Construction of nanozyme composite drug delivery system based on columbianadin for inflammatory bowel disease therapy

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Materials and Methods

1.1 Biocompatibility Evaluation of MXene/CBN@GelMA

To assess the biocompatibility of the synthesized MCG, an in vitro cytotoxicity assay was performed using the human normal colon epithelial cell line NCM 460. Cells were seeded uniformly in a 96-well plate at a density of 5,000 cells per well and incubated for 24 hours at 37 °C under a 5% CO_2 humidified atmosphere to allow cell attachment. The original culture medium was then carefully aspirated and replaced with fresh complete medium containing different concentrations of MCG (0, 10, 50, 100, 200, and 500 μg/mL), with six experimental groups in total. Each treatment group was tested in triplicate to ensure reproducibility. After further incubation for 24 hours, 10 μL of Cell Counting Kit-8 (CCK-8) solution was added to each well, and the plate was incubated for another 1.5 hours at 37 °C. The optical density (OD) of each well was measured at a wavelength of 450 nm using a microplate reader to evaluate cell metabolic activity.

1.2 Intracellular ROS Scavenging Assay of MXene/CBN@GelMA

NCM 460 cells were seeded in confocal dishes at a density of 20,000 cells per dish and stimulated with culture medium containing 3% dextran sulfate sodium (DSS). After induction, the cells were treated with MXene (23.5 μg/mL), MXene/CBN (26.1 μg/mL), or MCG (200 μg/mL) for 24 h. Subsequently, the cells were incubated in the dark with the ROS-sensitive fluorescent probe DCFH-DA (1 μM) at 37 °C for 20 minutes. After washing three times with PBS, cell nuclei were stained with DAPI (0.5 μg/mL) for 10 minutes. The cells were gently washed three times with PBS and immediately imaged using a confocal microscope for qualitative and quantitative analysis.

1.3 Intracellular Oxygen Generation Assay of MXene/CBN@GelMA

NCM 460 cells were plated in confocal dishes at 20,000 cells per dish and induced with 3% DSS. The cells were then treated with MXene (23.5 μg/mL), MXene/CBN (26.1 μg/mL), or MCG (200 μg/mL) for 24 h. Following treatment, the cells were incubated in the dark with the oxygen-sensitive probe $Ru(dpp)_3Cl_2$ (1 μM) at 37 °C for 20 minutes. After three washes with PBS, the nuclei were counterstained with DAPI (0.5 μg/mL) for 10 minutes. The cells were rinsed gently three times with PBS and promptly

visualized under a confocal microscope for image acquisition and analysis

1.4 *In vivo* experiment in IBD mice

Male C57BL/6 mice (6–8 weeks old, 20–22 g) were used for in vivo experiments. An inflammatory bowel disease (IBD) model was established by orally administering 2.5% dextran sulfate sodium (DSS) for 8 consecutive days. General health status was monitored daily, and disease activity index (DAI) was calculated to evaluate model establishment. The mice were randomly divided into five groups (n = 6 per group) based on body weight: healthy control group (normal drinking water, no treatment), IBD + PBS group, IBD + MXene (23.5 μg/mL) group, IBD + MXene/CBN (26.1 μg/mL) group, and IBD + MCG (200 μg/mL) group. Starting from the first day of modeling, the corresponding treatments (PBS, MXene, MXene/CBN, or MCG) were administered orally on days 1, 3, 5, and 7 at a uniform dose of 0.1 mg/kg. After 8 days, all mice were euthanized following humane endpoints. Colon tissues and major organs (heart, liver, spleen, lungs, and kidneys) were collected for subsequent histological and biochemical analyses. All experimental procedures were performed in strict accordance with animal welfare guidelines and were approved by the Institutional Animal Care and Use Committee of our institution, complying with national and regional regulations on animal experimentation.

The sample size (n = 6 per group) was determined based on preliminary exploratory experiments and previous studies using DSS-induced IBD mouse models, in which similar group sizes were sufficient to detect statistically significant differences in body weight change, disease activity index (DAI), colon length, and histopathological scores. This group size was considered adequate to ensure statistical reliability while adhering to the principles of ethical animal use.

