

Table S1. Products used in this study.

Products	Catalog number	Manufacturer
Complete Freund's adjuvant	F5881	Sigma-Aldrich
AMD3100	110078-46-1	TargetMol
NF- κ B activator 1	HY-134476	MedChemExpress
IL-17A	HY-P73174	MedChemExpress
IL-6	CG39	Novoprotein
TGF- β 1	CA59	Novoprotein
IL-23	CS31	Novoprotein
RNA extraction kit	220011	Fastagen
SYBR master qPCR mix and qPCR kit	22204	Tolobio
Malondialdehyde activity assay kit	S0131S	Beyotime
TUNEL apoptosis assay kit	RCT-50R	Recordbio
ROS assay kit	S0033S	Beyotime
Dihydroethidium assay kit	S0063	Beyotime
BCA protein assay kit	GK10009	GlpBio
PMA	CS0001	MultiSciences
Ionomycin	CS0002	MultiSciences
Monensin	CS0004	MultiSciences
Naïve CD4 ⁺ T cell isolation kit	130-104-453	Miltenyi Biotec
anti-CD3	BE0002	Bio X Cell
anti-CD28	BE0015-1	Bio X Cell
anti-IL-4	BE0045	Bio X Cell
anti-IFN- γ	BE0055	Bio X Cell
anti-CD4-FITC	553047	BD Biosciences
anti-IL-17A-PE	559502	BD Biosciences
anti-CCR6-PE	129804	Biolegend

Table S2. Antibodies used in WB, IHC and IF.

Name	Application	Catalog number	Manufacturer	Dilution ratio
anti-CXCR4	WB	CY5380	Abways	1:1000
anti-IL-17A	WB	DF6127	Affinity	1:1000
anti-ROR γ t	WB	ab207082	abcam	1:2000
anti-STAT3	WB	9139T	Cell Signaling Technology	1:1000
anti-phospho-STAT3	WB	9145T	Cell Signaling Technology	1:2000
anti-Bcl-2	WB	68103-1-Ig	Proteintech	1:1000
anti-Bax	WB	AF0120	Affinity	1:1000
anti-Cleaved caspase-3	WB	CY5501	Abways	1:1000
anti-GPX4	WB	DF6701	Affinity	1:1000
anti- γ -H2AX	WB	CY6572	Abways	1:1000
anti-p65	WB	8242T	Cell Signaling Technology	1:1000
anti-phospho-p65	WB	3033T	Cell Signaling Technology	1:1000
anti-GAPDH	WB	AF7021	Affinity	1:3000
anti-phospho-p65	IHC	CY6372	Abways	1:100
anti-8-OHdG	IHC	GB150115-50	Servicebio	1:200
anti-4-HNE	IF	bs-6313R	Bioss	1:200
anti-CD4	IF	GB15064-50	Servicebio	1:200
anti-IL-17A	IF	GB11110-1-50	Servicebio	1:200

Table S3. The primer sequences.

Gene	Forward primer sequence (5 ' to 3 ')	Reverse primer sequence (5 ' to 3 ')
<i>Cxcr4</i>	GACTGGCATAGTCGGCAATG	AGAAGGGGAGTGTGATGACAAA
<i>Il1b</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
<i>Il6</i>	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
<i>Tnf</i>	GAGTGACAAGCCTGTAGCC	CTCCTGGTATGAGATAGCAAA
<i>Il10</i>	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
<i>Il17a</i>	TCAGCGTGTCCAAACACTGAG	CGCCAAGGGAGTTAAAGACTT
<i>Il17f</i>	TGCTACTGTTGATGTTGGGAC	CAGAAATGCCCTGGTTTTGGT
<i>Rorc</i>	TCCACTACGGGGTTATCACCT	AGTAGGCCACATTACACTGCT
<i>Il21</i>	CGCCTCCTGATTAGACTTCG	GCCCCTTTACATCTTGTGGA
<i>Il22</i>	ATGAGTTTTTCCCTTATGGGGAC	GCTGGAAGTTGGACACCTCAA
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA

Figure S1. Representative hematoxylin-eosin stained prostate tissue sections illustrating the 0-3 grading system for prostatic inflammation.

Figure S2. CXCR4 is upregulated in the prostate tissues of EAP mice. (A) qPCR analysis of the expression level of *Cxcr4* in the prostate tissues from the Control and EAP groups (n = 5). (B) The expression level of CXCR4 in the prostate tissues from the Control and EAP groups (n = 3). ***P* < 0.01. CXCR4: C-X-C chemokine receptor type 4; EAP: experimental autoimmune prostatitis; qPCR: quantitative polymerase chain reaction.

