# Pooling Functional Genetic Screens to Identify Genetic Correlates of Tumor Immunotherapy Response

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**Abstract**

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**Purpose:** Recent advances in immunotherapy have resulted in unprecedented breakthroughs in cancer treatment, with previously devastating diseases like metastatic melanoma seeing substantial increases in expected survival. However, only some patients respond to immunotherapy, while others are either completely unresponsive or later develop resistance. Ongoing work has been directed at determining which patients are the best candidates for immunotherapy and, moreover, whether treatments can be developed to "rescue" immunotherapy response in patients who are resistant. So far, this work has uncovered several genetic correlates of immunotherapy response, which have provided some clues as to the fundamental mechanisms underlying the tumor immune response—especially the interferon-γ (IFN-γ), tumor necrosis factor (TNF), NF-kB, and antigen presentation pathways. But questions remain as to what might be actionable genetic biomarkers that can help stratify patients, guide treatment decisions, and develop new immunotherapy drugs. Part of the challenge lies in the fact that it can be difficult to discriminate signal from noise among genetic data, and many studies have used different experimental approaches to genetic screens, making it unclear how to compare their results. We carried out the first pooled analysis of *in vitro* and *in vivo* CRISPR screens in an effort to identify and prioritize genetic correlates of tumor immunotherapy response.

**Methods:** We pooled extended data from four *in vitro* and *in vivo* CRISPR screens to identify genes that were significantly altered following exposure to immune pressure across multiple studies.

**Results:** We identified 10 genes that were significantly enriched (i.e., candidate genes conferring sensitivity to immune pressure) following exposure to immune therapy in 3 or more CRISPR data sets, and we found 16 genes that were significantly depleted (i.e.,

candidate genes conferring resistance to immune pressure) following exposure to immune therapy in 3 or more CRISPR data sets. Several of these genes belong to pathways already implicated in immunotherapy response (e.g., NF-kB, IFN- γ, and antigen presentation pathways), but many others belong to pathways with less well known functions—including two candidate genes conferring sensitivity to immune pressure (*Nf1* and *Tjap1*) and ten candidate genes conferring resistance to immune pressure (*Calr, Ube2n, Ccs, Pigu, Gpaa1, Maea, Atg5, Larp4, Vps4b, Fitm2*).

**Conclusions:** Our results confirm and extend previous findings, emphasizing the importance of known immune pathways and also identifying several promising novel candidate genes that could represent new patient biomarkers or drug targets.

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**Glossary of Abbreviations**

Atg5 - Autophagy related 5

B2m - Beta-2-microglobulin

Birc2 - Baculoviral IAP repeat-containing protein 2 (aka cIAP1)

Calr - Calreticulin

Ccs - Copper chaperone for superoxide dismutase

Fitm2 - Fat storage-inducing transmembrane protein 2

Gpaa1 - Glycosylphosphatidylinositol anchor attachment 1 protein

IFN-γ - Interferon-gamma

Ifngr1 - Interferon Gamma Receptor 1

Ifngr2 - Interferon Gamma Receptor 2

Ikbkg - Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma (aka NEMO, NF-kappa-B essential modulator)

Larp4 - La-related RNA-binding protein

Maea - Macrophage erythroblast attacher

Nf1 - Neurofibromin 1

Pigu - Phosphatidylinositol glycan anchor biosynthesis class U protein

Ptpn2 - Tyrosine-protein phosphatase non-receptor type 2

Rnf31 - E3 ubiquitin protein ligase 31

Stat1 - Signal transducer and activator of transcription 1

Stub1 - STIP1 homology and U-Box containing protein 1

Tap1 - Transporter associated with Antigen Processing 1

Tap2 - Antigen peptide transporter 2

Tjap1 - Tight junction-associated protein 1

TNF-alpha - Tumor necrosis factor alpha

Ube2n - Ubiquitin conjugating enzyme E2N

Vps4b - Vacuolar protein sorting-associated protein 4B

## Introduction

In recent years, immunotherapy has revolutionized cancer treatment. For example, since 2011, eight new drugs (including three immunotherapy reagents) have been approved for the treatment of metastatic melanoma, which has led to an increase in median overall survival from 9 months to 2+ years.[1] Immunotherapy's strength comes from its ability to generate a lasting, durable response. It "teaches" the immune system how to mount an attack against tumor cells; because the immune system has memory, this attack can continue after the medication has been discontinued.

