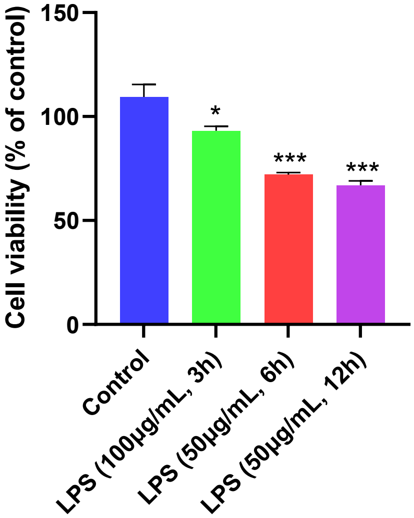

Figure S1. The cell viability induced by LPS in different conditions.

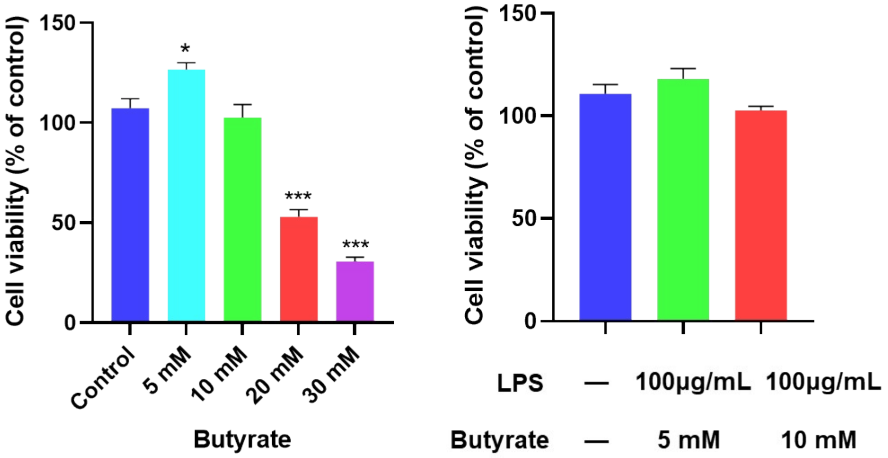

Figure S2. The cell viability induced by butyrate as well as butyrate and LPS treatment.

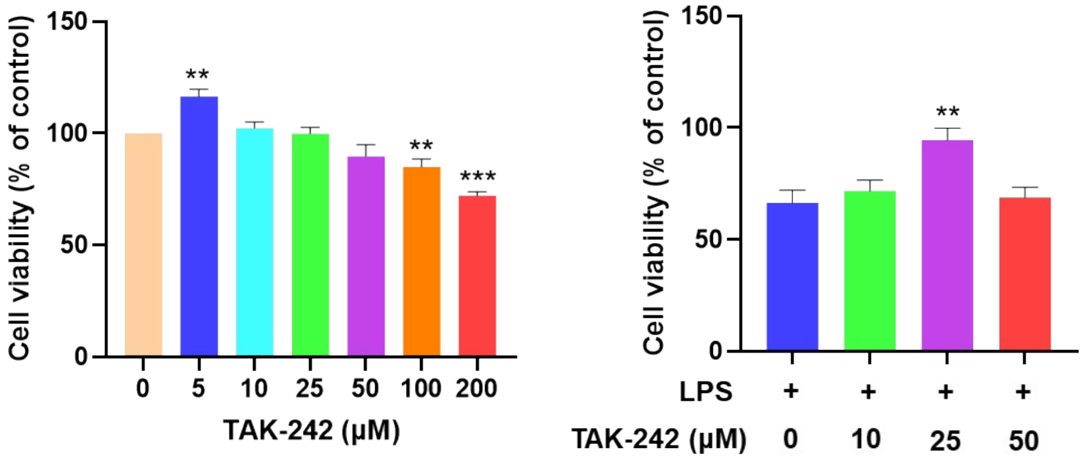

Figure S3. The cell viability induced by TAK-242 as well as TAK-242 and LPS treatment.

