Supporting Information

The authors declare that this manuscript complies with Wiley-stipulated guidelines for artificial-intelligence application. No unauthorized AIGC-generated content has been incorporated into this work, and any AI-assisted operations will be explicitly stated if relevant tools are utilized.

Preparation and characterization of SeMet-LP@RGD

DSPC (25.316 mmol,20 mgDSPE-PEG2k (,2.5 mmol, 7 mg) and DSPE-PEG2k-mal (1.744 mmol, 5 mg) were obtained from Xi'an Ruixi Biological Technology Co., Ltd. Cholesterol (Chol, 5 mg, 12.953 mmol) was obtained from Aladdin (Shanghai, China). The organic solvent used was ethanol (Meryer, Shanghai, China) and the anti solvent used was phosphate buffered saline (PBS). All other chemicals were analytical grade. Blank liposomes were synthetized via microfluidic-driven self-assembly. In brief, organic phase composed of DSPC, DSPE- PEG2k, DSPE-PEG2k- mal and cholesterol were dissolved in 1 mL ethanol; PBS was used as an aqueous anti-solvent phase. The two syringe pumps (Pump A: OEM020102ZY; Pump B: TYD010102 CE; Baoding Lead Fluid Technology Co., Ltd., China) were used for the delivery of the two separate phases, which were then immediately mixed in a staggered herringbone micromixer (Fluidic187, Microfluidic ChipShop, Germany) to trigger the lipid self-assembly. The flow rate of ethanol and PBS was 400 and 3.6 mL/min, respectively. In order to obtain a narrow particle size distribution, the freshly prepared liposome dispersion was extruded 21 times through polycarbonate-membranes (0.1 μm, Avanti Polar Lipids) at 60 °C using a hand-operated extruder, followed by the removal of remaining ethanol by ultrafiltration using 15 ml centrifugal filter devices at 4500 rpm for 30 min. The concentrated blank liposome suspension obtained was used for further experiments. Blank liposomes were filled with selenomethionine (SeMet) by multiple freeze thaw procedures. To sum it all up, 450 μL blank liposome suspension was combined with 50 μL SeMet stock solution, (10 mg/mL PBS). A ten-cycle freeze–thaw was used for the mixture where the freezing was performed in liquid nitrogen (10 s per freezing cycle) and the thawing in a 40 °C water bath (3 min per thawing cycle). This process created transient defects in lipid bilayers that allowed the incorporation of drugs into the vesicles. The free unloaded SeMet was removed by the use of the centrifugal filter unit with MWCO (0.5 mL, 100 kDa, Merck Millipore, 4500 rpm, 30 min). Liposome enriched retentate was washed with PBS and re-suspended to 500 μL to get SeMet-loaded liposomes (SeMet-LP). The 5mg RGD Kasım SH peptide powder was dissolved in 1mL PBS and the pH value was adjusted to 7.2 by adding 0.1mol/L NaOH (Aladdin, China) for peptide coupling. Then, 200 μL purified SeMet was co-incubated with 500 μL solution of RGD at 37 °C for 1 h to covalently conjugate SeMet with RGD. Unbound RGD SH peptide was removed from the system via the ultrafiltration (0.5 mL filter, 4500 rpm, 30 min). After diluting with PBS to 500 μL, the final

RGD modified drug loaded liposome product (SeMet- LP@RGD) was achieved. Liposomes were defined in this study as: LP (Empty blank liposomes), SeMet erythroid(Seine Met) encapsulated liposomes (SeMet-LP), RGD functionalized drug encapsulated liposomes (SeMet-LP@RGD).

