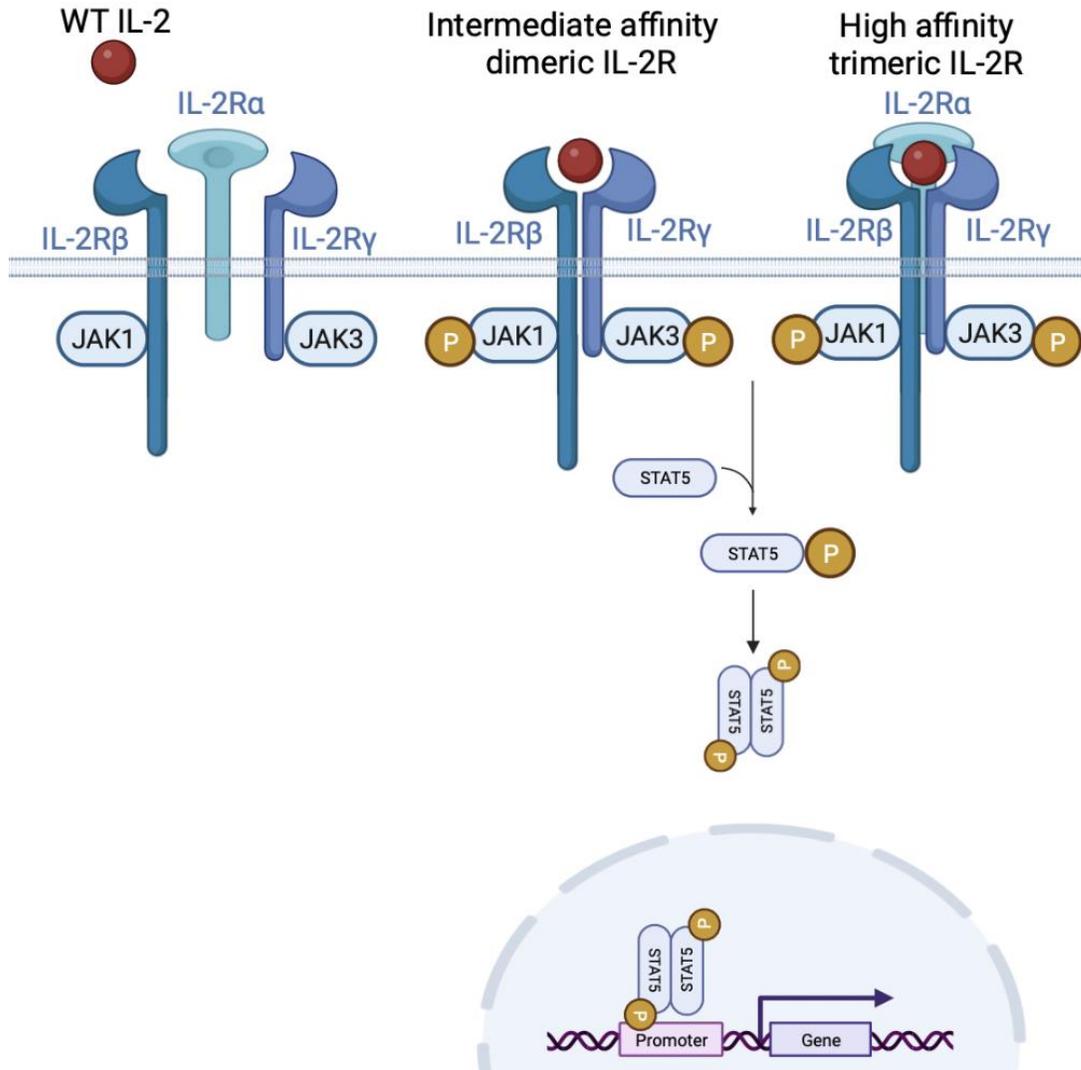


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# Engineered human IL-2/IL-2R $\beta$ orthogonal pairs selectively enhance CAR T cells to drive complete responses in hematological and solid epithelial tumor models

P.J. Aspuria  
Synthekine  
Menlo Park, California

# IL-2: A Potent Cytokine to Armor Adoptive T Cell Therapy



- IL-2 is a pleiotropic cytokine that positively influences the homeostasis and development of different T cell lineages and other immune cells (e.g. NK cells and eosinophils)
- IL-2 signals through the stepwise assembly of the IL-2R complex to primarily activate the JAK/STAT5 pathway
- Recombinant IL-2 (Proleukin) is used as a monotherapy and in combination with TCR and TIL therapies
  - Limited by significant, life-threatening toxicity (small therapeutic window)
    - Capillary leak syndrome (CLS), hypotension mediated by non-selective activation of immune cells

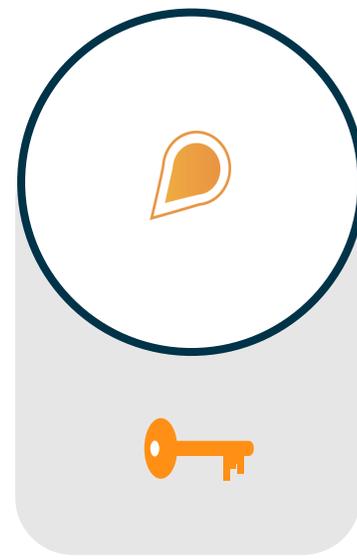
# Orthogonal Cytokine + Cell Therapy: A Lock and Key System to Stimulate ACTs Selectively In Vivo

**hoR $\beta$** , an engineered IL-2 receptor beta subunit

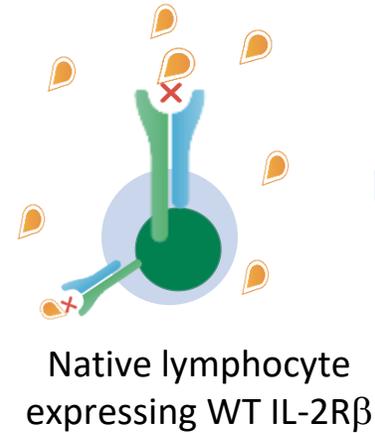


+

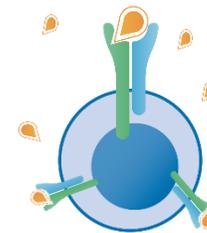
**STK-009**, an engineered IL-2 cytokine



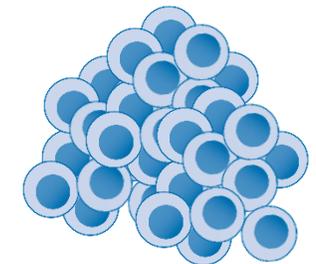
=



**No expansion or activation**



**Significant expansion and activation**



Potential to be incorporated into a wide range of ACTs, including CAR-Ts (SYNCAR-001, SYNCAR-002), TCRs, TILs, and Tregs

# Orthogonal IL-2 Armoring In a Hematological Tumor Setting

# Manufacturing in Ortho IL-2 (STK-009) And Specific Expansion of hoR $\beta$ Expressing CARs

**SYNCAR-001**

Anti-CD19  
scFv

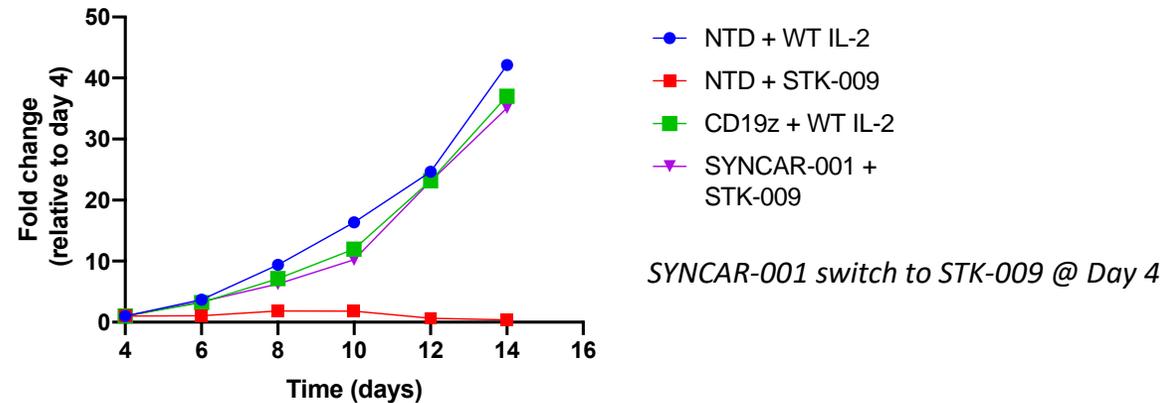
CD28

CD3z

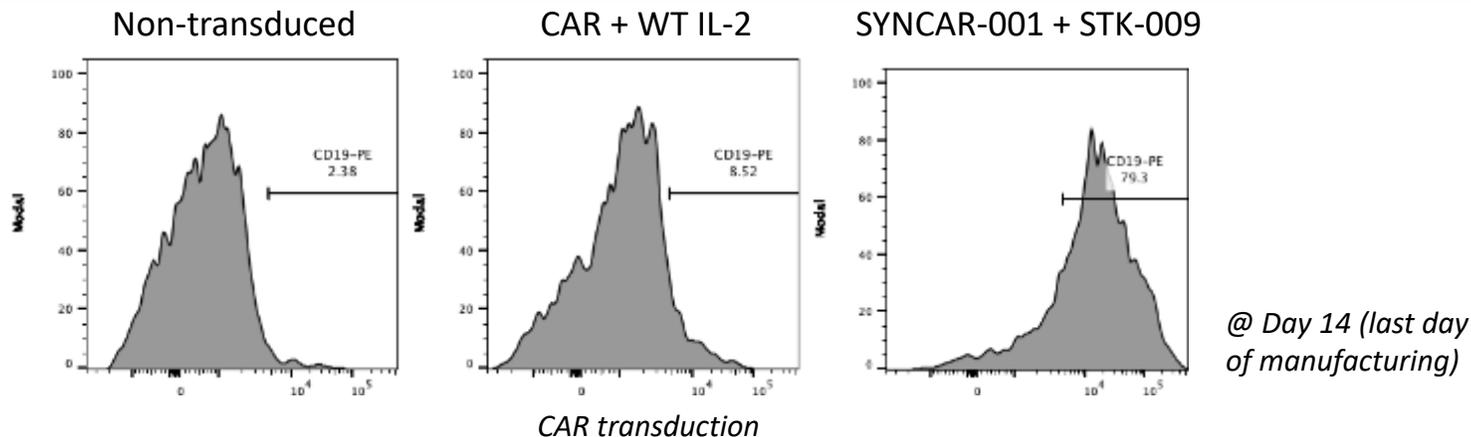
T2A

hoR $\beta$

**SYNCAR manufacturing in STK-009 has equivalent growth to conventional CAR manufacturing with WT IL-2**



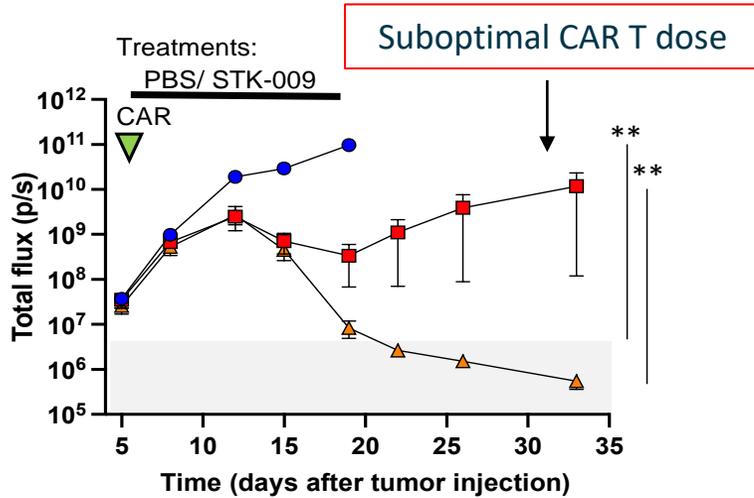
**SYNCAR manufacturing in STK-009 significantly enriches for CAR transduction vs traditional CAR manufacturing methods**



