Supporting Information

A Parallel and cascade control system: Magnetofection of miR125b for synergistic tumor-association macrophage polarization regulation and tumor cell suppression in breast cancer treatment

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Table S1 Primer nucleic acid sequence for detection miR-125b targeted gene and polarization related gene

Gene name	Primer sequence（5′→3′）
IRF4-Forward	CTCTTCAAGGCTTGGGCATT
IRF4-Reverse	TGCTCCTTTTTTGGCTCCCT
ETS1-Forward	CACCTCGGATTACTTCAT
ETS1-Reverse	ACGGCTCAGTTTCTCATA
CCNJ-Forward	CACCGTCTCACTGCCTACT
CCNJ-Reverse	GGGAAATGTTCGTTTATCTG
IL-1β-Forward	AGCACCTTCTTTTCCTTC
IL-1β-Reverse	TGCCGTCTTTCATTACAC
TNF-α-Forward	CTGAACTTCGGGGTGATC
TNF-α-Reverse	TCCTCCACTTGGTGGTTT
iNOS-Forward	CACGGACGAGACGGATAG
iNOS -Reverse	CACTGACACTTCGCACAAA
IL-10-Forward	CAAGGAGCATTTGAATTCCC

IL-10-Reverse	GGCCTTGTAGACACCTTGGTC
CD163-Forward	GCAAAAACTGGCAGTGGG
CD163-Reverse	GTCAAAATCACAGACGGAGC
IL-12-Forward	AGCACTCCCCATTCCTACTT
IL-12-Reverse	ACGCACCTTTCTGGTTACAC
IL-6-Forward	TCGTGGAAATGAGAAAAGAG
IL-6-Reverse	ATTGGAAATTGGGGTGAA
Arginase-Forward	AAGACAGCAGAGGAGGTG
Arginase-Reverse	AGTCAGTCCCTGGCTTAT
ERBB3-Forward	CCAAAGGTCCAATCTACA
ERBB3-Reverse	TCCTACTGTCACCGCTAT
MKNK2-Forward	CAGGAATATGCTGTCAAG
MKNK2-Reverse	AAACACCAGGTAGAAACG
MEGF9-Forward	GATCATTCCTCCTCTTCG
MEGF9-Reverse	ACATCCAAACTTCCAACC
CDK2-Forward	CTGGCAGACTTTGGACTA
CDK2-Reverse	GGGGAAACTTGGCTTATA

