



IL-18 Surrogate Cytokine Agonists (SCAs): Overcoming Limitations of IL-18 Cancer Immunotherapy

Sandro Vivona, PhD

Senior Director of Biochemistry and Biophysics

SyntheKine, Inc.

PEGS May 2024



Our vision: harnessing the potential of cytokine therapeutics

Solving the pleiotropy of cytokines to develop promising immunotherapies

Founded in 2019 based on pioneering research by Chris Garcia (Stanford)



Named 2022 Fierce15 biotech

World-class team and \$290M from leading investors

Partnerships with top pharmaceutical companies



Deep pipeline of cytokine therapeutics and cytokine-enabled cell therapies

	Disc. Stage	Pre-Clin.	IND Prep.	Phase 1/2
Oncology				
STK-012 (IL-2)	<div></div>			
STK-026 (IL-12)	<div></div>			
SYNCAR-001 (CD19 CAR-T) + STK-009 (IL-2)	<div></div>			
SYNCAR-002 (GPC3 CAR-T) + STK-009 (IL-2)	<div></div>			
Other programs	<div></div>			
Autoimmune/Inflammation				
SYNCAR-001 (CD19 CAR-T) + STK-009 (IL-2)	<div></div>			
IL-10 	<div></div>			
IL-22	<div></div>			
Undisclosed 	<div></div>			
Other programs	<div></div>			

Three-pronged approach to engineering cytokines



Cytokine Partial Agonists



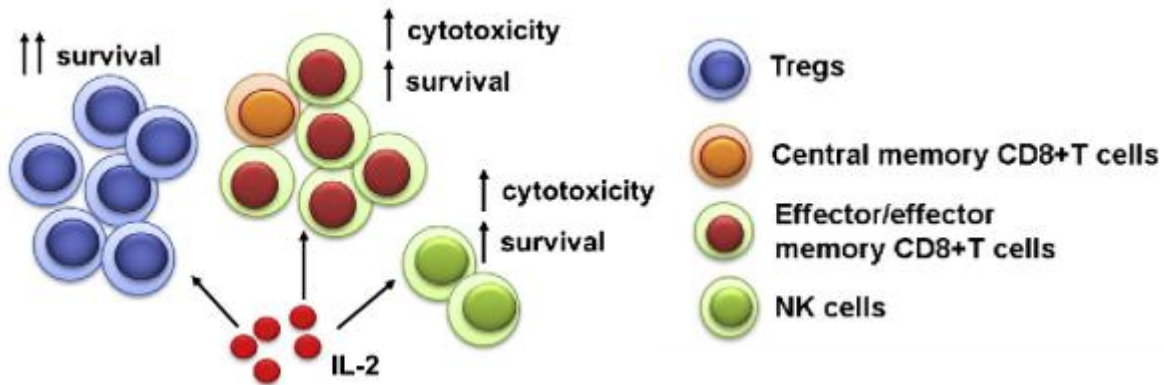
Orthogonal Cytokine Cell Therapy



Surrogate Cytokine Agonist

Cytokine therapeutics: limitations and potential

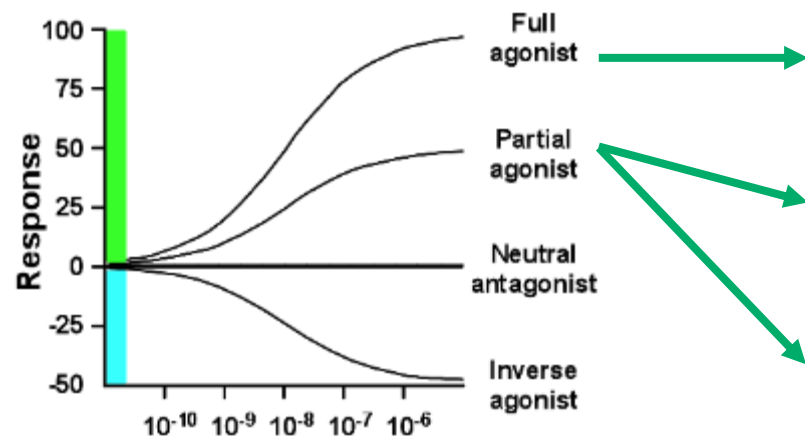
- **Approved drugs:** IL-2, type I IFNs, EPO, HGH, G-CSF, IL-15
- **In clinic:** IL-2 muteins, IL-12, IL-22, IL-18, IL-10, IL-7
- **Cytokine agonism within the immune system is pleiotropic**
leads to both positive and negative effects → partial agonism to decouple efficacy and toxicity



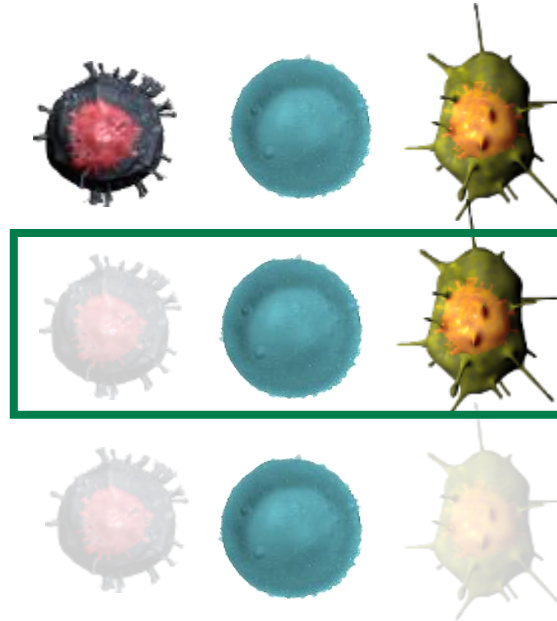
For example, the broad effects of IL-2 on multiple cell types limit therapeutic potential of wild type molecule

Partial agonism of engineered cytokines can elicit unique therapeutic properties

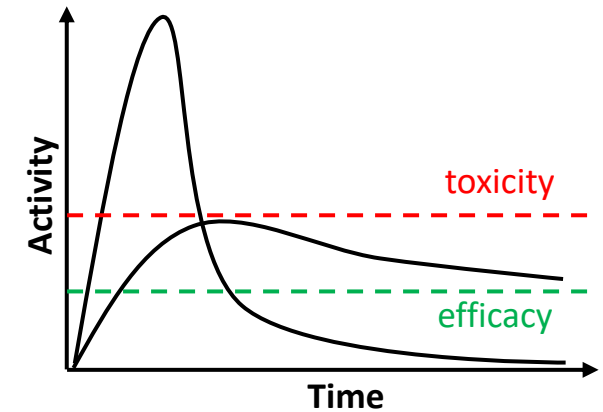
Engineering of wild type cytokine to alter response



Selection of optimized lead based on signaling in specific cell type(s)



