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Comprehensive analysis of a peripheral blood transcriptome signature in piglets infected with *Salmonella* Typhimurium: insight into immune responses

Eun-Yeong Bok¹, Seung-Won Yi¹, Han Gyu Lee¹, Jae Kyeom Kim^{2,3}, Kangwook Lee², Seungmin Ha¹, Bumseok Kim⁴, Young-Hun Jung¹ and Sang-Ik Oh^{4*} 

Abstract

Salmonella Typhimurium (ST) infection in pigs poses a significant threat to animal health and food safety; the intricate mechanisms underlying host-immune responses and pathogen persistence remain poorly understood. To address this knowledge gap, we comprehensively analyzed the peripheral blood transcriptome in piglets infected with ST. We performed histopathological evaluation, blood parameter analysis, advanced RNA-sequencing techniques, and quantitative reverse transcription PCR (RT-qPCR)-based validation. The increase in the monocyte counts at 2 days post-infection suggested its potential to serve as a hematological marker for ST infection in piglets. Functional and pathway enrichment analyses of the differentially expressed genes highlighted the pivotal roles of innate and adaptive immune responses, notably in pathways associated with Toll-like receptors, NIK/NF- κ B signaling, cytokine signaling, and T cell proliferation. RT-qPCR-based validation using peripheral blood mononuclear cells provided additional insights into the immune system dynamics in response to ST infection, revealing the marked elevation of the interleukin (*IL*)-15, *IL*-27, and *CXCL10* levels being significantly elevated in ST-infected piglets. Our comprehensive analysis underscores the multifaceted impact of ST infection on piglets and offers valuable insights into the host-pathogen interactions and the role of host immune system during ST infection.

Keywords *Salmonella* Typhimurium, Immune response, RNA-seq, Cytokine signaling, Cellular interaction

*Correspondence:

Sang-Ik Oh
sioh@jbnu.ac.kr

¹National Institute of Animal Science, Rural Development Administration, Wanju 55365, Republic of Korea

²Department of Food Science and Biotechnology, College of Science and Technology, Korea University, Sejong 55365, Republic of Korea

³Department of Behavioral Health and Nutrition, College of Health Sciences, University of Delaware, Newark, DE 19716, USA

⁴Laboratory of Veterinary Pathology and Biosafety Research Institute, College of Veterinary Medicine, Jeonbuk National University, Iksan 54596, Republic of Korea



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Introduction

Pigs infected with *Salmonella* spp. are significant zoonotic reservoirs for the transmission of salmonellosis to humans [1]. Among the various serovars of *Salmonella enterica*, *Salmonella enterica* serovar Typhimurium (ST) is particularly prevalent in pigs, affecting all age groups [2]. It is particularly prevalent in post-weaned pigs, in which it often leads to enterocolitis, manifesting as diarrhea. Post-weaned pigs are vulnerable to ST infection because of a temporary decrease in feed intake and reduced maternal antibody levels [3]. Several strategies, including vaccination, antibiotic administration, and regular health examinations, have been employed to mitigate *Salmonella* infection in post-weaned pigs [4].

Building on the efforts to reduce or eliminate *Salmonella* infection in post-weaned pigs, identifying key genes involved in the initial immune responses following ST infection represents a proactive strategy to control salmonellosis. Pathogenesis of ST has been reported in quantitative reverse transcription PCR (RT-qPCR) studies using specific immune tissues, such as Peyer's patches [5, 6] and mesenteric lymph nodes [7, 8]. *Salmonella* spp. typically enters the host through fecal-oral route, and this pathogen is detected by pattern recognition receptors (PRRs) of innate immune cells. Some bacteria penetrate the epithelium and M cells, while others are phagocytosed by macrophages in the submucosal layer [9, 10]. If these bacteria escape due to macrophage death triggered by the caspase-1 pathway, they can spread either directly or via lymphatic vessels and mesenteric lymph nodes into the bloodstream, causing systemic infection [11]. During this systemic infection phase, the immune response is mediated largely by white blood cells, such as macrophages, dendritic cells, and natural killer cells. In addition, peripheral blood mononuclear cells (PBMCs) contain a mixed population of various immune cells [12]. Therefore, the investigation of gene expression in blood samples from pigs could serve as a valuable window into understanding the immune responses in ST infection. Furthermore, recent studies based on advanced Next-Generation Sequencing technologies have established a foundational understanding of the peripheral blood transcriptomic responses to ST infections, providing critical insights into the dynamics of host–pathogen interactions. Huang et al. highlighted the roles of specific regulators, such as *TGFBI* and *TRP53*, in modulating intracellular ST replication, demonstrating the complexity of immune response modulation by these genes [13]. Another study by Huang et al. further outlined the influence of microRNA on the host immune system during ST infection, emphasizing the intricate network of gene regulation and signaling pathways involved in the response to ST [14].

However, previous studies have primarily focused on the roles of specific annotated pathway factors; other potential regulators and mechanisms that may influence *Salmonella*–host interactions remain unclear. This gap in literature underscores the need for continued research regarding the mechanisms underlying *Salmonella* infection and host immune responses. Thus, in the present study, we aimed to elucidate the important immune mechanisms associated with the early stages of ST infection in post-weaned piglets. To this end, we comprehensively analyzed blood parameters and peripheral blood transcriptome in ST-infected piglets at 0 and 2 days post-infection (dpi); these results were validated using RT-qPCR in PBMCs samples. Our findings will greatly enhance our understanding regarding the mechanisms underlying host responses to ST and aid the development of effective strategies to control ST infection in post-weaned piglets.

Materials and methods

Animals and sample collection

The animal experimental procedures were approved by the Animal Ethics Committee of National Institute of Animal Science in Republic of Korea (approval No. NIAS 2021–503). This study was part of a larger project aimed at developing novel alternative antibiotics for preventing *Salmonella* infection in piglets. The goal of the current study was to specifically elucidate the *Salmonella*–host interaction in ST-infected piglets using transcriptomic approaches. The analytical samples used in this study were obtained in our previous study [15], but the present study focused on elucidating the peripheral blood transcriptome in ST-infected piglets. In brief, castrated piglets (Landrace × Yorkshire, 25 days of age) were randomly assigned to an ST infection group (ST, $n=6$) and a negative control group (NC, $n=4$). To prevent cross-contamination, animals of each group were housed in a separate, sanitized room. At the age of 49 days, the piglets in the ST group were orally administered 1×10^8 colony-forming units of ST strain LT2 (ATCC 19585; American Type Culture Collection, Manassas, VA, USA), whereas those in the NC group received an equivalent volume of sterile phosphate-buffered saline, with the dosage selected based on previous studies [16, 17]. Comprehensive details on the methodologies used in the animal experiments, including fecal sampling, bacterial isolation, post-mortem examination at 14 dpi, and histopathological analysis, are provided in our previous study [15].

At 0, 2, 5, 8, 11, and 14 dpi, blood samples were collected from the jugular vein of each piglet before the morning feeding. Blood was collected in EDTA-vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, USA) for complete blood counts (CBCs) and in Tempus Blood RNA Tubes (Applied Biosystems, Seoul, Korea) for

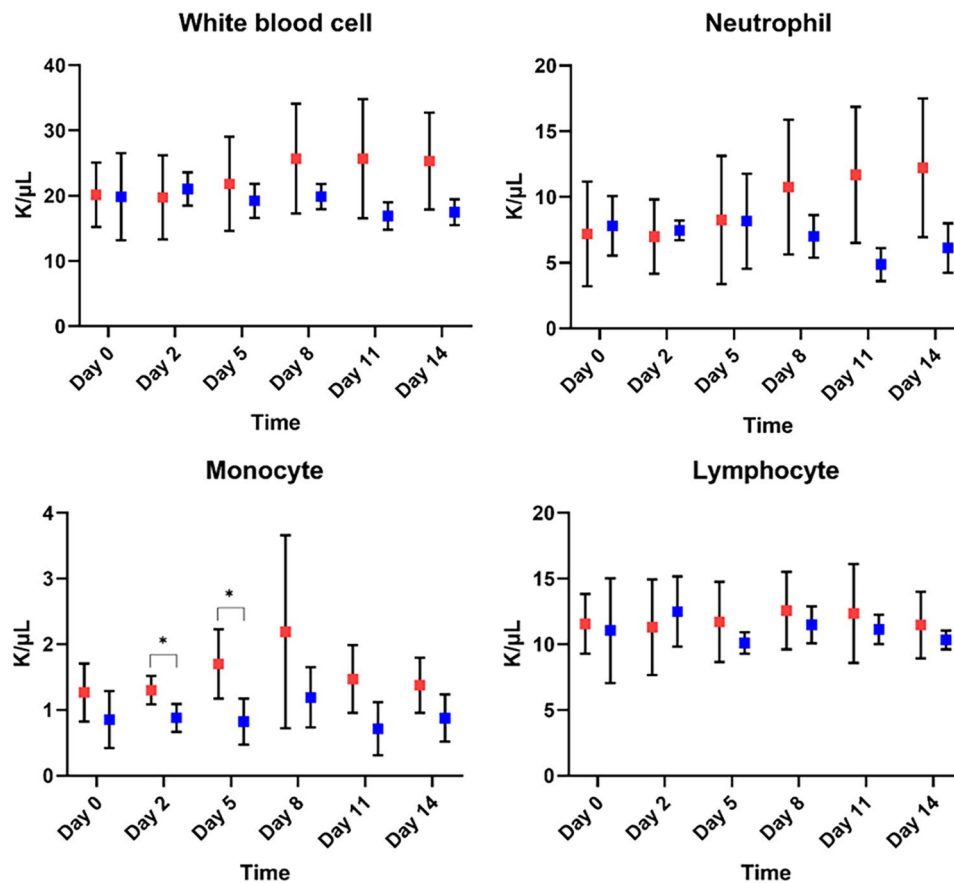


Fig. 1 Blood parameter analysis in ST-infected and NC piglets. WBC, neutrophil, monocyte, and lymphocyte counts at 0, 2, 5, 8, 11, and 14 dpi are shown. Red squares represent the average value of ST group, blue squares denote the NC group. * $P < 0.05$

transcriptome analysis of blood samples obtained at 2 dpi.

Analysis of blood parameters

The CBC test was conducted according to a previous study [18]. In brief, blood samples were analyzed using a ProCyte Dx hematology analyzer (IDEXX Laboratories, Westbrook, ME, USA) to quantify white blood cells (WBCs), neutrophils, monocytes, and lymphocytes.

RNA extraction, library construction, and sequencing

Total RNA was isolated from peripheral blood collected at 2 dpi from pigs of the ST and NC groups using an RNeasy Mini Kit (Qiagen, Hilden, Germany) per the manufacturer's guidelines. Potential genomic DNA contaminants were removed using DNase I (Qiagen) for on-column DNase digestion during RNA purification. RNA purity and structural integrity were validated using a NanoPhotometer (Implen, Munich, Germany). RNA quality was evaluated using a Nano 6000 Assay kit in a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Libraries were constructed using a TruSeq Stranded Total RNA Library Prep Globin kit (Illumina,

San Diego, CA, USA) per the manufacturer's instructions. In brief, the total RNA was fragmented, and cDNA was synthesized. The cDNA fragments were subjected to end repair, A-tailing, and indexed adapter ligation. The ligated fragments were PCR-amplified, followed by gel purification to eliminate leftover primers and adapters. Transcriptome sequencing was executed using the Illumina HiSeq 2000 system, producing 100-bp paired-end unprocessed reads.

