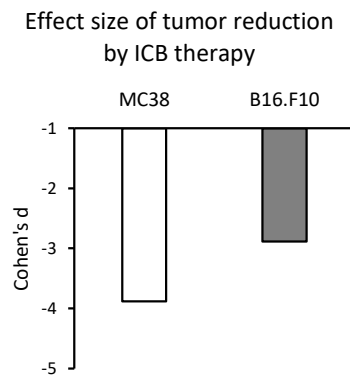
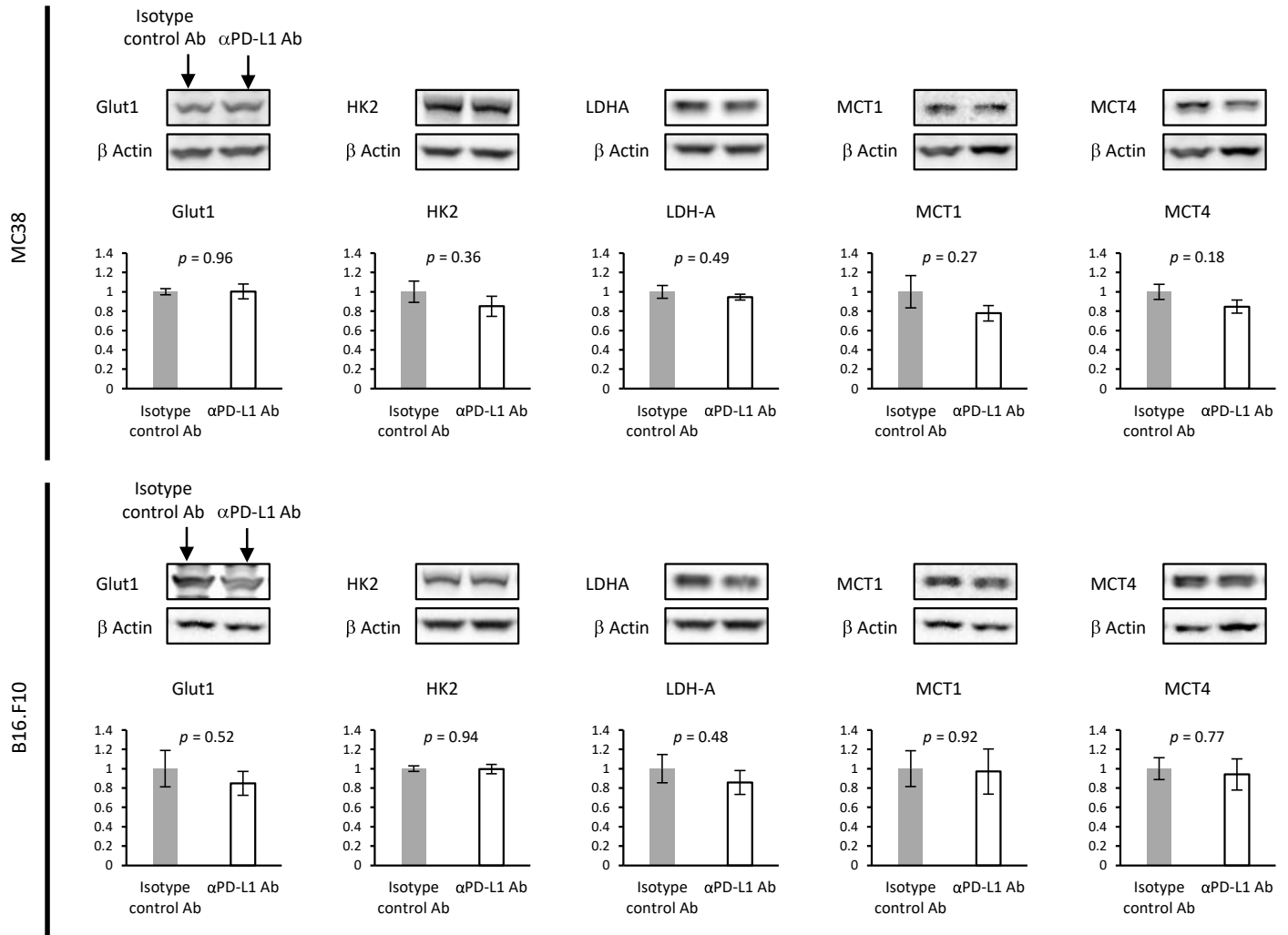


