Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTAL INFORMATION (SI)

TransFEr: a new device to measure the transfer of volatile and hydrophobic organic chemicals across an *in vitro* intestinal fish cell barrier

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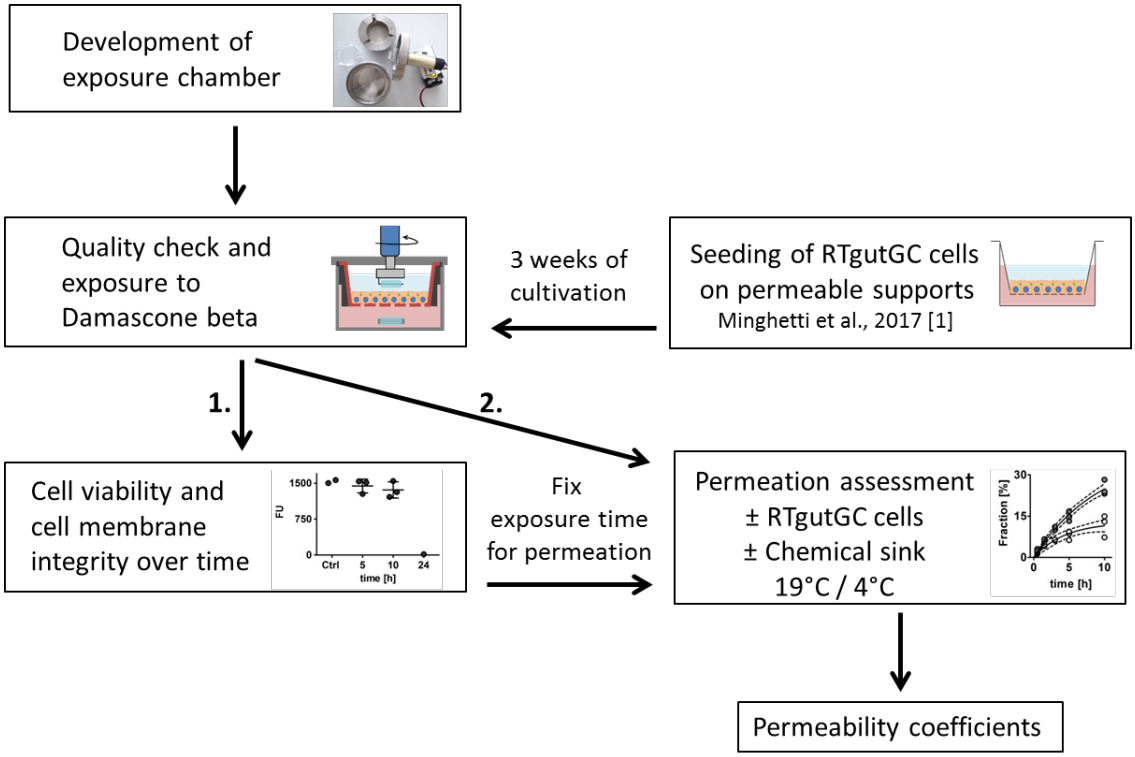


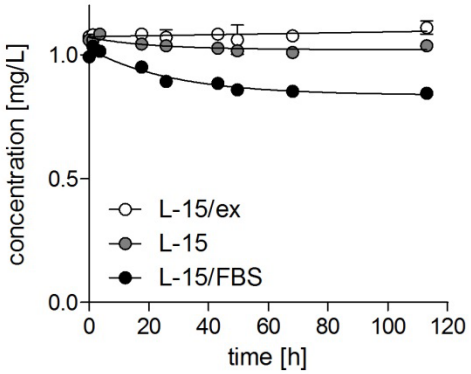
SI Figure 1 Workflow scheme

For the permeation assessment, the TransFEr exposure chamber was designed to maintain stable exposure concentrations for hydrophobic and volatile chemicals. The intestinal cell-barrier model was established on permeable supports as described in Minghetti et al.[1] and used after 3 weeks of cultivation. After the established epithelium was placed in the TransFEr exposure chamber, maintenance of cell viability and cell membrane integrity were verified over time of exposure to Damascone beta (1 mg/L) (step 1). In a second step (2.), the transfer of Damascone beta was measured over the permeable supports without (cell-free) and with RTgutGC cells, in the presence and absence of a chemical sink and at 4°C. Damascone beta concentration was quantified over time in all compartments in order to establish a mass balance. Transfer coefficients were calculated based on the Damascone beta mass quantified in the media of the apical and the basolateral compartment over time.

