Supplemental Materials

Inventory

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Table S1. Clinicopathological Features of Human NSCLC Patients

Patients and tumor characteristics	Value Mean ± SD (range) or No (%) N=16
Age (year)	61.25 ± 8.25
Gender	
Male	6 (37.5%)
Female	10 (62.5%)
Histology	
Adenocarcinoma	10 (62.5%)
Squamous cell carcinoma	6 (37.5%)
TNM stage	
I	11 (68.75%)
II	3 (18.75%)
III	2 (12.5%)
IV	0
T- factor	
T1	6 (37.5%)
T2	9 (56.25%)
T3	1 (6.25%)
T4	0
N- factor	
N0	13 (81.25%)
N1/2	3 (18.75%)
Smoking status	
Non-smoker	3 (18.75%)
Smoker	13 (81.25%)

Table S2. Primer sequences for qRT-PCR and ChIP-qPCR

Gene	Primers	
mc-Maf	Forward	5’-AGCAGTTGGTGACCATGTCG-3’
	Reverse	5’-TGGAGATCTCCTGCTTGAGG-3’
mCSF-1R CNS+3	Forward	CAGCAATGTGTTTCCGCCC
	Reverse	TCAAACCCCCTGTCAGGTCT
mCSF-1R CNS-5	Forward	TGAGAAGCGAGAGCCCTAAGT
	Reverse	CACCAGATTGTCCACCTCCAC
mCSF-1R CNS+0.6	Forward	CCTAGGCTTTGACTCACGGT
	Reverse	ACTCCCAGGTAACACCCCAA
mCSF-1R CNS+0.7	Forward	CTGGGGACAAGGGGACATAC
	Reverse	TCGAGTTGAGTTGACTGGCA
mIL-10	Forward	5’-AGTGGAGCAGGTGAAGAGTG-3’
	Reverse	5’-TTCGGAGAGAGGTACAAACG-3’
mArginase-1	Forward	5’-TTTTAGGGTTACGGCCGGTG-3’
	Reverse	5’-CCTCGAGGCTGTCCTTTTGA-3’
mIL-1β	Forward	5’- GCCACCTTTTGACAGTGATGAG-3’
	Reverse	5’- GACAGCCCAGGTCAAAGGTT-3’
mIL-6	Forward	5’- AAGACAAAGCCAGAGTCCTTCA-3’
	Reverse	5’- AGAGCATTGGAAATTGGGGT-3’
mTNF-α	Forward	5’- TGTAGCCCACGTCGTAGCAAA-3’
	Reverse	5’- GCTGGCACCACTAGTTGGTTGT-3’
miNOS	Forward	5’- GGTGAAGGGACTGAGCTGTTA-3’
	Reverse	5’- CAACGTTCTCCGTTCTCTTGC-3’
mIL-12p35	Forward	5’- CTCCTAAACCACCTCAGTTTGG-3’
	Reverse	5’- CATGATCGATGTCTTCAGCAGT-3’
mβ-MG	Forward	5’-CTTTCTGGTGCTTGTCTC-3’
	Reverse	5’-TCAGTATGTTCGGCTTCC-3’
mVEGF	Forward	5’-TTACTGCTGTACCTCCACC-3’
	Reverse	5’-ACAGGACGGCTTGAAGATG-3’

mIRF4	Forward	5’- CCATTGAGCCAAGCATAAGG-3’
	Reverse	5’- CTCGTCGTGGTCAGCTCTTT-3’
mIRF5	Forward	5’-AATACCCCACCACCTTTTGA-3’
	Reverse	5’-TTGAGATCCGGGTTTGAGAT-3’
mIRF8	Forward	5’-CTACTGCACAGCAACCGCAA-3’
	Reverse	5’-ACCACCCTGCTGTCAGGTA-3’
mTGF-β1	Forward	5’-TGCTAATGGTGGACCGCAA-3’
	Reverse	5’-CACTGCTTCCCGAATGTCTGA-3’
mCCL5	Forward	5’-CAATCTTGCAGTCGTGTTTG-3’
	Reverse	5’-GGAGTGGGAGTAGGGGATTA-3’
mCCR2	Forward	5’-GGAGAAAAGCCAACTCCTTC-3’
	Reverse	5’-AGGCAGTTGCAAAGGTACTG-3’
hB2M	Forward	5’- TCTTTCAGCAAGGACTGGTCT-3’
	Reverse	5’- ACATGTCTCGATCCCACTTAAC-3’
hc-Maf	Forward	5’- CAGCAAGGAGGAGGTGATCC-3’
	Reverse	5’- GGTTCTTCTCCGACTCCAGG-3’
hIL-10	Forward	5’-CCCACTTCCCAGGCAACCTGC-3’
	Reverse	5’-CCCAGGGAGTTCACATGCGCC-3’
hIL-12p35		Hs-IL12A-1-SG (Qiagen, Cat.no.: QT00000357)
hIL-23p19	Forward	5’- GCTTCATGCCTCCCTACTGG-3’
	Reverse	5’- TTGAAGCGGAGAAGGAGACG-3’
hTNF-α	Forward	5’-ACCCACGGCTCCACCCTCTC-3’
	Reverse	5’-CCCTCTGGGGGCCGATCACT-3’
hIL-6	Forward	5’-TCCCCTCCAGGAGCCCAGCTA-3’
	Reverse	5’-CAGGGCTGAGATGCCGTCGAG-3’

