Supplementary information:

Switching the polarity of mouse enteroids affects the epithelial interplay with prenylated phenolics from licorice (*Glycyrrhiza*) roots

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A. Optimization of cell cytotoxicity assay

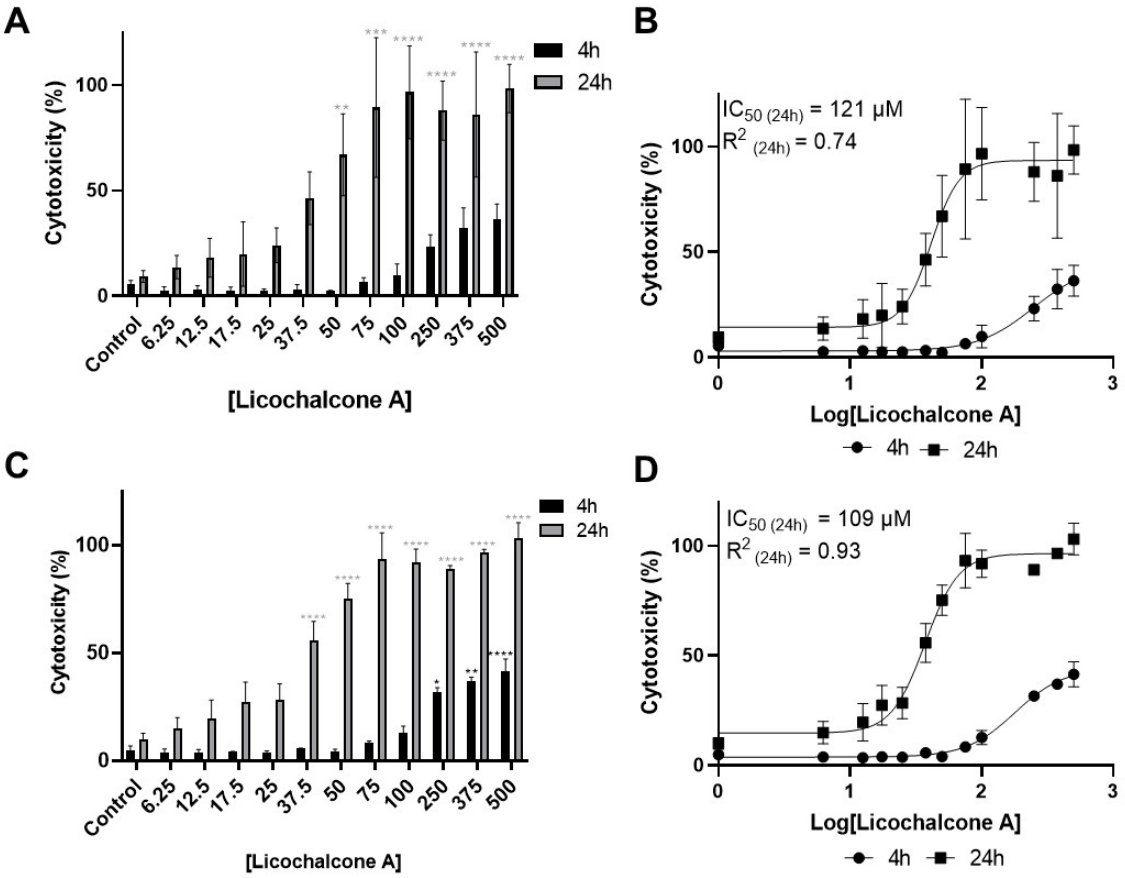

Figure A1. **Optimization of the cell cytotoxicity assay in apical-in enteroids with licochalcone A as an example**. In panels (A) and (B) cytotoxicity after 4 h and 24 h incubation and half-maximal cytotoxicity concentrations (IC_{50}) after 24 h incubation are shown without each enteroid dome treated as its own positive control, and in panels (C) and (D) with each enteroid dome treated as its own positive control. Data are expressed as the mean ± SEM of three biological replicates, measured in duplicate. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

