

# Supplementary Material

## **NMR spectroscopy-based metabolomic profile of tissue, perfusate, and bile from rat livers subjected to normothermic machine perfusion**

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**Supplementary material and methods**

**Supplementary Figure S1.** Schematic representation of the experimental workflow.

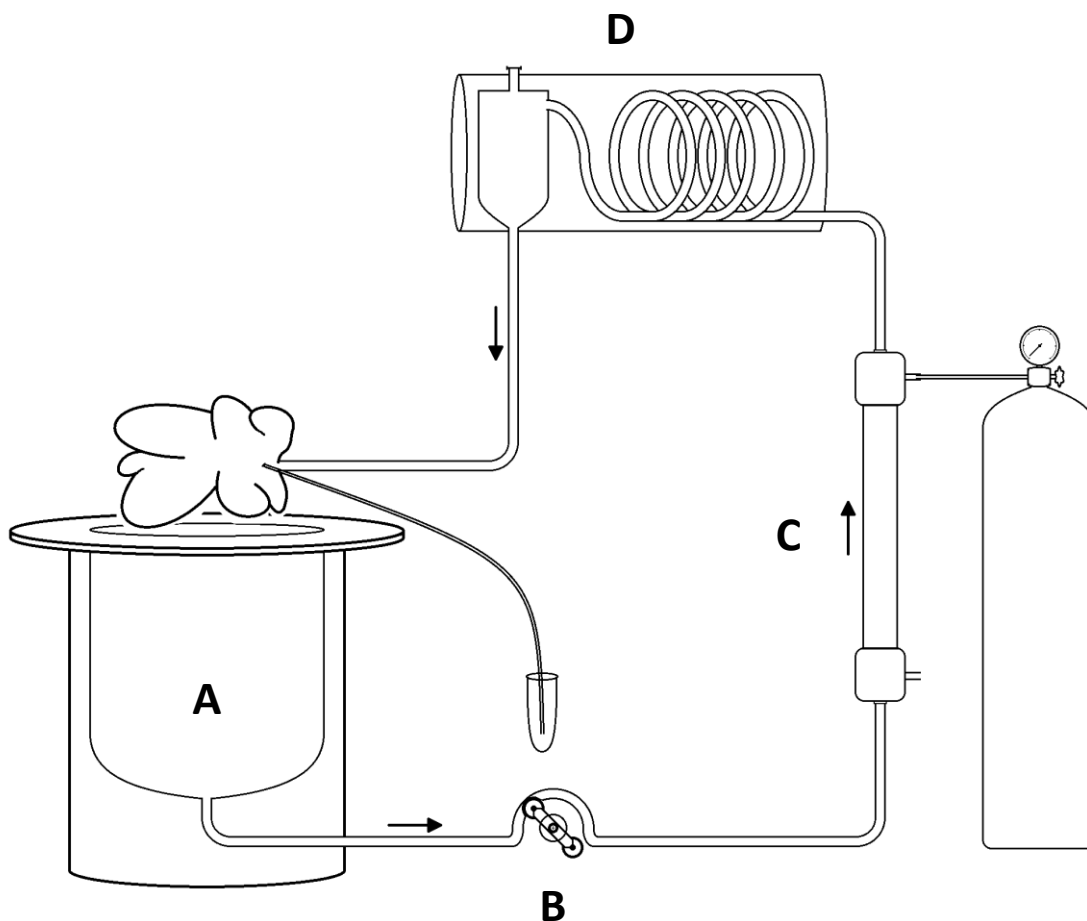
All groups received a surgery, further SCS, h-NMP and OxC-NMP had in-situ perfusion and SCS for 30 minutes. Finally, h-NMP and OxC-NMP groups as the name suggests had the NMP.