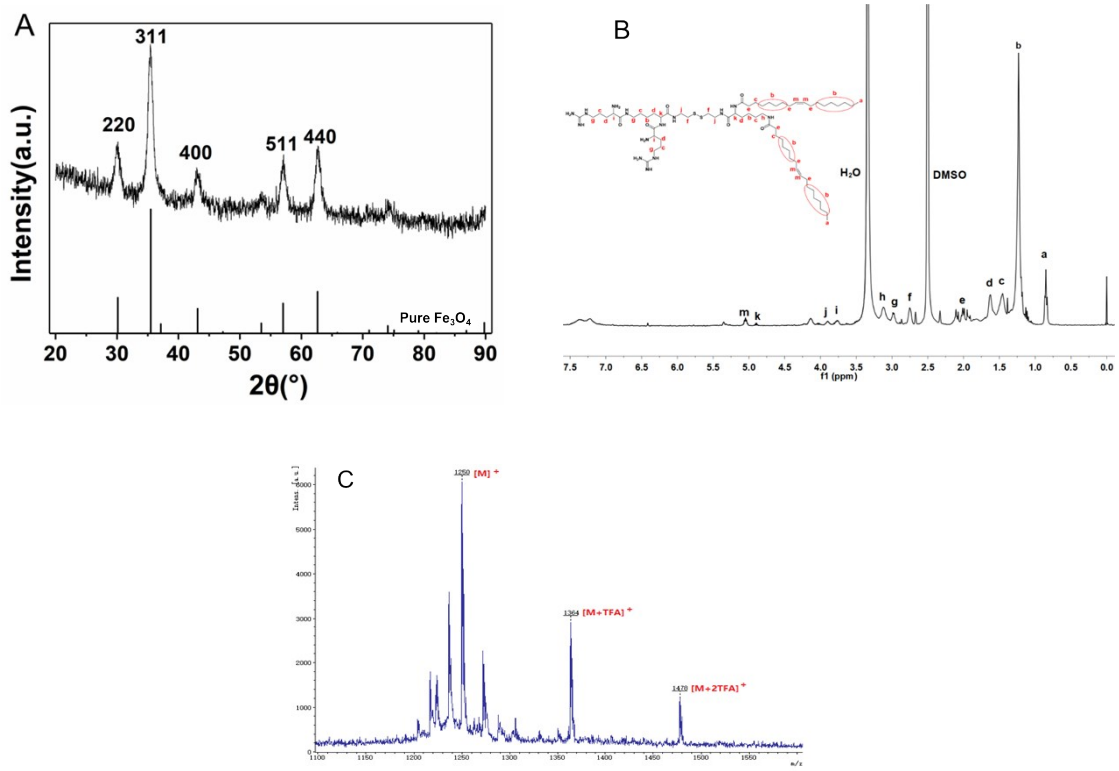

Figure S1 Characterization of MNPs and RLS. (A) XRD of MNPs; (B) [1] HNMR spectra of RLS and (C) mass spectrometry (MALDI-TOF-MS).

	Z-Average (d. nm)	PDI	Zeta potential (mV)
MNPs	32.11 ± 0.1	0.11 ± 0.006	-19.1 ± 0.6
RLS	96.8 ± 2.5	0.27 ± 0.011	27.5 ± 0.3
RG	139.9 ± 3.8	0.14 ± 0.036	25.4 ± 0.3
RMG	258.3 ± 12.2	0.32 ± 0.033	7.2 ± 1.0

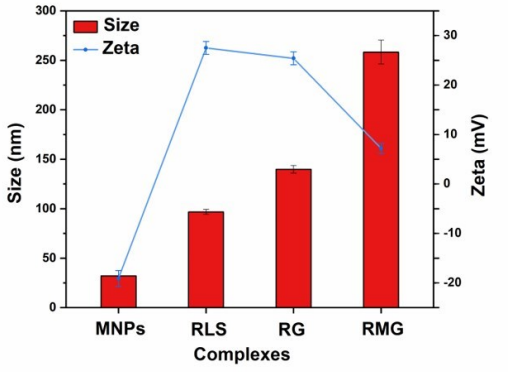

Figure S2 Size and zeta potential photographs of MNPs, RLS, RLS/gene (RG) with N/P ratio 30 and RLS/MNPs/gene (RMG) with N/P ratio of 30 and M/G ratio of 0.2.

