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IL-10 surrogate cytokine agonists (SCAs) tune receptor signaling and engineer cell type specificity

Martin Oft, MD
Chief Scientific Officer
Synthekine, Inc.
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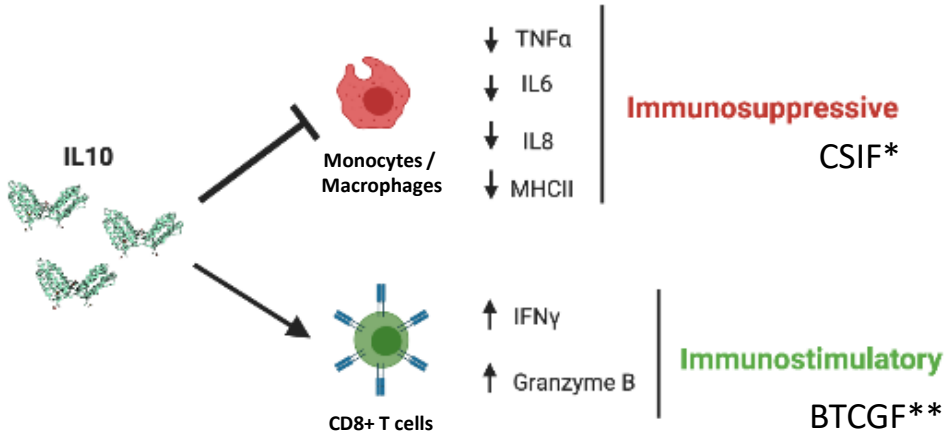
IL-10 Therapeutic Rationale for Inflammatory Disease

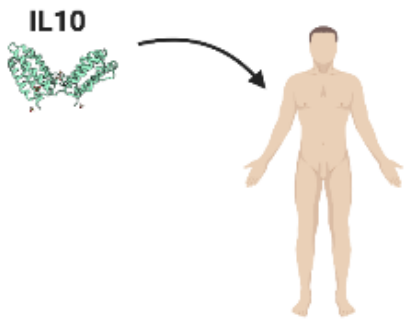


* Cytokine Secretion Inhibitory Factor
** B cell derived T cell growth Factor

Crohn's Disease

Ulcerative Colitis

Rheumatoid Arthritis



Efficacy in early clinical studies, but also side effects from T cell activation:

- *IFNγ* ↑
- *Macrophage Activation Syndrome / HLH*
- *CD163, CD64 FcgR1 scavenger receptors* ↑
- *Anemia / Thrombocytopenia*

Trials stopped

- IL-10 is genetically associated with IBD, RA, etc.
  - IL-10 deficient individuals develop severe very-early-onset IBD
- IL-10 is potently anti-inflammatory and was a promising therapy for autoimmune disease in the 1990's (Schering Plough)
  - Daily admin of WT IL-10 showed signs of efficacy (IBD, psoriasis, etc)
  - Anemia and thrombocytopenia limited exposures
  - Higher doses induced IFNγ and proinflammatory signals
  - Clinical investigation was stopped
- PEGylated IL-10 induces CD8 T cell mediated tumor rejections and increases IFNγ, Granzymes and proinflammatory cytokines (Armo / Lilly)
  - G3 anemia and thrombocytopenia (Monotherapy 27% ORR in RCC)

➢ T cell activation and anemia prevent anti-inflammatory efficacy of IL-10; An IL-10 ***without*** T cell activation may…
  - ➢ avoid IFNγ and hemophagocytosis and hematologic AEs (anemia)
  - ➢ allow for sustained exposures to drive anti-inflammatory effects

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HLH = hemophagocytic lymphohistiocytosis

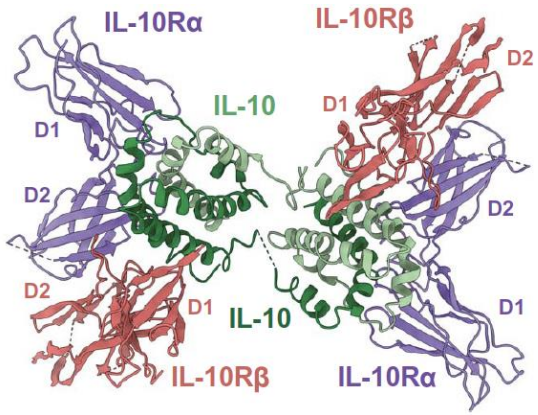
Engineering IL-10 for Reduced IL-10Rβ Binding Creates Myeloid Biased IL-10v

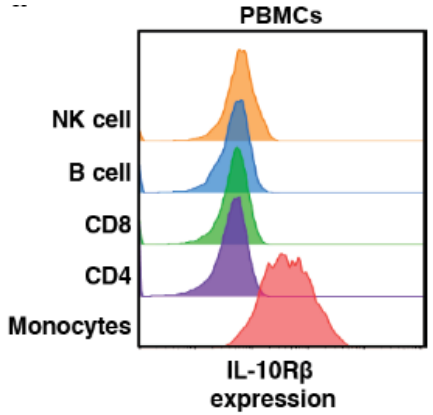
RESEARCH ARTICLE

IMMUNOLOGY

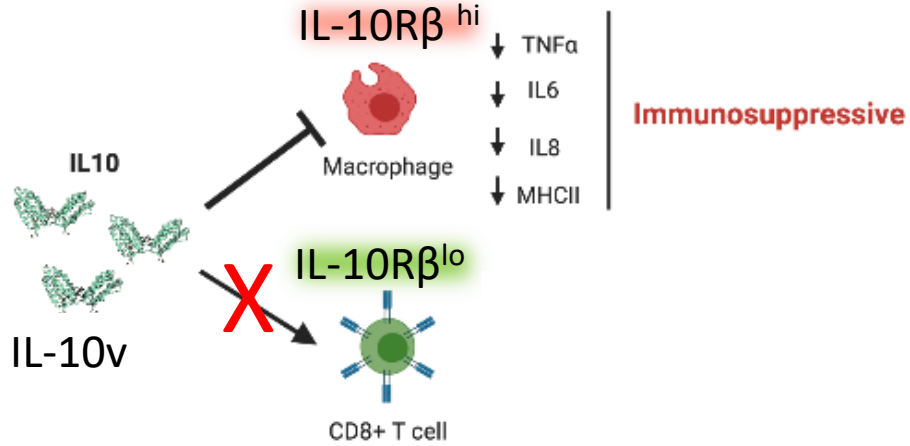
Structure-based decoupling of the pro- and anti-inflammatory functions of interleukin-10

