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Agonizing Over Treg Expansion to Treat Human Disease: An Update on Our TNFRSF25-Agonist, PTX-35

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VP of Research & Technology Acquisition
Treg Summit 2022

NIGHTHAWK Ecosystem

Streamlining Innovative Discoveries to Accelerate Clinical Development

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OUR ECOSYSTEM

SKUNKWORX BIO

Heat Biologics

Elusys THERAPEUTICS, INC.

PELICAN Therapeutics

SCORPION BIOLOGICAL SERVICES

Discovery → Preclinical/Clinical Development → Biomanufacturing

Conflict of Interest Disclosure

- The presenter is an employee of NightHawk Biosciences, Inc. (NYSE American: NHWK)

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Acknowledgments & Collaborators

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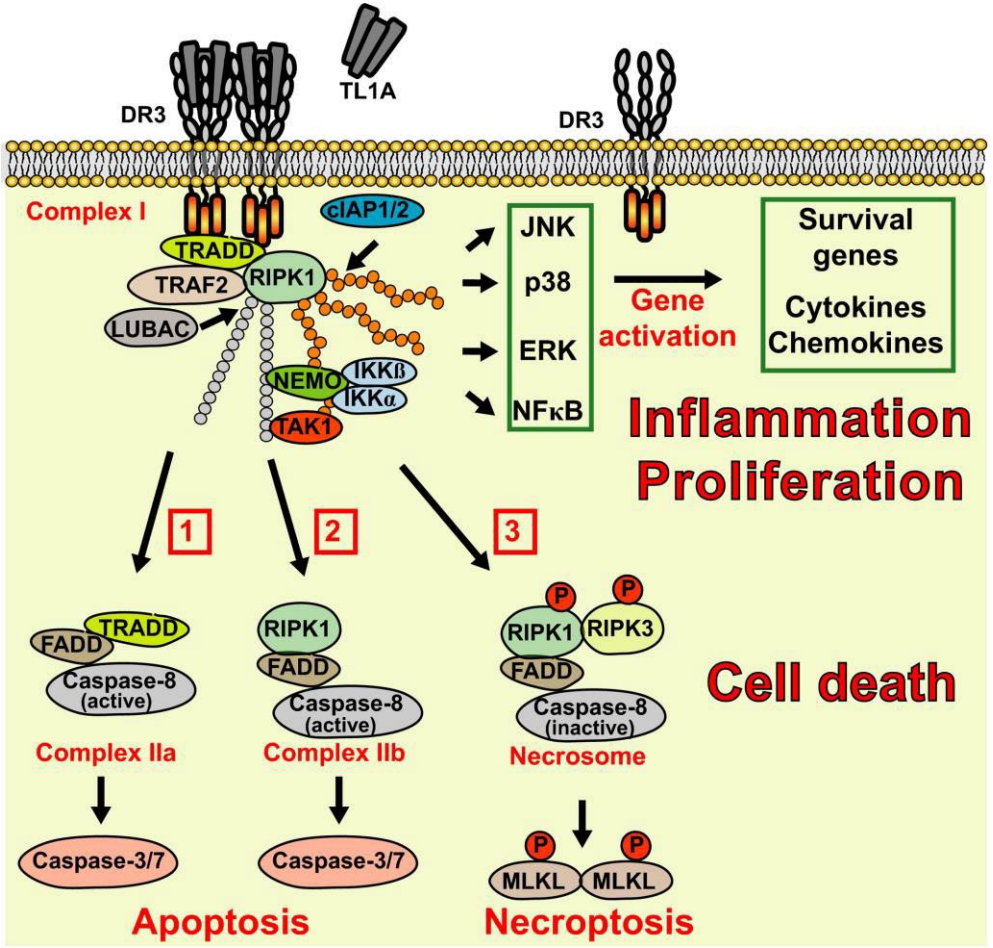
TNFRSF25/DR3 - Biology

- TNFRSF25 also known as DR3 (Death Receptor 3), and infrequently as TRAMP (TNF receptor-related apoptosis-mediated protein), WSL-1, Apo-3, LARD *Bodmer, et al 1997*
- First described in 1996, Type I membrane protein, 417 amino acids, 45kDa *Chinnaiyan et al 1996*
- DR3 designation based on death receptor domain – found by mining genome for sequences with similarity to the death domain contained in TNFR1
- Among all TNF receptors, TNFRSF25 has the highest homology to TNFR1, potentially implying early evolutionary divergence *Bodmer et al. Immunity. 1997, Kitson et al. Nature. 1996, Marsters et al. Curr Biology. 1996, Screaton et al. PNAS. 1997*



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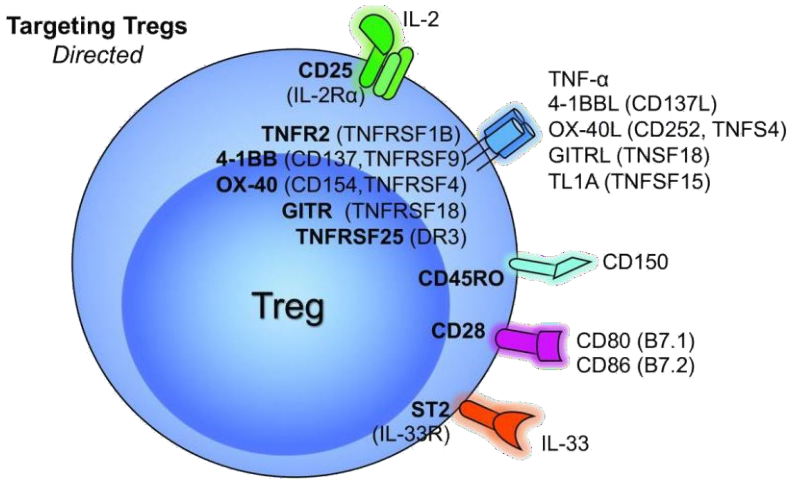
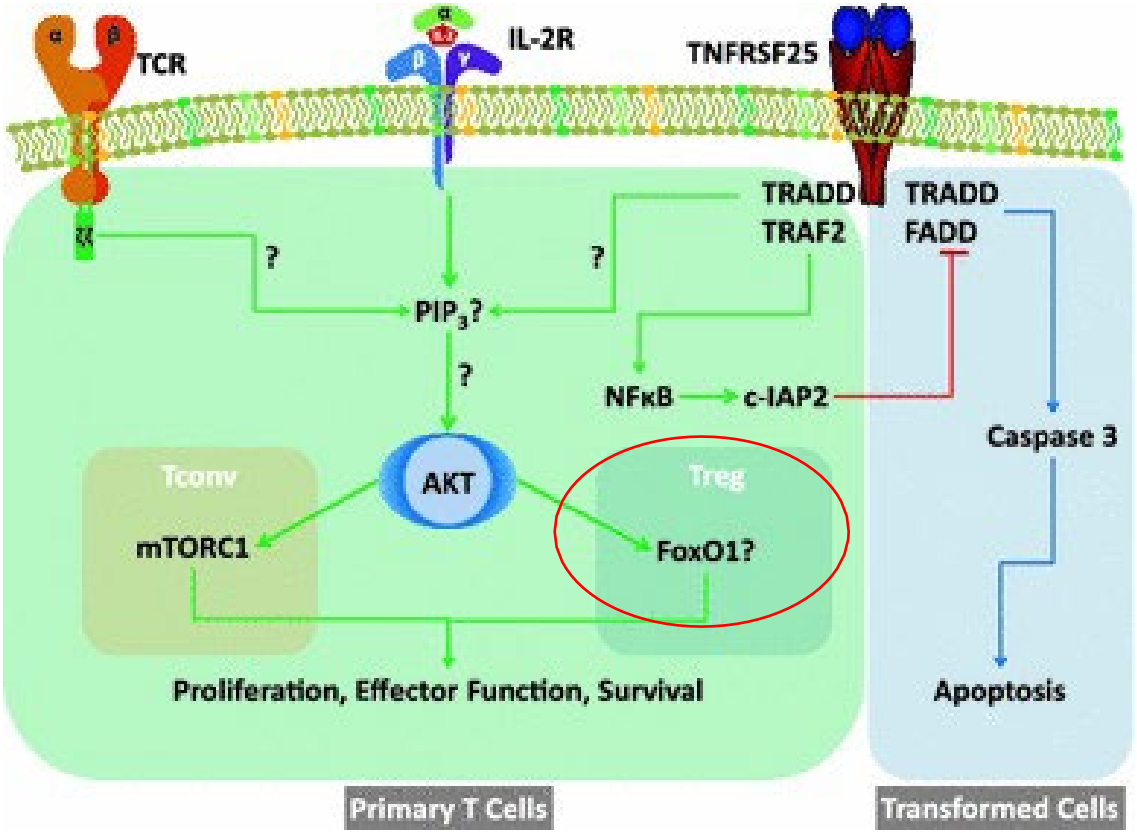
TNFRSF25 Signaling & Tregs

- The activation of the ***TNFRSF25-TL1A axis*** was initially described as an inflammatory disease pathway; its role in Treg and Tcon co-stimulation was discovered later by Schreiber & Podack *Schreiber & Podack 2013*
- In primary cells, TNFRSF25 ligation leads to recruitment of "complex 1"(TRADD/TRAF2), NF-κB activation, survival, proliferation, and effector function
  Warzocha and Salles 1998 ; Wen et al. 2003





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PTX-35 Mechanism of Action

Immunomodulatory Activity Dependent on Presence or Absence of Danger Signal

TNFRSF25

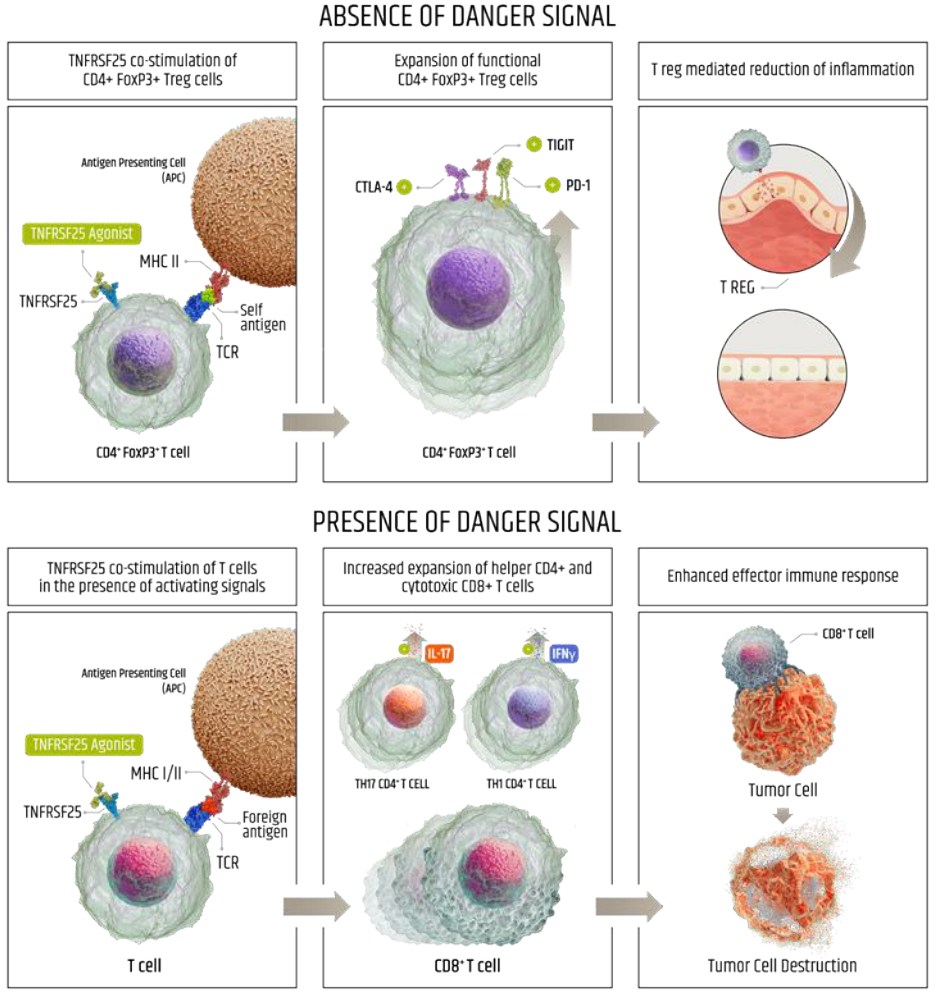
- Recognizes TNF-like ligand 1A secreted by several immune cell types
- Highly and constitutively expressed on $CD4^+$ $FoxP3^+$ regulatory T cells

Absence of an activating signal, co-stimulation of TNFRSF25 promotes

- Expansion of immunosuppressive Treg cells
- Treg expression of immunosuppressive markers CTLA4, TIGIT, and PD-1
- Minimal impact on resting $CD4^+$ and $CD8^+$ T cells

Presence of an activating signal, co-stimulation of TNFRSF25 promotes

- Enhanced expansion of activated $CD8^+$ effector T cells
- Increased percent of inflammatory $IFN\psi^+$ Th1 & $IL\text{-}17^+$ Th17 $CD4^+$ T cells
- Decreased Treg function characterized by reduced CTLA4 expression



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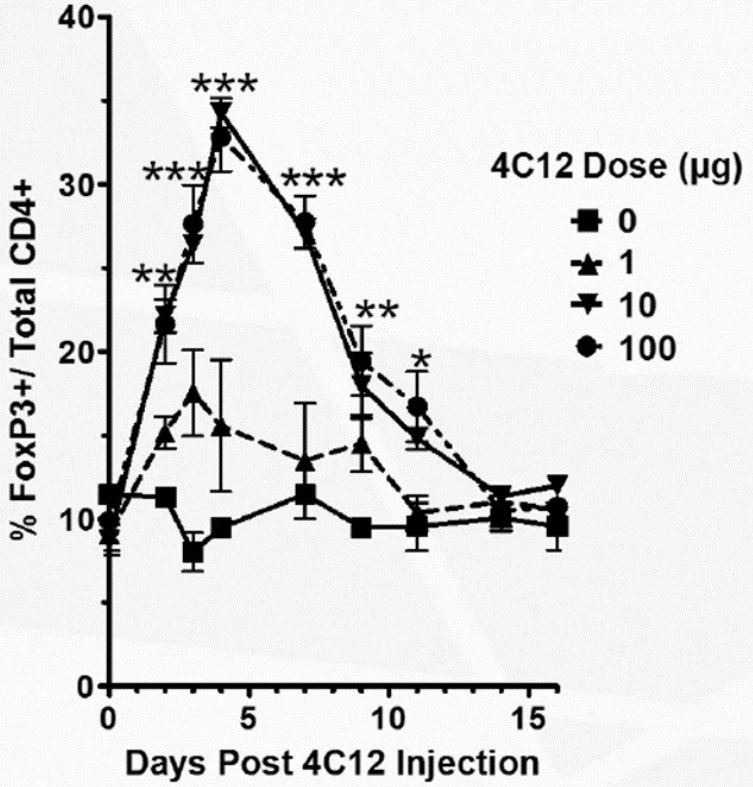
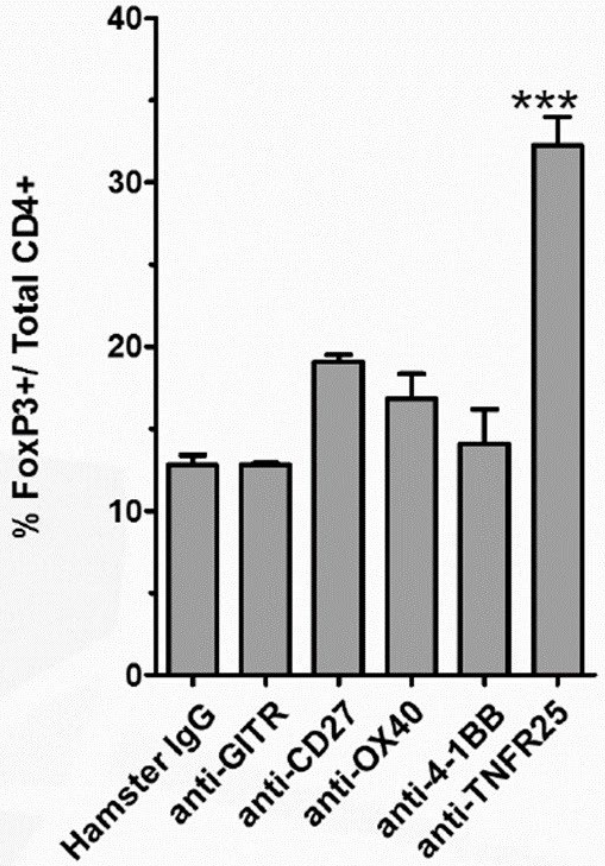
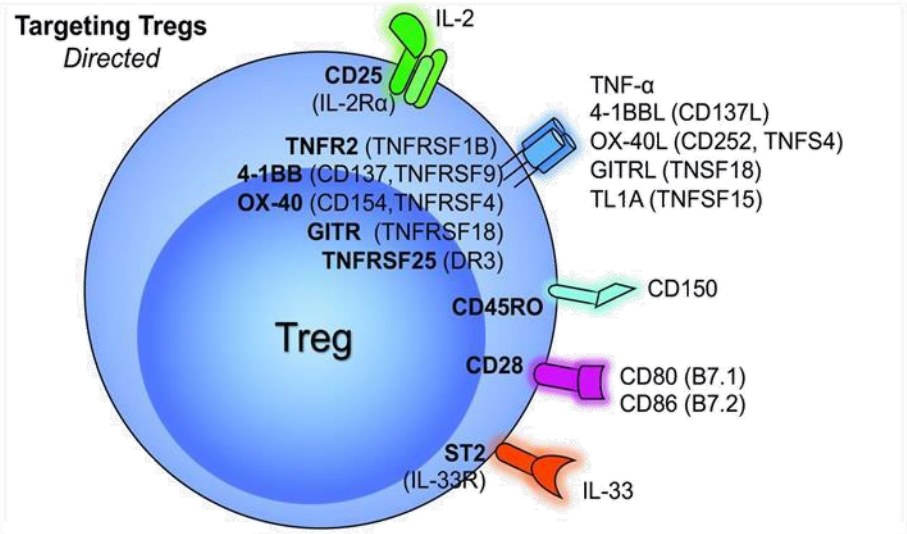
TNFRSF25 Engagement & Treg Expansion





Compared to other TNFR family co-stimulators, TNFRSF25 agonism is most potent in Treg expansion

Schreiber & Wolf *et al. J Clin Invest.* 2010;**120**(10):3629-3640



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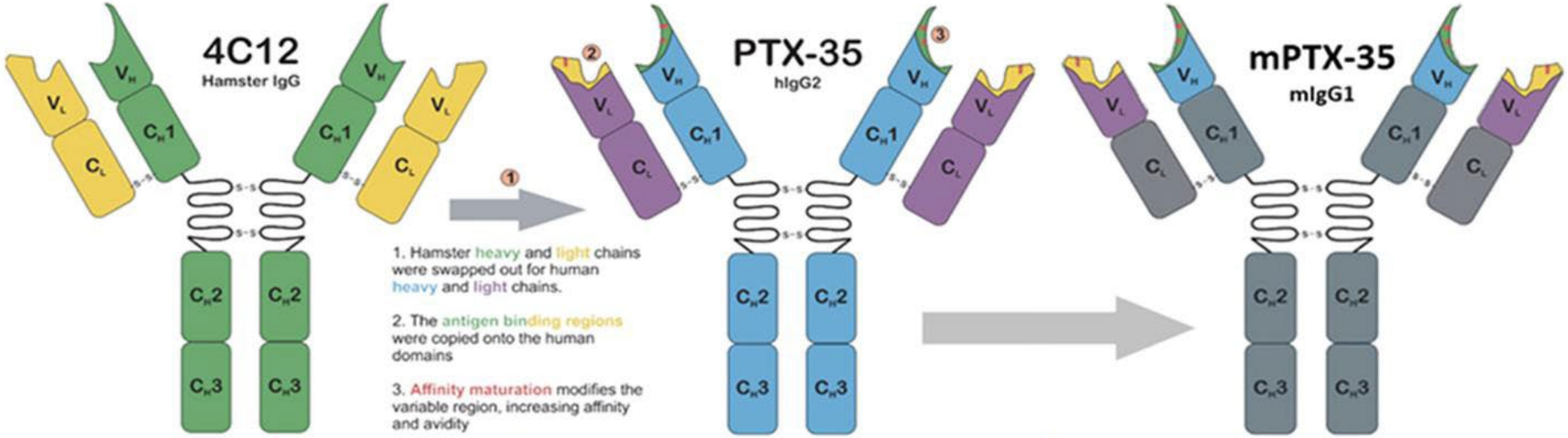
Generation of PTX-35 & Research Tools



