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Perlmutter Cancer Center

Preclinical Models to Elucidate Tumor Immune Microenvironment

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Division of Hematology and Medical Oncology

Integration of Bench and Bedside

Novel Cancer Genomic, Proteomic Immune and Metabolomic Discoveries

- Patient derived cancer cell lines
- Genetically Engineered cell lines harboring specific oncogenic mutations
- Xenografts and Primary Cancer Transplant Models
- Patient Derived cancer cell lines
- Syngeneic models
- **Genetically Engineered Mouse Models (GEMM)**

Elucidate the mechanism of primary and acquired resistance in the patient samples

Preclinical and Co-clinical Trials

Determine best single agent or combination therapy to bring forth to human trials with genetically stratified patients

Run Human Clinical Trials

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Inducible Mouse Lung Cancer Models

KRAS G12D
KRAS G12V
KRAS G12C
EGFR Del19
EGFR L858R
EGFR T790M
EGFR wild type
EGFR vIII
HER2 exon 20 insertion
HER2 wild type
BRAF V600E
p110 exon 20 (H1047R)
EML4-ALK
KRAS G12D/Tbk1 mutant

C-MET
EGFR T790M-Del19/c-MET
EGFR T790M-L858R/c-MET
EGFR T790M-Del19
EGFR T790M-L858R
EGFR T790M-L858R-C797S
EGFR T790M-Del19-C797S
EGFR T790M-L858R-L718Q
EGFR T790M-Del19-L718Q
EML4-ALK F1174L
EML4-ALK L1196M

DDR2 mutants
FGFR 1
FGFR 2 WT
FGFR2 W290C
FGFR2 S320C
FGFR2 K659N
FGFR3 WT
FGFR3 K652E
FGFR3 S249C
NRF2 WT
NRF2 G81S
SOX2
Pten/Lkb1

IDH1 R132H
IDH2 R140Q
IDH2 R172K
EZH2
EZH2 Y641N
RBP2
LSD2
LSD1
KDM5B
SETDB1
MINA53
KDM2a
JMJD2C
Histone H3.3 G34R

p53/Rb
p53/Rb/p130
p53/Rb/myc

Matching the right model to the right drug

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Genetically engineered mouse cancer models for Immunotherapy

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Current Conditional Mouse Lung Cancer Models

1. Genetically defined
2. Native Vasculature
3. Intact Immune System

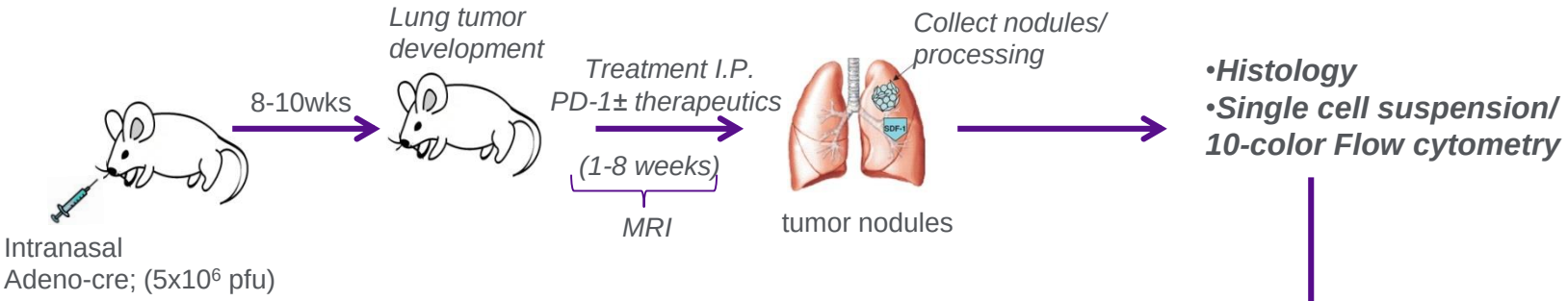
Potential limitations

1. Low Mutational Load (novel neo-antigens are present)
2. Low throughput for drug screening (large number of drug combinations)
3. Minor differences between mouse and human immune system

LSL-Kras^{G12D}

LSL-Kras^{G12D} Lkb1$^{f/f}$

LSL-EGFR-TL



- ***Histology***
- ***Single cell suspension/ 10-color Flow cytometry***

BAL for cytokines

Immune cell Quantification
-NK, B, CD4, CD8
Treg, TANs, TAMS

RNA Seq or single cell RNA Seq

T cell phenotype
-Inhibitory receptors:
PD-1, TIM-3, LAG-3
CTLA-4
-Proliferation/Survival markers: Ki67, Bcl-2
-Activation/Effector markers: CD62L/CD44
CD69

T cell function
-Effector cytokine production: IFN-γ

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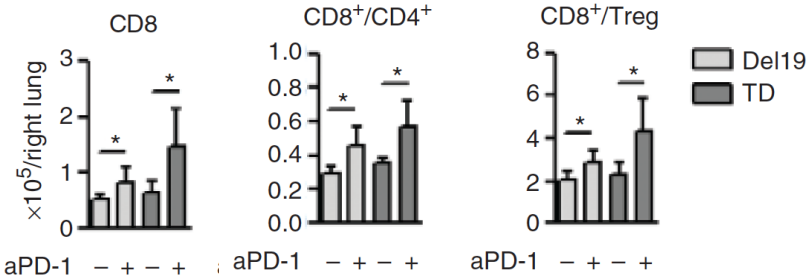
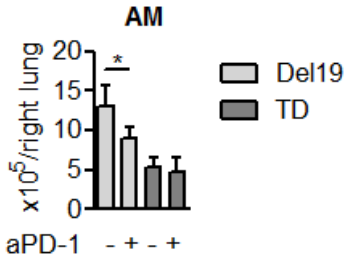
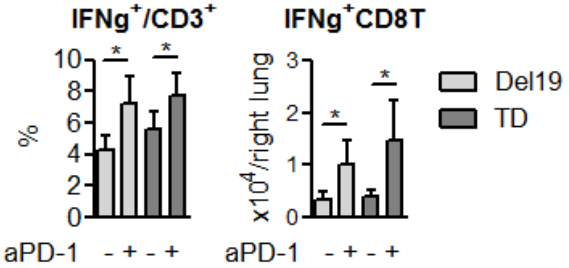
PD1 blockade decreases the immune suppressive factors in the microenvironment in EGFR driven tumors

T CELLS

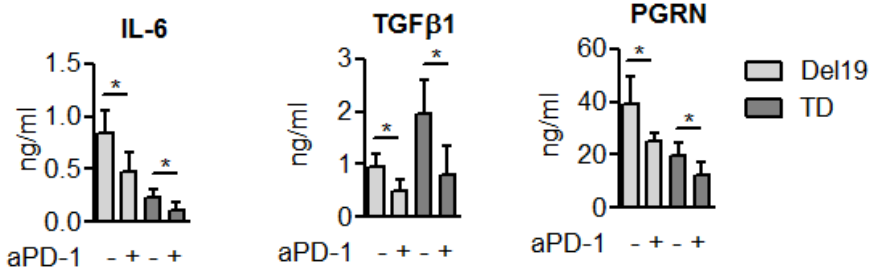


Macrophages





Cytokines



Akbay et al, *Cancer Discovery,* 2013

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Adaptive Resistance to chronic PD1 Blockade

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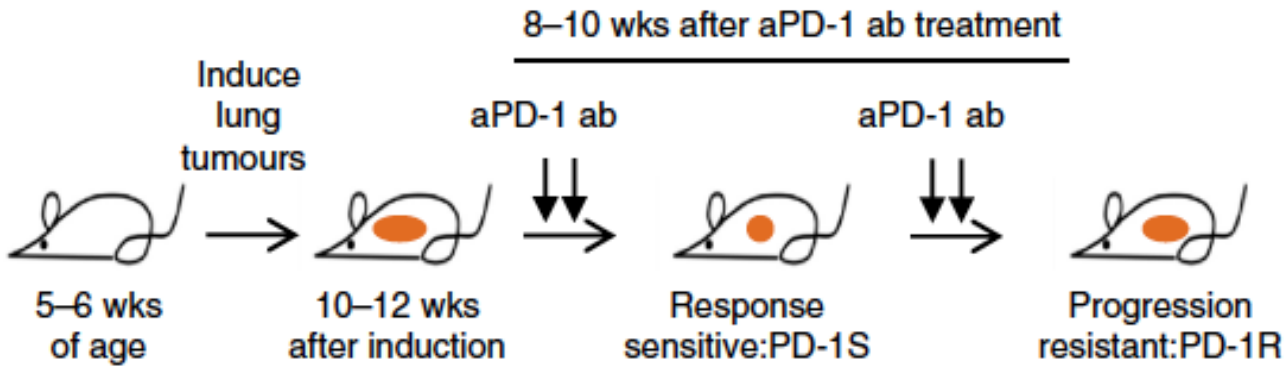
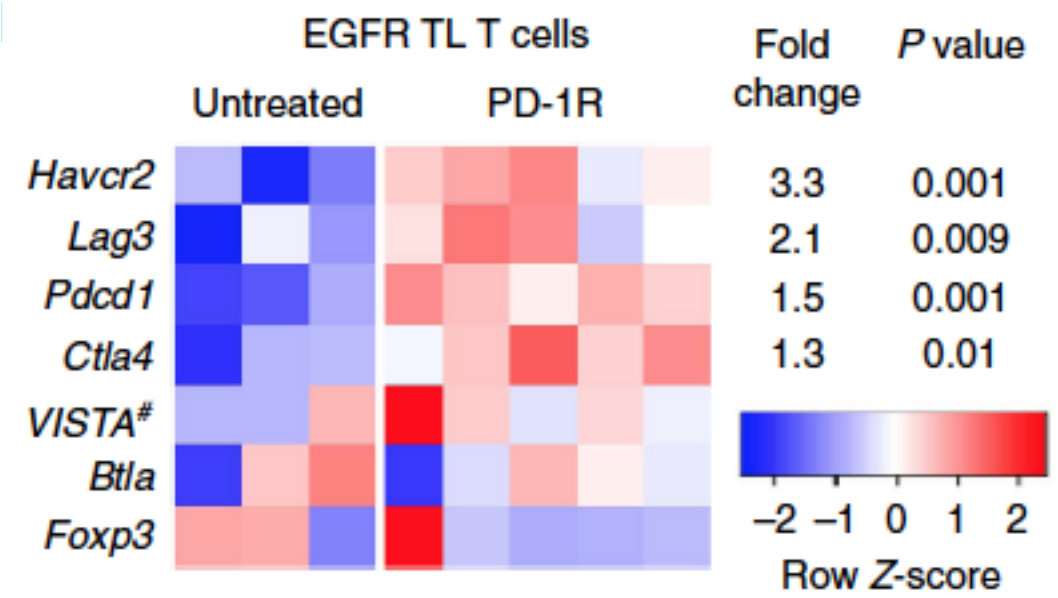
T cells in PD-1 resistant lung cancer



