

Supplemental information

**Chemical inhibition of oxygen-sensing prolyl
hydroxylases impairs angiogenic competence of human
vascular endothelium through metabolic reprogramming**

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SUPPLEMENTAL INFORMATION

Figure S1. DMOG treated endothelial cells maintain viability and stabilize HIF- α (related to Figure 1). (A) Representative images of immunostaining of HIF-1 α and HIF-2 α proteins in control and DMOG-treated cells. **Scale bars, 50 μ m.** (B) Immunoblot analysis of HIF-1 α and HIF-2 α in nuclear extracts isolated from control and DMOG-treated cells. (C) Shown are percentages of Annexin V/propidium iodide (PI) positive cells after 24 h treatment with vehicle (control) or DMOG (1 mM). Data are pooled from 3 independent experiments and represented as mean \pm SEM. Statistics were determined by two-tailed t-test. **, P<0.01.

Figure S2. DMOG induces broad transcriptional reprogramming in endothelial cells (related to Figure 2). (A) Volcano plot of RNA-seq data showing number of significantly up-regulated and down-regulated genes in DMOG-treated cells compared to control. (B) Heatmaps showing the top 50 up-regulated (top) and top 50 down-regulated genes (bottom) in the transcriptome of HPAECs treated with DMOG for 24h. (C) Pathway map illustrating transcriptional changes induced by DMOG in genes involved in glycolysis, TCA cycle and ETC. Up-regulated and down-regulated genes by DMOG are shown in red and blue color respectively, whereas genes in black color showed no significant transcriptional changes. TCA, tricarboxylic acid cycle; ETC, electron transport chain.

Figure S3. DMOG mimics transcriptional response to hypoxia (related to Figure 2) (A) Venn diagrams show the DEGs demonstrating uniquely or commonly up-regulation (left graph) or down-regulation (right graph) by exposure to hypoxia or DMOG (B). Bubble charts for Hallmark pathways of overlapped up-regulated (left) or down-regulated DEGs (right) in HPAEC by DMOG and hypoxia. (C) Table shows the up-regulated glycolytic genes (left table) and down-regulated genes of MC1 following hypoxia exposure.

Figure S4. Metabolomic analysis of DMOG treated ECs compared to control (related to Figure 3). (A) Experimental workflow for the metabolomic analysis of cellular lysates and media from control and DMOG treated HPAEC. Shown in (B) and (C) are biochemical importance plots generated by random forest classification of metabolites measured in cells and media, respectively from control and DMOG-treated HPAEC.

Figure S5. Supplementation of membrane permeable derivatives of pyruvate (methyl pyruvate) and aspartate (methyl aspartate) do not affect angiogenic defects induced by DMOG (related to Figure 4). (A) Effect of pyruvate supplementation (2 mM, 1 mM and 0.5 mM) on HPAEC proliferation assessed by MTT assay. (B) Representative images of 2D scratch wound assay of control, DMOG, DMOG + pyruvate and pyruvate (0.5mM) treated cells and semi-quantitative analysis of healed area after 24h. (C) Effect of aspartate supplementation (5mM, 2mM and 0.5mM) on HPAEC proliferation assessed by MTT assay. (D) Representative images of 2D scratch wound assay of control, DMOG, DMOG + aspartate and aspartate (0.5mM) treated cells and semi-quantitative analysis of healed area after 24 h. (E) Representative images of tubes formed at indicated time points in control, DMOG, DMOG + pyruvate, DMOG + aspartate, pyruvate (0.5 mM) and aspartate (0.5 mM) treated cells and semi-quantitative analysis of different parameters at 20 h time point. Data are pooled from 3 independent experiments and represented as mean \pm SEM. Statistics were determined by one-way ANOVA with Sidak correction for multiple comparisons. ***, P<0.001; ****, P< 0.0001; ns, not significant. Asterisks/ns above bars indicate significance level between control and treated group, whereas asterisks/ns above the lines indicate significance level between DMOG and DMOG + pyruvate or DMOG + aspartate treated groups. **Scale bars, 200 μ m.**

