

Mechanism of uptake and toxicity of a BCMA antibody drug conjugate with a MMAF payload by nonantigen expressing cells

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SUPPLEMENTARY METHODS

LC-MS/MS payload quantification in HCEC and RPTEC as a function of time

Preparation of analytical solutions of cys-mcMMAF

Two sets of analytical solutions (A/B) of cys-mcMMAF from independent weighings in silanized glass vials were prepared and dissolved in the required volume of 40/30/30 water/acetonitrile/methanol with 1% formic acid and 0.1% BSA to give a 1 mg/mL stock solution of the free compound; 30 µL of 1 mg/mL stocks were sub-aliquoted into 0.5 mL LoBind polypropylene tubes for single use aliquots and stored at -20°C for up to 92 days.

Preparation of internal standard solution of [15N1 13C3 2H3] cys-mcMMAF

[15N1 13C3 2H3] cys-mcMMAF was weighed in a silanized glass vial and dissolved in the required volume of 40/30/30 water/acetonitrile/methanol with 1% formic acid and 0.1% BSA to give a 1 mg/mL solution; 30 µL of 1 mg/mL and 100 µg/mL stocks were sub-aliquoted into 0.5 mL LoBind polypropylene tubes for single-use aliquots and at stored at -20°C.

Calibration standard preparation

Calibration standards were prepared in radioimmunoprecipitation assay (RIPA) buffer in 1.5 mL or 2 mL protein LoBind Eppendorf tubes and thoroughly mixed.

Sample preparation

Cells were thawed and kept on ice. RIPA buffer (500 µL) was added to the cells, thoroughly vortexed, and kept on wet ice for 30 minutes. Samples were centrifuged at approximately 18,000 relative centrifugal force for 10 minutes at 4°C, and the supernatant was removed to a fresh tube. The samples were dried down under nitrogen at 45°C, and when 50 µL of liquid remained, 200 µL of 10/90 (v/v) methanol/water +3.5% ammonium hydroxide was added.

Preparation for LC-MS/MS

A waters oasis WAX u-Elution SPE plate was conditioned with 200 µL of methanol followed by 200 µL of water, applying positive pressure at each stage. The samples were added to the plate and pressure was gently applied to draw the sample through slowly. Ammonium formate (100 mM, 200 µL) was added and pressure was applied, then 200 µL of methanol was added and once again

pressure was applied. The samples were eluted in 50 μ L of 50/50 (v/v) methanol/water+3.5% ammonium hydroxide and injected into the high-performance liquid chromatography system.

LC-MS/MS conditions

UHPLC conditions

Autosampler	Waters Acquity (Fixed Loop)
Strong Solvent Wash	40/40/20/0.1 (v/v/v/v) Acetonitrile/Isopropanol/Water/Formic acid: 500 μ L
Weak Solvent Wash	80/20/0.1 (v/v/v) Water/Acetonitrile/Formic Acid; 1000 μ L
Typical Injection Volume	20 μ L; (50 μ L loop fitted)
Sample Loop Injection Mode	Partial Loop
Load Ahead	Disabled
Loop Offline	Automatic
Air Gaps (pre aspirate)	Automatic
Air Gaps (post aspirate)	Automatic
Chromatography System	Waters Acquity UHPLC
Flow Rate	300 μ L/min
Analytical Column	100 x 2.1 mm Acquity PREMIER HSS T3 1.8 μ m, Waters
Column Temperature	40°C
Sample Temperature	10°C +/-5°C
Column divert	Connected but not active
LC run Time/MS Data Acquisition Time	5.0 minutes/5.0 minutes
Mobile Phase A	0.4 mM ammonium fluoride + 0.025% formic acid in water
Mobile Phase B	0.4 mM ammonium fluoride + 0.025% formic acid in acetonitrile

LC-MS/MS gradient table

Time (mins)	%A	%B	Curve
Initial	80	20	6
0.50	80	20	6
4.0	25	75	6
4.5	25	75	6
4.51	80	20	6
5.0	80	20	6

Mass spectrometer parameters

Mass Spectrometer	Waters Xevo TQ-S
Ionisation Interface and Source Temperature	Electrospray at 150°C
Desolvation Temperature	550°C
Capillary Voltage (kV)	3
Source offset (V)	50
Cone Gas Flow (L/hour)	150
Desolvation Gas Flow (L/hour)	800
Collision Gas Flow (mL/min)	0.15
Nebuliser Gas Flow (Bar)	7.0
Resolution Setting	Unit Mass
Cone Voltage (V)	15
Collision Energy Setting	12

Masses detected

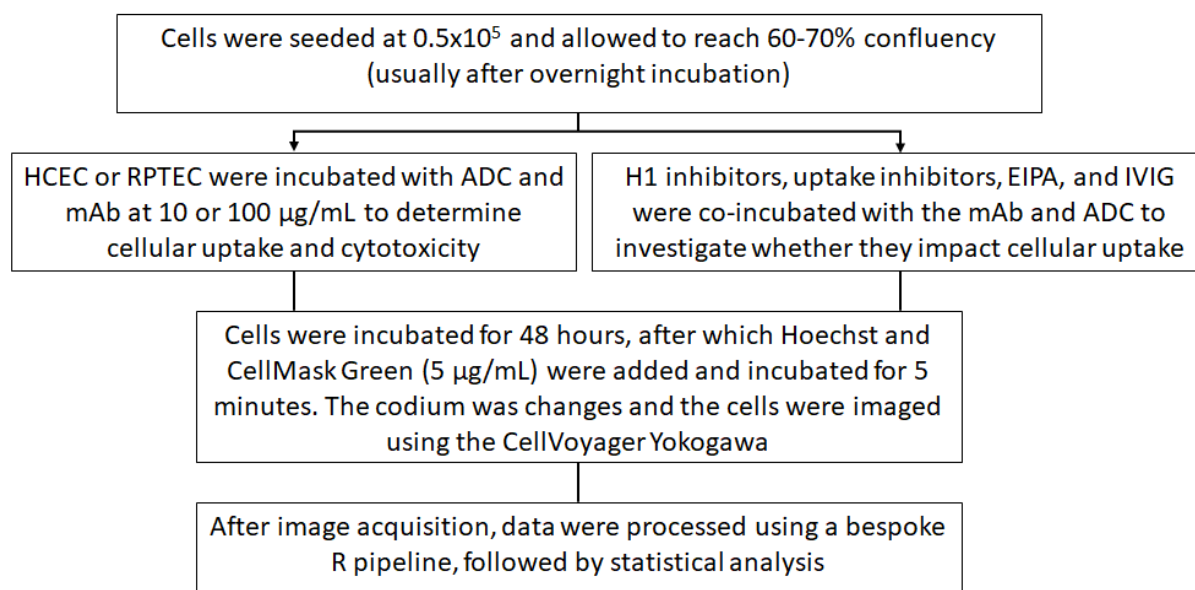
Analyte	Precursor ion (m/z)	Product Ion (m/z)	Dwell Time (msec)	Polarity	Typical R.T. (min)
CYS-MCMAAF	523.7	428.2	200	Positive	3.8
[¹⁵ N ¹³ C ₃ ² H ₃]-CYS-MCMAAF (I.S.)	527.3	435.3	200	Positive	3.8

Target Lynx integration parameters

	CYS-MCMMAF	[¹⁵ N ¹³ C ₃ ² H ₃]-MMAF (I.S.)
Retention Time Window (min) ±	0.20	0.20
Smoothing Enabled	☒ Yes	☒ Yes
Smoothing Method	Mean	Mean
Smoothing Iterations	1	1
Smoothing Width	2	2
Apex Track Enabled	☒ Yes	☒ Yes
Generate Noise and Width from Reference Peak	☒ No	☒ No
Peak-to-Peak Baseline Noise	☒ 34	☒ 51
Peak Width at 5% Height	☒ 0.24	☒ 0.24
Baseline Start Threshold %	0.0	0.0
Baseline End Threshold %	0.5	0.5
Detect Shoulder Peaks	☒ No	☒ No
Threshold Parameters	-	-
Threshold Relative Height	☒ 1.5	☒ 1.5
Threshold absolute Height	☒ 0	☒ 0
Threshold Relative Area	☒ 2	☒ 2
Threshold Absolute Area	☒ 0	☒ 0
Integration Window Extent	1	1

Microscopy Analyses

Flow of uptake experiments



Unless otherwise stated, fluorescence was detected using a Modified CQ1 Microscope (Yokogawa). Fluorophore imaging parameters were: 405 nm excitation and 447/60 nm emission filter for Hoechst nuclear stain; 488 nm excitation and 525/50 nm emission filter for Alexa488 and Cell Tracker Green; and 640 nm excitation and 685/40 nm emission filter for Vivotag680. The laser power for all lasers was set at 20% with 200 μ sec exposure time. A z-stack of 5 slices (over 8 μ m) or 6 slices (over 10 μ m) was performed, 9 fields of view were acquired, and a 10x objective was used.

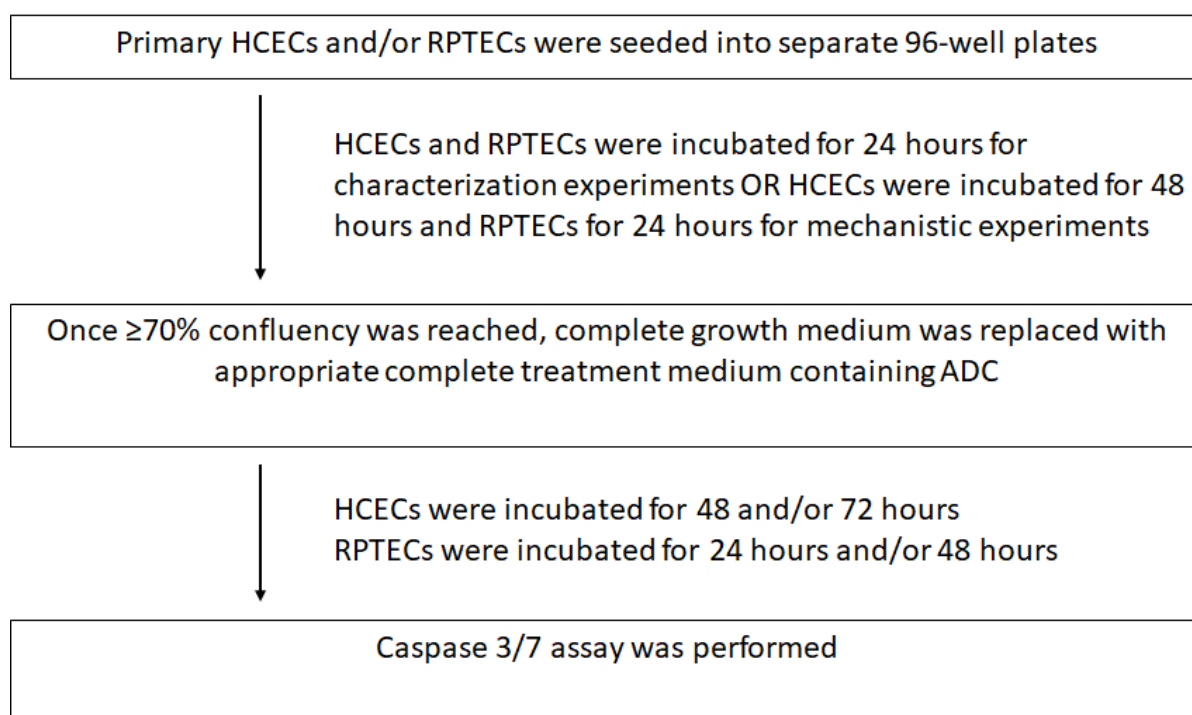
Micrographs were quantified using the Modified CQ1 software and processed using a bespoke Python-based pipeline (**Supplement File S1**). Briefly, brightfield images were maximum projected along the z-axis. Cell instance segmentation was performed using Cellpose (Stringer et al. 2021) to create a segmentation mask and instance mask to identify each individual cell. Cell profiler was used to compute image features using the instance mask as regions of interest and the brightfield and virtual nuclei as signals. Finally, feature filtering and selection was performed to find predictive features.

