Genetically Modified Human Renal Proximal Tubule Epithelial Cells (RPTEC/TERT1) – A New Model for Drug Toxicity Studies

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees

Established partner to global researchers and scientists

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Renal transport proteins

Play important roles for drug:

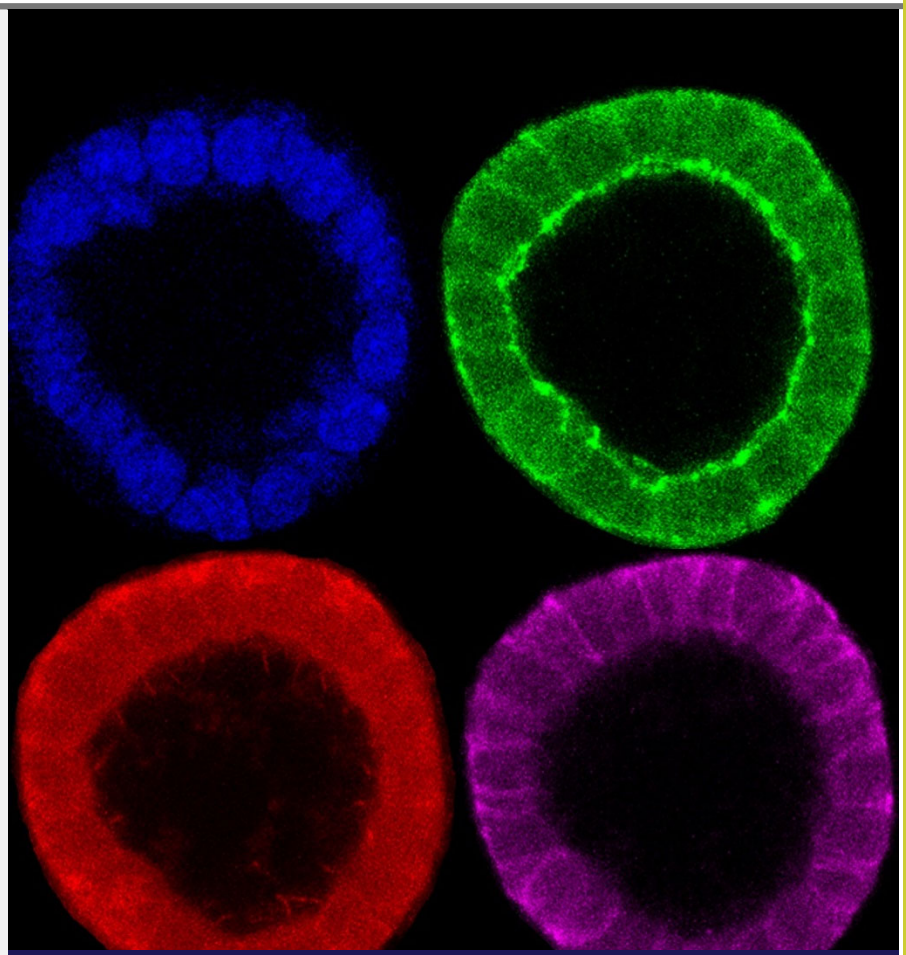
- Absorption
- Distribution
- Elimination

Can be divided into 2 classes:

- The ATP-binding cassette (ABC) family, most are efflux transporters
- The solute carrier (SLC) family, most are influx transporters, some are efflux and bidirectional

Expression and activities at the basolateral and apical side of transporting epithelia are significant determinants for:

- Drug disposition
- Drug-drug interactions
- Variability in drug response and toxicity



Kidney epithelial cells recapitulate *in vivo* tubule formation, image courtesy of Moe Mahjoub

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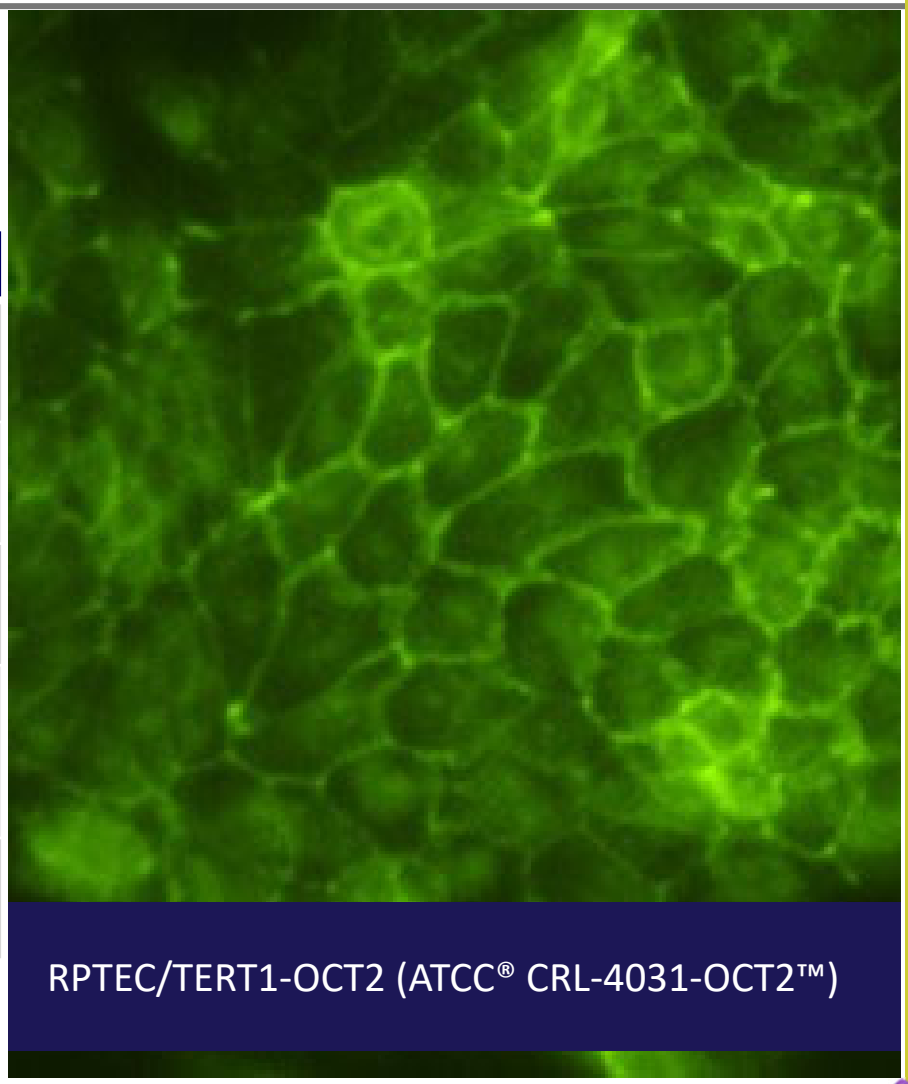
Toxicologically important transport proteins

Transporter/alias	Organs/cells
OATP1B1/OATP-C, OATP2, LST-1	Hepatocytes (sinusoidal)
OATP1B3/OATP-8 (SLCO1B3)	Hepatocytes (sinusoidal)
OAT1 (SLC22A6)	Kidney proximal tubule, placenta
OAT3 (SLC22A8)	Kidney proximal tubule, choroid plexus, blood–brain barrier
OCT2 (SLC22A2)	Kidney proximal tubule, neurons



RPTEC/TERT1-OCT2 (ATCC® CRL-4031-OCT2™)

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Renal transport protein substrates and inhibitors

