**Supplementary materials**

**Supplemental Table 1. Clinicopathological characteristics of patients with colon cancer.**

|  |  |  |
| --- | --- | --- |
|  | **n** | **%** |
| **Gender**  Male  Female | 25  16 | 61  39 |
| **Location of primary lesion**  Transverse colon  Right hemicolon  Left hemicolon  Missing | 5  13  15  8 | 12  32  37  20 |
| **TNM stage**  I  II  III  IV  Missing | 5  7  17  4  8 | 12  17  41  10  20 |
| **Differentiation**  Well  Moderately  Poorly and undifferentiated  Missing | 3  25  5  8 | 7  61  12  20 |
| **Tumor size**  ≤ 5 cm  > 5 cm  Missing | 17  8  16 | 41  20  40 |
| **Lymph node status**  Positive  Negative | 15  26 | 37  63 |

**Supplemental Table 2. Sequences of the primers and TaqMan® probes.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene (GenBank accession)** | | **Oligonucleotide sequence (5’-3’)** | |
| *ADIPOQ* (NM\_001177800) | |  | |
| Forward | | GGAGATCCAGGTCTTATTGGTCCTA | |
| Reverse | | CCTTGGATTCCCGGAAAGC | |
| TaqMan® Probe | | FAM-ACATCGGTGAAACCGGAGTACCCGG-TAMRA | |
| *ASC* (NM\_013258.4) | |  | |
| Forward | | AGCCAGGCCTGCACTTTATAGA | |
| Reverse | | CAGCAGCCACTCAACGTTTG | |
| TaqMan® Probe | | FAM-CACCGGGCTGCGCTTATCGC-TAMRA | |
| *CCL2* (NM\_002982) | |  |
| Forward | | GCTCATAGCAGCCACCTTCATT |
| Reverse | | TCTGCACTGAGATCTTCCTATTGGT |
| TaqMan® Probe | | FAM-TCGCTCAGCCAGATGCAATCAATGC-TAMRA |
| *CLDN1* (NM\_021101.5) | |  | |
| Forward | | AAAGTCTTTGACTCCTTGCTGAATCT | |
| Reverse | | TTCCAAGCACTTCATACACTTCATG | |
| TaqMan® Probe | | FAM-AGCAGCCACATTGCAAGCAACCCGT-TAMRA | |
| *FGF21* (NM\_019113) | |  | |
| Forward | | CACCTGGAGATCAGGGAGGAT | |
| Reverse | | ATTTGAATAACTCCCGGCTTCA | |
| TaqMan® Probe | | FAM-CTGCTGACCAGAGCCCCGAAAGTCTC-TAMRA | |
| *GLP1* (NM\_002054.5) | |  | |
| Forward | | CCCTTCAAGACACAGAGGAGAAA | |
| Reverse | | GCGCTTGTCCTCGTTCATCT | |
| TaqMan® Probe | | FAM-CCAGATCATTCTCAGCTTCCCAGGCA-TAMRA | |
| *GSDMD* (NM\_001166237.1) | |  | |
| Forward | | TGGCCCAGCTGGTTATTGAC | |
| Reverse | | CGCTCGTGGAACGCTTGT | |
| TaqMan® Probe | | FAM-GACTTGGACGTCCTTCTCTTCCCGG-TAMRA | |
| *HMGB1* (NM\_001313893.1) | |  | |
| Forward | | GAAGTTCAAGGATCCCAATGCA | |
| Reverse | | CTCCTTTGATTTTTGGGCGATAC | |
| TaqMan® Probe | | FAM-CTCCTTCGGCCTTCTTCCTCTTCTGCTC-TAMRA | |
| *IL1B* (NM\_000576) | |  | |
| Forward | | CAGTGGCAATGAGGATGACTTG | |
| Reverse | | GTAGTGGTGGTCGGAGATTCGTA | |
| TaqMan® Probe | | FAM-TGGCCCTAAACAGATGAAGTGCTCCTTCC-TAMRA | |
| *IL18* (NM\_001562) | |  | |
| Forward | | CCAAGGAAATCGGCCTCTATT | |
| Reverse | | CCTCTAGGCTGGCTATCTTTATACATACT | |
| TaqMan® Probe | | FAM-TTCTGACTGTAGAGATAATGCACCCCGGAC-TAMRA | |
| *IL32* (NM\_001012631) | |  |
| Forward | | GAGACAGTGGCGGCTTATTATGA |