Supplemental Figure 1

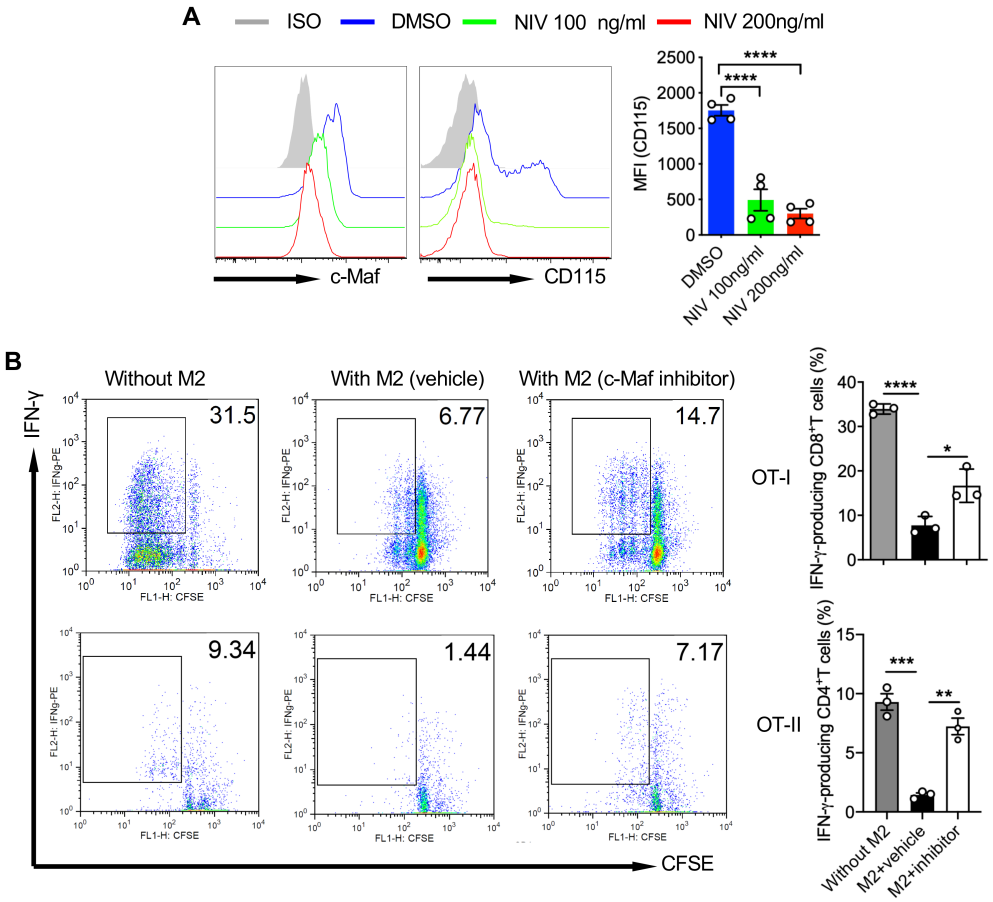


Figure S1. c-Maf inhibition abrogates CD115 expression and reduces M2 BMM-mediated T cell suppression. (**A**) M2 BMM were treated with c-Maf inhibitor or vehicle and c-Maf and CD115 expression levels were detected by flow cytometry. Representative histograms and summarized expression levels are shown. MFI: mean fluorescent intensity. Representative of at least three experiments. Data are shown as mean ± SEM. ****P <0.0001, by one-way ANOVA analysis with Dunnett's multiple comparisons test. (**B**) Splenocytes from OT-I or OT-II mice were labeled with CFSE and then co-cultured with polarized M2 BMM in the presence of c-Maf inhibitor or vehicle control for 3 days. Cells without M2 BMM were used as control. Representative dot plots and summarized IFN-γ-producing CD4 and CD8 T cells are shown. Data are representative of at least two independent experiments with similar results. Data are shown as mean ± SD (n=3). *P <0.05, **P <0.01, ***P <0.001, ****P <0.0001 by by one-way ANOVA with *post hoc t* test and Bonferroni's correction.