But, as with many other cancer treatments, responses to immunotherapy vary across individuals. Resistance limits responses in a large number of patients.[2] Some patients never respond to immunotherapy ("primary resistance"), whereas other patients initially respond to immunotherapy but then relapse ("adaptive resistance" or "acquired resistance").

The mechanisms underlying different forms of resistance are not well understood. Resistance appears to arise from both tumor-cell-intrinsic and tumor-cell-extrinsic factors.[2] Broadly speaking, resistance is likely due to tumor cells developing strategies of "hiding" from the immune system—e.g., absence of tumor antigens or alterations in antigen-presenting machinery.[3,4] Adaptive or acquired resistance could occur due to expansion of pre-existing resistant clones or, alternatively, development of new strategies to hide from the immune system.[4,5] Specifically, recent studies suggest that some modes of resistance include alterations in pathways involving the inflammatory cytokines IFN-γ (interferon-γ)[6-8] and TNF (tumor necrosis factor),[9] the NF-kB pathway, as well as antigen-presenting machinery.

Currently, we lack reliable methods to predict which patients will respond well to immunotherapy and which patients are beginning to develop resistance. A major research initiative is to identify biomarkers indicating whether a certain patient or tumor will be sensitive or resistant to immunotherapy. So far, efforts to uncover such biomarkers have

been driven by large-scale CRISPR-based screens in the laboratory. In general, these screens involve systematically silencing genes in tumor cells (using CRISPR-Cas9) and then determining how this gene silencing affects tumor immune response (e.g., when exposed to cytotoxic T cells).[9-12] Similar CRISPR screens have been repeated numerous times, in different cancer cell lines and with different forms of immune pressure, in an effort to uncover new clues as to what determines whether the immune system can kill tumor cells. Different papers have highlighted different gene hits, making it difficult to know which genes to prioritize when analyzing highly heterogeneous patient data.

To our knowledge, no one has combined the vast data from different CRISPR screens to determine whether different screens share gene hits. Shared gene hits between different CRISPR screens could help elucidate fundamental mechanisms of immune resistance (or sensitivity) that are common to different cancers and different forms of immune pressure, which could in turn prioritize novel drug targets as well as biomarkers for stratifying patients and guiding treatment decisions.

Here, we combine extended data from four published studies in order to identify shared gene hits. We confirm and extend previous findings indicating the importance of TNF-alpha, IFN-γ, NF-kB, and antigen presentation pathway genes. We also highlight several novel genes that could represent previously unknown genetic correlates of tumor immunotherapy response, including two candidate genes conferring sensitivity to immune pressure (*Nf1* and *Tjap1*) and ten candidate genes conferring resistance to immune pressure (*Calr, Ube2n, Ccs, Pigu, Gpaa1, Maea, Atg5, Larp4, Vps4b, Fitm2*). Future work will be necessary to determine whether these genes—or, alternatively, related genes belonging to the same pathways—have clinical importance in human patients.

**Student Role**

I carried out all of the work described here, with Python coding support from members of the Van Allen Lab.

## Methods

### Study Selection

Studies were selected from a pool of recently published CRISPR screens that all shared the goal of identifying genetic correlates of resistance or sensitivity to immunotherapy. To facilitate comparisons between papers, we selected only those studies using melanoma cell lines: Manguso et al., 2017; Patel et al., 2017; Pan et al., 2018; Kearney et al., 2018 (Table 1).