# STK-009 + SYNCAR-001 Demonstrates Improved Activity Versus a CD19 CAR Alone

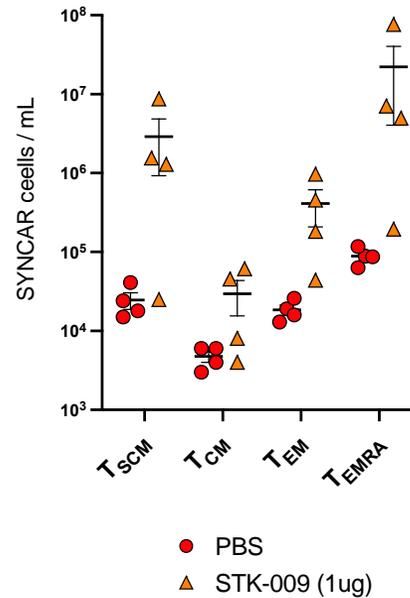
RAJI disseminated tumor model in mice, dosed with suboptimal SYNCAR-001 dose +/- STK-009

## Complete responses with STK-009

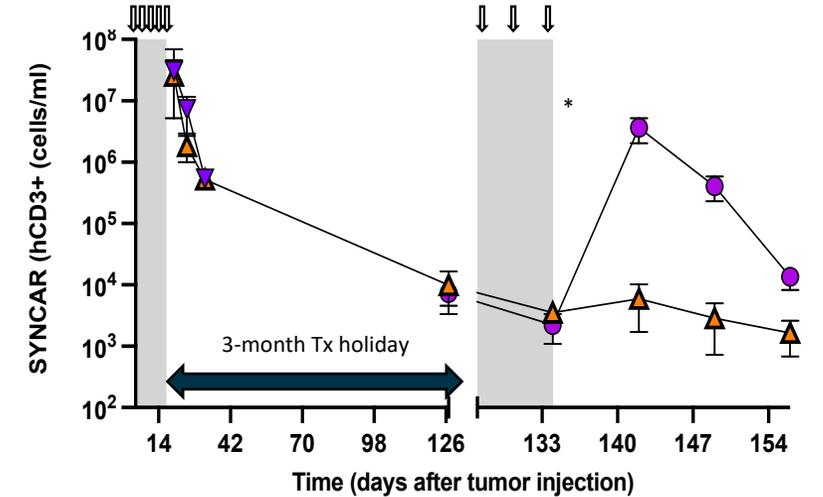


| Treatment                  | CR    |
|----------------------------|-------|
| PBS                        | 0 %   |
| SYNCAR + PBS               | 50 %  |
| SYNCAR + STK-009 1 $\mu$ g | 100 % |

## Unbiased expansion of immunophenotypes



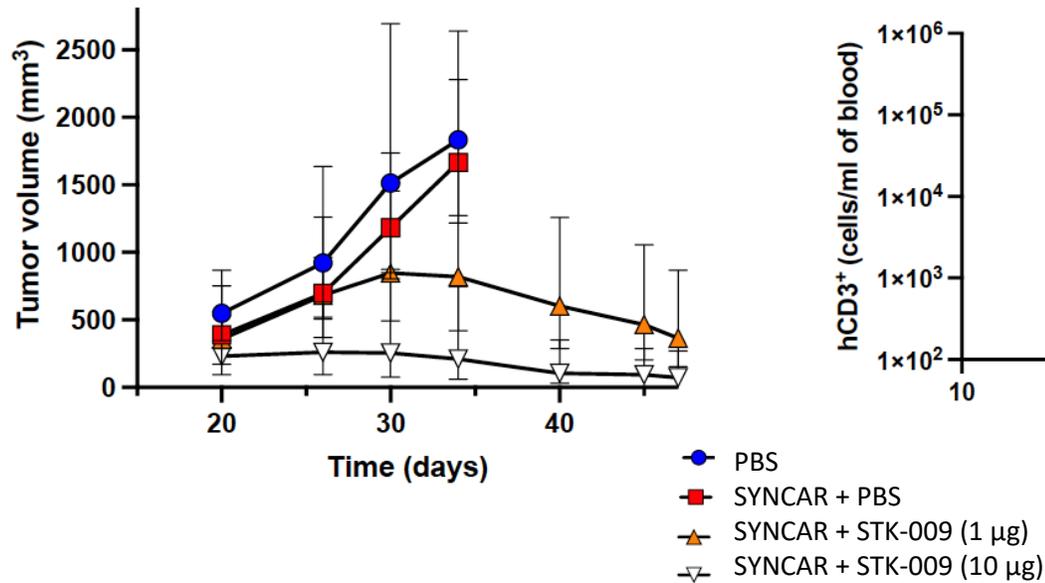
## STK-009 re-treatment drives T cell re-expansion



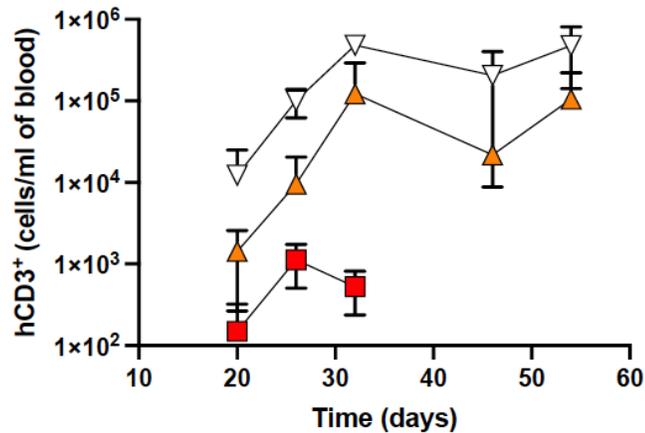
# STK-009 + SYNCAR-001 Induce Responses in a Subcutaneous RAJI NSG Model Characteristically Resistant to CAR Ts

RAJI subcutaneous tumor model in mice, dosed with **400,000 SYNCAR-001 cells** and with STK-009

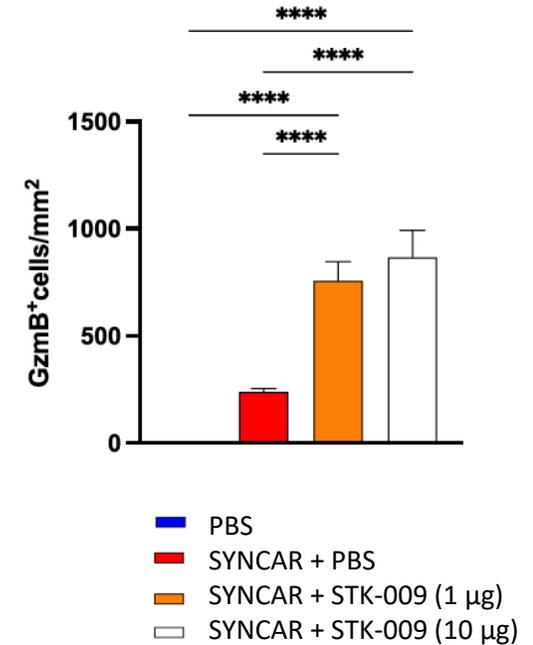
SYNCAR-001 + STK-009 induces tumor shrinkage



STK-009 expands SYNCAR-001 in systemic circulation



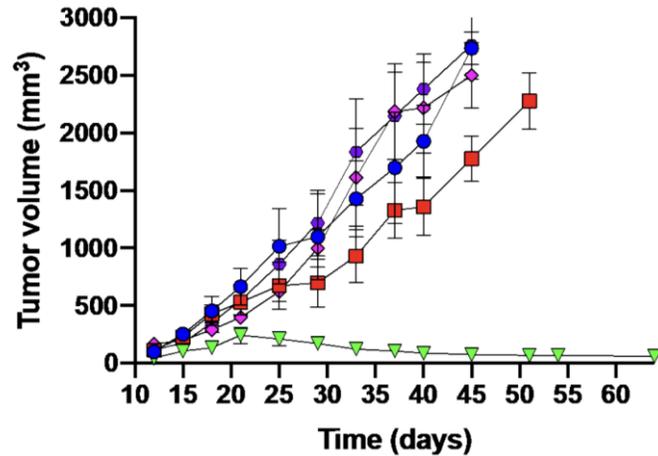
STK-009 activates SYNCAR-001 in the tumor



# STK-009 But Not Proleukin Enables Tumor Rejection and Eliminates Liver Metastases

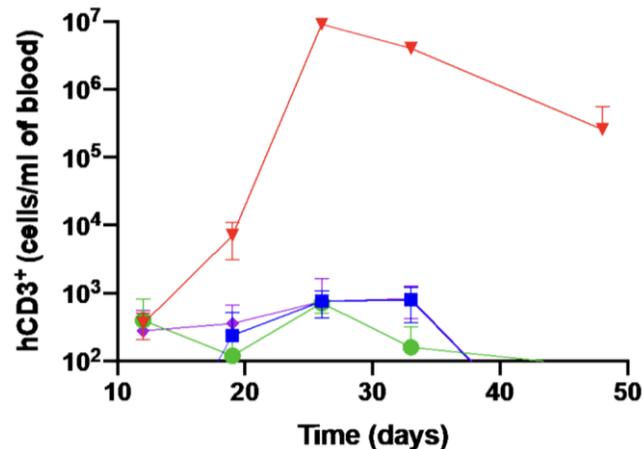
## RAJI subcutaneous tumor model

### Tumor volume



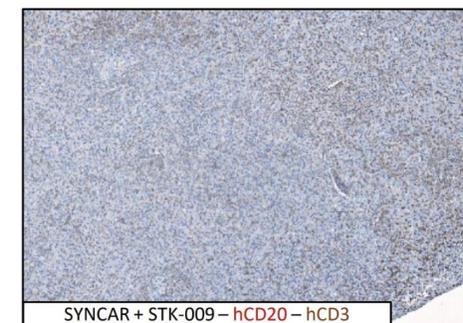
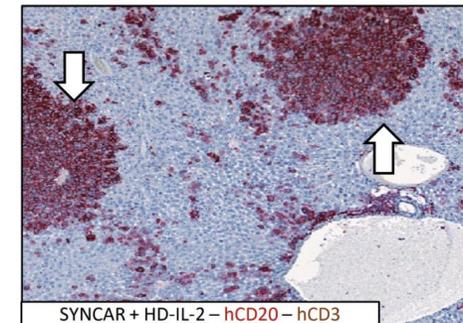
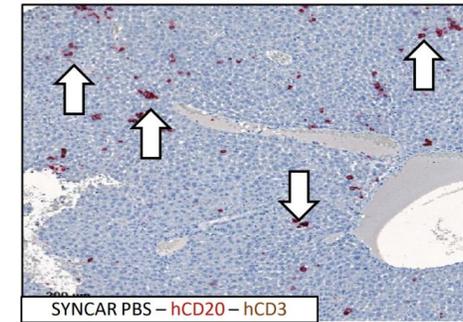
- PBS
- SYNCAR + PBS
- ▼ SYNCAR + STK-009
- ◆ SYNCAR + HD Proleukin
- SYNCAR + LD Proleukin