Supplemental Figure 2

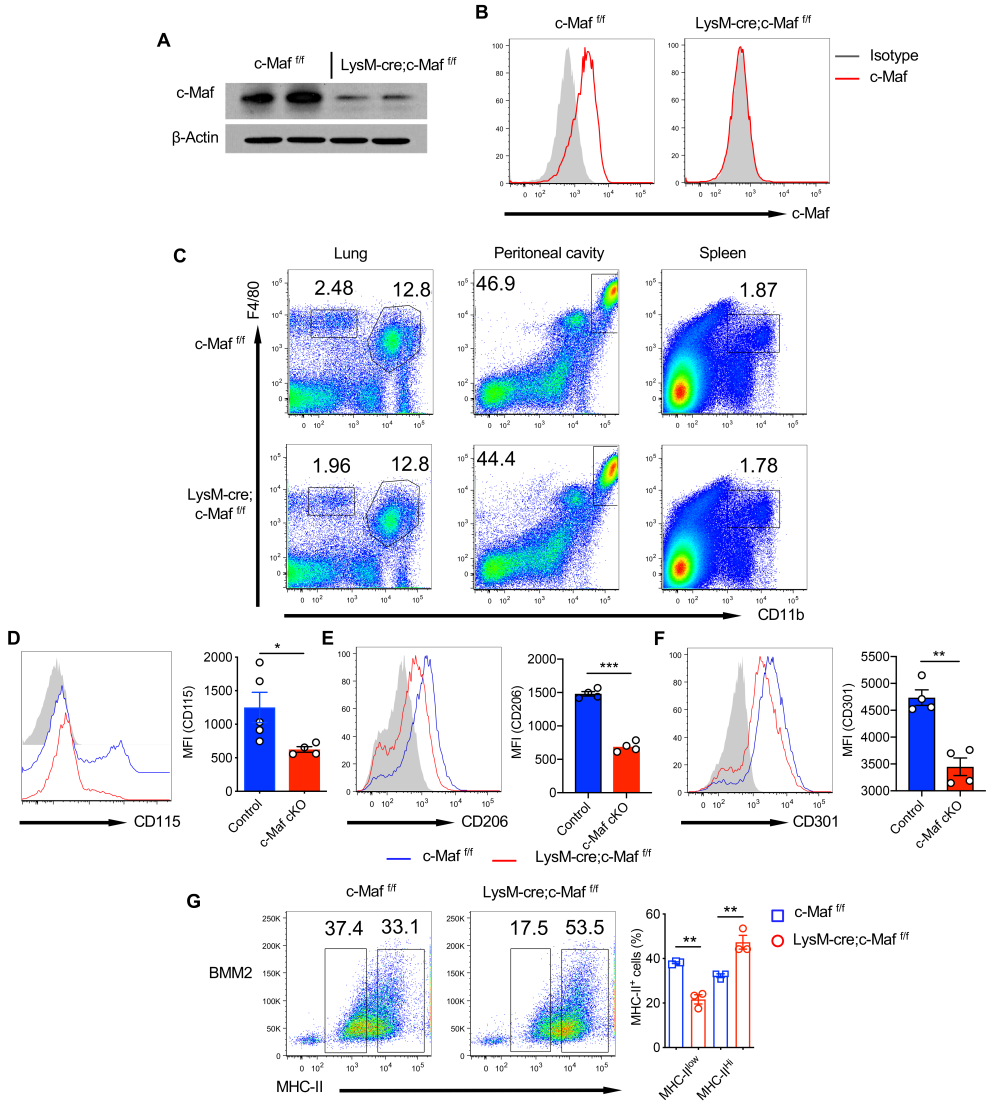


Figure S2. Conditional deletion of c-Maf in M2 macrophages leads to decreased CD115, CD206, and CD301 expression. (**A, B**) Polarized M2 BMM from c-Maf $^{f/f}$ control mice and LysM-cre;c-Maf $^{f/f}$ mice were lysed and c-Maf expression was determined by WB analysis (**A**) or intracellular flow analysis (**B**). (**C**) Single cell suspensions from lung, peritoneal cavity, and spleen were stained with CD11b and F4/80. Cells were gated on viable cells. (**D, E, F, G**). Polarized M2 BMM from c-Maf $^{f/f}$ control mice and LysM-cre;c-Maf $^{f/f}$ mice were stained for CD115 (**D**), CD206 (**E**), CD301 (**F**), and MHC II (**G**). Representative histograms or dot plots and summarized data are shown. Data are shown as mean ± SEM. **P* <0.05, ***P* <0.01, ****P* <0.001, by two-tailed, unpaired *t* test.