For fluorescent measurements, mean fluorescent intensity (MFI) per cell was analyzed per condition ensuring fluctuation in cell number did not affect fluorescence measurements. To display data from multiple experiments, average MFI per cell of single experiments is shown as MFI. Error bars display standard error of the mean.

Nuclei count measurements show average nuclei count and standard error of the mean. The number of control cells was set to 100% and the rest of the data were transformed accordingly.

For compound uptake analyses, several parameters were extracted including number of nuclei, number of cells, compound intensity, nuclear intensity, cell marker intensity, cell marker area, nuclear area, and compound area.

Flow of caspase 3/7 assay to measure apoptosis



Effect of siRNA depletion of endocytic pathway proteins on ADC uptake

To further evaluate ADC uptake in HCEC cells, key endocytic pathway proteins were depleted by siRNA, and ADC uptake evaluated by flow cytometry. HCEC cells were transfected with siRNA targeting AP2mu2, Arf6, Graf1, Caveolin-1, CtBP1, CDC42, Flotillin-1, Rabankyrin-5, and control siRNA using Opti-MEM and RNAiMAX. Knockdown was assessed by western blot, confirming protein depletion for all targets except Graf1, which was not taken forward for further studies.

HCEC were seeded at a density of 1×10^4 cells per well into 6 well plates (Western) or 24 well plates (flow cytometry) and allowed to adhere for 48 hours. Before transfection, Opti-MEM (Gibco) was pre-warmed to 37 °C. For Western analysis, per well of a 6-well plate, 8.1 µL RNAiMAX (Invitrogen) was gently mixed with 135 µL Opti-MEM, while separately, 3 µL siRNA was gently mixed with 150 µL

Opti-MEM. To 135 μ L of RNAiMAX/Opti-MEM 135 μ L of siRNA/Opti-MEM was added and mixed by pipetting and incubated for 5 minutes at room temperature. The media on HCEC was replaced with 750 μ L pre-warmed Opti-MEM and 250 μ L siRNA/RNAiMAX/Opti-MEM was added per well.

For flow cytometry, samples were run in duplicate so that for 2 wells, 4.2 μ L RNAiMAX was gently mixed with 70 μ L Opti-MEM, while separately, 1.5 μ L siRNA was gently mixed with 75 μ L OptiMEM. To 70 μ L of RNAiMAX/Opti-MEM 70 μ L of siRNA/Opti-MEM was added and mixed by pipetting and incubated for 5 minutes at room temperature. The media on HCEC was replaced with 435 μ L pre-warmed OptiMEM and 65 μ L siRNA/RNAiMAX/Opti-MEM was added per well.

For flow cytometry analyses, cells siRNA transfected for 48 hours were treated with mAb 10 μ g/mL-Vivotag680, 100 μ g/mL Dextran-Cascade Blue, and 10nM Transferrin-AlexaFluor488 in media without transferrin supplement and incubated for 1 hour. Cells were washed with PBS, acid washed (500 mM sodium chloride, 50 mM sodium MES, pH 4), washed again with PBS before being detached and re-suspended in ice cold trypsin neutralizing solution (5% FBS in PBS). Cell solutions were kept on ice and analyzed immediately by flow cytometry on a BD FACSverse. Cells were excited using 405 nm (Dextran-Cascade Blue), 488 nm (Transferrin-AlexaFluor488) and 633 nm (mAb-Vivotag680) lasers. Single cell populations were separated using a double gate of side scatter-area versus forward scatter-area and forward scatter-width versus forward scatter-height. Median fluorescence intensity was collected for each population, with the mean value taken from each duplicate to count as an independent experiment. Results are the mean of three independent experiments and statistics calculated using a one-way ANOVA.

For Western blot analysis, cells were transfected with siRNA for 48 hours were washed and lysed in 80 μ L ice cold NP40 lysis buffer containing Roche protease inhibitor cocktail. Cells were scraped, placed in sterile microcentrifuge tubes and centrifuged at 13,000 $\times g$ for 10 minutes at 4 $^{\circ}$ C. The supernatant was transferred to a fresh microcentrifuge tube and the protein content quantified using a bicinchoninic acid assay. Loading buffer (8% sodium dodecyl sulfate, 40% glycerol, 0.08% bromophenol blue in 0.25M Tris-HCl) was added to 30 μ g protein (1 part loading buffer to 3 parts protein) and samples were boiled at 96 $^{\circ}$ C for 3 minutes. Proteins were loaded onto an AnyKD SDS-PAGE gel (Biorad) and separated by electrophoresis. The samples were transferred onto polyvinylidene difluoride membrane by wet electrophoresis and blocked in 5% milk/PBS for 1 hour at room temperature prior to incubation with primary antibodies. AP2mu2 (1:2000), Cav1 (1:500), CtBP1 (1:2000), CDC42 (1:1000) and Flot1 (1:500) were incubated in 1% milk in PBS with 0.1% tween (PBST), Rabankyrin-5 (1:100) incubated in 0.5% milk in PBST and Arf6 (1:100) in PBST all incubated

overnight at 4 °C under gentle agitation. Samples were washed three times in PBST and the appropriate secondary HRP-conjugated (Santa Cruz) was incubated for 1 hour at 1:1000 in 1% milk in PBST. Samples were washed a further three times in PBST and imaged using Femto chemiluminescence reagent on a Biorad ChemiDoc XRS+.

Target	Species	Supplier	Catalogue Number
AP2mu2	Rabbit	Abcam	ab75995
Arf6	Mouse	Santa Cruz	sc-7971
Graf1	Rabbit	Abcam	ab137085
Caveolin-1	Rabbit	Cell Signalling	3267S
CtBP1	Mouse	BD Bioscience	612042
CDC42	Rabbit	Cell Signalling	2462S
Flotillin-1	Mouse	BD Bioscience	610821
Rabankyrin-5	Mouse	Santa Cruz	sc-393353

Statistical analysis

Apoptosis, nuclei count, compound uptake and compound area assays were all assessed using linear mixed-effects models, followed by post-hoc comparisons tests using estimated marginal means. The models included the assay endpoints as the response variables, almost always on the log₁₀ scale, with fixed effects for variables such as compound, concentration, assay duration, cell type, cell donor and plate (where applicable). Interactions between these factors may also have been included in these models, depending on the experiment, and some of these factors/interactions may have been treated as random effects. Comparisons between groups were made using Dunnett's multiple comparison tests, with *p*-values left unadjusted or adjusted upwards using Dunnett's method or the Benjamini Hochberg method. Minimum effective concentration (MEC) analyses were conducted by modelling dose-response relationships between intensity and compound concentration with four parameter logistic models. The MEC was evaluated as the concentration where the fitted curve crosses a 3 standard deviation threshold around the mean control response (if applicable), with standard deviations calculated using responses from the control.

SUPPLEMENTARY RESULTS

The uptake and cytoprotective effects of H1 inhibitors were inconsistent

HCEC and RPTEC were co-treated with H1 inhibitors (ketotifen 0.52 μ M, levocabastine 1.8 μ M, desloratadine 2.2 μ M, levocetirizine 1.2 μ M, and fexofenadine 34.1 μ M) and ADC (10 or 100 μ g/mL) or mAb (100 μ g/mL) for 48 hours. In HCEC, significant reduction in ADC uptake was demonstrated for co-treatment with desloratadine versus ADC alone (-20.0% for ADC 10 μ g/mL, $p=0.0187$; -34.0% for ADC 100 μ g/mL, $p<0.0001$), fexofenadine (-23.3% for ADC 10 μ g/mL, $p=0.0040$; -26.6% for ADC 100 μ g/mL, $p=0.0007$), and ketotifen (-13.4 for ADC 10 μ g/mL, $p=0.2108$ [non-significant]); -24.1% for ADC 100 μ g/mL, $p=0.0027$) (**Supplement Table S9**). Similar results were seen for uptake of mAb 100 μ g/mL co-treated with desloratadine versus mAb alone (-27.6%, $p=0.0004$), fexofenadine (-20.7%, $p=0.0141$), and ketotifen (-21.4%, $p=0.0103$). A significantly greater nuclei count was observed for ADC 10 μ g/mL (+158.4%, $p=0.0384$) and mAb 100 μ g/mL (+238.6, $p=0.0058$) co-treated with desloratadine versus control (**Supplement Table S10**). In RPTEC, there were no significant reductions in ADC uptake with H1 inhibitor co-treatment; significant reductions in uptake were seen for mAb 100 μ g/mL with desloratadine (-20.5%, $p=0.0443$) and fexofenadine (-26.3%, $p=0.0083$); there were no significant changes in nuclei count (cell number) for co-treatment of H1 inhibitors with ADC or mAb.

Co-treatment of ketotifen 0.52 μ M and fexofenadine 34.1 μ M with ADC (10 μ g/mL or 100 μ g/mL) or mAb (100 μ g/mL) were further evaluated. There was no significant reduction of ADC or mAb uptake with co-treatment compared with ADC or mAb alone in HCEC or RPTEC (**Supplement Fig. S8a**). There were significant changes in HCEC and RPTEC nuclei counts with co-treatment versus controls (**Supplement Fig. S8b**); generally, these were reductions in nuclei count, although RPTEC nuclei counts were significantly greater for mAb 100 μ g/mL with ketotifen (+40.8%, $p=0.0002$) and fexofenadine (+27.5%, $p=0.0059$) compared with controls.

