Supplementary Information for

Mesoporous silica nanoparticles inflame tumors to overcome anti-PD-1 resistance through TLR4-NFκB axis

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Figure legends

Figure S1. MSNs with IgG treatment do not elicit any anti-tumor response in αPD-1-resistant tumors. a, B16F10 cells were transfected with the *B2m* sgRNA and Cas9. The expression of B2m was analyzed by western blot. **b**, The expression of MHC-I was analyzed by flow cytometry. **c-h**, Tumor growth (**c-e**) and tumor weights (**f**-**h**) of *B2m*-sgRNA B16F10 (**c** and **f**), H22 (**d** and **g**) and CT26 (**e** and **h**) tumor-bearing mice after different MSNs treatment (top: sizes; bottom: L/Ds) prior to IgG treatment. **i**, Representative photographs of *B2m*-sgRNA B16F10, H22 and CT26 tumors after indicated treatments.

Figure S2. Administration of MSNs does not affect body weight of mice. a-**c**, Body weight changes of *B2m*-sgRNA B16F10 (**a**), H22 (**b**), or CT26 (**c**) tumor-bearing mice during indicated treatments.

Figure S3. The biodistribution of MSNs in mice. *B2m*-sgRNA B16F10 tumor-bearing mice were intraperitoneally injected with MSNs, and kidney, liver, lung, spleen and tumor tissue were harvested at 24 hours post-injection. The content of Si in each organ was detected by ICP-MS.

Figure S4. MSNs enhance the infiltration of CTLs in αPD-1-resistant tumors. a-c, Flow cytometry analysis quantifying the proportion of infiltrating $CD45^+$ cells in the TME of *B2m*-sgRNA B16F10 (**a**), H22 (**b**), and CT26 (**c**) tumor-bearing mice treated with different MSNs treatment (left: sizes; right: L/Ds) prior to αPD-1. **d-f**, Flow cytometry analysis quantifying the proportion of infiltrating $CD8^+$ T cells, $CD4^+$ T cells, G-MDSCs, M-MDSCs, macrophages, DCs and NKs in the TME of *B2m*-sgRNA B16F10 (**d**), H22 (**e**), and CT26 (**f**) tumor-bearing mice treated with different MSNs treatment (left: sizes; right: L/Ds) prior to αPD-1. Error bars represent mean ± SEM. *P* value was calculated by unpaired Student's *t*-test (**P*<0.05, ***P*<0.01, ****P*<0.001).

Figure S5. MSNs re-sensitize tumors to αPD-1 in a CTLs–dependent manner. Tumor growth of CT26 tumor-bearing mice treated with anti-CD8 antibody to deplete $CD8^+$ T cells.

Figure S6. Macrophages are the main source of Ccl5 and Cxcl9 in tumor tissues after MSNs treatment. The major cell types that produced Ccl5 and Cxcl9 in CT26 tumor tissues were analyzed by flow cytometry. The right graph quantified the percentage of Ccl5 and Cxcl9 producing cell types.

Figure S7. Macrophages are required for MSNs-induced anti-tumor effect. a, Growth of B16F10-OVA tumor in $Rag1^{-/-}$ mice after indicated treatment. **b**, The mRNA expression of CD8a, Ccl5, Cxcl9 and Cxcl10 in tumor tissues after indicated treatments was determined by qRT-PCR.

Figure S8. MSNs drive macrophage polarization towards M1. a,b, The expression of CD206 in macrophages from *B2m*-sgRNA B16F10 (**a**) and CT26 (**b**) tumors was analyzed by flow cytometry. The right graph quantified the M1/M2 macrophage ratio. **c,** Immunohistochemical staining of Arg-1 and iNOS in H22 tumors after MSNs treatment. Error bars represent mean ± SEM. *P* value was calculated by unpaired Student's *t*-test (**P*<0.05, ***P*<0.01, ****P*<0.001).

Figure S9. TNFR1 signaling is not responsible for MSNs-induced NF-κB activation and chemokines production in macrophages. a, Raw 264.7 cells were treated with 100 μg/mL MSNs for different times and the protein levels were determined by western blot. **b**, Raw 264.7 cells were treated with 100 μM anti-TNFR1 antibody for 3 hours and then 100 μg/mL MSNs treatment for 1 hour. The protein levels were determined by western blot. **c**, Agarose gel electrophoresis identifying mouse genotypes. The wild-type band is 780 bp, and the mutant band is 556 bp. **d**, BMDMs from WT and TNFR1-deficient ($TNFR1^{-/-}$) mice were treated with 100 μg/mL MSNs for 1 hour. The protein levels were determined by western blot. **e**, WT and $TNFR1^{-/-}$ BMDMs were treated with 100 μg/mL

MSNs for 12 hours. The mRNA expression of Ccl5, Cxcl9 and Cxcl10 was determined by qRT-PCR.

Table S1. Primers for qRT-PCR

Gene	Forward sequence	Reverse sequence
Actb	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT
CD27	CCCAACTCGACTGTCTATAGC	AGAACAAGATTGCACCCAGG
Tnfrsf9	CCTGTGATAACTGTCAGCCTG	TCTTGAACCTGAAATAGCCTGC
Tnfrsf4	GCTGTGATCATACCAGGGATAC	TCTGCTTGAGTTCACTTCCAC
Perforin1	AGCACAAGTTCGTGCCAGG	GCGTCTCTCATTAGGGAGTTTTT
Ifng	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
GranzymeB	CCACTCTCGACCCTACATGG	GGCCCCCAAAGTGACATTTATT
Tnf	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Mki67	TGCCCGACCCTACAAAATG	GAGCCTGTATCACTCATCTGC
Eomes	GCGCATGTTTCCTTTCTTGAG	GGTCGGCCAGAACCACTTC
Prdm1	ATTAAGCCTATCCCTGCCAAC	CTACTGTATTGCTTTGGGTTGC
Foxo1	CTACGAGTGGATGGTGAAGAG	TGTGAAGGGACAGATTGTGG
Batf	TGATGTGAGGAAAGTTCAGAGG	GCTGTTTGATCTCTTTGCGG
Foxp1	GCTTCTGCTGACTCTCCTGG	GGAGCCCTTTAGGCTAGCAG
Nfatc1	GACTTCGATTTCCTCTTCGAGTTC	CTCGATTCTCGGACTCTCCAG
Nr4a1	GCCTAGCACTGCCAAATTG	TCTGCCCACTTTCGGATAAC
Tox	GCTTGATGTGAGAGTGAAATGG	GCTCATATACATGTTCTCCCCG
Ccl5	TGCCCACGTCAAGGAGTA TT	CAGGACCGGAGTGGGAGTA
Cxcl9	AGTCCGCTGTTCTTTTCCTC	TGAGGTCTTTGAGGGATTTGTAG
Cxcl10	TCAGCACCATGAACCCAAG	CTATGGCCCTCATTCTCACTG
CD8a	CATCACTCTCATCTGCTACCAC	TTTTCTCTGAAGGTCTGGGC