Supplementary information

An AAV-based NF-κB-targeting Gene Therapy (rAAV-DMP-miR533) to Inflammation Diseases

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Supplementary Tables

Table S1 Target sequence of miRNA.

Name	miRNA target (5′-3′)
Human RELA	CAAAGATGGGATGAGAAAGGA
Mouse RELA	TACTCTTGAAGGTCTCATAGG
NT	GTCTCCACGCGCAGTACATTT

Table S2 Oligonucleotides sequences used to constructed miRNA expression vector.

Name	Sequence (5′-3′)
Human miRELA-F	TGCTGCAAAGATGGGATGAGAAAGGAGTTTTGGCCACTGACTGACTCCTTTCTTCCCATCTTTG
Human miRELA-R	CGTTTCTACCCTACTCTTTCCTCAAAACCGGTGACTGACTGAGGAAAGAAGGGTAGAAACGTCC
Mouse miRELA-F	TGCTGTACTCTTGAAGGTCTCATAGGGTTTTGGCCACTGACTGACCCTATGAGCTTCAAGAGTA
Mouse miRELA-R	CCTGTACTCTTGAAGCTCATAGGGTCAGTCAGTGGCCAAAACCCTATGAGACCTTCAAGAGTAC
miNT-F	TGCTGAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAGTACATTT
miNT-R	CCTGAAATGTACTGCGTGGAGACGTCAGTCAGTGGCCAAAACGTCTCCACGCGCAGTACATTTC

Table S3 Primer sequences used for qPCR.

Name	Primer sequence (5′-3′)	Name	Primer sequence (5′-3′)
AAV-F	TGCATGACCAGGCTCAGCTA	AAV-R	GACAGGGAAGGGAGCAGTG
Human RELA-F	CCTGGAGCAGGCTATCAGTC	Human RELA-R	ATGGGATGAGAAAGGACAGG
Mouse RELA-F	AGGCTTCTGGGCCTTATGTG	Mouse RELA-R	TGCTTCTCTCGCCAGGAATAC
Human GAPDH-F	ATTTGGTCGTATTGGGCG	Human GAPDH-R	CTCGCTCCTGGAAGATGG
Mouse GAPDH-F	AGGTCGGTGTGAACGGATTTG	Mouse GAPDH-R	TGTAGACCATGTAGTTGAGGTCA
Human BCL3-F	GAACACCGAGTGCCAAGAAACC	Human BCL3-R	GCTAAGGCTGTTGTTTTCCACGG
Human CD54-F	AGGTGACGCTGAATGGG	Human CD54-R	GCTCGGGCAATGGGTT
Human NFKBIA-F	TCCACTCCATCCTGAAGGCTAC	Human NFKBIA-R	CAAGGACACCAAAAGCTCCACG
Human NFKB1-F	GCAGCACTACTTCTTGACCACC	Human NFKB1-R	TCTGCTCCTGAGCATTGACGTC
Human NFKB2-F	GGCAGACCAGTGTCATTGAGCA	Human NFKB2-R	CAGCAGAAAGCTCACCACACTC
Human CCL2-F	AGAATCACCAGCAGCAAGTGTCC	Human CCL2-R	TCCTGAACCCACTTCTGCTTGG
Human CXCL1-F	AGCTTGCCTCAATCCTGCATCC	Human CXCL1-R	TCCTTCAGGAACAGCCACCAGT
Human PTGS2-F	CGGTGAAACTCTGGCTAGACAG	Human PTGS2-R	GCAAACCGTAGATGCTCAGGGA
Human MMP9-F	GCCACTACTGTGCCTTTGAGTC	Human MMP9-R	CCCTCAGAGAATCGCCAGTACT
Mouse BCL3-F	AGCAGTCGTCTCAGCTCCAATG	Mouse BCL3-R	AGGCAGGTGTAGATGTTGTGGG
Mouse CD54-F	GTGATGCTCAGGTATCCATC	Mouse CD54-R	CACAGTTCTCAAAGCACAGCG
Mouse NFKBIA-F	GCCAGGAATTGCTGAGGCACTT	Mouse NFKBIA-R	GTCTGCGTCAAGACTGCTACAC
Mouse NFKB1-F	GCTGCCAAAGAAGGACACGACA	Mouse NFKB1-R	GGCAGGCTATTGCTCATCACAG
Mouse NFKB2-F	TGCTGATGGCACAGGACGAGAA	Mouse NFKB2-R	GTTGATGACGCCGAGGTACTGA
Mouse CCL2-F	GCTACAAGAGGATCACCAGCAG	Mouse CCL2-R	GTCTGGACCCATTCCTTCTTGG
Mouse CXCL1-F	TCCAGAGCTTGAAGGTGTTGCC	Mouse CXCL1-R	AACCAAGGGAGCTTCAGGGTCA
Mouse PTGS2-F	GCGACATACTCAAGCAGGAGCA	Mouse PTGS2-R	AGTGGTAACCGCTCAGGTGTTG
Mouse MMP9-F	GCTGACTACGATAAGGACGGCA	Mouse MMP9-R	TAGTGGTGCAGGCAGAGTAGGA
Mouse TNF-α-F	CCCTCACACTCAGATCATCTTCT	Mouse TNF-α-R	GCTACGACGTGGGCTACAG
Mouse IL-6-F	TAGTCCTTCCTACCCCAATTTCC	Mouse IL-6-R	TTGGTCCTTAGCCACTCCTTC
Mouse IL-1β-F	TGGACCTTCCAGGATGAGGACA	Mouse IL-1β-R	GTTCATCTCGGAGCCTGTAGTG
Mouse IL-10-F	CGGGAAGACAATAACTGCACCC	Mouse IL-10-R	CGGTTAGCAGTATGTTGTCCAGC
Mouse IFN-γ-F	CAGCAACAGCAAGGCGAAAAAGG	Mouse IFN-γ-R	TTTCCGCTTCCTGAGGCTGGAT
Mouse IL-12A-F	ACGAGAGTTGCCTGGCTACTAG	Mouse IL-12A-R	CCTCATAGATGCTACCAAGGCAC
Mouse miRELA-F	CGCGCGCGACCGAAGG	Mouse miRELA-R	AGTGCAGGGTCCGAGGTATT

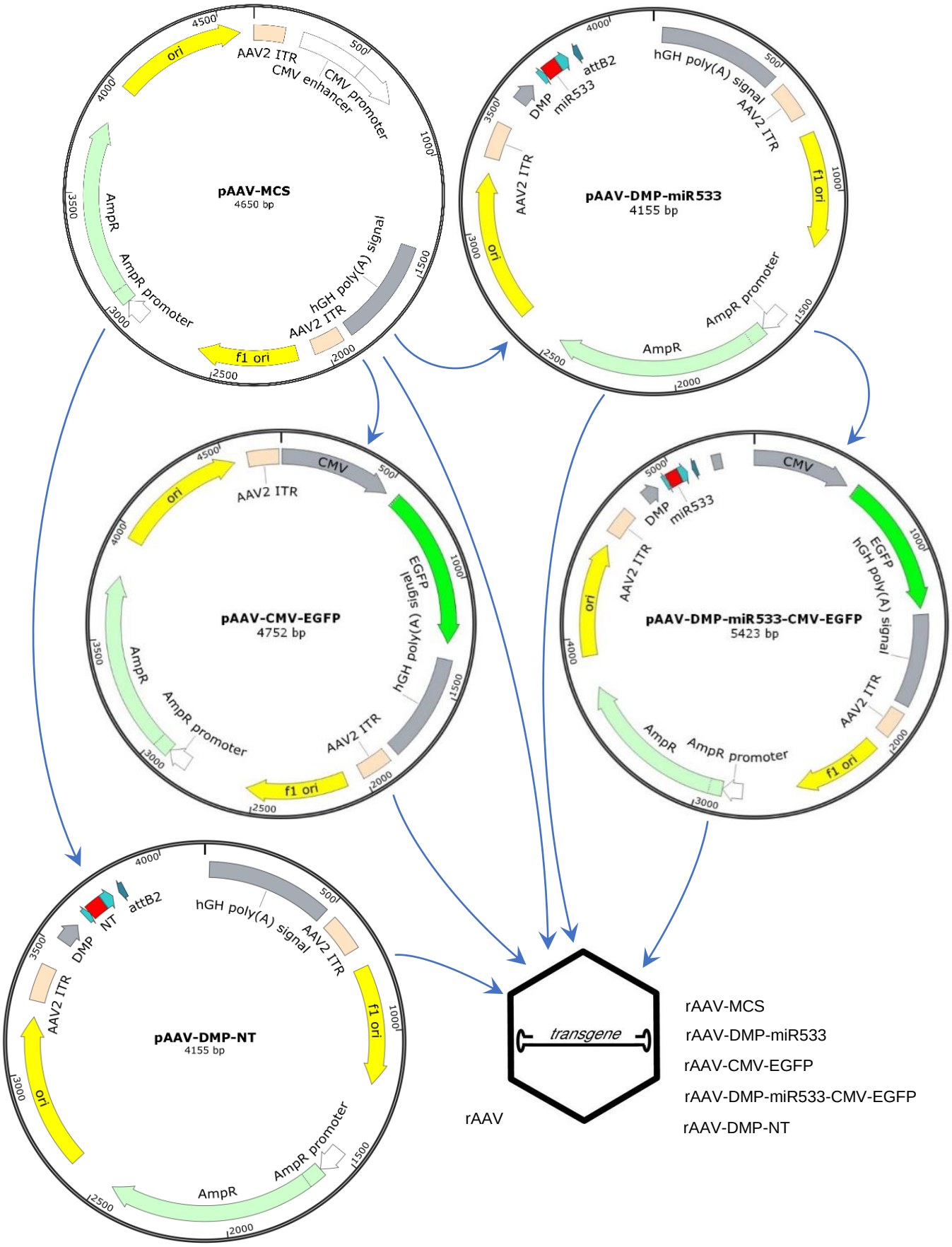

Figure S1 Plasmids and virus vectors. Plasmid map of pAAV-MCS, pAAV-DMP-NT, pAAV-DMP-miR533, pAAV-CMV-EGFP, pAAV-DMP-miR533-CMV-EGFP, and their package into recombinant AAV (rAAV). The prepared rAAVs were named as rAAV-MCS, rAAV-DMP-NT, rAAV-DMP-miR533, rAAV-CMV-EGFP, and rAAV-DMP-miR533-CMV-EGFP, respectively.