TIM-3



Nature Communications

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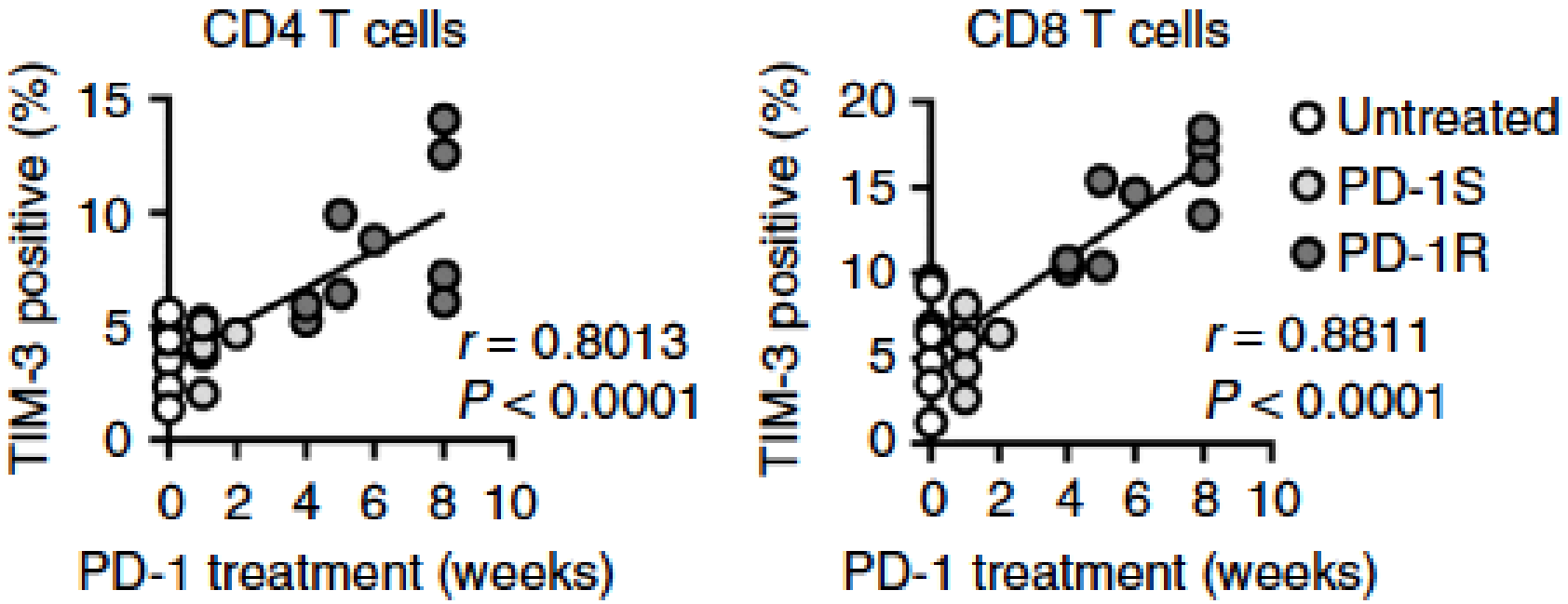
Increased TIM-3^{+} T cells in PD-1-resistant mouse lung cancer



Nature Communications

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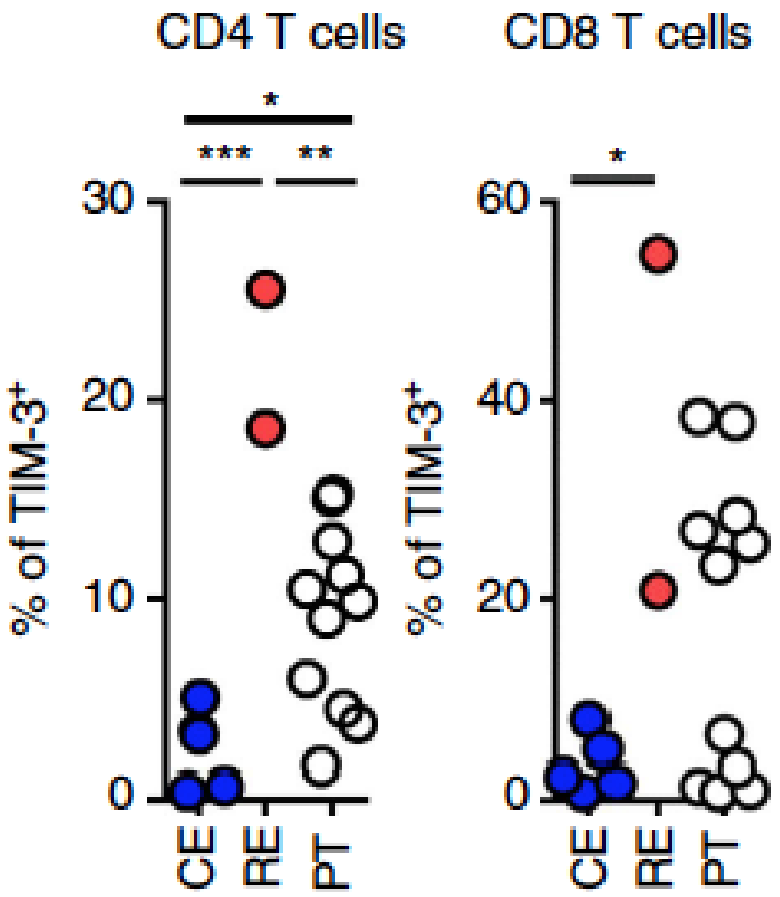
Lung cancer patients that develop resistance to PD-1 therapy express higher levels of TIM-3



CE = control effusion

RE = resistant effusion

PT = primary tumor

Nature Communications, 2016

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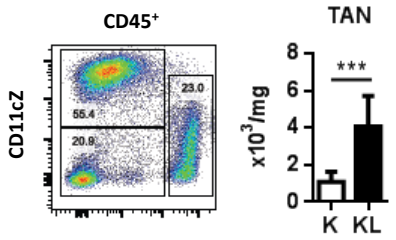
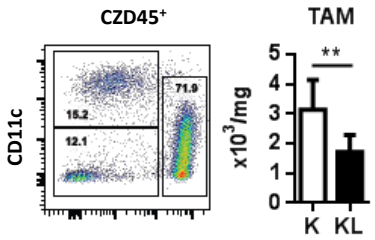
Tumor Suppressor loss leads to altering of TME

Lkb1 inactivation drives development of a distinct tumor immune microenvironment enriched with neutrophil inflammatory cytokines

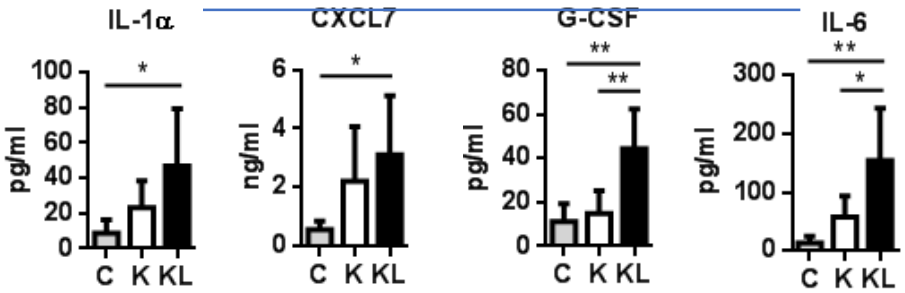
Kras



Kras/Lkb1



CYTOKINES IN BAL



Cancer Research, 2016

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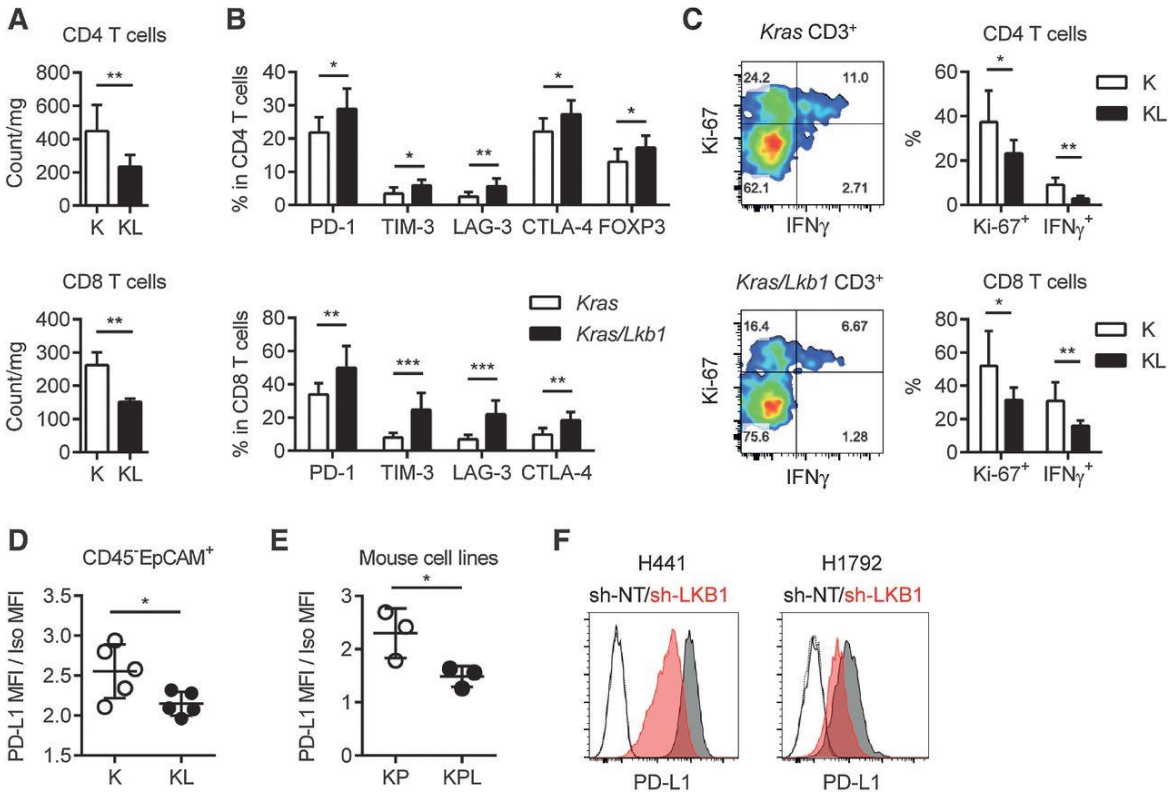
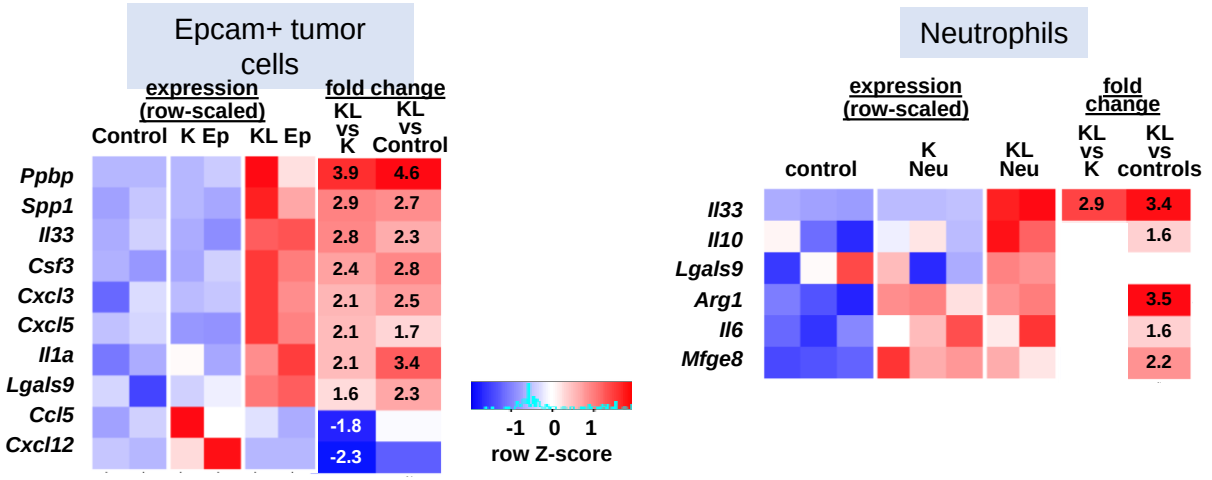
Lkb1 inactivation leads to a T-cell–suppressive tumor microenvironment with low PD-L1 expression in tumor cells.

