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Electronic Supplementary Information

Canalicular domain structure and function in matrix-free hepatic spheroids

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Supplementary figures

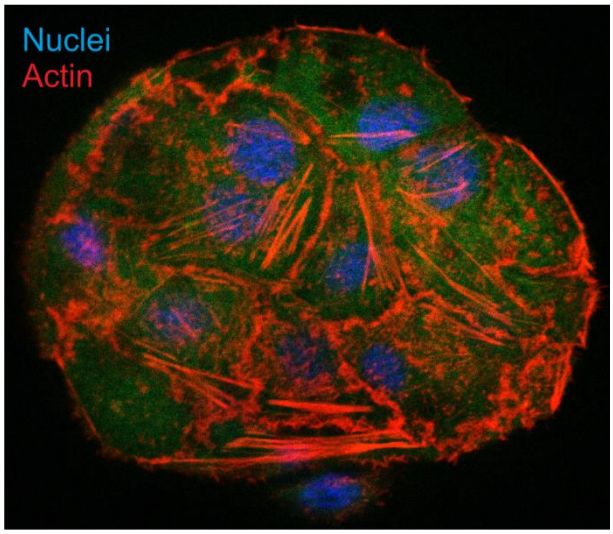

Fig. S1 Actin at cell adhesion sites. Single plan confocal of a spheroid on-chip, prepared as described in [1], stained for actin (red) and Hoechst (blue).

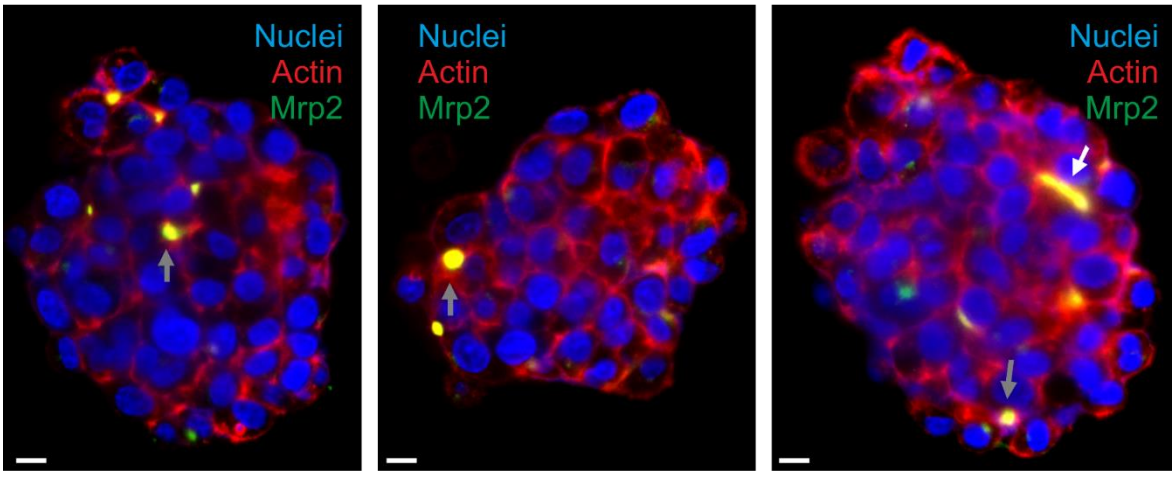

Fig. S2 HepG2 cells in matrix-free spheroids. Single plan LSFM of a matrix-free HepG2 spheroid stained for actin (red), Hoechst (blue) and Mrp2 (green). Grey arrows show focal point with actin and Mrp2 co-localized and the white arrow show elongated structure with actin and Mrp2 co-localized but without lumen. Scale bars correspond to 10 µm.