Robert A. Saxton[1,2], Naotaka Tsutsumi[1,2], Leon L. Su[1], Gita C. Abhiraman[1,3], Kritika Mohan[1]*, Lukas T. Henneberg[1], Nanda G. Aduri[4,5], Cornelius Gati[4,5], K. Christopher Garcia[1,2,4]†

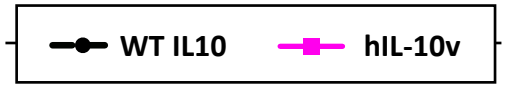




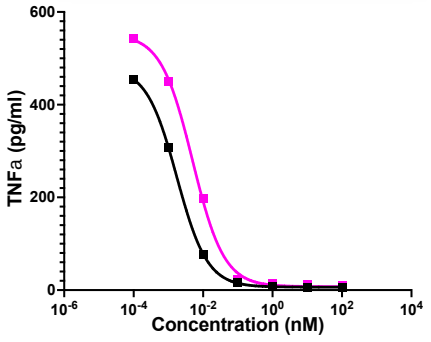
- Synthekine generated panel of myeloid-biased mutants
- IL-10v, has a single point mutation and reduced IL-10Rb binding



WT IL-10 vs IL-10v:

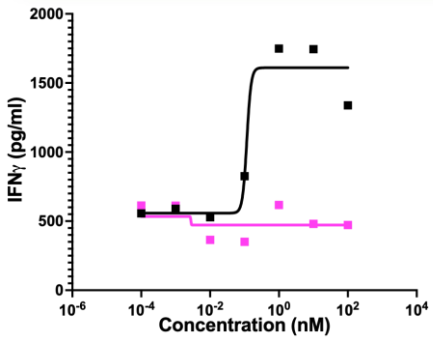


LPS act. Monocytes



Similar data obtained for IL-1β, IL-6

Activated CD8+ T cells



Similar data obtained for GzmA, GzmB

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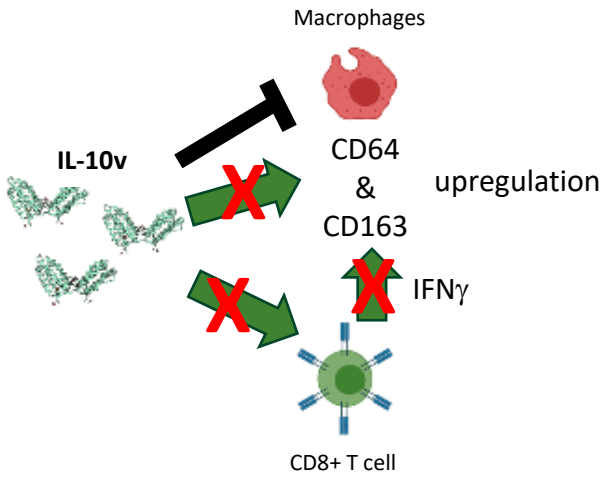
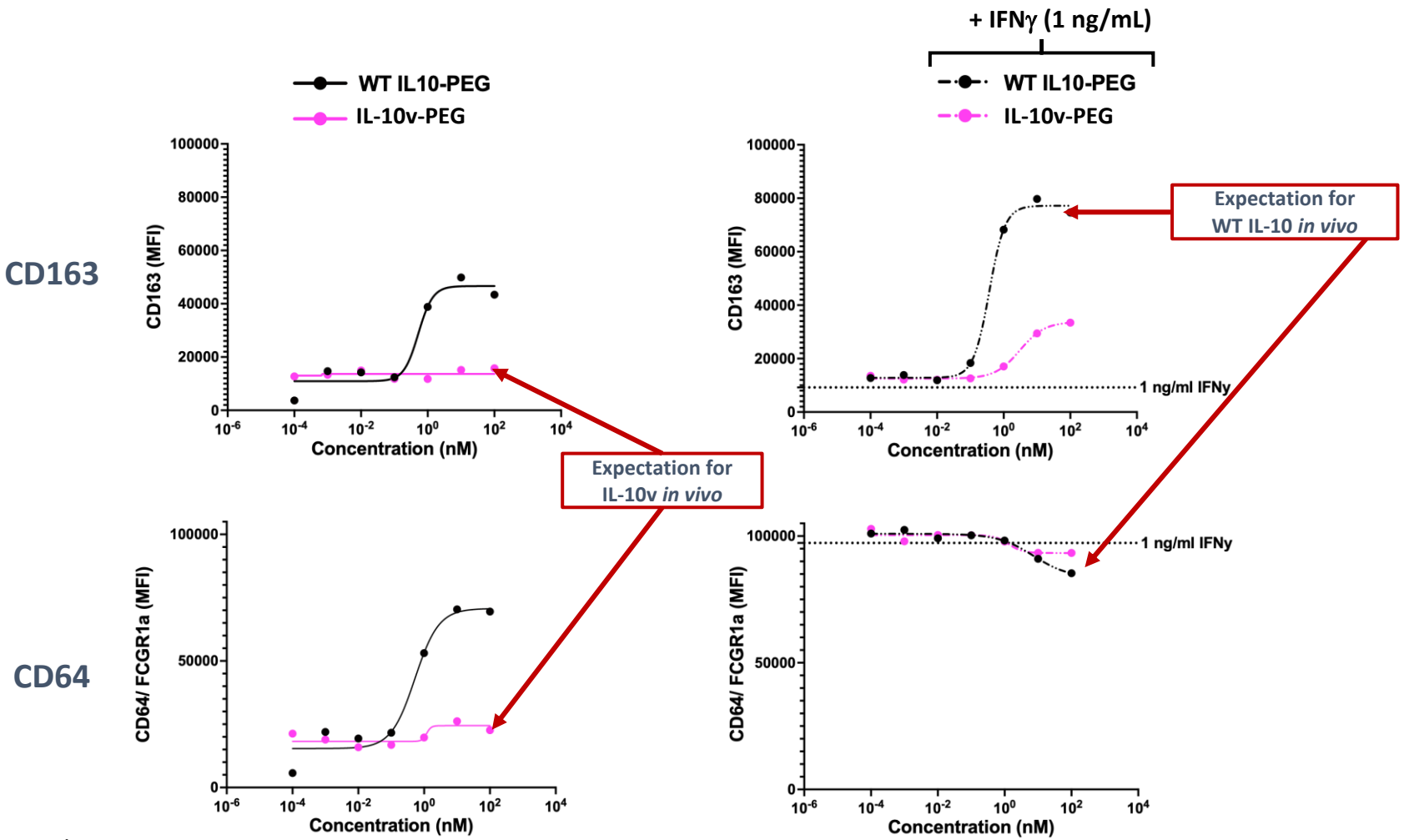
IL-10v Reduces the Induction of HLH-Associated Scavenger Receptors

