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SUPPLEMENTARY INFORMATION

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Supercooling extends preservation time of human livers

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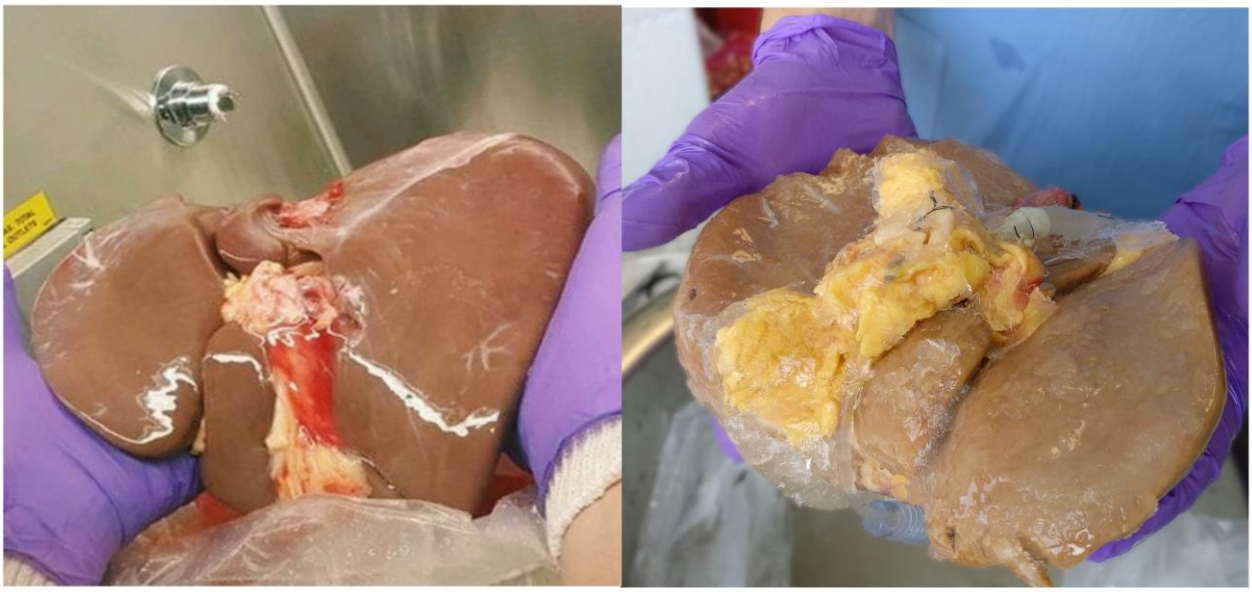
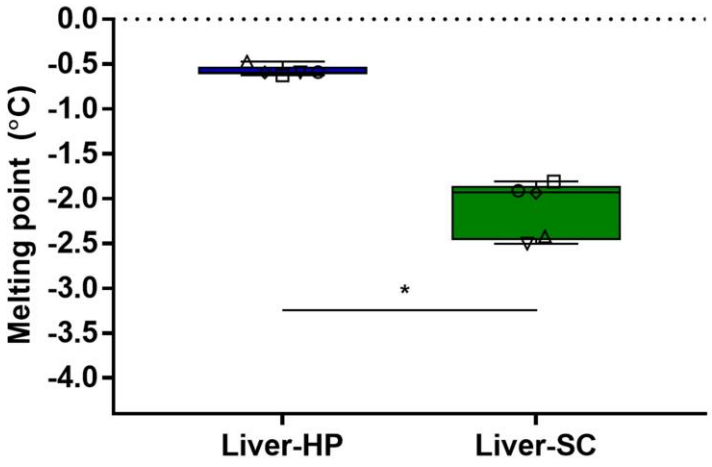


Supplementary Figure 1

First attempt at supercooling a human liver with the rat protocol.