1.5 H&E Staining

To examine histopathological changes in colon tissues, hematoxylin and eosin (H&E) staining was performed. After euthanasia, colon tissues were rapidly dissected, and lesion areas were carefully excised. The tissues were gently rinsed with physiological saline to remove luminal contents and immediately fixed in 4% paraformaldehyde solution for 24 h at 4 °C. Following fixation, samples were dehydrated through a graded

ethanol series (70%, 80%, 90%, 95%, and 100%), cleared in xylene, and embedded in paraffin.

Sections were prepared at a thickness of 7[19] μm using a microtome and stored at room temperature. Deparaffinization was carried out with xylene and a descending ethanol series. Staining was performed using an H&E staining kit: sections were stained with hematoxylin for 7 minutes, rinsed three times with water, differentiated in 1% acid alcohol for 10 seconds, rinsed again, counterstained with eosin for 2 minutes, and rinsed thoroughly. Finally, sections were dehydrated, cleared, and mounted. Images were acquired using a confocal microscope.

1.6 Transcriptome Sequencing

Upon completion of all experiments, colon tissue samples from each group were preserved in RNA stabilization solution and flash-frozen in liquid nitrogen for subsequent transcriptomic analysis (RNA-Seq). Total RNA was extracted from the tissues, and RNA integrity and concentration were assessed using a NanoDrop 2000 spectrophotometer and an Agilent 2100 Bioanalyzer.

Differential gene expression analysis was performed using two widely adopted R packages: DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html) and edgeR (https://bioconductor.org/packages/release/bioc/html/edgeR.html) within the R environment (https://www.r-project.org/). Statistically significant differentially expressed genes (DEGs) were identified based on adjusted p-values and log2 fold changes. To interpret the biological functions and pathways associated with the DEGs, Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted using the clusterProfiler package (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html). The entire workflow—from raw data processing, alignment, and quantification to differential expression analysis and functional annotation—constituted a comprehensive and reproducible RNA-Seq data analysis pipeline, ensuring the reliability and scientific rigor of the results.