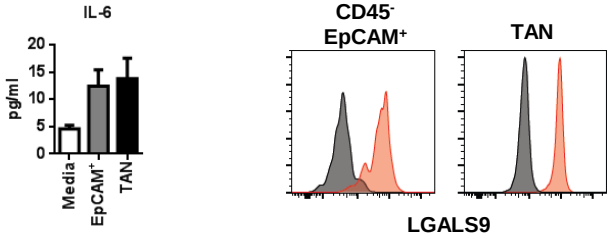


Shohei Koyama et al. Cancer Res 2016;76:999-1008

RNA sequencing of sorted tumor cell populations yields novel targets in different Kras vs Kras Lkb1 models

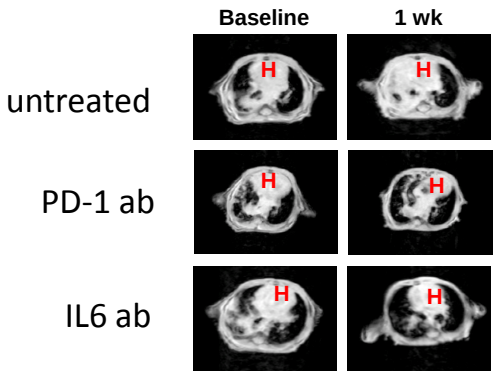


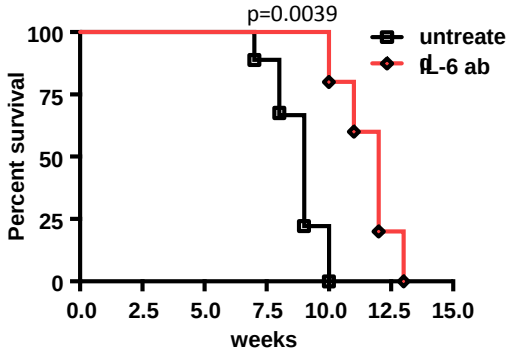
Validation of some targets at protein level in sorted tumor vs neutrophils

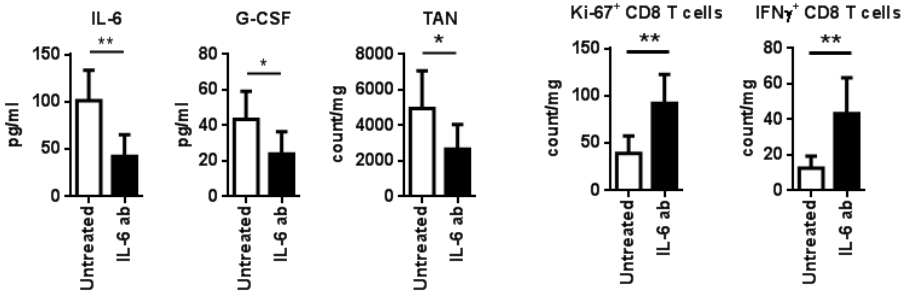


Shohei Koyama et al. Cancer Res 2016;76:999-1008

IL6 blockade but not PD-1 blockade is effective in Lkb1 mutant tumors







Shohei Koyama et al. Cancer Res 2016;76:999-1008

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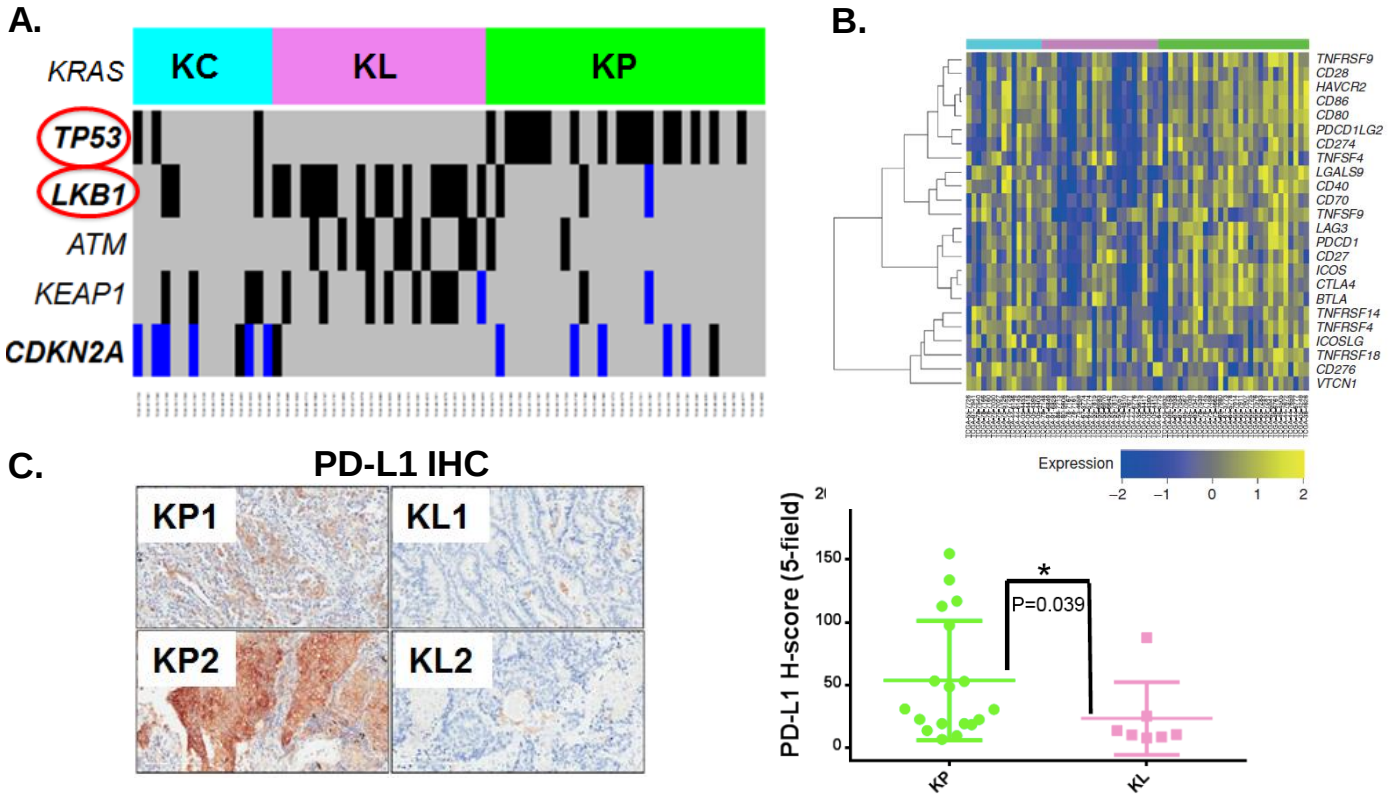
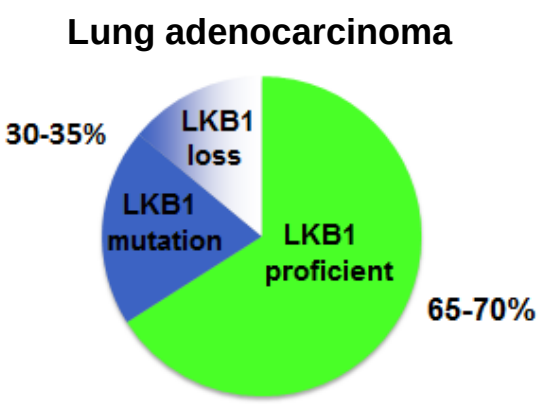
Co-mutations in *STK11/LKB1* (KL) and *TP53* (KP) define subgroups of *KRAS*-mutant LUAC with distinct immune profiles



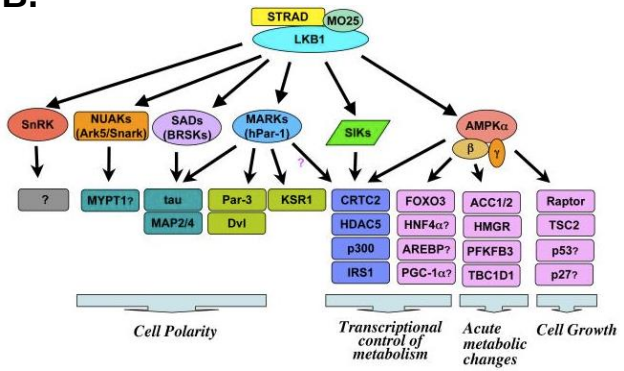
Skoulidis et al., *Cancer Discovery*, 2015

LKB1 inactivation is associated with a “cold” tumor immune microenvironment in LUAC

A.

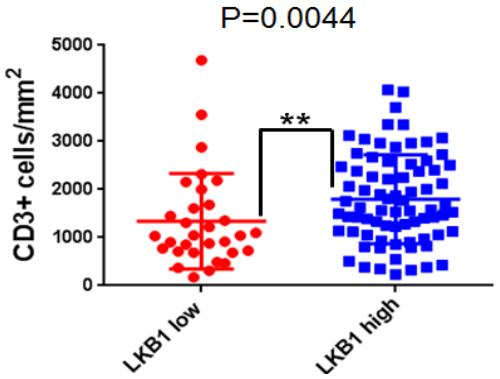


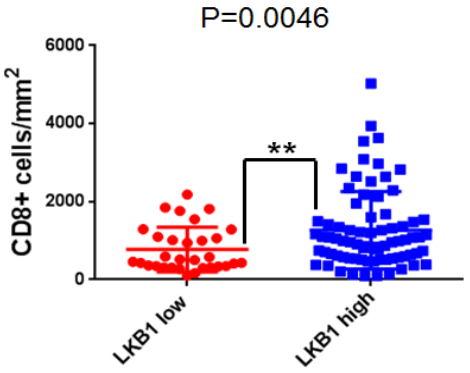
B.



Shackelford and Shaw, Nat Rev Cancer, 2009

C.





Skoulidis et al, ASCO Annual Meeting, 2015

Preclinical Models to understand the impact of small molecules on the lung cancer tumor immune microenvironment

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Epigenetics regulation in cancer: HDAC inhibitors

- Histone deacetylases (HDAC) catalyze the removal of acetyl groups from lysine residues in histones and non-histone proteins thereby regulating many cellular processes

- Pan or isozyme-specific HDACi have gained attention in oncologic applications due to their reported cytostatic effects in cancer models

- Emerging data highlight their immuno-regulatory properties in various inflammatory settings

Immunomodulatory properties of ACY1215 (Ricolinostat) in genetically engineered mouse models of NSCLC

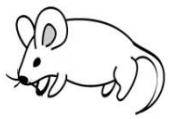
In vivo studies

LSL-*Kras*^{G12D}
p53$^{f/f}$ (KP)
LSL-*EGFR*$^{T790M/L858R}$
(TL)

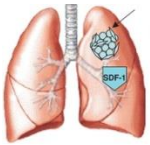
Intranasal
Adeno-cre; (5x10^6 pfu)

8-10wks

Lung tumor development



Treatment I.P.
Ricolinostat
(1-2 wks)



Collect nodules

t.n.;
tumor nodule

Immune Profiling

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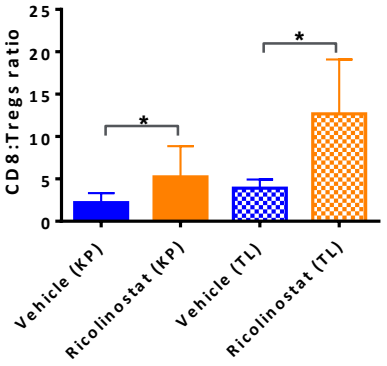
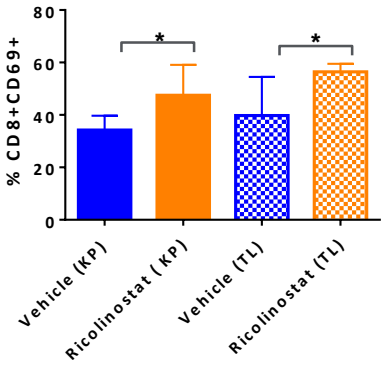
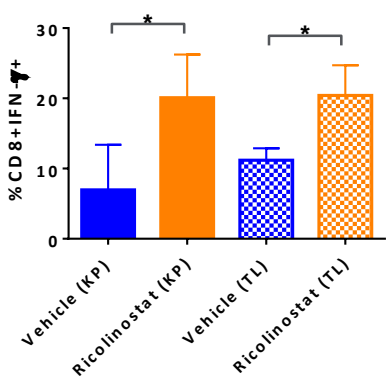
In vivo effects of ACY1215 in genetically engineered mouse models of NSCLC



Increased T cell activation

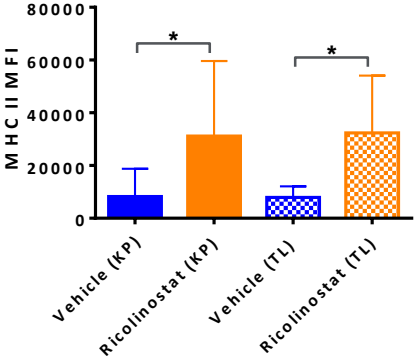


Increased T cell function

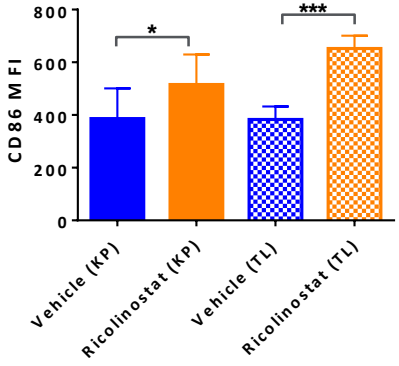


Effect on T cells

Increased MHC II



Increased CD86



Effect on APC (macrophages)