- Wild-type IL-10 treatment upregulates CD163 and CD64 on monocytes
- High doses of IL-10 induced IFNγ and anemia / thrombocytopenia in patients
- IFNγ directly induces anemia and leads to upregulation of CD64
- ➢ IL-10v does *not* induce IFNγ or upregulate CD64 and CD163



Expression of CD64 (FCGR1A) and CD163 by FACS on Monocytes Cultured in WT IL-10 PEG vs. IL-10v-PEG, ± IFNγ



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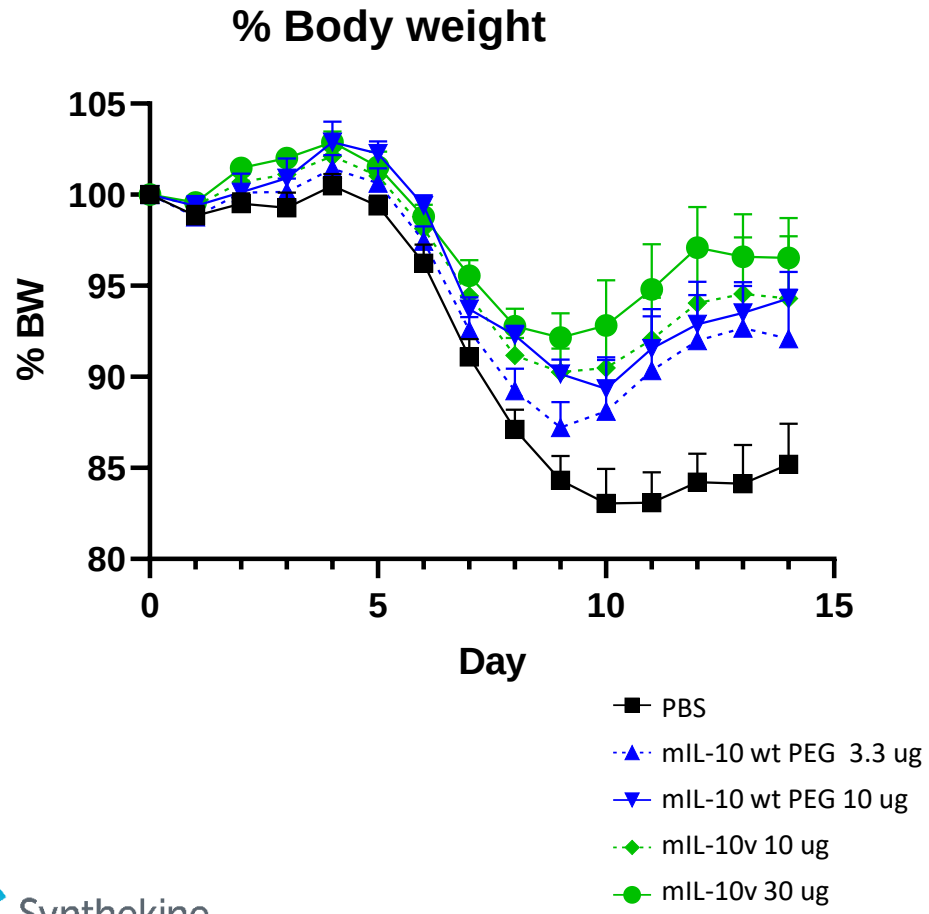
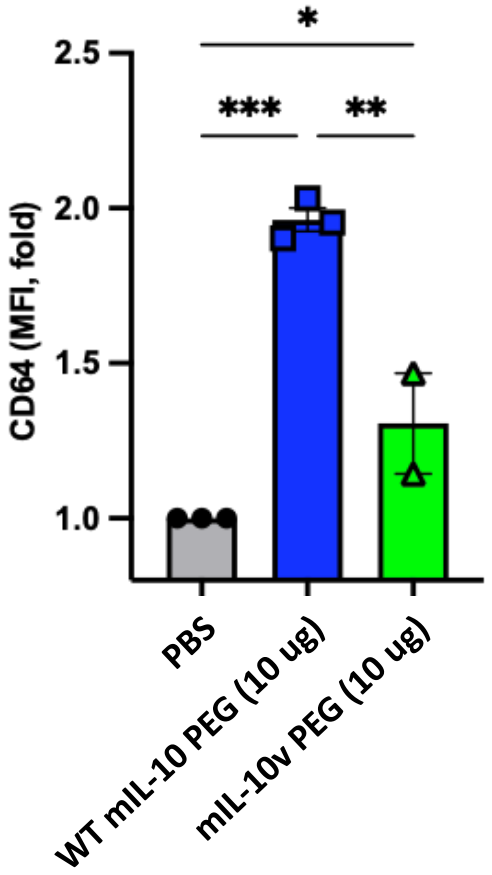
HLH = hemophagocytic lymphohistiocytosis