Parental
Hamster IgG anti-TNFRSF25

Original Ab
Good for early POC

Affinity Matured
Human IgG2 anti-Human TNFRSF25

Human Ab
Clinical Candidate

Affinity Matured
Mouse IgG1 anti-Human TNFRSF25

Human Surrogate Ab
Preclinical Testing in Mice

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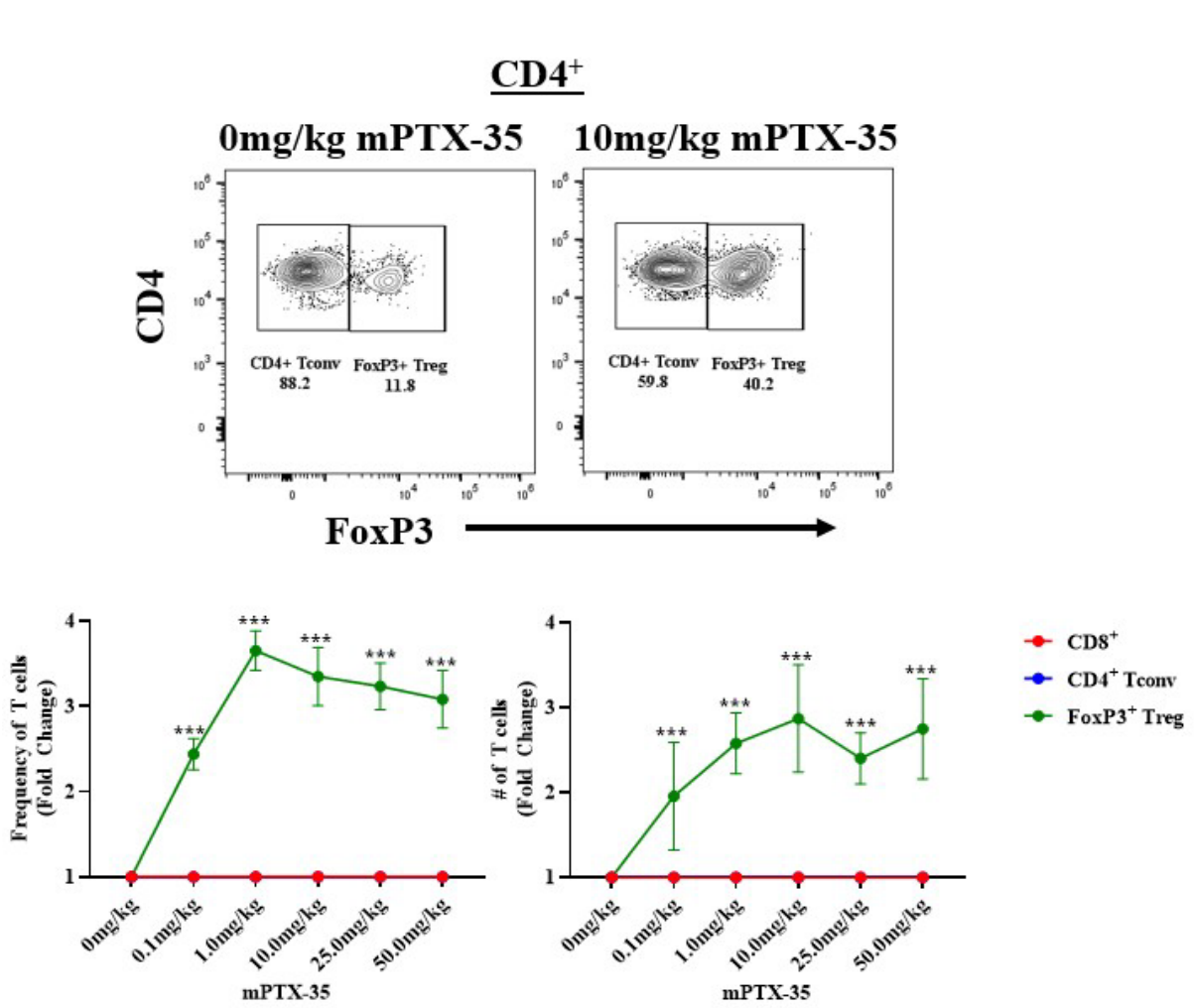
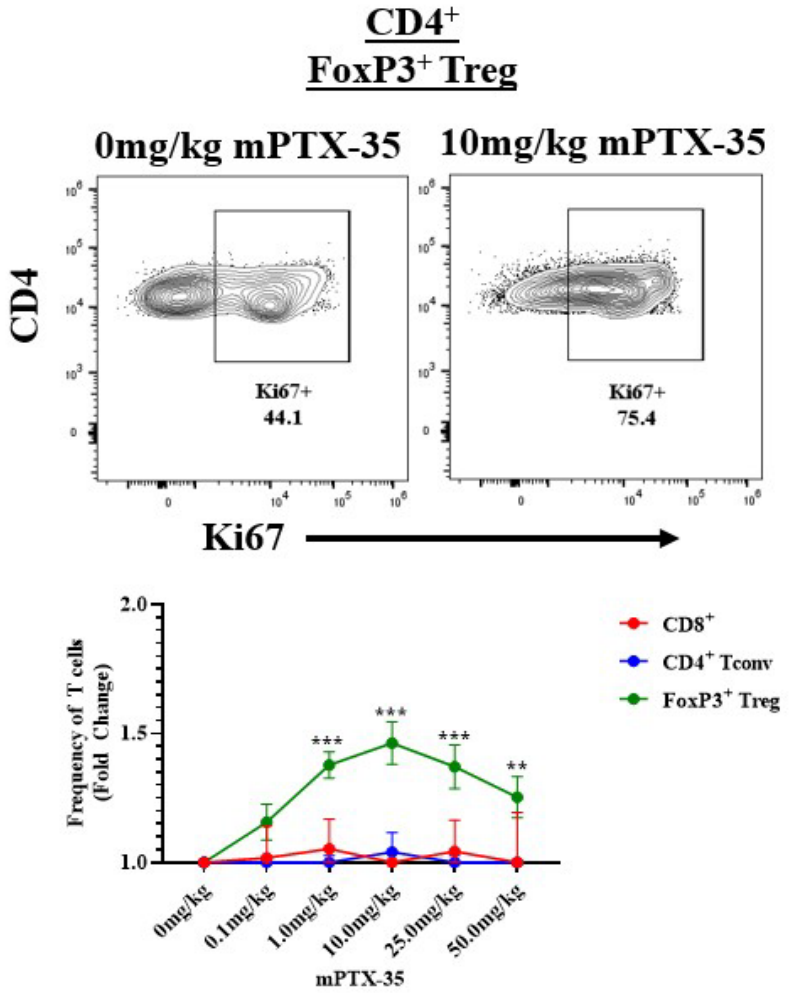
PTX-35 Preferentially Expands Tregs





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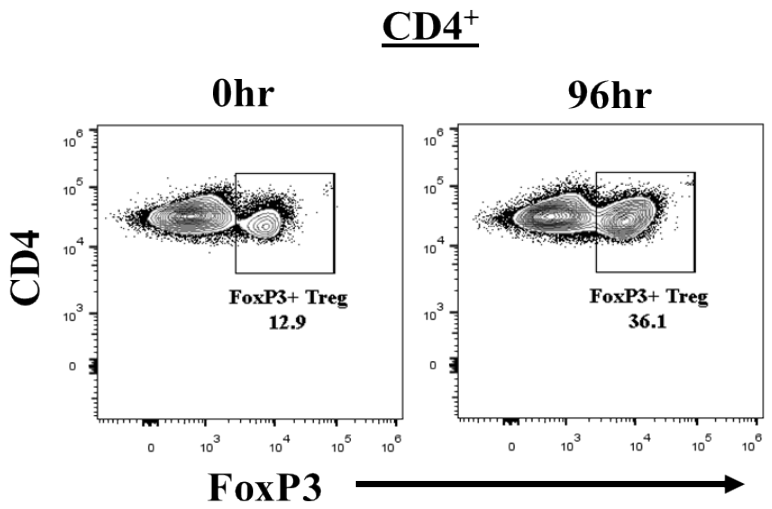
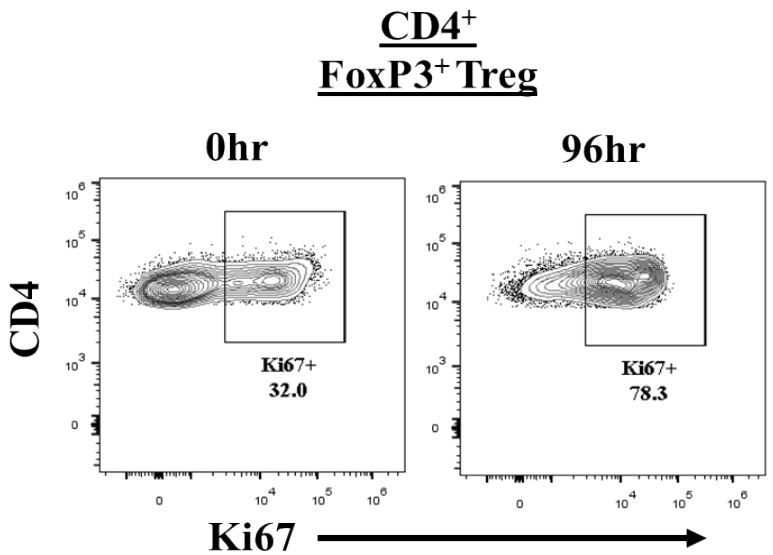
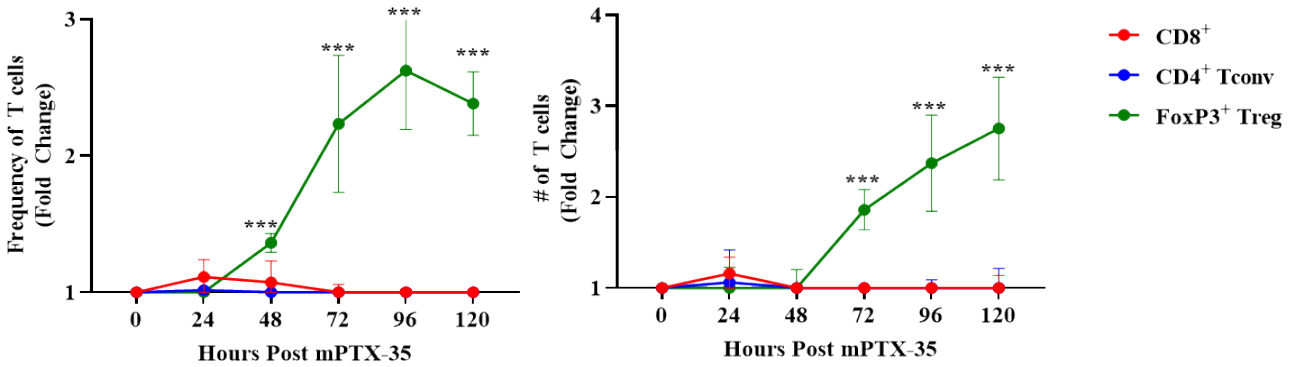
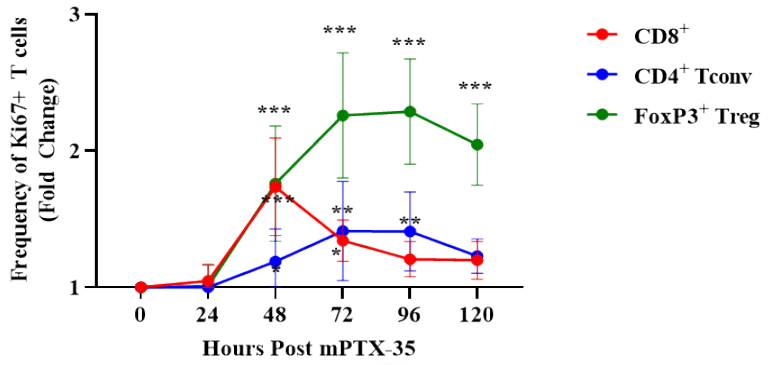
PTX-35 Treg Expansion Is Durable









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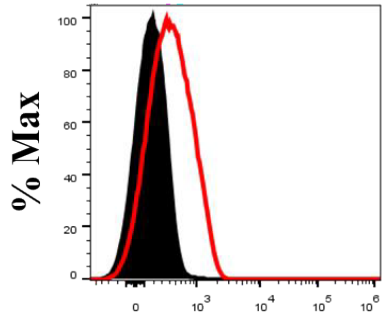
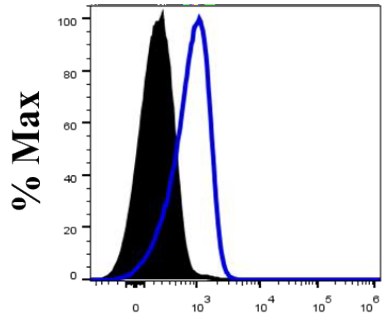
TNFRSF25 Expression Correlates with Activity

CD8+



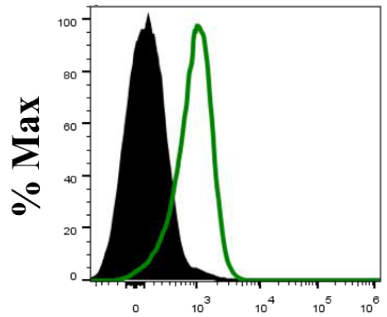
= Isotype
= mPTX-35

CD4+ Tconv

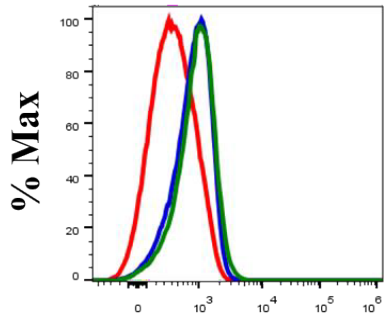


= Isotype
= mPTX-35

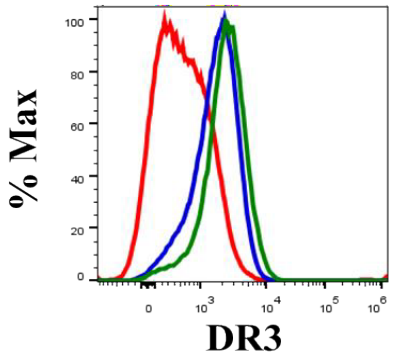
FoxP3+ Treg



= Isotype
= mPTX-35



= CD8+
= CD4+ Tconv
= FoxP3+ Treg



= CD8+
= CD4+ Tconv
= FoxP3+ Treg

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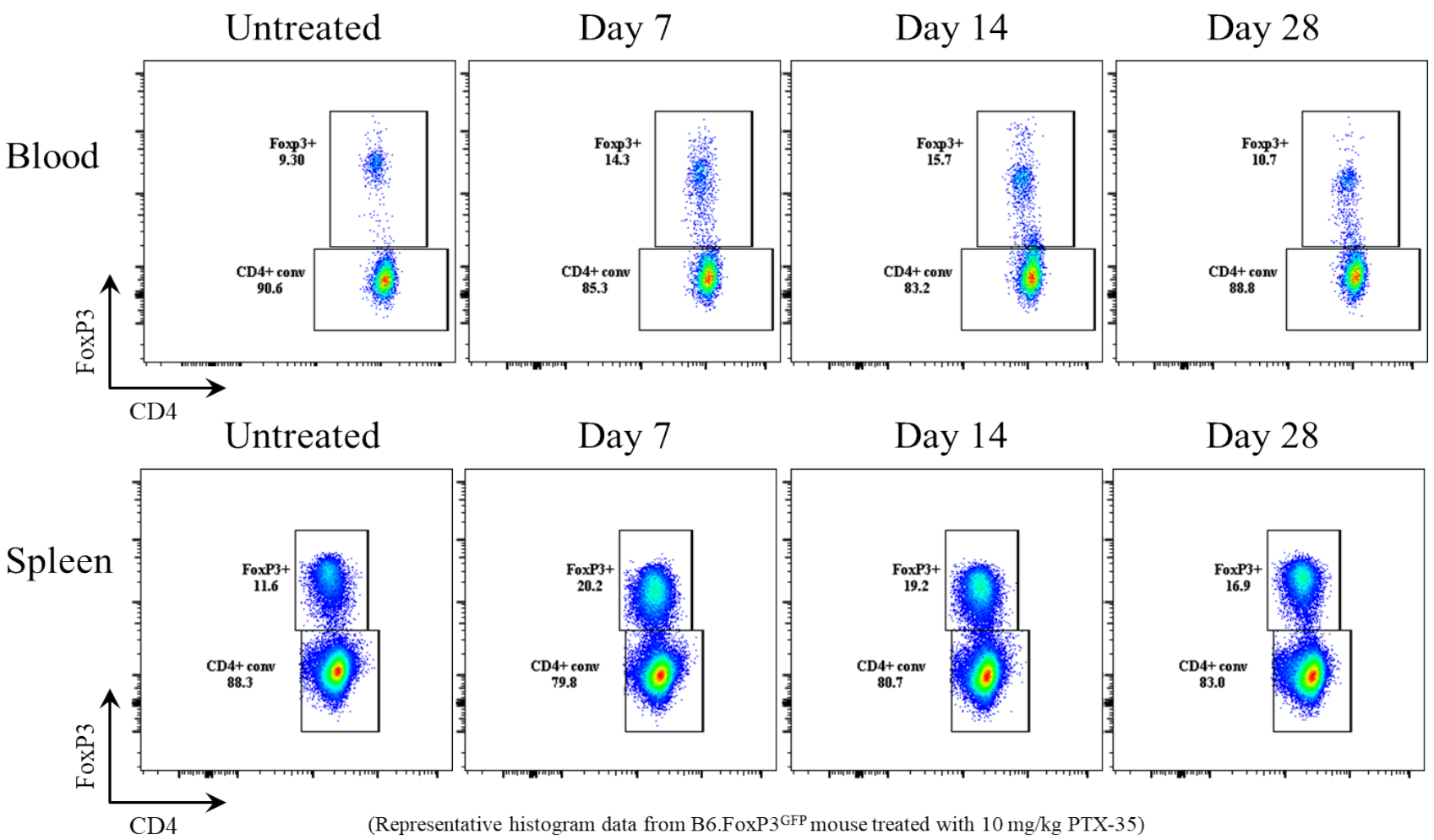
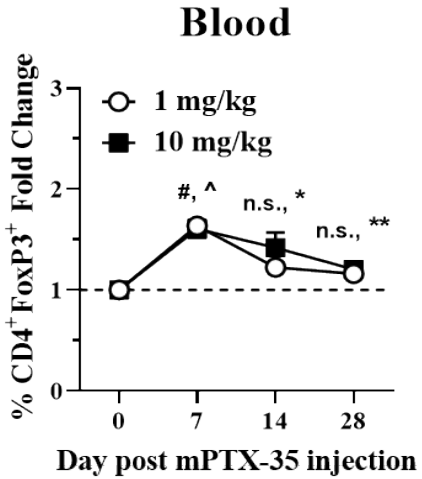
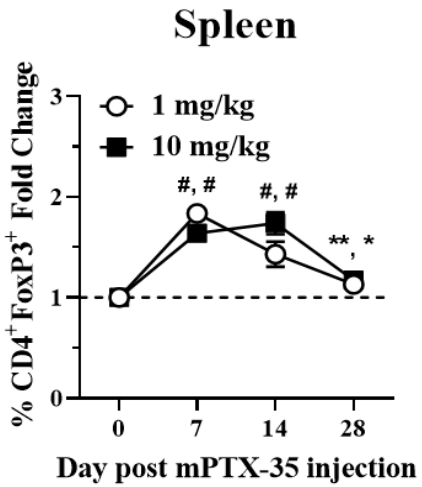
PTX-35 Treg Expansion is Observed in the Blood & Spleen for Weeks



(Representative histogram data from B6.FoxP3^{GFP} mouse treated with 10 mg/kg PTX-35)





