Supplemental Data

Figure S1

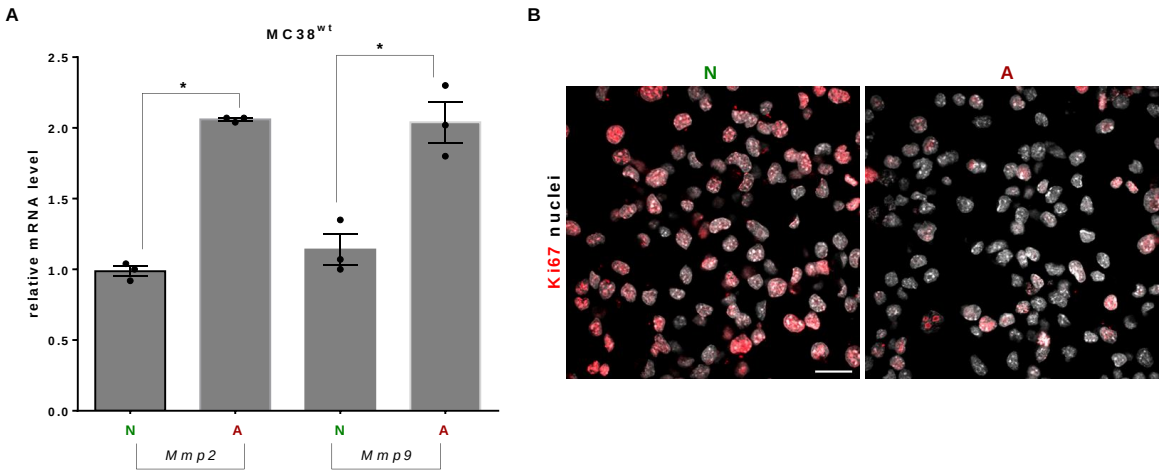

Fig. S1 Induction of surrogate acidosis markers in MC38^{wt} cells treated with acidic cell culture media *in vitro*. **(A)** Relative *Mmp2* and *Mmp9* mRNA expression normalized to *Gapdh*, *Aldolase* and *β-actin* (n = 3, statistics: two-tailed Student's t-test), both of which are surrogate markers for acidosis, and **(B)** fluorescent staining for Ki67 (red) and nuclei (gray) in MC38^{wt} cells treated with acidic cell culture media for 24 h. Scale bar: 20 µm. The data are presented as the means ± SEM. Abbreviations: N = neutral media, A = acidic media. Statistics: one-tailed Mann-Whitney test.

