SITC
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Society for Immunotherapy of Cancer

November 8-12 • NATIONAL HARBOR, MD

SITC 2017

Dual and Opposing Roles for Tumor Cell-Intrinsic Type-II Interferon Signaling in anti-Tumor Immunity

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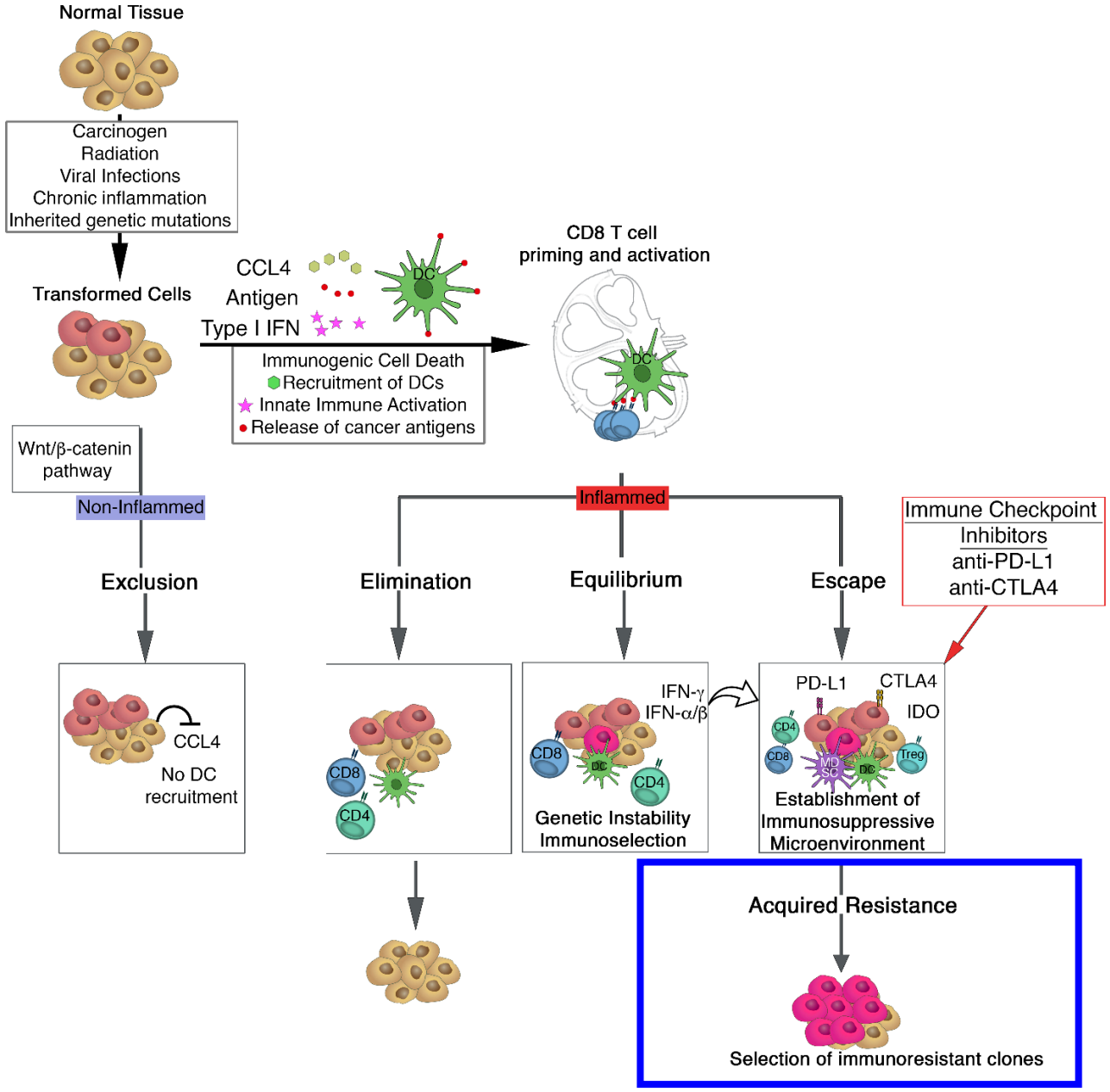
Presenter Disclosure Information

Jason Williams

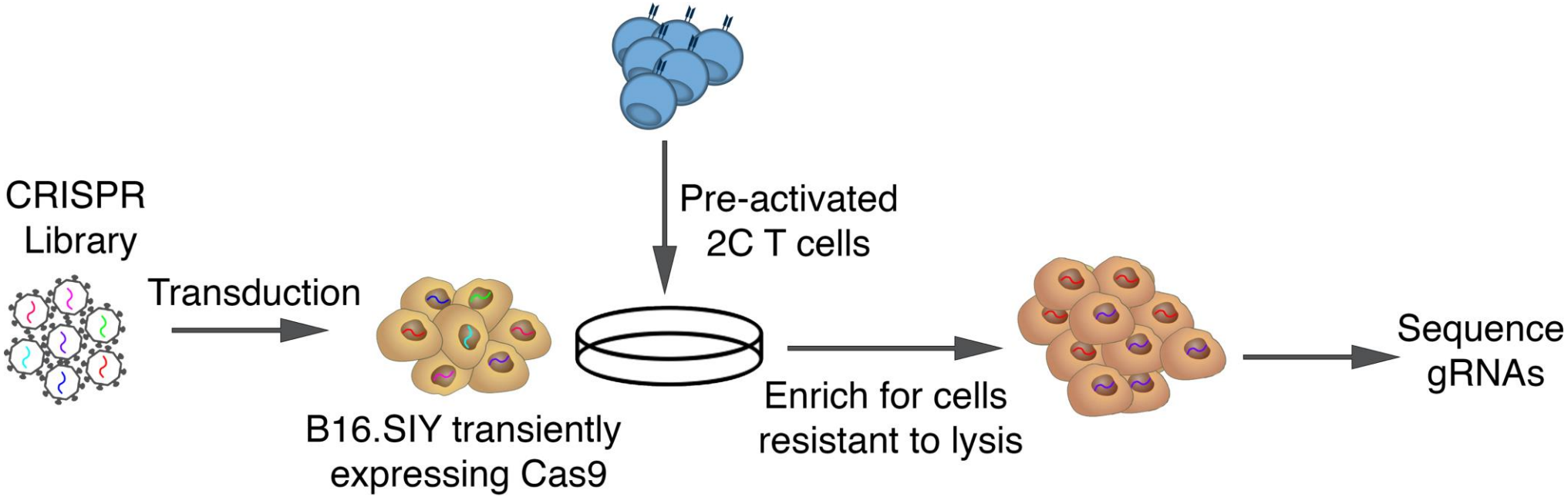
The following relationships exist related to this presentation:

No Relationships to Disclose

Secondary resistance can arise after initial successful immunotherapy

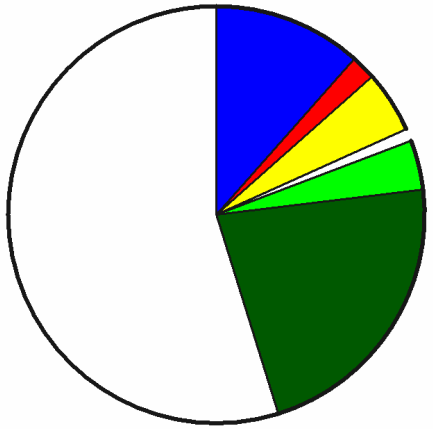


Genome-wide CRISPR screen to identify essential genes in tumor cells for elimination by CD8+ T cells



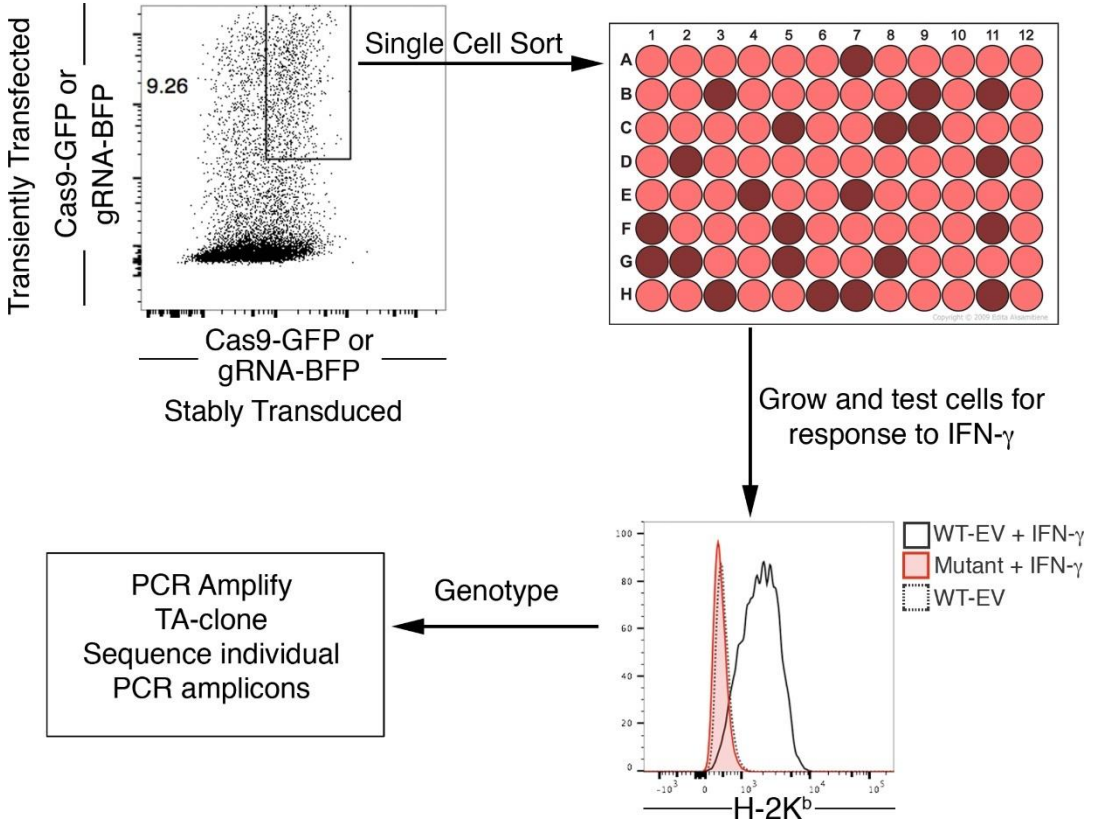
87,897 unique gRNAs targeting 19,150 mouse protein-coding regions

gRNAs targeting type II IFN pathway genes are enriched in B16.SIY cells resistant to T cell-mediated killing *in vitro*