Figure S3. Dose-response analysis of AMD3100 treatment in EAP mice. (A) A simple schematic diagram showed the whole process of AMD3100 intervention at doses of 1, 5, and 10 mg/kg in EAP mice. (B) HE staining revealed the inflammatory cell infiltration within the prostate tissues in the Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). (C) Inflammation scores of the prostate tissues in the Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). (D) Differences in the pain responses of the mice in the Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). (E, F) Proportion of CD4⁺IL-17A⁺ cells detected by flow cytometry in the splenic lymphocytes from the Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). (G-J) qPCR analysis of the expression levels of inflammatory cytokines in the prostate tissues from the Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). (K) Immunofluorescence was used to assess the levels of ROS in the prostate tissues from the Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). (L) Immunofluorescence was used to assess the levels of 4-HNE in the prostate tissues from the Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). (M) The expression levels of GPX4 and γ -H2AX in the prostate tissues from Control, EAP, EAP + AMD3100 (1mg/kg), EAP + AMD3100 (5mg/kg), and EAP + AMD3100 (10 mg/kg) groups (n = 5). **P* < 0.05, ***P* < 0.01, ****P* < 0.001. EAP: experimental autoimmune prostatitis; HE: hematoxylin-eosin; qPCR: quantitative polymerase chain reaction; ROS: reactive oxygen species; 4-HNE: 4-hydroxy-2-nonenal; GPX4: glutathione peroxidase 4.

Figure S4. Analysis of single-cell expression of CXCR4 in the prostate using the Human Protein Atlas database. CXCR4: C-X-C chemokine receptor type 4.

Figure S5. NF- κ B activator partially negates the beneficial effects of AMD3100 in EAP mice. (A-D) qPCR analysis of the expression levels of inflammatory cytokines in the prostate tissues from the EAP, EAP + AMD3100, and EAP + AMD3100 + NF- κ B activator groups (n = 5). (E-H) qPCR analysis of the expression levels of Th17-associated cytokines in the prostate tissues from the EAP, EAP + AMD3100, and EAP + AMD3100 + NF- κ B activator groups (n = 5). (I) Immunofluorescence was used to assess the levels of 4-HNE in the prostate tissues from the EAP, EAP + AMD3100, and EAP + AMD3100 + NF- κ B activator groups (n = 5). (J) The levels of MDA were measured in the prostate tissues from the EAP, EAP + AMD3100, and EAP + AMD3100 + NF- κ B activator groups (n = 5). * P < 0.05, ** P < 0.01, *** P < 0.001. EAP: experimental autoimmune prostatitis; qPCR: quantitative polymerase chain reaction; Th17: T helper 17; 4-HNE: 4-hydroxy-2-nonenal; MDA: malondialdehyde.