Figure S2

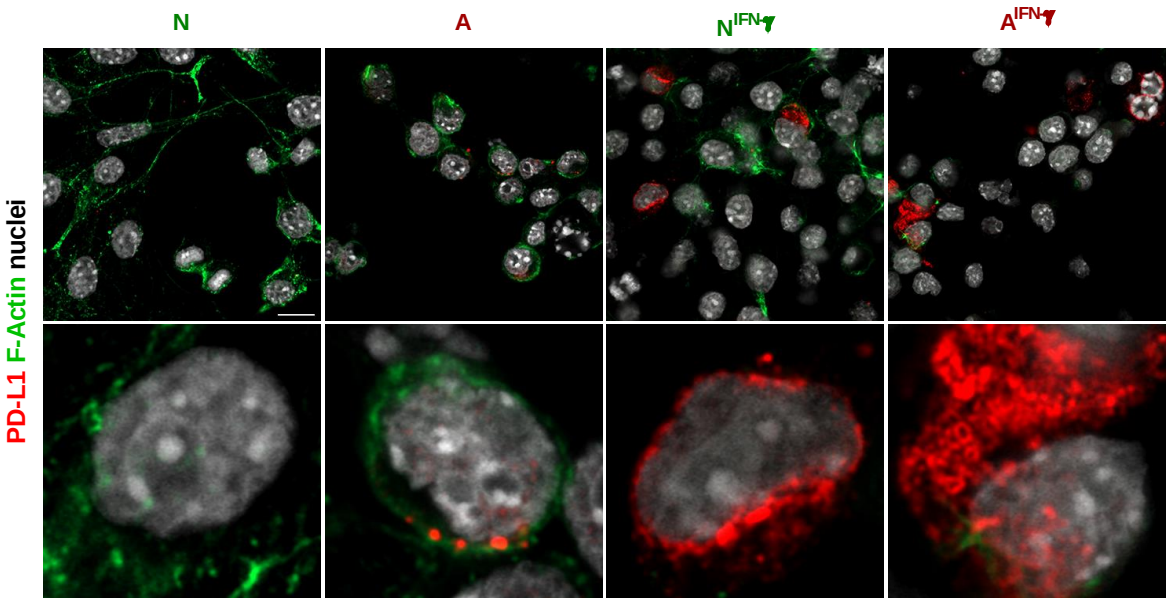

Fig. S2 $A^{IFN\text{-}\gamma}$ induces PD-L1 expression on cancer cells. Fluorescent staining for PD-L1 (red), F-actin (green) and nuclei (gray) in $MC38^{wt}$ cells cultured with neutral or acidic cell culture media in the presence or absence of IFN-γ (10 ng ml^{-1}) for 72 h. Scale bar for the upper row: 10 μm. The lower row presents magnified images of a single cell from the images in the upper row.

Figure S3

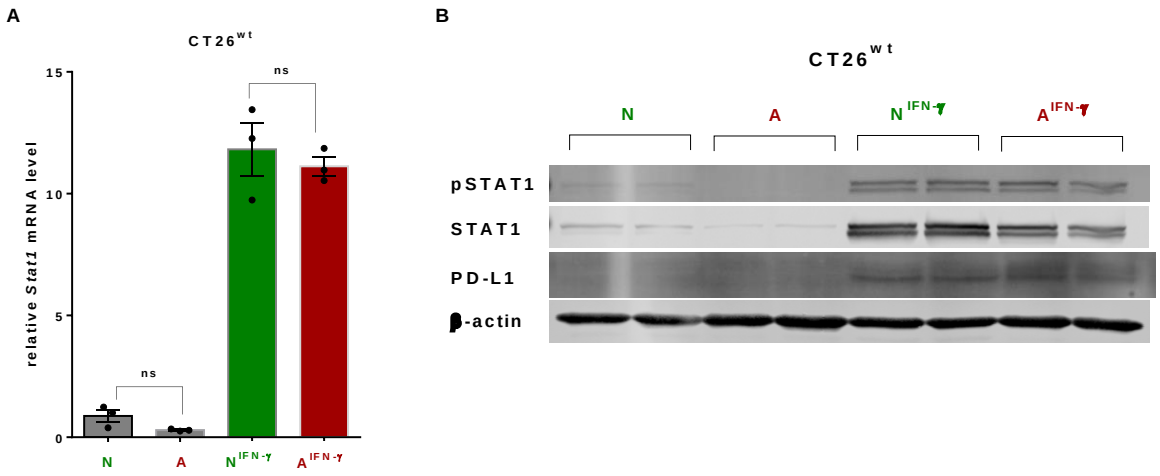

Fig.S3 IFN-γ induces *Stat1* and PD-L1 expression in CT26^{wt} cells. (A) Relative *Stat1* mRNA expression normalized to *Gapdh*, *Aldolase* and *β-actin* (n = 3, statistics: Tukey's multiple comparison test) and **(B)** Western blot analysis of PD-L1 and β-actin levels in CT26^{wt} cells treated with acidic media and/or IFN-γ (10 ng ml^{-1}) for 72 h (n = 2). Data are presented as the means ± SEM. N = neutral media, A = acidic media, N$^{IFN-\gamma}$ = neutral media plus IFN-γ, A$^{IFN-\gamma}$ = acidic media plus IFN-γ.

