Tumor Immune Microenvironment: A Holistic Approach Workshop

April 21-22, 2022 • San Diego and Virtually

#SITCworkshop

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Society for Immunotherapy of Cancer

Yale CANCER CENTER

A Comprehensive Cancer Center Designated by the National Cancer Institute

Decoding the Lung Cancer Immune Microenvironment Using Spatially-resolved Multiplexed Tissue Analysis

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Conflict of Interest disclosure (last 24 months)

Employee of: Yale University

Consultant for: Clinica Alemana Santiago, Shattuck Labs, AstraZeneca, EMD Serono, Takeda, Torque/Repertoire Therapeutics, Takeda, Agenus, Genmab, OnCusp, Therapeutics, Bristol-Myers Squibb. Parthenon Therapeutics and Merck.

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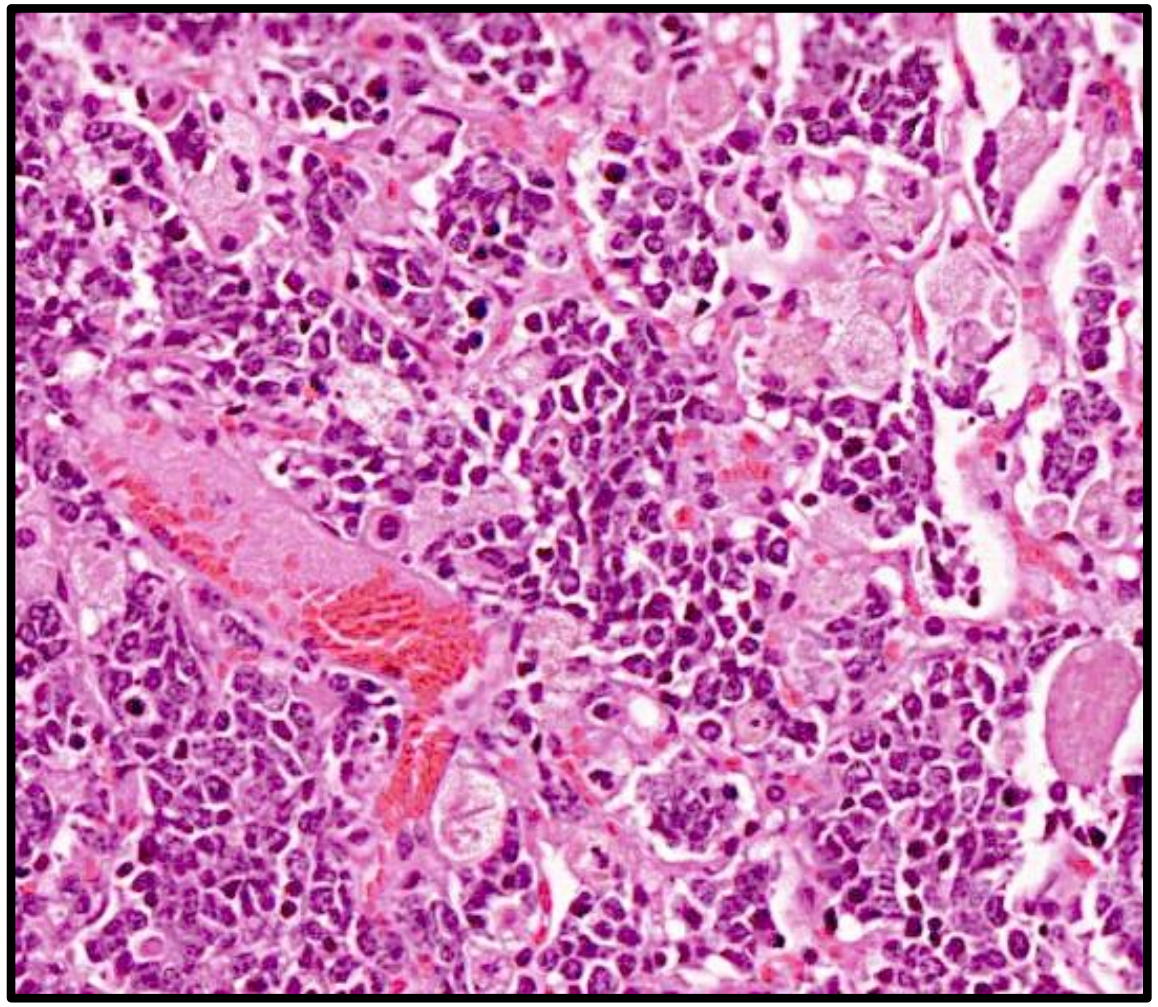
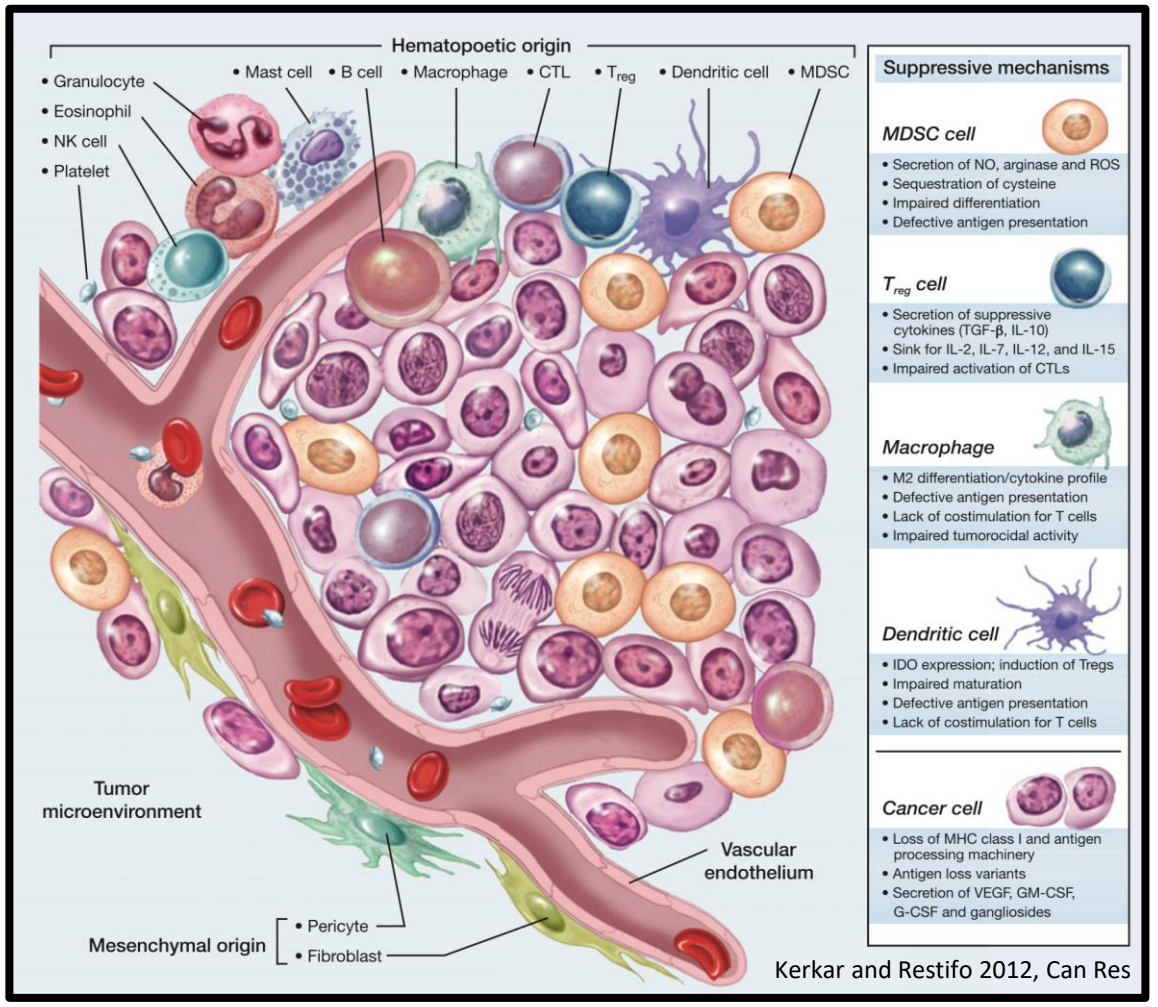
Why studying the tumor microenvironment?

- Major role in immunotherapy sensitivity & resistance
  - *Local tolerogenic factors and suppression of effector responses.*
- Complex problem with unexplored determinants
  - *Multiple parameters, spatial features (heterogeneity) and dynamic nature*
- Tumors can display multiple immune evasion pathways
  - *Earlier tumors may be less complex*
- Identify “dominant” signals mediating tolerance and resistance
  - *Multiple altered pathways, few may be dominant*
- Therapies based on correcting a specific defect may have higher activity
  - *Non-corrective immune enhancers vs “corrective” immunotherapy*

Composition of the tumor microenvironment (TME)

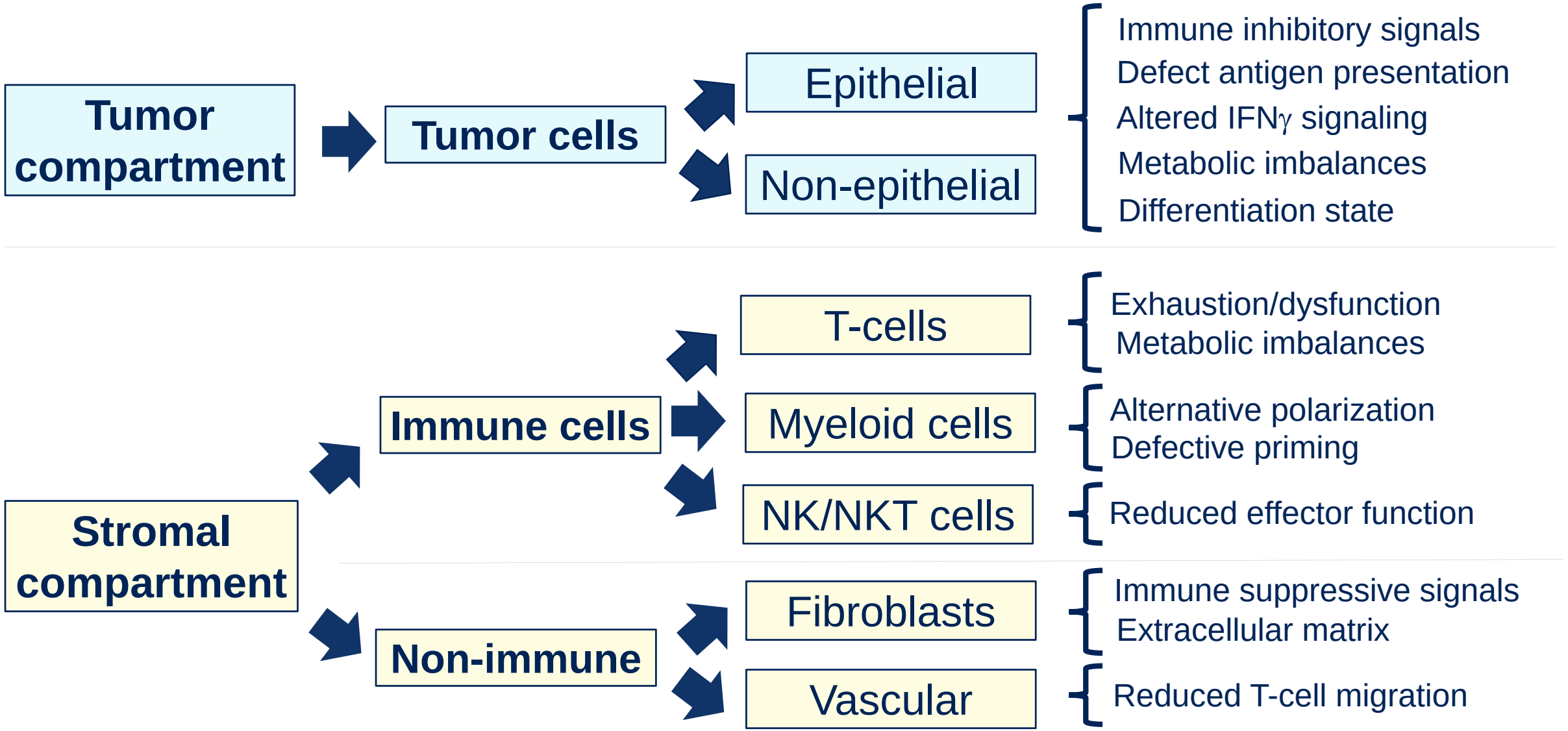




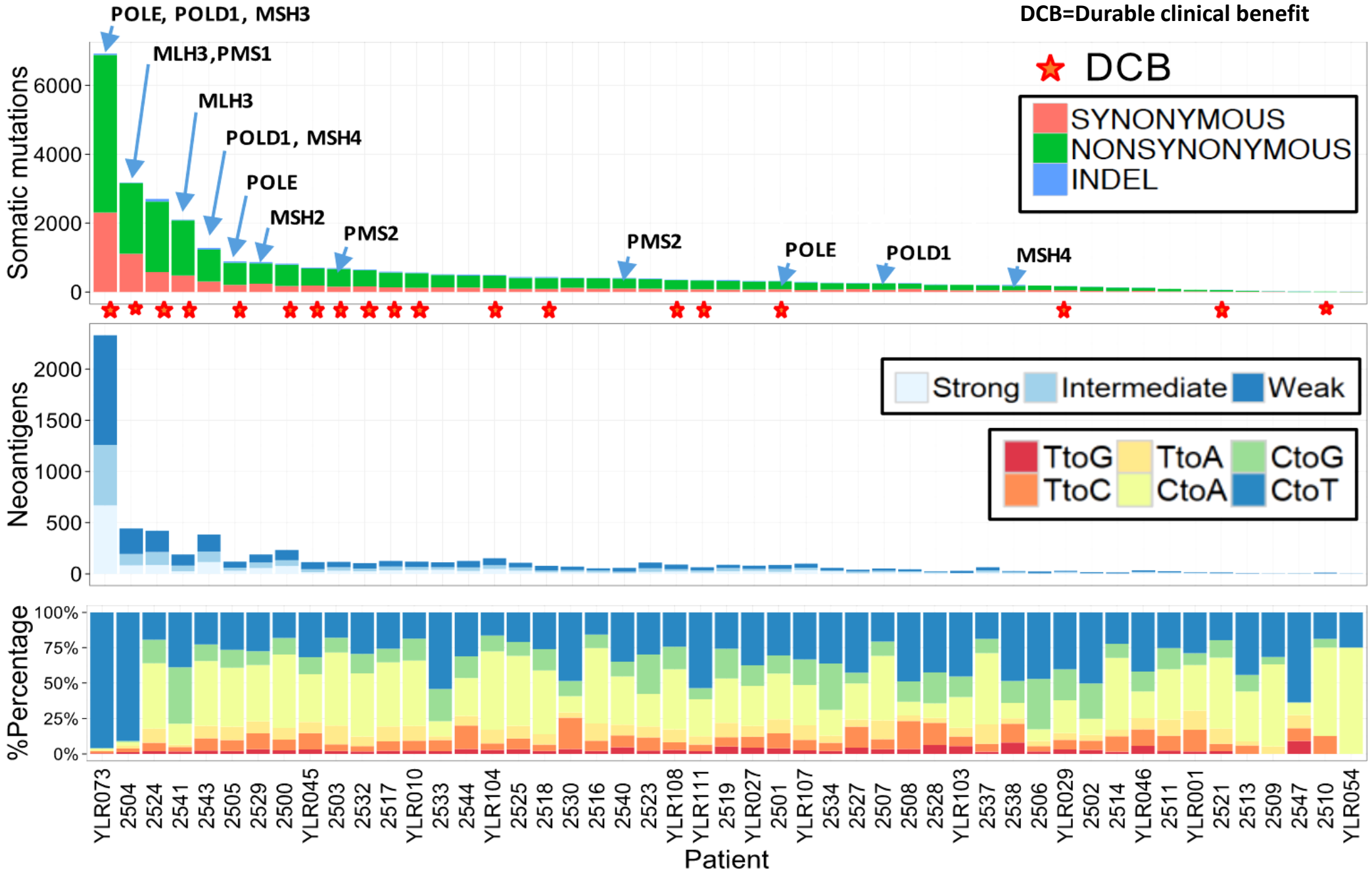
Kerkar and Restifo 2012, Can Res

1. <u>Tumor compartment</u>= Epithelial tumor cells
2. <u>Non-tumor compartment</u>= Immune and non-immune cells

