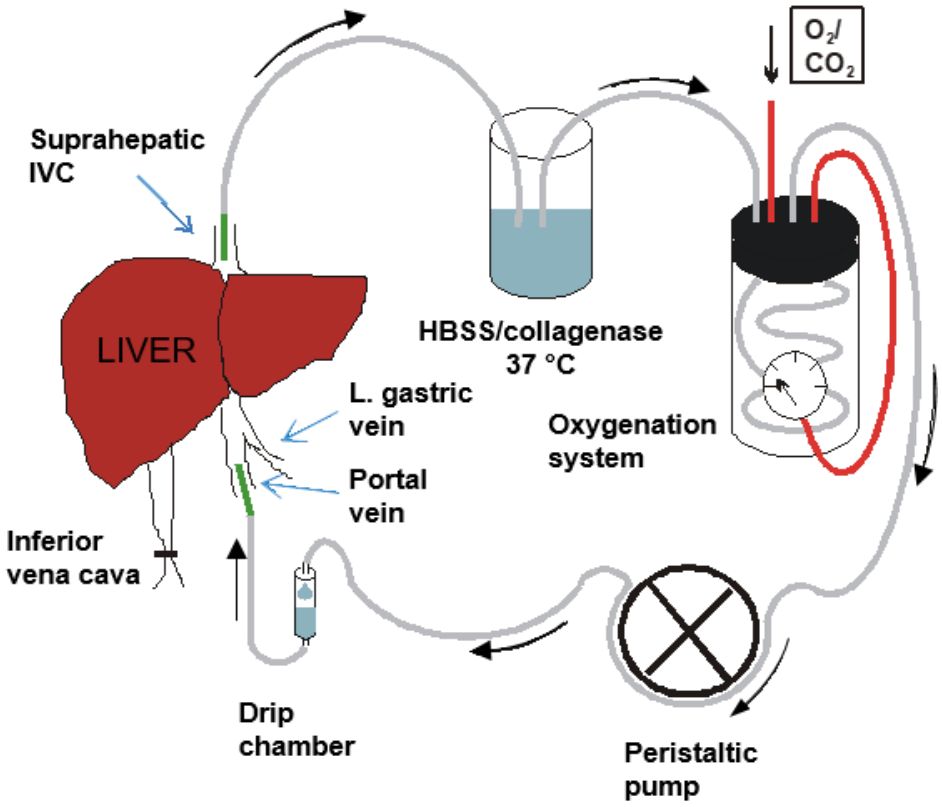

Figure S1: Routine protocol for rat hepatocyte isolation. Hepatocytes were isolated by collagenase perfusion using the procedure originally described by Seglen [P.O. Seglen, Preparation of isolated rat liver cells, *Methods Cell Biol* 13 (1976) 29–83.] and modified by us. Adult male Wistar rats weighing 250-300 g were used in all experiments. They were fed *ad libitum* and received humane care according to the principles and recommendations given by the National Academy of Sciences (Argentina). The School of Biochemical and Pharmaceutical Sciences Institutional Animal Care and Use Committee (Universidad Nacional de Rosario) approved all experimental procedures. Briefly, rats were heparinized and anesthetized and livers were perfused *in situ* for 5 min via the portal vein with 100 mL of a modified Hank's balanced salt solution (HBSS) supplemented with 5 mM TRIS and 0.5 mM EGTA, pH 7.40 at 37 °C. The perfusate was oxygenated by passing through oxygen-permeable tubing (Silicone Tubing, Baxter Healthcare Corporation, Irvine, CA, USA) inside an appropriate glass container with 95 % O_2 and 5 % CO_2, at a pressure of 80 mm Hg. Air bubbles were avoided by connecting a disposable drip chamber in line between the oxygenator and the inflow. After the initial flush out, the perfusion was followed in a recirculating buffer system with 150 ml of HBSS in the absence of EGTA, supplemented with 1.2 mM $CaCl_2$, 0.025 % collagenase type IV and 1 % BSA. The cell suspension was centrifuged (50 x *g* for 3 min) and the pellet was resuspended two times in HBSS solution containing 1 % BSA and 1.2 mM $CaCl_2$. Only viable cells, which excluded greater than 85 % of 0.2 % Trypan blue dye, were utilized for the experiments.