Supplemental Figure 3

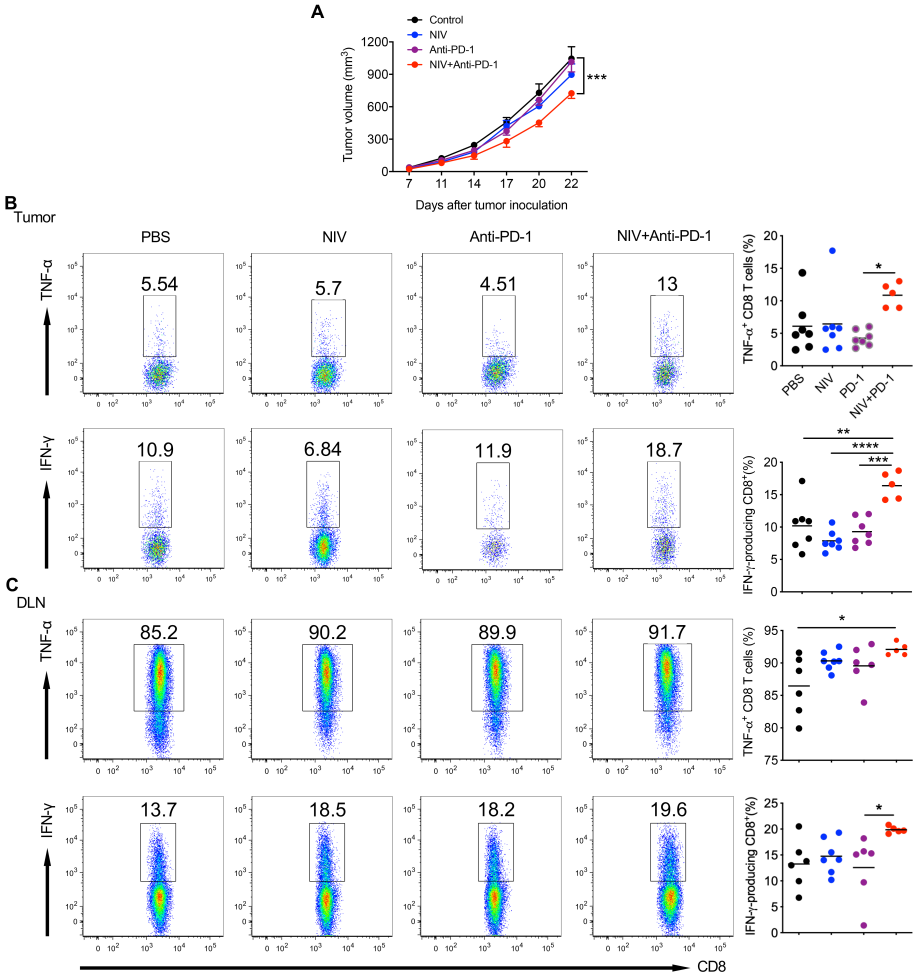


Figure S3. Inhibition of c-Maf promotes anti-PD-1 therapeutic efficacy in LLC s.c model. (**A**) LLC-bearing mice were treated with c-Maf inhibitor Nivalenol (NIV) with or without anti-PD-1. Tumor growth curve is shown. ***P <0.001 by mixed-effects two-way ANOVA with Sidak's multiple comparisons test. (**B, C**) Single cell suspensions from tumor and draining lymph node (DLN) were stimulated with PMA+ionomycin. Cells were stained for CD8 and intracellular TNF-α and IFN-γ production. Representative dot plots and summarized percentages of TNF-α- and IFN-γ-producing $CD8^+$ T cells are shown. Cells were gated on $CD8^+$ cells. Data are shown as mean ± SEM. *P <0.05, **P <0.01, ***P <0.001, ****P <0.0001, by one-way ANOVA with *post hoc t* test and Bonferroni's correction.

