Supplementary Materials for

CXCL12-ACKR3 Signaling Drives Fibrosis and Inflammation in Frozen Shoulder: A Single-Cell Guided Therapeutic Discovery

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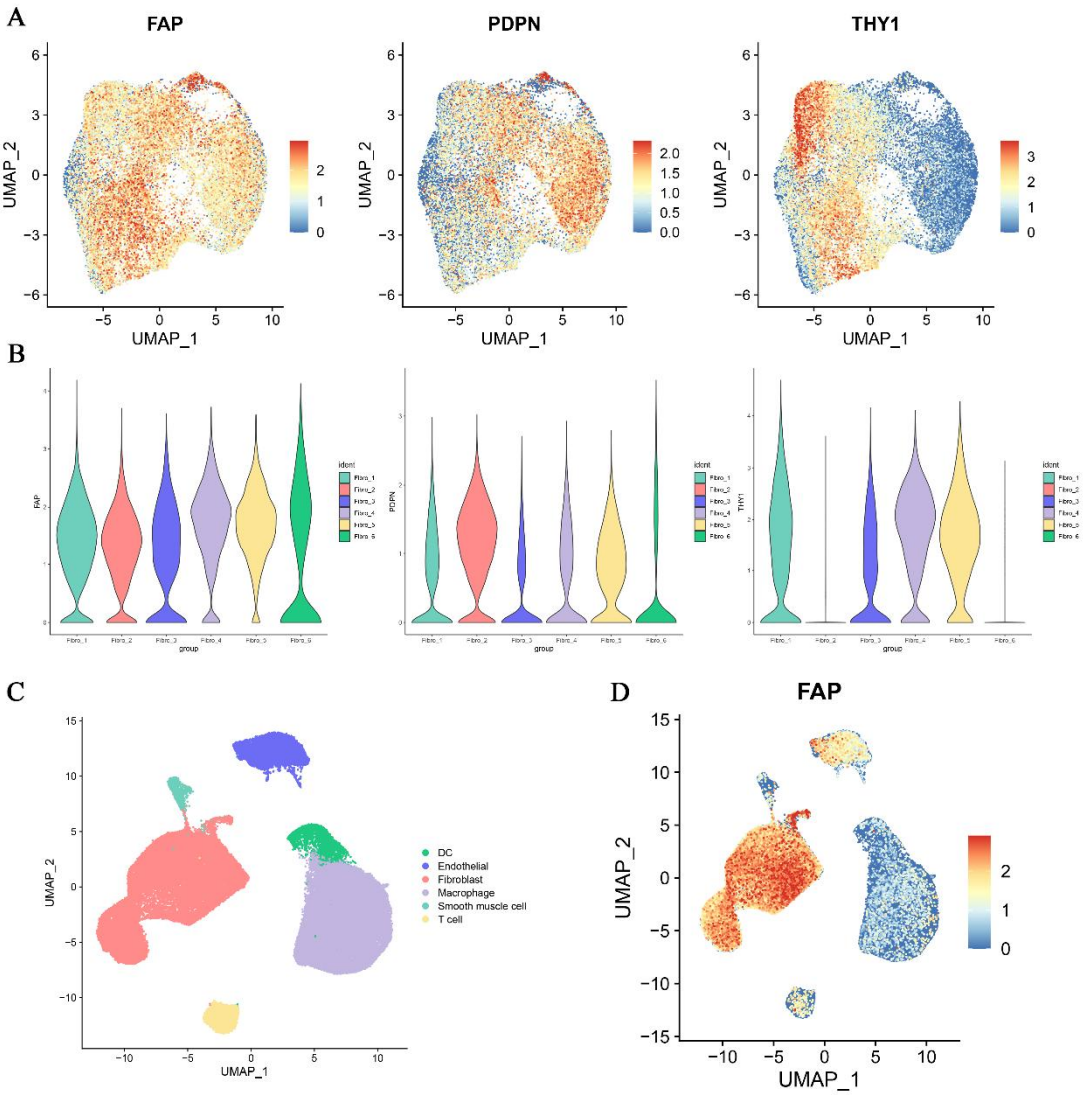

Fig. S1.

Definition of fibroblasts based on single-cell atlas. **(A)** Feature plot showing the expression of fibroblast marker genes (FAP, PDPN, THY1) in shoulder capsular fibroblasts. **(B)** Violin plots illustrating the expression of fibroblast marker genes across fibroblast subsets in shoulder capsular tissues. **(C)** Feature plot displaying FAP expression across different cell types. **(D)** UMAP visualization of the major cell types identified. The general identity of each cell type is indicated on the right.

