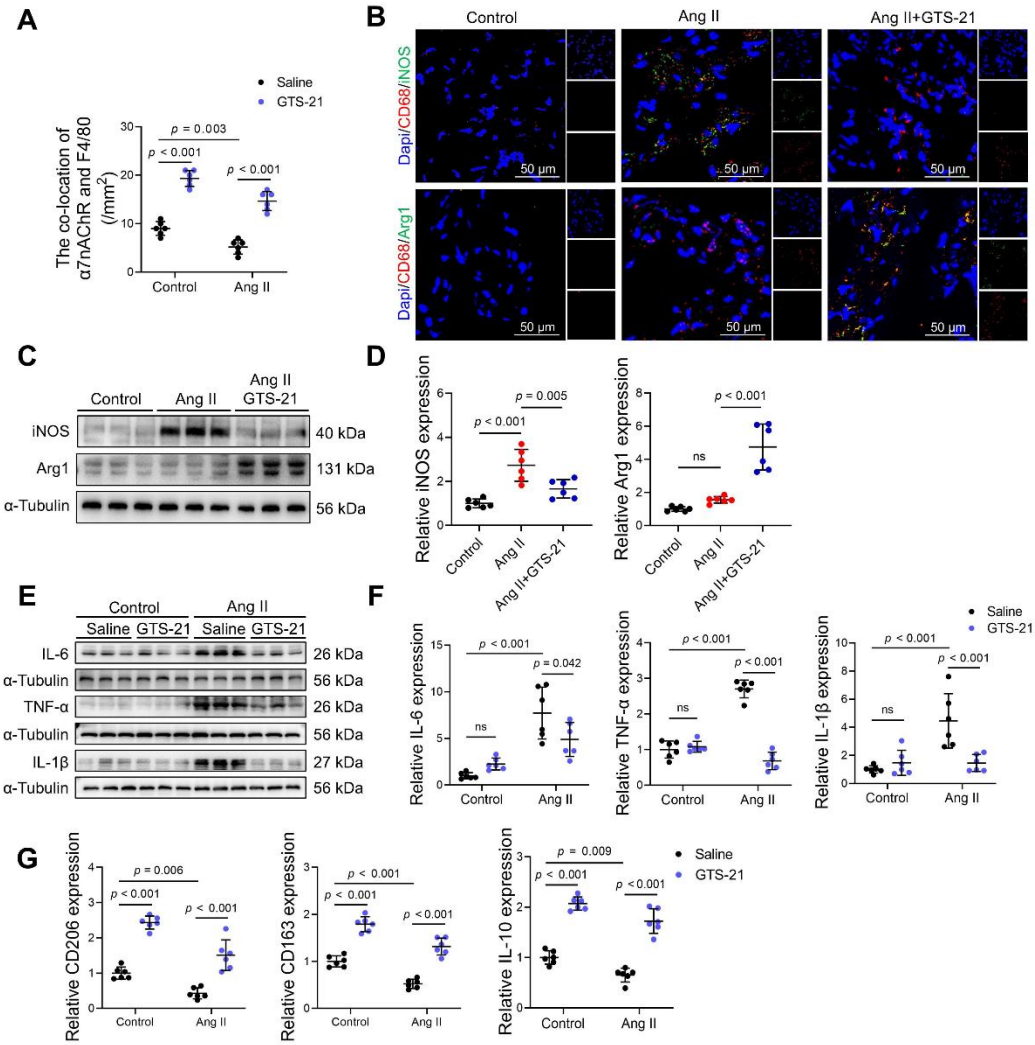
Supplementary Material

Supplementary Figure

Figure S1

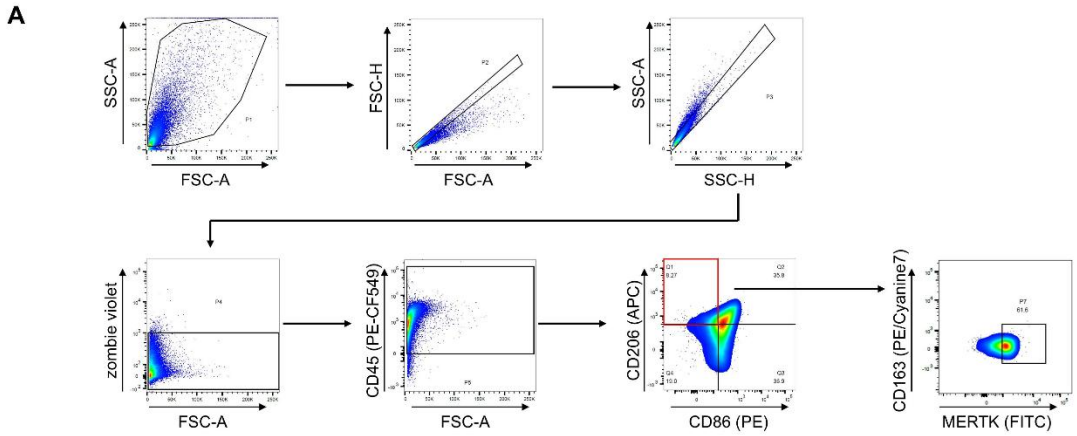


Effect of α7nAChR upregulation on Ang II-Induced macrophage polarization *in vivo*

(A) Quantitative analysis of the co-localization amount of F4/80 (red) and α7nAChR in Figure 2C (n = 6). (B) Representative immunofluorescence detection of CD68 (red) and iNOS or Arg1 (green) in WT, Ang II-stimulated and Ang II + GTS-21-stimulated mice (bar = 50 μm). (C-D) Representative immunoblots and quantification of iNOS and Arg1 