

Single-cell and bulk transcriptomic analyses identify HSP90AA1- associated oxidative stress adaptation during impaired erythroid differentiation in β -thalassemia

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Abstract

β -thalassemia is characterized by defective β -globin synthesis, chronic oxidative stress, and ineffective erythropoiesis. However, the cellular heterogeneity and molecular responses underlying these processes remain incompletely defined. In this study, we reanalyzed publicly available single-cell and bulk transcriptomic datasets to investigate oxidative stress–associated erythroid remodeling in β -thalassemia. By integrating murine single-cell RNA sequencing data with both murine and human bulk RNA-seq datasets, we identified conserved oxidative stress–related candidate genes and characterized erythroid-centered intercellular communication and differentiation dynamics. Oxidative stress signatures were assessed using AUCell scoring, stemness potential via CytoTRACE, intercellular communication using CellChat, and erythroid differentiation trajectories reconstructed by Monocle2. Oxidative stress–related genes were identified through GeneCards and validated across datasets. A total of 84,639 bone marrow cells were analyzed, revealing 13 major hematopoietic and immune cell populations. Erythroid cells were markedly expanded in β -thalassemia, accompanied by elevated oxidative stress and reduced stemness. Hsp90aa1 was the only candidate that passed the cross-dataset filtering strategy and showed increased expression in the human β -thalassemia cohort. Its expression was significantly higher in β -thalassemia across multiple lineages and progressively increased along the erythroid pseudotime trajectory. Cell–cell communication analysis suggested reduced inferred erythroid outgoing signaling and relatively enhanced incoming signals from macrophage, mesenchymal, and myeloid compartments. Pseudotime analysis revealed depletion of early erythroid progenitors and stress-dependent upregulation of Hsp90aa1 during differentiation. Our findings provide an integrative transcriptomic view of β -thalassemia, linking oxidative stress to disrupted erythroid maturation and altered intercellular communication. Together, these findings nominate Hsp90aa1/HSP90AA1 as a conserved oxidative-stress-associated candidate linked to erythroid maturation and inferred niche remodeling, warranting future functional validation.

1 Introduction

β -thalassemia is a hereditary hemoglobin disorder caused by mutations in the β -globin gene, resulting in reduced or absent β -globin chain synthesis [1, 2]. The consequent imbalance between α - and β -globin chains leads to excessive accumulation of unpaired α -globin subunits, which precipitate within erythroid precursors and trigger a cascade of oxidative and inflammatory responses [3, 4]. Despite decades of research and improvements in transfusion and chelation therapies, the molecular basis linking β -globin deficiency to cellular dysfunction remains incompletely elucidated [2, 5, 6].

A growing body of evidence indicates that oxidative stress serves as a central pathogenic driver in β -thalassemia [4, 7, 8]. Unstable α -globin chains undergo auto-oxidation and release free heme and iron, catalyzing the generation of reactive oxygen species (ROS) [9, 10]. Elevated ROS levels provoke membrane lipid peroxidation, protein oxidation, and DNA damage, thereby compromising erythrocyte integrity and exacerbating ineffective erythropoiesis [11, 12]. Moreover, chronic hemolysis and secondary

iron overload further perpetuate ROS accumulation through the Fenton reaction, establishing a self-sustaining cycle of oxidative injury [13, 14].

Beyond erythroid cells, oxidative stress extends its deleterious effects to the bone marrow microenvironment and peripheral tissues [15]. Dysregulated redox signaling perturbs the function of macrophages, stromal cells, and hematopoietic stem and progenitor cells (HSPCs), thereby disrupting erythroid maturation and promoting a pro-inflammatory milieu [16, 17]. These systemic redox imbalances also contribute to complications such as endocrine dysfunction, cardiac injury, and liver fibrosis commonly observed in β -thalassemia patients [18, 19]. However, the cell type-specific transcriptional and redox regulatory landscapes that underpin these pathological processes remain insufficiently characterized.

The emergence of single-cell RNA sequencing (scRNA-seq) technologies offers unprecedented resolution to dissect the cellular heterogeneity and redox-related transcriptional dynamics in β -thalassemia. By integrating scRNA-seq with bulk RNA-seq data [20], it becomes possible to capture both the fine-grained cellular perturbations and the global transcriptomic alterations driven by oxidative stress. Such integrative analyses not only deepen our mechanistic understanding of β -thalassemia pathophysiology but also hold promise for prioritizing redox-sensitive candidates for future mechanistic studies of erythroid homeostasis and for alleviating oxidative burden in affected individuals. Although the original study delineated the broad single-cell landscape of ineffective erythropoiesis in this murine model, the present reanalysis was designed to address a more specific question: whether oxidative stress-associated molecular signatures converge across murine single-cell, murine bulk, and human bulk transcriptomic datasets, and how these signatures relate to erythroid-centered intercellular communication and differentiation trajectories inferred through pseudotime analysis.

2 Methods

2.1 Data acquisition

Single-cell RNA sequencing (scRNA-seq) data were obtained from the National Genomics Data Center (NGDC: <https://ngdc.cncb.ac.cn/gsa/>) under accession number CRA003639 [21], which includes bone marrow samples from four β -thalassemia mice and four wild-type control mice. This dataset was generated using the 10x Genomics Chromium platform and contains high-quality single-cell transcriptomic profiles covering diverse hematopoietic cell populations. For bulk RNA sequencing (bulk RNA-seq) analysis, two datasets were utilized. First, the mouse erythroid cell bulk RNA-seq dataset (accession number CRA003641) was retrieved from the NGDC database, comprising three β -thalassemia mice and three control mice [21]. Second, the human bulk RNA-seq dataset (accession number GSE117221) was downloaded from the Gene Expression Omnibus (GEO: <https://www.ncbi.nlm.nih.gov/gds>) database, containing 32 β -thalassemia patients and 17 healthy controls. To explore oxidative stress-related molecular pathways, a curated list of oxidative stress-associated genes was extracted from the GeneCards database (<https://www.genecards.org/>). Genes

with a Relevance score greater than 7 were selected to ensure biological significance and literature-supported relevance to oxidative stress processes. A total of 2179 oxidative stress-related genes (OSRGs) were obtained. Because these datasets differ in species, sequencing resolution, sample source, and cohort composition, the human bulk RNA-seq cohort was used as an external expression-level validation dataset rather than as direct evidence of conserved single-cell mechanisms.

2.2 Data preprocessing

Raw sequencing files (FASTQ format) corresponding to the mouse bone marrow scRNA-seq dataset (CRA003639) were processed using the Cell Ranger (10x Genomics, version 6.1.2) pipeline. Reads were aligned to the *Mus musculus* reference genome (mm10) and gene–cell count matrices were generated using the cellranger count function with default parameters [22]. For the mouse erythroid cell bulk RNA-seq dataset (CRA003641), raw FASTQ files from β -thalassemia and control mice were aligned to the mm10 genome assembly using the STAR aligner (version 2.7.9a) with default settings [23]. Alignment quality metrics, including mapping rate and read distribution, were assessed using FastQC and MultiQC. Gene-level read counts were quantified using featureCounts (Subread package, version 2.0.3) [24]. Expression matrices were normalized as transcripts per million (TPM) and log2-transformed for subsequent differential expression analyses. The human β -thalassemia bulk RNA-seq dataset (GSE117221) was obtained directly from the Gene Expression Omnibus (GEO) database using the R package GEOquery (version 2.76.0) [25]. Expression data and corresponding metadata were downloaded, parsed, and normalized according to the processing pipeline described in the original GEO submission. Only samples annotated as β -thalassemia patients ($n = 32$) and healthy controls ($n = 17$) were included in the downstream analysis to maintain clinical comparability.

