Using Genomics to Elucidate Cancer Immunoediting and Guide Cancer Immunotherapy

SITC Annual Meeting Keynote
Bethesda, MD
October 26, 2012

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CONFLICT OF INTEREST

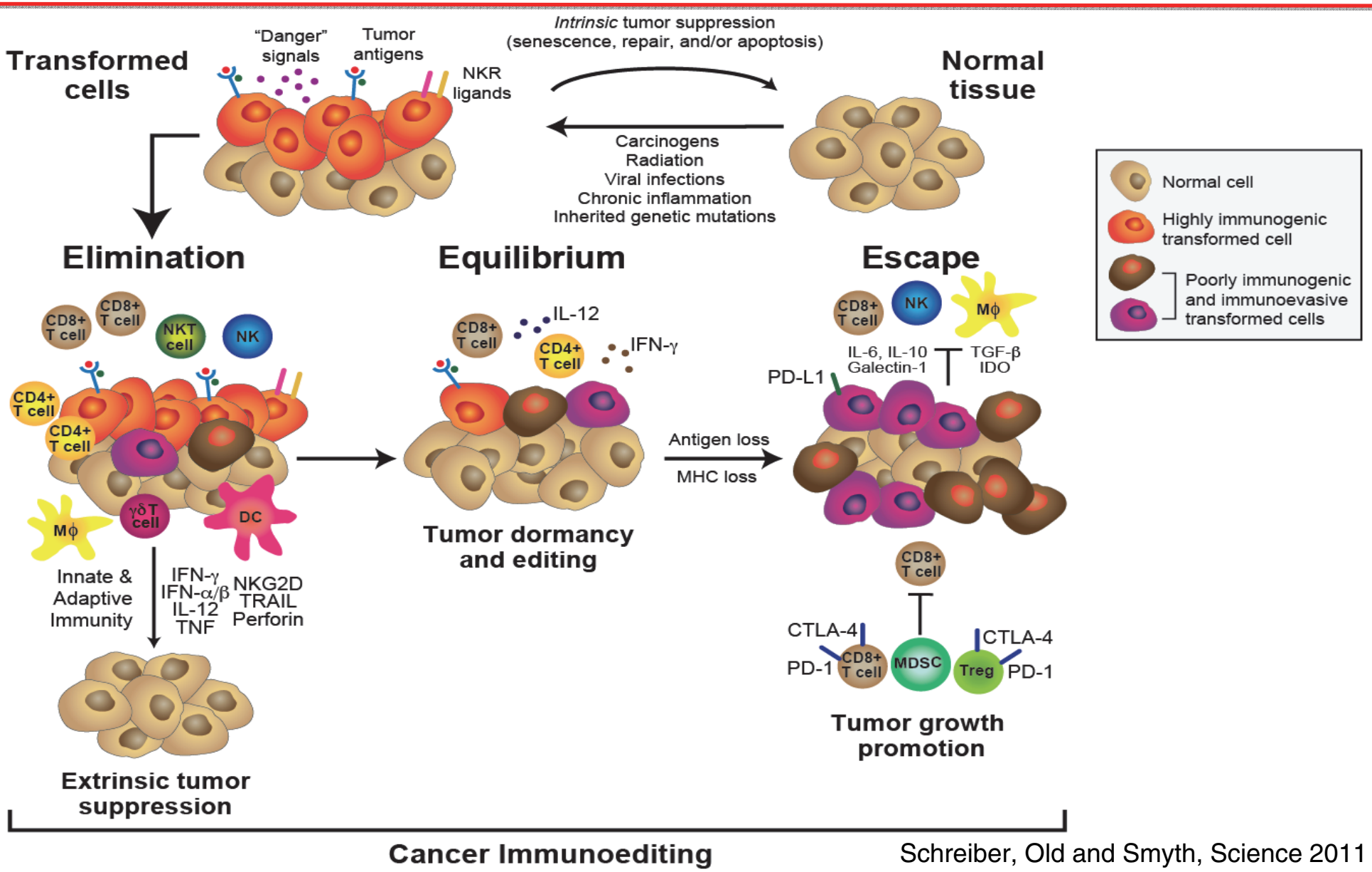
I have no conflicts that are directly related to this presentation.

However, in the spirit of full disclosure:

I am a co-founder of Igenica, Inc. a company interested in generating novel antibody cancer therapeutics

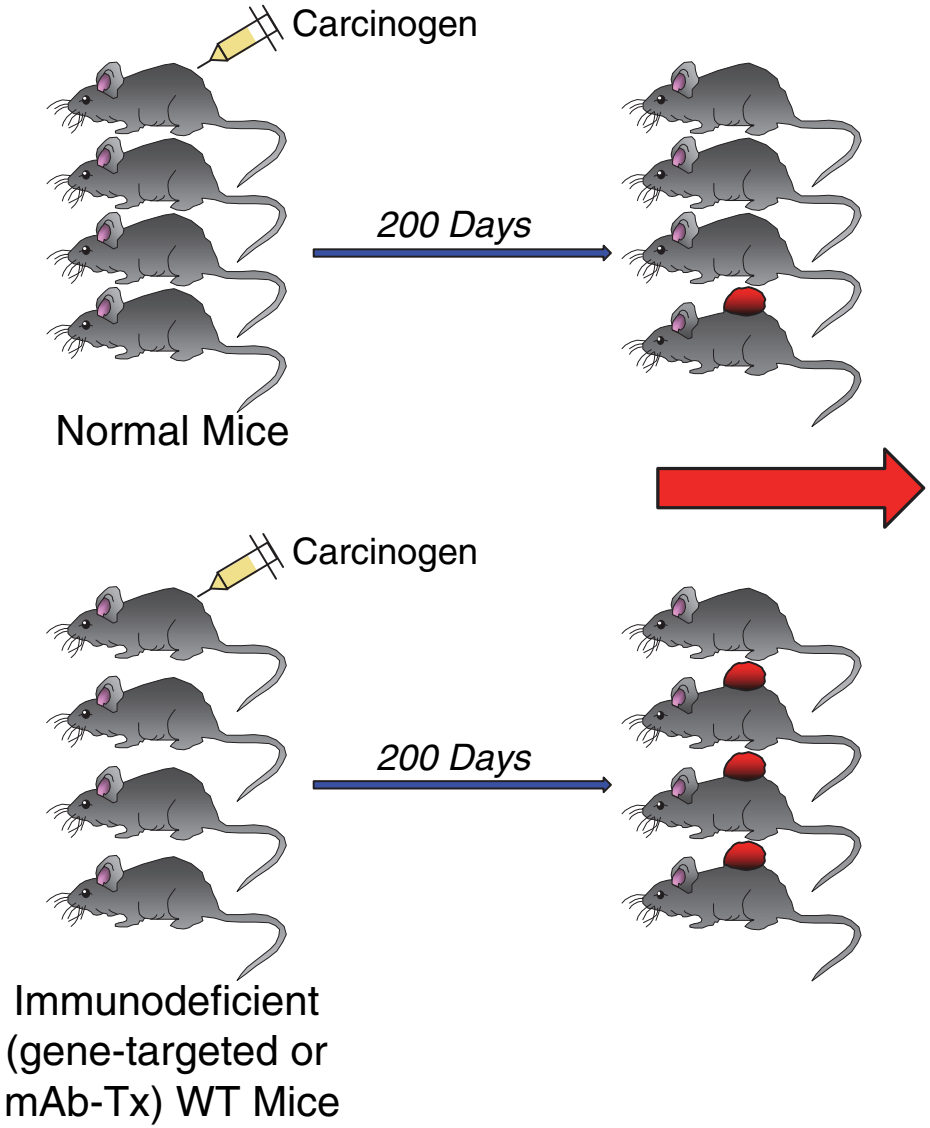
I receive research funding from Bristol Meyers Squibb for future work in this area

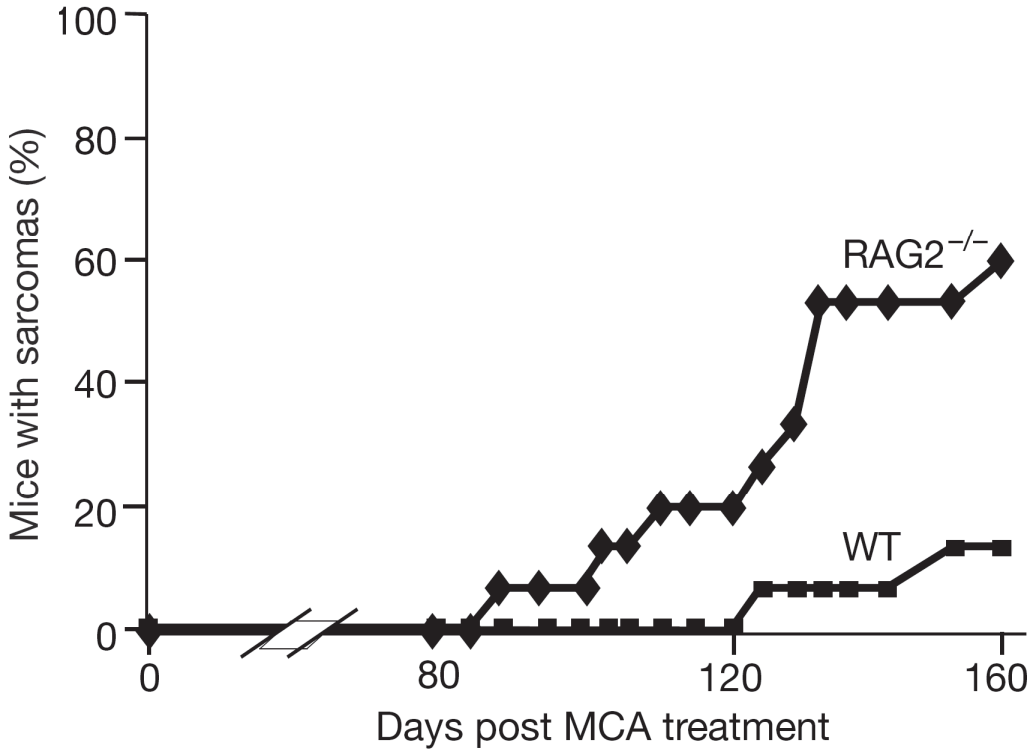
The 3 Es of Cancer Immunoediting



Schreiber, Old and Smyth, Science 2011

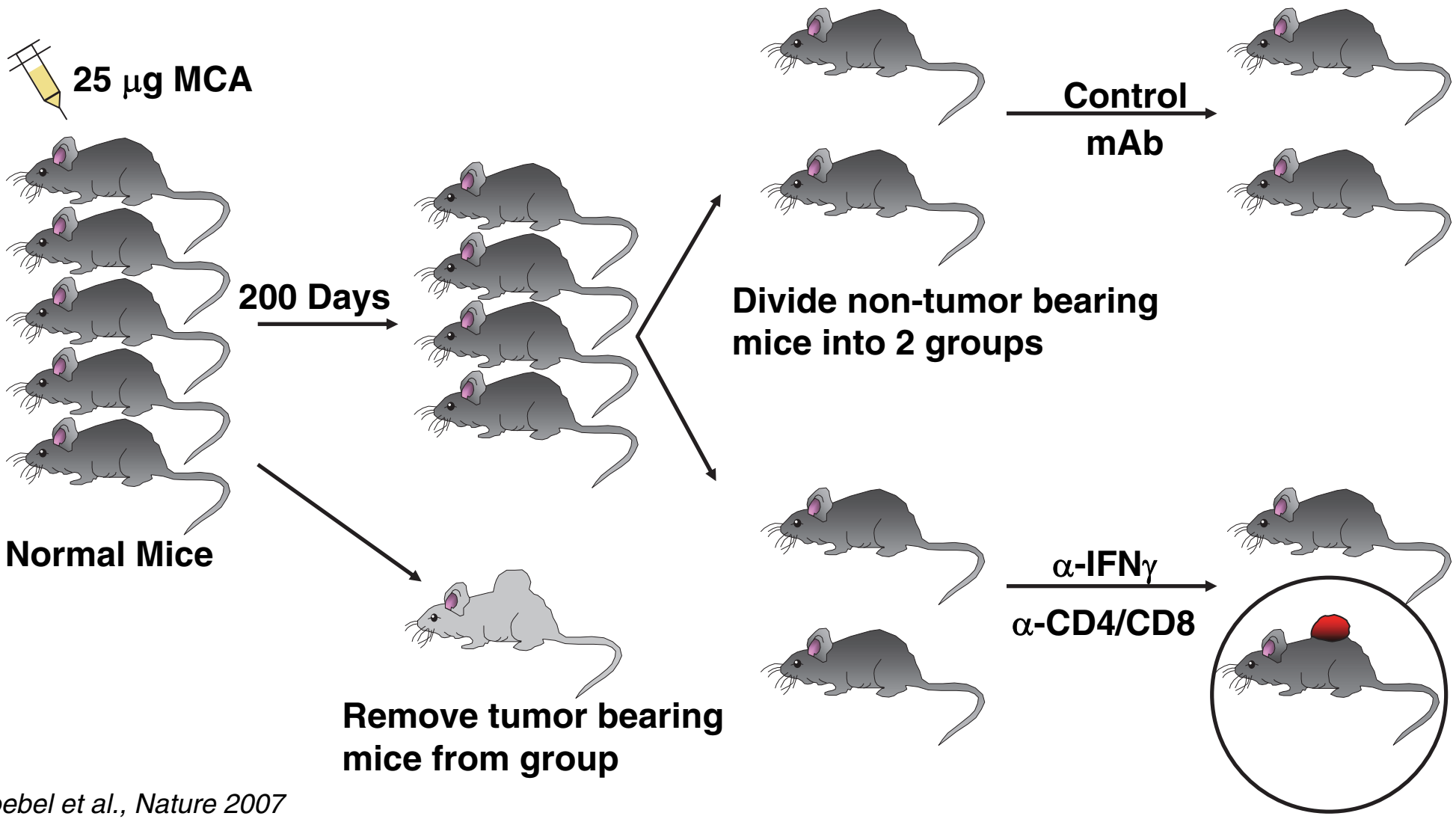
Experimental Proof for Cancer Immunosurveillance (Elimination Phase)