Supplemental Figure 4

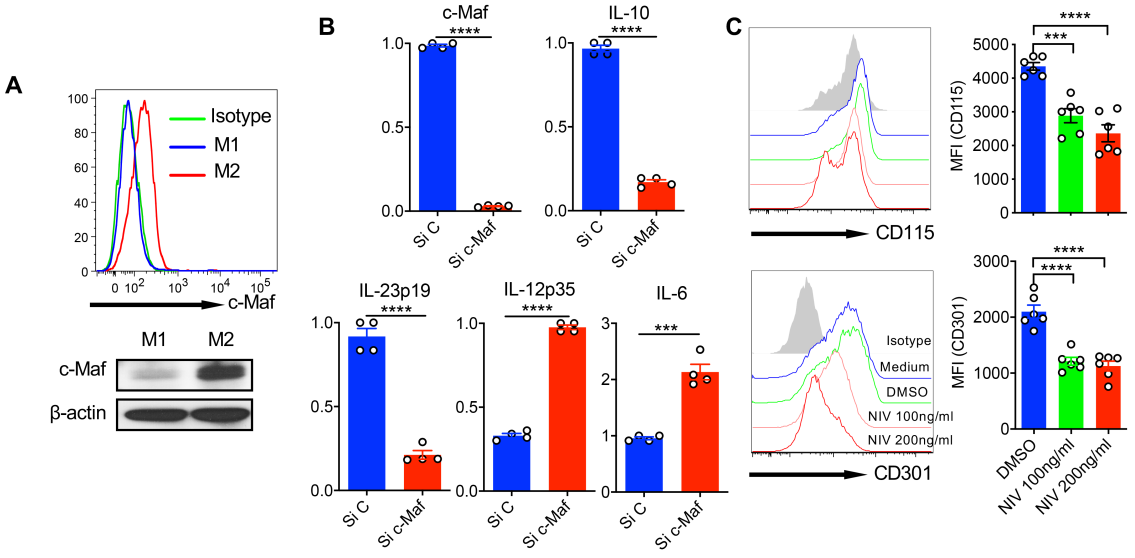


Figure S4. c-Maf is expressed in human M2 macrophages and regulates M2-related gene expression and CD115 and CD301 expression. (**A**) c-Maf expression in polarized human M1-like and M2-like macrophages determined by flow cytometry and WB analysis. Data are representative of at least three independent experiments with similar results. (**B**) Human M2 macrophages were transfected with c-Maf (Si c-Maf) or control siRNA (Si C) and the specific gene mRNA expression levels were determined by qPCR. ***P <0.001, ****P <0.0001, by two-tailed, unpaired t test. (**C**) Human M2 macrophages were treated with c-Maf inhibitor or vehicle control for 24 h and the expression levels of CD115 and CD301 were determined by flow cytometry. Representative histograms and summarized data are shown. Data are shown as mean ± SEM. ***P <0.001, ****P <0.0001, by one-way ANOVA with Dunnett's multiple comparisons test.

