NOD1 deficiency impairs CD44a/Lck as well as PI3K/Akt pathway

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Figure S1

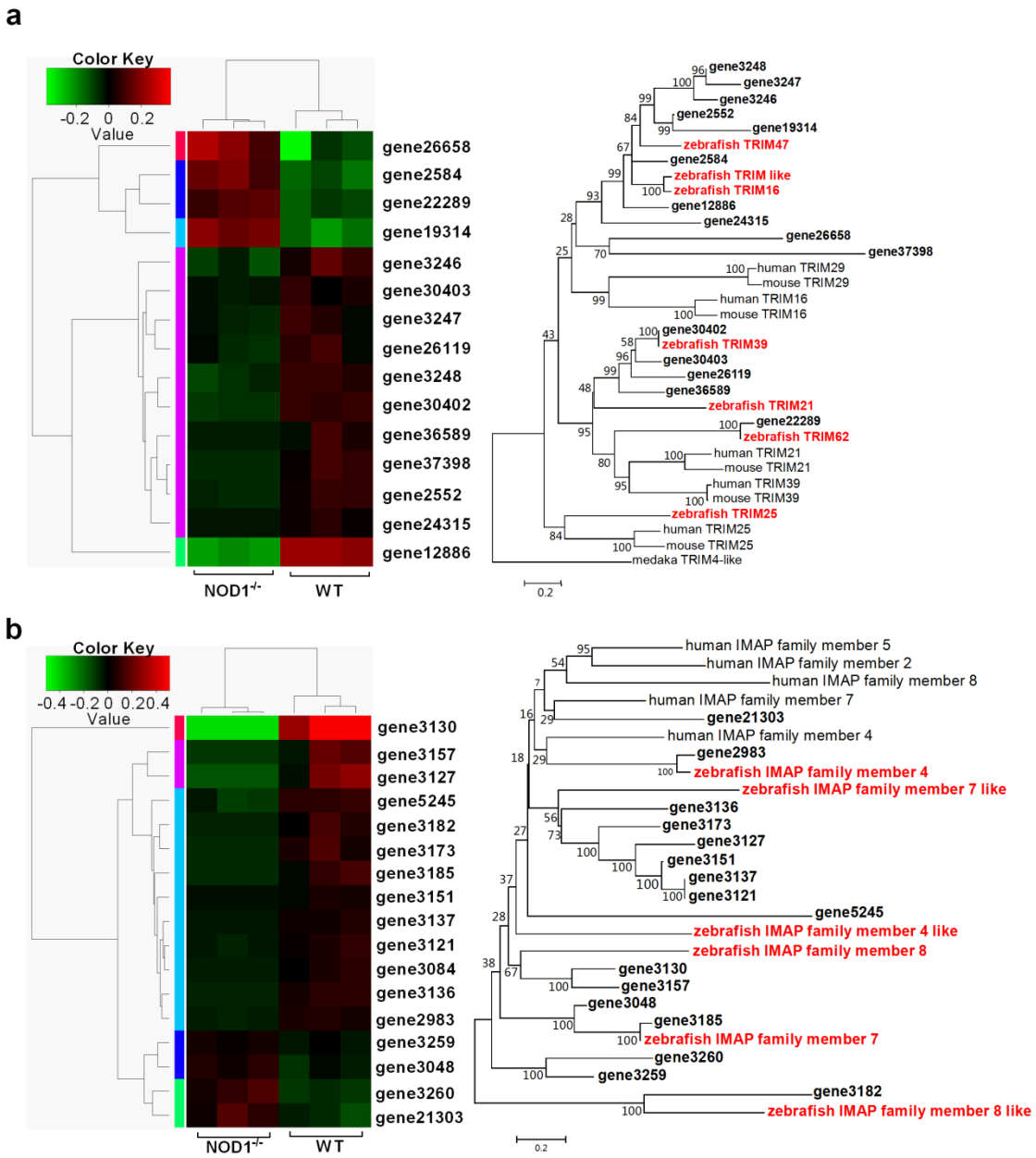


Fig. S1. Heatmap and phylogenetic analysis for differentially expressed genes involved in immune system. (**a**) Heatmap and phylogenetic analysis for tripartite motif proteins (TRIMs). Accession numbers of other TRIMs from NCBI database are as follows: human TRIM25, NP_005073; mouse TRIM25, NP_033572; zebrafish TRIM25, NP_956469; zebrafish TRIM62, NP_001074143; zebrafish TRIM47, XP_002660960; human TRIM29, NP_036233; mouse TRIM29, NP_076144; human TRIM21, NP_003132; mouse TRIM21, NP_001076021; zebrafish TRIM21, XP_691507; human TRIM39, NP_067076; mouse_TRIM39, NP_840065; zebrafish TRIM39, XP_009292249; human TRIM16, NP_006461; mouse TRIM16, NP_444399; zebrafish TRIM16, XP_009297237; medaka TRIM4-like, XP_011472583. (**b**) Heatmap and phylogenetic analysis for immunity-associated proteins (IMAPs). Accession numbers of other IMAPs from NCBI database are as follows: zebrafish IMAP family member 8 like, XP_002664923; zebrafish IMAP family member 8, XP_009294065; human IMAP family member 8, NP_783161; zebrafish IMAP family member 7, XP_009305485; zebrafish IMAP family member 7 like, XP_009301153; human IMAP family member 7, NP_694968; human IMAP family member 5, NP_060854; zebrafish IMAP family member 4 like, XP_001920359; zebrafish IMAP family member 4, XP_009297228; human IMAP family member 4, NP_060796; human IMAP family member 2 , NP_056475.

Figure S2

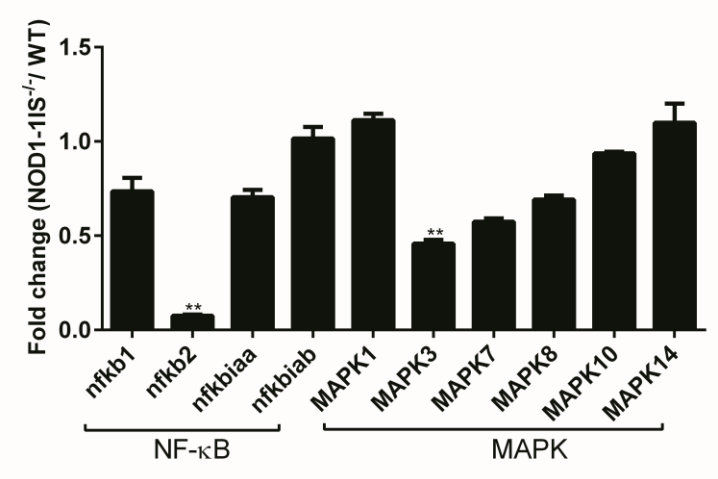


Fig. S2. The effect of NOD1 deficiency on the expression of those genes involved in NF-κB and MAPK pathways. 30~50 larvae from WT and *NOD1-1IS*$^{-/-}$ zebrafish were collected at 10 dpf , and used for qRT-PCR. Data represent the mean ± the SEM, and were tested for statistical significance using two-tailed Student's t-test. $^{**}p < 0.01$.