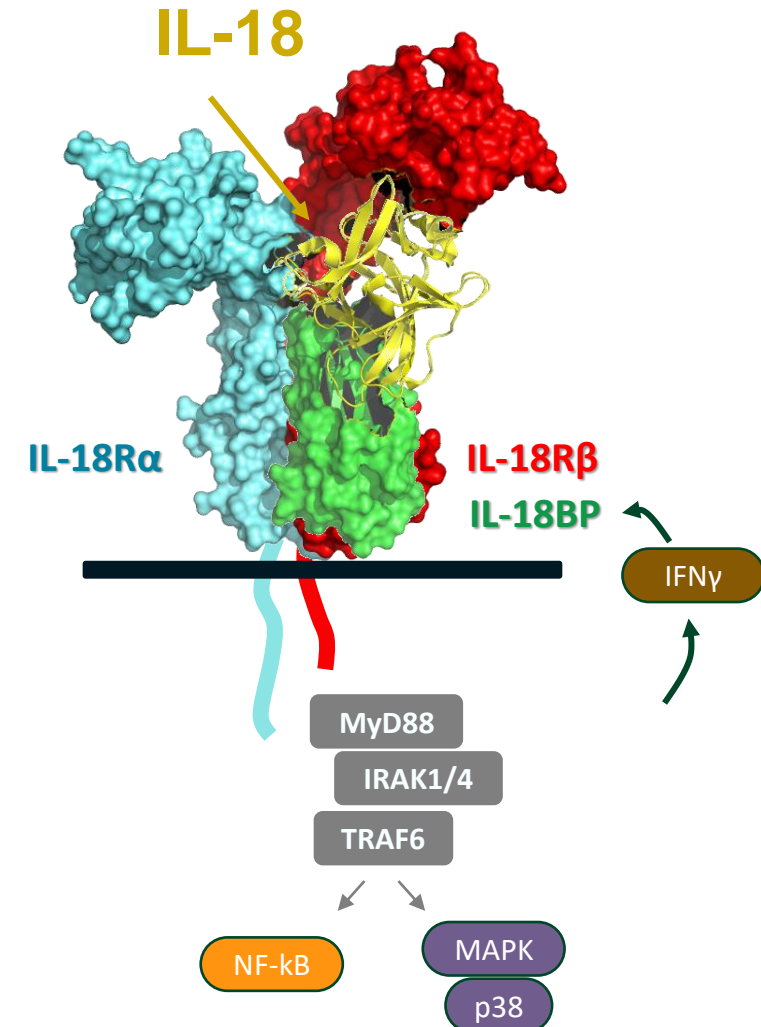
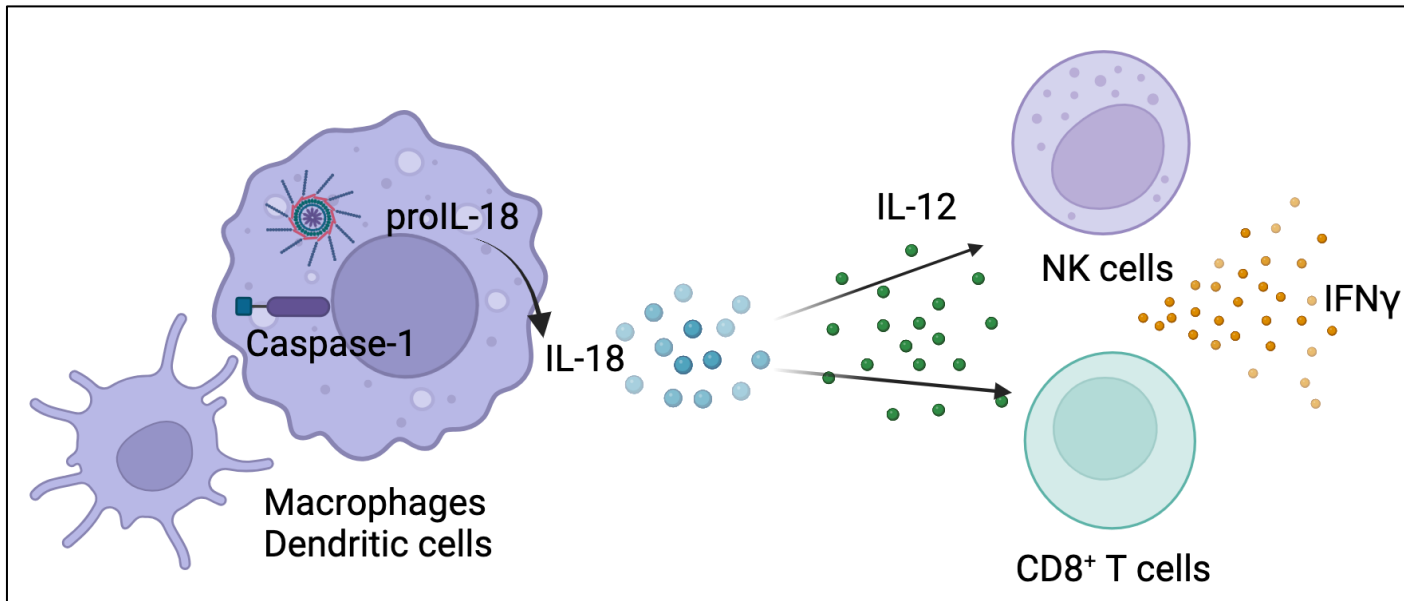
Potency tuning and half-life extension



- *Engineered cytokine partial agonists enable targeted activity on cell subtypes with high receptor expression and mitigation of adverse events*

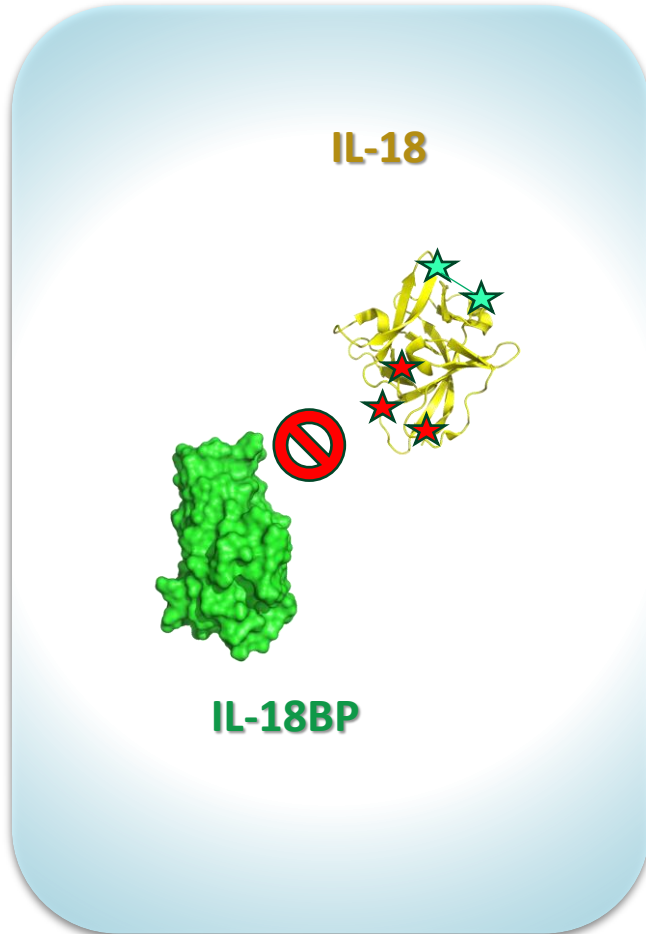
Can IL-18 broad immunostimulatory activity be leveraged in cancer therapy?

- IL-18 can boost both innate and adaptive anti-cancer immune responses by signaling on a broad range of cells
- IL-18 proinflammatory activity can alter the immunosuppressive state of the tumor micro-environment
- As a single agent in the clinical trials, IL-18 has been well tolerated but not efficacious, likely due to IL-18BP inhibition