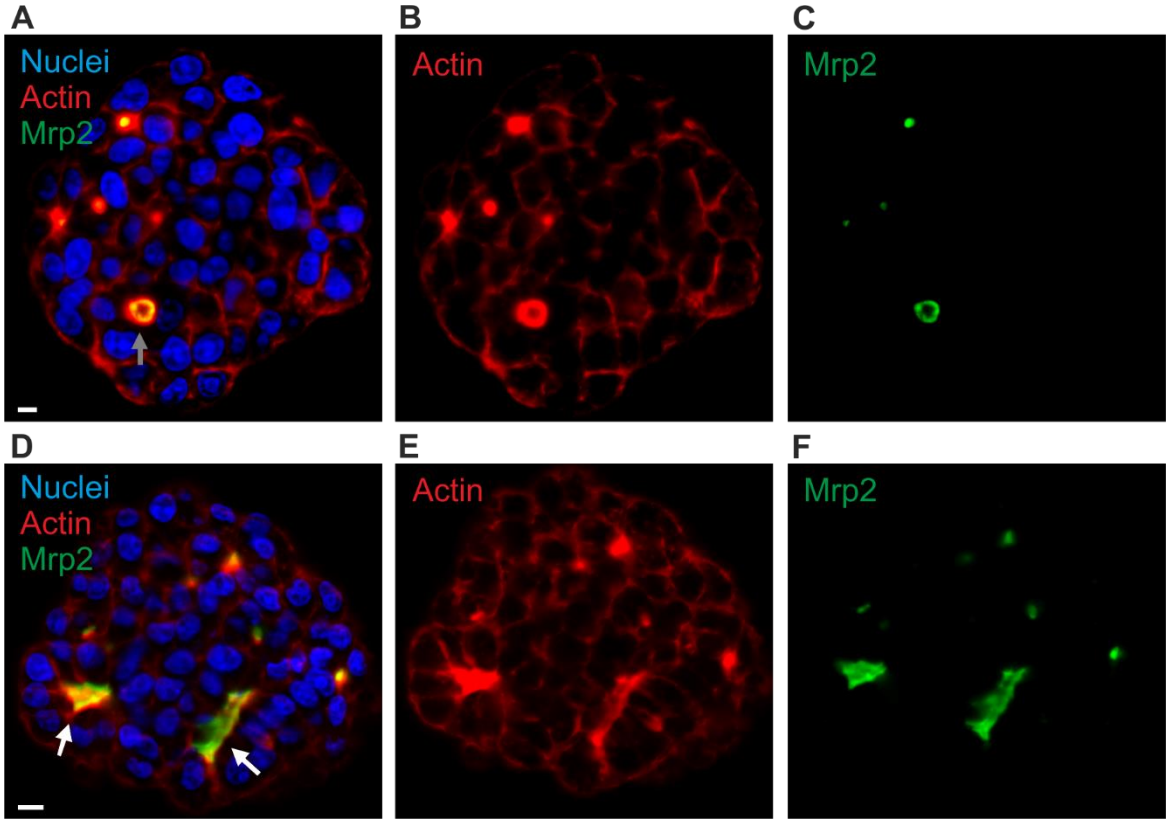

Fig. S3 Impact of matrigel on hepatocyte polarization in matrix-free HepG2/C3A spheroids. The spheroids were made as for Fig. 1 but in presence of 6% matrigel. Single plan LSFM of matrix-free HepG2/C3A spheroids stained for actin (red), Hoechst (blue) and Mrp2 (green). (A-C) Example of a spheroid with a donut shaped bile canaliculus, grey arrow. (D-F) Example of a spheroid with elongated bile canaliculi, white arrows. Scale bars correspond to 10 µm.