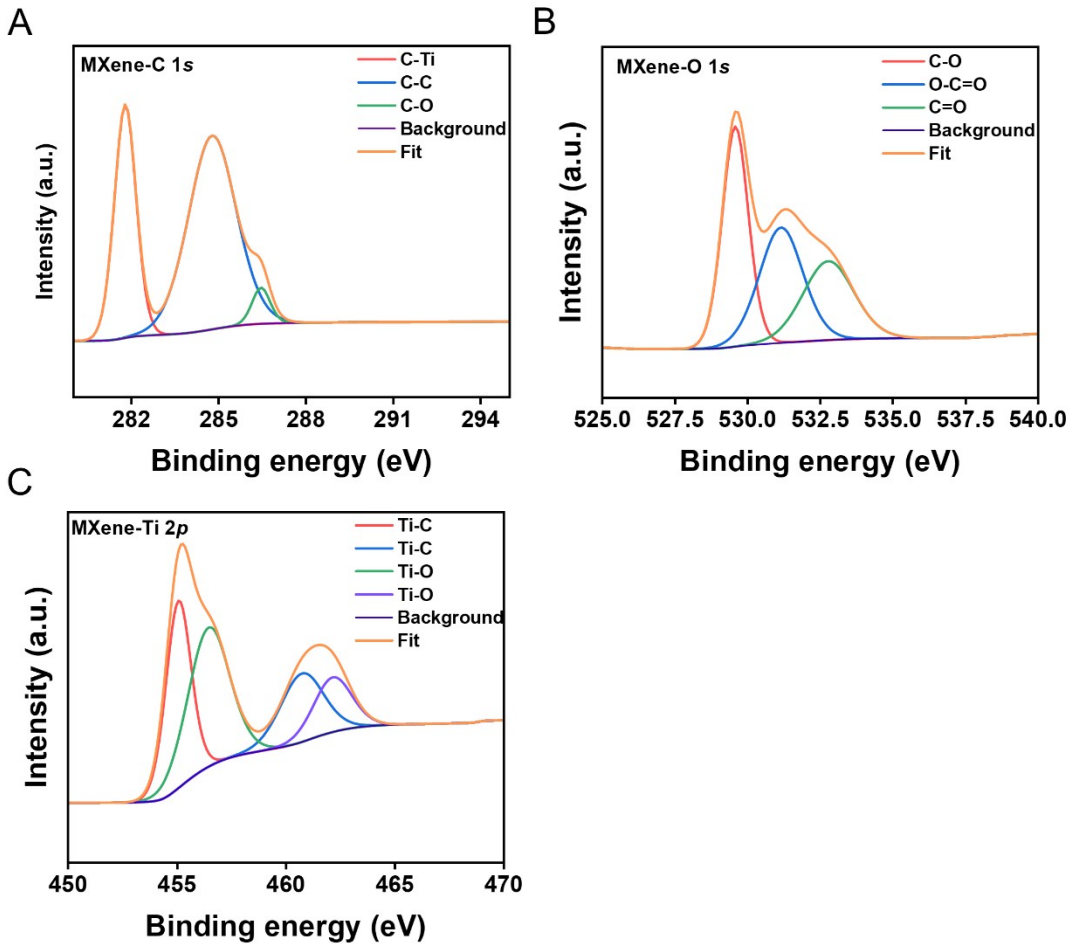

Figure S1. A) High-resolution XPS C1*s* spectrum of MXene. B) High-resolution XPS O1*s* spectrum of MXene. C) High-resolution XPS Ti 2*p* spectrum of MXene.

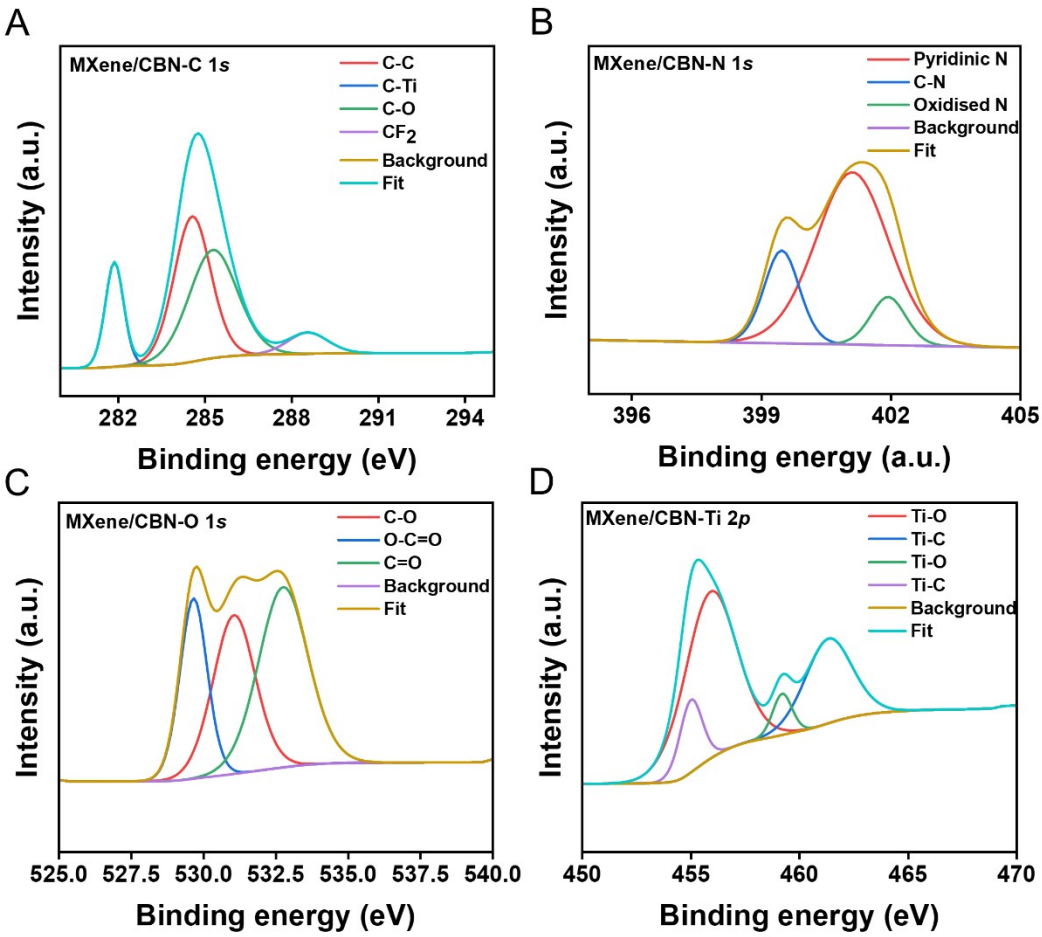

Figure S2. A) High-resolution XPS C 1*s* spectrum of MXene/CBN. B) High-resolution XPS N 1*s* spectrum of MXene/CBN. C) High-resolution XPS O 1*s* spectrum of MXene/CBN. D) High-resolution XPS Ti 2*p* spectrum of MXene/CBN.

