**SDC, Materials and Methods**

*Perfusion*

We used the SNMP model described previously by our group (Figure 1). Briefly, livers were procured as if for transplantation and stored in cold UW. On arrival to the lab, they were flushed with 2L of lactated Ringers (4°C) and then placed directly onto oxygenated SNMP (21°C) on a custom made machine for 3 hours. The perfusion fluid was based on Williams E Medium supplemented with dexamethasone (8 mg/L), insulin (5 U/L), heparin (5000 U/L) and sodium bicarbonate (2 g/L initially) to adjust the pH to 7.4. Additional sodium bicarbonate 8.4% was added during the perfusion as necessary. Our target pressure values were 7 mmHg for portal, and 70-80 mmHg for hepatic artery, throughout the perfusion. Please note that the system used in this work utilizes double roller pumps, which may affect the flow regime experienced by the endothelial cells; however in our past work with both this system and a clinical grade system that features centrifugal blood pumps we did not observe major differences in terms of resistance, flow, or histological evidence of endothelial injury (data not shown). Blood gases was controlled by oxygenators. 5% Carbon dioxide balanced oxygen (certified standard gas mixture) was used in the system. Oxygen partial pressure has been kept same across both portal and arterial inputs (between 600-650 mmHg for oxygenated media, right before entering the liver), and only the flow was regulated separately for arterial and portal branches. Oxygen saturation has been checked regularly throughout the perfusion with an i-STAT Handheld Blood Analyzer (Abbott) system (as well as other common analytes of the blood, pH, osmolarity, etc.).
**Real time measurements and sample taking**

Flow, pressure and biochemical profile were monitored at regular intervals. During preservation and perfusion liver tissue and common bile duct biopsies were taken for examination. Liver tissue biopsies were taken directly after the initial procedure (marked as time 0) and at 60, 120 and 180 minutes after the start of SNMP from the subcapsular region of the liver, which is the parenchymal tissue rich in capillaries, without reaching any major artery and/or portal branch. As a result only liver sinusoids associated endothelial cells are investigated. This was designed to assess the health of microcirculation. Liver sinusoids have dual blood supply, receiving blood flow from the portal vein (70%) and the hepatic artery (30%). Bile duct tissue biopsies were taken at the start and at the end of SNMP. All biopsies were taken in duplicates. One biopsy was preserved in 10% formaldehyde for paraffin embedding and the other was snap-frozen in liquid nitrogen and stored at -80°C. The paraffin embedded slides were prepared for H&E staining. Snap-frozen samples were used for Western Blotting and qRT-PCR. Samples of the perfusion fluid were taken every 10 minutes for the first 30 minutes and after that every 30 minutes. Samples of perfusion fluid were frozen on dry ice and used for ALT and NO-assays.

**qRT-PCR**

Housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-Actin (ACTB) and hypoxanthine phosphoribosyltransferase (HPRT-1) were used to standardize sample loading. Frozen tissue samples were crushed in liquid nitrogen with a mortar and pestle. Equal amounts of tissue powder was lysed with QIAzol (Qiagen catalog number: 217084) and homogenized by vortexing and passing it through a 1 ml syringe with a 22G needle several times. RNA was extracted using miRNeasy kit (Qiagen, catalog number: 217084). The concentration, purity and integrity of the RNA was determined by NanoDrop 2000
Spectrophotometer and 2% agarose gel electrophoresis. cDNA synthesis was performed in the Eppendorf Mastercycler thermal cycler, using SuperScript® VILO™ Master Mix (Thermofisher, catalog number: 11755). All qPCR runs were performed in triplicates using ViiA™7 Real-Time PCR System (Applied Biosystems). Power SYBR® Green PCR Master Mix (Thermofisher Scientific, catalog number: 4367659) was used with 200ng cDNA for PCR reaction in the presence of 10 µM primer mix. A final elongation cycle to create melting curves, to determine the purity of the amplicon, was also performed.

**Western Blotting**

Western blot analyses were performed with p-eNOS antibodies eNOS antibodies were used to determine exact location of the phosphorylated signal (Figure S1). Time wise normalized p-eNOS signal was calculated for every sample. eNOS activation was evaluated by serine phosphorylation at S-1177. Crushed tissue samples lysed in NP40 buffer supplemented with a phosphatase and protease inhibitors cocktail (Thermofisher Scientific, catalog number 78442) at 4°C. Beadbug homogenizer was used for fast and efficient lysis. Following lysis, homogenates incubated 1h at 4°C with constant agitation and centrifuged at 4°C at 10.000G for 30 minutes. Total protein concentration determined by BCA protein assay (Thermofischer Scientific, catalog number 23227). 50 µg of total protein per sample was used for SDS-PAGE on a 6.5% gel. Low fluorescent polyvinylidene (PVDF) membrane (EMD Millipore, catalog number: IPFL00010) was used for semi-dry transfer. Bovine Aortic Endothelial Cell (BAEC) lysates were used as a positive control and isolated human hepatocytes were used as a negative control. Blocked membranes were incubated overnight at 4°C with phospho-eNOS Ser1177 primary antibody (BD Transduction Laboratories, Mouse anti-Human mAb, catalog number: 612392). LI-COR BioSciences, IRDye® 680LT, 680RD, and 800CW anti-Rabbit, and anti-Mouse IgG secondary antibodies were used for detection as needed. Near-infrared
imaging performed with LI-COR CLX-1368 imaging station according to the manufacturer’s protocol. β-Actin and GAPDH was used for loading control.

**Nitric Oxide Assay**

Because NO rapidly degrades to both nitrate and nitrites, the total concentration of both was used to estimate NO levels with a commercially available kit (Biovision Nitric Oxide Colorimetric Assay, catalog number K262-200). Samples of perfusion fluid were thawed and subsequently transferred to a spin column with 10kDa cutoff filter (Biovision, catalog number 1997-25) and centrifuged for 25 minutes at 10.000G at 4°C to deproteinize the sample. Next, NO concentrations were determined using Griess reagents following manufacturer’s protocol.

Absolute values of NO concentration were calculated in uM and normalized per kilogram of tissue. Relative values were calculated as a fold change from the baseline (t=0) sample.