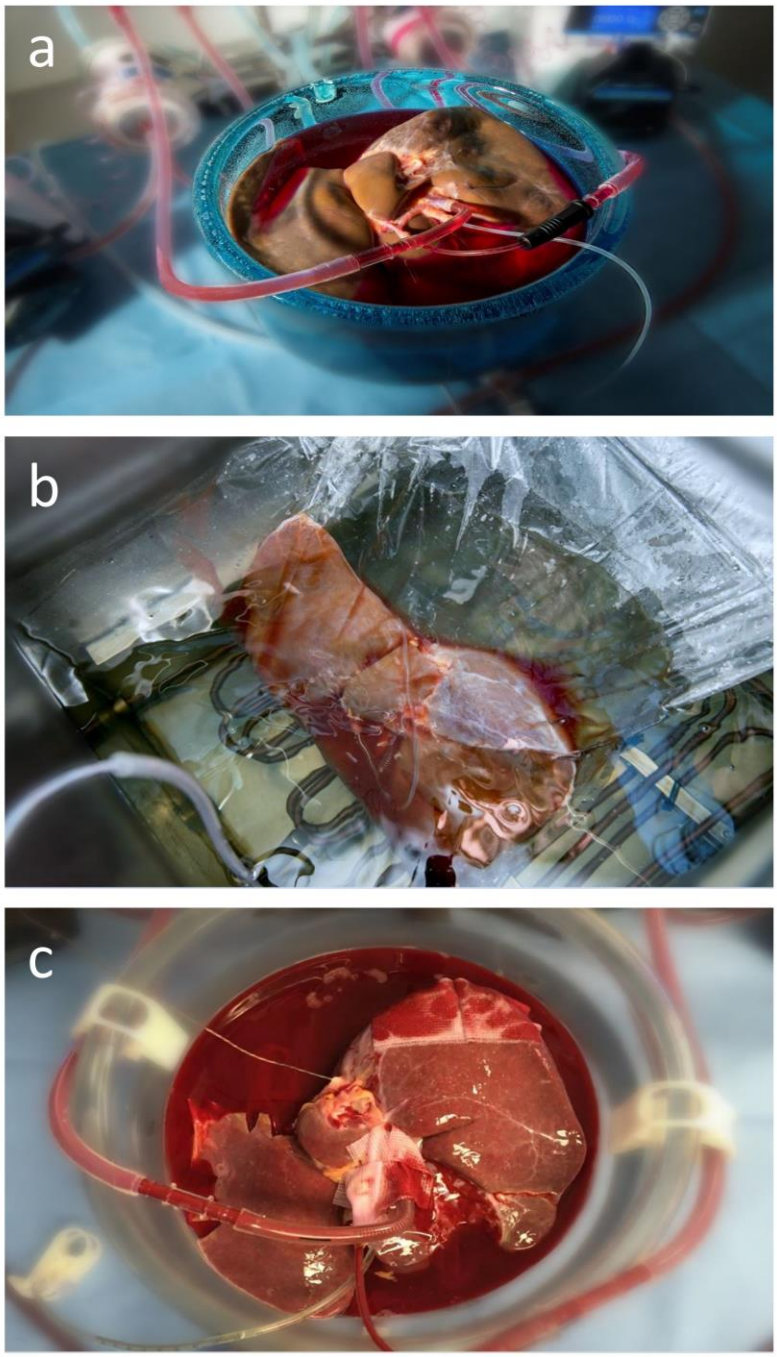
Left: A liver after convetntional hypothermic preservation (HP) in University of Winsconsin solution (UW). Right: A liver frozen at –6ºC after ealier failed attempts to supercool, shown after recovery from the chiller.



Supplementary Figure 2

Melting point depression of the liver grafts.