Substrates

OAT1

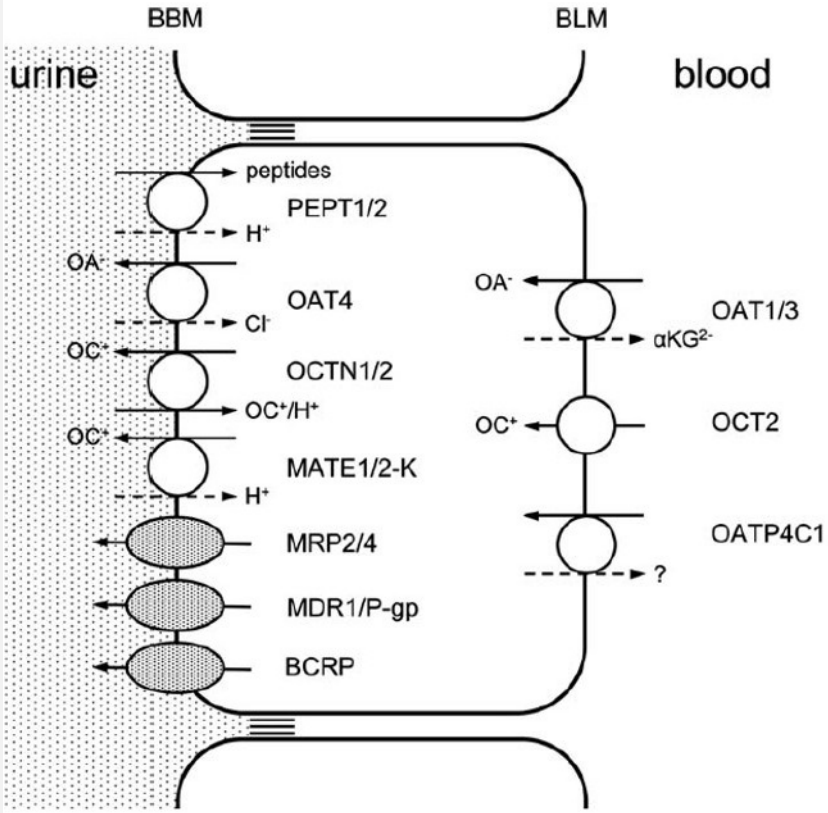
- Cipro
- Methotrexate
- Acyclovir
- Tenofovir

OAT3

- NSAIDS
- Cefaclor,
- Ceftizoxime
- Furosemide
- Bumetanide

OCT2

- Pindolol
- Amiloride
- Oxalliplatin
- Varenicline



Pang Ks, et al. Enzyme- and Transporter-Based Drug–Drug Interactions. DOI 10.1007/978-1-4419-0840-7_2,C Am Assoc Pharmaceut Sci 2010

Inhibitors

OAT1

- Probenecid
- Novobiocin

OAT3

- Probenecid
- Novobiocin

OCT2

- Cimetidine
- Pilsialnide
- Etrizine
- Testosterone
- Quinidine

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Focus on transporters in new regulatory documents

	FDA guidance 2006	ITC Recommendations 2010 FDA Guidance 2012	EMA Guidelines 2010	Tissue
P-gp/MDR1	+	+	+	Multi
BCRP		+	+	Multi
BSEP			+	Liver
OATP1B1		+	+	Liver
OATP1B3		+	+	Liver
OCT1			+	Liver
OAT1		+	+	Kidney
OAT3		+	+	Kidney
OCT2		+	+	Kidney

New (draft) regulatory documents published by FDA, EMA, and ITC recommended evaluate NME as substrate and drug interaction on the most important membrane transporters expressed in liver, intestine, and kidney

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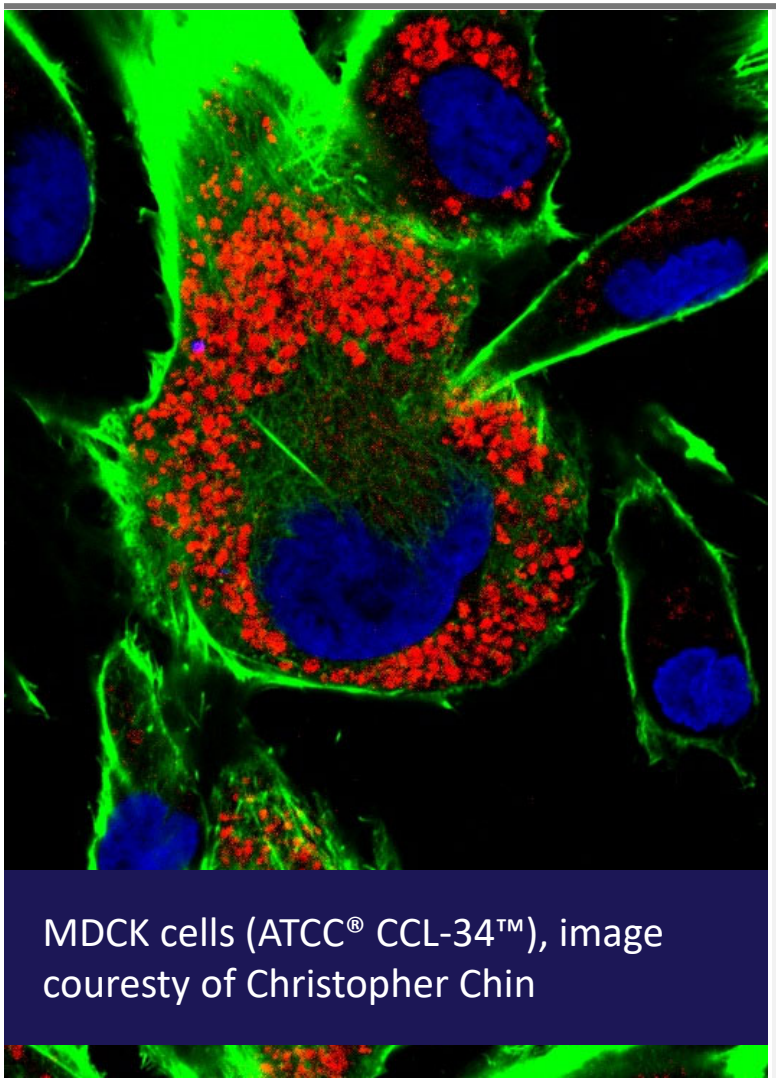
Cell line-based models



MDCK cells (ATCC® CCL-34™), image couresty of Christopher Chin

Current cell line-based models are available:

- MDCK (ATCC® CCL-34™)
- CHO-K1 (ATCC® CCL-61™)
- U-2 OS (ATCC® HTB-96™)
- Others

Problems with these lines:

- Do not have the human kidney tissue origination
- The cell line itself is a cancer line

Therefore, the clinical predictability is greatly compromised

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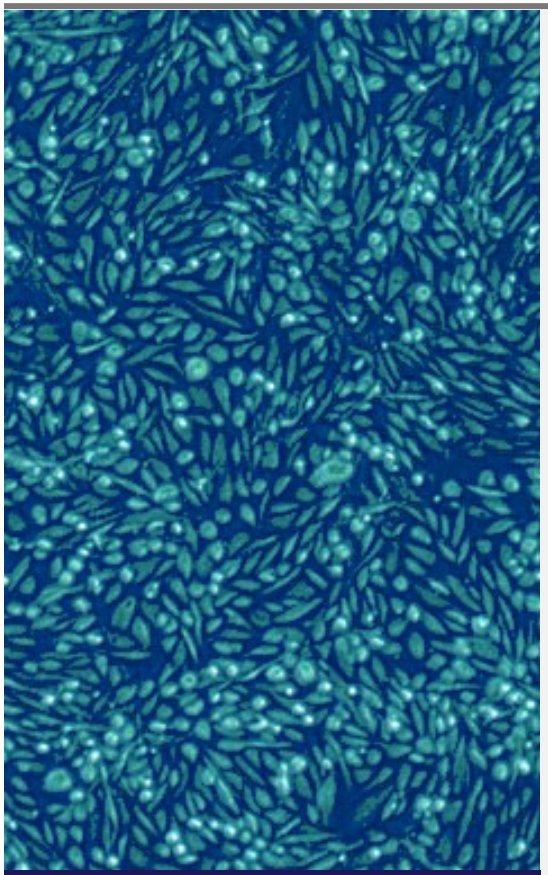
Primary cell-derived models



Primary Renal Cortical Cells (ATCC® PCS-400-011™)

Problems with primary kidney cell models:

- Obtaining primary cultures is difficult
  - The kidney comprises 15 cell types
  - The nephron comprises 20 cell types
  - Homogeneous cultures retaining physiological functions are hard to obtain
- Primary RPTEC lose OAT1, OCT2, and OAT3 expression in culture
- Transiently expressing transporters in primary RPTEC show large variations between production lots
  - Makes the data hard to interpret

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Renal cell lines

Cell Line	ATCC® No.	Species of origin	Nephron segment of origin
LLC-PK1	CL-101™	Yorkshire Pig	Proximal nephron
OK	CRL-1840™	North American Opossum	Proximal nephron
JTC-12	N/A	Monkey	Proximal nephron
MDCK	CCL-34™	Dog	Collecting duct
A6	CCL-102™	*Xenopus laevis*	Distal tubule
HK-2	CRL-2190™	Human	HPV16-transformed, Proximal/Distal?
Caki-1	HTB-46™	Human	Kidney carcinoma
HEK293/OAT1	CRL-11268G-1™	Human	Embryonic