Figure S3

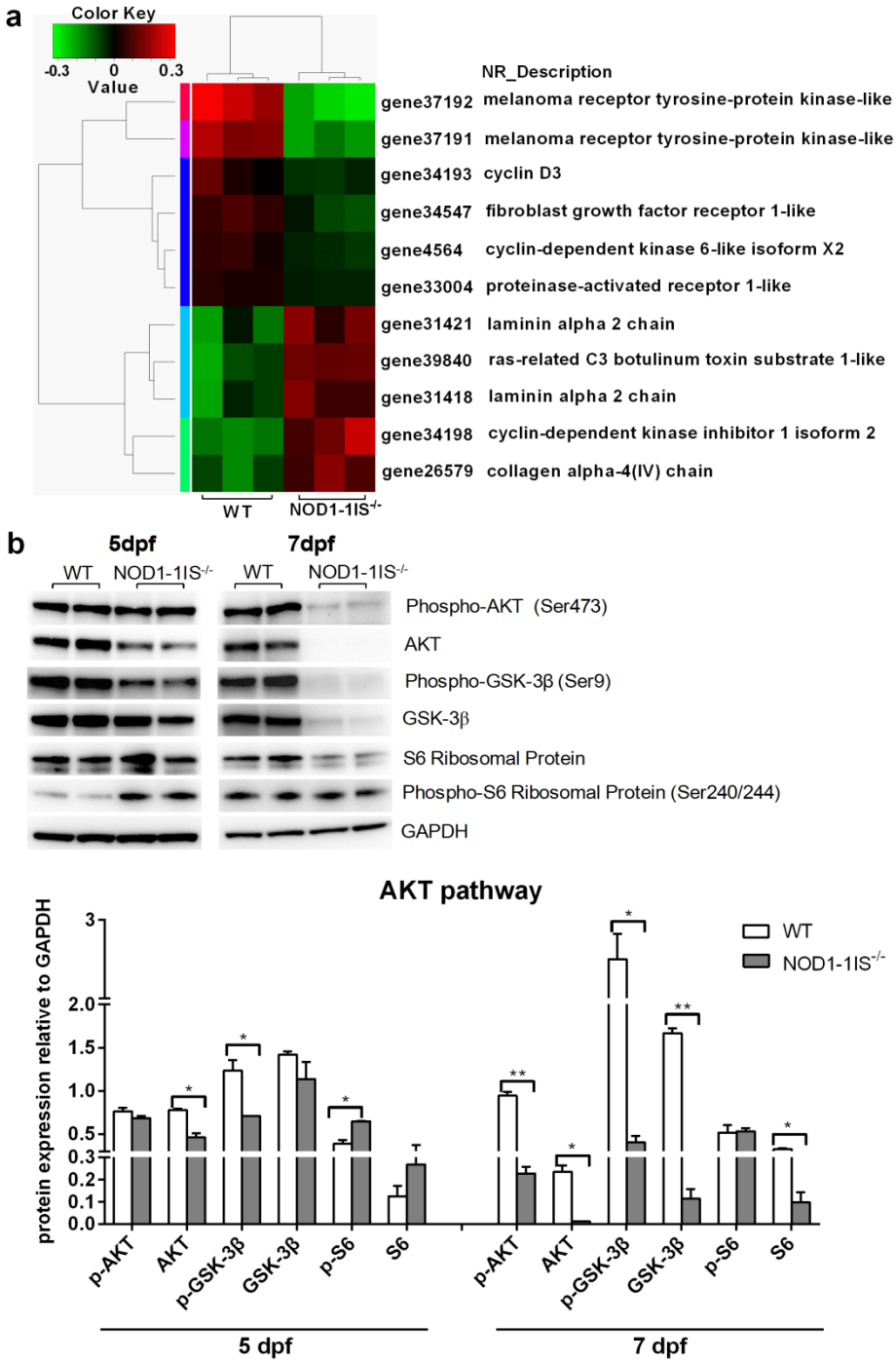