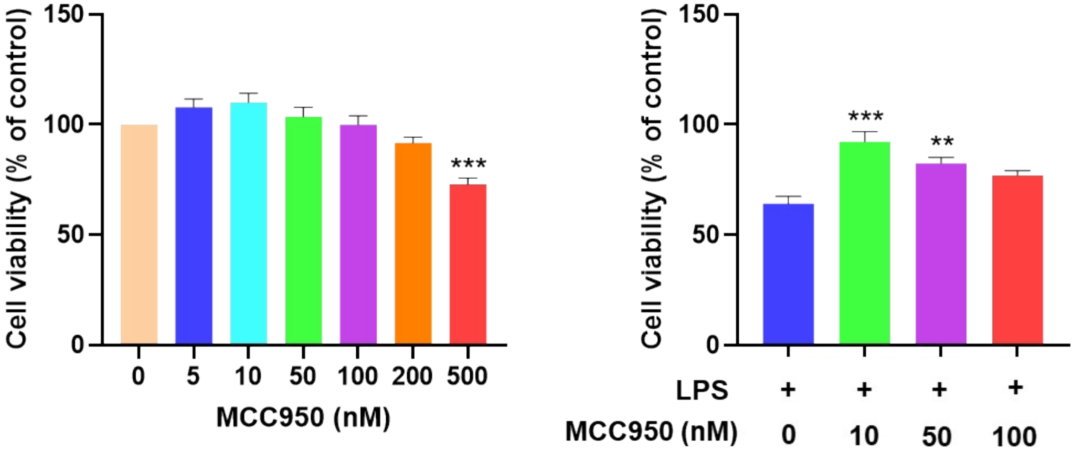

Figure S4. The cell viability induced by MCC950 as well as MCC950 and LPS treatment.

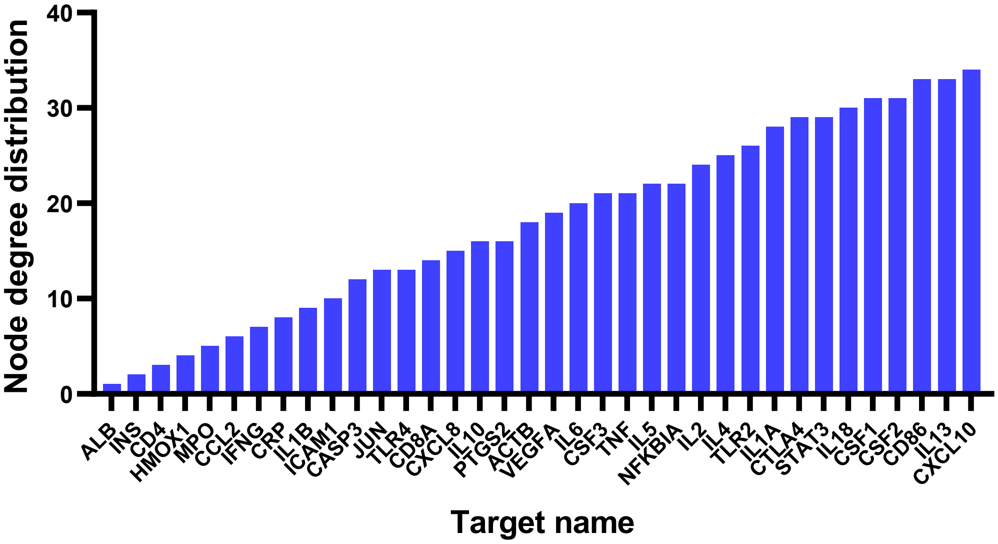

Figure S5. Node-degree distribution of top50 targets that interacted proteins below 35.

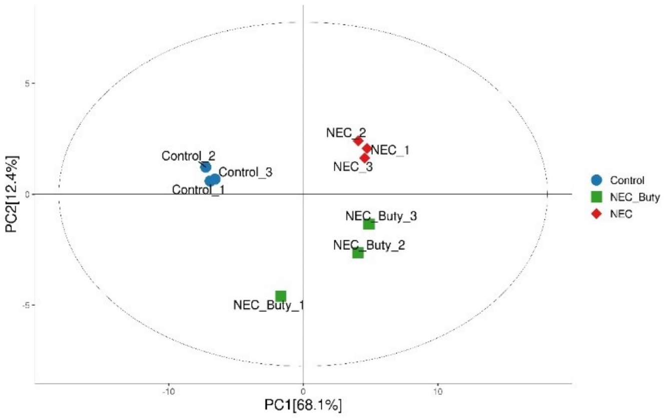

Figure S6. The PCA analyses of butyrate-treated NEC neonatal rats for the control, NEC, and butyrate + NEC groups.