None of the continuous renal epithelial cell lines fully recapitulate the functions of the parental cells *in vivo*

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Pros and cons of different cell models for tissue-relevant functional studies

	Primary cells	hTERT immortalized	Oncogene, virally immortalized	Cancer cell lines
Mimic *in vivo* tissue phenotype	++++	+++	++	+
Genotypic stability	Diploid	Diploid / Near diploid	Near diploid / Aneuploid	Aneuploid
Proliferative capacity	+	+++	+++	+++
Supply	+	+++	+++	+++
Inter-experimental reproducibility	Low	Good	Good	Good
Cost	High	Medium	Low	Low
Ease-of-use	+	++	++	+++

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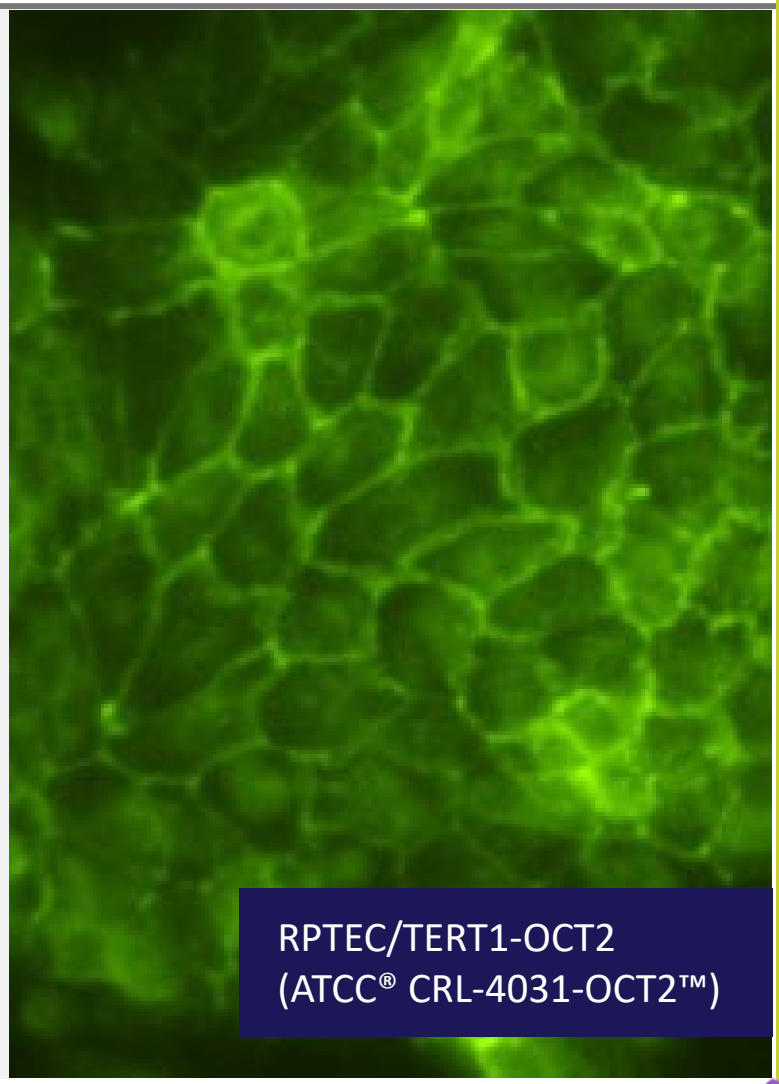
hTERT-immortalized cells provide unique tools

hTERT-immortalized cells combine:

- The *in vivo* nature of primary cells
- The ability to be cultured continuously

hTERT-immortalized cells avoid the limitations of primary cells and continuous cell lines while still reaping their benefits



RPTEC/TERT1-OCT2
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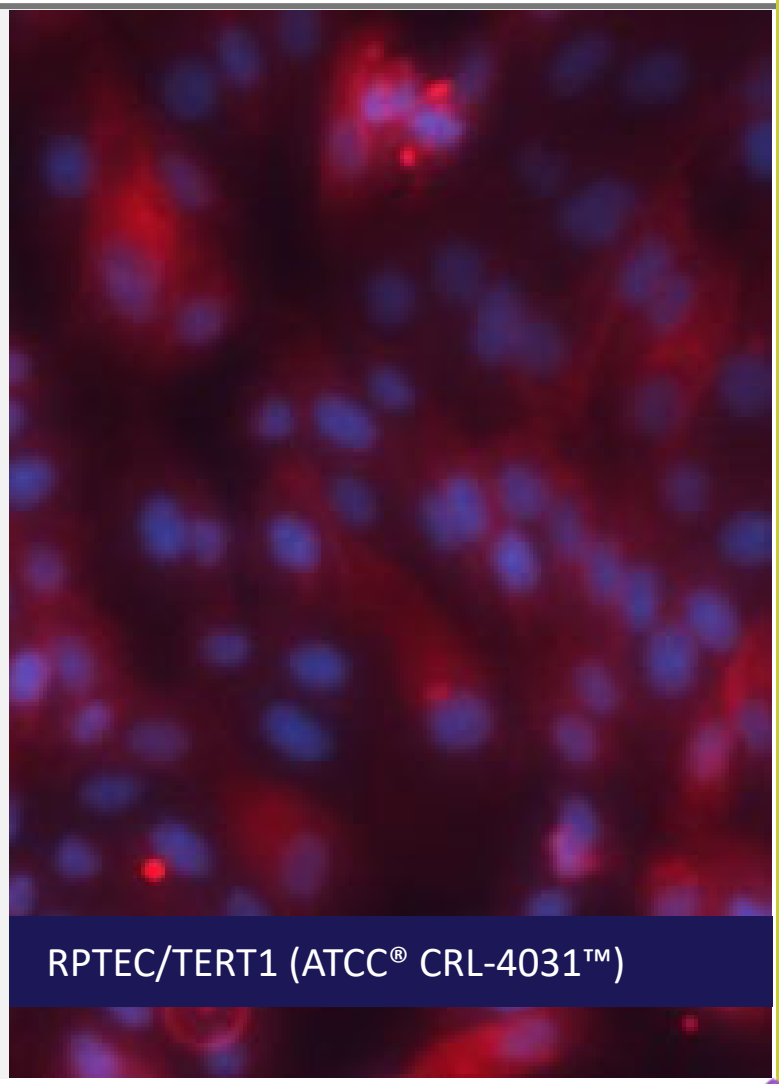
The parental cell

RPTEC/TERT1 (ATCC® CRL-4031™)

- An epithelial cell line
- Isolated from human renal proximal tubes
- Immortalized by hTERT only

RPTEC/TERT1 exhibit:

- Uniform expression of E-cadherin and CD13 (aminopeptidase N)
- Dome-like structures
- Stabilized TEER



RPTEC/TERT1 (ATCC® CRL-4031™)

