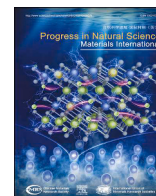


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A self-enhancing catalytic epigenetic nano-agonist for cancer immunotherapy

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ABSTRACT

Epigenetic abnormalities drive malignant tumor proliferation and shape a microenvironment characterized by hypoxia, acidification, and immunosuppression. Although the excessive H₂O₂ in this microenvironment provides the necessary substrate for catalytic therapy, conventional catalysts are inefficient, and the immunosuppressive barrier further limits therapeutic efficacy. To address these dual challenges, this study designed a nano-agonist, JV@FeRu, that achieves highly effective anti-tumor immunotherapy through the synergistic combination of self-enhancing catalysis and epigenetic regulation. This system utilizes FeRu nanoparticles as a core catalytic platform to efficiently decompose H₂O₂ into O₂ and ·OH, thereby alleviating hypoxia and inducing oxidative stress; VB124-mediated metabolic acidification amplifies the catalytic efficiency of FeRu, forming a self-reinforcing loop; the co-loaded JQ1 reverses immune suppression by inhibiting BRD4 function to downregulate oncogenes and reprogram immune-related genes. *In vivo* experiments demonstrated that JV@FeRu significantly promotes T cell infiltration, reduces the proportion of Tregs, and promotes dendritic cell maturation, achieving an 81.46% tumor suppression rate. Transcriptomic analysis confirmed that this system activates the antigen-presentation pathway, enhances cytokine signaling, thereby reshaping the tumor immune microenvironment. The synergistic strategy of self-enhancing catalysis, epigenetic regulation, microenvironment remodeling proposed in this study offers a novel approach to overcoming the dual barriers of the tumor microenvironment.

1. Introduction

The initiation and progression of tumors are complex pathological processes driven by the dynamic interaction between epigenetic reprogramming and the tumor microenvironment (TME) [1]. Epigenetic modifications include DNA methylation [2], histone acetylation and methylation [3], and non-coding RNA regulation [2–4], stably regulate the expression of oncogenes, tumor suppressor genes, and immune-related genes without altering DNA sequences [5]. These modifications constitute the core molecular basis for determining tumor

malignancy and immune evasion. Epigenetic dysregulation in tumor cells not only drives their own proliferation, invasion, and metastasis [6], but also actively shapes a hypoxic, acidic, highly oxidative, and immunosuppressive TME by regulating the secretion of cytokines, chemokines, immune checkpoint ligands, and matrix remodeling molecules [7]. This environment inhibits the infiltration and activation of CD8⁺ T cells while promoting the accumulation of Tregs, M2 macrophages, and myeloid-derived suppressor cells (MDSCs), thereby creating a microenvironment conducive to immune evasion [8]. Meanwhile, hypoxia [9], inflammatory signals, and metabolic dysregulation within the TME

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can further upregulate the expression and activity of epigenetic enzymes [10,11], such as DNA methyltransferases, histone deacetylases, and arginine methyltransferases, exacerbating epigenetic abnormalities and creating a malignant positive feedback loop [12] (see Scheme 1).

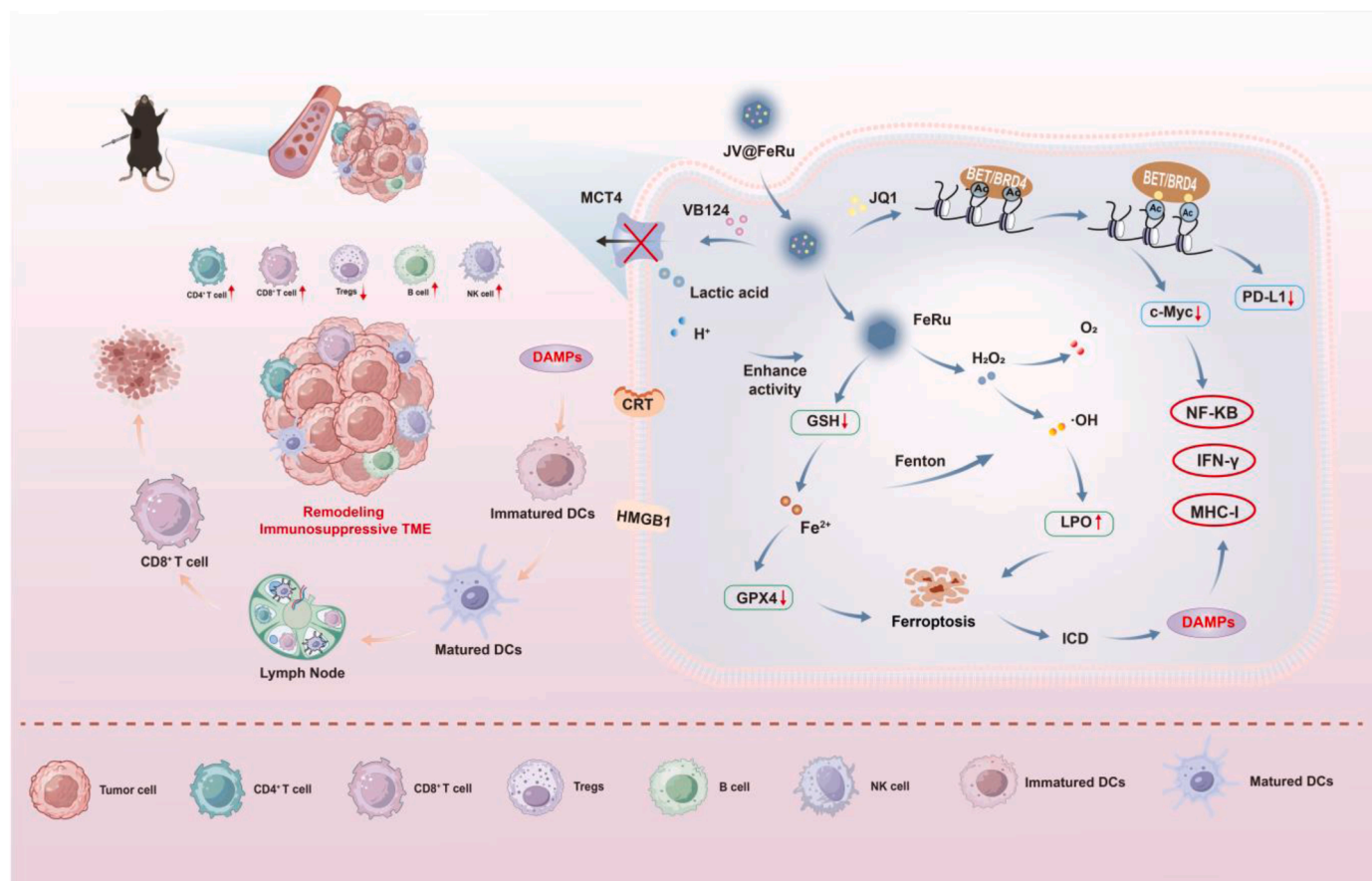
From the above, the effectiveness of epigenetic regulation is highly dependent on the tumor microenvironment, which is characterized by typical physicochemical features such as hypoxia [13], high hydrogen peroxide (H_2O_2) concentration [14], and acidity [15]. These characteristics are the key drivers of tumor malignancy progression and also provide potential targets for developing targeted therapeutic strategies. The high concentration of H_2O_2 has created favorable conditions for the development of H_2O_2 -activated catalytic therapy [14]. However, traditional Fenton catalysts (such as Fe^{2+}) have limited catalytic activity under the TME [16], making it difficult to efficiently utilize H_2O_2 [17]. Meanwhile, the highly expressed antioxidant defense system in tumor cells can rapidly eliminate reactive oxygen species (ROS), significantly weakening the efficacy of catalytic therapy [18]. Moreover, the spatio-temporal heterogeneity of the TME requires the catalytic system to have environmental responsiveness and feedback regulation capabilities [19], while traditional catalysts cannot achieve this [20]. On the other hand, given that immunotherapy relies on continuous immune activation signals [21], an ideal catalytic system should also possess the ability to provide sustained immune stimulation [22].

In this background, we developed a self-enhancing catalytic epigenetic nano-agonist for cancer immunotherapy achieved by synergistic self-enhancing catalysis and epigenetic regulation. Using FeRu nanoparticles as the core catalytic platform, this system efficiently catalyzes the decomposition of H_2O_2 into O_2 and hydroxyl radical ($\cdot OH$), thereby alleviating hypoxia condition and inducing oxidative stress. Metabolic acidification mediated by VB124 further amplifies the catalytic efficiency of FeRu, forming a self-reinforcing cycle. Given that BRD4

recognizes and binds to acetylated histones, maintaining chromatin openness and driving the high expression of oncogenes and immunosuppressive genes. The co-loading JQ1 in the nano-agonist competitively binds to BRD4, blocking its interaction with acetylated histones, downregulating oncogenes, and upregulating immune-related genes, ultimately achieving epigenetic reprogramming and reversing the epigenetic state of tumor immunosuppression. *In vivo* experiments demonstrated that JV@FeRu significantly enhances the infiltration of $CD8^+$ T cells, lowers fraction of regulatory T cells, and promotes dendritic cell maturation, enhances the activation of natural killer (NK) cells, and aids in the accumulation of B cells within the tumor microenvironment. In a mouse melanoma model, this strategy achieved a tumor suppression rate of 81.46%. Transcriptomic analysis further revealed that JV@FeRu systematically reshapes the tumor immune microenvironment by activating the antigen-presentation pathway (upregulating B2m, H2-K1, H2-D1, and dendritic cell co-stimulatory molecules CD80, CD86, and CD83), enhancing cytokine signaling, and regulating T-cell immune responses, while simultaneously downregulating the expression of the immune checkpoint gene CD274. The schematic diagram in Fig. 1 visually illustrates the synergistic therapeutic mechanism of JV@FeRu in the tumor microenvironment. The synergistic strategy of “self-enhancing catalytic epigenetic nano-agonist” proposed in this study provides new design concept and effective approach for overcoming the dual barriers of epigenetic imbalance and tumor immune suppression.