Figure S6. DCA treatment does not rescue angiogenic defects induced by DMOG (related to Figure 4). (A) Representative immunoblots of Phospho-PDHE1 α , total PDHE1 α and GAPDH following 24 h treatment with 2, 5 and 10 mM DCA. Quantitative analysis of the blots is shown in the lower graph. (B) Effect of DCA on HPAEC proliferation assessed by BrdU assay. **Scale bar, 50 μ m.** (C) Representative images of 2D scratch wound assay of control, DMOG, DMOG+DCA and DCA (10 mM) treated cells and semi-quantitative analysis of healed area after 24 hr. **Scale bar, 200 μ m.** (D) Representative images of tubes formed at indicated time points in control, DMOG, DMOG+DCA, and DCA (10mM) treated cells and semi-quantitative analysis of different parameters at 20 h time point. **Scale bar, 200 μ m.** Data are pooled from 3 independent experiments and represented as mean \pm SEM. Statistics were determined by one-way ANOVA with Sidak correction for multiple comparisons. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not significant. Asterisks/ns above bars indicate significance level between control and treated group, whereas asterisks/ns above the lines indicate significance level between DMOG and DMOG + DCA treated groups.

Figure S7. Effect of citrate on cell cycle progression, HIF- α protein and NAD⁺ levels (related to Figure 4). HPAEC were treated with different compounds for 24 h. (A) Histogram of cell cycle analysis for control, DMOG, DMOG + citrate, and citrate (0.5 mM) treated cells. (B) Representative images of immunostaining for HIF-1 α and HIF-2 α proteins in control, DMOG, DMOG + citrate and citrate treated cells. **Scale bars, 50 μ m.** (C) Immunoblot analysis of HIF-1 α and HIF-2 α in nuclear extracts isolated from control, DMOG, DMOG + citrate, and citrate treated cells. (D) Relative abundance of NAD⁺ levels in cellular extracts from control, DMOG, DMOG + citrate, and citrate treated cells. Data are represented as mean \pm SEM. Statistics were determined by one-way ANOVA with Sidak correction for multiple comparisons. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P< 0.0001; ns, not significant. Asterisks/ns above bars indicate significance level between control and treated group, whereas asterisks/ns above lines indicate significance level between DMOG and DMOG + citrate treated groups. D + Citr., DMOG + citrate treated group.

Figure S1. DMOG treated endothelial cells maintain viability and stabilize HIF- α .

