Electronic Supplementary Material (ESI) for Metallomics.

Supplemental Material

The impact of apical and basolateral albumin on intestinal zinc resorption in the Caco-2/HT-29-MTX co-culture model

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Supplemental Methods

Real time PCR

QPCR-analysis for ALP and MUC5AC was performed as described in the main text, using the primer listed in Suppl. Table S1.

Table S1. Oligonucleotide sequences used for real-time PCR

Primer	NCBI Reference Sequence	Sequence fwd 5'-3'	Sequence rev 5'-3'	Ref
ALP	NM_001631.4	CCGCTTTAACCAGTGCAACA	CCCATGAGATGGGTCACAGA	[S1]
MUC5AC	NM_001304359.1	CATCAACGGGACCCTGTACC	ACAGGTCGACTGGTTCTGGT	
β-actin	NG_007992.1	CGCCCCAGGCACCAGGGC	GCTGGGGTGTTGAAGGT	[S2]

Histological staining of mucins

The mucin-secretion of Caco-2/HT-29-MTX co-cultures was investigated by histological staining of anionic mucins, using alcianblue, and neutral mucins with the PAS (periodic acid-Schiff)-staining. Therefore, a total of 120.000 cells was cultivated in 6-well plates for 21d, whereas several ratios of Caco-2 and HT-29-MTX were co-cultivated (Caco-2/HT-29-MTX: 100/0, 90/10, 75/25, 50/50, 0/100). The

protocols for the alcianblue- and PAS-staining were adapted after [S3]. Prior to the staining, cells were washed carefully with PBS and fixed using 3.7% formaldehyde in PBS. For the alcianblue-staining, cells were washed again with PBS, incubated with 3% acetic acid for 3min cells and directly treated with 1% alcianblue 8GX (in 3% acetic acid) for 30min. Subsequently, the staining reagent was carefully removed, cells were washed with H_2O and macroscopic pictures were taken. Prior to the PAS-staining, cells were incubated with 5% periodic acid for 5min, washed carefully with PBS and incubated with Schiff-reagents for 15min. The Schiff reagent was generated following the method from Graumann [S4] using 5% pararosanilin. After successful incubation, Schiff-reagent was removed from the cells and pictures were taken instantly.

Alkaline phosphatase activity

The effect of cellular ratio on the activity of alkaline phosphatase (ALP) in co-cultures of Caco-2 and HT-29-MTX was investigated, examining several Caco-2/HT-29-MTX ratios: 100/0, 90/10, 75/25, 50/50 and 0/100. Therefore, two days prior to the HT29-MTX cells, Caco-2 cells were transferred to 96-well plates (a total of 5,000 cells) and ALP-activity after 21d of cultivation was analyzed as described [S1].

