SENTI BIO™

# Discovery of Novel CARs for Solid Tumors Using Senti REVEAL™

## A High Throughput Technology Platform Comprising Pooled Screening, Machine Learning, and Lab Automation

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**Senti Biosciences, Inc.**

American Society for Gene and Cell Therapy, 2024

# Disclosures

Employee of Senti Biosciences, and receive salary and benefits from the company

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# Senti Bio's Internal Programs Focus on Oncology, Partnering to Support Non-Oncology Indications

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| Program | Target/Disease Candidate | Preclinical | Early Stage Clinical | Late Stage Clinical | Collaborator |
|---|---|---|---|---|---|
| SENTI-202 | AML, MDS and other blood cancers | | Clinical Trial ID NCT06325748<br>https://www.clinicaltrials.gov/study/NCT06325748<br>Initial clinical data by YE 2024 | | |
| SENTI-301A | HCC and other solid tumors | | Enroll first patient in 1H 2024 | | CELEST 晟临生物 |
| Multiple Gene Therapy Programs | Eye, CNS and liver diseases | | | | Spark Therapeutics<br>Roche |
| Multiple iPSC Cell Therapy Programs | Regenerative medicine | | | | BlueRock Therapeutics<br>Bayer |

AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; HCC: Hepatocellular carcinoma; CNS: Central nervous system

# Senti's Gene Circuit Platform to Enable Next-Gen Cell and Gene Therapies

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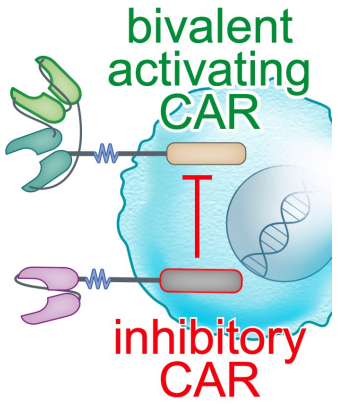
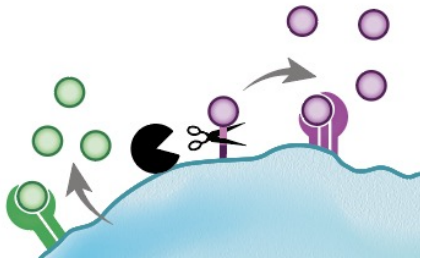
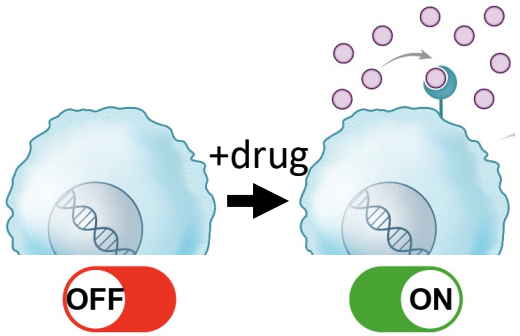
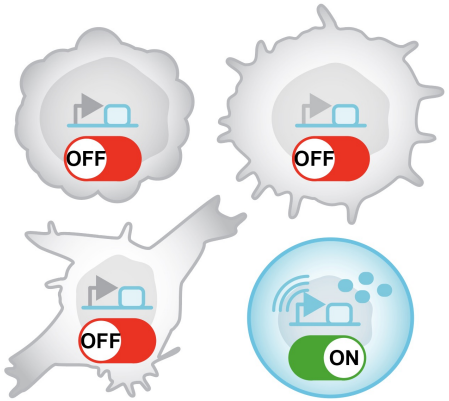
| | Logic Gating | Multi-Arming | Regulator Dial | Smart Sensor |
|---|---|---|---|---|
| Platform Technology | | | | |
| Example Implementation |  |  |  |  |
| Components and Variants | Multivalent CARs for heterogeneous targets<br><br>Inhibitory CARs for reducing off-target<br><br>Potent CAR signaling domains | Calibrated Release (cr) system<br><br>Potent immune-activating payloads<br><br>Multi-payload combinations | Payload expression induced by FDA approved drugs at physiological levels<br><br>High sensitivity safety/kill switches | Tissue and cell type specific promoters<br><br>Cell activation inducible promoters<br><br>Potent constitutive promoters |