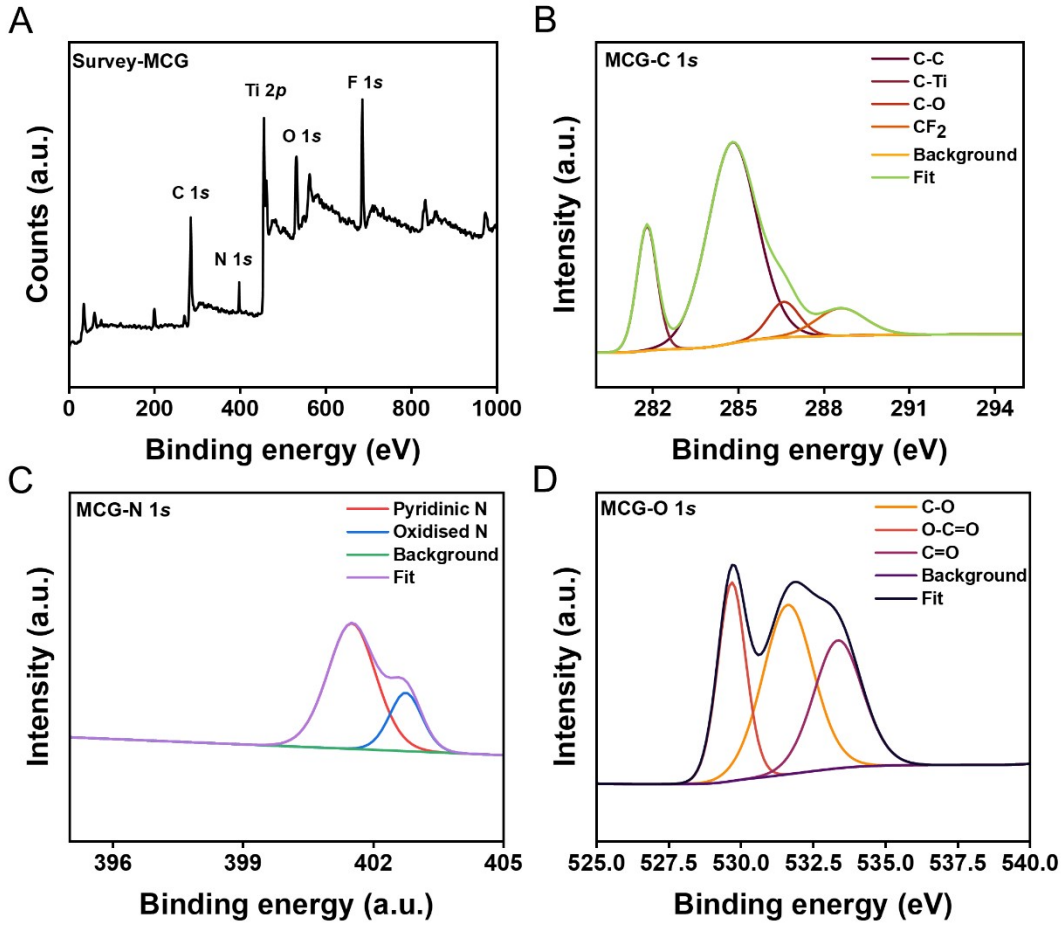

Figure S3. A) High-resolution XPS C 1*s* spectrum of MCG. B) High-resolution XPS N 1*s* spectrum of MCG. C) High-resolution XPS O 1*s* spectrum of MCG. D) High-resolution XPS Ti 2*p* spectrum of MCG.