Deionized water was used to dilute liposome samples a hundredfold. The hydrodynamic particle diameter and polydispersity index (PDI) were measured by dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Instruments). The morphology of liposomes was observed by TEM (Hitachi HT 7800). Ultrafiltration-centrifugation combined with HPLC was used to calculate SeMet encapsulation efficiency (EE%). SeMet stock solution (10 mg/mL) was prepared in ≥99.9% methanol (Sigma Aldrich) and serially diluted to create calibration standards ranging from 0-175 μg/mL (0, 25, 50, 75, 100, 125, 150, 175 μg/mL). For chromatographic analysis, we adopted the Agilent 1260 Infinity II HPLC apparatus. A C18 reversed-phase column (InfinityLab Poroshell 120 EC-C18, 4.6 mm × 100 mm, 2.7 μm particle size) was assembled onto this system. For gradient-mode separation, acetonitrile functioned as mobile phase A, and mobile phase B corresponded to an aqueous solution containing 0.05% trifluoroacetic acid.The elution program used was: 0–15 min, A/B ratio was linearly shifted from 5:95 to 80:20 (v/v); 15–20 min, A/B ratio was linearly changed from 80:20 to 95:5 (v/v); 20–30 min, A/B ratio fixed at 95:5 (v/v); 30–40 min, re-equilibration to start conditions. A constant flow of 1.0 mL/min was applied. The column temperature was kept at 35 °C, while the detection wavelength was adjusted to 214 nm. The peak areas of standard samples were measured and a linear calibration was developed, and then used to determine the content of SeMet in the test samples. The samples containing the drug loaded liposomes were poured into 0.5 mL MWCO 100 kDa (Merck Millipore) ultrafiltration centrifugal tubes and centrifuged for 30 min at 4500 rpm to differentiate liposome entrapped drug from free SeMet for EE% measurement. The retentate with liposome was collected and the inner wall of the tube was washed with PBS buffer. The retentate fraction was made up to 500 μL with PBS. Then 50 μL liposome sample was thoroughly vortexed with 250 μL anhydrous methanol and filtered through 0.45 μm microporous membranes before the HPLC measurement. W1 and W2 were determined based on the data in chromatograms, where W1 is the total amount of SeMet, and W2 is the amount of SeMet encapsulated by liposome. The EE% was calculated according to the following formula: $EE\% = (W2 / W1) \times 100\%$. The physical stability of SeMet-LP liposomal formulation was assessed. Samples were kept in dark amber glass vials at 4 °C. Aliquots were withdrawn at pre-defined time intervals for the recording of the changes in the important physicochemical parameters such as particle dimension, PDI value and encapsulation efficiency.

Cell culture

Cell culture of Murine RAW264.7 macrophages and aortic vascular smooth muscle cells

(VSMCs) was performed in DMEM medium and supplemented with 10% FBS and 1% penicillin-streptomycin double antibiotics. VSMCs used were MOVAS cells, an immortalized mouse aortic vascular smooth muscle cell line. MOVAS cells were purchased from BNCC(Item:BNCC338213), and cells at passages 3-5 were used for all experiments. Before experiments, we detected classic VSMC contractile markers including α-SMA and SM-MHC. ells were grown in humidified 5% CO_2 atmosphere at 37 °C in incubators. At confluences of about 80% cells were collected for further experimental manipulation.

Analysis of Intracellular Uptake by Confocal Laser Scanning Microscopy (CLSM).

RAW264.7-macrophages were cultured at a 2×10^5 cell per dish on 15mm-glass-bottom dishes for 12 h for complete adherence. Cells underwent 24h stimulation: incubation with 1 μg/mL lipopolysaccharide (LPS) elicited M1 polarization, while 20 ng/mL interleukin-4 (IL-4) treatment promoted M2 phenotypic switching. Cells were incubated in fresh culture medium after polarization induction with either DiI labelled LP or LP@RGD nanoparticles for an additional 24 h. Once incubation finished, PBS was used to wash the cells. Cells were fixed in 4% paraformaldehyde solution at 25℃for 15 min. Afterwards, 0.2% Triton X-100 was applied for 10 min to permeabilize cell membranes, and the cells were blocked with 5% BSA solution for 1 h. The prepared samples were kept at 4 °C overnight together with primary antibody against CD68. Following three rounds of PBS washing, samples were incubated for one hour with FITC-conjugated secondary antibody. Before CLSM observation and image acquisition, cell nuclei were counterthestained with DAPI containing antiaktadır mounting medium.

VSMC uptake assessment: VSMCs were seeded onto 15 mm glass bottom dishes (1×10^5 per dish) and allowed to attach for 12 h. Synthetic phenotypical transition was then performed by stimulating the cells for 24 h with 1 μM angiotensin II (AngII). Afterwards, the cells were co-cultured with DiI labeled LP or LP@RGD for 24 h. Cells were treated after completion, with PBS rinse, 4% paraformaldehyde (PFA) fixation, 0.2% Triton X - 100 permeabilization, and then PBS rinse again. The intrinsic DiLfluorescence in the smooth muscle actin signals was used to detect them, and the DAPI dye was used to mark the cell nuclei.