| Reverse | | GGCACCGTAATCCATCTCTTTCT |
| TaqMan® Probe | | FAM-CAGCACCCAGAGCTCACTCCTCTACTTGAA-TAMRA |
| *IL33* (NM\_033439) | |  | |
| Forward | | CACTGAAAACAGGTAGAAAGCACAAA | |
| Reverse | | CCCCTGATATACCAAAGGCAAAG | |
| TaqMan® Probe | | FAM-CTGGTACTCGCTGCCTGTCAACAGCAG-TAMRA | |
| *KLF4* (NM\_001314052.1) | |  |
| Forward | | ACCTACACAAAGAGTTCCCATCTCA |
| Reverse | | GTTTACGGTAGTGCCTGGTCAGTT |
| TaqMan® Probe | | FAM-CCTGCGAACCCACACAGGTGAGAAA-TAMRA |
| *MUC2* (NM\_002457) | |  | |
| Forward | | ACGGCCTGCAGAGCTATTCA | |
| Reverse | | TGATCTTCTGCATGTTCCCAAAC | |
| TaqMan® Probe | | FAM-ATTCCTCTCTGACGGCGTGCTCTTCAGT-TAMRA | |
| *NGAL* (NM\_005564) | |  |
| Forward | | CCCAGCCCCACCTCTGA |
| Reverse | | CTTCCCCTGGAATTGGTTGTC |
| TaqMan® Probe | | FAM-CAAGGTCCCTCTGCAGCAGAACTTCCA-TAMRA |
| *NLRP1* (NM\_033004.3) | |  | |
| Forward | | CCCTGGAGCAGGAGAAACCT | |
| Reverse | | CGAGTCTCTGCCGCTTGAGT | |
| TaqMan® Probe | | FAM-CTCATCTTCAGCAGACGGAAACCAAGTGTG-TAMRA | |
| *NLRP3* (NM\_001079821.2) | |  | |
| Forward | | AAGCTTCAGGTGTTGGAATTAGACA | |
| Reverse | | GTTGCCCAGGCTCAGCTTT | |
| TaqMan® Probe | | FAM-CACACTGCTGCTGGGATCTTTCCACA-TAMRA | |
| *NLRP6* (NM\_138329.2) | |  | |
| Forward | | TCCCTTCTTCATCCACTCTTTCAG | |
| Reverse | | CAGACCGCGTCAGGGAGTT | |
| TaqMan® Probe | | FAM-CTGAGCAGCCTCACGCTGTCCCA-TAMRA | |
| *NOD2* (NM\_001293557) | |  |
| Forward | | TTCAGGAATTACCAGTCCCATTG |
| Reverse | | GGTCCTCAGCTTGGCCATATACT |
| TaqMan® Probe | | FAM-CCCTGCCTTTGGAAGCTGCCACA-TAMRA |
| *OCLN* (NM\_001205254.2) | |  | |
| Forward | | TGGACTCTACGTGGATCAGTATTTGTA | |
| Reverse | | AGCAAAAGCCACAATAATCATGAAC | |
| TaqMan® Probe | | FAM-TCCCCAGGAGGCCATTGCCAT-TAMRA | |
| *S100A9* (NM\_002965) | |  |
| Forward | | CTCAAGAAGGAGAATAAGAATGAAAAGG |
| Reverse | | TCAGCTGCTTGTCTGCATTTG |
| TaqMan® Probe | | FAM-CATAGAACACATCATGGAGGACCTGGAC-TAMRA |
| *SPP1* (NM\_000582) | |  |
| Forward | | CATCCAGTACCCTGATGCTACAGA |
| Reverse | | GGCCTTGTATGCACCATTCAA |
| TaqMan® Probe | | FAM-ACATCACCTCACACATGGAAAGCGAGGA-TAMRA |
| *TGFB* (NM\_000660) | |  |
| Forward | | GCCCAGCATCTGCAAAGC |
| Reverse | | TCCTTGCGGAAGTCAATGTACA |
| TaqMan® Probe | | FAM-CACCAACTATTGCTTCAGCTCCACGGA-TAMRA |
| *TJP1* (NM\_003257.5) | |  | |
| Forward | | GCACAGCAATGGAGGAAACAG | |
| Reverse | | TCTCGTCCACCAGATATTGCAAT | |
| TaqMan® Probe | | FAM-ACAACATACAGTGACGCTTCACAGGGCTC-TAMRA | |
| *TLR4* (NM\_003266) | |  |
| Forward | | CTGCGTGGAGGTGGTTCCTA |
| Reverse | | CAGGTCCAGGTTCTTGGTTGAG |
| TaqMan® Probe | | FAM-TTTCTACAAAATCCCCGACAACCTCCCCT-TAMRA |
| *TNFAIP9* (NM\_024636) | |  |
| Forward | | TCTCCAGTCAGGAGCACTGGAT |
| Reverse | | CAATTTCTTTGGCTGCCATGA |
| TaqMan® Probe | | FAM- CAAGTCGGCAGGTGTTTGTGTGTGGA-TAMRA |