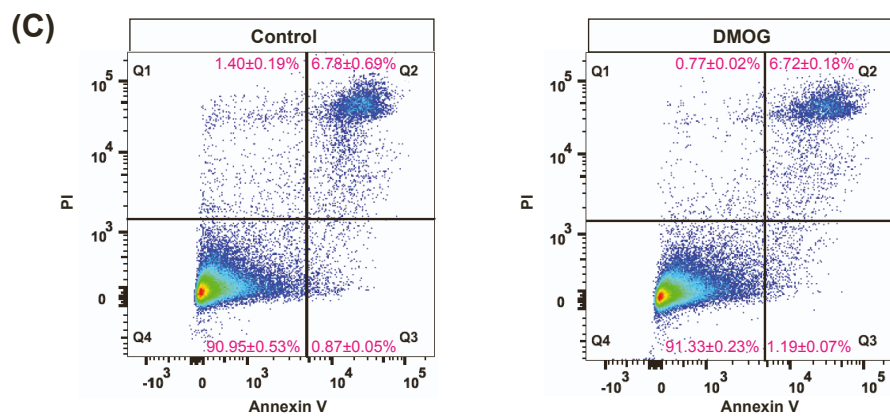
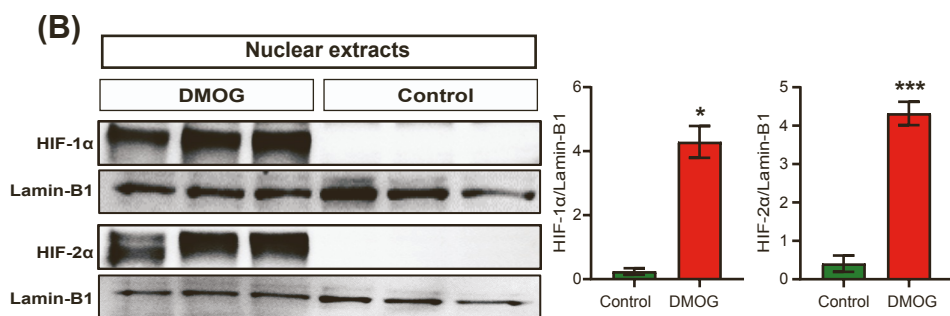
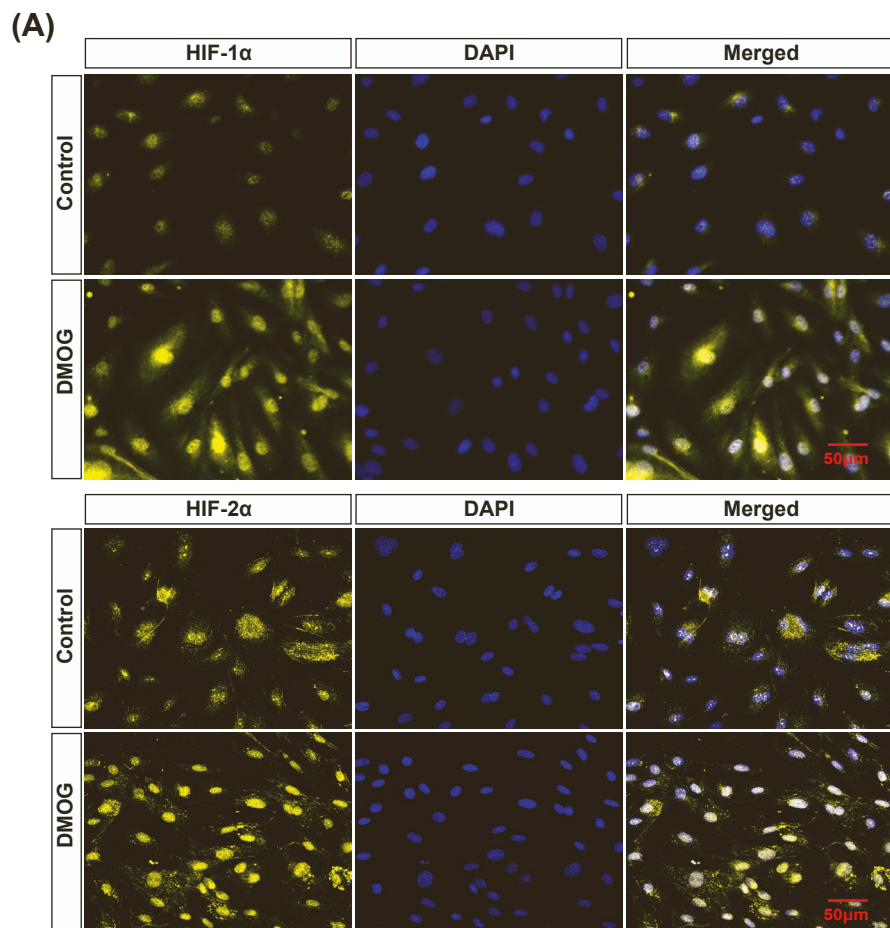


Figure S2. DMOG induces broad transcriptional reprogramming in endothelial cells.