Flow cytometric determination of cellular uptake DiI labeled LP or LP@RGD was cultured with M0, M1 or M2 macrophages for 24 h.Then, the fluorescent intensity of DiI in the intracellular space was measured and analyzed by Attune NxT flow cytometer.

Animals

Animal studies were carried out after approval by the Animal Ethics Committee of Enshi Prefecture Central Hospital (Approval No. W202507001). Male $ApoE^{-/-}$ mice (8–10 weeks old) and male C57BL/6 mice (< 12g) were obtained from Wuhan Hualianke Biotechnology Co., Ltd. the laboratory animals were raised under conventional breeding conditions

(25 ± 2 °C, 50 ± 10 %RH, 12 /12 h light-dark cycle, ad libitum diet).

An abdominal aortic aneurysm (AAA) model was created in mice. Male ApoE$^{-/-}$ mice 8-10 weeks of age were anesthetised with isoflurane for the creation of the AAA animal model. Subcutaneous implantation of mini-osmotic pumps (Alzet model 2004; Sigma, USA) pre-loaded with AngII solution was performed. AngII was infused through the osmotic pump at the constant rate of 1000 μg/kg/min for 4 weeks. Mice received high-fat diet throughout the whole experimental period. Successful AAA model establishment was considered to be an increase in aortic diameter over 50% as to the base line measurements. Animals were randomly assigned into five groups: Control group, AngII + saline group, AngII + SeMet group, AngII + SeMet-LP group and AngII + SeMet-LP@RGD group. In AngII + SeMet group, SeMet solution was injected monthly to the tail vein at a dosage of 7.5 μg/20 g body weight of mice. The dosage of liposome preparations used for injection in SeMet LP and SeMet LP @ RGD groups was based on their respective SeMet encapsulation efficiency, and the amount of SeMet injection was adjusted to obtain a same dosage (7.5 μg/20 g BW) in all experimental groups.

Preparation of mouse thoracic aortic dissection (TAD) model. We used 3–4 weeks old male C57BL/6 mice fed on a normal chow diet to develop the TAD model using β Reinforced Amino Propionitrile monofumarate (BAPN). BAPN (Sigma Aldrich, USA) was provided in a drinking water with a concentration of 0.25% for several weeks. Mice were randomly assigned to the Control, saline + BAPN, SeMet + BAPN, SeMet + BAPN and SeMet + BAPN groups. Dosages of free and liposomal SeMet formulations were similar to those used in the animal studies related to AAA.

EVG scoring criteria

Semi-quantitative evaluation for elastin degradation within mouse aortic tissues was carried out following standardized scoring rules: Score 1: elastic lamellae without any degradation; Score 2: mild degradation of elastic fibers; Score 3: severe elastin damage; Score 4: occurrence of aortic rupture.

***In vivo* looking at LP@RGD performance evaluation**

DiR-labeled LP@RGD nanoparticles were intravenously injected into the tail vein on day 1 of the induction of the AAA or TAD model. Mice were anaesthetized using isoflurane and imaged by the optical imaging system IVIS Spectrum, PerkinElmer at fixed time points (1, 7, 14 and 28 DPI) post administration. Animals were sacrificed following last imaging at day 28. Aortic tissues (especially aneurysm- or dissection-affected segments) were dissected for ex-vivo fluorescence imaging to map DiR nanoparticle distribution. The expression of MFI (Mean Fluorescence Intensity) was measured by Living Image supporting software. Dissected aortic tissue specimens were mounted within optimal cutting temperature (OCT) medium,

followed by cryosectioning using a cryostat instrument.

Immunofluorescent staining was done as follows: the tissue slices were first stained with the primary antibodies: α SMA (marker for smooth muscle cells) and CD68 (macrophage marker) and then stained with the corresponding Alexa Fluor 488 conjugated fluorescent secondary antibody. DAPI-containing mounting medium was applied to stain cell nuclei. CLSM was then used for the analysis of signal co-localisation between the red DiR signal and some biomarker green cellular signals (to verify the cell type targeting characteristics of the nanoliposomes).

