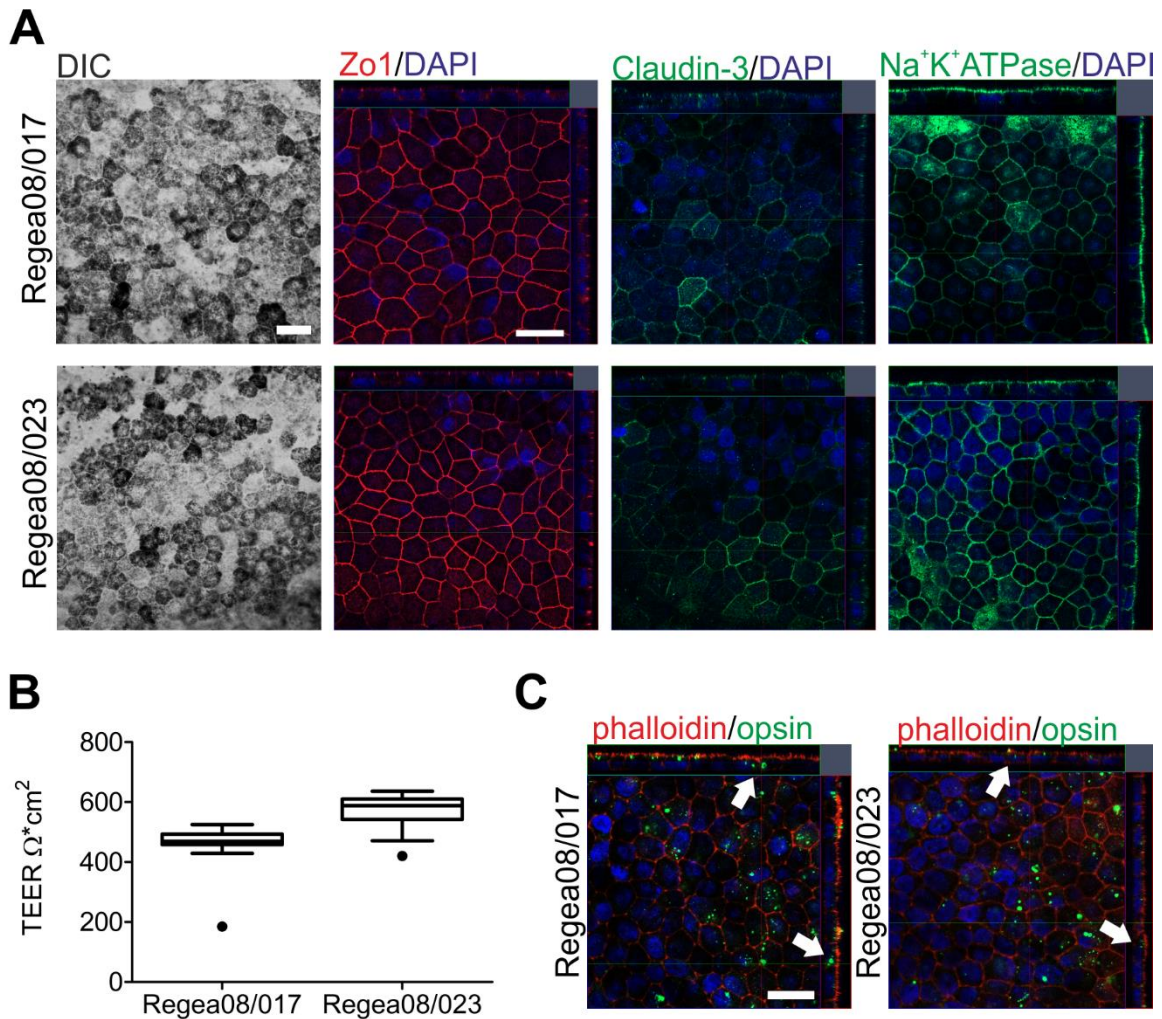


## Supplementary material

Drug flux across RPE cell models: the hunt for an appropriate outer blood-retinal barrier model for use in early drug discovery



