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Single-cell RNA sequencing reveals divergent responses in feline peripheral blood immune cells exposed to Toxoplasma gondii

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Abstract

Toxoplasma gondii is an obligate intracellular apicomplexan parasite of major public health importance, and felids are the only known definitive hosts in which sexual reproduction occurs. Despite the central role of cats in parasite transmission, the single-cell immune responses of feline peripheral blood immune cells to *T. gondii* exposure remain poorly characterized. In this study, feline peripheral blood immune cells isolated from a healthy donor cat were exposed *in vitro* to *T. gondii* tachyzoites (Pru strain) for 24 h and analyzed by single-cell RNA sequencing. After quality control, 15,474 high-quality cells were retained for downstream analysis. Exposed cells were further classified as infected or bystander according to the detection of parasite-derived transcripts. Five major immune cell populations were identified, including T cells, B cells, monocytes, neutrophils, and megakaryocytes. Although lymphocytes dominated the overall cellular landscape, the most prominent transcriptional remodeling occurred in myeloid populations, particularly monocytes and neutrophils. Monocytes exhibited a shared cytokine-centered inflammatory program in both infected and bystander states, but differed in functional emphasis; whereas neutrophils displayed more distinct state divergence, with bystander cells enriched for stress and remodeling associated features and infected cells showing a stronger inflammatory effector-like profile. Cell-cell communication analysis further identified a central monocyte-neutrophil interaction axis, with IL-1 and CSF2 related signaling emerging as prominent pathways. This study provides the first single-cell framework for understanding the early feline immune response to *T. gondii* exposure and offers a basis for future research of host-parasite interactions in the definitive host.

Introduction

Toxoplasma gondii is an obligate intracellular apicomplexan parasite with a broad host range and the capacity to infect virtually all warm-blooded animals, including humans, thereby posing a major public health concern [1, 2]. Humans acquire *T. gondii* infection primarily through ingestion of food or water contaminated with oocysts, consumption of raw or undercooked meat containing tissue cysts, or vertical transmission from mother to fetus [3]. In most immunocompetent individuals, infection is asymptomatic or causes only mild, self-limiting manifestations. However, in patients with acquired immunodeficiency syndrome (AIDS), *T. gondii* infection can cause severe complications, including toxoplasmic encephalitis, pneumonia, and disseminated infection [4]. In pregnant women, primary infection may lead to adverse pregnancy outcomes, including miscarriage, preterm birth, fetal malformation, and stillbirth [5].

Although *T. gondii* undergoes asexual replication in a wide range of intermediate hosts, felids are the only known definitive hosts [6]. Sexual development occurs exclusively in feline intestinal epithelial cells, where oocysts are formed and subsequently shed in the feces [7, 8]. After sporulation in the environment, these oocysts become infectious and serve as an important source of transmission to other hosts [9]. In addition to the oocyst stage, *T. gondii* exists in the host as tachyzoites and bradyzoites. Tachyzoites are the rapidly replicating form responsible for acute infection, whereas bradyzoites are enclosed within tissue cysts and are mainly associated with chronic infection and long-

term persistence [10]. Therefore, understanding immune cell responses in the feline definitive host is important for elucidating both parasite transmission biology and host-pathogen interactions.

T. gondii can infect multiple nucleated host cell types and disseminate throughout the host via the circulatory system, making peripheral blood immune cells an important cellular context for host-pathogen interactions [11]. Upon interaction with host phagocytic cells, the parasite may actively invade and establish a parasitophorous vacuole (PV), be transiently retained within a phagosome after phagocytic uptake, or inject effector proteins into host cells without completing invasion [12–14]. These findings indicate that *T. gondii* infection is not a single homogeneous event, but rather a dynamic process involving multiple host-pathogen interaction outcomes. Previous single-cell transcriptomic studies have shown that human monocytes upregulate chemokines such as CCL2 and CXCL5 in response to *T. gondii* exposure [15]. More recently, single-cell profiling of human peripheral blood mononuclear cells demonstrated that *T. gondii* preferentially infects dendritic cells and induces markedly heterogeneous transcriptional responses among infected cells, bystander cells, and distinct dendritic cell subclusters. Meanwhile, monocytes may acquire a dendritic cell-like transcriptional state during infection [16].

Despite these advances, current knowledge of host-*T. gondii* interactions at single-cell resolution has been derived mainly from intermediate hosts such as mice and humans. As the only known definitive hosts of *T. gondii*, cats remain poorly characterized with respect to changes in peripheral immune cell composition and cell type-specific transcriptional responses following parasite exposure. Single-cell RNA sequencing (scRNA-seq) provides a powerful approach for dissecting host-pathogen interactions at cellular resolution. Here, we used scRNA-seq to systematically characterize the single-cell transcriptomic features of feline peripheral blood immune cells following *T. gondii* exposure, thereby providing single-cell-level insight into the early immune response of the feline definitive host to *T. gondii* and the host-pathogen interaction mechanisms relevant to parasite transmission.

Materials and methods

Ethical approval

The study received ethical clearance from the Ethical Committee of the College of Veterinary Medicine, Yunnan Agricultural University (Permit number: 202504014).

Isolation of feline peripheral blood immune cells

Peripheral blood was collected by venipuncture from a healthy 6-month-old male cat purchased from a local breeder and housed in a controlled environment. Prior to the experiment, the cat was confirmed to be negative from T. gondii using the modified agglutination test (MAT) and free of major viral infections (including feline immunodeficiency virus, feline parvovirus, feline coronavirus and feline leukemia virus) based on serological examination. Cats received commercial cat diets (Chuncui, Shanghai, China) and water ad libitum during the two weeks prior to experimentation in order to minimize any potential dietary impact on the study results. Feline peripheral blood mononuclear cells (PBMCs) were isolated from whole

blood using the feline peripheral blood mononuclear cell isolation kit (Solarbio, Beijing, China) according to the manufacturer's instructions.

Parasites and infection

Tachyzoites of *Toxoplasma gondii* Pru strain were maintained by serial passage on human foreskin fibroblasts (HFFs) in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher) supplemented with 2% fetal bovine serum (FBS) without antibiotics, at 37°C in 5% CO2. For infection experiments, tachyzoites were released from heavily infected HFFs by scraping and syringe lysis, pelleted by centrifugation at 3000 rpm for 5 min, washed in PBS, and filtered through a 3 μm membrane to remove host cell debris. Purified parasites were counted and added to 1×10^6 feline PBMCs in 6-well plates at an MOI of 3. After brief centrifugation to promote parasite-cell contact, cultures were incubated in fresh RPMI 1640 medium at 37 °C in 5% CO_2 for 24 h prior to single-cell RNA sequencing. Parallel cultures without parasite exposure served as uninfected controls.

