Discovery & Characterization of an IKZF2 Selective Molecular Glue Degrader with Best In-Class Potential

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proteovant THERAPEUTICS

Degrading Proteins, Defeating Disease

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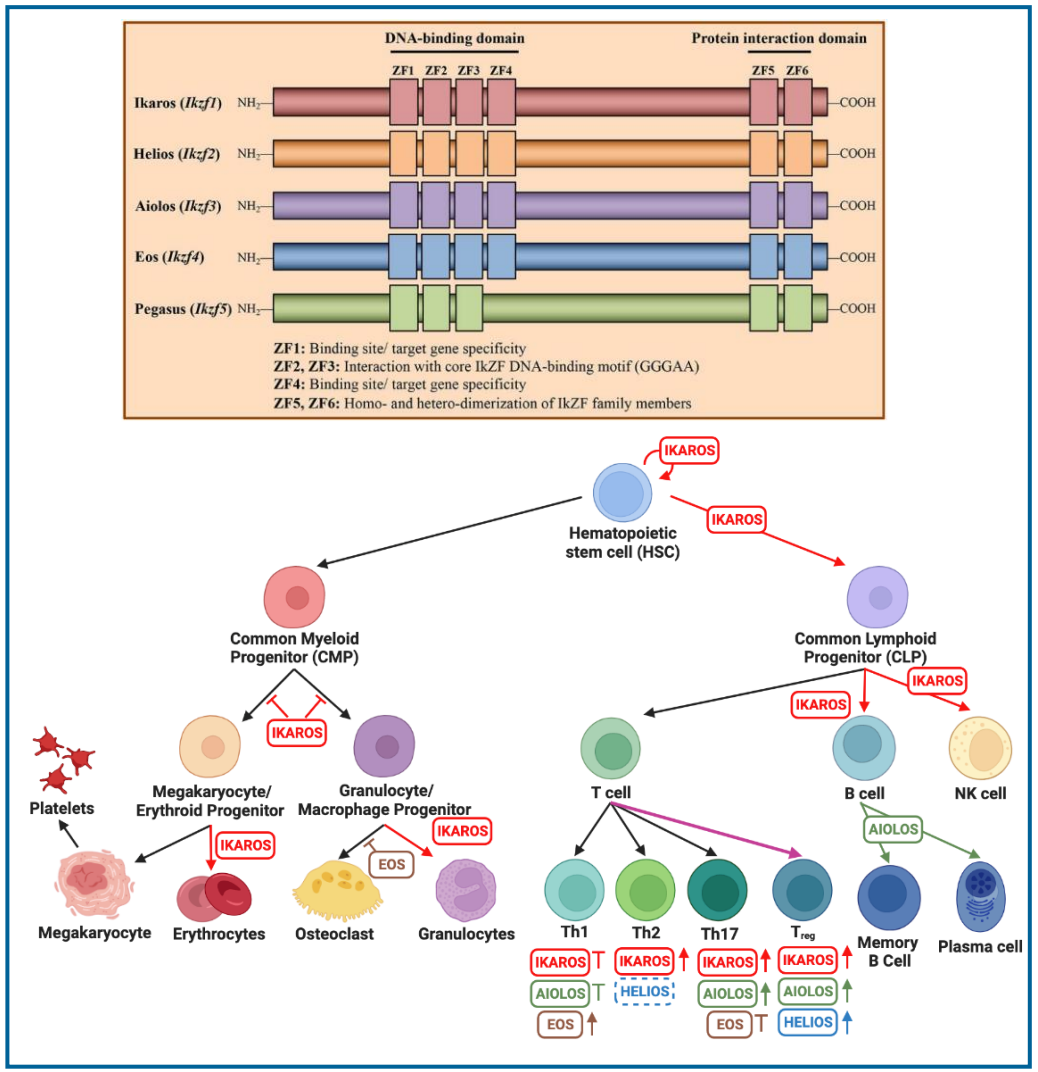
IKZF2 is an Ikaros Zinc Finger Family Transcription Factor Highly Expressed in Regulatory T-Cells

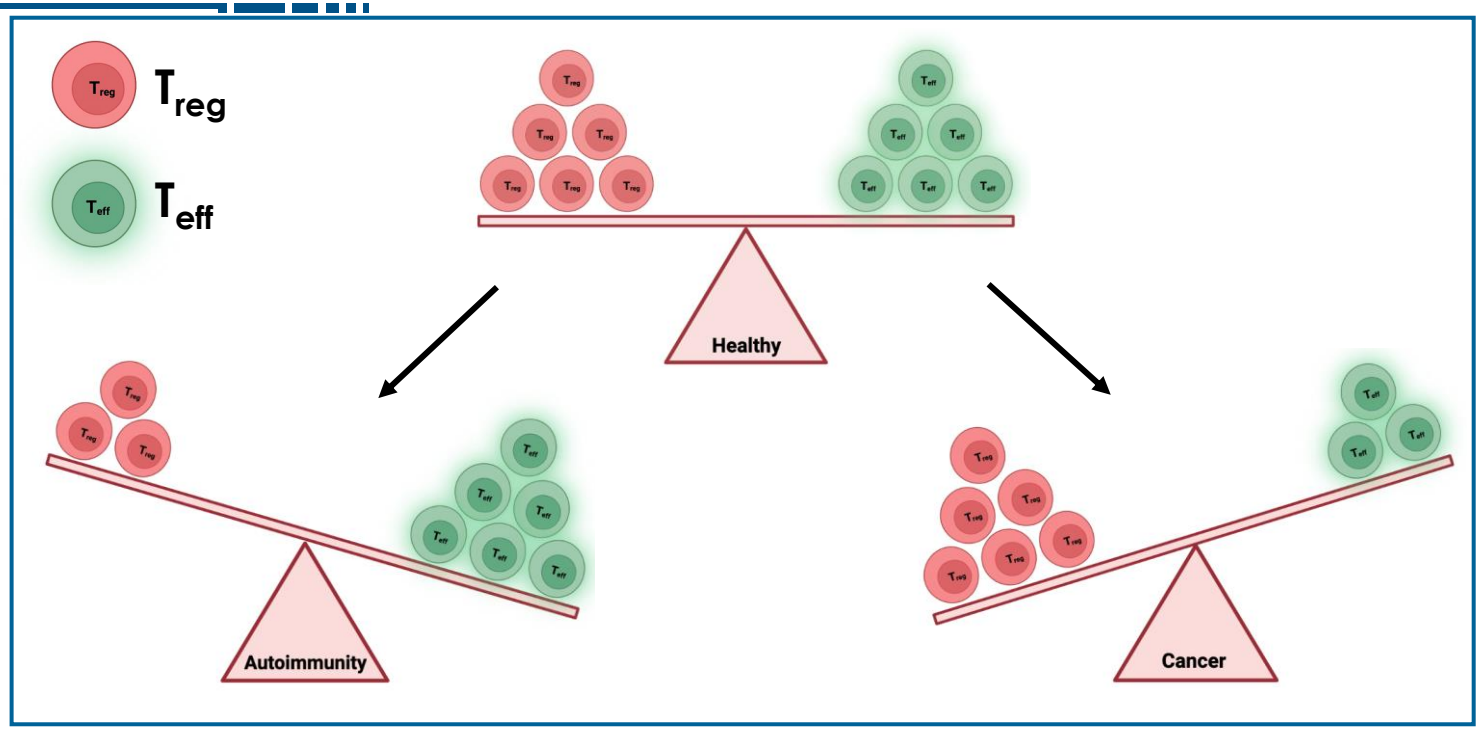


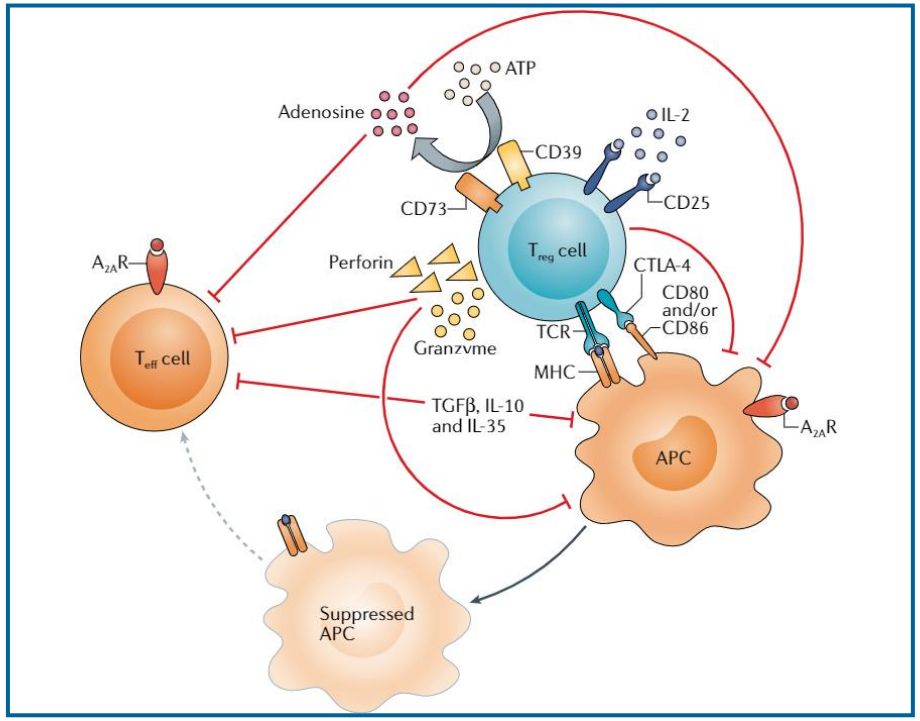
- IKZF2 (Helios) is a member of a family of five transcriptional regulators that include IKZF1, IKZF3, IKZF4, and IKZF5
- IKZF2 is comprised of four N-terminal zinc finger (ZF) DNA-binding domains and two C-terminal ZF protein-protein interaction domains
- IKZF2 expression is largely restricted to select lymphoid cells including T Helper 2 (T_H2) cells and regulatory T-cells (T_{regs})

Powell, M. D., Read, K. A., Sreekumar, B. K. & Oestreich, K. J. *Front Immunol* **10**, 1299 (2019).
Cai, Q., Dierich, A., Oulad-Abdelghani, M., Chan, S. & Kastner, P. J Immunol 183, 2303–2311 (2009).
Kim, H.-J. et al. Science 350, 334–339 (2015).
Shahin, T. et al. Sci Immunol 6, eabe3981 (2021).
John, L. B. & Ward, A. C. *Mol Immunol* **48**, 1272–1278 (2011).