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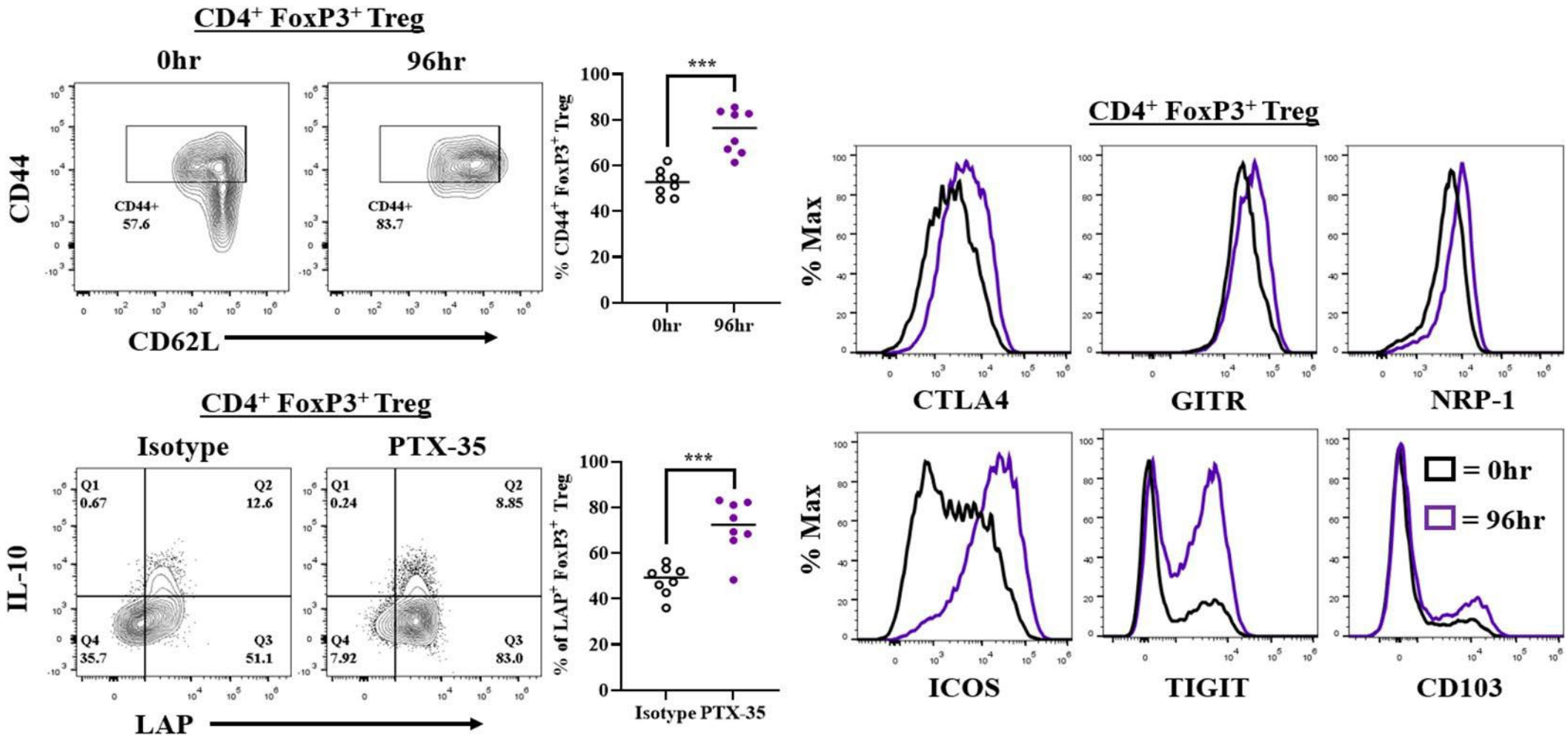
PTX-35 Expanded Treg Subsets Express Suppressive Molecules



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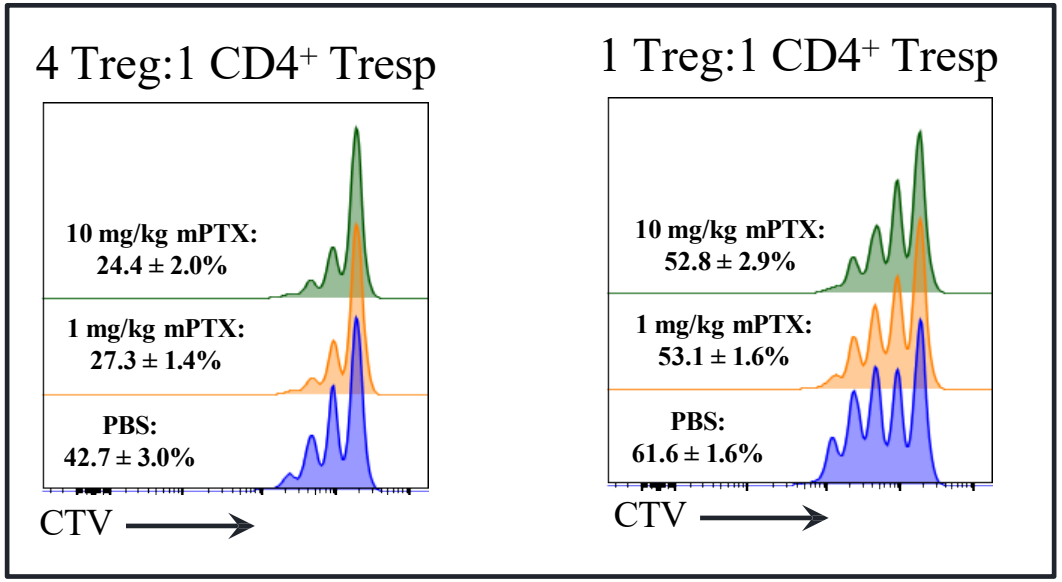
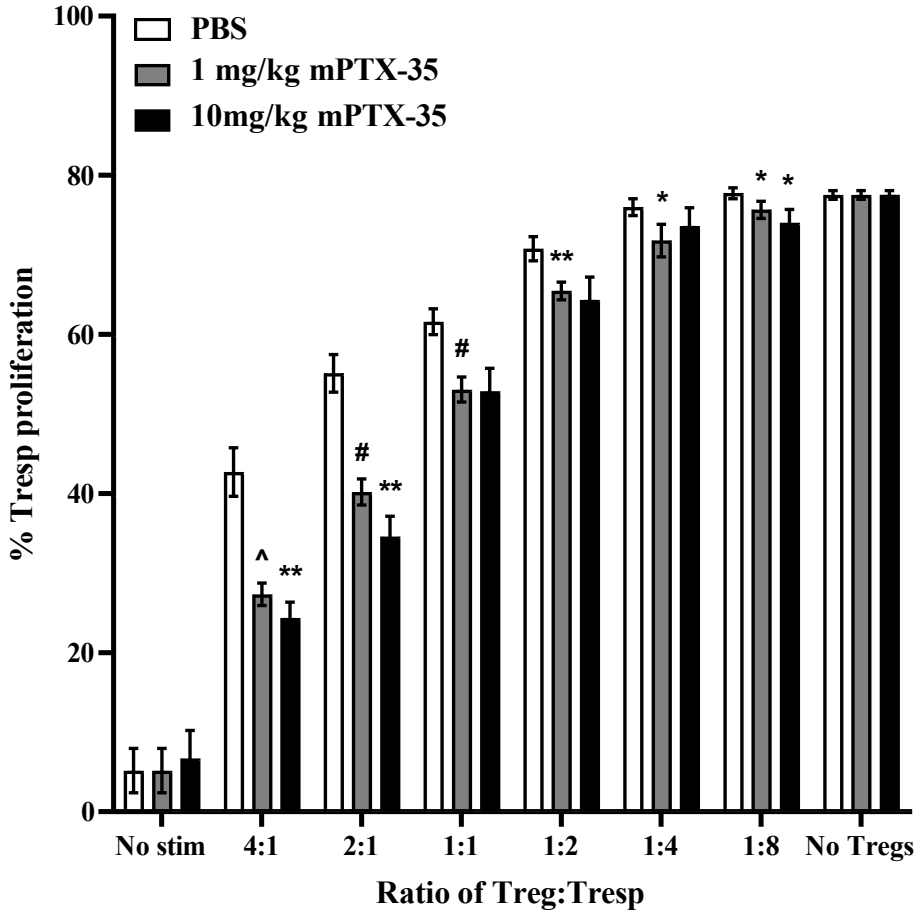
PTX-35-Expanded Treg Subsets are Functional and Retain Suppressive Activity





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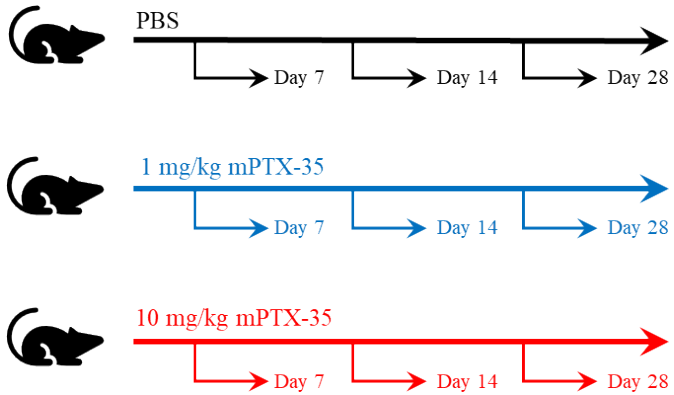
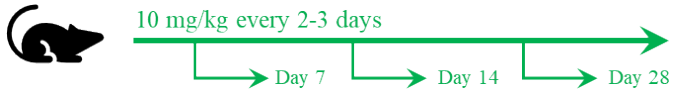
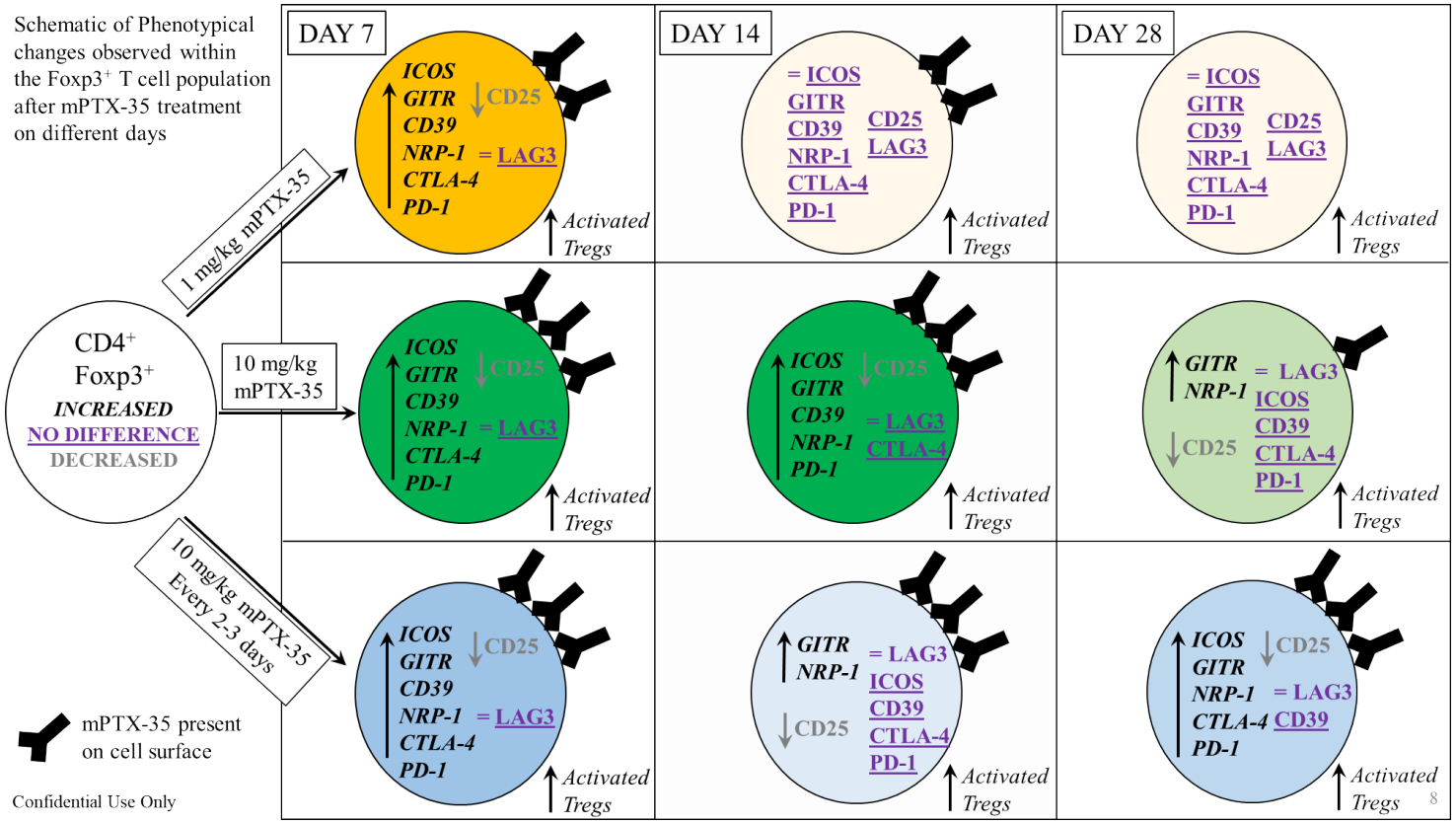
Repeat Dosing of PTX-35 is Not Required for Enhanced Suppressive Treg Phenotype

Single PTX-35 injection



Multiple PTX-35 injections





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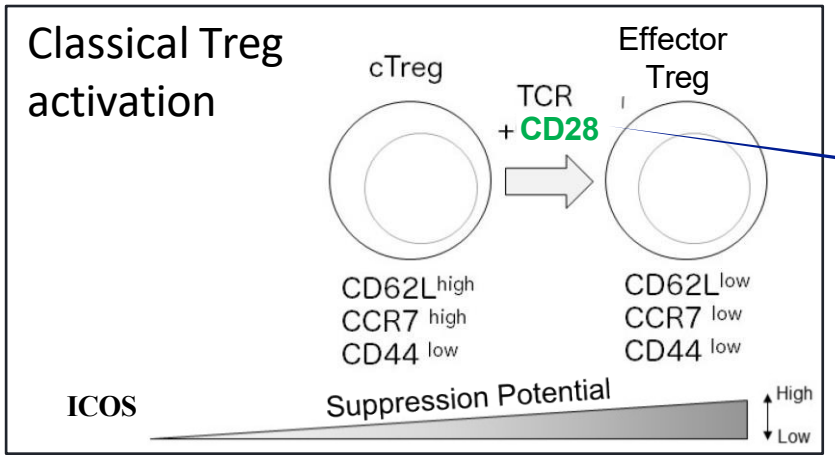
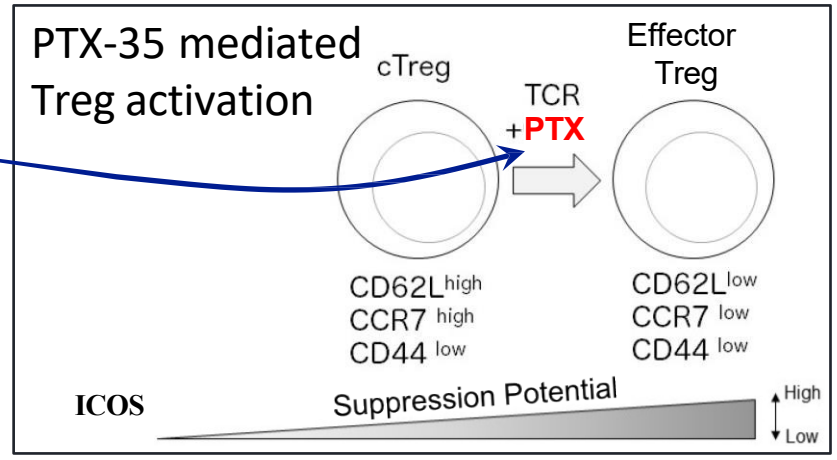
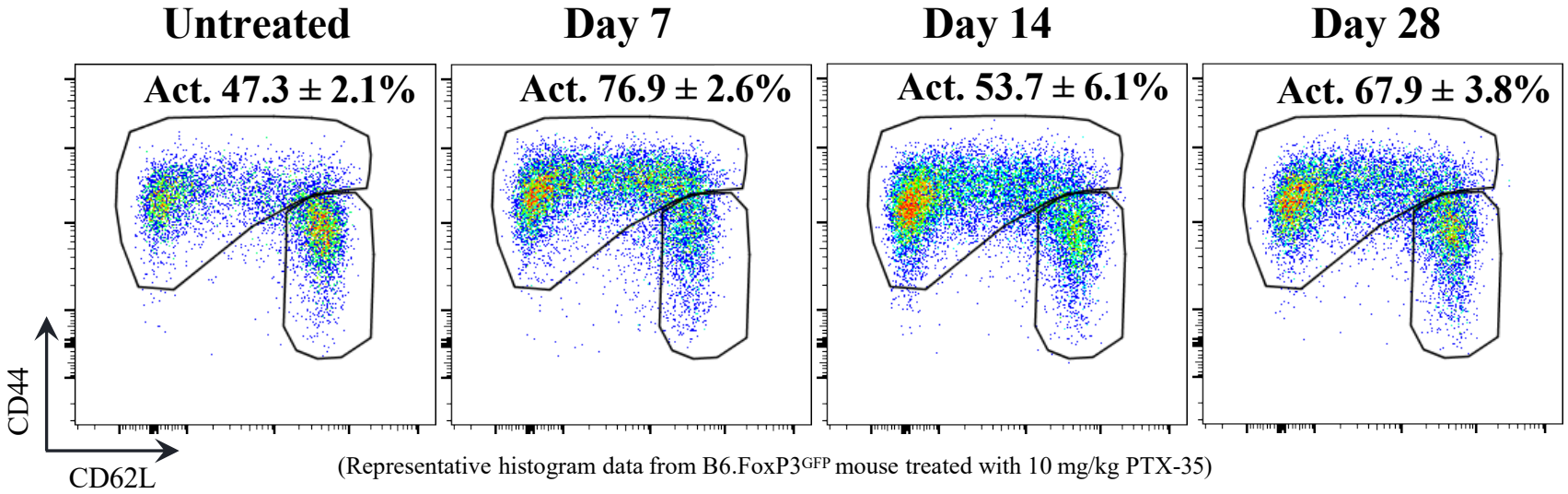
PTX-35 Durability – Single Dose Produces Lasting, Suppressive, Phenotype In Vivo

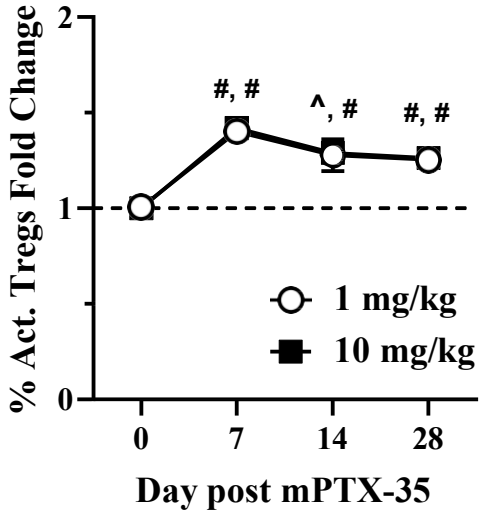






(Representative histogram data from B6.FoxP3^{GFP} mouse treated with 10 mg/kg PTX-35)