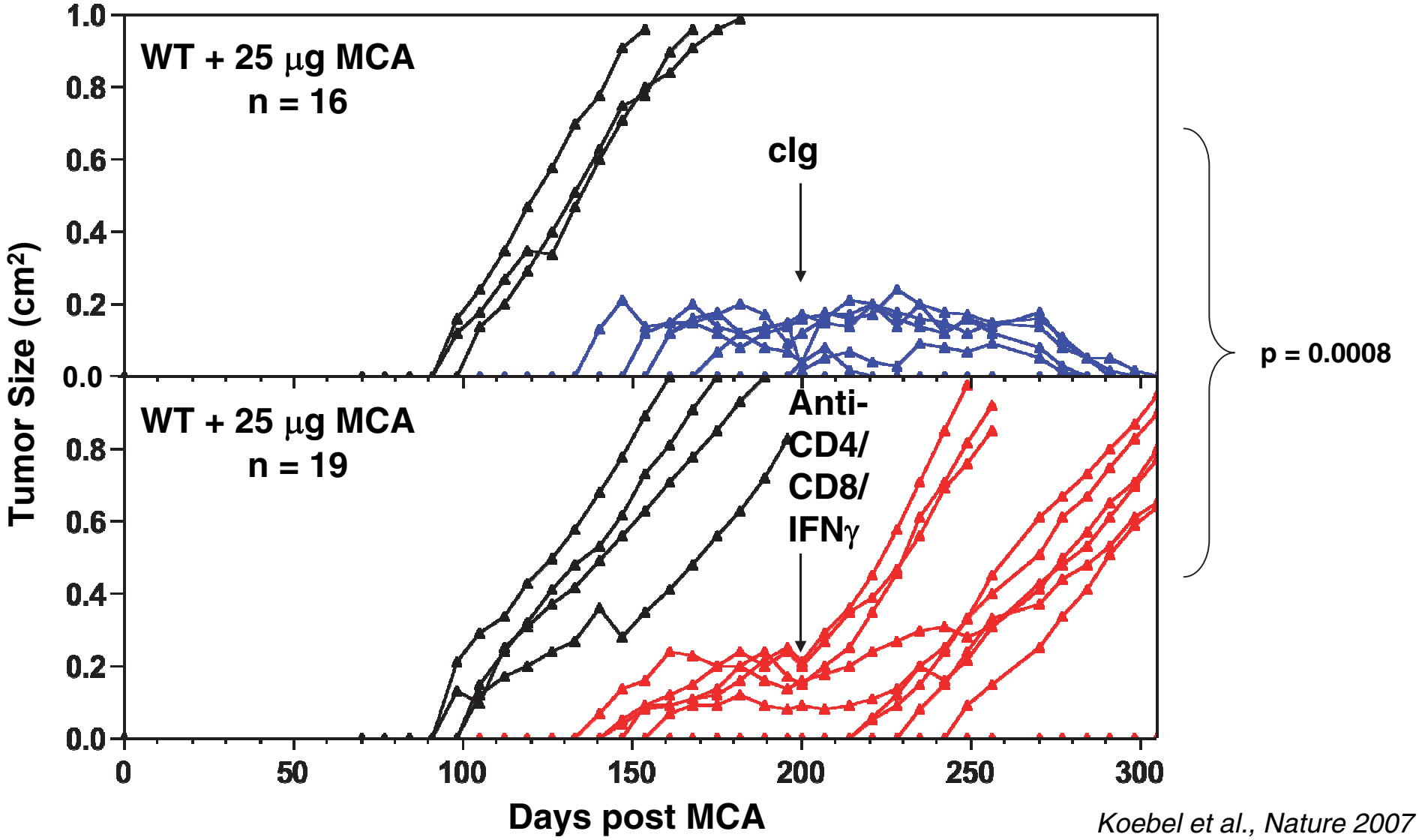
Shankaran et al. Nature 2001

Experimental Protocol to Assess Whether the Equilibrium Phase Occurs



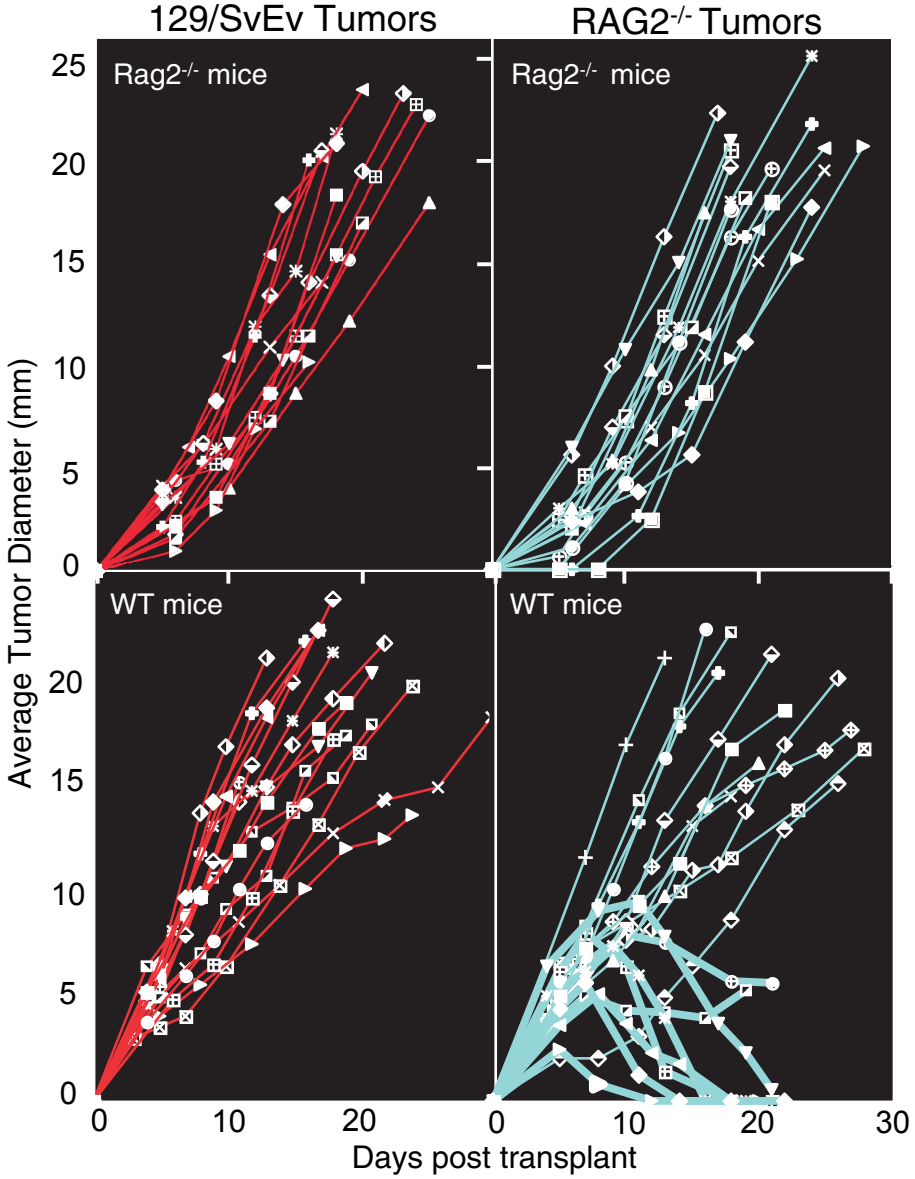
Koebel et al., Nature 2007

Experimental Demonstration of Equilibrium



Koebel et al., Nature 2007

Experimental Proof That Editing of Tumor Cell Immunogenicity Promotes Tumor Outgrowth



Tumors from WT mice are "edited" (display reduced immunogenicity) and thus are models of clinically apparent, mature tumors.

Tumors from RAG2$^{-/-}$mice are "unedited" (display high immunogenicity) and thus are good models of nascent tumors.

Shankaran et al, Nature 2001

Cancer Immunoediting

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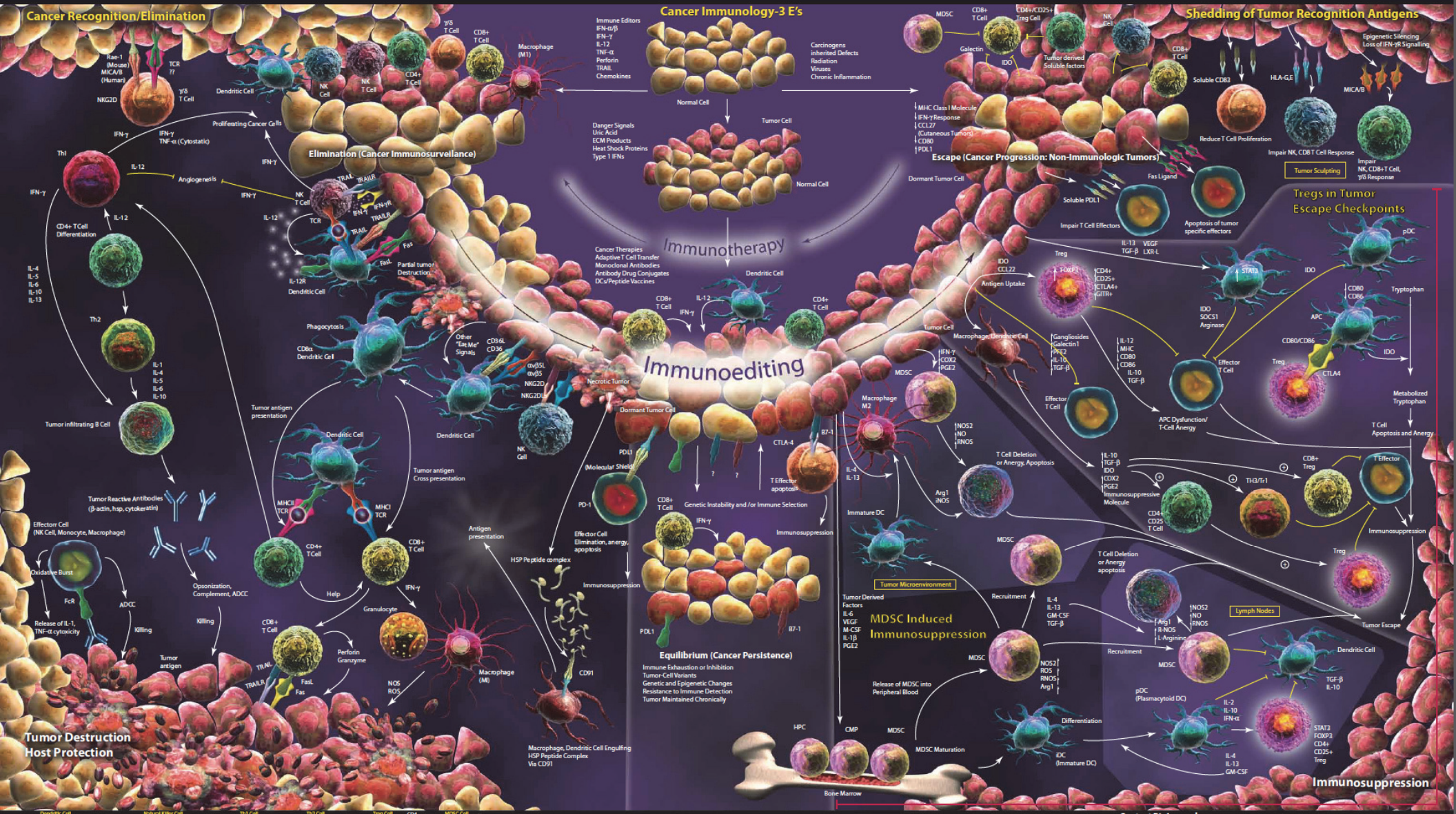

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We would like to thank Dr. Robert Schreiber of Washington University - School of Medicine for his contributions to this poster.

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04-0017-00

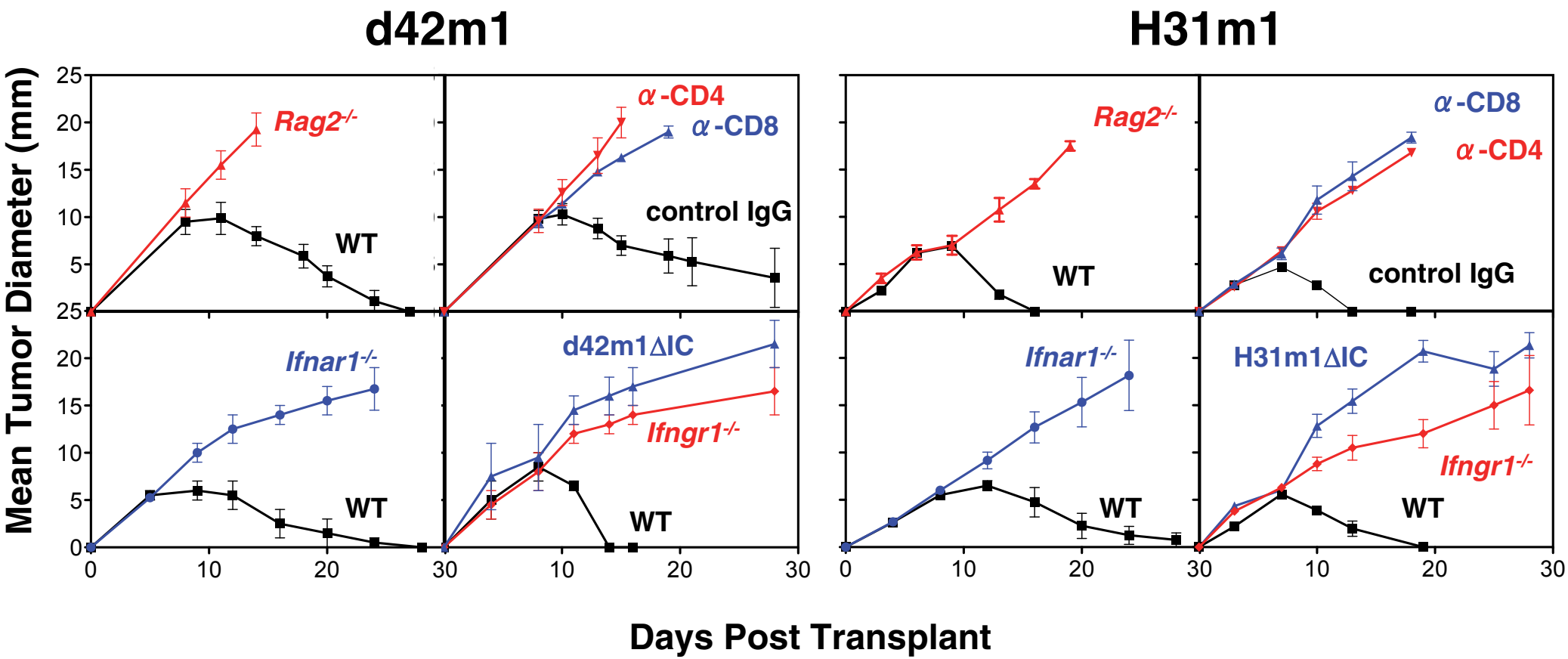
Focus of Today's Talk

- What are the targets of cancer immunoediting? (Tumor Antigens? MHC? IFN-γ signaling? Other?)
- By what mechanism(s) does immunoediting occur?
- Are edited tumors still immunogenic?
- Can edited tumors still be targets for cancer immunotherapy?

d42m1 and H31m1: Typical Unedited Rag2$^{-/-}$ Regressor Tumors

d42m1 and H31m1 rejection in naïve syngeneic hosts requires:

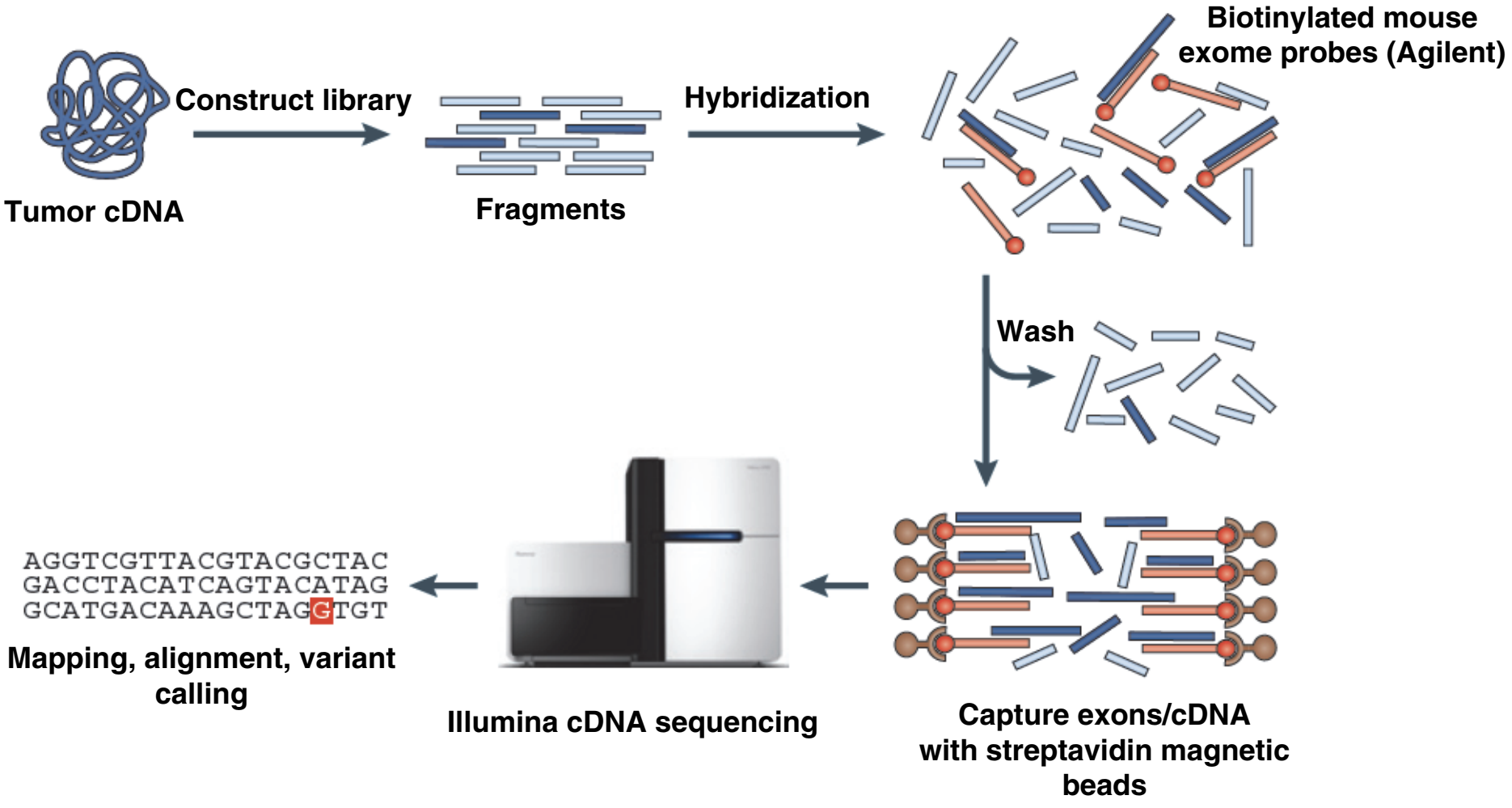
Lymphocytes (CD4 and CD8), IFNα/β responsiveness (host), and IFNγ responsiveness (tumor and host cells)



Rationale for Using Exome Sequencing Approaches for Antigen Profiling of Tumors

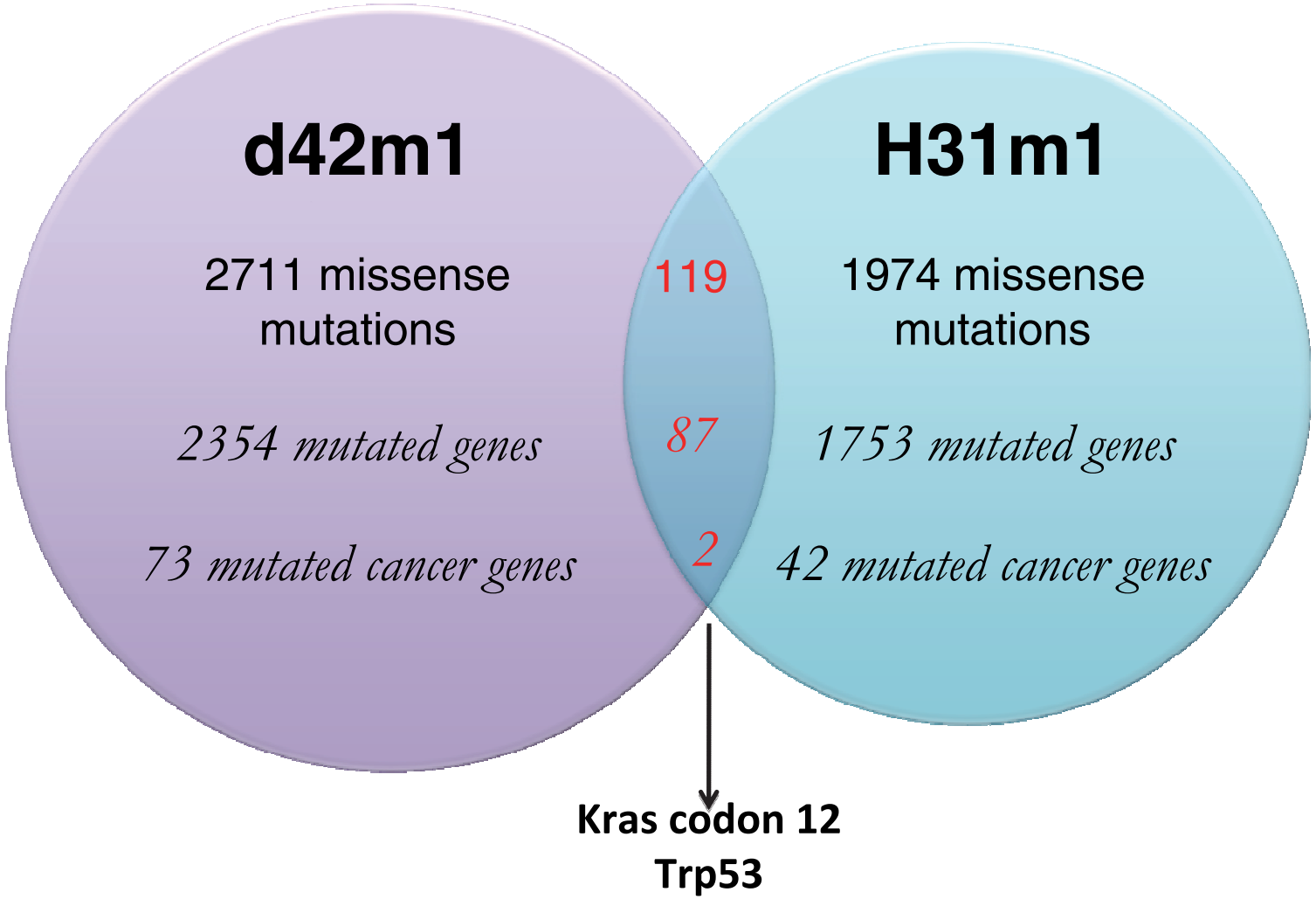
- Current methods of tumor antigen identification are work intensive and time consuming and are not suitable for “antigen profiling”.
- Genome sequencing of cancers reveals that all cancers express somatic mutations.
- Some tumors (melanoma, lung, colorectal) express a large number of mutations while others (AML) express only a few.
- Most studies of cancer genomes are focused on identifying novel driver mutations\signaling pathways that cause cancer.
- We wondered whether defining mutations in the tumor exome, when combined with *in silico* immunoepitope analysis could rapidly identify tumor neoantigens for $CD8^+$ T cells.

cDNA Capture Sequencing (cDNA CapSeq) Methodology and *Immuno*epitope Prediciton



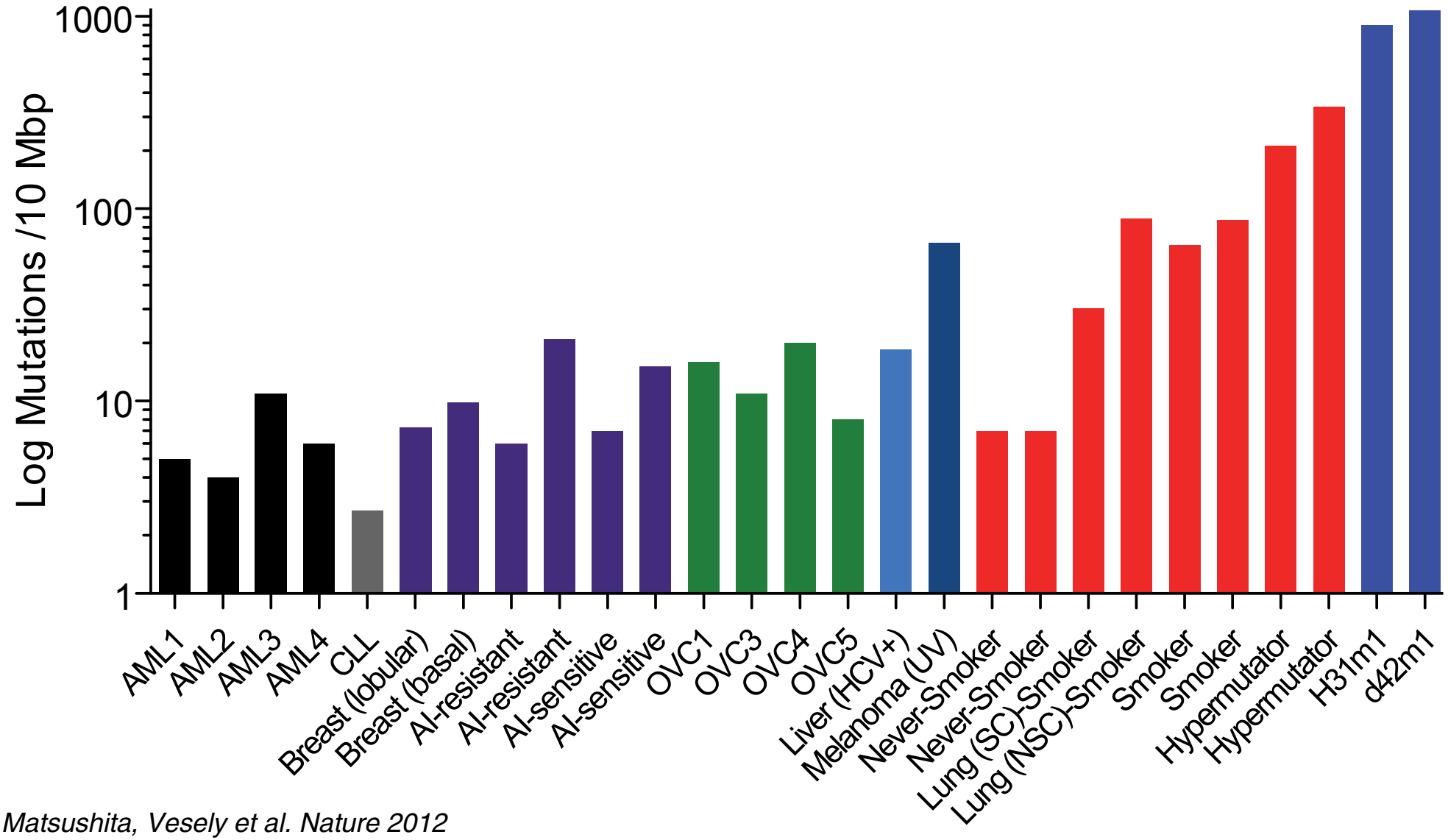
Bamshad et al. *Nat Rev Gen*. 2011

d42m1 and H31m1 MCA Sarcomas Display Limited Overlapping Missense Mutations *(20X Coverage only)*



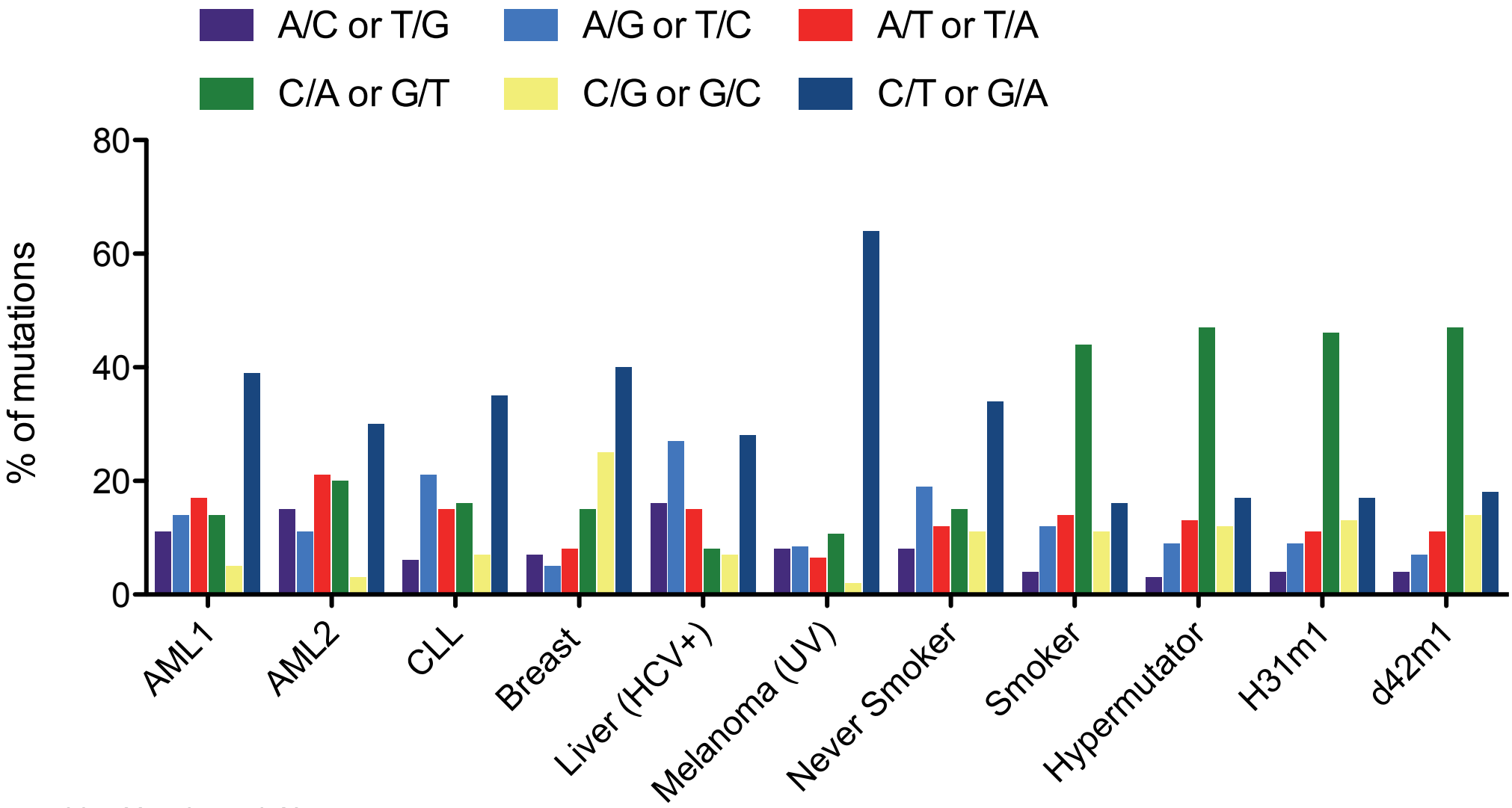
Matsushita, Vesely et al. Nature 2012

MCA Sarcomas Display Mutation Rates Similar to Carcinogen Induced Human Cancers



Matsushita, Vesely et al. Nature 2012

MCA Sarcomas Display Carcinogen Signatures Similar to Smoking-induced Lung Cancers