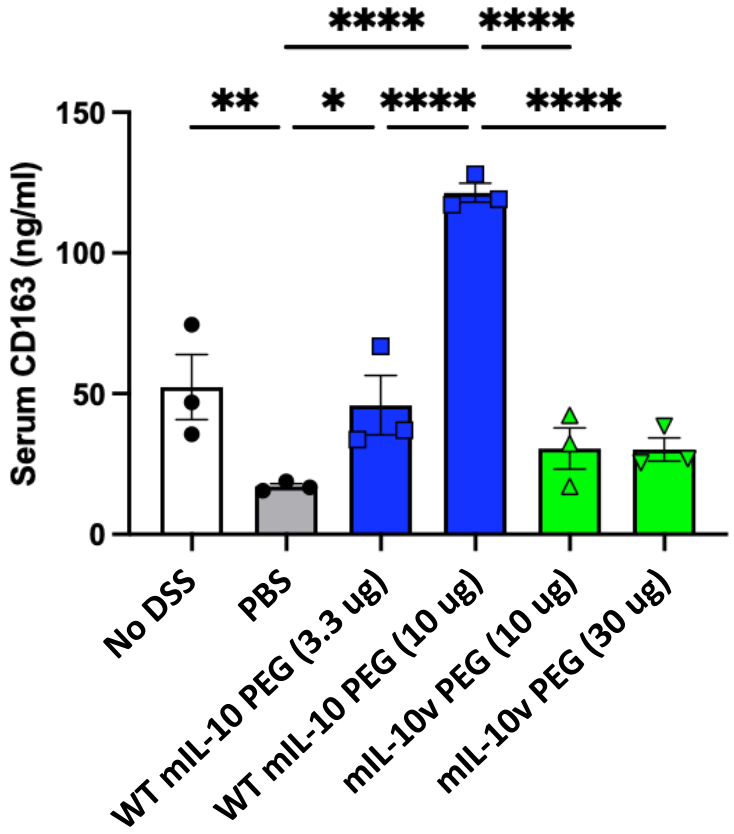
Mouse Surrogate of IL-10v Reduces Colitis and Does Not Induce HLH-Associated Markers In Vivo

Murine IL-10v (mIL-10v) ameliorates DSS colitis in mice at least as effectively as wild-type mIL-10



Unlike wild-type mIL-10, mIL-10v does not induce increase in cell surface CD64 or in soluble CD163





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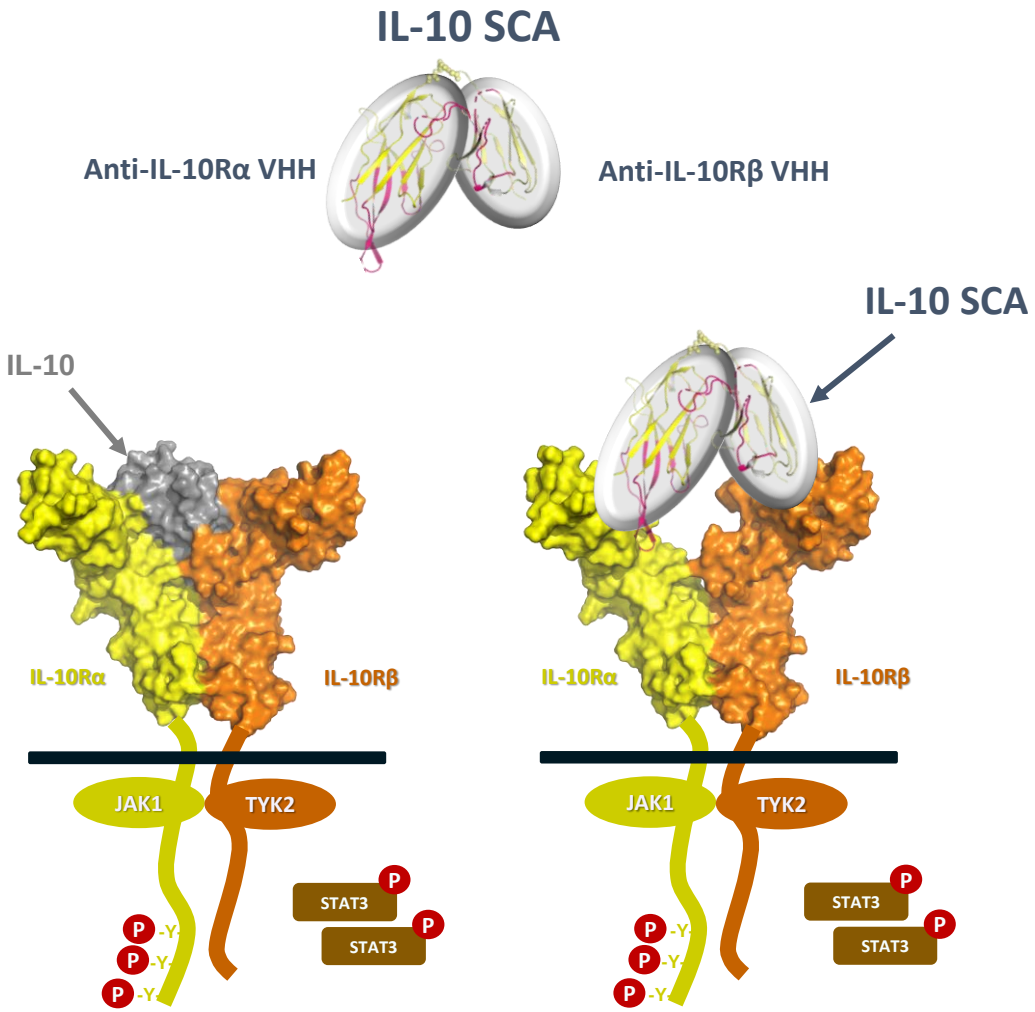
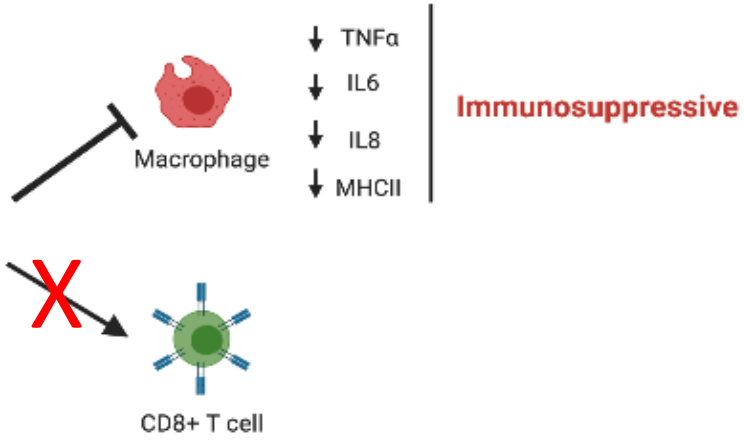
Surrogate Cytokine Agonists (SCAs): Novel Structures that Signal Through Cytokine Receptors to Screen for Biased Activity



- Surrogate Cytokine Agonists (SCAs) have antibody-like development properties
- Their activity and half-life are tunable through engineering
- IL-10 SCA Panel:
  - 7 Anti-hIL10Rα VHHs x 7 Anti-hIL10Rβ VHHs
  - Broad range of affinities and binding epitopes
  - Made in both N- and C-terminal orientations (98 SCAs)
  - Screened in vitro for signaling and function

Q: Can IL-10 SCAs also exhibit myeloid-biased activity?



