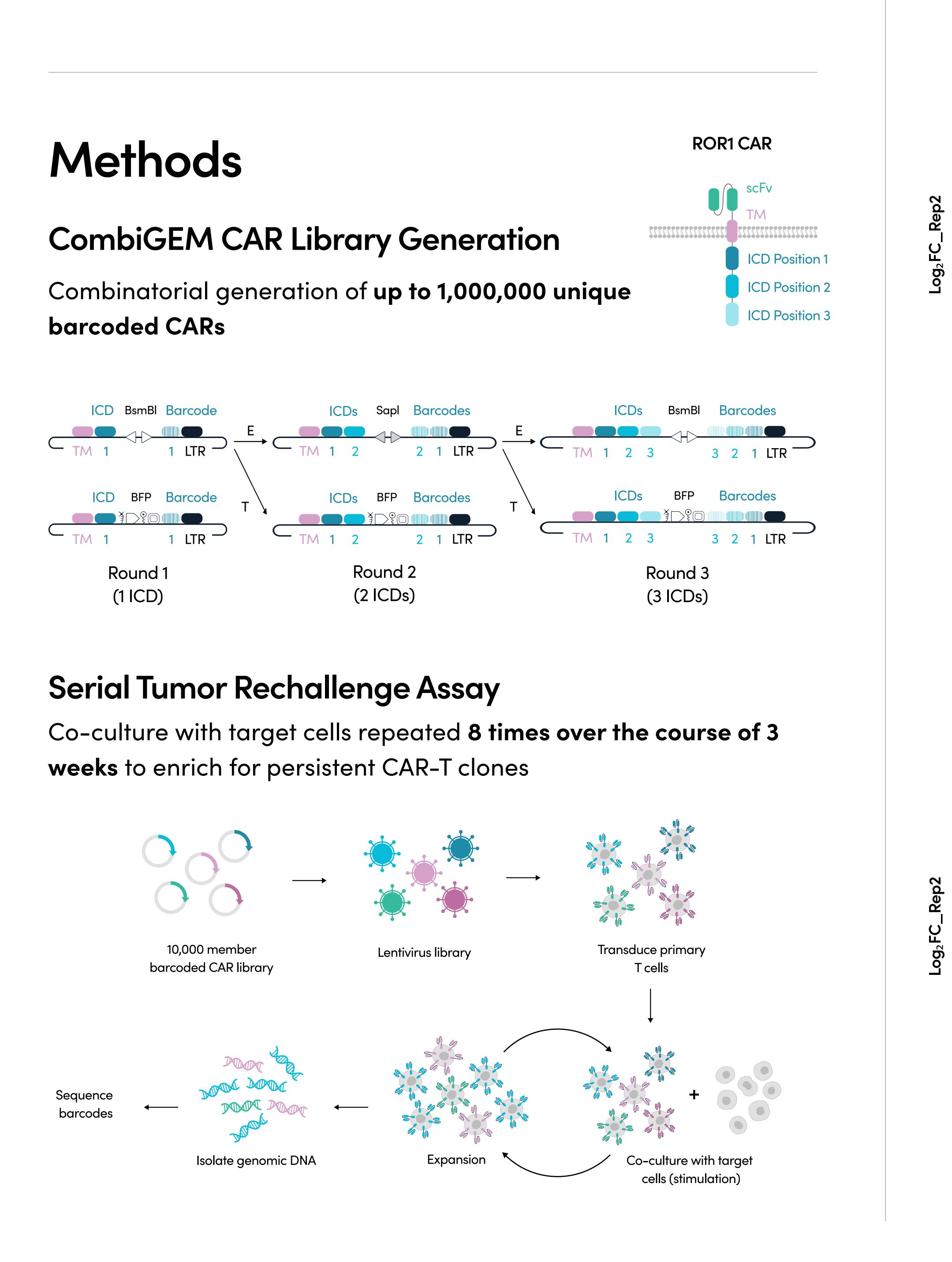
# **Pooled screening platform** for discovering the next generation of CARs

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# Background

- CAR-T cell behavior and fate are differentially driven by the type of intracellular domains (ICDs) upon antigen recognition
- 2. Canonical CAR ICD combinations have achieved limited success against challenging tumor indications
- 3. Systematic discovery of novel CAR ICD designs have been onerous due to technical constraints in screening and scaling