### Data Analysis

Extended data from each paper were loaded into Python and processed using the Pandas Data Analysis Library. Each paper contributed one data set with the exception of Pan et al., 2018, which contributed two data sets. This is because Pan et al. carried out two different screens using two different forms of immune pressure—Pmel-1 T cells (with low affinity for T cell receptors) and OT-1 cells (with high affinity for T cell receptors). Of note, Patel et al., 2017 did not contribute a data set to the comparison of genes whose deletion conferred sensitivity to immune pressure (i.e., candidate resistance genes) because their extended data were not clearly annotated for that condition.

Gene hits from each paper were selected for based on significance criteria used in each paper: FDR (Pan et al., 2018); q-value (Manguso et al., 2017); MAGeCK score (Kearney et al., 2018; MAGeCK algorithm described in Li et al., 2014); Empirical RIGER p-value (Patel et al., 2017). Significance thresholds were set to less than or equal to 0.1 for all studies, and only those genes that met this threshold were included in subsequent analysis. Genes meeting this threshold from each paper were compared across studies.

For genes conferring sensitivity to immune pressure: Pan et al. contributed 76 genes from the Pmel-1 condition and 22 genes from the OT-1 condition. Patel et al. contributed 864 genes. Manguso et al. contributed 38 genes. Kearney et al. contributed 2182 genes.

For genes conferring resistance to immune pressure: Pan et al. contributed 249 genes from the Pmel-1 condition and 152 genes from the OT-1 condition. Patel et al. contributed zero genes (as described above). Manguso et al. contributed 62 genes. Kearney et al. contributed 4534 genes.

**Results**

Shared enriched genes: Candidate genes conferring sensitivity to immune pressure

We first assessed genes that were found to be relatively enriched following exposure to immune pressure. These represent candidate genes conferring sensitivity to immune pressure—that is, their deletion (via CRISPR-Cas9) made tumor cells more resistant to immune pressure. For each study, significantly enriched genes were selected as described in Methods. Then, these genes were compared across studies.

In order to determine which genes represent shared hits across multiple studies, we selected for only those genes that were significantly enriched in three or more data sets (Figure 1A). The resulting list consisted of 10 shared genes, several of which have been well described in the literature as influencing tumor susceptibility to immune attack.[2] Among the well known genes are five that are required for sensing and signaling through the IFN-γ pathway: *Stat1, Jak1*, and *Ifngr2* (enriched in 5/5 data sets) as well as *Ifngr1* and *Jak2* (enriched in 4/5 data sets). Another gene known to influence tumor cell killing, beta-2-microglobulin (*B2m*), was also enriched in 4/5 data sets; it is required for HLA class I folding and transport to the cell surface, and its genetic deficiency allows the cell to evade CD8 T cell recognition.[2]

Other enriched genes included *Tap1* and *Tap2*, which are involved in antigen processing and presentation (enriched in 3/5 studies) and are also known to correlate with tumor cell immune response.[2]

The remaining two shared genes—*Nf1* and *Tjap1*—have less well established relationships with tumor immune response. *Nf1* was enriched in 3/5 studies and is known to be a negative regulator of the Ras/MAPK (mitogen-activated protein kinase) pathway.[14] Ras/MAPK mutations are crucial in the pathogenesis of multiple types of cancers, and a particular *Nf1* mutation causes neurofibromatosis, a disease characterized by the growth of numerous benign tumors. However, the mechanism by which *Nf1* loss of function contributes to immune resistance is less clear. *Tjap1*, a tight junction-associated protein, was also enriched in 3/5 studies and has no known association with tumor immune response. But, of note, decreased tight junction expression is a key step in the epithelial-to-mesenchymal transition that drives metastasis.[15]

Shared depleted genes: Candidate genes conferring resistance to immune therapy

We next turned our attention to genes that were found to be relatively depleted following exposure to immune pressure. These represent candidate genes conferring resistance to immune pressure—that is, their deletion (via CRISPR-Cas9) facilitated tumor cell killing. Importantly, these genes represent possible drug targets for enhancing the response to immunotherapy. For each data set, significantly depleted genes were selected as described in Methods and then compared across data sets. This approach identified 16 genes that were significantly depleted across three or more data sets (Figure 1B). Here, we have roughly grouped them based on what is already known about their function with relation to tumor immunotherapy response, the tumor immune microenvironment, and the overall immune system.