B. Influence of passage number on glabridin cytotoxicity in apical-in mouse enteroids

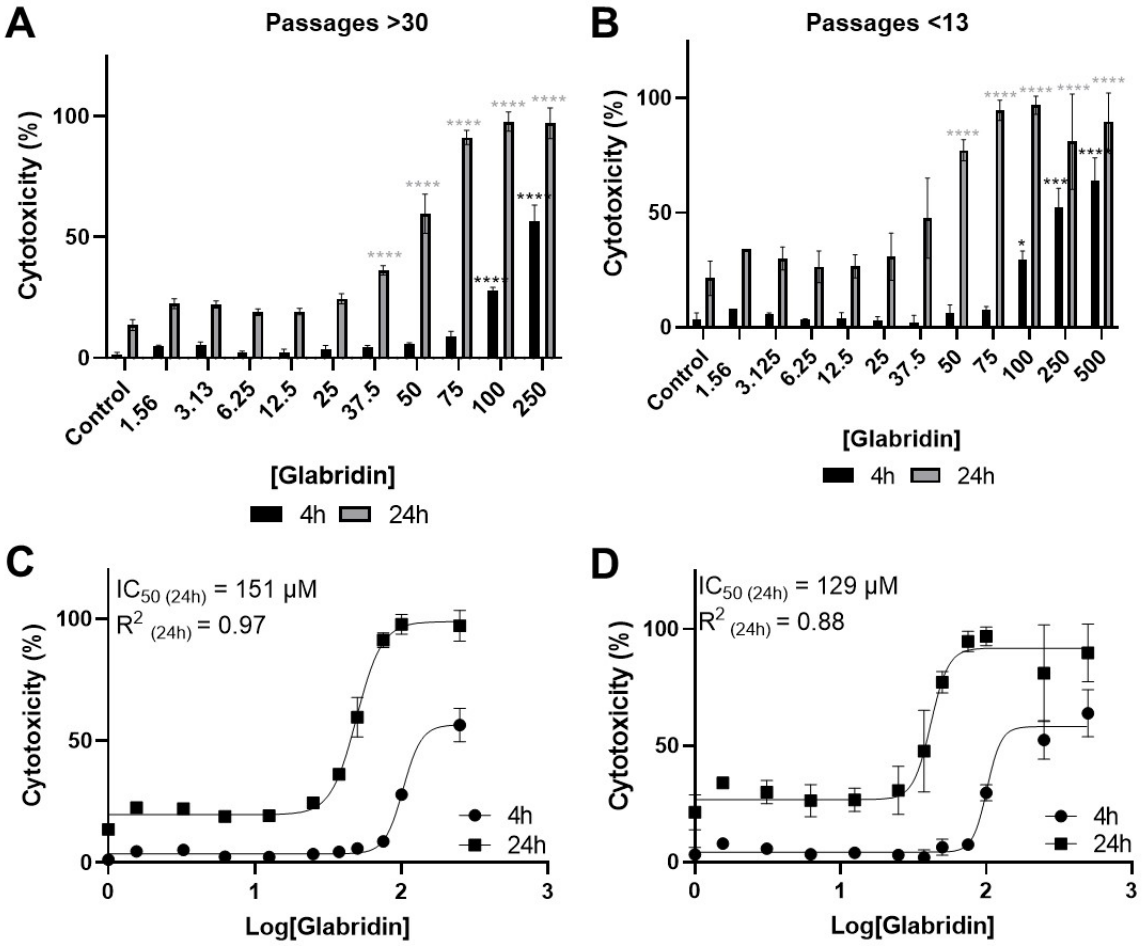

Figure B2. **Influence of passage number of mouse apical-in enteroids on glabridin cytotoxicity**. Panels (A) and (B) show glabridin cytotoxicity after 4 h and 24 h incubation in high (> 30) and low (< 13) passages, respectively, and panels (C) and (D) show half-maximal cytotoxicity concentrations (IC_{50}) of glabridin after 24 h incubation in high (> 30) and low passages (< 13), respectively. Data are expressed as the mean ± SEM of three biological replicates, measured in duplicate. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

