Fig. 1: Exemplary ADME-N chip footprint (top) and isometrical view on separate layers (bottom) exemplifying the positioning of a membrane between the two circuits.

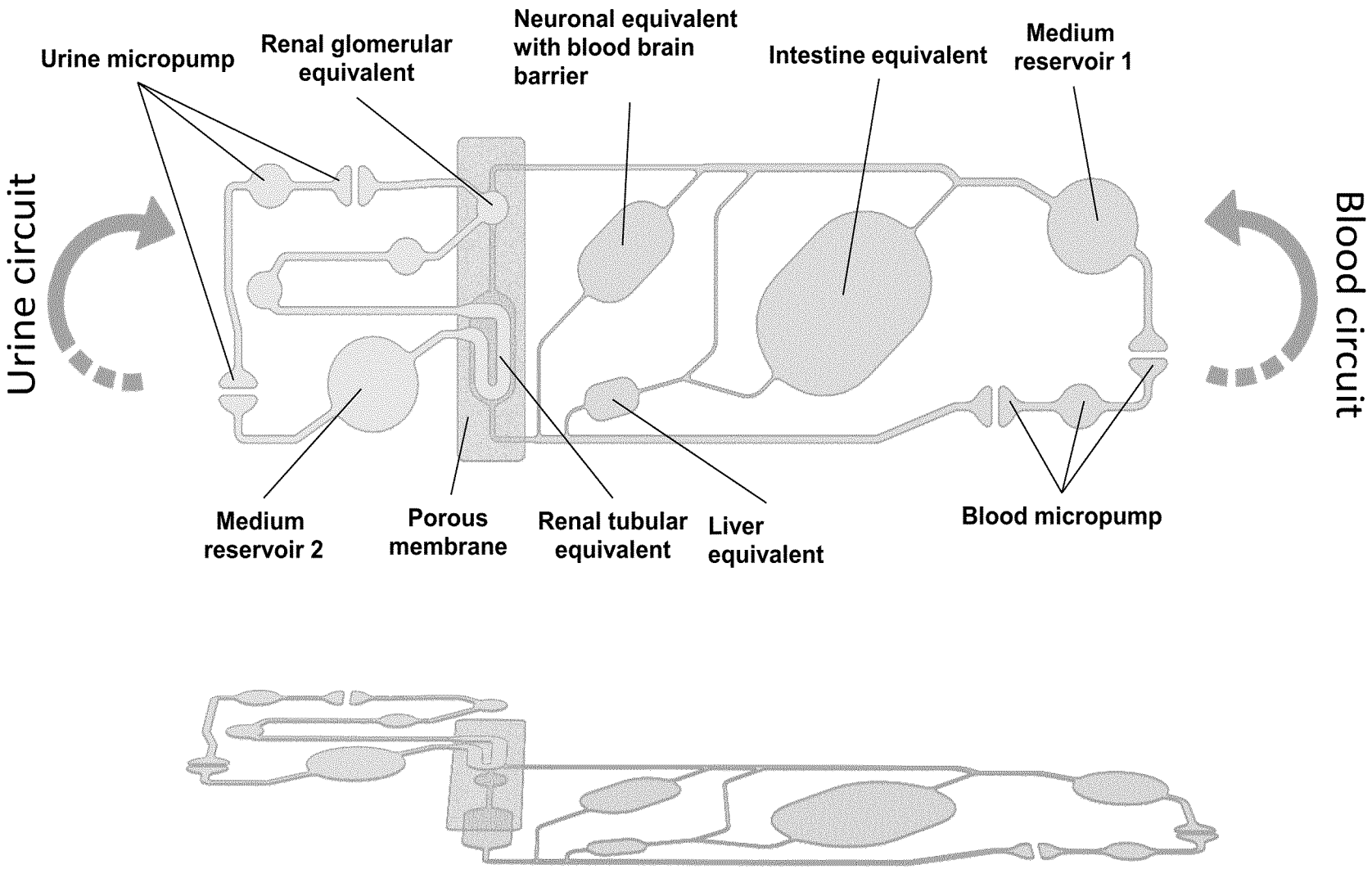


Fig. 2: Experimental design of iPSC chips and primary tissue chips



Fig. 2A: Experiment 1.1: iPSC chips – 3 chips

- iPSC-derived liver equivalent
- iPSC-derived intestine equivalent
- iPSC neuronal equivalent with blood brain barrier printed
- RPTECs kidney

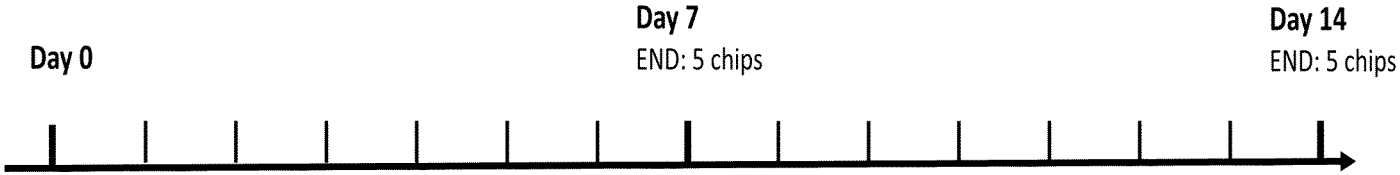


Fig. 2A: Experiment 1.2: Primary tissue chips – 10 chips

- Primary liver tissue
- Primary small intestine
- iPSC-derived neuronal equivalent with blood brain barrier printed
- RPTECs kidney

Fig. 2 (*cont'd*): Experimental design of iPSC chips - Experiment 2.

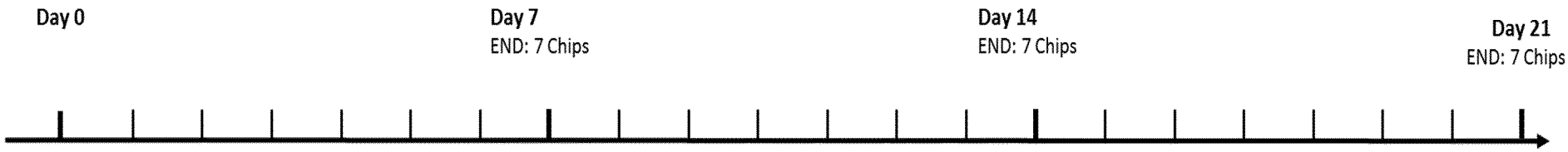


Fig. 2B: Experiment 2: iPSC chips – 21 chips

- iPSC-derived liver equivalent
- iPSC-derived intestine equivalent
- iPSC-derived neuronal equivalent with blood brain barrier
- iPSC-derived renal equivalent

Fig. 3: Glucose concentration and LDH activity of Experiments 1.1 and 1.2, measured daily in the apical compartment of the intestinal equivalent, in the "blood" medium reservoir, and in the "urine" medium reservoir.

Fig. 3A: Experiment 1.1: iPCS chips – 3 chips

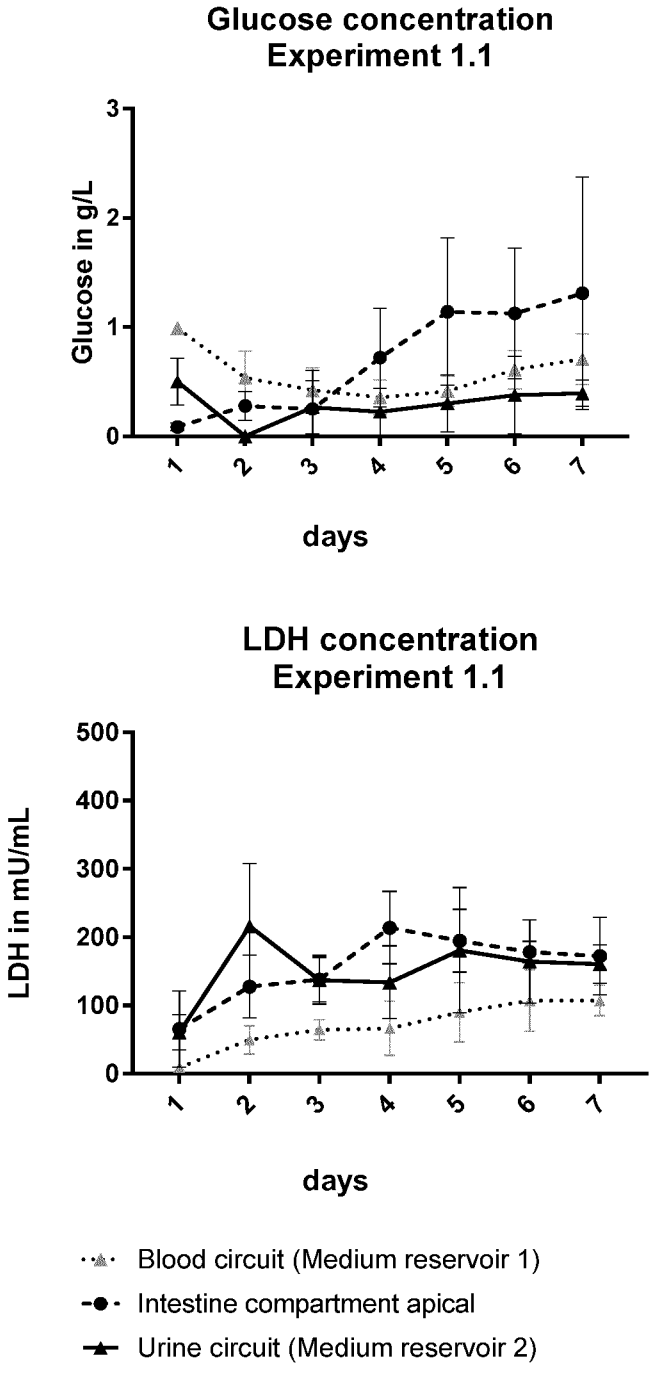


Fig. 3 (*cont'd*): Glucose concentration and LDH activity of Experiments 1.1 and 1.2, measured daily in the apical compartment of the intestinal equivalent, in the "blood" medium reservoir and "urine" medium reservoir.

