

Bioinspired IDO1-loaded Cu/ZIF for alopecia areata therapy via remodeling immune privilege

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Abstract

Alopecia areata (AA) is an autoimmune disease driven by the collapse of hair follicle immune privilege. Currently available treatments showed limited efficacy and high recurrence rates. Indoleamine 2,3-dioxygenase 1 (IDO1) has been recognized as critical enzyme by catalyzing tryptophan-to-kynurenine conversion to induce immunosuppression and maintain immune privilege. Aberrant IDO1 inactivation disrupts hair follicle immune privilege, causing inflammatory follicular damage and subsequent hair loss. Herein, we designed an IDO1-loaded Cu-doped zeolitic imidazolate framework (IDO@Cu/ZIF) to restore tryptophan-to-kynurenine metabolism, thereby rebuilding hair follicle immune privilege for long-term AA management. By mimicking the Cu/Zn centers in natural antioxidant enzymes, Cu/ZIF nanozymes deliver high capability to eliminate excessive reactive oxygen species in AA lesions. Meanwhile, Cu atom-induced upshift of the d-band center enhances substrate adsorption, thereby improving antioxidant performance. *In vitro* and *in vivo* experiments confirmed that IDO@Cu/ZIF reconstructs hair follicle immune privilege by restoring the tryptophan/kynurenine axis, thereby promoting hair follicle regeneration and achieving approximately 75% hair coverage. More importantly, IDO@Cu/ZIF treatment maintains long-term immune privilege and effectively suppresses AA recurrence. This study offers mechanistic insights into the rational design of high-performance antioxidant nanozymes and expands their potential applications in autoimmune diseases of AA.

Introduction

The collapse of immune privilege is a common core trigger of autoimmune diseases, which typically involve immune dysregulation and aberrant attacks on normal self-tissues and cells, thereby causing disease. Alopecia areata (AA) is a common autoimmune skin disease characterized by recurrent, non-scarring hair loss, imposing a substantial psychosocial burden on patients^{1,2}. The collapse of hair follicle immune privilege is a defining feature of AA pathogenesis. In healthy hair follicles, cells are maintained in a state of local immune tolerance, protecting them from recognition and attack by the autoimmune system²⁻⁴. Under physiological conditions, regulatory T cells (Tregs) actively maintain this immune privilege, suppress autoreactive lymphocytes and protect the hair follicle stem-cell niche from autoimmune insult^{5,6}. When hair follicle immune privilege collapses, cytotoxic CD8⁺T cells infiltrate the anagen bulb and inflict damage by secreting interferon- γ (IFN- γ), perforin and granzyme^{2,3,7,8}. Therefore, restoring Treg-supported hair follicle immune privilege represents a rational therapeutic strategy for AA^{5,6,9}. Although Janus kinase (JAK) inhibitors (e.g., baricitinib) can effectively suppress inflammatory signaling and alleviate CD8⁺T cell-mediated follicular damage, they fail to restore the intrinsic immune privilege of hair follicles, leading to frequent recurrence of AA after drug withdrawal¹⁰⁻¹⁴. Moreover, JAK inhibitors are administered orally, posing systemic toxicity and an increased risk of infection¹⁵⁻¹⁸. Therefore, there is an urgent need to develop localized skin immunotherapeutic strategies to reconstruct hair follicle immune privilege and improve therapeutic outcomes in AA¹⁹⁻²².

Indoleamine 2,3-dioxygenase 1 (IDO1) is a key metabolic checkpoint for immune tolerance, converting tryptophan (Trp) to immunosuppressive kynurenine (Kyn) to activate aryl hydrocarbon receptor (AHR)-dependent regulatory programs that support Treg differentiation and restrain effector T-cell responses, thereby inducing tissue immune privilege^{23–28}. Impairment of the IDO1-Trp/Kyn-AHR axis can therefore weaken Treg function, promote cytotoxic CD8⁺T-cell activation and contribute to collapse of hair follicle immune privilege^{23,24,27,28}. Meanwhile, excessive reactive oxygen species (ROS) generated during AA inflammation further enhance the activity of cytotoxic CD8⁺T cells and exacerbating hair follicle damage^{29,30}. Moreover, excessive ROS inhibit the catalytic activity of IDO1, creating a vicious cycle between inflammatory injury and immune privilege collapse. Consistent with this mechanism, transcriptomic analysis of AA lesional scalp revealed concurrent suppression of the IDO1-Trp/Kyn-AHR axis and activation of oxidative stress responses (Fig. 1A). Therefore, restoring IDO1-mediated Trp-Kyn conversion and alleviating oxidative stress provide a rational basis for reconstructing immune privilege through localized antioxidant-immunometabolic therapy, enabling long-term and safe treatment of AA^{24,26–31}. However, direct administration of free IDO1 may be limited by its structural instability, susceptibility to proteolytic degradation and poor lesion retention, which may reduce its local availability and catalytic efficacy^{32,33}.

Nanozyme-engineered metal-organic frameworks (MOFs) provide a rational platform for constructing multifunctional nanoreactors that integrate antioxidant catalysis with enzyme-mediated biochemical reactions^{33,34}. ZIF-8, a Zn-imidazolate framework, is well suited for this purpose because Zn²⁺ nodes and 2-methylimidazole linkers self-assemble into porous particles under mild and biomacromolecule-compatible conditions³⁵. Moreover, biomimetic mineralization enables enzymes and other biomacromolecules to be encapsulated within ZIF-based matrices, thereby improving protein stability while preserving catalytic activity³³. Beyond its role as a carrier of natural enzymes and biomolecules, the ZIF-8 framework also provides a structurally relevant basis for constructing a superoxide dismutase (SOD)-mimetic nanozyme. Natural Cu, Zn-superoxide dismutase contains a Cu/Zn bimetallic center coordinated by histidine-derived imidazole nitrogen atoms, in which Cu undergoes redox cycling during superoxide dismutation, whereas Zn contributes to stabilization of the local coordination environment^{36,37}. Inspired by this structural motif, incorporation of Cu into the Zn-imidazolate framework can generate a Cu/Zn-N coordination environment with SOD-like catalytic activity^{36–38}. Copper-based nanozymes can recapitulate key redox features of natural Cu, Zn-SOD, enabling catalytic superoxide dismutation rather than stoichiometric ROS scavenging^{36–39}. Recent studies further demonstrate that Cu-based MOF nanozymes can provide broad antioxidant activity in inflammatory disease models^{37,38,40}, while Cu/Zn bimetallic catalytic designs support SOD-like superoxide conversion^{36,37,39}. Thus, Cu-incorporated ZIF can be engineered as a dual-functional nanoreactor that couples catalytic superoxide dismutation with enzyme-mediated immunometabolic conversion.

To address these pathological features, we developed IDO@Cu/ZIF, an IDO1-loaded Cu-incorporated ZIF nanozyme designed for topical reconstruction of hair follicle immune privilege through coordinated

antioxidant and immunometabolic regulation (Fig. 1B). Cu incorporation modulates the electronic structure of ZIF and creates Cu-N and Zn-N coordination environments inspired by natural Cu, Zn-SOD. The resulting Cu/ZIF exhibits optimal SOD-like activity, while biomimetic mineralization incorporates catalytically active IDO1 to construct a dual-functional nanoreactor. *In vitro* and *in vivo* experiments demonstrated that IDO@Cu/ZIF reduced oxidative stress, restored Trp-Kyn metabolism and AHR-related transcriptional responses, thereby rebalancing CD8⁺T cells and Tregs and remodeling hair follicle immune privilege (Fig. 1C). *In vivo* AA experiments confirmed that topical application of IDO@Cu/ZIF achieved approximately 75% hair coverage after treatment and maintained over 90% hair coverage in an AA recurrence model after treatment cessation, indicating that hair follicle immune privilege was reconstructed and maintained over a long-term period. Collectively, this study demonstrates that remodeling immune privilege through IDO1-based immunometabolic reprogramming may provide a safe and long-acting universal therapeutic strategy for AA and other autoimmune diseases.

Results

Transcriptomic profiling identifies Trp metabolic dysregulation and oxidative stress response hyperactivation in AA

Impaired IDO1-Trp/Kyn-AHR signaling is closely associated with the collapse of immune privilege and drives the progression of various autoimmune diseases^{24,27}. AA, an autoimmune hair loss disease, is characterized by the collapse of hair follicle immune privilege, IFN- γ -driven inflammation, and infiltration of cytotoxic CD8⁺T cells²⁻⁹. Subsequently, we analyzed publicly available transcriptomic data from AA and healthy scalp biopsies to determine whether impaired Trp/Kyn immunometabolism and oxidative stress injury coexist in AA lesions. Gene Ontology (GO) enrichment network analysis using differentially expressed genes (DEGs) revealed the enriched modules included immune activation and antigen presentation, leukocyte migration and chemotaxis, interferon-driven inflammation, hair follicle morphogenesis, melanin metabolism, amino acid metabolic reprogramming and oxidative stress responses (Fig. 2A). Consistently, representative CD8-associated inflammatory genes, including *CD8A*, *CD8B*, *NKG7*, *KLRK1*, *IFNG*, *CXCL9*, *CXCL10*, *STAT1*, *STAT3* and *TNF*, were upregulated, whereas follicle developmental and structural genes, including *LEF1*, *WNT10B*, *SHH*, *KRT83* and *DSG4*, were reduced in AA samples (Fig. 2B). These findings confirmed the characteristic follicular inflammatory injury in AA and demonstrated that aberrant amino acid metabolism and dysregulated oxidative stress play critical roles in the pathogenesis of AA.

To further analyze metabolic alterations, we examined GO terms related to amino acid metabolism and identified Trp/Kyn metabolism as the most significantly altered pathway in AA, indicating that aberrant Trp/Kyn metabolism is closely associated with AA (Fig. 2C). We then examined enzymes involved in Trp-Kyn conversion (*IDO1*, *IDO2* and *TDO2*) and downstream Kyn metabolism (*AFMID*, *KMO* and *KYNU*). Heatmap analysis showed broad remodeling across this cascade (Fig. 2D), whereas boxplot

quantification identified *IDO1* as the only significantly downregulated enzyme in AA lesions, with no significant changes in *IDO2*, *TDO2*, *AFMID*, *KMO* or *KYNU* (Fig. 2E). Among the enzymes capable of initiating Trp-to-Kyn conversion, *IDO1* was therefore selectively reduced in AA lesions, indicating an IDO1-centred impairment of the local immunoregulatory Trp/Kyn axis.

Given that Kyn serves as a key endogenous ligand for the AHR, diminished IDO1 expression would be expected to compromise Kyn-AHR signaling and downstream immunoregulatory responses. Consistent with this, single-sample gene set enrichment analysis (ssGSEA) showed that the integrated IDO1-Trp/Kyn-AHR axis score was significantly reduced in AA compared to control scalp tissue (Fig. 2F), confirming comprehensive pathway suppression rather than isolated IDO1 downregulation. Additionally, ssGSEA revealed that oxidative stress pathways were significantly elevated in AA lesional skin (Fig. 2F). These alterations provide a pathological basis for improving AA treatment by restoring hair follicle immune privilege through intervention in Trp/Kyn metabolism. Together, the transcriptomic results define two convergent pathological features in AA lesions: suppression of the IDO1-Trp/Kyn-AHR immunometabolic axis and hyperactivation of oxidative stress responses. This dual signature guided the construction of a nanozyme platform combining ROS scavenging with exogenous IDO1 loading to restore Trp/Kyn-AHR signaling, rebalance CD8⁺T/Treg immunity and support follicle regeneration.

Cu, Zn-SOD-inspired synthesis, characterization and enzyme-like activity of Cu/ZIF

To reconstruct hair follicle immune privilege by restoring IDO1-mediated Trp/Kyn metabolism while alleviating oxidative stress, we designed an IDO@Cu/ZIF nanoreactor that combines SOD-like antioxidant capability with IDO1-mediated Trp/Kyn conversion. Inspired by the Cu/Zn active centers of natural SOD, Cu was introduced into the ZIF-8 framework to enhance ROS catalytic scavenging activity. Meanwhile, Cu/ZIF was used as a biomineralization matrix to incorporate catalytically active IDO1. To optimize SOD-like activity, we synthesized a series of Cu_x/ZIF nanozymes with nominal Cu precursor fractions of 5%, 10%, and 20%, denoted as Cu_{0.5}/ZIF, Cu₁/ZIF, and Cu₂/ZIF, respectively, *via* aqueous self-assembly (Fig. 3A). Transmission electron microscopy (TEM) showed that all Cu_x/ZIF samples retained the typical polyhedral morphology of ZIF-8, with clear outlines and an average size of approximately 300 nm (Fig. 3B, Supplementary Fig. 1). High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and elemental mapping revealed homogeneous Zn and Cu distributions without obvious Cu-rich domains or isolated Cu nanoparticles in Cu₁/ZIF (Fig. 3C). Energy-dispersive X-ray spectroscopy (EDS) spectra showed the presence of C, Zn and Cu signals, including Zn-K and Cu-K peaks at approximately 8.6 and 8.0 keV (Supplementary Fig. 2). ICP-OES results showed that the Cu content in Cu_{0.5}/ZIF, Cu₁/ZIF, and Cu₂/ZIF was 1.25, 2.42, and 3.92 wt.%, respectively, gradually increasing with the increase of Cu feed ratio. These results confirm the successful incorporation of Cu into the ZIF framework and the controlled preparation of samples with graded Cu contents, thereby enabling subsequent screening and optimization of SOD-like catalytic activity (Supplementary Table 1).