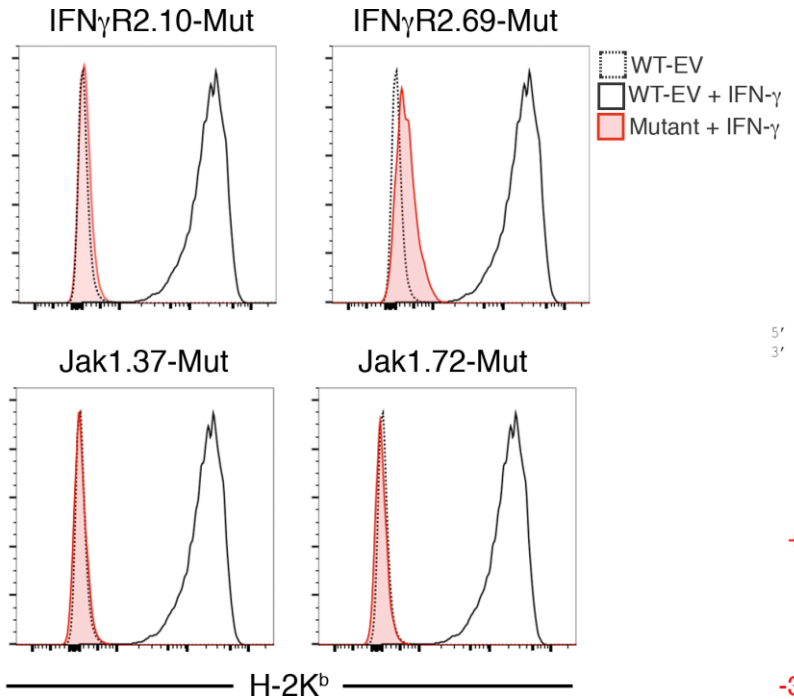


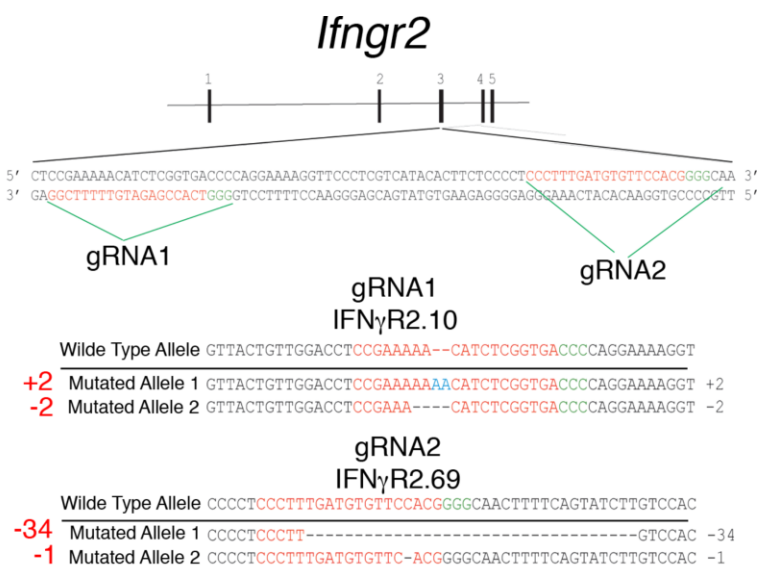
Total = 104

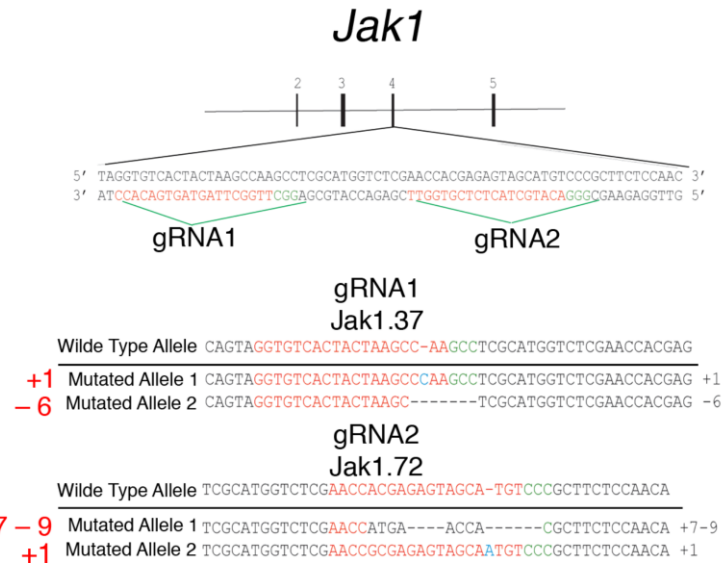
- Jak1 gRNA1
- Jak1 gRNA2
- Ifngr2 gRNA1
- Ifngr2 gRNA2
- H2-K1 gRNA1
- H2-K1 gRNA2
- Others



IFNγR2- and Jak1-mutant B16.SIY cells do not respond to IFN-γ stimulation *in vitro*

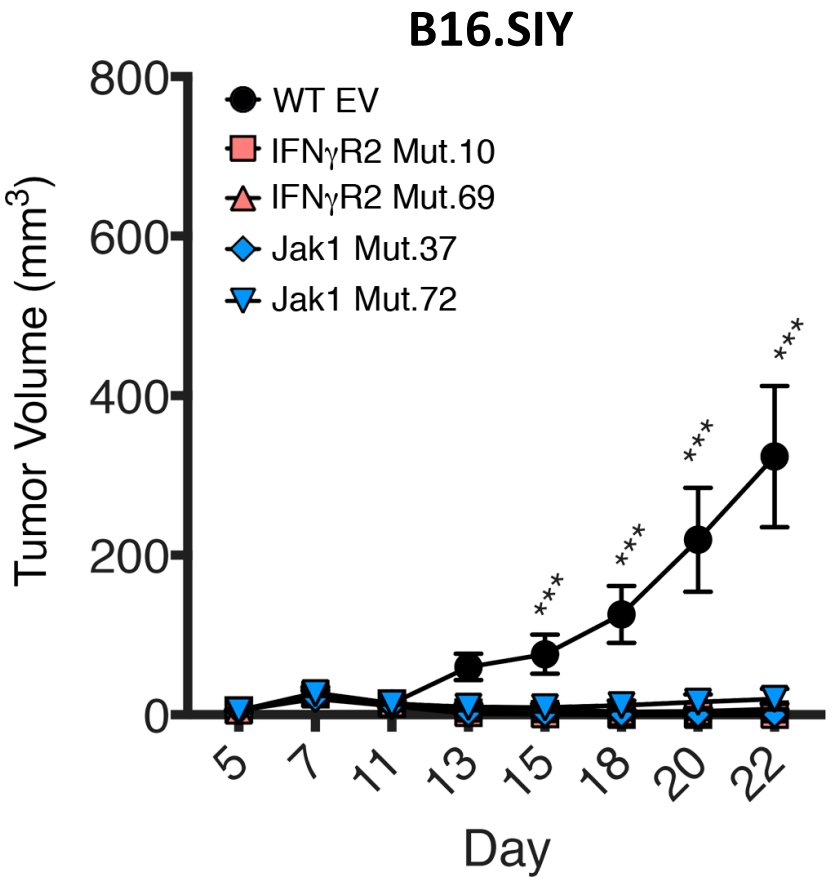


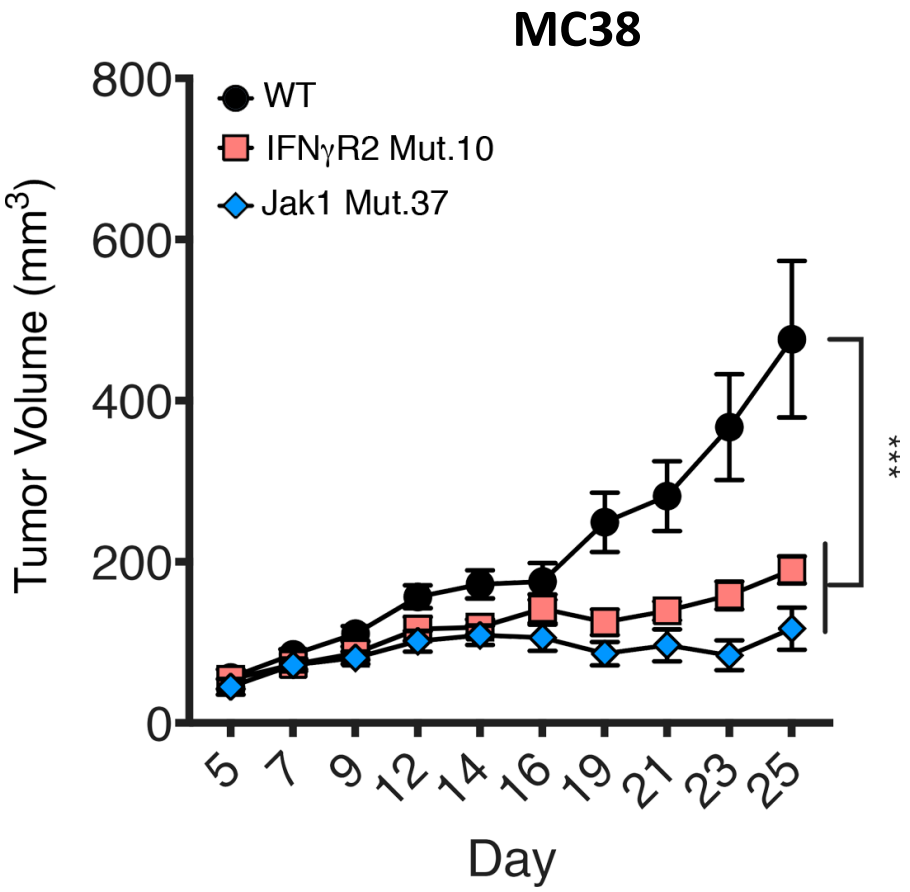




What is the behavior of these tumor cells in vivo?

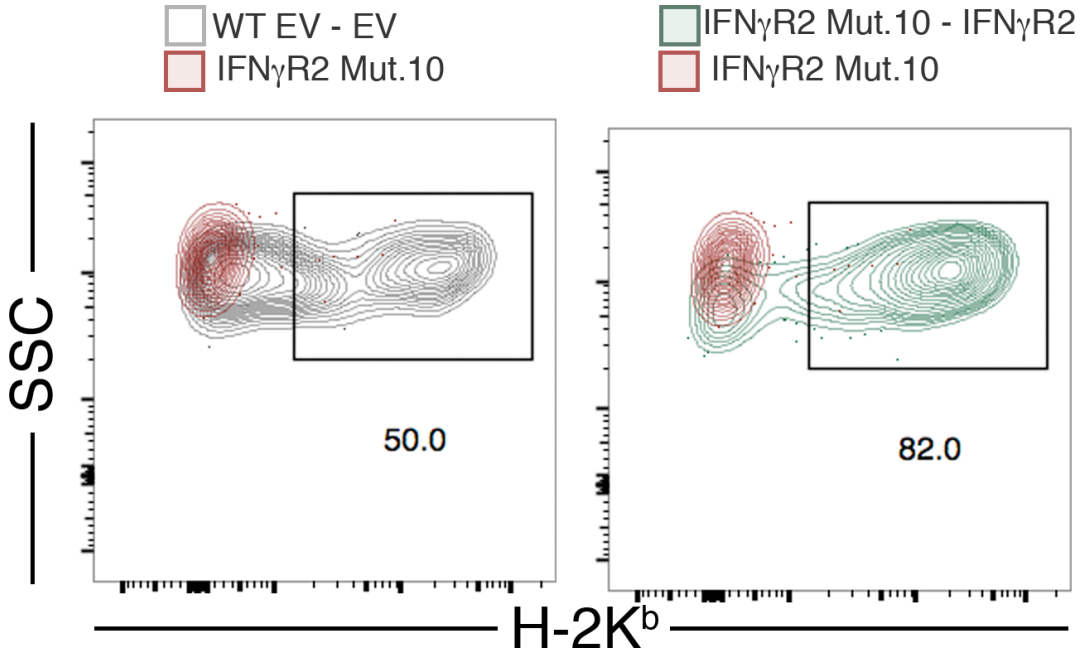
Paradoxically, IFNγR2- and Jak1-mutant tumors show retarded tumor growth *in vivo*

