**Supplementary document**

**1 Inclusion and exclusion criteria**

**1.1 AS Patient Group**

**Inclusion Criteria:**

1. Diagnosed with ankylosing spondylitis (AS) per the 1984 Revised New York Criteria .
2. No recent immunosuppressive treatment or related therapies.
3. Completed erythrocyte sedimentation rate (ESR) or complete blood count (CBC) testing.
4. Patients meeting all inclusion criteria and none of the exclusion criteria were enrolled.

**Exclusion Criteria:**

1. Comorbid immune disorders (e.g., systemic lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, or Crohn’s disease).
2. Severe cardiovascular/cerebrovascular diseases, hepatic/renal insufficiency, or hematologic disorders.
3. Pregnancy, lactation, or active systemic/local infections.
4. Non-infectious conditions (e.g., malignancy, major trauma, or recent surgical history).
5. Absence of ESR or CBC testing.

Patients meeting any exclusion criterion were excluded.

**1.2 Control Patient Group**

**Inclusion Criteria:**

1. Confirmed healthy status via physical examination, or diagnosis of non-immune conditions (e.g., traumatic fracture, degenerative joint disease, sports injury, spinal degeneration).
2. No history of immunosuppressants, glucocorticoids, or immunomodulatory drugs.
3. Absence of autoimmune diseases (e.g., rheumatoid arthritis, ulcerative colitis, systemic lupus erythematosus).
4. Completed ESR or CBC testing.
5. Subjects fulfilling all criteria were enrolled as controls.

**Exclusion Criteria:**

1. Comorbid autoimmune diseases (e.g., RA, SLE, UC).
2. Severe cardiovascular/cerebrovascular diseases, hepatic/renal insufficiency, or hematologic disorders.
3. Pregnancy, lactation, or active systemic/local infections.
4. Malignancy, major trauma, or recent surgery.
5. Recent use of immunosuppressants, biologics (e.g., TNF-α inhibitors), or glucocorticoids.
6. Absence of ESR or CBC testing.

Subjects meeting any exclusion criterion were excluded.

**2 Single-Cell Library Construction**

The samples employed for single cell sequencing emanated from spinal vertebral bone marrow blood derived from patients undergoing surgical procedures. This cohort encompassed three patients diagnosed with AS. Fresh specimens, harvested during surgical resection, were promptly placed in MACS tissue storage solution (Miltenyi Biotec, Germany), and expedited to our laboratory without delay.

**2.1 Single-Cell Library Construction**

Firstly, the single-cell suspension was obtained, and its activity was tested. The cell activity was >85% and the monodispersity was good and the impurity content was low. Then the microfluidic channel oil-in-water droplet library was prepared and operated on the machine. In order to ensure the effective capture efficiency of single cells, the cell concentration is generally required to be controlled within the range of 700-1200cell/uL. In the process of library preparation, a single Gel Bead and a single cell were coated with a single oil-coated water drop to form a GEM, and each Gel Bead has a unique Barcode and UMI sequence and Poly-dT primer sequence for initiating reverse transcription reaction. Subsequently, in this GEM reaction system, the cells are broken and lysed to release mRNA Full cDNA was generated by reverse transcription reaction with Poly-dT primer sequence under the action of reverse transcriptase. The cDNA was then amplified and the library was constructed.

**2.2 Processing of scRNA-Sequencing Data**

After the completion of the library construction, Qubit3.0 was used for preliminary quantification, and the library was diluted to 1ng/uL. Then Agilent 2100 was used to detect the insert size of the library. After the insert size met the expectation, The StepOnePlus Real-Time PCR System fluorescent quantitative PCR instrument was used for Q-PCR, and the effective concentration of the library was accurately quantified (the effective concentration index of the library was not less than 10nM) to ensure the quality of the library. Qualified libraries were sequenced using Illumina platform. Raw reads were obtained. First, data were intercepted according to the 10x transcriptome library structure, and Read1 and target Read2 containing arcode and UMI sequence information were obtained. Then Cell Range, the official 10x software, was used for data analysis and processing.