Figure S4

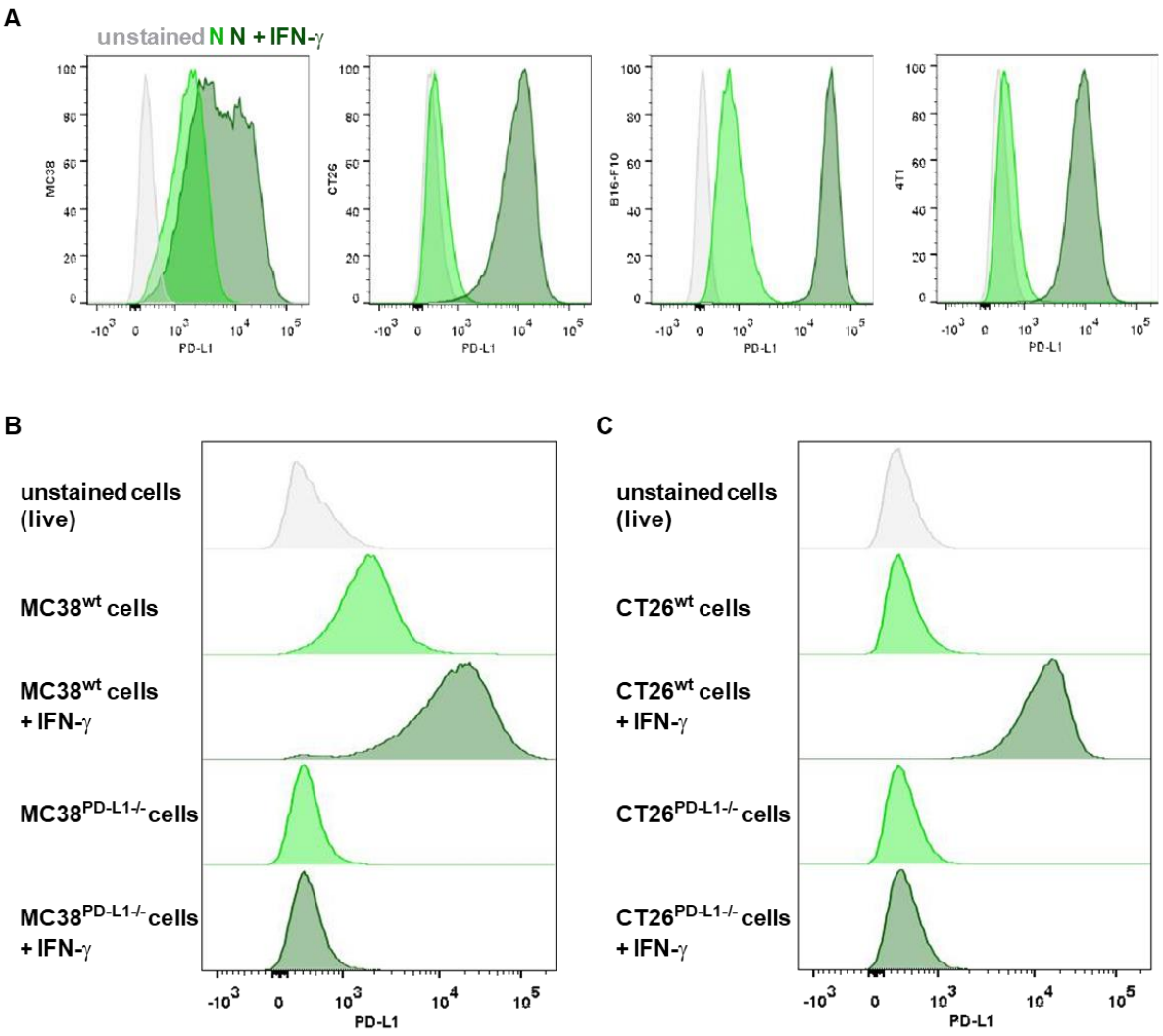

Fig. S4 Basal and IFN-γ-induced PD-L1 expression in murine wild-type cell lines and CRISPR/Cas9-generated PD-L1 knockout cells. (A) Basal (light green) and IFN-γ-induced (10 ng ml^{-1}; 24 h; dark green) PD-L1 expression in MC38^{wt}, CT26^{wt}, B16-F10^{wt} and 4T1^{wt} cells visualized using flow cytometry. Unstained live cells are shown in gray. Histograms of PD-L1 expression on **(B)** MC38^{wt} and MC38$^{PD\text{-}L1\text{-}/\text{-}}$ and **(C)** CT26^{wt} and CT26$^{PD\text{-}L1\text{-}/\text{-}}$ cells cultured in the absence (light green) or presence (dark green) of IFN-γ (100 ng ml^{-1}). Cells were stimulated for 24 h (MC38^{wt} and MC38$^{PD\text{-}L1\text{-}/\text{-}}$) or 48 h (CT26^{wt} and CT26$^{PD\text{-}L1\text{-}/\text{-}}$) with IFN-γ; unstained living cells (gray) served as controls.

