Electronic Supplementary information

Targeted ferritin nanoparticle encapsulating CpG oligodeoxynucleotides induces tumor-associated macrophage M2 phenotype polarization into M1 phenotype and inhibits tumor growth†

Hui Shan,[a] Wenlong Dou,[b] Yu Zhang,[a] Mi Qi*[a]

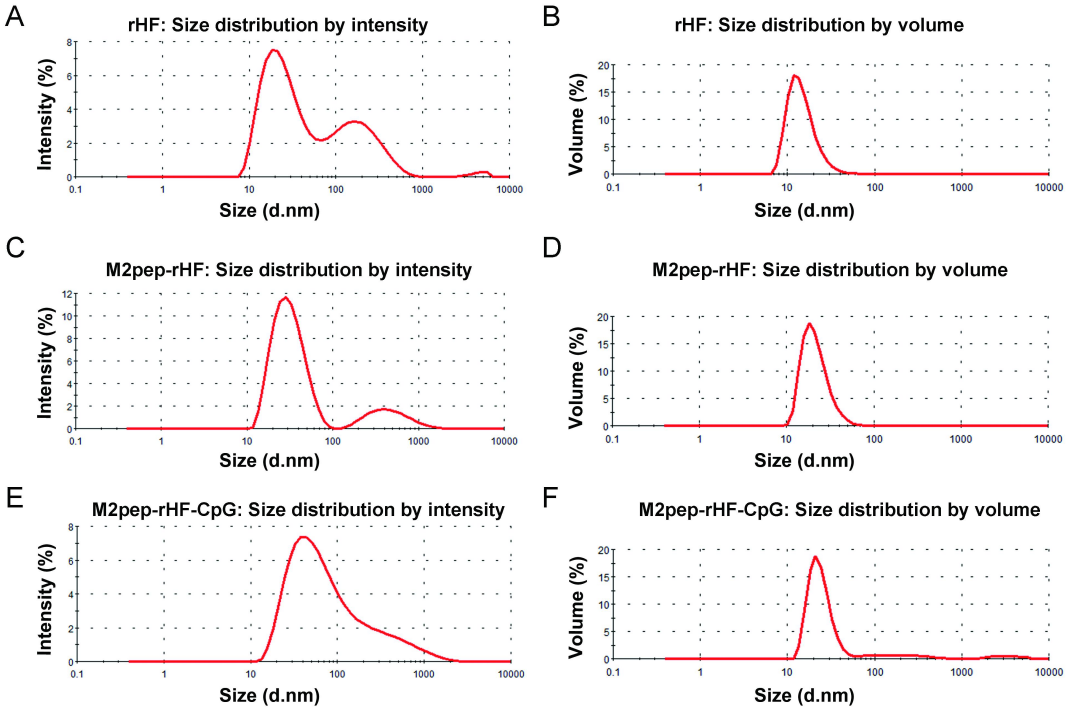

Fig. S1 Size distribution of nanoparticles analyzed via DLS. (A) DLS analysis in intensity for rHF nanoparticles. (B) DLS analysis in volume for rHF nanoparticles. (C) DLS analysis in intensity for M2pep-rHF nanoparticles. (D) DLS analysis in volume for M2pep-rHF nanoparticles. (E) DLS analysis in intensity for M2pep-rHF-CpG nanoparticles. (F) DLS analysis in volume for M2pep-rHF-CpG nanoparticles.