Supplemental Figure 5

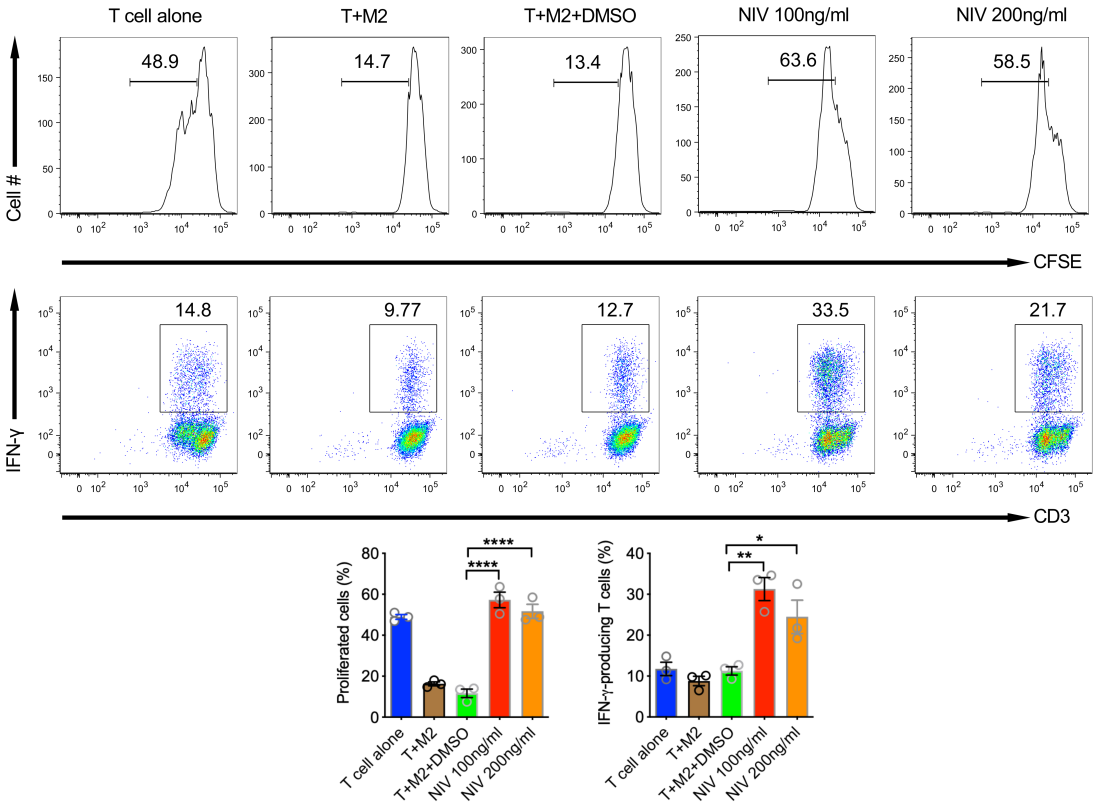
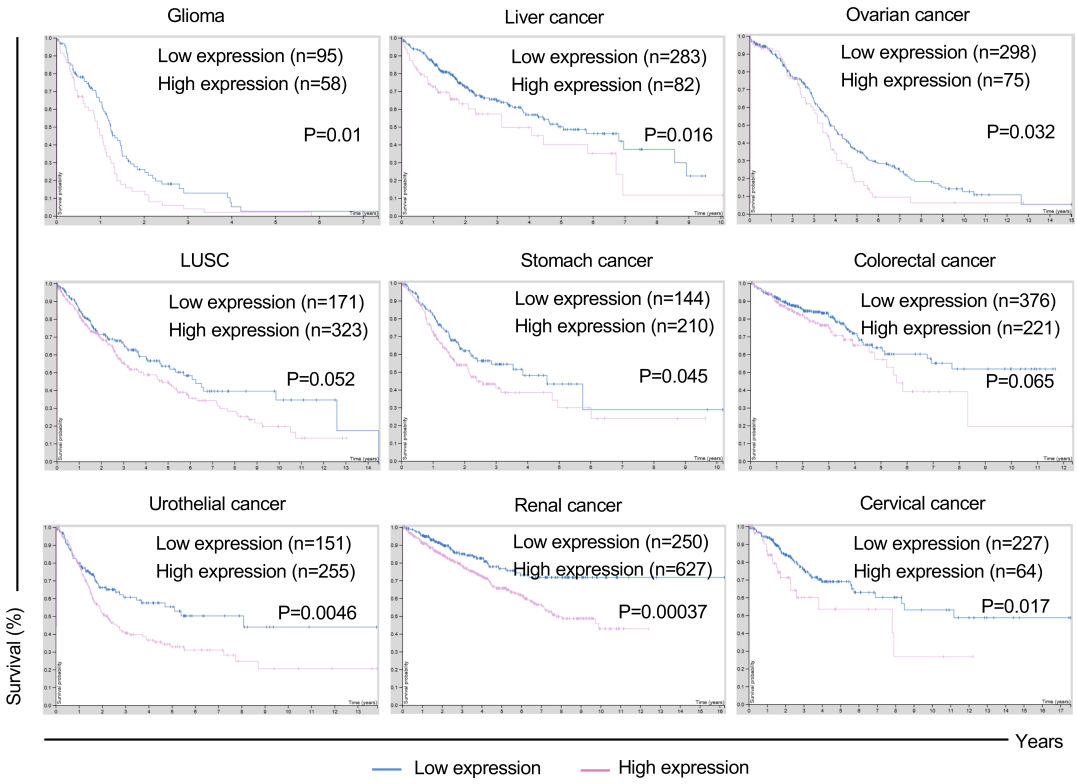


Figure S5. Inhibition of c-Maf in human M2 macrophages abrogates its immunosuppressive activity. Human polarized M2 macrophages were co-cultured with CFSE-labeled autologous T cells stimulated with CD3/CD28 beads in the presence of c-Maf inhibitor or vehicle control DMSO for three days. Cell proliferation and intracellular IFN-γ production were determined by flow cytometry. Representative histograms, dot plots, and summarized data are shown. Data are shown as mean ± SEM. **P* <0.05, ***P* <0.01, *****P* < 0.0001 by one-way ANOVA with *post hoc t* test and Bonferroni's correction.

