Harnessing NK cell functions in settings of infections and disease

Amir Horowitz, PhD
Assistant Professor
Department of Oncological Sciences
Precision Immunology Institute
Tisch Cancer Center
Icahn School of Medicine at Mount Sinai

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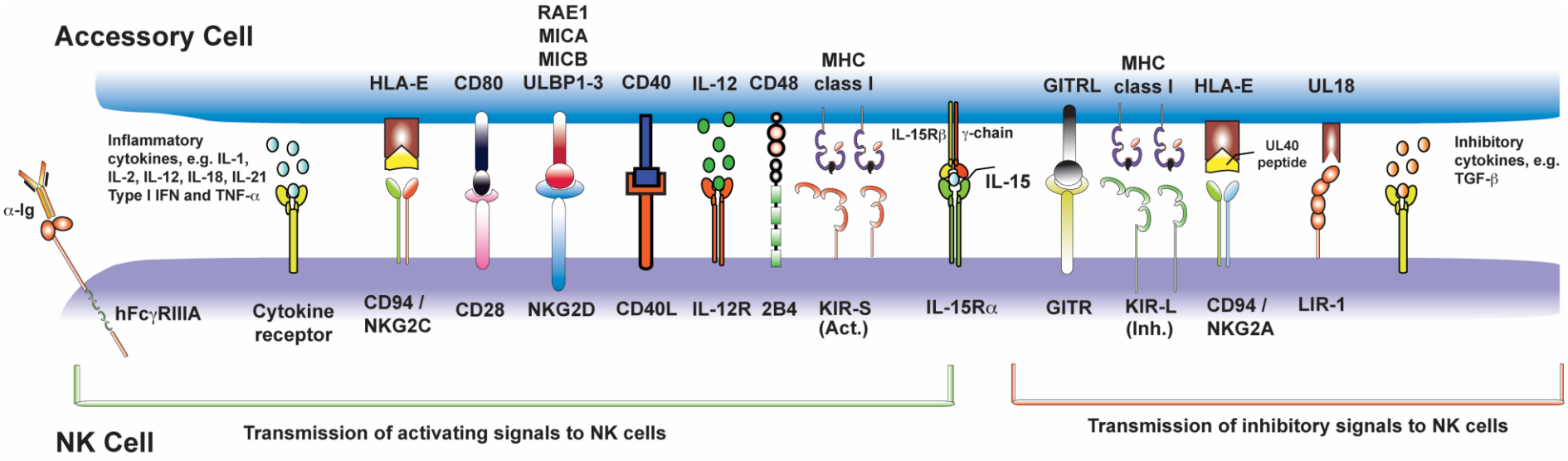
MetroFlow annual meeting

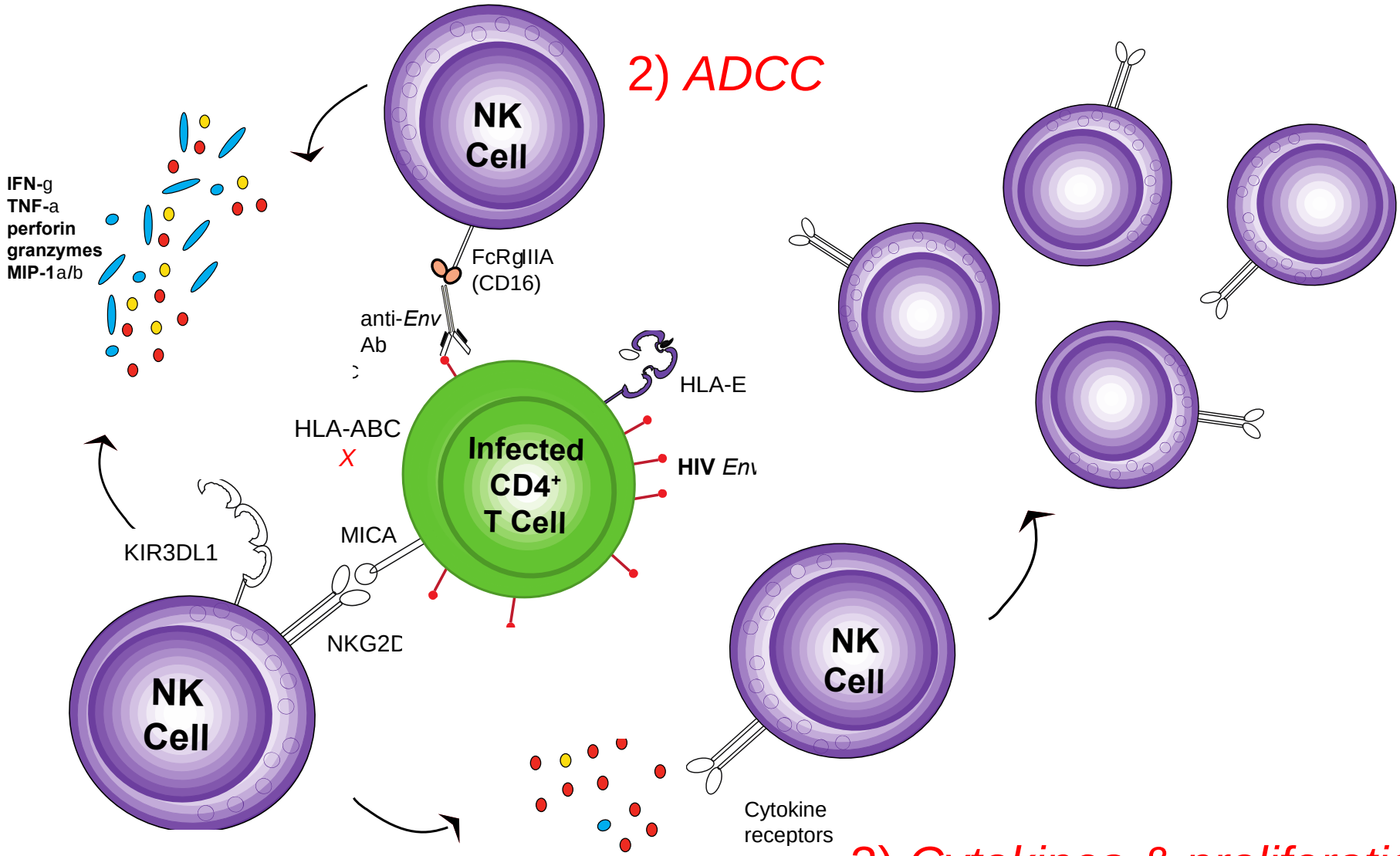
Mount Sinai

NK cell activation is regulated by the collective strength of inhibitory and activating signals

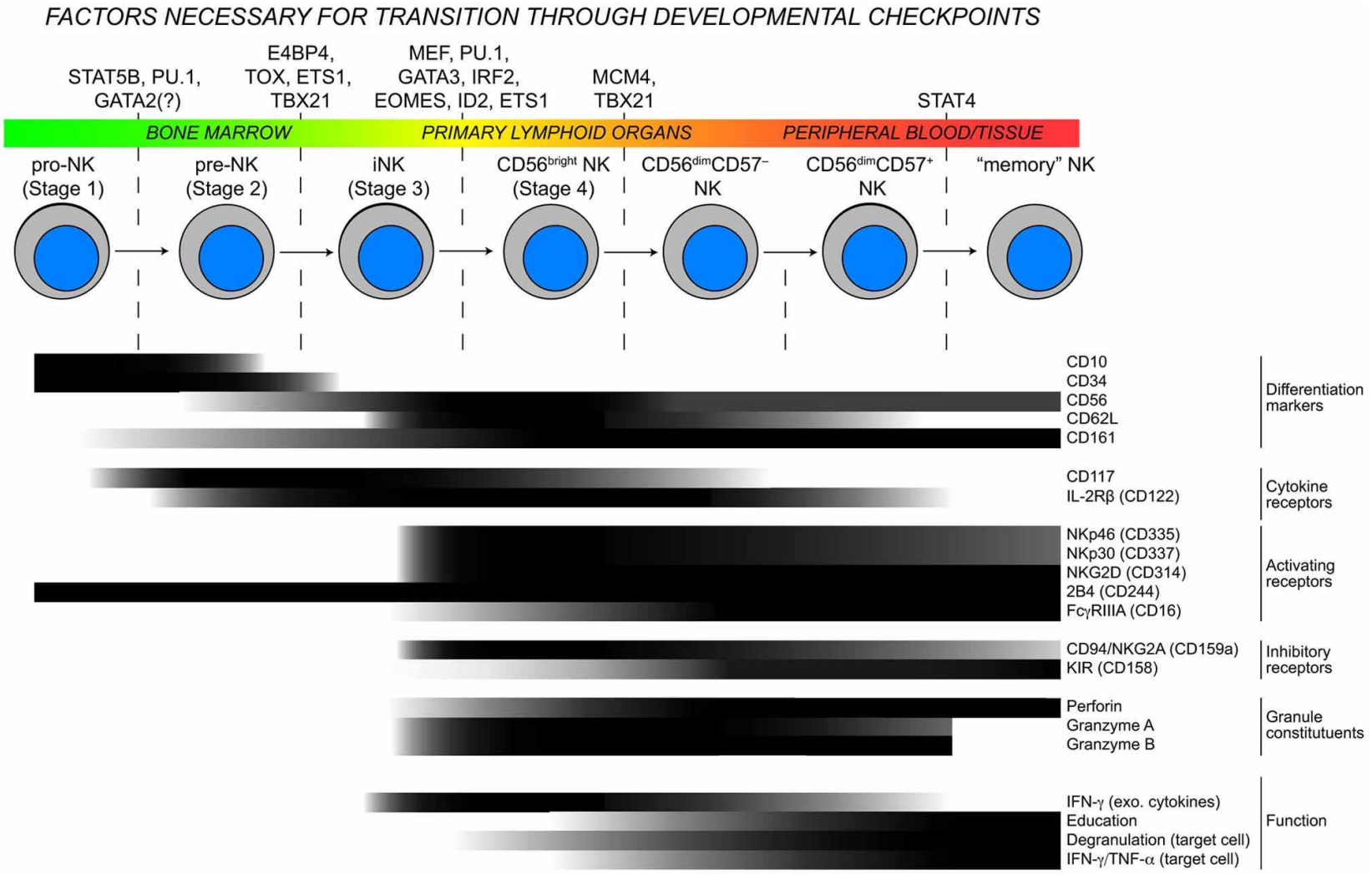


Horowitz, 2012 *Front Immunol*

NK cell functions are coordinated across specialized subsets - Example: Viral infection

