

# **Rediscovering T cell dysfunction in the context of lung tumors.**

SITC TME workshop  
San Diego, April 2022

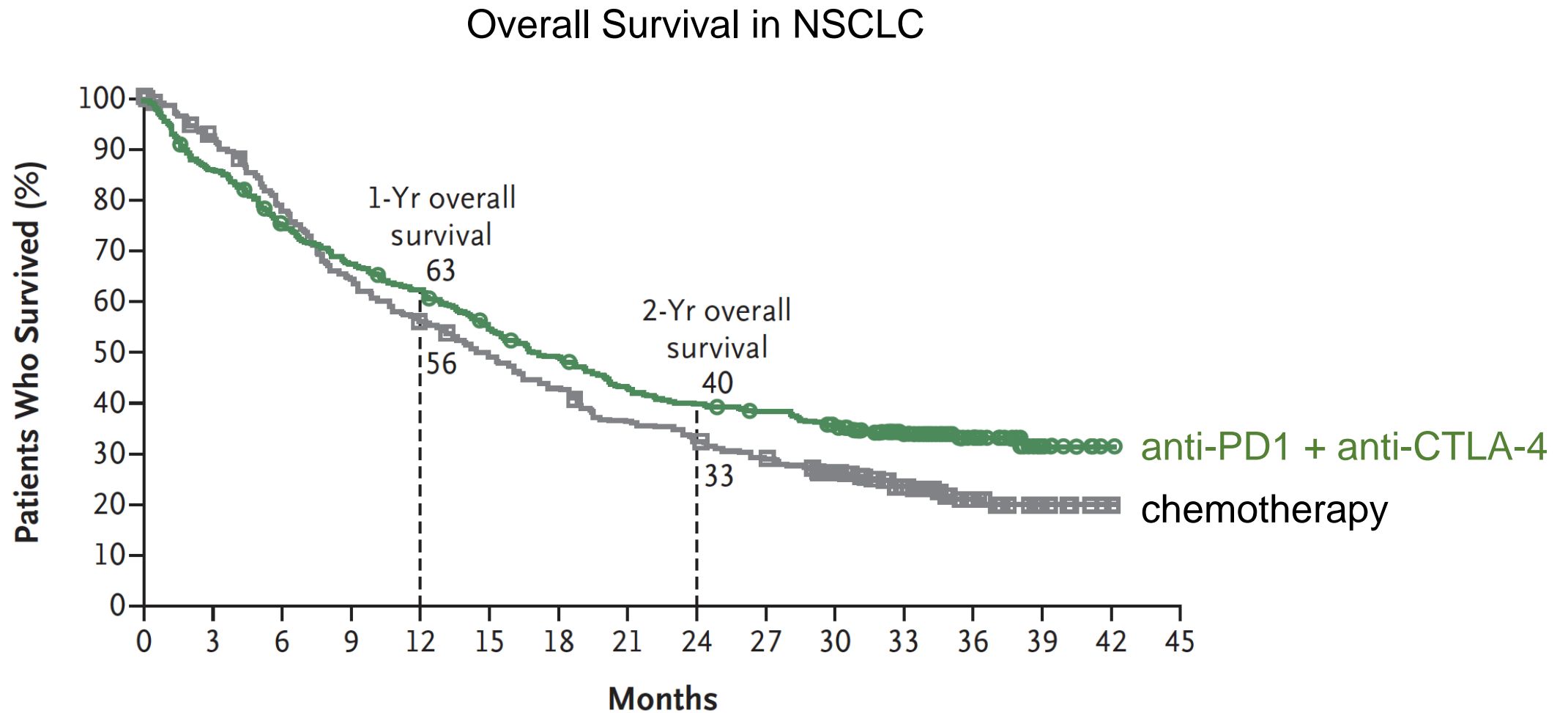
# Disclosures

Co-founder and SAB member  
**Danger Bio**

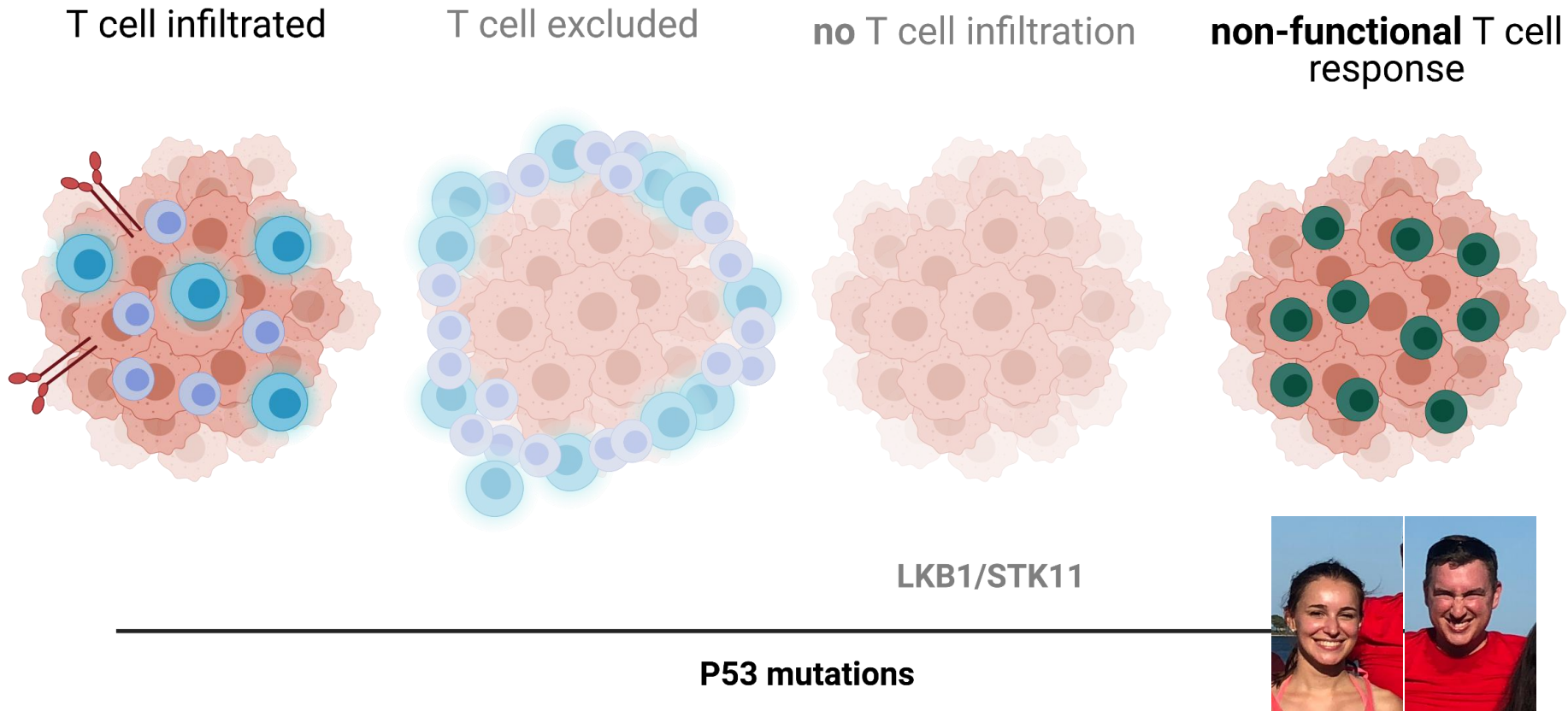
SAB member or consultant for  
**Ankyra Therapeutics**  
**Arcus Biosciences**  
**Takeda**  
**Ribon**  
**Dragonfly**  
**Merck**

Research support from  
**Leap Therapeutics**

# Immune checkpoint blockade can induce durable responses in a subset of lung cancer patients



# Immunotherapy is highly effective but only in a fraction of cancer patients

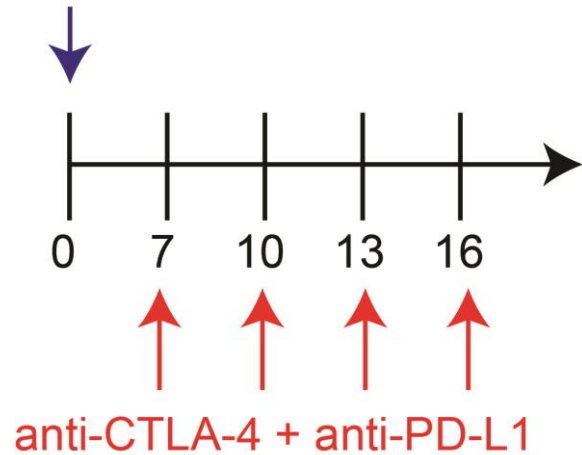


Herbst et al. 2014 Nature  
Chen and Mellman, 2017 Nature

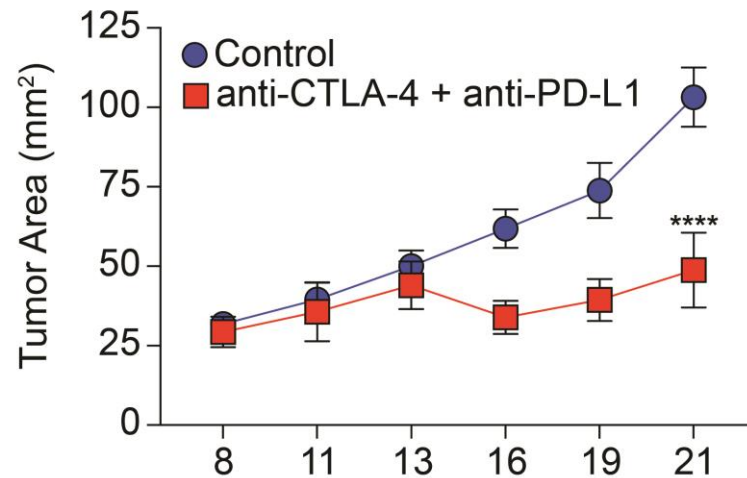
Skoulidis et al. 2015 Can. Disc.  
Koyama et al. 2016 Can. Res.

# Immunotherapy controls flank tumors but not lung tumors

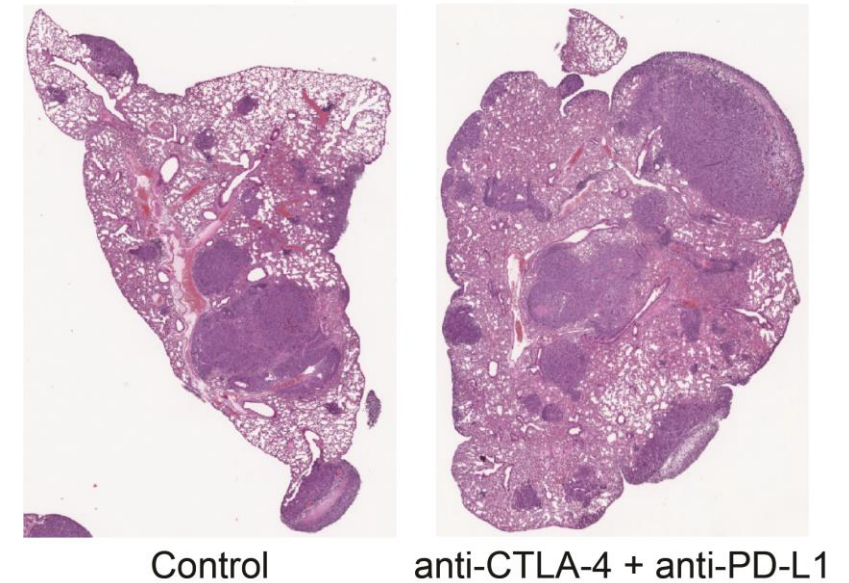
Lung Tumor KP Cell Line  
Subcutaneous or Intravenous



Subcutaneous Tumor Outgrowth



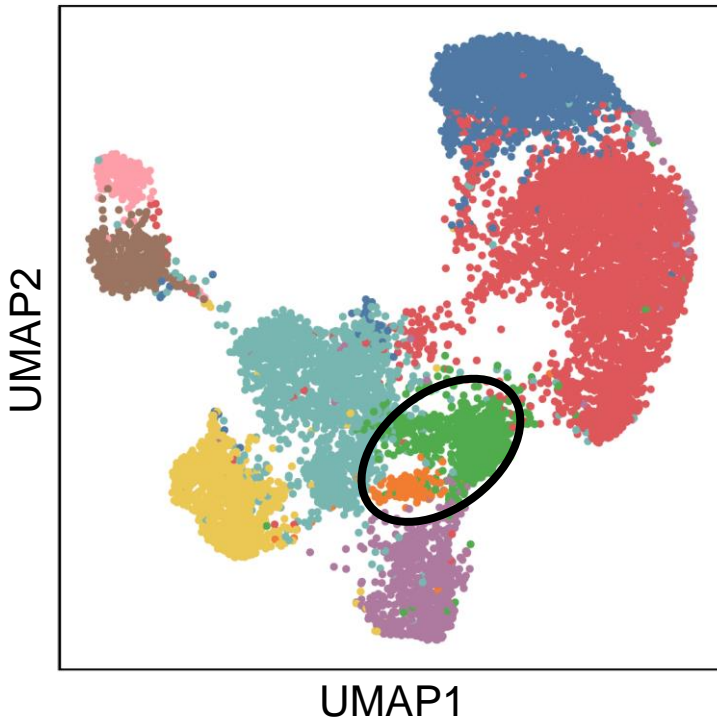
Lung Tumor Outgrowth



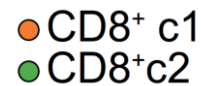
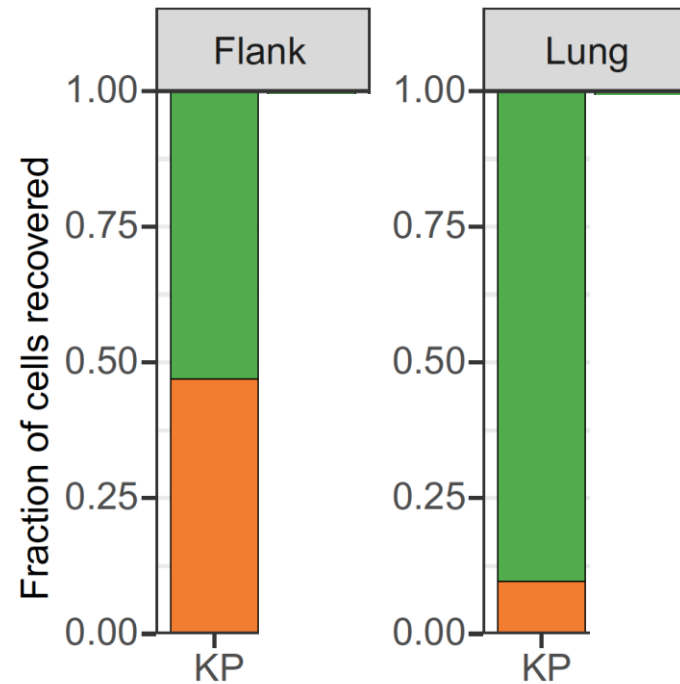