X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) further confirmed preservation of the ZIF-8 framework. All Cu_x/ZIF nanozymes retained the major diffraction peaks of ZIF-8, without detectable peaks corresponding to CuO, Cu₂O or metallic Cu (Fig. 3D). After Cu doping, the ZIF-8 peaks shifted slightly toward higher angles, indicating alterations in the interplanar spacing and framework-node bonding of the ZIF-8 structure rather than surface adsorption of Cu atoms. FTIR spectra showed characteristic 2-methylimidazole vibrations, including C = N, C-N, C = C and M-N modes, indicating that the linker skeleton and metal-imidazolate environment were largely maintained (Fig. 3E). N₂ adsorption-desorption measurements confirmed that Cu-doped samples remained porous. ZIF-8 showed a surface area of 1860 m² g⁻¹ and pore volume of 0.839 cm³ g⁻¹. Cu_{0.5}/ZIF, Cu₁/ZIF and Cu₂/ZIF showed surface areas of 1738, 1710 and 1558 m² g⁻¹, with pore volumes of 0.749, 0.749 and 0.732 cm³ g⁻¹, respectively (Supplementary Fig. 3A and Supplementary Table 1). The moderate decrease in porosity indicates pore-environment modulation while preserving the ZIF architecture (pore width ~ 1.1 nm, Supplementary Fig. 3B). Thermogravimetric analysis (TGA) showed comparable thermal stability among samples, with weight loss around 200 °C and framework decomposition between 500 and 650 °C (Supplementary Fig. 4).

X-ray photoelectron spectroscopy (XPS) revealed that Cu incorporation regulated the electronic structure of the metal nodes. High-resolution Cu 2p XPS spectra showed mixed Cu⁺ and Cu²⁺ species in Cu_{0.5}/ZIF, Cu₁/ZIF and Cu₂/ZIF, with Cu⁺ 2p_{3/2} and Cu²⁺ 2p_{3/2} peaks at approximately 932 and 934 eV, respectively (Fig. 3F). The Cu⁺/Cu²⁺ ratios were 6:4, 5:5 and 3:7 respectively, indicating that Cu doping introduced redox-active mixed-valence sites favorable for electron transfer during ·O₂⁻ dismutation. Meanwhile, the Zn 2p binding energy also shifted upon the Cu incorporation. Compared with ZIF-8, Cu_{0.5}/ZIF, Cu₁/ZIF and Cu₂/ZIF showed shifts of 0.04, 0.10 and 0.18 eV, respectively, suggesting electronic perturbation of Zn nodes through the metal-imidazolate network (Supplementary Fig. 5A). XPS survey spectra and metal-N components in N 1s spectra further supported the retention of the ZIF framework and the presence of Cu and Zn sites (Supplementary Fig. 5B-D). The Auger electron spectroscopy of Cu LMM and Zn LMM provides additional evidence for the chemical states of Cu and Zn (Supplementary Fig. 6).

We then evaluated whether the Cu-induced electronic modulation could improve SOD-like activity. In the xanthine/xanthine oxidase-WST-8 system, ZIF alone barely scavenged ·O₂⁻, whereas Cu_{0.5}/ZIF, Cu₁/ZIF and Cu₂/ZIF achieved ·O₂⁻ scavenging rates of 60%, 97% and 77%, respectively (Fig. 3G). Although the Zn 2p binding-energy shift increased progressively with the Cu incorporation, their catalytic activity peaked in Cu₁/ZIF, which exhibited an approximately balanced Cu⁺/Cu²⁺ state. Cu₁/ZIF was therefore selected for subsequent studies. It further showed a concentration-dependent ·O₂⁻ inhibition, increasing from 6% to 94% as the Cu₁/ZIF concentration increased from 2.5 to 25 µg mL⁻¹ (Supplementary Fig. 7). Electron paramagnetic resonance (EPR) spin-trapping confirmed its radical-scavenging capacity, with markedly reduced DMPO·O₂⁻ and DMPO·OH signals compared with the control and ZIF groups (Fig. 3H, I). These

results indicate that Cu₁/ZIF is an effective antioxidant nanozyme with potentials to suppress ROS-related damage in the AA inflammatory microenvironment.

After identifying Cu₁/ZIF as the optimal SOD-like nanozyme, IDO1 was introduced by biomimetic mineralization to construct IDO@Cu₁/ZIF. The resulting platform integrates Cu₁/ZIF-driven ROS scavenging with IDO1-targeted Trp-Kyn metabolic regulation. Protein quantification by bicinchoninic acid assay showed an IDO1 loading efficiency of 72% and a loading content of 1.8 wt.% (Supplementary Fig. 8). IDO@Cu₁/ZIF retained the polyhedral morphology and characteristic diffraction pattern of Cu₁/ZIF, indicating that IDO1 incorporation did not appreciably alter the morphology or crystalline framework of the carrier (Supplementary Figs. 9 and 10). The appearance of characteristic protein amide bands in the FTIR spectrum further supported the incorporation of IDO1 (Supplementary Fig. 11). Compared with an equivalent amount of free IDO1, IDO@Cu₁/ZIF retained approximately 70% of its catalytic activity toward Trp conversion, whereas Cu₁/ZIF alone showed negligible IDO1-like activity (Fig. 3J). Importantly, IDO1 loading did not measurably compromise the SOD-like activity of Cu₁/ZIF, indicating that the antioxidant catalytic function of the framework was retained after the biomineralization (Supplementary Fig. 12). These results indicate that IDO1 remained catalytically active after the incorporation into Cu₁/ZIF, supporting the dual functionality of IDO@Cu₁/ZIF for ROS scavenging and Trp-Kyn metabolic regulation.

Atomic coordination structure and antioxidant catalytic mechanism of Cu/ZIF

With Cu₁/ZIF selected as the optimal antioxidant component, we next investigated the structural and electronic origins of its enhanced SOD-like activity. X-ray absorption fine structure (XAFS) spectroscopy was employed to determine the local coordination structures of Cu and Zn. X-ray absorption near-edge structure (XANES) results showed that the Zn K-edge of Cu₁/ZIF was similar to that of ZnO but markedly different from that of Zn foil, indicating a Zn²⁺-like coordination environments (Fig. 4A). Cu K-edge XANES showed an absorption edge between Cu₂O and CuO, consistent with mixed Cu⁺/Cu²⁺ oxidation states observed by XPS spectra (Fig. 4B). Fourier-transformed extended X-ray absorption fine structure (FT-EXAFS) spectra of ZIF and Cu₁/ZIF displayed a major metal N-O coordination peak at approximately 1.5 Å in R space, whereas metal-foil-like M-M scattering was absent, indicating that Zn and Cu mainly existed as coordination nodes rather than metallic clusters or nanoparticles (Fig. 4C-E). In the ZIF-8 structure, this peak is mainly assigned to M-N coordination. The Zn-N coordination shell characteristic of the parent ZIF framework was preserved after the Cu incorporation, while an additional Cu-N coordination contribution was identified. Together with the absence of detectable M-M scattering, these results indicate that Cu was incorporated as framework-coordinated sites rather than metallic aggregates, generating a Cu/Zn bimetallic imidazolate coordination environment. Together, these results show that Cu₁/ZIF captures key structural features of the natural Cu,Zn-SOD active center, including its

Cu/Zn composition and imidazole-derived N coordination. This biomimetic coordination environment provides a structural basis for the SOD-like activity of Cu₁/ZIF.

To determine how this experimentally identified Cu/Zn-imidazolate coordination environment altered the electronic properties and substrate interaction of the framework, we constructed the pristine ZIF and Cu-doped ZIF models and evaluated them by projected density of states (PDOS), Bader charge and adsorption-energy calculations. PDOS analysis showed that the electronic states of Zn, N and C in pristine ZIF were mainly below the Fermi level, with a d-band center of -4.708 eV (Fig. 4F). In Cu doped ZIF model, Cu-related states appeared and the d-band center shifted upward to -4.149 eV (Fig. 4G), suggesting stronger electronic coupling with ROS. Valence-band photoelectron spectroscopy showed d-band centers of -2.94, -2.62 and -1.96 eV for Cu_{0.5}/ZIF, Cu₁/ZIF and Cu₂/ZIF, respectively, consistent with the calculated Cu-induced d-band upshift (Supplementary Fig. 13). Bader charge analysis showed that Zn in pristine ZIF exhibited an electron depletion of approximately 1.17 e (Fig. 4H), which remained nearly unchanged after Cu incorporation. In contrast, Cu exhibited a smaller electron depletion of approximately 1.00 e, indicating that Cu retained relatively more electron density and induced local charge redistribution within the framework. This electronic modulation was accompanied by a stronger substrate binding. The calculated $\cdot\text{O}_2^-$ adsorption energy decreased from -4.05 eV on pristine ZIF to -4.49 eV on Cu/ZIF (Fig. 4I), indicating the enhanced interaction with $\cdot\text{O}_2^-$. Thus, Cu incorporation creates a more favorable electronic and adsorption environment for the enhanced SOD-like performance.

Having established a biomimetic Cu/Zn-imidazolate coordination environment and a relatively electron-rich Cu site in Cu₁/ZIF, we next sought to clarify how these structural and electronic features influence the $\cdot\text{O}_2^-$ dismutation pathway. Therefore, the Gibbs free energy changes of $\cdot\text{O}_2^-$ conversion were compared on pristine ZIF and Cu doped ZIF (Fig. 4J). In this model, $\cdot\text{O}_2^-$ first forms an adsorbed $\ast\text{OOH}$ intermediate on the metal site, followed by conversion of $\ast\text{OOH}$ to $\ast\text{OH}$ and $\cdot\text{O}_2^-$ related species. The rate-determining barrier decreased from 0.42 eV on ZIF to 0.22 eV on Cu₁/ZIF, with $\ast\text{OOH}$ -to- $\ast\text{OH}$ conversion as the limiting step. Collectively, Cu incorporation endows Cu₁/ZIF with a favorable electronic and coordination environment for SOD-like catalysis. The relatively electron-rich Cu sites, upward-shifted d-band center, and Cu/Zn-N coordination motifs jointly strengthen $\cdot\text{O}_2^-$ adsorption and promote $\ast\text{OOH}$ activation, thereby lowering the rate-determining barrier and enhancing ROS-scavenging activity. These findings identify Cu₁/ZIF as a SOD-inspired antioxidant nanozyme and provide a material basis for its integration with IDO1-mediated immune privilege remodeling.

IDO@Cu/ZIF relieves IFN- γ -induced immunoinflammatory damage and modulates Trp/Kyn metabolism

AA is characterized by collapse of hair follicle immune privilege, evidenced by cytotoxic CD8⁺T-cell infiltration into the perifollicular niche and concurrent loss of Tregs that normally maintain immune privilege^{2,5,7,8}. Therefore, the IFN- γ -stimulated human peripheral blood mononuclear cells (PBMCs) were

used as an AA-related *in vitro* inflammatory model to evaluate whether IDO@Cu₁/ZIF could restore this protective CD8⁺T/Treg balance and reconstruct immune privilege. Before conducting immunomodulatory experiments, we first assessed cellular internalization by co-incubating human PBMCs with Cy5.5-labelled IDO@Cu₁/ZIF. Confocal images showed clear cytoplasmic red fluorescence, confirming efficient internalization of IDO@Cu₁/ZIF PBMCs (Supplementary Fig. 14). Biocompatibility assays showed that ZIF, Cu₁/ZIF and IDO@Cu₁/ZIF maintained cell viability above 99% from 10 to 100 µg mL⁻¹ (Supplementary Fig. 15). Supplementary Fig. 16 revealed that IDO@Cu₁/ZIF reached a plateau in scavenging IFN-γ-induced ROS at 50 µg mL⁻¹. Therefore, all subsequent cellular experiments were performed at 50 µg mL⁻¹.

The ROS fluorescence staining and quantitative analysis showed that ROS accumulation was significantly observed in PBMCs after the IFN-γ stimulation (Fig. 5A, B). ZIF achieved only partial ROS scavenging, whereas Cu₁/ZIF and IDO@Cu₁/ZIF strongly inhibited ROS accumulation, confirming that Cu-enhanced SOD-like activity of Cu₁/ZIF and IDO@Cu₁/ZIF effectively alleviates inflammation-induced oxidative stress. Baricitinib, included as a clinically relevant JAK inhibitor control for AA, also attenuated IFN-γ-induced ROS accumulation, likely by suppressing cytokine-driven inflammatory signaling. Consistent with improved redox status, IDO@Cu₁/ZIF restored cell survival. IFN-γ reduced PBMC viability to 59.1%, and ZIF alone partially rescued it to 70.3% (Fig. 5C, D). In contrast, Cu₁/ZIF, IDO@Cu₁/ZIF and baricitinib restored viability to 96.1%, 97.5% and 96.9%, respectively, approaching the 99.5% survival of normal controls.