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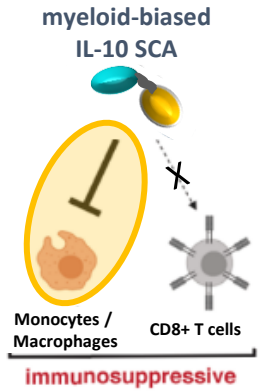
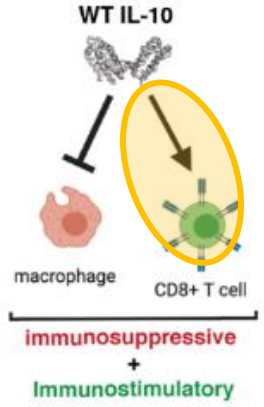
Multiple Human IL-10 SCAs Demonstrate Myeloid Bias In Vitro

Immunosuppressive



Monocytes

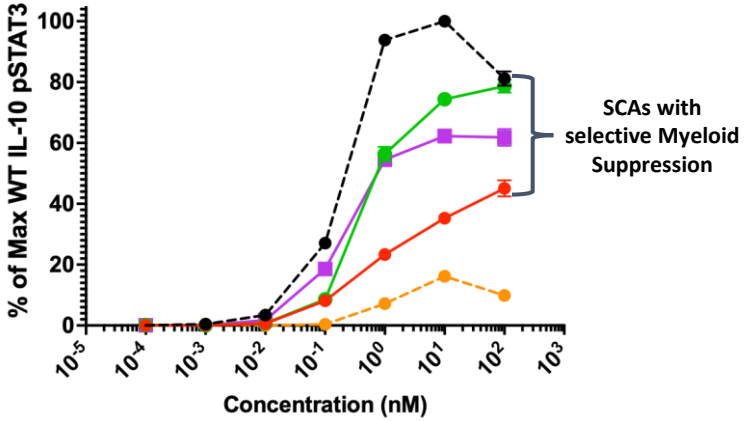
Uncouple immune stimulation



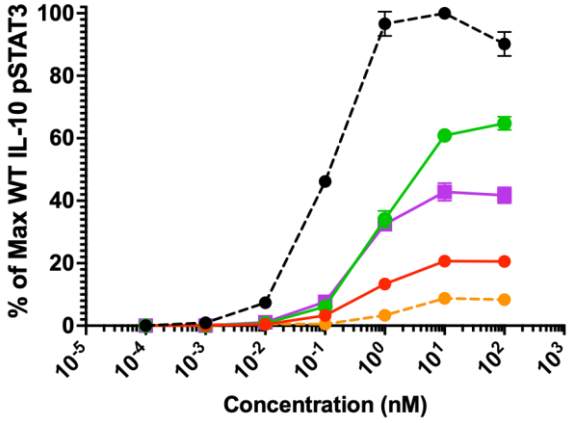
CD8 T cells

Signaling

Blood

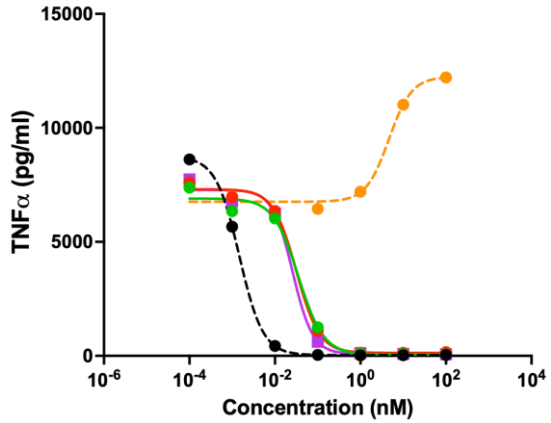


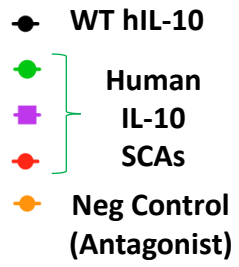
Blood



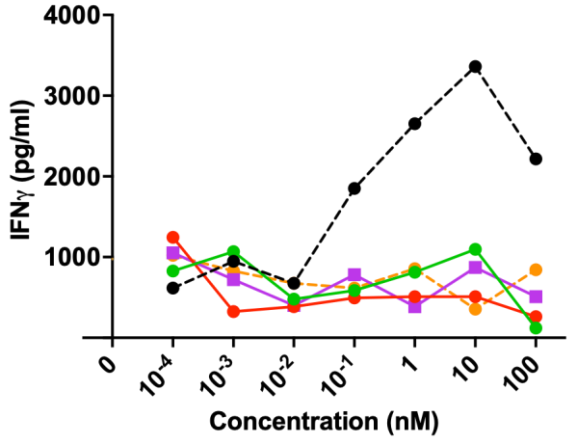
Functional Assay

Monocytes treated with LPS





CD3/CD28 stimulated 8 T cells



(not shown) **IL-10 SCA, like IL-10v, strongly reduces upregulation of HLH-associated scavenger receptors in vitro**

