

*Select slides adapted with permission for the BE Comm Lab  
“Defense Season Wrapped: What Makes a Thesis Defense Great?” Blog Post*

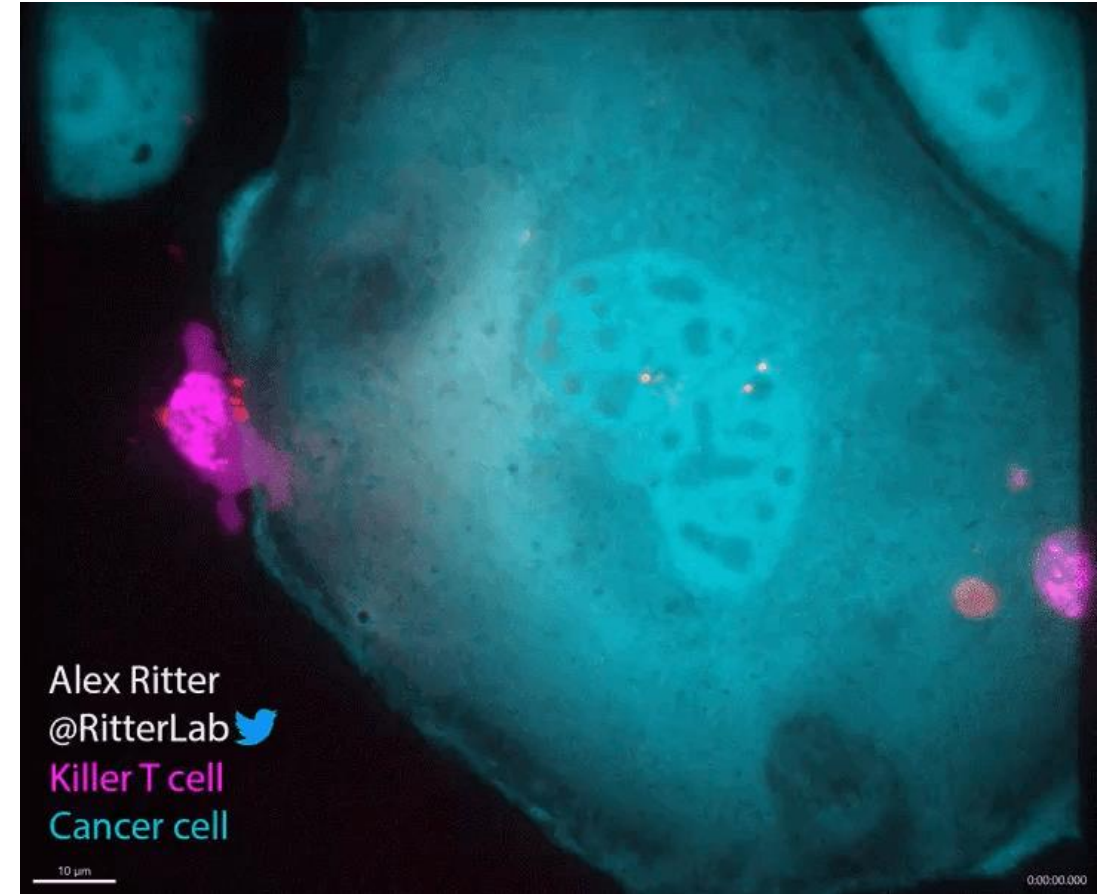
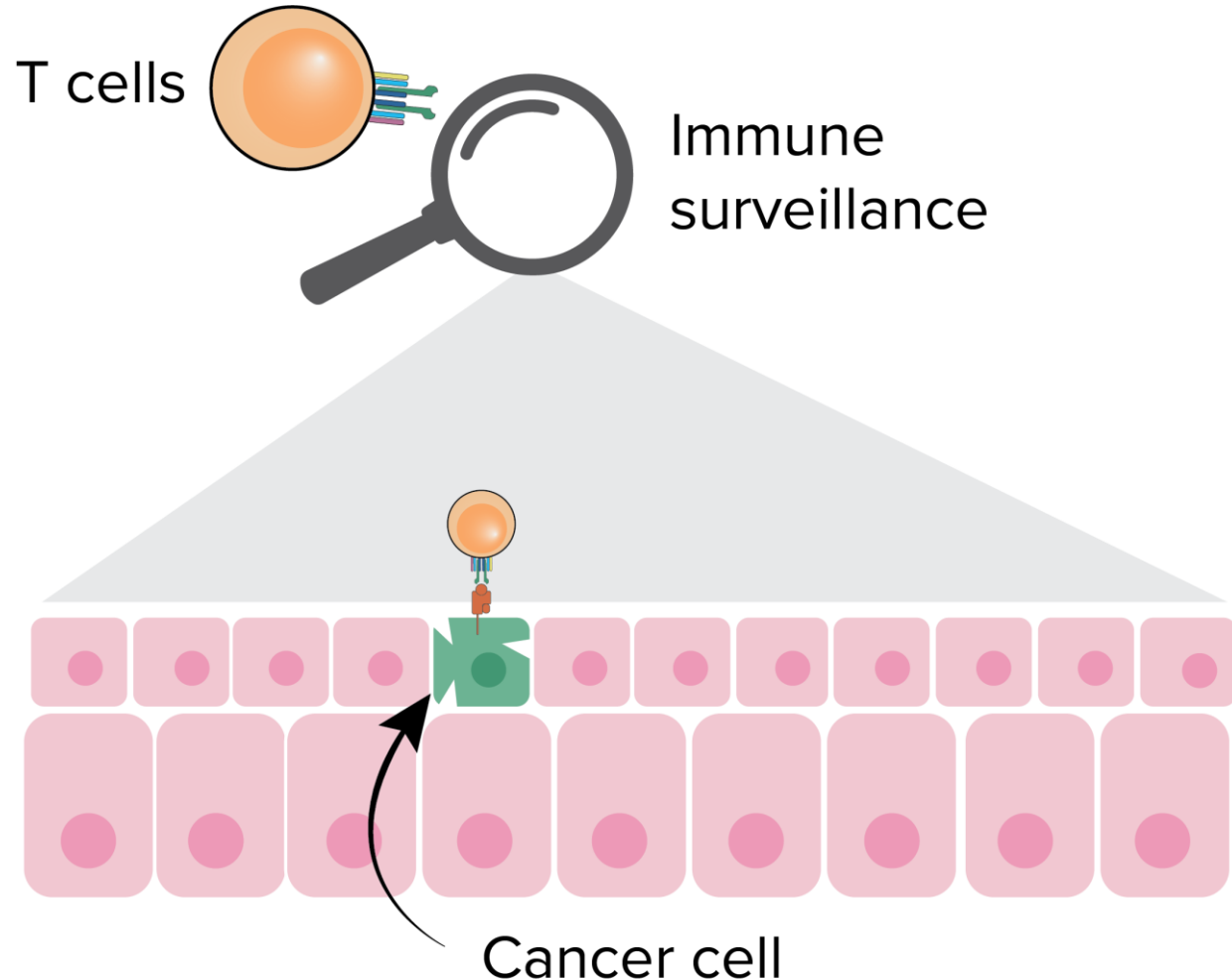
# Deconstructing the CAR signaling landscape to identify drivers of engineered T cell function