C. Influence of passage number and medium on gene expression of epithelial cell markers in apical-out and apical-in enteroids

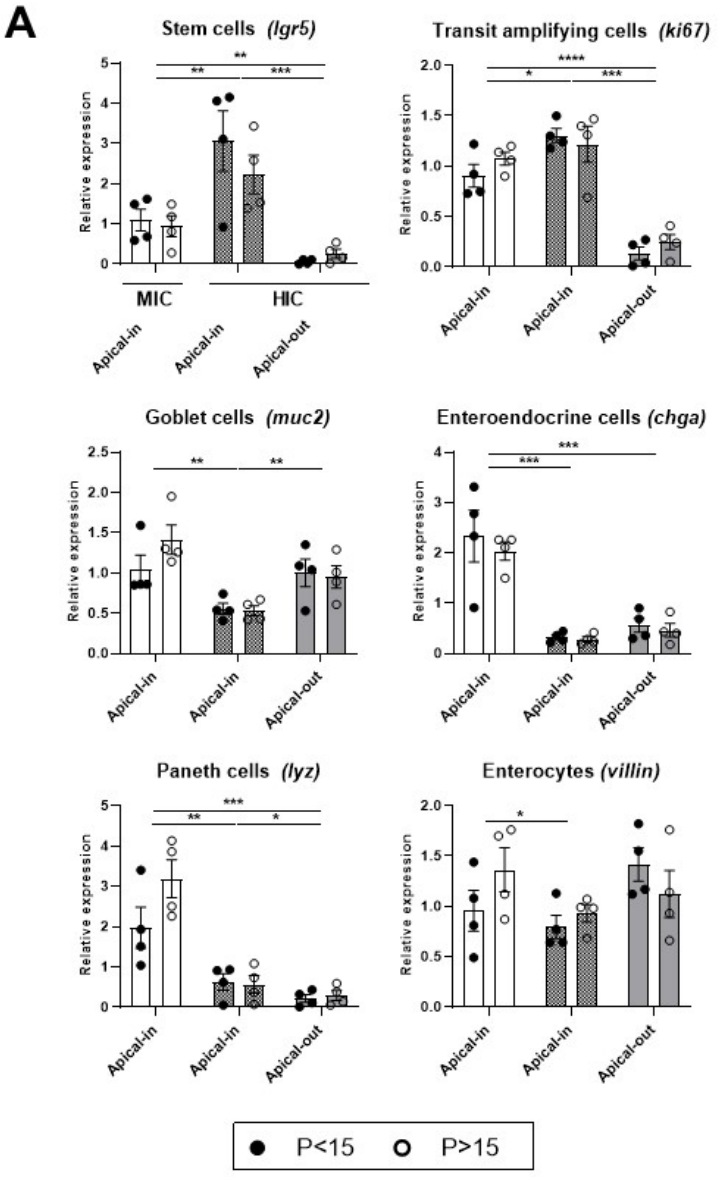

Figure C3. Gene expression of epithelial cell markers in apical-in and apical-out enteroids. Panel (A) shows qRT-PCR gene expression analysis of epithelial cell markers for stem cells (*lgr5*), transit amplifying cells (*ki67*), goblet cells (*muc2*), enteroendocrine cells (*chga*), Paneth cells (*lyz*), and enterocytes (*villin*) between low (P < 15, black dots) and high passages (P > 15, white dots) in apical-in enteroids grown in mouse (mIC) and human (hIC) growth medium, respectively, and apical-out enteroids (grown in hIC). For the figures where the medium legend is not shown (*e.g.* mIC and hIC), these are similarly to those in the upper left figure. Panel (B) shows the ratio between gene expression of epithelial markers (*lgr5*, *ki67*, *muc2*, *chga*, *lyz*) compared to *villin* gene expression in (1) jejunal scrapings from C57BL/6 mice[1] and apical-in enteroids grown in (2) mIC and (3) hIC, respectively. Data are expressed as the mean ± SEM of four biological replicates, measured in duplicate (pooled gene expression analysis of apical-in and apical-out organoids is shown in **Figure 5A**). *In vivo* data from (B) were measured in three biological replicates. For the statistical analyses in (A) the pooled gene expression data was used ($n = 8$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

