

STAT6: A Transcriptional Driver In Tyepe 2 Immunity And A Rising Therapeutic Target

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Abstract

Signal Transducer and Activator of Transcription 6 (STAT6) is a pivotal transcription factor activated downstream of the interleukin-4 (IL-4) and interleukin-13 (IL-13) signaling pathways. It governs the expression of key genes involved in type 2 immune responses, including Th2 cell differentiation, IgE class switching, and M2 macrophage polarization. Dysregulated STAT6 activity has been implicated in a range of diseases, from asthma and atopic dermatitis to fibrosis and immune-evasive tumors.

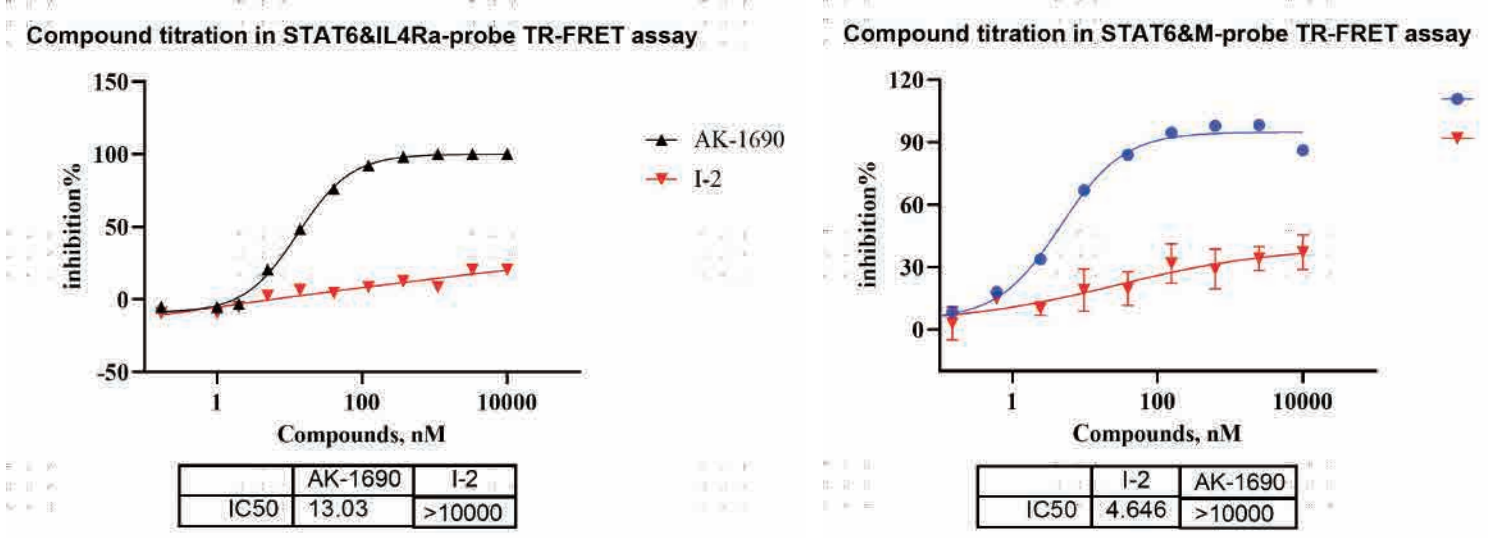
With the clinical advancement of STAT6-targeting drugs like Kymera's KT-621 and Recludix's REX-8756, the demand for precise STAT6 binding profiling is rising—supporting target validation, mechanism studies, and therapeutic development.

Representative STAT6 Inhibitors:

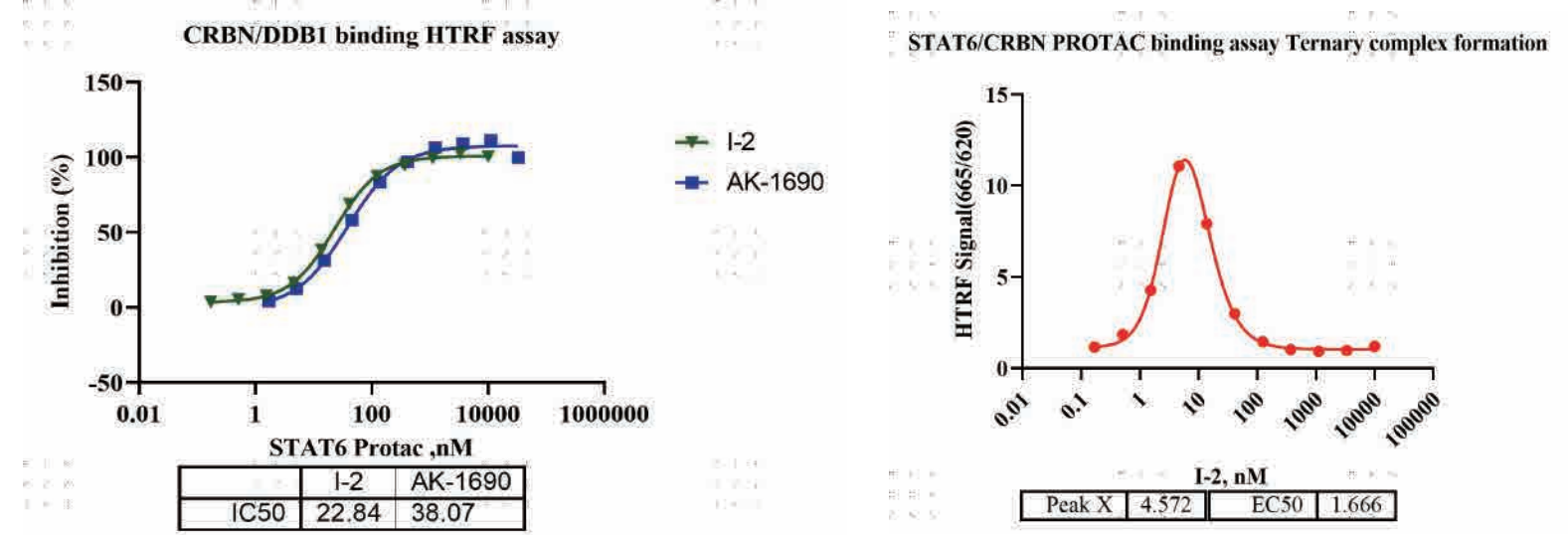
STAT6-IN-3: A small-molecule inhibitor blocking STAT6 phosphorylation or DNA binding for Th2-driven diseases.
AK-1690: A highly selective STAT6 PROTAC degrader with a DC50 of 1 nM, developed by the University of Michigan/ShaoMeng Wang group.
KT-621: An oral STAT6 PROTAC degrader with picomolar potency against IL-4/IL-13 signaling, developed by Kymera Therapeutics for atopic dermatitis and asthma.
I-2: The positive control in this article is molecule 2 from the Kymera Therapeutics patent (WO2025049820).