Supplemental Figures

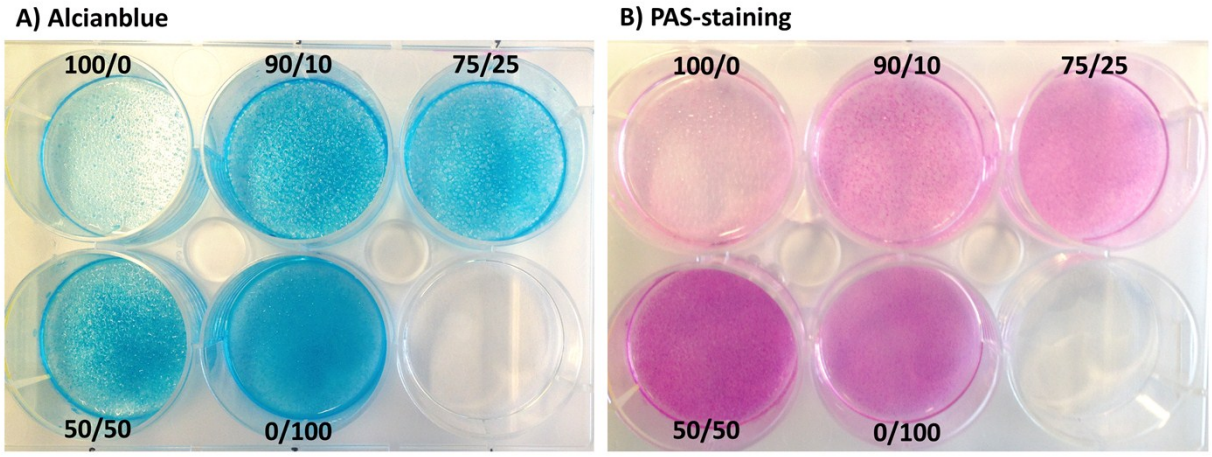

Figure SF1: Histological analysis of secreted mucins by Caco-2/HT-29-MTX co-cultures. Co-cultures of various ratios of Caco-2 and HT-29-MTX cells were cultured for 21d and mucin secretion was investigated using alcianblue- (A) and PAS-staining (B).

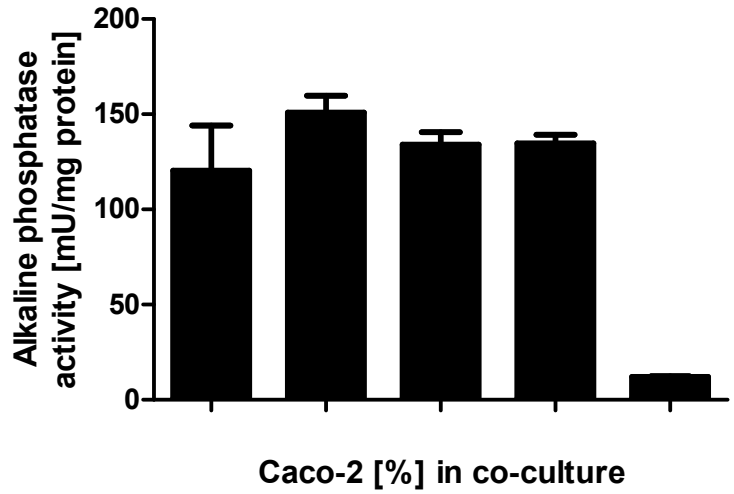

Figure SF2: Activity of Alkaline phosphatase (ALP) in Caco-2 and HT-29-MTX co-cultures. Shown is the ALP-activity in Caco-2/HT-29-MTX co-cultures with different cellular ratios depicted as the relative amount of Caco-2 cells. Enzyme activity was measured after 21d of cultivation using the ALP-assay and is displayed relative to cellular protein. Data are displayed as means + SD of three independent experiments.