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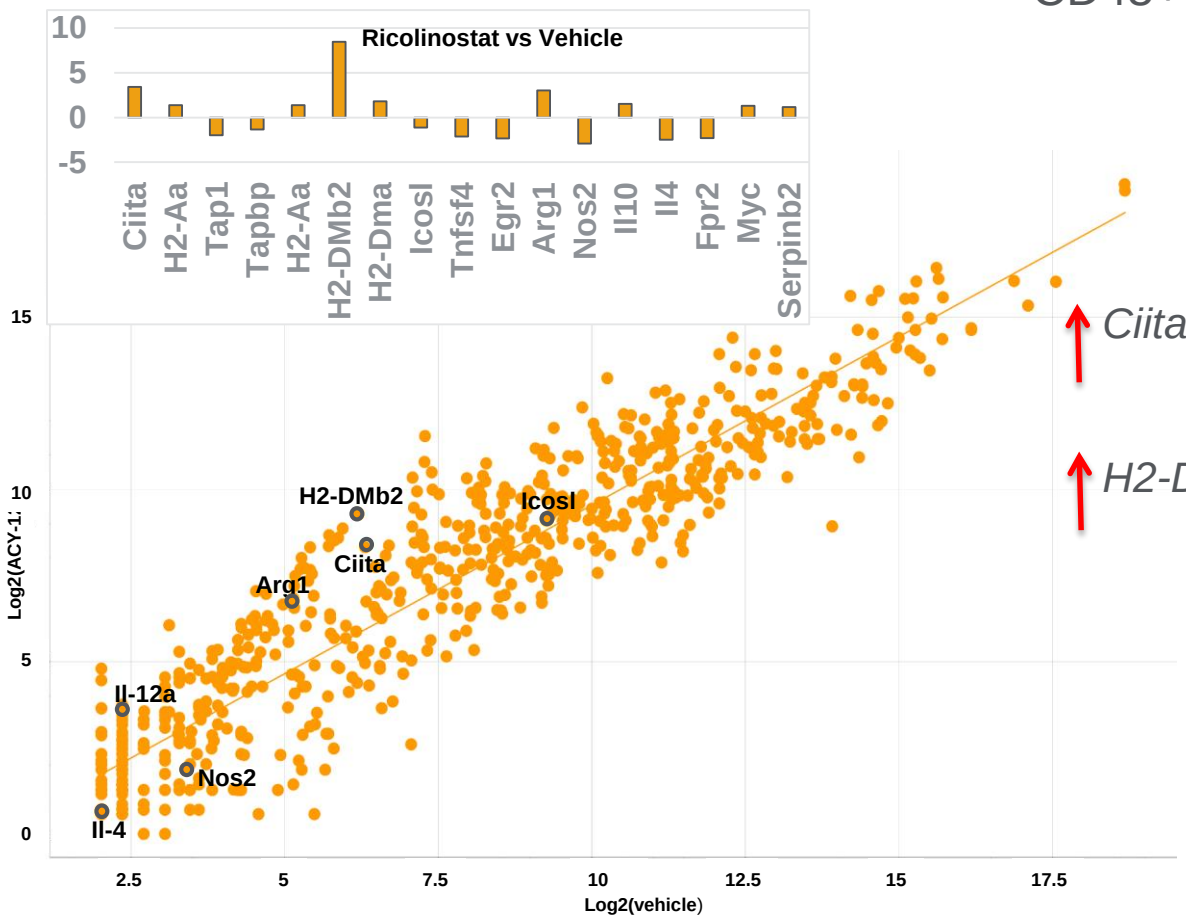
Upregulation of MHC II assembly genes under ACY1215 treatment



CD45+CD11c+CD11b low TAMs

↑ *Ciita*; MHC class II transactivator

↑ *H2-DMb2;* MHC II beta chain

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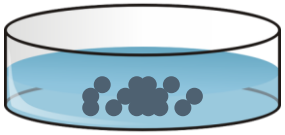
Human immunomodulatory properties of ACY1215

In vitro studies

Tumor
NSCLC Patient tumor specimen (fresh)

gentle dissociation

DMSO
Ricolinostat (2.5uM)
(72 hrs)



Flow cytometry Analysis

CD4/CD8/Tregs
Proportion/Phenotype
•Activation marker; CD69

Tumor-macrophages (CD45+CD68+CD11b+)
Phenotype
•MHC expression; HLA-ABC, HLA-DR
•Co-stimulatory molecules;CD80, CD86

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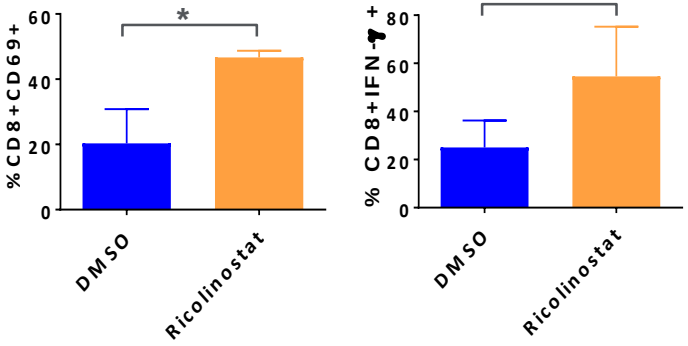
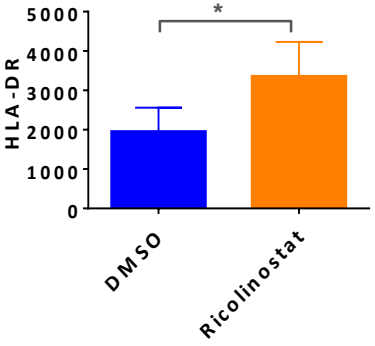
ACY1215 promotes immune-enhancing phenotypic and functional changes in human tumor-immune cell subsets

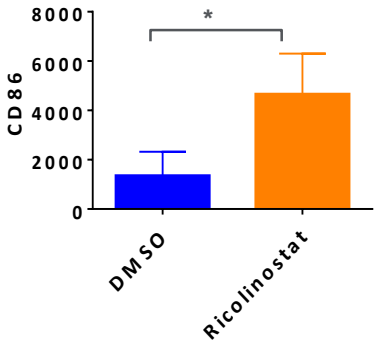
Increased T cell Activation and function

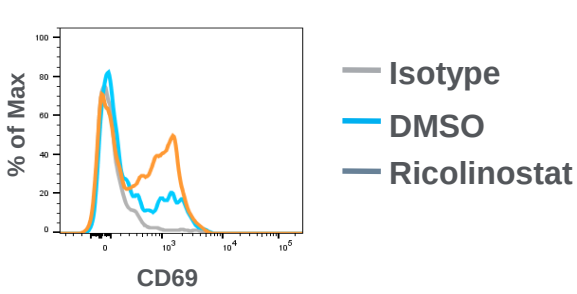


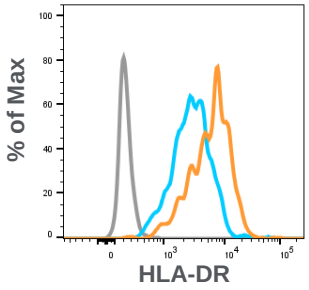
Increased MHC II (antigen presentation)

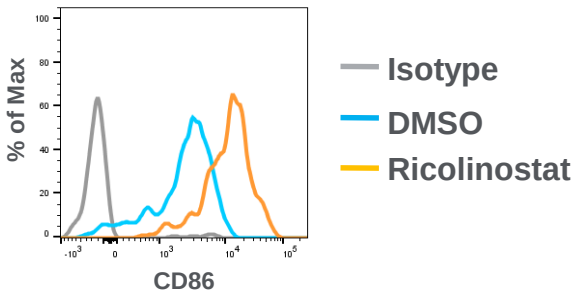


Increased CD86 (co-stimulation)









CD45+CD68+CD11b+
Tumor macrophages

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Epigenetics regulation in cancer: Bromodomain inhibitors

- Bromodomains are unique amino acid domains which act as readers of lysine acetylation thus are involved in epigenetic regulation

- The utility of inhibitors of bromodomain proteins (BrDi) are also being explored in many cancer indications
  - JQ1, an inhibitor of the BET family of bromodomain proteins (BRD2,3,4, and BRDT) has shown efficacy in hematologic malignancies such as AML and multiple myeloma

- A number of ongoing clinical trials are exploring therapeutic efficacy of BrDi in solid cancers

- There is paucity of data on their effects on tumor-associated immune cells

Evaluation of JQ1 effects on tumor-immune suppressive T cells

LSL-Kras^{G12D}
p53$^{f/f}$ (KP)

Intranasal
Adeno-cre;
(5x10^6 pfu)

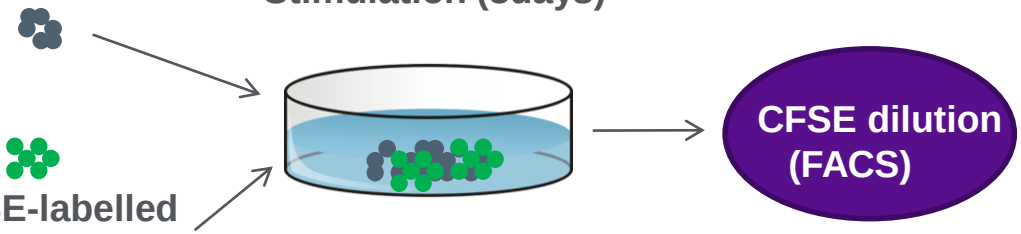
Treatment I.P.
JQ1 (1-2 wks)

Excise lung
Tumors

- ***Evaluate Treg phenotype***
- ***Isolate tumor Tregs (CD4+CD25hi)***
- ***Test function in vitro***

Sorted CD4+CD25+ Tregs (tumor)

α-CD3/CD28 Stimulation (3days)



CFSE-labelled T cells

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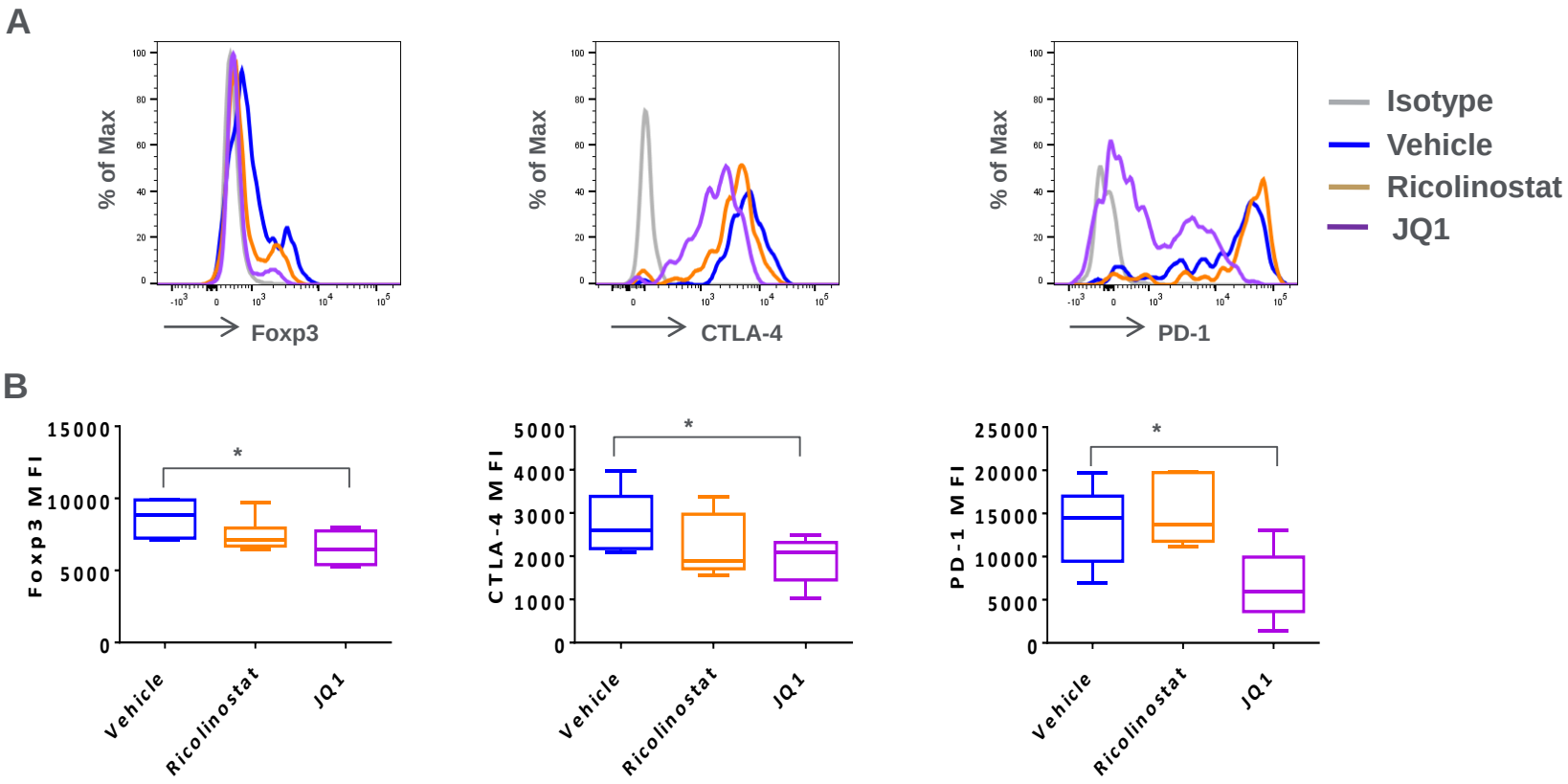
JQ1 alters the phenotype of suppressive Treg cells



