

Silencing IL2RB attenuates abdominal aortic aneurysm-related pathology in experimental models by partially restoring mitochondrial homeostasis

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Abstract. Abdominal aortic aneurysm (AAA) remains a life-threatening vascular disease with limited therapeutic options. The role of interleukin-2 receptor subunit beta (IL2RB) in AAA *via* mitochondrial dysfunction is unclear. In this study, integrated bioinformatics analyses identified IL2RB as a key gene. In a rat AAA model and angiotensin II-stimulated vascular smooth muscle cells (VSMCs), IL2RB was upregulated and associated with mitochondrial impairment. IL2RB knockdown attenuated aortic dilation, reduced elastic fiber degradation (decreased by 45%), restored adenosine triphosphate (ATP) production (increased by 126.9%), suppressed interleukin-6 (decreased by 49.3%) and tumor necrosis factor-alpha (decreased by 51.8%) release, and decreased malondialdehyde (reduced by 47.7%) and reactive oxygen species (reduced by 38.5%) production in the rat model. In VSMCs, silencing IL2RB rescued angiotensin II-induced ATP depletion, preserved mitochondrial membrane potential, and reduced apoptosis. Mechanistically, IL2RB knockdown downregulated dynamin-related protein 1, and upregulated mitofusin 1 and parkin RBR E3 ubiquitin protein ligase. IL2RB knockdown mitigates AAA progression by restoring mitochondrial homeostasis and suppressing inflammation, identifying IL2RB as a potential therapeutic target for AAA.

Key words: IL2RB — Abdominal aortic aneurysm — Mitochondrial homeostasis

Abbreviations: AAA, abdominal aortic aneurysm; Ang-II, angiotensin II; AUC, area under the curve; DEGs, differentially expressed genes; DRP1, dynamin-related protein 1; ELISA, enzyme-linked immunosorbent assay; GEO, Gene Expression Omnibus; GO, Gene Ontology; H&E, hematoxylin and eosin; IL2RB, interleukin-2 receptor subunit beta; IL-6, interleukin-6; KEGG, Kyoto Encyclopedia of Genes and Genomes; LASSO, least absolute shrinkage and selection operator; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MFN1, mitofusin 1; Parkin, Parkin RBR E3 ubiquitin protein ligase; ROC, receiver operating characteristic; ROS, reactive oxygen species; RT-qPCR, real-time quantitative polymerase chain reaction; SVM-RFE, support vector machine-recursive feature elimination; TNF- α , tumor necrosis factor-alpha; VSMCs, vascular smooth muscle cells; VVG, Verhoeff-Van Gieson; WGCNA, weighted gene co-expression network analysis.

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Introduction

Abdominal aortic aneurysm (AAA) is a localized pathological dilatation of the abdominal aorta, typically defined by a maximum diameter ≥ 30 mm on imaging (Wanhainen et al. 2019; Golledge et al. 2023). It represents a significant global health burden, causing approximately 170,000 deaths annually worldwide (Wei et al. 2021). AAAs are frequently asymptomatic until rupture, which carries catastrophic mortality without prompt surgical intervention (Wanhainen et al. 2019). Current management relies on imaging surveillance for small AAAs (30–55 mm in men; 30–50 mm in women) and surgical repair (open or endovascular) for larger, symptomatic, or ruptured aneurysms (Wanhainen et al. 2019; Golledge et al. 2023). However, while advances in repair techniques exist, their durability remains suboptimal, and rupture risk persists post-intervention (Hensley et al. 2022). Critically, no pharmacological therapy has convincingly demonstrated efficacy in limiting AAA growth or rupture in randomized controlled trials despite testing diverse agents, such as antibiotics, antihypertensives, and statins, across 13 placebo-controlled trials (Pinchbeck et al. 2018; Baxter et al. 2020; Golledge et al. 2020; Wanhainen et al. 2020). This persistent clinical challenge underscores the urgent need to identify novel molecular targets and pathways driving AAA pathogenesis.

The interleukin-2 receptor subunit beta (IL2RB), also known as CD122, is a type I transmembrane glycoprotein that forms the core signaling component of the interleukin-2 (IL-2) and IL-15 receptor complexes (Kubota et al. 2023). As a critical mediator of cytokine signaling, IL2RB harbors conserved intracellular tyrosine residues that activate downstream pathways essential for immune cell survival, proliferation, and effector function (Keller et al. 2020). Dysregulation of IL2RB signaling has been implicated in diverse pathological conditions. In graft-versus-host disease, blockade of CD122 reduces skin-resident memory T cell accumulation by disrupting IL-15-mediated survival signals, thereby attenuating sclerodermatous lesions (Kubota et al. 2023). Conversely, IL2RB downregulation in septic patients correlates with impaired Th1 responses and skewed Th1/Th2 balance, highlighting its role in maintaining immune homeostasis (Zhou et al. 2023). In cancer, IL2RB-targeted therapies have shown promise. For example, engineered IL-2 variants that preferentially bind CD122 enhance anti-tumor activity by activating natural killer cells and cytotoxic T lymphocytes while minimizing Treg cell activation (Noh et al. 2024). Furthermore, IL2RB-dependent signaling drives B cell apoptosis in Peyer's patches, suggesting its involvement in mucosal immune regulation (Singh et al. 2020). Despite these advances, the role of IL2RB in AAA remains unknown.

Mitochondria, the cellular powerhouses, are dynamic organelles responsible for ATP production, calcium homeo-

stasis, and regulation of apoptotic signaling (Tavris et al. 2023). Mitochondrial dysfunction, characterized by impaired oxidative phosphorylation, increased reactive oxygen species (ROS) generation, and disrupted mitochondrial morphology, has been implicated in diverse pathological conditions, including cardiovascular diseases (Ouyang et al. 2021). In vascular smooth muscle cells (VSMCs), mitochondrial dysfunction drives phenotypic switching from a contractile to a synthetic state, promoting extracellular matrix degradation and inflammation, hallmarks of AAA progression (Liu et al. 2025). Mitochondrial dysfunction in VSMCs correlates with increased DNA damage and premature senescence, further contributing to aortic wall weakening (Summerhill et al. 2021; Liu et al. 2025). Whether IL2RB modulates mitochondrial dynamics in the aorta remains entirely uncharacterized. Elucidating this mechanism could uncover IL2RB as a potential therapeutic target for mitigating AAA-associated mitochondrial dysfunction.

In this study, utilizing integrated bioinformatics and experimental validation in both rat AAA models and VSMCs with AAA induction, we demonstrated that IL2RB knock-down could attenuate AAA progression by ameliorating mitochondrial dysfunction, reducing inflammation, and suppressing VSMC apoptosis, identifying IL2RB as a novel regulator of AAA development through mitochondrial pathways and highlighting its potential as a therapeutic target.

Materials and Methods

Integrated dataset analysis

Gene expression profiles analyzed in this study were retrieved from the Gene Expression Omnibus GEO database hosted by the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/geo/>). We systematically queried the database using keywords including “abdominal aortic aneurysm”, “human”, and “array”. Datasets were included if they met the following criteria: (1) contained at least 10 samples in total; (2) included both AAA tissues and normal aortic tissues as controls; (3) each group (AAA and control) contained no fewer than 5 samples. Following rigorous screening criteria, four microarray datasets meeting the requirements were selected, among which GSE7084, GSE47472, and GSE98278 were designated as the training cohort, while GSE57691 served as the independent validation cohort. Mitochondrial homeostasis-related genes were compiled from the GeneCards database.

Raw data for datasets GSE7084, GSE47472, and GSE98278 were acquired. Data preprocessing was performed as follows: probes (rows) and samples (columns) exhibiting missing values exceeding 50% were excluded. Subsequently, missing value imputation was conducted utilizing the *impute.knn*

function of the R package *impute*, employing a predefined number of nearest neighbors (k) for estimation. Processed expression values were then subjected to $\log_2(x + 1)$ transformation, yielding normalized expression matrices.

Robust cross-dataset critical batch effect mitigation was achieved through sequential processing: Initial dataset merging was performed using the *inSilicoMerging* R package, followed by application of specialized batch correction algorithms. The efficacy of batch effect removal was rigorously assessed and visualized *via* density distribution plots and Uniform Manifold Approximation and Projection (UMAP) analysis, confirming appropriate sample distribution in the resultant harmonized expression matrix.

Differential expression analysis using limma

Following dataset integration, differential gene expression analysis between AAA samples and normal control (NC) samples was performed using the *limma* package in R. Briefly, Expression profiles were fitted using the *lmFit* function to implement a multiple linear regression model. Moderated test statistics were computed using the *eBayes* function. This step performed empirical Bayes moderation of the standard errors towards a common value, yielding Moderated t -statistics, Moderated F -statistics, and Log-odds of differential expression. The criteria for defining DEGs were set as an adjusted p -value ($\text{adj. } p$) < 0.05 and $|\log_2 \text{fold-change}| (|\log_2 \text{FC}|) \geq 1$ (Oren et al. 2023; Bernabei et al. 2025; Shetty et al. 2025). The adjusted p -value threshold was applied to control the false discovery rate in multiple hypothesis testing, while the $|\log_2 \text{FC}|$ threshold was chosen to ensure the identification of genes with both statistical significance and substantial biological relevance (Oren et al. 2023; Bernabei et al. 2025; Shetty et al. 2025). To characterize the identified DEGs, hierarchical clustering analysis was conducted. Results were visualized using a volcano plot, depicting statistical significance *versus* magnitude of change, and a heatmap, illustrating expression patterns across samples and DEGs.

