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Research Article

Keywords: CD74, RNA-seq, Antigen presentation, Transcriptomic profiling, Differential Expression Analysis, Functional analysis, Immune signaling, B-cell lymphoma

Posted Date: August 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10325671/v1

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Additional Declarations: No competing interests reported.

Transcriptomic Consequences of CD74 Knockout in Raji and Meljuso Tumor Cells.

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Abstract

The invariant chain (CD74) plays a crucial role in MHC-II antigen presentation and intracellular trafficking, as well as immunological signaling and cell survival. Although its canonical function is well established, the global transcriptional consequences of CD74 loss remain unclear. Here, we used RNA sequencing to characterize the impact of CD74 knockout in Raji and Meljuso cells and compared with their respective wild-type counterparts. CD74 depletion induced extensive transcriptional reprogramming in both models, as revealed by principal component and differential expression analyses. Functional enrichment and gene set enrichment analyses showed that loss of CD74 affects antigen processing and presentation pathways. In parallel, immune signaling and intracellular trafficking networks were significantly altered. Notably, the transcriptional response was highly context dependent. In Raji cells, CD74 knockout led to suppression of antigen presentation pathways and activation of stress-response and protein-folding programs. In contrast, Meljuso cells exhibited robust induction of interferon signaling and antiviral response pathways. Comparative analysis identified both shared and divergent gene expression programs, including genes displaying inverse expression patterns between the two cell lines. Pathway enrichment analysis further revealed remodeling of key signaling networks, including B cell receptor signaling and immune regulatory pathways. Together, these results indicates that CD74 works as a context-dependent regulator of transcriptional and signaling networks. Its loss triggers distinct compensatory responses depending on cellular background, with potential implications for immune regulation and tumor biology. This study expands the understanding of CD74 beyond its

original role in antigen presentation and highlights its broader impact on cellular homeostasis.

Keywords

CD74, RNA-seq, Antigen presentation, Transcriptomic profiling, Differential Expression Analysis, Functional analysis, Immune signaling, B-cell lymphoma

Background

CD74, the invariant chain, is a non-polymorphic type II transmembrane protein that plays a crucial role in the biology of major histocompatibility complex class II (MHC-II) molecules [1,2]. Within endoplasmic reticulum, CD74 associates with MHC-II α and β chains that are newly synthesized, stabilizing the complex and preventing premature peptide binding [2]. MHC-II molecules are further directed by CD74 to lysosomal and endosomal compartments, where antigenic peptides are processed and loaded prior to surface presentation. Through these functions, CD74 is essential for antigen presentation and the activation of adaptive immune responses [3,4].

Apart from its traditional function as an MHC-II chaperone, CD74 has evolved into a multifunctional protein involved in intracellular signaling and cellular homeostasis [1]. CD74 functions as a receptor for macrophage migration inhibitory factor (MIF), triggering downstream signaling pathways involved in cell survival, proliferation, and inflammation [5]. Additionally, CD74 has been implicated in endosomal trafficking, proteostasis, and regulation of immune signaling pathways. These observations suggest that CD74 may influence broader cellular programs extending beyond antigen presentation alone [6].

CD74 is highly expressed in B cell–derived malignancies and has been associated with tumor progression and immune modulation [7]. Its established role in antigen presentation positions CD74 as a potential regulator of tumor immunogenicity, whereas its signaling functions may contribute to cell survival and resistance to stress. Despite these insights, the global transcriptional consequences of CD74 loss still not clearly understood, in particular when it comes to malignant B cells [8].

Prior studies has mostly focused on the molecular mechanisms of MHC-II trafficking and peptide loading, with limited attention given to the genome-wide regulatory effects of CD74 loss [1,2]. It remains unclear whether CD74 depletion leads to uniform changes across different cellular contexts or whether these effects are shaped by cell-specific regulatory networks.

To address this question, we used bulk RNA sequencing approach following genetic knockout of CD74 in two Raji and Meljuso and compared the resulting transcriptomic profiles with their respective wild-type controls. Our objectives were to (i) characterize the global transcriptional alterations induced by CD74 loss, (ii) identify disrupted biological pathways and signaling networks, and (iii) determine whether CD74-dependent responses are conserved or context dependent across distinct cellular backgrounds.

Our comparative transcriptomic analysis reveals that CD74 loss induces extensive transcriptional reprogramming, impacting antigen presentation, immune signaling, and intracellular homeostasis. Notably, the response to CD74 loss differed between Raji and Meljuso cells, revealing both shared and cell-specific regulatory programs. This study sheds new lights into the multifaceted functions of CD74 and its potential implications in immune regulation.

Materials and Methods

Cell Lines and Culture Conditions

The Raji and Meljuso human cell lines were used in this study. The cells were kept in a humidified incubator with 5% CO2 at 37°C under standard conditions. 10% fetal bovine serum (FBS) and a suitable antibiotic were added to the RPMI-1640 medium used to culture Raji and Meljuso cells.

RNA Extraction and Quality Control

As instructed by the manufacturer, a standard RNA isolation kit was used to bulk RNA from both wild-type (WT) and CD74 KO cells. Spectrophotometry was used to measure RNA concentration and purity, and an automated electrophoresis system was used to evaluate RNA integrity. Sequencing only applied to samples with excellent RNA integrity (RIN > 7).

Library Preparation and RNA Sequencing

RNA sequencing libraries were constructed using a poly(A) enrichment protocol to capture messenger RNA. For paired end reads, libraries were built according to the manufacturer's protocol and sequenced with Illumina platform. Sequencing depth and read length were sufficient to ensure comprehensive transcriptome coverage.

Preprocessing and Alignment

Five independent biological replicates were prepared for each WT and CD74 KO condition in both Raji and Meljuso cell lines. Following quality control and principal component analysis (PCA), one Meljuso sample was identified as a transcriptional outlier

and excluded from downstream analyses. FastQC v0.11.9. is used to evaluate the quality of raw sequencing reads, adapter content, and sequence duplication levels [9]. To trim and remove adapter and low-quality bases, trimmomatic v0.39 tool employed [10]. STAR v2.7 used to aligned cleaned reads with the human reference genome (e.g., GRCh38) [11]. Quantification was done using featureCounts based on annotated gene models [12].

Differential Gene Expression Analysis

Differential expression analysis between CD74 KO and WT samples was performed using DESeq2 [13]. Count data were normalized, and dispersion estimates were calculated using the default DESeq2 pipeline. Filtered significantly expressed genes with the adjusted *p*-value < 0.05 and $\log_2$ fold change ≥ 1. Principal component analysis (PCA) method was performed to cluster samples based on variation.

Functional Enrichment Analysis

Functional annotation of DEGs was done using Gene Ontology (GO), KEGG pathway, and Reactome pathway analysis. Over-representation analysis was performed separately for upregulated and downregulated gene sets to identify significantly enriched molecular functions, biological processes, and pathways. Adjusted *p*-values of < 0.05 used to determine statistical significance.

