Hold-to-Kill: RIPTAC Expanding Induced Proximity from Target Engagement to Therapeutic Window

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Abstract

RIPTAC (Regulated Induced Proximity Targeting Chimeras) introduces a novel strategy that leverages induced proximity to achieve selective cancer cell killing. Using AR–BRD4 RIPTAC II-5 as a tool compound, we integrated biochemical screening, computational docking, and multi-level cellular and in vivo assays to establish a direct link between target engagement and therapeutic efficacy. Biochemical evaluation confirmed binary and ternary affinity, supported by computational simulations that predicted novel AR–BRD4 ternary protein–protein interactions (neoPPIs) consistent with cooperativity observed experimentally. II-5's distinct slow on/off binding kinetics stabilize ternary complexes, translating into superior activity in ternary formation and downstream signaling modulation. Cellular assays demonstrated that II-5 induces ternary complexes in HEK293T AR OE, VCaP, and LNCaP cells, correlating with strong inhibition of BRD4-driven signaling (c-Myc) while only moderately inhibit AR signaling (reporter and PSA). In vivo CDX models further validated ternary complex formation, PSA reduction, and pharmacodynamic biomarker responses, showing that ternary assembly is preserved across biochemical, cellular, and tumor tissue contexts. II-5 was highly potent in AR-high prostate cancer models, with efficacy scaling across AR mutants and expression levels, highlighting a mechanistic correlation between AR expression and therapeutic effect. This enables RIPTAC to address resistance mechanisms such as AR amplification, point mutations, etc. Safety panel profiling indicates an overall favorable profile with limited off-target activity. Beyond AR–BRD4, RIPTAC expands the induced proximity landscape, aligning with TCIP paradigms and demonstrating broader applicability across oncogenic drivers. Together, computational and experimental evidence converge to highlight ternary complex stability and AR expression dependence as the mechanistic drivers of RIPTAC's therapeutic window. Importantly, our induced proximity platform, built on extensive expertise in targeted protein degradation (TPD), can be rapidly migrated to other target pairs, enabling first-in-class drug discovery programs and supporting both domestic and international partners.