TME components and IO sensitivity & resistance

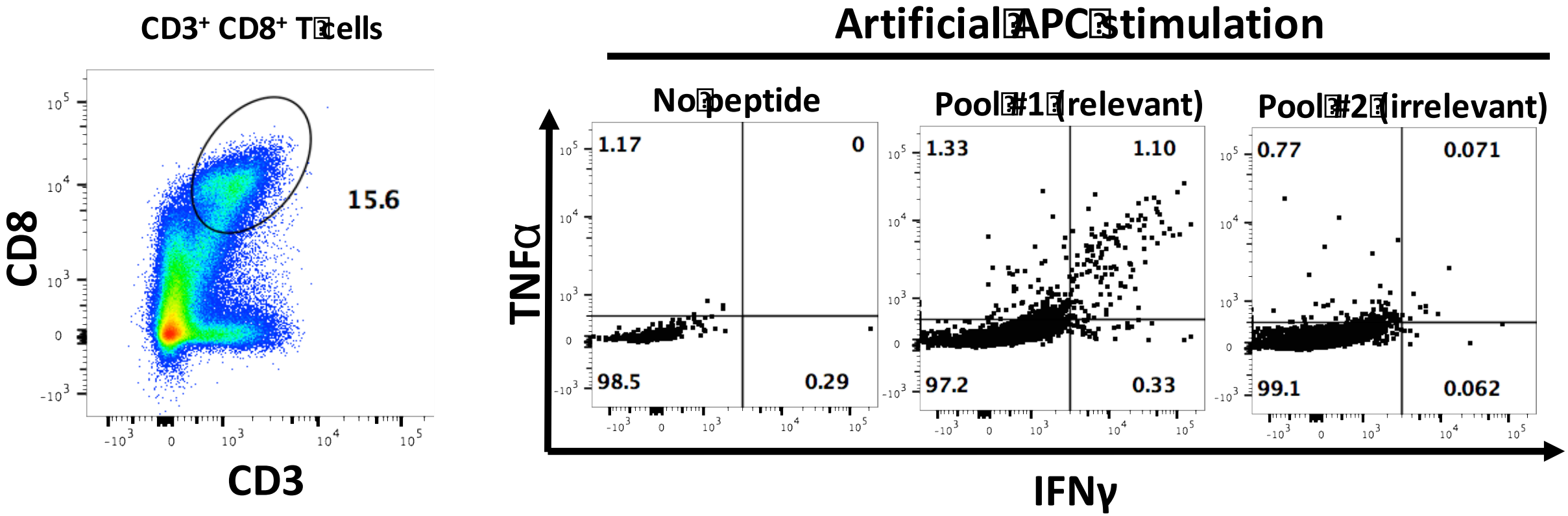


Tumor cells



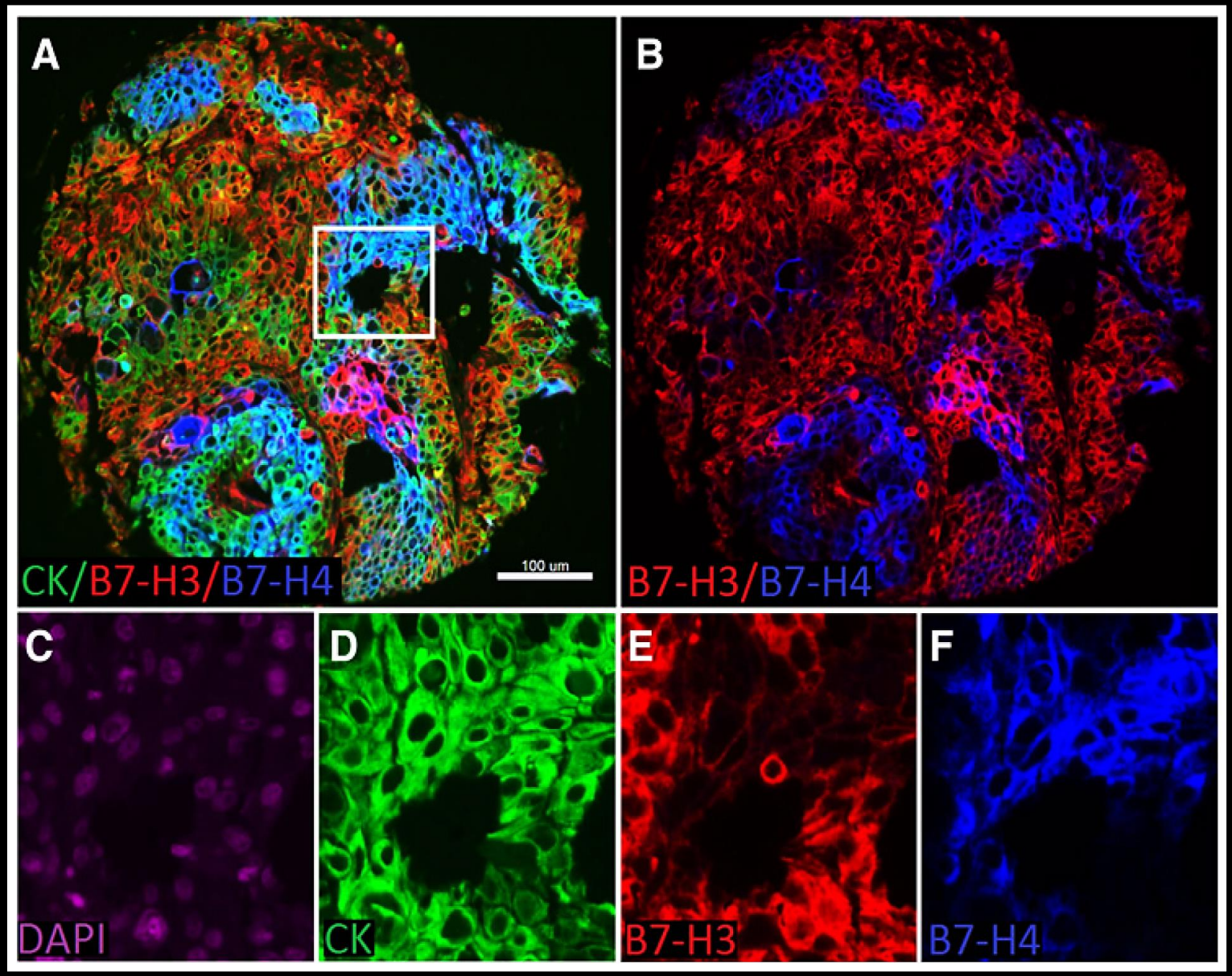
Gettinger et al., 2018 Nat Comm

In vitro CD8 T-cell stimulation by mutant neopeptides

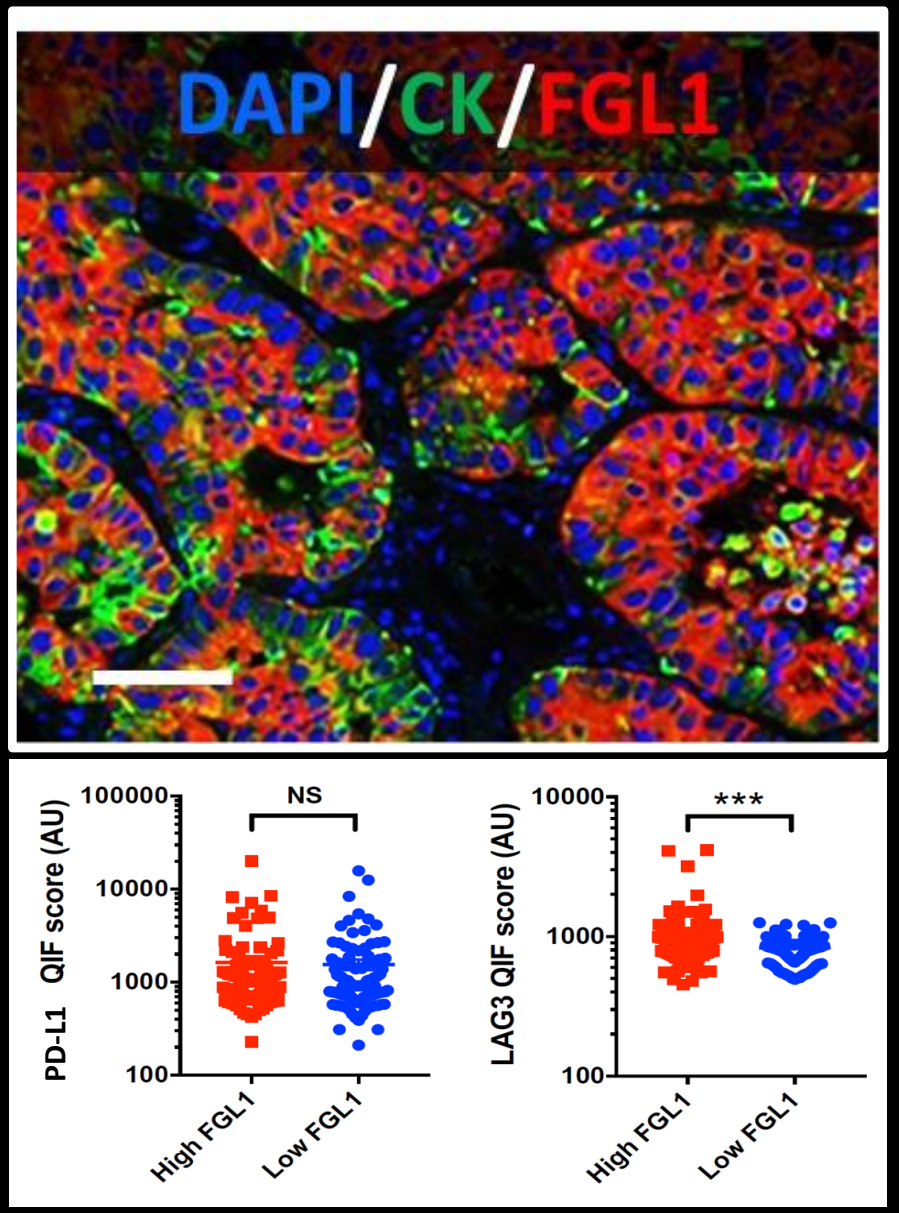


Pepitde pool #1: TMOD4, TENM2, MDM2, NXPE1, RHOT2(A), RHOT2(I), KIF5A, ARHGAP9

Gettinger et al., 2018 Nat Comm

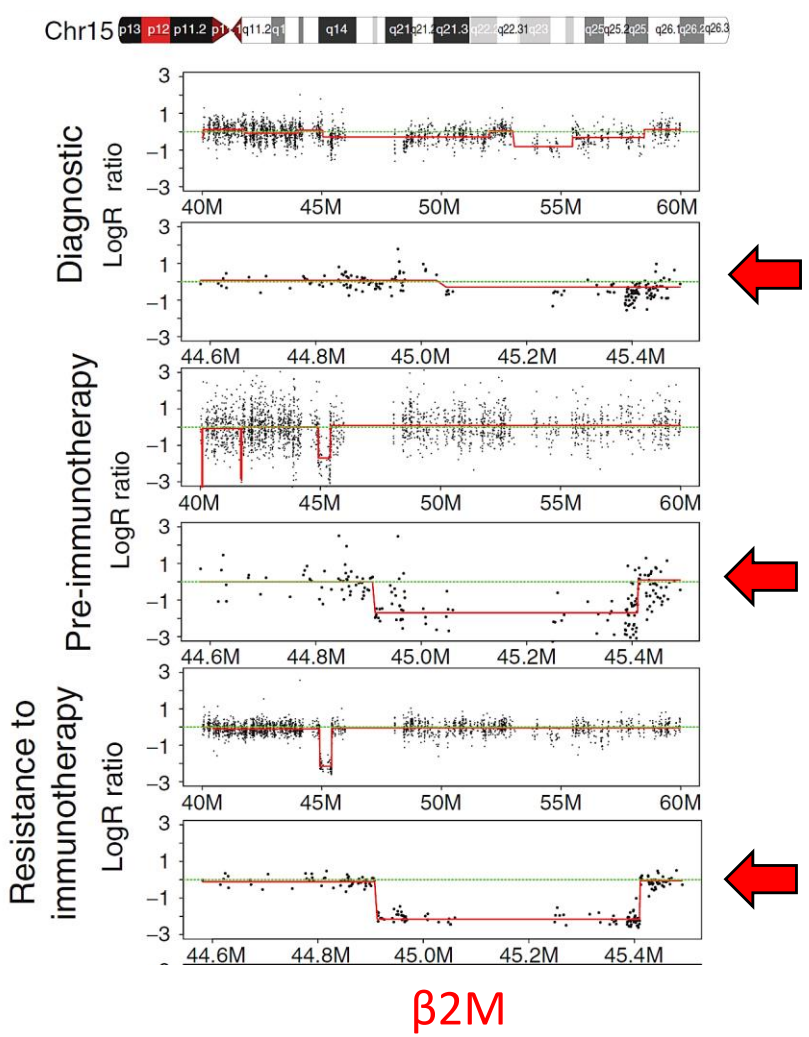


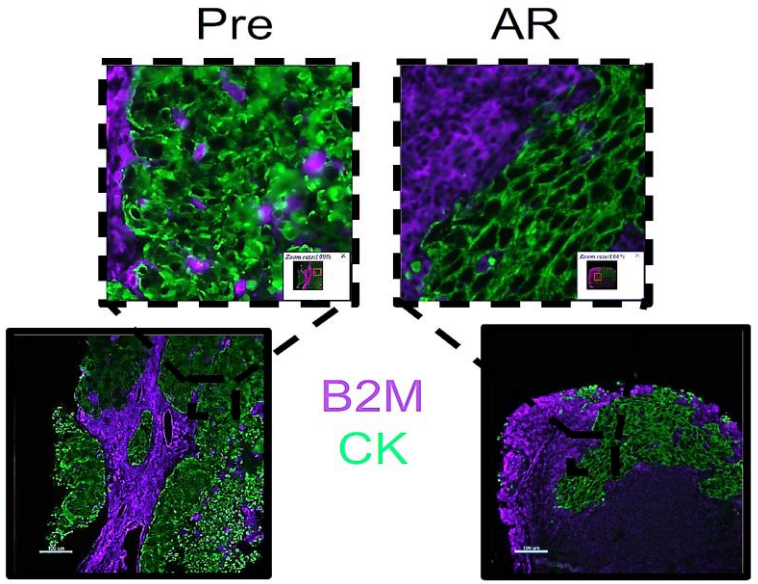
Altan et al., 2017 Clin Can Res

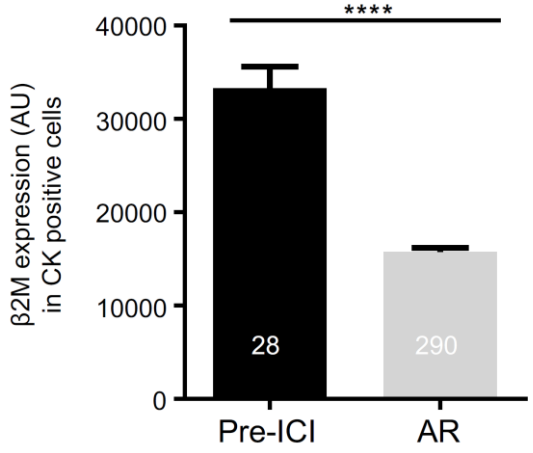


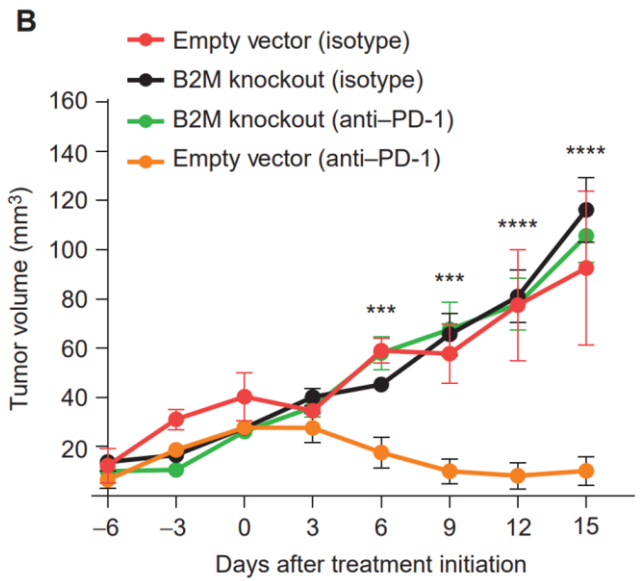
Wang et al. 2019, Cell

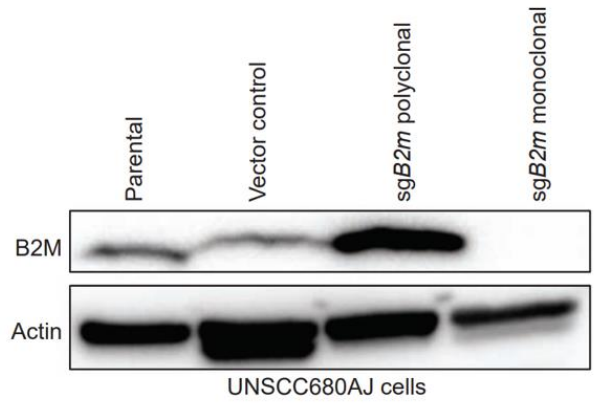
β2M loss and acquired resistance to IO in NSCLC



