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Supplemental information

Deficiency of miRNA-149-3p shaped gut microbiota and enhanced dextran sulfate sodium-induced colitis

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Table S1. The information of antibodies

Antibodies	Source	Identifier	Dilution	Origin
p-I κ B α (Ser32/36)	Cell Signaling Technology	9246s	1:1000	Danvers, USA.
I κ B α	Cell Signaling Technology	9242s	1:1000	Danvers, USA.
p-AMPK α (Thr172)	Cell Signaling Technology	2531s	1:1000	Danvers, USA.
AMPK α	Cell Signaling Technology	2532s	1:1000	Danvers, USA.
β -actin	TransGen Biotech	HC201	1:1000	Beijing, China
Anti-Rabbit IgG	LABLEAD	S0101	1:2000	Beijing, China
Anti-Mouse IgG	LABLEAD	S0100	1:2000	Beijing, China

Table S2. Primers for qRT-PCR

Species	Gene	Forward primer	Reverse primer
Human	<i>ACTB</i>	ACTGGGACGACATGGAGAAA	CTGGATAGCAACGTACATGG
	<i>CCL22</i>	GCGTGGTGTGCTAACCTTC	GAGGTGACCAAGGGTGACAG
	<i>CCL5</i>	CCAGCAGTCGTCTTTGTCAC	CTCTGGGTTGGCACACACTT
	<i>COX2</i>	CAATCATTTCCTCCTGTGCCTGATGATTG	GTGCTGGGCAAAGAATGCAA
	<i>CXCL17</i>	GATTGGTTCCTGAGAGCCCC	GGTGCCTTTGGTGTCTTGTTT
	<i>ICAM1</i>	GAGCTGTTTGAGAACACCTC	ATAGGTGACTGTGGGGTTCA
	<i>IL17A</i>	ATCTCCACCGCAATGAGGAC	CTTGCTGGATGGGGACAGAG
	<i>IL1A</i>	GGAGCTTGTCAACCCAAACT	TCCGAAGTCAAGGGGCTAGA
	<i>IL1B</i>	AACCTCTTCGAGGCACAAGG	CATTGCCACTGTAATAAGCCATCA
	<i>IL6</i>	GATGAGTACAAAAGTCCTGA	GCCCATGCTACATTTGCCGA
	<i>CXCL8</i>	CTTTCAGAGACAGCAGAGCAC	GTTCTTTAGCACTCCTTGGC
	<i>NOS2</i>	GATAAGTGACATAAGTGACC	CTGAGGTTGTGATACTGAAG
	<i>CXCL10</i>	TCCACGTGTTGAGATCATTGCT	TGCATCGATTTTGCTCCCCT
	<i>CCL2</i>	TAGCAGCCACCTTCATTCCC	TGCTTGGGGTCAGCACAGAT
	<i>MMP1</i>	TTCAACCAGGCCAGGTATT	TGTCATCCTGAGCTAGCTGA
	<i>MMP12</i>	TGGCAAAGGTGGAATCCTAG	CATTACGGCCTTTGGATCAC
	<i>MMP2</i>	AATACCATCGAGACCATGCG	GATCAGGTGTGTAGCCAATG
	<i>MMP7</i>	AGGCATGAGTGAGCTACAGT	CACATCTGGGCTTCTGCATT
<i>TNFA</i>	GCGCTC CCAAGAAGA CAGG	GCTGATTAGAGAGAGGTCCC	
Mouse	<i>36b4</i>	GCCCTGCACTCTCGCTTCT	CAACTGGGCACCGAGGCAACAGTTG
	<i>Ptgs2</i>	TGGTGCCTGGTCTGATGATG	GCAATGCGGTTCTGATACTG
	<i>Icam1</i>	TGTGACCAGCCCAAGTTGTT	TGGAGTCCAGTACACGGTGA