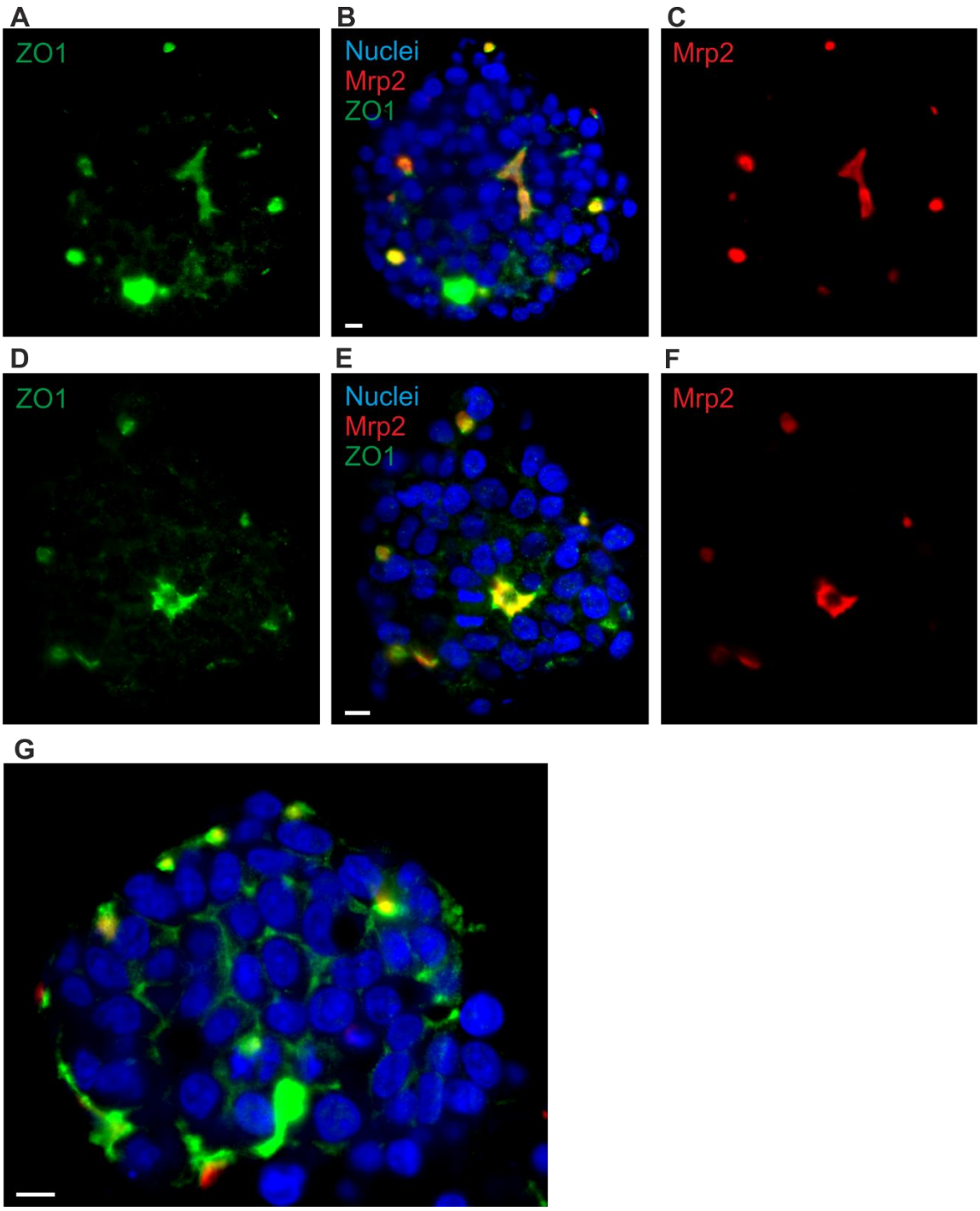

Fig. S4 Hepatocyte polarization in matrix-free HepG2/C3A spheroids. Single plans LSFM of matrix-free HepG2/C3A spheroids stained for ZO1 (green), Hoechst (blue) and Mrp2 (red). (A-C and D-F) Two examples of spheroids with staining of both Mrp2 and ZO1 at bile canaliculi. (G) Contrast enhanced image that shows low intensity ZO1 staining highlighting hepatocyte plasma membranes. Scale bars correspond to 10 µm.

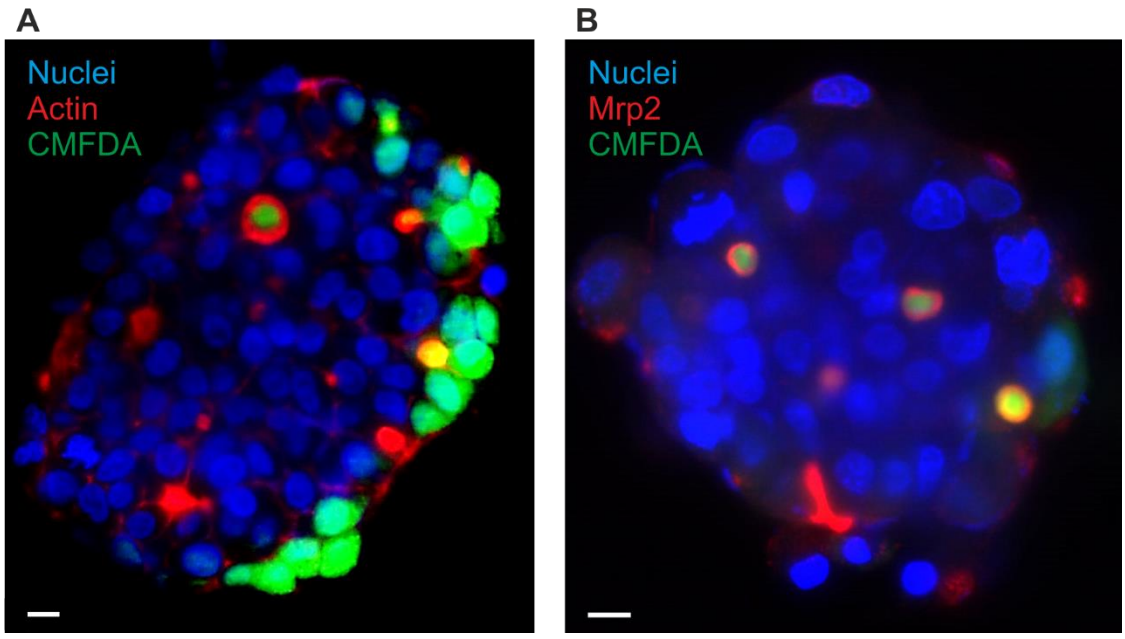

Fig. S5 Biliary excretion of CMFDA in matrix-free spheroids. (A) Single plan LSFM of a matrix-free hepatic spheroid stained for actin (red), Hoechst (blue) and CMFDA (green). Peripheral cells showed intense CMFDA staining trapped in their cytosol probably due to the lack of bile canaliculi for the outer layer of cells in this spheroid. (B) Single plan LSFM of a matrix-free hepatic spheroid stained for Mrp2 (red), Hoechst (blue) and CMFDA (green). Scale bars correspond to 10 µm.