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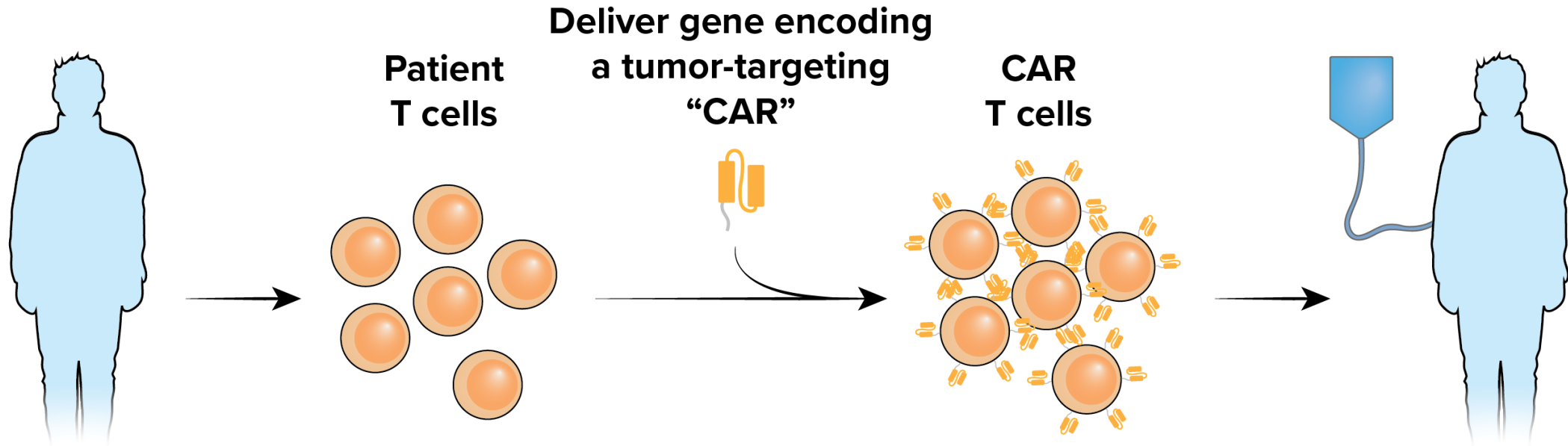
Caleb Perez  
Thesis Defense  
May 14, 2024

# The immune system can be leveraged as a powerful cancer therapeutic

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# Chimeric antigen receptors (CARs) synthetically reprogram T cells to attack cancers



Unprecedented response rates in liquid tumors  
(**>62% complete response** in relapsed/refractory B-ALL)

“Living” therapeutic that **expand within the patient** and  
can **persist for >10 years**

Limited success observed in **solid tumors**

Significant risk of **relapse** and **toxicity**

# Chimeric antigen receptors (CARs) synthetically reprogram T cells to attack cancers

Deliver gene encoding  
a tumor-targeting  
“CAR”

Patient  
T cells

CAR  
T cells

Optimizing the safety and efficacy profiles of CAR-T therapy remains an **important clinical need**.

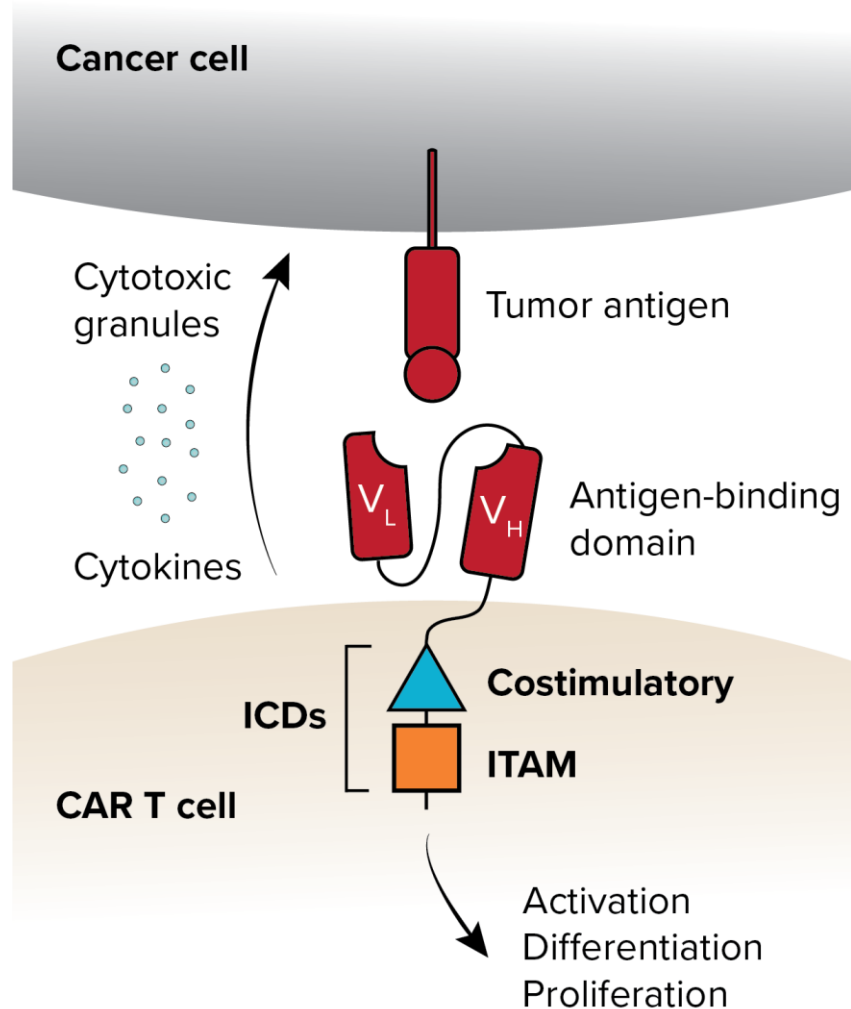
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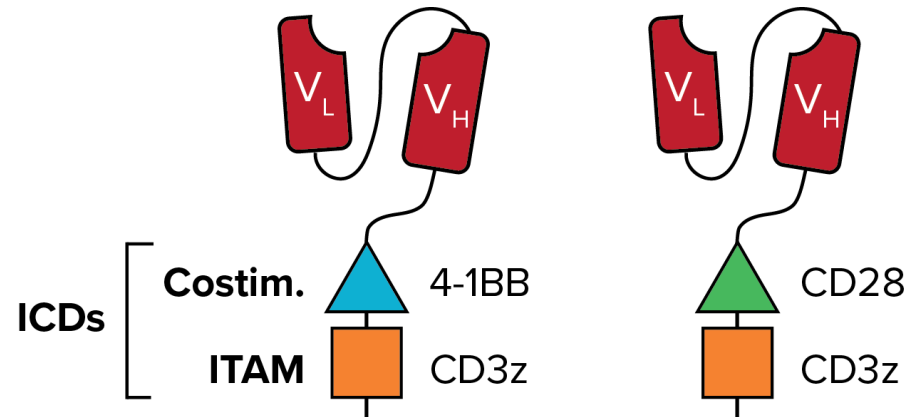
# Intracellular signaling domains (ICDs) reprogram CAR T cells to attack tumor cells