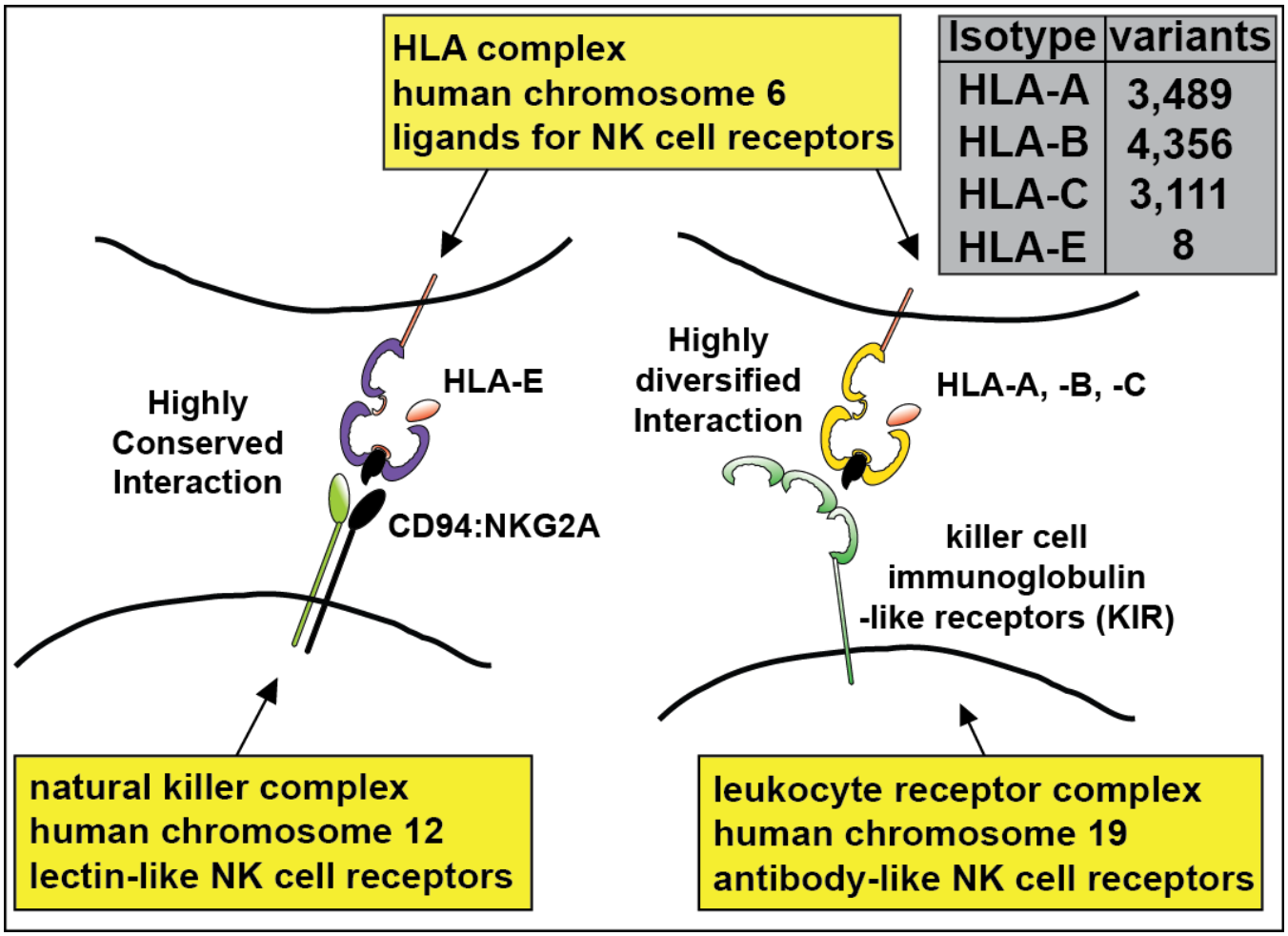


NK cell functions are acquired, regulated and differentiated as NK cells mature



Cichocki, 2013 *Front Immunol*

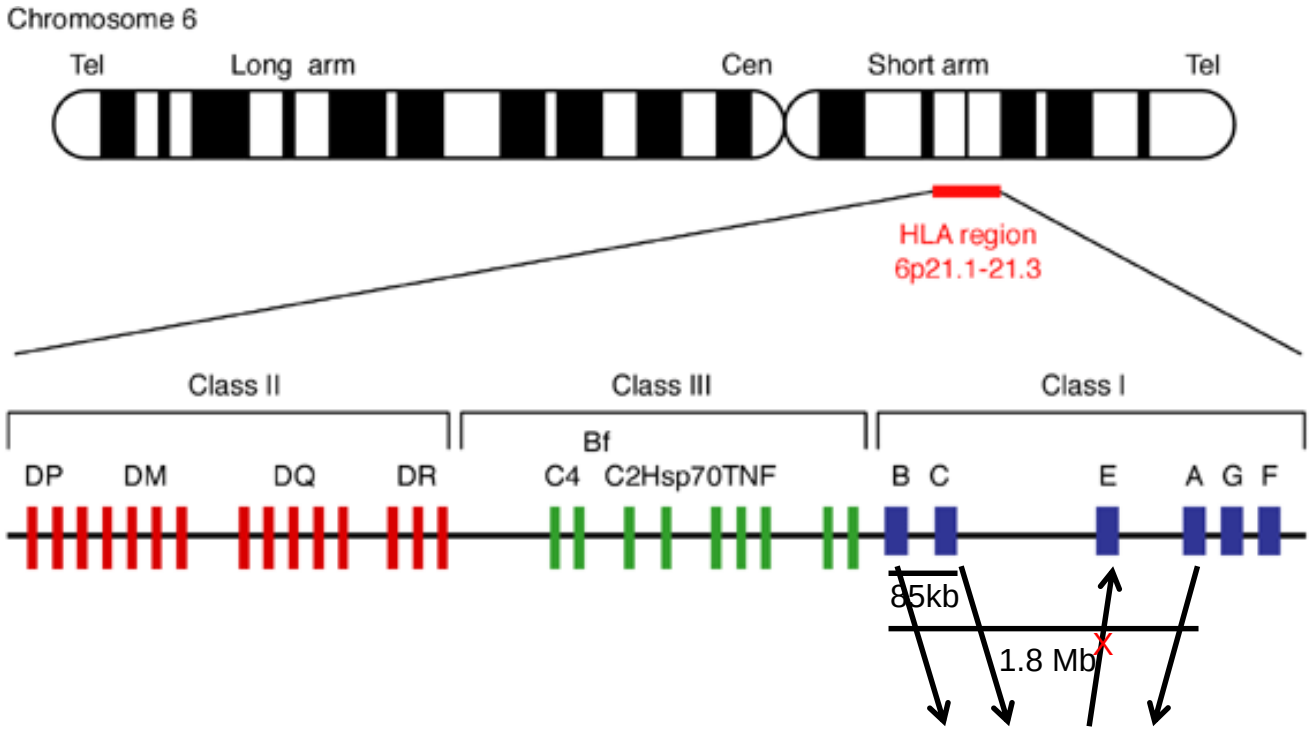
Education is regulated by a bipartite system of Immunogenetics



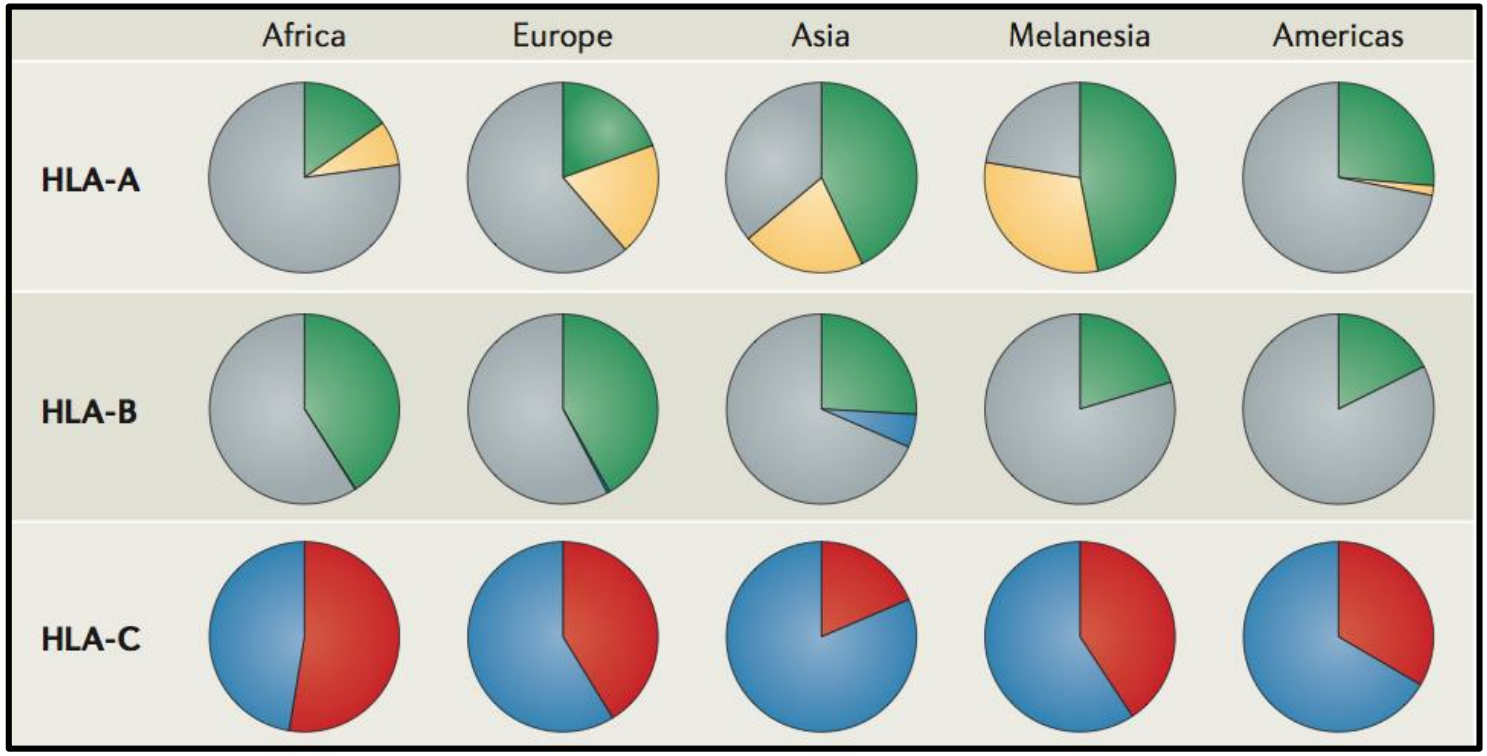
Adapted from: Parham, 2012 *Phil. Trans. R. Soc. B;* Horowitz, 2016, *Sci Immunol*.

Robinson, 2017 *PLoS Genetics*
(IPD: Up to date list of HLA alleles)

HLA-A, -B and –C contribute leader sequence-derived peptides to HLA-E

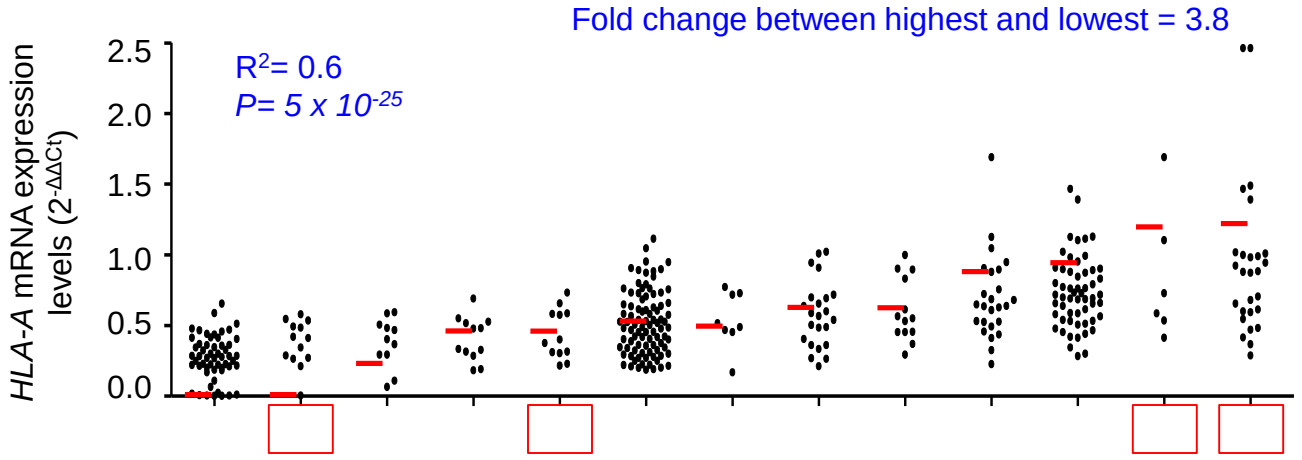


KIR ligands on HLA class I are distributed differently across human populations

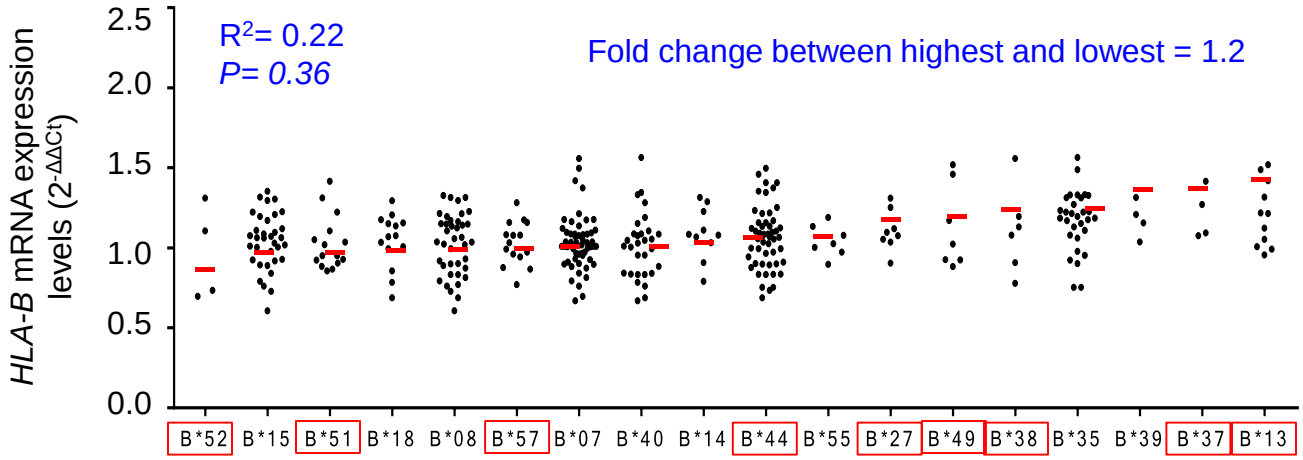


Parham and Moffett, 2013 *Nat Rev Immunol*

HLA-A alleles are differentially transcribed, but *HLA-B* alleles are uniformly transcribed



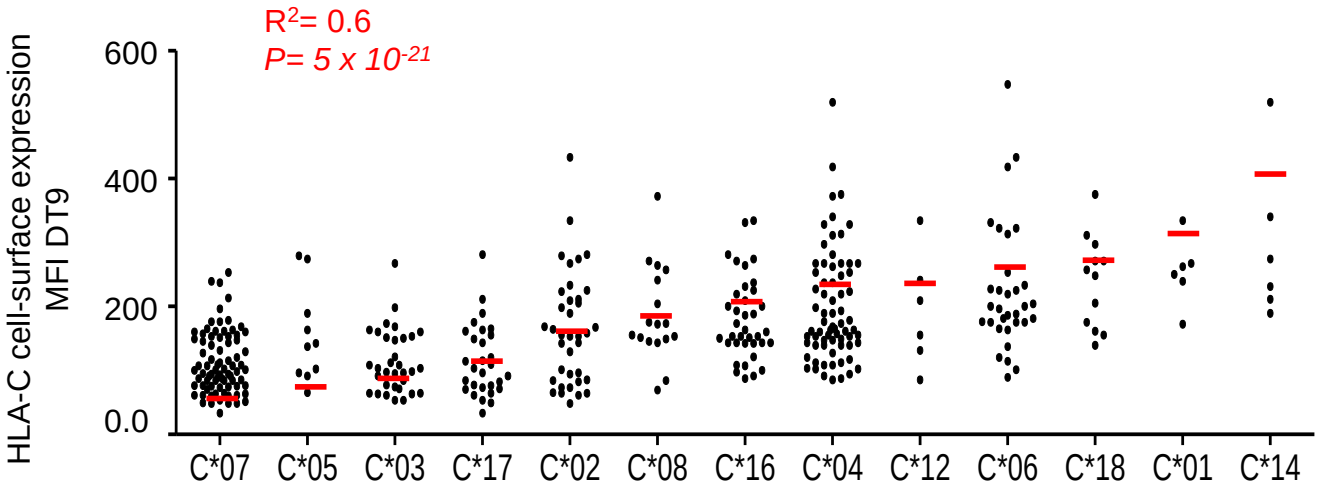
Ramsuran, 2015 *Am J Hum Genet*



Ramsuran, 2017 *J Immunol*

Bw4 Allele

DT9 antibody distinguishes between alleles of HLA-C and demonstrates differential expression