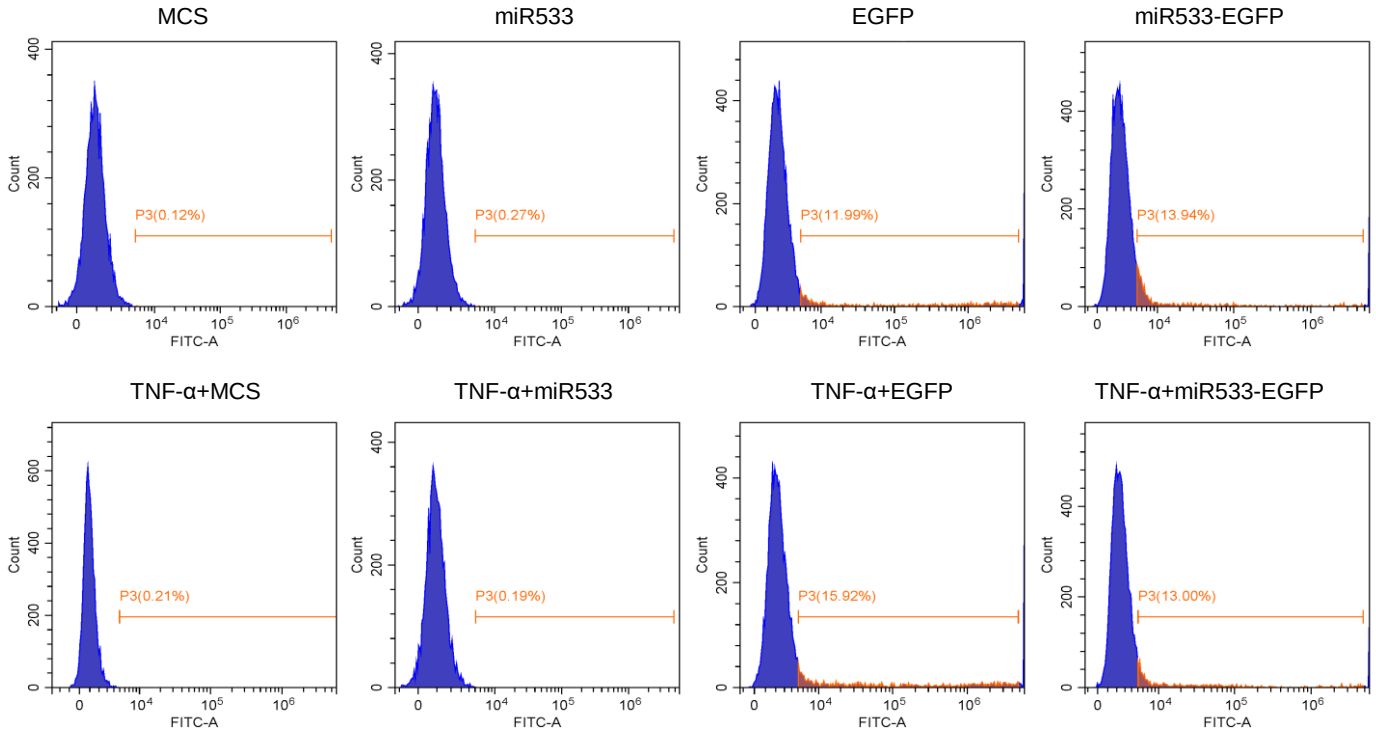

Figure S2 Flow cytometry analysis of EGFP fluorescence intensity. HL7702 was first stimulated with or without TNF-α at a final concentration of 10 ng/mL for 1 h and then infected by various viruses for 48 h. MCS, miR533, EGFP, and miR533-EGFP: cells infected with phosphate buffered saline (PBS), rAAV-MCS, rAAV-DMP-miR533, rAAV-CMV-EGFP, and rAAV-DMP-miR533-CMV-EGFP, respectively; TNF-α+MCS, TNF-α+miR533, TNF-α+EGFP, TNF-α+miR533-EGFP: TNF-α-stimulated cells infected with rAAV-MCS, rAAV-DMP-miR533, rAAV-CMV-EGFP, and rAAV-DMP-miR533-CMV-EGFP, respectively.

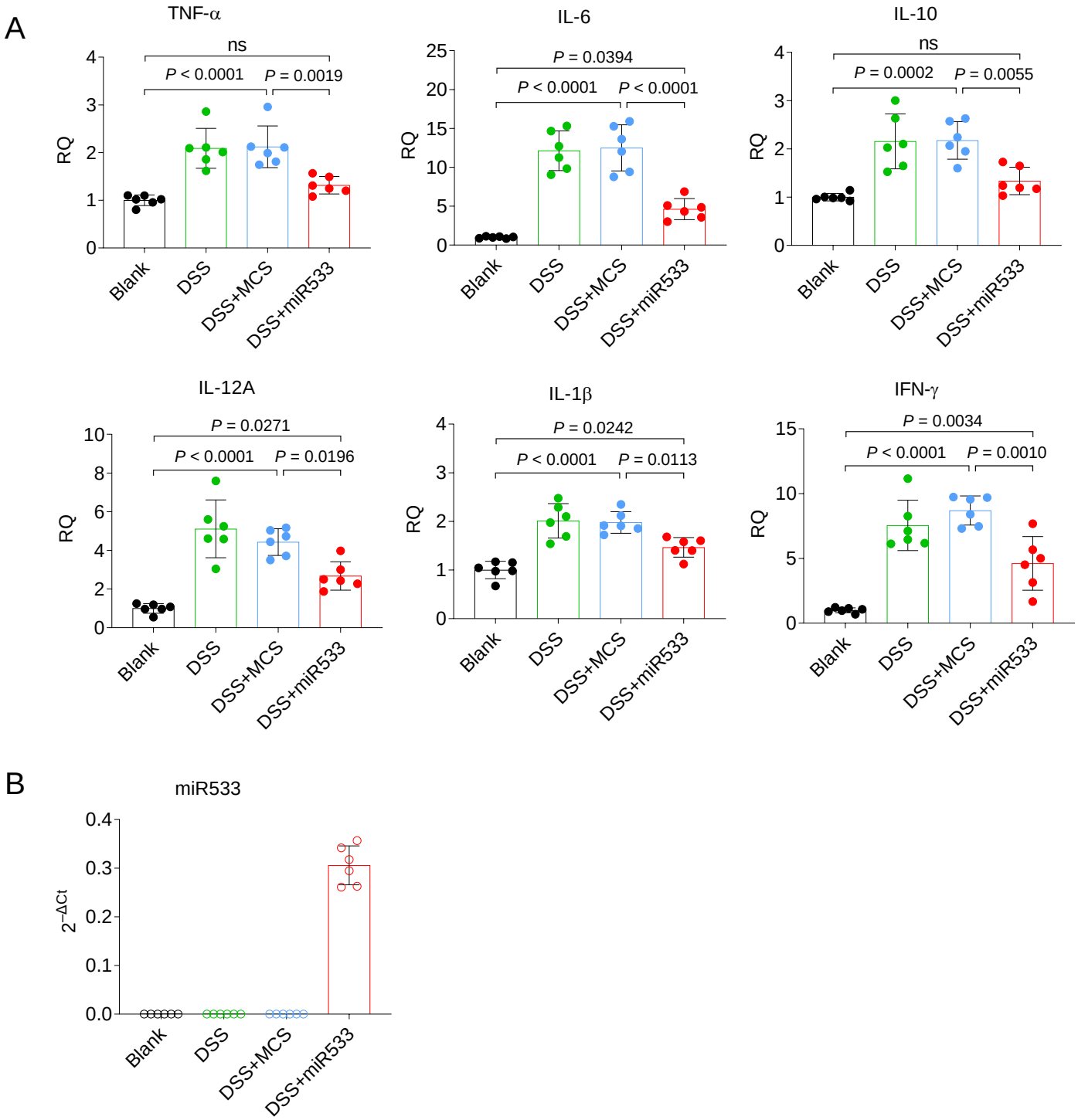

Figure S3 Expression of inflammatory cytokines and miR533 in the dextran sulphate sodium (DSS)-induced acute colitis mouse model. **(A)** Expression of TNF-α, IL-6, IL-10, IL-1β, IFN-γ, and IL-12A. **(B)** Expression of miR533 in colon tissue (n = 6 mice). Gene expression was detected by qPCR. Blank, mice drinking water and treated with PBS; DSS, mice drinking 3% DSS and treated with PBS; MCS, DSS-induced mice treated with rAAV-MCS (i.v.). MiR533, DSS-induced mice treated with rAAV-DMP-miR533 (i.v.).

