#### **Supplementary Figure 1**



**Supplementary Figure 1: Generation and characterization of checkpoint blockade immunotherapy resistant tumor cells through serial *in vivo* passage. (A)** Tumor growth was monitored inmice challenged with parental or resistant tumor cells with and without immunotherapy treatment in wild type and (**B)** Rag-/- mice. 2.5 x 104 resistant and parental tumor cells were implanted in wild type and Rag-/- mice. Tumor growth was monitored with and without treatment. Error shown is mean +/- SD.

**Supplementary Figure 2**

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**Supplementary Figure 2: Evolution of checkpoint resistant gene expression across generations. (A)** Cell lines were generated at each serial passage from the 1st (3I-F1) through the 4th (3I-F4). RNA was purified from these cell lines using the RNAasy kit (Qiagen) and used to probe a MouseRef-8 microarray (Illumina). Ingenuity pathway analysis was performed comparing each of these generations to the parental B16-tdTomato cell line with a cutoff of ±0.5 log2 fold. The number of pathways over this cutoff at each generation is shown in the header line, and the -log (p-value) for a selected group of key pathways is shown across all four generations. **(B)** Cell line microarray data, as above, was plotted as a Venn diagram showing the number of genes whose expression increased or decreased by at least 0.5 log2 fold change between the indicated generations.

**Supplementary Figure 3**



**Supplementary Figure 3: NMR metabolite profile and hypoxia immunofluorescence indicate alterations in metabolism of resistant versus parental melanoma. (A)** Heat map ofrelative NMR metabolite intensities inresistant cell line (3I-F4) compared to parental cell line (B16/BL6-tdTomato). Cell lines were washed with PBS twice and flash frozen on liquid nitrogen. The intensities of metabolites were measured with respect to NMR reference compounds. The heat map was generated using Z score, which represents relative intensity of metabolites in resistant cell line lysate compared to parental cell line lysate. **(B)** Resistant and parental tumors were implanted in mice and treated on days 3, 6, and 9 or **(C)** untreated. Tumors were collected on day 12-14 for confocal microscopy. Hypoxia (green) was imaged using Hypoxyprobe and tumor cells (red) were visualized based on their expression of tdTomato fluorescent protein.

#### **Supplementary Figure 4**

**Supplementary Figure 4: Flow cytometry gating of T cells.** Example gating used to segment lymphoid and myeloid populations and profile their phenotype. Also shown are overexpression levels for PGAM2 and ADH7 in transduced B16/BL6 and Panc02 cell lines.

#### **Supplementary Figure 5**

**Supplementary Figure 5: Effects of metabolic adaptation by resistant tumors on T cell infiltration and function. (A)** CD4 T effector cell density per tumor weight was determined using flow cytometry analysis.Resistant and parental tumors were implanted in mice and treated on day 3, 6 and 9. Tumors were weighed before harvest for flow cytometric analysis. Data are expressed as the total number of CD4 effector cells per gram of tumor. CD4 T effector cells were gated as CD3 and CD4 positive and FoxP3 negative. **(B)** Quantification of CD8/Treg ratios within the tumor were calculated by dividing the number of CD8+CD3+ cells by the number of CD4+FoxP3+ Treg cells. CD4 effector and Treg cell phenotypic data was measured using multi-parametric flow cytometry as mean fluorescent intensity of **(C)** Glut1 receptor expression for Teff and **(D)** Treg, **(E)** PD-1 expression by Teff and **(F)** Treg. Data were pooled from ≥ 2 experiments with 5 mice per group. Bars represent mean ± SD. Statistical significance was calculated using a Student’s t test. ns, not significant; \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

**Supplementary Figure 6: **

**Supplementary Figure 6: Effects of metabolic adaptation by resistant tumors on infiltration and function of myeloid-derived suppressor cells. (A)** The myeloid derived suppressor cell (MDSC)fraction of total tumor infiltrating CD45+CD3- cells is shown. MDSC were gated on CD11b+ and Gr1+ positive populations. The suppressive enzyme expression of tumor MDSC was analyzed using multicolor flow cytometric analysis and data was presented as mean fluorescent intensity of **(B)** Indoleamine-pyrrole 2,3-dioxygenase (IDO) and **(C)** Arginase I. Data were pooled from ≥ 2 experiments with 5 mice per group. Bars represent mean ± SD. Statistical significance was calculated using a Student’s t test. ns, not significant; \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

**Supplementary Figure 7:**

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**Supplementary Figure 7: Monogenic assessment of the candidate immunotherapy resistance genes PGAM2 and ADH7 in the Panc02 model of pancreatic cancer.**

**(A)** Mice were implanted with either PGAM2 or empty vector-overexpressing Panc02 cells and treated, or not, on days 3, 6, and 9 with the αCTLA-4 antibody 9H10 (100ug i.p.). Survival and **(B)** tumor growth were monitored inthese mice with and without treatment. **(C)** Mice were implanted as above and treated with the αPD-1 antibody RMP1-14 (250ug i.p.) and survival and **(D)** tumor growth were measured (mean +/- SD shown). **(E)** Mice were implanted with either ADH7 or empty vector-overexpressing Panc02 cells and treated, or not, on days 3, 6, and 9 with the αCTLA-4 antibody 9H10 (100ug i.p.). Survival and **(F)** tumor growth were monitored inthese mice with and without treatment. **(G)** Mice were implanted as above and treated with the αPD-1 antibody RMP1-14 (250ug i.p.) and survival and **(H)** tumor growth were measured (mean +/- SD shown). Statistical significance was calculated using the log-rank (Mantel-Cox) test. ns, not significant; \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001.

**Supplementary Figure 8:**

**A close up of a map

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**Supplementary Figure 8:** **Knockdown of ADH7 does not sensitize PGAM2-knockout 3I-F4 melanoma to triple checkpoint blockade. (A)** Taqman (Invitrogen) gene expression analysis of Pgam2 in 3I-F4 cells in which PGAM2 was gene edited by CRISPR/CAS9, of Adh7 in 3I-F4 cells in which PGAM2 was gene edited by CRISPR/CAS9, and of Adh7 in 3I-F4-Pgam2-KO cells in which Adh7 was knocked down by shRNA. **(B)** Mice were implanted with resistant cells as indicated and prepared using scrambled guide and control hairpin RNAs (sg/shScramble), PGAM2 guide and scramble hairpin RNAs (sgPGAM2/shScramble), or PGAM2 guide and ADH7 targeting hairpin RNAs (sgPGAM2/shADH7). The mice were then treated or not with immunotherapy on days 3, 6, and 9 and survival and tumor growth were monitored in these mice with and without treatment.

**Supplementary Figure 9:**



**Supplementary Figure 9:** **Therapeutic modulation of tumor metabolism to reverse immunotherapy resistance.** Experimental design and treatment strategies for tumor survival experiments are depicted in the figure. Wild type mice on day 0 were challenged with therapy resistant 3I-F4 tumors and control parental tumors. Mice were then treated with FVAX, αCTLA-4, αPD-1 and αPD-L1 on days 3, 6 and 9 in combination with various therapeutic agents or control vehicle as described in the Methods section. **(A)** Survival of mice treated as indicated with inhibitors of glycolysis, 2DG or GSK2837808A (LDH-A) is shown. **(B)** Survival of mice treated as indicated with Metformin as an inhibitor of oxidative phosphorylation either via i.p. injection or orally in the drinking water is shown. Statistical significance was calculated using the log-rank (Mantel-Cox) test. ns, not significant; \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001.