Schematic created with biorender.com

Regulatory T-cells are Key Contributors to Immune Evasion by Cancer Cells



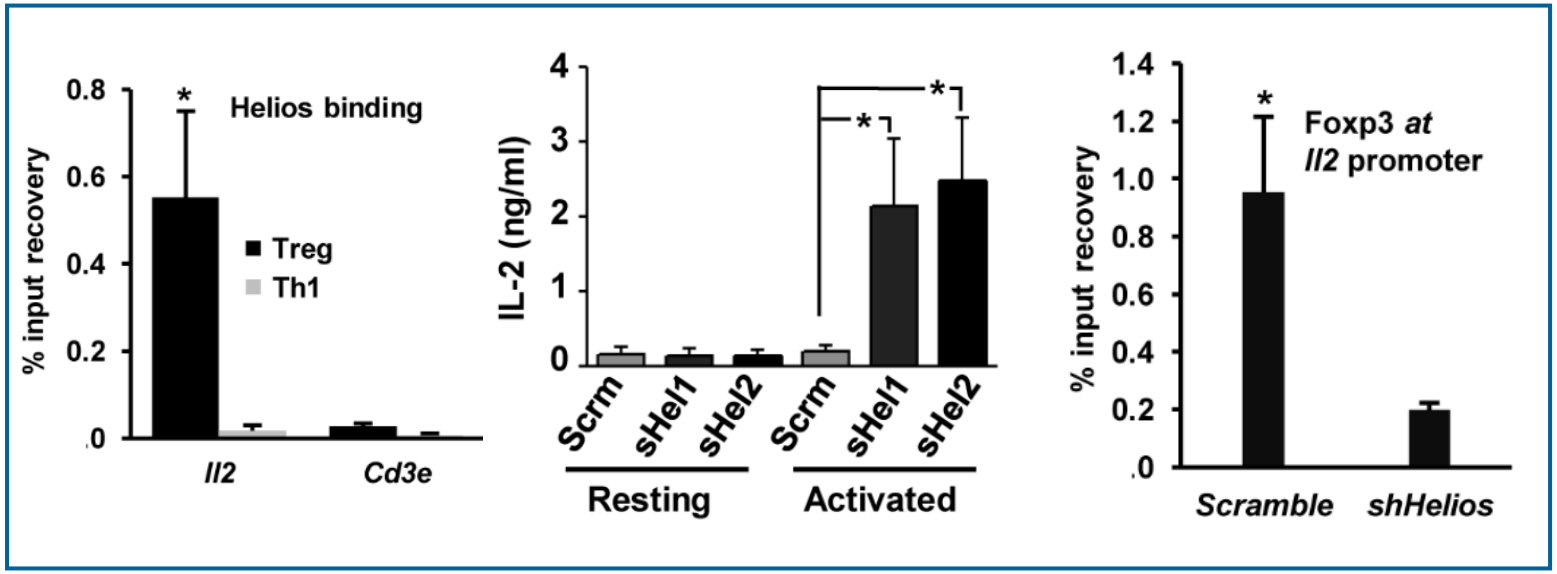


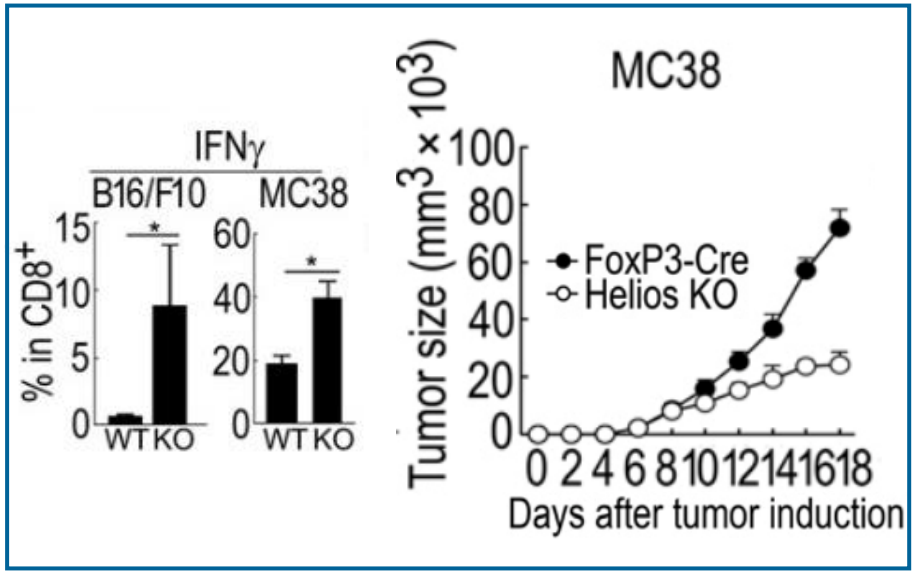
- T_{reg} cells are an immunosuppressive subset of CD4+ T cells that play essential roles in self-tolerance
- High relative abundance of T_{reg} cells in the tumor microenvironment (TME) is associated with poor prognosis in various cancer types
- Evading immune surveillance and destruction is fundamental to progression of many cancers

- T_{reg} cells exert their immunosuppressive activity through various mechanisms
  - serving as an IL-2 sink in the TME
  - suppressing inflammatory response

Rakebrandt, N., Littringer, K. & Joller, N.. Swiss Med Wkly 146, w14343 (2016).
Togashi, Y. Shitara, K. & Nishikawa, H. *Nat Rev Clin Oncol* **16**, 356–371 (2019).
Schematic created with biorender.com

IKZF2 is Important for Immunosuppressive Activity of T_{regs}, Making it an Attractive Immuno-oncology Target



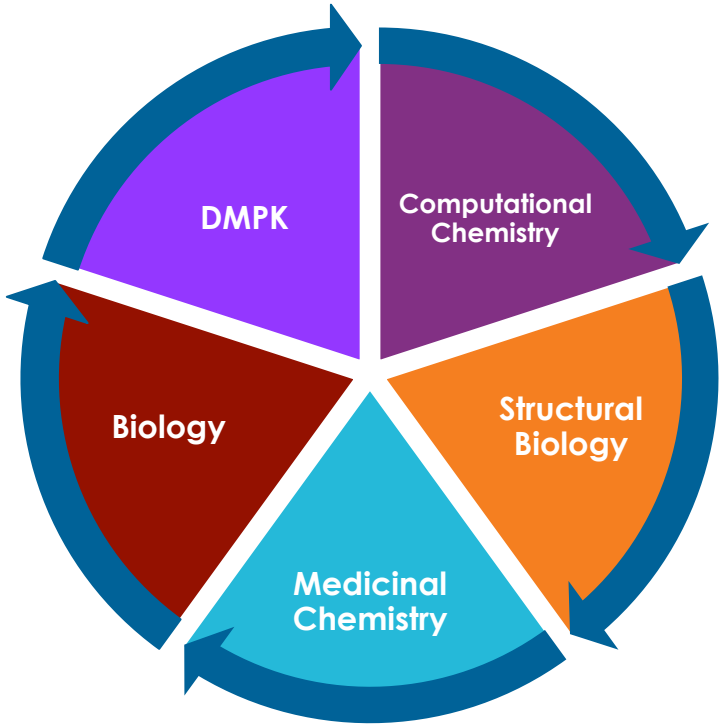


- Stable inhibitory activity of T_{regs} is linked to IL-2 repression
- IKZF2 binds to the IL-2 promoter in T_{reg} cells and suppresses transcriptional activation
  - IKZF2 KD results in higher IL-2 expression upon stimulation
- IKZF2 KD suppresses FoxP3 binding to IL-2 promoter

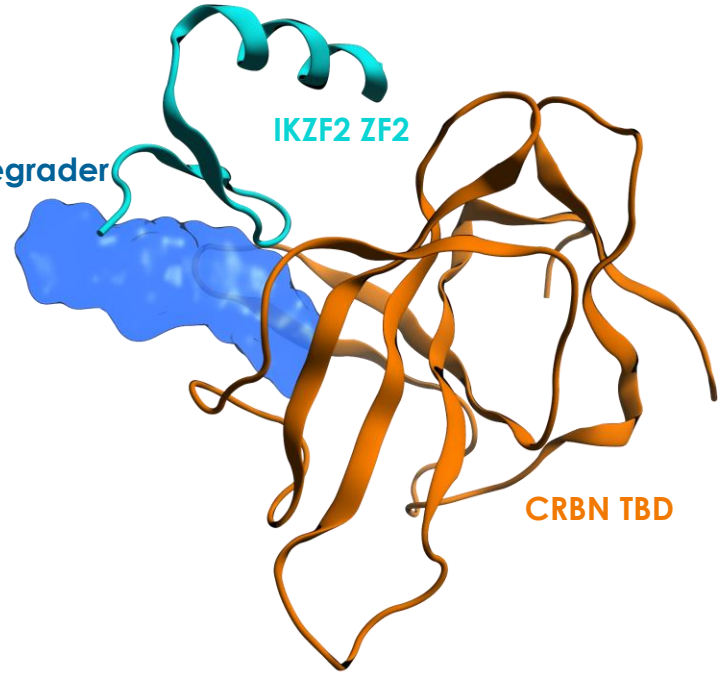
- IKZF2 KO leads to an unstable CD4 T_{reg} phenotype marked by production of effector cytokines
- IKZF2 KO in T_{regs} suppresses tumor growth

Baine, I., Basu, S., Ames, R., Sellers, R. S. & Macian, F. J Immunol 190, 1008–1016 (2013).
Nakagawa, H. et al. Proc National Acad Sci 113, 6248–6253 (2016).
Yates, K., Bi, K., Haining, W. N., Cantor, H. & Kim, H.-J. Proc National Acad Sci 115, 201720447 (2018).