2.3 Single-cell RNA-seq analysis

All downstream single-cell analyses were conducted in R (version 4.5.1) using the Seurat package (version 5.3.0) [26, 27]. Raw expression matrices were imported using the Read10X function and converted into Seurat objects via CreateSeuratObject. To ensure data quality, doublet removal was performed with DoubletFinder (version 2.0.6) prior to filtering [26]. Cells were retained according to the following quality control criteria: $300 < \text{nFeature_RNA} < 7000$, $500 < \text{nCount_RNA} < 25000$, $\text{percent.mt} < 5\%$, and $\text{percent.rb} < 20\%$. Filtered datasets were normalized using the NormalizeData function, and 2,000 highly variable genes (HVGs) were identified with FindVariableFeatures. Cell cycle phase scoring was computed using CellCycleScoring, after which the data were scaled using ScaleData while regressing out unwanted sources of variation, including cell cycle effects and mitochondrial gene content. Principal component analysis (PCA) was performed using RunPCA, and the top 30 principal components (PCs) were selected for downstream analysis. Cell-to-cell relationships were constructed using FindNeighbors, followed by clustering via FindClusters. Batch effects across biological replicates were corrected with Harmony (RunHarmony) (Supplementary Fig. 2A) [28], and a two-dimensional embedding was generated using Uniform Manifold Approximation and Projection (UMAP) implemented by RunUMAP [29]. A resolution of 1.0 was chosen for clustering, yielding distinct and biologically meaningful cellular populations. Cell-type annotation was carried out based on canonical marker genes

and validated using the CellMarker2 database (<http://117.50.127.228/CellMarker/index.html>). To investigate the influence of oxidative stress in β -thalassemia, orthologous gene conversion between mouse and human was performed using the homologue package (version 1.4.68.19.3.27), followed by gene set activity scoring using AUCell (version 1.30.1). To further evaluate the differentiation potential of erythroid and progenitor populations, CytoTRACE2 (version 1.1.0) was employed to infer cellular developmental trajectories based on transcriptional diversity. Higher CytoTRACE2 scores indicated less differentiated, stem-like cellular states, whereas lower scores represented more mature phenotypes. Differential gene expression at the single-cell level was determined using the FindMarkers function in Seurat. Data cleaning, statistical summarization, and visualization were accomplished using the tidyverse package (version 2.0.0). Statistical comparisons between groups were performed using the ggpubr package (version 0.6.2), applying the Wilcoxon test for two-group comparisons. A p-value < 0.05 was considered statistically significant unless otherwise specified.

2.4 Bulk RNA-seq analysis

Differential expression analysis of bulk RNA-seq data was performed using the DESeq2 package (version 1.48.2) in R (version 4.3.1) [30]. Raw count matrices were imported and normalized using the DESeqDataSetFromMatrix function. Differentially expressed genes (DEGs) between β -thalassemia and control groups were identified based on the following thresholds: adjusted p-value (Benjamini–Hochberg FDR) < 0.05 and $|\log_2$ fold change| > 1. The results were visualized using the ggplot2 package (version 4.0.0), and volcano plots were generated to depict significantly upregulated and downregulated genes. To further explore the expression patterns of DEGs, a heatmap was generated using the pheatmap package (version 1.0.13). Gene expression values were Z-score normalized across samples, and hierarchical clustering was applied using Euclidean distance and complete linkage to group genes and samples based on transcriptional similarity. Functional enrichment analyses were subsequently conducted using the clusterProfiler package (version 4.16.0) in conjunction with the org.Mm.eg.db annotation database (version 3.21.0) [31, 32]. Both Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on the DEGs. Terms or pathways with an adjusted p-value < 0.05 were considered significantly enriched. The results were visualized through bar plots and dot plots to illustrate the top enriched biological processes and signaling pathways associated with β -thalassemia.

2.5 Immuno-infiltration analysis

Immune cell deconvolution was performed on the human bulk RNA-seq cohort (GSE117221) using the IOBR R package (v0.99.0) with the CIBERSORT algorithm. Gene-level counts were transformed to TPM and $\log_2(\text{TPM} + 1)$ for visualization; raw TPM matrices were used for deconvolution. Prior to analysis, gene symbols were harmonized (duplicated symbols collapsed by median), and samples with > 15% missing genes in the signature were excluded. CIBERSORT was run with the LM22 leukocyte gene signature to estimate the relative fractions of 22 immune cell subsets. Group comparisons between β -thalassemia (TH) and healthy controls (WT) were performed on cell-type fractions using the Wilcoxon rank-sum test (two-sided).

2.6 Cell–Cell communication analysis

To explore intercellular signaling networks and potential communication alterations in β -thalassemia, cell–cell communication analysis was performed using the CellChat package (version 1.6.1) [33]. The processed Seurat objects of β -thalassemia and control groups were converted into CellChat objects using the createCellChat function. The built-in CellChatDB.mouse ligand–receptor database was employed to infer intercellular communication probability based on known ligand–receptor interactions. Each dataset was normalized and subjected to computation of communication probabilities using the computeCommunProb function, followed by aggregation of significant interactions via computeCommunProbPathway. Communication networks were visualized using netVisual_circle and netVisual_chord_gene functions to display both global and cell type–specific signaling relationships. Comparative analysis between β -thalassemia and control groups was conducted using the compareInteractions and rankNet functions to identify differentially active signaling pathways. Key signaling networks exhibiting significant gain or loss of communication strength were further analyzed to determine which ligand–receptor pairs contributed most to these changes. The inferred communication networks were quantified by computing the information flow score for each signaling pathway, allowing for the identification of dominant regulatory axes in erythroid–immune–hematopoietic interactions.

2.7 Pseudotime analysis

To investigate the dynamic differentiation trajectory of erythroid cells in β -thalassemia, pseudotime analysis was performed using the monocle package (version 2.36.0) [34]. A subset of erythroid cells was selected based on Seurat clustering, focusing on the major erythroid populations identified in both β -thalassemia and control samples. These cells were subsequently used to infer their differentiation trajectories, providing insights into the aberrations in erythroid development that characterize β -thalassemia. The gene expression data were normalized and reduced in dimensionality using the DDRTree algorithm, which constructs a tree-like structure to represent cell fate decisions. The estimateSizeFactors and estimateDispersions functions were used for pre-processing, followed by the application of reduceDimension to generate the low-dimensional representation of the data. A single-cell trajectory was then inferred by the orderCells function, which organizes cells along a pseudotime axis that represents the erythroid cells.

2.8 Validation of HSP90 protein expression by ELISA

To validate the transcriptomic upregulation of HSP90AA1 at the protein level, serum HSP90 concentrations were measured by ELISA in peripheral blood samples collected from β -thalassemia patients (TH group, n = 10) and age- and sex-matched healthy controls (WT group, n = 10). All samples were collected following informed consent and stored at -80 °C until analysis. HSP90 protein levels were quantified using the Human HSP90 ELISA Kit (Proteintech, Cat# KE00054) according to the manufacturer's instructions. Briefly, standard samples were serially diluted and added to a 96-well microplate (100 μ L/well), followed by overnight incubation at 4 °C to allow adsorption onto the solid-

phase carrier. After blocking with 1% bovine serum albumin (BSA) at room temperature for 1 hour, the test samples (diluted as required) were added at 100 μ L per well and incubated for 2 hours to allow specific binding of the target molecule. Subsequently, an enzyme-labeled secondary antibody was added and incubated at 37 °C for 1 hour to form an immune complex with the target molecule. The plate was washed three times to remove unbound components. A substrate solution was added and incubated in the dark for 30 minutes, after which a stop solution was added to terminate the colorimetric reaction. The optical density (OD) of each well was measured at 450 nm using a multi-mode microplate reader (BioTek Synergy HTX). All samples and standards were assayed in triplicate. All samples were within the detection range of the assay.

2.9 Data analysis

Data analysis mainly involves one-way analysis of variance (ANOVA) and t-tests to compare differences between treatment groups. ANOVA is used for comparing multiple groups, while t-tests are used for comparing two groups. A significance level of ($p < 0.05$) is typically used, indicating that a (p)-value below this threshold suggests a significant difference between the experimental and control groups.

3 Results

3.1 Cellular landscape and differential population analysis in β -thalassemia

Single-cell transcriptomic analysis revealed a highly heterogeneous cellular landscape in the β -thalassemia bone marrow. After doublet removal (Supplementary Fig. 1A) and quality control filtering (Supplementary Fig. 1B), 84,639 cells were retained for downstream analysis. Unsupervised clustering and UMAP-based dimensionality reduction identified 32 distinct clusters at a resolution of 1 (Fig. 1A; Supplementary Fig. 2B). These clusters were subsequently annotated into 13 major cell populations, including B cells, erythroid cells, granulocytic cells, hematopoietic stem and progenitor cells (HSPCs), macrophages, mast cells, megakaryocytes, mesenchymal cells, monocytes, natural killer (NK) cells, plasma cells, T cells, and plasmacytoid dendritic cells (pDCs) (Fig. 1B,C). Differential cell population analysis between the β -thalassemia group and the control group revealed significant differences in the proportions of several cell types (Fig. 1D, E). Specifically, the proportion of erythroid cells was significantly increased in the β -thalassemia group compared to controls ($p < 0.05$), reflecting the dysregulated erythropoiesis characteristic of the disease (Fig. 1F, G). In contrast, the relative proportions of granulocytic cells, macrophages, megakaryocytes, mesenchymal cells, monocytes, NK cells, plasma cells, and T cells were significantly reduced in the β -thalassemia group, with all differences reaching statistical significance ($p < 0.05$) (Fig. 1F, G). These findings suggest a shift in the hematopoietic landscape in β -thalassemia, with an overrepresentation of erythroid cells at the expense of other immune and stromal cell populations. The increase in erythroid cells likely reflects a compensatory response to ineffective erythropoiesis and chronic hemolysis, which are hallmarks of β -thalassemia. On the other hand, the reduction in various immune and stromal cell types may be indicative of dysregulated

hematopoietic differentiation and an impaired bone marrow microenvironment, which may be influenced by oxidative stress and chronic inflammation. These results were used primarily to confirm the cellular composition of the public dataset and to provide the annotated framework for downstream oxidative-stress, communication, and pseudotime analyses.