With the caspase-Glo 3/7 apoptosis assay, pre-treatment (30 minutes) and co-treatment (48 hours) of HCEC with sub-cytotoxic concentrations of ketotifen (52 μ M), fexofenadine (341 μ M), or desloratadine (2.2 μ M) with ADC (1–100 μ g/mL) resulted in significantly reduced apoptosis in HCEC versus ADC alone in a concentration-dependent manner, with maximal effects generally at ADC 25 μ g/mL (-56.8%, $p<0.0001$ for ketotifen; -56.6%, $p<0.0001$ for fexofenadine; -47.8%, $p<0.0001$ for desloratadine; -18.9%, $p=0.0104$ for levocetirizine) (**Supplement Fig. S8c**).

Synopsis

Uptake inhibition of ADC in HCEC and cytoprotective effects with H1 inhibitors were inconsistent at fixed concentrations.

Table S1 Cell lines

Cell line	Source	Origin	Donor number	Growth medium
HCEC	MatTek	Progenitor-rich limbal region of whole human corneas	CE435B1 and CE9060216B1	NHCE-GM + growth supplements
	ScienCell Research Laboratories	Human cornea	20885	CEpiCM
	ATCC	Corneal-scleral rim	80915170 80721171 70022729 70021863 70036732 70035457 81202211	Proprietary CECBM + growth supplements
	Merck	Cornea	1001231/NRG1000212	EpiGRO Human Ocular Epithelia Complete Media kit
RTPEC	Lonza		362300 400592 682573 400592 664995 203150	REGM BulletKit
NCI-H929	BioCat	Myeloma B lymphocyte cell line derived from human bone marrow		RPMI 1640 medium supplemented with FBS (10%), 2-mercaptoethanol (0.1%), L-glutamine (1%) and sodium pyruvate (1%), all supplied from Life Technologies

CECBM, proprietary cell culture medium; CEpiCM, proprietary corneal epithelial cell medium; FBS, fetal bovine serum; HCEC, human corneal epithelial cells; NHCE-GM, normal human corneal epithelial cell growth medium; REGM, renal epithelial cell growth medium; RPTEC, renal proximal tubule epithelial cells.

Table S2 Gene expression levels in cell lines used in this study

The cycle threshold (CT) value for BCMA for GAPDH (housekeeper gene) was 26.8, whereas for HCEC, the CT value was 36.4; this indicated the gene expression for BCMA was negligible for the HCECs. For the NCI-H929 cells, the CT value for BCMA expression was 23.6, and for RPTECs, this was 40.

Cell Line	BCMA expression (copy number per 50 ng input cDNA)
HCEC	
MatTek	20
ATCC	9
ScienCell	0
RPTEC	
Lonza	0
H929 (multiple myeloma cell line)	47,872

BCMA, B-cell maturation antigen; CT, cycle threshold; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HCEC, human corneal epithelial cells; RPTEC, renal proximal tubule epithelial cells.

Table S3 Uptake of mAb and ADC in HCEC and RPTEC

	HCEC					RPTEC			
	% change	95% CI		<i>p</i> -value		% change	95% CI		<i>p</i> -value
mAb 10 µg: 20 vs 44 hr	-0.58	-22.71	27.88	0.9561		-2.71	-18.53	16.18	0.7175
mAb 100 µg: 20 vs 44 hr	-10.68	-30.56	14.89	0.3096		-11.86	-26.19	5.25	0.1322
ADC 10 µg: 20 vs 44 hr	-11.67	-31.33	13.62	0.2688		-3.28	-19.01	15.49	0.6613
ADC 100 µg: 20 vs 44 hr	-20.63	-38.30	2.09	0.0654		-0.92	-17.03	18.31	0.9023
20 hr: ADC 10 µg vs mAb 10 µg	-15.43	-27.95	-0.74	0.0412		-10.99	-22.11	1.73	0.0829
ADC 100 µg vs mAb 100 µg	-27.90	-38.58	-15.38	0.0004		-35.04	-43.16	-25.76	<0.0001
mAb 100 µg vs mAb 10 µg	49.33	27.22	75.27	0.0001		78.44	56.14	103.93	<0.0001
ADC 100 µg vs ADC 10 µg	27.31	8.47	49.43	0.0054		30.22	13.95	48.82	0.0007
44 hr: ADC 10 µg vs mAb 10 µg	-4.82	-18.91	11.72	0.5252		-10.46	-21.65	2.33	0.0981
ADC 100 µg vs mAb 100 µg	-18.86	-30.87	-4.76	0.0135		-42.21	-49.44	-33.96	<0.0001
mAb 100 µg vs mAb 10 µg	66.21	41.61	95.09	<0.0001		96.97	72.35	125.10	<0.0001
ADC 100 µg vs ADC 10 µg	41.69	20.72	66.31	0.0002		27.12	11.23	45.28	0.0016

Uptake based on estimated changes in compound intensity fluorescence of Vivotag680-labeled mAb and ADC (10 or 100 µg) at 20 and 44 hours; *p*-values for the test of no change relative to baseline.

ADC, antibody-drug conjugate; CI, confidence interval; HCEC, human corneal epithelial cells; hr, hours; mAb, monoclonal antibody; min, minutes; RPTEC, renal proximal tubule epithelial cells.

Table S4 Changes in nuclei counts of HCEC and RPTEC treated with mAb or ADC

	HCEC					RPTEC			
	% change	95% CI		<i>p</i> -value		% change	95% CI		<i>p</i> -value
Untreated Ctrl: 20 vs 44 hr	-52.92	-68.50	-29.62	0.0053		-36.40	-64.06	12.54	0.0773
mAb 10 µg: 20 vs 44 hr	-44.28	-62.60	-16.98	0.0119		-35.58	-62.71	11.27	0.0769
mAb 100 µg: 20 vs 44 hr	-55.33	-70.02	-33.45	0.0028		-38.00	-64.10	7.10	0.0654
ADC 10 µg: 20 vs 44 hr	-26.48	-50.65	9.54	0.1070		-33.40	-61.44	15.04	0.0893
ADC 100 µg: 20 vs 44 hr	-2.22	-34.37	45.68	0.8937		-14.78	-50.66	47.21	0.3637
20 hr: mAb 10 µg vs Ctrl	3.07	-12.61	21.55	0.7104		3.02	-3.40	9.87	0.3602
mAb 100 µg vs Ctrl	-1.68	-16.63	15.96	0.8352		1.77	-4.58	8.53	0.5899
ADC 10 µg vs Ctrl	-23.34	-35.00	-9.60	0.0026		9.67	2.83	16.96	0.0055
ADC 100 µg vs Ctrl	-49.61	-57.27	-40.57	<0.0001		-8.69	-14.39	-2.62	0.0062
ADC 10 µg vs mAb 10 µg	-25.62	-37.76	-11.13	0.0018		6.45	-1.17	14.67	0.0980
ADC 100 µg vs mAb 100 µg	-48.75	-57.11	-38.76	<0.0001		-10.28	-16.71	-3.35	0.0048
44 hr: mAb 10 µg vs Ctrl	-12.91	-26.15	2.71	0.0971		1.71	-4.63	8.48	0.6011
mAb 100 µg vs Ctrl	3.65	-12.11	22.24	0.6596		4.38	-2.13	11.32	0.1890
ADC 10 µg vs Ctrl	-50.91	-58.37	-42.11	<0.0001		4.73	-1.80	11.69	0.1575
ADC 100 µg vs Ctrl	-75.73	-79.42	-71.38	<0.0001		-31.86	-36.11	-27.33	<0.0001
ADC 10 µg vs mAb 10 µg	-43.63	-52.83	-32.65	<0.0001		2.96	-4.42	10.91	0.4371
ADC 100 µg vs mAb 100 µg	-76.59	-80.41	-72.02	<0.0001		-34.72	-39.40	-29.68	<0.0001

Estimated changes in cells counts of HCEC and RTEC treated with Vivotag680-labeled mAb or ADC at 20 and 44 hours; *p*-values for the test of no change relative to baseline.

ADC, antibody-drug conjugate; CI, confidence interval; Ctrl, control; HCEC, human corneal epithelial cells; hr, hours; mAb, monoclonal antibody; RPTEC, renal proximal tubule epithelial cells.

Table S5 Comparison of uptake and nuclei counts between HCEC and RPTEC

Time	Contrast	% change*	95% CI†		p-value†
Uptake					
20 hours	ADC – mAb 10 µg/mL	-5.00	-24.35	19.30	0.6487
20 hours	ADC – mAb 100 µg/mL	10.99	-11.62	39.38	0.3569
44 hours	ADC – mAb 10 µg/mL	6.30	-15.35	33.49	0.5847
44 hours	ADC – mAb 100 µg/mL	40.41	11.81	76.33	0.0049
Nuclei count					
20 hours	mAb 10 µg/mL - control	0.68	-21.93	29.84	0.9572
20 hours	mAb 100 µg/mL - control	-2.27	-24.22	26.04	0.8553
20 hours	ADC 10 µg/mL - control	-28.55	-44.59	-7.85	0.0112
20 hours	ADC 100 µg/mL - control	-44.62	-57.06	-28.58	<0.0001
44 hours	mAb 10 µg/mL - control	-7.19	-28.03	19.69	0.5545
44 hours	mAb 100 µg/mL - control	0.45	-22.11	29.54	0.9715
44 hours	ADC 10 µg/mL - control	-49.26	-60.66	-34.57	<0.0001
44 hours	ADC 100 µg/mL - control	-62.28	-70.75	-51.36	<0.0001

*% change represents the change in uptake or nuclei counts with HCEC compared to RPTEC as the baseline. †95% CIs and *p*-values represent the test for a difference between HCEC and RPTEC.

