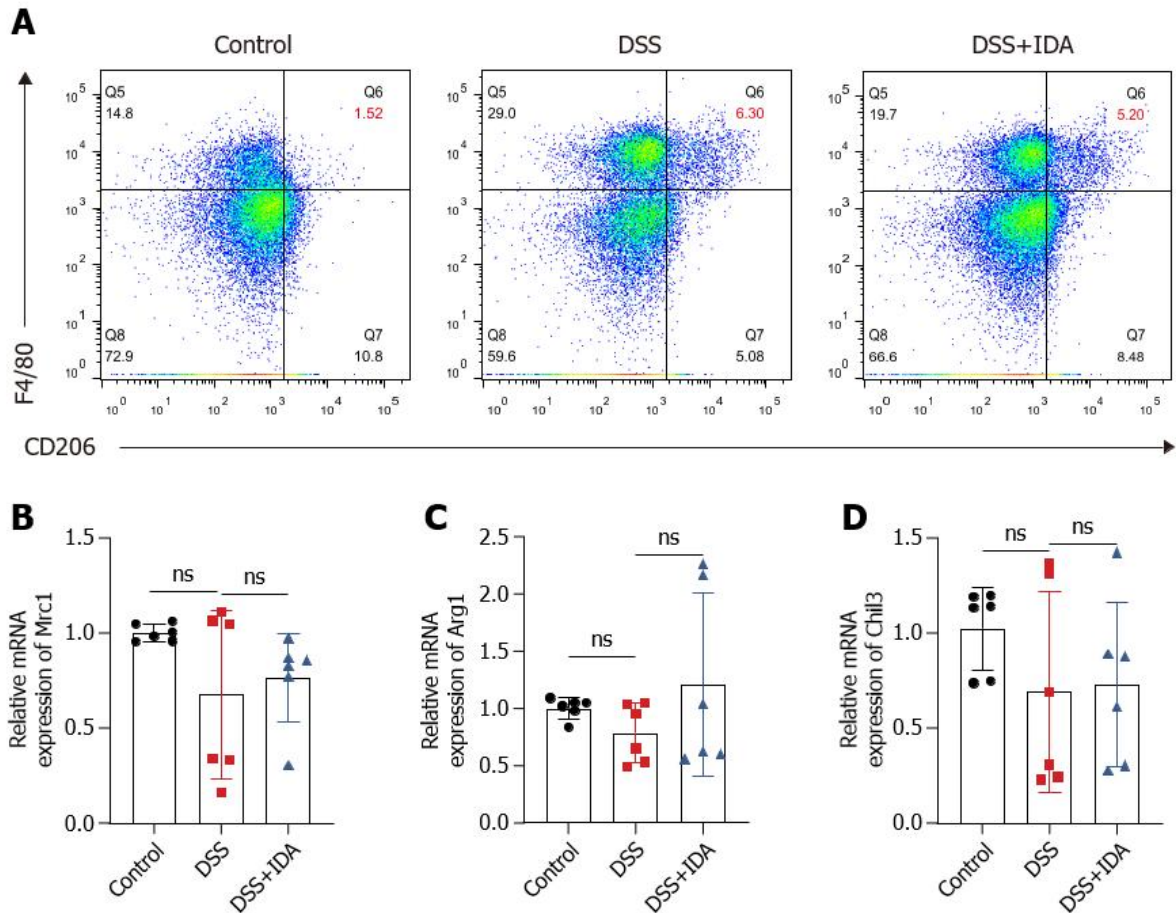
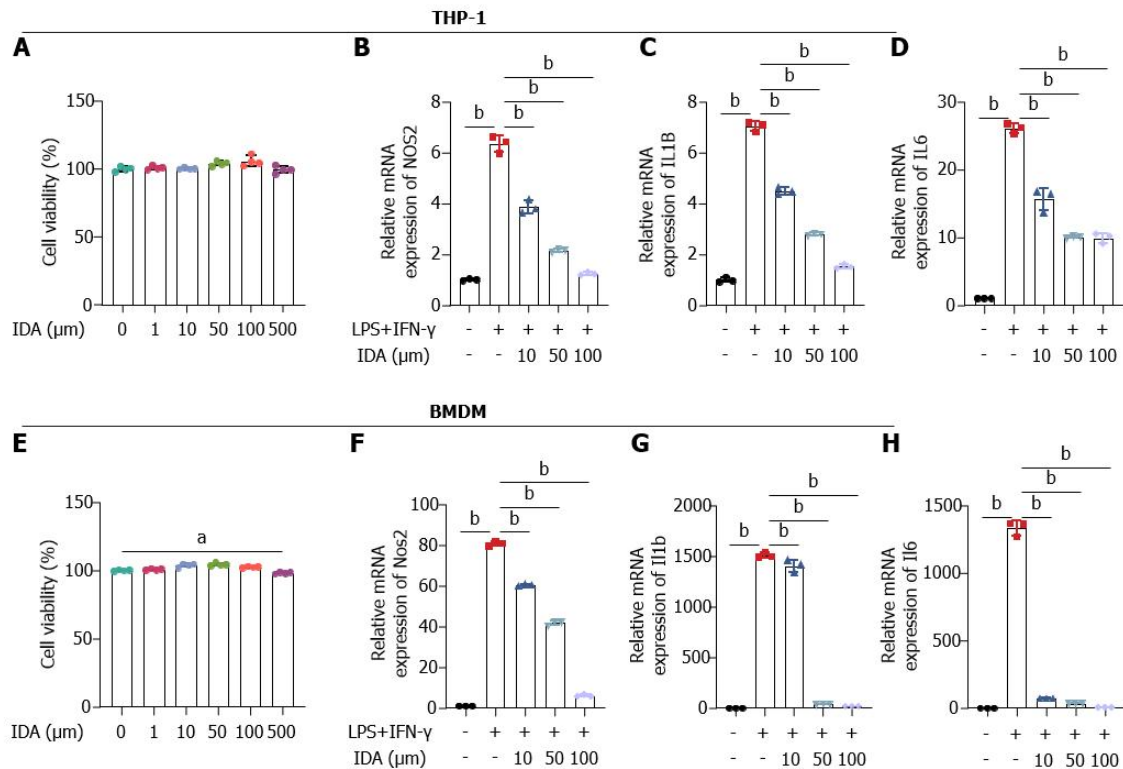
