

Supplemental information

**Inhibition of IKK β /NF- κ B signaling facilitates
tendinopathy healing by rejuvenating
inflamm-aging induced tendon-derived
stem/progenitor cell senescence**

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Supporting Information

Inhibition of IKK β /NF- κ B signaling facilitates tendinopathy healing by rejuvenating
inflamm-aging induced tendon-derived stem/progenitor cell senescence

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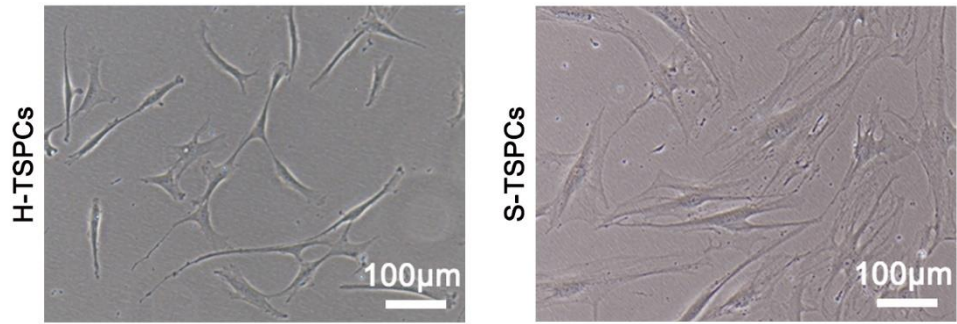


Fig. S1. Cell morphology of S-TSPCs and H-TSPCs. H-TSPCs, TSPCs isolated from degenerative supraspinatus tendons. S-TSPCs, TSPCs isolated from healthy hamstring tendons.