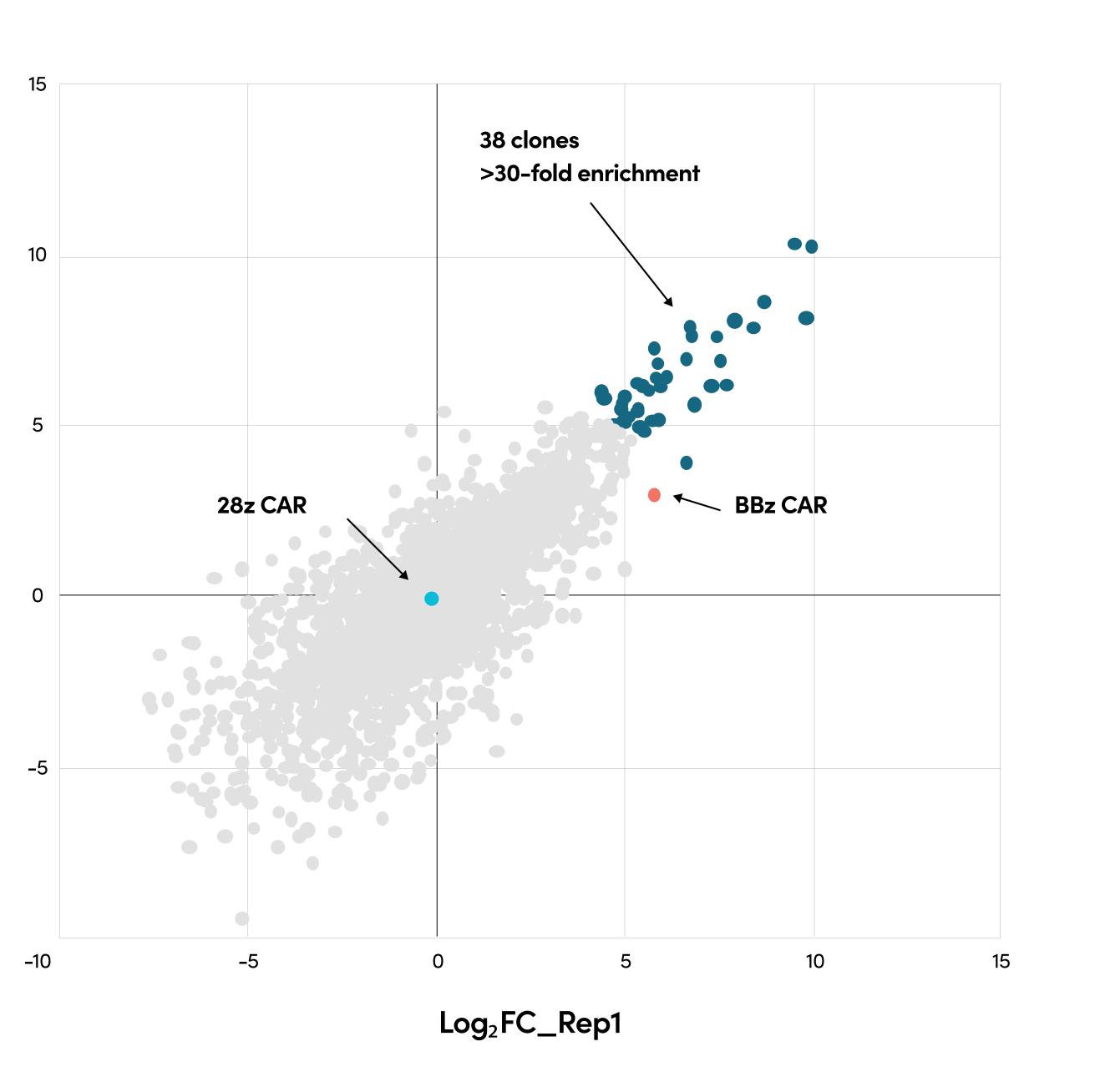


# Results: A Case Study with a 10,000-member Library with 2 ICDs

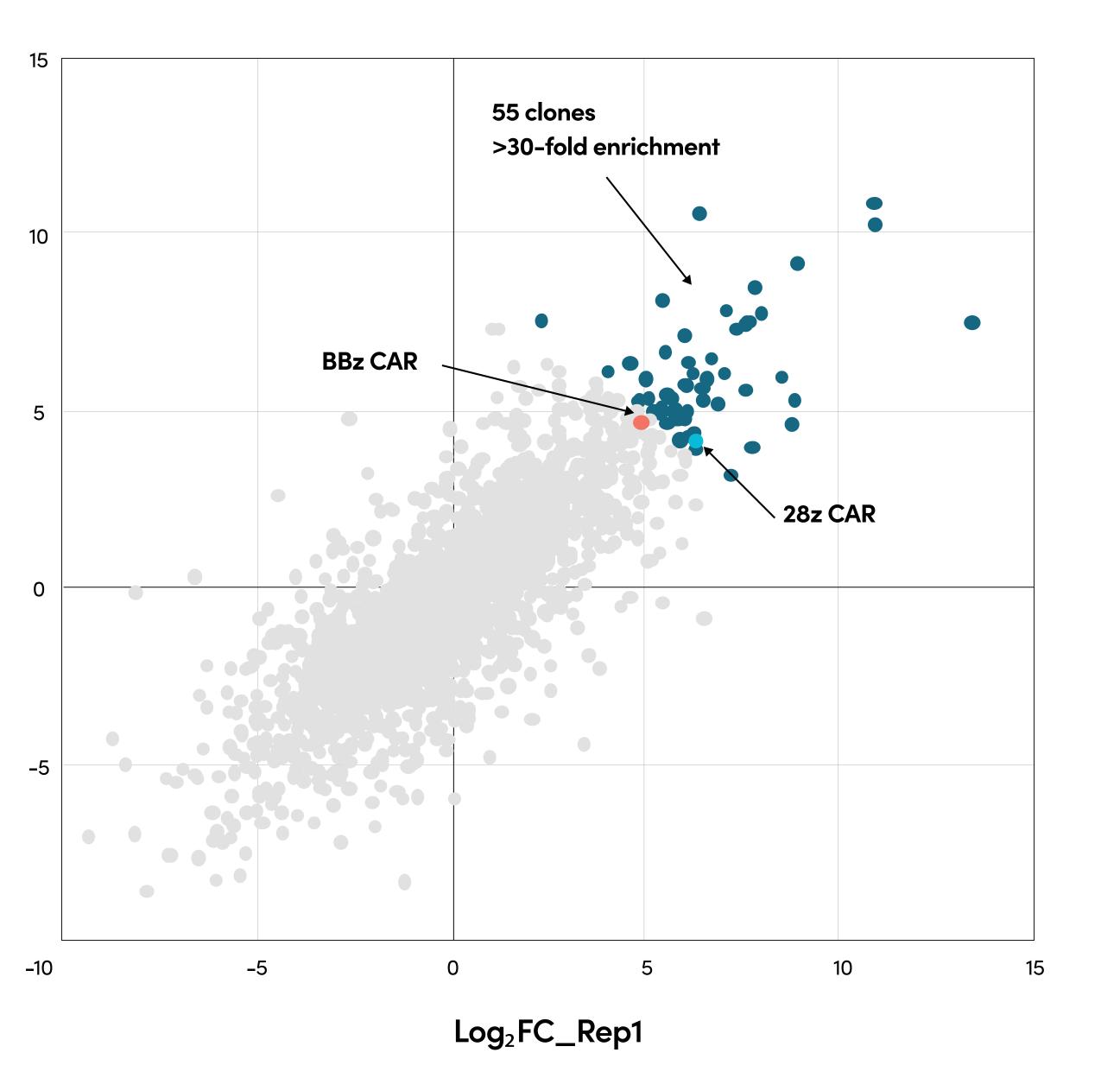
Automated high throughput Foundry allows parallel screening of multiple libraries. Novel CAR clones are enriched more that BBz and CD28c canonical CAR designs over 20 days of tumor cell co-culture.