	Surgery	In-situ flushing	SCS (30 min)	NMP
Native				
SCS				
h-NMP				
OxC-NMP				

**Supplementary Figure S2.** Schematic representation of the ex-situ normothermic machine perfusion system.

The system was derived from an isolated lung perfusion system (Hugo Sachs Elektronik, Harvard Apparatus, March-Hugstetten, Germany) with disposable PVC tubes and membrane oxygenator (Micro-1 rat oxygenator, Kewei Rising Medical, Shenzhen, China). The perfusate flow was generated by a peristaltic pump and the membrane oxygenator received a gas mixture of 95% oxygen and 5% carbon dioxide (CO<sub>2</sub>). The liver, connected to the circuit using the portal vein cannula, was placed on a double layer of Parafilm® (Merck, KGaA, Darmstadt, Germany), previously modelled and perforated to allow the drainage of the perfusate to the reservoir. Recirculating water bath was set to maintain graft temperature at 37°C that was measured with a custom-made thermocouple. Pressures along the circuit were monitored downstream the peristaltic pump and upstream the portal cannula.

A: Water Jacked reservoir, B: Peristaltic Pump, C: Membrane oxygenator, D: Heating Coil/bubble trap.



**Supplementary Table S1.** Perfusion fluid composition

	<b>h-NMP</b>		<b>OxC-NMP</b>	
	<b>mL</b>	<b>Concentration</b>	<b>mL</b>	<b>Concentration</b>
<b>DMEM</b>	76		37.5	
<b>Oxyglobin® (13 g/dL hemoglobin)</b>	0	0	38.5	5 g/dL
<b>Human Albumin (20 %)</b>	20	4%	20	4%
<b>Pen-Strep (10000 U/mL – 10 mg/mL)</b>	1	100 U/mL – 0.1 mg/mL	1	100 U/mL – 0.1 mg/mL
<b>L-Glutamine (200 mM)</b>	1	0.292 g/L	1	0.292 g/L
<b>Human Insulin (100 UI/mL)</b>	2	2 U/mL	2	2 U/mL
<b>Total Volume</b>	100		100	

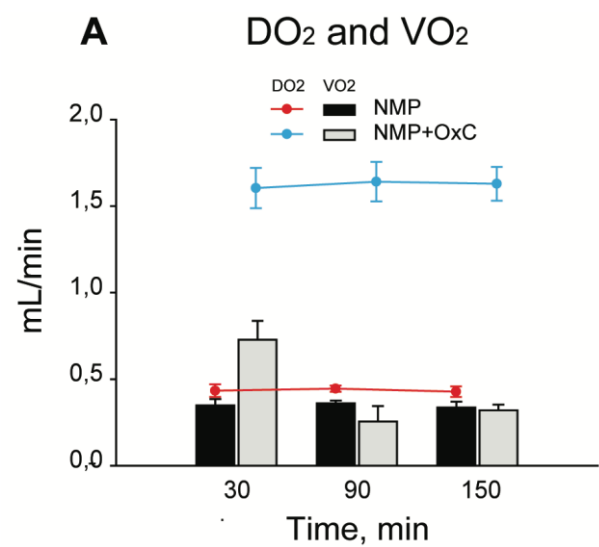
## Supplementary results

**Supplementary Table S2.** Baseline perfusate composition

	<b>h-NMP (n=5)</b>	<b>OxC-NMP (n=5)</b>	<b>P value</b>
<b>pH</b>	7.38 ± 0.09	7.16 ± 0.27	0.118
<b>Hb, g/dL</b>	-	5.2 ± 0.2	-
<b>Htc, %</b>	-	16 ± 1	-
<b>Gluc, g/dL</b>	80.00 ± 0.50	39.00 ± 1.00	<0.001
<b>Lac, mmol/L</b>	-	4.2 ± 0.2	-
<b>K<sup>+</sup>, mmol/L</b>	4.1 ± 0.1	3.6 ± 0.1	<0.001
<b>Na<sup>+</sup>, mmol/L</b>	149 ± 1	148 ± 2	0.166
<b>Ca<sup>2+</sup>, mmol/L</b>	0.72 ± 0.28	0.85 ± 0.10	0.429
<b>Cl<sup>-</sup>, mmol/L</b>	121 ± 2	113 ± 1	<0.001
<b>HCO<sub>3</sub><sup>-</sup>, mmol/L</b>	23 ± 2	13 ± 1	<0.001