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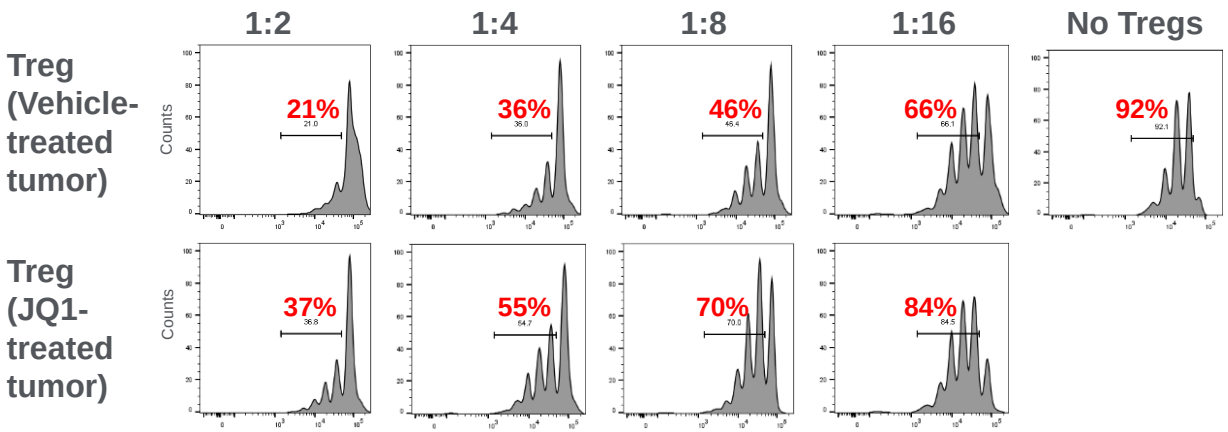
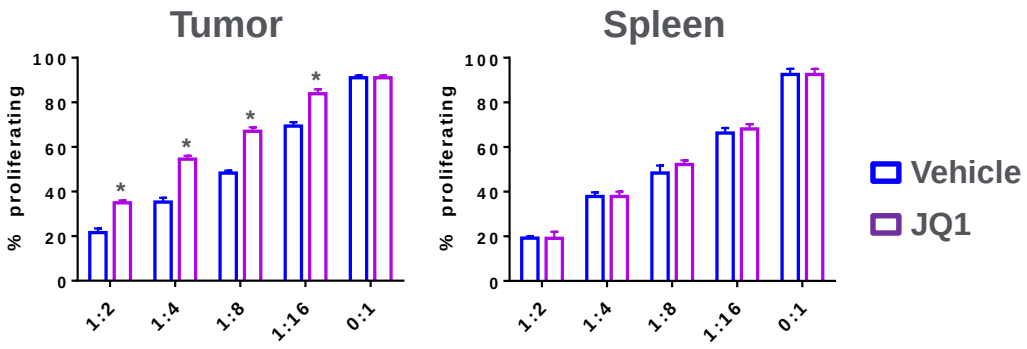
JQ1 disrupts the suppressive function of Tregs

A



B



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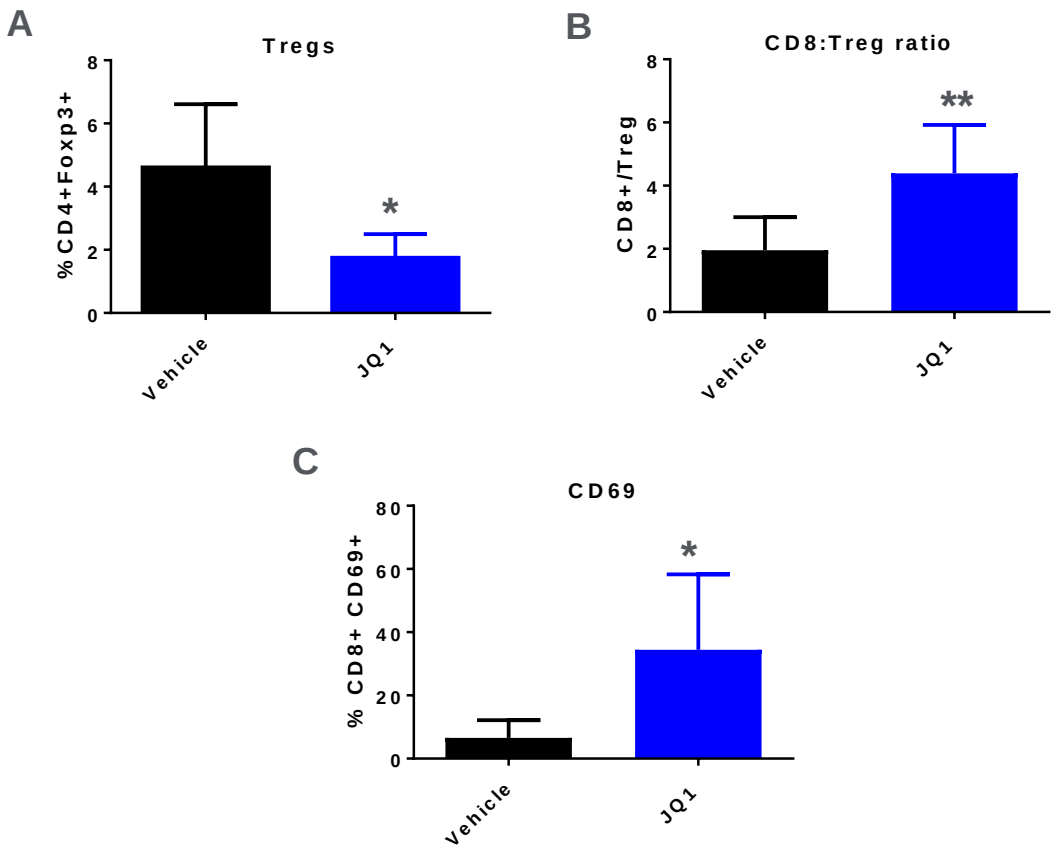
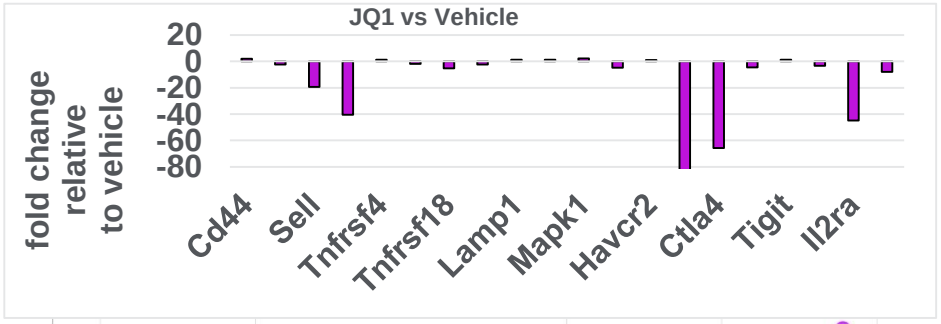
Effects of JQ1 on tumor-infiltrating immune cells in GEM (KP)