2. Methods

Materials: Ferrous chloride ($FeCl_2$), ruthenium chloride trihydrate ($RuCl_3 \cdot 3H_2O$), JQ-1 ($C_{23}H_{25}ClN_4O_2S$), methylene blue (MB) were from Macklin (Shanghai, China). Sodium hydroxide (NaOH) was from Damao



Scheme 1. The synergistic therapeutic effects of the JV@FeRu nanocomposite within the tumor microenvironment.

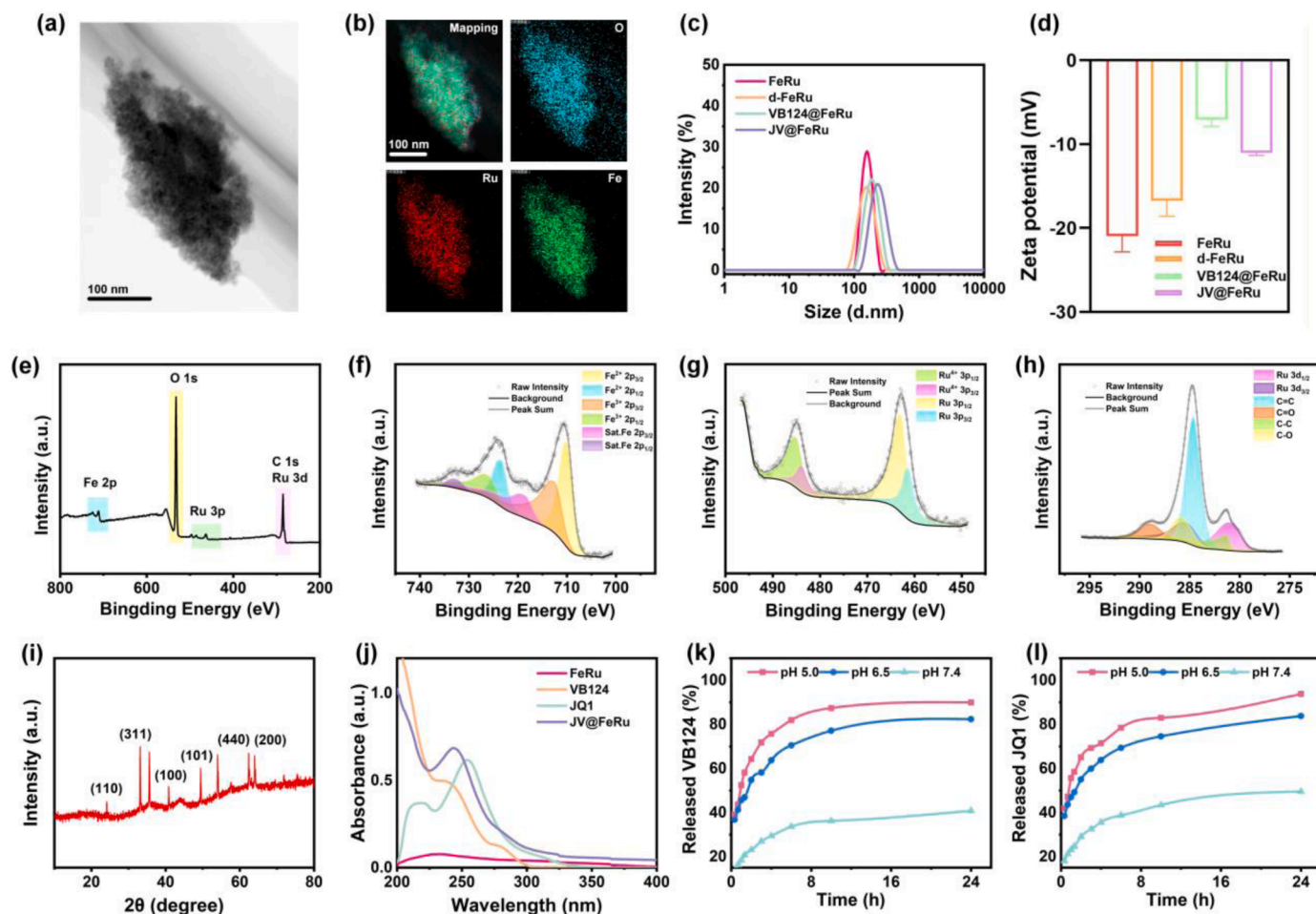


Fig. 1. Characterization of JV@FeRu. (a) TEM images of FeRu. (b) Element mapping of FeRu. Particle size distribution (c) and zeta potentials (d) of FeRu, VB124@FeRu and JV@FeRu by DLS analysis. (e) Survey XPS spectra of FeRu. XPS spectra of (f) Fe 2p, (g) Ru 3p and (h) C 1s. (i) XRD analysis of FeRu. (j) UV-Vis spectra of FeRu, VB124@FeRu and JV@FeRu. The release profiles of JQ1 (k) and VB124 (l) from JV@FeRu at different pH values.

Chemical Reagent Factory (Tianjin, China). D- α -tocopherol acid polyethylene glycol succinate (TPGS) and VB124 were from Yuancheng Technology (Wuhan, China) and Chuchang Biomedical Technology (Wuhan, China), respectively. Chlorpromazine and amiloride were from Jizhi Biochemical (Shanghai). Genistein, idebenone (IDB), deferoxamine (DFO) and VE were from Macklin Biotech (Shanghai). RPMI 1640 medium, fetal bovine serum (FBS), CCK-8, MDA kits, calcein AM/PI, DCFH-DA, GSH kits were from Meilun Biotechnology (Dalian, China). Actin-Tracker Red-555, γ -H2AX immunofluorescence kit, 7-AAD, BCECF, Ferro FarRed, FITC were from Beyotime Biotechnology (Haimen, China). [Ru(dpp)₃] Cl₂, C11-BODIPY581/591 were from Maokang Biotechnology (Shanghai, China). Primary antibodies (anti-CD45-PerCPy5.5, anti-CD4-FITC, anti-CD3-FITC, anti-CD8-APC, anti-NK1.1-PE, anti-CD19-AOC etc.) were from BioLegend (Beijing, China).

Characterization: FeRu morphology and elements were characterized by TEM (Tecnai G20, FEI, USA). XRD was used for crystal structure. DLS (Malvern Zetasizer Nano, UK) measured size distribution and zeta potential of FeRu, VB124@FeRu, JV@FeRu. JV@FeRu drug loading efficiency (LE) was detected by UV-Vis at 254 nm (JQ1) and 234 nm (VB124), calculated as $LE\ wt\% = (m_0 - m) / (m_s + m_0 - m)$ (m_0 : initial drug mass; m : supernatant drug mass; m_s : initial nanoparticle mass).

The synthesis of FeRu: FeRu: Liquid A (0.2 mM FeCl₂, 0.1 mM RuCl₃·3H₂O in 10 mL deionized water) was added dropwise to liquid B (6 mM NaOH in 40 mL deionized water) under stirring. The mixed solution was reacted at room temperature for 30 min, then transferred to a Teflon-lined reactor (120 °C, 24 h). Afterwards, the product was washed three times (12000 rpm, 8 min), dispersed in 2 mL deionized water, and

freeze-dried to obtain FeRu nanoparticles.

The synthesis of JV@FeRu: First, FeRu nanoparticles (1 mg/mL) were etched in PBS (pH = 4) for 6 h. Then, JQ1 and VB124 (1 mg/mL, 200 μ L) were added, and the mixture was stirred for 2 h, sonicated (40 kHz, 100 W) for 10 min, and centrifuged (12,000 rpm, 15 min) to remove unloaded drugs. Then the precipitate was washed twice with deionized water and redispersed in 2 mL deionized water. Subsequently, the dispersion was mixed with TPGS (50 mg/mL, 2 μ L) as a stabilizer, stirred for 1 h to obtain JV@FeRu.

The stability of JV@FeRu: Appropriate amount of JV@FeRu was immersed into PBS, RPMI-1640 medium without serum, and RPMI-1640 medium with 10% fetal bovine serum, respectively, and stored at room temperature for one week. Samples were taken every day, and the particle size change of JV@FeRu was determined by particle size analyzer.

The released of drug: Drug release: 1 mL JV@FeRu (1 mg/mL) was sealed in dialysis tubing (MWCO = 3500 Da) in PBS (pH 5.5, 6.5, 7.4) at 37 °C, with release detected by UV-Vis at 0, 5, 10, 20, 30 min, 1, 2, 3, 4, 6, 8, 12, 24 h.

O₂ generation: FeRu (100 μ g/mL) was mixed with H₂O₂ (0, 5, 10, 40, 100 mM) and [Ru(dpp)₃] Cl₂ (5 μ M), incubated 20 min, detected by *in vivo* imager (Ex = 470 nm, Em = 600 nm) and microplate reader (620 nm); FeRu (0, 25, 50, 100, 150, 300 μ g/mL) with 100 mM H₂O₂ was tested similarly.

ROS generation: FeRu (250 μ g/mL) was mixed with H₂O₂ (100 mM) and MB (25 μ g/mL) (pH 6.5), UV-Vis-NIR spectra (400-800 nm) were measured at 0, 10, 30 min, and 1, 2, 3, 4, 12 h; pH (5.5, 6.5, 7.4), FeRu

(0, 50, 100, 250, 500 $\mu\text{g/mL}$) and H_2O_2 (0–300 mM) were optimized.

Enzymatic kinetics: O_2 generation (FeRu 100 $\mu\text{g/mL}$, H_2O_2 0, 10, 20, 30, 40, 50 mM, dissolved oxygen recorded every 10 s); ROS generation (FeRu 250 $\mu\text{g/mL}$, MB 25 $\mu\text{g/mL}$, H_2O_2 0, 25, 50, 100, 200, 300 mM, MB absorbance at 664 nm measured at 0, 10, 20, 30 min, and 1, 2, 3, 4 h, v_0 calculated by Beer-Lambert law ($A = \epsilon bc$, $\epsilon = 8.74 \times 10^4 \text{ L/mol}\cdot\text{cm}$), data fitted to Michaelis-Menten/Lineweaver-Burk plots for K_m and V_{max} .

$$A = \epsilon bc \quad (1)$$

$$V_0 = V_{\text{max}} \cdot [S] / (K_m + [S]) \quad (2)$$

$$1 / V_0 = K_m / V_{\text{max}} \cdot 1 / [S] + 1 / V_{\text{max}} \quad (3)$$

Cell experiments: B16 cells (Chinese Academy of Sciences, Shanghai) were cultured in RPMI 1640 with 10% FBS and 1% penicillin-streptomycin (37 °C, 5% CO_2).