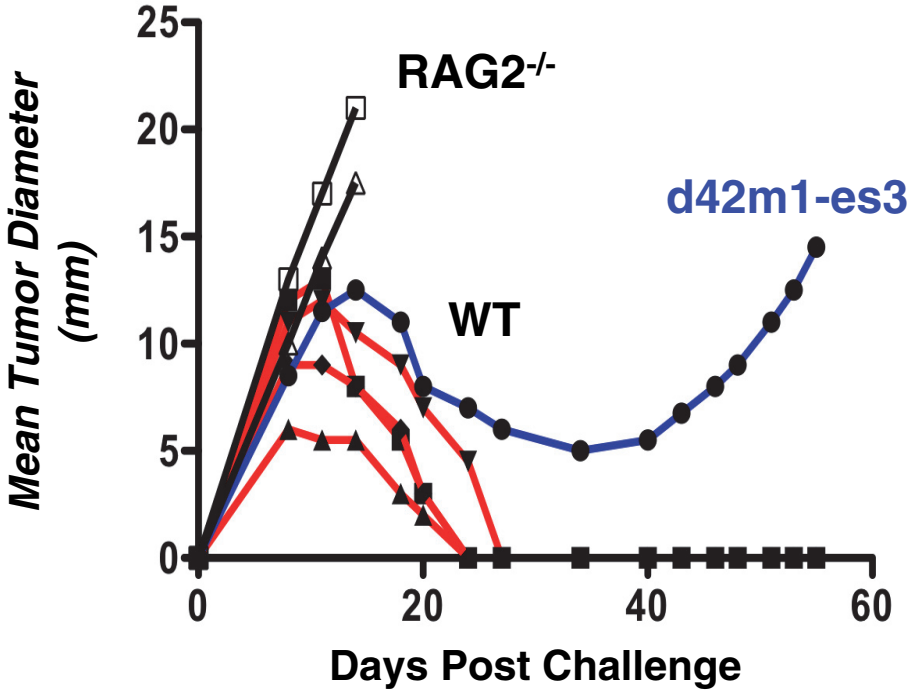


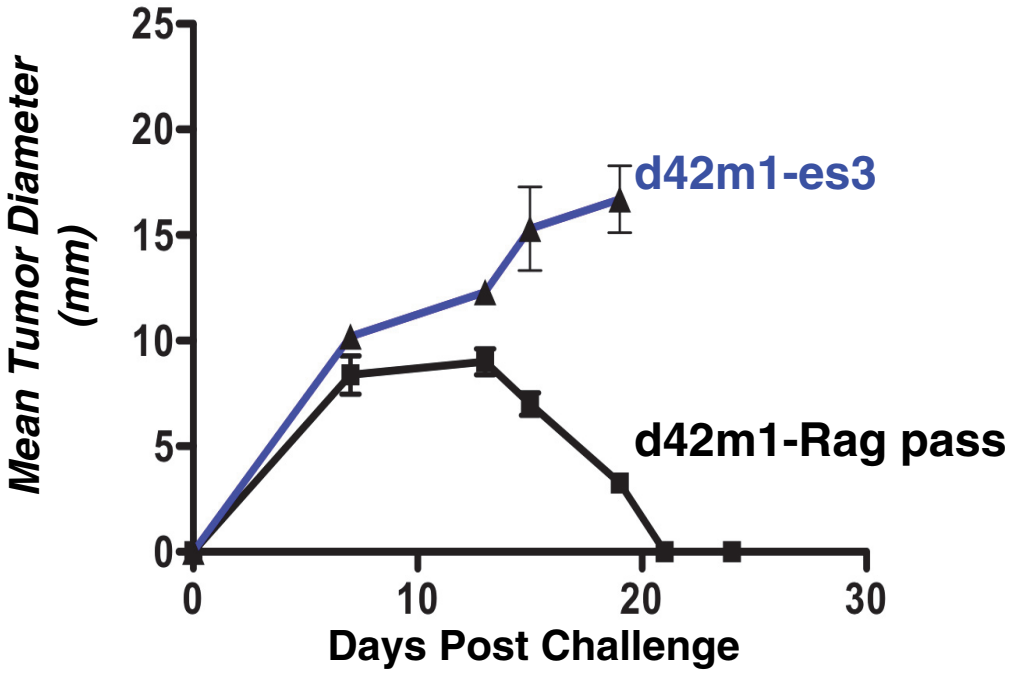
Matsushita, Vesely et al. Nature 2012

MCA sarcomas display nearly unique mutational patterns—a result that might explain their individualistic immunogenicities.

The MCA sarcoma model resembles human carcinogen induced cancers at the genomic level.

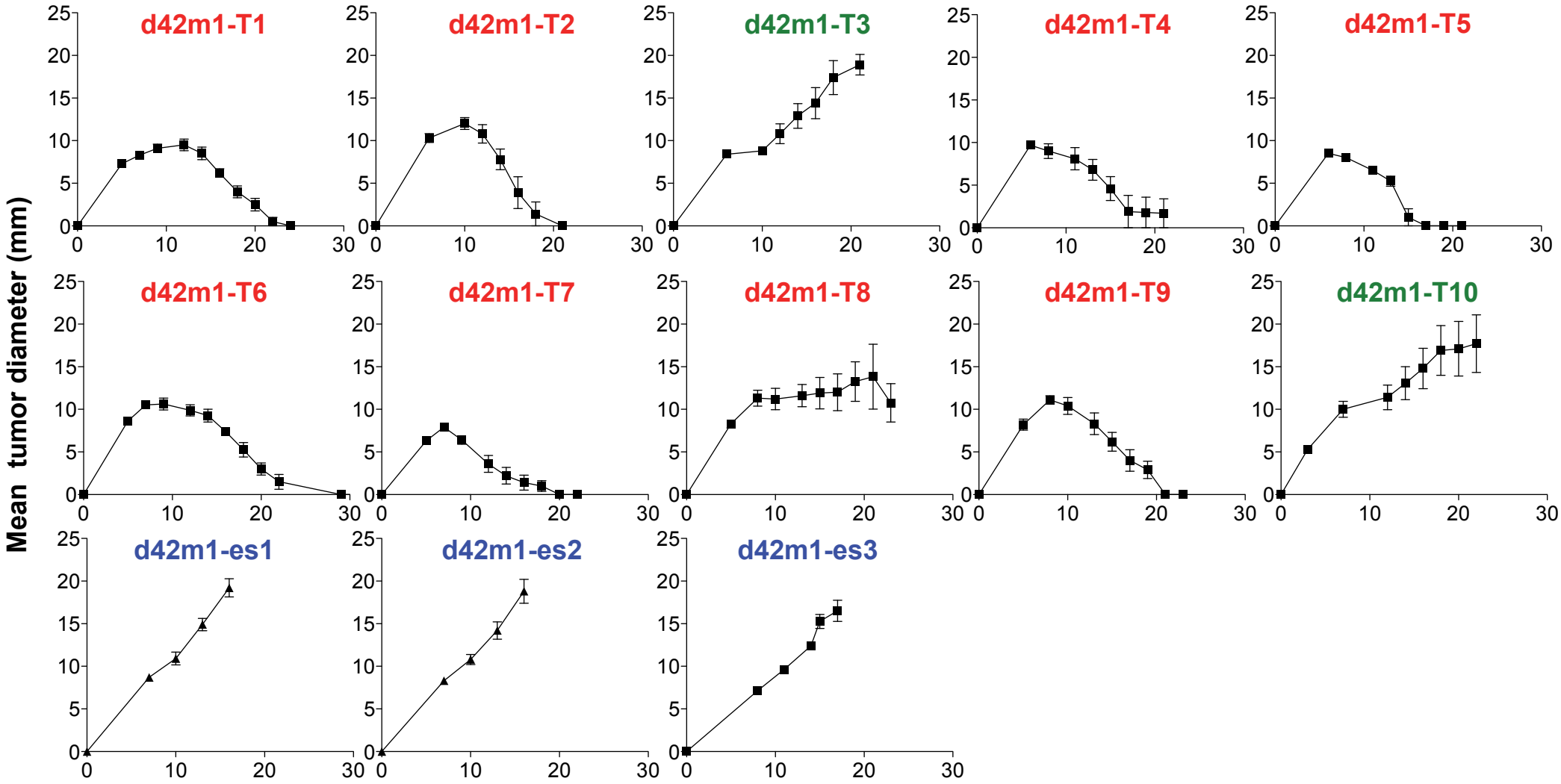
Passage of d42m1 through WT Mice Produces Escape Tumors With Lower Immunogenicity





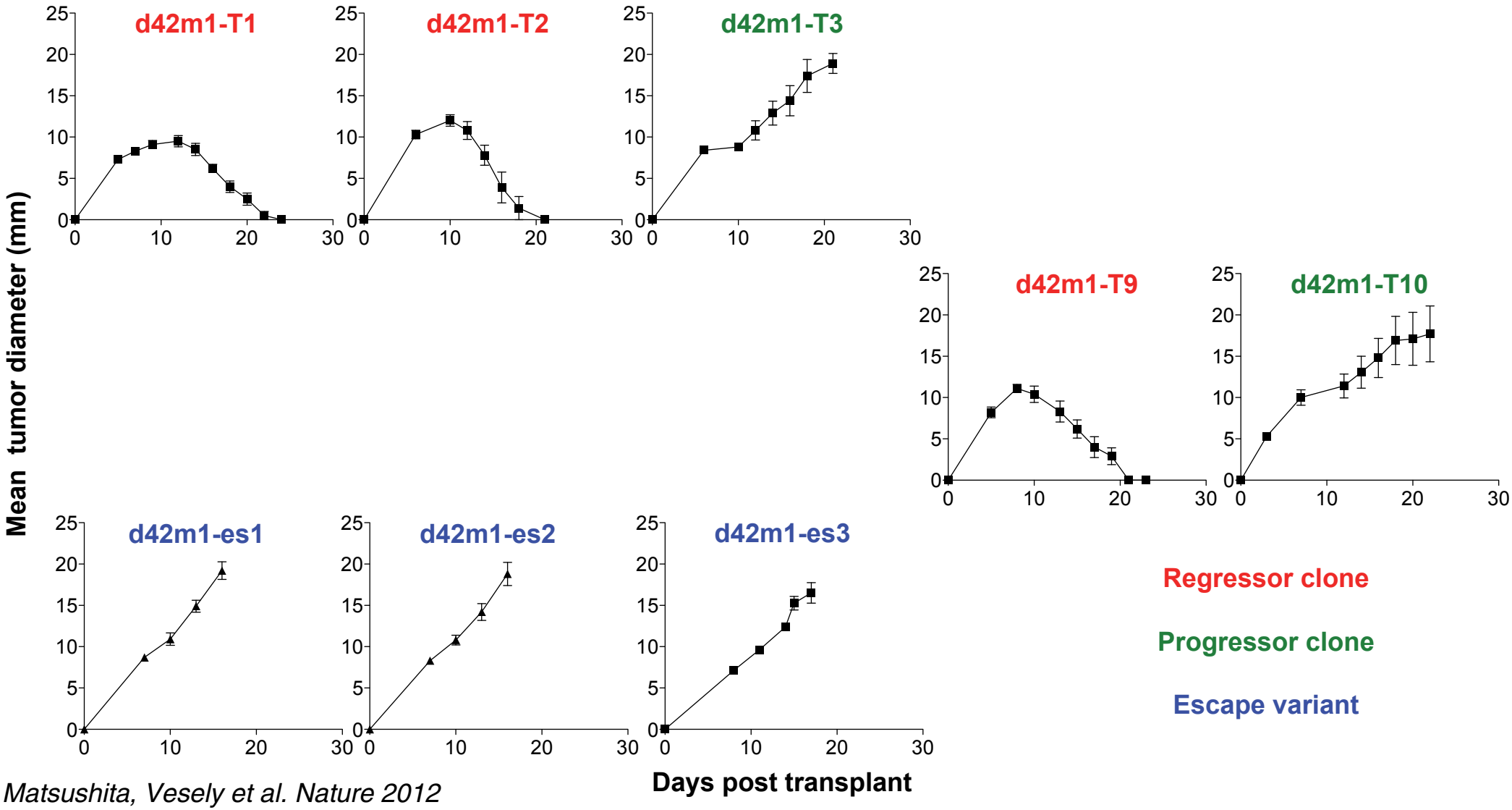
Matsushita, Vesely et al. Nature 2012

d42m1 is a Heterogeneous Tumor Composed of Highly and Poorly Immunogenic Tumor Clones