# Lung-tumor specific dysfunction program in CD8<sup>+</sup> T cells drives resistance

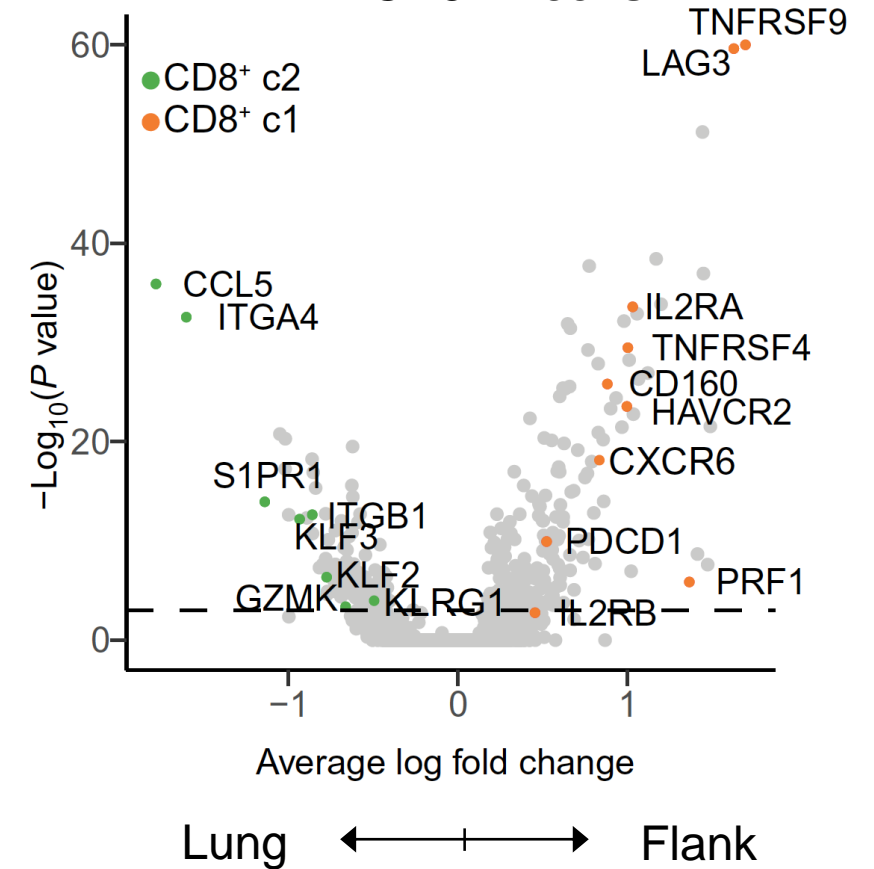
Single-cell RNA sequenced tumor-infiltrating T cells



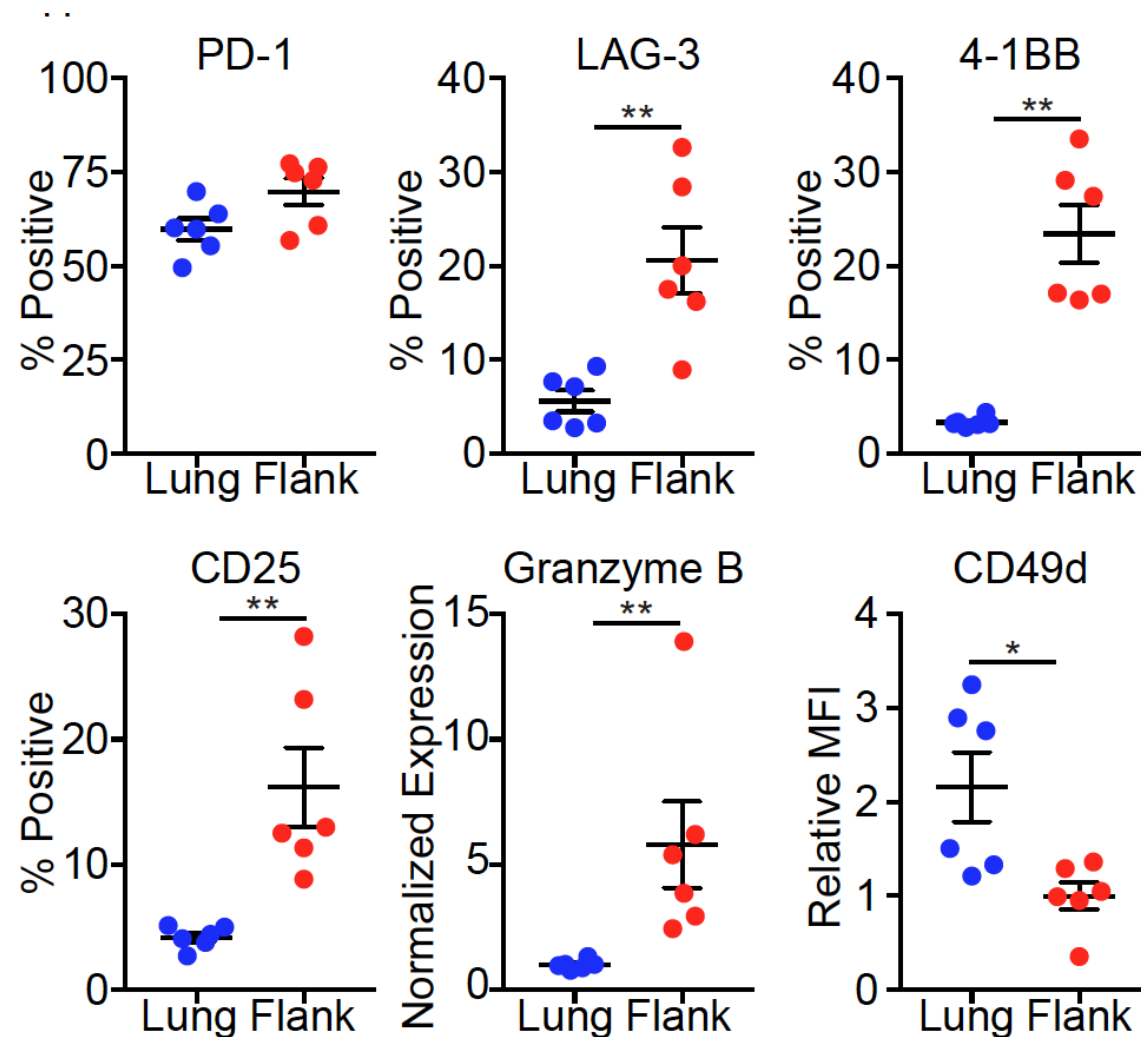
CD8<sup>+</sup> T cell composition



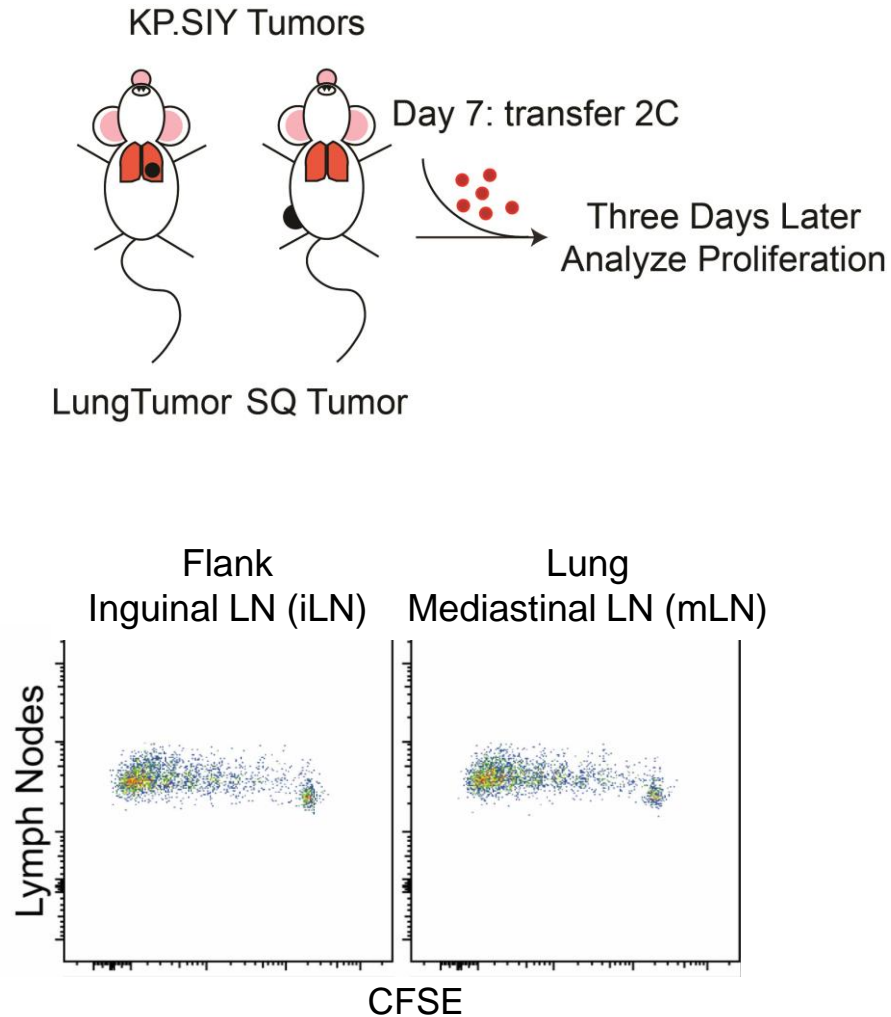
Differentially expressed genes in CD8<sup>+</sup> T cells