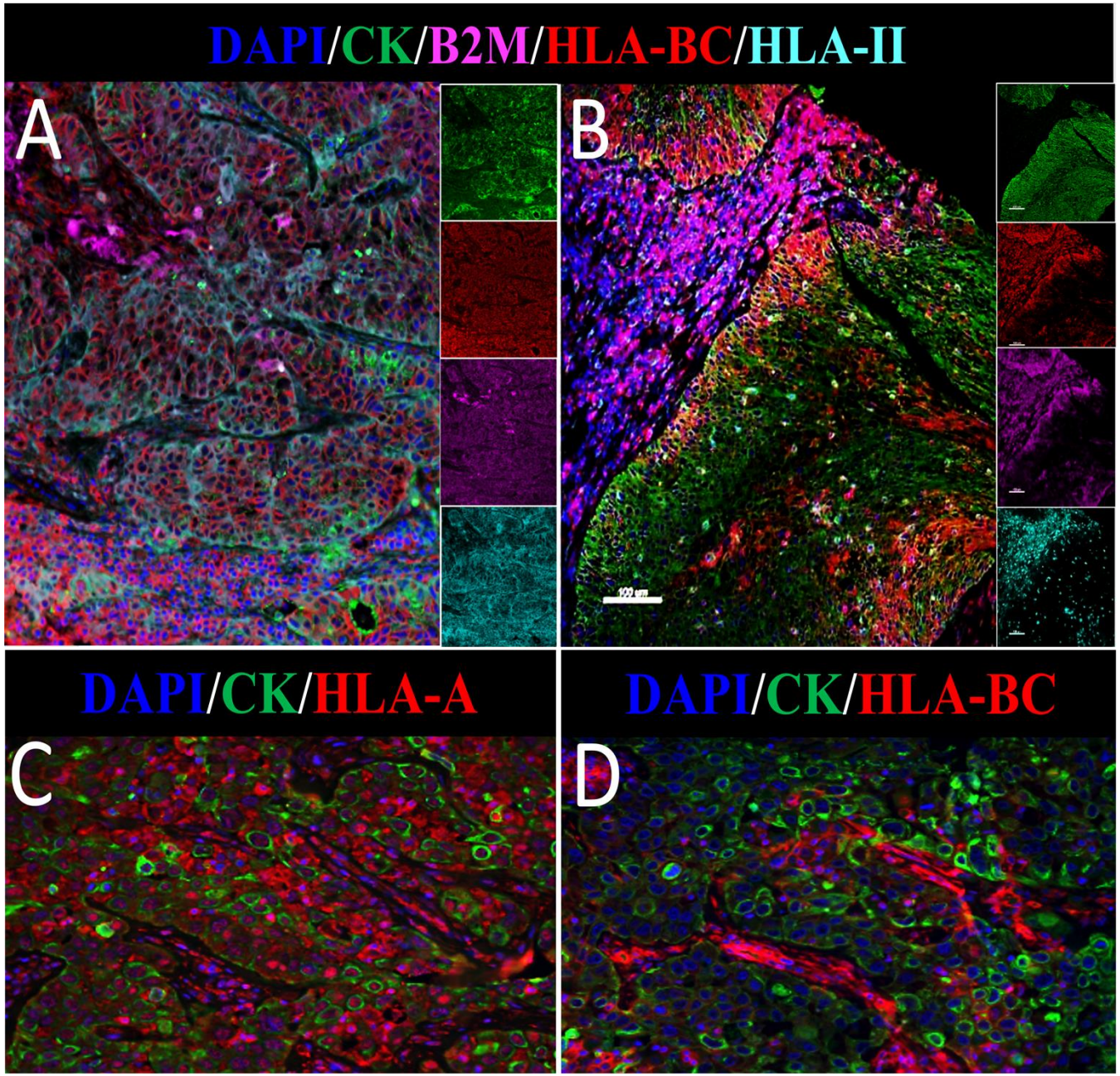




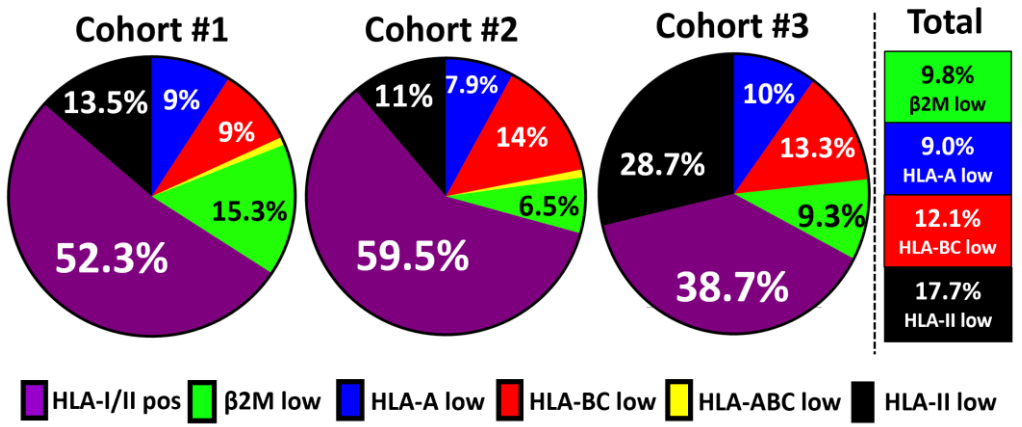




Gettinger et al., Cancer Discovery 2017

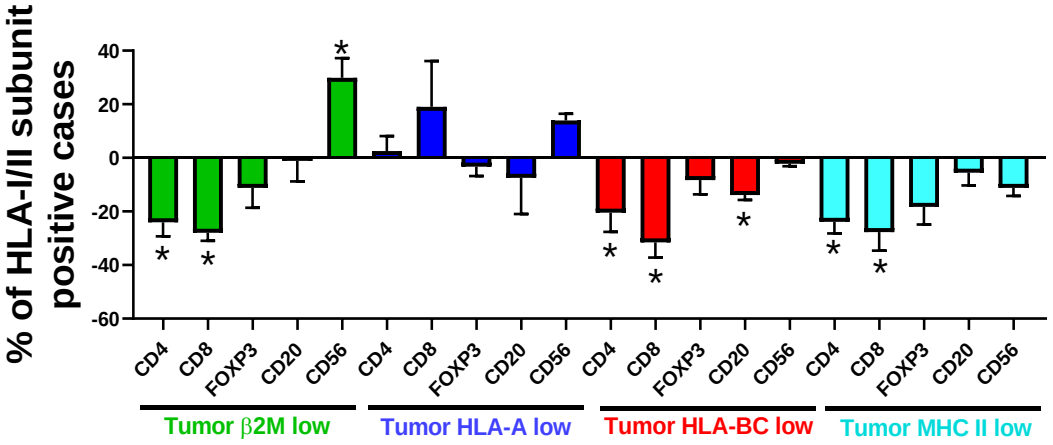


HLA class-I/II APM defects in IO naïve NSCLC



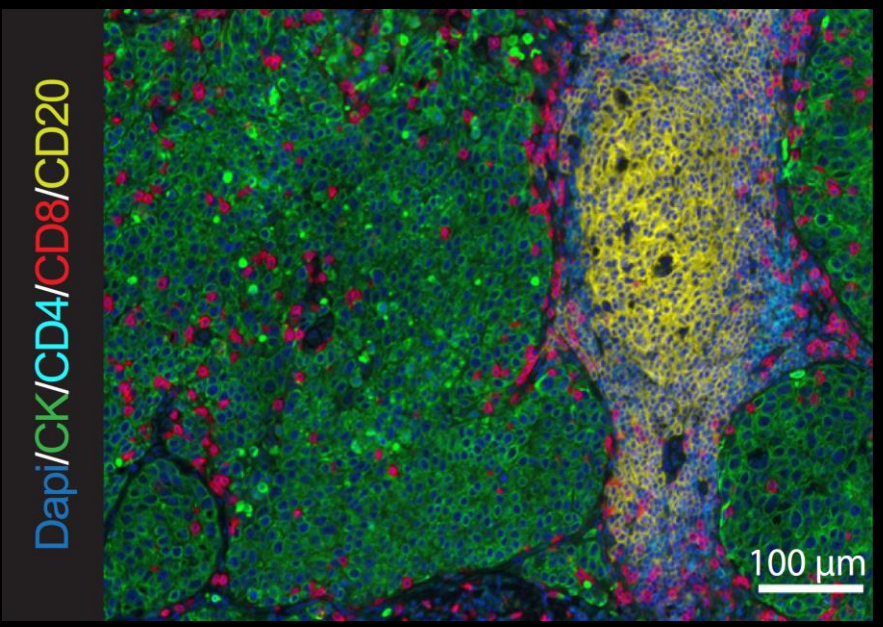
Deleterious mutations in <5% of NSCLCs

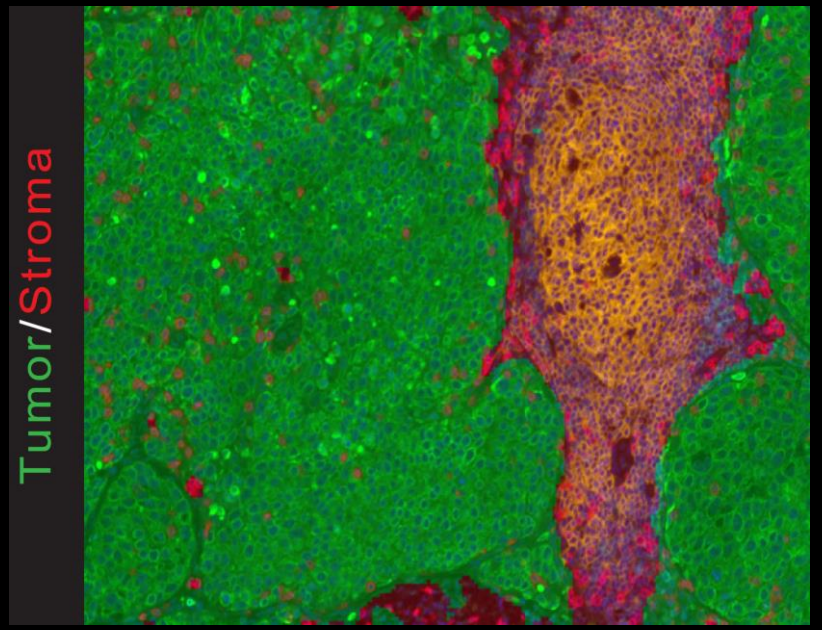
Immune contexture of HLA-I/II deficient NSCLS

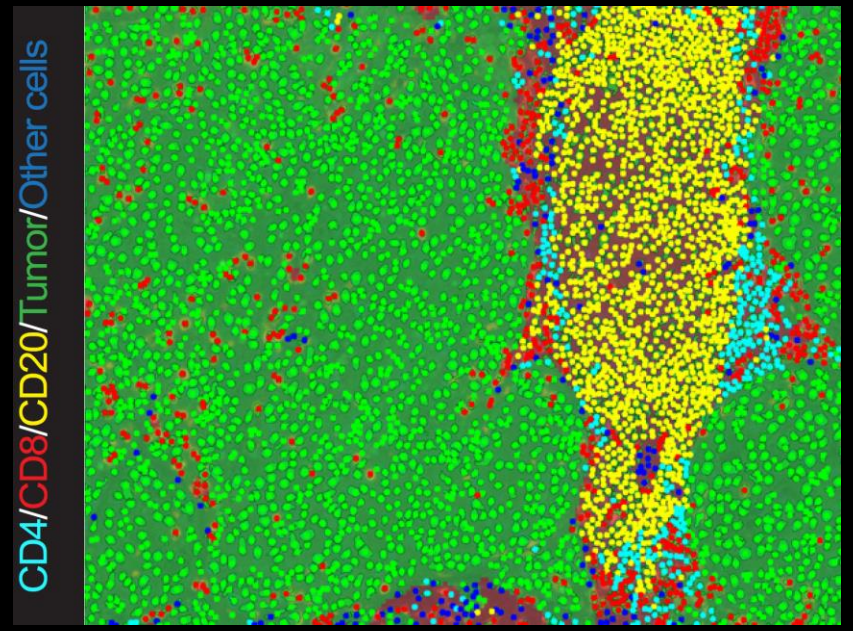


Datar I., Hauc S. et al., 2021 Clin Can Res

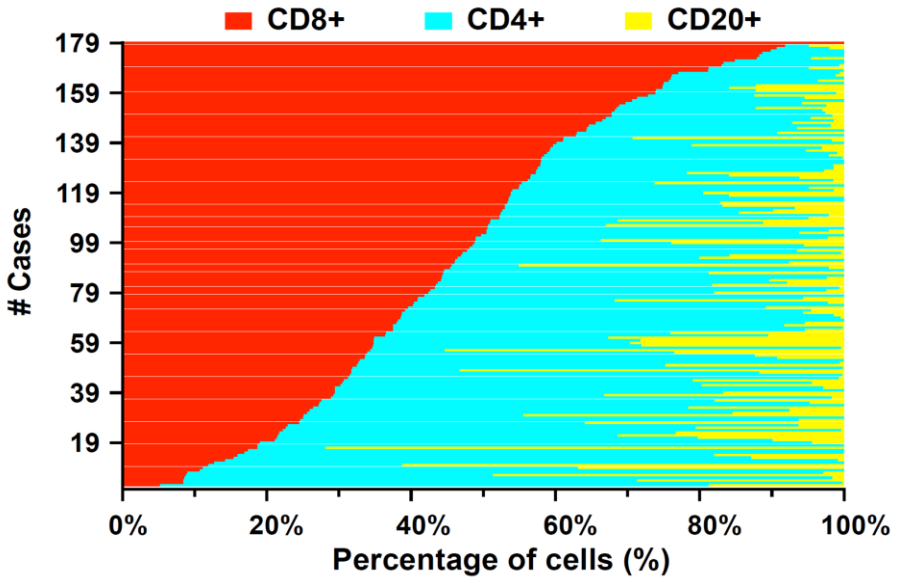
Non-tumor cells (T-cells)