Supplemental Figure 1



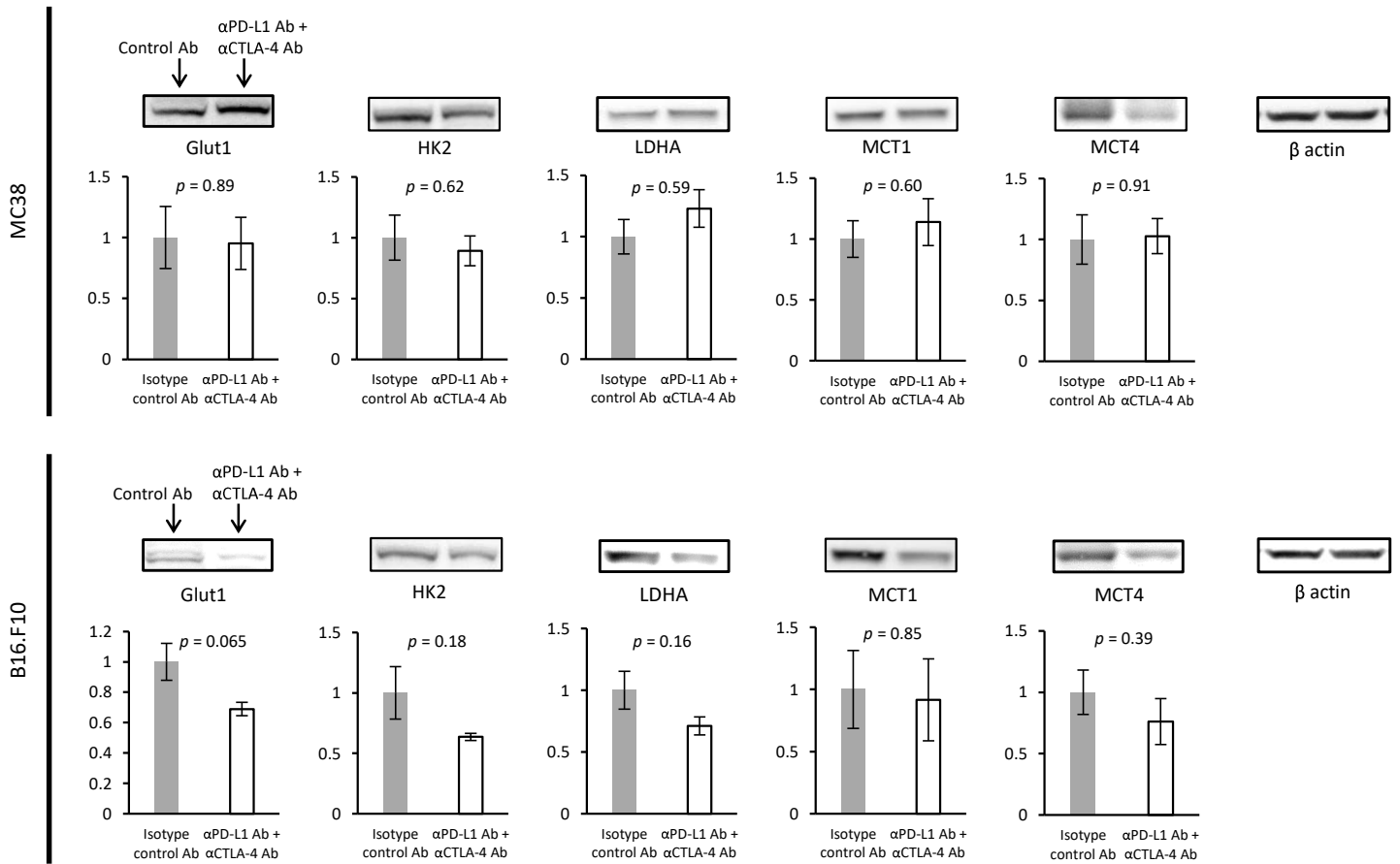
Supplemental Figure 1. Effect size of tumor reduction by Immune checkpoint blockade therapy. MC38 or B16.F10 inoculated mice were treated with either Isotype control Ab, anti-PD-L1 Ab, or anti-PD-L1 Ab + anti-CTLA-4 Ab on days 9, 12, and 15 post tumor inoculation (n = 5 per group). Tumor size of the control group and ICB group on day 17 were used for calculation of Cohen's d.