1 Binary/Ternary binder screening and orthogonal validation

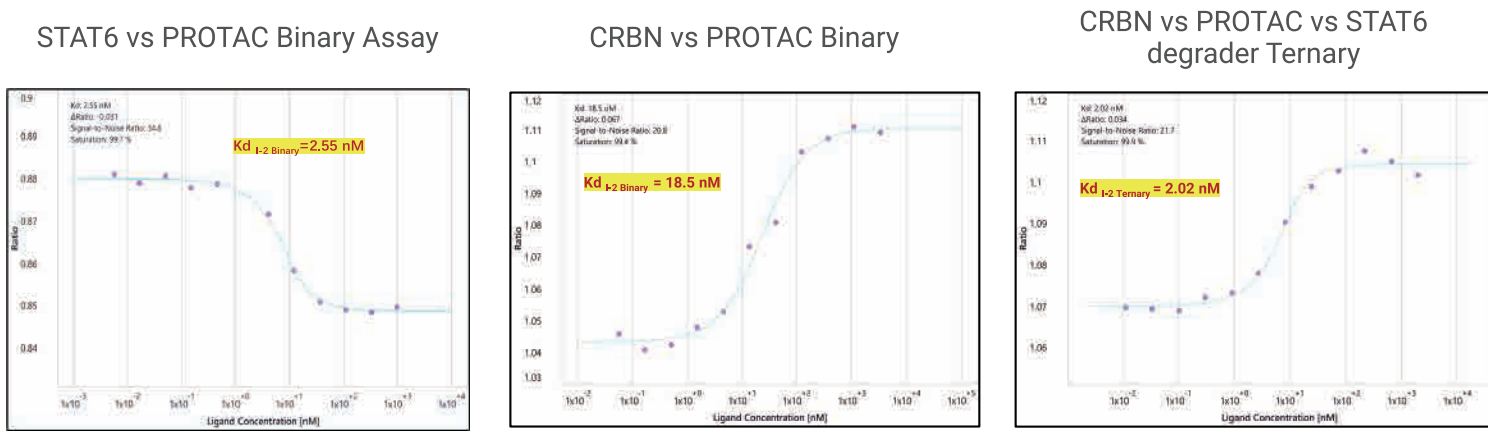
A HTRF binding assay



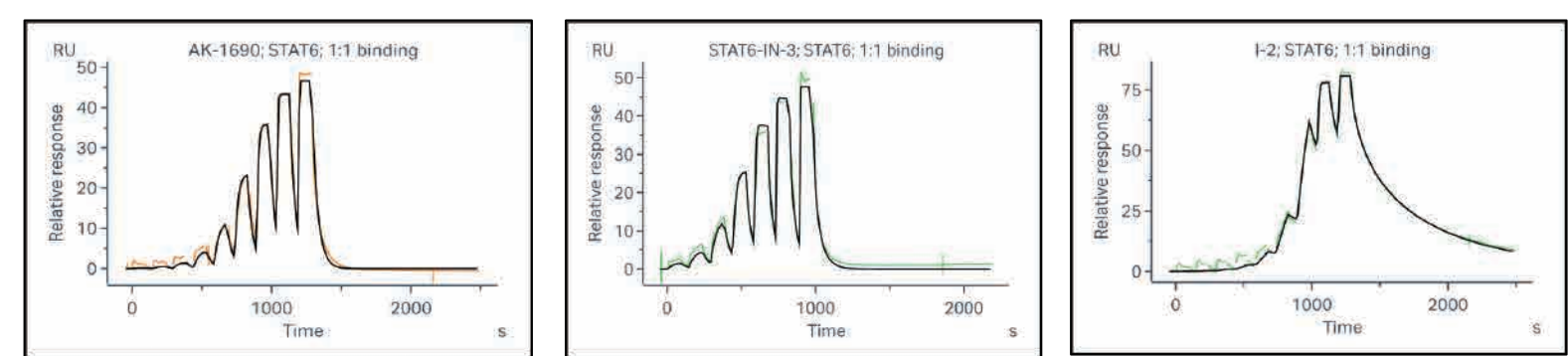
B Binary/Ternary complex formation



C Spectrum Shift (SpS) Assay



D STAT6 SPR binding

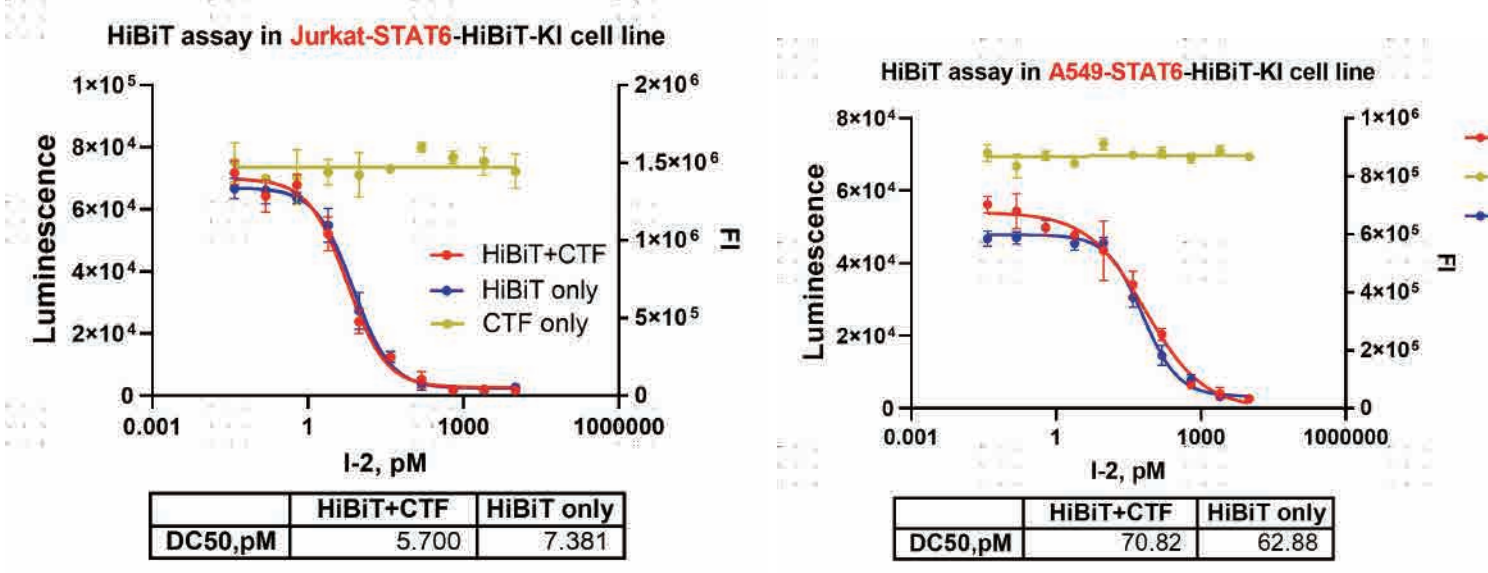


Immobilized ligand	Analyte	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)
STAT6	AK-1690	6.43E+05	2.53E-02	3.94E-08
STAT6	STAT6-IN-3	3.76e+06	1.30e-01	3.47e-08
STAT6	I-2	2.87E+06	2.56E-02	8.90E-09