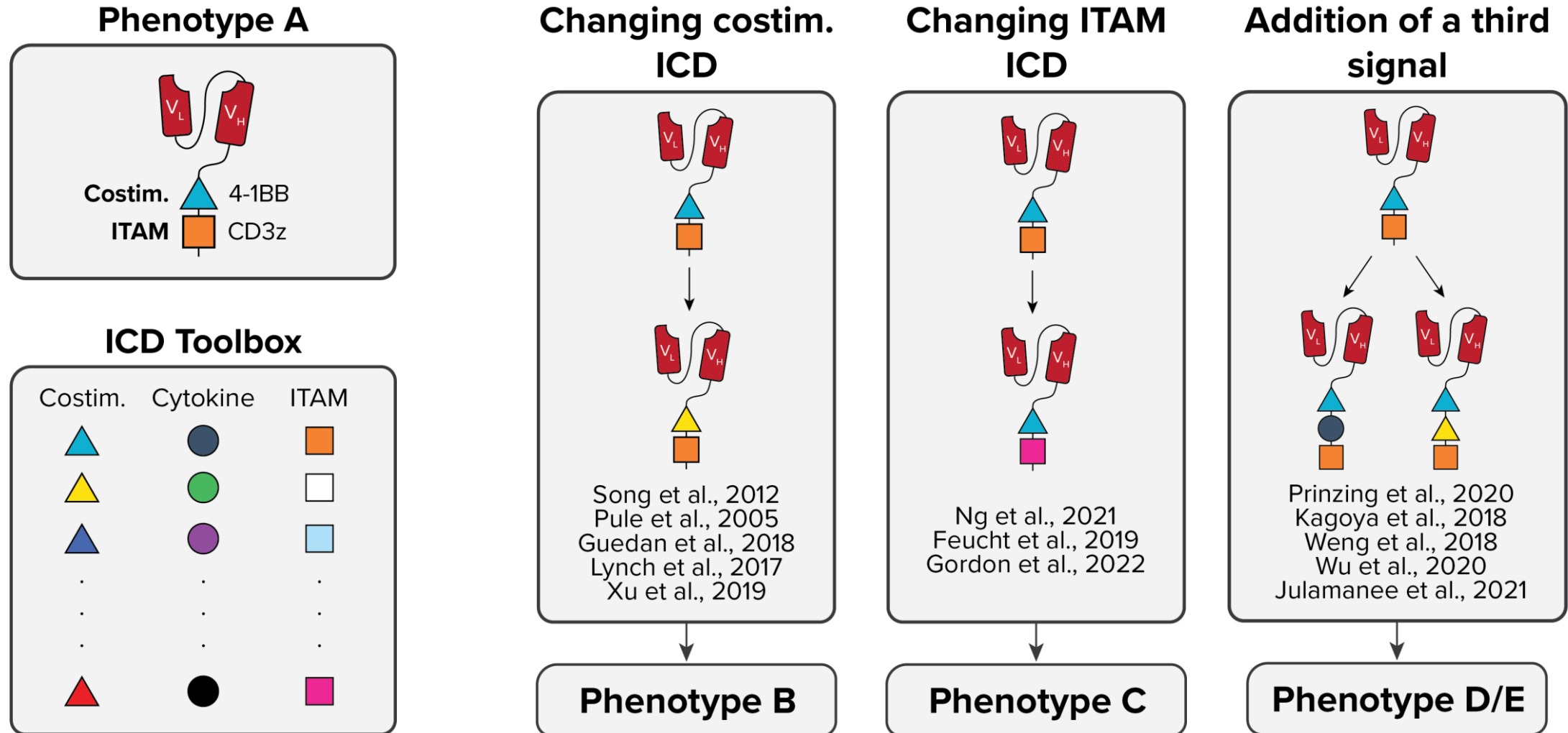
## Clinically approved ICD architectures