Raw data processing

Raw Illumina sequencing data were obtained after base calling and stored in FASTQ format. A series of data pre-processing steps were undertaken to ensure sequencing data quality and reliability. FastQC v0.11.7 (www.bioinformatics.babraham.ac.uk) was used to assess data quality. Trimmomatic v0.38 (www.usadellab.org) was used to eliminate N bases from both 5'- and 3'-end reads. Reads shorter than 36 bp were removed. The trimmed sequences were aligned to the pig reference genome (Sscrofa11.1) using the Hisat2 v2.1.0 mapping program (www.ccb.jhu.edu/software/hisat2). Using the StringTie program, we conducted transcript assembly based on a

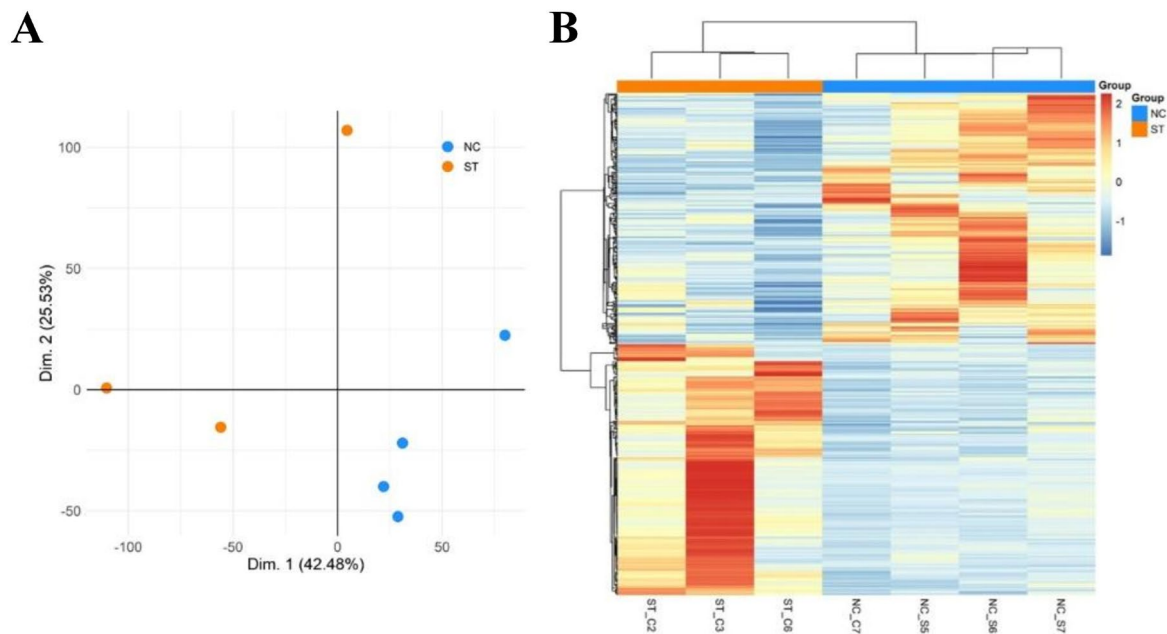


Fig. 2 (A) PLS-DA plot (supervised classification) and (B) heatmap of the DEGs. The PLS-DA plot shows a robust separation of the groups (NC vs. ST). HCA of the DEGs between the two study groups is represented in the heatmap (red color indicates high abundance, blue indicates low abundance). NC ($n=4$), ST ($n=3$)

reference gene model for known transcripts. Read count values for known genes were used for subsequent analysis of differentially expressed genes (DEGs).

Partial least squares-discriminant analysis (PLS-DA)

PLS-DA is a supervised classification method that builds on the PLS algorithm to determine axes (latent variables) that explain the most variance in both predictors and response variables. We conducted PLS-DA using the R package (v4.3.1). Processed transcriptome data were scaled to ensure equal variance across all measured transcripts. Then, a PLS-DA model was constructed to differentiate between the ST and NC groups.

DEG analysis and hierarchical clustering analysis (HCA)

Prior to DEG analysis, low-quality genes across samples were removed and the read counts were normalized. DEGs were identified using the DESeq2 R package tool, which assumes that no genes are differentially expressed. The data were normalized using the median of ratios, which is calculated as counts divided by sample-specific size factors determined by median ratio of gene counts relative to the geometric mean per gene. P -values were adjusted using the Benjamini & Hochberg method. DEGs were identified using a false discovery rate (FDR)-adjusted $P < 0.05$ and \log_2 (fold change) ≥ 2.0 as thresholds. In total, 495 DEGs were identified and used in subsequent analyses. DEGs between the ST and NC groups were visualized in a heatmap generated using HCA.

Bioinformatics analyses

Initial exploratory steps involved enrichment analyses of the DEGs, using the Gene Ontology (GO) framework. GO provides a systematic nomenclature that uniformly characterizes gene functions, along with the properties of their protein products, across multiple databases. We assessed enrichment in the three primary GO categories: biological processes (BP), cellular components (CC), and molecular functions (MF). In addition, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

It is noteworthy that variations may arise in pathway enrichment across different databases, even for the same DEGs, because of the unique datasets and algorithmic processes employed in each database. Therefore, the same set of DEGs was analyzed using the Ingenuity Pathway Analysis (IPA) platform (Qiagen) to identify potential upstream regulators and corresponding canonical pathways affected by ST inoculation. The upstream regulators were predicted based on the well-curated relationships documented in the Ingenuity Knowledge Base. The significance of associations identified by Upstream Regulator Analysis in IPA was determined using Fisher's exact test, providing statistical rigor to the inferred biological relationships.

PBMC isolation

At 2 dpi, blood samples were collected from animals of the ST and NC groups in EDTA tubes and mixed at a 1:1 ratio with Dulbecco's PBS (Life Technologies, Carlsbad,

CA, USA). The mixture was deposited on the porous membrane in a Leucosep tube (Greiner Bio-One, Kremsmünste, Austria) pre-filled with Ficoll-Paque (GE Healthcare, Chicago, IL, USA). Following centrifugation at $800 \times g$ for 15 min, the PBMCs were carefully harvested from the Ficoll-Paque interface, washed twice with PBS, and stored at -80°C until use in RT-qPCR analysis.

RT-qPCR validation

We used a combination of literature-derived and custom-designed primers to target a comprehensive panel of genes of interest (Additional file 1: Table S1). For genes, such as *TLR2*, *TLR4*, and *CXCL10*, primer sequences were adopted from previously published studies and normalized by *ACTB* [19–21]. Primers for *IL15* and *IL27* were designed using the IDT Oligo Primer Design Tool (Integrated DNA Technologies, Coralville, IA, USA) and synthesized at TNT Biotech Co., Ltd. (Jeonju, Korea). RT-qPCR assays were conducted to validate the transcriptomic findings. Total RNA was extracted from PBMCs using a RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. Five hundred nanograms of total RNA was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) per the supplier's instructions. qPCRs were run in a 7500 Real-Time PCR System (Applied Biosystems). The amplification protocol was as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Target gene expression levels were quantified relative to the *ACTB* reference gene using the comparative $\Delta\Delta\text{CT}$ method [22] and analyzed using the StepOne software v2.1 (Applied Biosystems).

Statistical analysis

Statistical analyses were performed using the SPSS software version 25.0 (IBM, Armonk, NY, USA). A generalized linear mixed model with Bonferroni correction was used to evaluate repeated measurements of hematological and serum biochemical parameters. Within this model, "time" and "group" were treated as fixed effects, whereas "piglets nested within groups" was considered a random effect. The Mann–Whitney U test with Bonferroni correction was employed for non-parametric comparisons and to avoid type-1 errors. Data are presented as mean \pm standard deviation. Results with P -values < 0.05 were considered statistically significant. RT-qPCR results were analyzed using Welch's t -test to accommodate any potential variance in gene expression between the ST and NC groups.

Results and discussion

Confirmation of phenotypic observations and blood parameters in ST-infected piglets

In our previous study [15], we reported ST isolation from all fecal samples (6 out of 6 piglets, 100%) at 2 dpi, along with histopathological changes indicative of ST infection at 14 dpi. Piglets in the ST group showed moderate to severe lesions representative of *Salmonella* infection, including villous atrophy, inflammatory cell infiltration, and ST presentation, in immunohistochemical staining tissue sections (Additional file 1: Fig. S1). To evaluate the cellular immune responses to ST infection in piglets, the WBC, neutrophil, monocyte, and lymphocyte counts were determined at 0, 2, 5, 8, 11, and 14 dpi (Fig. 1). The WBC, neutrophil and lymphocyte counts at 5 dpi tended to be higher in the piglets from ST group than in those from NC group; this finding is consistent with the results of a previous study [23]. This previous study reported that the WBC and neutrophil counts in the ST-infected piglets increased significantly by 7 dpi, and a genetic variant in the *CCT7* gene was associated with higher levels of these immune cells. Our findings also revealed that ST-infected piglets showed significantly higher monocyte counts (1.30 ± 0.22 K/gL and 1.70 ± 0.53 K/gL, respectively) at 2 and 5 dpi than the piglets in NC group (0.88 ± 0.21 K/gL and 0.83 ± 0.35 K/gL, respectively). These results implied that the monocyte count in ST-infected piglets may serve as a valuable hematology-based marker for ST infection. Monocytes play a crucial role in controlling ST infection in the early and late phases and are key to both innate and adaptive immunity. They originate from progenitors in the bone marrow and migrate via the bloodstream to peripheral tissues, where they can differentiate into macrophages and dendritic cells, which are essential for combating ST infection [24, 25]. The recruitment of monocytes into tissues during persistent ST infection is critical; the depletion of monocytes during early infection increases the susceptibility to salmonellosis, underscoring their importance in the host defense mechanism against ST infection.

RNA-sequencing quality control

To assess the quality of our sequencing data, the sequence error rate was calculated based on the Phred score, with Phred scores 10, 20, 30, and 40 corresponding to 10%, 1%, 0.1%, and 0.01% error rates, respectively. Following paired-end transcriptome sequencing analysis of seven samples, the outcomes for all samples, except three from the ST group, aligned with the anticipated range. Additional file 1: Tables S2 and S3 show the raw and processed reads for each sample, categorized by total data volume and Q30 (Phred score for base quality), highlighting metrics that exceeded a value of 30 (Additional file 1: Tables S2–S4).

Table 1 GO terms enriched in peripheral blood samples from *Salmonella* Typhimurium-infected piglets

Term	Count	%	PValue	Genes	Fold Enrichment	Bonferroni	Benjamini	FDR
Terms for biological process								
inflammatory response	21	4.1	0.000000	SPHK1, SLC11A1, PTGER3, IL18, IL27, CXCR6, AIF1, CXCL10, LTB4R2, TLR8, S100A12, CD14, CCR5, TLR4, TLR2, CMKLR1, IDO1	4.95	0.00001	0.00001	0.00001
cellular response to cytokine stimulus	8	1.6	0.000000	LOC100523310, LOC102161784, LOC100523492, CXCR4, LOC100155195, GBP2, LOC100523668, GBP1	15.49	0.00068	0.00031	0.00031
positive regulation of NIK/NF-kappaB signaling	10	2.0	0.000001	IL1RL1, TNFSF14, SPHK1, CD14, NMI, MMP8, TLR4, SASH1, HAVCR2, TLR2	9.68	0.00094	0.00031	0.00031
immune response	21	4.1	0.000001	CD274, GHSR, TNFSF14, IL15, TNFSF13, IL18, LOC110260749, CXCR4, CXCR6, CTSV, LOC100511343, TNFSF13B, CXCL10, SLPI, CCR9, TLR8, TNFSF11, XCL1, FAS, CCR5, TLR4	3.74	0.00126	0.00031	0.00031
positive regulation of interferon-gamma production	10	2.0	0.000003	IL1RL1, KLRK1, CLEC7A, SLC11A1, IL18, TLR8, IL27, CD14, TLR4, HAVCR2	8.15	0.00426	0.00085	0.00085
innate immune response	20	3.9	0.000007	FCN1, BLK, IL27, NOD1, C2, ISG20, KLRK1, VNN1, SLPI, TIFA, TLR8, S100A12, CD14, TLR4, S100A8, TLR2	3.40	0.00962	0.00161	0.00160
toll-like receptor 2 signaling pathway	5	1.0	0.000039	TLR2	23.24	0.05244	0.00744	0.00737
MyD88-dependent toll-like receptor signaling pathway	7	1.4	0.000044	TLR8, TLR4, TLR2	10.49	0.05776	0.00744	0.00737
positive regulation of T cell proliferation	7	1.4	0.000222	SPTA1, CD274, IL15, PDCD1LG2, AIF1, HAVCR2, TNFSF13B	7.93	0.26172	0.03371	0.03341
positive regulation of interleukin-6 production	8	1.6	0.000350	IFNG, CLEC7A, TLR8, IL1RAP, MMP8, AIF1, TLR4, TLR2	6.00	0.38031	0.04785	0.04743
Terms for cellular component								
external side of plasma membrane	21	4.1	0.000000	CD274, CD163, HHLA2, LOC110257900, LOC110260749, IL5RA, CXCR4, ECE1, CXCR6, FCER2, CXCL10, KLRK1, CLEC2B, CLEC7A, CCR9, FAS, CD14, CCR5, LOC100523789, TLR4, ABCG1	4.14	0.00005	0.00005	0.00004
extracellular space	46	9.1	0.000005	FCN1, FCN2, ASAH1, TINAGL1, TNXB, SPARC, TNFAIP6, HP, IL27, C4BPA, RETN, CTSV, ERFE, TSKU, TNFSF13B, LOC100156248, RELN, PLAUI, SERPINH1, S100A12, GHSR, IGFBP4, IL15, CD180, TNFSF13, IL18, NRG1, NMI, MERTK, MMP8, EREG, COL1A1, CHIT1, CXCL10, COL3A1, COL1A2, SLPI, IFNG, TCN1, SERPING1, XCL1, S100A9, S100A8, LTF	2.06	0.00115	0.00058	0.00057
cell surface	19	3.7	0.000078	FZD1, TNFSF18, PRNP, GHSR, GPR37, SPARC, CXCR4, ACVR1B, SLC4A4, LOC100511343, OSCAR, KLRK1, CLEC7A, PLAUI, LY6D, CCR9, HAVCR2, TLR2, LTF	2.98	0.01815	0.00611	0.00600
phagocytic vesicle membrane	6	1.2	0.000441	SLC11A1, TLR2	9.19	0.09878	0.02384	0.02344
membrane raft	10	2.0	0.000505	PRNP, GHSR, SERPINH1, FAS, CD14, TLR2	4.34	0.11240	0.02384	0.02344
Terms for molecular function								
transmembrane signaling receptor activity	16	3.2	0.000000	THBD, FCMR, CD180, CD300LB, CHRNA9, FCER1A, TLR8, FAS, FCGR2B, LOC100523789, TLR4, TLR2	7.31	0.000002	0.000002	0.000002
NAD+ nucleosidase activity	9	1.8	0.000002	IL1RL1, TLR8, IL1RAP, TLR4, TLR2	10.61	0.000731	0.000365	0.000363