We then examined the proportions of cytotoxic CD8⁺T cells and Tregs, two opposing immune populations that respectively mediate hair follicle immune injury and immune privilege maintenance. Flow cytometric analysis revealed that IFN-γ increased cytotoxic CD8⁺CD44⁺T cells from 20.3% to 31.0%. ZIF and Cu₁/ZIF did not significantly reduce this population, which remained at 30.2% and 29.8%, respectively (Fig. 5E). Comparatively, baricitinib and IDO@Cu₁/ZIF greatly reduced this population to 26.1% and 24.9%, respectively (Fig. 5F). Meanwhile, Tregs (CD4⁺CD25⁺Foxp3⁺) collapsed from 5.86% to 0.46% after IFN-γ stimulation. ZIF, Cu₁/ZIF and baricitinib did not restore Tregs, which remained near 0.43–0.46%. Importantly, IDO@Cu₁/ZIF increased Tregs to 6.31%, comparable to controls (Fig. 5E, F). These results indicate that IDO@Cu₁/ZIF suppresses cytotoxic CD8⁺ T-cell activation while restoring Tregs abundance, unlike baricitinib-mediated cytokine blockade which suppresses inflammation without reconstructing immune privilege. To probe the molecular basis of these immunological effects, we measured anti- and pro-inflammatory gene expression by reverse-transcription quantitative PCR (RT-qPCR). RT-qPCR analysis showed that IFN-γ stimulation increased *IFNG* and *TNF* while reducing *IL10* and *TGFB1* (Fig. 5G). IDO@Cu₁/ZIF reversed this pattern, suppressing pro-inflammatory transcripts and restoring anti-inflammatory gene expression, consistent with normalization of T cell subsets and immune homeostasis.

Given that IDO1 catalyzes the conversion of Trp to Kyn, we further examined Trp/Kyn metabolic axis changes. ELISA analysis showed that IDO@Cu₁/ZIF decreased Trp and increased Kyn in culture supernatants relative to the IFN- γ -stimulated controls (Fig. 5H). This metabolic shift was absent in baricitinib-treated cells, indicating that IDO@Cu₁/ZIF uniquely coupled immunosuppression with the activated Trp catabolism. The increased Kyn functionally engaged AHR signaling, as *CYP1A1*, *CYP1B1* and *AHRR* were significantly upregulated after the IDO@Cu₁/ZIF treatment (Fig. 5I). *CYP1A1* induction in particular confirms the AHR pathway activation, which drives Treg differentiation and represents the molecular basis for immune privilege reconstruction. Thus, Cu₁/ZIF-mediated ROS scavenging limits cytotoxic CD8⁺ T cell activation, whereas IDO1-driven Kyn production activates AHR signaling to promote Tregs recovery, together reconstructing the CD8⁺T/Treg balance required to maintain hair follicle immune privilege and thereby enabling long-term AA treatment.

IDO@Cu/ZIF promotes hair regrowth and restores follicle structure

Prior to evaluating therapeutic efficacy, the *in vivo* safety of IDO@Cu₁/ZIF after the repeated topical administration was assessed. Histopathological examination of major organs (heart, liver, spleen, lung, kidney, stomach, colon, and skin) revealed normal tissue architecture with no evidence of necrosis, inflammatory infiltration, or fibrosis in any treatment group (Supplementary Fig. 17). Hematological parameters including white blood cell count, red blood cell count, hemoglobin, and platelet counts remained within normal ranges, as did serum biochemistry markers of hepatic and renal function (Supplementary Fig. 18). Furthermore, analysis of spleen and draining lymph nodes showed unchanged CD8⁺T cells and Tregs proportions across all groups, confirming that IDO@Cu₁/ZIF achieves localized immunomodulation without perturbing systemic immune homeostasis (Supplementary Fig. 19). Taken together, these findings have verified the high safety of IDO@Cu₁/ZIF and support further evaluation of the therapeutic effect.

Therapeutic efficacy was evaluated in an imiquimod (IMQ)-induced AA model in C3H/HeJ mice. After a 3-week IMQ induction period, AA-like lesions were established, and mice were randomized to topical PBS, ZIF, Cu₁/ZIF, IDO@Cu₁/ZIF or baricitinib. Treatment was initiated immediately after successful modeling, and hair regrowth was monitored on Days 0, 7, 14 and 21 (Fig. 6A). IDO@Cu₁/ZIF achieved approximately 20% hair coverage by Day 14, which further increased to approximately 75% by Day 21 (Fig. 6B, C). By Day 21, PBS alone showed no apparent regrowth; the ZIF treatment produced minimal improvement; the Cu₁/ZIF group led to moderate regrowth; and baricitinib achieved approximately 50% coverage. Scanning electron microscopy (SEM) analysis at day 21 showed that IDO@Cu₁/ZIF regenerated hair shafts with near-normal diameter (~ 51 μ m) and organized cuticular scales, whereas baricitinib and Cu₁/ZIF produced thinner shafts of approximately 42 and 37 μ m, respectively, with less complete cuticular restoration (Fig. 6B, D).

Hematoxylin and eosin (H&E) stain of PBS-treated AA mice revealed typical disease-associated histopathological changes, including miniaturized follicles and dense perifollicular inflammatory infiltrates (Fig. 6E). The therapeutic effect of ZIF group was not significant, while the Cu₁/ZIF and baricitinib treatment groups showed partial recovery effects. In contrast, the IDO@Cu₁/ZIF group exhibited organized anagen hair follicle, intact dermal papillae and minimal perifollicular inflammation. Hair follicle counting confirmed the highest follicle density after the IDO@Cu₁/ZIF treatment (Supplementary Fig. 20A). Body weight remained stable during the 21-day treatment period (Supplementary Fig. 20B). These data show that IDO@Cu₁/ZIF safely promotes both the quantity and quality of hair regrowth, outperforming its single components and baricitinib.

IDO@Cu1/ZIF reshapes local CD8 + T/Treg balance by alleviating oxidative stress and restoring Trp/Kyn metabolism

To confirm that the superior therapeutic efficacy of IDO@Cu₁/ZIF derives from immune privilege reconstruction *in situ*, we examined the perifollicular immune microenvironment in treated skin. CD8 immunofluorescence revealed that the untreated AA skin exhibited dense cuff-like perifollicular infiltration, whereas IDO@Cu₁/ZIF nearly eliminated CD8⁺T cell accumulation, approaching the levels observed in the normal skin tissue (Fig. 7A and Supplementary Fig. 20C). Foxp3 staining revealed sparse Treg signals in the PBS-treated group, whereas IDO@Cu₁/ZIF markedly restored perifollicular Treg density, surpassing the therapeutic outcome of baricitinib (Fig. 7B and Supplementary Fig. 20D). TUNEL staining revealed extensive apoptotic damage in follicular epithelial and dermal papilla cells of the PBS-treated group, consistent with cytotoxic T-cell-mediated injury; IDO@Cu₁/ZIF markedly reduced TUNEL-positive cells, indicating protection of follicular structure through immune-privilege reconstruction (Supplementary Fig. 20E). Flow cytometry confirmed these changes. CD8⁺T cells increased from 26.8% in controls to 40.8% in AA mice, and IDO@Cu₁/ZIF reduced to 29.0%, below that of baricitinib at 31.1% (Fig. 7C, D). Tregs declined from 10.5% to 5.76% in AA mice, whereas IDO@Cu₁/ZIF restored it to 11.5%; baricitinib showed no significant Tregs recovery (Fig. 7C, D). Consequently, the CD8⁺T/Treg ratio increased to 6.8 in AA mice but normalized to 2.5 after the IDO@Cu₁/ZIF treatment, compared with 3.9 under baricitinib (Supplementary Fig. 20F). Effector-function analysis showed that CD8⁺CD44⁺ cytotoxic T cells decreased from 32.7% in AA group to 19.7% after the treatment with IDO@Cu₁/ZIF, which was also below baricitinib at 21.2% (Fig. 7E, F). In the IDO@Cu₁/ZIF group, the proportions of CD8⁺IFN-γ⁺ cells and CD8⁺TNF-α⁺ cells decreased from 25.7% and 9.43% to 9.67% and 4.84%, respectively, significantly outperforming the baricitinib-treated group (13.2% and 5.27%) (Fig. 7E, F). Overall, these results indicate that IDO@Cu₁/ZIF suppresses CD8⁺T-cell infiltration and activation while restoring Tregs representation, thereby alleviating hair follicle cell damage through reconstruction of immune privilege.

Mechanistically, dihydroethidium (DHE) staining showed intense ROS accumulation in AA skin, consistent with oxidative stress as a characteristic damage phenotype of the inflammatory follicular microenvironment. Cu₁/ZIF and IDO@Cu₁/ZIF both reduced ROS, with IDO@Cu₁/ZIF achieving near-normalization. Notably, Cu₁/ZIF-mediated ROS scavenging could not achieve immune privilege reconstruction and satisfactory hair regeneration, confirming that oxidative stress scavenging serves a supportive rather than a primary therapeutic role (Fig. 7G and Supplementary Fig. 20G).

The core immunometabolic mechanism was verified by ELISA of skin tissue lysates, which showed decreased Trp and increased Kyn in IDO@Cu₁/ZIF-treated skin, confirming sustained IDO1 enzymatic activity (Fig. 7H). Immunofluorescence for CYP1A1, a canonical AHR target gene, showed minimal expression in AA + PBS skin but strong, widespread induction throughout the epidermis and perifollicular regions after the IDO@Cu₁/ZIF treatment (Supplementary Fig. 20H). CYP1A1 upregulation demonstrates functional engagement of the AHR pathway by IDO1-generated Kyn, providing the molecular link between Trp catabolism and Treg-mediated immune privilege reconstruction. In summary, these findings establish that IDO@Cu₁/ZIF reconstructs follicular immune privilege through a hierarchical mechanism: Cu₁/ZIF-mediated ROS scavenging alleviates oxidative stress and follicular damage to create a permissive microenvironment for immune rebalancing, while IDO1-driven Trp-Kyn conversion activates AHR signaling to promote Treg differentiation. The restored Tregs population, together with the suppressed cytotoxic CD8⁺ T cells, re-establishes the protective immune privilege that shields hair follicles from autoimmune attack, ultimately enabling hair cycle re-entry and sustained regrowth.

IDO@Cu/ZIF remodels the AA skin immune transcriptome and metabolic signaling networks

To define the molecular basis of immune remodeling, we performed RNA sequencing of lesional skin from the IMQ-induced AA model. Differential expression analysis revealed broad transcriptional changes after the IDO@Cu₁/ZIF treatment, as shown by the volcano plot (Fig. 8A). Heatmap analysis showed various reduced cytotoxic lymphocyte genes (e.g., *Cd8a*, *Cd8b1*, *Nkg7* and *Klrk1*) and inflammatory mediators including (e.g., *Ifng*, *Il15*, *Cxcl9*, *Cxcl10*, *Stat1*, *Stat3* and *Tnf*, Fig. 8B). In contrast, hair follicle growth and differentiation genes including *Lef1*, *Wnt10b*, *Shh*, *Krt83* and *Dsg4* were increased. GO enrichment highlighted the effective T cell activation, type II interferon production and response, antigen processing and presentation, natural killer cell-mediated cytotoxicity, inflammatory response, positive regulation of hair follicle development and hair cycle processes (Fig. 8C). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis further enriched antigen processing and presentation, cell adhesion molecules, NF-κB signaling, natural killer cell-mediated cytotoxicity, Th17 differentiation, Th1 and Th2 cell differentiation, cytokine-cytokine receptor interaction, Toll-like receptor and JAK-STAT pathways (Fig. 8D). These changes indicate that IDO@Cu₁/ZIF shifts the lesional skin transcriptome from an autoimmune attack program toward immune-privileged hair follicle regeneration. Gene set enrichment analysis (GSEA) refined these changes without applying a fold-change cutoff. The T cell receptor

signaling pathway, CD8 positive alpha beta T cell gene set and natural killer cell-mediated cytotoxicity gene set were negatively enriched in IDO@Cu₁/ZIF-treated lesions (Fig. 8E), indicating a coordinated suppression of TCR-proximal and cytotoxic programs. Conversely, the hair follicle development gene set was positively enriched (Fig. 8F), supporting reactivation of regenerative programs. Immune infiltration deconvolution also showed decreased CD8⁺T cells and increased Tregs after the IDO@Cu₁/ZIF treatment, consistent with the results of flow cytometry (Fig. 8G).

Beyond immune and hair follicle-related gene programs, we further leveraged the RNA-seq data to validate the effects of IDO@Cu₁/ZIF on the oxidative stress and Trp/Kyn metabolism at the transcriptional level. ssGSEA showed that the ROS generation score was significantly reduced in those IDO@Cu₁/ZIF-treated mice compared with AA + PBS controls, corroborating DHE staining (Fig. 8H). Meanwhile, the IDO1/AHR and Trp/Kyn metabolism scores were significantly increased, further confirming that both upstream Trp metabolic conversion and downstream AHR signaling were transcriptionally activated (Fig. 8I and Supplementary Fig. 21A). In addition, GO analysis revealed concurrent enrichment of Trp degradation and de novo NAD synthesis (Supplementary Fig. 21B), indicating that IDO1 activity is closely linked to broader metabolic network remodeling in the skin after treatment. Collectively, these transcriptomic analyses independently validate at the gene expression level that IDO@Cu₁/ZIF reconstructs immune privilege at AA lesion sites through synergistic antioxidant and immunometabolic mechanisms.