Novel CAR clones are enriched more than BBz and 28z canonical CAR designs

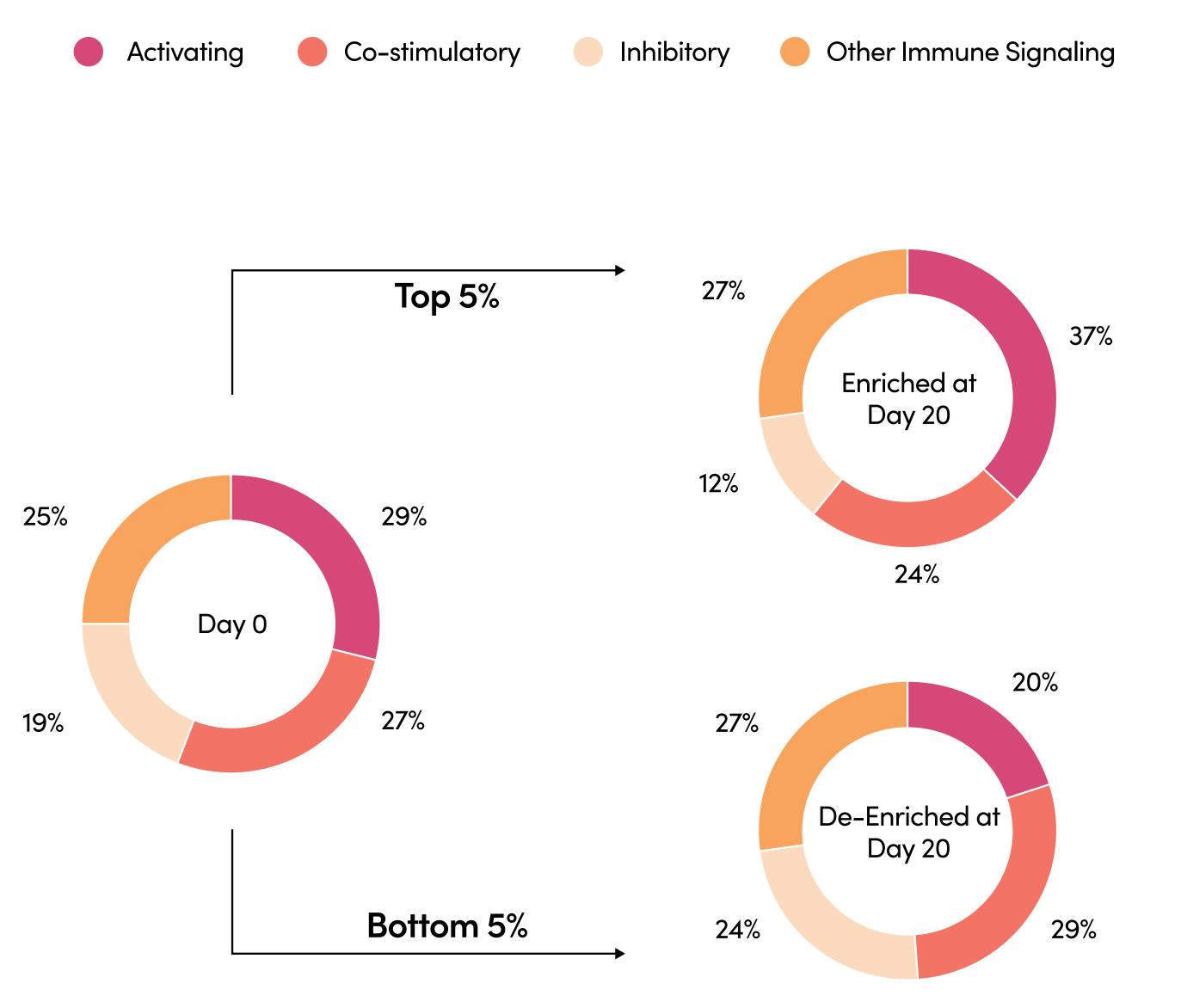
#### **Higher Affinity scFv**



### Lower Affinity scFv