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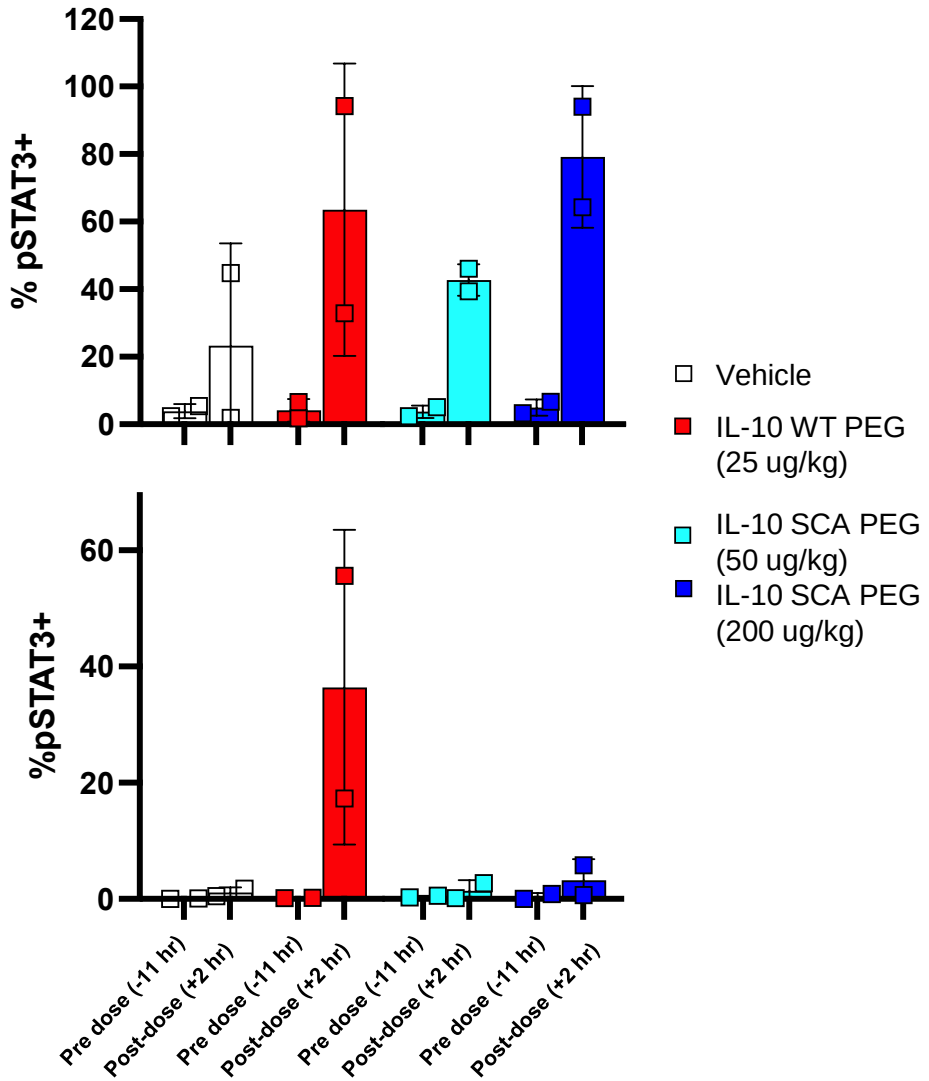
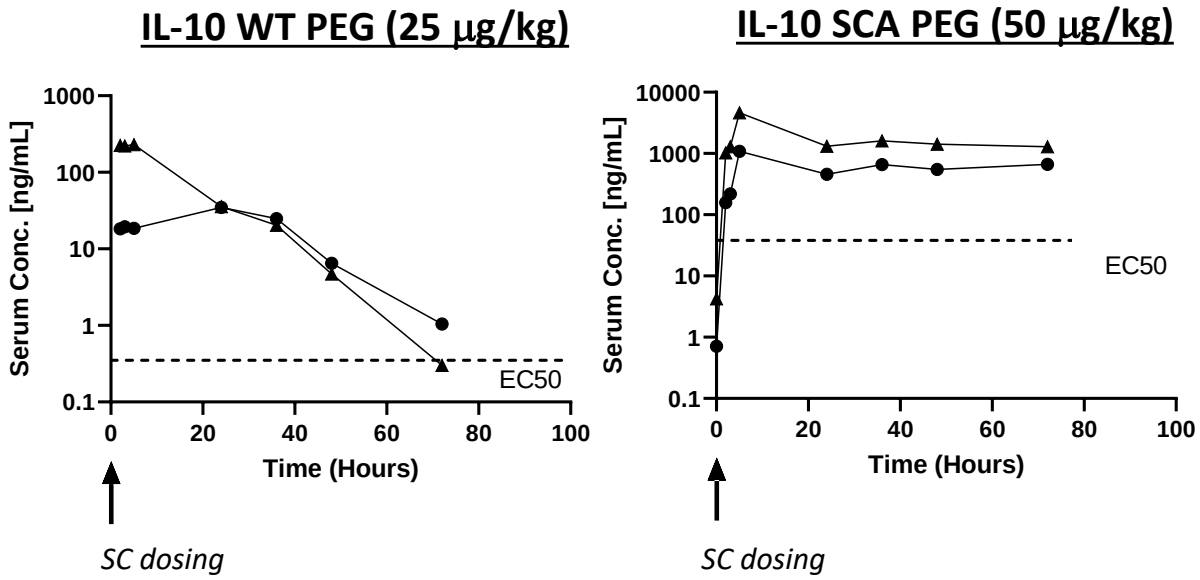
PEGylated hIL-10 SCA Shows Selective STAT3 on Monocytes and High and Durable Exposure in Cyno

Monocytes

CD8 T cells



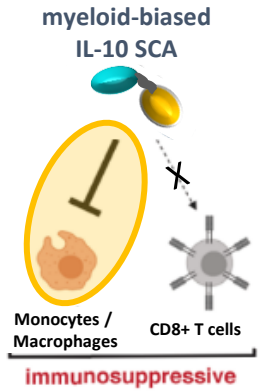
- PEGylated IL-10 SCA showed preferential signaling on monocytes at both the high and low concentrations
- IL-10 SCA PEG have a stable PK profile