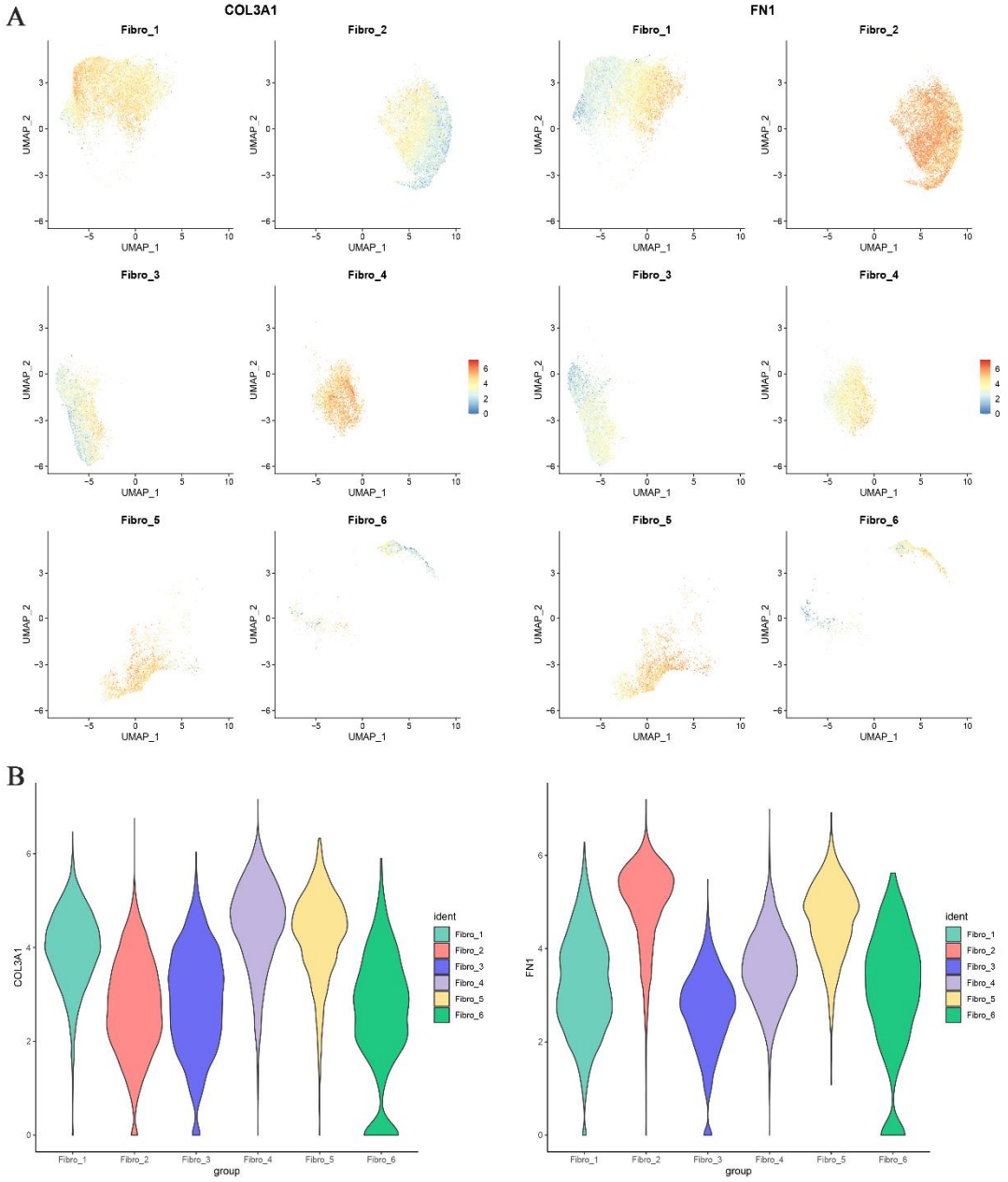

Fig. S2.

Expression of extracellular matrix-related genes in fibroblast subsets. (A) Feature plot showing the expression of extracellular matrix-related genes across fibroblast subsets. **(B)** Violin plots illustrating the expression of extracellular matrix-related genes in fibroblast subsets.

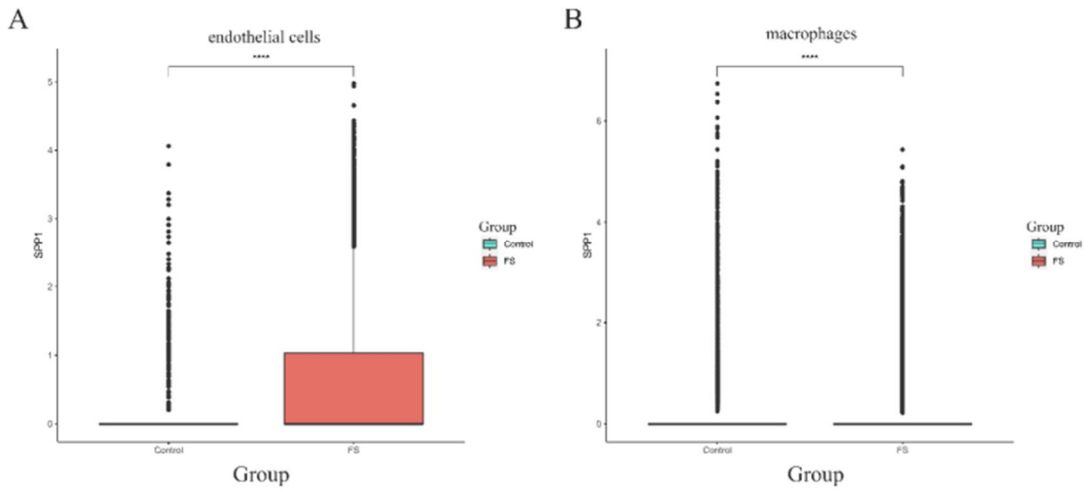

Fig. S3.

Expression of SPP1 in endothelial cells and macrophages in frozen shoulder and control tissues. (A) Box plot showing SPP1 expression in endothelial cells from FS and control tissues. Two-sided unpaired t-test, ****P < 0.0001. **(B)** Box plot showing SPP1 expression in macrophages from FS and control tissues. Two-sided unpaired t-test, ****P < 0.0001.

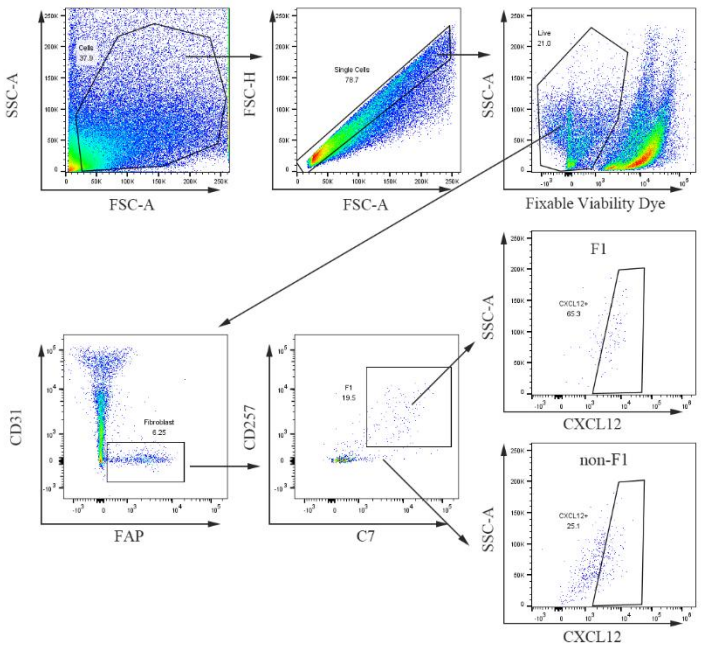

Fig. S4.

Gating strategy for detection of CXCL12 expression in fibroblasts.

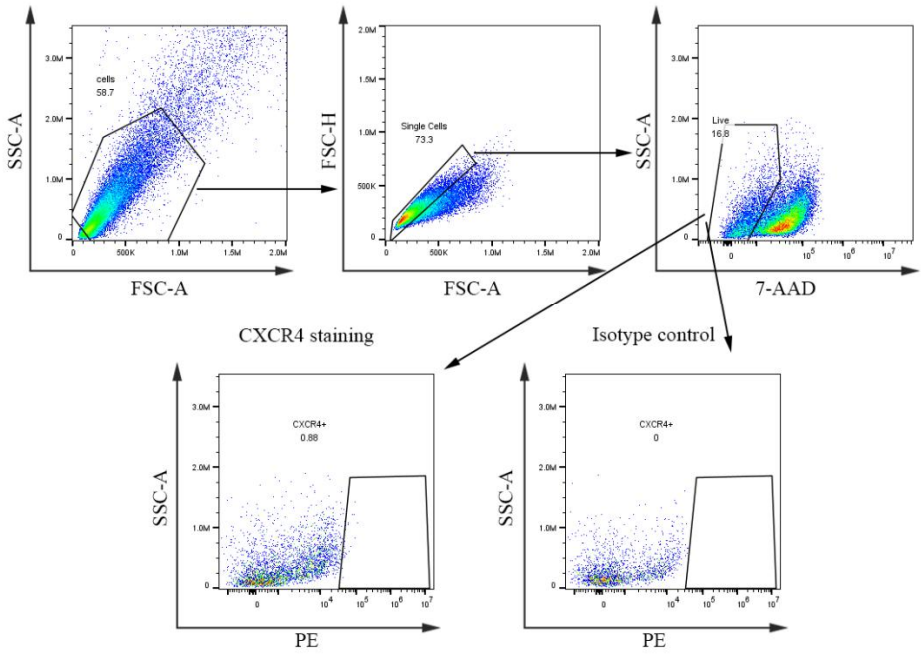

Fig. S5.