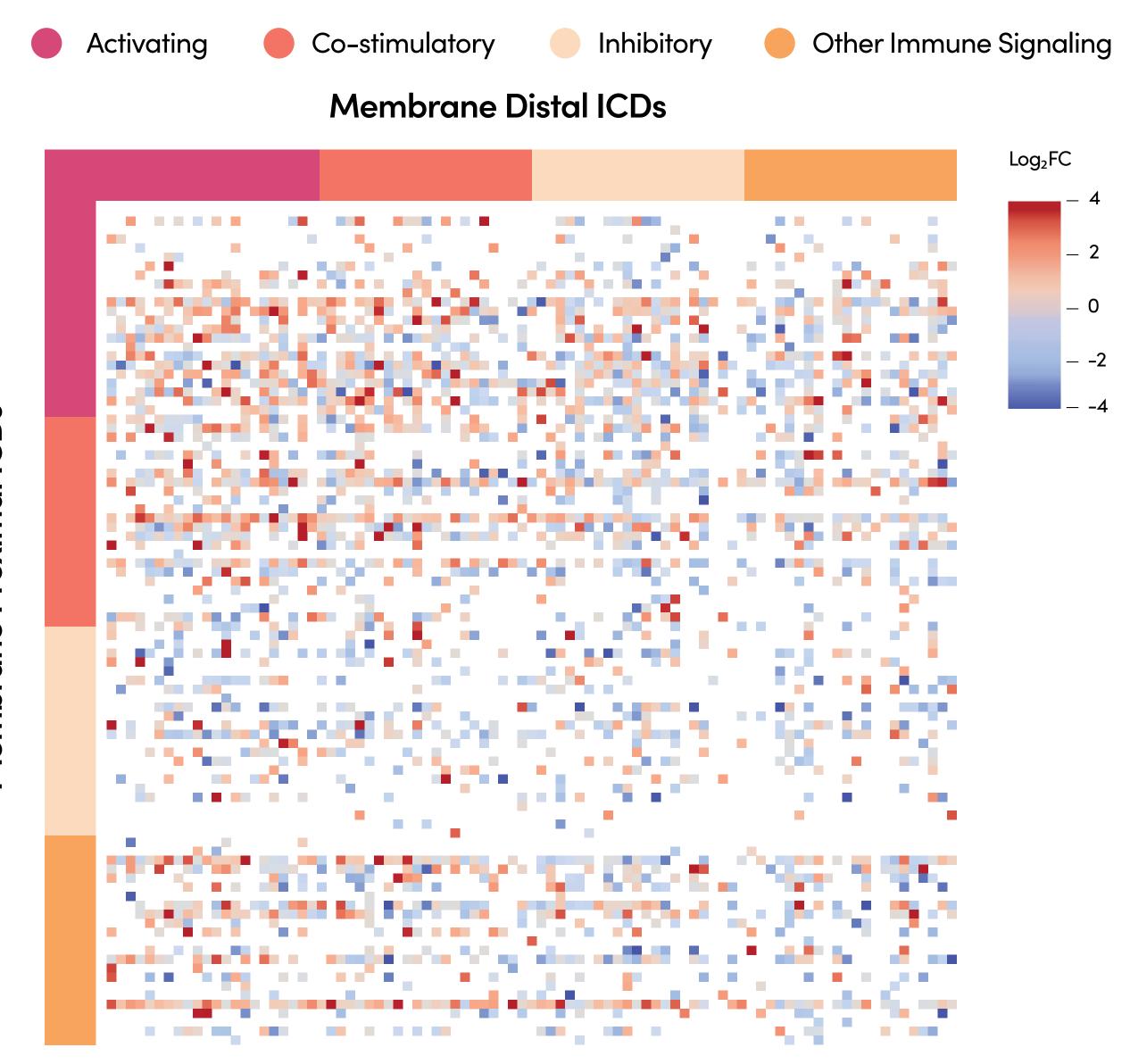


### **Composition of ICD Subtypes**



Along with activating and co-stimulatory ICDs, non-T cell-native ICDs make up novel CAR hits