*Genes that have previously been shown to promote sensitivity to tumor immunotherapy*

As with the shared enriched genes (described above), the shared depleted genes included several that have previously been implicated in cancer and in immunity, but only two of these have been shown to influence tumor immunotherapy response: *Ptpn2* and *Stub1* (both enriched in 3/4 data sets). The role of these two genes in tumor immunotherapy response was first described by one of the CRISPR screens that contributed to the current study, Manguso et al. 2017. They described a role for *Ptpn2* in enhancing IFN-γ-mediated effects on antigen presentation and growth suppression. *Stub1*, on the other hand, is an E3 ubiquitin ligase involved in the regulation

of the unfolded protein response. Loss of either *Ptpn2* or *Stub1* sensitized tumors to immunotherapy in *in vivo*. Of note, their deletion did not alter cell growth in the absence of immune pressure, suggesting that the change in immunotherapy response is due to an interaction with immune machinery rather than an overall change in cell fitness.

*Genes that have previously been linked to tumor immune response*

*Calr* (calreticulin) has been implicated in the tumor immune environment but has not been shown to directly affect tumor immunotherapy response. Calr is a calcium binding protein that is found in the lumen of the endoplasmic reticulum, in the nucleus, and on the cell surface. It was relatively depleted in 3/4 CRISPR screens, suggesting its deletion confers sensitivity to immune pressure. However, previous studies have suggested the opposite effect of calreticulin: that its upregulation on tumor cells—specifically on the cell surface—promotes phagocytosis.[16,17] Moreover, a previous study suggested that blockade or knockdown of cell surface calreticulin suppressed phagocytosis of anthracyclin-treated tumor cells. These contradictory findings might be related to where, exactly, a particular form of calreticulin is expressed; perhaps decreased calreticulin expression in the endoplasmic reticulum or nucleus promotes sensitivity to immune pressure, whereas decreased cell surface calreticulin diminishes sensitivity to immune pressure. Future studies will be required to reconcile these contradictory findings.

*Genes that have been implicated in cancer and have known immune function, but no known effect on tumor immune response*

Several other shared depleted genes have previously been implicated in cancer and, separately, have some known interaction with the immune system. However, their effect on tumor immunity is unknown.

Rnf31 and Birc2 (depleted in 3/4 data sets) are both components of the linear ubiquitin assembly complex (LUBAC), which stabilizes Ripk1, a known NF-kB survival pathway mediator.[18] Moreover, *Rnf31* has been linked to certain breast cancers, where it is thought to stabilize estrogen receptor-stimulated cell proliferation[19] and promote p53 degradation.[20] It has also been linked to prostate cancer, where its amplification is associated with an increased risk of metastasis, and its suppression has been shown to diminish cell proliferation and metastasis.[21] *Birc2*, on the other hand, has been shown to be altered in lymphoid and epithelial cell cancers.[22]

However, neither *Rnf31* nor *Birc2* has previously been shown to correlate with cancer immunotherapy response.

Like *Rnf31* and *Birc2*, Ikbkg (aka NEMO, depleted in 3/4 data sets) is part of the NF-kB pathway and has been implicated in some cancers. For example, it was found to be significantly upregulated in Inflammatory Breast Cancer, a rare and aggressive form of breast cancer, relative to non-inflammatory breast cancer.[23] In addition, previous studies have suggested that *Ikbkg* deletion triggers an inflammatory reaction that leads to cell death.[24] However, it has not previously been implicated in mediating cancer immunotherapy response.