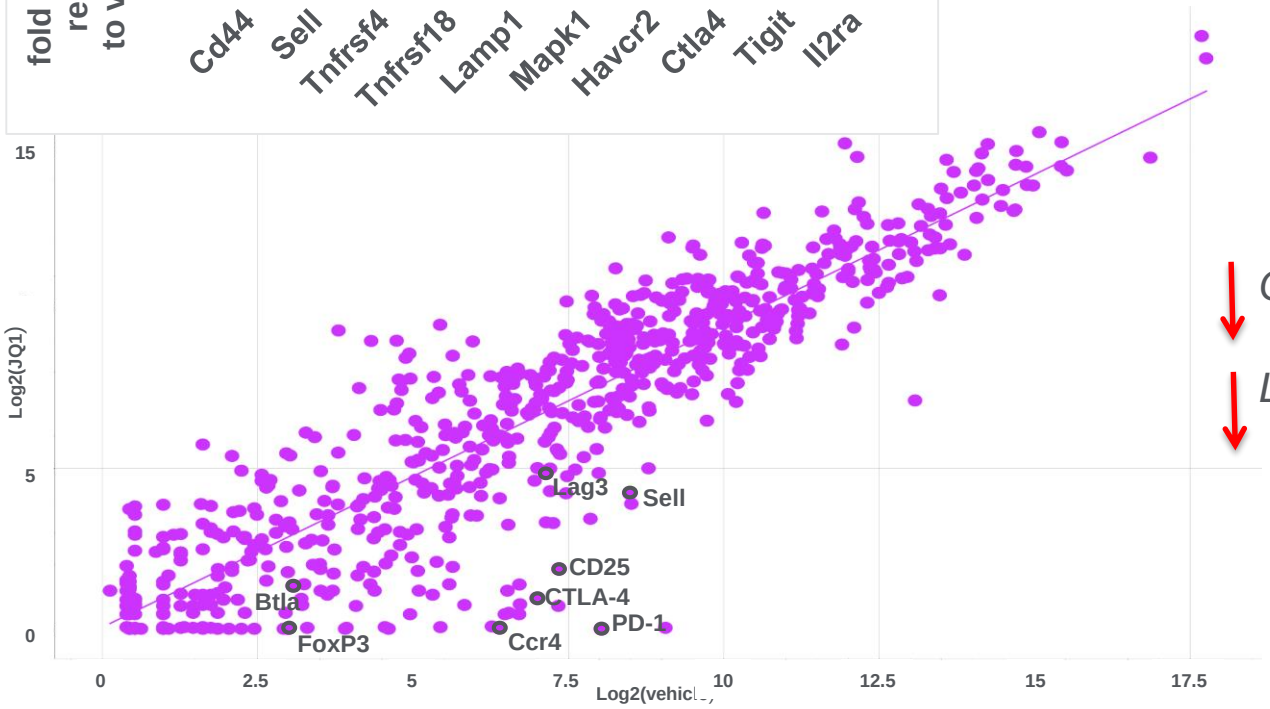


NYU Langone Health

Down regulation of Treg-associated/immune checkpoint genes under JQ1 treatment

CD45+CD3+CD25lo/- T cells





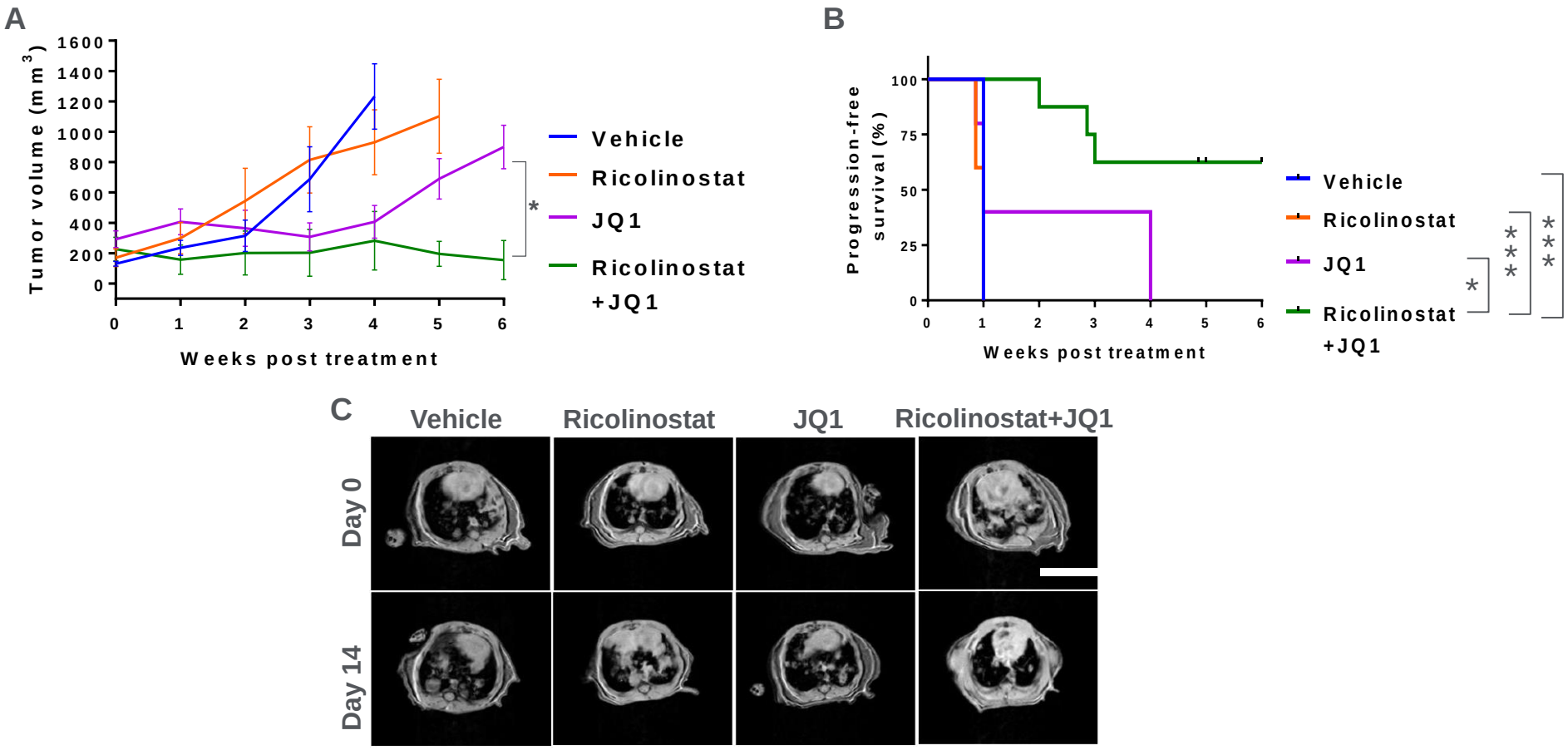
Cd25, Ctla-4, Foxp3

Lag3, Pd-1, Ccr4

Cancer Discovery 2017

NYU Langone Health

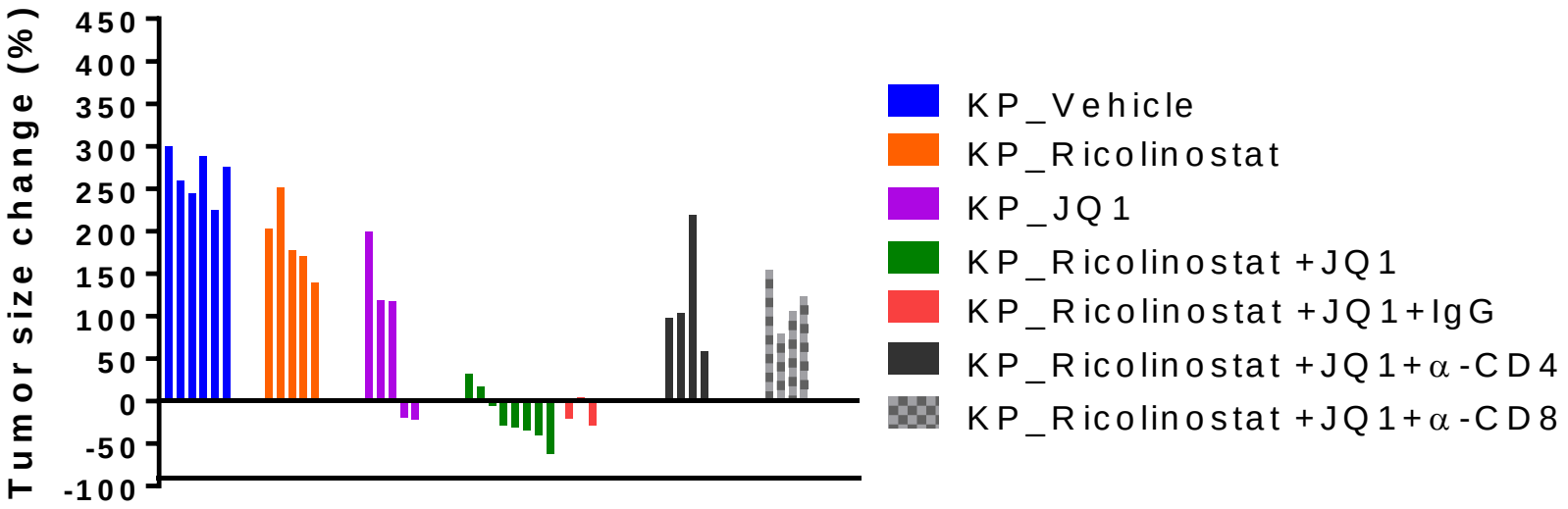
Combination of ACY1215 and JQ1 promotes durable anti-tumor response in GEM (KP)



Cancer Discovery 2017

NYU Langone Health

Immunotherapeutic effect of ACY1215 and JQ1 is dependent on both CD4+ and CD8+ T cell presence



Cancer Discovery 2017

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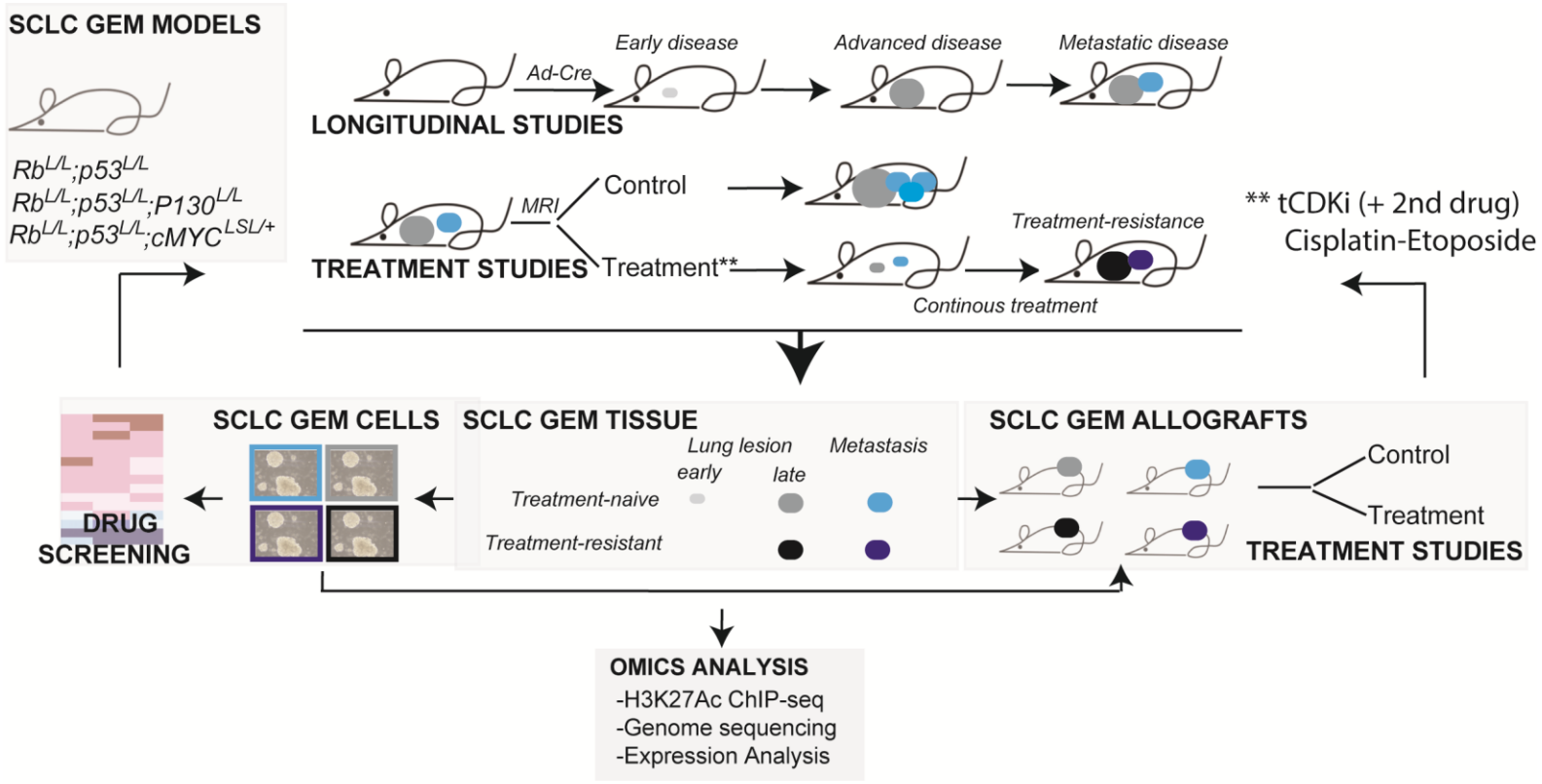
Metastatic murine small cell lung cancer preclinical models and new genomic technologies to interrogate TME

NYU Langone Health

Investigation of the SCLC treatment-naïve and resistant tumor microenvironment - SCLC GEM model-based platform



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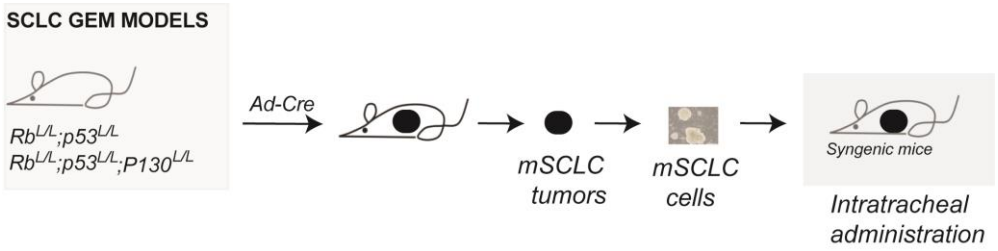
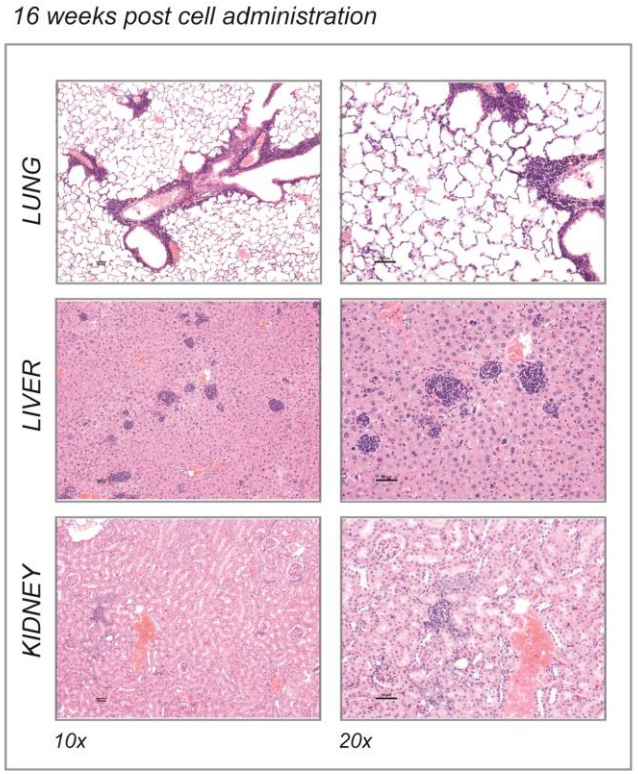
Investigation of the SCLC treatment-naïve and resistant tumor microenvironment - SCLC GEM model-based platform





