

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	B	
	(0.1% Phosphoric acid H <sub>2</sub> O, %)	(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.

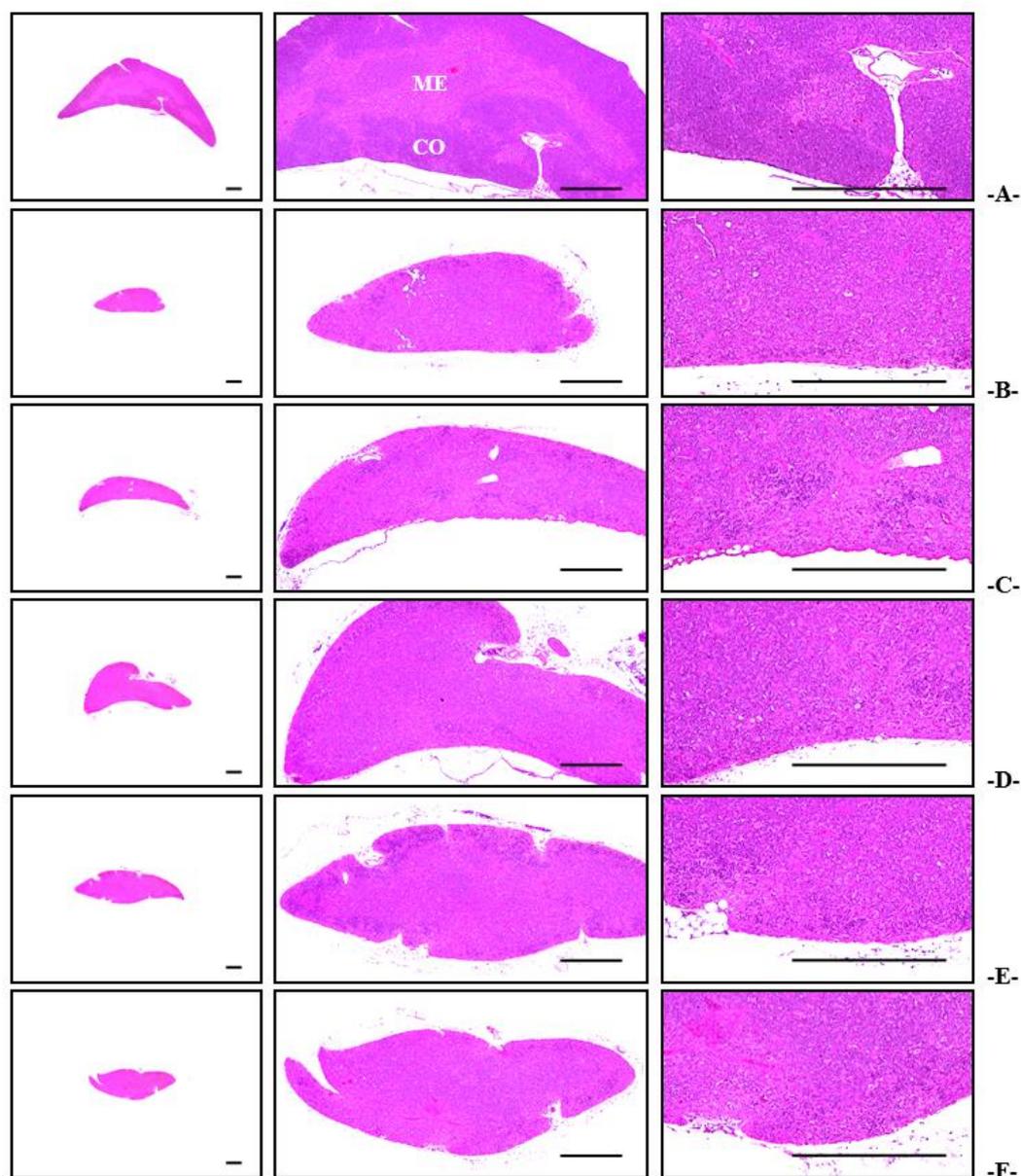
Target	5' – 3'	Sequence	NCBI accession No.
NF-κB	Sense	CAATGGCTACACAGGACCA	NM_008689
	Antisense	CACTGTCACCTGGAACCAGA	
Interferon-γ	Sense	GTTACTGCCACGGCACAGTCATTG	NM_008337
	Antisense	ACCATCCTTTTGCCAGTTCCTCCAG	
TNF-α	Sense	CCTGTAGCCCACGTCGTAGC	NM_013693
	Antisense	TTGACCTCAGCGCTGAGTTG	
Interleukin-1β	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 = Glyceraldehyde 3-phosphate dehydrogenase