# CD8<sup>+</sup> T cells from lung and flank tumor environments are phenotypically distinct

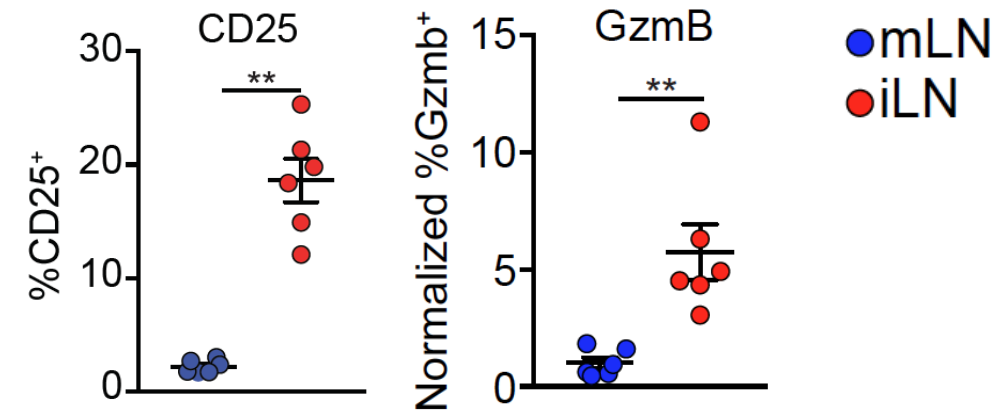
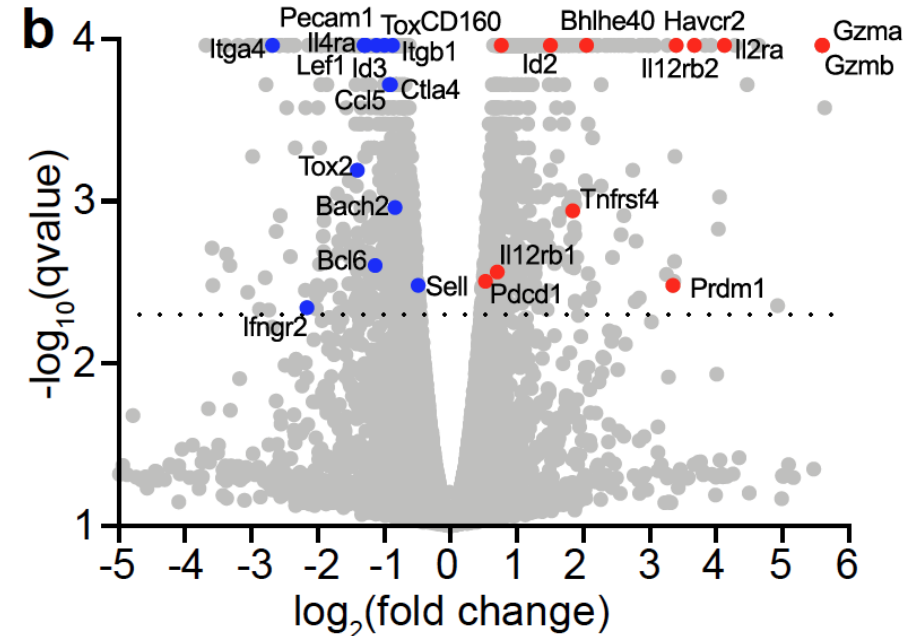
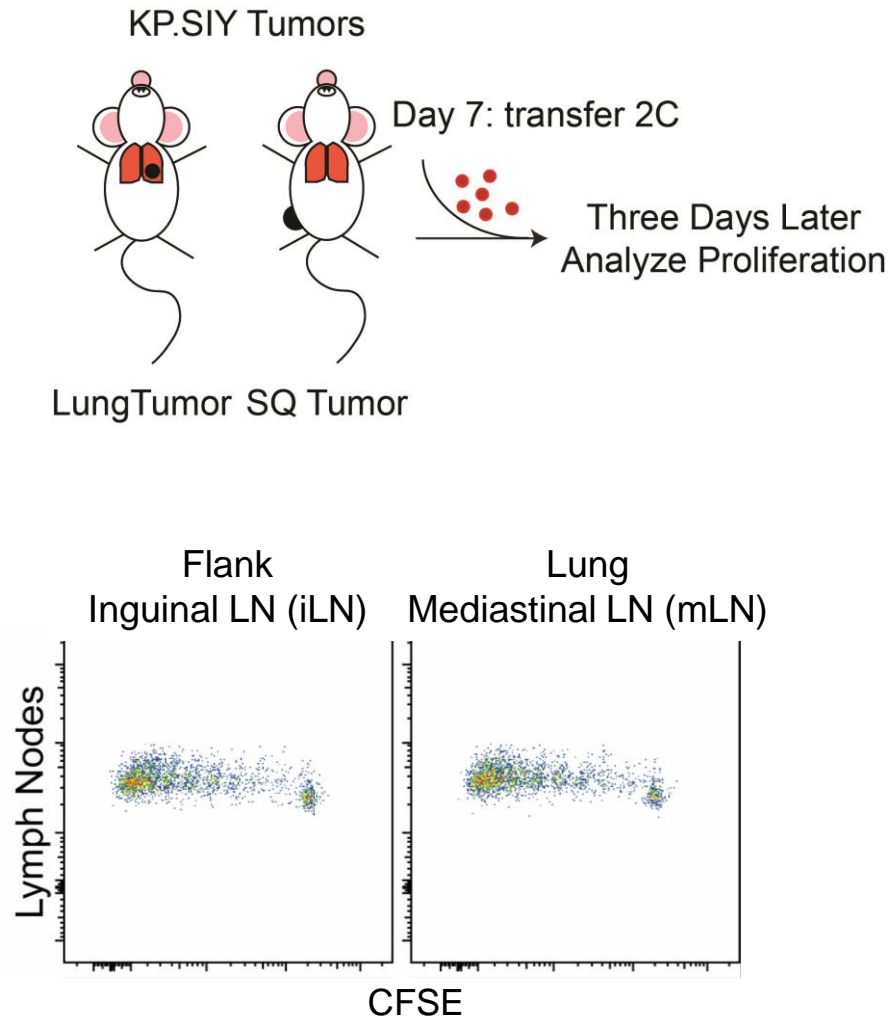


# Qualitative differences are induced during T cell priming in the draining LN

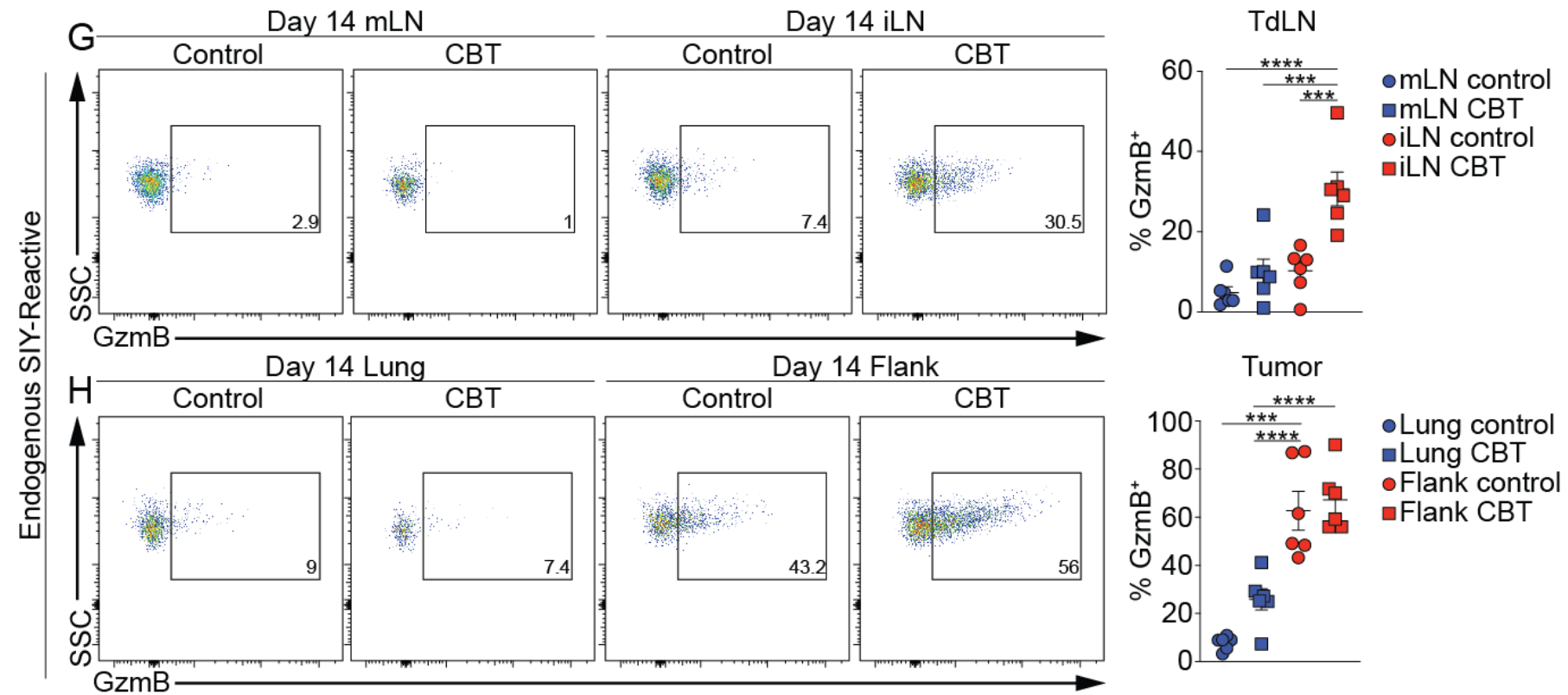




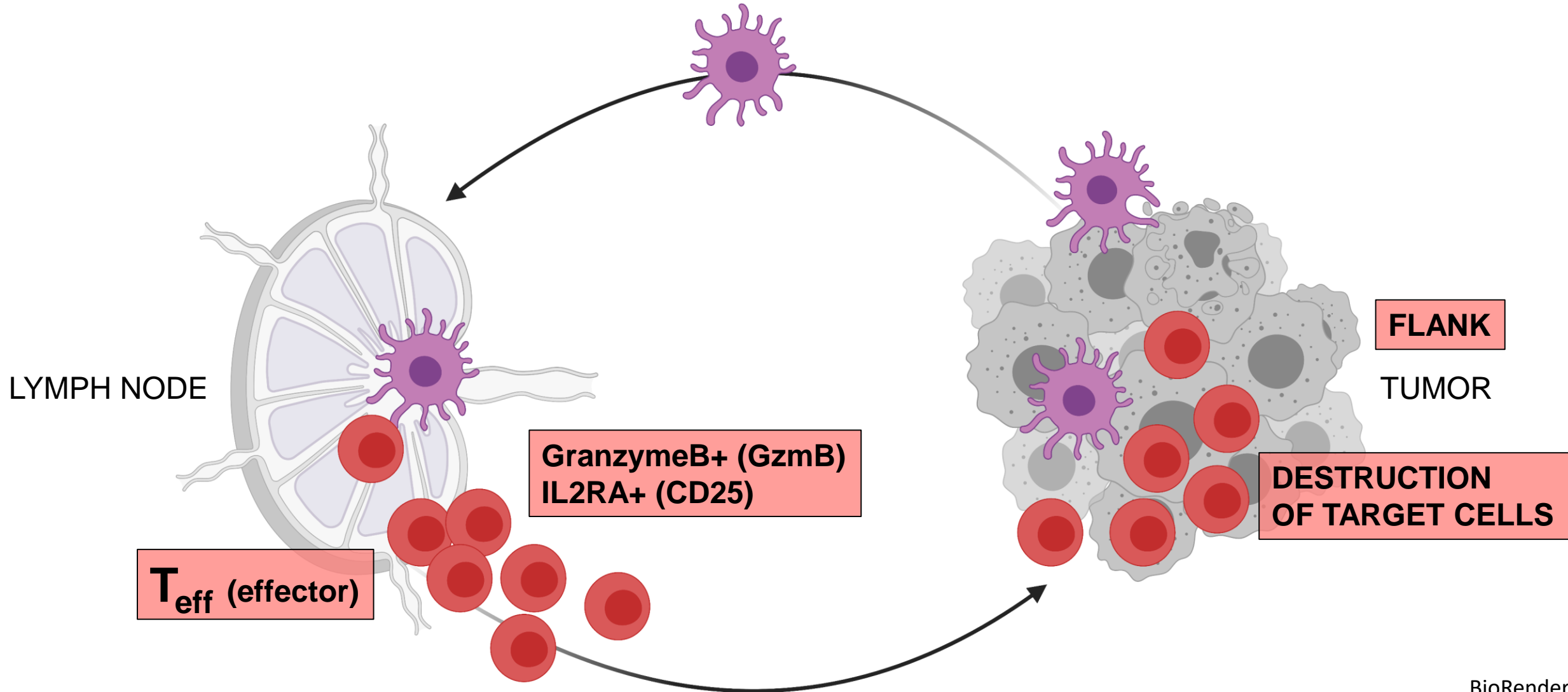
# Qualitative differences are induced during T cell priming in the draining LN



# T cells in the lung tumor-specific dysfunctional state are refractory to anti-PD-1/PD-L1 and anti-CTLA4



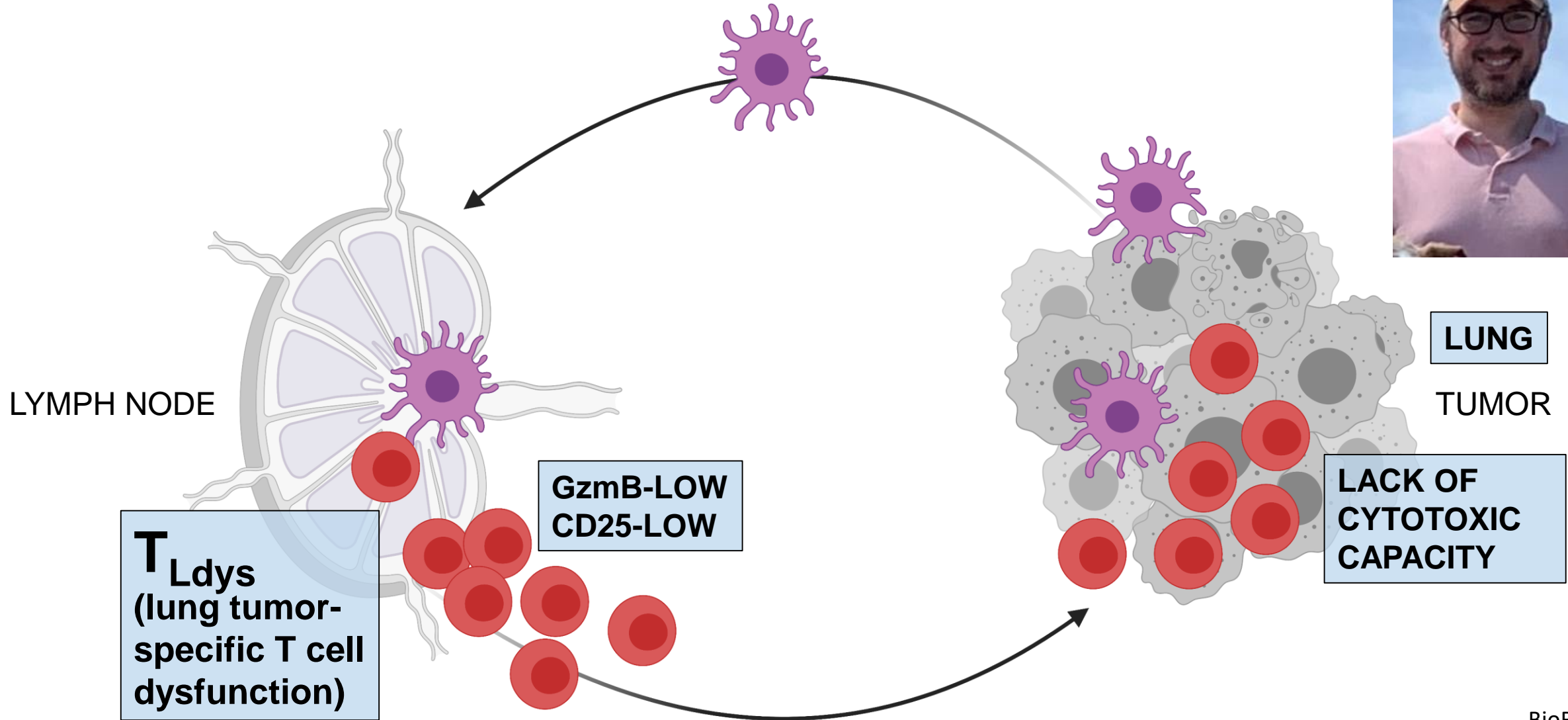
# T cell activation in the lymph node determines downstream T cell functions in the tumor



