

# Supplementary Materials: Reactive Oxygen Species Differentially Modulate the Metabolic and Transcriptomic Response of Endothelial Cells

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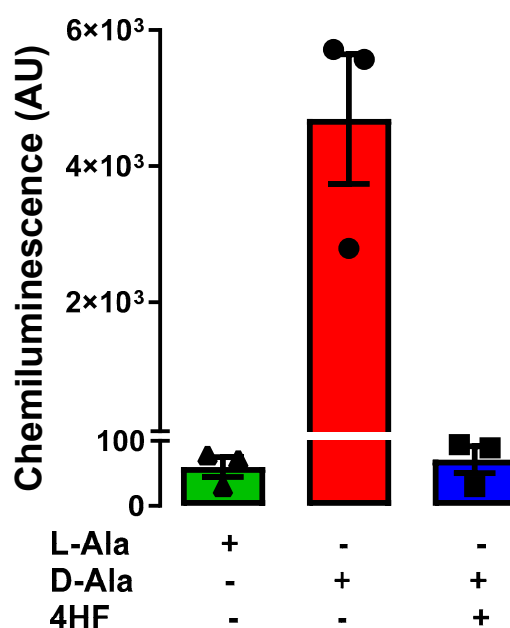
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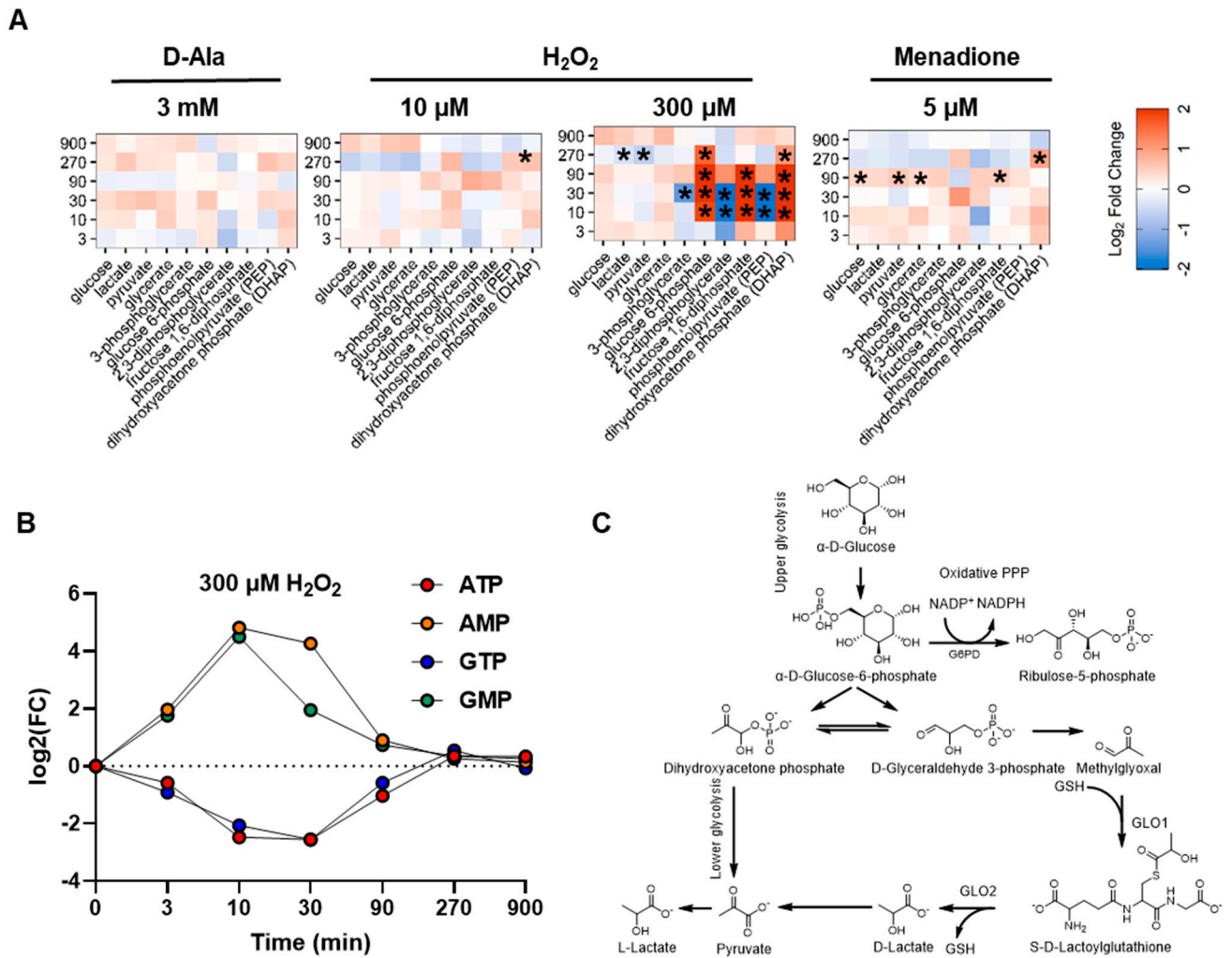
