

Supplementary Material

Anti-Mutagenic and Immunomodulatory Effects of Astragali Radix Extract on a Cyclophosphamide-Induced Immunosuppressed Mouse Model

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Table S1. Specific conditions for HPLC analysis.

| Item | HPLC Analysis Condition | | |
|------------|---|---|--------|
| Column | Capcellpak C18 UG120 (Osakasoda Co., Japan), 4.6×250 mm, 5 μm | | |
| Injection | 10 μL | Column temp. | 35 °C |
| Flow | 1.0 mL/min | Wavelength | 260 nm |
| Time (min) | Mobile Phase | | |
| | A (0.1% Phosphoric acid H ₂ O, %) | B (0.1% Phosphoric acid Acetonitrile, %) | |
| 0 | 87 | 13 | |
| 30 | 87 | 13 | |
| 31 | 0 | 100 | |
| 41 | 0 | 100 | |
| 42 | 87 | 13 | |
| 52 | 87 | 13 | |

Table S2. Oligonucleotides used for real time RT-PCR.

| Target | 5' – 3' | Sequence | NCBI accession No. |
|----------------|-----------|---------------------------|--------------------|
| NF-κB | Sense | CAATGGCTACACAGGACCA | NM_008689 |
| | Antisense | CACTGTCACCTGGAACCAGA | |
| Interferon-γ | Sense | GTTACTGCCACGGCACAGTCATTG | NM_008337 |
| | Antisense | ACCATCCTTTTGCCAGTTCCTCCAG | |
| TNF-α | Sense | CCTGTAGCCCACGTCGTAGC | NM_013693 |
| | Antisense | TTGACCTCAGCGCTGAGTTG | |
| Interleukin-1β | Sense | AGCTGTGGCAGCTACCTGTG | NM_008361 |
| | Antisense | GCTCTGCTTGTGAGGTGCTG | |
| Interleukin-6 | Sense | TCTTGGGACTGATGCTGGTGAC | NM_031168 |
| | Antisense | GCAGCTCCCTCTTGTGT | |
| Interleukin-12 | Sense | CAGGTGTCTTAGCCAGTCC | NM_008354 |
| | Antisense | CATAACGCACTAGGTTTGCCGA | |
| GAPDH | Sense | CATCTTCCAGGAGCGAGACC | NM_008084 |
| | Antisense | TCCACCACCCTGTTGCTGTA | |

NCBI = National Center for Biotechnology Information; real time RT-PCR = Real time reverse transcription polymerase chain reaction; NF-κB = Nuclear factor kappa B; TNF = Tumor necrosis factor; GAPDH = Glycer-aldehyde 3-phosphate dehydrogenase

Table S3. Primary antisera and immunohistochemistry detection kits used in this study.

| Antisera or detection kits | Code | Source | Dilution |
|--|-----------|--|----------|
| Primary antisera* | | | |
| Anti-tumor necrosis factor- α antibody | sc-130349 | Santa Cruz Biotechnology ¹⁾ | 1:200 |
| Anti-nitric oxide synthase2 (N-20) polyclonal antibody | sc-651 | Santa Cruz Biotechnology | 1:100 |
| Anti-interleukin-1 β (H-153) polyclonal antibody | sc-7884 | Santa Cruz Biotechnology | 1:100 |
| Anti-CD3 (PC3/188A) antibody | sc-20047 | Santa Cruz Biotechnology | 1:100 |
| Anti-CD4 (H-370) antibody | sc-7219 | Santa Cruz Biotechnology | 1:100 |
| Anti-CD8 polyclonal antibody | ab4055 | Abcam ²⁾ | 1:100 |
| Detection kits | | | |
| Vectastain Elite ABC Kit | PK-6200 | Vector Lab. Inc. ³⁾ | 1:50 |
| Peroxidase substrate Kit | SK-4100 | Vector Lab. Inc. | 1:50 |