Western blotting assay

Protein expression profiles in were detected by blot experiments. RAW264.7 macrophages and VSMCs. Cells were plated in 6-well culture wells Seeding densities (1×10^6 cells/well) were used and the cells were treated with various treatments. To the complete medium (control), RAW264.7 macrophages were added and treated with: LPS, LPS and free SeMet (1 μM), LPS and SeMet-LP or LPS and SeMet-LP@RGD for 24 h. The concentrations used were SeMet-LP and SeMet-LP@RGD applied. Normalized to encapsulation efficiency to ensure equal amount of SeMet No differences in the number of surviving cells or concentration (1 μM) between experimental groups. For VSMC groups, cells received:In the VSMC groups, cells were treated with: with bound SeMet, and a blank (no AngII).complete medium, AngII (1 μM), AngII with free SeMet, AngII with bound SeMet, and a blank (no AngII). Cells received 24h exposure to AngII alone, AngII combined with SeMet-LP, or AngII plus SeMet-LP@RGD. Once the incubation period concluded, total cellular protein was isolated with RIPA lysis buffer (Beyotime, China) pre-added with both protease-inhibitor cocktail and phosphatase inhibitors. Protein concentrations of the lysate samples were quantified utilizing the BCA assay kit from Yeasen (China). Following quantification, protein specimens were blended with 5 × SDS loading buffer (Beyotime, China) and subjected to heat denaturation at 100 °C lasting 10 min. Denatured proteins were fractionated via SDS-PAGE electrophoresis and subsequently blotted onto 0.45 μm PVDF membranes (Millipore). Afterwards, these membranes underwent 1-h blocking at room temperature within TBST solution containing 5% non-fat skim milk. Afterwards, membranes were incubated Overnight at 4 °C with primary antibodies: iNOS (1:1000, Proteintech), Arg-1 (1:1000, Proteintech) and GAPDH (1:1000, Proteintech). (1:1000, ABclonal), α-SMA (1:2000, ABclonal), SM-MHC (1:2000, ABclonal), Vimentin (1:1000, ABclonal), OPN (1:1000, ABclonal), and β-actin (1:3000, CST). HRP-conjugated secondary antibody (1:2000, ABclonal) was to which were added for 1-h incubation at 25℃. Visualization of protein bands was accomplished by ECL chemiluminescent substrate (EpiZyme, China), and images were captured by Bio-Rad ChemiDoc imaging system (USA). Therapeutic Evaluation of efficacy of the different nanoliposome preparations. After Mice were perfused transcardially with PBS solution

(20 mL). Thoracic and abdominal aorta tissues were carefully removed in surgery after which they were under control. For morphological analysis, a stereomicroscope was used. Maximum diameter of abdomen is The aorta diameter was measured and AAA and TAD incidence rates were documented. Aortic tissues were fixed for 18h n 4% paraformaldehyde and embedded in. paraffin blocks, and were then cut into 5-um slices for histology staining. Hematoxylin-eosin (H&E) staining was performed on rectangular fragments of full-length aortic tissue. EVG (elastin-Van-Gieson) staining was used to assess elastic-fiber integrity. To etermine the in-situ polarization state of macrophages, tissue slices were stained with immunofluorescent antibodies to the marker of M1-phenotype, CD86 (1:100; Abcam, USA) and M2-phenotype marker CD206 (1:100; Abcam, USA). Alexa-Fluor-series Signals were developed with secondary antibodies. Sections were sealed with DAPI-supplemented mounting medium for nuclear staining. All stained tissue sections were observed under OLYMPUS BX15 optical microscope (Tokyo, Japan), For subsequent analysis, photographs of both the and representative fields were taken. Cellular One-Step TUNEL apoptosis detection kit was used to identify the apoptosis status. BeyouTec (Beyotime, China) strictly abide by the operating procedure given by the manufacturer. instructions.