*ADIPOQ,* adiponectin; *ASC*, apoptosis-associated speck-like protein containing a CARD; *CCL2*,monocyte chemoattractant protein-1; *CLDN1*, claudin 1; *FGF21*, fibroblast growth factor 21; *GLP1*, glucagon-like peptide-1; *GSDMD*, gasdermin D; *HMGB1*, high mobility group box 1; *IL*, interleukin; *KLF4*, kruppel like factor 4; *MUC2*, mucin 2; *NGAL*, lipocalin 2; *NLRP*, nucleotide-binding oligomerization domain, leucine rich repeat and pyrin; *NOD2*, nucleotide binding oligomerization domain containing 2; *OCLN*, occludin; *S100A9*, S100 calcium-binding A9; *SPP1*,osteopontin; *TGFB1*, transforming growth factor-; *TLR4*, toll-like receptor-4; *TJP1*, tight junction protein 1; *TNFAIP9*, tumor necrosis factor- induced protein 9.

**Supplemental Table 3. Univariate analysis of the correlations between mRNA levels of different components of the inflammasome in VAT.**

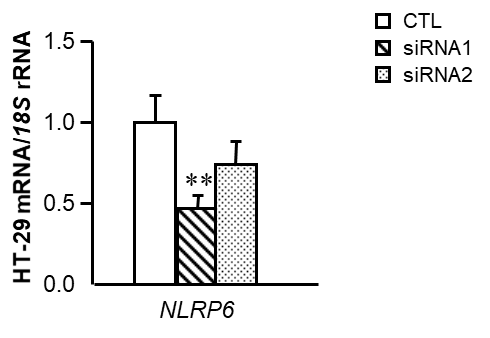
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **mRNA *NLRP1*** | | **mRNA *NLRP3*** | | **mRNA *NLRP6*** | |
|  | r | *P* | r | *P* | r | *P* |
| **mRNA *NLRP1*** | - | - | 0.32 | 0.008 | 0.04 | 0.771 |
| **mRNA *NLRP3*** | 0.32 | 0.008 | - | - | 0.56 | <0.001 |
| **mRNA *NLRP6*** | 0.04 | 0.771 | 0.56 | <0.001 | - | - |
| **mRNA *IL1B*** | 0.04 | 0.784 | 0.59 | <0.001 | 0.50 | <0.001 |
| **mRNA *IL18*** | 0.25 | 0.041 | 0.41 | <0.001 | 0.09 | 0.448 |
| **mRNA *ASC*** | 0.40 | <0.001 | 0.33 | 0.006 | 0.15 | 0.238 |
| **mRNA *NOD2*** | 0.50 | <0.001 | 0.40 | <0.001 | 0.28 | 0.025 |
| **CRP** | 0.20 | 0.270 | 0.44 | 0.012 | 0.209 | 0.115 |
| **Monocyte (%)** | 0.30 | 0.043 | 0.44 | 0.003 | 0.24 | 0.120 |
| **Eosinophil (%)** | -0.06 | 0.669 | -0.30 | 0.044 | -0.44 | 0.002 |

