

1 Supplementary Materials for

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3 **Neuropeptide substance P attenuates colitis by suppressing**
4 **inflammation and ferroptosis via the cGAS-STING signaling**
5 **pathway**

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14 **This PDF file includes:**

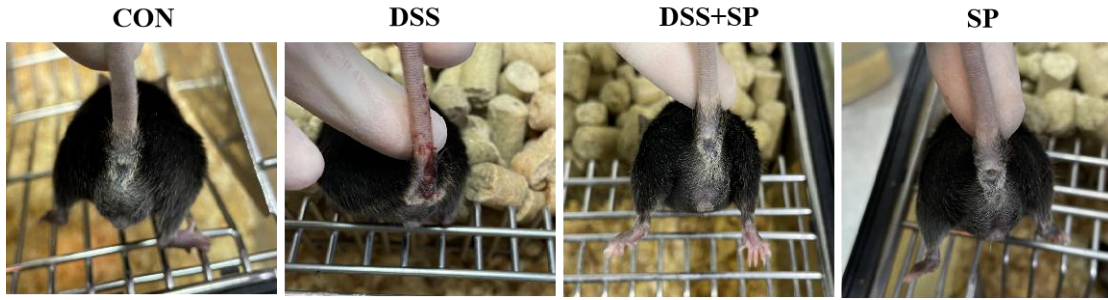
15 Figs. S1 to S5

16 **Other Supplementary Materials for this manuscript include the following:**

17 Table S1 to S2

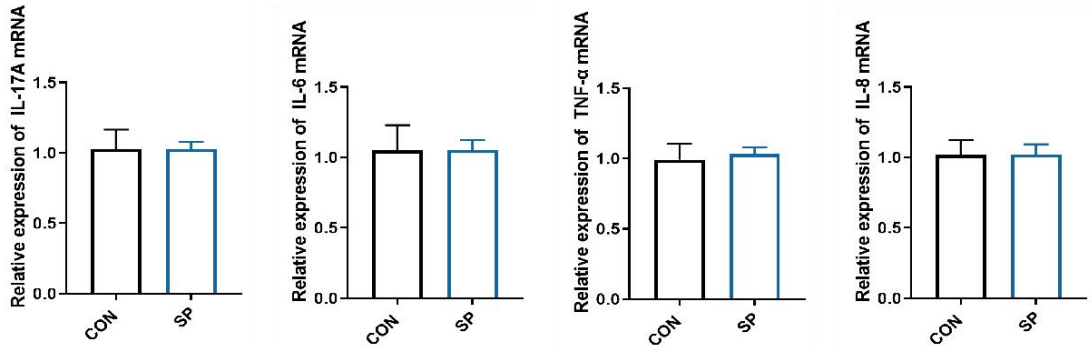
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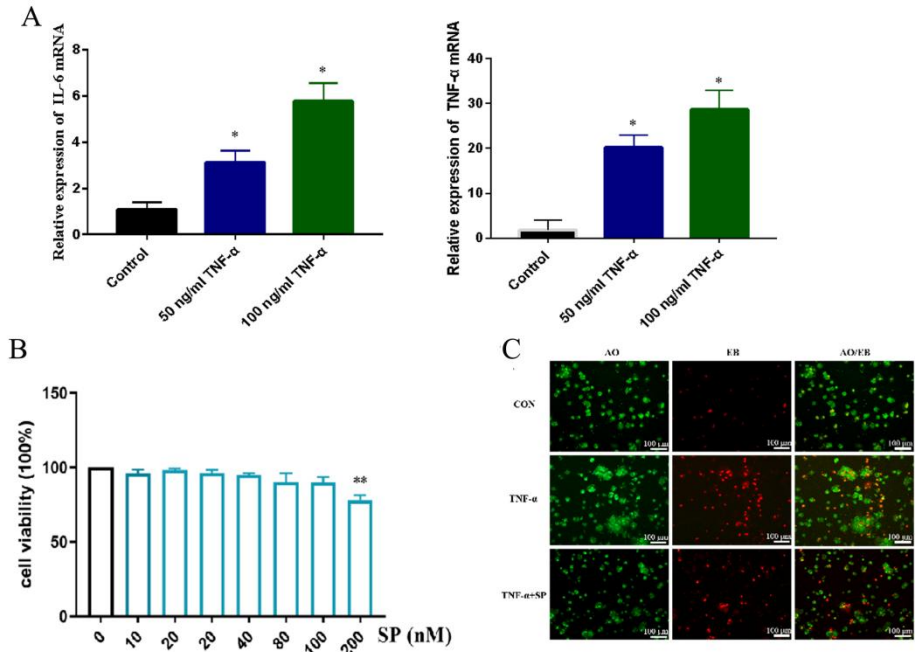
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Figure S1 The representative clinical observation and anatomical observation in CON group, DSS group, DSS+SP group, and SP group.