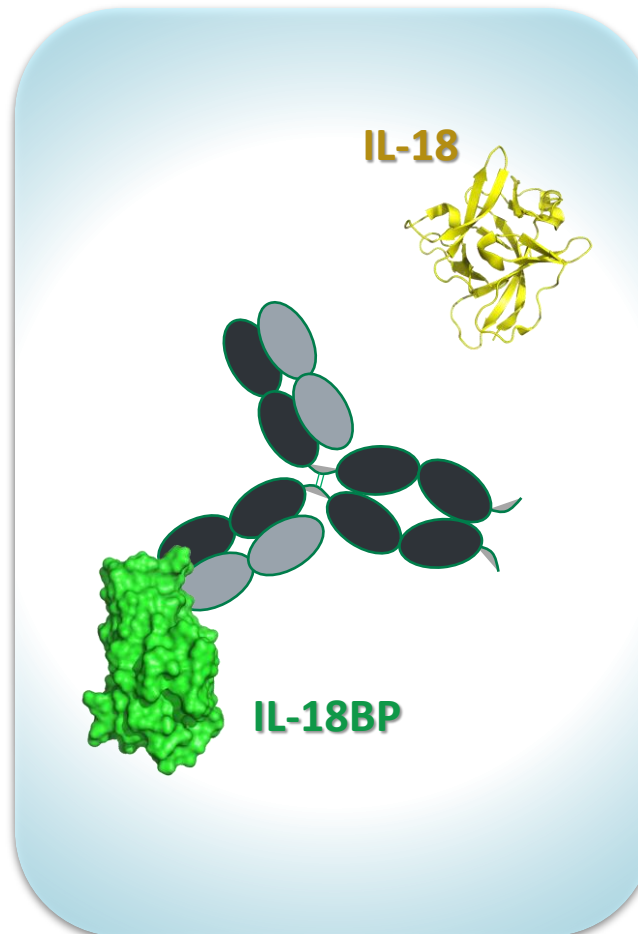


Approaches to overcome IL-18BP inhibition

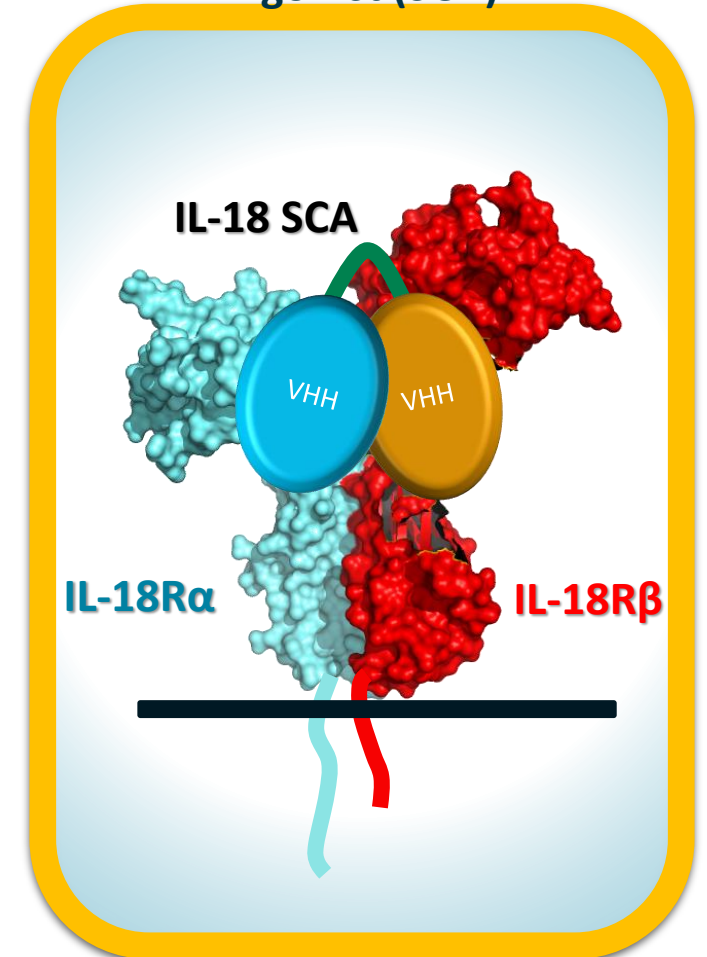
Stabilize IL-18 and disrupt binding to IL-18BP



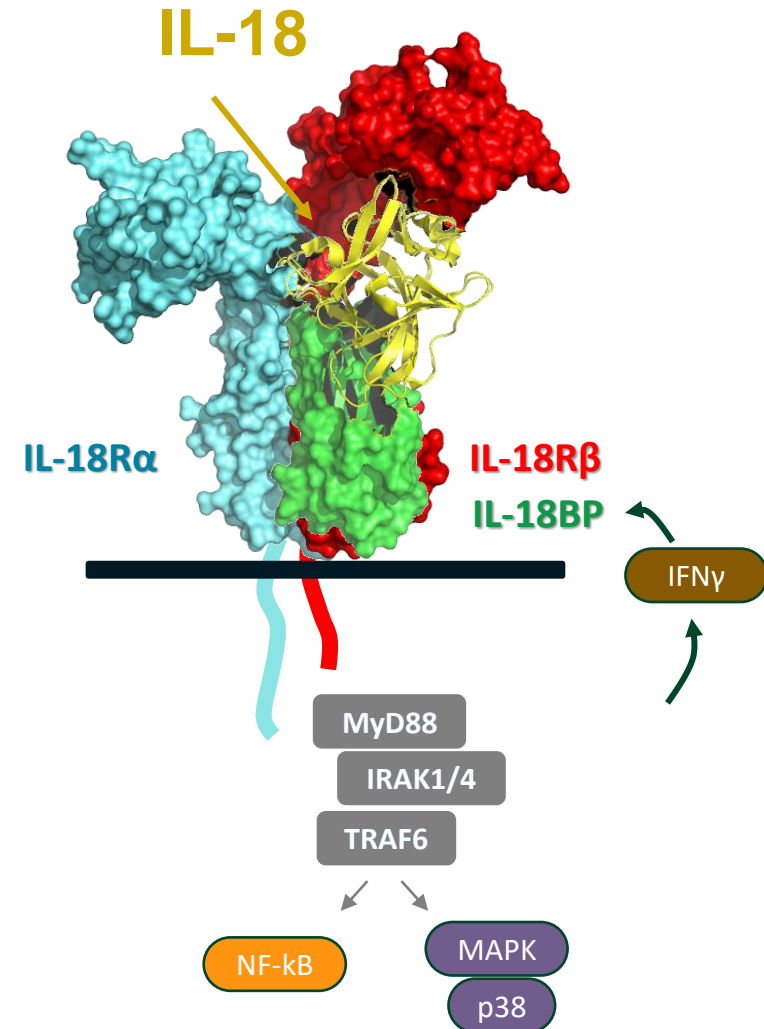
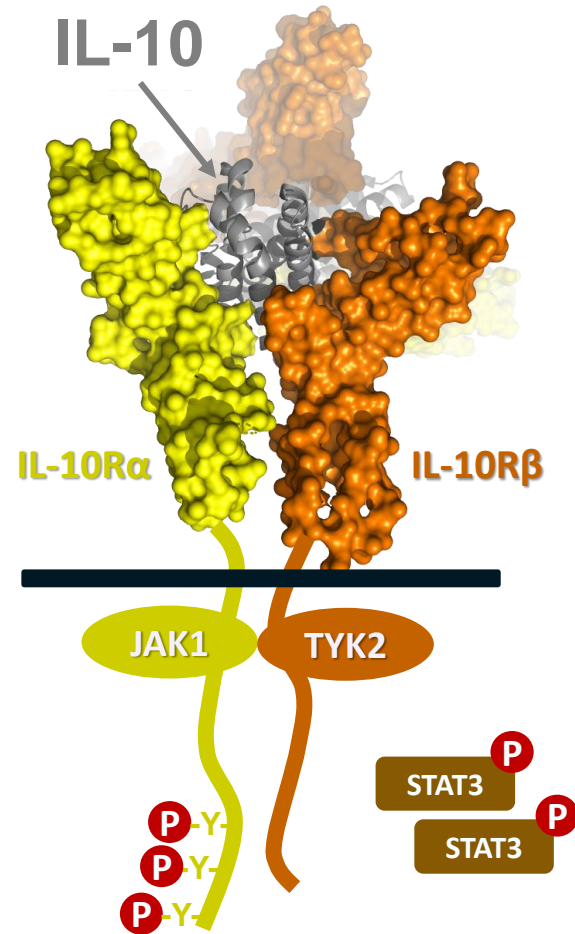
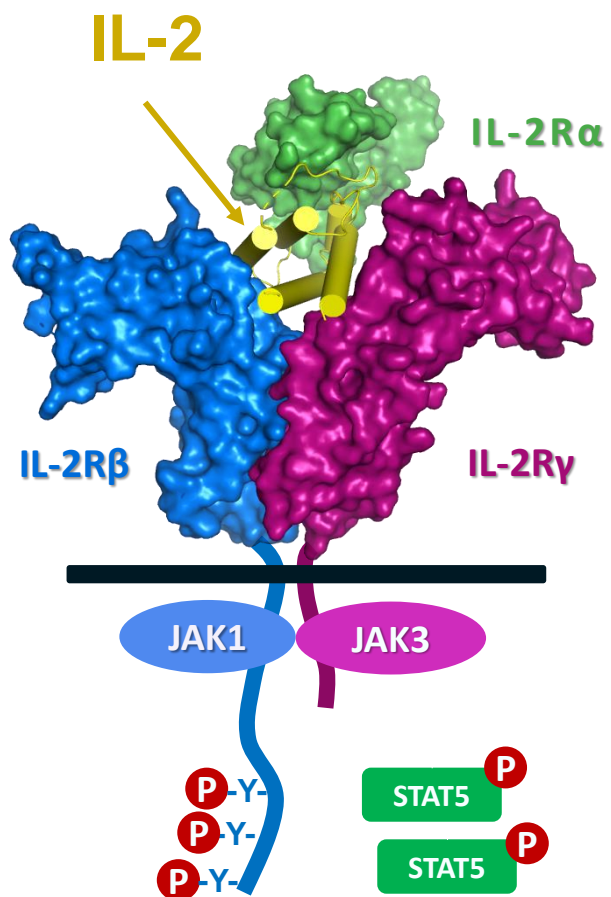
Block IL-18BP with therapeutic mAbs



Surrogate Cytokine Agonist (SCA)