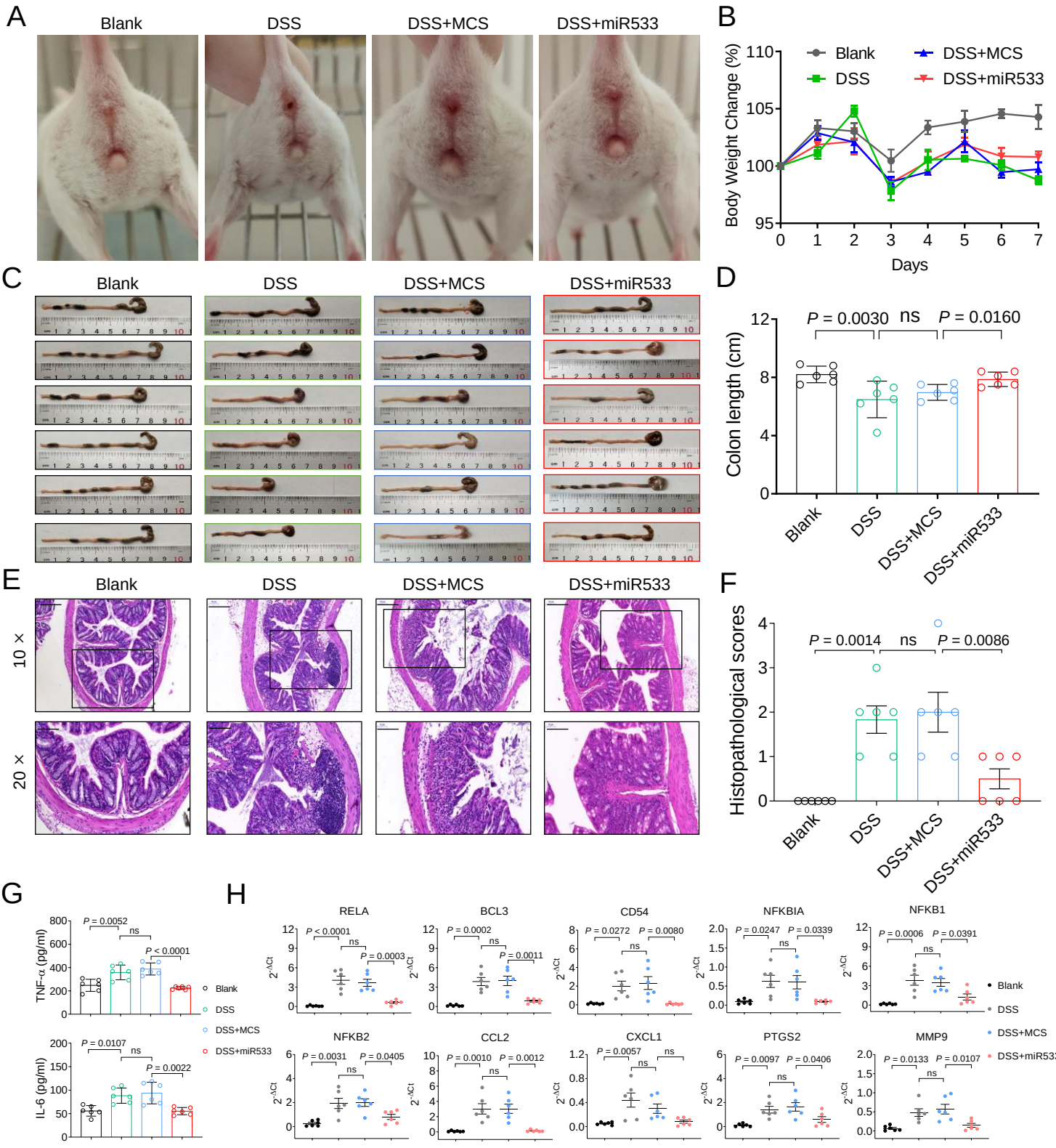

Figure S4 Treatment of the dextran sulphate sodium (DSS)-induced acute colitis in mouse with rAAV-DMP-miR533 (Biological replicate 2). **(A)** Bloody traces around mice anus. **(B)** Body weight of mice**.** **(C)** Colons of mice**.** **(D)** Colons length of mice (n = 6 mice). **(E)** Representative H&E-stained sections of colon tissue. The amplified area in black box is showed below. Scale bar: 200 μm (10×) and 100 μm (20×). **(F)** Histopathological scores of colon tissue (n = 6 mice). **(G)** TNF-α and IL-6 level in serum detected by ELISA (n = 6 mice). **(H)** Expression of NF-κB and its target genes in colon tissue detected by qPCR (n = 6 mice). Blank, mice drinking water and treated with PBS; DSS, mice drinking 3% DSS and treated with PBS; MCS, DSS-induced mice treated with rAAV-MCS (i.v.); MiR533, DSS-induced mice treated with rAAV-DMP-miR533 (i.v.).

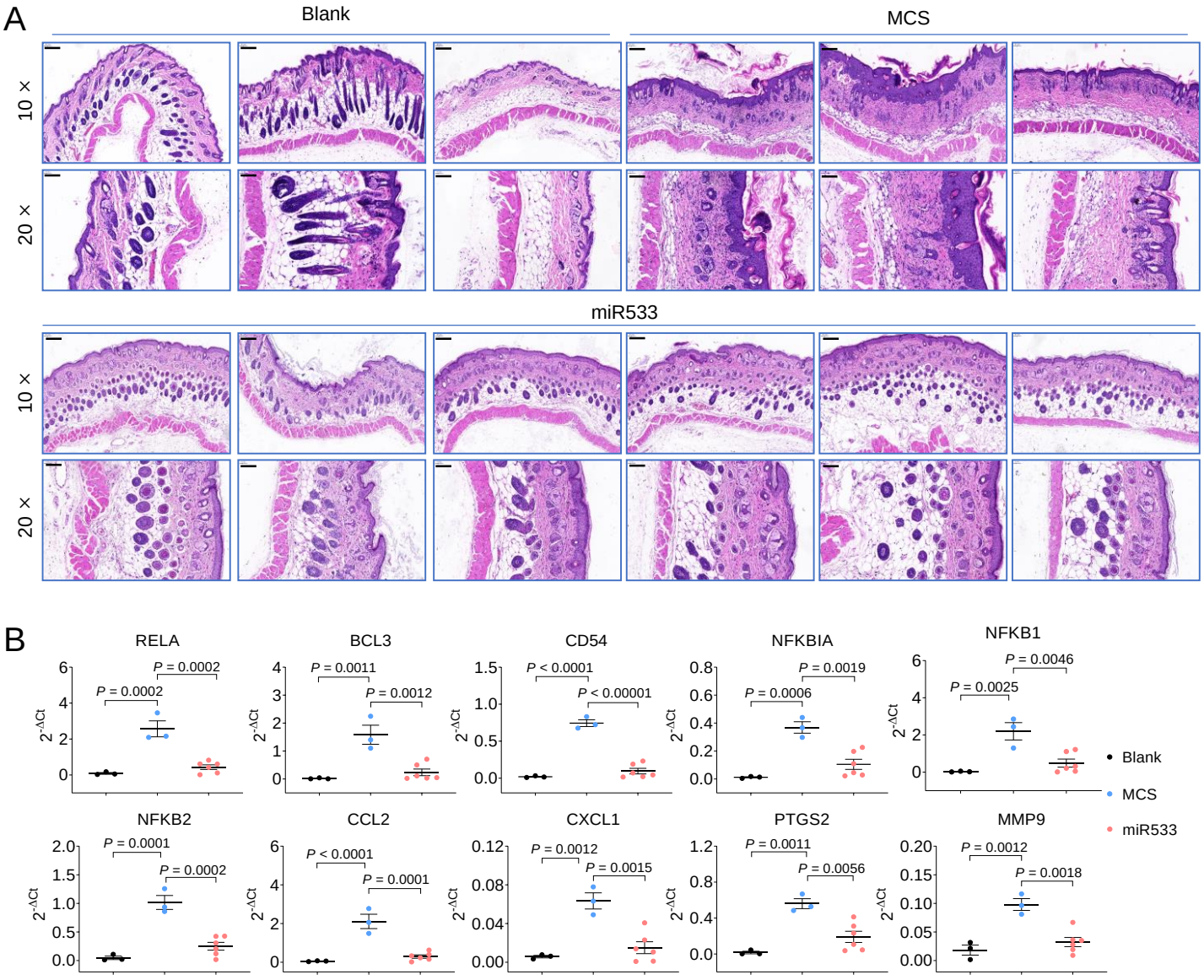

Figure S5 Treatment of psoriasis mice with rAAV-DMP-miR533. The psoriasis mice model was established by the Imiquimod (IMQ) inducement and treated by intravenous injection (i.v.) of rAAV-DMP-miR533. **(A)** Representative H&E-stained sections of skin tissue of all mice. Scale bar: 200 μm (10×) and 100 μm (20×). **(B)** Expression of NF-κB and its target genes in skin tissue detected by qPCR (n =3 mice). Blank, Vaseline-induced mice treated with phosphate buffered saline (PBS) (n =3 mice); MCS, IMQ-induced mice treated with rAAV-MCS (n = 3 mice) (i.v.); miR533, IMQ-induced mice treated with rAAV-DMP-miR533 (i.v.) (n = 6 mice).