Gating strategy for detection of CXCR4 expression in fibroblasts.

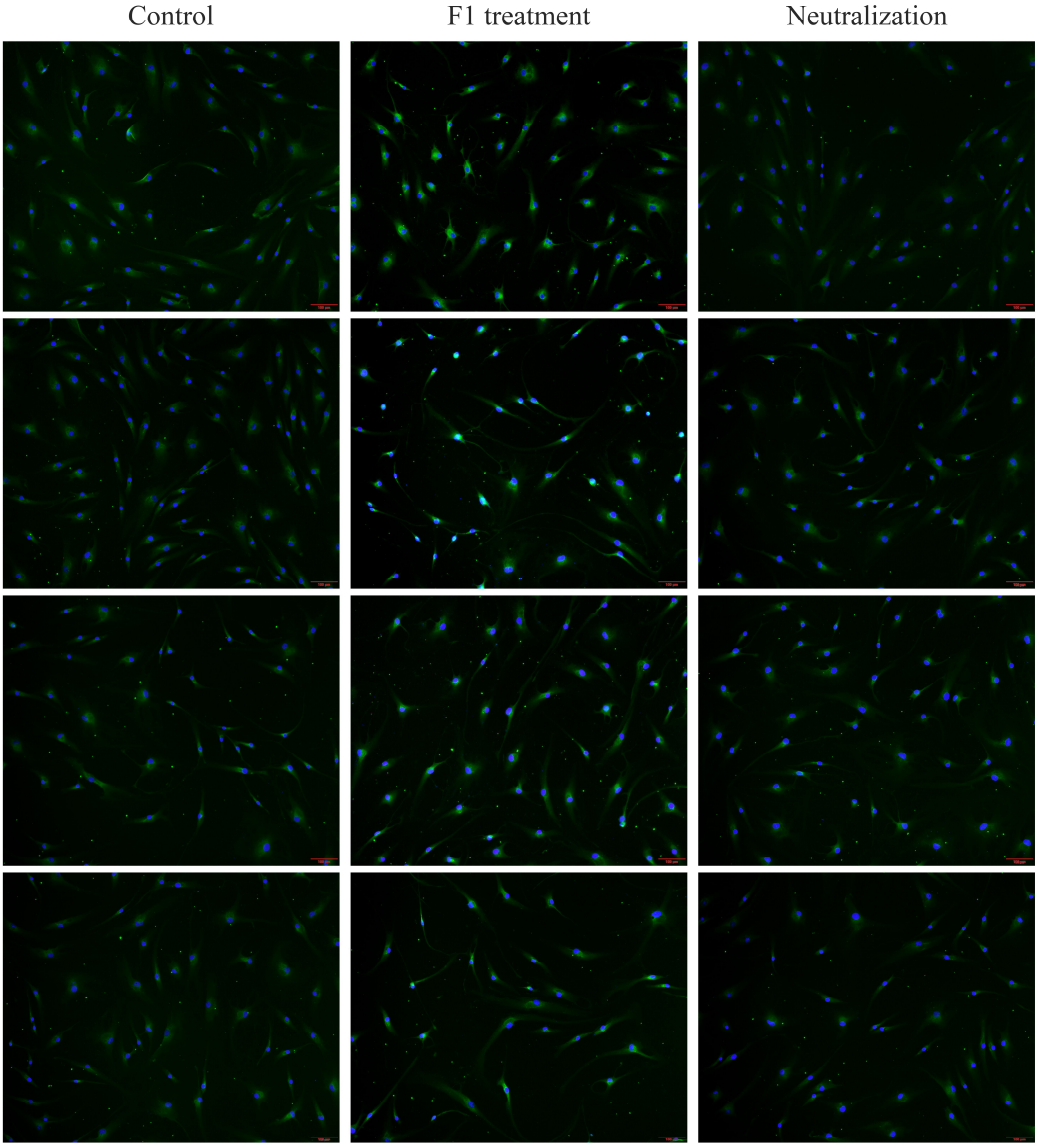

Fig. S6.

Expression of COL3A1 in fibroblasts treated with CXCL12 neutraligand (LIT-927). Immunofluorescence staining of COL3A1 (green) in non-F1 fibroblasts treated with normal culture medium, the supernatant of F1 fibroblasts, or the supernatant of F1 fibroblasts with 100 ng/ml LIT-927. Scale bar = 100 μm. Experiments were repeated four times with fibroblasts from four different donors.