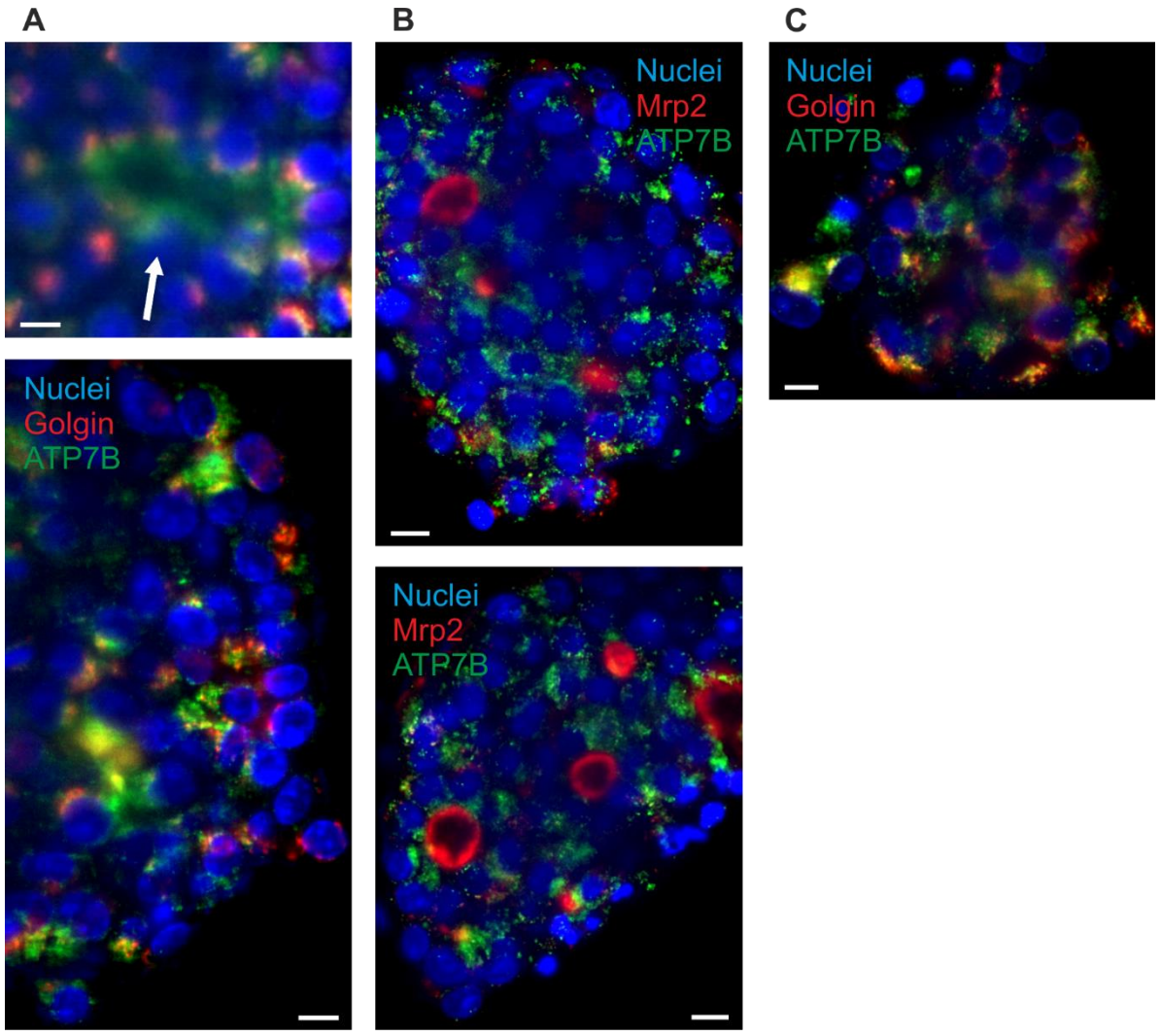

Fig. S6 ATP7B trafficking within spheroids in low Cu conditions. (A) Single plan LSFM of matrix-free hepatic spheroids in basal conditions stained for Golgin-97 (red), Hoechst (blue) and ATP7B (green). White arrow show diffuse ATP7B staining between nuclei and a probable bile canaliculus proving the nice polarization of hepatocytes within the spheroid. (B) Single plan LSFM of matrix-free hepatic spheroids in basal conditions stained for Mrp2 (red), Hoechst (blue) and ATP7B (green). (C) Single plan LSFM of a matrix-free hepatic spheroid in Cu-deprived conditions (200 µM BCS) stained for Golgin-97 (red), Hoechst (blue) and ATP7B (green). Scale bars correspond to 10 µm.