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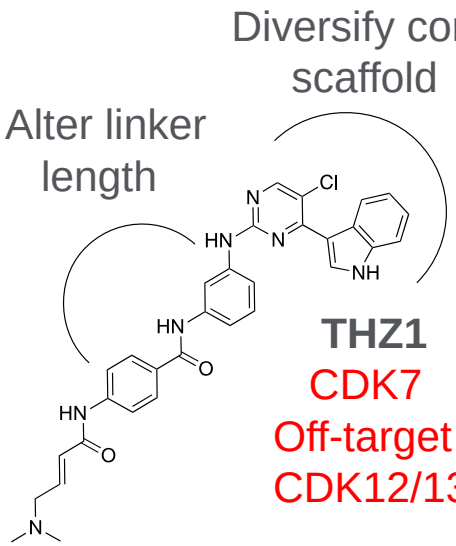
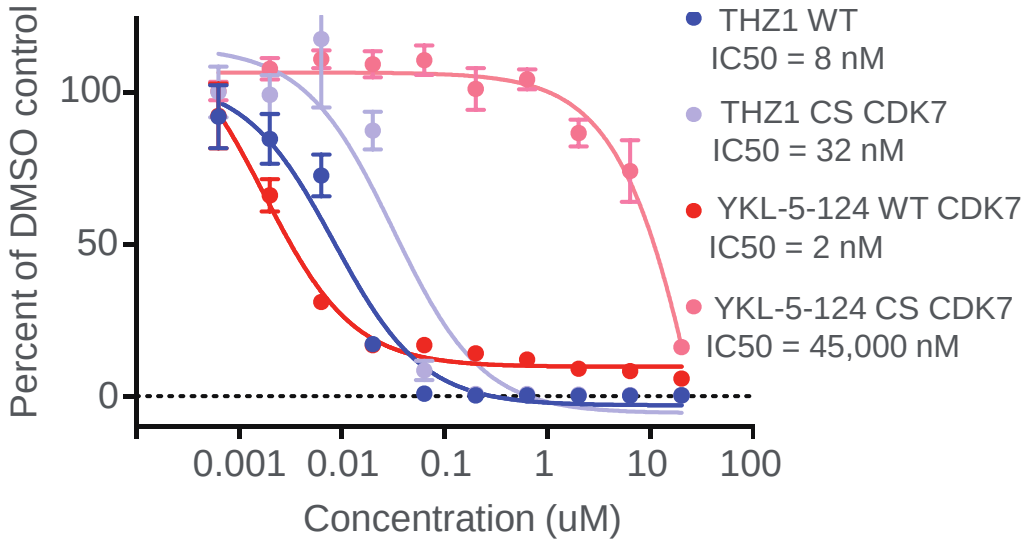
Medical chemistry beyond THZ1

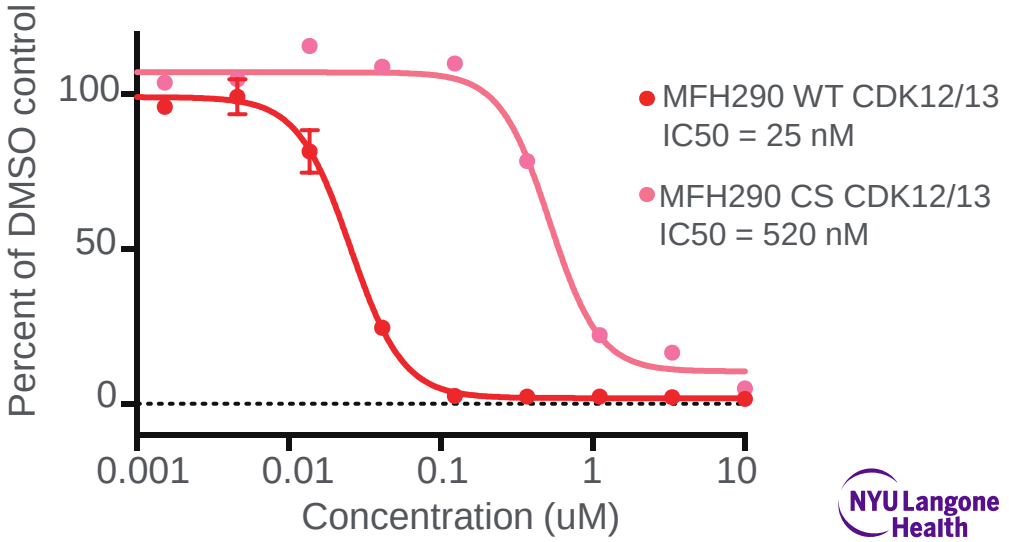


THZ1
CDK7
Off-target
CDK12/13

YKL-5-124
CDK7

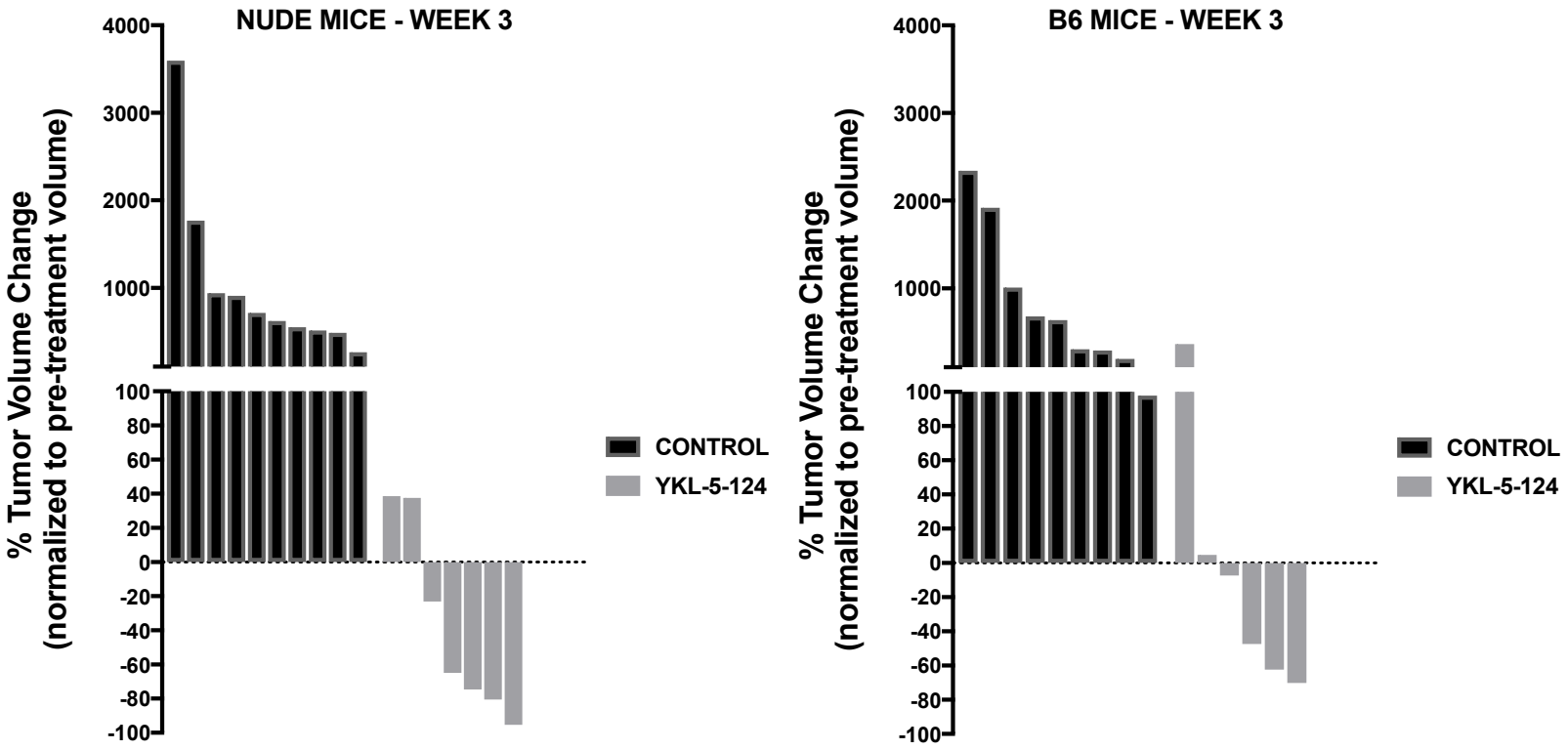


MFH290
CDK12/13



NYU Langone Health

Investigation of the SCLC treatment-naïve and resistant tumor microenvironment - YKL5-124 treatment



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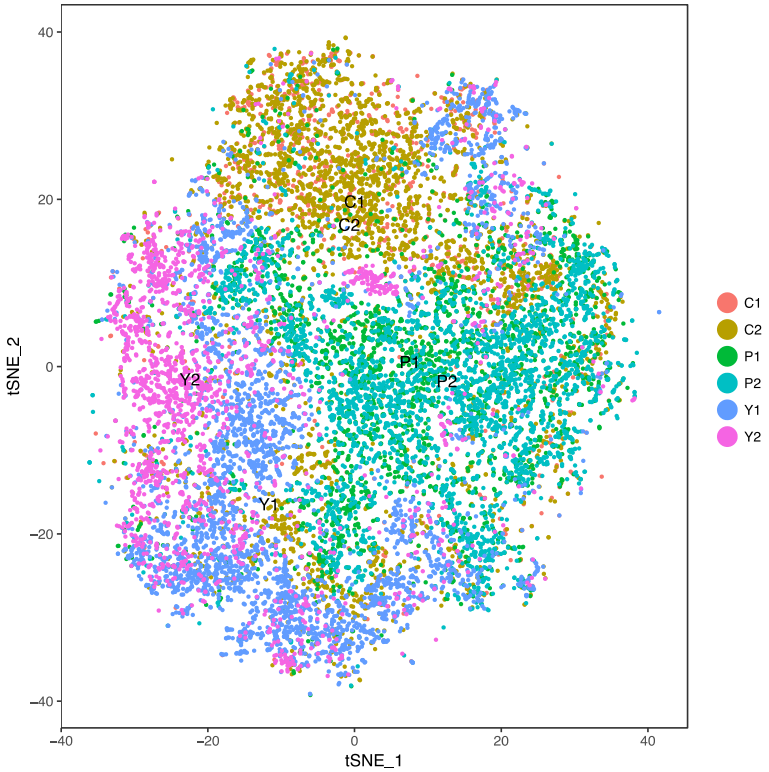
Cancer Cells