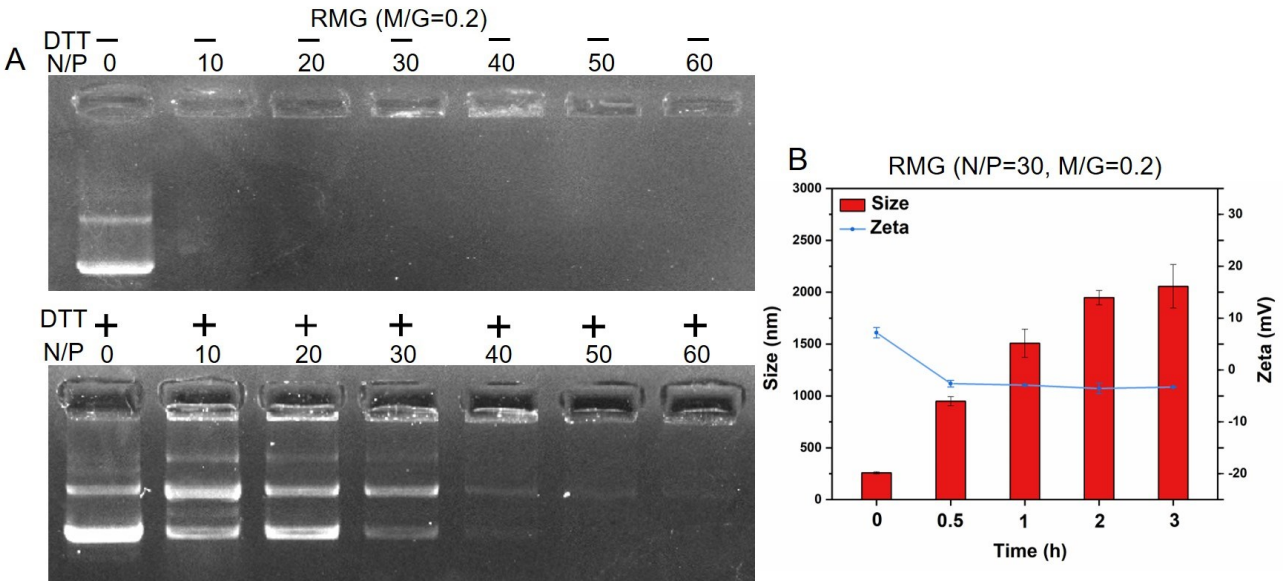

Figure S3 Stability study of RMG complexes by size and zeta potential changes determination after incubation with 10 mM DTT. (A) Gel retardation assay of RMG complexes with different N/P ratios and identical M/G ratio of 0.2 in the absence or presence of reductive agent (10 mM DTT) for 1 h at 37°C. (B) Size and zeta potential of RMG complexes (N/P of 30 and M/G of 0.2) after incubation with 10 mM DTT for 0, 0.5, 1, 2 and 3 h.

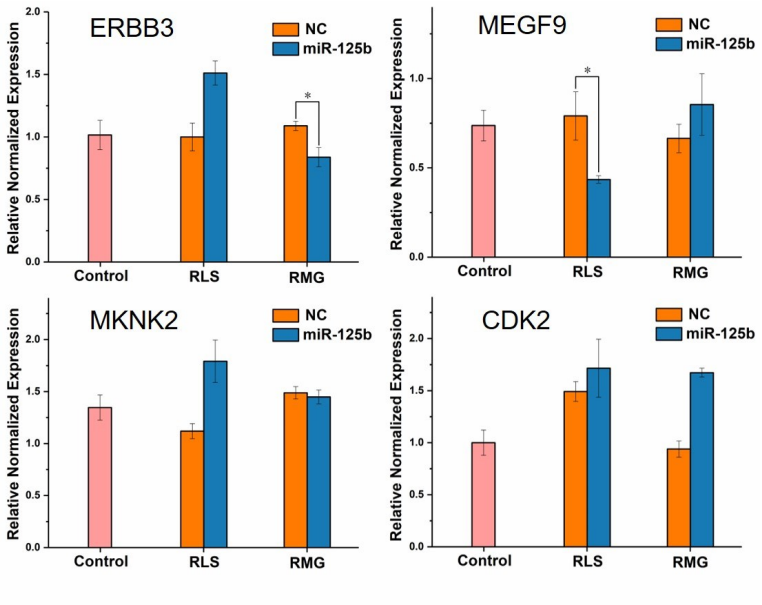

Figure S4 Potential target genes (ERBB3, MEGF9, MKNK2 and CDK2) of miR-125b in 4T1 cells.

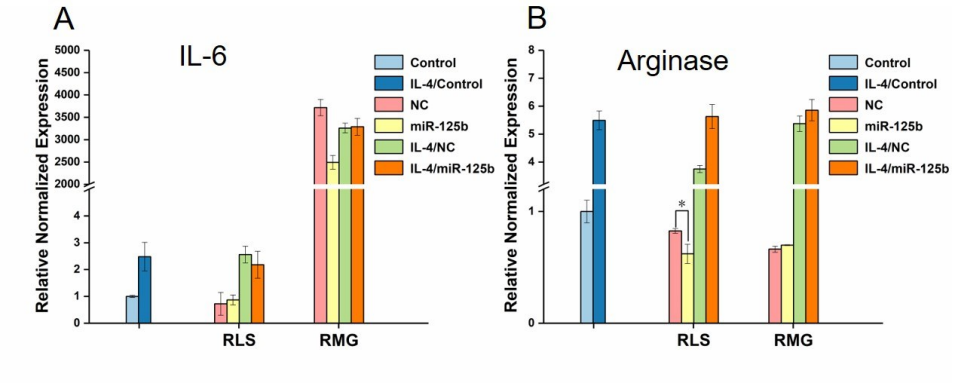

Figure S5 Polarization genes of miR-125b in RAW264.7 cells measured by Q-PCR. (A) Expression of M1-associated gene (IL-6) for pro-inflammatory macrophages activation. (B) Expression of M2-associated genes (Arginase) for anti-inflammatory macrophages activation.