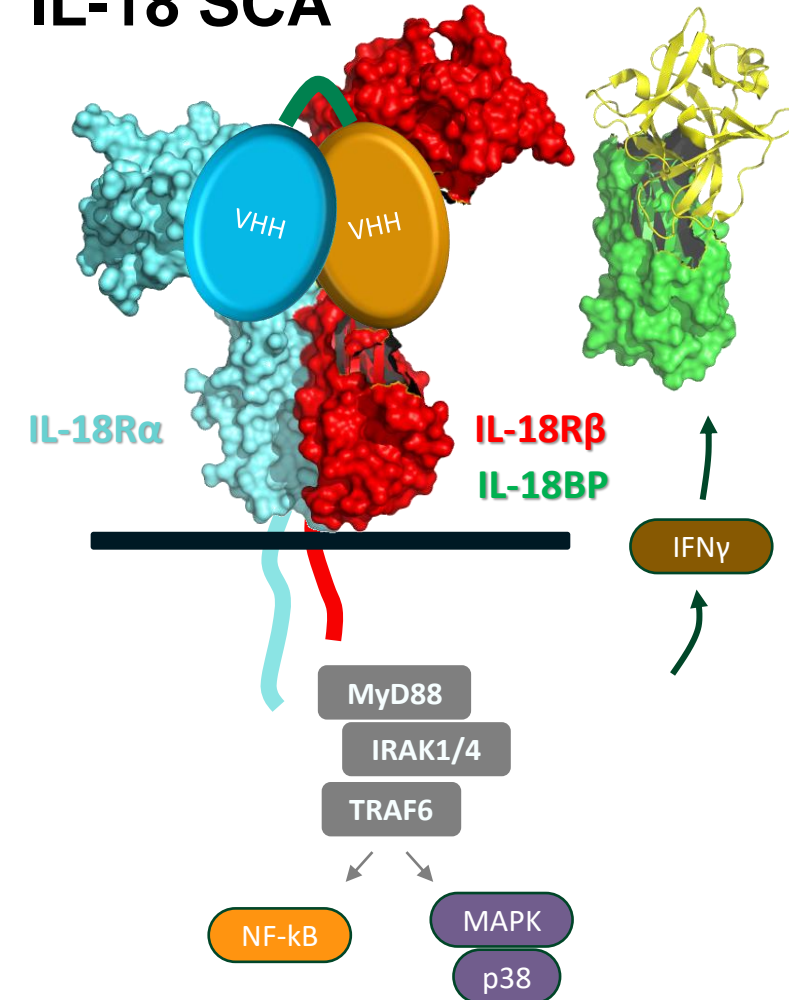


Cytokines enable diverse signaling pathways via pairing of receptors



An IL-18 SCA could stimulate the IL-18 pathway and avoid inhibition by IL-18BP

IL-18 SCA



“Med Chem” approach to discovery of SCAs at Synthekine

Discover/select individual binders

- Llama immunization
- Yeast/phage display with cytokine receptor
- Sequence and sort binders (VHH)
- Biophysical Characterization funnel

Generate panels of dimers

- Produce panels containing an all-by-all matrix of VHH dimers
- Express/purify protein

In vitro screening for desired signaling activity or cell type bias

- ### Tune activity
- linker engineering
 - Valency (Fc)
 - Half life extension

**Advance to
in vivo PoC**

Human IL-18R β
0.1-185 nM, 4 epitope bins

C-terminus →

	VHH 1	VHH 2	...	VHH 12
VHH 1				
VHH 2				
...				
VHH 7				

84 Dimers of varying selectivity / activity

X

Human IL-18R α
0.3-26 nM, 3 epitope bins

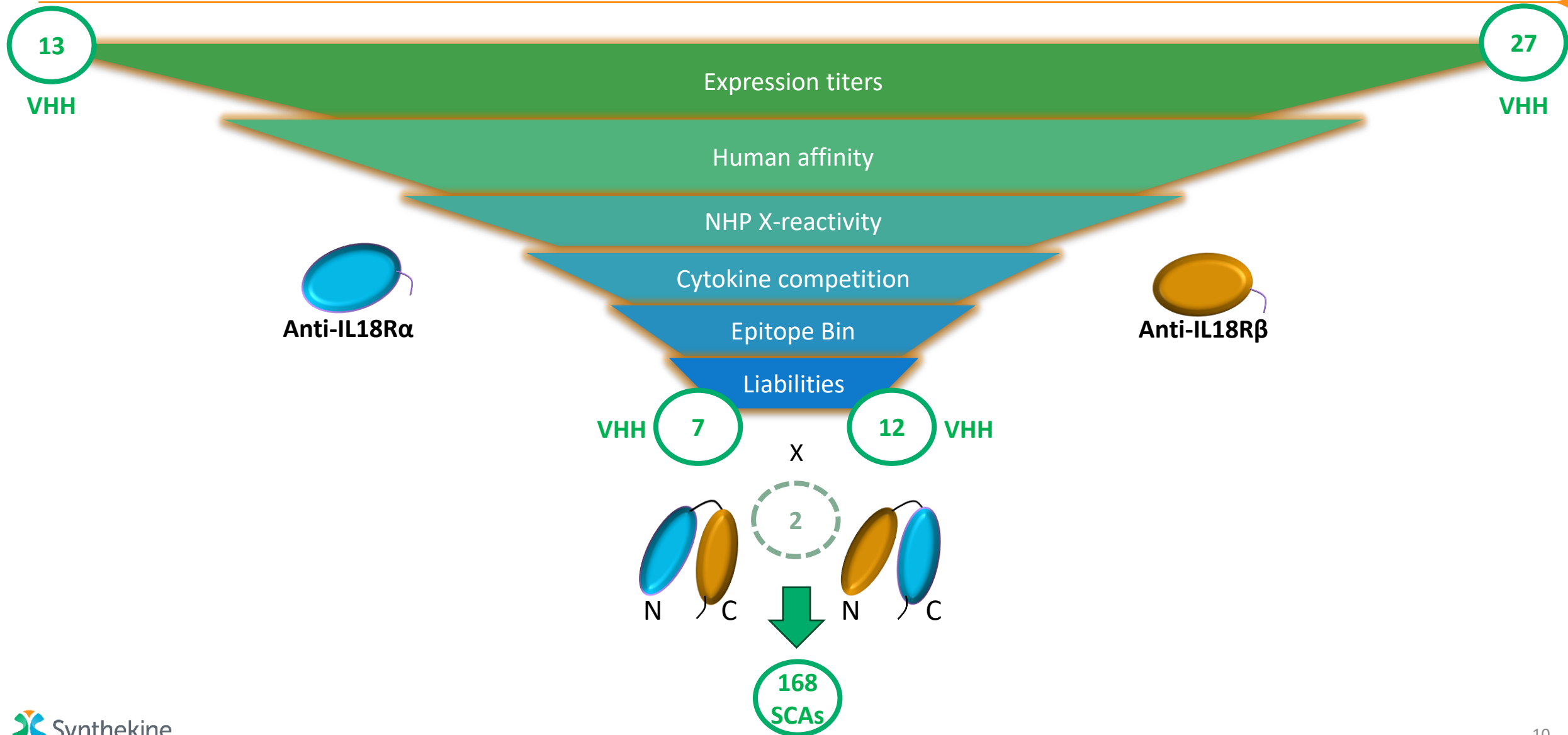
C-terminus →

	VHH 1	VHH 2	...	VHH 7
VHH 1				
VHH 2				
...				
VHH 12				

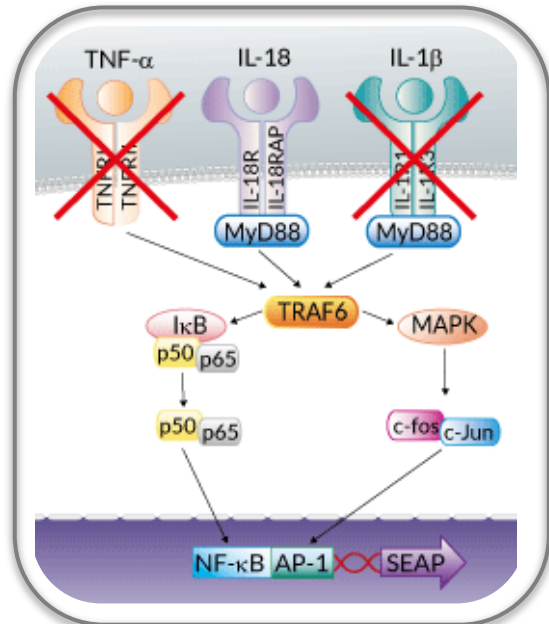
84 Dimers of varying selectivity / activity