<i>Cxcl1</i>	ACTCAAGAATGGTCGCGAGG	GTGCCATCAGAGCAGTCTGT
<i>Tgfb1</i>	GTCACTGGAGTTGTACGGCA	GGGGCTGATCCCGTTGATTT
<i>Il6</i>	AAAACAATCTGAAACTTCCA	CAGAAGACCAGAGGAAATTT
<i>Il2</i>	GGAACCTGAAACTCCCCAGG	AATCCAGAACATGCCGCAGA
<i>Ccl2</i>	ATGCTTCTGGGCCTGCTGTT	CAGCTTCTTTGGGACACCTG
<i>Tgfb2</i>	CCCCGGAGGTGATTTCCATC	ATGGCATTTCGGAGGGGAG
<i>Cxcl10</i>	ATGACGGGCCAGTGAGAATG	GAGGCTCTCTGCTGTCCATC
<i>Mmp12</i>	TGATGGCAAAGGTGGTACAC	CCAAGGAATGGCCAAGTTCA
<i>Mmp13</i>	GGCTCCGAGAAATGCAATCT	CCACTTCAGAATGGGACATA
<i>Ccl5</i>	GCTGCTTTGCCTACCTCTCC	TCGAGTGACAAACACGACTGC
<i>Il1a</i>	TCCCTCAACCAAATATAT	ACGGGCTGGTCTTCTCCTTG
<i>Tnfa</i>	CATCAGTTCTATGGCCAGAC	GGAGTAGACAAGGTACAACCC
<i>Mmp2</i>	CATCGCCCATCATCAAGTTC	ATGGTCTCGATGGTGTCTG

