CUE BIOPHARMA

Immune Responses, On Cue™

Directly Targeting Autoantigen-Specific T cells with Immuno-STATs

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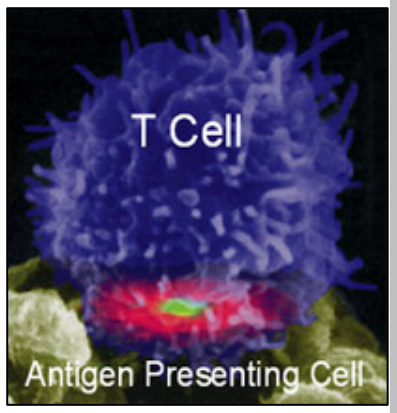
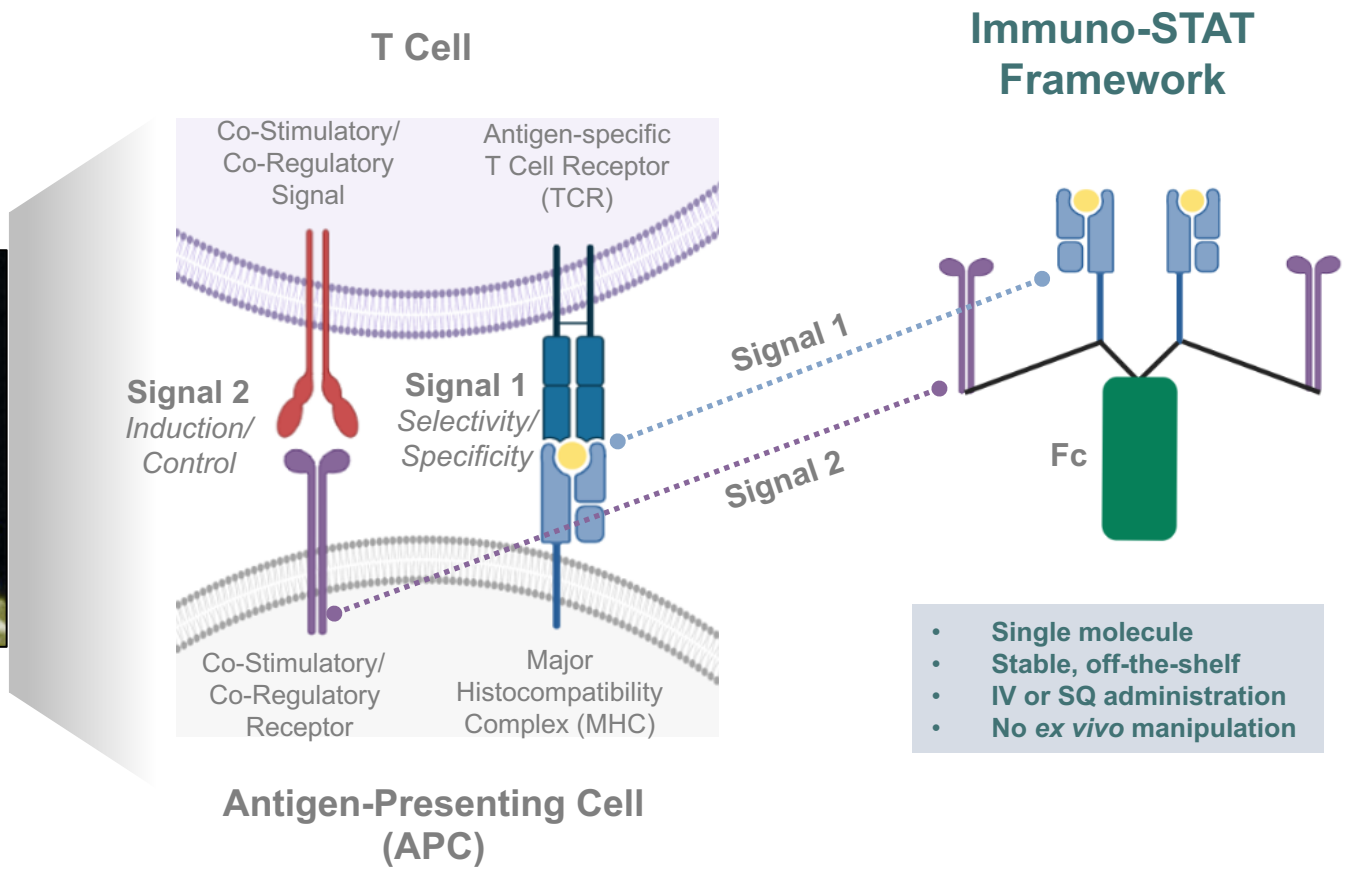
VP and Head of Discovery and Translational Immunology

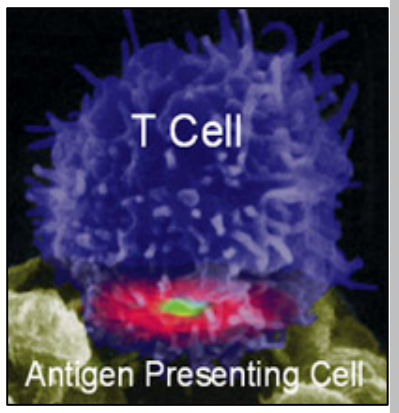
Emulating Nature's Cues to Selectively Modulate T Cells

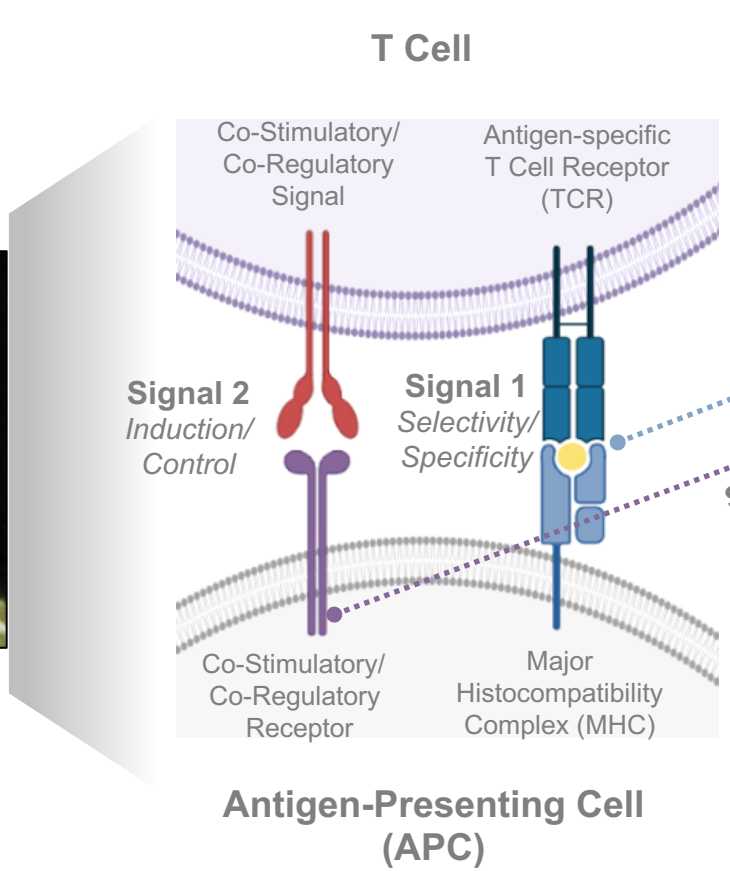


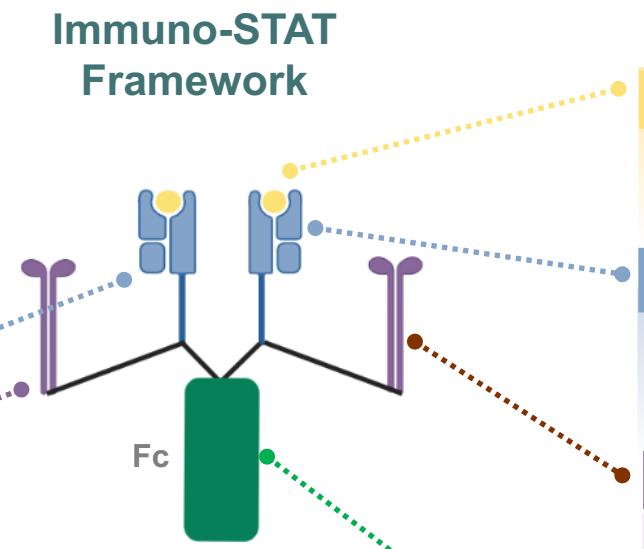


The Immuno-STAT™ platform can generate a wide diversity of therapeutic molecules for many diseases to selectively target and modulate the activity of disease-relevant T cells

Emulating Nature's Cues to Selectively Modulate T Cells







- **Single molecule**
- **Stable, off-the-shelf**
- **IV or SQ administration**
- **No *ex vivo* manipulation**

Platform Modularity

Peptide Antigen

Peptide epitopes to target different diseases

MHC

Different HLA alleles to address global patient populations

Co-stim/Co-reg

Distinct biological signals, including cytokines; cell-surface receptors; and/or other targeting modalities (e.g., scFv, etc.)