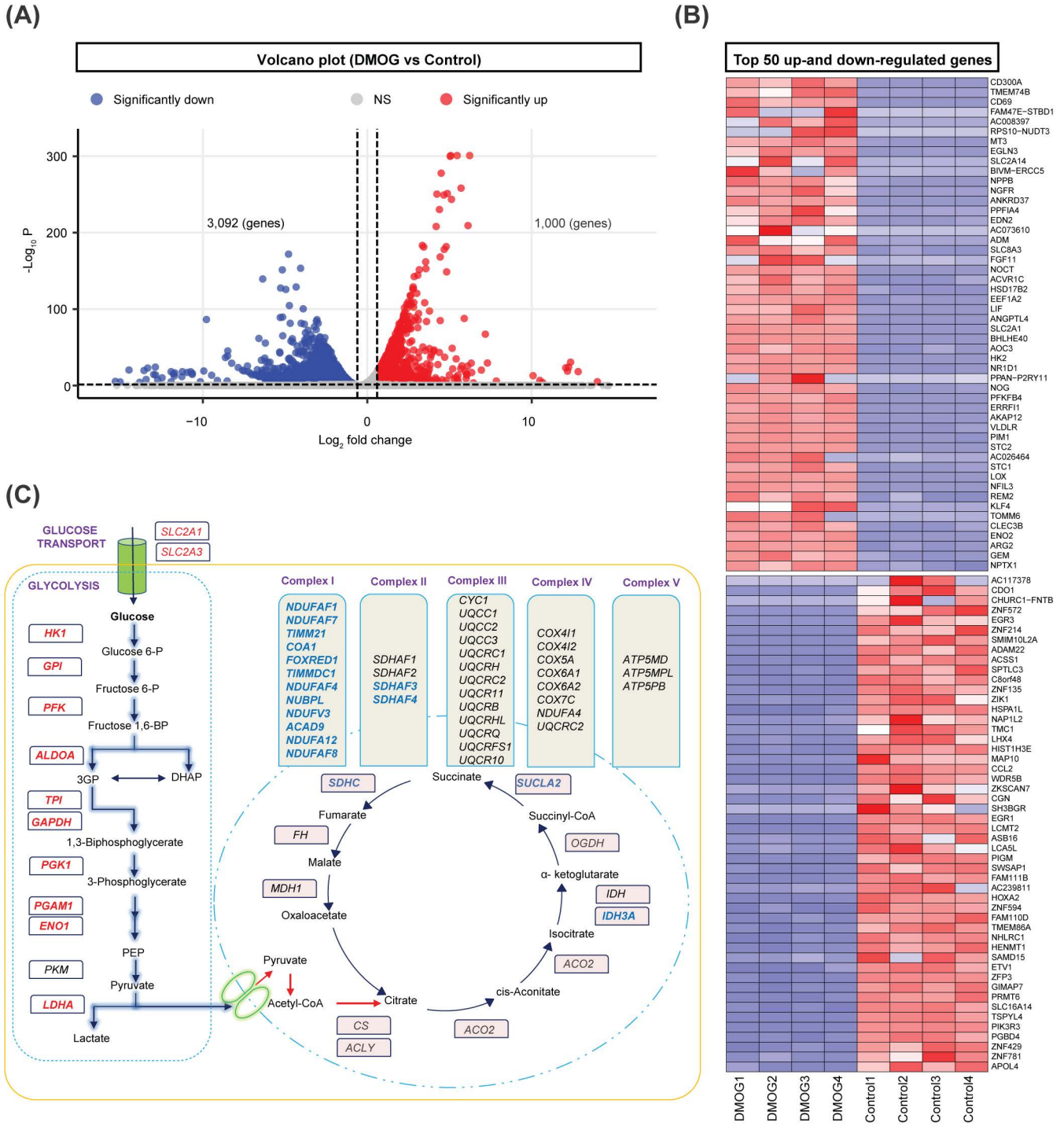
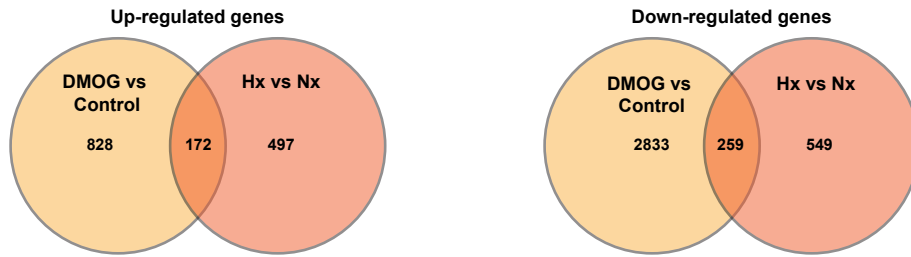