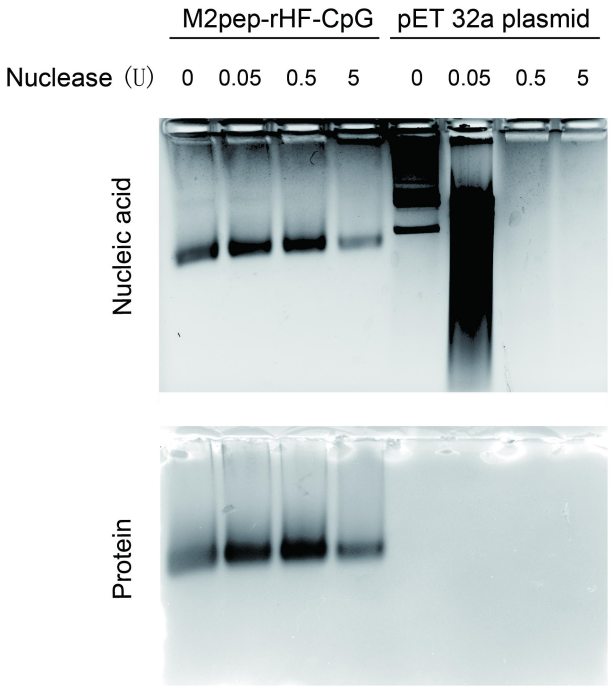

Fig. S2 M2pep-rHF-CpG shows good resistance to nuclease digestion. Upper panel: nucleic acid imaging after M2pep-rHF-CpG or pET32a plasmids digested with different concentrations (0 U, 0.05 U, 0.5 U and 5 U) of universal nuclease. Lower panel: protein staining after M2pep-rHF-CpG or pET32a plasmids treated with different concentrations (0 U, 0.05 U, 0.5 U and 5 U) of universal nuclease.