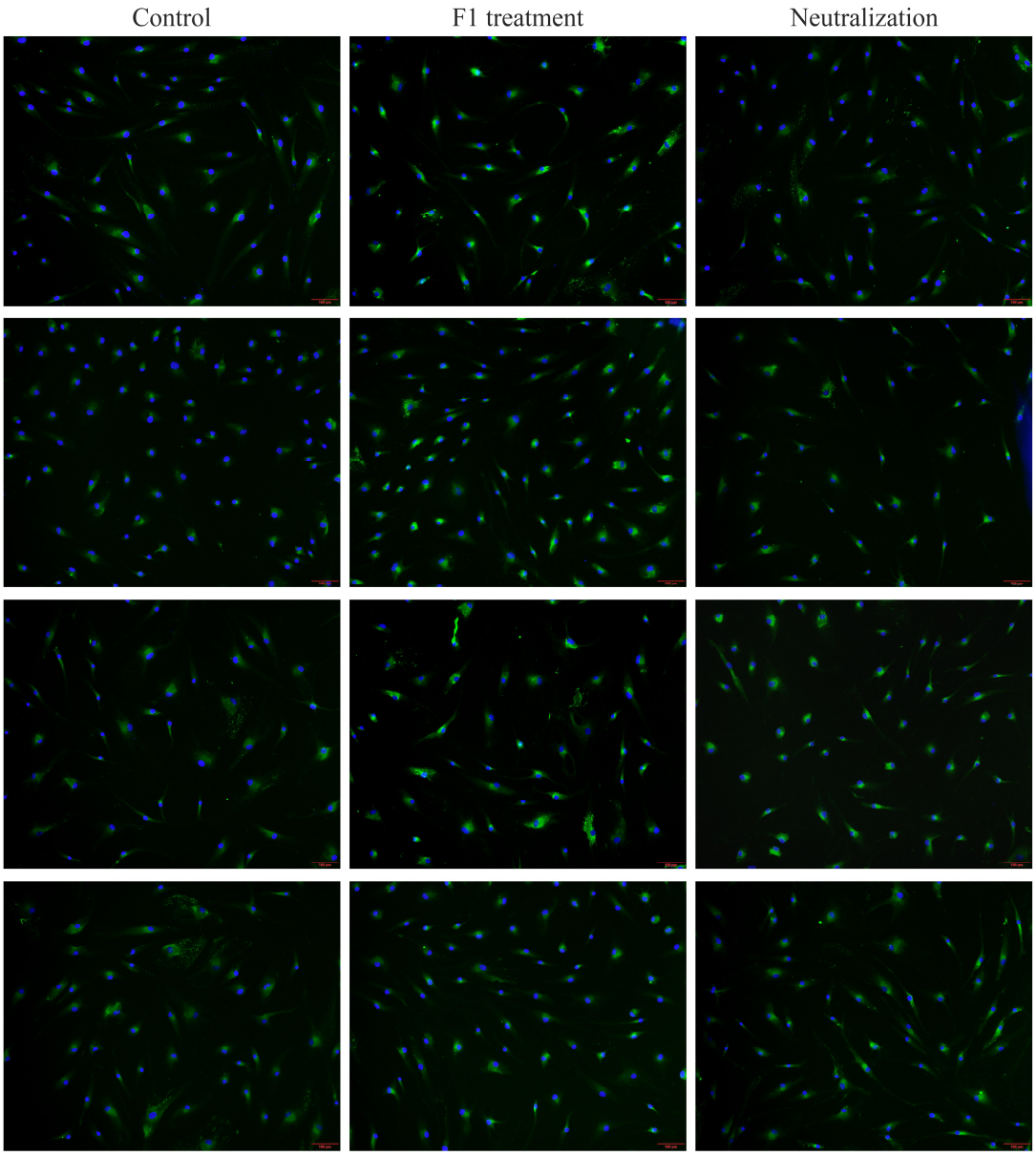

Fig. S7.

Expression of FN1 in fibroblasts treated with CXCL12 neutraligand (LIT-927). Immunofluorescence staining of FN1 (green) in non-F1 fibroblasts treated with normal culture medium, the supernatant of F1 fibroblasts, or the supernatant of F1 fibroblasts with 100 ng/ml LIT-927. Scale bar = 100 μm. Experiments were repeated four times with fibroblasts from four different donors.

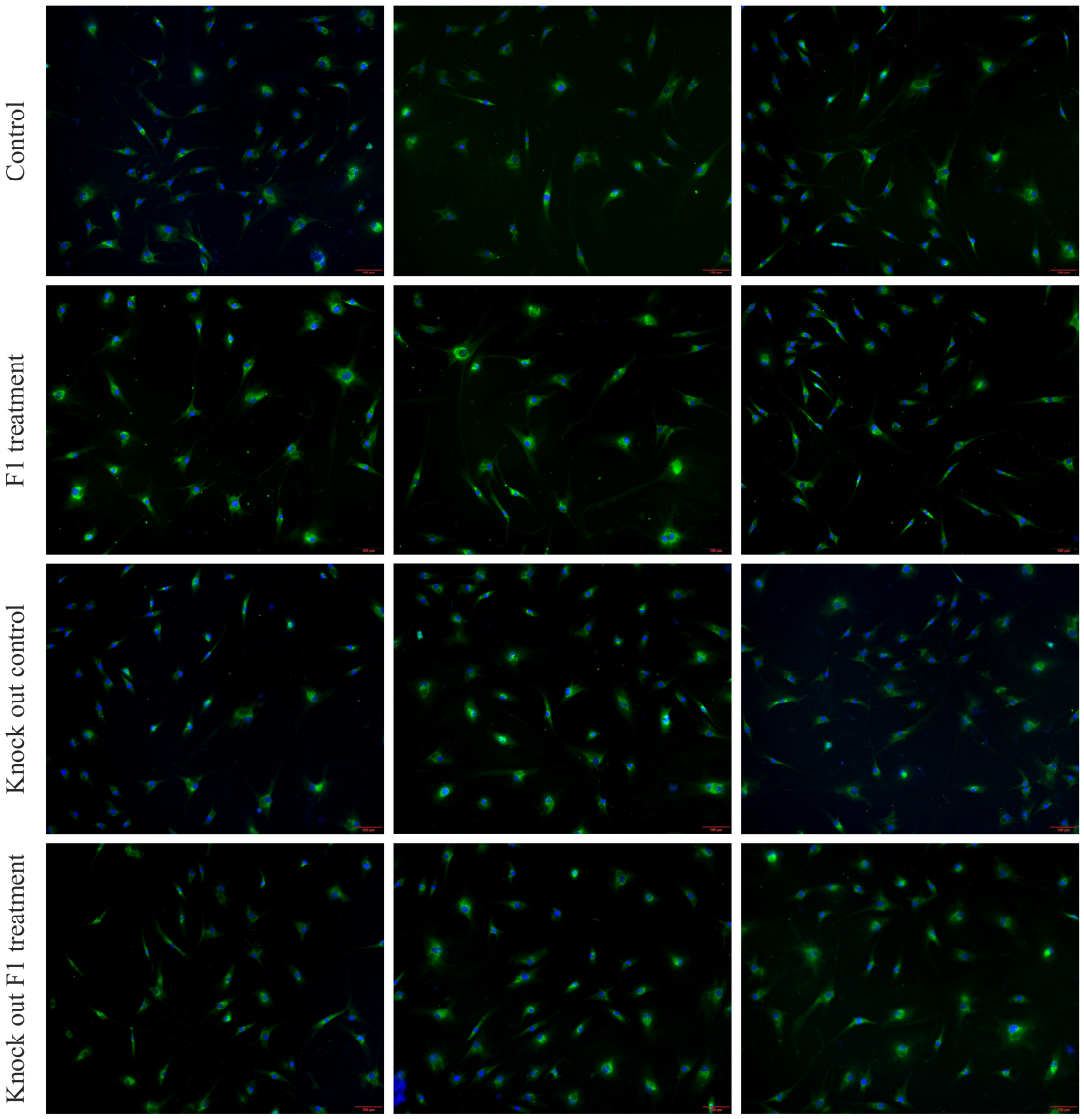

Fig. S8.

Expression of COL3A1 in ACKR3-knockout fibroblasts. Immunofluorescence staining of COL3A1 (green) in non-F1 fibroblasts and ACKR3-knockout non-F1 fibroblasts treated with normal culture medium or the supernatant of F1 fibroblasts. Scale bar = 100 μm. Experiments were repeated three times with fibroblasts from three different donors.