Supplemental Figure 6



P score: Log-rank P value for Kaplan-Meier plot showing results from analysis of correlation between mRNA expression level and patient survival

Figure S6. High expression of c-Maf correlates with worse survival in human cancer patients. Human protein atlas data show that high mRNA expression level of c-Maf is correlated with poor survival of human cancers. Log-rank P value is shown for Kaplan-Meier plots.

Supplemental Figure 7

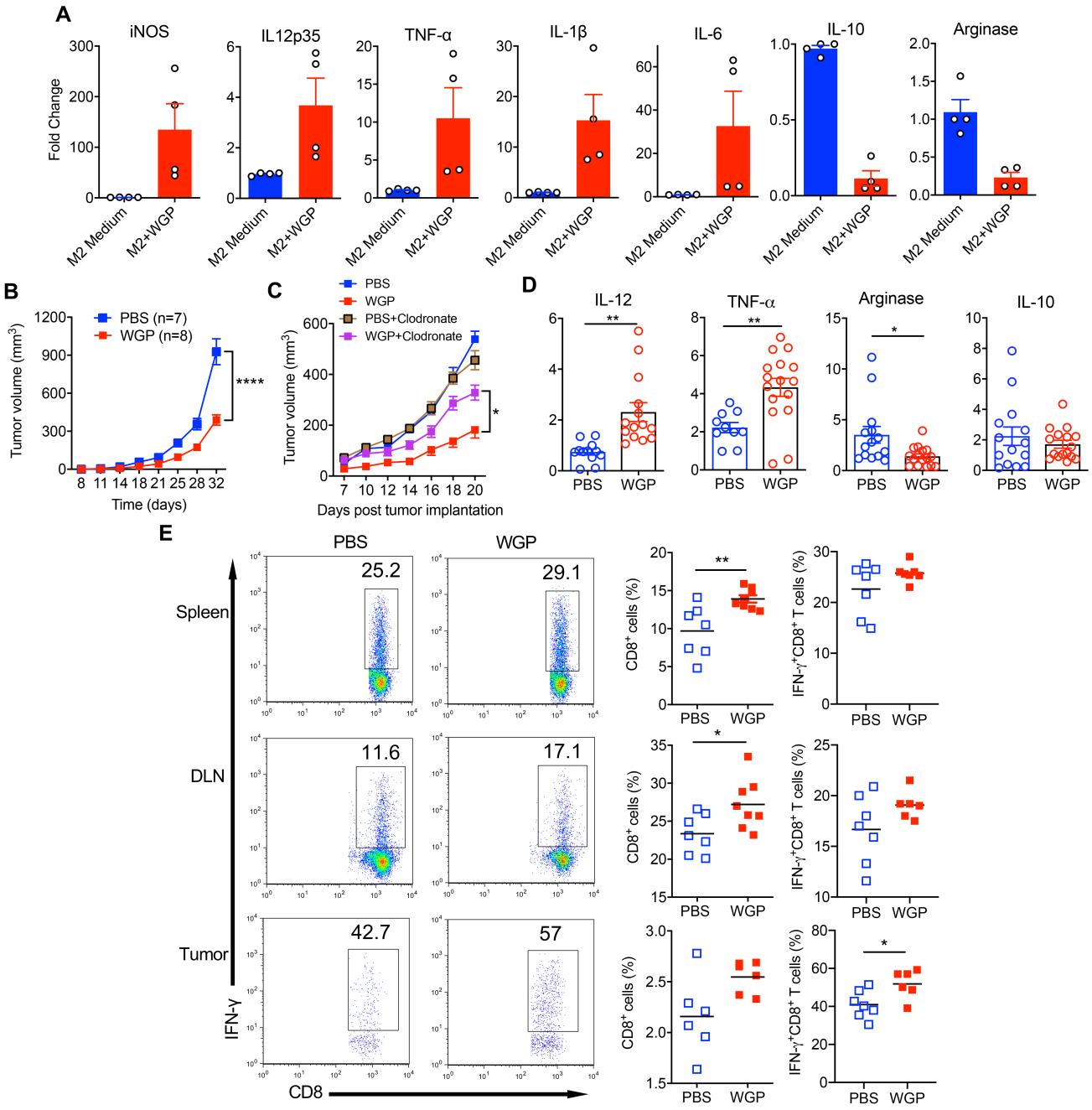


Figure S7. WGP treatment converts M2 BMM and immunosuppressive TAM into an M1-like phenotype leading to reduced tumor progression with enhanced antitumor T cell response. (**A**) Polarized mouse M2 BMM were treated with WGP β-glucan (150 μg/ml) for 24 h and the mRNA expression levels of indicated genes were determined by qPCR analysis. Data are representative of at least three independent experiments. (**B**) LLC-bearing mice (n=7-8) were treated with or without oral WGP β-glucan daily. Tumor growth curve is shown. ****P <0.0001, by two-way repeated measures ANOVA with Sidak's multiple comparisons test. (**C**) Mice (n=6-10) depleted with or without macrophages using Clodronate were injected with LLC cells. Mice were treated with or without oral WGP β-glucan daily when tumors were palpable. Tumor growth was recorded. *P <0.05, by two-way repeated measures ANOVA with Sidak's multiple comparisons test. (**D**) TAM were sorted from WGP β-glucan treated mice