Table 2 KEGG pathways enriched in peripheral blood samples from *Salmonella* Typhimurium-infected piglets

Term	Count	%	PValue	Genes	Fold Enrichment	Bonferroni	Benjamini	FDR
Cytokine-cytokine receptor interaction	24	4.7	0.00000	TNFSF18, IL15RA, TNFSF14, IL15, IL1R2, TNFSF13, IL18, IL5RA, CXCR4, IL27, IL1RAP, CXCR6, ACVR1B, TNFSF13B, CXCL10, IL1RL1, IFNG, CCR9, TNFSF11, XCL1, FAS, TNFRSF25, CCR5, IL9R	3.93	0.00001	0.00001	0.00001
Intestinal immune network for IgA production	8	1.6	0.00011	IL15RA, IL15, TNFSF13, CCR9, CXCR4, SLA-DOB, AICDA, TNFSF13B	7.15	0.02758	0.01399	0.01382
Rheumatoid arthritis	10	2.0	0.00021	IFNG, IL15, TNFSF13, IL18, TNFSF11, CTSV, SLA-DOB, TLR4, TLR2, TNFSF13B	4.81	0.05396	0.01450	0.01433
Pertussis	9	1.8	0.00022	C1R, CASP1, SERPING1, C4BPA, NOD1, CD14, TLR4, C2	5.44	0.05637	0.01450	0.01433

Table 3 Top 10 predicted canonical pathways in peripheral blood samples from *Salmonella* Typhimurium-infected piglets

Canonical pathways	$-\log(p\text{-value})$	Ratio	z-score	Molecules
Pathogen Induced Cytokine Storm Signaling Pathway	11.4	0.0759	1.512	CASP1,CCR5,CD163,CLEC7A, COL1A1,COL1A2,COL3A1,COL5A2,CXCL10,CXCR4,IFNG, IL15,IL18,IL1R2,IL1RAP, IL1RL1,NOD1,RYR1,SOCS3,STX11,TLR2,TLR4,TLR8,TNFSF11,TNFSF13,TNFSF13B, TNFSF14,XCL1
Activin Inhibin Signaling Pathway	7.38	0.0787	0.728	ACVR1B, CEBPB, COL1A1,COL1A2,COL3A1,CXCR4,IL18,IL1R2,IL1RAP, IL1RL1,SMAD9,TCF7,TCF7L2,TLR2,TLR4,TLR8,TNFSF13B
Wound Healing Signaling Pathway	7.14	0.0714	0.943	ACVR1B, CEBPB, COL1A1,COL1A2,COL3A1,COL5A2,IFNG, IL15,IL18,IL1R2,IL1RAP, IL1RL1,MMP8,MRAS, TNFSF11,TNFSF13,TNFSF13B, TNFSF14
NOD1/2 Signaling Pathway	6.69	0.0802	2.324	CASP1,HSPA1L, IFNG, IL15,IL18,NOD1,SLC15A3,TLR2,TLR4,TLR8,TNFSF11,TNFSF13, TNFSF13B, TNFSF14,TNFSF18
S100 Family Signaling Pathway	6.47	0.0416	3.182	ADGRE1,ADGRE3,ADGRL1,CCR5,CCR9,CMKLR1,CXCR6,EREG, FCGR2B, FFAR2,FZD1,GHSR, GPR160,GPR171,GPR179,GPR27,GPR37,HTR7,IL18,LPAR3,LPAR6 ,LTB4R2,MMP8,PLCL1,PTGER3,S100A12,S100A8,S100A9,SMAD9,TCF7,TCF7L2,TLR4
Multiple Sclerosis Signaling Pathway	6.46	0.0721	3	C2,CAPN3,CASP1,FAS, IFNG, IL15,IL18,PLAU, SLC8A1,TLR2,TLR4,TLR8,TNFSF11,TNFSF13,TNFSF13B, TNFSF14
Phagosome Formation	6.45	0.0432	2.921	ADGRE1,ADGRE3,ADGRL1,CCR5,CCR9,CD14,CLEC7A, CMKLR1,CXCR6,FCER1A, FCER2,FCGR2B, FFAR2,FZD1,GHSR, GPR160,GPR171,GPR179,GPR27,GPR37,HTR7,LPAR3,LPAR6,LTB4R2,MRAS, PTGER3,SPHK1,TLR2,TLR4,TLR8
Osteoarthritis Pathway	6.13	0.0681	2.714	CASP1,CEBPB, FZD1,IL1R2,IL1RAP, IL1RL1,PRKAA2,S100A8,S100A9,SLC39A8,SMAD9,SPHK1,TCF7,TCF7L2,TLR2,TLR4
IL-10 Signaling	6.13	0.0844	0.277	ARG2,CCR5,FCGR2B, FFAR2,IFNG, IL18,IL1R2,IL1RAP, IL1RL1,MAFB, PRKAA2,SOCS3,TLR4
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.07	0.0833	2	CASP1,CLEC7A, IFNG, IL15,IL18,NOD1,TLR2,TLR4,TLR8,TNFSF11,TNFSF13,TNFSF13B, TNFSF14

PLS-DA was used to reduce the dimensionality of the transcriptome dataset. The PLS-DA plot in Fig. 2A shows a clear separation of the samples from the NC and ST groups, with dimensions 1 and 2 explaining 42.48% and 25.53% of the classification, respectively. The distinct clustering of the ST and NC groups indicates the presence of pronounced differences between their respective RNA expression profiles. This separation, with dimensions 1 and 2 explaining a substantial portion of

the variance, suggests that the identified biomarkers are potentially strong predictors of the respective group characteristics. The robustness of these findings points to the utility of PLS-DA in discerning subtle yet crucial biological variations in understanding ST infections.

DEGs in *Salmonella*-infected piglets

Using the DESeq2 package in R, we identified a total of 495 DEGs between the ST and NC groups based on

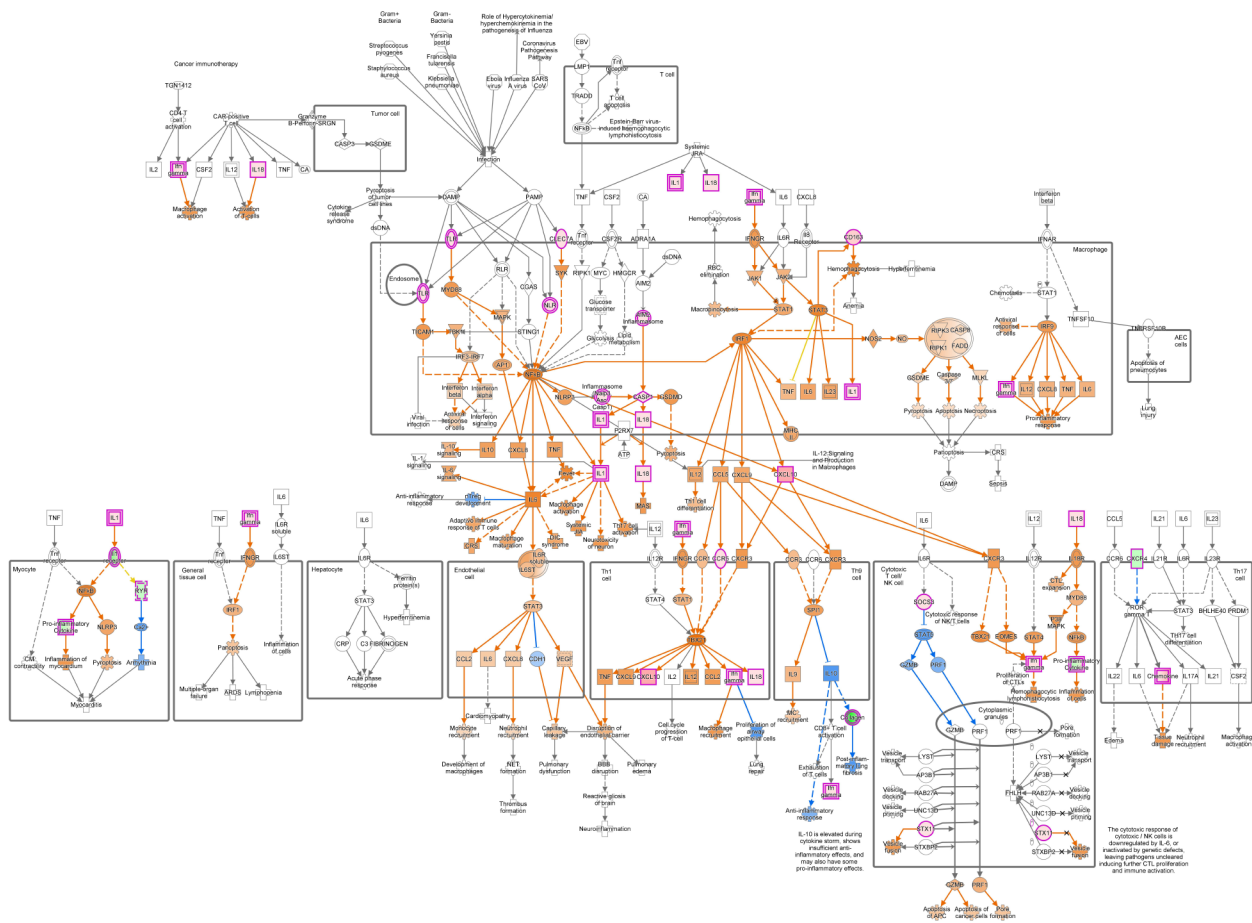


Fig. 3 Predicted most significantly activated canonical pathway: Pathogen-induced cytokine storm signaling pathway

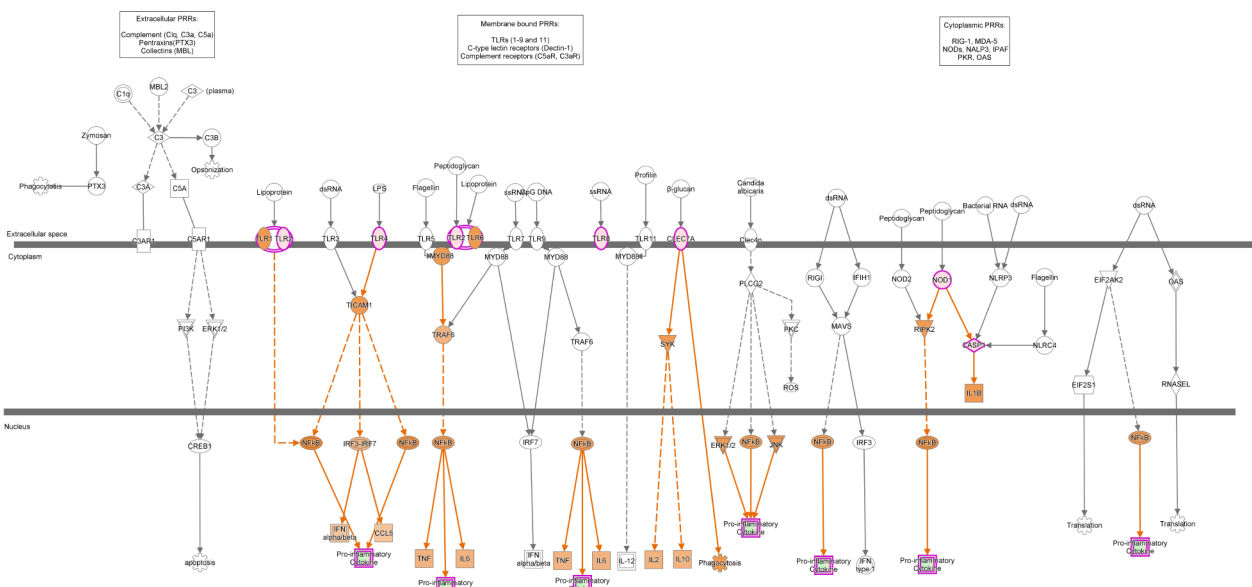


Fig. 4 Predicted activated canonical pathway that is possibly relevant to the study design: Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses

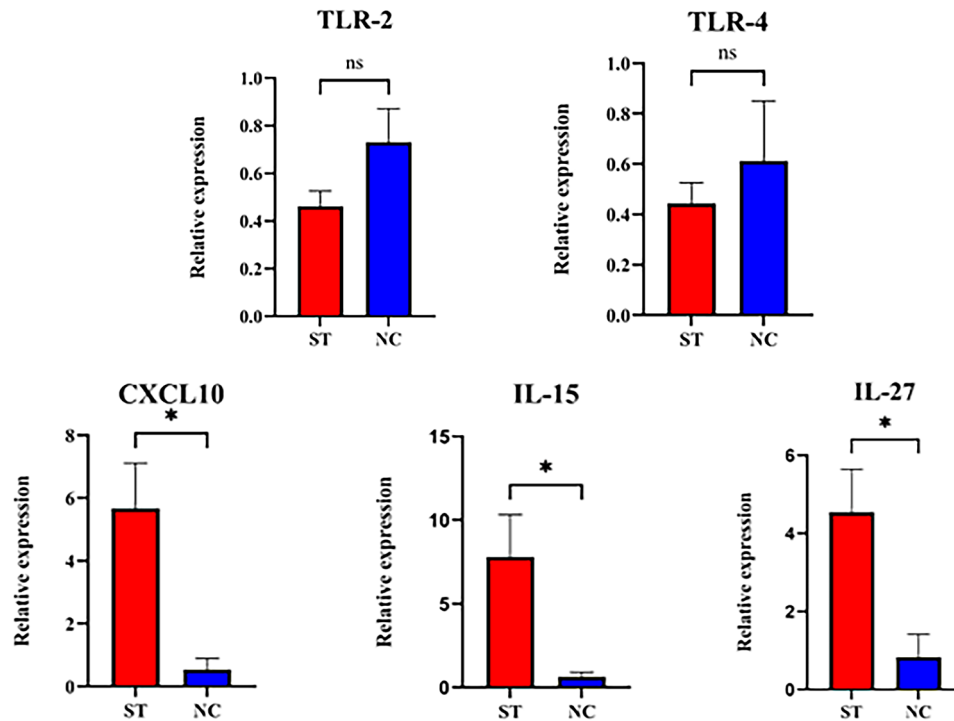


Fig. 5 RT-qPCR validation of key genes. Transcript levels of *TLR2*, *TLR4*, *CXCL10*, *IL15*, and *IL27* in peripheral blood mononuclear cells isolated from experimental piglets at 2 dpi relative to *ACTB* expression are shown. * $P < 0.05$

thresholds of $P < 0.05$ and \log_2 (fold change) ≥ 2.0 . Among the 495 DEGs, 247 genes were upregulated, and 248 genes were downregulated in the ST group compared to the NC group (Additional file 1: Table S5). A hierarchical clustering-based heatmap is shown in Fig. 2B. In the heatmap, the ST and NC groups are clearly delineated, reflecting the differences between the groups as well as the homogeneity within each group. This pattern indicates a consistent biological response to ST infection, as evidenced by the specific gene expression alterations.

Enrichment analyses

GO term enrichment

Table 1 lists the significantly ($FDR < 0.05$) enriched GO terms specific for BPs, CCs, and MFs. In the BP category, “inflammatory response,” “cellular response to cytokine stimulus,” “positive regulation of NIK/NF- κ B signaling,” “immune response,” “positive regulation of interferon-gamma production,” “innate immune response,” “toll-like receptor 2 signaling pathway,” “MyD88-dependent toll-like receptor signaling pathway,” “positive regulation of T cell proliferation,” and “positive regulation of interleukin-6 production” were enriched.

The above mentioned terms have strong implications in the immune response and inflammatory processes. Particularly, the enrichment of DEGs related to pathway associated with toll-like receptors (TLRs), such as “toll-like receptor 2 signaling pathway” and

“MyD88-dependent toll-like receptor signaling pathway”, indicates that TLRs are crucial PRRs in the initial immune response against ST infection in pigs. Huang et al. [13] also reported the upregulation of the genes associated with Toll-like receptors and the MyD88 pathway in the innate immune system at 2 dpi of ST infection and the attenuation of these gene expression at 7 dpi. Our findings indicated that the specific genes associated with the TLR2 signaling pathway were highly expressed during ST infection. In general, the lipopolysaccharides (LPSs) of gram-negative bacteria, such as *Salmonella* spp., are primarily recognized by TLR4, which utilizes MyD88 as a key adaptor protein for signal transduction [26, 27]. In contrast, TLR2 could recognize pathogen-associated molecular patterns (PAMPs), such as lipoteichoic acid and lipoproteins from gram-positive bacteria [28]. However, a recent study suggested that low-endotoxic atypical LPSs can induce TLR4/TLR2 interaction and heterodimer formation [29]. Therefore, our results suggested the possibility that atypical LPSs or other components of *Salmonella* spp. (i.e., cell wall constituents or lipoproteins) stimulate TLR2. The observed increase in NF- κ B expression is attributed to the stimulation of TLRs by the PAMPs of *Salmonella* spp. [30]. The results a study by Huang et al. [13] also support our results, which highlight the overexpression of the NF- κ B regulon and inhibitory subunit I κ B (NFKB1A) in ST-infected pigs classified as persistent shedders [13]. Activated NF- κ B translocates

to the nucleus, regulating the expression of various immune-related genes and generating pro-inflammatory cytokines. Our study also reported the enrichment of specific terms, such as “cellular response to cytokine stimulus,” “positive regulation of interferon-gamma production,” and “positive regulation of interleukin-6 production.” These findings were also agreement with the aforementioned previous study by Huang et al. [13], who analyzed the alterations of both microRNAs and mRNAs in the peripheral blood from ST-infected pigs at 2 dpi. The findings of this previous study suggested that the miR-146a regulates the levels of the targets of *IFN-γ* and *IL-6*, thereby increasing fecal bacterial shedding; overall, these findings underscore the significance of *IL-6* and *IFN-γ* in response to *Salmonella* infection [14]. The term “positive regulation of T cell proliferation” indicates a potential activation of adaptive immunity [31]. Collectively, these terms highlight a profound involvement of both innate and adaptive immune responses in ST infection, with potential crosstalk among cytokine signaling, TLRs, and T cell activation. The presence of these interconnected terms underscores the complexity and integrated nature of the immune response to ST infection captured in the dataset.

In the CC category, the significantly enriched terms included “external side of the plasma membrane,” “extracellular space,” “cell surface,” “phagocytic vesicle membrane,” and “membrane raft.” All these terms are primarily associated with the cellular boundary or its immediate surroundings.

The enrichment of “external side of the plasma membrane,” “extracellular space,” and “cell surface” were in agreement with the findings of previous study in piglets [32]; this previous study reported a significant upregulation of genes (*BGN*, *DCN*, *ZFPM2*, and *BPI*) associated with the extracellular mechanisms underlying the adhesion of *Salmonella* spp. to host cells. Our results showed that these interactions are not confined to intestinal epithelial cells, but also involve blood cellular components, indicating a systemic response to ST infection. The term “phagocytic vesicle membrane” suggests the involvement of immune cells, such as macrophages [33]. Membrane rafts are specialized microdomains in the plasma membrane that are rich in cholesterol and sphingolipids and play essential roles in signal transduction and cellular trafficking [34]. Overall, these results suggest a critical role of cell surface interactions in the initial stages of the colonization of, and infection with, *Salmonella* spp. The term “phagocytic vesicle membrane” underscores the importance of cellular boundaries and interactions with the external environment in the response to ST infection.

In the MF category, the significantly enriched terms included “transmembrane signaling receptor activity” and “NAD⁺ nucleosidase activity.” “Transmembrane

signaling receptor activity” is associated with proteins that span the cell membrane and transmit signals from the external environment to the cell's interior. These receptors play pivotal roles in immune responses and cell-to-cell communication [35]. These findings were agreement with the terms enriched under the BP category, suggesting that ST infection could lead to the activation of specific genes associated with intracellular immune responses through the stimulation of cell membrane receptors. “NAD⁺ nucleosidase activity” pertains to the enzymatic breakdown of nicotinamide adenine dinucleotide (NAD⁺), a crucial coenzyme in cellular metabolism. The activity of NAD⁺ is responsible for various cellular processes, including energy production, DNA repair, and cell signaling. While this term appears to be more related to metabolism, NAD⁺ and NAD⁺-associated pathways are being increasingly recognized for their roles in immune responses. For instance, the role of NAD⁺ in immune responses was evidenced by a study on *de novo* NAD⁺ synthesis in macrophages [36], revealing that the cell-autonomous generation of NAD⁺ via the kynurenine pathway regulates macrophage immune function in aging and inflammation. This underscores the crucial role of NAD⁺ in mediating macrophage effector responses and suggests that a decrease in *de novo* NAD⁺ synthesis underlies the decline NAD⁺ levels and enhancement of innate immune dysfunction in aging and age-associated diseases.

Overall, the GO enrichment results emphasized the roles of immune responses and inflammatory processes in ST infection, particularly highlighting the recognition of PAMPs by TLRs, including key innate immune components, such as TLR2 and MyDD88-dependent toll-like receptors. This recognition is followed by NIK/NF-κB signaling and cytokine signaling, leading to T cell proliferation as a part of adaptive immunity. These findings highlight the involvement of both innate and adaptive immune mechanisms in the response to ST infection. The enrichment of the MF-related terms “transmembrane signaling receptor activity” and “NAD⁺ nucleosidase activity” underscored the importance of signal transduction and metabolic processes in cellular health and immune function, and particularly highlighting the role of NAD⁺ role in immune responses.