Single-cell RNA sequencing

The scRNA-Seq libraries were generated using the 10× Genomics Chromium Controller Instrument and Chromium Single Cell 3' V3 Reagent Kits (10× Genomics, Pleasanton, CA). Briefly, cells were concentrated to approximately 1000 cells/uL and loaded into each channel to generate single-cell Gel Bead-In-Emulsions (GEMs). After the RT step, GEMs were broken and barcoded-cDNA was purified and amplified. The amplified barcoded cDNA was fragmented, A-tailed, ligated with adaptors and index PCR amplified. The final libraries were quantified using the Qubit High Sensitivity DNA assay (Thermo Fisher Scientific) and the size distribution of the libraries were determined using a High Sensitivity DNA chip on a Bioanalyzer 2200 (Agilent). All libraries were sequenced by illumina Novaseq6000 (Illumina, San Diego, CA) on a 150 bp paired-end run.

Single-cell data analysis

Raw scRNA-seq data were processed using Cell Ranger v7.0.0 with a merged reference containing the feline genome (Felis catus 9.0) and the Toxoplasma gondii genome (ToxoDB-64). Subsequent downstream analyses were carried out in R v4.3.3 using Seurat v5 [17]*. Following quality control, clean reads were aligned to the combined feline and T. gondii reference, and feature-barcode matrices were generated for downstream single-cell analysis.* Cells were retained for downstream analysis if they met the following quality control criteria: more than 200 detected genes, fewer than 4000 detected genes, a mitochondrial transcript percentage below 10%, and fewer than 30,000 total RNA counts. The filtered expression matrix was normalized and scaled in Seurat, and principal component analysis (PCA) was performed using highly variable genes. Significant principal components were then used for graph-based unsupervised clustering and UMAP visualization. Cell types were manually annotated on the basis of canonical marker genes, with reference to the published feline single-cell dataset [18]. To distinguish parasite-positive from parasite-negative exposed cells, cells with ≥ 3 detected *T. gondii* genes were defined as infected, whereas exposed cells with < 3 detected parasite genes were classified as bystander cells. Differential

expression analysis was performed using the FindMarkers function in the Seurat package. Genes with avg $\log_2$FC ≥ 0.25 and adjust *p* value < 0.05 were defined as upregulated, whereas genes with avg $\log_2$FC ≤ −0.25 and adjust *p* value < 0.05 were defined as downregulated. GO and KEGG functional enrichment analysis was performed on differentially expressed genes (DEGs) to reveal pathways significantly associated with the genes specifically expressed [19].

Cell communication analysis

Cell-cell communication analysis was performed using CellChat v2.2.0. Single-cell transcriptomic data were first preprocessed, and overexpressed genes and ligand-receptor interactions were identified. Based on these results, intercellular communication probabilities were inferred. Low-confidence interactions were then filtered using the filterCommunication function with a minimum cell threshold of min.cells = 10. Next, pathway-level communication probabilities were calculated using computeCommunProbPathway, and the overall cell-cell communication network was constructed with aggregateNet. Finally, ligand-receptor interaction data (df.net) and pathway-level communication results (df.netP) were extracted using the subsetCommunication function for downstream analysis and visualization.

Data visualization and statistical analysis

Data visualization was mainly performed in R using Seurat and ggplot2 v.3.5.2, including UMAP plots, stacked bar plots, volcano plots, heatmaps, Venn diagrams, GO/KEGG enrichment plots, and cell-cell communication network plots. Unless otherwise specified, *p* values were adjusted for multiple testing, and an adjusted *p* value < 0.05 was considered statistically significant.

Results

Single-cell transcriptomic profiling identifiedmajor immune cell populations infeline peripheral blood immune cells after *T.gondii*exposure

To characterize the transcriptional responses of feline peripheral blood immune cells to *T. gondii* infection, we performed single-cell RNA sequencing (10× Genomics) on peripheral blood immune cells collected from a healthy cat and exposed to in vitro *T. gondii* tachyzoites, with unexposed cells used as controls (Fig. 1A). After quality control, normalization, and scaling to minimize potential technical bias, a total of **15,474 high-quality cells** were retained for downstream analysis, including **7,956 cells** in the exposed group and **7,518 cells** in the unexposed group. Unsupervised clustering and UMAP visualization resolved these cells into **14 transcriptionally distinct clusters**, which were subsequently annotated into five major immune cell populations on canonical marker genes and cluster-specific transcriptional signatures, including **T cells, B cells, megakaryocytes, monocytes and** neutrophils (Fig. 1B). T cells were identified by the expression of markers such as **CD3D, CD3E, TCF7 and LCK, whereas** B cells expressed **MS4A1, CD79A,** and **CD79B** [20]. Monocytes showed enriched expression of myeloid-associated genes such as **CSF1R, TYROBP and CD163** [21], whereas neutrophils were distinguished by strong expression

of **S100A8** and **S100A9** [22]. Megakaryocytes were defined by platelet-lineage markers including **PPBP, GP9,** and **GP1BB** [23, 24] (Fig. 1C). The overall cellular distribution was similar between the exposed and unexposed groups (Fig. 1D), indicating that short-term parasite exposure did not dramatically alter the global gene expression landscapes of peripheral blood immune cells. In both groups, **T cells constituted the dominant cell population**, followed by **B cells**, while **neutrophils, monocytes, and megakaryocytes** accounted for smaller fractions of the total cells (Fig. 1E).

Distinct transcriptional responses of feline peripheral blood immune cell populations to *T. gondii*infection

To further distinguish directly infected cells from non-infected bystander cells within the *Toxoplasma gondii*-exposed peripheral immune cells, parasite-derived transcripts were mapped back to the host single-cell dataset. Based on the presence or absence of *T. gondii* transcripts, cells were classified into three categories: infected cells, bystander cells, and unexposed control cells. UMAP visualization showed that both infected and bystander cells were broadly distributed across the major immune cell populations, whereas unexposed cells showed no detectable *T. gondii*-derived transcripts (Fig. 2A).

We next compared the abundance of *T. gondii* transcripts among different host cell populations. Parasite transcript levels differed significantly across cell types, indicating marked heterogeneity in intracellular parasite burden (Fig. 2B). Among all annotated immune cell populations, **neutrophils showed the highest overall parasite transcript abundance**, suggesting that they harbored the strongest detectable parasite signal. In comparison, **T cells, B cells, monocytes, and megakaryocytes displayed significantly lower levels of parasite-derived transcripts**. These results indicate that although *T. gondii* exposure affected multiple peripheral blood immune cells populations, the extent of detectable intracellular parasite signal was not uniform across cell types.

To further assess cellular susceptibility at the population level, we calculated the proportion of infected cells within each annotated immune cell type (Fig. 2C). **Neutrophils exhibited the highest infection proportion**, with **49.8%** of all neutrophils containing detectable parasite transcripts. This was followed by **monocytes (36.0%)**, whereas **T cells (29.5%)**, **megakaryocytes (29.0%)**, and **B cells (28.3%)** showed comparatively lower infection proportions. In terms of absolute infected cell numbers, **T cells contributed the largest number of infected cells (n = 1,887)**, reflecting their predominance in the total peripheral blood immune cells compartment, followed by **B cells (n = 288)**, **neutrophils (n = 154)**, **monocytes (n = 40)**, and **megakaryocytes (n = 36)** (Fig. 2C). However, when normalized to the total number of cells in each population, neutrophils and monocytes remained the higher infected cell type.