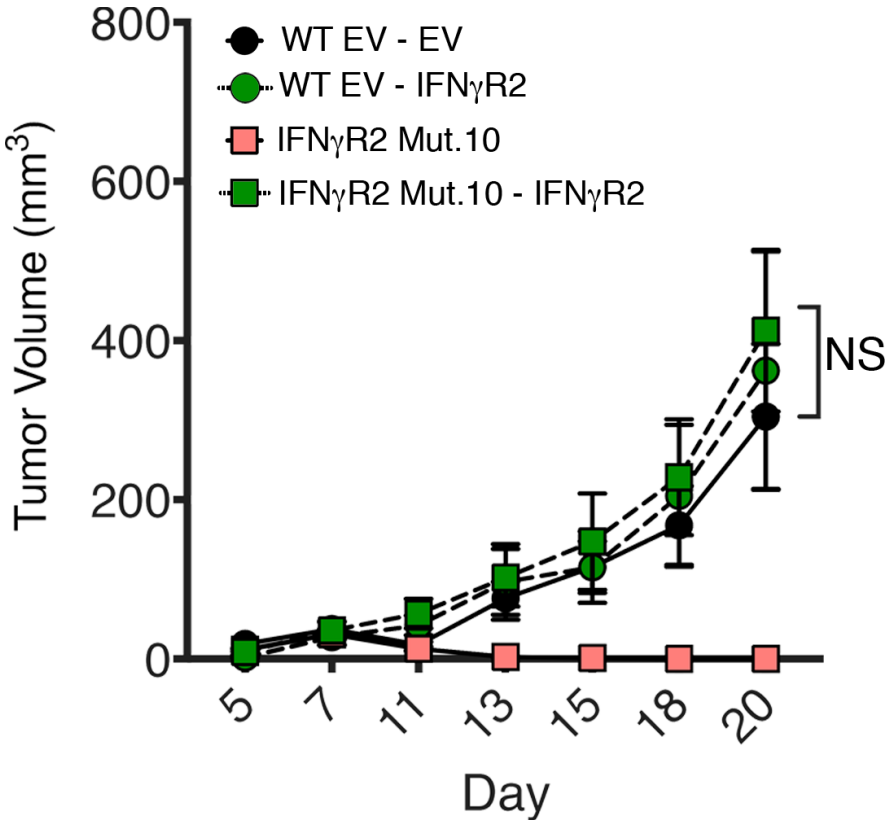




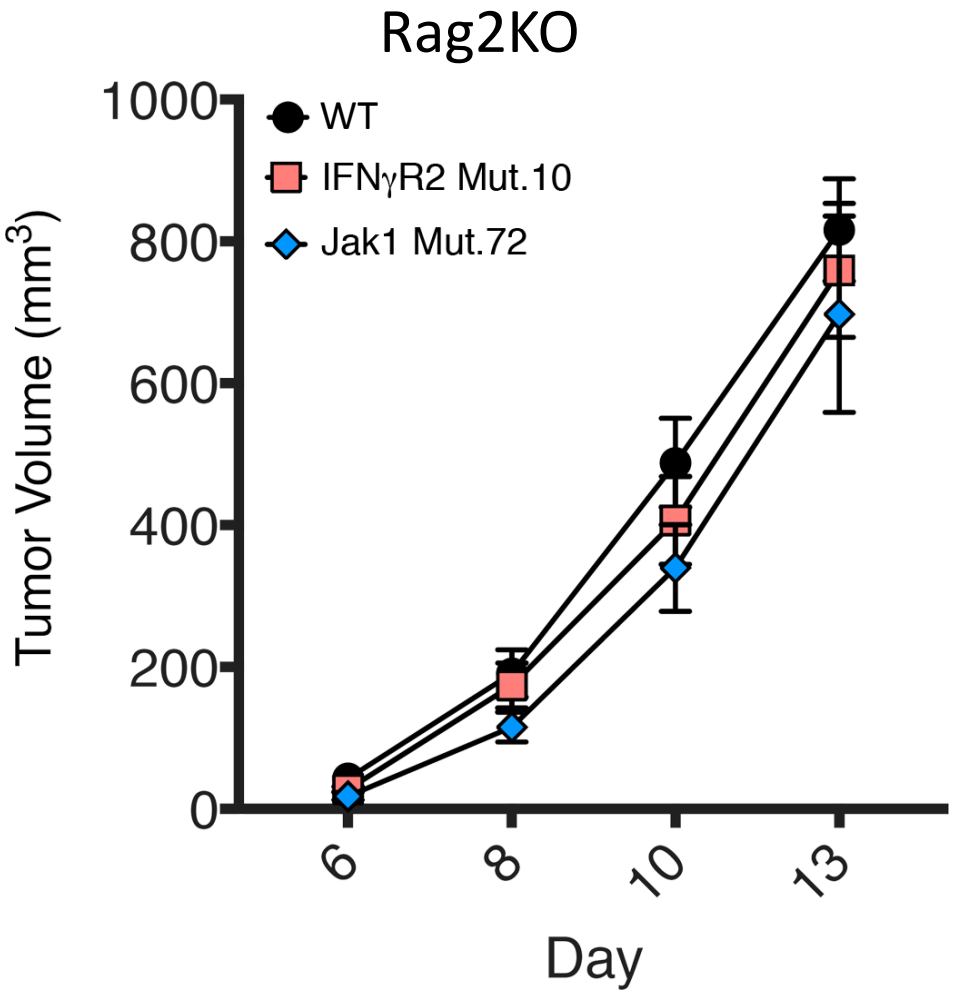
Re-introduction of IFNγR2 restores progressive tumor growth *in vivo*

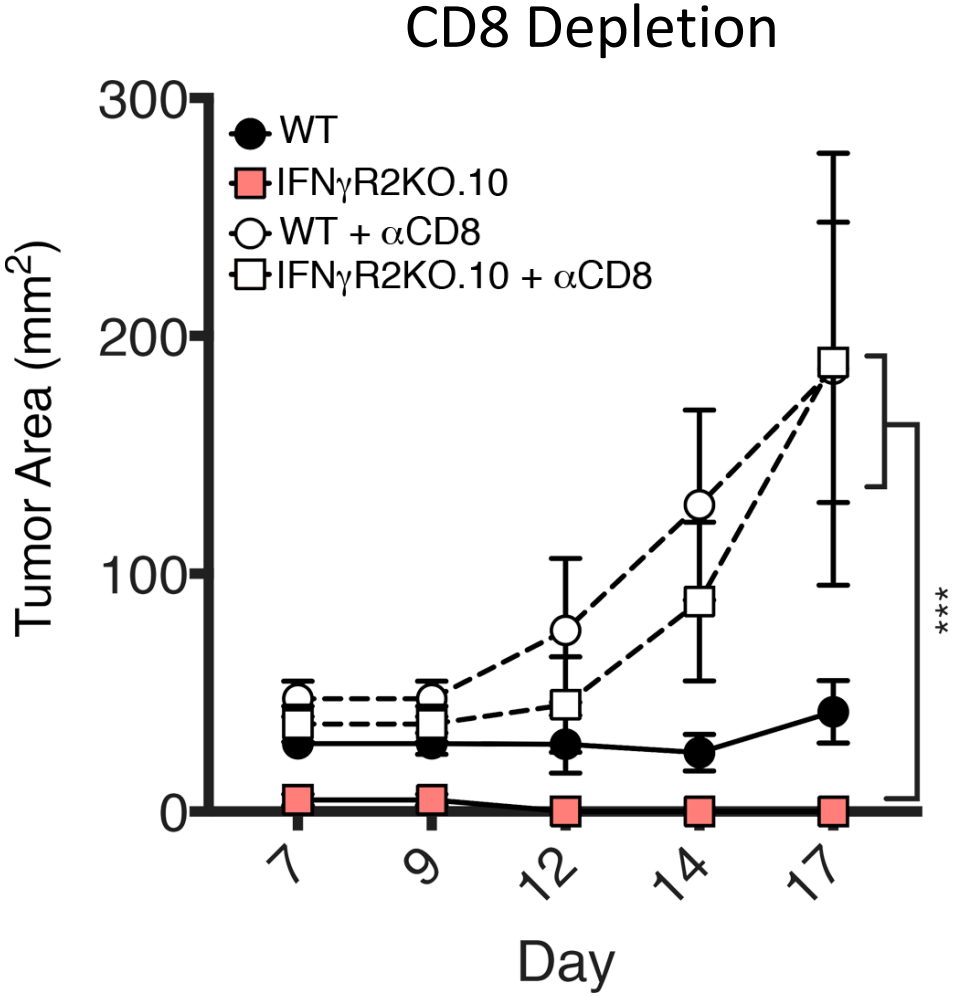
In vivo



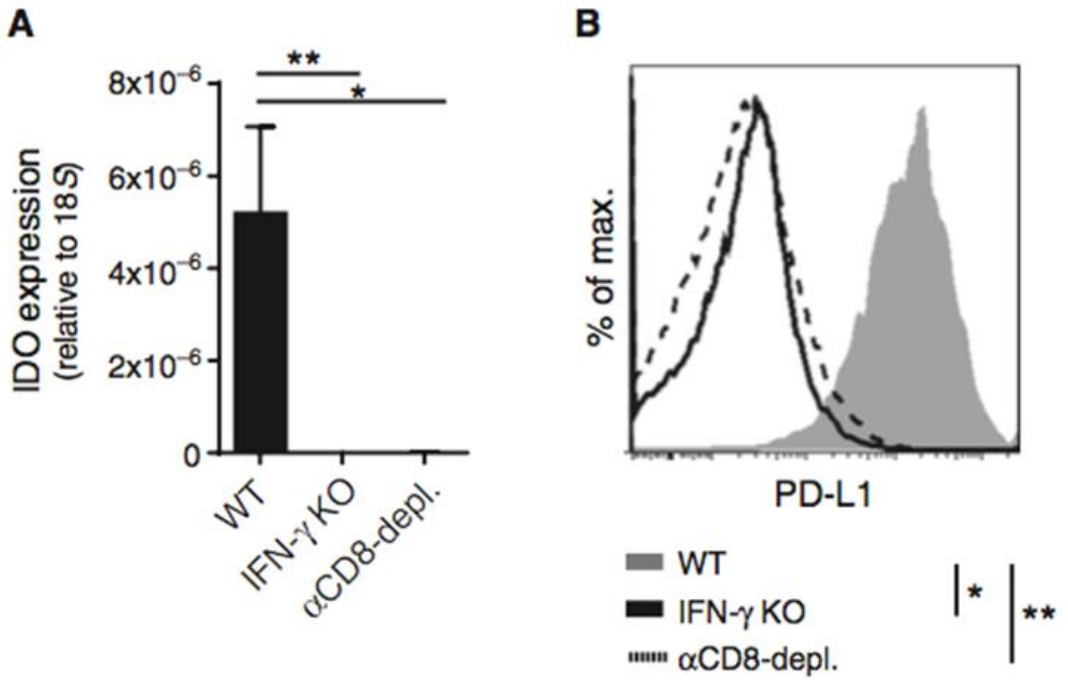


CD8+ T cells are required for spontaneous regression of IFNγR2-mutant B16.SIY tumors





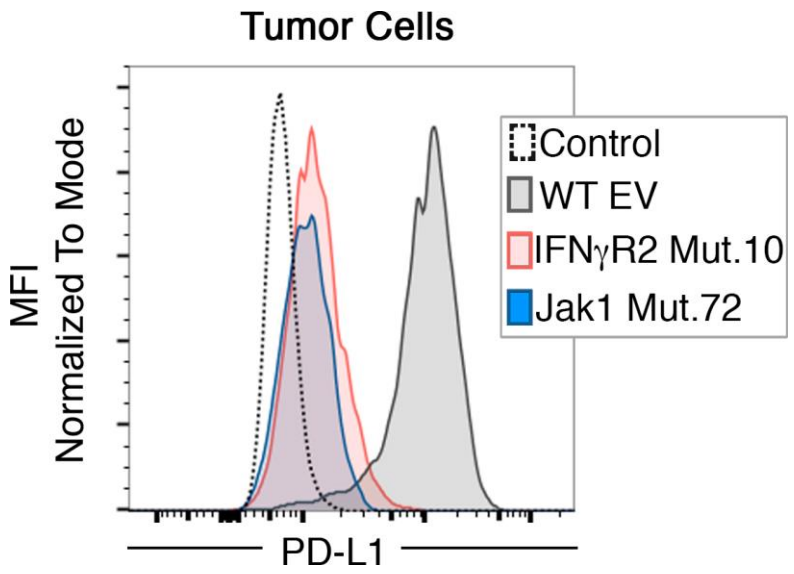
Why might blunted IFN-γ signaling in tumor cells lead to improved immune-mediated tumor control *in vivo*?