Figure Legends

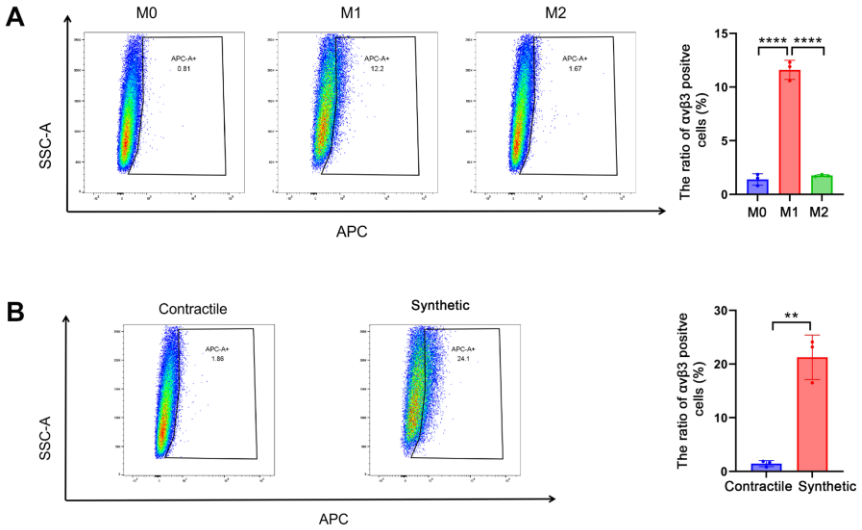


Figure S1 (A) Flow-cytometry histograms and quantitative statistics of αvβ3 expression levels in M0, M1 and M2 macrophage subsets (n=3). (B) Flow-cytometry profiles and quantitative analysis of αvβ3 abundance in contractile-type and synthetic-type VSMCs (n=3).

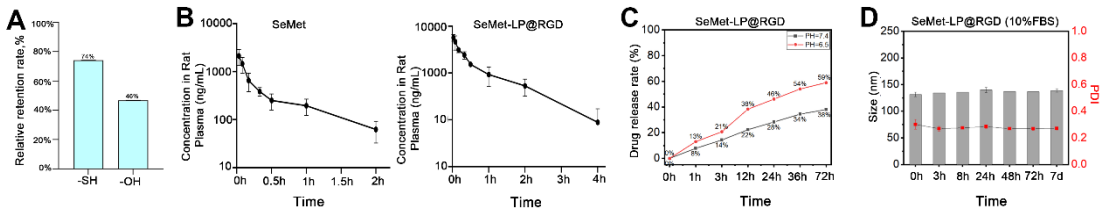


Figure S2 (A) Thiol-modified fluorescent probe FITC-PEG2k-SH (MW=2000 Da) and non-thiol-containing control probe FITC-PEG2k-OH (MW = 2000 Da) were incubated with drug-loaded liposomes under identical experimental conditions (pH 7.2, 37 °C, 1 h, dark environment). Unbound small-molecule probes were removed by purification procedures. Absorbance retention ratio at 490 nm was calculated as absorbance after filtration divided by absorbance value before filtration. (B) Semi-logarithmic plots showing plasma SeMet concentration-time curves in SD rats after tail-vein injection of free SeMet or SeMet-LP@RGD (n=3).(C) In vitro cumulative drug release profiles of SeMet-LP@RGD at pH 7.4 and pH 6.5 over 72 h.(D) Time-dependent changes in particle size (grey bars) and PDI (red dots) of SeMet-LP@RGD in 10% FBS during 7 days.

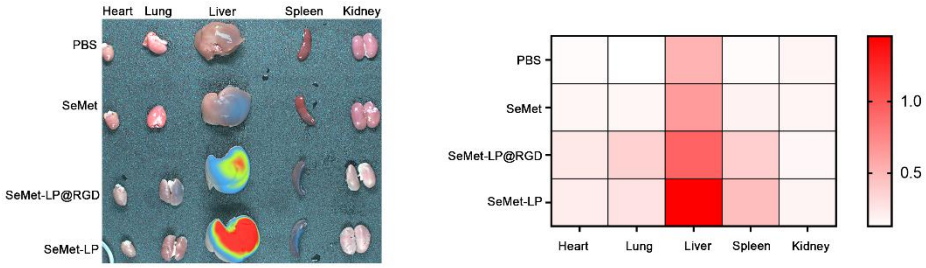


Figure S3 Representative ex-vivo fluorescence imaging photographs of major organs (heart, lung, liver, spleen and kidney) harvested from mice treated with PBS, free SeMet, SeMet-LP@RGD or SeMet-LP (n=3).