ADC, antibody-drug conjugate; CI, confidence interval; mAb, monoclonal antibody

Table S6 Effect of endocytosis inhibitors on nuclei count in HCEC and RPTEC treated with mAb, ADC, and dextran

	HCEC					RPTEC			
	% change	95% CI		p-value		% change	95% CI		p-value
Untreated control Ctrl ± inhibitor									
Ctrl + wortmannin vs Ctrl	-7.6	-54.1	86.1	0.9870		7.8	-99.0	11810.1	0.9998
Ctrl + chlorpromazine vs Ctrl	-65.9	-83.1	-31.4	0.0014		-46.1	-99.5	5857.4	0.9557
Ctrl + nystatin vs Ctrl	0.9	-49.9	103.3	1.0000		-85.8	-99.9	1471.2	0.4968
Ctrl + imipramine vs Ctrl	-3.9	-52.3	93.5	0.9979		-0.4	-99.1	10899.5	1.0000
Ctrl + phenoxybenzamine vs mAb	-13.3	-56.9	74.7	0.9394		0.5	-99.1	10997.6	1.0000
Ctrl + co-dosing vs mAb	-24.7	-37.9	-8.7	0.0088		-49.1	-66.7	-22.1	0.0173
mAb 100 µg/mL ± inhibitor									
mAb + wortmannin vs mAb	20.5	-40.2	142.8	0.8801		10.4	-99.0	12090.0	0.9997
mAb + chlorpromazine vs mAb	-48.9	-74.6	2.9	0.0635		-31.1	-99.4	7513.0	0.9881
mAb + nystatin vs mAb	33.8	-33.6	169.6	0.6689		-87.6	-99.9	1269.8	0.4479
mAb + imipramine vs mAb	33.2	-33.9	168.3	0.6797		25.1	-98.9	13711.9	0.9969
mAb + phenoxybenzamine vs mAb	3.9	-48.4	109.2	0.9982		32.5	-98.8	14533.1	0.9943
mAb + co-dosing vs mAb	-17.3	-31.8	0.3	0.0530		-60.2	-74.0	-39.0	0.0082
ADC 100 µg/mL ± inhibitor									
ADC + wortmannin vs ADC	30.5	-35.2	162.9	0.7264		-5.9	-99.1	10292.4	0.9999
ADC + chlorpromazine vs ADC	7.2	-46.8	116.0	0.9907		-4.3	-99.1	10471.8	1.0000
ADC + nystatin vs ADC	109.8	4.1	322.6	0.0352		-73.0	-99.8	2883.2	0.7476
ADC + imipramine vs ADC	81.4	-9.9	265.5	0.1166		56.6	-98.6	17197.1	0.9805
ADC + phenoxybenzamine vs ADC	49.4	-25.8	201.0	0.4132		44.7	-98.7	15884.0	0.9883
ADC + co-dosing vs ADC	42.8	17.8	73.2	0.0024		10.9	-27.6	69.7	0.4641
Dextran 200 µg/mL ± inhibitor									
Dextran + wortmannin vs dextran	13.0	-43.9	127.7	0.9587		-0.4	-99.1	10900.8	1.0000
Dextran + chlorpromazine vs dextran	-53.1	-76.7	-5.4	0.0307		-37.9	-99.4	6758.1	0.9772
Dextran + nystatin vs dextran	30.8	-35.1	163.6	0.7208		-81.6	-99.8	1933.2	0.5954
Dextran + imipramine vs dextran	16.8	-42.0	135.2	0.9246		41.7	-98.7	15547.7	0.9900
Dextran + phenoxybenzamine vs dextran	-11.1	-55.9	79.1	0.9629		6.3	-99.0	11640.9	0.9999
Dextran + co-dosing vs dextran	-22.9	-36.4	-6.5	0.0139		-43.3	-63.0	-13.3	0.0260

Estimated changes in nuclei count of HCEC and RTEC at 48 hours; treated with Vivotag680-labeled mAb, ADC, or Alexa488-labeled dextran with or without co-treatment with endocytosis inhibitors; *p*-values for the test of no change relative to baseline.

Inhibitor concentrations: wortmannin 400 nM, chlorpromazine 10 μ M, nystatin 100 μ M, imipramine 10 μ M, phenoxybenzamine 10 μ M

Co-dosing with chlorpromazine 5 μ M, nystatin 50 μ M, and imipramine 5 μ M.

ADC, antibody-drug conjugate; CI, confidence interval; Ctrl, control; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; RPTEC, renal proximal tubule epithelial cells.

Table S7 Uptake: HCEC and RPTEC were treated with ADC or mAb alone or co-treated with human immunoglobulin solution

Cell	Compound	Contrast	% change	95% CI		<i>p</i> -value
HCEC	mAb 100 µg/mL	Human immunoglobulin solution 8 mg/mL - undosed	-25.1	-42.0	-3.4	0.0203
HCEC	mAb 100 µg/mL	Human immunoglobulin solution 15 mg/mL - undosed	-40.7	-54.0	-23.5	<0.0001
HCEC	mAb 100 µg/mL	Human immunoglobulin solution 20 mg/mL - undosed	-47.6	-59.4	-32.4	<0.0001
HCEC	ADC 10 µg/mL	Human immunoglobulin solution 8 mg/mL - undosed	-18.6	-36.9	5.1	0.1523
HCEC	ADC 10 µg/mL	Human immunoglobulin solution 15 mg/mL - undosed	-30.1	-45.8	-9.9	0.0028
HCEC	ADC 10 µg/mL	Human immunoglobulin solution 20 mg/mL - undosed	-36.0	-50.4	-17.4	0.0002
HCEC	ADC 100 µg/mL	Human immunoglobulin solution 8 mg/mL - undosed	-26.3	-42.8	-4.9	0.0134
HCEC	ADC 100 µg/mL	Human immunoglobulin solution 15 mg/mL - undosed	-34.1	-48.9	-15.0	0.0004
HCEC	ADC 100 µg/mL	Human immunoglobulin solution 20 mg/mL - undosed	-37.8	-51.7	-19.7	0.0001
RPTEC	mAb 100 µg/mL	Human immunoglobulin solution 8 mg/mL - undosed	-15.9	-26.2	-4.2	0.0061

RPTEC	mAb 100 µg/mL	Human immunoglobulin solution 15 mg/mL - undosed	-25.8	-34.9	-15.5	<0.0001
RPTEC	mAb 100 µg/mL	Human immunoglobulin solution 20 mg/mL - undosed	-35.3	-43.2	-26.3	<0.0001
RPTEC	ADC 10 µg/mL	Human immunoglobulin solution 8 mg/mL - undosed	-18.0	-28.1	-6.6	0.0015
RPTEC	ADC 10 µg/mL	Human immunoglobulin solution 15 mg/mL - undosed	-29.2	-37.8	-19.3	<0.0001
RPTEC	ADC 10 µg/mL	Human immunoglobulin solution 20 mg/mL - undosed	-38.8	-46.2	-30.3	<0.0001
RPTEC	ADC 100 µg/mL	Human immunoglobulin solution 8 mg/mL - undosed	-9.6	-20.7	2.9	0.1699
RPTEC	ADC 100 µg/mL	Human immunoglobulin solution 15 mg/mL - undosed	-20.6	-30.3	-9.5	0.0003
RPTEC	ADC 100 µg/mL	Human immunoglobulin solution 20 mg/mL - undosed	-31.4	-39.7	-21.8	<0.0001

ADC, antibody-drug conjugate; CI, confidence interval; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; RPTEC, renal proximal tubule epithelial cells.

Table S8 Nuclei counts: HCEC and RPTEC were treated with ADC or mAb alone or co-treated with human immunoglobulin solution

Cell	Condition	Contrast	% change	95% CI		p-value
HCEC	Undosed	mAb 100 µg/mL - Ctrl	9.7	-34.3	83.4	0.9105
HCEC	Undosed	ADC 10 µg/mL - Ctrl	-57.0	-74.3	-28.1	0.0014
HCEC	Undosed	ADC 100 µg/mL - Ctrl	-70.8	-82.5	-51.2	<0.0001
HCEC	Human immunoglobulin solution 8 mg/mL	mAb 100 µg/mL - Ctrl	17.7	-29.6	96.7	0.7334
HCEC	Human immunoglobulin solution 8 mg/mL	ADC 10 µg/mL - Ctrl	-46.7	-68.1	-11.0	0.0147
HCEC	Human immunoglobulin solution 8 mg/mL	ADC 100 µg/mL - Ctrl	-70.1	-82.1	-50.1	<0.0001
HCEC	Human immunoglobulin solution 15 mg/mL	mAb 100 µg/mL - Ctrl	13.5	-32.1	89.7	0.8344
HCEC	Human immunoglobulin solution 15 mg/mL	ADC 10 µg/mL - Ctrl	-27.0	-56.3	22.0	0.3014
HCEC	Human immunoglobulin solution 15 mg/mL	ADC 100 µg/mL - Ctrl	-59.4	-75.7	-32.1	0.0007
HCEC	Human immunoglobulin solution 20 mg/mL	mAb 100 µg/mL - Ctrl	-4.2	-42.7	60.0	0.9812
HCEC	Human immunoglobulin solution 20 mg/mL	ADC 10 µg/mL - Ctrl	-41.3	-64.9	-1.9	0.0412
HCEC	Human immunoglobulin solution 20 mg/mL	ADC 100 µg/mL - Ctrl	-47.4	-68.5	-12.2	0.0127

RPTEC	Undosed	mAb 100 µg/mL - Ctrl	33.7	12.3	59.3	0.0013
RPTEC	Undosed	ADC 10 µg/mL - Ctrl	-1.9	-17.6	16.8	0.9674
RPTEC	Undosed	ADC 100 µg/mL - Ctrl	-36.0	-46.2	-23.7	<0.0001
RPTEC	Human immunoglobulin solution 8 mg/mL	mAb 100 µg/mL - Ctrl	13.2	-4.9	34.8	0.2013
RPTEC	Human immunoglobulin solution 8 mg/mL	ADC 10 µg/mL - Ctrl	19.2	0.1	42.0	0.0486
RPTEC	Human immunoglobulin solution 8 mg/mL	ADC 100 µg/mL - Ctrl	-22.3	-34.7	-7.4	0.0044
RPTEC	Human immunoglobulin solution 15 mg/mL	mAb 100 µg/mL - Ctrl	6.2	-10.9	26.4	0.6954
RPTEC	Human immunoglobulin solution 15 mg/mL	ADC 10 µg/mL - Ctrl	22.3	2.7	45.7	0.0223
RPTEC	Human immunoglobulin solution 15 mg/mL	ADC 100 µg/mL - Ctrl	-14.2	-28	2.2	0.0930
RPTEC	Human immunoglobulin solution 20 mg/mL	mAb 100 µg/mL - Ctrl	8.4	-9.0	29.1	0.5088
RPTEC	Human immunoglobulin solution 20 mg/mL	ADC 10 µg/mL - Ctrl	13.6	-4.6	35.3	0.1844
RPTEC	Human immunoglobulin solution 20 mg/mL	ADC 100 µg/mL - Ctrl	-7.7	-22.5	10.0	0.5140

ADC, antibody-drug conjugate; CI, confidence interval; Ctrl, control; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; RPTEC, renal proximal tubule epithelial cells.