**= 168 human
IL-18 SCAs
for screening**

VHH biophysical funnel leads to the selection of a functional screening panel

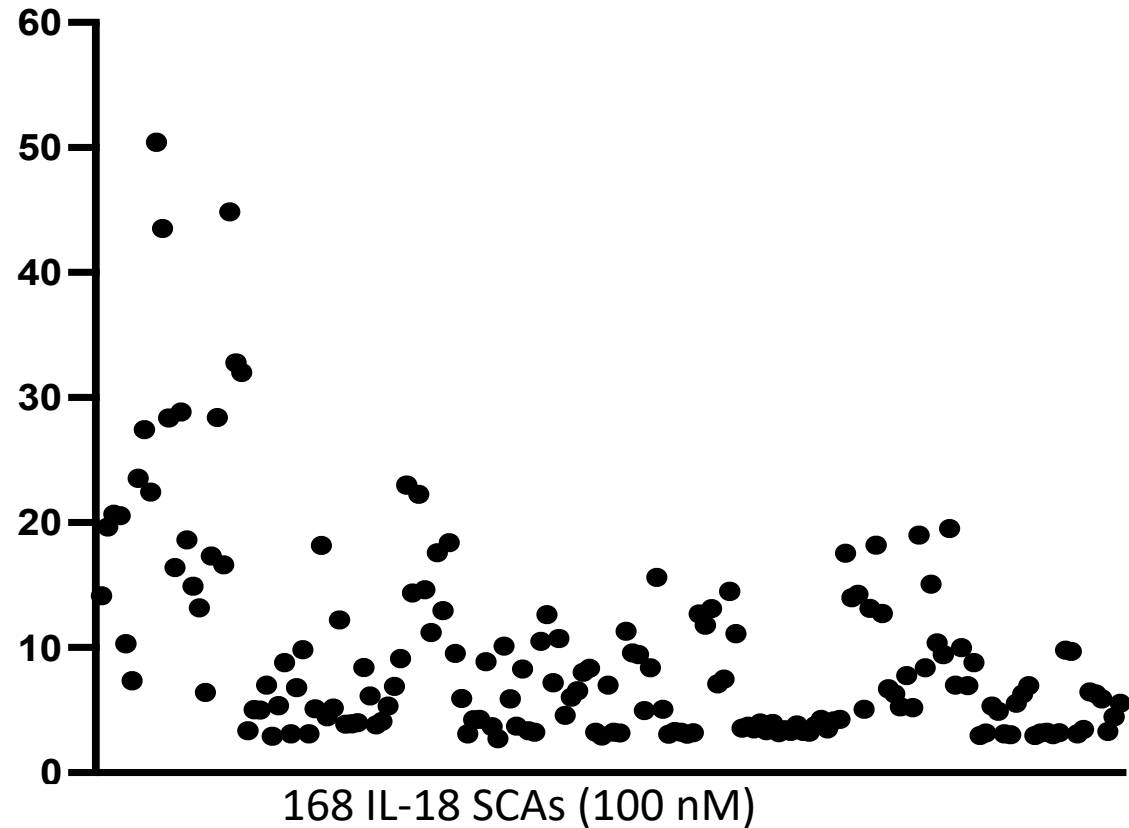


30% of IL-18 SCAs show detectable NF- κ B signaling in HEK-Blue reporter assay

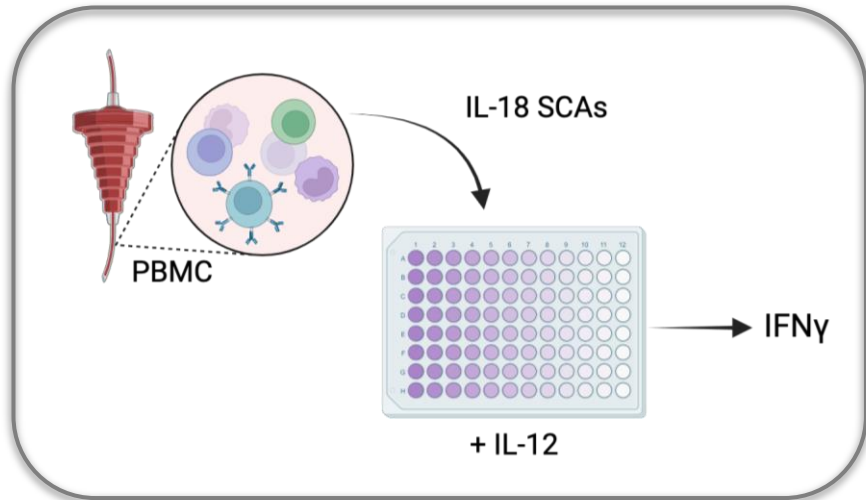


NF- κ B/AP-1 \rightarrow Secreted Embryonic Alkaline Phosphatase (SEAP).
IL-18 / IL-18 SCA, 24 hours
(Quanti Blue absorbance)

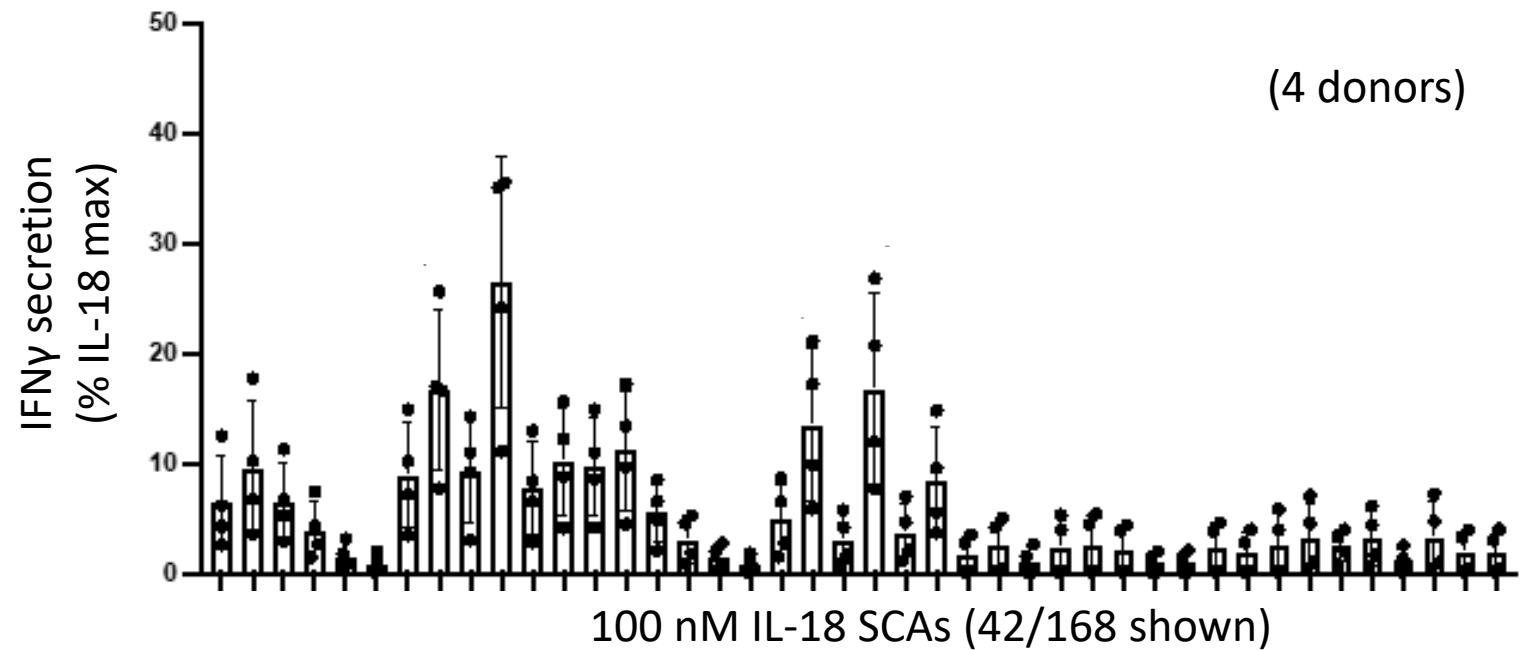
NF- κ B reporter activity
(% IL-18 max)