Differential expression gene and function analyses of monocyte cells after *T. gondii* exposure

To further define the monocyte response alterations post *T. gondii* exposure, we compared **bystander monocytes** and **infected monocytes** with **unexposed monocytes**. Differential expression overlap analysis showed that the two exposed monocyte states shared a substantial proportion of upregulated genes, including **39 commonly upregulated genes**, together with **12 bystander-specific** and **20 infected-**

specific upregulated genes. In contrast, fewer common downregulated genes were detected, with only **6 shared downregulated genes**, whereas **31** and **3** genes were uniquely downregulated in bystander and infected monocytes, respectively (Fig. 3A). These findings indicate that exposed monocytes share a common transcriptional activation framework but retain state-specific features.

Volcano plot analysis further demonstrated that both bystander and infected monocytes upregulated genes associated with inflammatory signaling, cytokine/chemokine communication, and immune regulation, although the transcriptional response was generally stronger in infected monocytes (Fig. 3B, C). In bystander monocytes, genes such as **IL-6, CSF2, CSF3, CCL5, CCL14, IL2RA, IFNLR1, EBI3, IL-10,** and **CD274** were upregulated (Fig. 3B), suggesting that exposure to the parasite-associated environment alone was sufficient to induce a coordinated inflammatory and immunoregulatory state. In infected monocytes, these changes were further intensified (Fig. 3C).

GO biological process enrichment analysis revealed distinct functional emphases between the two monocyte states (Fig. 3D). Infected monocytes were more strongly enriched in processes related to mononuclear cell proliferation, lymphocyte proliferation, regulation of T cell activation, and T cell proliferation, suggesting a stronger role in immune coordination and lymphocyte-related regulation. In contrast, bystander monocytes preferentially enriched processes such as response to interleukin-1, regulation of leukocyte proliferation, leukocyte chemotaxis, and regulation of lymphocyte proliferation, consistent with functions in inflammatory sensing, leukocyte recruitment, and peripheral immune modulation.

Despite these differences at the GO-BP level, KEGG enrichment analysis showed that infected and bystander monocytes shared a largely overlapping signaling framework (Fig. 3E). The common pathways were mainly related to **cytokine-cytokine receptor interaction, TNF signaling, IL-17 signaling, JAK-STAT signaling,** and **Toll-like receptor signaling**, indicating that *T. gondii* exposure induced a shared inflammatory and cytokine-centered transcriptional backbone regardless of direct parasite transcript detection. Representative heatmaps further supported this interpretation (Fig. 3F, G).

Bystander and infected neutrophils exhibit distinct transcriptional programs following *T. gondii* exposure

To further examine neutrophil responses after *T. gondii* exposure, we compared bystander neutrophils and infected neutrophils with unexposed neutrophils. UMAP visualization showed that both bystander and infected neutrophils occupied the neutrophil compartment but represented distinct exposure-associated states (Fig. 4A). Differential expression overlap analysis revealed that the two neutrophil states shared a subset of transcriptional changes, including 39 commonly upregulated genes and 6 commonly downregulated genes, while 12 and 20 genes were uniquely upregulated in bystander and infected neutrophils, respectively. Additionally, 31 and 3 genes were uniquely downregulated in bystander and infected neutrophils, respectively (Fig. 4B). These findings indicate that bystander and infected neutrophils share a partial exposure-induced response program but also diverge substantially in their transcriptional remodeling.

Volcano plot analysis showed that both bystander and infected neutrophils underwent substantial transcriptional remodeling compared with unexposed neutrophils. Bystander neutrophils upregulated genes preferentially associated with inflammatory priming, pathogen sensing, oxidative stress adaptation, and apoptosis-related regulation, including CXCL8, IL-1β, TXNRD1, PRDX1, CLEC4E, FCGR1A, and CD274 (Fig. 4C). In contrast, infected neutrophils displayed a more pronounced inflammatory effector-like profile, with enhanced expression of TNFAIP3, TRAF1, BIRC3, GADD45B, MMP9, FCGR1A, and CLEC4E (Fig. 4D). Both bystander and infected neutrophils also downregulated several genes linked to canonical neutrophil identity and baseline granulocyte programs, including CSF3R, PGLYRP1, S100A8, S100A9, ELANE, and CLEC12A.

GO enrichment analysis further highlighted the functional divergence between the two neutrophil states. Bystander neutrophils were enriched in biological processes related to **cellular response to biotic stimulus, response to oxidative stress, regulation of leukocyte apoptotic process,** and **regulation of myeloid cell apoptotic process**, together with molecular functions such as **pattern recognition receptor activity** and **antioxidant activity** (Fig. 4E). These results support a model in which bystander neutrophils are primarily characterized by environmental sensing, oxidative stress adaptation, and cell fate regulation. By contrast, infected neutrophils were enriched in biological processes associated with **regulation of nucleotide-binding oligomerization domain-containing signaling, death receptor-mediated extrinsic apoptotic signaling, canonical NF-kappa B signal transduction,** and **regulation of apoptotic signaling**, as well as molecular functions related to **cytokine receptor binding** and **antioxidant activity** (Fig. 4F), indicating a more inflammatory and effector-oriented state.

KEGG enrichment analysis was consistent with these observations in GO analysis. In bystander neutrophils, the enriched pathways included **IL-17 signaling, NF-kappa B signaling, necroptosis, p53 signaling,** and **cellular senescence** (Fig. 4G), suggesting a state characterized by inflammatory priming, stress responsiveness, and cell fate remodeling. In infected neutrophils, the dominant pathways were **NF-kappa B signaling, NOD-like receptor signaling, apoptosis, TNF signaling, necroptosis, IL-17 signaling,** and **toxoplasmosis** (Fig. 4H), consistent with a strongly activated inflammatory effector program accompanied by pathogen-sensing activation and cell death/survival remodeling.

Cellular communication analysis identifies a monocyte-neutrophil signaling axis in feline peripheral blood immune cellsexposed *Toxoplasma gondii infection*

To further investigate the major communication nodes in feline peripheral blood immune cells following *T. gondii* exposure and to determine how these immune populations respond in a coordinated manner, we performed ligand-receptor-based cell-cell communication analysis on the major immune cell populations identified in our dataset. The overall interaction network revealed extensive intercellular communication among peripheral blood immune cell populations, with monocytes and neutrophils emerging as major communication hubs within the *T. gondii*-exposed immune network (Fig. 5A). In particular, monocytes displayed strong outgoing and incoming interactions with multiple cell populations, supporting their central role in organizing the myeloid immune response.

We next focused on signaling pathways most relevant to the transcriptional reprogramming observed in monocytes and neutrophils. Analysis of the **IL-1 signaling network** showed that IL-1-associated communication was largely concentrated in **myeloid cells**, with the strongest interaction observed along the **monocyte-to-monocyte** axis and an additional prominent signal from **neutrophils to monocyte** (Fig. 5B). These findings suggest that monocytes may serve as a central integrative node for IL-1 mediated inflammatory signaling, while neutrophils may provide feedback inflammatory input to monocytes. This result is consistent with the marked upregulation of inflammatory genes such as **IL-1β** observed in infected neutrophils. To further define the functional roles of different cell populations in specific signaling pathways, we performed signaling role analysis for the IL-1 and CSF2 pathways. In the IL-1 signaling pathway, monocytes showed the highest importance as both signal receivers and overall central nodes, whereas neutrophils contributed as an additional signaling component within the myeloid inflammatory network (Fig. 5C). In contrast, the CSF2 signaling pathway showed a distinct pattern, in which monocytes exhibited prominent sender-like characteristics, whereas neutrophils showed stronger receiver-associated roles (Fig. 5C). These results support the notion that different signaling pathways underlie distinct aspects of myeloid coordination in exposed immune cells.