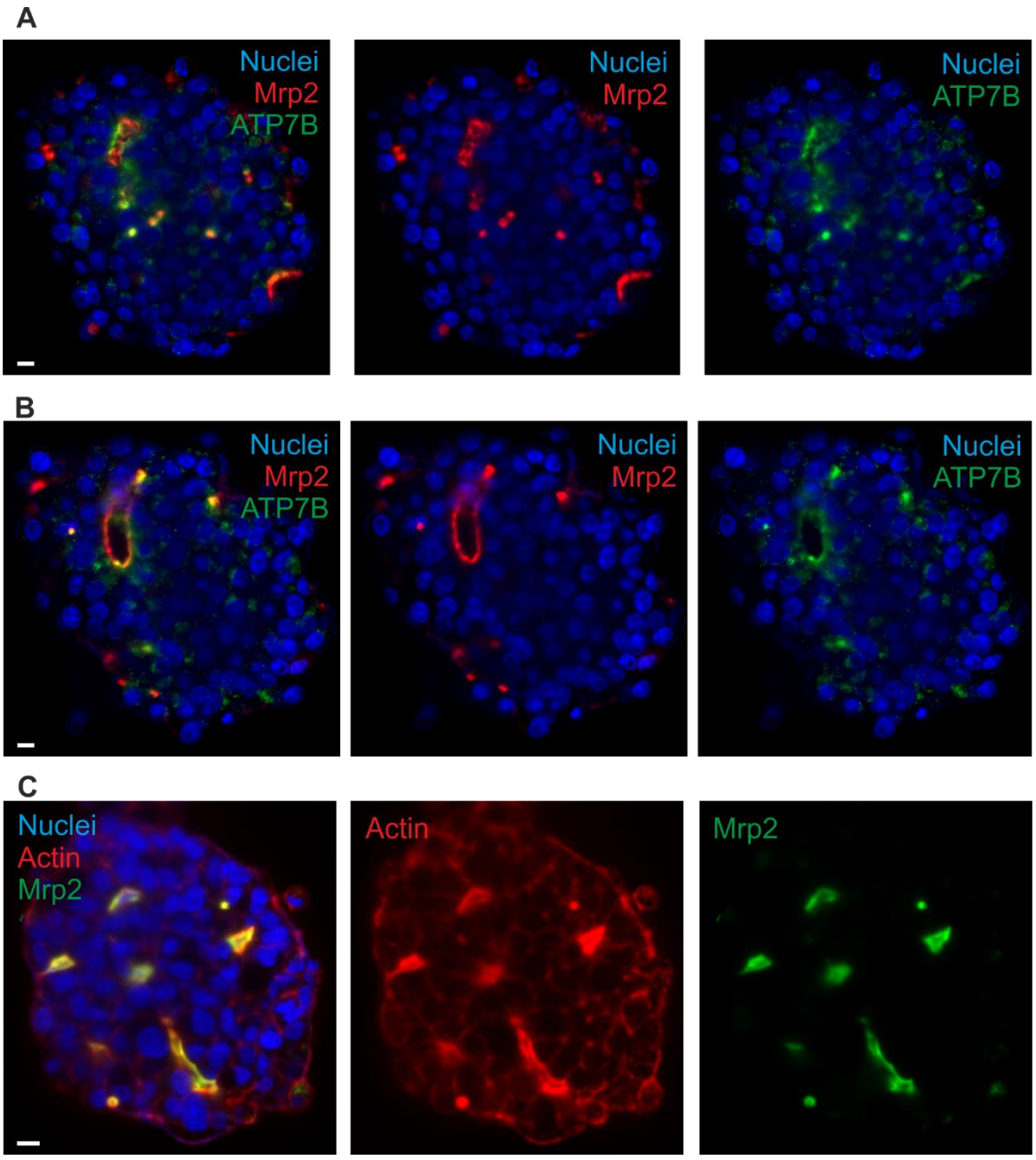

Fig. S7 ATP7B trafficking within spheroids in intermediate Cu conditions. (A-B) Single plan LSFM of matrix-free hepatic spheroids exposed to 10 µM Cu stained for Mrp2 (red), Hoechst (blue) and ATP7B (green). Same plan is presented with different combination of colors for a better visualization of the co-localization between ATP7B and Mrp2. (C) Single plan LSFM of a matrix-free HepG2/C3A spheroid exposed to 10 µM Cu stained for actin (red), Hoechst (blue) and Mrp2 (green). Scale bars correspond to 10 µm.