Figure S1

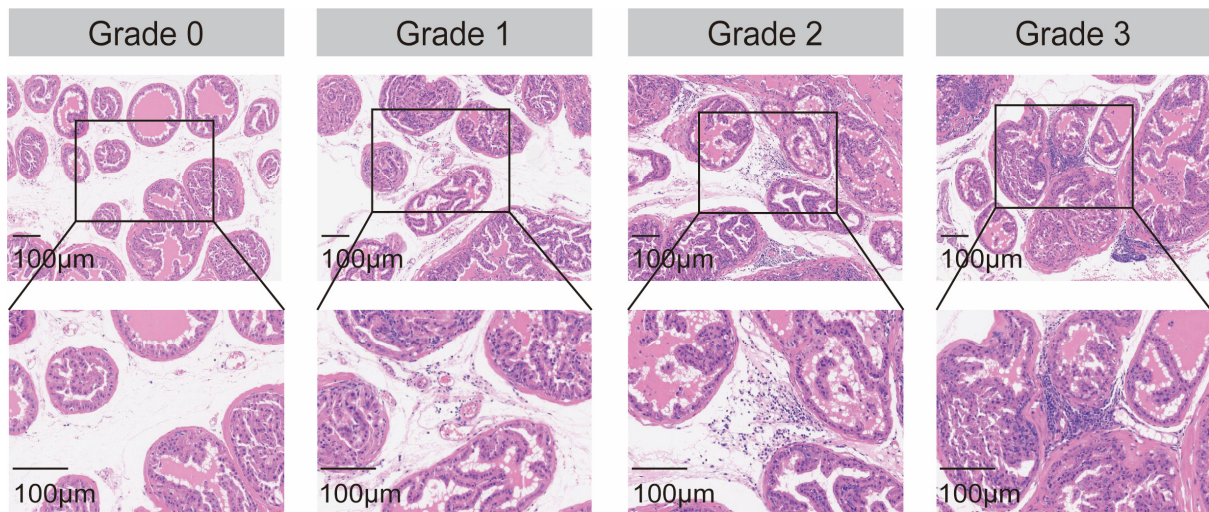


Figure S2

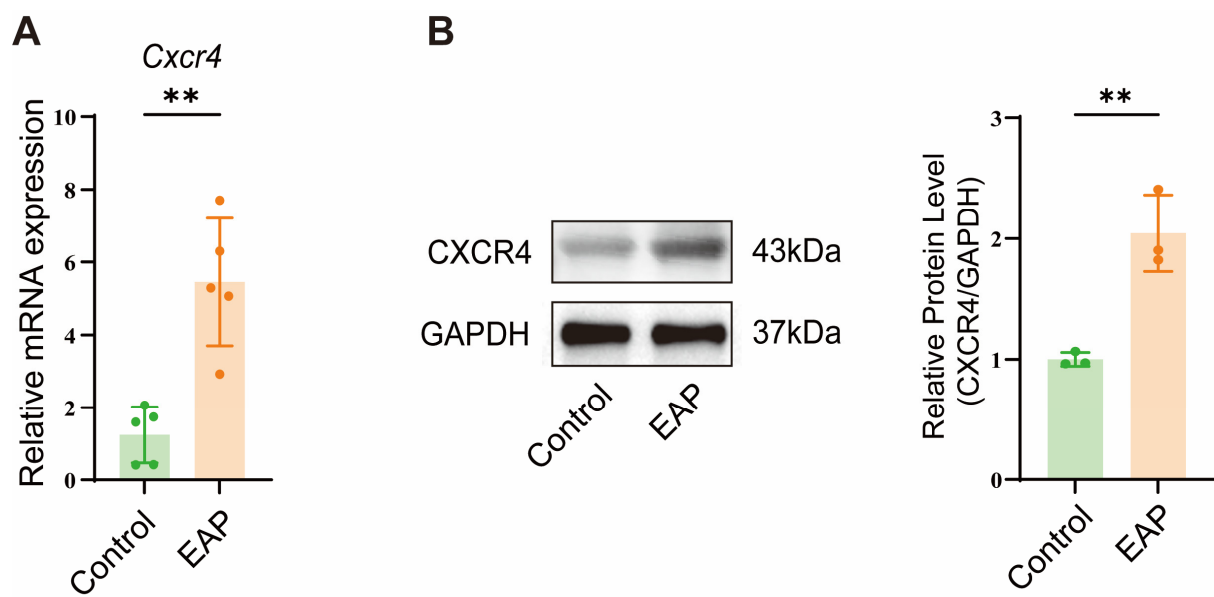