Gene Set Enrichment Analysis (GSEA)

Gene set enrichment analysis was done using GSEA framework [14]. Pre-ranked gene lists based on differential expression statistics were used as input. Enrichment was assessed using the Molecular Signatures Database (MSigDB) for curated gene sets, including hallmark and canonical pathway collections (C2:CP) [15]. Normalized enrichment scores

(NES) and False discovery rate (FDR) values were calculated to determine the significance.

Canonical Pathway Analysis

Canonical pathway enrichment analysis was performed using MSigDB C2:CP gene sets to identify signaling pathways affected by CD74 knockout. Enrichment results were used to show pathways related to immune signaling, stress response, and cellular regulation [16].

Comparative Analysis Between Cell Lines

To identify shared and distinct transcriptional responses, differentially expressed genes from Raji and Meljuso datasets were compared. Overlapping genes were classified into commonly upregulated, commonly downregulated, and oppositely regulated categories. This analysis enabled identification of conserved and context-specific transcriptional programs.

Data Visualization

Data visualization, including PCA plots, volcano plots, enrichment plots, and comparative analyses, was performed using ggplots, EnhancedVolcano, pheatmap, clusterProfiler, and enrichplot packages in R [17]. Figures were generated to illustrate global transcriptomic changes, differential gene expression, and pathway enrichment.

Statistical Analysis

R studio (version 4.3) was used for all statistical analyses. The DESeq2 package, with negative binomial models to calculate a dispersion and differential expression, was used

to perform differential gene expression analysis. The Benjamini–Hochberg false discovery rate (FDR) correction method was used for adjusted P-values for multiple hypothesis testing. Filtered significantly expressed genes with the adjusted p-value < 0.05 and $\log_2$ fold change ≥ 1. For functional enrichment and gene set enrichment analyses, pathways with adj p-values < 0.05 were considered significantly enriched.

Results

Global Transcriptomic Separation Between WT and CD74 Knockout Cells

With Principal component analysis (PCA), observed good separation between wild-type (WT) and CD74 knockout (KO) samples in both Raji and Meljuso cell lines (Figure 1A-B). In Raji cells, the first principal component (PC1) accounted for 76% of the variance, while in Meljuso cells PC1 explained 82% of the variance, indicating that CD74 loss is a major driver of transcriptional variation. Replicates clustered tightly within each condition, supporting high data quality and reproducibility.

Differential Gene Expression Following CD74 Knockout

Volcano plot analysis revealed extensive transcriptional remodeling upon CD74 knockout in both cell lines (Figure 1 C-D). Using an FDR < 0.05 and |log2FC| ≥ 1 threshold, 377 genes were significantly upregulated, and 872 genes were downregulated in Raji cells, indicating a broad impact of CD74 loss on gene expression. Similarly, Meljuso cells exhibited extensive differential expressions, with a notable distribution of significantly 3340 altered genes, in which 791 were upregulated and 2549 were downregulated with large positive and negative fold changes.

These findings indicate that CD74 depletion affects diverse immune trafficking, and stress-associated pathways. Importantly, several of the most significantly downregulated genes are consistent with components of antigen presentation pathways, while upregulated genes may reflect compensatory or stress-related responses.

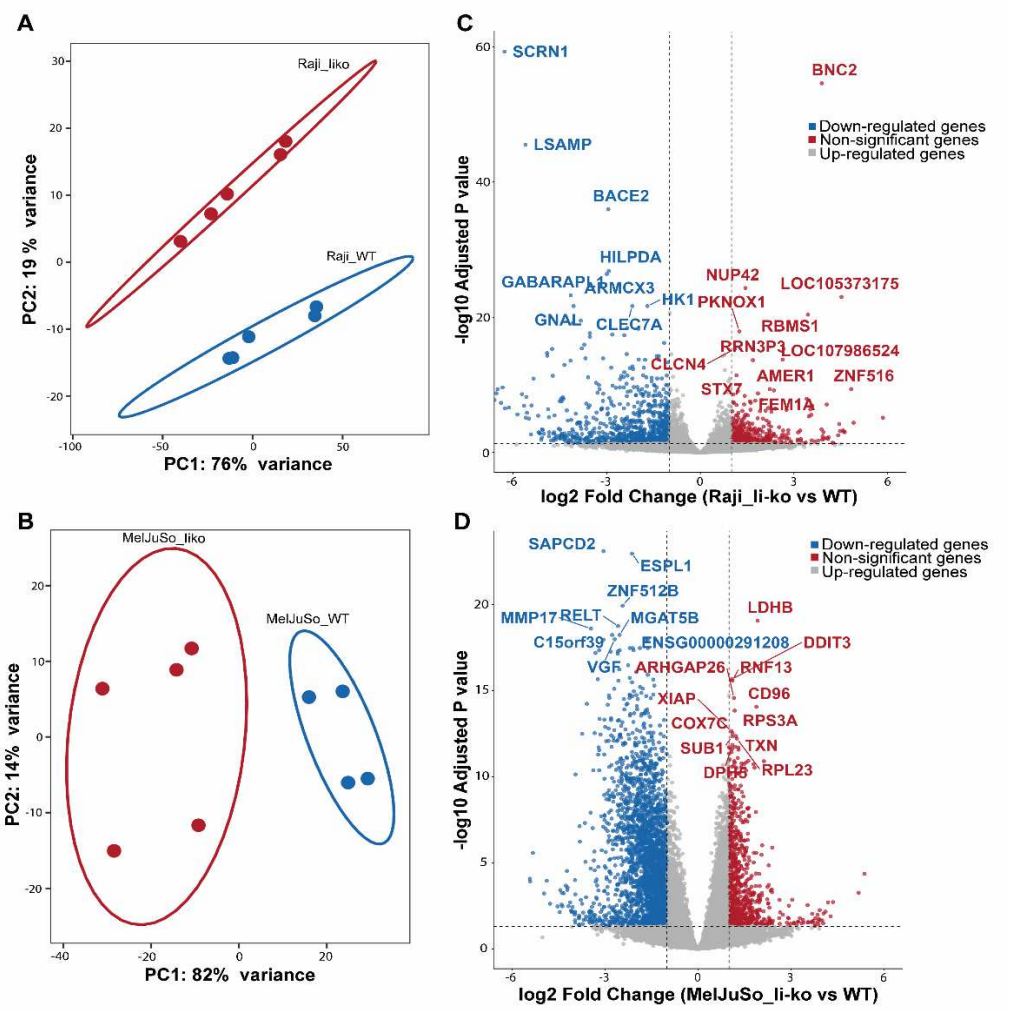


Figure 1. Transcriptomic alteration following CD74 knockout in Raji and Meljuso cell lines. (A–B) Principal component analysis (PCA) of bulk RNA-seq data showing clear separation between wild-type (WT) and CD74 knockout (KO) samples in Raji (A) and Meljuso (B) cells. PC1 explained 76% and 82% of the variance in Raji and Meljuso cells, respectively, indicating substantial transcriptomic differences following CD74 depletion. Each point represents an independent biological replicate. One Meljuso outlier

sample identified during quality control was excluded from downstream analyses. (C–D) Volcano plots depicting differentially expressed genes in Raji (C) and Meljuso (D) cells (KO vs WT). Significantly upregulated genes are shown in red, downregulated genes in blue, and non-significant genes in grey. Differentially expressed genes were defined using thresholds of adjusted $p < 0.05$ and $|\log_2 \text{fold change}| \geq 1$.