IDO@Cu/ZIF prevents AA recurrence and maintains follicle immune homeostasis

Having established therapeutic activity against active AA, we then evaluated whether IDO@Cu₁/ZIF could prevent recurrence after inflammatory re-exposure. We established a relapse-prevention model consisting of an initial 3-week IMQ induction phase followed by 3 weeks of treatment, a 5-week recovery period, and a subsequent IMQ re-challenge without further treatment to induce relapse (Fig. 9A). This design simulates recurrent AA after exposure to environmental or immunological triggers, which examines the capability of prior IDO@Cu₁/ZIF treatment for the sustained hair follicle immune privilege to prevent relapse after treatment withdrawal. Imaging results showed that untreated AA recurrence mice began to exhibit progressive hair loss from the first week, with near-complete loss of dorsal hair by Day 21. In contrast, mice previously treated with IDO@Cu₁/ZIF maintained over 90% skin hair coverage throughout the 21-day observation period after the IMQ re-challenge, outperforming both the untreated and baricitinib groups at all time points (Fig. 9B,C). Baricitinib provided partial hair maintenance but remained significantly less effective than IDO@Cu₁/ZIF. Body weights remained stable across all groups during the 21-day study (Supplementary Fig. 22), indicating favorable systemic tolerability under both therapeutic and preventive regimens. At Day 14 after IMQ re-challenge, H&E staining and hair follicle quantification revealed that IDO@Cu₁/ZIF preserved ordered anagen hair follicles, intact follicular basal cells, and higher follicle density, whereas untreated mice exhibited atrophic follicles, telogen/catagen

transition, dense perifollicular inflammation, and reduced follicle numbers (Fig. 9D,E). In contrast, baricitinib provided only partial protection.

Histological staining results revealed that on Day 14 after IMQ re-challenge, the untreated recurrence group exhibited marked perifollicular CD8⁺T cell infiltration and aggregation (Fig. 9F). In contrast, prior treatment with IDO@Cu₁/ZIF significantly reduced the CD8⁺T cell infiltration. Moreover, Foxp3⁺ Treg levels in the IDO@Cu₁/ZIF group were maintained at values comparable to those in healthy skin, again outperforming baricitinib in preserving local Treg proportions (Fig. 9G). TUNEL staining showed extensive apoptotic damage in follicular epithelial cells and dermal papilla cells of untreated recurrent mice, whereas TUNEL-positive cells were rare in IDO@Cu₁/ZIF-treated skin, indicating sustained protection of follicular structures through the maintenance of immune privilege (Fig. 9H). Collectively, these data confirm that IDO@Cu₁/ZIF is a multifunctional immunomodulatory nanomedicine capable of both reversing established AA and preventing recurrence by reconstructing the CD8⁺ T/Treg balance upon which immune privilege depends.

Discussion

AA is an autoimmune disease driven by the collapse of hair follicle immune privilege, accompanied by inflammation induced oxidative stress injury. To address these issues, IDO@Cu₁/ZIF was designed to synergistically restore Trp/Kyn metabolism, reconstruct hair follicle immune privilege, and scavenge excessive ROS, thereby achieving long-term improvement of AA. By incorporating Cu into the ZIF framework, a Cu/Zn active center mimicking the SOD catalytic site was constructed, significantly enhancing the antioxidant performance of Cu₁/ZIF. Mechanism investigations further reveal that Cu incorporation induces an upshift of the d-band center, thereby strengthening superoxide adsorption and lowering the rate-determining reaction barrier. IDO@Cu₁/ZIF restored Kyn production and activated downstream AHR signaling, promoting Treg differentiation, suppressing detrimental CD8⁺T cell activation, and reconstructing hair follicle immune privilege to improve AA treatment. IDO@Cu₁/ZIF achieved 75% hair regrowth *in vivo*. More importantly, IDO@Cu₁/ZIF effectively prevented AA recurrence after treatment cessation. Compared with existing therapeutic agents, IDO@Cu₁/ZIF provides a durable therapeutic strategy by reconstructing the intrinsic immune privilege state. These findings indicate that IDO@Cu₁/ZIF is a promising immunometabolic therapeutic nanomedicine that integrates catalytic microenvironment modulation with immune privilege reconstruction, offering a therapeutic strategy for AA that extends beyond conventional immunosuppression.

Methods

Material characterization

TEM, HAADF-STEM, and EDS elemental mapping were performed using an FEI Themis Z microscope to examine particle morphology and the spatial distribution of Zn and Cu. XRD patterns were recorded on a

D8 Advance diffractometer using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$). XPS was performed using a Kratos AXIS SUPRA+ spectrometer equipped with a monochromatic Al K α X-ray source. Binding energies were calibrated to the C 1s peak at 284.8 eV. Nitrogen adsorption-desorption isotherms were measured at 77 K using a Micromeritics TriStar II Plus 3030 analyzer. Before measurement, samples were degassed under vacuum for 12 h. The specific surface area was calculated using the Brunauer-Emmett-Teller method, and the pore size distribution was obtained using the nonlocal density functional theory (NLDFT) model. TGA was performed under N $_2$ using HITACHI STA200. Samples were heated from 30 to 800 °C at a heating rate of 10 °C min $^{-1}$. FTIR spectra were collected in attenuated total reflection mode over the range of 400–4000 cm $^{-1}$.

Determination of Cu/Zn content by inductively coupled plasma analysis

The actual Cu and Zn contents in ZIF-8, Cu $_{0.5}$ /ZIF, Cu $_1$ /ZIF, and Cu $_2$ /ZIF were determined by ICP-OES analysis using a PerkinElmer Avio 220 Max system after acid digestion. Briefly, each sample was subjected to acid digestion, diluted with deionized water to an appropriate concentration range, and analyzed according to the instrument protocol. Each sample was measured three times, and the results were reported as mean $\pm$ SD.

X-ray absorption spectroscopy (XAS) and EXAFS analysis

XAS at the Cu K-edge and Zn K-edge was performed at the BL14W1 beamline in transmission mode. Cu foil, Cu $_2$ O, CuO, CuPc, Zn foil, and ZnO were used as reference samples for energy calibration and chemical-state comparison, as appropriate. The XAS data were processed using the Athena program within the Demeter software package⁴¹. After background subtraction and normalization, the EXAFS spectra were converted from energy space to k space, and k ^{3} -weighted EXAFS oscillations were Fourier transformed into R space for local coordination analysis. The resulting R-space spectra were used to qualitatively identify the dominant coordination-shell features and compare the corresponding peak positions among the samples and reference materials.

SOD-like activity assay

SOD-like activity was evaluated using a Total Superoxide Dismutase Assay Kit with WST-8 (Beyotime, S0101M, 250T) according to the manufacturer's protocol. Briefly, 20 μ L of nanozyme suspension within a defined concentration range was added to 160 μ L of WST-8 working solution. The reaction was initiated by adding 20 μ L of enzyme working solution and incubated at 37 °C for 30 min. The absorbance at 450 nm was measured using an Infinite microplate reader (Tecan). The inhibition rate was calculated

according to the kit protocol. Each sample was measured three times, and the results were reported as mean $\pm$ SD.

EPR detection of radical-scavenging activity

EPR spectroscopy was used to evaluate radical-scavenging activity using DMPO as the spin-trapping agent. EPR spectra were recorded using an EMXplus spectrometer (Bruker) at room temperature.

DFT calculations

All calculations were performed using the CP2K 2025 package. Geometry optimizations were carried out at the DZVP basis set level within the framework of DFT, while single-point energy calculations were subsequently performed using the larger TZV2P basis set to ensure higher accuracy and reliability for the final energies. The vibrational frequencies were computed at the same theoretical level as the geometry optimizations to confirm that all optimized structures correspond to true minima on the potential energy surface without any imaginary frequencies, and the thermal corrections to the Gibbs free energy were extracted using the Shermo program⁴². For all metal-containing species, various spin states were carefully examined, and the corresponding magnetic moments were explicitly included in the calculations. The Gibbs free energy (G) at 298.15 K and 1 atm was calculated as $G = E + \text{ZPE} + G_{\text{corr}}$, where E is the electronic energy obtained from the TZV2P single-point calculation, ZPE is the zero-point energy derived from the vibrational analysis, and G_{corr} represents the thermal correction to Gibbs free energy, including vibrational, translational, and rotational entropy contributions obtained from Shermo. Difference charge density analyses were conducted to investigate charge redistribution upon adsorption, and the resulting data were processed and visualized using Multiwfn⁴³.

Acquisition and analysis of public transcriptomic data

The publicly available transcriptomic dataset GSE68801 was obtained from the Gene Expression Omnibus (GEO) database. GSE68801 contains gene-expression profiles of scalp biopsies from patients with AA and healthy controls generated using the Affymetrix Human Genome U133 Plus 2.0 Array (GPL570)⁴⁴. Before downstream analysis, the expression matrix and corresponding sample annotation files were organized. For the normalized expression matrix, differential expression analysis was performed using the limma package in R. DEGs were identified using adjusted $P < 0.05$ and $|\log_2 \text{fold change}| > 0.5$ as the thresholds. DEGs were further used for GO enrichment analysis. Trp/Kyn metabolism and IDO1-AHR pathway activity were evaluated by ssGSEA.

In vitro cytocompatibility assay

To evaluate the cytocompatibility of ZIF, Cu₁/ZIF and IDO@Cu₁/ZIF toward PBMCs, cells were seeded into 96-well plates and treated with the corresponding materials at 10, 20, 30, 50 or 100 µg mL⁻¹. Cells were further cultured at 37 °C with 5% CO₂ for 24 h. After treatment, cells were collected, washed with PBS, and stained with 7-AAD for 10 min at room temperature in the dark. Samples were analyzed by flow cytometry.

IFN-γ-induced in vitro inflammatory model and optimization of material concentration

To mimic the AA-associated inflammatory microenvironment, PBMCs were stimulated with recombinant human IFN-γ. Cells were divided into the following groups: Control, IFN-γ, IFN-γ + ZIF, IFN-γ + Cu₁/ZIF, IFN-γ + IDO@Cu₁/ZIF and IFN-γ + baricitinib. Except for the control group, all groups were treated with IFN-γ (100 ng mL⁻¹) for 48 h.

To determine the appropriate working concentration for subsequent experiments, ZIF, Cu₁/ZIF and IDO@Cu₁/ZIF were added at 10, 20, 30, 50 or 100 µg mL⁻¹ under IFN-γ stimulation. After 48 h of treatment, intracellular ROS was detected using the DCFH-DA fluorescent probe, which was incubated at 37 °C for 30 min in the dark. Based on the ROS staining results, 50 µg/mL was selected as the working concentration of ZIF, Cu₁/ZIF and IDO@Cu₁/ZIF for subsequent functional experiments. Baricitinib was used as a positive control at a concentration of 100 nM. In the functional experiments, the corresponding materials or drug were added simultaneously with IFN-γ, and cells were cultured for 48 h before being used for ROS detection, cell viability assessment, immune cell subset analysis by flow cytometry, inflammatory factor detection by RT-qPCR, and Trp/Kyn measurement.

RT-qPCR analysis of inflammatory factors and AHR target genes

After treatment, PBMCs were lysed with TRIzol, and total RNA was extracted according to the manufacturer's instructions. After RNA concentration and purity were assessed, cDNA was synthesized using a reverse transcription kit. RT-qPCR was used to detect inflammatory factors and AHR downstream target genes. Relative expression was calculated using the 2^{-ΔΔCt} method and normalized to GAPDH. Primer sequences are listed in Supplementary Table 2.

Animals

Fifteen-week-old female C3H/HeJ mice were purchased from Shanghai Model Organisms Center, Inc. Mice were housed in a specific pathogen-free animal facility under the following conditions: 22 ± 2 °C, 50–60% relative humidity, and a 12 h light/dark cycle, with free access to food and water. Animals were

acclimatized for 1 week before experiments and were randomly assigned to different treatment groups. All animal experiments were approved by the Animal Ethics Committee of Air Force Medical University (approval number: 20260056).

IMQ-induced AA-like model in C3H/HeJ mice

The IMQ-induced AA-like mouse model was adapted from previously reported protocols^{45,46}. Before modelling, dorsal hair was removed using an electric clipper. IMQ cream was then topically applied once every other day to a 1.5 cm × 1.5 cm area of the cervicodorsal skin for 3 consecutive weeks. Normal control mice received the same amount of vehicle cream on the corresponding skin area. Skin and hair changes were monitored during modelling. When obvious patchy hair loss appeared on the dorsal skin, the AA-like model was considered successfully established, and the mice were used for therapeutic experiments.

Therapeutic treatment of AA mice

After the development of IMQ-induced AA-like hair loss, mice were randomly divided into the following groups: Control, AA + PBS, AA + ZIF, AA + Cu₁/ZIF, AA + IDO@Cu₁/ZIF and AA + baricitinib. Before each administration, the dorsal lesional area was pretreated with a microneedle roller with a needle length of 0.5 mm, followed by topical application of the corresponding treatment.