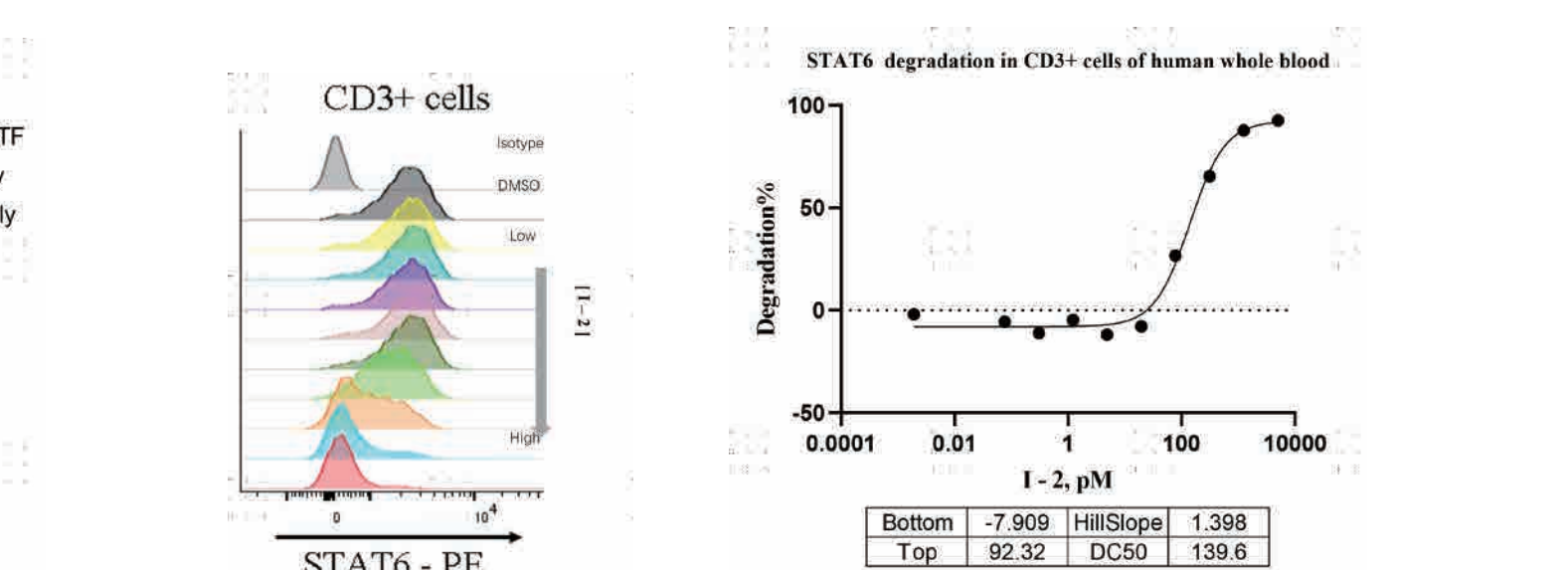
Figure 1. Assay portfolio for STAT6 binder screening and ternary complex profiling. (A) HTRF assay for STAT6 binding screen. The IL-4Ra-probe can screen for small molecules and PROTACs that target the IL-4Ra binding site, such as STAT6-IN3 and AK1690. The M-probe can screen for small molecules and PROTACs that bind to the allosteric site, such as the KT-621 series. (B) HTRF ternary complex profiling for STAT6 PROTAC (hook effect found). (C) Spectrum Shift (SpS) assay for cost-effective HTS of STAT6 binary and ternary binding. (G) SPR as the gold standard for STAT6 binder screening.