**Supplementary Figure S3.** Oxygen delivery/consumption during NMP.

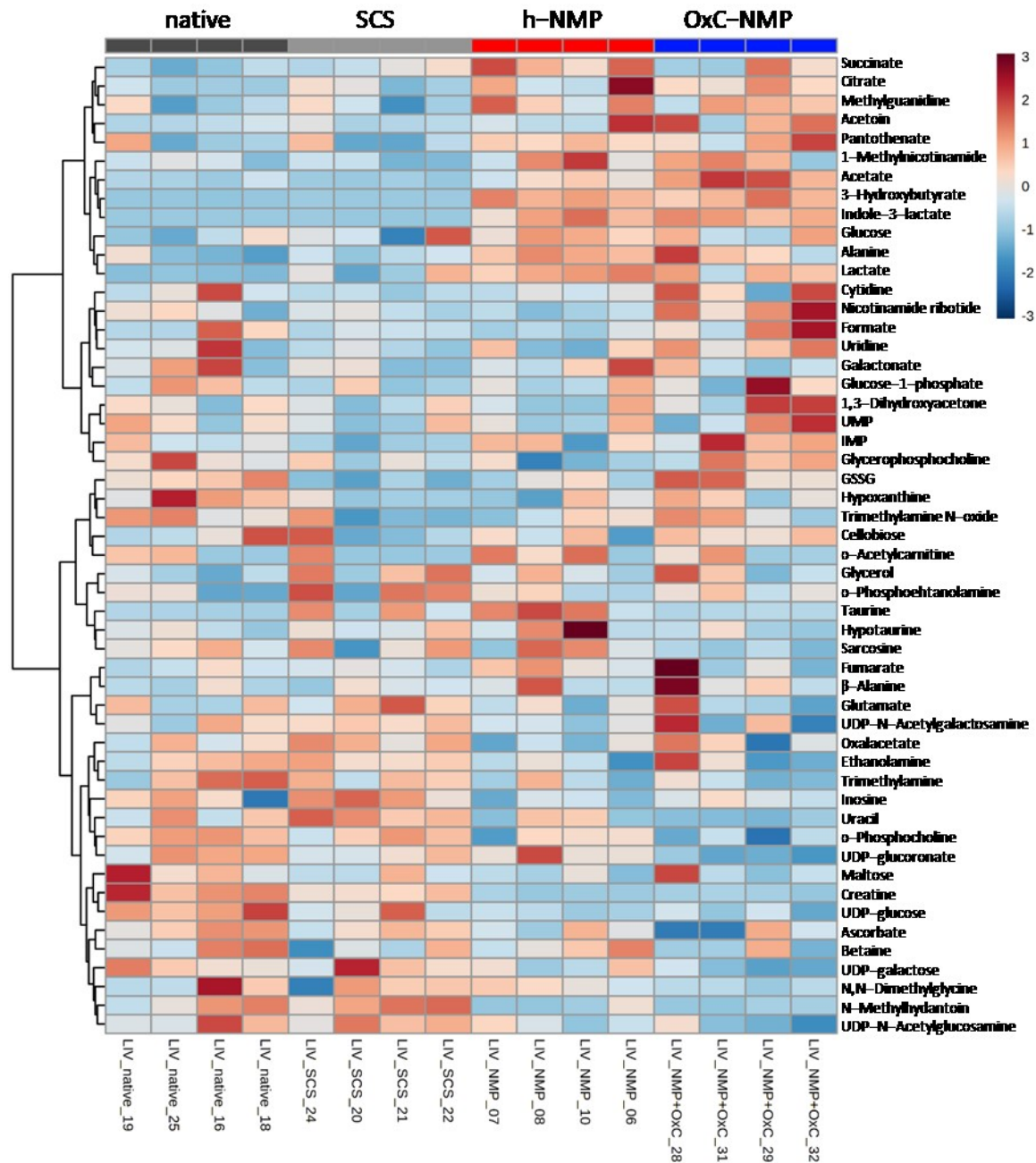
Two-way repeated measures ANOVA; Tukey’s post hoc test. DO<sub>2</sub>: p<0.001; VO<sub>2</sub>: p<0.05 at 5 min.