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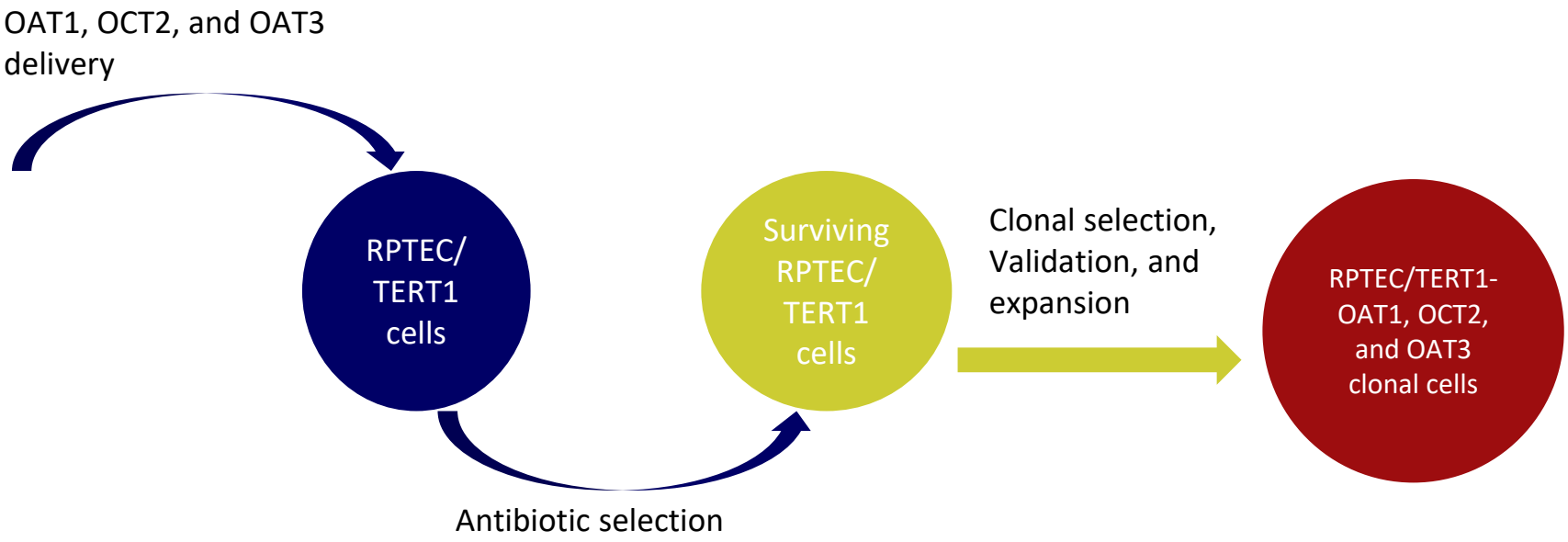
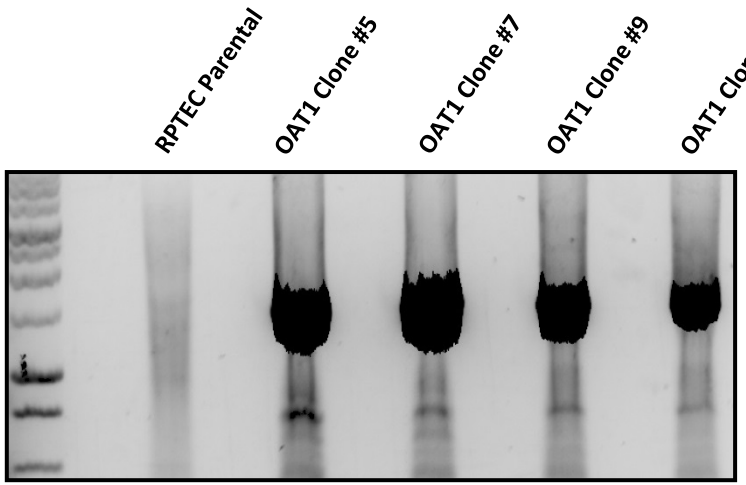
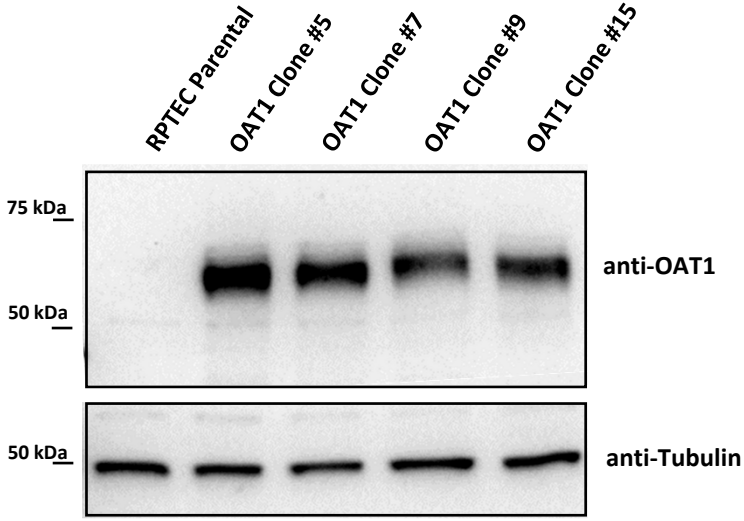
Stable cell line generation