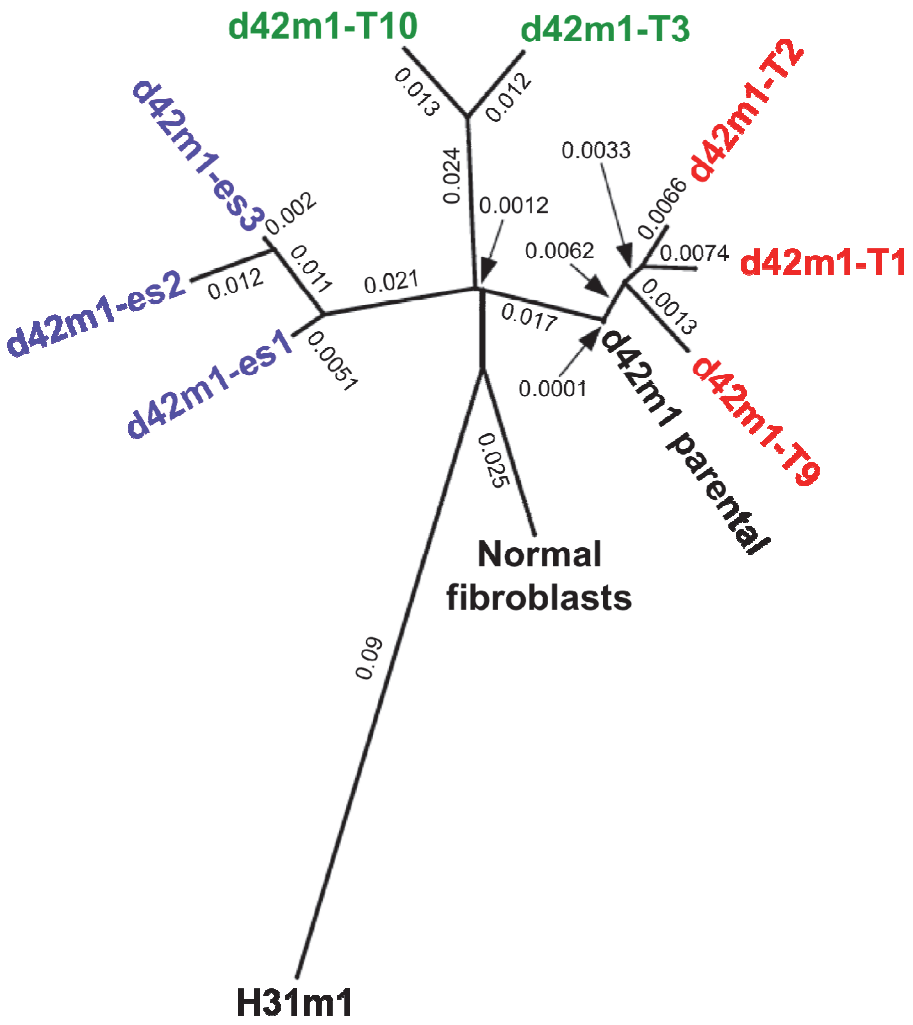
Matsushita, Vesely et al. Nature 2012

d42m1 is a Heterogeneous Tumor Composed of Highly and Poorly Immunogenic Tumor Clones

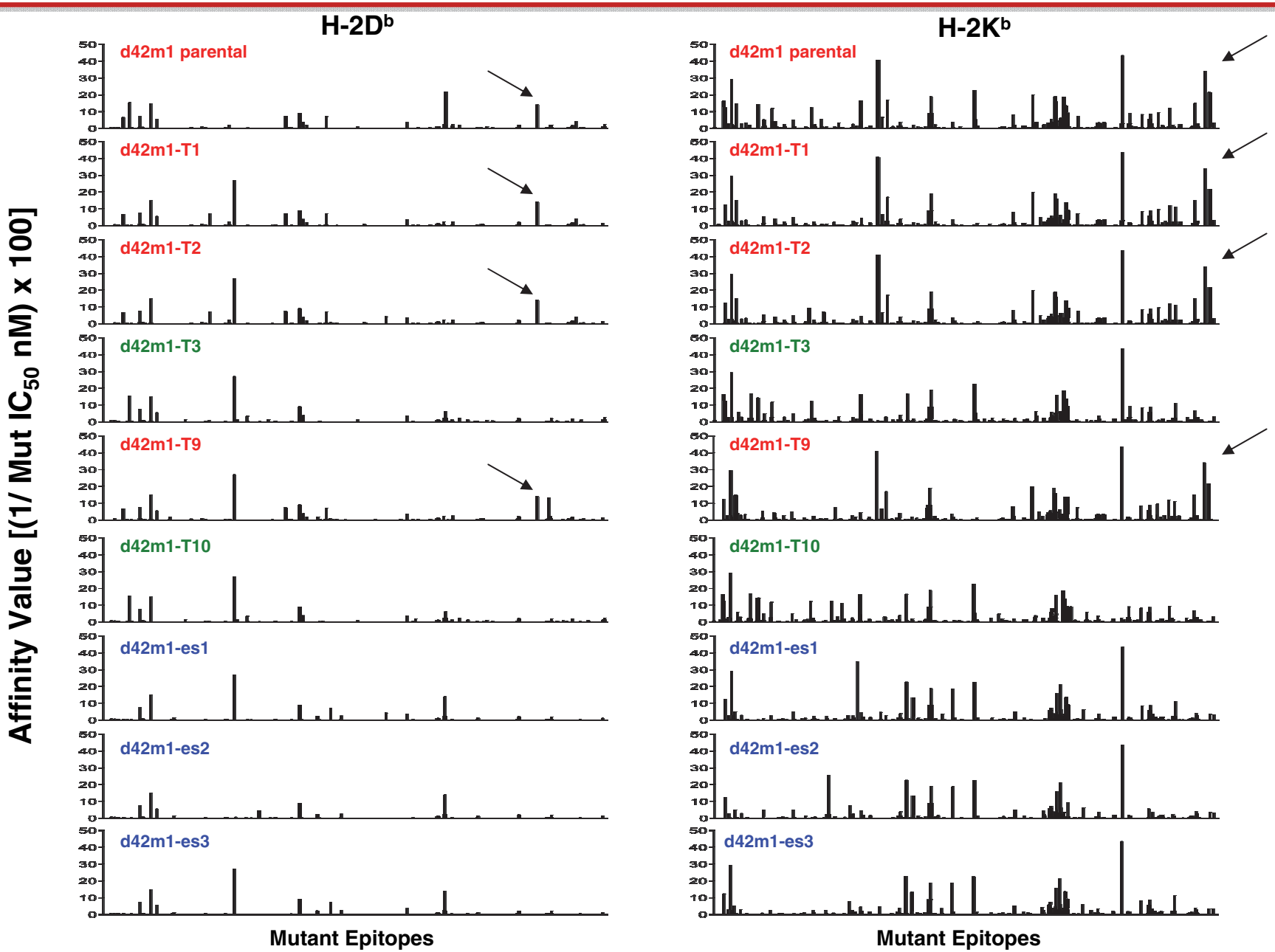


Matsushita, Vesely et al. Nature 2012

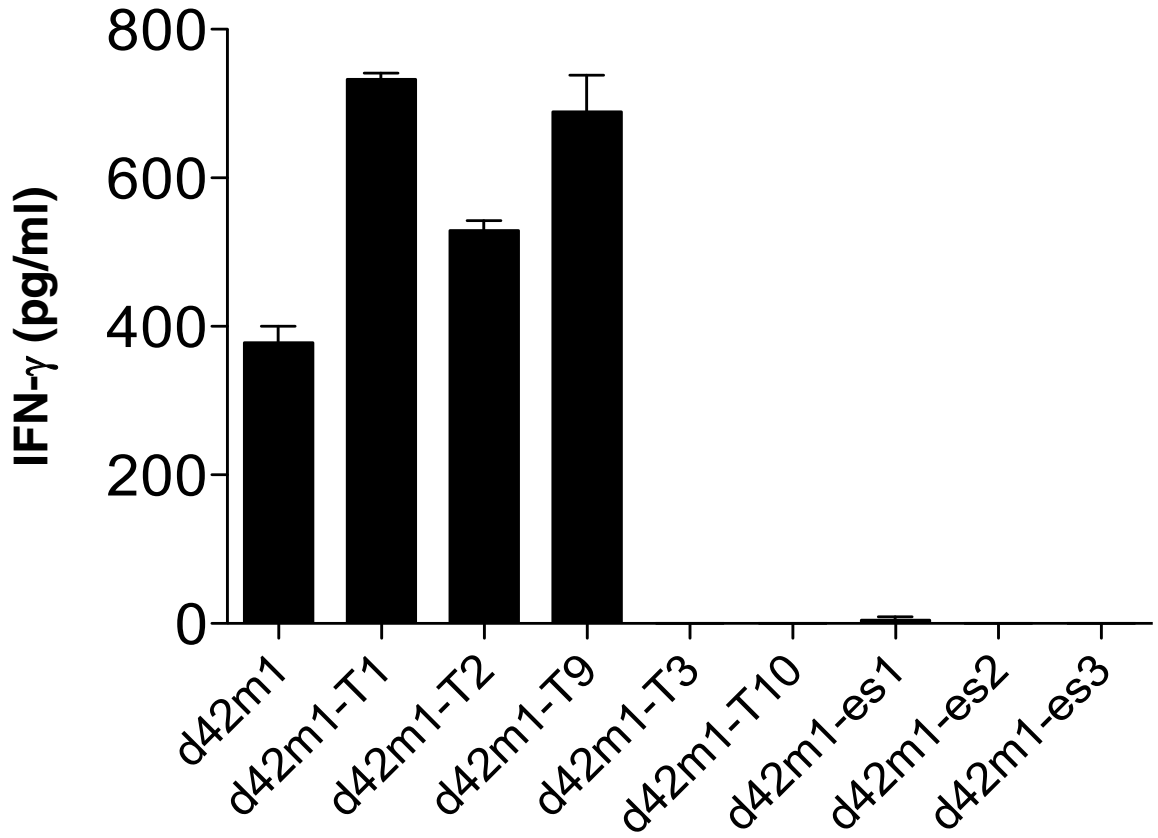
Relatedness Between d42m1 Tumor Cell Derivatives and d42m1 Parental Cells But Not to H31m1 Cells



Predicted Class I Epitopes Based on Expressed Mutations

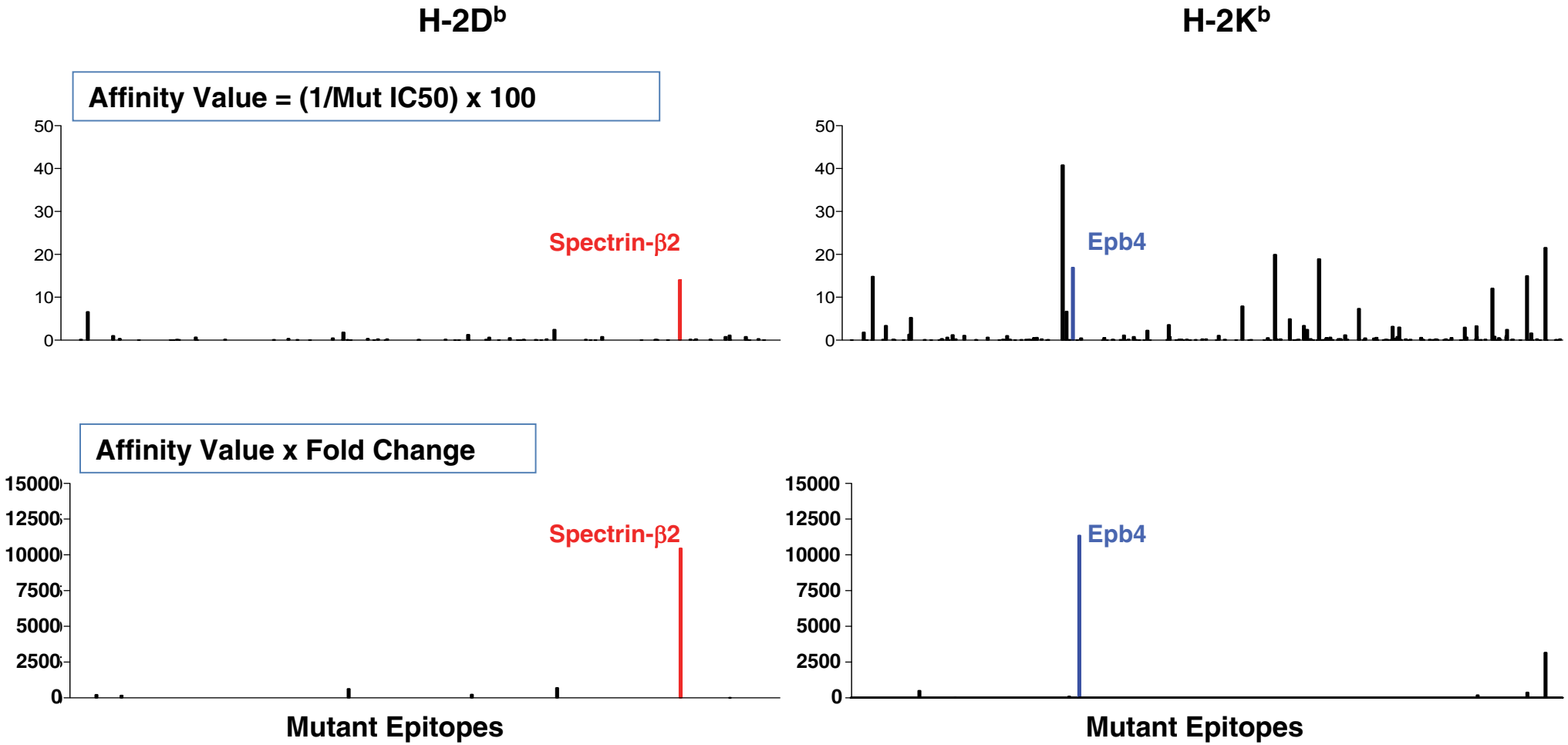


The C3 d42m1-Specific CTL Clone Recognizes d42m1 Regressors But Not d42m1 Progressors



Epitopes Present in All d42m1 Regressor Cells But Not in Any d42m1 Progressor Cells

Regressor = d42m1 parental, -T1, -T2, and -T9
Progressor = d42m1-T3, -T10, -es1, -es2, and -es3



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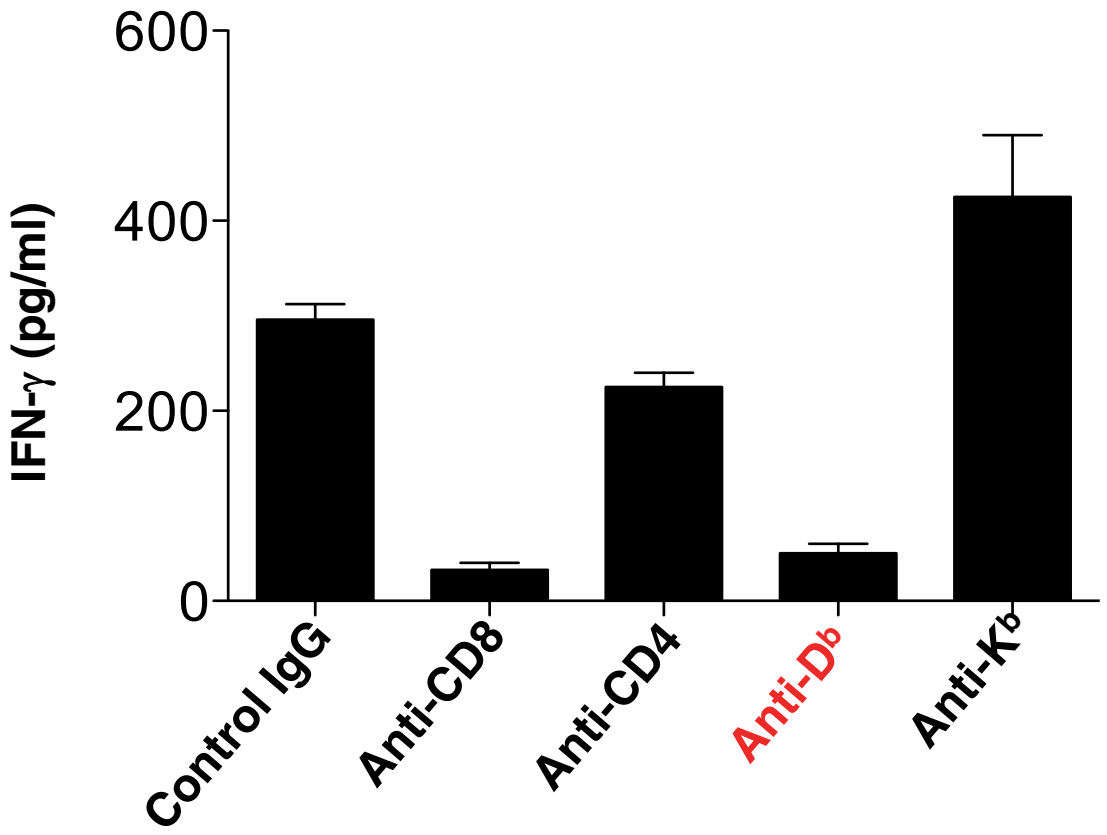
The C3 d42m1-Specific CTL Clone Recognizes Regressor Clones and Shows H2-D^b Restriction