Consistent with this observation, analysis of the CSF2 signaling network demonstrated that CSF2-related communication was primarily centered on interactions between monocytes and neutrophils, with monocytes acting as the major signaling source for neutrophils (Fig. 5D). Given that CSF2 was among the upregulated genes identified in exposed/infected monocytes, this pathway may constitute a key mechanism by which monocytes coordinate and amplify neutrophil-associated immune responses after *T. gondii* exposure.

Discussion

As the definitive host of *T. gondii*, the cat occupies a central position in parasite transmission and environmental dissemination [25]. However, compared with intermediate hosts and classical murine models, the immune response of the feline host to *T. gondii* remains insufficiently characterized, particularly at the level of individual immune cell populations. In the present study, we used single-cell RNA sequencing to generate an initial transcriptional map of feline peripheral blood immune cells after *T. gondii* exposure. Notably, neutrophils were identified within the feline peripheral blood immune cells fraction, which is consistent with previous reports [18]. Our results showed that although the overall peripheral immune landscape remained dominated by lymphocytes, the most prominent early transcriptional remodeling occurred in myeloid populations, especially monocytes and neutrophils. These findings suggest that the early peripheral response of cats to *T. gondii* exposure is primarily characterized by innate immune remodeling driven by myeloid cells.

ScRNA-seq is a powerful technique for providing a transcriptional atlas of immune cells and for analyzing the heterogeneity of cell populations [26, 27]. With its high resolution, scRNA-seq has been widely applied in the research of various parasitic diseases [28–30]. Studies have shown that individual human monocytes from the same donor exhibit significant transcriptional heterogeneity in their

responses to parasites, and the exposure and non-exposure of *T. gondii* can be distinguished by cell subpopulations, including both infected cells and potential bystander cells [15]. One of the main advantages of this study lies in the use of single-cell resolution to distinguish between infected cells and bystander cells within the exposed feline peripheral blood immune cells compartment. Host-pathogen interactions occur at the single-cell level, and under the same exposure condition some cells contain detectable parasite transcripts whereas others do not, despite being exposed to the same inflammatory environment. Averaging all exposed cells would therefore obscure important cellular heterogeneity. In our dataset, both monocytes and neutrophils showed clear transcriptional divergence between infected and bystander states, highlighting the importance of resolving host-parasite interactions at single-cell resolution in the feline definitive host context.

Monocytes are important myeloid defensive cells during *T. gondii* infection and can contribute to host control of the parasite through phagocytosis, production of inflammatory mediators and chemokines, and antiparasitic effector mechanisms [31, 32]. In our dataset, monocytes exhibited a relatively coherent cytokine-coordinating response framework. Although infected and bystander monocytes differed at the level of GO biological processes, both states shared enrichment of KEGG pathways related to cytokine-cytokine receptor interaction, TNF, IL-17, JAK-STAT, and Toll-like receptor signaling (Fig. 3E), indicating that *T. gondii* exposure induces a common inflammatory and cytokine-centered signaling backbone in feline monocytes. Notably, in contrast to the classical host defence model of toxoplasmosis, in which type 1 immunity is largely driven by the **IL-12** [13], feline monocytes in our study did not display a predominantly **IL-12** or **IFN-γ-associated** transcriptional profile. Instead, they preferentially upregulated **IL-1β, IL-6, CSF2, CSF3, CCL20, CCL5, CXCL8, IL-10, IL-27,** and **EBI3 (**Fig. **3B, C)**. Previous studies have shown that *T. gondii* infection can rapidly induce an **IL-1β-associated inflammatory response** in primary human monocytes [33], suggesting that monocyte responses to the parasite are not always restricted to a simple **IL-12** pattern, but may instead reflect a more complex state of inflammatory coordination and immunoregulation. Further comparison of infected and bystander monocytes showed that, although the two populations shared a common exposure-associated transcriptional backbone, they diverged in their functional emphases (Fig. **3D**). Infected monocytes were more strongly associated with processes such as **mononuclear cell proliferation, lymphocyte proliferation,** and **regulation of T cell activation**, suggesting a greater involvement in immune coordination and lymphocyte-related regulation. In contrast, bystander monocytes were more strongly associated with **response to interleukin-1, leukocyte chemotaxis,** and **regulation of leukocyte proliferation**, indicating a greater role in inflammatory sensing and leukocyte recruitment. These findings suggest that even in the absence of detectable parasite transcripts, monocytes can still undergo functional remodeling under the paracrine or microenvironmental signals.

From a general innate immunology perspective, neutrophils do not usually respond to pathogens by entering a single uniform “activated” state. Rather, they initiate a set of interconnected response programs, including activation of pathogen-recognition receptors, upregulation of inflammatory cytokines and chemokines, degranulation and tissue remodeling, ROS/oxidative stress regulation, and cell fate remodeling [34, 35]. At the same time, accumulating evidence indicates that neutrophil

transcriptional responses to pathogens are markedly heterogeneous [34]. These responses may manifest either as inflammatory effector states driven by direct pathogen-associated stimulation or as stress-adaptive and remodeling-associated states shaped by the local inflammatory microenvironment [36, 37]. In our dataset, neutrophils displayed a clearer state dichotomy than monocytes. Infected neutrophils were characterized by upregulation of **IL-1β, CXCL8, TNFAIP3, TRAF1, BIRC3, MMP9** and **GADD45B**, together with enrichment of **NF-kappa B, TNF, NOD-like receptor signaling, apoptosis** and **necroptosis**, consistent with a classical inflammatory effector state (Fig. 4D, F, H). In contrast, bystander neutrophils preferentially enriched pathways related to **biotic stimulus response, oxidative stress adaptation** and **apoptotic regulation**, suggesting a stress-responsive and remodeling-associated state rather than a quiescent phenotype (Fig. 4C, E, G). These findings are in line with previous studies showing that neutrophils are rapidly recruited during early *T. gondii* infection, contribute to initial host protection, and can be directly activated by parasite-derived stimuli [38]. Moreover, *T. gondii* has been reported to delay spontaneous neutrophil apoptosis [39], and in cats specifically, tachyzoites can induce neutrophil extracellular trap release, supporting a direct early innate response of feline neutrophils to the parasite [40].