Table S9 Effect of H1 inhibitors on uptake of mAb and ADC

Cell	Compound	Contrast	% change	95% CI		p-value
HCEC	mAb 100 µg/mL	Desloratadine - undosed	-27.6	-40.4	-12.0	0.0004
HCEC	mAb 100 µg/mL	Fexofenadine - undosed	-20.7	-34.7	-3.6	0.0141
HCEC	mAb 100 µg/mL	Ketotifen - undosed	-21.4	-35.3	-4.5	0.0103
HCEC	mAb 100 µg/mL	Levocabastine - undosed	-11.1	-26.9	7.9	0.3769
HCEC	mAb 100 µg/mL	Levocetirizine - undosed	-13.2	-28.6	5.4	0.2226
HCEC	ADC 100 µg/mL	Desloratadine - undosed	-34.0	-45.7	-19.8	<0.0001
HCEC	ADC 100 µg/mL	Fexofenadine - undosed	-26.6	-39.6	-10.8	0.0007
HCEC	ADC 100 µg/mL	Ketotifen - undosed	-24.1	-37.5	-7.7	0.0027
HCEC	ADC 100 µg/mL	Levocabastine - undosed	4.0	-14.4	26.4	0.9432
HCEC	ADC 100 µg/mL	Levocetirizine - undosed	-8.4	-24.6	11.2	0.6237
HCEC	ADC 10 µg/mL	Desloratadine - undosed	-20.0	-34.2	-2.9	0.0187
HCEC	ADC 10 µg/mL	Fexofenadine - undosed	-23.3	-36.9	-6.8	0.0040
HCEC	ADC 10 µg/mL	Ketotifen - undosed	-13.4	-28.7	5.2	0.2108
HCEC	ADC 10 µg/mL	Levocabastine - undosed	-1.1	-18.6	20.1	0.9981
HCEC	ADC 10 µg/mL	Levocetirizine - undosed	-5.3	-22.0	15.1	0.8752
RPTEC	mAb 100 µg/mL	Desloratadine - undosed	-20.5	-36.5	-0.5	0.0443
RPTEC	mAb 100 µg/mL	Fexofenadine - undosed	-26.3	-41.1	-7.8	0.0083
RPTEC	mAb 100 µg/mL	Ketotifen - undosed	-16.5	-33.3	4.5	0.1303
RPTEC	mAb 100 µg/mL	Levocabastine - undosed	16.9	-6.6	46.4	0.2120
RPTEC	mAb 100 µg/mL	Levocetirizine - undosed	-3.0	-22.5	21.4	0.9722

RPTEC	ADC 100 µg/mL	Desloratadine - undosed	-2.3	-21.9	22.3	0.9867
RPTEC	ADC 100 µg/mL	Fexofenadine - undosed	-13.5	-30.8	8.3	0.2676
RPTEC	ADC 100 µg/mL	Ketotifen - undosed	4.5	-16.5	30.7	0.9283
RPTEC	ADC 100 µg/mL	Levocabastine - undosed	27.8	2.1	59.9	0.0316
RPTEC	ADC 100 µg/mL	Levocetirizine - undosed	15.9	-7.4	45.1	0.2518
RPTEC	ADC 10 µg/mL	Desloratadine - undosed	6.0	-15.3	32.7	0.8556
RPTEC	ADC 10 µg/mL	Fexofenadine - undosed	-2.7	-22.3	21.8	0.9783
RPTEC	ADC 10 µg/mL	Ketotifen - undosed	6.6	-14.8	33.5	0.8196
RPTEC	ADC 10 µg/mL	Levocabastine - undosed	25.6	0.3	57.2	0.0463
RPTEC	ADC 10 µg/mL	Levocetirizine - undosed	10.0	-12.1	37.6	0.5950

ADC, antibody-drug conjugate; CI, confidence interval; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; RPTEC, renal proximal tubule epithelial cells.

Table S10 Effect of H1 inhibitors on nuclei counts (cell number) of mAb and ADC

Cell	Inhibitor	Contrast	% change	95% CI		p-value
HCEC	Undosed	mAb 100 µg/mL - Ctrl	12.9	-54.5	180.1	0.9562
HCEC	Undosed	ADC 100 µg/mL - Ctrl	-78.4	-91.3	-46.4	0.0005
HCEC	Undosed	ADC 10 µg/mL - Ctrl	-64.4	-85.7	-11.8	0.0219
HCEC	Desloratadine	mAb 100 µg/mL - Ctrl	238.6	36.5	739.8	0.0058
HCEC	Desloratadine	ADC 100 µg/mL - Ctrl	23.0	-50.4	205.0	0.8707
HCEC	Desloratadine	ADC 10 µg/mL - Ctrl	158.4	4.2	541.0	0.0384
HCEC	Fexofenadine	mAb 100 µg/mL - Ctrl	17.4	-52.7	191.2	0.9226
HCEC	Fexofenadine	ADC 100 µg/mL - Ctrl	-82.7	-93.0	-57.0	0.0001
HCEC	Fexofenadine	ADC 10 µg/mL - Ctrl	-53.9	-81.4	14.4	0.1112
HCEC	Ketotifen	mAb 100 µg/mL - Ctrl	10.2	-55.6	173.4	0.9723
HCEC	Ketotifen	ADC 100 µg/mL - Ctrl	-79.4	-91.7	-48.9	0.0003
HCEC	Ketotifen	ADC 10 µg/mL - Ctrl	-57.3	-82.8	5.9	0.0712
HCEC	Levocabastine	mAb 100 µg/mL - Ctrl	25.6	-49.4	211.5	0.8435
HCEC	Levocabastine	ADC 100 µg/mL - Ctrl	-78.3	-91.3	-46.2	0.0005
HCEC	Levocabastine	ADC 10 µg/mL - Ctrl	-56.4	-82.4	8.1	0.0801
HCEC	Levocetirizine	mAb 100 µg/mL - Ctrl	32.8	-46.4	229.5	0.7614
HCEC	Levocetirizine	ADC 100 µg/mL - Ctrl	-76.7	-90.6	-42.3	0.0009
HCEC	Levocetirizine	ADC 10 µg/mL - Ctrl	-54.1	-81.5	13.7	0.1076
RPTEC	Undosed	mAb 100 µg/mL - Ctrl	-1.0	-50.5	97.9	0.9993
RPTEC	Undosed	ADC 100 µg/mL - Ctrl	-55.6	-77.8	-11.3	0.0256

RPTEC	Undosed	ADC 10 µg/mL - Ctrl	9.9	-45.0	119.6	0.9290
RPTEC	Desloratadine	mAb 100 µg/mL - Ctrl	7.0	-46.4	113.9	0.9632
RPTEC	Desloratadine	ADC 100 µg/mL - Ctrl	-8.1	-54.0	83.6	0.9424
RPTEC	Desloratadine	ADC 10 µg/mL - Ctrl	12.3	-43.8	124.4	0.8925
RPTEC	Fexofenadine	mAb 100 µg/mL - Ctrl	2.4	-48.7	104.7	0.9956
RPTEC	Fexofenadine	ADC 100 µg/mL - Ctrl	-31.5	-65.7	37.0	0.3088
RPTEC	Fexofenadine	ADC 10 µg/mL - Ctrl	19.4	-40.3	138.6	0.7615
RPTEC	Ketotifen	mAb 100 µg/mL - Ctrl	26.7	-36.6	153.3	0.6127
RPTEC	Ketotifen	ADC 100 µg/mL - Ctrl	-23.7	-61.8	52.5	0.5305
RPTEC	Ketotifen	ADC 10 µg/mL - Ctrl	60.3	-19.8	220.4	0.1812
RPTEC	Levocabastine	mAb 100 µg/mL - Ctrl	-0.8	-50.4	98.3	0.9995
RPTEC	Levocabastine	ADC 100 µg/mL - Ctrl	-38.9	-69.4	22.1	0.1602
RPTEC	Levocabastine	ADC 10 µg/mL - Ctrl	23.4	-38.2	146.7	0.6787
RPTEC	Levocetirizine	mAb 100 µg/mL - Ctrl	-5.9	-52.9	88.1	0.9708
RPTEC	Levocetirizine	ADC 100 µg/mL - Ctrl	-38.6	-69.3	22.7	0.1649
RPTEC	Levocetirizine	ADC 10 µg/mL - Ctrl	15.6	-42.2	131.0	0.8347

ADC, antibody-drug conjugate; CI, confidence interval; Ctrl, control; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; RPTEC, renal proximal tubule epithelial cells.

Fig. S1 Fluorescence microscopy showing nuclei, cytoplasmic labelling, and antibody/ADC uptake across treatment conditions. Representative images of Control, mAb 10 $\mu\text{g/mL}$, mAb 100 $\mu\text{g/mL}$, ADC 10 $\mu\text{g/mL}$, and ADC 100 $\mu\text{g/mL}$.

Column 1 (cyan): Nuclear staining used for nuclei identification and counting.

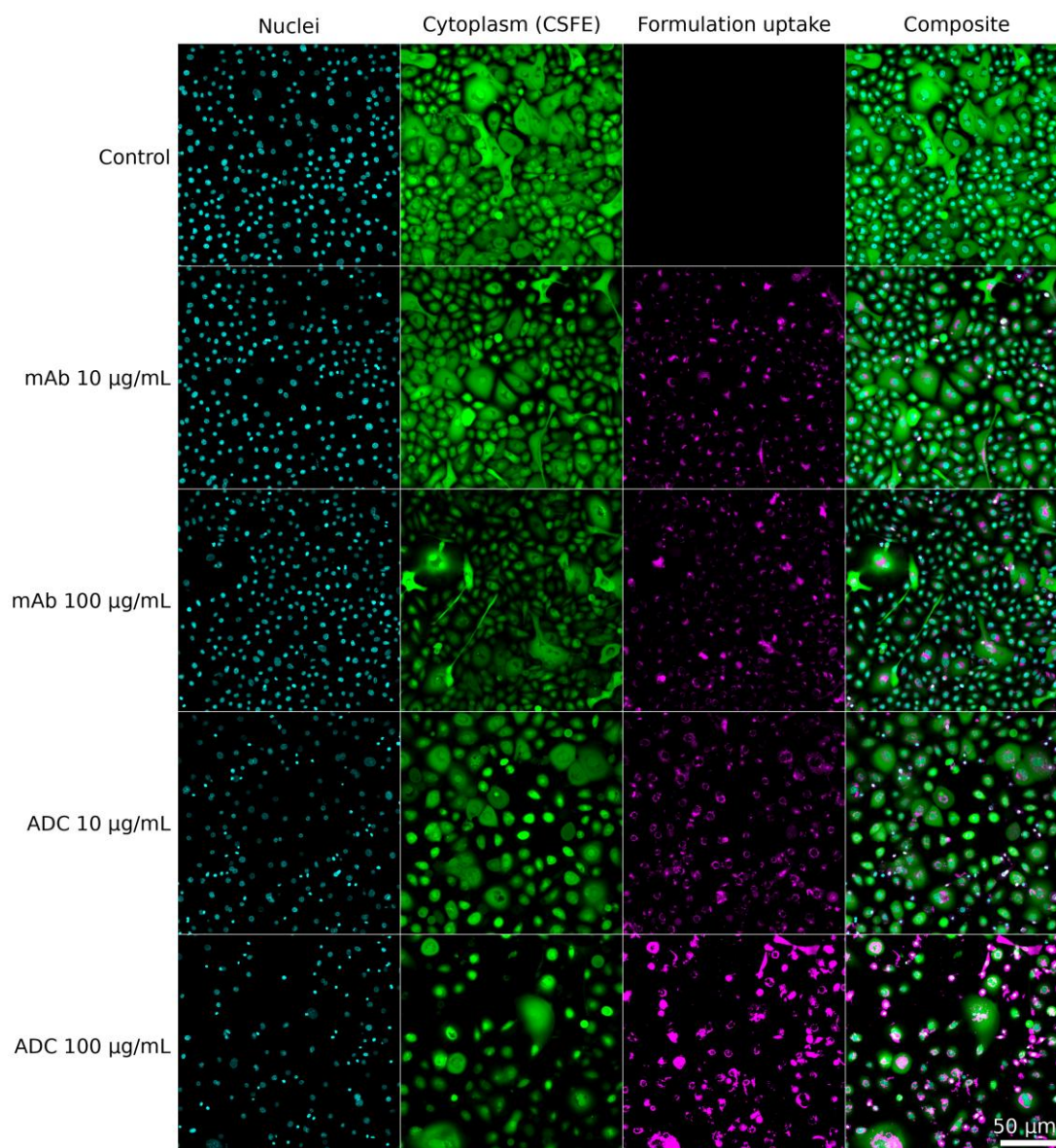
Column 2 (green): CFSE-labeled cytoplasm used to outline total cell area and visualize the full cell population.