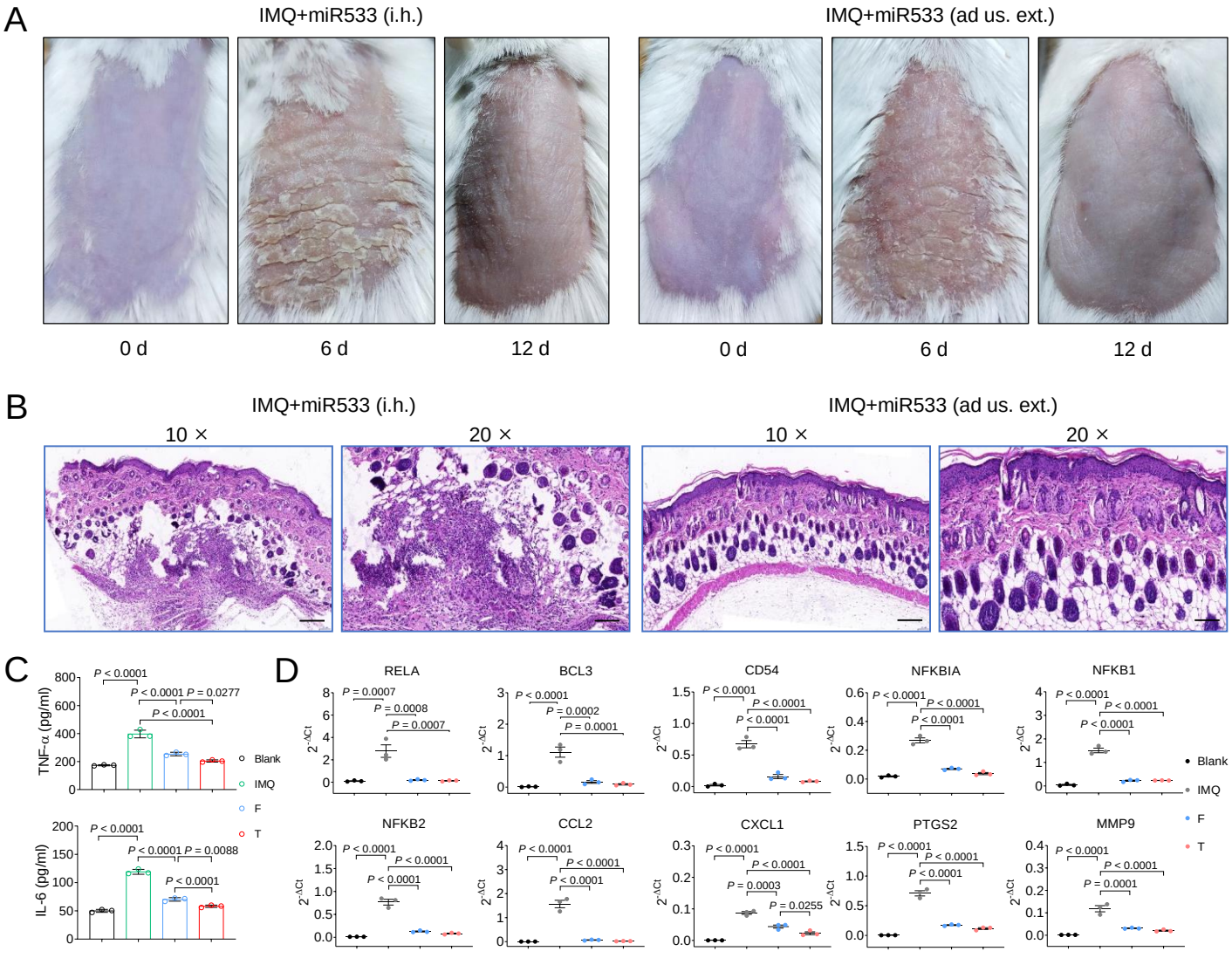

Figure S6 Treatment of psoriasis mice with rAAV-DMP-miR533. The psoriasis mice model was established by the Imiquimod (IMQ) inducement and treated by subcutaneous injection (i.h.) and administration usum externum (ad us. ext.) (for external use) of rAAV-DMP-miR533. Only one mouse was tried to treat with rAAV-DMP-miR533 by subcutaneous injection (i.h.) and usum externum (ad us. ext.), respectively. **(A)** Imaging of back skins of mice on 0, 6, and 12 d. **(B)** Representative H&E-stained sections of skin tissue. Scale bar: 200 μm (10×) and 100 μm (20×). **(C)** TNF-α and IL-6 level in serum detected by ELISA (n = 3 technical replicates). (D) Expression of NF-κB and its target genes in skin tissue detected by qPCR (n = 3 technical replicates). F, subcutaneous injection (i.h.); T, administration usum externum (ad us. ext.) (for external use). IMQ+miR533, IMQ-induced mice treated with rAAV-DMP-miR533.

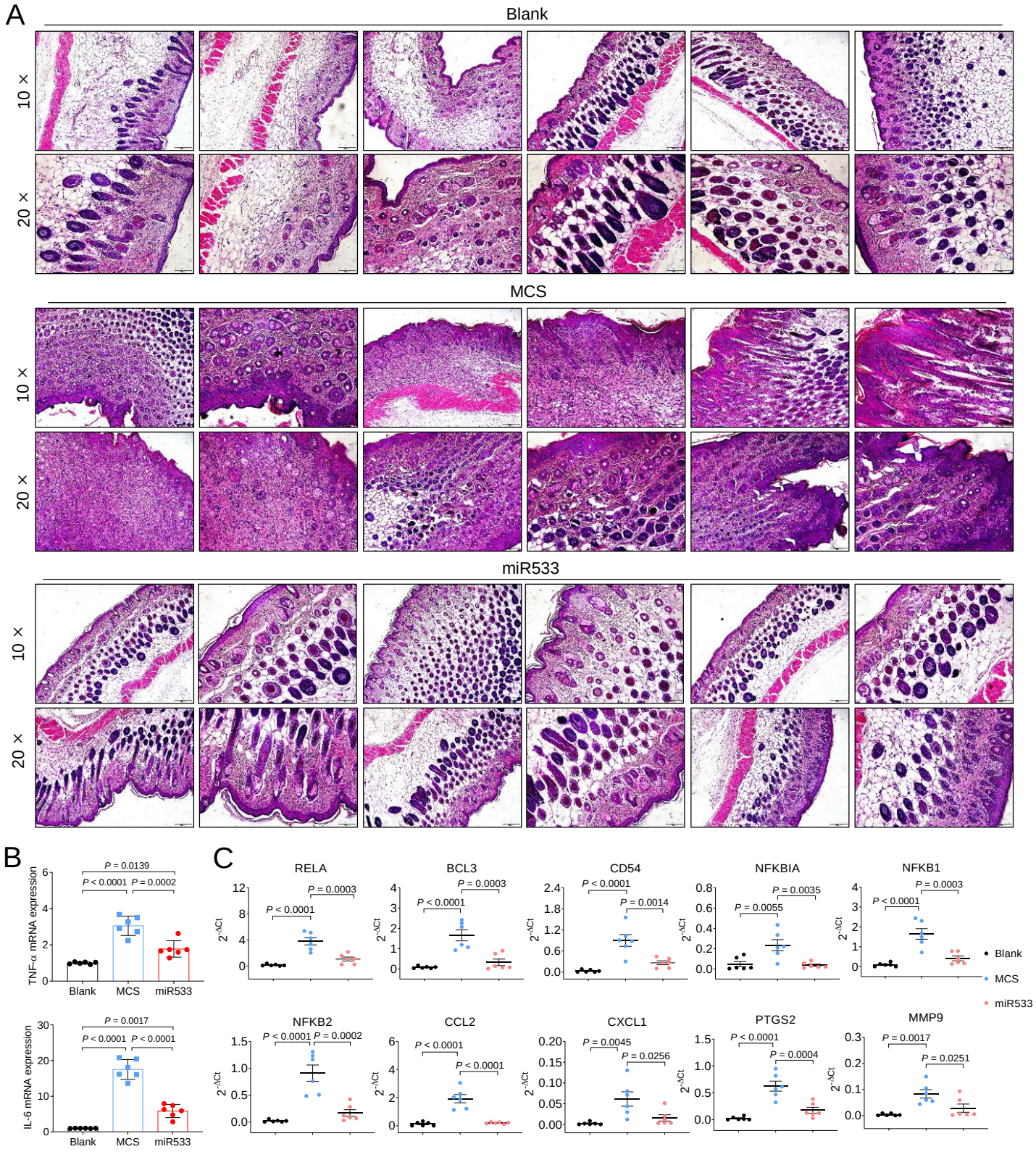

Figure S7 Treatment of psoriasis mice with rAAV-DMP-miR533. The psoriasis mice model was established by the Imiquimod (IMQ) inducement and treated by administration usum externum (ad us. ext.) (for external use) of rAAVs. **(A)** Representative H&E-stained sections of skin tissue of all mice. Scale bar: 200 μm (10×) and 100 μm (20×). **(B)** TNF-α and IL-6 expression in skin samples (n = 6 mice). **(C)** Expression of NF-κB and its target genes in skin tissue (n = 6 mice). Gene expression was detected by qPCR. Blank, Vaseline-induced mice treated with phosphate buffered saline (PBS); MCS, IMQ-induced mice treated with rAAV-MCS; miR533, IMQ-induced mice treated with rAAV-DMP-miR533.

