Supporting Information

Figure S1

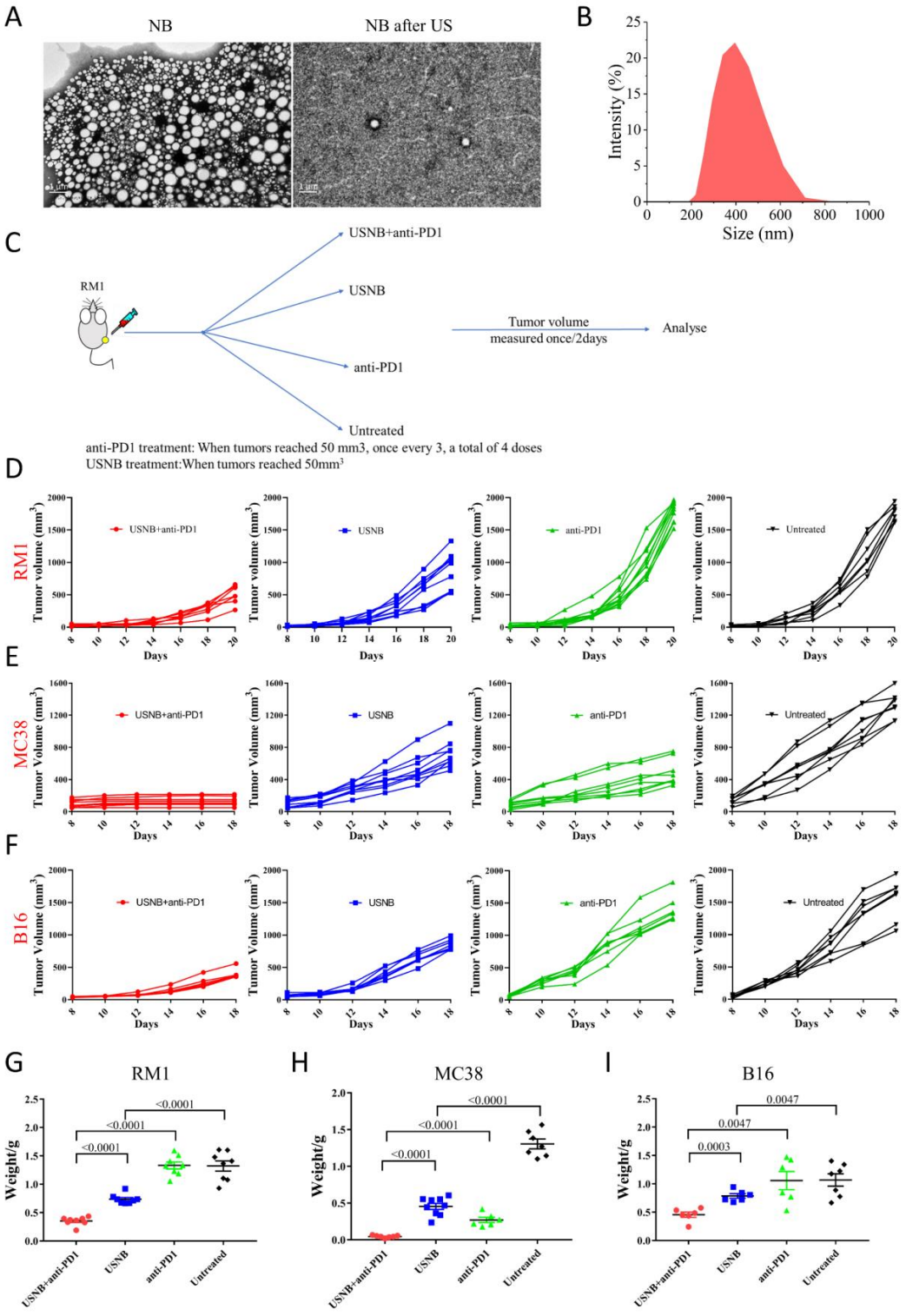

Figure S1

(A) Transmission electron microscopy images of NB before (left) and after (right) US stimulation; the scale bar represents 1 µm. (B) Size distribution of NB. (C) Schematic diagram of the combination of USNB and anti-PD1 treatment in mice. (D-F) The tumor growth curves of individual animals bearing RM1, MC38 and B16 tumors after each treatment. (G-I) The weights of RM1, MC38 and B16 tumors at the endpoint of the study. The bars represent the mean±SEM. The *p* value was calculated by unpaired t test (G-I). The data are representative of two independent experiments with at least five mice per group.