Unpaired Student's t-test: *, p<.05, ^ p<0.001, # <0.0001, N= ≥6 mice



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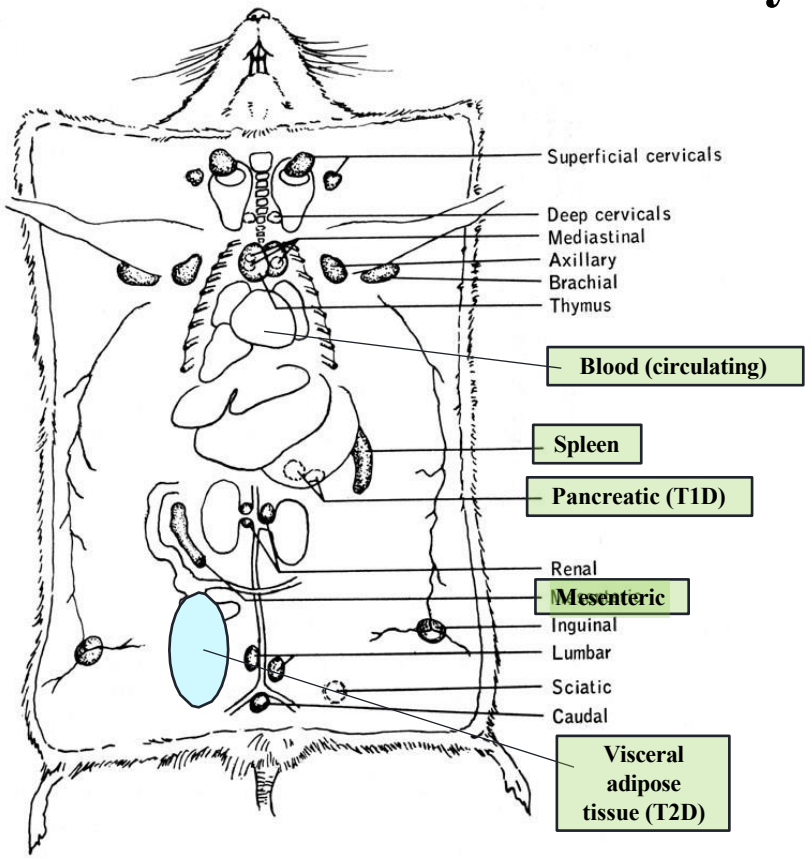
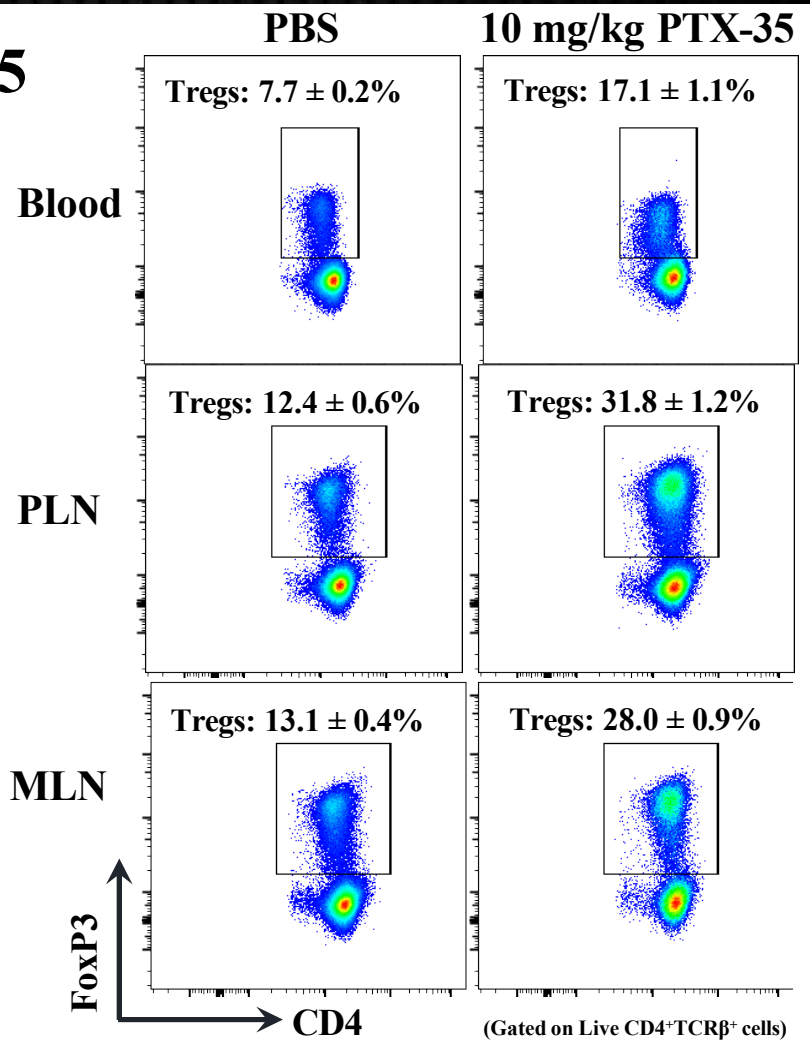
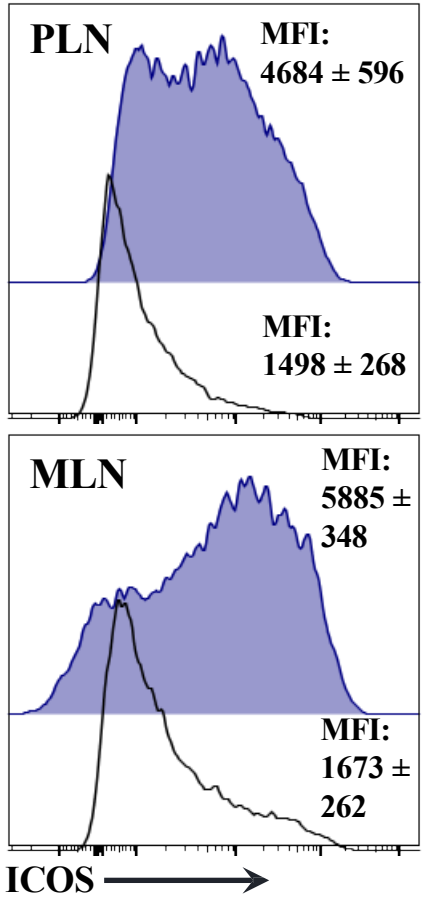
PTX-35 Enhanced Treg Phenotype is Observed in Multiple Tissues







PBS
10 mg/kg PTX-35

N= 4-5 mice

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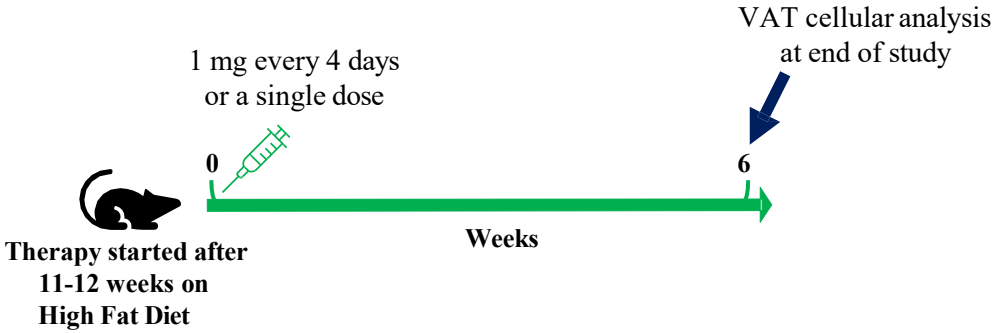
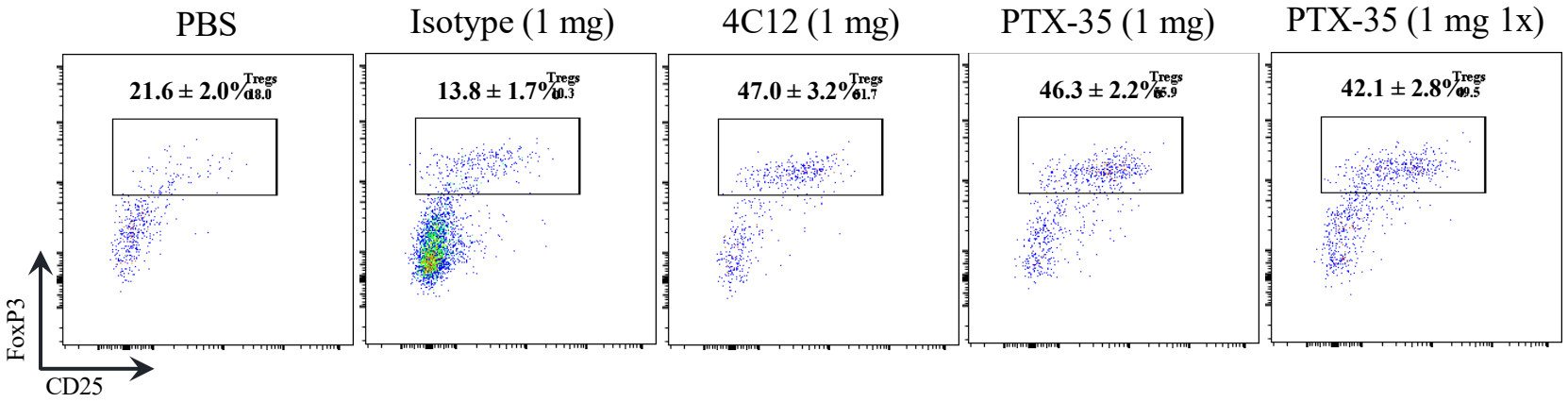
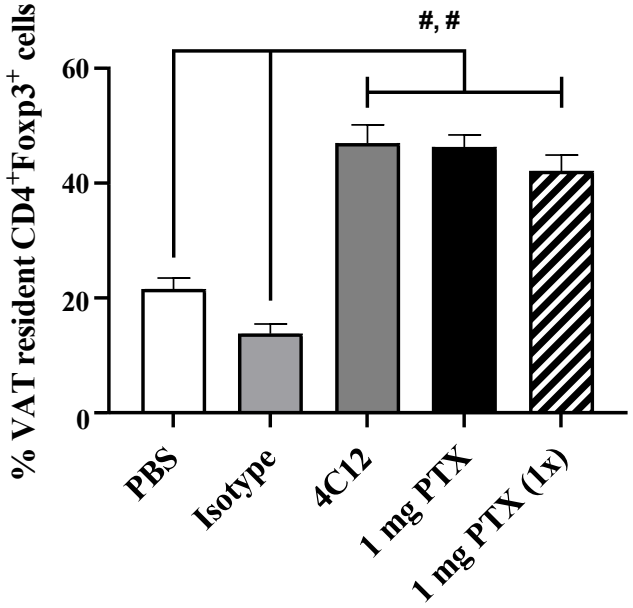
PTX-35 Expands Visceral Adipose Tissue Tregs





Unpaired Student's t-test: # <0.0001, N= 14-15 mice



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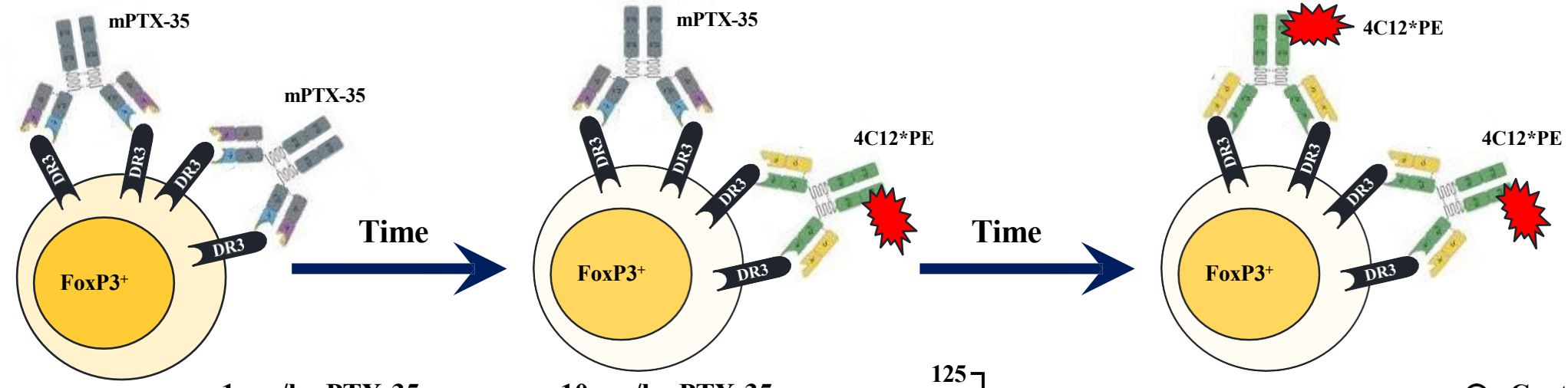
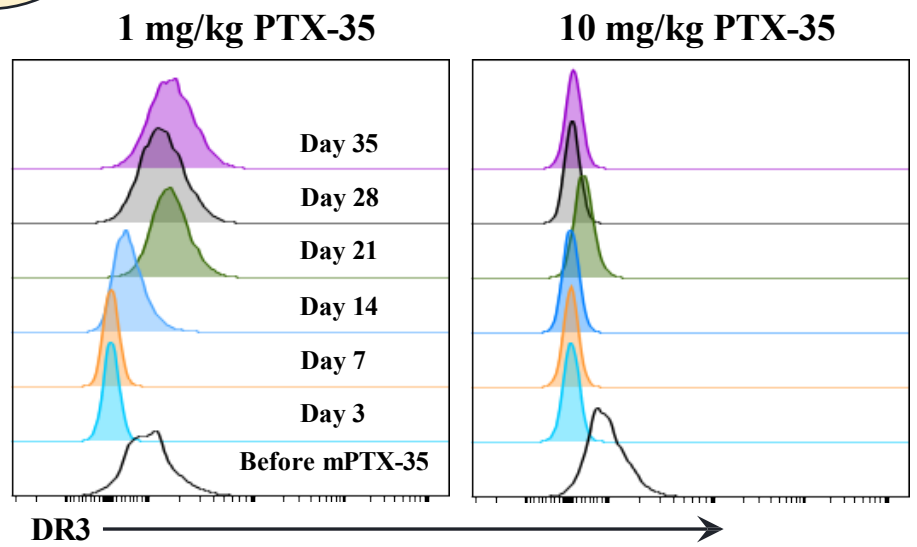
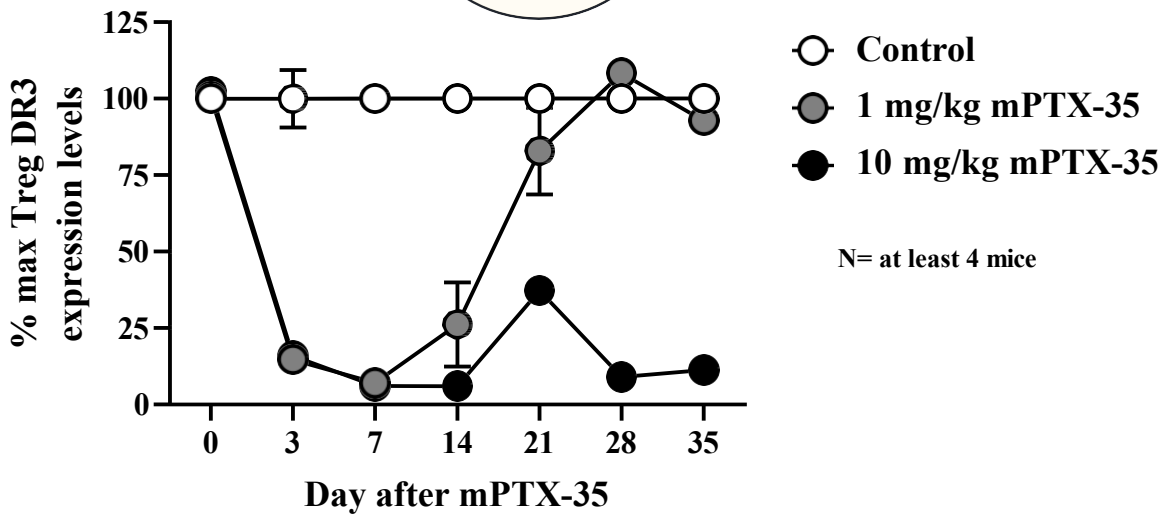
Sustained Receptor Occupancy of PTX-35 In Vivo, Explains Single Dose Pharmacodynamics





(Representative histogram gated on live CD4+FoxP3+ cells)



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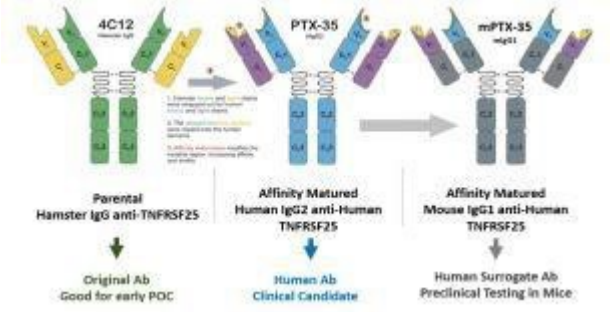
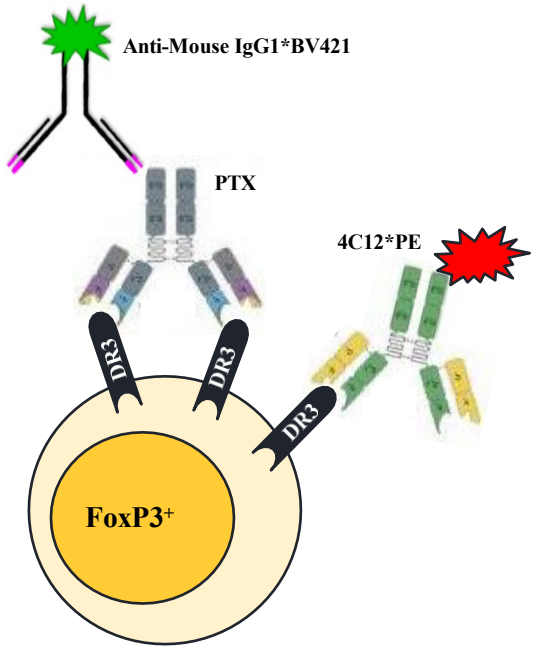
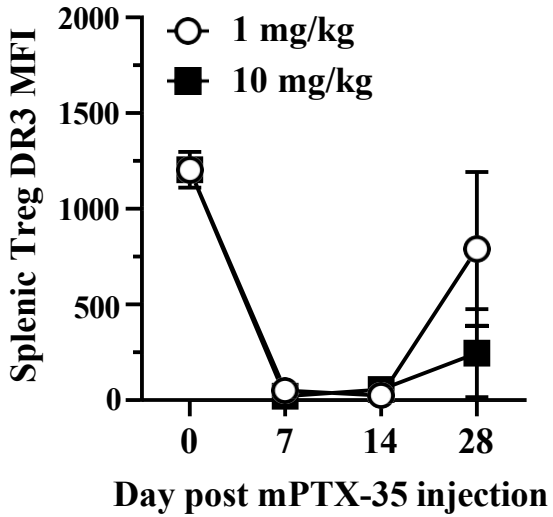
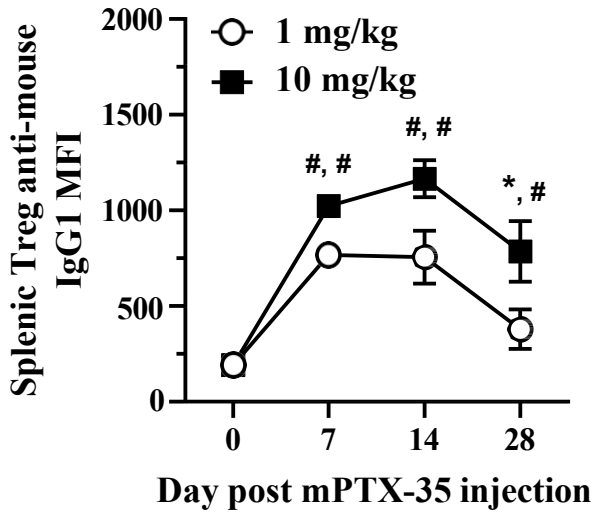
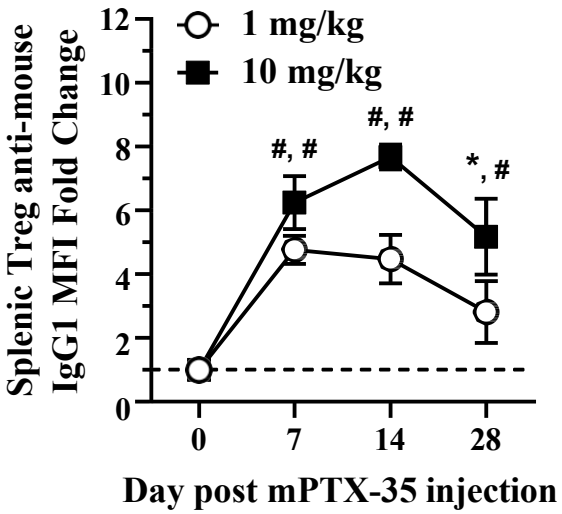
PTX-35 Can Be Detected Bound to Blood TNFRSF25, Four Weeks Post Single-Dose Administration

