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Supplementary Figures and Tables

Modification of the antibiotic, colistin, with dextrin causes enhanced cytotoxicity and triggers apoptosis in myeloid leukemia

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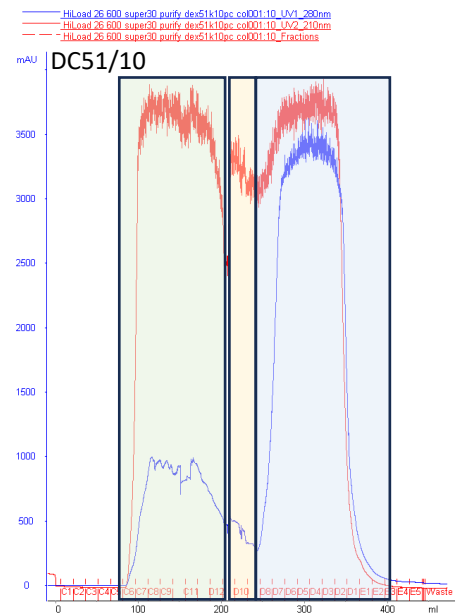
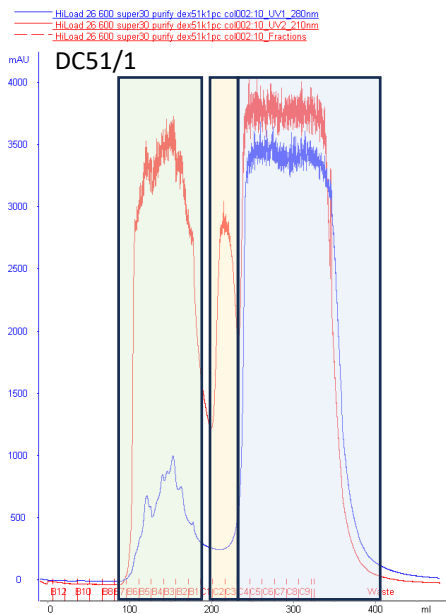
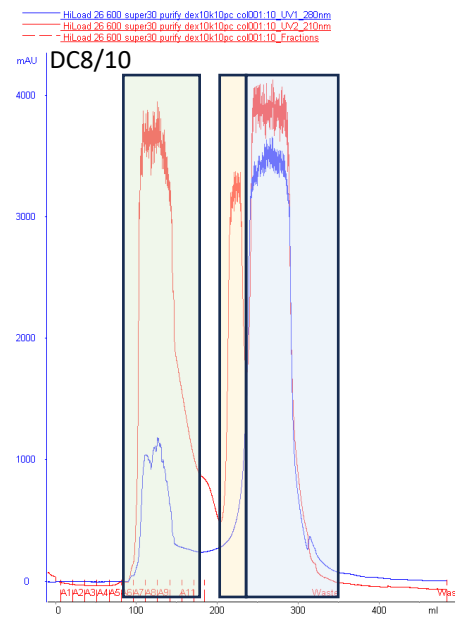
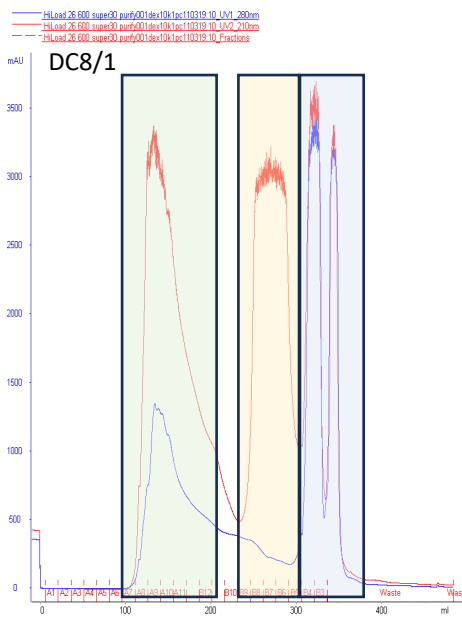
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Table S1. Characteristics of cell lines used in this study.