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

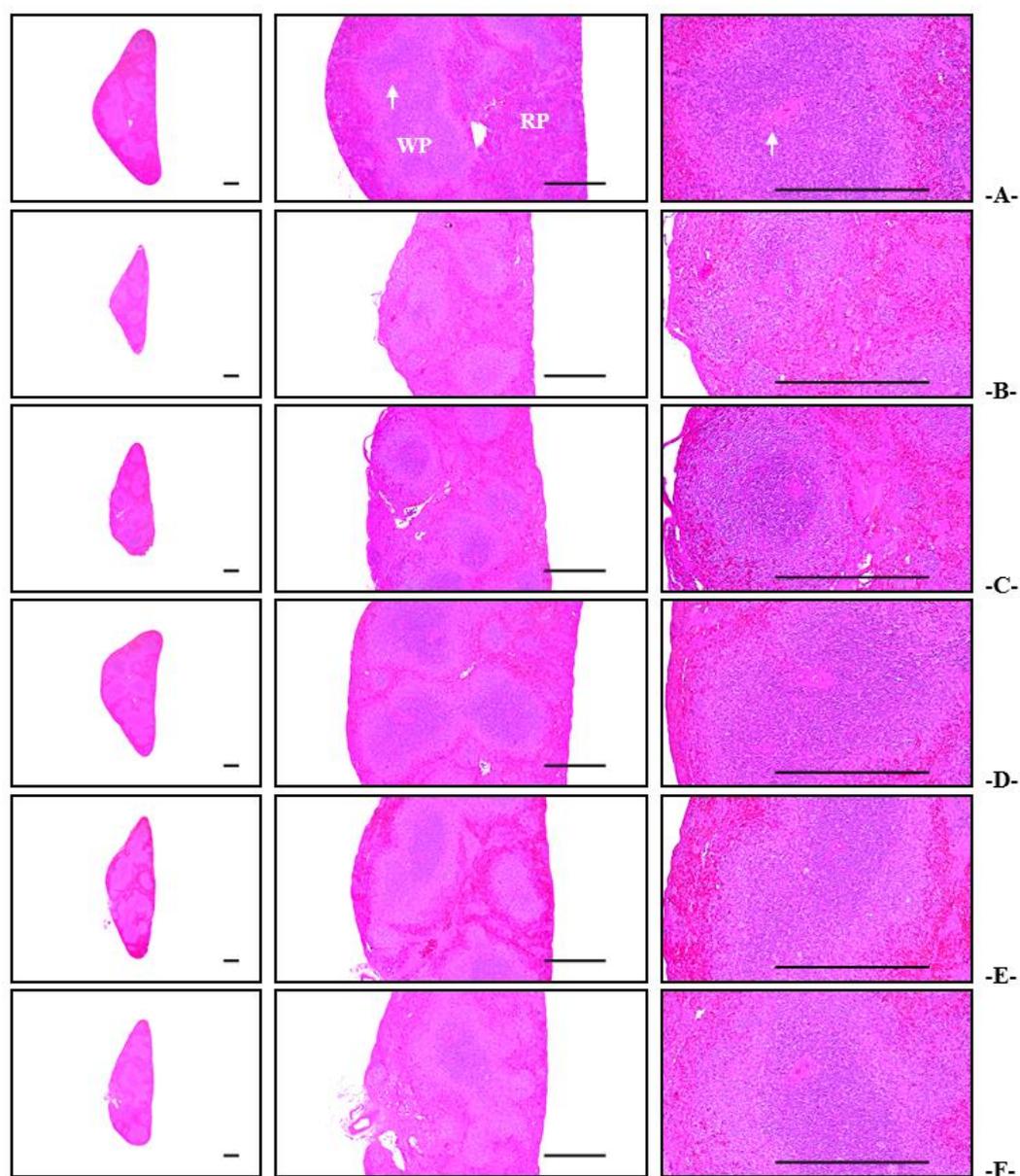
Antisera or detection kits	Code	Source	Dilution
<b>Primary antisera*</b>			
Anti-tumor necrosis factor- $\alpha$ antibody	sc-130349	Santa Cruz Biotechnology <sup>1)</sup>	1:200
Anti-nitric oxide synthase2 (N-20) polyclonal antibody	sc-651	Santa Cruz Biotechnology	1:100
Anti-interleukin-1 $\beta$ (H-153) 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 <sup>2)</sup>	1:100
<b>Detection kits</b>			
Vectastain Elite ABC Kit	PK-6200	Vector Lab. Inc. <sup>3)</sup>	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; <sup>1)</sup> Santa Cruz Biotechnology (Santa Cruz, CA, USA); <sup>2)</sup> Abcam, Cambridge, UK; <sup>3)</sup> Vector Lab. Inc., Burlingame, CA, USA)