*ASC*, apoptosis-associated speck-like protein containing a CARD; CRP, C-reactive protein; *IL*, interleukin; *NLRP*, nucleotide-binding oligomerization domain, leucine rich repeat and pyrin; *NOD2*, nucleotide binding oligomerization domain containing 2; VAT, visceral adipose tissue. Associations between variables were analyzed by Pearson’s correlation coefficients.

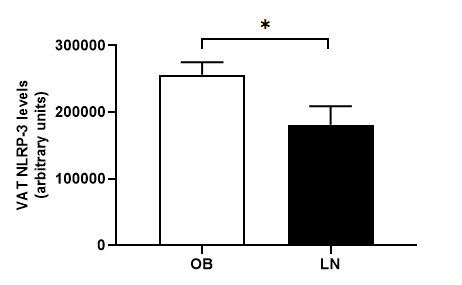
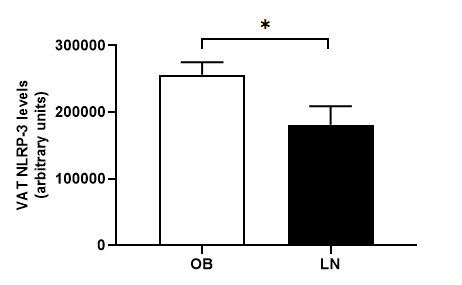
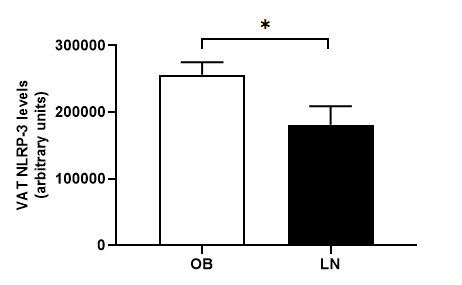
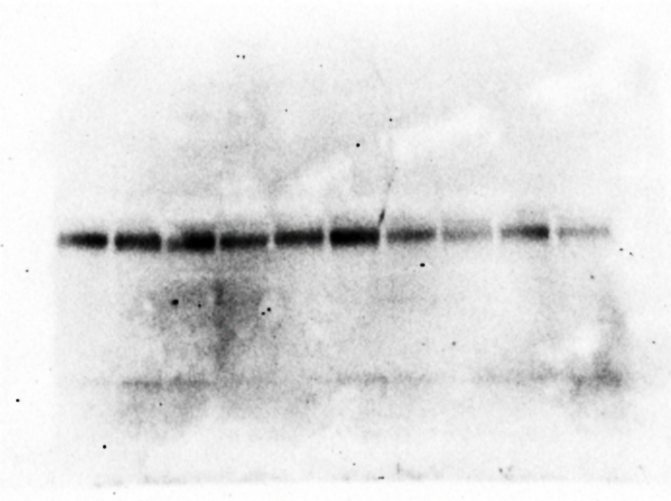
**Supplemental Table 4. Analysis of gene expression levels of inflammation- and intestinal integrity-associated markers in colon cancer cells after the incubation with *Akkermansia muciniphila*.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Gen** | **CTL** | ***A. muciniphila* Heat-inactivated** | ***A. muciniphila***  **Alive** | **CTL-BM**  **20%** | **BCM**  **20%** | **CTL-BM**  **40%** | **BCM**  **40%** |
| ***NLRP1*** | 1.00  0.08 | 0.72  0.12 | 0.43  0.08 | 0.98  0.12 | 3.15  0.68\*\*,††,§§,‡‡ | 1.09  0.12 | 4.21  0.57\*\*,††,§§,‡‡ |
| ***NLRP3*** | 1.00  0.22 | 1.12  0.21 | 13.84  1.20\*\*,††,‡‡,## | 0.81  0.02 | 0.21  0.02 | 0.87  0.07 | 0.39  0.05 |
| ***NLRP6*** | 1.00  0.13 | 0.61  0.06 | 1.37  0.20 | 0.51  0.08 | 2.00  0.44†,‡‡ | 0.92  0.18 | 3.01  0.34\*\*,†,§,‡‡ |
| ***ASC*** | 1.00  0.08 | 0.96  0.18 | 1.66  0.18\*,†,‡‡,# | 0.72  0.05 | 0.92  0.21 | 0.67  0.05 | 0.74  0.04 |
| ***IL1B*** | 1.00  0.17 | 1.40  0.17## | 2.93  0.28\* | 1.26  0.17 | 4.06  0.94\*\*,††,§,‡ | 1.89  0.29 | 3.40  0.45\*,††,§,‡ |
| ***IL6*** | 1.00  0.34 | 1.38  0.30 | 1.32  0.38 | 1.47  0.29 | 1.00  0.39 | 1.10  1.14 | 0.59  0.16 |
| ***IL32*** | 1.00  0.12 | 7.96  0.88\*\* | 1.13  0.17 | 0.83  0.10 | 27.97  3.75\*\*,††,§§,‡‡ | 0.70  0.10 | 22.01  3.19\*\*,††,§§,‡‡ |
| ***TLR4*** | 1.00  0.11 | 0.99  0.08 | 0.68  0.03 | 0.98  0.10 | 0.40  0.04 | 1.12  0.17 | 1.03  0.06 |
| ***HMGB1*** | 1.00  0.12 | 1.46  0.22 | 1.97  0.27\* | 0.99  0.07 | 0.52  0.09 ††,§§,‡‡ | 0.92  0.15 | 0.49  0.07 ††,§§,‡‡ |
| ***GSDMD*** | 1.00  0.07 | 0.95  0.11 | 0.84  0.11 | 0.98  0.08 | 0.77  0.07 | 0.82  0.08 | 1.00  0.21 |
| ***MUC2*** | 1.00  0.14 | 2.51  0.30\* | 3.38  0.51\*\* | 0.79  0.02 | 2.29  0.29\*,‡ | 0.88  0.01 | 2.49  1.18\*,‡ |
| ***CLDN1*** | 1.00  0.12 | 1.73  0.23 | 1.85  0.20\* | 0.87  0.02 | 1.47  0.32 | 0.83  0.03 | 1.21  0.23 |
| ***OCLN1*** | 1.00  0.13 | 1.50  0.70 | 1.13  0.10 | 0.73  0.03 | 1.30  0.32 | 0.75  0.05 | 1.38  0.17 |
| ***TJP1*** | 1.00  0.20 | 0.95  0.18 | 1.17  0.21 | 0.86  0.03 | 0.79  0.07 | 0.73  0.04 | 0.85  0.20 |