3.2 Differential oxidative stress and stemness signatures across cell types in β -thalassemia

To further elucidate the cellular heterogeneity of oxidative stress responses in β -thalassemia, we calculated oxidative stress-related gene activity scores at the single-cell level using the AUCell algorithm. Overall, most cell populations exhibited higher oxidative stress scores in the TH group compared to the WT group, suggesting a widespread elevation of redox imbalance across hematopoietic and immune lineages (Fig. 2A). Quantitatively, B cells ($p < 0.0001$), erythroid cells ($p < 0.0001$), granulocytic cells ($p < 0.0001$), HSPCs ($p < 0.05$), monocytes ($p < 0.0001$), NK cells ($p < 0.05$), pDCs ($p < 0.0001$), plasma cells ($p < 0.001$), and T cells ($p < 0.0001$) displayed significantly elevated oxidative stress scores in β -thalassemia compared with controls (Fig. 2C). These results underscore a systemic enhancement of oxidative stress not only in erythroid lineages—where globin imbalance directly contributes to ROS accumulation—but also in non-erythroid immune compartments, reflecting a more generalized disruption of redox homeostasis in the disease state. To complement these findings, cellular stemness potential was evaluated using CytoTRACE (Fig. 2B). Interestingly, β -thalassemia cells exhibited distinct alterations in differentiation potential across cell types. The erythroid cells ($p < 0.0001$) and B cells ($p < 0.0001$) from β -thalassemia samples displayed markedly lower CytoTRACE scores relative to controls, indicating a loss of stem-like characteristics and accelerated differentiation. Conversely, granulocytic cells ($p < 0.0001$), HSPCs ($p < 0.0001$), macrophages ($p < 0.05$), pDCs ($p < 0.01$), and plasma cells ($p < 0.001$) demonstrated significantly higher CytoTRACE scores in β -thalassemia, implying a retention of progenitor-like features and potential blockage in terminal maturation (Fig. 2D). Thus, the signature-scoring analysis served as a cell-type-resolved context for prioritizing oxidative-stress-associated candidates, rather than as an independent claim that oxidative stress is newly discovered in β -thalassemia.

3.3 Integrative transcriptomic analysis reveals oxidative stress-associated upregulation of Hsp90aa1 in erythroid cells

We next applied a stringent prioritization strategy requiring concordance among erythroid scRNA-seq upregulation, mouse bulk RNA-seq upregulation, oxidative-stress annotation, and human bulk RNA-seq validation. Given the unique characteristics of erythroid cells—the only population showing an increased proportion, elevated oxidative stress score, and reduced stemness potential in β -thalassemia at the single-cell level—we further conducted differential expression analyses in erythroid cells using both scRNA-seq and bulk RNA-seq data (Fig. 3A, B, C). In the single-cell dataset, a total of 27 upregulated and

67 downregulated genes were identified in β -thalassemia erythroid cells compared with controls (Fig. 3A). In the mouse bulk RNA-seq dataset, 405 genes were upregulated and 165 genes were downregulated in β -thalassemia relative to wild-type mice (Fig. 3B, F). To delineate the biological processes associated with these transcriptional changes, GO and KEGG enrichment analyses were performed on the upregulated genes (Fig. 3D, E). At the single-cell level, enriched GO terms were predominantly associated with protein homeostasis and stress adaptation, including protein folding, protein refolding, cellular response to heat, response to temperature stimulus, erythrocyte development, oxygen transport, intracellular iron ion homeostasis, oxidoreductase activity, peroxidase activity, heme binding, antioxidant activity, and cellular detoxification. Similarly, KEGG pathway analysis revealed enrichment in Protein processing in endoplasmic reticulum, Ferroptosis, Necroptosis, and Lipid and atherosclerosis, all of which are tightly linked to oxidative stress and redox imbalance (Fig. 3D). Consistent results were observed in the bulk RNA-seq dataset, where enriched terms included heme metabolic process, erythrocyte homeostasis, cellular detoxification, response to oxygen radical, cellular oxidant detoxification, iron ion transport, porphyrin metabolism, Ferroptosis, and ABC transporters. Collectively, these findings indicate that β -thalassemia erythroid cells undergo transcriptional reprogramming toward oxidative stress adaptation and iron-dependent redox regulation (Fig. 3E).

By intersecting the upregulated gene set with the oxidative stress–related gene list obtained from GeneCards (Relevance score > 7), we identified three oxidative stress–associated upregulated genes common to both datasets: Hsp90aa1, Pnpo, and Glo1 (Fig. 3G). No downregulated genes overlapped with oxidative stress–related genes (Fig. 3H). To validate these findings at the human level, we examined their expression using the GSE117221 β -thalassemia cohort (32 patients vs. 17 controls). Among the three candidates, only Hsp90aa1 exhibited a statistically significant increase in expression ($p < 0.05$), consistent with the murine transcriptomic findings (Fig. 3I). At the single-cell resolution, Hsp90aa1 expression was markedly elevated in the β -thalassemia group across multiple hematopoietic and immune lineages (Fig. 3J), including B cells ($p < 0.0001$), erythroid cells ($p < 0.0001$), granulocytic cells ($p < 0.0001$), HSPCs ($p < 0.0001$), macrophages ($p < 0.0001$), mesenchymal cells ($p < 0.01$), monocytes ($p < 0.0001$), NK cells ($p < 0.001$), and T cells ($p < 0.0001$) (Fig. 3K). These consistent cross-platform results highlight Hsp90aa1 as a pivotal oxidative stress–responsive gene, potentially reflecting chaperone-dependent stress adaptation in β -thalassemia.

The limited number of cross-dataset candidates likely reflects the stringent filtering criteria and differences between murine single-cell data and human bulk transcriptomes. Therefore, Hsp90aa1/HSP90AA1 should be interpreted as a conserved candidate signal rather than proof that the mouse model fully captures all molecular features of human β -thalassemia.

3.4 Altered immune cell composition in β -thalassemia revealed by CIBERSORT deconvolution

To investigate alterations in the immune landscape of β -thalassemia, immune cell infiltration analysis was conducted on the human bulk RNA-seq dataset (GSE117221) using the CIBERSORT algorithm

implemented in the IOBR framework (Supplementary Fig. 3A). The relative proportions of 22 immune cell subsets were estimated for each sample, and comparisons between TH and WT groups were subsequently performed. Among the immune populations analyzed, two subsets exhibited statistically significant differences between groups. Specifically, the Macrophages_M0 fraction was significantly reduced in β -thalassemia compared with controls ($p < 0.05$), while the Monocyte fraction was significantly elevated in β -thalassemia ($p < 0.05$) (Supplementary Fig. 3B). These results indicate a remodeling of the myeloid compartment in β -thalassemia, characterized by a relative depletion of undifferentiated macrophages and an expansion of circulating monocytes.

3.5 Erythroid-centered cell–cell communication networks in β -thalassemia

The main observation from the CellChat analysis was a shift in inferred erythroid-centered communication: erythroid cells showed reduced outgoing communication but received relatively stronger incoming signals from macrophage, mesenchymal, and myeloid compartments. To elucidate the remodeling of erythroid-centered signaling in β -thalassemia, cell–cell communication analysis was conducted to compare interaction networks between TH and WT groups (Fig. 4A, B). When erythroid cells were designated as signal senders, the total number of outgoing interactions was consistently reduced in β -thalassemia, indicating an overall attenuation of erythroid-derived signaling output (Fig. 4C). Conversely, when erythroid cells were considered signal receivers, they exhibited increased incoming communications from macrophages, mesenchymal cells, and monocytes, whereas signals from granulocytic cells were decreased relative to controls (Fig. 4D). Analysis of communication strength further revealed distinct cell-type–specific changes. As signal senders, erythroid cells displayed enhanced communication intensity toward macrophages, mesenchymal cells, NK cells, plasmacytoid dendritic cells (pDCs), and plasma cells, but reduced signaling toward HSPCs, monocytes, T cells, and B cells (Fig. 4E). When acting as signal receivers, erythroid cells exhibited stronger incoming signals from macrophages, mesenchymal cells, monocytes, and T cells, as well as from granulocytic cells, megakaryocytes, and NK cells (Fig. 4F). At the level of ligand–receptor pair analysis, several key alterations were observed. With erythroid cells as senders, the adhesion-related pair Vcam1–(Itga4 + Itgb1) showed markedly reduced signaling toward both macrophages and mesenchymal cells, suggesting impaired cell–cell adhesion and niche anchoring in β -thalassemia. Similarly, Lgals9–Ighm, Lgals9–Cd45, and Lgals9–Cd44 interactions exhibited weakened communication strength, reflecting reduced immunomodulatory crosstalk between erythroid and immune compartments. In contrast, MHC class I–related interactions, including H2-t23, H2-t24, and H2-d1, displayed enhanced activity toward CD8⁺ T cells (Cd8a, Cd8b1) and NK cell receptors (Klrd1, Klrc1, Klrc2), implying the activation of stress-induced immune surveillance pathways in β -thalassemia erythroid cells (Fig. 4G). When erythroid cells were defined as receivers, ligand–receptor analysis revealed strengthened adhesion and survival-related signaling from other lineages. Specifically, Granulocytic_Cell→Erythroid_Cell and Megakaryocyte→Erythroid_Cell interactions via Thbs1–Cd47 and Thbs1–Cd36 were significantly enhanced in β -thalassemia (Fig. 4H). Taken together, these results indicate that β -thalassemia is characterized by global attenuation of erythroid-derived signaling output but selective amplification of

incoming stress- and adhesion-related pathways, particularly those mediated by macrophages, mesenchymal cells, and myeloid lineages.