Cell line	Source	Patient	Pathology	Culture medium	KDM1A mRNA expression (RNAseq)*
MV-4-11	Peripheral blood	Caucasian male, 10yrs	biphenotypic B myelomonocytic leukemia	IMDM with GlutaMAX™, 10% v/v heat-inactivated (HI)-FBS	5.08
THP-1	Peripheral blood	Japanese male, 1yr	acute monocytic leukemia	RPMI 1640 with GlutaMAX™, 10% HI-FBS	5.40
TF-1	Bone marrow	Japanese male, 35yrs	erythroleukemia	RPMI-1640, 10% v/v HI-FBS, 2ng/mL rhGM-CSF	6.11
HK-2	Kidney proximal tubule	Caucasian male, adult	Normal	K-SFM with L-glutamine, epidermal growth factor, bovine pituitary extract	N.D.

* from Barretina *et al.*³⁸ RNAseq TPM gene expression data for just protein coding genes using RSEM. Log2 transformed, using a pseudo-count of 1.
N.D., not determined



Conjugate
Free colistin
Unreacted sulfo-NHS, DMAP

Figure S1. FPLC chromatograms of dextrin-colistin conjugate purification, where the blue line shows absorbance at 280nm and the red line shows absorbance at 210 nm. Fractions (15 mL) are marked along the x-axis. Shaded areas correspond to conjugated colistin, free colistin and unreacted sulfo-NHS and DMAP.

