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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	For RNA seq analysis, we used BCL2fasq tool (Illumina), STAR (2.5.3a) for alignment and zUMIs for quantification. For proteomics analysis, we used MaxQuant (1.6.0.16) for identification and quantification. For miRNA microarrays analysis, we used Agilent Feature Extraction software. we used BD FACSDiva™ Software (BD Biosciences-US) for sorting and MetaMorph software for microscope image acquisition.
Data analysis	Data analysis Data were analyzed using MATLAB (R2018) and RStudio (v1.2, using R v3.5). R packages used for analysis are "dplyr",tidyr","biomaRt" and "edgeR". Additional tools employed are mfinder, CIBERSORT and Proteomap.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA sequencing data that support the findings of this study have been deposited in NCBI Sequence Read Archive (SRA) with the BioProject accession code PRJNA556572, and the SRA identifiers SAMN12360372- SAMN12360382. Supplementary Table 2 summarizes the UMI counts per million for each sample. Supplementary Table 11 summarizes the zUMI barcodes used per each sample and the corresponding zUMI settings.
LC-MS/MS proteomic data was uploaded to ProteomeXchange via the PRIDE database, with the project identifier PXD014512. Processed data can be found in Supplementary Table 3.
MiRNA microarray data have been deposited in NCBI Gene Expression Omnibus (GEO) with the primary accession code GSE134827. Supplementary Table 6

summarizes processed miRNA data.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	n=5 mice in RNA-seq and MS/MS experiments. sample size of 5 was chosen to allow power for non-parametric statistical tests (e.g Kruskal-Wallis), especially given the noise of the methods and the inter-mouse variability. m=3 mice in miRNA microarray. Here, the internal control embedded in microarray mothod helps with inter-mouse variability and allowed us to use 3 mice.
Data exclusions	two populations (out of 40) were excluded from RNA-seq, due to insufficient number of reads. Number of reads threshold was set a-priori, in line with sequencing depth limitations. Stated in the Methods section.
Replication	We have used 3-5 biological replicate for each experiment - all attempts at replication were successful as there was no sample which was excluded duo to outliers.
Randomization	No allocation for experimental groups in our study. All five mice underwent same procedure.
Blinding	N/A - all mice were treated the same.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Immune-fluorescence antibodies:
Alexa Fluor 647 rat anti-mouse CD73 (BD, cat: 561543, lot: 7128541, clone: TY/23, dilution: 1:50),
Alexa Fluor 555 mouse anti-Ecadherin (BD, cat: 560064, lot: 6279687, clone: 36/Ecadherin, dilution: 1:50),
Aldolase b (Thermo-Fisher, cat: PA5-30218, lot: UD2748636B, polyclonal, dilution: 1:50),
Glutamine Synthetase (Thermo-Fisher, cat: MA5-27749, lot: UD2749352F, clone:GT1055, dilution: 1:200) ,
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody conjugated to Cy5 (Thermo-Fisher, cat: A10523, dilution: 1:500)
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody conjugated to Cy3 (Thermo-Fisher, cat: A10521, dilution: 1:200)
HNf4a antibody (R&D, cat: PPH1415-00, lot: A-2, dilution: 1:350),
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, conjugated to Alexa Fluor 594 (Thermo-Fisher, cat: A-21203, Ref: A21203, dilution: 1:700).

FACS antibodies:

PE-anti-E-cadherin (BioLegend, cat: 147304, lot: B238754, clone: DECMA-1, dilution: 1:300)
APC-anti-CD73 (BioLegend, cat: 127210 lot: B265946, clone: TY/11.8, dilution: 1:300)
PE-Cy7-anti-CD31 (BioLegend, cat: 102418, lot: B212262, clone: 390, dilution: 1:300)
APC-Cy7-anti-CD45 (BioLegend, cat: 103116, lot: B257634, clone: 30-F11, dilution: 1:300)