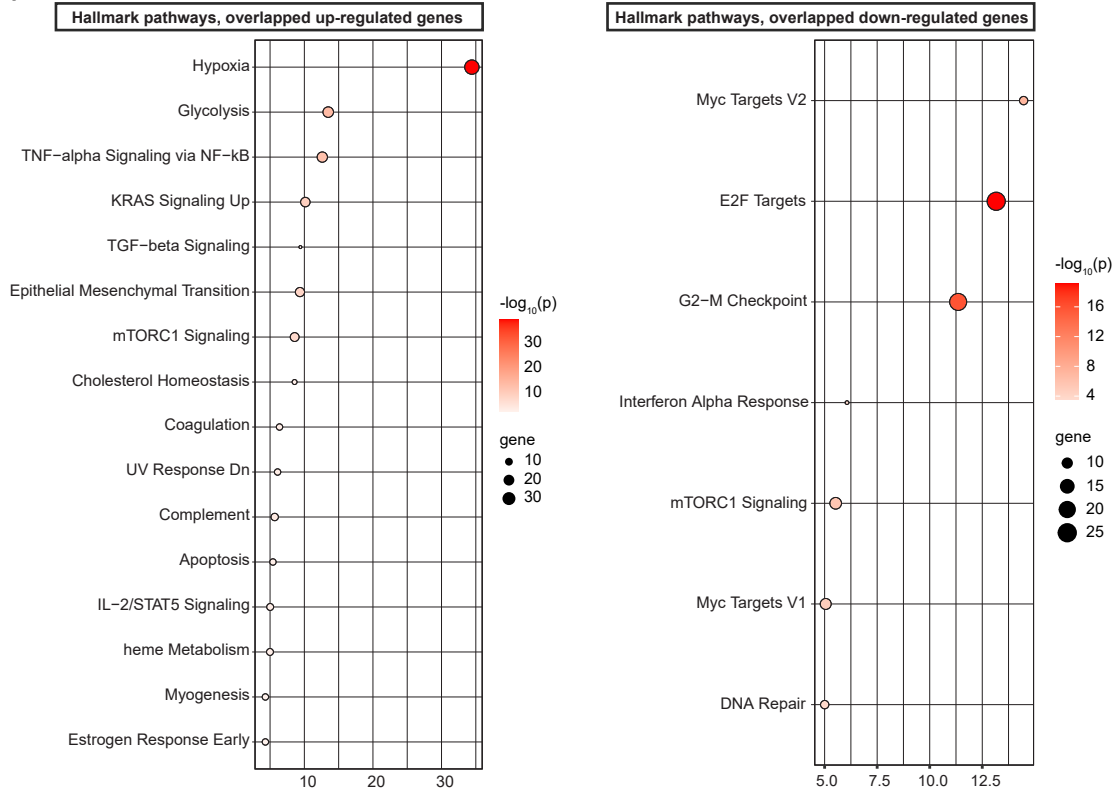