Cell-cell communication analysis further suggested that monocytes and neutrophils form a central myeloid signaling axis in feline peripheral blood immune cells following *T. gondii* exposure, with IL-1 and CSF2 representing the two most prominent communication pathways (Fig. 5). This finding is biologically consistent with previous studies in other hosts. In primary human peripheral blood monocytes, *T. gondii* infection has been shown to rapidly induce IL-1β production, accompanied by transcriptional upregulation of IL-1**β**, IL1RN, and NLRP3, and this release is dependent on inflammasome-associated mechanisms, indicating that IL-1 signaling is an important component of the myeloid inflammatory response to *T. gondii* [33]. At the same time, murine studies have shown that IL-1R-related pathways contribute to inflammatory pathology and disease tolerance during toxoplasmosis, suggesting that the IL-1 axis is not merely an inflammatory marker but may also influence infection outcome [41]. In the context of our data, the upregulation of IL-1β in infected neutrophils together with the increased Neutrophil to Monocyte IL-1 signaling suggests that, in feline peripheral blood, neutrophils may amplify monocyte-associated inflammatory networks through IL1-mediated feedback, thereby contributing to the shaping of the early innate immune response.

Similar to the IL-1 axis, **CSF2 signaling** is also supported by previous studies in other host systems during *T. gondii* infection. Classical studies have shown that parasite-infected host cells can induce secretion of **GM-CSF/CSF2** and **G-CSF**, thereby significantly delaying spontaneous neutrophil apoptosis [42]. In addition, GM-CSF has been shown to modulate the anti-*Toxoplasma* functional state of monocytes, suggesting that **CSF2** is not simply a pro-inflammatory factor, but may also participate in myeloid cell survival, activation, and functional remodeling [43]. In our dataset, monocytes showed clear upregulation of **CSF2**, and cell-cell communication analysis further demonstrated enhanced **Monocyte to Neutrophil** CSF2 signaling, which is consistent with previous cross-species observations that GM-CSF contributes to neutrophil survival and regulation of myeloid responses. Together with the increased expression of **IL-6, CSF2, CSF3, CCL5, CCL20, IL-10, IL-27,** and **EBI3** in monocytes, these findings

suggest that monocytes in the exposed feline peripheral blood immune cells compartment are not merely inflammatory cells, but rather function as signaling coordinators embedded within a broader immune interaction network. Monocytes are a key component of innate immunity, broadly involved in inflammatory responses and capable of shaping subsequent immune responses through cytokine and chemokine networks, including effects on T-cell activation [44]. More importantly, monocytes do not act in isolation; instead, they are embedded within a dynamic network shaped by immune cell interactions, local inflammatory conditions, and tissue niche-derived signals, all of which continuously influence their functional state [45].

This myeloid communication pattern is broadly consistent with observations in other feline inflammatory diseases. Feline infectious peritonitis (FIP/FCoV), another macrophage/monocyte-centered inflammatory disease of cats, has been associated with increased IL-1, IL-6, and GM-CSF-related inflammatory responses [46, 47]. Although *T. gondii* and FIP/FCoV differ fundamentally in pathogen type and tissue tropism, these parallel observations support the biological plausibility of the IL-1 and CSF2-centered myeloid signaling network identified here.

Although we revealed the differential immune response of feline peripheral blood immune cells to *T. gondii* infection, this study has several limitations. First, it was based on an **in vitro tachyzoite exposure model using feline** peripheral blood immune cells, with a limited number of samples and time points; therefore, it can't interpret the **dynamic transcriptional responses** of cat peripheral blood immune cells to *T. gondii* exposure. Second, this study focused on **peripheral blood immune cells**, whereas, as the definitive host, the cat is likely to exhibit key host-parasite interactions not only in blood but also within the **intestinal mucosa and local tissue microenvironment**.

Conclusions

In summary, this study provides a single-cell transcriptional framework for understanding the early immune response of feline peripheral blood immune cells to *T. gondii* exposure. Although the overall immune landscape remained dominated by lymphocytes, the most prominent transcriptional remodeling occurred in myeloid populations, particularly monocytes and neutrophils. At single-cell resolution, both monocytes and neutrophils displayed clear divergence between parasite-positive and bystander states, indicating that direct parasite association and indirect exposure to the inflammatory microenvironment can drive distinct response programs. Monocytes exhibited a shared cytokine-centered inflammatory backbone with state-specific functional biases, whereas neutrophils showed a more pronounced dichotomy between stress/remodeling-associated bystander states and inflammatory effector-like infected states. Cell-cell communication analysis further identified a central monocyte-neutrophil signaling axis, with IL-1 and CSF2 related pathways emerging as prominent components of the early myeloid interaction network. Collectively, these findings expand current understanding of host-*T. gondii* interactions in the feline definitive host and provide a valuable resource for future studies aimed at dissecting the molecular basis of feline anti-*Toxoplasma* immunity and parasite transmission biology.

Declarations

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Authors' contribution

JM, JJH, FCZ and XQZ conceived and designed the study, and critically revised the manuscript. YDW, YCZ, QYL, THG, ZWL and JLZ performed the experiments, YDW analyzed the data and drafted the manuscript. FCZ, JJH and XQZ secured the funds. All the authors read and approved the final manuscript.

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Consent for publication

Not applicable

Data availability

The data supporting the findings of this article are included within the article and its additional files. The raw single-cell RNA-seq data have been deposited at China National Center for Bioinformation with the access number: CRA042857.

Ethics approval and consent to participate

This study was approved by the Ethical Committee of the College of Veterinary Medicine, Yunnan Agricultural University (Permit number: 202504014).

Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

Supplementary Information

The online version contains supplementary material available at XXX

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Figures

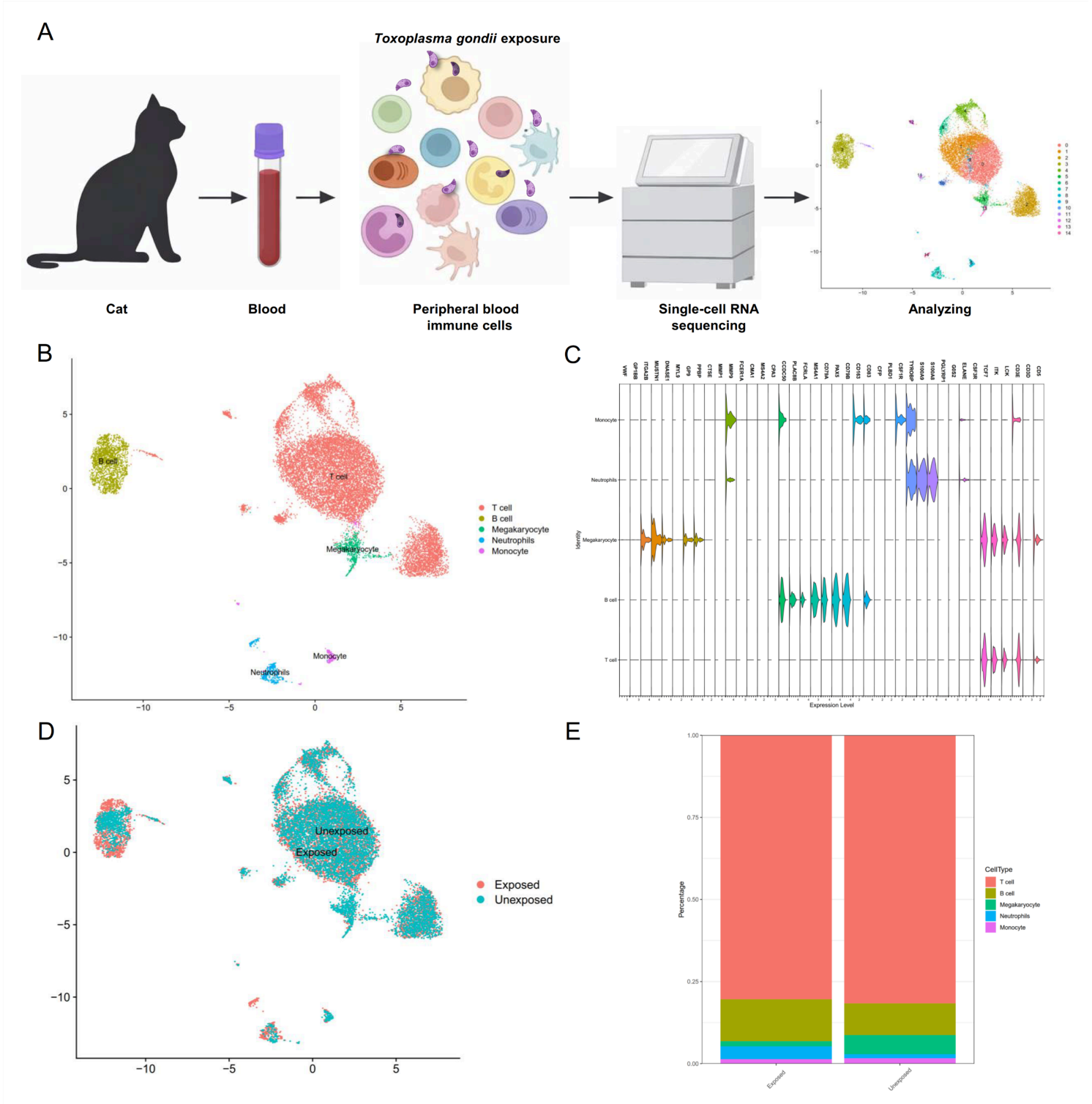


Figure 1

Single-cell transcriptomic profiling of feline peripheral blood immune cells.

(**A**) Experimental design and analysis workflow. (**B**) UMAP plot showing the distribution of all high-quality cells and annotation of the major immune cell populations including T cells, B cells, monocytes, neutrophils and megakaryocytes. (**C**) Violin plots of representative marker genes used to define T cells, B cells, megakaryocytes, neutrophils, and monocytes. (**D**) UMAP plot showing the distribution of exposed

and unexposed cells. (**E**) Relative abundance of the five major immune cell populations in the exposed and unexposed groups.

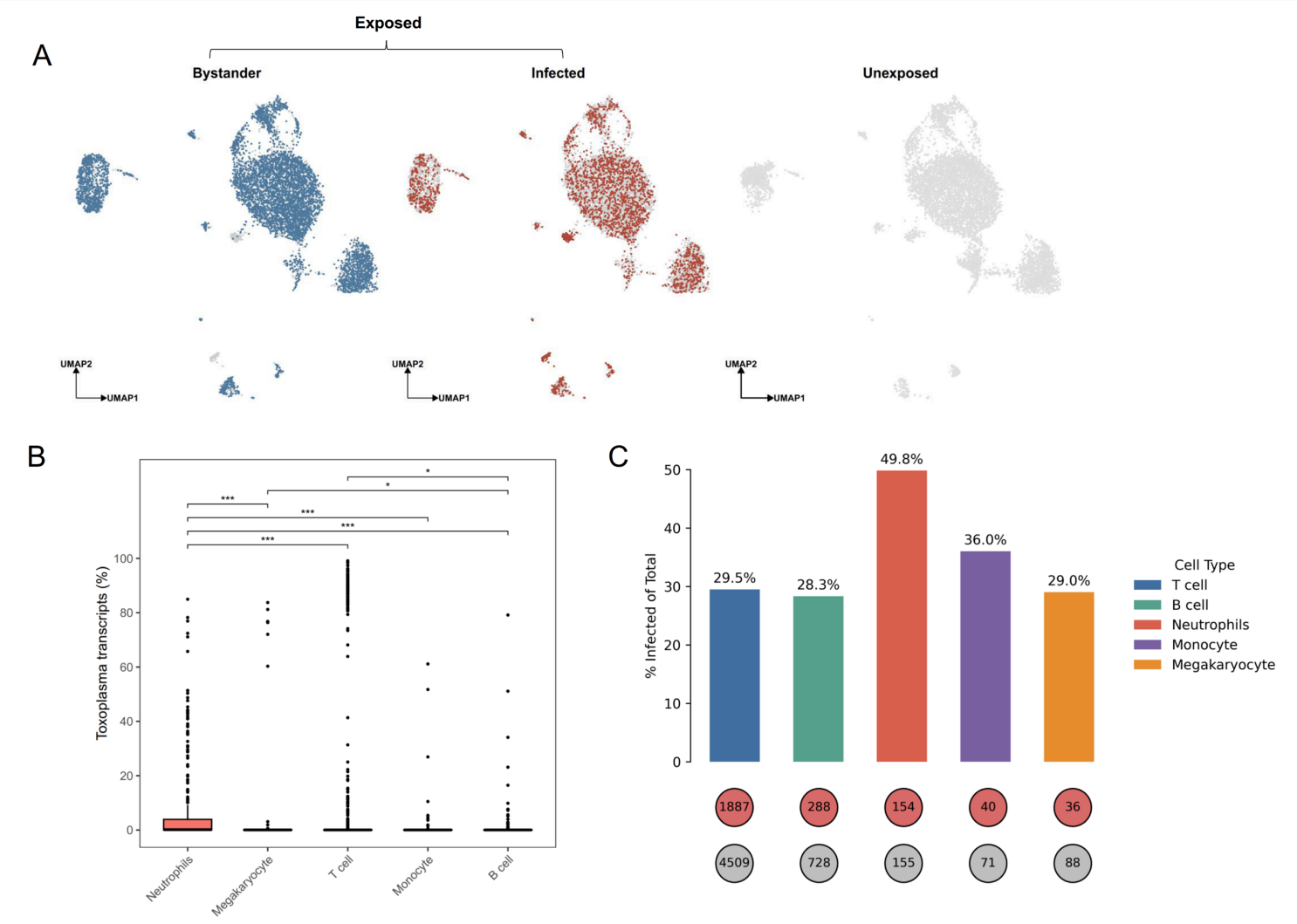


Figure 2

Distribution of *Toxoplasma gondii* infection across feline peripheral blood immune cells. (**A**) UMAP visualization of bystander, infected and unexposed cells. (**B**) Distribution of parasite transcript abundance across feline peripheral blood immune cell populations. (**C**) Infection rate across different feline peripheral blood immune cell types.