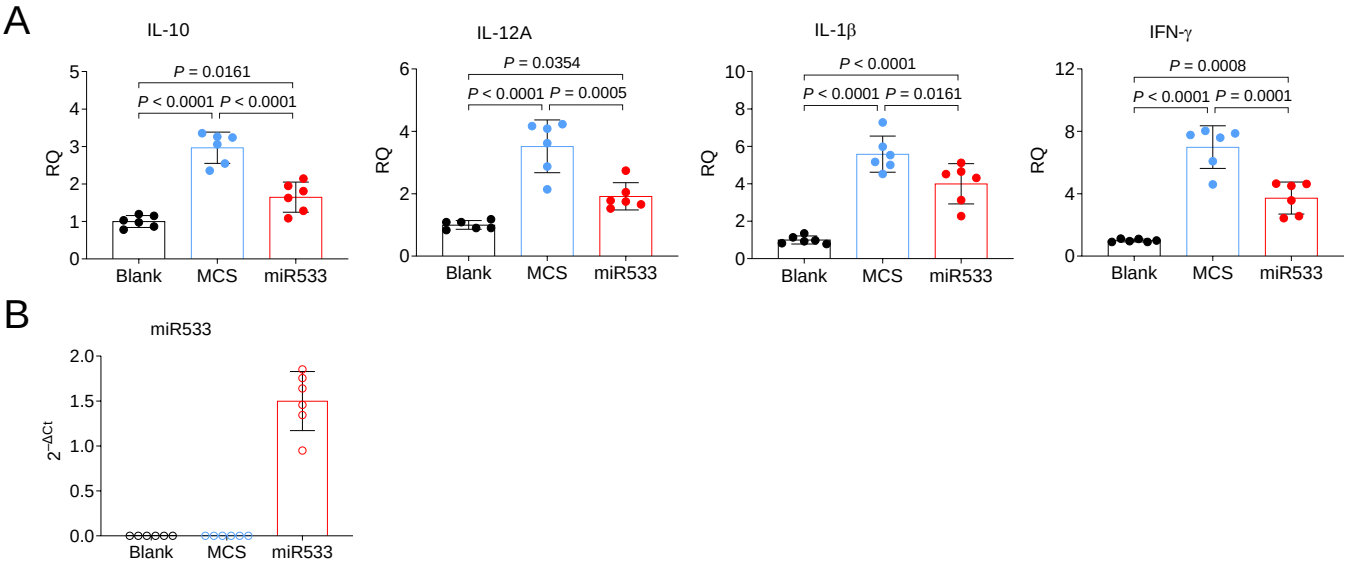

Figure S8 Expression of other inflammatory cytokines and miR533 in psoriasis mouse model. **(A)** Expression of IL-10, IL-1β, IFN-γ in skin tissue (n = 6 mice). **(B)** Expression of miR533 in skin tissue (n = 6 mice). Gene expression was detected by qPCR. Blank, Vaseline-induced mice treated with phosphate buffered saline (PBS); MCS, IMQ-induced mice treated with rAAV-MCS; miR533, IMQ-induced mice treated with rAAV-DMP-miR533.

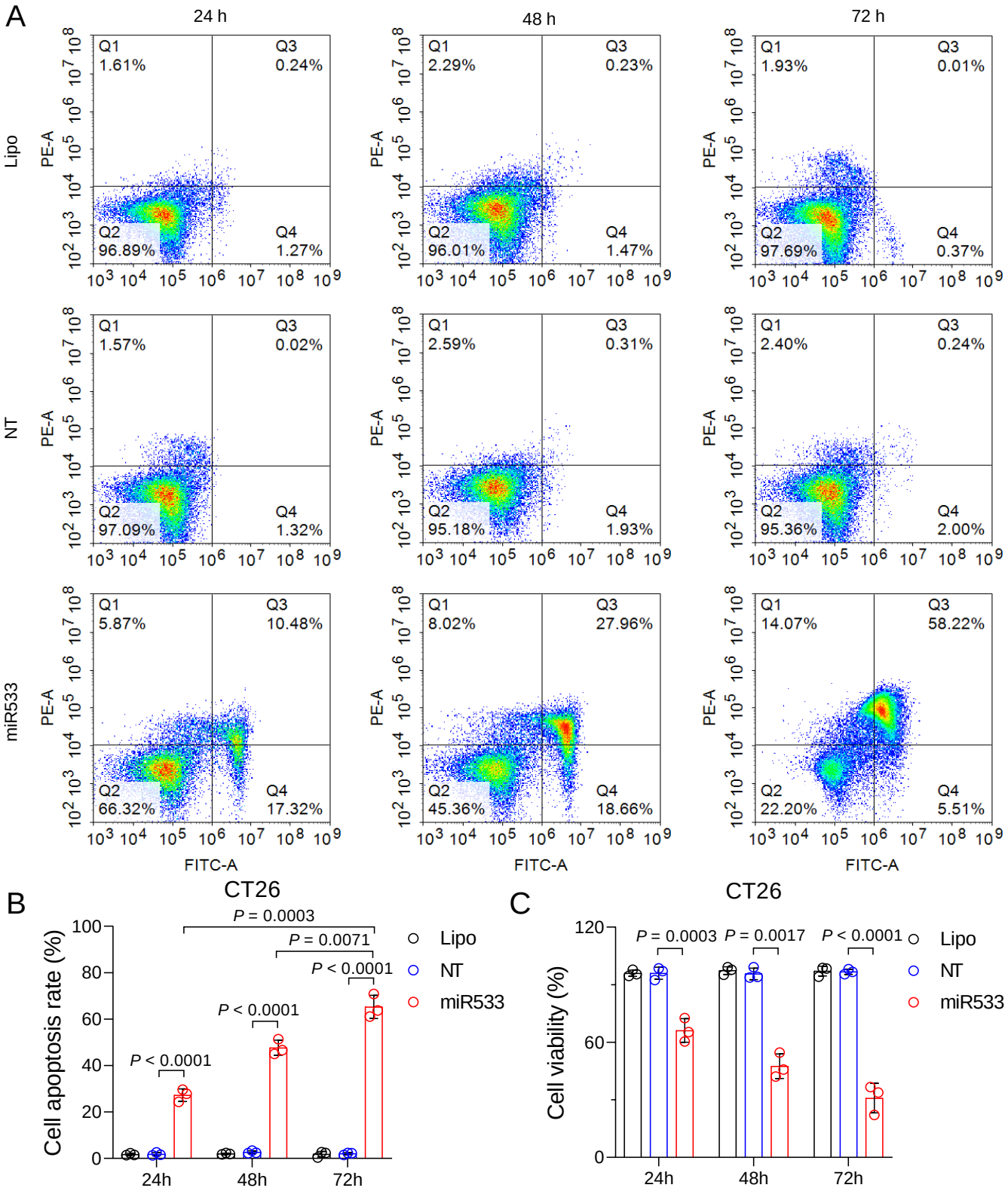

Figure S9 Effect of pAAV-DMP-miR533 on apoptosis and viability of CT-26 cell. CT-26 cell was transfected by various plasmids and then cultured for 24, 48, and 72 h, respectively. **(A)** Representative images of Flow Cytometry analysis of cell apoptosis. **(B)** Cell apoptosis (n = 3 wells). **(C)** Cell viability (n = 3 wells). Lipo, cells treated with Lipofectamine 2000. NT, cells transfected with pAAV-DMP-NT; miR533, cells transfected with pAAV-DMP-miR533.

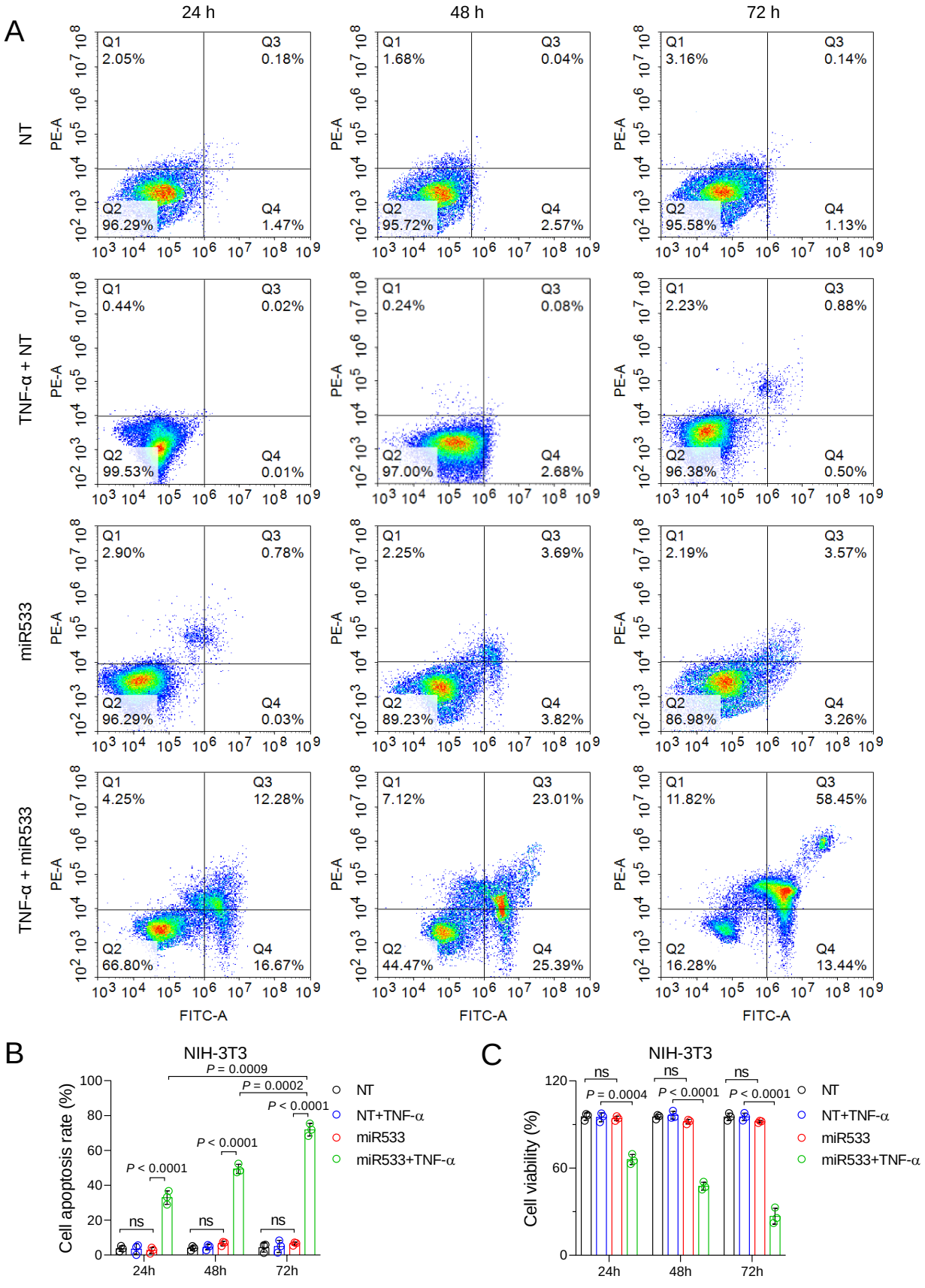

Figure S10 Effect of pAAV-DMP-miR533 on apoptosis and viability of NIH-3T3 cell. NIH-3T3 was stimulated with or without TNF-α at a final concentration of 10 ng/mL for 1 h before transfection. NIH-3T3 cell was then transfected by various plasmids and then cultured for 24, 48, and 72 h, respectively. **(A)** Representative images of flow cytometry analysis of cell apoptosis. **(B)** Cell apoptosis (n = 3 wells). **(C)** Cell viability (n = 3 wells). NT, cells transfected with pAAV-DMP-NT; miR533, cells transfected with pAAV-DMP-miR533.