**Supplementary Figure 1. Human ESC-RPE cells display characteristic RPE properties prior to the permeation studies.** A) Illustration of the hexagonal RPE morphology and mosaic pigmentation revealed in differential interference contrast (DIC). Immunofluorescence labeling and confocal z-stack images for tight junction markers zonula occludens-1 (ZO-1) and claudin-3 and polarized apical labeling for Na<sup>+</sup>K<sup>+</sup>ATPase pump. Scale bar 20  $\mu\text{m}$  valid for all images. B) Boxplots for transepithelial electrical resistance (TEER) measurements from 18 inserts for both cell lines. Outliers (accidentally punctured insert for Regea08/017) were not included in the permeability studies. Confocal z-stack images for hESC-RPE after *in vitro* phagocytosis of porcine photoreceptor outer segments (POS) and immunolabeling for opsin. Arrows pointing to internalized POS. Phalloidin for filamentous actin and DAPI to label nuclei. Scale bar 20  $\mu\text{m}$  valid for all images.

**Table S1. Apparent permeation coefficients ( $P_{app}$  values)**

$P_{app}$ values ( $\times 10^{-6}$ cm/sec) average $\pm$ standard deviation	ARPE19		ARPE19mel		LEPI		hfrPE		hESC-RPE (Regea08/017)		hESC-RPE (Regea08/023)		bRPE-choroid <sup>1</sup>	
	Outward n=3	Inward n=3	Outward n=2-3	Inward n=3	Outward n=5	Inward n=3-5	Outward n=4	Inward n=5	Outward n=5	Inward n=5	Outward n=5	Inward n=5	Outward n=8-9	Inward n=8-9
<b>Aztreonam</b>	6.5 $\pm$ 0.3	11.0 $\pm$ 2.5	7.6 $\pm$ 1.3	8.0 $\pm$ 0.8	1.9 $\pm$ 0.8	0.40 $\pm$ 0.2	12.0 $\pm$ 0.7	9.3 $\pm$ 0.8	n.a.	n.a.	n.a.	n.a.	5.4 $\pm$ 5.2	4.5 $\pm$ 2.6
<b>Ciprofloxacin</b>	10.2 $\pm$ 1.4	16.9 $\pm$ 1.6	5.2 $\pm$ 3.1	9.4 $\pm$ 0.9	2.3 $\pm$ 0.8	0.59 $\pm$ 0.2	n.a.	15.5 $\pm$ 3.7	5.6 $\pm$ 0.2	3.0 $\pm$ 0.2	5.4 $\pm$ 0.3	4.8 $\pm$ 0.5	9.5 <sup>****</sup> $\pm$ 5.3	1.4 <sup>****</sup> $\pm$ 0.8
<b>Dexamethasone</b>	n.a.	21.7 $\pm$ 21.1	n.a.	23.4 $\pm$ 2.2	5.6 $\pm$ 1.1	5.0 $\pm$ 1.6	n.a.	26.4 $\pm$ 1.7	n.a.	n.a.	n.a.	n.a.	n.d.	n.d.
<b>Fluconazole</b>	n.a.	17.8 <sup>*</sup> $\pm$ 11.6	n.a.	24.7 $\pm$ 1.6	8.0 $\pm$ 1.1	5.4 $\pm$ 1.2	n.a.	21.9 $\pm$ 1.7	23.4 $\pm$ 2.5	20.4 $\pm$ 0.9	23.5 $\pm$ 3.3	21.1 $\pm$ 0.4	15.6 $\pm$ 4.7	13.0 $\pm$ 13.0
<b>Ganciclovir</b>	n.a.	20.1 <sup>*</sup> $\pm$ 6.4	11.4 <sup>*</sup> $\pm$ 6.8	15.4 $\pm$ 1.9	2.3 $\pm$ 0.8	0.87 $\pm$ 0.4	n.a.	18.7 $\pm$ 2.3	2.8 $\pm$ 0.7	0.99 $\pm$ 0.1	1.2 $\pm$ 0.2	1.0 $\pm$ 0.2	9.7 $\pm$ 7.9	6.5 $\pm$ 4.0
<b>Ketorolac</b>	14.6 $\pm$ 1.0	18.4 $\pm$ 5.1	n.a.	25.1 $\pm$ 10.3	3.8 $\pm$ 0.8	1.3 $\pm$ 0.4	n.a.	17.9 $\pm$ 1.9	12.1 $\pm$ 0.8	6.9 $\pm$ 1.7	12.2 $\pm$ 0.3	9.6 $\pm$ 0.4	69.2 $\pm$ 31.9	4.8 $\pm$ 4.0
<b>Methotrexate</b>	8.4 $\pm$ 1.0	12.9 $\pm$ 2.8	8.3 $\pm$ 0.3	10.3 $\pm$ 1.6	1.3 $\pm$ 0.4	0.44 $\pm$ 0.2	n.a.	13.0 $\pm$ 1.7	1.6 $\pm$ 0.5	0.37 $\pm$ 0.05	0.7 $\pm$ 0.1	0.4 $\pm$ 0.1	9.4 $\pm$ 2.7	4.5 $\pm$ 3.0
<b>Quinidine</b>	n.a.	n.a.	n.a.	n.a.	n.a.	20.7 <sup>***</sup> $\pm$ 3.2	n.a.	19.6 $\pm$ 1.5	12.8 $\pm$ 1.2	14.3 $\pm$ 3.0	11.2 $\pm$ 1.6	16.5 $\pm$ 1.0	n.a.	n.a.
<b>Voriconazole</b>	n.a.	n.a.	n.a.	n.a.	n.a.	29.4 <sup>**</sup> $\pm$ 3.0	n.a.	12.5 $\pm$ 14.6	31.7 $\pm$ 4.7	27.8 $\pm$ 3.6	27.2 $\pm$ 6.6	26.2 $\pm$ 0.8	25.0 $\pm$ 6.1	21.0 $\pm$ 4.2