3.6 Pseudotime trajectory analysis reveals aberrant erythroid differentiation and dynamic upregulation of Hsp90aa1 in β -thalassemia

To examine whether Hsp90aa1 expression changed along inferred erythroid maturation, pseudotime analysis was performed on the erythroid cell subset using Monocle (Fig. 5A). Notably, erythroid cells from the β -thalassemia (TH) group appeared less represented in the early portion of the inferred trajectory compared with the control (WT) group, indicating an impaired commitment or reduced abundance of early erythroid progenitors. This observation aligns with the characteristic ineffective erythropoiesis seen in β -thalassemia, where early-stage differentiation is often disrupted by oxidative and metabolic stress (Fig. 5B). In examining the expression dynamics of Hsp90aa1 along the pseudotime axis, a distinct divergence between the two groups was observed. In the TH group, Hsp90aa1 expression exhibited a progressive upregulation trend as differentiation advanced, suggesting an increasing dependence on molecular chaperone activity during late-stage erythroid maturation under stress conditions. In contrast, the WT group displayed a gradual downregulation trend, consistent with normal erythropoiesis where stress response mechanisms are attenuated upon maturation (Fig. 5C).

3.7 Elevated HSP90 protein levels in β -thalassemia confirmed by ELISA

Given that our integrative transcriptomic analyses consistently identified HSP90AA1 as a significantly upregulated oxidative stress-associated gene in erythroid cells across mouse scRNA-seq, mouse bulk RNA-seq, and human bulk RNA-seq datasets, we sought to validate these findings at the protein level. Serum HSP90 concentrations were quantified by ELISA in an independent cohort comprising TH group and WT group. As shown in Fig. 6A, HSP90 protein levels were significantly elevated in the TH group compared with the WT group.

4 Discussion

In this study, we observed a consistent up-regulation of HSP90AA1 in erythroid cells of TH at single-cell resolution, along with a gradually increasing expression trend along pseudotime—contrasting sharply with the decreasing trend seen in WT erythroid maturation [35]. These findings support HSP90AA1 as a stress-associated chaperone candidate in the erythroid lineage of β -thalassemia. HSP90, the cytosolic 90 kDa heat-shock protein, plays a pivotal role in cellular proteostasis and stress tolerance [36]. It supports the folding, stabilization and activation of a wide array of client proteins and is up-regulated in response to oxidative stress, inflammation and proteotoxic condition. In the erythroid context specifically, Ghosh et al. demonstrated that HSP90 associates with immature, heme-free β - or γ -globins

and facilitates heme insertion, thereby assisting maturation of functional hemoglobin tetramers [37]. In β -thalassemia, reduced or absent β -globin synthesis, accumulation of unpaired α -globin chains, iron overload and reactive oxygen species (ROS) formation underpin ineffective erythropoiesis [38]. The upregulation of HSP90AA1 observed in this study may represent an adaptive cellular response to oxidative stress, whereby erythroid progenitors increase HSP90AA1 expression to stabilize misfolded globin proteins, facilitate residual heme incorporation, and mitigate ROS-induced proteotoxic stress. The increasing expression across pseudotime suggests that this chaperone response becomes more pronounced as erythroid maturation proceeds under stress-conditions [39]. However, this compensatory up-regulation may be double-edged. Persistent overexpression of HSP90AA1 could lock erythroid cells in a state of chronic stress adaptation, delaying proper maturation and undermining normal differentiation kinetics—consistent with our observed depletion of early-stage progenitors and altered stemness scores. Moreover, HSP90 chaperones have been implicated in ferroptosis and necroptosis regulation under oxidative stress conditions [40]. Furthermore, our cell–cell communication findings show erythroid cells in TH receive stronger inputs from macrophages, mesenchymal and myeloid populations while their outgoing signaling is suppressed [41]. Elevated HSP90AA1 expression may also modulate the extrinsic niche-interactions of erythroid cells by stabilizing surface receptors, adhesion molecules and stress-responsive signalling complexes, thereby contributing to the rewired marrow ecosystem observed in β -thalassemia [42]. Oxidative stress represents a major pathogenic driver in β -thalassemia, arising from the imbalance between α - and β -globin synthesis, excess unpaired α -chains, iron overload, and repeated transfusions, all of which generate reactive oxygen species (ROS) that damage erythroid precursors and disrupt bone marrow homeostasis [43]. Chronic oxidative injury impairs mitochondrial function, alters redox signaling, and induces apoptosis of developing erythroblasts, leading to ineffective erythropoiesis a hallmark of β -thalassemia [44]. In summary, our data position HSP90AA1 as a key stress-adaptive chaperone in β -thalassemia erythropoiesis up-regulated under oxidative stress, dynamically increasing during abnormal differentiation, and potentially influencing erythroid-microenvironmental communication. This axis may provide a focused hypothesis for future functional studies of ineffective erythropoiesis in β -thalassemia.

HSP90 protein levels elevation were consistent with the transcriptomic upregulation of HSP90AA1 observed in both the mouse and human datasets, providing experimental validation that the stress-adaptive chaperone response identified through bioinformatic analysis translates to measurable differences in circulating HSP90 protein abundance. Together with the pseudotime analysis showing progressive Hsp90aa1 upregulation during aberrant erythroid differentiation, these results further support the role of HSP90 as a stress-associated chaperone that is upregulated at both the transcriptional and translational levels in β -thalassemia, reinforcing its potential as a candidate biomarker for oxidative stress-driven ineffective erythropoiesis.

Several limitations should be acknowledged. This study is a reanalysis of public datasets, and the initial single-cell annotation analyses provide the cellular framework for the subsequent integrative and trajectory-based analyses. The integration of mouse single-cell data with mouse and human bulk RNA-seq supports expression-level convergence but cannot prove conserved mechanisms across species.

CellChat, CIBERSORT, and pseudotime analyses are computational inferences. Although HSP90 protein upregulation was validated by ELISA in an independent patient cohort, HSP90AA1 was not experimentally perturbed or validated at the protein level. Future functional studies are needed to determine whether HSP90AA1 is a driver, consequence, or compensatory marker of oxidative stress during ineffective erythropoiesis.

5 Conclusions

This study combined single-cell and bulk RNA sequencing to outline the cellular and molecular landscape of β -thalassemia. Erythroid cells showed a marked expansion and signs of oxidative stress, accompanied by decreased stemness and transcriptional shifts toward stress adaptation. Among oxidative stress-related genes, Hsp90aa1 emerged as a consistently elevated marker across both datasets and species. Communication analysis revealed that erythroid cells had weaker outgoing signals but received stronger inputs from macrophages, mesenchymal, and myeloid populations, reflecting disturbed marrow interactions. Pseudotime reconstruction further indicated fewer early erythroid cells and a steady rise in Hsp90aa1 expression along differentiation in the β -thalassemia group. Importantly, ELISA validation in an independent patient cohort confirmed that HSP90 protein concentrations were significantly elevated in β -thalassemia relative to healthy controls, bridging the transcriptomic findings to a measurable protein-level phenotype. Together, these findings suggest that oxidative-stress-associated transcriptional remodeling is linked to erythroid maturation and inferred niche communication in β -thalassemia. Hsp90aa1/HSP90AA1 may serve as a conserved candidate marker for future mechanistic validation and clinical exploration.

Declarations

Data availability statement

The datasets supporting the conclusions of this article are publicly available in the Genome Sequence Archive (GSA) hosted by the National Genomics Data Center (NGDC) at <https://ngdc.cncb.ac.cn/gsa/> under accession numbers CRA003639 and CRA003641, and in the Gene Expression Omnibus (GEO) repository at <https://www.ncbi.nlm.nih.gov/geo/> under accession number GSE117221.