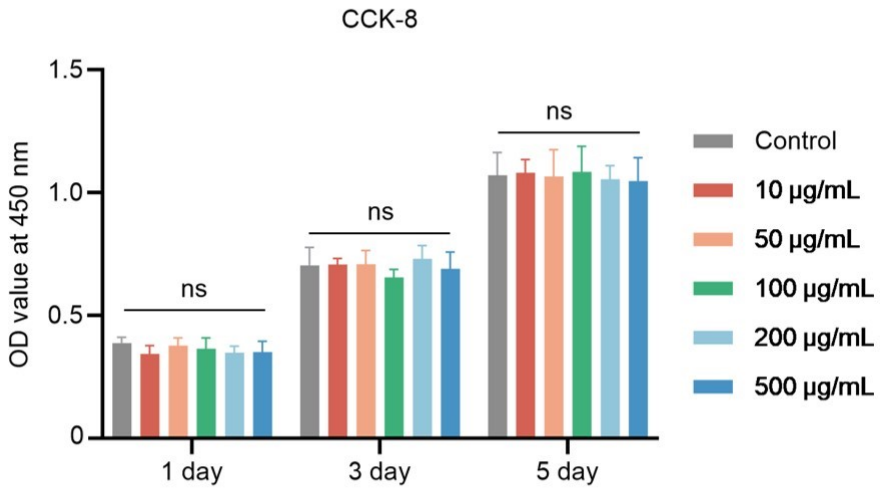

Figure S4. The effects of different treatment methods on the viability of NCM460. Data are presented as mean ± SD, n = 3, ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

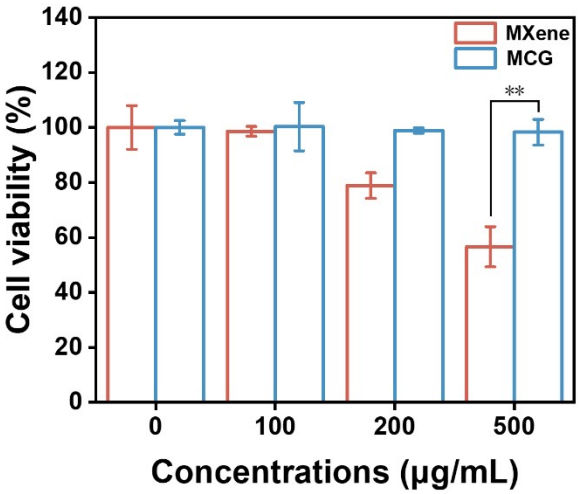

Figure S5. The effects of different concentrations of MXene and MCG on the viability of NCM460. Data are presented as mean ± SD, n = 3, ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

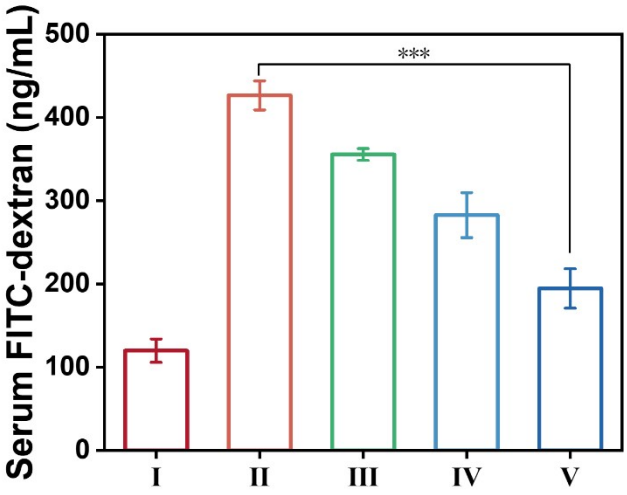

Figure S6. Serum FITC-dextran levels in Control, DSS-induced IBD model, and MCG-treated groups. Data are presented as mean ± SD, n = 6, ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. I: Control, II: DSS + PBS, III: DSS + MXene, IV: DSS + MXene/CBN, V: DSS + MCG.