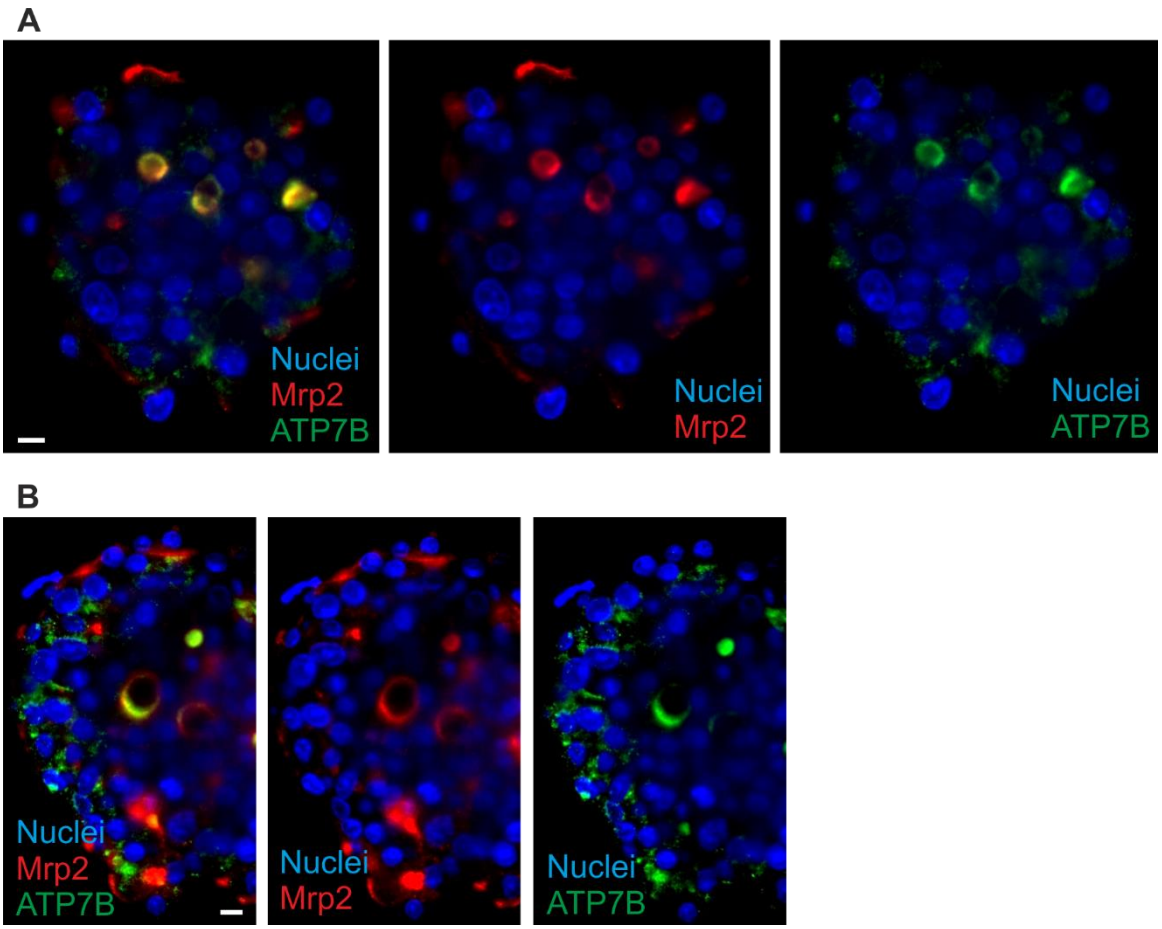

Fig. S8 ATP7B trafficking within spheroids in high Cu conditions. (A-B) Single plan LSFM of matrix-free hepatic spheroids exposed to 100 µM Cu stained for Mrp2 (red), Hoechst (blue) and ATP7B (green). Same plan is presented with different combination of colors for a better visualization of the co-localization between ATP7B and Mrp2. Scale bars correspond to 10 µm.