Comparative Patterns Across Cell Lines

While both Raji and Meljuso cells exhibited clear transcriptional shifts upon CD74 knockout, differences in the distribution and magnitude of differentially expressed genes suggest cell line specific responses. These differences likely reflect underlying biological variation between the two models, including differences in baseline transcriptional programs and regulatory dependencies.

Functional Enrichment Reveals Disruption of Immune and Stress-Response Pathways in Raji Cells

To further investigate the biological significance of transcriptional changes induced by CD74 knockout, functional enrichment analyses were performed on differentially expressed genes in Raji cells (Figure 2). Gene Ontology (GO) biological processes revealed that downregulated genes were significantly enriched in immune-related pathways, including immune response, antigen processing and presentation, and response to external stimuli (Figure 2A). In contrast, upregulated genes were associated with processes related to cellular stress, protein folding, and metabolic regulation. Consistent with these findings, GO molecular function analysis highlighted enrichment of protein binding and chaperone-related activities among upregulated genes, while

downregulated genes were associated with binding functions relevant to immune signaling (Figure 2B).

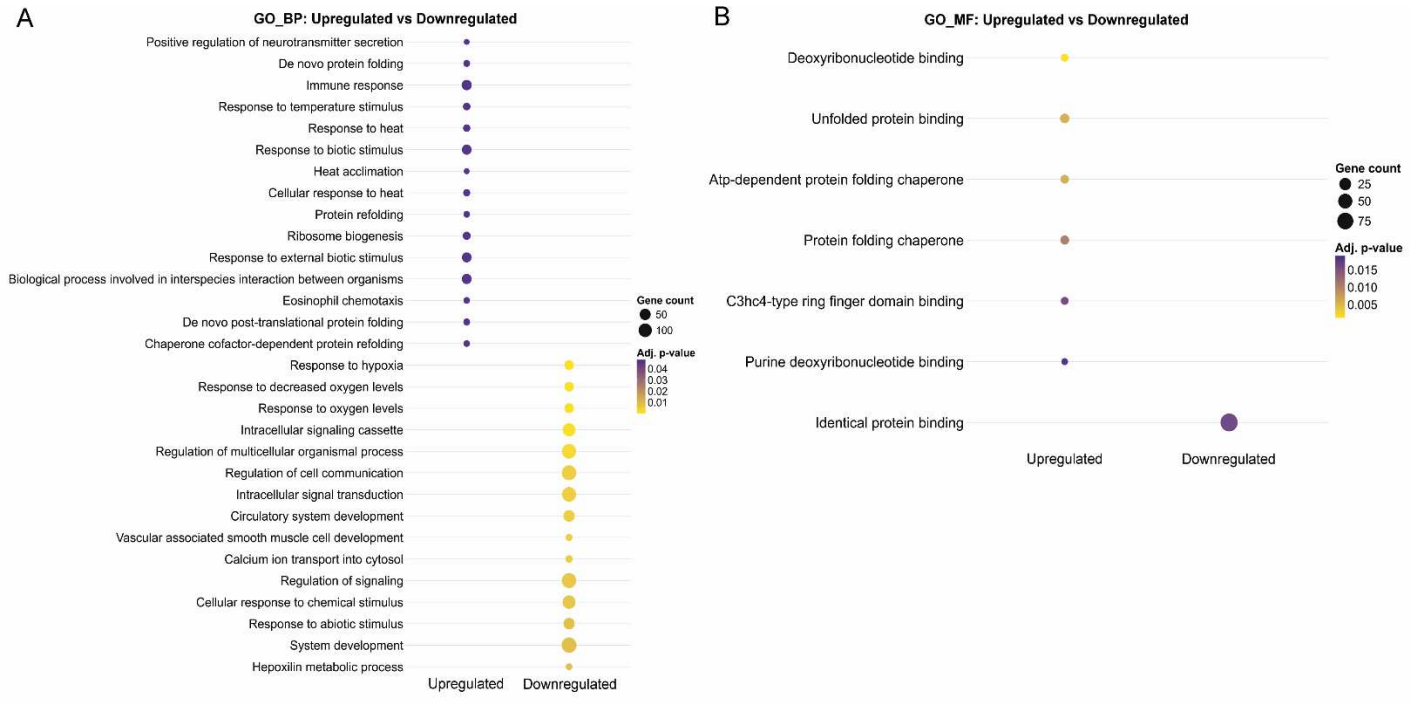


Figure 2. Functional enrichment analysis of differentially expressed genes in Raji CD74 knockout cells. (A) Gene Ontology biological process (GO-BP) enrichment analysis of upregulated and downregulated genes following CD74 knockout. Downregulated genes are primarily associated with immune-related processes, including antigen processing and immune response. (B) GO molecular function (GO-MF) enrichment analysis highlighting enrichment of chaperone-related activities, unfolded protein binding, ATP-dependent protein folding chaperone activity, and protein-binding functions among differentially expressed genes.

KEGG pathway analysis further demonstrated that CD74 knockout impacts key signaling pathways, including antigen processing and presentation, cytokine-cytokine receptor interaction, and multiple signaling cascades such as MAPK and NF-κB pathways (Figure 3A). Notably, several pathways related to cellular stress and apoptosis were enriched among downregulated genes.

Reactome pathway analysis reinforced these observations, revealing significant enrichment of pathways involved in heat shock response, chaperone-mediated protein folding, and stress-induced signaling (Figure 3B). Additionally, pathways related to innate immune responses and host defense mechanisms were altered, indicating broad immunological consequences of CD74 depletion. These results suggest that loss of CD74 not only disrupts antigen presentation machinery but also induces compensatory activation of stress-response pathways and remodeling of immune signaling networks.