Fig. 3B: Experiment 1.2: Primary tissue chips – 10 chips

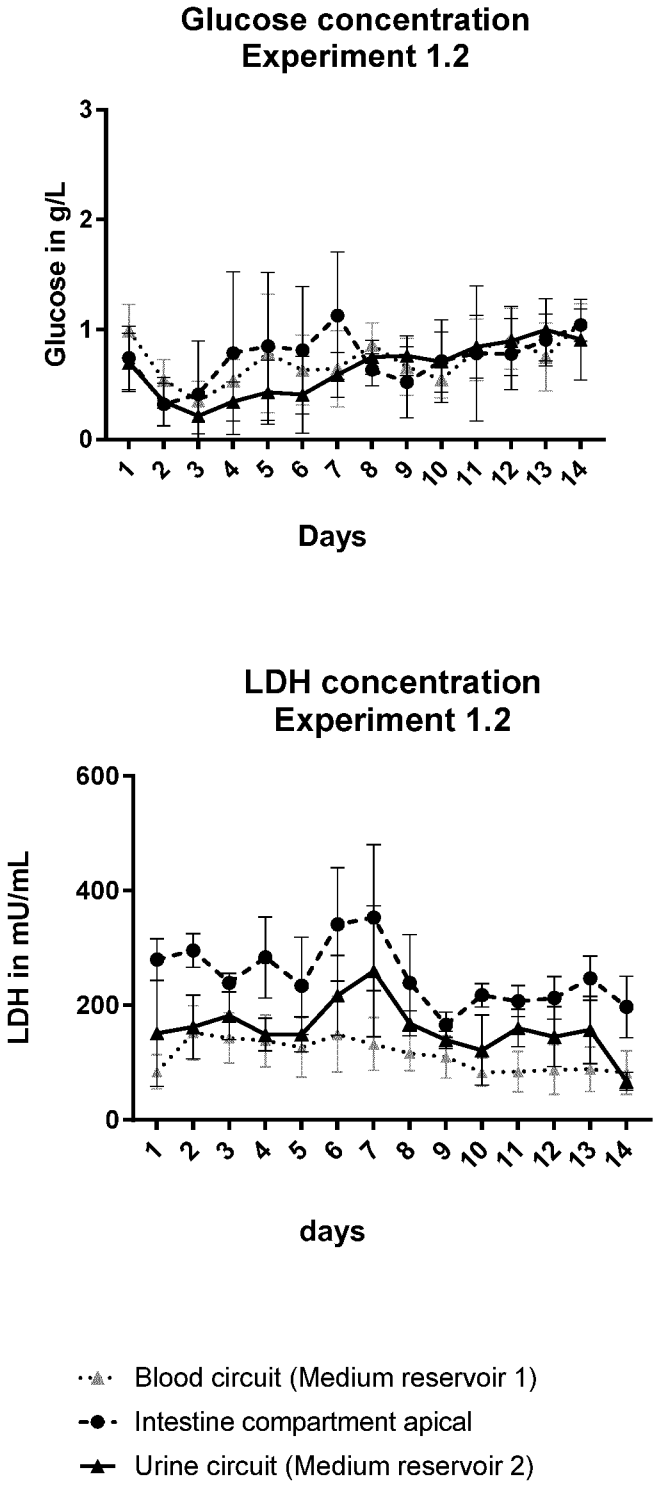
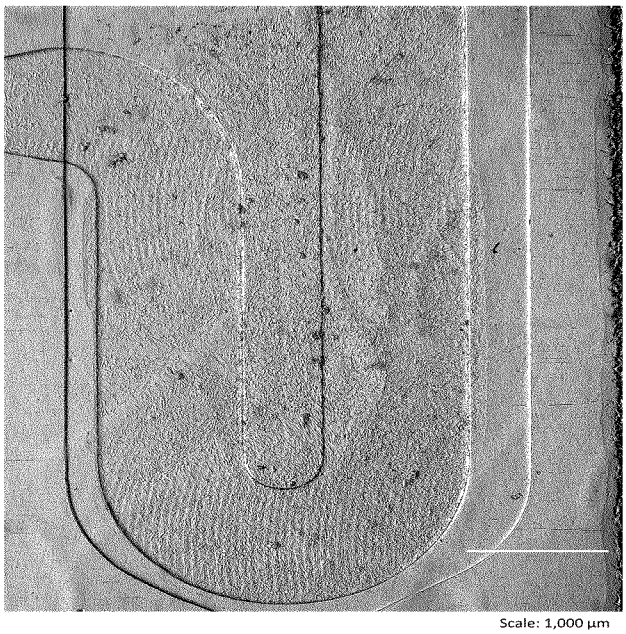
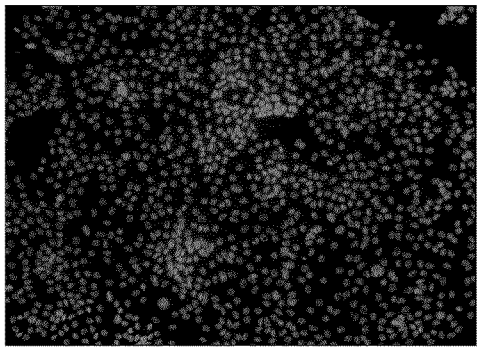


Fig. 4: Renal tubular equivalent fully seeded with human RPTECs (Experiment 1.1, day 7). **A:** bright field (BF) image on day 7 of culture. **B-D:** immunohistolgy stainings of RPTECs on day 14 (Experiment 1.2) **B:** nucleus stained with DAPI; **C:** cell membranes stained with NaK-ATPase; **D:** cytoskeleton stained with cytokeratin 8/18.

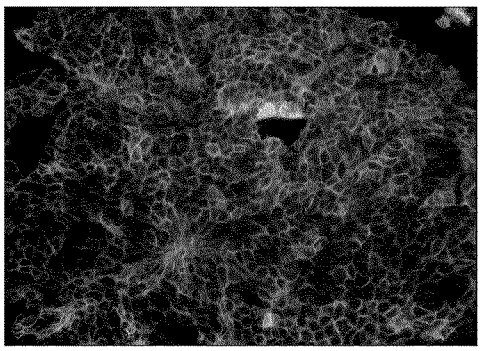
A: BF



B: DAPI



C: NaK-ATPase



D: Cytokeratin 8/18

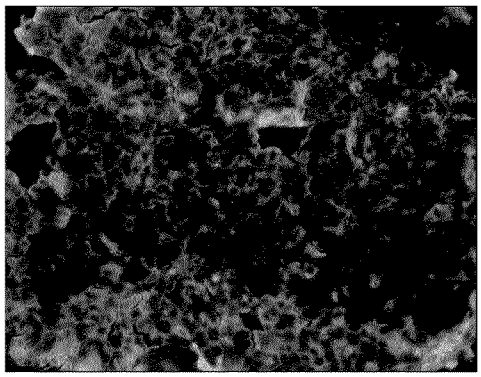


Fig. 5: Aspartate transaminase (AST) concentration measured in the "blood" circuit in medium reservoir 1, and in the "urine" circuit in medium reservoir 2.

