

Supplementary Materials: *Astragalus* Polysaccharide RAP Induces Macrophage Phenotype Polarization to M1 via Notch Signaling Pathway

Wei Wei, Zhi-Peng Li, Zhao-Xiang Bian and Quan-Bin Han

Table S1. Primers used in RT-PCR assay.

Gene name		Primers
TNF- α	forward	5'-ATGAGCACAGAAAGCATGATC-3'
	reverse	5'-TACAGGCTTGTCACCTCGAATT-3'
IL-6	forward	5'-GATGCTACCAAACCTGGATATAATC-3'
	reverse	5'-GGTCCTTAGCCACTCCTTCTGTG-3'
iNOS	forward	5'-GCCGTGGCCAACATGCTACT-3'
	reverse	5'-GGTCTTCCTGGGCTCGATCTG-3'
CXCL10	forward	5'-CCAAGTGCTGCCGTCATTTTC-3'
	reverse	5'-GGCTCGCAGGGATGATTTCAA-3'
Mannose receptor	forward	5'-CAAGGAAGGTTGGCATTGT-3'
	reverse	5'-CCTTTCAGTCCTTTGCAAGC-3'
Arginase 1	forward	5'-TGGCTTGCGAGACGTAGAC-3'
	reverse	5'-GCTCAGGTGAATCGGCCTTTT-3'
Notch 1	forward	5'-AGAATGGCATGGTGCCAG-3'
	reverse	5'-TGGTGGAGAGGCTGCTGTGTAG-3'
Notch 2	forward	5'-GATGGAGGTGACTGTTCCCTCA-3'
	reverse	5'-CGTCTTGCTATTCCCTCTGGCAC-3'
Notch 3	forward	5'-GATTTCCCATACCCACTTCGG-3'
	reverse	5'-TGTGTAATGCAAAACCCTCAGG-3'
Notch 4	forward	5'-GTTGAAGAATTGATCGCAGCC-3'
	reverse	5'-AGGAAAAGCGGCGTCTGTT-3'
Jaddge 1	forward	5'-AGAAGTCAGAGTTCAGAGGCGTCC-3'
	reverse	5'-AGTAGAGGCTGTCACCAAGCAAC-3'
Dll 1	forward	5'-GGACCTCAGTGAGAGGCATATGG-3'
	reverse	5'-GGCAATTGGCTAGGTTGTTTCATG-3'
β -actin	forward	5'-TGTCCACCTTCCAGCAGATGT-3'
	reverse	5'-AGCTCAGTAACAGTCCGCCTAGA-3'