Fc Backbone

Fc engineering to dial in or out biological and effector functions

The Immuno-STAT™ platform can generate a wide diversity of therapeutic molecules for many diseases to selectively target and modulate the activity of disease-relevant T cells

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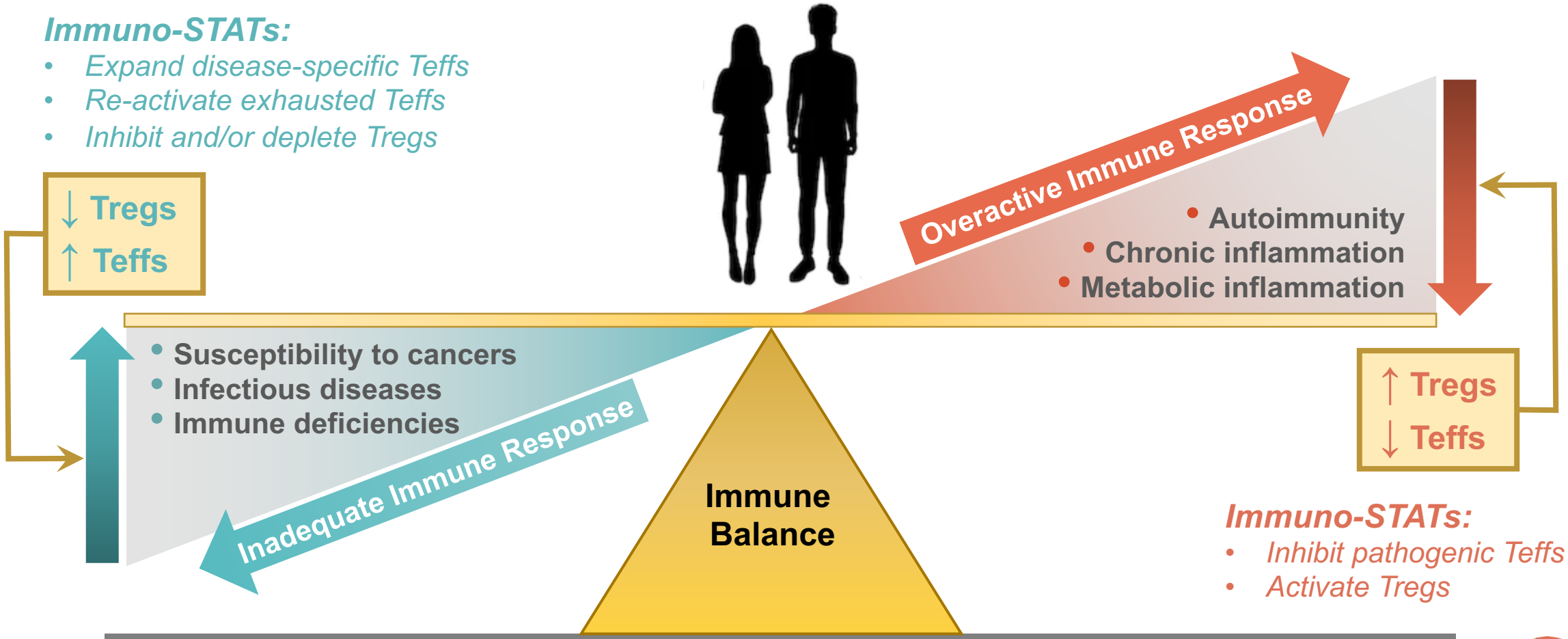
Immuno-STATs are Designed to Restore Immune Balance

Immuno-STATs:
- *Expand disease-specific Teffs*
- *Re-activate exhausted Teffs*
- *Inhibit and/or deplete Tregs*

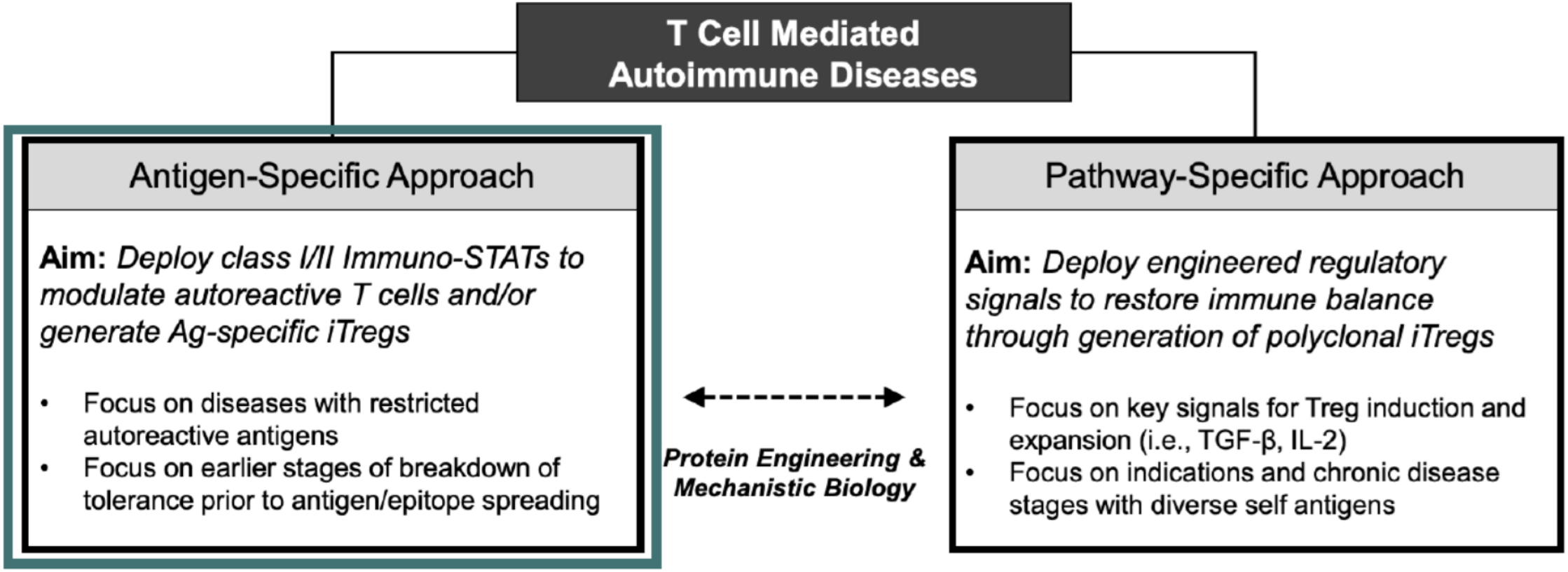


Immuno-STATs:
- *Inhibit pathogenic Teffs*
- *Activate Tregs*

Restoration of immune balance is a key pillar of human health

Key: Teffs, effector T cells; Tregs, regulatory T cells

AI Focus



Target Rationale and Therapeutic Hypothesis

Target rationale:

- Type 1 diabetes (T1D) is a chronic autoimmune disease resulting in severe loss of pancreatic β cells due to the targeting of islet cell autoantigens
  - Imbalances in the number and/or function of antigen-specific (AgS) autoreactive T cells (Teff) and/or regulatory T cells (Tregs) are considered the main effectors of β cell destruction
- ~50% of the genetic risk for T1D derives from specific HLA alleles involved in the presentation of peptide antigens to T cells
  - Allelic associations with disease are known (DR4, DQ8)
  - Critical peptide-HLA class II T cell epitopes are known (e.g., Proinsulin, GAD65, InsB, etc)
- Intervention in T1D may be achieved by:
  - (1) deletion or functional attenuation of AgS pathogenic Teff and/or
  - (2) expansion or functional enhancement of AgS Treg

Therapeutic hypothesis:

- Peptide-HLA class II-based Immuno-STATs (ISTs) can target disease-relevant CD4+ T cells for therapeutic effect in T1D through their deletion/functional attenuation

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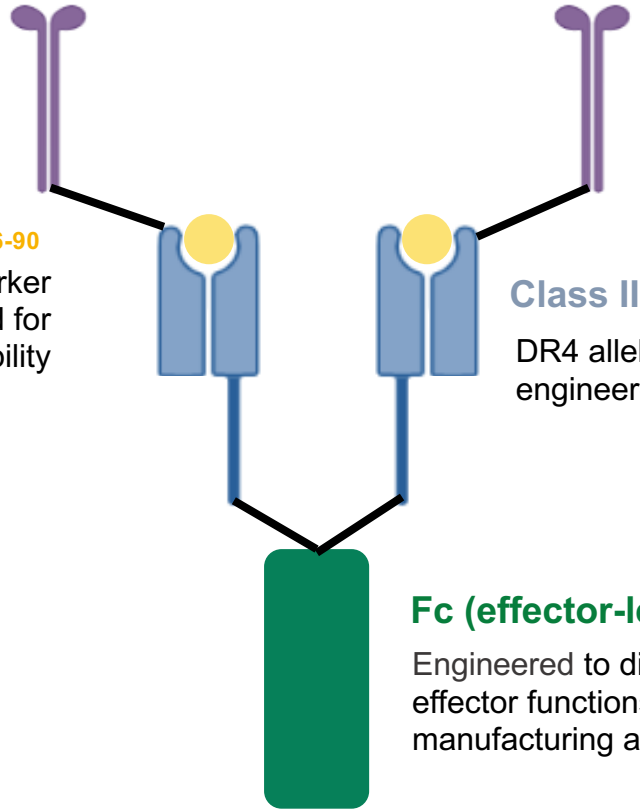
Design of the Proins$_{76\text{-}90,\ K88S}$/DR4-PDL1 Immuno-STAT (IST)