**BBz**

**28z**

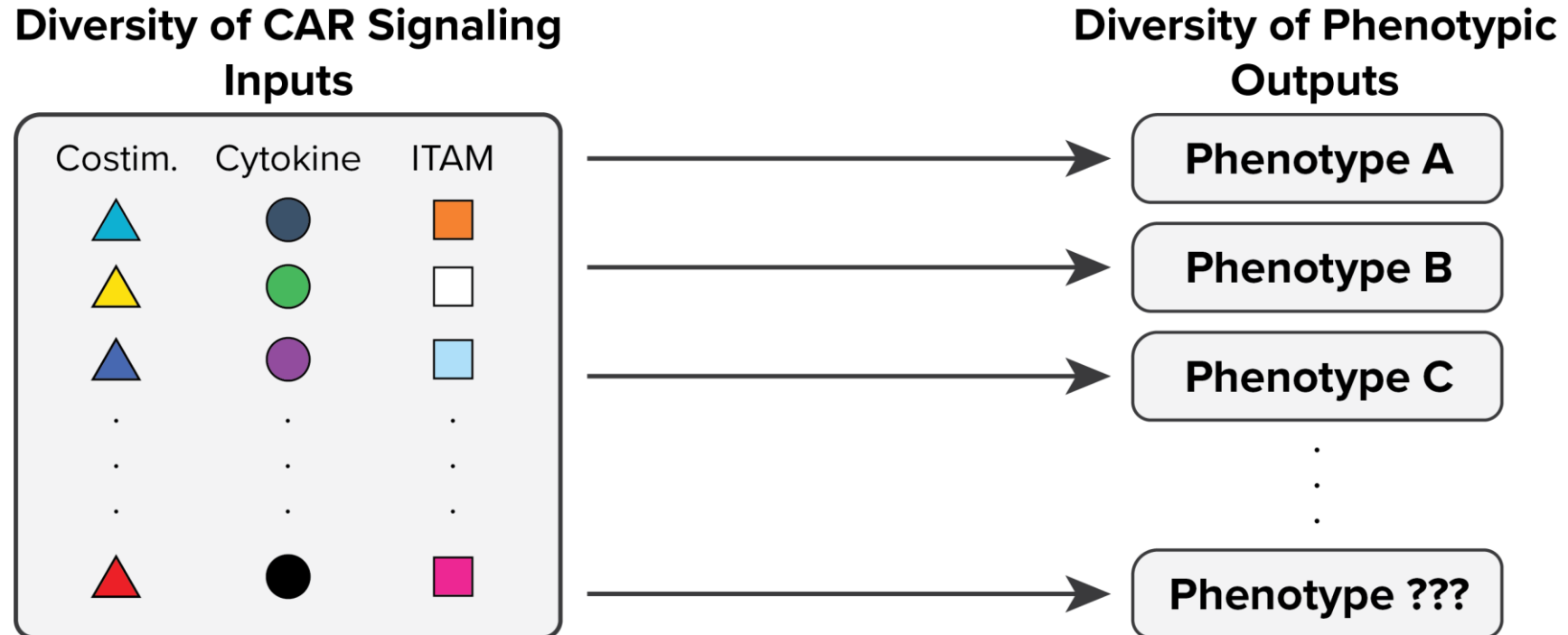


# Modulating CAR signaling is a promising engineering tool to boost therapeutic efficacy

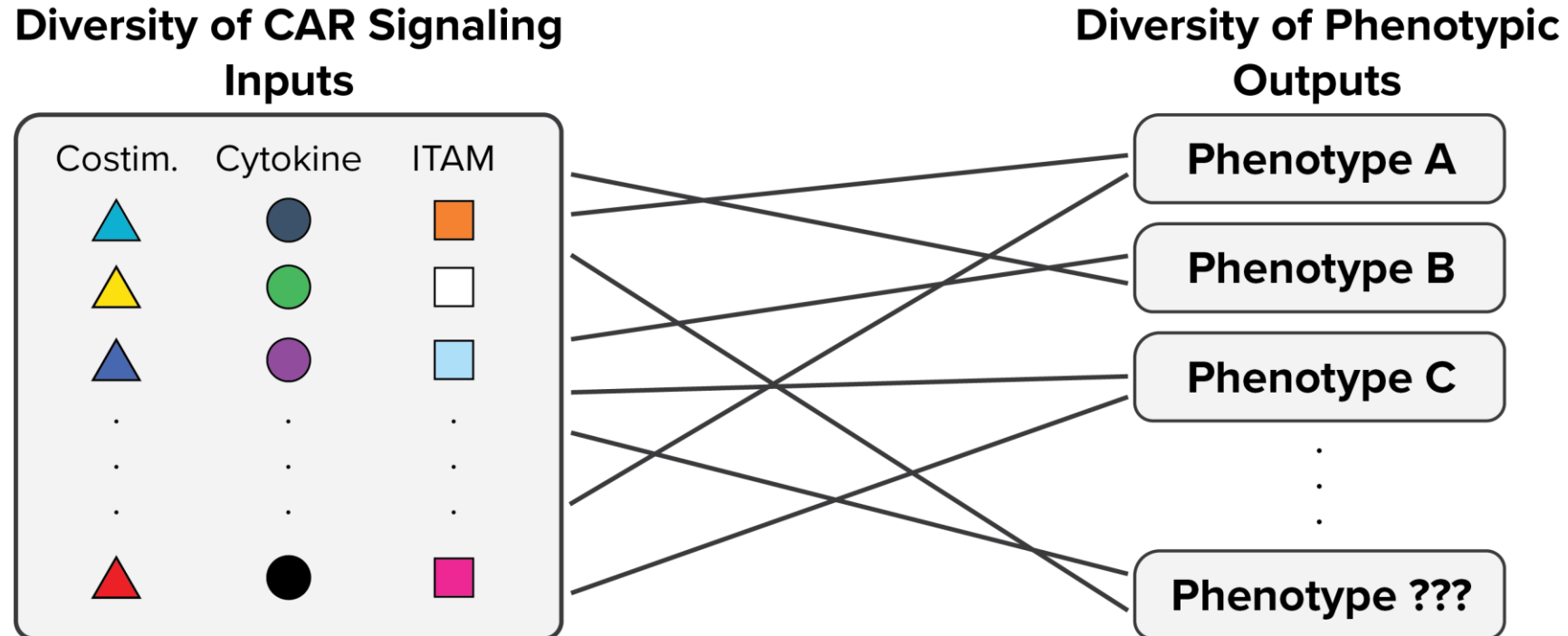


# The CAR signaling landscape is poorly characterized

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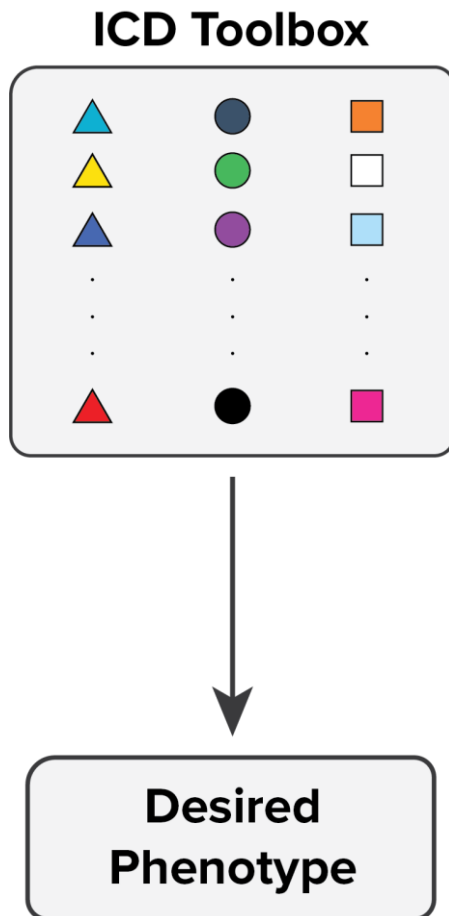
# How signaling inputs drive functional outputs is not well understood, limiting rational CAR design



**Objective:** Build comprehensive maps from diverse signaling inputs to phenotypic outputs, toward predictive tools for novel ICD architectures with enhanced efficacy.

# Outlook: Laying the groundwork for a comprehensive, predictive understanding of CAR signaling

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**caRNA-seq** presents a **high-throughput platform** for the systematic characterization of CAR signaling space

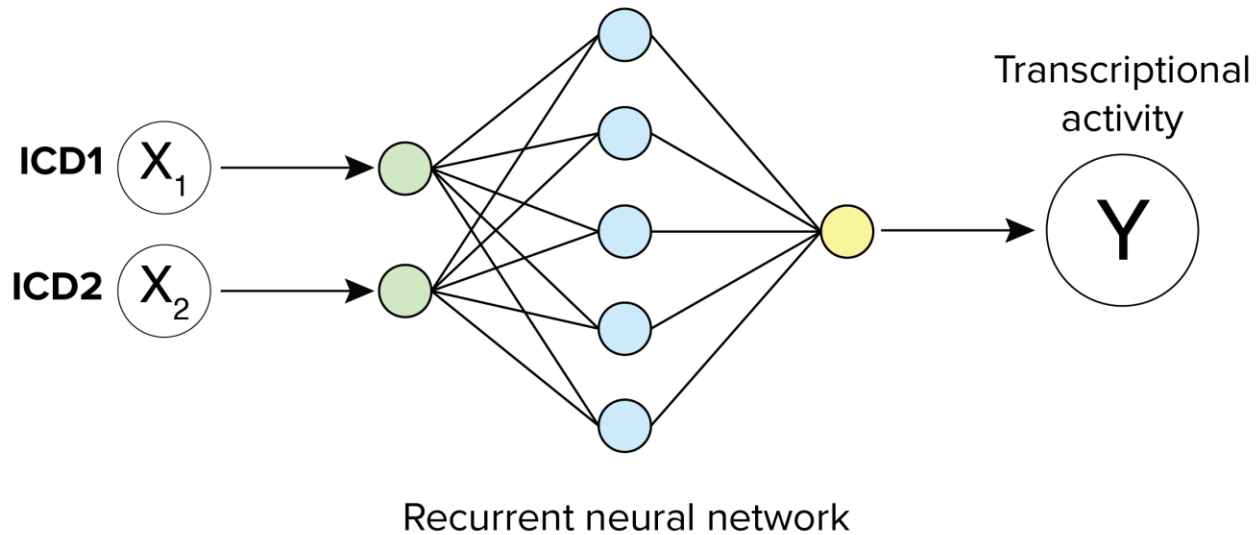
**Deep transcriptional profiling of CAR signaling libraries** via **caRNA-seq** can identify **functionally distinct** ICD architectures, and provide insight into what drives that response

Modulating ICDs can program **unique T cell biology**, which can be leveraged for **therapeutic benefit**

A **comprehensive understanding** of CAR signaling is within reach, which would be **invaluable** for the **design of more efficacious CARs**

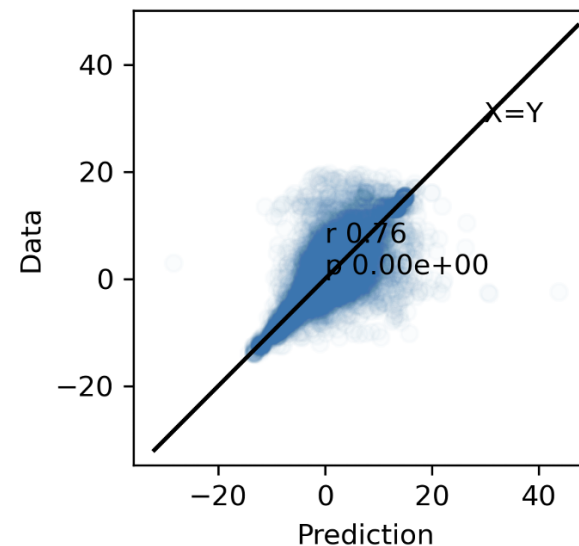
# CARML: Our **caRNA**-seq datasets are suitable training sets for predictive computational models

Large-scale knowledge-**EMB**bedded **Artificial Signaling networks** (LEMBAS)