Experiment 2: iPSC chips – 21 chips

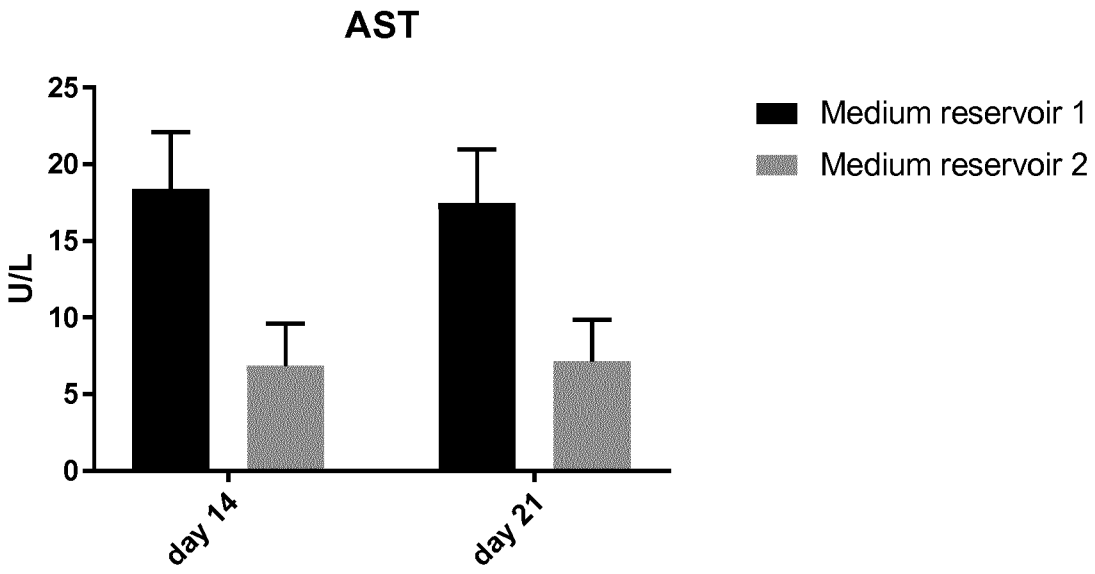
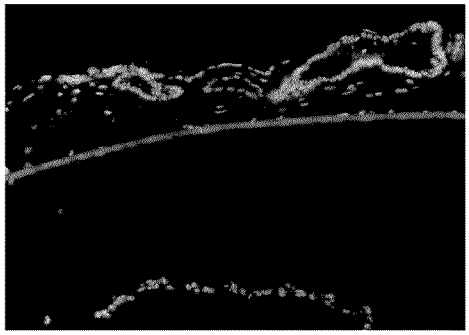
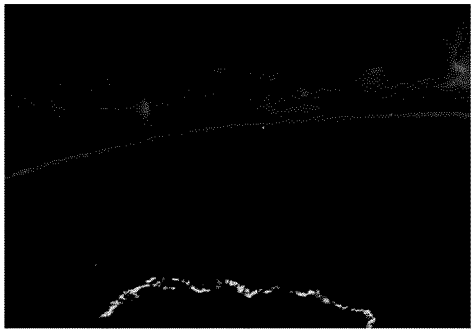


Fig. 6: Exemplary immunohistological examination of an iPSC-derived intestine equivalent with primary endothelial cells on day 7 of ADME-N chip culture (Experiment 1.1). **A:** nucleus stained with DAPI; **B:** primary endothelial cells stained for CD31; **C:** iPSC-derived intestinal organoids stained for NaK-ATPase. **D:** nucleus stained with DAPI; **E:** iPSC-derived intestinal organoids stained for Cyp3A4; **F:** iPSC-derived fibroblasts stained for vimentin.

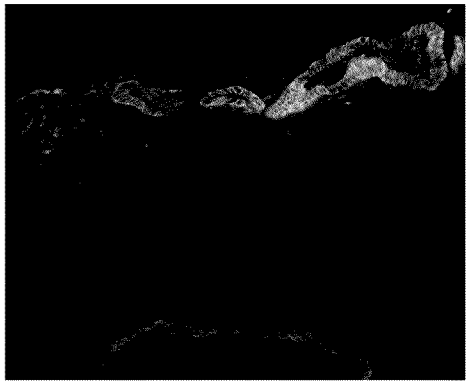
A: DAPI



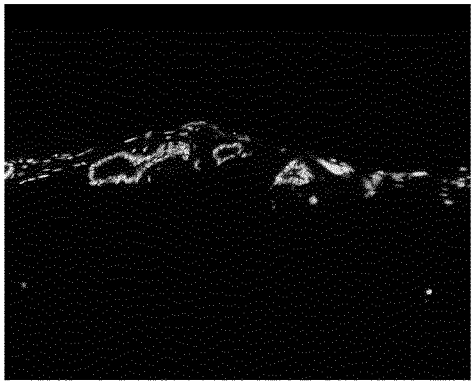
B: CD31



C: NaK-ATPase



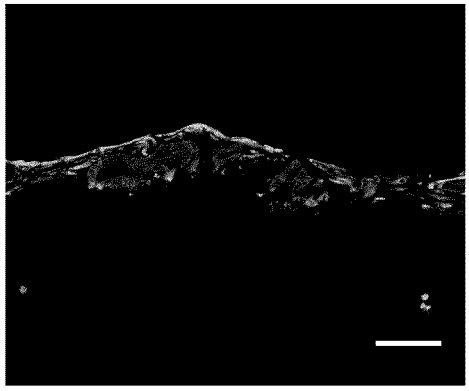
D: DAPI



E: Cyp3A4



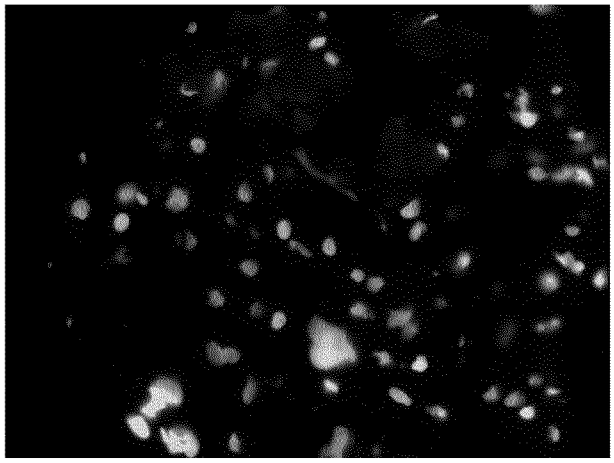
F: Vimentin



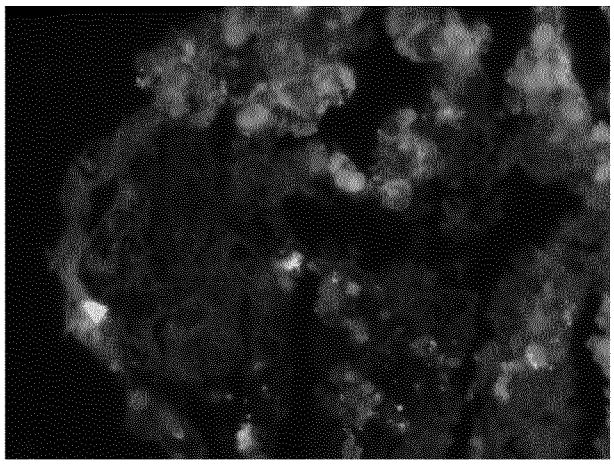
Scale: 100µm

Fig. 7: Exemplary immunohistological examination of primary liver equivalents (from InSphero) cultivated for two weeks in the ADME-N chip (Experiment 1.2). **A:** nucleus stained with DAPI; **B:** albumin; **C:** Cyp3A4.

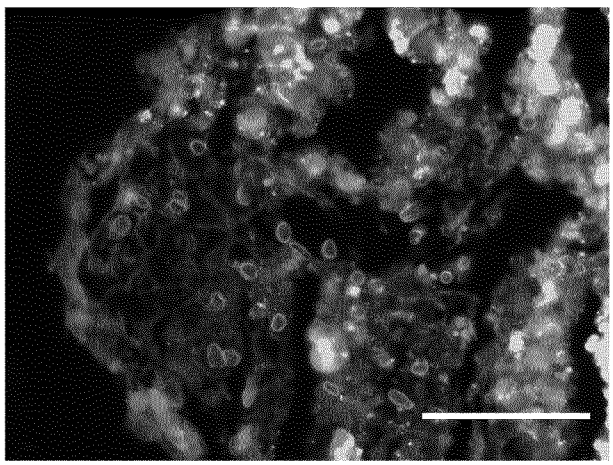
A: DAPI



B: Albumin



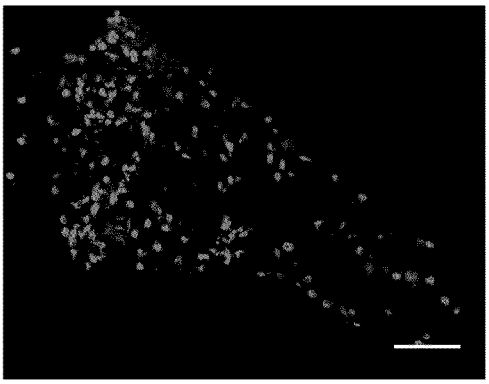
C: Cyp3A4



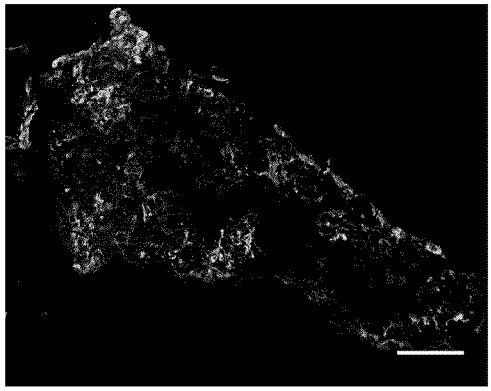
Scale: 100µm

Fig. 8: Exemplary immunohistological examination of iPSC-derived liver equivalent cultivated for three weeks in the ADME-N chip (Experiment 2). All cells derived from the same donor. **A:** nucleus stained with DAPI; **B:** iPSC-derived fibroblasts stained for vimentin; **C:** cytoskeleton of hepatocytes stained with cytokeratin 8 /18, **D:** nucleus stained with DAPI; **E:** tight junctions stained with ZO-1; **F:** albumin.