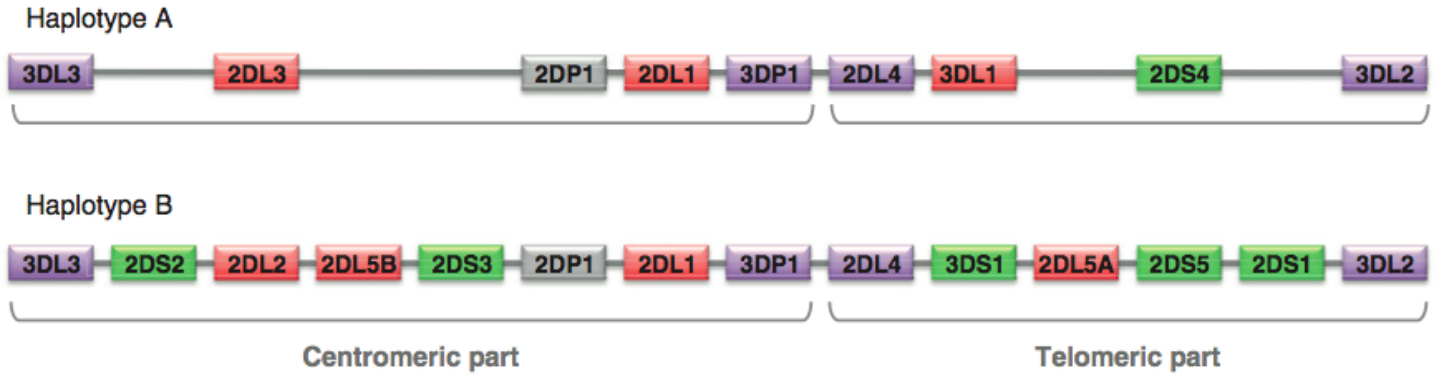


Apps, 2013 *Science*
Apps*, 2016, Cell Host Microbes*
Horowitz, 2015 *J Immunol*
Horowitz, 2016 *Science Immunology*

KIR genes diversify both in gene content as well as organization on a haplotype

KIR gene	*3DL3*	*2DL4*	*2DL5*	*3DL1/3DS1*	*3DL2*	*2DL1*	*2DL2/2DL3*	*2DS1*	*2DS2*	*2DS3/5*	*2DS4*	*2DP1*	*3DP1*
Alleles	111	52	48	110/30	112	48	30/55	16	22	15/18	31	28	27
Allotypes	57	28	20	66/17	82	28	13/31	8	8	6/12	14	0	0
Ligand	?	G?	?	Bw4	A3/11	C2	C1/C2	C2	A11?	?	A11	exC1/C2	NA

Guethlein, *Immunol Rev*, 2015



Thielens, *Curr Opin Immunol*, 2012

Immunogenetics and environment determine the extent of diversity of NK cells

RESEARCH ARTICLE

IMMUNOLOGY

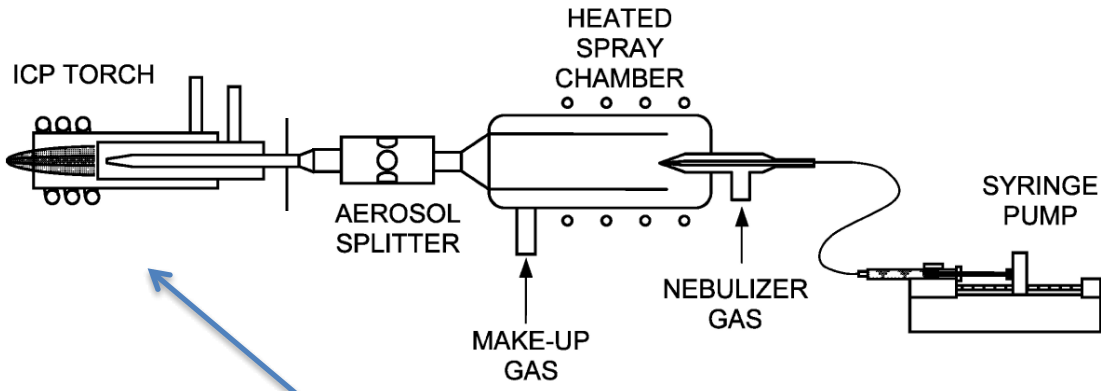
Genetic and Environmental Determinants of Human NK Cell Diversity Revealed by Mass Cytometry

Amir Horowitz,[1,2,3] Dara M. Strauss-Albee,[3,4] Michael Leipold,[2] Jessica Kubo,[4] Neda Nemat-Gorgani,[1] Ozge C. Dogan,[4] Cornelia L. Dekker,[5] Sally Mackey,[5] Holden Maecker,[2] Gary E. Swan,[6] Mark M. Davis,[2,3] Paul J. Norman,[1] Lisbeth A. Guethlein,[1] Manisha Desai,[4] Peter Parham,[1,2,3] Catherine A. Blish[3,4]*

Science Trans Med, 2013

Single Cell Analysis - CyTOF™ Machine

Fluidigm, Inc.



7,500 K

Mass Cytometry: Surface Panel

Surface phenotyping panel:

Lineage Markers

- CD3, (Vδ2)TCR-γδ, CD4/CD8 — T cells
- CD33 — Myeloid cells
- CD16, CD56 — NK cells
- CD19 — B cells
- CCR7 — LN homing
- HLA-DR — Mθ, DCs, B cells

MHC — All PBMC

HLA-C (DT9)

HLA-E (3D12)

Live/Dead

Cisplatin

Activating Receptors	Inhibitory Receptors
KIR2DS1	KIR2DL1
KIR2DS2	KIR2DL2
KIR2DS4	KIR2DL3
KIR2DL4	KIR2DL5
KIR3DS1	KIR3DL1
CD2	LILRB1 (ILT-2)
CD16	CD94/NKG2A
CD27	CD161
CD56	
CD57	**NK Development**
CD94/NKG2C	CD94
NKG2D	CD117
CD7	CD122
HLA-DR	
NKp30	
NKp44	N = 28 markers
NKp46	
2B4	

N = 40 markers

Mass Cytometry: Intracellular / Functional Panel

Functional/ICS panel:

Lineage Markers

- CD3, (Vδ2)TCR-γδ, CD4/CD8 — T cells
- CD33 — Myeloid cells
- CD16, CD56 — NK cells
- CD19 — B cells
- HLA-DR — Mθ, DCs, B cells,

Cytokines/ Chemokines — Upon activation

- IFN-γ
- TNF-α
- MIP-1β
- CD107a
- Granzyme B
- GM-CSF
- PLZF
- Eat-2
- Syk
- FcεRIγ

Live/Dead

- Cisplatin

Activating Receptors	Inhibitory Receptors
KIR2DS1	KIR2DL1
KIR2DS2	KIR2DL2
KIR2DS4	KIR2DL3
KIR2DL4	KIR2DL5
KIR3DS1	KIR3DL1
CD2	LILRB1 (ILT-2)
CD16	CD94/NKG2A
CD27	CD161
CD56	
CD57	**Exhaustion**
CD94/NKG2C	CTLA-4
NKG2D	PD-1
CD7	TIGIT
HLA-DR	Tim-3
NKp30	
NKp44	
NKp46	
2B4	
CD122	

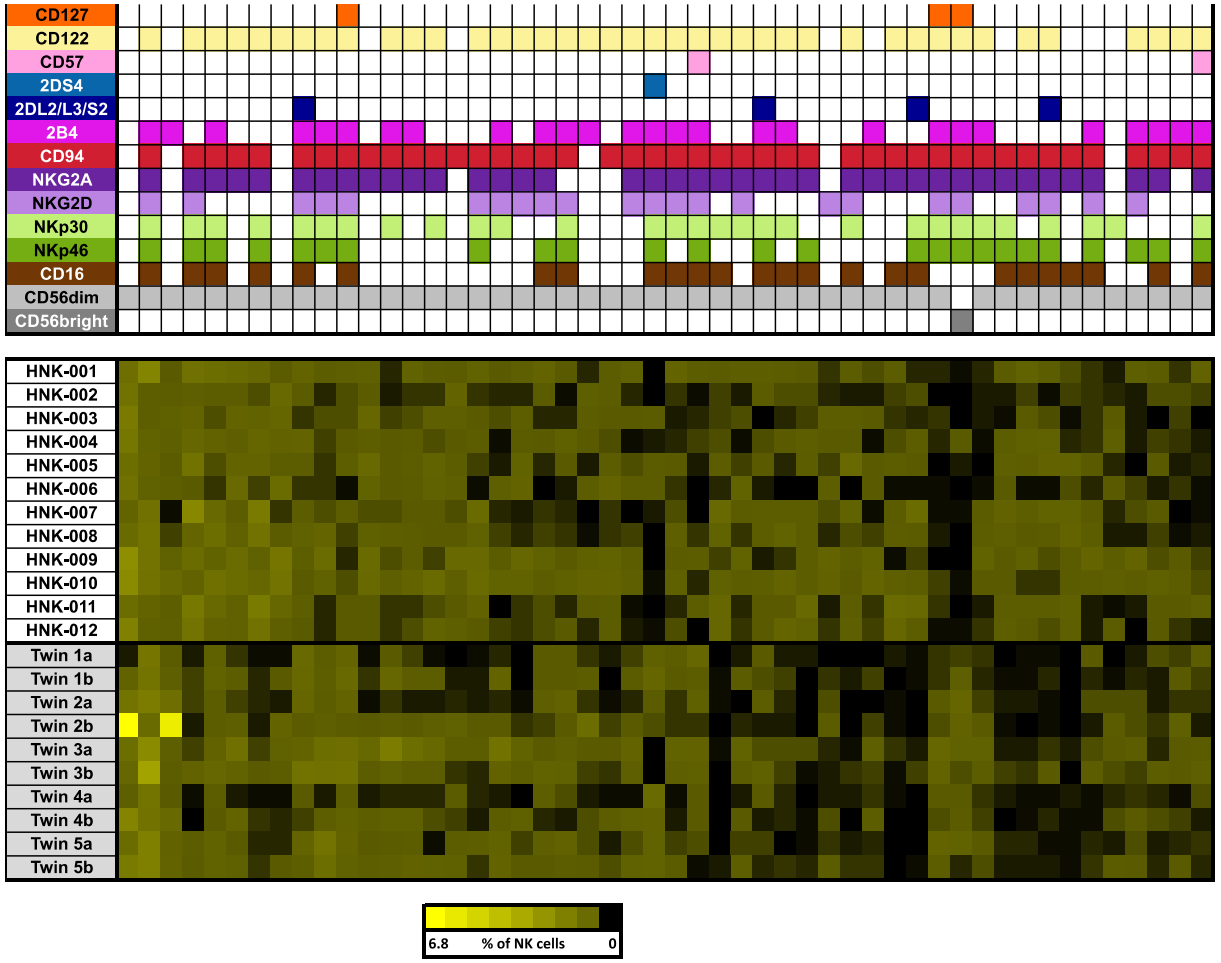
N = 42 markers

Boolean gating: ‘logics’ equations based on “AND”, “OR” and “NOT” for sorting cells based on phenotypes and functions

Question: If clustering on 28 markers, how many possible phenotype combinations can be predicted?

$2^{28} = 268,435,456$

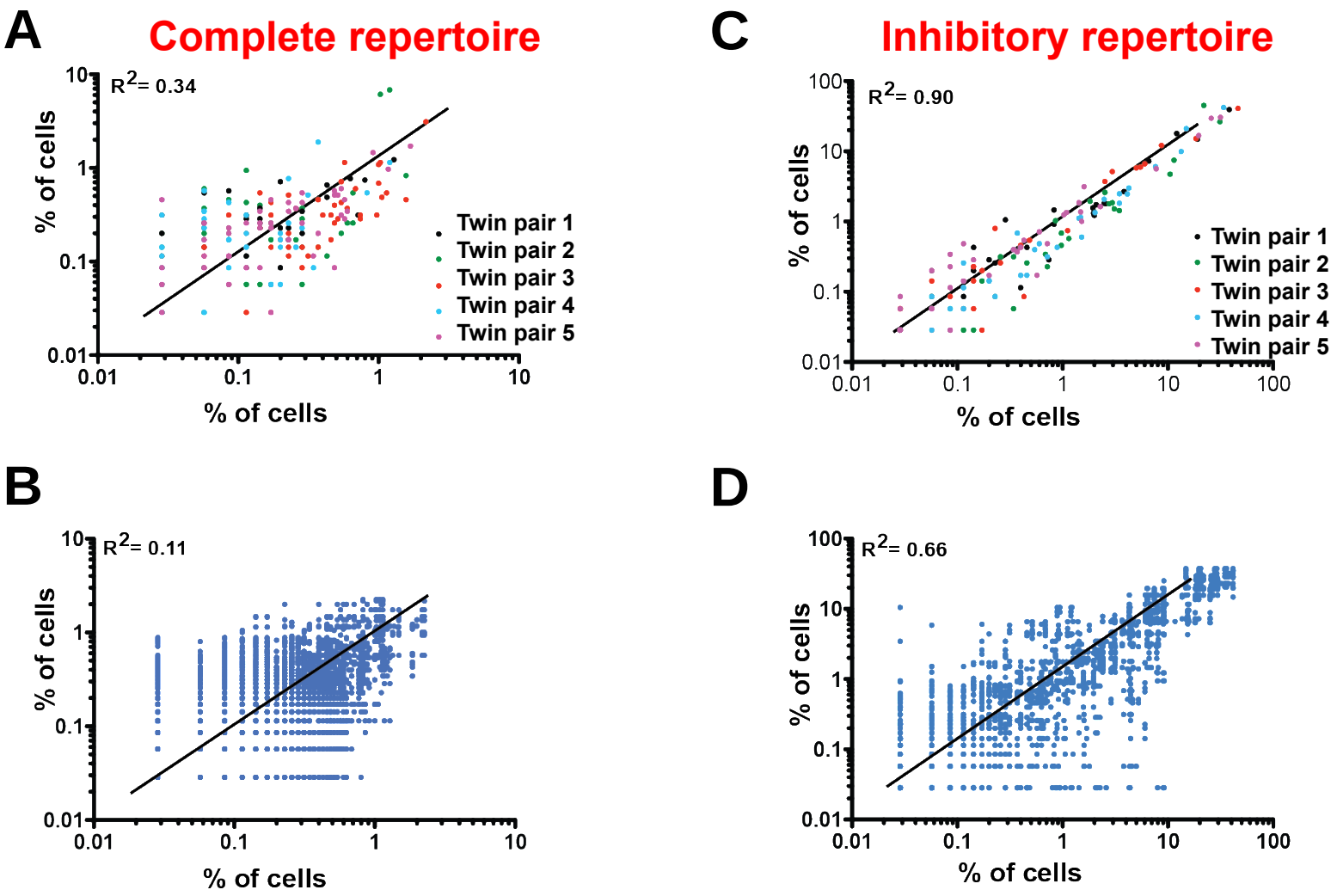
Boolean gating reveals thousands of phenotypes