Discovery of Selective IKZF2 Molecular Glue Degraders



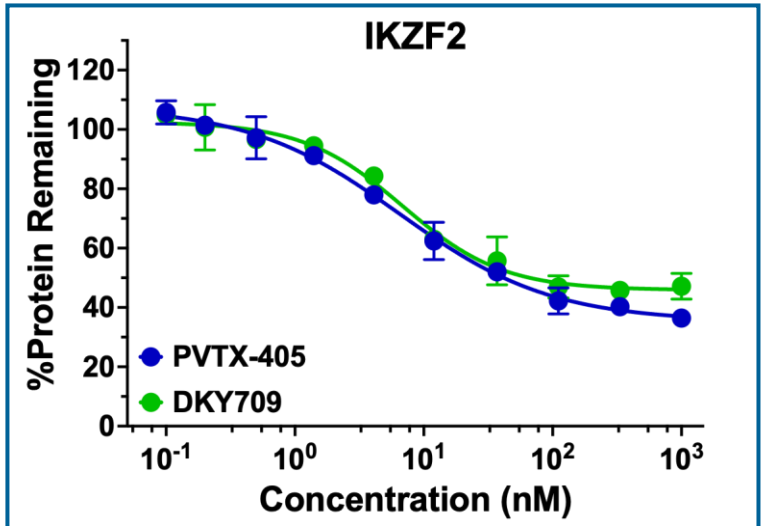
X-ray of CRBN TBD: PVT Degrader: IKZF2 (ZF2) Complex (~2 Å)



PVTX-405

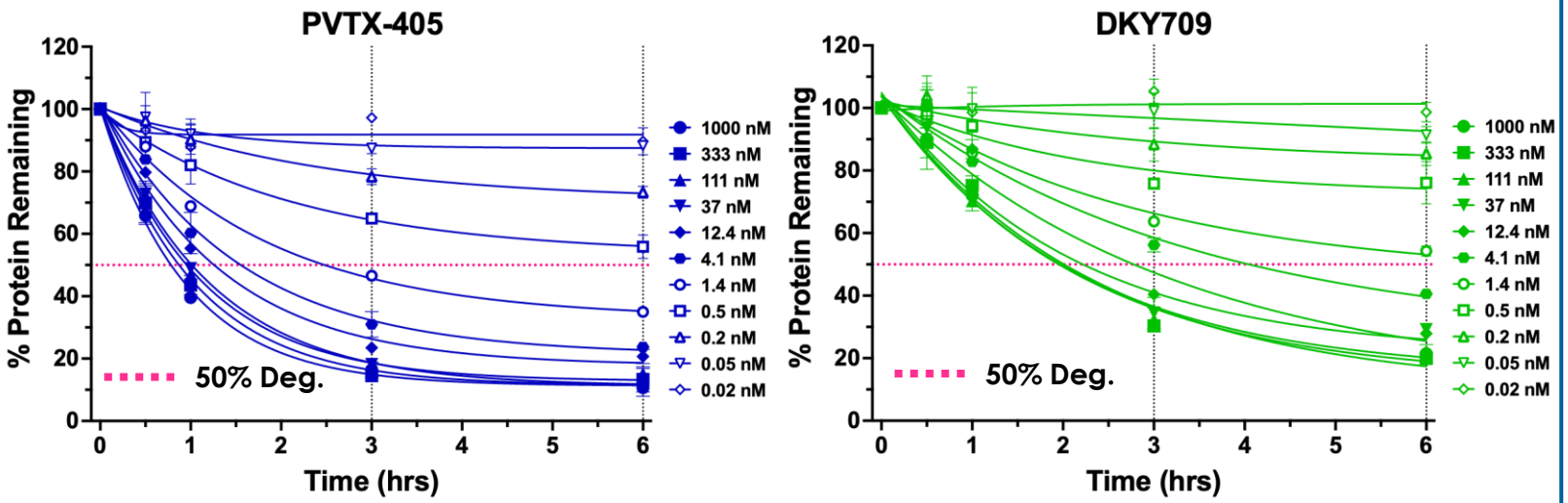
- Fully integrated discovery team applying a multi-disciplinary approach to drug hunting
- Multiple cycles of SBDD using ternary complex structures to guide lead optimization

PVTX-405 Induces Potent and Rapid IKZF2 Degradation



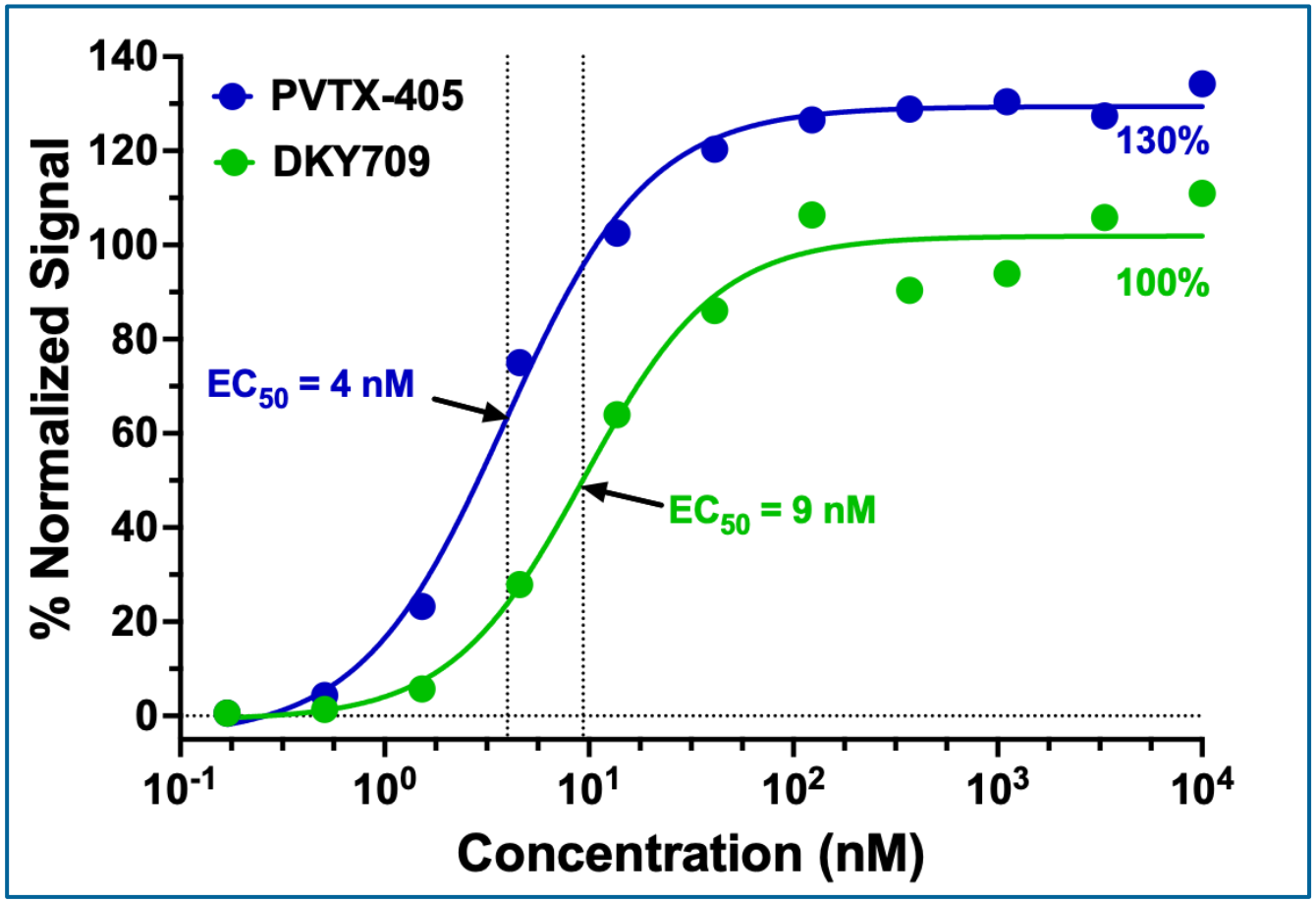
Compound	DC_{50} (nM)	D_{max} (%)
PVTX-405	6.3	65
DKY709	8.1	59