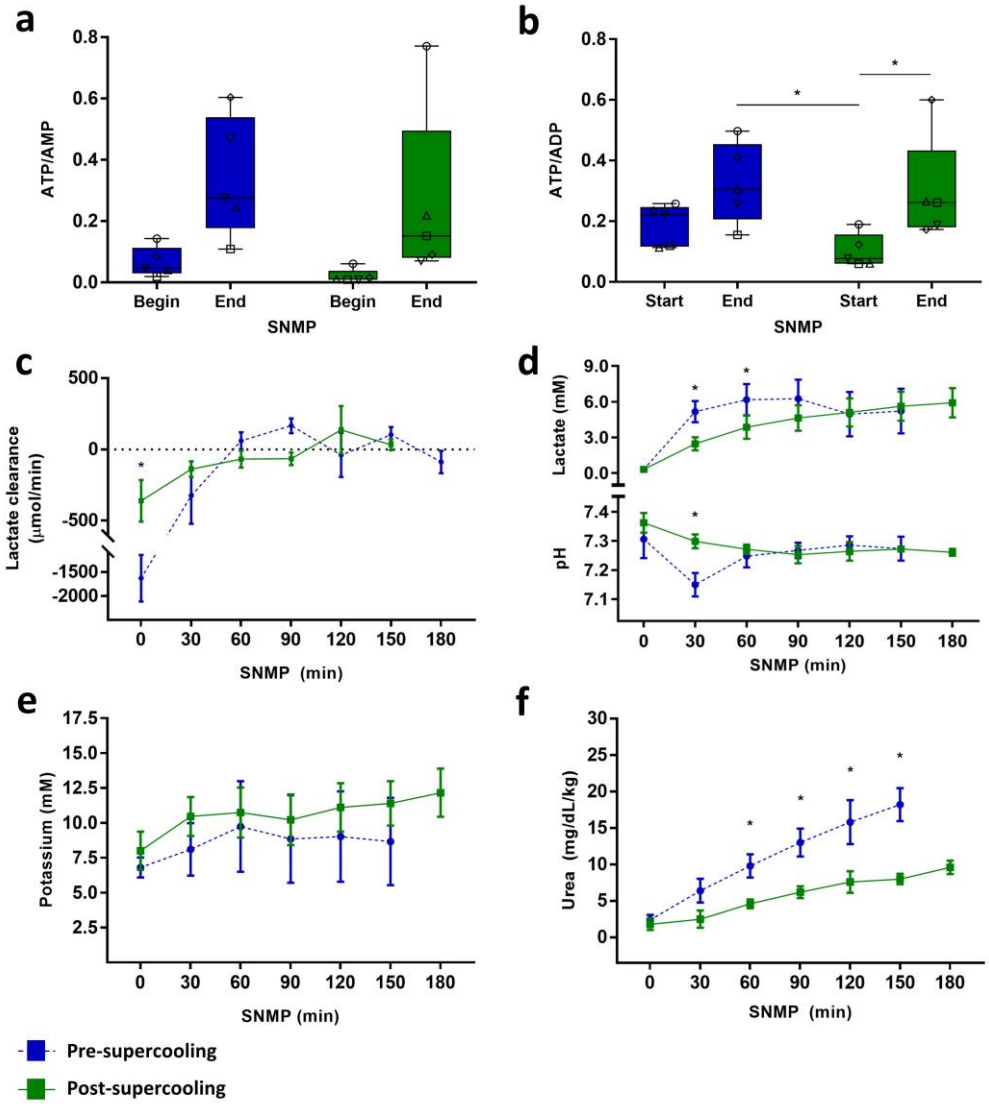
The symbols of the dot plot overlay correspond to the unique independent biological replicates (n = 5) and match between Figures 2 and 3, Supplementary Figures 2, 4, and 5, and Supplementary Table 2. Left: melting point of the human livers preserved University of Wisconsin hypothermic preservation (HP) solution. Right: melting point of the same human livers preserved in our supercooling (SC) preservation solution. Star denotes statistical significance (p = 0.001, paired two-tailed student's t tests).



Supplementary Figure 3

Photos of the livers during the supercooling protocol.