**Outward** (apical-to-basolateral), **Inward** (basolateral-to-apical) <sup>1</sup>Ramsay et al. 2019

**n.a.** not applicable:  $P_{app}$  value could not be calculated due the problems in analytics (hESC-RPE cells: dexamethasone and aztreonam) or rapid drug flux (sink conditions were not maintained for the first hour)

**n.d.** not determined \*n=2, \*\*n=3, \*\*\*n=4, \*\*\*\*n=8

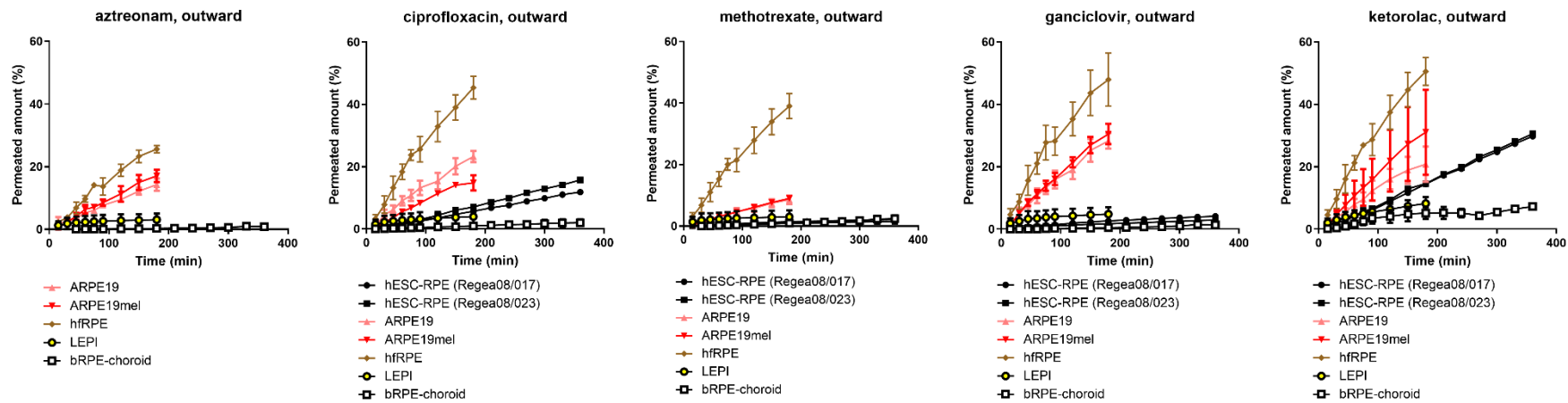
**Table S2. Efflux ratios**

Efflux ratios (AB/BA)	ARPE19	ARPE19mel	LEPI	hfrPE	hESC-RPE (Regea08/017)	hESC-RPE (Regea08/023)	bovine RPE- choroid <sup>1</sup>
<b>Aztreonam</b>	0.6	0.9	4.8	1.3	n.a.	n.a.	1.2
<b>Ciprofloxacin</b>	0.6	0.5	3.9	n.a.	1.9	1.1	6.7
<b>Dexamethasone</b>	n.a.	n.a.	1.1	n.a.	n.a.	n.a.	n.d.