2 Assessment of PROTAC-Mediated STAT6 Degradation

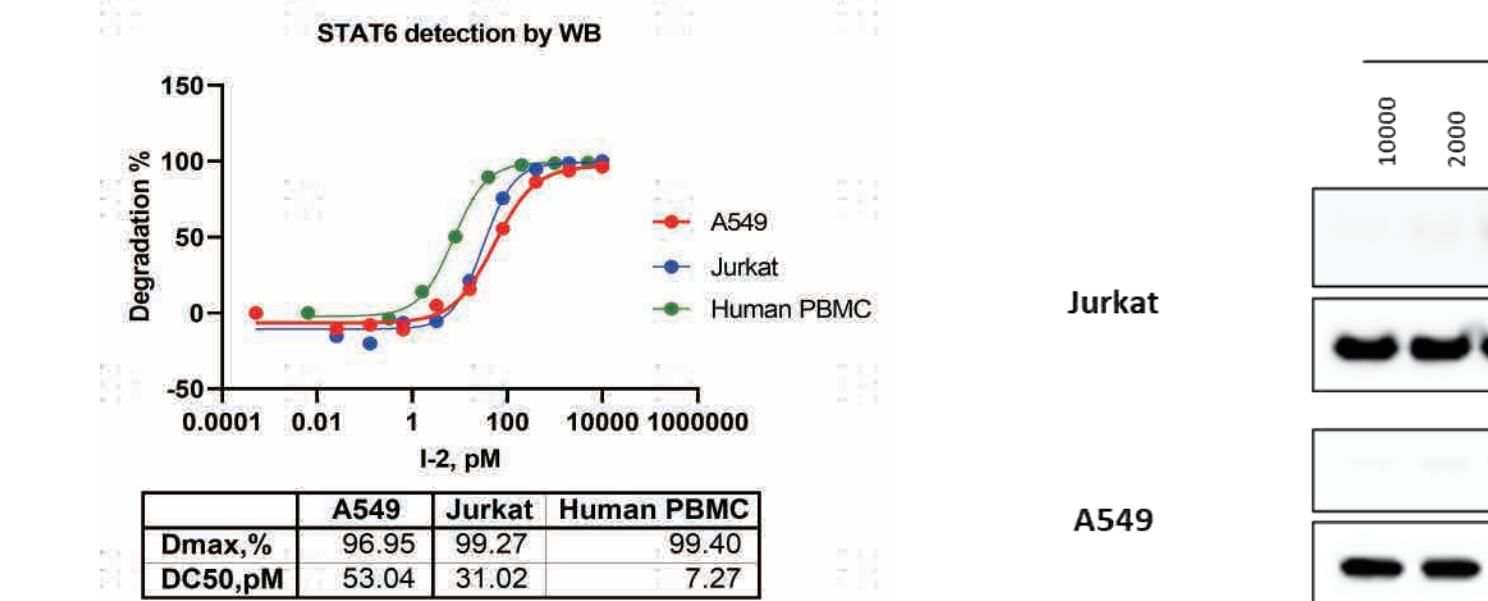
A STAT6 degradation_HiBiT assay



C STAT6 degradation_Human whole blood_FACS



B STAT6 degradation_Different cells_WB



D STAT6 degradation_Different cells_WB

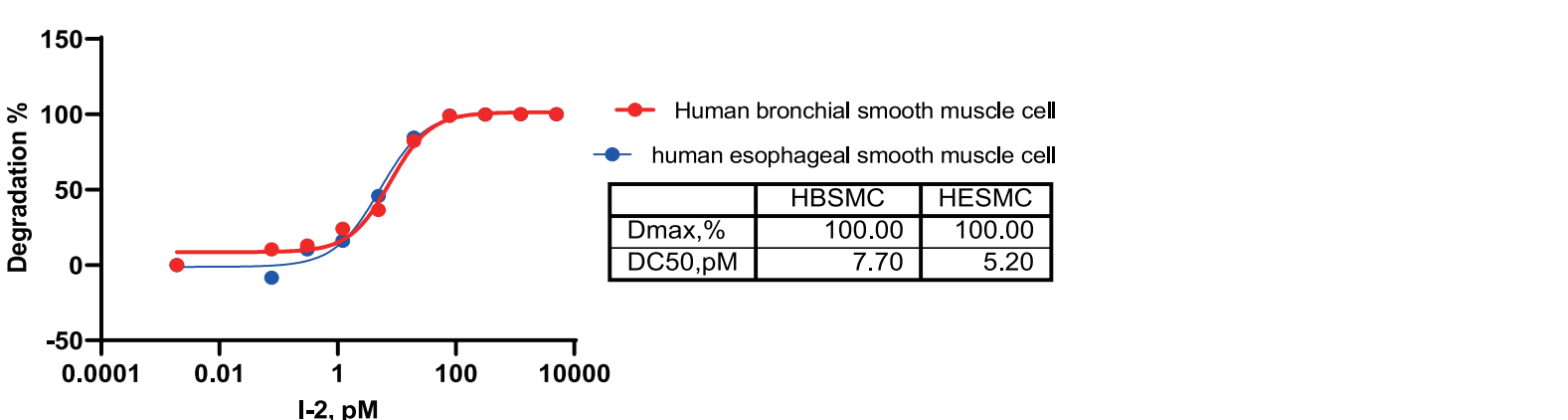
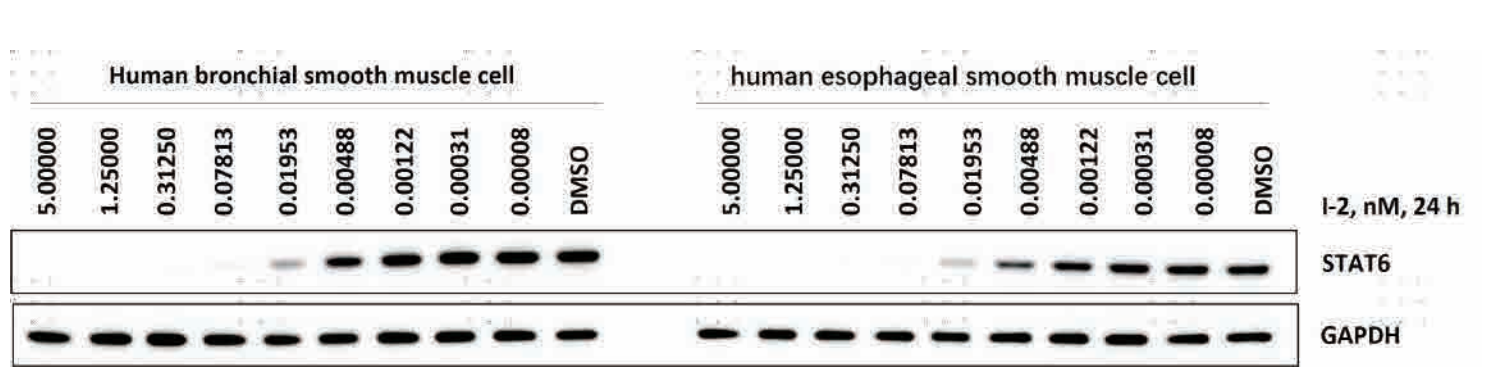
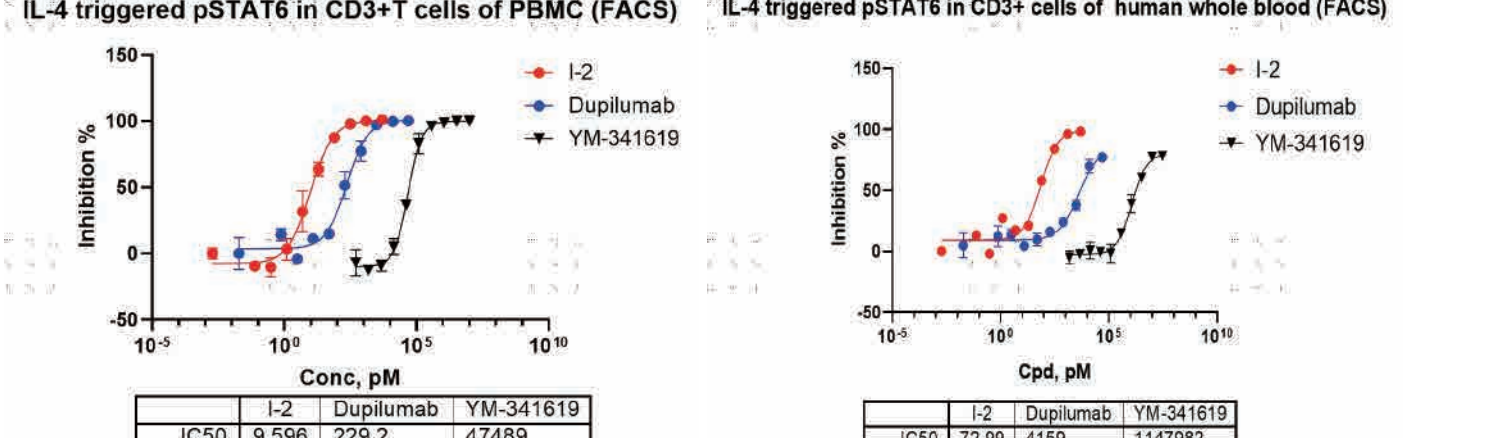


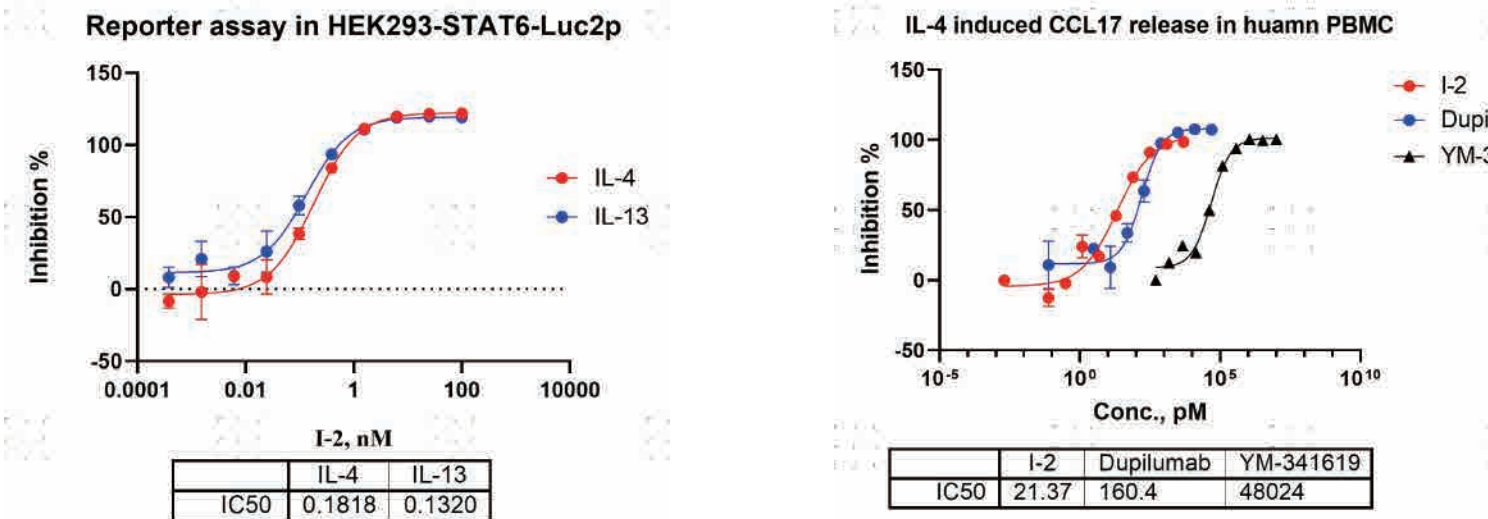
Figure 2. PROTAC-mediated STAT6 degradation assessed by HiBiT, WB, and FACS assays. (A) HiBiT-based STAT6 degradation, with degradation and viability measured in a single assay. (B) STAT6 Degradation evaluated by Western blot. (C) FACS-based STAT6 degradation in human T cells (whole blood sample). (D) Western blot analysis in human primary smooth muscle cells.