Figure S3. DMOG mimics transcriptional response to hypoxia.

(A)



(B)



(C)

Glycolysis genes	Padj	Fold change (Hx/Nx)
<i>LDHA</i>	1.60E-06	1.37
<i>PGAM1</i>	1.87E-02	1.15
<i>PGK1</i>	7.98E-03	1.13
<i>GAPDH</i>	2.29E-08	1.37
<i>TPI1</i>	8.70E-13	1.39
<i>ALDOA</i>	1.04E-39	1.92
<i>PFKFB3</i>	4.73E-04	1.25
<i>SLC2A3</i>	1.21E-73	2.77
<i>SLC2A1</i>	1.87E-196	6.12

MC1 genes	Padj	Fold change (Hx/Nx)
<i>NDUFA12</i>	2.06E-02	0.83
<i>ACAD9</i>	1.00E-06	0.71
<i>NUBPL</i>	4.02E-02	0.74
<i>NDUFAF4</i>	1.01E-02	0.74
<i>FOXRED1</i>	3.15E-03	0.70
<i>TIMM21</i>	7.97E-05	0.71
<i>NDUFAF1</i>	2.42E-02	0.79

Figure S4. Metabolomic analysis of DMOG treated ECs compared to control.

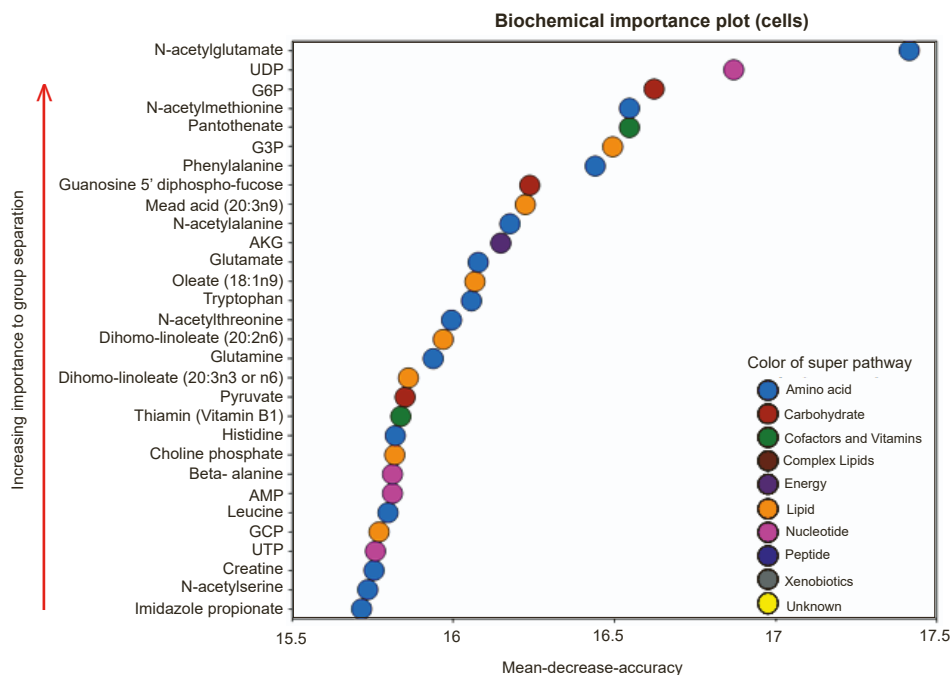
(A)

Groups	Matrix (n)	
	Cells	Media
Control	5	5
DMOG	5	5

UPLC-MS/MS ↓ GC/MS

Welch's Two-sample t-test	DMOG/Control			
	Cells		Media	
Total biochemicals (p ≤ 0.05)	126		45	
Biochemicals (▲▼)	70	56	19	26

(B)



(C)

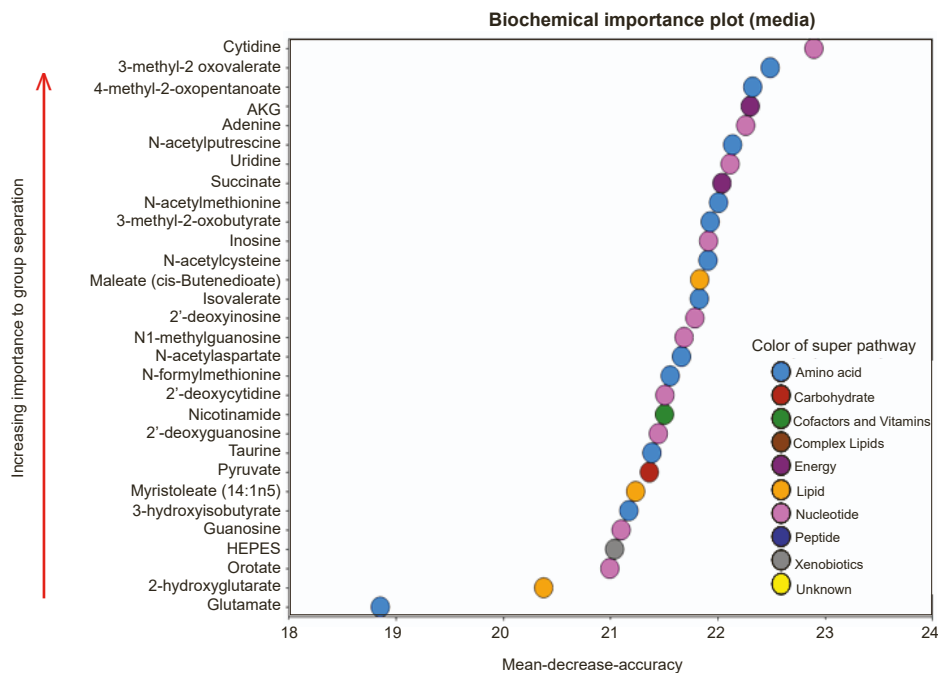


Figure S5. Supplementation of pyruvate and aspartate do not affect angiogenic defects induced by DMOG.