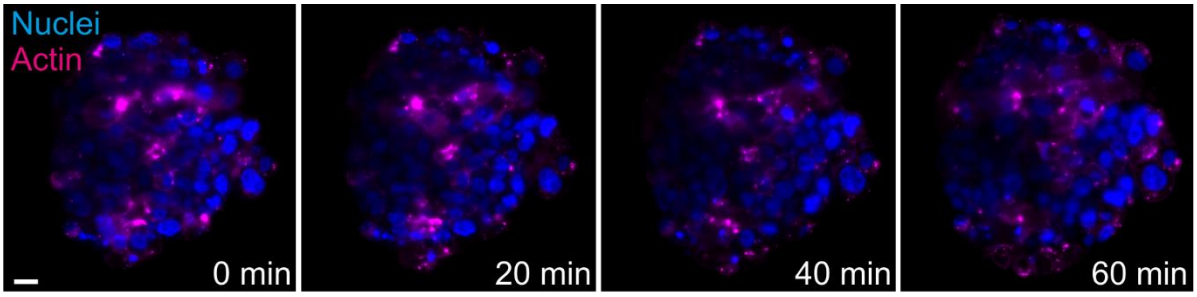

Fig. S9 Actin network dynamics in presence of CDCA. Live LSFM imaging of SiR-actin (pink) and Hoechst (blue). Selected time-points of a single plan LSFM within a spheroid exposed to 80 µM CDCA. The full video corresponds to 70 minutes with one acquisition every 2 minutes (Video S9). The scale bar corresponds to 10 µm.

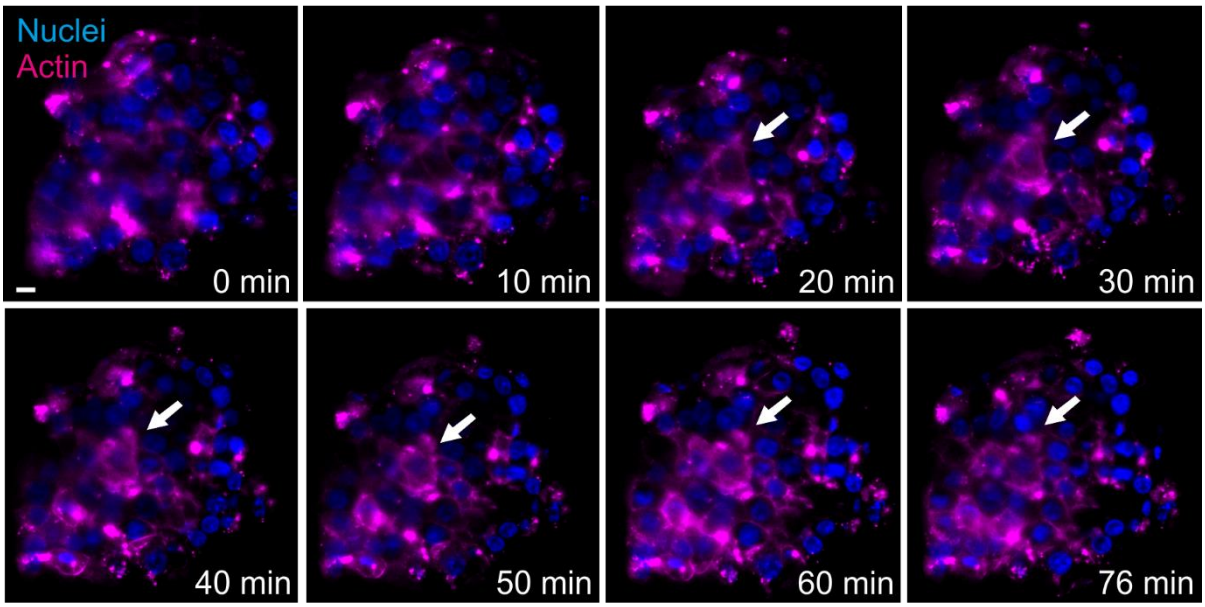

Fig. S10 Actin network dynamics in Cu conditions. Live LSFM imaging of SiR-actin (pink) and Hoechst (blue). Selected time-points of a single plan LSFM within a spheroid exposed to 100 µM Cu. The full video corresponds to 120 minutes with one acquisition every 2 minutes (Video S11). White arrows highlight the location of a site of actin re-organization upon cell division process. The scale bar corresponds to 10 µm.