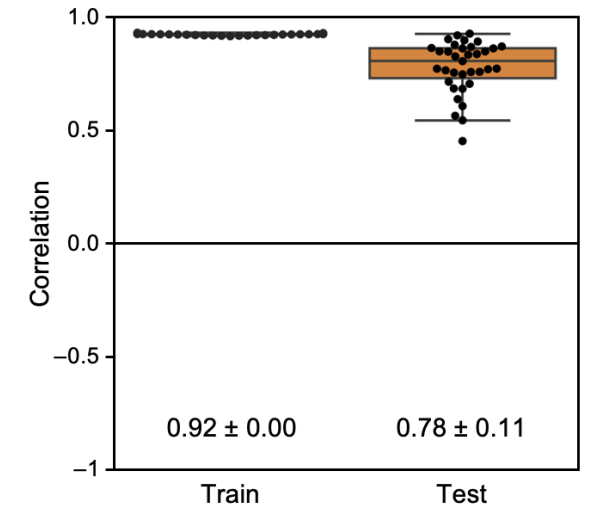


## Testing model performance

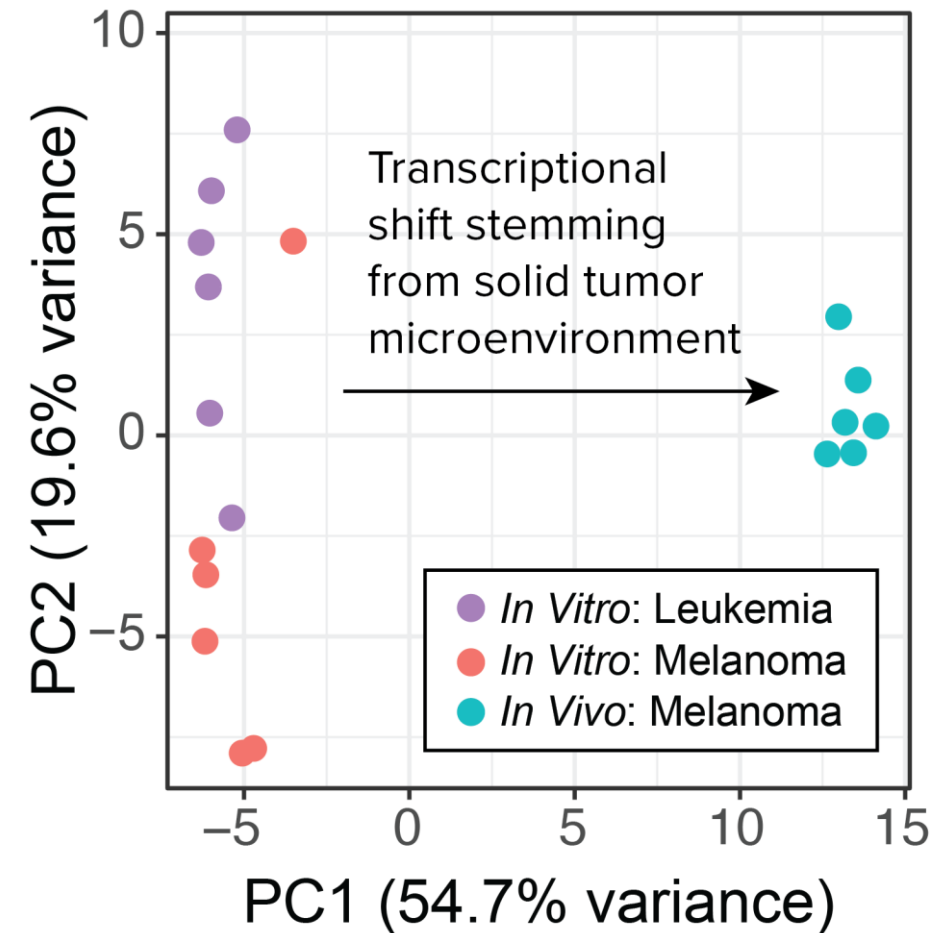
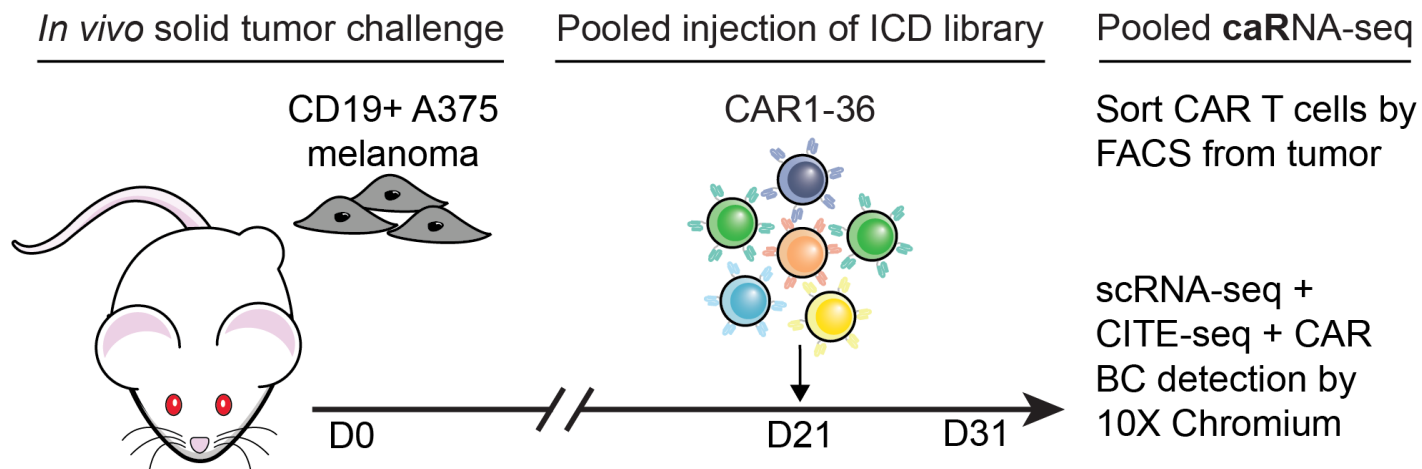
Predicted TF Activity