Figure S2

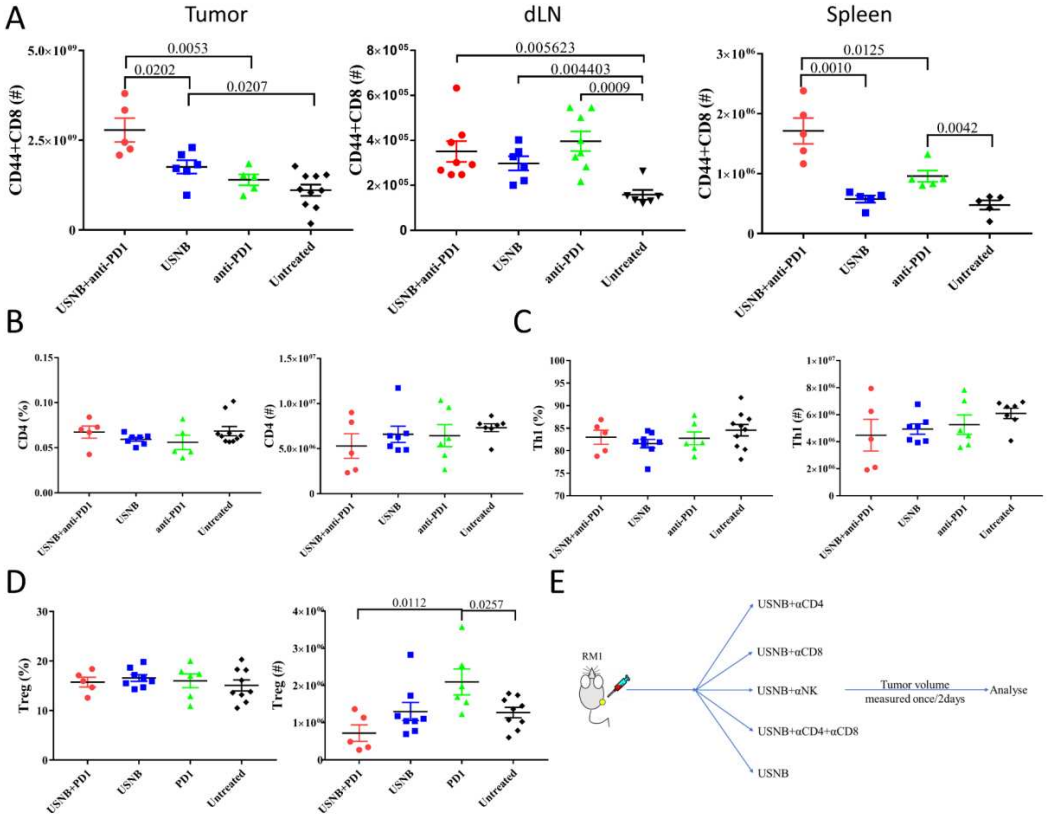

Figure S2

C57BL/6 mice implanted with MC38 cells were left untreated or treated with anti-PD1, USNB, or USNB+anti-PD1. The mice were sacrificed after the treatment schedule. (A) Flow cytometry analysis of the number of CD44+CD8+ T cells in the tumors, draining lymph nodes and spleens. The percentage and absolute number of CD4+ T cells (B), Th1 (C) and Treg cells (D) in the tumors. (E) Schematic diagram of the combination of USNB with antibodies to deplete CD4+ T cells, CD8+ T cells and NK cells in mice. The bars represent the mean±SEM. The p value was calculated by unpaired t test. The data are representative of two independent experiments with at least five mice per group.