Validation	Alexa Fluor 647 rat anti-mouse CD73: validated in https://doi.org/10.1016/j.cell.2018.08.063, antibody registry: AB_11218786 Alexa Fluor 555 mouse anti-Ecadherin: validated in previous refs, reported in manufacturer's page: http://www.bdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/human/alexa-fluor-555-mouse-anti-e-cadherin-36e-cadherin/p/560064 Aldolase b: validated in DOI: 10.1016/j.cmet.2018.04.003 Glutamine Synthetase: validated in the supplier's page for the same animal (mouse) and on the same method (IF): https://www.thermofisher.com/antibody/product/Glutamine-Synthetase-Antibody-clone-GT1055-Monoclonal/MA5-27749 HNF4A: validated in DOI: 10.1016/j.jhep.2019.02.003 All FACS antibodies were validated using FMO experiments.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	C57BL/6JOlaHsd Three-months old male mice, obtained from Envigo laboratories (Israel)
Wild animals	the study did not involve wild animals.
Field-collected samples	the study did not involve field collected samples
Ethics oversight	Mouse experiments were approved by the Institutional Animal Care and Use Committee of the Weizmann Institute of Science and performed in accordance with institutional guidelines.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Liver Perfusions and hepatocytes dissociation Five mice were anaesthetized and livers were perfused as previously previously described, with a few adjustments. A 27G syringe, connected to the perfusion line and pump, was inserted into the vena cava. 25ml of pre-warmed to 37oC EGTA buffer followed by 25ml of pre-warmed to 37oC EBS buffer with 2.3U of Liberase Blendzyme 3 recombinant collagenase (Roche Diagnostics) were cannulated into the vena cava. Shortly after the beginning of the perfusion, the portal vein was cut to allow drainage of the blood. After perfusion, livers were explanted into a Petri dish with 25ml of pre-warmed EBS and gently minced using forceps. Dissociated liver cells were collected and filtered through a 100um cell strainer. Cells were spun down at 30rcf for 3 min at 4oC to get hepatocytes enriched pellet. Pellet was resuspended in 25ul cold EBS. Cells Staining To discard dead hepatocytes, 22.5ml Percoll (Sigma) mixed with 2.5ml 10x PBS was added to the cells. Cells were centrifuged at 600rpm for 10 minutes. Supernatant containing the dead cells was aspirated and cells were resuspended in pre-warmed Hoechst buffer (DMEM + 10% FBS + 10mM Hepes). After counting, concentration was adjusted to 2x106 cells in 1ml. To determine ploidy of hepatocytes, DNA was stained with Hoeschst (15ug/ml). Resperine (5uM) was also supplemented to the cells to prevent Hoechst expulsion from the cells. Cells were incubated 30min at 37oC. Hepatocytes were centrifuged for 5min in 1000rpm at 4oC and supernatant was discarded. Next, cells were stained with Alexa fluor 488 Zombie green (BioLegend) to later enable the detection of viable cells by FACS. Cells were resuspended in cold PBS in a concentration of 106 cells in 100ul. Zombie-green was added in a dilution of 1:500. Cells were kept in a rotator in the dark at room temperature for 15min. After spinning down (1000rpm, 5min, 4oC), cells were resuspended in FACS buffer (2mM EDTA pH 8 and 0.5% BSA in 1xPBS), in a concentration of 106 cells in 100ul. Cells were stained with PE-anti-E-cadherin (BioLegend, cat: 147304), APC-anti-CD73 (BioLegend, cat: 127210), PE-Cy7-anti-CD31 (BioLegend, cat: 102418) and APC-Cy7-anti-CD45 (BioLegend, cat: 103116), in a dilution of 1:300. FcX blocking solution (BioLegend) was added in a dilution of 1:50.
Instrument	SORP-FACSAriaII sorter (BD)
Software	BD FACSDiva™ Software | BD Biosciences-US
Cell population abundance	Abundances of sorted populations were 2%-6% from the total recorded events. Purity of the sorted populations was validated computationally by comparing RNA-seq data of the sorted populations with positive and negative controls.
Gating strategy	Cells were sorted by SORP-FACSAriaII sorter (BD) using a 130 µm nozzle and 1.5 natural density (ND) filter. Lasers compensation

Gating strategy

was corrected manually. In order to collect eight populations, each enriched with spatially-stratified hepatocytes with equal viability and ploidy levels, events were screened through the following five nested gates (Fig. 3a-b): (1) hepatocytes gate from all events – set by plotting FSC-A against SSC-A and excluding large clusters and small debris; (2) singlets FSC – set by excluding the margins of FSC-A and FSC-W plot; (3) singlets SSC – excluding upper margins of SSC-W when plotted against SSC-A; (4) live cells gates according to the Zombie-488 negative cells, comparable to unstained cells; (5) hepatocytes only, by depleting CD31 and CD45 (markers of NPCs), and (6) tetraploid hepatocytes, inferred by Hoechst histogram (Fig. 3a-b, Supplementary Fig. 1b-c). Hepatocyte size and overall protein content scale with ploidy, thus creating spurious correlations between the zonated surface markers (Supplementary Fig. 1). Sorting without ploidy stratification would result in inclusion of hepatocytes from different lobule layers, reducing spatial accuracy (Supplementary Fig. 1).
We then plotted PE-intensity for E-cadherin staining and APC-intensity for CD73 staining. The positively stained cells were determined by measuring the intensities for unstained cells. The highest intensity for unstained cells was the threshold for the positively stained cells. Each population, CD73 positive and E-cadherin positive, was further gated to four equal subpopulations, representing graded intensities of the marker. Thus, subpopulations 1, 2, 3 and 4 had equal amount of events, 1 had the highest APC-CD73 intensity while 2, 3, 4 had gradually decreasing intensities of APC. Likewise, subpopulations 5, 6, 7 and 8 were equally distributed, 8 having the highest PE-E-cadherin intensity while 7,6,5 had gradually decreasing PE intensities. Populations 4 and 5 contained cells from below positive intensity threshold, to accurately resemble mid lobule hepatocytes, in which both CD73 and E-cadherin abundances are very low (Fig. 2). All gates were set for each of the five experiments independently, with a large overlap.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.