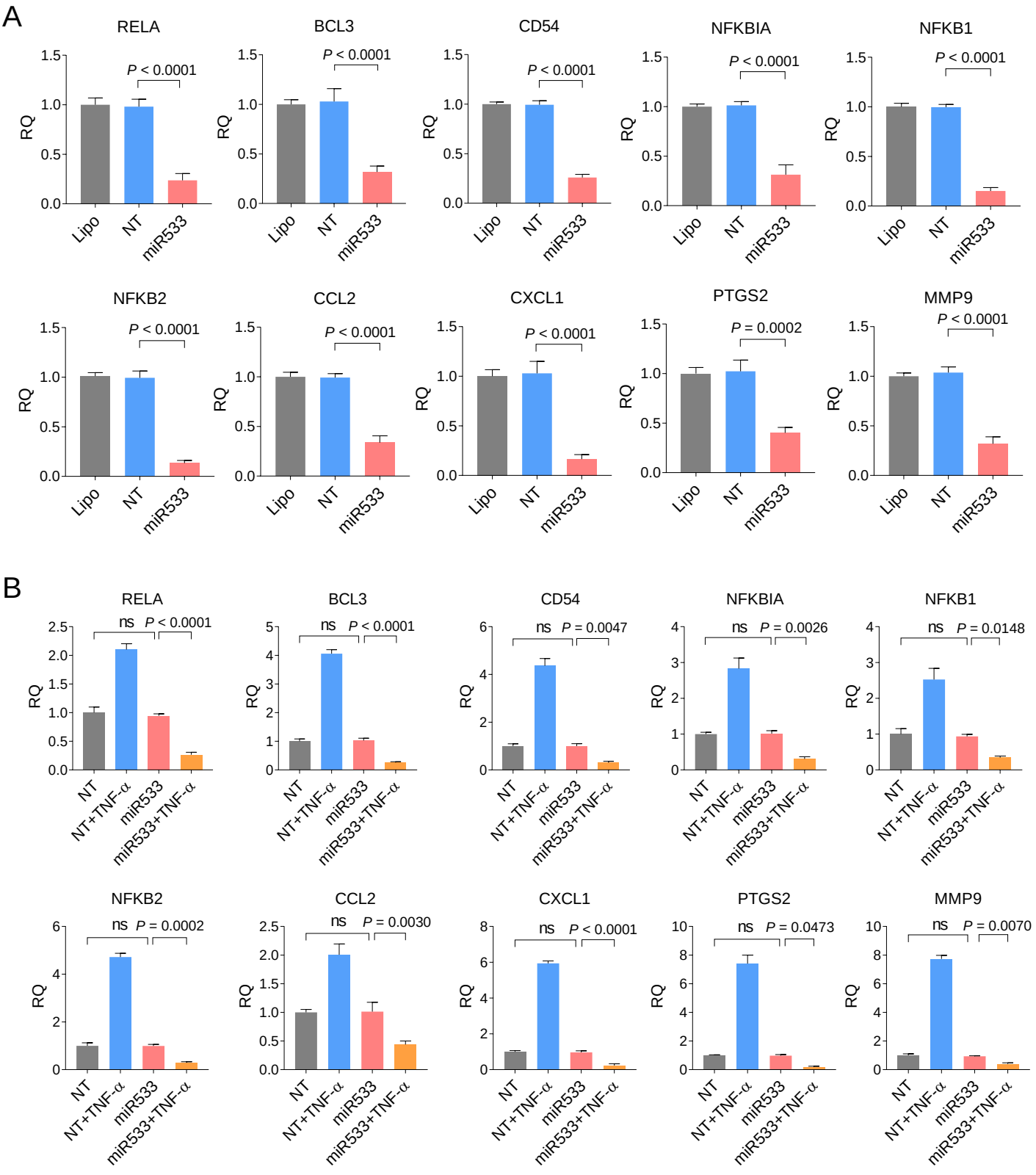

Figure S11 Expression of NF-κB and its target genes in CT26 **(A)** and NIH-3T3 **(B)** treated with pAAV-DMP-miR533 and cultured for 48 h. NIH-3T3 was stimulated with or without TNF-α at a final concentration of 10 ng/mL for 1 h before transfection. Gene expression was detected by qPCR. RQ $=2^{-\Delta\Delta Ct}$. RQ, relative quantity. Lipo, cells treated with Lipofectamine 2000. NT, cells transfected with pAAV-DMP-NT. miR533, cells transfected with pAAV-DMP-miR533.

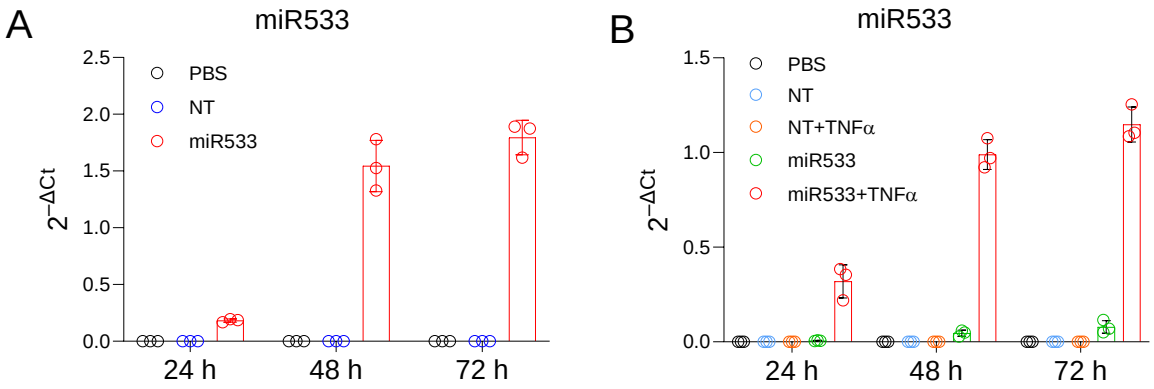

Figure S12 Expression of miR533 in CT26 **(A)** and NIH-3T3 **(B)** treated with rAAV-DMP-miR533 and rAAV-DMP-NT at variant time points. NIH-3T3 was stimulated with or without TNF-α at a final concentration of 10 ng/mL for 1 h before infection. Gene expression was detected by qPCR. PBS, cells treated with PBS; NT, cells infected with rAAV-DMP-NT. miR533, cells infected with rAAV-DMP-miR533.

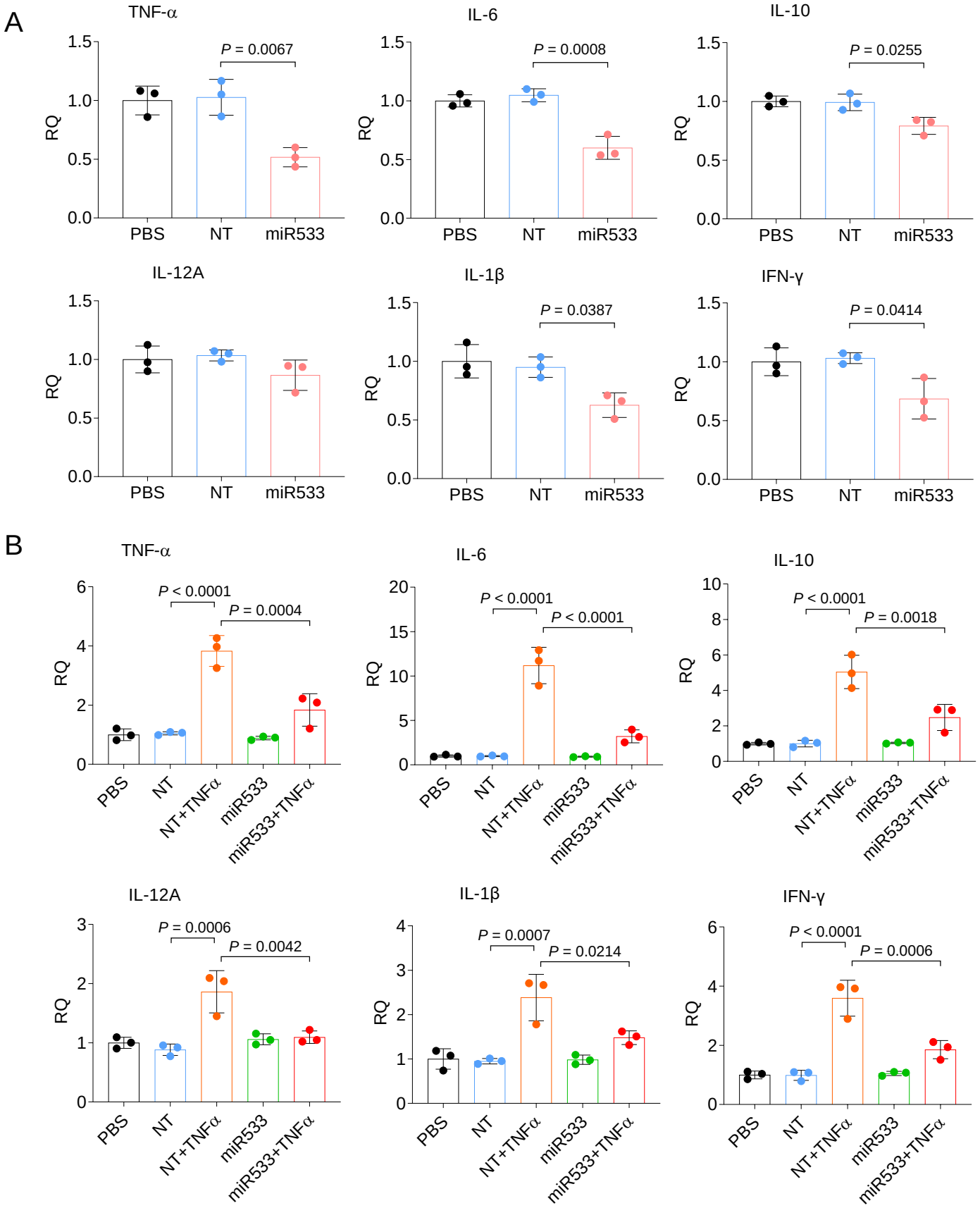

Figure S13 Expression of inflammatory cytokines in CT26 **(A)** and NIH-3T3 **(B)** treated with rAAV-DMP-miR533 and rAAV-DMP-NT for 48 h. NIH-3T3 was stimulated with or without TNF-α at a final concentration of 10 ng/mL for 1 h before transfection. Gene expression was detected by qPCR. PBS, cells treated with PBS; NT, cells infected with rAAV-DMP-NT. miR533, cells infected with rAAV-DMP-miR533.