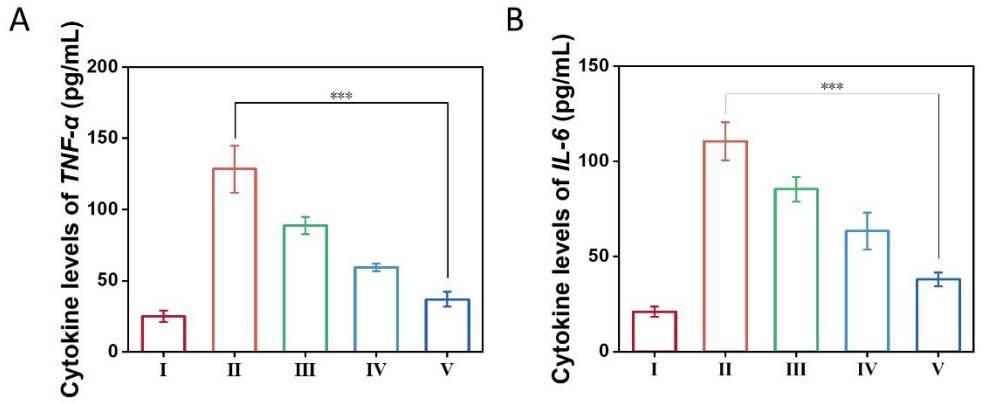

Figure S7. TNF-α and IL-6 levels in serum or colon tissue from different groups. Data are presented as mean ± SD, n = 6, ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. I: Control, II: DSS + PBS, III: DSS + MXene, IV: DSS + MXene/CBN, V: DSS + MCG.

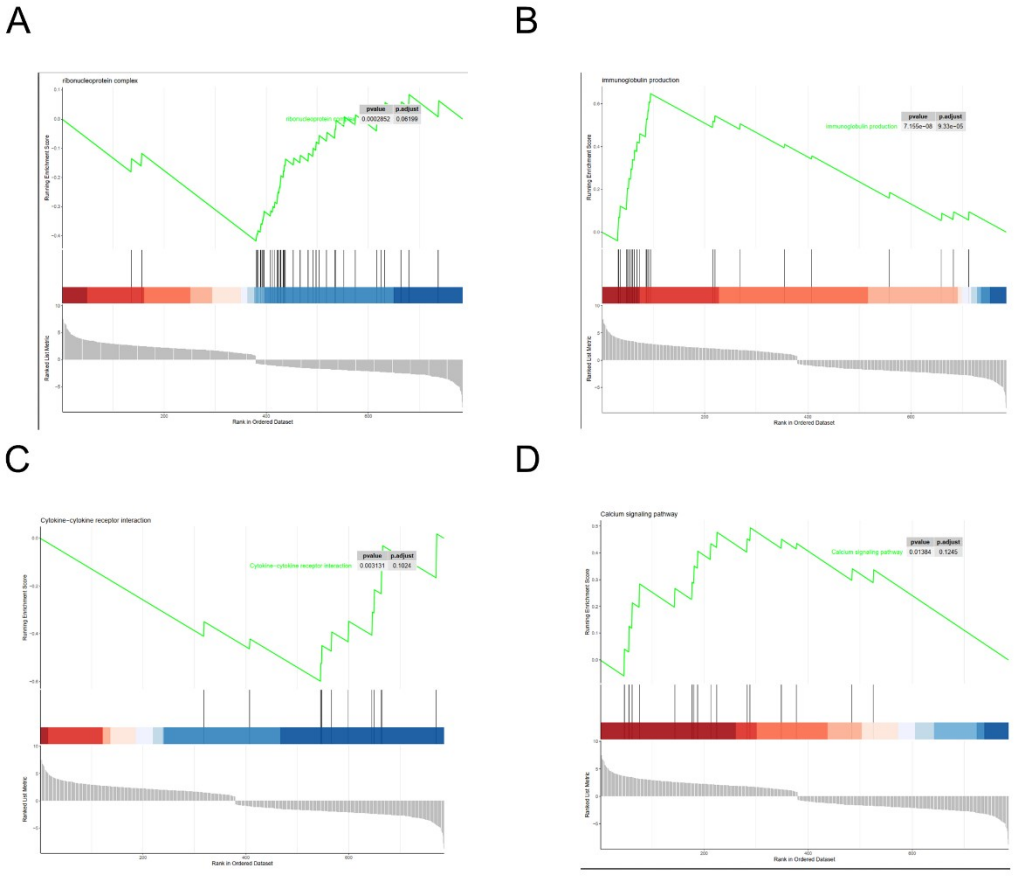

Figure S8. A-D) Enrichment Map Presentation.