Figure S3

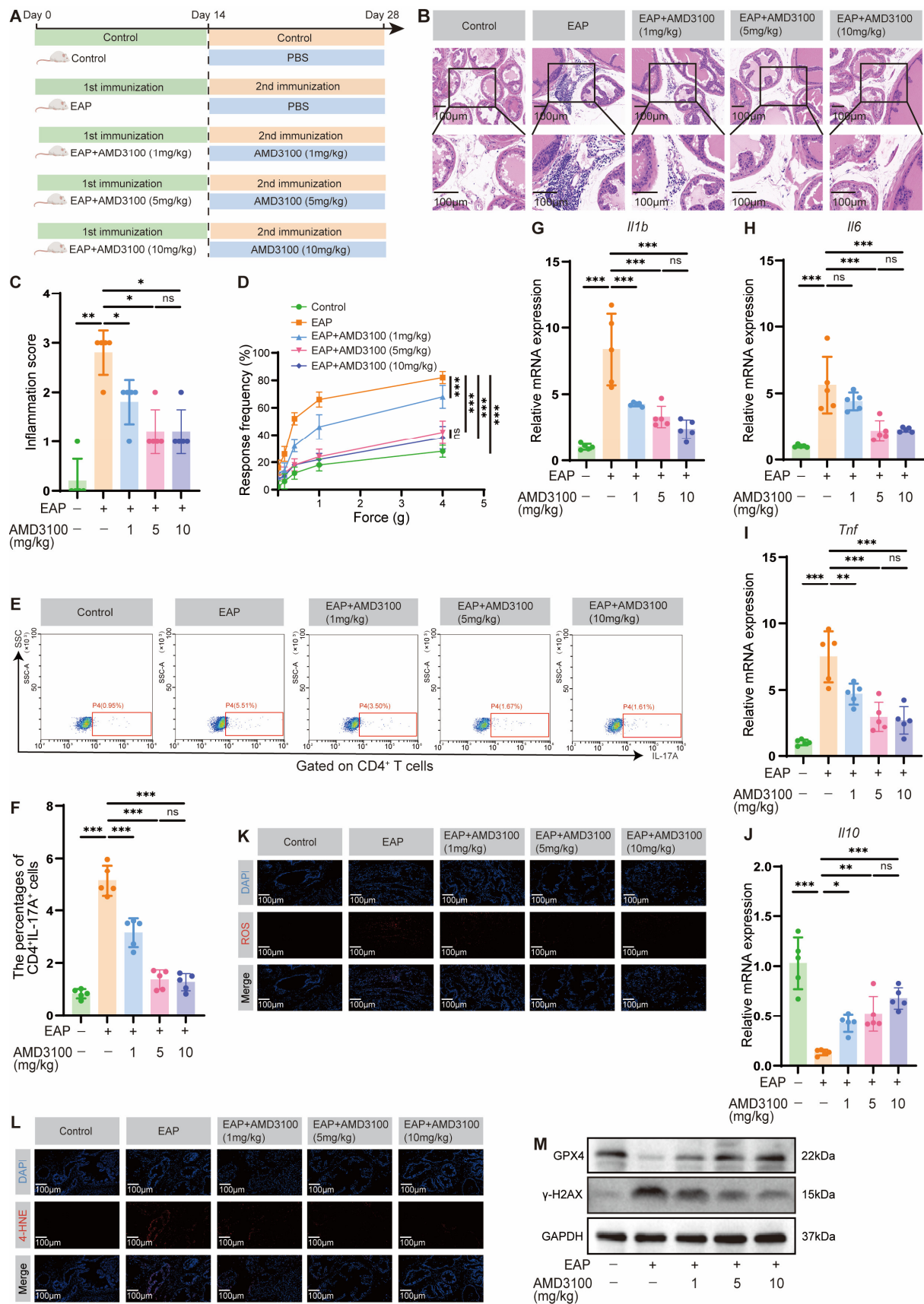


Figure S4

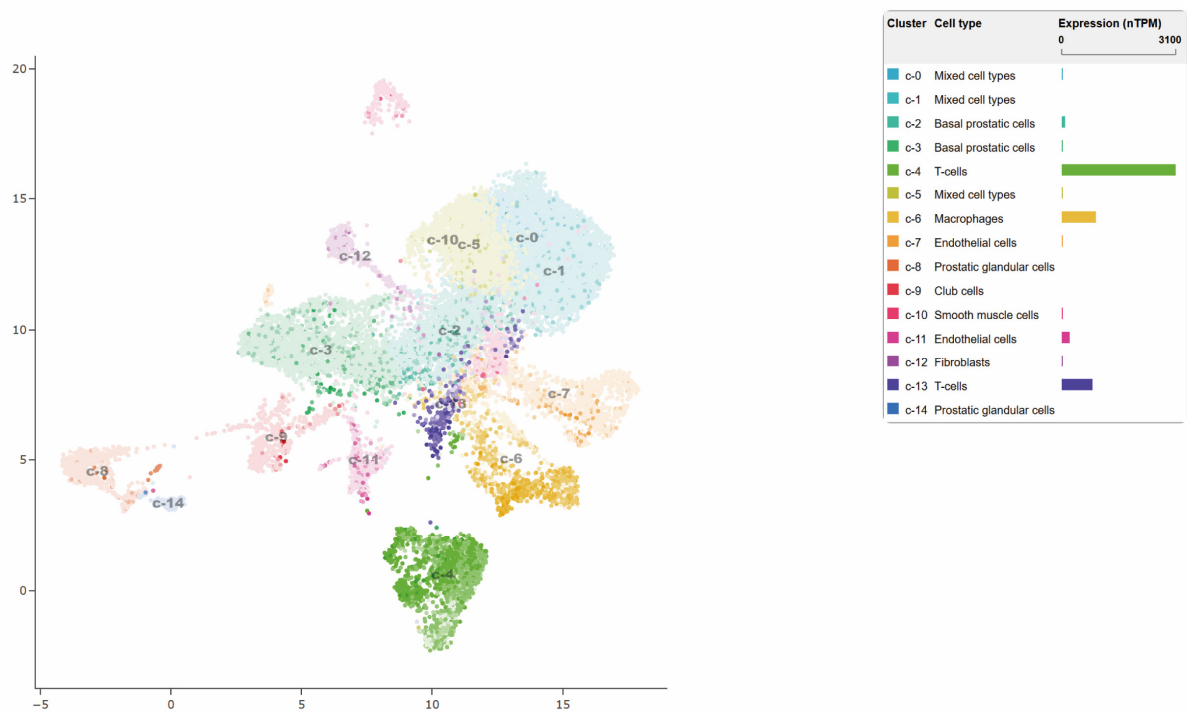


Figure S5