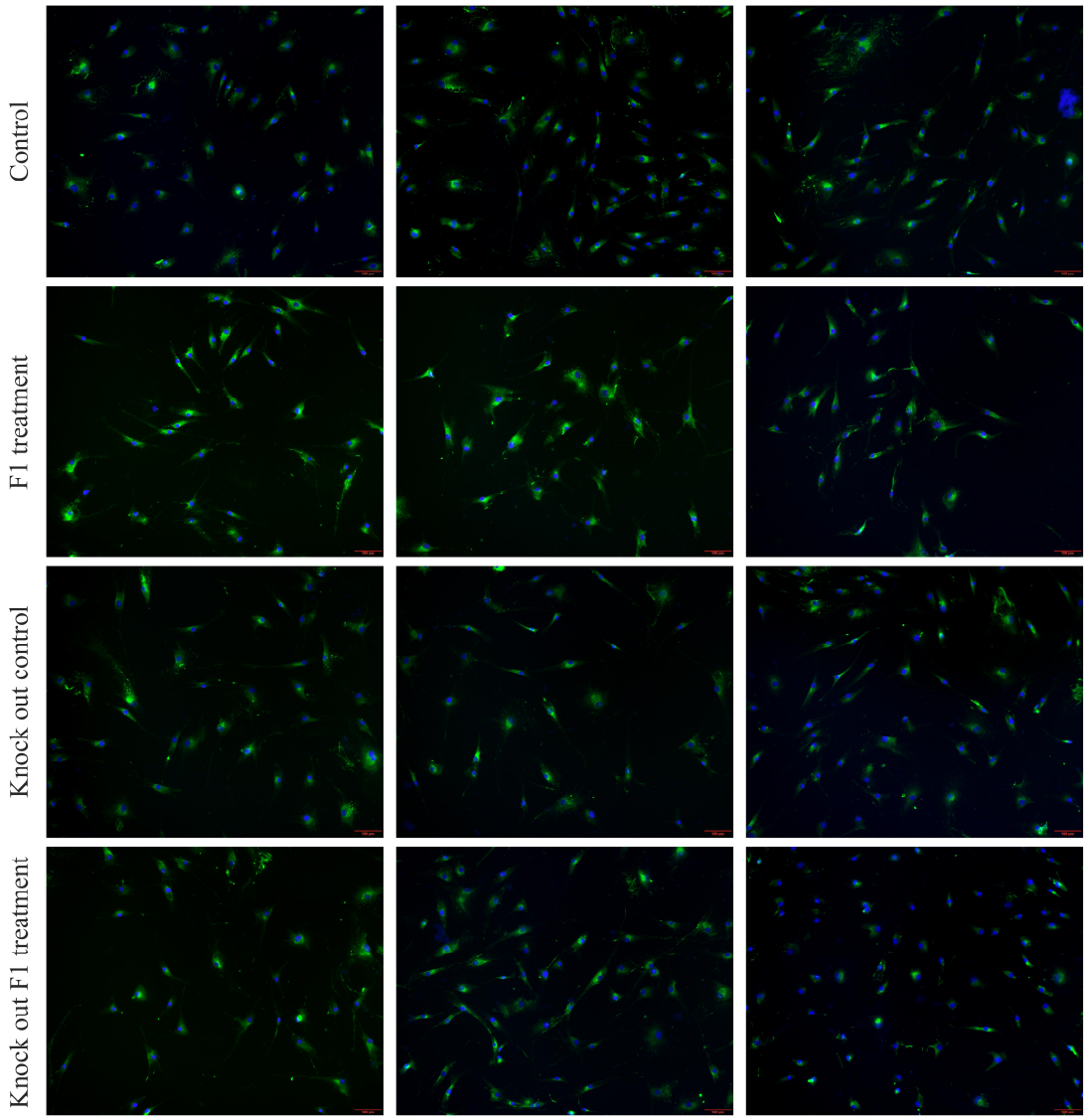

Fig. S9.

Expression of FN1 in ACKR3-knockout fibroblasts. Immunofluorescence staining of FN1 (green) in non-F1 fibroblasts and ACKR3-knockout non-F1 fibroblasts treated with normal culture medium or the supernatant of F1 fibroblasts. Scale bar = 100 μm. Experiments were repeated three times with fibroblasts from three different donors.