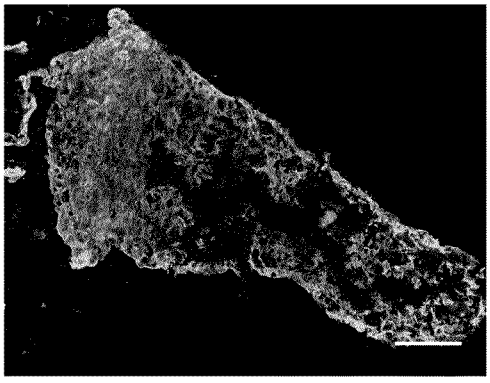
A: DAPI



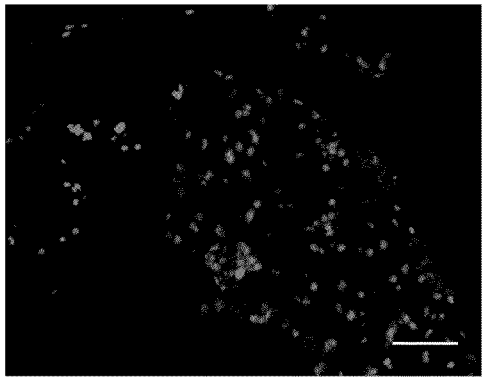
B: Vimentin



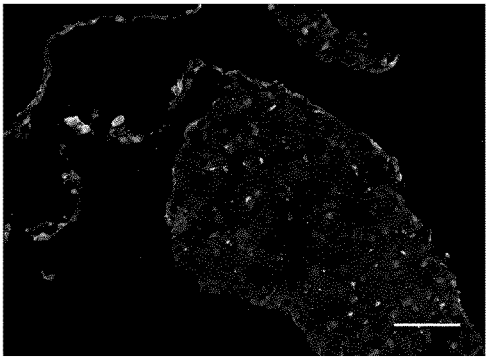
C: Cytokeratin 8 /18



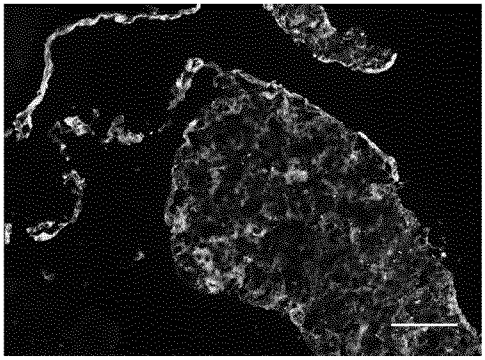
D: DAPI



E: ZO-1



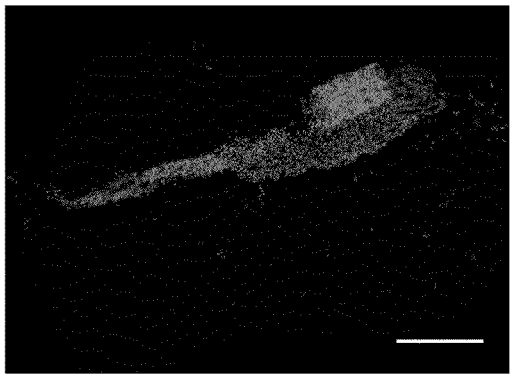
F: Albumin



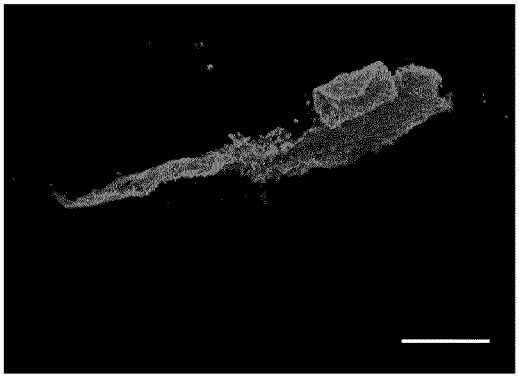
Scale: 100µm

Fig. 9: Exemplary immunohistological examination of iPSC-derived neuronal equivalents cultivated for two weeks in the ADME-N chip (Experiment 1.2). **A:** nucleus stained with DAPI; **B:** neuronal cells stained with b Tubulin III; **C:** primary endothelial cell stained with vWF (Von-Willebrand-Faktor); **D:** bright field image of neuronal spheroids lying on printed hydrogel including primary endothelial cells.

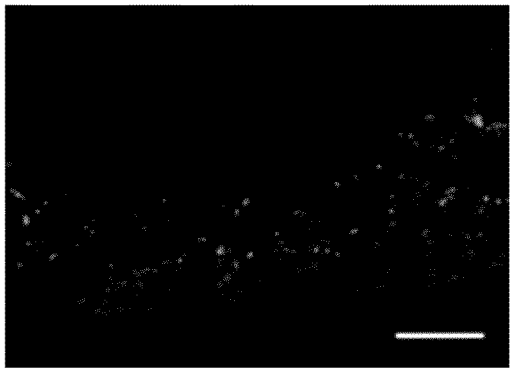
A: DAPI



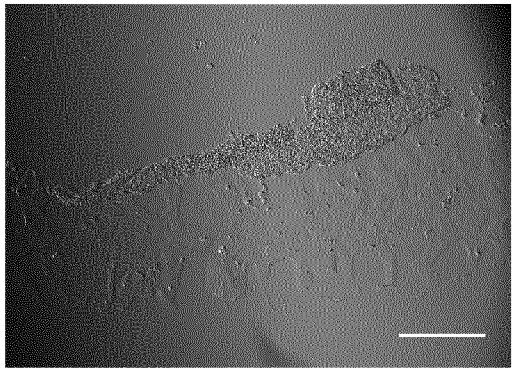
B: B Tubulin III



C: vWF



D: BF



Scale: 250µm

Fig. 10: LDH concentration measured in the apical compartment of the intestinal equivalent, in the blood circuit in medium reservoir 1, and in the urine circuit in medium reservoir 2. For Experiment 2. The barrier function of the intestine equivalent and the renal equivalent is shown by different concentrations of LDH in all three compartments.

Experiment 2: iPSC chips – 21 chips

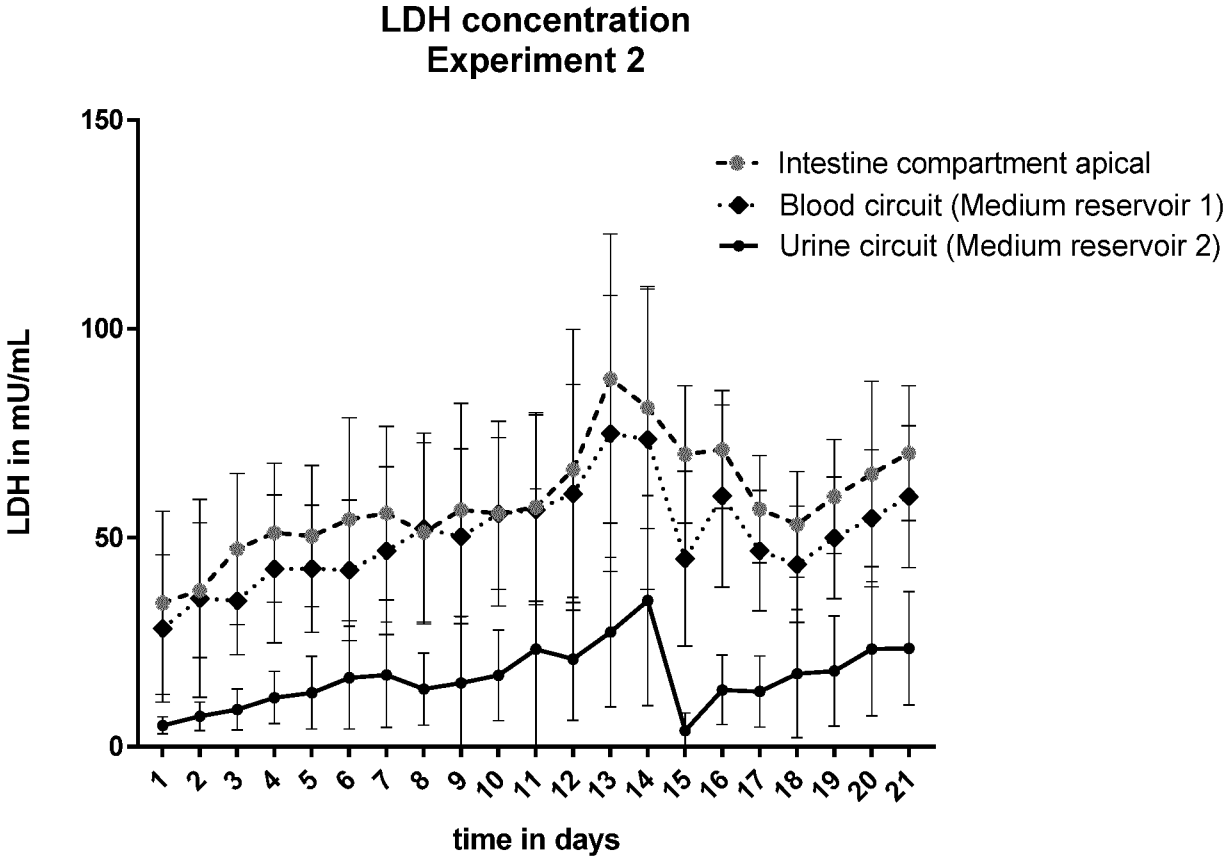
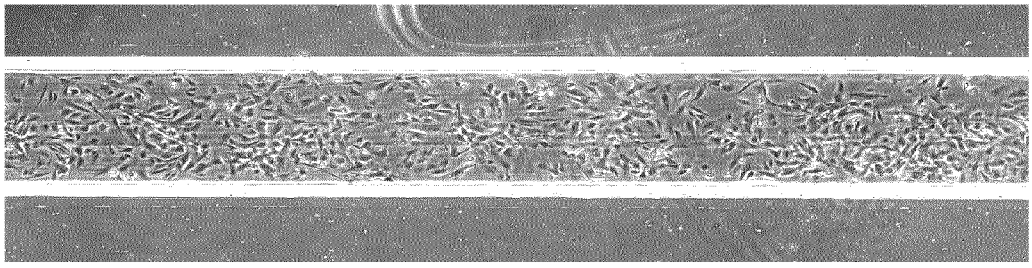
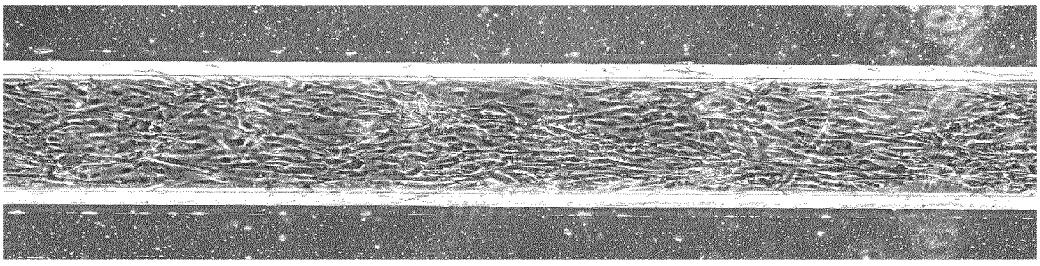