PI-DR4-PDL1



PD-L1

Engineered to provide functional attenuation of targeted cells

Proinsulin$_{76\text{-}90}$

Major proinsulin epitope, marker of autoreactivity, engineered for enhanced binding affinity/stability

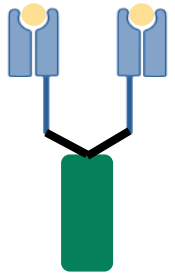
Class II DRB1*04

DR4 allele highly associated with T1D, engineered for enhanced stability

Fc (effector-less)

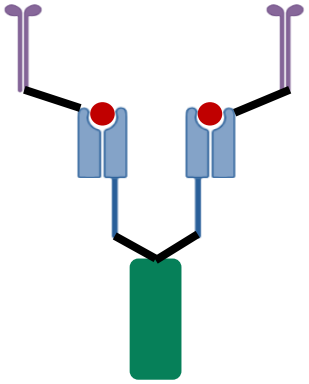
Engineered to dial out biological and effector functions while maintaining manufacturing and stability advantages

Control molecules



MOD-less

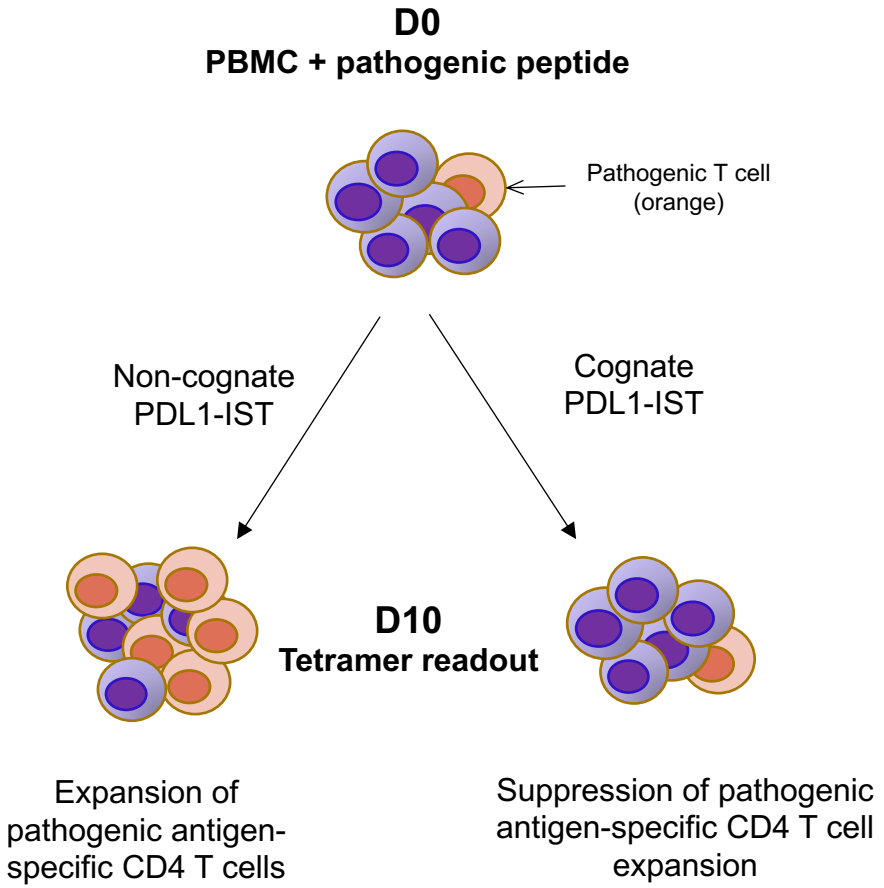
"PI/DR4"
Generated as a control for PD-L1 function