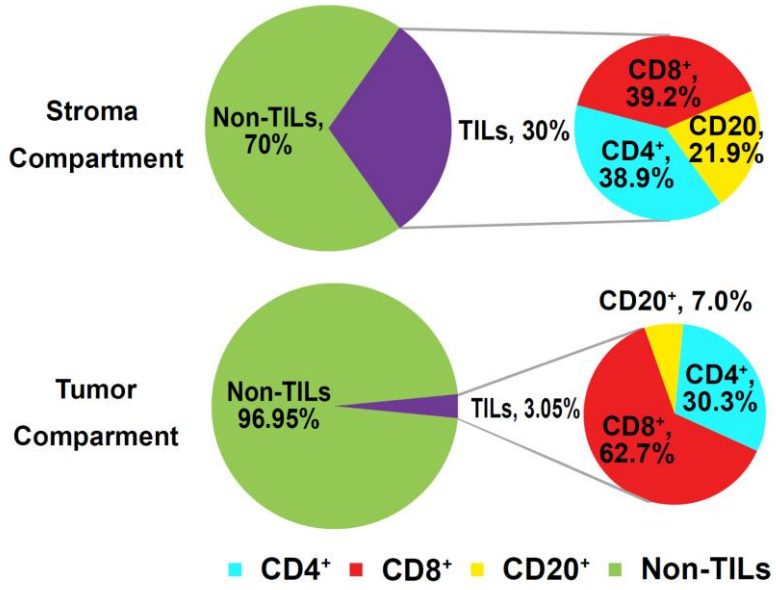




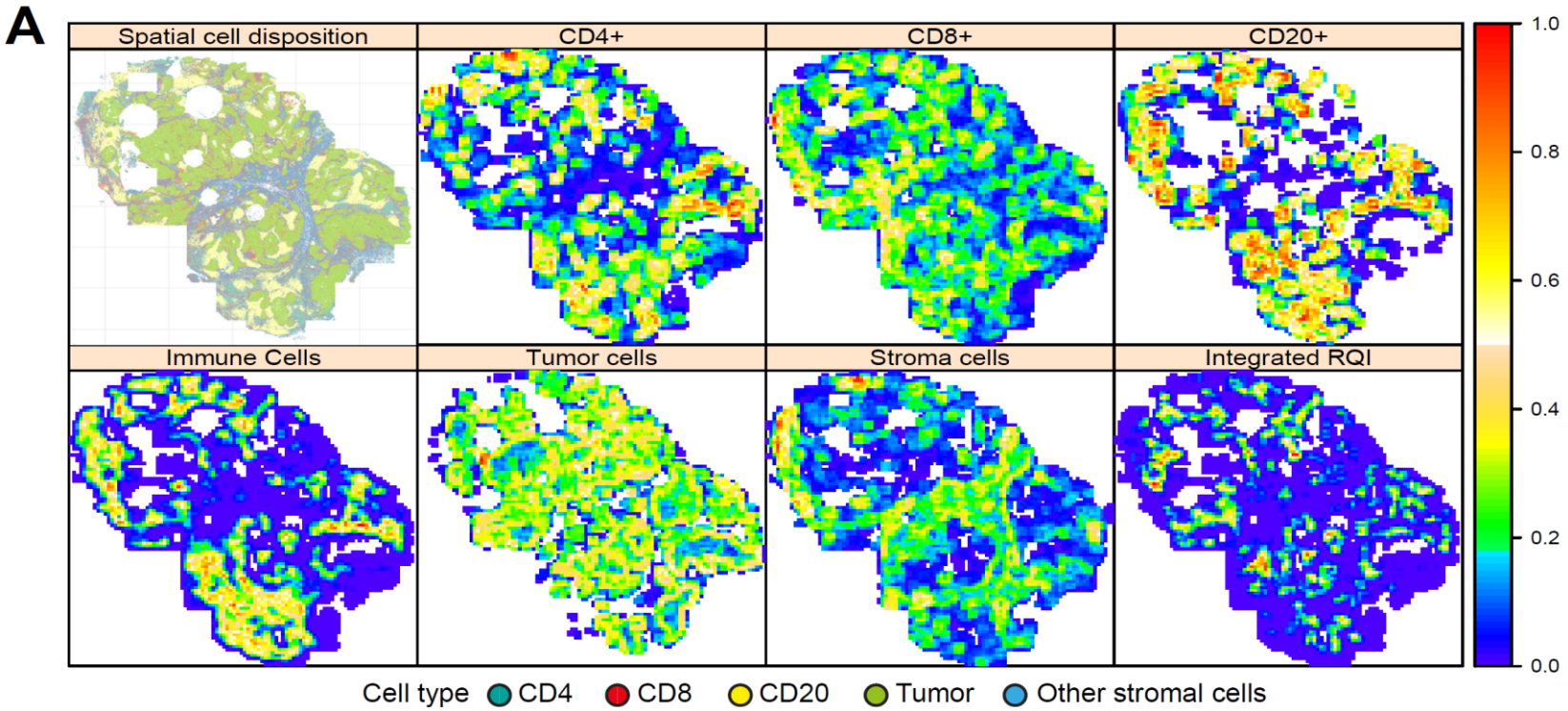
TILs in 179 NSCLCs

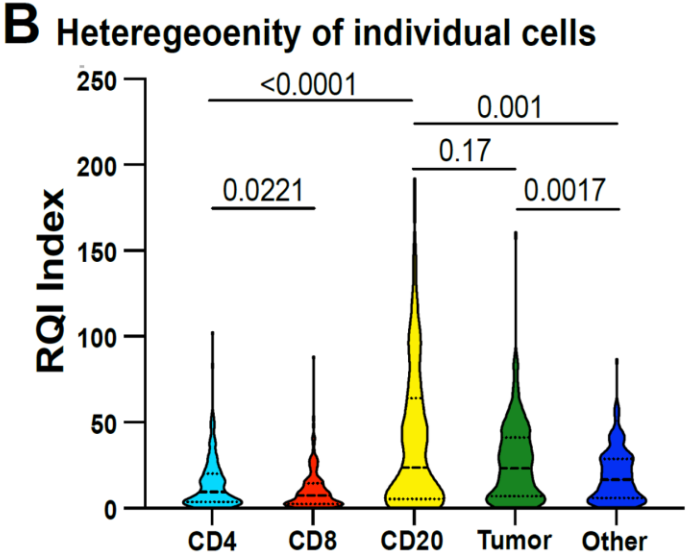


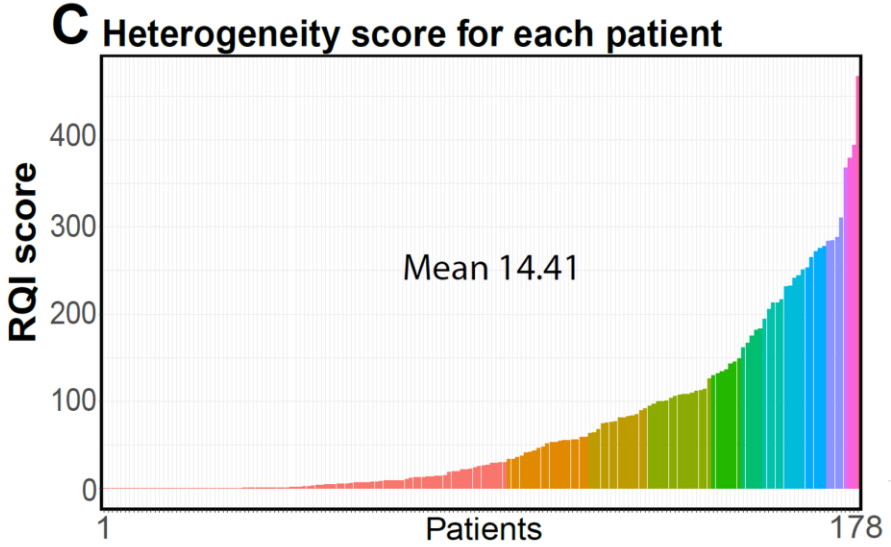
Spatial distribution

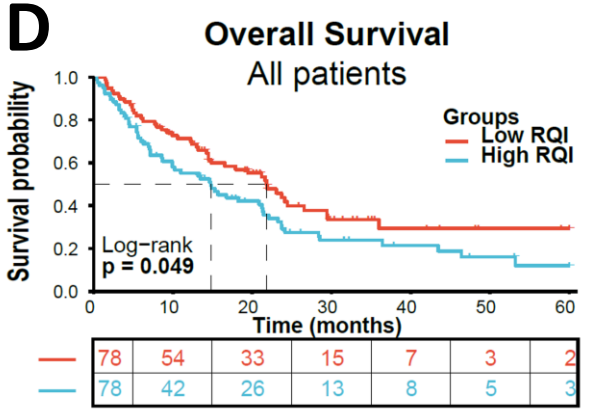


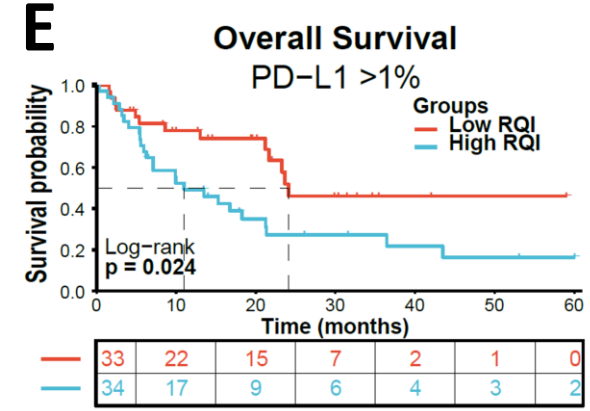
Lopez de Rodas et al., 2022 In Revision

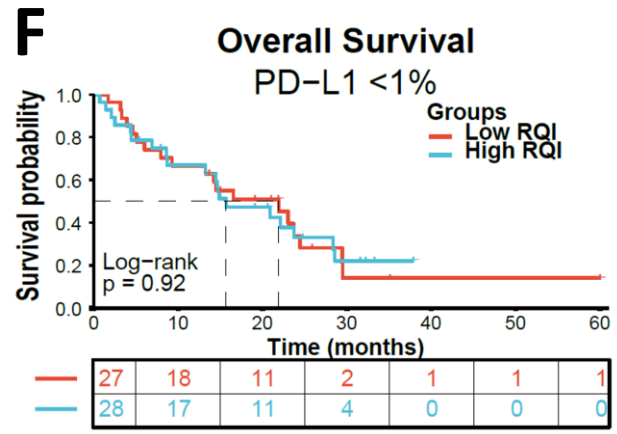




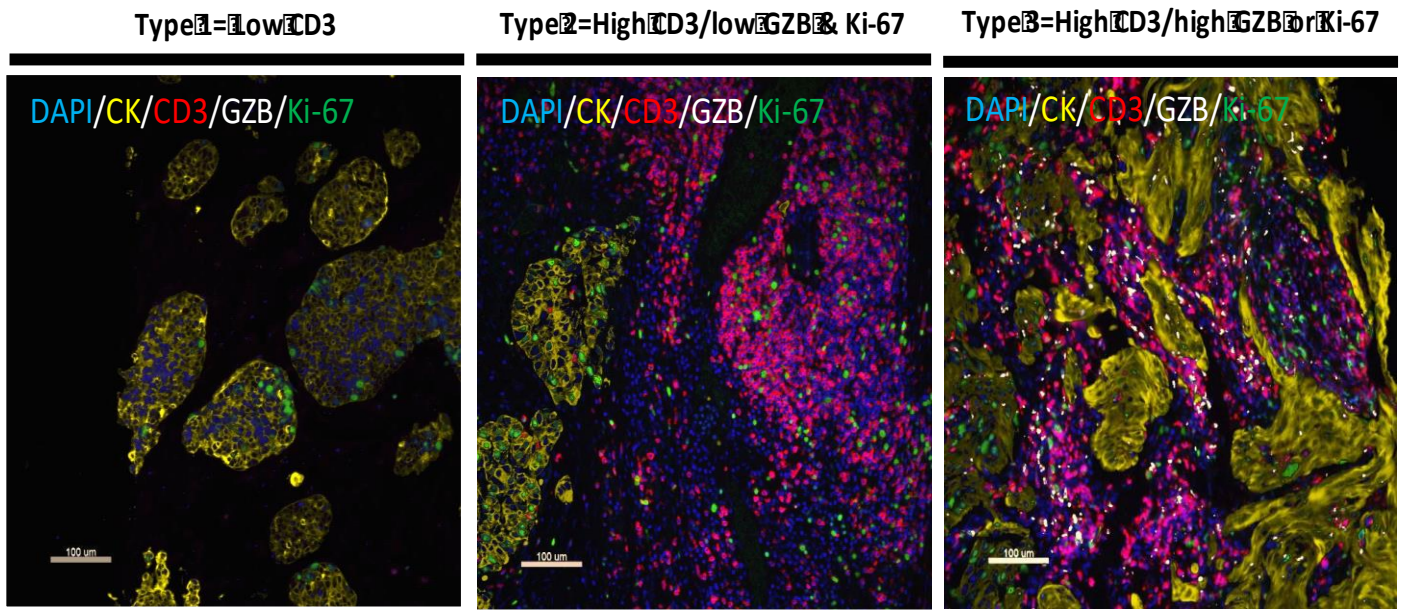




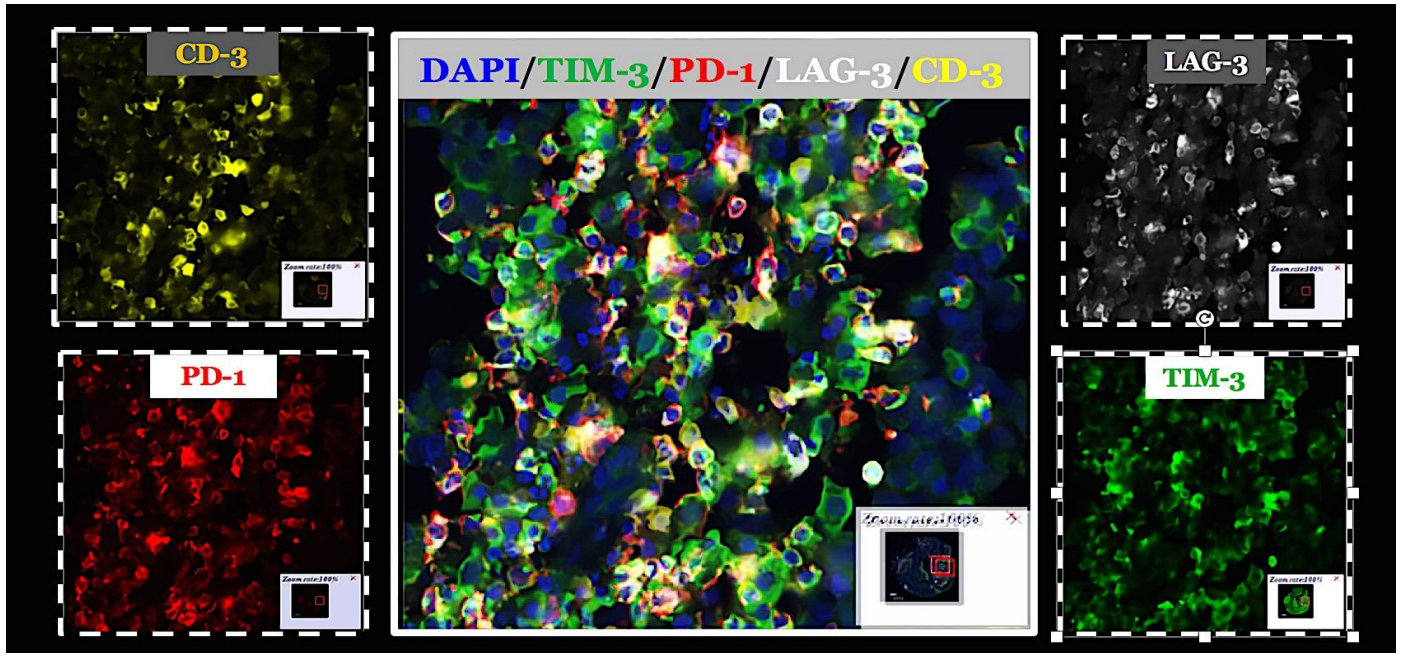




Lopez de Rodas et al., 2022 In Revision

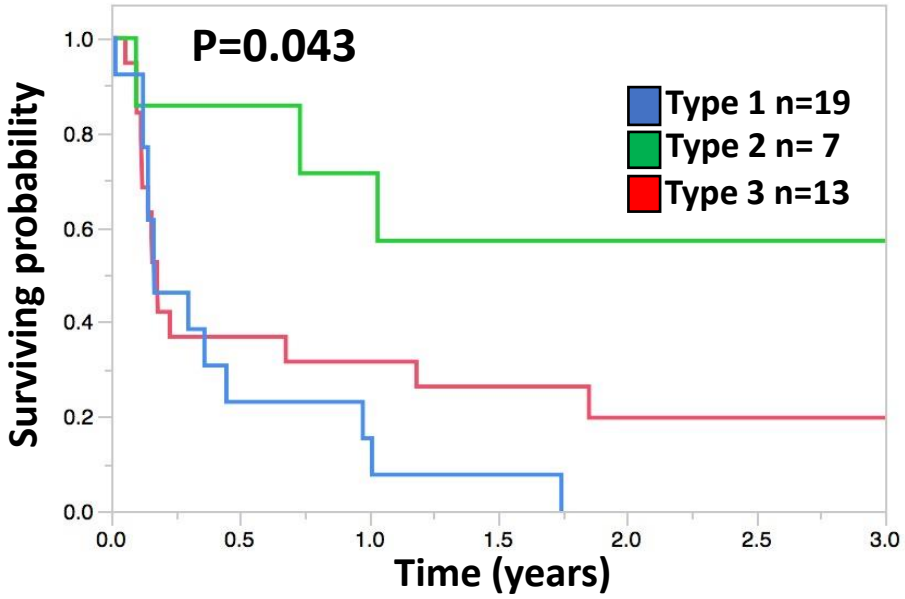


Gettinger et al., 2018 Nat Comm

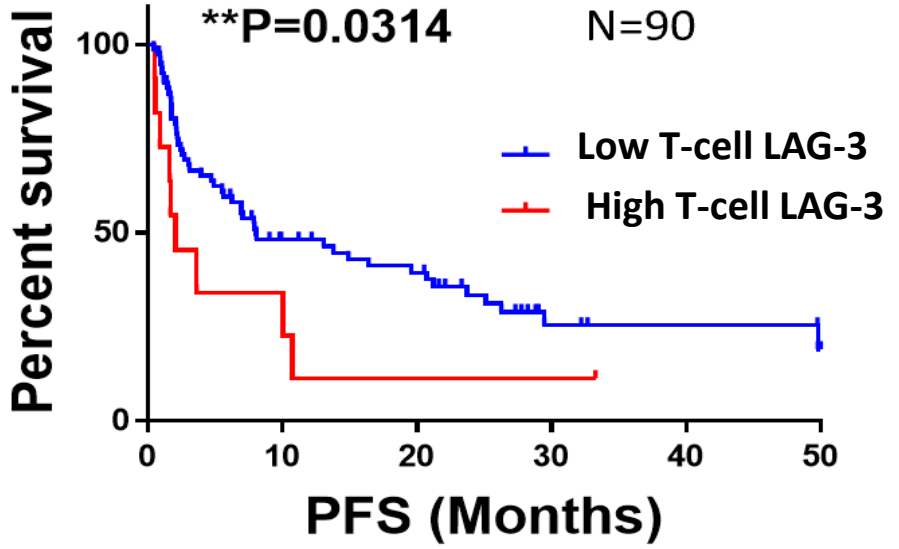


Datar et al., 2018 Clin Can Res

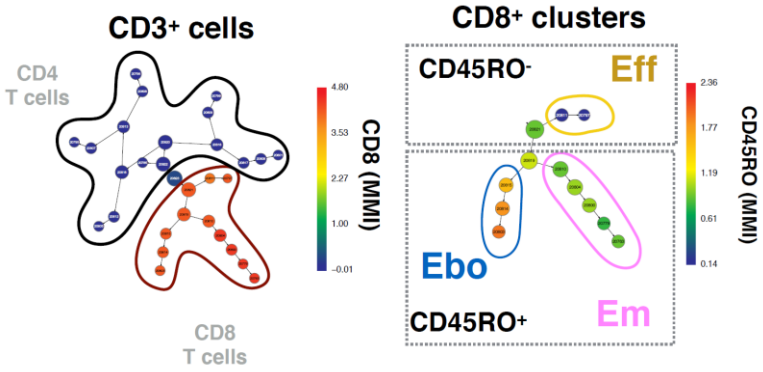
Progression-free survival

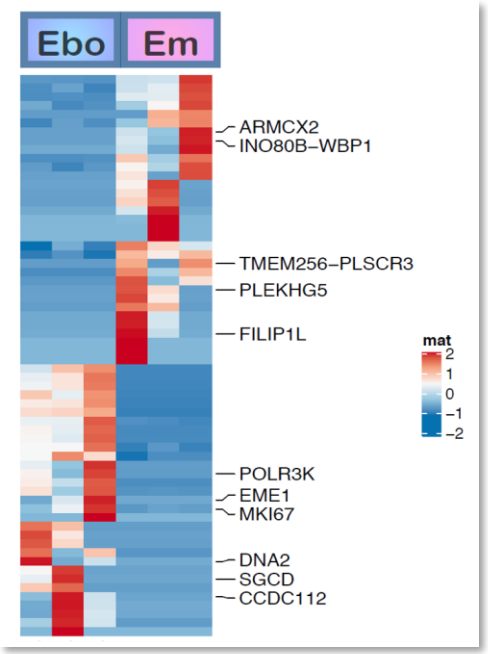


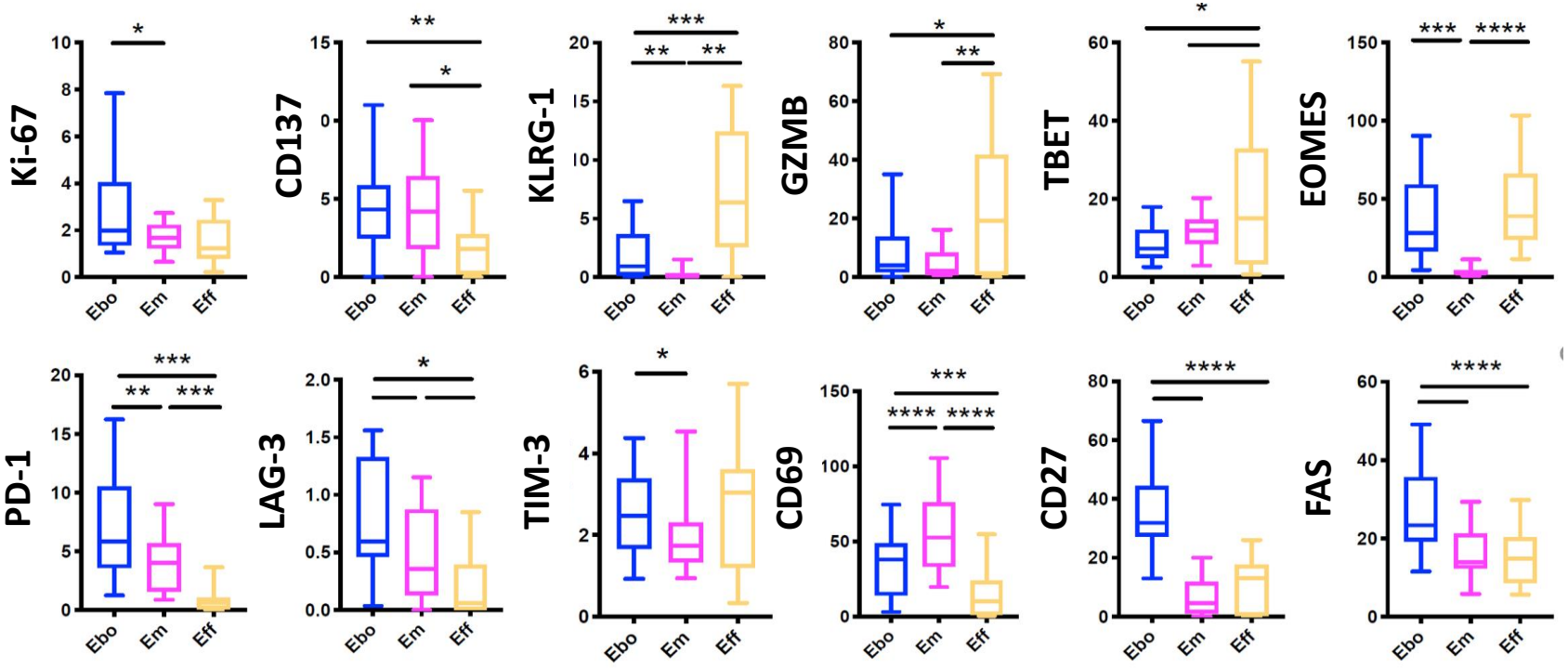
Progression-free survival

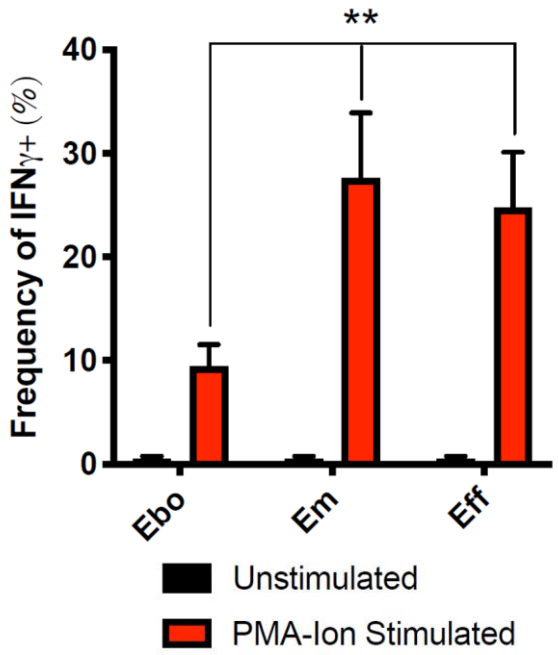


T-cell dysfunction in human NSCLC (Ebo)