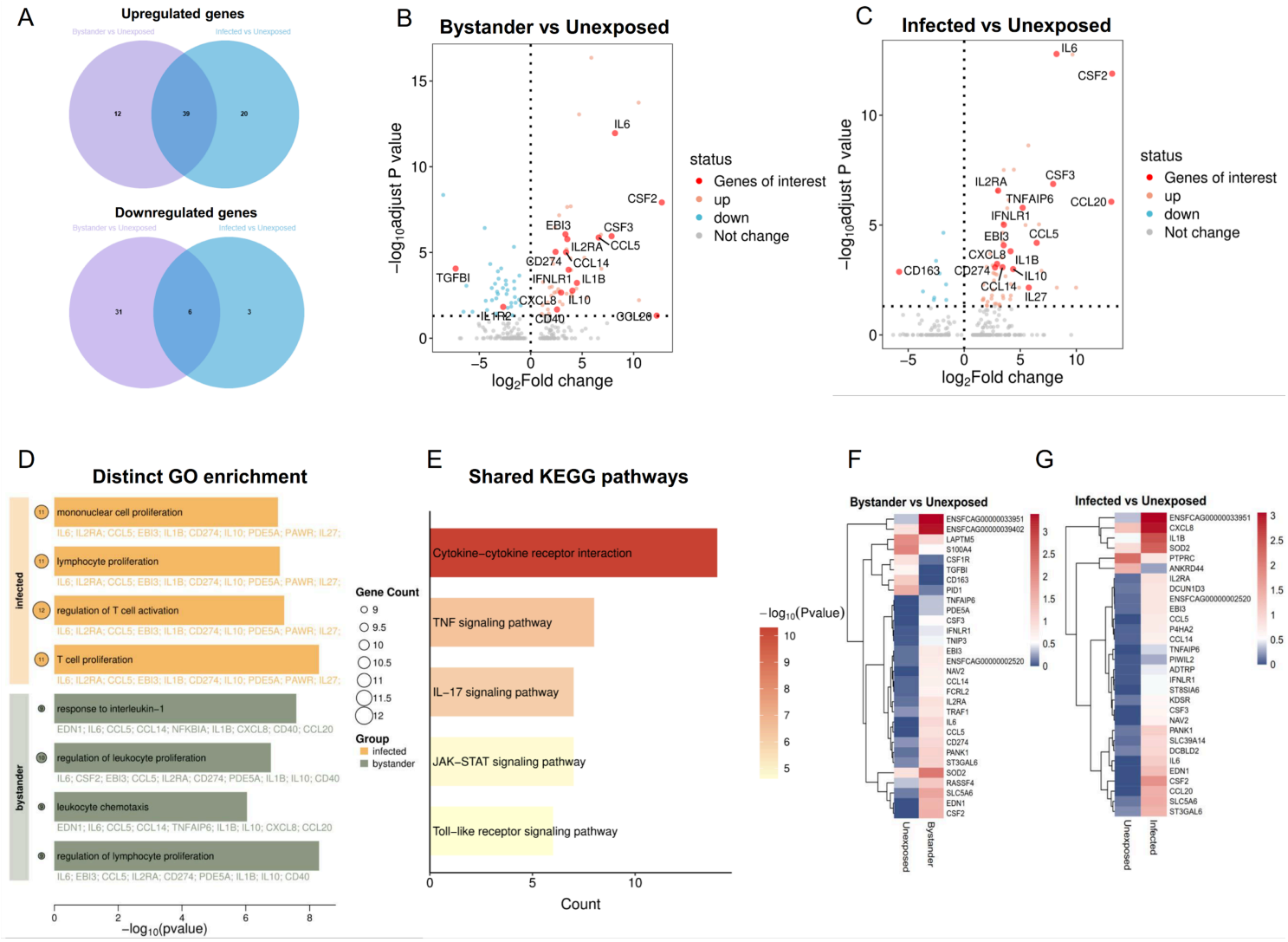


Figure 3

Infected and bystander monocytes share a common inflammatory signaling backbone but display distinct functional biases after *T. gondii* exposure. (**A**) Venn diagrams showing the overlap of upregulated genes and downregulated genes in bystander monocytes versus unexposed monocytes and infected monocytes versus unexposed monocytes. (**B**) Volcano plot showing differentially expressed genes in bystander monocytes versus unexposed monocytes. (**C**) Volcano plot showing differentially expressed genes in infected monocytes versus unexposed monocytes. (**D**) GO biological process enrichment analysis showing distinct functional biases between bystander and infected monocytes. (**E**) Shared KEGG pathways enriched in both bystander and infected monocytes, indicating a common inflammatory signaling framework. (**F**) Heatmap showing representative upregulated genes in bystander monocytes versus unexposed monocytes. (**G**) Heatmap showing representative upregulated genes in infected monocytes versus unexposed monocytes.