Functional enrichment analysis of DEGs

To elucidate the biological functions and pathways associated with the identified DEGs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted. The latest GO annotations, comprising biological process BP, molecular function MF, and cellular component CC, and KEGG pathway annotations, were retrieved using the R package *org.Hs.eg.db* and KEGG REST API (<https://www.kegg.jp/kegg/rest/keggapi.html>), respectively. These consolidated annotations served as the comprehensive reference set. The DEGs were then functionally annotated against this

reference set prior to enrichment analysis using the R package *clusterProfiler*. Results for each GO category (BP, MF, CC) were categorized separately, and for each category, the top 10 most significantly enriched terms were selected for presentation. The top 10 most significantly enriched KEGG pathways (ranked by p -value) were presented graphically using a bubble plot, illustrating the relationship between enrichment significance ($-\log_{10} p$ -value), the number of DEGs mapped to the pathway (count), and the enrichment ratio for the key pathways.

Weighted gene co-expression network analysis (WGCNA)

WGCNA was performed using the integrated gene expression matrix to identify functionally related gene modules associated with AAA. The Median absolute deviation (MAD) was computed for each gene across the integrated dataset. Genes exhibiting the lowest 50% MAD values were excluded to retain genes with sufficient expression variability. The *goodSamplesGenes* function within the WGCNA R package was employed to remove outlier genes and samples failing quality thresholds. A scale-free co-expression network was built using the WGCNA framework. To determine the appropriate soft-thresholding power (β), we analyzed the scale-free topology fit index (R^2) and the mean connectivity for a range of β values (1 to 30). The optimal β value was selected as the lowest power at which the scale-free topology fit index reached a plateau and exceeded 0.85, while maintaining a relatively high mean connectivity to preserve biological information. Based on this analysis, the soft-thresholding power was set to $\beta = 22$ to achieve an approximate scale-free topology ($R^2 = 0.85$). Genes were clustered using average linkage hierarchical clustering based on a Topological Overlap Matrix (TOM)-based dissimilarity measure. Dynamic tree cutting was performed with a minimum module size (gene) of 30 and a sensitivity parameter (*deepSplit*) of 3 to define initial co-expression modules. Modules exhibiting high similarity (module eigengene distance < 0.25) were subsequently merged to reduce complexity. Modules demonstrating significant correlation with the AAA phenotype were identified as hub modules for downstream analysis. Gene Significance (GS) was calculated as the correlation between gene expression and the sample trait, and Module Membership (MM) was calculated as the correlation between gene expression and the module eigengene (ME). Intramodular hub genes were defined as genes meeting stringent criteria: $|MM| > 0.8$ and $|GS| > 0.5$, indicating strong association with both their module and the disease trait. Using a Venn diagram, overlap analysis was performed between DEGs identified *via* *limma* analysis and intramodular hub genes derived from significant WGCNA modules. Genes common to both sets were designated as candidate key genes for subsequent mechanistic investigation.

Machine learning analysis

To pinpoint core regulatory genes among the candidate key genes, machine learning-based feature selection was employed. Least Absolute Shrinkage and Selection Operator (LASSO) regression analysis was performed using the *glmnet* R package to identify a minimal set of highly predictive features within the training cohort. A 10-fold cross-validation procedure was implemented to optimize the regularization parameter (λ), selecting the value that minimized the binomial deviance. Genes with non-zero coefficients in the final model were retained as significant features. SVM-RFE analysis was conducted using the *e1071* R package to independently identify the most discriminative subset of genes from the candidate pool capable of classifying AAA versus control samples. Features (genes) were recursively eliminated based on their ranking importance derived from the SVM model. The optimal feature subset size was determined as the point yielding the lowest classification error rate during the elimination process. The Random Forest algorithm was implemented using the “randomForest” package in R. This ensemble learning method enhances prediction accuracy and stability by constructing a multitude of decision trees and outputting the mode of their classes. Following model training, the Mean Decrease Gini index for each gene was extracted as a metric of its importance in the classification. A higher value indicates a greater contribution of the gene to distinguishing between the disease and control groups. Genes with a Mean Decrease Gini index > 2 were retained as significant features selected by the Random Forest algorithm, a stringent empirical cutoff consistent with prior bioinformatics studies (Liang et al. 2022). The significant genes selected by LASSO regression and those identified as the optimal feature subset by SVM-RFE were independently intersected with the predefined list of mitochondrial homeostasis-related genes.

Validation and diagnostic performance assessment

The differential expression levels of the final key genes identified through the integrated approach were validated by generating box plots using both the integrated training dataset matrix and the independent validation dataset (GSE57691). This visually confirmed the expression patterns between AAA and NC samples. The diagnostic utility of the key genes in distinguishing AAA from NC samples was rigorously evaluated. Receiver Operating Characteristic (ROC) curves were constructed, and the area under the curve (AUC) was calculated for each key gene using both the integrated training data and the external validation dataset (GSE57691).

Immunocyte infiltration analysis

To investigate disparities in the immune microenvironment composition between AAA samples and NC samples, we

performed immunocyte infiltration analysis. Specifically, the relative abundances of 22 distinct leucocyte subpopulations within each sample of the integrated dataset were quantified using the IBERSORT algorithm. Subsequently, a stacked bar plot was generated to visualize the proportional distribution of these immune cell subsets across all samples, delineating patterns unique to the AAA and NC cohorts. Furthermore, to assess the potential association between the expression level of the target gene, IL2RB, and specific immune cell infiltration, correlation analysis was performed. Statistical comparisons of immune cell infiltration levels stratified by high versus low IL2RB expression groups (dichotomized based on median expression) were visualized using box plots.

Construction of rat AAA model

Male Sprague-Dawley rats (12 weeks old, body weight 350–370 g), from SPF (Beijing) Biotechnology Co., Ltd. (Beijing, China), were utilized for this study. Lentivirus encoding IL2RB short hairpin RNA (LV-sh-IL2RB) was generated by GeneChem Co., Ltd. (Shanghai, China), and lentivirus expressing scrambled shRNA (LV-sh-NC) used as a negative control was also obtained from GeneChem. All animal experimental procedures were rigorously reviewed and approved by our institution's Animal Care and Use Committee, adhering to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Rats were housed under standard conditions (12-hour light/dark cycle, $22 \pm 1^\circ\text{C}$, $50 \pm 10\%$ humidity) with ad libitum access to food and water. After 7 days of environmental acclimation, rats were divided into Sham, AAA, AAA+sh-NC, and AAA+sh-IL2RB groups ($n \geq 6/\text{group}$). To induce AAA, rats were anesthetized by inhalation of isoflurane (induction: 4–5% in oxygen; maintenance: 1.5–2.5%). Depth of anesthesia was confirmed by the absence of pedal reflex. A midline laparotomy incision was performed under aseptic conditions. The abdominal aorta segment between the renal arteries and the iliac bifurcation was carefully exposed and isolated from surrounding connective tissue using sterile rubber loops. Except for the Sham group, the isolated aortic segment was circumferentially wrapped with a sterile cotton swab saturated in 0.8 mol/l calcium chloride (CaCl_2) solution (Guo et al. 2022). The aorta of the Sham group was similarly wrapped with a sterile cotton swab saturated in sterile phosphate-buffered saline (PBS). Following the incubation period, the CaCl_2 or PBS-soaked swab was removed. The surgical site was copiously irrigated with warm sterile saline (37°C) to remove residual chemical irritants. The abdominal cavity was closed in layers using appropriate absorbable sutures. LV-sh-IL2RB and LV-sh-NC were used to intervene in the expression of IL2RB in rats treated with AAA induction by injecting 5×10^7 infectious lentiviral particles *per rat via* the tail vein (Yu et al. 2021). The rats in the Sham and AAA groups were injected

with normal saline in the same manner. Animals were monitored daily for signs of distress, infection, or complications for 14 days post-surgery and had free access to standard chow and water. On postoperative day 28, rats were anesthetized by inhalation of 5% isoflurane for blood collection and were then euthanized by cervical dislocation. The entire aorta from the aortic arch to the iliac bifurcation was carefully excised. To confirm the efficiency of lentiviral transduction and IL2RB knockdown specifically in the abdominal aortic tissue, the expression levels of IL2RB mRNA and protein were assessed by Real-time Quantitative Polymerase Chain Reaction (RT-qPCR) and Western blotting, respectively.

RT-qPCR

The mRNA expression levels of IL2RB, inflammatory factors interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and monocyte chemoattractant protein-1 (MCP-1) were detected by RT-qPCR. Briefly, total RNA was isolated from cell cultures and aortic tissues using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA), with RNA integrity verified electrophoretically. First-strand complementary DNA (cDNA) was synthesized from 1 μ g total RNA using the SweScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (VAZYME, Nanjing, China) according to the manufacturer's protocols. qPCR reactions were performed in triplicate using the ChamQ Universal SYBR® qPCR Master Mix (2 $\times$ concentration; Vazyme) on an ABI 7500 FAST Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The thermal cycling profile consisted of initial denaturation at 95°C for 30 s and 40 amplification cycles (denaturation at 95°C for 10 s and annealing at 60°C for 30 s). GAPDH served as the endogenous reference gene for normalization. Relative target gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. Primer sequences were provided Table S1 in Supplementary material.