<b>Fluconazole</b>	n.a.	n.a.	1.5	n.a.	1.1	1.1	1.2
<b>Ganciclovir</b>	n.a.	0.7	2.7	n.a.	2.9	1.3	1.5
<b>Ketorolac</b>	0.8	0.6	3.1	n.a.	1.8	1.3	14.5
<b>Methotrexate</b>	0.7	0.8	3.0	n.a.	4.4	1.8	2.1
<b>Quinidine</b>	n.a.	n.a.	n.a.	n.a.	0.9	0.7	n.a.
<b>Voriconazole</b>	n.a.	n.a.	n.a.	n.a.	1.1	1.0	1.2

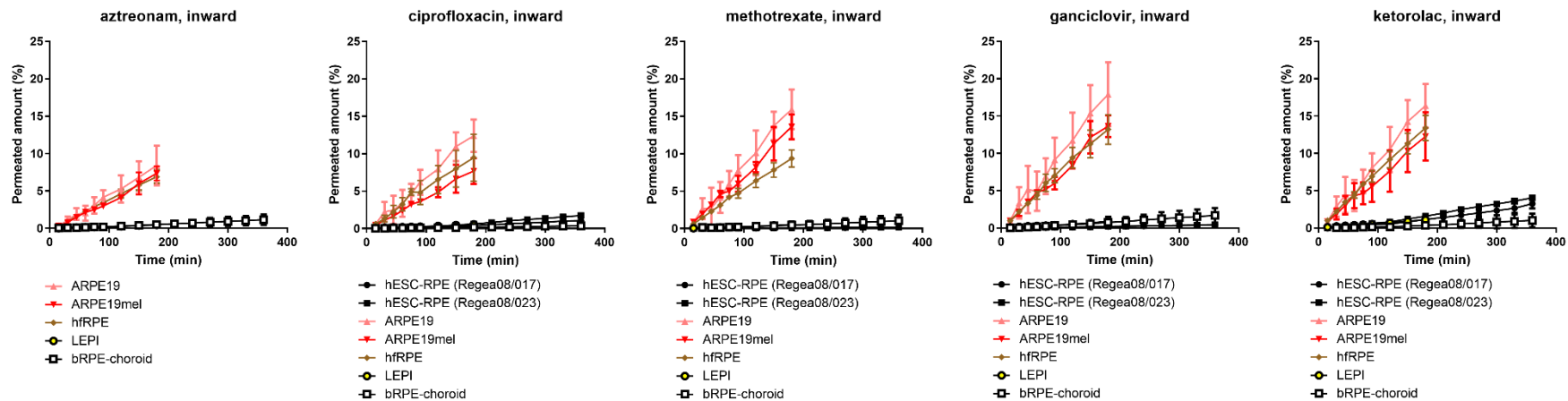
**n.a.** not applicable:  $P_{app}$  value could not be calculated due the problems in analytics (hESC-RPE cells: dexamethasone and aztreonam) or rapid drug flux (sink conditions were not maintained for the first hour)

**n.d.** not determined

## Outward permeation

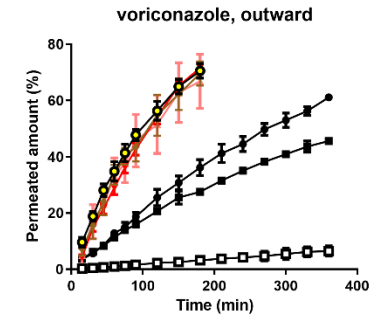
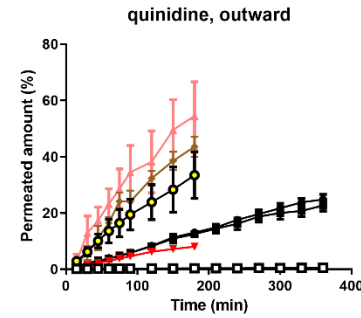
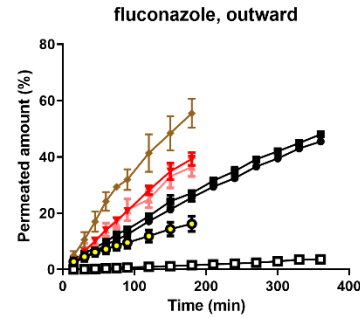
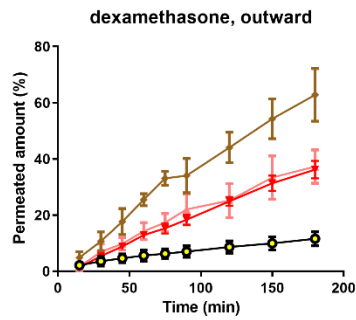