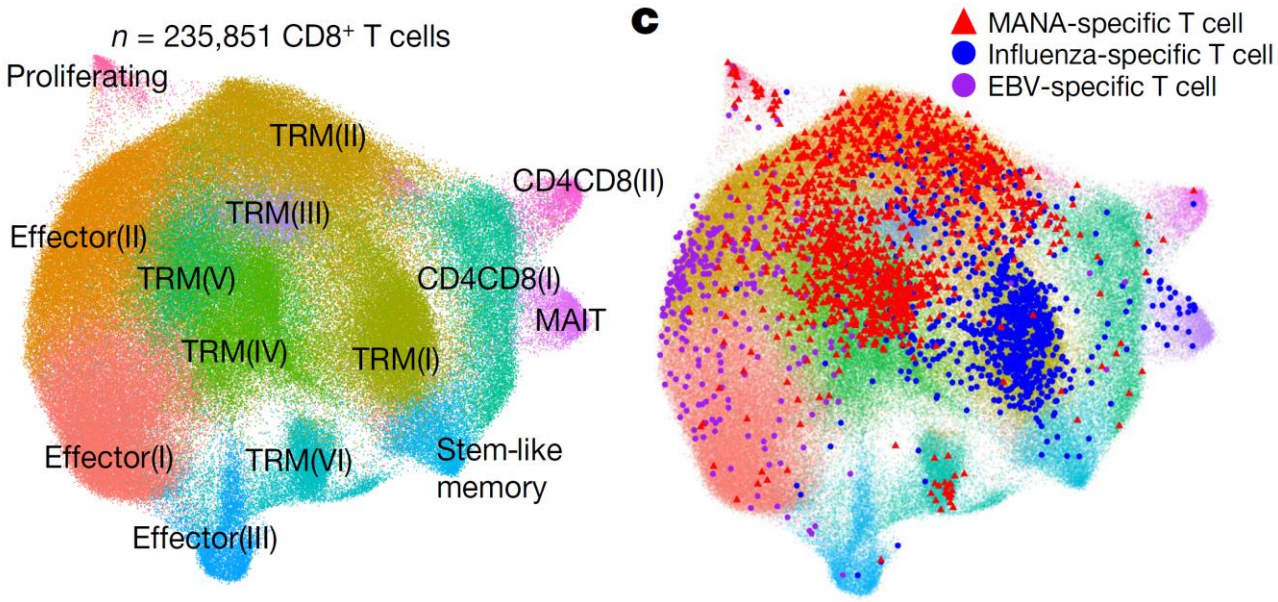


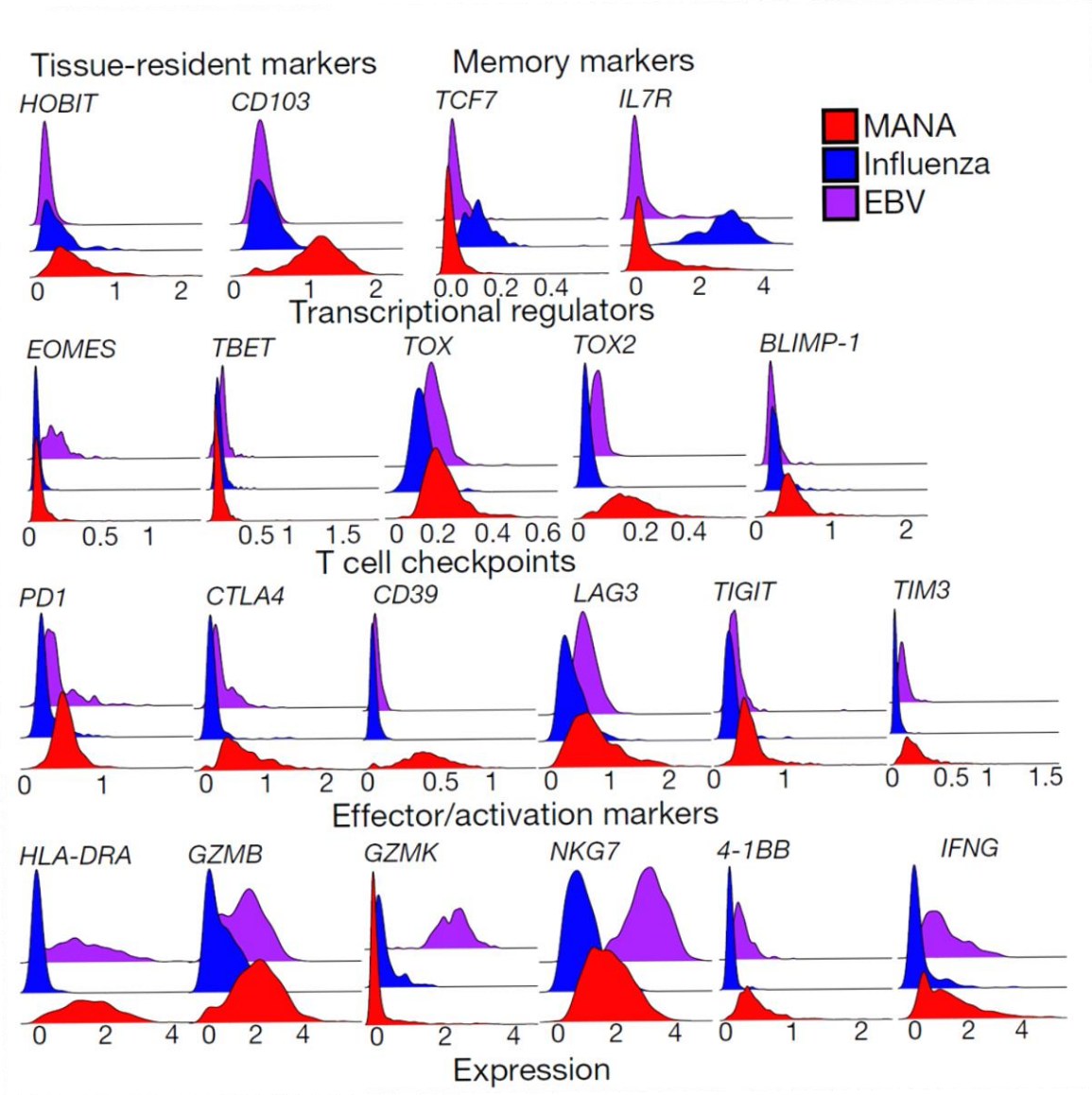




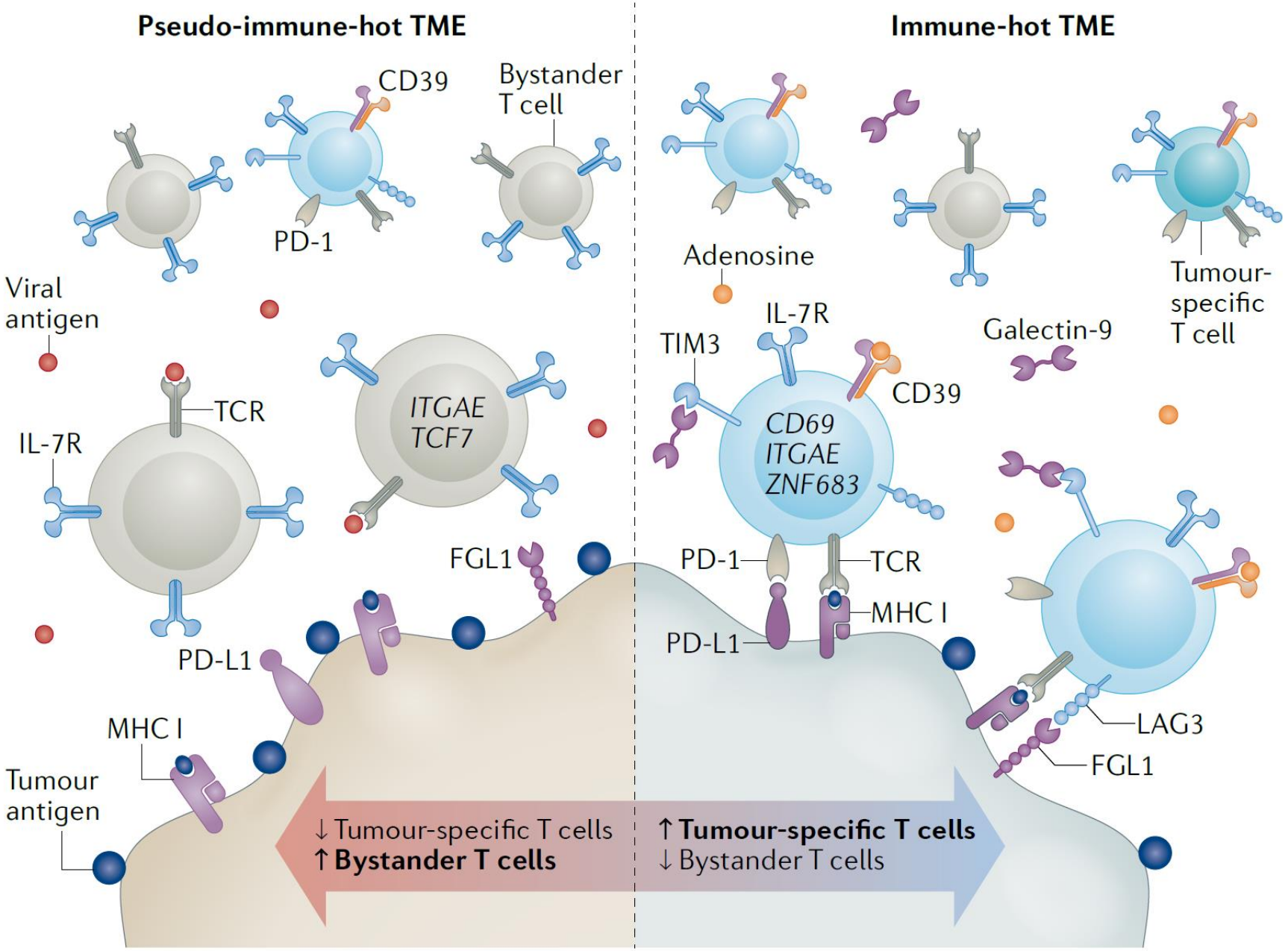
Sanmamed, Nie, Desai et al., 2021 Can Discovery

Tumor-antigen specific CD8+ T-cells in NSCLC by scRNAseq



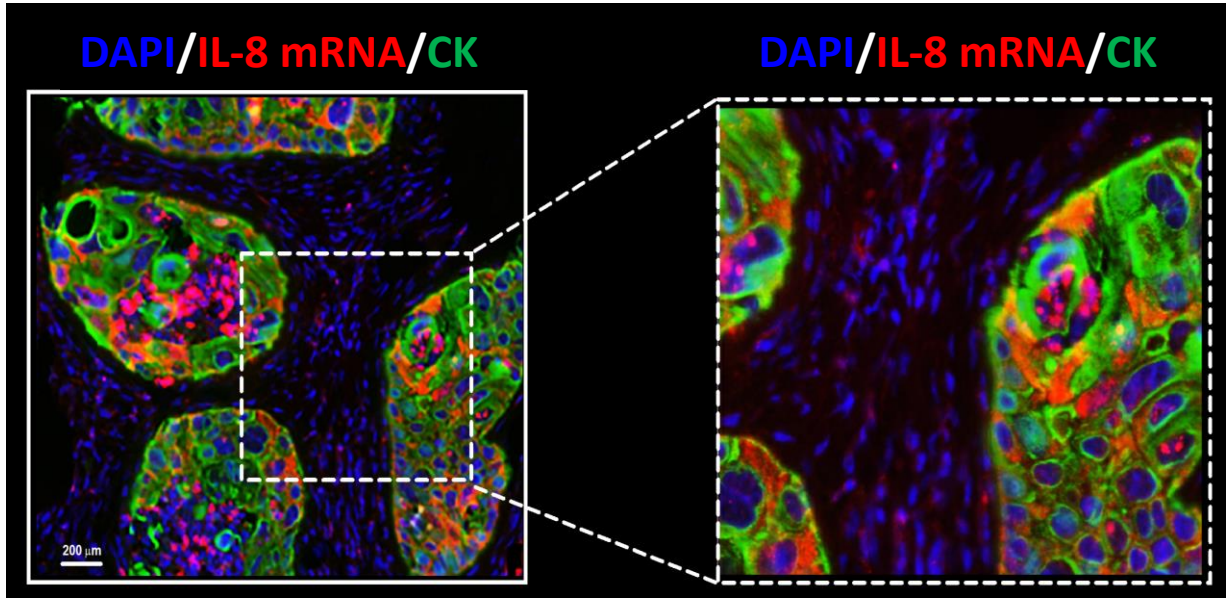


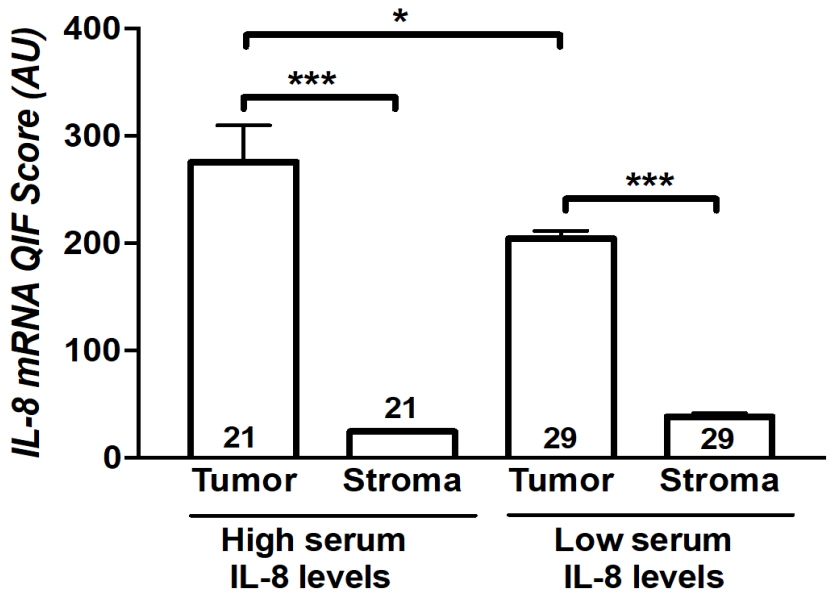
Caushi et al., 2021, Nature

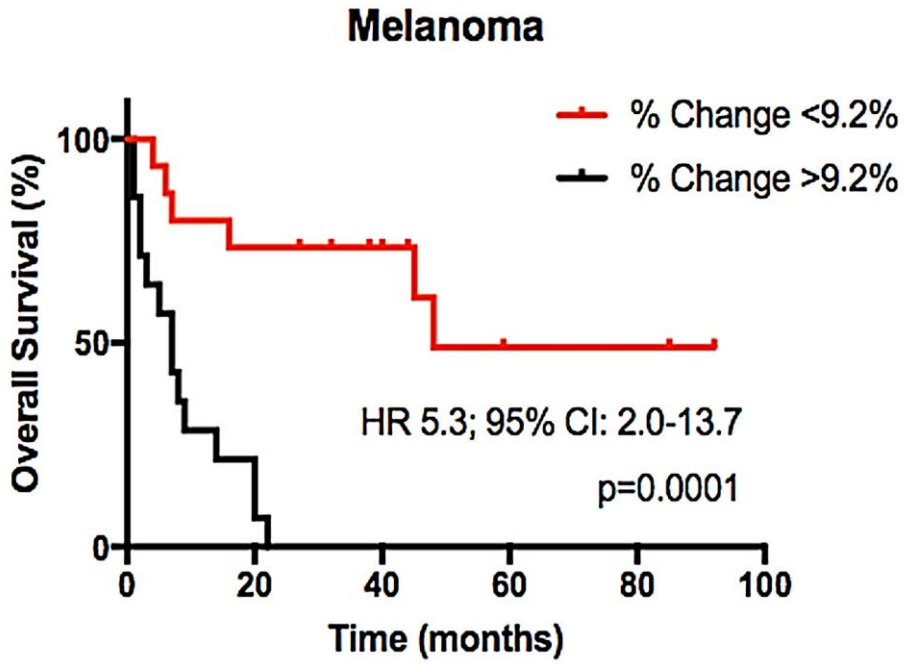


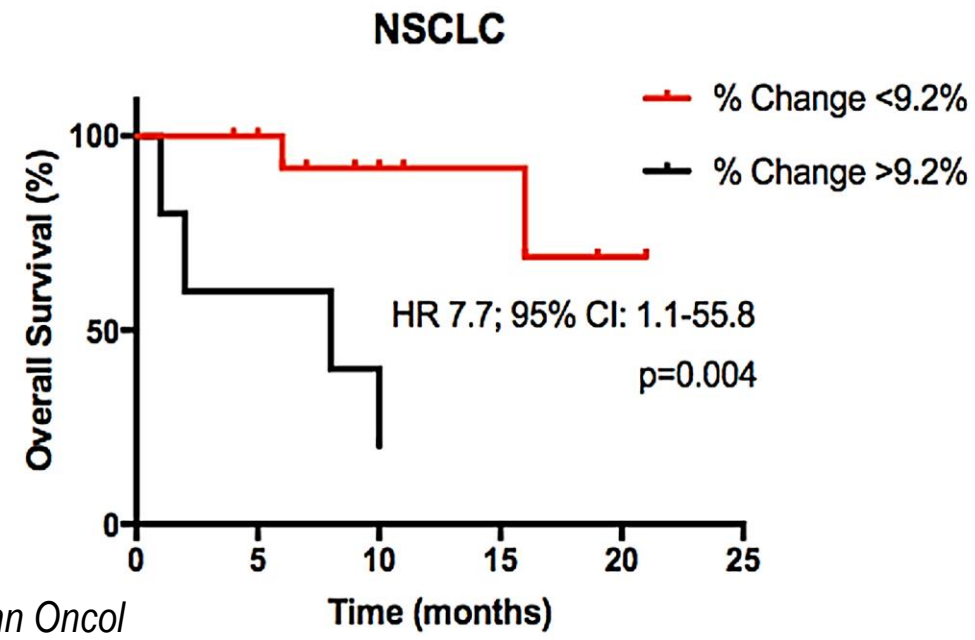
Lopez de Rodas and Schalper. 2021 Nat Rev Clin Onc

Non-tumor cells (Myeloid cells)





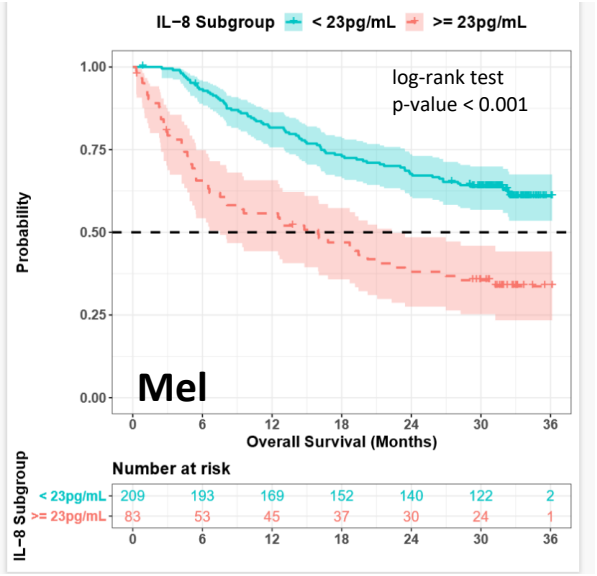




Sanmamed et al., 2017 Ann Oncol

A

CM067-Nivo only



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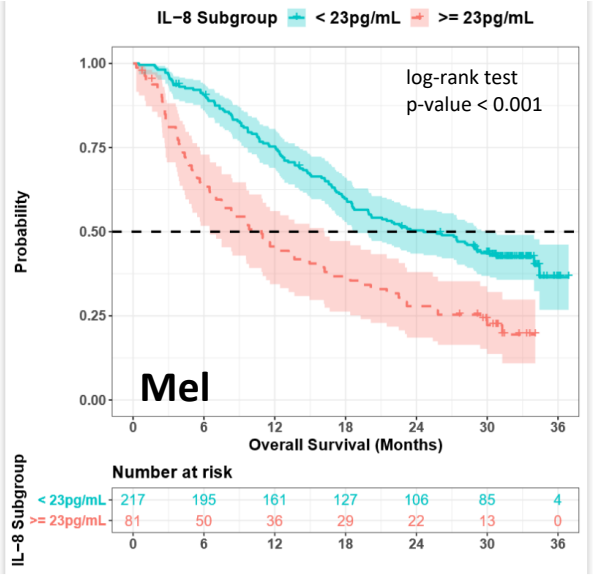
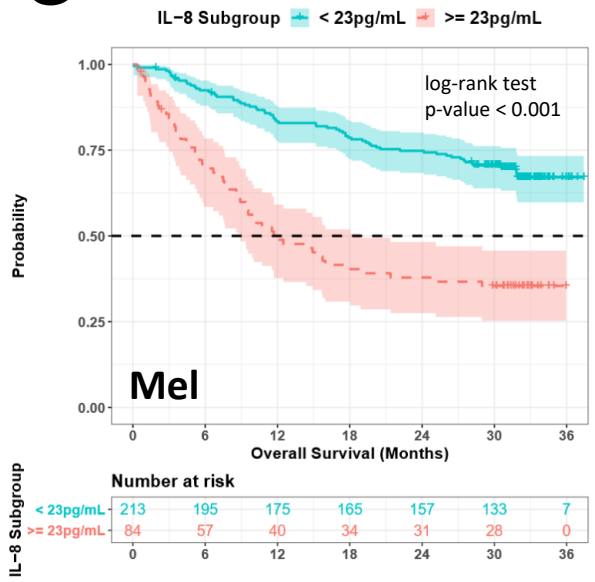
CM067-Ipi only