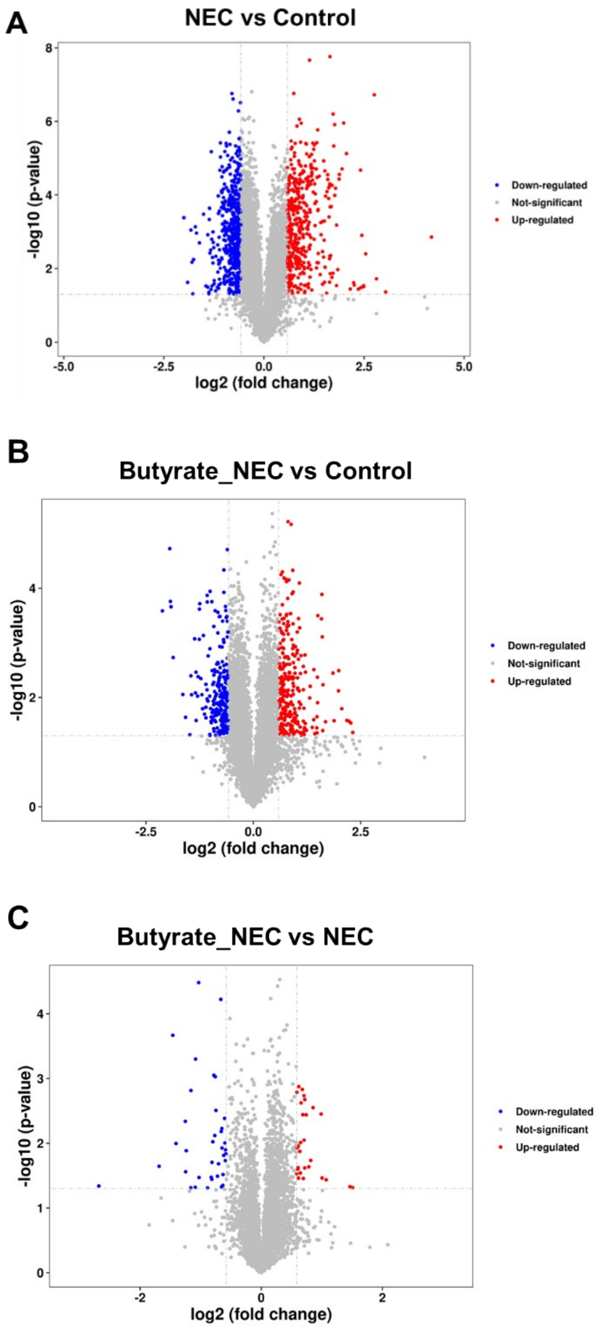

Figure S7. Differentially expressed proteins identified in neonatal rats. (A-C) The volcano diagram showing the differentially expressed proteins in NEC vs control, sodium butyrate + NEC vs control, and sodium butyrate + NEC vs NEC, respectively. Bule represents the down-regulated proteins, red represents the up-regulated proteins, and grey represents the proteins with no significant difference. The criteria for significance are *p*-value < 0.05 and fold change > 1.5.