Figure S1

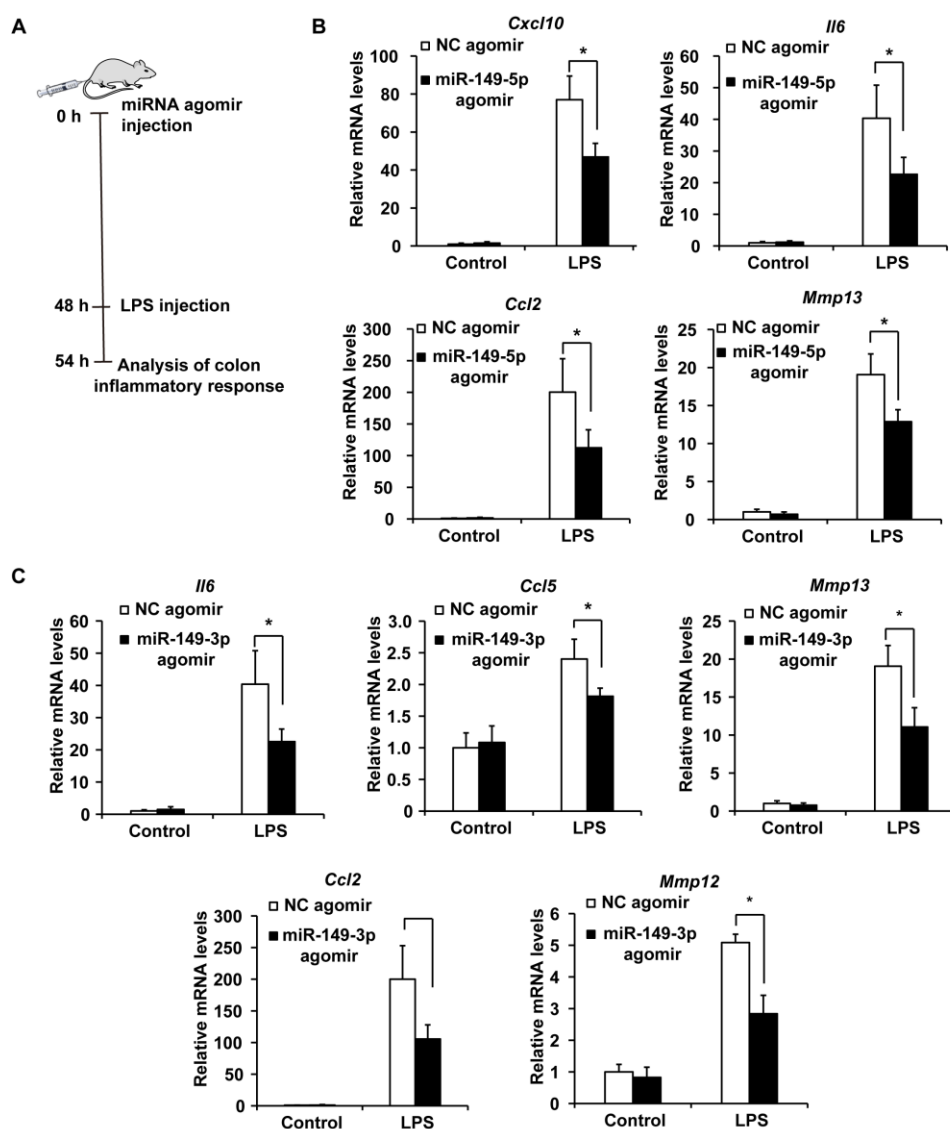


Figure S1. miR-149-5p and miR-149-3p regulate LPS-induced colon inflammatory response *in vivo*. (A) Schematic diagram of the experimental process. (B) After LPS administration for 6h, relative mRNA levels of pro-inflammatory genes from pretreated mouse colon with miRNA negative control and miR-149-5p agomirs ($n=6$). (C) miR-149-3p agomirs repressed LPS-induced inflammatory gene levels in mouse colon tissues ($n = 6$). $*P < 0.05$.

Figure S2

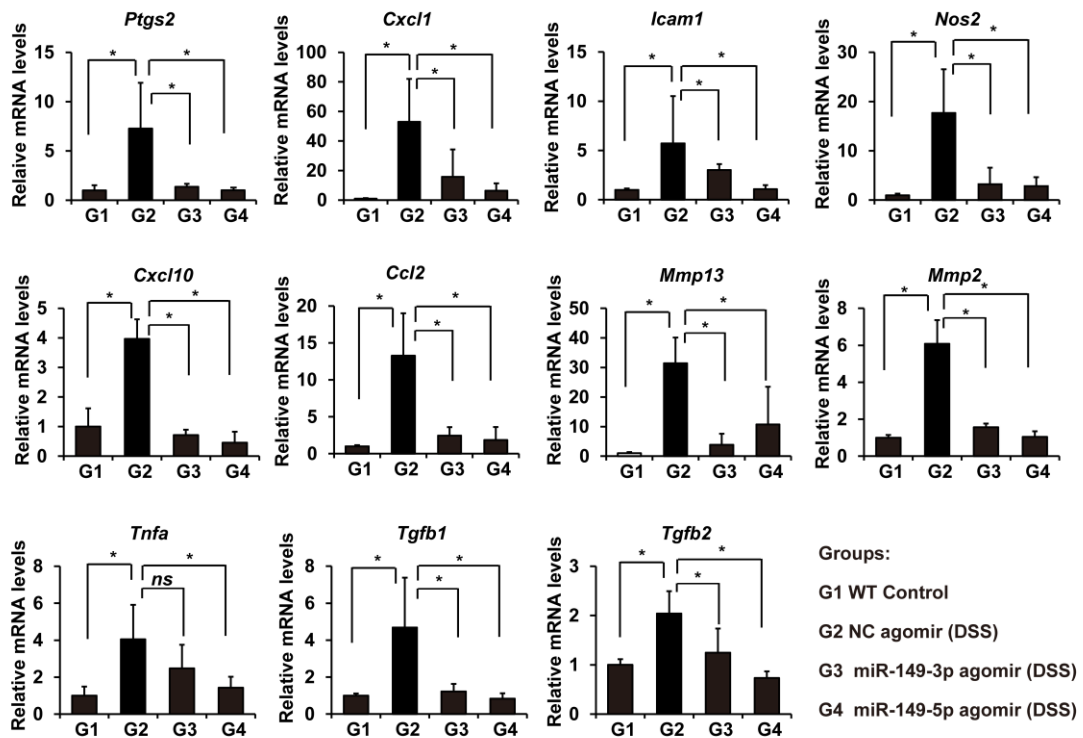


Figure S2. miR-149-5p and miR-149-3p agomirs regulate DSS-induced proinflammatory genes in mouse colons ($n = 5$), $*P < 0.05$; *ns*, no significant difference.

