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Appendix

Metabolism Guides Definitive Lineage Specification During Endothelial to Hematopoietic Transition

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Appendix Figure S1. Modulation of pyruvate metabolism affects lineage specification in vivo

(a) Pregnant mice were injected with UK5099 or DCA at E9.5 and fetal livers were analyzed at E14.5 by flow cytometry. FL, fetal liver. (b, c) Levels of HPC-1, HPC-2 (b) and erythroid progenitors (c) as percentages ± SEM in fetal liver are shown for control (n=10 biological replicates), UK5099-treated (n=14 biological replicates) and DCA-treated (n=16 biological replicates) conditions (one-way ANOVA test). (d) Erythroid differentiation stages according to CD71/Ter119 staining are shown on the control plot. Representative plots showing percentages of cells in each stage are shown for control, UK5099- or
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DCA-treated conditions. (e) Gating strategy is depicted for sorting LT-HSCs in E14.5 embryos. (f) Percentages of colonies obtained from sorted LT-HSCs are shown for control (n=4 biological replicates), UK5099-treated (n=8 biological replicates) and DCA-treated (n=10 biological replicates) conditions (one-way ANOVA test). (g) Sorted HE cells were kept in co-culture with OP9-DL1 stroma for 3 days, with or without DCA (3 mM) before transplantation into irradiated NSG mice together with bone marrow (BM) support cells. Human cells in peripheral blood (PB) or BM were assessed on weeks 4, 8 or 12. (h) Engraftment levels ± SEM in PB as percentages of huCD45+ cells are shown (Control, n=6 biological replicates; DCA, n=7 biological replicates). (i) Thymi were harvested on week 12 after transplantation and representative plots showing CD4/CD8 expressing cells are presented for control and DCA-treated conditions. (j) Representative plots and percentages ± SEM of CD19+ cells (B cells) in huCD45+ cells from PB at week 8 are shown (Control, n=6 biological replicates; DCA, n=6 biological replicates; unpaired t test). (k) Percentages ± SEM of human HSCs in huCD45+ cells from the BM are shown (Control, n=6 biological replicates; DCA, n=7 biological replicates; unpaired t tests). (l) Pregnant mice were injected with DCA at E8.5 and AGM regions of embryos at E10.5 were dissected, stained with anti-cKit/anti-CD45 antibodies and analyzed by flow cytometry. (m) Numbers ± SEM of cKit’CD45+ cells in the AGM region of control or DCA-treated embryos are shown. (Control, n=9 biological replicates; DCA, n=10 biological replicates; from 2 independent experiments).

Data information: ns, not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
Appendix Figure S2. Expression of endothelial and hematopoietic genes in differentiating HE cells

(a, b) Single-cell RNaseq was performed on control, UK5099-treated and DCA-treated HE cells at day 2 of subculture. Feature plots showing the expression of endothelial (a) or hematopoietic (b) genes on the UMAP in Fig. 4a. (c) 10 x 10 dot plots showing the percentages of cells belonging to clusters 6 and 7 in each condition. (d) Numbers of GPA⁺ clones obtained from single HE cells co-cultured on OP9-DL1 stroma, treated with the indicated compounds for 14 days (n=6 independent experiments, with a total of 552 wells screened for each condition).
Appendix Figure S3. Mechanistic analyses of pyruvate catabolism during EHT

(a) Fold change of expression of LSD1 ± SEM relative to HPRT1 in shRNA-transduced cells compared to shScr (shScr) are shown (n=3 biological replicates, unpaired t tests). Untr, untransduced. (b-c) FACS-sorted HE cells were subcultured with TCP (300 nM), DCA (3 mM) or both. Day 6 CD43⁺CD45⁺ cell frequencies ± SEM (b) and Day 6 CD43⁺CD45⁺CD33⁺CD11b⁺ cell frequencies ± SEM (c) relative to the control are shown (n=5 biological replicates, one-way ANOVA test). (d) Dot plots showing gene expression levels of cholesterol efflux pathway genes detected by scRNAseq and based on percent expressed (size of the dots) and average level of expression (color intensity).

Data information: ns, not significant, *p<0.05, **p<0.01