Ethics approval

This study was approved by the Medical Ethics Committee of Hainan General Hospital (Approval No. 2023-229). Written informed consent was obtained from all participants prior to sample collection.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contribution

Author Contributions Statement Single-cell and bulk transcriptomic analyses identify HSP90AA1-associated oxidative stress adaptation during impaired erythroid differentiation in β -thalassemia Xunqi Ji: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, and Writing – original draft. Jia Li: Conceptualization, Supervision, Project administration, and Writing – review and editing. Xiaowei Feng, Yuwen Chen, Ning Zhang, Zefu Chen, Hong Liang, and Yuehua Fu: Investigation, Data curation, Validation, and Writing – review and editing. All authors reviewed and approved the final manuscript.

References

1. Ho PJ, Thein S (2000) Gene regulation and deregulation: a β globin perspective. *Blood Rev* 14(2):78–93
2. Makis A, Voskaridou E, Papassotiriou I, Hatzimichael E (2021) Novel therapeutic advances in β -thalassemia. *Biology* 10(6):546
3. Hirsch RE, Sibmooh N, Fucharoen S, Friedman JM, Signaling R (2017) HbE/ β -thalassemia and oxidative stress: the key to pathophysiological mechanisms and novel therapeutics. *Antioxidants* 26(14):794–813
4. Vinchi F, Vercellotti GM, Belcher JD, Fibach E, Zreid H, Rasras R, Ghoti H, Muckenthaler MU, Rachmilewitz EA (2016) Systemic heme and iron overload results in depletion of serum hemopexin, haptoglobin and transferrin and correlates with markers of endothelial activation and lipid oxidation in beta thalassemia major and intermedia. *Blood* 128(22):2469
5. Maia de Oliveira, da Silva JP, Brugnerotto AF, Romanello S, KL Teixeira K, Lanaro K, Duarte CS, Costa AGL, da Silva Araújo G, Bezerra AC, de Farias Domingos MA I (2019) Global gene expression reveals an increase of HMGB1 and APEX1 proteins and their involvement in oxidative stress, apoptosis and inflammation pathways among beta-thalassaemia intermedia and major phenotypes. *J Haematol* 186(4):608–619
6. Lidonnici MR, Chianella G, Tiboni F, Barcella M, Merelli I, Scaramuzza S, Rossi C, Crippa S, Bernardo ME, Ciceri F (2020) Hematopoietic Stem Cells Are Endowed with Erythroid Signature in Beta-Thalassemia. *Blood* 136:31
7. Mirlohi MS, Yaghooti H, Shirali S, Aminasnafi A, Olapour S (2018) Increased levels of advanced glycation end products positively correlate with iron overload and oxidative stress markers in patients with β -thalassemia major. *Ann Hematol* 97(4):679–684

8. Penglong T, Saensuwanna A, Jantapaso H, Phuwakanjana P, Jearawiriyapaisarn N, Paiboonsukwong K, Wanichsuwan W, Srinoun K (2024) miR-214 aggravates oxidative stress in thalassemic erythroid cells by targeting ATF4. *PLoS ONE* 19(4):e0300958
9. Kattamis A, Lazaropoulou C, Kanavakis E, Ladis V, Paleologos G, Stamoulakatou A, Kattamis C, Papassotiriou I (2006) Non-Transferrin-Bound Iron and Oxidative Stress Are Related to the Degree of Erythropoiesis in Patients with Thalassemia Intermedia. *American Society of Hematology*
10. De Luca C, Filosa A, Grandinetti M, Maggio F, Lamba M, Passi S (1999) Blood antioxidant status and urinary levels of catecholamine metabolites in β -thalassemia. *Free Radic Res* 30(6):453–462
11. Pan L, Xie Y, Yin X, Huang Y, Yang G, Li C, Chen Y, Liu F, Zhang N, Liang X (2023) Oxidative stress damage of Iron overload on bone marrow erythropoiesis, heart and liver in Non-Transfusion dependent thalassemia. *Blood* 142:5245
12. Matte A, Federti E, Kung C, Kosinski PA, Narayanaswamy R, Russo R, Federico G, Carlomagno F, Desbats MA, Salviati L (2021) The pyruvate kinase activator mitapivat reduces hemolysis and improves anemia in a β -thalassemia mouse model. *The Journal of clinical investigation* 131 (10)
13. Walter PB, Slater N, Harmatz P, Higa A, Ng V, Weyhmiller M, Evans P, Porter JB, Sweeters N, Price J (2015) Iron Level and Monocyte Morphology Predict TLR4 Expression and Reactive Oxygen Species Production Which Influences Chronic Inflammation in β -Thalassemia. *Blood* 126(23):950
14. Fibach E, Dana M (2019) therapy Oxidative stress in β -thalassemia. *Molecular diagnosis therapy* 23 (2):245–261
15. Allen A, Perera S, Mettananda S, Rodrigo R, Perera L, Darshana T, Moggach F, Crawford AJ, Heirene L, Fisher C (2021) Oxidative status in the β -thalassemia syndromes in Sri Lanka; a cross-sectional survey. *Free Radical Biology Med* 166:337–347
16. Crippa S, Rossella V, Aprile A, Silvestri L, Ravis S, Scaramuzza S, Pirroni S, Avanzini MA, Basso-Ricci L, Hernandez RJ (2019) Bone marrow stromal cells from β -thalassemia patients have impaired hematopoietic supportive capacity. *J Clin Investig* 129(4):1566–1580
17. Aprile A, Gulino A, Villa I, Beretta S, Merelli I, Rubinacci A, Ponzoni M, Marktel S, Tripodo C, Lidonnici MR (2019) Hematopoietic Stem Cell Function in β -Thalassemia Is Impaired and Is Rescued By Targeting the Bone Marrow Niche. *Blood* 134:967
18. Walter PB, Macklin EA, Porter J, Evans P, Kwiatkowski JL, Neufeld EJ, Coates T, Giardina PJ, Vichinsky E, Olivieri N (2008) Inflammation and oxidant-stress in β -thalassemia patients treated with iron chelators deferasirox (ICL670) or deferoxamine: an ancillary study of the Novartis CICL670A0107 trial. *Haematologica* 93(6):817–825
19. Makis A, Hatzimichael E, Papassotiriou I, Voskaridou E (2016) 2017 Clinical trials update in new treatments of β -thalassemia. *Am J Hematol* 91(11):1135–1145
20. Wang H, Tian RF, Liang X, Fan J, Duan ZC, Fan XY, Zhang JJ, Yao DS, Chen ZN, Li L (2022) A four oxidative stress gene prognostic model and integrated immunity-analysis in pancreatic adenocarcinoma. *Front Oncol* 12:1015042. 10.3389/fonc.2022.1015042

21. Peng Y, Liang L, Zhang H, Liu H, Zhang G, Sun S, Guo X, Wang Y, Hu B, Liu R (2023) Single-cell profiling of ineffective erythropoiesis in a mouse model of β -thalassaemia intermedia. *Br J Haematol* 201(5):982–994
22. Zheng GX, Terry JM, Belgrader P, Ryvkin P, Bent ZW, Wilson R, Ziraldo SB, Wheeler TD, McDermott GP, Zhu J, Gregory MT, Shuga J, Montesclaros L, Underwood JG, Masquelier DA, Nishimura SY, Schnall-Levin M, Wyatt PW, Hindson CM, Bharadwaj R, Wong A, Ness KD, Beppu LW, Deeg HJ, McFarland C, Loeb KR, Valente WJ, Ericson NG, Stevens EA, Radich JP, Mikkelsen TS, Hindson BJ, Bielas JH (2017) Massively parallel digital transcriptional profiling of single cells. *Nat Commun* 8(1):14049. 10.1038/ncomms14049
23. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1):15–21. 10.1093/bioinformatics/bts635
24. Liao Y, Smyth GK, Shi W (2019) The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. *Nucleic Acids Res* 47(8):e47–e47
25. Davis S, Meltzer PS (2007) GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. *Bioinformatics* 23(14):1846–1847
26. McGinnis CS, Murrow LM, Gartner ZJ (2019) DoubletFinder: Doublet Detection in Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. *Cell Syst* 8(4):329–337e324. 10.1016/j.cels.2019.03.003
27. Hao Y, Stuart T, Kowalski MH, Choudhary S, Hoffman P, Hartman A, Srivastava A, Molla G, Madad S, Fernandez-Granda C, Satija R (2024) Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat Biotechnol* 42(2):293–304. 10.1038/s41587-023-01767-y
28. Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, Wei K, Baglaenko Y, Brenner M, Loh PR, Raychaudhuri S (2019) Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat Methods* 16(12):1289–1296. 10.1038/s41592-019-0619-0
29. Healy J, McInnes L (2024) Uniform manifold approximation and projection. *Nat Reviews Methods Primers* 4(1):82
30. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550
31. Yu G, Wang L-G, Han Y, He Q-Y (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 16(5):284–287
32. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo X, Yu G (2021) clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innov (Camb)* 2(3):100141. 10.1016/j.xinn.2021.100141
33. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan CH, Myung P, Plikus MV, Nie Q (2021) Inference and analysis of cell-cell communication using CellChat. *Nat Commun* 12(1):1088. 10.1038/s41467-021-21246-9

34. Trapnell C, Cacchiarelli D, Grimsby J, Pokharel P, Li S, Morse M, Lennon NJ, Livak KJ, Mikkelsen TS, Rinn JL (2014) Pseudo-temporal ordering of individual cells reveals dynamics and regulators of cell fate decisions. *32*(4):381
35. Matte A, De Falco L, Iolascon A, Mohandas N, An X, Siciliano A, Leboeuf C, Janin A, Bruno M, Choi SY, signaling r (2015) The interplay between peroxiredoxin-2 and nuclear factor-erythroid 2 is important in limiting oxidative mediated dysfunction in β -thalassemic erythropoiesis. *Antioxidants* *23*(16):1284–1297
36. Wei H, Zhang Y, Jia Y, Chen X, Niu T, Chatterjee A, He P, Hou G (2024) Heat shock protein 90: biological functions, diseases, and therapeutic targets. *MedComm* (2020) *5* (2):e470. 10.1002/mco2.470
37. Ghosh A, Garee G, Sweeny EA, Nakamura Y, Stuehr DJ (2018) Hsp90 chaperones hemoglobin maturation in erythroid and nonerythroid cells. *Proceedings of the National Academy of Sciences* *115* (6):E1117-E1126
38. Spinelli S, Straface E, Gambardella L, Caruso D, Dossena S, Marino A, Morabito R, Remigante A (2025) Iron Overload-Related Oxidative Stress Leads to Hyperphosphorylation and Altered Anion Exchanger 1 (Band 3) Function in Erythrocytes from Subjects with β -Thalassemia Minor. *Int J Mol Sci* *26*(4):1593
39. Duong LD, West JD, Morano KA (2024) Redox regulation of proteostasis. *J Biol Chem* *300*(12):107977
40. Lee S, Hwang N, Seok BG, Lee S, Lee S-J, Chung SW (2023) disease Autophagy mediates an amplification loop during ferroptosis. *Cell death* *14* (7):464
41. Li W, Guo R, Song Y, Jiang Z, BIOLOGY D (2022) Erythroblastic Island Macrophages Shape Normal Erythropoiesis and Drive Associated Disorders in Erythroid Hematopoietic Diseases (vol 8, 613885, 2021). *Frontiers in Cell Developmental Biology* *10*
42. Gu J, He Y, He C, Zhang Q, Huang Q, Bai S, Wang R, You Q, Wang L (2025) therapy t Advances in the structures, mechanisms and targeting of molecular chaperones. *Signal transduction targeted therapy* *10* (1):84
43. Sadiq IZ, Abubakar FS, Usman HS, Abdullahi AD, Ibrahim B, Kastayal BS, Ibrahim M, Hassan HA (2024) Thalassemia: Pathophysiology, diagnosis, and advances in treatment. *14*(4):81–102
44. Lin S, Zheng Y, Chen M, Xu L, Huang H (2024) The interactions between ineffective erythropoiesis and ferroptosis in β -thalassemia. *Front Physiol* *15*:1346173

Figures

B. UMAP plot visualizing cell types, annotated by major cell type groups, including B cells, erythroid cells, granulocytic cells, HSPCs, macrophages, mast cells, megakaryocytes, mesenchymal cells, monocytes, NK cells, plasma cells, T cells, and pDCs.

C. Bubble plot displaying expression levels of marker genes across different cell types. The size of each bubble corresponds to the percentage of cells expressing a given marker, and the color intensity represents the expression level.

D. Radar plot comparing the proportion of each cell type across TH and WT groups. The plot highlights differential abundance between groups.

E. Stacked bar plots showing the proportion of each cell type within samples (left) and across experimental groups (right). Each bar represents the distribution of cell types within the group.

F. Bar plot showing the differential proportion of cell types between TH and WT groups. Error bars represent standard deviation.

G. Bar plot showing the log₂ fold change (log₂FC) in cell type abundance between TH and WT groups. Positive values indicate higher proportions in the TH group, and negative values indicate lower proportions in TH compared to WT.

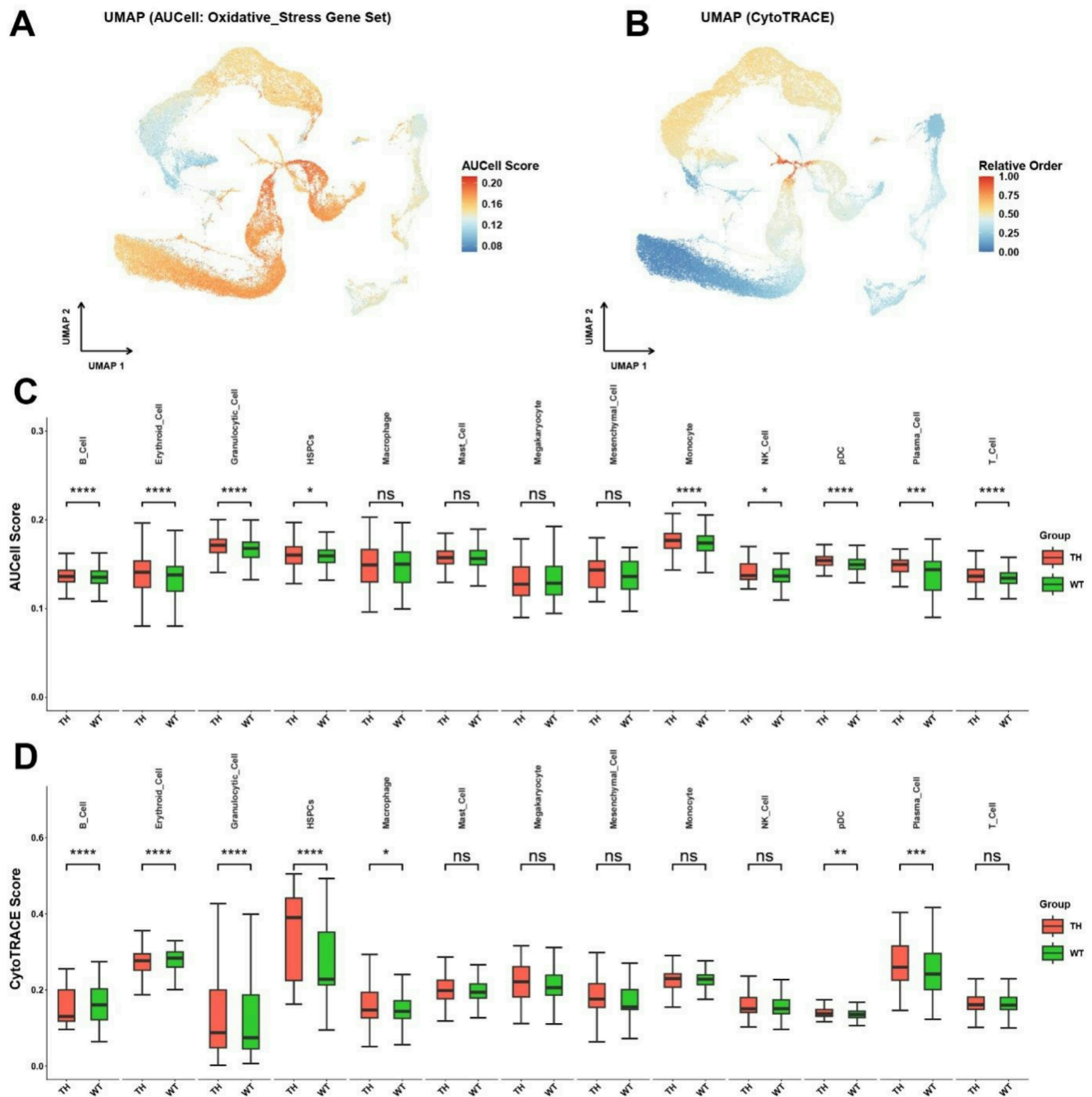


Figure 2

Single-cell profiling of oxidative stress and stemness signatures in β -thalassemia

A. UMAP visualization of oxidative stress activity across all cells, as quantified by AUCell scores derived from the oxidative stress–related gene set.

B. UMAP plot illustrating CytoTRACE-derived stemness scores, representing the inferred differentiation potential of individual cells.

C. Box plots comparing AUCell oxidative stress scores across major cell populations between TH and WT groups (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$).

D. Box plots comparing CytoTRACE stemness scores among the same cell populations (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$).

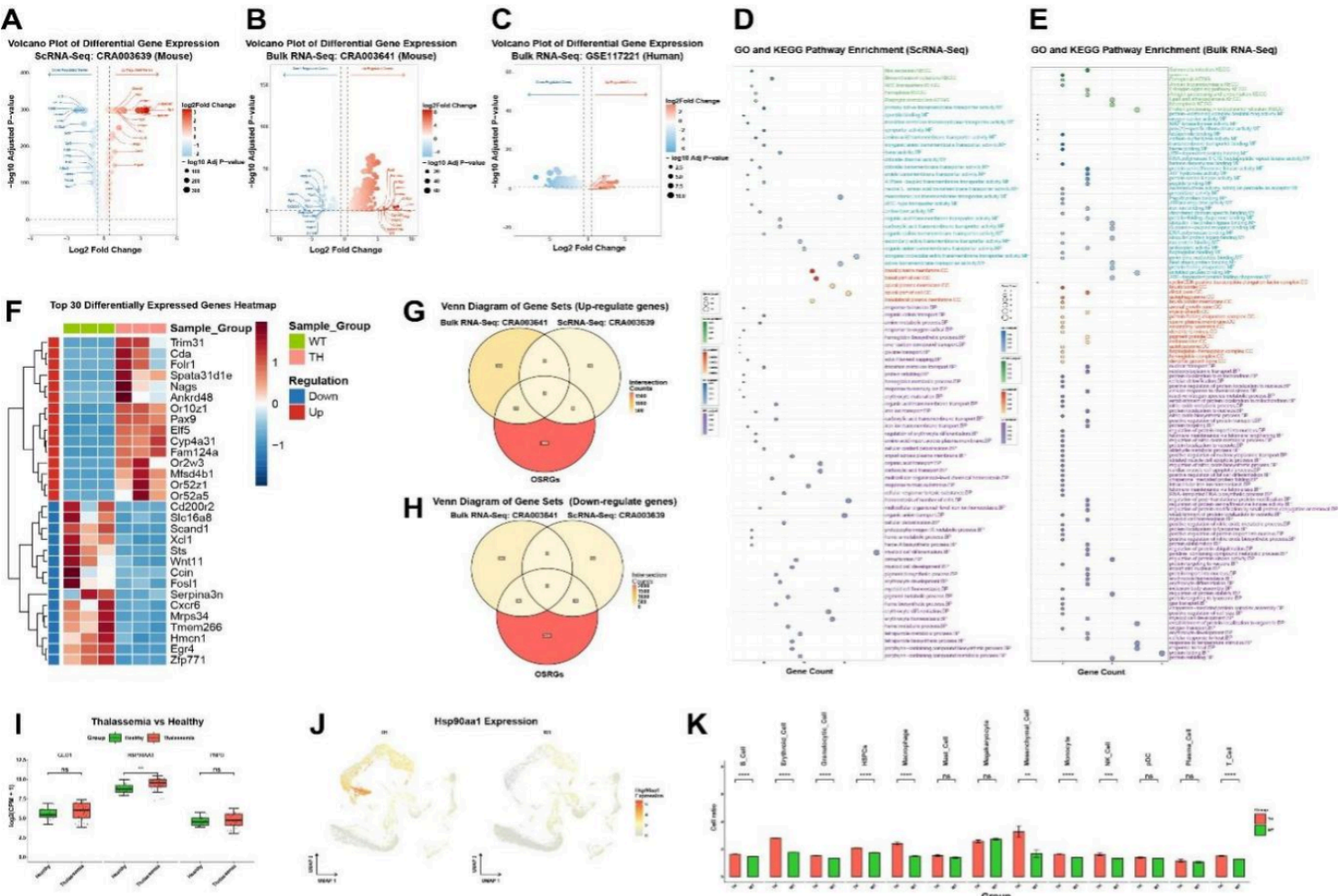


Figure 3

Integrative transcriptomic analysis identifies oxidative stress–related upregulation of Hsp90aa1 in erythroid cells of β -thalassemia

- A. Volcano plot of differentially expressed genes (DEGs) in erythroid cells from the mouse scRNA-seq dataset (CRA003639). Red and blue points represent significantly upregulated and downregulated genes, respectively ($|\log_2FC| > 1$, adjusted $p < 0.05$).
- B. Volcano plot showing DEGs in erythroid cells from the mouse bulk RNA-seq dataset (CRA003641) with the same threshold criteria.
- C. Volcano plot illustrating DEGs in erythroid cells from the human bulk RNA-seq dataset (GSE117221).

- D. GO and KEGG enrichment analysis of upregulated genes identified in the scRNA-seq dataset.
- E. GO and KEGG enrichment analysis of upregulated genes from the bulk RNA-seq dataset
- F. Heatmap of the top 30 DEGs in erythroid cells from the mouse bulk RNA-seq dataset
- G. Venn diagram displaying the overlap between upregulated genes from the scRNA-seq and bulk RNA-seq datasets and OSRGs.
- H. Venn diagram illustrating the overlap of downregulated genes with OSRGs
- I. Validation of Hsp90aa1, Pnpo, and Glo1 expression in the human β -thalassemia cohort (GSE117221) (**: $p < 0.01$).
- J. UMAP visualization of Hsp90aa1 expression in the mouse scRNA-seq dataset
- K. Box plots showing differential expression of Hsp90aa1 across major cell subpopulations (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$).

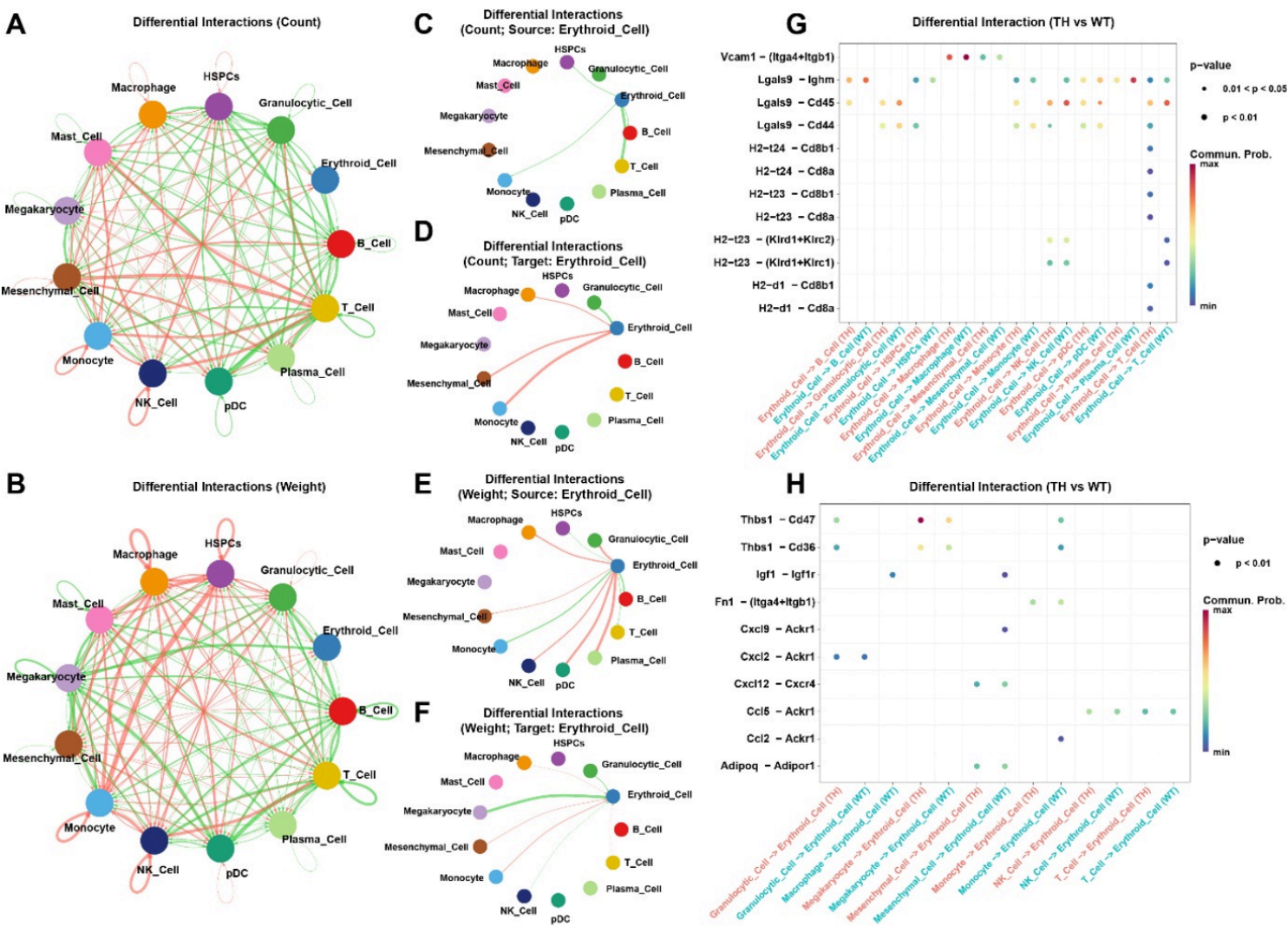


Figure 4

Comparative cell–cell communication networks centered on erythroid cells in β -thalassemia and control groups

A. Network diagram showing the overall number of intercellular communications among all cell populations. Red edges indicate interactions upregulated in TH, whereas green edges denote interactions upregulated in WT samples.