Supplemental Figure 2



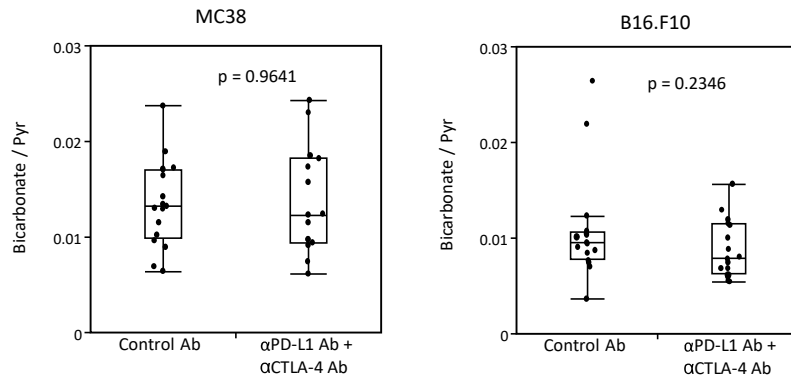
Supplemental Figure 2. Western blot analysis for glycolytic enzymes and transporters (Glut1, HK2, LDHA, MCT1, and MCT4) on tumor cells in vitro. MC38 and B16.F10 tumor cells pre-treated with IFN- γ for 24 h followed by anti-PD-L1 Ab or isotype control Ab for 24 h were collected and processed. Bar plot shows the quantification of protein bands. Data are shown as mean \pm SE. The mean of the control group is set as 1 (n = 4 per group). Statistical significance between groups was determined by Student's t test.

Supplemental Figure 3



Supplemental Figure 3. Western blot analysis for glycolytic enzymes and transporters (Glut1, HK2, LDHA, MCT1, and MCT4) on in vivo ICB treated tumor. MC38 or B16.F10 tumor bearing mice were treated with isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab on day 9 and 12. Tumors were harvested and processed on day 13. Bar plot shows the quantification of protein bands. Data are shown as mean \pm SE. The mean of the control group is set as 1 (n = 5 per group). Statistical significance between groups was determined by Student's t test.