SI Table 1 Details on chemical quantification method of different sample extracts

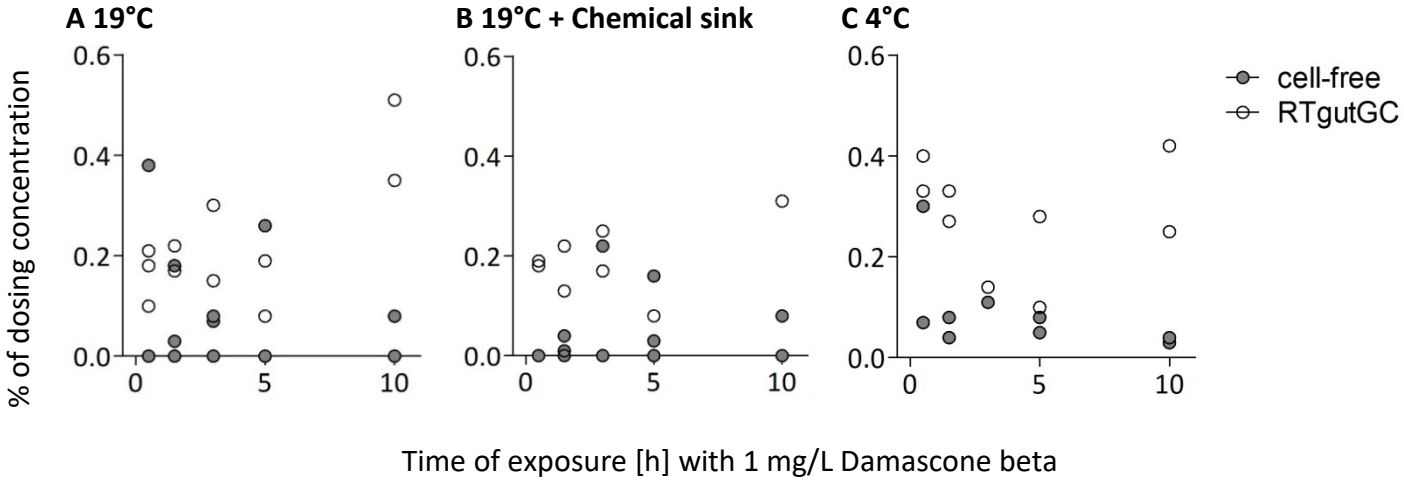
All quantifications were done by external calibration with an internal standard. Samples were thawed at ambient temperature during 2 hours, vigorously shaken and filtered through 0.2 µm regenerated cellulose filters (OlimPeak TR200430, diam 13mm, Teknokroma, Spain).

Sample extract	Analytical instrument	Method and instrumental details
Apical and basolateral compartment, cell fraction	LC-MS/MS	• 2 µl injection volume • Two binary pumps (LC-20AD): operation in gradient mode (total flow = 0.5 mL/min, mobile phase A = H2O + 0.1 % formic acid, mobile phase B = Methanol + 0.1 % formic acid, gradient program = 50 % B up to 95 % B in 4 min, maintained 0.5 min, then decreased to 50 % B in 0.2 min, maintained 1.3 min, total run time = 6 min) • Autosampler, SIL-30AC (temperature 15 °C) • Column oven CTO-20AC (temperature 30 °C) with Kinetex C18 2.6 µ 100 A° 50 mm x 2.1 mm column (ref: 00B-4462-AN, Phenomenex, USA) and diverter valve to limit the injection of interfering compounds into the ESI source of the QTrap (temperature= 550 °C, voltage= 3000 V, declustering potential= 60 V) • Qtrap: operation in MRM mode (Damascone beta transitions = 193.2/137.0 and 193.2/69.0, ISTD (Tonalide) transition = 259.3/175.1)
Plastic, membrane	GC-MS	• 1 µl injection volume • Carrier gas: purified helium • Split/splitless injector (liner Agilent, split 1/20, initial flow = 1 mL/min) • Operation in gradient mode (gradient from 60 °C to 280 °C at 20 °C/min, total run time = 12min, column DB-XLB 30 m x 0.25 mm x 0.25 µm, ref122-0132). • Operation in SCAN and SIM mode (Damascone beta ions = 177.1/192.0, Dibromobenzene (DBB) ions = 233.8 transfer line temperature = 280°C).
PDMS from stir bars	GC-FID	• Carrier gas: purified helium • Split/splitless injector (liner Agilent ref210-4022-5, split 1/20, initial flow = 1 mL/min) • Operation in gradient mode (70°C maintained 0.5 min, then gradient at 30°C/min until 220°C, total run time = 6.5min, column DB-XLB 10m x 0.18mm x 0.18um, ref121-1222