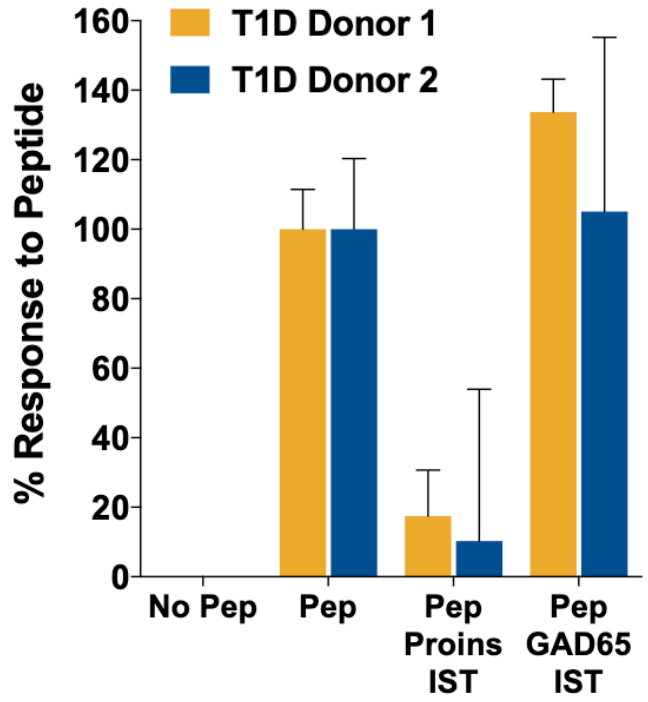


GAD65$_{555\text{-}567}$

"GAD/DR4-PDL1"
Generated as a specificity control

PI-DR4-PDL1 IST Inhibits Expansion of Proinsulin-Specific T cells In Vitro

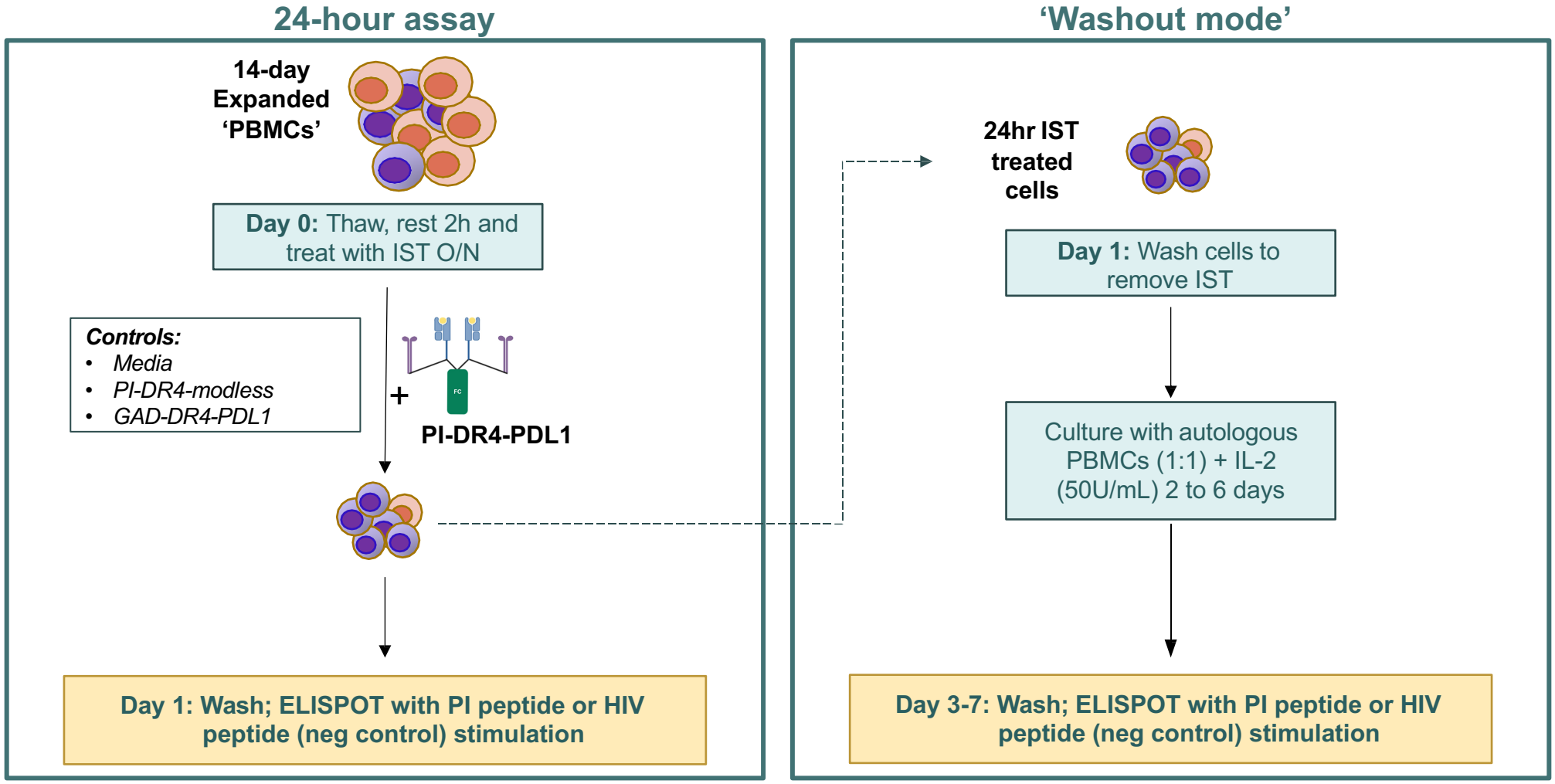




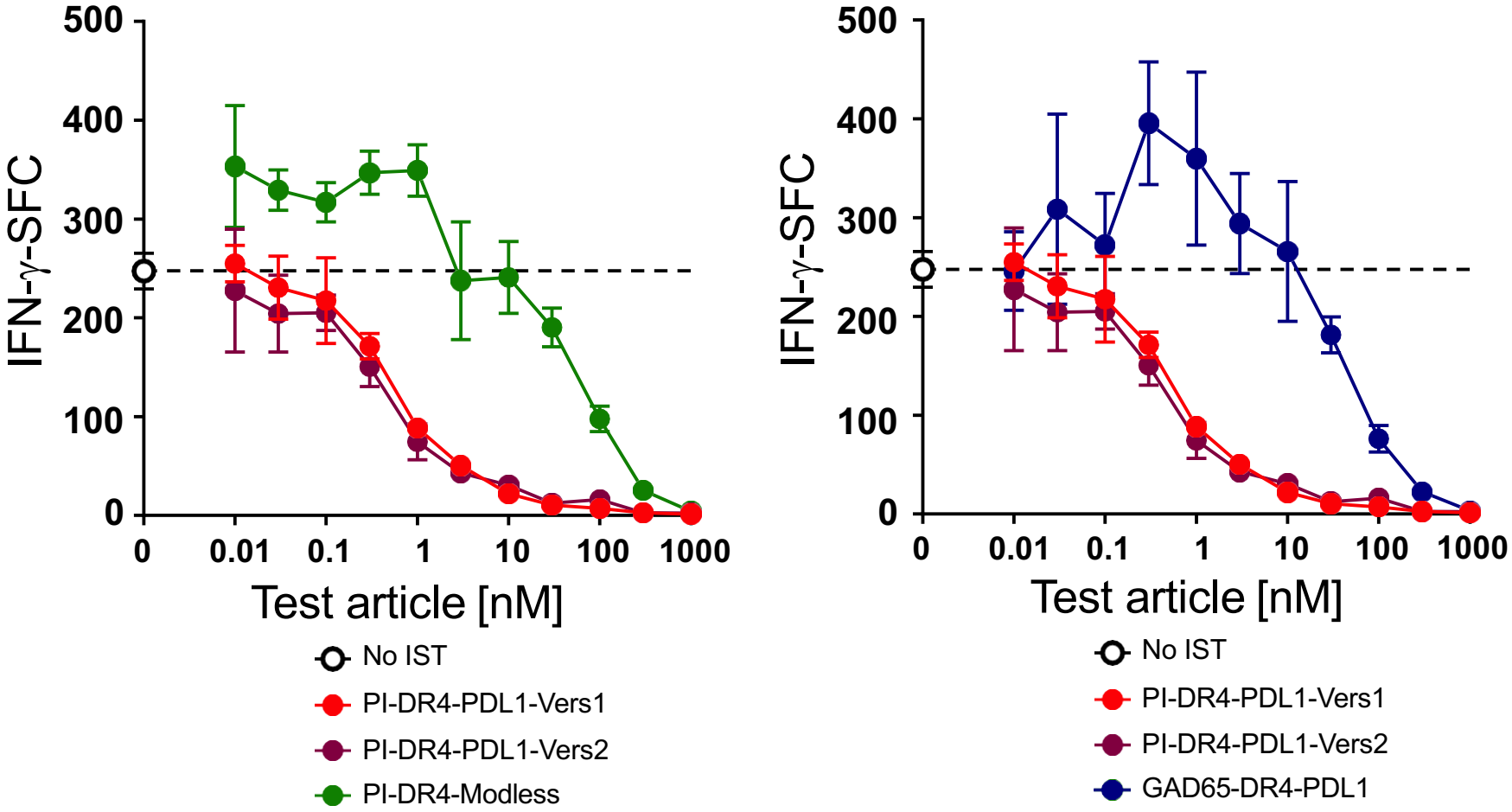
PI-DR4-PDL1 IST selectively inhibits antigen-specific CD4+ T cells from T1D donor PBMCs

Potency and Duration of Effect of PI-DR4-PDL1 ISTs In Vitro: In Vitro Assay Format #1

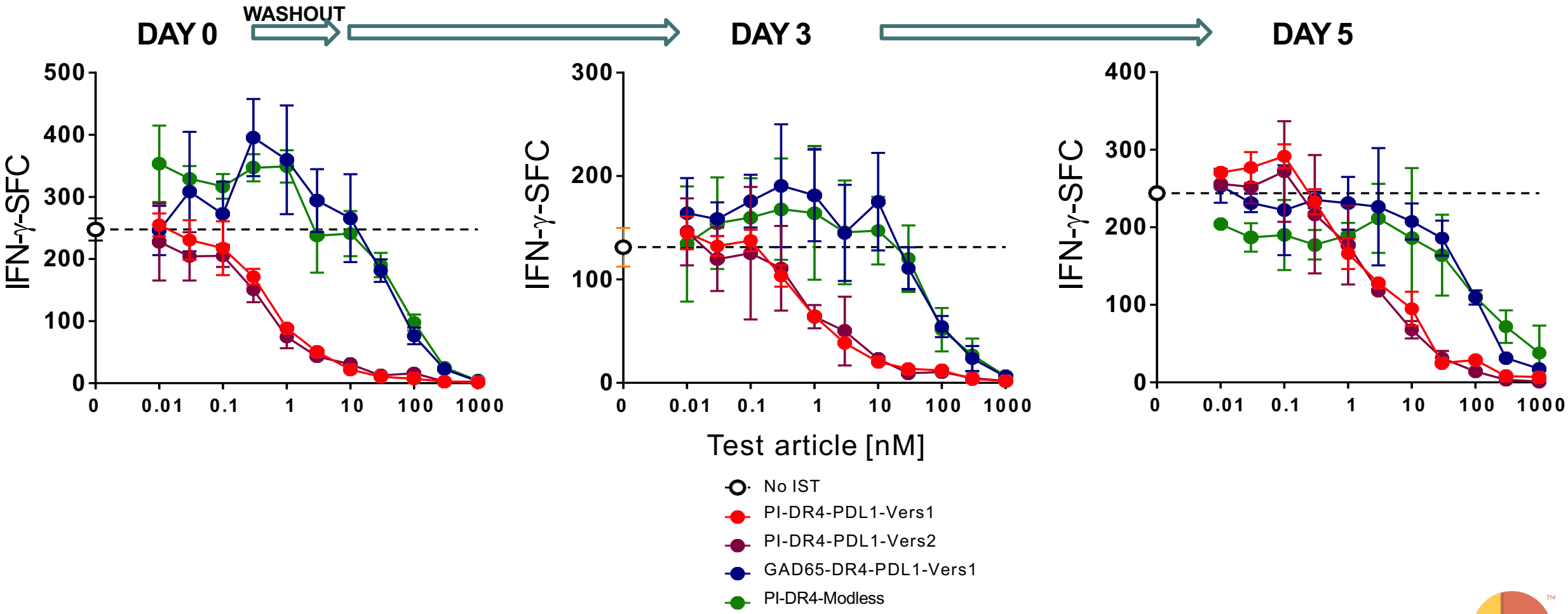
- Assays are based on PBMCs expanded for 14 days with peptide and rhIL-2