Cytotoxicity: The toxicity of drugs to cells was determined using Cell Counting Kit-8 (CCK-8). Inoculate B16 cells into a 96 well plate at a density of 6×10^4 cells/mL, with 100 μL cell suspension per well, and set up 3 replicate wells. Incubate in a 37 °C incubator containing 5% CO_2 for 24 h. Replace the culture medium and add 10 μL of different groups of drugs to each well: Blank group, Control group, VB124, FeRu, VB124@FeRu, JV@FeRu. The VB124 concentrations were 5, 10, 20, 30, 40, 60, 80, 100 $\mu\text{g/mL}$. The other three experimental groups had concentrations of 20, 40, 60, 80 and 100 $\mu\text{g/mL}$, and were cultured in an incubator for 12 h. 10 μL of CCK-8 enhanced solution was added to each well, and the absorbance was measured at 450 nm after 1 h of incubation in the incubator.

For ferroptosis identification, cells were pretreated with idebenone, deferoxamine, VE, and GSH for 2 h, followed by JV@FeRu (80 $\mu\text{g/mL}$) incubation for 12 h. The cell viability was determined using the same CCK-8 protocol as described for the cytotoxicity evaluation.

Live/dead cell staining: In order to observe the cell state and quantity more intuitively after drug treatment, Calcein/PI cell viability and cytotoxicity detection kit was used to stain the cells. B16 cells were seeded at a density of 5×10^4 cells/mL in a 96 well plate and cultured in a cell culture incubator for 24 h. Different groups of drugs were added, including the control group, FeRu, VB124@FeRu, JV@FeRu (100 $\mu\text{g/mL}$). Incubate together for 12 h; Suck off the drug containing culture medium, then wash once with PBS, add 100 μL Calcein AM/PI detection working solution to each well, incubate in the dark for 30 min at 37 °C, and use confocal laser scanning microscope to capture fluorescence images (CLSM). Intracellular O_2 , ROS, LPO, pH, Fe^{2+} detection, DNA damage were all determined according to the same method. The contents of GSH and MDA were determined according to the instructions of the kits.

Cellular uptake: The uptake behavior of the nanosystem was studied by using FITC as a fluorescent probe instead of drug during the preparation process (FITC@FeRu). B16 cells (1×10^5 cells/mL) were seeded in 12-well plates for 24 h, then incubated with FITC@FeRu (80 $\mu\text{g/mL}$) for 1, 2, or 4 h. Then cells were washed with PBS, stained with Actin-Tracker Red-555 (1:500) and Hoechst 33342 (1 $\mu\text{g/mL}$) for 30 min at 37 °C, and imaged by CLSM.

For endocytic pathway analysis, cells were pretreated with chlorpromazine, genistein and amiloride for 30 min and the untreated group was the control group, followed by FITC@FeRu incubation for 4 h. After that, fluorescence images were taken by CLMS to evaluate the effect of different inhibitors on uptake.

Immunofluorescence assay: To evaluate JV@FeRu impact on immune response was investigated by inoculating B16 cells into a 12 well plate containing cell slides, and after 24 h of adherent growth, different groups of test drugs were added: control PBS, FeRu, VB124@FeRu, JV@FeRu (50 $\mu\text{g/mL}$). After incubating for 20 h, the cells were incubated with primary (1:200 dilution) and fluorescent secondary (1:500 dilution) antibodies, and then stained with DAPI for 5 min. And finally,

capture fluorescence images using CLSM.

Western blot: B16 cells were seeded in 6-well plates and cultured at 37 °C for 24 h. The cells were then exposed to PBS, FeRu, VB124@FeRu, or JV@FeRu for 6 h, followed by total protein extraction. The collected proteins were separated via electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes, which were subsequently blocked with 5% non-fat milk. After incubation with primary (1:1000 dilution) and secondary antibodies (1:5000 dilution), protein bands were visualized and quantified using ImageJ software.

Animal and tumor models: All animal experiments were approved by the Animal Research Committee of Shenyang Pharmaceutical University (Ethical approval No. SYP-U-IACUC-S2025-0912-103). C57BL/6 mice were obtained from Changsheng Biotechnology Co. Ltd (Benxi, China). B16 xenograft tumor models were established in female C57BL/6 mice via subcutaneous injection of B16 cells (1×10^7 cells/mL).

In vivo biocompatibility analysis: Healthy KM mice were randomly assigned to seven groups and subcutaneously administered saline (control), FeRu, VB124@FeRu, or JV@FeRu (10 mg/kg). Mice were euthanized on day 7 post-injection. Major organs were harvested for hematoxylin and eosin (H&E) staining, and blood samples were collected for biochemical analysis.

Evaluation of in vivo antitumor efficacy: Once tumor volumes reached 70–100 mm^3 , B16 tumor-bearing mice were randomly divided into four groups. Each group ($n = 4$) received intratumoral injections of saline (control), FeRu, VB124@FeRu, or JV@FeRu (10 mg/kg) on days 0, 3, 6, and 9. Body weight and tumor volume were recorded every other day. Tumors were collected for H&E staining, Ki67/PD-L1 immunohistochemistry, TUNEL assay, and CD4/CD8 immunofluorescence analysis after cervical vertebra removal. Tumor homogenate supernatants were collected to measure TNF- α , IL-6, TGF- β , and IFN- β levels via enzyme-linked immunosorbent assay (ELISA). Additionally, tumors were digested into single-cell suspensions (0.1% collagenase IV, incubated at 37 °C for 1 h, with shaking every 15 min), and the frequencies of CD4 $^+$ T cells, CD8 $^+$ T cells, Treg cells, mature DCs, NK cells, and B cells were analyzed by flow cytometry.

In vivo RNA-seq analysis: After completion of treatment, the tumors underwent a series of experimental procedures including RNA sample extraction, RNA detection, sequencing library construction, and on-machine sequencing, ultimately obtaining mRNA sequencing data for bioinformatics analysis.

Statistical analysis: Data were processed with Origin, GraphPad Prism and ImageJ, expressed as mean $\pm$ SD. One-way ANOVA was used for comparison, with $P < 0.05$ significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

3. Results and discussion

3.1. Synthesis and characterization

FeRu nanoparticles were synthesized through the hydrothermal method. Transmission electron microscopy (TEM) imaging clearly demonstrated the structure (Fig. 1a), while energy-dispersive X-ray spectroscopy (EDS) elemental mapping confirmed the uniform distribution of Fe, Ru, and O within the material (Fig. 1b). AFM images (Fig. S1) confirmed the morphology of the FeRu. Dynamic light scattering (DLS) analysis revealed a hydrodynamic diameter of 167 nm (Fig. 1c). The nanoparticles exhibited lateral sizes of 100–200 nm and a thickness of approximately 40 nm. The X-ray diffraction (XRD) pattern showed characteristic diffraction peaks at the (200), (311), (440), (100), (101), and (110) crystal planes (Fig. 1i), indicating the presence of Fe and Ru [23]. The sharpness of the diffraction peaks suggests excellent crystallinity. These results collectively validate the successful preparation of FeRu nanoparticles. Furthermore, X-ray photoelectron spectroscopy (XPS) analysis was conducted to identify the chemical states present in the FeRu nanoparticles (Fig. 1e). In the Fe 2p spectrum, two characteristic doublets were observed at 723.48/710.00 eV and

726.12/712.50 eV (Fig. 1f), corresponding to Fe^{2+} and Fe^{3+} [24], respectively. Quantitative analysis conducted by peak area integration revealed that the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio was 2.3:1. Fe^{2+} with high proportion forms an efficient initial redox center, ensuring rapid ROS generation. In the Ru 3p spectrum, two characteristic doublets were observed at 483.86/461.40 eV and 485.13/462.98 eV (Fig. 1g), corresponding to Ru and Ru^{4+} respectively [25]. After peak area integration analysis, the ratio of Ru/Ru^{4+} was 2.7:1. The dominant presence of Ru^0 serves as a crucial electron buffer pool, enabling the rapid restoration of consumed Fe^{2+} through Ru^0 mediate electron transfer, thereby achieving long-lasting catalytic activity maintenance [26]. Notably, the Ru 3d orbital signal overlaps with the C 1s peak in the XPS spectrum. After peak separation processing, characteristic Ru signals were observed at 280.87 and 285.27 eV (Fig. 1h). Next, we analyzed the particle size and potential of the nonsystem. To co-load the BRD4 inhibitor JQ1 and the MCT4 inhibitor VB124, FeRu were etched in PBS (pH 4.0) for 6 h. The average particle size or zeta potential of the FeRu did not change significantly before and after etching (Fig. 1c and d). The synthesis process of JV@FeRu has been provided in Fig. S2. After loading the two drugs, JV@FeRu showed an increase in particle size of 234.09 nm and a decrease in the absolute value of zeta potential (Fig. 1c and d). The UV-Vis absorption spectrum further confirmed successful loading of the drug (Fig. 1j). Furthermore, in the 7-day test, the JV@FeRu nanosystem did not show any significant changes in particle size, indicating its excellent stability (Fig. S3). Based on the UV standard curves (Fig. S4),

the calculated loading efficiency of JQ1 was 20.54%, and that of VB124 was 13.79%. Moreover, to evaluate the pH-responsive drug release profiles, JV@FeRu was incubated in PBS with different values (pH 5.5, 6.5, and 7.4). Under acidic conditions (pH 5.5), JV@FeRu exhibited pronounced pH-responsive release behavior. The 48-h cumulative release rates were 93.81% for JQ1 (Figs. 1k) and 89.91% for VB124 (Fig. 1l), which were significantly higher than those observed under neutral conditions (pH 7.4). This pH-dependent release behavior is mainly attributed to the acid-responsive properties of the FeRu nanocarrier, which enables JV@FeRu to achieve effective therapeutic agent release within the acidic tumor microenvironment.