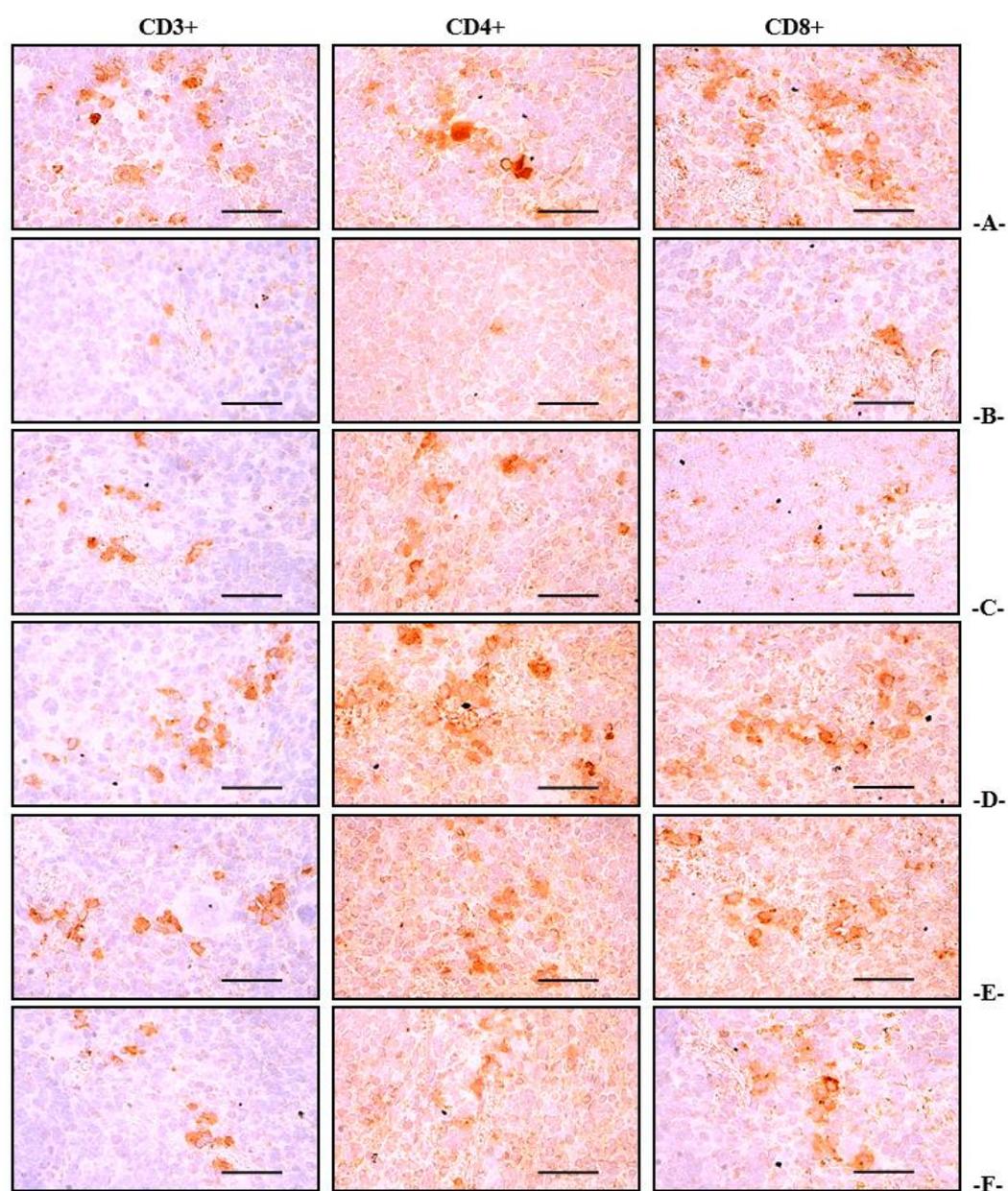
**Figure S1.** Representative histopathological images of the thymus obtained from intact or CPA-induced immunosuppressed mice.