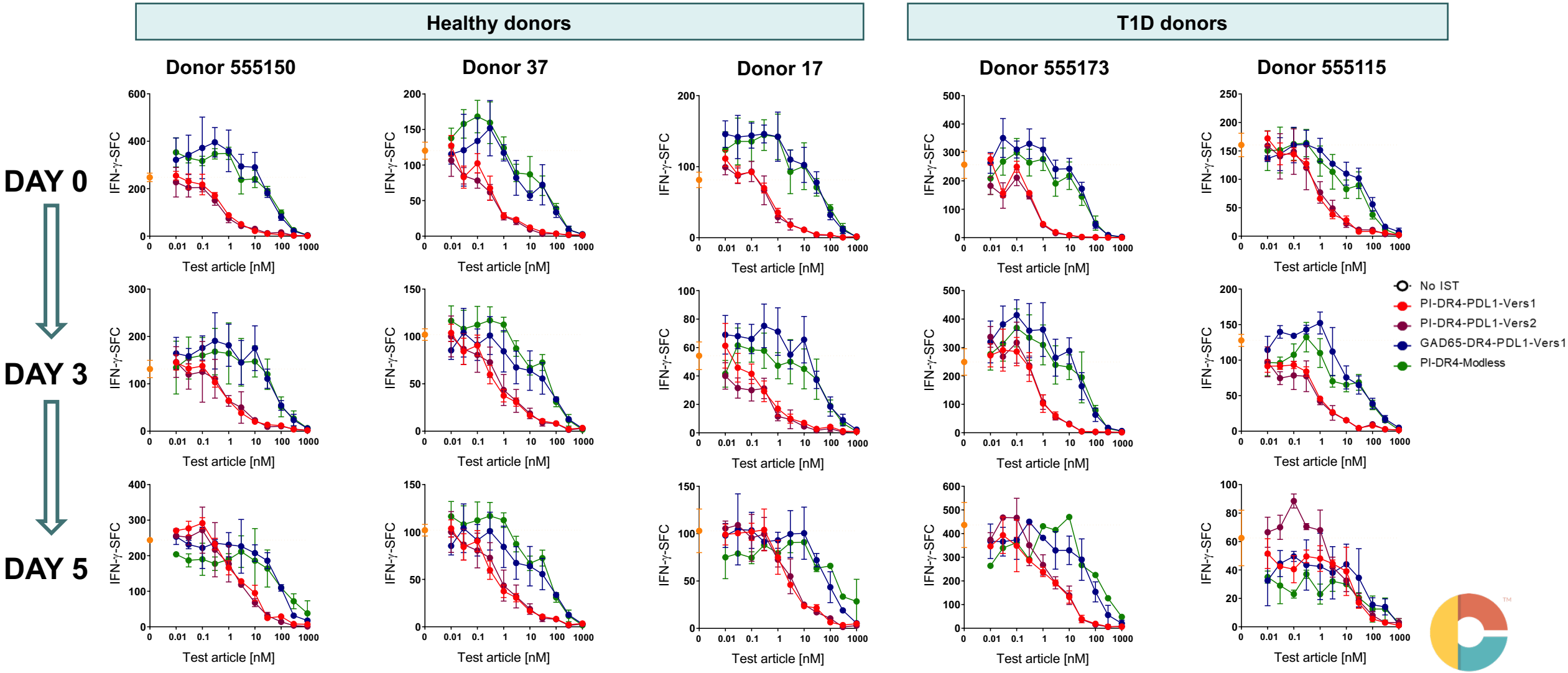
Overnight Treatment with PI-PDL1 ISTs Inhibits Antigen-Specific CD4+ T Cell IFN-γ-Responses in a PDL1-Dependent, Antigen-Specific Manner



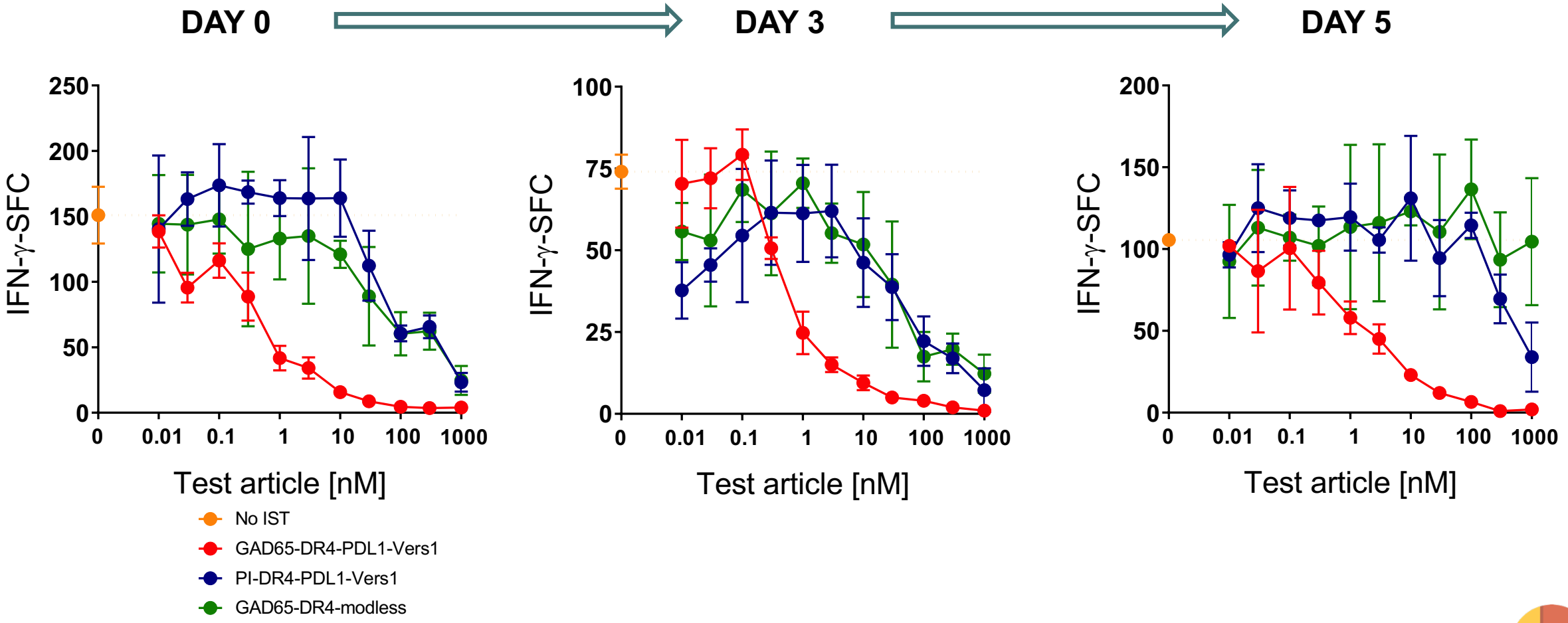
Functional Responses of Proinsulin-Specific CD4+ T cells Remain Suppressed 3-5 Days Post Washout



The Antigen-Specific, PDL1-Dependent Suppression of PI-Specific CD4+ T Cells is Observed Across Multiple Tested Donors

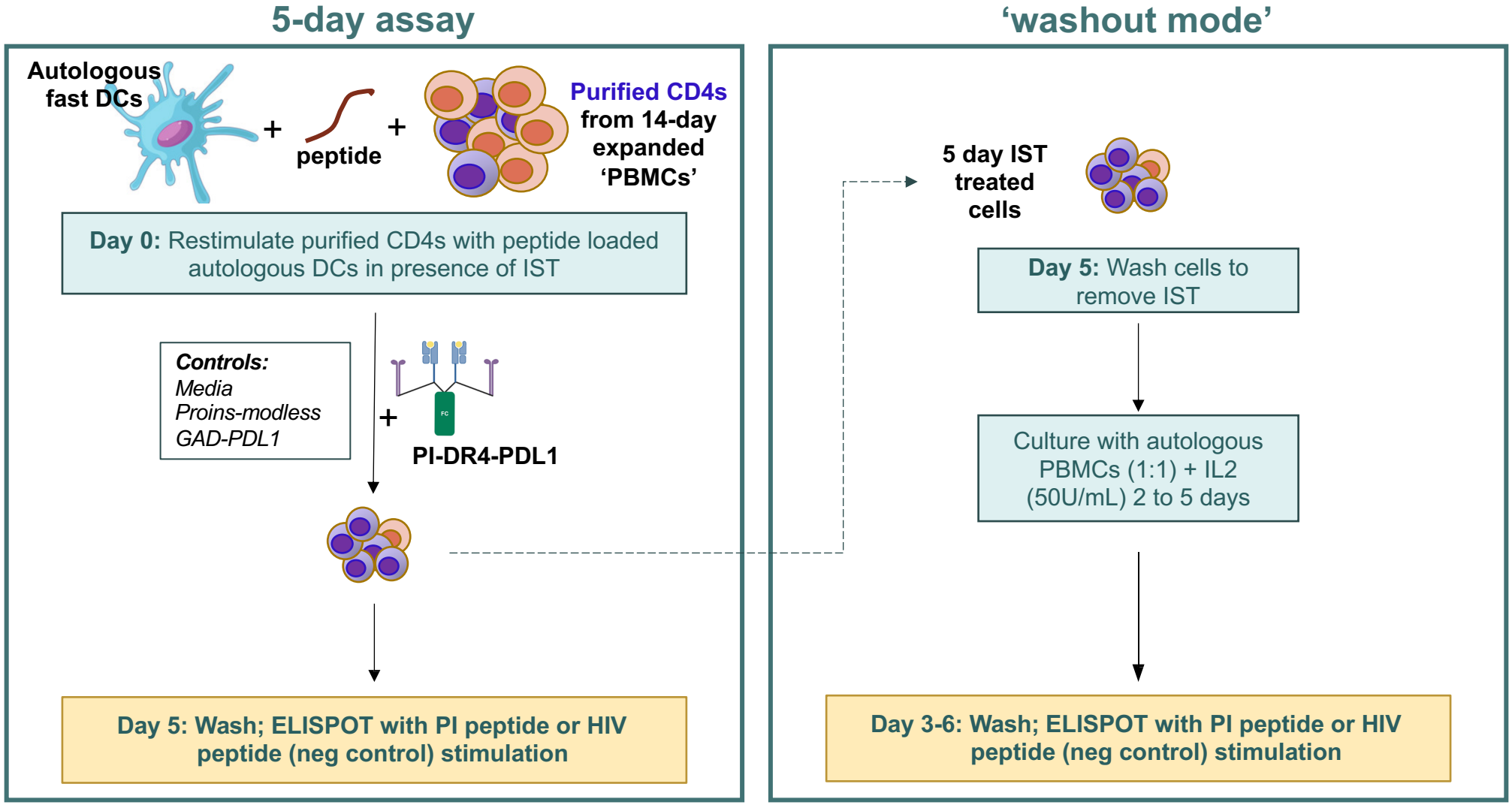


Observed Mechanism is Not Unique to PI-PDL1: Similar Effect is Seen with GAD65-Specific Donors and a GAD65-PDL1 IST