3 Functional Readouts in Primary Human Cells

A STAT6 phosphorylation



B Reporter assay



E CD23 expression in B cell

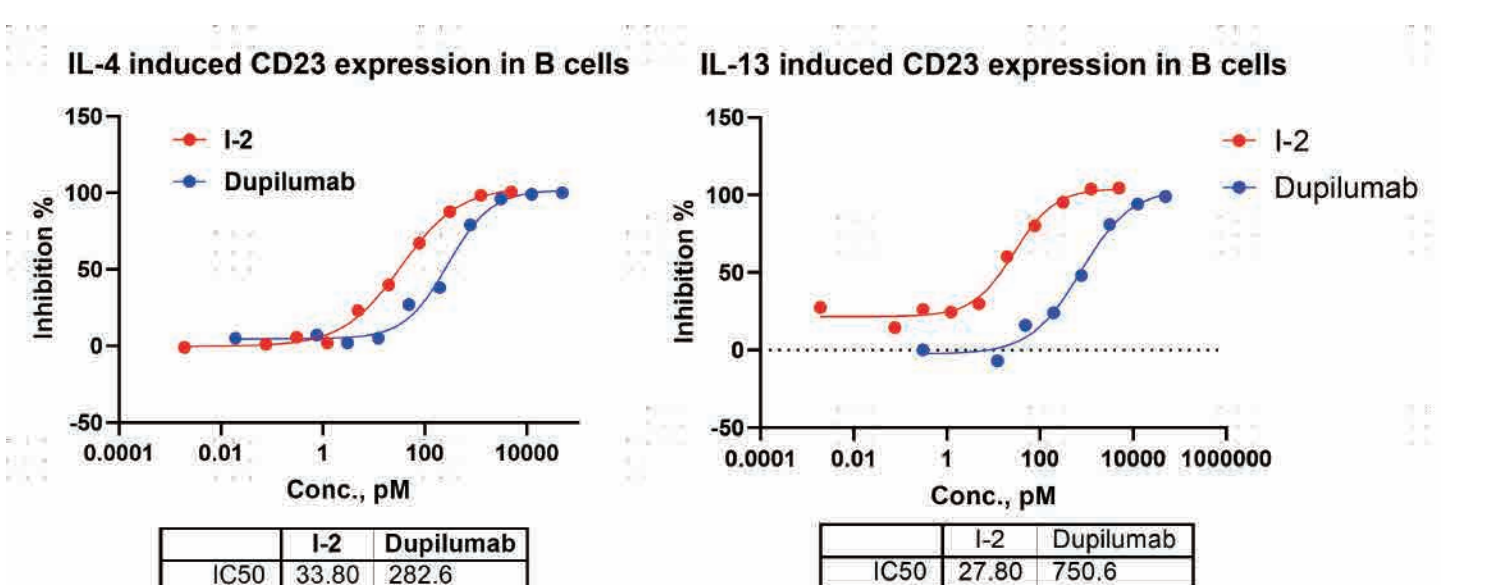
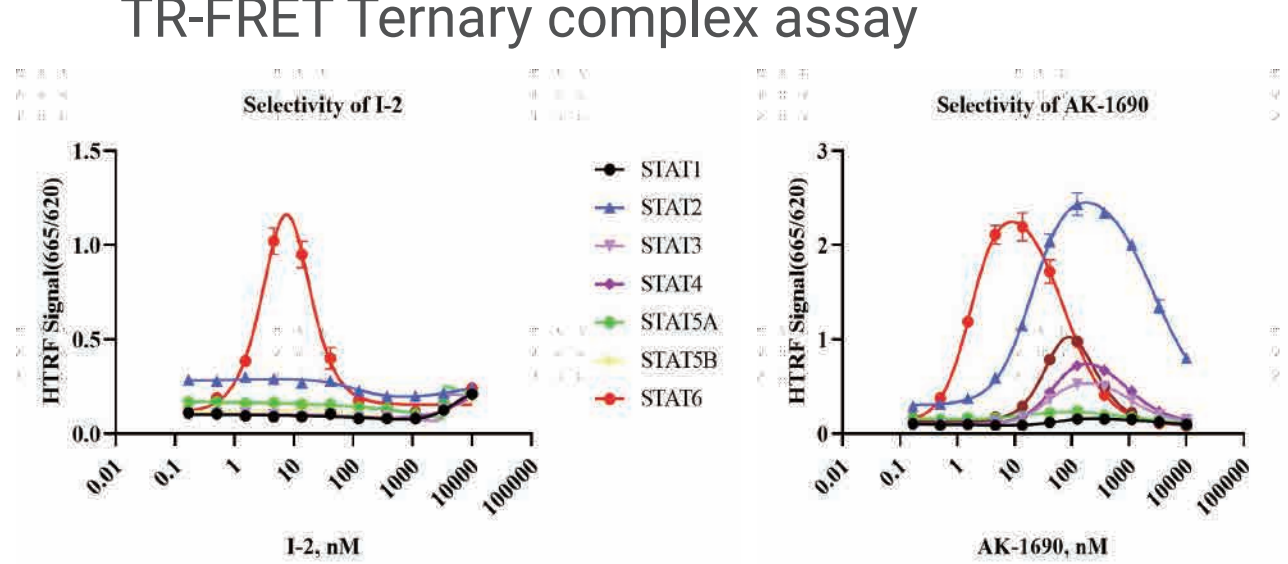


Figure 3 : Functional assays in primary human cells. (A) FACS-based analysis of STAT6 phosphorylation following stimulation in T cells. (B) Luciferase reporter assay measuring STAT6 transcriptional activity. (C) Periotrist release from human bronchial smooth muscle cells (HBSMC) upon stimulation. (D) TARC secretion from PBMCs upon stimulation. (E) CD23 expression in B cells under different stimulation conditions.