BioRender

Conclusions from Horton *et al.* (2021) Science Immunology

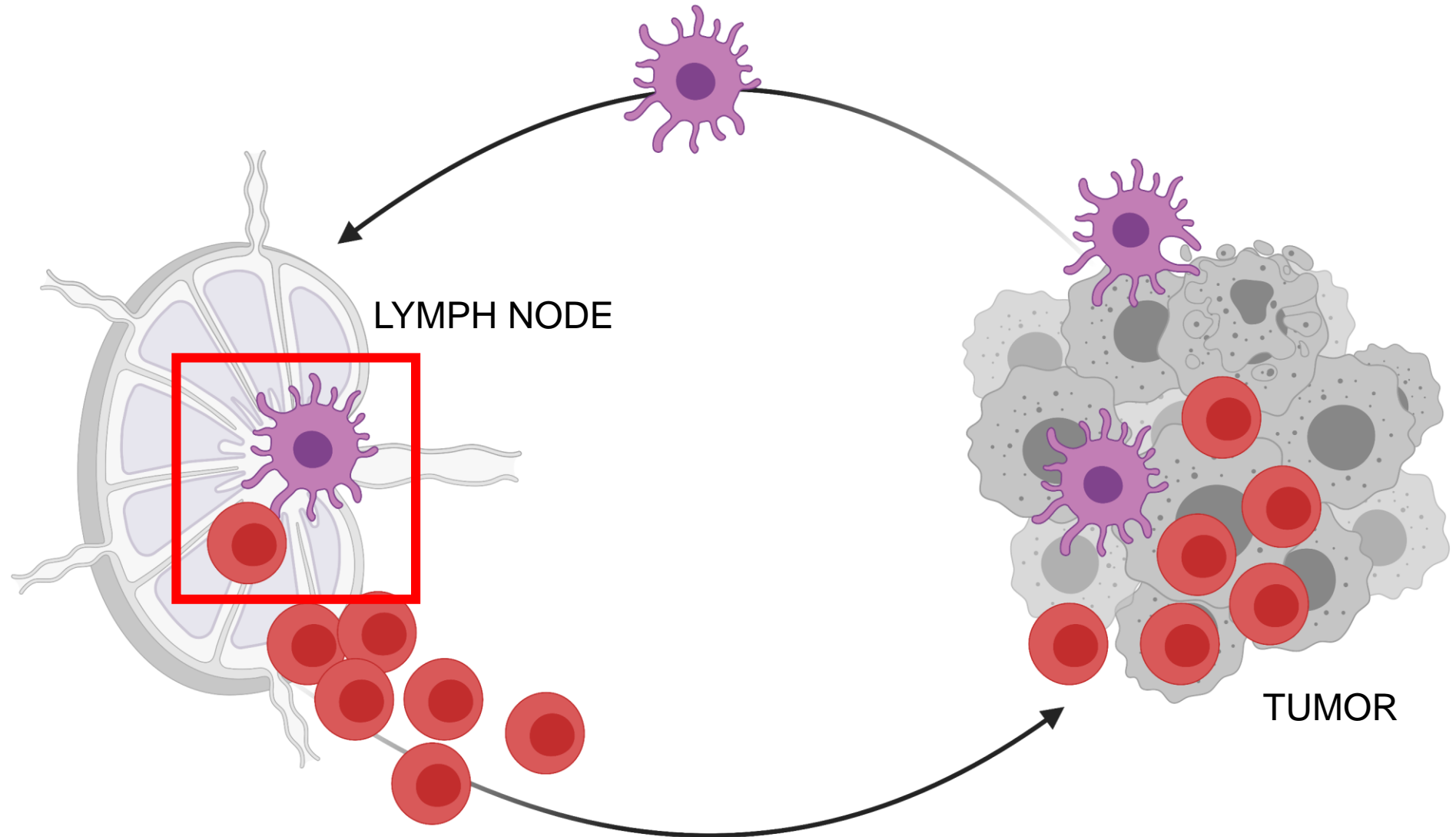
# T cell activation in the lymph node determines downstream T cell functions in the tumor



BioRender

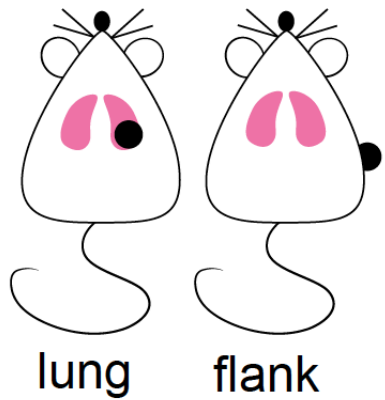
Conclusions from Horton *et al.* (2021) Science Immunology

# What factors control T cell activation phenotype?



# Lung lymph node DC1 have high signal 1 but low signals 2 and 3

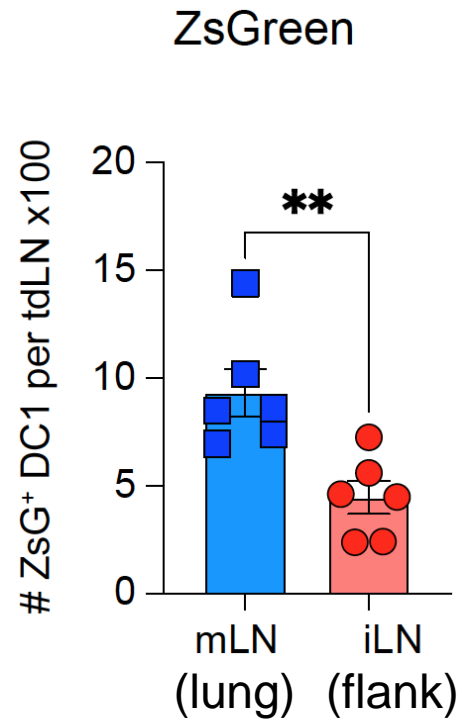
KP-zSIIN tumors



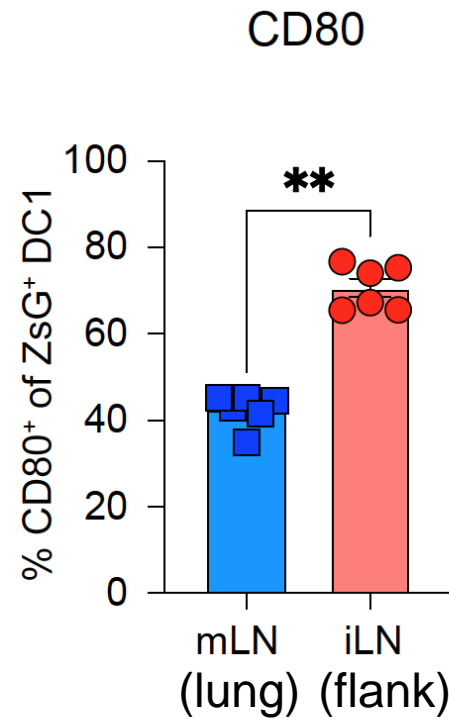
7 days

analyze  
ZsG<sup>+</sup> DC1  
in tdLN

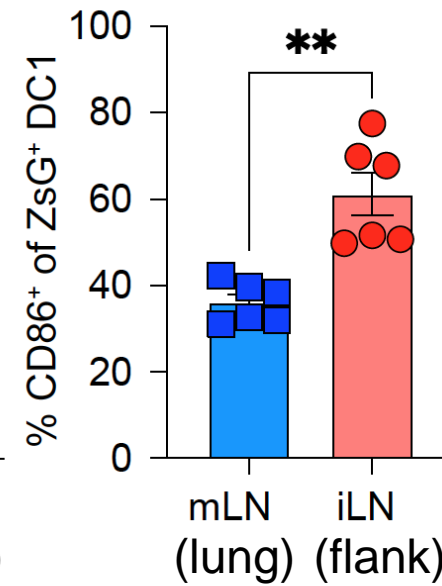
Signal 1 (antigen)



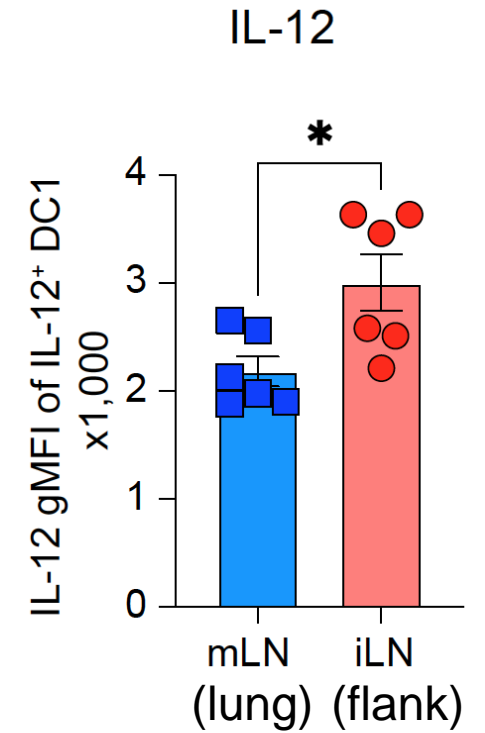
Signal 2 (costimulation)



CD86



Signal 3 (cytokines)

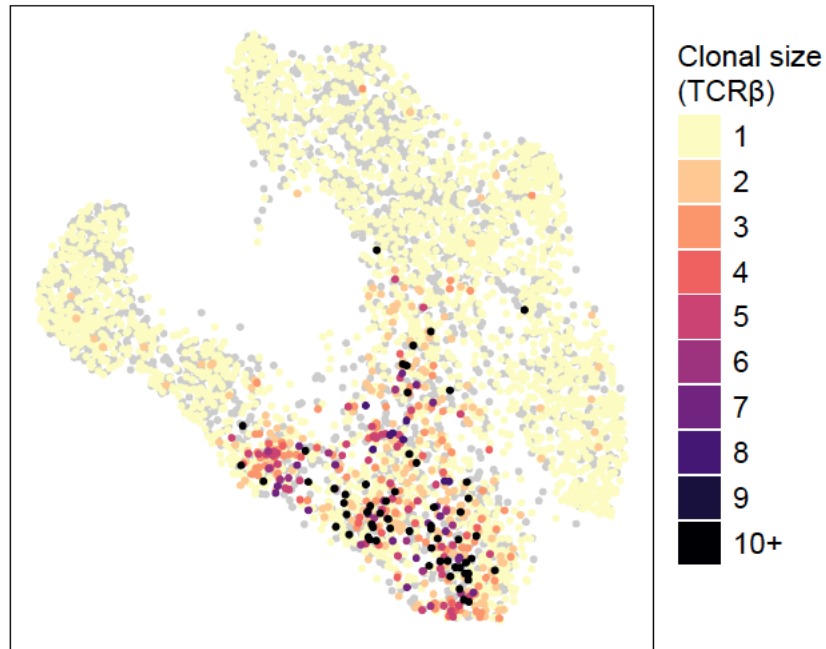




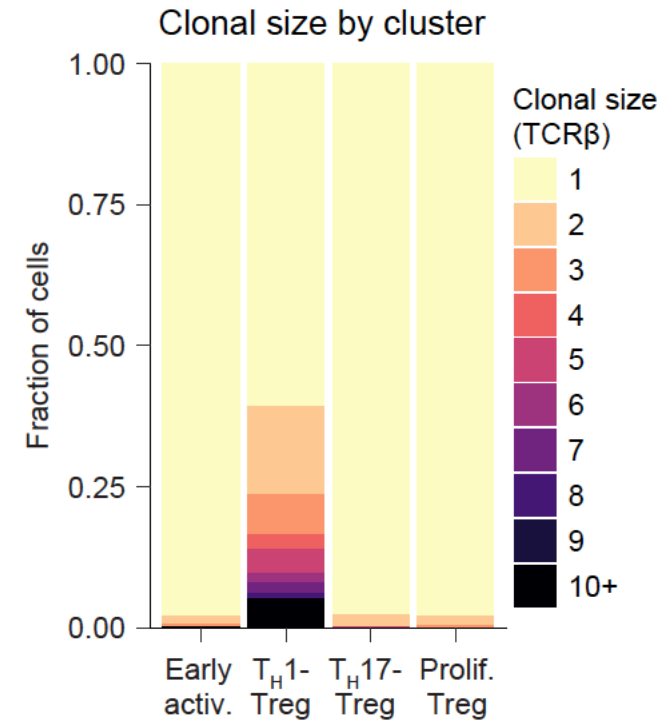
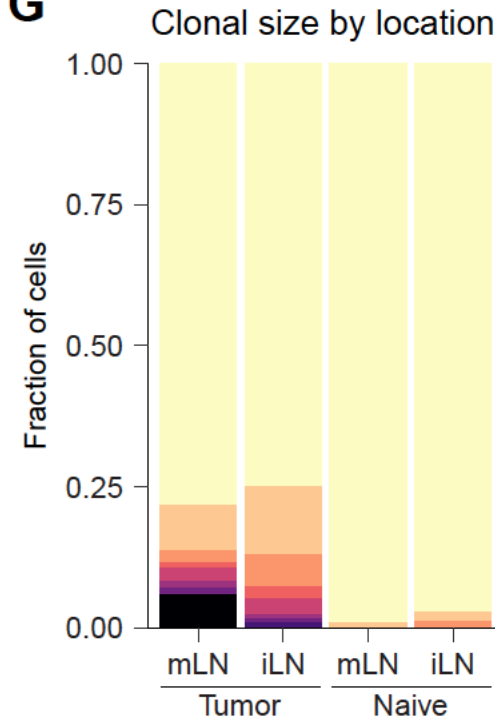
# TCR sequencing reveals similar levels of Treg clonal expansion in tumor-draining mLN and iLN