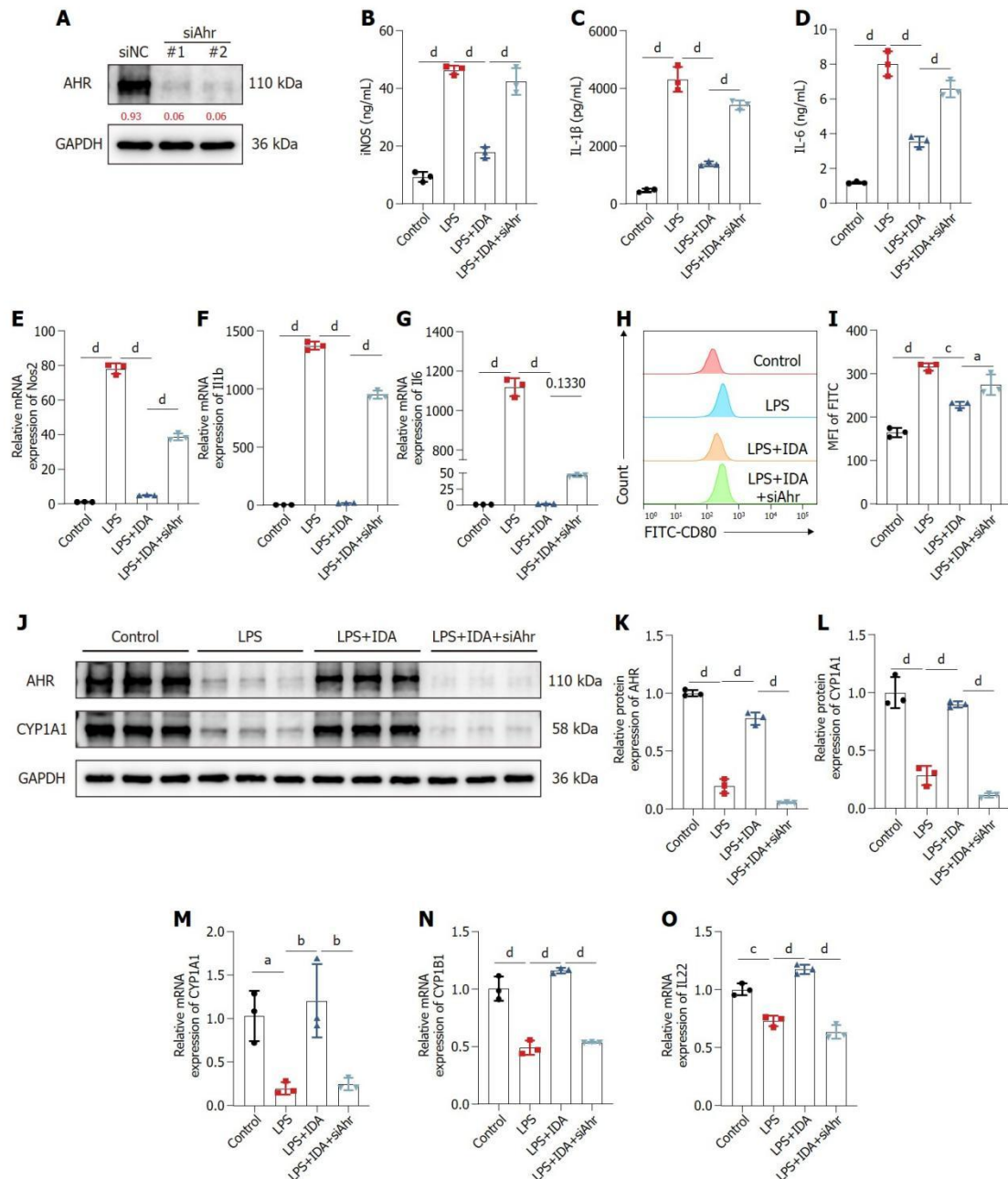