4 Profiling STAT Family Selectivity and Cross-Species Specificity

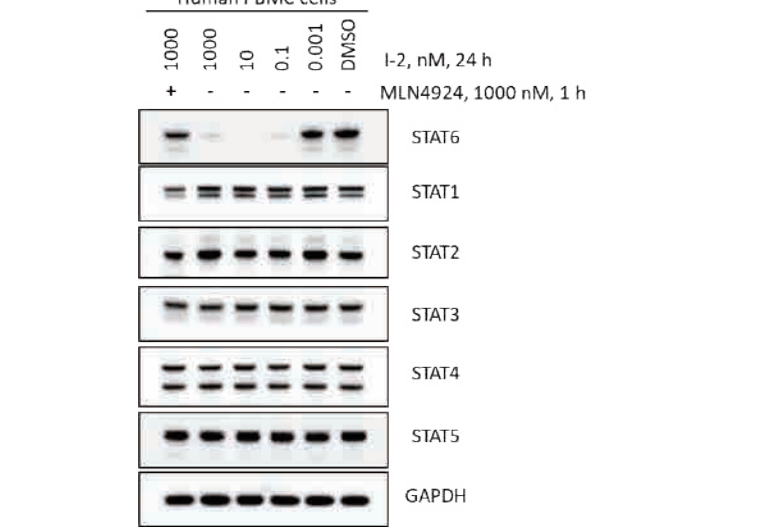
A STAT6 vs protac vs CRBN



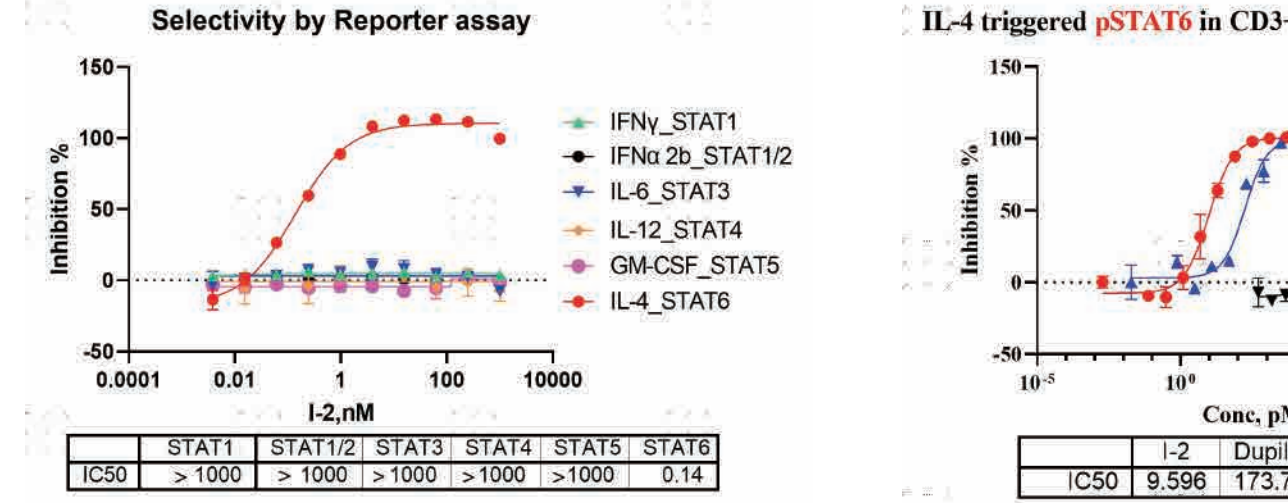
B SPR binary binding assay

Target	I-2	AK-1690
STAT1	/	11400
STAT2	/	1820
STAT3	/	2920
STAT4	/	2360
STAT5A	/	6020
STAT5B	/	186
STAT6	7.97	33.7

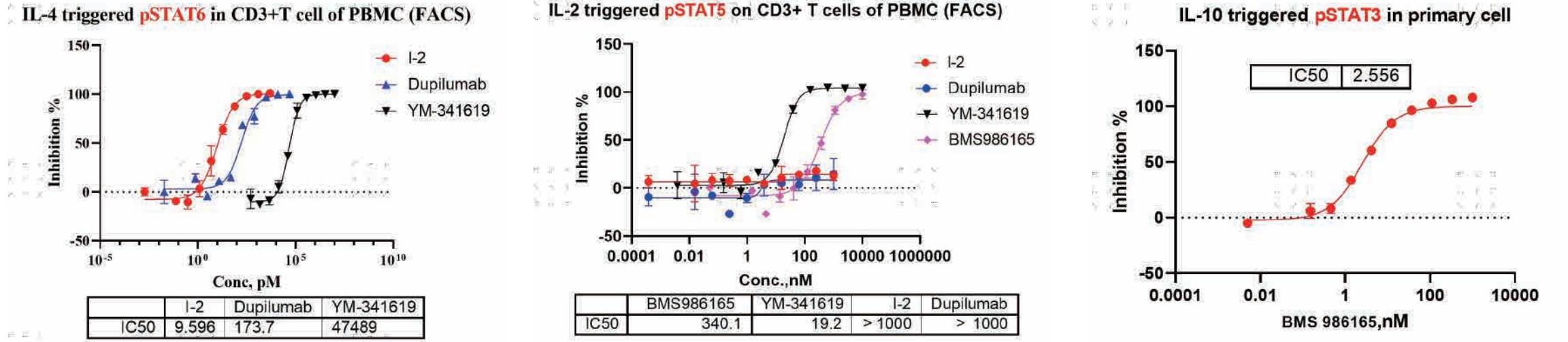
C WB analysis of STAT family selectivity