# SENTI-202: Logic-Gating and Multi-Arming to Address Clinical Needs in AML

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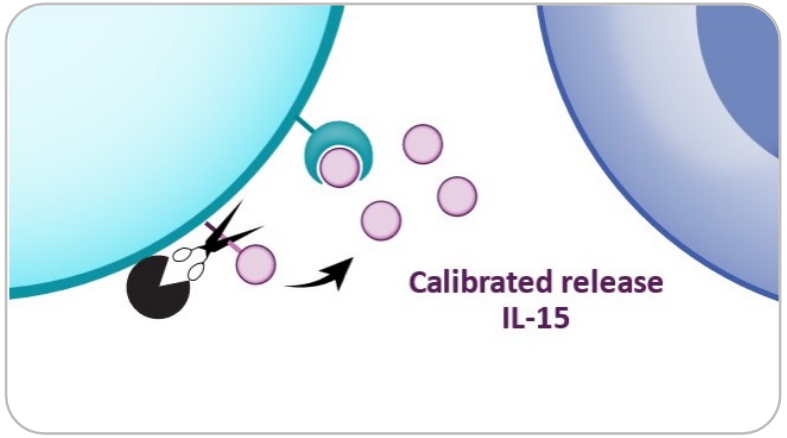
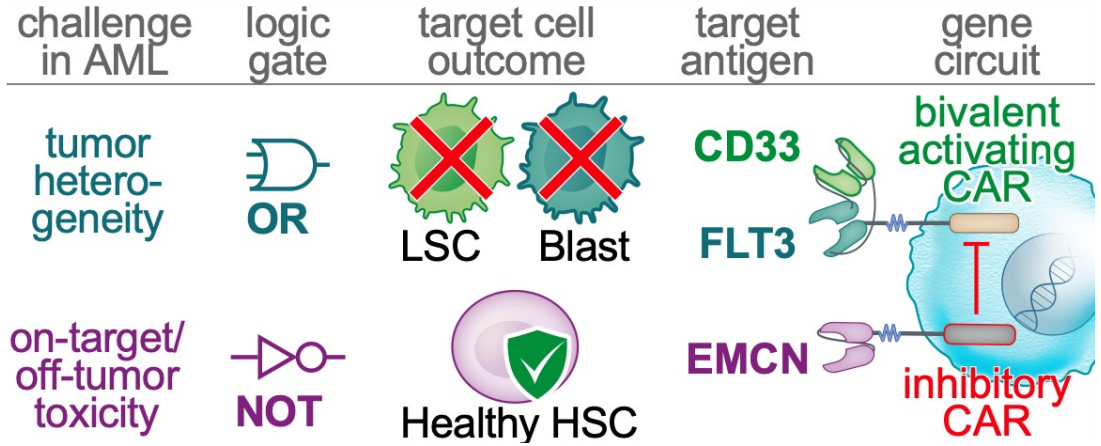
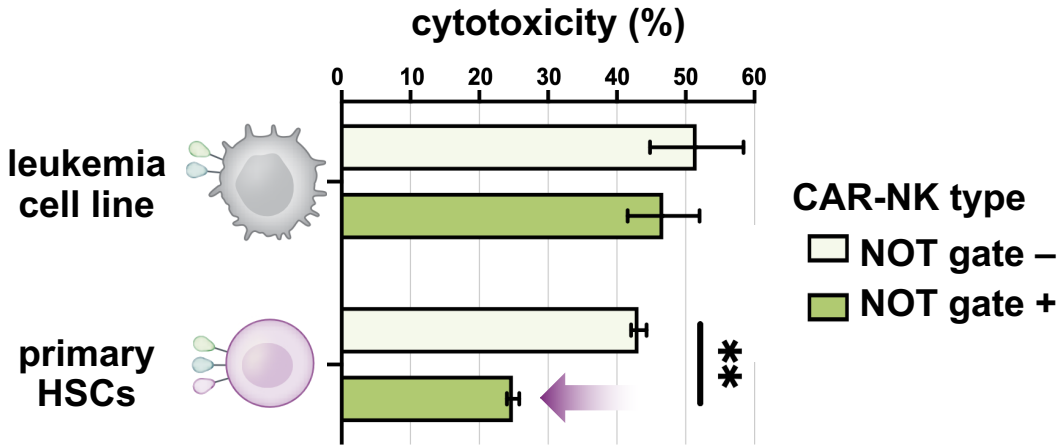
## Calibrated release of IL-15 for persistence