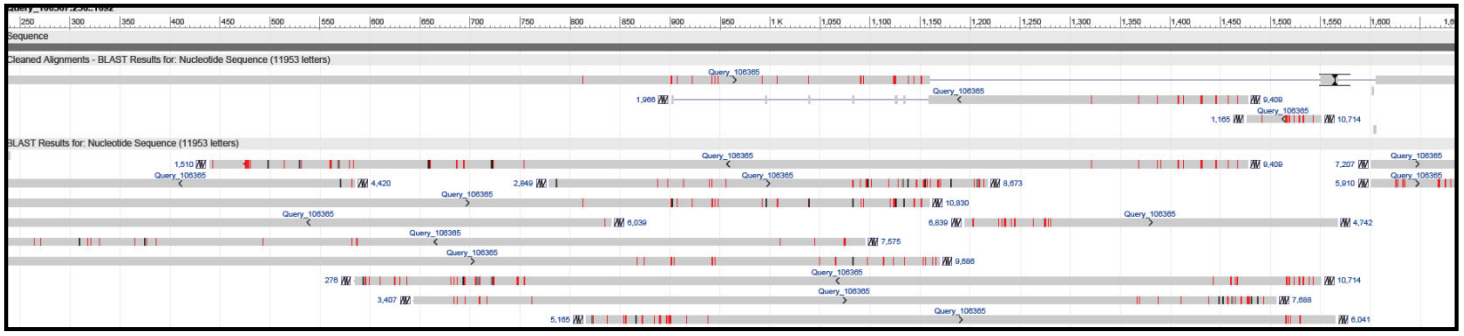
RPTEC/TERT1-OAT1



RT-PCR

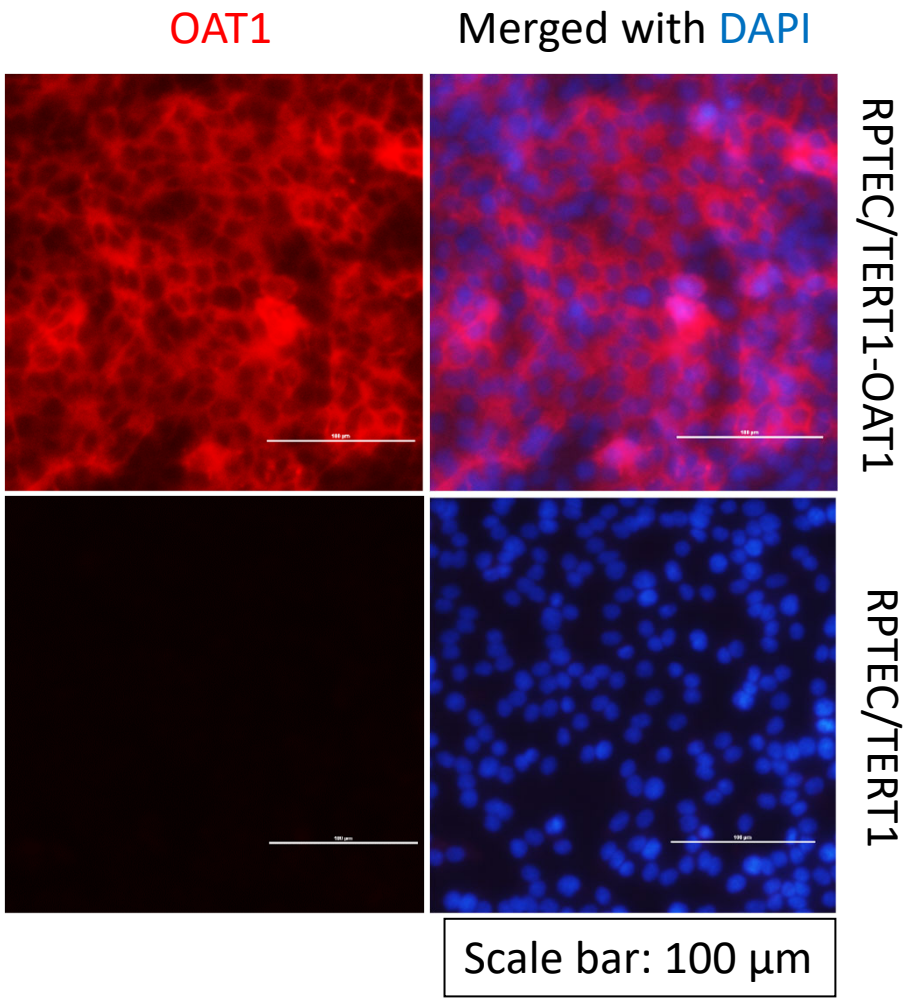


Western Blot



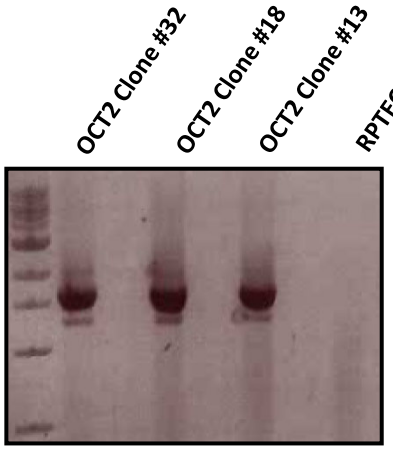
Sequencing: no mutation

OAT1 correctly localizes to the cell membrane in RPTEC/TERT1



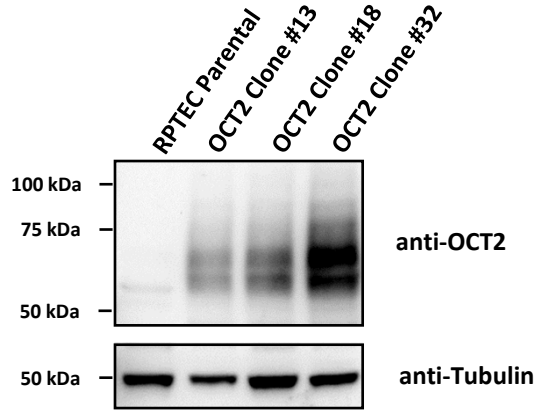
RPTEC/TERT1-OCT2

A.



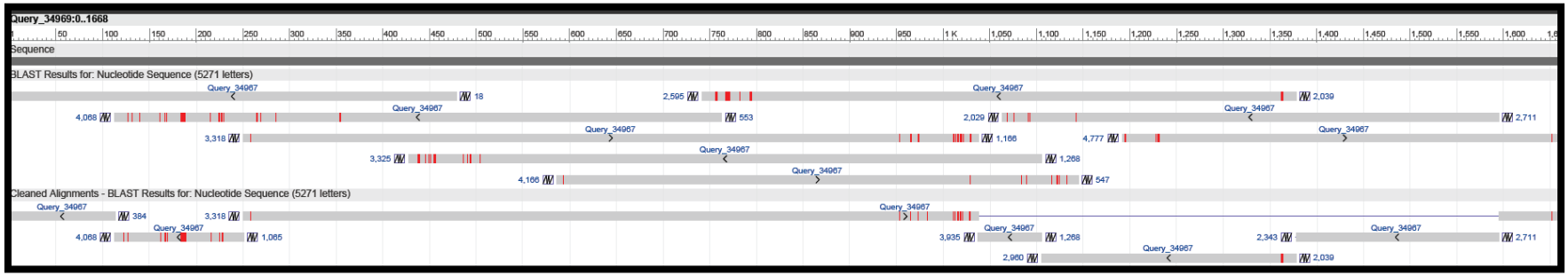
RT-PCR

B.



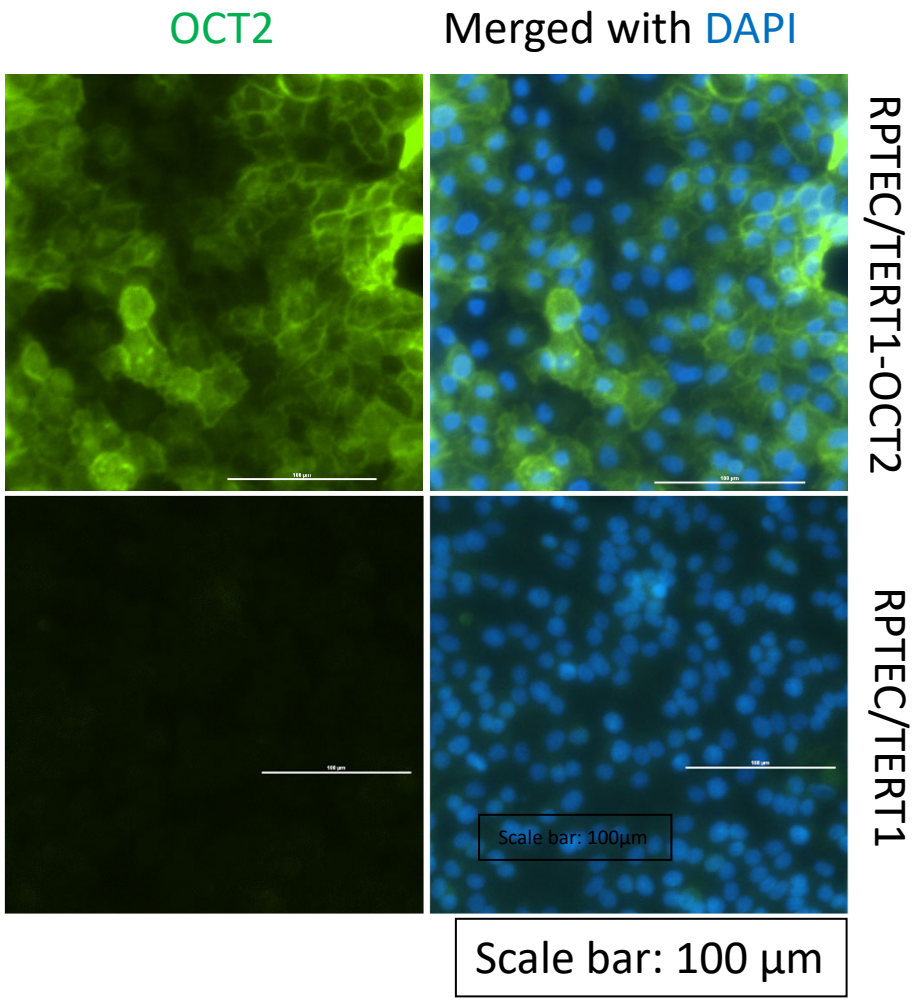
Western Blot

C.