- PVTX-405 shows similar potency as DKY709 with higher D_{max}



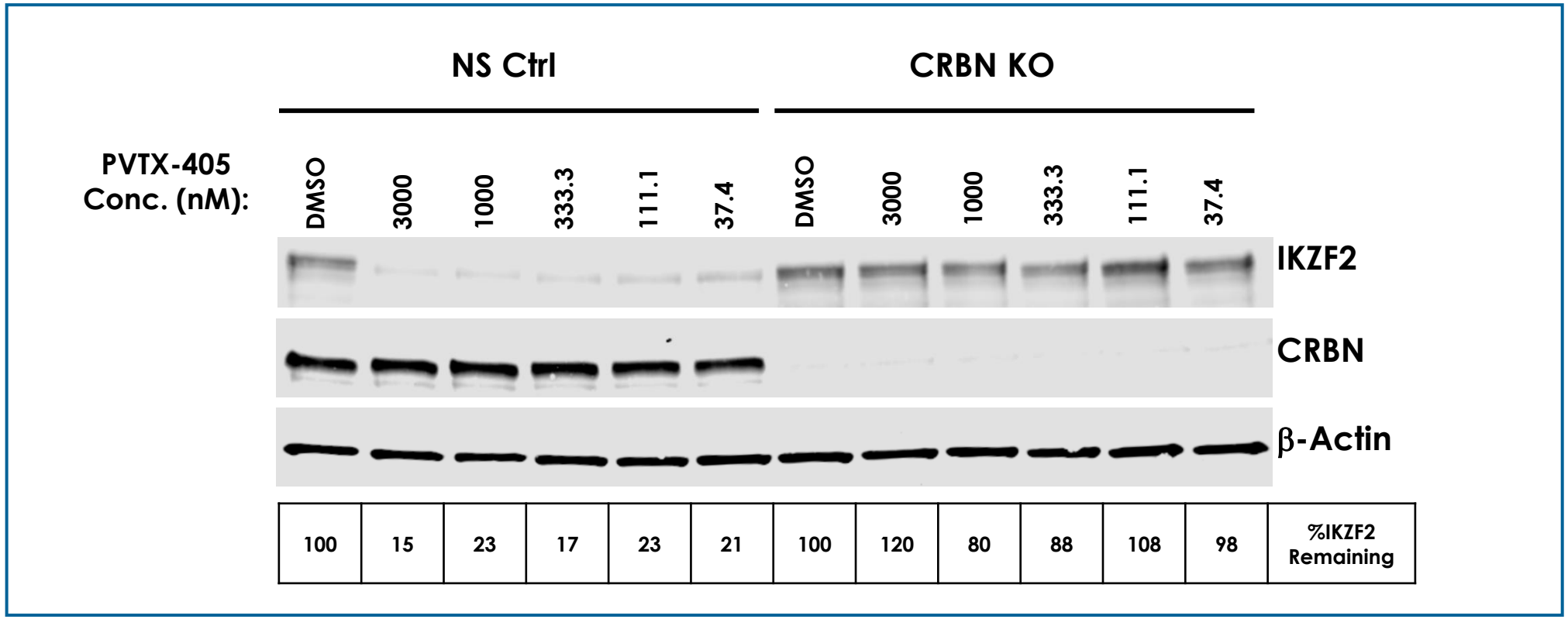
- PVTX-405 shows more rapid degradation kinetics than DKY709
- PVTX-405 achieves maximal degradation by 6 hrs while DKY709 requires 18 hours to reach D_{max} plateau

PVTX-405 Demonstrates Robust CRBN/IKZF2 Ternary Complex Formation



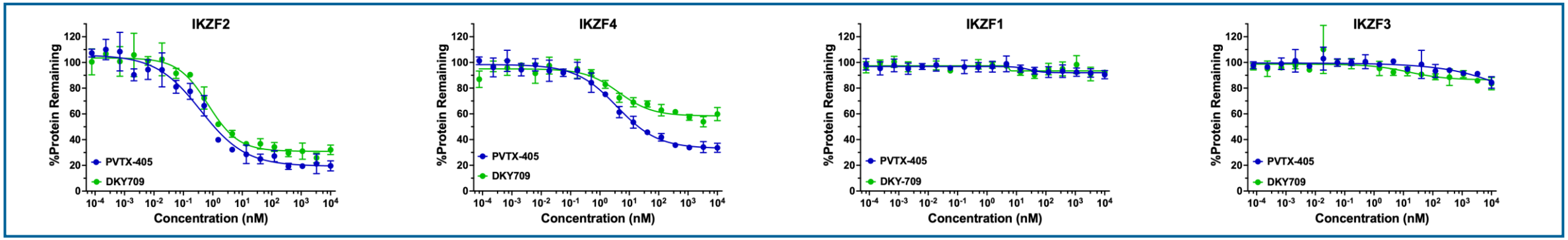
- A greater level of ternary complex is formed in the presence of PVTX-405 than DKY709
  - Higher max signal and higher signal at each concentration of PVTX-405 than DKY709 are evident
- EC_{50} values are similar for the two compounds suggesting similar stability of the complex

PVTX-405 Mediated IKZF2 Degradation is CRBN Dependent



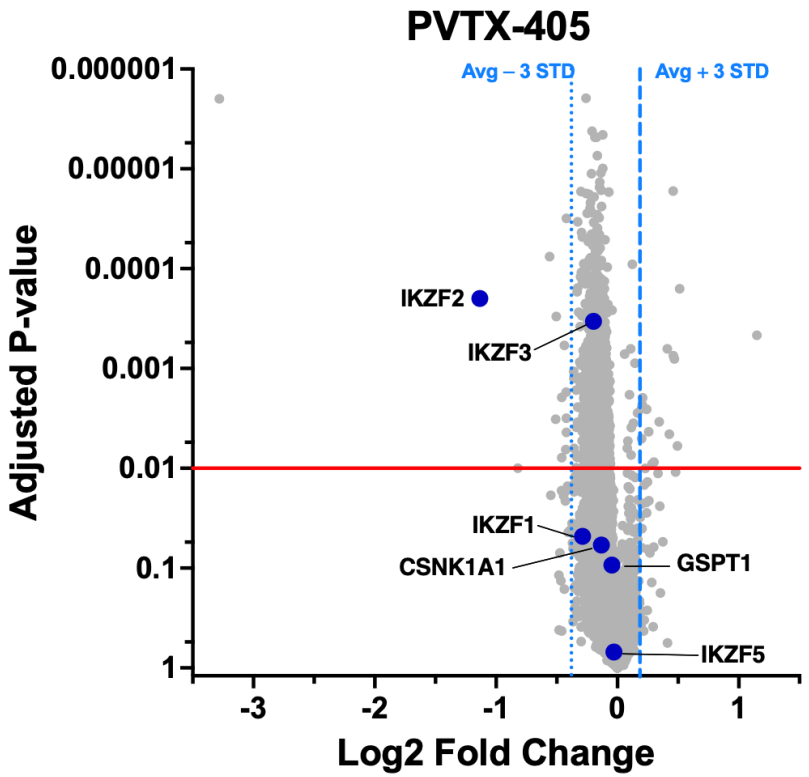
- CRISPR/Cas9 was utilized to engineered CRBN knockout in Jurkat cells
- CRBN KO abrogates IKZF2 degradation by PVTX-405

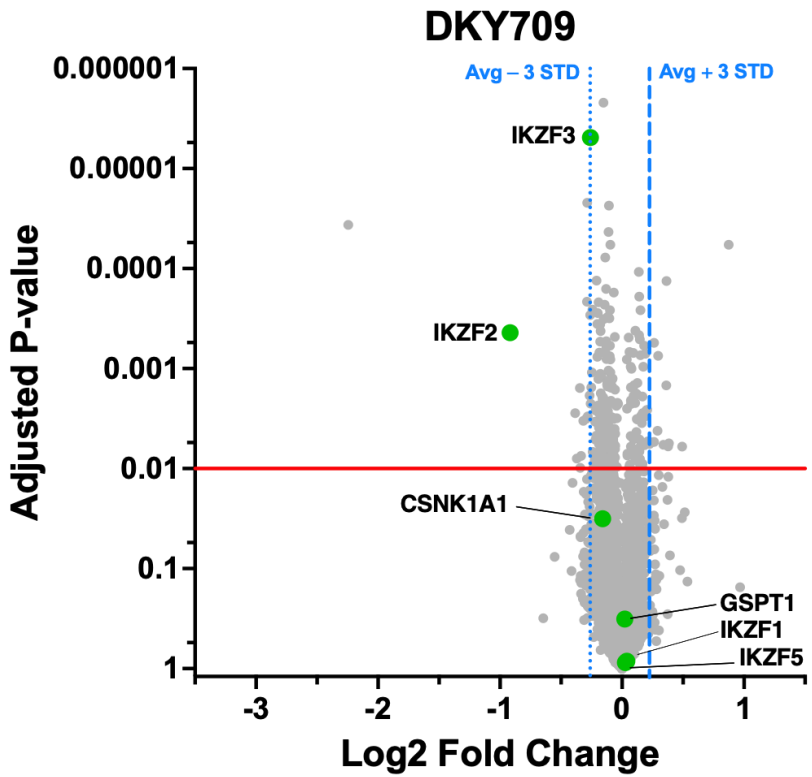
PVTX-405 Shows Selective Degradation of IKZF2



Compound	DC_{50} in nM ($\%D_{max}$)						
	IKZF2 HiBit	IKZF4 HiBit	IKZF1 HiBit	IKZF3 HiBit	SALL4 HiBit	GSPT1 HiBit	CK1a HiBit
PVTX-405	1.0 (84)	3.8 (66)	>10000 (ND)	>10000 (ND)	45 (30)	>10000 (ND)	>10000 (ND)
DKY709	1.5 (73)	4.4 (39)	>10000 (ND)	>10000 (ND)	4.9 (55)	>10000 (ND)	>10000 (ND)