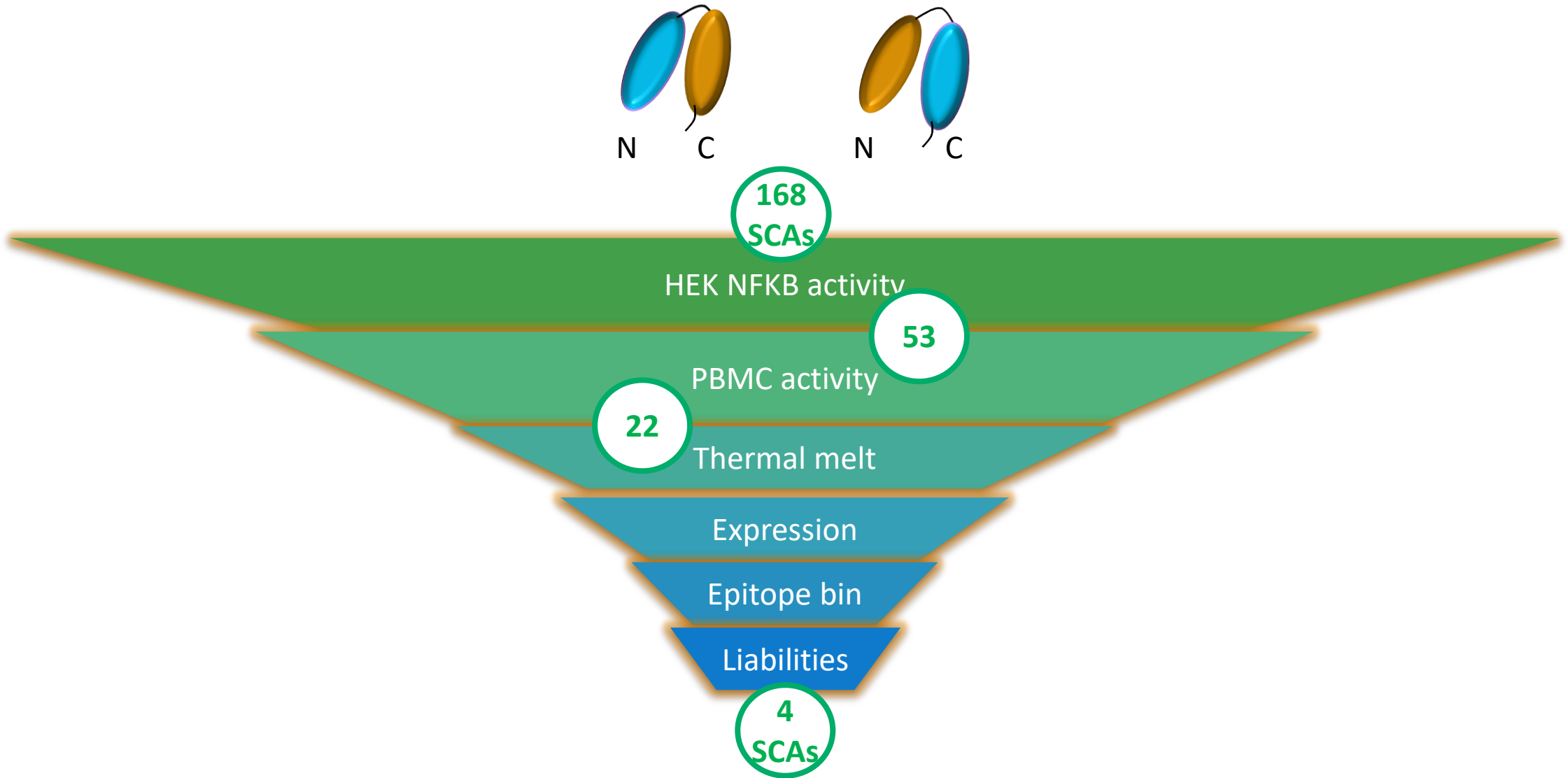
13% of IL-18 SCAs induce IFN γ secretion on human PBMCs



human PBMC incubated 24 hours with IL-18 or IL-18 SCA in the presence of 10 ng/mL IL-12. IFN γ concentration in supernatant measured by MSD and shown as % of IL-18 wt induced secretion.



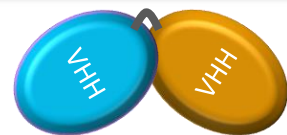
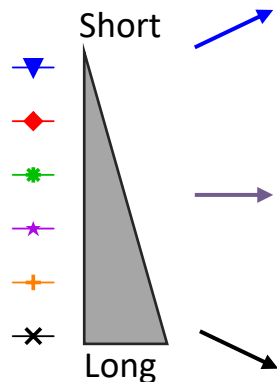
Dual VHH screening funnels lead to the selection of 4 SCAs



Protein engineering can tune IL-18 SCA potency and partial agonism

Linker length

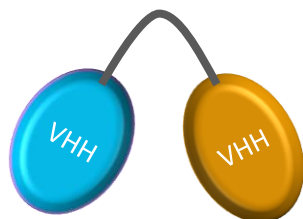
SCA1
Linker length:



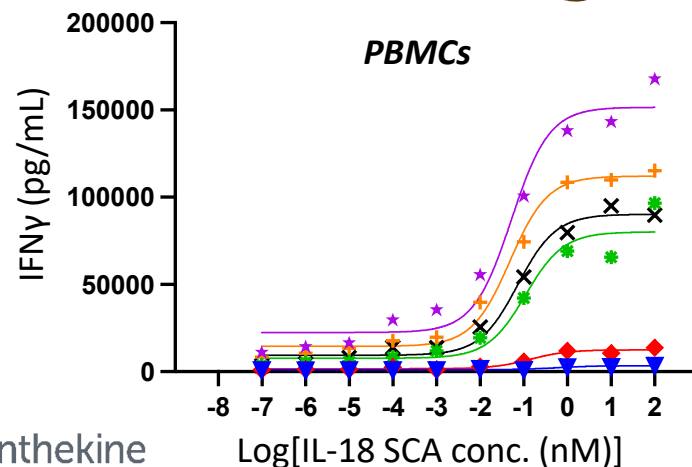
✗ Restrained geometry



✓ Maximum activity

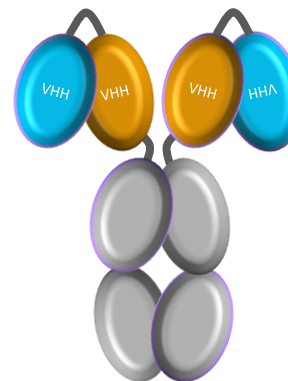


✓ Loose engagement

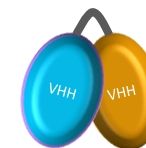


Format

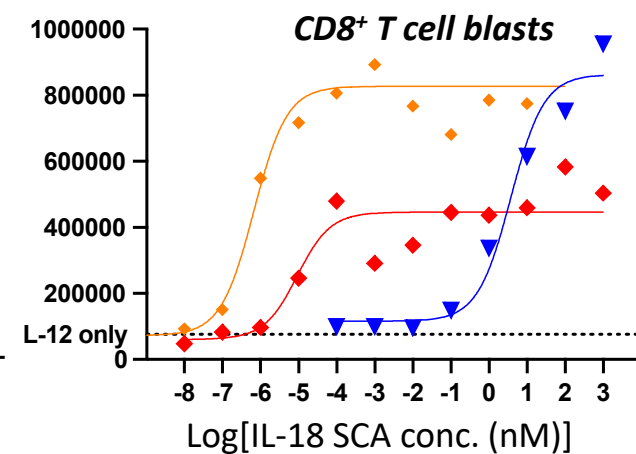
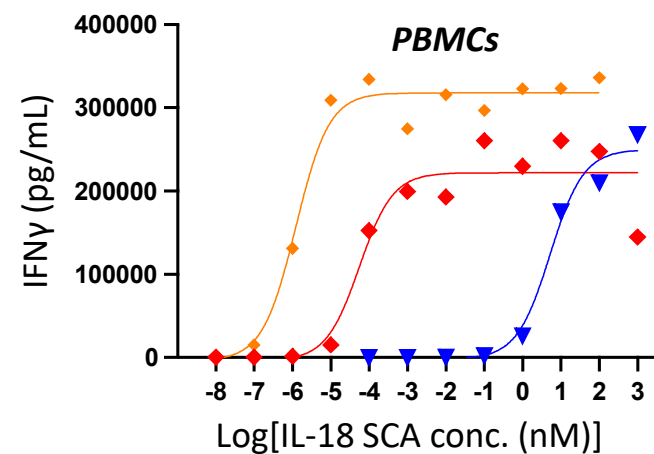
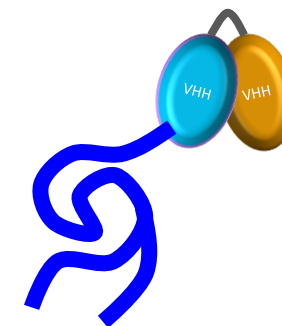
— SCA1-Fc



