

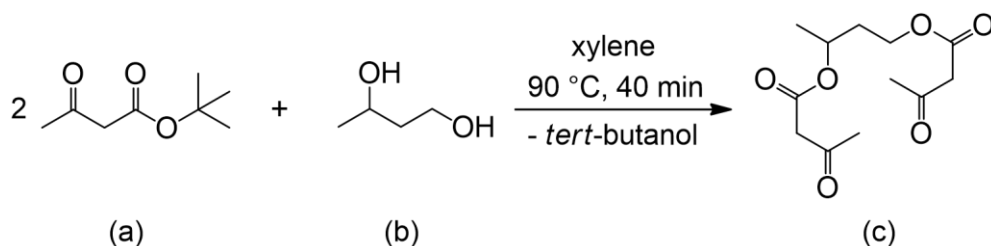
**Table S1 Detailed description of dietary composition.**

| <b>Ingredient (g/kg diet)</b> | <b>CD</b>    | <b>CD+KE</b> | <b>KD</b>    |
|-------------------------------|--------------|--------------|--------------|
| Protein, of which             | 183,5        | 183.5        | 198.5        |
| <i>Casein</i>                 | <i>178.5</i> | <i>178.5</i> | <i>193.5</i> |
| <i>L-Cystein</i>              | <i>2</i>     | <i>2</i>     | <i>2</i>     |
| <i>DL-Methionine</i>          | <i>3</i>     | <i>3</i>     | <i>3</i>     |
| Carbohydrates, of which       | 618          | 568          | -            |
| <i>Corn starch</i>            | <i>309</i>   | <i>284</i>   | -            |
| <i>Sucrose</i>                | <i>309</i>   | <i>284</i>   | -            |
| Fat, of which                 | 116          | 116          | 724          |
| <i>Corn oil</i>               | <i>100</i>   | <i>100</i>   | <i>100</i>   |
| <i>Pork lard</i>              | <i>11</i>    | <i>11</i>    | <i>429</i>   |
| <i>Butter fat</i>             | <i>5</i>     | <i>5</i>     | <i>195</i>   |
| Choline bitartrate            | 5            | 5            | 5            |
| Cellulose                     | 32           | 32           | 27           |
| Tert-butylhydroquinone        | 0.5          | 0.5          | 0.5          |
| Vitamin mix, AIN93G           | 10           | 10           | 10           |
| Mineral mix, AIN93G           | 35           | 35           | 35           |
| Ketone ester                  | -            | 50           | -            |