*All antisera were diluted using 0.01 M phosphate buffered saline; CD = Cluster of differentiation; ABC= avidin-biotin-peroxidase complex; ¹⁾ Santa Cruz Biotechnology (Santa Cruz, CA, USA); ²⁾ Abcam, Cambridge, UK; ³⁾ Vector Lab. Inc., Burlingame, CA, USA)

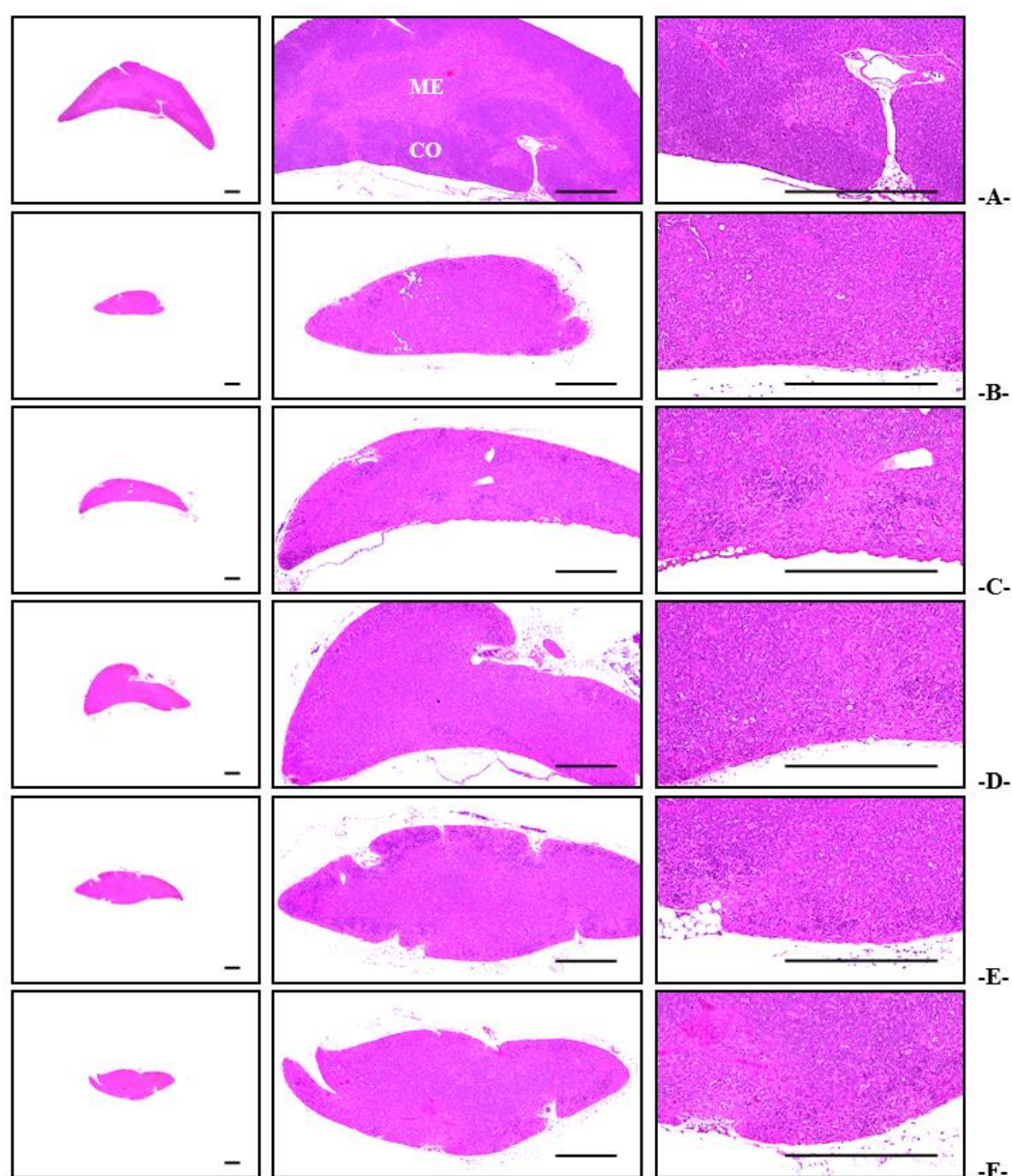


Figure S1. Representative histopathological images of the thymus obtained from intact or CPA-induced immunosuppressed mice.

A = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP₂₀₀: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR₄₀₀: CPA treated and AR (400 mg/kg) administered mice; **E** = AR₂₀₀: CPA treated and AR (200 mg/kg) administered mice; **F** = AR₁₀₀: CPA treated and AR (100 mg/kg) administered mice; AR = Astragali Radix (Roots of *A. membranaceus* Bunge) extracts; CO = cortex; ME = medulla; All Hematoxylin-Eosin stain; Scale bar = 400 μ m

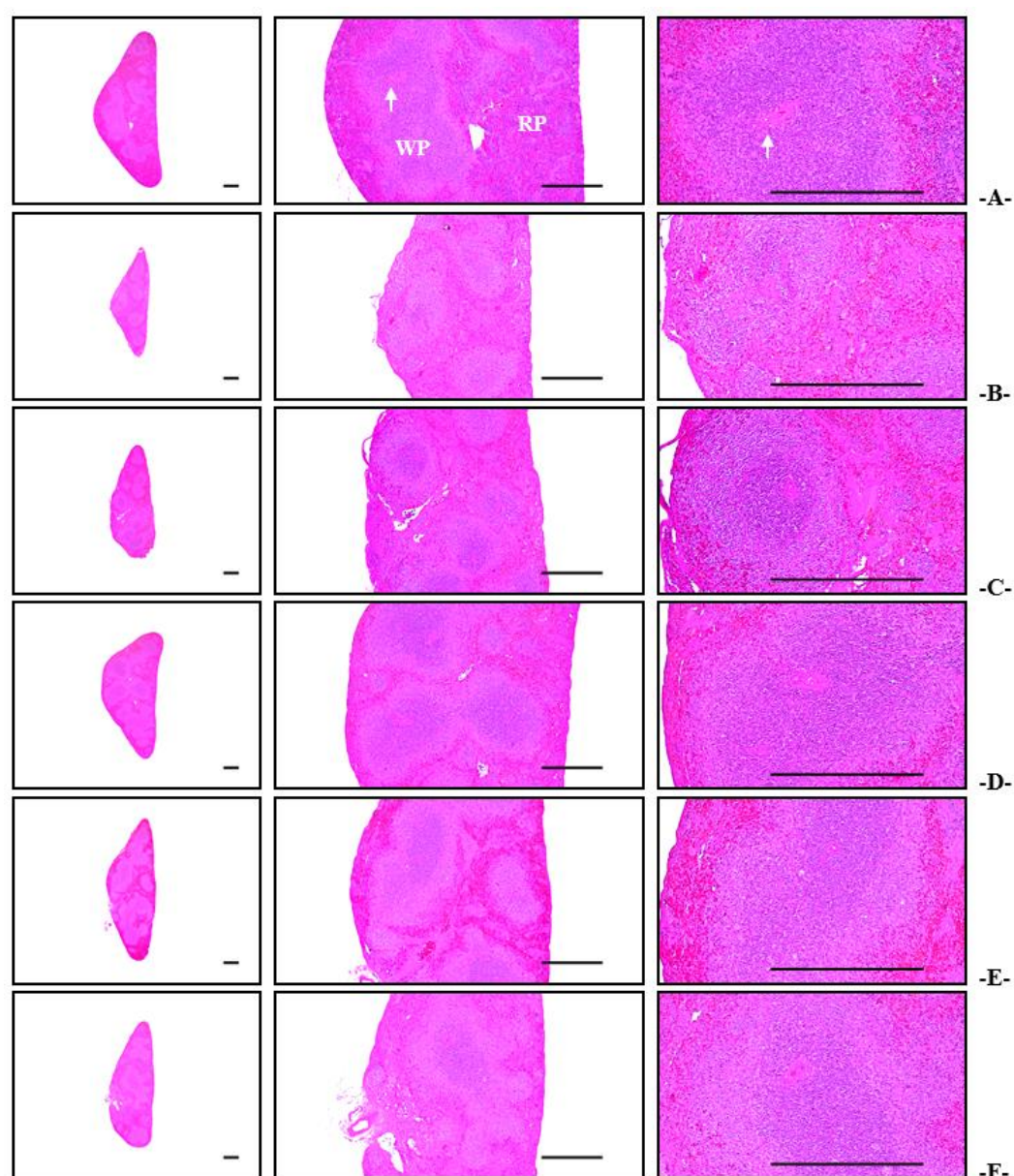


Figure S2. Representative histopathological images of the spleen, taken from intact or CPA-induced immunosuppressed mice.

A = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP₂₀₀: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR₄₀₀: CPA treated and AR (400 mg/kg) administered mice; **E** = AR₂₀₀: CPA treated and AR (200 mg/kg) administered mice; **F** = AR₁₀₀: CPA treated and AR (100 mg/kg) administered mice; AR = Astragali Radix (Roots of *A. membranaceus* Bunge) extracts; WP = white pulp; RP = red pulps; Arrow = central arteriole; All Hematoxylin-Eosin stain; Scale bar = 400 μ m