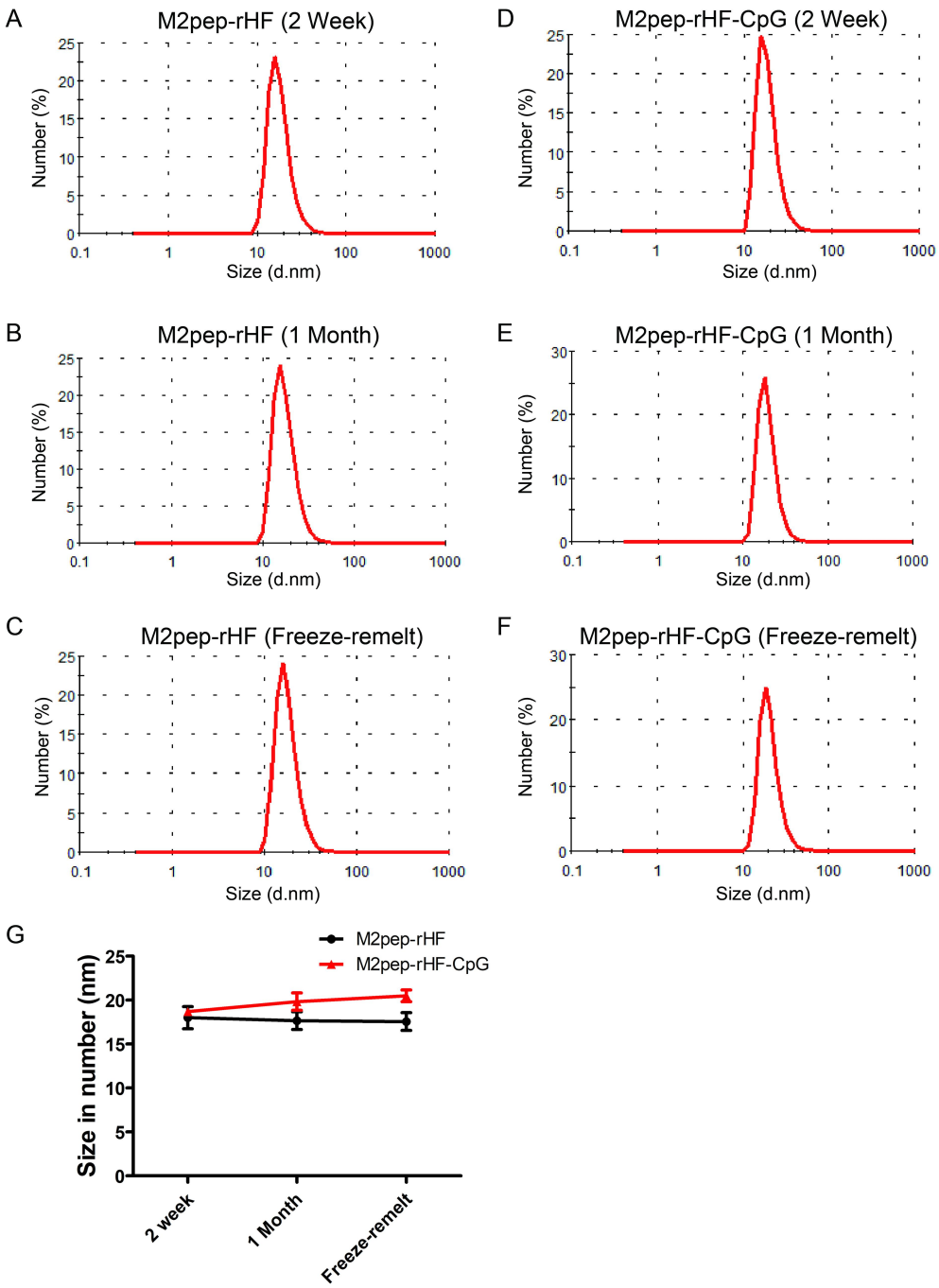

Fig. S3 Stability of M2pep-rHF and M2pep-rHF-CpG nanoparticles *in vitro*. (A) DLS analysis in number for M2pep-rHF nanoparticles after being stored at 4 °C for 2 weeks. (B) DLS analysis in number for M2pep-rHF nanoparticles after being stored at 4 °C for 1 month. (C) DLS analysis in number for M2pep-rHF nanoparticles after being stored at -80 °C for 1 month, and thawed quickly in 37 °C water bath. (D) DLS analysis in number for M2pep-rHF-CpG nanoparticles after being stored at 4 °C for 2 weeks. (E) DLS analysis in number for M2pep-rHF-CpG nanoparticles after being stored at 4 °C for 1 month. (F) DLS analysis in number for M2pep-rHF-CpG nanoparticles after being stored at -80 °C for 1 month, then thawed quickly at 37 °C. (G) Size analysis of M2pep-rHF and M2pep-rHF-CpG nanoparticles under different storage conditions.