*Ube2n* (aka *UBC13*, depleted in 3/4 data sets) is amplified in many cancers,[25] but its role in immune function is less well characterized. A recent paper suggested that it has a role in the innate immune response to viral infection,[26] and another study described a potential role for Ube2n in cytokine signaling as part of a large, multi-component signaling complex along with Ikbkg (described above).[27]

Ccs, or copper chaperone for superoxide dismutase (depleted in 3/4 data sets), delivers copper to superoxide dismutase (SOD1). Although Ccs itself has not been directly implicated in immune responses, it is indirectly related via its relationship with SOD1. Previous studies suggest that SOD1 activity levels are directly related to TNF-alpha activation—that is, increased SOD1 activity leads to increased TNF-alpha activity and vice-versa.[28] Moreover, SOD1 is overexpressed in some cancers and thus has been targeted in some early experimental cancer treatments.[29] For example, inhibition of SOD1 by a small molecule was shown to decrease colony formation in a non-small cell lung cancer cell line and decrease tumor burden in a non-small cell lung cancer mouse model.[30] However, the role of *Ccs* in mediating tumor immune responses is unknown.

*Genes that have been implicated in cancer but have no known immune function*

Other shared depleted genes have previously been associated with certain cancers, but their immune function remains unknown.

Pigu (depleted in 3/4 data sets) is a subunit of the GPI transamidase complex that attaches GPI-anchors to proteins. It has been implicated as an oncogene in both bladder cancer[31] and prostate cancer,[32] and it is overexpressed in lymphoma and lung adenocarcinoma.[31] Interestingly, another

shared depleted gene, Gpaa1 (also depleted in 3/4 data sets), is a subunit of this same GPI transaminase complex.[33] It has been implicated as an oncogene in breast cancer and is overexpressed in prostate cancer and lung adenocarcinoma.[31] Neither *Pigu* nor *Gpaa1* has a known role in immune function or tumor immune response.

Maea, or macrophage erythroblast attacher (depleted in 3/4 data sets), plays an important role in erythroblast maturation. It has also been found to be amplified in pancreatic adenocarcinoma[34,35] and hypomethylated in malignant peripheral nerve sheath tumors,[36] but its role in interacting with the immune system remains unknown.

Atg5 is required for autophagy, and autophagy-deficient cells are less tolerant of metabolic stress.[37] But, paradoxically, autophagy defects have been associated with increased tumorigenesis.[37] Indeed, Atg5 is strongly downregulated in sporadic cases of colon cancer.[38] This is somewhat at odds with the CRISPR screens studied here, 3/4 of which showed that *Atg5* deletion increased sensitivity to immune pressure. Future work will be necessary to disambiguate the role of *Atg5* in the tumor immune response.

Larp4 (depleted in 3/4 data sets) is an RNA-binding protein that is thought to affect cancer cell migration and invasion via changes in cell morphology.[39] Specifically, *Larp4* deletion results in tumor cell elongation and increased migration and invasion in prostate and breast cancer cell lines. Its role in immunity remains unknown.

Vps4b (vacuolar sorting protein 4B, depleted in 3/4 data sets) is responsible for vesicular trafficking and the maturation of autophagosomes. It has been shown to be enriched in chronic myelogenous leukemia,[40] and its overexpression has been associated with accelerated cell proliferation and poor prognosis in human hepatocellular carcinoma.[41] Others have hypothesized that it might promote tumorigenesis by prolonging the duration of EGFR signaling.[42] Its interaction, if any, with the immune system remains unknown.

Last, Fitm2 (fat storage-inducing transmembrane protein 2, depleted in 3/4 data sets) has little known role in cancer or in immunity, although copy number loss has been observed in a small cell ovarian carcinoma cell line.[43]

*Genes have a known role in immunity but no known role in cancer*

Otulin (depleted in 3/4 data sets) is a deubiquitinase with a well known role as a negative immune regulator. Specifically, it suppresses systemic TNF-alpha activation and NF-kB activation (via LUBAC regulation, see *Rnf31* and *Birc2* above).[44] However, its role in cancer and cancer immunity remains less clear.