**A** = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP<sub>200</sub>: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR<sub>400</sub>: CPA treated and AR (400 mg/kg) administered mice; **E** = AR<sub>200</sub>: CPA treated and AR (200 mg/kg) administered mice; **F** = AR<sub>100</sub>: 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  $\mu$ m



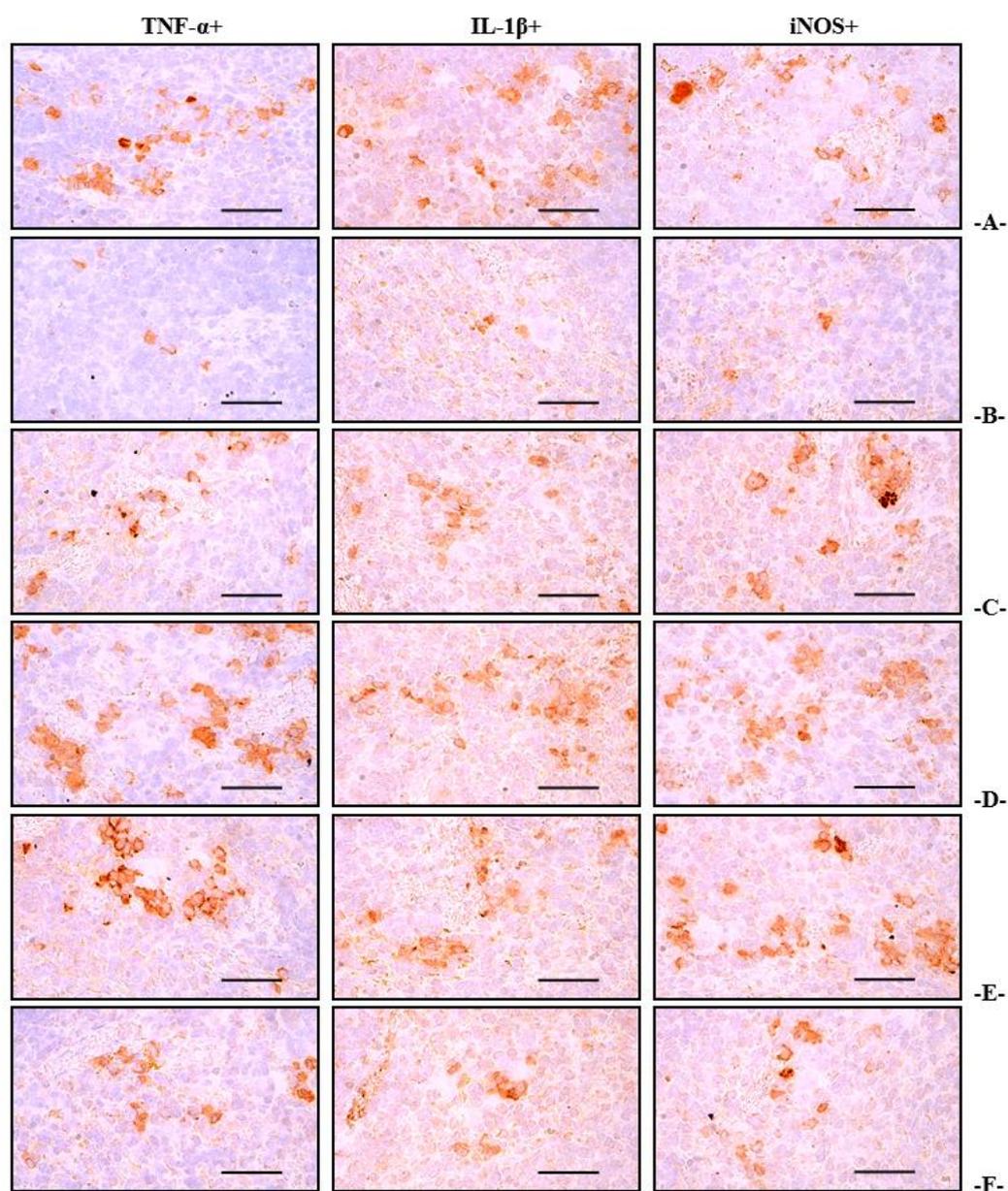
**Figure S2.** Representative histopathological images of the spleen, taken from intact or CPA-induced immunosuppressed mice.

**A** = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP<sub>200</sub>: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR<sub>400</sub>: CPA treated and AR (400 mg/kg) administered mice; **E** = AR<sub>200</sub>: CPA treated and AR (200 mg/kg) administered mice; **F** = AR<sub>100</sub>: 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  $\mu$ m