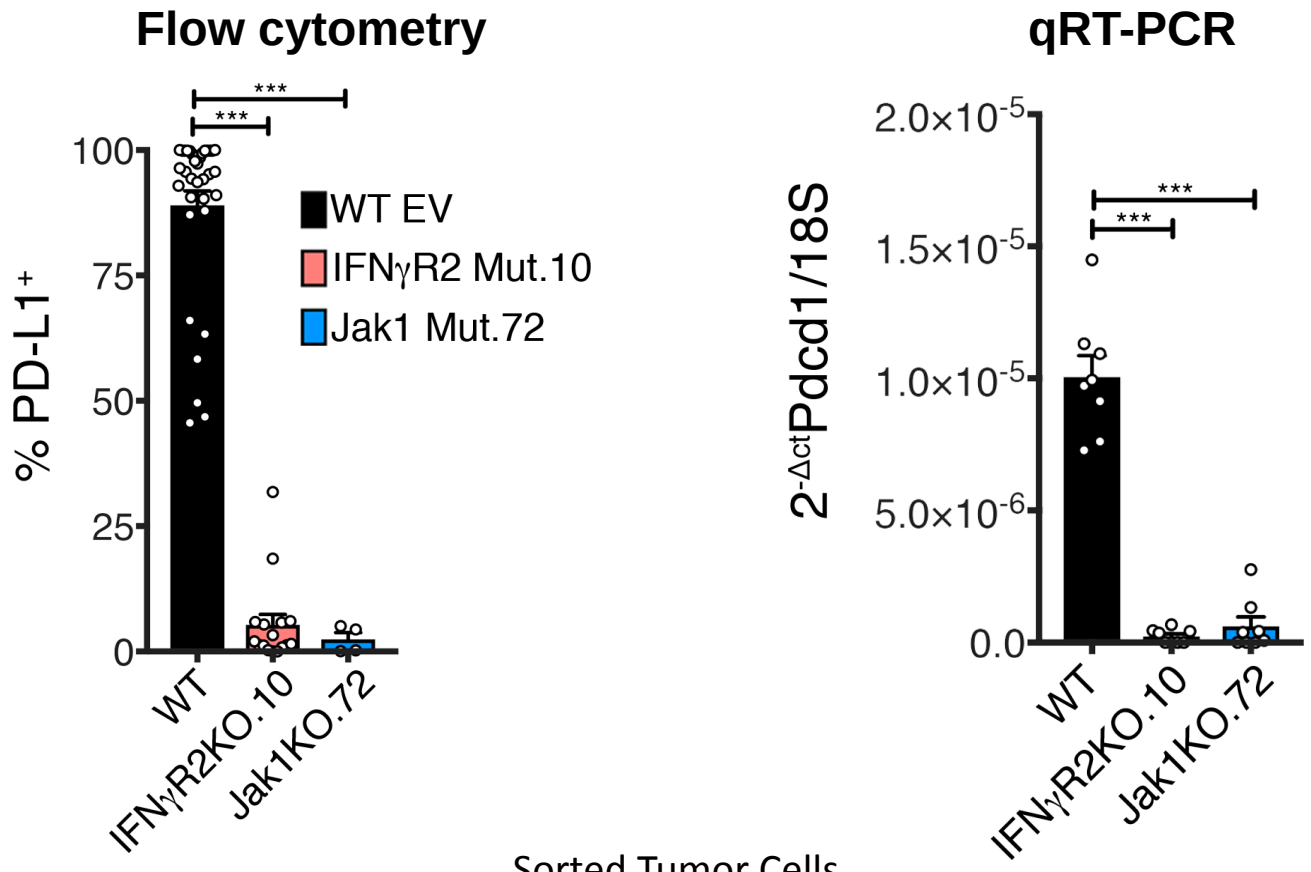


Spranger S. et al., *Sci. Transl. Med.* 2013

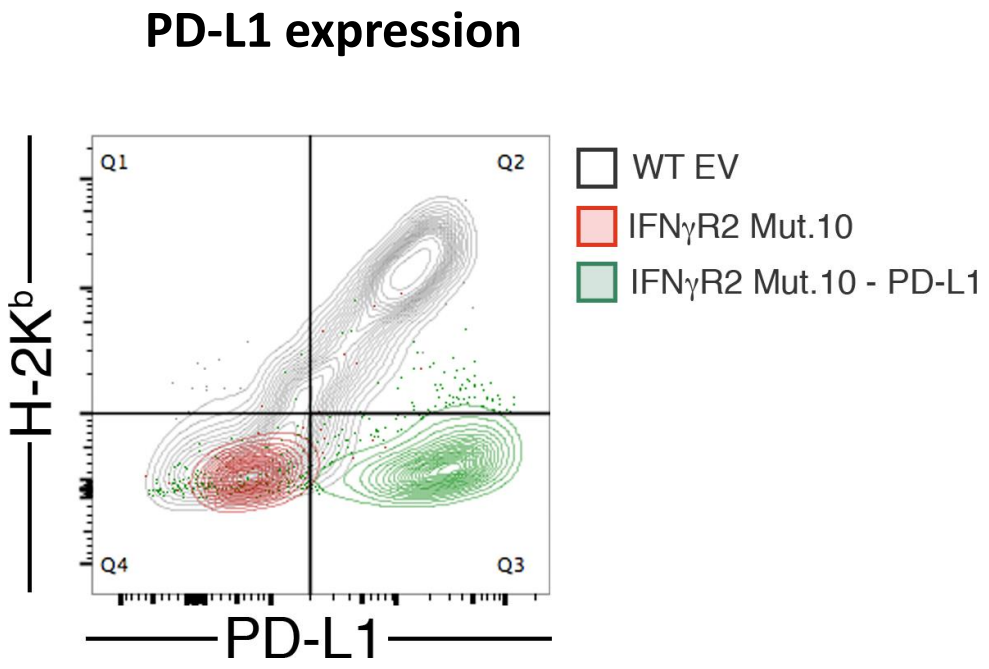
Hypothesis: Tumor cells deficient in IFN-γ signaling may fail to upregulate PD-L1, which in some tumor models could be dominant

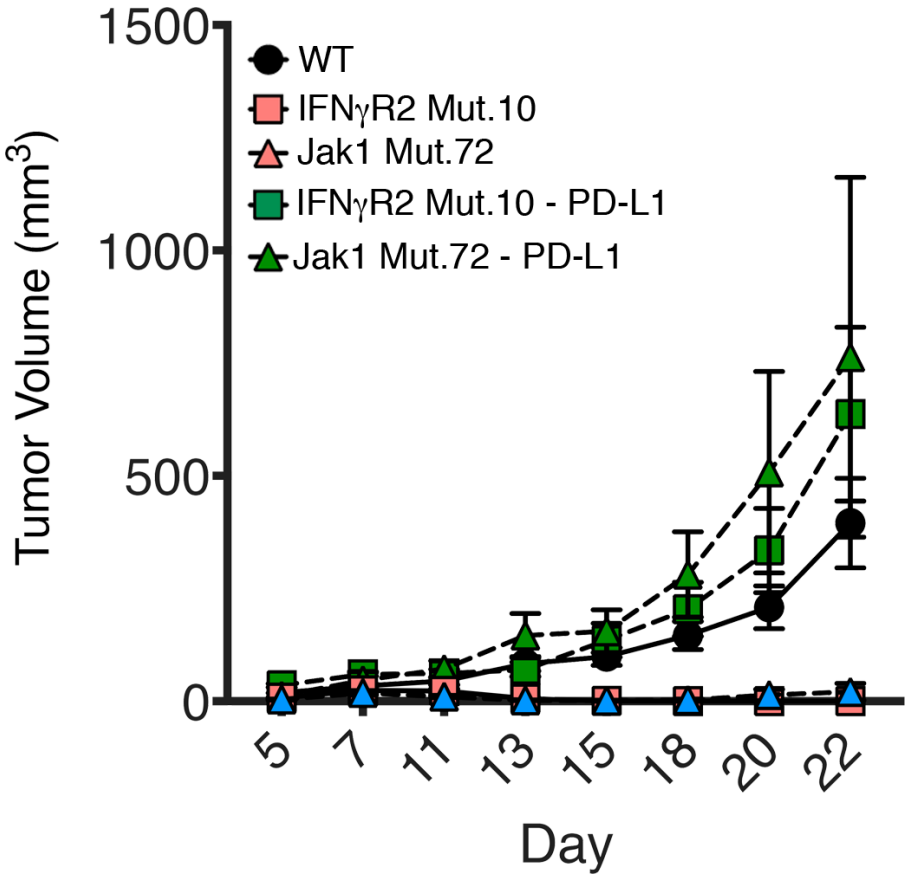
Deficient IFN-γ signaling in tumor cells leads to lack of PD-L1 upregulation *in vivo*





Restoration of PD-L1 expression in IFNγR2- and Jak1-mutant tumors is sufficient to restore progressive tumor growth *in vivo*





Conclusions

- In a genome wide CRISPR screen, tumor cells with mutation in Jak1 or in IFNγR2 arose as resistant to T cell-mediated killing *in vitro.*
- Paradoxically, IFNγR2- and Jak1-mutant tumors were better controlled *in vivo* in two independent tumor models, in a $CD8^+$ T cell-dependent fashion.
- Re-introduction of IFNγR2 restored progressive tumor growth, proving an on-target effect.
- Mutant tumors failed to upregulate PD-L1 *in vivo,* which likely explained improved immune-mediated tumor control.
- Restoration of PD-L1 expression was sufficient to restore tumor progression, confirming a critical role of PD-L1 on tumor cells in mediating this effect.
- Together, these data imply that while IFN-γ can have positive immune effects on tumor cells, in some settings the upregulation of immune-inhibitory molecules such as PD-L1 can dominate leading to tumor progression.

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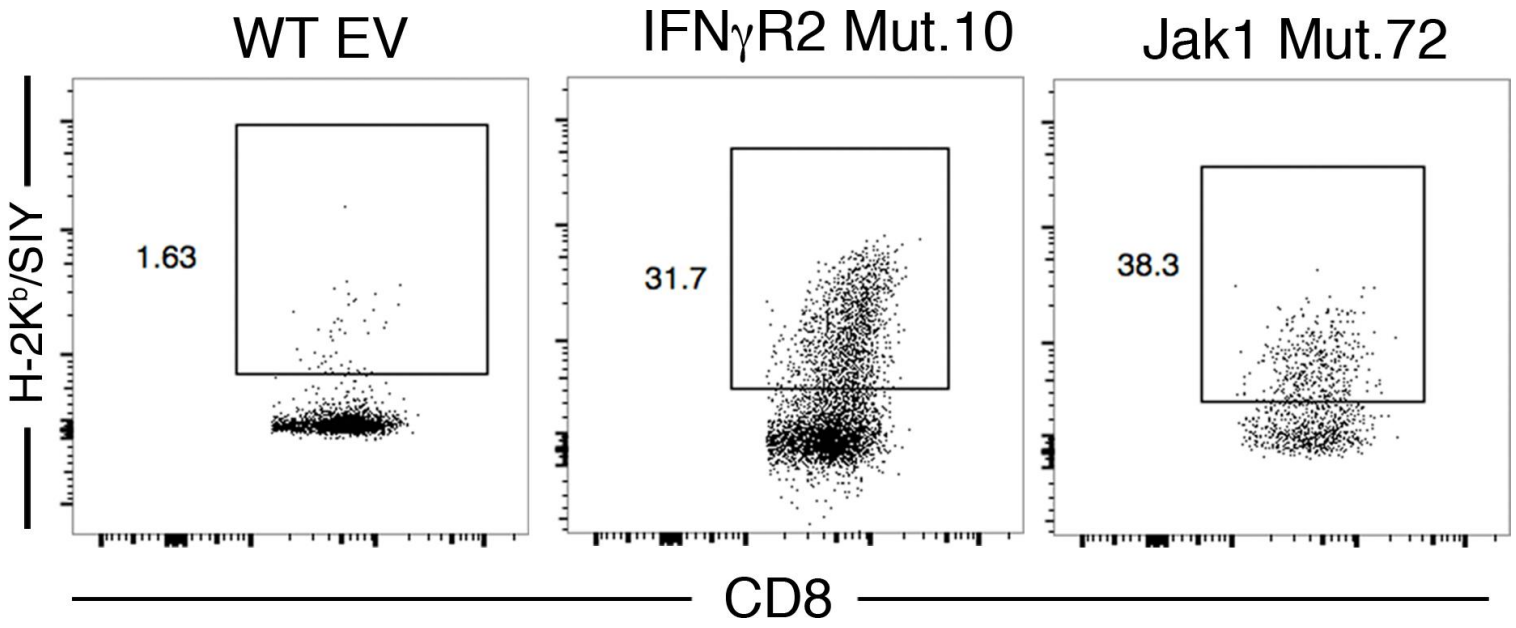
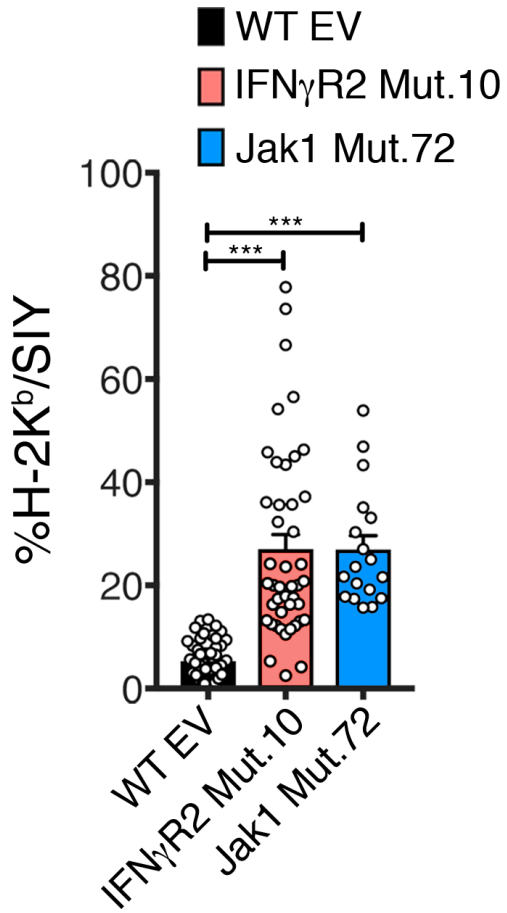
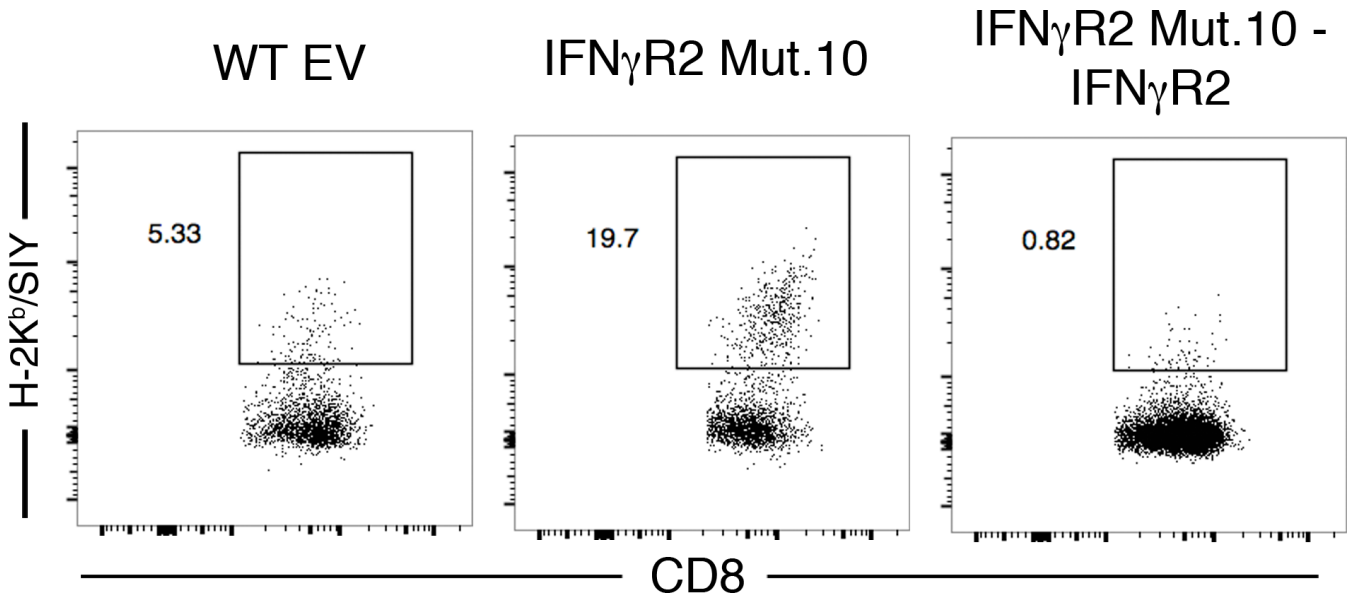
Ayelet Sivan
Leticia Corrales
Stefani Spranger
Seng-Ryong Woo