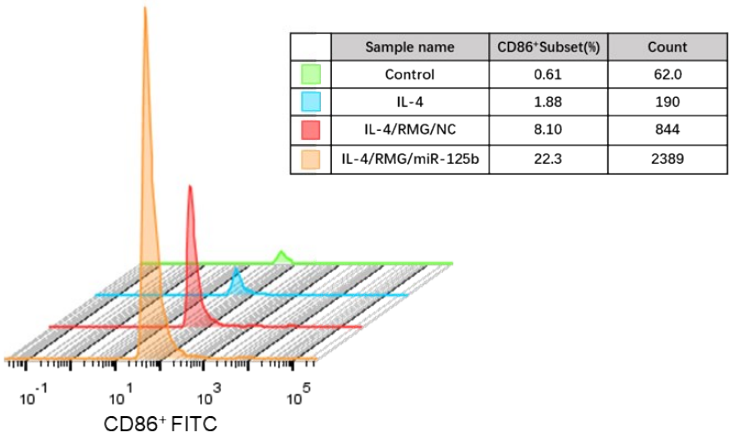

Figure S6 FACS analysis of CD86 expression in RAW264.7 cells with various formulations treatment.

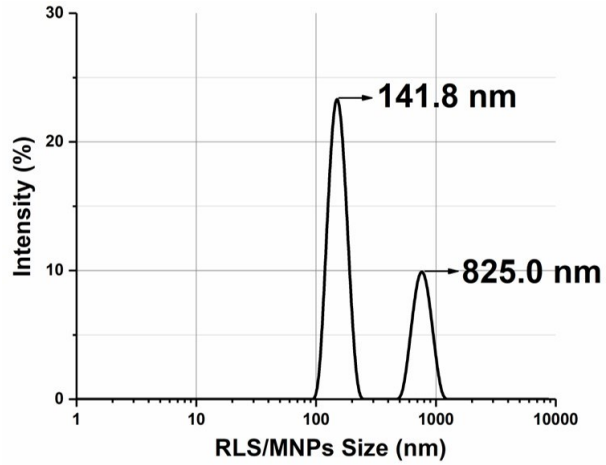

Figure S7 Size distribution photograph of RLS/MNPs without genes

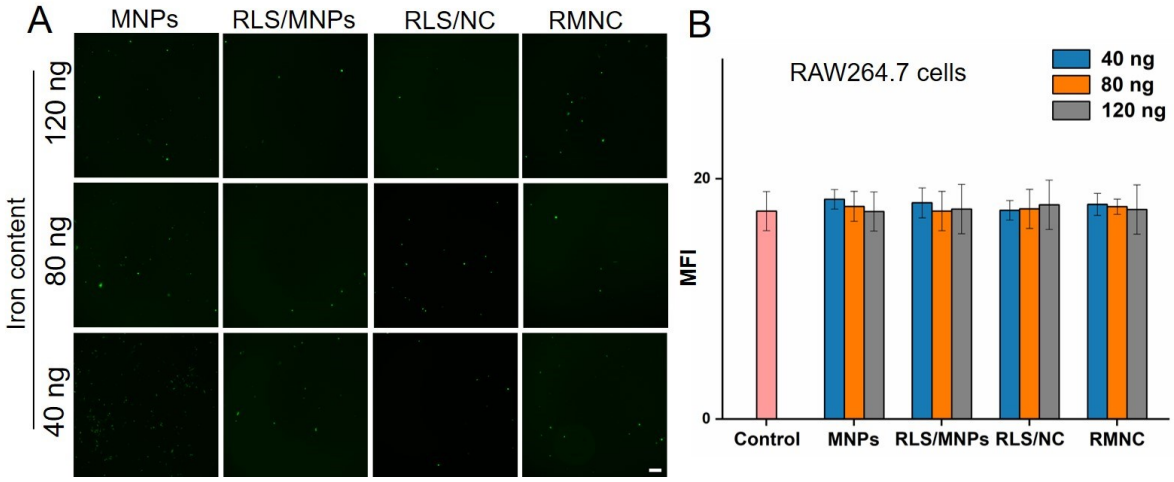

Figure S8 Reactive oxygen species (ROS) measurement of RAW264.7 cells after various treatment. (A) The fluorescence microscopy images of ROS assay on RAW264.7 cells after MNPs, RLS/MNPs, RLS/NC and RMNC treatment with various iron content. ROS generation was monitored by 2,7-dichlorofluorescin diacetate (DCFH-DA, green fluorescence). The scale bar was 200 μm. (B) Semi-quantitative analysis of ROS generation by mean fluorescence intensity (MFI) in RAW264.7 cells.