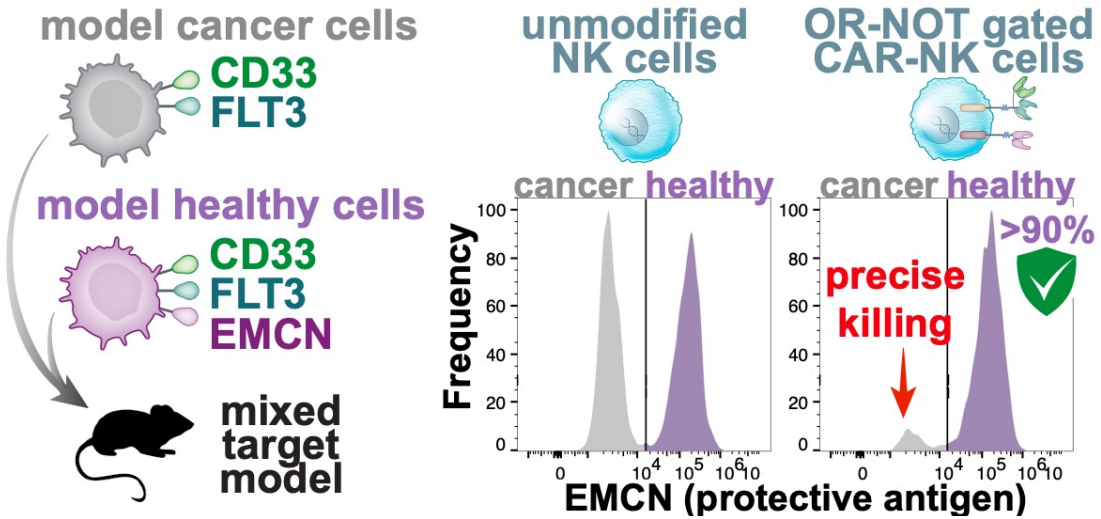

## Logic gating for efficacy and safety




## Protecting HSCs without compromising efficacy




## Precision killing in vivo




Adapted from Frankel et al., Cell Reports, 2024, https://doi.org/10.1016/j.celrep.2024.114145

# Senti's Discovery and Optimization Engine for Novel Protein and DNA Assets

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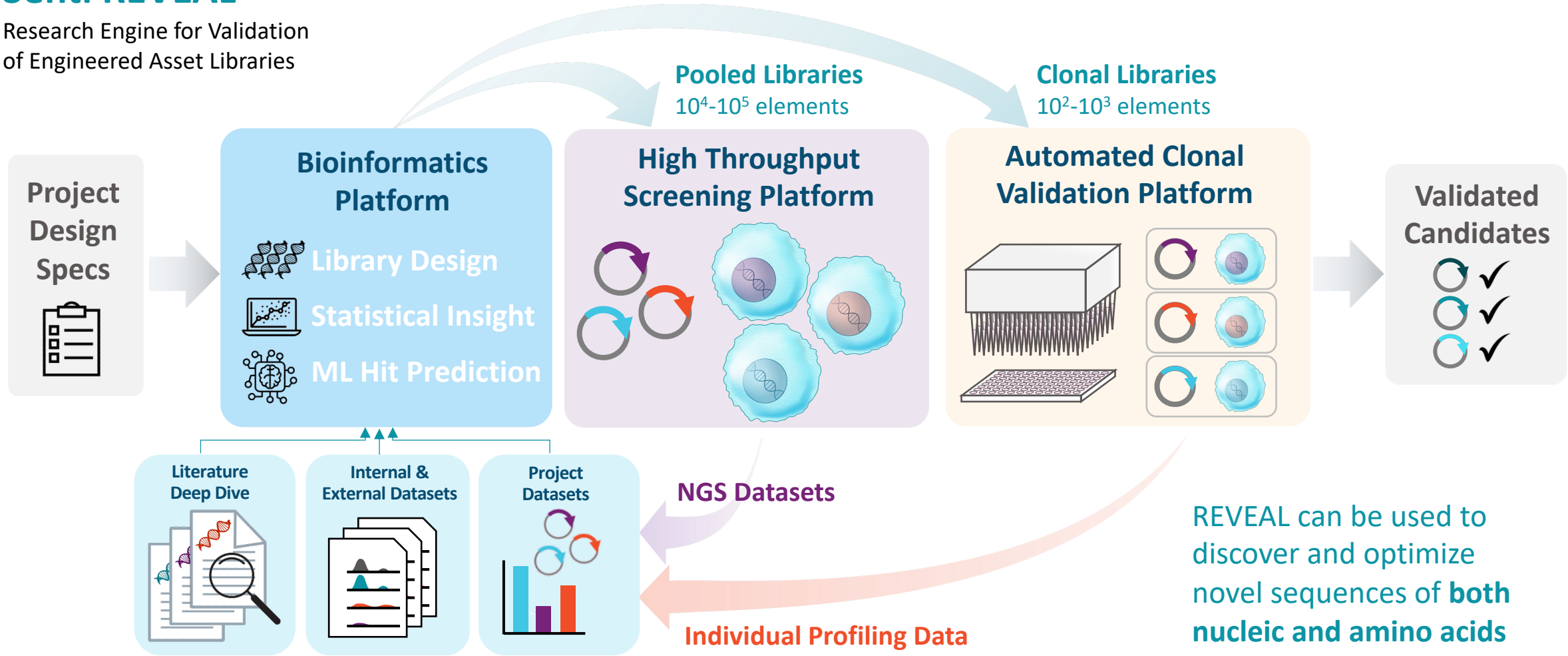
## Senti REVEAL™

