

Supplementary Figure 1: Representative images of lung metastases in mice with or without concomitant MFP tumors. Images of India ink stained lungs with 67NR tumors from BALB/c mice with MFP and lung tumors (MFP + Lung) or lung tumors along (Lung) as described in Figure 1F and G. Lungs appear black with metastases in white. A) All images from representative experiment shown in Figure 1F. B) Representative images from one of three independent experiments shown in Figure 1G.



Supplementary Figure 2: Isoflurane does not decrease lung metastases in IV injected mice.

BALB/c mice were injected with 67NR cells IV and returned to their cage (IV only) or put into a chamber with isoflurane for 5-10 minutes (IV + isoflurane) to simulate anaesthetic conditions of mice injected with concomitant MFP tumors. A) Enumeration of lung metastases and B) representative images of each group. Data points represent whole lungs of individual mice pooled from 2 independent experiments with mean \pm SEM (n = 4 – 5 mice per group, per experiment). Unpaired *t*-test. ns $P \ge 0.05$.



Supplementary Figure 3: Gating strategy for flow cytometry of the immune TME. MFP and lung tumors from mice injected with 67NR tumor cells (as outlined in Figure 1) were harvested and processed to a single cell suspension by collagenase digestion and 70 µm filtration. Samples were stained with antibodies as indicated and analysed by flow cytometry. Diagrams show flow cytometry gating strategy from 3 different panels to identify indicated cell populations within tumors. **A)** T cell panel. **B)** NK cell/B cell panel. **C)** Myeloid panel.



Supplementary Figure 4: Immune cell frequencies by flow cytometry of MFP and lung tumors at treatment commencement. MFP and lung tumors were harvested at 10 days post tumor injection and analysed by flow cytometry as indicated in Supplementary Figure 3. Data points represent individual tumors with n = 4 mice per group. Bars represent mean \pm SEM. Mann-Whitney test. ns $P \ge 0.05$; *P < 0.05.



Supplementary Figure 5: CD206⁺ Macrophages are decreased by concomitant MFP tumor growth in treated lung tumors. MFP and lung tumors were harvested at 7 days post treatment with α PD-1/ α CTLA4 and analysed by flow cytometry as indicated in Figure 2 and Supplementary Figure 3. A) Immune cell populations analysed during treatment. B) Analysis of CD206 expression in macrophages within lung tumors with representative plots alongside quantitation. Data points represent whole lungs from individual mice from 2 independent experiments with mean ± SEM (n = 4 – 6 mice per group, per experiment). Unpaired *t*-test. ns $P \ge 0.05$; *P < 0.05; *** $P \le 0.001$.