**Table S2 Primer sequences used for quantitative PCR analysis.**

| <b>Target</b>          | <b>Primer sequences</b>                                                                             |
|------------------------|-----------------------------------------------------------------------------------------------------|
| <b>Human</b>           |                                                                                                     |
| <b><i>ATOH1</i></b>    | forward: 5'-CCA GCT GCG CAA TGT TAT CC-3'<br>reverse: 5'-TGC TGT TTT CCT CCT GCA CT-3'              |
| <b><i>B-ACTIN*</i></b> | forward: 5'-ACA TCC GCA AAG ACC TGT ACG-3'<br>reverse: 5'-TTG CTG ATC CAC ATC TGC TGG-3'            |
| <b><i>B2M*</i></b>     | forward: 5'-GAG GCT ATC CAG CGT ACT C-3'<br>reverse: 5'- CGG CAG GCA TACT CA TCT-3'                 |
| <b><i>KLF4</i></b>     | forward: 5'-CCA TCT TTC TCC ACG TTC G-3'<br>reverse: 5'-ATC GGA TAG GTG AAG CTG CA-3'               |
| <b><i>MUC2</i></b>     | forward: 5'-GAC GGA GCT GAA GTT GGA AG-3'<br>reverse: 5'-GGA CAC GGA GAT GTT GGA GT-3'              |
| <b><i>MUC5AC</i></b>   | forward: 5'-CTG TGT CAA AGT GTG CCT GC-3'<br>reverse: 5'-TTG ATC ACC ACC ACC GTC TG-3'              |
| <b><i>SPDEF1</i></b>   | forward: 5'-GAT TCA CTA CTG TGC CTC GAC-3'<br>reverse: 5'-ATG TCT GGC TTC CGG ATG AT-3'             |
| <b><i>YWHAZ*</i></b>   | forward: 5'-ACT TTT GGT ACA TTG TGG CTT CAA-3'<br>reverse: 5'-CCG CCA GGA CAA ACC AGT AT-3'         |
| <b>Mouse</b>           |                                                                                                     |
| <b><i>Atoh1</i></b>    | forward: 5'-GTG GGG TTG TAG TGG ACG AG-3'<br>reverse: 5'-GTT GCT CTC CGA CAT TGG G-3'               |
| <b><i>Eef2*</i></b>    | forward: 5'-CTG CCT GTC AAT GAG TCC-3'<br>reverse: 5'-AGT ATC AGG CTG CCT ACA GT-3'                 |
| <b><i>Hprt1*</i></b>   | forward: 5'-TGG ATA CAG GCC AGA CTT TGT T-3'<br>reverse: 5'-CAG ATT CAA CTT GCG CTC ATC-3'          |
| <b><i>Il1β</i></b>     | forward: 5'-CAA CCA ACA AGT GAT ATT CTC CAT G-3'<br>reverse: 5'-GAT CCA CAC TCT CCA GCT GCA-3'      |
| <b><i>Il6</i></b>      | forward: 5'-TGA GAA AAG AGT TGT GCA ATG GC-3'<br>reverse: 5'-GCA TCC ATC ATT TCT TTG TAT CTC TGG-3' |
| <b><i>Klf4</i></b>     | forward: 5'-GGT AAG GTT TCT CGC CTG TG-3'<br>reverse: 5'-GAT TAA GCA AGA GGC GGT-3'                 |
| <b><i>Muc2</i></b>     | forward: 5'-GCT GAC GAG TGG TTG GTG AAT G-3'<br>reverse: 5'-GAT GAG GTG GCA GAC AGG AGA C-3'        |
| <b><i>Rpl13a*</i></b>  | forward: 5'-GCT TAC CTG GGG CGT CTG-3'<br>reverse: 5'-ACA TTC TTT TCT GCC TGT TTC C-3'              |
| <b><i>Spdef1</i></b>   | forward: 5'-GGA GAA GGC AGC ATC AGG A-3'<br>reverse: 5'-CCA GGG TCT GCT GTG ATG T-3'                |

\*housekeeping gene

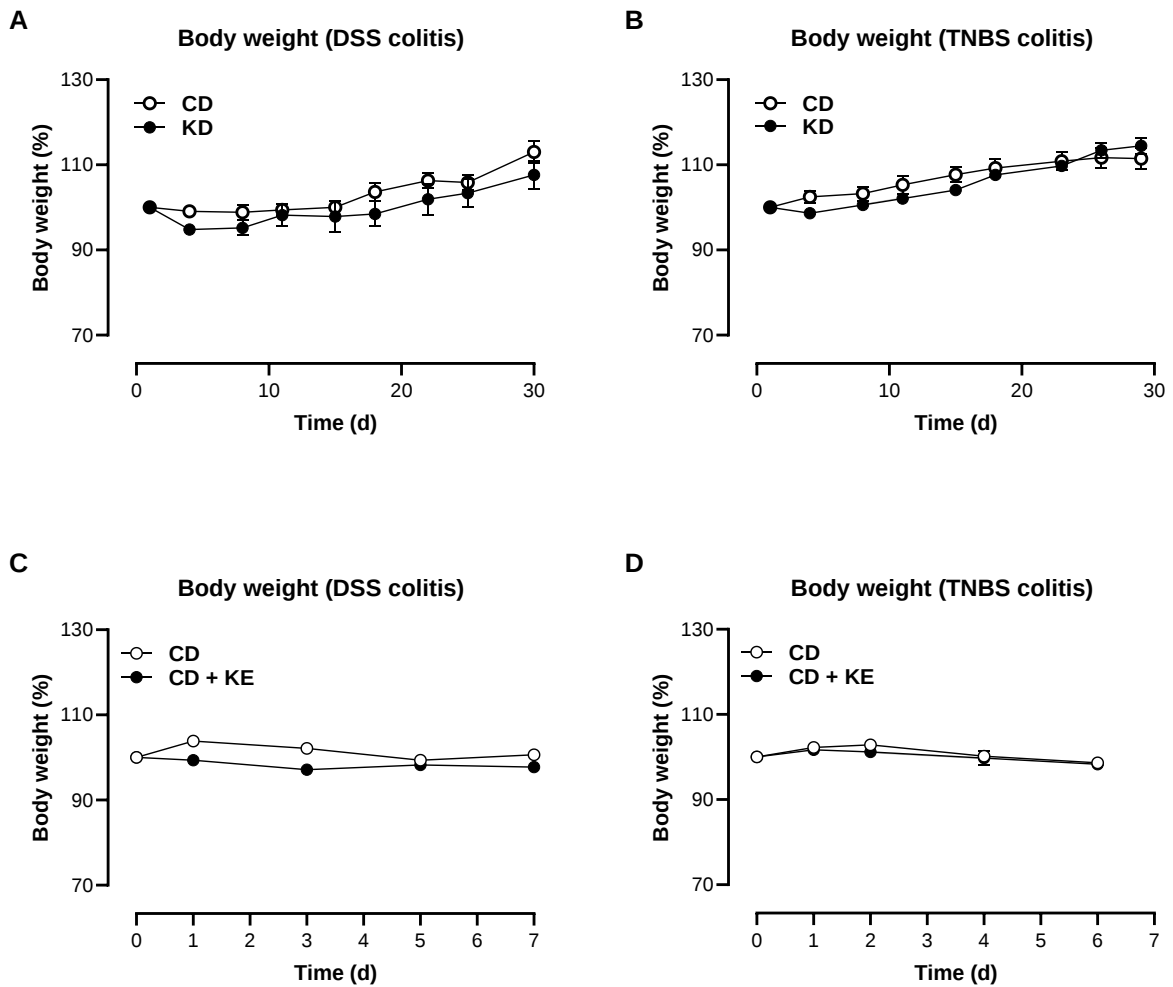


**Supplementary Figure S1 Synthesis of 1,3-butanediol diacetoacetate via transacetoacetylation.** 1,3-butanediol diacetoacetate (c) was synthesized from *tert*-butylacetoacetate (a) and 1,3-butanediol (b) (according to (Desrochers et al., 1995)). In a round bottom flask equipped with a distillation bridge the educts were heated up to 90 °C for 40 min in xylene and *tert*-butanol was removed. The reaction mixture was distilled under reduced pressure to remove remaining educts.

**$^1\text{H}$  and  $^{13}\text{C}$  NMR data of 1,3-butanediol diacetoacetate:**

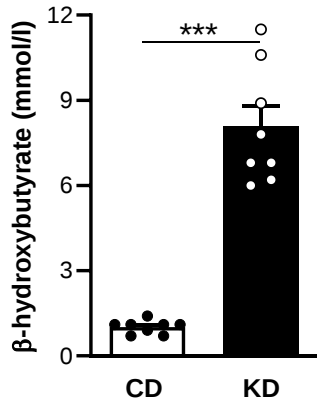
**$^1\text{H}$  NMR** (600 MHz,  $\text{DMSO-d}_6$ ): :  $\delta$  = 1.22 (d,  $J$  = 1.0 Hz, 3H), 1.86 (m, 2H), 2.18 (d,  $J$  = 2.5 Hz, 6H), 3.58 (d,  $J$  = 1.3 Hz, 4H), 4.11 (m, 2H), 4.94 (m, 1H).

**$^{13}\text{C}$  NMR** (150 MHz,  $\text{DMSO-d}_6$ ): :  $\delta$  = 19.6, 29.9, 34.0, 49.4, 49.7, 61.0, 68.3, 166.7, 167.1, 201.5.

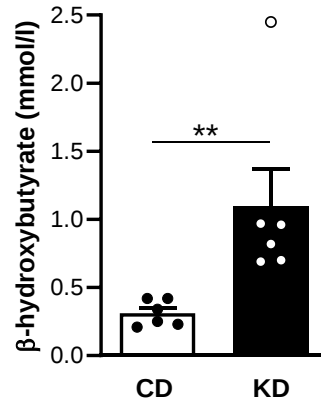


**Supplementary Figure S2 Body weight is not affected by administration of the ketogenic diet (KD) or ketone ester (KE) supplementation before colitis induction.** (A and B) Body weight change during four-week feeding of the KD in female (A,  $n = 4-6$ /group, DSS model) and male (B,  $n = 6$ /group, TNBS model) C57BL/6J mice before induction of colitis. (C and D) Body weight change during one-week KE supplementation in female (C,  $n = 10-11$ /group, DSS model) and male (D,  $n = 6$ /group, TNBS model) C57BL/6J mice before induction of colitis. Results are shown as mean  $\pm$  SEM.

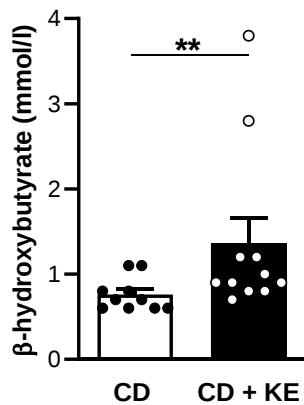
**A Blood ketones (DSS colitis)**



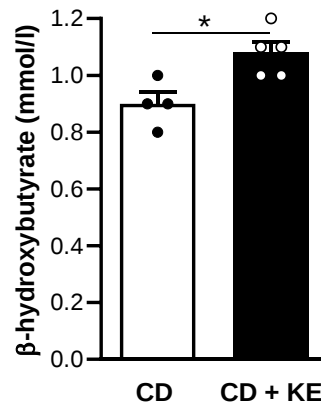
**B Blood ketones (TNBS colitis)**



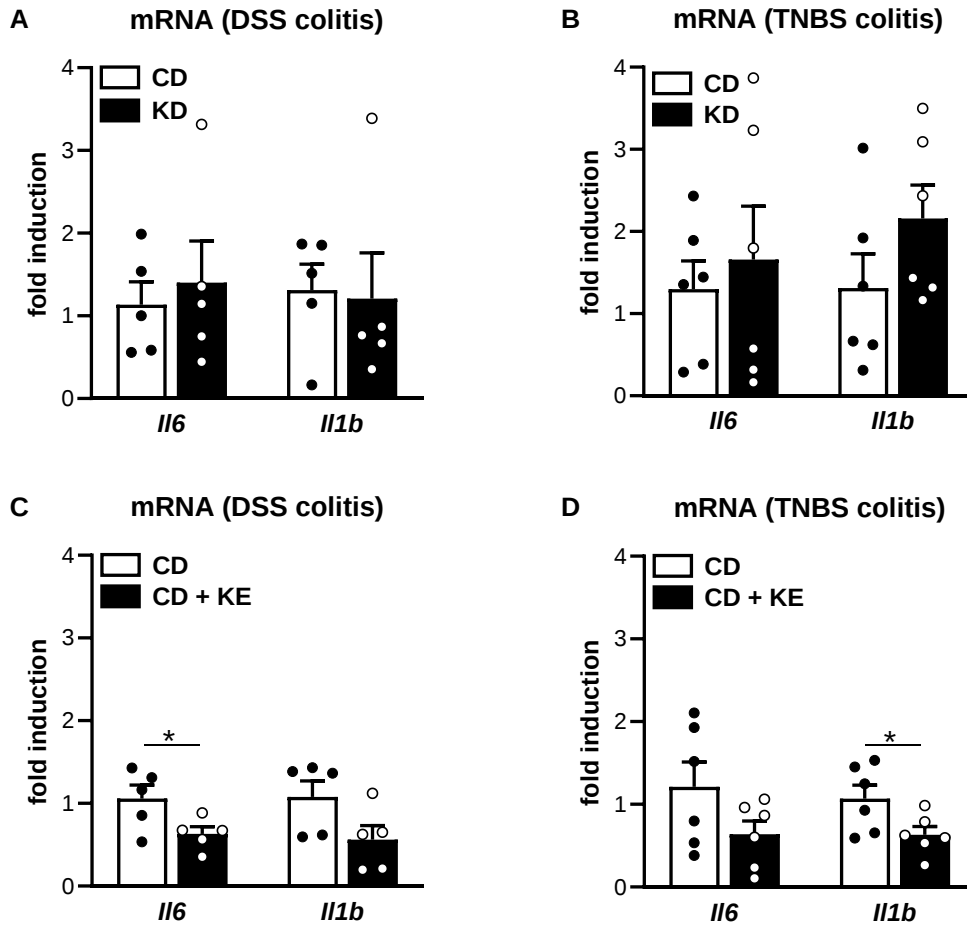
**C Blood ketones (DSS colitis)**



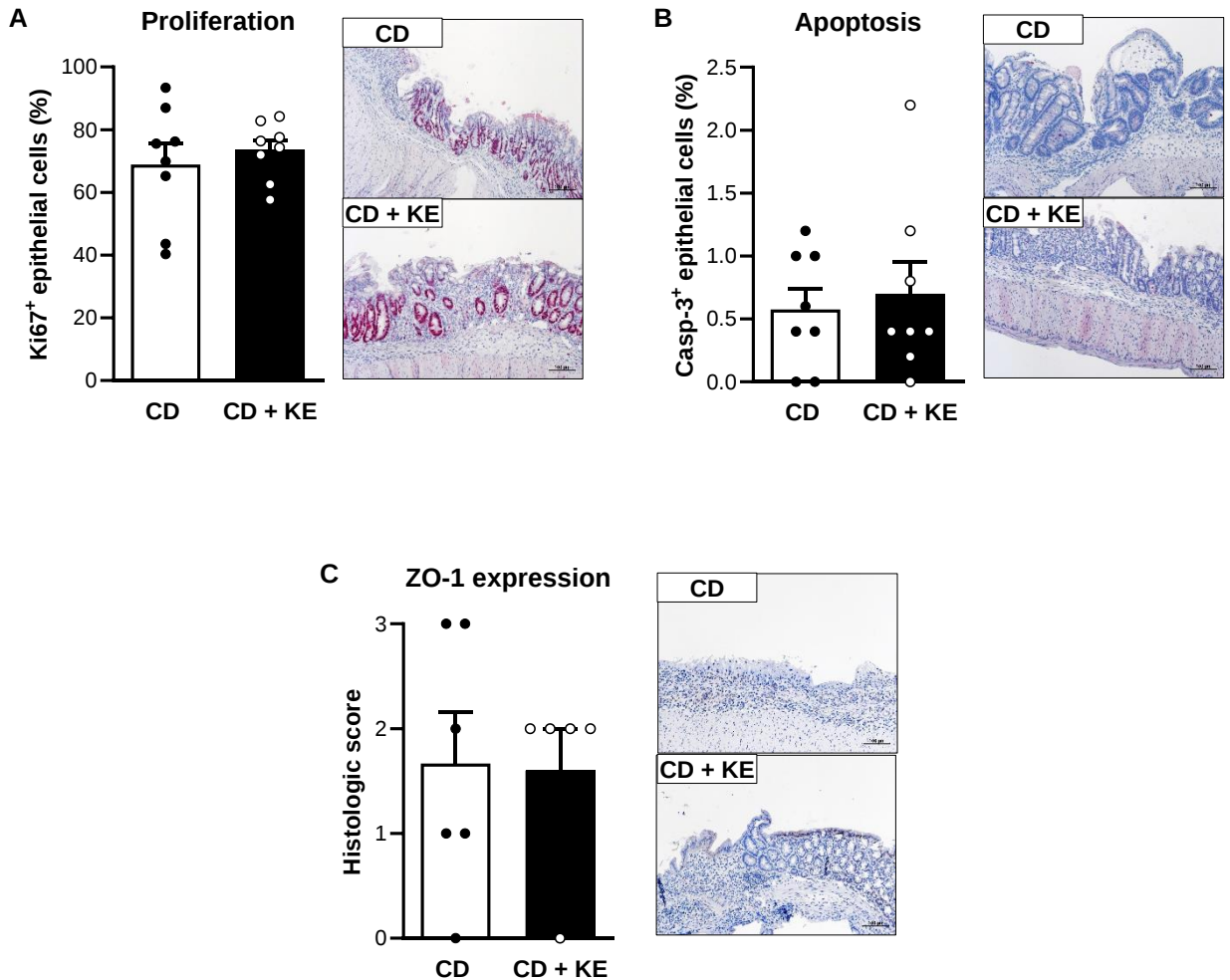
**D Blood ketones (TNBS colitis)**



**Supplementary Figure S3 Administration of the ketogenic diet (KD) and ketone ester (KE) supplementation increases blood  $\beta$ -hydroxybutyrate levels.** (A and B) Blood  $\beta$ -hydroxybutyrate concentration in female (A,  $n = 8/\text{group}$ , DSS model) and male (B,  $n = 6/\text{group}$ , TNBS model) C57BL/6J mice following four-week administration of the KD. (C and D) Blood  $\beta$ -hydroxybutyrate concentration in female (C,  $n = 10\text{-}11/\text{group}$ , DSS model) and male (D,  $n = 4\text{-}5/\text{group}$ , TNBS model) C57BL/6J mice following one-week KE supplementation. Results are shown as mean  $\pm$  SEM. Statistical significance was determined using two-tailed unpaired Student's  $t$  test (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).



**Supplementary Figure S4 Ketone ester (KE) supplementation, but not a ketogenic diet (KD), reduces mRNA levels of cytokines in the acute DSS- and TNBS-induced colitis model.** (A and B) mRNA levels of cytokines *Il6* and *Il1b* in colon tissues of control diet (CD)- and KD-fed mice following DSS (A, n = 5/group) or TNBS (B, n = 6/group) treatment. (C and D) mRNA levels of cytokines *Il6* and *Il1b* in colon tissues of CD- and CD+KE-fed mice following DSS (C, n = 5/group) or TNBS (D, n = 6/group) treatment. Results are shown as mean  $\pm$  SEM. Statistical significance was determined using two-tailed unpaired Student's t test (\* $p$  < 0.05).

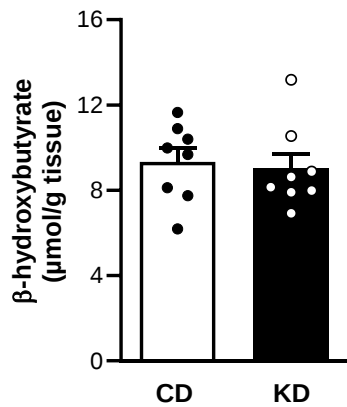


**Supplementary Figure S5 Ketone ester supplementation does not affect proliferation, apoptosis and tight junction protein ZO-1.** (A – C) Immunohistochemical analysis of Ki67 (A, proliferation, n = 8/group), cleaved caspase-3 (B, apoptosis, n = 8/group) and ZO-1 (C, tight junction, n = 5-6/group) staining of colon sections from CD- and CD+KE-fed mice after DSS treatment. Representative images of stained colon sections are shown next to the graphs (magnification x100). Results are shown as mean  $\pm$  SEM.

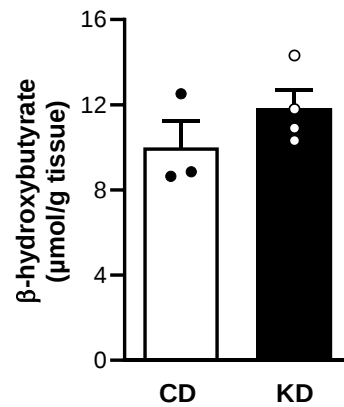




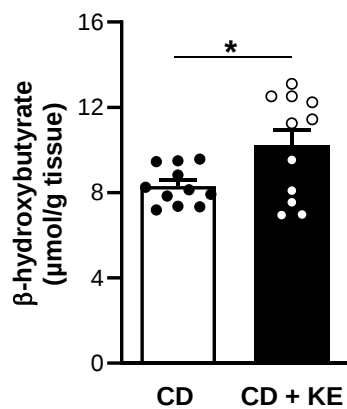
**A Colon ketones (DSS colitis)**



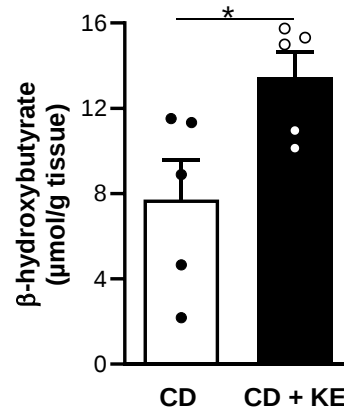
**B Colon ketones (TNBS colitis)**



**C Colon ketones (DSS colitis)**



**D Colon ketones (TNBS colitis)**



**Supplementary Figure S8 Preventive ketone ester supplementation (KE), but not a ketogenic diet (KD), increases  $\beta$ -hydroxybutyrate levels in colon tissues.** (A and B) Colonic  $\beta$ -hydroxybutyrate concentration in female (A,  $n = 8/\text{group}$ , DSS model) and male (B,  $n = 3\text{-}4/\text{group}$ , TNBS model) C57BL/6J mice following four-week administration of the KD. (C and D) Colonic  $\beta$ -hydroxybutyrate concentration in female (C,  $n = 11/\text{group}$ , DSS model) and male (D,  $n = 5/\text{group}$ , TNBS model) C57BL/6J mice following one-week KE supplementation. Results are shown as mean  $\pm$  SEM. Statistical significance was determined using two-tailed unpaired Student's  $t$  test ( $*p < 0.05$ ).