3.2. In vitro evaluation of FeRu catalytic activity

To evaluate the catalytic activity of FeRu, ammonium titanate oxalate (ATO) was employed as a chromogenic indicator to monitor H_2O_2 decomposition under weakly acidic conditions (pH 5.5). UV-vis spectroscopy revealed a concentration-dependent decrease in the characteristic ATO absorption peak at 365 nm (Fig. S5a), indicating efficient H_2O_2 consumption. Time-dependent experiments further demonstrated that the absorbance of ATO gradually decreased at 365 nm (Fig. S5b), confirming the continuous catalytic degradation of H_2O_2 by FeRu. Similar to native catalase (CAT), FeRu also efficiently catalyzes the decomposition of H_2O_2 into O_2 . Using the oxygen-sensitive fluorescent probe $[\text{Ru}(\text{pdd})_3] \text{Cl}_2$, we observed significant fluorescence quenching

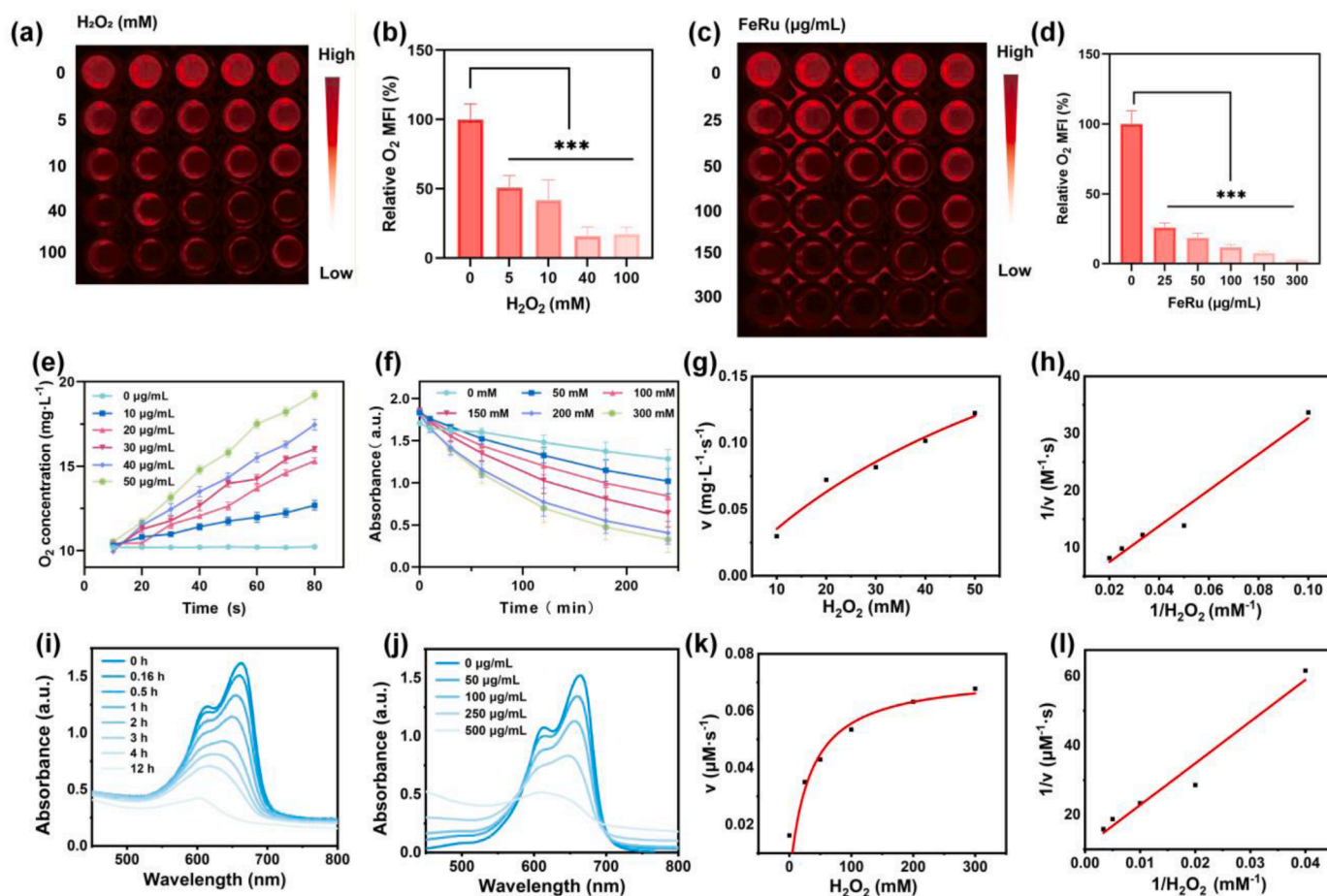


Fig. 2. Multienzymatic catalytic activity evaluations of FeRu. (a) Detection of FeRu-catalyzed O_2 generation and (b) quantitative determination of the relative fluorescence intensities with different concentrations of H_2O_2 . (c) Detection of O_2 generation and (d) quantitative determination of the relative fluorescence intensities with different concentrations of FeRu. (e) The O_2 concentrations catalyzed by FeRu of different concentrations. (f) The determination of H_2O_2 consumption at different times using ATO. (g) Michaelis-Menten curve and (h) Lineweaver-Burk plotting of CAT-like activity. The $\cdot\text{OH}$ production ability at (i) different times and (j) different concentrations of FeRu. (k) Michaelis-Menten curve and (l) Lineweaver-Burk plotting of POD-like activity. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns, no significant.

of $[\text{Ru}(\text{pdd})_3] \text{Cl}_2$ due to a concentration-dependent increase in O_2 production (Fig. 2a and b). Similarly, an increase in FeRu concentration can also enhance O_2 production (Fig. 2c and d), further confirming its outstanding CAT-like activity. The measurement of dissolved oxygen quantitatively verified the positive correlation between FeRu concentration and O_2 generation (Fig. 2e), further validating its efficient H_2O_2 catalytic capability. Enzymatic kinetic analysis revealed that the CAT-like activity of FeRu conformed to the Michaelis-Menten model (Fig. 2g). The Michaelis constant ($K_m = 76.329 \text{ mM}$) indicates that its affinity for the substrate is moderate, while the maximum reaction rate ($V_{\max} = 0.304 \text{ mg/L}\cdot\text{s}$) is superior to that of some natural enzymes (Fig. 2h), demonstrating its highly efficient catalytic performance

(Table S1) [27–30].

We further evaluated the peroxidase-like activity of FeRu using methylene blue (MB) as a hydroxyl radical ($\cdot\text{OH}$) indicator. The $\cdot\text{OH}$ generated by H_2O_2 decomposition oxidized MB, causing a decrease in its characteristic absorption peak (664 nm). The experimental results confirmed that in a weakly acidic environment, FeRu exhibits significant POD-like activity. Both the increase in H_2O_2 and FeRu concentration accelerated MB decolorization due to the enhanced $\cdot\text{OH}$ generation (Fig. 2f–j). Notably, even after 12 h, the absorbance of MB continued to decrease (Fig. 2i), indicating the continuous ability of FeRu to generate $\cdot\text{OH}$. The kinetic study revealed that the POD-like activity of FeRu follows Michaelis-Menten kinetics ($K_m = 112.208 \text{ mM}$, $V_{\max} = 1.553 \cdot 10^{-7} \text{ M/s}$)