B. Network diagram illustrating the overall communication strength among all cell populations. Red edges indicate interactions upregulated in TH, whereas green edges denote interactions upregulated in WT samples.

C. Network depicting the number of cell–cell communication events when erythroid cells function as signal senders. Red edges represent interactions that are upregulated in TH samples, whereas green edges indicate interactions upregulated in WT samples.

D. Network of communication counts when erythroid cells act as signal receivers. Red edges indicate interactions upregulated in TH, whereas green edges denote interactions upregulated in WT samples.

E. Network of communication strength (weight) when erythroid cells are senders. Red edges indicate interactions upregulated in TH, whereas green edges denote interactions upregulated in WT samples.

F. Network of communication strength when erythroid cells act as receivers. Red edges indicate interactions upregulated in TH, whereas green edges denote interactions upregulated in WT samples.

G. Bubble plot showing differential ligand–receptor interactions when erythroid cells serve as signal senders. Red edges indicate interactions upregulated in TH, whereas blue edges denote interactions upregulated in WT samples.

H. Bubble plot showing differential ligand–receptor interactions when erythroid cells act as receivers. Red edges indicate interactions upregulated in TH, whereas blue edges denote interactions upregulated in WT samples.

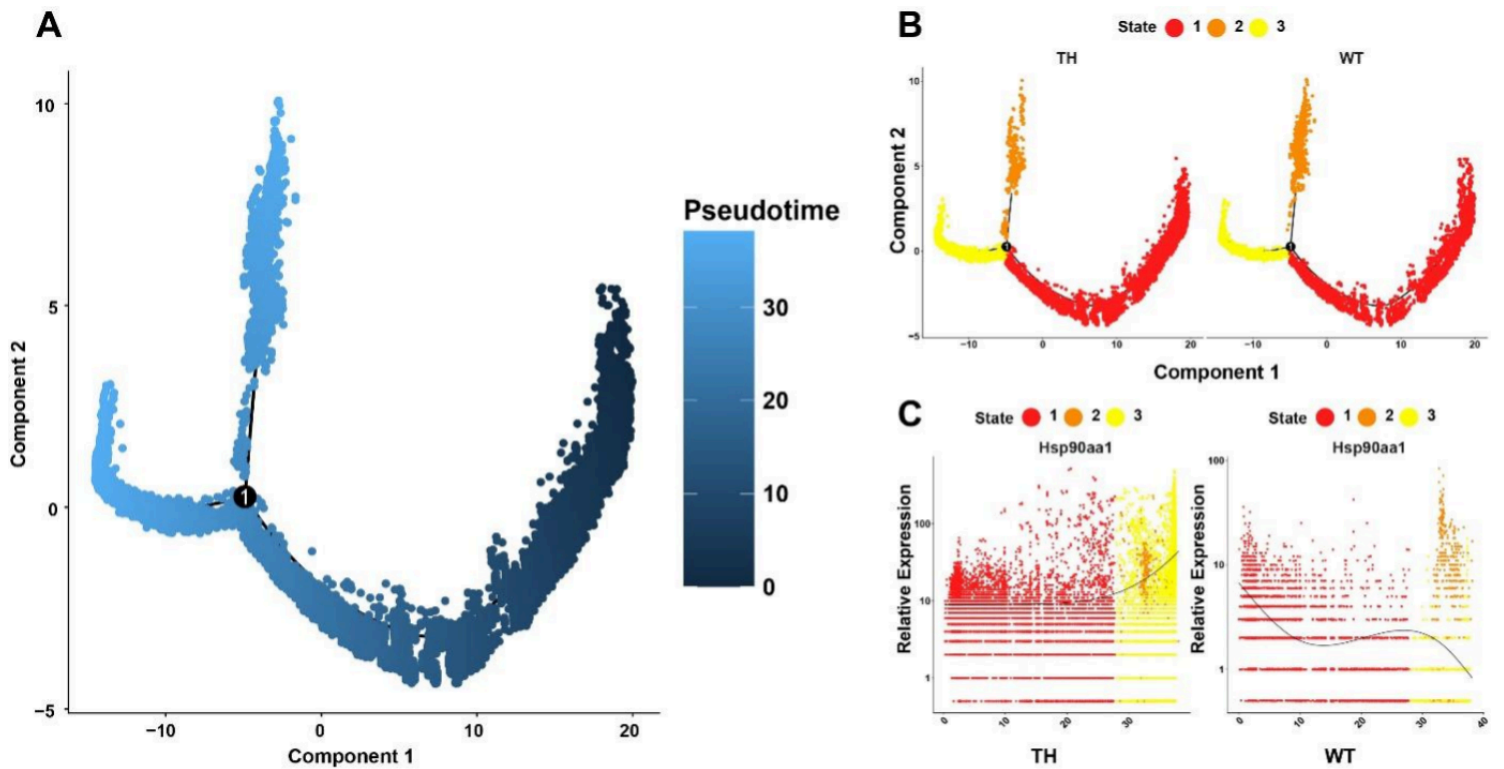


Figure 5

Pseudotime trajectory analysis of erythroid differentiation and dynamic expression of Hsp90aa1 in β -thalassemia

A. Pseudotime trajectory reconstructed by Monocle, illustrating the continuous differentiation path of erythroid cells from progenitor to mature states. Each dot represents a single cell, colored by pseudotime progression.

B. Comparison of pseudotime differentiation states between the β -thalassemia (TH) and wild-type (WT) groups.

C. Expression trends of Hsp90aa1 along the pseudotime trajectory in TH and WT groups.

A

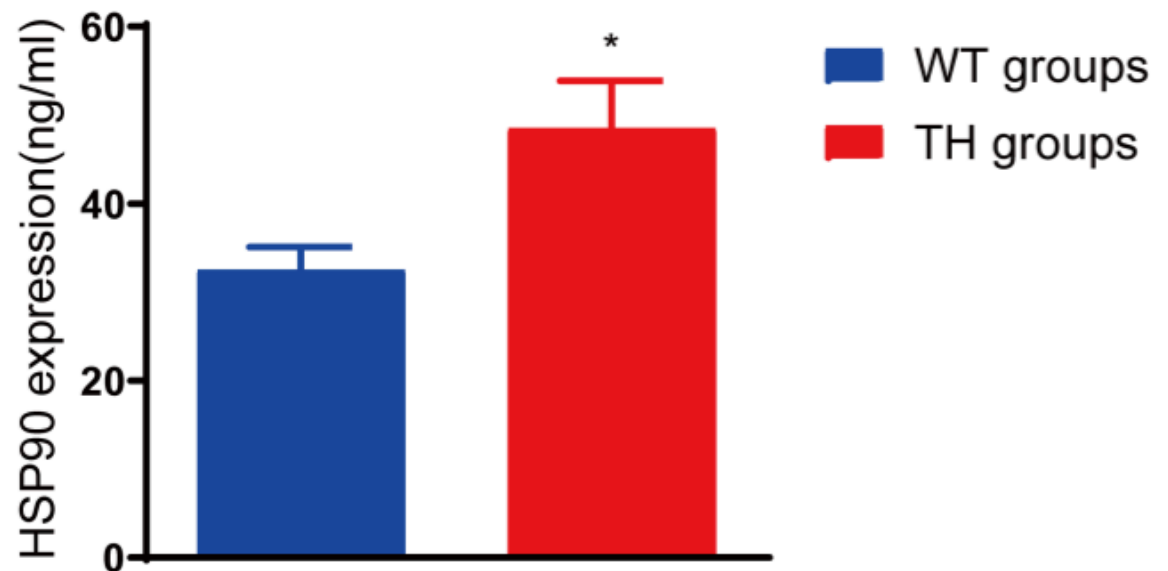


Figure 6

Validation of HSP90 protein expression by ELISA.

A. Serum HSP90 concentrations measured by ELISA in healthy controls (WT, n = 10) and β -thalassemia patients (TH, n = 10). All samples were measured in triplicate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.pdf](#)