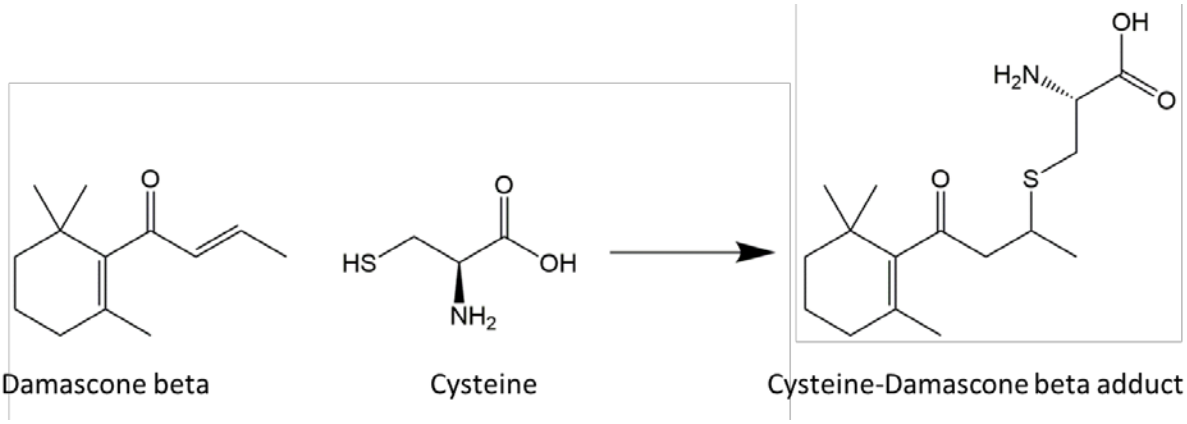
SI Figure 2 Stability of 1 mg/L Damascone beta in different media

The stability of 1 mg/L Damascone beta was assessed in exposure medium L-15/ex, Leibovitz medium L-15 and L-15 supplemented with 5 % FBS (L-15/FBS) over the course of 5 days.



SI Figure 3 Percentage of Damascone beta mass in the cell fraction over time.

Percentage of Damascone beta mass in the cell fraction in experiments conducted at 19°C (A), at 19°C in the presence of a chemical sink (B) and at 4°C (C) in the presence and absence of RTgutGC cells. In the absence of RTgutGC cells, the extraction procedure was conducted similar as if cells would have been present and quantified for the Damascone beta mass. The analysed mass for cell-free (grey-filled circles) and permeable supports seeded with RTgutGC (white circles) was normalized to the introduced mass for each experiment. All data are shown as individual replicates with four biological replicates for experiments at 19°C, three biological replicates at 19°C in the presence of a chemical sink and two biological replicates at 4°C.



67 **SI Figure 4 Potential reaction of Damascone beta with the thiol-groups present in the amino acid cysteine**

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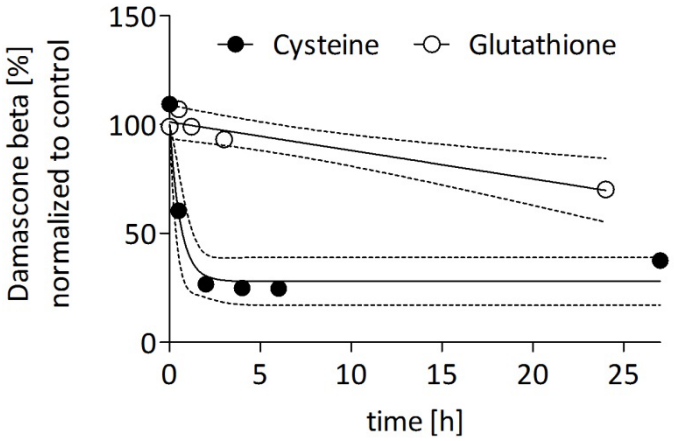


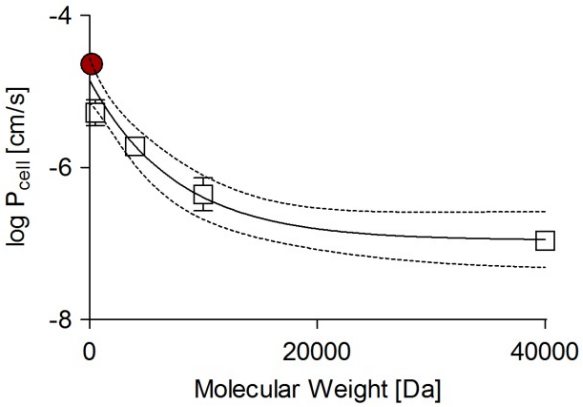
SI Figure 5 Change of Damascone beta mass in a mixture with L-cysteine or glutathione

Simplified excess experiments were conducted by mixing 5 ml of a 260 µM Damascone beta solution with 5 ml of a 1310 µM L-cysteine (black circles) or 1100 µM glutathione (white circles) solution. This represents a Damascone beta to cysteine ratio of about 1:5, and a Damascone beta to glutathione ratio of about 1:4. To work significantly above the limit of quantification, the Damascone beta concentration introduced in the excess experiments was about 50 times higher than in the transfer experiments. The solutions were incubated at 19°C and after different time points, samples were taken and analysed for the Damascone beta mass. As control, Damascone beta solution without the addition of cysteine and glutathione was treated identically. The results presented in the graph are normalized to the control samples. Solid lines represent a non-linear fit for cysteine and linear fit for glutathione and are plotted along with 95 % confidence intervals.