or control mice. The mRNA expression levels of indicated genes were determined by qRT-PCR. *P <0.05, **P <0.01, by two-tailed, unpaired *t* test. (**E**) Single cell suspensions from spleen, draining lymph node (DLN), and tumor were stimulated with PMA+ionomycin. Cells were stained for CD8 and intracellular IFN-γ production. Representative dot plots are shown. Cells were gated on $CD8^+$ cells. Summarized frequencies of total CD8 T cells (gated on the total viable cells) and IFN-γ-producing $CD8^+$ T cells are also shown. Data are shown as mean ± SEM. *P <0.05, **P <0.01, by two-tailed, unpaired *t* test.

Supplemental Figure 8

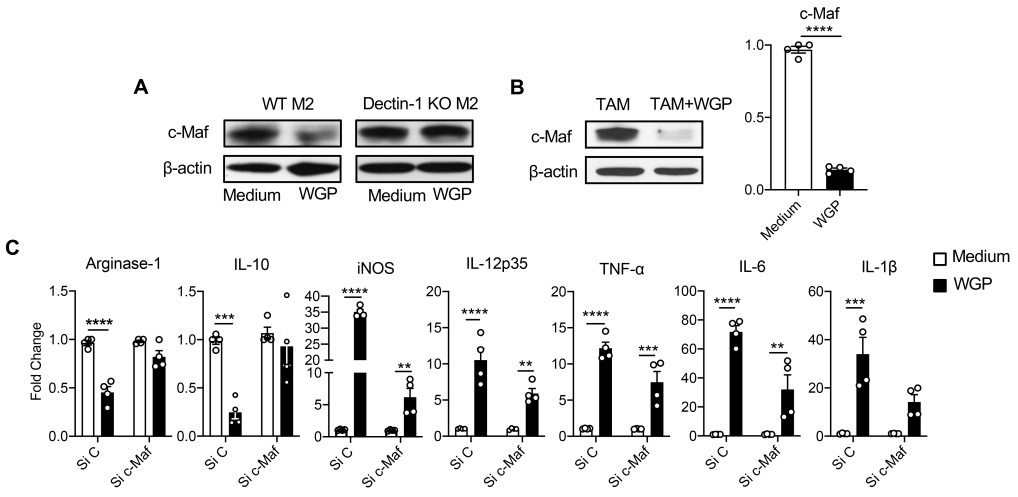


Figure S8. WGP β-glucan treatment downregulates c-Maf expression in murine M2 BMM and TAM. (**A**) M2 BMM from WT or dectin-1 KO mice were treated with WGP β-glucan (150 μg/ml) for 24 h and c-Maf was detected by WB. (**B**) TAM sorted from LLC-bearing mice were treated with WGP β-glucan for 24 h and c-Maf was determined by WB and qRT-PCR analysis. ****P <0.0001, by two-tailed, unpaired t test. (**C**) M2 BMM transfected with c-Maf (Si c-Maf) or control siRNA (Si C) were stimulated with WGP β-glucan for 6 h and then assayed for the specific gene mRNA expression levels by qRT-PCR. The relative fold increase was calculated using corresponding medium alone (Si-c-Maf or Si C) as baseline. **P <0.01, ***P <0.001, ****P <0.0001, by two-way ANOVA with Sidak's multiple comparisons test.