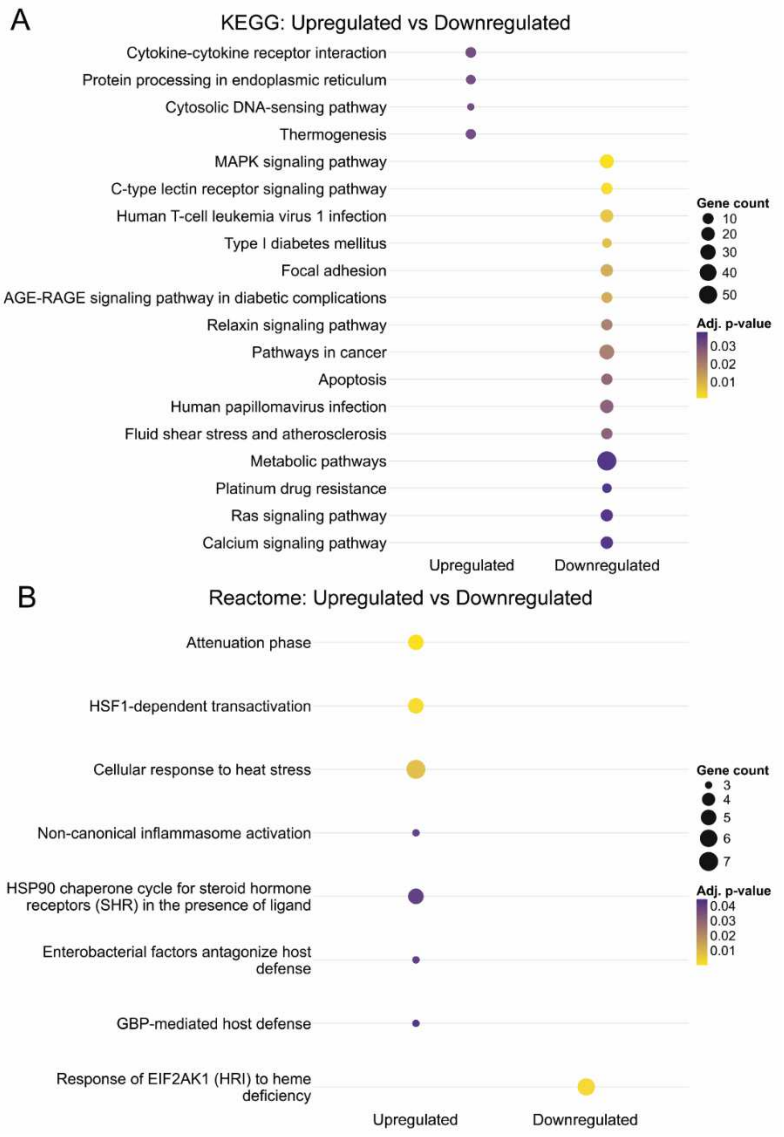


Figure 3. Pathway enrichment analysis of differentially expressed genes in Raji CD74 knockout cells. (A) KEGG pathway enrichment analysis showing significant enrichment of pathways associated with cytokine–cytokine receptor interaction, MAPK signaling, apoptosis, calcium signaling, protein processing in the endoplasmic reticulum, and immune-related signaling pathways. (B) Reactome pathway enrichment analysis demonstrating enrichment of stress-response and proteostasis-associated pathways, including heat shock response, HSF1-dependent transactivation, chaperone cycle pathways, inflammasome-related signaling, and host defense mechanisms. Dot size represents gene count, and color indicates adjusted p-value.

Distinct Functional Reprogramming in Meljuso Cells Following CD74 Knockout

To determine whether the transcriptional consequences of CD74 loss are conserved across cell types, we performed functional enrichment analysis on DEGs in Meljuso cells.

Gene Ontology (GO) analysis revealed that upregulated genes in Meljuso KO cells were enriched in pathways associated with immune activation, interferon signaling, and antiviral responses (Figure 4). In contrast, downregulated genes were linked to cellular metabolic processes and regulatory functions, suggesting a shift in cellular priorities following CD74 loss.



Figure 4. Gene Ontology (GO) biological process enrichment analysis of upregulated and downregulated genes following CD74 knockout in Meljuso cells.

KEGG pathways further supported these findings, identifying enrichment of pathways related to immune signaling, including cytokine signaling and infection-related pathways, among upregulated genes (Figure 5A). Downregulated pathways included metabolic and signaling pathways involved in cellular homeostasis.

Reactome pathway analysis highlighted strong enrichment of interferon signaling, antiviral defense mechanisms, and innate immune responses among upregulated genes

(Figure 5B). These results suggest that, unlike Raji cells, Meljuso cells respond to CD74 knockout by activating robust immune and antiviral transcriptional programs.

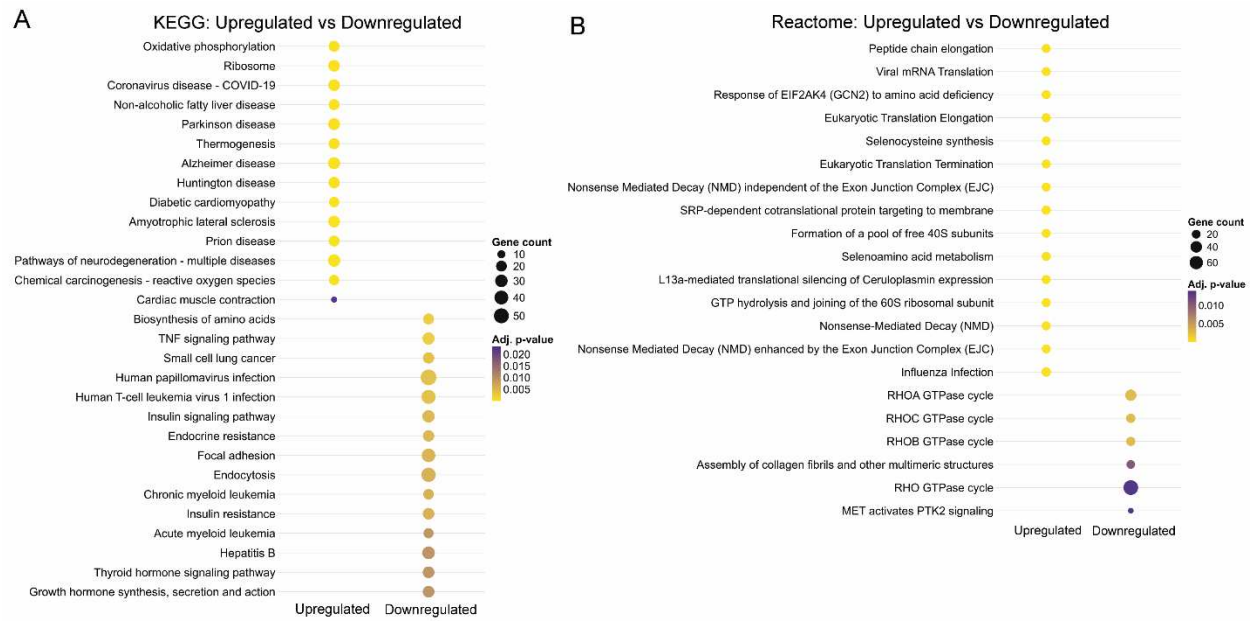


Figure 5. Pathway enrichment analysis of differentially expressed genes in Meljuso CD74 knockout cells. (A) KEGG pathway enrichment analysis showing significant enrichment of pathways associated with viral infection, cytokine signaling, oxidative phosphorylation, thermogenesis, ribosome function, insulin signaling, and metabolic regulation. (B) Reactome pathway enrichment analysis demonstrating enrichment of pathways related to viral RNA processing, translational regulation, nonsense-mediated decay, RHO GTPase signaling, MET signaling, and innate immune–associated pathways. Dot size represents gene count, and color indicates adjusted p-value.

Comparative Analysis Reveals Divergent Responses to CD74 Loss

Comparison of enrichment profiles between Raji and Meljuso cells reveals both shared and distinct transcriptional responses to CD74 knockout. While Raji cells predominantly exhibit downregulation of antigen presentation pathways coupled with activation of stress-response mechanisms, Meljuso cells show a pronounced activation of interferon and antiviral pathways. These differences highlight the context-dependent role of CD74 and

suggest that its loss may trigger distinct compensatory mechanisms depending on the cellular background. Such divergence may have important implications for tumor immunogenicity and therapeutic targeting in different lymphoma subtypes.