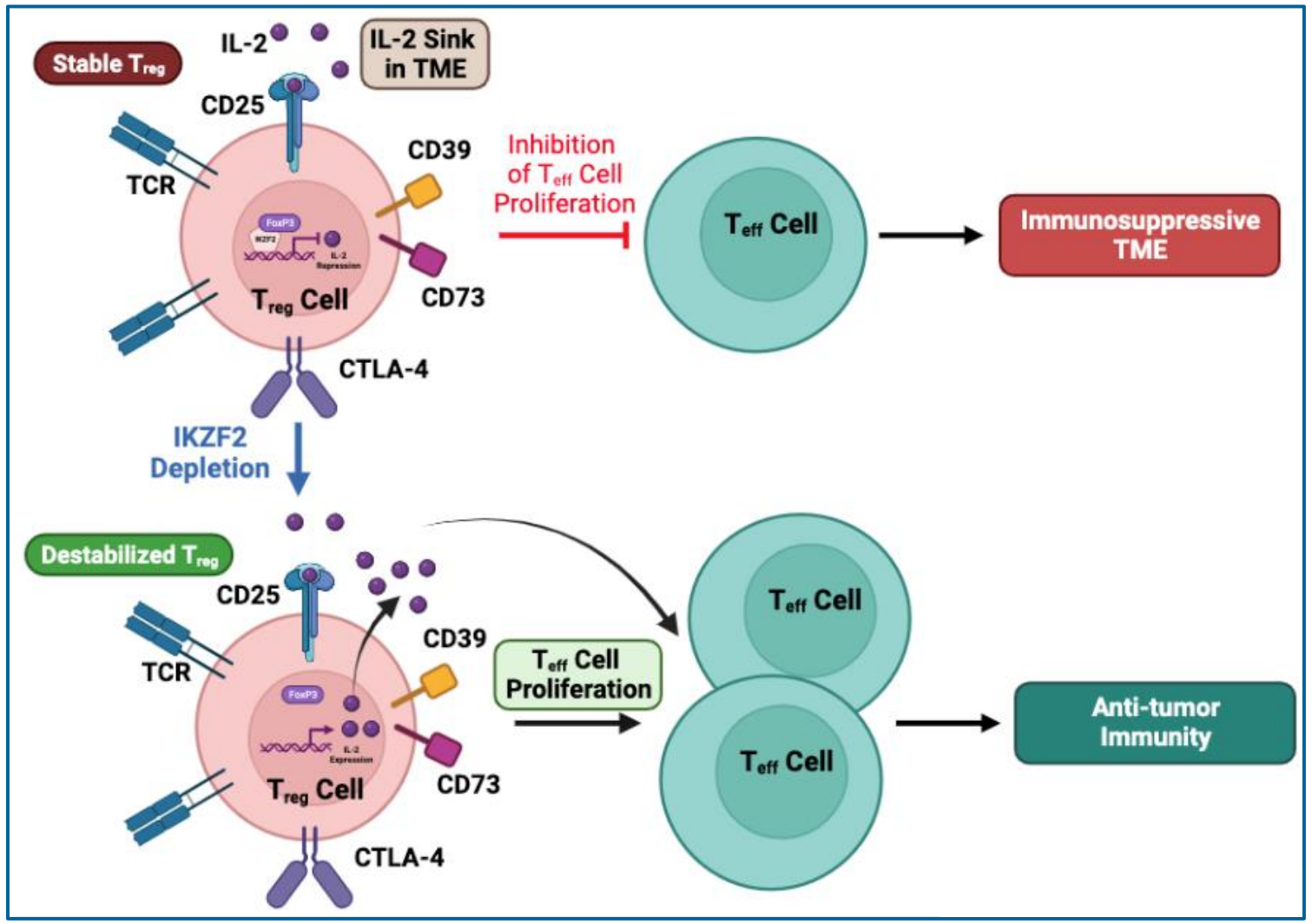
Proteomics Confirms Selective Degradation of IKZF2 by PVTX-405





- Relative protein abundance was determined using shotgun-MS proteomics
- PVTX-405 demonstrates high selectivity for IKZF2 relative to other IKZF family members, GSPT1, and other CRBN neo-substrates

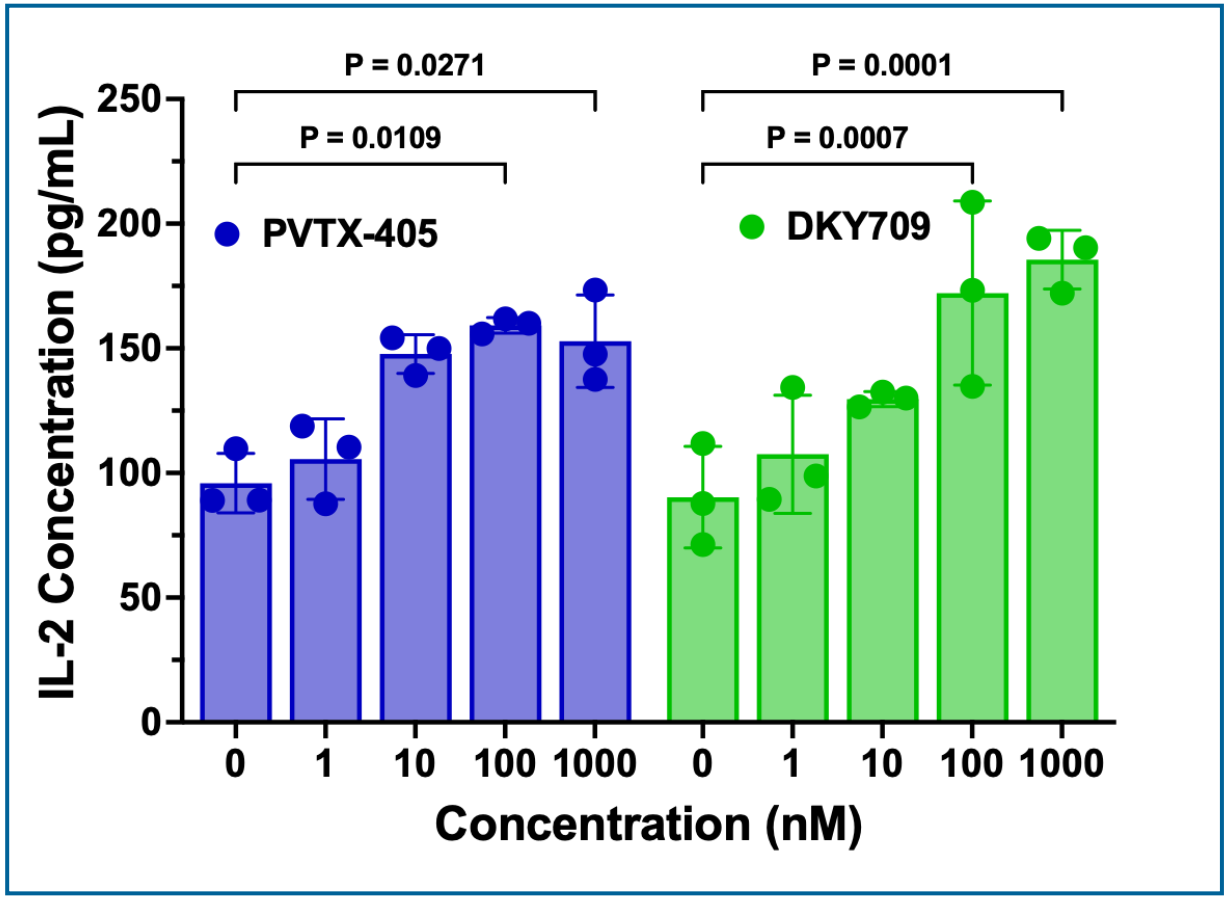
IKZF2 Depletion in T_{regs} Should Lead to Increases in Effector Cytokine Production



- T_{regs} reduce inflammatory responses by consuming IL-2 and suppressing effector T-Cell (T_{eff}) proliferation
- IKZF2 depletion should destabilize T_{regs} and induce production of effector cytokines IL-2 and IFNγ
- Increased effector cytokine production can induce T_{eff} cell proliferation and anti-tumor immunity