Figure S3

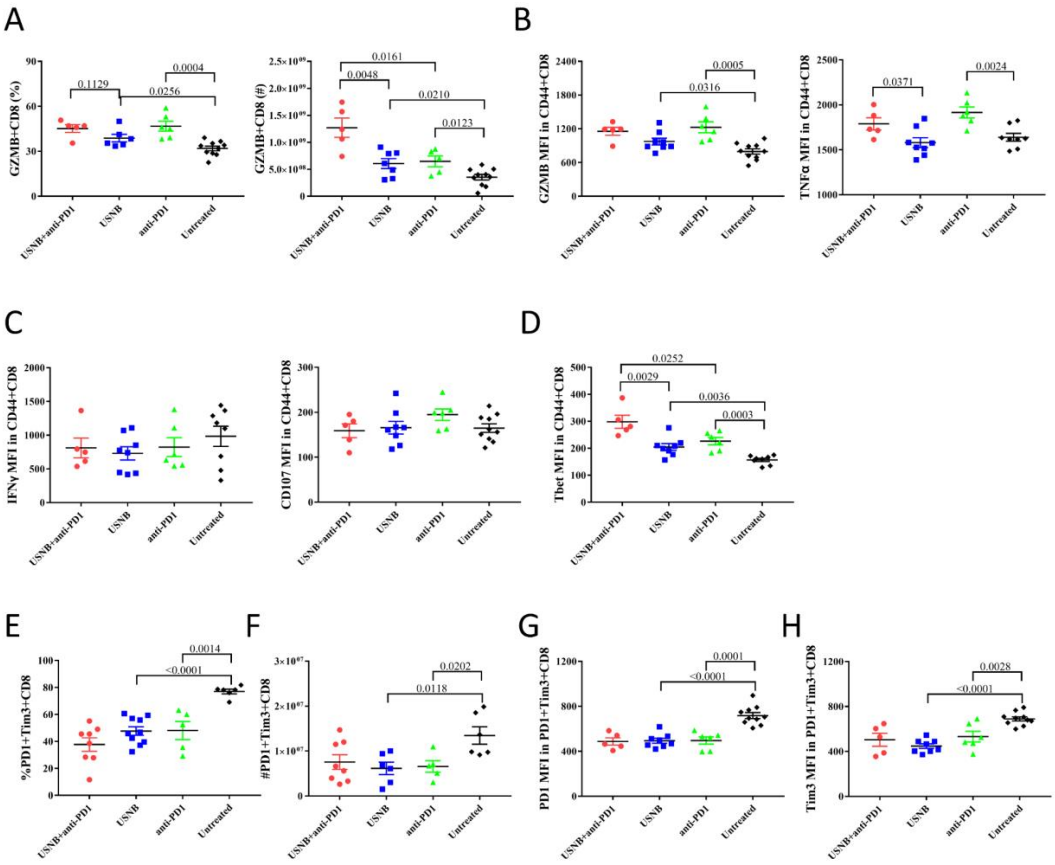

Figure S3

C57BL/6 mice implanted with MC38 cells were left untreated or treated with anti-PD1, USNB, or USNB+anti-PD1. The mice were sacrificed after the treatment schedule. (A) The percentage and absolute number of GZMB+CD8+ T cells in tumors. (B) MFI of GZMB and TNFα in CD44+CD8+ T cells. (C) MFI of IFNγ and CD107 in CD44+CD8+ T cells. (D) MFI of Tbet in CD44+CD8+ T cells. (E) and (F) show the percentage and absolute number of PD1+Tim3+CD8+ T cells in tumor tissues, respectively. (G) and (H) show the mean fluorescence intensity (MFI) of PD1 and Tim3 in PD1+Tim3+CD8+ T cells, respectively. The bars represent the mean±SEM. The *p* value was calculated by unpaired t test (A-H). The data are representative of two independent experiments with at least five mice per group.