CAR-level Predictions

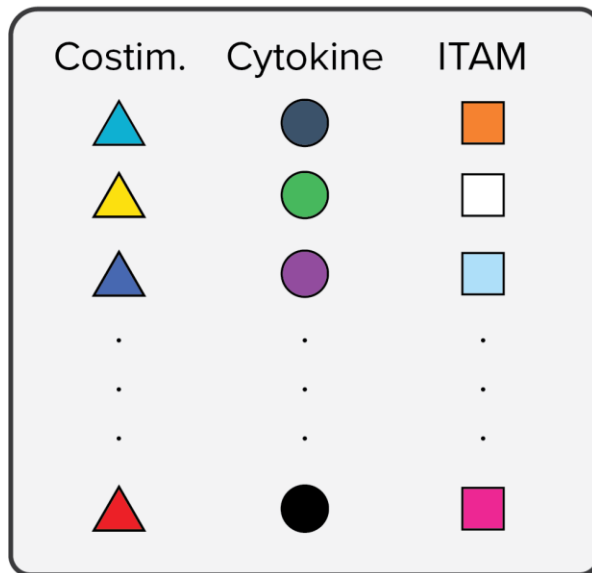


# caRNA-seq can be extended to directly characterize the signaling landscape within *in vivo* solid tumors

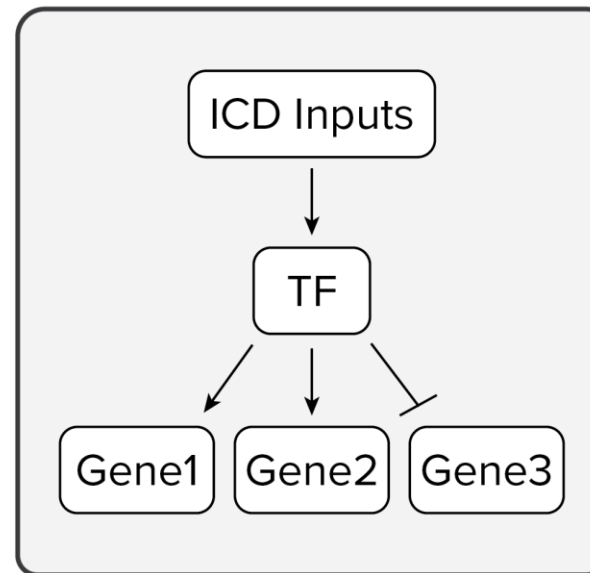


# Building datasets that systematically map diverse signaling inputs to phenotypic outputs

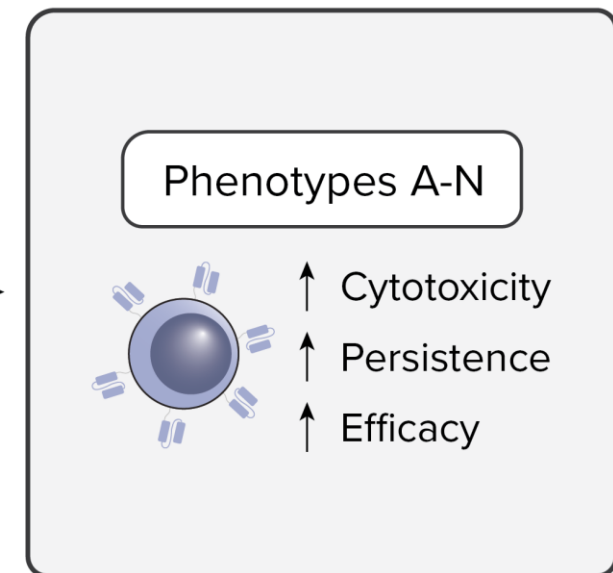
## 1. Create diversity in signaling inputs



## 2. Map each signaling input to global transcriptional response

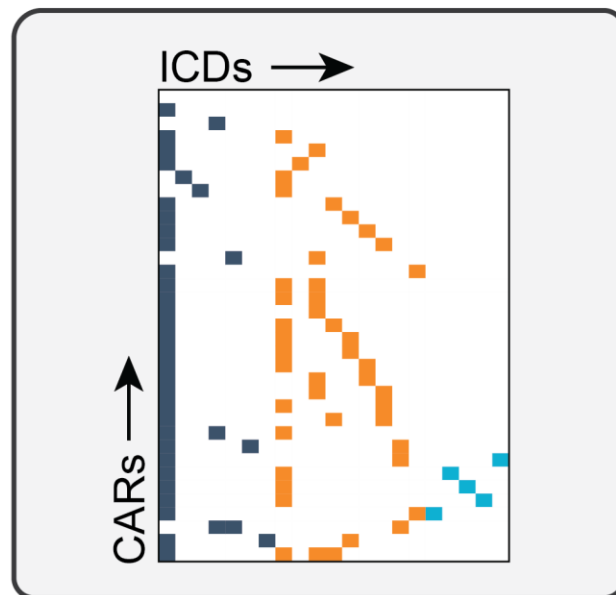


## 3. Assess phenotypic outputs of each transcriptional profile



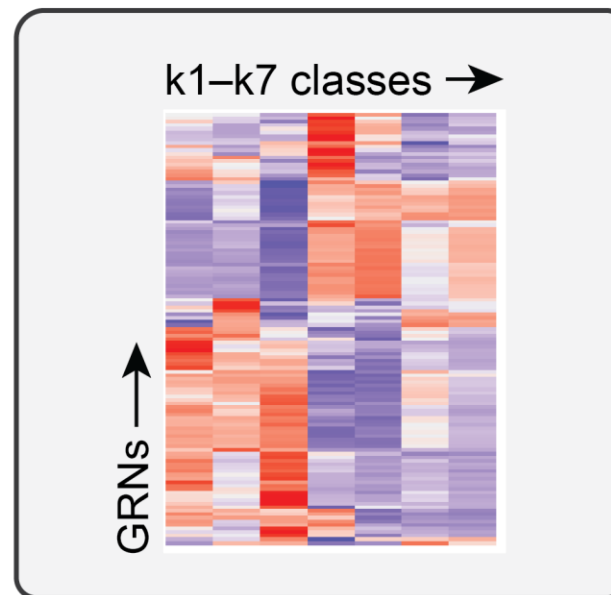
# Systematic characterization of CAR signaling space reveals ICD-specific drivers of CAR-T efficacy

1. Constructed library of diverse ICD architectures in clinical development



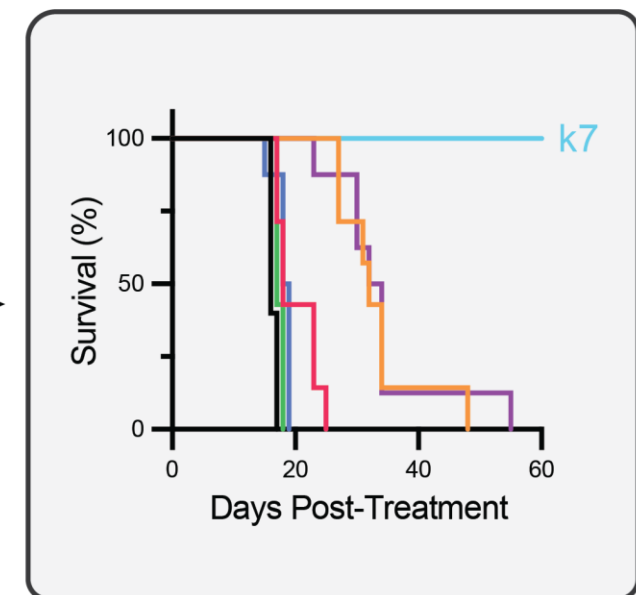
CD40 (mostly)