**F**

Activated Tregs from LN



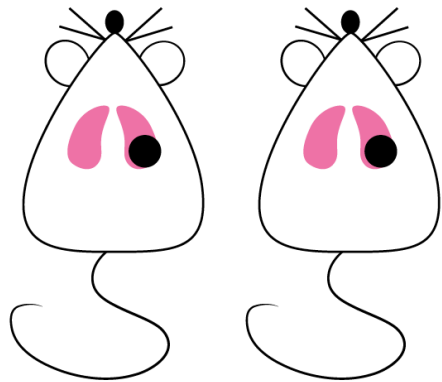
**G**



# Tregs are required for inducing dysfunctional CD8<sup>+</sup> T cells in the lung tumor-draining lymph node

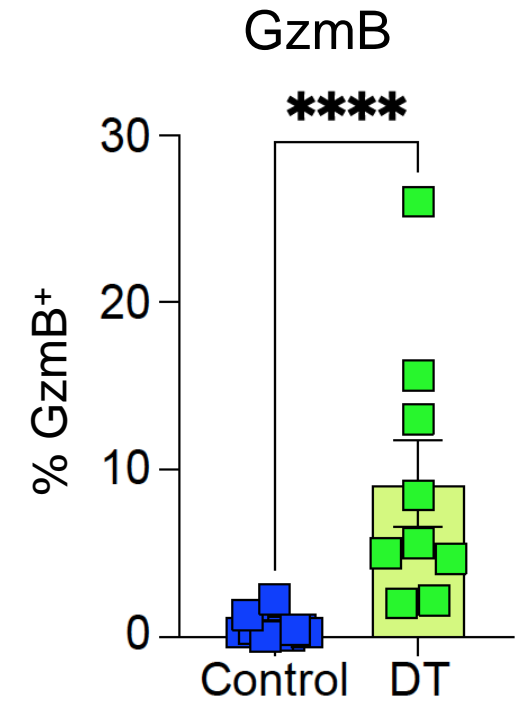
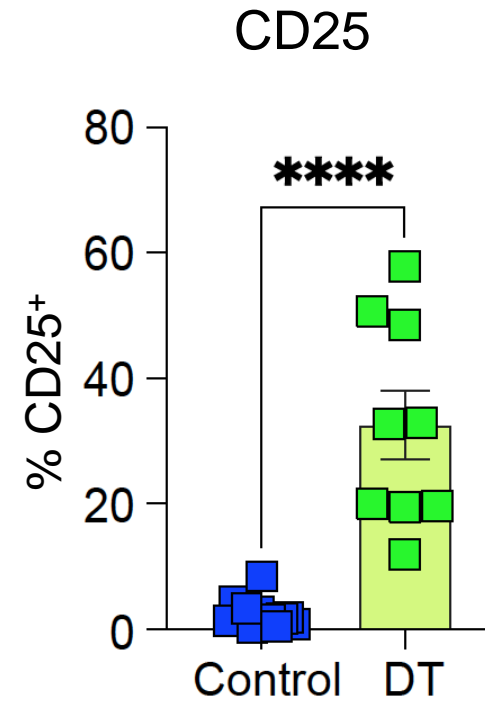
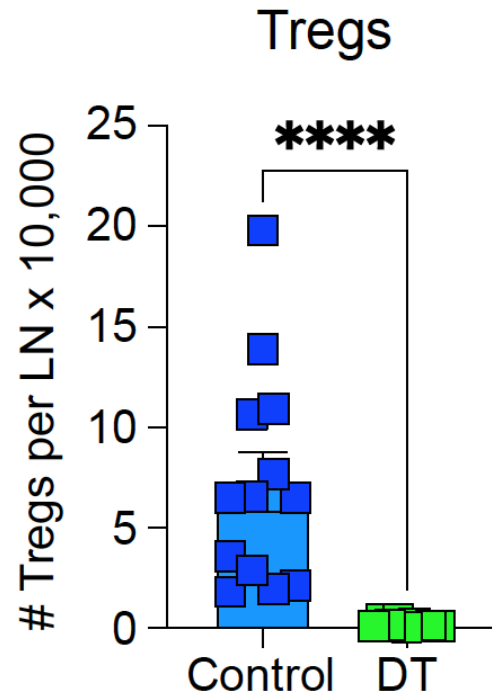
## Tumor-reactive CD8<sup>+</sup> T cells

KP-zSIIN lung tumors

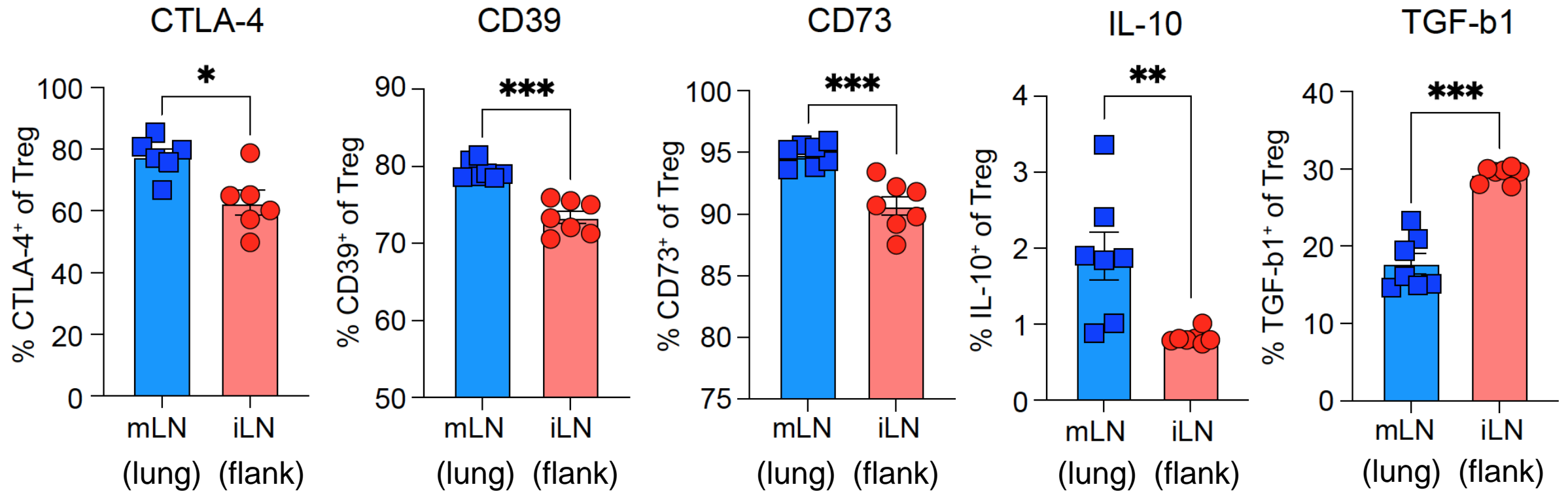


*FoxP3<sup>DTR</sup>*

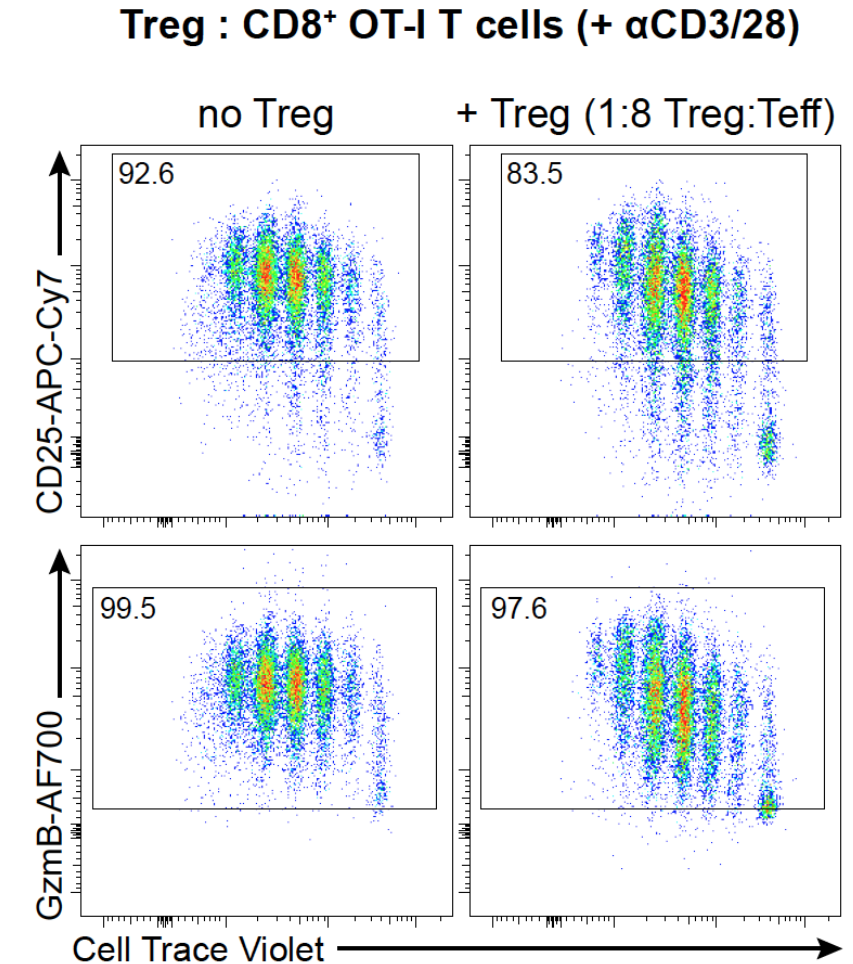
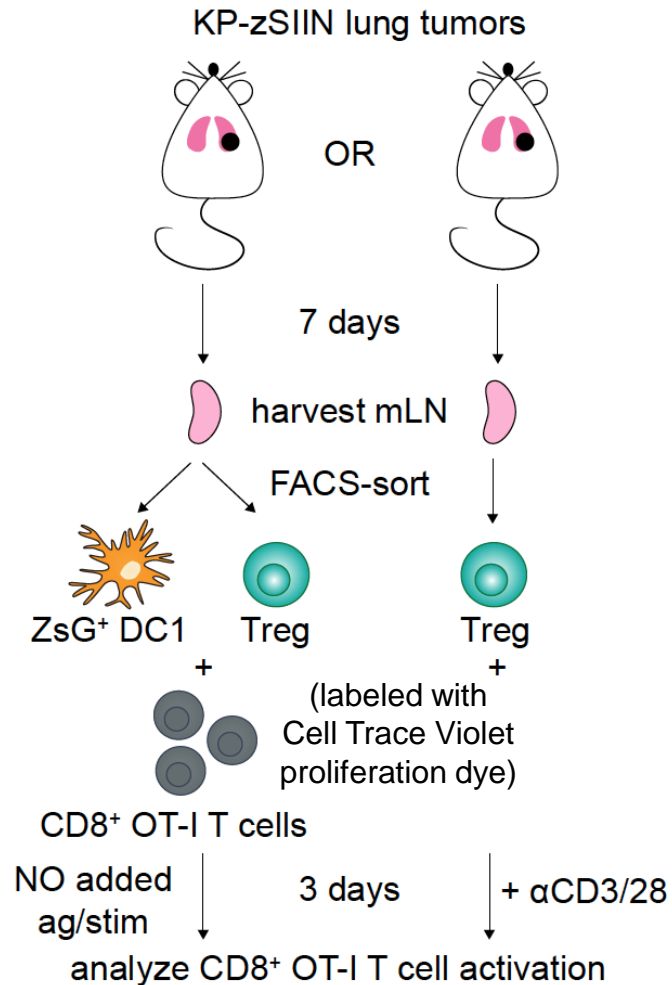
Control | DT-treated