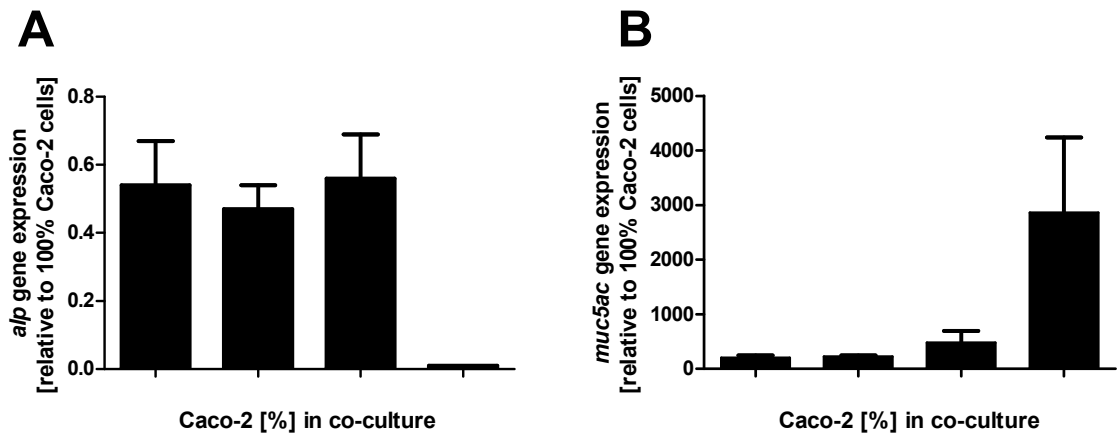

Figure SF3: Expression of characteristic genes for Caco-2 and HT-29-MTX in the co-cultures. Gene expression of differentiation marker *alp* [S1] and *muc5ac*, which is one of the characteristic mucins secreted by HT-29-MTX [S5], was analyzed in Caco-2/HT-29-MTX co-cultures after 21d of cultivation using qPCR (A) *Alp*-expression is shown relative to Caco-2 monocultures. (B) *Muc5ac*-expression is depicted relative to Caco-2 monocultures. Data are shown as means + SD of three replicates.