D Reporter assay



E STAT6 phosphorylation



F Different species specificity

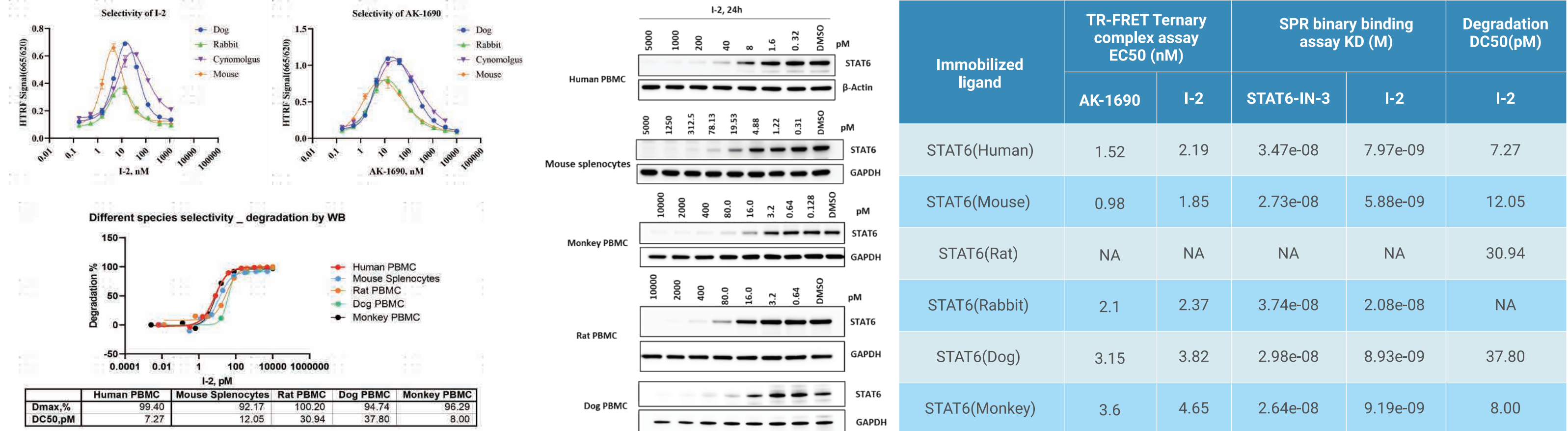


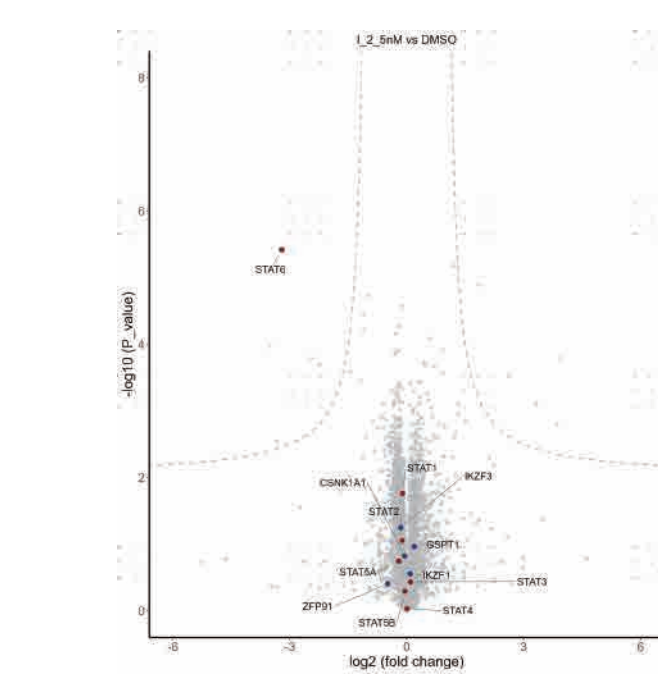
Figure 4. STAT family selectivity and cross-species specificity profiling. (A) HTRF evaluation of I-2-mediated STAT/CRBN ternary complex formation. (B) SPR analysis of STAT family selectivity. (C) Western blot-based assessment of STAT degradation selectivity. (D) Reporter assays across different STAT family members. (E) STAT phosphorylation measured by FACS. IFN-γ-pSTAT1/2 and IL-12-pSTAT4 assays were ongoing. (F) Cross-species specificity evaluated by HTRF ternary complex formation, SPR binding, and degradation assays using several known STAT6 inhibitors/PROTACs.

5 Off-Target Profiling: Western, Proteomics, ICESTP Safety Panel & HiBiT

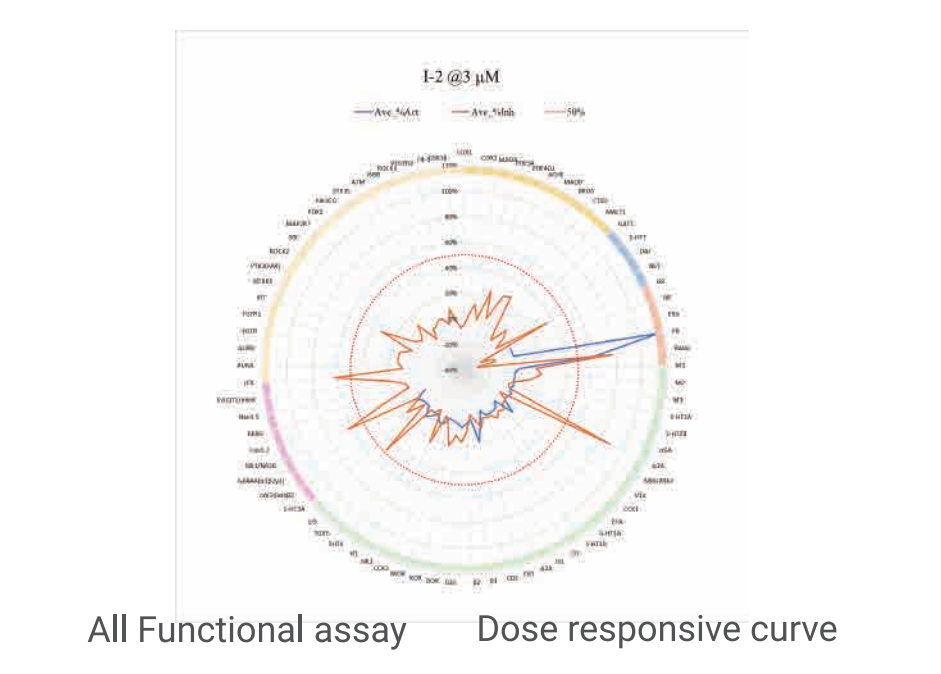
A Identification of off-target CRBN substrates by WB