Fig. 11: iPSC derived endothelial cells elongate in the channels of the multi-organ-chip. High expression of the endothelial marker CD31 on day 8 of chip culture.

Day 0

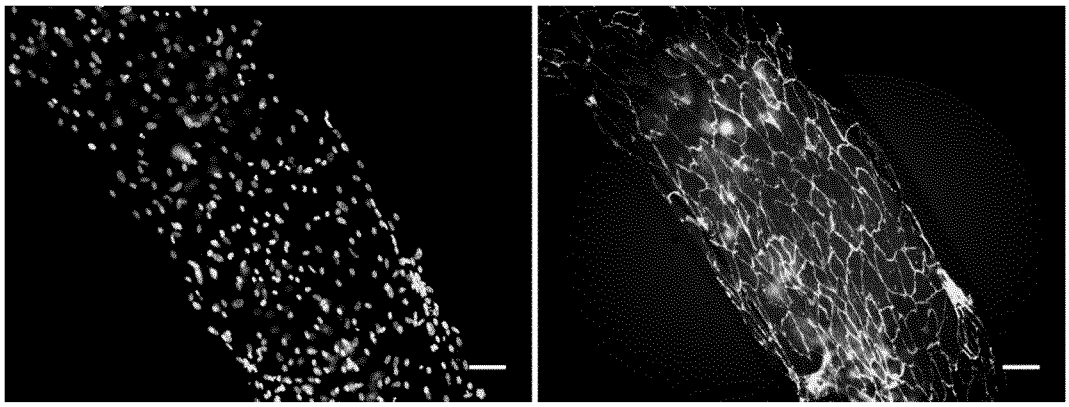


Day 8



Day 8:

DAPI **CD31 (Scale 100µm)**



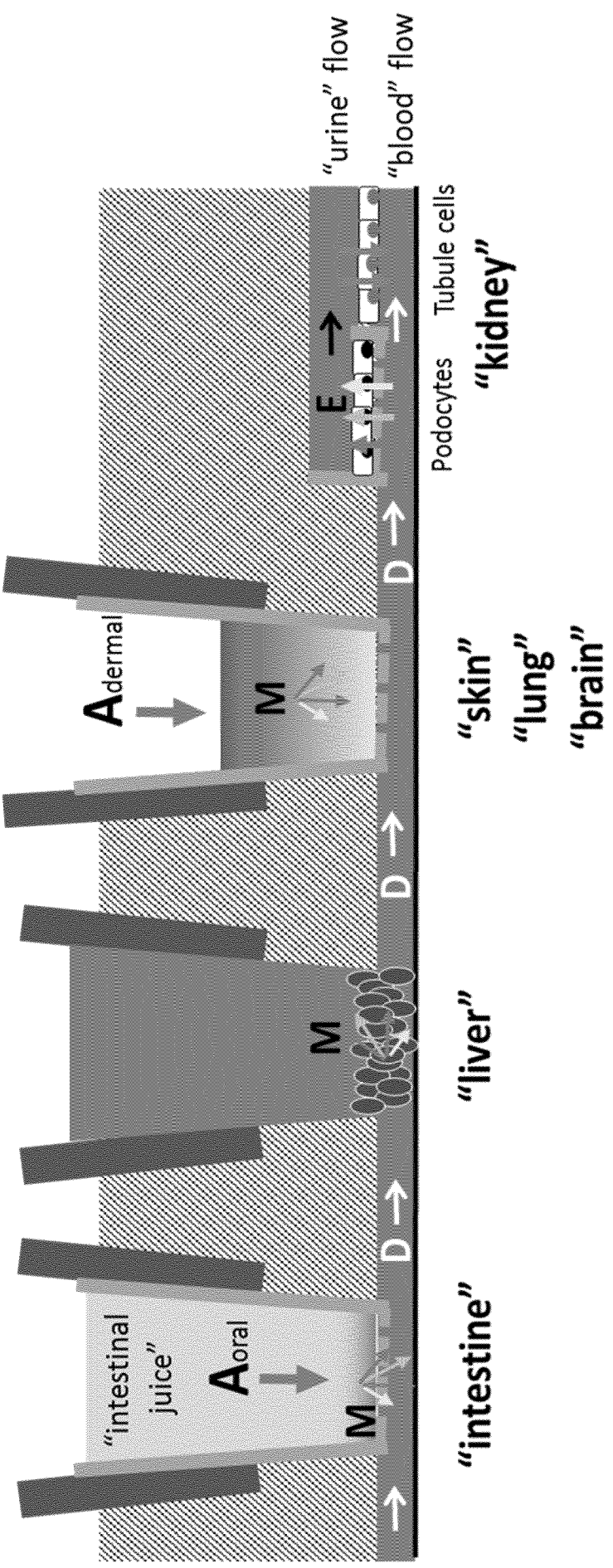
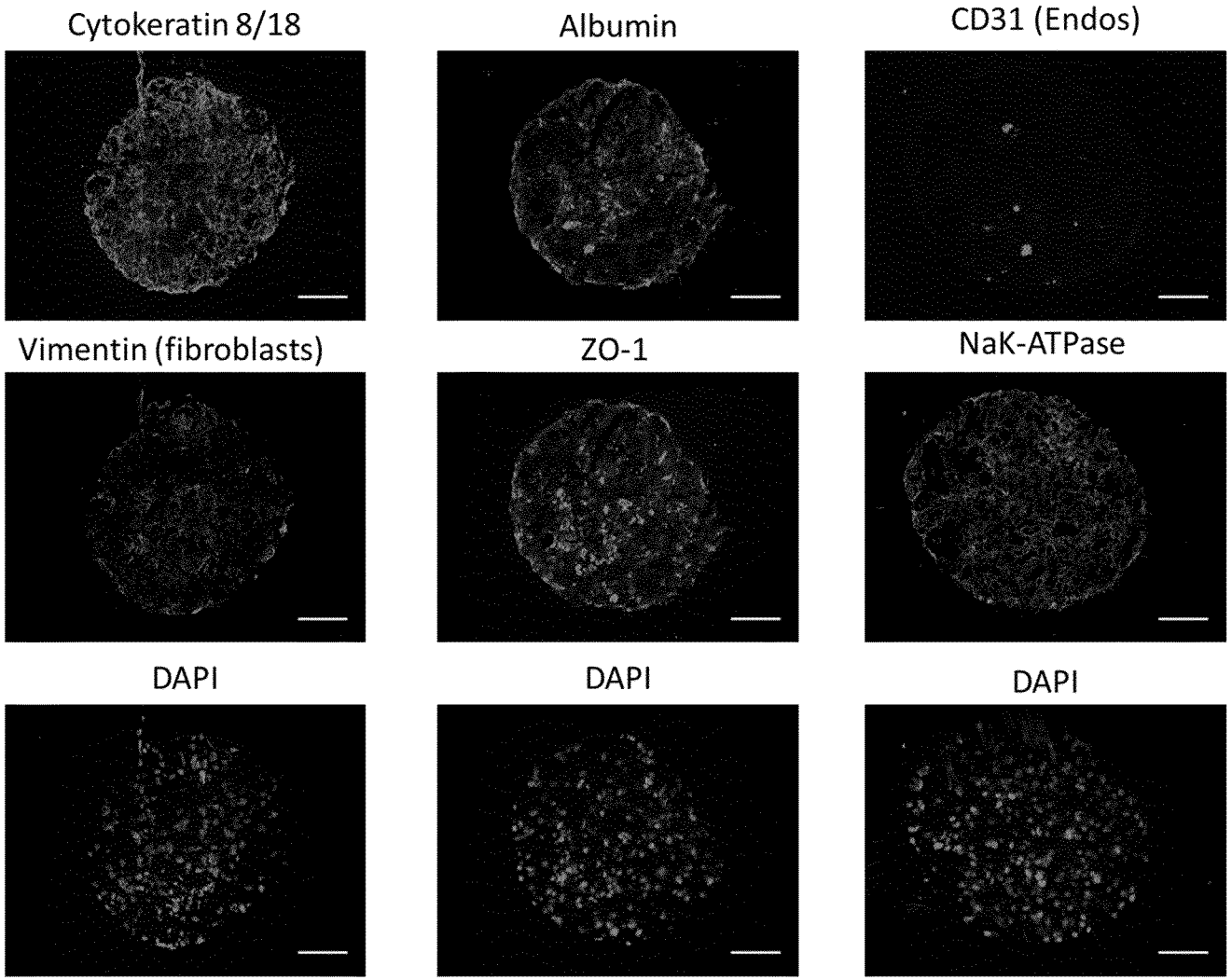


Fig. 12: Partial cross-sectional schematic view of ADME-Chip. A = Administration, D = Distribution, M = Metabolism, E = Excretion.