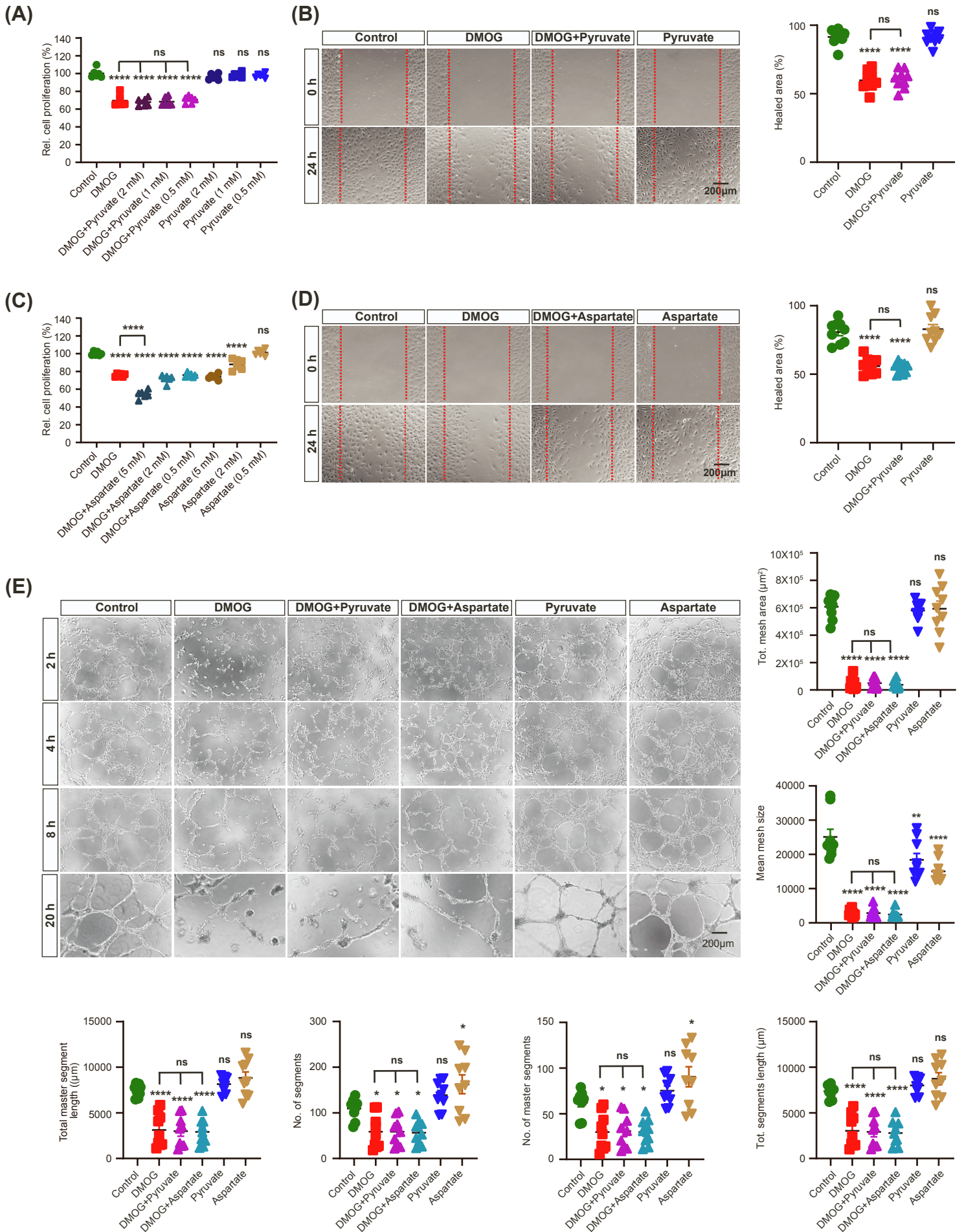


Figure S6. DCA treatment does not rescue angiogenic defects induced by DMOG.