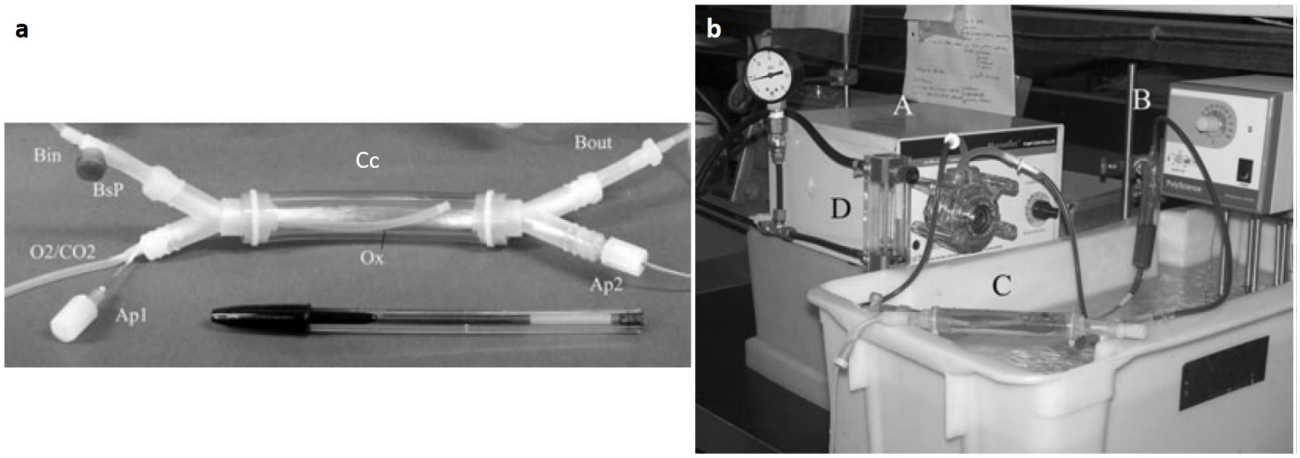

Figure S2: Cylindrical BAL operation. <u>Panel a</u>: The different components of the cylindrical BAL for hepatocytes. It offers a cell compartment (Cc) separated from the blood inflow and outflow compartment by commercially available hollow fibers. Two Cc access ports (Ap1 and Ap2) enable easy filling of the Cc with a hepatocyte suspension and could serve to take samples of fluid during the operation. In addition, a thermocouple is inserted to measure the internal temperature of the device during operation. A plastic housing is made of 100 mm of a Nalgene 580 tube (12.8-mm i.d., 19.2-mm o.d., and 3.2-mm wall), two Y polypropylene connectors (Nalgene cat. 6152–0375), S/P silicone tubes (6.4-mm i.d., 11.2-mm o.d., and 2.4-mm wall), two Teflon large catheters (14 gauge, 2-mm i.d.), an oxygenator (Ox) made of oxygen permeable tube (silicone tubing, 0.078-in. i.d., 0.125-in. o.d.; cat. no. T5715-9, Baxter Healthcare Corp., Deerfield, IL, USA), transparent epoxy glue (PARSECS, Buenos Aires, Argentina) for assembling the fibers to the Y connectors and self-locking ties (nylon 6/6-length 100 mm) for locking the Nalgene 580 tube to the Y connectors. The 140 hollow fibers (Cuprophan capillary membranes, Nikkiso Co., Ltd., Tokyo, Japan) are then fixed and sealed to one end of the Y connectors with the epoxy glue and cut to their final length. **Bin**: Blood inflow; **Bout**: Blood outflow; **Bsp**: Blood sampling port. **<u>Panel b</u>:** Photograph of the perfusion system. It consists of a Masterflex peristaltic pump (A) (7554-60, Cole Parmer, Vernon Hills, IL, USA), a plastic device with a nylon filter–bubble trap (B) and the BAL (C). Prior to loading the BAL with hepatocytes, the system has to be filled with goat blood via the inlet tube (Bin). For pressure adjustment, one of the two Cc access ports is opened during the filling and hepatocyte charging process. The cell suspension is then inoculated into the Cc via the Cc access port 1 (Ap1), and it is charged with 70–90 x 10^6 cells (viability 82–91 %) in a volume of 9.7 ± 1.1 mL. After freeing the system from the remaining trapped air, the BAL is operated in the horizontal position immersed in the temperature-controlled bath at 37 °C. The pump ensures the circulation of the blood at a flow of 9 mL/min. Temperature and gas flow rates are monitored on a continuous basis. The temperature inside the BAL was measured using a thermocouple probe connected to an electronic thermometer, through a thermocouple inserted in the access port 2 (Ap2). During perfusion, carbogen (95% O_2:5% CO_2) is delivered to the BAL via the oxygenator tube at a constant pressure (D) of 85 mm Hg. The pH of the blood in the reservoir was monitored d maintained at 7.40 ± 0.50 with 8.4 % $NaHCO_3$. The recirculating blood volume was 20 mL. Depending on the individual needs, the system can be operated in recirculating or single-pass perfusion modes.