Supplemental Figure 1 IDA does not significantly affect M2 macrophage polarization in colitis mice. A: Representative flow cytometry plots of macrophages from the colon lamina propria. Cells were pre-gated on the CD11b⁺ population. The upper right quadrant (Q2) indicates F4/80⁺CD206⁺ macrophages (M2 macrophages); B-D: Relative mRNA expression of *Mrc1*, *Arg1*, and *Chil3* in colonic tissues. The data are shown as the means $\pm$ SDs (n = 6 mice per group). Group comparisons were analyzed using one-way ANOVA with Sidak's multiple comparisons test. DSS: Dextran sulfate sodium; IDA: *trans*-3-Indoleacrylic acid; F4/80: EGF-like module-containing mucin-like hormone receptor-like 1; CD206: Cluster of differentiation 206; *Mrc1*: Mannose receptor C-type 1; *Arg1*: Arginase 1; *Chil3*: Chitinase-like protein 3; ns: Not significant.

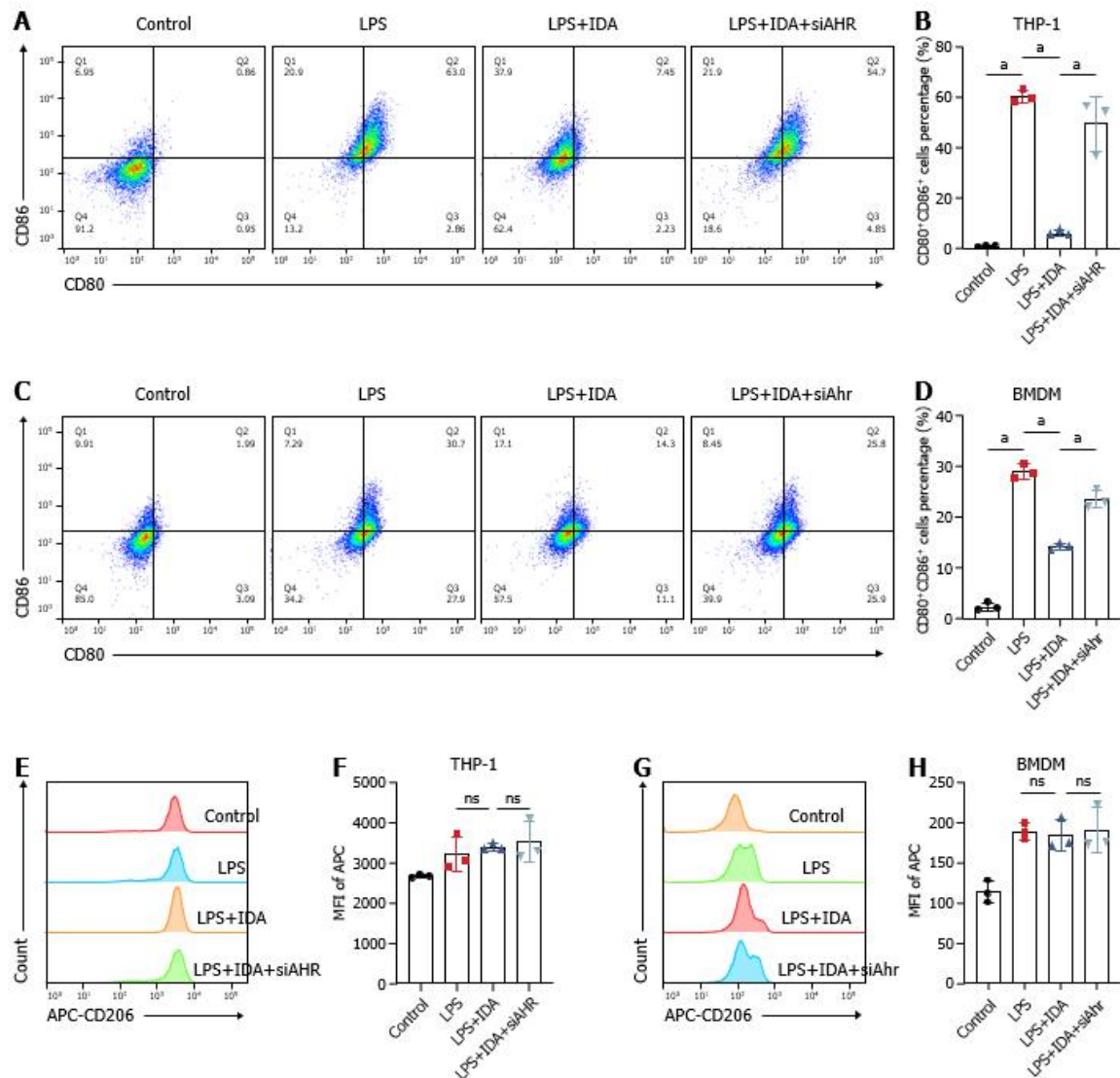


Supplemental Figure 2 IDA exerts anti-inflammatory effects in macrophages *in vitro*. A: Cell viability of THP-1 macrophages treated with IDA for 36 h; B–D: Relative mRNA expression of *NOS2*, *IL1B*, and *IL6* in