Supplementary information for

DNA Methylome and Transcriptome Alterations and Cancer Prevention by Curcumin in Colitis-accelerated Colon Cancer in Mice

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Table S1. Genes and primers for qPCR validation (5' – 3').

Gene	Forward	Reverse
GAPDH	AACAGCAACTCCCACTCTTC	CCTGTTGCTGTAGCCGTATT
Itgb2	GTGGTAGGTGTCGTACTGATTG	GGGACTTGAGTTTCTCCTTCTC
Lgals9	GAGTGAGGAAGAGAGCATTGG	CTCCTTGGATTGGTCCAGTAAA
Igfbp4	CTGCAGGAGAGAGGAAAGAATG	GGTGGGCAACTAGAAAGATAGAG
Duoxa2	ATGTCCATGCCTTGGAGAAG	CCAGGTGATATTGGTGGTACAG
PF4	GGGCAGGCAGTGAAGATAAA	GCCACTGGACCCAAAGATAA
Icam1	CCAGTACTGCTGGTCATTGT	TCCTCCTGAGCCTTCTGTAA
Gja1	GGTGTCTCTCGCTCTGAATATC	GTAAGGATCGCTTCTTCCCTTC
Tnf	TTGTCTACTCCCAGGTTCTCT	GAGGTTGACTTTCTCCTGGTATG
Nt5e	CTGGAACAGAGTGGTCCAATTA	GCTTGGGAGGGTCACTTTATAC

Table S2. Primers used in pyrosequencing and vector construction (5' – 3').

|	
Amplicon A	
Forward	ATGGAGAAGAAATYGAGATAGAGGTGTAGG
Reverse	TCCCTCCTAACTAATCCCTTACTAT-biotin
Sequencing	ATAGAGGTGTAGGGT
Amplicon B	
Forward	GAGAGTGTGGAGGGGATAGAGTT
Reverse	CCCCTTCATCTTCCTCCTTAT-biotin
Sequencing	GGGGATAGAGTTAGGAT
Amplicon B (Vector construction)	
Forward	AGTCAAGCTTCGGGGTGATCGGTCC
Reverse	TGACCCATGGAACTGATGAGAGGGAGGCCA

Table S3. Regulated pathways from Ingenuity Pathway analysis with RNA-seq data.

Ingenuity Canonical Pathways	AOM+DSS vs Control		AOM+DSS+Cur. vs AOM+DSS	
	-log (p-value)	activation z score	-log (p-value)	activation z score
PKC Signaling in T Lymphocytes	6.8	1.512	4.41	-4.146
iCOS-iCOSL Signaling in T Helper Cells	6.45	1.46	4.04	-3.357
Aldosterone Signaling in Epithelial Cells	6.06	-2.524	2.54	-0.632
PI3K Signaling in B Lymphocytes	6.01	1.877	5.13	-3.273
Leukocyte Extravasation Signaling	5.47	1.257	2.41	-3.962
NRF2-mediated Oxidative Stress Response	5.47	0.832	6.11	-0.243
Role of NFAT in Regulation of the Immune Response	5.45	1.826	3.59	-4.707
Mitotic Roles of Polo-Like Kinase	4.59	1.941	3.52	-1.265
IGF-1 Signaling	4.38	-0.728	2.29	-1.897
p70S6K Signaling	4.34	-0.426	2.41	-2.183
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	4.24	1.257	2.32	-3.266
LPS/IL-1 Mediated Inhibition of RXR Function	4.16	0.775	6.81	-2.84
Th1 Pathway	4.12	1.279	5.19	-3.128
IL-6 Signaling	4.1	-0.426	2.21	-2
Calcium-induced T Lymphocyte Apoptosis	4	1.807	6.01	-3.153
ERK/MAPK Signaling	3.62	-0.186	2.44	-2.6
Dendritic Cell Maturation	3.61	2.268	3.05	-4.2
Estrogen-Dependent Breast Cancer Signaling	3.22	0.535	2.37	-3
Cdc42 Signaling	3.07	2.673	5.25	-2.183
Actin Cytoskeleton Signaling	2.36	0.408	2.48	-3.4