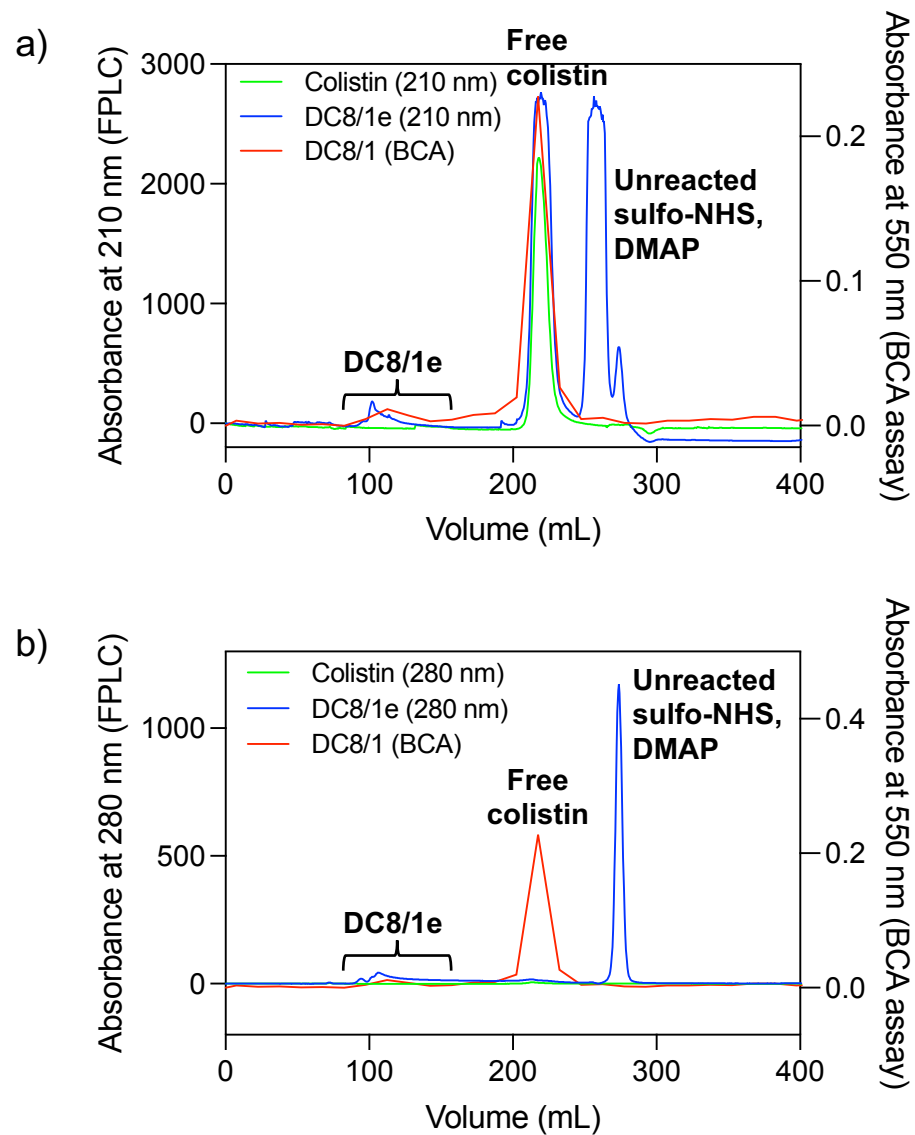
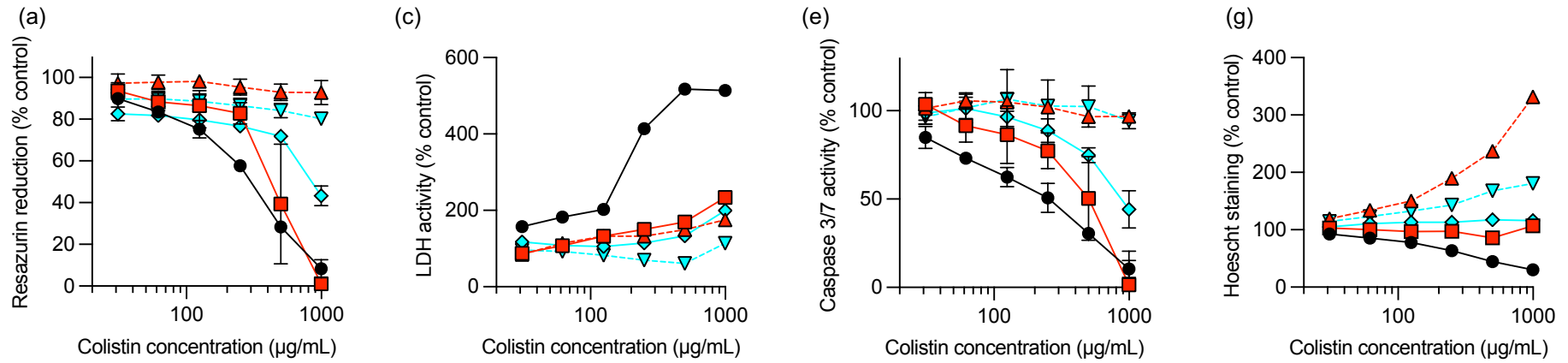


Figure S2. Overlay of FPLC chromatograms of colistin sulfate and DC8/1 purification at a) 210 nm and b) 280 nm, with protein content (by BCA assay) of each 15 mL fraction, confirming conjugation of dextrin and colistin.

HK-2 (kidney) cells (72 h incubation)



TF-1 (leukemia) cells (72 h incubation)