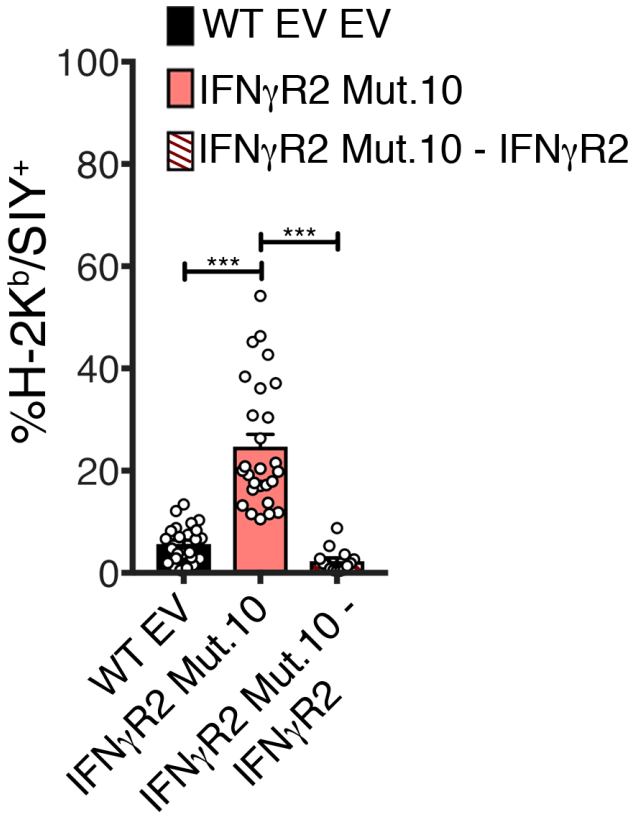
SIY-specific CD8+ T cell responses are augmented in the setting of IFNγR- and Jak1-mutant tumors



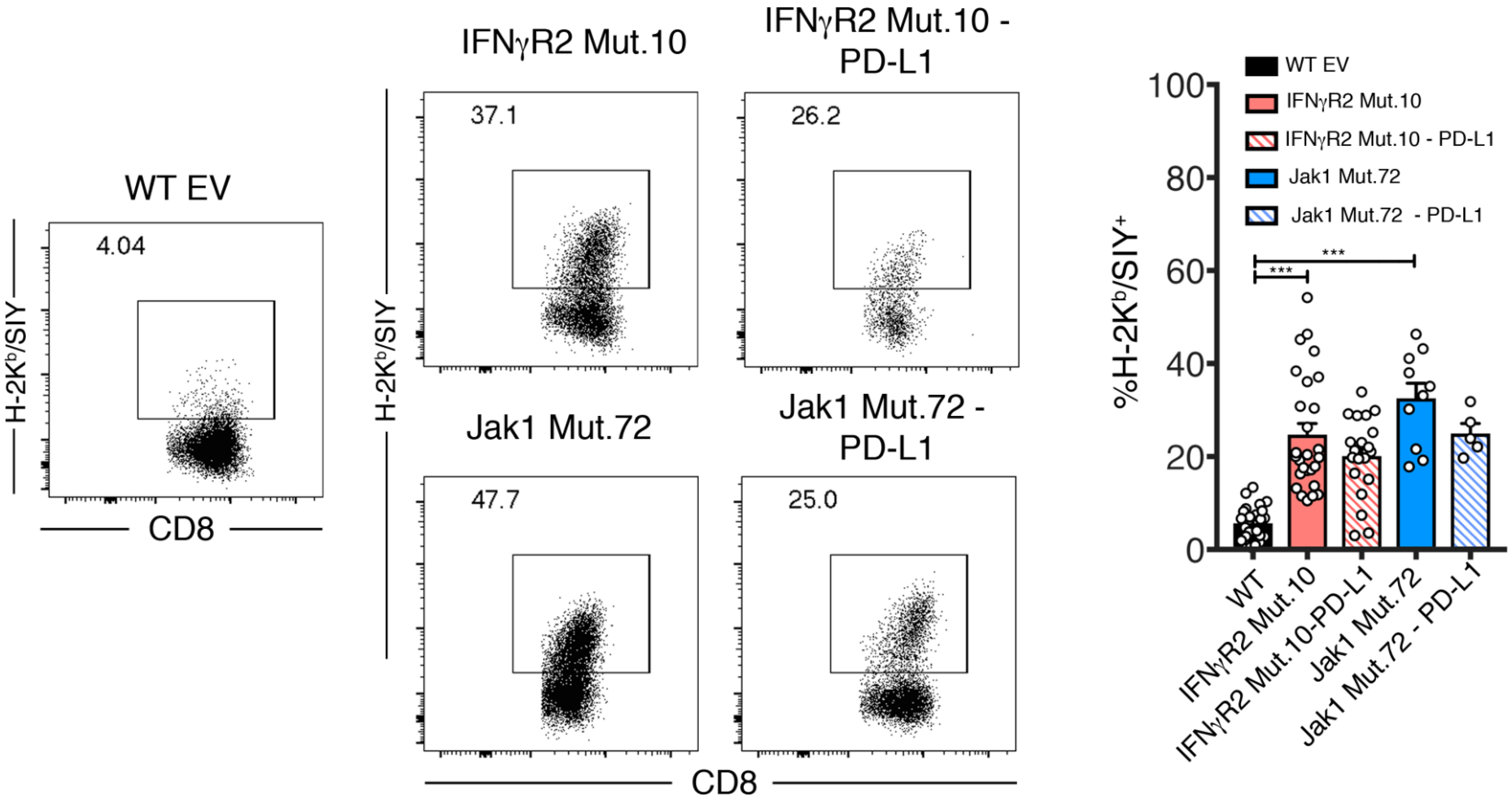


Restoration of tumor cell-intrinsic IFN-γ signaling is sufficient to revert the anti-tumor T cell response to wild-type levels

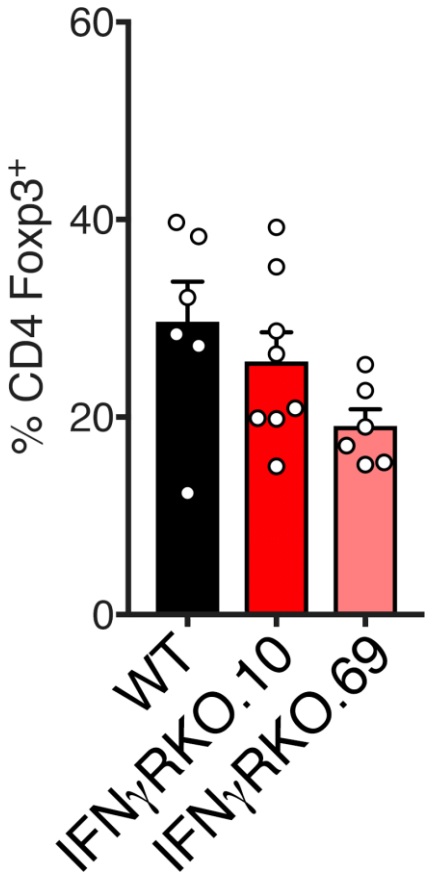




PD-L1 overexpression does not fully normalize the augmented anti-tumor response against IFNγR2- and Jak1-mutant tumors

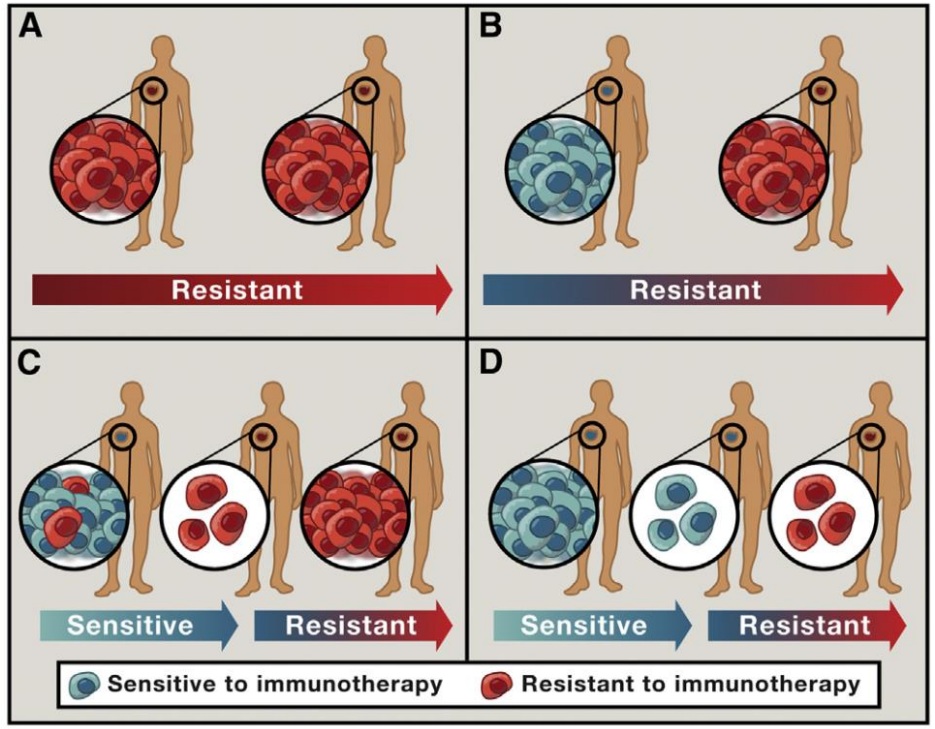


Regulatory T cells are recruited efficiently to IFNγRKO tumors



Clinical scenarios of primary, adaptive, and acquired resistance to immunotherapy