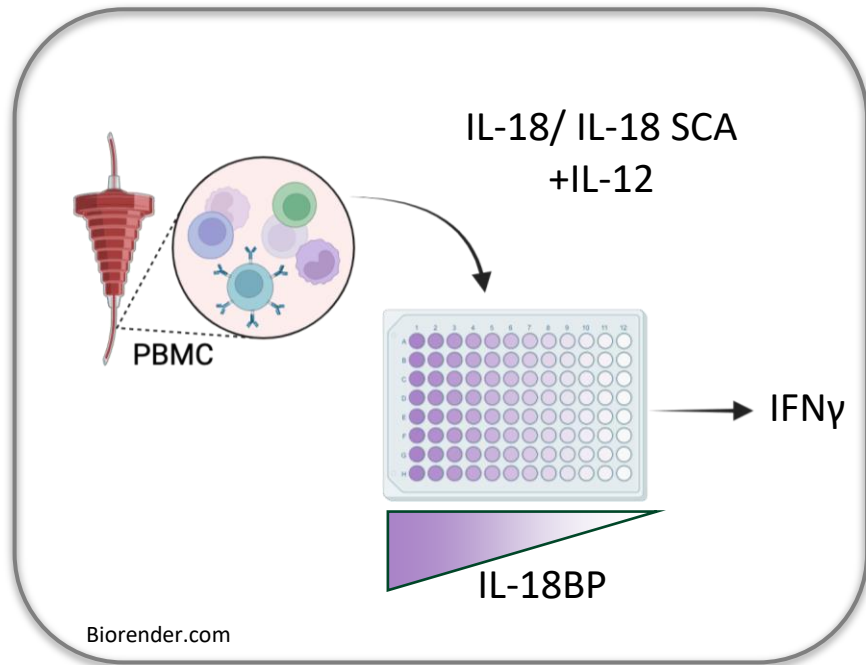
— SCA1



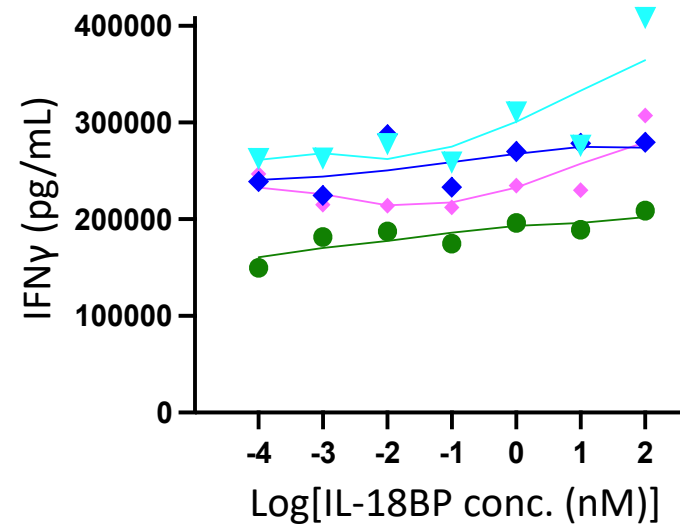
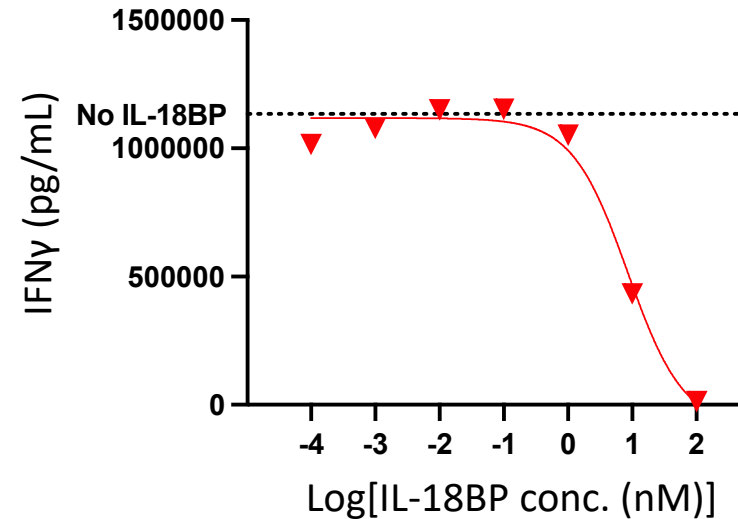
— SCA1-PEG



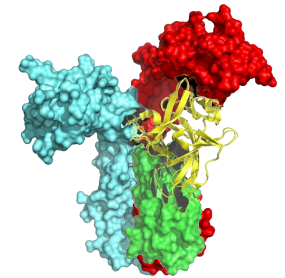
Unlike IL-18, IL-18 SCAs are not inhibited by IL-18BP



Human PBMCs incubated 48 hours with 10 nM IL-18 or 100 nM IL-18 SCA in the presence of 10 ng/mL IL-12 and **variable concentrations of IL-18BP**. IFN γ concentration measured by MSD