ZIF, Cu₁/ZIF and IDO@Cu₁/ZIF were administered at a concentration of 0.5 mg/mL, with 100 µL applied per mouse per administration. Baricitinib was used as a positive control at a dose of 1 µg per mouse¹⁹. Treatments were administered once daily for 21 d. Dorsal skin photographs were taken on D0, D7, D14 and D21 to evaluate hair regrowth, and body weight was recorded simultaneously to monitor general condition and treatment tolerance. At the indicated sampling time points, dorsal skin, spleens and skin-draining lymph nodes were collected for histology, immunofluorescence staining, flow cytometry, Trp/Kyn measurement and transcriptomic analysis.

Quantification of hair coverage area

Hair coverage area was quantified using ImageJ. The dorsal treatment area was defined as the total region of interest. Hair-covered and exposed-skin regions were semi-automatically segmented by thresholding, and the percentage of hair coverage was calculated using the following formula:

Hair coverage area (%) = hair-covered area / total analysis area × 100%.

SEM observation and diameter analysis of hair shafts

At the end of treatment, hair samples were collected from the dorsal treatment area. Samples were gently washed with deionized water, air-dried, mounted on conductive adhesive tape, and sputter-coated with gold. Hair shaft morphology was observed and imaged by SEM.

H&E staining and hair follicle counting

Dorsal skin tissues were collected and fixed in 4% paraformaldehyde for 24 h. Fixed tissues were dehydrated through a graded ethanol series, cleared in xylene and embedded in paraffin. Sections with a thickness of 4 μm were prepared, deparaffinized, rehydrated and stained with H&E. Images were acquired using a digital slide scanning system, and hair follicles were counted in each field of view.

Detection of ROS in skin tissues by DHE fluorescence staining

To evaluate oxidative stress levels in AA skin, dorsal skin samples were embedded in OCT and cryosectioned at a thickness of 4 μm . Sections were rehydrated with PBS to remove residual OCT and incubated with the DHE fluorescent probe at 37 °C for 30 min in the dark. After three washes with PBS, sections were mounted with DAPI-containing mounting medium. Fluorescence images were acquired using a confocal laser scanning microscope.

Immunofluorescence staining of skin tissues

To evaluate CD8⁺T-cell infiltration, changes in Foxp3⁺ Tregs and AHR downstream signalling in AA skin, immunofluorescence staining was performed on paraffin sections of dorsal skin. Sections were deparaffinized, rehydrated and subjected to antigen retrieval, followed by blocking with 5% goat serum for 1 h at room temperature. Sections were then incubated with primary antibodies overnight at 4 °C. The primary antibodies used included anti-CD8 (Abcam, ab22378, 1:300), anti-Foxp3 (Abcam, ab36607, 1:1000) and anti-CYP1A1 (Proteintech, 13241-1-AP, 1:200). The next day, sections were washed three times with PBST and incubated with the corresponding fluorophore-conjugated secondary antibodies for 1 h at room temperature in the dark. Sections were then mounted with DAPI-containing mounting medium. Images were acquired using a confocal laser scanning microscope.

TUNEL staining

Apoptotic cells in paraffin-embedded skin sections were detected using a One-Step TUNEL Cell Apoptosis Detection Kit (CX107; Epizyme Biotech) according to the manufacturer's instructions. After three washes with PBS, nuclei were counterstained with DAPI, and the sections were mounted for fluorescence imaging.

Flow cytometric analysis of immune cells in skin tissues

To analyze local immune cell subsets in AA skin, single-cell suspensions were stained with Zombie UV™ Fixable Viability Kit and fluorophore-conjugated surface antibodies. The antibodies used included anti-CD45 (BioLegend, 103125), anti-CD4 (BioLegend, 100431), anti-CD8 (BioLegend, 100731/100713), anti-CD25 (BioLegend, 102037) and anti-CD44 (BioLegend, 103029). Cells were incubated at 4 °C for 30 min in the dark. After surface staining, cells were fixed and permeabilized. Cells were then stained with anti-Foxp3 (Invitrogen, MA5-16224) overnight at 4 °C in the dark.

To detect IFN- γ and TNF- α expression in CD8⁺T cells, skin single-cell suspensions were stimulated with Cell Activation Cocktail (with Brefeldin A) for 6 h before surface staining. After stimulation, cells underwent viability staining and surface staining as described above. After fixation and permeabilization, cells were stained with anti-IFN- γ (BioLegend, 505807) and anti-TNF- α (BioLegend, 506333). After staining, samples were analyzed by flow cytometry.

Measurement of Trp and Kyn levels in skin tissues

At the end of treatment, dorsal skin tissues were collected and immediately stored in liquid nitrogen. Before detection, tissues were weighed and homogenized in cold PBS. The supernatants of tissue homogenates were collected for measurement. Trp and Kyn levels were measured using mouse Trp ELISA and mouse Kyn ELISA kits, respectively, according to the manufacturer's instructions. Trp and Kyn levels were normalized to tissue weight and expressed as nmol/g tissue.

RNA sequencing of skin tissues

To investigate the transcriptomic mechanisms by which IDO@Cu₁/ZIF improves AA, RNA sequencing was performed on dorsal skin tissues from the AA + PBS and AA + IDO@Cu₁/ZIF groups, with n = 3 biological replicates per group. Tissues were ground in liquid nitrogen, and total RNA was extracted using TRIzol reagent. After RNA quality assessment and library construction, sequencing was performed by LC-Bio Technology Co., Ltd. (Hangzhou, China) using the Illumina Novaseq™ X Plus platform. Raw sequencing data were processed using standard procedures for quality control and alignment. DEGs were identified using adjusted $p < 0.05$ and $|\log_2FC| > 0.5$ as the thresholds. Pathway enrichment analysis and GSEA were subsequently performed.

The raw sequencing data generated in this study have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics^{47,48}, Chinese Academy of Sciences (GSA: CRA044347), and are publicly available at <https://ngdc.cncb.ac.cn/gsa>.

In vivo biosafety evaluation

To evaluate the *in vivo* biosafety of IDO@Cu₁/ZIF, unmodelled C3H/HeJ mice were used for material safety assessment. Mice were randomly divided into Control, ZIF, Cu₁/ZIF and IDO@Cu₁/ZIF groups. The Control group received an equal volume of PBS, whereas the other groups received ZIF, Cu₁/ZIF or IDO@Cu₁/ZIF using the same administration route and dose as those used in the therapeutic experiment. Treatments were administered continuously for 21 d.

At the experimental endpoint, peripheral blood and major organs, including the heart, liver, spleen, lung, kidney, stomach, colon and treated skin, were collected. Peripheral blood was used for routine blood tests and liver and kidney function assays. Major organs were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned and subjected to H&E staining to evaluate potential tissue toxicity.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 10.1.2. Bioinformatic analyses were performed using R 4.3.3. Flow cytometry data were analyzed using FlowJo 10.8.1. Fluorescence and histological images were quantified using ImageJ. Data are presented as mean ± SD. The numbers of biological replicates or independent experiments are indicated in the corresponding figure legends.

Pairwise comparisons between two groups were performed using unpaired two-tailed Student's t-tests. Data involving multiple time points or multiple factors were analyzed using two-way analysis of variance (two-way ANOVA). $P < 0.05$ was considered statistically significant. Statistical significance was indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

A complete list of abbreviations used in this study is provided in Supplementary Table 3.

Declarations

Data availability

The data supporting the findings of this study are available within the article and its Supplementary Information files. The raw bulk RNA-sequencing data are available in the GSA, under accession number CRA044347.

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Author contributions

Ling Liu, Yiting Lin, Zhimin Tian, and Yongquan Qu conceived the project, secured overall supervision, and directed the study. Mingzhu Jin, Jiahao Ma, Zhenhua Liu and Yaoyao Li performed the majority of the experiments, analyzed the data, and prepared the initial manuscript draft. Xiaolei Su, Yuqi Wang, Haoran Song, Bingquan Zong, Yajie Lv, and Da Zhai contributed to specific experimental procedures, including immunofluorescence staining, biosafety assessment, flow cytometric analysis and related validation experiments. All authors contributed to the interpretation of the results, critically revised the manuscript, and approved the final version.

Competing interests

The authors declare no competing interests.

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Figures

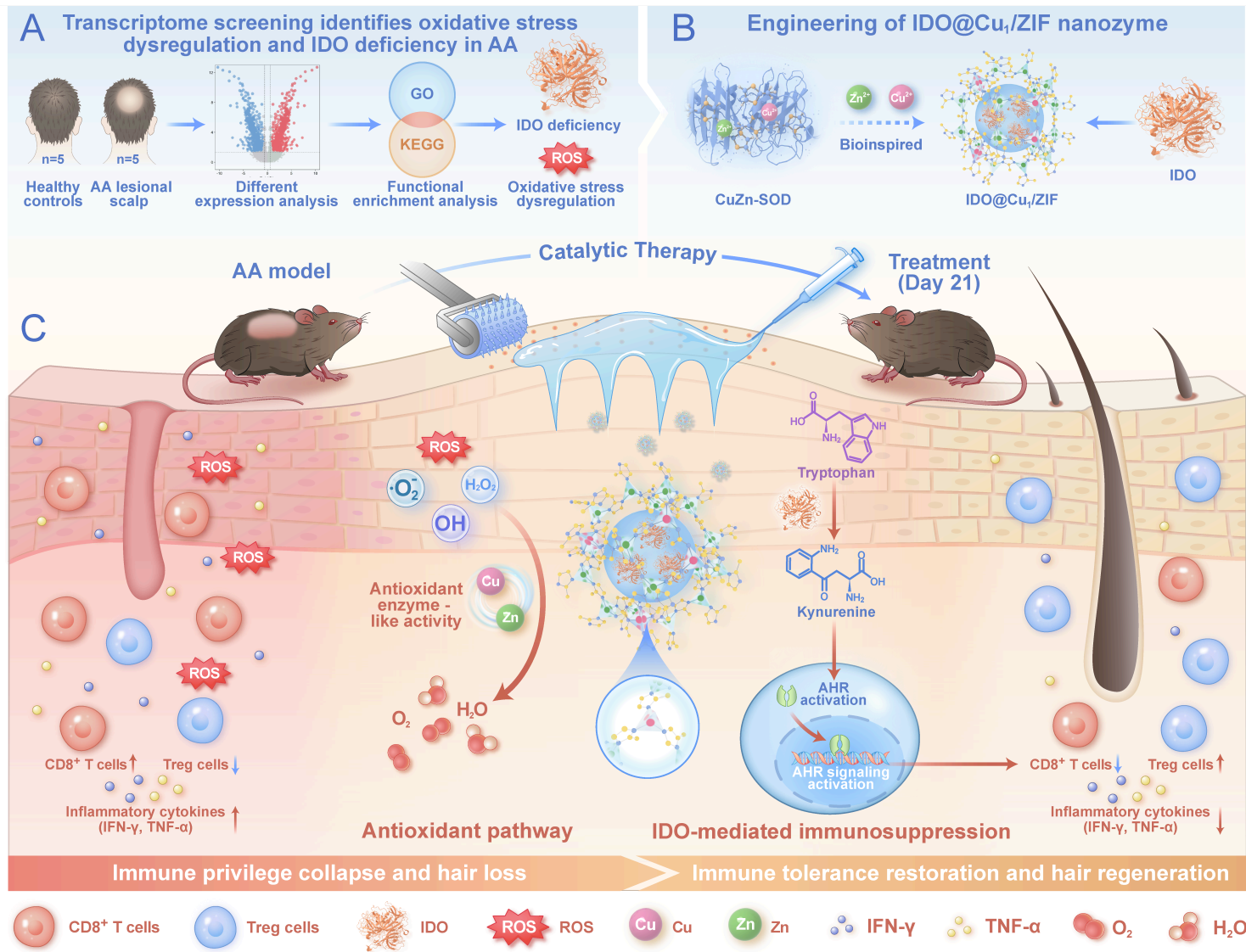


Figure 1

Design and proposed mechanism of IDO@Cu₁/ZIF for catalytic immunotherapy of AA. (A) Transcriptomic screening of AA lesions identifies oxidative stress dysregulation and insufficient IDO1-

associated signaling. Public transcriptomic analysis of scalp samples from healthy controls and patients with AA revealed oxidative stress dysregulation together with attenuated IDO1-associated Trp/Kyn signaling in AA lesions. (B) Design and engineering of the IDO@Cu₁/ZIF nanozyme. Inspired by the catalytic center of the natural Cu, Zn-SOD enzyme, Cu and Zn were selected to construct a biomimetic ZIF-based nanozyme with antioxidant enzyme-like activity, followed by incorporation of IDO1 to generate IDO@Cu₁/ZIF. (C) Proposed mechanism of IDO@Cu₁/ZIF in AA treatment. Following local administration to AA lesions, the Cu₁/ZIF framework scavenges excessive ROS through antioxidant enzyme-like activity, thereby reducing oxidative stress in the inflammatory microenvironment. Meanwhile, loaded IDO1 catalyzes the conversion of Trp into Kyn, leading to activation of downstream AHR-associated signaling. Coordinated redox regulation and Trp/Kyn metabolic rewiring suppress CD8⁺T cells-driven inflammation, decrease inflammatory cytokine production, restore Treg-associated immune tolerance, and ultimately promote hair follicle regeneration.