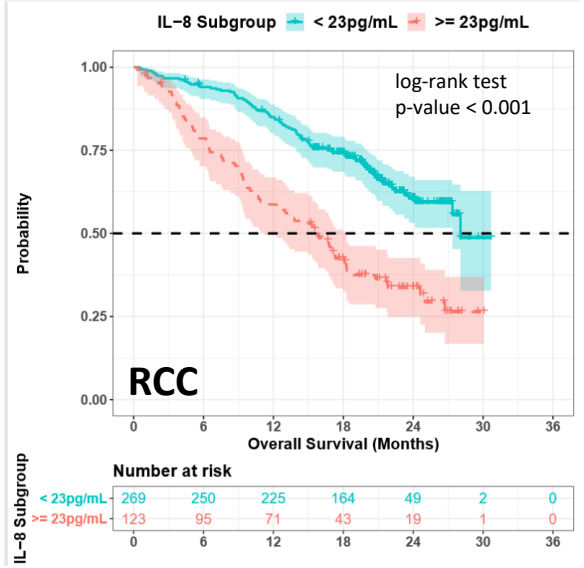
C

CM067-Ipi + Nivo



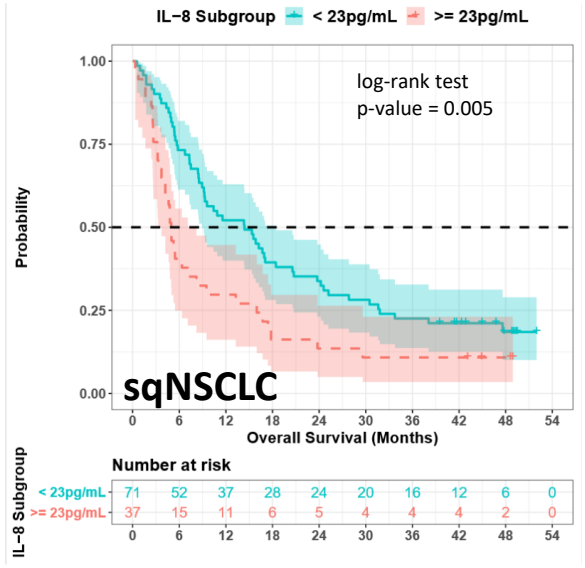
D

CM025-Nivo only



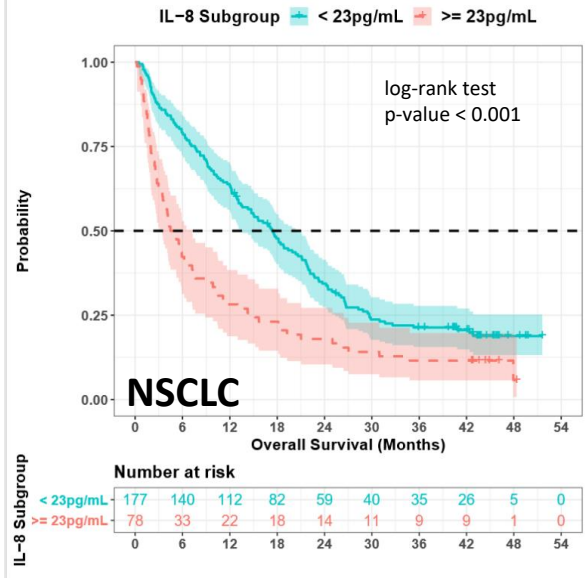
E

CM017-Nivo only



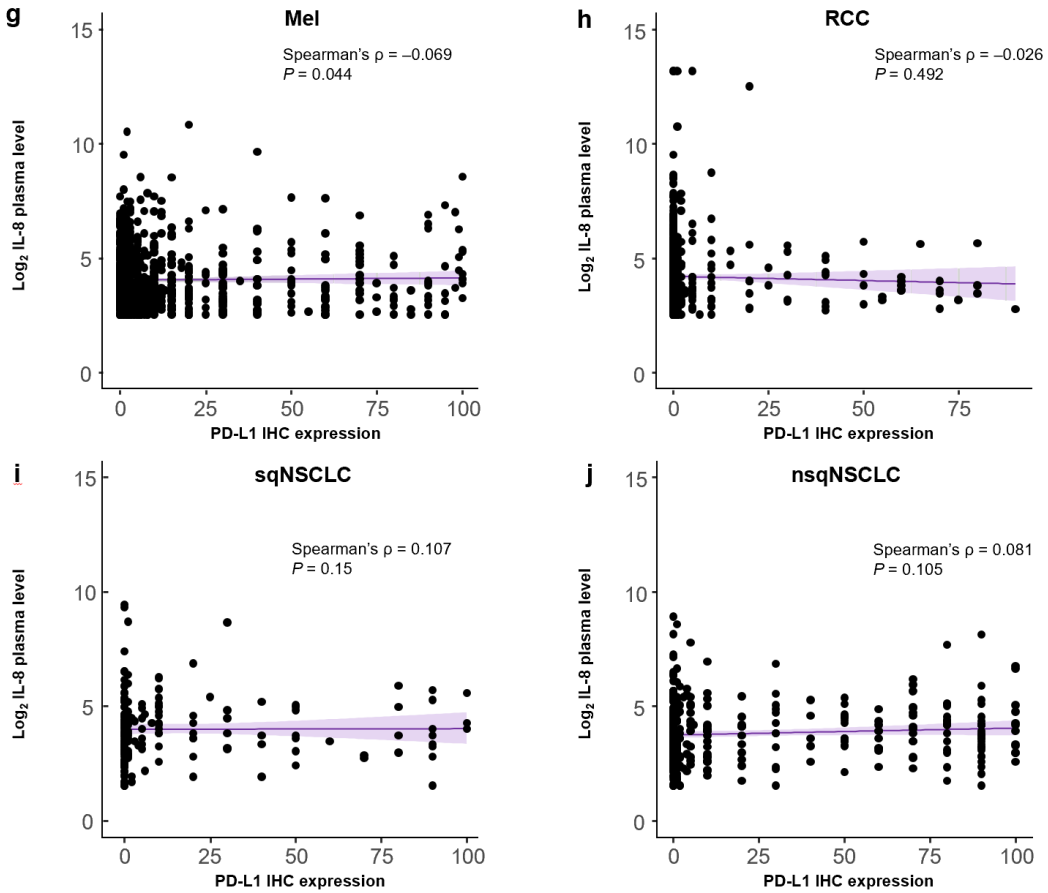
F

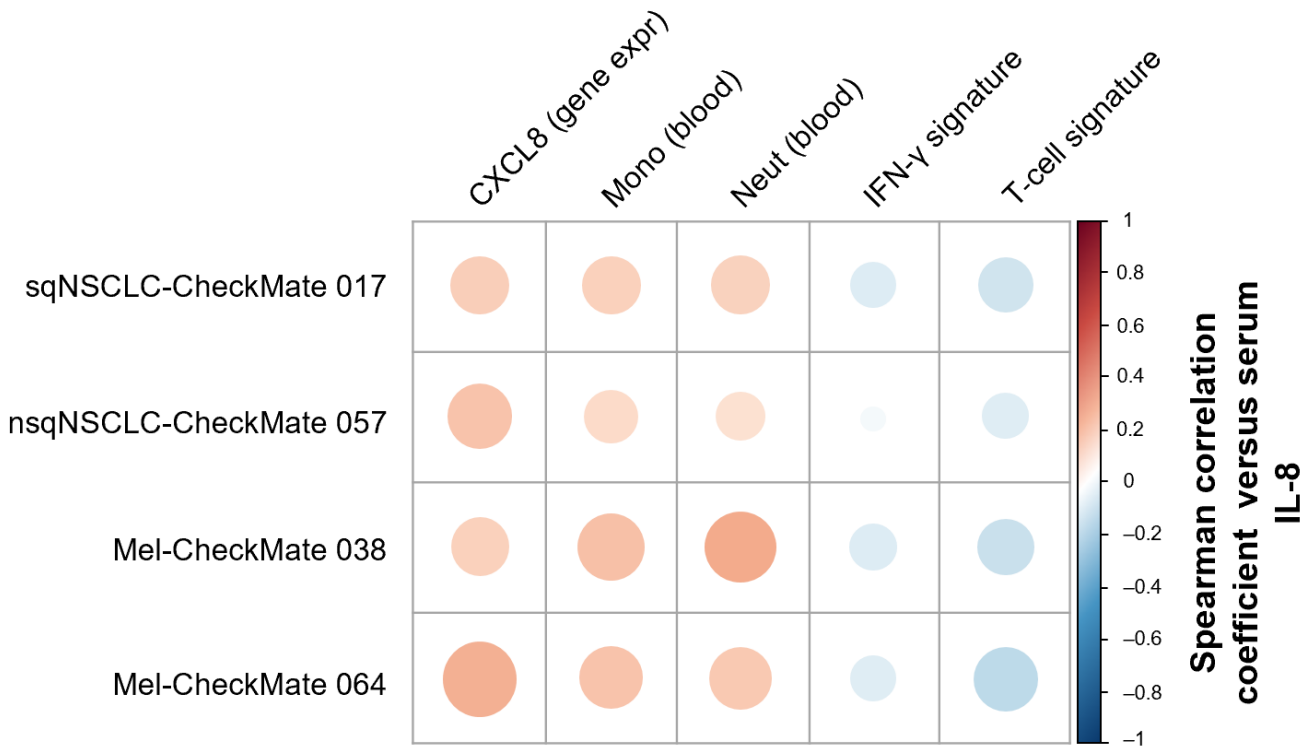
CM057-Nivo only



Schalper et al., 2020 Nat Med

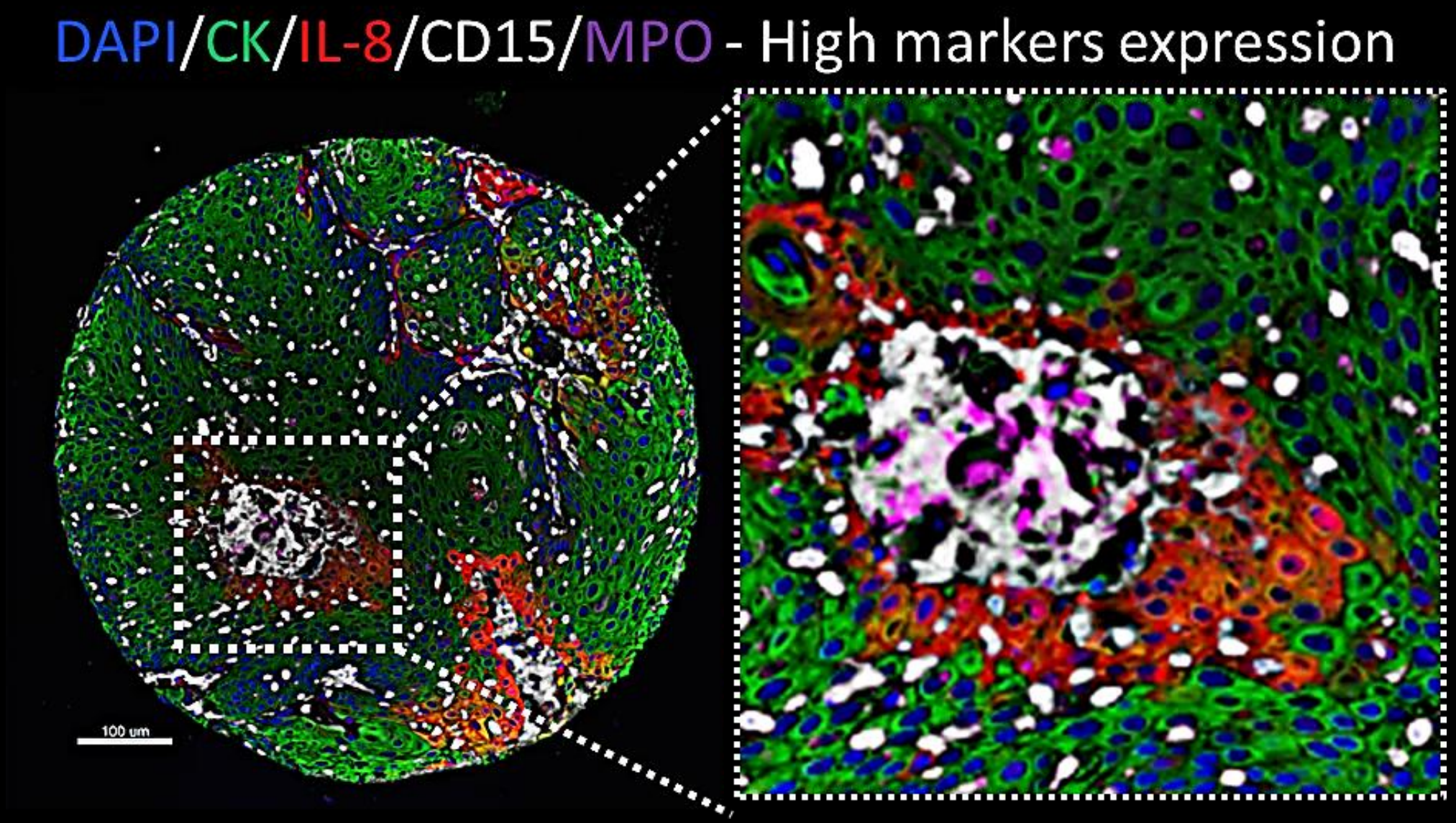
Effect of IL-8 is independent from PD-L1 expression and IFNγ responses

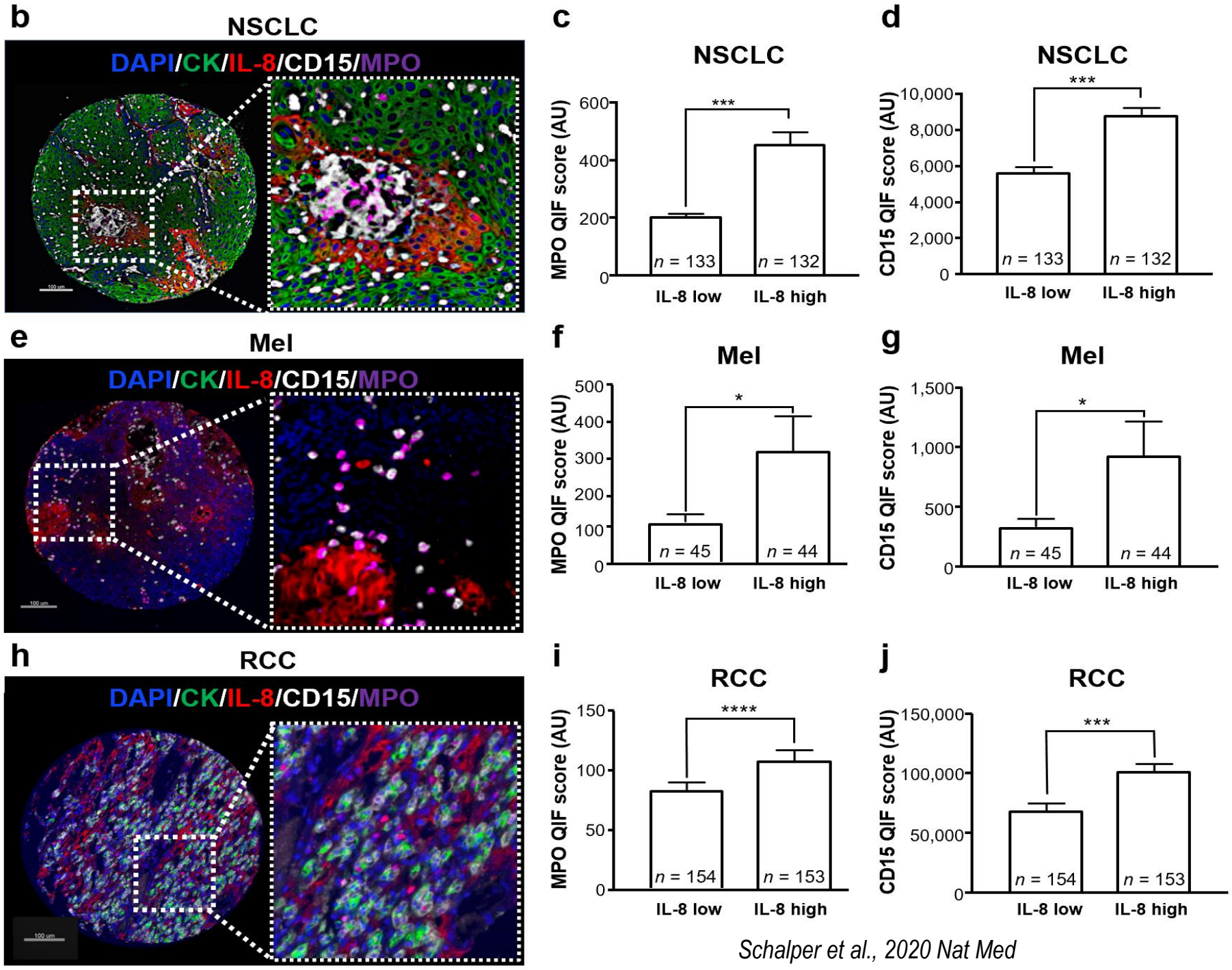




Schalper et al., 2020 Nat Med

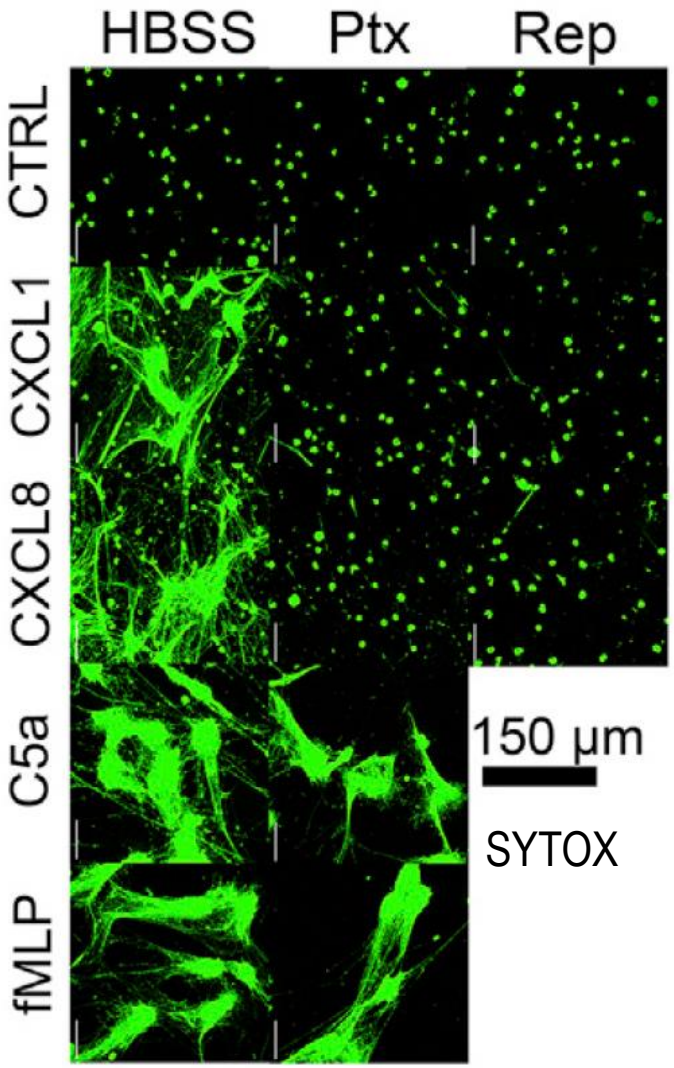
Measuring IL-8 and granulocytes in tumor tissue