**Table S4.** Regulated pathways from Ingenuity Pathway analysis with methyl-seq data.

| Ingenuity Canonical Pathways	AOM+DSS vs Control		AOM+DSS+Cur. vs AOM+DSS	
	-log (p-value)	activation z score	-log (p-value)	activation z score
Role of NFAT in Regulation of the Immune Response	5.26	2.6	3.15	-1.886
Angiopoietin Signaling	4.7	0.333	2.03	-0.816
Tec Kinase Signaling	4.52	2.041	2.76	-3
Neuropathic Pain Signaling in Dorsal Horn Neurons	4.44	1.091	2.44	-1.604
Gαq Signaling	4.37	2.041	2.56	-1.698
Dendritic Cell Maturation	4.33	2.887	3.42	-1.528
Type II Diabetes Mellitus Signaling	4.19	1.606	2.02	-0.905
IL-8 Signaling	4.18	1.512	2.06	-1.147
CD40 Signaling	4.05	2	2.45	-2.714
Mouse Embryonic Stem Cell Pluripotency	3.91	0.229	2.74	0.535
Lymphotoxin β Receptor Signaling	3.78	1.732	2.01	-1.414
FcγRIIB Signaling in B Lymphocytes	3.6	1.155	2.09	-1.414
Renin-Angiotensin Signaling	3.58	1.147	2.2	-1.604
Fc Epsilon RI Signaling	3.38	1.5	4.25	-1.291
Glioma Signaling	3.14	1	2.1	-0.905
LPS-stimulated MAPK Signaling	3.09	1.291	2.17	-0.905
Colorectal Cancer Metastasis Signaling	2.99	0.539	2.36	-1.225
Nitric Oxide Signaling in the Cardiovascular System	2.8	1.213	2.16	-1.155
B Cell Activating Factor Signaling	2.73	1.633	2.81	-0.816
Estrogen-Dependent Breast Cancer Signaling	2.63	0.905	2.06	-0.707
Melanocyte Development and Pigmentation Signaling	2.59	0.775	2.68	-1.387
B Cell Receptor Signaling	2.43	1.46	2.68	-0.894
TNFR2 Signaling	2.39	1.342	2.28	0
CXCR4 Signaling	2.31	1.147	3.22	-1.698