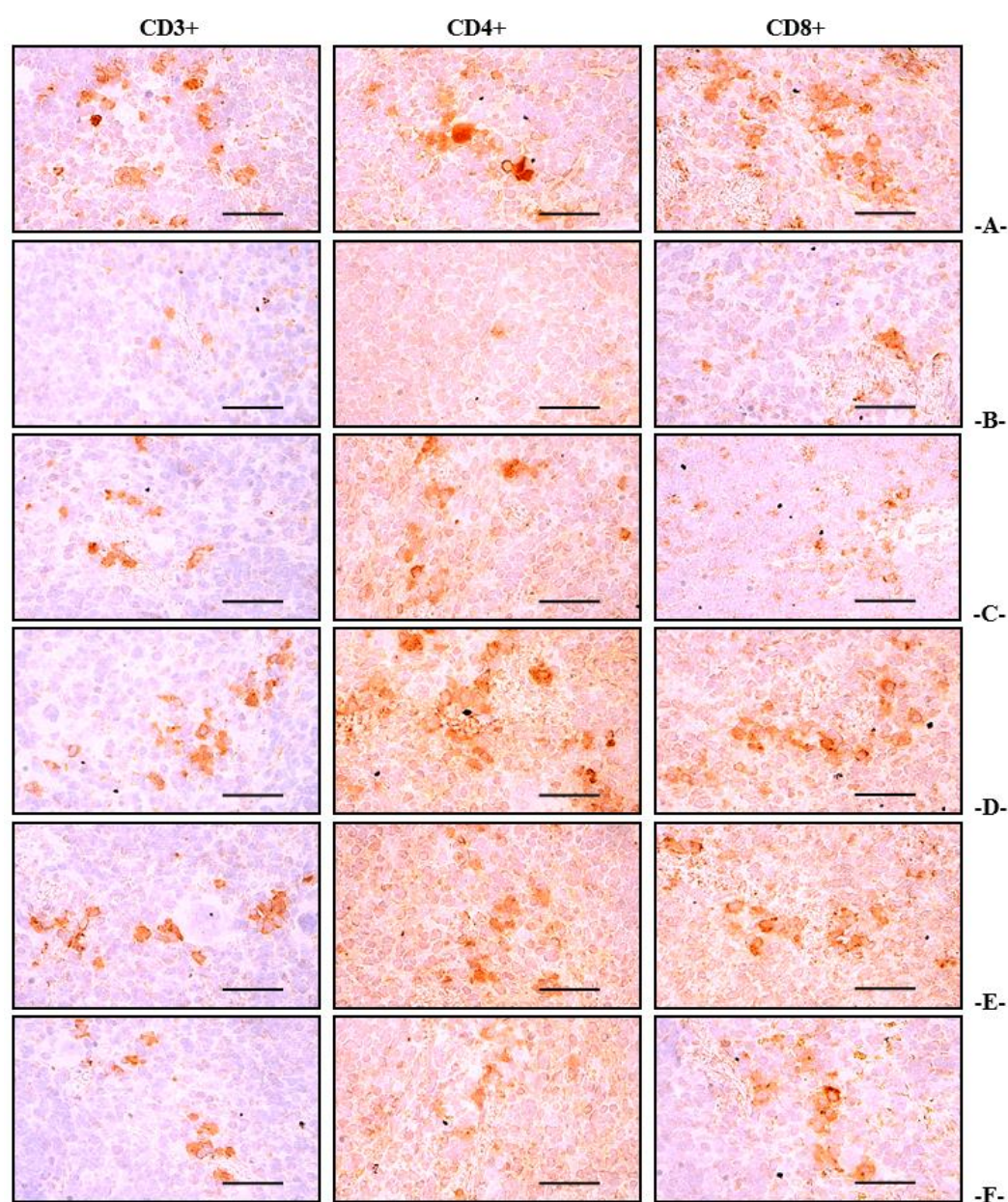


Figure S3. Representative immunohistochemical images of the CD3+, CD4+ and CD8+ Cells on the spleen obtained from intact or CPA-induced immunosuppressed mice.

A = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP₂₀₀: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR₄₀₀: CPA treated and AR (400 mg/kg) administered mice; **E** = AR₂₀₀: CPA treated and AR (200 mg/kg) administered mice; **F** = AR₁₀₀: CPA treated and AR (100 mg/kg) administered mice; AR = Astragali Radix (Roots of *A. membranaceus* Bunge) extracts; CD = Cluster of differentiation; ABC = Avidin-biotin-peroxidase complex; All ABC-based immunohistochemical stain; Scale bar = 50 μ m

