

Supplement

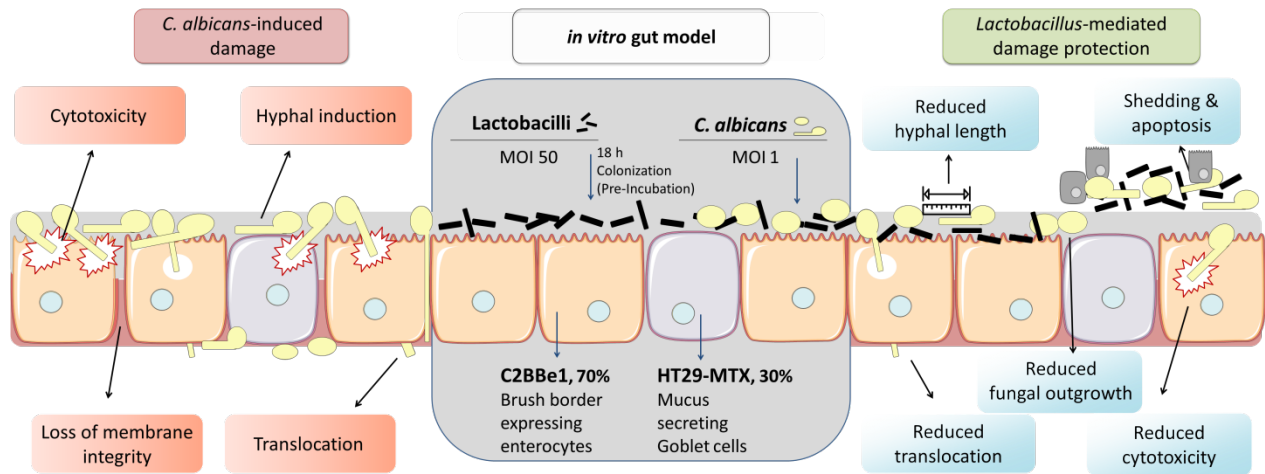


Figure S1: graphical abstract to visualize the written abstract.

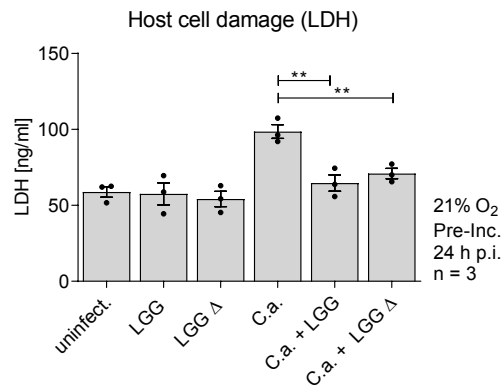


Figure S2: The protective effect of *L. rhamnosus* is independent of exopolysaccharides. LDH release at 24 h post-infection of IECs left untreated, colonized either with the *L. rhamnosus* GG mutant ((LGG Δ) lacking exopolysaccharides), or the corresponding WT strain (LGG) and infected with *C. albicans* (MOI 1) or not. The results were normalized to *C. albicans* single infection. Results shown are the mean values \pm SEM, * p < 0.05, *** p < 0.005.