THP-1 macrophages. E: Cell viability of BMDMs treated with IDA for 36 h; F–H: Relative mRNA expression of *Nos2*, *Il1b*, and *Il6* in BMDMs. The data are shown as the means $\pm$ SDs ($n = 3$ cell per group). Group comparisons were analyzed using one-way ANOVA with Sidak's multiple comparisons test. ^a $P < 0.05$, ^b $P < 0.0001$. IDA: *trans*-3-Indoleacrylic acid; LPS: Lipopolysaccharide; IFN- γ : Interferon gamma; *NOS2*/*Nos2* Nitric oxide synthase 2; *IL1B*/*Il1b*: Interleukin 1 beta; *IL6*/*Il6*: Interleukin 6.



Supplemental Figure 3 IDA attenuates LPS-stimulated M1 polarization through AHR signaling *in vitro*. A: Representative immunoblots of AHR in *Ahr* knockdown BMDMs; B-D: Concentration of iNOS, IL-1 β , and IL-6 in BMDMs culture supernatants; E-G: Relative mRNA expression of *Nos2*, *Il1b*, and *Il6* in BMDMs; H: Representative flow cytometry histograms of the CD80 expression levels in BMDMs; I: Quantification of FITC-CD80 mean fluorescence intensity; J-L: Representative immunoblots and the relative protein expression of AHR and CYP1A1 in BMDMs; M-O: Relative mRNA