**Supplementary Figure S4.** Heatmap representing the metabolite concentrations in liver tissue homogenates.

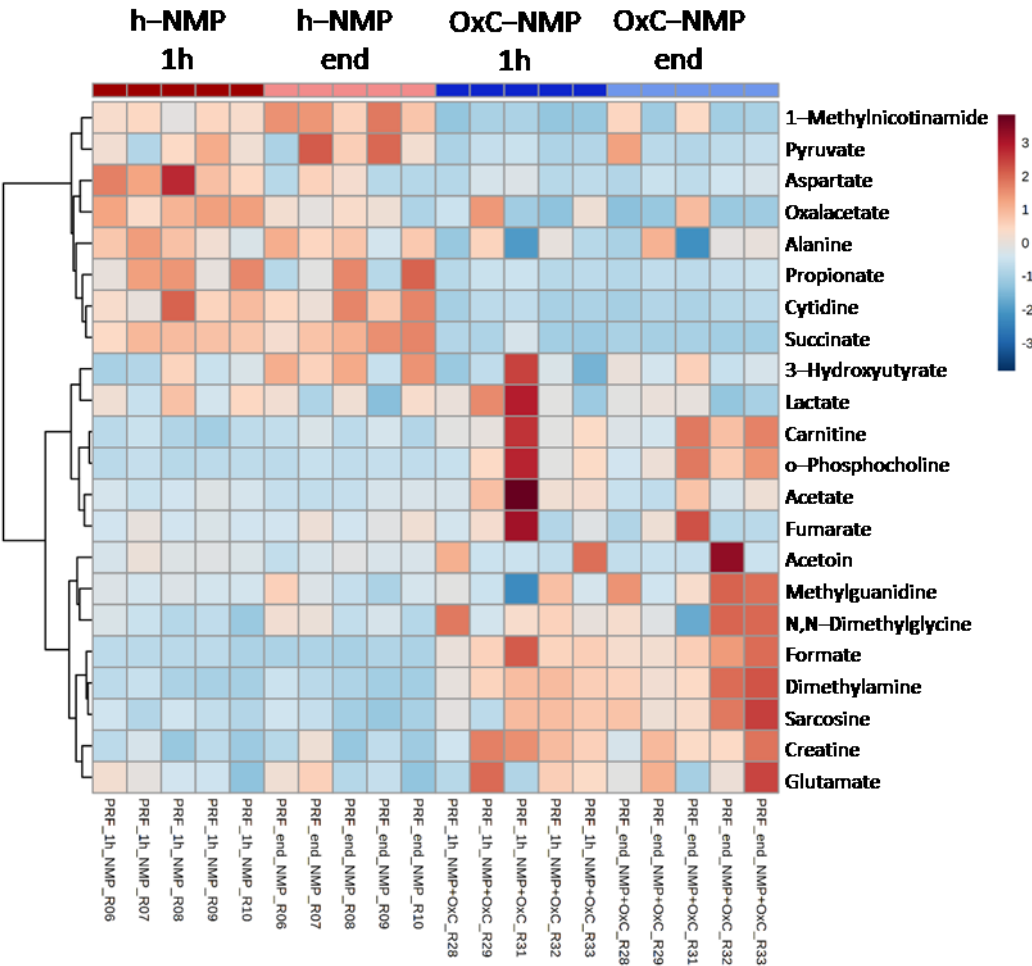
Abbreviations: SCS – static cold storage, NMP – normothermic machine perfusion, h-NMP – hypoxic NMP, OxC-NMP – oxygen carrier NMP, UMP – uracil monophosphate, IMP – inosine monophosphate, GSSG – glutathione disulfide, UDP – uracil diphosphate.