- These top 50 subpopulations account for only 15% of the total NK cells
- Sampled between 6,000 – 25,000 unique phenotypes/donor
- Sampled ~125,000 unique phenotypes across all donors

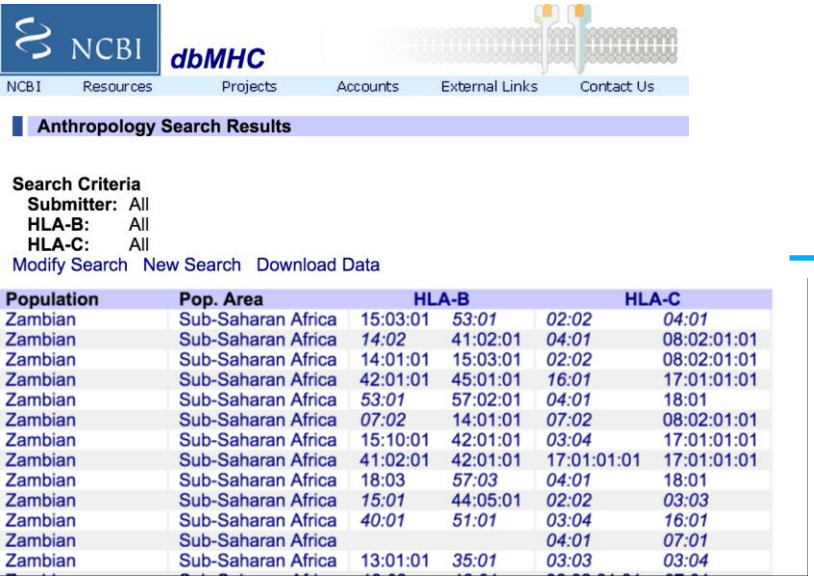
Horowitz, *Sci Transl Med*, 2013

Genetics vs Environmental determinants of repertoire diversity



Horowitz, *Sci Transl Med*, 2013

Population analyses of HLA class I: Alleles of HLA-A, -B and -C segregate in human populations to modulate education through HLA-E



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Anthropology Search Results

Search Criteria
Submitter: All
HLA-B: All
HLA-C: All
Modify Search New Search Download Data

Population	Pop. Area	HLA-B		HLA-C	
Zambian	Sub-Saharan Africa	15:03:01	53:01	02:02	04:01
Zambian	Sub-Saharan Africa	14:02	41:02:01	04:01	08:02:01:01
Zambian	Sub-Saharan Africa	14:01:01	15:03:01	02:02	08:02:01:01
Zambian	Sub-Saharan Africa	42:01:01	45:01:01	16:01	17:01:01:01
Zambian	Sub-Saharan Africa	53:01	57:02:01	04:01	18:01
Zambian	Sub-Saharan Africa	07:02	14:01:01	07:02	08:02:01:01
Zambian	Sub-Saharan Africa	15:10:01	42:01:01	03:04	17:01:01:01
Zambian	Sub-Saharan Africa	41:02:01	42:01:01	17:01:01:01	17:01:01:01
Zambian	Sub-Saharan Africa	18:03	57:03	04:01	18:01
Zambian	Sub-Saharan Africa	15:01	44:05:01	02:02	03:03
Zambian	Sub-Saharan Africa	40:01	51:01	03:04	16:01
Zambian	Sub-Saharan Africa			04:01	07:01
Zambian	Sub-Saharan Africa	13:01:01	35:01	03:03	03:04

Sampled 51 populations:

- Curated from Anthropological studies

HLA-B	HLA-C
B*15:03 / B*53:01	C*02:02 / C*04:01
B*14:02 / B*41:02	C*04:01 / C*08:02

N = 8,204 genotypes

HLA-B	HLA-C
P2T/ P2T	C2/ C2
P2M/ P2T	C2 / C1

Gap package

R

- # of haplotypes
- Haplotype frequencies
- D' values
- X^2 values (P values)

N = 16,408 haplotypes

Horowitz, 2016 *Science Immunology*

C2^{+}*HLA-C* and -21M *HLA-B* alleles segregate on different HLA haplotypes in Eurasian populations

5,439 haplotypes

Population group	Proportion of total *HLA B–C* haplotypes (%)			
	HLA-C2		HLA-C1	
	HLA-B -21M	HLA-B -21T	HLA-B -21M	HLA-B -21T
African	0.13	0.39	0.13	0.36
European	0.01	0.39	0.27	0.33
Asian	0.01	0.18	0.19	0.61
Australian	0.0	0.56	0.05	0.39
Oceanian	0.0	0.25	0.22	0.53
North American	0.0	0.36	0.21	0.40
South American	0.0	0.35	0.31	0.35

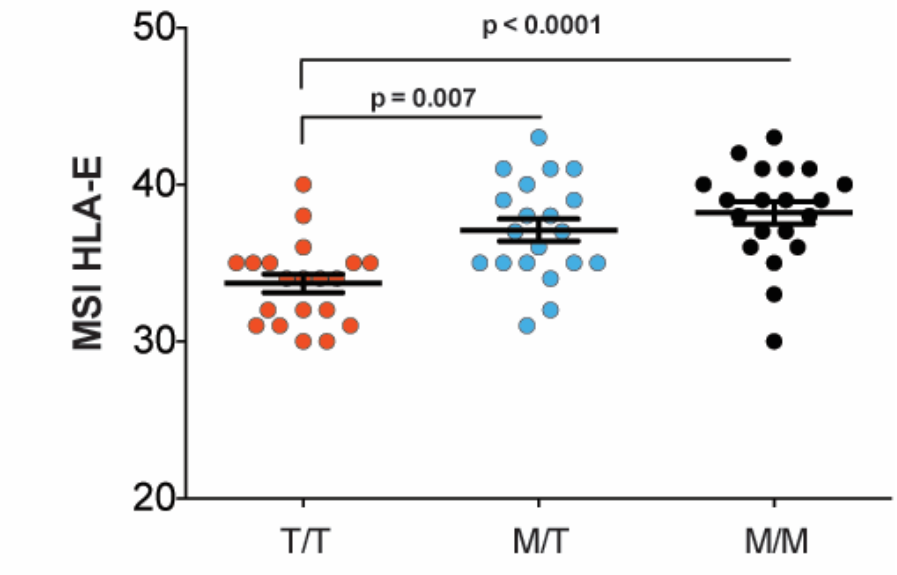
10,945 haplotypes

Five HLA-B -21M — C2^{+}HLA-C haplotypes			
Haplotype group	Haplotype	Population group	Group characteristics
I	B*42:01 — C*17:01	Africans	African-specific HLA-B and HLA-C alleles
	B*81:01 — C*18:01		
II	B*81:01 — C*04:01		African-specific HLA-B allele and widespread HLA-C allele
III	B*73:01 — C*15:05	Asians	All or part of the haplotype arising by introgression from archaic humans
	B*07:05 — C*15:05		

Hypothesis: -21M/T dimorphism divides HLA haplotypes into two schools of education:

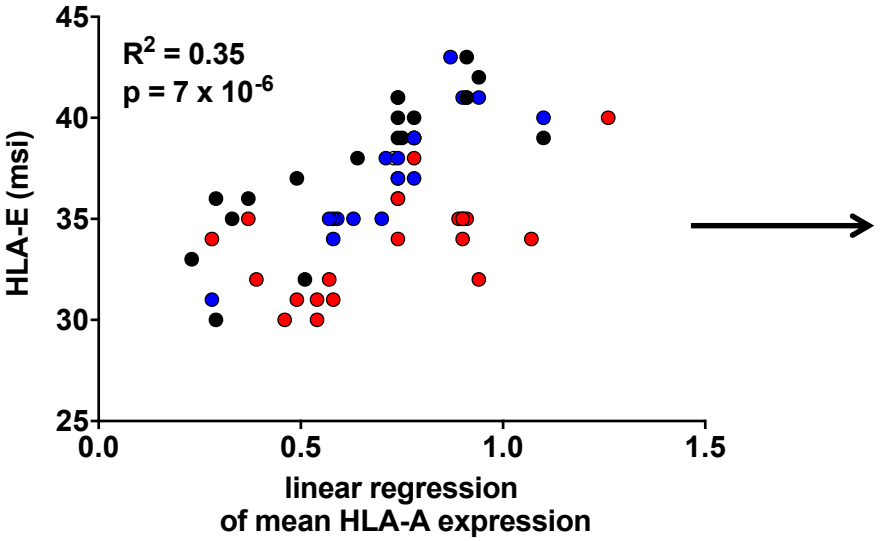
- One biased to providing CD94:NKG2A ligands
- One biased towards providing KIR ligands

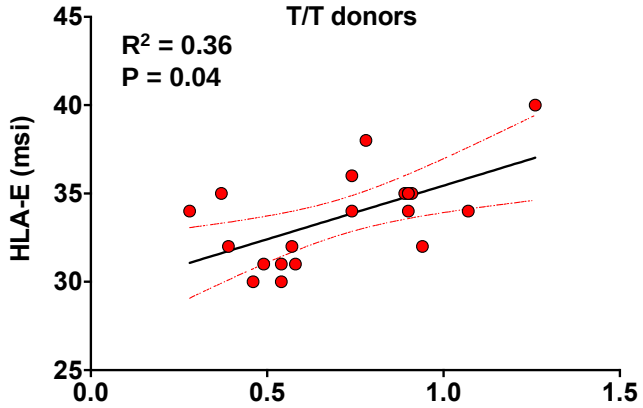
-21 *HLA-B* genotype correlates with cell-surface expression of HLA-E

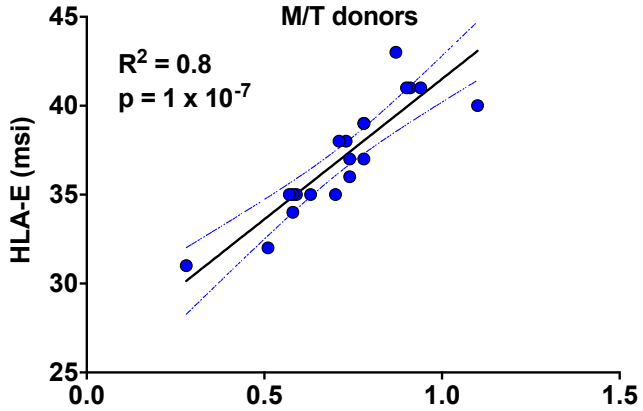


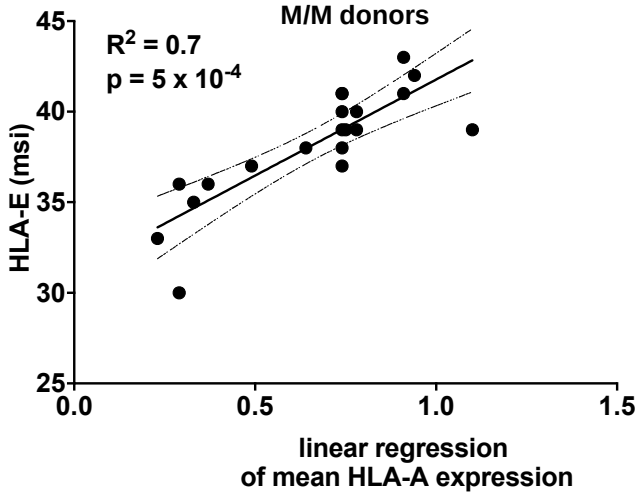
Horowitz, 2016 *Science Immunology*

HLA-A and HLA-E expression correlates with HLA-B -21 amino acid variation







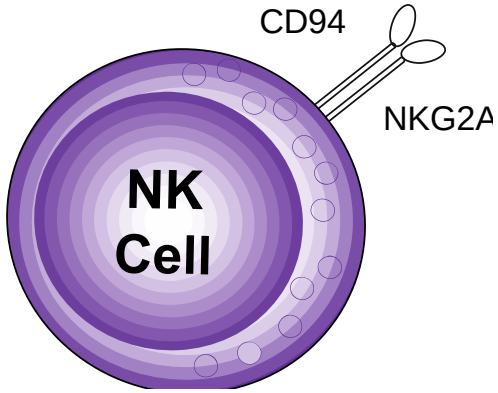


HLA-B -21 variant

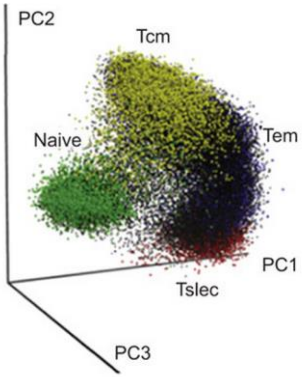
- M/M
- M/T
- T/T

Horowitz, 2016 *Science Immunology*
Ramsuran*, Naranbhai*, Horowitz, *In revision*

Why are the two most polymorphic genes in our genome cooperating to regulate expression on a very conserved gene?