Figure S1
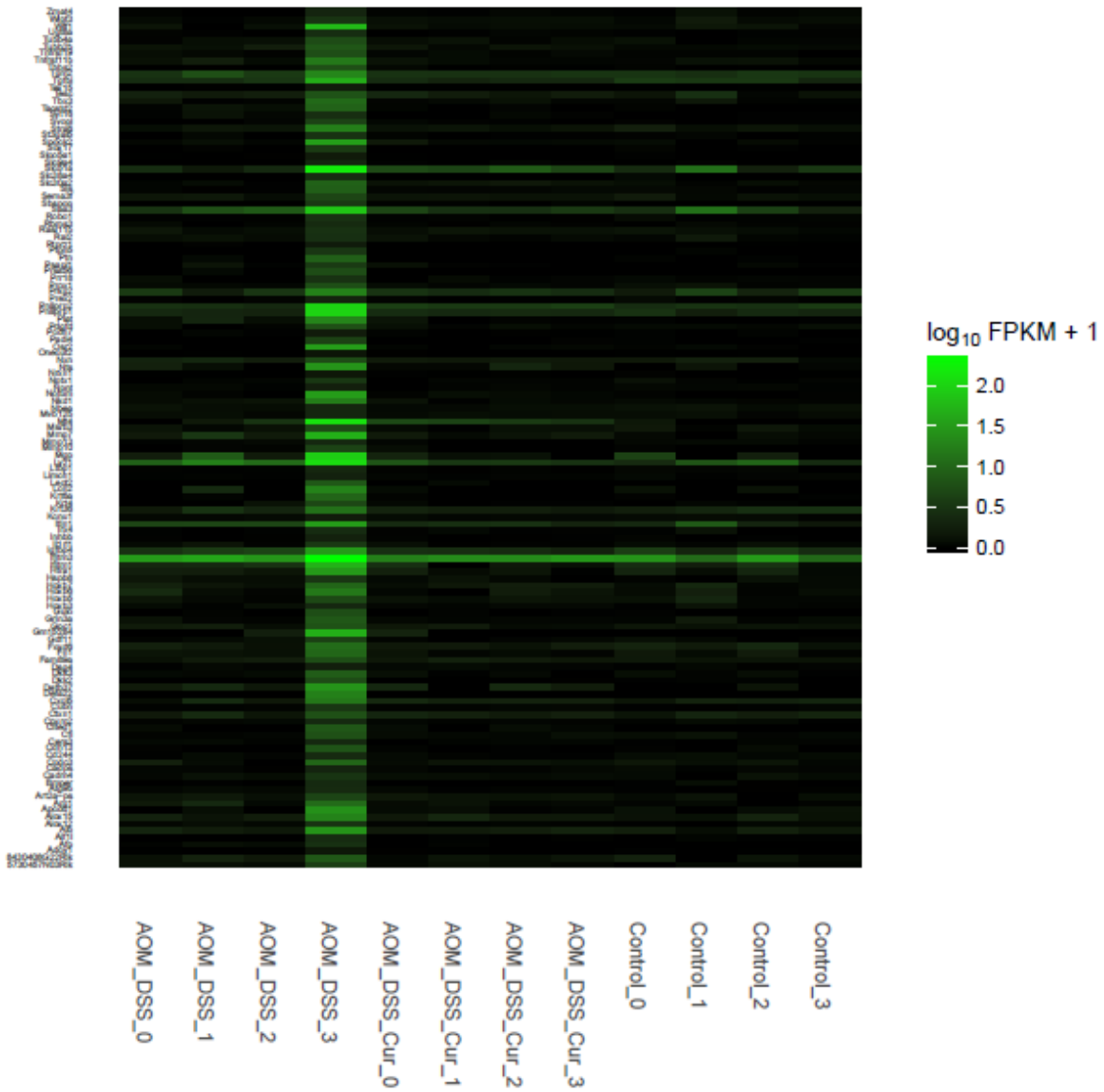
Figure S1: The mRNA expression of selected genes in all the three groups. One animal from the AOM+DSS group showed different expression pattern than others. This sample was considered as an outlier and excluded from this study.