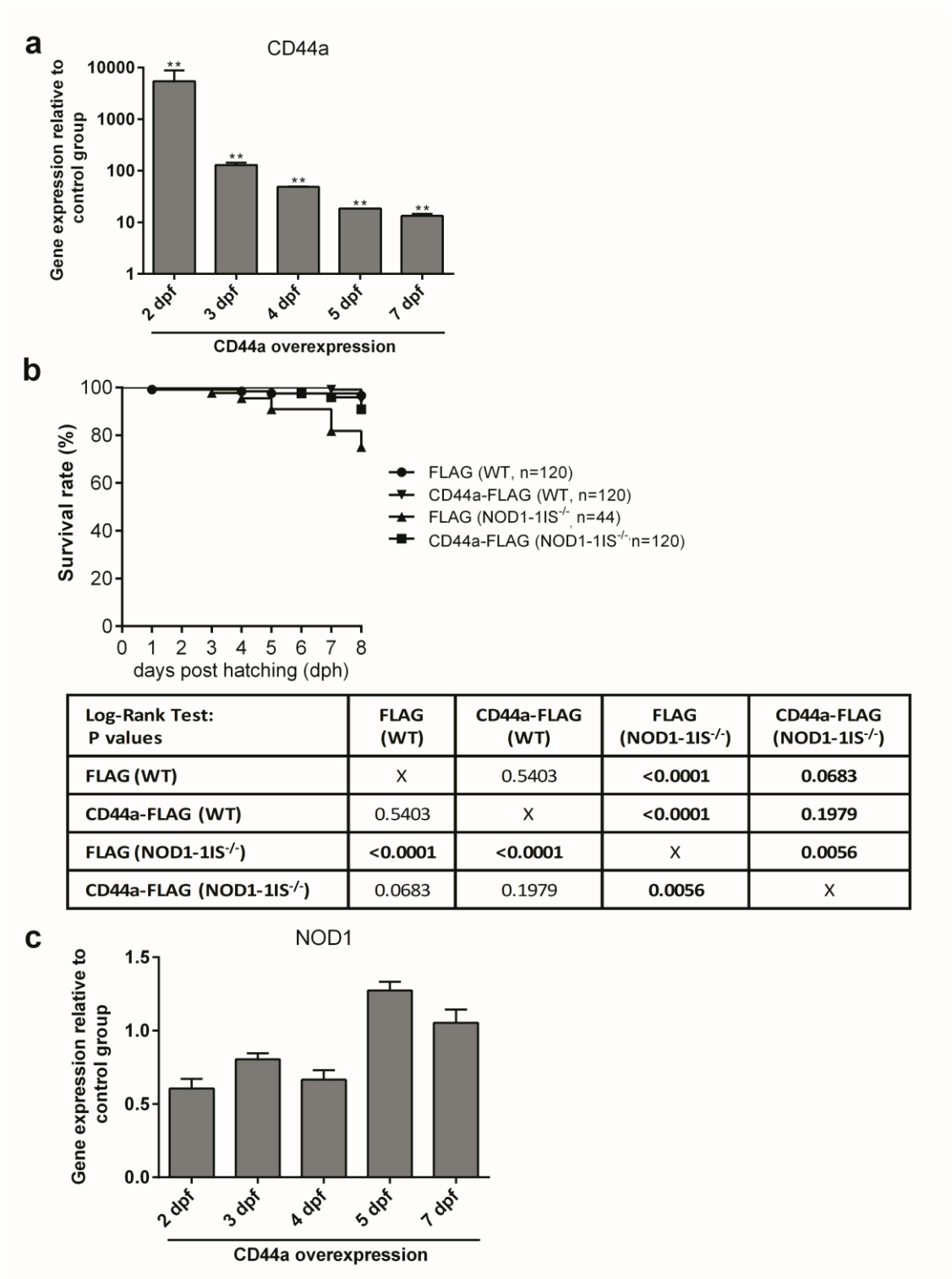