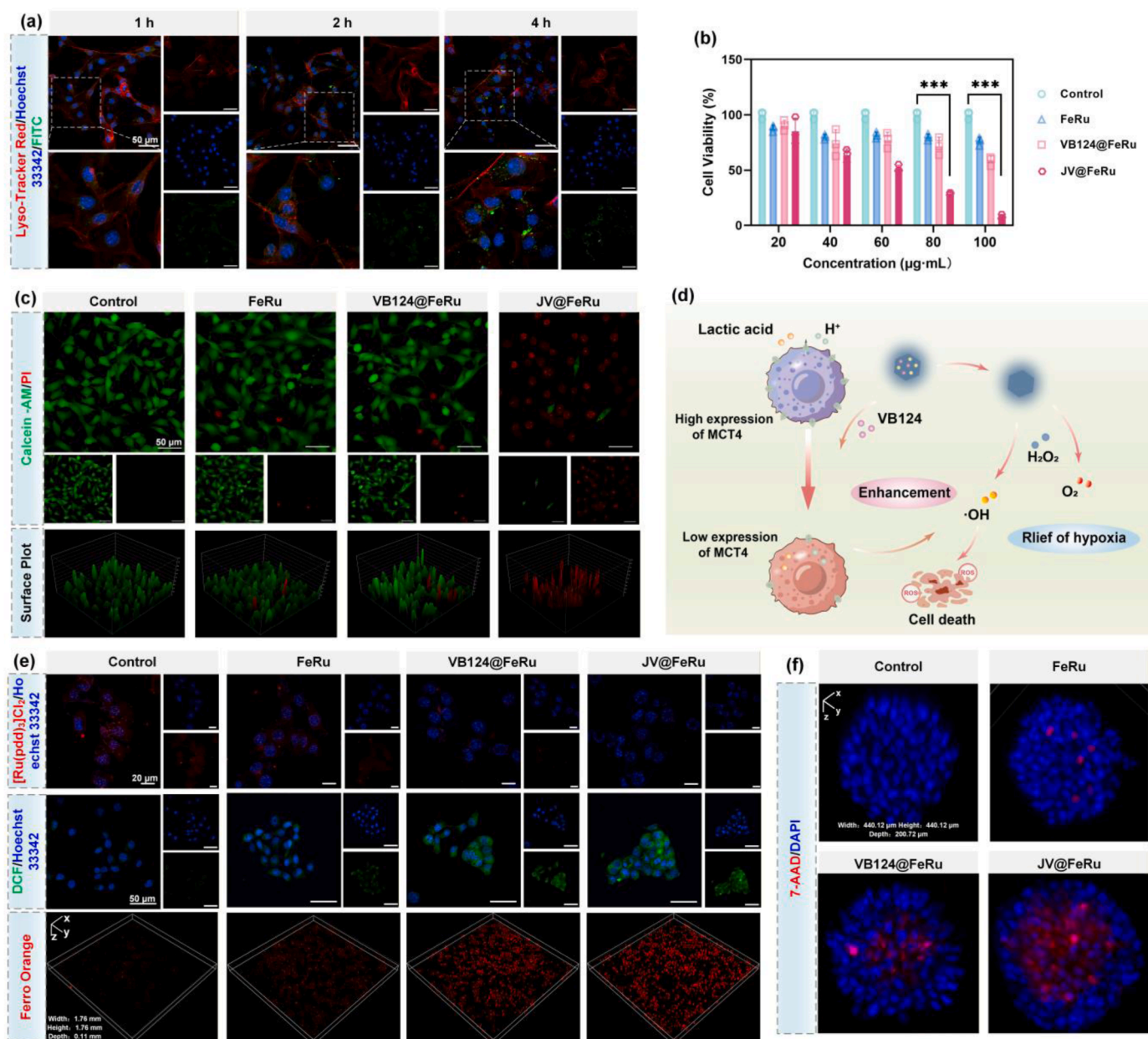


Fig. 3. In vitro cellular internalization, viability assessment and mechanistic investigation of JV@FeRu. (a) Confocal laser scanning microscopy (CLSM) images of B16 cells following incubation with FITC-conjugated FeRu (green) for 1, 2, and 4 h. Cytoskeletal actin and nuclei were counterstained with phalloidin (red) and Hoechst 33342 (blue). (b) Quantitative analysis of B16 cell viability across different treatment groups. (c) Fluorescence micrographs of B16 cells subjected to different treatments after dual staining with Calcein-AM (green, live cells) and propidium iodide (PI, red, dead cells). (d) Schematic diagram illustrating the proposed working mechanism of the VB124@FeRu nanocomplex. (e) Multiplex fluorescence imaging of intracellular O_2 , ROS, and Fe^{2+} levels in B16 cells under various treatments. Detection was performed using $[\text{Ru}(\text{dpp})_3] \text{Cl}_2$ (red, O_2), DCFH-DA (green, ROS), Ferro Orange (red, Fe^{2+}), with nuclear counterstaining by Hoechst 33342 (blue). (f) CLSM images of 3D-cultured B16 tumor spheroids after different treatments, stained with the viability dye 7-AAD (red) and DAPI (blue). $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(Fig. 2k and l). Although its substrate affinity and catalytic rate are lower than those of some natural enzymes (Table S2) [31–34], its prolonged catalytic stability endow it with an excellent capacity to generate $\cdot\text{OH}$.

3.3. *In vitro* efficacy evaluation

Inspired by the above research findings, we systematically evaluated the antitumor efficacy of JV@FeRu *in vitro* using mouse melanoma (B16) cells. Cell uptake experiment confirmed that JV@FeRu could be efficiently internalized by tumor cells within a short period of time (Fig. 3a). The endocytosis inhibition experiment showed that, compared with the control group, chlorpromazine pretreatment significantly reduced the cellular uptake of JV@FeRu. These results indicate that JV@FeRu is mainly internalized through clathrin-mediated endocytosis (Fig. S6). The cytotoxicity of FeRu, VB124@FeRu, and JV@FeRu against B16 cells was evaluated using the CCK-8 assay. As expected, cells treated with VB124 alone maintained a survival rate of over 90% after 12-h incubation (Fig. S7), indicating limited efficacy. In contrast, FeRu alone induced a concentration-dependent decrease in cell viability, which was mainly attributed to its catalytic production of H_2O_2 and reactive oxygen species (ROS). Notably, VB124@FeRu exhibited stronger cytotoxicity, which validates our hypothesis that VB124 enhances the catalytic efficiency of FeRu by inhibiting lactate efflux and elevating intracellular acidity. JV@FeRu exhibited outstanding antitumor efficacy (Fig. 3b), indicating that the epigenetic regulation mediated by JQ1 synergistically enhances the cytotoxicity of VB124@FeRu. The rescue experiment with ferroptosis inhibitors further confirmed that JV@FeRu predominantly triggers ferroptosis, as the cell survival rate significantly recovered after pretreatment with the inhibitors (Fig. S8). Furthermore, the live/dead cell staining using calcein AM (green) and propidium iodide (PI, red) was performed and microscopic visualization was achieved through confocal microscopy to confirm this conclusion (Fig. 3c).

To better reflect the actual situation of solid tumor, we established a 3D spheroid model. After treatment, the cell condition was examined through 7-AAD staining (Fig. 3f), which conclusively proved that JV@FeRu could cause the extensive tumor cell death, highlighting its outstanding therapeutic potential. To further evaluate the impact of JV@FeRu on the long-term tumor proliferation capacity, we conducted a cell colony formation assay. The results demonstrated that JV@FeRu effectively inhibited the colony formation of B16 cells (Fig. S9), further confirming its significant efficacy in blocking tumor proliferation.

To evaluate intracellular catalytic products, we used the oxygen-sensitive probe $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ to measure O_2 levels in B16 cells. A significant quenching of red fluorescence was observed in the FeRu group (Fig. 3c–S10a), indicating the generation of O_2 . VB124@FeRu exhibited a more pronounced reduction in fluorescence, confirming that the enhanced acidity of VB124 further promoted the CAT-like activity of FeRu to alleviate tumor hypoxia. Subsequently, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was used to assess the generation of $\cdot\text{OH}$. The most significant green fluorescence in the VB124@FeRu group indicated the synergistic increase of $\cdot\text{OH}$ (Fig. 3c–S10b). Flow cytometry was also employed to analyze intracellular ROS levels, and the results were consistent with the above findings (Fig. 4c). Overall, these data indicate that FeRu possesses intrinsic CAT and POD activities in the cellular environment, and the acidic amplification mediated by VB124 significantly enhances both the generation of O_2 and $\cdot\text{OH}$.

To gain mechanistic insight into how VB124 enhances the catalytic activity of FeRu, we next investigated its acidification effect and its downstream impacts. A schematic diagram illustrating the self-enhancing mechanism of FeRu after VB124 loading is shown in Fig. 3d. We first used the intracellular pH indicator BCECF to measure pH changes within cells (Fig. S11). The results showed that VB124@FeRu significantly reduced the intracellular pH, as evidenced by pronounced weakening of green fluorescence, which was attributed to the inhibitory effect of VB124 on the monocarboxylate transporter 4 (MCT4). Meanwhile, Western blot results confirmed the downregulated MCT4 protein expression

(Fig. 4e–S14b), indicating that VB124 indeed inhibited MCT4 function. This acidification effect serves dual roles, it not only creates an optimal acidic environment that enhances the catalytic efficiency of FeRu, but it also promotes the release of Fe^{2+} from the nanoparticle platform. Next, we used the Ferro Orange indicator to detect intracellular ferrous ion release. Compared to the control group, the Fe^{2+} indicator exhibited significantly enhanced red fluorescence in both the FeRu and VB124@FeRu groups (Fig. 3e). These results collectively validate that VB124 establishes a self-enhancing feedback loop by augmenting intracellular acidity, simultaneously boosting the activity of FeRu and accelerates the release of Fe^{2+} , ultimately triggering a potent ROS burst.

After establishing the self-enhancing catalytic mechanism, we further investigated whether this oxidative stress would cause persistent damage to tumor cells. We first assessed GSH depletion as a direct indicator of oxidative damage, which was most pronounced in the JV@FeRu group (Fig. S12). ROS overproduction is known to induce DNA damage; accordingly, we assessed DNA double-strand breaks via $\gamma\text{-H2AX}$ immunofluorescence. The control group showed negligible signals. VB124@FeRu caused strong intracellular oxidative stress, which disrupted double-stranded DNA and resulted in green fluorescence, while JQ1 contributed to DNA damage by inhibiting the function of BRD4 (Fig. 4b–S14a). By selectively binding to and downregulating BRD4, JQ1 suppresses BRD4 dependent MYC transcription, which consequently leads to the decreased expression of c-myc (Figs. S13 and S14e). As expected, the strongest green fluorescence was observed in the JV@FeRu group. Furthermore, we examined GPX4 expression after different treatments. Both Western blotting and immunofluorescence revealed that GSH depletion caused by JV@FeRu significantly downregulated GPX4 expression (Figs. S13 and S14c), weakening the antioxidant defense capability of the cells. More importantly, the C11-BODIPY^{581/591} probe was used to detect ROS accumulation-induced lipid peroxidation. FeRu induced strong green fluorescence, and JV@FeRu produced the highest lipid peroxidation level (Fig. 4a), and the quantitative results were consistent with the aforementioned findings (Fig. S10c). In summary, the amplified oxidative stress is manifested by GSH depletion, DNA damage, GPX4 downregulation, and lipid peroxidation. It not only directly kills tumor cells but also creates conditions for the release of damage-associated molecular patterns (DAMPs), which is a critical prerequisite for initiating antitumor immunity.

In addition to the powerful multienzyme activity exhibited by JV@FeRu within cells, we also aim to investigate whether it can initiate an immune response within tumors, thereby reshaping the tumor-suppressive microenvironment. We found that the release of DAMPs from B16 cells treated with JV@FeRu was significantly enhanced. First, in untreated cells, high-mobility group box-1 protein (HMGB1) was highly expressed, and obvious red fluorescence was observed via CLSM (Fig. 4d), as showed by the mean fluorescence intensity (MFI) in Fig. 4f. In contrast, the red fluorescence signal disappeared in JV@FeRu-treated cells, indicating a large amount of HMGB1 was released into the extracellular space. Meanwhile, the cells treated with JV@FeRu exhibited showed a greater exposure of CRT on the tumor cell surface compared to the other groups (Fig. 4d–g), suggesting that this system can enhance immunogenicity and activate the immune system. Additionally, the cells treated with FeRu nanozymes released a large amount of ATP, as reflected by elevated ATP content in the cell culture medium (Fig. S15). To further analyze the impact of this system on the efficacy of immunotherapy, we examined the PD-L1 expression in B16 cells after different treatments. The results showed that the JV@FeRu group significantly downregulated PD-L1 level through epigenetic regulation effect of JQ1, as reflected by the WB and immunofluorescence results (Fig. 4d–h, S13, S14d). This effect is expected to effectively enhance the enrichment level of effector CD8⁺T cells and their ability to kill tumor cells *in vivo*. Combined with the oxidative stress induced by VB124@FeRu, JV@FeRu synergistically disrupts the antioxidant defense system, thereby alleviating immune suppression.