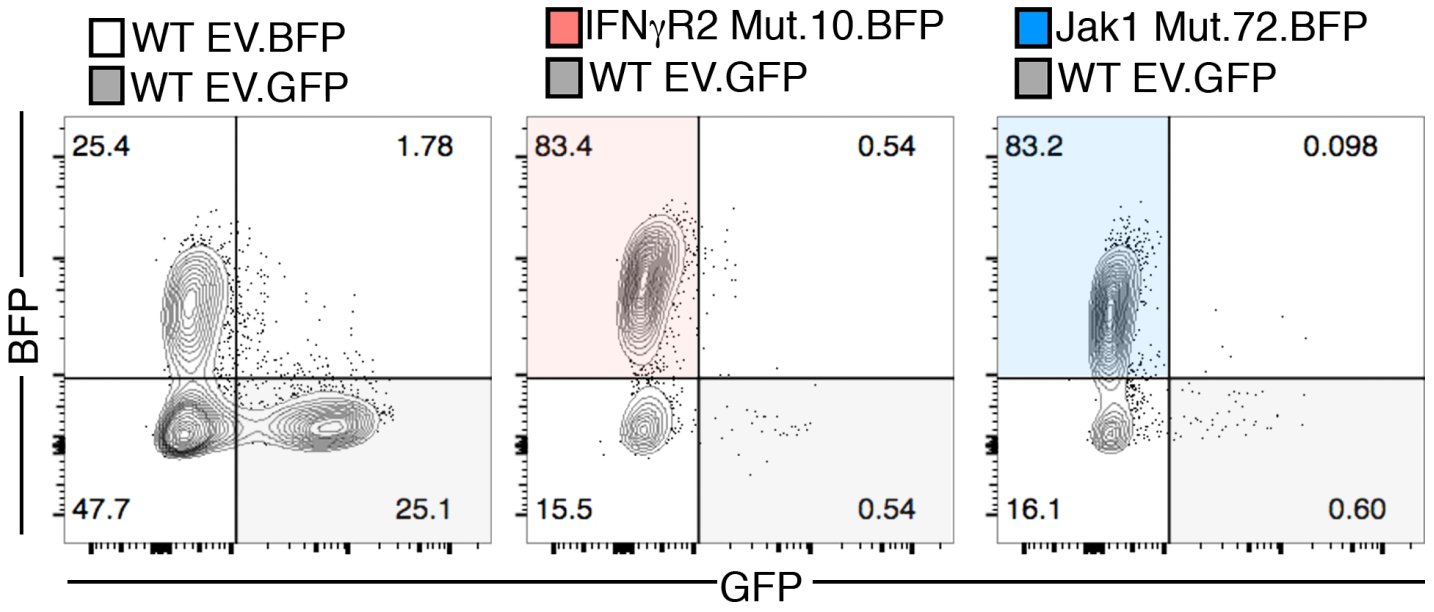
No active immune response	Adaptive Resistance

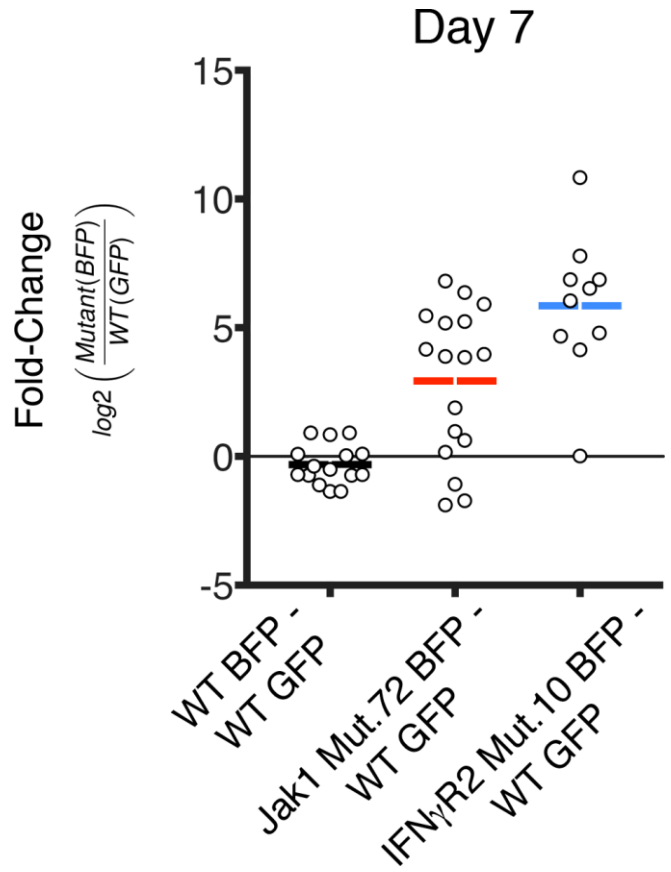


Acquired Resistance Resistant clones present before treatment	Acquired Resistance Resistant clones arise from treatment

What might occur with a mixture of WT and IFN-pathway mutants?

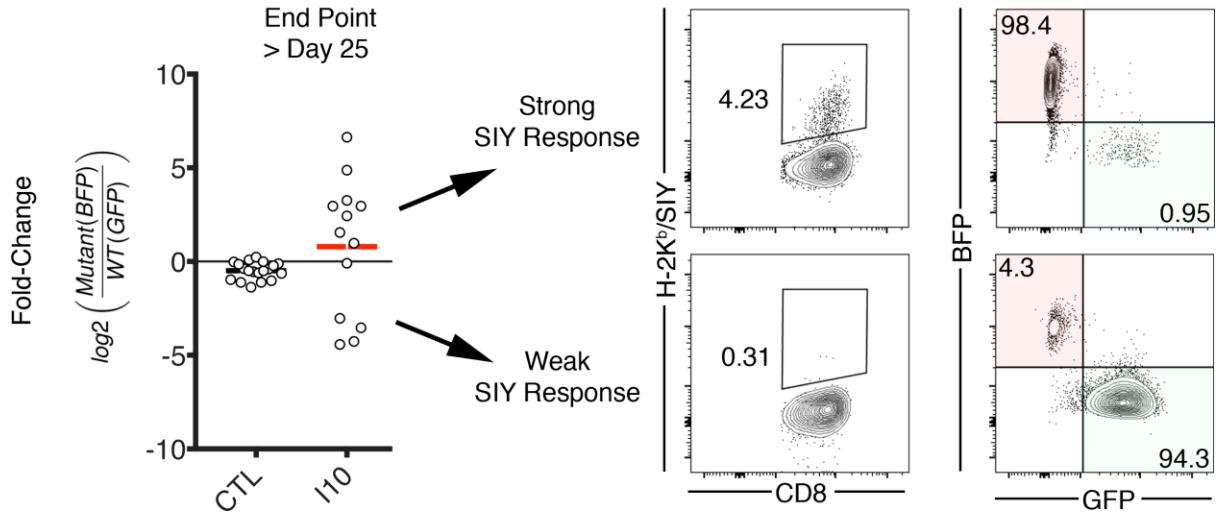
IFNγR2- and Jak1- mutant tumor cells are selected for in a mixture setting *in vivo*

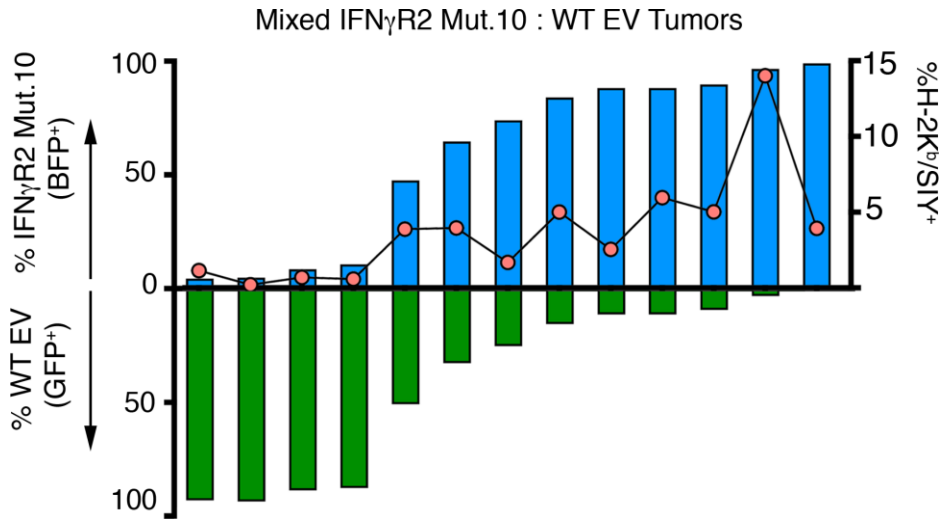




A strong anti-tumor T cell response correlates with selection of mutant tumors over time