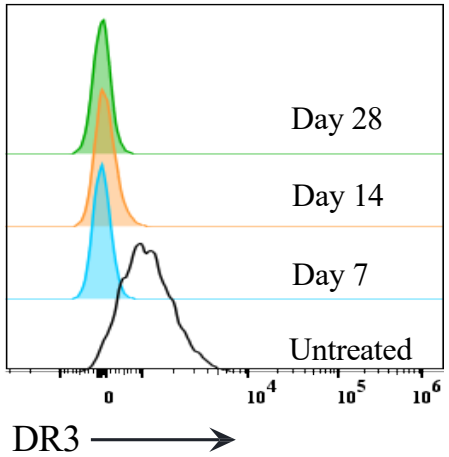


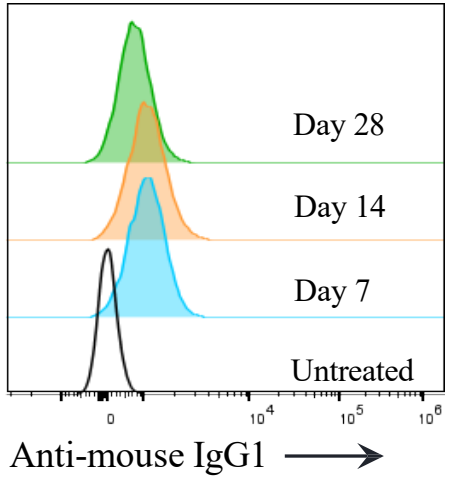








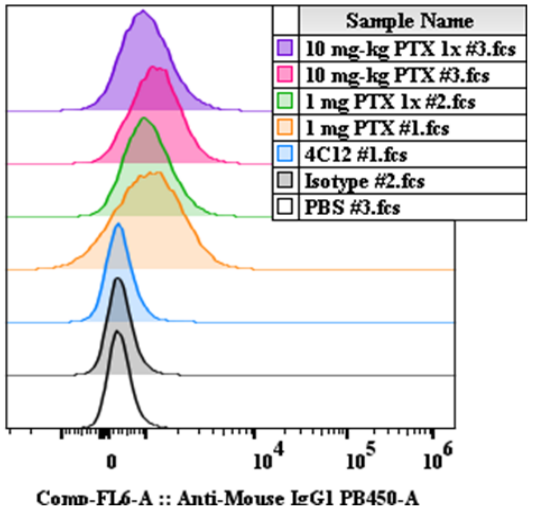


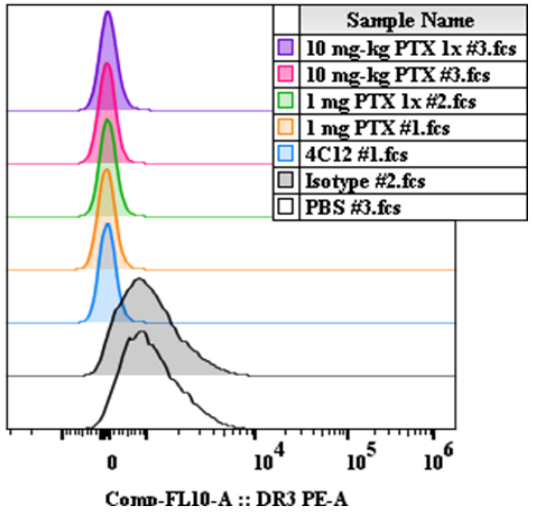


(Representative histogram data from B6.FoxP3^{GFP} mouse treated with 10 mg/kg PTX-35)

NIGHTHAWK BIOSCIENCES

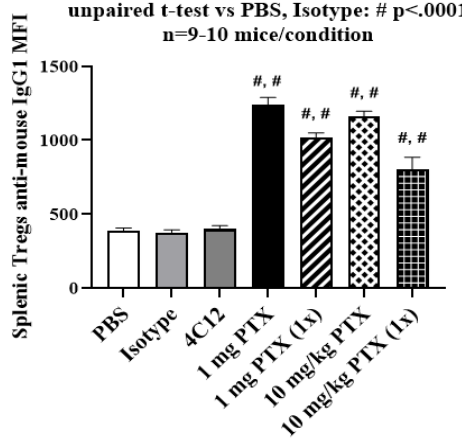
PTX-35 Can Be Detected Bound to Splenic TNFRSF25, Six Weeks Post Single-Dose Administration



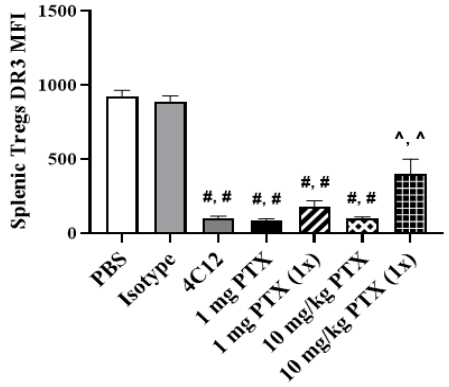


(Representative histogram data from T2D reversion experiment #2 study)

Splenic Tregs anti-mouse IgG1 MFI (to detect PTX-35) 6 weeks after starting antibody treatment and receiving injections either every 4 days or only one time (1x)
unpaired t-test vs PBS, Isotype: # p<.0001
n=9-10 mice/condition

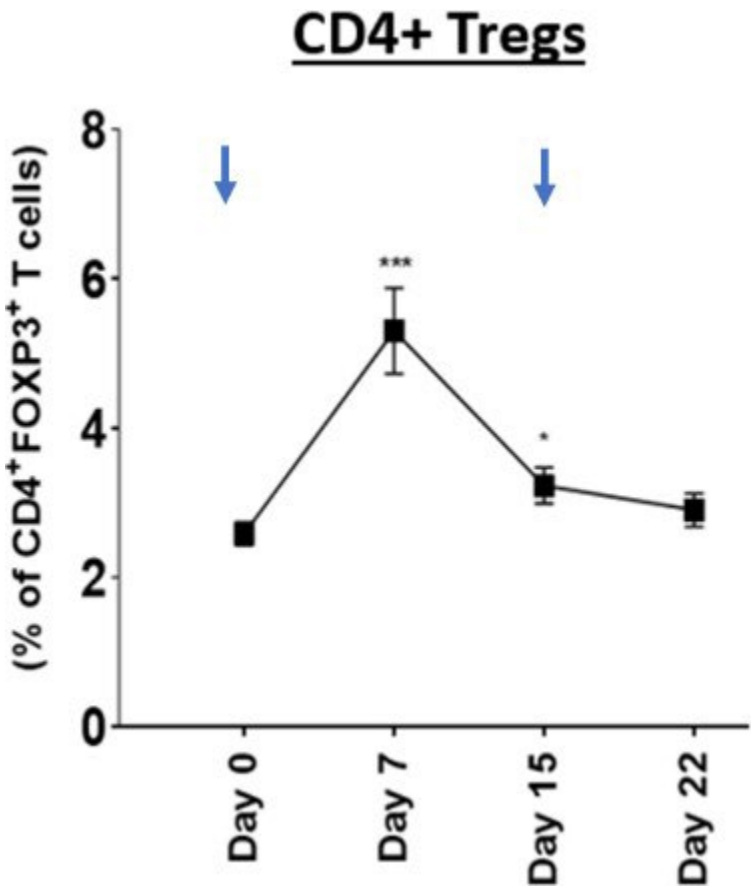


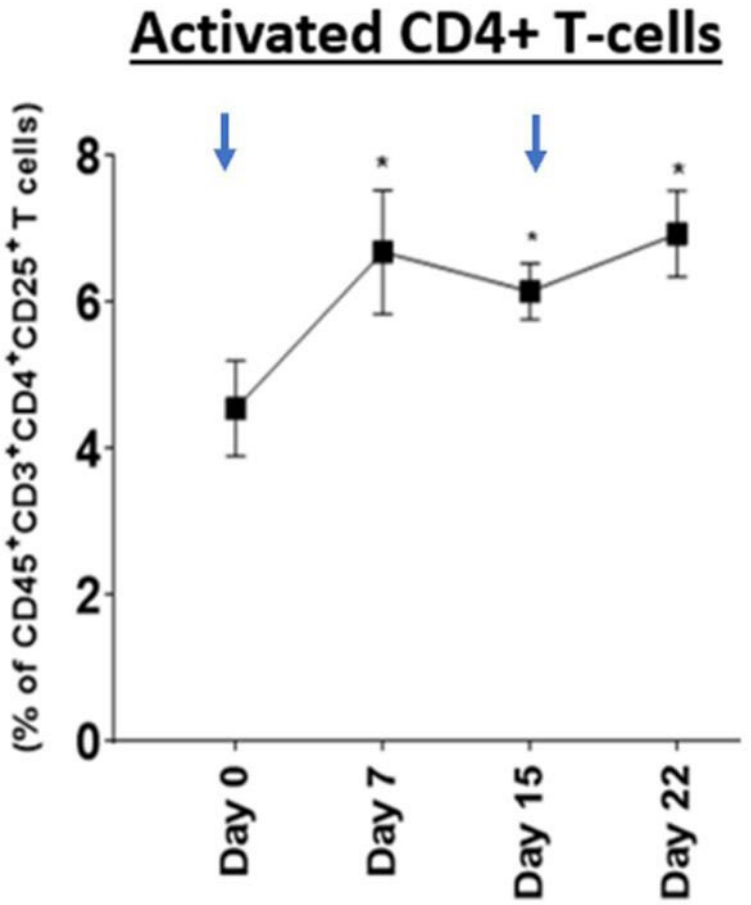
Splenic Treg "free" DR3 antibody binding site expression levels 6 weeks after starting antibody treatment and receiving injections either every 4 days or only one time (1x)
unpaired t-test vs PBS, Isotype: # p<.0001
n=9-10 mice/condition



NIGHTHAWK BIOSCIENCES

PTX-35 Can Expand Tregs in Primates





Human PTX-35

- Cynomolgus monkeys dosed with 100 mg/kg as part of a 4-week acute tox study
- Arrows show dose points for human PTX-35, as i.v. bolus

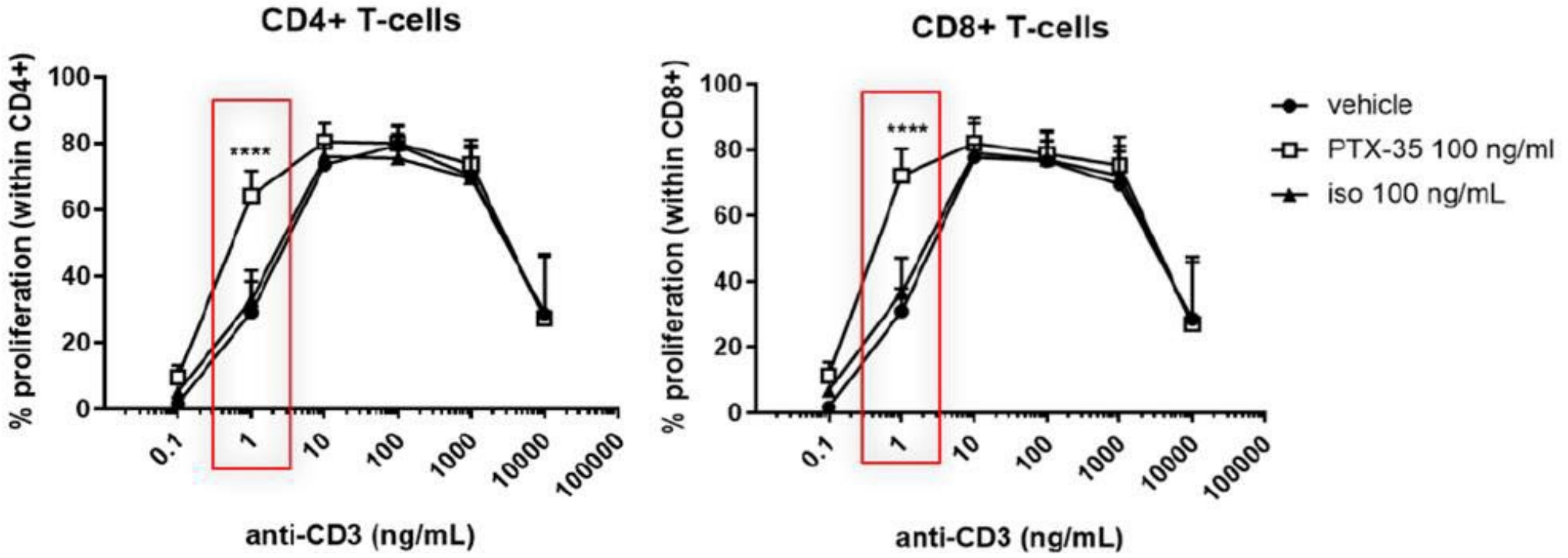
NIGHTHAWK BIOSCIENCES

TCR-Engagement Is Required for TNFRSF25 Agonism

PTX-35 on Human Peripheral Blood Mononuclear Cells (PBMCs)

- CFSE-labeled human PBMCs (n=4 donors) were stimulated with plate-bound anti-CD3 for 72 hours then analyzed by flow cytometry. Graphs show mean + SEM; ****p<0.0001 by two-way ANOVA comparing PTX-35 to isotype control group

Human PTX-35

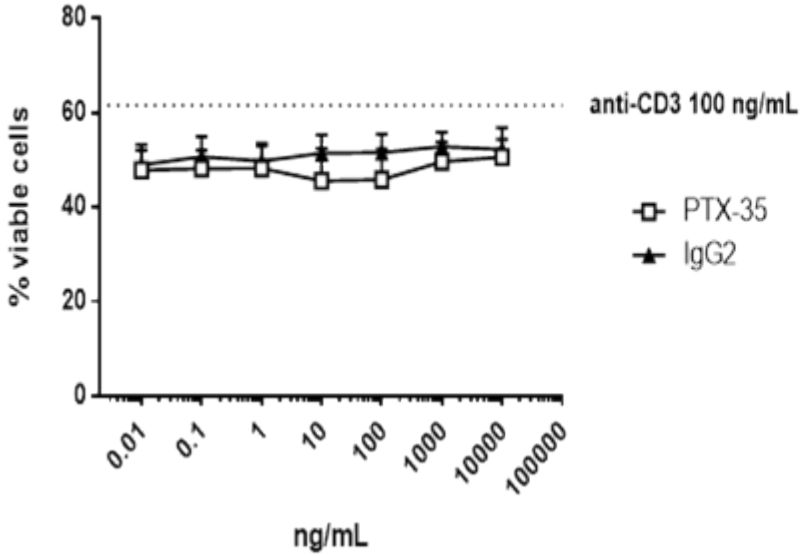


NIGHTHAWK BIOSCIENCES

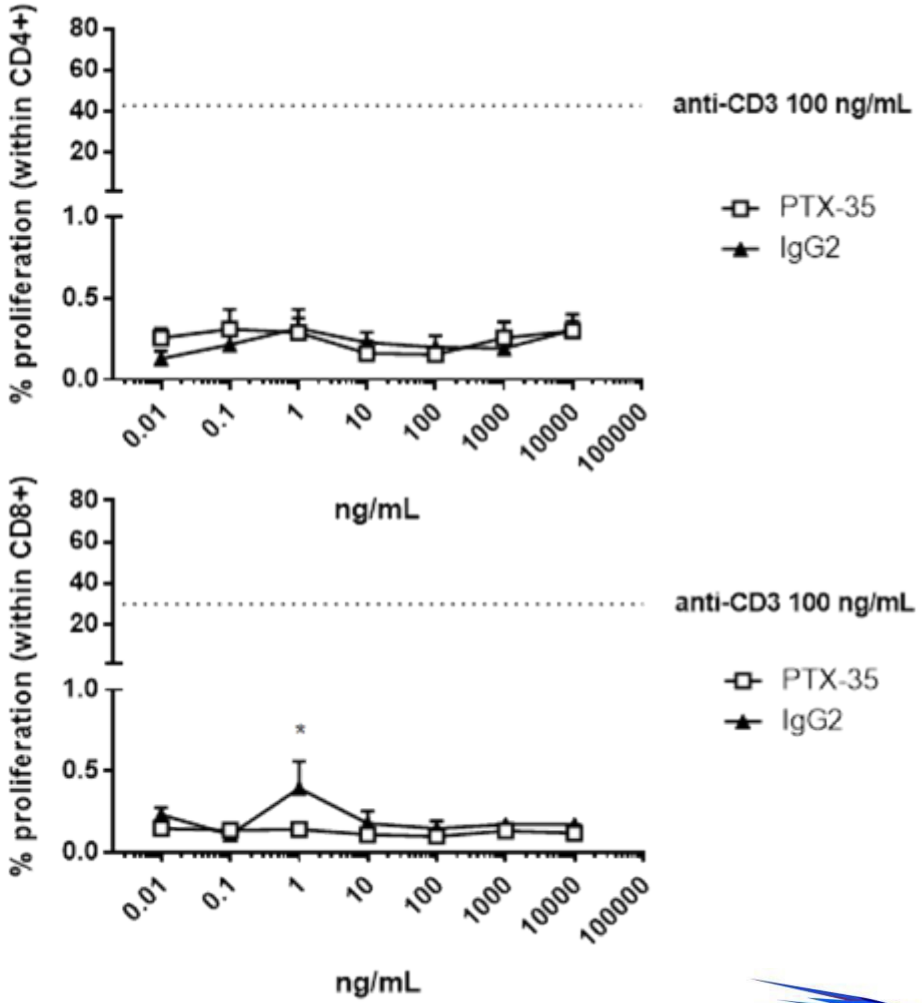
No Non-Specific Cellular Responses or Cytokines

PTX-35 Engagement without TCR-Stimulation

Human PTX-35



- **Cytokines:**
  - **Tested 34 cytokines**
  - **Zero elevated above isotype control**
  - **Positive control worked**



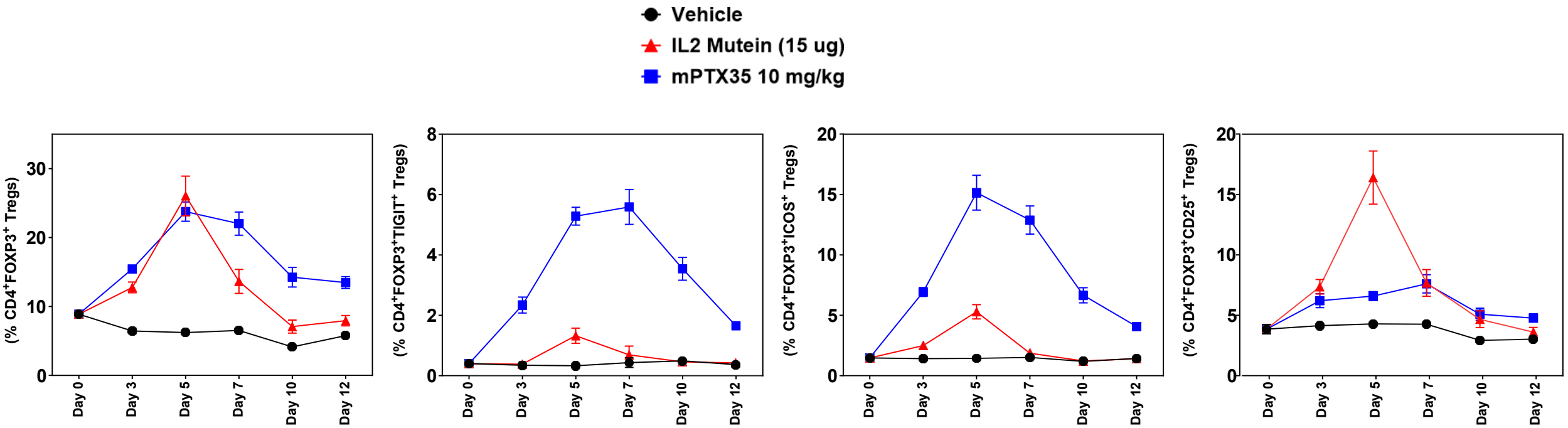
NIGHTHAWK BIOSCIENCES

PTX-35 Nonclinical Product Profile

Type		Measured Parameter	Value
Pharmacology		K_D for human TNFRSF25	305 pM
		EC_{50} on human Jurkat-TNFRSF25 cells	760 ng/mL
		MABEL in mice	0.01 mg/kg
		NOEL in mice	0.001 mg/kg
		PAD Range in mice	0.1 – 10 mg/kg
Safety Pharmacology		Human PBMCs stimulated with anti-CD3 and PTX-35	No increase in deleterious cytokine release (35 tested)
Tissue cross-reactivity		Cytoplasmic and membrane	Human, monkey, mouse
Toxicology & Toxicokinetics	28-day DRF in mouse i.v. bolus	NOAEL	200 mg/kg
		C_{max}	2,440 µg/mL
		T_{max}	24 hrs
		$AUC_{(0\text{-}216\ hrs)}$	390,000 hr x µg/mL
	2-week DRF in NHP i.v. bolus	NOAEL	96 mg/kg/dose
		C_{max}	2,590 µg/mL
		$AUC_{(0\text{-}360\ hrs)}$	261,500 hr x µg/mL
	8-week in NHP i.v. bolus	NOAEL	100 mg/kg
		C_{max}	2,660 µg/mL
		$AUC_{(0\text{-}336\ hrs)}$	298,000 µg x hr/mL