Phospho-flow of two cynos (1M, 1F) per treatment arm

Murine IL-10 SCAs Also Demonstrate Myeloid Bias In Vitro

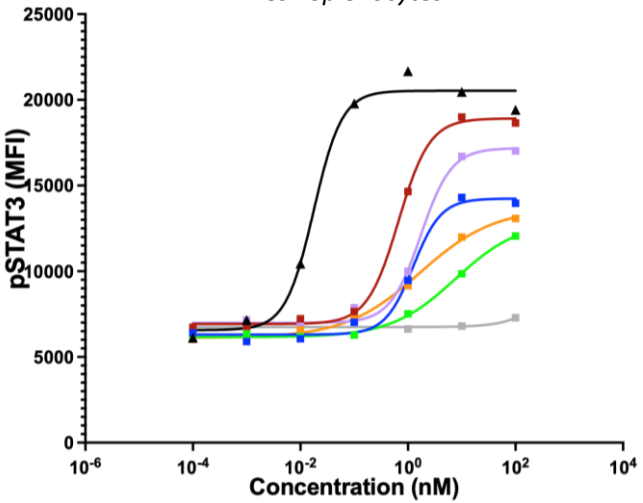
Immunosuppressive



Monocytes

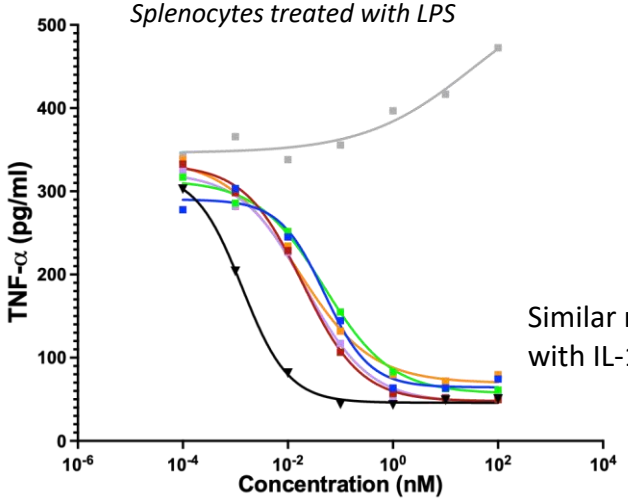
Signaling

Fresh Splenocytes

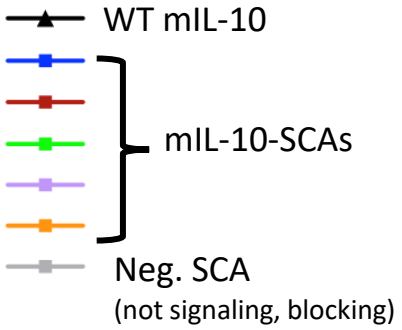


Functional Assay

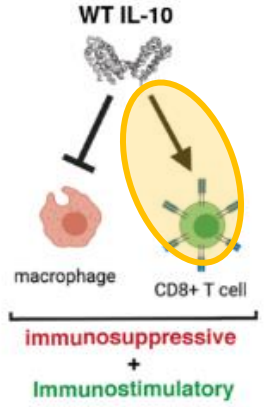
Splenocytes treated with LPS



Similar results
with IL-1b or IL-6

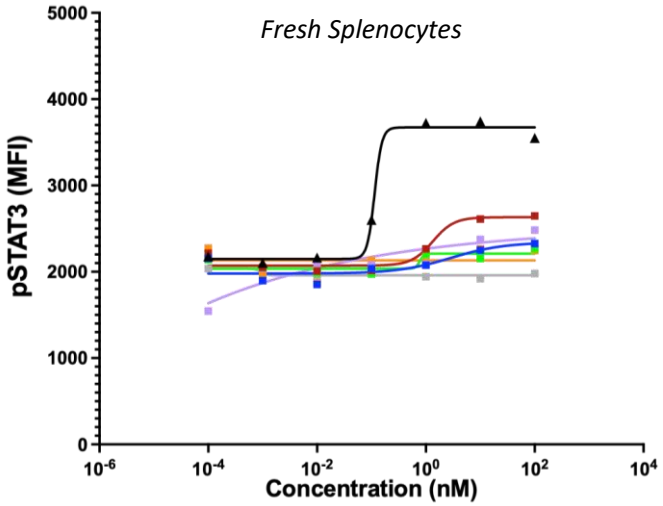


Uncouple immune stimulation

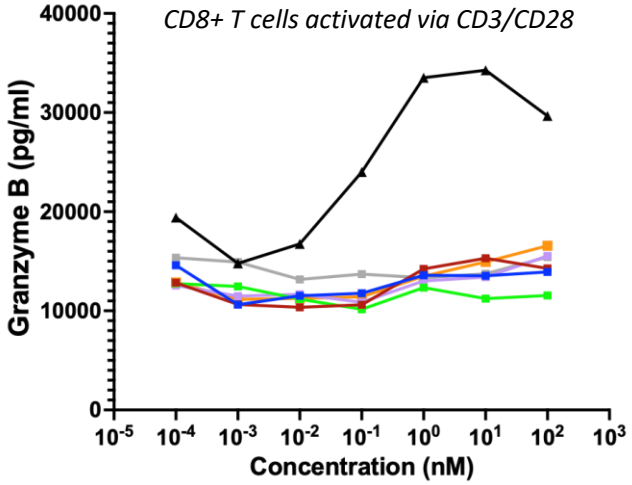


CD8 T cells

Fresh Splenocytes



CD8+ T cells activated via CD3/CD28



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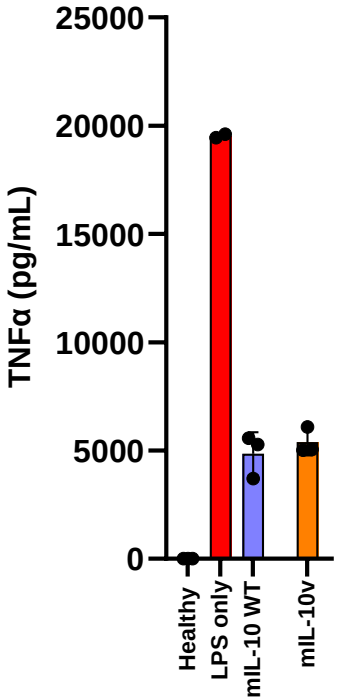
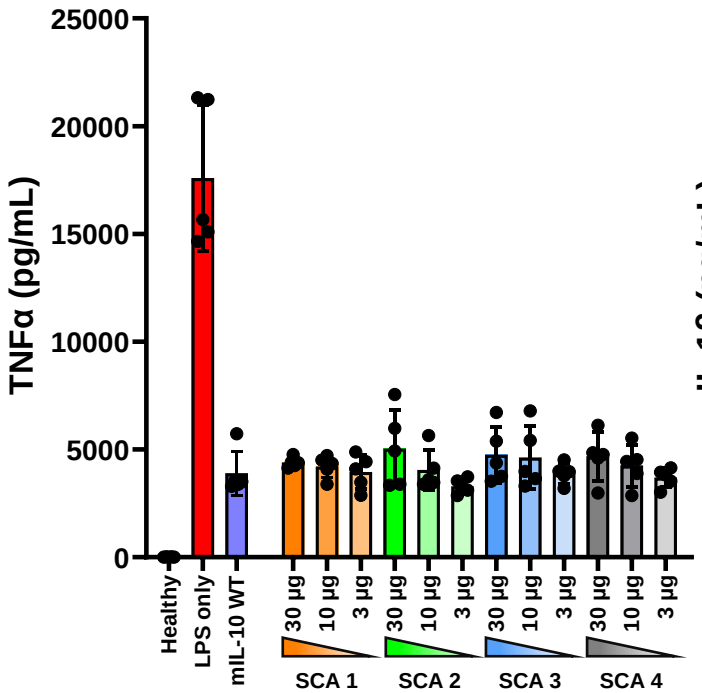
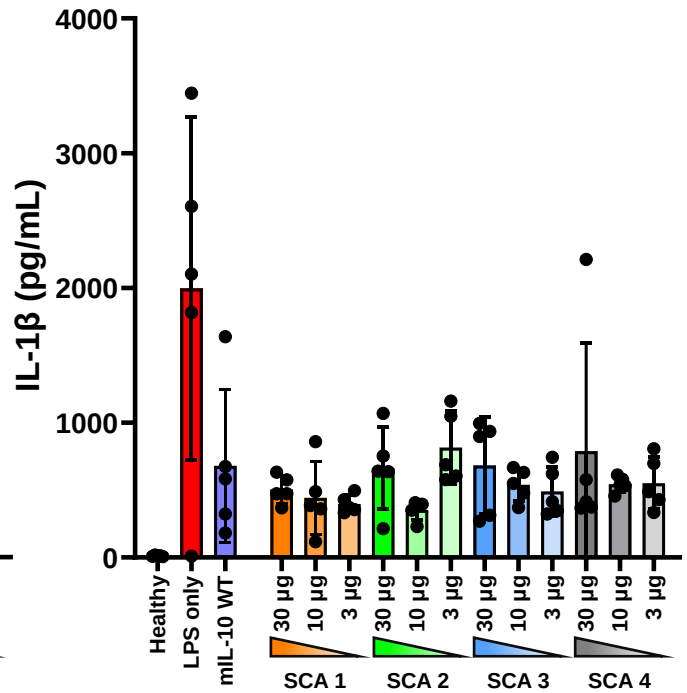
Murine IL-10 SCAs Have Potent Immunosuppressive Activity in Vivo

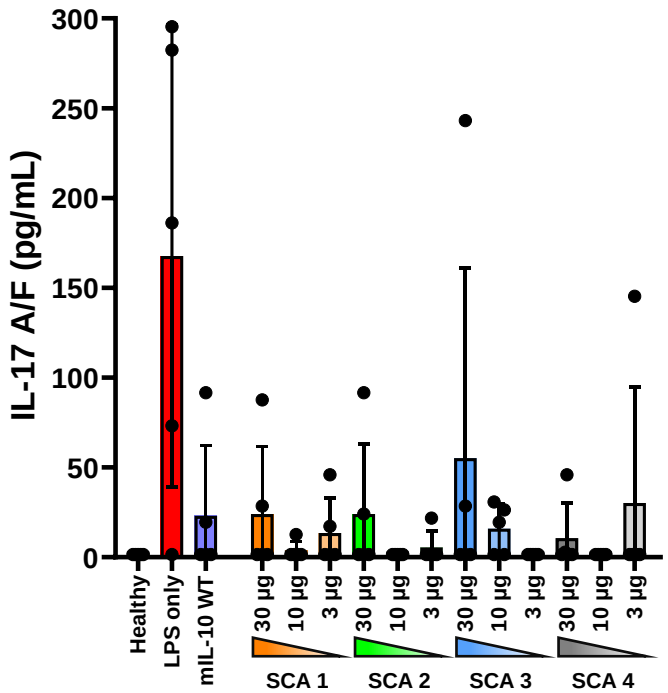
mIL-10v suppresses the proinflammatory cytokine response to LPS shock in mice



Several mouse IL-10 SCAs also suppress the proinflammatory cytokine response to LPS shock in mice