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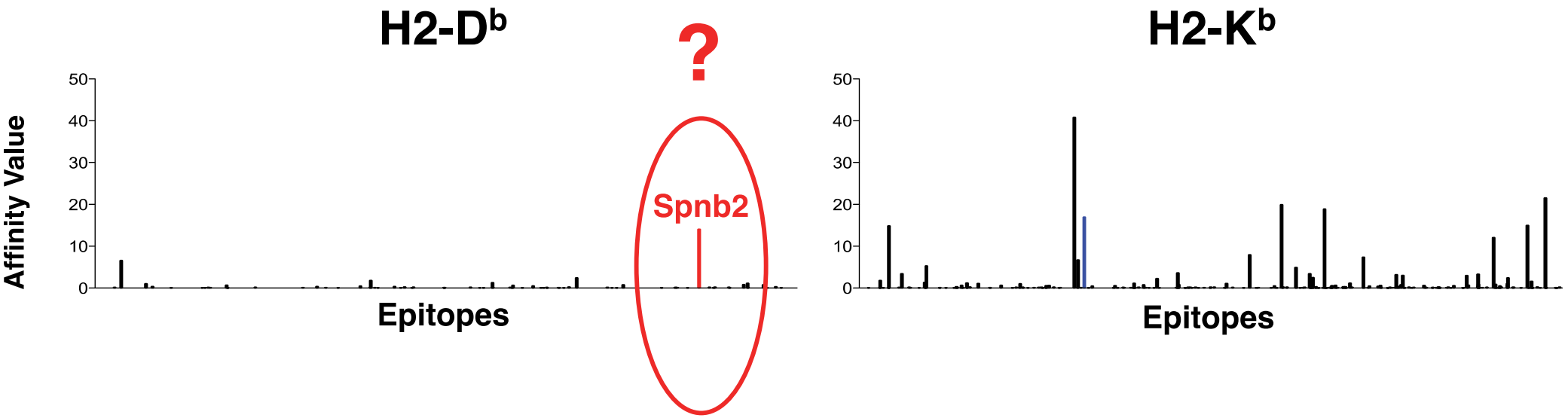
HYPOTHESIS

Mutant ($R_{913}L$) Spectrin-β2 is the major rejection antigen of the d42m1 MCA Sarcoma



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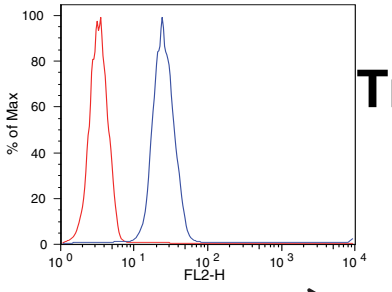
cDNA Expression Cloning of d42m1 Antigens

d42m1 cDNA library
100-400 colonies/pool
(120,000-160,000 colonies)
plasmids extraction

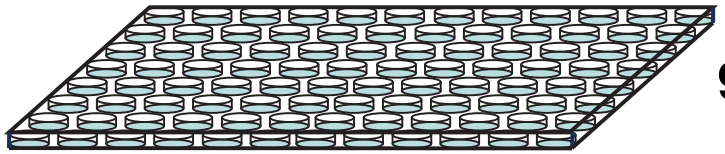


H-2D^b

Transfection into
COS-D^b cells

48h

d42m1 specific, H-2D^b restricted
T cell clone (C3)

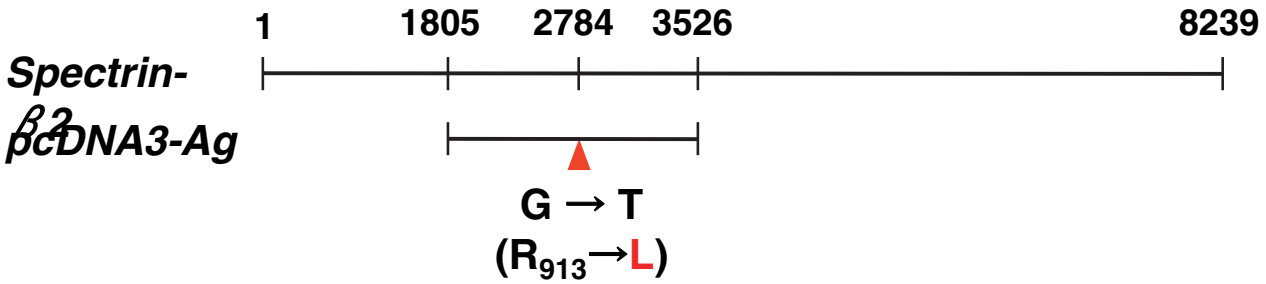


96-well plate

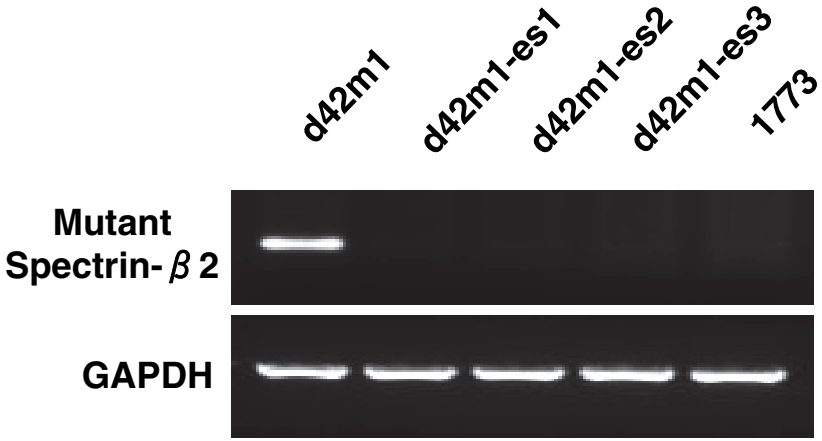
Overnight

IFN-γ ELISA or Killing of ^{51}Cr-labeled d42m1 cells

The Immunodominant D^b-Restricted d42m1 Tumor Rejection Antigen is a Mutated Form of Spectrin-β2*



	Peptide	#Score ($t_{½}$)	*IC_{50} (nM)
Spectrin-β 2	VAVVNQIAR (wild type)	8	5305
	VAVVNQIAL (mutated)	2177	7

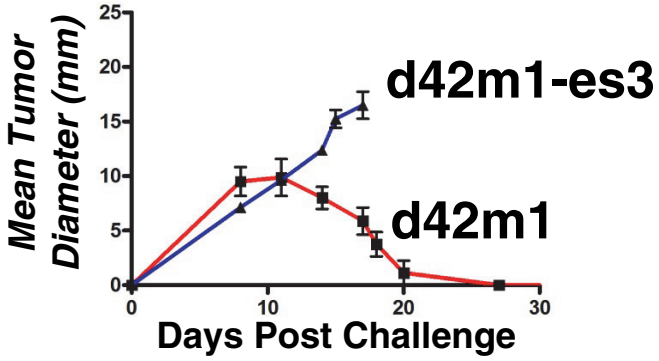


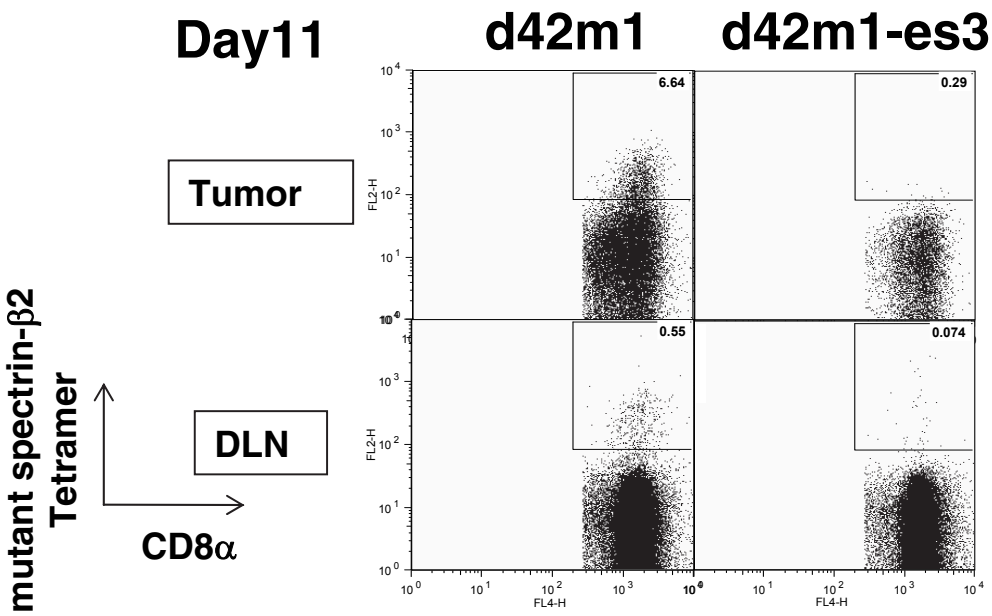
#BIMAS (BioInfomatics and Molecular Analysis Section)
*Immunoepitope Database

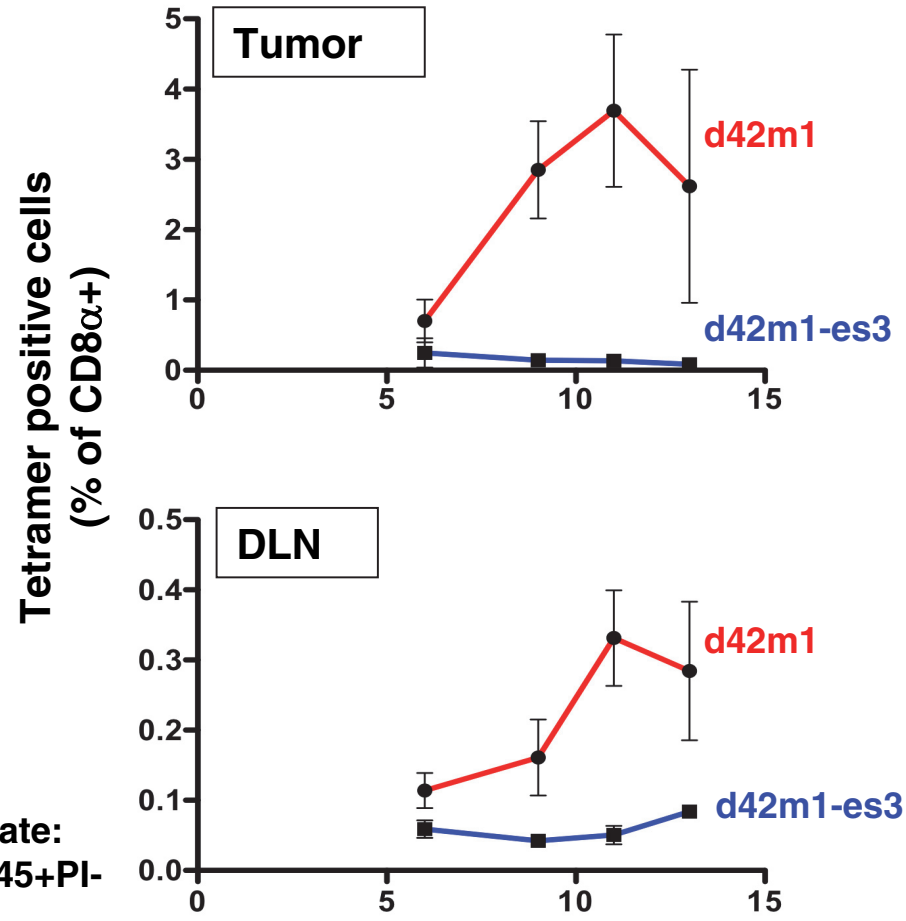
**** Mutant proteins are not found in normal cells from same mouse***