2. Used caRNA-seq to classify CARs by transcriptional activity



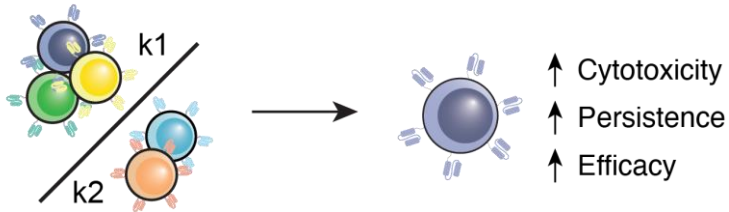
k7 tonic signaling

3. Identified a transcriptional program that drives strong efficacy



# Assessment of candidate CARs from each class reveals striking functional differences

## CAR classes Candidate phenotyping



k1, CAR1: Non-signaling control

k2, CAR21: CD28.ICOS.CD3z

k3, CAR33: DNAM1.2B4.CD3z

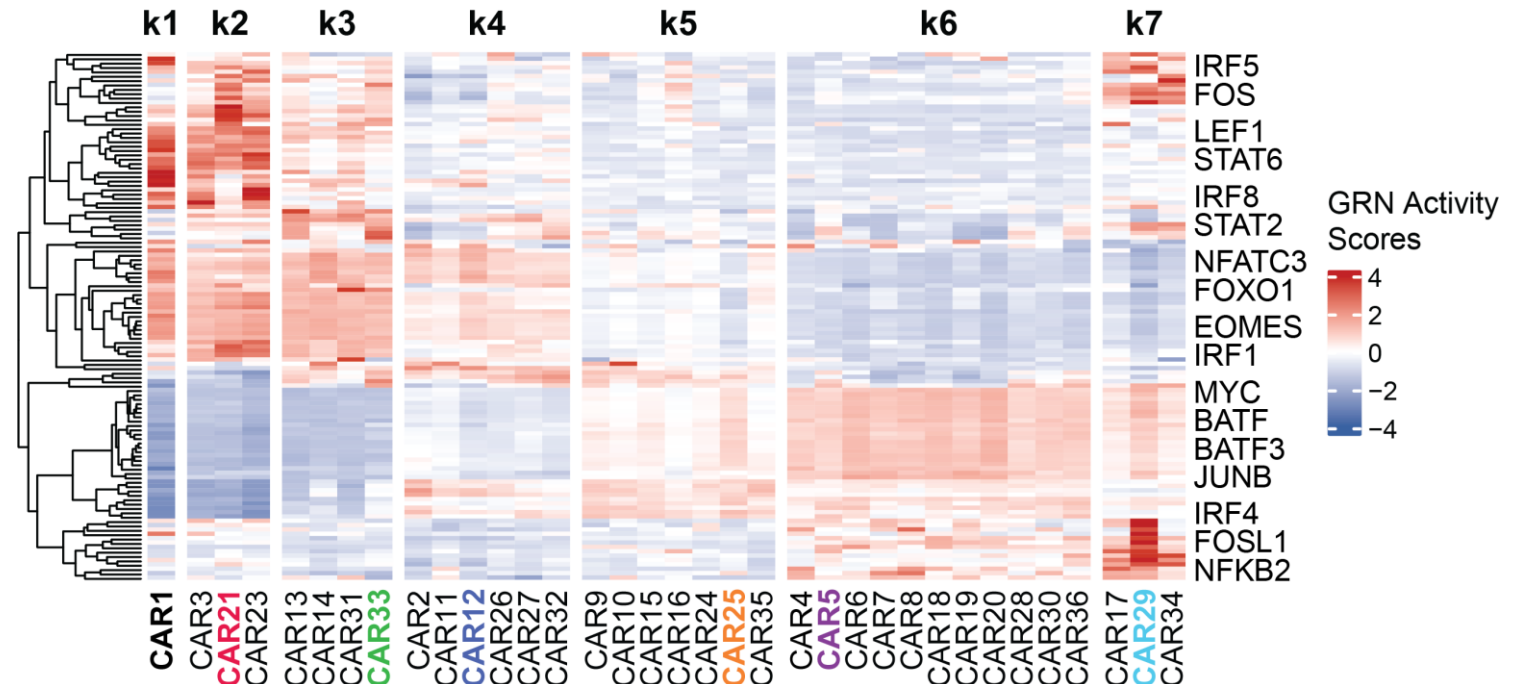
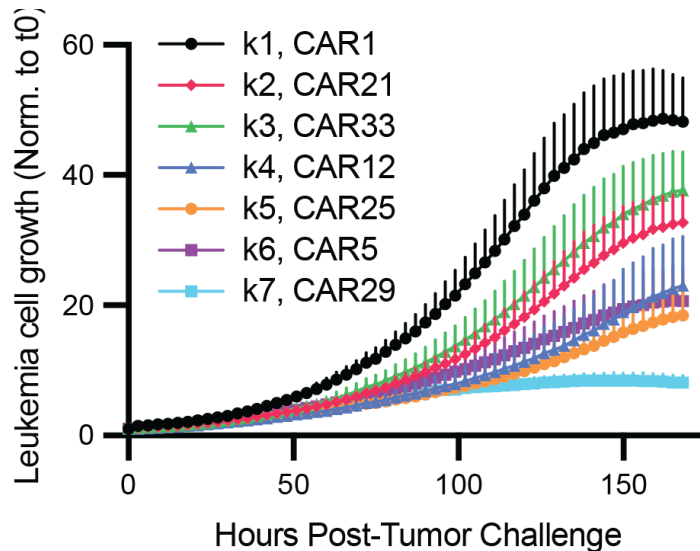
k4, CAR12: DAP10.CD3z