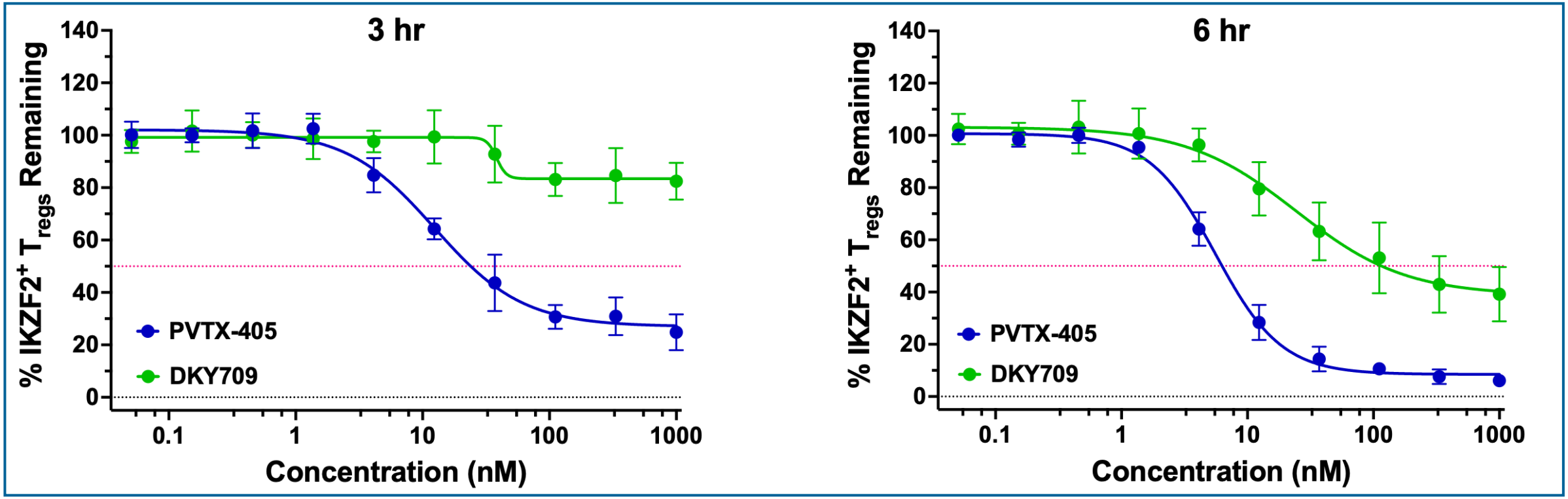
Schematic created with biorender.com

IKZF2 Degradation Results in Increased IL-2 Production



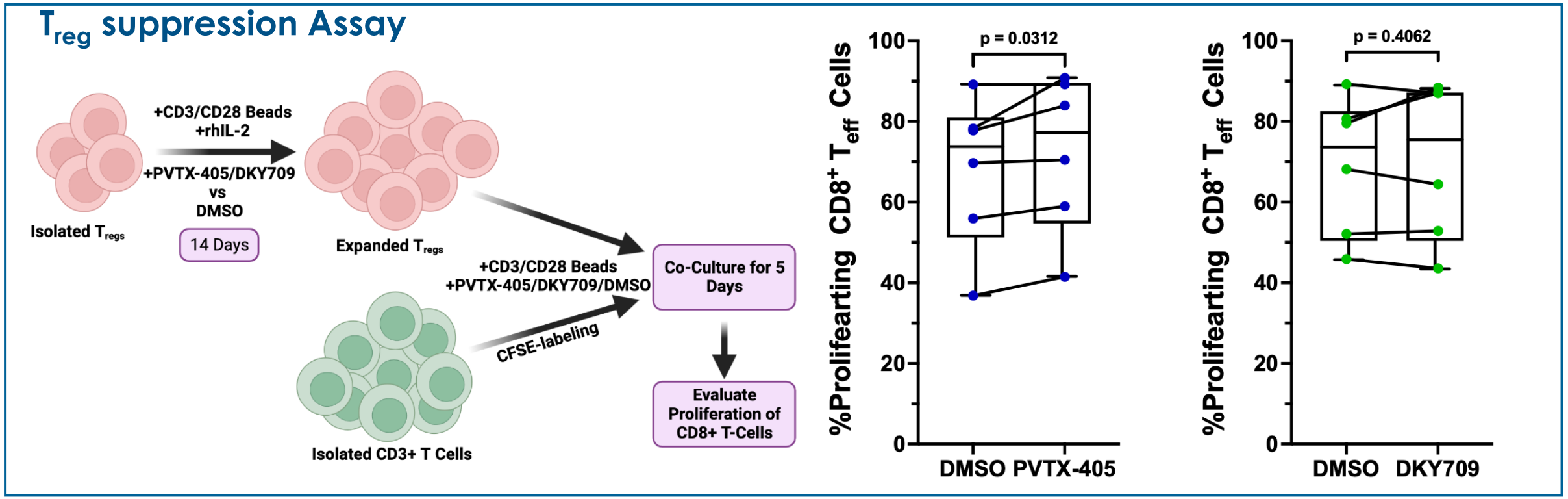
- PVTX-405 treatment of Jurkat cells results in increased IL-2
- IL-2 induction is comparable to DKY709
- Increased IL-2 production demonstrates functional consequence associated with predicted increased anti-tumor immunity

PVTX-405 Induces Rapid, Potent, and Selective IKZF2 Degradation in Primary Human T_{regs}



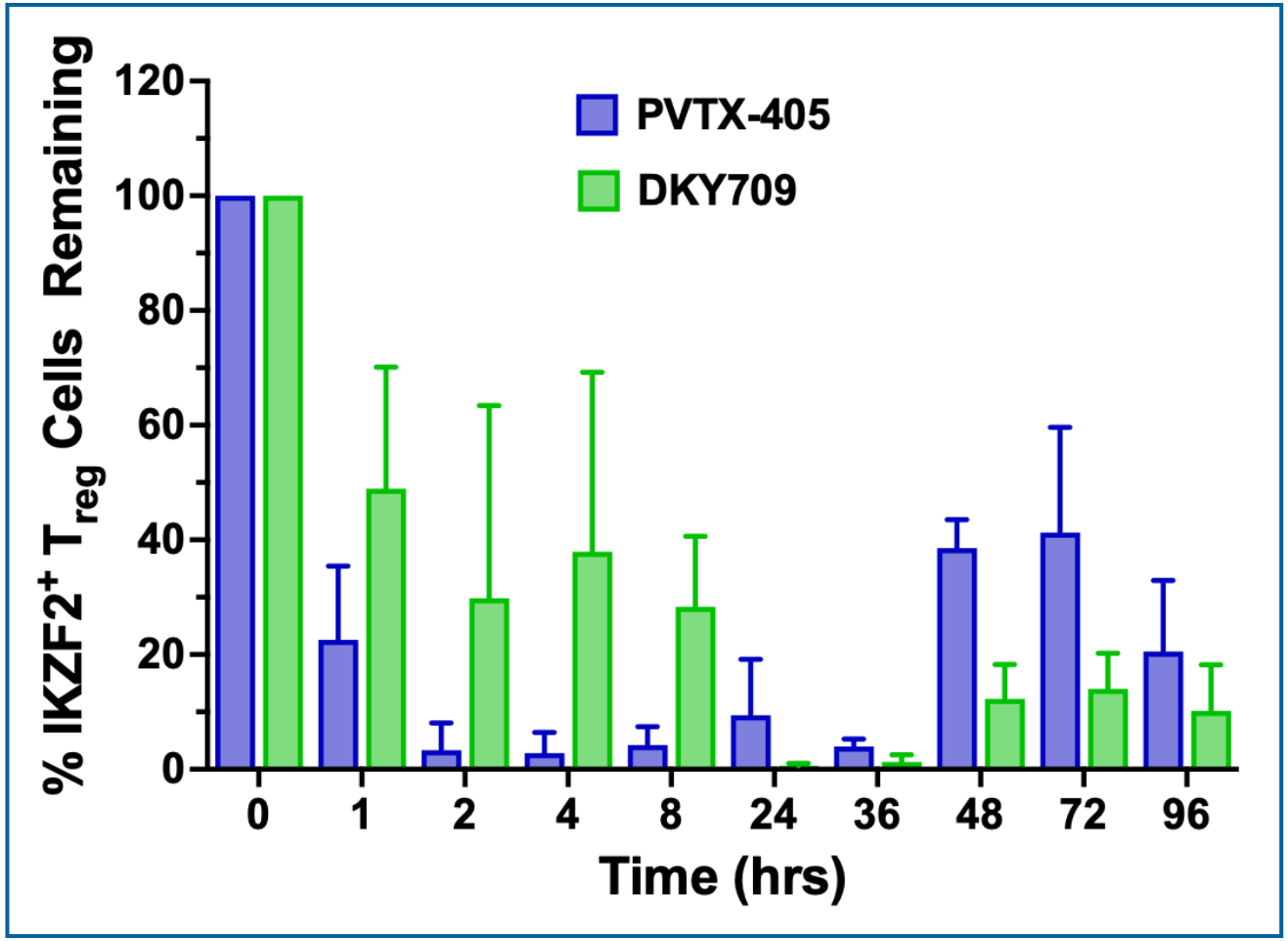
- Human PBMC cells were assessed using multiparameter FACS to measure effects on T_{regs}
- PVTX-405 demonstrates more rapid and potent degradation of IKZF2 than DKY709

Suppression of T_{regs} by IKZF2 Enhances T_{eff} Cell Proliferation



- Impact of PVTX-405 and DKY709 on T_{reg} induced suppression of effector T cell (T_{eff}) proliferation was evaluated in 6 donors
- PVTX-405 treatment showed significant increases in T_{eff} cell proliferation in T_{reg}:T_{eff} cell co-culture assays

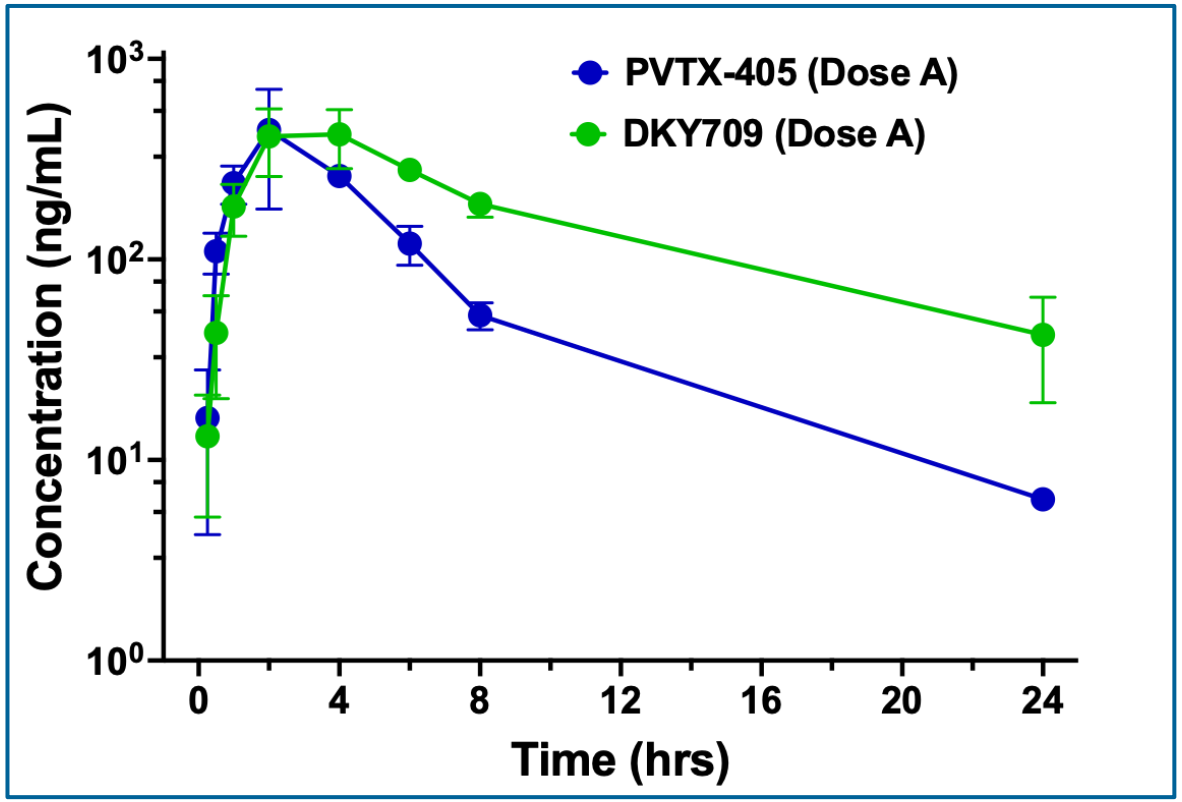
PVTX-405 Shows Robust IKZF2 Degradation in Cyno *In Vivo*