Gene Set Enrichment Analysis Reveals Coordinated Pathway-Level Changes

To further validate pathway-level alterations and capture coordinated transcriptional programs, we performed Gene Set Enrichment Analysis (GSEA) on both Raji and Meljuso datasets (Figure 6-7).

In Raji cells, GSEA revealed strong enrichment of gene sets associated with antigen processing and presentation, vesicular trafficking, and immune-related biological processes in wild-type cells, which were depleted upon CD74 knockout (Figure 6). Conversely, CD74-deficient Raji cells showed enrichment of pathways related to protein folding, chaperone activity, and cellular stress responses, reinforcing findings from over-representation analyses.

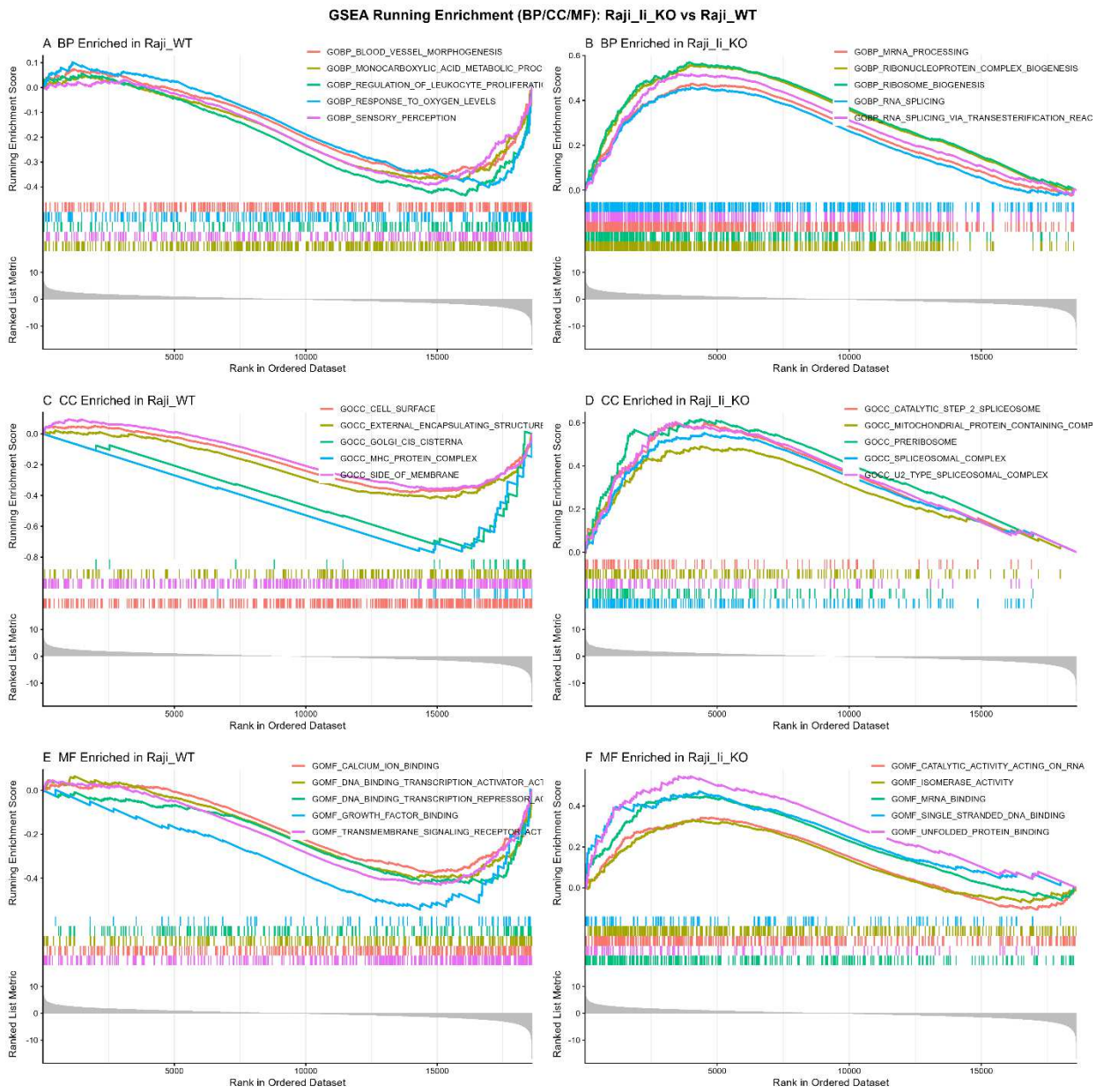


Figure 6. Gene Set Enrichment Analysis (GSEA) of transcriptomic alterations in CD74 knockout Raji cell. GSEA enrichment plots for Raji CD74 knockout (KO) versus wild-type (WT) cells showing enrichment of pathways associated with RNA processing, ribosome biogenesis, and unfolded protein binding, together with depletion of vesicle-mediated transport and membrane-associated signaling pathways.

In Meljuso cells, GSEA highlighted a distinct transcriptional program characterized by enrichment of interferon signaling, antiviral responses, and immune activation pathways

in CD74 knockout cells (Figure 7). Wild-type Meljuso cells, in contrast, were enriched for gene sets associated with baseline cellular homeostasis and metabolic processes. Across both cell lines, GSEA confirmed that CD74 loss induces coordinated, pathway-level reprogramming rather than isolated gene expression changes. Importantly, these results reinforce the divergence observed between Raji and Meljuso cells, with Raji cells exhibiting loss of antigen presentation capacity and induction of stress pathways, while Meljuso cells activate robust immune and interferon-driven responses.