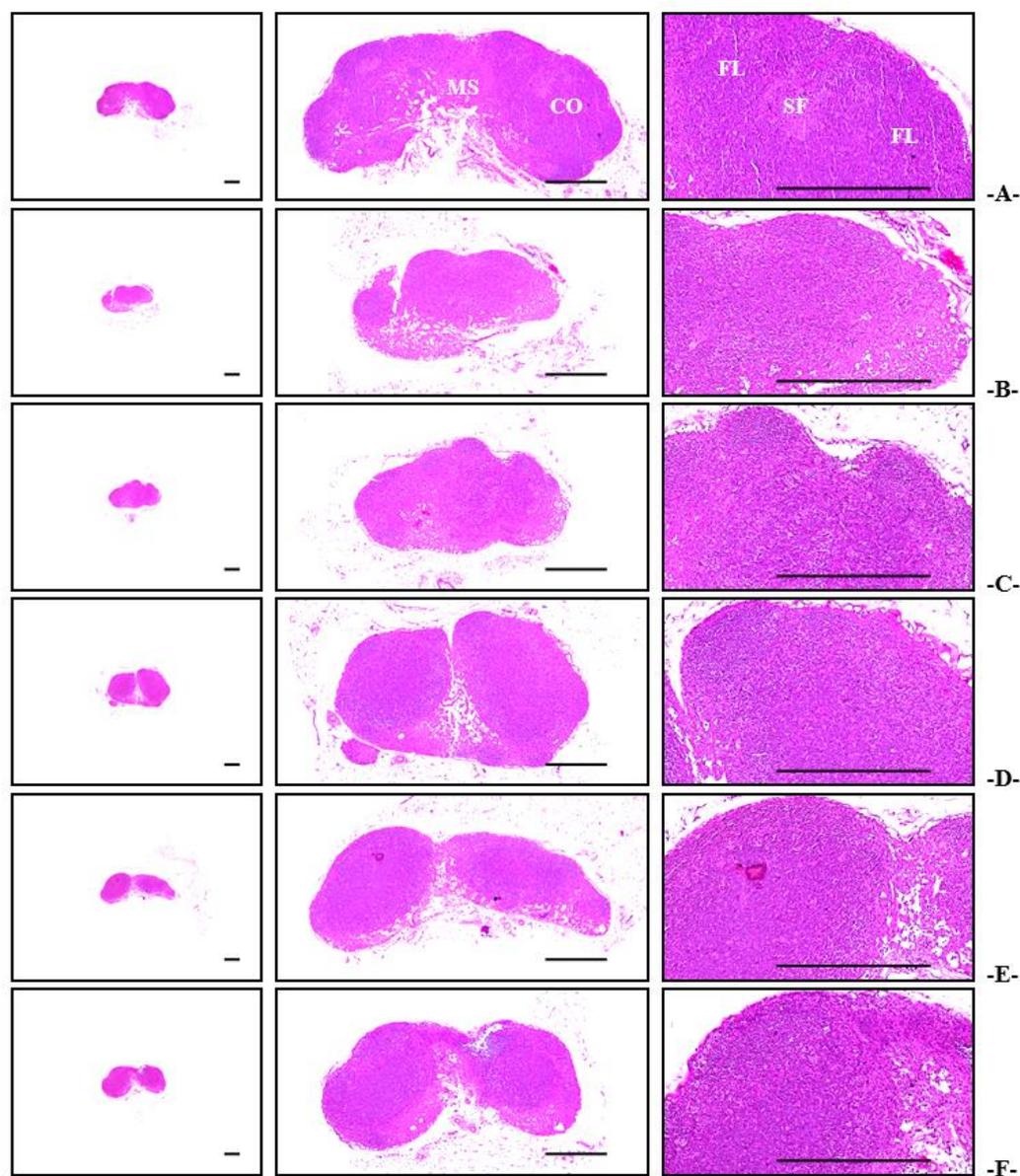
**Figure S3.** Representative immunohistochemical images of the CD3+, CD4+ and CD8+ Cells on the spleen obtained from intact or CPA-induced immunosuppressed mice.

**A** = Intact vehicle control: Distilled water administered intact mice; **B** = CPA control: Cyclophosphamide treated and distilled water administered control mice; **C** = EAP<sub>200</sub>: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR<sub>400</sub>: CPA treated and AR (400 mg/kg) administered mice; **E** = AR<sub>200</sub>: CPA treated and AR (200 mg/kg) administered mice; **F** = AR<sub>100</sub>: 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  $\mu$ m



**Figure S4.** Representative immunohistochemical images of the TNF- $\alpha$ +, IL-1 $\beta$ + 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** = EAP<sub>200</sub>: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR<sub>400</sub>: CPA treated and AR (400 mg/kg) administered mice; **E** = AR<sub>200</sub>: CPA treated and AR (200 mg/kg) administered mice; **F** = AR<sub>100</sub>: 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  $\mu$ 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** = EAP<sub>200</sub>: CPA treated and *A. pullulans* purified exopolymers (EAP at a dose of 200 mg/kg) administered mice; **D** = AR<sub>400</sub>: CPA treated and AR (400 mg/kg) administered mice; **E** = AR<sub>200</sub>: CPA treated and AR (200 mg/kg) administered mice; **F** = AR<sub>100</sub>: 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  $\mu$ m