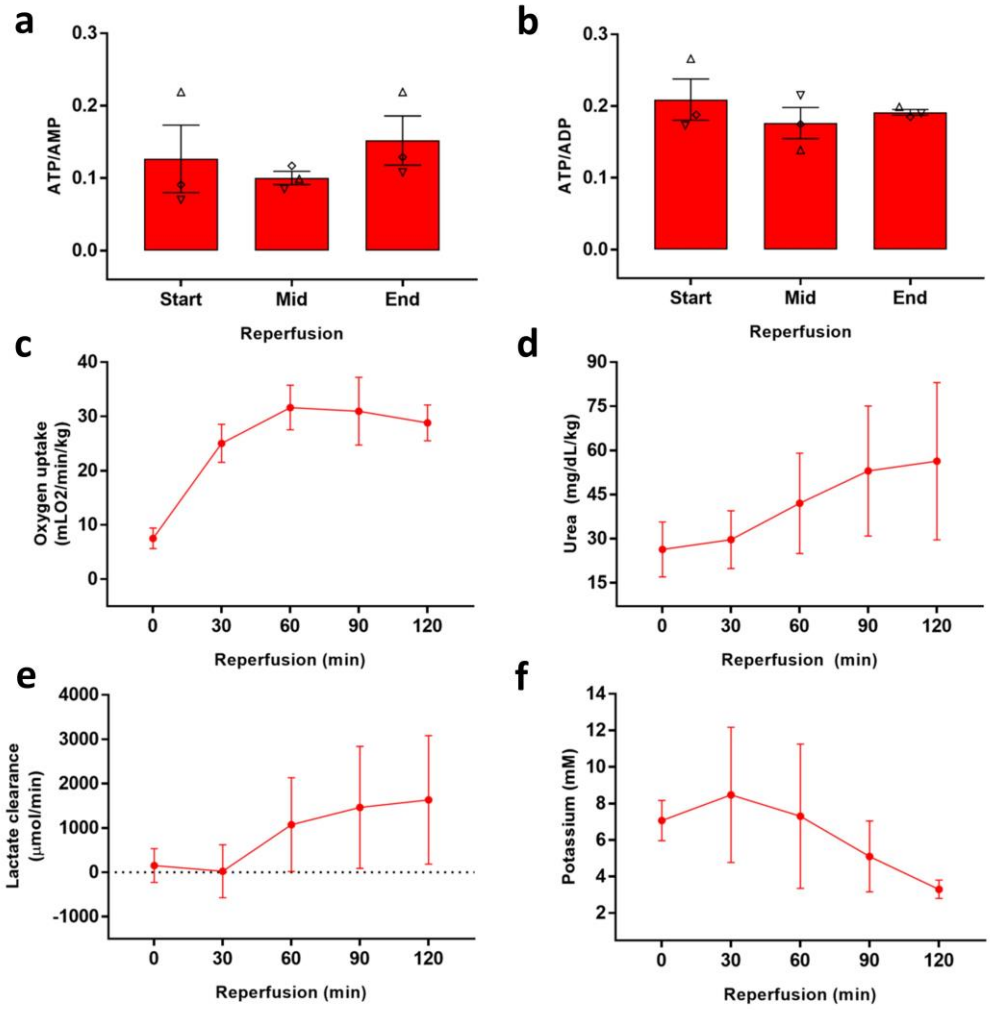
(a) Liver during the cooling phase of subnormothermic machine perfusion (SNMP). **(b)** Liver submerged in the chiller basin during ice-free subzero supercooled storage. **(c)** Liver during reperfusion.



Supplementary Figure 4

Important *ex vivo* viability parameters and histology during pre- and post-supercooling subnormothermic machine perfusion.