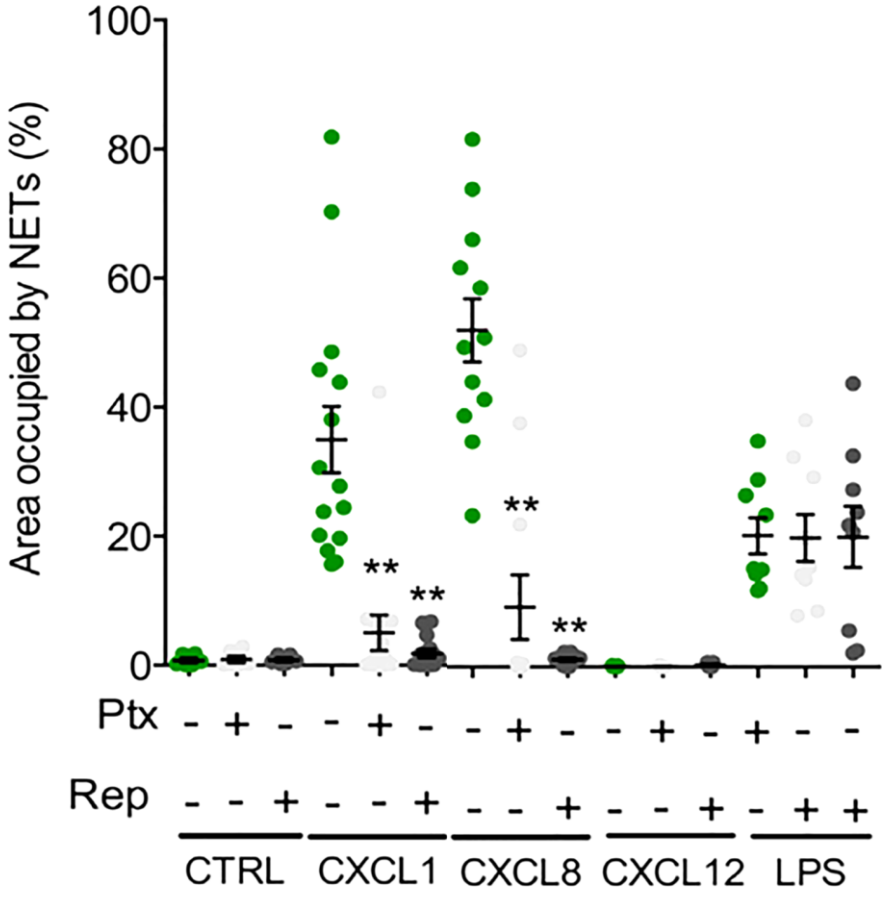




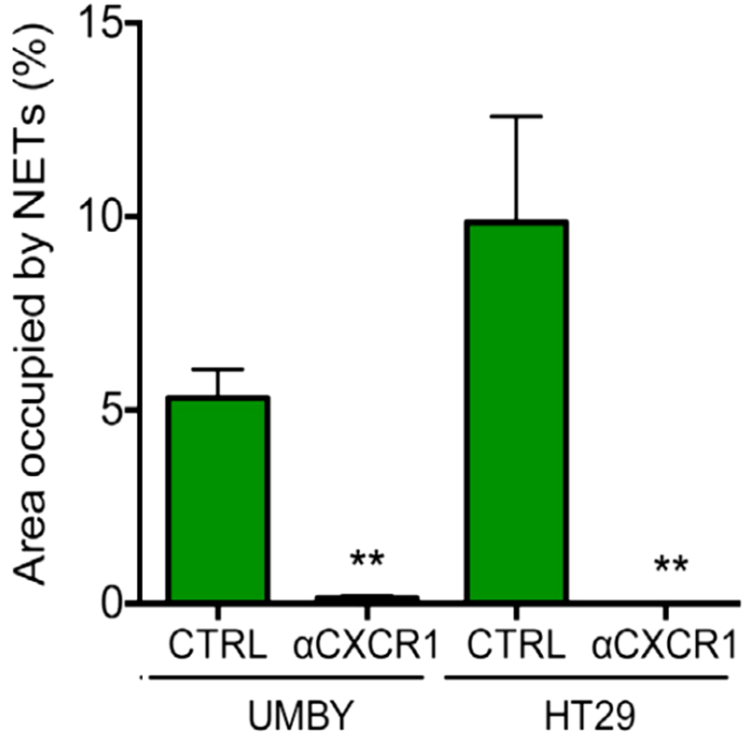
Schalper et al., 2020 Nat Med

Neutrophils and NETosis in human granulocytes

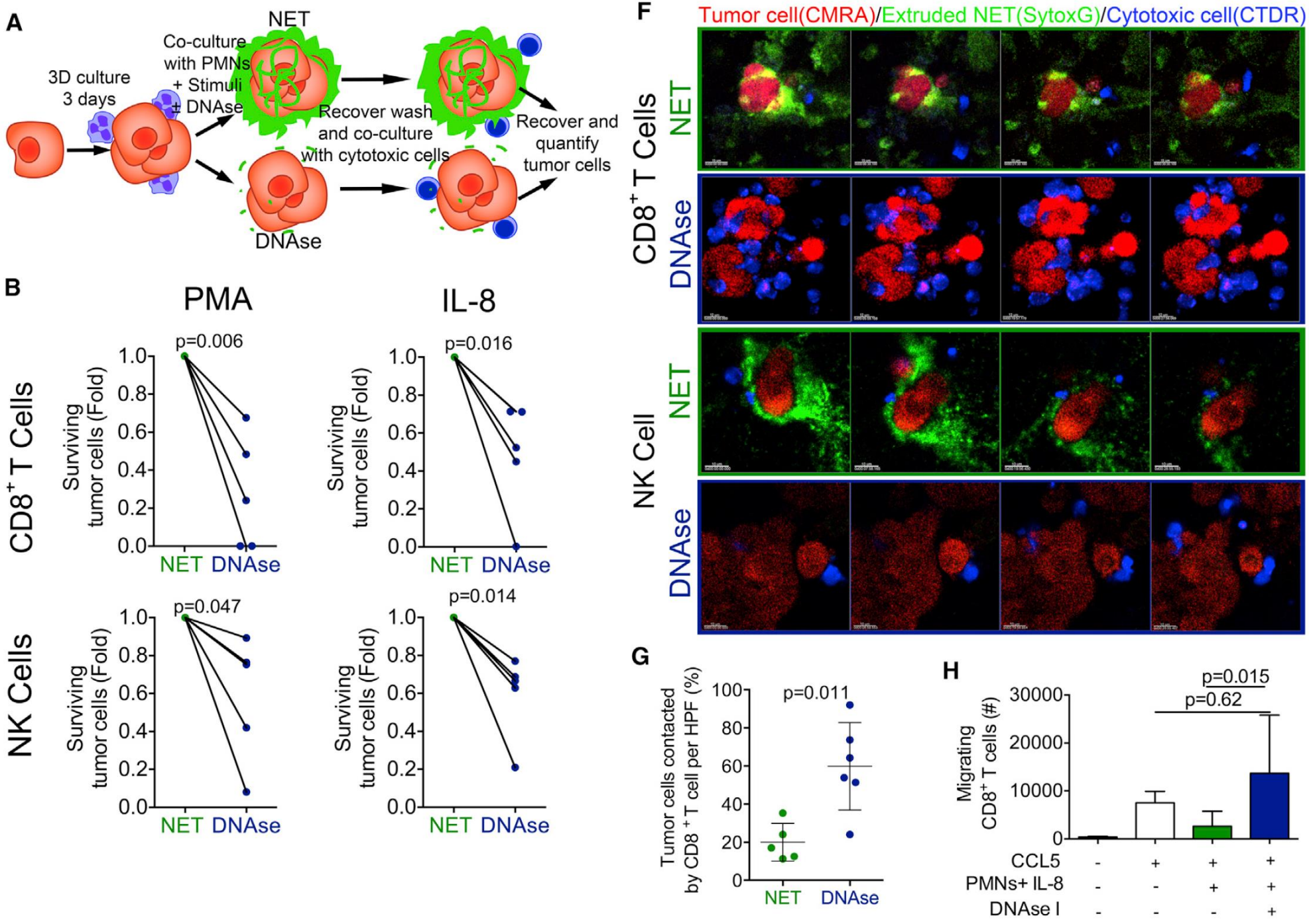




Ptx= Pertussis toxin; Rep: Reparixin



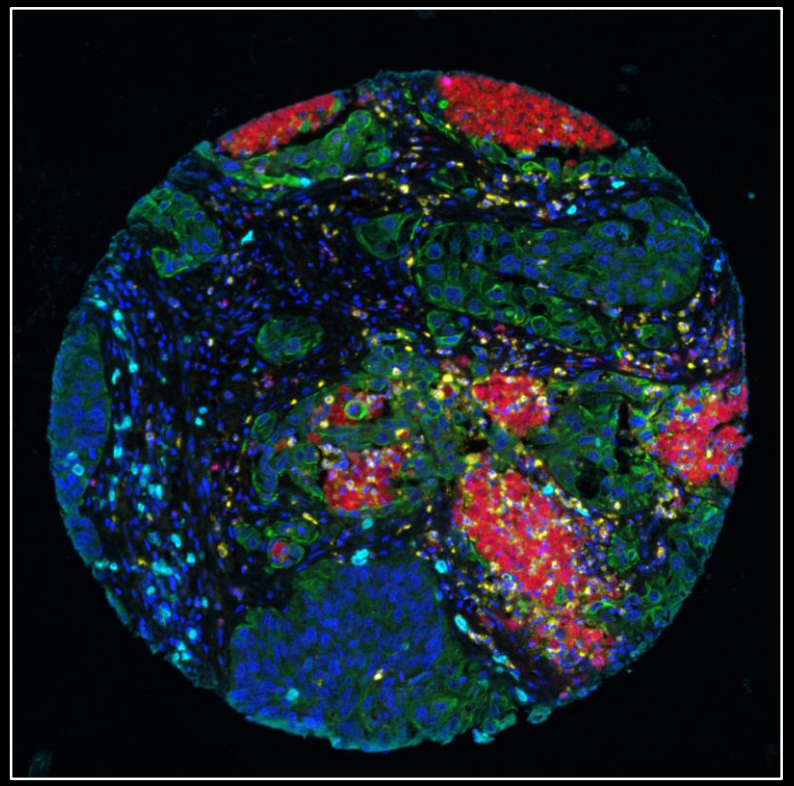
Teijeira et al., 2020 Immunity


A
NET
Co-culture
with PMNs
+ Stimuli
± DNAse
3D culture
3 days
Recover wash
and co-culture
with cytotoxic cells
Recover and
quantify
tumor cells
DNAse
B
PMA
IL-8
CD8+ T Cells
NK Cells
Surviving
tumor cells (Fold)
p=0.006
p=0.016
p=0.047
p=0.014
NET DNAse
F
Tumor cell(CMRA)/Extruded NET(SytoxG)/Cytotoxic cell(CTDR)
CD8+ T Cells
NK Cell
NET
DNAse
G
Tumor cells contacted
by CD8 + T cell per HPF (%)
p=0.011
H
Migrating
CD8+ T cells (#)
p=0.015
p=0.62
CCL5
PMNs+ IL-8
DNAse I

Automated NET measurement

Immunofluorescence

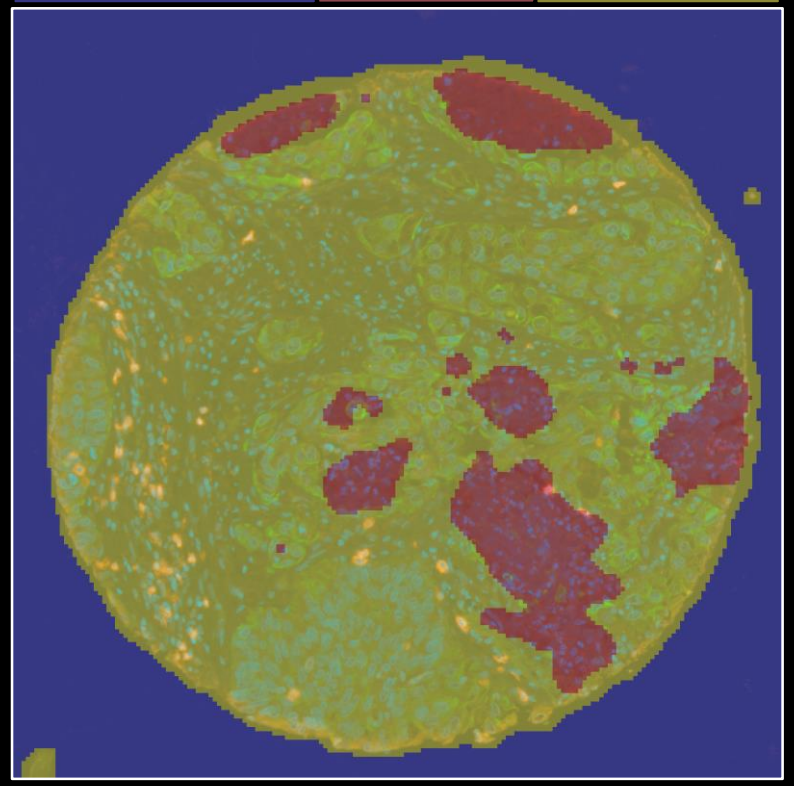
Dapi/CK/citH3/CD66b/CD8



MULTISPECTRAL IMAGING

Compartment segmentation

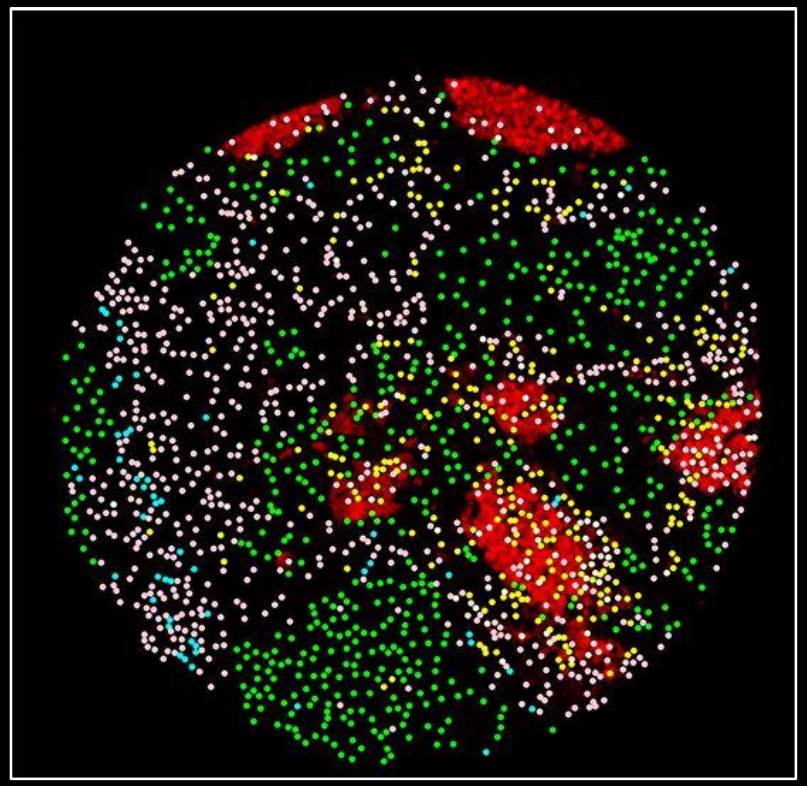
Background | NET | Tissue



AREA (mm^2)

Cells phenotype

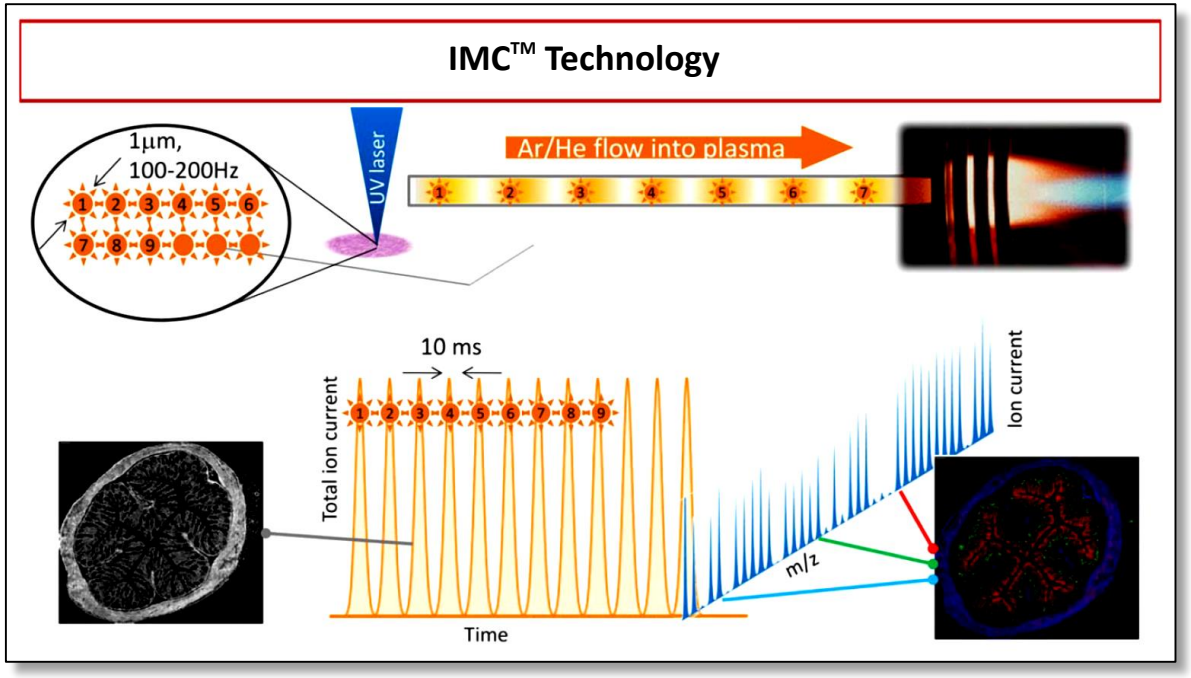
CK+/Stromal+/CD66b+/CD8+



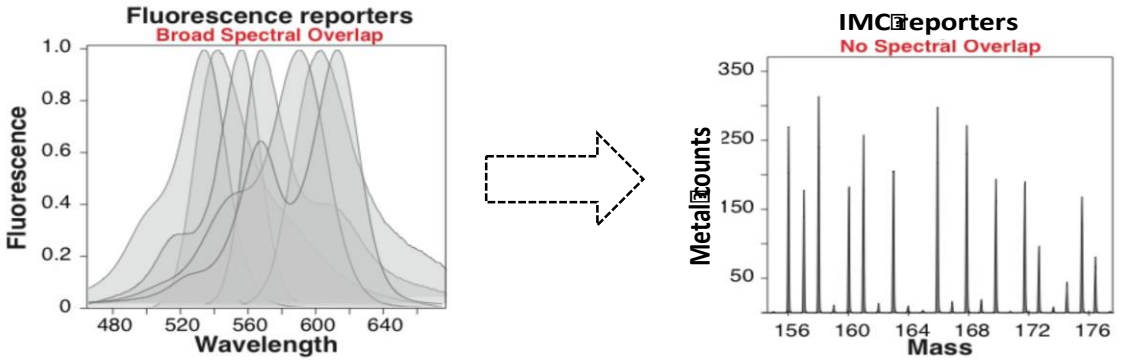
Cell Density (Cells/mm^2)

Integrated TME analysis

Yale IMC Immuno-oncology panel



Chang et al., 2017. Cytometry A. 91(2):160-169



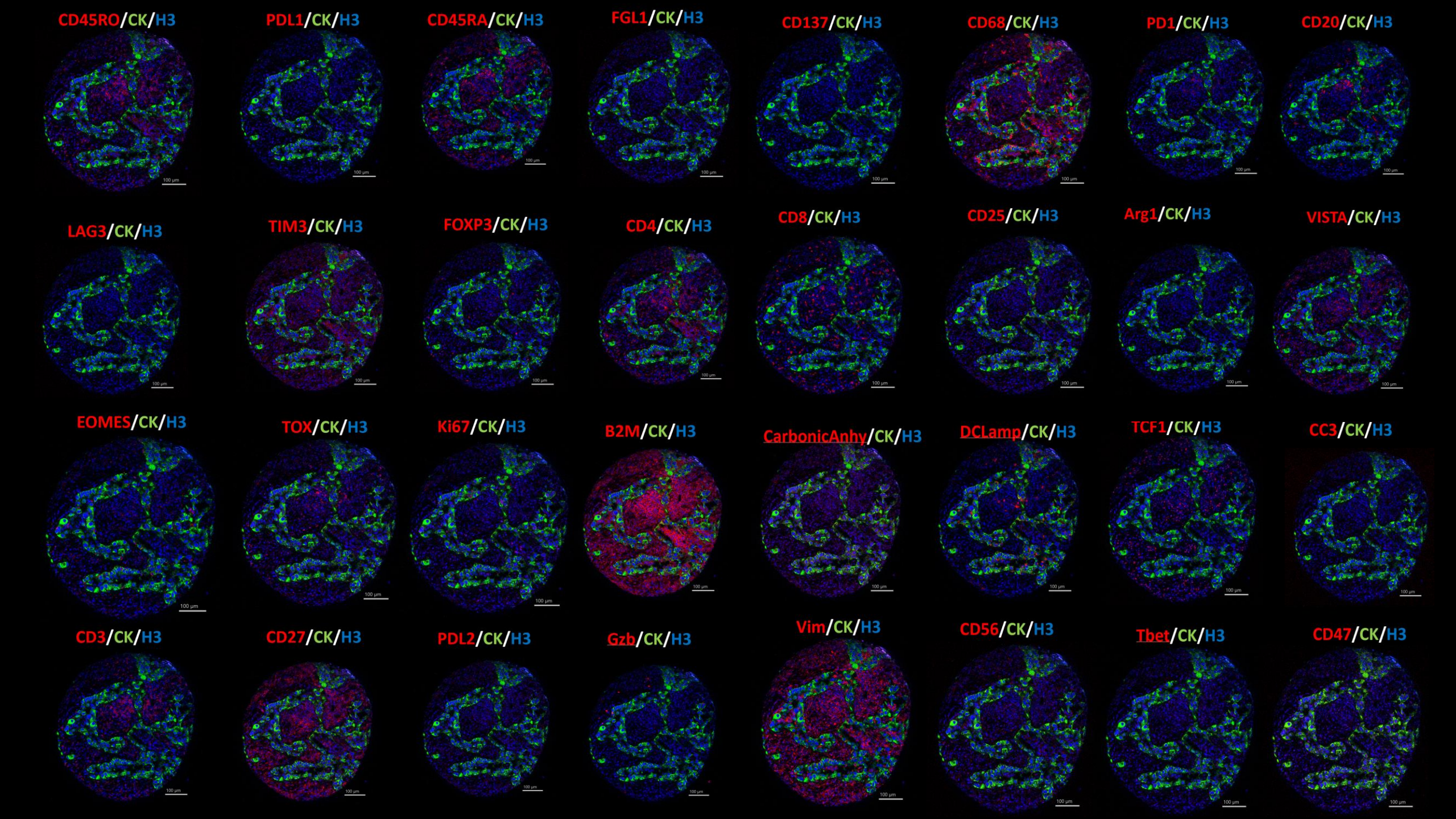
Isotope	Target	Dilution	Category
Im115	LipoR	1:100	Membrane
Eu150	GAPDH	1:50	Structure
Yb176	Histone-3	1:500	Nuclei
Nd148	Pancytokeratin	1:50	Epithelial
Gd156	Vimentin	1:50	Stromal
Er170	CD3	1:50	T-lymphocytes
Gd156	CD4	1:50	T-helper
Dy162	CD8	1:50	T-cytotoxic
Gd155	FoxP3	1:25	T-regulatory
Dy161	CD20	1:50	B-lymphocytes
Tb159	CD68	1:50	Macrophages
Sm149	CD45RO	1:50	Memory T-cells
Yb173	Granzyme-B	1:25	Cytotoxicity
Er168	Ki-67	1:50	Proliferation
Nd145	T-bet	1:25	Effector function
Dy163	CD25	1:50	Activation
Tm169	B2-microglobulin	1:50	Antigen presentation
Nd150	PD-L1	1:50	B7-Immune regulation
Yb172	PD-L2	1:50	B7-Immune regulation
Sm152	B7-H3	1:50	B7-Immune regulation
Gd158	B7-H4	1:50	B7-Immune regulation
Yb171	IDO-1	1:50	Immune regulation
Gd160	PD-1	1:50	Immune regulation
Eu153	LAG-3	1:25	Immune regulation
Sm154	TIM-3	1:25	Immune regulation
Nd146	CD47	1:25	Immune regulation
Ho165	VISTA	1:50	Immune regulation