Research Engine for Validation of Engineered Asset Libraries




REVEAL can be used to discover and optimize novel sequences of **both nucleic and amino acids** and is cell type agnostic.

# High-Throughput Engineering of Enhanced Constitutive Promoters to Drive Multi-Payload Constructs for Next-Gen Cell Therapies

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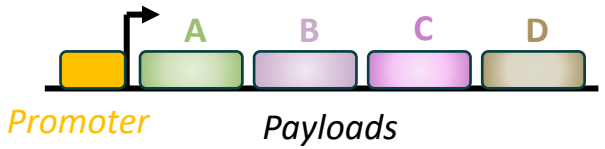
## Challenge: driving expression of 4 therapeutic payloads from a single promoter in NK cells



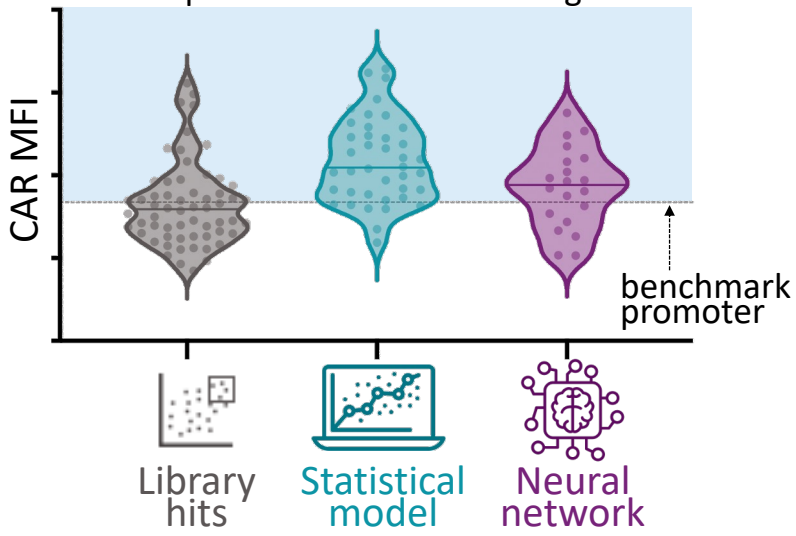

A. Secreted cytokine
B. Calibrated release cytokine
C. activating CAR
D. inhibitory CAR

## Senti REVEAL with three iterative design cycles and tens of thousands of promoters.

Start-to-finish under 9 months

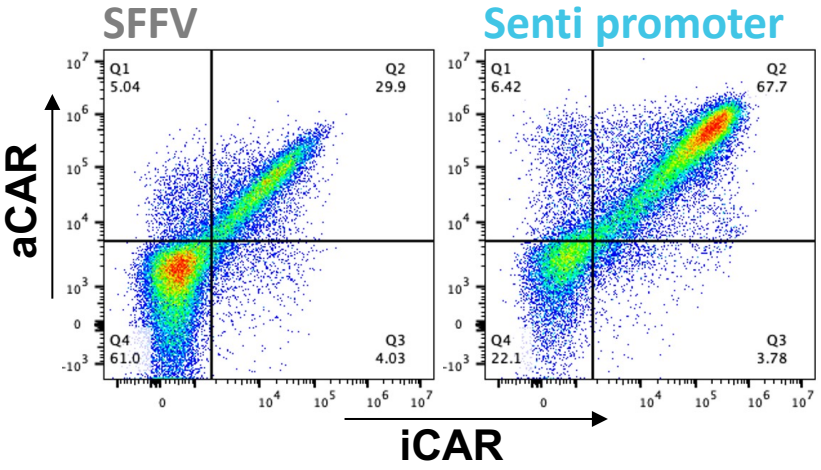
## Automated clonal validation

100s of candidates validated in parallel
Models predict robust hits at a high rate