Sequencing: No mutation

OCT2 correctly localizes to cell membrane in RPTEC/TERT1



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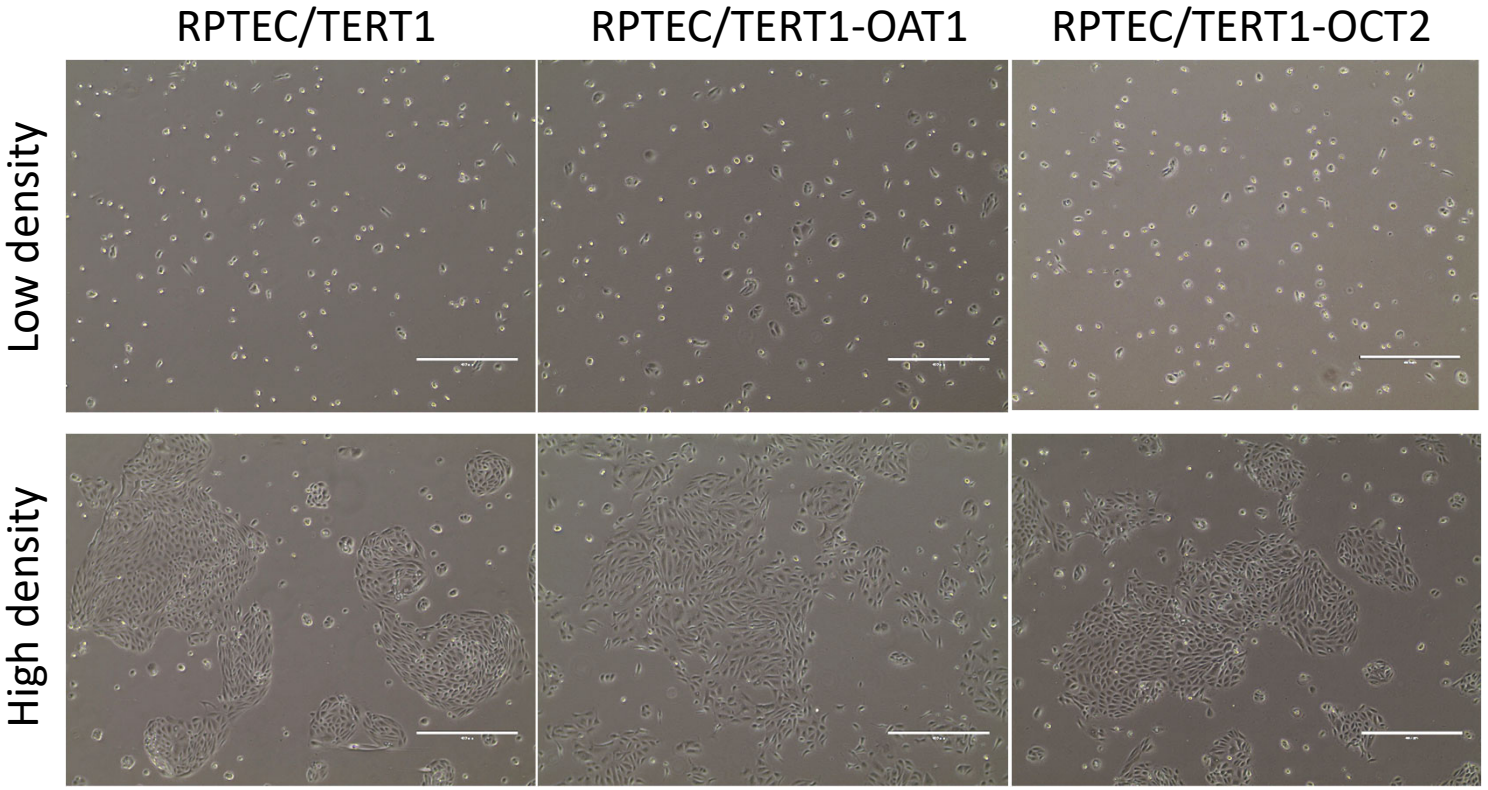
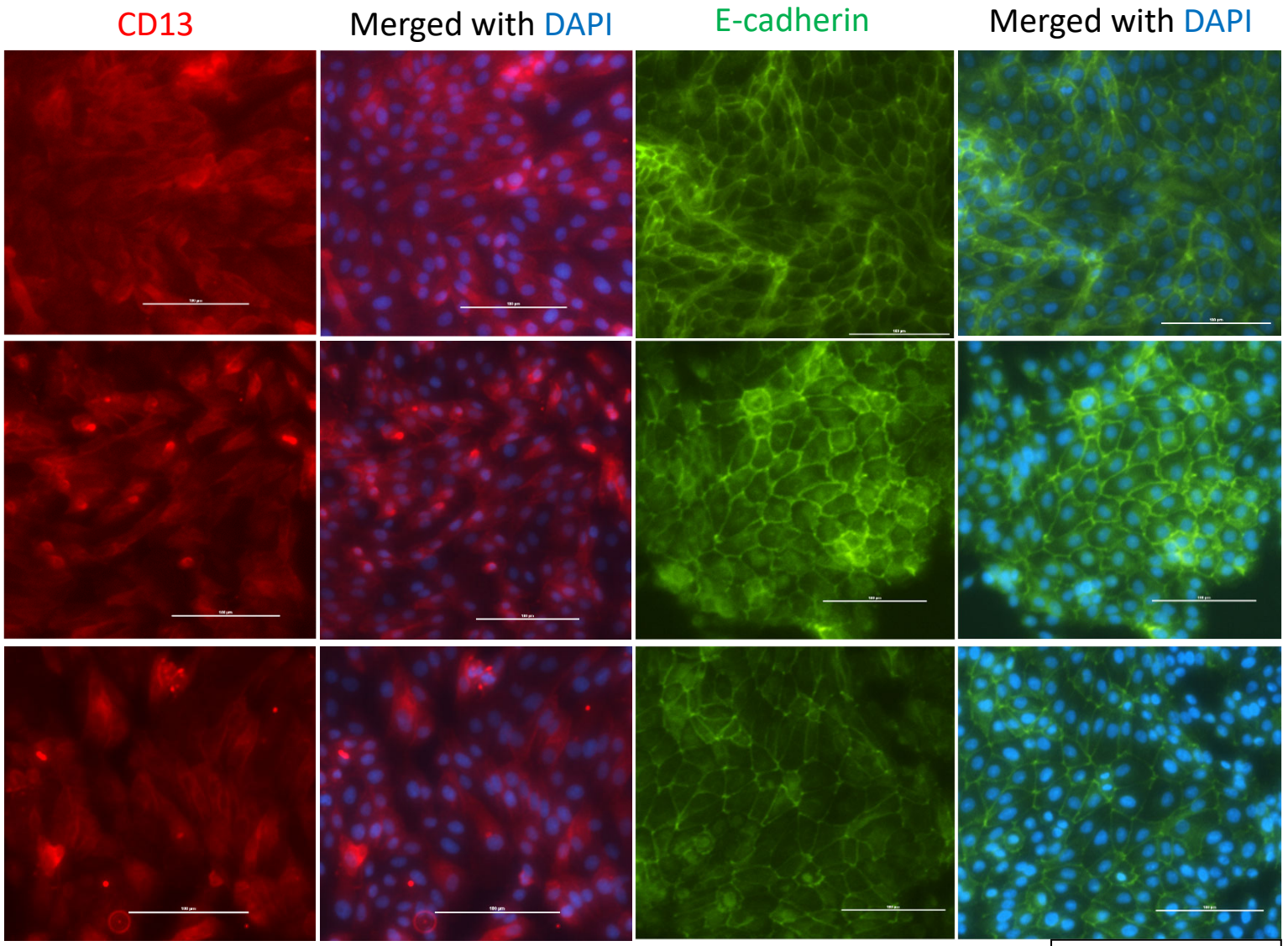
Growth characteristics of stably transfected RPTEC/TERT1