Figure S3.

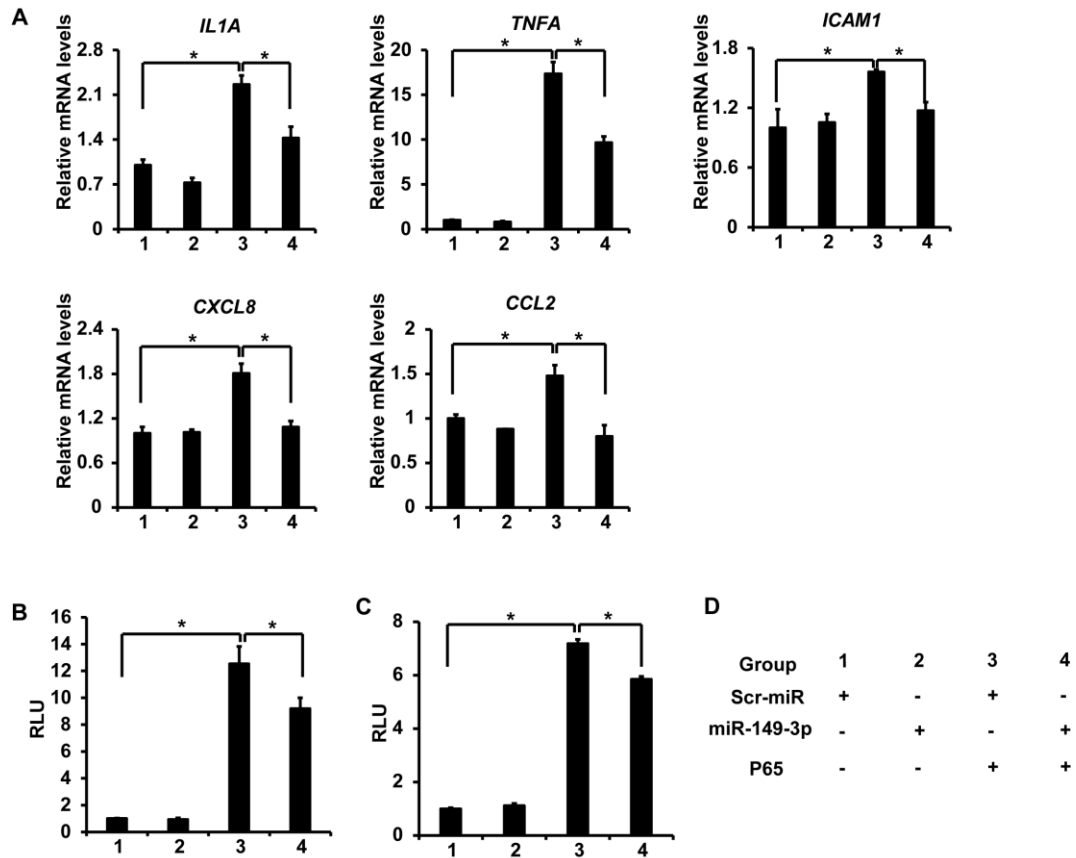


Figure S3. miR-149-3p antagonized NF- κ B cell signaling pathway induced by p65 overexpression in colon adenocarcinoma cells. (A) Relative mRNA levels of NF- κ B-mediated inflammatory cytokines induced by p65 overexpression are reduced upon miR-149-3p mimics in Caco2 cells (n=3). (B-C) miR-149-3p mimics lessened the luciferase signal of NF- κ B activity induced by p65 overexpression in Caco2 (B) and SW480 cells (C) (n=3). RLU, relative luciferase units. (D) Group information. * $P < 0.05$.

Figure S4.