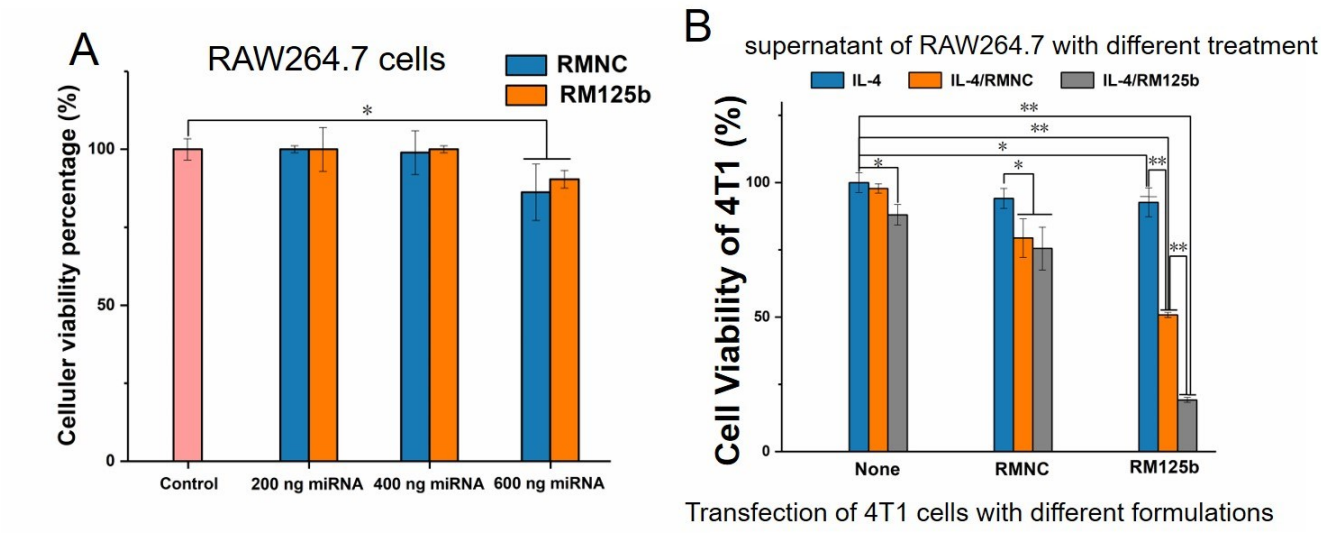

Figure S9 The cytotoxicity of various formulations. (A) RM125b complexes against RAW264.7 cells. (B) CCK-8 analysis of gene complexes pre-transfected 4T1 cells after treating with condition medium from macrophage for 24 h.

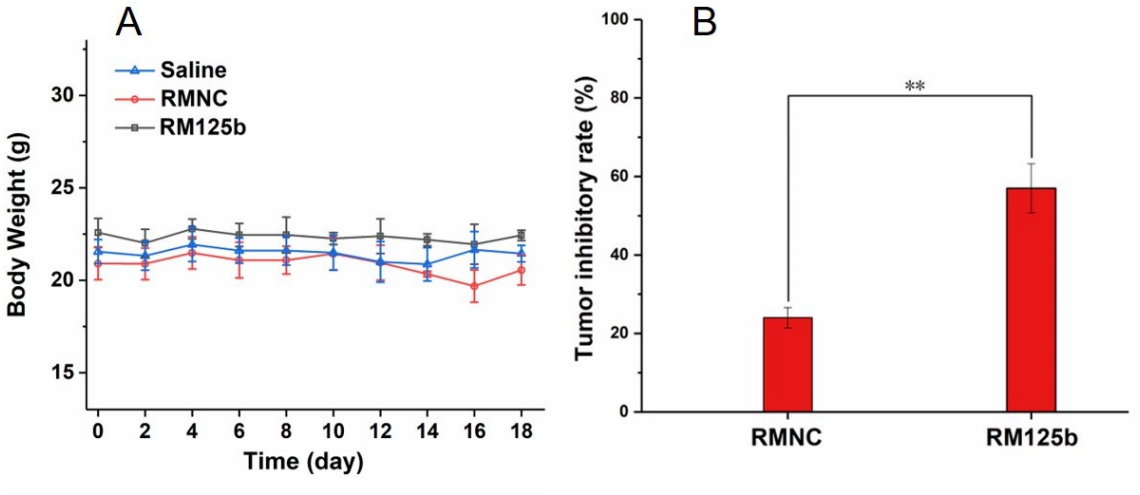

Figure S10 In vivo therapeutic effect of various treatment. (A) Body weight change curves of mice in different groups (Saline, RMNC and RM125b). (B) Tumor inhibition rates of different formulations. ** $P < 0.01$.