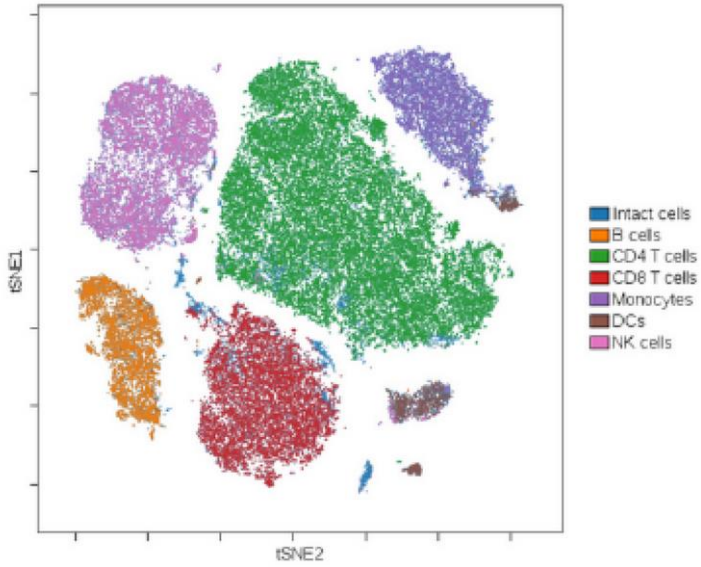


Visual tools for reducing data dimensionality are critical!



3D PCA

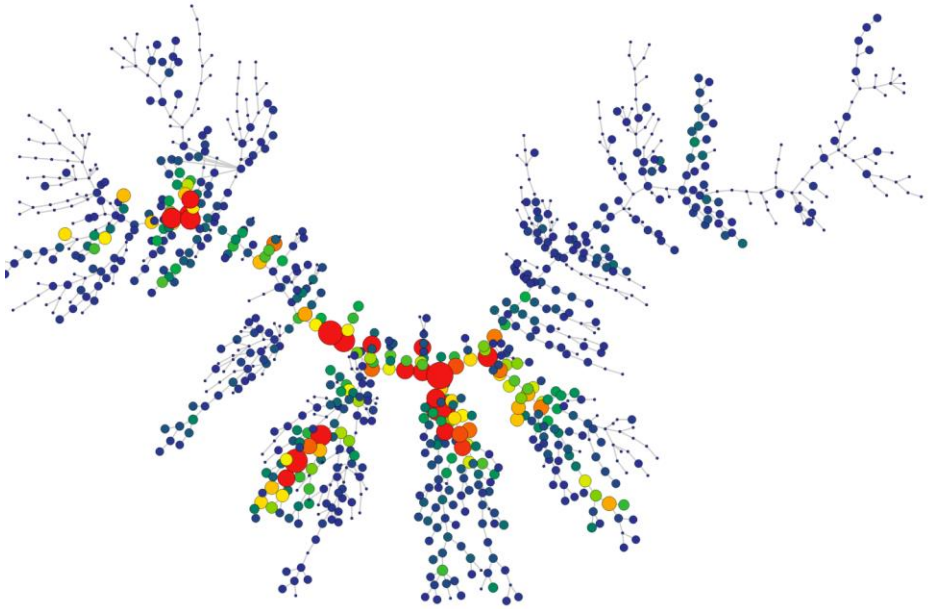
SPADE



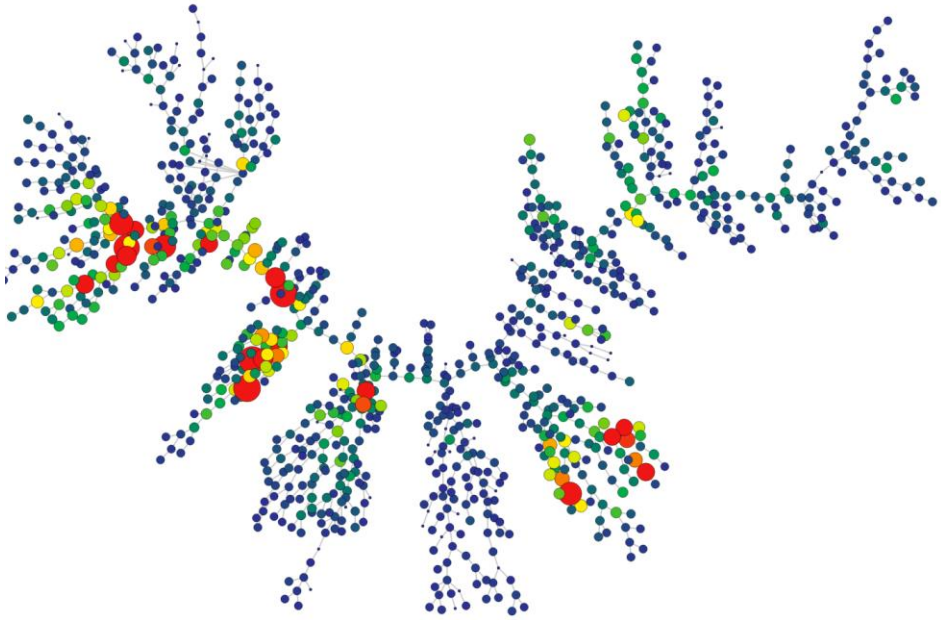
viSNE

Spanning-tree Progression Analysis of Density-normalized Events (SPADE): *requires transformation to reveal informative patterns*

T/T



M/M

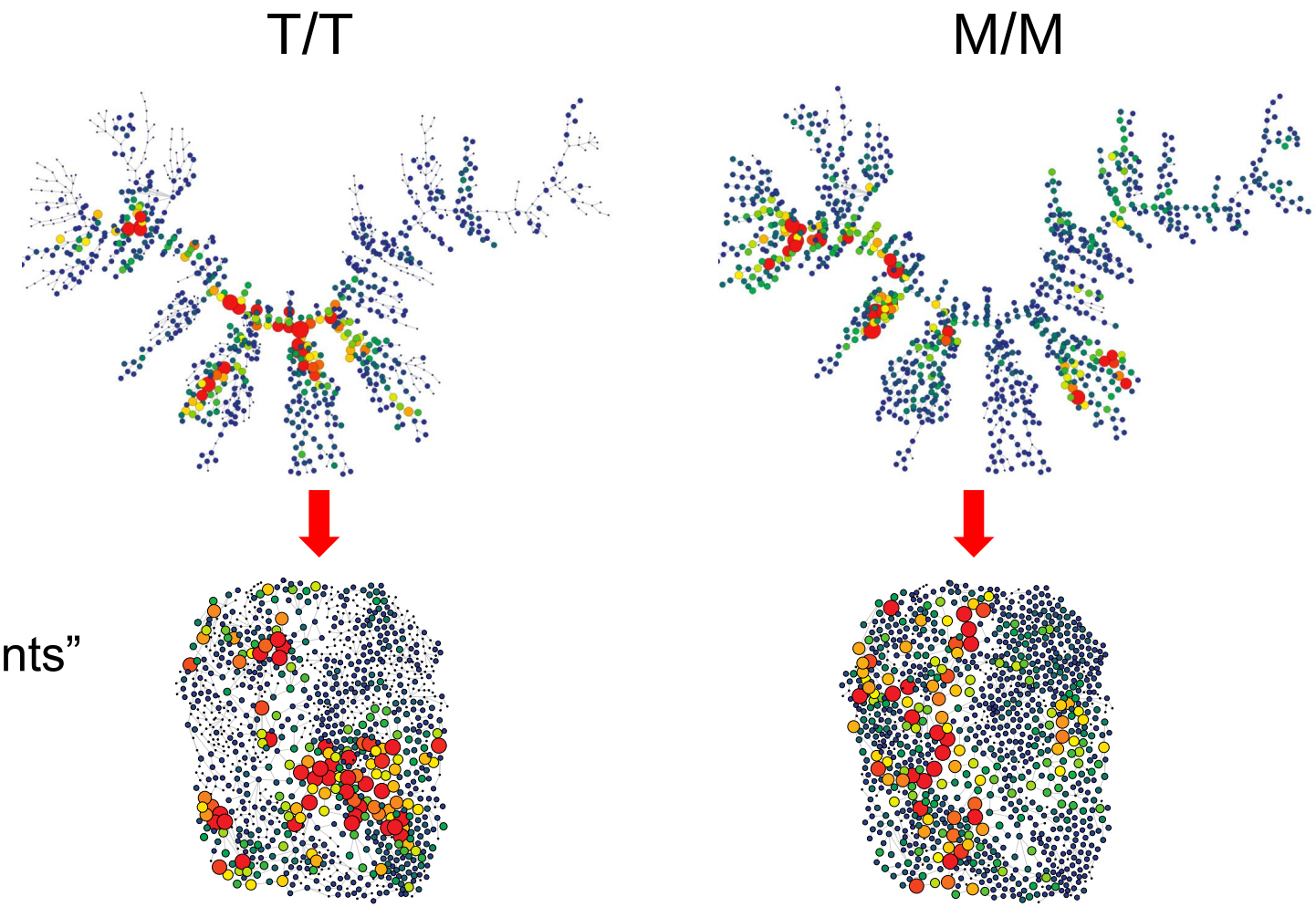


SPADE analysis built on:

~20,000 CD94:NKG2A^{+} NK cells from 60 donors
Cluster size range: 1-163 cells per cluster

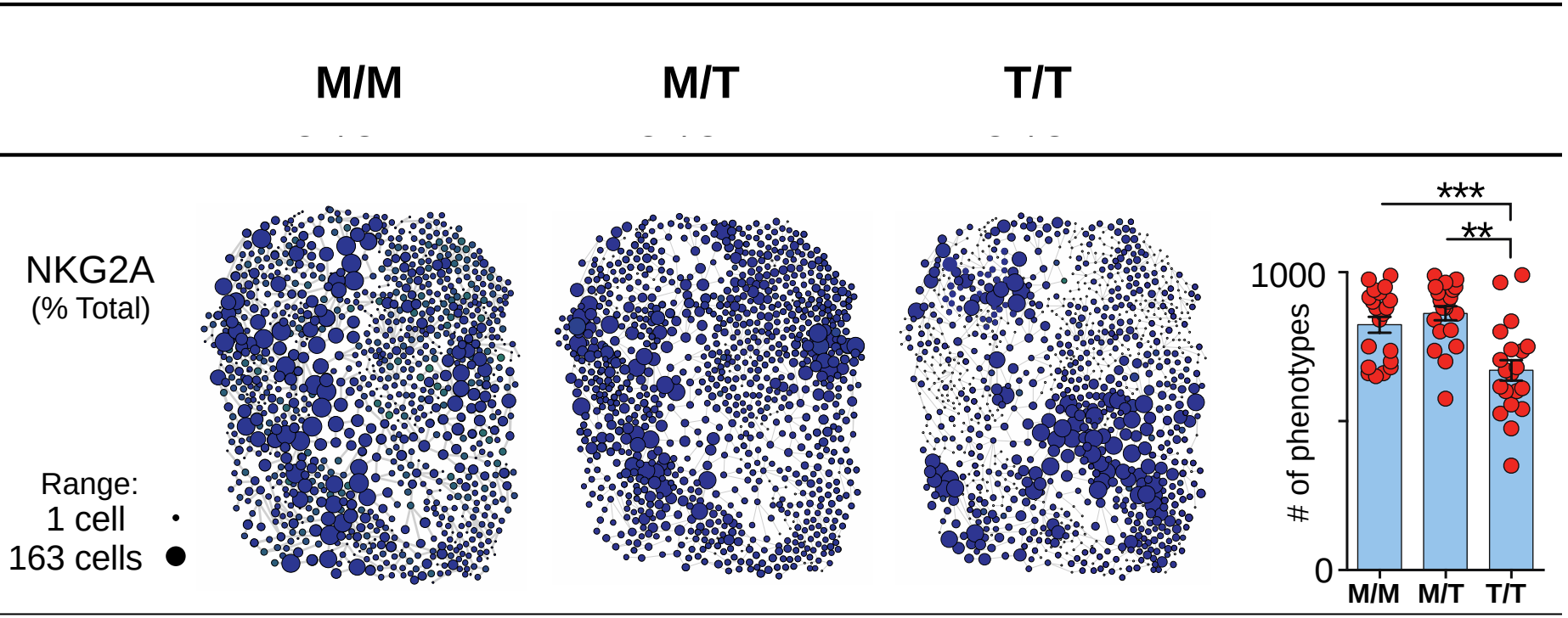
Horowitz, 2016 *Science Immunology*

Transforming SPADE trees into ‘Immune fingerprints’