▼ IL-18

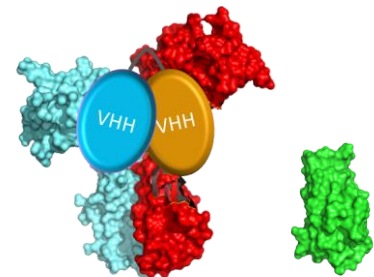


▼ SCA1

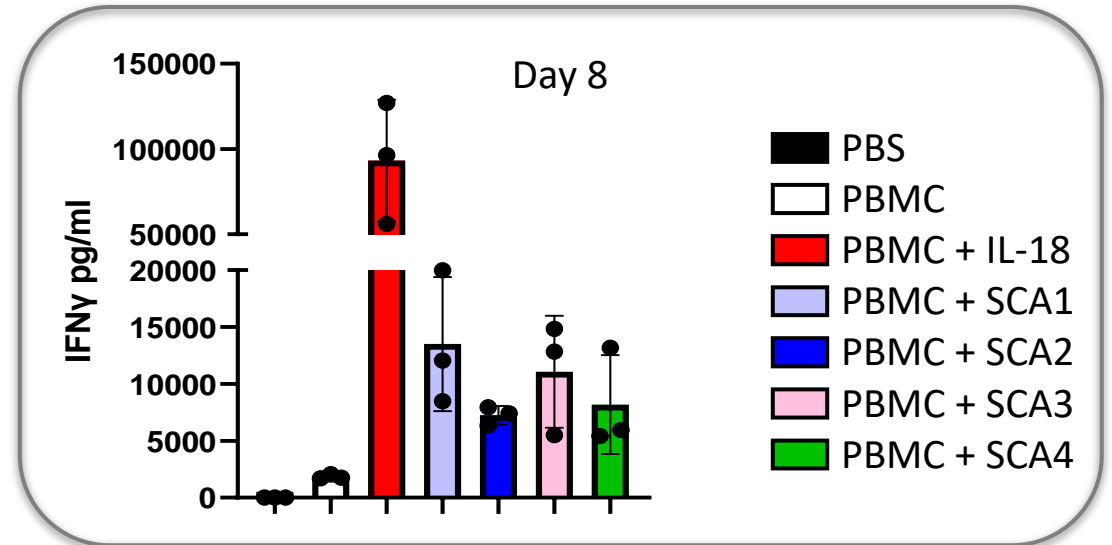
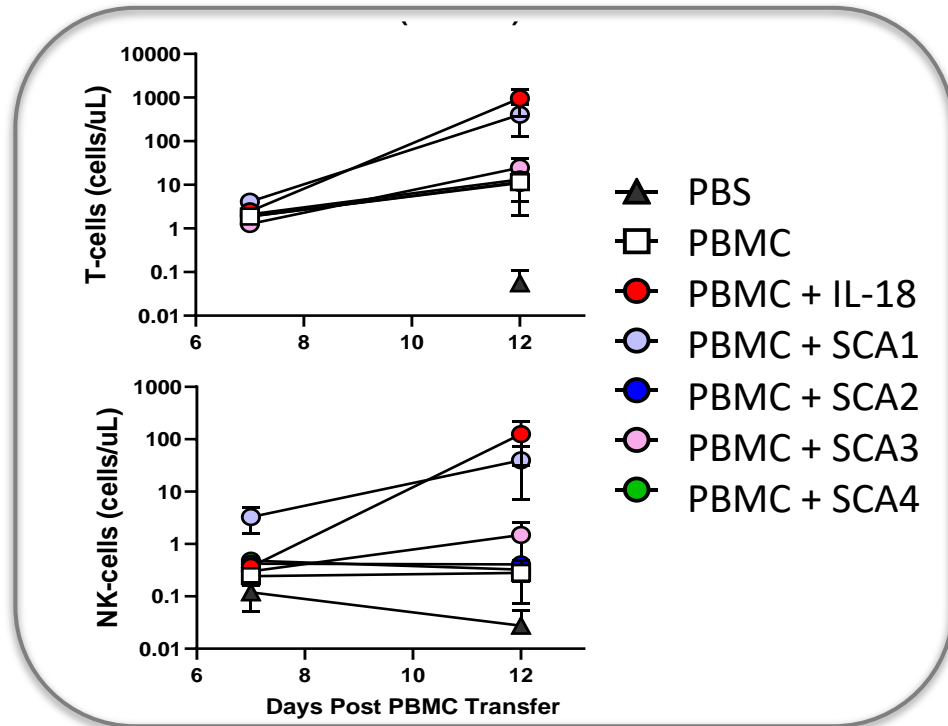
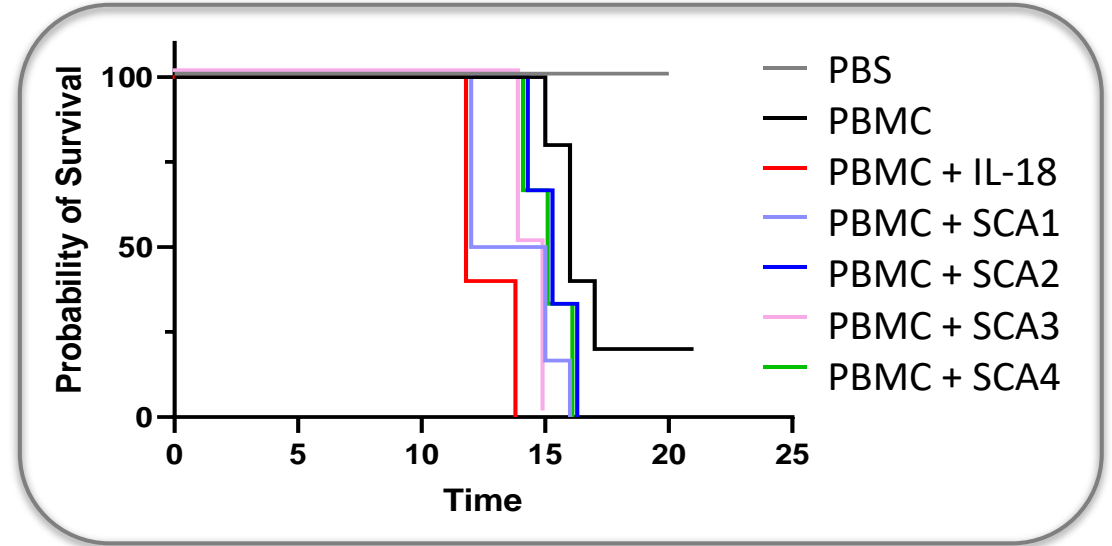
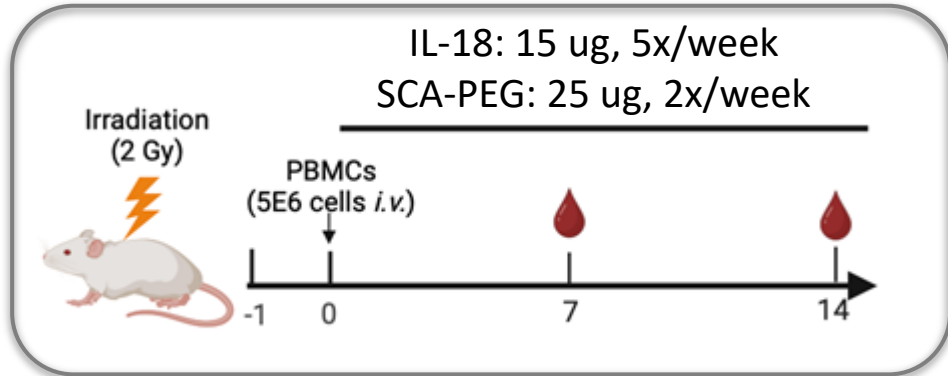
▼ SCA2

▼ SCA3

▼ SCA4

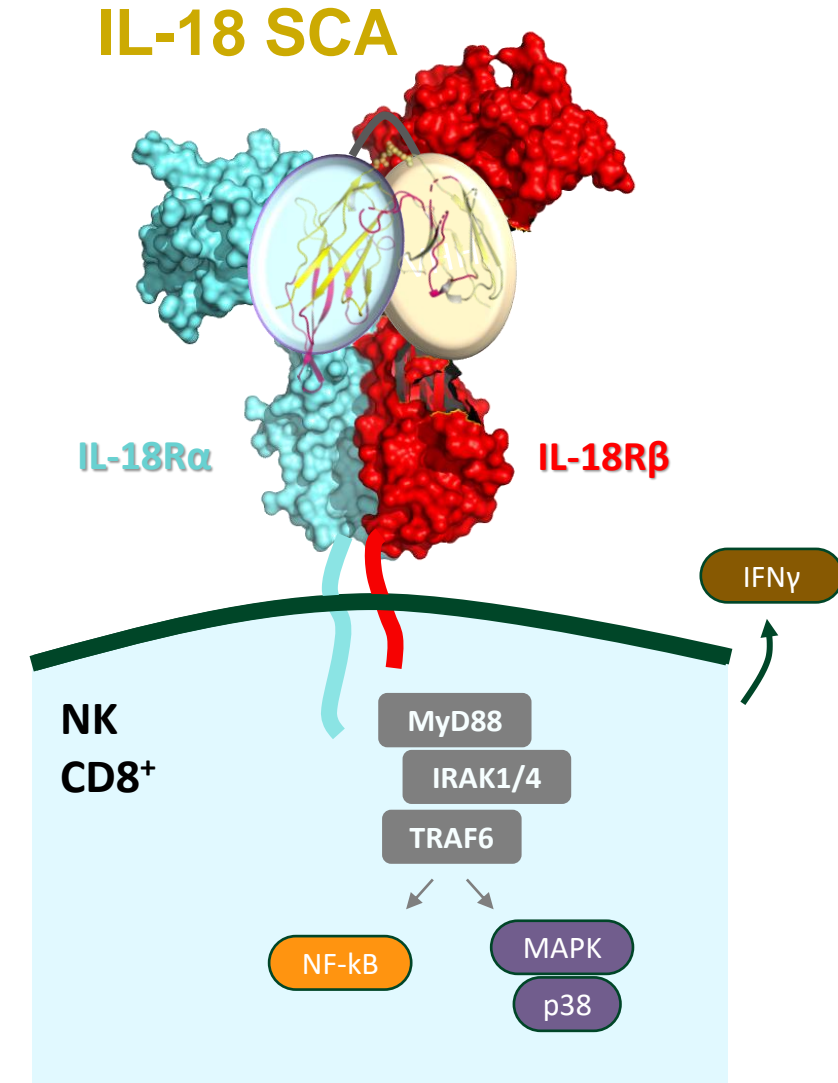


IL-18 SCAs activate human NK and T cells in NSG mice and accelerate GvHD



Conclusions

1. Surrogate cytokine agonists (SCAs) composed of two receptor binding VHH can mimic cytokine signaling
2. Combinatorial screens of VHH pairs and format engineering can identify leads with a wide range of agonist activity
3. IL-18 SCAs expand and activate NK and CD8⁺ *in vitro* and *in vivo*, while bypassing the IL-18BP checkpoint and addressing poor drug-like properties of IL-18





Harnessing the power of cytokines

with a **world class team** and using **multiple engineering platforms** to build novel, selective cytokine therapeutics for cancer and inflammatory diseases as part of a **rapidly maturing pipeline** with **emerging partnerships**

Acknowledgements

Raphael Trenker, Verenice Paredes, Deepti Rokkam, Priyanka Balasubrahmanyam, PJ Aspuria, Ethan Jung, Helena Silva, Zhenya Koliesnik, Kim Tran, David Rosen, Kunal Patel, Patrick Lupardus, Martin Oft