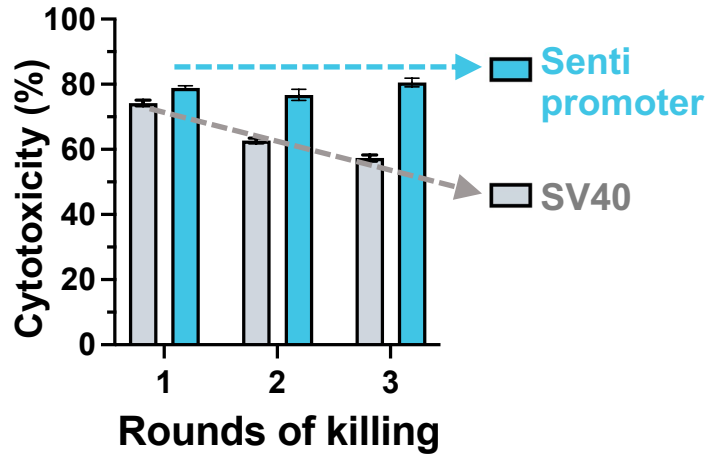

## Enhanced multi-payload expression

Showing payloads 3 and 4. Payloads 1 and 2 also higher.
2-4x higher expression than SFFV or SV40




## Expression drives performance

Persistence in serial re-challenge in vitro killing assay (solid tumor cell line)




*Mean fluorescence intensity (arbitrary fluorescence units)

*2-4x depending on metric. Not due to copy number differences

Each round 72 hrs, E:T 1:4, IncuCyte readout

# Discovery of Novel CAR Signaling Domains for Tackling Solid Tumors by Screening Libraries of Tens of Thousands of CAR Signaling Domains

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## CAR Library Design

Collection of 87 sequences from different signaling pathways

Fixed domains

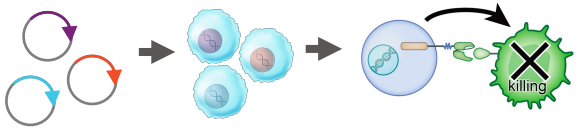
Variable intracellular domain

- ✓ Intracellular domains are combinatorial assemblies containing from 1 to 5 subdomains
- ✓ Subdomains sizes range from isolated motifs <10 AA to full-size domains >300 AA
- ✓ Increased diversity by building multiple sub-libraries with different design principles and assembly methods
- ✓ **Over 50,000 CARs in total, works with T or NK cells**

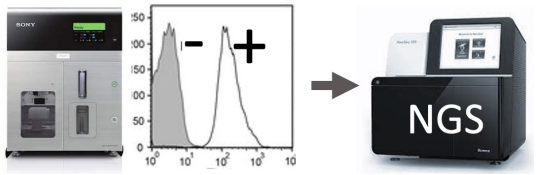
Illustrative diagram, not drawn to scale