Fig. 13: Immunofluorescence images after differentiation of iPSC-derived hepatocytes and 3D spheroid formation with iPSC-derived fibroblast and iPSC-derived endothelial cells (liver equivalents). "*Vimentin*": iPSC-derived hepatocytes with iPSC-derived fibroblasts (vimentin staining). "*CD31*": iPSC-derived hepatocytes with iPSC-derived endothelial cells (CD31 staining). All cells are derived from the same iPSC donor. Scale = 100 µm.



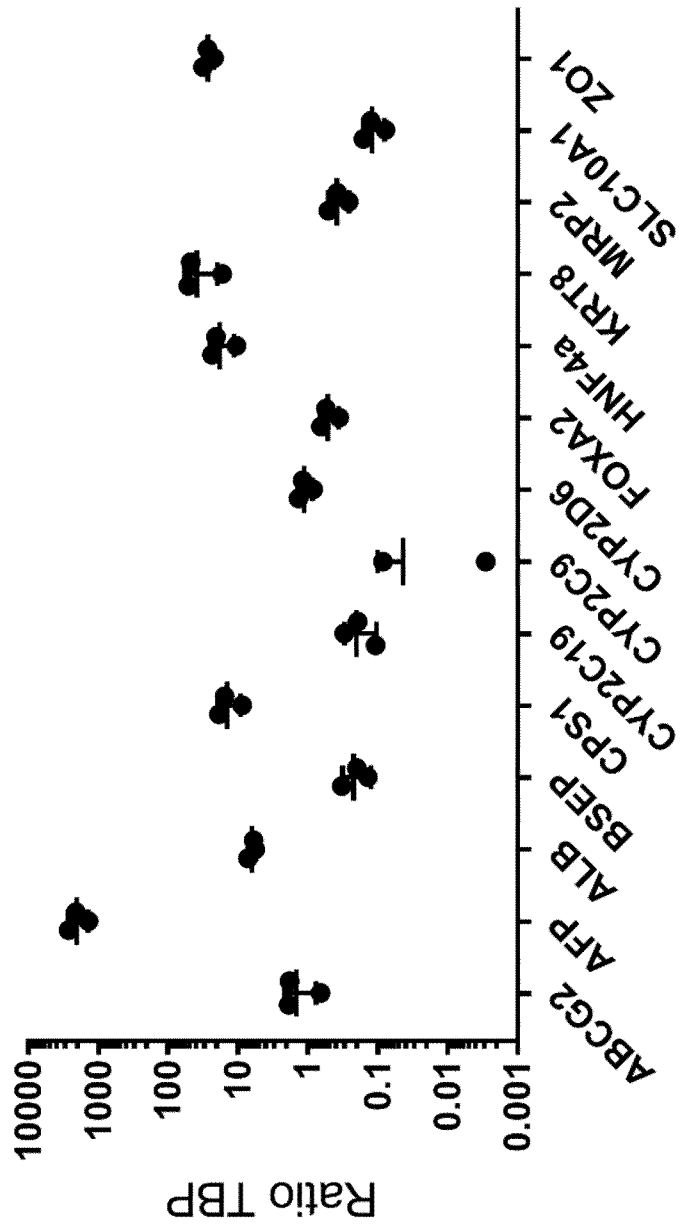
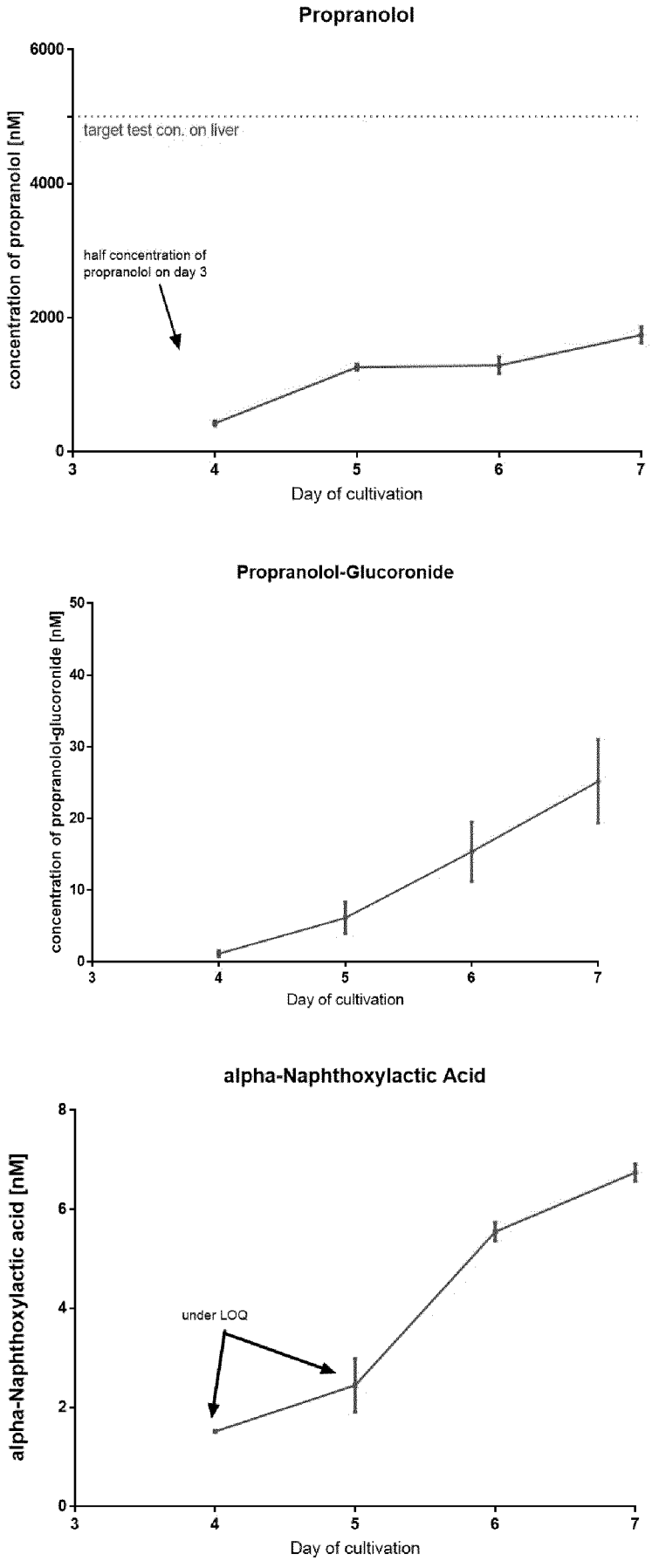


Fig. 14: Transcription levels of selected hepatic marker genes in iPSC-derived liver equivalents (iPSC-derived hepatocytes). Transcription levels analyzed by qPCR.

Fig. 15: Metabolization capacity of iPSC-derived 3D liver equivalents to metabolize propranolol into propranolol-glucoronide, and alpha-naphthoxylactic acid in single culture.