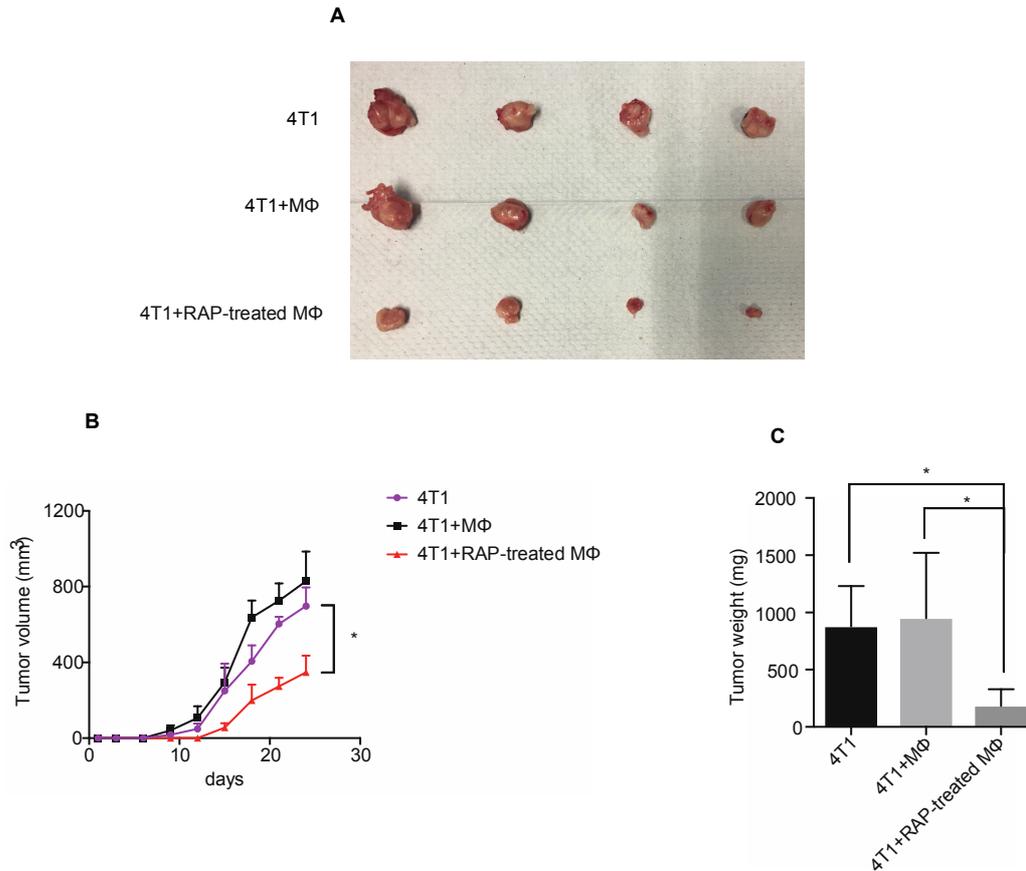


Figure S1. Tumoricidal effect of RAP-stimulated BMDMs in 4T1-induced animal model.

The BALB/c mice (4 mice/group) were injected with 4T1 cells, 4T1 cells plus BMDMs, and 4T1 cells plus RAP-treated BMDMs in mammary grand fat pad. Tumor volumes were measured every 3 days (B), and tumor weight were determined after 26 days. A significant difference of tumor weight was detected by one way ANOVA test ($p < 0.05$) (A and C).

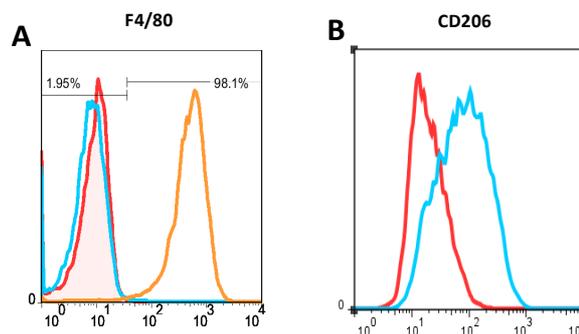


Figure S2. Histogram of F4/80 and CD206 fluorescent intensity of BMDMs induced by MCSF and IL-4 was analyzed by FCM.

(A) The bone marrow cells were treated with or without 10 ng/mL of MCSF for 7 days. Then the cells were stained with APC anti-F4/80 antibody and analyzed with FCM. The red line represents the isotype control. The orange line represents the bone marrow cells treated with 10 ng/mL of MCSF for 7 days. (B) BMDMs treated with or without 20 ng/mL of IL-4 for 24 h were stained with

FITC anti-CD206 antibody and analyzed with FCM. The red line represents the BMDMs. The blue line represents the BMDMs treated with 20 ng/mL of IL-4 for 24 h.

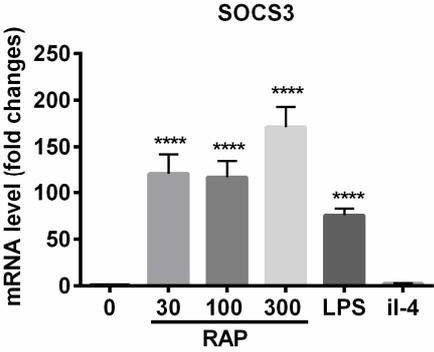


Figure S3. SOCS3 gene expression induced by RAP.