## Sort-seq Workflow

Transduce CAR library into immune cells and challenge with target cells

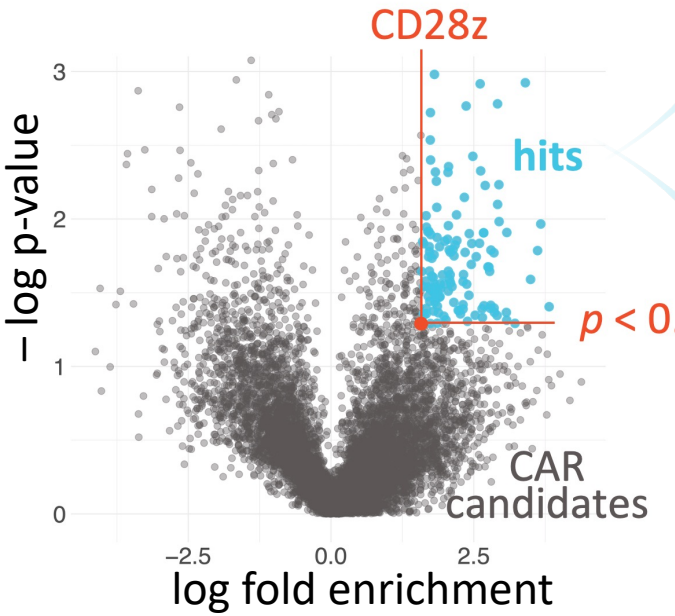



Sort activated from non-activated immune cells and sequence CARs




## HTS Results (sub-library)

>100 significant CAR hits above the CD28z benchmark




CAR read freq. in active vs. inactive

Bioinformatics platform

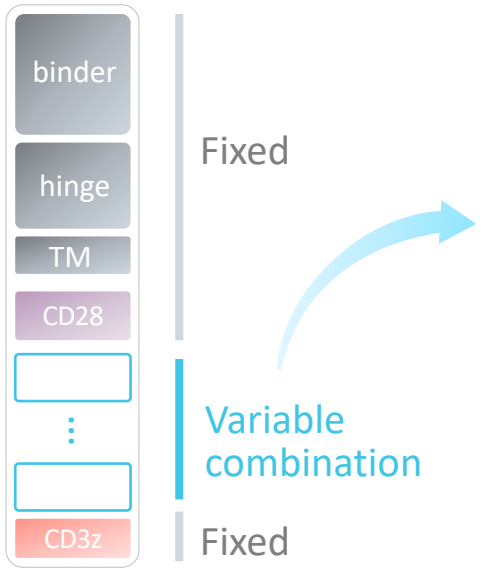
predicted structures

Clonal validation

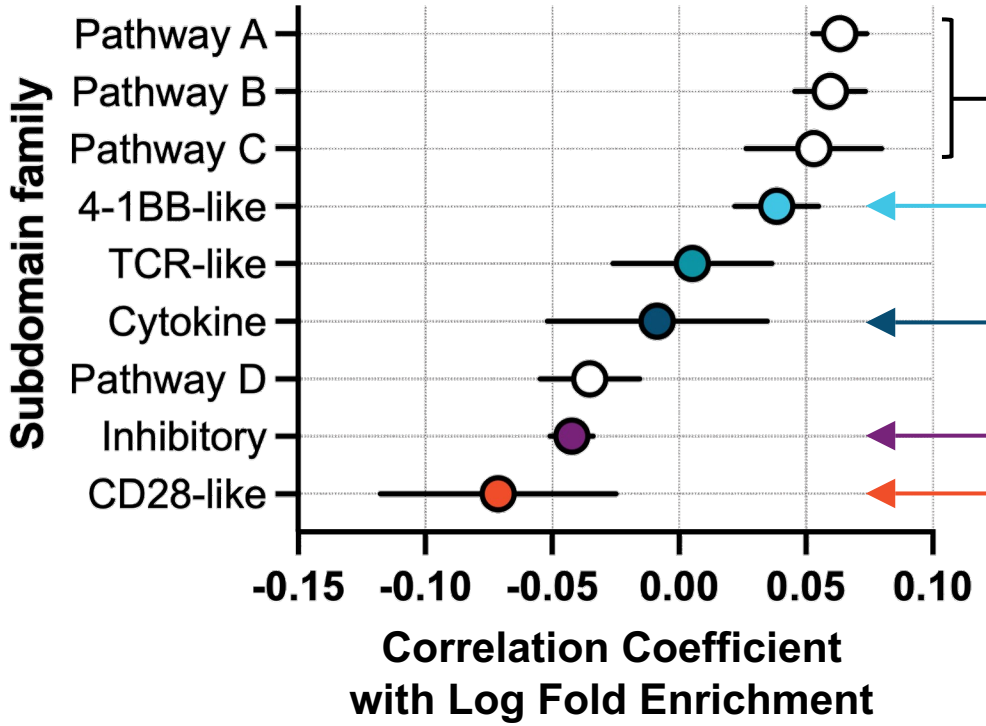
# Certain Subdomain Families Are Enriched in High Performing CAR Intracellular Domains in the Context of a 3rd Generation CAR architecture

**Trends at the sub-library level**

## Sub-library with fixed CD28 and CD3z plus variable domain (3rd gen)




## Summary of CAR performance by subdomain family




Mean +/- standard deviation across all sequences in family and across all positions. Correlation of each sequence in each position is calculated over all combinations in sub-library.