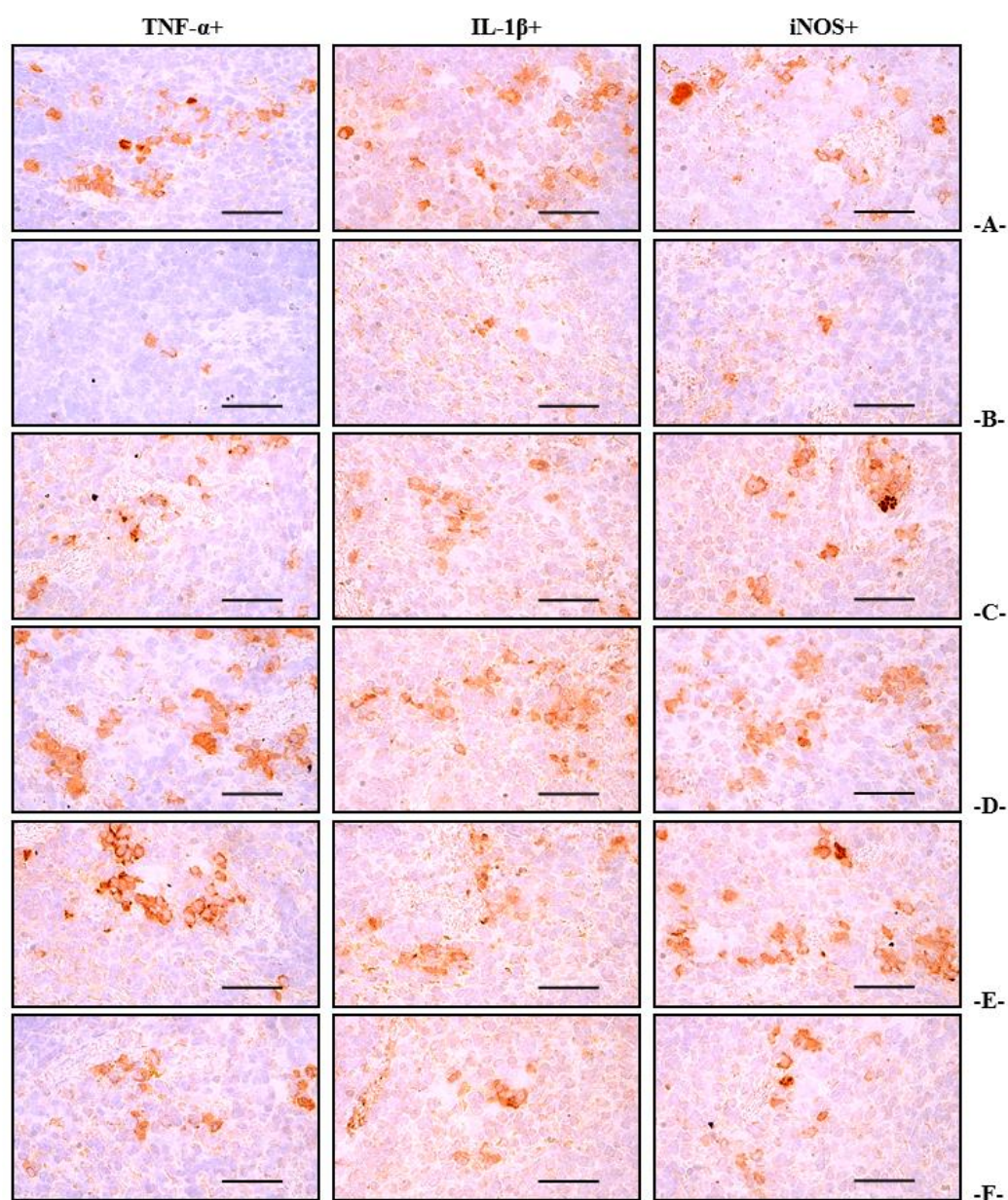


Figure S4. Representative immunohistochemical images of the TNF- α +, IL-1 β + and iNOS+ cells on the spleen obtained from intact or CPA-induced immunosuppressed mice.

A = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP₂₀₀: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR₄₀₀: CPA treated and AR (400 mg/kg) administered mice; **E** = AR₂₀₀: CPA treated and AR (200 mg/kg) administered mice; **F** = AR₁₀₀: CPA treated and AR (100 mg/kg) administered mice; AR = Astragali Radix (Roots of *A. membranaceus* Bunge) extracts; TNF = Tumor necrosis factor; IL = Interleukin; iNOS = Inducible nitric oxide synthases; ABC = Avidin-biotin-peroxidase complex; All ABC-based immunohistochemical stain; Scale bar = 100 μ m