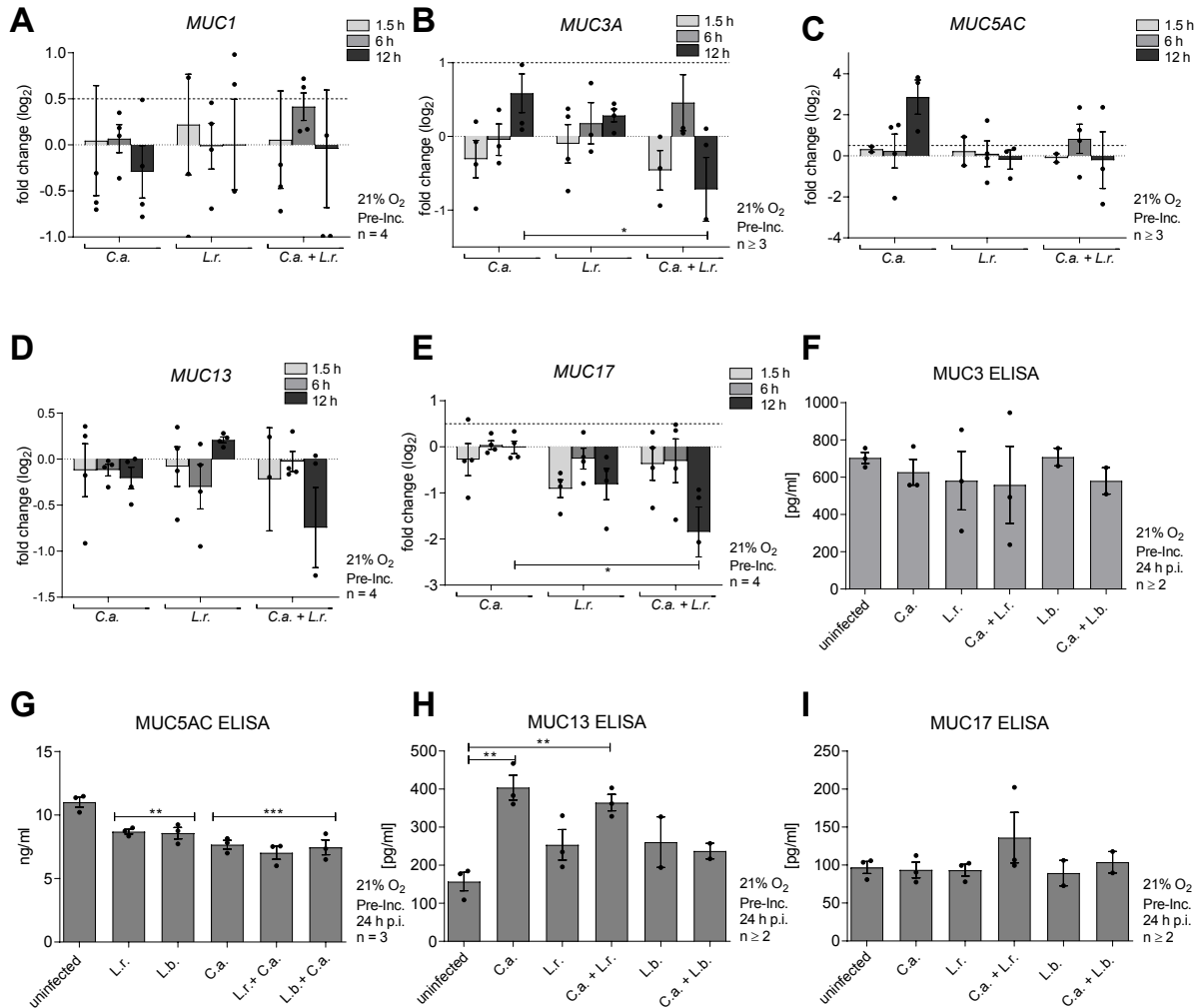


Figure S3: The protective effect of lactobacilli does not correlate with altered mucus secretion. The relative mRNA expression of (A) *MUC1*, (B) *MUC3A*, (C) *MUC5AC*, (D) *MUC13*, and (E) *MUC17* in IECs either left untreated or colonized with *L. rhamnosus* (MOI 50) and infected or not with *C. albicans* (MOI 1) for 1.5, 6, or 12 h. Expression levels were normalized to the reference genes *ACT1* and *GAPDH*. Results shown are the mean values \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. (F-I) Protein concentrations of (F) MUC3, (G) MUC5AC, (H) MUC13, and (I) MUC17 quantified in whole cell lysate of IECs left untreated or colonized with lactobacilli (*L. rhamnosus* or *L. brevis*; MOI 50) and infected or not with *C. albicans* for 24 h. Results shown are the mean values \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ compared to uninfected host cells.

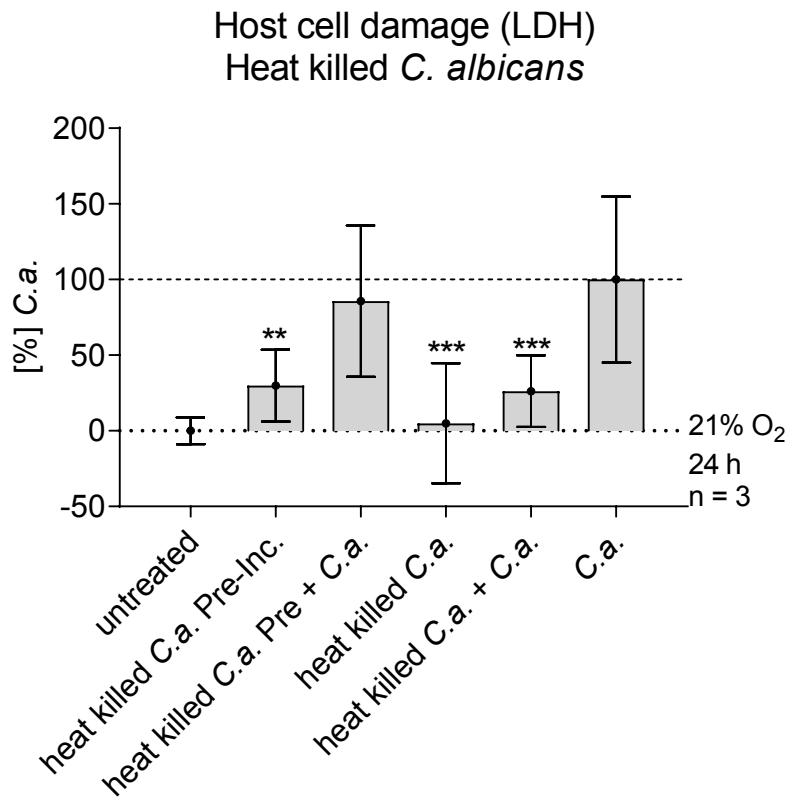


Figure S4: Protective effect through high biomass of heat-killed *C. albicans*. LDH release at 24 h post-infection of IECs colonized or not with heat-killed *C. albicans* (MOI 100) and infected with *C. albicans* (MOI 1) or not. The results were normalized to *C. albicans* single infection.