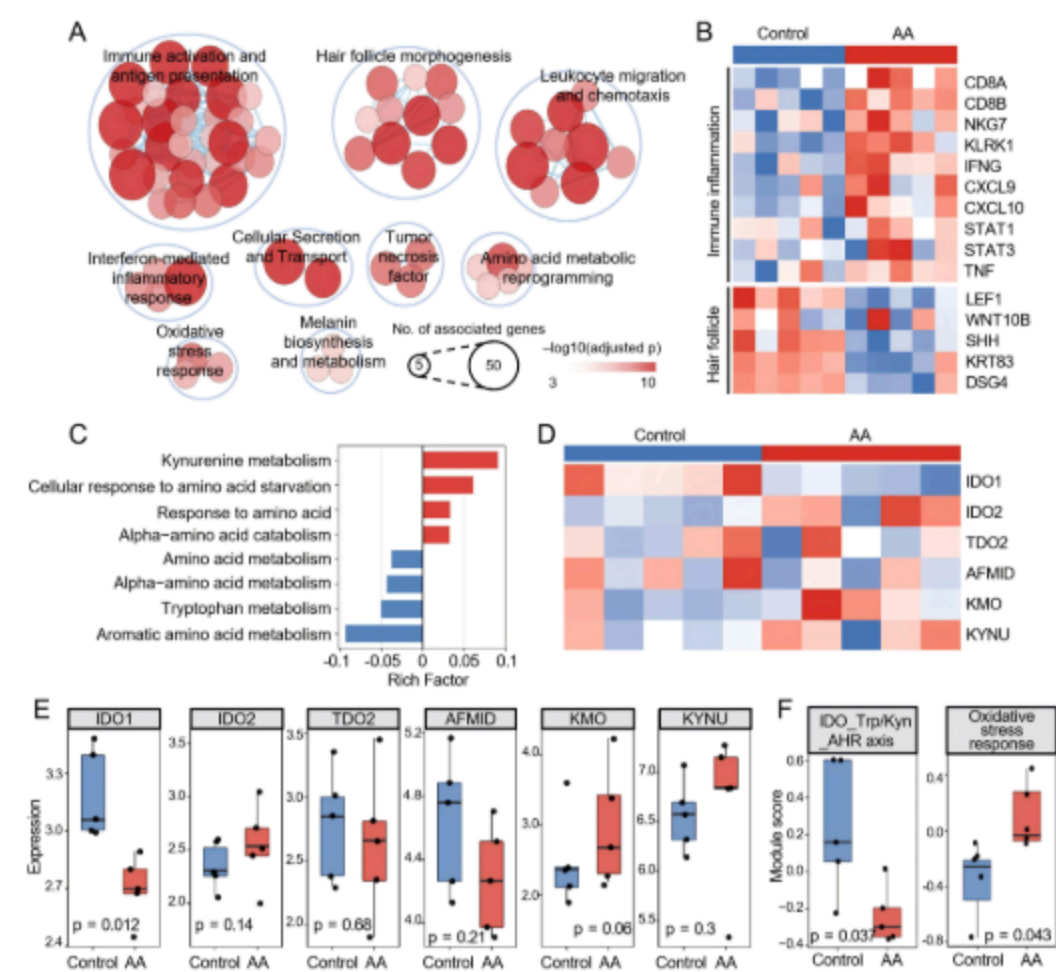


Figure 2

Transcriptomic profiling of human AA scalp lesions reveals impaired Trp/Kyn signaling and metabolic perturbation. (A) GO network showing the major biological processes enriched in DEGs from AA scalp samples. (B) Heatmap of representative genes associated with cytotoxic immune activation and hair

follicle biology. (C) GO enrichment analysis highlighting amino acid metabolism-related processes altered in AA lesions. (D) Heatmap showing the expression profiles of genes involved in Trp/Kyn metabolism. (E) Expression levels of selected enzymes in the Trp/Kyn pathway. (F) Pathway activity scores for the IDO1-Trp/Kyn-AHR axis and oxidative stress response. Public transcriptomic analyses in (A-F) were performed using scalp samples from 5 healthy controls and 5 patients with AA. For the indicated pairwise comparisons, P values were calculated using two-sided unpaired Student's t-tests in R 4.3.3.

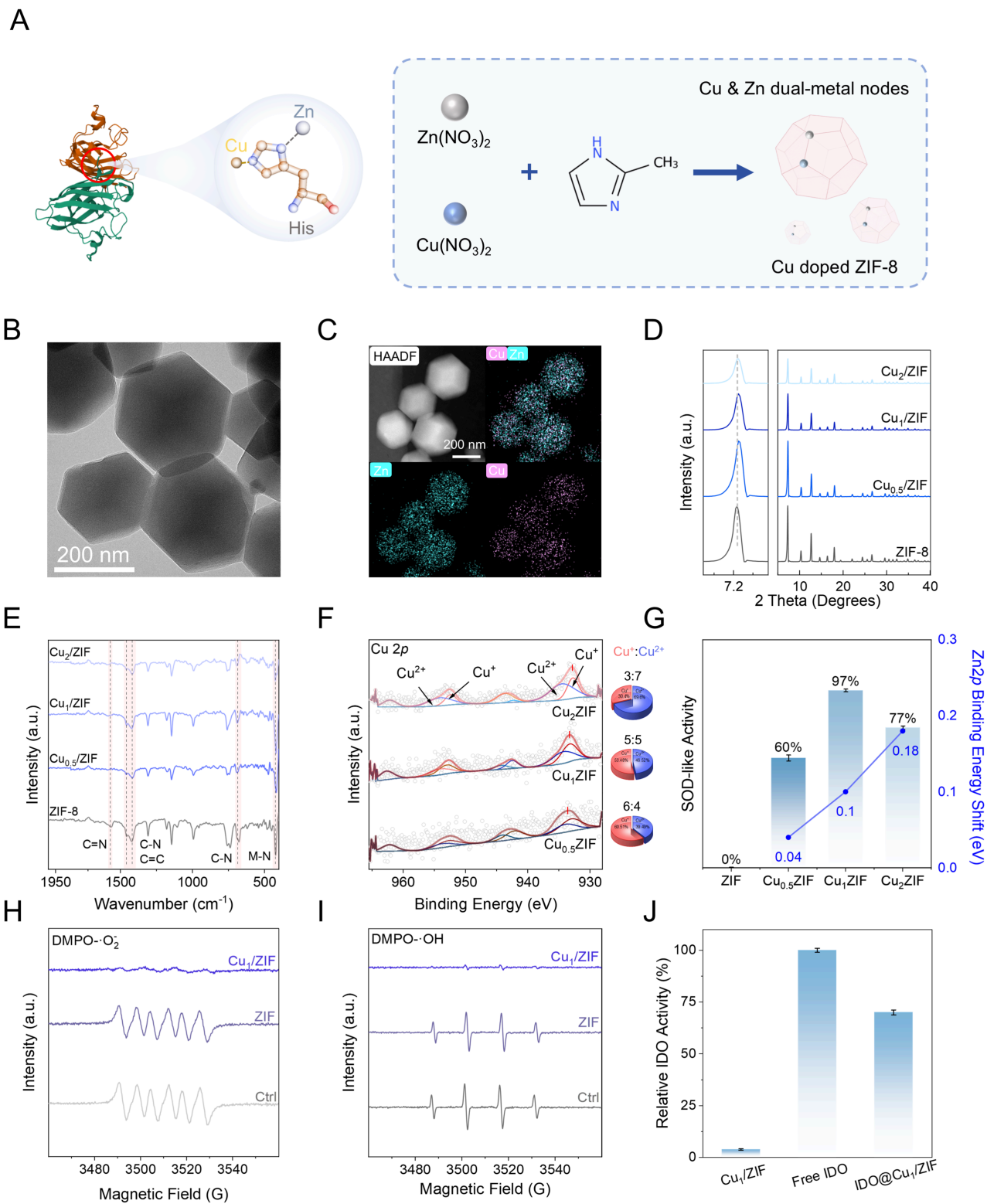


Figure 3

Biomimetic construction and electronic modulation of Cu-doped ZIF-8 nanozymes. (A) Schematic illustration of the Cu, Zn-SOD-inspired design and aqueous synthesis of Cu-doped ZIF-8. Inspired by the Cu/Zn bimetallic active center coordinated by histidine residues in natural Cu, Zn-SOD, Cu and Zn ions were co-assembled with 2-methylimidazole to construct Cu/Zn dual-metal nodes in the ZIF framework. (B) Representative TEM image of Cu₁/ZIF. Scale bar, 200 nm. (C) HAADF-STEM image and corresponding elemental mapping of Zn and Cu in Cu₁/ZIF, showing the homogeneous distribution of Cu and Zn within the particles. Scale bar, 200 nm. (D) XRD patterns of ZIF-8, Cu_{0.5}/ZIF, Cu₁/ZIF, and Cu₂/ZIF. The inset shows the enlarged diffraction region around the characteristic low-angle peak of ZIF-8. (E) FTIR characterization of ZIF-8 and Cu-doped ZIF-8. (F) High-resolution Cu 2p XPS spectra of Cu_{0.5}/ZIF, Cu₁/ZIF, and Cu₂/ZIF, showing the coexistence of Cu⁺ and Cu²⁺ species. (G) SOD-like activity of ZIF and Cu-doped ZIF samples and the corresponding Zn 2p binding energy shift. Data are presented as mean ± SD. n = 3 independent measurements. (H, I) EPR spectra of DMPO-trapped ·O₂⁻ and ·OH in control, ZIF, and Cu₁/ZIF groups. (J) Relative IDO1 activity of Cu₁/ZIF, free IDO1, and IDO@Cu₁/ZIF. Data are presented as mean ± SD. n = 3 independent measurements.

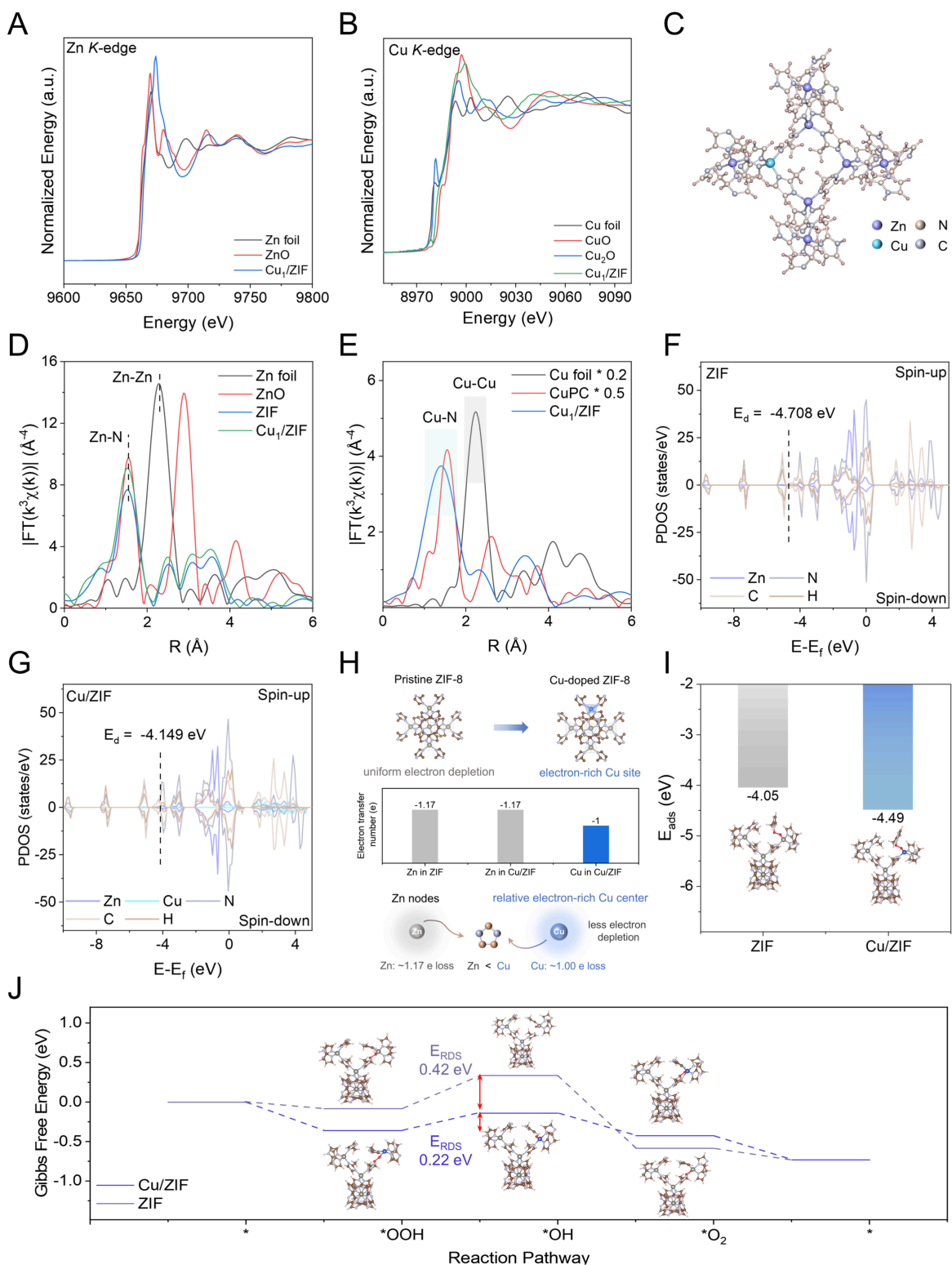


Figure 4

Atomic coordination structure and antioxidant catalytic mechanism of Cu₁/ZIF. (A, B) Zn K-edge and Cu K-edge XANES spectra of Cu₁/ZIF and reference samples. (C) Proposed local coordination model of Cu₁/ZIF consistent with XAFS analysis, showing the Cu/Zn-N coordination environment within the ZIF framework. (D) Fourier-transformed EXAFS spectra at the Zn K-edge of ZIF and Cu₁/ZIF, compared with

Zn foil and ZnO references. (E) Fourier-transformed EXAFS spectra at the Cu K-edge of Cu₁/ZIF, compared with Cu foil and CuPc references. (F, G) Spin-polarized projected density of states (PDOS) of pristine ZIF and Cu/ZIF models. (H) Bader charge analysis showing the electron-transfer characteristics of Zn and Cu sites in ZIF and Cu doped ZIF models. (I) Calculated adsorption energies of ·O₂⁻ on ZIF and Cu/ZIF models. (J) DFT-calculated Gibbs free energy profiles for the SOD-like catalytic reaction pathway on ZIF and Cu doped ZIF models.