Figure S5

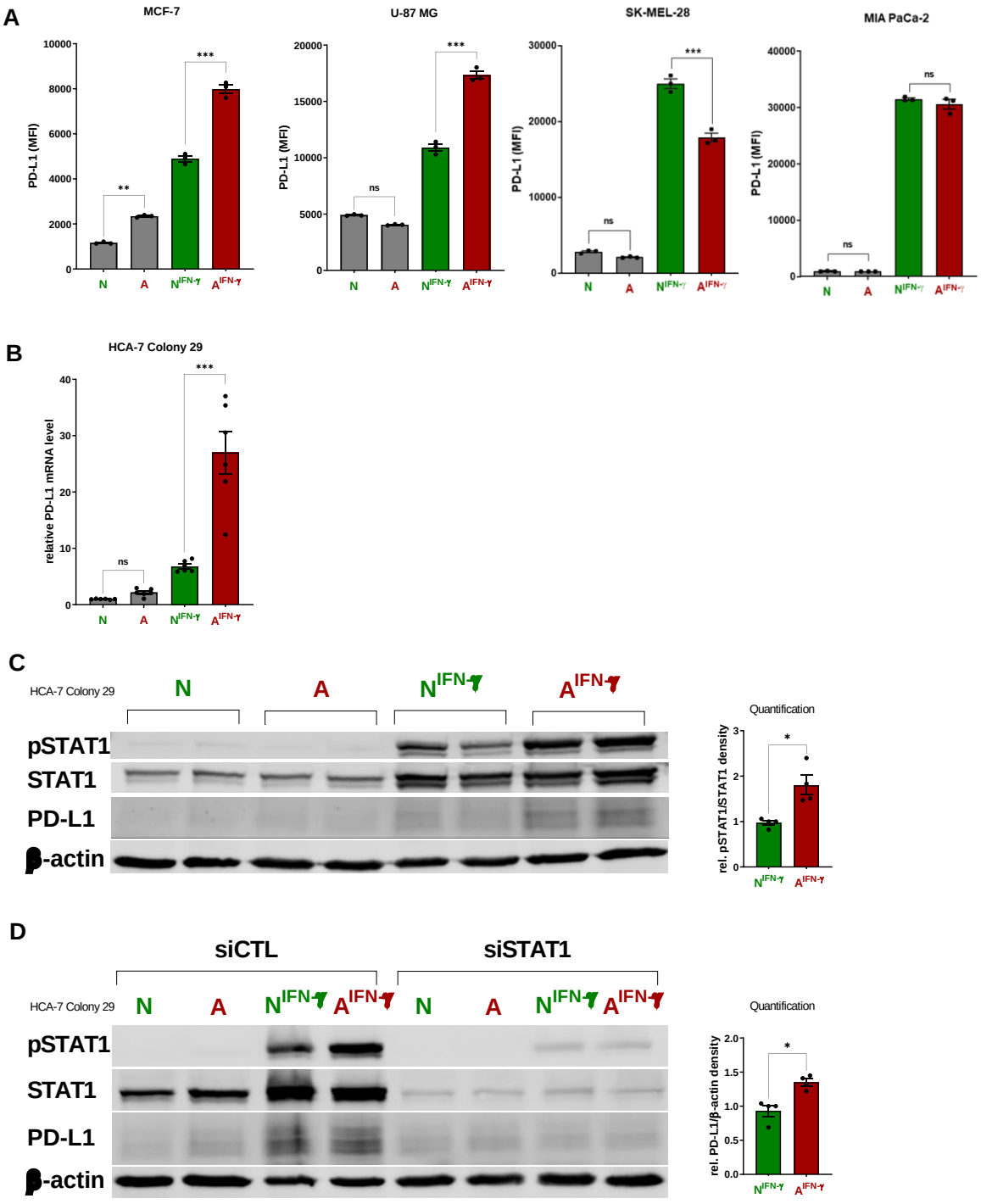

Fig. S5 $A^{IFN-\gamma}$ induces total and cell surface PD-L1 expression in different human cancer cell lines. (A) PD-L1 MFI measured using flow cytometry in human MCF-7, U-87 MG, SK-MEL-28 and MIA PaCa-2 cells treated with acidic media and/or IFN-γ (10 ng ml^{-1}) for 72 h