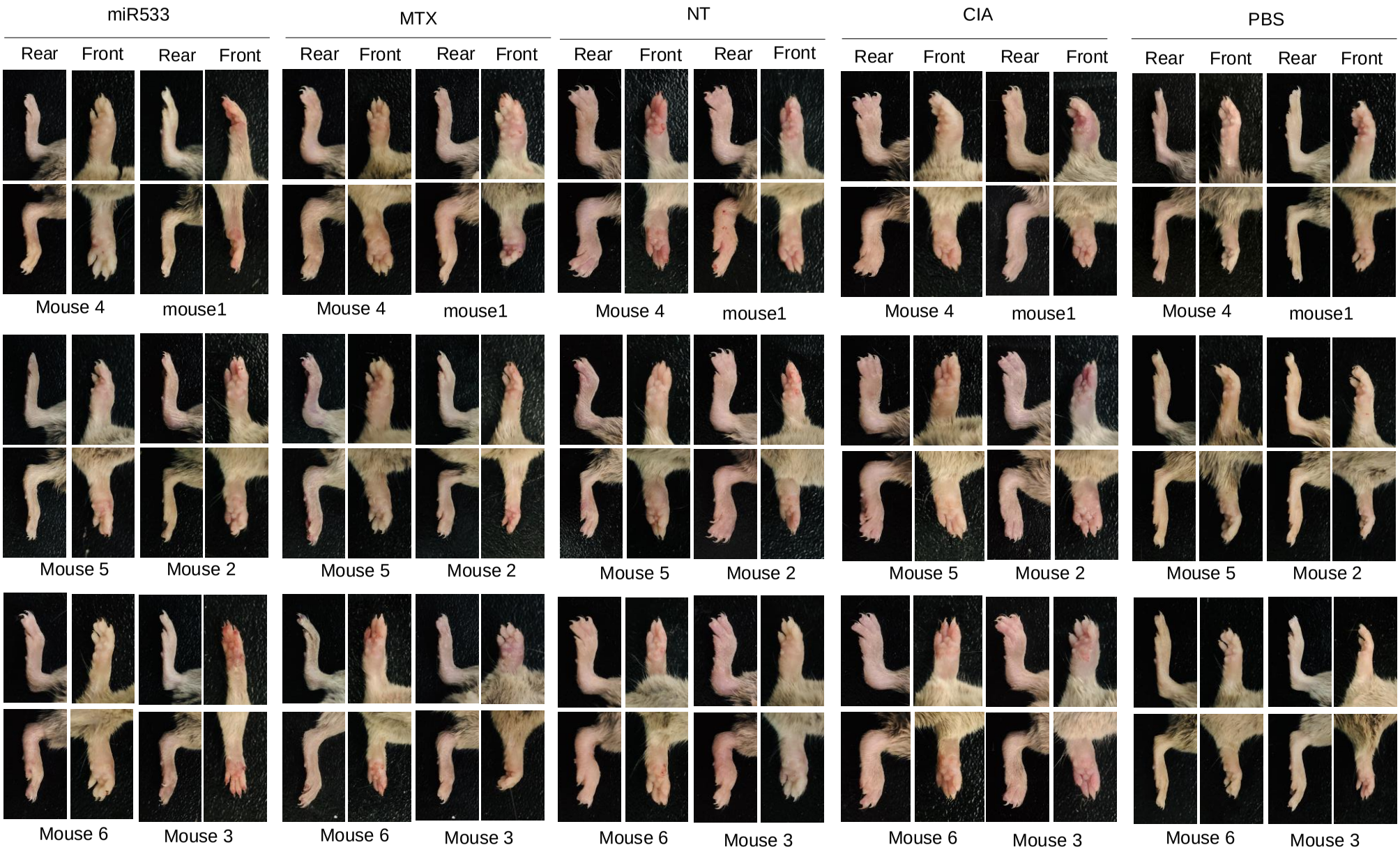
miR533
MTX
NT
CIA
PBS
Rear
Front
Rear
Front
Mouse 4
mouse1
Mouse 5
Mouse 2
Mouse 6
Mouse 3

Figure S14 Treatment of the arthritis mice with rAAV-DMP-miR533. The collagen-induced arthritis (CIA) mouse model was established by the collagen inducement and treated by intravenous injection (i.v.) of rAAVs. Photos of forepaws and rearpaws of all mice in different groups (PBS, CIA, NT, MTX, miR533) (n = 6 mice). PBS, normal mice treated with phosphate buffered saline (PBS); CIA, CIA mice treated with PBS; MTX, CIA mice treated with Methotrexate (MTX); NT, CIA mice treated with rAAV-DMP-NT; miR533, CIA mice treated with rAAV-DMP-miR533. ns, no significance. NT, no target.

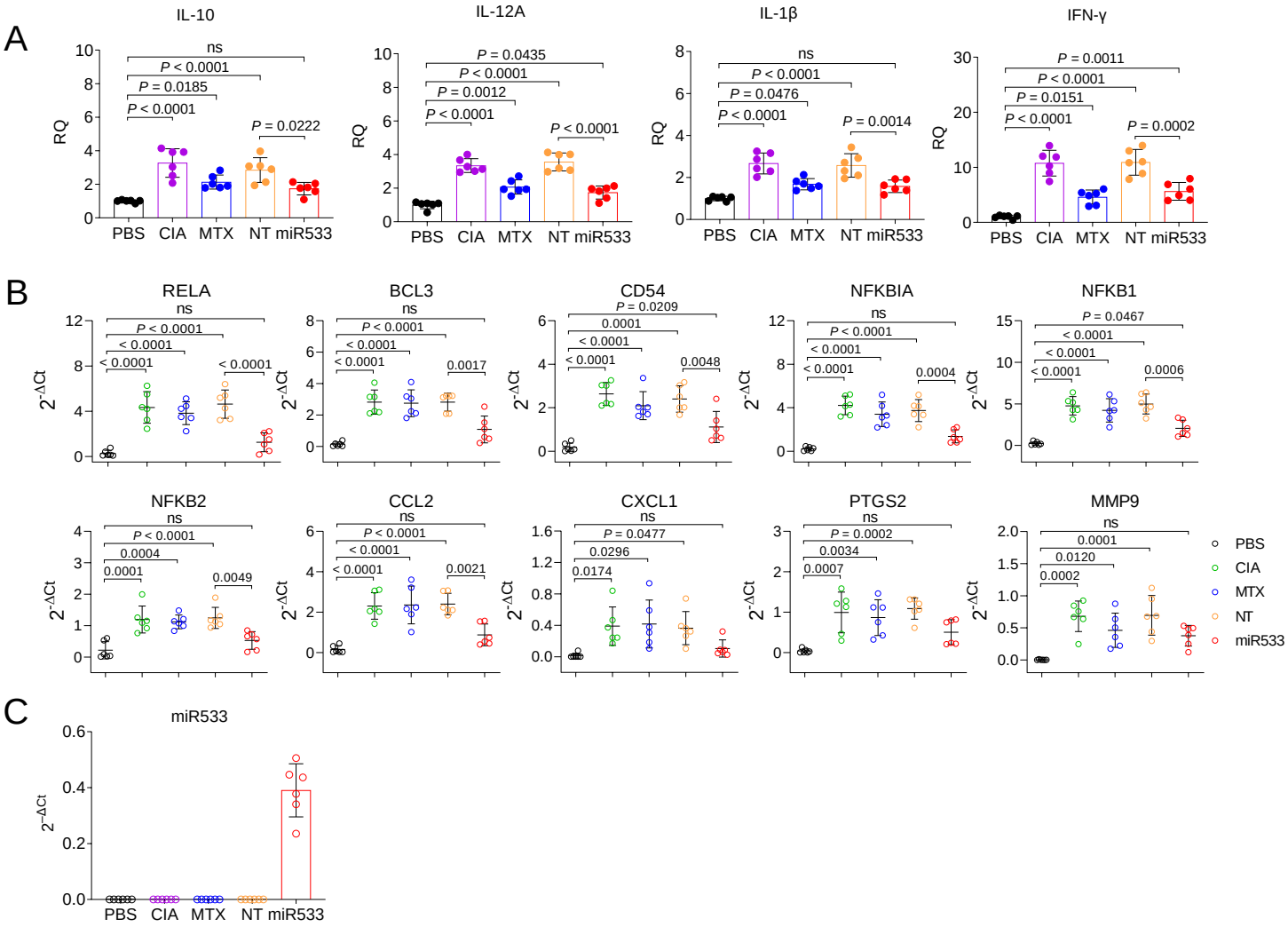

Figure S15 Expression of inflammatory cytokines, NF-κB and its target genes, and miR533 in the arthritis mice model. **(A)** Expression of IL-10, IL-1β, IFN-γ, IL-12A in rear paws (n = 6 mice). **(B)** Expression of NF-κB and its target genes in rear paws (n = 6 mice). **(C)** Expression of miR533 in rear paws (n = 6 mice). Gene expression was detected by qPCR. PBS, normal mice treated with PBS; CIA, CIA mice treated with PBS; MTX, CIA mice treated with Methotrexate (MTX); NT, CIA mice treated with rAAV-DMP-NT; miR533, CIA mice treated with rAAV-DMP-miR533. ns, no significance. NT, no target.

