

GO:0006457: protein folding
R-HSA-109582: Hemostasis
hsa00500: Starch and sucrose metabolism
GO:0045055: regulated exocytosis
GO:0031110: regulation of microtubule polymerization or depolymerization GO:0007420: brain development
GO:0035966: response to topologically incorrect protein
GO:0009112: nucleobase metabolic process
GO:0005975: carbohydrate metabolic process
GO:0031589: cell-substrate adhesion
GO:0021932: hindbrain radial glia guided cell migration
GO:0006650: glycerophospholipid metabolic process
GO:1903441: protein localization to ciliary membrane
GO:0045744: negative regulation of G protein-coupled receptor signaling pathway ko04142: Lysosome
GO:0030258: lipid modification
CORUM:5232: TNF-alpha/Nf-kappa B signaling complex
GO:1901137: carbohydrate derivative biosynthetic process hsa04931: insulin resistance
GO:1901615: organic hydroxy compound metabolic process
GO:0071214: cellular response to abiotic stimulus
GO:0010975: regulation of neuron projection development
GO:0042058: regulation of epidermal growth factor receptor signaling pathway
GO:0030100: regulation of endocytosis
R-HSA-202733: Cell surface interactions at the vascular wall
GO:0060337: type I interferon signaling pathway
GO:0032787: monocarboxylic acid metabolic process
GO:0098739: import across plasma membrane
GO:0071560: cellular response to transforming growth factor beta stimulus GO:0006869: lipid transport
GO:0042573: retinoic acid metabolic process
GO:0042391: regulation of membrane potential
GO:0030032: lamellipodium assembly
hsa05020: Prion disease
GO:0051235: maintenance of location
hsa04928: parathyroid hormone synthesis, secretion and action hsanan01: drug metabolism
R-HSA-1989781: PPARA activates gene expression
GO:0006979: response to oxidative stress
R-HSA-8955332: Carboxyterminal post-translational modifications of tubulin GO:1901652: response to peptide
GO:0033674: positive regulation of kinase activity
GO:0010634: positive regulation of epithelial cell migration
M238: PID THROMBIN PAR1 PATHWAY
GO:0021762: substantia nigra development
R-HSA-425397: Transport of vitamins, nucleosides, and related molecules hsa00260: Glycine, serine and threonine metabolism

Fig. S1 Enriched biological pathways due to propionate in Caco-2 cells.
Caco-2 cells were incubated with and without 20 mM propionate for 24 h . Total RNA was extracted from Caco-2 cells and DNA microarray analysis of all expressed RNA performed. The 300 genes upregulated by propionate were subjected to gene ontology analysis by Metascape in order to identify the enriched ontology clusters.


#### Abstract

-800 CTTTCСССАA GTGCTCСTCC TACCCGGATC AGCCAACGCC CACATACCTC AGGCTTAAAC - 740 CAACTAGGGA ACTTTCCAGT ACTTTCCCAA ACAAGGACCT ACTGAGCCTT TCAGGTTCAC - 680 AATCAATCAG ATCCCTACTG GCTCACCTAG TCTCCCGACG CCTTCGCTTC AGTTTGGAAA - 620 CGTCCAGATT ACGCAGCCCC AGCGAGTAGG TGGGGGCTCC CTCAATATCA AACTGCACAA - 560 CCGGGGTCCC CCCACCCCCC ACCCCGTCCC TCCCTGCAAA TTTGAGACGG CTCCAACTCA - 500 GTAATCTTTT TCCAAACTGG CCCATGAGGT CAGAGACAGT ATCTCCATTG TAACGTGGCC -440 GGGCGGTGTC AACACAAACG CCCCCACCCT CCCCTGGACG CGCGTAACCC GCTCCCCGCA - 380 CCAGCCCCCT GCCCACAACT GCGCAGGCCC AGCAAGCCCC CACAATTAAA AGCCCAGCGC - 320 CGACCCTTCC TGTCAATTAG GCGCTGAAGC GCAGGCGGTC AGCATCGCCA TGGAGACCAA - 260 CAСССТТССС ACCGCСAСTC ССССТТССТС TCAGGGTCCC TGTCCCCTCC AGTGAATCCC - 200 AGAAGACTCT GGAGAGTTCT GAGCAGGGGG CGGCACTCTG GCCTCTGATT GGTCCAAGGA -140 AGGCTGGGGG GCAGGACGGG AGGCGAAAAC CCTGGAATAT TCCCGACCTG GCAGCCTCAT - 80 CGAGCTCGGT GATTGGCTCA GAAGGGAAAA GGCGGGTCTC CGTGACGACT TATAAAAGCC - 20 CAGGGGCAAG CGGTCCGGAT AACGGCTAGC CTGAGGAGCT GCTGCGACAG TCCACTACCT 41 TTTTCGAGAG TGACTCCCGT TGTCCCAAGG CTTCCCAGAG CGAACCTGTG CGGCTGCAGG 101 CACCGGCGCG TCGAGTTTCC GGCGTCCGGA AGGACCGAGC TCTTCTCGCG GATCCAGTGT 161 TCCGTTTCCA GCCCCCAATC TCAGAGCGGA GCCGACAGAG AGCAGGGAAC CGGC


Fig. S2 Nucleotide sequence of human Hspa1 promoter cloned in pGL3 plasmid.
Numbers indicate the nucleotide positions -800 to +214 , relative to the major transcriptional start site, which is underlined.




Fig. S3 Effect of propionate on regulation of the tight junction barrier in Caco-2 cells.
(A) Caco-2 cells were incubated with and without propionate (10 and 20 mM ) for 24 h. (B, C) MEK ( $10 \mu \mathrm{M}$ U0126, B) and mTOR ( $0.5 \mu \mathrm{M}$ rapamycin, C) inhibitors were added to cell cultures 1 h before propionate administration, and cells were incubated with and without propionate ( 10 mM ) for 24 h . The tight junction barrier was evaluated by measuring transepithelial electrical resistance (TER). Data are presented as the mean $\pm$ SEM, $n=6$. Means without a common letter are significantly different ( $p<0.05$ ).


Fig. S4 Effects of pharmacological inhibition of GPR41 on the propionate-mediated upregulation of HSP70 in Caco-2 cells.

A selective antagonist for $\mathrm{Ga}_{\mathrm{i} / \mathrm{o}}$ protein, NF023 (10 and $20 \mu \mathrm{M}$ ), was added to cell cultures 1 h before propionate administration, and cells were incubated with and without propionate ( 10 mM ) for 24 h . HSP70 protein levels in the cells were determined by immunoblot analysis. Representative immunoblot images of six samples are shown. Data are presented as the mean $\pm$ SEM, $n=6$. Means without a common letter are significantly different ( $p<0.05$ ).

Supplemental Table S1. Primers Used in qRT-PCR.

| Target <br> gene | Forward (5' to $\left.3^{\prime}\right)$ | Reverse (5' to 3') |
| :---: | :---: | :---: |
| Human <br> Gapdh | CAACGGATTTGGTCGTATTGGG | AAGGGGTCATTGATGGCAAC |
| Human | CGCAGAACACCGTGTTTGAC | AAAGGCCAGTGCTTCATGTC |
| Hspa1a |  |  |

Supplemental Table S2. Primers Used for Constructing the Hspa1a Promoter Plasmids.

| Forward (5' to $\left.3^{\prime}\right)$ | Reverse (5' to 3') |
| :---: | :---: |
| CGCACGCGTCTTTCCCCAAGTGCTC | CCGAGATCTGCCGGTTCCCTGCTCTC |
| CTCCTACCCGG | TGTCGGCTCC |

