Physiological oxygen causes the release of volatile organic compounds from human pluripotent stem cells with possible roles in maintaining self-renewal and pluripotency

Sara Barreto [1,†], Mohammed A. Al-Zubaidi [1,2,†], Tina P. Dale [1], Amy J. Worrall [1], Zoher Kapace [3], Susan J. Kimber [3], Josep Sulé-Suso [1,4], Nicholas R. Forsyth [1,*] and Abigail V. Rutter [1,*]

[1] School of Pharmacy and Bioengineering, Keele University, Stoke-on-Trent, Staffordshire, UK; s.barreto-francisco@keele.ac.uk (S.B.); m.al_zubaidi@uomustansiriyah.edu.iq/mohmsc82@gmail.com (M.A.A.-Z.); t.p.dale@keele.ac.uk (T.P.D.); a.j.worrall1@keele.ac.uk (A.J.W.); zoher.kapacee@manchester.ac.uk (Z.K.); sue.kimber@manchester.ac.uk (S.J.K.); josep.sule-suso@uhnm.nhs.uk (J.S.-S.); n.r.forsyth@keele.ac.uk (N.R.F.); a.v.rutter@keele.ac.uk (A.V.R.)

[2] Department of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

[3] Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, University of Manchester, Oxford Rd, Manchester M13 9PT

[4] Cancer Centre, University Hospital of North Midlands, Newcastle Road, Stoke-on-Trent, Staffordshire, UK

* Correspondence: a.v.rutter@keele.ac.uk; n.r.forsyth@keele.ac.uk

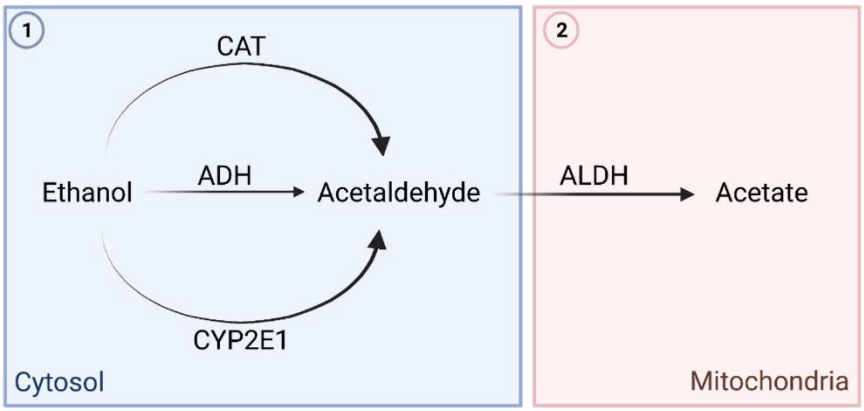
† Co-first author, these authors contributed equally to this work

Supplementary Materials:

Table S1. Primer design and annealing temperature of the genes investigated in this study.

Gene of interested	Forward Primer	Reverse Primer	Annealing temperature (°C)
ACTB	5′ GCCACGGCTGCTTCCAGC 3′	5′ AGCCATGCCAATCTCATCTT 3′	57
POU5F1	5′ GCAATTTGCCAAGCTCCTGAAGCAG 3′	5′ CATAGCCTGGGGTACCAAAATGGGG 3′	55
NANOG	5′ GGTGGCAGAAAAACAACTGGC 3′	5′ TGCAGGACTGCAGAGATTC 3′	56
SOX1	5′ CCAGGAGAACCCCAAGAGGC 3′	5′ CGGCCAGCGAGTACTTGTCC 3′	56
AFP	5′ AAGGATACCAGGAGTTATTGG 3′	5′ GTTGGCATATGAAGAAGTGC 3′	56
Brachyury	5′ GCATAAGTATGAGCCTCGAA 3′	5′ GTTGTCAGAATAGGATTGGGA 3′	56
OTX2	5′ CTCGCCACATCTACTTTGATA 3′	5′ GGCGGTTGCTTAAGATAAGA 3′	57
SOX17	5′ GCAAGATGCTGGGCAA 3′	5′ GCCGGTACTTGTAGTTGG 3′	56
hTERT	5′ GCAGCTCCCATTTCATCAGC 3′	5′ CAGGATGGTCTTGAAGTCTG 3′	55
ADH4	5′ CGCATTCAGATCATTGCTAC 3′	5′ ACTGGTTCCAAAGAAATGGT 3′	56
ADH5	5′ CGAATCAAGATCATTGCCAC 3′	5′ CTGGCATTAATCCTTTCCCT 3′	56
CYP2E1	5′ CTGGCTCCAGCTTTACAATA 3′	5′ AGAATCAGGAGCCCATATCT 3′	56
ALDH1A1	5′ TCATTCCTTGGAATTTCCCG 3′	5′ GCCCATAACCAGGAACAATA 3′	57
ALDH1A3	5′ GAAGAAGGAGATAAGCCCG 3′	5′ CTGCAAAGTATCTGAGGGTT 3′	56
ALDH6A1	5′ CTTGCTCCGCTATCAACAA 3′	5′ AGGAAGGTATTTCCACACAC 3′	57