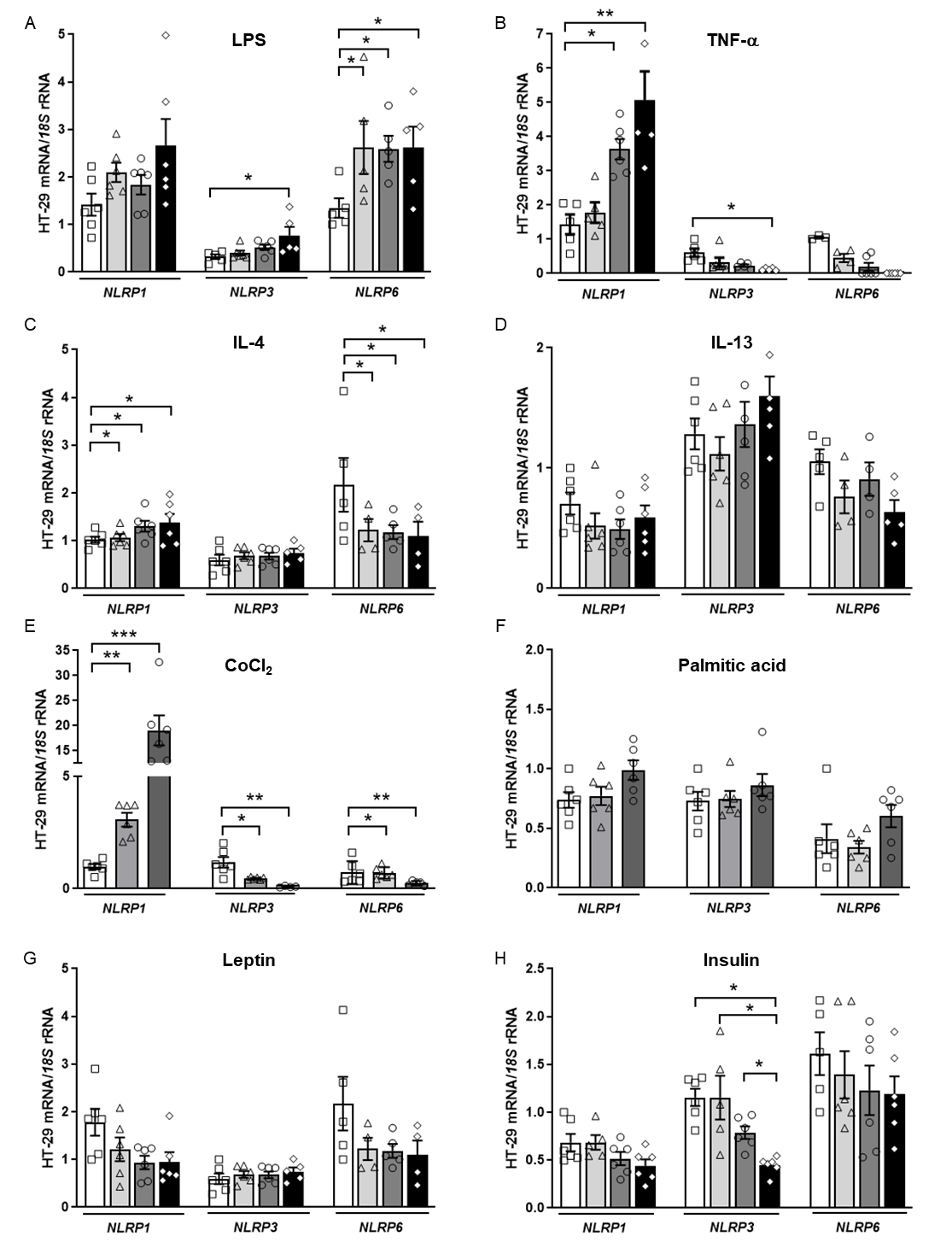
Analysis of mRNA levels in HT-29 colon cancer cells (n=6 per group). Data represent the mean  SEM of the ratio between the gene expressionto *18S* rRNA. Differences between groups were analyzed by one-way ANOVA followed by Tukey’s *post-hoc* tests. \**P*<0.05 and \*\**P*<0.01 *vs* CTL. †*P*<0.05 and ††*P*<0.01 *vs* heat-inactivated *A. muciniphila*. §*P*<0.05 and §§*P*<0.01 *vs* *A. muciniphila* alive. ‡*P*<0.05 and ‡‡*P*<0.01 vs corresponding CTL-BCM. #*P*<0.05 and ##*P*<0.01 vs BCM. *ASC*, apoptosis-associated speck-like protein containing a CARD; *CLDN1*, claudin 1;*GSDMD*, gasdermin D; *HMGB1*, high mobility group box 1; *IL*, interleukin; *MUC2*, mucin 2; *NLRP*, nucleotide-binding oligomerization domain (NOD)-like receptors; *OCLN*, occludin; *TJP1*, tight junction protein 1; *TLR4*, toll-like receptor-4.CTL-BM, brain heart infusion medium diluted in McCoy’s 5a medium.