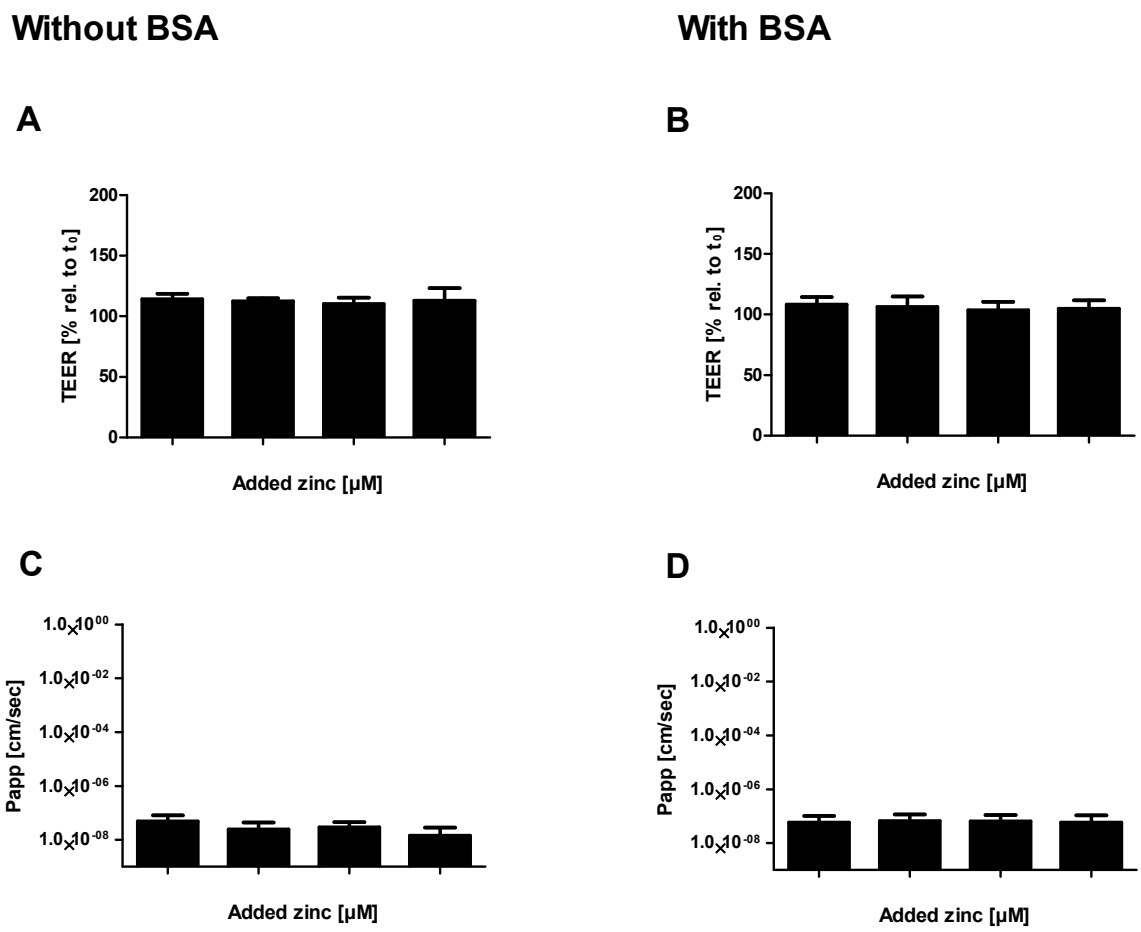

Figure SF4: Integrity of Caco-2/HT-29-MTX cell monolayers used for the transport studies depicted in Fig. 6 measured as TEER (A, C) and paracellular permeability (C, D). Shown is the TEER of cell-monolayers after the transport-experiment relative to TEER measured before incubation with zinc and 0mg/mL (A) or 30mg/mL albumin, respectively. The permeability of the cell monolayer during the transport assay without (C) or with albumin (D) is depicted as the apparent permeability (P_{app}) of a 20kDa FITC-Dextran. Data are shown as means + SD of three replicates.

	WST		MTT		NRU		SRB	
	0% FCS	10% FCS	0% FCS	10% FCS	0% FCS	10% FCS	0% FCS	10% FCS
Best-fit values								
Bottom	21,97	33,95	9,525	-42,44	0.0	0.0	3,663	1,599
Top	97,04	87,61	109,7	104,1	111,6	105,1	108,6	103,3
Hill slope	-2,315	-4,216	2,473	2,881	-4,163	-4,165	-5,034	-5,012
95% Confidence Interval of LC_{50}	162.5 to 567.7	315.4 to 761.2	247.4 to 356.3	214.1 to 2694	397.7 to 486.8	648.2 to 741.6	239.1 to 306.4	451.2 to 506.9
Goodness of Fit								
Degree of Freedom	17	17	17	17	18	18	17	17
R^2	0.7939	0.6740	0.9664	0.9453	0.9601	0.9761	0.9719	0.9860
Absolute Sum of Squares	4,033	3,543	1,094	1,294	1,294	456.2	1,219	402.1
Standard deviation of residuals	15.40	14.44	8.023	8.723	8.479	5.034	8.467	4.863

Table ST2: Parameters of the non-linear regression analysis applied in the zinc cytotoxicity study in Fig. 3. Shown are parameters of the applied non-linear regression using a sigmoidal dose-response curve with variable slope as a function of the logarithm of zinc concentration. Data were obtained in three independent experiments and analyzed with GraphPad Prism software version 5.01 (GraphPad Software Inc., CA, USA).

Supplemental References

[S1] M. Maares, C. Keil, S. Thomsen, D. Günzel, B. Wiesner, H. Haase, Characterization of Caco-2 cells stably expressing the protein-based zinc probe eCalwy-5 as a model system for investigating intestinal zinc transport, Journal of Trace Elements in Medicine and Biology (2018).
[S2] K. Wolf, C. Schulz, G.A.J. Riegger, M. Pfeifer, Tumour necrosis factor-α induced CD70 and interleukin-7R mRNA expression in BEAS-2B cells, European Respiratory Journal 20(2) (2002) 369-375.
[S3] H. Denk, H. Künzele, H. Plenk, J. Rüschoff, W. Seller, Romeis Mikroskopische Technik. 17., neubearbeitete Auflage, Urban und Schwarzenberg, München-Wien. Baltimore (1989) 439-50.
[S4] W. Graumann, Zur Standardisierung des Schiffschen Reagens, S HIRZEL VERLAG Stuttgart, 1953, pp. 225-226.
[S5] G. Nollevaux, C. Deville, B. El Moualij, W. Zorzi, P. Deloyer, Y.J. Schneider, O. Peulen, G. Dandrifosse, Development of a serum-free co-culture of human intestinal epithelium cell-lines (Caco-2/HT29-5M21), BMC cell biology 7 (2006) 20.