Characterization of the scavenger cell proteome in mouse and rat liver

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**Supplementary material**
Supplementary Figure 1: Isolation of GS⁺ and GS⁻ mouse hepatocytes by FACS sorting. Upper row: Schematic illustration of FACS sorting of GS⁺ and GS⁻ hepatocytes. Hepatocytes isolated from mouse liver were immediately fixed using 4% formaldehyde and stained with anti-glutamine synthetase (anti-GS) and FITC labeled secondary antibodies. Cell nuclei were counterstained using Hoechst 34580 (left panel). Gating strategy for the selection of GS⁺ cells within isolated hepatocytes. Cell debris was excluded (right panel). Lower row: GS⁺ hepatocytes were discriminated from GS⁻ hepatocytes by FITC-labelling and separated as described in materials and methods. Samples were compared with an unlabeled control (grey) and a secondary antibody labeling control (blue).
Supplementary Figure 2: Comparison of proteins higher abundant in GS⁺ or GS⁻ hepatocytes (HCs) in rat and mouse, respectively. Venn diagram illustrating the numbers of proteins found to be in significantly higher abundances in GS⁺ HCs, GS⁻ HCs or which were found in comparable amounts in mouse and rat, respectively.
A) Protein networks more abundant GS⁺ hepatocytes (mouse)

B) Protein networks more abundant GS⁺ hepatocytes (rat)

- organic acid metabolic process
- lipid metabolic process
- glutathione metabolic process
- glutamine family amino acid metabolic process
- urea cycle
- ATP metabolic process
Supplementary Figure 3 (continued)

C) Protein networks more abundant GS⁺ hepatocytes (mouse)

D) Protein networks more abundant GS⁻ hepatocytes (rat)
E) Protein networks more abundant GLT1⁺ hepatocytes (mouse)

F) Protein networks more abundant E-Cad⁺ hepatocytes (mouse)
**Supplementary Figure 3:** Network analysis of proteins showing significant abundance differences between (A-D) GS⁺ and GS⁻ hepatocytes from mouse and rat as well as in (E-F) E-Cad⁺ and GLT1⁺ hepatocytes from mouse. The network analysis was carried out with STRING version 11 and proteins showing a higher abundance in hepatocyte subpopulations were separately analyzed. An analogous analysis was applied for GLT1⁺ and E-Cad⁺ hepatocytes. Relevant gene ontology terms regarding biological processes were manually selected and proteins associated to GO terms were color-coded.
**Supplementary Figure 4:** Immunofluorescence analyses on isolated mouse hepatocytes. Isolated hepatocytes were stained with antibodies directed against glutamine synthetase (GS), glutaminase 2 (GLS2), carbamoylphosphate synthetase 1 (CPS1), glutamate/aspartate transporter II (GLT1), ammonium transporter Rh type B (RhBG) and E-cadherin (E-Cad) as described in materials and methods. Cell nuclei were counterstained with Hoechst 34580.
Supplementary Figure 5: Isolation of GLT1\(^+\) and E-Cad\(^+\) mouse hepatocytes by FACS sorting. Upper row: Illustration of FACS sorting strategy for GLT1\(^+\) and E-Cad\(^+\) hepatocytes. Hepatocytes isolated from mouse liver were stained with anti-GLT1 and anti-E-cadherin and labeled with secondary antibodies conjugated to FITC (GLT1) or Cy3 (E-cadherin). Cell nuclei were counterstained using Hoechst 34580. Middle row: Gating strategy for the selection of GLT1\(^+\) and E-Cad\(^+\) cells. Cell debris was excluded (left panel) and labeled hepatocytes were separated as described in materials and methods. Hepatocytes were separated into GLT1\(^+\) (green) and E-Cad\(^+\) (red) cells. Lower row: The samples were compared with a secondary antibody labeling control (blue).
Supplementary Figure 6: Comparison of GO terms regarding biological processes in perivenous and in GS- and periportal hepatocytes. GO term analysis was conducted using STRING as described in Materials and Methods. (A) Venn diagram illustrating the number of GO terms enriched in perivenous hepatocytes isolated with antibodies directed against GS (GS+ HCs) or against GLT1 (GLT1+ HCs). (B) Venn diagram showing the number of GO terms enriched in GS- hepatocytes or in periportal E-Cad+ hepatocytes.
Supplementary Figure 7: Composition of high and low protein diet as well as standard chow used for feeding experiments in mouse.
Supplementary Figure 8: Uncropped pictures of Western Blots from whole liver lysates of mouse fed with standard chow, low protein or high protein diet.