Matsushita, Vesely et al. Nature 2012

Detection of Mutant Spectrin-β2 Tetramer+ T Cells in Mice Rejecting d42m1

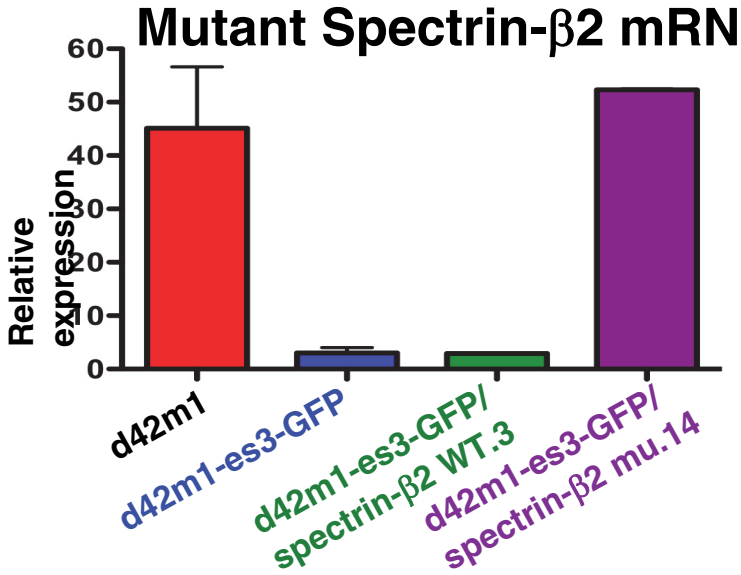


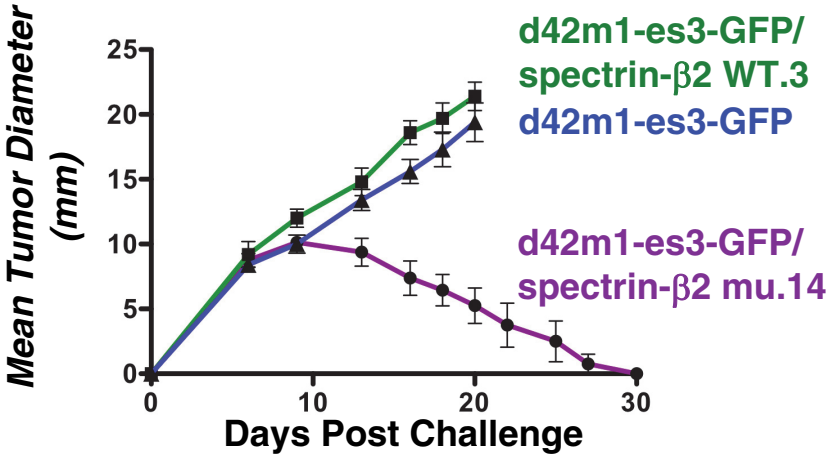


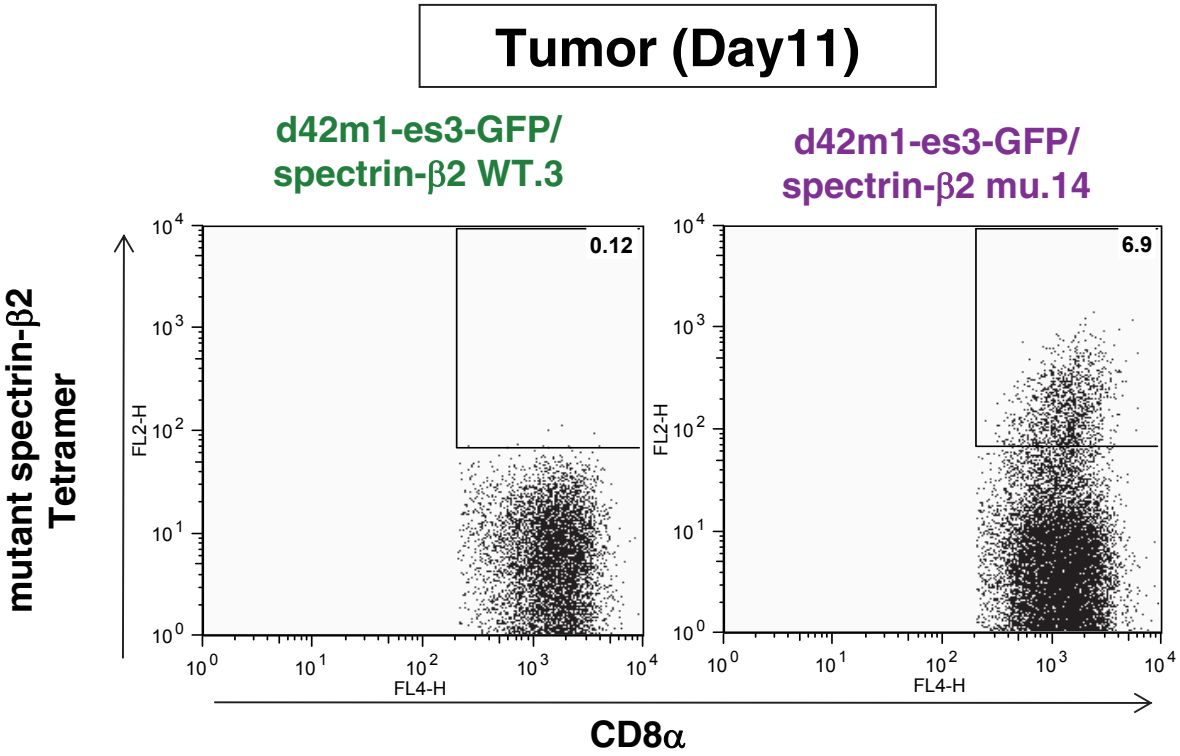


Matsushita, Vesely et al. Nature 2012

Enforced Expression of Mutant Spectrin-β2 in d42m1 Escape Tumor Cells Results in Their Rejection







Gate: CD45+PI-

Matsushita, Vesely et al. Nature 2012

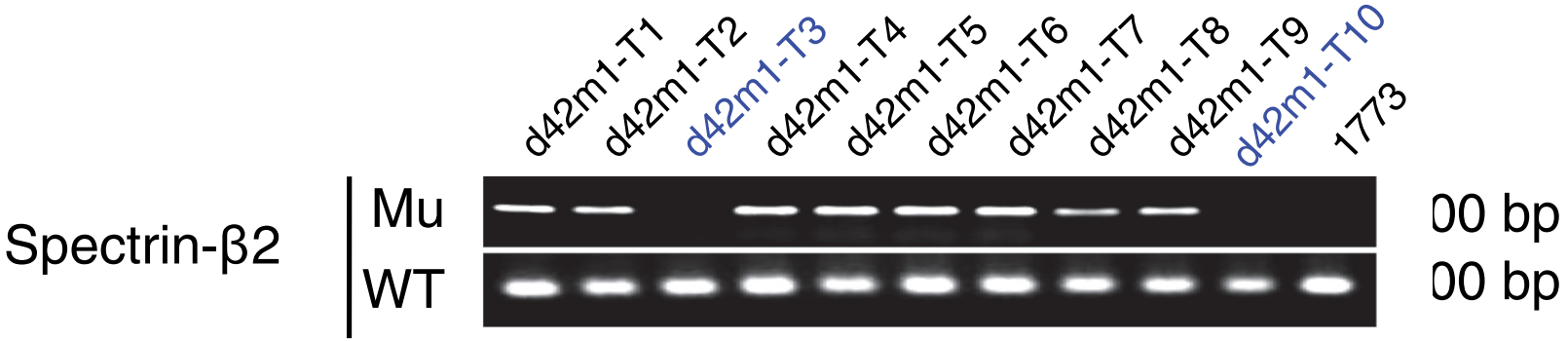
CONCLUSIONS

Mutant $R_{913}L$-Spectrin-β2 is a physiologically relevant, major rejection antigen of the d42m1 MCA Sarcoma.

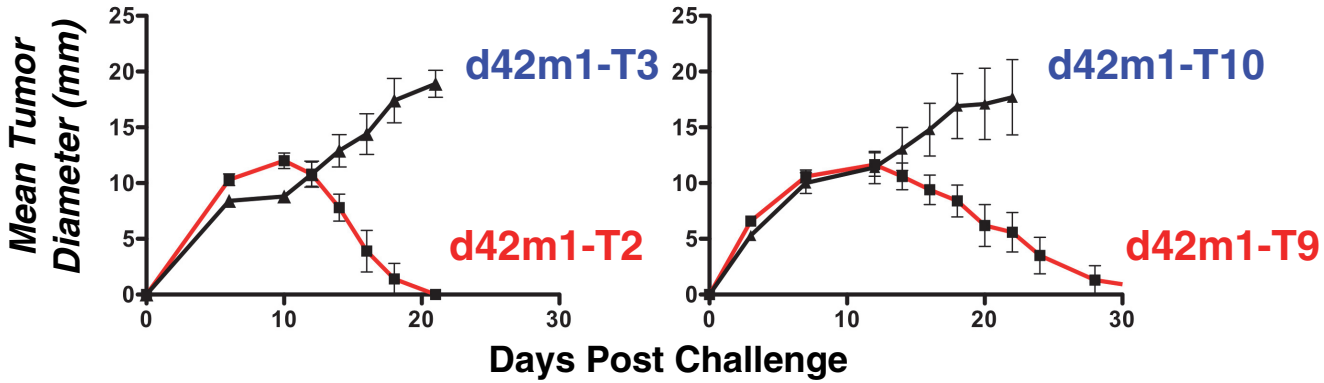
Therefore: Immunoediting can produce tumor cells that lack immunodominant tumor-specific rejection antigens.

Mutant Spectrin-β2 Expression is Heterogeneous in Parental d42m1 Tumor Cells

- Only 14/17 (82%) tumor cell clones express mutant spectrin-β2



- Tumor cell clones expressing mutant spectrin-β2 are rejected in WT mice
- Clones lacking mutant spectrin-β2 (d42m1-T3 and -T10) grow progressively



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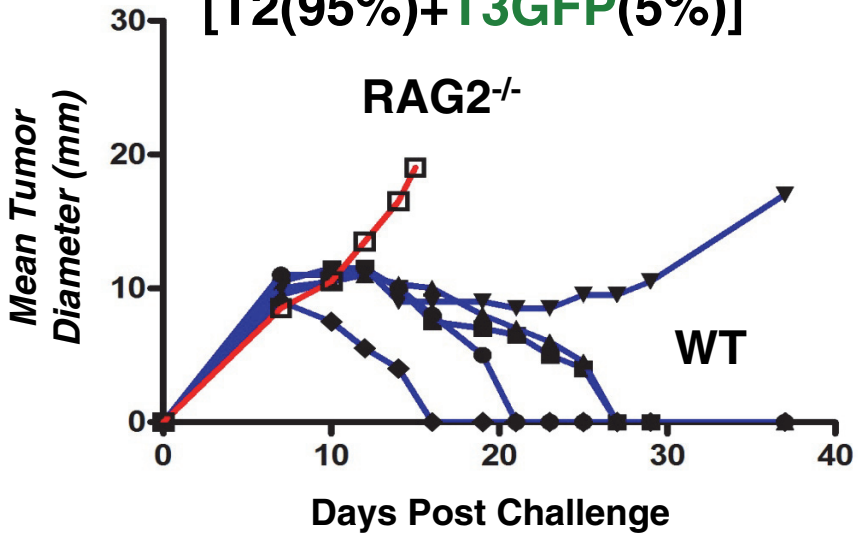
Immunoselection Produces d42m1 Clones That Do Not Express Mutant Spectrin-β2

in vivo growth

d42m1 mixture injected:

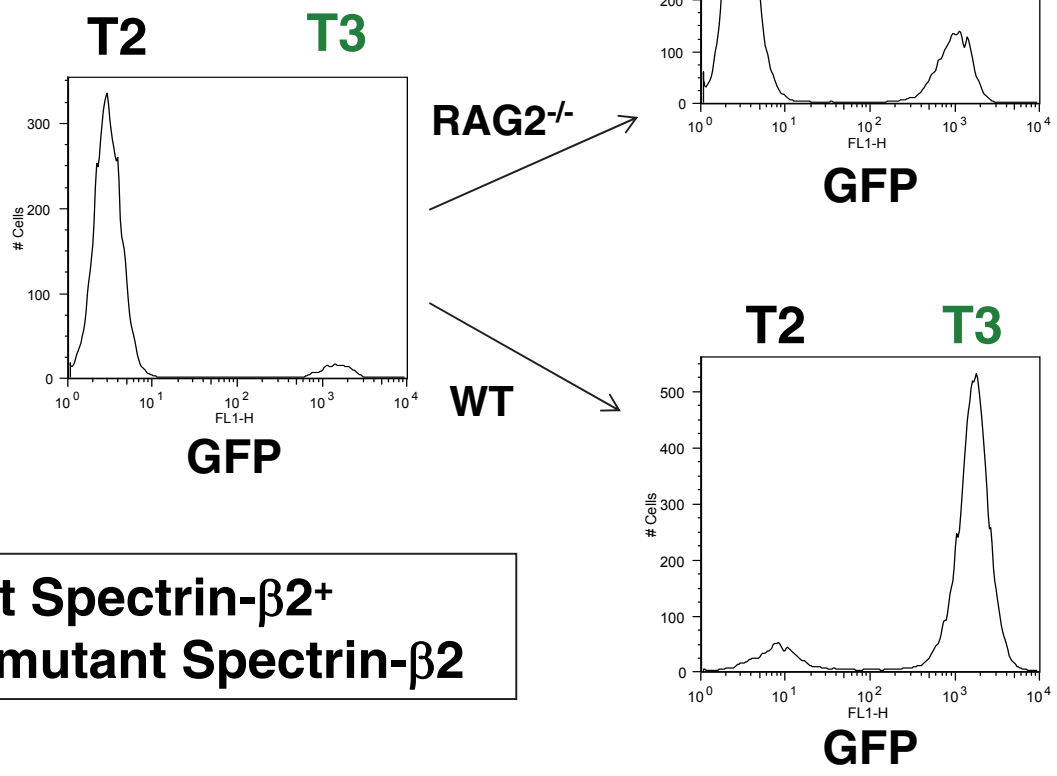
[T2(95%)+T3GFP(5%)]



FACS before injection

d42m1 mixture:

[T2(95%)+T3GFP(5%)]



T2: Mutant Spectrin-β2+
T3: Lacks mutant Spectrin-β2

Matsushita, Vesely et al. Nature 2012

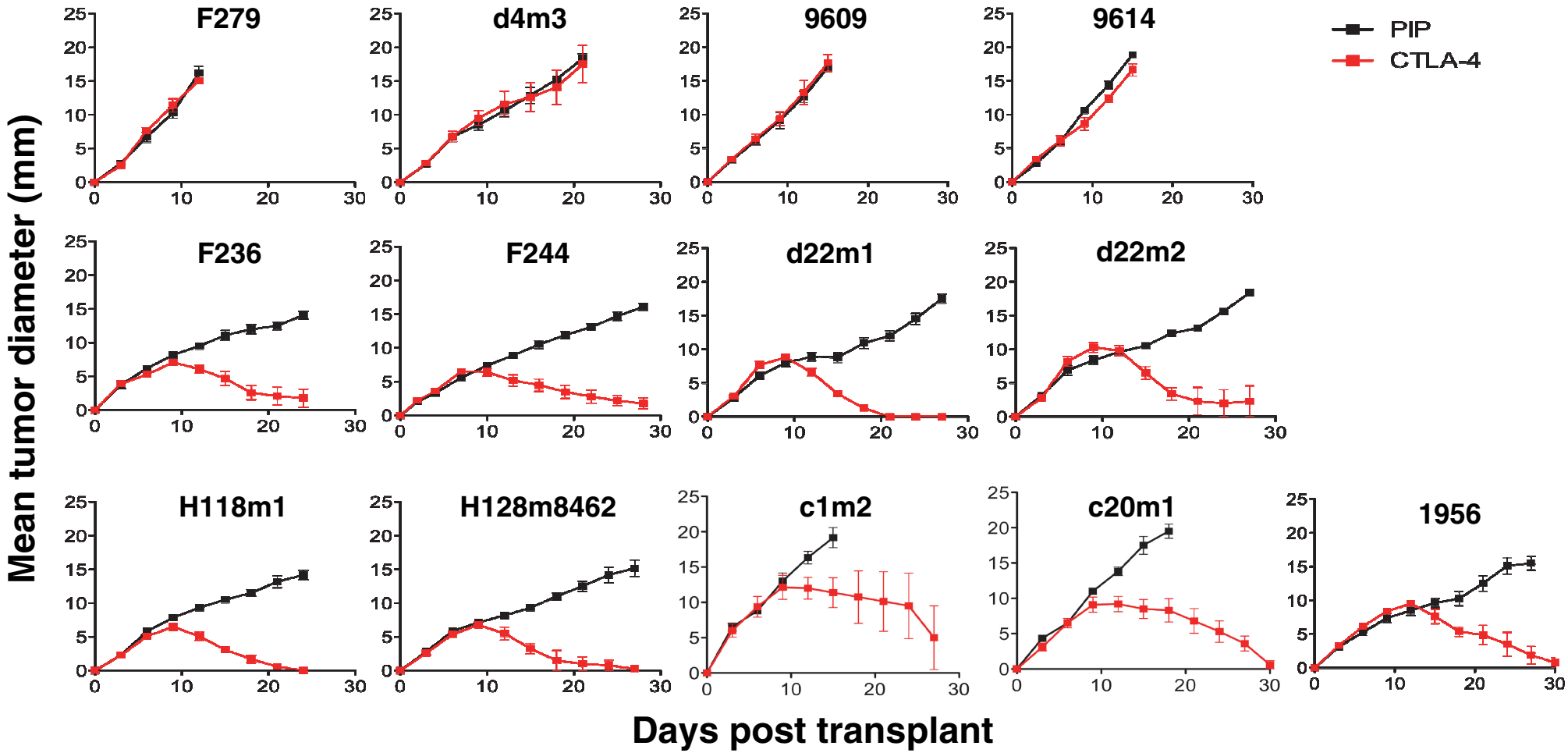
CONCLUSION

Cancer immunoediting of d42m1 is the result of a Darwinian immunoselection process acting on a tumor cell population that displays heterogeneous expression of strong antigens resulting in evolution of tumor cell variants lacking major rejection antigens that display reduced immunogenicity.*