**Discussion**

To date, efforts to uncover genetic correlates of tumor immunotherapy response have largely been siloed, with different labs carrying out their own variations on a similar type of study. Because of these between study differences, it has been unclear to what extent their data could be pooled. In parallel, efforts have also been ongoing in the clinical realm, scouring large quantities of patient data for clues as to who will respond to immunotherapy and who will not. But highly variable patient data can make it difficult to discriminate signal from noise. We pooled results from large-scale *in vitro* and *in vivo* CRISPR screens to identify shared genetic correlates of cancer immunotherapy response.

Overall, our findings confirmed and extended previous work regarding genetic correlates of cancer immunotherapy response. In particular, genes that were relatively enriched following exposure to immune pressure—i.e., those genes whose deletion decreased tumor sensitivity to immune pressure—corroborated the previously described importance of several IFN-γ pathway genes (*Stat1, Jak1, Jak2, Ifngr1, Ifngr2*), antigen presentation pathway genes (*Tap1, Tap2*), and *B2m*. That these genes repeatedly correlated with immune response across multiple different experiments highlights their importance to the fundamental mechanisms underlying the tumor immune response. We also identified *Nf1* and *Tjap1* as having a possible role. Future work is necessary to determine whether loss of function mutations in these genes correlate with a poor response to immunotherapy in human patients.

Our findings also identified over one dozen genes that were relatively depleted following exposure to immune pressure across multiple different studies. These genes represent candidate resistance genes—that is, their deletion increased tumor sensitivity to immune pressure. Taken together, these genes confirm the importance of the NF-kB pathway and TNF-alpha. They also

reveal a potential role for the GPI transamidase complex that attaches GPI-anchors to proteins (*Pigu, Gpaa1*). Two of the shared depleted genes, *Ptpn2* and *Stub1*, were recently shown to increase tumor cell sensitivity to immunotherapy both *in vitro* and *in vivo* in mice.[11] But less is known about most of the other genes uncovered. Several are promising candidate genes based on their known immune functions and their correlation with certain cancers (*Rnf31, Birc2, Ccs, Ikbkg, Ube2n*). Others have previously been implicated in cancer but not in immune function (*Calr, Pigu, Gpaa1, Maea, Atg5, Larp4, Vps4b, Fit2m*), and one gene is a well known negative immune regulator but has an unclear role in cancer (*Otulin*).

Overall, our study has identified several promising candidate genetic correlates of tumor response to immunotherapy, which could represent actionable biomarkers for use in patient care.

Potential drug targets

Generally speaking, it is easier to design drugs that result in loss of function rather than gain of function. Therefore, the shared depleted genes—i.e., genes whose deletion resulted in increased sensitivity to immune pressure—represent the best potential drug targets. Since the Manguso et al., 2017 study was the only *in vivo* CRISPR screen included here, it is reasonable to prioritize shared depleted genes that were hits in that study. These genes include: *Rnf31, Birc2, Maea, Otulin, Calr, Ube2n, Ptpn2, Stub1, and Gpaa1*.

Within these genes, *Rnf31, Birc2*, and *Gpaa1* are particularly promising. Rnf31 and Birc2 are both members of the same complex (LUBAC), which stabilizes Ripk1, a known NF-kB survival pathway mediator, and both genes have been altered in certain cancers. *Gpaa1* is closely related to another shared depleted gene, *Pigu* (although, *Pigu* was not significantly depleted in the Manguso et al. study); both are members of the same GPI transamidase complex that attaches GPI-anchors to proteins. Both *Gpaa1* and *Pigu* have been named as possible oncogenes in several different cancer types. And so, targeting *Gpaa1* or some other part of the GPI transaminase complex is enticing.