NIGHTHAWK BIOSCIENCES

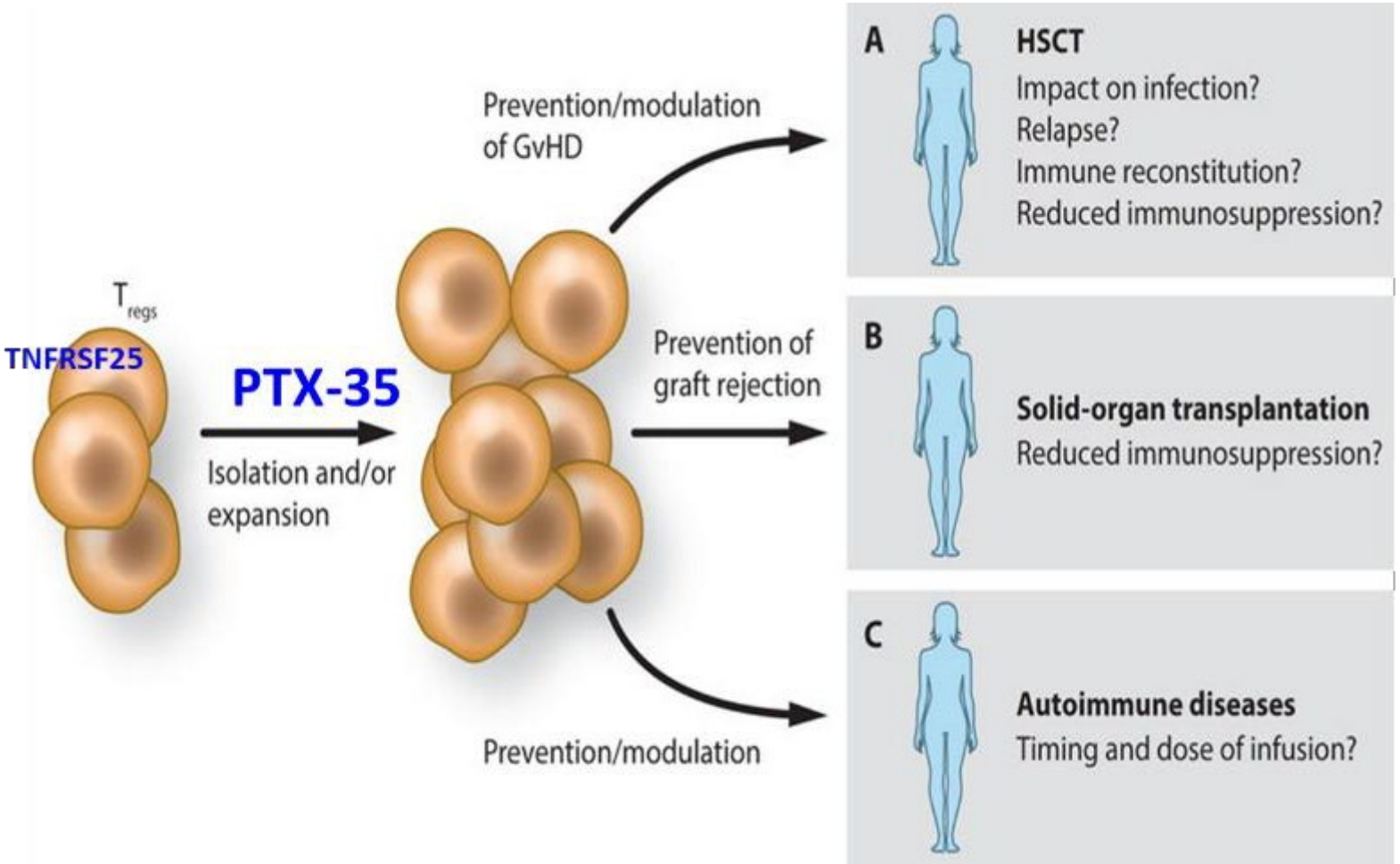
mPTX-35 vs. mIL-2 Mutein* – Treg Expansion, Mice



**In-house synthesized IL-2 mutein reference control. Mutated (N103R, V106D, P51T, & C140A) mouse IL-2 fused to mouse IgG2a with N297G mutation in Fc domain to abolish effector function. References: Khoryati Sci. Immunology 2020; Peterson J Autoimmunity 2018; US Patent 10,174,092*

NIGHTHAWK BIOSCIENCES

Treg Modulation as a Therapy

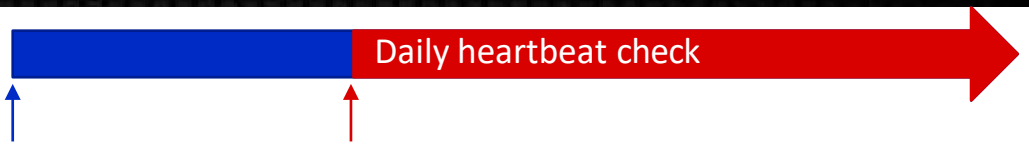


NIGHTHAWK BIOSCIENCES

TNFRSF25 Agonism Prolonged Allograft Survival

Prophylactic Treatment of Recipient Mice by 4C12 in a Cardiac Allograft Transplantation Mode

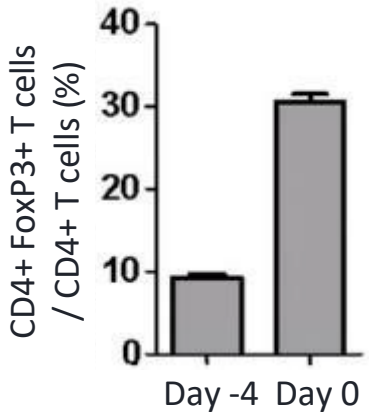
Eckhard Podack, MD, PhD
University of Miami



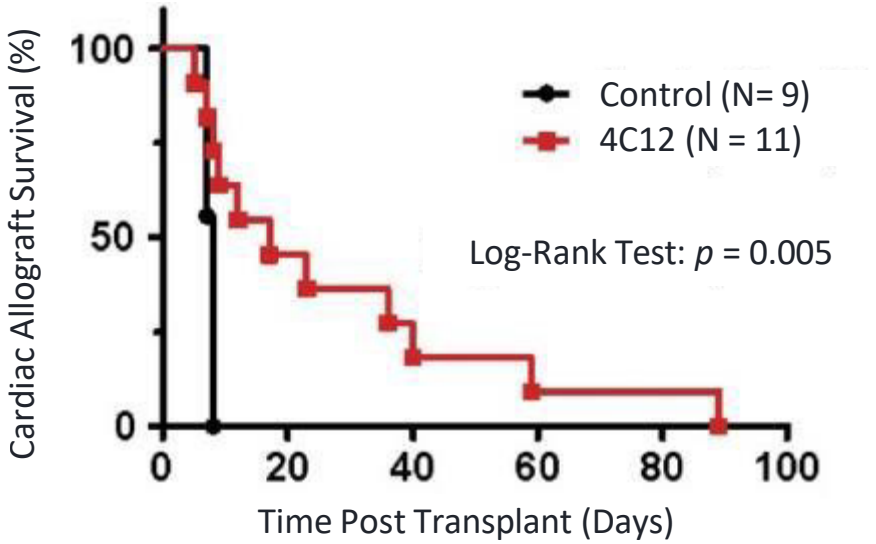
Day -4
Recipient received 4C12 or control (IgG)

Day 0
Allograft cardiac transplant performed on Day 0

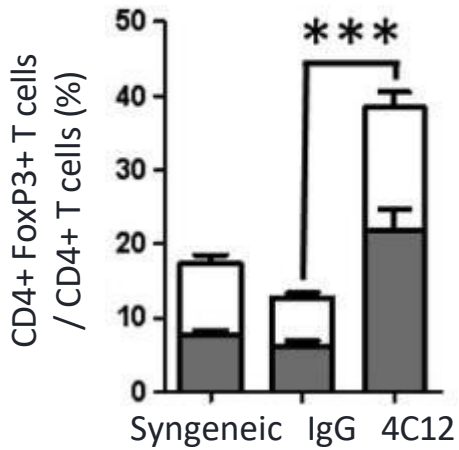
Peripheral Blood



Allograft Survival



Heart



Ki67+ (Proliferating Cells)
Ki67- (Non-proliferating Cells)

Syn = Syngeneic transplant performed on control mice
***P < 0.001

Source: Wolf D et al. 2012 Transplantation

NIGHTHAWK BIOSCIENCES

TNFRSF25 Agonism Delays GvHD in MHC-Mismatched Transplant

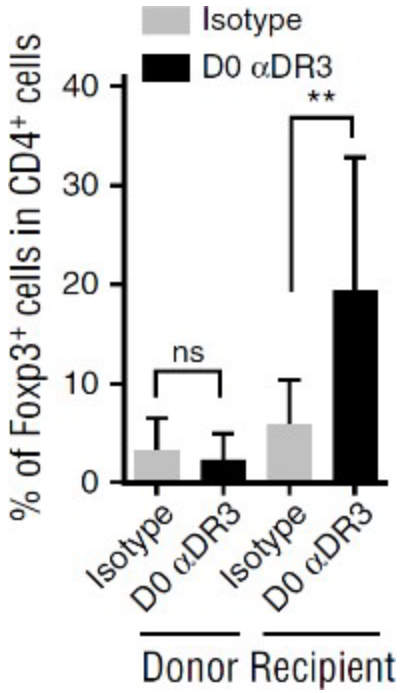
Prophylactic Treatment of Recipient Mice by 4C12 in Hematopoietic Stem Cell Transplantation

Robert Negrin, MD
Stanford University

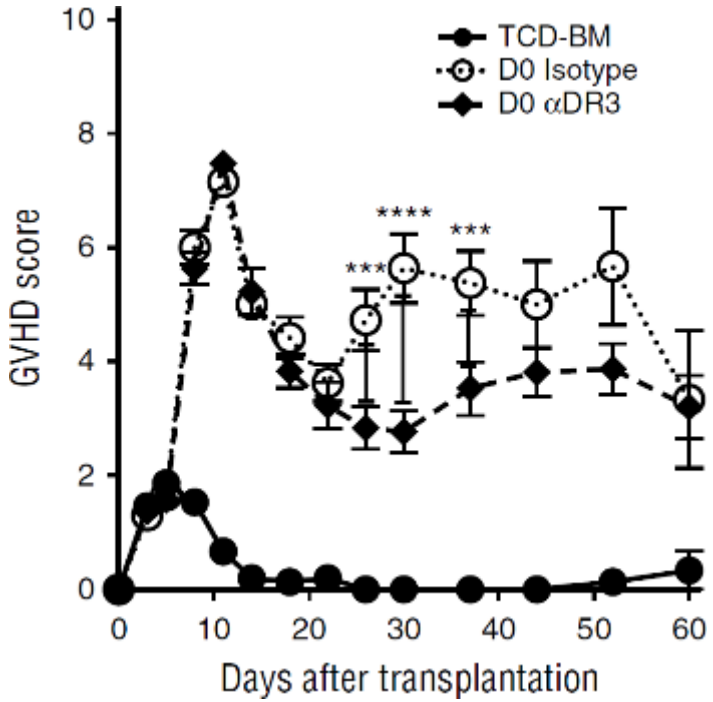
MHC-Mismatched Allogeneic Hematopoietic Stem Cell Transplantation

Donor: C57BL/6 mice ($H2^b$), Recipient: Balb/c mice ($H2^d$)

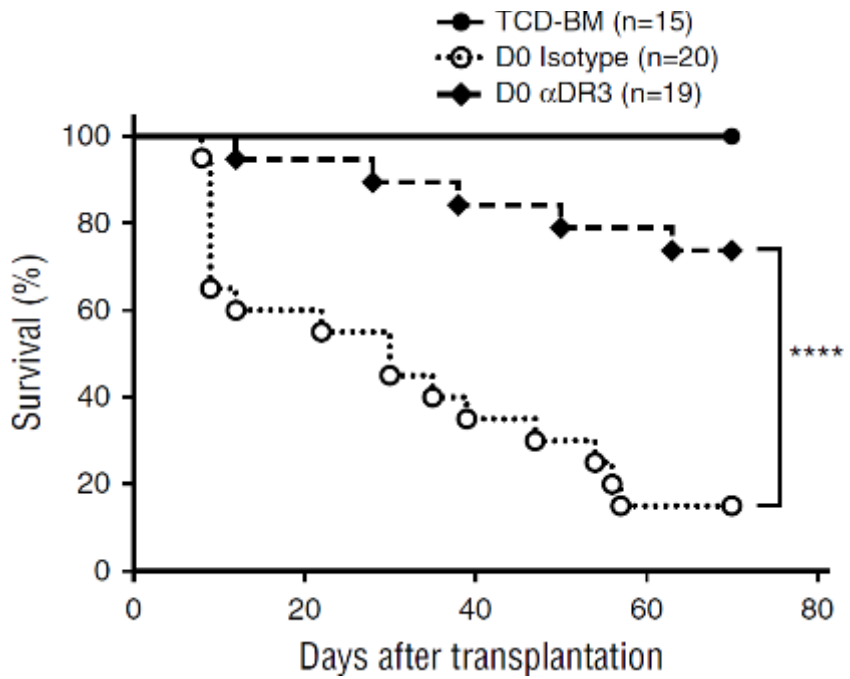
Spleen



GvHD Clinical Score



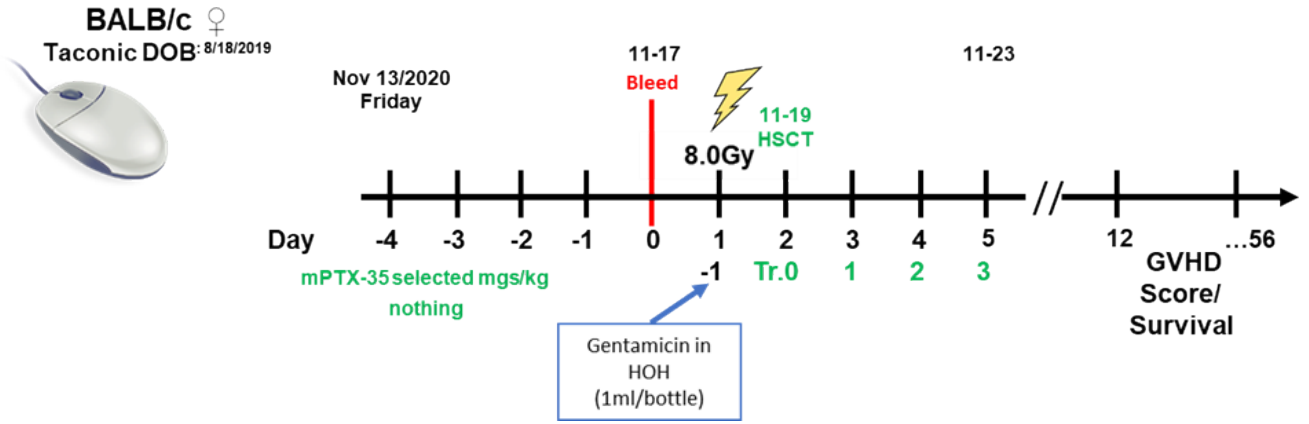
Survival

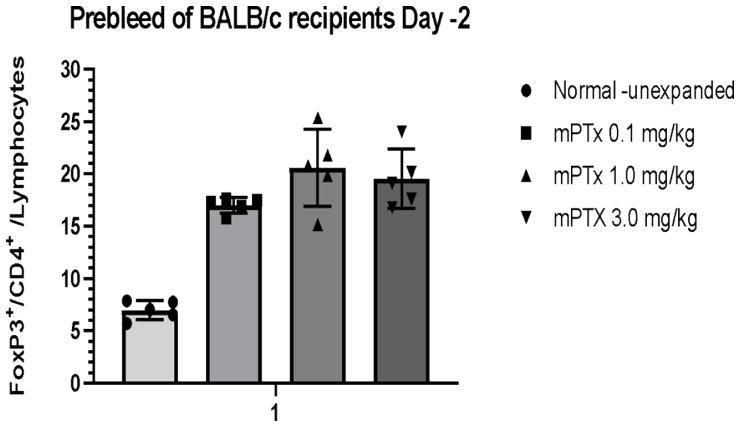


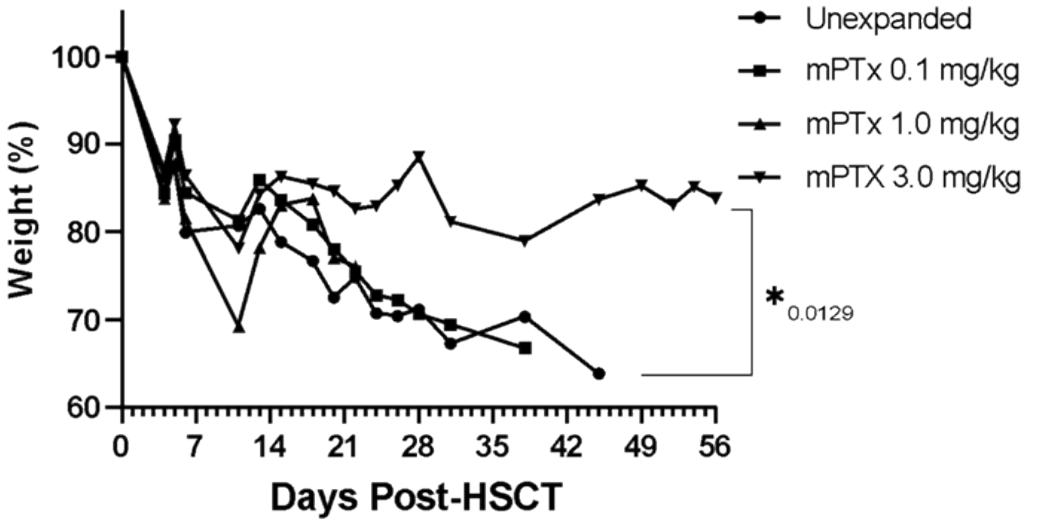
Source: Nishikii and Kim et al. 2016 Blood

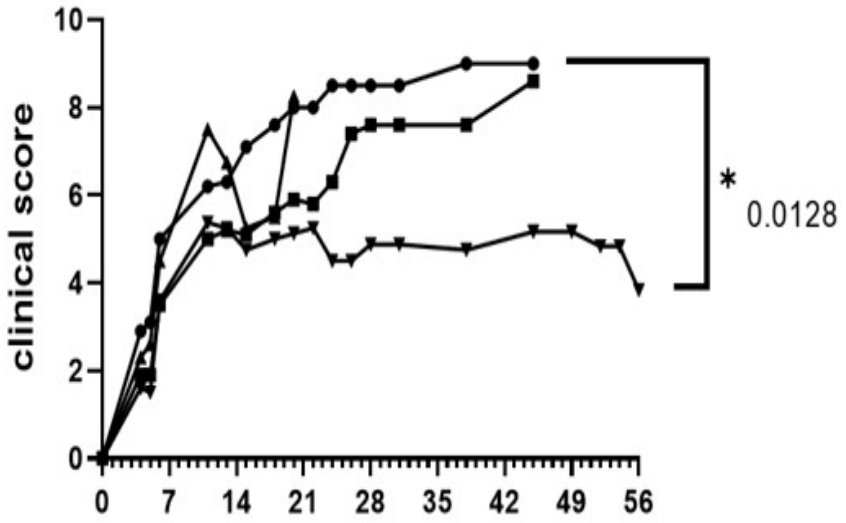
NIGHTHAWK BIOSCIENCES

PTX-35 Protects Allo-HSCT Rejection





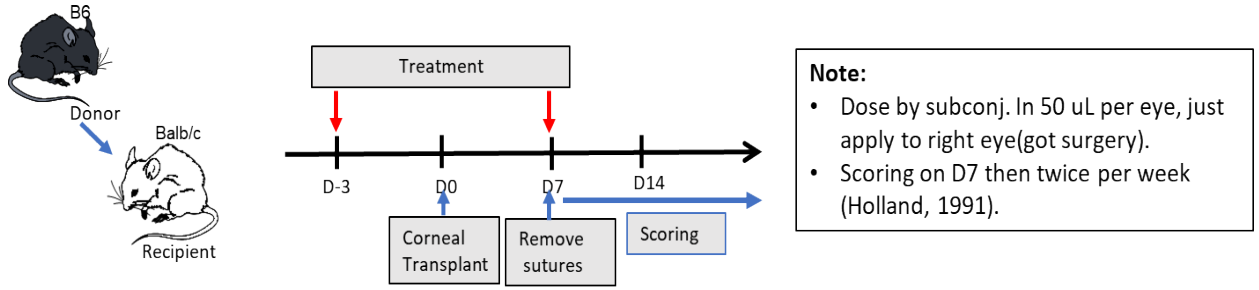




Robert Levy, PhD
University of Miami

NIGHTHAWK BIOSCIENCES

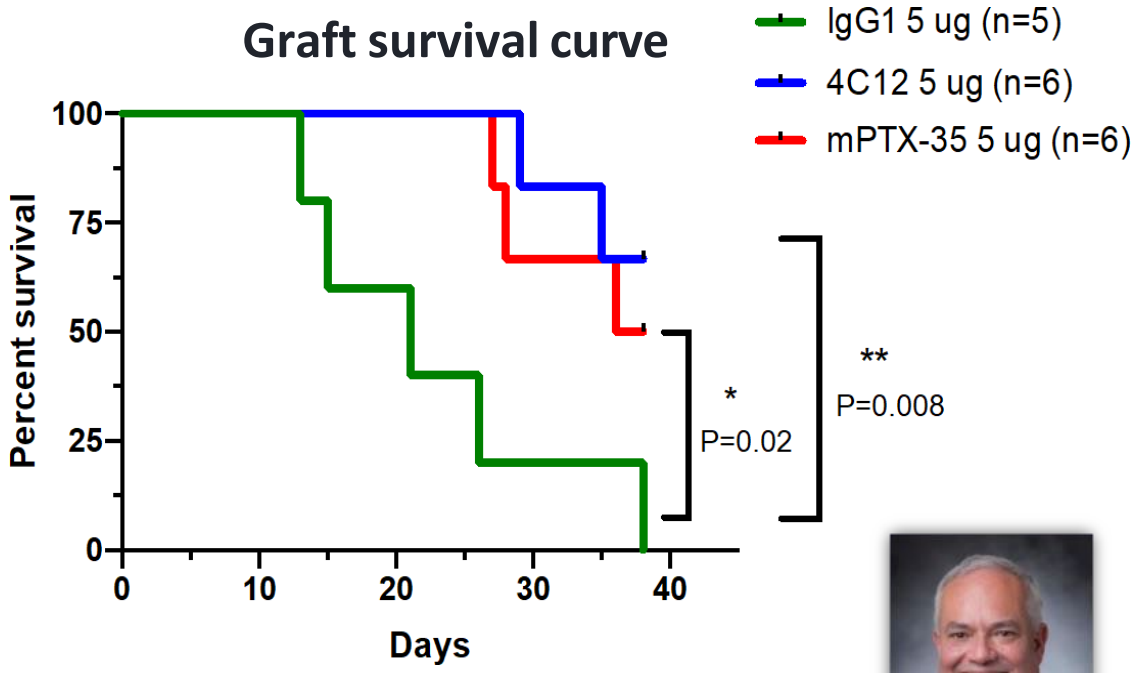
PTX-35 Delays Corneal Allograft Rejection in Mice



Note:
- Dose by subconj. In 50 uL per eye, just apply to right eye(got surgery).
- Scoring on D7 then twice per week (Holland, 1991).