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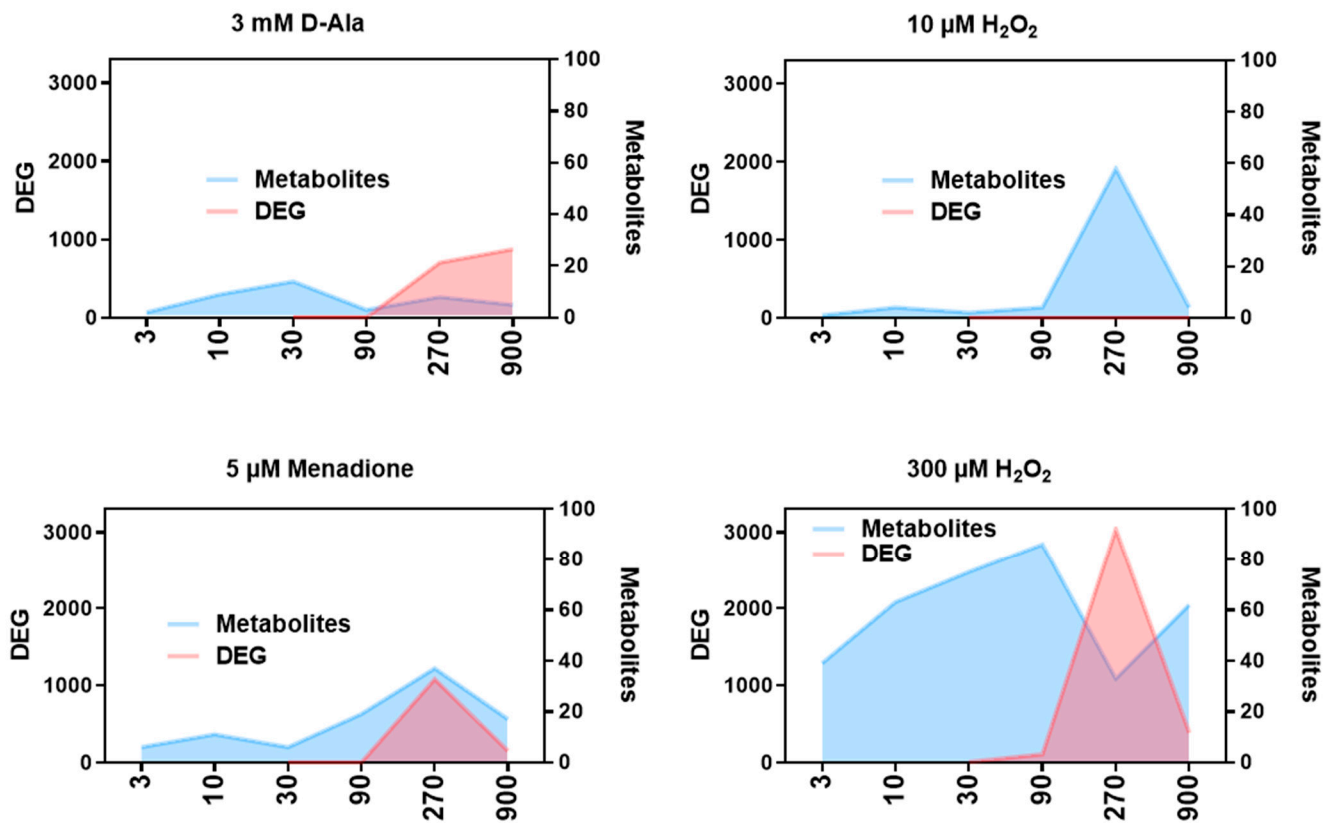
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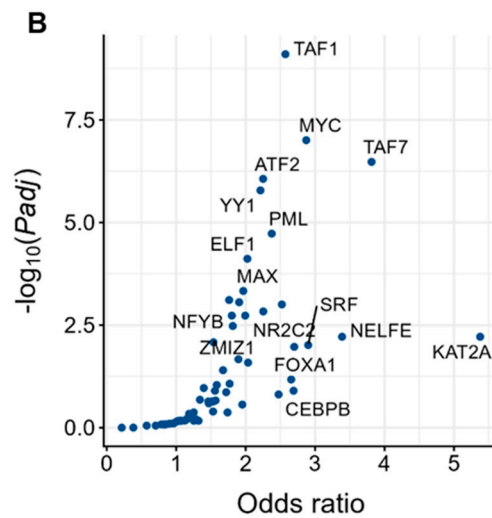
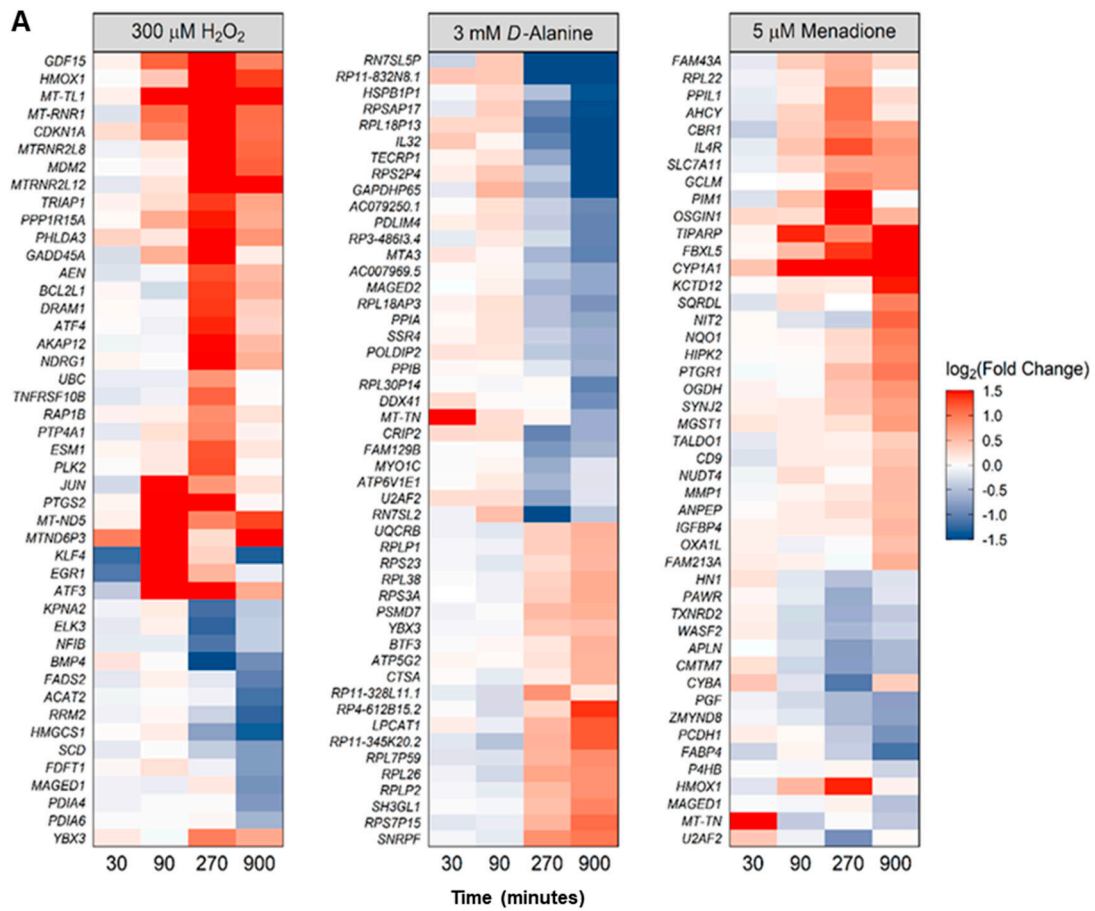
**Supplementary Figure S1: H<sub>2</sub>O<sub>2</sub> production by HUVEC-DAO.** H<sub>2</sub>O<sub>2</sub> (n=3) assessed by chemiluminescence with luminol (100 μM)/ HRP (1 U/mL) after exposure to D- or L-alanine with or without pre-incubation (10 min) with the DAO inhibitor 4HF (1 μmol/L) (n=3).