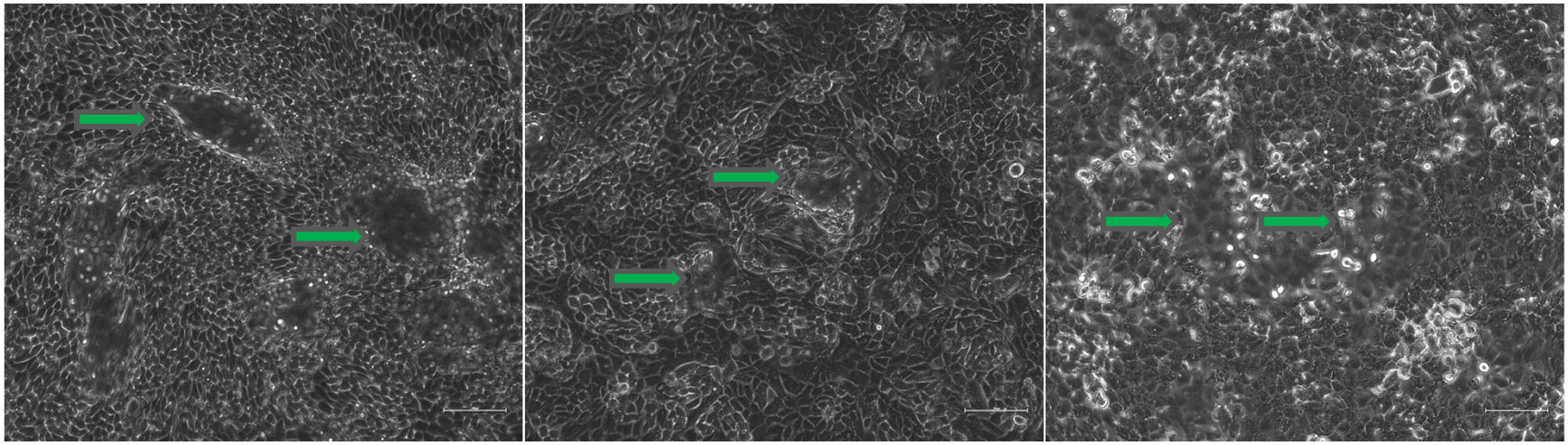
Scale bar: 400 µm

RPTEC/TERT1 renal uptake key marker staining



Dome formation

RPTEC/TERT1 | RPTEC/TERT1-OAT1 | RPTEC/TERT1-OCT2



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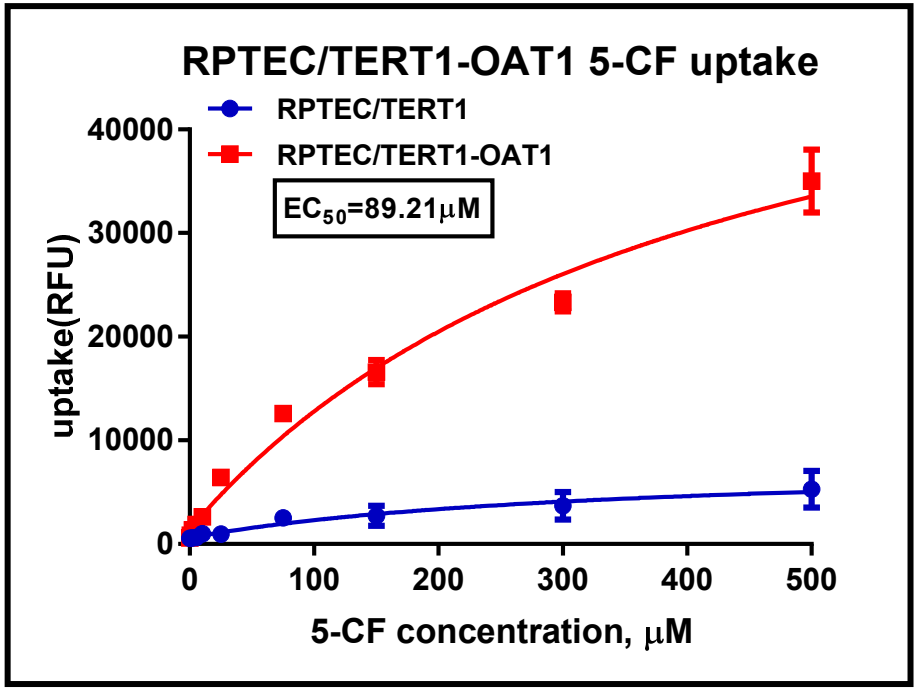
RPTEC/TERT1-OAT1 drug kinetic profile



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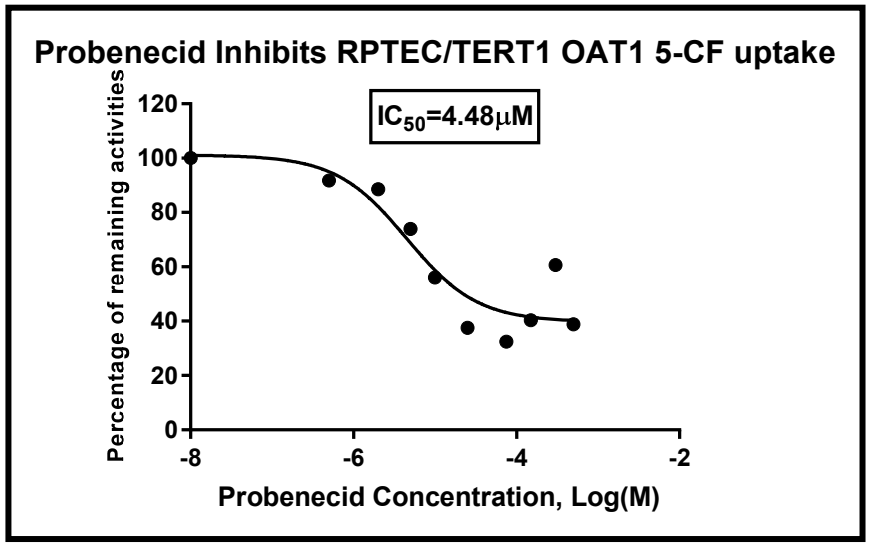
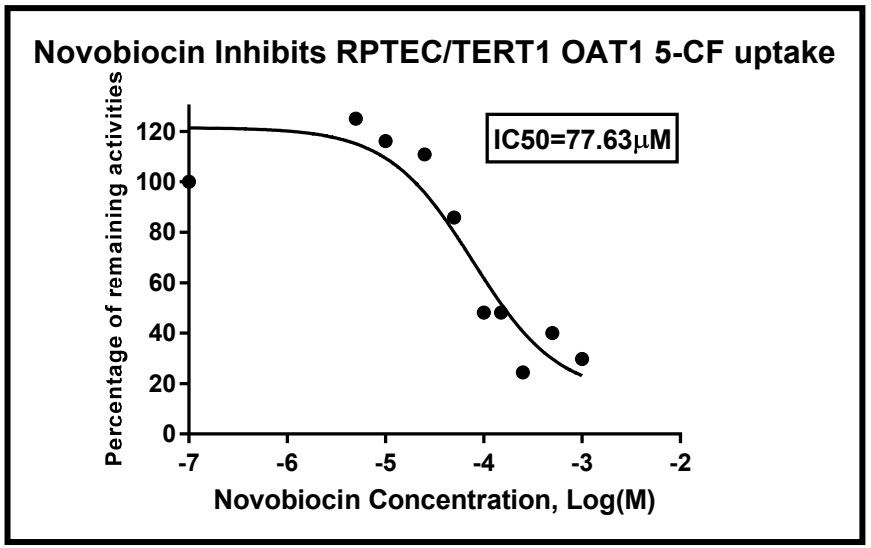
Known OAT1 inhibitors block the RPTEC/TERT1-OAT1 5-CF uptake





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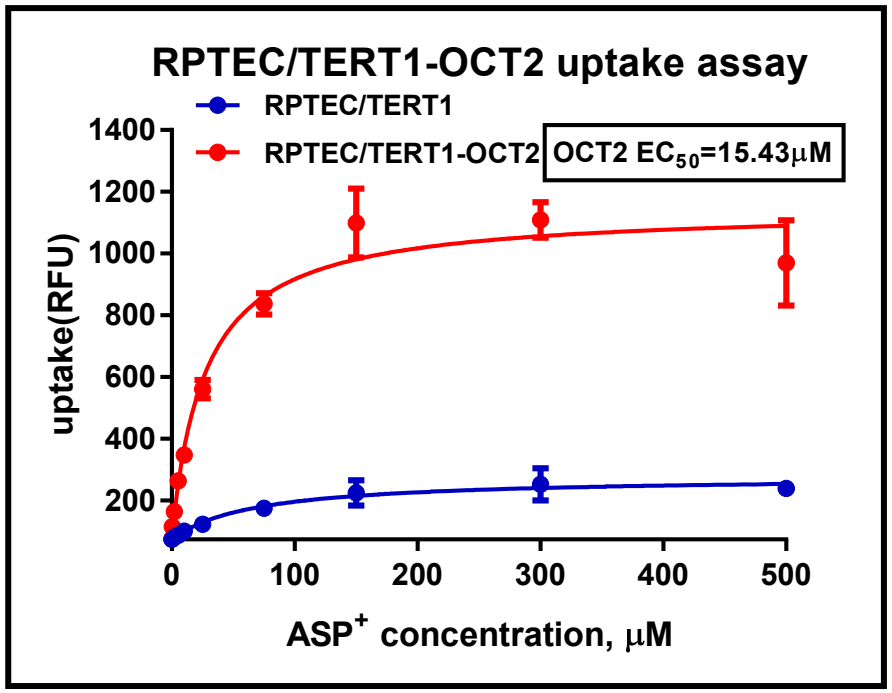
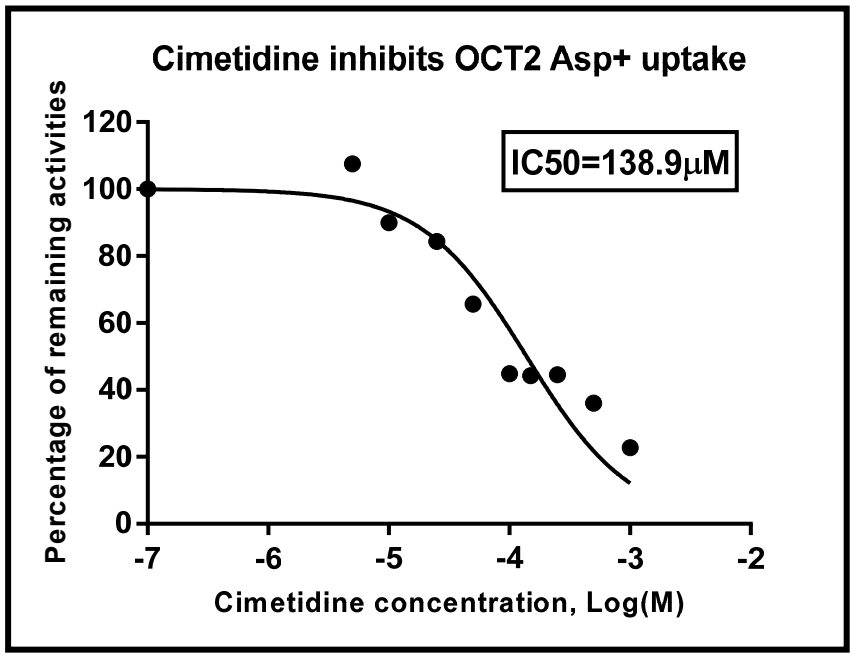
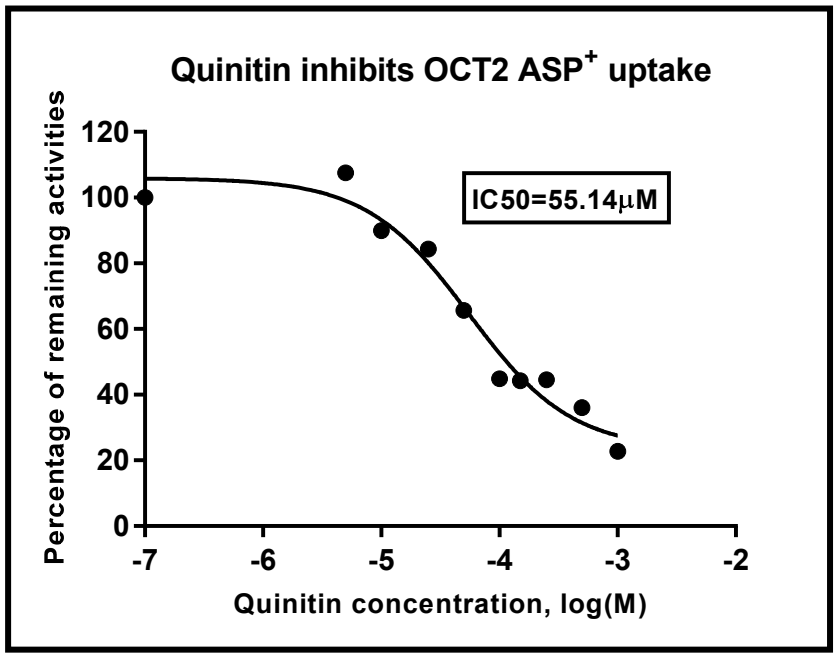
RPTEC/TERT1-OCT2 drug kinetic profile