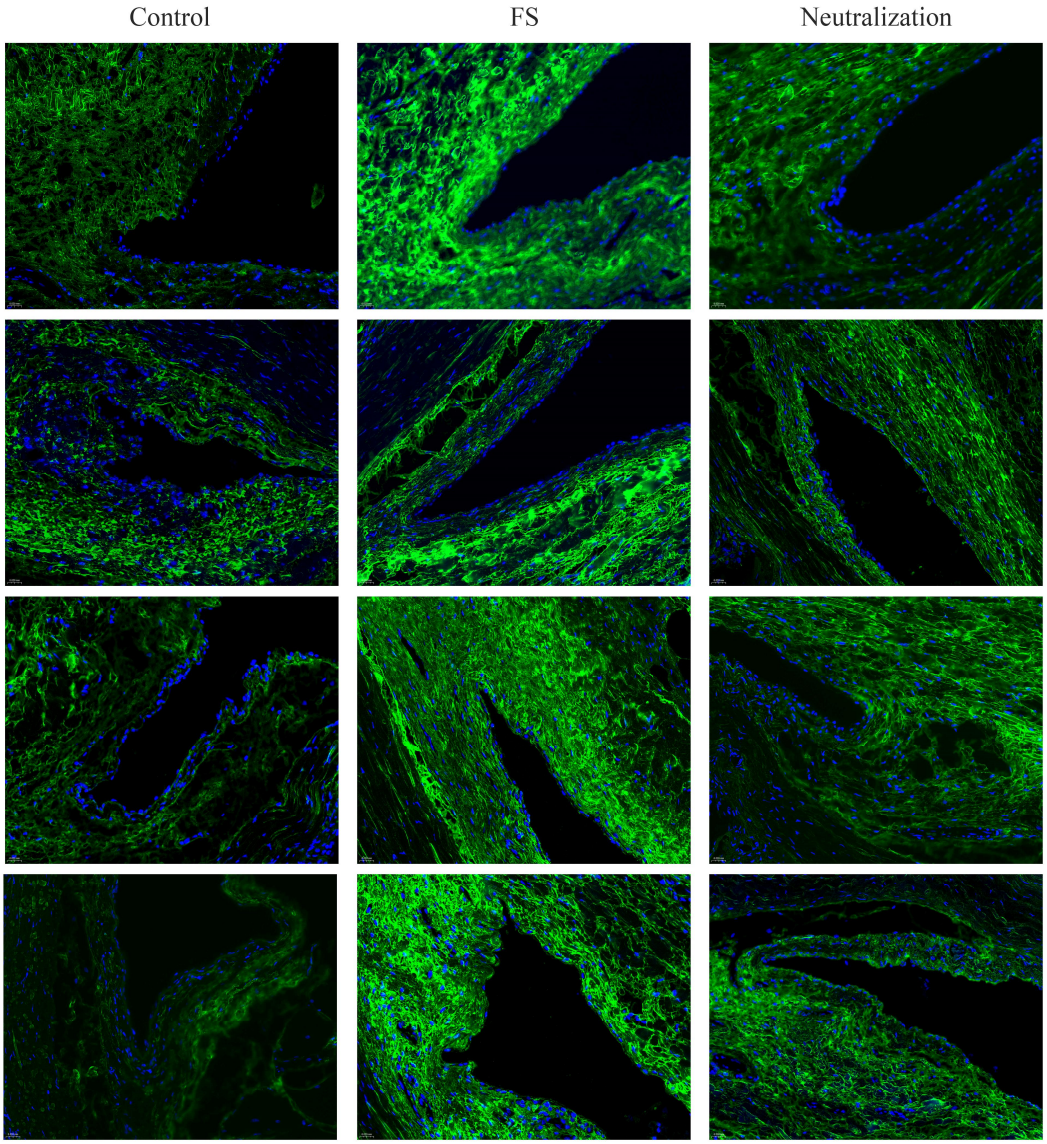

Fig. S10.

Expression of COL3A1 in rat model. Immunofluorescence staining of COL3A1 (green) in rat models groups: Control, FS, and Neutralization, scale bar = 20 μm. The experiments were repeated four times with four different rats in each group.

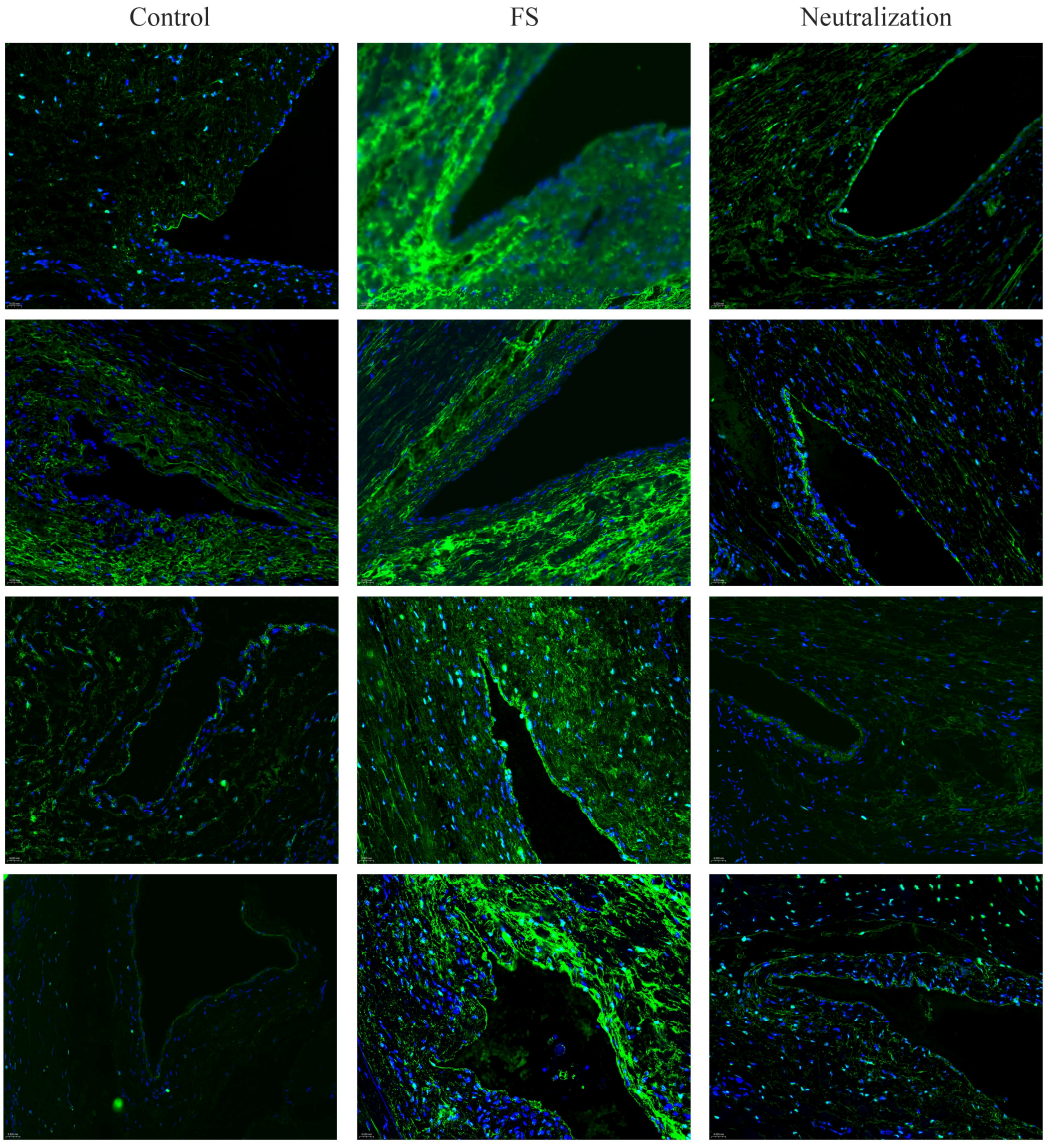

Fig. S11.

Expression of FN1 in rat model. Immunofluorescence staining of FN1 (green) in rat models groups: Control, FS, and Neutralization, scale bar = 20 μm. The experiments were repeated four times with four different rats in each group.