A



B

Metabolic reaction	Enzyme	Gene
1	Alcohol dehydrogenase (ADH)	ADH1B ADH1C ADH1A ADH5 ADH4 ADH7 ADH6
1	Cytochrome P450 family 2 subfamily E member 1	CPYP2E1
1	Catalase	CAT
2	Aldehyde dehydrogenase (ALDH)	ALDH2 ALDH1A1 ALDH1B1 ALDH7A1 ALDH3A1 ALDH1L1 ALDH3A2 ALDH5A1 ALDH1A2 ALDH1A3 ALDH18A1 ALDH4A1 ALDH9A1 ALDH3B1 ALDH6A1 ALDH1L2 ALDH16A1 ALDH8A1 ALDH3B2

Figure S1. Genes involved in ethanol metabolism that were selected for expression analysis. **(A)** Diagram of ethanol metabolism. In the cytosol, ethanol is oxidized by various enzymes, such as alcohol dehydrogenase (ADH), cytochrome P450 family 2 subfamily E member 1 (CYP2E1) and catalase (CAT), into acetaldehyde, which is then further oxidize into acetate by aldehyde dehydrogenase (ALDH) enzymes in the mitochondria. **(B)** Table of the selected genes known to be involved in stages of ethanol metabolism.

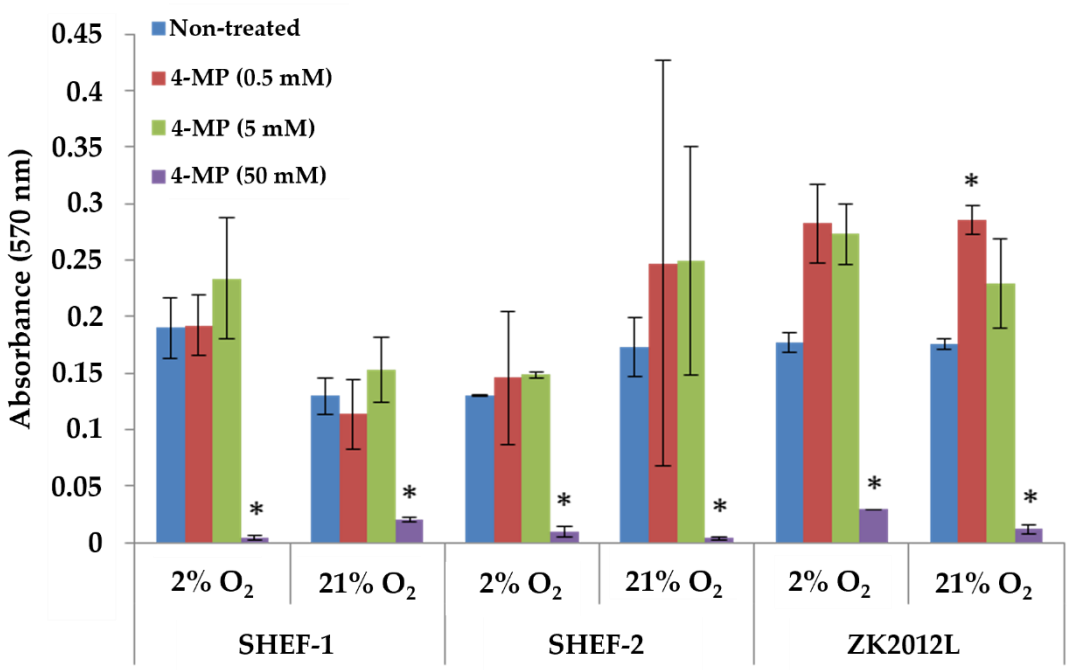


Figure S2. Viability of hPSCs exposed to different concentrations of the ADH and CYPE1 inhibitor 4-methyl pyrazole (4-MP) cultured in both 21% and 2% O_2. Viability was assessed using the MTT assay. MTT data demonstrated that the concentrations 0.5 mM and 5 mM had no significant effect on the viability of the hPSCs, except for hiPSC ZK2021L treated with 0.5 mM at 21%, which showed a significant increase in MTT reduction and consequently, higher number of viable cells were present. The concentration of 50 nM was toxic to all hPSCs. X-axis shows each hPSC line (SHEF-1, SHEF-2 and ZK2021L) cultured in both 2% O_2 and 21% O_2 conditions. Y-axis represents MTT absorbance at 570 nm. Blue bars indicate untreated cells (control), while red, green, and purple bars represent 0.5, 5 and 50 mM 4-MP concentrations, respectively. Errors bar are +/-SD. Asterisk indicates significant difference ($p<0.01$) be-tween non-treated and 4-MP treated samples.