protein expression in WT, Ang II-stimulated and Ang II + GTS-21 co-stimulated mice (n = 6). (E-F) Representative immunoblots and densitometric quantification of IL-6, TNF-α and IL-1β protein expression in mice treated by GTS-21 or Saline with/without Ang II infusion (n = 6).

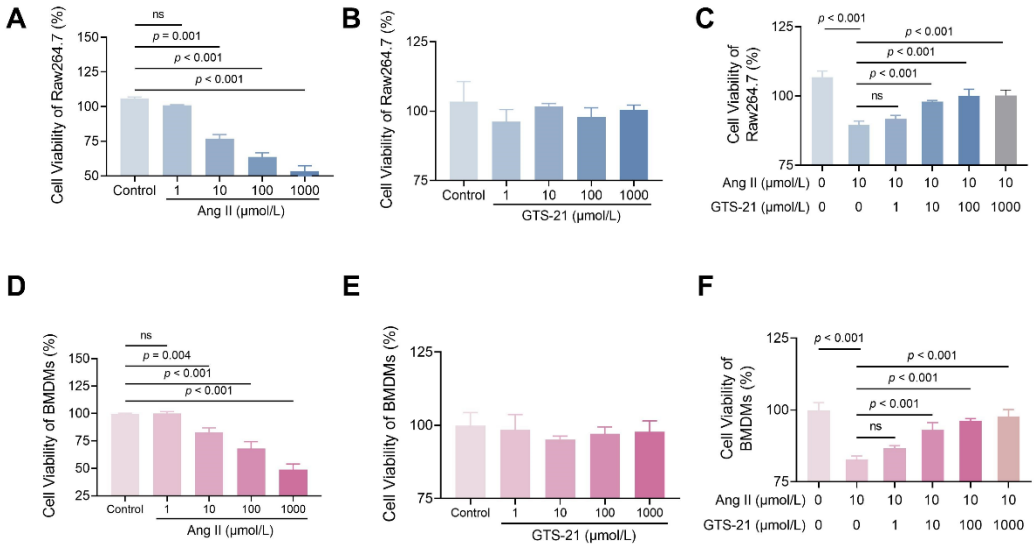
The IL-1β loading control reused that in Figure 3F (COL3A1 and α-SMA); both are from the same blot. Two-way (A,F) or one-way (D) ANOVA with Tukey's post hoc test was applied; error bars represent S.D. Significance: $p < 0.05$; ns, not significant.