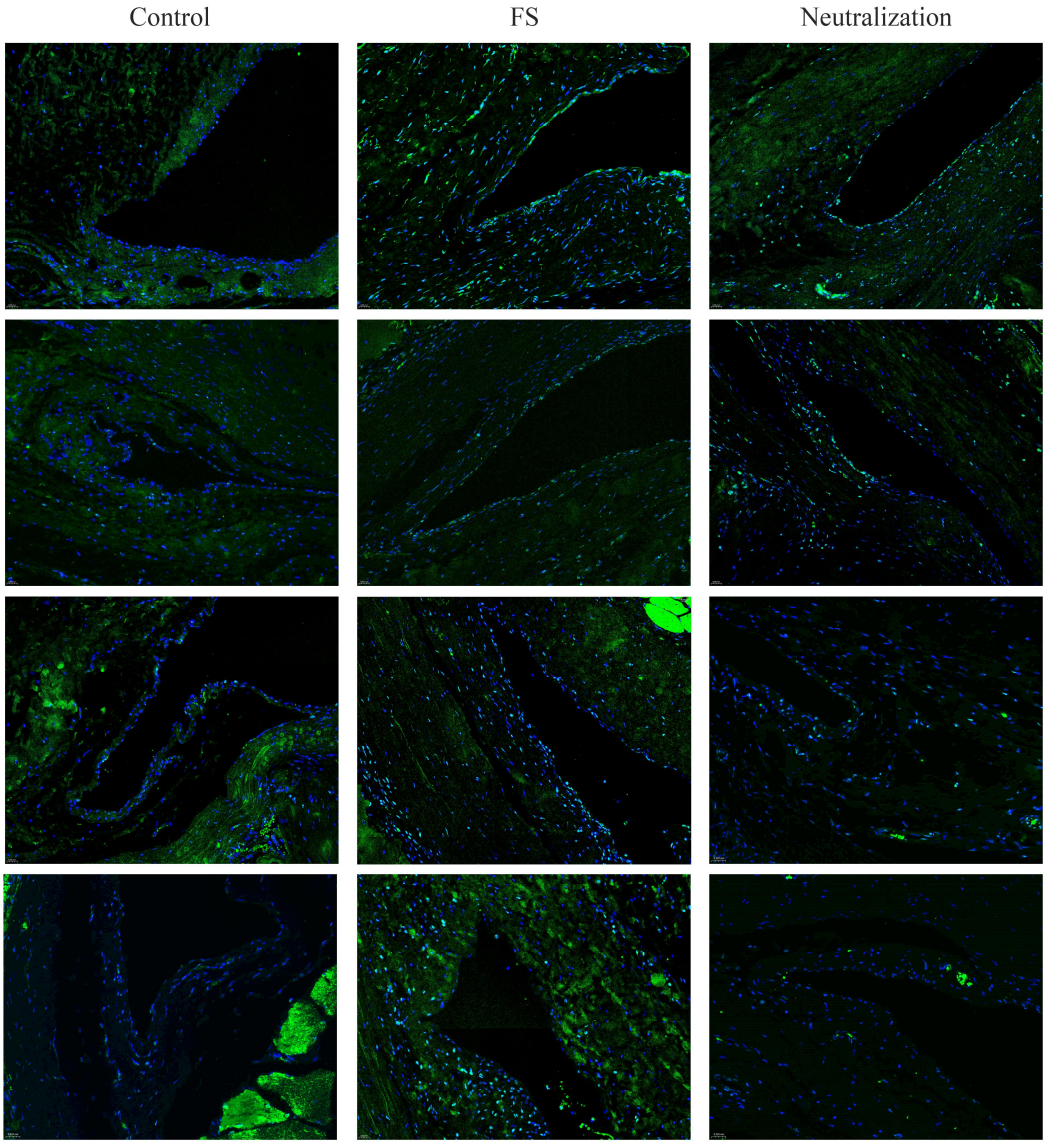

Fig. S12.

Expression of MMP1 in rat model. Immunofluorescence staining of MMP1 (green) in rat models groups: Control, FS, and Neutralization, scale bar = 20 μm. The experiments were repeated four times with four different rats in each group.

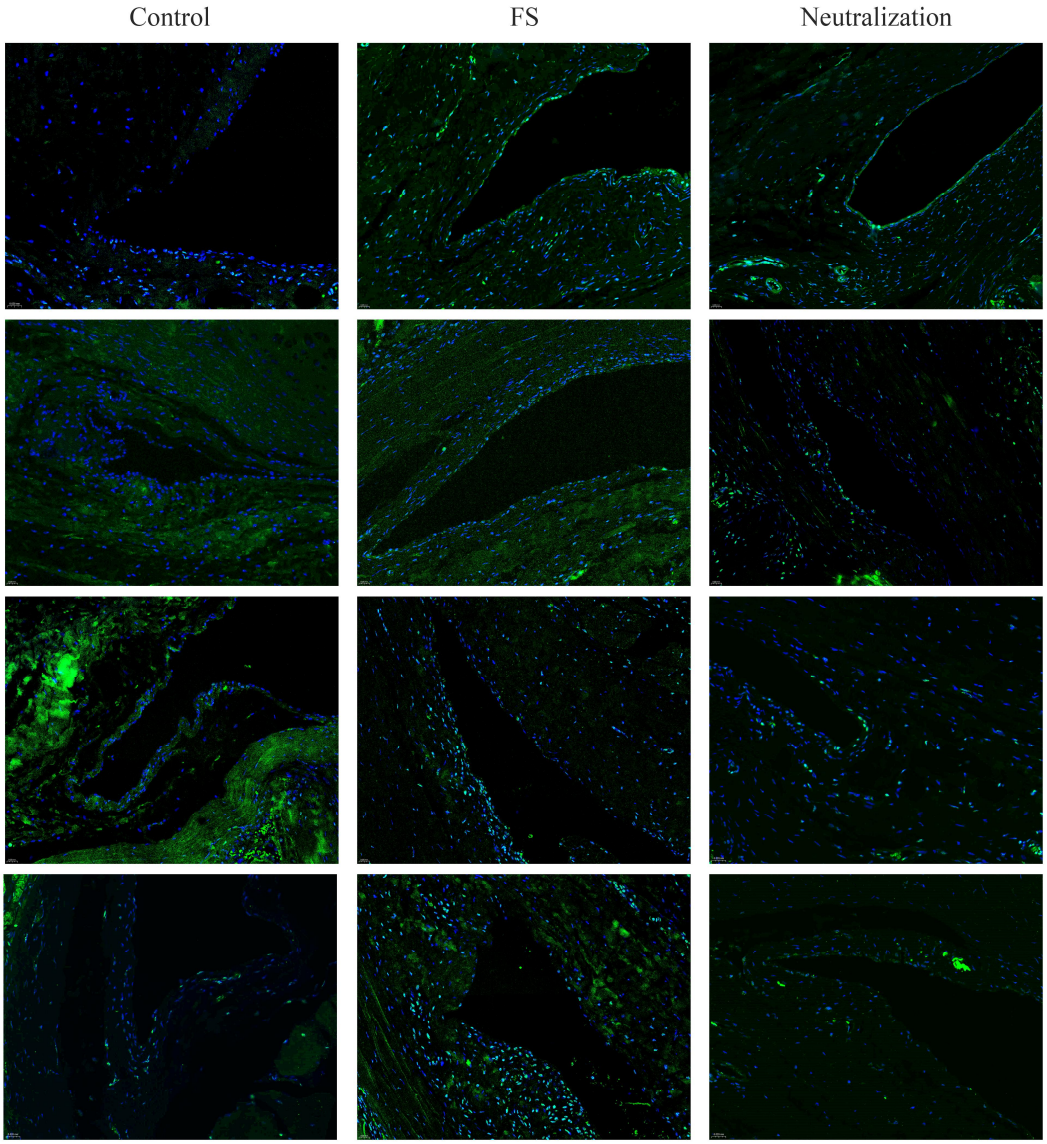

Fig. S13.