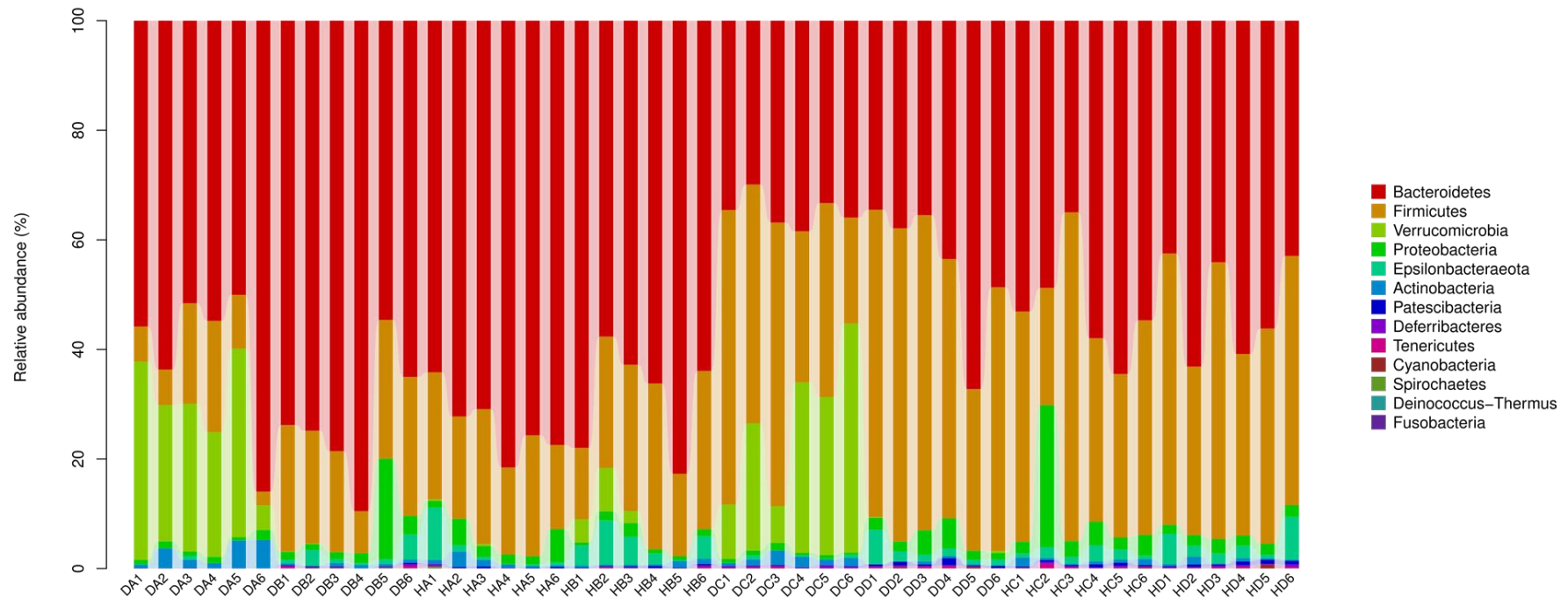


Figure S4. Fecal microbiota taxa analysis from WT and miR-149^{*-/} mice at phylum. To allow natural transfer of the microbiota, WT and miR-149^{*-/} males at 4-weeks were co-housed. As a control, single type control group was fed at the same conditions when the mice reached 8 weeks, then 2% of DSS was given for 7 days, fecal microbiota were analyzed based on 16S RNA sequencing (n=6). DA1-DA6, WT control group (WT), DB1-DB6, miR-149^{*-/} control group (KO), HA1-HA6, WT mice co-housed with miR-149^{*-/} mice (CH-WT), HB1-HB6, miR-149^{*-/} mice co-housed with WT mice (CH-KO), DC1-DC6, DSS treated WT

control group (WT (DSS)), DD1-DD6, DSS-treated miR-149*^{-/-} control group (WT (DSS)), HC1-HC6, DSS-treated CH-WT group (CH-WT (DSS)), HD1-HD6, DSS-treated CH-KO group (CH-KO (DSS)).

Figure S5.