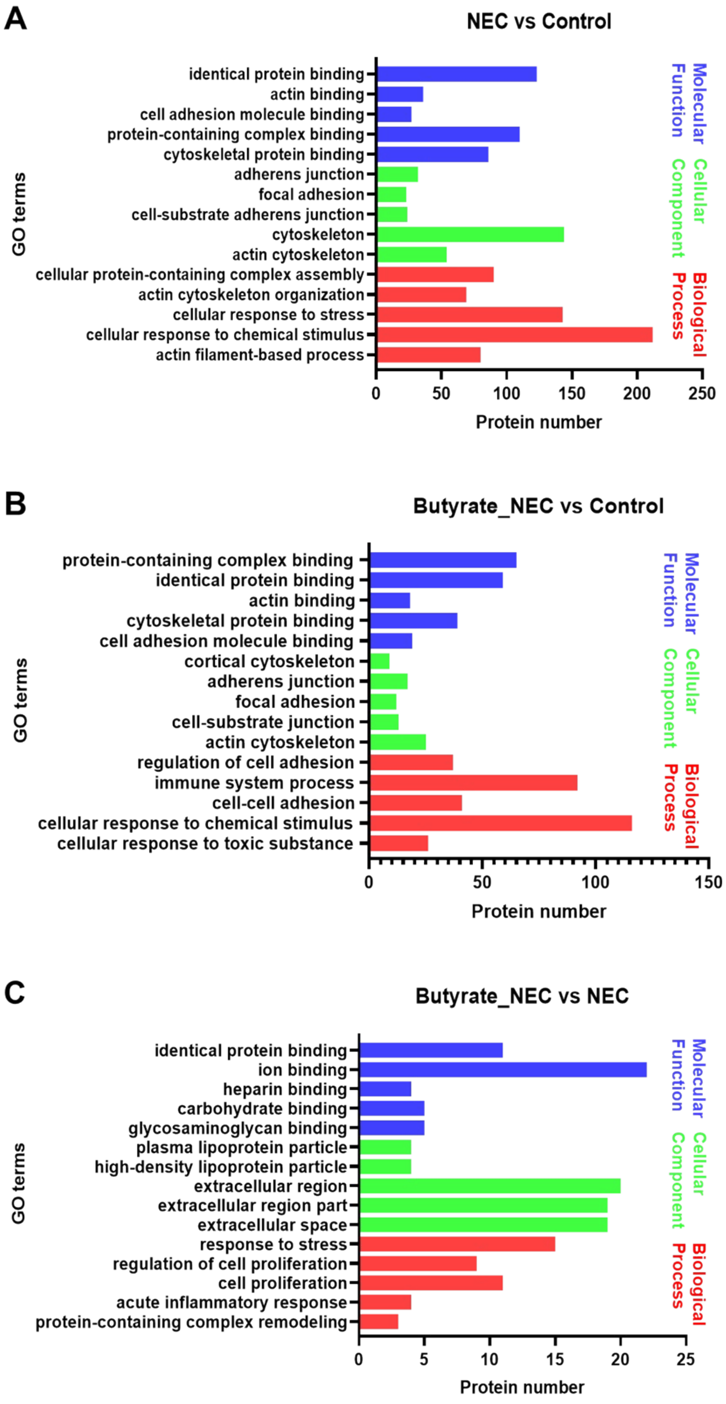

Figure S8. The intestinal barrier-related gene ontology (GO) analysis of the differentially expressed proteins in neonatal rats. (A) The GO analysis between NEC and control group. (B) The GO analysis between sodium butyrate + NEC and control group. (C) The GO analysis between sodium butyrate + NEC and NEC group. Red, green, and blue represents the terms of biological process, cellular component, and molecular function, respectively.

Table S1. Primers used for RT-qPCR analysis

Genes	Primer sequences（5'－3'）	
GAPDH	F	TCAAGAAGGTGGTGAAGCAG
	R	AAGGTGGAAGAGTGGGAGTTG
IL-1β	F	AATCTCACAGCAGCATCTCGACAAG
	R	TCCACGGGCAAGACATAGGTAGC
IL-6	F	ACTTCCAGCCAGTTGCCTTCTTG
	R	TGGTCTGTTGTGGGTGGTATCCTC
Claudin-3	F	ATTCATCGGCAGCAGCATC
	R	CCAGCAGCGAGTCGTACATC
Claudin-7	F	CATCGTGGCAGGTCTTGCTG
	R	GTGCACGGTATGCAGCTTTG
Occludin	F	CCAATGGCCTACTCCTCCAA
	R	CATCCACGGACAAGGTCAGA
ZO-1	F	CAGGCCATTACGAGCCTCTC
	R	AGGCTGTGGCTTGGTAGCTG
Bax	F	CTGCAGAGGATGATTGCTGA
	R	GATCAGCTCGGGCACTTTAG
Bcl-2	F	CTGGCATCTTCTCCTTCCAG
	R	CGGTAGCGACGAGAGAAGTC
Caspase-6	F	AGACAAGCTGGACAACGTGACC
	R	CCAGGAGCCATTCACAGTTTCT
Caspase-9	F	GTCACGGCTTTGATGGAGAT
	R	CAGGCCTGGATGAAGAAGAG
Atg5	F	AGATGGACAGCTGCACACAC
	R	GCTGGGGGACAATGCTAATTA
Atg7	F	TCCGTTGAAGTCCTCTGCTT
	R	CCACTGAGGTTCACCATCCT
Atg12	F	AACAAAGAAATGGGCTGTGG
	R	TTGCAGTAATGCAGGACCAG
ULK1	F	CCCAGAGTACCCGTACCAGA
	R	GTGTAGGGTTTCCGTGTGCT
ULK2	F	GCAGACAGGAGGACGAAGAC
	R	CTGGAGAACCTGAGGAGTGC
Beclin1	F	GTGCTCCTGTGGAATGGAAT