Figure S2
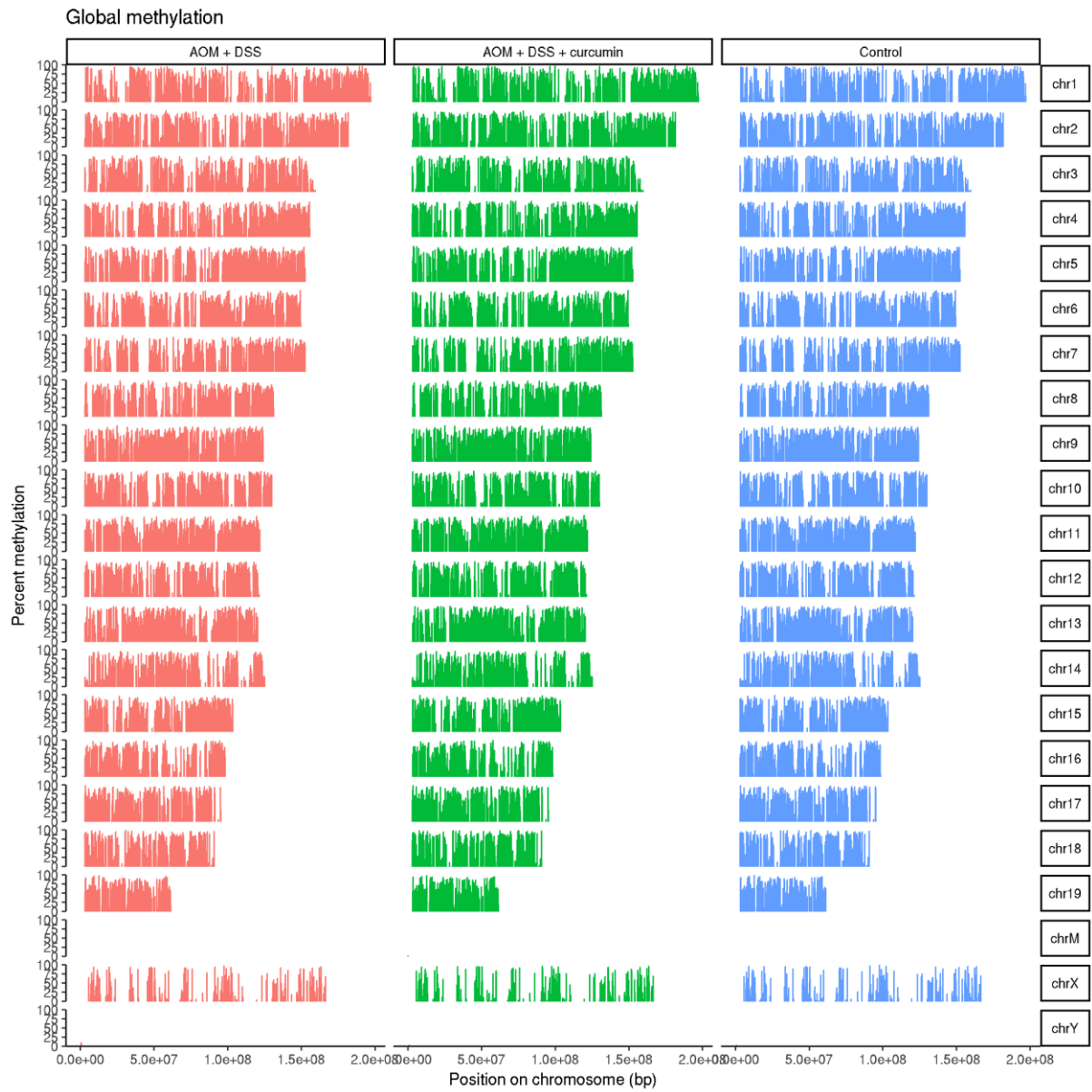
Figure S2: Global methylation level in control, AOM+DSS, and AOM+DSS+Curcumin groups.

