

## Supplementary Materials

**Table S1.** Characteristics of pristine ENM as described by Bredeck et al. [50].

| ENM <sup>1</sup> | Name, Source              | Mean Diameter (nm) | Mode Diameter (nm) | Sigma (fit) <sup>2</sup> |
|------------------|---------------------------|--------------------|--------------------|--------------------------|
| TiO <sub>2</sub> | NM105, JRC                | 26.2 ± 10.7        | 21.0               | 1.39                     |
| Ag               | #576832-5G, Sigma-Aldrich | 40.2 ± 17.6        | 32.0               | 1.40                     |
| CeO <sub>2</sub> | NM212, JRC                | 35.4 ± 17.0        | 28.7               | 1.38                     |
| SiO <sub>2</sub> | #S5130, Sigma-Aldrich     | 12.9 ± 4.9         | 11.0               | 1.32                     |

<sup>1</sup> Engineered nanomaterials (ENM) used in the respective studies were analyzed by scanning electron microscopy as described by Bredeck et al. [50]. <sup>2</sup> Standard deviation of logarithmic values.

**Table S2.** Primer sequences for human genes.

| Gene    |    | Sequence (5' → 3')      | Working Conc. (nM) | Amplicon Length (bp) |
|---------|----|-------------------------|--------------------|----------------------|
| β-Actin | fw | CCTGGCACCCAGCACAAT      | 60                 | 70                   |
|         | rv | GCCGATCCACACGGAGTACT    | 60                 |                      |
| IL-8    | fw | ACTCCAAACCTTTCCACCC     | 60                 | 168                  |
|         | rv | CCCTCTTCAAAAACCTTCTCCAC | 60                 |                      |
| MUC1    | fw | AGACGTCAGCGTGAGTGATG    | 37.5               | 139                  |
|         | rv | GACAGCCAAGGCAATGAGAT    | 37.5               |                      |
| MUC2    | fw | GTCCGTCTCCAACATCACCT    | 60                 | 287                  |
|         | rv | GCTGGCTGGTTTTCTCCTCT    | 60                 |                      |
| MUC5AC  | fw | CAGCACAACCCCTGTTTCAA    | 60                 | 100                  |
|         | rv | GCGCACAGAGGATGACAGT     | 37.5               |                      |
| MUC13   | fw | CAGAGACAGCCAGATGCAAA    | 60                 | 126                  |
|         | rv | CGGAGGCCAGATCTTTACTG    | 37.5               |                      |
| MUC20   | fw | GTGCAGGTGAAAATGGAGGT    | 60                 | 299                  |
|         | rv | ACGCAGTAAGGAGACCTGGA    | 37.5               |                      |

**Table S3.** Primer sequences for murine genes.

| Gene   |    | Sequence (5' → 3')            | Working Conc. (nM) | Amplicon Length (bp) |
|--------|----|-------------------------------|--------------------|----------------------|
| Rplp0  | fw | CCACGAAAATCTCCAGAGGC          | 100                | 164                  |
|        | rv | AATGCTGCCGTTGTCAAACA          | 375                |                      |
| Muc1   | fw | GCTGGACATCTTTCCAACCC          | 100                | 137                  |
|        | rv | TACTGCCATTACCTGCCGAA          | 100                |                      |
| Muc2   | fw | CCATTGAGTTTGGGAACATGC         | 100                | 104                  |
|        | rv | TTCGGCTCGGTGTTTCAGAG          | 375                |                      |
| Muc5AC | fw | AAAGACACCAGTAGTCACTCAGC<br>AA | 375                | 139                  |
|        | rv | CTGGGAAGTCAGTGTCAAACCA        | 100                |                      |
| Muc13  | fw | TCGTCATGTGTGAAAGGAACG         | 100                | 228                  |
|        | rv | CTTTGTAGCATCACGCATTGC         | 100                |                      |
| Muc20  | fw | GCACCAGCAAAAACCCAAAC          | 60                 | 153                  |
|        | rv | TCAGCCGTACAAGGAGGAAG          | 60                 |                      |

**Table S4.** Estimated constitutive gene expression in pre-confluent and confluent E12 cells as well as in stable and inflamed triple cultures.

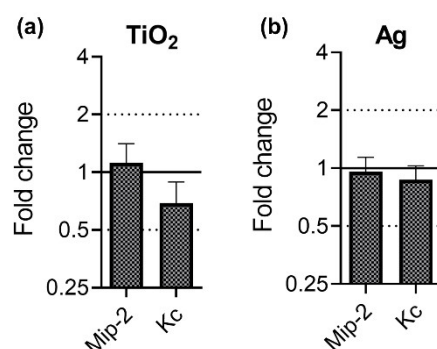
|                         | IL-8 | MUC1 | MUC2 | MUC5AC | MUC13 | MUC20 |
|-------------------------|------|------|------|--------|-------|-------|
| Pre-confluent E12       | +    | -    | -    | ++     | +     | +     |
| Confluent E12           | -    | -    | +    | ++     | +     | ++    |
| Triple culture stable   | -    | -    | -    | ++     | +     | +     |
| Triple culture inflamed | +    | +    | -    | +      | +     | +     |

According to the mean of the measured  $C_T$ -values of non-exposed cells, the gene expression was classified into the following categories: very high gene expression at  $C_T$ -values 19–25 (++); high gene expression at  $C_T$ -values 25–31 (+); low gene expression at  $C_T$ -values 31–37 (-); very low gene expression at  $C_T$ -values >37 (--).

**Table S5.** Estimated constitutive gene expression in murine ileal tissue.

| Mip-2 | Kc | Muc1 | Muc2 | Muc5AC | Muc13 | Muc20 |
|-------|----|------|------|--------|-------|-------|
| -     | -  | -    | +    | --     | +     | -     |

According to the mean of the measured  $C_T$ -values of non-exposed mice, the gene expression was classified into the following categories: very high gene expression at  $C_T$ -values 19–25 (++); high gene expression at  $C_T$ -values 25–31 (+); low gene expression at  $C_T$ -values 31–37 (-); very low gene expression at  $C_T$ -values >37 (--).



**Figure S1.** Gene expression of Mip-2 and Kc in murine ileal tissues after ENM exposure as published previously [52]. Female and male C57BL/6J mice were fed with feed pellets containing either no additive, 1.0% TiO<sub>2</sub> (a) or 0.2% Ag (b) for 28 days. The relative gene expression was assessed by qRT-PCR. The results were normalized to the unexposed negative controls and Rplp0 as reference gene. The depicted fold changes with SEM were derived from the mean and SEM of the  $\Delta\Delta C_T$ -values of  $N \geq 5$  independent experiments ( $t$ -test; \*  $p \leq 0.05$ ).