	R	GCTGCACACAGCCAGAAAA
NF-κB	F	GGCCGGAAGACCTATCCTACT
	R	CTACAGACACAGCGCACACT
Caspase-1	F	AAAGACAAGCCCAAGGTGATC
	R	CCAAGTCACAAGACCAGGCATA
NLRP3	F	AGAAGAGACCACGGCAGAAG
	R	CCTTGGACCAGGTTCAGTGT
TLR4	F	CTCACAACTTCAGTGGCTGGATTTA
	R	GTCTCCACAGCCACCAGATTCTC
GSDMD	F	AGTGCTCCAGAACCAGAACCG
	R	TGGAGGCACTGGAACTGTCA
MyD88	F	TATACCAACCCTTGCACCAAGTC
	R	TCAGGCTCCAAGTCAGCTCATC
IL-18	F	TGAAGTAAGAGGACTGGCTGTGA
	R	TTGGCAAGCAAGAAAGTGTCC
IL-4	F	GGTCTCAACCCCCAGCTAGT
	R	GCCGATGATCTCTCTCAAGTGAT
TNF-α	F	GACGTGGAACTGGCAGAAGAG
	R	TTGGTGGTTTGTGAGTGTGAG
IFN-γ	F	CTTGAAAGACAATCAGGCCATC
	R	CTTGGCAATACTCATGAATGCA

Table S2. The information of top50 target hub gene

Target Name	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Neighborhood Connectivity
CSF3	0.012207	0.75	0.493237	48.06522
CTLA4	0.003627	0.914286	0.494715	48.15909
IFNB1	0	0.924528	0.494444	48.13333
IL13	0.012046	1	0.486395	47.69388
IL6	0.010899	0.675	0.486395	47.69388
CASP1	1.35E-04	0.979167	0.488032	47.79167
CSF2	0.008548	1	0.486395	47.69388
TNF	0.010899	0.710526	0.486395	47.69388
NFKBIA	0.007331	0.75	0.488032	47.79167
ITGAM	1.35E-04	1	0.486395	47.69388
ICAM1	0.007442	1	0.486395	47.69388
VEGFA	0.010899	0.642857	0.486395	47.69388
PTGS2	0.146559	0.473684	0.486395	47.69388
CD40LG	5.30E-04	0.928571	0.494444	48.13333
IL1B	0.007442	1	0.486395	47.69388
ACTB	0.010899	0.613636	0.486395	47.69388
FOXP3	3.31E-04	1	0.486395	47.69388
MYD88	3.31E-04	1	0.486395	47.69388
CRP	0.003385	1	0.490338	47.93478
IL10	0.023995	0.574468	0.486395	47.69388
IL18	0.008548	1	0.486395	47.69388
CXCL1	3.31E-04	1	0.486395	47.69388
IFNG	0.006074	1	0.486395	47.69388
TLR9	2.62E-04	0.933333	0.492271	48.02174
STAT3	0.008548	1	0.486395	47.69388
CCL5	3.31E-04	1	0.486395	47.69388
IL17A	0.012537	1	0.486395	47.69388
IL1A	0.008548	1	0.486395	47.69388
MPO	0.004476	1	0.491304	47.97826
HMGB1	0.001229	0.95122	0.491304	47.97826
CCL2	0.006074	1	0.486395	47.69388
CXCL8	0.023995	0.55102	0.486395	47.69388
NLRP3	0.006444	0.948718	0.490338	47.93478
TLR2	1.248556	0.964286	0.486395	47.69388
INS	0.001721	1	0.487145	47.75
CD8A	0.023995	0.529412	0.486395	47.69388
ALB	0.002021	1	0.486395	47.69388
HMOX1	0	1	0.5	48.41463

CD86	0.010443	0.923077	0.493237	48.06522
JUN	0.023995	0.509434	0.486395	47.69388
CSF1	0.006058	0.897436	0.493129	48.09091
CCL3	0.001229	1	0.486395	47.69388
PPARG	0	0	0.497096	48.28571
CD4	0.00229	1	0.486395	47.69388
IL5	0.004539	0.84375	0.494186	48.13636
TLR4	1.348429	0.658537	0.486395	47.69388
CASP3	0.017502	0.490909	0.489824	47.89362
IL4	0.018367	0.9	0.486395	47.69388
IL2	0.018367	0.84375	0.486395	47.69388
CXCL10	0.012046	1	0.486395	47.69388