Figure S4

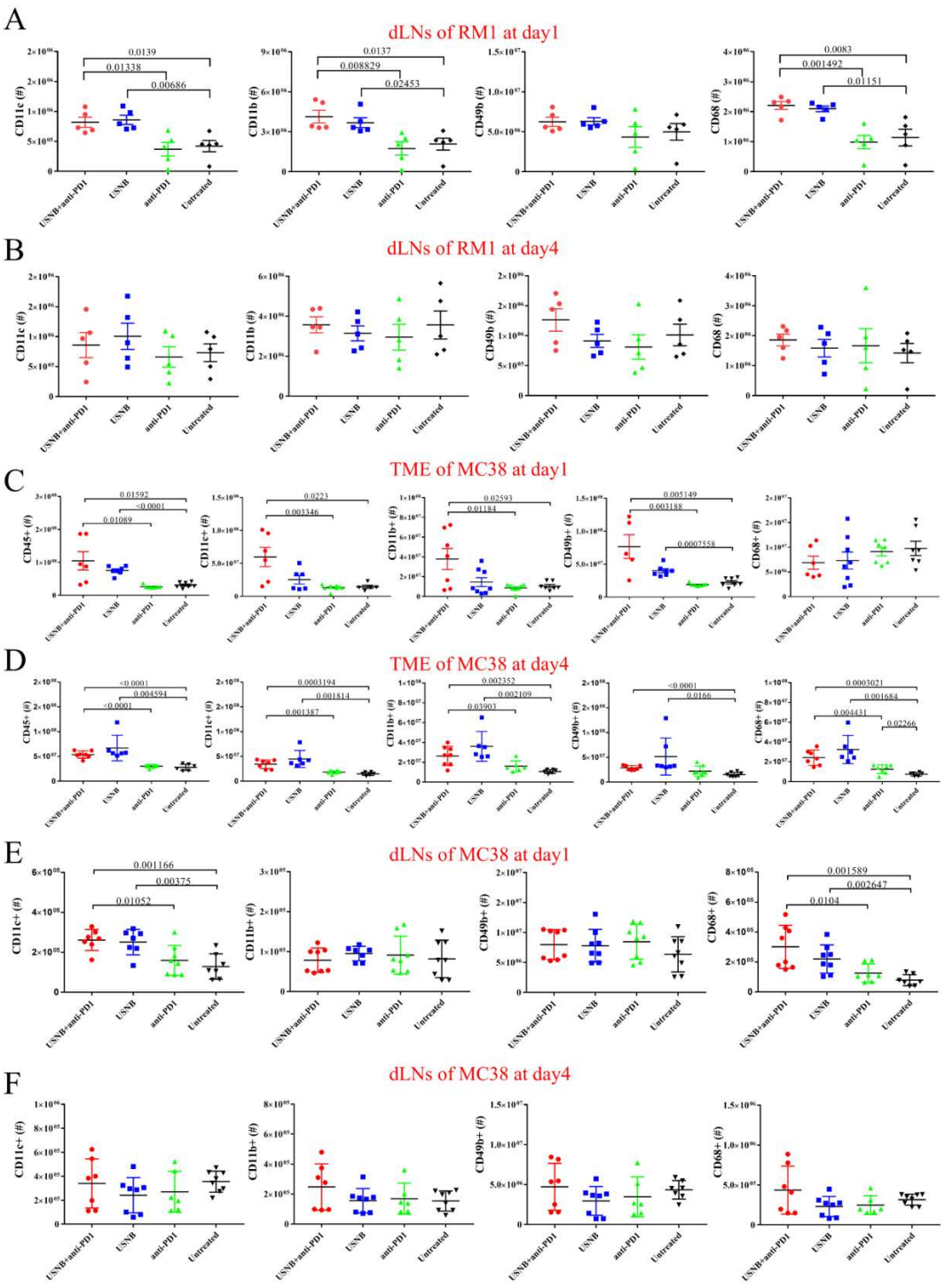

Figure S4

One day and 4 days after the mice were inoculated with RM1 cells, the mice were treated with

USNB+anti-PD1, USNB or anti-PD1, the absolute numbers of DCs (CD11c+), monocytes (CD11b+), NK cells (49b+) and macrophages (CD68+) In the dLNs were analyzed by flow cytometry (A and B). One day and 4 days after the mice inoculated with 0.5×10^6 MC38 cells, the mice were treated with USNB+anti-PD1, USNB or anti-PD1, the absolute numbers of leukocytes (CD45+), DCs (CD11c+), monocytes (CD11b+), NK cells (49b+) and macrophages (CD68+) in tumor tissue (C and D) and DCs (CD11c+), monocytes (CD11b+), NK cells (49b+) and macrophages (CD68+) in dLNs (E and F) were analyzed by flow cytometry. The bars represent the mean±SEM. The *p* value was calculated by unpaired t test (A-F). The data in A-F are representative of two independent experiments with at least five mice per group.