Calreticulin (encoded by *Calr*) is also a promising drug target because it has been related to the tumor immune environment. However, it could present a challenge in that it might have opposite effects depending on where it is expressed. Indeed, previous studies suggest that decreased expression of cell surface calreticulin could suppress phagocytosis.

Limitations

Large-scale functional screens using CRISPR technology have the benefit of being high throughput, with infinite tumor cells in a controlled laboratory environment, which allows for improved signal-to-noise relative to patient data. But CRISPR screens are limited by a lack of clinical correlation and an unrealistic immune environment. Indeed, three out of the four studies included here tested cancer cell lines *in vitro*, which hardly captures the complexity of the human immune system. Manguso et al. come the closest to approximating a human immune system with their *in vivo* mouse model, but lab mice have notoriously weak immune systems and have been reared in unusually clean lab environments, and so it is unclear whether they are at all comparable to human immune systems.

Much remains to be understood about how, exactly, the different approaches used by these CRISPR screens affect their results and our ability to compare across studies. Although we selected for only those studies using melanoma cell lines, cancer cell lines vary in their responses to immunotherapy even within cancer type. Indeed, Pan et al., 2018 chose the B16F10 melanoma cell line because it is resistant to checkpoint blockade with antibodies targeting the PD-1 and/or CTLA-4 receptors. Moreover, it is still difficult to anticipate how different forms of immune pressure will affect these CRISPR screen outcomes. For example, Pan et al. conducted two experiments in parallel, exposing B16F10 melanoma cells to two different T-cell lines: either Pmel-1 CD8 T cells (with low affinity for the TCR T cell receptor) or OT-1 CD8 T cells (with high affinity for the TCR T cell receptor). The screen hits from these different experiments varied (see Figure 1) and thus were not pooled for the analysis shown here. It is unclear to what extent this is due to the effect of using different T cells versus expected between-screen variability. Last, certain studies contributed many more gene hits than others (e.g., Manguso et al. contributed only 62 candidate resistance genes, whereas Kearney et al. contributed 4534

genes), suggesting that future pooled analyses should consider using different significance thresholds for different studies instead of striving for uniform significance thresholds across studies, as we have done here. However, it is encouraging that overall, our results were consistent with some of the best known genetic correlates of cancer immunotherapy—*Stat1, Jak1, Jak2, Ifngr1, Ifngr2, Tap1, Tap2,* and *B2m*—which appeared as shared hits among the different studies.

Another challenge of identifying genetic correlates of tumor immunotherapy response is that it does not clarify how or where the protein of interest is expressed. As described above with calreticulin, different studies suggest opposing effects on tumor immune response, which could be related to whether the protein is intracellular or on the cell surface.

Last, because these CRISPR studies are loss-of-function screens, it is unclear whether gain of function mutations in the genes discussed here would have the expected (opposite) effect. That is, it is unclear whether gain of function mutations in putative resistance genes would predict poor response to immunotherapy; likewise, it is unclear whether gain of function mutations in putative genes conferring sensitivity would predict a good response to immunotherapy. In the future, clinical correlation will help determine how, exactly, these CRISPR screen hits should be interpreted.

## Future Directions

### *Describing and predicting how different CRISPR screen protocols affect gene hits*

As described above, different cancer cell lines and different forms of immune pressure appear to lead to different CRISPR screen hits. But it remains unclear how to anticipate the changes that might come with these differences. For example, does a cell line known to be resistant to checkpoint inhibitors (like B16F10) acquire a unique genetic signature in response to immune pressure? Or do certain forms of immune pressure lead to certain genes being significantly enriched or depleted (e.g., Pmel-1 vs OT-1 CD8 T cells)? Gaining a better understanding of how

these differences in experimental design affect our results will in turn help us improve future genetic screens and make more informed comparisons across studies.