Table S3. The detailed information of GO terms related with inflammatory responses enriched by top50 target hub gene

term description	observed gene count	background gene count	FDR	matching proteins in network
Biological Process				
Cytokine-mediated signaling pathway	37	678	2.41E-36	CD4, TNFRSF1A, HMOX1, IL2RB, NFKBIA, CSF3, CCL2, IL2, IFNG, IL4, IL5, IL1A, IL1B, STAT3, ICAM1, IL18, CSF2, IL13, CXCL10, CXCL8, CASP3, CSF1, CD86, IL17A, PTGS2, CD40LG, IFNB1, CXCL1, IL6, TNF, MYD88, IL10, CASP1, ITGAM, CCL5, CCL3, VEGFA
Inflammatory response	34	515	1.47E-35	TNFRSF1A, HMOX1, MEFV, CCL2, IFNG, IL4, IL5, CRP, TLR2, IL1A, IL1B, STAT3, ICAM1, IL18, IL13, CXCL10, CXCL8, CSF1, NLRP3, IL17A, HMGB1, TLR9, PTGS2, CD40LG, JUN, TLR4, CXCL1, INS, IL6, TNF, MYD88, ITGAM, CCL5, CCL3
Response to cytokine	41	1101	1.47E-35	CD4, TNFRSF1A, HMOX1, IL2RB, NFKBIA, MEFV, CSF3, CCL2, IL2, IFNG, IL4, IL5, TLR2, IL1A, IL1B, STAT3, ICAM1, IL18, CSF2, IL13, CXCL10, CXCL8, CASP3, CSF1, CD86, IL17A, PTGS2, CD40LG, JUN, TLR4, IFNB1, CXCL1, IL6, TNF, MYD88, IL10, CASP1, ITGAM, CCL5, CCL3, VEGFA
Immune system process	49	2481	5.89E-34	CD4, HMOX1, MEFV, FCGRT, MPO, CSF3, CCL2, IL2, IFNG, IL4, IL5, CRP, TLR2, IL1A, IL1B, STAT3, ICAM1, IL18, PPARG, CSF2, CTLA4, IL13, CXCL10, CXCL8, CASP3, CSF1, CD86, NLRP3, IL17A, HMGB1, ACTB, TLR9, CD40LG, JUN, TLR4, FOXP3, IFNB1, CXCL1, INS, IL6, CD8A, TNF, MYD88, IL10, CASP1, ITGAM, CCL5, CCL3, VEGFA

Cellular response to cytokine stimulus	39	1013	5.89E-34	CD4, TNFRSF1A, HMOX1, IL2RB, NFKBIA, CSF3, CCL2, IL2, IFNG, IL4, IL5, TLR2, IL1A, IL1B, STAT3, ICAM1, IL18, CSF2, IL13, CXCL10, CXCL8, CASP3, CSF1, CD86, IL17A, PTGS2, CD40LG, TLR4, IFNB1, CXCL1, IL6, TNF, MYD88, IL10, CASP1, ITGAM, CCL5, CCL3, VEGFA
Regulation of cell death	39	1696	1.79E-26	TNFRSF1A, HMOX1, IL2RB, NFKBIA, MPO, CSF3, CCL2, IL2, IFNG, IL4, IL1A, IL1B, STAT3, ICAM1, PPARG, ALB, CSF2, CTLA4, IL13, CXCL10, CASP3, CSF1, NLRP3, HMGB1, PTGS2, CD40LG, JUN, TLR4, IFNB1, INS, IL6, TNF, MYD88, IL10, CASP1, ITGAM, CCL5, CCL3, VEGFA
Regulation of cell population proliferation	30	1642	1.24E-25	CD4, HMOX1, NFKBIA, CSF3, CCL2, IL2, IFNG, IL4, IL5, CRP, TLR2, IL1A, IL1B, STAT3, IL18, PPARG, CSF2, CTLA4, IL13, CXCL10, CXCL8, CASP3, CSF1, CD86, HMGB1, TLR9, PTGS2, CD40LG, JUN, TLR4, FOXP3, CXCL1, INS, IL6, TNF, IL10, CCL5, VEGFA
Regulation of apoptotic process	35	1550	1.32E-22	TNFRSF1A, HMOX1, IL2RB, NFKBIA, MPO, CCL2, IL2, IFNG, IL4, IL1A, IL1B, ICAM1, PPARG, ALB, CSF2, CTLA4, IL13, CXCL10, CASP3, NLRP3, HMGB1, PTGS2, CD40LG, JUN, IFNB1, INS, IL6, TNF, MYD88, IL10, CASP1, ITGAM, CCL5, CCL3, VEGFA
Regulation of nik/nf-kappab signaling	10	110	6.13E-11	NFKBIA, TLR2, IL1B, IL18, CD86, NLRP3, HMGB1, TLR9, TLR4, TNF
Apoptotic signaling pathway	13	286	7.91E-11	TNFRSF1A, HMOX1, IL2, IFNG, IL4, IL1A, IL1B, CASP3, JUN, TLR4, TNF, CASP1, ITGAM