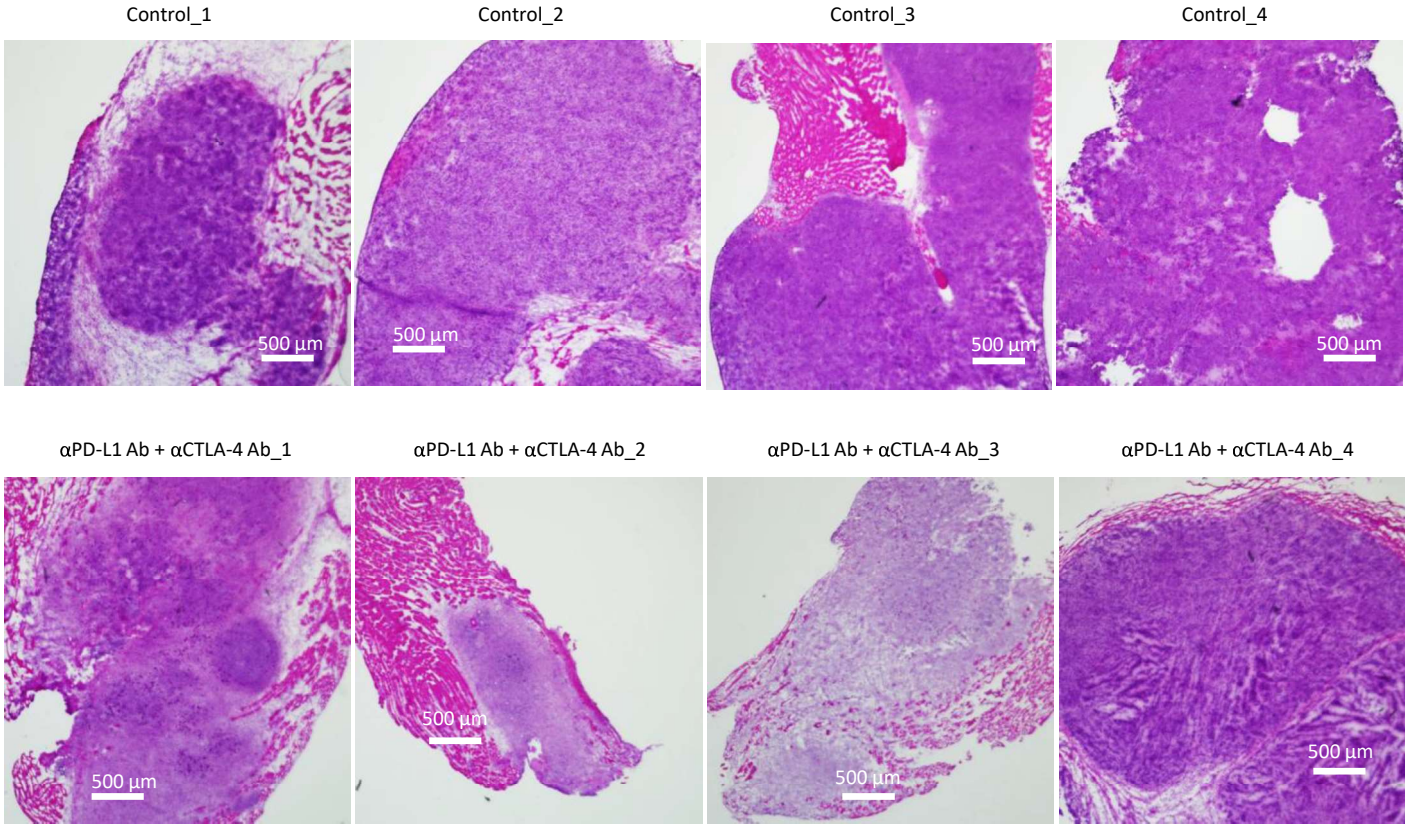
Supplemental Figure 4



Supplemental Figure 4. Bicarbonate/Pyr ratio examined with hyperpolarized ^{13}C MRI using $[1-^{13}\text{C}]$ pyruvate in Figure 3.