D. Intrinsic LDH release in apical-out mouse enteroids

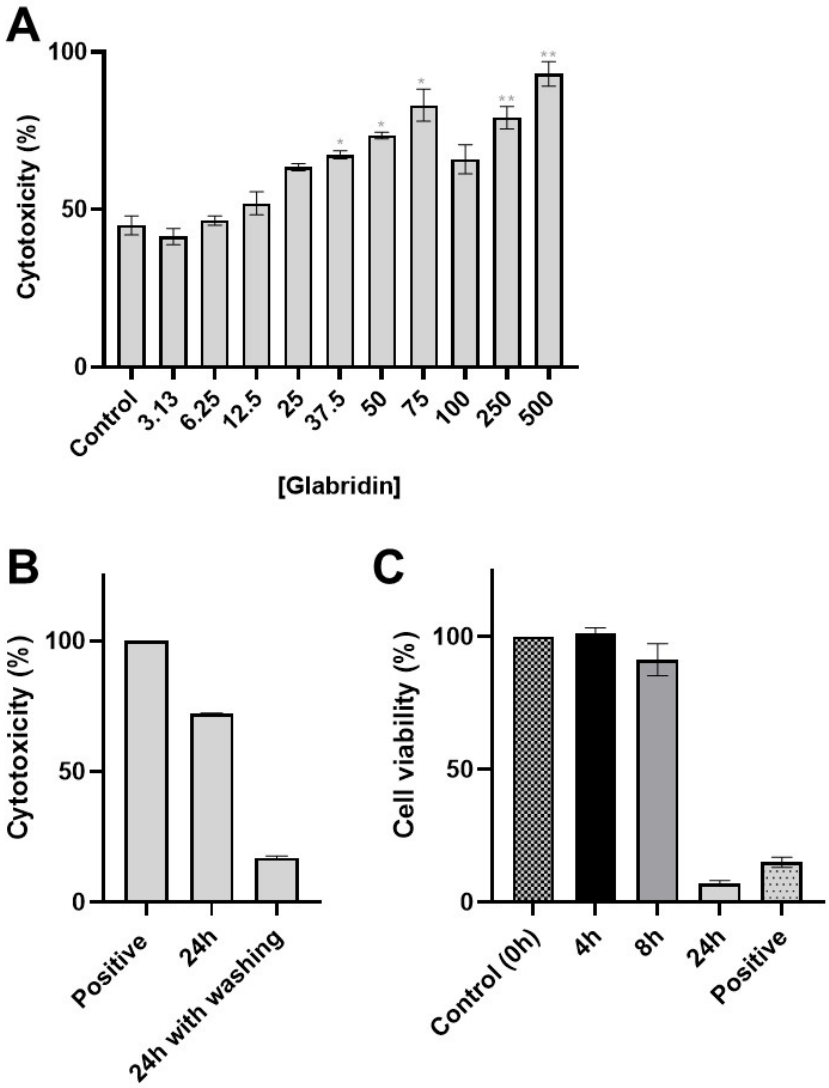

Figure D4. Cytotoxicity and cell viability of mouse apical-out enteroids. (A) Enteroid cytotoxicity after 24 h glabridin incubation in µg mL^{-1}, (B) apical-out enteroid cell cytotoxicity after 24 h without prenylated phenolics incubation, and (C) cell viability at different time points without prenylated phenolics incubation. Data in (A) are expressed as the mean ± SEM of three biological replicates, measured in duplicate and in (B) and (C) are expressed as the mean ± SEM of one independent biological replicate, measured in duplicate. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

E. (Tentative) identification of glabridin, licochalcone A, and glycycoumarin and metabolites by RP-UHPLC-PDA-ESI-IT-MS^n

Table E1. (Tentative) identification of glabridin, licochalcone A, and glycycoumarin and metabolites. (Tentative) identification of metabolites in apical-in and apical-out jejunal enteroids and the spectroscopic and spectrometric data obtained in negative ionization (NI) and positive ionization (PI) mode with RP-UHPLC-PDA-ESI-IT-MS^n.