expression of *Cyp1a1*, *Cyp1b1*, and *Il22* in BMDMs. The data are shown as the means $\pm$ SDs ($n = 3$ cell per group). Group comparisons were analyzed using one-way ANOVA with Sidak's multiple comparisons test. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$, ^d $P < 0.0001$. AHR/*Ahr*: Aryl hydrocarbon receptor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NC: Negative control; LPS: Lipopolysaccharide; IDA: *trans*-3-Indoleacrylic acid; iNOS: Inducible nitric oxide synthase; IL-1 β /*Il1b*: Interleukin 1 beta; IL-6/*Il6*: Interleukin 6; *Nos2*: Nitric oxide synthase 2; CD80: Cluster of differentiation 80; MFI: Mean fluorescence intensity; CYP1A1/*Cyp1a1*: Cytochrome P450, family 1, subfamily a, polypeptide 1; *Cyp1b1*: Cytochrome P450, family 1, subfamily b, polypeptide 1; *Il22*: Interleukin 22.



Supplemental Figure 4 IDA suppresses M1 macrophage polarization without affecting M2 polarization *in vitro*. A: Representative flow cytometry plots showing CD80 and CD86 expression in THP-1 macrophages after different treatments. The upper right quadrant (Q2) represents CD80⁺CD86⁺ macrophages; B: Quantitative analysis of the percentage of CD80⁺CD86⁺ macrophages; C: Representative flow cytometry plots showing CD80 and CD86 expression in BMDMs after different treatments. The upper right quadrant (Q2) represents CD80⁺CD86⁺ macrophages; D: Quantitative analysis of the percentage of CD80⁺CD86⁺ macrophages; E: Representative flow cytometry histograms of the CD206 expression levels in THP-1 macrophages; F: Quantification of FITC-CD206 mean fluorescence intensity; G:

Representative flow cytometry histograms of the CD206 expression levels in BMDMs; H: Quantification of FITC-CD206 mean fluorescence intensity. The data are shown as the means $\pm$ SDs ($n = 3$ cell per group). Group comparisons were analyzed using one-way ANOVA with Sidak's multiple comparisons test. ^a $P < 0.0001$. LPS: Lipopolysaccharide; IDA: *trans*-3-Indoleacrylic acid; AHR/*Ahr*: Aryl hydrocarbon receptor; CD80: Cluster of differentiation 80; CD86: Cluster of differentiation 86; CD206: Cluster of differentiation 206; MFI: Mean fluorescence intensity; ns: Not significant.

Supplementary Table 1 Histological scoring criteria for DSS-induced colonic damage

Score	Severity of inflammation	Crypt damage	Depth of injury
0	No or rare inflammatory cells in the lamina propria layer	none	Absence of mucosal damage
1	Increased numbers of inflammatory cells in the lamina propria layer	Less than 5% involvement	Discrete epithelial lesions
2	Inflammatory cells extending into the submucosa	33% damage	Mucosal erosion or ulceration
3	Transmural extension of inflammatory cell infiltration	66% damage	Transmural damage
4		Complete loss of entire crypt and epithelium	

The total histological score is calculated as the sum of the individual scores for the severity of inflammation, crypt damage, and depth of injury, with a maximum possible score of 10.

DSS: Dextran sulfate sodium.

Supplementary Table 2 siRNA sequences used for AHR knockdown in THP-1-derived macrophages

<i>Target gene</i>	<i>siRNA</i>	<i>Sense (5' - 3')</i>	<i>Antisense (5' - 3')</i>
<i>AHR</i>	siAHR#1	CUGAAGAGCUUAGACACAU UUAUCUUGCCAAUUUCUGCA	AUGUGUCUAAGCUCUUCAG UAUUUAUGUUAUUUAAUUCA
<i>AHR</i>	siAHR#2	U	G

Supplementary Table 3 siRNA sequences used for AHR knockdown in mouse bone marrow-derived macrophages (BMDMs)

<i>Target gene</i>	<i>siRNA</i>	<i>Sense (5' - 3')</i>	<i>Antisense (5' - 3')</i>
<i>Ahr</i>	siAhr#1	GUAUCUUCAUGGACAGAACA ATT CAUCGACAUAAACGGACGAAA	UUGUUCUGUCCAUGAAGAU CTT AUUUCGUCCGUUAUGUCGAU
<i>Ahr</i>	siAhr#2	UTT	GTT

