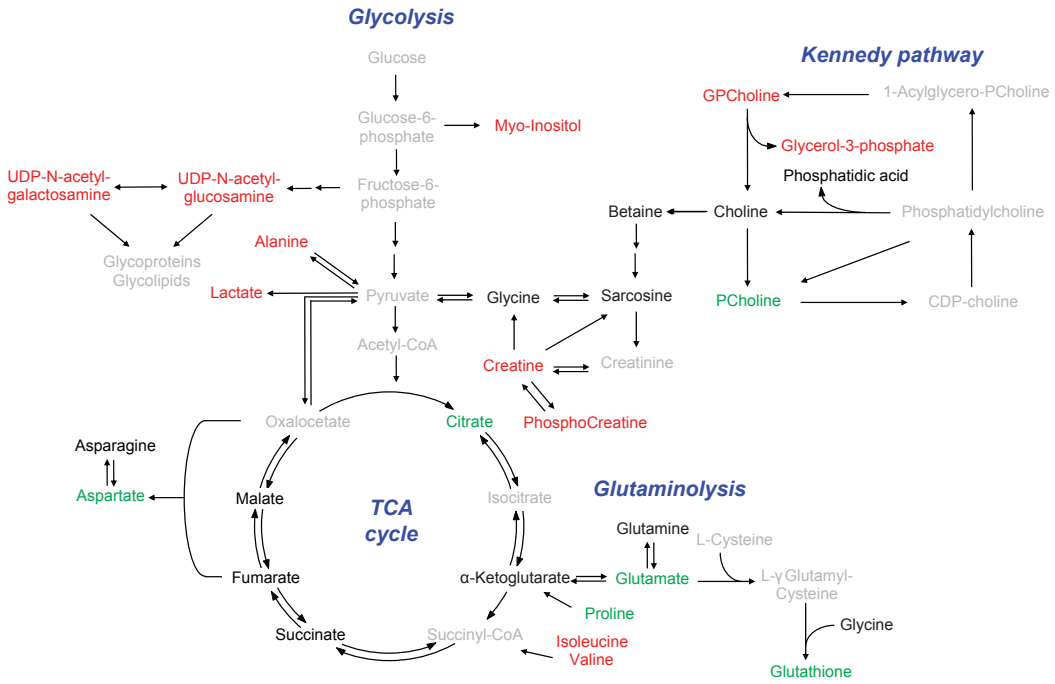
FAX: +44 (0)121 4145925

Email: M.Viant@bham.ac.uk



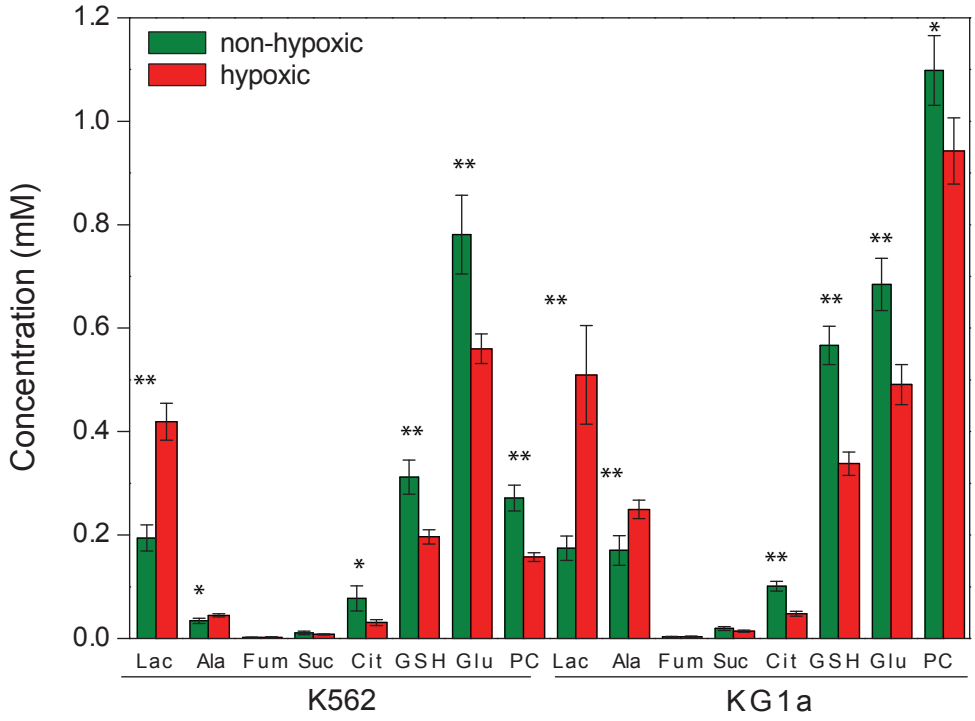

**Supplementary Figure 1.** Metabolic differences between acute myeloid leukemia cell lines grown in hypoxic and non-hypoxic environments.