Cellular Component				
Cell surface	18	824	2.79E-09	CD4, TNFRSF1A, IL2RB, FCGRT, TLR2, ICAM1, CTLA4, IL13, CXCL10, CD86, IL17A, HMGB1, CD40LG, TLR4, CD8A, TNF, ITGAM, VEGFA
Receptor complex	8	381	0.002	CD4, TNFRSF1A, IL2RB, TLR2, TLR4, IL6, CD8A, ITGAM
Cytoplasmic vesicle	18	2386	0.009	CD4, IL2RB, MEFV, FCGRT, MPO, TLR2, IL1B, ALB, CTLA4, HMGB1, TLR9, TLR4, CXCL1, INS, TNF, MYD88, ITGAM, VEGFA
Whole membrane	14	1715	0.0235	CD4, TNFRSF1A, HMOX1, FCGRT, TLR2, ICAM1, CASP3, TLR9, PTGS2, TLR4, CD8A, TNF, MYD88, ITGAM
NLRP3 inflammasome complex	2	8	0.0284	NLRP3, CASP1
Molecular Function				
Cytokine receptor binding	27	264	1.05E-31	CSF3, CCL2, IL2, IFNG, IL4, IL5, IL1A, IL1B, STAT3, IL18, CSF2, IL13, CXCL10, CXCL8, CASP3, CSF1, TLR9, CD40LG, IFNB1, CXCL1, IL6, TNF, MYD88, IL10, CCL5, CCL3, VEGFA
Cytokine activity	25	233	1.01E-29	CSF3, CCL2, IL2, IFNG, IL4, IL5, IL1A, IL1B, IL18, CSF2, IL13, CXCL10, CXCL8, CSF1, IL17A, HMGB1, CD40LG, IFNB1, CXCL1, IL6, TNF, IL10, CCL5, CCL3, VEGFA

Signaling receptor binding	38	1581	8.24E-26	CD4, CSF3, CCL2, IL2, IFNG, IL4, IL5, CRP, TLR2, IL1A, IL1B, STAT3, ICAM1, IL18, PPARG, CSF2, IL13, CXCL10, CXCL8, CASP3, CSF1, IL17A, HMGB1, TLR9, CD40LG, TLR4, IFNB1, CXCL1, INS, IL6, CD8A, TNF, MYD88, IL10, ITGAM, CCL5, CCL3, VEGFA
Binding	54	12516	1.91E-07	CD4, TNFRSF1A, HMOX1, IL2RB, NFKBIA, MEFV, FCGRT, MPO, CSF3, PFN1, CCL2, IL2, IFNG, IL4, IL5, CRP, TLR2, IL1A, IL1B, STAT3, ICAM1, IL18, PPARG, ALB, CSF2, IL13, CXCL10, CXCL8, CASP3, CSF1, CD86, NLRP3, IL17A, HMGB1, ACTB, TLR9, PTGS2, CD40LG, JUN, TLR4, FOXP3, IFNB1, CXCL1, INS, IL6, CD8A, TNF, MYD88, IL10, CASP1, ITGAM, CCL5, CCL3, VEGFA
G protein-coupled receptor binding	8	294	0.00038	CCL2, IL2, STAT3, CXCL10, CXCL8, CXCL1, CCL5, CCL3