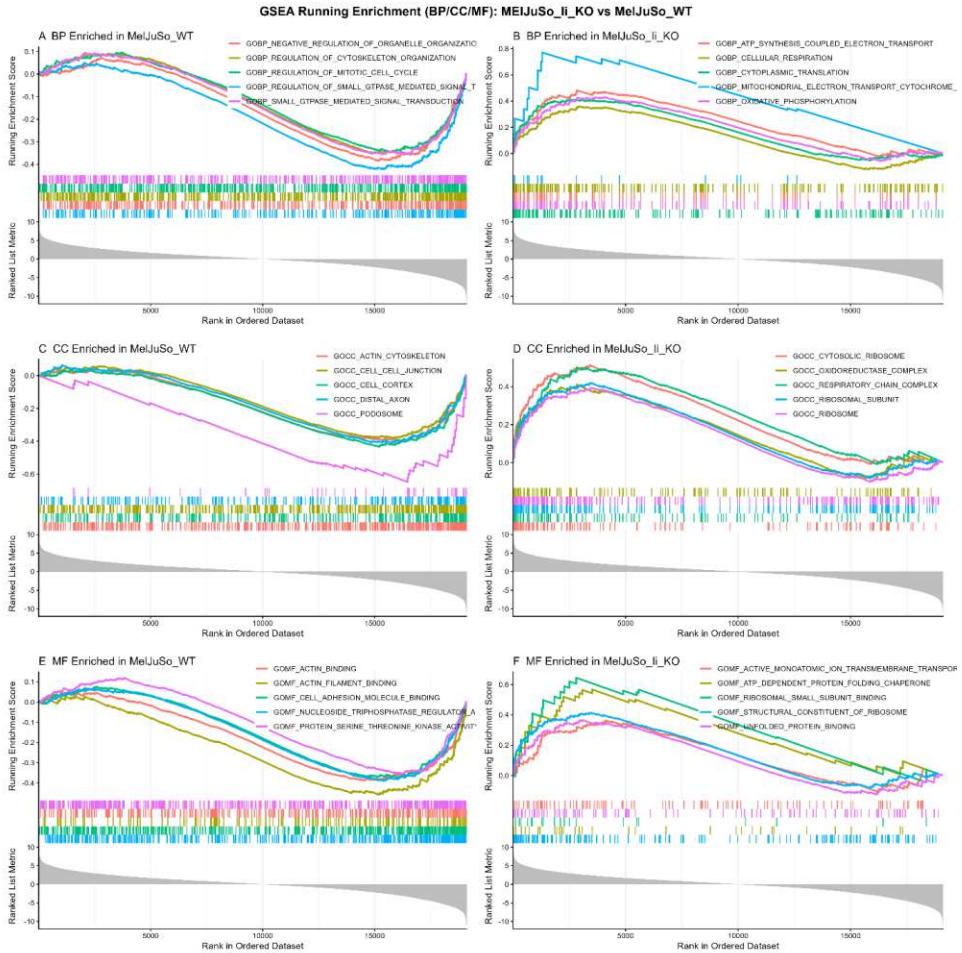


Figure 7. Gene Set Enrichment Analysis (GSEA) of transcriptomic alterations in CD74 knockout Meljuso cell. GSEA enrichment plots for Meljuso KO versus WT cells demonstrate enrichment of oxidative

phosphorylation, cytoplasmic translation, and protein-folding pathways, alongside depletion of pathways related to cytoskeletal organization and membrane trafficking.

Canonical Pathway Analysis Highlights Signaling Rewiring Following CD74 Loss

To further delineate signaling pathways affected by CD74 depletion, we performed GSEA using curated canonical pathways from the MSigDB C2:CP collection (Figure 8-9). In Raji cells, canonical pathway analysis revealed that wild-type cells were enriched for B cell receptor (BCR) signaling, immune receptor signaling, and cytoskeletal regulation pathways (Figure 8). In contrast, CD74 knockout cells showed enrichment of pathways associated with apoptosis, signaling cascades linked to cellular stress, and pathways related to cytoskeletal remodeling. These findings further support the notion that CD74 loss disrupts immune receptor signaling while promoting stress-associated and compensatory pathways.

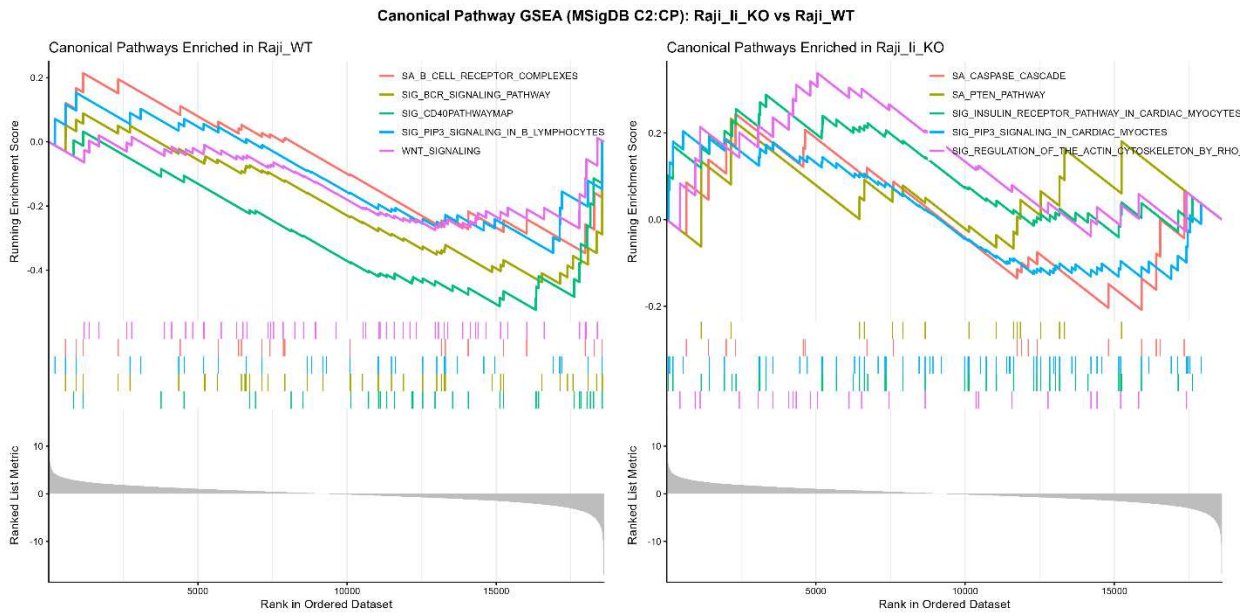


Figure 8. Canonical pathway enrichment analysis using GSEA (MSigDB C2:CP) in CD74 knockout in Raji cell. Canonical pathways enriched in Raji WT and CD74 KO cells. Wild-type cells show enrichment

of B cell receptor (BCR) signaling and immune receptor pathways, whereas knockout cells display enrichment of pathways associated with apoptosis, cytoskeletal remodeling, and stress-related signaling.

In Meljuso cells, canonical pathway enrichment demonstrated a distinct profile, with wild-type cells enriched for baseline signaling pathways including BCR and TRAF-mediated signaling (Figure 9). Upon CD74 knockout, there was a shift toward pathways associated with immune activation and signaling reprogramming, consistent with the interferon-driven phenotype observed in earlier analyses. Collectively, these results highlight that CD74 loss leads to substantial rewiring of canonical signaling pathways, with both shared and cell line–specific effects. Notably, disruption of B cell receptor–related signaling appears to be a common feature, while downstream responses diverge depending on cellular context.