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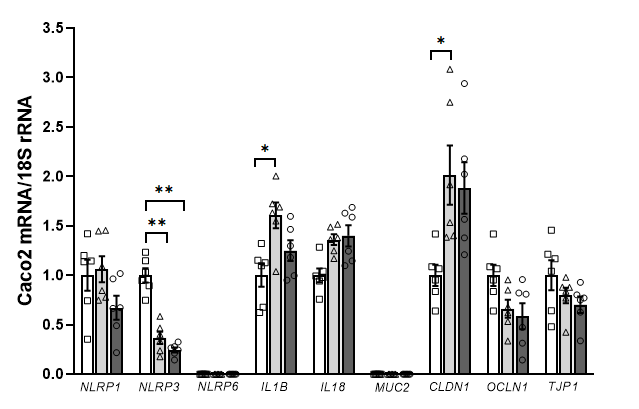
**Supplemental Figure 1.** mRNA expression levels of *NLRP6* in HT-29 cells after transfection with two siRNAs (s128674 and s128675) at 100 pM siRNA *NLRP6*/2x105 cells for 24 h. Gene expression levels in scrambled siRNA cells (CTL) were assumed to be 1. Values are the mean ± SEM (n=6 per group). Differences between groups were analyzed by one-way ANOVA followed by Dunnet’s tests. \*\**P*<0.01 *vs* CTL.