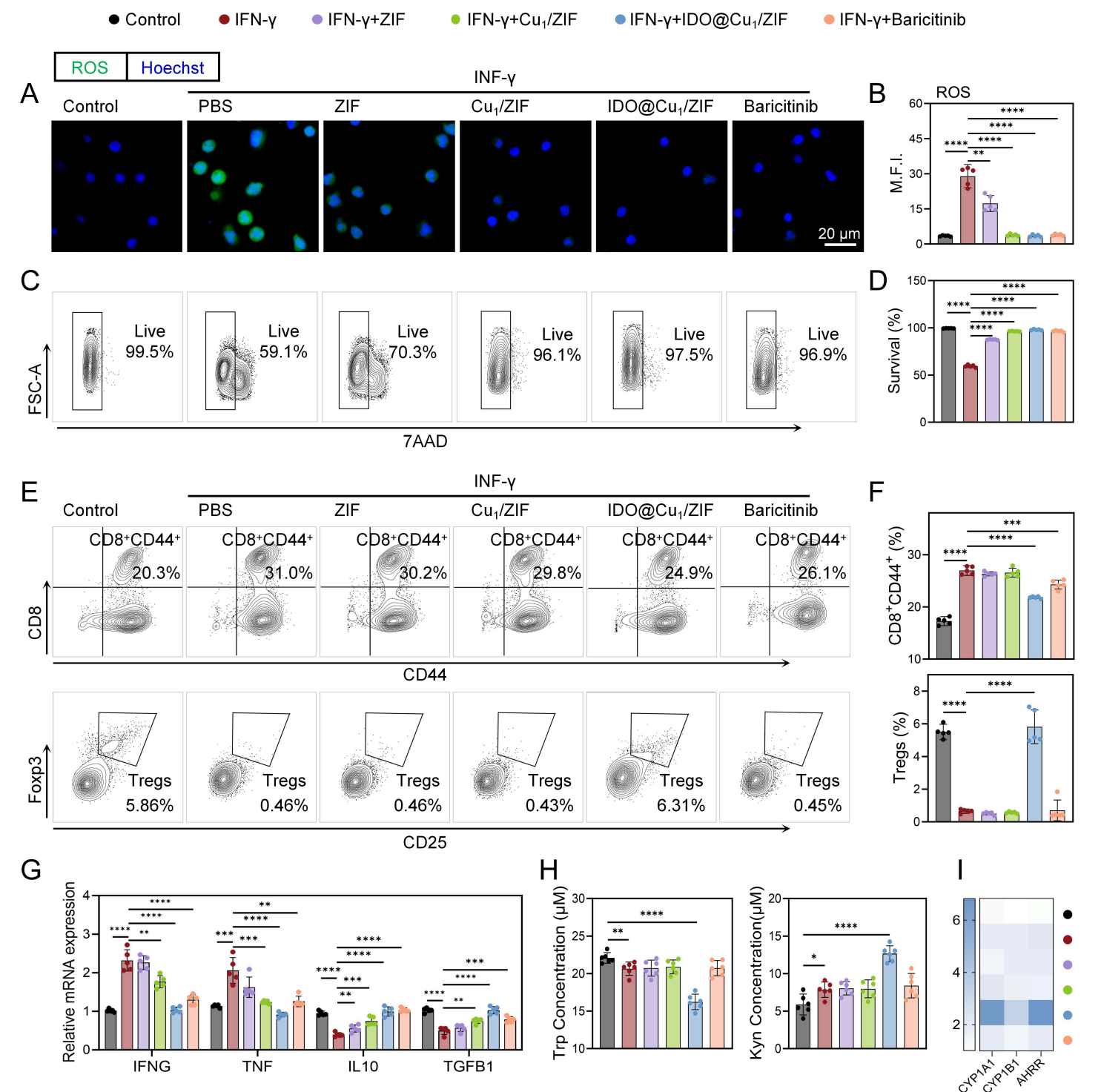


Figure 5

IDO@Cu₁/ZIF mitigates inflammatory activation and rewires Trp metabolism in IFN- γ -stimulated PBMCs. (A, B) Representative fluorescence images and quantification of intracellular ROS in PBMCs. ROS was detected using a fluorescent probe (green), and nuclei were counterstained with Hoechst (blue). Quantitative analysis is shown in (B). Scale bar, 20 μ m. (C, D) Representative flow cytometry plots and quantification of PBMC viability assessed by 7-AAD staining. The percentage of live cells is indicated in each panel in (C), and the corresponding quantitative analysis is shown in (D). (E, F) Flow cytometric analysis of immune cell subsets. (E) Representative contour plots showing CD8/CD44 gating for activated CD8⁺T cells (upper panels) and CD25/Foxp3 gating for Tregs (lower panels). (F) Quantification of CD8⁺CD44⁺ activated T cells and Tregs. (G) Relative mRNA expression of inflammatory and immunoregulatory factors, including *IFNG*, *TNF*, *IL10*, and *TGFB1*. (H) Quantification of Trp and Kyn concentrations in PBMC culture supernatants. (I) Heatmap showing the relative expression of AHR pathway-related genes, including *CYP1A1*, *CYP1B1*, and *AHRR*, across different treatment groups. Data are presented as mean $\pm$ SD. n = 5 independent experimental replicates per group. Statistical significance for the indicated pairwise comparisons was determined using unpaired two-tailed Student's t-tests. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

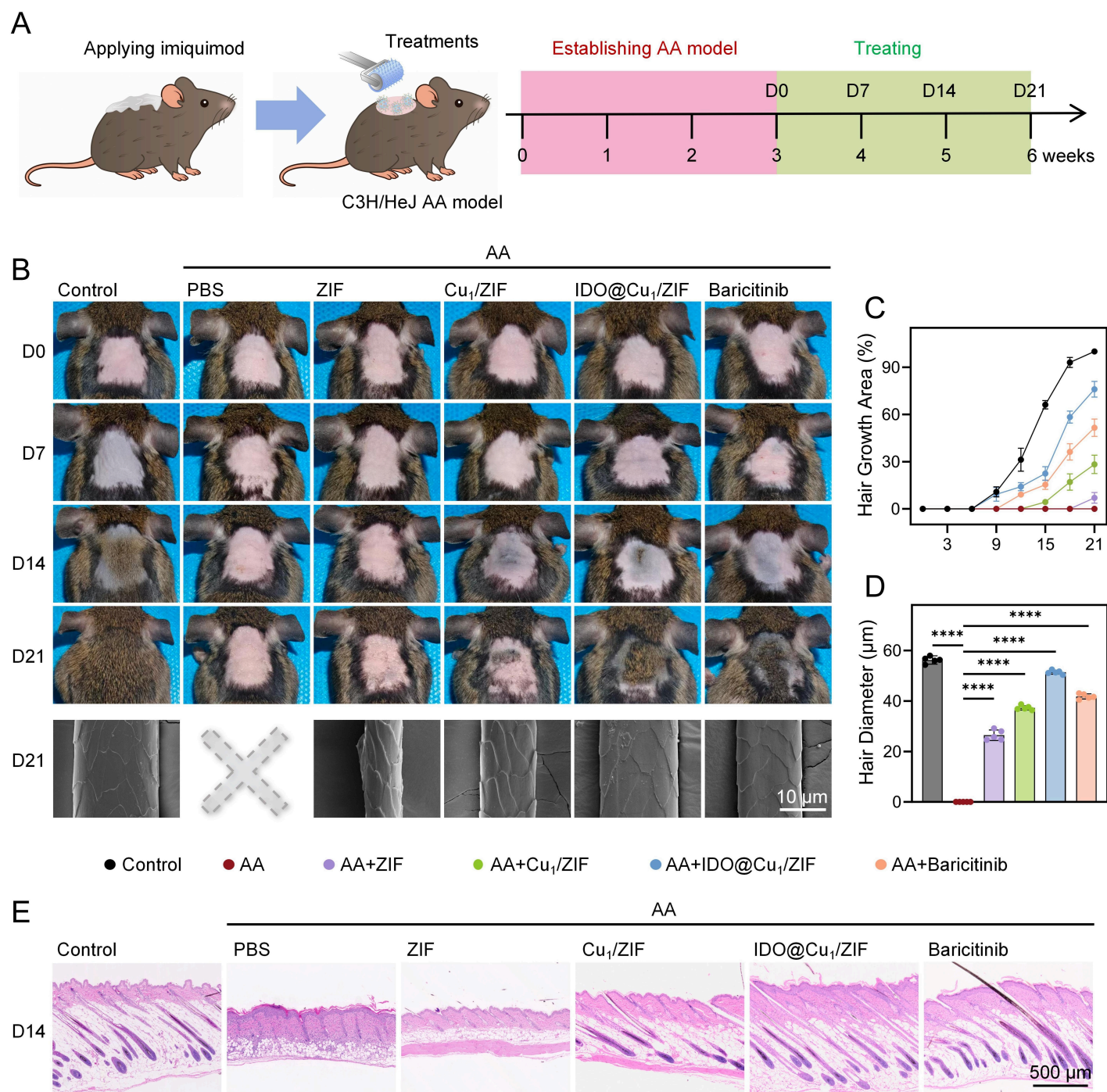


Figure 6

IDO@Cu₁/ZIF promotes hair regrowth and preserves follicular architecture in AA mouse model. (A) Schematic illustration of the therapeutic treatment of AA mouse model. AA-like lesions were induced on the dorsal skin of C3H/HeJ mice by topical IMQ, followed by treatment with PBS, ZIF, Cu₁/ZIF, IDO@Cu₁/ZIF, or baricitinib. Hair regrowth and tissue responses were evaluated at the indicated time points. (B) Representative photographs of dorsal skin collected during the treatment course and SEM images of regenerated hair shafts. Scale bar, 10 μ m. (C, D) Quantitative evaluation of hair regeneration. (C) Time-dependent changes in hair regrowth area. (D) Quantification of hair shaft diameter. (E)

Representative H&E staining of dorsal skin sections. Scale bar, 500 μ m. Data are presented as mean $\pm$ SD. n = 5 biologically independent mice per group. Statistical significance for the hair-regrowth curves in (C) was determined using two-way ANOVA. Statistical significance for the indicated pairwise comparisons in the remaining quantitative analyses was determined using unpaired two-tailed Student's t-tests. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