KEGG pathway enrichment

The significantly (FDR < 0.05) DEG-enriched KEGG pathways included “Cytokine-cytokine receptor interaction,” “Intestinal immune network for IgA production,” “Rheumatoid arthritis,” and “Pertussis,” shedding light on the comprehensive impact of ST infection on host immune mechanisms (Table 2). The “Cytokine-cytokine receptor interaction” pathway underscores the pivotal role of cytokines in mediating immune responses to ST infection,

Table 4 Predicted activated and inhibited upstream regulators with target molecules in peripheral blood samples from *Salmonella* Typhimurium-infected piglets

Upstream Regulator	Molecule Type	Activation z-score	p-value of overlap	Target Molecules in Dataset	Mechanistic Network
<i>E. coli</i> B5 lipopolysaccharide	chemical - endogenous non-mammalian	3.054	1.24E-07	CD274,CXCL10,GBP2,IDO1,IFNG, IL18,IL27,TLR2,TLR4,XCL1	107 (20)
<i>E. coli</i> lipopolysaccharide	chemical - endogenous non-mammalian	2.78	3.3E-06	CXCL10,IFNG, IL18,IL27,S100A8,S100A9,SOCS3,TLR4	90 (20)
lipid A	chemical toxicant	2.433	2.75E-05	ACOD1,AICDA, C2,CD14,CXCL10,FCGR2B	107 (17)
<i>E. coli</i> B4 lipopolysaccharide	chemical toxicant	2.21	0.000313	ACOD1,CXCL10,FAS, IFNG, IL27,PADI4,SOD2,TLR4	103 (18)
hydrocortisone	chemical - endogenous mammalian	2.213	0.000997	CD163,CEBPB, COL1A1,COL1A2,FAS, S100A8	73 (11)
cardiotoxin	chemical - other	2.333	2.81E-06	CASP1,CCR5,CD180,COL1A1,CXCR4,HP, IL18,MERTK, S100A8,S100A9	
<i>Salmonella minnesota</i> R595 lipopolysaccharides	chemical - endogenous non-mammalian	2.607	0.000232	ACOD1,ACSL1,CXCL10,IFNG, PSTPIP2,SOCS3,TFEC, TLR2	99 (18)
IFN alpha/beta	group	3.052	1.09E-07	CXCL10,GAS7,IFNG, IL15,IL15RA, IL27,SLPI, TLR2,TNFSF13,TNFSF13B	101 (17)
NFkB (family)	group	2.828	4.16E-05	CASP1,CXCL10,DRAM1,IFNG, IL18,S100A8,S100A9,TGM2	88 (11)
meds433	chemical reagent	2.333	1.51E-05	EREG, FAS, GBP2,ITF, OSCAR, S100A8,S100A9,TFEC, TGM2	
Ifn gamma	complex	2.195	0.00435	CASP1,CXCL10,IGFBP4,SOCS3,TLR4	109 (19)
GC-GCR dimer	complex	2.219	6.88E-05	CD163,FBP1,HP, IL1R2,SLPI	
Interferon alpha	group	4.037	1.97E-10	CASP1,CCR5,CD274,CXCL10,FAS, FCER1A, GBP1,GVINP1,IDO1,IFNG, IGFBP4,IL15,IL15RA, IL27,ISG20,LAP3,MERTK, NMI, SOCS3,TGM1,TLR2,TLR4,TLR8,TNFSF13B, WARS1	115 (19)
IL12 (complex)	complex	3.784	1.06E-12	ADGRE1,CCR5,CXCL10,FAS, GADD45G, GZMA, HAVCR2,HLX, IDO1,IFNG, IL15,IL18,IL27,ISG20,KLRB1,KLRC4-KLRK1/KLRK1,PLAU, SOCS3,TLR2,TLR4,TNFRSF25	122 (19)
<i>Salmonella enterica</i> serotype abortus equi lipopolysaccharide	chemical toxicant	2.33	2.88E-08	ATL1,CD274,EREG, FFAR2,GADD45A, GBP1,IDO1,IL15RA, IL18,MCF2L2,PLAU, SGPP2,SOCS3,TIFA, TNIP3	97 (11)
ZBTB10	transcription regulator	3.317	1.38E-05	ACOD1,ARID5A, BATF3,CD274,CXCL10,IDO1,IL15,IL27,ISG20,NOD1,NR4A3	
Tlr	group	2.569	0.000313	CLEC2B, CXCL10,IDO1,IFNG, SOCS3,SPHK1,TNFSF13,TNFSF13B	99 (20)
IFN Beta	group	3.303	5.78E-08	ACOD1,ANKRD22,C2,CD163,CD274,CEBPB, CXCL10,GBP2,IDO1,IFNG, IL15,IL15RA, IL27,ISG20,KLRB1,PPP1R3B	119 (18)
ZC3H14	other	2.236	3.49E-06	BATF, IL15,IL15RA, MERTK, NMI	
DOCK8	other	2.828	8.33E-06	ARID5A, CXCL10,IL15,IL15RA, IL18,ISG20,MERTK, NMI	
TLR7	transmembrane receptor	2.97	6.14E-05	CD274,CEBPB, CXCL10,FCMR, IDO1,IFNG, IL27,ISG20,SOD2	100 (15)
Ifn	group	2.773	6.55E-05	CXCL10,IDO1,IL15,IL15RA, IL27,ISG20,S100A8,TLR2,TNFSF13B	65 (9)
Tnf (family)	group	2.034	4.7E-08	CCR5,CD163,CD274,CXCL10,FCER2,FFAR2,GBP1,IFNG, IL18,IL1R2,LAP3,PTGER3,SLPI, SOCS3,TGM2,TLR2,TNFAIP6,TNFSF11	88 (15)
CSF	group	2.121	2.25E-06	CD274,FCER2,FFAR2,IL1R2,SLPI, SOCS3,TGM2,TNFAIP6	
vidutolimod	chemical drug	3.592	7.34E-10	CALHM6,CD274,CD68,CXCL10,GBP1,IDO1,IFNG, IL27,ISG20,LAP3,SERPING1, SOD2,TNFAIP6,TNFSF13B	
STAT1	transcription regulator	3.159	6.82E-10	ACOD1,BATF2,C1R, CASP1,CD274,CXCL10,FAS, GBP1,GBP2,HAVCR2,IFNG, IL15,IL15RA, IL18,IL1R2,PCD1LG2,SLC8A1,SOCS3,TLR4,TNFSF11,TNFSF13B, WARS1	109 (19)
RIPK2	kinase	2.216	0.000317	ACOD1,ACSL1,CXCL10,IFNG, SOD2	84 (11)
NR4A3	ligand-dependent nuclear receptor	2.401	0.0845	ADGRE1,COL1A1,FHL2,IDO1,SH2D1B, TRIB3	

Table 4 (continued)

Upstream Regulator	Molecule Type	Activation z-score	p-value of overlap	Target Molecules in Dataset	Mechanistic Network
PPP2CA	phosphatase	2	0.00528	CXCL10,GZMA, SOCS3,UPP1	80 (7)
IL27	cytokine	3.5	4.7E-14	ADGRE1,CD14,CD163,CD274,CXCL10,FAS, GADD45G, GBP1,GBP2,IDO1,IFNG, IL15,IL18,ISG20,PDCD1LG2,SOCS3,TNFSF11,TNFSF13 ,TNFSF13B,TNFSF14,TNFSF18	136 (19)
ALDH1A2	enzyme	2	0.000117	COL1A2,COL3A1,IGFBP4,SPARC	74 (7)
TLR4	transmembrane receptor	3.262	2.48E-07	ARID5A, BATF, CCR5,CXCL10,EREG, FAS, IFNG, IL15,IL15RA, IL18,IL27,ISG20,KDM6B, MERTK, NMI, S100A8,SOCS3,TLR2,TLR4	89 (18)
IL5	cytokine	2.514	9.98E-09	AICDA, CLEC2B, CXCR4,FAS, FCGR2B, GADD45A, GADD45G, GBP2,HLX, IFNG, IL1R2,IL1RL1,IL5RA, PSAT1,RBPMS, SGK1,SLC39A8,SNB1,UPP1	114 (20)
CEBPE	transcription regulator	2.141	4.44E-06	CD14,CTSV, IL18,IL5RA, LTF, LYZ, TNFSF13B	
CEBPA	transcription regulator	2.728	8.66E-08	ACSL1,CD14,CEBPB, COL1A1,COL1A2,CPT1A, CXCR4,GADD45A, GFI1B, HP, LTF, RETN, S100A8,S100A9,SOCS3,TNFAIP6	33 (3)
IFNAR1	transmembrane receptor	2.225	9.42E-08	ACOD1,BCL2L14,CD274,CXCL10,IDO1,IFNG, IL15,IL18,IL27,SOCS3,TGM2	100 (14)
BHLHE40	transcription regulator	3.43	8.6E-08	CASP1,CD14,CD274,CTSV, EREG, FAS, FCGR2B, FFAR2,GBP2,HAVCR2,IFNG, IL1R2,LAP3,MERTK, SOCS3,SOD2,TGM2,TNFSF11,TNFSF14,UPP1	114 (16)
LEP	growth factor	2.242	3.94E-05	CASP1,CD14,CPT1A, CXCL10,GHSR, IFNG, IL1R2,NPR2,RETN, SCD, SOCS3,SOD2,TNFSF11	102 (16)
SPI1	transcription regulator	2.209	8.99E-09	ADGRE1,BCL7A, CD68,CHIT1,FCER1A, FCGR2B, HDAC7,ID3,IL18,IL1R2,IL27,LTF, LYZ, OSCAR, PDCD1LG2,TFEC, TLR4	85 (7)
miR-338-3p (miRNAs w/seed CCAGCAU)	mature microRNA	2.236	0.000169	COL1A1,COL1A2,COL5A2,NID1,SPARC	
miR-29b-3p (and other miRNAs w/seed AGCACCA)	mature microRNA	2.392	0.000606	COL1A1,COL1A2,COL3A1,COL5A2,NID1,SPARC	51 (7)
miR-335-3p (miRNAs w/seed UUUUCAU)	mature microRNA	2.236	4.86E-05	COL1A1,COL1A2,COL5A2,NID1,SPARC	
miR-30c-5p (and other miRNAs w/seed GUAACA)	mature microRNA	2.438	0.0162	COL1A1,COL1A2,COL5A2,NID1,SLC12A4,SPARC	
let-7a-5p (and other miRNAs w/seed GAGGUAG)	mature microRNA	2.244	0.00119	BCL7A, COL1A1,COL1A2,COL3A1,COL5A2,NID1,SCD, SPARC, TLR4	
S100A9	other	2.054	9.95E-08	ABCG1,CASP1,CD274,CXCL10,FAS, IL1RL1,NR4A3,NRG1,PLAU, RAB20,S100A8,S100A9,SGK1,SOCS3	110 (19)
CSF3	cytokine	2.565	1.32E-09	ADGRE1,BATF, CEBPB, CXCR4,GADD45A, GADD45G, GNLY, IFNG, LTF, LYZ, MMP8,PDCD1LG2,SOCS3,TLR2,TLR4,TLR8	98 (19)
SAMSN1	other	2.887	8.99E-09	ARID5A, BATF, CXCL10,FCER2,IFNG, IL15,IL15RA, IL18,ISG20,MERTK, NMI, SOCS3	94 (7)
CSF2	cytokine	2.784	2.41E-15	BATF3,CASP1,CCR5,CD14,CD163,CD274,CLEC7A, CXCL10,CXCR4,FAS, FCGR2B, FFAR2,ID3,IDO1,IFNG, IL15,IL1RL1,IL5RA, MMP8,NR4A3,PLAU, PRNP, SGK1,SNB1,SOCS3,SOD2,SPHK1,TGM2,TIFA, TLR2,TLR4,TNFSF14,UPP1	122 (18)
IL2	cytokine	3.233	1.17E-12	ACVR1B, AICDA, BATF, CASP1,CCR5,CCR9,CD274,CMKLR1,CXCL10,CXCR4,CXCR6,FAS, FCMR, GADD45G, GBP2,GZMA, HAVCR2,HOPX, IDO1,IFNG, IGFBP4, IL18,IL1R2,IL1RL1,KLRB1,KLRC4-KLRK1/KLRK1,NMI, NR4A3,PSAT1,PTPN3,S100A8,SOCS3,TLR2,TNFSF11,TRIB3,UPP1,XCL1	126 (17)
TNF	cytokine	3.458	3.99E-19	ACOD1,CALHM6,CASP1,CCR5,CD14,CD163,CD163L1,CD274,CEBPB, CMKLR1,COL1A1,COL1A2,COL3A1,CXCL10,CXCR4,EREG, FAS, FCER2,FCGR2B, FRMD4A, GADD45A, GBP1,GBP2,HP, ID3,IDO1,IFNG, IGFBP4,IL15,IL18,IL1R2,IL1RL1,IL27,ISG20,KLRB1,LY6D, MERTK, MMP8,OSCAR, PLAU, PRNP, RETN, S100A8,S100A9,SAMD4A, SLC11A1,SLC12A4,SLPI, SOCS3,SOD2,SPARC, TBXAS1,TGM2,THBD, TIFA, TINAGL1,TLR2,TLR4,TNFAIP6,TNFRSF25,TNFSF11 ,TNFSF14	136 (19)

Table 4 (continued)

Upstream Regulator	Molecule Type	Activation z-score	p-value of overlap	Target Molecules in Dataset	Mechanistic Network
XRCC5	enzyme	2	0.000802	CD274,CXCL10,PDCD1LG2,TGM2	56 (7)
MET	kinase	2.57	5.03E-05	ARID5A, HLX, IL15,IL15RA, IL18,MERTK, SOCS3	
KRAS	enzyme	2.619	1.29E-05	ARG2,BCL2L14,CD274,COL1A1,COL1A2,COL3A1,CXCL10,CXCR4,EREG, ETV1,FBLN2,FCGR2B, GAS1,IDO1,KLRB1,PAWR, PPA1,RAB20,SPARC, TGM2,THBD, TLR2,UPP1	103 (16)
KLF4	transcription regulator	2.608	3.23E-06	ADGRE1,AIF1,CD14,COL1A2,GADD45A, ID3,IL15,SERPINH1,SOD2,SPHK1,TNFSF11	60 (7)
IRF7	transcription regulator	3.658	8.49E-08	CALHM6,CXCL10,FZD1,GBP1,IDO1,IL15,IL15RA, IL27,ISG20,NMI, S100A8,TLR4,TLR8,TNFSF13B	83 (17)
MYD88	other	3.897	6.44E-09	ACOD1,ACSL1,BATF, CD274,CXCL10,HP, IFNG, IL18,IL1RAP, IL27,KDM6B, LTB4R2,MMP8,PSTPIP2,S100A8,SOCS3,TFEC, TLR2,TNFSF11,TNFSF13B	101 (20)
TICAM1	other	3.383	4.93E-07	ACOD1,ACSL1,CXCL10,IFNG, IL15,IL15RA, IL18,ISG20,PSTPIP2,SOCS3,TFEC, TLR2	87 (18)
FCGR2A	transmembrane receptor	2.596	9.02E-06	ACOD1,CXCL10,FAS, GBP2,IFNG, IL18,ISG20	110 (19)
IRF1	transcription regulator	2.643	2.38E-08	ACOD1,C1R, CASP1,CD274,CXCL10,FAS, GBP2,IDO1,IFNG, IL15,IL18,IL27,PDCD1LG2,SLPI	106 (16)
IL18	cytokine	2.697	6.71E-07	CCR5,CXCL10,FAS, GADD45G, GZMA, HAVCR2,HLX, IFNG, IL18,KLRC4-KLRK1/KLRK1,TLR4,TNFRSF25,TNFSF11	124 (18)
MEF2A	transcription regulator	2.383	0.000121	CXCL10,GBP2,IL15,IL27,ISG20,NOD1	52 (6)
F2	peptidase	2.207	0.0464	CD68,CXCR4,ECE1,EREG, IGFBP4,THBD	
SASH1	other	2.828	9.27E-06	ARID5A, CXCL10,IL15,IL15RA, IL18,ISG20,MERTK, NMI	
TLR9	transmembrane receptor	2.042	5.01E-07	ACOD1,AICDA, CD274,CXCL10,FCER2,FCMR, IDO1,IFNG, IL18,ISG20,KDM6B, TLR4,TNFSF13B	98 (19)
IRF3	transcription regulator	2.739	0.000633	CALHM6,CD274,CXCL10,FAS, IFNG, IL15,IL27,ISG20,TLR4	106 (16)
PLK2	kinase	2	0.000522	CXCL10,IL15,IL15RA, MERTK	
pembrolizumab	biologic drug	2.813	3.13E-07	CD274,CMKLR1,CXCR6,GZMA, HAVCR2,IDO1,KLRB1,PDCD1LG2	
NFATC2	transcription regulator	2.667	2.42E-10	BATF, CCR5,CD274,CXCL10,FAS, FCER2,HAVCR2,IFNG, IL15,IL18,IL27,ISG20,KDM6B, MERTK, NMI, SOCS3,TLR2,TNFSF13B	123 (20)
IFNB1	cytokine	2.078	6.69E-08	ACOD1,CASP1,CCR5,CD14,CD274,CXCL10,FBLN2,GAS1,GBP1,GBP2,IDO1,IFNG, IL18,IL27,KDM6B, KLRC4-KLRK1/KLRK1,NOD1,SH2D1B	109 (16)
IRF9	transcription regulator	2.374	8.33E-06	BCL2L14,CXCL10,GBP1,IL18,IL27,ISG20,NMI, SOCS3	71 (10)
IFNG	cytokine	5.327	2.56E-24	ACOD1,AIF1,ARG2,C1R, C2,CASP1,CCR5,CD14,CD163,CD163L1,CD274,CEBPB, CLEC7A, COL1A1,COL1A2,COL5A2,CXCL10,CXCR4,ECE1,ETV7,FAS, FBP1,FCER2,FCGR2B, FCN1,FHL2,GBP1,GBP2,HP, IDO1,IFNG, IGFBP4,IL15,IL15RA, IL18,IL1RL1,IL27,ISG20,KLRC4-KLRK1/KLRK1,LZTS1,MERTK, NMI, PDCD1LG2,PLAU, RAB20,S100A8,S100A9,SERPIN1,SERPINH1,SLC11A1,SLC15A3,SLC8A1,SOCS3,SOD2,STX11,STX3,TBXAS1,TCF7L2,TGM1,TLR2,TLR4,TLR8,TNFAIP6,TNFSF13,TNFSF13B, WARS1	124 (18)
TBK1	kinase	2.387	0.000529	CXCL10,IL15,IL15RA, IL18,ISG20,NMI, TLR2	99 (15)
IFNA2	cytokine	2.588	0.000599	BCL2L14,CXCL10,FAS, IDO1,IFNG, SOCS3,TGM2	80 (17)
ARHGAP21	other	3	1.01E-08	ARID5A, BATF, CXCL10,IL15,IL15RA, IL18,ISG20,MERTK, NMI	
ERBB2	kinase	2.536	0.00139	CEBPB, COL5A2,CXCL10,CXCR4,CXCR6,FBLN2,FFAR2,HP, IFNG, IGFBP4,NID1,NPR2,NRG1,SLPI, SPARC, TGM1,TSPAN13	116 (22)
TYK2	kinase	2.752	8.93E-07	CCR5,CD274,CXCL10,GBP2,GZMA, IDO1,IFNG, PDCD1LG2,SOCS3,UPP1	102 (16)
IRF5	transcription regulator	2.186	0.00158	COL1A1,COL3A1,CXCL10,CXCR4,ISG20	87 (10)
IL1B	cytokine	3.209	6.16E-10	CCR5,CD274,CEBPB, COL1A1,CXCL10,CXCR4,FAS, HP, IFNG, IL15,IL18,IL1R2,IL1RAP, IL27,KLRB1,MMP8,NMI, PLAU, S100A8,S100A9,SOCS3,SOD2,SPARC, TGM1,THBD, TLR2,TLR4,TNFSF11,TNFSF13B	120 (19)
EBI3	cytokine	2.599	1.99E-06	CD274,FAS, GADD45G, GBP2,HAVCR2,IFNG, PDCD1LG2	111 (14)

Table 4 (continued)

Upstream Regulator	Molecule Type	Activation z-score	p-value of overlap	Target Molecules in Dataset	Mechanistic Network
PLK4	kinase	2	0.000447	CXCL10,IL15,IL15RA, MERTK	
cigarette smoke	chemical toxicant	2.101	0.0171	IFNG, IL27,LTB4R2,NRG1,TLR4	
resiquimod	chemical drug	3.213	1.4E-10	AICDA, AIF1,BATF, CD180,CEBPB, CTSV, CXCL10,FCER2,FCGR2B, FCN1,GBP1,GPR160,IFNG, IL15,IL18,IL27,LAP3,SOCS3,SOD2,TLR2,TLR4,TLR8,WARS1	111 (18)
Pam3-Cys	chemical toxicant	2.205	0.00025	CD163,CXCL10,IFNG, SOD2,TLR2	99 (17)
imiquimod	chemical drug	2.4	0.000677	CXCL10,IFNG, IL18,ISG20,PPP1R3B, SOCS3,SOD2	86 (18)
MALP-2s	chemical reagent	2.211	2.21E-05	IFNG, IL15,IL15RA, TLR2,TLR4	98 (18)
PAX3-FOXO1	fusion gene/product	2	0.439	CXCR4,IFNG, LY6D, TCF7L2,TMEM47	
Pam3-Cys-Ser-Lys4	chemical reagent	2.395	5.45E-10	BATF, CCR5,CD14,CD274,CXCL10,FCGR2B, HAVCR2,IDO1,IFNG, IL15,IL18,IL27,KDM6B, TLR2,TLR4,TNFSF13B	107 (21)
CpG ODN 1826	chemical reagent	2.195	0.00784	CD274,CXCL10,FCGR2B, IFNG, XCL1	109 (20)
poly rl: rC-RNA	biologic drug	4.936	1.59E-15	ACOD1,ARG2,BATF2,C2,CD14,CD163,CD274,CLEC7A, CXCL10,ETV7,FAS, FCGR2B, FCN1,GBP1,GBP2,HAVCR2,IDO1,IFNG, IL15,IL15RA, IL18,IL27,ISG20,LAP3,MAFB, MERTK, NR4A3,PDCD1LG2,PLAU, S100A8,SOCS3,SOD2,TCF7L2,TFEC, THBD, TLR2,TLR4,TNFAIP6,WARS1	128 (18)
CpG oligonucleotide	chemical drug	3.662	5.36E-08	AICDA, C2,CD14,CD274,CEBPB, CXCL10,FCGR2B, IDO1,IFNG, IL15,IL18,SOD2,TLR2,TNFSF13,TNFSF13B	113 (21)
lipopolysaccharide	chemical drug	5.883	3.09E-34	ABCG1,ACOD1,ACSL1,AICDA, AIF1,ANKRD22,ARG2,BACH2,BATF, BATF3,BCL7A, C1R, CALHM6,CASP1,CCR5,CCR9,CD14,CD163,CD163L1,CD180,CD274,CD68,CEBPB, CLEC7A, COL1A1,COL1A2,COL3A1,COL5A2,CPT1A, CTSV, CUBN, CXCL10,CXCR4,DHHDH, DRAM1,ECL2,EREG, ETV7,FAS, FBLN2,FCER2,FCGR2B, FFAR2,FZD1,GAS1,GBP1,GBP2,GZMA, HOPX, HP, ID3,IDO1,IFNG, IGFBP4,IL15,IL15RA, IL18,IL1R2,IL1RAP, IL27,IL5RA, ISG20,KCNJ15,LAP3,LTB4R2,LTF, LY6D, LYZ, MERTK, MMP8,NID1,NOD1,NR4A3,OSCAR, PDCD1LG2,PLAU, RBMS2,RBPMS, RETN, S100A12,S100A8,S100A9,SERPINH1,SGPP2,SLC11A1,SLC15A3,SLC39A8,SLPI, SOCS3,SOD2,SPARC, SPHK1,TCF7L2,TFEC, TGM2,THBD, TLR2,TLR4,TNFAIP6,TNFSF11,TNFSF13,TNFSF13B, TNFSF14,TNFSF18,TNIP3,TRIB3,UPP1,VPS37C, WARS1,XCL1	162 (18)
laminaran	chemical drug	2.121	8.06E-08	ACOD1,CXCL10,EREG, IDO1,SGPP2,SLC39A8,TNFAIP6,TNIP3	
cytokine	group	3.031	8.23E-11	AICDA, FAS, IDO1,IFNG, KLRC4-KLRK1/KLRK1,PDCD1LG2,PLAU, RETN, SGK1,SOCS3,SOD2,SPARC, TLR2,TLR4,TNFAIP6,TNFSF11,UPP1	124 (21)
D-galactosamine	chemical - endogenous mammalian	2.412	1.32E-06	CASP1,CD14,IFNG, IL18,SOD2,TLR4	108 (15)
tetradecanoylphorbol acetate	chemical drug	3.049	3.39E-08	CD14,CD163,CD68,ECE1,EEF1AKMT3,FAS, HTR7,ID3,IFNG, IL18,LYZ, MMP8,PLAU, PRNP, RETN, S100A8,S100A9,SLC39A8,SLPI, SOCS3,SOD2,SPARC, SPHK1,TBXAS1,TGM1,TLR2,TNFSF14,XCL1	112 (19)
IZUMO1R	other	-2.429	1.51E-07	CD14,CLEC7A, IL18,S100A8,S100A9,SLPI	
MRTFB	transcription regulator	-2.333	2.79E-06	COL3A1,COL5A2,CXCR4,FCN1,GPR37,LTF, NUAK1,S100A8,S100A9,SGK1,SLPI, TBXAS1	
fontolizumab	biologic drug	-2	9.23E-05	FAS, IFNG, IL15RA, TGM3	
resolvin D1	chemical - endogenous mammalian	-2	0.00248	CXCR4,GBP2,IFNG, IL18	105 (19)
NR1H3	ligand-dependent nuclear receptor	-2.2	3.46E-06	ABCG1,ADGRE1,ARG2,CCR5,CD274,CD68,CXCL10,IFNG, LYZ, MERTK, NOD1,PLAU, SCD	99 (11)
SRF	transcription regulator	-2.333	9.19E-05	BATF, CXCR4,ETV1,FCN1,FHL2,GADD45A, LTF, S100A8,S100A9,SLC8A1,SLPI, TBXAS1,TCF7	

Table 4 (continued)

Upstream Regulator	Molecule Type	Activation z-score	p-value of overlap	Target Molecules in Dataset	Mechanistic Network
MRTFA	transcription regulator	-2.236	0.0035	CXCR4,FCN1,LTF,S100A8,S100A9,SLPI,TBXAS1	13 (2)
IL10RA	transmembrane receptor	-3	1.1E-12	ABCG1,ACOD1,ACSL1,ADGRL2,ARG2,BATF2,CALHM6,DRAM1,ETV1,FAS,GBP2,GPR171,HTR7,IFNG,IL15RA,NOD1,PDCCD1LG2,SCIN,SLCO2B1,SOCS3,SPARC,TFEC,TGM2,TGM3,TLR2,UPP1	73 (7)
LILRB4	other	-2.2	0.000194	CXCL10,CXCR6,FAS,IFNG,PDCCD1LG2	83 (10)
JQ1	chemical reagent	-2.121	0.000372	BCL2L14,CD274,CXCR4,ERG,HAVCR2,IFNG,KLRB1,TLR2	91 (14)
IL1RN	cytokine	-2.449	0.0045	GBP1,IFNG,ISG20,S100A9,SLC15A3,TCF7L2	73 (7)
STAG2	other	-2.219	0.000317	BATF2,CD274,CXCL10,GBP2,ISG20	
MEF2C	transcription regulator	-2	0.000242	BACH2,BLK,CCR5,CXCR4,MMP8,MYOM2	
TGFB1	growth factor	-2.252	2.81E-11	ABCG1,CASP1,CCR5,CD163,CEBPB,CHMP7,COL1A1,COL1A2,COL3A1,CXCL10,CXCR4,ECE1,FAS,FCER1A,FCER2,GADD45A,GAS1,GBP1,GYG1,GZMA,HOPX,ID3,IDO1,IFNG,IGFBP4,IL1RL1,KDM6B,KLRB1,KLRC4-KLRK1/KLRK1,MLCF2L2,MGAT3,NR4A3,NUAK1,PLAU,PLCL1,PSAT1,PSPH,RBPMS,SCD,SGK1,SLC12A4,SLC39A8,SOCS3,SPARC,SPHK1,TGM2,TLR2,TLR4,TNFAIP6,TNFSF11,TRIB3,XCL1	127 (20)
SSTR2	G-protein coupled receptor	-2.63	2.14E-06	CEBPB,ETV1,GADD45A,IFNG,IL18,ISG20,SLPI,TLR2,WARS1,XCL1	
TGFBR2	kinase	-3.639	3.57E-08	ACOD1,ADGRE1,BATF,CASP1,DGAT2,GADD45G,HP,ID3,IFNG,IL1R2,KLRB1,LPAR3,LY6D,MCF2L2,PDCCD1LG2,S100A8,S100A9,SLC12A4,SOCS3,UPP1	83 (7)
MYC	transcription regulator	-2.017	0.000174	COL1A1,CTSV,CXCL10,CXCR4,FAS,FCGR2B,GADD45A,GAS1,GBP2,GPT2,HP,ID3,IL1RAP,ISG20,LYZ,NIBAN1,PEG3,PLAU,PSAT1,SGK1,SLC11A1,SPARC,TNFSF11,TNFSF13B	102 (17)
Bay 11-7082	chemical - kinase inhibitor	-2.219	0.00281	CASP1,IL15,IL15RA,IL18,SOD2	112 (17)
ETV6-RUNX1	fusion gene/product	-2.828	0.000394	FSCN2,GBP2,IL15,IL15RA,ISG20,NMI,STX11,TLR4	52 (6)
epinephrine	chemical - endogenous mammalian	-2.19	0.00115	CLEC2B,CXCR4,ERFE,IFNG,IL27	67 (9)
tyrphostin AG490	chemical drug	-2	0.0265	CD68,IFNG,OSCAR,SOCS3	
IZUMO1R	other	-2.429	1.51E-07	CD14,CLEC7A,IL18,S100A8,S100A9,SLPI	
MRTFB	transcription regulator	-2.333	2.79E-06	COL3A1,COL5A2,CXCR4,FCN1,GPR37,LTF,NUAK1,S100A8,S100A9,SGK1,SLPI,TBXAS1	

aligning with the emphasis on the term “cellular response to cytokine stimulus” in the BP category. This suggests that ST infection triggers a cytokine-mediated signaling cascade crucial for initiating and regulating both innate and adaptive immune responses [37]. The identification of the “Intestinal immune network for IgA production” pathway emphasizes the specific impact of ST infection on mucosal immunity. IgA is the predominant immunoglobulin class in mucosal areas, such as the gut, playing a crucial role in neutralizing pathogens and toxins; this indicates that ST infection triggers an adaptive immune response aimed at boosting mucosal defenses [38]. Further, a previous study reported that the genes associated with the intestinal immune network for IgA production were upregulated in chickens after ST infection [39], demonstrating the significant activation of pathways

involved in Toll-like receptor signaling, cytokine–receptor interactions, and IgA production. These common pathways highlight the fundamental mechanisms underlying innate immunity that are preserved across various species to combat bacterial infections. The identification of disease-specific pathways, such as “Rheumatoid arthritis” and “Pertussis”, suggests that the immune response to ST infection shares common pathways with these conditions. Rheumatoid arthritis is characterized by chronic inflammation and autoimmunity; therefore, the activation of this pathway may suggest that ST infection may induce or exacerbate inflammatory and autoimmune processes [40]. The enrichment of the “Pertussis” pathway underscores the relevance of general mechanisms underlying pathogen recognition, immune activation, and defense mounted by the host against bacterial

challenges, suggesting that a broad immune response is elicited against bacterial infections [41].

Canonical pathway enrichment

The IPA analysis results were consistent with the findings of the GO term and KEGG analyses, emphasizing the importance of PRRs, such as TLRs and cytokine interactions. The top 10 pathways identified by IPA canonical pathway analysis encompassed various biological processes (Table 3). The most significant pathway was “Pathogen-induced cytokine storm signaling pathway” (Fig. 3), underscoring the role of genes involved in inflammatory responses during pathogenic infections. Similarly, the “Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses” (Fig. 4) highlighted specific genes that play critical roles in the initial immune response to microbial pathogens, thereby emphasizing the importance of innate immunity. Overall, these findings underscore the significant role of immune responses, particularly those triggered by pathogenic challenges, and suggest the involvement of a complex interplay of genes in the immediate response to pathogens.

Upstream regulators and target molecules

The activated upstream regulators (Table 4) suggest a significant influence of external chemical entities, particularly those associated with bacterial components, which elicit strong immune responses. The inhibited upstream regulators included diverse entities, ranging from transcription factors (e.g., MRTFB) to responses on drugs (e.g., fontolizumab), indicating the potential downregulating of a range of processes, including transcriptional activities and specific drug responses. Specifically, the activated regulators indicated a pronounced influence of bacterial components (i.e., lipopolysaccharides derived from *Escherichia coli* and *Salmonella*), suggesting the importance of immune responses, particularly those elicited by bacterial challenges. Several of the inhibited regulators (e.g., fontolizumab) were related to therapeutic interventions, hinting at the potential impacts of drugs or treatments on these genes. Responses to chemicals, such as hydrocortisone and cholesterol, further underscored the diversity of the dataset, emphasizing both endogenous processes (e.g., lipid metabolism) and external interventions (e.g., hydrocortisone treatment). Together, these findings suggested that our dataset captured a wide range of biological processes, from transcriptional regulation to immune responses to therapeutic interventions.

RT-qPCR validation using PBMCs

Analyzing genes in PBMCs can be highly informative for several reasons, primarily because of the diverse

roles and origins of PBMCs in the immune system. As the transcriptomic signatures collectively indicated significant enrichment of immune-related signaling pathways, we analyzed the expression of a few key genes in PBMCs isolated from the piglets. Specifically, major players involved in the aforementioned GO terms and KEGG pathways were included: *TLR2*, *TLR4*, *CXCL10*, *IL-15*, and *IL-27*. While *TLR2* and *TLR4* transcript levels did not significantly differ between the groups, *CXCL10*, *IL15*, and *IL27* mRNA levels were all consistently higher in the ST group than in the NC group (Fig. 5).

Our findings that *TLR2* and *TLR4* expression did not differ between the study groups is in line with previous findings that the baseline levels of these receptors do not markedly fluctuate in response to infection or disease states, indicating a possible steady state of readiness for pathogen recognition [42]. In contrast, *CXCL10*, *IL15*, and *IL27* transcript levels were significantly higher in the ST group than in the NC group. These cytokines and chemokines are critical in the recruitment and activation of immune cells, indicating a more pronounced immune response in the ST group. *CXCL10* has chemotactic properties, directing the migration of immune cells to sites of inflammation or infection [43]. *IL-15* and *IL-27* are pivotal for T cell proliferation and differentiation and inflammatory response regulation, respectively [44]. The elevated levels of these molecules underscore a heightened state of immune activation, potentially reflecting the piglets' response to ST infection. In summary, the differential expression of *CXCL10*, *IL-15*, and *IL-27* in contrast to the stable expression of *TLR2*, and *TLR4* reflects the dynamic nature of the immune response to *Salmonella* infection in piglets.

Our study revealed the complex interactions between ST infection and the immune response in piglets, advancing our understanding of the mechanisms underlying host–pathogen dynamics. We discovered significant alterations in both innate and adaptive immune strategies through the identification of DEGs and GO term and KEGG pathway enrichment analyses. Notably, the genes associated with Toll-like receptors, NIK/NF- κ B signaling, cytokine signaling, and T cell proliferation pathways were upregulated, consistent with the elevated expression of *CXCL10*, *IL-15*, and *IL-27*, indicating robust immune activation. Furthermore, the increase in the monocyte counts at the early stages of infection suggested its potential to serve as a hematological marker for ST infection in post-weaned piglets. Our integrative approach combined phenotypic, biochemical, and molecular data to provide a holistic view of the response of piglets to ST infection. However, this study had a few limitations.

We utilized RNA-seq technology, which may not adequately reflect the genetic profiles of rare cell populations, compared to single-cell sequencing, which is a more advanced. In addition, the present study's findings do not reflect about the clinical stage-dependent effects of *Salmonella* infection. Therefore, future studies are essential to explore the subtle changes in cells across different phases of disease progression. Despite these limitations, the overall results of this study could enhance our understanding of the mechanisms underlying, and contribute to the development of novel therapeutic strategies for, ST infection in post-weaned piglets.

Abbreviations

BP	Biological pathway
CC	Cellular component
DEG	Differentially expressed gene
dpi	Days post-infection
FDR	False discovery rate
GO	Gene Ontology
IPA	Ingenuity Pathway Analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
MF	Molecular function
NC	Negative control
PAMPs	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cell
PRRs	Pattern recognition receptors
PLS-DA	Partial least squares-discriminant analysis
RT-qPCR	Quantitative reverse transcription PCR
ST	<i>Salmonella enterica</i> serovar Typhimurium
TLR	Toll-like receptor
WBC	White blood cell

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-024-00924-4>.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

Conceptualization, E-YB and S-IO; data curation, E-YB, S-WY, HGL, and S-IO; funding acquisition, E-YB and S-IO; formal analysis, E-YB, JKK, KWL, and S-IO; investigation, S-WY, HGL, and S-IO; methodology, E-YB, S-WY, HGL, and S-IO; project administration, E-YB; resources, BK and Y-HJ; supervision, S-IO; validation, E-YB, SH, and S-IO; visualization, E-YB, JKK, KWL, and S-IO; writing—original draft preparation, E-YB, JKK, KWL, and S-IO; writing—review and editing, E-YB and S-IO. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) and Korea Smart Farm R&D Foundation (KosFarm) through the Smart Farm Innovation Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) and Ministry of Science and ICT (MSIT), Rural Development Administration (RDA) (421042-04). This paper was also supported by "Research Base Construction Fund Support Program" funded by Jeonbuk National University in 2023.

Data availability

The transcriptome data from this study was deposited in the NCBI Sequencing Read Archive database under the accession number PRJNA1108035.

Competing interests

The authors declare no potential conflict of interest.