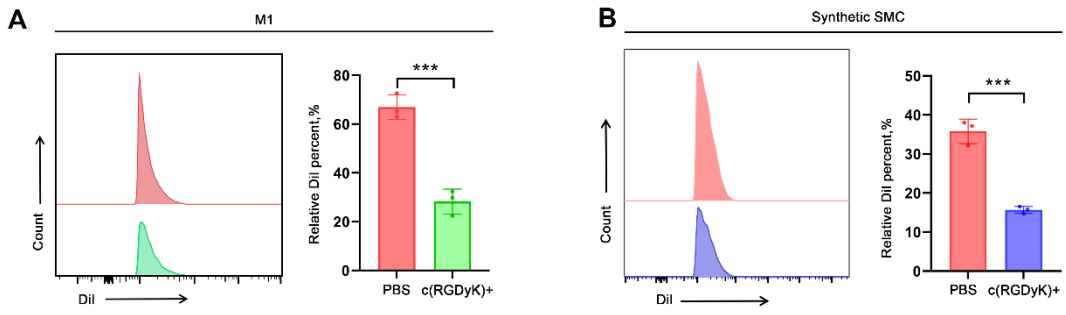


Figure S4 (A) Flow-cytometry diagrams and quantitative statistics for DiI-positive cell proportion in M1 macrophages with or without pre-treatment of c(RGDyK) peptide. (B) Flow-cytometry data and quantitative comparison for DiI-positive percentages in synthetic-phenotype VSMCs with/without c(RGDyK) pre-incubation (n=3).