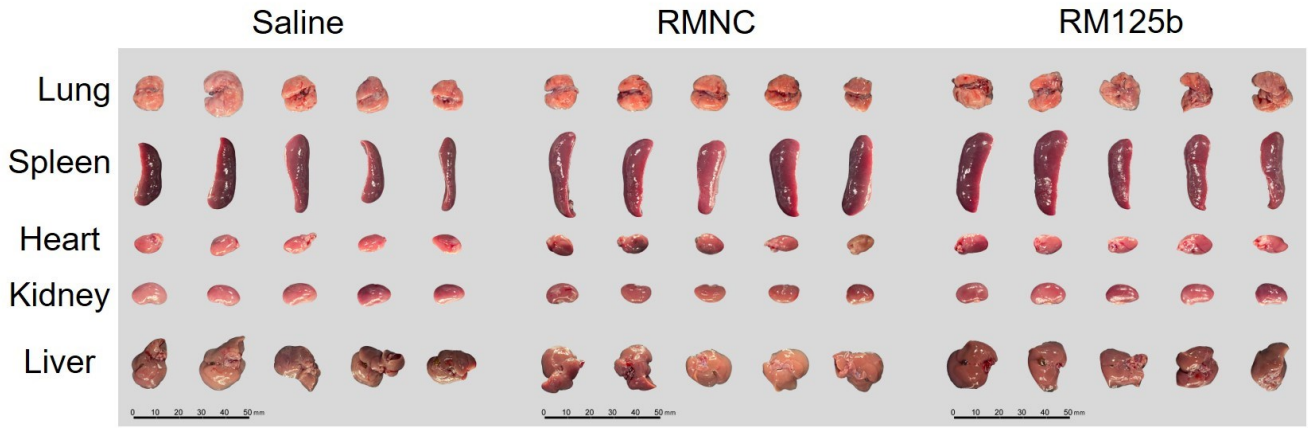

Figure S11 The main organs photographs (heart, liver, spleen, lung and kidney) of mice after treatment with saline, RMNC and RM125b (Scale bar is 50 mm).

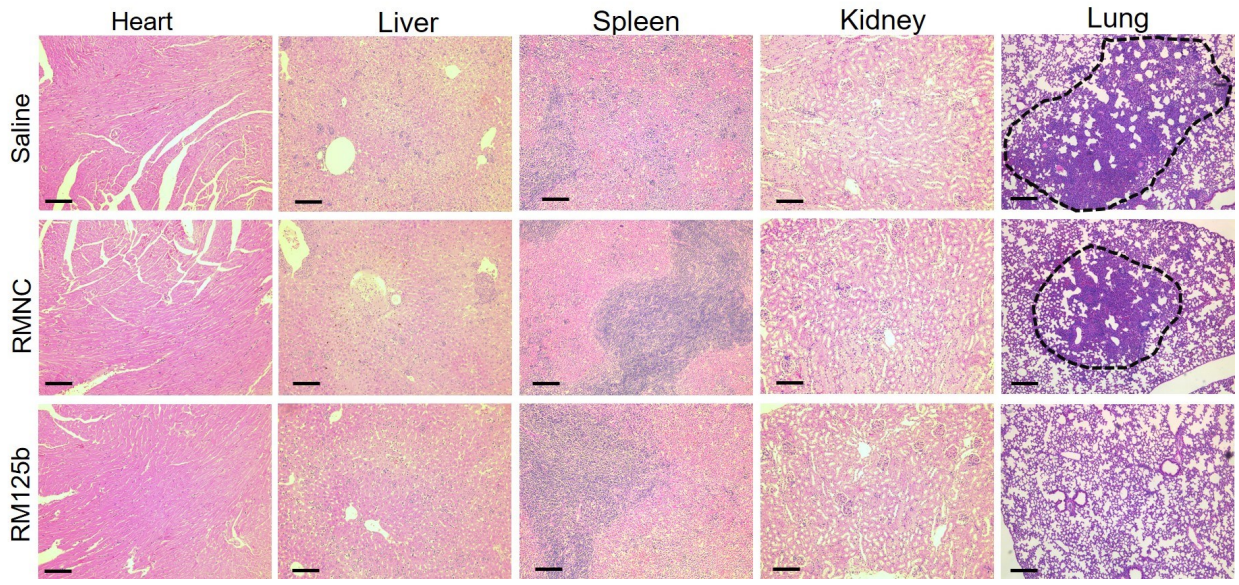

Figure S12 Hematoxylin-eosin stained slice of main organs at day 18 post treatment. Metastatic lesions were indicated by black cycles. Scale bar is 100 μm.