Scores plot obtained from the principal component analysis of the $^{1}$H projected *J*-resolved NMR datasets of KG1a and K562 cells grown either in non-hypoxic (12 replicates per cell line) or in hypoxic (~1% oxygen environments; 6 replicates per cell line) conditions.




**Supplementary Figure 2.** Metabolic differences between acute myeloid leukemia cell lines grown in hypoxic and non-hypoxic environments.

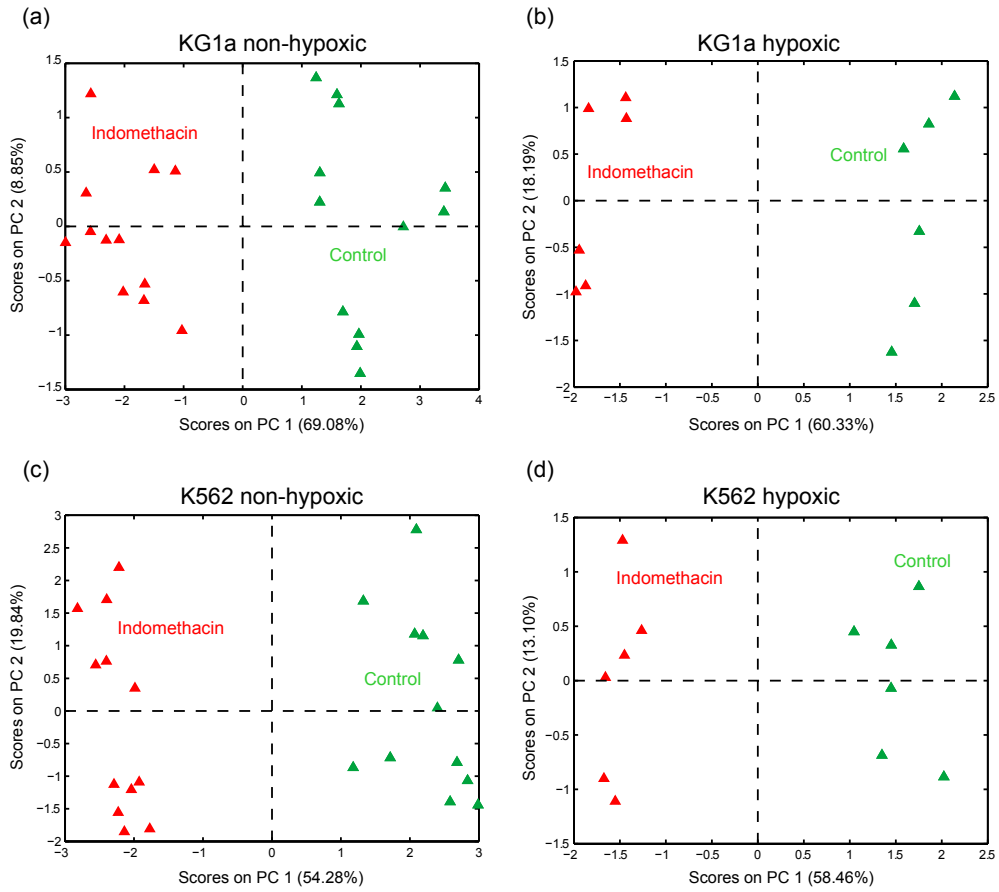
Schematic representation of the metabolic pathways showing the most relevant metabolic changes induced by hypoxia and common to untreated KG1a and K562 AML cell lines. Metabolites in green/red have *significantly* increased concentrations in both KG1a and K562 cell lines grown in a non-hypoxic/hypoxic environment; metabolites in black are detected/identified in the NMR spectra but change differently or non-significantly in the two cell lines; metabolites in grey were not detected in the NMR spectra.




