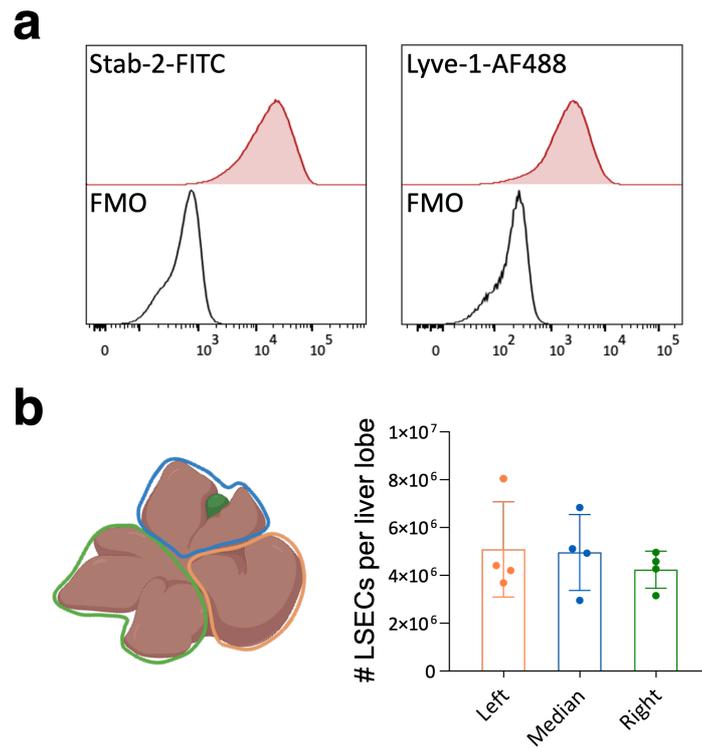


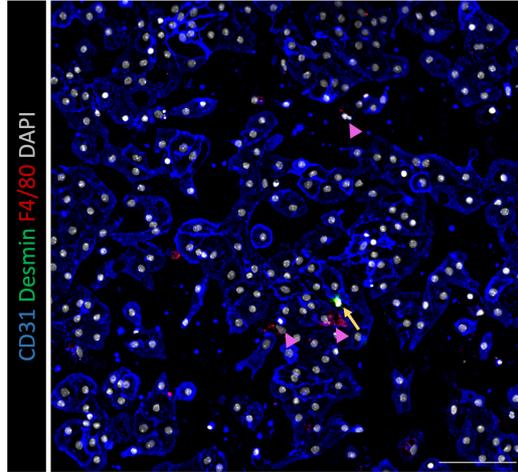
## **A perfusion-independent high-throughput method to isolate liver sinusoidal endothelial cells**

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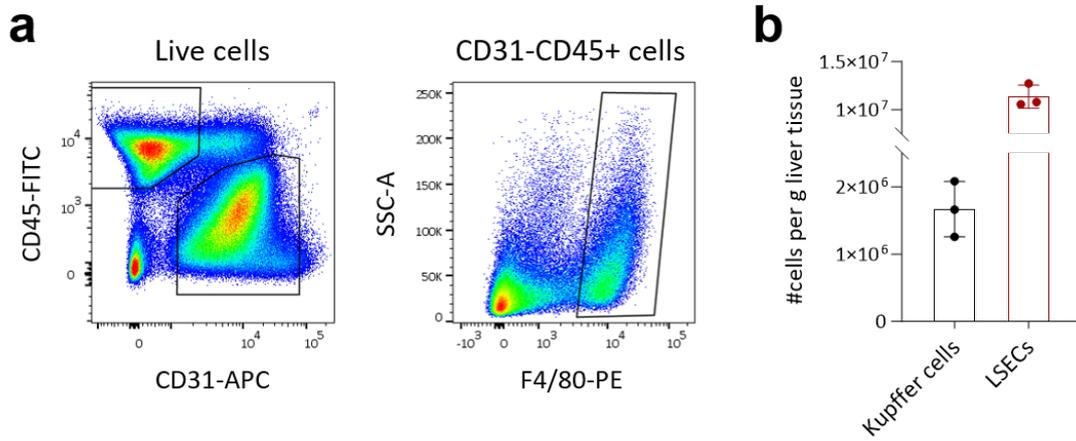
Supplemental Figures



**Supplementary Figure S1. Validating LSECs and isolating them from different lobes of the liver.** (a) Liver endothelial cells isolated with perfusion-independent digestion method were stained for Stabilin-2 and Lyve-1 and analyzed with flow cytometry. Fluorescence minus one (FMO) stained samples served as corresponding controls. (b, left) The liver tissue was divided into three parts as shown in the picture. (b, right) Each part was processed to isolate LSECs. The dot plot shows the total count of LSECs isolated from each part of the liver [mean  $\pm$  SD, n = 4 mice].



**Supplementary Figure S2. Analyzing the primary LSEC cultures for contaminations.** The image shows primary LSEC culture stained with for an endothelial cell-specific (CD31, in blue), stellate cell-specific (Desmin, in green), macrophage-specific (F4/80, in red), and nuclear marker (DAPI, in grey). Pink arrowheads highlight F4/80-positive macrophages, and the yellow arrow indicates a Desmin-positive stellate cell. Scale bar = 100  $\mu\text{m}$ .



**Supplementary Figure S3. Isolating Kupffer cells and LSECs from the liver.** (a) FACS gating strategy to analyze and sort LSECs and Kupffer cells. All live cells were stratified based on the expression of CD45 and CD31. CD31+ cells were gated as LSECs. CD31-CD45+ cells were further analyzed for the expression of F4/80. CD31-CD45+F4/80+ cells were isolated as Kupffer cells. (b) The dot plot shows the number of isolated LSECs and Kupffer cells from the liver. [mean  $\pm$  SD, n = 3 mice].