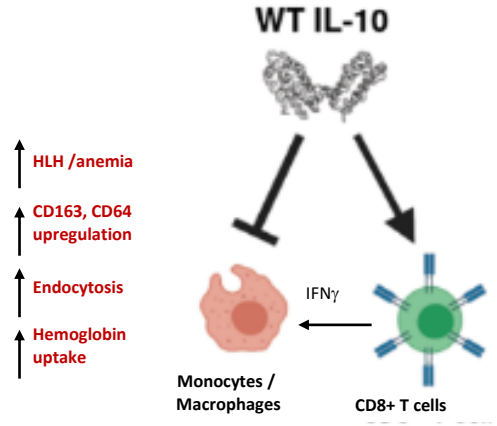




TNFα is from 1.5 hr post-challenge serum samples. IL-1β and IL-17A/F are from 6 hr post-challenge serum samples. Values at or below the LLOQ are represented as LLOQ

Myeloid-Biased Signaling Overcome Clinical Limitations for Treatment with IL-10

- Our goal is to discover IL-10 partial agonists that:
  - Retain anti-inflammatory IL-10 properties, suppressing pro-inflammatory cytokines (TNFα, IL-1β, IL-6) and IL12/23p40 (IL-17)
  - Avoid stimulation of T cells and induction of anemia/HLH associated markers
- An IL-10 variant (IL-10v) shows this is possible with structure-guided engineering of the native cytokine
- Here we demonstrate that Surrogate Cytokine Agonists (SCAs), composed of IL10Rα and IL10Rβ binding VHHs, can also accomplish this goal:
  - Combinatorial screens of IL-10 SCAs show a diverse range of IL-10 agonist signaling and activity
  - Multiple IL-10 SCAs have myeloid-biased activity in vitro and in vivo
  - IL-10 SCAs show good development and pharmacokinetic properties



Optimized IL-10 Agonists for Immunosuppression (IL-10v and IL-10 SCA)