SI Table 2 Permeability coefficients of Damascone beta

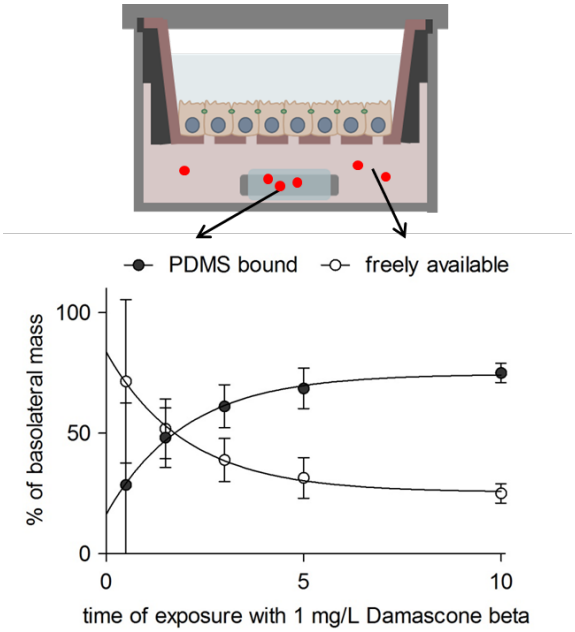
The total permeability P_{total} [cm/s] of Damascone beta across cell layer and the membrane of the permeable support without cells with 95 % confidence intervals (CI) was calculated by non-linear fitting (Eq. 3) and the permeability coefficient across the cell was calculated according to Equation 2.

Temperature [°C]	**Inserts + / - cells**	**P_{total} x 10^{-6} [cm/s]** (95% CI)	**P_{cells} x 10^{-6} [cm/s]** (95% CI)
19°C	Cell-free	9.38 (8.58 – 10.2)	0.25 (0.16 – 0.34)
	RTgutGC	6.66 (6.09 – 7.24)	
19°C + Chemical sink	Cell-free	11.65 (11.03 – 12.29)	0.61 (0.34 – 0.88)
	RTgutGC	9.64 (8.47 – 10.9)	
4°C	Cell-free	7.9 (6.15 – 9.81)	0.15
	RTgutGC	5.19 (4.31 – 6.11)	



SI Figure 6 Transfer (permeability) coefficients across the cells for molecules with different sizes.

The logarithm of the transfer coefficients for Damascone beta (red circle) and four fluorescent integrity markers (squares) are plotted according to the molecular weight of the molecules: Damascone beta (192 Da), Lucifer Yellow (520 Da), Dextran-FD4 (4 kDa), Rhodamine-Dextran (10 kDa) and Dextran-FD40 (40 kDa). The data are shown as mean ± SD for three biological replicates and the fitted line against the mean (solid line) with 95 % confidence intervals (dashed lines). For better comparability, all coefficients were calculated using non-linear fitting. Transfer of Damascone beta, Lucifer Yellow and Rhodamine-Dextran was measured in 6-well inserts as described in the experimental section. Dextran-FD4 and -FD40 were measured in 12-well inserts and are taken from Minghetti et al.[1] In previous experiments carried out in our laboratory, no difference in permeation coefficients obtained from experiments in 12- or 6-well inserts was detected (data not shown).



SI Figure 7 Adding a chemical sink to the basolateral compartment

The fraction of basolateral Damascone beta mass which is absorbed to the PDMS of the stir bar (dark filled circles) and which is freely available in the basolateral solution (white circles) was calculated in percentage of total Damascone beta mass measured in the basolateral compartment. The results from experiments in the presence and absence of RTgutGC cells were used and the data are shown as mean ± SD of 6 biological replicates and the fitted line against the mean.

SI References

1. Minghetti, M.; Drieschner, C.; Bramaz, N.; Schug, H.; Schirmer, K., A fish intestinal epithelial barrier model established from the rainbow trout (Oncorhynchus mykiss) cell line, RTgutGC. *Cell Biology and Toxicology* **2017**, 1-17.