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Figure S2 The effects of SP on inflammatory factors in colon tissue. The mRNA levels of IL-17A, IL-6, TNF-α and IL-8 in CON and SP alone group. Data are expressed as the mean ± SEM; n = 10.

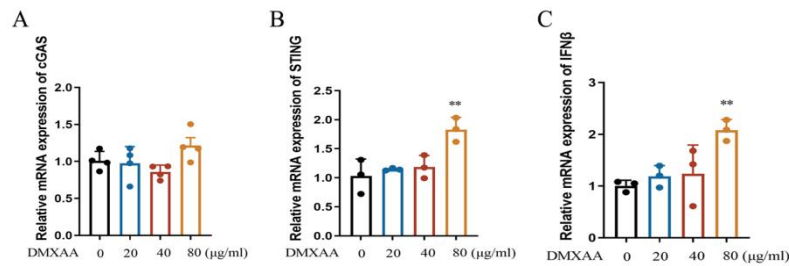


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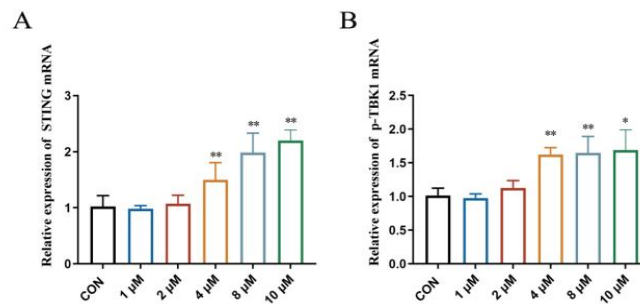
Figure S3 The effect of SP on cell viability and apoptotic cells induced by TNF-α in Caco-2 cells. (A) The mRNA levels of IL-6 and TNF-α in Caco-2 cells treated with TNF-α at the concentration of 50 and 100 ng/ml. (B) The effect of SP on cell viability.

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33 (C) Apoptotic cells were examined by AO/EB staining in Caco-2 cells. Data are
 34 expressed as the mean± SEM; n = 4. * $p < 0.05$, ** $p < 0.01$ vs. the control group.
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 37 **Figure S4** Caco-2 cells were treated with different doses of DMXAA. (A-C) Relative
 38 expression of cGAS, STING and IFNβ after DMXAA treatment at the concentration
 39 of 20, 40 and 80 μg/mL. Data are expressed as the mean ± SEM; n = 4. ** $p < 0.01$ vs.
 40 the control group.
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 43 **Figure S5** Caco-2 cells were treated with different concentrations of SR717. (A, B)
 44 Relative expression of STING and p-TBK1 after SP717 treatment at the concentration
 45 of 1, 2, 4, 8 and 10 μM. Data are expressed as the mean ± SEM; n = 4. * $p < 0.05$ and
 46 ** $p < 0.01$ vs. the control group.
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Table S1 Sequence information of primers for mice colon tissue