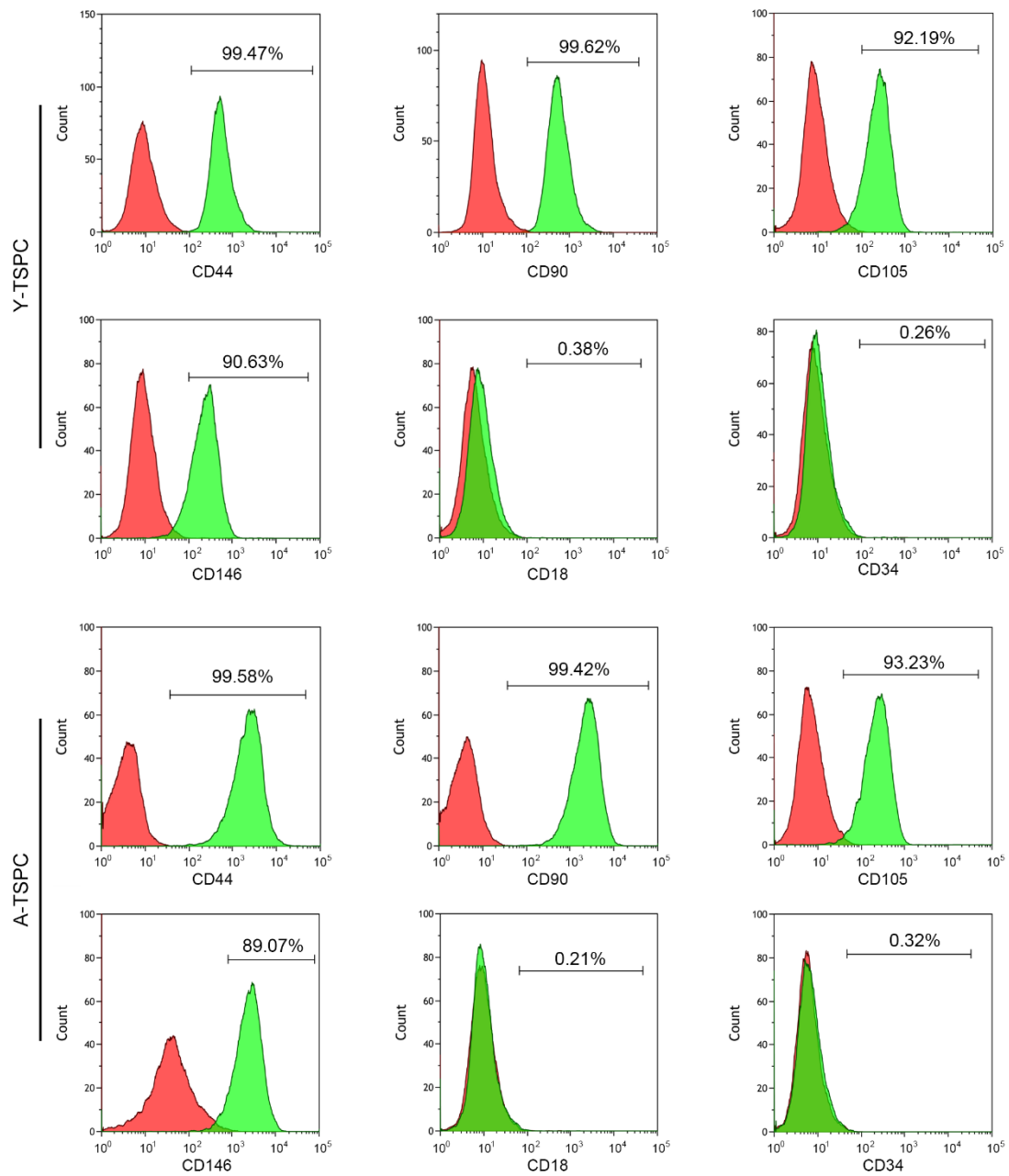


Fig. S2. Flow cytometry analysis of TSPCs surface markers. The red curves represent isotype controls, and the green curves represent measured surface markers (CD44, CD90, CD105, CD146, CD18, and CD34). H-TSPCs, TSPCs isolated from healthy hamstring tendons. S-TSPCs, TSPCs isolated from degenerative supraspinatus tendons.

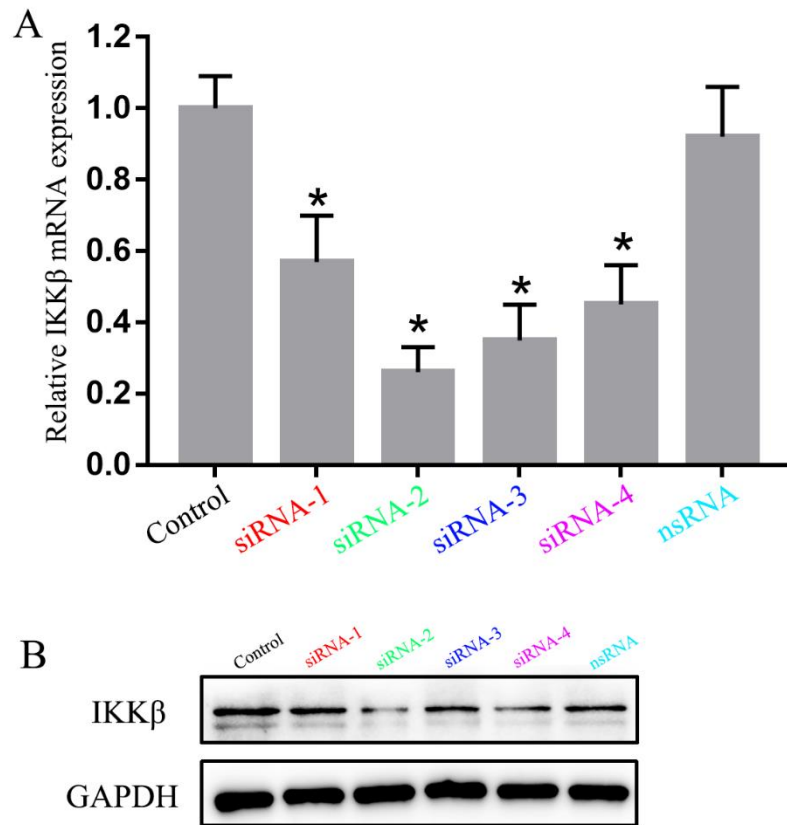


Fig. S3. IKK β gene silencing in TSPCs with different siRNA sequences. (A) Expression of IKK β mRNA in TSPCs. (B) Levels of IKK β protein in TSPCs.