Structural components segmentation

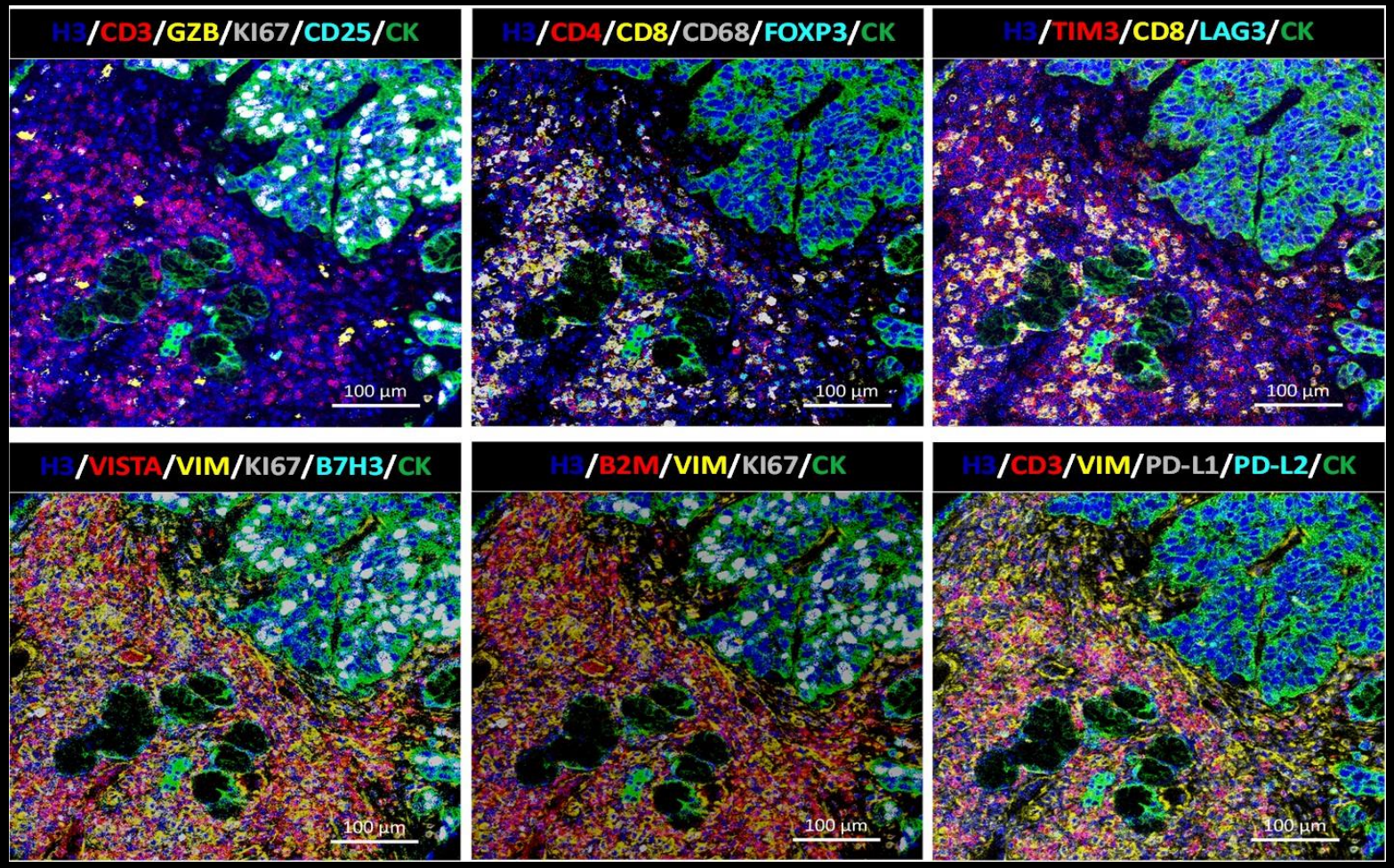
Cell phenotype compartments

Functional markers immune

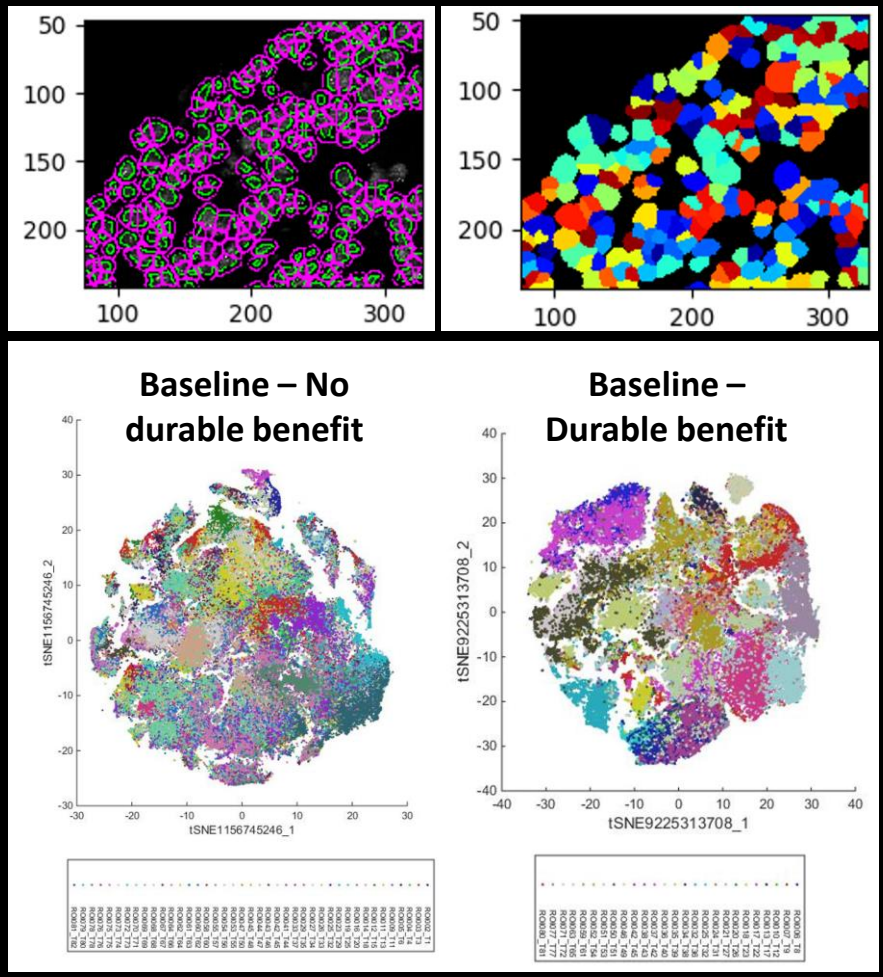
Immune regulation candidate targets


CD45RO/CK/H3
PDL1/CK/H3
CD45RA/CK/H3
FGL1/CK/H3
CD137/CK/H3
CD68/CK/H3
PD1/CK/H3
CD20/CK/H3
LAG3/CK/H3
TIM3/CK/H3
FOXP3/CK/H3
CD4/CK/H3
CD8/CK/H3
CD25/CK/H3
Arg1/CK/H3
VISTA/CK/H3
EOMES/CK/H3
TOX/CK/H3
Ki67/CK/H3
B2M/CK/H3
CarbonicAnhy/CK/H3
DCLamp/CK/H3
TCF1/CK/H3
CC3/CK/H3
CD3/CK/H3
CD27/CK/H3
PDL2/CK/H3
Gzb/CK/H3
Vim/CK/H3
CD56/CK/H3
Tbet/CK/H3
CD47/CK/H3

Integrated spatial visualization of selected markers

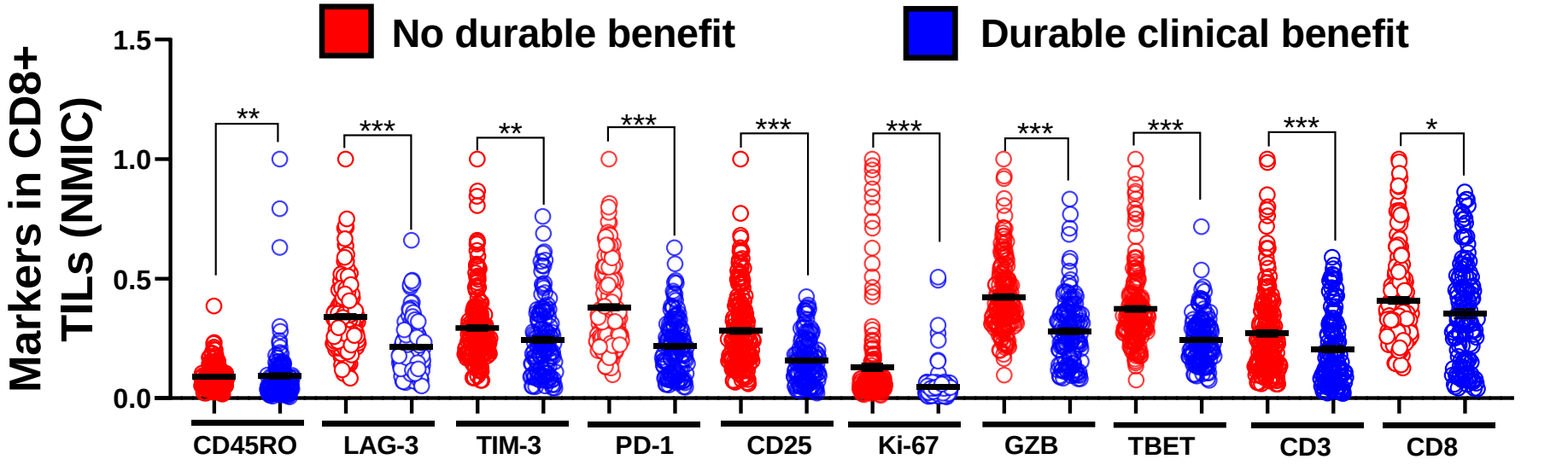


Segmentation and single-cell analysis

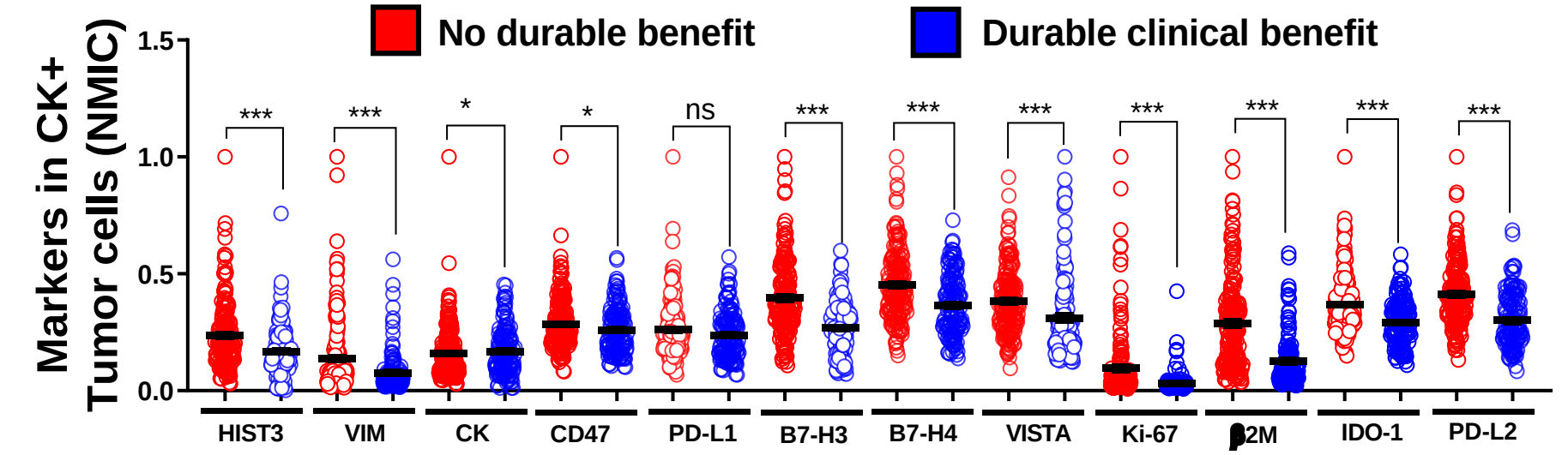


Unpublished

CD8+ TILs

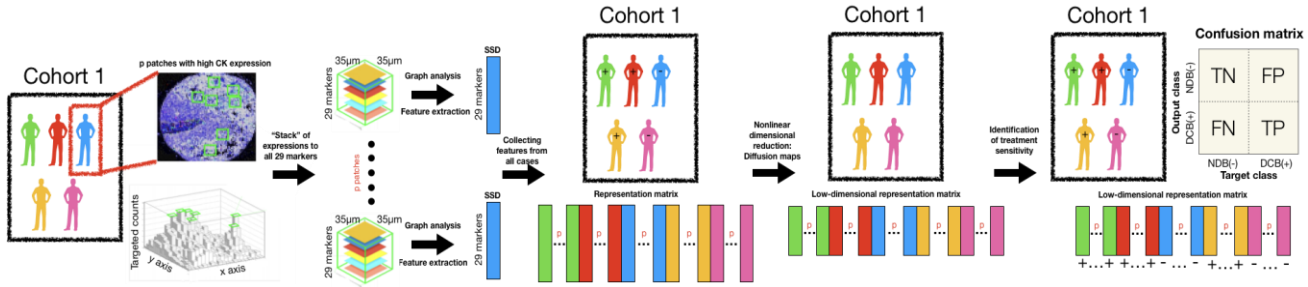


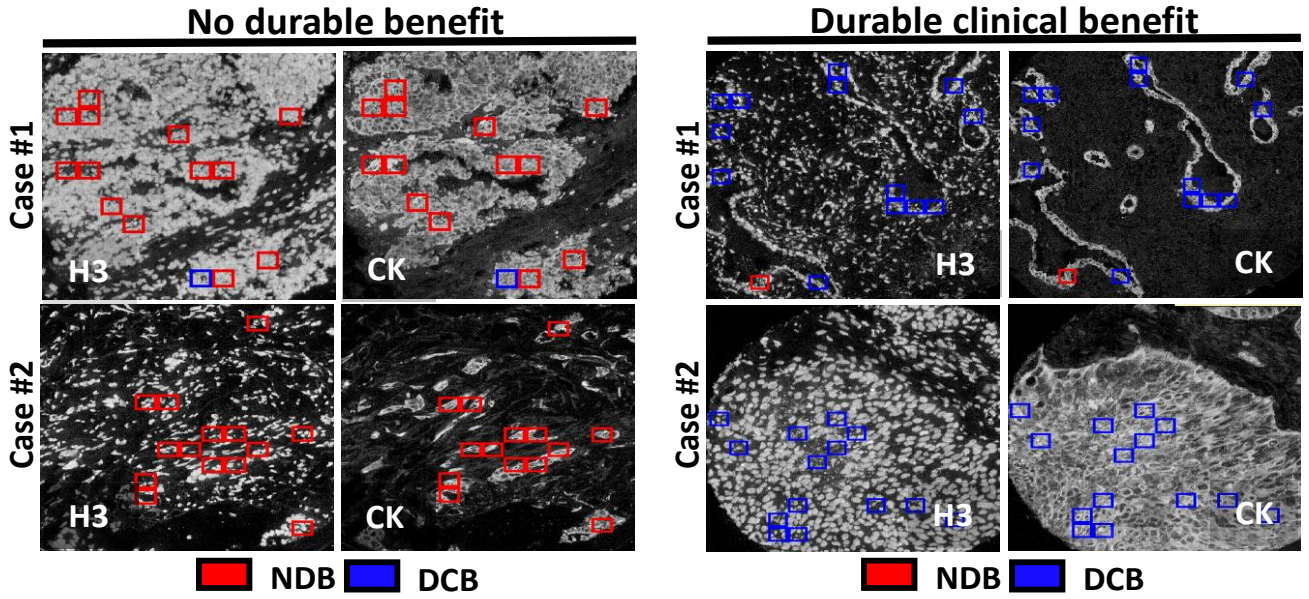
CK+ Tumor cells

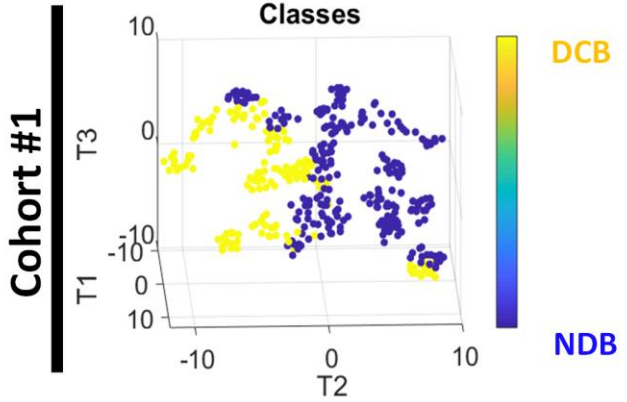


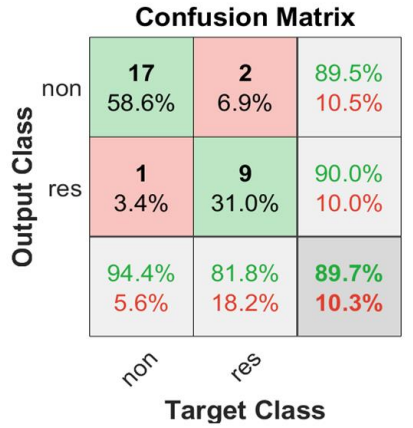
Unpublished

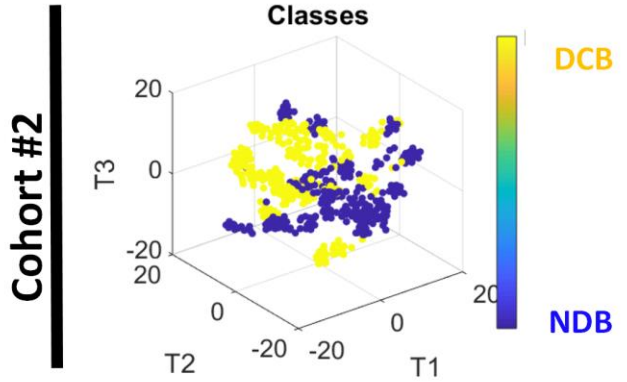
Integration of TME components using manifold learning

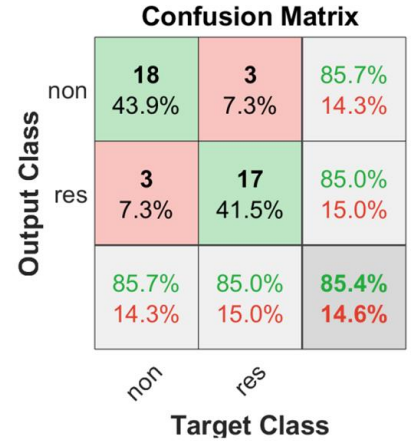












Lin et al., 2021, PLOS Comp Biol

Conclusions & take home message

- Resistance to immune checkpoint blockade can be tumor and immune-cell mediated.
- T-cell dysfunction in the TME is complex and associated with tumor antigenic exposure.
- Tumor-cells can express dominant immunoregulatory ligands in cancer subsets.
- Tumors can abrogate antigen presentation proteins associated with immune evasion and immunotherapy resistance in NSCLC.
- Developments allow deep and integrated spatial analysis of intact tumor specimens.
- Interaction of TME with patient features may provide additional context and value.

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