Column 3 (magenta): Internalized mAb/ADC fluorescence representing formulation uptake.

Column 4: Composite merged channels using floating-point screen blending to preserve dynamic range.

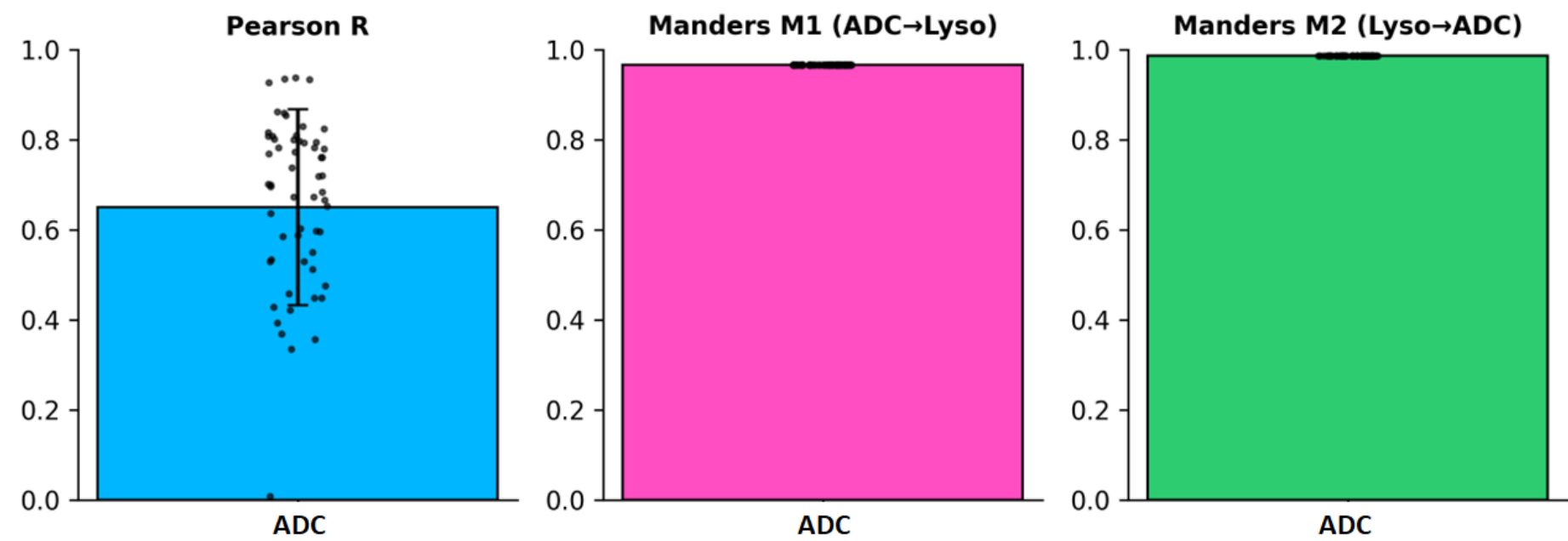
All channels were displayed using medium contrast stretching (0.5–99.5 percentile) applied uniformly across conditions to enhance visibility without saturation. Scale bars represent 50 μm .

Images are shown with identical display settings for valid comparison across treatments.



ADC, antibody-drug conjugate; CFSE, carboxyfluorescein diacetate succinimidyl ester; HCEC, human corneal epithelial cells; mAb, monoclonal antibody.

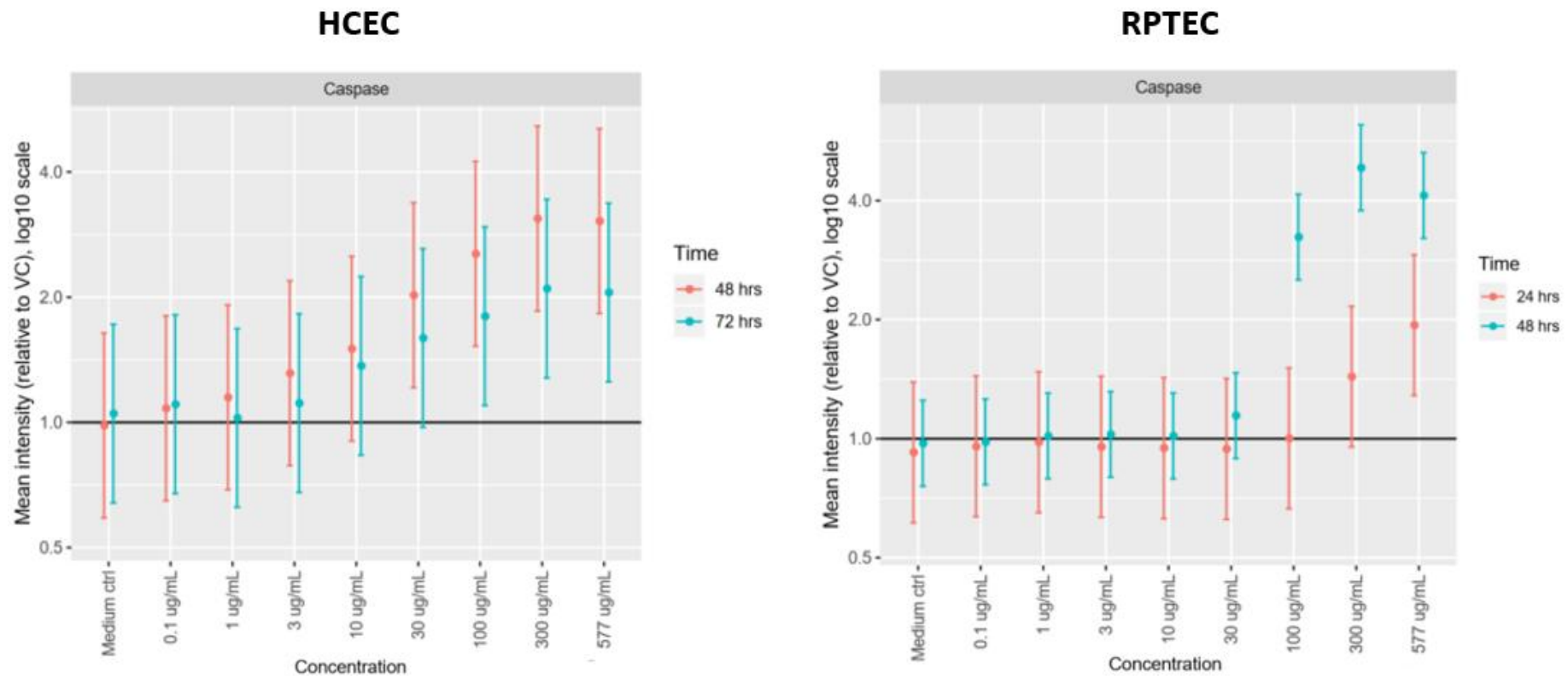
Fig. S2 Pearson correlation coefficient and Manders colocalization coefficient between ADC (10 $\mu\text{g/mL}$) and lysotracker.



ADC, antibody-drug conjugate

Fig. S3 ADC-mediated apoptosis in HCEC and RPTEC

HCEC and RPTEC treated with ADC (0.1–577 $\mu\text{g/mL}$). Apoptosis was assessed at 48 and 72 hours for HCEC and 24 and 48 hours for RPTEC using a caspase 3/7 assay.