Potency and Duration of Effect of PI-DR4-PDL1 ISTs In Vitro: In Vitro Assay Format #2

- All assays are based on CD4+ T cells expanded for 14 days with peptide and rhIL-2



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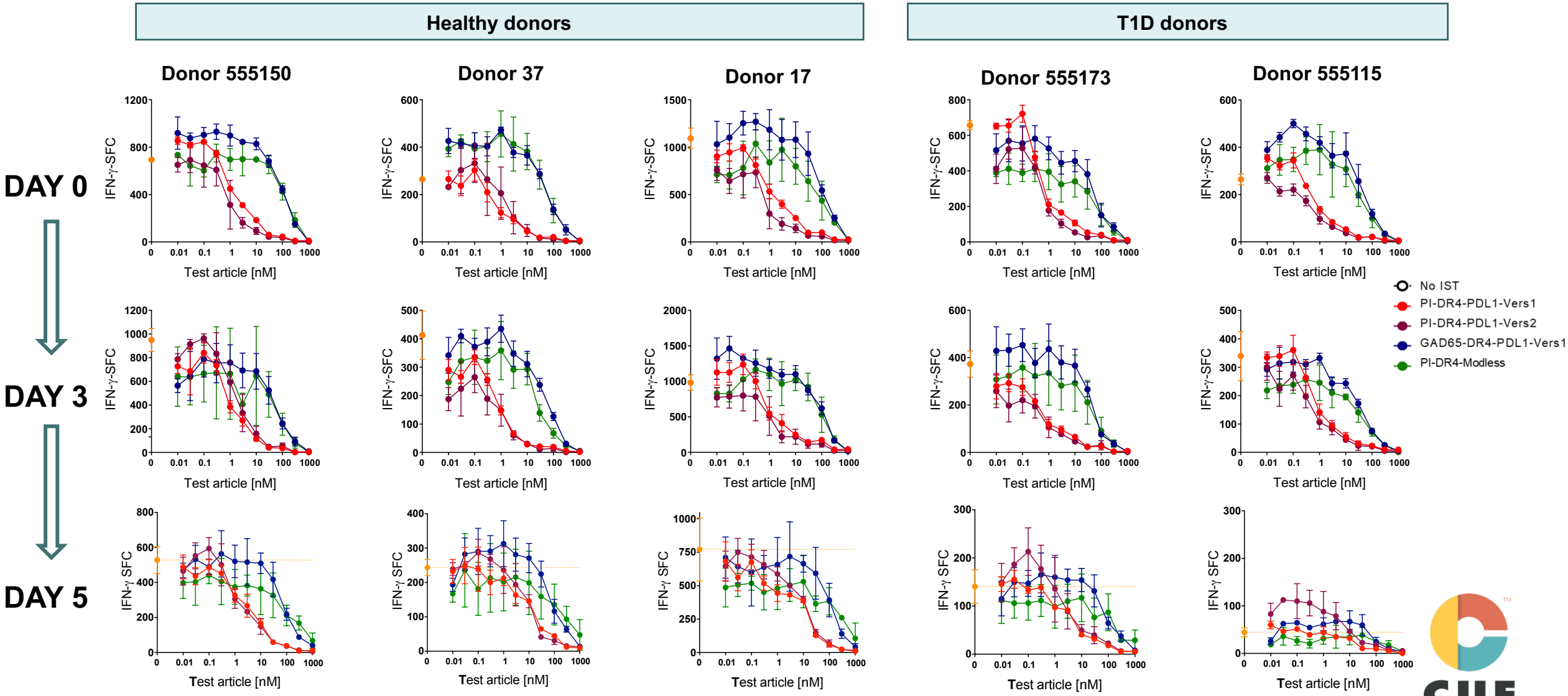
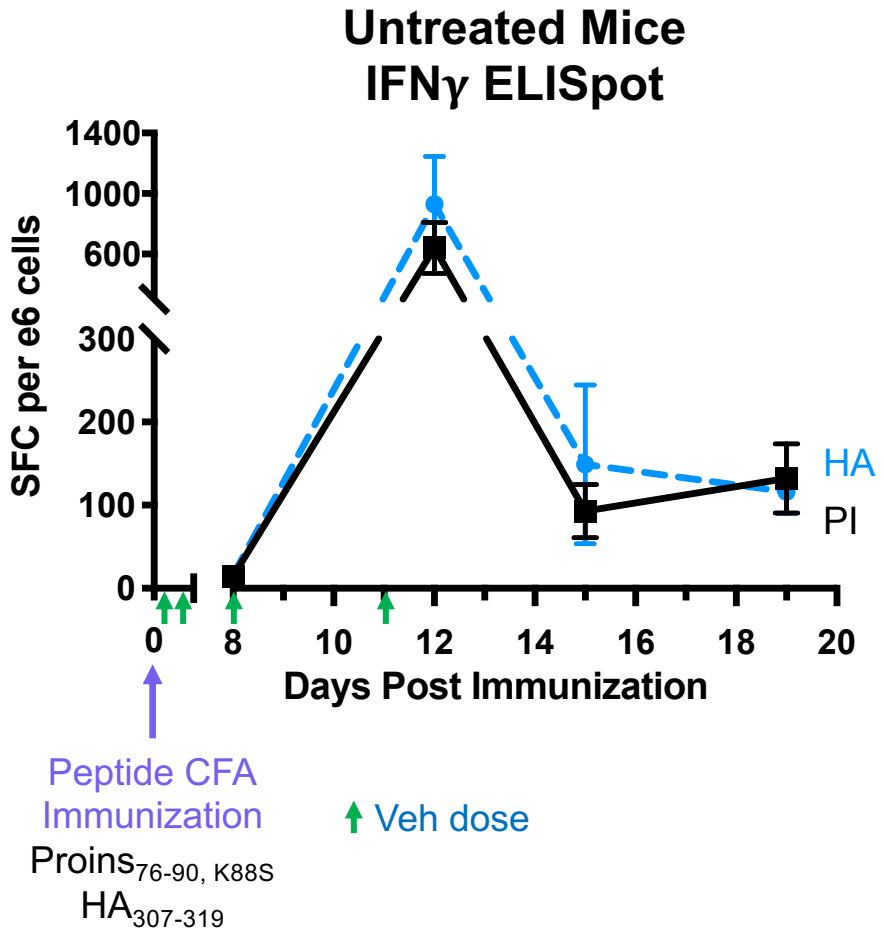
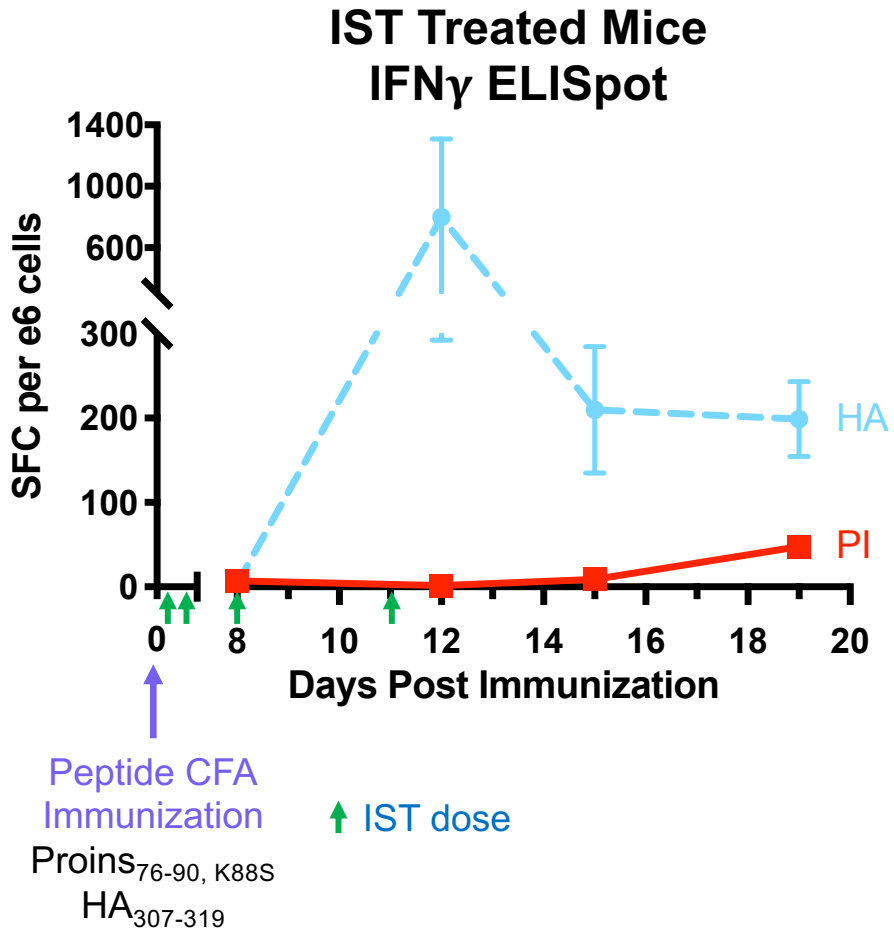