Figure S2



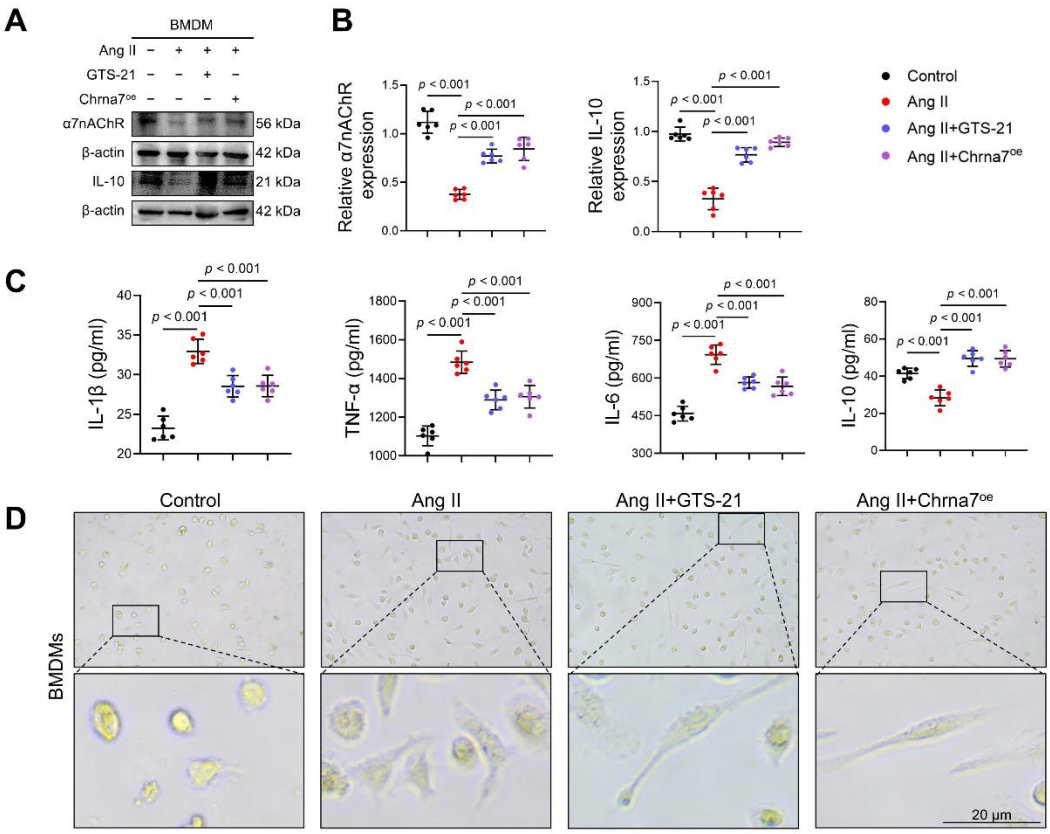
The effect of α7nAChR on atrial macrophages polarization (A) Flow cytometric gating strategy for detecting atrial macrophages polarization.

Figure S3



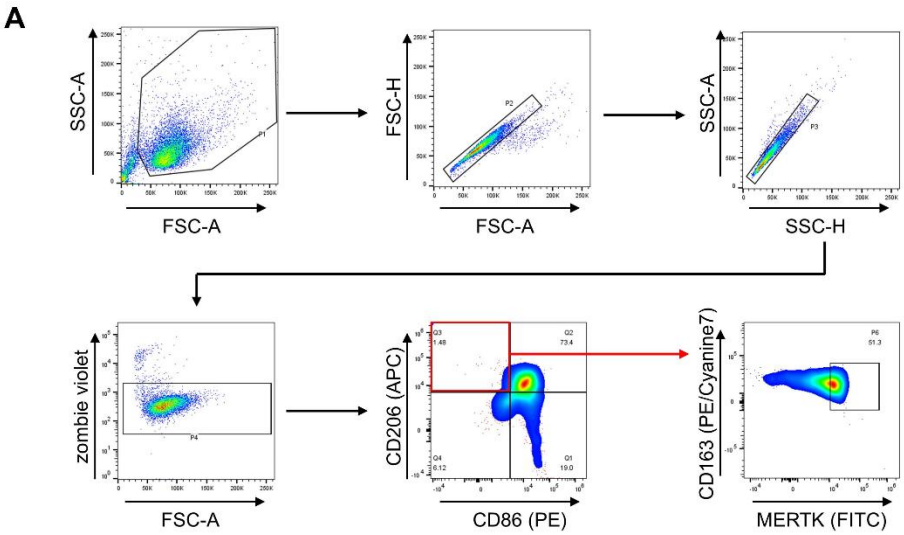
Cell viability of Raw264.7 macrophages and BMDMs under GTS-21 and/or Ang II treatment (A) Cell viability (CCK-8 assay) of Raw264.7 macrophages cultured with/without Ang II (0-1000 μmol/L) for 24h. (B) CCK-8 assay of Raw264.7 macrophages treated with/without GTS-21 (0-1000 μmol/L) for 24h. (C) CCK-8 assay of Raw264.7 macrophages treated with/without Ang II (10 μmol/L) plus GTS-21 (0-1000 μmol/L) for 24h. (D) Cell viability of BMDMs treated with/without Ang II (0-1000 μmol/L) for 24h. (E) CCK-8 assay of BMDMs treated with/without GTS-21 (0-1000 μmol/L) for 24h. (F) CCK-8 assay of BMDMs treated with/without Ang II (10 μmol/L) plus GTS-21 (0-1000 μmol/L) for 24h. One-way ANOVA with Tukey's test; error bars, S.D.; $p < 0.05$; ns, not significant.