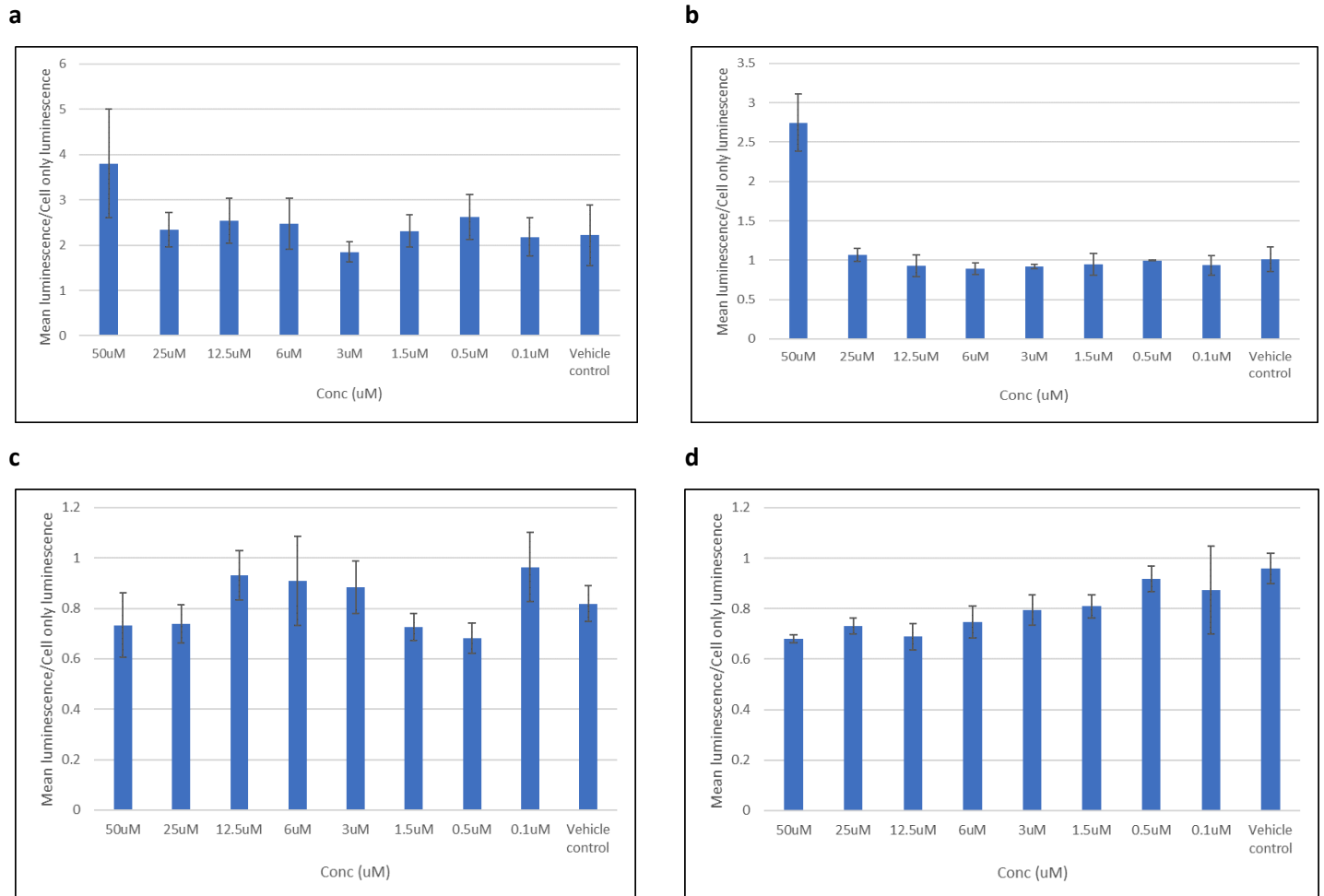


Error bars represent 95% CIs.

ADC, antibody-drug conjugate; HCEC, human corneal epithelial cells; hrs, hours; RPTEC, renal proximal tubule epithelial cells.

Fig. S4 Sub-cytotoxic concentration of macropinocytosis inhibitor EIPA in HCEC and RPTEC

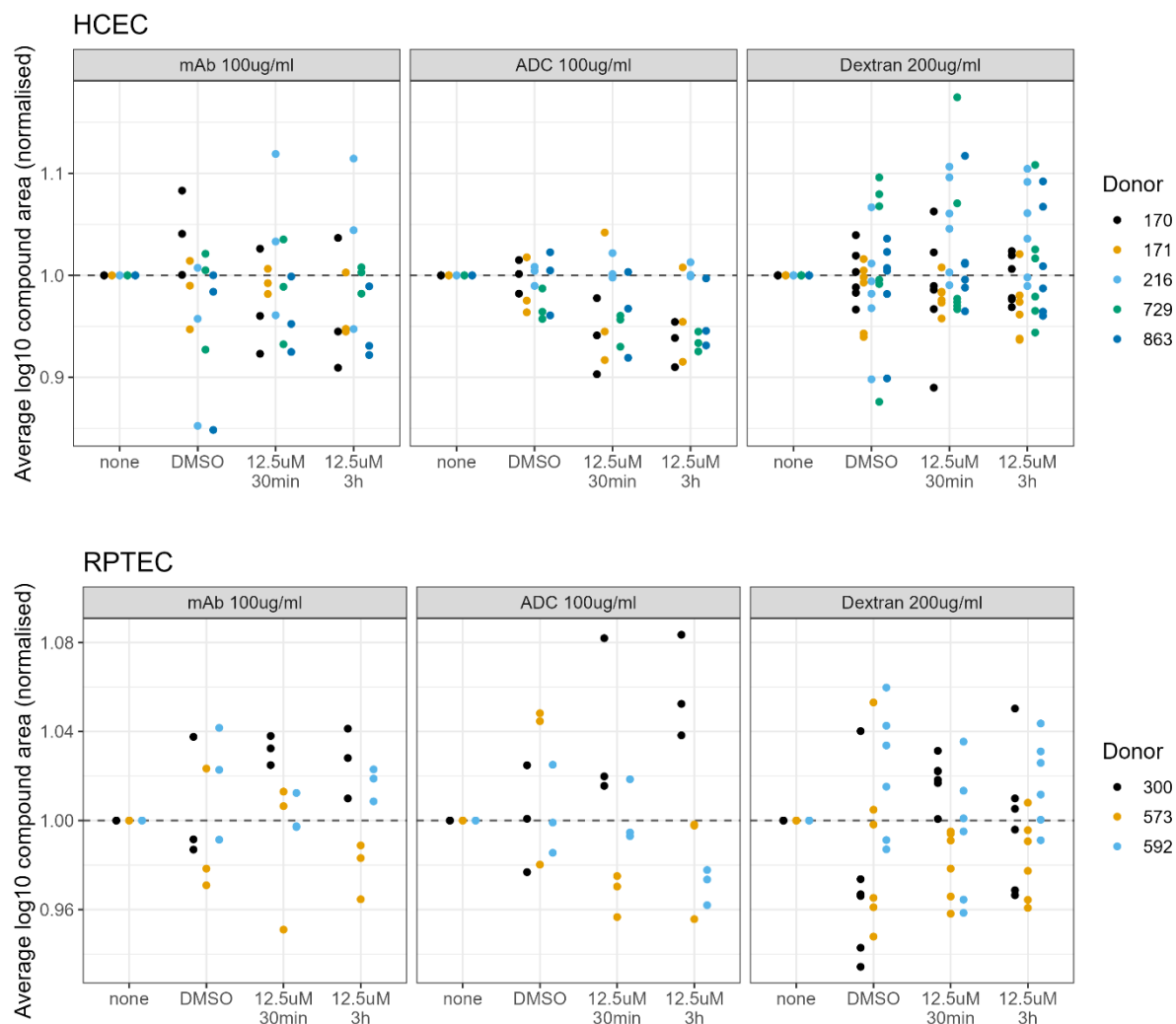
The sub-cytotoxic concentration of EIPA was determined by measuring apoptosis at 3 and 48 hours using a caspase 3/7 assay. Exposure of HCEC and RPTEC to EIPA (0.1–50 μ M) did not increase apoptosis relative to vehicle control at concentrations ≤ 25 μ M in HCEC and ≤ 50 μ M in RPTEC. In HCEC (single donor), there was an increase in apoptosis with EIPA 50 μ M at 3 hours (**a**) and at 48 hours (**b**). In RPTEC (single donor), there was no increase in apoptosis with EIPA up to 50 μ M at 3 hours (**c**) and at 48 hours (**d**).



Conc, concentration; EIPA, 5-(N-Ethyl-N-isopropyl)amiloride; HCEC, human corneal epithelial cells; RPTEC, renal proximal tubule epithelial cells.

Fig. S5 Effect of EIPA on uptake of mAb, ADC, and dextran in HCEC and RPTEC

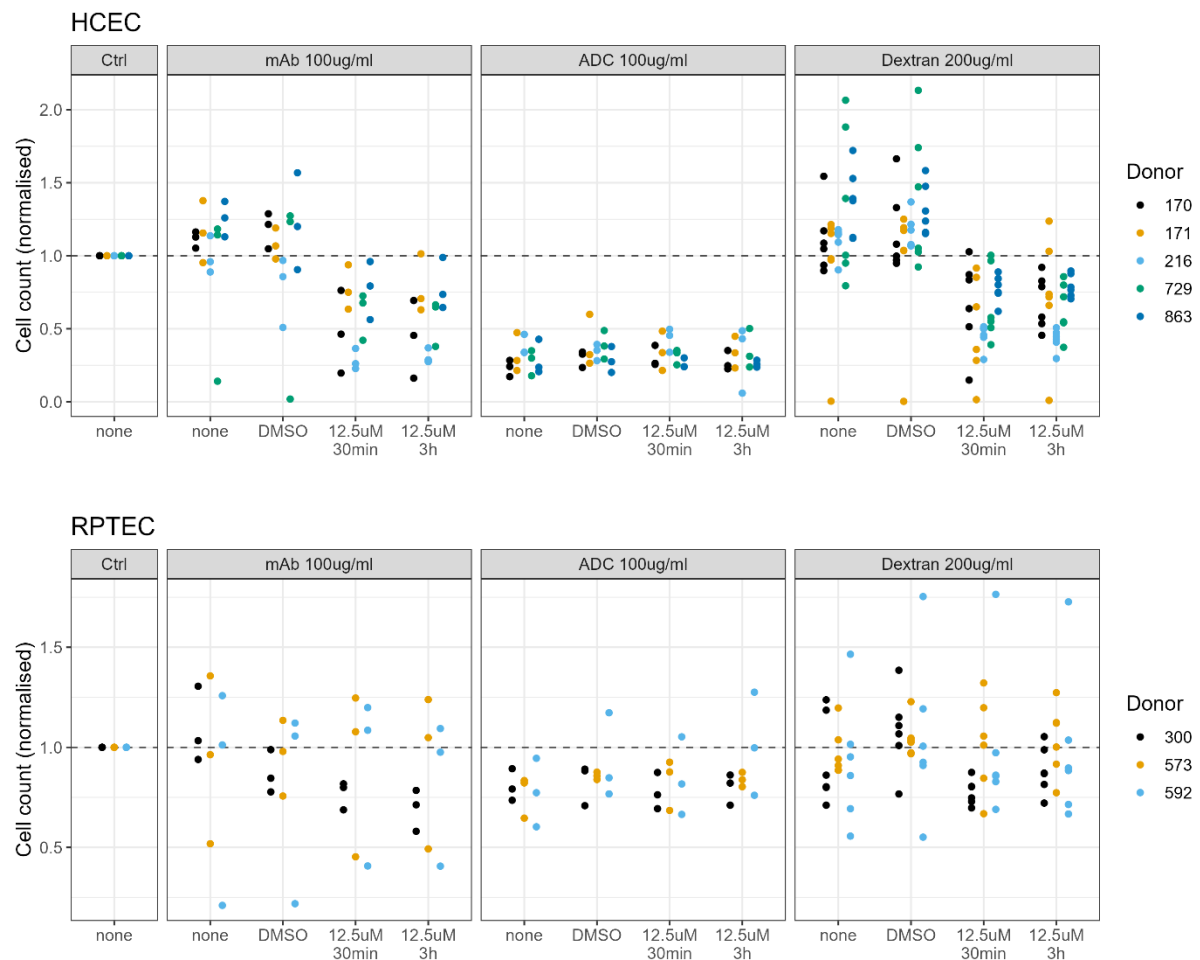
Uptake based on estimated changes in compound area fluorescence of Vivotag680-labeled mAb, ADC, or Alexa488-labeled dextran at 47 hours of co-treatment with EIPA; HCEC and RPTEC were pre-treated with EIPA for 30 minutes or 3 hours.