Expression of MMP3 in rat model. Immunofluorescence staining of MMP3 (green) in rat models groups: Control, FS, and Neutralization, scale bar = 20 μm. The experiments were repeated four times with four different rats in each group.

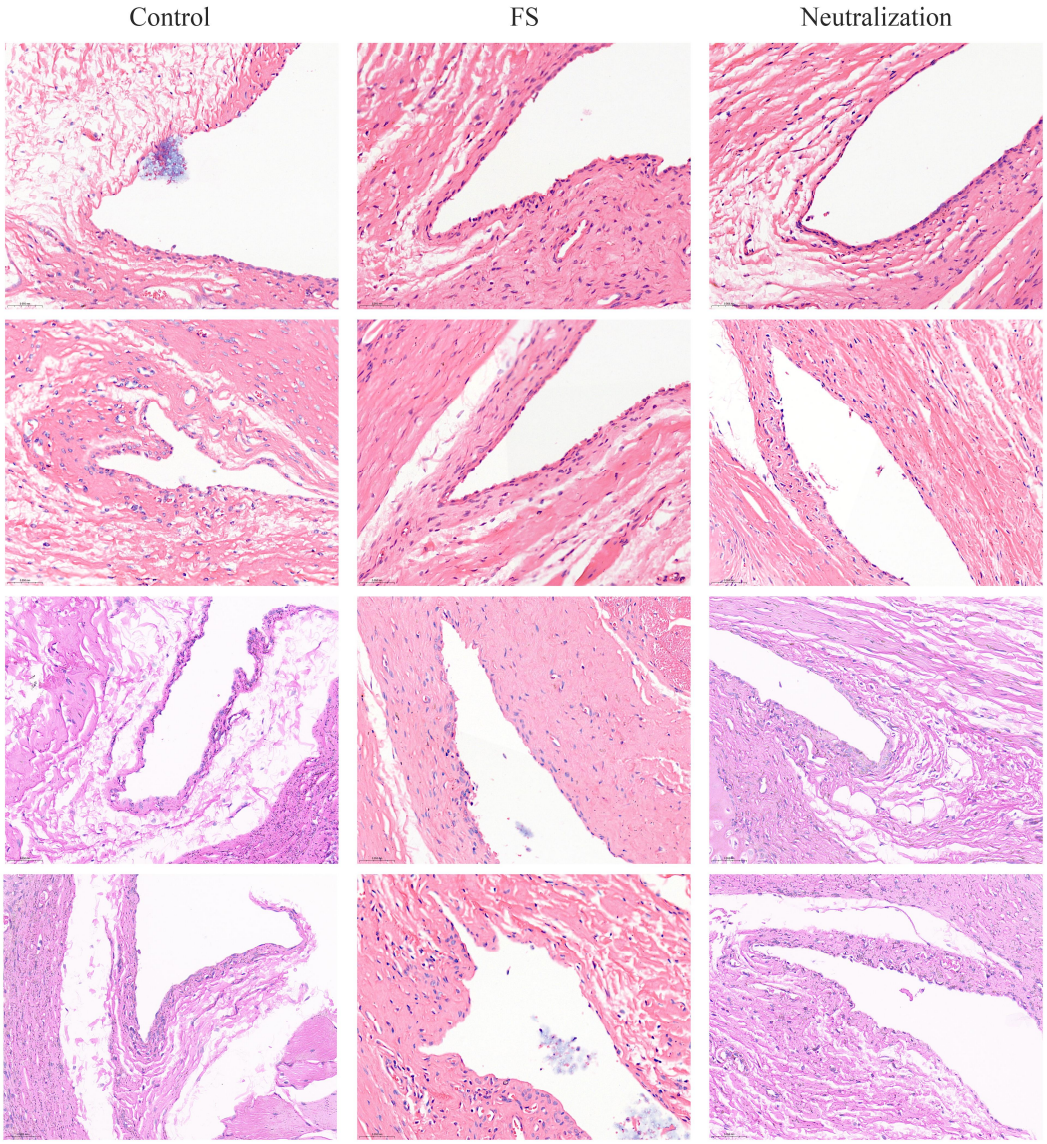

Fig. S14.

Haematoxylin & Eosin (H&E) staining of the rat shoulder capsule. H&E staining of the rat shoulder capsule in rat models groups: Control, FS, and Neutralization, scale bar = 50 μm. The experiments were repeated four times with four different rats in each group.

Table S1.

Information of FS and control donors whose biospecimens were used for scRNA-seq experiments.

Sample ID	Gender	Age	Sample ID	Gender	Age
FS.1	F	60	Control.1	M	60
FS.2	F	53	Control.2	F	59
FS.3	M	62	Control.3	F	51

Table S2.

Information of FS and control donors whose biospecimens were used for immunohistochemistry (IHC) and histological characterization (Hematoxylin & Eosin staining and Masson' s trichrome staining).

Sample ID	Gender	Age	Experiment	Sample ID	Gender	Age	Experiment
FS.1	F	73	IHC	Control.1	M	56	IHC
FS.2	F	69	IHC	Control.2	F	64	IHC
FS.3	M	64	IHC	Control.3	F	61	IHC
FS.4	F	57	IHC	Control.4	M	63	IHC
FS.5	F	58	histology	Control.5	F	48	histology

Table S3.

Information of FS donors whose biospecimens were used for CXCL12 stimulation and flow cytometry analysis of CXCR4.

Sample ID	Gender	Age
FS.1	M	48
FS.2	F	46
FS.3	F	61
FS.4	M	61

Table S4.

Information of FS and control donors whose biospecimens were used for WB of ACKR3.

Sample ID	Gender	Age	Sample ID	Gender	Age
FS.1	F	61	Control.1	M	65
FS.2	M	56	Control.2	F	62
FS.3	F	68	Control.3	F	63

Table S5.

Information of FS and control donors whose biospecimens were used for flow cytometry analysis.

Sample ID	Gender	Age	Sample ID	Gender	Age
FS.1	F	59	Control.1	M	50
FS.2	F	51	Control.2	F	61
FS.3	M	61	Control.3	F	59
FS.4	F	59			

Table S6.

Information of FS and control donors whose biospecimens were used for Flow Cytometry Sorting.

Sample ID	Gender	Age	Experiment	Sample ID	Gender	Age	Experiment
FS.1	F	52	CXCL12 Neutralization and Bulk RNA-Seq	Control.1	F	56	-
FS.2	F	63	CXCL12 Neutralization and Bulk RNA-Seq	Control.2	F	62	-
FS.3	M	48	CXCL12 Neutralization and Bulk RNA-Seq	Control.3	M	49	-
FS.4	M	50	CXCL12 Neutralization and ACKR3 Knockout	Control.4	M	61	-
FS.5	F	46	ACKR3 Knockout	Control.5	M	60	-
FS.6	F	65	ACKR3 Knockout	Control.6	M	39	-