### hCD3 cells



- PBS
- SYNCAR + PBS
- ▲ SYNCAR + STK-009
- SYNCAR + HD Proleukin
- ◆ SYNCAR + LD Proleukin

### Liver IHC



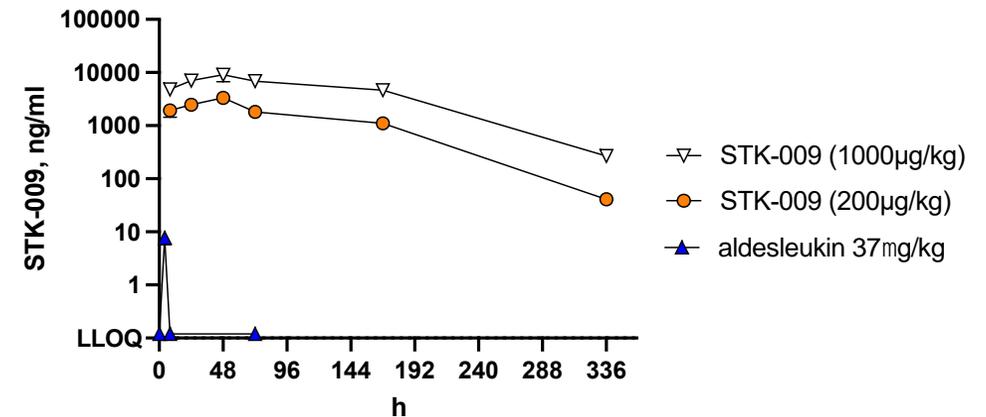
**Proleukin  
enhances  
liver met growth**

↓ Liver Metastasis

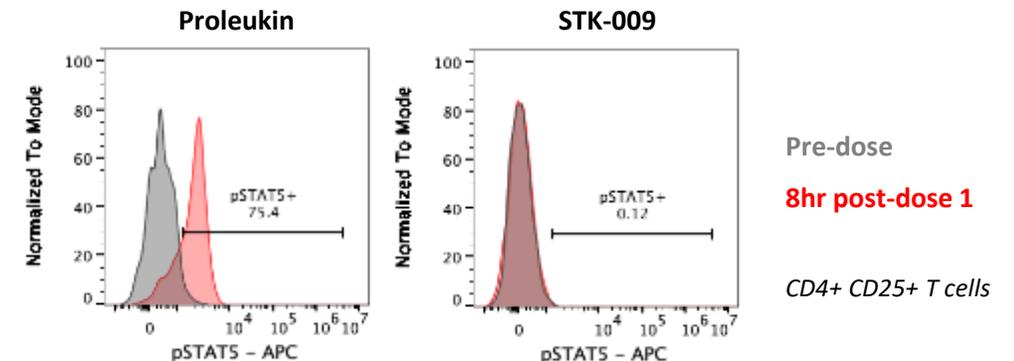
# STK-009 Demonstrates Extended PK and No Native Lymphocyte Activation in NHPs

- **STK-009 had high, sustained exposures in NHP**
  - PEG: decreased renal clearance
  - Ortho: decreased target mediated clearance
- **No clinical or pathological changes observed**
  - No increase in eosinophils
  - No change in lymphocyte / white blood cells
- **STK-009 treatment for up to 2 weeks did not induce IL-2 related cellular or cytokine/chemokine changes**
  - No induction of STAT-5 phosphorylation
  - No NK cell proliferation
  - No change to T cell populations
- **STK-009 does not activate the host IL-2 pathway in the absence of ortho-IL-2R $\beta$**

Single dose of STK-009 in NHPs has >2 week exposure



WT IL-2 but not STK-009 activates T cells in NHPs



# SYNCAR-001 + STK-009: Phase I Clinical Trials

**Oncology: CD19+ Heme malignancies**

**Cohort A**

**Cy/Flu  
Lymphodepletion**

**Cohort B**

**No lymphodepletion**

**ClinicalTrials.gov Identifier: NCT05665062**

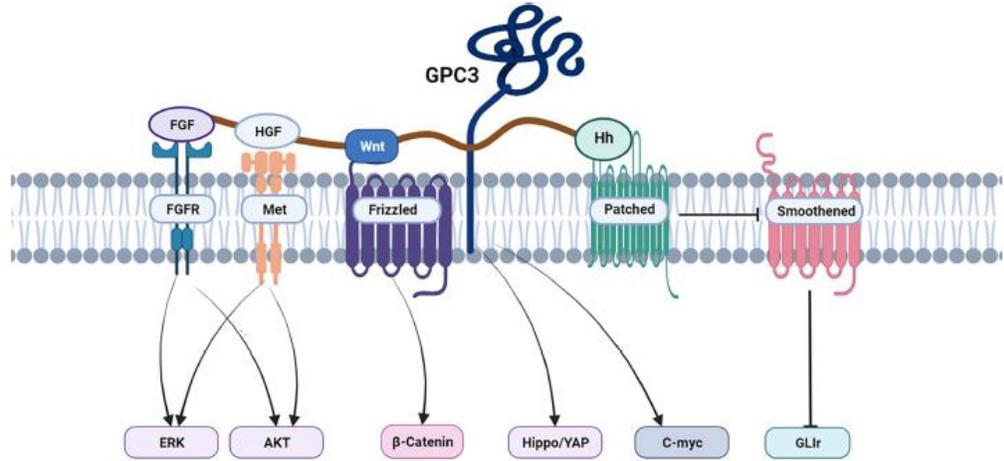
**Autoimmunity: Non-renal systemic lupus erythematosus and lupus nephritis**

**No lymphodepletion**

**IND Approval in March '24**

# Orthogonal IL-2 Armoring In a Solid Epithelial Tumor Setting

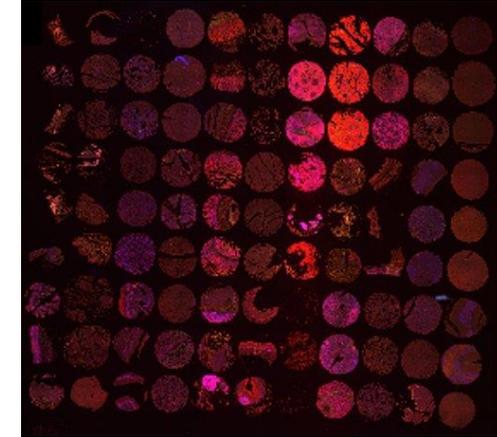
# GPC3 is an Attractive CAR T Target in Hepatocellular Carcinoma



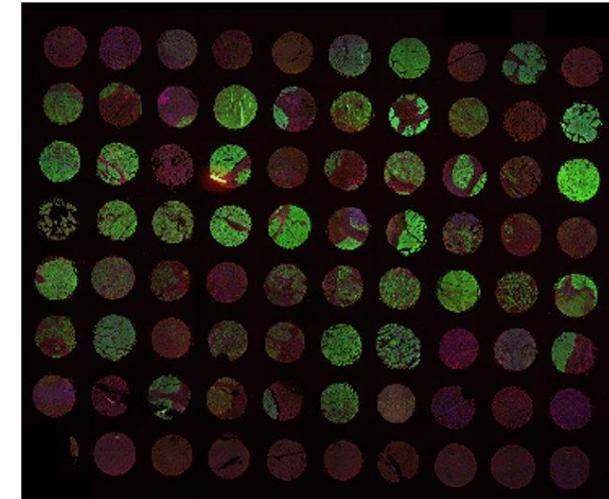
- Glypican 3 (GPC3) is a heparan sulfate proteoglycan and cell surface oncofetal protein
- It is implicated in a variety of processes, including cell growth, differentiation, and migration
- Significant expression on normal adult tissue limited to placenta and largely devoid in other tissues
- GPC3 is expressed in approximately 2/3 of HCC
- Numerous anti-GPC3 CAR T programs are currently in clinical trials

## GPC3/CD3 multiplex IHC

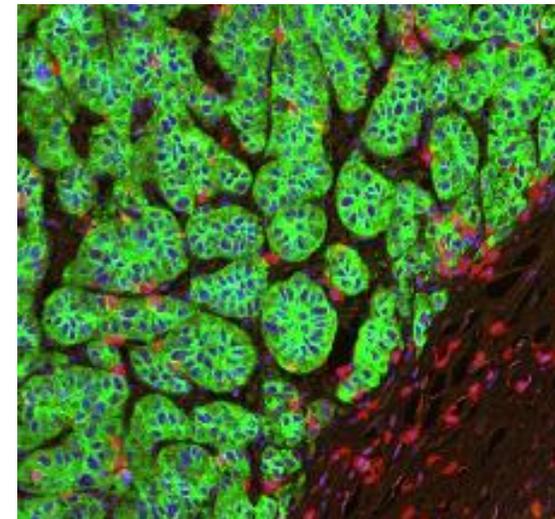
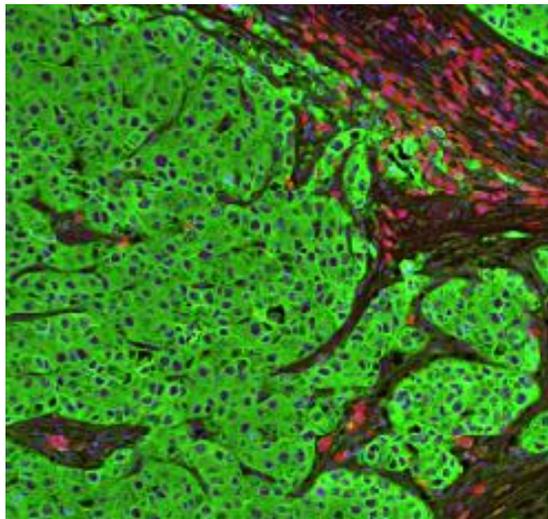
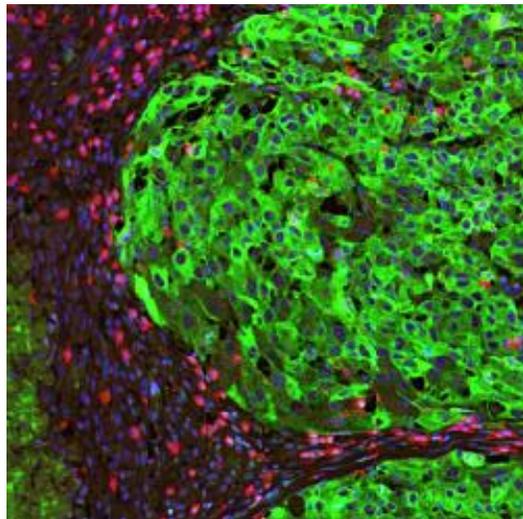
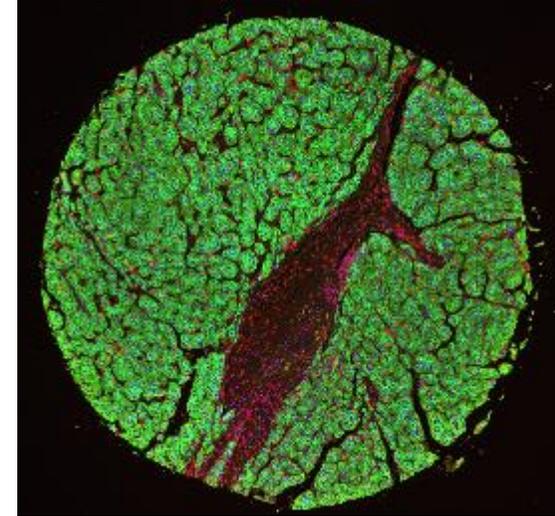
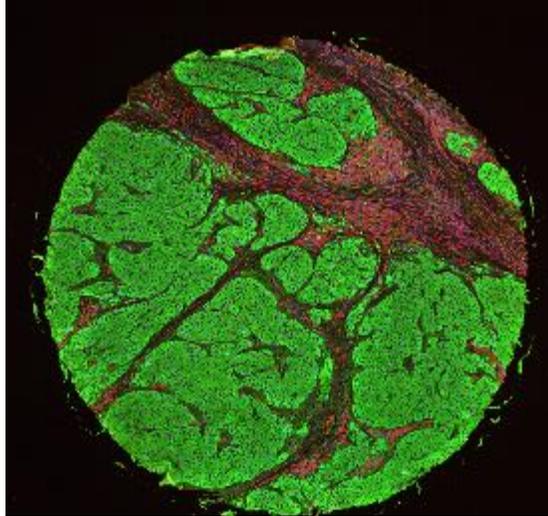
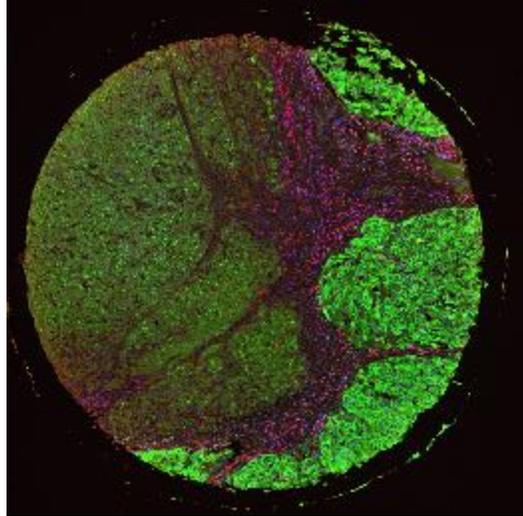
### Normal Tissue