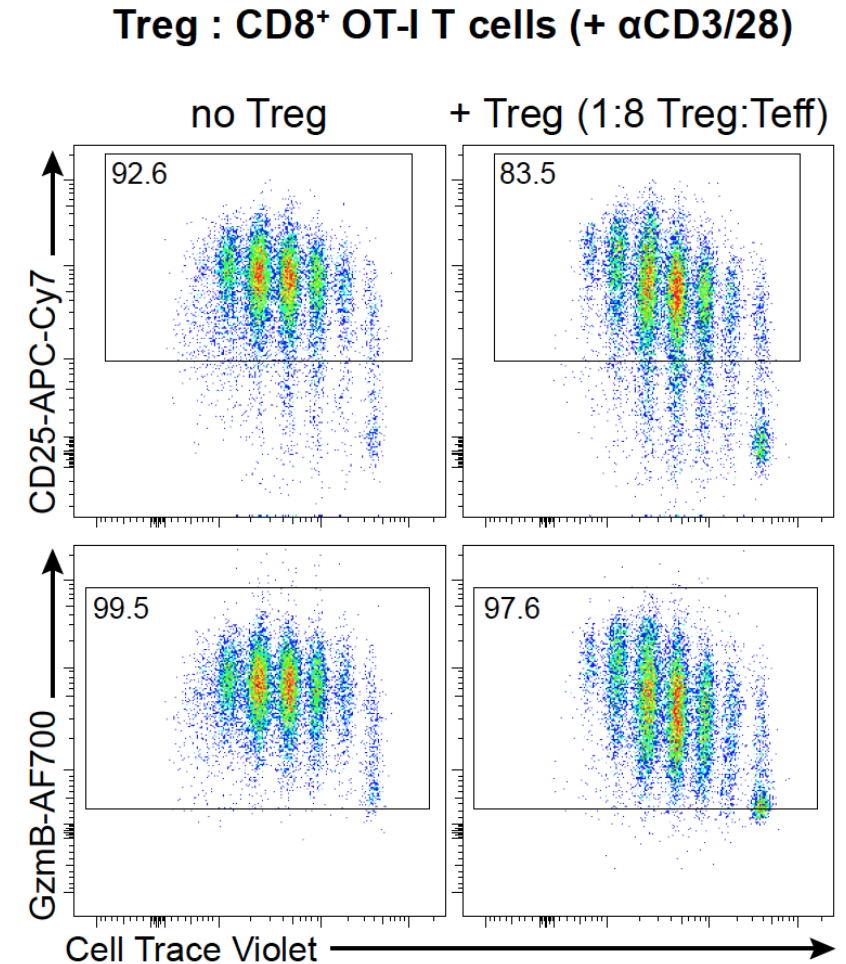
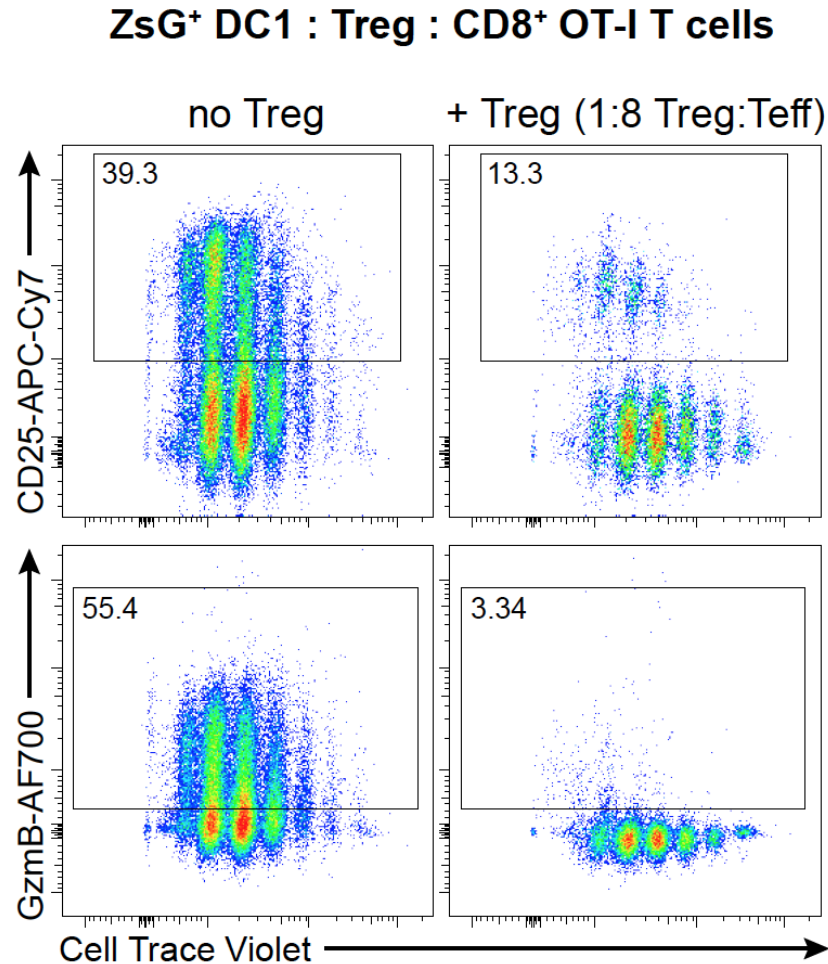
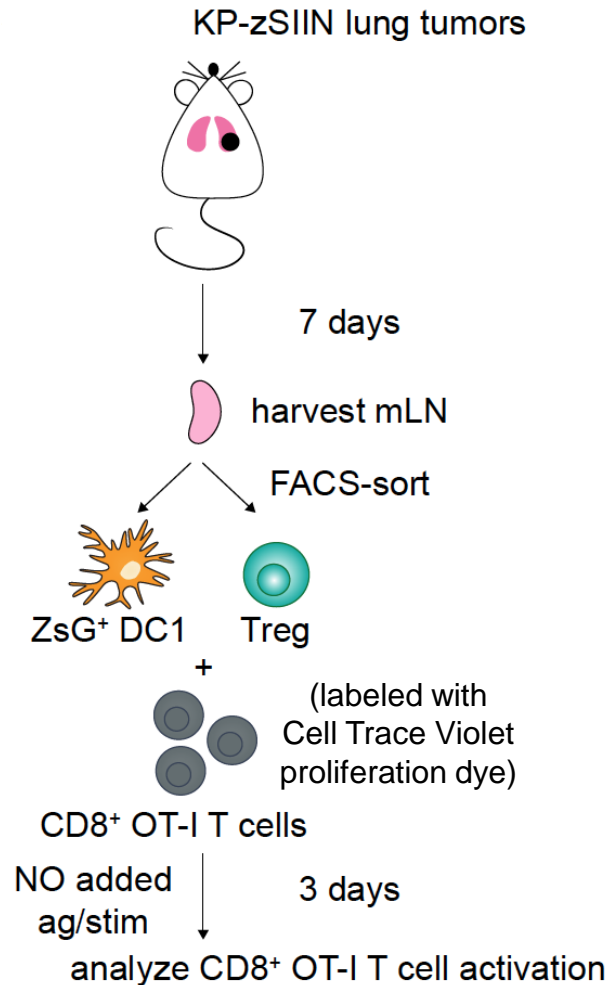
# Tregs in the lung and flank tumor-draining lymph nodes are phenotypically distinct



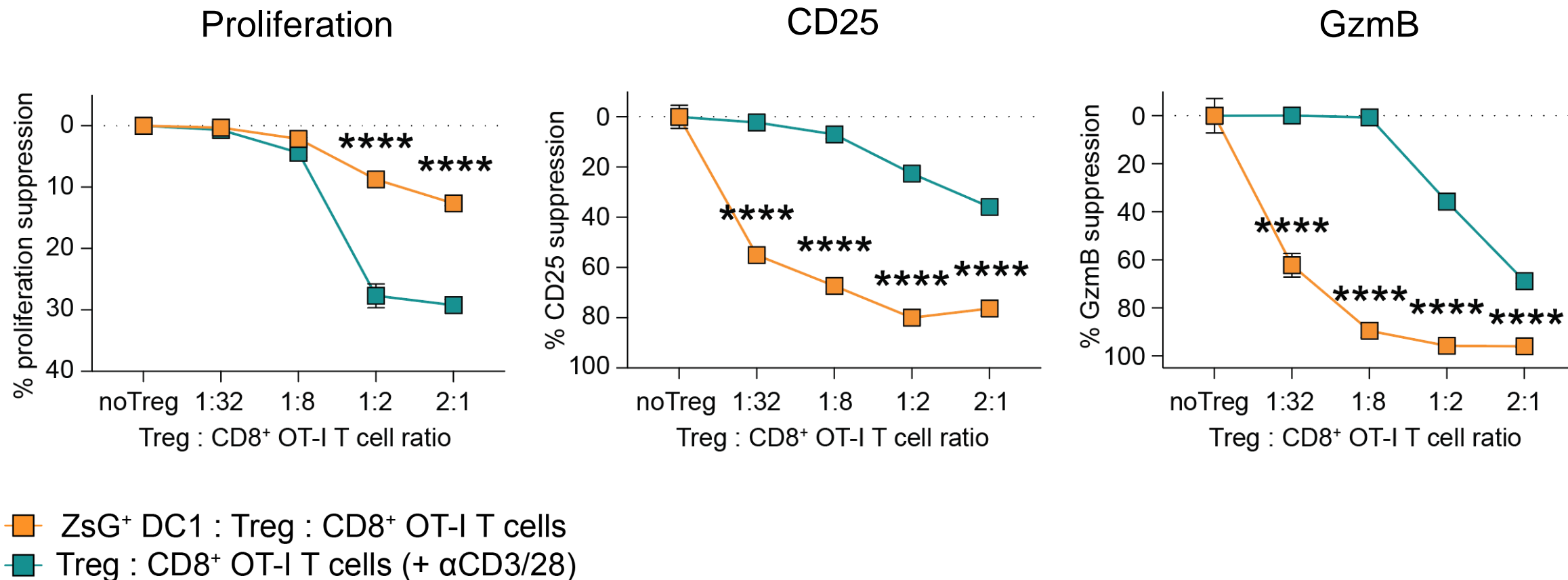
# DC1 and Treg from lung tumor-draining lymph node are sufficient to induce CD8<sup>+</sup> T cell dysfunction