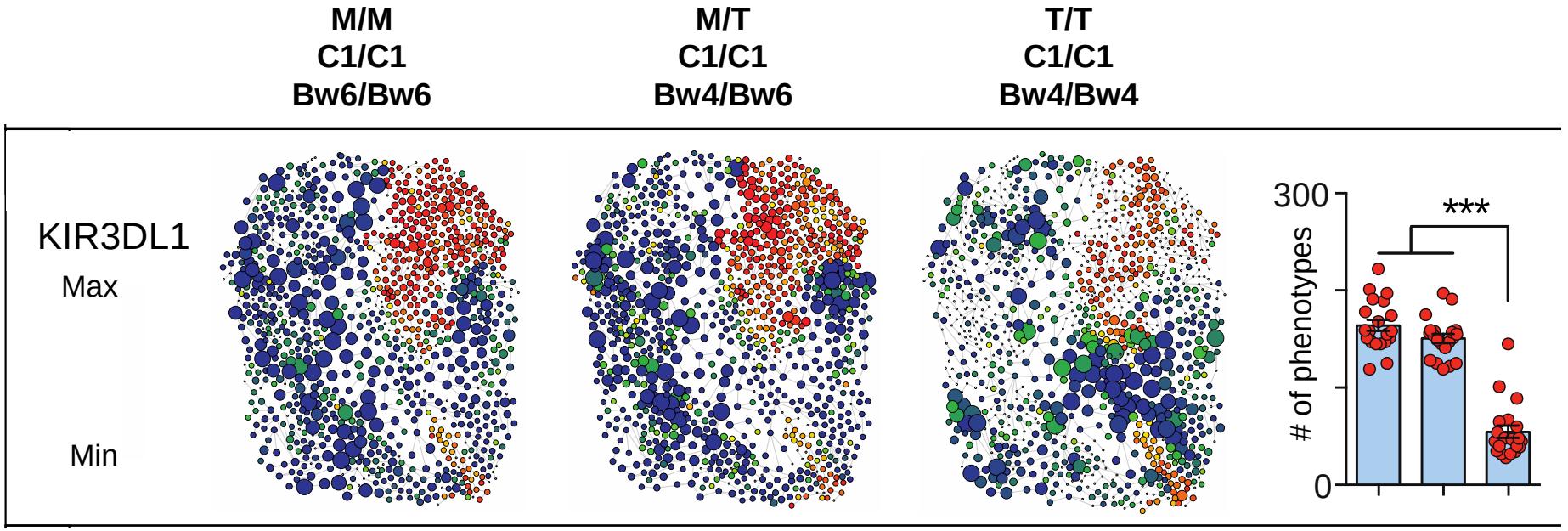
Horowitz, 2016 *Science Immunology*

SPADE reveals greater diversity in the CD94:NKG2A^{+} NK repertoire in individuals with -21M HLA-B



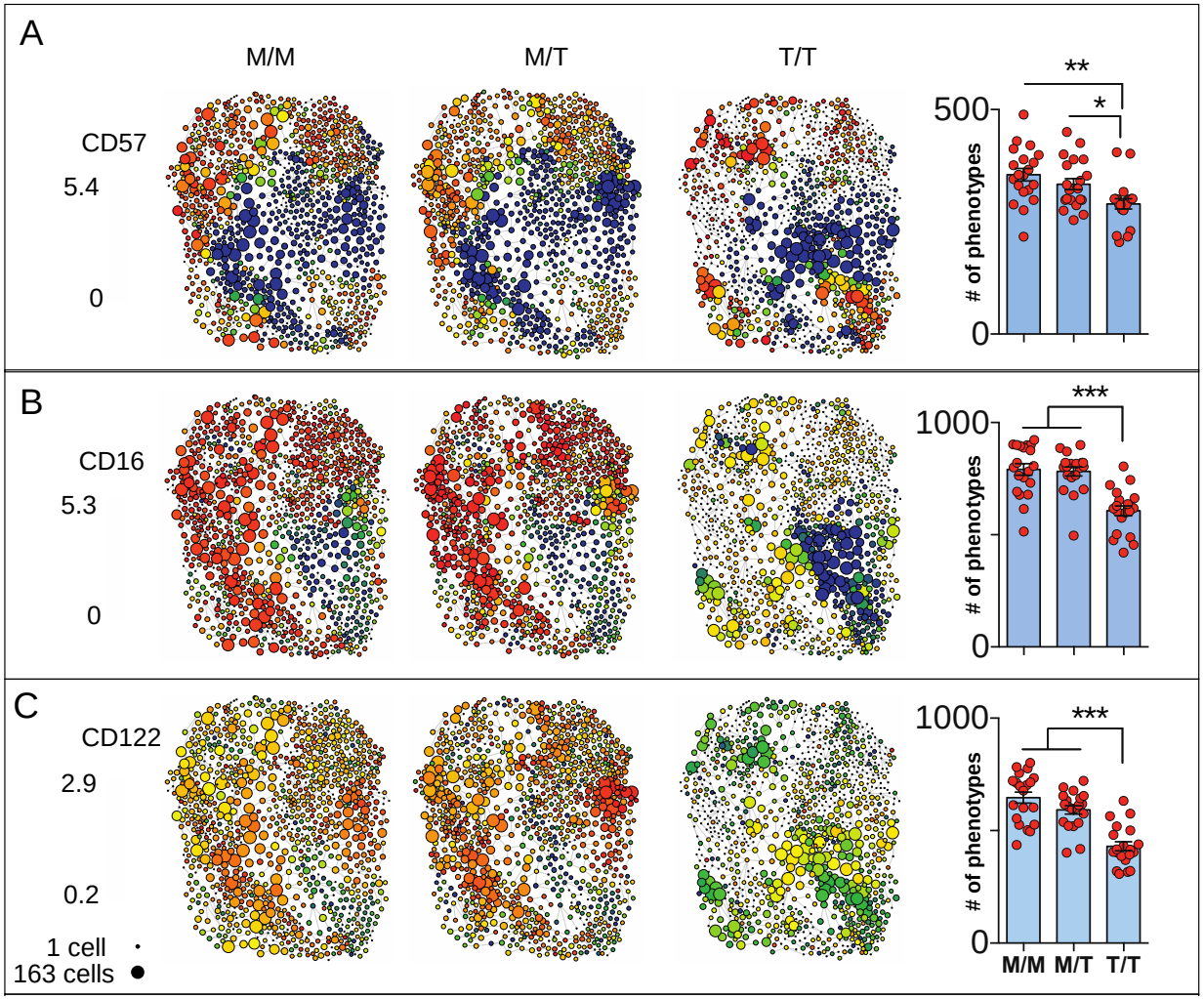
Horowitz, 2016 *Science Immunology*

-21M *HLA-B* correlates with increased levels of inhibitory NK receptors on CD94:NKG2A^{+} NK cells



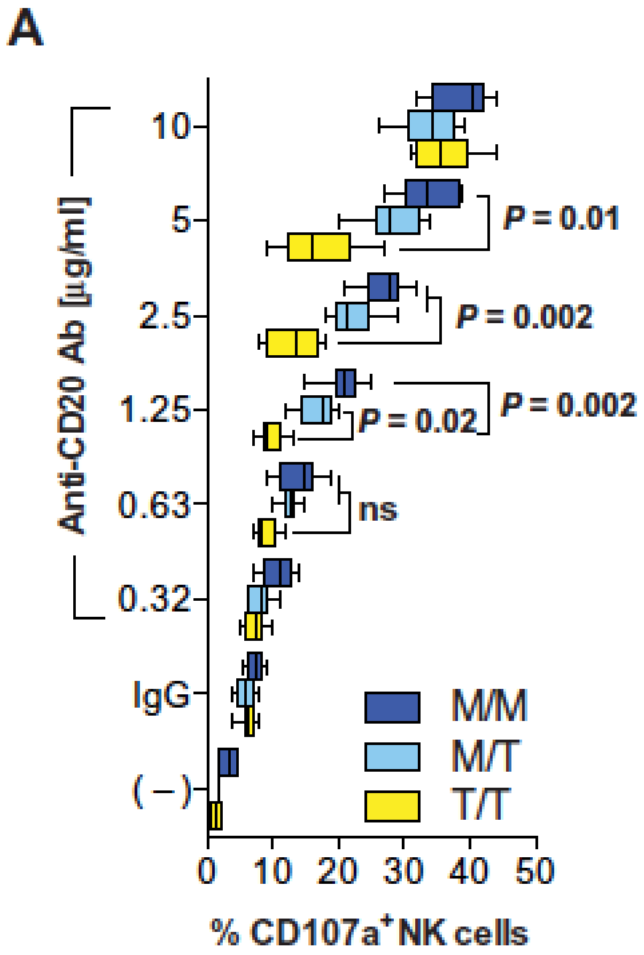
Horowitz, 2016 *Science Immunology*

-21M *HLA-B* correlates with increased proportion of adaptive CD94:NKG2A^{+} NK cells



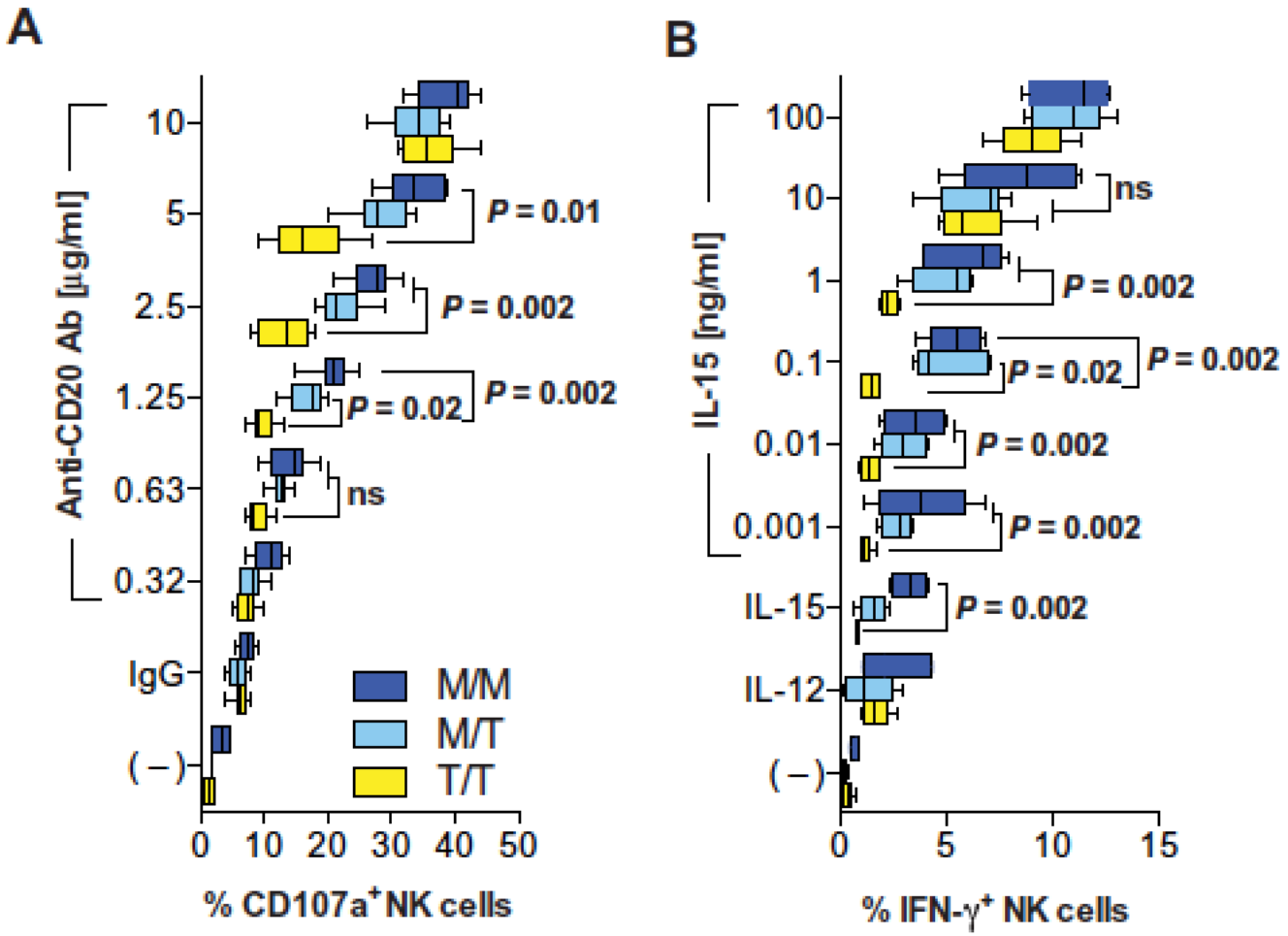
Horowitz, 2016 *Science Immunology*

-21M *HLA-B* correlates with increased sensitivity to signaling through FcγRIIIA



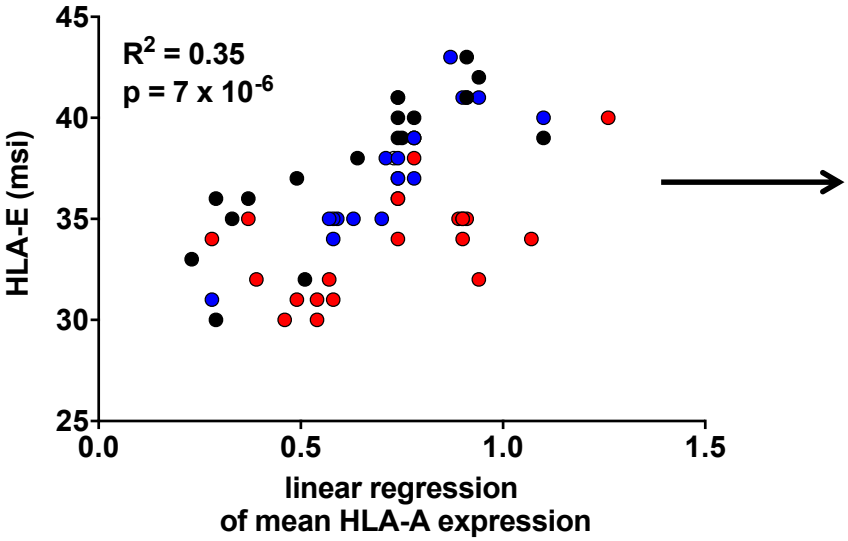
Horowitz, 2016 *Science Immunology*

-21M *HLA-B* correlates with increased sensitivity to signaling through IL-2/15Rβ