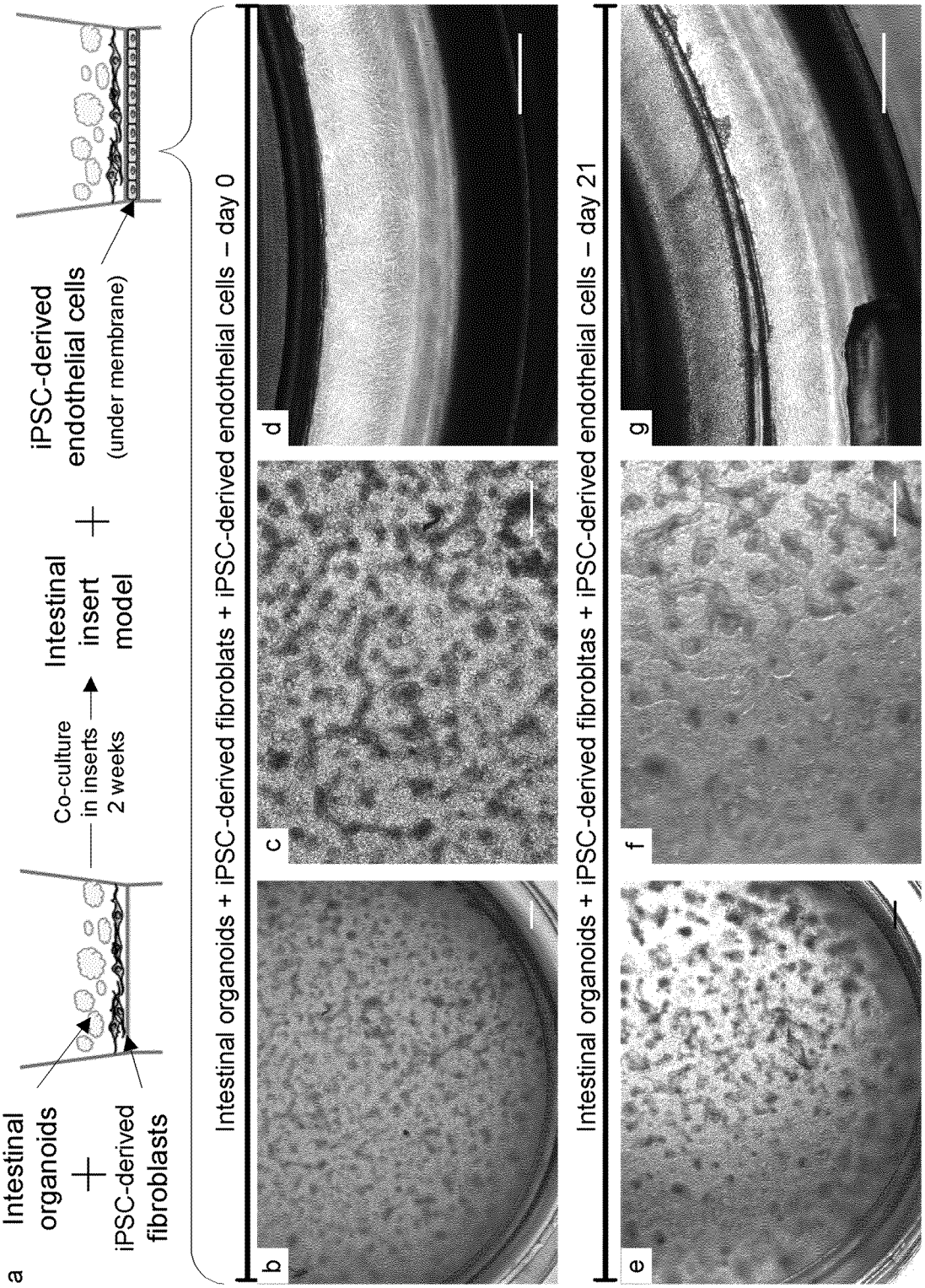


Fig. 16: Intestinal insert model development. All cells are derived from the same iPSC donor. Scale: 500 µm.

Fig. 17: Bioprinted hydrogel barrier with iPSC-derived intestinal organoids, iPSC-derived fibroblasts, and primary endothelial cells.

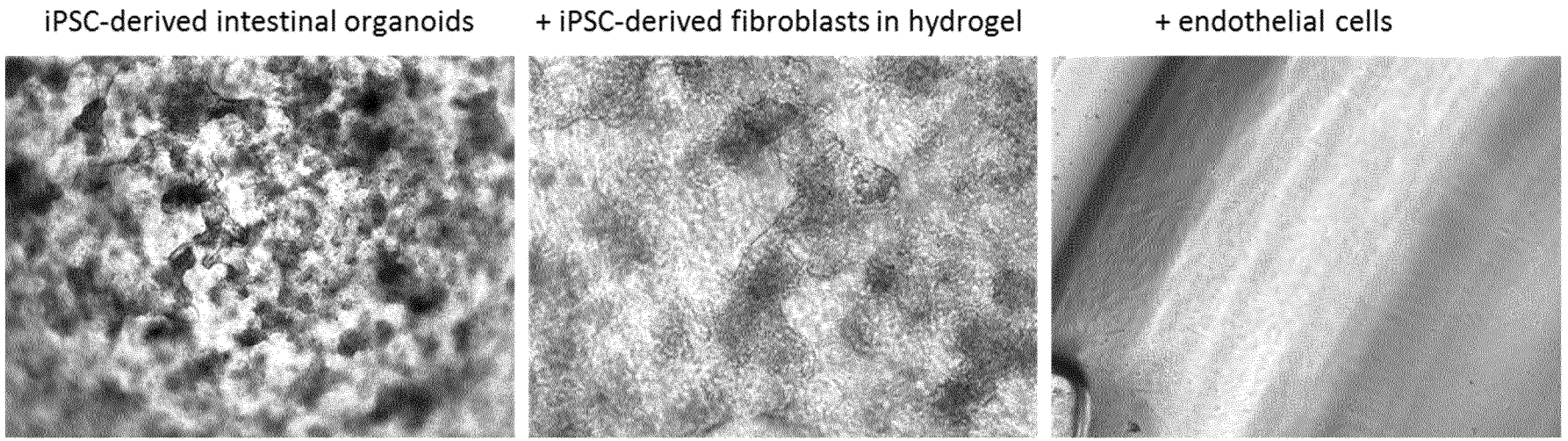


Fig. 18: iPSC-derived blood-brain-barrier made up of neurospheres and endothelial cells. All cells are derived from the same iPSC donor.

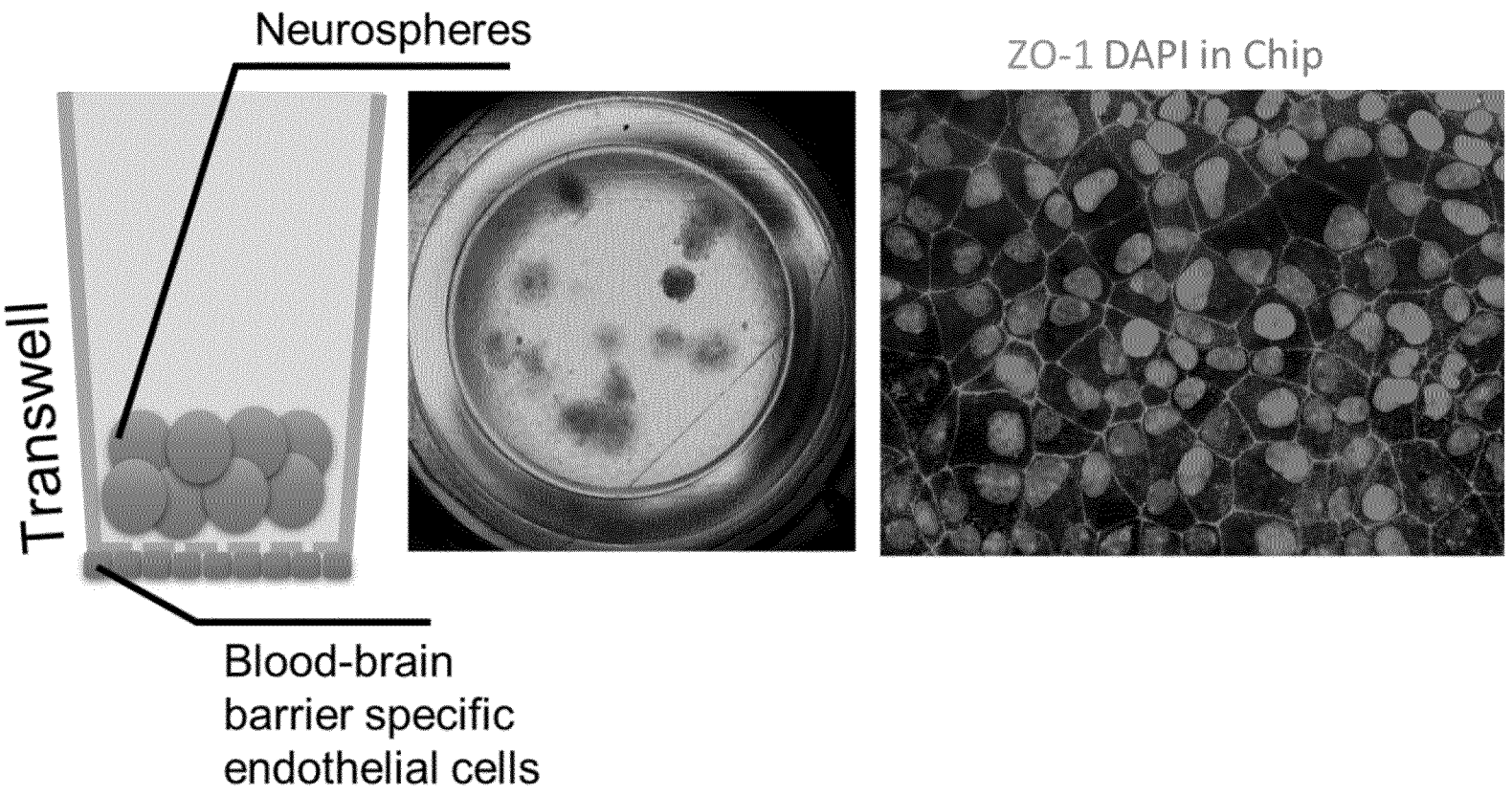


Fig. 19: Setup and timeline of the iPSC differentiation into kidney organoids, liver spheroids, neurospheres and intestinal organoids, transfer and co-cultivation in ADME-N Chip. All cells are derived from the same iPSC donor. DE: definitive endoderm; TW: Transwell.