(**a**) Tissue adenylate triphoshate (ATP) and adenylate monophosphate (AMP) ratio. Blue denotes pre-supercooling and green denotes post-supercooling throughout the figure. The symbols of the dot plot overlay correspond to the unique independent biological replicates (n = 5 throughout the figure) and match between Figures 2 and 3, Supplementary Figures 2, 4, and 5, and Supplementary Table 2. (**b**) Tissue adenylate triphosphate (ATP) and adenylate diphosphate (ADP) ratio. (**c**) Lactate clearance derived from in and outflow measurements. (**d**) Lactate concentration (top) and pH (below) of the arterial inflow. (**e**) Perfusate potassium concentrations. (**f**) Urea concentrations in the perfusate. Stars denote statistical significance ($p < 0.05$, repeated measures two-way ANOVA followed by the Sidak multiple comparisons test). Boxes: median and IQR. Whiskers: min and max. Error bars of line graphs: mean ± SEM.



Supplementary Figure 5

Important *ex vivo* viability parameters during simulated transplantation by normothermic blood reperfusion.

(**a**) Tissue adenylate triphosphate (ATP) and adenylate monophosphate (AMP) ratio. The symbols of the dot plot overlay correspond to the unique independent biological replicates (n = 3 throughout the figure) and match between Figures 2 and 3, Supplementary Figures 2, 4, and 5, and Supplementary Table 2. (**b**) Tissue adenylate triphosphate (ATP) and adenylate diphosphate (ADP) ratio. (**c**) Oxygen uptake. (**d**) Plasma urea concentrations. (**e**) Lactate clearance derived from in and outflow measurements. (**f**) Plasma potassium concentrations. Error bars: mean ± SEM.

Supplementary Table 1 Melting points of the supercooling preservation solution.

	Additive concentration		Melting point (°C)	
	% w/v	Molarity	Mean	SEM
H2O			0.00	0.01
UW			0.59	0.01
UW + ***PEG***	5.00	1.43×10^{-6}	0.58	0.11
UW + PEG + ***TRE***	3.78	0.10	0.81	0.02
UW + PEG + TRE + ***GLY***	12.57	1.36	3.03	0.05

Note: H2O = Milli-Q water (n (independent melting point measurement replicates) = 5), UW = University of Wisconsin solution (n = 5), PEG = 35 kDa Polyethylene glycol (50g/L) (n = 7), TRE = D-(+)-Trehalose dihydrate (37.83 g/L) (n = 7), GLY = glycerol (125.7 g/L) (n = 10).

Supplementary Table 2 Donor and graft characteristics.

	Liver no. (symbol)				
	1 (○)	2 (□)	3 (◊)	4 (^)	5 (v)
Donation type	DCD	DCD	DCD	DBD	DCD
Age	57 yr	32 yr	53 yr	57 yr	60 yr
Sex	female	male	female	female	female
BMI	29	28	36	23	30
Liver Weight	2.26 kg	2.59 kg	1.78 kg	1.62 kg	1.21 kg
Ischemia					
WIT	00:46	>0:30	00:34	00:00	00:26
CIT	05:47	09:04	06:50	15:05	07:44
Steatosis	0%	0%	0%	0%	20%

Liver symbols match the dot plot overlay symbols in (supplementary) figures. DCD: donation after cardiac death. DBD: donation after brain death. WIT: warm ischemia time. CIT: cold ischemia time. BMI: body mass index. Steatosis: Histological grade of steatosis.

Supplementary Table 3 Blood count values of the blood prior to reperfusion.

	Absolute count		Relative count	
White blood cells	1.9	10^3/µL		
Lymphocytes	0.9	10^3/µL	45.7	%
Mid cells	0.7	10^3/µL	35.9	%
Granulocytes	0.3	10^3/µL	18.4	%
Red blood cells	3.0	10^6/µL		
Platelets	87	10^3/µL		

Supplementary Table 4 Solution compositions.