In Vitro Study Conclusions

- Functional responses of Proinsulin-specific CD4+ T cells are strongly suppressed by overnight PI-PDL1 IST treatment and remain suppressed at 3-5 days post-washout
- Functional responses of PI-specific CD4+ T cells re-stimulated for 5 days in the presence of PI-PDL1 ISTs are strongly suppressed and remain suppressed at 3 days post washout
- The observed effects are antigen-specific and PDL1-dependent and are observed across multiple tested donors (both healthy donors and T1D patients)
- Similar results are obtained with GAD65-specific CD4+ T cells treated with a GAD65-PDL1 IST → the mechanism and duration of PDL1-mod-induced suppression is not limited to a single antigen specificity

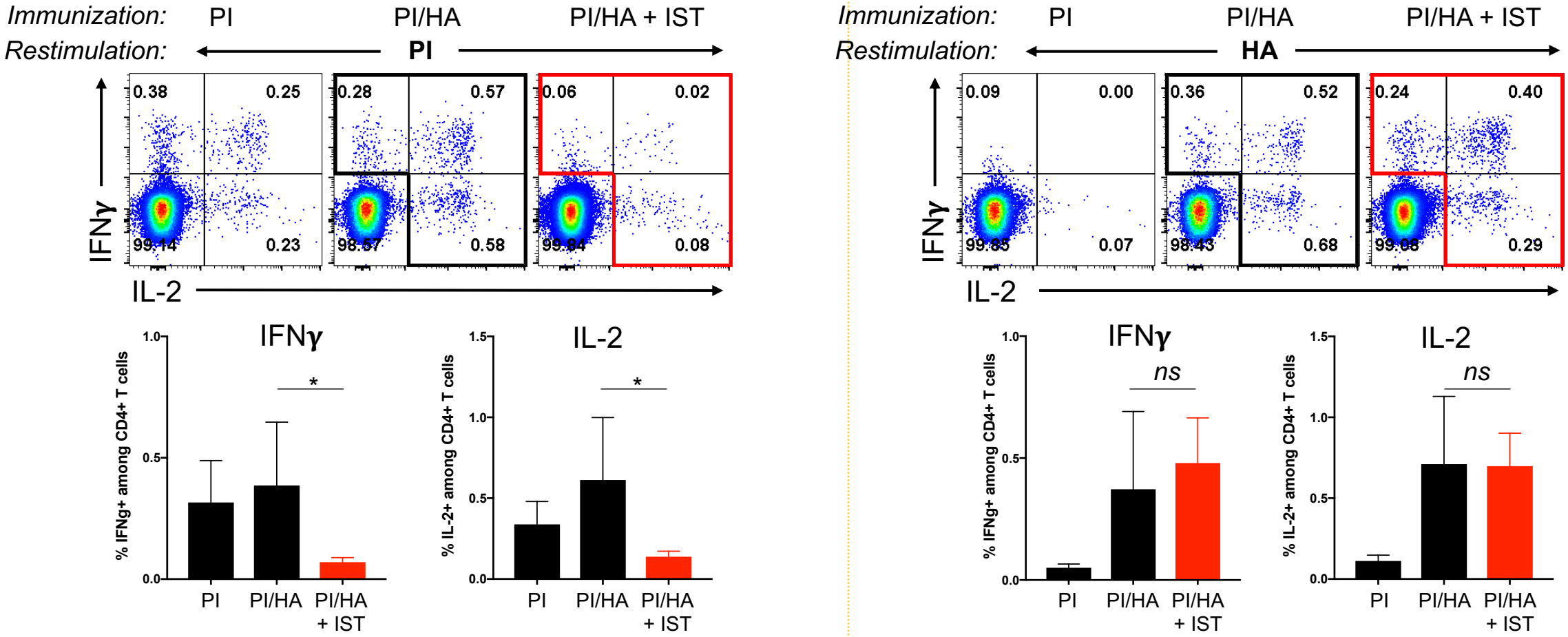
Early Intervention with Immuno-STAT Selectively Reduces the Number of Proinsulin (PI) Responsive CD4+ T Cells in Transgenic Mice





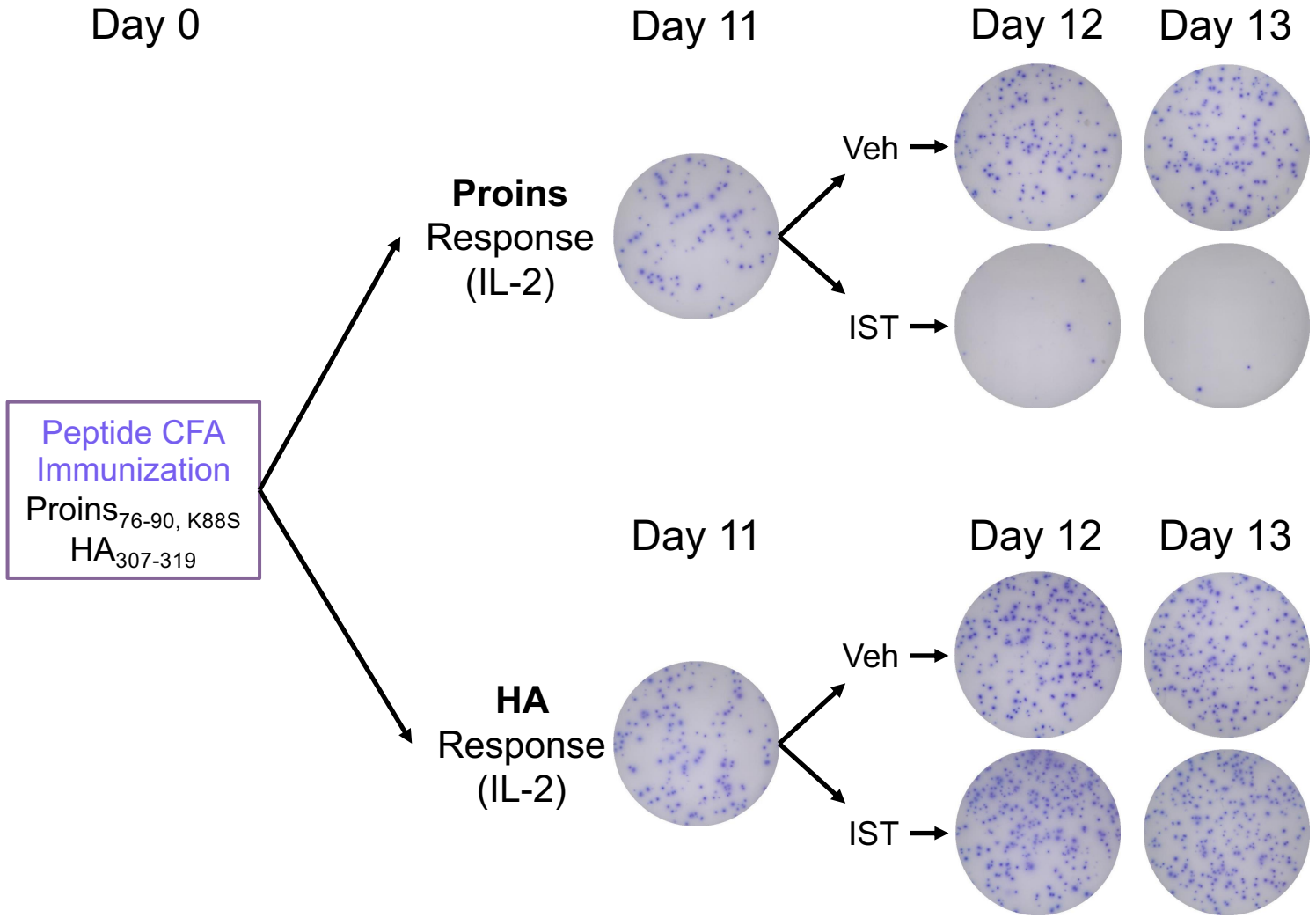
Treatment with Proins-DR4-PDL1 IST starting on Day 1 post immunization selectively suppresses expansion of PI-reactive cells without inhibiting expansion of HA-reactive cells