Experiment	Format	initial No of IECs/well (volume)	CFU C.a./well (volume)	CFU lactobacilli/well [excess compared to C.a.] (volume)	total medium	incubation period
Cytotoxicity (LDH assay)	96 well plate	2×10 ⁴ (50 µl)	2×10 ⁴ (50 µl)	1×10 ⁵ [5x] (50 µl) 1×10 ⁶ [50x] (50 µl) 5×10 ⁶ [250x] (50 µl)	150 µl	24 or 48 h
Adhesion of <i>C. albicans</i>	24 well plate coverslips	1×10 ⁵ (125 µl)	5×10 ⁴ (125 µl)	2.5×10 ⁶ [50x] (125 µl)	375 µl	1 h
Hypheal length	24 well plate coverslips	1×10 ⁵ (125 µl)	5×10 ⁴ (125 µl)	2.5×10 ⁶ [50x] (125 µl)	375 µl	4 h
Translocation, TEER	transwell inserts	2×10 ⁴ (50 µl)	2×10 ⁴ (50 µl)	1×10 ⁶ [50x] (150 µl)	250 µl	24 h
cell-free supernatants	96 well plate	2×10 ⁴ (0 µl)	2×10 ⁴ (10 µl)	- undiluted SN 140 µl	150 µl	24 h
killed lactobacilli (pre-incubation)	96 well plate	2×10 ⁴ (50 µl)	2×10 ⁴ (50 µl)	1×10 ⁶ [50x] (50 µl)	150 µl	24 h
killed lactobacilli (simult. infection), displacement	96 well plate	2×10 ⁴ (50 µl)	2×10 ⁴ (50 µl)	1×10 ⁶ [50x] (50 µl) 5×10 ⁶ [250x] (50 µl) 1×10 ⁷ [500x] (50 µl)	150 µl	24 h
Growth of lactobacilli in IECs	24 well plate	1×10 ⁵ (125 µl)	5×10 ⁴ (0 µl)	2.0×10 ³ [-10x] (1000 µl)	1000 µl	24 h
Western Blot	6 well plate	4×10 ⁵ (1300 µl)	4×10 ⁵ (1300 µl)	2.0×10 ⁷ [50x] (1300 µl)	3900 µl	6 h, 12 h, 24 h
Glucose- and Lactate measurement	6 well plate	4×10 ⁵ (1300 µl)	4×10 ⁵ (1300 µl)	2.0×10 ⁷ [50x] (1300 µl)	3900 µl	0 h, 6 h, 12 h, 24 h
<i>C.a.</i> shedding	6 well plate	4×10 ⁵ (1300 µl)	4×10 ⁵ (1300 µl)	2.0×10 ⁷ [50x] (1300 µl)	3900 µl	1 h, 3 h, 6 h, 24 h
SEM	24 well plate coverslips	1×10 ⁵ (125 µl)	5×10 ⁴ (125 µl)	2.5×10 ⁶ [50x] (125 µl)	375 µl	6 h
ELISA	24 well plate	1×10 ⁵ (125 µl)	5×10 ⁴ (125 µl)	2.5×10 ⁶ [50x] (125 µl)	375 µl	24 h
RNA isolation	6 well plate	4×10 ⁵ (1300 µl)	4×10 ⁵ (1300 µl)	2.0×10 ⁷ [50x] (1300 µl)	3900 µl	1.5 h, 6 h, 12 h
Apoptosis	96 well plate	2×10 ⁴ (50 µl)	2×10 ⁴ (50 µl)	1×10 ⁶ [50x] (50 µl)	150 µl	0 h-24 h

Table S1: Overview of the exact number of host cells, *Lactobacillus* species, *C. albicans*, and incubation periods used in the respective experiments.

target gene	sequence	target gene	sequence
<i>β-ACTIN</i>	F: AAATGCTTCTAGGCGGACTAT R: AAGGGACTTCTGTAACAACG	<i>MUC13</i>	F: GAGCTTGCAACCTAGCCTCA R: AGAGTGCACCCCATAGTGGA
<i>GAPDH</i>	F: TTGCCCTCAACGACCACTTT R: GGTGGTCCAGGGGTCTTACT	<i>MUC17</i>	F: TTGTCAGCACCACACTTCCA R: GGCAGTGTCTAGTAGAAGGGG
<i>MUC1</i>	F: CAGTGCCGCCGAAAGAACTA R: TAGGGGCTACGATCGGTACT	<i>cFOS</i>	F: GGAGGAGGGGAGCTGACTGAT R: GCTGCCAGGATGAACCTAGT
<i>MUC2</i>	F: CACTGCCCTCGACAGCTTTA R: TGGAAGCAAGGACTGAACAA	<i>DUSP1</i>	F: CTGCAGTTTGAGTCCCAGGT R: AGACGGGGAAGTTGAACACG
<i>MUC3A</i>	F: TCACATCCTGGTCCCTAGCA R: TGAATGGACGGGCTCAGAAG	<i>DUSP5</i>	F: TGGAAGACAGCCACACGG R: GAGATCCCAGCCTCACAGTG
<i>MUC4</i>	F: CAGCTCCTGGGCTGAACATT R: CTCGTGTGAAGTCCGATGCT	<i>NFκB-IA</i>	F: ACCTCACCTTTGTGGGGTTT R: ACAGGATACCACTGGGGTCA
<i>MUC5AC</i>	F: GTACTCGCTCGAGGGCAACA R: TTCCACCTCGGTGTAGCTGA		

Table S2: Primers used for gene expression analyses