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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

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	N/A
Data analysis	N/A

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 and 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

The authors declare that all data supporting the findings of this study are available within the paper.

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	No sample size calculation was performed and three or more independent differentiations were used.
Data exclusions	No data were excluded for the analysis.
Replication	All attemps of replication were successful and for each differentiation we have replicates.
Randomization	N/A
Blinding	N/A

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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

PE Mouse Anti-Human CD140b Clone 28D4 (BD PharMingen, Cat#558821). RRID: AB_397132
PE Mouse IgG2a, Isotype Control Clone MOPC-173 (Biolegend, Cat#400214). RRID: AB_ 326460
Recombinant Anti-PDGFR beta antibody [Y92] - C-terminal (Abcam, Cat#ab32570). RRID: AB_777165
Anti-PCDH7 antibody [OTI2G6] (Abcam, Cat#ab139274). RRID: AB_2868608
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Invitrogen, Cat#A-11001). RRID: AB_2534088
Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L) (Jackson Immuno Research, Cat#711-545-152). RRID: AB_2313584

Validation

PE Mouse Anti-Human CD140b Clone 28D4 Human (QC Testing) Application Flow cytometry (Routinely Tested) provided by the manufacturer website.
In addition, iPSC-HSCs can be co-cultured with hepatocyte cell lines, such as HepaRGs, obtaining complex 3D spheroids14. Here in this protocol a ratio of 2:1 (iPSC-HSCs-HepaRG) was used to form liver spheroids following Leite et al (2016) and Figure 3A. This ratio allows to maintain iPSC-HSCs in a less activated state. A different cell ratio may be reacquired according to the final objective of the investigators. Spheroids can be characterized by immunostaining and gene expression analysis of both cell types (Table 5 and 6) and, as discussed in the Anticipated Results section can be used in assays looking at liver fibrosis, inflammation and toxicity (Table 3).
PE Mouse IgG2a, Isotype Control Clone MOPC-173 Mouse conjugated with PE, Flow Cytometry (Quality tested) provided by the manufacturer website.
Recombinant Anti-PDGFR beta antibody [Y92] - C-terminal (Human, host in rabbit) suitable for IHC-Fr, Flow Cyt, IHC-FoFr, WB, IHC-P, ICC/IF, IP, IHC-FrFl applicaitons provided by manufacturer website.
Anti-PCDH7 antibody [OTI2G6] (Human, host in mouse) suitable for WB, IHC-P and ICC/IF provided by manufacturer website.
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (target mouse, host in goat) suitable for Flow Cytometry, ICC, IF, IHC F, IHC P, WB and IHC provided by manufacturer website.
Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L) (target rabbit, host in donkey) polyclonal antibody suitable for ELISA, FACS, IFIHC and WB provided by manufacturer website.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Human BJ1 iPSCs line: The BJ1 line is generated in Catherine Verfaillie's Lab, from fibroblast cell line (ATCC® CRL-2522™, 28). Made in house cell line. Details on how to generate this cell line is described in Ordovas L et al (2015). Differentiated HepaRG cells: HPR116215-TA08 purchased from Biopredic.
Authentication	Human BJ1 iPSCs line was authenticated following the stablished stem cells characterization procedures (evaluate pluripotency markers by gene expression, FACS and IF, formation of EBs, SNP profiling). HepaRG were pruchased and no authentication procedure was performed.
Mycoplasma contamination	iPSC Cell line tested negatively for mycoplasma contatmination.
Commonly misidentified lines (See ICLAC register)	N/A

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	Once the differentiation is finished, wash iPSC-HSCs with DPBS (-/-) for 1min, remove it and add 0.5mL of Trypsin-EDTA 0.25% to each well. Incubate cells at 37ºC during 2-3min in order to detach them. Check the cells under microscopy and when cells start detaching, add an equal amount of medium containing 10% Fetal Bovine Serum (FBS) as trypsin solution in order to stop the reaction. Collect the cells by gently pipetting the medium. Centrifuge at 300g for 5min at RT. After centrifugation, resuspend cells in 3mL of cold DMEM Glutamax and distribute them in 3 FACS tubes: Autofluorescence, Isotype and PDGFRb. Add DPBS (-/-) to each tube in order to wash the cells. Centrifuge at 300g for 5min at 4ºC. Discard the supernatant and add 100uL of cold FACS Buffer to each tube. Resuspend cells and add 1uL of each antibody to Isotype and PDGFRb tubes. Incubate for 30min at RT under photo-protected conditions. At the end of the incubation add DPBS (-/-) to wash the cells. Centrifuge at 300g for 5min at 4ºC. Discard the supernatant and resuspend cells in 200uL of FACS Buffer and perform FACS analysis.
Instrument	FACSCanto II Cytometer
Software	BD FACS DIVA
Cell population abundance	After the differentiation, 60% of the cells are positive for PDGFRb.
Gating strategy	Comparison between SSCA vs FSCA to find our interest population. Use 488-530/30-502LP-A vs PDGFRb-PE to locate the positive fraction of the population.

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