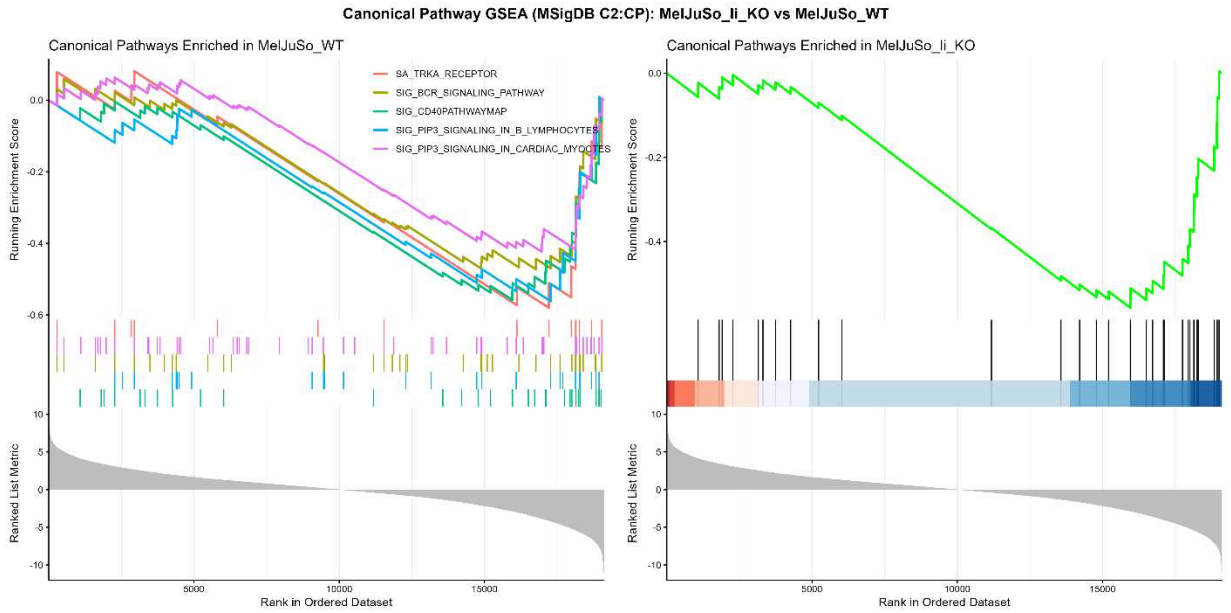


Figure 9. Canonical pathway enrichment analysis using GSEA (MSigDB C2:CP) in CD74 knockout in Meljuso cell. Canonical pathways enriched in Meljuso WT and CD74 KO cells. WT cells are enriched in baseline signaling pathways including BCR and TRAF-mediated signaling, while CD74 knockout cells exhibit pathway signatures consistent with immune activation and signaling reprogramming.

Comparative Gene-Level Analysis Reveals Shared and Opposing Transcriptional Programs

To directly compare transcriptional responses between Raji and Meljuso cells, we performed an integrated analysis of differentially expressed genes across both models (Figure 10). A subset of genes was consistently upregulated in both Raji and Meljuso CD74 knockout cells, indicating shared transcriptional responses to CD74 loss (Figure 10A). These genes likely represent core pathways affected by invariant chain depletion, potentially including stress-response and compensatory mechanisms. Similarly, a group of genes was commonly downregulated across both cell lines (Figure 10B), suggesting conserved suppression of specific biological processes, including components of antigen presentation and immune-related pathways.

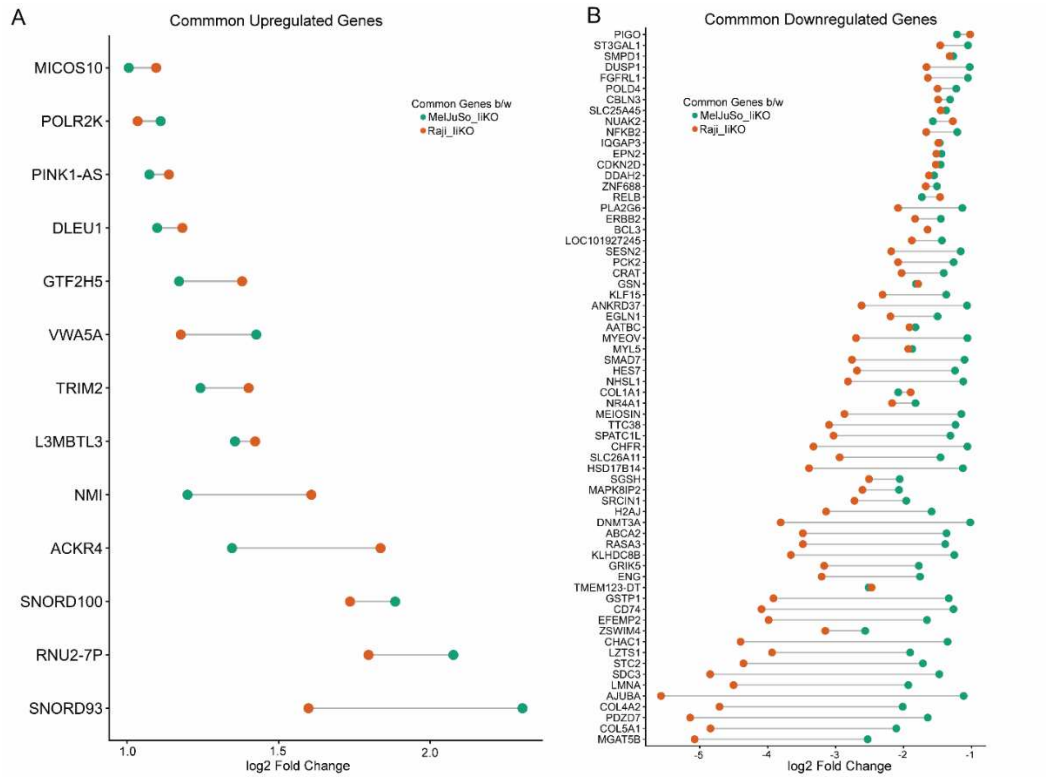


Figure 10. Comparative analysis of differentially expressed genes between Raji and Meljuso CD74 knockout cells. (A) Genes commonly upregulated in both Raji and Meljuso CD74 knockout (KO) cells. (B) Genes commonly downregulare commonlyonlyonlyss both cell lines.

Notably, a significant number of genes display opposing regulation between the two cell lines (Figure 11). Several genes were upregulated in Meljuso KO cells but downregulated in Raji KO cells, and vice versa, highlighting fundamentally different transcriptional rewiring in response to CD74 loss. These opposing patterns reinforce the context-dependent nature of CD74 function and suggest that distinct regulatory networks are engaged in each cellular background.

Together, these findings demonstrate that while CD74 knockout induces a core set of shared transcriptional changes, a substantial proportion of the response is cell line–specific, with divergent and even opposing gene expression programs.