Known OCT2 inhibitors block the RPTEC/TERT1-OCT2 Asp+ uptake





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Summary

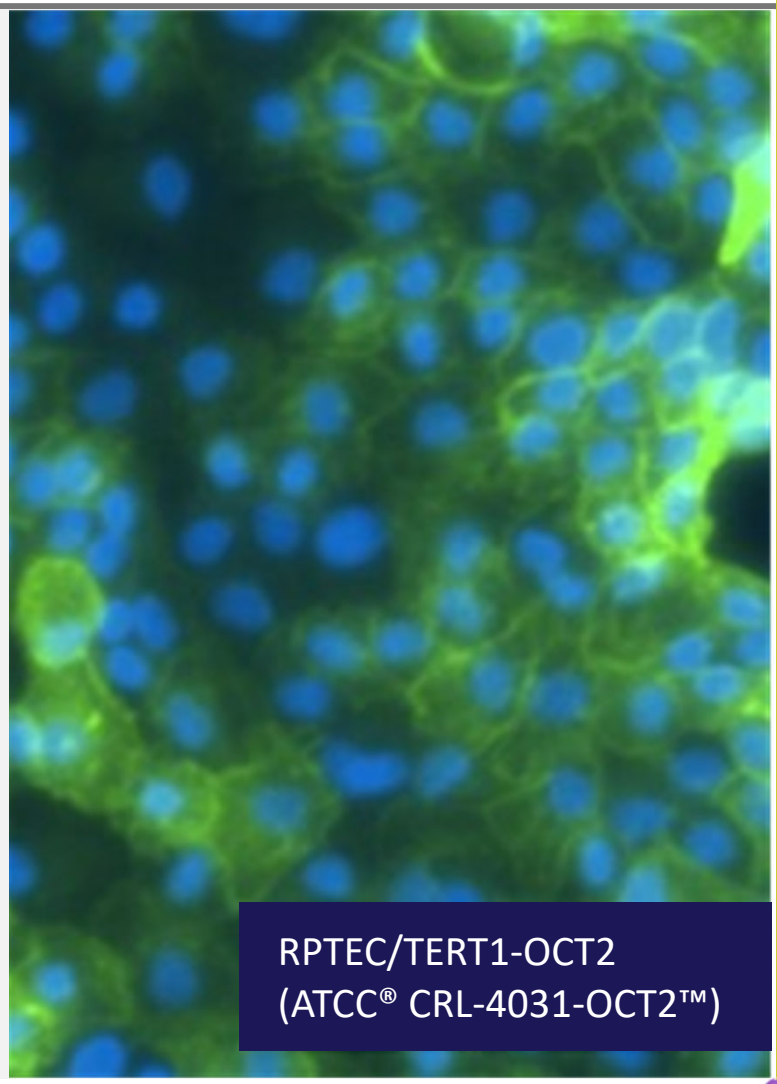
We generated clonal RPTEC/TERT1 renal uptake cell models by stably expressing OAT1 and OCT2 proteins

- Expression has been confirmed by:
  - PCR
  - Western blot
  - Immunocytochemistry

The clonal stable cells keep the original characteristics of the RPTEC/TERT1 cells

The performance of these stable cells are well characterized by:

- 5-CF uptake assays
- ASP uptake assays
- Inhibitor assays



RPTEC/TERT1-OCT2
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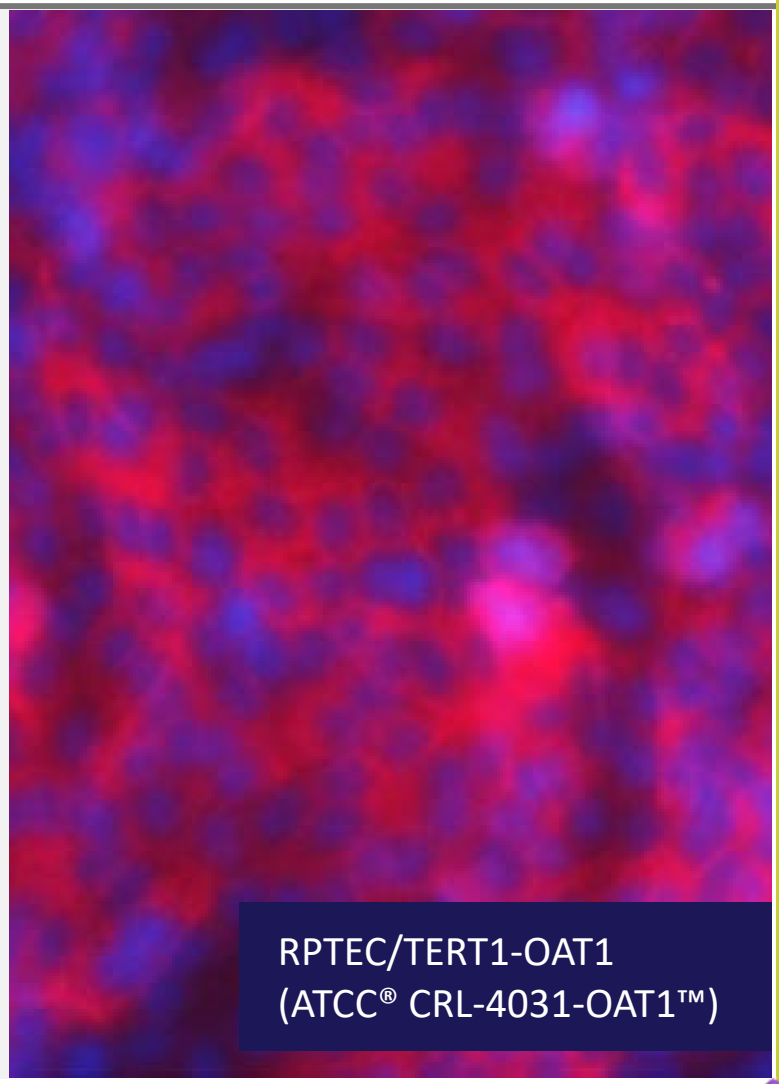
Disclaimers



RPTEC/TERT1-OAT1
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