**Supplementary Figure 3.** Metabolic differences between acute myeloid leukemia cell lines grown in hypoxic and non-hypoxic environments.

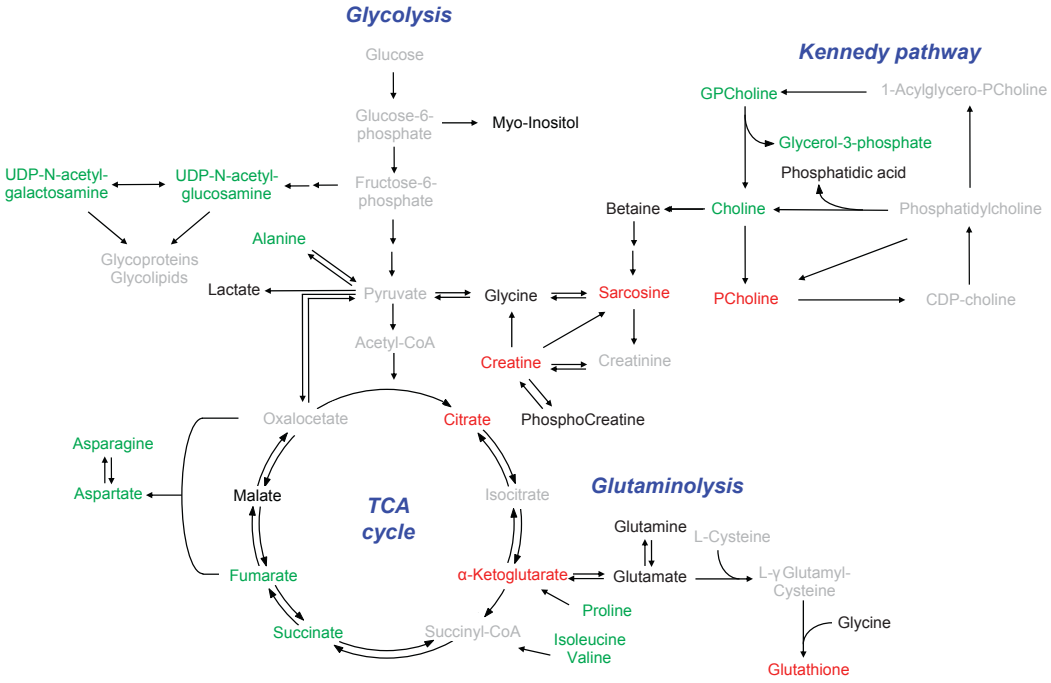
Average concentrations of selected metabolites in untreated KG1a and K562 AML cells grown in non-hypoxic (average of 12 samples) or in hypoxic (average of 6 samples) conditions.

(Val, valine; Ile, isoleucine; Leu, leucine; Lac, lactate; Ala, alanine; Ace, acetate; Pro, proline; UDP-GlcNAc, uridine-5'-diphospho-N-acetyl-glucosamine; Glu, glutamate; GSH, glutathione; Gln, glutamine; Suc, succinate; a-KG, a-ketoglutarate; Cit, citrate; Asp, aspartate; Fum, fumarate; PC, phosphocholine; *: $p<0.01$; **: $p<0.0001$).




**Supplementary Figure 4.** Metabolic differences induced by treatment with indomethacin in acute myeloid leukemia cell lines grown under non-hypoxic or hypoxic conditions.

Scores plot obtained from the principal component analysis of the $^{1}$H projected *J*-resolved NMR datasets of untreated (green symbols) and indomethacin treated (red symbols) KG1a cells grown in **a)** non-hypoxic and **b)** hypoxic conditions and K562 cells grown in **c)** non-hypoxic and **d)** hypoxic conditions.




**Supplementary Figure 5.** Metabolic differences induced by treatment with indomethacin in acute myeloid leukemia cell lines grown in non-hypoxic conditions. Schematic representation of the metabolic pathways showing the most relevant metabolic changes induced by treatment with indomethacin and common to both KG1a and K562 cell lines grown under non-hypoxic conditions. Metabolites in green/red have *significantly* increased/decreased concentrations in both KG1a and K562 cell lines treated with indomethacin and grown in a non-hypoxic environment; metabolites in black are detected/identified in the NMR spectra but change differently or not-significantly in the two cell lines; metabolites in grey were not detected in the NMR spectra.