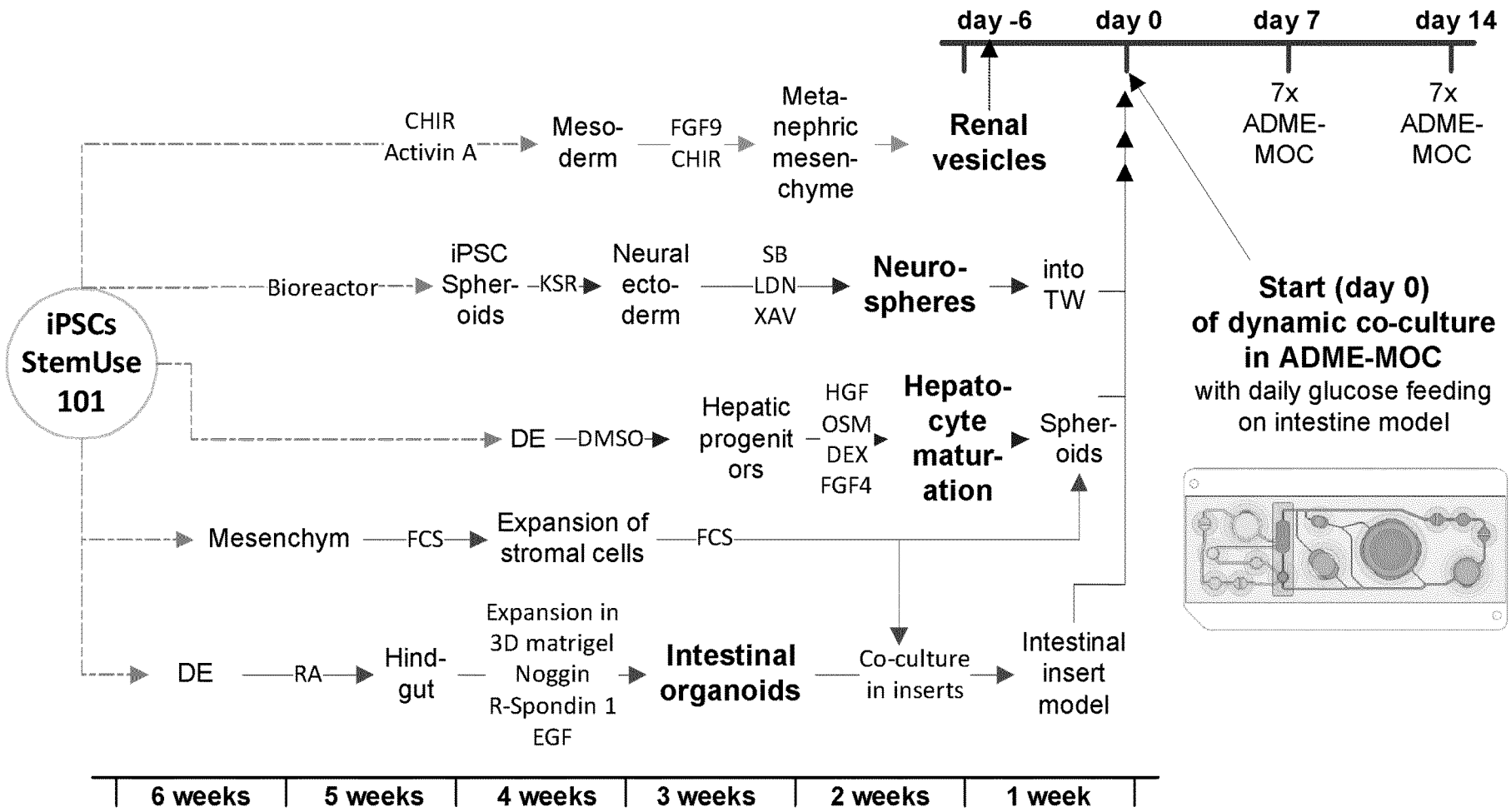


Fig. 20: Immunostaining characterization of the liver, intestinal, renal and neuronal model co-cultivated in the ADME-N Chip for 14 days (cf. Exp. 2). All cells are derived from the same iPSC donor. **a – c:** Liver model: a: Albumin, ZO-1, b: HNF4α, SLC10A1, and c: Ki67, TUNEL; **d – f:** Intestinal model: d: CDX2, Na+/K+-ATPase, e: Cytokeratin 8/18, vimentin, and f: Ki67, TUNEL; **g – i:** Renal model: g: Cytokeratin 8/18, vimentin, h: Aquaporin1, Na+/K+-ATPase, and i: Ki67, TUNEL; **j – l:** Neuronal model: j: TUBB3, PAX6, k: Nestin, and l: Ki67, TUNEL. Scale = 50 µm. The dotted lines illustrate the permeable insert membrane.

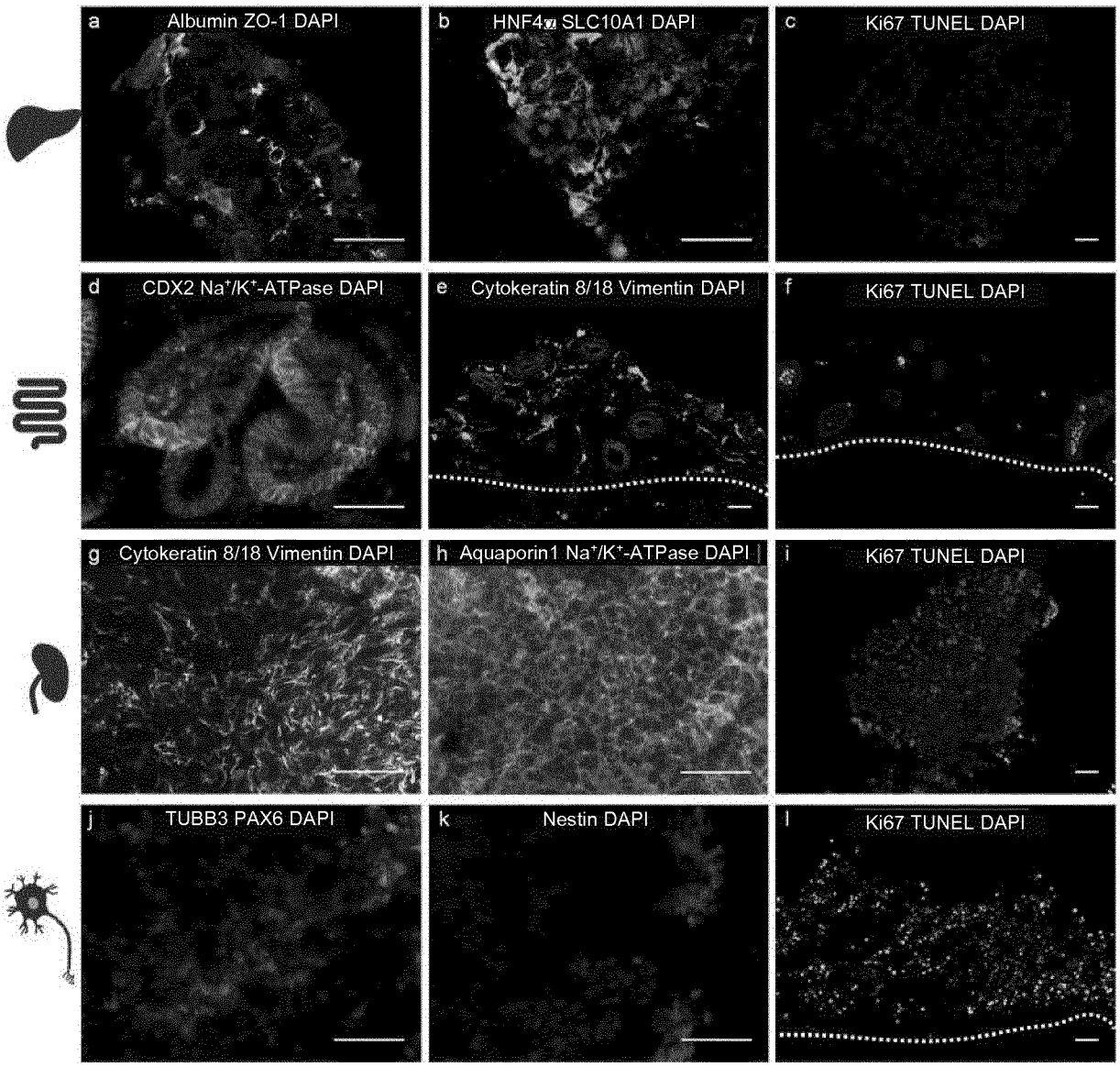


Fig. 21: qPCR results of the co-culture in the ADME-N Chip of the iPSC-derived intestinal, liver, renal and brain model. All cells are derived from the same iPSC donor. Data are normalized to the housekeeper EF1 (a, c) or TBP (b, d). N = 3 - 6 for day 0, 7 and 14; N = 1 – 3 for iPSCs, and N = 1 for the positive controls represented. ND: no signal detected. Ordinary one-way ANOVA with Tukey's multiple comparisons test was used for statistical analysis. In each of a) to d): bars from left to right are "iPSCs", "Day 0", "Day 7", "Day 14", and the respective "positive control".