### Hepatocellular carcinoma



# GPC3 is Localized to the Cell Membrane and T Cells are Mostly Localized in the Stroma in HCC samples



# STK-009 Specifically Enriches and Stimulates SYNCAR-002 Ex Vivo

**SYNCAR-002**

Anti-GPC3  
scFv

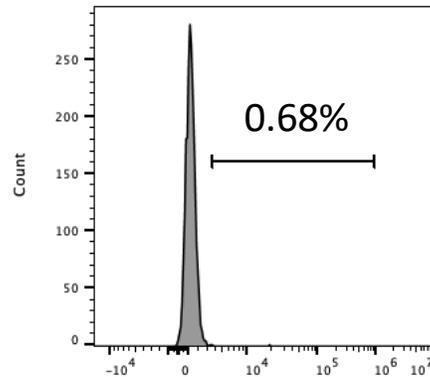
CD28

CD3z

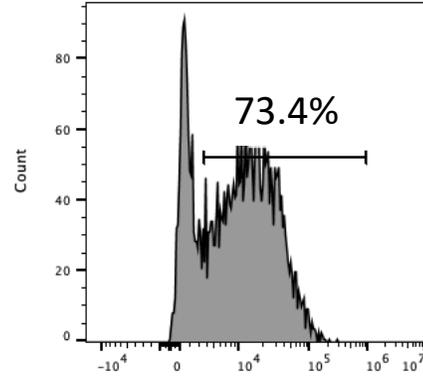
T2A

hoRβ

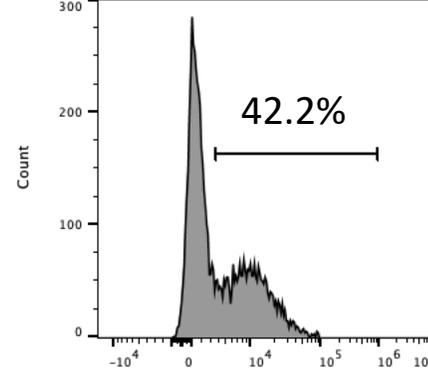
**Non-transduced  
WT IL-2**



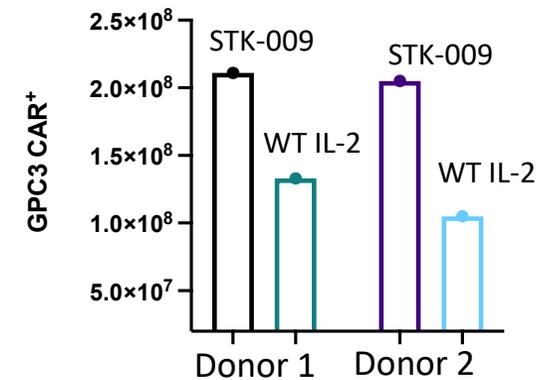
**SYNCAR-002  
STK-009**



**SYNCAR-002  
WT IL-2**



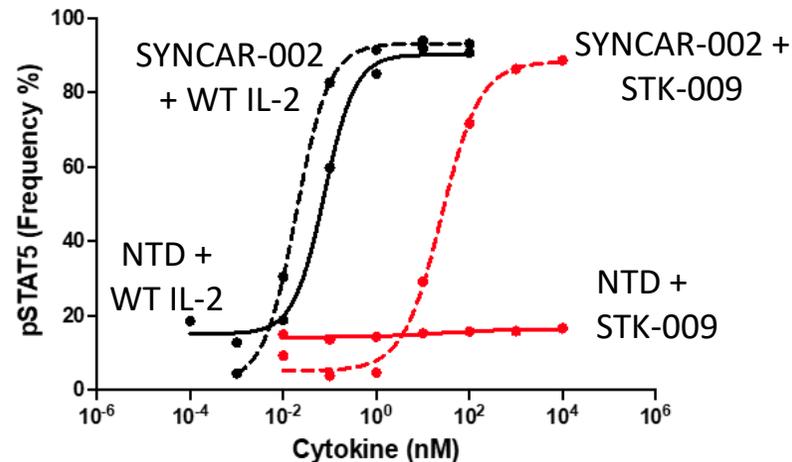
**Total GPC3 CAR+ cells  
on harvest**



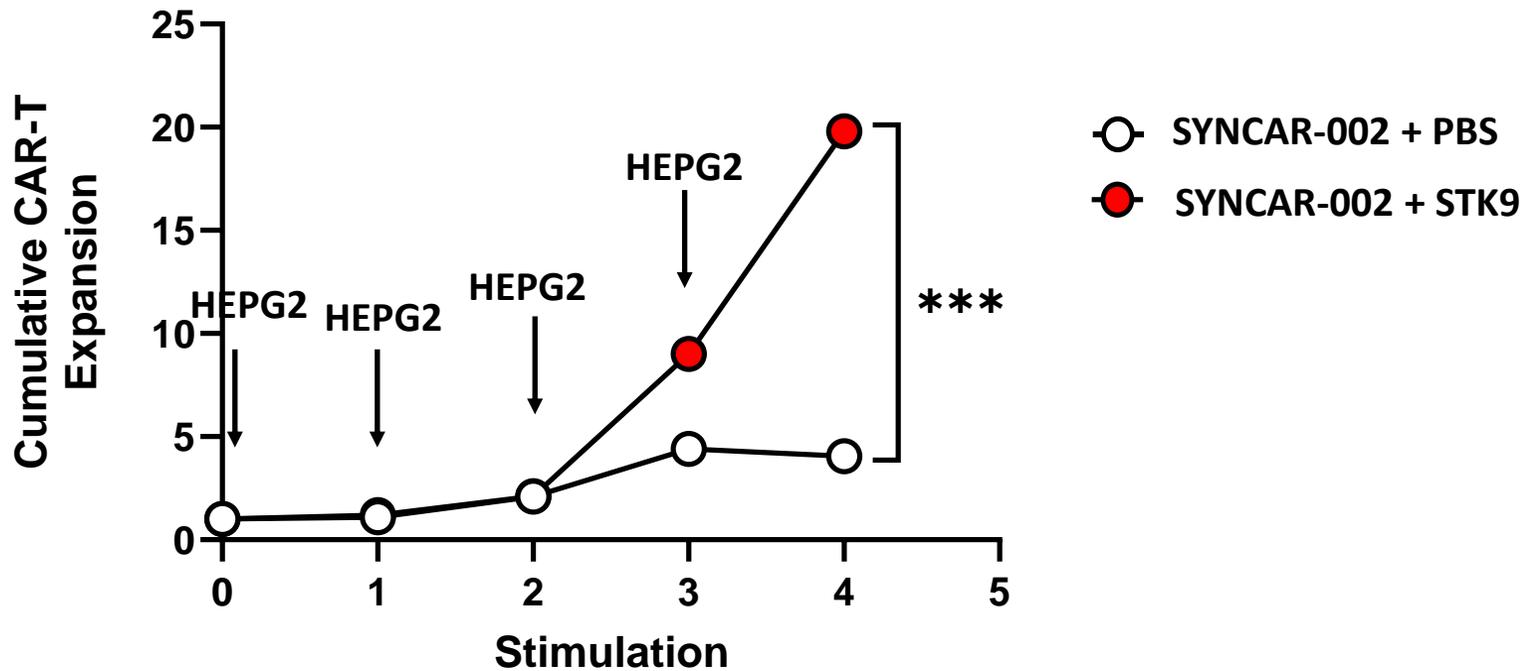
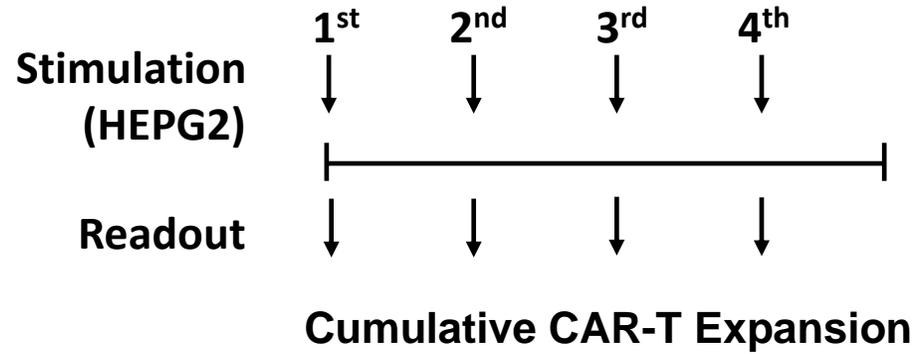
**GPC3 CAR**

**Cells transduced in  
WT IL-2 and switched  
to STK-009 or  
maintained in WT IL-2  
on Day 4 until end of  
manufacturing**

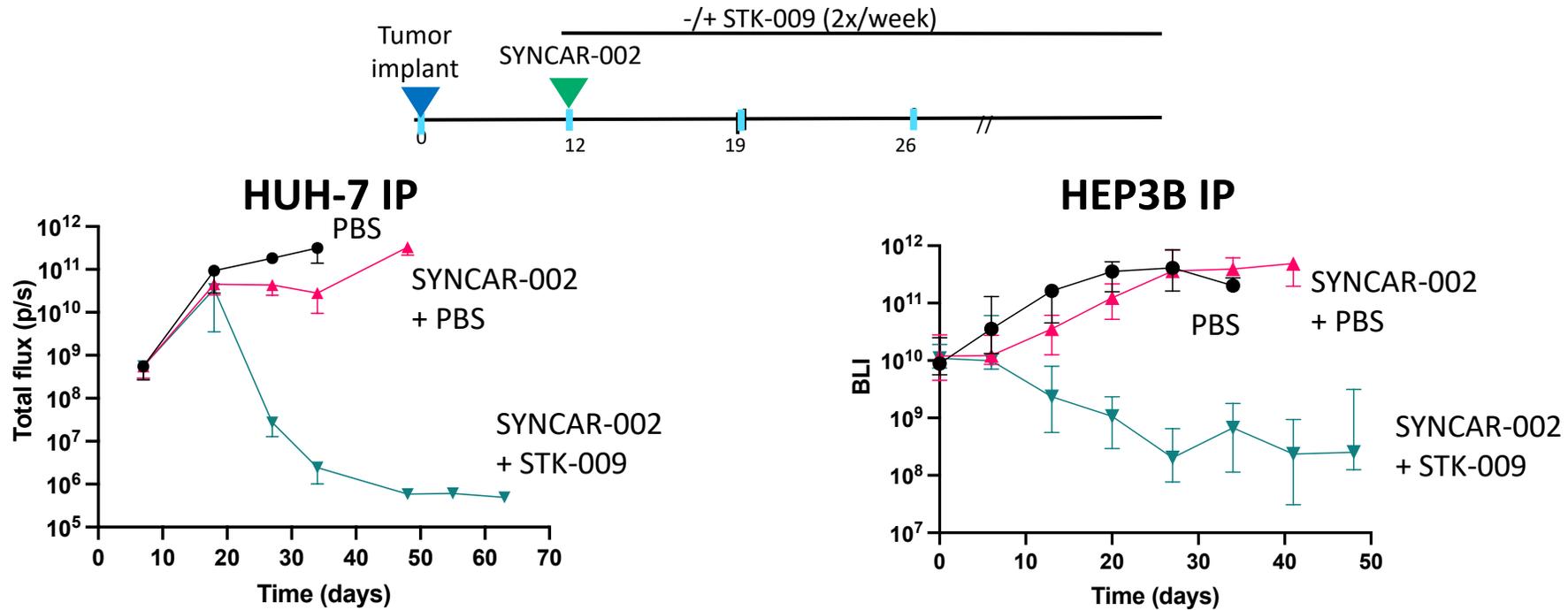
**pSTAT5 assay**



# STK-009 Enhances Proliferative Capacity After Repeat Stimulation

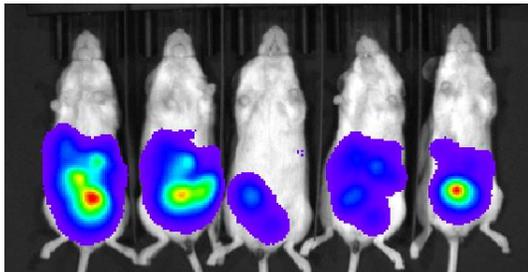


# STK-009 + Low Dose SYNCAR-002 Treatment of Intraperitoneal HCC Xenograft Models Results in Tumor Control

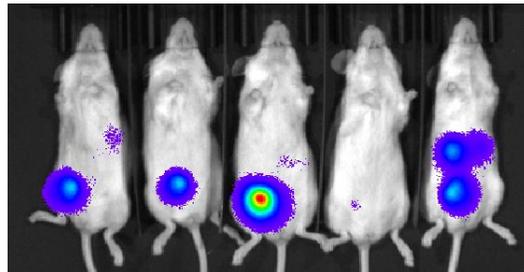


## HEP3B IP (Day 20)

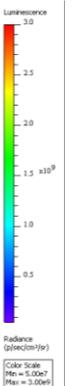
PBS



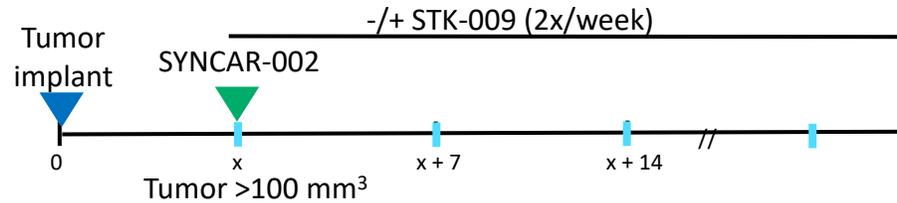
SYNCAR-002 + PBS



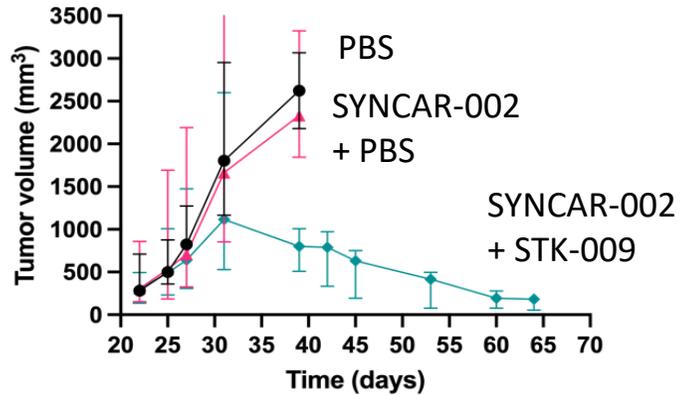
SYNCAR-002 + STK-009



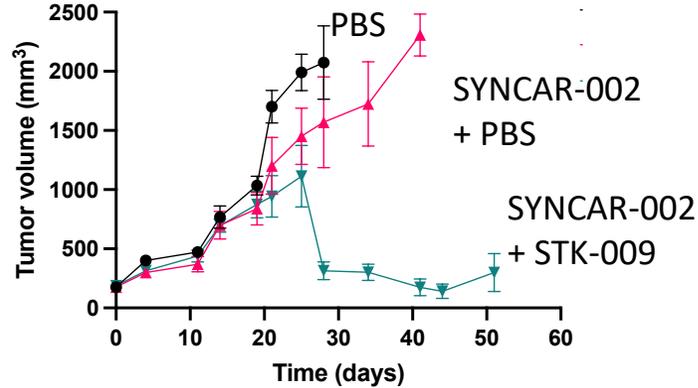
# STK-009 + Low Dose SYNCAR-002 Treatment of Various Subcutaneous HCC Xenograft Models Results in Tumor Control



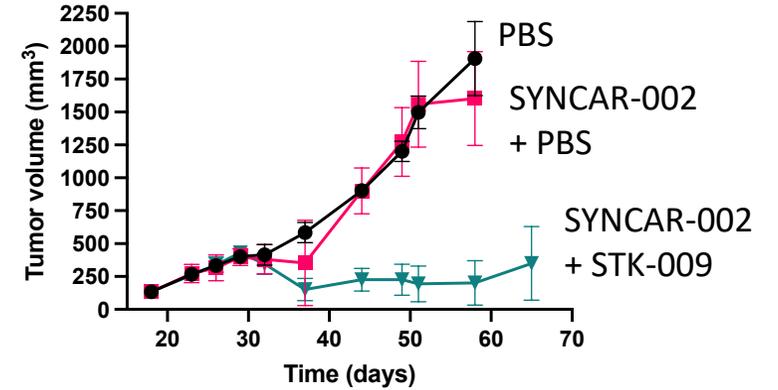
### HUH-7 SQ (GPC3<sup>med</sup>)



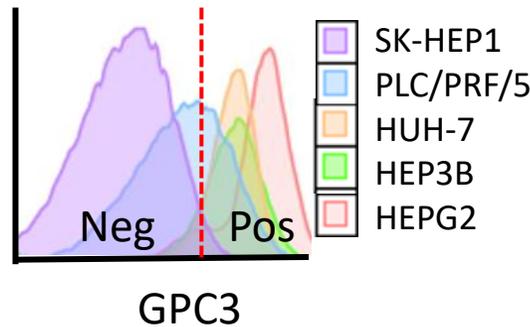
### HEP3B SQ (GPC3<sup>med</sup>)



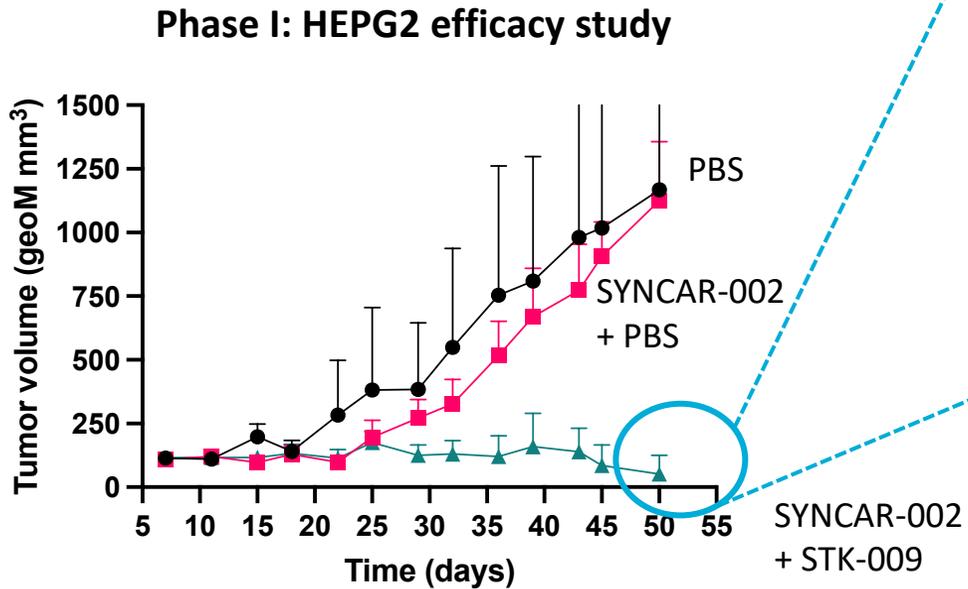
### HEPG2 SQ (GPC3<sup>high</sup>)