Western blotting

Protein abundance of IL2RB, mitofusin 1 (MFN1), dynamin-related protein 1 (DRP1), and Parkin RBR E3 ubiquitin protein ligase (Parkin) was determined by Western blot analysis, using β -actin for normalization. Cellular proteins and aortic tissue proteins were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to PVDF membranes (Millipore, Danvers, MA, USA). Blocking was achieved with 5% skim milk. Primary antibodies applied overnight at 4°C included: anti-IL2RB (Invitrogen PA5-86323, 1:1000), anti-MFN1 (Abcam, ab221661, Cambridge, UK; 1:1000), anti-DRP1 (Abcam, ab184247; 1:1000), anti-Parkin (Abcam, ab77924; 1:1000), and anti- β -actin (Abcam, ab8227; 1:1000). After washing, membranes were exposed to horseradish peroxidase-conjugated secondary antibodies: goat anti-rabbit (Abcam ab288151, 1:5000) or goat anti-mouse

(Abcam ab205719, 1:2000), incubated at room temperature. Visualization of protein bands utilized chemiluminescence on the Tanon 5200 imager (Tanon, Shanghai, China).

Tissue processing and histochemical staining

Fixed abdominal aortic specimens (4% paraformaldehyde) underwent paraffin embedding. Serial cross-sections (5 μ m thickness) encompassing maximal aortic dilation were mounted on glass slides for histomorphometric analysis. For structural evaluation, sections underwent Hematoxylin and Eosin (H&E) staining and Verhoeff-van Gieson (VVG) staining. For H&E staining, following deparaffinization in xylene and rehydration through graded ethanols, nuclei were stained using Mayer's hematoxylin (5 min), rinsed in running water, briefly differentiated in acidified ethanol (0.7% hydrogen chloride), and blued in alkaline water (15 min). Cytoplasmic counterstaining employed eosin Y (30 s). For VVG staining, sections were incubated in Verhoeff's iron hematoxylin solution (30 min, RT), rinsed, differentiated to achieve elastic fiber specificity, dehydrated in 95% ethanol, and counterstained with eosin Y (30 s). Post-staining, sections were dehydrated in absolute ethanol, cleared in xylene, and permanently mounted with synthetic resin. Histoarchitectural features were examined under bright-field microscopy. Elastin degradation was graded as follows: Grade 1 = almost intact, well-organized elastic laminae, wavy morphology; Grade 2 = mild elastin degradation, flattening, and thinning of elastic laminae; Grade 3 = high elastic breakdown, fragmentation, and decreasing of elastic laminae; and Grade 4 = severe elastin destruction or loss (Watanabe et al. 2012).

Culture and treatment of VSMCs

Primary rat VSMCs were cultured in Dulbecco's modified eagle medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 4.5 g/l glucose, 100 U/ml penicillin, and 100 μ g/ml streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂. For experimental treatments, VSMCs were seeded into 6-well plates at 50–70% confluence and serum-starved in basal DMEM for 24 h to synchronize growth. Transient transfection with small interfering RNA (siRNA) targeting IL2RB (si-IL2RB) or scrambled control siRNA (si-NC) was performed using Lipofectamine 3000 reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. Following a 12-h post-transfection incubation, the medium was replaced with complete DMEM (containing 10% FBS and antibiotics). To establish AAA cell models, after an additional 24-h culture period, cells were exposed to 1 μ M angiotensin II (Ang-II; Sigma-Aldrich) for 24 h (Sulistiyowati et al. 2023). Therefore, cells were assigned

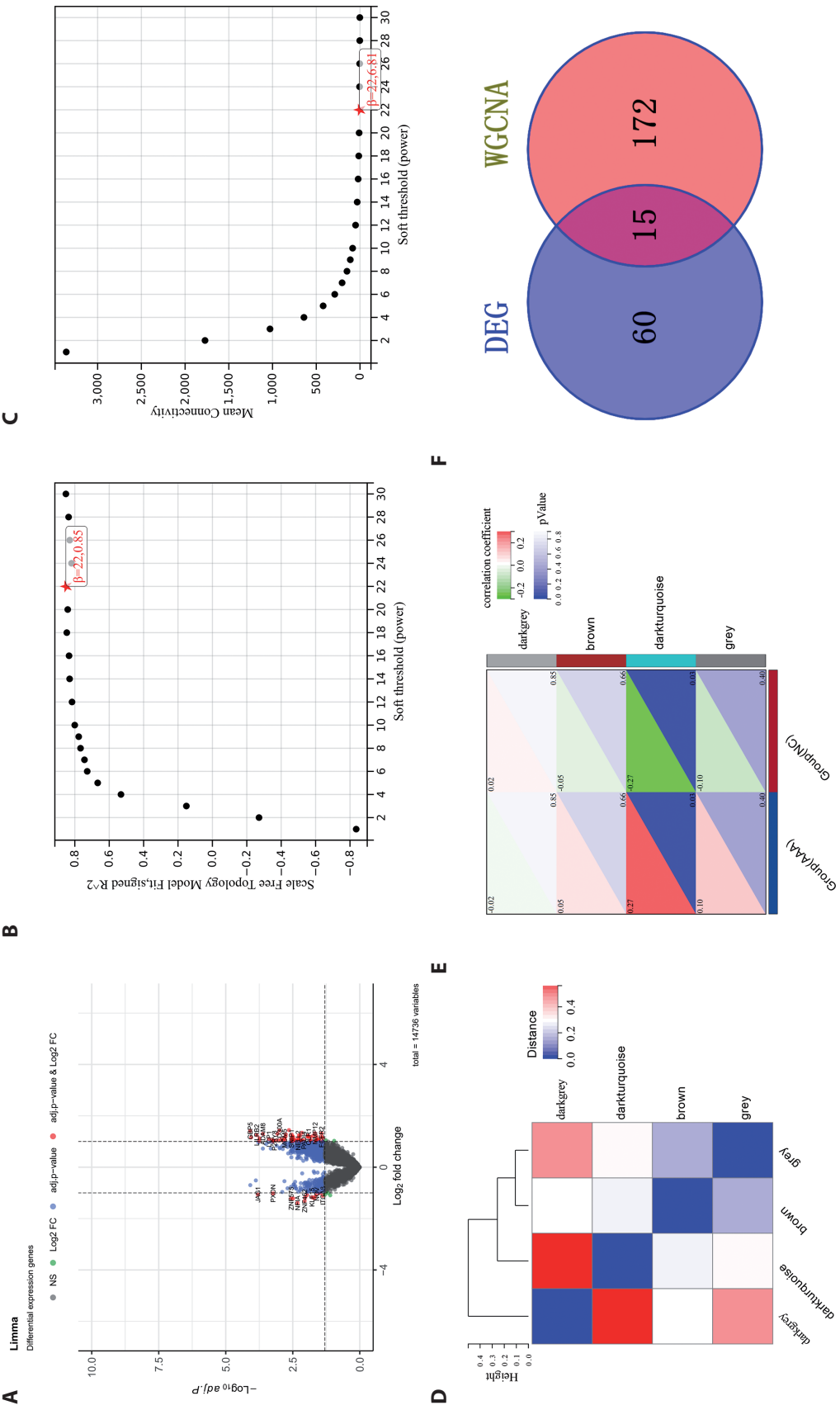


Figure 1. Identification of key genes and co-expression networks in AAA. **A.** A volcano plot depicting differential gene expression between AAA and normal control samples. The x-axis represented $\log_2$ (fold-change); the y-axis represented $-\log_{10}$ (adj. p -value). Red points denoted differentially expressed genes (DEGs) (adj. p -value < 0.05 , $|\log_2$ (fold-change)| ≥ 1 ; $n = 75$). **B.** Assessment of scale-free topology fit for WGCNA. The soft-thresholding power ($\beta = 22$) was selected. **C.** Dendrogram generated by average linkage hierarchical clustering of genes based on topological overlap matrix (TOM) dissimilarity, with assigned module colors indicated by the color band below. Module merging was performed for dissimilarity < 0.25 . **D.** Heatmap depicting module-trait associations between module eigengenes and the AAA phenotype. Color scale represented the correlation coefficient. The darkturquoise module exhibited the strongest significant association ($p < 0.05$) with AAA status. **E.** Venn diagram identifying 15 high-confidence candidate genes *via* intersection analysis between the 75 DEGs (Limma) and 187 intramodular hub genes within the AAA-associated darkturquoise module (WGCNA).

to four groups: Control (untreated cells), Ang-II (cells stimulated with Ang-II), Ang-II+si-NC (cells transfected with non-targeting control siRNA prior to Ang-II stimulation), and Ang-II+si-IL2RB (cells transfected with IL2RB-specific siRNA prior to Ang-II stimulation).

Assessment of cellular apoptosis

Apoptotic rates were quantified using flow cytometry with a dual-staining approach employing fluorescein isothiocyanate (FITC)-conjugated Annexin V and propidium iodide (PI) (Beyotime, Shanghai, China). Following experimental treatments, cells were harvested, washed twice with ice-cold PBS, and resuspended in Annexin V binding buffer containing FITC-Annexin V (5 μ l/ml) and PI (5 μ g/ml). After incubation for 15 min in the dark at ambient temperature, samples were diluted with additional binding buffer and immediately analyzed using a BD FACSCanto II flow cytometer (Becton Dickinson, Ashland, OR, USA). A sequential gating strategy was applied to ensure the analysis of single, viable cells. First, the main cell population was gated based on forward scatter-area (FSC-A) and side scatter-area (SSC-A) parameters to exclude debris (P1 gate, live cells). Subsequently, single cells were discriminated from doublets by gating on FSC-A versus forward scatter-height (FSC-H) within the P1 population (P2 gate). Finally, apoptotic cells were analyzed within the P2 gate on a FITC-Annexin V (*x*-axis) versus PI (*y*-axis) dot plot. Apoptotic cells were defined as follows: early apoptotic cells (Annexin V⁺/PI⁻) and late apoptotic/necrotic cells (Annexin V⁺/PI⁺). The total apoptotic rate was calculated as the sum of the percentages of early and late apoptotic/necrotic cells. Data were processed using FlowJo software.