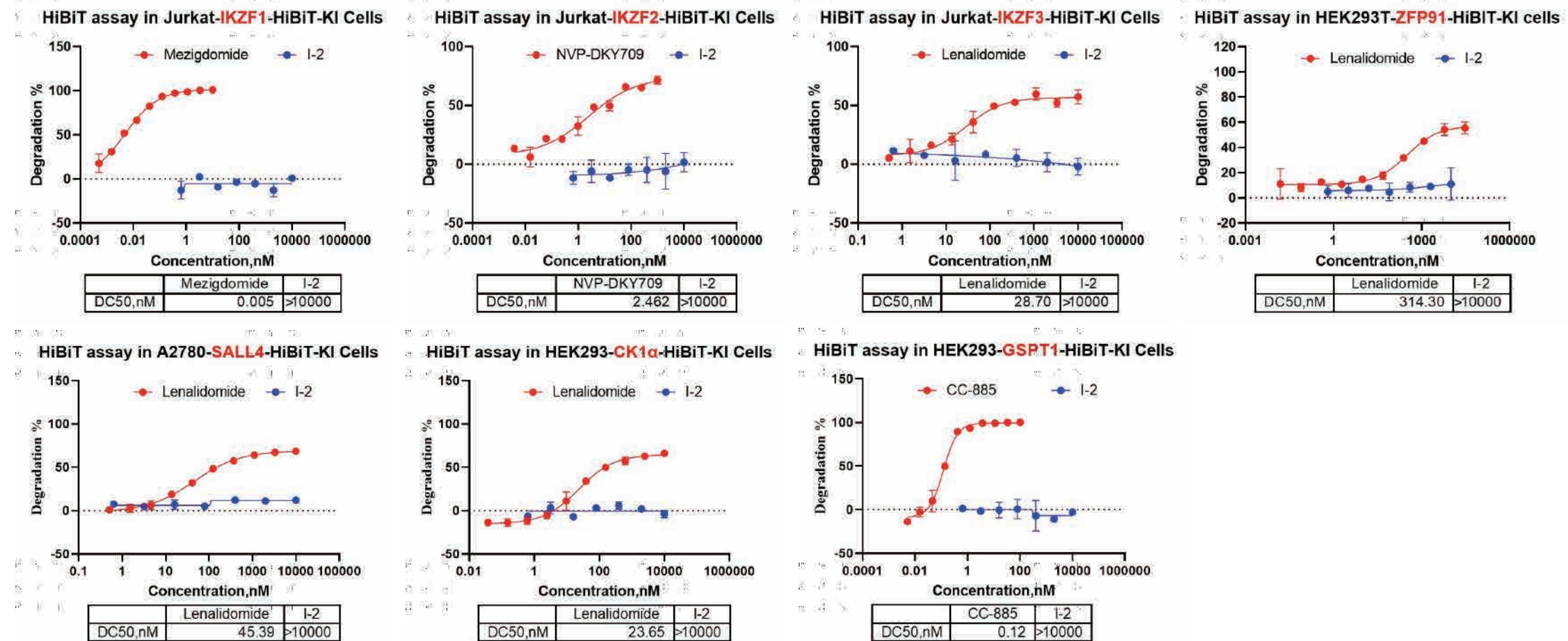
B DIA-based proteomics profiling



C ICESTP SAFETY PANEL @ 77



D Identification of off-target CRBN substrates by HiBiT



CRBN off-target	Status
HEK293-GSPT1-HiBiT-KI cell line	ready
Jurkat-IKZF1-HiBiT-KI cell line	ready
Jurkat-IKZF2-HiBiT-KI cell line	ready
Jurkat-IKZF3-HiBiT-KI cell line	ready
HEK293-CK1α-HiBiT-KI cell line	ready
A2780-SALL4-HiBiT KI cell line	ready
HEK293-ZFP91-HiBiT-KI cell line	ready

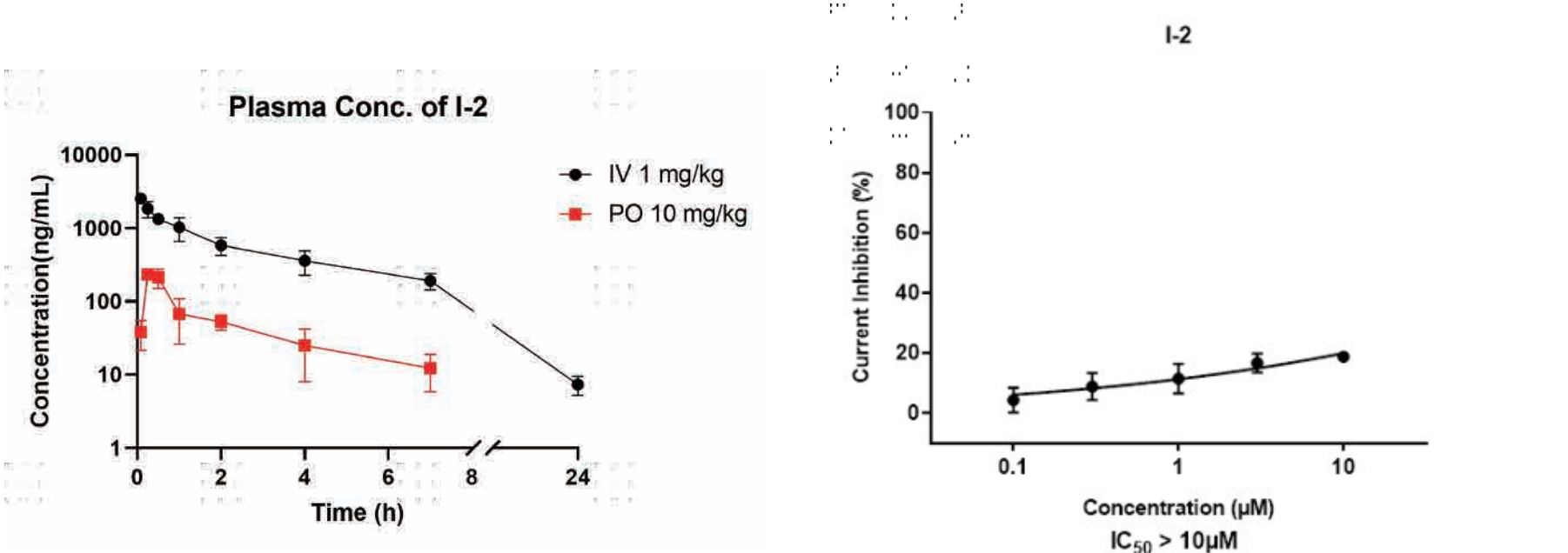
Figure 5. Off-target profiling of STAT6 degraders (A) I-2 did not show off-target degradation of common CRBN substrates. (B) DIA-based proteomics profiling of I-2 off-targets. (C) ICE-STP safety panel revealed off-target effects of I-2 on LOK, AZA, PR, RARα and NR1/NR2B.

6 Developability: ADME, PK & HERG

A PK parameters

PK parameters	IV 1mg/kg	PO 10mg/kg
t1/2(h)	3.58±0.104	2.40±0.429
Tmax(h)	0.083±0.00	0.333±0.144
C0/Cmax(ng/mL)	3030±145	249±39.2
AUClast(h*ng/mL)	5838±1395	344±126
AUCinf(h*ng/mL)	5876±1404	389±157
AUC0-∞(h*ng/mL)	0.645±0.104	10.8±3.31
MRTinf(h)	3.81±0.247	2.75±0.563
AUClast/D(h*kg*ng/mL/mg)	5838±1395	34.4±12.6
Vd(L/kg)	0.920±0.263	/
CLobs(mL/min/kg)	2.96±0.760	/
F(%)	/	0.662±0.268

B Cardio-one®—hERG study



C In vitro ADME assay

Parameters	ICE data
Kinetic solubility (FaSSGF/FaSSiF/ FeSSiF, PBS pH= 7.4)	FaSSGF(pH=1.2): 31.99 μM FaSSiF(pH=6.5): 19.64 μM FeSSiF(pH=5.0): 161.29 μM PBS(pH= 7.4): 0.58 μM
LogD	3.98
LM Stability (Human/Mouse)	t1/2 (min) Human:16.55; Rat:40.02; Mouse: 78.82 Dog: 25.24; Monkey: 2.72
Hepatocyte Stability (Human/Mouse)	t1/2 (min) Human: 83.76; Rat: 34.63; Mouse: 17.58; Dog: 54.92 Monkey: 509.76
Plasma Protein Binding (human)	fu/% fu/%: 7.91% (Human)
Plasma Stability(≥120min) (Human/Rat/Mouse/Dog/Monkey)	t1/2 (min) Human > 372.67 min; Rat: 215.24 min Mouse: 239.90 min; Dog: 209.96 min Monkey: 351.64 min
CYP Inhibition (1A2/2C9/2C19/2D6/3A4M/3A4T)	@5uM Inhibition/% 9.19%/18.86%/14.15%/8.25%/ 38.82%/42.06%
Caco-2 permeability	Papp(ER)(E-6 cm/s) Papp A-B: 5.42; Papp B-A: 11.96 ER: 2.21

Figure 6. Developability Profiling of STAT6 Degraders. (A) Pharmacokinetic profile of the STAT6 degrader following IV and PO administration. (B) hERG current measured by manual patch clamp. I-2 showed no significant inhibition of hERG current. (C) In vitro ADME properties of the STAT6 Degraders.

Summary

ICE Biosciences' discovery platform integrates HTS-compatible screening, orthogonal biochemical and cellular assays, and mechanistic studies. Supported by a broad suite of established assays and customizable modules, it generates high-quality activity and safety data to advance drug discovery pipelines. Incorporating human primary cell assays, PK/PD, and safety profiling ensures translational relevance, accelerating the development of next-generation STAT6 therapeutics with clinically meaningful insights.