k5, CAR25: CD28.CD3z.DAP10

k6, CAR5/BBz: 4-1BB.CD3z

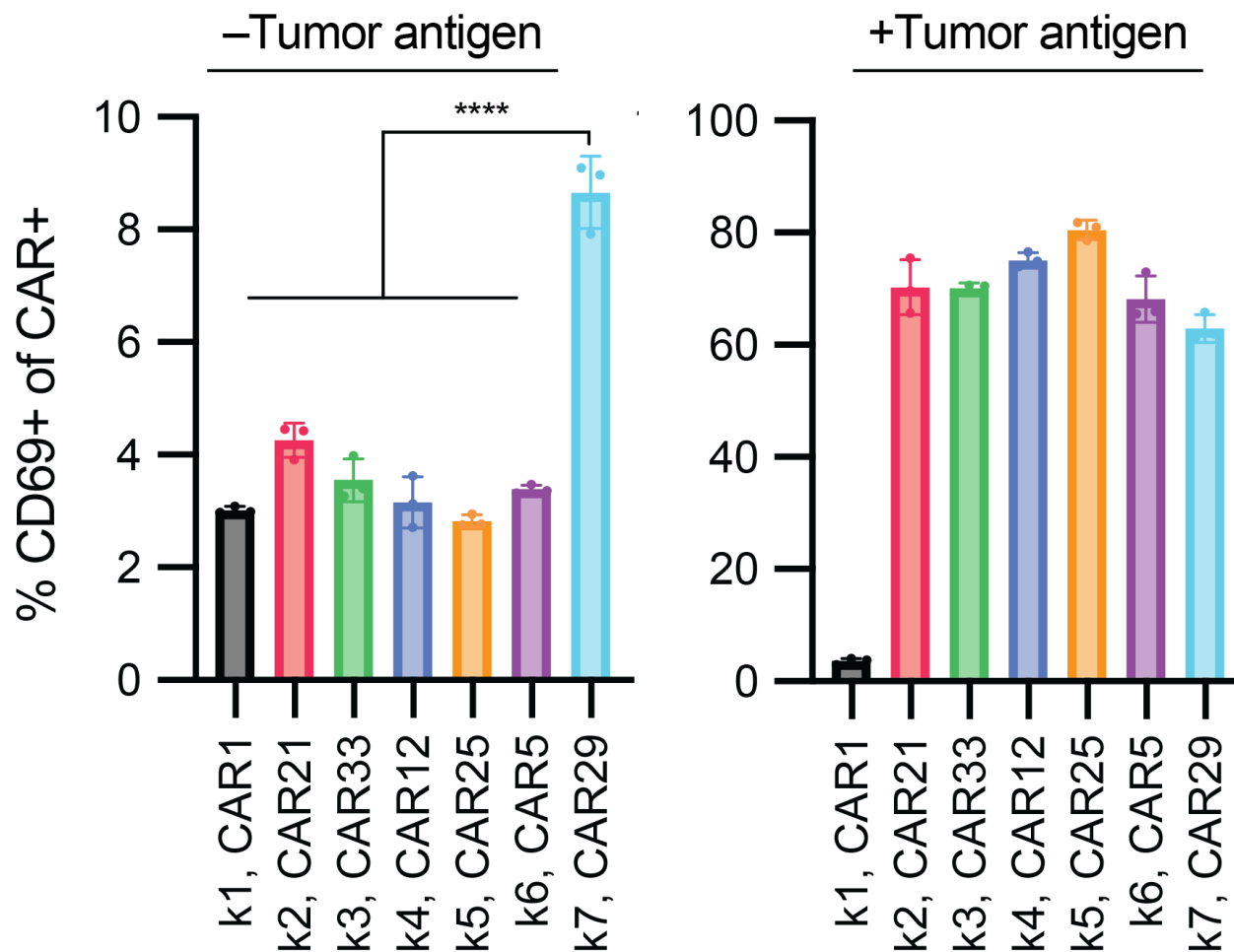
k7, CAR29: MYD88.CD40.CD3z

### In vitro cytotoxicity

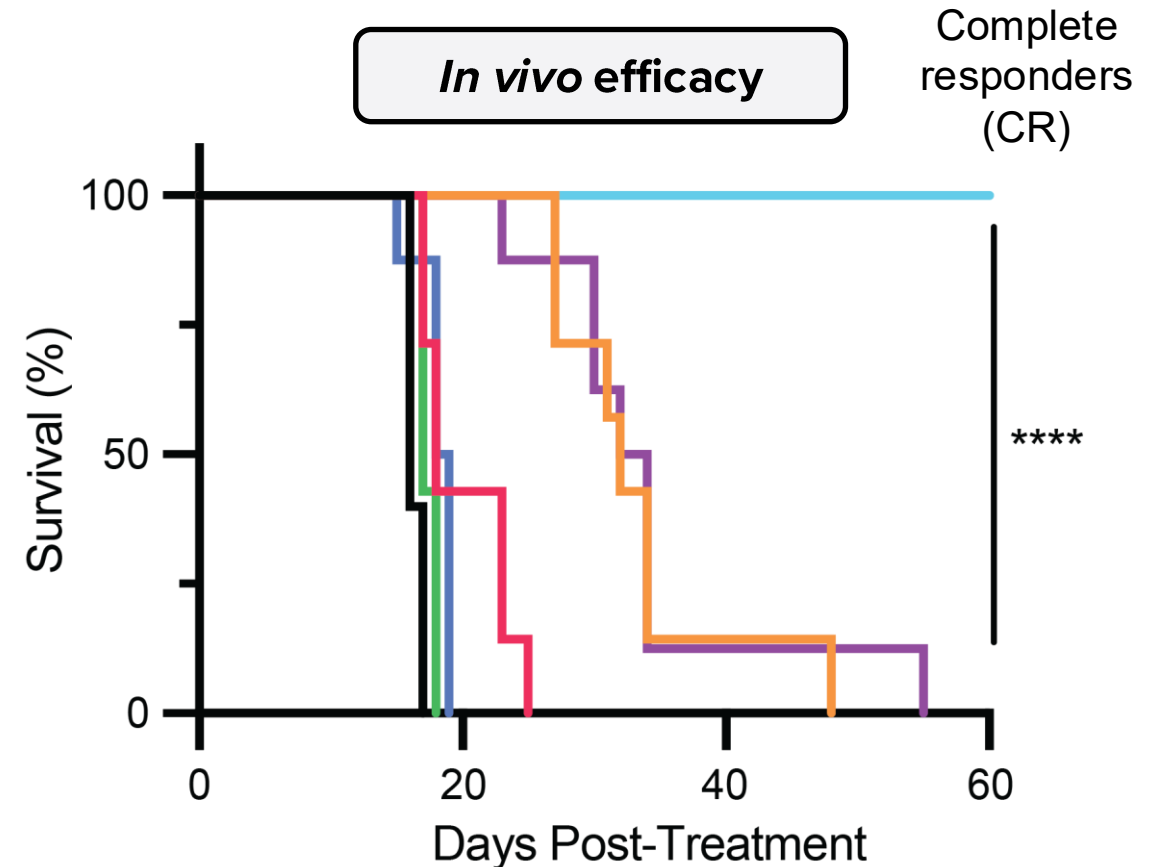
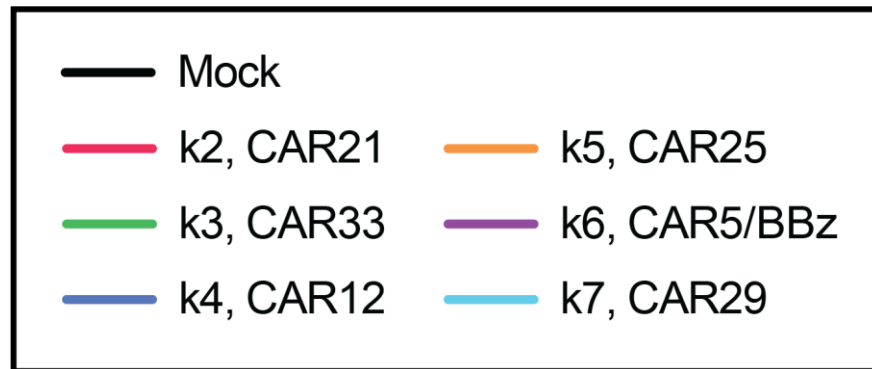
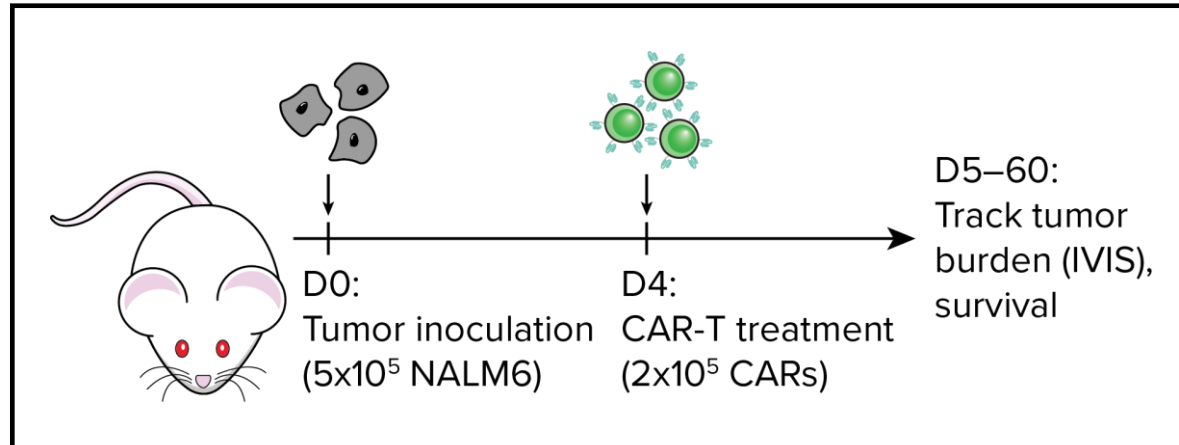


# The k7 candidate CAR shows evidence of increased levels of baseline activation in the absence of antigen

- k1, CAR1: Non-signaling control
- k2, CAR21: CD28.ICOS.CD3z
- k3, CAR33: DNAM1.2B4.CD3z
- k4, CAR12: DAP10.CD3z
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- k7, CAR29: MYD88.CD40.CD3z

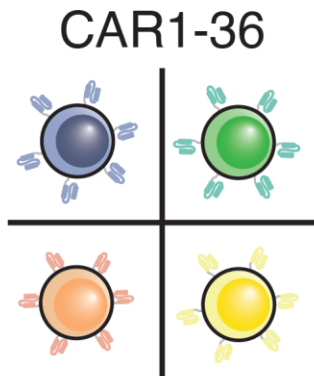


# Treatment with k7 candidate CARs induce months of durable cures in mouse models of leukemia



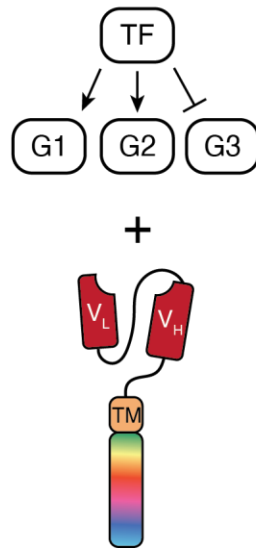
# Do k7 CARs exhibit a distinct transcriptional signature in the absence of antigen?

Express ICD library in resting CD3+ T cells



Pooled  
**caRNA-seq**

Gene expression +  
CAR BCs



Principal component  
analysis (PCA)

Tonic signaling profiles  
by CAR class