Measurement of intracellular ATP levels

Cellular ATP content was determined using a bioluminescent ATP assay kit (Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's protocol. Briefly, treated VSMCs were lysed in ice-cold ATP extraction buffer. Cell lysates were centrifuged (12,000 $\times$ g, 10 min, 4°C), and supernatants were collected. Equal volumes of supernatant were mixed with the luciferase/luciferin working solution, and bioluminescence intensity was measured immediately using a microplate reader. ATP concentrations were interpolated from a concurrently generated ATP standard curve (0.1–10 μ M) and normalized to total protein concentration determined by bicinchoninic acid assay.

Evaluation of mitochondrial membrane potential ($\Delta\Psi_m$)

Alterations in $\Delta\Psi_m$ were assessed using the cationic fluorescent probe JC-1 (Beyotime). Post-treatment, VSMCs (seeded at 1×10^4 cells/well in 6-well plates) were incubated with JC-1

staining solution (5 μ g/ml in serum-free medium) for 20 min at 37°C in a CO₂ incubator. Cells were subsequently rinsed twice with pre-warmed PBS. Mitochondrial depolarization was indicated by a shift in JC-1 fluorescence from red aggregates (intact $\Delta\Psi_m$; excitation/emission 585/590 nm) to green monomers (depolarized $\Delta\Psi_m$; excitation/emission 514/529 nm). Fluorescent images were captured using a microscope equipped with a 40 $\times$ oil immersion objective. The red/green fluorescence intensity ratio *per cell* was quantified using ImageJ software, with decreased ratios signifying loss of $\Delta\Psi_m$.

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were obtained by centrifugation of the blood samples. We used corresponding ELISA kits (Esebio, Shanghai, China) to determine the content of malondialdehyde (MDA) and ROS in the serum of rats, in strict accordance with the instructions in the manual.

Statistical analysis

Data were presented as multiple groups of repeated data or means $\pm$ standard deviation and were processed by GraphPad Prism 7.0 statistical software (GraphPad, San Diego, CA, USA). The comparisons between two groups were determined by Student's *t*-test, and those among multiple groups were determined by one-way ANOVA with Tukey's post hoc analysis. A *p*-value less than 0.05 was considered statistically significant.

Results

Bioinformatic screening of DEGs

Raw gene expression data from datasets GSE7084, GSE47472, and GSE98278 were acquired from GEO. Following data integration and batch effect correction, density distribution plots and UMAP projection confirmed the successful removal of technical variability, showing convergent expression profiles and intermingled sample clustering without discernible batch-specific separation (Fig. S1A,B in Supplementary material). This spatial integration further confirmed the effective removal of batch effects. Applying stringent thresholds of adj *p* < 0.05 and $|\log_2\text{FC}| \geq 1$, we identified 75 DEGs. Among these, 61 genes were significantly upregulated and 14 genes were significantly downregulated in AAA tissues compared to NC controls. Unsupervised hierarchical clustering analysis of the 75 DEGs clearly distinguished AAA samples from NC controls. This differential expression signature was visualized using a volcano plot (Fig. 1A) and a heatmap (Fig. S2), illustrating the distinct expression patterns of the DEGs across all samples.

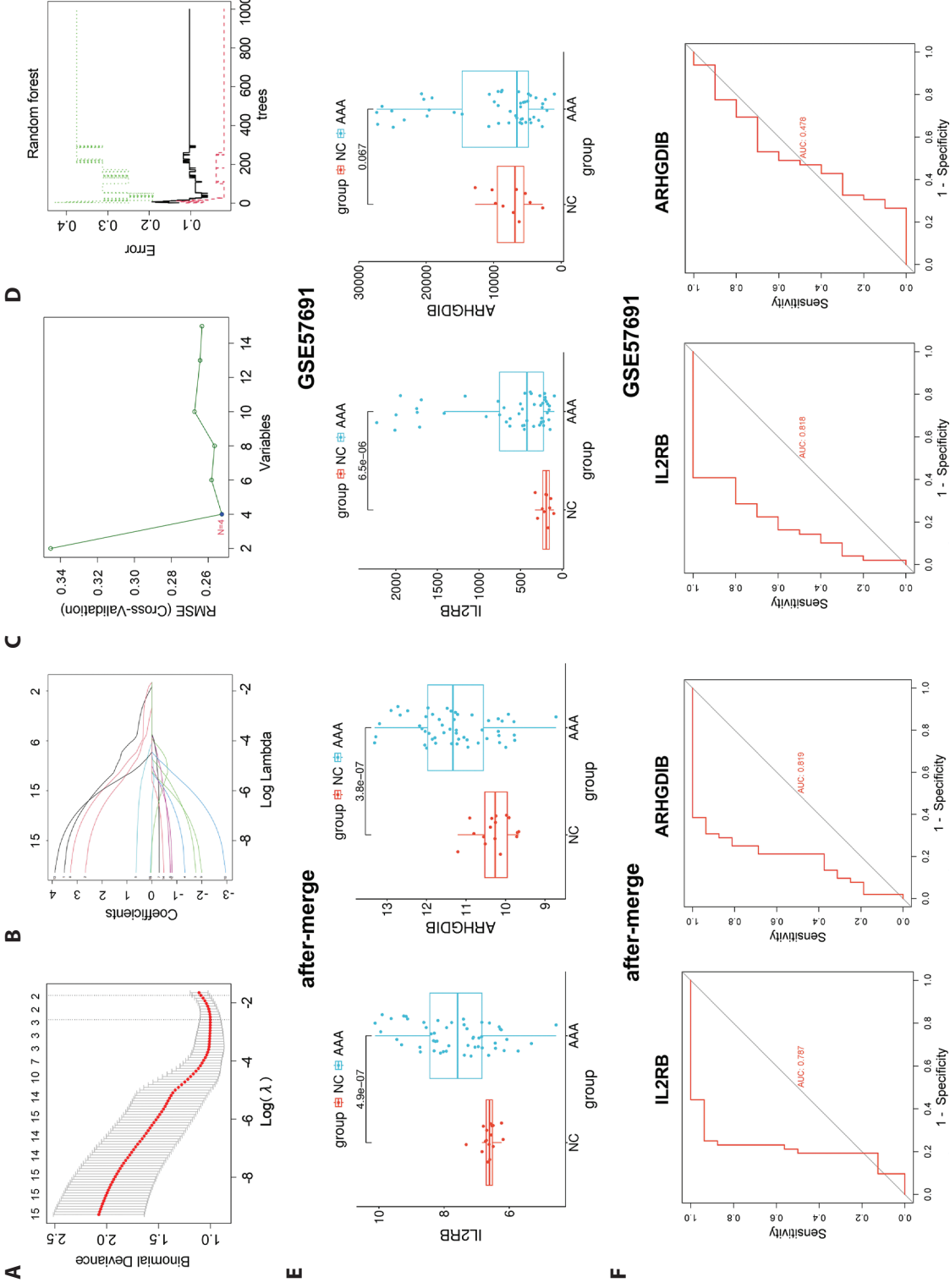


Figure 2. Machine learning-based gene prioritization and experimental validation. **A.** LASSO regression coefficient trajectories across regularization parameters. The vertical dotted line indicated the optimal $\lambda_{\min}$ selected by 10-fold cross-validation. **B.** Feature selection profile for LASSO analysis. Genes with non-zero coefficients at $\lambda_{\min}$ (dashed line) were retained. **C.** SVM-RFE classification error rate versus feature subset size. **D.** Random forest variable importance ranked by mean decrease in Gini index. The dashed horizontal line denoted the significance threshold (importance > 2). **E.** Differential expression validation of candidate genes. Boxplots depicted normalized expression values (y-axis) across AAA and control groups (x-axis); center lines represented medians. **** $p < 0.0001$. **F.** Diagnostic performance evaluation using ROC analysis.

To elucidate the biological significance of the 75 DEGs, we performed functional enrichment analysis. Significantly enriched GO terms were categorized into three ontologies: BP, CC, and MF. Within each ontology, we identified the top 10 most significantly enriched terms. Immune system process and response to stress were predominant BP terms (Fig. S3A). Plasma membrane part, cytoplasmic vesicle, and intracellular vesicle were enriched in CC (Fig. S3B). Identical protein binding, signaling receptor activity, and molecular transducer activity were key MF terms (Fig. S3C). The top 10 most significantly enriched KEGG pathways were visualized using a multidimensional bubble plot (Fig. S3D), showing prominent enrichment in the chemokine signaling pathway and cytokine-cytokine receptor interaction.