Fig. S3. PI3K-Akt pathway is impaired in NOD1-deficient zebrafish. (**a**) Heatmap analysis for differentially expressed genes involved in PI3K/Akt signaling pathway from transcriptomic data. (**b**) Immunoblot analysis of phospho-Akt, Akt, phospho-GSK3β, GSK3β, phospho-S6 and S6 in larvae homogenate from WT and *NOD1-1IS*$^{-/-}$ zebrafish at 5 and 7 dpf. Western blotting results were quantified using Quantity One software. Data represent the average of two independent experiments. *$p < 0.05$, **$p < 0.01$.

Figure S4



Log-Rank Test: P values	FLAG (WT)	CD44a-FLAG (WT)	FLAG (NOD1-1IS$^{-/-}$)	CD44a-FLAG (NOD1-1IS$^{-/-}$)
FLAG (WT)	X	0.5403	**<0.0001**	**0.0683**
CD44a-FLAG (WT)	0.5403	X	**<0.0001**	**0.1979**
FLAG (NOD1-1IS$^{-/-}$)	**<0.0001**	**<0.0001**	X	**0.0056**
CD44a-FLAG (NOD1-1IS$^{-/-}$)	0.0683	0.1979	**0.0056**	X

Fig. S4. CD44a overexpression in *NOD1-1IS*$^{-/-}$ zebrafish rescues larval survival. (**a**) The expression of CD44a in WT zebrafish microinjected with p3×FLAG or CD44a-FLAG using qRT-PCR (mean ±SEM, n=3). Samples were collected at 2, 3, 4, 5 and 7dpf, and used for RNA extraction. Data were tested for statistical significance using two-tailed Student's t-test. $^{**}p < 0.01$. (**b**) Survival of WT and *NOD1-1IS*$^{-/-}$ zebrafish microinjected with p3×FLAG or CD44a-FLAG (n=44~120 larvae each group). The survival curves lasting for 8 dph were compared statistically significant difference using the Log-Rank Test. (**c**) The expression of NOD1 in WT zebrafish microinjected with p3×FLAG or CD44a-FLAG using qRT-PCR (mean ±SEM, n=3). Samples were collected at 2, 3, 4, 5 and 7dpf, and used for RNA extraction. Data were tested for statistical significance using two-tailed Student's t-test.