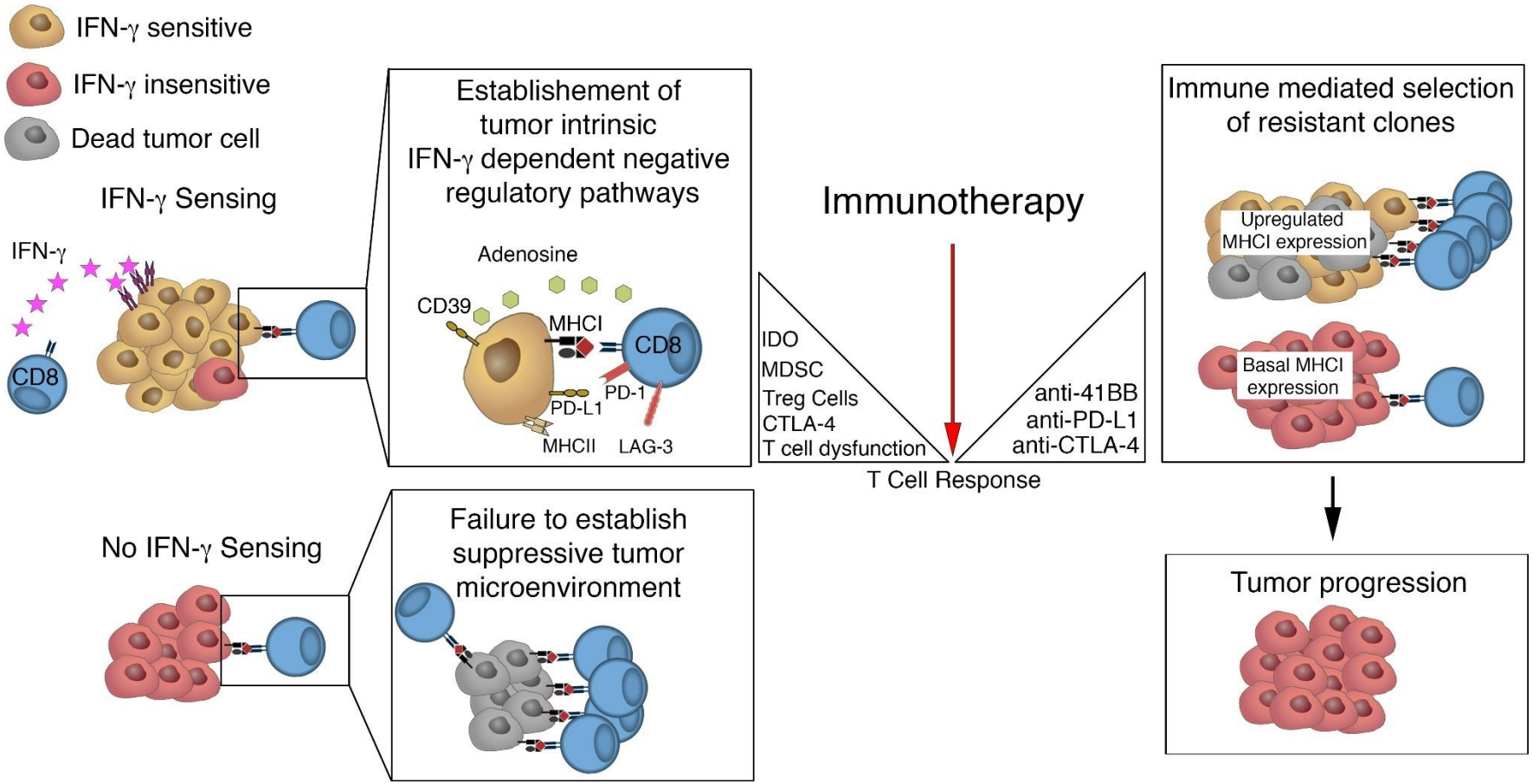
Acquired Resistance

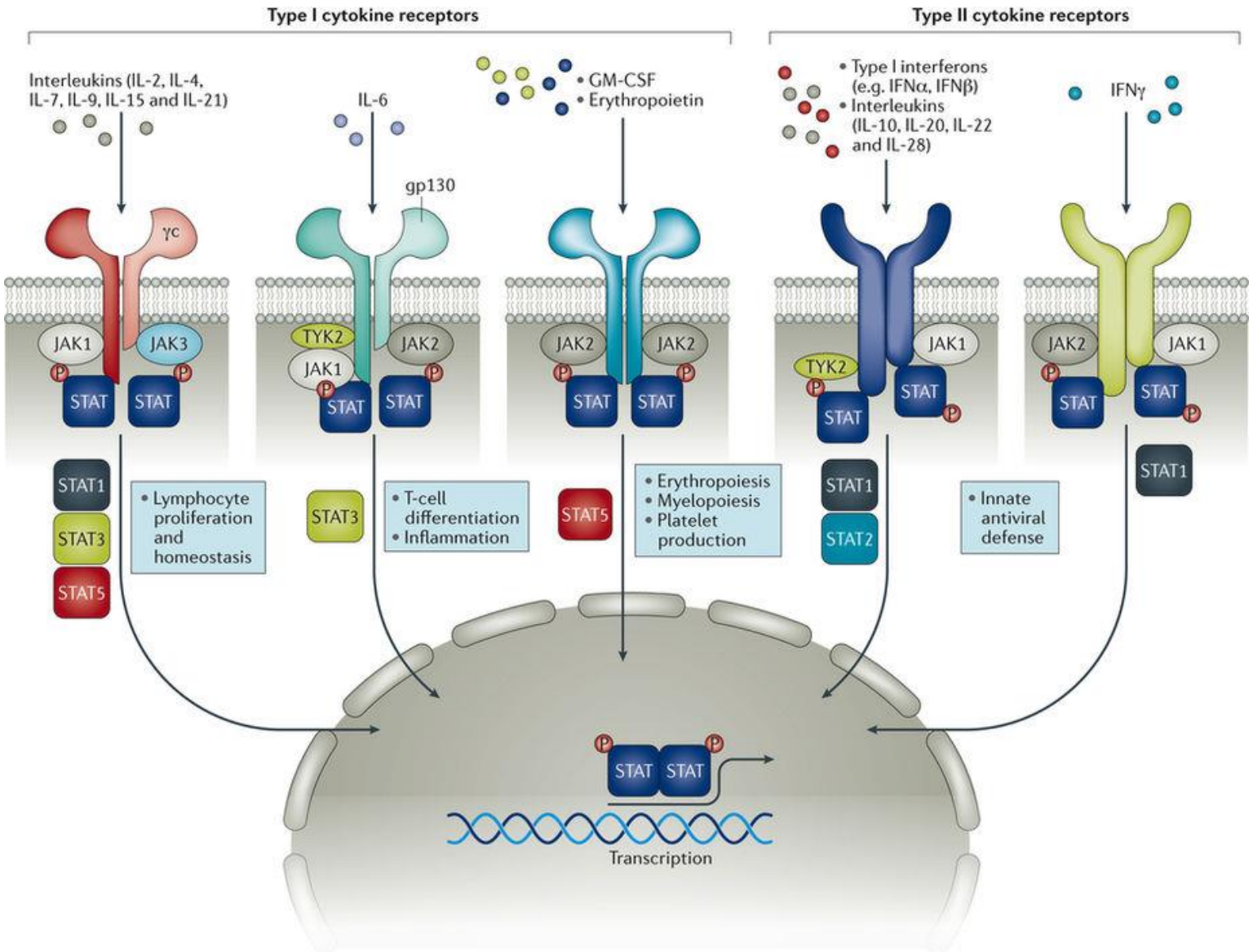




Adaptive Resistance

Working Model





Kevin Winthrop Nat. Rev. Rheu 2017

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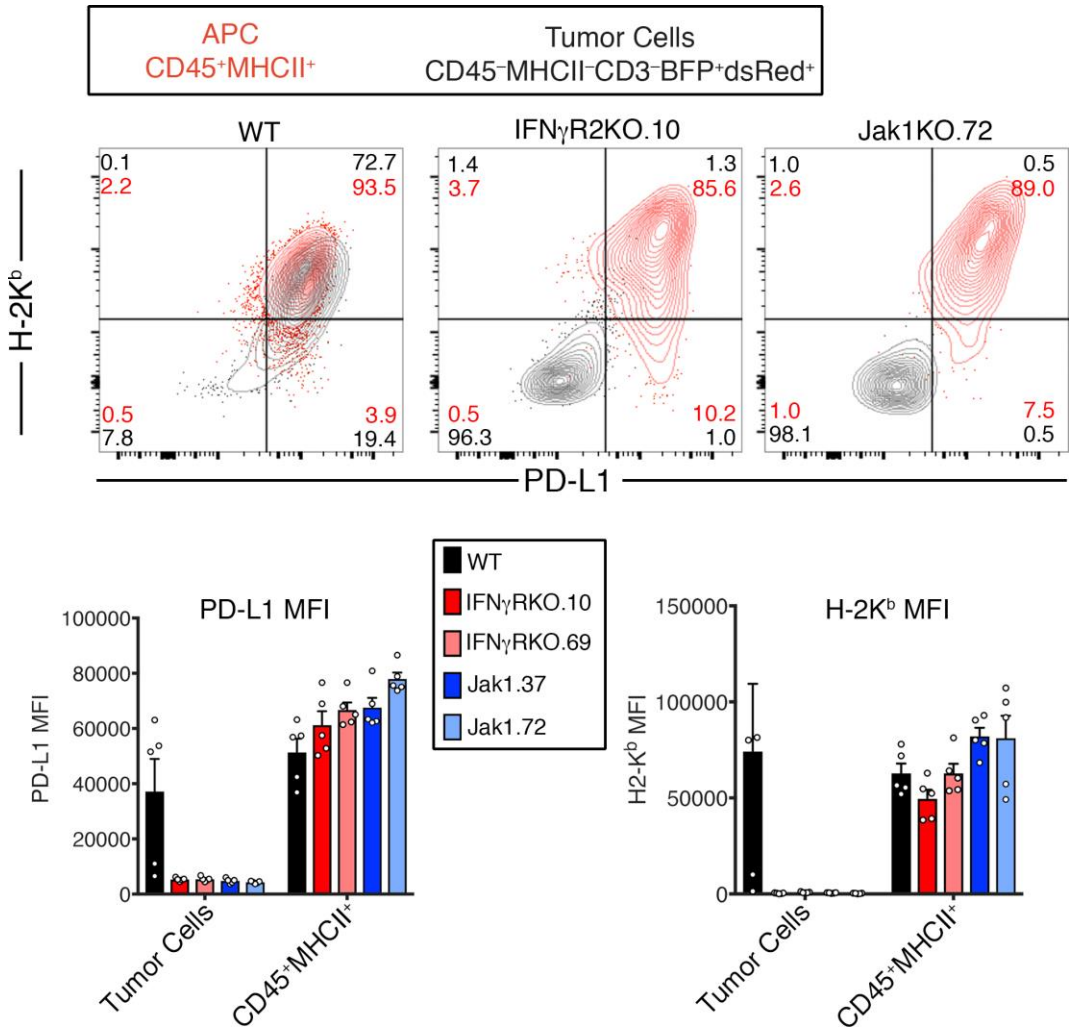
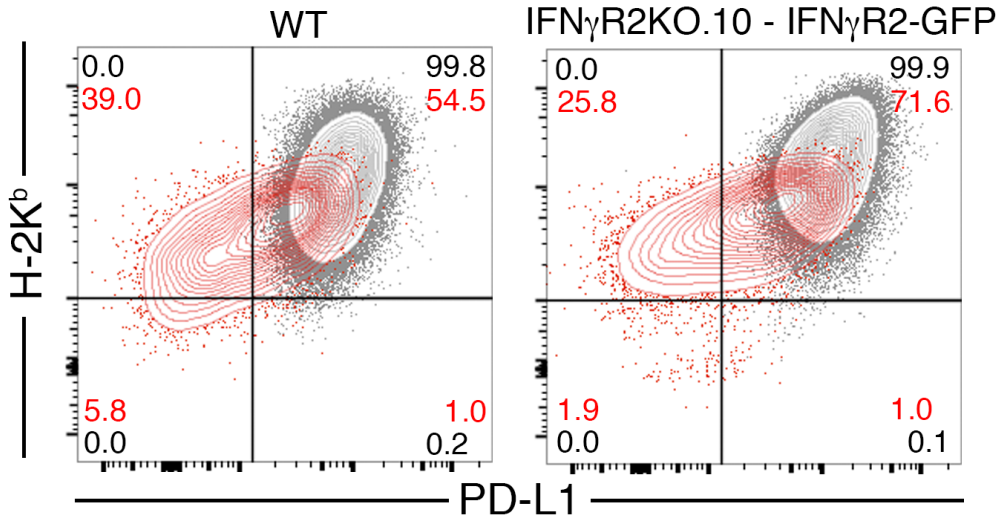
IFNγR2$^{-/-}$ B16.SIY cells fail to upregulate H-2K^{b} and PD-L1

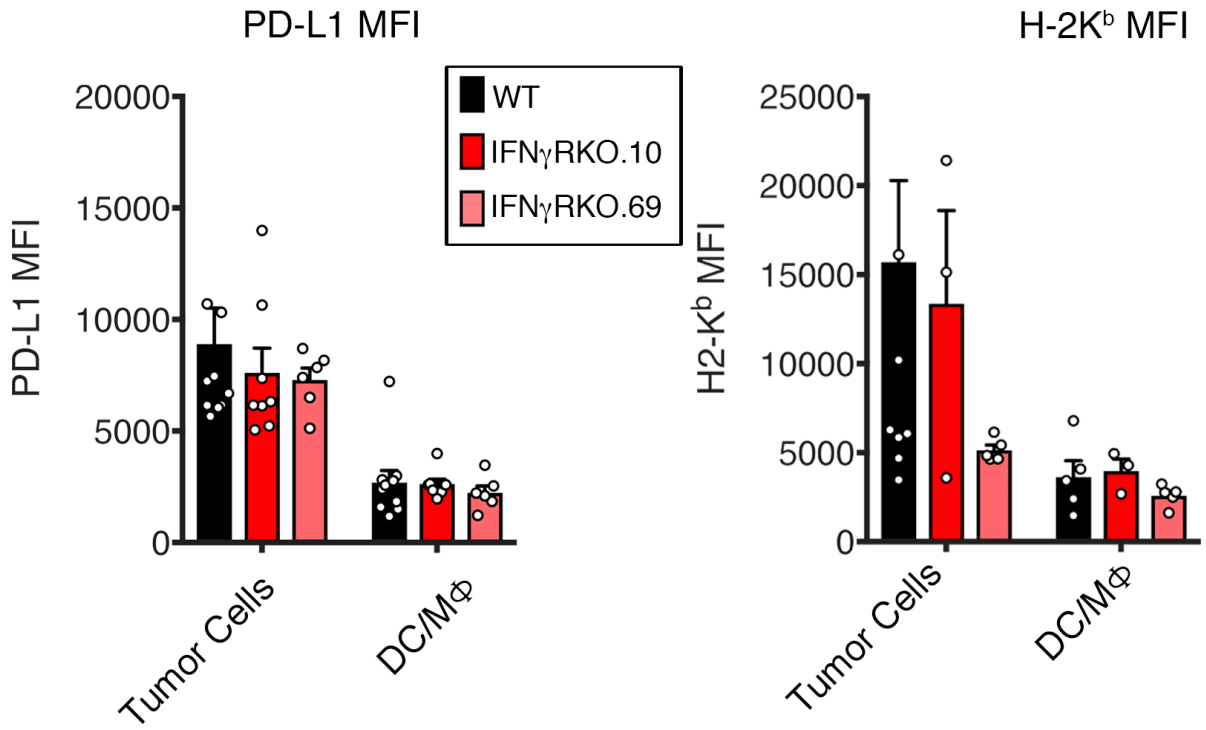


In the absence of tumor sensing IFNγ, PD-L1, as well as H-2K^{b}, are not upregulated.