Pre-supercooling recovery solution (4 liter)	
40 U of regular insulin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
32 mg Dexamethasone	Sigma Aldrich, St. Louis, MO, USA
20,000 U of sodium heparin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
32 mL penicillin-streptomycin	Thermo Fisher Scientific, Waltham, MA, USA
200 mL of 25% Human albumin	Massachusetts General Hospital Pharmacy, Boston, MA
80 g 35 kDa Polyethylene Glycol	Sigma Aldrich, St. Louis, MO, USA
Williams' medium E	Sigma Aldrich, St. Louis, MO, USA
to a total volume of 4000 mL	
77.68 g 3-O-methyl-D-glucose	Sigma Aldrich, St. Louis, MO, USA
added during perfusion	
380 U regular insulin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
added during perfusion	
Loading Solution 1 (1 liter)	
40 U regular insulin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
8 mg dexamethasone	Sigma Aldrich, St. Louis, MO, USA
50 g 35 kDa Polyethylene Glycol	Sigma Aldrich, St. Louis, MO, USA
50 mL glycerol	Thermo Fisher Scientific, Waltham, MA, USA
37.83 g D-(+)-Trehalose dihydrate	Sigma Aldrich, St. Louis, MO, USA
University of Wisconsin Solution	Bridge to Life Ltd., Columbia, SC, USA
to a total volume of 1000 mL	
Loading Solution 2 (3 liter)	
120 U regular insulin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
24 mg dexamethasone	Sigma Aldrich, St. Louis, MO, USA
150 g 35 kDa Polyethylene Glycol	Sigma Aldrich, St. Louis, MO, USA
300 mL glycerol	Thermo Fisher Scientific, Waltham, MA, USA
113.49 g D-(+)-Trehalose dihydrate	Sigma Aldrich, St. Louis, MO, USA
University of Wisconsin Solution	Bridge to Life Ltd., Columbia, SC, USA
to a total volume of 3000 mL	
Unloading Solution (1 liter)	
10 U regular insulin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
8 mg dexamethasone	Sigma Aldrich, St. Louis, MO, USA
8 ml penicillin-streptomycin	Thermo Fisher Scientific, Waltham, MA, USA
50 g 35 kDa Polyethylene Glycol	Sigma Aldrich, St. Louis, MO, USA
500 mg Trolox	Cayman Chemical Company, Ann Arbor, MI, USA
50 ml 25% Human albumin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
50 ml Glycerol	Thermo Fisher Scientific, Waltham, MA, USA
37.83g D-(+)-Trehalose dihydrate	Sigma Aldrich, St. Louis, MO, USA
Wiliams' medium E	Sigma Aldrich, St. Louis, MO, USA
To a total volume of 100 mL	
Post-supercooling recovery solution (4 liter)	
40 U of regular insulin	Massachusetts General Hospital Pharmacy, Boston, MA, USA

32 mg Dexamethasone	Sigma Aldrich, St. Louis, MO, USA
20,000 U of sodium heparin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
32 mL penicillin-streptomycin	Thermo Fisher Scientific, Waltham, MA, USA
2 g Trolox	Cayman Chemical Company, Ann Arbor, MI, USA
200 mL of 25% Human albumin	Massachusetts General Hospital Pharmacy, Boston, MA
80 g 35 kDa Polyethylene Glycol	Sigma Aldrich, St. Louis, MO, USA
Williams' medium E	Sigma Aldrich, St. Louis, MO, USA
to a total volume of 4000 mL	

Blood (2 liter)

3 U packed red blood cells.	Research Blood Components LLC, Boson, MA, USA
type O Rh+ non-leuko reduced	
3 U fresh frozen plasma	Research Blood Components LLC, Boson, MA, USA
non-leuko reduced	
20 U regular insulin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
32 mg dexamethasone	Sigma Aldrich, St. Louis, MO, USA
10,000 U of sodium heparin	Massachusetts General Hospital Pharmacy, Boston, MA, USA
16 mL penicillin-streptomycin	5,000 U/ml) (Thermo Fisher Scientific, Waltham, MA, USA
30 ml 8.4% sodium bicarbonate	Massachusetts General Hospital Pharmacy, Boston, MA, USA
Williams' medium E	Sigma Aldrich, St. Louis, MO, USA
to a total volume of 2000 mL	