WGCNA and key gene identification

To construct a biologically meaningful co-expression network, we first determined the optimal soft-thresholding power. Analysis of network topology for various soft-thresholding powers (β) indicated that $\beta = 22$ was the lowest value that achieved a scale-free topology fit index (R^2) of 0.85 (Fig. 1B), while retaining a mean connectivity of 6.81 (Fig. 1C), thus satisfying the standard scale-free network criterion. Module-trait associations were calculated correlating each module's eigengene with the AAA phenotype. The darkturquoise module demonstrated the strongest and statistically significant association with AAA status ($p < 0.05$) and was consequently designated as the hub module for downstream analysis (Fig. 1D). Within the darkturquoise module, 187 intramodular hub genes exhibiting high connectivity and strong phenotypic association were identified, with stringent thresholds, $|MM| > 0.8$ and $|GS| > 0.5$ (Fig. 1E). To pinpoint genes central to AAA pathogenesis, an intersection analysis was performed between the 75 DEGs and the 187 hub genes identified within the darkturquoise module *via* WGCNA, yielding 15 candidate key genes exhibiting both differential expression and high intramodular connectivity/phenotypic association (Fig. 1F). These candidate genes were prioritized for subsequent functional validation and mechanistic studies.

Machine learning-driven identification of core mitochondrial regulators

To refine the 15 candidate key genes into core regulators of mitochondrial dysfunction in AAA, we implemented a tripartite machine learning framework. This approach identified Rho GDP-dissociation inhibitor beta (ARHGDIB) through LASSO regression (Fig. 2A,B) and IL2RB *via* SVM-RFE analysis (Fig. 2C). In parallel, random forest analysis ranked variable importance based on the mean decrease Gini, with no genes meeting the importance threshold of >2 (Fig. 2D). The intersection of these results with a predefined

list of mitochondrial homeostasis-related genes established ARHGDIB and IL2RB as prime candidates linking IL2RB signaling to mitochondrial dysregulation in AAA pathogenesis.

To validate the differential expression and diagnostic efficacy of ARHGDIB and IL2RB, we performed comprehensive analyses across integrated and independent cohorts. Boxplot analysis of the harmonized training dataset revealed significant transcriptional upregulation of both ARHGDIB and IL2RB in AAA compared with controls (Fig. 2E). External validation using dataset GSE57691 showed that IL2RB maintained significant overexpression, but ARHGDIB showed non-significant expression trends (Fig. 2E). ROC curve analysis demonstrated that the AUC of ARHGDIB was 0.819 in the integrated dataset and 0.478 in GSE57691, while the AUC of IL2RB was 0.787 and 0.818 respectively (Fig. 2F).

Analysis of immune cell infiltration

We employed the CIBERSORT algorithm to quantify the relative abundances of 22 immune cell subsets within each sample of the integrated dataset. The results were visualized as a stacked bar plot (Fig. 3A), demonstrating distinct distribution patterns of immune infiltrates between groups. Specifically, macrophages and CD4+ T cells emerged as the predominant cell populations in AAA samples, highlighting their potential role in disease pathogenesis. Further analysis *via* boxplot (Fig. 3B) revealed significant correlations between IL2RB expression and infiltration of CD4+ T cells, macrophages, dendritic cells (DCs), eosinophils, and neutrophils. Notably, IL2RB expression was associated with increased infiltration of CD4+ memory activated T cells, Monocytes, M0 macrophages, resting DCs, eosinophils, and neutrophils, suggesting that IL2RB may modulate the immune landscape in AAA by regulating immune cell recruitment.

Expression validation in rat AAA model

To visualize structural alterations in the abdominal aorta, H&E staining was performed. The Sham group exhibited intact aortic walls with well-ordered laminar structures, while the AAA group showed disrupted vascular architecture, including rupture of elastic lamellae, disorganized thinning, sparse arrangement, and increased medial elastin degradation (Fig. 4A). VVG staining further revealed fragmented elastic fibers, disordered collagen arrangement, fibrous hyperplasia, extensive elastic fiber loss, and thinning of the vessel wall in AAA tissues compared to Sham controls (Fig. 4B). The aortic elastin degradation score was significantly elevated in the AAA group, indicating severe elastin disruption (Fig. 4B). RT-qPCR analysis showed that mRNA levels of inflammatory factors TNF- α , IL-6, and MCP-1 were

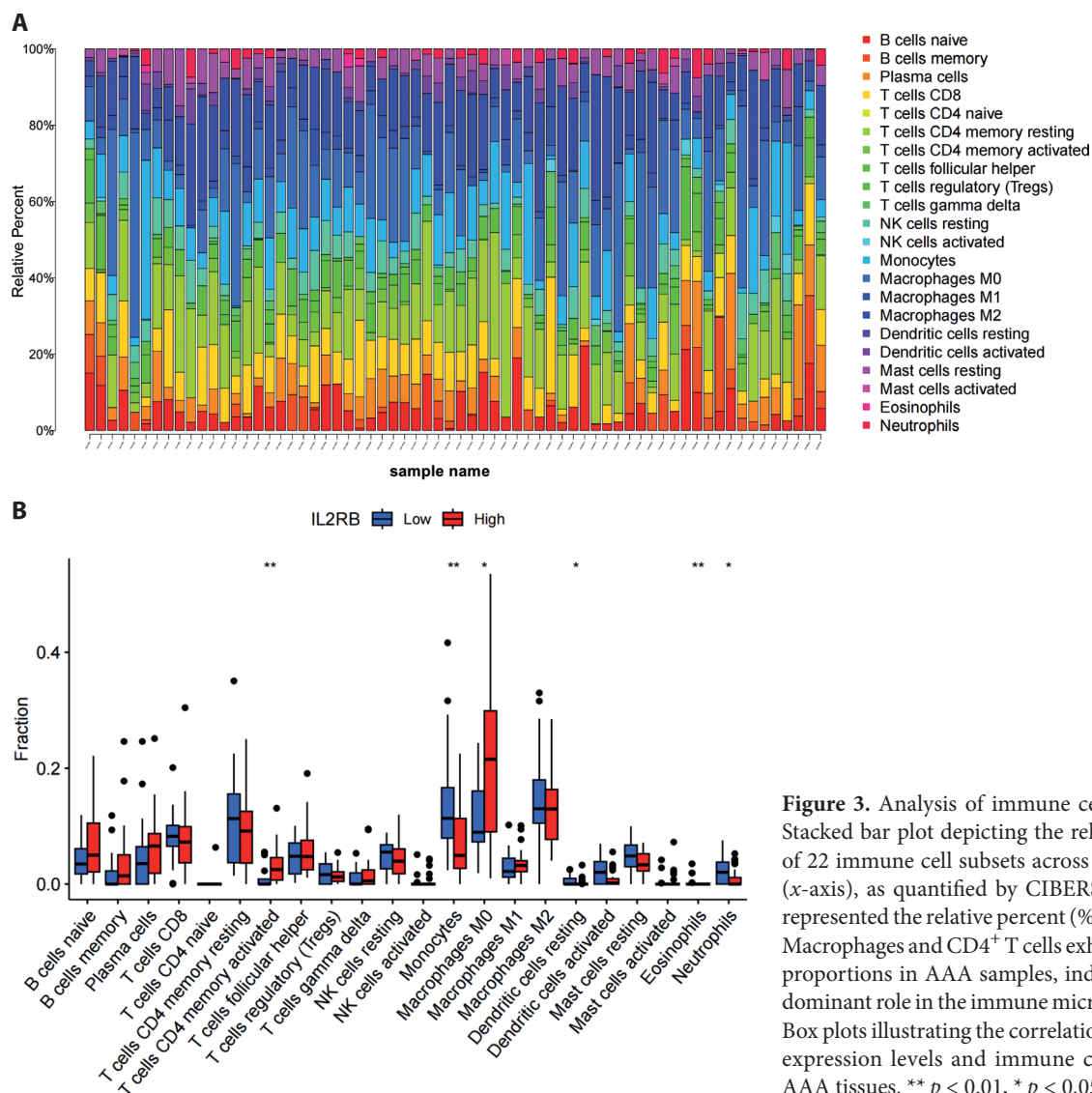


Figure 3. Analysis of immune cell infiltration. **A.** Stacked bar plot depicting the relative abundances of 22 immune cell subsets across different samples (*x*-axis), as quantified by CIBERSORT. The *y*-axis represented the relative percent (%) of each cell type. Macrophages and CD4⁺ T cells exhibited the highest proportions in AAA samples, indicating their predominant role in the immune microenvironment. **B.** Box plots illustrating the correlation between IL2RB expression levels and immune cell infiltration in AAA tissues. ** $p < 0.01$, * $p < 0.05$.

markedly upregulated in AAA tissues relative to the Sham group (Fig. 4C). Western blotting assays for mitochondrial homeostasis-related proteins demonstrated reduced expression of MFN1 and Parkin, but increased DRP1 expression in the AAA group compared to the Sham group (Fig. 4D). Consistently, both RT-qPCR and western blotting analyses revealed significantly higher IL2RB expression at both mRNA and protein levels in AAA tissues (Fig. 4E,F).