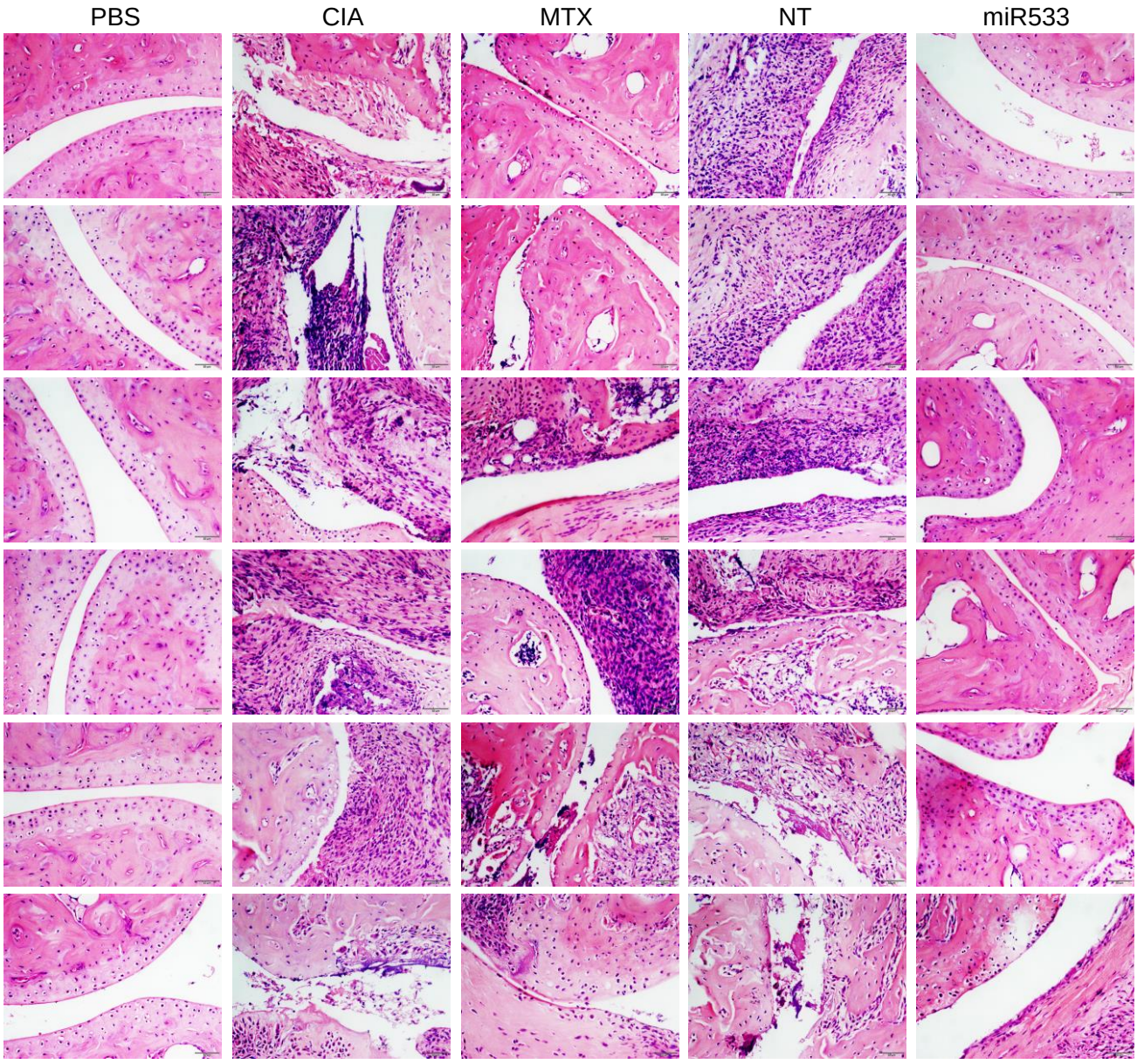

Figure S16 Treatment of the arthritis mice with rAAV-DMP-miR533. The collagen-induced arthritis (CIA) mouse model was established by the collagen inducement and treated by intravenous injection (i.v.) of rAAVs. Representative H&E-stained sections of ankle joints of all mice are shown. Scale bar: 50 μm. PBS, normal mice treated with phosphate buffered saline (PBS); CIA, CIA mice treated with PBS; MTX, CIA mice treated with Methotrexate (MTX); NT, CIA mice treated with rAAV-DMP-NT; miR533, CIA mice treated with rAAV-DMP-miR533. ns, no significance. NT, no target.

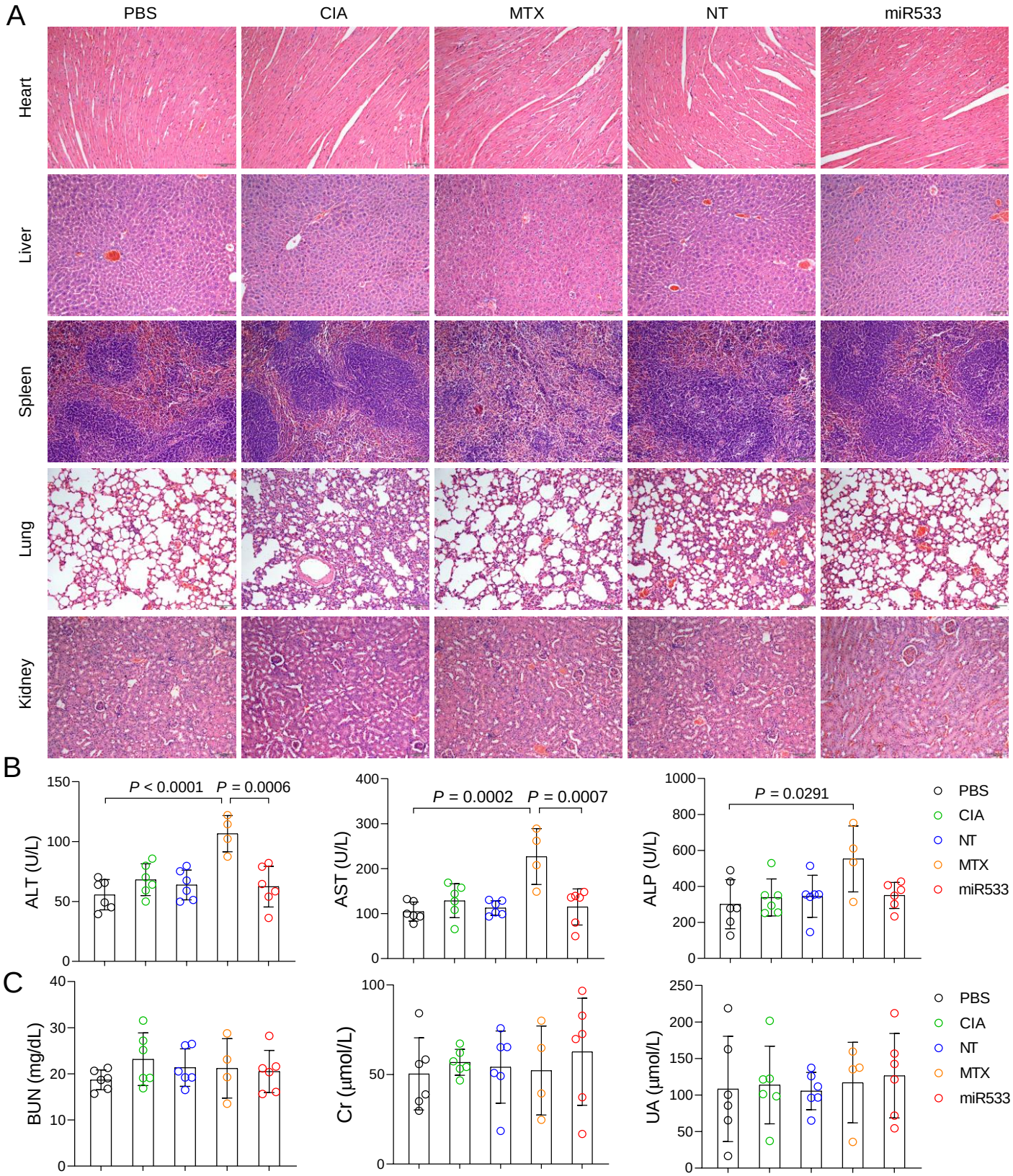

Figure S17 Treatment of the arthritis mice with rAAV-DMP-miR533. The collagen-induced arthritis (CIA) mouse model was established by the collagen inducement and treated by intravenous injection (i.v.) of rAAVs. **(A)** Representative H&E-stained sections of major organs. Scale bar: 100 μm. **(B)** Serum biochemical indices of liver (n = 6 mice). **(C)** Serum biochemical indices of kidney (n = 6 mice). PBS, normal mice treated with phosphate buffered saline (PBS); CIA, CIA mice treated with PBS; MTX, CIA mice treated with Methotrexate (MTX); NT, CIA mice treated with rAAV-DMP-NT; miR533, CIA mice treated with rAAV-DMP-miR533. ns, no significance. NT, no target. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid.