Supplementary Materials: Keratins Are Altered in Intestinal Disease-Related Stress Responses

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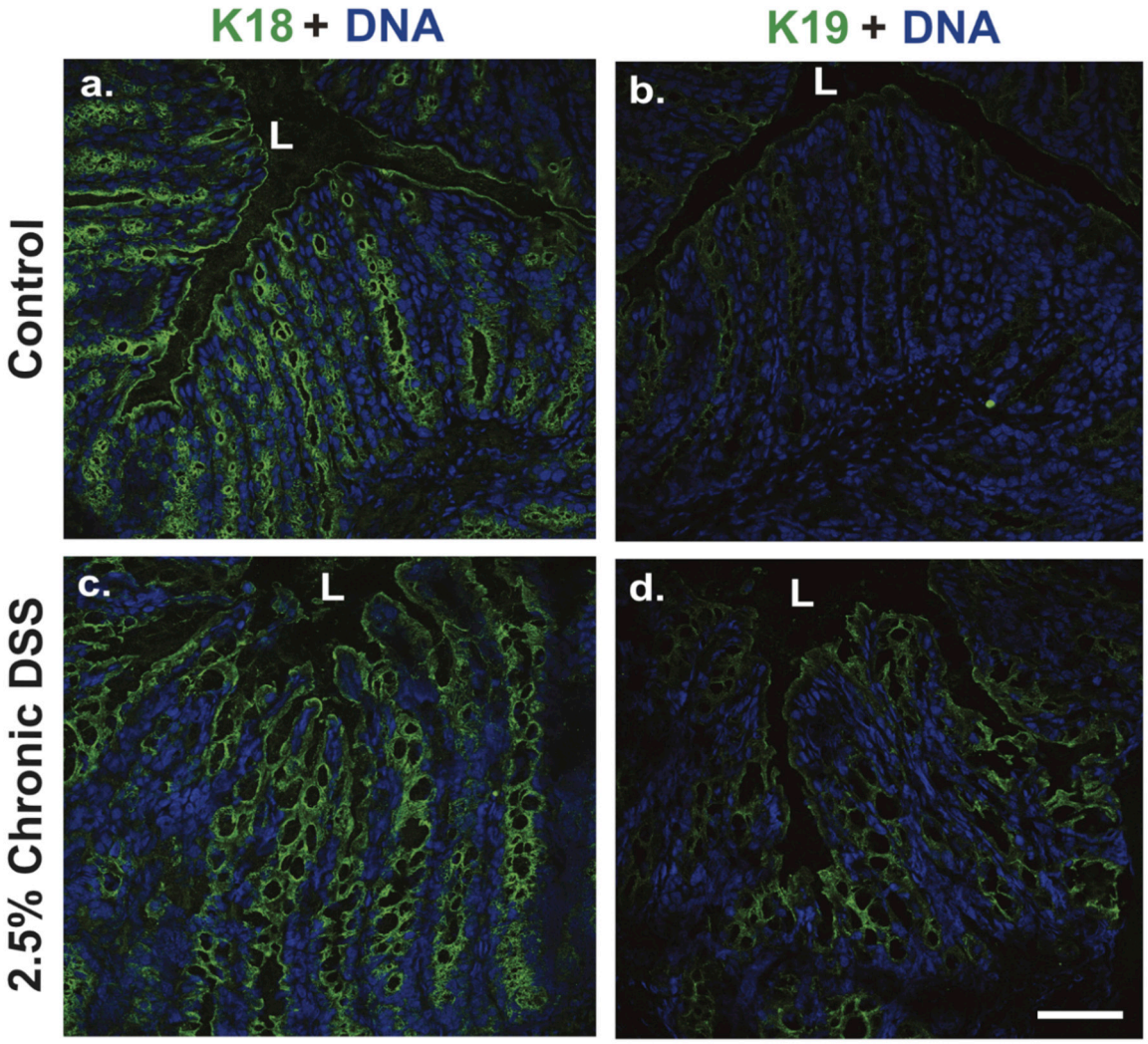

Figure S1. K18 and K19 distribution is not altered in response to chronic DSS-treatment. To induce chronic colitis, mice were given two one-week cycles of 2.5% DSS with two weeks of recovery with normal drinking water after each cycle. Tissue section samples were analyzed for K18 (**a**, **c** in green) and K19 (**b**, **d** in green) by immunofluorescence staining and confocal microscopy. Nuclei (DNA) are stained blue. Baseline crypt-base to -top distribution of K18 and K19 was not altered after 2.5% chronic DSS-treatment. L = Lumen, scale bar = 50 μm.

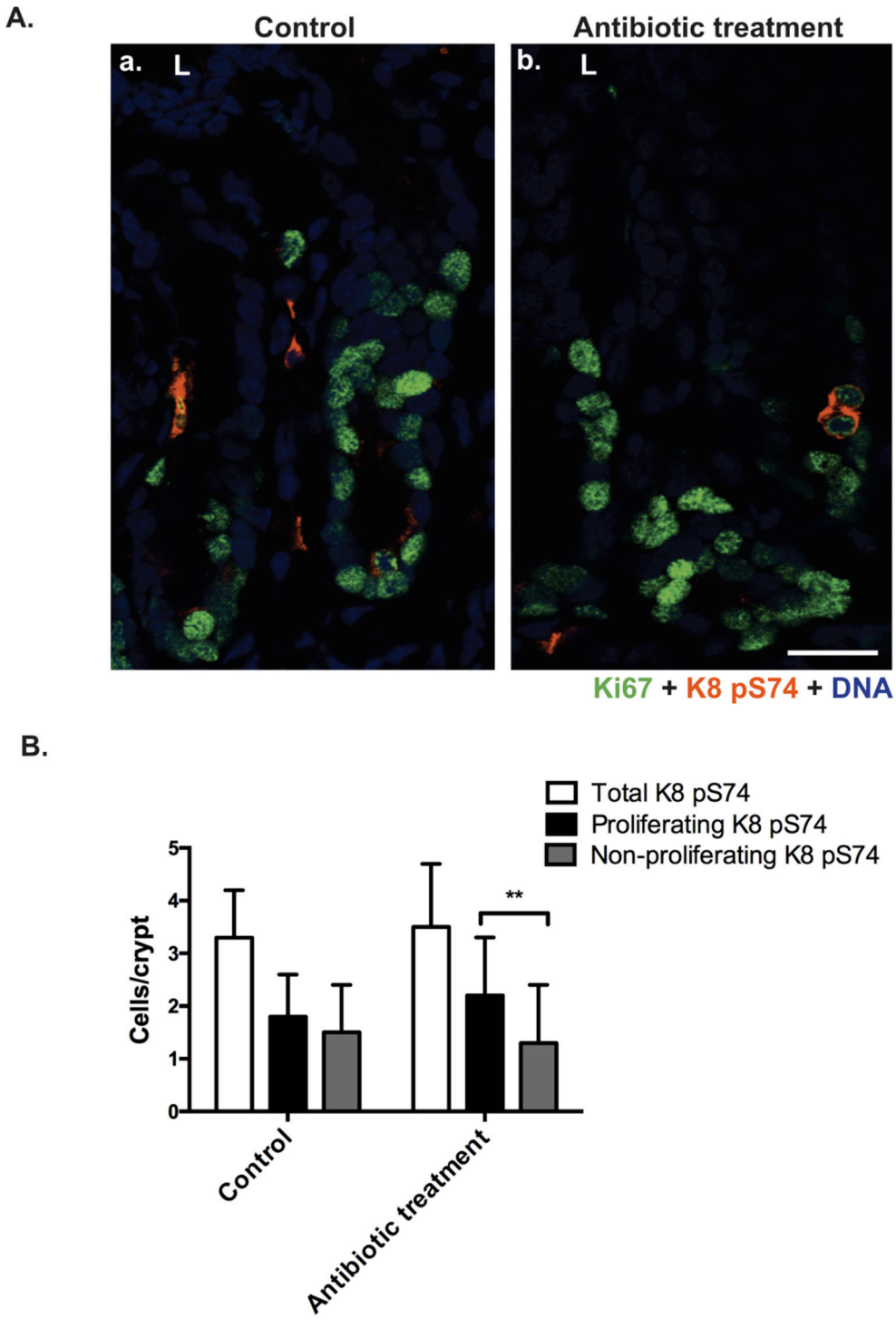

Figure S2. A slight increase in K8 pS74 was seen in proliferating cells compared to non-proliferative cells following antibiotic treatment. Control mice (Aa) were treated with oral broad-spectrum antibiotics (**A**b) for 56 days, samples for immunofluorescence analysis were collected at the end of the treatment, and (**A**) immunofluorescence staining of K8 pS74 (red) and the proliferation marker Ki67 (green) were analyzed. Proliferating (red and green cells) and non-proliferating K8 pS74-positive cells (red only cells) in the bottom of the crypts were imaged and quantified (**B**). The number of proliferating K8 pS74-positive cells/crypt (black bars) is increased compared to K8 pS74-positive non-dividing cells (gray bars). The total number of K8 pS74 cells is shown in white bars. The data is shown as averages ± SD and ** $p < 0.01$. Statistical significance was determined by t-test. Nuclei (DNA) are shown in blue. L = Lumen, scale bar = 50 μm

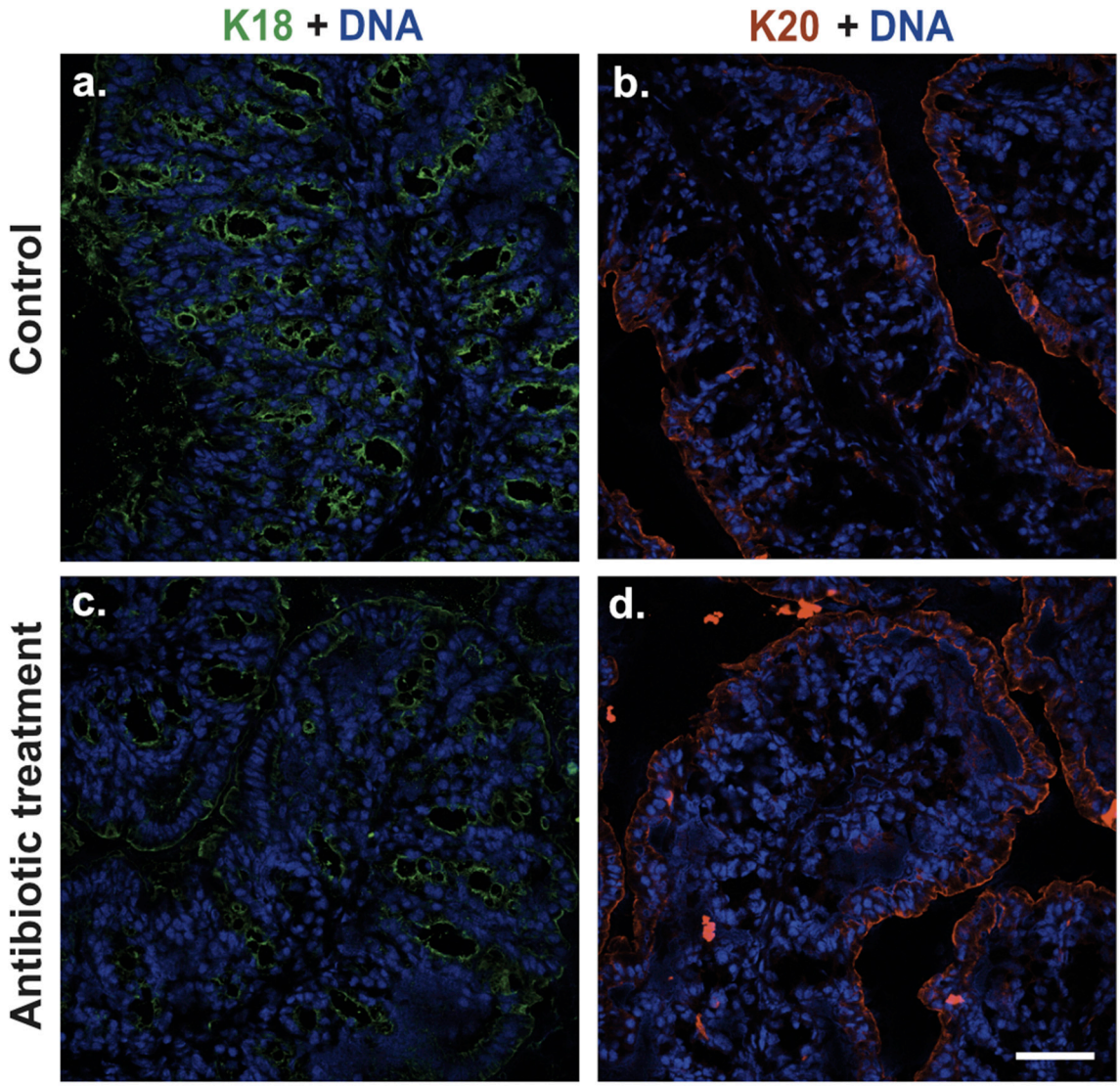

Figure S3. The distribution of K18 and K20 is unaltered in response to antibiotic treatment. Mice (n = 3 for controls, n = 4 for antibiotic-treated) were treated with oral broad-spectrum antibiotics for 56 days. Samples for immunofluorescence analysis were collected at the end of the treatment, and keratin distribution was analyzed in response to the depletion of microbiota. (**a**,**b**) The wide distribution of K18 (green) is unchanged in response to antibiotic treatment. (**c**,**d**) K20 (red) is found at the top of the crypts both under baseline conditions and after antibiotic treatment. Nuclei (DNA) are shown in blue. L = Lumen, scale bar = 50 μm.