IL2RB knockdown modulates mitochondrial homeostasis to attenuate VSMC dysfunction

The results of RT-qPCR revealed that siRNA-mediated silencing (si-IL2RB-1, -2, -3) achieved a significant reduction in IL2RB mRNA compared to si-NC controls (Fig. 5A), with si-IL2RB-1 exhibiting the highest silencing efficiency

and used in subsequent experiments. Compared with the Control group, Ang-II stimulation induced significant IL2RB upregulation, while co-transfection with si-IL2RB markedly reduced IL2RB mRNA levels compared to the Ang-II+si-NC group (Fig. 5B). Western blotting confirmed consistent protein expression trends, with Ang-II-induced IL2RB upregulation reversed by si-IL2RB transfection (Fig. 5C). Flow cytometry analysis showed that Ang-II induced a marked increase in VSMC apoptosis (Fig. 5D). A lower apoptosis rate was observed in the Ang-II+si-IL2RB group compared to the Ang-II+si-NC group (Fig. 5D). RT-qPCR further demonstrated elevated mRNA levels of inflammatory factors IL-6, TNF- α , and MCP-1 in Ang-II-treated cells, whereas si-IL2RB transfection significantly reduced their expression relative to the Ang-II+si-NC group (Fig. 5E). Mitochondrial functional assessment showed IL2RB

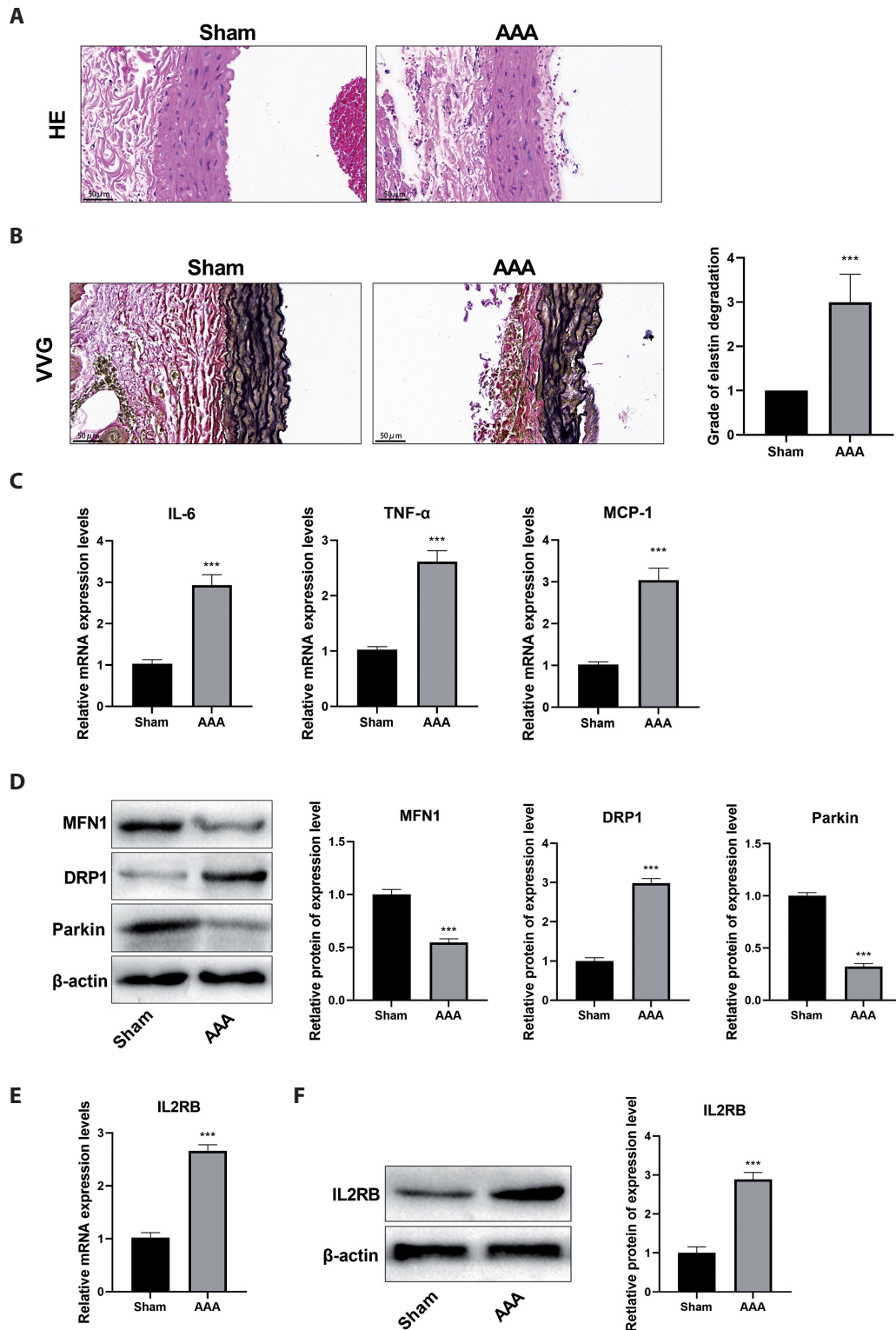


Figure 4. Expression validation in the rat AAA model. **A.** H&E staining for morphological assessment of abdominal aortic structure (magnification: 400 $\times$, scale bar: 50 μ m). **B.** VVG staining for elastic fiber visualization and aortic elastin degradation scoring (magnification: 400 $\times$, scale bar: 50 μ m). **C.** RT-qPCR analysis of mRNA levels for inflammatory factors IL-6, TNF- α , and MCP-1. **D.** Western blot detection of mitochondrial homeostasis-related proteins, MFN1, DRP1, and Parkin. **E.** RT-qPCR quantification of IL2RB mRNA expression. **F.** Western blotting analysis of IL2RB protein expression. *** $p < 0.001$ vs. Sham group.

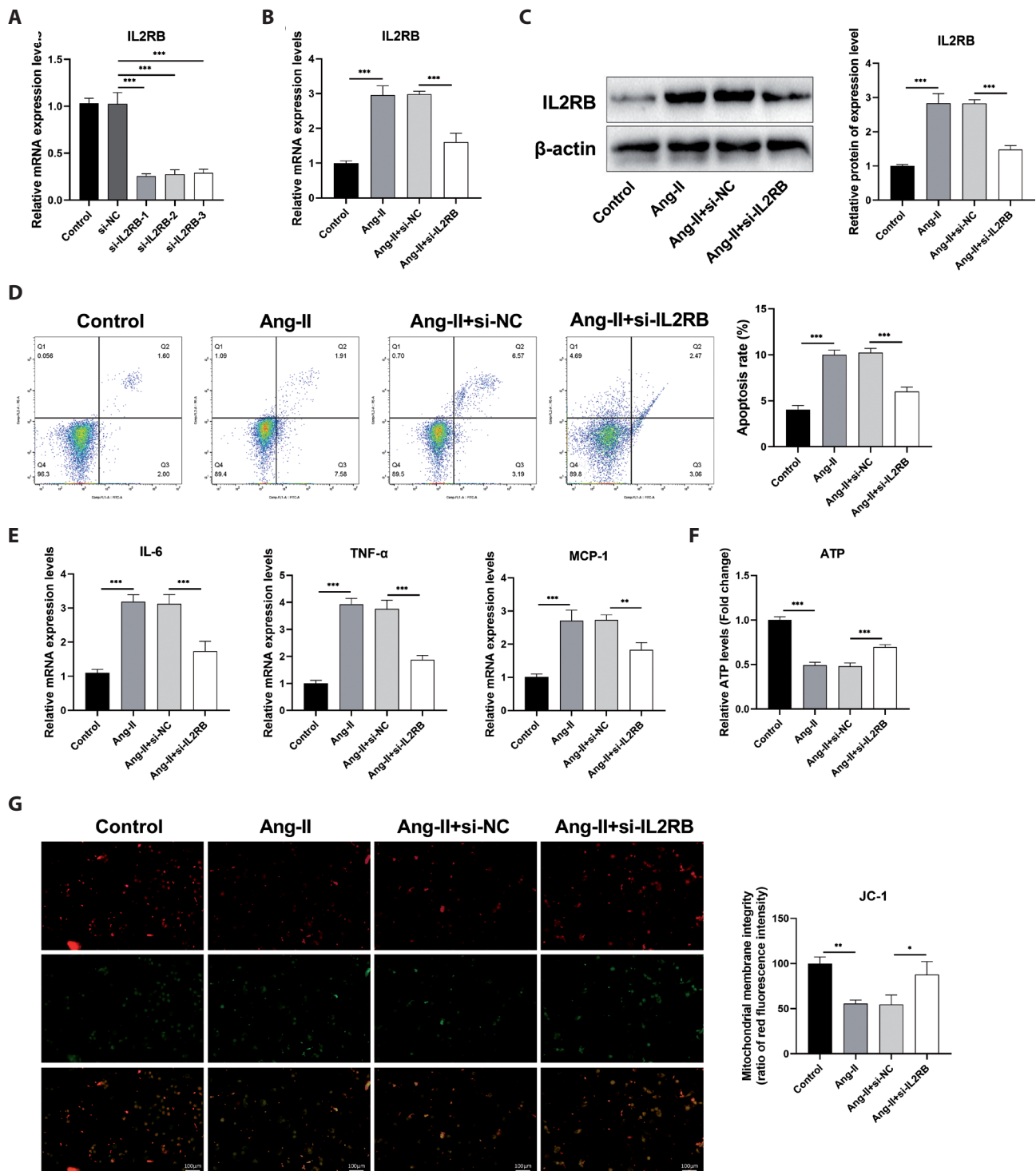


Figure 5. IL2RB knockdown modulated mitochondrial homeostasis to attenuate the dysfunction of VSMCs. **A.** RT-qPCR analysis for IL2RB transfection efficiency in VSMCs. **B.** RT-qPCR quantification of IL2RB mRNA expression levels. **C.** Western blotting analysis of IL2RB protein expression. **D.** Flow cytometry analysis of cell apoptosis rate using Annexin V/Propidium iodide staining. **E.** RT-qPCR detection of inflammatory factors (IL-6, TNF- α , MCP-1) mRNA levels. **F.** Mitochondrial functional assessment by detecting the intracellular ATP concentration. **G.** Fluorescence microscopy images of JC-1 staining in VSMCs (magnification: 200 $\times$, scale bar: 100 μ m), with quantification of the red/green fluorescence ratio reflecting mitochondrial membrane potential. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

knockdown counteracted Ang-II-induced ATP depletion (Fig. 5F). JC-1 staining indicated that relative to the Control group, Ang-II caused mitochondrial membrane potential depolarization, according to the reduced red/green fluorescence ratio. IL2RB silencing prevented Ang-II-triggered mitochondrial depolarization, suggesting IL2RB knockdown preserved membrane integrity (Fig. 5G).