- Non naïve cynomolgus monkeys were treated with either a single dose of PVTX-405 or DKY709
- Whole blood was analyzed using multiparameter FACS assay to measure IKZF2 degradation in T_{regs}
- PVTX-405 shows >90% suppression of $IKZF2^+$ T_{regs} in Cyno

PVTX-405 and DKY709 Share Similar Oral Exposure Profiles in Cyno

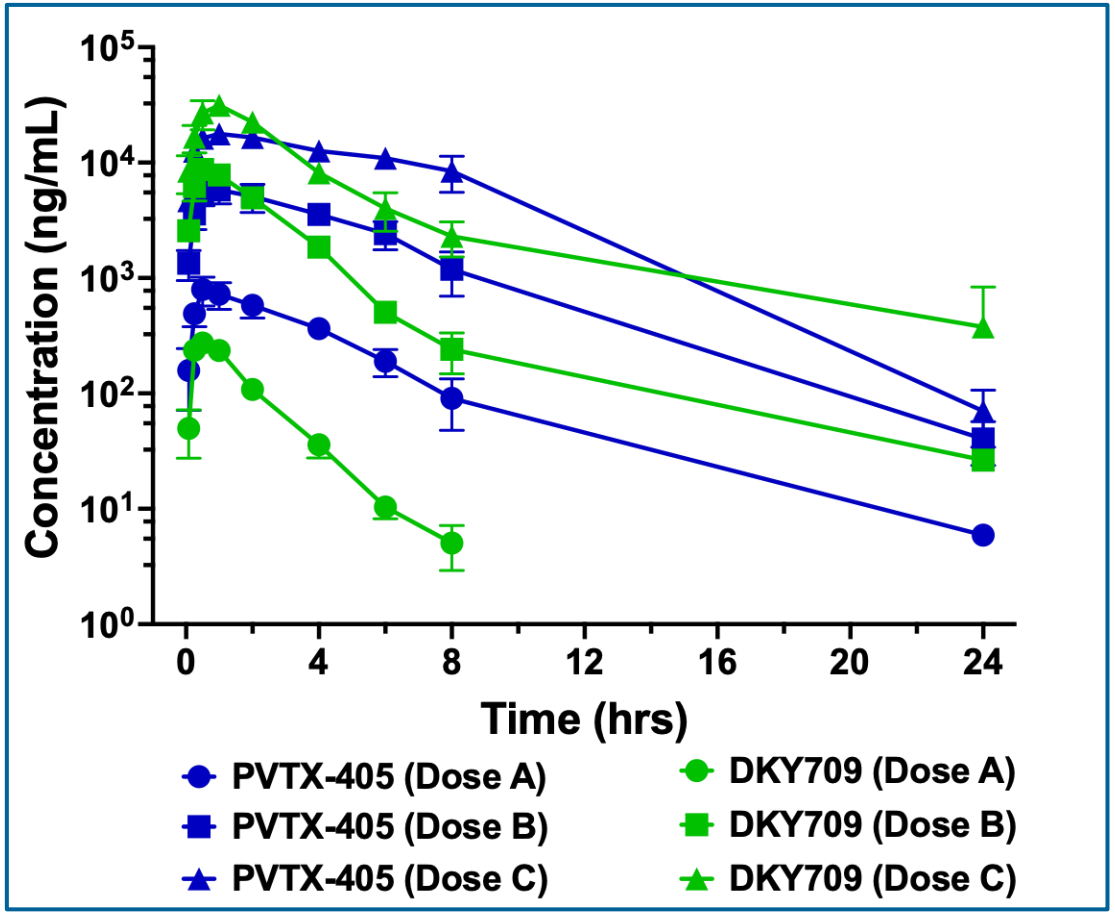
Cyno Pharmacokinetics



Compound	PO Dose, QD	Mean AUC_{0-24} ng*hr/mL	Mean C_{max} ng/mL
PVTX-405	Dose A	2174	442
DKY709	Dose A	4210	451

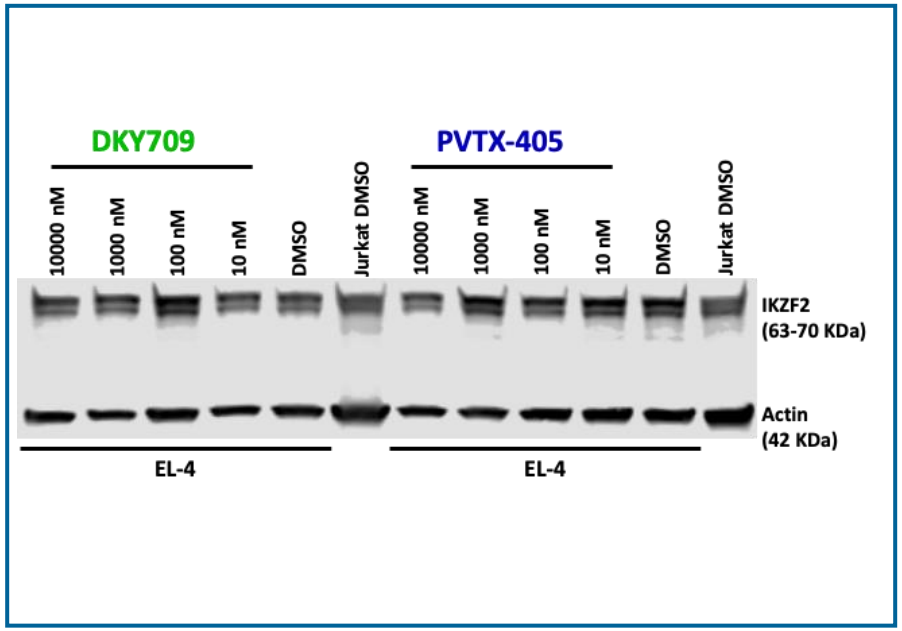
PVTX-405 and DKY709 Show Similar Oral Exposure Profiles in Mice

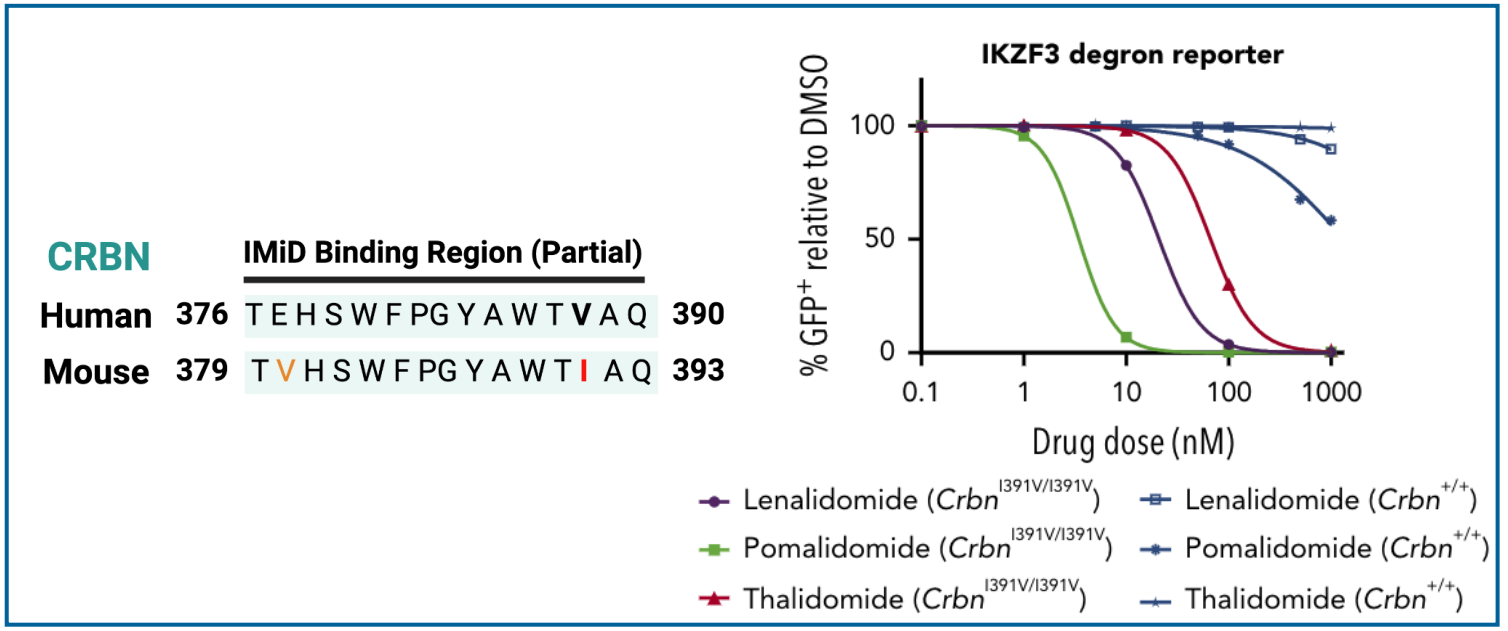
Mouse Pharmacokinetics



Compound	PO Dose, QD	Mean AUC_{0-24} ng*hr/mL	Mean C_{max} ng/mL
PVTX-405	Dose A	3421	884
	Dose B	37957	5916
	Dose C	170989	18032
DKY709	Dose A	594	273
	Dose B	24601	9078
	Dose C	119770	31349