*Comparison with patient data*

In the immediate future, a priority will be to validate the shared gene hits described here using a patient cohort. Unlike laboratory CRISPR screens, analysis of patient data facilitates comparisons between germline and somatic mutations, pre- and post-treatment, primary and metastatic lesions, all under realistic clinical conditions. But clinical data are inherently more variable and less plentiful compared to the lab, making these analyses generally less well powered and more difficult to interpret. To our knowledge, no group has combined CRISPR and patient data to identify and prioritize novel biomarkers for immunotherapy response. But doing so could help make important steps towards stratifying patients to guide treatment decisions, which can rapidly be incorporated into clinical practice.

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## Tables and Figures

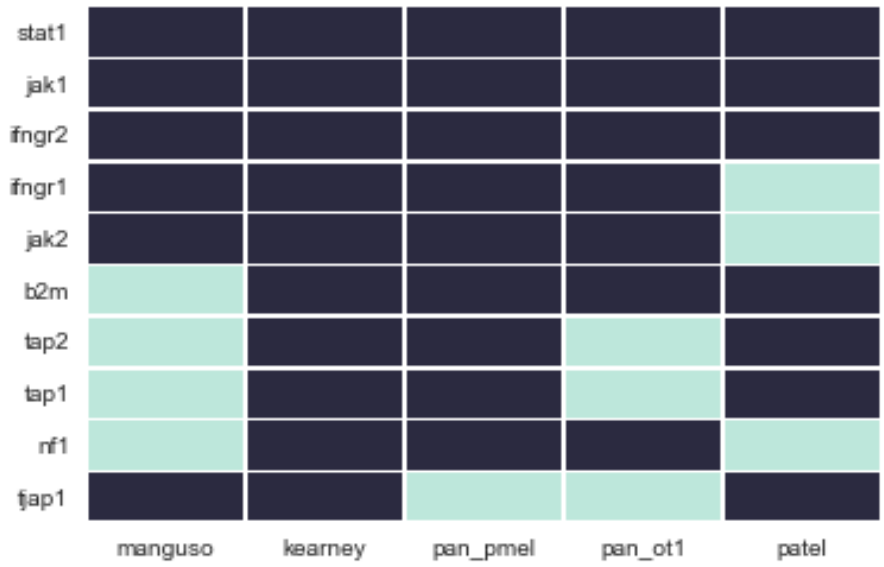
### Table 1: Study characteristics

| | Model system | Immune pressure (Test condition) | Control | Significance criterion |
|---|---|---|---|---|
| **Manguso et al., 2017** | mice transfected with B16 melanoma cell line | anti-tumor vaccine (GVAX) + checkpoint inhibitor (anti-PD1) | T-cell deficient mice (TCRa -/-) not exposed to immune pressure | q-value <= 0.1 |
| **Patel et al., 2017** | Mel624 melanoma cell line | transgenic CD8 T cells (human T cells engineered to detect antigens presented in an HLA class I-restricted manner on Mel624 cells) | no immune pressure | Empirical RIGER p-value <= 0.1 |
| **Kearney et al., 2018** | B16 cell line | OT-1 CD8 T cells | no immune pressure | MAGeCK score <= 0.1 |
| **Pan et al., 2018** | B16F10 melanoma cell line | 1) Pmel-1 CD8 T Cells (low affinity, 3-day exposure)<br>2) OT-1 CD8 T cells (high affinity, 1-day exposure) | T cells not specific for tumor cells | FDR <= 0.1 |

**Figure 1: CRISPR screen hits compared across studies.**

Gene names (rows) represented in each data set. Dark blue boxes represent genes present in a given study. Light blue boxes represent genes absent from a given study. Shown in panels A and B are those genes that met significance in 3 or more studies. **A.** Genes whose deletion conferred decreased sensitivity to immune pressure (i.e., candidate genes conferring immune sensitivity). **B.** Genes whose deletion conferred increased sensitivity to immune pressure (i.e., candidate genes conferring immune resistance).

**A**




**B**