Identified compound	Rt[a] (min)	UV-$_{max}$[b] (nm)	$[M-H]^-$ (*m/z*)	MS^2 NI mode *m/z* (R.A.)[c]	$[M+H]^+$ (*m/z*)	MS^2 PI mode *m/z* (R.A.)	Ref.
Glabridin	28.87	278	323	109 (23), 121 (38), 135 (100), 147 (31), 175 (29), 187 (16), 201 (82), 213 (43), 253 (13), 279 (24), 305 (24), 308 (18)	325	123 (36), 189 (100), 203 (24)	
Glabridin glucuronide	24.50	278	499	175 (66), 323 (100), 431 (19), 437 (24), 481 (44)	501	189 (13), 325 (98), 367 (15), 465 (22), 483 (100)	2
Glabridin sulfate	28.38	n.d.	403	323 (100)	405	n.d.	2
Licochalcone A	26.84, 27.67, 28.17	314, **378**	337	243 (55), 268 (44), 305 (100), 306 (14), 307 (15)	339	121 (26), 219 (11), 245 (21), 271 (32), 297 (100)	
Licochalcone A glucuronide	20.15, 20.30, 22.57	362, 370, 382	513	337 (100)	515	339 (100)	
Saturated licochalcone A glucuronide	21.24, 22.99	280	515	175 (16), 339 (100), 497 (26)	517	205 (100), 341 (10)	
Saturated licochalcone A	28.16	n.d.	339	135 (100), 270 (31)	341	205 (100), 273 (10)	
Licochalcone A sulfate	25.54	n.d.	417	337 (100)	419	n.d.	
Glycycoumarin	25.85	350	367	284 (11), 297 (38), 309 (100), 352 (16)	369	285 (48), 313 (100), 327 (15), 341 (11)	
Glycycoumarin glucuronide	18.85	346	543	367 (100)	545	369 (100)	3
Glycycoumarin sulfate	24.23	n.d.	447	367 (100)	449	n.d.	
Hydrated glycycoumarin	25.38	n.d.	n.d.	n.d.	387	119 (33), 127 (25), 145 (10), 233 (27), 245 (15), 263 (56), 265 (29), 281 (100), 369 (31)	3
Glycyrol	28.09	362	365	294 (13), 295 (49), 307 (100), 349 (25), 350 (21)	367	283 (14), 311 (100), 339 (26)	3
Glycycoumarin dimer	32.06	362	733	309 (21), 365 (33), 367 (100), 715 (65)	735	311 (10), 369 (78), 395 (40), 667 (10), 679 (100)	
Unknown	31.24	n.d.	721	527 (100)	723	n.d.	

[a] Retention time in MS; [b] Bold numbers indicate the main UV absorbance peak; [c] R.A. = relative abundance.