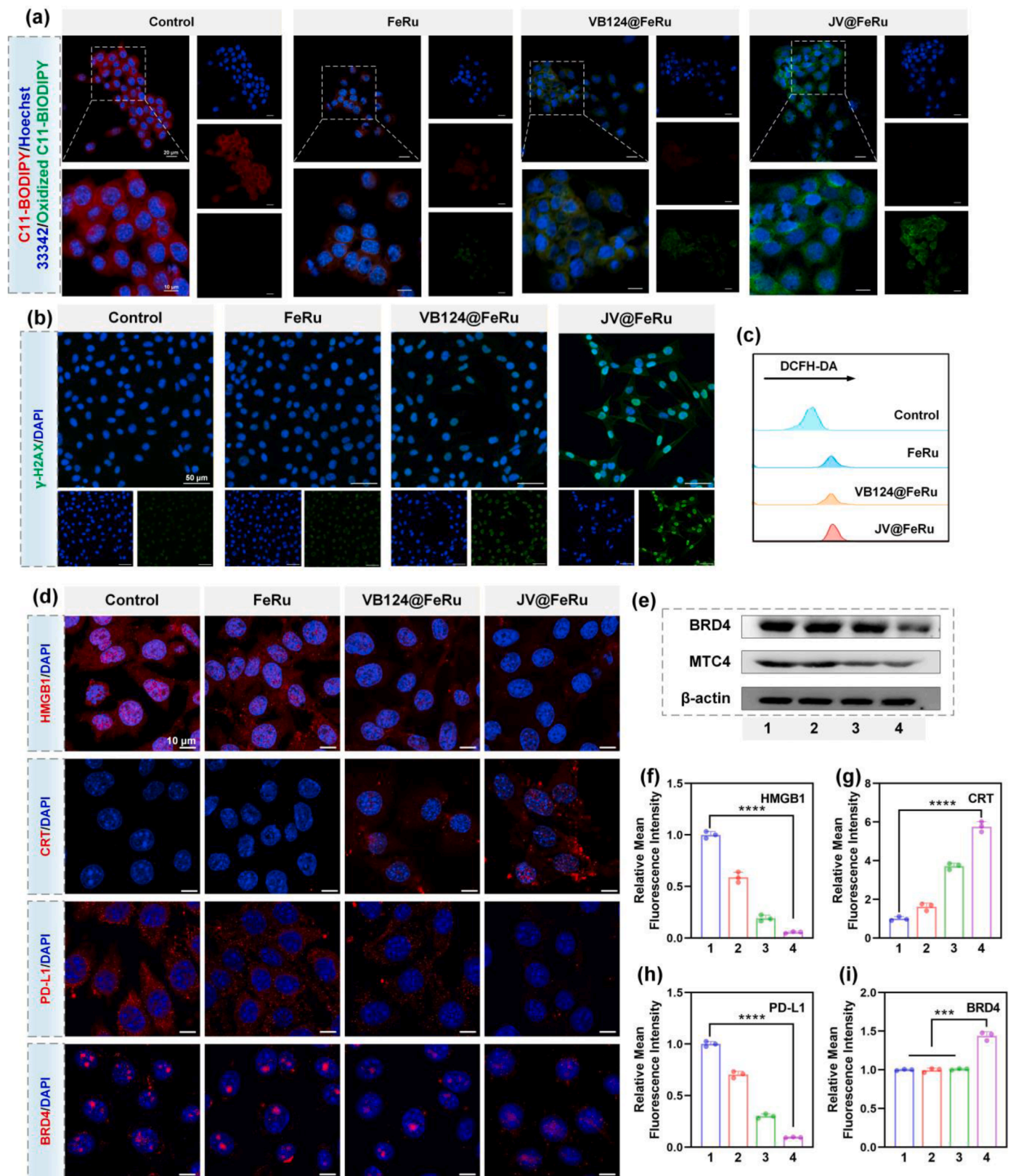


Fig. 4. Mechanisms of immunogenic modulation by JV@FeRu in B16 cells. (a) Confocal microscopy images depicting lipid peroxidation levels in B16 cells under different treatments. (b) Immunofluorescence imaging of DNA double-strand breaks (γ-H2AX, green) in B16 cells across treatment groups. (c) Flow cytometric quantification of intracellular reactive oxygen species (ROS) levels in differently treated B16 cells. (d) Immunofluorescence staining of HMGB1, CRT, BRD4, and PD-L1 in B16 cells following various treatments. (e) Western blot analysis of BRD4 and MCT4 protein expression in B16 cells under different treatment conditions. Quantitative analysis of fluorescence intensity for (f) HMGB1, (g) CRT, (h) PD-L1, and (i) BRD4 from immunofluorescence images. (1. Control; 2. FeRu; 3. VB124@FeRu; 4. JV@FeRu.) n = 3, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

3.4. *In vivo* antitumor efficacy and activation of immune responses

The promising antitumor effects of JV@FeRu *in vitro* prompted us to further investigate its antitumor activity *in vivo*. First, C57 tumor-bearing mouse model was established. When the tumor volume reached 100 mm³, DiR and DiR@FeRu were injected intratumorally into

B16-bearing mice to investigate the biodistribution of this system. Compared to the free DiR group, DiR@FeRu exhibited stronger fluorescence intensity within the tumor within 72 h (Fig. 5a, S16b), which was further confirmed by fluorescence imaging and quantitative detection of *ex vivo* tumor tissues (Fig. S16a and c), demonstrating the superior accumulation at the tumor site. In addition, the other tumor-

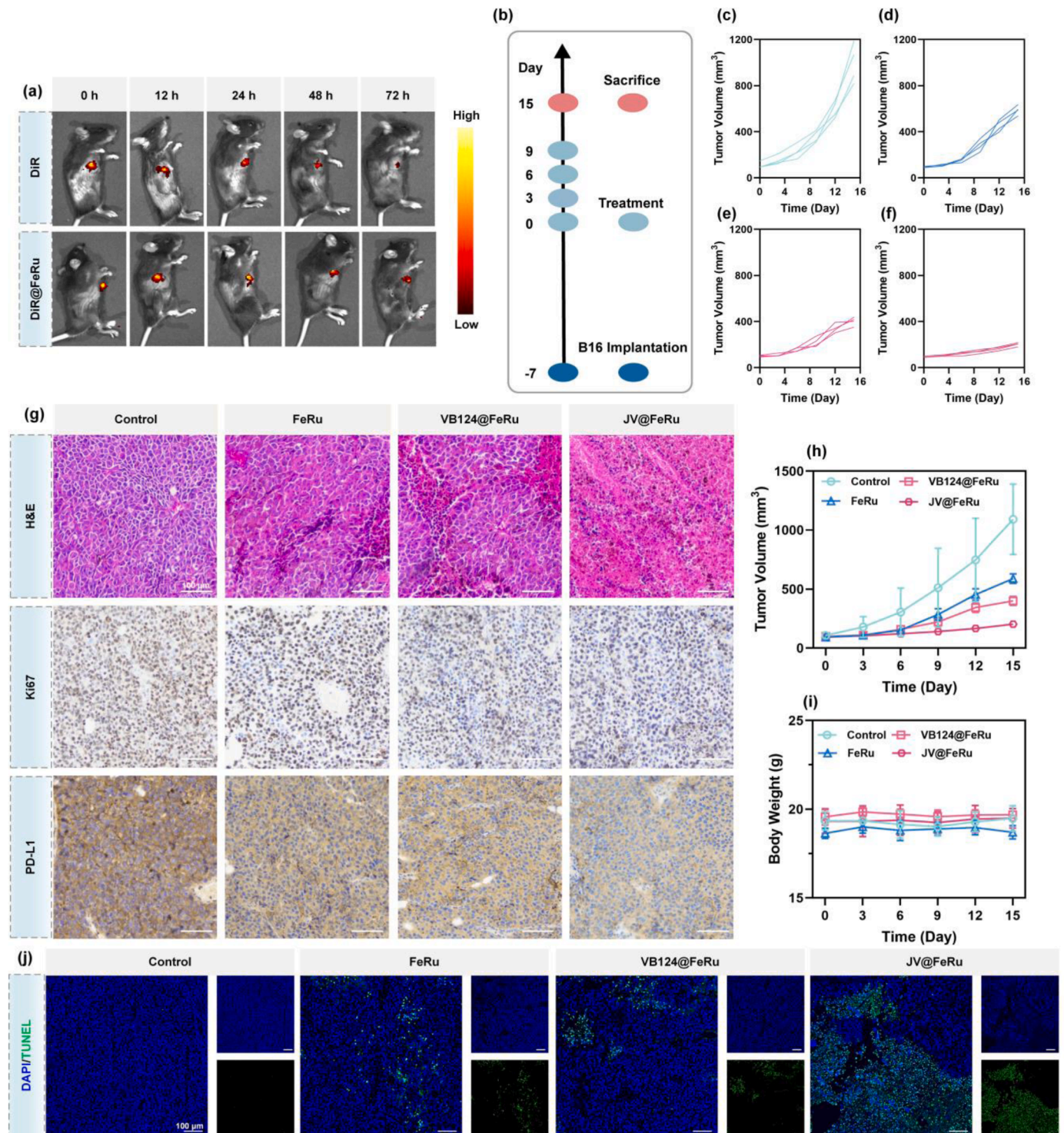


Fig. 5. *In vivo* therapeutic evaluation and histological analysis of JV@FeRu. (a) Near-infrared fluorescence imaging of tumor-localized DiR signal following intratumoral injection. (b) Schematic representation of the therapeutic regimen and experimental timeline. (c-f) Individual tumor growth kinetics across different treatment cohorts. (g) Histopathological assessment of tumor tissues by H&E staining and immunohistochemical analysis of proliferation marker Ki67 and immune checkpoint protein PD-L1. (h) Tumor volume progression curves for each treatment group. (i) Body weight dynamics as an indicator of systemic toxicity. (j) Detection of apoptotic cells in tumor sections by TUNEL assay.

bearing mice was divided into four groups upon reaching appropriate tumor volume: i) PBS (control), ii) FeRu, iii) VB124@FeRu, iv) JV@FeRu. Each group received four intratumoral injections at different time points ($n = 4$), with tumor volume and body weight monitored every three days (Fig. 5b). The results showed that throughout the entire treatment, no significant changes in body weight were observed in any mice (Fig. 5i), indicating negligible adverse reactions brought by JV@FeRu *in vivo*.