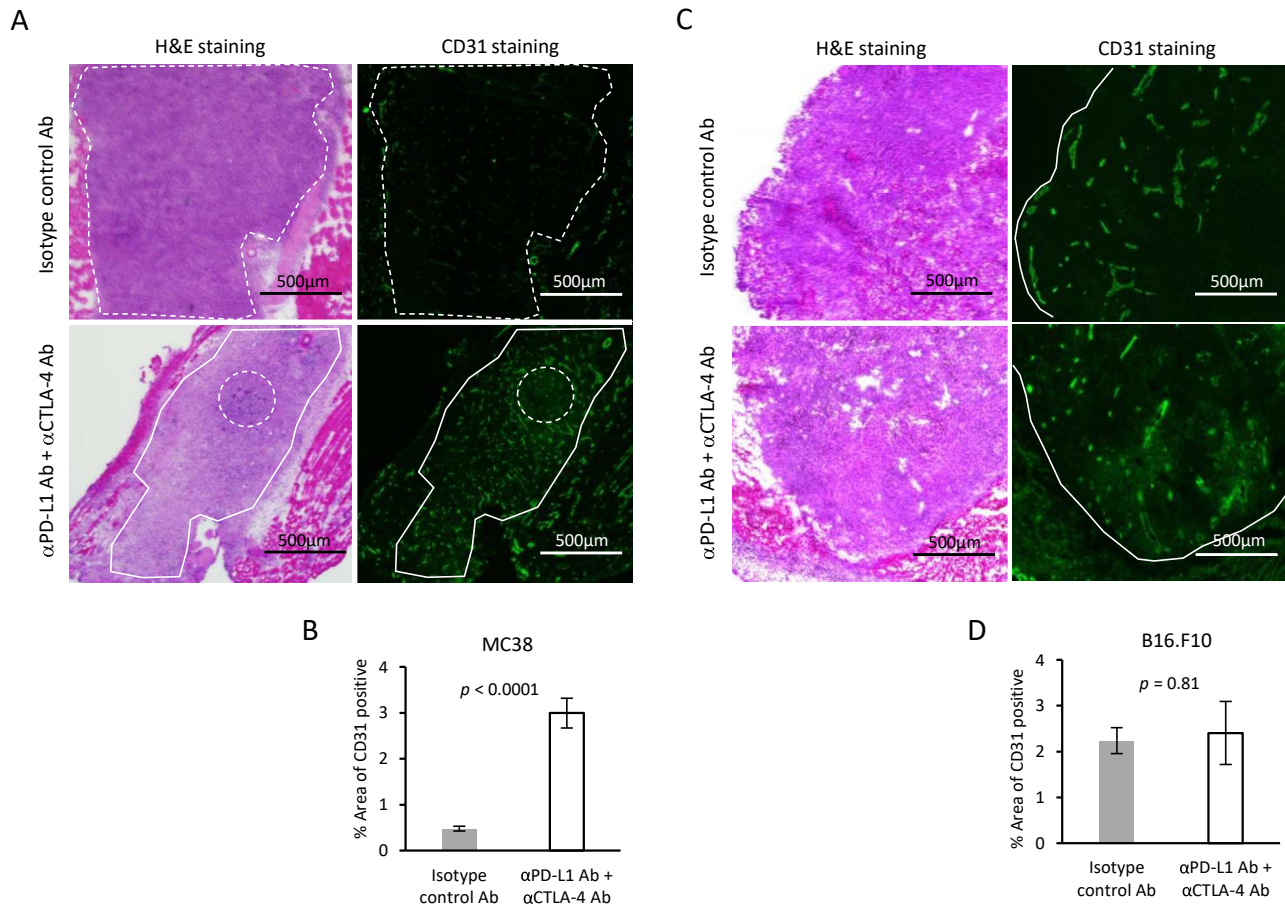
MRI of hyperpolarized ^{13}C pyruvate metabolism in two murine tumor models. Tumor bearing mice treated with isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab were scanned after 2nd injection of antibodies (MC38; $n = 23$, $n = 16$ each group, B16.F10; $n = 17$, $n = 16$ each group). (A) Bicarbonate to Pyruvate ratio of MC38 or B16.F10 sorted by treatment. Data are shown as box-and-whisker plot (median, maximum, minimum, first quartile, and third quartile); individual values are shown. Statistical significance between groups was determined by Student's t test.

Supplemental Figure 5



Supplemental Figure 5. Hematoxylin and eosin staining (HE) of MC38 tumors. MC38 tumor bearing mice were treated with each Ab on day 9 and 12. Tumors were harvested on day 13 (scale bar = 500 μm).

Supplemental Figure 6



Supplemental Figure 6. Histological assessment of CD31 staining. (A and C) Immunofluorescence staining of CD31 in MC38 (A) and B16.F10 (C) tumor. MC38 or B16.F10 tumor bearing mice were treated with each Ab on day 9 and 12. Tumors were harvested on day 13 (MC38; n = 4 per group, B16.F10; n = 5 per group). (A and C) Representative HE and CD31 staining of same section in MC38 and B16.F10 tumor (scale bar = 500 μ m). (B and D) Quantification of CD31 positive area. Data are shown as mean \pm SE. Statistical significance between groups was determined by Student's *t* test for B and D.