Table S4. The detailed information of KEGG pathways related with inflammatory responses enriched by top50 target hub gene

term description	observed gene count	background gene count	FDR	matching proteins in network
Cytokine-cytokine receptor interaction	26	282	1.69E-30	CD4, TNFRSF1A, IL2RB, CSF3, CCL2, IL2, IFNG, IL4, IL5, IL1A, IL1B, IL18, CSF2, IL13, CXCL10, CXCL8, CSF1, IL17A, CD40LG, IFNB1, CXCL1, IL6, TNF, IL10, CCL5, CCL3
Inflammatory bowel disease	17	60	1.27E-26	IL2, IFNG, IL4, IL5, TLR2, IL1A, IL1B, STAT3, IL18, IL13, IL17A, JUN, TLR4, FOXP3, IL6, TNF, IL10
IL-17 signaling pathway	18	92	7.11E-26	NFKBIA, CSF3, CCL2, IFNG, IL4, IL5, IL1B, CSF2, IL13, CXCL10, CXCL8, CASP3, IL17A, PTGS2, JUN, CXCL1, IL6, TNF
TNF signaling pathway	16	112	3.66E-21	TNFRSF1A, NFKBIA, CCL2, IL1B, ICAM1, CSF2, CXCL10, CASP3, CSF1, PTGS2, JUN, IFNB1, CXCL1, IL6, TNF, CCL5
Toll-like receptor signaling pathway	15	101	4.85E-20	NFKBIA, TLR2, IL1B, CXCL10, CXCL8, CD86, TLR9, JUN, TLR4, IFNB1, IL6, TNF, MYD88, CCL5, CCL3
NOD-like receptor signaling pathway	16	174	1.57E-18	NFKBIA, MEFV, CCL2, IL1B, IL18, CXCL8, NLRP3, JUN, TLR4, IFNB1, CXCL1, IL6, TNF, MYD88, CASP1, CCL5
T cell receptor signaling pathway	13	101	8.90E-17	CD4, NFKBIA, IL2, IFNG, IL4, IL5, CSF2, CTLA4, CD40LG, JUN, CD8A, TNF, IL10
Th17 cell differentiation	12	101	3.81E-15	CD4, IL2RB, NFKBIA, IL2, IFNG, IL4, IL1B, STAT3, IL17A, JUN, FOXP3, IL6

NF-kappa B signaling pathway	11	101	1.43E-13	TNFRSF1A, NFKBIA, IL1B, ICAM1, CXCL8, PTGS2, CD40LG, TLR4, CXCL1, TNF, MYD88
JAK-STAT signaling pathway	12	160	4.79E-13	IL2RB, CSF3, IL2, IFNG, IL4, IL5, STAT3, CSF2, IL13, IFNB1, IL6, IL10
Necroptosis	11	149	6.32E-12	TNFRSF1A, IFNG, IL1A, IL1B, STAT3, NLRP3, HMGB1, TLR4, IFNB1, TNF, CASP1
PI3K-Akt signaling pathway	11	350	2.63E-08	IL2RB, CSF3, IL2, IL4, TLR2, CSF1, TLR4, IFNB1, INS, IL6, VEGFA
MAPK signaling pathway	10	288	5.70E-08	TNFRSF1A, IL1A, IL1B, CASP3, CSF1, JUN, INS, TNF, MYD88, VEGFA
HIF-1 signaling pathway	7	106	1.72E-07	HMOX1, IFNG, STAT3, TLR4, INS, IL6, VEGFA
Chemokine signaling pathway	8	186	3.75E-07	NFKBIA, CCL2, STAT3, CXCL10, CXCL8, CXCL1, CCL5, CCL3
Cell adhesion molecules	7	137	8.53E-07	CD4, ICAM1, CTLA4, CD86, CD40LG, CD8A, ITGAM
RIG-I-like receptor signaling pathway	5	70	1.09E-05	NFKBIA, CXCL10, CXCL8, IFNB1, TNF
Apoptosis	6	132	1.19E-05	TNFRSF1A, NFKBIA, CASP3, ACTB, JUN, TNF
Rap1 signaling pathway	6	202	0.00012	PFN1, CSF1, ACTB, INS, ITGAM, VEGFA

Table S5. Results of docking between butyrate and its key targets

Molecular name	Targets	PBD ID	Residue involved in H bonding	H-bond length (Å)	Docking score (kcal/mol)
butyrate	TLR4	3VQ1	LYS341; LYS367	1.86; 3.69	-3.37
butyrate	NF-κB	1VKX	LYS218	2.02	-3.57