(n = 3, statistics: Tukey's multiple comparison test). **(B)** Relative *Pdl1* mRNA expression normalized to *Gapdh*, *Aldolase* and *β-actin* (pooled data from 2 experiments, n = 5-6, statistics: Tukey's multiple comparison test) and **(C)** Western blot analyses and densitometry of pSTAT1, STAT1, PD-L1 and β-actin levels in the HCA-7 colony 29 cell line treated with acidic media and/or IFN-γ (10 ng ml^{-1}) for 72 h (pooled data from 2 experiments, n = 4, statistics: nonparametric Mann-Whitney test). HCA-7 colony 29 cells were transfected with a control (siCTL) or STAT1-specific siRNA and treated with acidic media and/or IFN-γ (10 ng ml^{-1}) for 24 h. **(D)** Western blot analyses of pSTAT1, STAT1, PD-L1 and β-actin levels were performed to determine the STAT1 knockdown efficiency and absence of PD-L1 induction following treatment with the combination of IFN-γ and acidic media (n = 2). Data are presented as the means ± SEM. N = neutral media, A = acidic media, $N^{IFN-\gamma}$ = neutral media plus IFN-γ, $A^{IFN-\gamma}$ = acidic media plus IFN-γ.

Figure S6

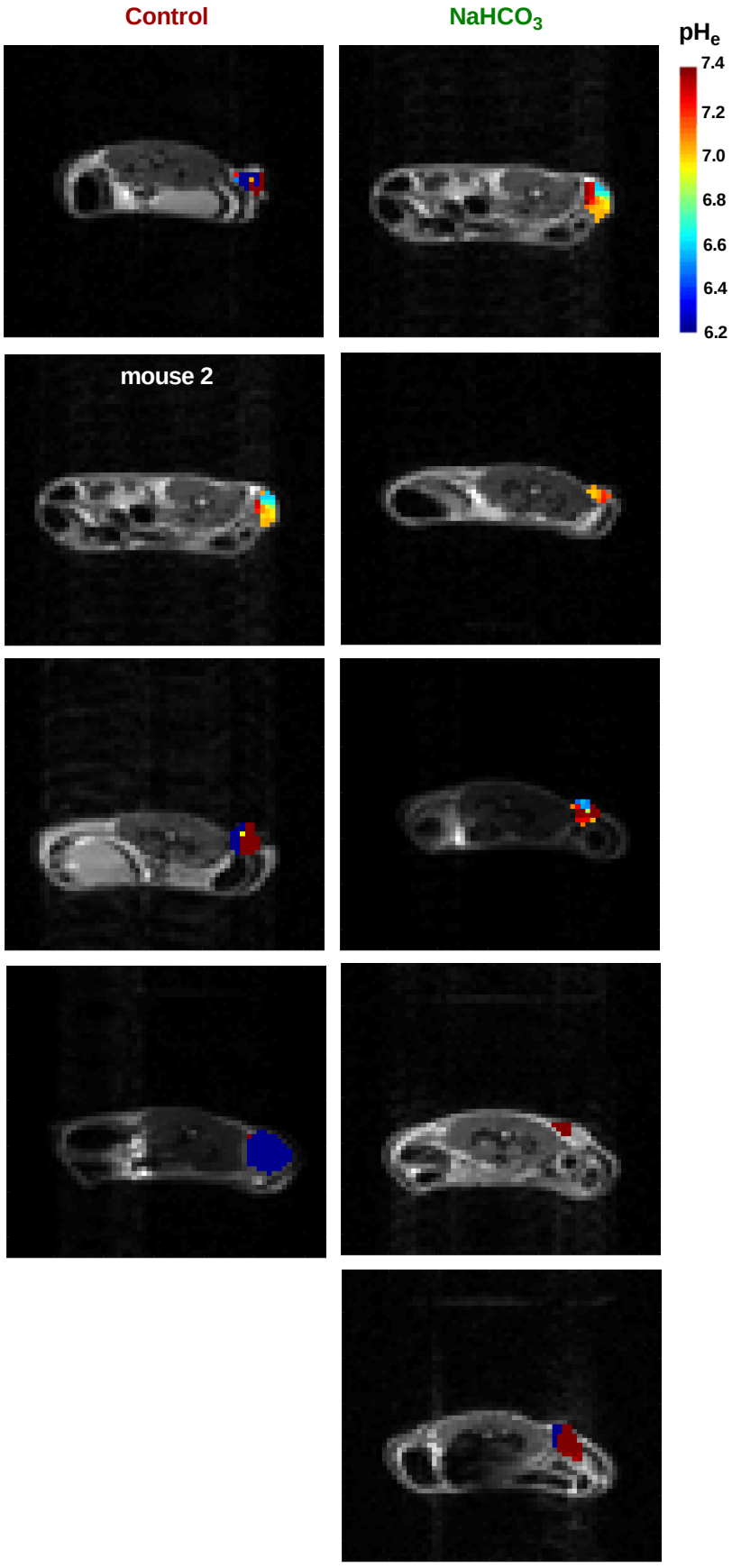

Fig. S6 Imaging of tumor pH_e neutralization by $NaHCO_3$ treatment using noninvasive *in vivo* acidoCEST MRI. $NaHCO_3$-enriched drinking water increased the tumor pH_e in mice ($NaHCO_3$) compared to mice receiving regular drinking water, where the tumor pH_e is acidic (control). Representative pH_e maps overlaid on T_2-weighted axial MRI images of $MC38^{wt}$ tumor-bearing mice injected with iopamidol (*i.v.*) at day 10 after the cancer cell injection. Mice received either $NaHCO_3$ (200 mM) water or regular drinking water (control) three days prior to cancer cell inoculation. Mouse 4 from the $NaHCO_3$ group and mouse 4 from the control group were excluded from the analysis, as the measured pH_e value was out of the calibration range.