Figure S5

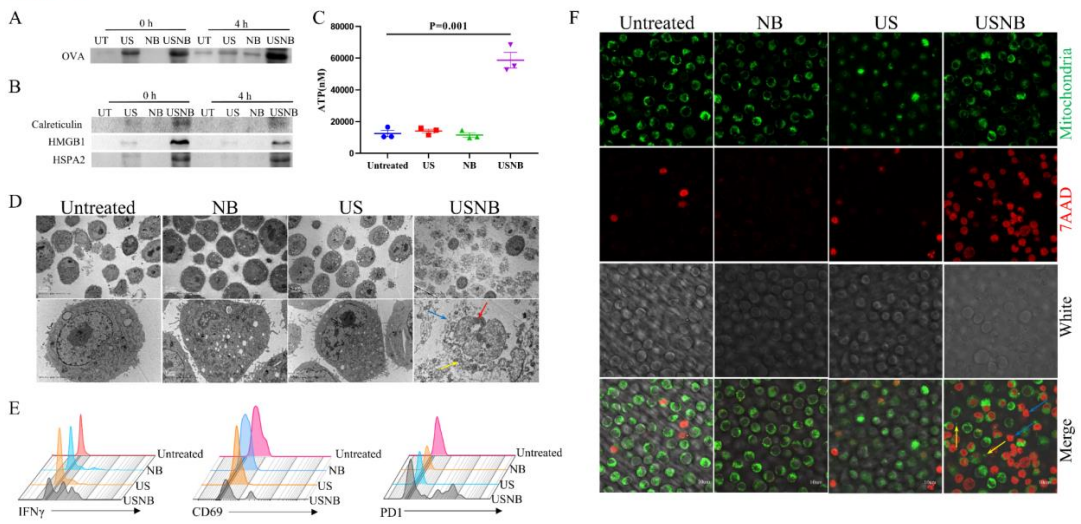

Figure S5

MC38-OVA cells were treated as before, and the supernatant was collected and analyzed. Western blotting of tumor antigen OVA levels (A) and immunogenic cell death marker expression (B). (C) ATP concentration in the supernatant after different treatments. (D) Transmission electron microscopy images of MC38 cells obtained immediately after treatment with US, NB, or USNB. The blue arrow indicates the fragmented cell membrane, the yellow arrow indicates swollen mitochondria, and the red arrow indicates fragmented nuclei. The scale bars in the global and magnified images represent 5 µm and 1 µm, respectively. The data are from three independent experiments, and representative results are shown. (E) Flow cytometry analysis of the IFNγ, CD69 and PD1 expression levels in CD8+ T cells after ex vivo coculture assay. (F) Confocal microscopy images of MC38 cells treated as indicated and stained with MitoTracker (indicated as green) and 7AAD (indicated as red). The blue arrows and yellow arrows indicate nuclear fragments and extracellular mitochondria, respectively. The scale bars in the images represent 10 µm. Representative images from three independent experiments are shown. The bars represent the mean±SEM (n=3 per group in C). The *p* value was calculated by unpaired t test (C). The data in A-F are from three independent experiments, and representative results are shown.