**Supplementary Figure S2: Changes in glycolysis and nucleotide metabolism in HUVEC in response to different oxidative stimuli. (A): Heat maps of glucose metabolism. (B): Changes in ATP, AMP, GTP and GMP after exposure to 300 μM H<sub>2</sub>O<sub>2</sub>. (C): Fates of glucose and S-lactoylglutathione pathway.**

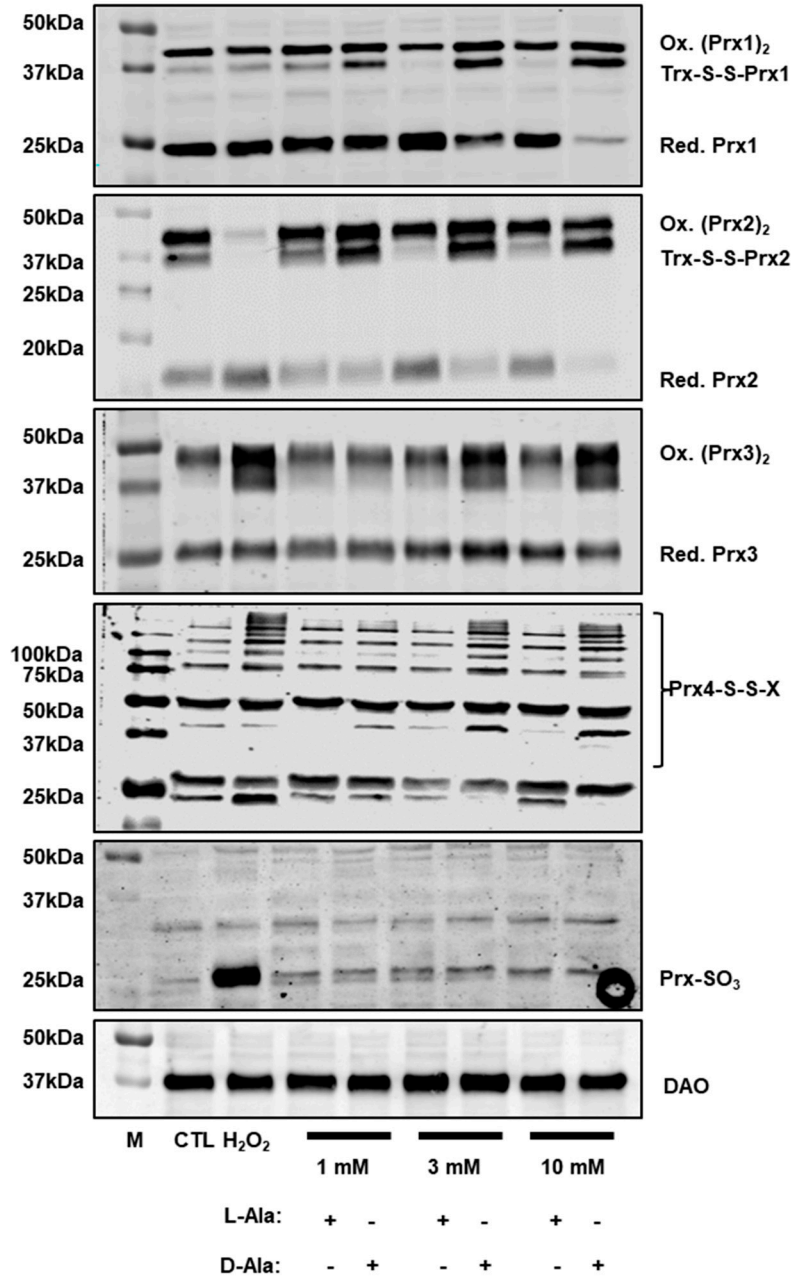


**Supplementary Figure S3: High degree of dissimilarity between effects of different ROS exposures to HUVEC.** Time course changes in metabolomics and transcriptomics of HUVEC in response to different oxidative stimuli.

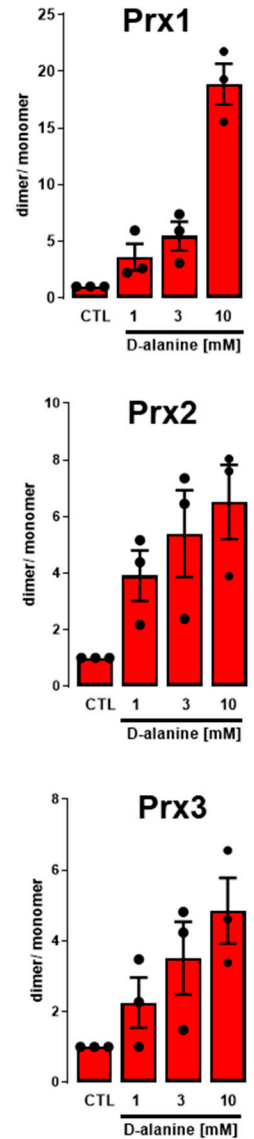


**Supplementary Figure S4: Differentially expressed genes in HUVEC in response to different ROS. (A): Heatmap of DEGs. (B): Transcription factor analysis performed with ENCODE and CheA for the genes commonly regulated by 300  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , 3 mM *D*-Ala and 5  $\mu\text{M}$  menadione.