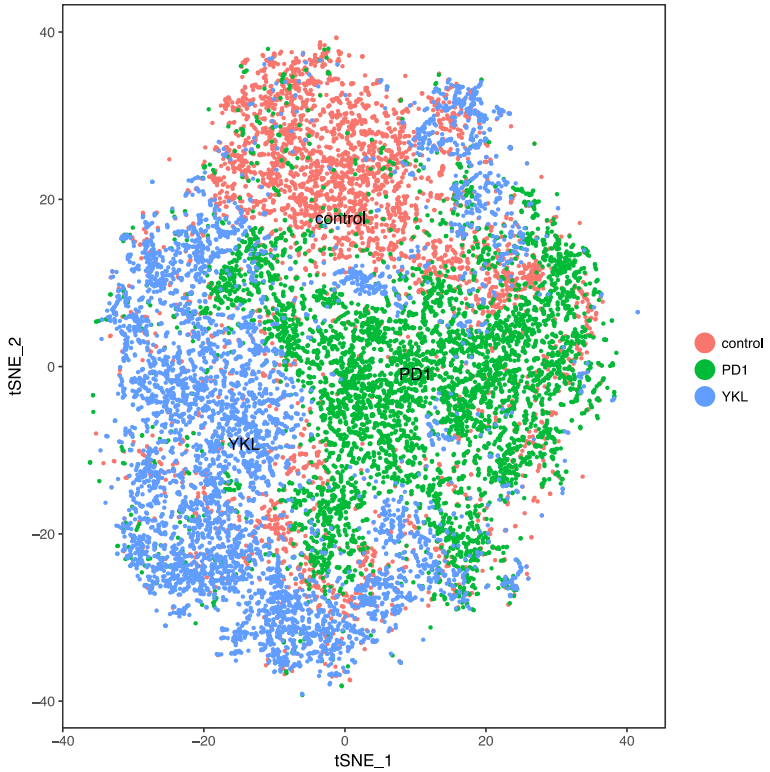
Biological replicates cluster together

- limited batch effects
- reproducible effects



Conditions (treatments + control) cluster together

- treatment results in strong transcriptional effect



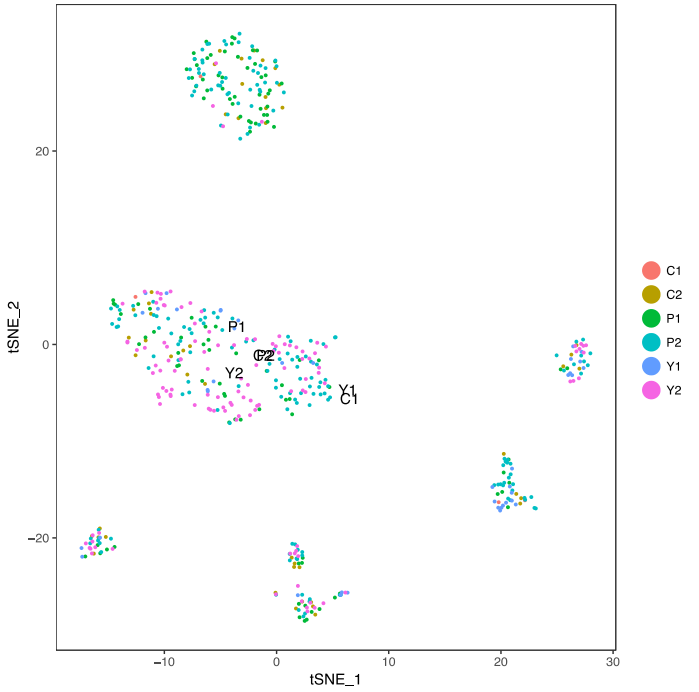
Ruben Dries, PhD

NYU Langone Health

Immune Cells

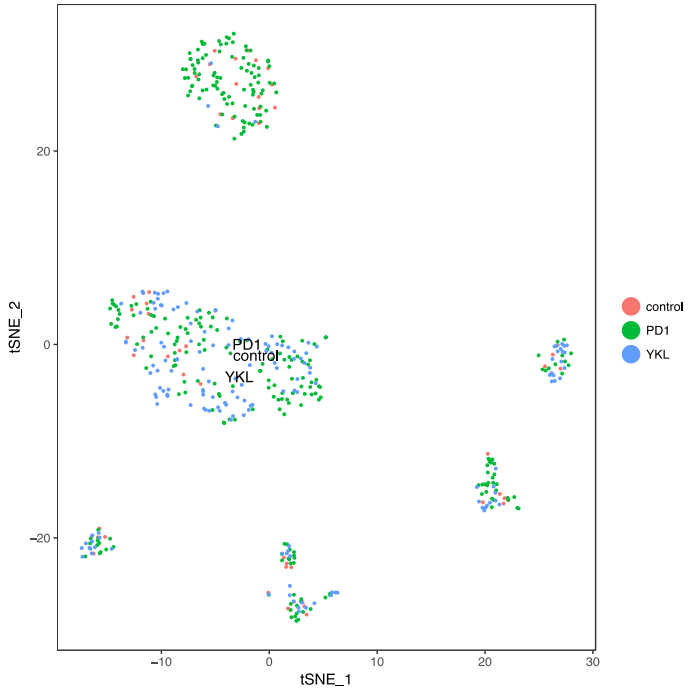
Biological replicates cluster together

- limited batch effects
- reproducible effects



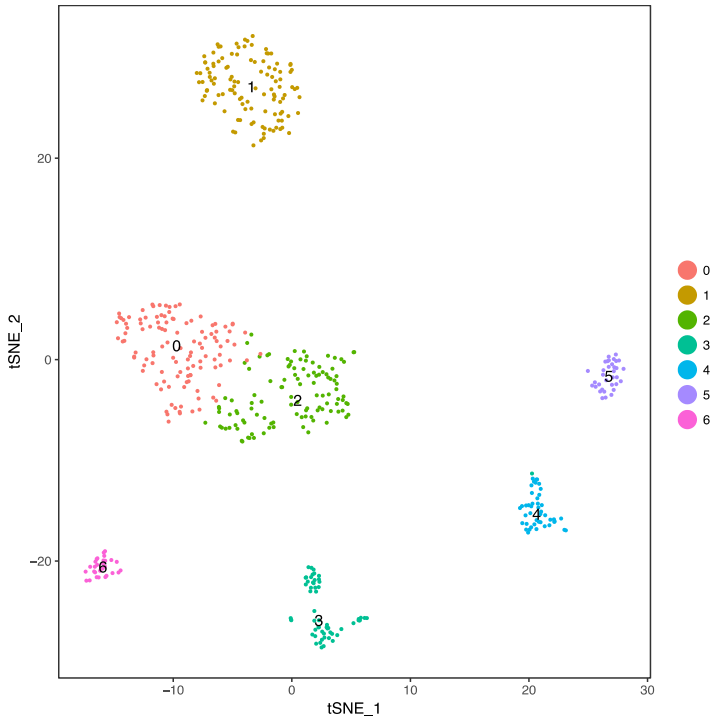
Conditions (treatments + control) do NOT cluster together

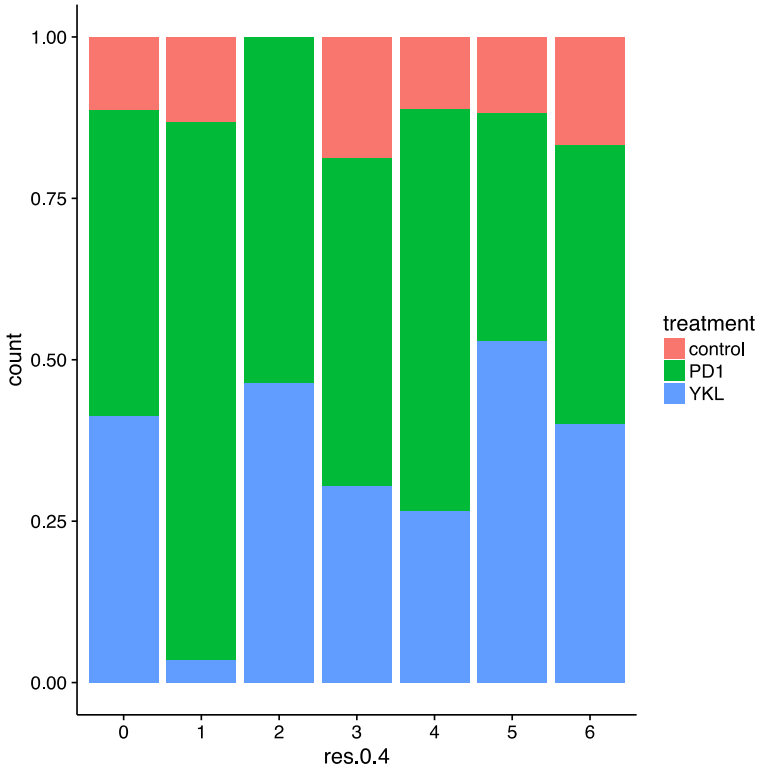
- major differences in mRNA abundance is due to different cell types



Immune Cells

- Distribution of conditions in different identified clusters:
  - most clusters contain single-cells from all conditions, except cluster 2
  - cluster is enriched for single-cells from the PD1 treated samples

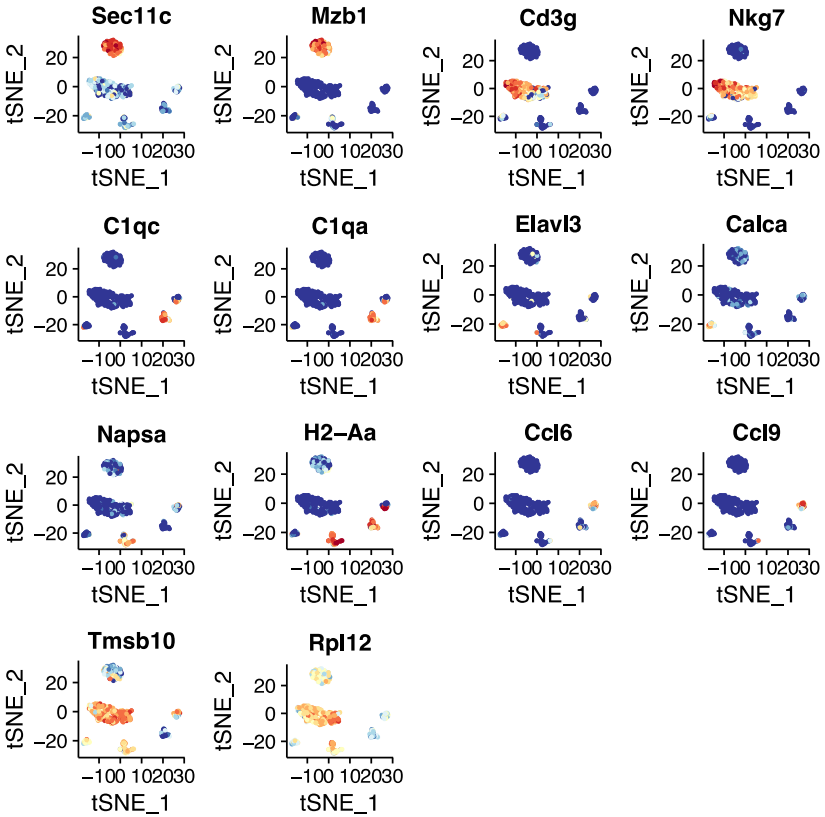




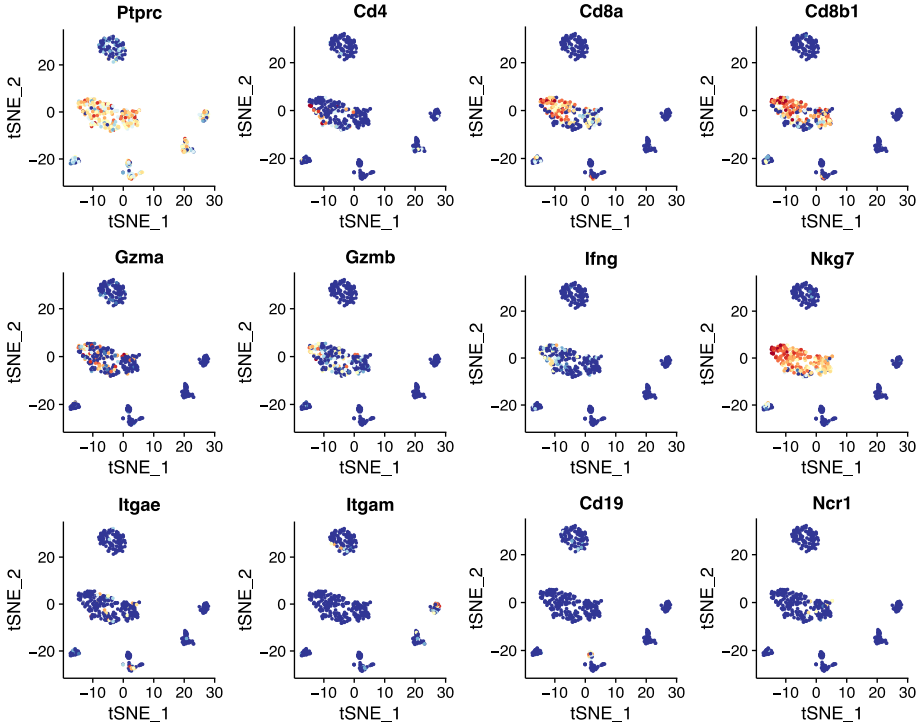
Immune Cells

- Based on New and Known markers, plus gene enrichment analysis, we could identify different types of immune cells: T cells, B cells, Dendritic cells, Macrophages, Monocytes, ... (fine-tuning in progress)

New markers



Known markers



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