IL2RB knockdown attenuates AAA progression via mitochondrial pathway regulation

Histopathological analysis by H&E staining revealed well-organized aortic wall architecture in the Sham group. AAA-induced rats exhibited severe structural disruption, including elastic lamina fragmentation, medial thinning, and increased external diameter. IL2RB knockdown (AAA+sh-IL2RB) substantially restored aortic wall organization and reduced dilatation compared to the AAA+sh-NC group (Fig. 6A). Quantitative analysis demonstrated that the maximum external aortic diameter was significantly increased in the AAA

group compared to the Sham group (Fig. 6A). In contrast, IL2RB knockdown markedly reduced the aortic diameter in the AAA+sh-IL2RB group, representing a 32.47% decrease compared to the AAA+sh-NC group (Fig. 6A). VVG staining demonstrated intact parallel elastic fibers (black) in Sham controls (Fig. 6B). AAA and AAA+sh-NC groups showed extensive elastic fiber fragmentation, collagen disorganization, and neointimal hyperplasia (Fig. 6B). The elastin degradation score was significantly elevated in the AAA group compared to the Sham group (Fig. 6B). IL2RB knockdown partially restored elastic fiber integrity, showing a 45% reduction in degradation score compared to the AAA+sh-NC group (Fig. 6B). These quantitative changes in aortic diameter and elastin degradation were further detailed in Table S2. RT-qPCR and Western blotting analyses confirmed AAA-induced IL2RB up-regulation relative to Sham controls, while sh-IL2RB delivery effectively reversed this induction compared to the AAA+sh-NC group at both mRNA (Fig. 6C) and protein (Fig. 6D) levels.

RT-qPCR analysis of inflammatory factors revealed significantly upregulated IL-6, TNF- α , and MCP-1 mRNA

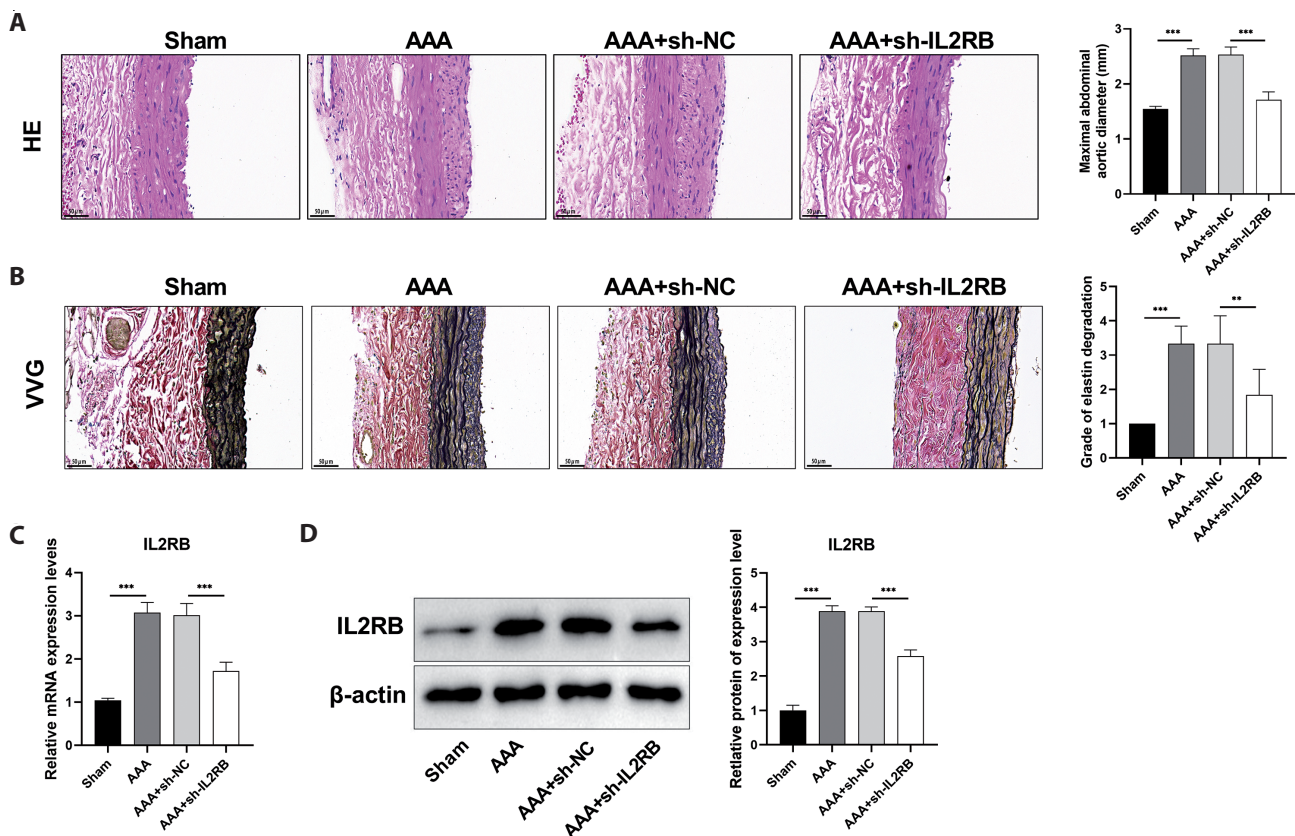


Figure 6. IL2RB knockdown attenuated aortic wall degeneration in AAA rat models. **A.** Representative H&E-stained sections demonstrating histopathological alterations in abdominal aortas (magnification: 400 $\times$, scale bar: 50 μ m) and quantitative analysis of maximum external aortic diameters. **B.** VVG staining evaluating elastic fiber integrity and corresponding elastin degradation scores (magnification: 400 $\times$, scale bar: 50 μ m). **C.** IL2RB mRNA expression quantified by RT-qPCR. **D.** Western blotting analysis confirmed IL2RB protein expression patterns consistent with the mRNA level. *** $p < 0.001$, ** $p < 0.01$.

levels in AAA tissues compared to the Sham controls (Fig. 7A). In contrast, AAA+sh-IL2RB rats exhibited marked downregulation of these cytokines compared to AAA+sh-NC rats (Fig. 7A). ATP assay showed a significant reduction in aortic ATP levels in the AAA group relative to the Sham group, while AAA+sh-IL2RB rats displayed restored ATP production relative to the AAA+sh-NC group (Fig. 7B). ELISA results demonstrated elevated MDA and ROS levels in AAA tissues, indicative of oxidative stress, which were

significantly attenuated by IL2RB knockdown (Fig. 7C,D). Quantitative data for inflammatory cytokines, oxidative stress markers, and mitochondrial function, and the percentage changes relative to the AAA+sh-NC group, were provided in Table S3. Western blotting analysis of mitochondrial homeostasis proteins revealed increased DRP1 expression and decreased MFN1/Parkin expression in AAA tissues compared to the Sham group (Fig. 7E). Conversely, rats in the AAA+sh-IL2RB group showed reduced DRP1