# DC1 and Treg from lung tumor-draining lymph node are sufficient to induce CD8<sup>+</sup> T cell dysfunction

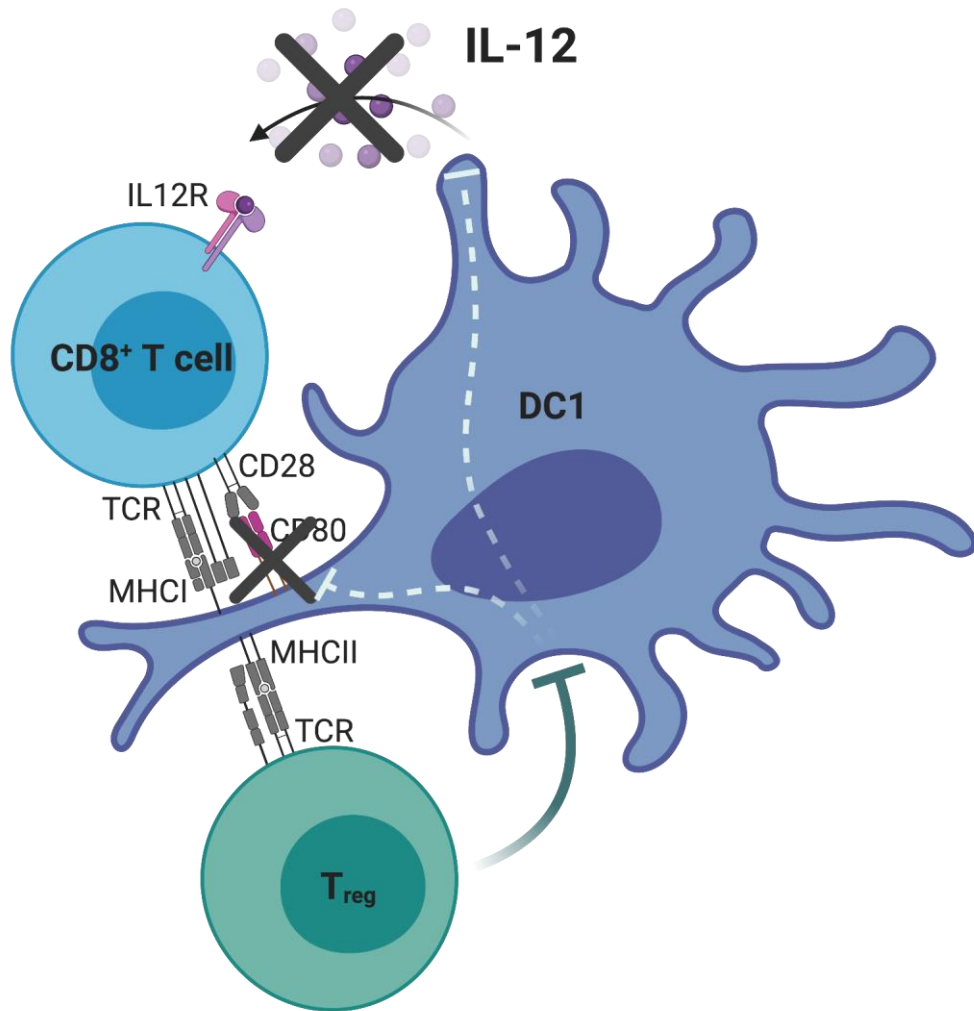


# Tregs can only induce CD8<sup>+</sup> T cell dysfunction when DC1 are present

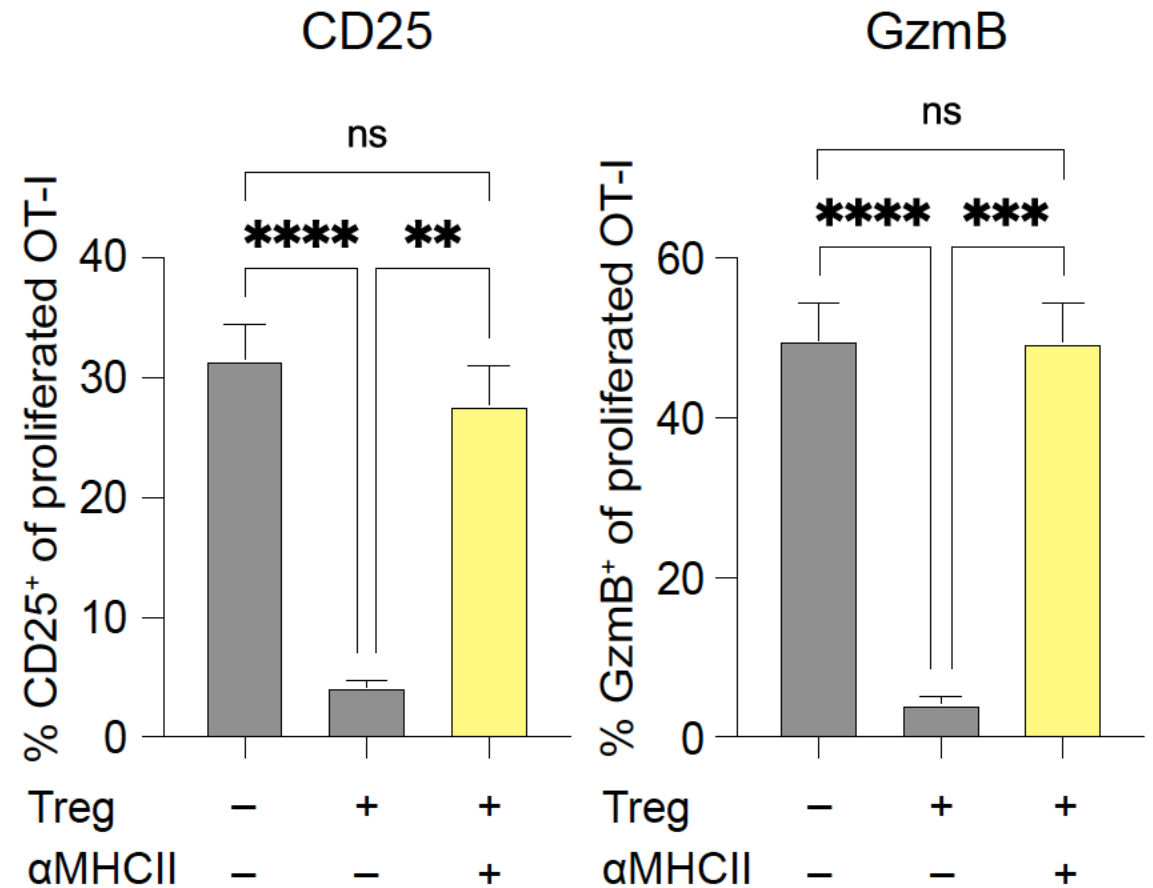
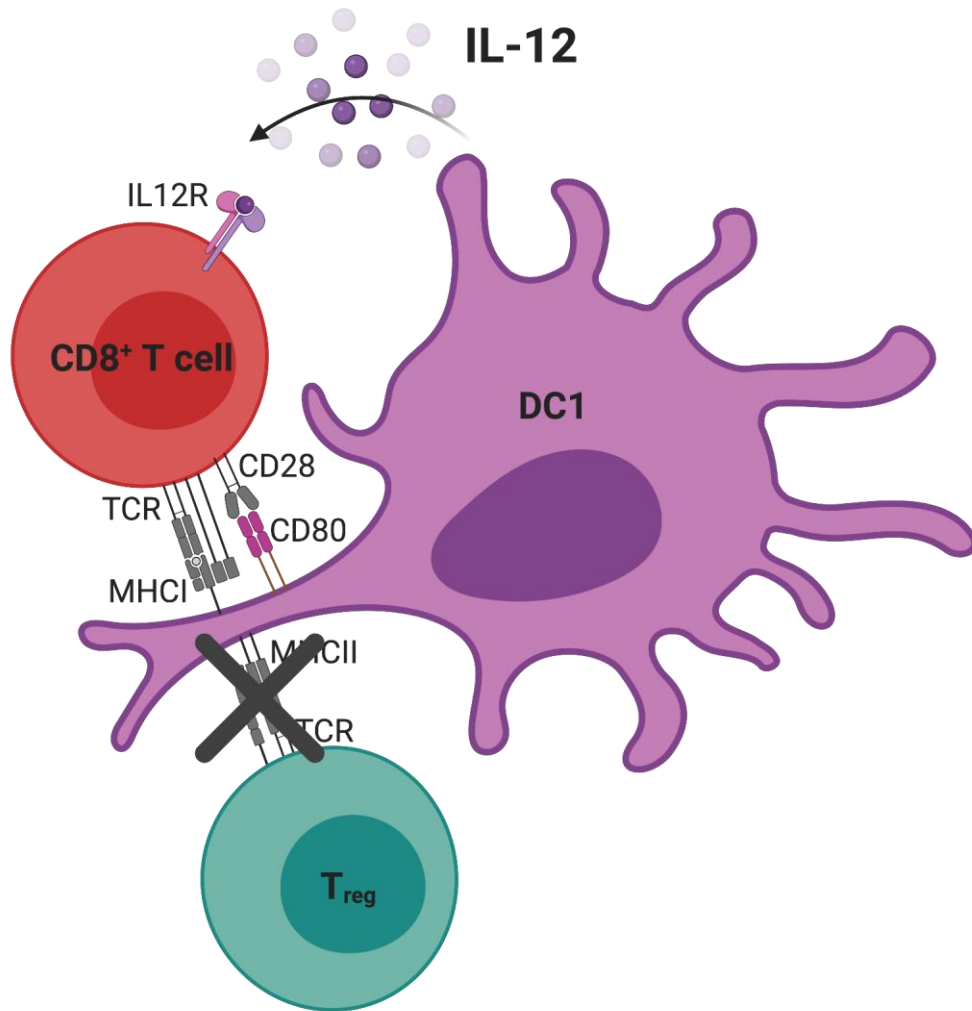




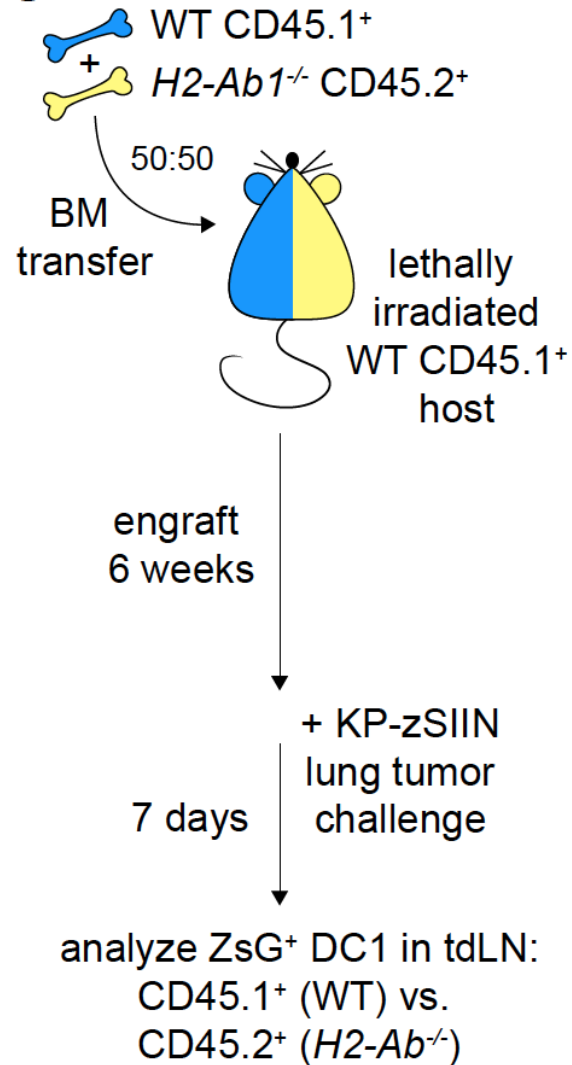
# Is direct contact of Tregs and DC1 necessary for suppression?



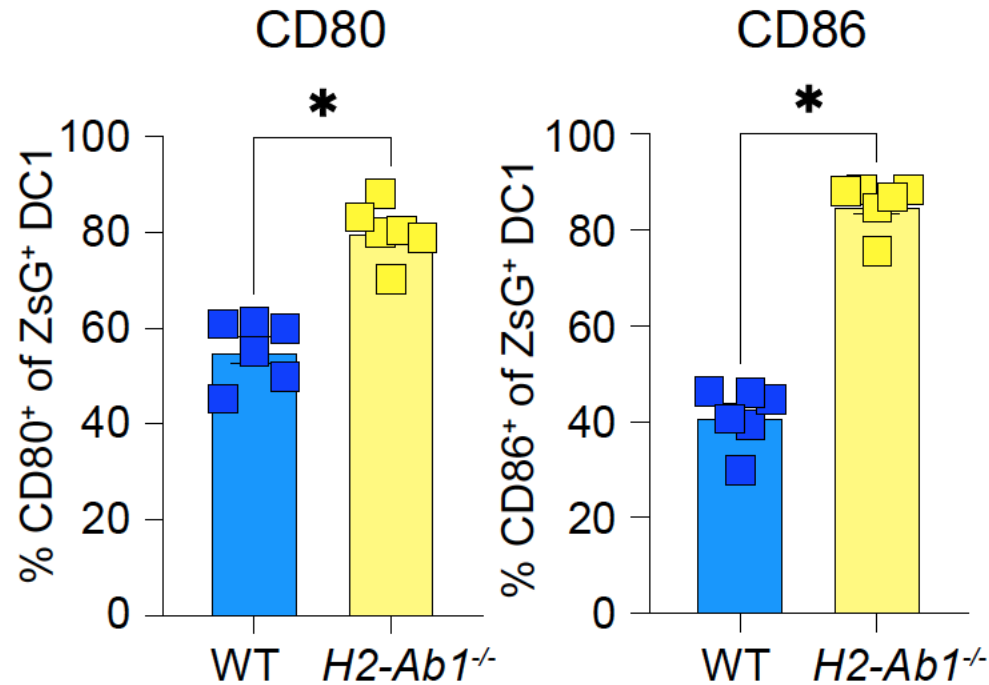
# MHCII blockade prevents ex vivo Treg suppression suggesting that direct contact with DC1 is needed



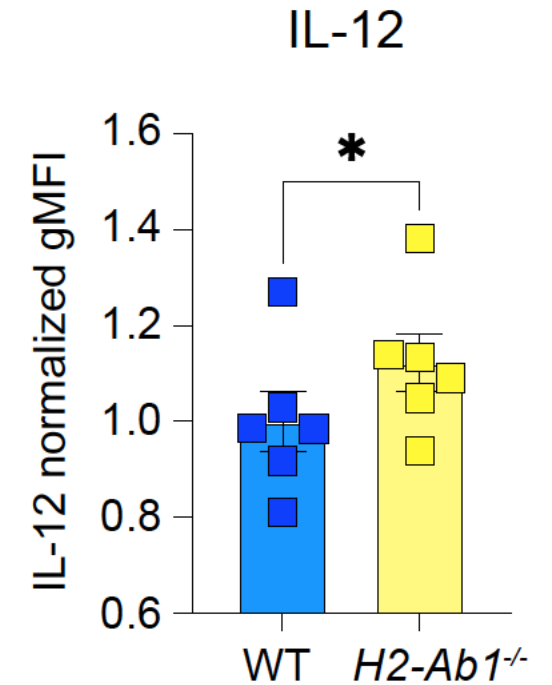
# Abrogating MHCII-mediated DC1:Treg interaction causes upregulation of CD80, CD86 and IL-12 on DC1