**

**A**



**B**



**Supplementary Figure S5: DAO-derived H<sub>2</sub>O<sub>2</sub> results in a dose-dependent oxidation of peroxiredoxins in HEK-DAO. (A):** Representative redox western blot for Prx1, Prx2, Prx3, Prx4 and Prx-SO<sub>3</sub> after exposure to different concentration of D- or L-Ala. **(B):** Quantification of redox western blotting by densitometry (n=3).

3 mM D-Ala/CTL						
	3'	10'	30'	90'	270'	900'
↑	1	9	14	1	6	3
↓	1	0		2	2	2
<b>Total</b>	2	9	14	3	8	5

	10 $\mu$ M H <sub>2</sub> O <sub>2</sub> /CTL						300 $\mu$ M H <sub>2</sub> O <sub>2</sub> /CTL					
	3'	10'	30'	90'	270'	900'	3'	10'	30'	90'	270'	900'
↑	0	4	1	4	2	3	23	39	44	56	23	60
↓	1	0	1	0	56	1	16	24	31	30	10	2
<b>Total</b>	1	4	2	4	58	4	39	63	75	86	33	62

5 $\mu$ M Menadione/CTL						
	3'	10'	30'	90'	270'	900'
↑	1	6	3	16	18	4
↓	5	5	3	3	19	13
<b>Total</b>	6	11	6	19	37	17

**Supplementary Table S1:** Summary of altered metabolites.