Figure S7

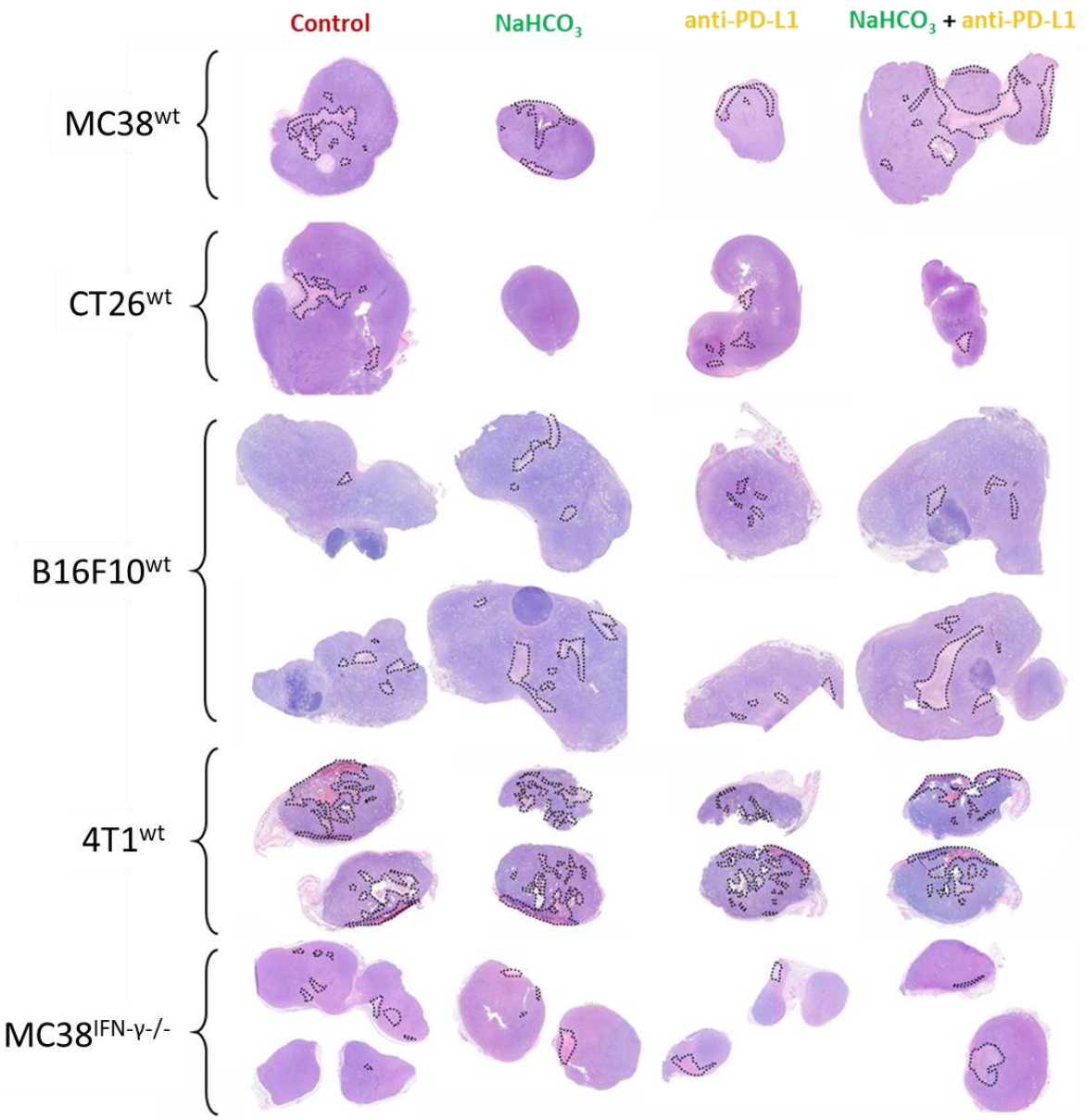

Fig. S7 Histopathology of murine MC38^{wt}, CT26^{wt}, B16F10^{wt}, 4T1^{wt} and MC38$^{IFN\text{-}\gamma\text{-}/\text{-}}$ tumors. Representative H&E staining of *s.c.* MC38^{wt}, CT26^{wt}, B16F10^{wt} 4T1^{wt} and MC38$^{IFN\text{-}\gamma\text{-}/\text{-}}$ tumors derived from C57BL/6J wild-type mice after the different treatment conditions. Necrotic areas are encircled by a dashed black line. Animals received regular drinking water (control), $NaHCO_3$ in water ($NaHCO_3$), anti-PD-L1 mAb or $NaHCO_3$ & anti-PD-L1 mAb. Tumors from n = 7 – 8 animals per treatment group underwent histopathological analysis by a professional pathologist.