ADC, antibody-drug conjugate; DMSO, dimethyl sulfoxide; EIPA, 5-(N-Ethyl-N-isopropyl)amiloride; h, hours; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; min, minutes; RPTEC, renal proximal tubule epithelial cells.

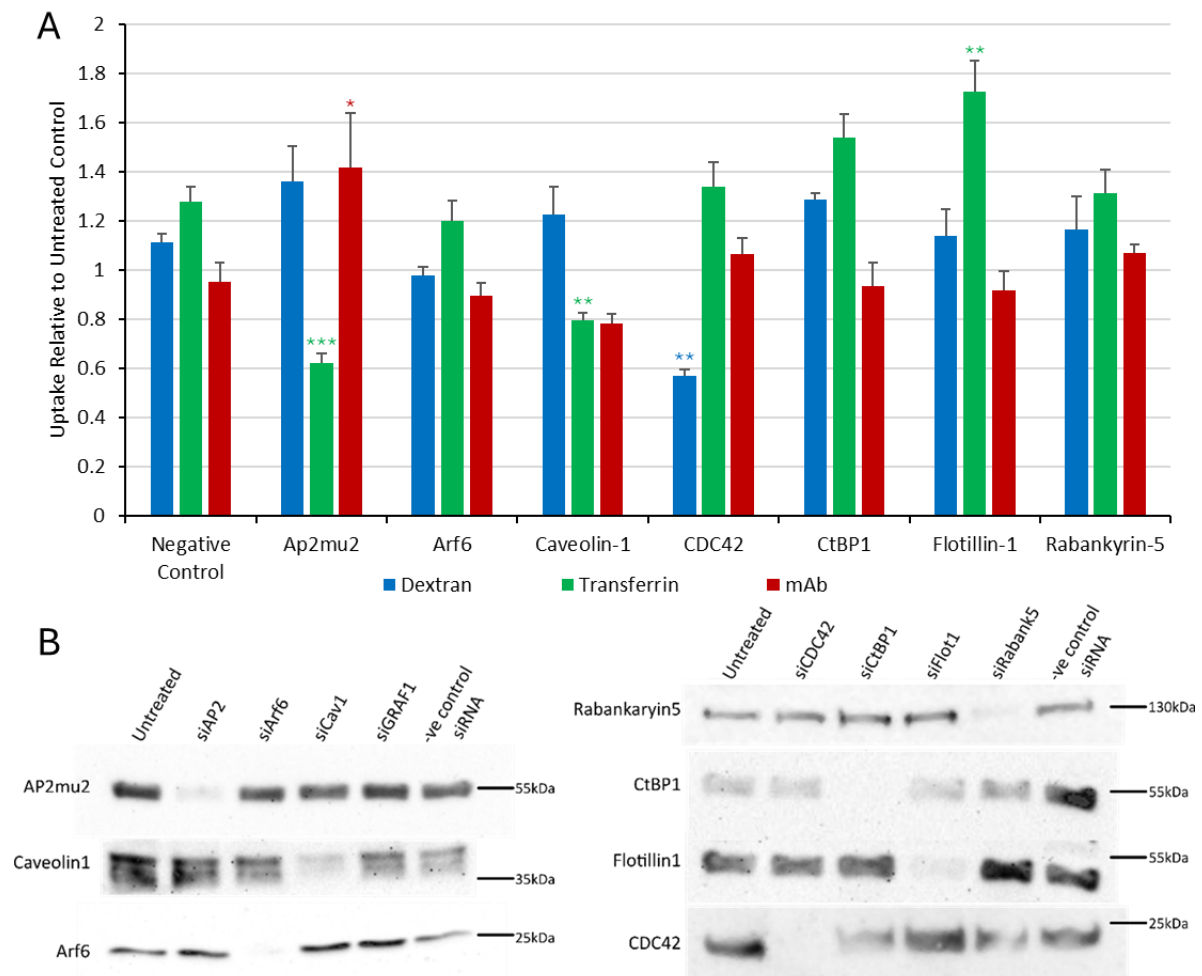
Fig. S6 Effect of EIPA on nuclei counts of HCEC and RPTEC treated with mAb, ADC, and dextran

Estimated changes in nuclei count of HCEC and RTEC at 48 hours; treated with Vivotag680-labeled mAb, ADC, or Alexa488-labeled dextran with or without EIPA co-treatment; HCEC and RPTEC were pre-treated with EIPA for 30 minutes or 3 hours.



ADC, antibody-drug conjugate; Ctrl, control; DMSO, dimethyl sulfoxide; EIPA, 5-(N-Ethyl-N-isopropyl)amiloride; h, hours; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; min, minutes; RPTEC, renal proximal tubule epithelial cells.

Fig. S7 (A) Flow cytometry analysis of mAb, dextran, and transferrin uptake in HCEC following siRNA protein depletion. **(B)** Western blot analysis of protein knockdown following siRNA treatment.



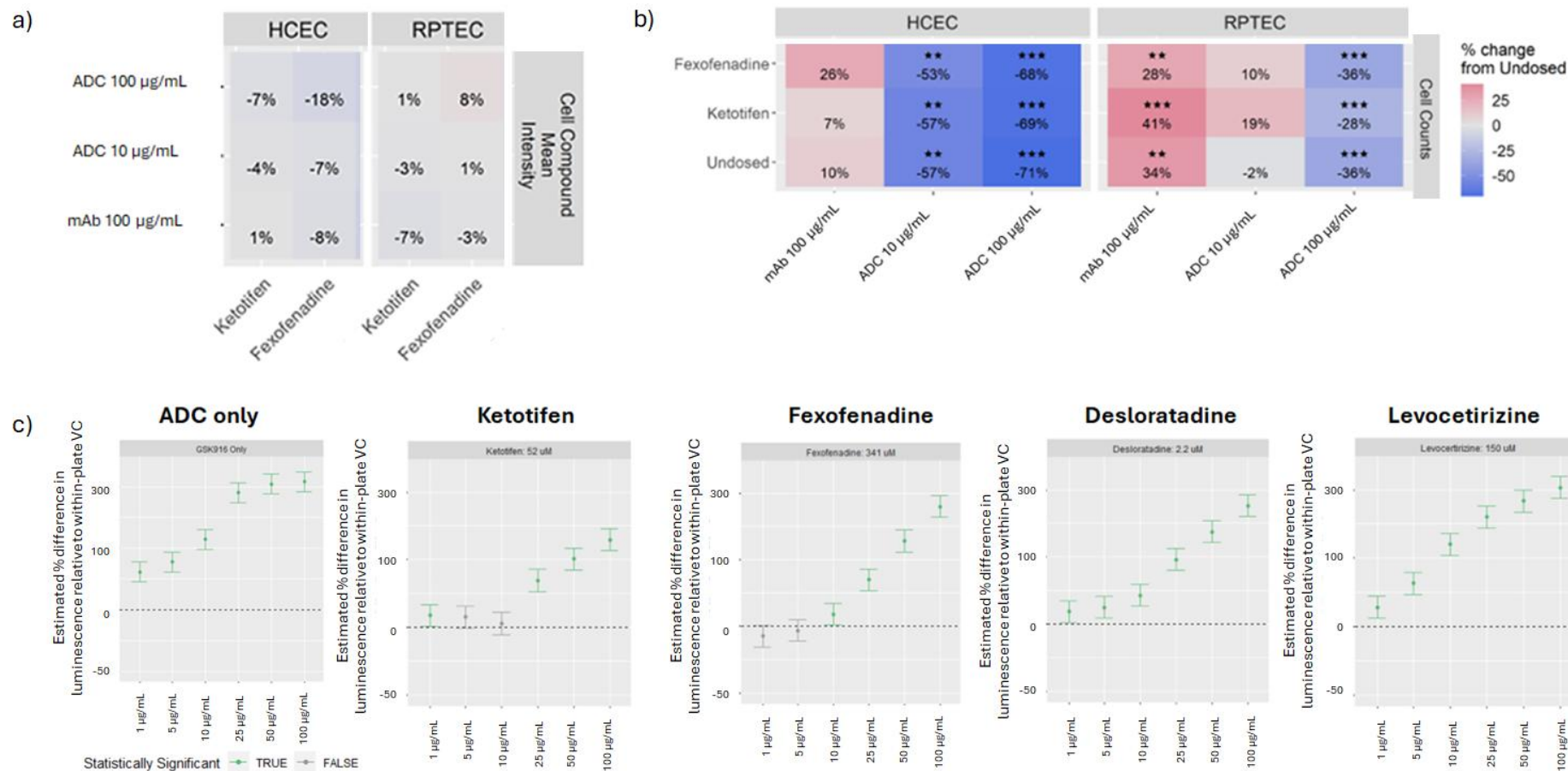
Dunnett's multiple comparison test of siRNA treatment versus negative control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

HCEC, human corneal epithelial cells; mAb, monoclonal antibody.

Fig. S8 Effect of H1 inhibitors on uptake of mAb and ADC **(a)** and nuclei counts **(b)** in HCEC and RPTEC, and ADC-mediated apoptosis in HCEC **(c)**

Uptake and nuclei counts: HCEC and RPTEC were treated with ADC or mAb alone or co-treated with ADC and H1 inhibitors for 48 hours. Concentrations were 10 and 100 µg for ADC; 100 µg for mAb; and 34.1 µM for fexofenadine; and 0.52 µM for ketotifen. Uptake based on estimated changes in compound intensity fluorescence of Vivotag680-labeled ADC or mAb. Dunnett adjusted *p*-values for the test of no change from control: ****p*<0.001, ** *p*<0.01.

Apoptosis: HCEC were treated with ADC alone or co-treated with ADC and H1 inhibitors. Concentrations were: 1–100 µg for ADC; 150 µM for levocetirizine, 2.2 µM for desloratadine, 341 µM for fexofenadine, and 52 µM for ketotifen. HCEC were pre-treated with H1 inhibitors for 30 minutes. Apoptosis was measured using the Caspase-Glo 3/7 apoptosis assay (a luminescence test) at 48 hours.



ADC, antibody-drug conjugate; HCEC, human corneal epithelial cells; mAb, monoclonal antibody; RPTEC, renal proximal tubule epithelial cells.

File S1 Pipeline code



Supplement File 1
(pipeline code).txt

References

Stringer C, Wang T, Michaelos M, Pachitariu M. Cellpose: a generalist algorithm for cellular segmentation. Nature Methods. 2021;18:100-6. <https://doi.org/10.1038/s41592-020-01018-x>