## Inward permeation



**Supplementary Figure S2.** Flux of hydrophilic compounds across RPE cell models and bovine RPE-choroid (Ramsay et al. 2019). The upper panel displays the outward permeation and lower the lower panel refers to inward permeation. Replicate numbers as stated in the Table S1 for the compounds that  $P_{app}$  value was successfully determined. For other compounds with successful LC-MS/MS analysis replicate numbers were: ARPE19 and ARPE19mel,  $n=3$ ; LEPI and hfRPE cells,  $n=5$ ; bovine RPE-choroid,  $n=5$  (quinidine).

Outward permeation



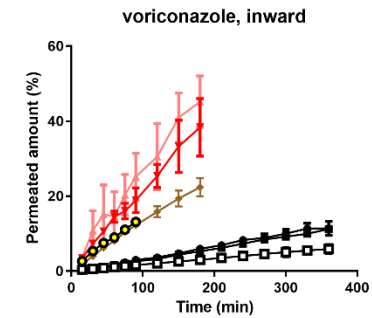
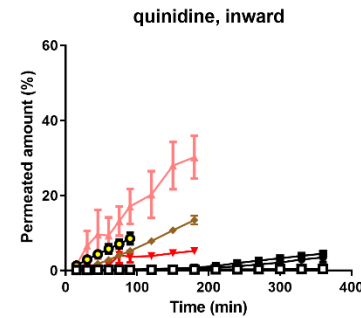
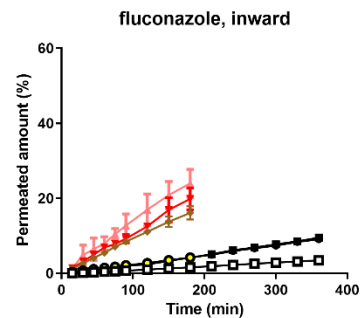
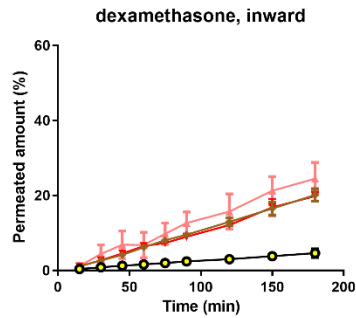
- ARPE19
- ARPE19mel
- hfRPE
- LEPI

- hESC-RPE (Regea08/017)
- hESC-RPE (Regea08/023)
- ARPE19
- ARPE19mel
- hfRPE
- LEPI
- bRPE-choroid

- hESC-RPE (Regea08/017)
- hESC-RPE (Regea08/023)
- ARPE19
- ARPE19mel
- hfRPE
- LEPI
- bRPE-choroid

- hESC-RPE (Regea08/017)
- hESC-RPE (Regea08/023)
- ARPE19
- ARPE19mel
- hfRPE
- LEPI
- bRPE-choroid

Inward permeation



- ARPE19
- ARPE19mel
- hfRPE
- LEPI

- hESC-RPE (Regea08/017)
- hESC-RPE (Regea08/023)
- ARPE19
- ARPE19mel
- hfRPE
- LEPI
- bRPE-choroid

- hESC-RPE (Regea08/017)
- hESC-RPE (Regea08/023)
- ARPE19
- ARPE19mel
- hfRPE
- LEPI
- bRPE-choroid

- hESC-RPE (Regea08/017)
- hESC-RPE (Regea08/023)
- ARPE19
- ARPE19mel
- hfRPE
- LEPI
- bRPE-choroid

**Figure S3.** Flux of lipophilic compounds across RPE cell models and bovine RPE-choroid (Ramsay et al. 2019). The upper panel displays the outward and the lower panel refers to inward permeation. Replicate numbers as stated in the Table S1 for the compounds that  $P_{app}$  value was successfully determined. For other compounds with successful LC-MS/MS analysis replicate numbers were: ARPE19 and ARPE19mel, n=3; LEPI and hfRPE cells, n=5; bovine RPE-choroid, n=5 (quinidine).