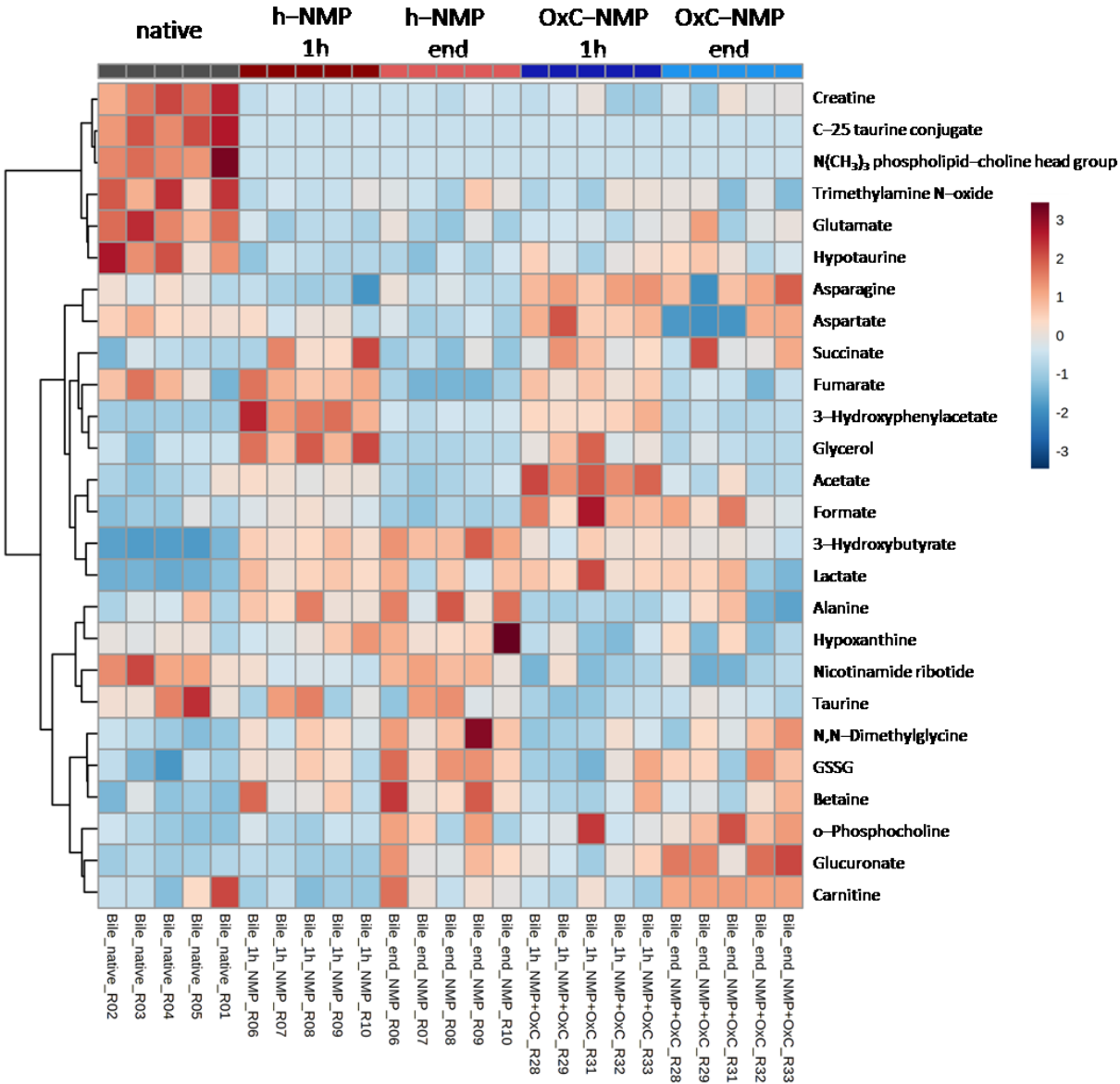


**Supplementary Figure S5.** Heatmap representing the metabolite concentrations in the perfusate samples.

Abbreviations: SCS – static cold storage, NMP – normothermic machine perfusion, h-NMP – hypoxic NMP, OxC-NMP – oxygen carrier NMP.



**Supplementary Figure S6.** Heatmap representing the metabolite concentrations in bile samples. Abbreviations: SCS – static cold storage, NMP – normothermic machine perfusion, h-NMP – hypoxic NMP, OxC-NMP – oxygen carrier NMP, c – carbon, GSSG – glutathione disulfide.



**Supplementary Figure S7.** Averaged heatmaps representing differentially expressed and statistically significant metabolites.

(a) liver biopsies; (b) perfusate samples. One-way ANOVA,  $p < 0.05$ .