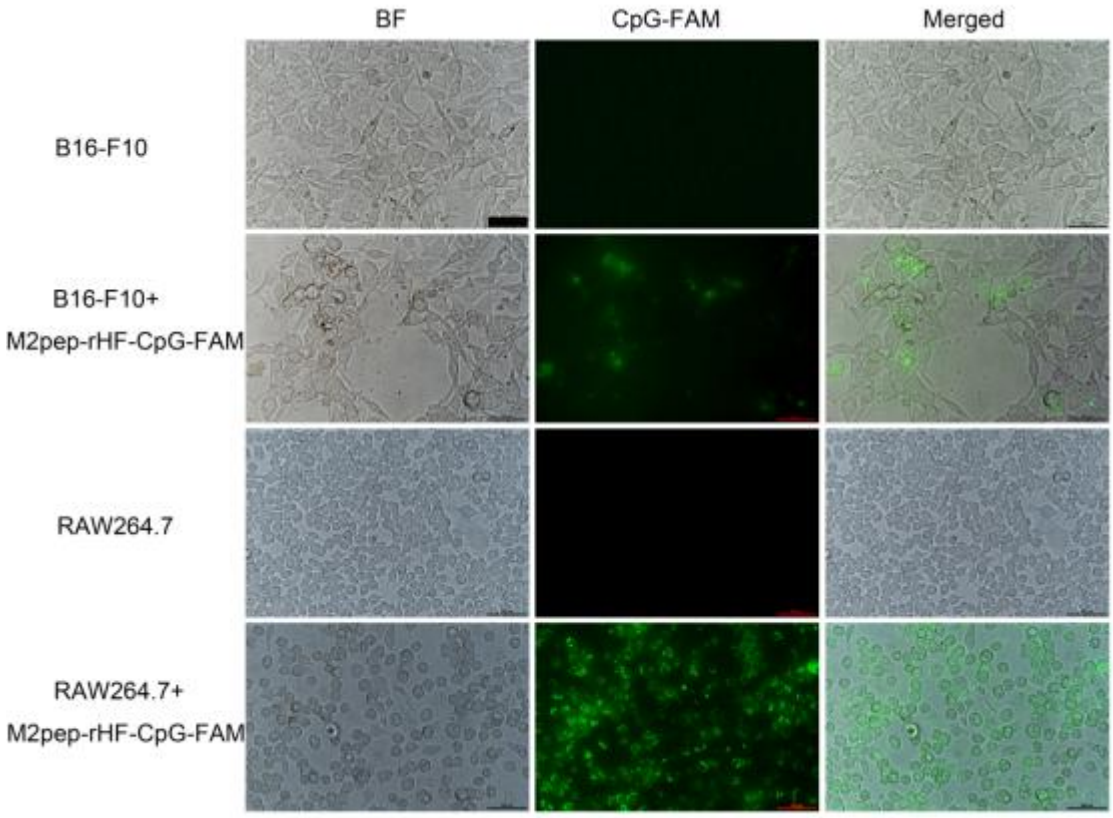

Fig. S4 Ability of M2pep-rHF-CpG nanoparticles to penetrate cell membranes. Representative images showing the detection of CpG-FAM in mouse B16-F10 and RAW264.7 cells (Scale bar = 500 μm).