Group	Treatment		Mice (Female)	
1	mPTX-35	5 ug/eye	Balb/c	6
2	4C12	5 ug/eye	Balb/c	6
3	IgG1 control	5 ug/eye	Balb/c	5

Highlights:
- Dose is 5 ug/eye.
- 2x treatments: (-3day, day7)

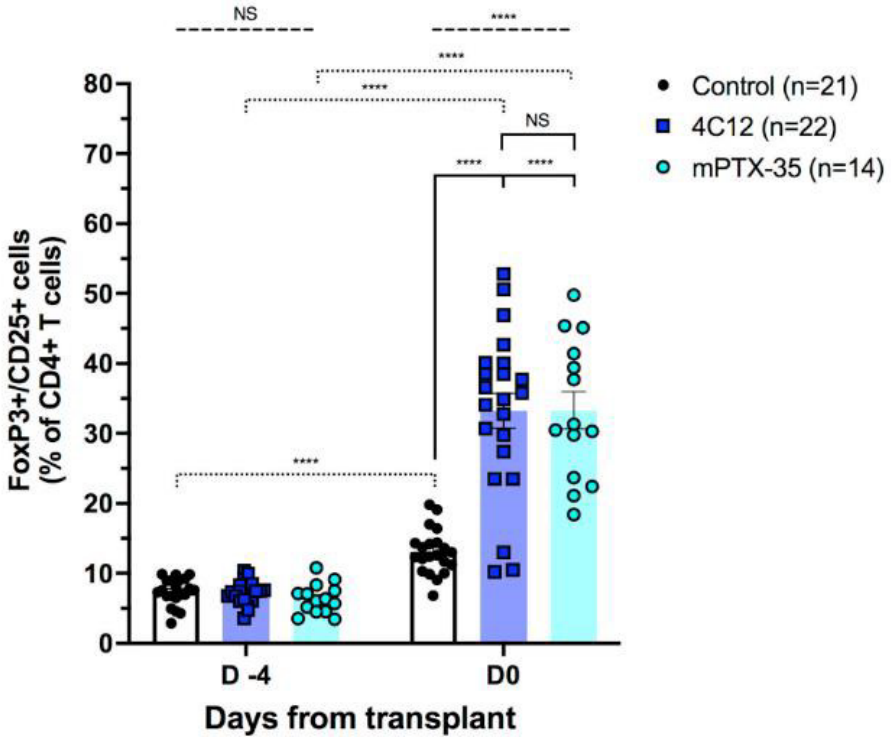


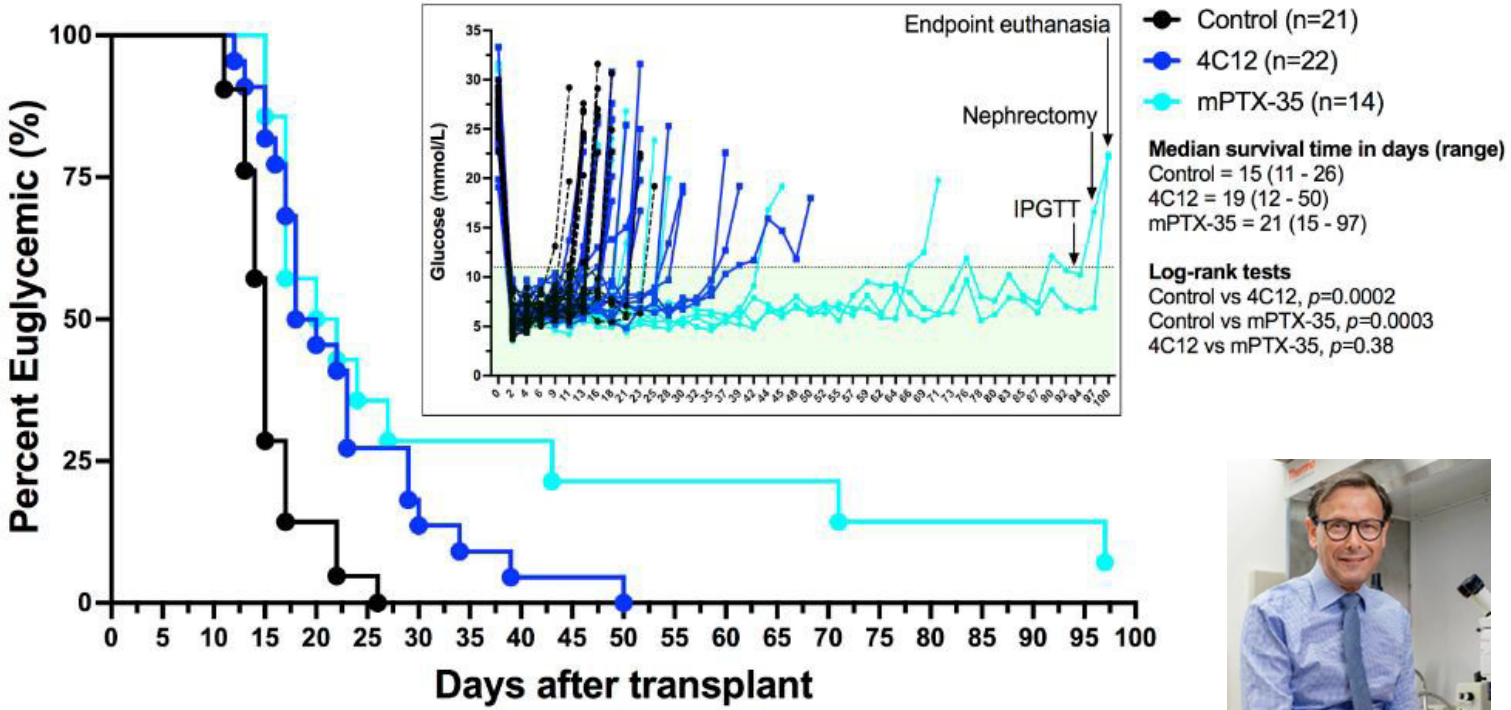
- **Subconjunctival injection results in local Treg cell expansion and protection**
- **Topical application fails to produce an efficacious result**

Victor Perez, MD
Duke University

NIGHTHAWK BIOSCIENCES

Beta-Islet Cell Allo-Transplantation Protection



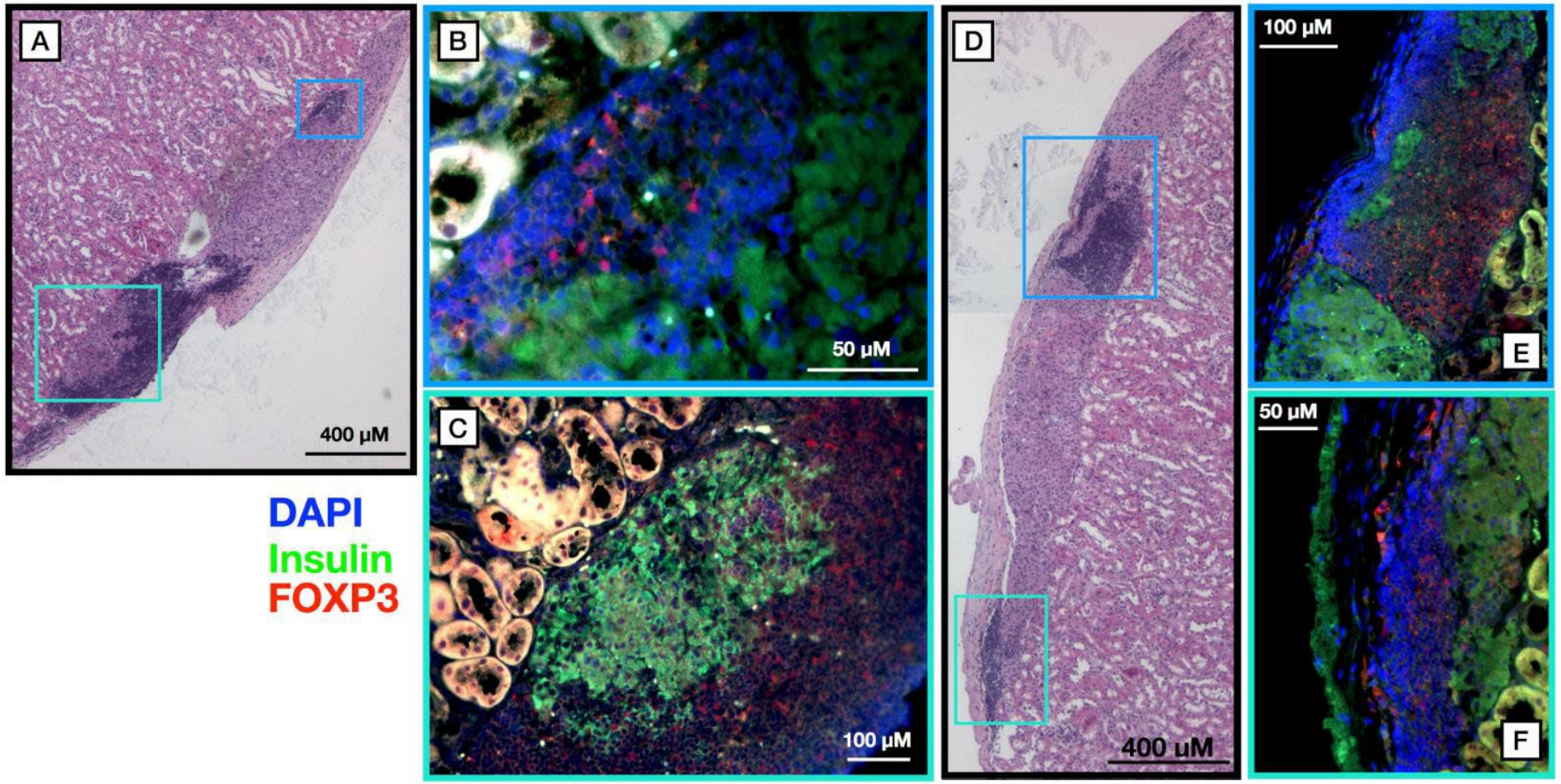


James Shapiro, MD
University of Alberta

Marfil-Garza BA et al., American Journal of Transplantation, December 2021, https://onlinelibrary.wiley.com/doi/10.1111/ajt.16940

NIGHTHAWK BIOSCIENCES

PTX-35 Expanded Treg Subsets within the Pancreatic Allograft

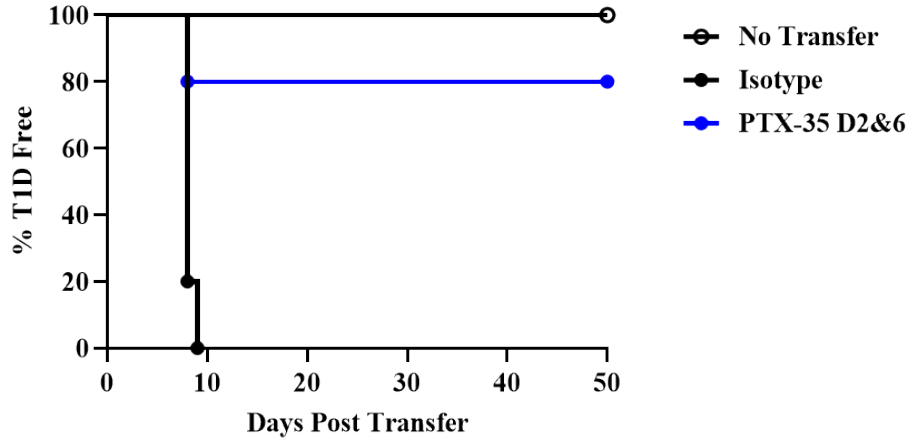


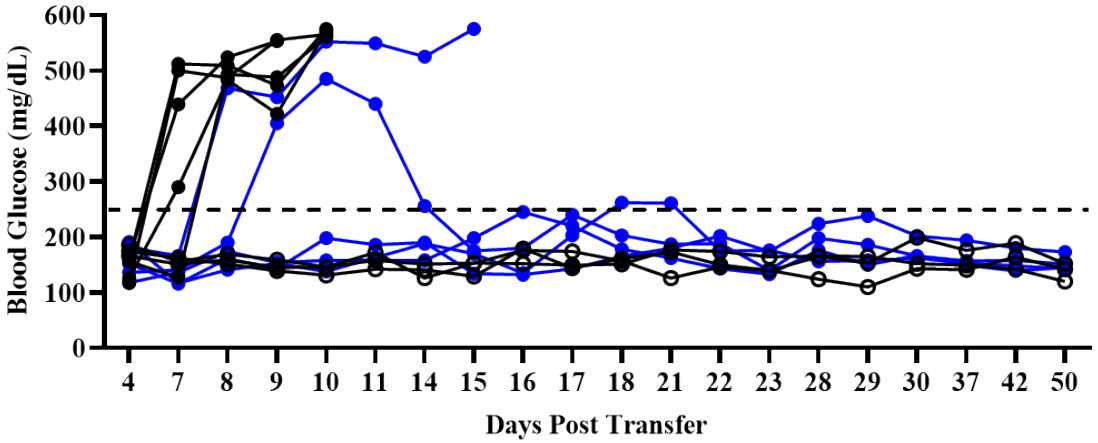
James Shapiro, MD
University of Alberta

Marfil-Garza BA et al., American Journal of Transplantation, December 2021, https://onlinelibrary.wiley.com/doi/10.1111/ajt.16940

NIGHTHAWK BIOSCIENCES

PTX-35 Prevents Adoptive Transfer of T1D in Mice



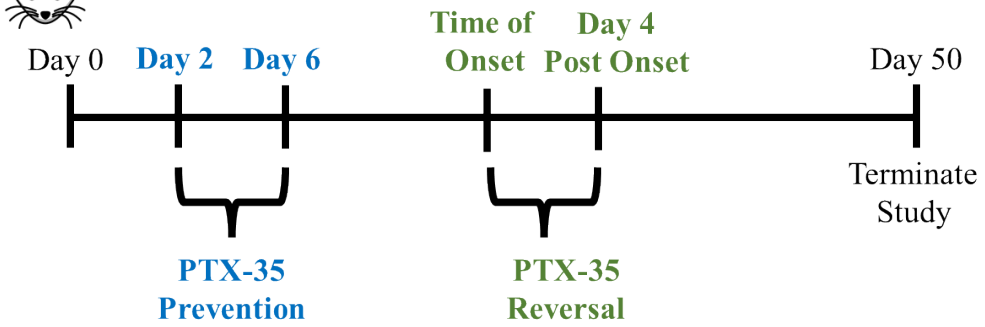


Adoptive Transfer of OT-I.Rag CD8$^+$ T cells into B6.RIP-mOva mice

Table 1. *Autoimmune Diabetes in RIP-mOVA Mice Depends on the Number of Adoptively Transferred OT-I Cells*

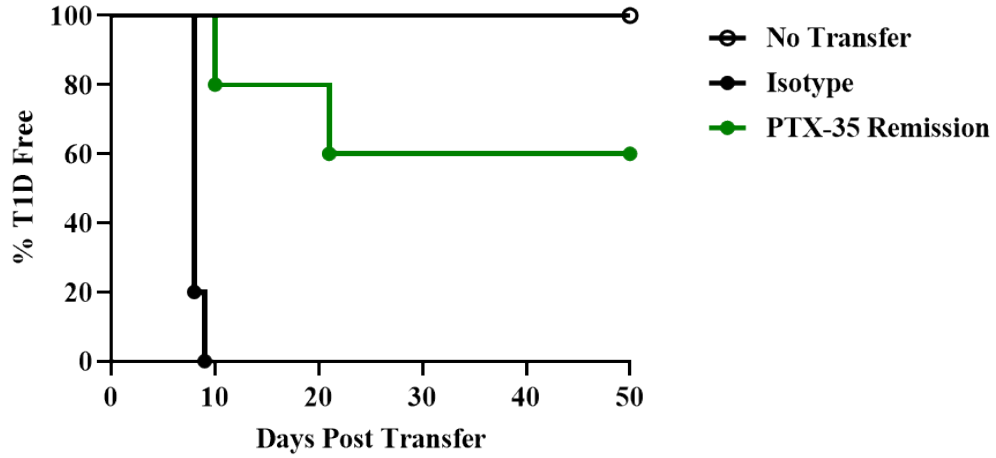
No. of OT-I cells transferred	No. mice diabetic/ total No. mice	(%)
10×10^6	3/3	(100%)
5×10^6	18/18	(100%)
2×10^6	9/10	(90%)
1×10^6	4/8	(50%)
0.5×10^6	1/12	(8%)
0.25×10^6	0/26	(0%)
0.10×10^6	0/7	(0%)

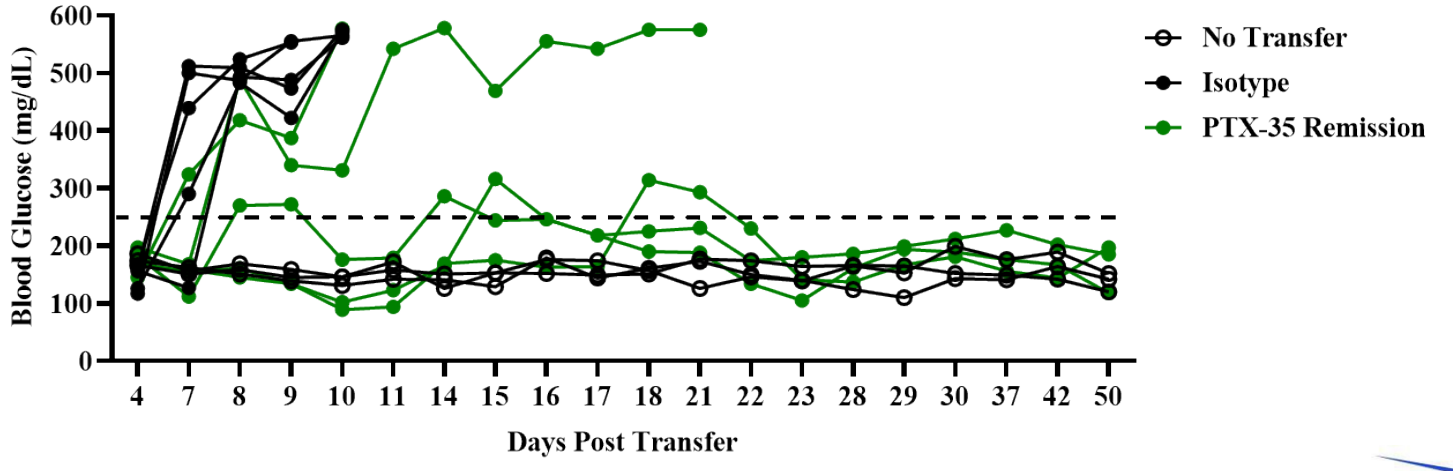
Diabetes is observed 5 to 8 days post transfer



NIGHTHAWK BIOSCIENCES

PTX-35 Can Reverse Established T1D in Mice



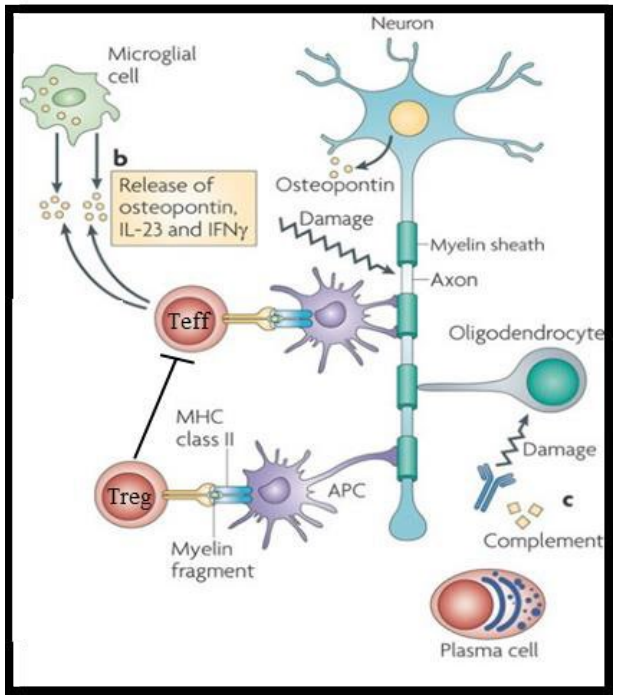


NIGHTHAWK BIOSCIENCES

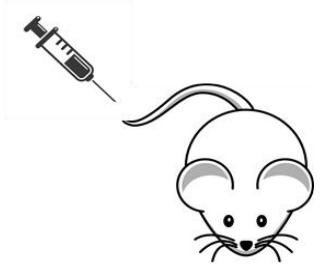
What if we intervene during an active immune response?