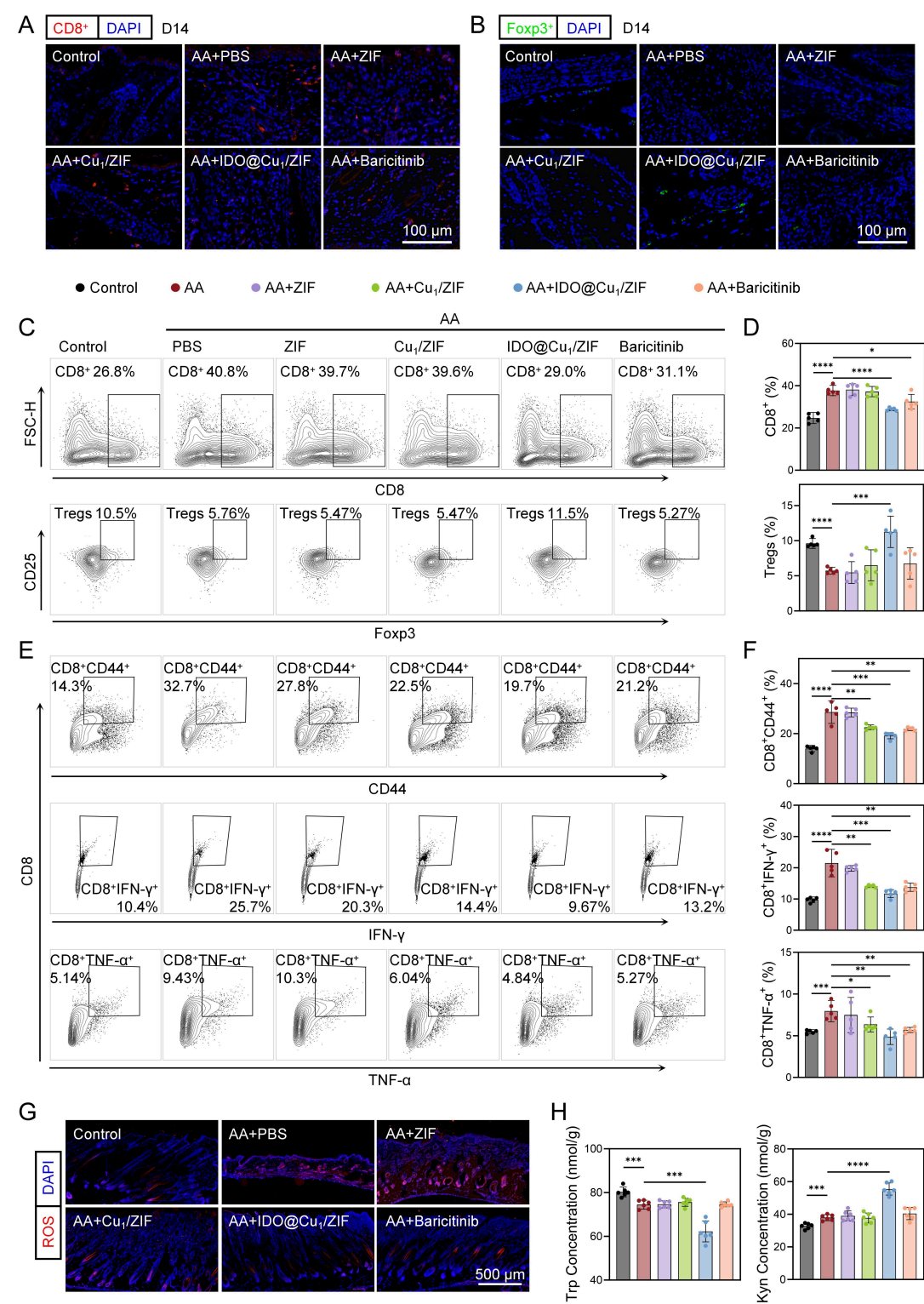


Figure 7

IDO@Cu₁/ZIF restores local CD8⁺T/Treg balance in AA lesions through redox regulation and Trp/Kyn-AHR signaling. (A, B) Immunofluorescence analysis of immune cell infiltration in skin lesions. (A) Representative images of CD8 staining (red), showing perifollicular accumulation of inflammatory T cells in lesional skin. (B) Representative images of Foxp3 staining (green), indicating Tregs. Scale bar, 100 μ m. (C, D) Flow cytometric analysis of total CD8⁺T cells and Tregs in skin. (C) Representative gating plots showing CD8⁺T cells and Tregs in different groups. (D) Quantification of CD8⁺T cells and Tregs frequencies. (E, F) Flow cytometric profiling of activated and effector CD8⁺T-cell subsets. (E) Representative plots showing CD8⁺CD44⁺, CD8⁺IFN- γ ⁺, and CD8⁺TNF- α ⁺ populations. (F) Quantification of these inflammatory CD8⁺ subsets. (G) Representative fluorescence images of ROS in skin sections. Scale bar, 500 μ m. (H) Quantification of Trp and Kyn concentrations in skin lesions. Data are presented as mean $\pm$ SD. n = 5 biologically independent mice per group. Statistical significance for the indicated pairwise comparisons was determined using unpaired two-tailed Student's t-tests. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

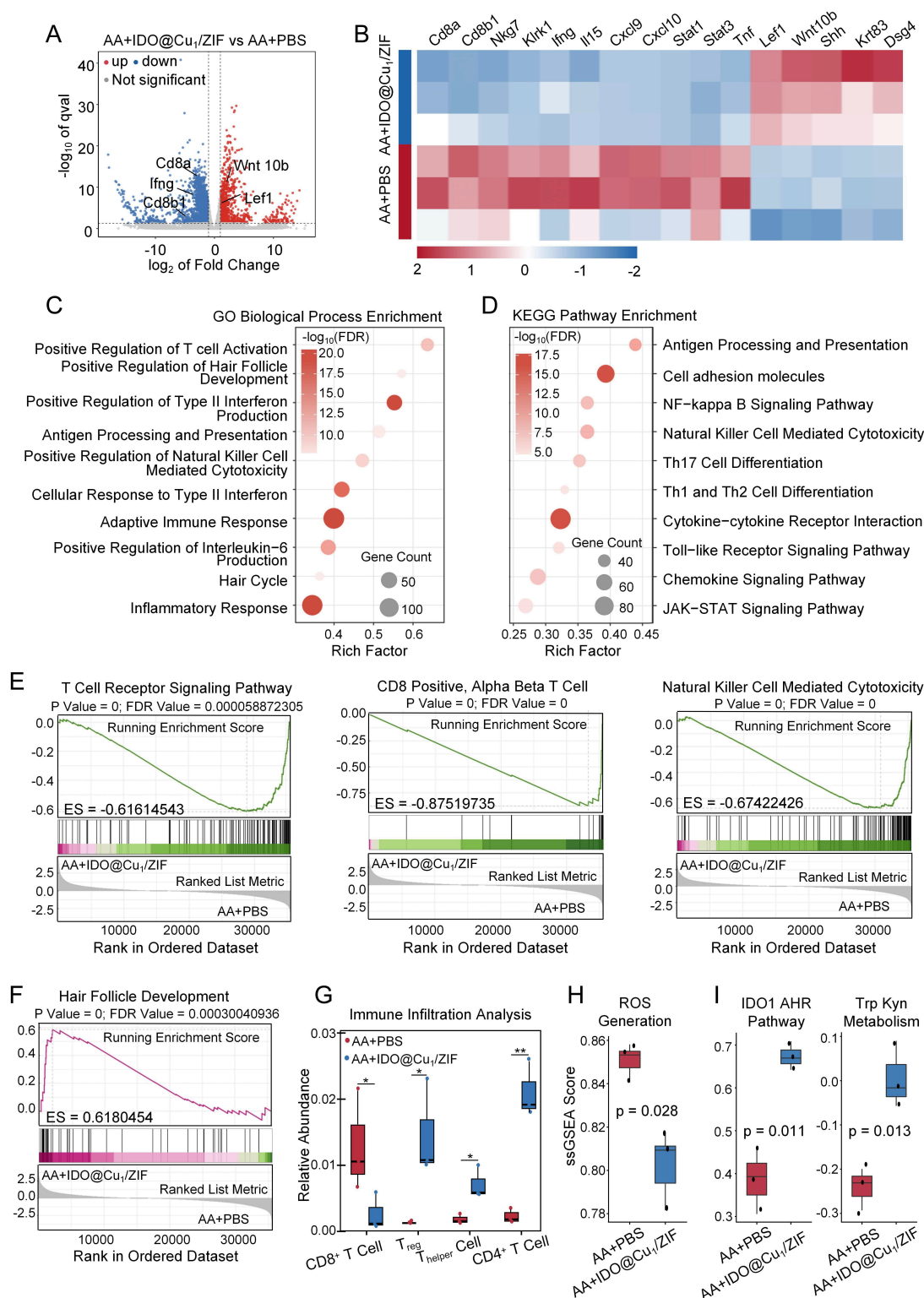


Figure 8

Transcriptomic analyses identify cytotoxic immune, follicle developmental, and Trp/Kyn-associated changes in AA lesions following IDO@Cu₁/ZIF treatment. (A) Volcano plot showing DEGs between AA+IDO@Cu₁/ZIF and AA+PBS skin lesions. (B) Heatmap of representative DEGs associated with cytotoxic immune activation and hair follicle development. (C, D) Functional enrichment analysis of DEGs. (C) GO biological process enrichment analysis. (D) KEGG pathway enrichment analysis. (E, F)

GSEA. (E) Enrichment plots of the T cell receptor signaling pathway, CD8 positive, alpha beta T cell gene set, and natural killer cell mediated cytotoxicity gene set. (F) Enrichment plot of the hair follicle development gene set. (G) Immune infiltration analysis showing the relative abundance of infiltrating immune cell subsets in AA+PBS and AA+IDO@Cu₁/ZIF samples. (H, I) ssGSEA. (H) ROS generation score. (I) IDO1-AHR pathway and Trp/Kyn metabolism scores. RNA-seq analyses in (A-I) were performed using three samples per group. For the indicated pairwise comparisons, P values were calculated using two-sided unpaired Student's t-tests in R 4.3.3. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

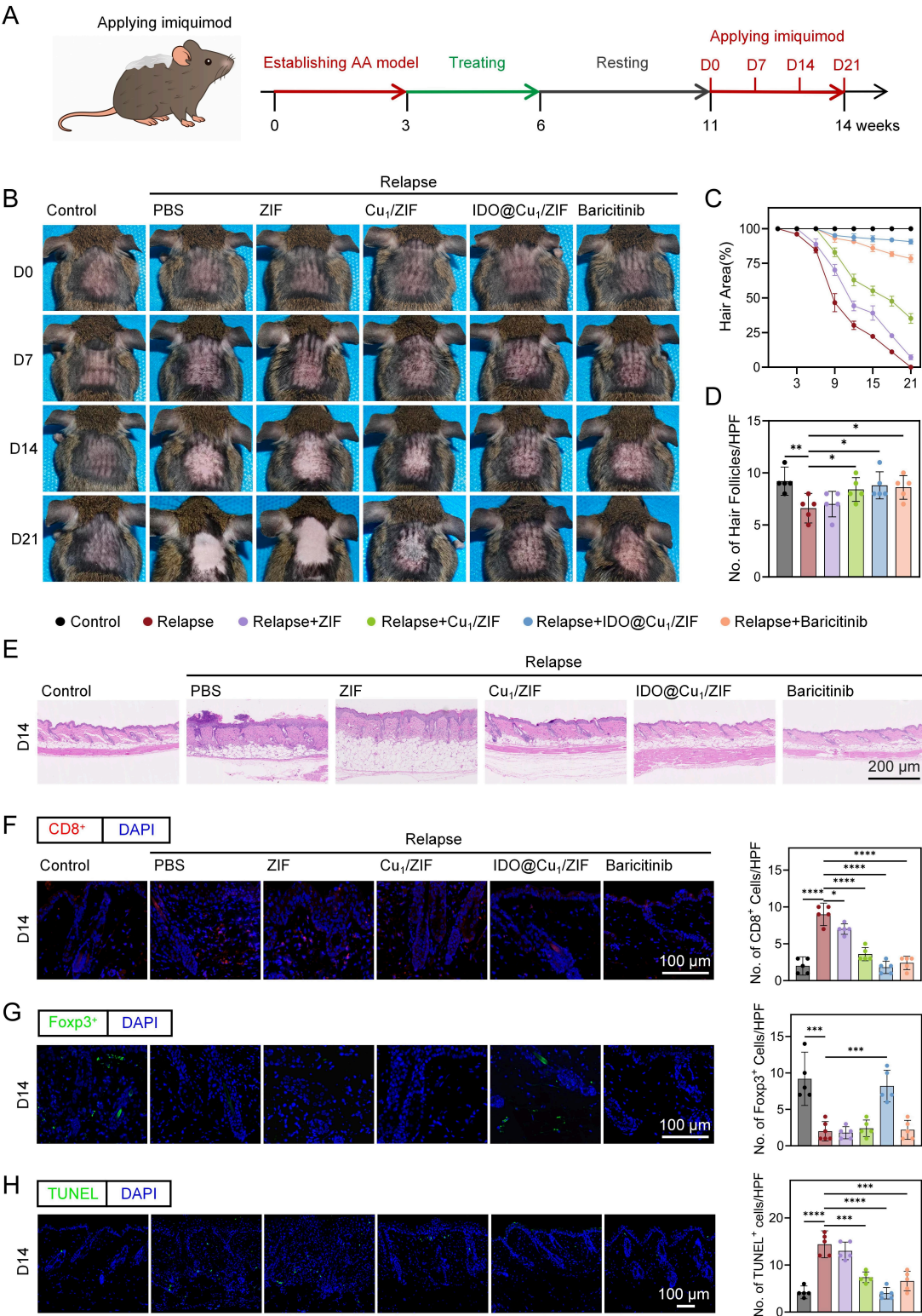


Figure 9

IDO@Cu₁/ZIF prevents AA relapse and maintains the cutaneous immune microenvironment during IMQ re-challenge. (A) Schematic illustration of the experimental design for relapse prevention in AA. (B, C) Representative photographs and quantitative analysis of hair loss during relapse induction. (B) Representative dorsal skin images collected at the indicated time points. (C) Quantification of hair covered area. (D, E) Histological evaluation of skin sections. (D) Quantification of hair follicle numbers per field. (E) Representative H&E staining images showing follicular morphology in different groups. Scale bar, 200 μ m. (F, G) Immunofluorescence staining and quantification of immune cell subsets in skin lesions. (F) CD8 staining (red) showing inflammatory T-cell infiltration. (G) Foxp3 staining (green) showing Tregs. Scale bar, 100 μ m. (H) Representative TUNEL staining (green) and quantification of TUNEL-positive cells per high-power field in skin sections. Scale bar, 100 μ m. Data are presented as mean $\pm$ SD. n = 5 biologically independent mice per group. Statistical significance for hair area in (C) was determined by two-way ANOVA. Statistical significance for the indicated pairwise comparisons in the remaining quantitative analyses was determined using unpaired two-tailed Student's t-tests. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Supplementary Files

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