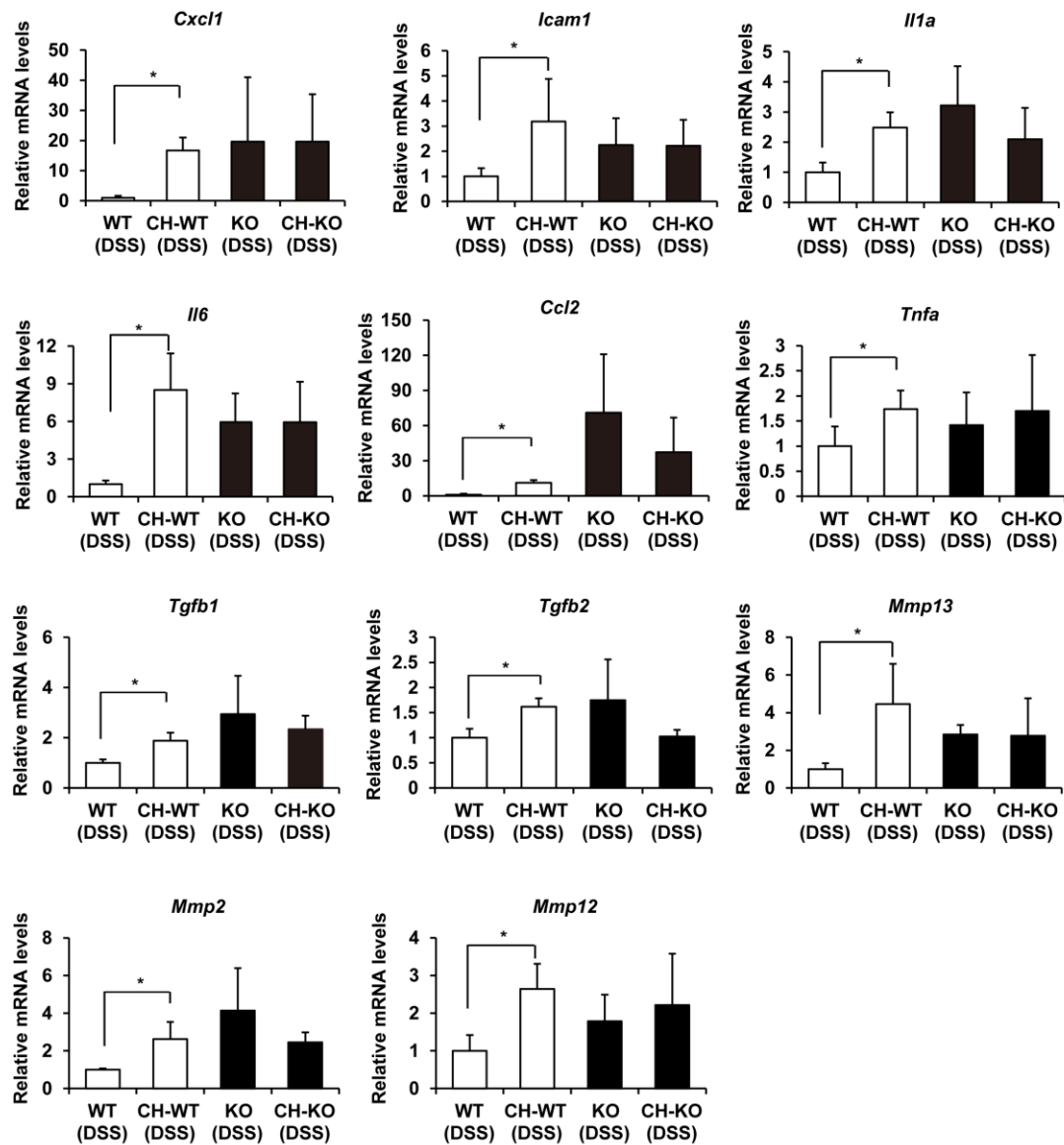


Figure S5. Relative mRNA levels of inflammatory cytokines induced by DSS administration are increased in CH-WT mice (n= 5). * $P < 0.05$.