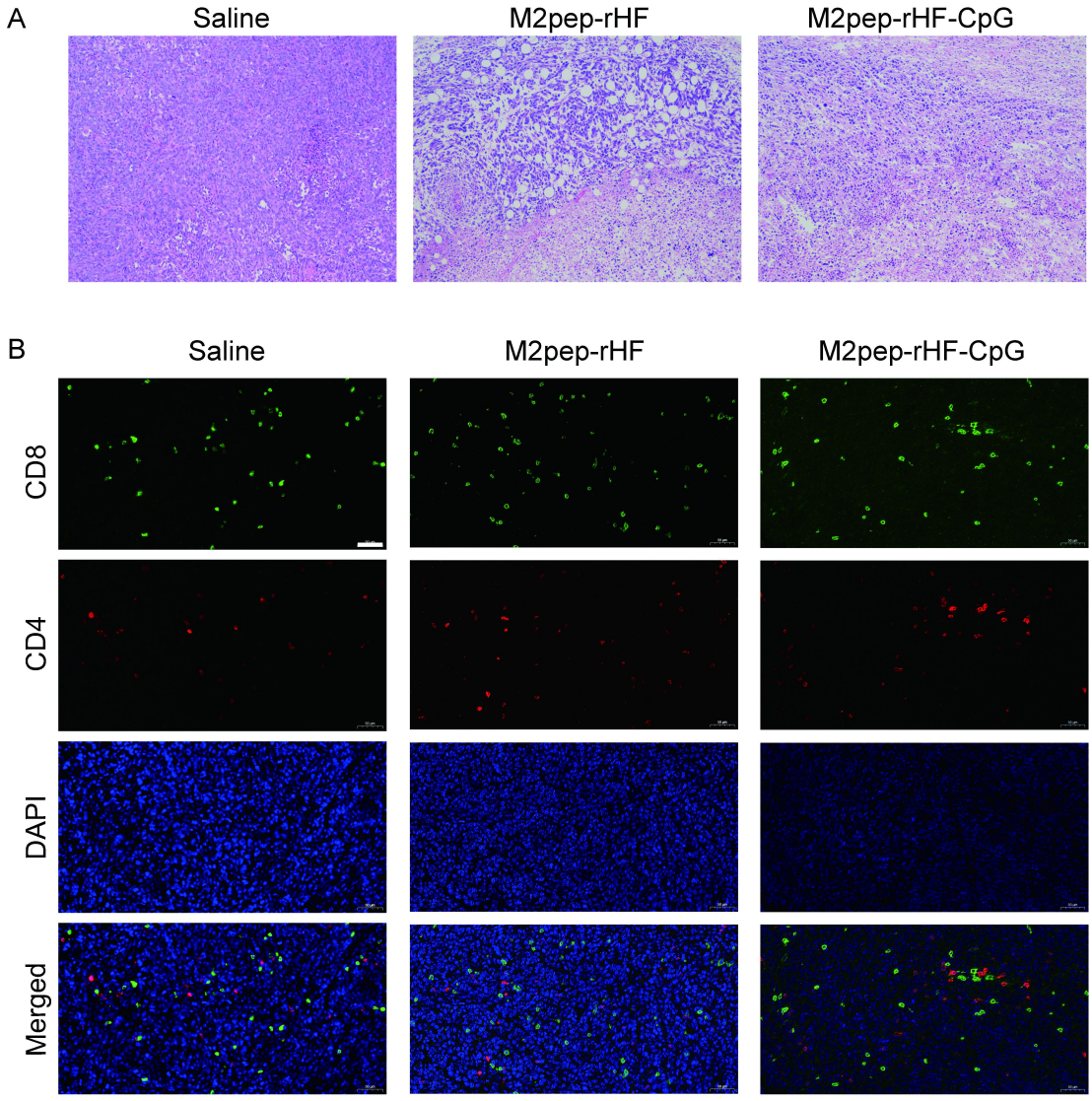

Fig. S5 Histological characterization of tumor tissues from saline, M2pep-rHF, and M2pep-rHF-CpG-treated mice. (A) Representative images of H&E-stained tumor tissue (100×). (B) Immunofluorescence images showing CD4/CD8 T lymphocyte cells in 4T1 tumor tissues (Green: CD8, Red: CD4, and Blue: DAPI. Scale bar = 50 μm).

Nucleotide sequence of rHF (528bp):

ACGACCGCGTCCACCTCGCAGGTGCGCCAGAACTACCACCAGGACTCAGAGGCCGCC
ATCAACCGCCAGATCAACCTGGAGCTCTACGCCTCCTACGTTTACCTGTCCATGTCTTA
CTACTTTGACCGCGATGATGTGGCCTTGAAGAACTTTGCCAAATACTTTCTTCACCAAT
CTCATGAGGAGAGGGAACATGCTGAGAAACTGATGAAGCTGCAGAACCAACGAGGTG
GCCGAATCTTCCTTCAGGATATCAAGAAACCAGACTGTGATGACTGGGAGAGCGGGCT
GAATGCGATGGAGTGTGCATTACATTTGGAAAAAAATGTGAATCAGTCACTACTGGAA
CTGCACAAACTGGCCACTGACAAAAATGACCCCCATTTGTGTGACTTCATTGAGACAC
ATTACCTGAATGAGCAGGTGAAAGCCATCAAAGAATTGGGTGACCACGTGACCAACTT
GCGCAAGATGGGAGCGCCCGAATCCGGCTTGGCGGAATATCTCTTTGACAAGCACACC
CTGGGA.

Nucleotide sequence of M2pep-rHF (603bp):

TATGAACAGGATCCGTGGGGCGTGTTTTGGTGGTATGGCTCAGGTACGACC
GCGTCCACCTCGCAGGTGCGCCAGAACTACCACCAGGACTCAGAGGCCGC
CATCAACCGCCAGATCAACCTGGAGCTCTACGCCTCCTACGTTTACCTGTCC
ATGTCTTACTACTTTGACCGCGATGATGTGGCCTTGAAGAACTTTGCCAAAT
ACTTTCTTCACCAATCTCATGAGGAGAGGGAACATGCTGAGAAACTGATGA
AGCTGCAGAACCAACGAGGTGGCCGAATCTTCCTTCAGGATATCAAGAAA
CCAGACTGTGATGACTGGGAGAGCGGGCTGAATGCGATGGAGTGTGCATT
ACATTTGGAAAAAAATGTGAATCAGTCACTACTGGAACTGCACAAACTGG
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