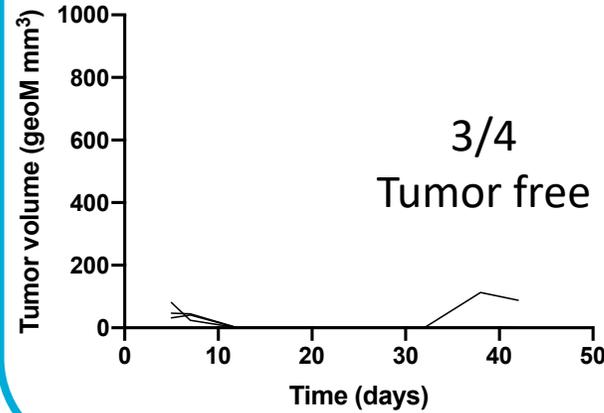
### GPC3 expression on HCC cell lines



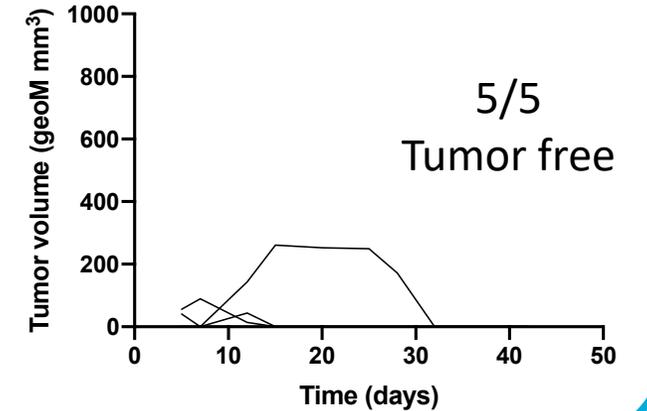
# Mice Previously Cured by STK-009 + SYNCAR-002 Withstand HEPG2 Tumor Rechallenge



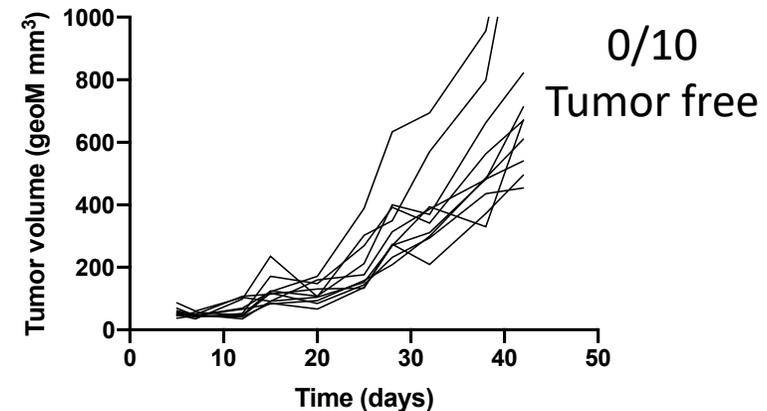
### Phase II (rechallenge): PBS



### Phase II (rechallenge): STK-009



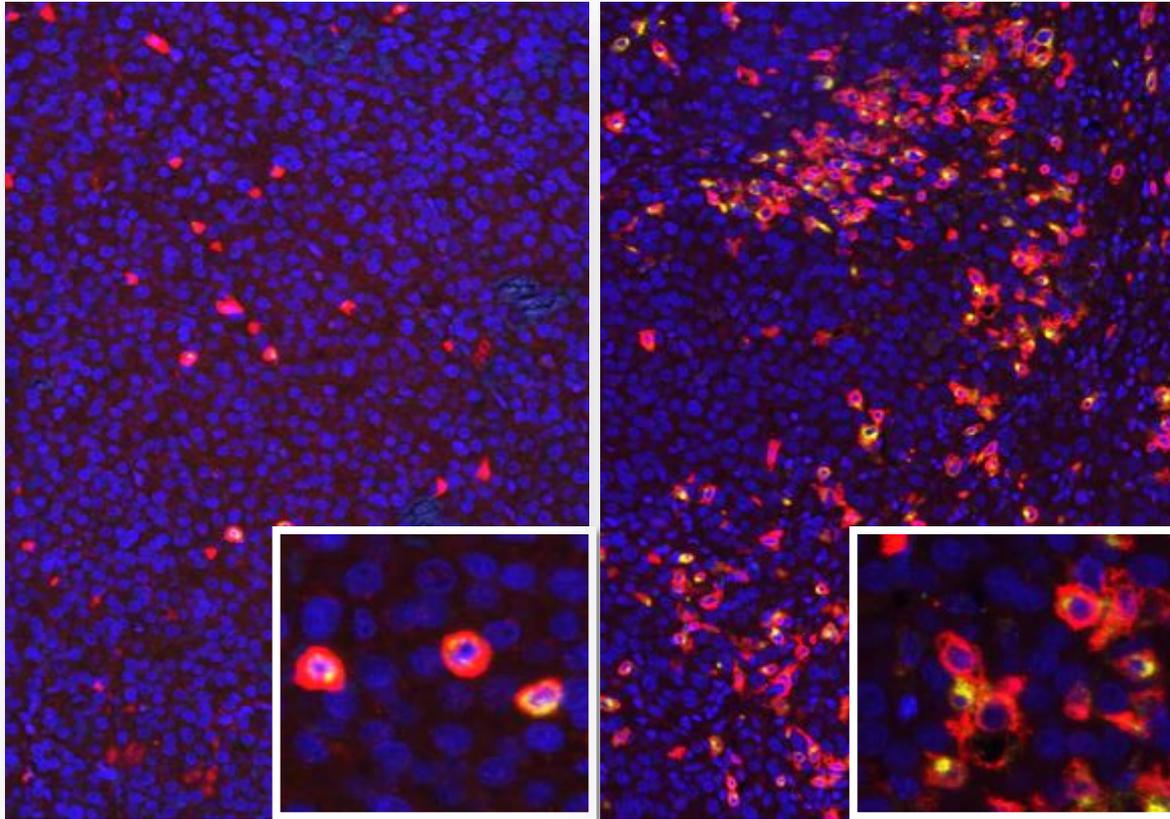
### Phase II: Naïve mice implanted with HEPG2



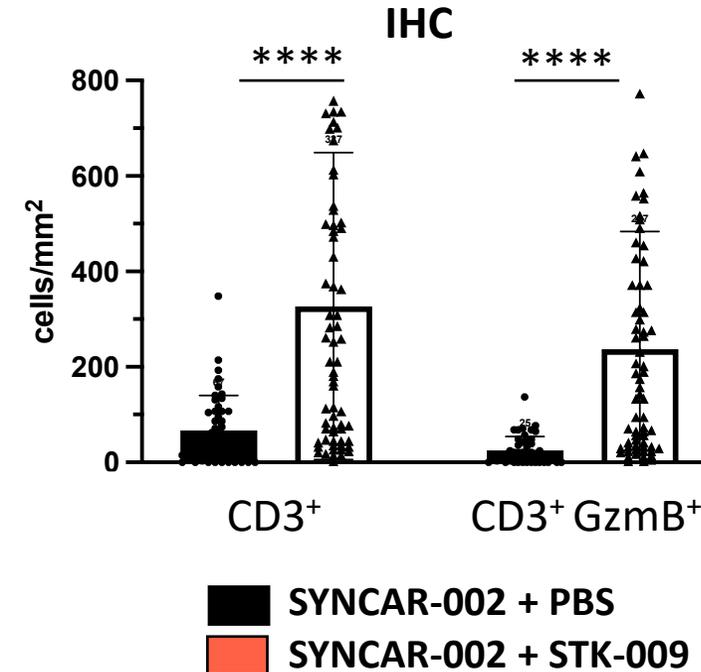
# STK-009 Significantly Increases Intratumoral SYNCAR-002 Expansion and Activation in HEPG2 Tumors

SYNCAR-002 + PBS

SYNCAR-002 + STK-009

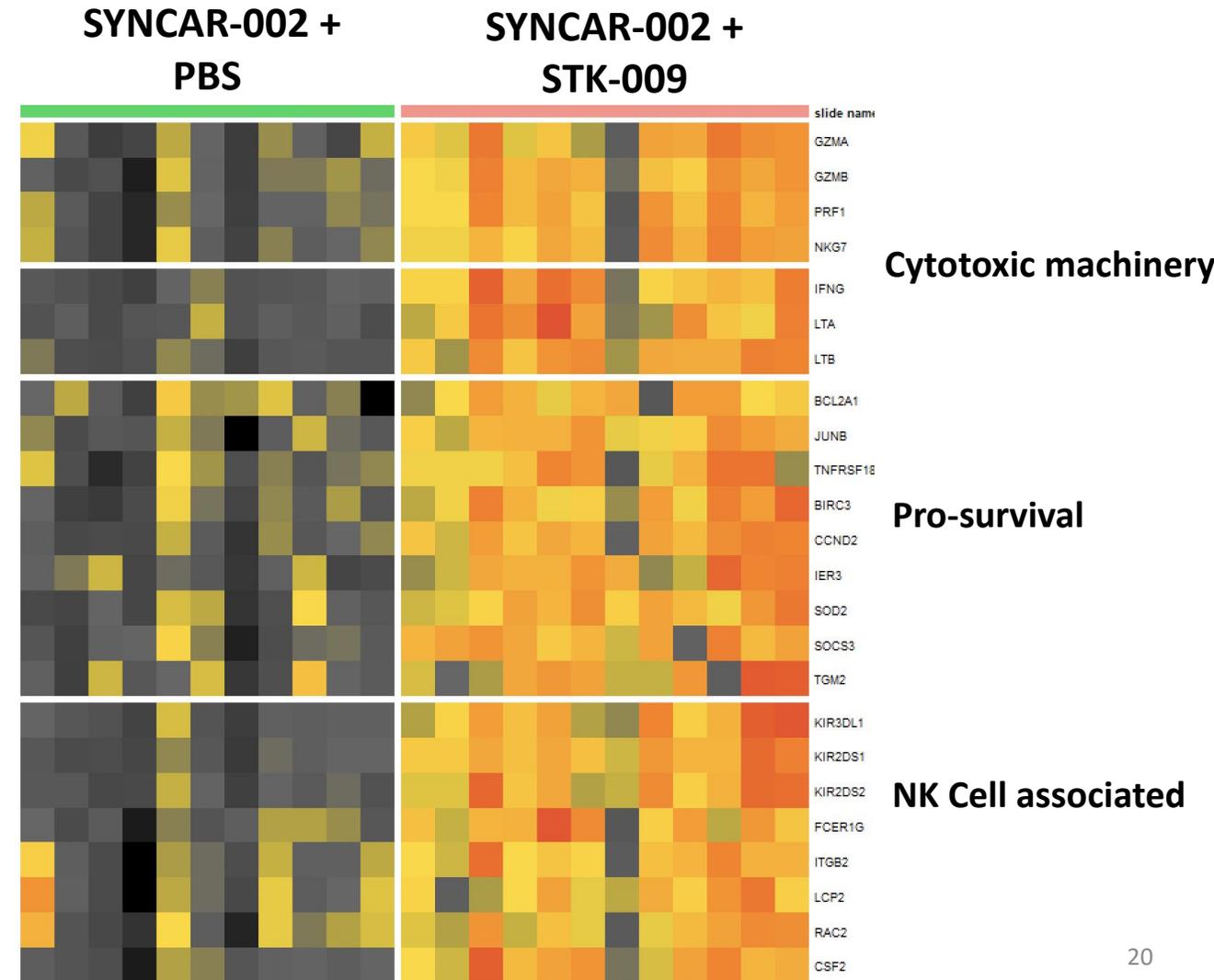
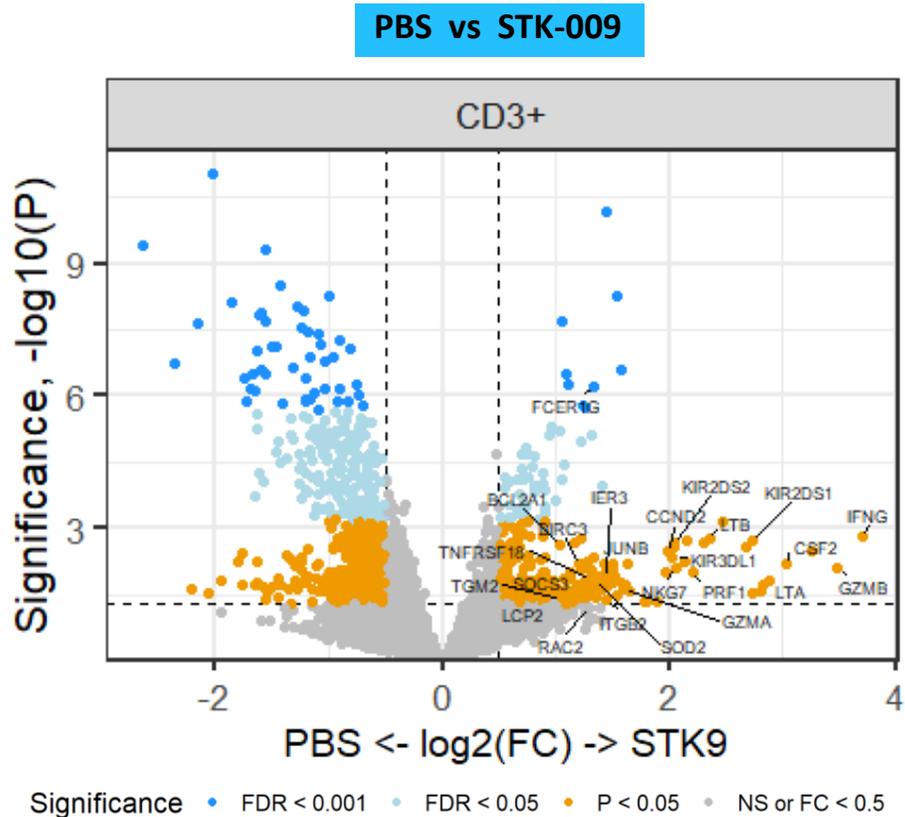


CD3/GranzymeB: Day 14 post-ACT



# STK-009 Induces Effector Molecules, Pro-Survival, and NK Cell Associated Markers in Intratumoral SYNCAR-002 Cells

Spatial Transcriptomics (GeoMx) performed on HEPG2 tumors treated with SYNCAR-002 +/- STK-009

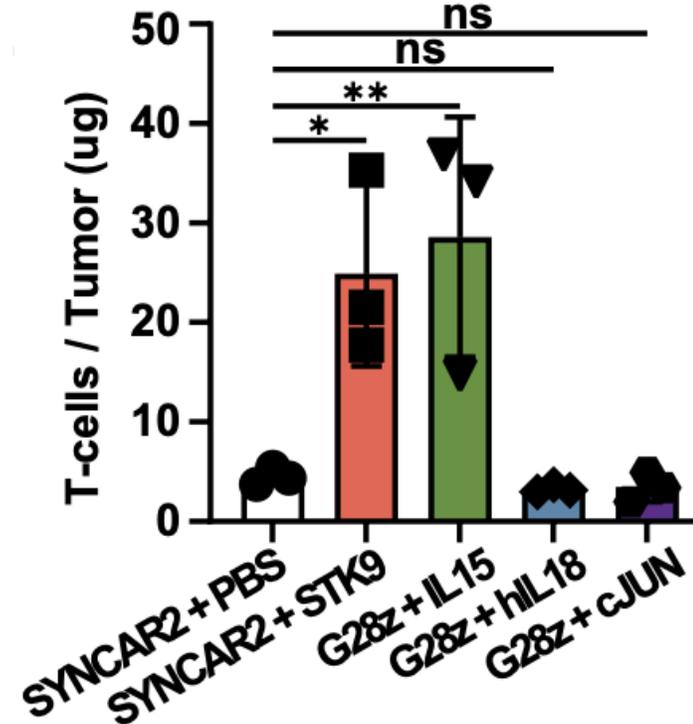
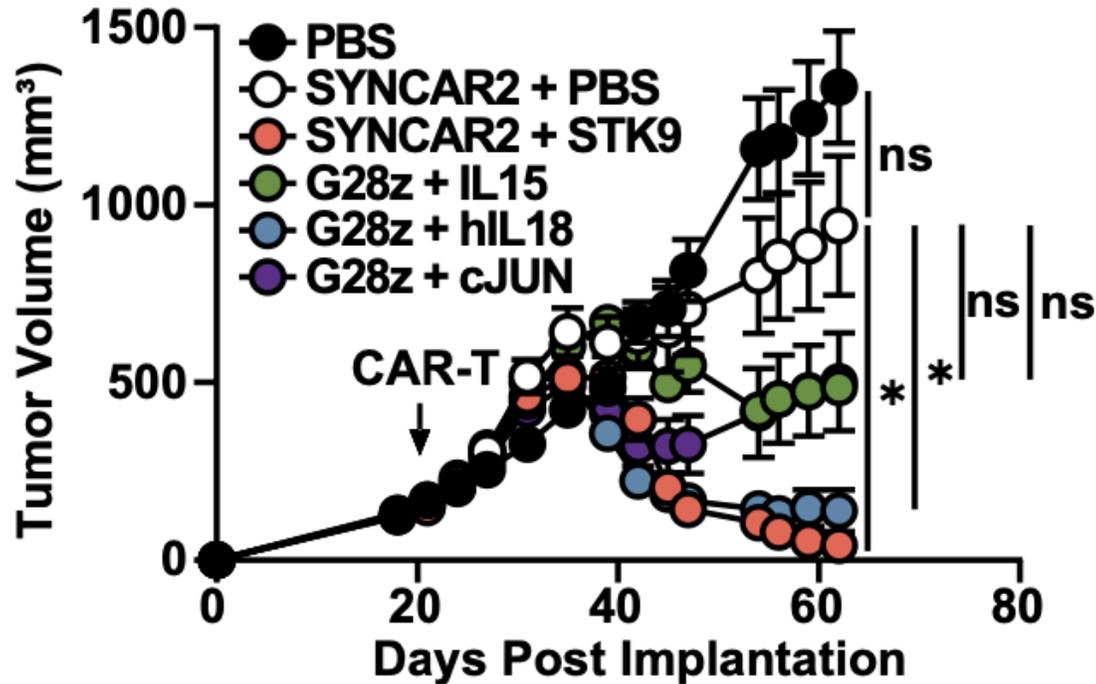
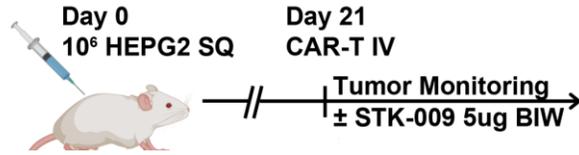


# Orthogonal IL-2 Armoring Versus Other Cell Engineering Approaches

# STK-009 Outperforms Other CAR Armoring Approaches from Enhanced Potency and Intratumoral Proliferation

SYNCAR-002 + STK-009 has potency equal to IL-18 armoring, and superior to other armoring approaches

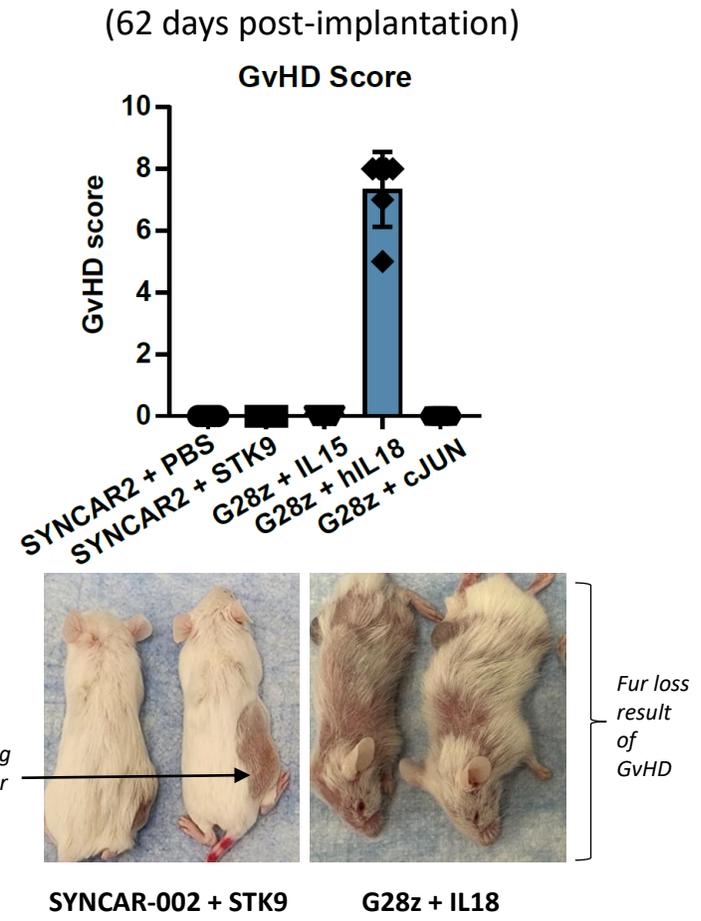
SYNCAR-002 + STK-009 has similar proliferative effects to IL-15, but enhanced potency



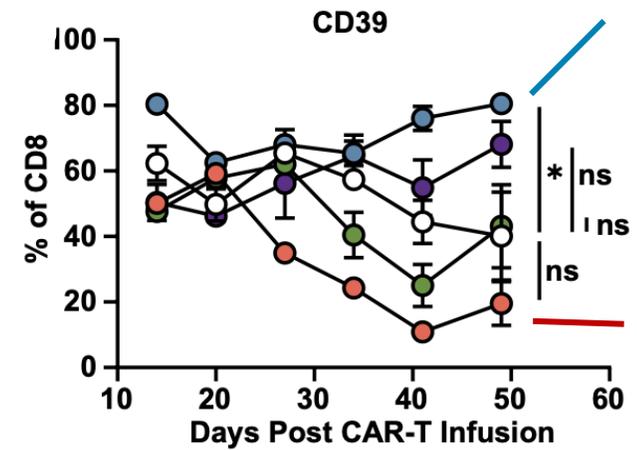
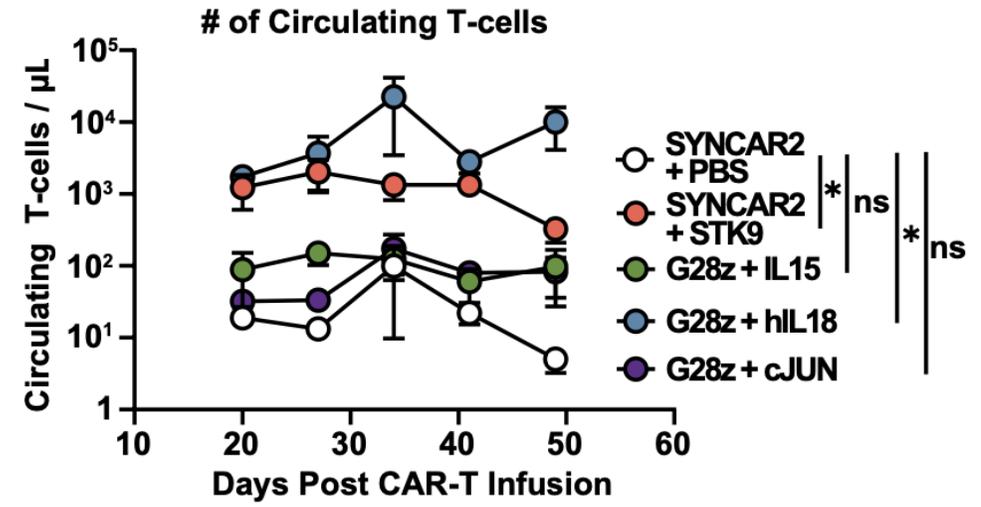
G28z = GCP3-CD28-CD3 CAR T