| Gene (mice) | Forward Primer (5' - 3') | Reverse Primer (5' - 3') |
|---------------|-----------------------------|---------------------------|
| SP | GCGACCAGATAAAGGAGGAGCTGC | CTGCTGAGGCTTGGGTCTCCG |
| IL-1 β | GCTGCTTCCAAACCTTTGAC | AGCTTCTCCACAGCCACAAT |
| IL-6 | TAGTCCTTCTACCCCAATTTCC | TTGGTCCTTAGCCACTCCTTC |
| IL-17A | CACAGCCTCAGAGTCCTTCAT | ACCTCCGCATTAGCACAGA |
| TNF- α | CCGATGGGTTGTACCTTGTC | AGATAGCAAATCGGCTGACG |
| IL-8 | GCAGAGGGTTGTGGAGAAGT | CCCTACAACAGACCCACACA |
| IL-10 | ACTGGCTGTTGCCTTCTTG | TGGGGTGGAAAGGTGTGGAA |
| IL-4 | CCCCAGCTAGTTGTCATCCTG | CAAGTGATTTTTGTCGCATCCG |
| ZO-1 | CCACCTCTGTCCAGCTCTTC | CACCGGAGTGATGGTTTTCT |
| Claudin-2 | GTCATCGCCCATCAGAAGAT | ACTGTTGGACAGGGAACCAG |
| Occludin | ATGTCCGGCCGATGCTCTC | TTTGGCTGCTCTTGGGTCTGTAT |
| cGAS | ATGTTTAAACTGGAAGTCCCC | AAGAAGTGTTACAGCAGGGCT |
| STING | GGTCACCGCTCCAAATATGTAG | CAGTAGTCCAAGTTCGTGCGA |
| p-IRF3 | CGAAGTGGGAGACAGGACGC | GAGCAGCTCCTCGCTCACTG |
| IFN β | TGGGTGGAATGAGACTATTGTTGTACG | CAAGTGGAGAGCAGTTGAGGACATC |
| CXCL10 | AAGTGCTGCCGTCATTTTCT | GTGGCAATGATCTCAACACG |
| CCL5 | CCTCTATCCTAGCTCATCTCCA | ATTTTCTGCCTCATCCTGCT |
| GPX4 | TTCTCAGCCAAGGACATCG | CACTCAGCATATCGGGCAT |
| FTH1 | GCCGAGAAACTGATGAAGCTGC | GCACACTCCATTGCATTCAGCC |
| SLC7A11 | GATGCTGTGCTTGGTCTTGA | GCCTACCATGAGCAGCTTTC |
| PTGS2 | AAATGCTGGTGTGGAAGGT | TTGTTGCTCTAGGCTTTGCT |
| mtDNA | CTAGCAGAAACAAACCGGGC | CCGGCTGCGTATTCTACGTT |
| 18S rRNA | CCGCCGCCATGTCTCTAGT | CTTTCCTCAACACCACATGAGC |
| mtCytb1 | AATCCACTAAACACCCACCC | GCTTCGTTGCTTTGAGGTATGA |
| mtCOXII | ATAACCGAGTCGTTCTGCCAAT | TTTCAGAGCATTGGCCATAGAA |
| mtND1 | GGATCCGAGCATCTTATCCA | GGTGGTACTCCCTCTGTAAA |
| GAPDH | TTCCTACCCCAATGTATCCG | CATGAGGTCCACCACCCTGTT |

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Table S2 Sequence information of primers for Caco-2 cells

| Gene (Human). | Forward Primer (5' - 3') | Reverse Primer (5' - 3') |
|----------------------|---------------------------------|----------------------------------|
| IL-6 | CATCACTGGTCTTTTGGAG | TGGATCAGGACTTTTGTAC |
| IL-17A | AATCTCCACCGCAATGAGGA | ACGTTCCCATCAGCGTTGA |
| TNF- α | TCTCTCAGCTCCACGCCATT | CCCAGGCAGTCAGATCATCTTC |
| IL-1 β | AAACAGATGAAGTGCTCCTTCCAGG | TGGAGAACACCACTTGTTGCTCCA |
| IL-8 | AGGAACCATCTCACTGTGTG | ATTGGGGTGGAAAGGTT |
| cGAS | AAGGATAGCCGCCATGTTTCT | TGGCTTTCAGCAAAAGTTAGG |
| STING | GCGGCTGTATATTCTCCTCCCATTG | TGCTGTTGCTGTAAACCCGATCC |
| p-IRF3 | CTGGGGCCCTTCATTGTAGAT | GGCACAACCTTGACCATCAC |
| IFN β | TGTCGCCTACTACCTGTTGTGC | AACTGCAACCTTTCGAAGCC |
| CXCL10 | CTTCCAAGGATGGACCACAC | AGCAGGGTCAGAACATCCAC |
| CCL5 | CCAGCAGTCGTCTTTGTAC | CTCTGGGTTGGCACACACTT |
| mtND1 | GCTACGACCAACTCATA | GAATGCTGGAGATTGTAATG |
| mtND2 | CACAGAAGCTGCCATCAAGTA | CCGGAGAGTATATTGTTGAAGAG |
| mtCOXII | ATGACCCACCAATCACATGC | ATCACATGGCTAGGCCGGAG |
| COX-2 | CGGTGAAACTCTGGCTAGACAG | GCAAACCGTAGATGCTCAGGGA |
| PTGS2 | CCTGTGCCTGATGATTGC | CTGATGCGTGAAGTGCTG |
| SLC7A11 | CCCAGATATGCATCGTCCTT | CCTGGGTTTCTTGTTCCATA |
| ACSL4 | GCTTCCTATCTGATTACCAGTGTTGA | GTCCACATAAATGATATGTTTAAACACAACCT |
| GPX4 | AGATCCACGAATGTCCCAAG | CCTCCTCCTTAAACGCACAC |
| FTH1 | CCATCAACCGCCAGATCAACCTG | GTTTCTCAGCATGTTCCCTCTCCTC |
| GAPDH | TGGAGCTGCAGAGGATGATTG | CCCAGTTGAAGTTGCCGTCAG |