Figure S4



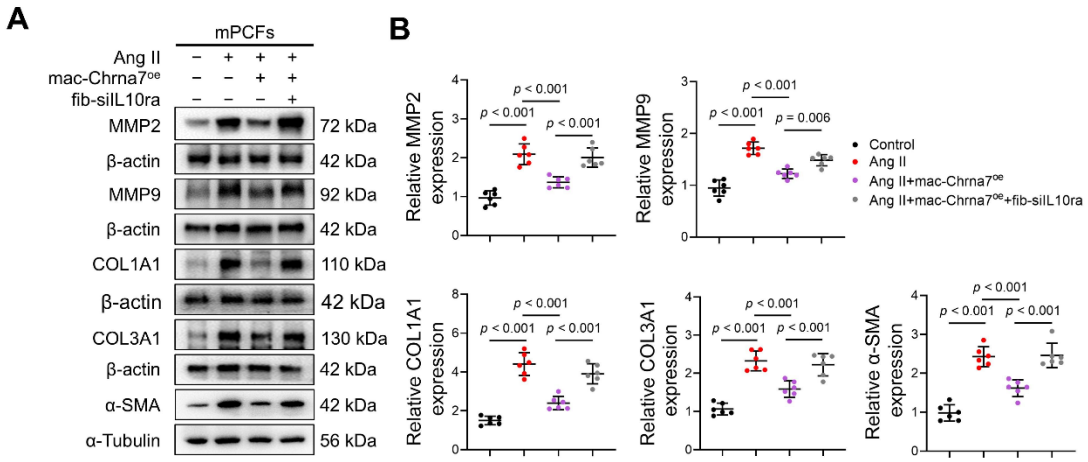
The effect of α7nAChR of BMDMs on cytokine secretion and macrophage polarization *in vitro* (A-B) Immunoblotting and quantification of α7nAChR and IL-10 protein levels in BMDMs treated with/without Ang II, GTS-21, or Chrna7 overexpression (Chrna7^{oe}) (n = 6). (C) Measurement of IL-1β, TNF-α, IL-6 and IL-10 levels in conditioned medium of BMDMs stimulated with/without Ang II, GTS-21, or Chrna7^{oe} (n = 6). (D) Morphology of BMDM cells (bar = 50 μm). One-way ANOVA with Tukey's test; error bars, S.D.; $p < 0.05$; ns, not significant.

Figure S5



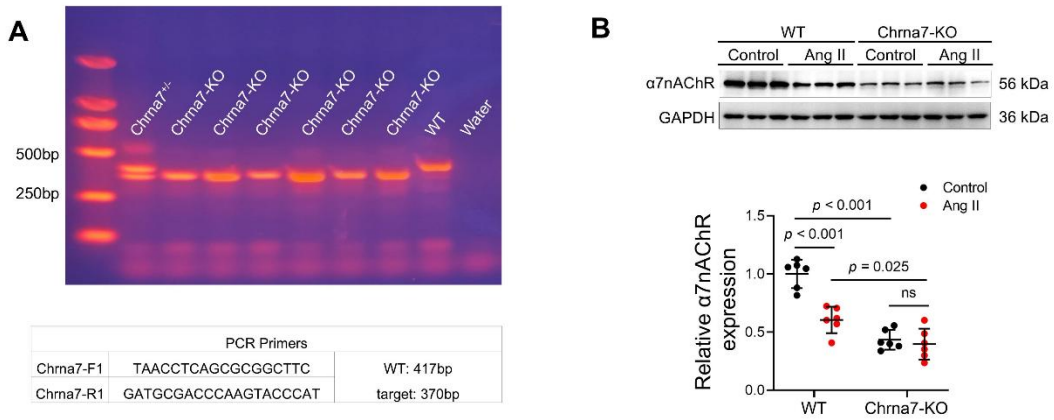
The effect of α7nAChR on Raw264.7 macrophage polarization *in vitro* (A) Gating strategy for the flow cytometric detection of Raw264.7 cell polarization in vitro.

Figure S6



The protective effect of BMDMs α7nAChR on mPCFs against fibrotic markers mediated by IL-10 *in vitro* (A-B) Representative immunoblots and quantification of MMP2, MMP9, COL1A1, COL3A1 and α-SMA in siIL10ra or NC infected mPCFs in the Transwell bottom chamber of the co-culture system with Chrna7^{oe} and Ang II treated BMDMs. Any reused loading control images were derived from the same blot. The α-SMA loading control reused that in Figure S9H (ANKRD1); both are from the same blot. One-way ANOVA with Tukey's test; error bars, S.D.; $p < 0.05$; ns, not significant.

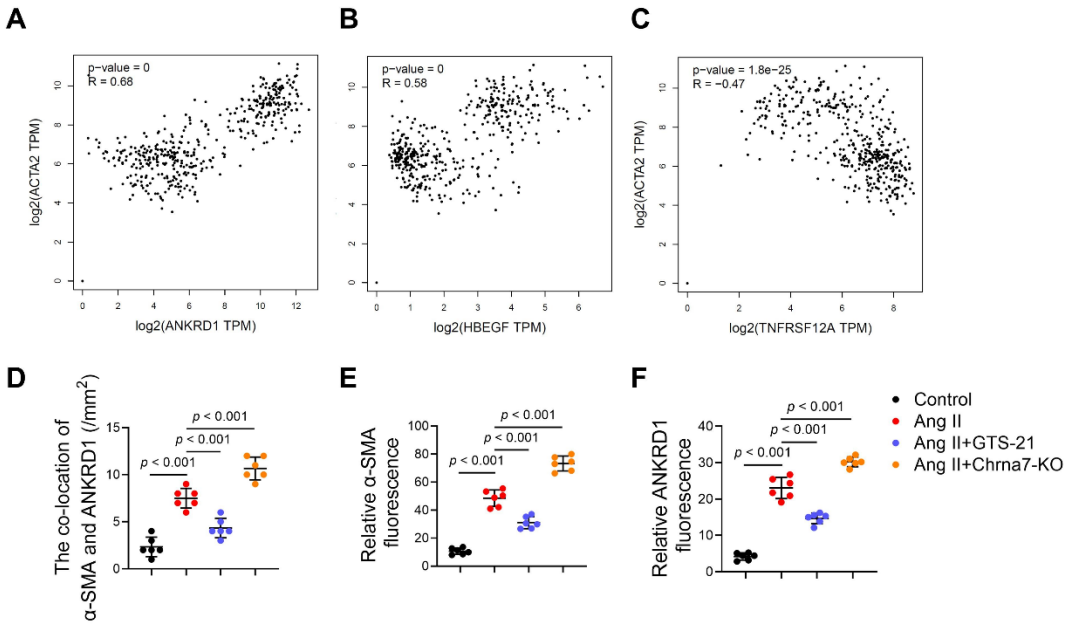
Figure S7



PCR Primers		
Chrna7-F1	TAACCTCAGCGCGGCTTC	WT: 417bp
Chrna7-R1	GATGCGACCCAAGTACCCAT	target: 370bp

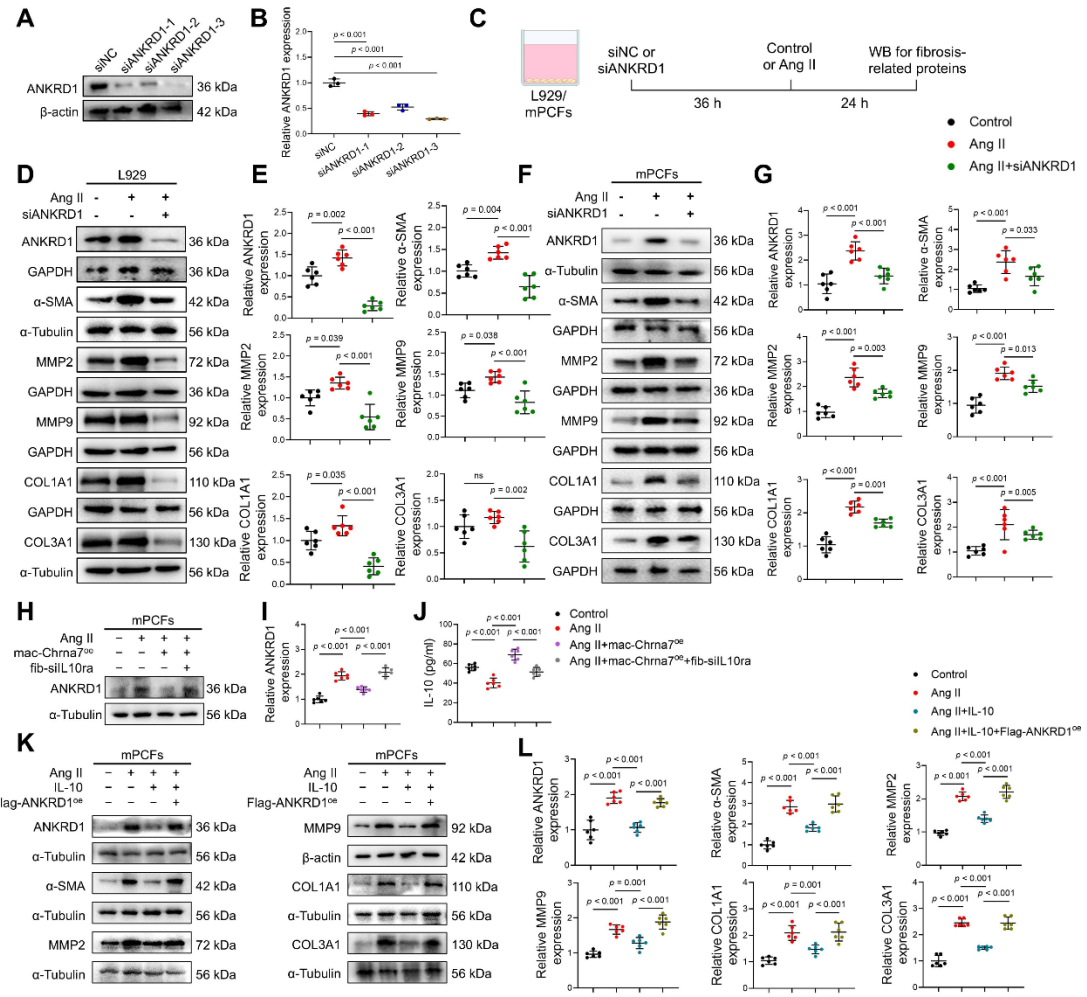
Chrna7-KO mice were predisposed to Ang II-induced AF (A) Representative image of WT, Chrna7$^{+/-}$ and Chrna7-KO mice genotype identification and primers. (B) Immunoblot analysis and quantification of α7nAChR protein expression in WT or Chrna7-KO mice with/without Ang II infusion (n = 6). One-way ANOVA with Tukey's test; error bars, S.D.; $p < 0.05$; ns, not significant.

Figure S8



The correlations between *Acta2* and shared DEGs in GTEx database, and location of ANKRD1 in fibroblasts (A-C) Correlation analysis of *Ankrd1*, *Tnfrsf12a*, and *Hbegf* with *Acta2* using atrial appendage and fibroblast data from the GTEx database. (D-F) Quantification of α-SMA and ANKRD1 expression levels and their co-localization in atria of WT, Ang II-stimulated, Ang II + GTS-21-stimulated and Ang II + Chrna7-KO mice (n = 6). One-way ANOVA with Tukey's test; error bars, S.D.; $p < 0.05$; ns, not significant.

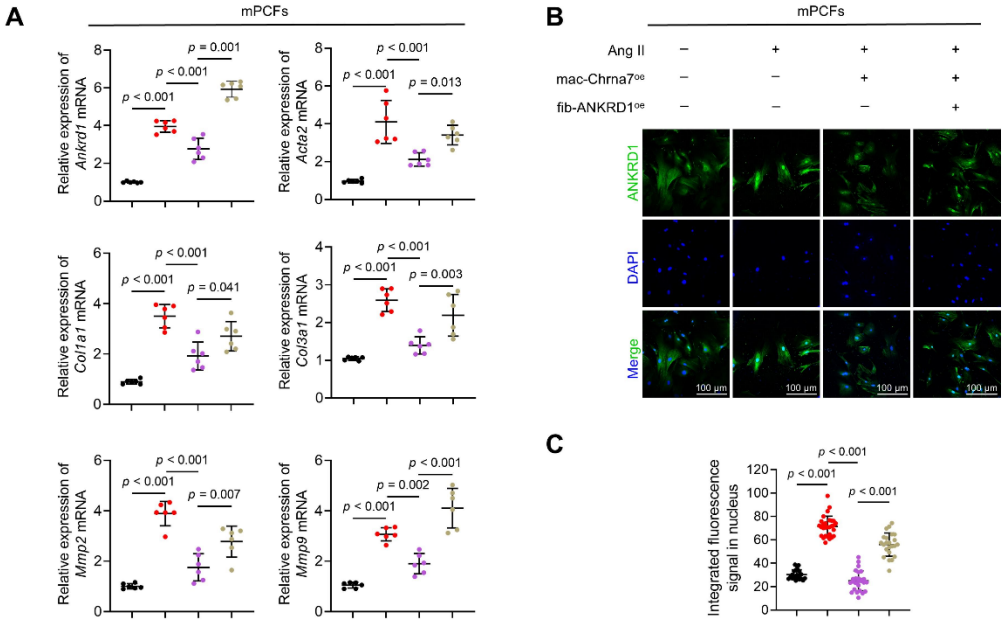
Figure S9



The effects of ANKRD1 on fibrosis mediated by mac-α7nAChR and IL-10 (A-B) Immunoblotting and quantification of ANKRD1 in three siANKRD1 types (n = 3). (C) Schematic diagram illustrating the treatment of siANKRD1 and Ang II in L929 cells or mPCFs. (D-E) Immunoblotting and quantification of COL1A1, COL3A1, α-SMA, MMP2 and MMP9 in L929 fibroblasts incubated with Ang II and/or ANKRD1 knockout (n = 6). (F-G) Immunoblotting and quantification of COL1A1, COL3A1, α-SMA, MMP2 and MMP9 in mPCFs treated with Ang II and/or ANKRD1 knockout (n = 6). (H-I) Immunoblotting and quantification of ANKRD1 in siIL10ra or NC infected mPCFs in the Transwell bottom chamber of the co-culture system with BMDMs Chrna7^{oe} and Ang II treatment (n = 6). The ANKRD1 loading control reused that in Figure S6A (α-SMA); both are from the same blot. (J) Quantification of IL-10 in culture medium (n = 6). (K-L) Immunoblotting and