Received: 9 May 2024 / Accepted: 24 July 2024

Published online: 19 August 2024

References

- Bonardi S (2017) *Salmonella* in the pork production chain and its impact on human health in the European Union. *Epidemiol Infect* 145(8):1513–1526. <https://doi.org/10.1017/S095026881700036X>
- Oh S-I, Kim JW, Chae M, Jung J-A, So B, Kim B, Kim H-Y (2016) Characterization and antimicrobial resistance of *Salmonella* Typhimurium isolates from clinically diseased pigs in Korea. *J Food Prot* 79(11):1884–1890. <https://doi.org/10.4315/0362-028X.JFP-16-131>
- Zheng L, Duarte ME, Sevarolli Loftus A, Kim SW (2021) Intestinal health of pigs upon weaning: challenges and nutritional intervention. *Front Veterinary Sci* 8:628258. <https://doi.org/10.3389/fvets.2021.628258>
- Wales A, Cook A, Davies R (2011) Producing *Salmonella*-free pigs: a review focusing on interventions at weaning. *Vet Rec* 168(10):267–276. <https://doi.org/10.1136/vr.d1125>
- Meurens F, Berri M, Auray G, Melo S, Virlogeux-Payant I et al (2009) Early immune response following *Salmonella enterica* subspecies *enterica* serovar typhimurium infection in porcine jejunal gut loops. *Vet Res* 40(1). <https://doi.org/10.1051/vetres:2008043>
- Martins RP, Lorenzi V, Arce C, Lucena C, Carvajal A, Garrido JJ (2013) Innate and adaptive immune mechanisms are effectively induced in ileal Peyer's patches of *Salmonella* typhimurium infected pigs. *Dev Comp Immunol* 41(1):100–104. <https://doi.org/10.1016/j.dci.2013.04.020>
- Martins RP, Collado-Romero M, Arce C, Lucena C, Carvajal A, Garrido JJ Exploring the immune response of porcine mesenteric lymph nodes to *Salmonella enterica* Serovar Typhimurium: an analysis of transcriptional changes, morphological alterations and pathogen burden. *Comp Immunol Microbiol Infect Dis*. <https://doi.org/10.1016/j.cimid.2012.11.003>
- Utthe JJ, Royae A, Lunney JK, Stabel TJ, Zhao S-H, Tuggle CK, Bearson SM (2007) Porcine differential gene expression in response to *Salmonella enterica* serovars Choleraesuis and Typhimurium. *Mol Immunol* 44(11):2900–2914. <https://doi.org/10.1016/j.molimm.2007.01.016>
- Osvaldova A, Stepanova H, Faldyna M, Matiasovic J (2017) Gene expression values of pattern-recognition receptors in porcine leukocytes and their response to *Salmonella enterica* Serovar Typhimurium infection. *Res Vet Sci* 114:31–35. <https://doi.org/10.1016/j.rvsc.2017.02.026>
- Mani V, Weber TE, Baumgard LH, Gabler NK (2012) Growth and development symposium: endotoxin, inflammation, and intestinal function in livestock. *Journal of animal Science*. :90(5):1452-65. <https://doi.org/10.2527/jas2011-4627>
- Wang M, Qazi IH, Wang L, Zhou G, Han H (2020) *Salmonella* virulence and immune escape. *Microorganisms* 8(3):407. <https://doi.org/10.3390/microorganisms8030407>
- Kirthika P, Ali MA, Behera P, Subudhi PK, Tolenkhomba TC, Gali JM (2017) Dynamics of cytokine gene expression in peripheral blood mononuclear cells of indigenous and exotic breeds of pigs in India. *Anim Sci J* 88:1794–1800. <https://doi.org/10.1111/asj.12827>
- Huang T, Huang X, Shi B, Wang F, Feng W, Yao M (2018) Regulators of *Salmonella*-host interaction identified by peripheral blood transcriptome profiling: roles of TGFB1 and TRP53 in intracellular *Salmonella* replication in pigs. *Vet Res* 49:1–14. <https://doi.org/10.1186/s13567-018-0616-9>
- Huang T, Huang X, Chen W, Yin J, Shi B, Wang F, Feng W (2019) MicroRNA responses associated with *Salmonella enterica* serovar typhimurium challenge in peripheral blood: effects of miR-146a and IFN- γ in regulation of fecal bacteria shedding counts in pig. *BMC Vet Res* 15:195. <https://doi.org/10.1186/s12917-019-1951-4>
- Yi S-W, Lee HG, Kim E, Jung Y-H, Bok E-Y, Cho A et al (2023) Gut microbiota alteration with growth performance, histopathological lesions, and immune responses in *Salmonella* Typhimurium-infected weaned piglets. *Veterinary Anim Sci* 22:100324. <https://doi.org/10.1016/j.vas.2023.100324>

16. Yi S-W, Lee HG, Kim E, Jung Y-H, Bok E-Y, Cho A, Do YJ, Hur T-Y, Oh S-I (2023) Raw potato starch diet supplement in weaned pigs could reduce *Salmonella* Typhimurium infection by altering microbiome composition and improving immune status. *Front Veterinary Sci* 10:1183400. <https://doi.org/10.3389/fvets.2023.1183400>
17. Lessard M, Talbot G, Bergeron N, Verso L, Morissette L, Yergeau B, Matte É, Bissonnette JJ, Blais N, Gong M, Wang J, Quessy Q, Guay S, F (2023) Weaning diet supplemented with health-promoting feed additives influences microbiota and immune response in piglets challenged with *Salmonella*. *Vet Immunol Immunopathol* 255:110533. <https://doi.org/10.1016/j.vetimm.2022.110533>
18. Ha S, Kang S, Jung M, Kim SB, Lee HG, Park H-T et al (2023) Comparison of blood parameters according to fecal detection of *Mycobacterium avium* subspecies *paratuberculosis* in subclinically infected Holstein cattle. *J Vet Sci* 24(5). <https://doi.org/10.4142/jvs.23111>
19. Badia R, Brufau MT, Guerrero-Zamora AM, Lizardo R, Dobrescu I, Martin-Venegas R et al (2012) β -Galactomannan and *Saccharomyces cerevisiae* var. *Boulardii* modulate the immune response against *Salmonella enterica* Serovar Typhimurium in porcine intestinal epithelial and dendritic cells. *Clin Vaccine Immunol* 19(3):368–376. <https://doi.org/10.1128/CVI.05532-11>
20. Tang Q, Yi H, Hong W, Wu Q, Yang X, Hu S et al (2021) Comparative effects of *L. Plantarum* CGMCC 1258 and *L. Reuteri* LR1 on growth performance, anti-oxidant function, and intestinal immunity in weaned pigs. *Front Veterinary Sci* 8:728849. <https://doi.org/10.3389/fvets.2021.728849>
21. Bouwhuis M, McDonnell M, Sweeney T, Mukhopadhyaya A, O'Shea C, O'Doherty J (2017) Seaweed extracts and galacto-oligosaccharides improve intestinal health in pigs following *Salmonella* Typhimurium challenge. *Animal* 11(9):1488–1496. <https://doi.org/10.1017/S1751731117000118>
22. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25(4):402–408. <https://doi.org/10.1006/meth.2001.1262>
23. Utthe J, Wang Y, Qu L, Nettleton D, Tuggle C, Bearson S (2009) Correlating blood immune parameters and a CCT7 genetic variant with the shedding of *Salmonella enterica* Serovar Typhimurium in swine. *Vet Microbiol* 135(3–4):384–388. <https://doi.org/10.1016/j.vetmic.2008.09.074>
24. Kempf F, Cordoni G, Chaussé A-M, Drumo R, Brown H, Horton DL et al (2023) Inflammatory responses induced by the monophasic variant of *Salmonella* typhimurium in pigs play a role in the high shedder phenotype and fecal microbiota composition. *MSystems* 8(1):e00852–e00822. <https://doi.org/10.1128/msystems.00852-22>
25. Wijburg OL, Simmons CP, Van Rooijen N, Strugnell RA (2000) Dual role for macrophages in vivo in pathogenesis and control of murine *Salmonella enterica* var. Typhimurium infections. *Eur J Immunol* 30(3):944–953. [https://doi.org/10.1002/1521-4141\(200003\)30:3%3C944::AID-IMMU944%3E3.0.CO;2-1](https://doi.org/10.1002/1521-4141(200003)30:3%3C944::AID-IMMU944%3E3.0.CO;2-1)
26. Kawasaki T, Kawai T (2014) Toll-like receptor signaling pathways. *Front Immunol*. <https://doi.org/10.3389/fimmu.2014.00461>
27. Mazgaen L, Gurung P (2020) Recent advances in Lipopolysaccharide Recognition Systems. *Int J Mol Sci* 21(2):379. <https://doi.org/10.3390/ijms21020379>
28. Oliveira LG et al (2012) The role of TLR2 in lipoteichoic acid recognition. *J Immunol*. <https://doi.org/10.4049/jimmunol.1103188>
29. Francisco S et al (2022) TLR4/TLR2 Interaction Induced by Low-Endotoxic atypical LPS. *Microb Pathog*. <https://doi.org/10.1016/j.micpath.2022.105404>
30. Andreakos E, Smith C, Monaco C, Brennan FM, Foxwell BM, Feldmann M (2003) I κ B kinase 2 but not NF- κ B-inducing kinase is essential for effective DC antigen presentation in the allogeneic mixed lymphocyte reaction. *Blood* J Am Soc Hematol 101(3):983–991. <https://doi.org/10.1182/blood-2002-06-1835>
31. Lacher SM, Thurm C, Distler U, Mohebiany AN, Israel N, Kitic M et al (2018) NF- κ B inducing kinase (NIK) is an essential post-transcriptional regulator of T-cell activation affecting F-actin dynamics and TCR signaling. *J Autoimmun* 94:110–121. <https://doi.org/10.1016/j.jaut.2018.07.017>
32. Won K, Kim D, Shin D, Hur J, Lee H-K, Heo J, Oh J-D (2022) High-throughput sequencing-based metagenomic and transcriptomic analysis of intestine in piglets infected with *salmonella*. *J Anim Sci Technol* 64(6):1144. <https://doi.org/10.5187/jast.2022.e73>
33. Kunzelmann-Marche C, Freyssinet J-M, Martínez MC (2002) Loss of plasma membrane phospholipid asymmetry requires raft integrity: role of transient receptor potential channels and ERK pathway. *J Biol Chem* 277(22):19876–19881. <https://doi.org/10.1074/jbc.M200324200>
34. Patel H, Murray F, Insel P G-protein-coupled receptor-signaling components in membrane raft and caveolae microdomains. *Protein-Protein Interact as New Drug Targets*. 2008:167–184. https://doi.org/10.1007/978-3-540-72843-6_7
35. Chervitz SA, Lin CM, Falke JJ (1995) Transmembrane signaling by the aspartate receptor: engineered disulfides reveal static regions of the subunit interface. *Biochemistry* 34(30):9722–9733. <https://doi.org/10.1021/bi00030a010>
36. Minhas PS, Liu L, Moon PK, Joshi AU, Dove C, Mhatre S et al (2019) Macrophage de novo NAD⁺ synthesis specifies immune function in aging and inflammation. *Nat Immunol* 20(1):50–63. <https://doi.org/10.1038/s41590-018-0255-3>
37. Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* 124(4):783–801. <https://doi.org/10.1016/j.cell.2006.02.015>
38. Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F (2008) Allergy and the gastrointestinal system. *Clin Experimental Immunol* 153(Supplement1):3–6. <https://doi.org/10.1111/j.1365-2249.2008.03713.x>
39. Dar MA, Singh R, Rather MA et al (2022) Disentangling the innate immune responses of intestinal epithelial cells and lamina propria cells to *Salmonella* Typhimurium infection in chickens. *Front Immunol* 13:123456. <https://doi.org/10.3389/fimmu.2022.123456>
40. Firestein GS (2003) Evolving concepts of rheumatoid arthritis. *Nature* 423(6937):356–361. <https://doi.org/10.1038/nature01661>
41. Fedele G, Cassone A, Ausiello CM (2015) T-cell immune responses to *Bordetella pertussis* infection and vaccination. *Pathogens Disease* 73(7):ftv051. <https://doi.org/10.1093/femspd/ftv051>
42. Ozato K, Tsujimura H, Tamura T (2002) Toll-like receptor signaling and regulation of cytokine gene expression in the immune system. *Biotechniques* 33:S66–S75. <https://doi.org/10.2144/Oct0208>
43. McInnes IB, Gravallese EM (2021) Immune-mediated inflammatory disease therapeutics: past, present and future. *Nat Rev Immunol* 21(10):680–686. <https://doi.org/10.1038/s41577-021-00603-1>
44. Choi YH, Lim EJ, Kim SW, Moon YW, Park KS, An H-J (2019) IL-27 enhances IL-15/IL-18-mediated activation of human natural killer cells. *J Immunother Cancer* 7:1–12. <https://doi.org/10.1186/s40425-019-0652-7>

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