Tumor growth curves demonstrated that both FeRu and VB124@FeRu treatments partially inhibited tumor growth, while the JV@FeRu group exhibited the most effective suppression, with a tumor inhibition rate of 81.46%. Furthermore, in order to visually compare the therapeutic efficacy, mice were euthanized and tumor tissues were harvested 15 days after treatment. H&E staining revealed that the tumor tissues in the JV@FeRu group possess extensive structural disorganization, nuclear condensation, and cell lysis, all of which are indicative of cell necrosis (Fig. 5g). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) further confirmed the significant increase in genomic DNA breaks in this group (Fig. 5j–S17c). Meanwhile Ki-67 staining revealed a reduced proportion of proliferating cells in the JV@FeRu group (Fig. 5g–S17a), confirming its potent inhibition of tumor regrowth. Importantly, JV@FeRu treatment significantly reduced the expression level of PD-L1 on tumor cell membranes (Fig. 5g–S17b), suggesting that it may reverse the immunosuppressive microenvironment through epigenetic or signaling pathway regulation. Moreover, CD4/CD8 immunofluorescence images revealed that the number of CD8⁺ T and CD4⁺ T cells in the JV@FeRu group has significantly increased (Fig. 6a) and the immune cells have deeply infiltrated and moved towards the core area of the tumor.

The antitumor immune mechanism *in vivo* is illustrated in Fig. 6b. Therefore, at the experimental endpoint, single-cell suspensions were prepared from the excised tumor tissues and labeled with multiple-color antibodies for flow cytometric analysis. First, we quantitatively evaluated the maturation status of dendritic cells (DCs).

As the most efficient professional antigen-presenting cells, DCs play a crucial role in immune activation through efficient antigen uptake, processing, and presentation to naive T cells. Flow cytometry revealed that JV@FeRu induced the most pronounced DC maturation (Fig. 6c and d), effectively promoting the occurrence of related events. Next, we focused on analyzing CD4⁺ helper T cells (CD3⁺CD4⁺) and CD8⁺ cytotoxic T lymphocytes (CD3⁺CD8⁺), which play a pivotal role in adaptive immunity. The proportion of CD8⁺ T cells increased to 18.8% (Fig. 6e and f) and CD4⁺ T cells rose to 20.2% in the JV@FeRu group (Fig. 6g and h), indicating that effective tumor-specific adaptive immune activation was achieved. In the immunosuppressive tumor microenvironment (TME), regulatory T cells (Tregs) induce antigen-specific tolerance through the secretion of inhibitory cytokines, thereby promoting tumor immune escape. As a key mediator of tumor immune suppression, we systematically evaluated the dynamic changes of Tregs. As expected, JV@FeRu treatment reduced the proportion of intratumoral Tregs (CD4⁺/FOXP3⁺) to 34.1% (Fig. 6i and j), indicating effective reversal of immunosuppression. In addition to the adaptive immune response, the therapeutic effect of JV@FeRu also extends to the innate and humoral immune systems. Flow cytometry analysis revealed that the proportion of tumor-infiltrating NK cells (NK1.1⁺) (Fig. S19) in the JV@FeRu treatment group significantly increased to 10.8% compared with the control group, indicating that NK cells were effectively recruited and activated. Meanwhile, JV@FeRu promoted the infiltration of tumor-associated B cells (CD19⁺), with their proportion increasing to 21.5% (Fig. S20), suggesting that the initial stage of anti-tumor humoral immunity might have been triggered. Fig. S18 shows gating strategies for DCs, CD8⁺ T cells, CD4⁺ T cells, Tregs, NK cell and B cell from flow cytometric analysis.

The analysis of cytokine further demonstrated that JV@FeRu significantly elevated pro-inflammatory factors reduced immunosuppressive TGF- β (Fig. S21), which confirmed its ability to transform

immunosuppressive TME into a pro-inflammatory microenvironment. Overall, these data demonstrate that JV@FeRu is capable of reshaping the tumor immune microenvironment by fundamentally coordinating the rebalancing of immune cells and cytokines.

In addition, to evaluate the biological safety of JV@FeRu, healthy female C57 mice were subcutaneously injected with PBS or JV@FeRu, respectively. After 15 days of observation, the mice were euthanized. Compared to the PBS group, the functional markers (including complete blood count and blood biochemical indicators) in the JV@FeRu group remained within normal range and showed no significant differences (Fig. S22). H&E staining of major organs (heart, liver, spleen, lung, kidney) further confirmed histological integrity of the tissues (Fig. S23), and no damage was observed. All these results demonstrate the excellent biosafety of JV@FeRu.

3.5. Transcriptome sequencing analysis

To investigate the epigenetic and immune regulatory mechanism of JV@FeRu on tumor-bearing mice, we conducted a whole transcriptome sequencing (RNA-seq) analysis on the tumor tissues of mice treated with JV@FeRu (Treat). Principal component analysis (PCA) revealed significant differences in gene expression between the Control and Treat groups (Fig. 7a). From the volcano plot and clustering analysis heatmap (Fig. 7b and c), it was observed that compared with the control group, 252 genes were upregulated (red) and 225 genes were downregulated (green) in the Treat group. Through heatmap analysis, it was found that the triple mechanisms of ferroptosis, epigenetic regulation, and immune activation synergistically induced a strong anti-tumor immune response.

As shown in Fig. 7i, the lipid peroxidation driver ACSL4 and the key marker PTGS2 were significantly upregulated, while GPX4 and SLC7a11 that maintain the redox balance at the tumor site were significantly downregulated. Meanwhile, the downregulation of Fth1 and the upregulation of oxidative stress genes Hmox1 and Nfe2l2 indicated the occurrence of ferroptosis and intense oxidative stress in tumor cells. Besides, JV@FeRu triggered profound epigenetic regulation. The inhibition of BRD4 function by JQ1 effectively downregulated the oncogene MYC and its downstream target BCL2 (Fig. 7d), thereby suppressing the proliferation and survival signals of tumor cells. Furthermore, the system also had an impact on the metabolism-related genes, the downregulation of SLC2a1 and LDHA indicated a significant inhibition of the glycolytic pathway in tumor cells. Ultimately, JV@FeRu successfully reversed the immunosuppressive microenvironment to an immune-activated state. The genes encoding the co-stimulatory molecules necessary for dendritic cell maturation and T cell activation, such as CD80, CD86, and CD83 (Fig. 7f), were significantly upregulated. Crucially, the core components of the antigen presentation mechanism, including B2M, H2-K1, and H2-D1, were constantly upregulated. This greatly enhanced the immunogenicity of tumor cells and enables them to be more effectively recognized by T cells. Meanwhile, the significant downregulation of the immune checkpoint molecule encoding gene CD274 confirmed that JV@FeRu was able to reverse T cell immune escape.

To further explore the biological processes regulated by JV@FeRu in the anti-tumor immune responses, we performed gene enrichment analysis using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The GO enrichment results (Fig. 7j) showed that at the biological process (BP) level, the differentially expressed genes were significantly enriched in antigen processing and presentation of peptide antigen via MHC class I molecules, response to type I interferon, and positive regulation of T cell-mediated immunity. This suggests that JV@FeRu can systematically activate antigen presentation-related biological processes and enhance T cell-dependent immune responses, which is highly consistent with the abovementioned upregulation of MHC I molecules (B2M, H2-K1, H2-D1) and the enhanced infiltration of CD8⁺ T cells. At the cellular component (CC) level, MHC class I peptide loading complex was significantly enriched, confirming that JV@FeRu