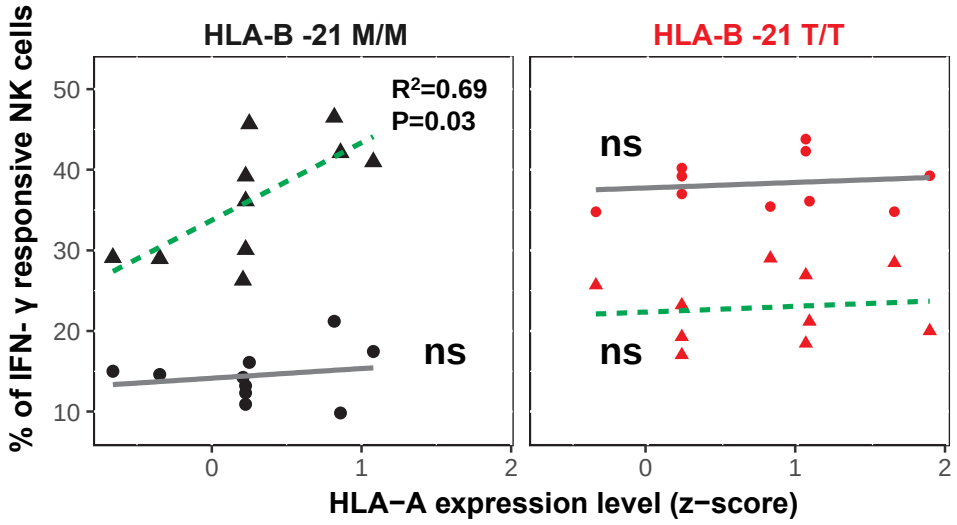
Horowitz, 2016 *Science Immunology*

HLA-A and HLA-E expression correlates with direction of education and breadth of activation



Low dose

α-CD20 Ab [2.5μg/ml]



HLA-B -21 variant

- M/M
- M/T
- T/T

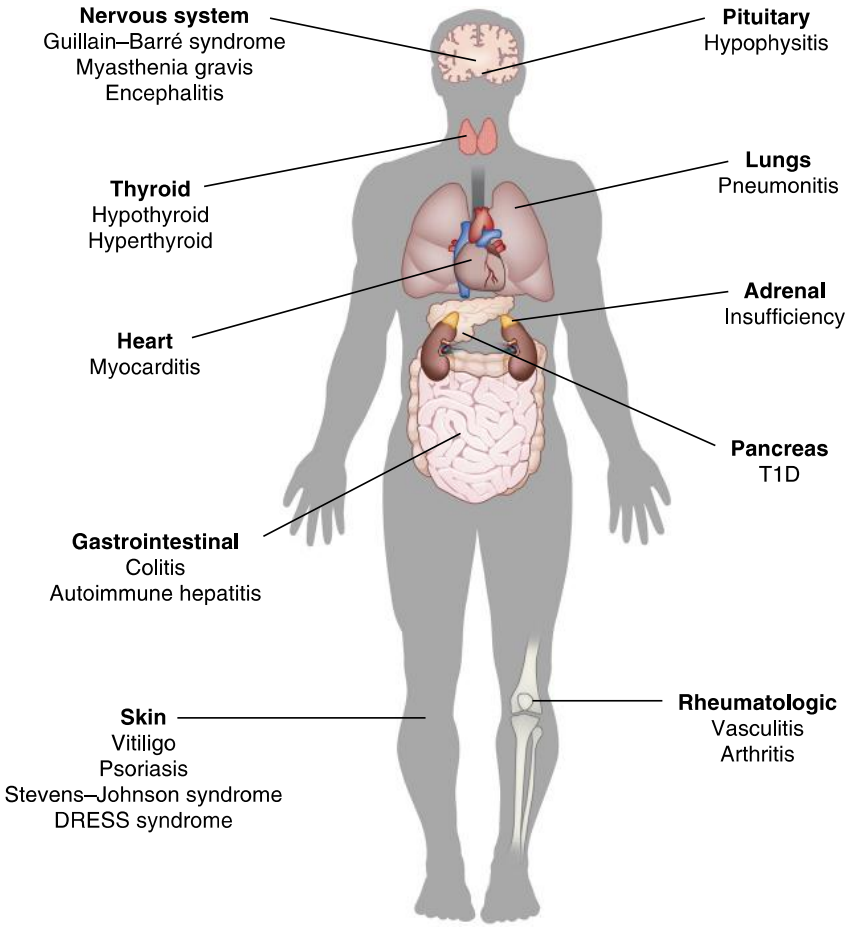
NK cell subset

- NKG2A+
- KIR+

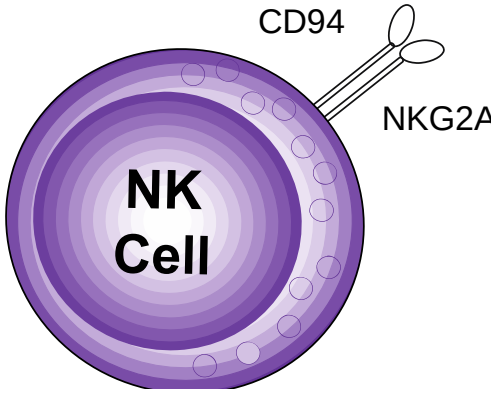
Horowitz, 2016 *Science Immunology*

When can a protective response turn bad?

Examples of autoimmune and immune-related adverse events associated with cancer immunotherapies and NK cells

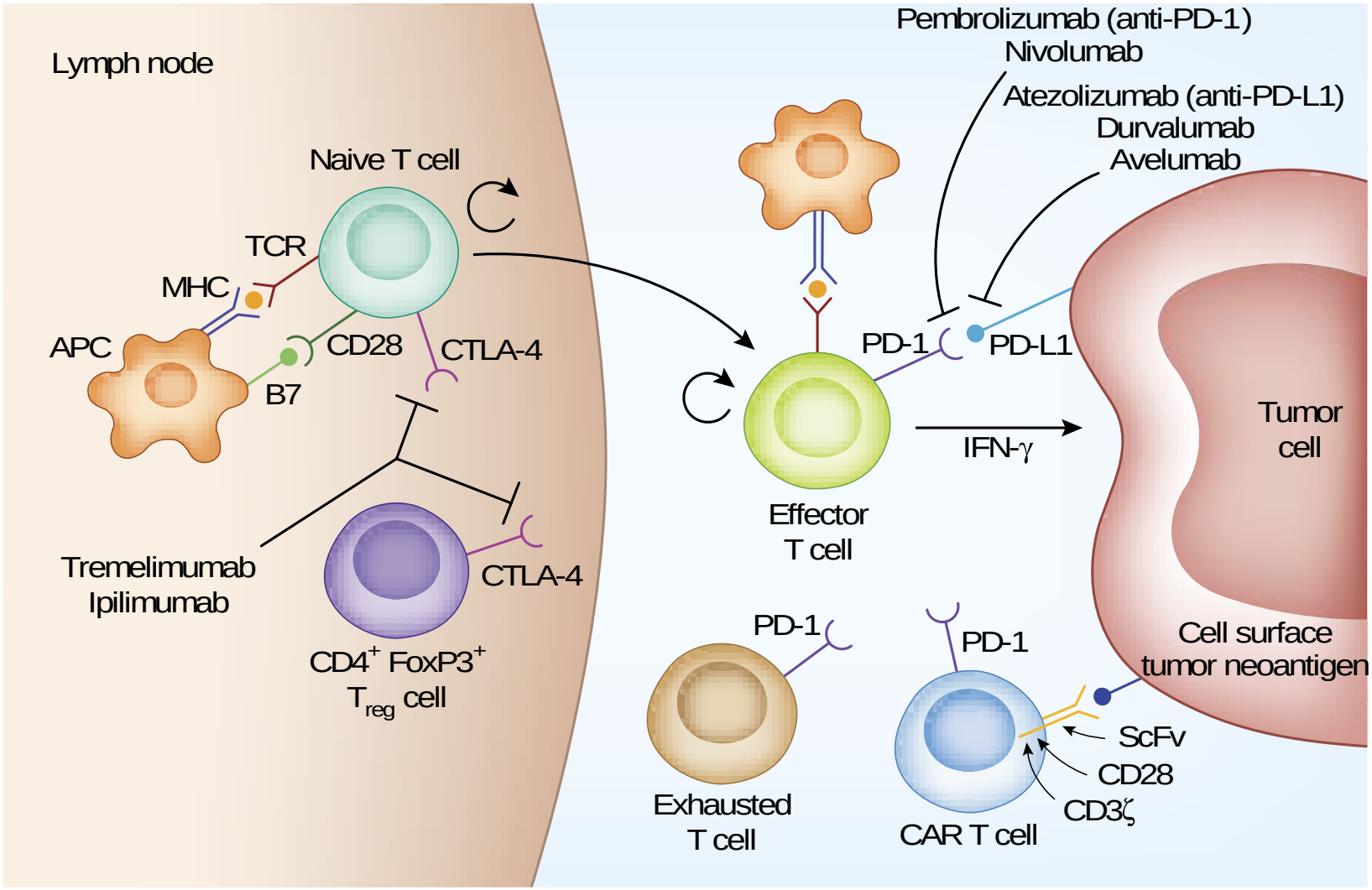


Immature NK cells



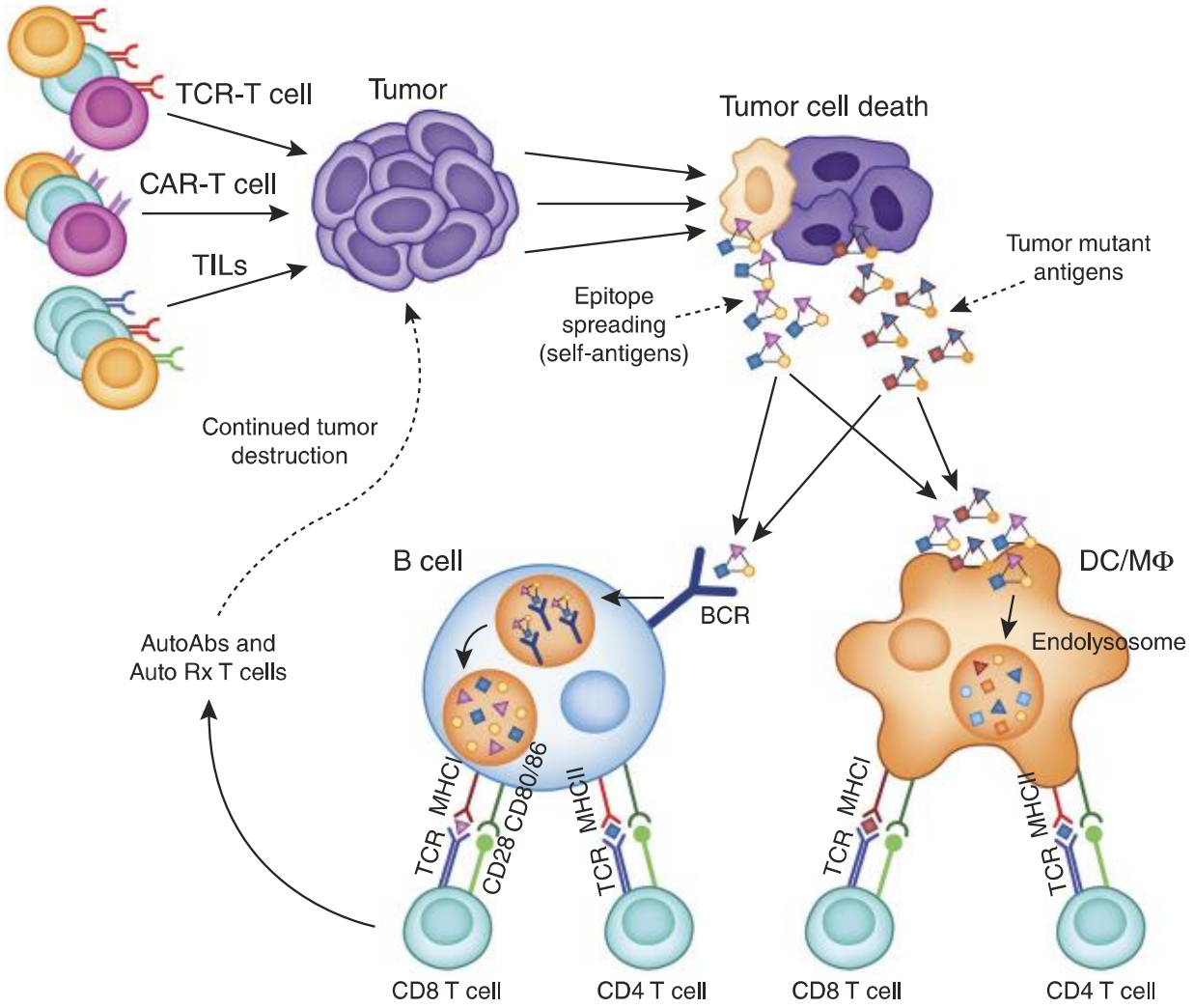
June, Warshauer & Bluestone 2017 *Nat Medicine*

Differential effects of Immune checkpoint blockade on T cells at different stages of differentiation/localization