F. Biotransformation of glabridin, licochalcone A, and glycycoumarin in mouse jejunal apical-in enteroids

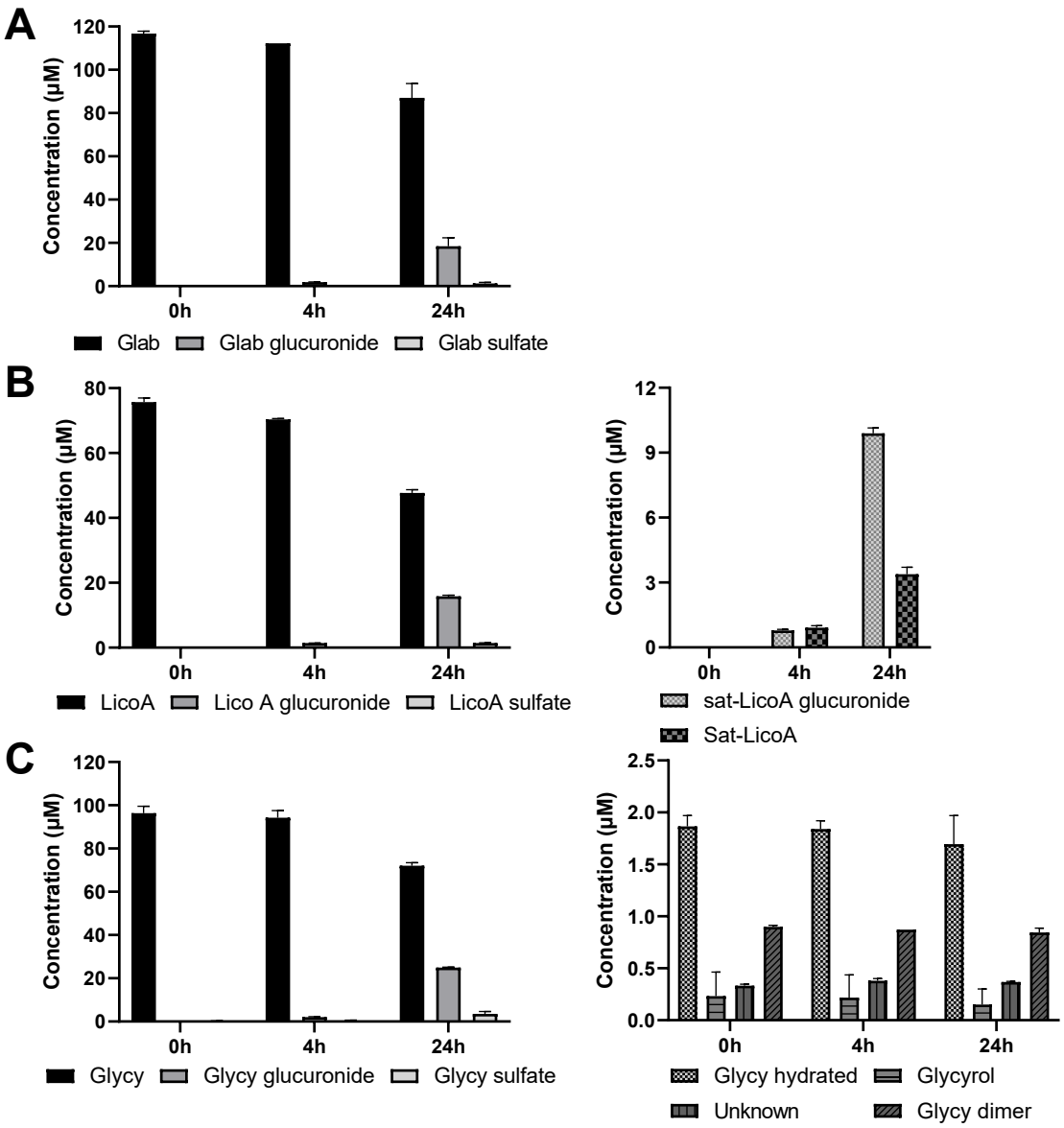

Figure F5. Biotransformation of glabridin (glab, A), licochalcone A (licoA, B), and glycycoumarin (glycy, C) in apical-in mouse jejunal enteroids. Sat-LicoA = tentatively identified as saturated licochalcone A, glycy hydrated = tentatively identified as hydroxylation at prenyl group [3]. Identifications are shown in **Table E1**. Data are expressed as the mean ± SEM of two biological replicates, measured in duplicate.

References

1. N. van der Wielen, M. van Avesaat, N. J. de Wit, J. T. Vogels, F. Troost, A. Masclee, S. J. Koopmans, J. van der Meulen, M. V. Boekschoten, M. Muller, H. F. Hendriks, R. F. Witkamp and J. Meijerink, Cross-species comparison of genes related to nutrient sensing mechanisms expressed along the intestine, *PLoS One*, 2014, **9**, e107531.
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3. Q. Wang, X. Qiao, C. F. Liu, S. Ji, L. M. Feng, Y. Qian, D. A. Guo and M. Ye, Metabolites identification of glycycoumarin, a major bioactive coumarin from licorice in rats, *J. Pharm. Biomed. Anal.*, 2014, **98**, 287-295.