## Take-homes

- Novel costimulatory subdomains from alternate families
- TRAF-binding adds to efficacy to make a classical 3rd gen architecture
- Some CARs successfully combine canonical signals 1, 2, and 3
- Negative controls inhibit function
- Poor performance likely because CD28 is already in the fixed region

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# Clonal Validation Using Automated Liquid Handling

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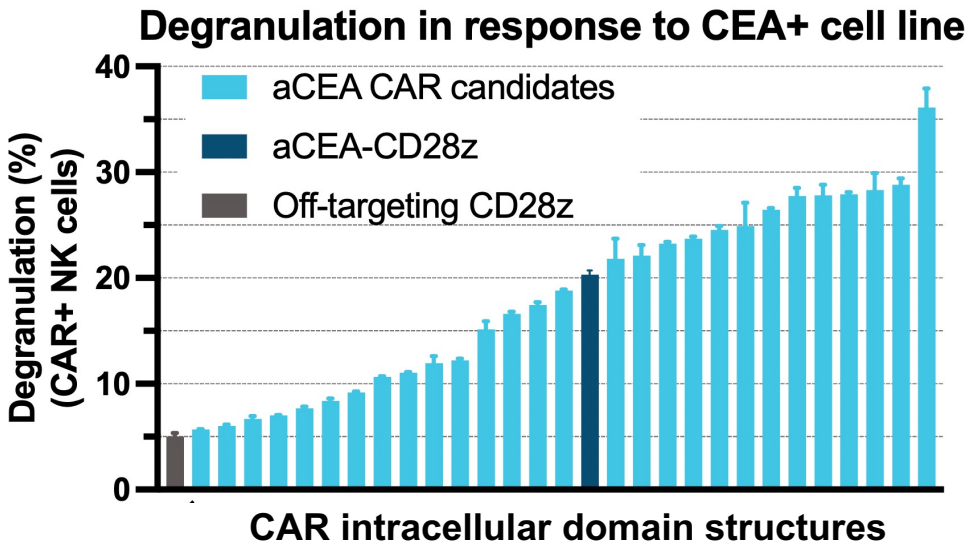
**Individual hit validation**

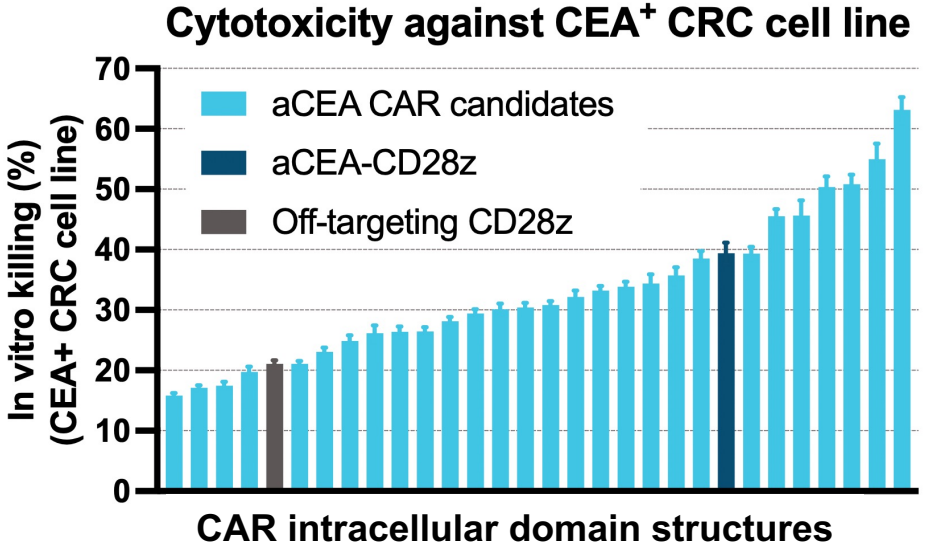
## Degranulation (CD107a)




~50% hit validation rate using the same assay used as in the pooled screen sort-seq protocol.

*24 hours, 1:4 E:T ratio, flow cytometry readout*

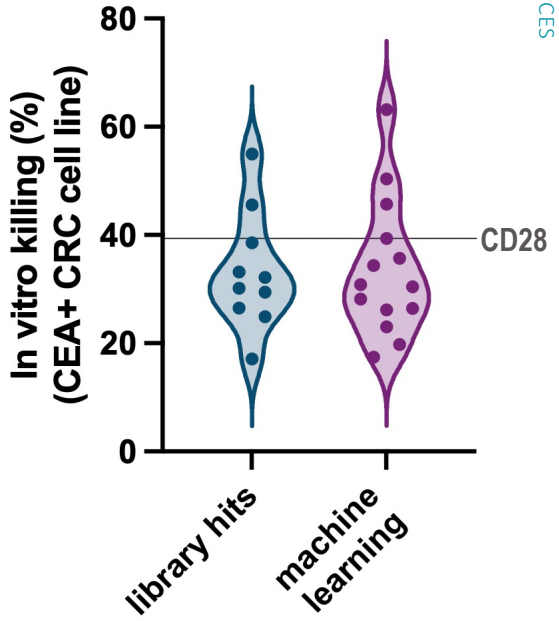
## Cytotoxicity (Solid tumor cell line)




Top CAR exhibits >50% higher cytotoxicity than CD28z in killing a solid tumor cell line

*72 hours, 1:4 E:T ratio, imaging readout*

## Design Method




ML model trained on HTS data predicts potent CARs.