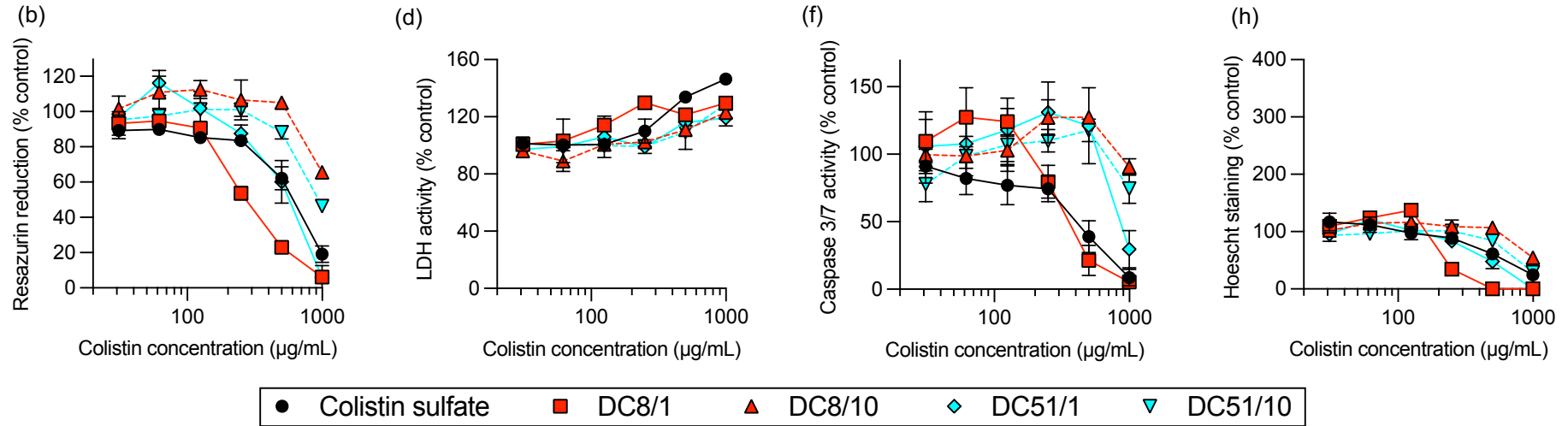


Figure S3. Detection of resazurin reduction (metabolic activity), LDH leakage (cell-membrane integrity, necrosis), caspase 3/7 activity (apoptosis) and Hoechst 33342 staining (DNA content) under multiplex conditions of (a,c,e,g) HK-2 and (b,d,f,h) TF-1 cells incubated for 72 h with colistin sulfate and dextrin-colistin conjugates. Data represent mean (± 1 SD, $n = 3$). Where error bars are invisible, they are within size of data points.