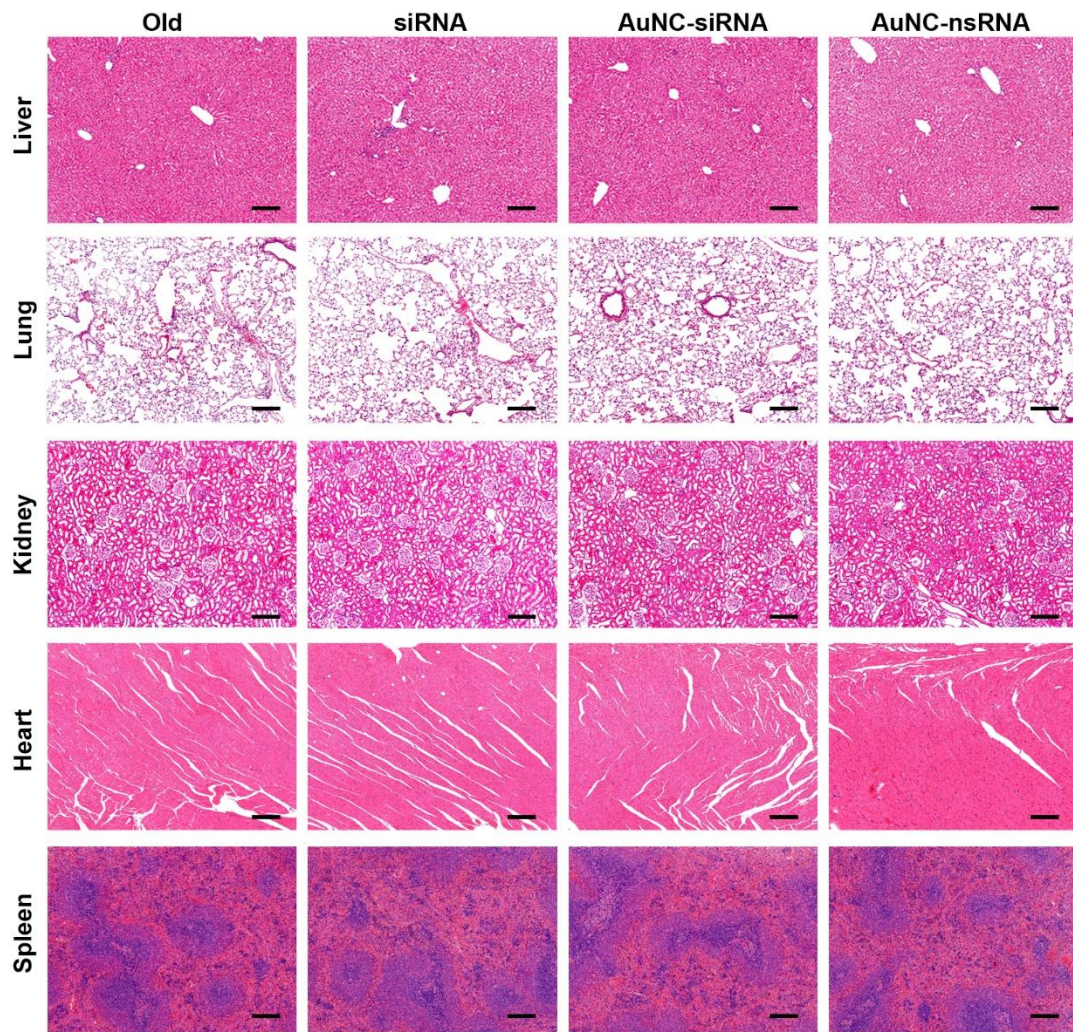


Fig. S4. (A) In vivo toxicity of AuNCs. Representative photomicrographs of H&E staining on the major organs including liver, lung, kidney, heart and spleen. Scale bars represent 200 μm.

Table S1. The exclusion criteria for patients to isolate torn supraspinatus tendons

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|--|
| Previous significant shoulder trauma |
| Systemic steroid use or tobacco smoking |
| Previous or current shoulder problems other than rotator cuff tendinopathy |
| Previous shoulder surgery |
| Local glucocorticoid injections in the last 6 weeks |
| Significant systemic medical conditions, e.g., diabetes or a rheumatological condition |

Table S2. List of primers used for human TSPCs RT-PCR analysis.

| Target | Specie | Forward primer (5' → 3') | Reverse primer (5' → 3') |
|--------------|--------|--------------------------|--------------------------|
| IL6 | Human | AGACAGCCACTCACCTCTTCAG | TTCTGCCAGTGCCTCTTTGCTG |
| IL1A | Human | TGTATGTGACTGCCCAAGATGAAG | AGAGGAGGTTGGTCTCACTACC |
| CXCL1 | Human | AGCTTGCCTCAATCCTGCATCC | TCCTTCAGGAACAGCCACCAGT |
| CXCL2 | Human | GGCAGAAAGCTTGCTCAACCC | CTCCTTCAGGAACAGCCACCAA |
| CDKN2A (p16) | Human | CTCGTGCTGATGCTACTGAGGA | GGTCGGCGCAGTTGGGCTCC |
| SCX | Human | AGACCGCACCAACAGCGTGAAC | CAGGTGCGAGATGTAGCTGGAG |
| BGN | Human | TTGAACCTGGAGCCTTCGATGG | TTGGAGTAGCGAAGCAGGTCCT |
| TNMD | Human | GGACTGGTGTTTGGTATCCTGG | CTCCATTGCTGTAGAAAGTGTGC |
| DCN | Human | GCTCTCCTACATCCGCATTGCT | GTCCTTTCAGGCTAGCTGCATC |
| COL1 | Human | GATTCCTGGACCTAAAGGTGC | AGCCTCTCCATCTTTGCCAGCA |
| COL3 | Human | TGGTCTGCAAGGAATGCCTGGA | TCTTCCCTGGGACACCATCAG |
| IKK β | Human | ACTTGGCGCCCAATGACCT | CTCTGTTCCTTGCTGCA |
| GAPDH | Human | CGACCACTTTGTCAAGCTCA | AGGGGTCTACATGGCAACTG |

Table S3. Sequences of different IKK β siRNA.

| Sequence | Forward primer (5' → 3') | Reverse primer (5' → 3') |
|-----------------|---------------------------------|---------------------------------|
| siRNA-1 | GGAAGUACCUGAACCAGUUTT | AACUGGUUCAGGUACUUCCTT |
| siRNA-2 | GGACAUUGUUGUUAGCGAATT | UUCGCUAACAACAAUGUCCTT |
| siRNA-3 | CCAUGUCUCAGCAGCUCAATT | UUGAGCUGCUGAGACAUGGTT |
| siRNA-4 | GCUGCUGCUUCAGGCAAUUTT | AAUUGCCUGAAGCAGCAGCTT |
| nsRNA | UUCUUCGAACGUGUCACGUTT | ACGUGACACGUUCGGAGAATT |

Table S4. List of primers used for rat TSPCs RT-PCR analysis.

| Target | Specie | Forward primer (5' → 3') | Reverse primer (5' → 3') |
|---------------|---------------|---------------------------------|---------------------------------|
| Sex | Rat | CAGCGGCACACGGCGAAC | CGTTGCCCAGGTGCGAGATG |
| Tnmd | Rat | GCGACAATGTGACTATGTAC | GTCTTCTCCACCTTCACTTG |
| Col1 | Rat | AGAGGCATAAAGGGTCATCGTG | AGACCGTTGAGTCCATCTTGC |
| Gapdh | Rat | CTGGAGAAACCTGCCAAGTATG | GGTGAAGAATGGGAGTTGCT |

Table S5. List of antibodies for flow cytometry analysis of TSPCs surface markers.

| Antibodies | Source | Cat# |
|---|----------------|--------|
| PE-CF594 Mouse Anti-Human CD105 | BD Biosciences | 562380 |
| PE-CF594 Mouse IgG1, κ Isotype Control RUO | BD Biosciences | 562292 |
| Hu CD90 APC 5E10 25ug | BD Biosciences | 561971 |
| APC Mouse IgG1, κ Isotype Control | BD Biosciences | 550854 |
| BV421 Mouse Anti-Human CD146 | BD Biosciences | 566226 |
| BV421 Mouse IgG1, κ Isotype Control RUO | BD Biosciences | 562438 |
| BV786 Mouse Anti-Human CD44 | BD Biosciences | 564942 |
| BV786 Mouse IgG2b, κ Isotype Control RUO | BD Biosciences | 564090 |
| FITC Mouse Anti-Human CD18 | BD Biosciences | 557156 |
| FITC Mouse IgG1, κ Isotype Control | BD Biosciences | 556649 |
| APC Mouse Anti-Human CD34 | BD Biosciences | 560940 |
| APC Mouse IgG1, κ Isotype Control | BD Biosciences | 550854 |