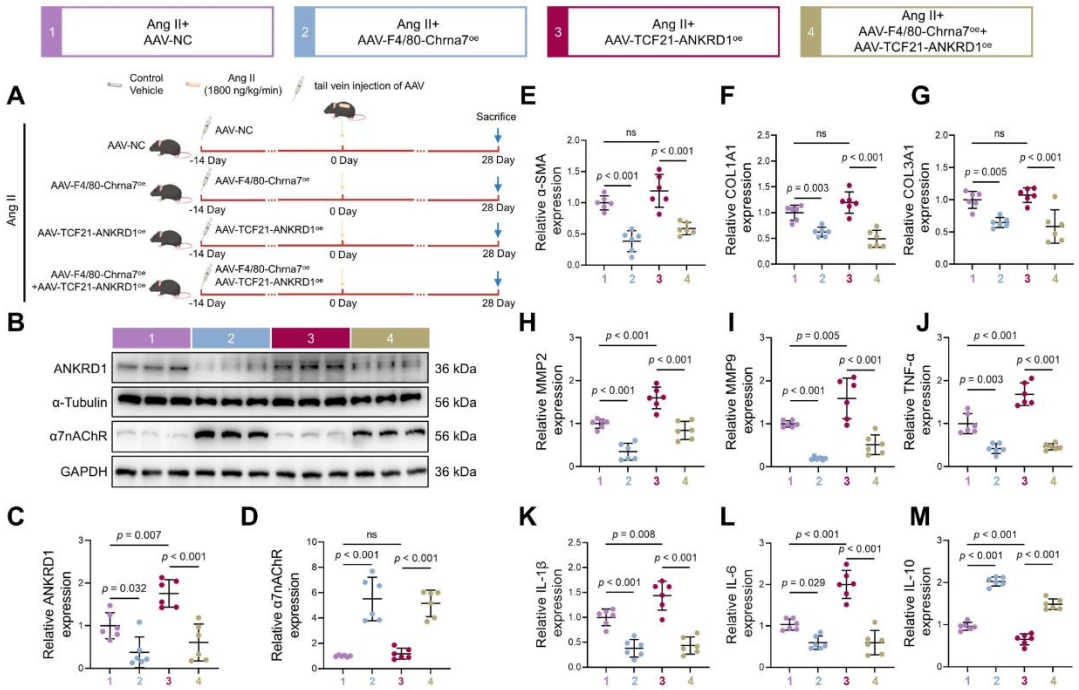
quantification of ANKRD1, α-SMA, MMP2, MMP9, COL1A1 and COL3A1 in mPCFs treated with Ang II and ANKRD1 overexpression with/without IL-10 protein (n = 6). Statistics: one-way ANOVA with Tukey's post hoc test; error bars, S.D.; $p < 0.05$; ns, not significant.

Figure S10



Macrophage α7nAChR attenuates fibrotic signaling by suppressing ANKRD1 nuclear translocation in mPCFs (A) Quantitative analysis of *Ankrd1*, *Acta2*, *Col1a1*, *Col3a1*, *Mmp2* and *Mmp9* mRNA levels of mPCFs in the Transwell bottom chamber of the co-culture system with/without BMDMs Chrna7^{oe}, fibroblast Flag-ANKRD1^{oe} or Ang II treatment (n = 6). (B) Representative immunofluorescence detection and quantification of ANKRD1 (green) and DAPI (blue) in mPCFs in the Transwell bottom chamber of the co-culture system (n = 23-29) (bar = 100 μm). (C) Quantification of integrated fluorescence signal in mPCFs nuclei. Statistics: one-way ANOVA with Tukey's post hoc test; error bars, S.D.; $p < 0.05$ was regarded as significant; ns, not significant.

Figure S11



The quantitative analysis of fibrotic markers and cytokines in AAV-F4/80-Chrna7^{oe} and/or AAV-TCF21-ANKRD1^{oe} injected mice treated with Ang II (A) Schematic depicting the experimental procedure. (B-D) Representative immunoblots and quantification of ANKRD1 and α7nAChR (n = 6). The ANKRD1 loading control reused that in Figure 7N (TNF-α); both are from the same blot. (E-I) Quantification of fibrotic markers in Figure 7M (n = 6). (J-M) Quantification of cytokines in Figure 7N (n = 6). Statistics: one-way ANOVA with Tukey's post hoc test; error bars, S.D.; $p < 0.05$ was regarded as significant; ns, not significant.

Supplementary Table

Table. S1 Mouse Primers for RT-PCR

Gene	Sense primers	Antisense primers
Ankrd1	GTGGATGTGCCGAGGTTTCT	GTGGAGTGCCGTCCGTTTAT
Acta2	GTACCACCATGTACCCAGGC	GAAGGTAGACAGCGAAGCCA
Mmp2	ACCTACACCAAGAACTTCCGATT	CAAAGACAATGTCCTGTTTGCAGA
Mmp9	GCTGGCAGAGGCATACTTGTAC	CAGTATGTGATGTTATGATGGTCCC
Col1a1	CTCCCAGAACATCACCTATCACT	GGAGGTCTTGGTGGTTTTGTATT
Col3a1	GTGGCAATGTAAAGAAGTCTCTGAAG	GGGTGCGATATCTATGATGGGTAG
Gapdh	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT