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

March 30, 2023

Harshil Dhruv

3rd TPD Europe Conference

proteovant THERAPEUTICS

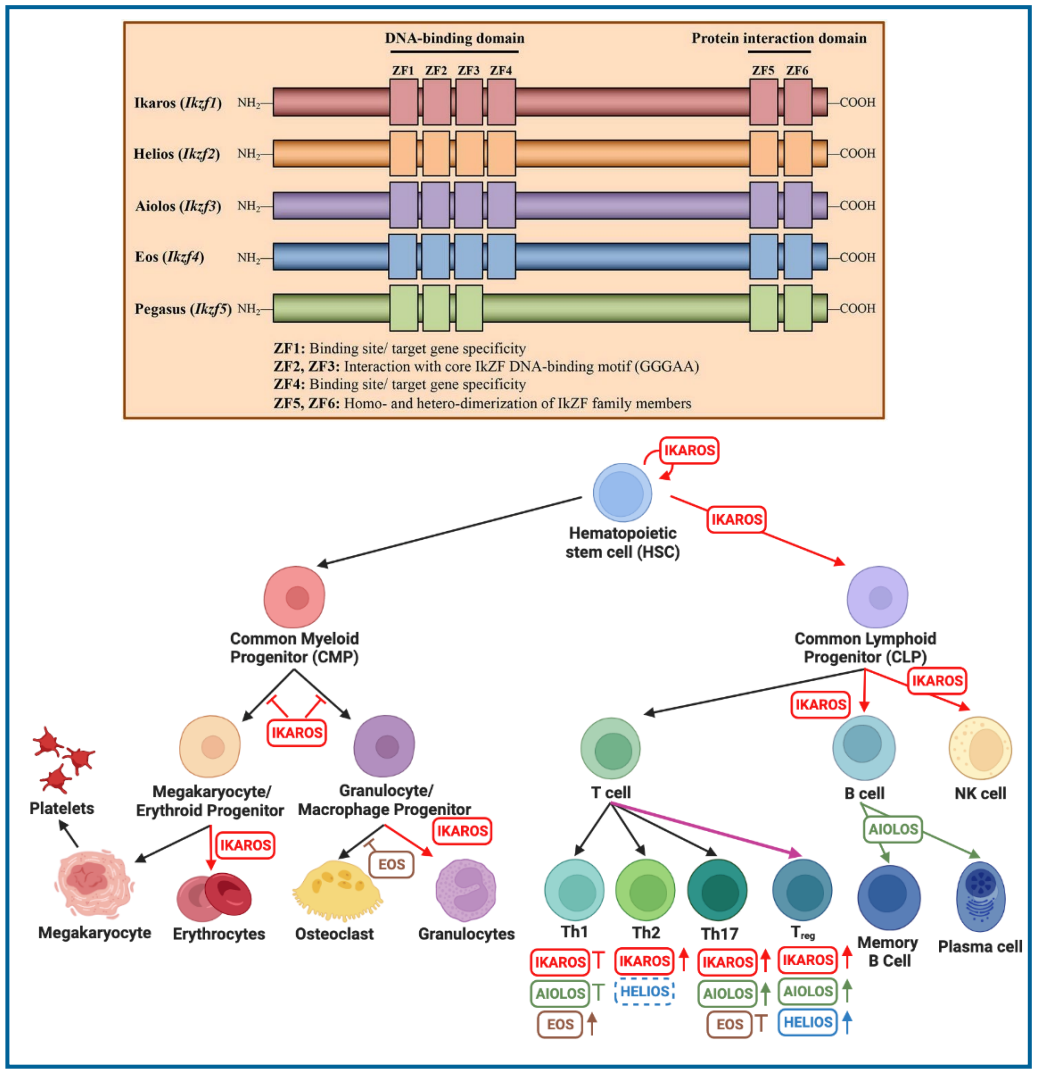
Degrading Proteins, Defeating Disease

Forward Looking Statements

This Presentation was prepared by Proteovant Therapeutics ("Proteovant") solely for presentation to a limited number of potential business partners of Proteovant, and may contain forward-looking statements for the purposes of the safe harbor provisions under The Private Securities Litigation Reform Act of 1995 and other federal securities laws. This document includes forward-looking statements that represent our opinions, expectations, beliefs, intentions, estimates or strategies regarding the future, which may not be realized. These statements may be identified by the use of words like "anticipate," "believe," "estimate," "expect," "intend," "may," "plan," "potential," "will," "should," "seek," and similar expressions. The forward-looking statements reflect our views and assumptions with respect to future events as of the date of this document and are subject to risks and uncertainties. Actual and future results and trends could differ materially from those described by such statements due to various factors that are beyond our ability to control or predict. Given these uncertainties, you should not place undue reliance on the forward-looking statements. Proteovant does not undertake any obligation to update or revise any forward-looking statements, whether as a result of new information, future events or otherwise.

No representation or warranty, express or implied, is made by Proteovant or its affiliates, or Proteovant or such affiliates' respective directors, officers, employees or advisers or any other person as to the accuracy or completeness of the information contained herein, and no responsibility or liability whatsoever is accepted for the accuracy or sufficiency thereof or for any errors, omissions or misstatements or otherwise, relating thereto. Without limiting the generality of the foregoing, no audit or review has been undertaken by an independent third party of the financial assumptions, data, results, calculations and forecasts contained, presented or referred to in this document. You should conduct your own independent investigation and assessment as to the validity of the information contained in this document and the economic, financial, regulatory, legal, taxation, stamp duty and accounting implications of that information.

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

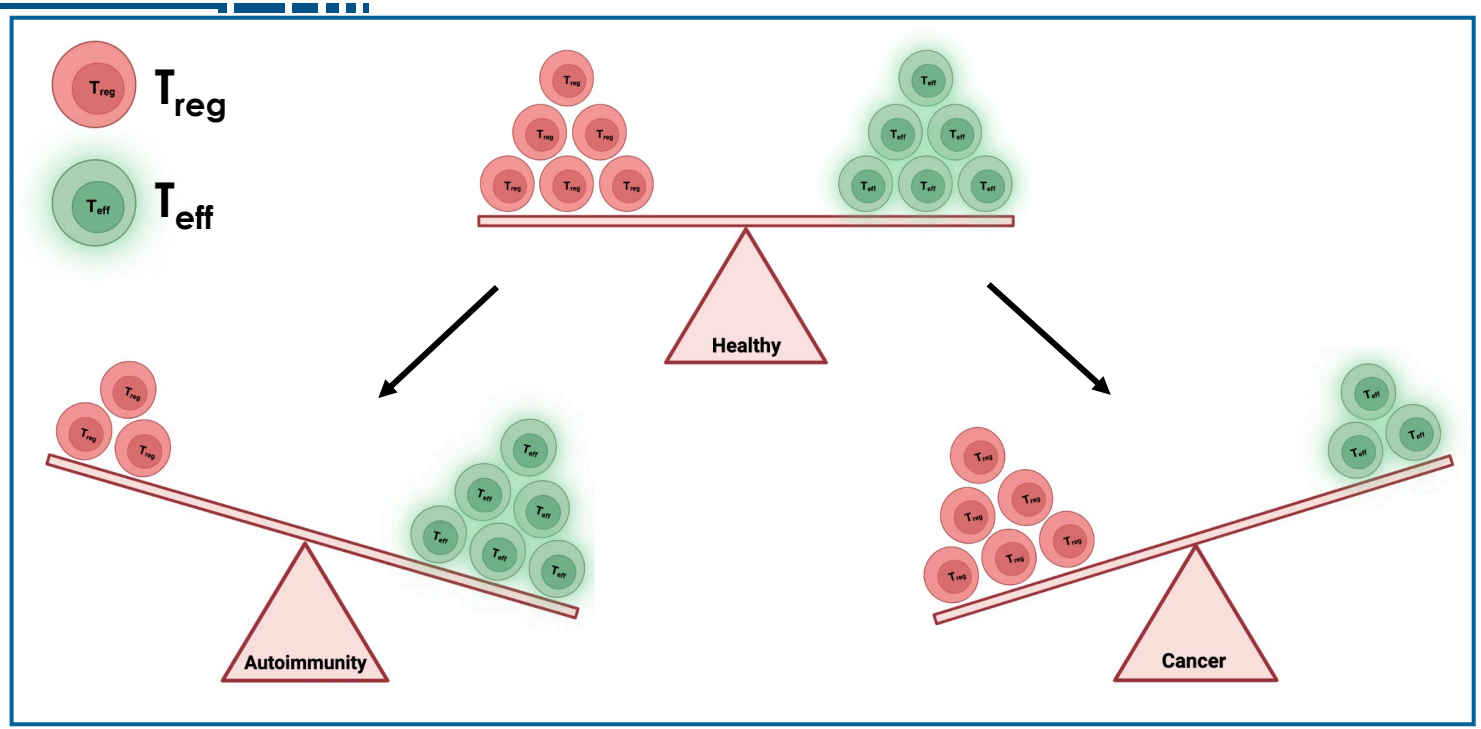


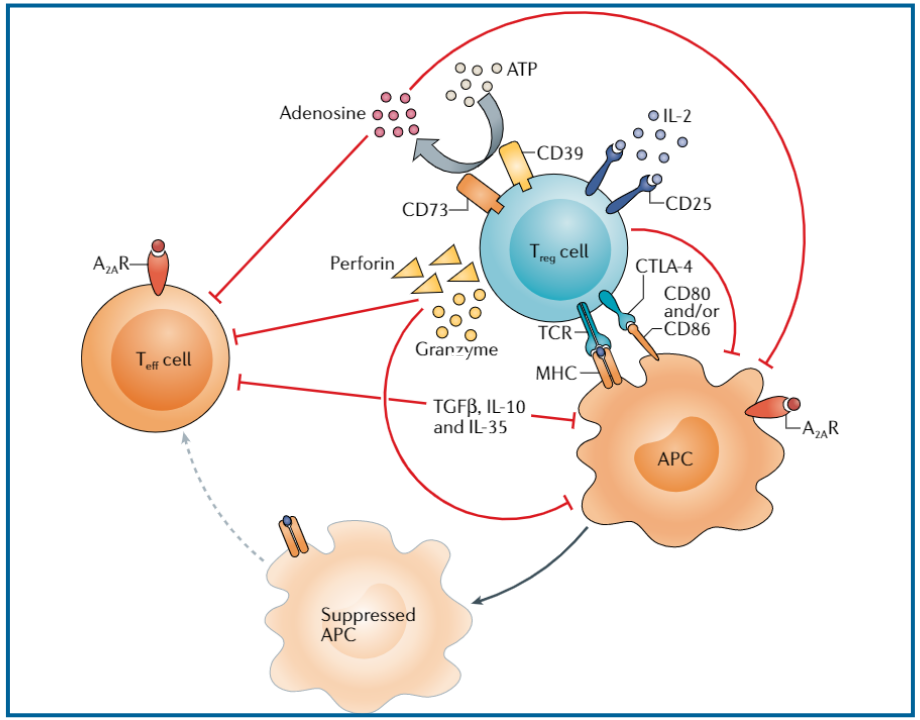
- IKZF2 (Helios) is a member of a family of five transcriptional regulators that include IKZF1, IKZF3, IKZF4, and IKZF5
- IKZF2 is comprised of four N-terminal zinc finger (ZF) DNA-binding domains and two C-terminal ZF protein-protein interaction domains
- IKZF2 expression is largely restricted to select 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



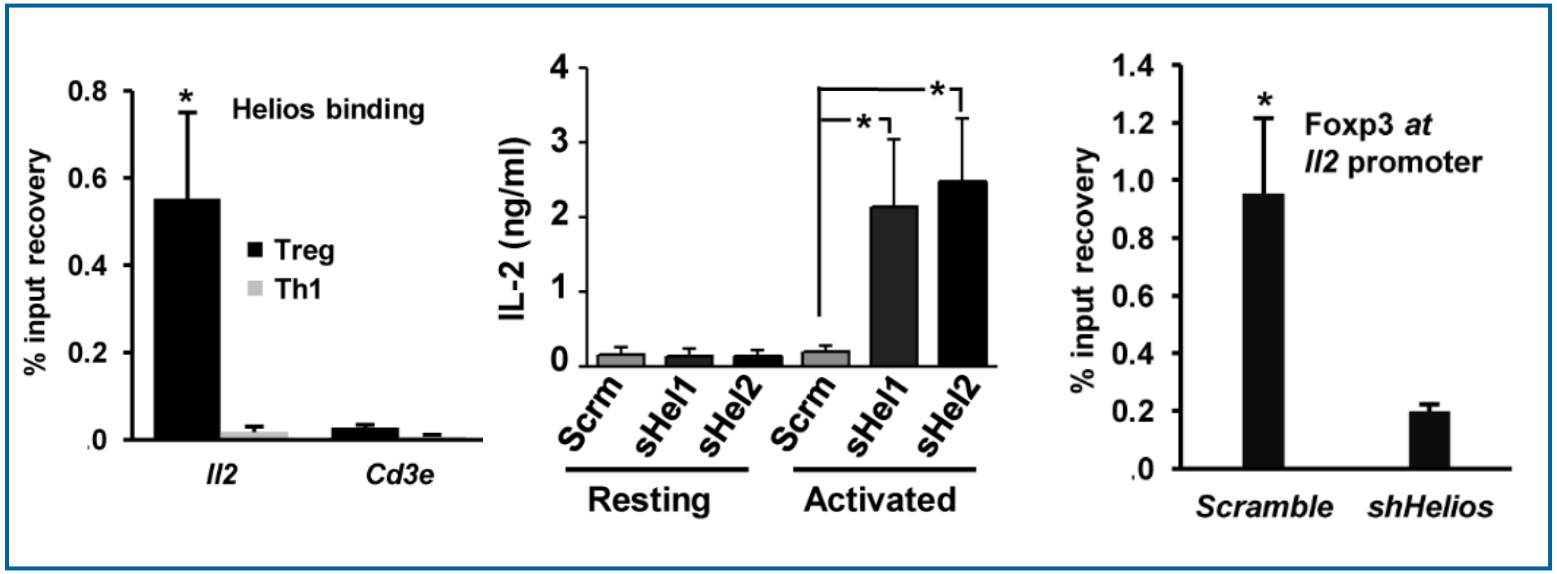


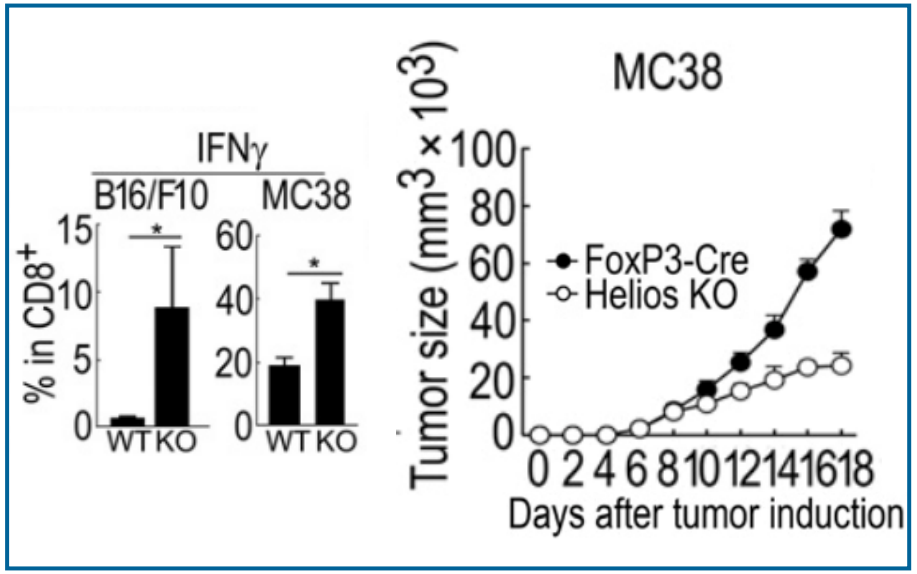
- T_{reg} cells are an immunosuppressive subset of CD4+ T cells that play essential roles in self-tolerance
- High relative abundance of T_{reg} cells in the tumor microenvironment (TME) is associated with poor prognosis in various cancer types
- Evading immune surveillance and destruction is fundamental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



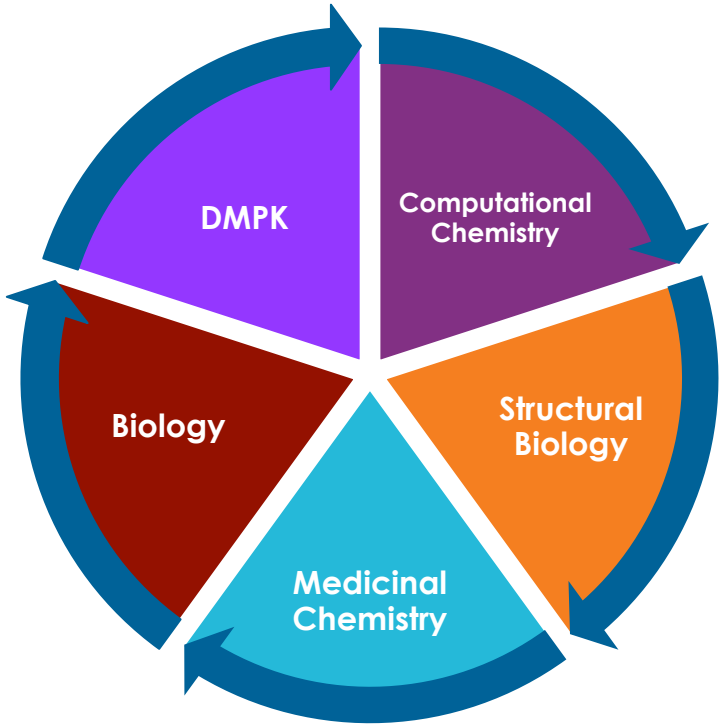


- Stable inhibitory activity of T_{regs} is linked to IL-2 repression
- IKZF2 binds to the IL-2 promoter in T_{reg} cells and suppresses transcriptional activation
  - IKZF2 KD results in higher IL-2 expression upon stimulation
- IKZF2 KD suppresses FoxP3 binding to IL-2 promoter

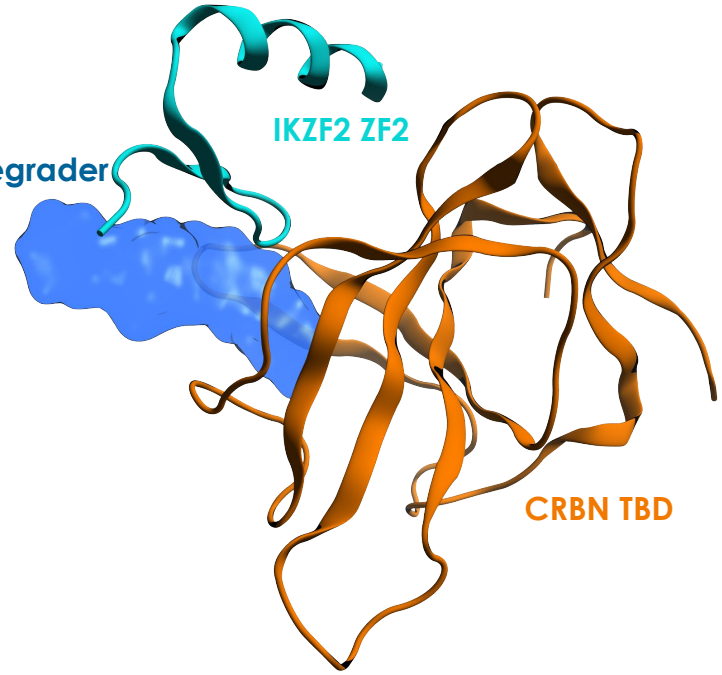
- IKZF2 KO leads to an unstable CD4 T_{reg} phenotype marked by production of effector cytokines
- IKZF2 KO in T_{regs} suppresses tumor growth

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



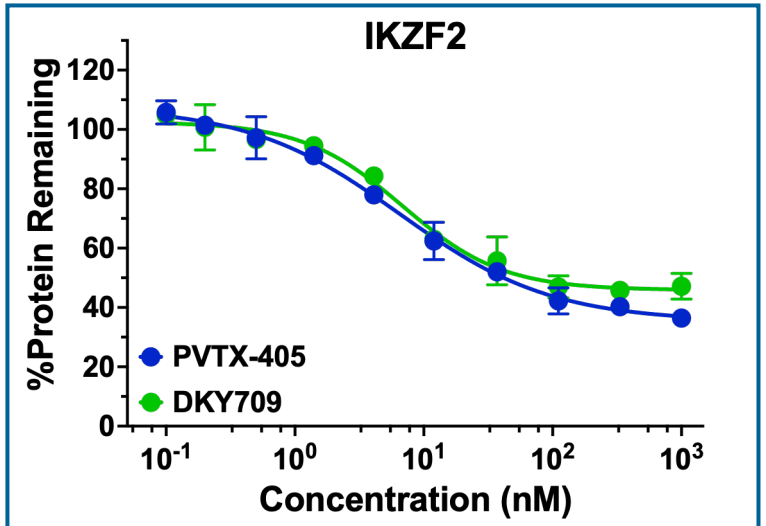
X-ray of CRBN TBD: PVT Degrader: IKZF2 (ZF2) Complex (~2 Å)



PVTX-405

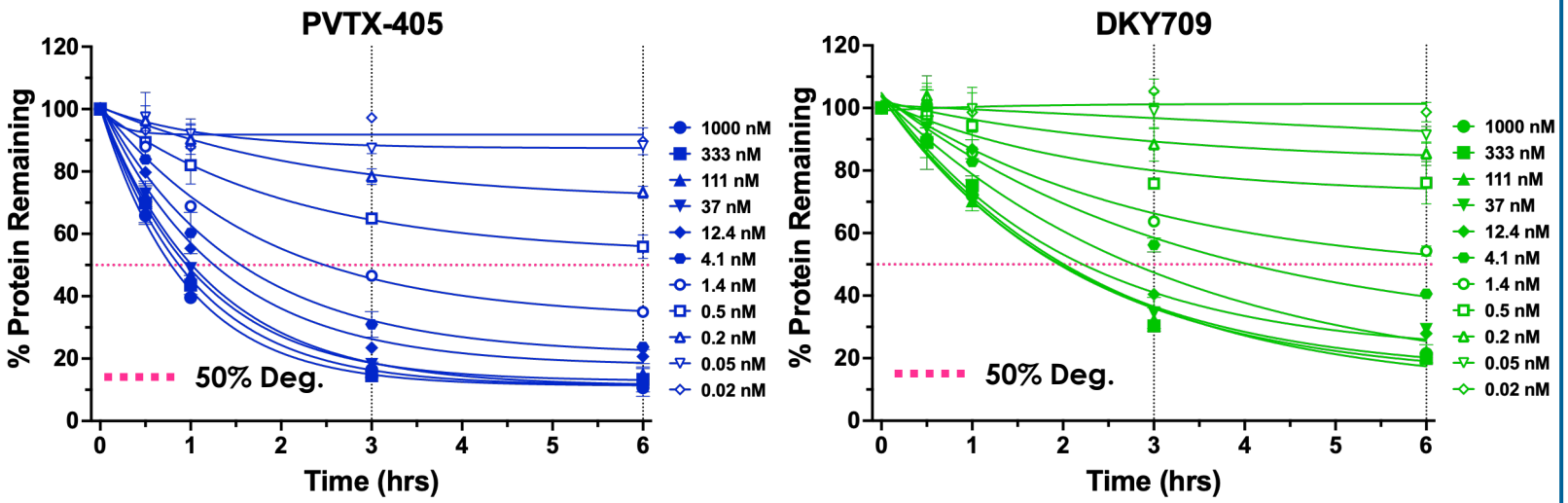
- Fully integrated discovery team applying a multi-disciplinary approach to drug hunting
- Multiple cycles of SBDD using ternary complex structures to guide lead optimization

PVTX-405 Induces Potent and Rapid IKZF2 Degradation



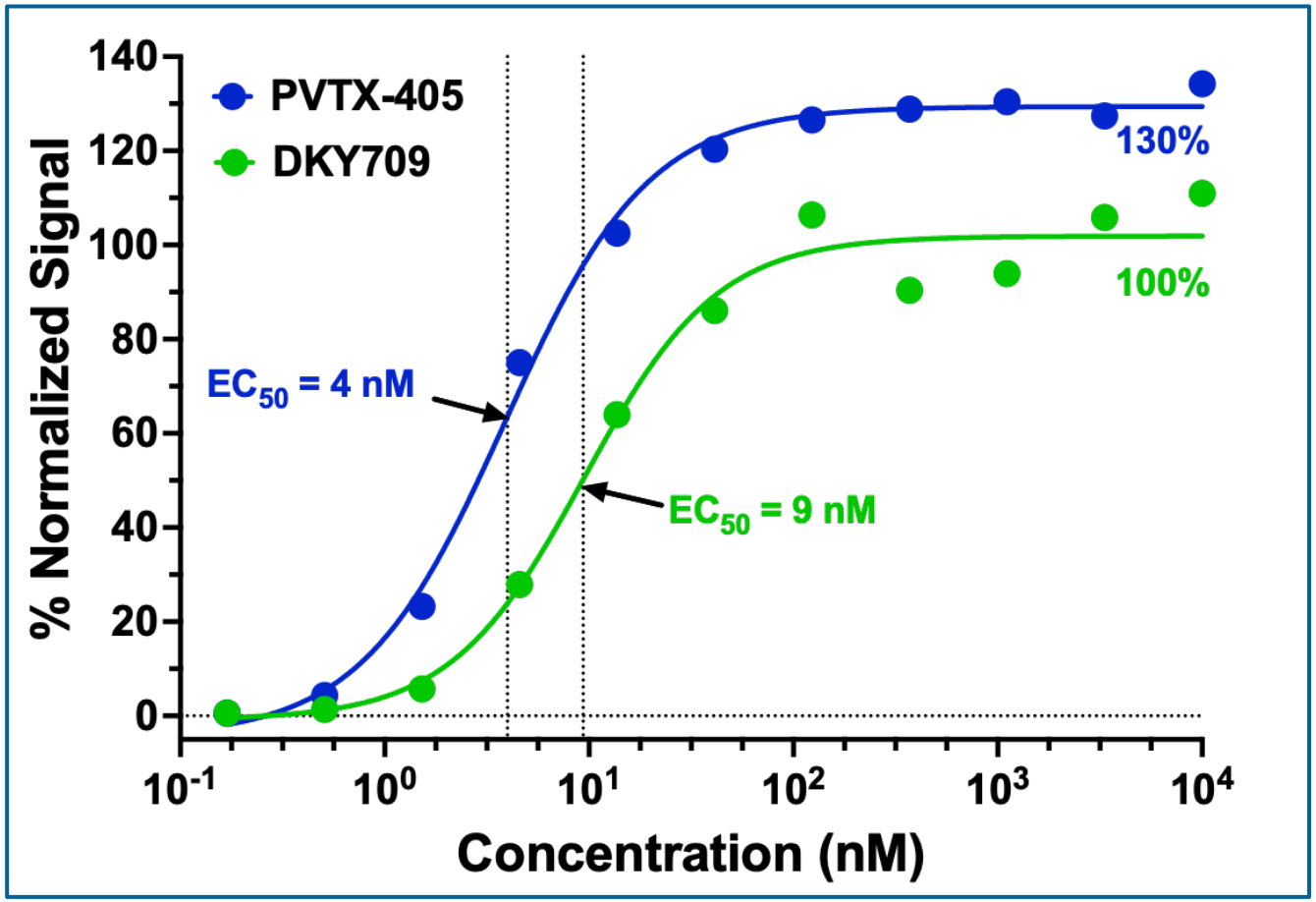
Compound	DC_{50} (nM)	D_{max} (%)
PVTX-405	6.3	65
DKY709	8.1	59

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



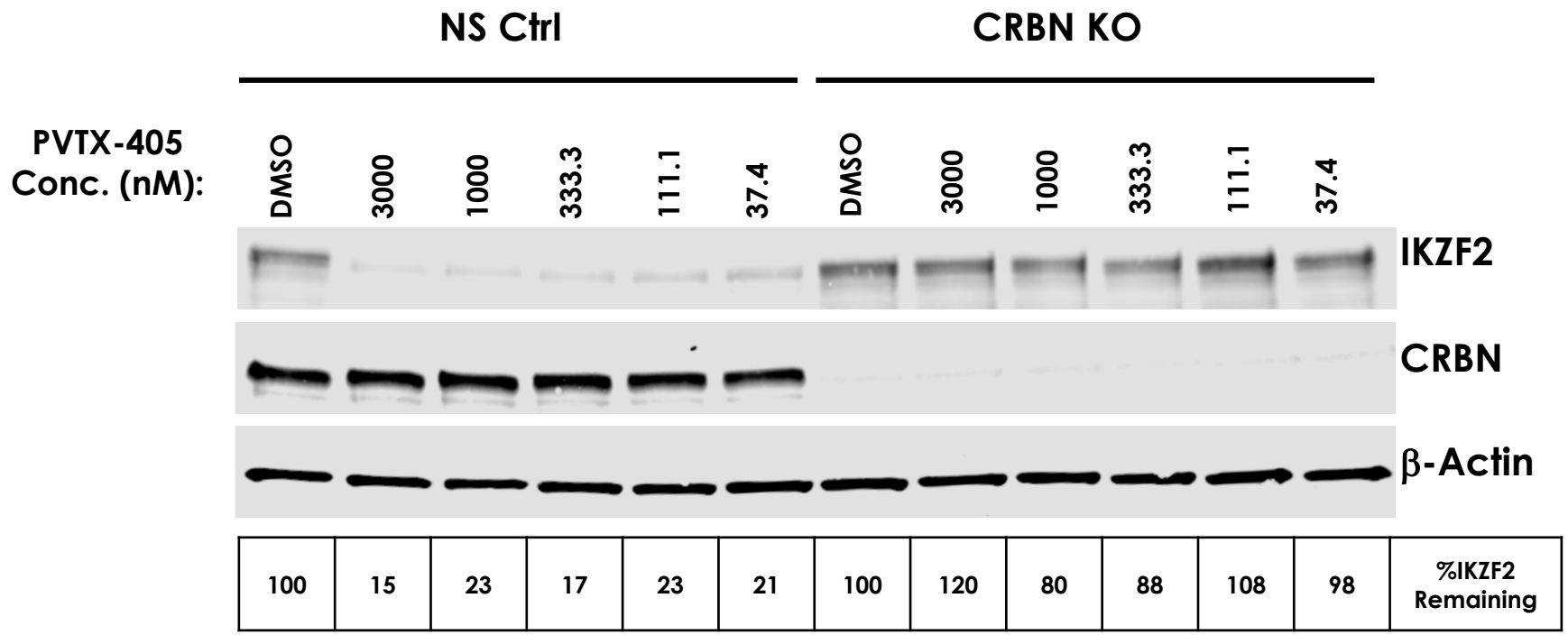
- PVTX-405 shows more rapid degradation kinetics than DKY709
- PVTX-405 achieves maximal degradation by 6 hrs while DKY709 requires 18 hours to reach D_{max} plateau

PVTX-405 Demonstrates Robust CRBN/IKZF2 Ternary Complex Formation



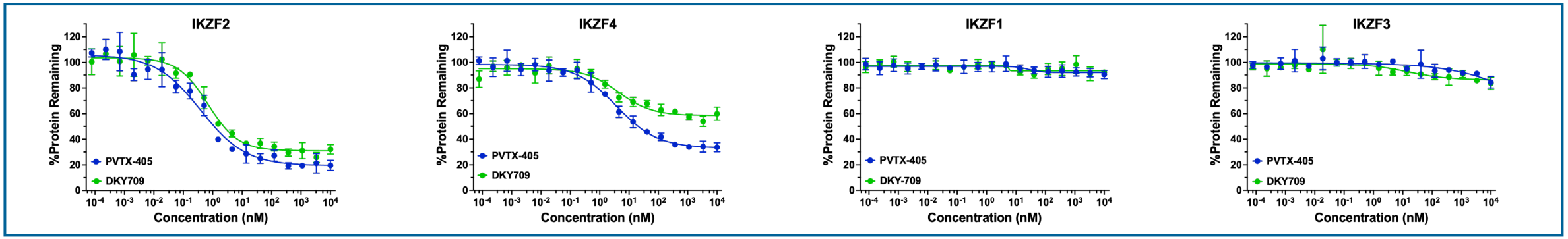
- A greater level of ternary complex is formed in the presence of PVTX-405 than DKY709
  - Higher max signal and higher signal at each concentration of PVTX-405 than DKY709 are evident
- EC_{50} values are similar for the two compounds suggesting similar stability of the complex

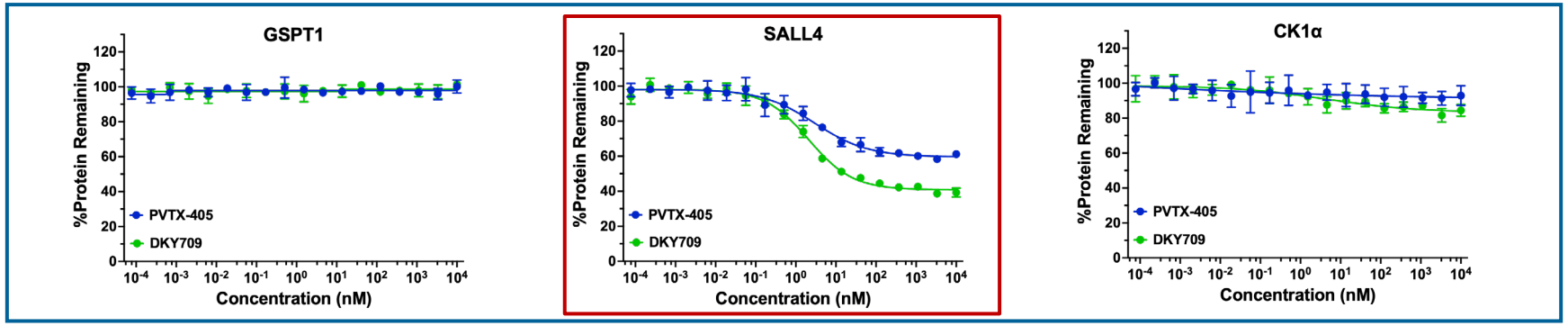
PVTX-405 Mediated IKZF2 Degradation is CRBN Dependent



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

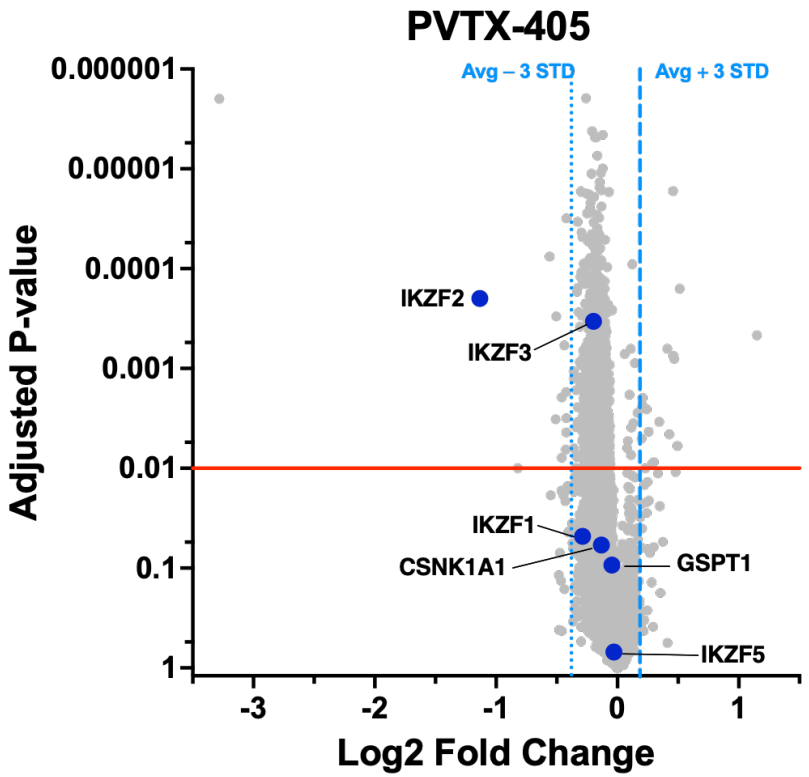
PVTX-405 Shows Selectivity Against Neosubstrates of Concern

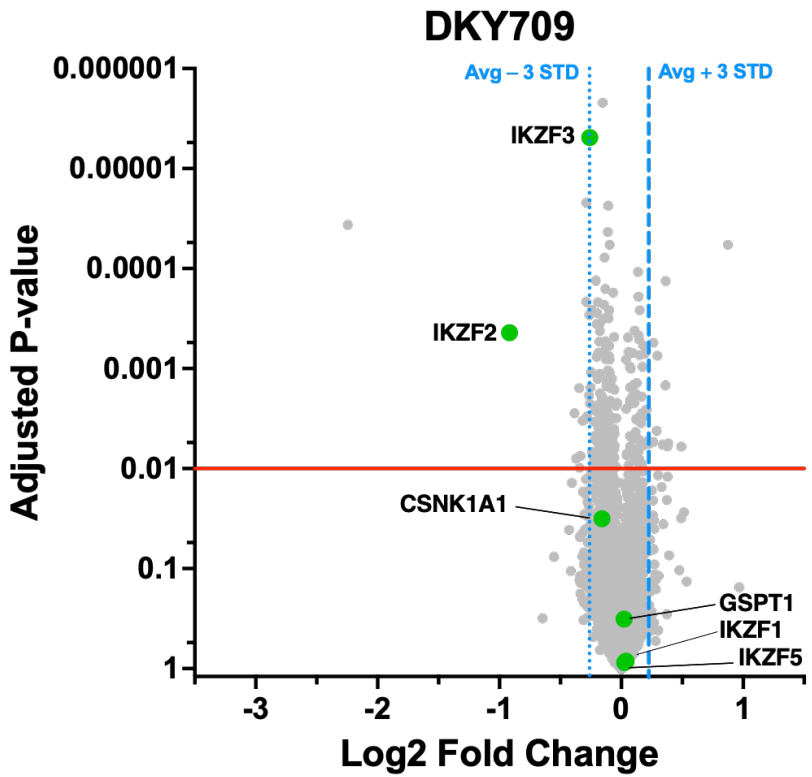




Compound	DC_{50} in nM ($\%D_{max}$)						
	IKZF2 HiBit	IKZF4 HiBit	IKZF1 HiBit	IKZF3 HiBit	SALL4 HiBit	GSPT1 HiBit	CK1a HiBit
PVTX-405	1.0 (84)	3.8 (66)	>10000 (ND)	>10000 (ND)	45 (30)	>10000 (ND)	>10000 (ND)
DKY709	1.5 (73)	4.4 (39)	>10000 (ND)	>10000 (ND)	4.9 (55)	>10000 (ND)	>10000 (ND)

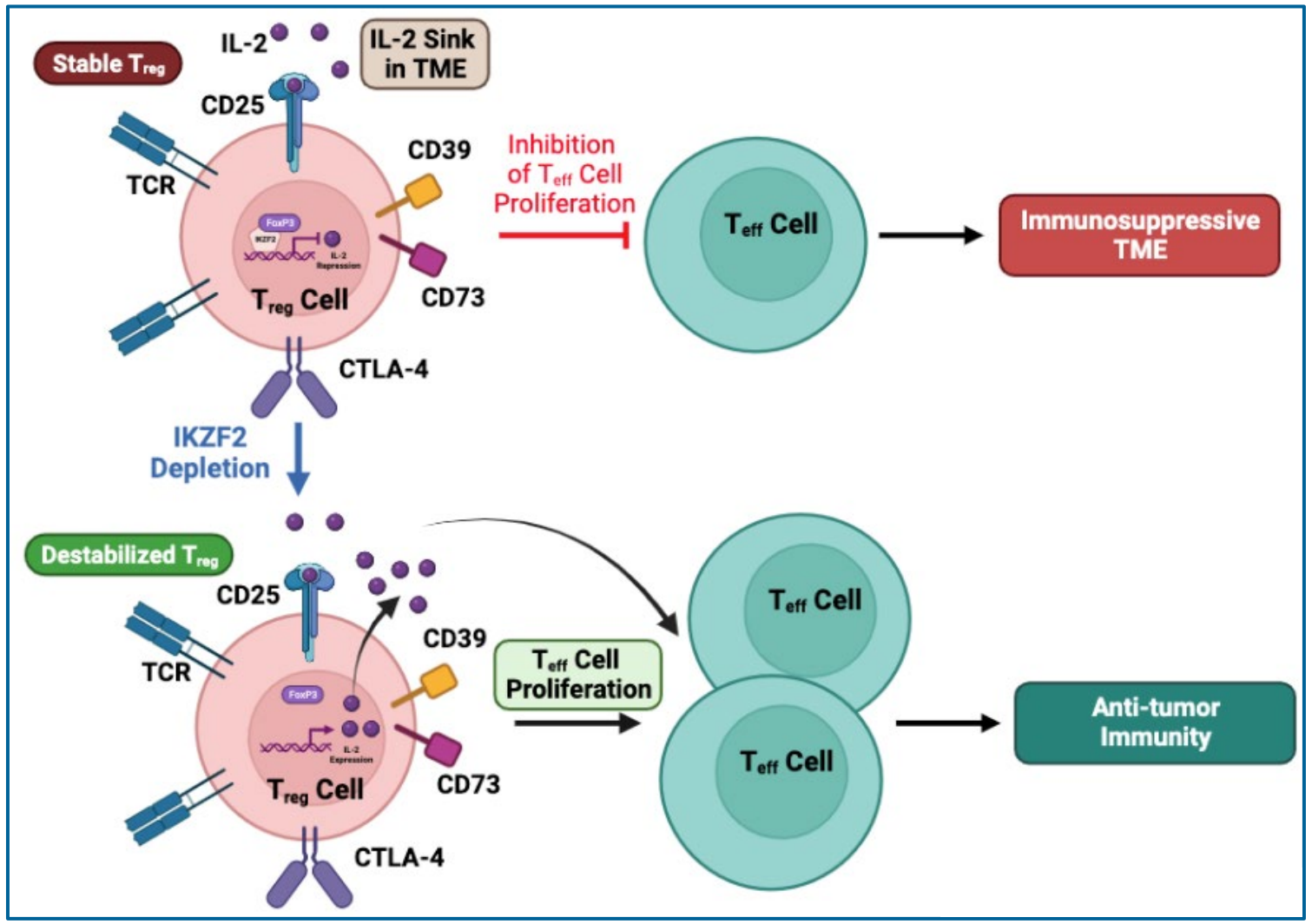
Proteomics Confirms Selective Degradation of IKZF2 by PVTX-405





- 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

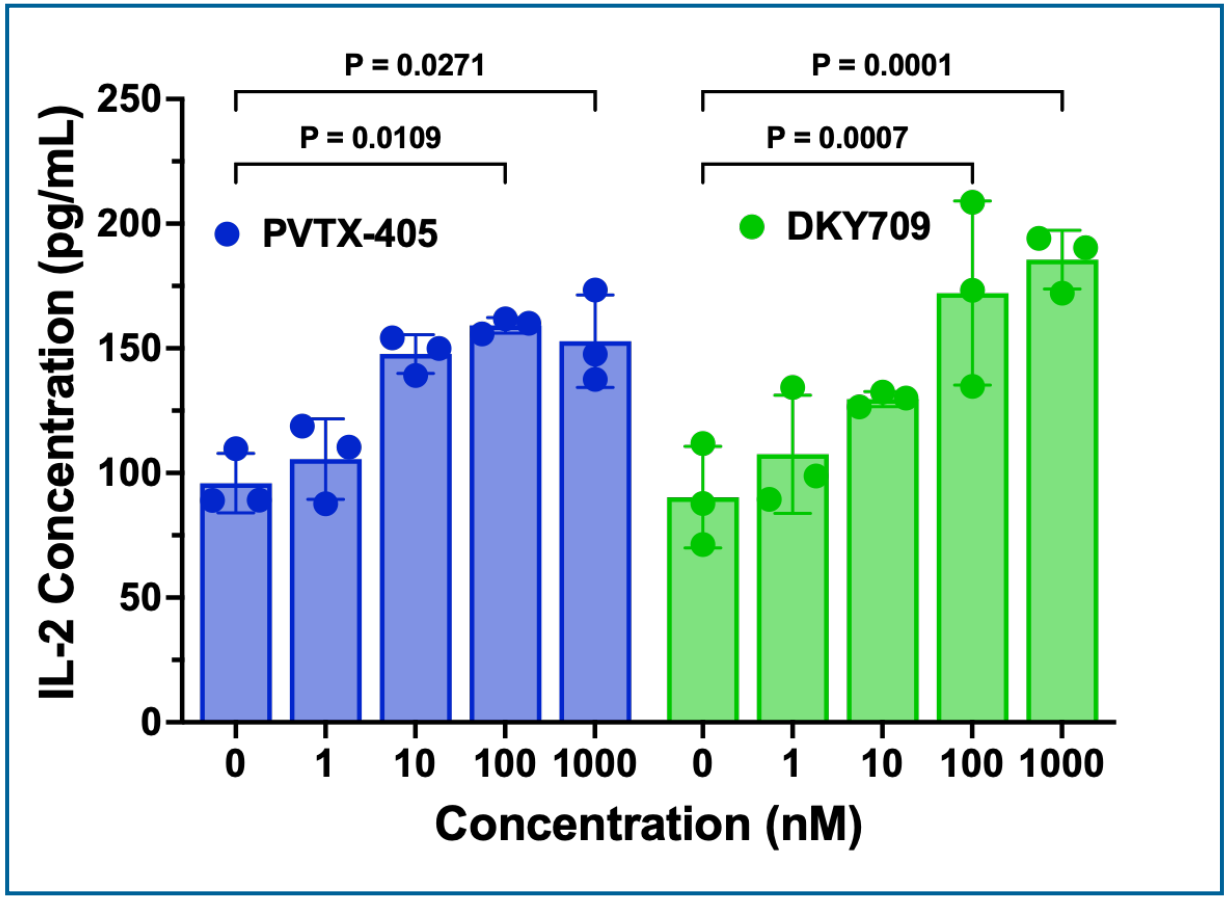
IKZF2 Depletion in T_{regs} Should Lead to Increases in Effector Cytokine Production



- 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

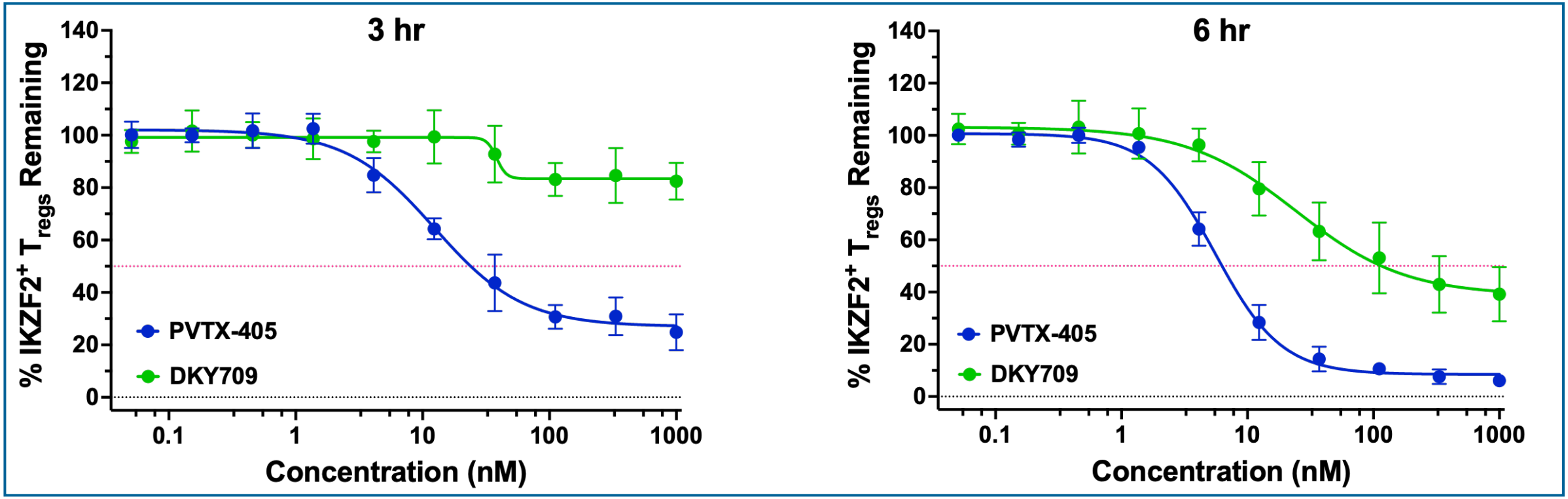
Schematic created with biorender.com

IKZF2 Degradation Results in Increased IL-2 Production



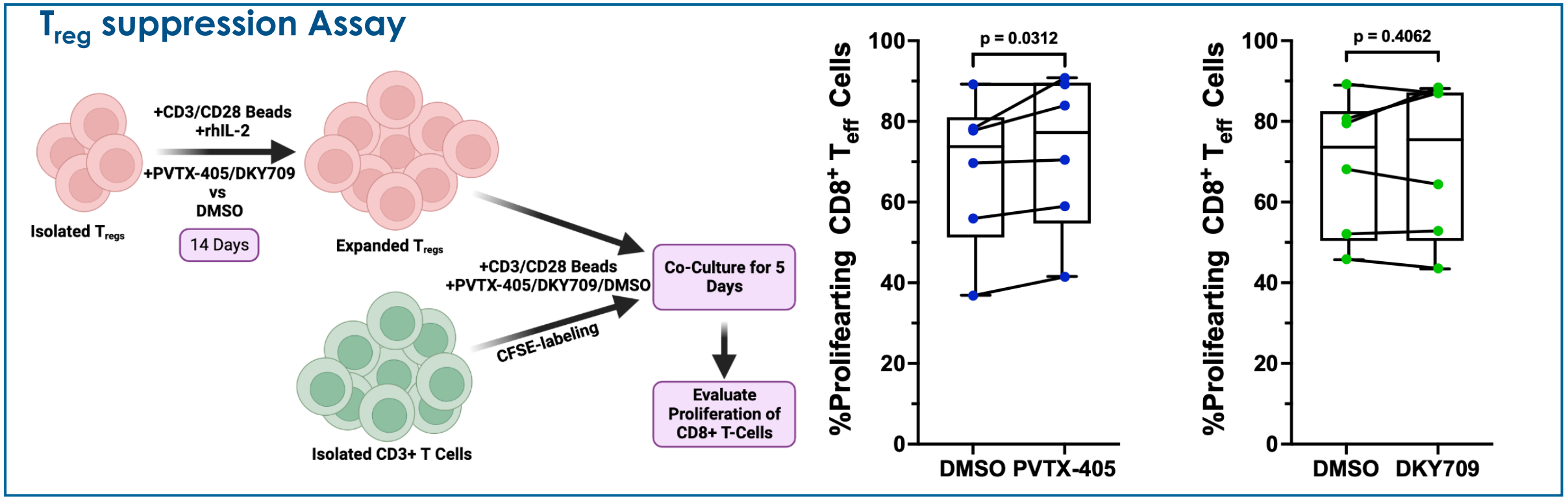
- PVTX-405 treatment of Jurkat cells results in increased IL-2
- IL-2 induction is comparable to DKY709
- Increased IL-2 production demonstrates functional consequence associated with predicted increased anti-tumor immunity

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



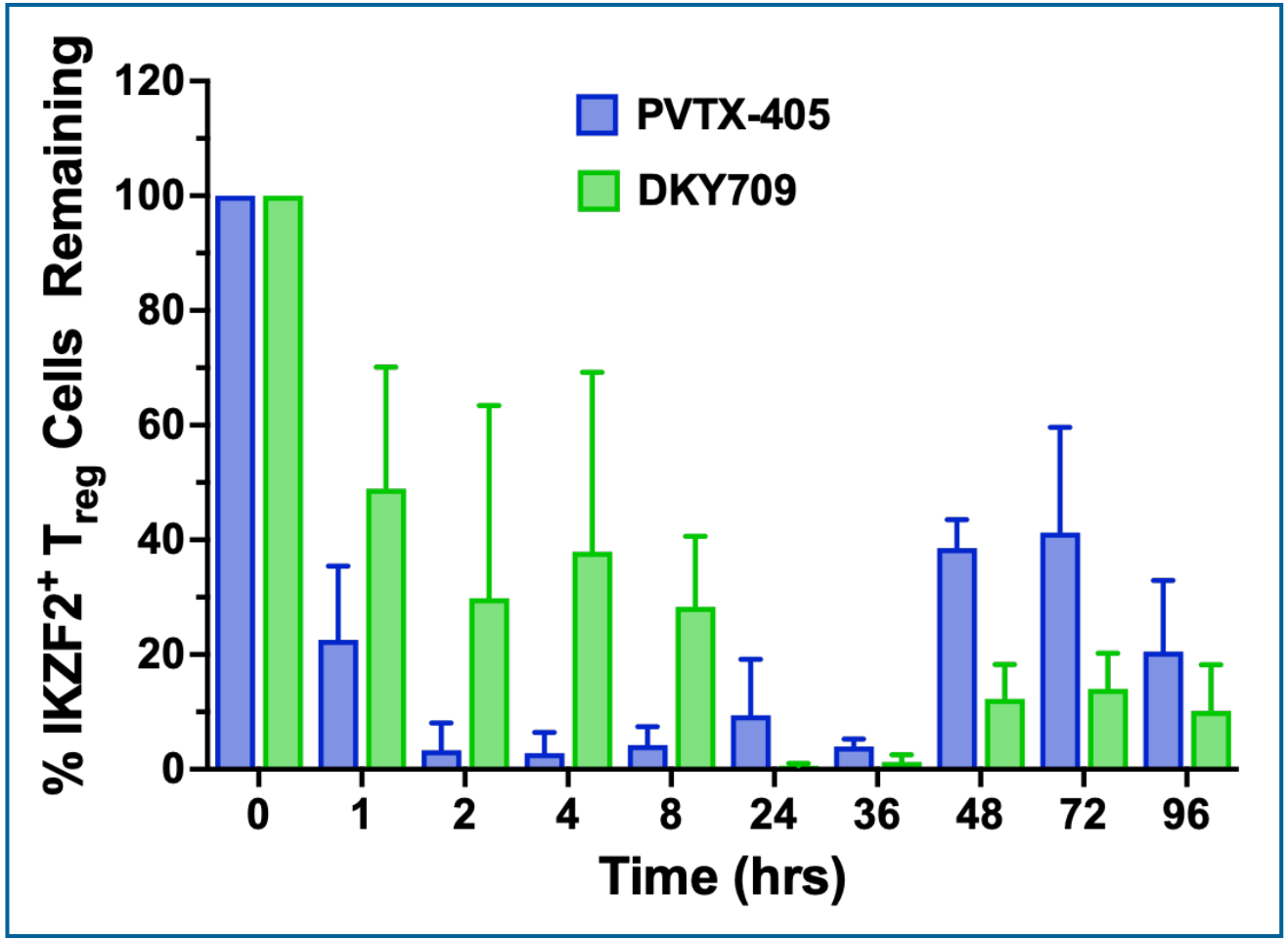
- Human PBMC cells were assessed using multiparameter FACS to measure effects on T_{regs}
- PVTX-405 demonstrates more rapid and potent degradation of IKZF2 than DKY709

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

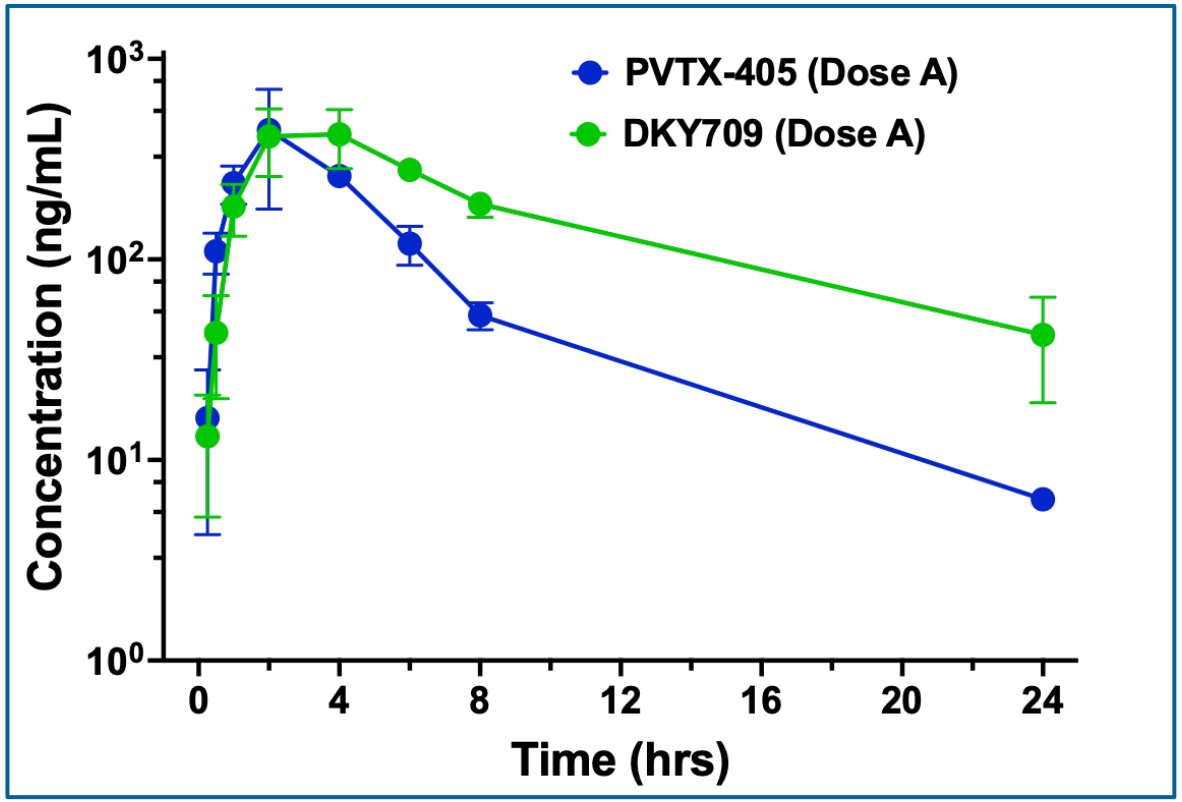
PVTX-405 Shows Robust IKZF2 Degradation in Cyno *In Vivo*



- 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

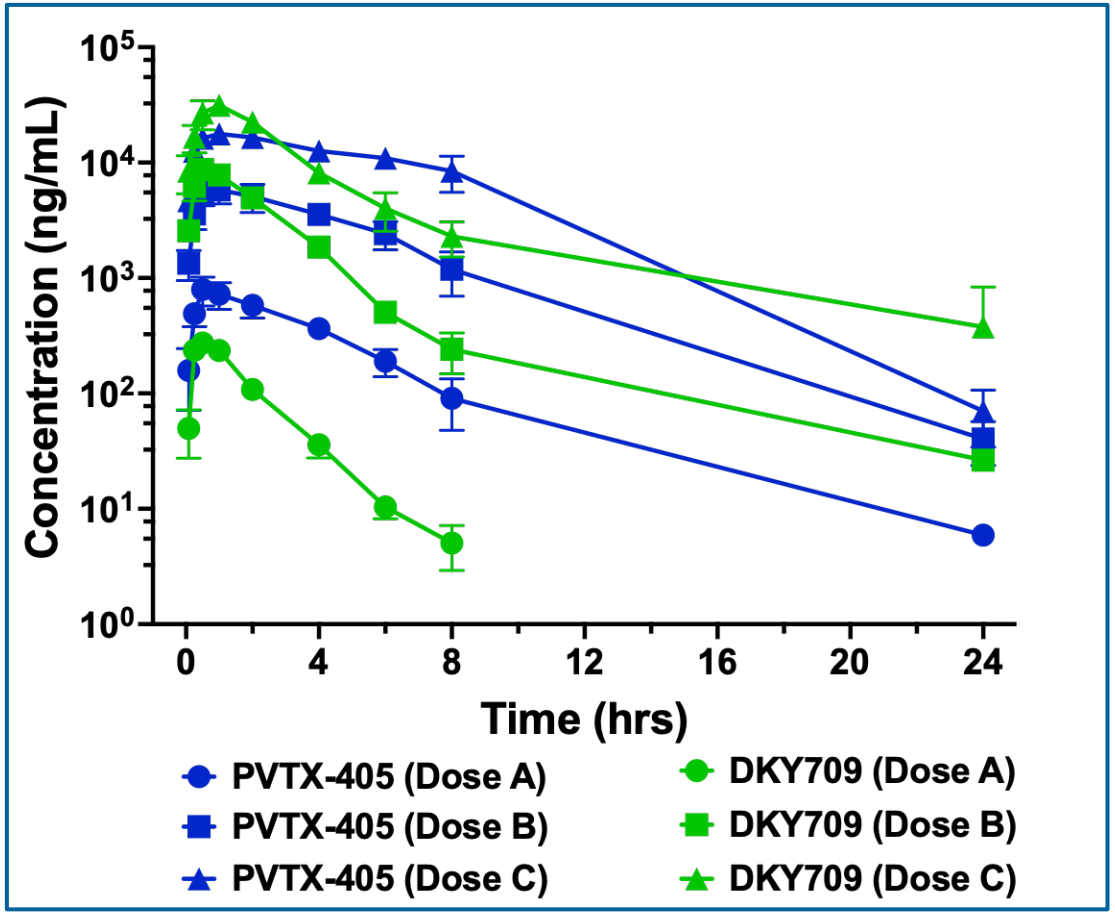
Cyno Pharmacokinetics



Compound	PO Dose, QD	Mean AUC_{0-24} ng*hr/mL	Mean C_{max} ng/mL
PVTX-405	Dose A	2200	440
DKY709	Dose A	4200	450

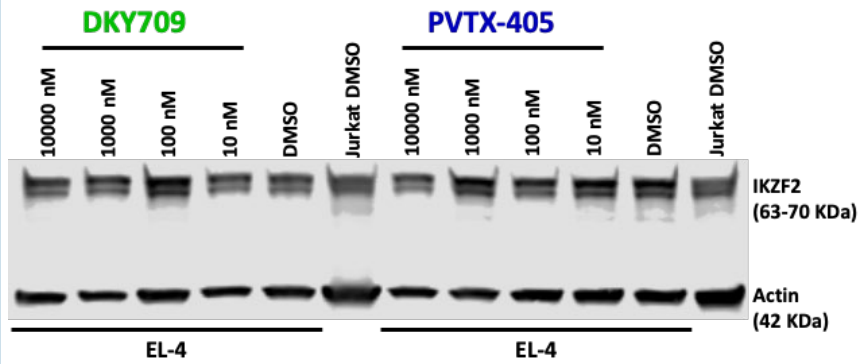
PVTX-405 and DKY709 Show Similar Oral Exposure Profiles in Mice

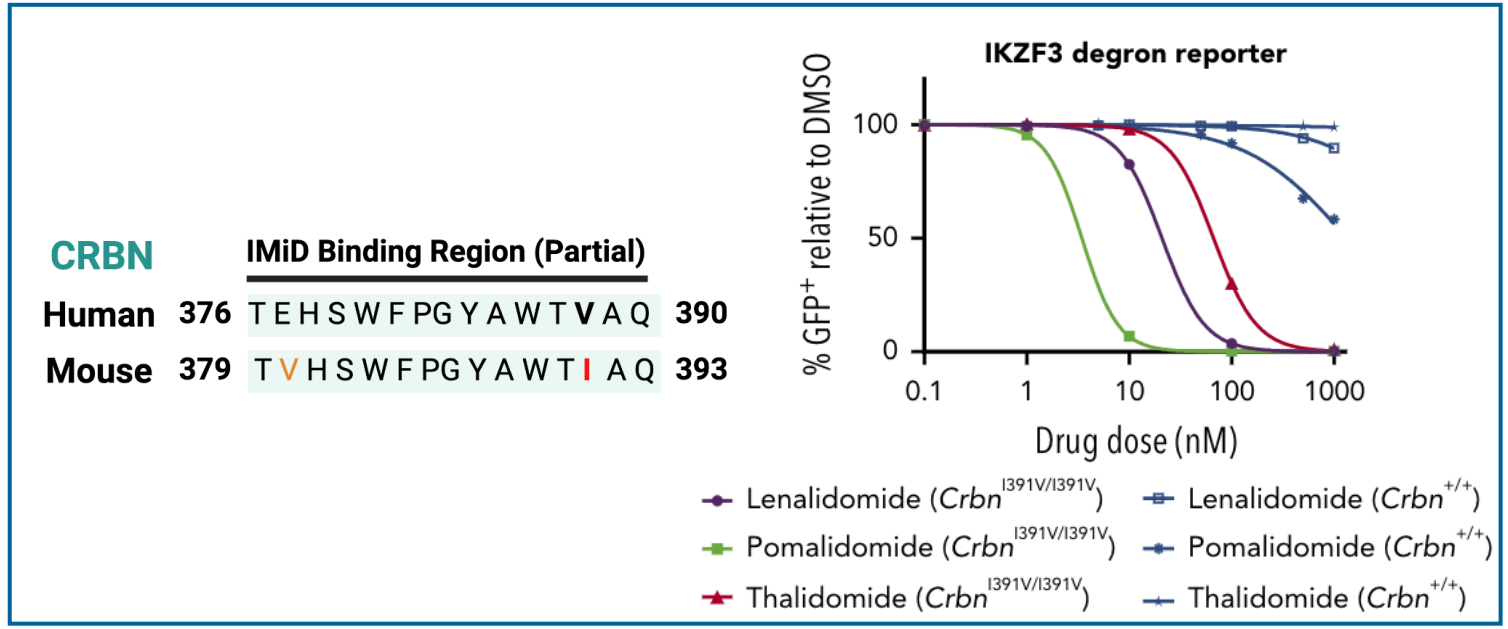
Mouse Pharmacokinetics



Compound	PO Dose, QD	Mean AUC_{0-24} ng*hr/mL	Mean C_{max} ng/mL
PVTX-405	Dose A	3400	880
	Dose B	38000	5900
	Dose C	171000	18000
DKY709	Dose A	590	270
	Dose B	24600	9080
	Dose C	120000	31000

Mouse CRBN is Resistant to PVTX-405 Glue Activity



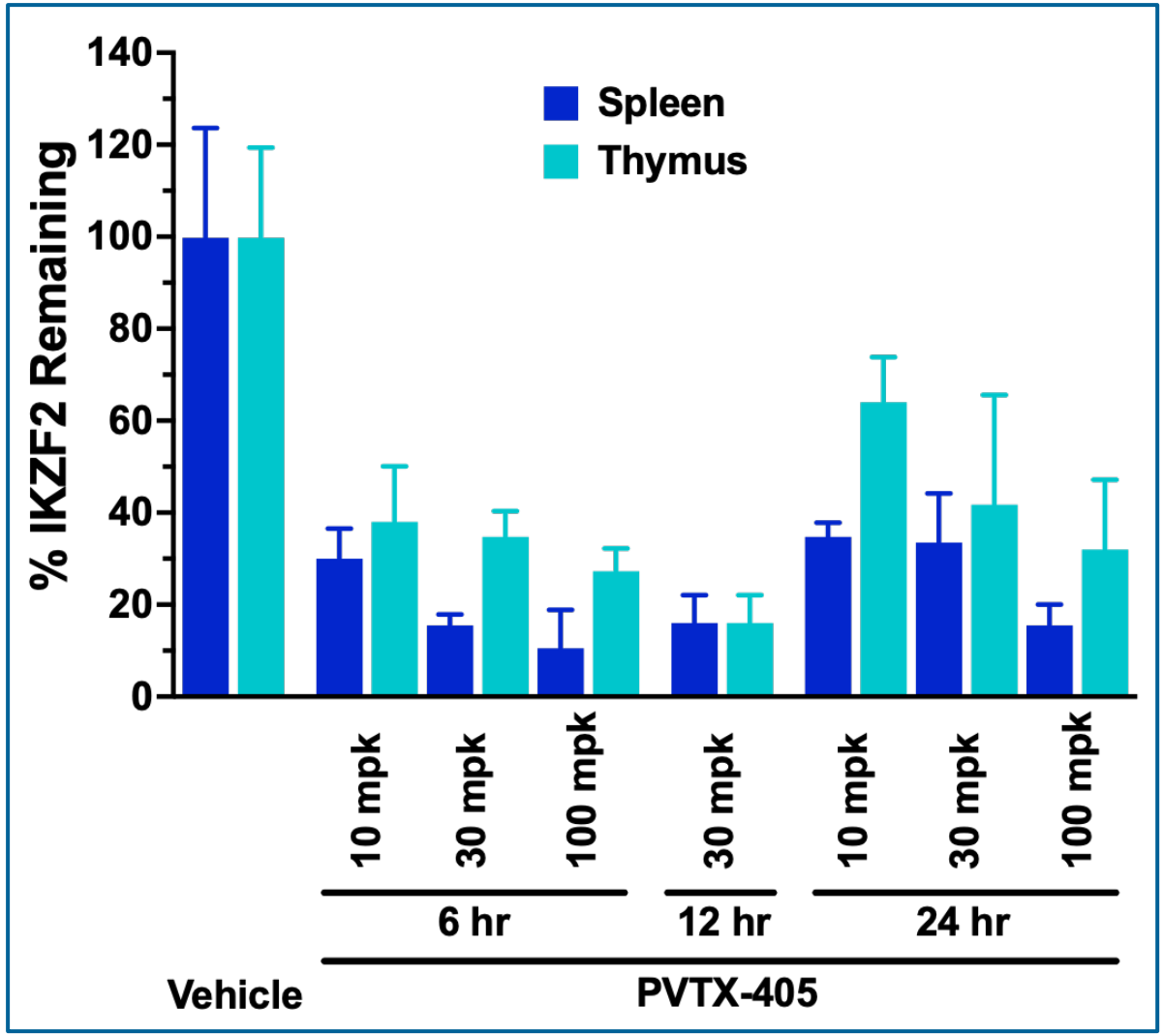


- Neither PVTX-405 or DKY709 treatment induces degradation of IKZF2 in mouse cells

- 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

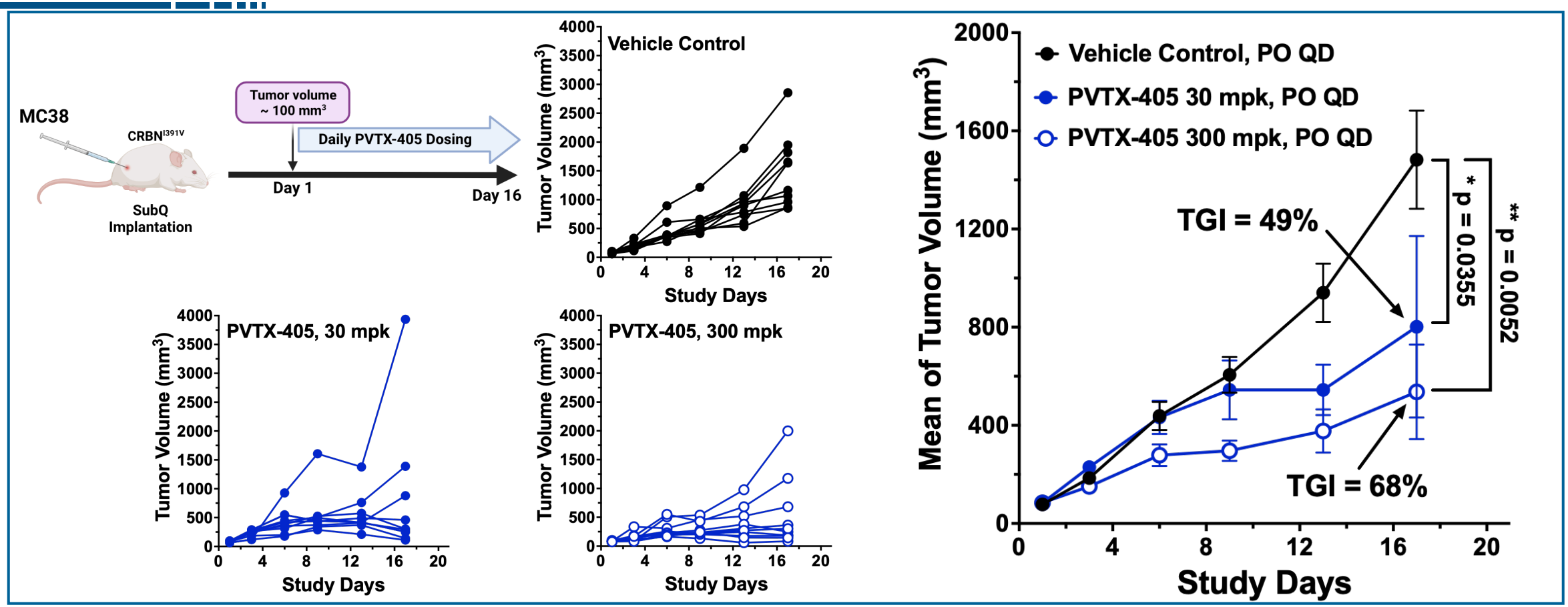
Fink, E. C. et al. Blood 132, 1535–1544 (2018).
Gemechu, Y. et al. P Natl Acad Sci Usa 115, 11802–11807 (2018).

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 ▪ **Non-GLP rat and cyno toxicology studies completed**
Efficacy	▪ **Single agent efficacy against novel MC38 syngeneic model *in vivo***

Acknowledgements

Biology
Harshil Dhruv
Cassandra Lowenstein
Michael Rossi
Niu Shin
Pramod Thekkat

Chemistry
Xuqing Zhang
Matt Tudor
Qiaolin Deng

DMPK
Hsuan-Ming Yao
Rakesh Nagilla
Ted Quin

Biochemistry and Structural Biology
Elham Behshad
Peter Orth

Proteomics
Bomie Han
Pankaj Dwivedi

Project Management
Christine Stuhlmiller
Melissa Yordy

Strategy
Jack Kabrich

Discovery Leadership
Corey Strickland
Helai Mohammad
Larry Jolivette
Scott Priestley
Winston Wu
Zhihua Sui

Prof. Shaomeng Wang
Zhixiang Chen
Rohan Rej
Donna McEachern
Longchuan Bai
Paul Kirchhoff

partnering@proteovant.com