Supplementary Table 4 Primers and full gene names for qRT-PCR of mouse tissue samples and mouse bone marrow-derived macrophages (BMDMs)

<i>Code</i>	<i>Genes</i>	<i>Forward (5' - 3')</i>	<i>Reverse (5' - 3')</i>
<i>Actb</i>	actin beta	TATGCTCTCCCTCACGCCATCC	GTCACGCACGATTTCCTCTCA

			G
		AAGCAACACTAGCAGGAAAG	GCTTGAAGGAGGACACAGATA
<i>Ahr</i>	aryl hydrocarbon receptor	A	G
		ATGTACACGATGTCTTTGGCAG	
<i>Arg1</i>	Arginase 1	ATA	AGCTCTGGGAATCTGCATGG
<i>Chil3</i>	chitinase-like 3	CAGGGTAATGAGTGGGTTGG	CACGGCACCTCCTAAATTGT
<i>Cldn1</i>	claudin 1	TGCCCCAGTGGAAGATTTACT	CTTTGCGAAACGCAGGACAT
	cytochrome P450, family 1, subfamily a,	GTGAGCAAGGAGGCTAACTAT	
<i>Cyp1a1</i>	polypeptide 1	C	GGCTACTGACACGACCAAATA
	cytochrome P450, family 1, subfamily b,	CTAGCCAGCAGTGTGATGATA	CGAGGATGGAGATGAAGAGA
<i>Cyp1b1</i>	polypeptide 1	TT	AAC
<i>Il1b</i>	interleukin 1 beta	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
		ATGAGTTTTTCCCTTATGGGGA	
<i>Il22</i>	interleukin 22	C	GCTGGAAGTTGGACACCTCAA
			AGTGGTATAGACAGGTCTGTTG
<i>Il6</i>	interleukin 6	CTGCAAGAGACTTCCATCCAG	G
<i>Mrc1</i>	mannose receptor C-type 1	CTCTGTTCAGCTATTGGACGC	TGGCACTCCCAAACATAATTTG

			A
<i>Nos2</i>	nitric oxide synthase 2	GGAGTGACGGCAAACATGACT	TCGATGCACAACCTGGGTGAAC
		TGAAAGTCCACCTCCTTACAG	CCGGATAAAAAGAGTACGCTG
<i>Ocln</i>	occludin	A	G
<i>Tjp1</i>	tight junction protein 1	GCCGCTAAGAGCACAGCAA	GCCCTCCTTTTAACACATCAGA

Supplementary Table 5 Primers and full gene name for qRT-PCR of THP-1-derived macrophages

<i>Code</i>	<i>Genes</i>	<i>Forward (5' - 3')</i>	<i>Reverse (5' - 3')</i>
<i>ACTB</i>	actin beta	GAGCACAGAGCCTCGCCTTT	TCATCATCCATGGTGAGCTGG
			CCTTTGGCATCACAACCAATA
<i>AHR</i>	aryl hydrocarbon receptor	CTTCAGCCACCATCCATACTT	G
<i>CYP1A1</i>	cytochrome P450, family 1, subfamily a, polypeptide 1	CCAGCTGACTTCATCCCTATTC	GTAGTGCTCCTTGACCATCTTC
<i>CYP1B1</i>	cytochrome P450, family 1, subfamily b, polypeptide 1	GGCCACTATCACTGACATCTTC	CGAGTCTGCACATCAGGATAC
<i>IL1B</i>	interleukin 1 beta	AAAGCTTGGTGATGTCTGGTC	GGACATGGAGAACACCACTTG
<i>IL6</i>	interleukin 6	ACTCACCTCTTCAGAACGAATT	CCATCTTTGGAAGGTTTCAGGTT

		G	G
<i>IL22</i>	interleukin 22	GCTTGACAAGTCCAACTTCCA	GCTCACTCATACTGACTCCGT
		TTCAGTATCACAACTCAGCA	TGGACCTGCAAGTTAAAATCC
<i>NOS2</i>	nitric oxide synthase 2	AG	C