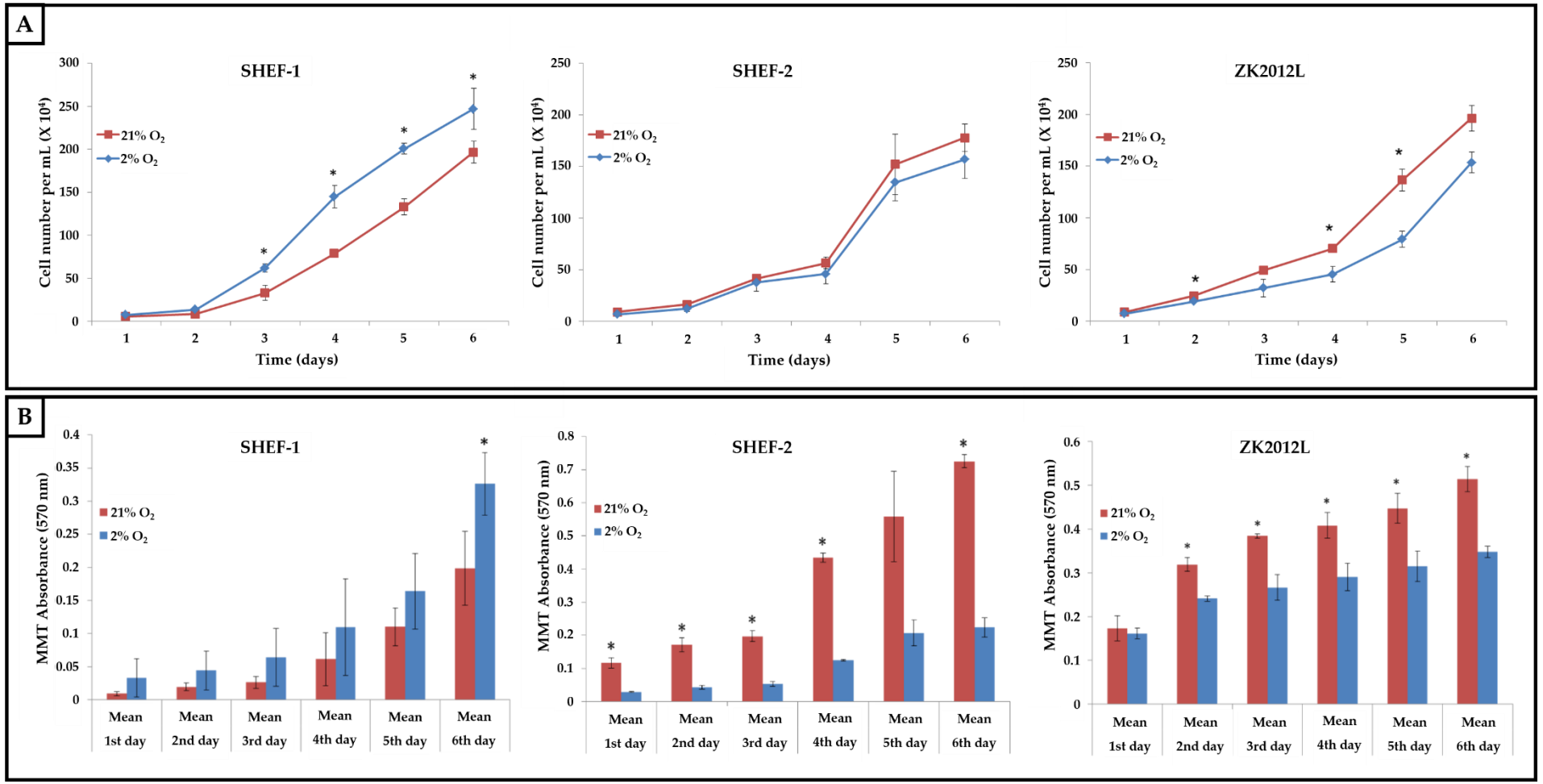


Figure S3. Influence of physioxia (2% O_2) and air oxygen (21% O_2) on the proliferation and metabolic activity of hPSCs. **(A)** Effects of O_2 on the proliferation of hPSCs. Cell counts were performed daily over a 6-day period. SHEF-1 cell numbers increased significantly after day 3 in 2% versus 21% O_2 ($p<0.05$). In contrast, both SHEF-2 and ZK2012L numbers raised from day 2 onwards in 21% versus 2%, and this was significant for ZK2012L at days 2, 4 and 5 ($p<0.05$). X-axis indicates time (in days), while y-axis shows cells x 10^4/mL. **(B)** Effects of O_2 on the mitochondrial activity of hPSCs assessed using MTT assay. MTT was performed at each day for a period of 6-days post-seeding. SHEF-1 displayed a significant increase in MTT at day 6 in 2% O_2, whereas both SHEF-2 and ZK2012L exhibited significant MTT declines across all time points tested (except for SHEF-2 at day 5 and ZK2012L at day 1). X-axis indicates time (in days), whereas y-axis indicates MTT absorbance (570 nm). Red and blue represent hPSCs cultured at 21% O_2 and 2% O_2, respectively. Error bars represent +/- SD. Asterisk (*) indicates $p<0.05$ between conditions.