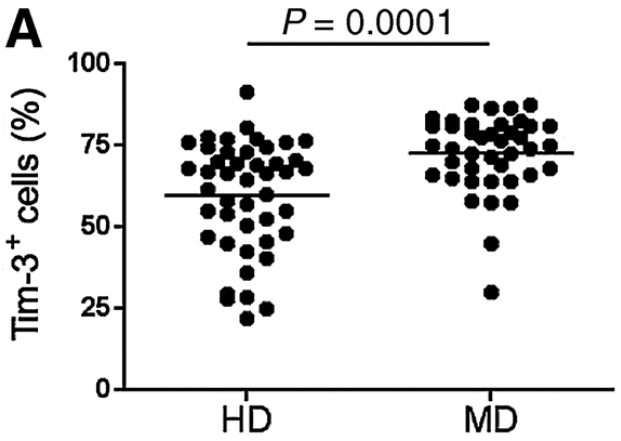
June, Warshauer & Bluestone 2017 *Nat Medicine*

Potential mechanism of epitope spreading promoting autoimmunity



June, Warshauer & Bluestone 2017 *Nat Medicine*

NK cell exhaustion: A potential mediator of immune dysregulation and autoimmunity

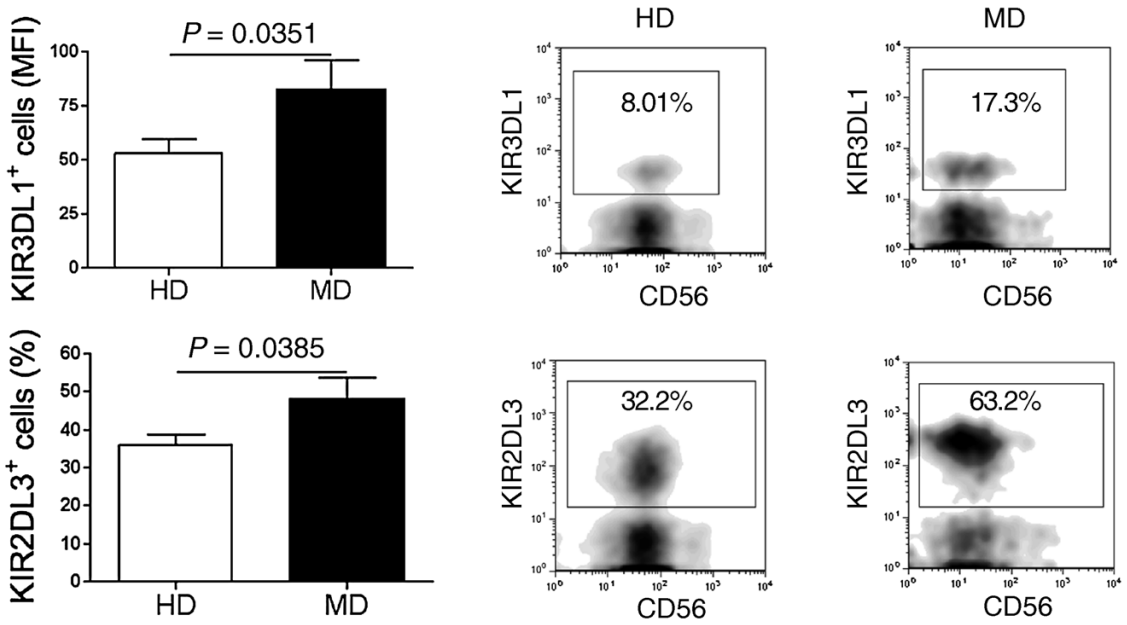


Nina Bhardwaj, MD, PhD

Elena González-Gügel, PhD

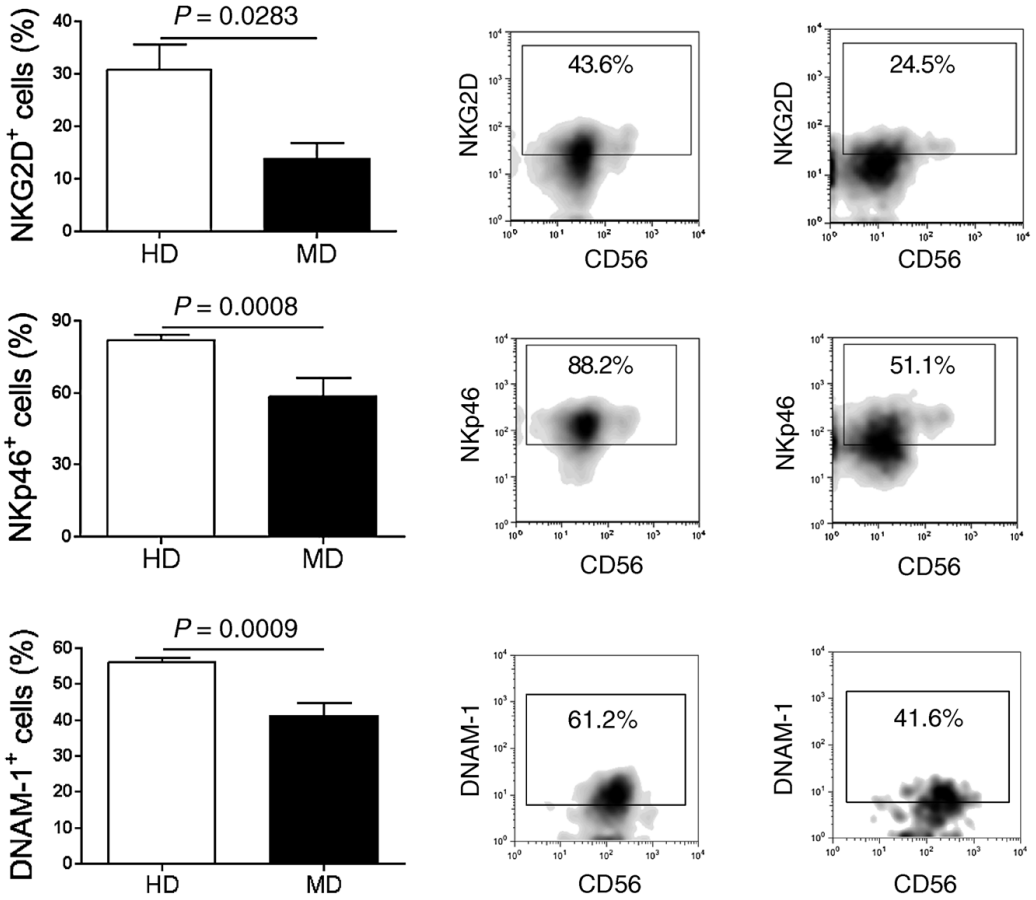
Adam Farkas, PhD

Melanoma patients upregulate inhibitory receptors, which may function similar to checkpoint inhibitors



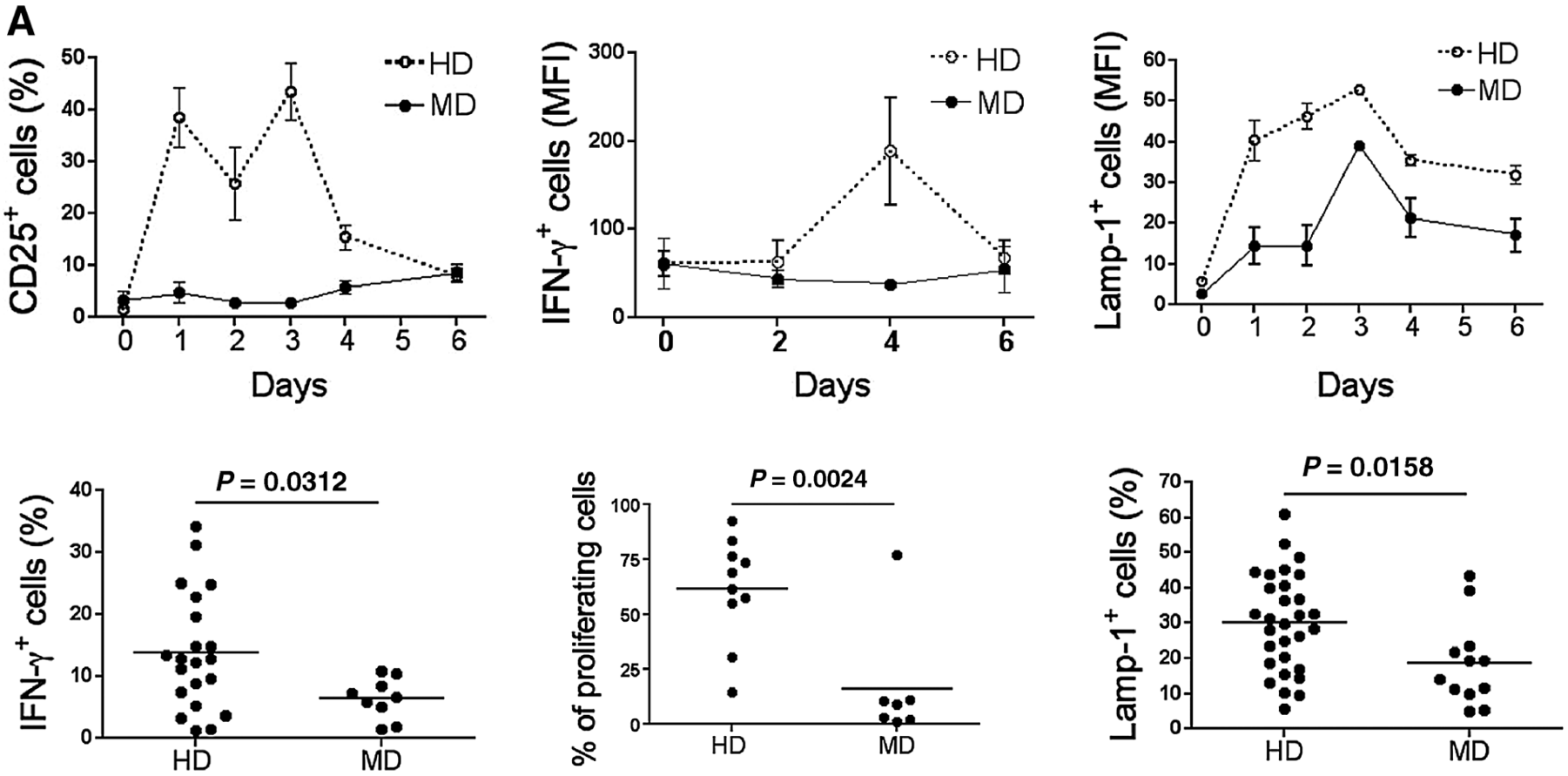
Di Silva 2014 *Cancer Immunol Res*

Melanoma patients downregulate activating receptors, decreasing sensitivity to tumor target cells



Di Silva 2014 *Cancer Immunol Res*

Melanoma patients display reduced sensitivity to IL-2 as well as activation and proliferation



Di Silva 2014 *Cancer Immunol Res*

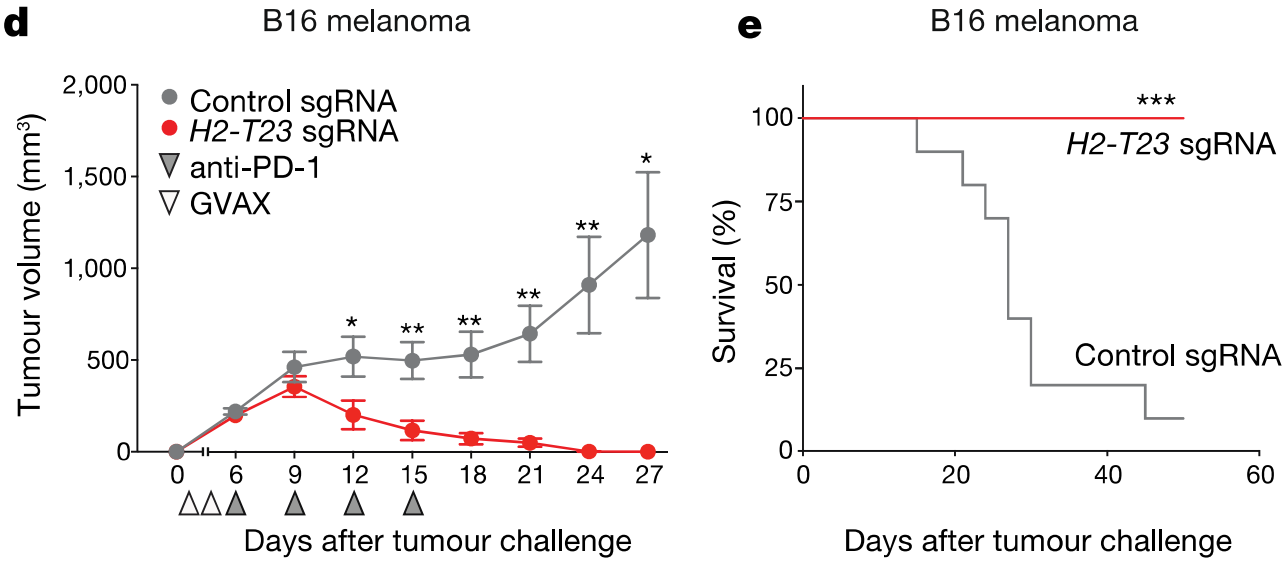
Loss-of-function screening identifies target that increases efficacy of immunotherapy

ARTICLE

doi:10.1038/nature23270

In vivo CRISPR screening identifies *Ptpn2* as a cancer immunotherapy target

Robert T. Manguso[1,2,3], Hans W. Pope[1,3], Margaret D. Zimmer[1,3], Flavian D. Brown[1,2], Kathleen B. Yates[1,3], Brian C. Miller[1,3,4], Natalie B. Collins[1,3,5], Kevin Bi[1,3], Martin W. LaFleur[1,2], Vikram R. Juneja[6], Sarah A. Weiss[1], Jennifer Lo[7], David E. Fisher[7], Diana Miao[2,3], Eliezer Van Allen[2,3], David E. Root[3], Arlene H. Sharpe[5,8], John G. Doench[3] & W. Nicholas Haining[1,3,5]



*Study did not report on treatment-related toxicities or autoimmunty assoicated with knocking down MHC-E!

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Contact:
amir.horowitz@mssm.edu

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