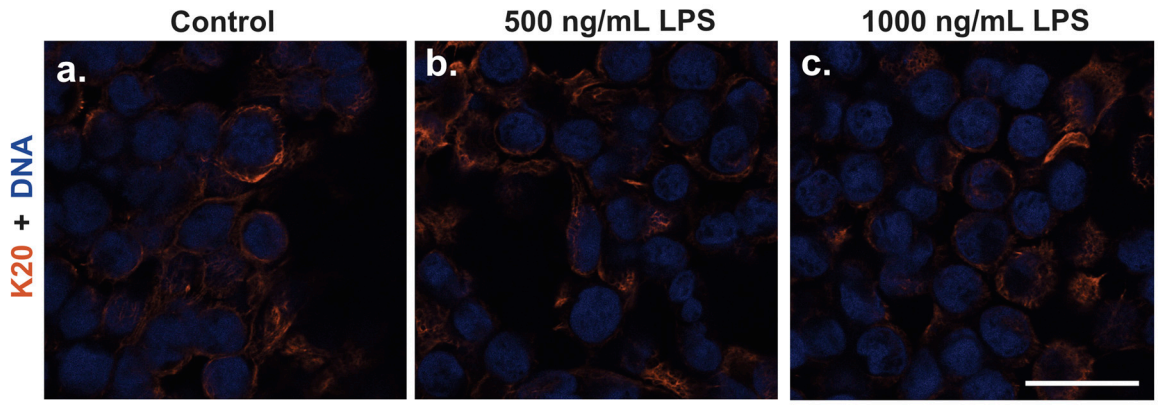

Figure S4. The amount and distribution of K20 in LPS-treated HT-29 cells is unchanged. Colorectal cancer HT-29 cells were treated with 500 ng/mL or 1000 ng/mL LPS for 48 h to induce inflammatory stress signaling prior to sample collection. The levels and distribution of K20 assessed by immunofluorescence staining and confocal microscopy in LPS-treated HT-29 cells (**b**,**c**) were unchanged compared to control cells (**a**). Nuclei (DNA) are shown in blue. Scale bar = 25 μm.