Figure S3
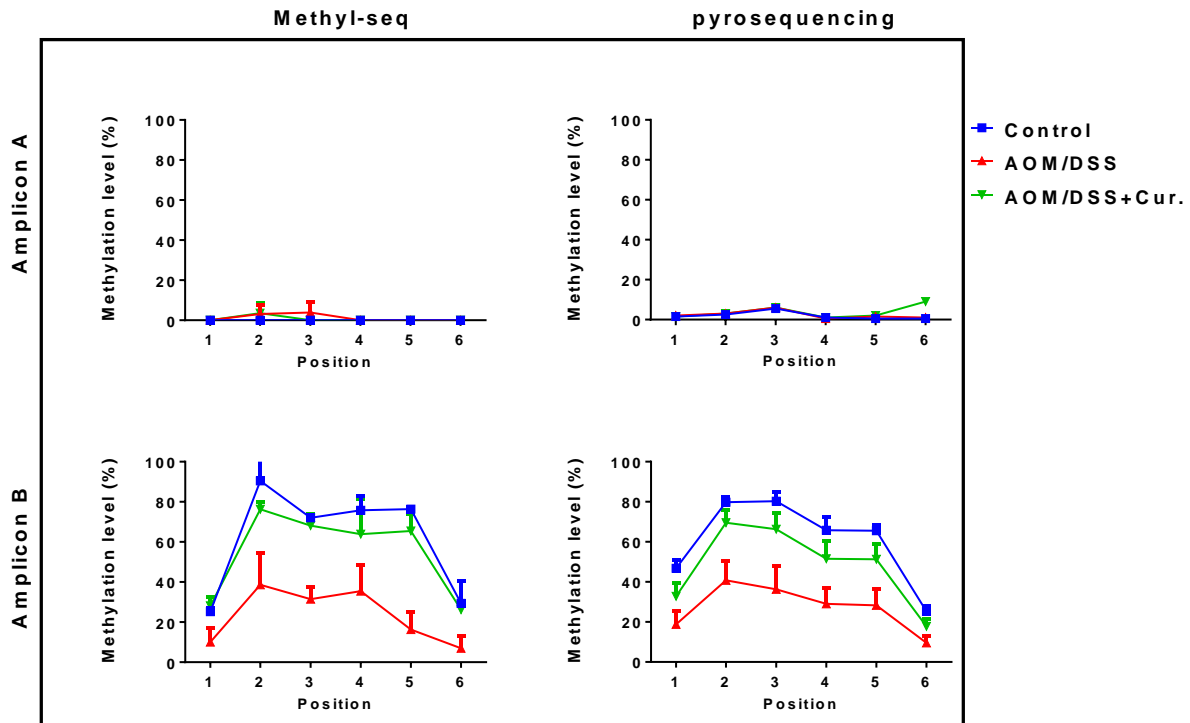
Figure S3: Single resolution methylation ratio of Tnf in amplicon A (upper) and B (lower). Each group has 2 replicates for Methy-seq (left panel) and 3 replicates for pyrosequencing. Data are presented as mean ± SD.
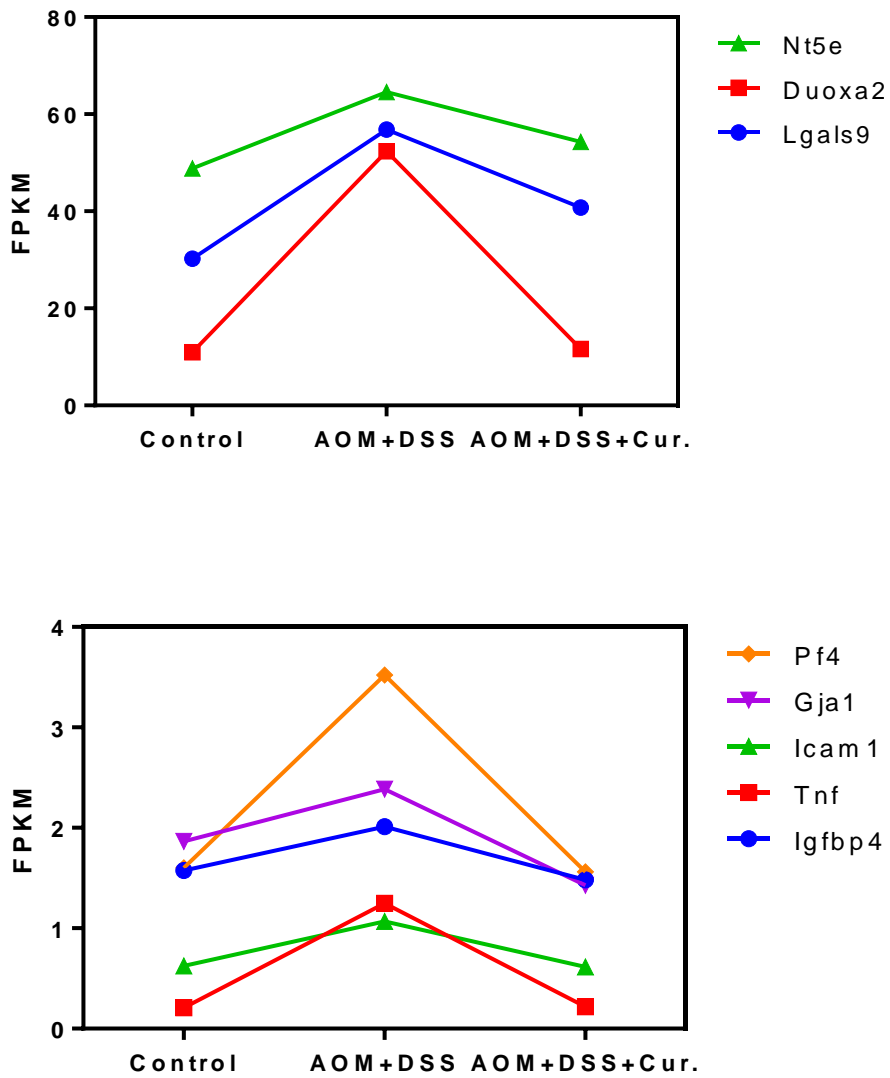
Figure S4

Figure S4: The FPKM value of selected genes from RNA-seq as validated in Figure 5.