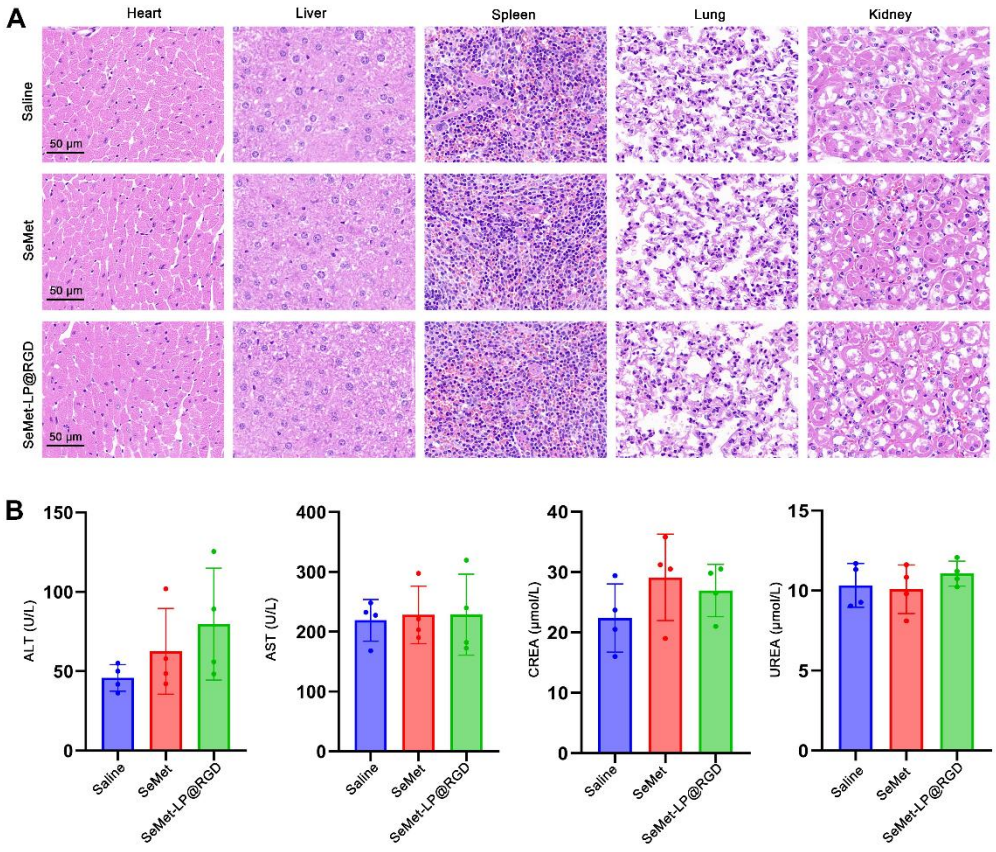


Figure S5 Histopathological observation and serum biochemical detection of major organs from mice receiving different therapeutic formulations. (A) Representative H&E-stained micrographs of heart, liver, spleen, lung and kidney sections derived from saline-treated, free-SeMet-treated and SeMet-LP@RGD-treated animal groups. Scale bar=50 μm. (B) Quantitative results of serum biochemical markers, including alanine transaminase (ALT), aspartate transaminase (AST), creatinine (CREA) and urea (UREA) across different cohorts (n=4).

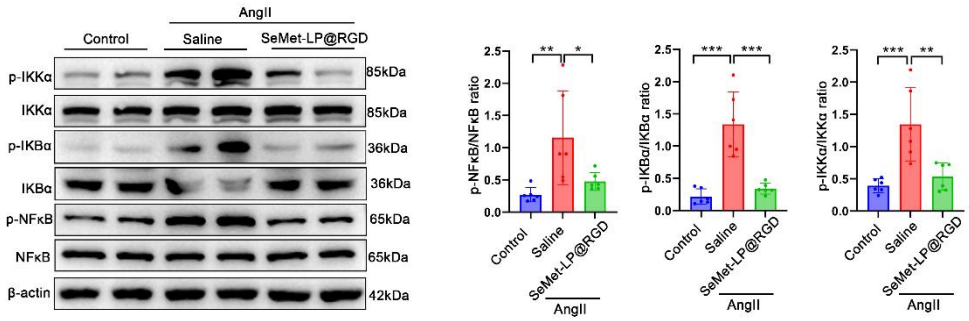


Figure S6 Representative western-blot bands and quantitative statistical results for IKKα, p-IKKα, IκBα, p-IκBα, NF-κB and p-NF-κB protein levels in AngII-induced AAA mouse model (n=6).

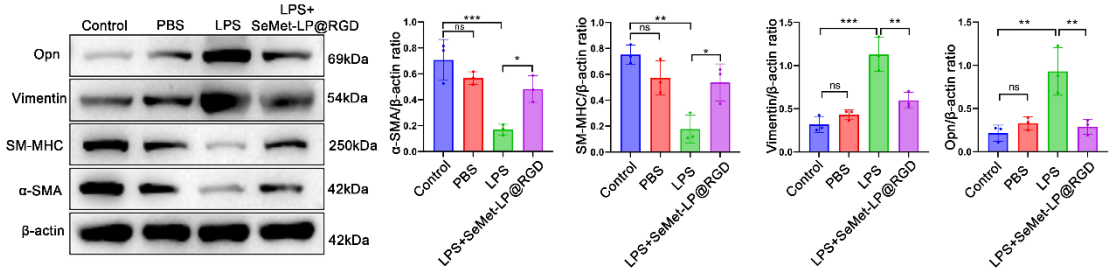


Figure S7 Representative western-blot images and quantitative analysis of α-SMA, SM-MHC, vimentin and OPN protein expression among control, PBS-treated, LPS-stimulated and LPS + SeMet-LP@RGD cell groups (n=3).

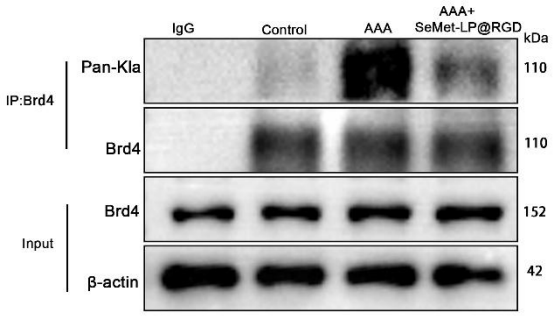


Figure S8 Co-immunoprecipitation (Co-IP) detection of Pan-Kla modification on Brd4 protein in aortic tissues collected from control mice, AAA-model mice and AAA mice receiving SeMet-LP@RGD intervention. IgG served as negative immunoprecipitation control, and β-actin was loaded as internal reference protein (n=3).