Figure S6

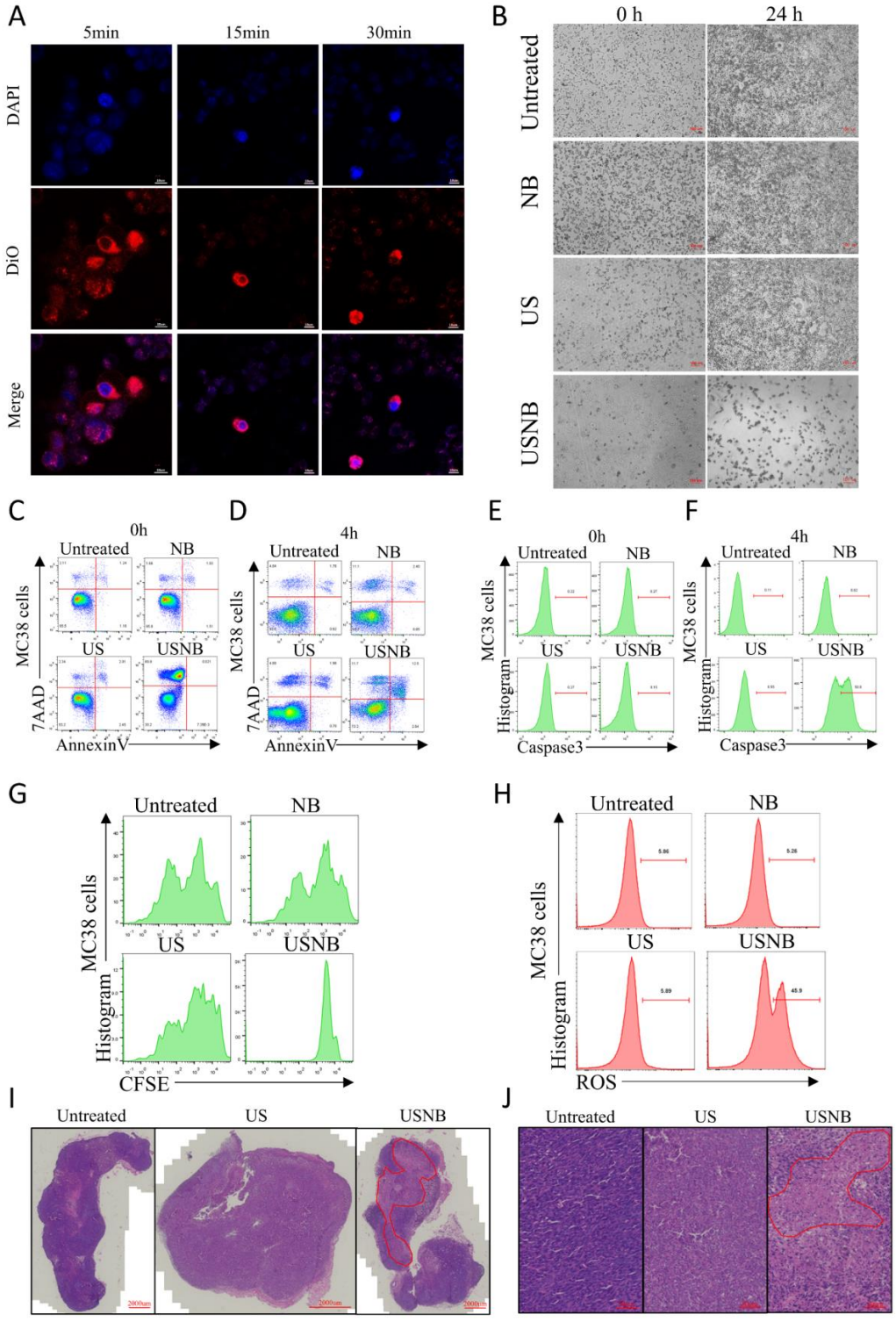

Figure S6

(A) Confocal microscopy images of MC38 cells after NB treatment for 5 min, 15 min and 30 mins. The nuclei were stained with DAPI, and NB were stained with DiO. The scale bar in the image represents 10 μm. (B) USNB was used to treat MC38 cells in vitro, and the morphology was observed by microscopy 0 h (left) and 24 h (right) after treatment. The scale bars in the images represent 100 μm. At 0 h and 4 h after MC38 cells were treated as indicated, the cells were stained with 7AAD and annexinV (C and D) or Caspase3 (E and F) and analyzed by flow cytometry. (G) Histogram of CFSE staining, indicating the proliferation ability of MC38 cells 24 h after treatment. (H) Flow cytometry analysis of ROS levels in MC38 cells 4 h after treatment. (I) and (J) Hematoxylin and eosin staining of tumor tissues after the mice were sacrificed. The red zone indicates the necrosis region. The scale bars in the global and magnified images represent 2000 μm and 100 μm. Representative images of three independent experiments are shown.

Figure S7

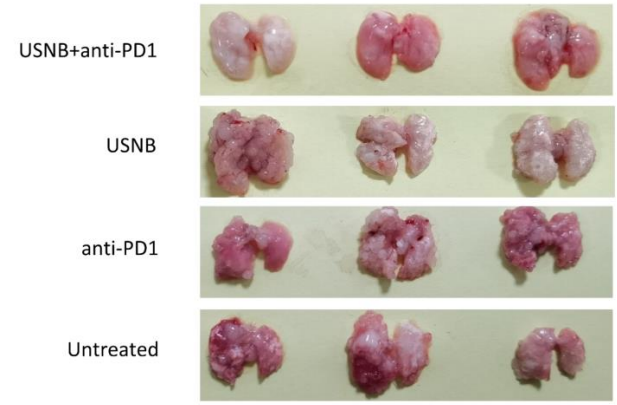

Figure S7

Representative image of lungs with metastatic tumor nodules at the end of the experiment.