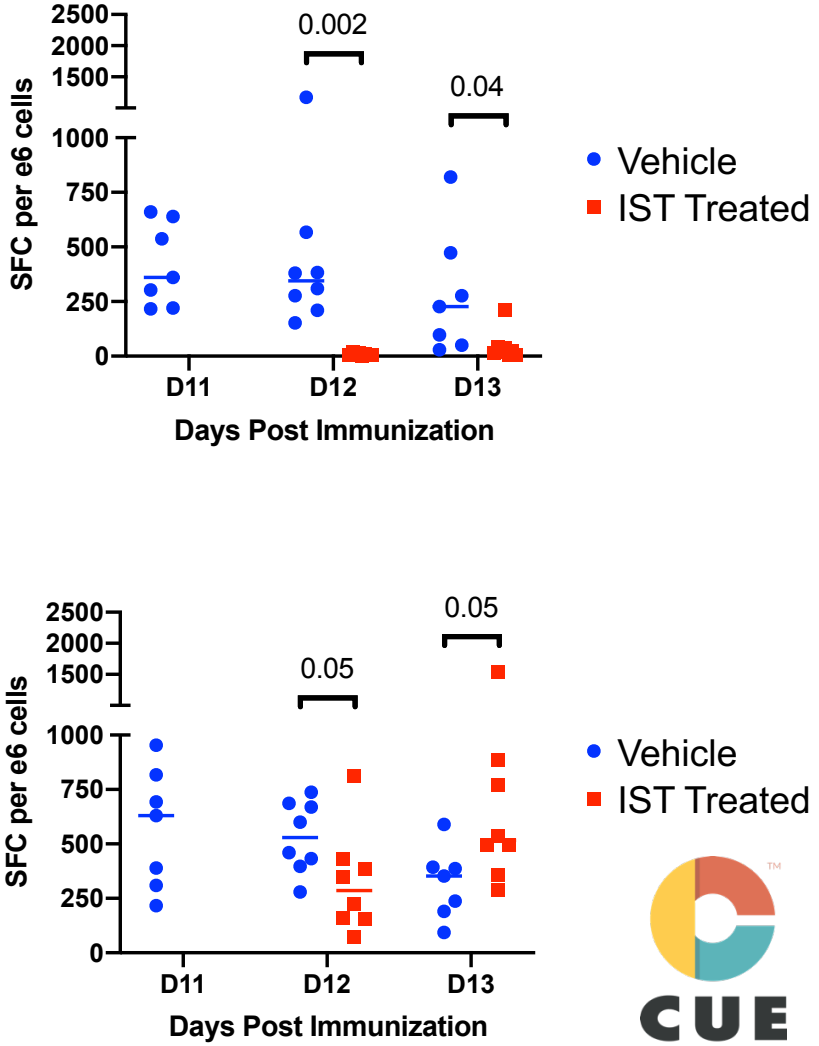
In Vivo Treatment with Proins$_{76\text{-}90,\ K88S}$/DR4-PDL1 Immuno-STAT Suppresses Cytokine Production by Proins (PI)-Specific CD4+ T Cells



Treatment with ProIns-DR4-PDL1 IST suppresses cytokine production of PI-specific CD4+ T cells (IL2, IFNγ, TNFα, IL-17) without affecting HA-reactive cells

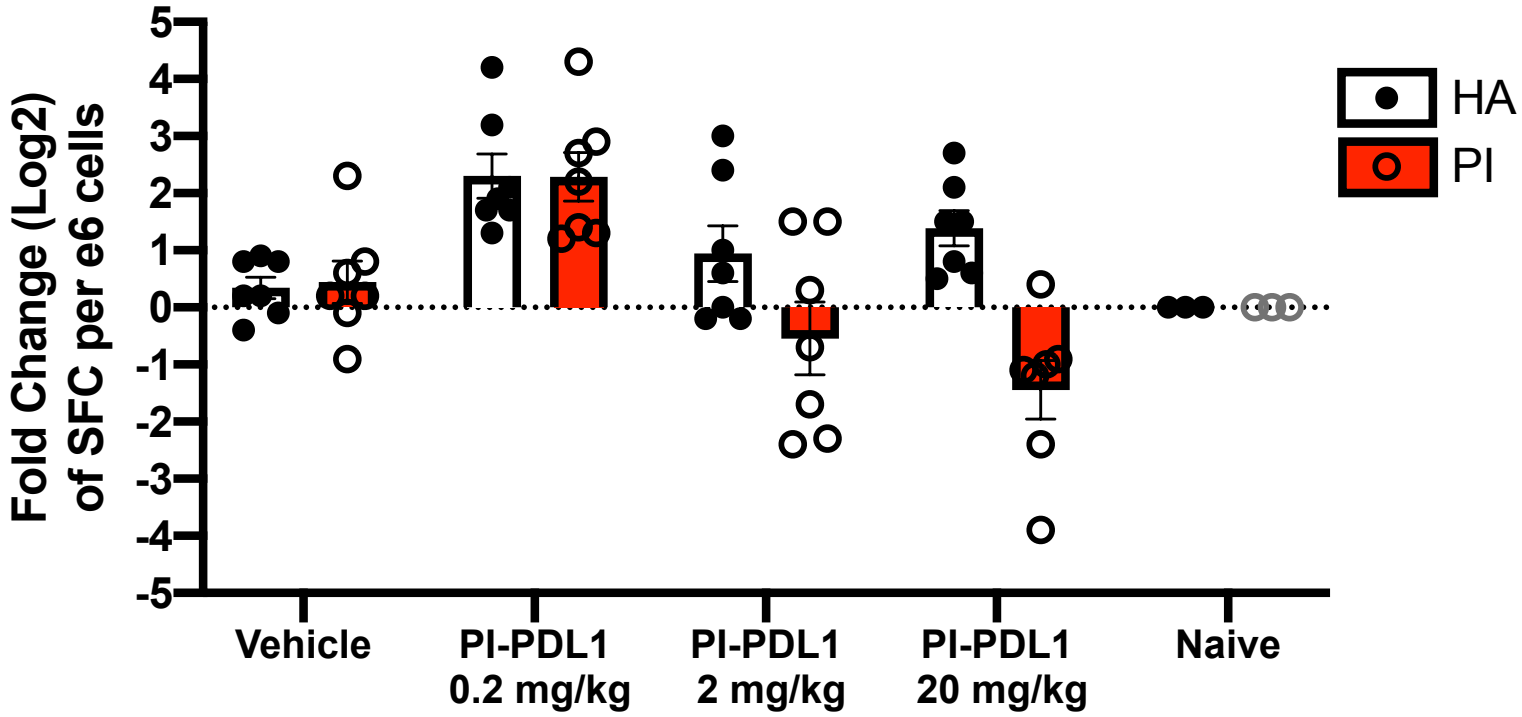
Late Intervention with Immuno-STAT Selectively Reduces the Number of Proins (PI) Responsive CD4+ T Cells In Vivo





Intra-Animal ELISpots Confirm Dose-Dependent and Antigen-Specific Suppression of Proins Response Following Single Administration of IST

Mean ± SEM



- Per animal data confirms dose-dependent inhibition of Proins response relative to pre-dose
- Inhibition of HA response was not observed upon IST administration

In Vivo Conclusions

- Treatment with $Proins_{76\text{-}90,\ K88S}$/DR4-PDL1 Immuno-STAT significantly suppressed expansion of Proins-specific CD4+ T cells in transgenic mice

- The effect of $Proins_{76\text{-}90,\ K88S}$/DR4-PDL1 Immuno-STAT treatment was antigen specific as treatment did not result in a reduction of HA-specific CD4+ T cells

- A single in vivo administration of $Proins_{76\text{-}90,\ K88S}$/DR4-PDL1 Immuno-STAT at the peak of immunization response significantly reduced the frequency of Proins-responsive T cells in a dose-dependent manner

Overall Summary

- Successful generation of Immuno-STAT molecules comprised of Class II HLA molecules (DRB1*04) along with PDL1 inhibitory ligand to selectively dampen autoreactive CD4+ T cells
- Proins-DR4-PDL1 IST exhibits favorable in vitro stability and manufacturability
- Proins-DR4-PDL1 IST potently and selectively inhibits Proins-specific responses in expanded human PBMCs
  - Significant suppression is maintained at 96 hours after wash-out, confirming prolonged pharmacodynamic effect from IST exposure
- Proins-DR4-PDL1 IST selectively inhibits Proins-specific responses in vivo within 24 hours of administration
  - Dose-dependent activity was observed
  - Selective inhibition of Proins response was confirmed using 2 assay formats
- ProIns-DR4-PDL1 IST and the Immuno-STAT platform could provide a promising therapeutic option for T1D patients

Acknowledgement

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