# Conclusions and future directions

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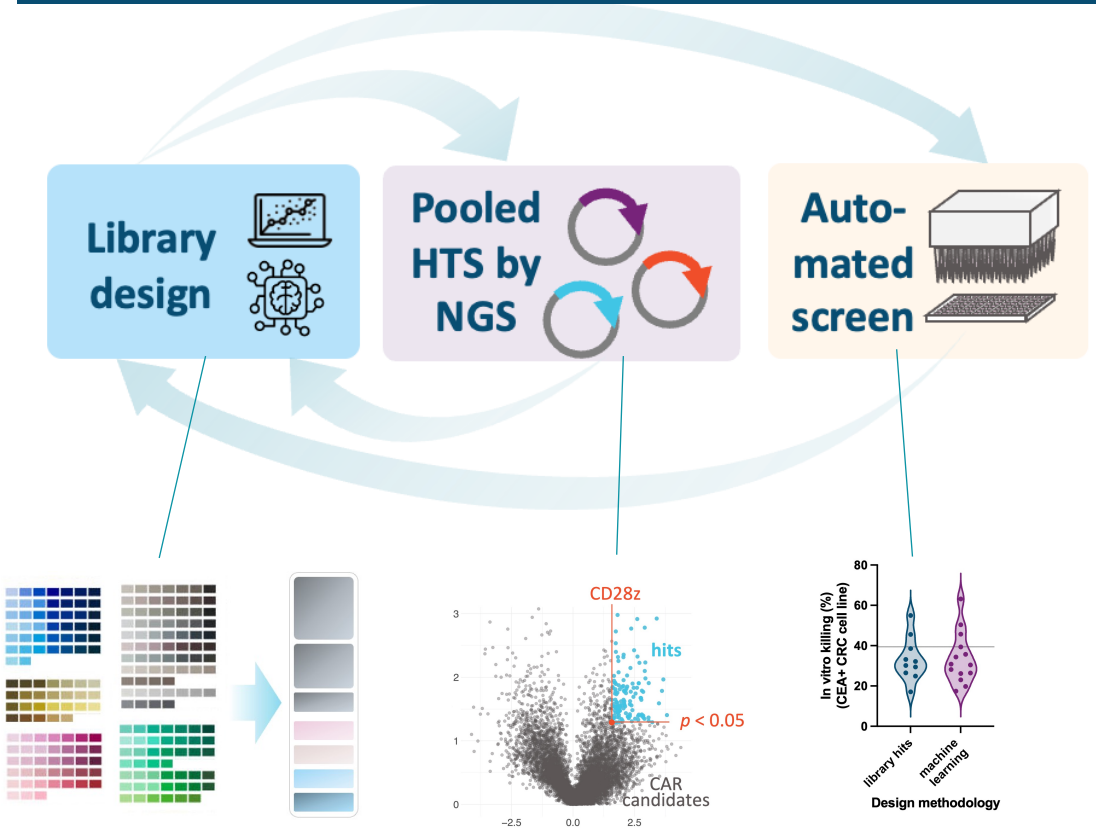
## Discovery of novel potent CARs using Senti's REVEAL™ platform




- Senti's gene circuit platform includes potent and specific promoters, logic-gated CARs, calibrated release cytokines, and inducible systems.
- Our discovery engine, REVEAL, combines pooled screening, machine learning, and automated liquid handling
- We used REVEAL to discover CARs with greater degranulation, cytotoxicity, and cytokine release (not shown) than CD28z, but with novel structures.

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# Acknowledgements

## Gene Circuit Discovery @ Senti

Researchers: Tony Hua, PhD, Marcus Gainer

Brian Garrison, PhD, VP of Research

Kanya Rajangam, MD, PhD, CMO and Head of Research and Development

Tim Lu, MD, PhD, CEO and Co-Founder