Table S1. Enrichment analysis of the KEGG pathways for differentially expressed genes in WT vs *NOD1-1IS*$^{-/-}$ zebrafish larvae

#Term	Id	Sample number	Background number	P-Value	typeII
Selenocompound metabolism	ko00450	4	22	0.000649471	Metabolism of other amino acids
Antigen processing and presentation	ko04612	10	97	0.001201018	Immune system
Drug metabolism - cytochrome P450	ko00982	4	47	0.008010135	Xenobiotics biodegradation and metabolism
Steroid hormone biosynthesis	ko00140	4	47	0.008010135	Lipid metabolism
Metabolism of xenobiotics by cytochrome P450	ko00980	4	51	0.010417054	Xenobiotics biodegradation and metabolism
Primary bile acid biosynthesis	ko00120	3	29	0.013136656	Lipid metabolism
Retinol metabolism	ko00830	4	55	0.013247944	Metabolism of cofactors and vitamins
NOD-like receptor signaling pathway	ko04621	4	73	0.031766049	Immune system
Carbon fixation pathways in prokaryotes	ko00720	2	21	0.04889759	Energy metabolism

Table S2. Primer information

Name	Sequence	Application
CD44a-FLAGF	GTCAAGCTTACCATGTGGACTTTGTTATTTGTAG	Ligated to p3xFLAG-CMV™-14 vector
CD44a-FLAGR	GAAGGTACCATTAAATATTCTTTTTCGTGTTCATC	
NOD1-FLAGF	GTGGAATTCTGAAATGAAATTAAATATGG	
NOD1-FLAGR	GAAGGATCCGCTGACTCCCTCTCGTTG	
NOD2-FLAGF	CCGGAATTCAACTATGAACGCTCAACAGT	
NOD2-FLAGR	CGCGGATCCGAAGGTCAATCTGCATTC	
NOD1F	AGCAACTGCGACCCTCTTTCA	Quantitative real-time PCR
NOD1R	TGGCGAGATATTTCCCACCTACA	
CD44aF	GAAAGTAATGCGAAGGAG	
CD44aR	TCATCAGTGCCACAATCT	
lckF	TACGTAAACATGGGGAACTG	
lckR	TCTTCTCCCCTTTCTCAAAC	
NALPL1F:	TCGTTCTCCACGGTGACTC	
NALPL1R	AGCAGGGCACAAACAATGAC	
mhc1umaF	ACGGTGCAAAGGACATACAACAAC	
mhc1uma R	ATCAGCCAGTAAATACATCCAAGT	
mhc1ulaF	AAGAGTCACATGGGTTTG	
mhc1ulaR	CTCATTTGGCTTTAGTTC	
similar to mhc1uba-F	GGATGGAAGATAAACTTGGGACA	
similar to mhc1uba-R	CAGCCACTCAATGCACTCAGACC	
mhc1ubaF	AAAACGCTCACCTGGACTG	
mhc1ubaR	ATACTTCTGCACCCACTCA	
MHC-IIβF	TGAGGAGATGGCTGATGGAGAC	
MHC-IIβR	CATAGATGATGGGCTTAGTTGA	
mhc2dabF	CTCTGTGGGGAAGTTTGTG	
mhc2dabR	CCAGATCCGAGCATTATGTC	
gene26712F	GTGGCATTGATGTGGCAGAAGC	
gene26712R	CGACTGACAAACCCAGAAGAAA	
gene38616F	AAAACTACTTCGCTCTTCCCTAA	
gene38616R	TACAGACTCCTGGATTTCTTGAT	
gene26713F	AGGTTGTGCCGCTTTGACTT	
gene26713R	CATTCCTTTAGATTGTGGGT	
gene2604F	TTTCTTGAGCGGGAATACAGT	
gene2604R	ATGAGGGATGGAAGTTTGGAC	
gene39147F	GCTCTATTGGCACGATGTTA	
gene39147R	CAGACGATTGTTGCGTAGGT	
gene4944F	GCATTCAGTGATGGGTCT	
gene4944R	ATCCAGGTGTCCAGTTTT	
gene12406F	CCTGACACAAGTCGACAAAAGAGAA	
gene12406R	ATCCAGATGAGGGAAGAACGAAGC	
gene12839F	ACCTCTTTCTGCGTTTCCTGCTT	
gene12839R	GCTTCTCCTCCGAGTGTTTGTCT	
gene4933F	GAGGACAAAAAAACAACATCAGTC	
gene4933R	CTCACAGCGGGCACCAGTCT	
gene8198F	TCTTTAGAAGTGGAGGAC	
gene8198R	ATGTGGCTGATGATGTTT	
gene39994F	TGACTTTGTGGAGGAACT	
gene39994R	GCTGAGCGGTGACATTAC	