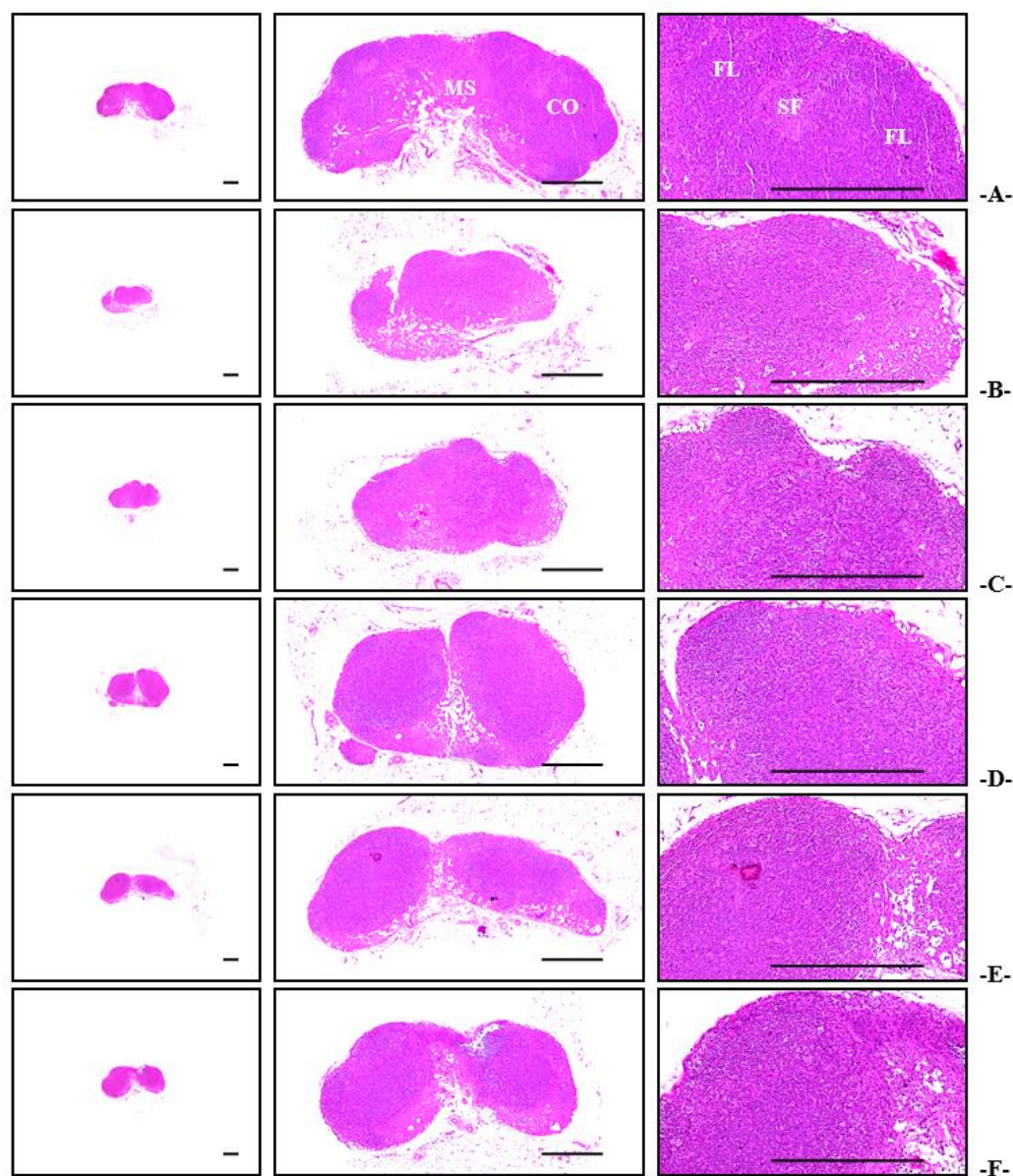


Figure S5. Representative histopathological images of the left submandibular LN, taken from intact or CPA-induced immunosuppressed mice.

A = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP₂₀₀: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR₄₀₀: CPA treated and AR (400 mg/kg) administered mice; **E** = AR₂₀₀: CPA treated and AR (200 mg/kg) administered mice; **F** = AR₁₀₀: CPA treated and AR (100 mg/kg) administered mice; AR = Astragali Radix (Roots of *A. membranaceus* Bunge) extracts; LN = Lymph node; CO = Cortex; MS = Medullary sinus; SF = Secondary follicle; FL = Follicle; All Hematoxylin and eosin stain; Scale bar = 400 μ m