

Evaluation of megalin activity using three-dimensional culture of human proximal tubular epithelial cells

ヒト近位尿細管上皮細胞三次元培養モデル3D-RPTEC®のメガリン活性評価

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Introduction

The kidney is an important organ that regulates the balance of fluid and electrolytes in the body and excretes waste products, with proximal tubule cells playing a particularly important role in renal structure and function. Megalin is expressed in the apical membrane of renal proximal tubular epithelial cells, and it plays a crucial role in the endocytic uptake of various ligands. We have previously developed three-dimensional cultured human proximal tubular epithelial cells (3D-RPTEC) with transporter activity and other functions similar to the human native kidney and conducted an evaluation of their function. In this study, we report on the evaluation of megalin activity using 3D-RPTEC.

Conclusion

3D-RPTEC exhibited megalin activity.

3D-RPTECはメガリン活性を示した。

- ①Megalin was expressed on the cell membrane in 3D-RPTEC.
3D-RPTEC細胞膜にメガリンが発現していた。
- ②The uptake of megalin-mediated substances in 3D-RPTEC was confirmed.
3D-RPTECのメガリン介在性物質の取り込みを確認した。

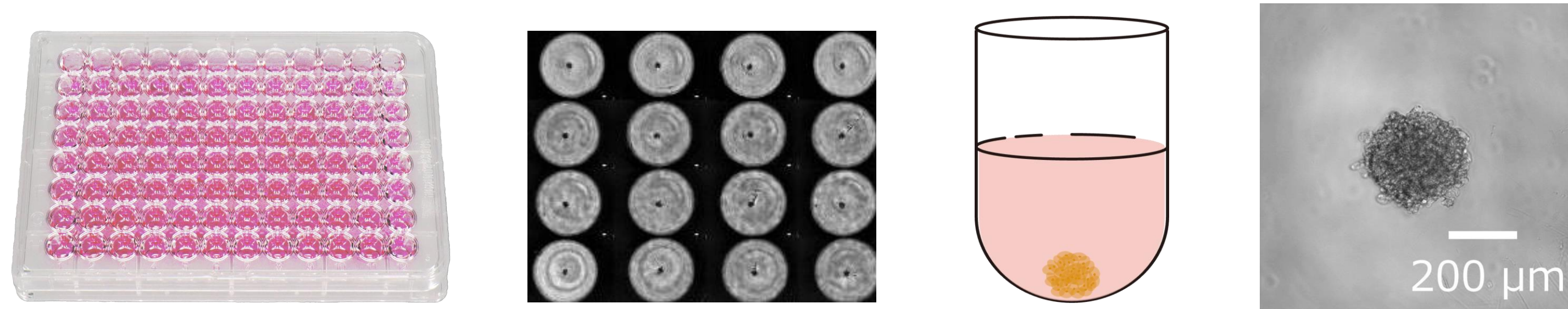
3D-RPTECのお問合せ： 日機装株式会社

企業出展ブース42（ドリンクコーナー正面）で製品展示をしております。
是非お立ち寄りください。



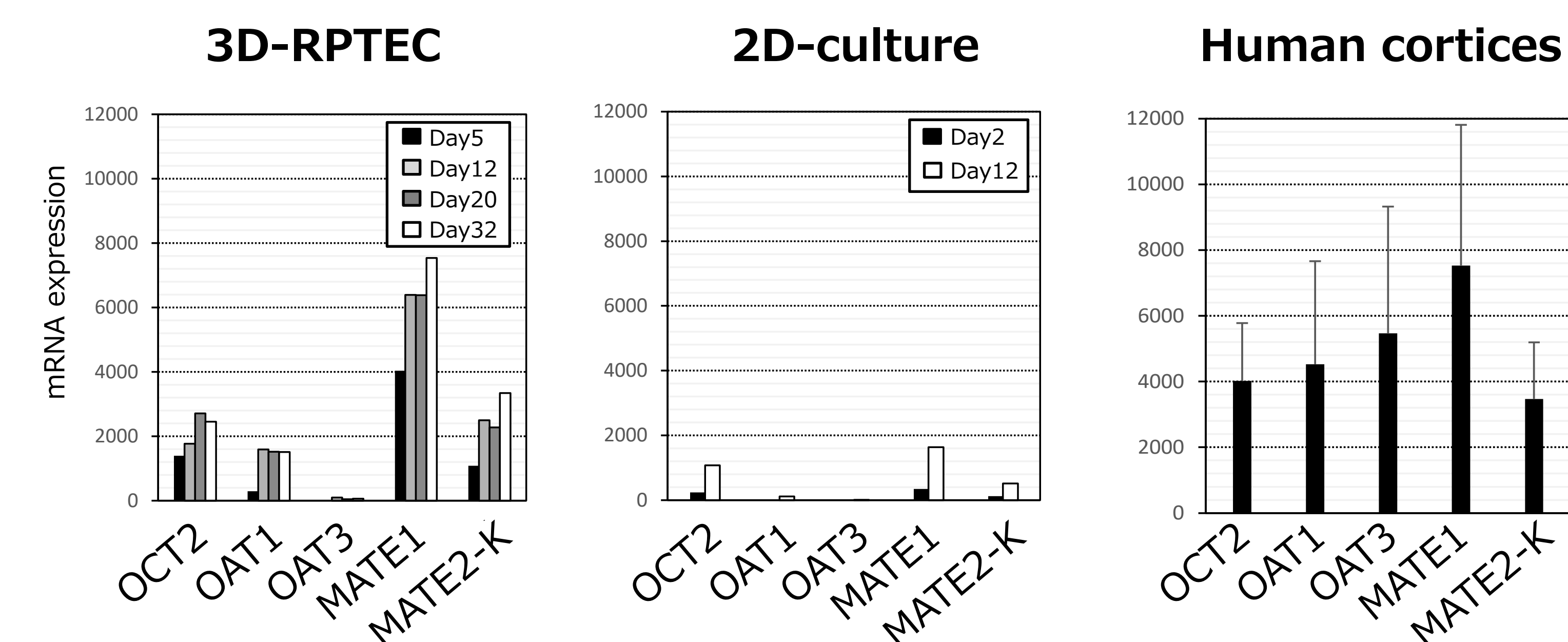
Features of 3D-RPTEC®

Sample (Product) form



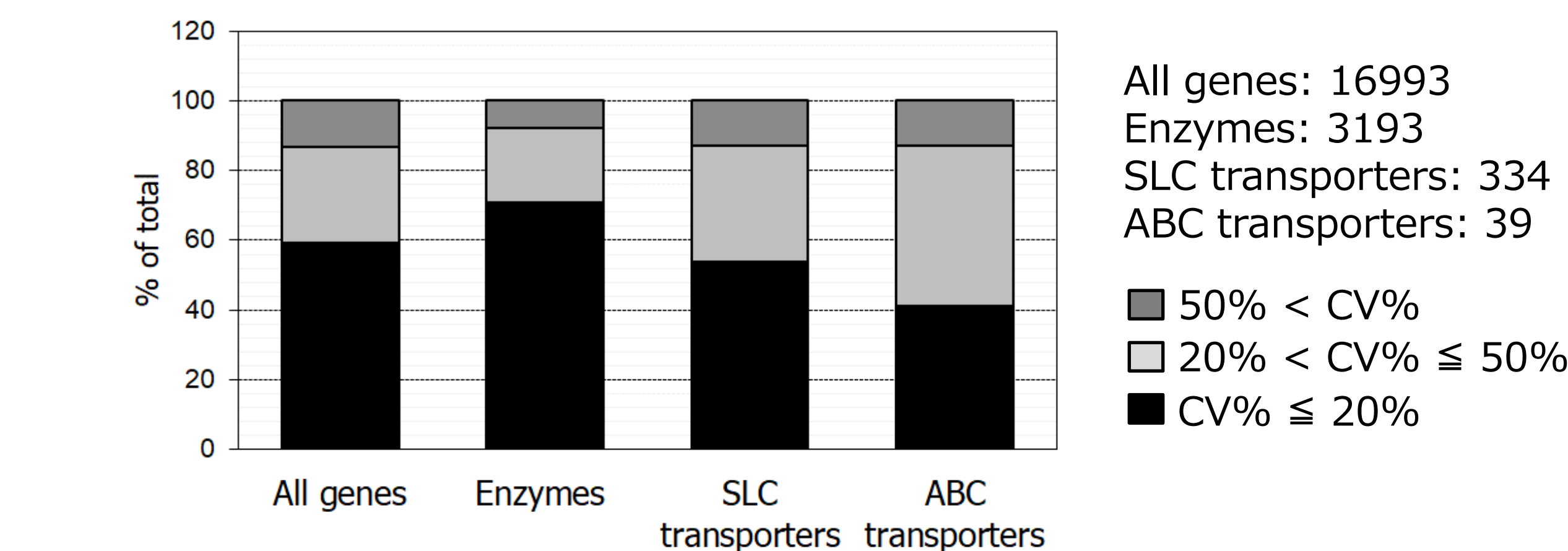
3D-RPTEC is a three-dimensional culture of human proximal tubule cells. Each well contains one spheroid in a 96-well plate, and the size of each spheroid is approximately 200 μm.

Drug transporter (Microarray)



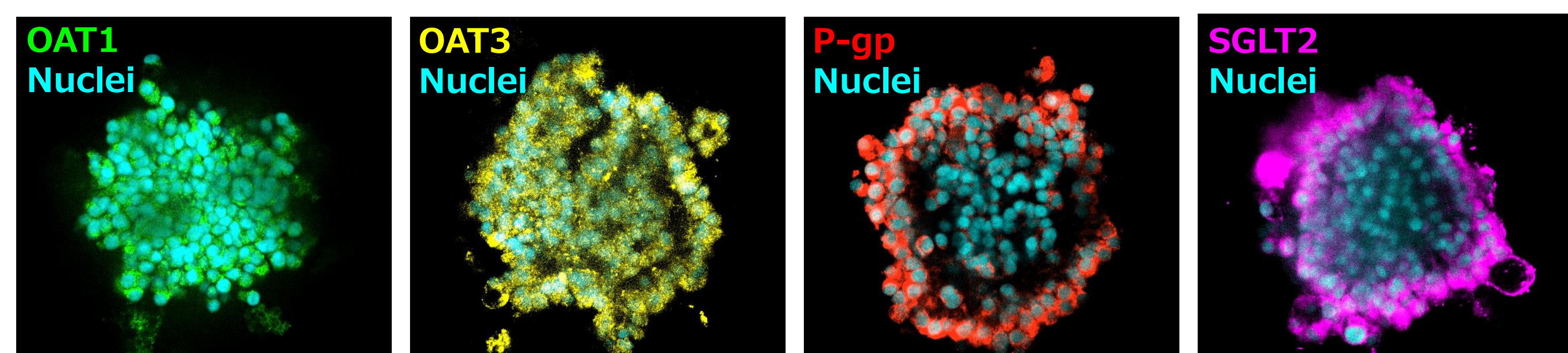
3D-RPTEC exhibited higher gene expression of drug transporters compared to 2D cultures. Furthermore, they also exhibited expression levels of some genes similar to those in the cortex. Additionally, 3D-RPTEC maintained gene expression for approximately a month.

Donor-to-donor variability



Approximately 50% of the SLC transporters and 40% of the ABC transporters in 3D-RPTEC have a coefficient of variation within 20%, indicating minimal donor-to-donor variation.

Transporter immunostaining



3D-RPTEC exhibited the expression of drug transporters in immunocytochemistry. Basolateral transporters (OAT1 and OAT3) were expressed throughout the spheroid, whereas apical transporters (P-gp and SGLT2) were more strongly localized to the outer surface of the spheroid.

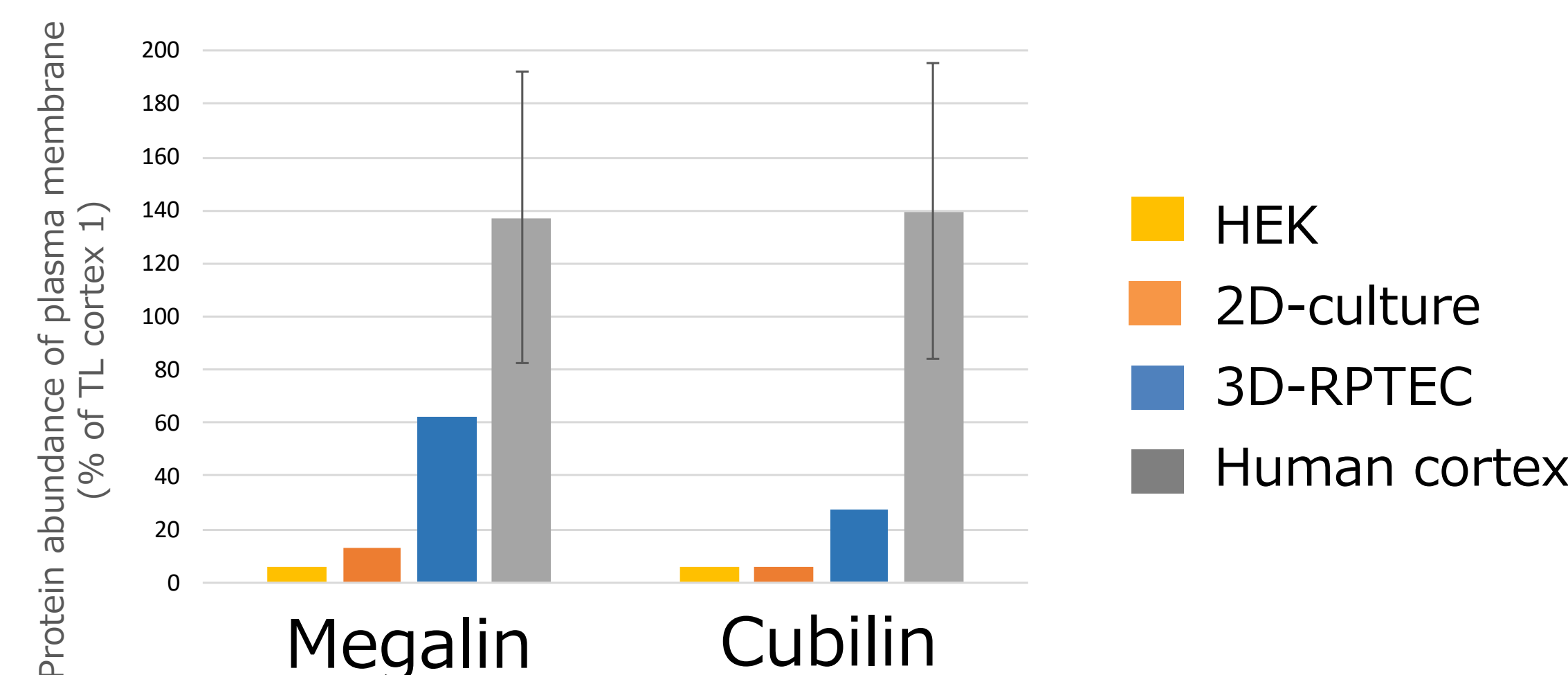
The following primary antibodies were used: anti-OAT1 (Transgenic, KE038), anti-OAT3 (Transgenic, KE032), anti-P-gp (Abcam, ab129450), and anti-SGLT2 (Proteintech, 24654-1-AP).

Conflict of interest

Hiroshi Arakawa declares that this study was supported by funding from NIKKISO CO., LTD. and a potential conflict of interest exists as a result of this financial support. The other authors have no conflicts of interest to disclose.

Endocytosis receptors

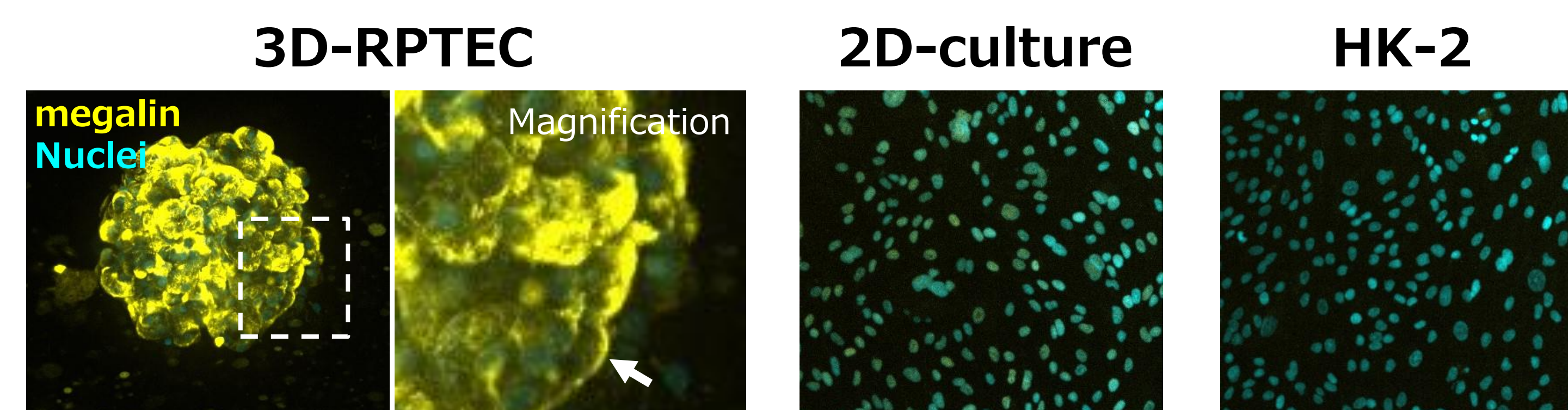
Proteome analysis



Proteome analysis of plasma membranes showed higher expression of megalin and cubilin, which are responsible for endocytosis in the kidney, compared to HEK, a kidney-derived cell line, and 2D-culture.

For detailed proteome analysis methods, refer to the following: *Drug Metab Dispos*, 51(9):1177-1187 (2023)

Megalin immunostaining

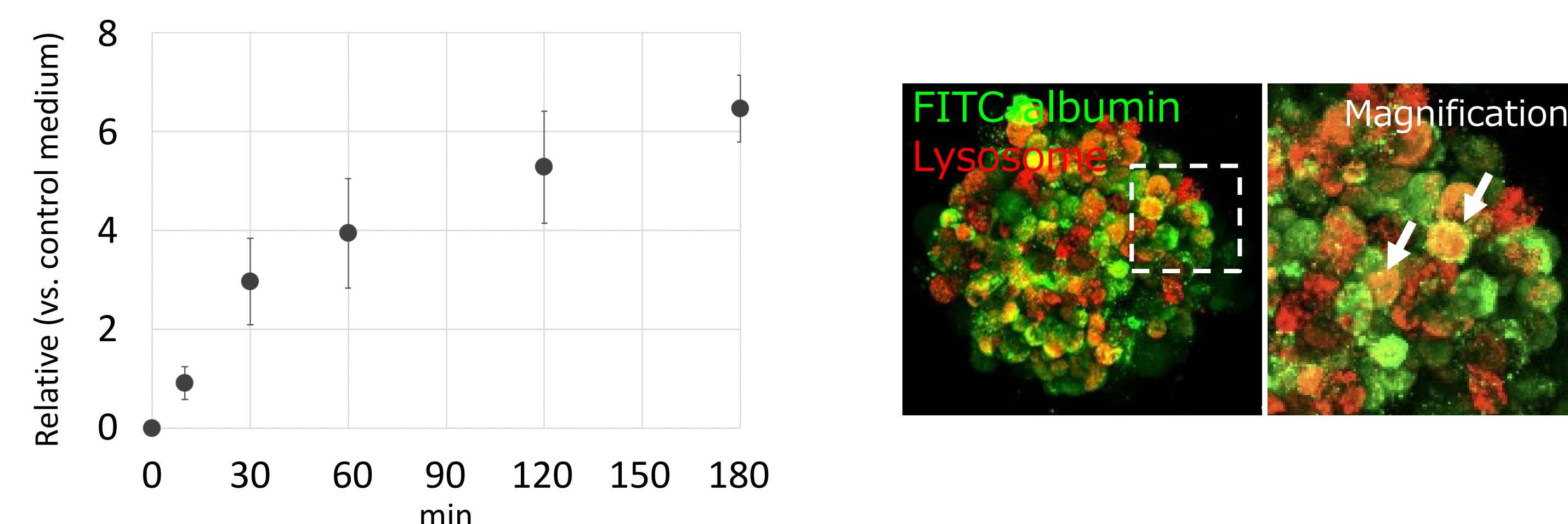


Megalin was expressed in 3D-RPTEC and was predominantly localized on the cell membrane (white arrow). In contrast, its expression was barely detected in 2D-culture and in HK-2, a kidney-derived cell line.

The primary antibody used was the LRP2 Antibody (Novus Biologicals, NBP1-85292).

Evaluation of megalin activity

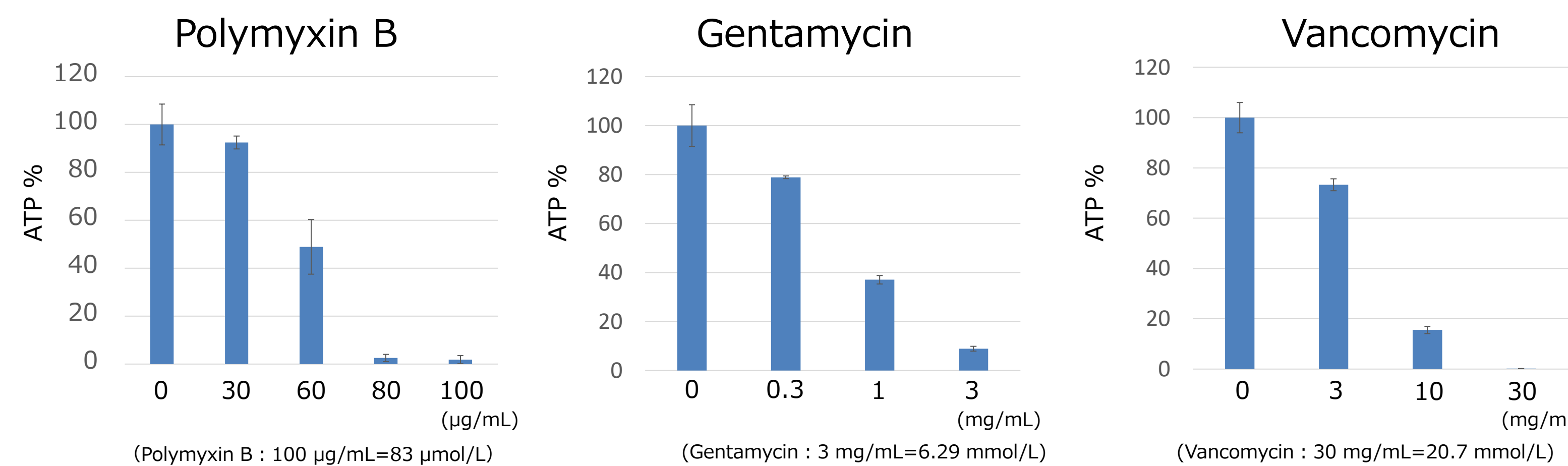
Albumin uptake



FI-TC albumin was taken up in a time-dependent manner. The internalized albumin indicated localization within lysosomes (white arrows).

Experimental Method: FI-TC albumin was dissolved in the 3D-RPTEC-specific medium at a concentration of 1000 μg/mL. The prepared medium was used to replace the original medium. Samples were collected at each time point, and fluorescence intensity was quantified using the CQ1 confocal microscope (Yokogawa Electric Corporation). Lysosome staining was used by LysoTracker™.

ATP assay



Exposure of 3D-RPTEC to megalin-mediated nephrotoxic drugs (polymyxin B, gentamicin, and vancomycin) led to a concentration-dependent decrease in ATP levels.

Experimental Method: The megalin-mediated nephrotoxic drugs were dissolved in a 3D-RPTEC-specific medium and used to replace the original medium. Thereafter, the medium was changed every two days with a drug-containing medium. After seven days of culture, ATP levels were measured using luminescent assay with CellTiter-Glo 3D.