**A similar selection process can also result in variants with other defects that compromise immunogenicity (e.g., MHC class I loss, defects in IFNγ receptor signaling) or that favor induction of immunosuppressive responses.*

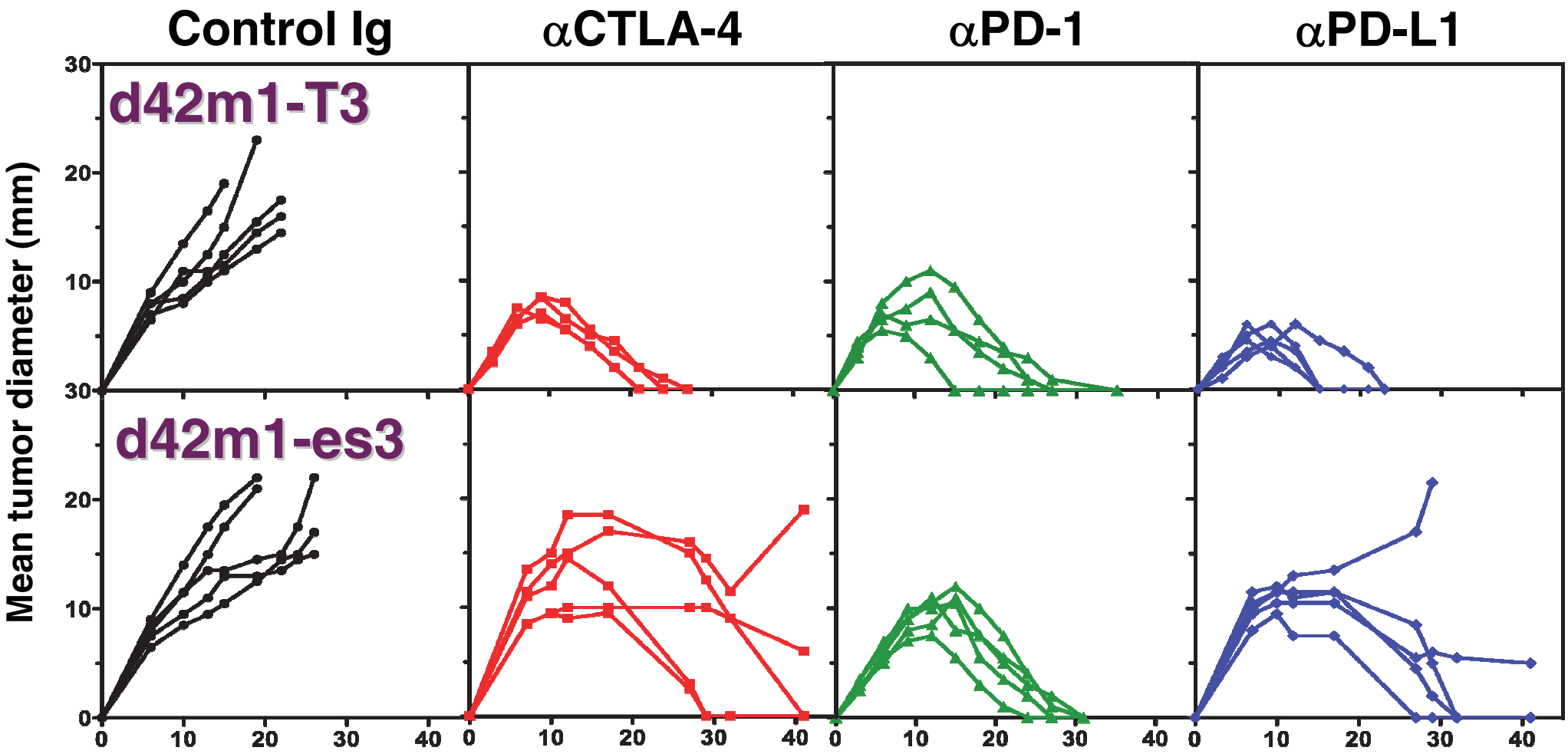
Is an edited tumor really non-immunogenic or does it express an immunogenicity that is just below the immunologic radar (i.e., can cancer immunotherapy effectively control an edited tumor)?

Edited MCA Sarcomas From WT Mice Show Varied Responses to Checkpoint Blockade Immunotherapy



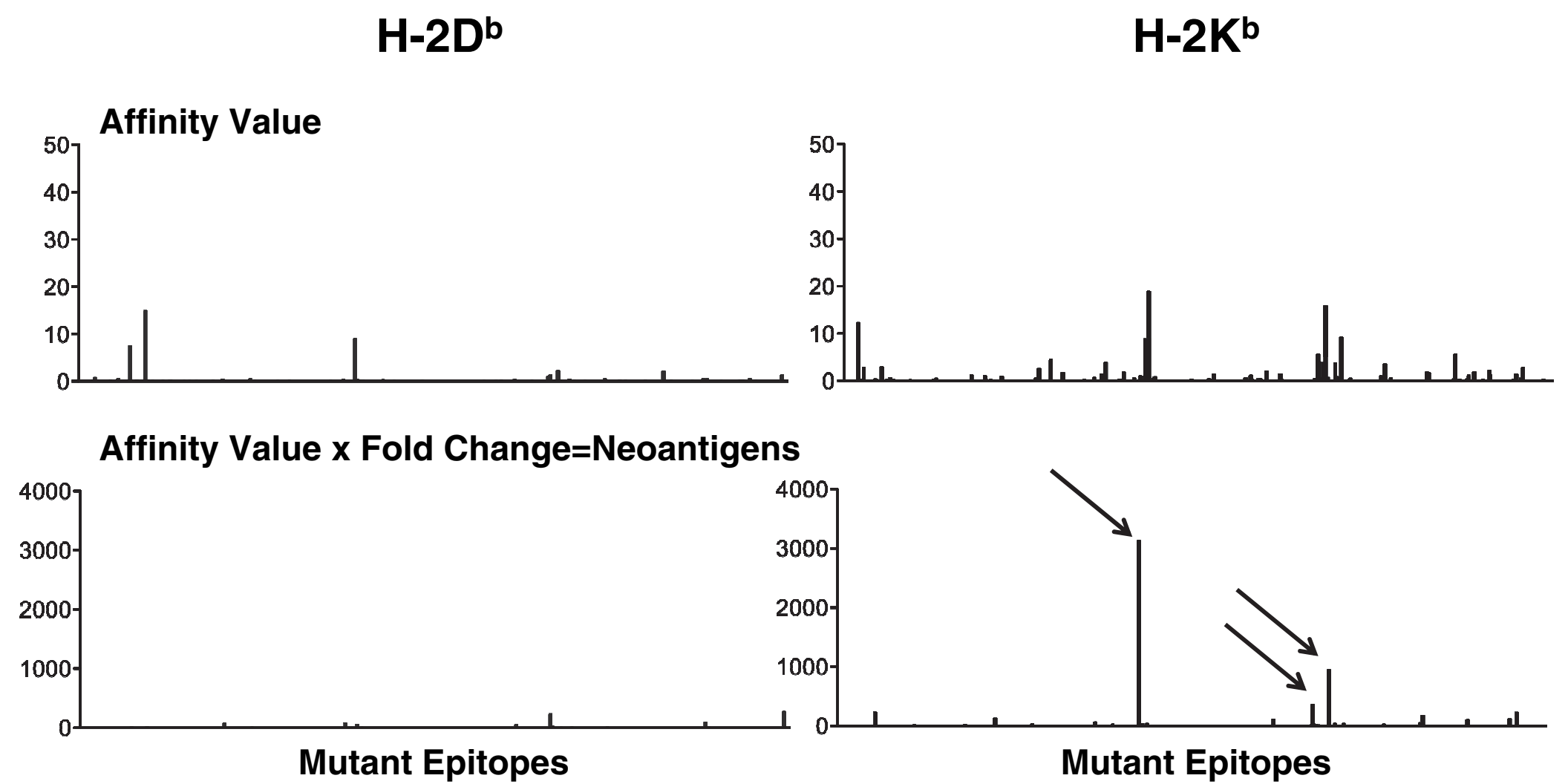
9/13 (70%) WT sarcomas respond to anti-CTLA-4 therapy

Progressor Derivatives of d42m1 Respond to Checkpoint Blockade Immunotherapy

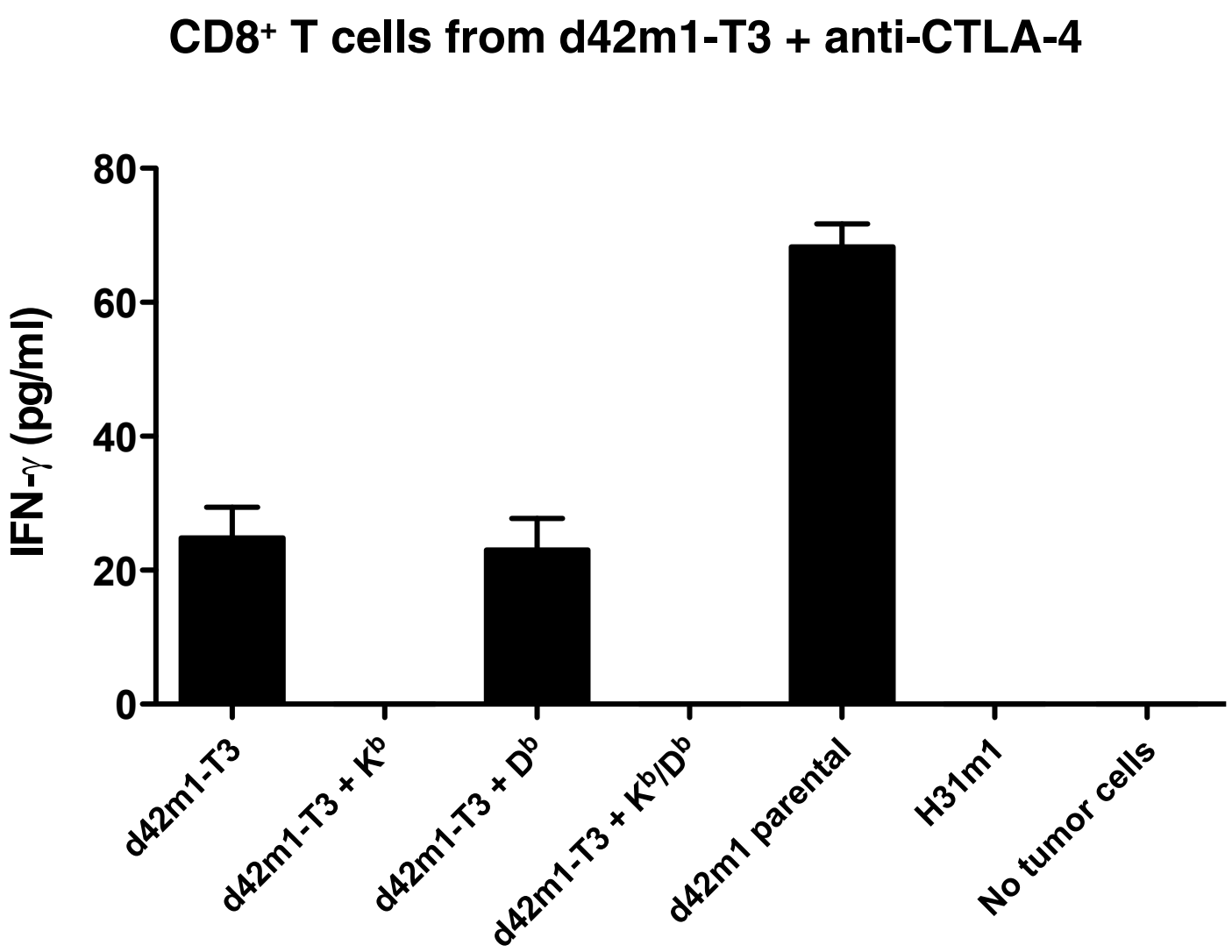


Similar results for d42m1-T10 and for ES-1 and ES-2

Shared Epitopes Among All Spectrin β2⁻ d42m1 Tumor Variants: Common Antigens



H-2 K^b Restriction of $CD8^+$ T Cells Raised to Edited Spectrin $\beta 2^-$ d42m1-T3 After anti-CTLA-4 Therapy



On-Going Research

- Which (if any) of the mutations in Spectrin β2^{-} d42m1 are the antigenic targets of anti-CTLA-4 therapy?
- Are the antigenic targets of Spectrin β2^{-} d42m1 tumors the same for other types of checkpoint blockade immunotherapies (e.g., anti-PD-1)?
- Can a genomics approach be used to identify T cell antigens in fully edited sarcomas from WT mice?
- Are tumor specific mutational antigens “better” cancer immunotherapy targets than other types of tumor antigens?

Using Cancer Genome Sequencing to Analyze Cancer Immunoediting and Guide Cancer Immunotherapy

- Identification of novel driver mutations that may be uniquely retained in unedited tumors.
- Longitudinal analysis of the extent of immunoediting that tumors undergo either naturally or as a consequence of therapy.
- Identification of the antigenic targets of Adoptive T cell Therapy (ACT) or Checkpoint Blockade Therapy.
- Identification of patients who may best benefit from cancer immunotherapy.
- Rapid identification of specific antigens expressed in tumors making possible individualized tumor immunotherapy.

ACKNOWLEDGEMENTS

Kazuhiro Matsushita | Matthew Vesely | Elaine Mardis | Mark Smyth | Lloyd Old

Samuel Lam
Ravi Uppaluri
Cora Arthur
Mike White
Jessica Archambault

Dan Koboldt
Vince Magrini
Ryan Demeter
Jasreet Hundal
Todd Wylie

James Alison
Alan Korman
Arlene Sharp
Gordon Freeman

Funding: *NCI; NHGRI; Cancer Research Institute; WWWW Foundation; Bristol Meyers Squibb*

Schreiber Lab Members Making Major Contributions to Our Understanding of Cancer Immunoediting

Kathleen Sheehan
Anand Dighe
Dan Kaplan
Vijay Shankaran
Hiroaki Ikeda
Allen Bruce
Gavin Dunn
S. Ruby Chan
Katherine Koebel
William Vermi
Ravi Uppaluri
Mark Diamond
Kazuhiro Matsushita
Matt Vesely
Charles Rickert
Sang Hun Lee
Su Kit Lam
Michelle Kinder
Matt Gubin
Alex Miceli