# STK-009 Outperforms Other CAR Armoring Approaches With Controllable T cell Persistence and Target Dependent Activity

SYNCAR-002 + STK-009 has no GvHD vs. IL-18 armoring



SYNCAR-002 + STK-009 offers controllable persistence and activation T cells



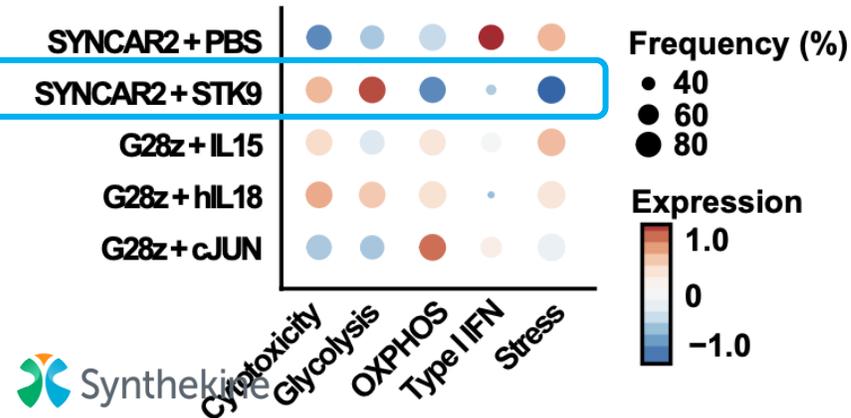
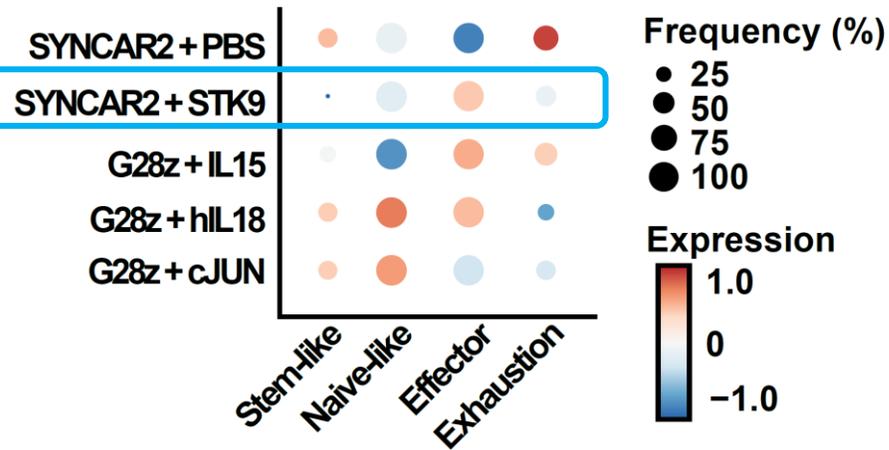
IL-18: Remains high as tumor is cleared

STK-009: Cell activation decreases as tumor is cleared

# STK-009 Promotes a Clinically Favorable Effector State

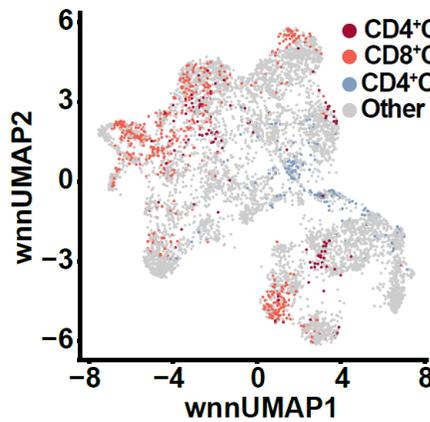
scRNAseq analysis of chronically stimulated CAR T cells

STK-009 suppresses exhaustion phenotype while maintaining an effector state with no stress response

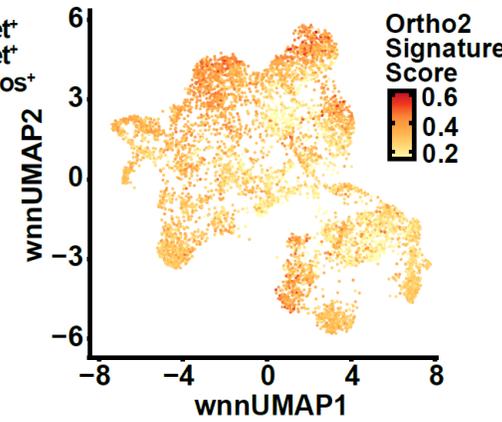


Orthogonal IL-2 Gene Signature Overlaps with Clinically Favorable Effectors

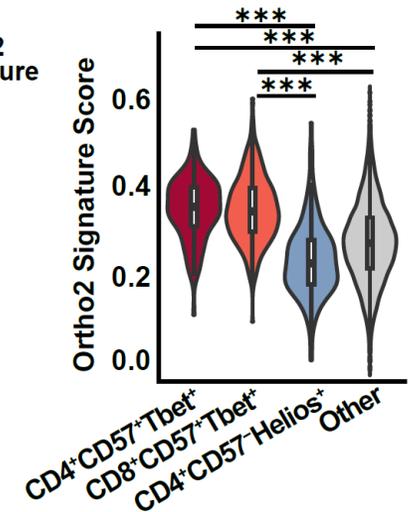
Clinically Favorable Effectors<sup>1</sup>



Orthogonal IL-2 Signature Score



Overlap between datasets

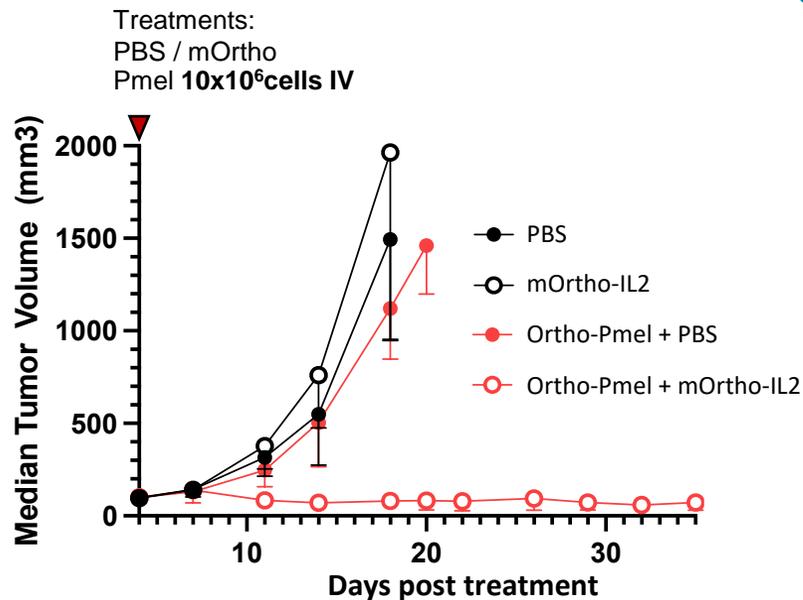


# Orthogonal IL-2 Armoring to Overcome the Need for Lymphodepletion

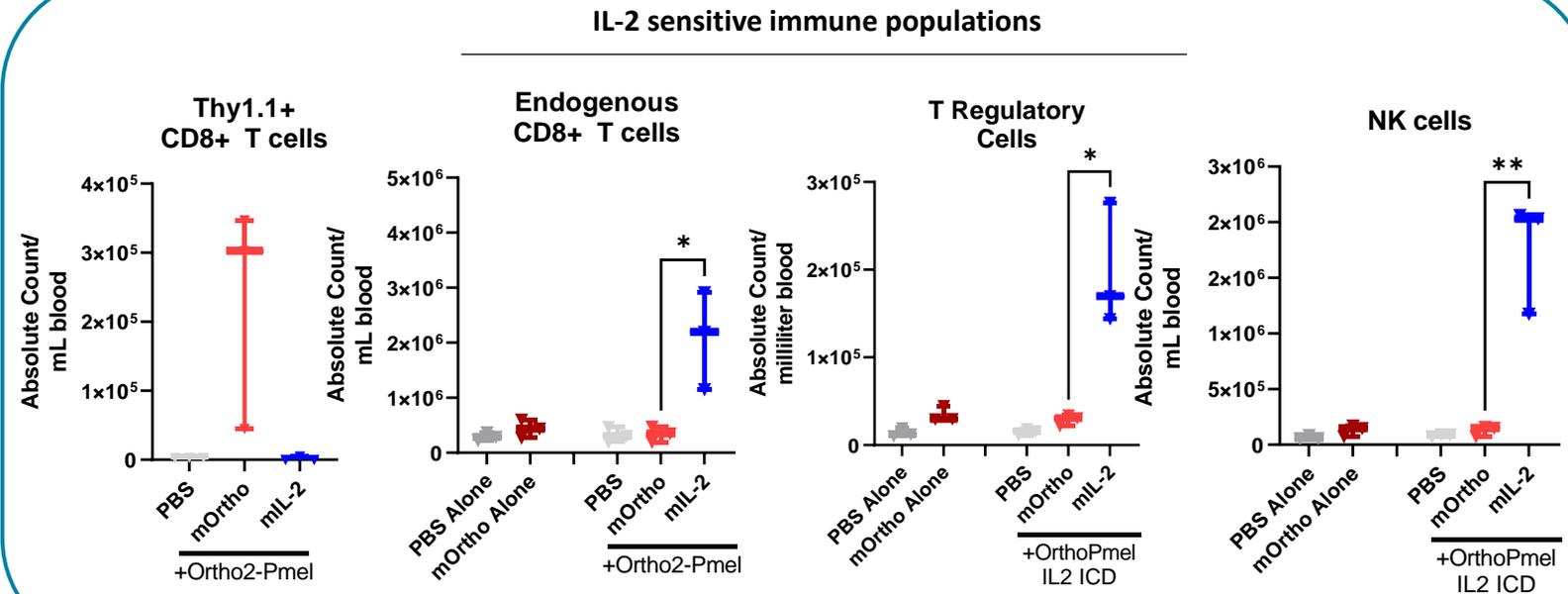
# Murine Orthogonal IL-2 System Provides Adoptive Cell Therapy Efficacy Without Lymphodepletion

B16 mouse melanoma model with Pmel-TCR Transgenic T-cells and a mouse surrogate of STK-009

OrthoPmel + mOrtho shows tumor growth inhibition in a *non-lymphodepleted* model



mOrtho *specifically* expands orthoPmel T cells *in vivo*



# STK-009 + SYNCAR Pre-clinical Summary

- STK-009 provides a **private IL-2 signal** to orthoIL-2 receptor expressing T cells **in vitro** and **in vivo**
- NHP study showed **sustained PK** of STK-009 and no activity on native lymphocytes
- SYNCAR T cells can be expanded **dramatically and at will** by STK-009 **in vivo**
- Potential to **obviate lymphodepletion** with STK-009
- STK-009 + SYNCAR T cells drive **deep and durable responses** in heme and solid epithelial tumor models



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