Figure S8

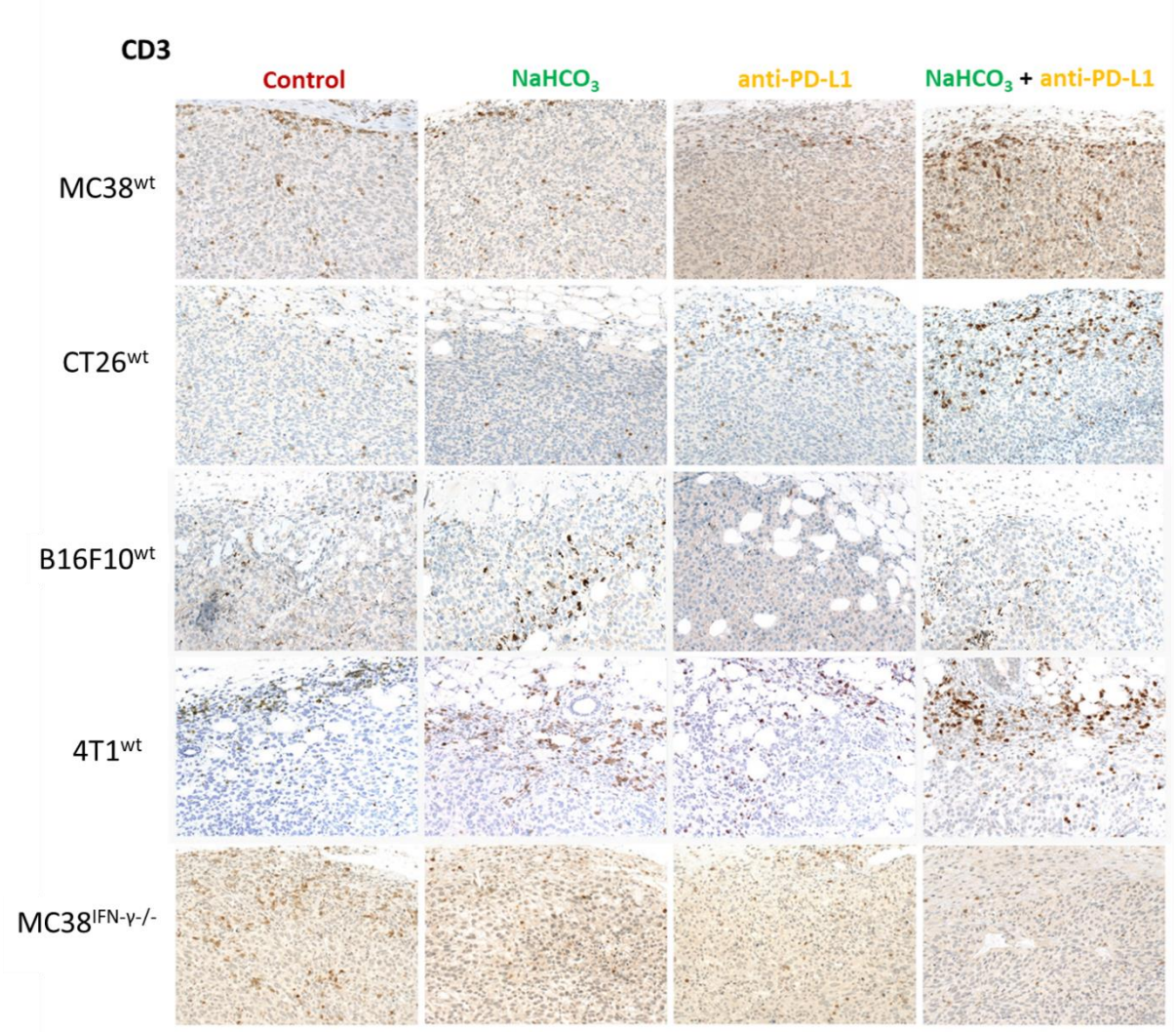

Figure S8 CD3 Immunohistochemistry of murine MC38^{wt}, CT26^{wt}, B16F10^{wt}, 4T1^{wt} and MC38$^{IFN\text{-}\gamma\text{-}/\text{-}}$ tumors. Representative CD3 immunohistochemistry of *s.c.* MC38^{wt}, CT26^{wt}, B16F10^{wt}, 4T1^{wt} and MC38$^{IFN\text{-}\gamma\text{-}/\text{-}}$ tumors derived from wild-type C57BL/6J mice at day 18 (MC38^{wt}), day 17 (CT26^{wt}), day 14 (B16F10^{wt}), day 20 (4T1^{wt}) and day 17 (MC38$^{IFN\text{-}\gamma\text{-}/\text{-}}$) after different treatment conditions. Animals received regular drinking water (control), $NaHCO_3$ in water ($NaHCO_3$), anti-PD-L1 mAb or $NaHCO_3$ & anti-PD-L1 mAb. N = 3 – 4 representative tumors of each experimental group were subjected to CD3 staining which was analyzed by a professional pathologist.

Supplementary References

1. Mall, C., et al., *Repeated PD-1/PD-L1 monoclonal antibody administration induces fatal xenogeneic hypersensitivity reactions in a murine model of breast cancer*. Oncoimmunology, 2016. **5**(2): p. e1075114.