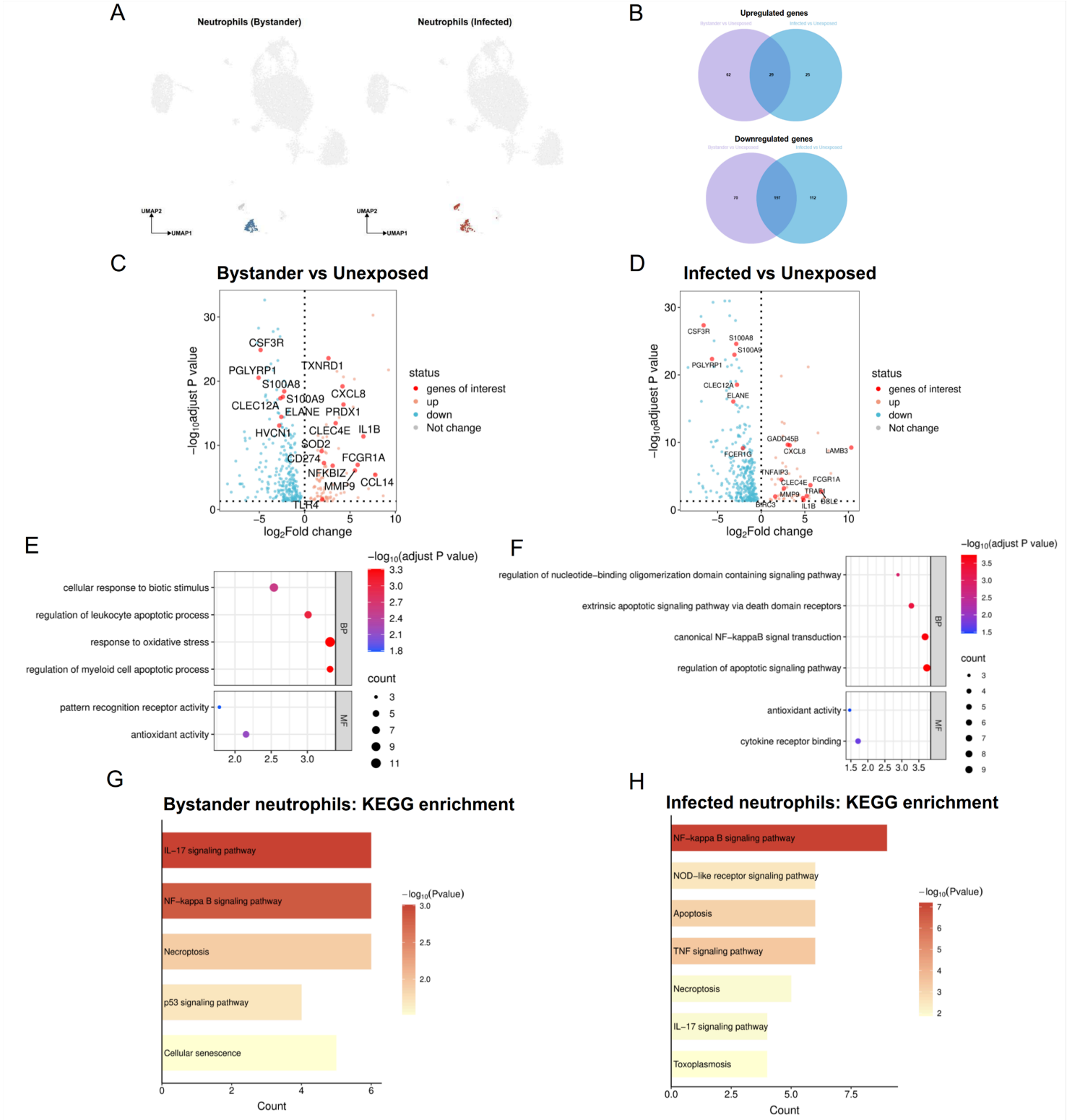


Figure 4

Bystander and infected neutrophils exhibit distinct transcriptional programs following *T. gondii* exposure. (**A**) UMAP visualization of neutrophils showing the distribution of bystander and infected cells within the neutrophil population. Highlighted cells indicate bystander neutrophils (left) and infected neutrophils (right), respectively. (**B**) Venn diagrams showing the overlap of upregulated and downregulated differentially expressed genes (DEGs) in bystander neutrophils and infected neutrophils

relative to unexposed neutrophils. (**C**) Volcano plots of DEGs in bystander neutrophils versus unexposed neutrophils. (**D**) Volcano plots of DEGs in infected neutrophils versus unexposed neutrophils. (**E**) GO enrichment analysis of DEGs in bystander neutrophils. The size of each dot represents gene count. (**F**) GO enrichment analysis of DEGs in infected neutrophils. The size of each dot represents gene count. (**G**) KEGG pathway enrichment analysis of DEGs in bystander neutrophils. Bar length indicates gene count. (**H**) KEGG pathway enrichment analysis of DEGs in infected neutrophils. Bar length indicates gene count.

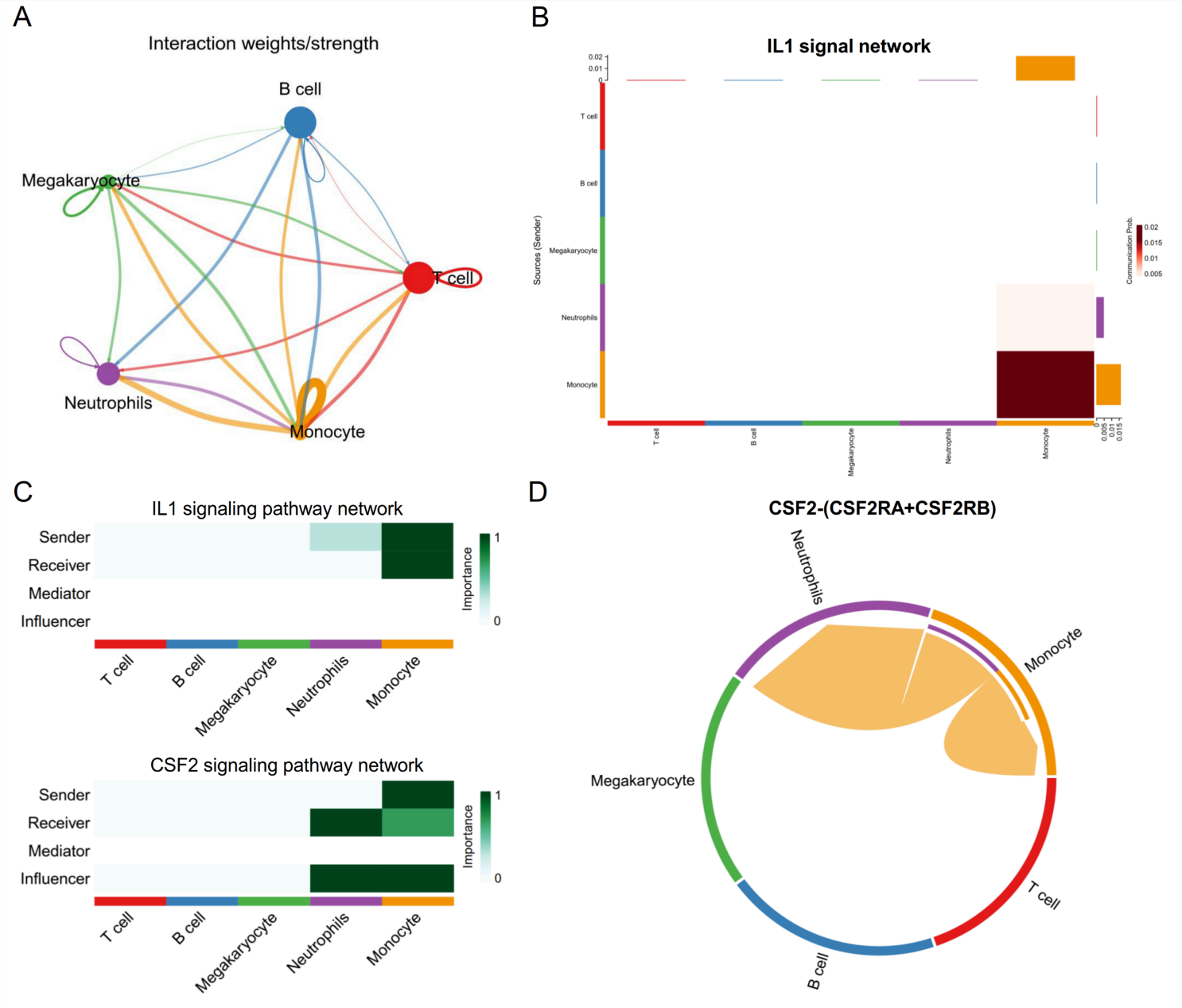


Figure 5

Cell-cell communication analysis identifies a central monocyte-neutrophil signaling axis in feline peripheral blood immune cells following *T. gondii* exposure. (**A**) Overall cell-cell communication network based on interaction strength among major immune cell populations in *T. gondii*-exposed feline peripheral blood immune cells. Circle size represents the relative contribution of each cell population to

the communication network, and edge width indicates interaction strength. (**B**) IL-1 signaling network showing communication probability among major immune cell populations. The strongest IL-1 associated interactions were centered on Monocyte to Monocyte, with additional Neutrophils to Monocyte signaling. (**C**) Signaling role heatmaps for the IL-1 and CSF2 pathways. Darker color indicates higher importance of a given cell population as sender, receiver, mediator, or influencer in the corresponding signaling network. (**D**) CSF2 signaling network showing that CSF2-associated communication was primarily mediated between monocytes and neutrophils, with monocytes acting as the major signaling source.

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