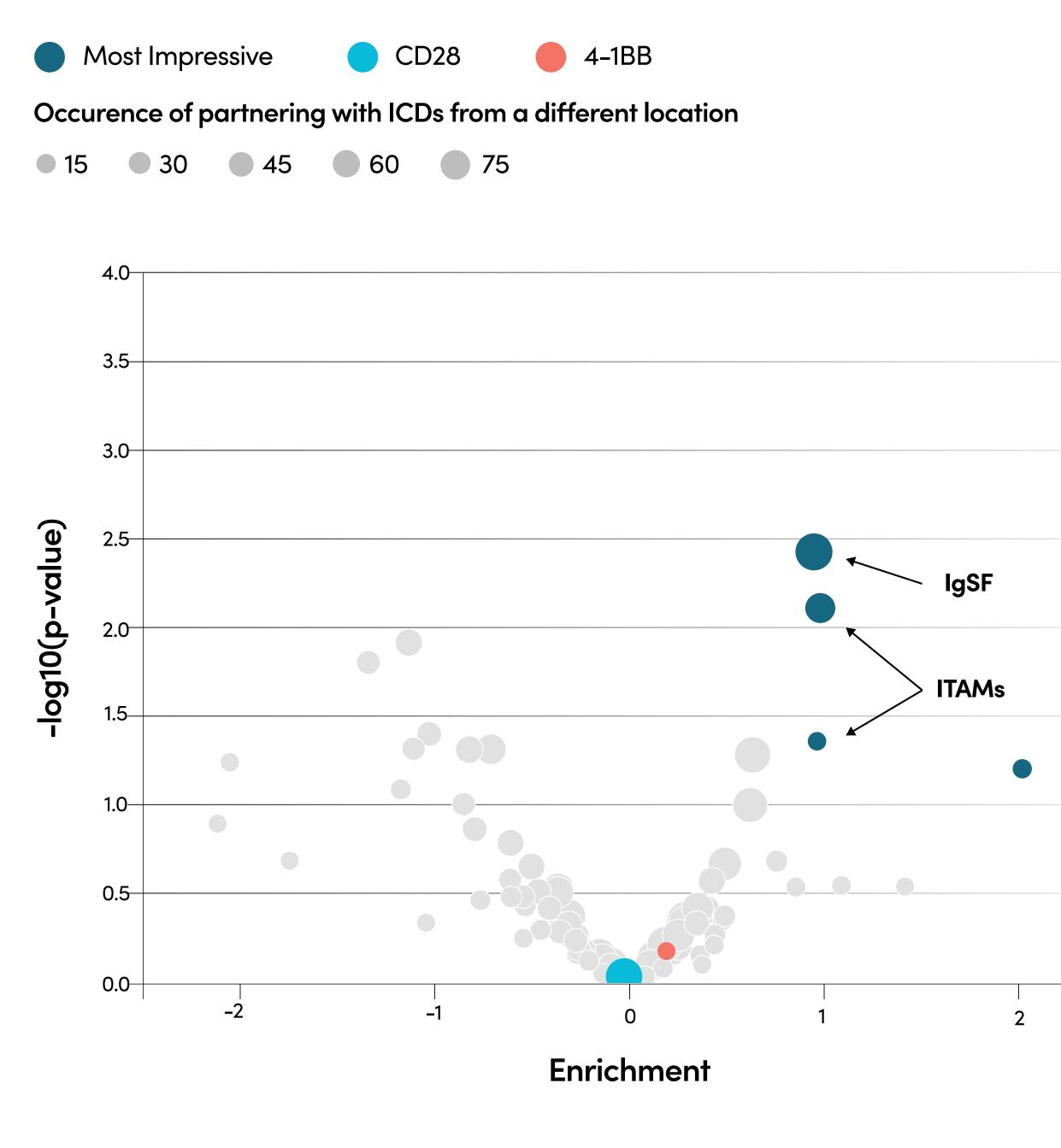
#### **ICD Positional Preference**



Repeated tumor challenge preferentially enriches CARs with T cell activating moieties but not suppressing components

Enriched ICDs from different protein families show universal features at specific intracellular location of CAR