gene3642F	GATTCCTGATGTCTGGGTC	
gene3642R	AGGGTGTAAACATTTCGGT	
gene12370F	TCCTGGGCTTTAATCTGCTACG	
gene12370R	GCGGCTCAGTGAATGTGGTTTG	
gene1131F	CCACGGACCCTCCACAGAACTAT	
gene1131R	CAGCGACTCCCTTCGTCAGCACA	
gene1132F	AAATCCATCAACCTGTTCCACT	
gene1132R	ACAAAGACCACCGCTGACCACT	
gene1133F	AGATGAAAACCTGCCCAAA	
gene1133R	GCTAGAGCCGTGACAAGTG	
gene12961F	TGCTAGAGTTTCTGCTGGAC	
gene12961R	AAAGTTTATTGGTGGACGC	
gene26693F	CAATGAGAAGTTTGCGGAGTT	
gene26693R	ATGACAGCAGATGCGGTTATG	
gene33648F	GACCAATCTCATCAGAGGGAA	
gene33648R	CGGCGAGTAGCACATAATAAA	
gene26589F	AAGTTGGTGGGTTGTGGT	
gene26589R	CTCGCAGATTTGAGAAGT	
gene6751F	GAGAATACACAGAAGCAAGGTGCAG	
gene6751R	GAACGGCAGTGGAAATATGAAGAC	
gene23645F	AGAAGCTCGTGCCCGTAGT	
gene23645R	ATGCCAAATCAGCAGAACC	
nfkb1F	TGTGGTTCGGCTGATGTTC	
nfkb1R	GGTTCGCTCGTCTCGTTGT	
nfkb2F	AAGATGAGAACGGAGACACGC	
nfkb2R	TCTACCAGCAATCGCAAACAA	
nfkbiaaF	CATTCACGAGGCAGAGGATTAT	
nfkbiaaR	TCCGCTGTAGTTAGGGAAGGTA	
nfkbiabF	TGTCATTGTTGGAAAGCCTCAT	
nfkbiabR	TAATCCTCGTCGCTATCCTCAC	
MAPK1F	ATTACCTGCTGTCCCTTCC	
MAPK1R	CTTCCTCCACCTCAATCCTC	
MAPK3F	GACCTGAAGCCCTCAAACC	
MAPK3R	GCATCCCACAGACCAAATA	
MAPK7F	CCTCAGGGAAAGTCACAATG	
MAPK7R	TCAGGTATCAGGAAGCGTAG	
MAPK8F	TGACCCGTTACTACAGAGCC	
MAPK8R	CATCCACCGAGATTCGTTTT	
MAPK10F	GGGGCTCAGGGAATAGTGT	
MAPK10R	AATCTTCAGCGTGCAGTCG	
MAPK14F	CGTGACGGTGGACATTTGGT	
MAPK14R	TGGGCATCTGAGGAAGTGAG	
GAPDHF	GTAACTCCGCAGAAAAGCCAGAC	
GAPDHR	CAAAAGAAACTAACACACACACA	