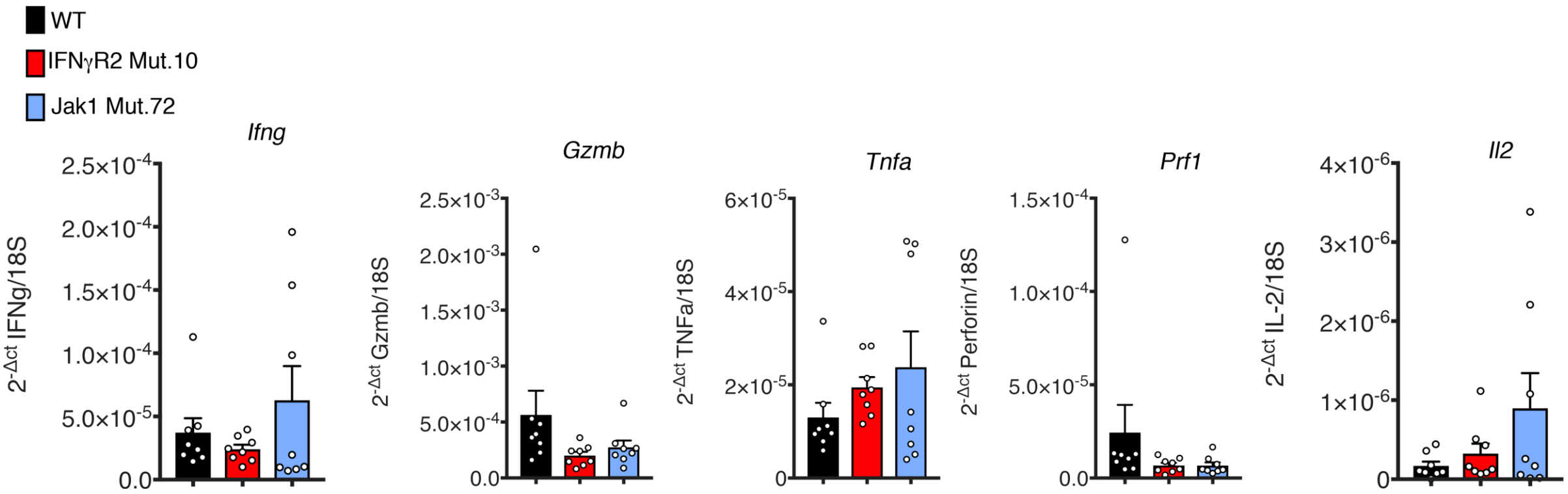
Reintroduction of IFNγR2 expression in IFNγR2KO tumors is sufficient to restore PD-L1 upregulation

APC	Tumor Cells
CD45⁺MHCII⁺	CD45⁻MHCII⁻CD3⁻
CD11b+CD11c+	BFP⁺dsRed⁺

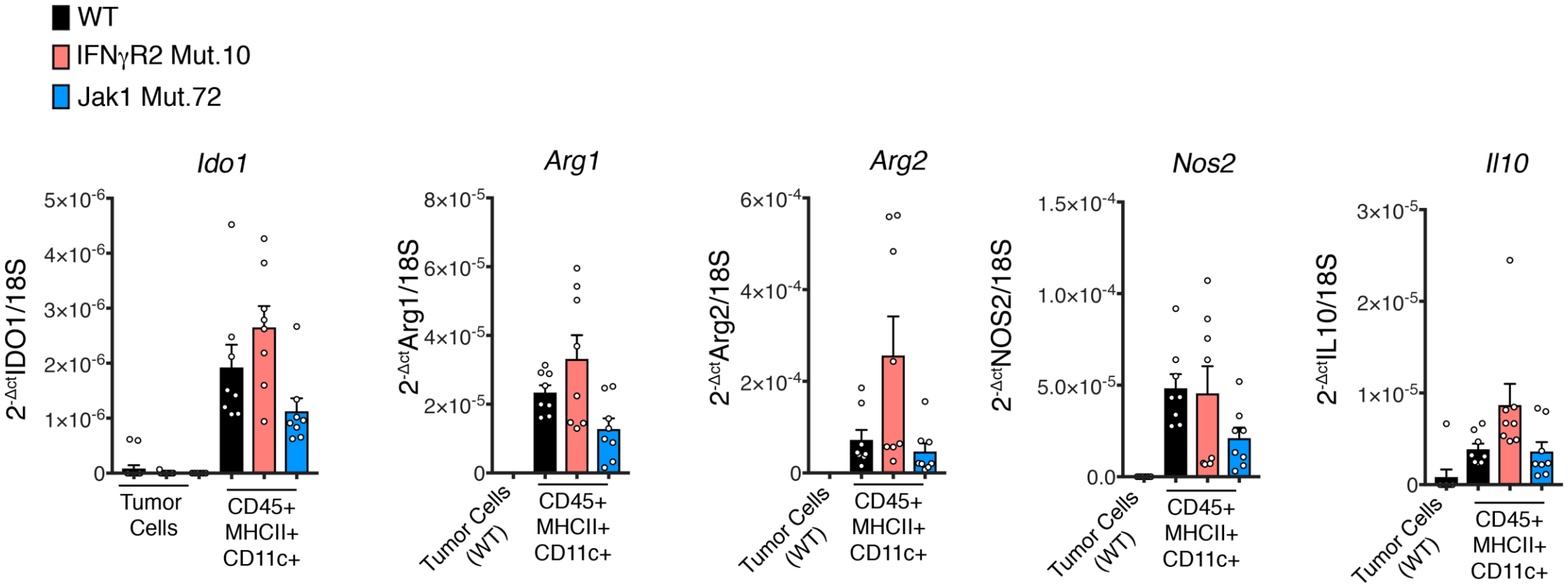




No substantial difference in CD8+ effector molecules is observed



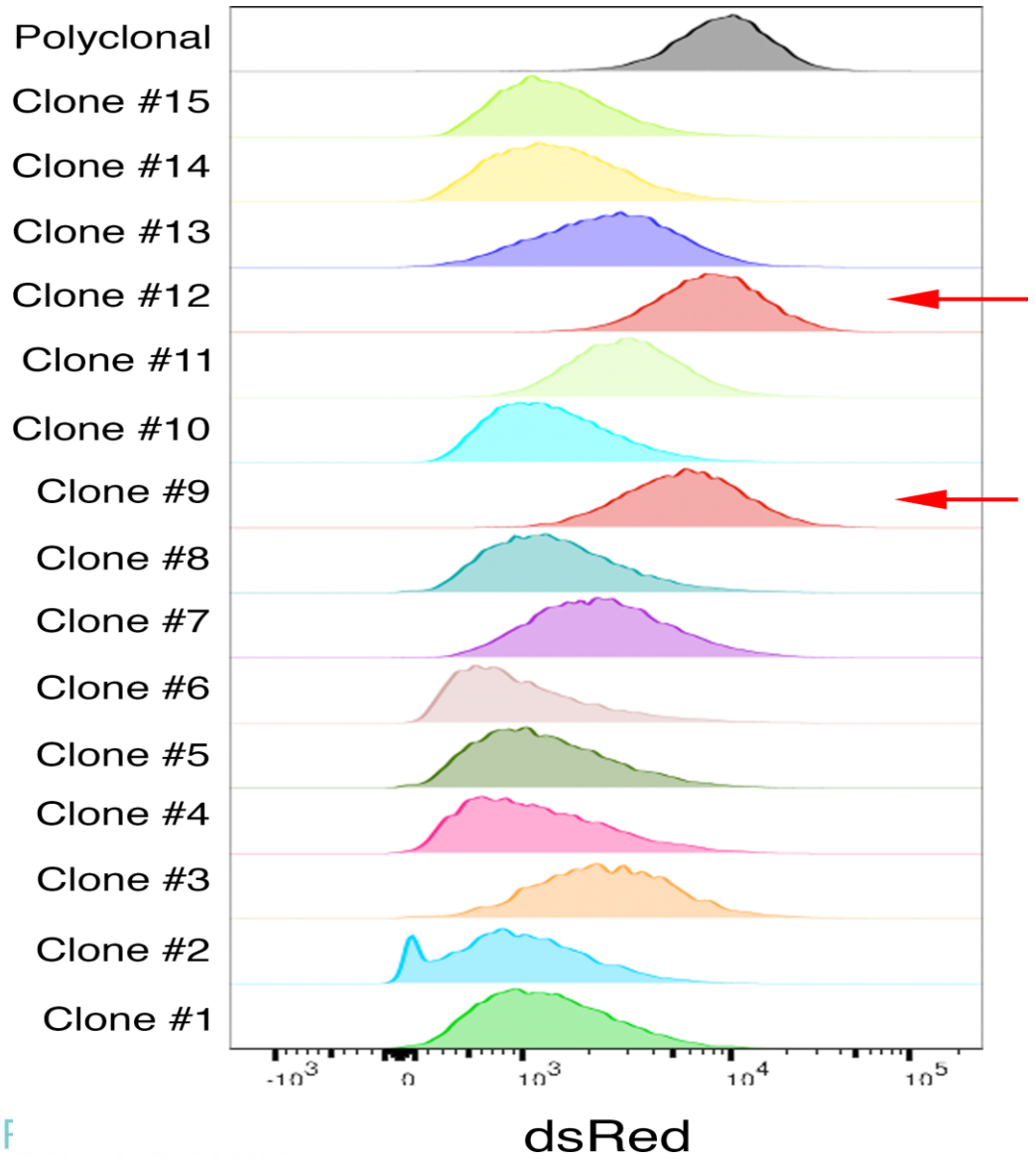
Other potential negative regulators are not substantially different



Selecting a single B16.SIY clone to normalize starting genetic composition

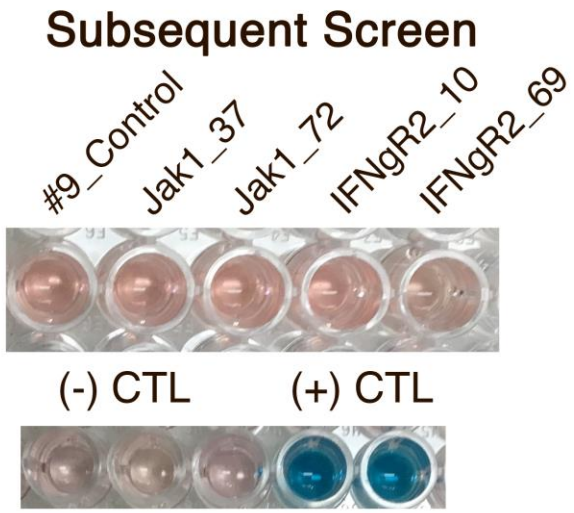
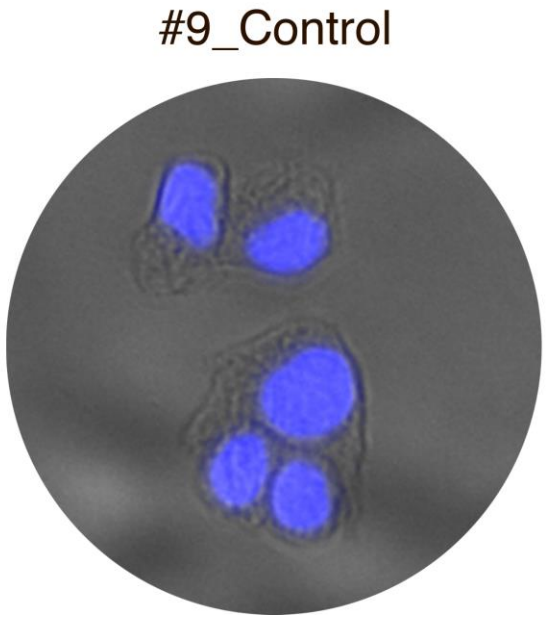
Criteria for clone

- Similar dsRed expression to polyclonal population
  - dsRed is a readout for SIY expression
- Tumors Grows progressively
- Tumors respond to checkpoint blockade



Cell lines were tested negative for mycoplasma

Mycoplasma Testing by TLR2 Activation



Mycoplasma Testing by DAPI Stain