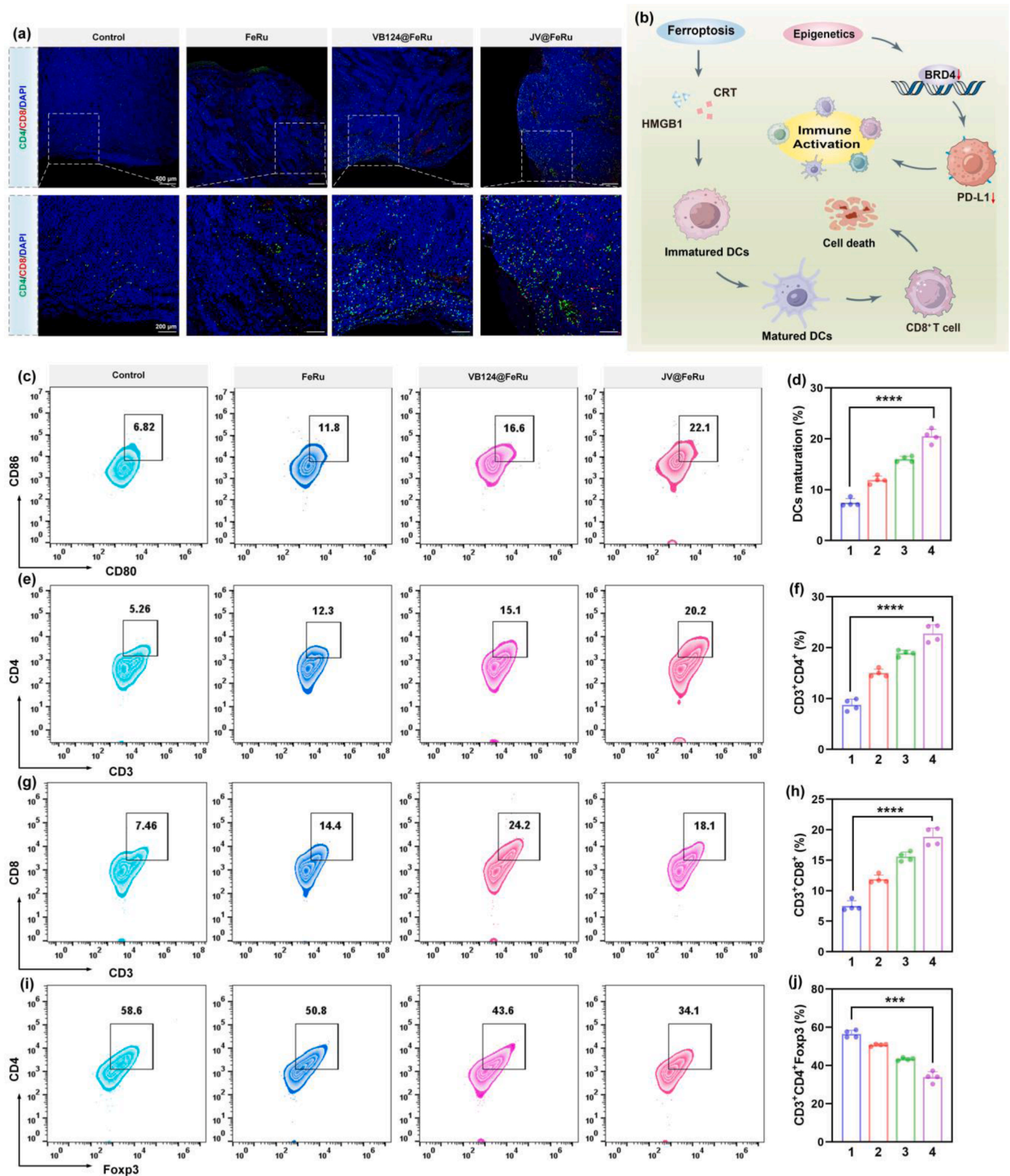


Fig. 6. In vivo immunomodulatory profiling following JV@FeRu treatment. (a) Confocal co-localization imaging of CD4⁺ (green) and CD8⁺ (red) T cell infiltration in tumor tissues across treatment groups. (b) Schematic illustration of the proposed immunomodulatory mechanism mediated by JV@FeRu. (c) Flow cytometric analysis of mature DC in different treatment groups, with (d) quantitative results. (e) Flow cytometric analysis of CD4⁺ T cell with (f) quantitative results. (g) Flow cytometric analysis of CD8⁺ T cell with (h) quantitative results. (i) Flow cytometric analysis of regulatory Treg cell with (j) quantitative results.

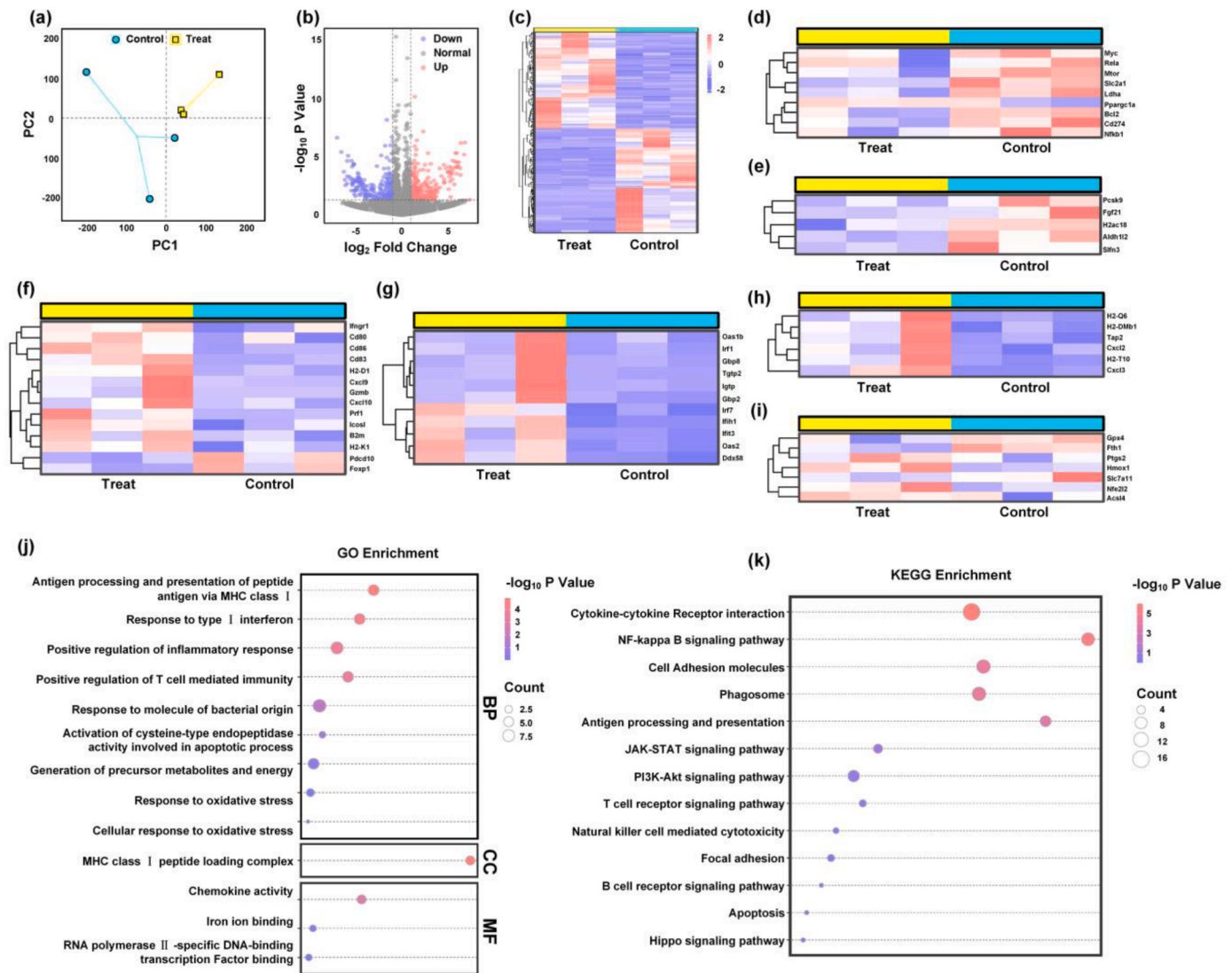


Fig. 7. Transcriptomic Analysis of JV@FeRu Treatment. (a) Principal component analysis (PCA) of differentially expressed genes (DEGs) between the control and JV@FeRu-treated groups. (b) Volcano plot and (c) overall expression heatmap of DEGs. (d, h) Heatmaps showing the expression of DEGs related to antigen presentation and immune response. (e) Heatmap displaying genes associated with epigenetic regulatory pathways. (f) Heatmap of DEGs involved in T cell immunity and (g) interferon (IFN) signaling pathway. (i) Heatmap of genes related to ferroptosis. (j) Gene Ontology (GO) functional enrichment analysis of DEGs. (k) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs.

provides a structural basis for the effective presentation of tumor antigens by regulating the assembly of antigen presentation-related molecular complexes. At the molecular function (MF) level, the "chemokine activity" was significantly enriched, suggesting that the differentially expressed genes may enhance the biological function of chemokines, promote the recruitment of immune cells such as dendritic cells and T cells to the tumor microenvironment, and form a synergistic effect with the activation of immune cells mediated by the upregulation of co-stimulatory molecules such as CD80 and CD86.

KEGG pathway analysis further revealed the core signaling networks involved in the differentially expressed genes (Fig. 7k). The NF- κ B significant enrichment of the signaling pathway indicates that this pathway may act as a cross-regulatory hub for metabolic stress and immune activation. Through the ROS generated by FeRu catalysis, it activates the NF- κ B transcription factor, further amplifies the inflammatory immune response and the oxidative stress signals related to ferroptosis.

The antigen processing and presentation pathway indicates the activation of the MHC class I antigen presentation; the high enrichment of the cytokine-cytokine receptor interaction pathway suggests that JV@FeRu may enhance the cross-cellular transmission of immune signals by

regulating cytokines, providing a molecular bridge for T cell activation and effector function. In summary, the expression of differentially expressed genes, as well as GO and KEGG functional enrichment analysis, systematically confirmed that JV@FeRu can reshape the tumor immune microenvironment by activating antigen presentation, enhancing cytokine signal transduction, and regulating T cell immunity. This provides functional and pathway evidence at the transcriptomic level for its "self-enhancing catalysis-epigenetic regulation-microenvironment remodeling" synergistic anti-tumor mechanism.

4. Conclusion

This study successfully constructed and validated a self-enhancing nano-agonist, JV@FeRu, which integrates tumor-specific catalysis and epigenetic regulatory functions. The system forms a positive feedback loop through the efficient catalytic activity of FeRu and the acidic microenvironment induced by VB124, substantially enhancing continuous ROS generation and effectively inducing ferroptosis in tumor cell. Meanwhile, the co-loading of JQ1 inhibits BRD4 function at the epigenetic level, thereby down-regulating the expression of PD-L1 and

reversing the immunosuppressive state of the tumor. Comprehensive *in vitro* and *in vivo* experiments demonstrated that JV@FeRu not only exhibits robust direct tumor cell-killing capacity but also systemically reshapes the tumor immune microenvironment, manifested as deep infiltration and activation of effector T cells, reduction of regulatory T cells, and maturation of dendritic cells. This fundamental reprogramming of the immune microenvironment transforms “cold” tumors into “hot” tumors. Transcriptomic analysis revealed the multi-level synergistic mechanism of JV@FeRu at the molecular level, providing comprehensive molecular evidence for the tripartite therapeutic mode of catalytic therapy, epigenetic intervention and immune remodeled. In summary, through the integration of the mechanisms, JV@FeRu is able to intelligently respond and actively reshape the tumor microenvironment, demonstrating anti-tumor effects that surpass those of monotherapies. This work not only offers an innovative nanotechnology solution, but also plays an important theoretical and experimental foundation for the development of more precise and effective combined immunotherapeutic strategies in the future.

CRediT authorship contribution statement

Yake Qi: Writing – original draft, Project administration. **Jie Ma:** Writing – original draft, Methodology. **Ouyang Su:** Methodology. **Lili Lu:** Methodology. **Min Ge:** Conceptualization. **Zhou Li:** Conceptualization. **Zhaoxu Meng:** Writing – review & editing, Funding acquisition. **He Lian:** Writing – review & editing, Funding acquisition, Conceptualization.

Supporting information

Supplementary materials include detailed experimental procedures and additional data.

Notes

The authors declare no competing financial interest.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pnsc.2026.08.013>.

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