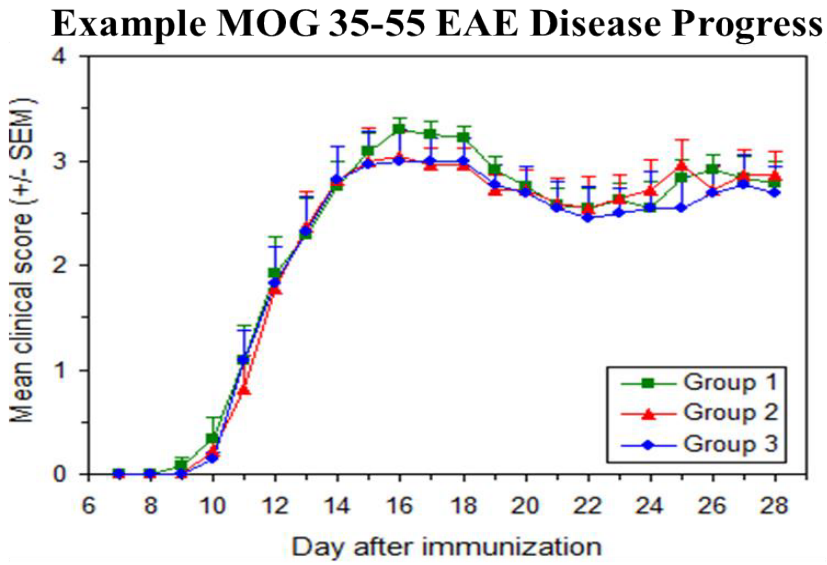
During an active autoimmune flare?

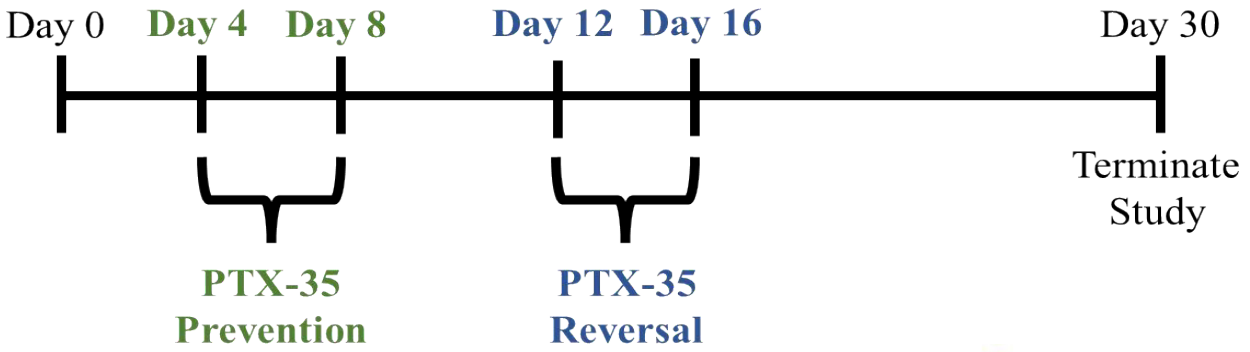
Classic EAE Model – Chronic MS Model



Immunization with MOG 35-55 C57BL/6

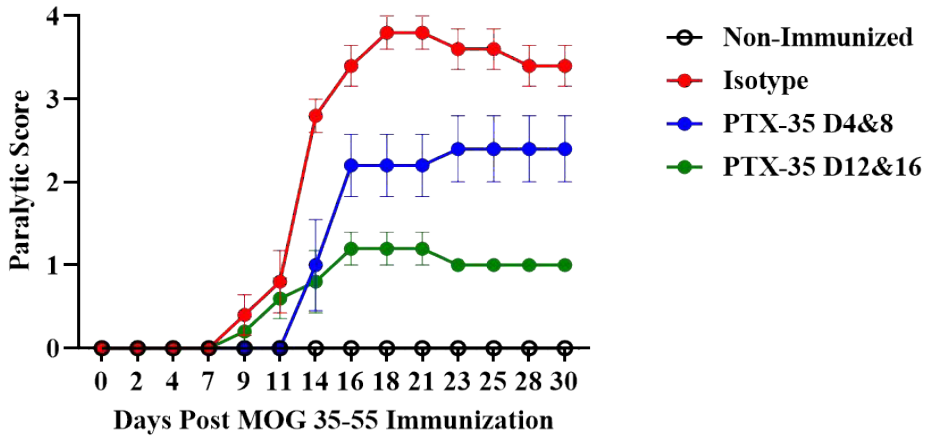


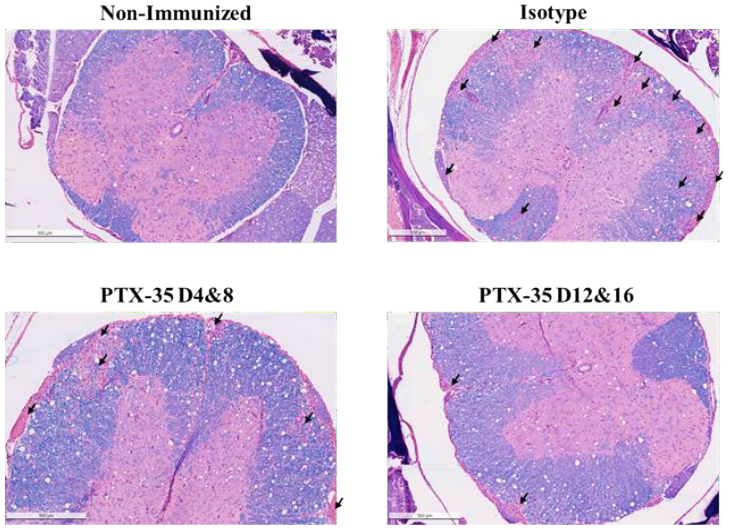


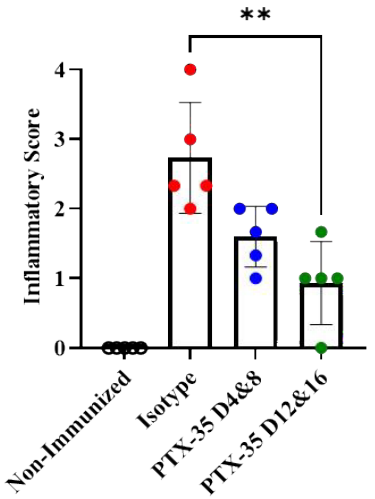


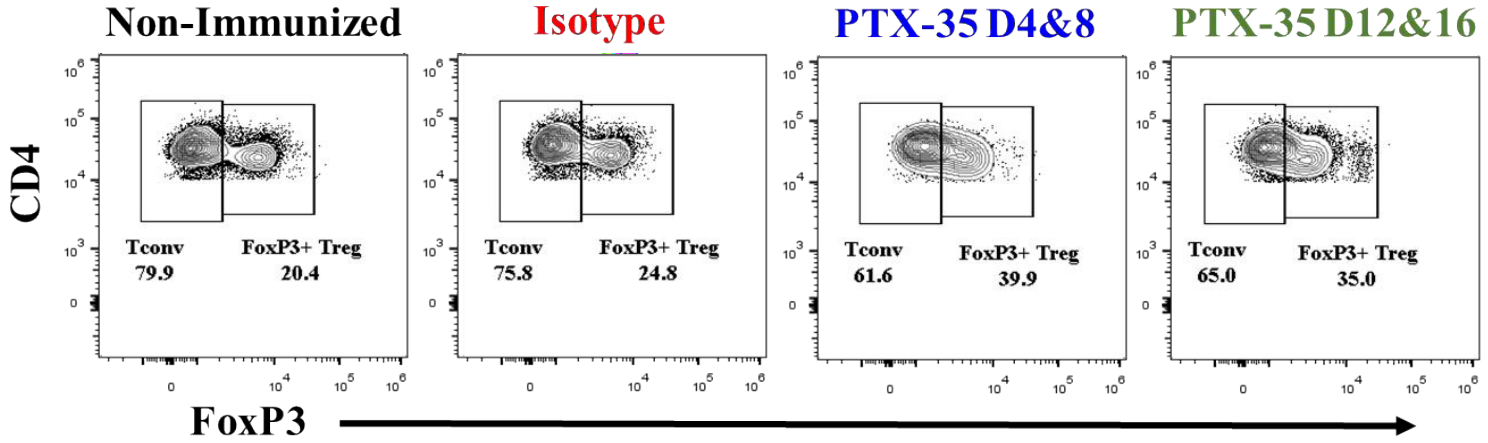
NIGHTHAWK BIOSCIENCES

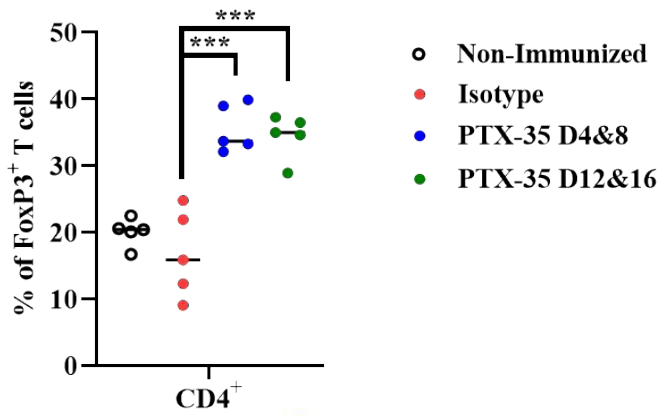
Reversal of Established EAE Disease





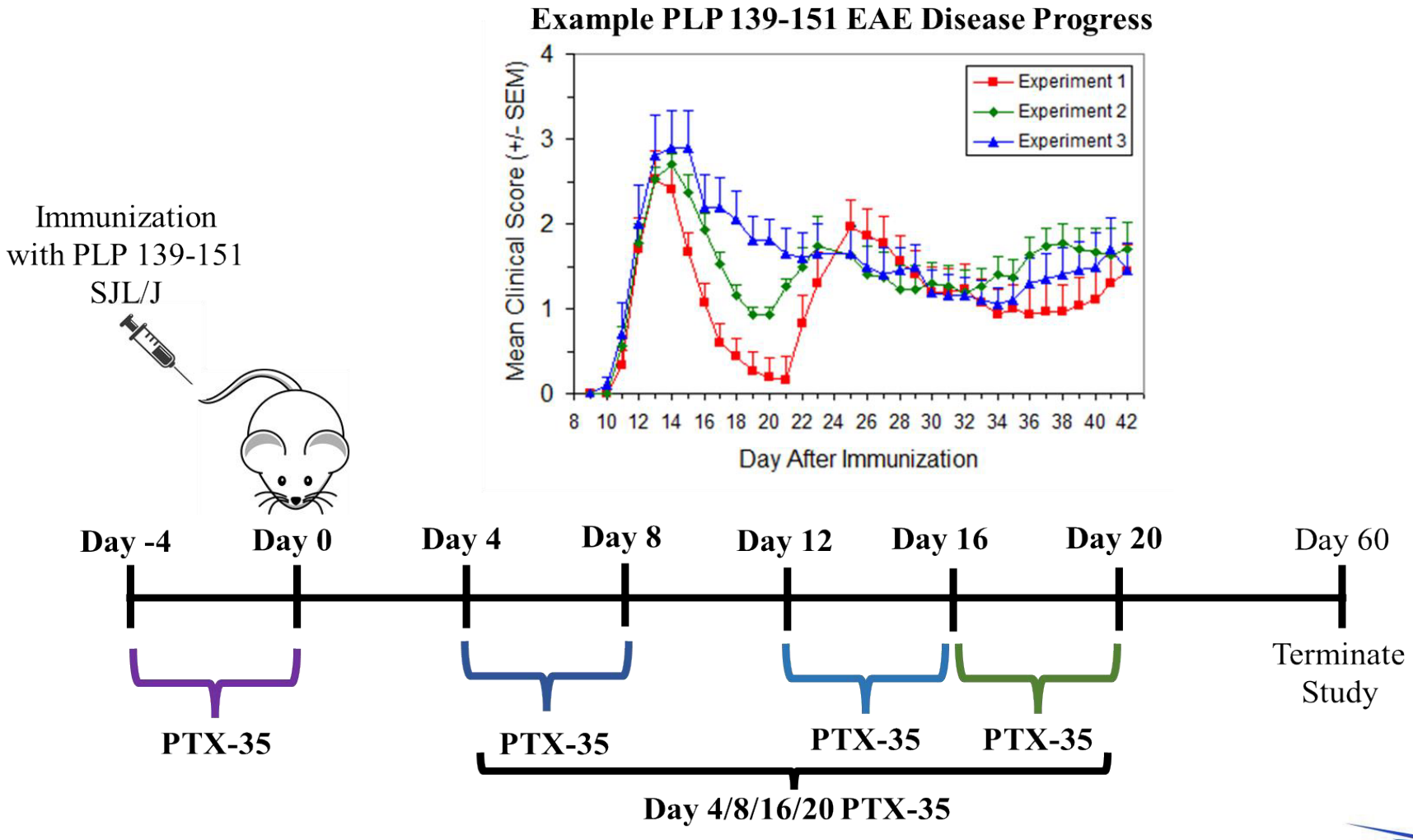






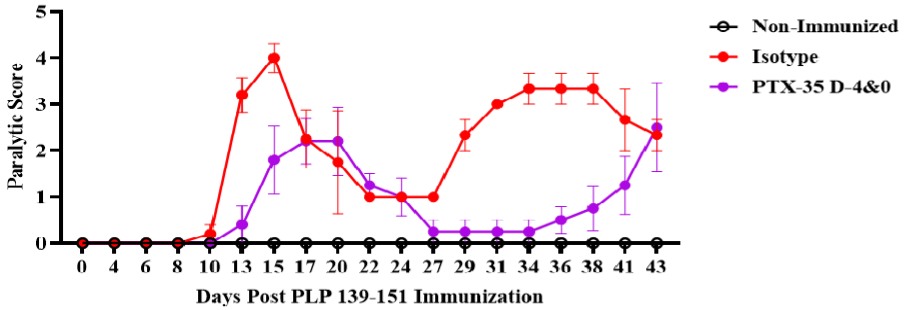
NIGHTHAWK BIOSCIENCES

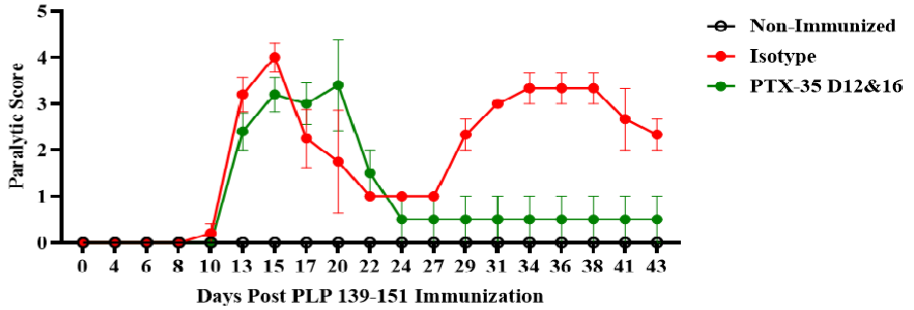
RRMS Model – Relapse-Remitting Disease

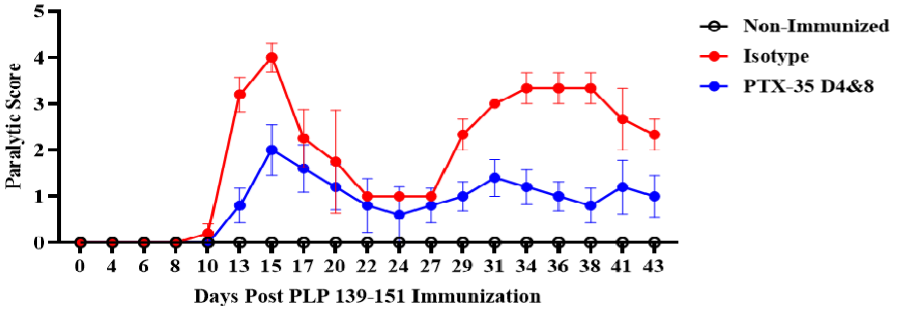


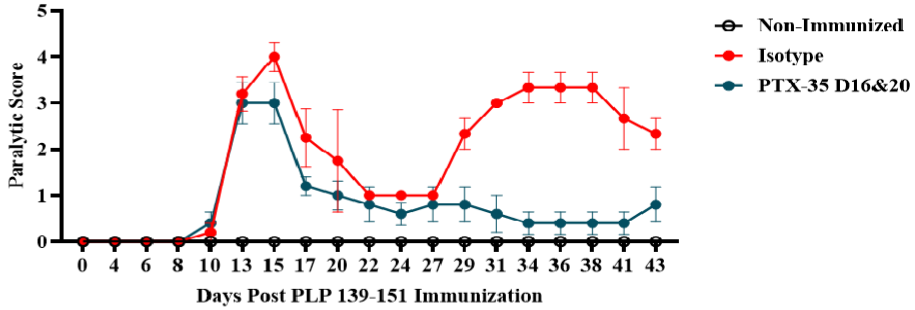
NIGHTHAWK BIOSCIENCES

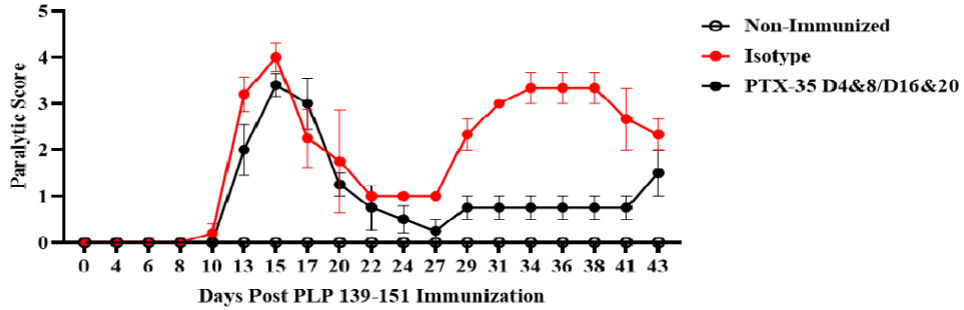
Control & Some Reversal of Established RRMS











NIGHTHAWK BIOSCIENCES

PTX-35 is a Potent Treg Cell Expander

- PTX-35 stimulation of T-cell expansion is highly TCR-dependent
- Preclinical modeling of PTX-35 shows applications in several inflammatory disorders
- Expansion of Tregs by PTX-35 and other TNFRSF25 agonists demonstrate utility in solid organ transplantation and GVHD
- Full GLP safety package completed, open IND in oncology, Ph1 study ongoing (NCT04430348)
- **Next Steps**
  - Various humanized mouse model systems to expand possible indications and better understand mechanism of action
  - Seeking new clinical applications in the Treg manipulation space
  - Open to partnerships

Acknowledgments & Collaborators

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