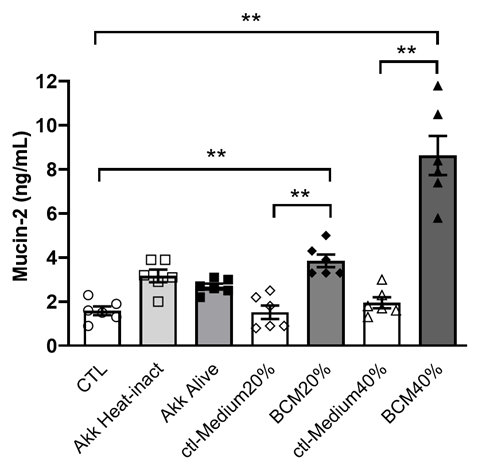
**Supplemental Figure 2.** Impact of obesity on protein expression levels of NLRP-3 in visceral active tissue (VAT). NLRP-3 levels in patients with obesity (OB) and lean volunteers (LN). Bars represent the mean  SEM. Differences between groups were analyzed by unpaired two-tailed Student’s *t* test. \**P*<0.05.



**Supplemental Figure 3. Impact of hypoxia and inflammation-related factors in the gene expression levels of the main inflammasome components in HT-29 colon cancer cells.** Bar graphs show the effect of (A) LPS, (B) TNF-, (C) IL-4, (D) IL-13 (E) CoCl2, (F) palmitic acid, (G) leptin and (H) insulin incubated for 24 h on the transcript levels of *NLRP1, NLRP3* and *NLRP6* in HT-29 cells. Values are the mean ± SEM (n=6 per group). Differences between groups were analyzed by one-way ANOVA followed by Dunnett’s tests. \**P*<0.05, \*\**P*<0.01 and \*\*\**P*<0.001.



**Supplemental Figure 4. Effect of adipocyte-conditioned media (ACM) on the gene expression levels of the inflammasome as well as on intestinal integrity markers in Caco-2 cells. Bar graphs show the effect of ACM (20 and 40%) from obese subjects incubated for 24 h on the transcript levels of inflammasome- and intestinal integrity-associated genes in Caco2 cells. Values are the mean ± SEM (n=6 per group). Differences between groups were analyzed by one-way ANOVA followed by Tukey’s tests.** \**P*<0.05 and \*\**P*<0.01**.** *CLDN1*, claudin 1; *IL*, interleukin; *NLRP*, nucleotide-binding oligomerization domain, leucine rich repeat and pyrin; *OCLN*, occludin; *TJP1*, tight junction protein 1.



**Supplemental Figure 5. Secreted levels of mucin-2 after *Akkermansia muciniphila*** **incubation in HT-29 colon cancer cells.** Mucin-2 concentrations in the culture media of HT-29 cells incubated in the presence of *A. muciniphila* heat-inactivated (Akk Heat-inact), *A. muciniphila* alive (Akk Alive) and with the bacteria-conditioned medium (BCM) (20% and 40%) for 24 h. Values are the mean ± SEM (n=6 per group). Differences between groups were analyzed by one-way ANOVA followed by Tukey’s tests. \*\**P*<0.01.