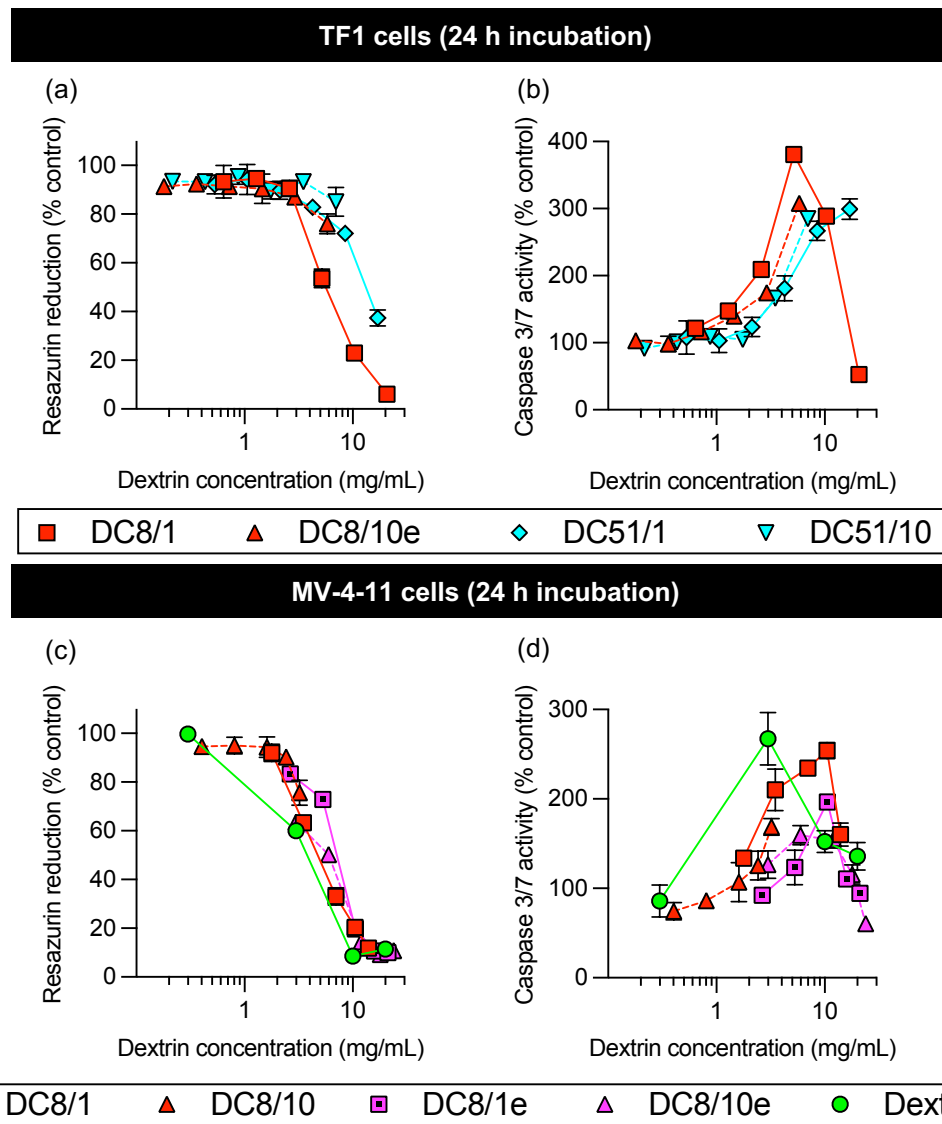


Figure S4. Detection of resazurin reduction (metabolic activity) (panels (a) and (c)) and caspase 3/7 activity (apoptosis) (panels (b) and (d)) under multiplex conditions of MV-4-11 cells incubated for 24 h with colistin sulfate and dextrin-colistin conjugates containing amide or ester linkers. Data represent mean ($\pm 1SD$, $n = 3$). Where error bars are invisible, they are within size of data points.

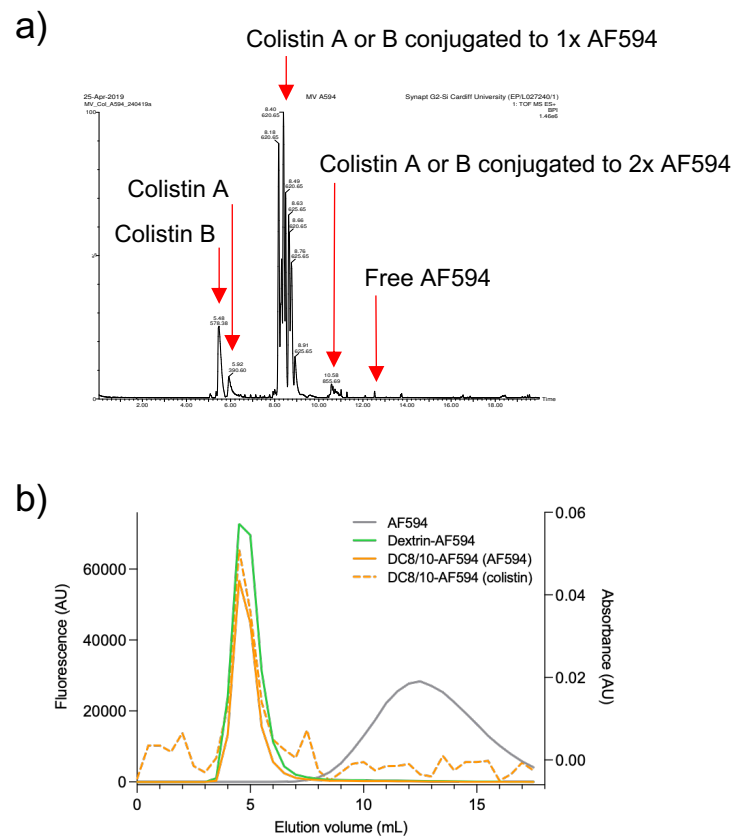


Figure S5. Representative characterization of AF594-labelled conjugates using (a) LC-MS and (b) PD-10 column separation and analysis of fractions using fluorescence (AF594 content) and absorbance (colistin content).