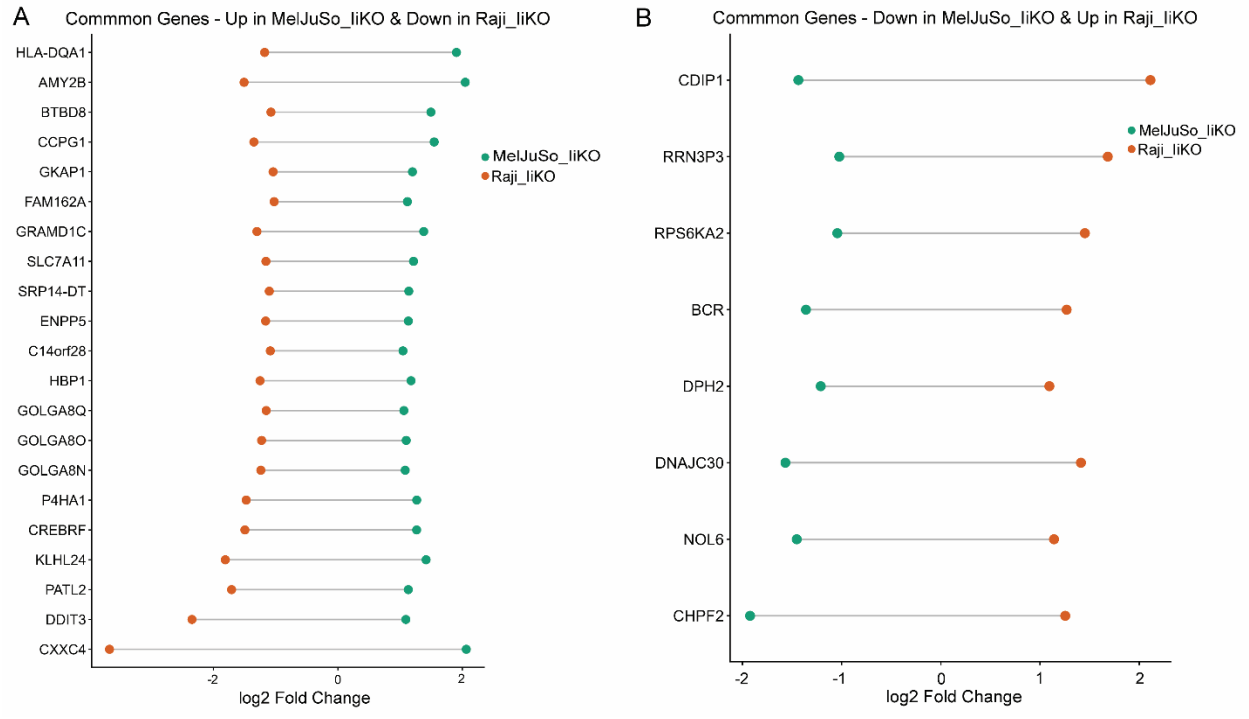


Figure 11. Comparative analysis of differentially expressed genes between Raji and Meljuso CD74 knockout cells. (A) Genes are upregulated in Meljuso KO but downregulated in Raji KO cells. (B) Genes downregulated in Meljuso KO but upregulated in Raji KO cells.

Discussion

In this study, we investigated the global transcriptional consequences of invariant chain (CD74) loss in two human cell lines, Raji and Meljuso. Through integrative analysis of bulk RNA sequencing data, we demonstrate that CD74 knockout induces extensive transcriptional reprogramming, affecting antigen presentation, intracellular trafficking, immune signaling, and cellular stress pathways. Importantly, while a subset of responses is shared between the two models, the overall transcriptional outcomes are highly context-dependent, with striking divergence and even opposing regulation between cell lines.

Consistent with the established role of CD74 as a chaperone for MHC class II molecules, its loss resulted in suppression of antigen processing and presentation pathways, particularly evident in Raji cells. This finding reinforces the canonical function of CD74 in stabilizing MHC-II complexes and facilitating their trafficking to endosomal compartments. The downregulation of immune-related pathways observed here suggests that CD74 depletion compromises antigen presentation capacity, which may have important implications for tumor immunogenicity and immune evasion.

Beyond antigen presentation, our data reveal that CD74 loss has broader effects on intracellular organization and signaling. In Raji cells, knockout of CD74 was associated with activation of stress-response pathways, including protein folding and chaperone-mediated processes. This likely reflects disruption of endosomal lysosomal trafficking and

accumulation of misfolded or improperly processed proteins. Such compensatory activation of proteostasis mechanisms highlights an underappreciated role of CD74 in maintaining intracellular homeostasis.

In contrast, Meljuso cells exhibited a markedly different response to CD74 depletion, characterized by robust activation of interferon signaling and antiviral pathways. This suggests that, in certain cellular contexts, loss of CD74 may trigger innate immune sensing mechanisms, potentially through altered endosomal signaling or accumulation of nucleic acid derived ligands. The induction of interferon-stimulated genes and host defense pathways points to a shift toward an immune-activated state, in stark contrast to the immunosuppressive signature observed in Raji cells.

Gene set enrichment and canonical pathway analyses further support this divergence, revealing that CD74 loss disrupts core signaling networks, including B cell receptor (BCR) signaling and immune receptor pathways. While disruption of these pathways appears to be a shared feature, the downstream consequences differ substantially. Raji cells preferentially activate stress and survival pathways, whereas Meljuso cells engage immune and antiviral programs. These findings suggest that CD74 functions as a regulator of signaling network balance, and its loss leads to distinct compensatory responses depending on the cellular background.

A key finding of this study is the identification of genes that are oppositely regulated between the two cell lines. This opposing transcriptional behavior underscores the context-dependent nature of CD74 function and suggests that its role extends beyond a simple linear pathway. Instead, CD74 appears to act as a hub that integrates antigen

presentation, intracellular trafficking, and signaling pathways, with its loss exposing underlying differences in regulatory circuitry between cell types.

Taken together, we propose a model in which CD74 maintains immune and cellular homeostasis by coordinating antigen presentation with intracellular signaling and proteostasis. Upon CD74 loss, this balance is disrupted, leading to compensatory rewiring of transcriptional networks. The direction of this rewiring toward stress adaptation or immune activation is determined by the intrinsic properties of the cellular context.

These findings have important implications for understanding the role of CD74 in cancer biology. Given its involvement in both antigen presentation and signaling, CD74 may influence tumor immune recognition as well as cell survival pathways. The context-dependent effects observed here suggest that therapeutic targeting of CD74 or related pathways may have variable outcomes depending on tumor type and molecular background.

In conclusion, our study demonstrates that CD74 is a central regulator of transcriptional and signaling networks. Its loss induces both shared and divergent transcriptional programs, highlighting a previously underappreciated context-dependent role. These findings expand our understanding of CD74 biology and provide a foundation for future studies exploring its role in immune regulation and cancer.

Conclusions

CD74 isprimarily viewed as a key regulator of MHC-II antigen presentation; however, our transcriptomic analyses reveal a broader role in coordinating immune signaling, intracellular trafficking, and cellular homeostasis. CD74 knockout induced extensive but

highly context-dependent transcriptional changes in Raji and Meljuso cells, resulting in distinct activation of stress-response, proteostasis, interferon, and antiviral pathways. Comparative analyses identified both shared and divergent gene expression programs, highlighting the influence of cellular background on CD74-dependent regulation. Together, these findings expand the current understanding of CD74 function and highlight its role as an important modulator of immune and signaling networks with potential relevance to tumor biology and immune regulation.

Declarations

Funding. This work was supported by the Norwegian Research Council under Grants 230779, 244388 and 254817 and ICMR-Intermediate from Indian Council of Medical Research under Grant DDR/01/IIRP-IG2025/01060.

Competing interests. The authors declare no competing interests.

Ethics approval and consent to participate. Not applicable.

Consent for publication. Not applicable.

Availability of data and materials

The bulk mRNA -Seq dataset used in this study are deposited in ENA (https://www.ebi.ac.uk/ena/browser/view/PRJEB115466) with accession number PRJEB115466.

Authors' contributions

NBP, SP and OB conceptualized the project. NBP and SP designed and performed the experiments. ST and MKBS performed the bioinformatics analysis. NBP, SP and ST

interpreted the data. NBP wrote the manuscript and all authors were involved in its editing.

Acknowledgements. We thank Prof. Oddmund Bakke, University of Oslo for providing the cell lines and offering lab facility.

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