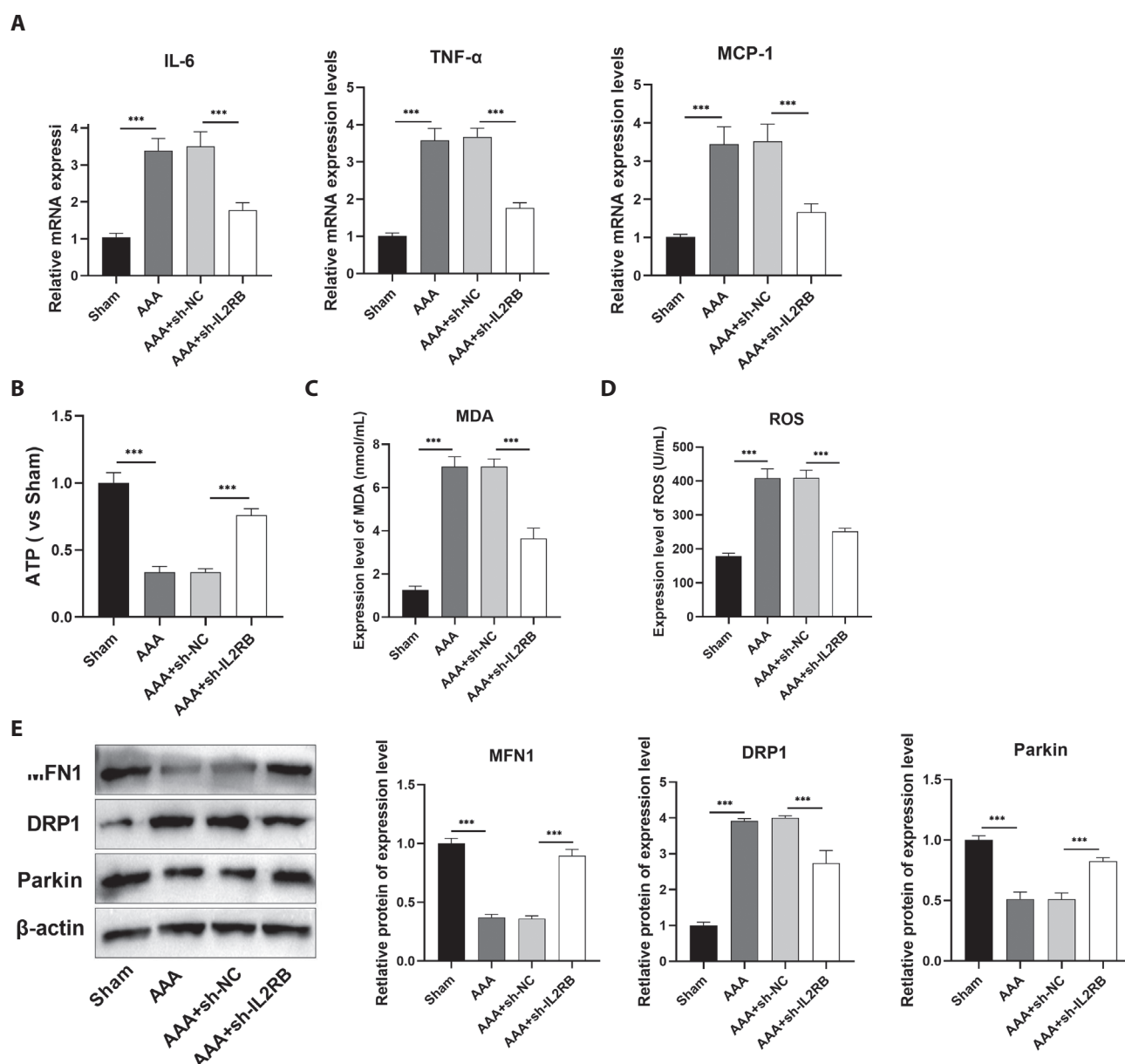


Figure 7. IL2RB knockdown attenuated AAA Progression *via* mitochondrial pathway regulation. **A.** RT-qPCR analysis of mRNA levels for inflammatory cytokines IL-6, TNF- α , and MCP-1. **B.** Mitochondrial functional assessment by detecting the intracellular ATP concentration. **C,D** Detection of oxidative stress markers MDA and ROS. **E.** Western blotting analysis of MFN1, DRP1 and Parkin. *** $p < 0.001$.

and restored MFN1/Parkin expression compared with the AAA+sh-NC group (Fig. 7E), suggesting that IL2RB knockdown could mitigate oxidative stress, rescue mitochondrial dysfunction, and suppress inflammatory responses in AAA, likely by regulating mitochondrial dynamics proteins.

Discussion

AAA remains a critical vascular pathology characterized by progressive aortic dilation and high rupture-related mortality, yet lacks effective pharmacotherapies to halt its progression (Baman et al. 2022). Current interventions, limited to surgical repair for advanced cases, are challenged by suboptimal durability and persistent post-operative rupture risk (Golledge 2019), underscoring an urgent need for novel molecular targets. Mounting evidence implicates mitochondrial dysfunction as a pivotal driver of AAA pathogenesis, wherein VSMC apoptosis, oxidative stress, and impaired energy metabolism contribute to aortic wall degeneration (Cooper et al. 2021; Yang et al. 2025). In this study, through integrated bioinformatics analysis of human AAA datasets, GSE7084, GSE47472, and GSE98278, combined with WGCNA and machine learning LASSO and SVM-RFE, we identified IL2RB as a key regulator intersecting mitochondrial homeostasis and AAA development. Experimental validation in rat AAA models and Ang-II-stimulated VSMCs demonstrated that IL2RB knockdown could attenuate AAA progression by ameliorating mitochondrial dysfunction, reducing inflammation, and suppressing VSMC apoptosis.

Bioinformatic approaches have become indispensable for identifying novel therapeutic targets in complex diseases like AAA (Xiong et al. 2022; Su et al. 2024). In recent years, machine learning approaches have emerged as a prominent novel paradigm for analyzing large-scale medical data, capturing nonlinear dependencies, and extracting subtle insights from continuous variables, thereby enabling the identification of critical determinants and enhancing predictive accuracy (Li et al. 2023). Notably, these algorithms demonstrate exceptional proficiency in deciphering high-dimensional patterns within genomic datasets, outperforming traditional statistical methodologies in this domain (Beam et al. 2018). As another pivotal bioinformatics methodology, WGCNA enables the investigation of associations between gene regulatory networks and diseases, inference of gene co-expression linkages, and detection of highly interconnected gene modules (Wang et al. 2023). In this study, we integrated Limma-based differential expression analysis with WGCNA, followed by dual machine learning feature selection, identifying ARHGDIB and IL2RB as core regulators intersecting mitochondrial homeostasis and AAA pathogenesis. Although ARHGDIB was selected from the training cohort due to its strong association with the disease trait and

mitochondrial pathways, its lack of significant expression and poor diagnostic performance (AUC = 0.478) in the independent validation cohort underscore the inherent risk of false-positive discoveries in high-throughput analyses and highlight the critical importance of external validation. In contrast, IL2RB maintained consistent upregulation and robust diagnostic accuracy (AUC = 0.818) across both cohorts, suggesting its superior reliability as a candidate biomarker. This multilayered computational strategy, culminating in rigorous validation, effectively prioritizes the most translationally promising targets for experimental investigation, which is why we subsequently focused our mechanistic studies on IL2RB.

Mitochondrial dysfunction has emerged as a pivotal therapeutic target for AAA, as evidenced by its involvement in VSMC dysfunction and aortic wall degradation (Summerhill et al. 2021; Yang et al. 2025). In AAA, mitochondrial dysfunction is characterized by impaired oxidative phosphorylation, excessive ROS production, and disrupted mitochondrial dynamics characterized by fission and fusion imbalance (Yuan et al. 2015; Jin et al. 2021; Tokuyama et al. 2023). This dysfunction arises from multiple mechanisms, including AngII-induced DRP1 phosphorylation at Ser616, which promotes mitochondrial fragmentation and reduces mitochondrial membrane potential (Lim et al. 2015). Concurrently, reduced peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1 α) expression – mediated by transforming growth factor- β signaling in Fibulin-4-deficient models – disrupts mitochondrial biogenesis and respiration, exacerbating energy metabolism deficits in VSMCs (van der Pluijm et al. 2018). The consequences of mitochondrial dysfunction in AAA include VSMC phenotypic switching from contractile to synthetic states, enhanced apoptosis, and extracellular matrix degradation. Excessive ROS generated by dysfunctional mitochondria activates NF- κ B signaling, promoting inflammatory cytokine release, such as IL-6, TNF- α , and MCP-1, and matrix metalloproteinase expression, which degrade elastic fibers and collagen (Sies et al. 2017; Summerhill et al. 2021). Additionally, impaired mitophagy evidenced by reduced Parkin-mediated clearance of damaged mitochondria further perpetuates oxidative stress and VSMC senescence (Zamorano-Leon et al. 2020). Current therapeutic strategies targeting mitochondrial dysfunction include pharmacological inhibition of DRP1, such as mitochondrial division inhibitor 1, which attenuates AAA progression by preserving mitochondrial fusion (Cooper et al. 2021), and genetic modulation of PGC1 α to restore mitochondrial respiration (van der Pluijm et al. 2018). In this study, IL2RB knockdown significantly attenuated aortic dilation in rat models, concurrently restoring mitochondrial homeostasis *via* DRP1 downregulation and MFN1/Parkin upregulation. In VSMCs, IL2RB silencing reversed Ang-II-induced ATP depletion, suppressed $\Delta\Psi_m$ collapse,

and reduced inflammatory cytokine secretion, mitigating VSMC apoptosis. This positions IL2RB as a novel regulator of mitochondrial homeostasis in AAA, offering a mechanistic basis for therapies targeting immunometabolic crosstalk in aortic remodeling.

Although our findings indicate that IL2RB knockdown modulates the expression of mitochondrial dynamics regulators such as DRP1, MFN1, and Parkin, the precise molecular pathways linking IL2RB to these proteins remain to be fully elucidated. Based on existing literature, we speculate that IL2RB may exert its effects indirectly through the PI3K/AKT signaling pathway, which is known to regulate DRP1 phosphorylation and mitochondrial fission (Gao et al. 2020; Chen et al. 2024). Furthermore, IL2RB has been shown to activate AKT in other cellular contexts (Meng et al. 2024), supporting the plausibility of this pathway. Future studies are warranted to experimentally validate whether IL2RB influences mitochondrial dynamics *via* PI3K/AKT activation or other yet unidentified mechanisms.

In conclusion, this study identifies IL2RB as a novel and critical regulator of AAA pathogenesis, mechanistically linking it to mitochondrial dysfunction in VSMCs. We demonstrated that IL2RB silencing significantly attenuated AAA progression *in vivo* and mitigated VSMC apoptosis and inflammation *in vitro*, which were mediated through restoring mitochondrial dynamics. These findings provide new insights into AAA pathogenesis and highlight the potential of mitochondrial pathway modulation for disease prevention and treatment.

Ethics approval. The experiments conformed to the Guide for the Care and Use of Laboratory Animals. The animal study has been approved by the Animal Ethics Committee of Guangzhou Red Cross Hospital, Jinan University. All methods are reported in accordance with ARRIVE guidelines.

Availability of data and materials. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest. The authors declare that they have no competing interests.

Author contribution. PL: conceptualization, formal analysis, methodology, writing-original draft, validation, resources; HZ and JH: formal analysis, methodology, validation, editing; YG: data curation, investigation, software, review and editing. All authors have read and approved the manuscript.

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