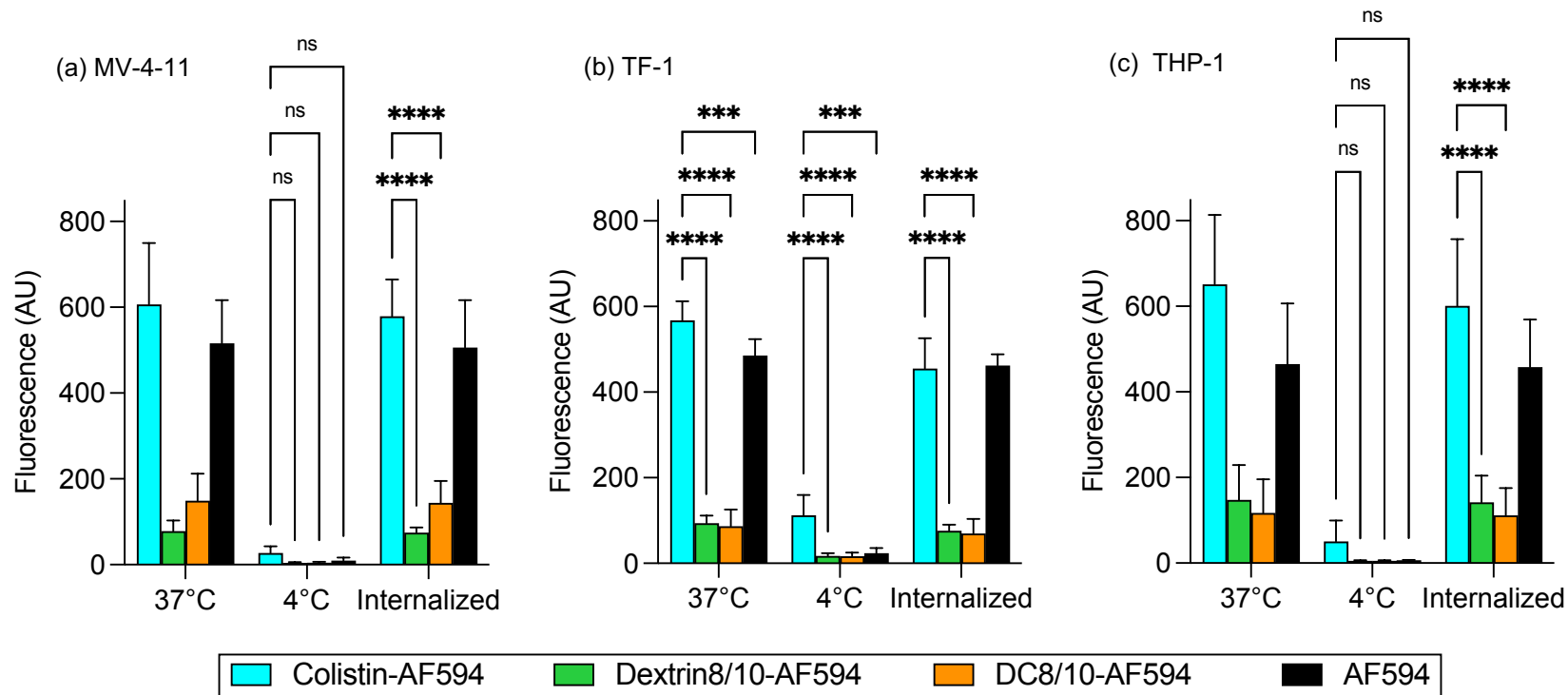


Figure S6. Cell-associated fluorescence at 37°C (total association) and 4°C (external binding) of AF594-labelled colistin, dextrin and dextrin-colistin conjugate (DC8/10) by (a) MV-4-11, (b) TF-1 and (c) THP-1 cells after 1 h incubation at 4 and 37°C (± 1 SD, $n = 5$ to 8), where *** indicates significance $p < 0.001$ and **** indicates significance $p < 0.0001$ compared to colistin-AF594. Where significance is not shown, $p > 0.05$ (*ns*, not significant).