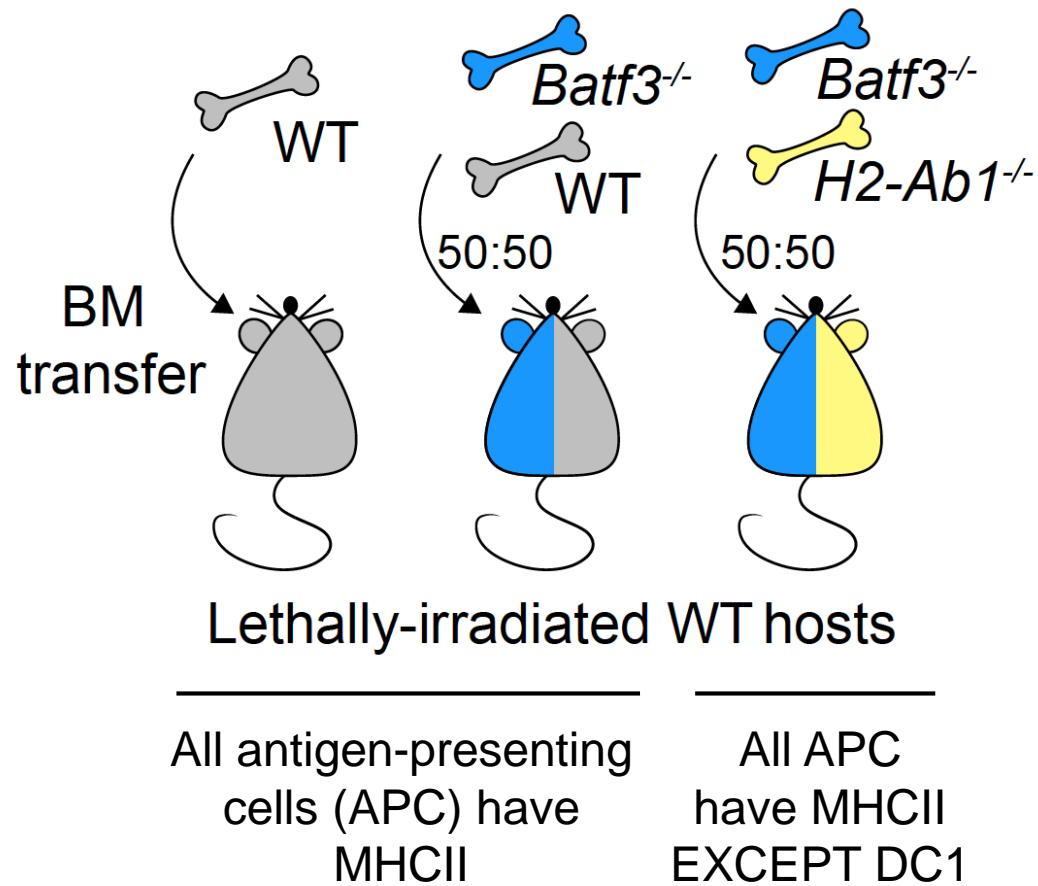
## Signal 2 (costimulation)



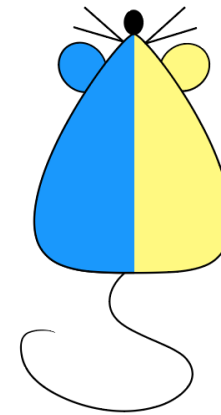
## Signal 3 (cytokines)



# Does abrogating MHCII-mediated DC1:Treg interaction rescue cytotoxic T cell priming in the lung tumor-draining lymph node?



*Batf3*<sup>-/-</sup> / *H2-Ab1*<sup>-/-</sup>  
bone marrow chimera



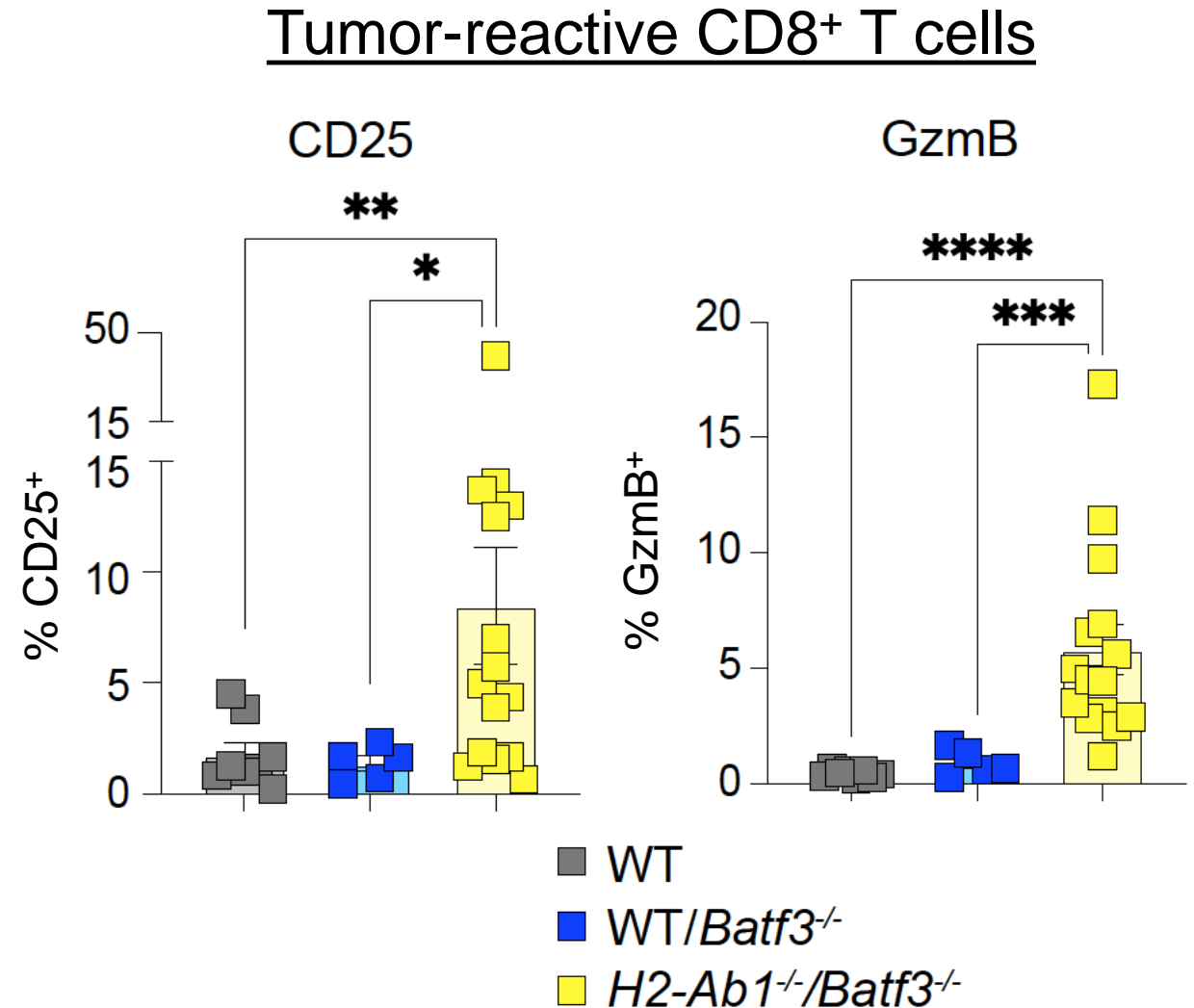
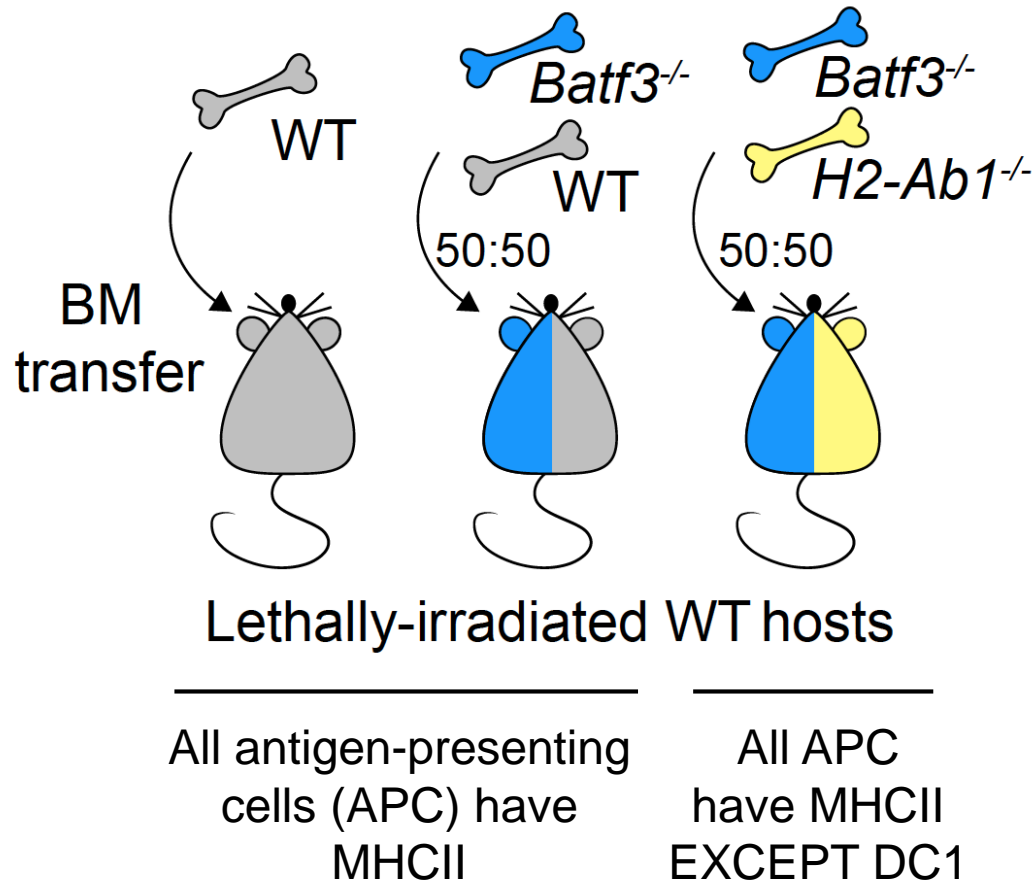
50% *Batf3*<sup>-/-</sup>    50% *H2-Ab1*<sup>-/-</sup>

	50% <i>Batf3</i> <sup>-/-</sup>	50% <i>H2-Ab1</i> <sup>-/-</sup>
DC1	No DC1	MHCII- DC1
Other APC	WT (MHCII <sup>+</sup> ) APC	MHCII- APC

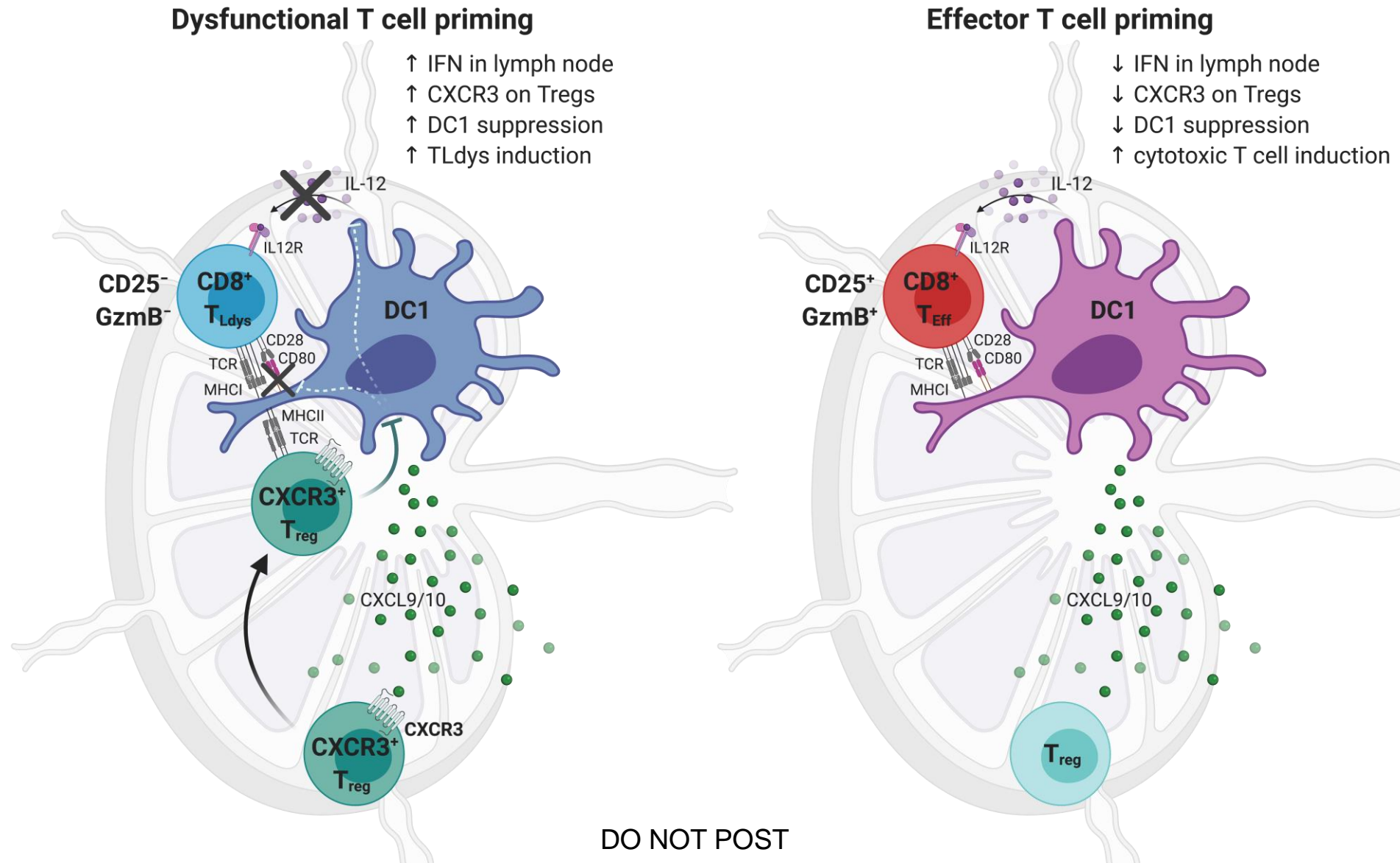
DO NOT POST

*Batf3*<sup>-/-</sup> → no DC1 development; *H2-Ab1*<sup>-/-</sup> → MHCII deficiency

# Abrogating MHCII-mediated DC1:Treg interaction rescues cytotoxic T cell priming in the lung tumor-draining lymph node



# We hypothesize that IFN sensing causes Tregs in the mLN to become more suppressive







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