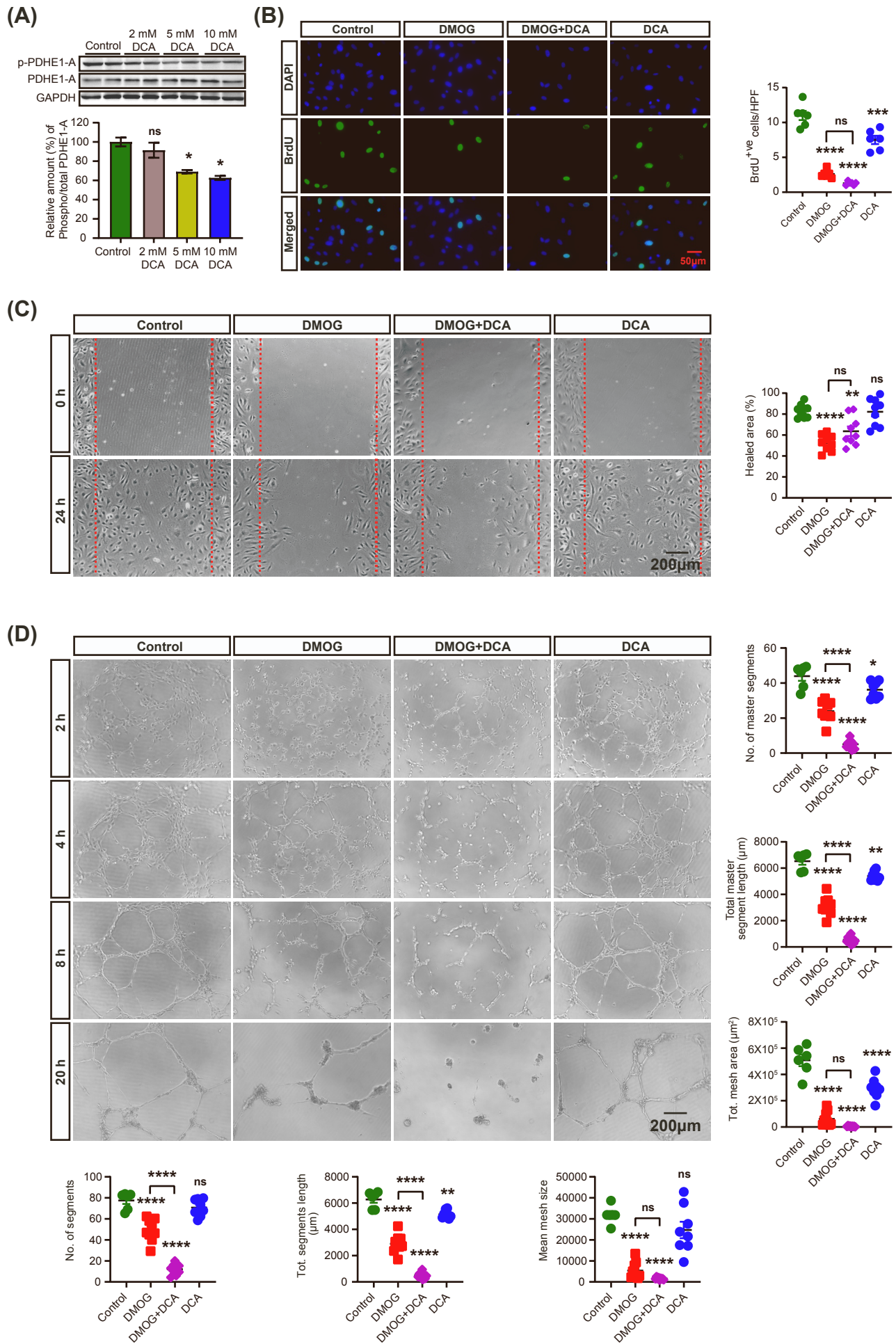


Figure S7. Effect of citrate on cell cycle progression, HIF- α protein and NAD⁺ levels.