Supplemental Table 1A. Multi-regression analysis of the treatment group (isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab) in MC38 tumors examined with ¹³C pyruvate MRI.

Coefficients	Estimate	Std. Error	t value	p value
(Intercept)	-2.18	1.46	2.22	0.136
Tumor size (mm ³)	0.00127	0.00334	0.15	0.703
Lac / Pyr	2.71	1.79	2.28	0.131

Supplemental Table 1B. Multi-regression analysis of the treatment group (isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab) in B16.F10 tumors examined with ¹³C pyruvate MRI.

Coefficients	Estimate	Std. Error	t value	p value
(Intercept)	-4.94	2.14	5.30	* 0.0213
Tumor size (mm ³)	0.00115	0.00437	0.07	0.793
Lac / Pyr	6.67	2.82	5.57	* 0.0182

Supplemental Table 1C. Multi-regression analysis of the treatment group (isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab) in MC38 tumors examined with ¹³C fumarate MRI.

Coefficients	Estimate	Std. Error	t value	p value
(Intercept)	5.30	2.32	5.20	* 0.0226
Tumor size (mm ³)	-0.00098	0.0089	0.01	0.912
Mal / Fum	-70	28.3	6.11	* 0.0134

Supplemental Table 1D. Multi-regression analysis of the treatment group (isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab) in B16.F10 tumors examined with ¹³C fumarate MRI.

Coefficients	Estimate	Std. Error	t value	p value
(Intercept)	3.35	2.23	2.25	0.133
Tumor size (mm ³)	-0.00826	0.00627	1.74	0.188
Mal / Fum	-17.3	21.8	0.63	0.429

Supplemental Table 1E. Multi-regression analysis of the treatment group (isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab) in MC38 tumors examined with DCE-MRI.

Coefficients	Estimate	Std. Error	t value	p value
(Intercept)	7.28	2.85	6.53	* 0.0106
Tumor size (mm ³)	-0.0117	0.00589	3.96	* 0.0466
K _{trans}	-199	79.1	6.3	* 0.0121

Supplemental Table 1F. Multi-regression analysis of the treatment group (isotype control Ab or anti-PD-L1 Ab + anti-CTLA-4 Ab) in B16.F10 tumors examined with DCE-MRI.

Coefficients	Estimate	Std. Error	t value	p value
(Intercept)	-1.29	1.55	0.7	0.403
Tumor size (mm ³)	-12.2	14.6	0.7	0.404
K _{trans}	0.00546	0.00405	1.81	0.178

Supplemental Table 2A. Single-regression analysis of imaging biomarker vs survival in MC38 tumor.

MC38	Lac/Pyr			Mal/Fum			K ^{trans}			Ve		
	Control	ICB	Whole	Control	ICB	Whole	Control	ICB	Whole	Control	ICB	Whole
<i>p</i> value	0.093	0.345	0.059	0.243	0.13	** 0.0033	0.488	0.35	* 0.0206	* 0.0422	0.249	** 0.0046
<i>R</i> ²	0.462	0.179	0.266	0.188	0.339	0.449	0.127	0.175	0.399	0.533	0.254	0.533

Supplemental Table 2B. Single-regression analysis of imaging biomarker vs survival in B16.F10 tumor.

B16.F10	Lac/Pyr			Mal/Fum			K ^{trans}			Ve		
	Control	ICB	Whole	Control	ICB	Whole	Control	ICB	Whole	Control	ICB	Whole
<i>p</i> value	0.246	* 0.0258	** 0.0023	0.533	0.966	0.279	0.526	* 0.0238	** 0.0017	0.069	* 0.0038	***<0.0001
<i>R</i> ²	0.186	0.591	0.473	0.0824	0.000502	0.105	0.107	0.759	0.641	0.604	0.901	0.9