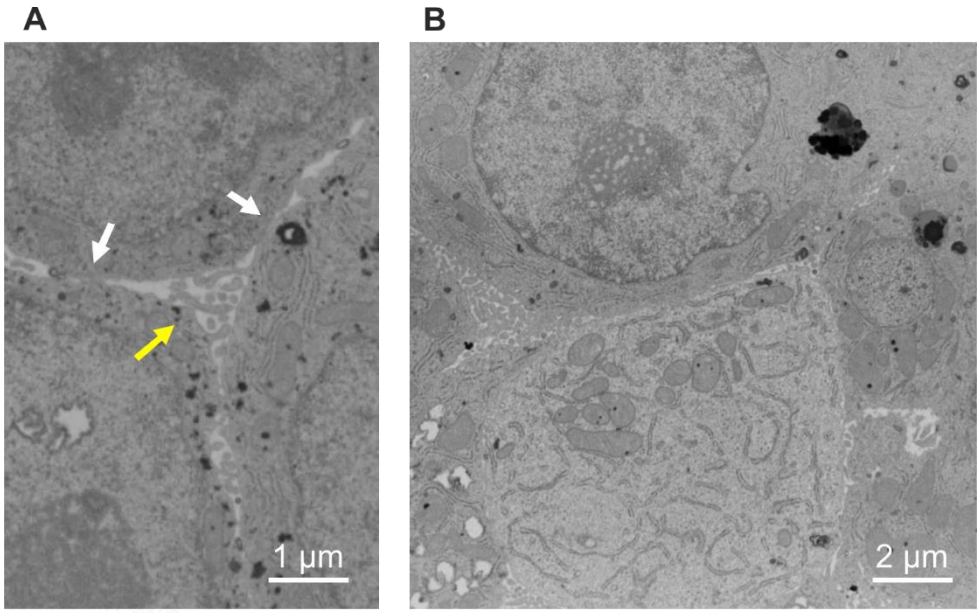

Fig. S11 Ultrastructure within a chemically-fixed spheroid. Electron micrographs extracted from FIB-SEM stack of images of a spheroid on-chip, prepared as described in [1]. (A) Zoom in on a bile canaliculus

region (yellow arrow) defined by microvilli in between hepatocytes and delimited by cell membranes juxtaposition (white arrows). (B) The more central hepatocyte contains an area full of mitochondria (middle of the micrograph) with surrounding endoplasmic reticulum.

Captions for Videos.

Video S1. 3D reconstructed spheroid stained for actin (red), Hoechst (blue) and Mrp2 (green). The video shows the full volume in 3-color, followed by sections through the volume in 3-color and then in red-green only and finally green alone. This enables to see several elongated bile canaliculi stained red and green that appeared yellow.

Video S2. 3D reconstructed spheroid stained for actin (red), Hoechst (blue) and CMFDA (green). The video shows the full volume in 3-color, followed by sections through the volume, which showed bile canaliculi (ref) filled with CMFDA (green).

Video S3. A bile canaliculus segmented from the spheroid presented in **Video S2.** The video shows a canaliculus with surrounding actin in red and encapsulated CMFDA in green.

Video S4. Live LSFM imaging of SiR-actin only (pink). Single plan LSFM within a spheroid in basal conditions. The full video corresponds to 2 minutes with one acquisition every 10-15 seconds.

Video S5. Live LSFM imaging of SiR-actin only (pink). Single plan LSFM within a spheroid in basal conditions. The full video corresponds to 2 minutes with one acquisition every 10-15 seconds.

Video S6. Live LSFM imaging of SiR-actin only (pink). Single plan LSFM within a spheroid exposed to 100 µM Cu. The same plan than Video S5 was imaged. The full video corresponds to 2 minutes with one acquisition every 10-15 seconds.

Video S7. Live LSFM imaging of SiR-actin (pink) and Hoechst (blue). Single plan LSFM within a spheroid in basal conditions. The full video corresponds to 80 minutes with one acquisition every 2 minutes.

Video S8. Live LSFM imaging of SiR-actin (pink) and Hoechst (blue). Single plan LSFM within a spheroid in basal conditions. The full video corresponds to 78 minutes with one acquisition every 2 minutes.

Video S9. Live LSFM imaging of SiR-actin (pink) and Hoechst (blue). Single plan LSFM within a spheroid exposed to 80 µM CDCA. The full video corresponds to 70 minutes with one acquisition every 2 minutes.

Video S10. Live LSFM imaging of SiR-actin (pink) and Hoechst (blue). Single plan LSFM within a spheroid exposed to 100 µM Cu. The full video corresponds to 80 minutes with one acquisition every 2 minutes.

Video S11. Live LSFM imaging of SiR-actin (pink) and Hoechst (blue). Single plan LSFM within a spheroid exposed to 100 µM Cu. The full video corresponds to 120 minutes with one acquisition every 2 minutes.

Video S12. The video presents a full FIB-SEM stack of micrographs within a matrix-free hepatic spheroid following high-pressure freezing preparation.

Video S13. The video shows the 3D reconstruction segmented with nuclei (pink), mitochondria (green), vesicles (blue) and bile canaliculi (grey). The different segments are also shown sequentially to visualize the different cellular compartments.

Video S14. The video shows the structure of the bile canaliculus segmented from the 3D reconstruction. Then, the sequential segmentation of the different cell membranes that are part of the canaliculus are shown in different colors and removed step-by-step to visualize clearly the microvilli and the contribution of each cell for this canaliculus.

Supplementary reference

1 V. Tardillo Suárez, E. Karepina, M. Chevallet, B. Gallet, C. Cottet-Rousselle, P. Charbonnier, C. Moriscot, I. Michaud-Soret, W. Bal, A. Fuchs, R. Tucoulou, P.-H. Jouneau, G. Veronesi and A.Deniaud, *BioRχiv*, 2019, doi: 10.1101/825919.