#### ICD Position 1, Membrane-proximal

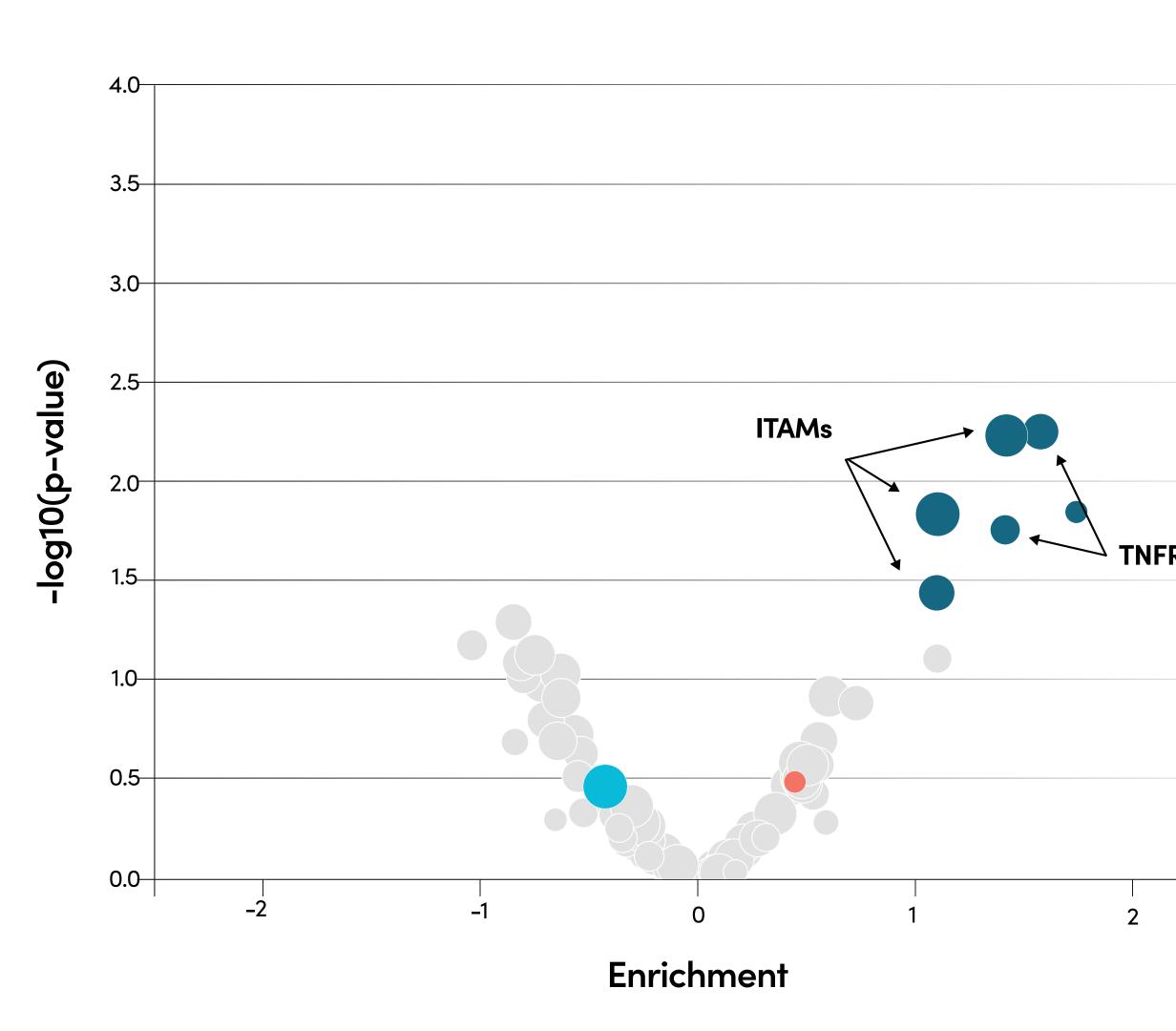


### **ICD Position 2, Membrane-distal**

CD28 **4–1BB** Most Impressive

Occurence of partnering with ICDs from a different location







## Conclusion

- Unbiased pooled screening approach at scale increases the chance of rapidly discovering novel synthetic molecules that drive favorable T cell phenotypes
- 2. Our approach is readily applicable to diverse immune cell types as well as optimizing CAR structural components and armouring strategies in addition to ICDs

# About Ginkgo Bioworks



Ginkgo Bioworks (\$DNA) is the world's leading horizontal platform for cell programming, providing flexible, end-to-end services solving challenges for biopharma companies, from discovery through manufacturing and across diverse therapeutic modalities. Leveraging cutting edge automation, large scale DNA synthesis, high throughput analytics, and machine learning, Ginkgo's platform enables partners to explore biological design space to discover and optimize their targets and processes in mammalian and microbial cells, with applications in cell, gene, RNA, microbiome and biologic therapeutic modalities.