Mouse CRBN is Resistant to PVTX-405 Glue Activity



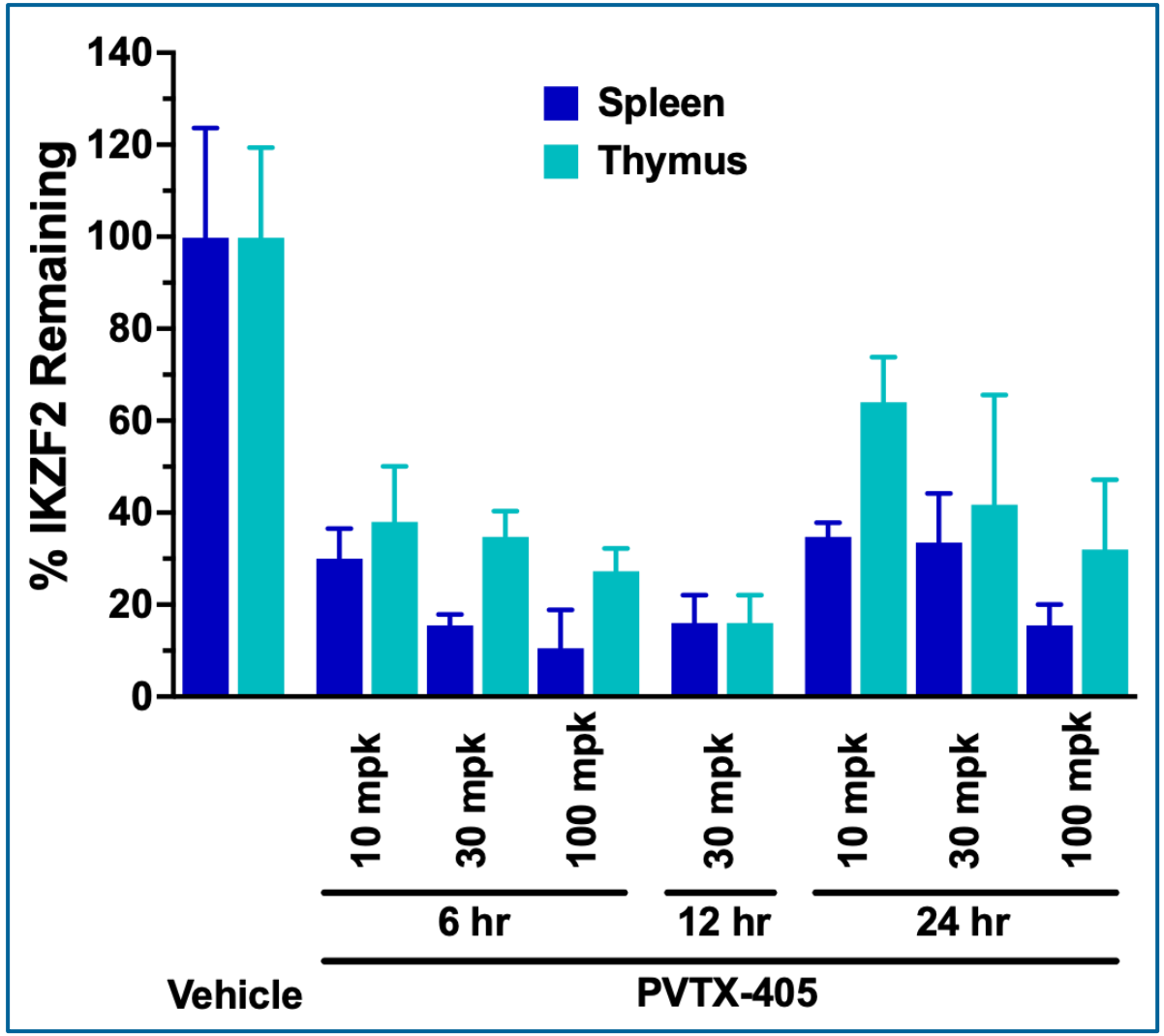


- Neither PVTX-405 or DKY709 treatment induces degradation of IKZF2 in mouse cells

- A single amino acid difference within the CRBN–Immunomodulatory drug (IMiD) binding region renders mouse CRBN resistant to degradation by IMiDs
- A change from Ile 391 to Val in mouse CRBN restores IMiD-induced degradation of IKZF3

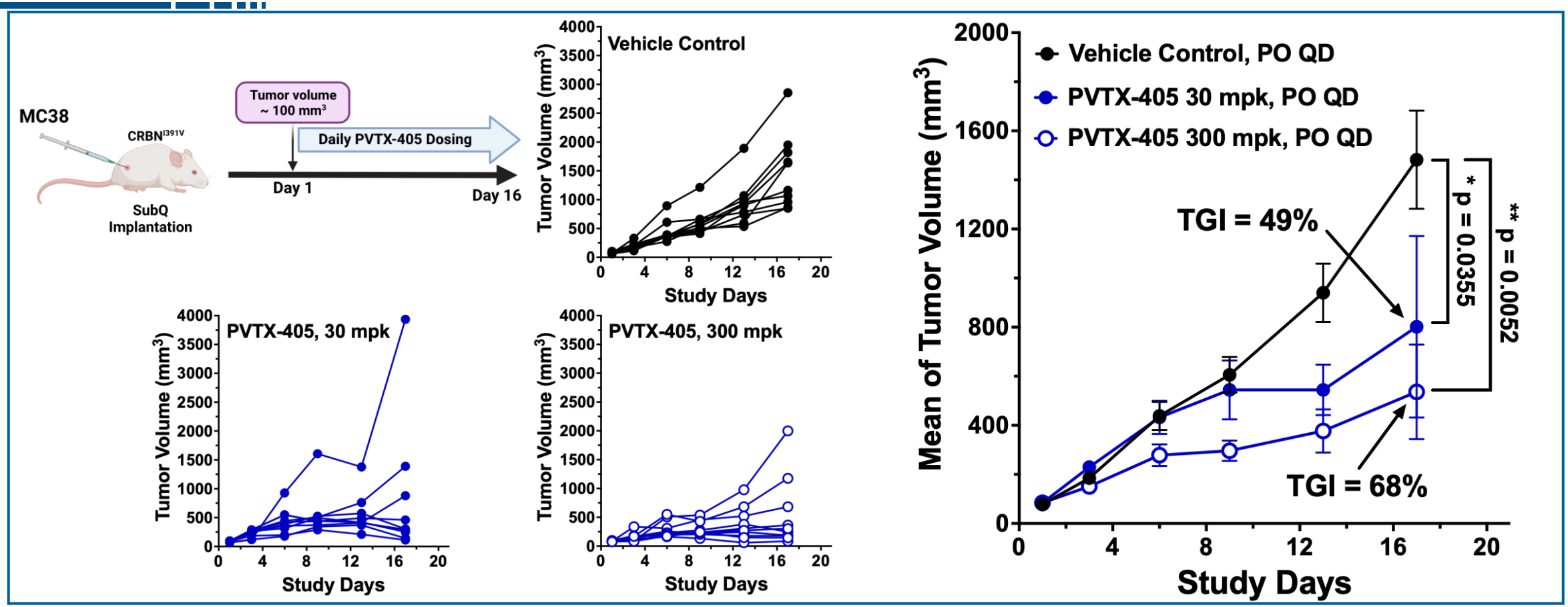
Fink, E. C. et al. Blood 132, 1535–1544 (2018).
Gemechu, Y. et al. P Natl Acad Sci Usa 115, 11802–11807 (2018).

PVTX-405 Administration Leads to Robust IKZF2 Degradation in CRBN^{I391V} Mice



- CRBN^{I391V} mice were administered a single oral dose of PVTX-405
- PVTX-405 shows dose dependent degradation of IKZF2 in spleen and thymus of CRBN^{I391V} mice

PVTX-405 Shows Significant Suppression of MC38 Tumor Growth in Immune-competent Mice



- MC38 xenograft model was established in CRBN^{I391V} mice
- PVTX-405 inhibits MC38 tumor growth *in vivo*

PVTX-405 is a Development Candidate Stage Molecular Glue Degrader of IKZF2 with Potential to be Best-in-Class

Development Candidate	▪ A potent, selective molecular glue degrader of IKZF2 ▪ Demonstration of target pharmacology including IL-2 induction ▪ *In vivo* degradation in multiple species
Developability	▪ **Low hERG liability; 5-fold improvement in hERG IC_{50} compared to DKY709** ▪ **Low plasma clearance and good oral bioavailability** across preclinical species ▪ Low risk for DDI ▪ **Excellent *in vitro* safety profile**: AMES and micronucleus negative, low potential for CV and DILI risk, no reactive metabolite formation, no human-specific metabolites ▪ Good off-target and neo-substrate profile ▪ **In-life portion of rat and cyno non-GLP toxicology studies completed**
Efficacy	▪ **Single agent efficacy against novel MC38 syngeneic model *in vivo***

Acknowledgements

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