Binary and Ternary Binding of RIPTAC II-5: Cooperative and High Affinity

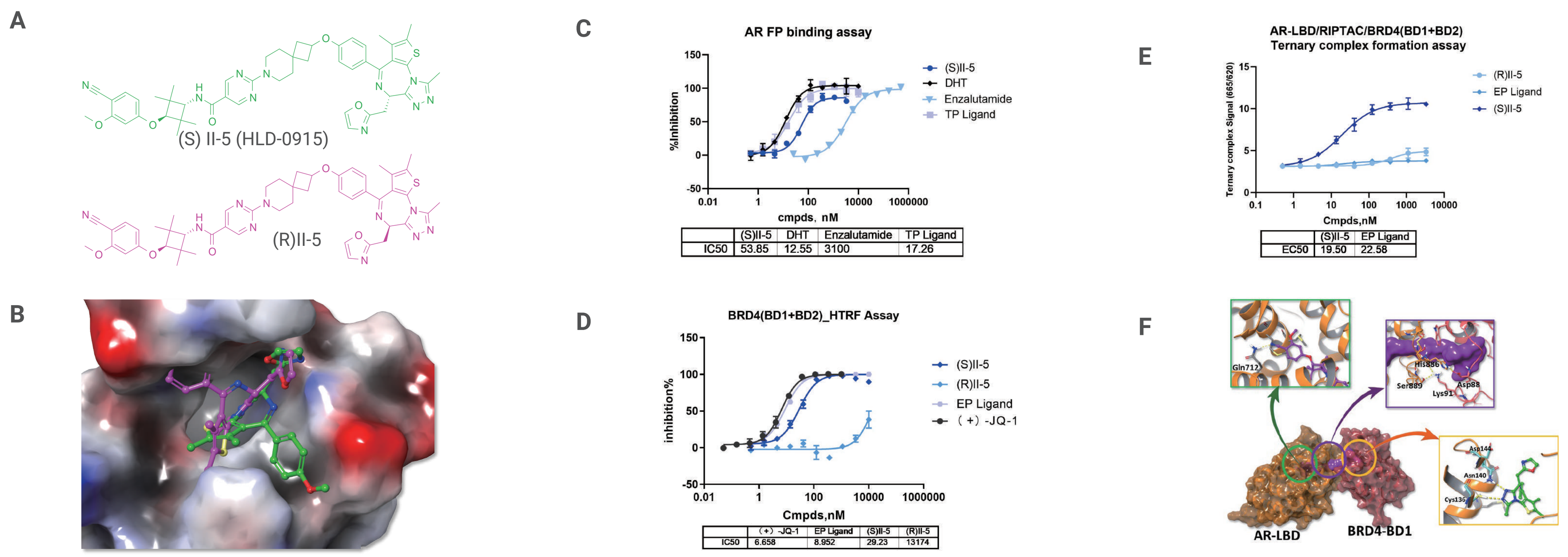


Figure 1. Biochemical evaluation of RIPTAC binary and ternary affinity(A) Structure of AR RIPTAC II-5 (S isomer, HLD-0915; R isomer, negative control).(B) Docking in BRD4 BD: (S)II-5 fits, (R)II-5 clashes (C) II-5 TP ligand shows similar potency as DHT, while II-5 also show strong potency in AR FP assays.(D) II-5 is highly active in BRD4 HTRF binding assays.(E) II-5 promotes AR-LBD/BRD4 ternary complex formation.(F) Ternary complex docking model shows neoPPI induced by II-5.

II-5 Engages BD1 and BD2 with Slow Binding Dynamics

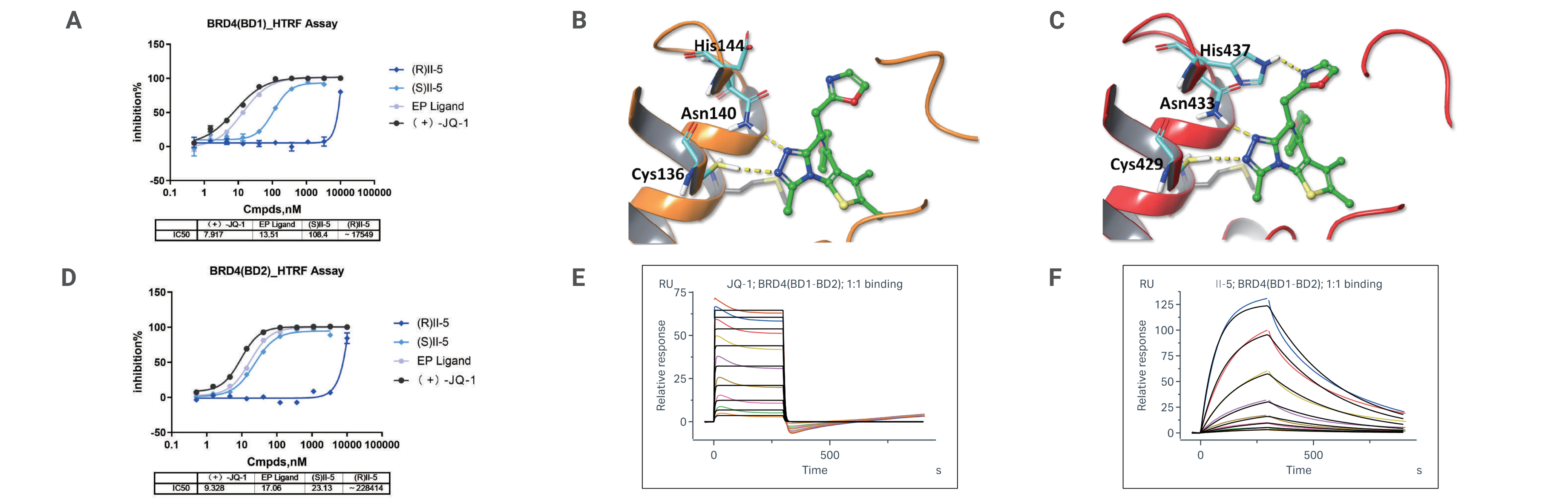


Figure 2. RIPTAC BRD4 binding model. (A) II-5 binds to the BRD4 BD1 domain with ~100 nM affinity; its EP ligand shows affinity comparable to JQ-1.(B–C) Docking results show II-5 binds BD1 and BD2 with similar poses.(D) II-5 also binds BD2.(E) SPR sensorgrams indicate JQ-1 is a fast on/off binder.(F) II-5 displays a distinct slow on/off binding mode, which may facilitate ternary complex formation.

II-5 Ternary Complex Formation Drives Cellular Response (Hold to Kill)

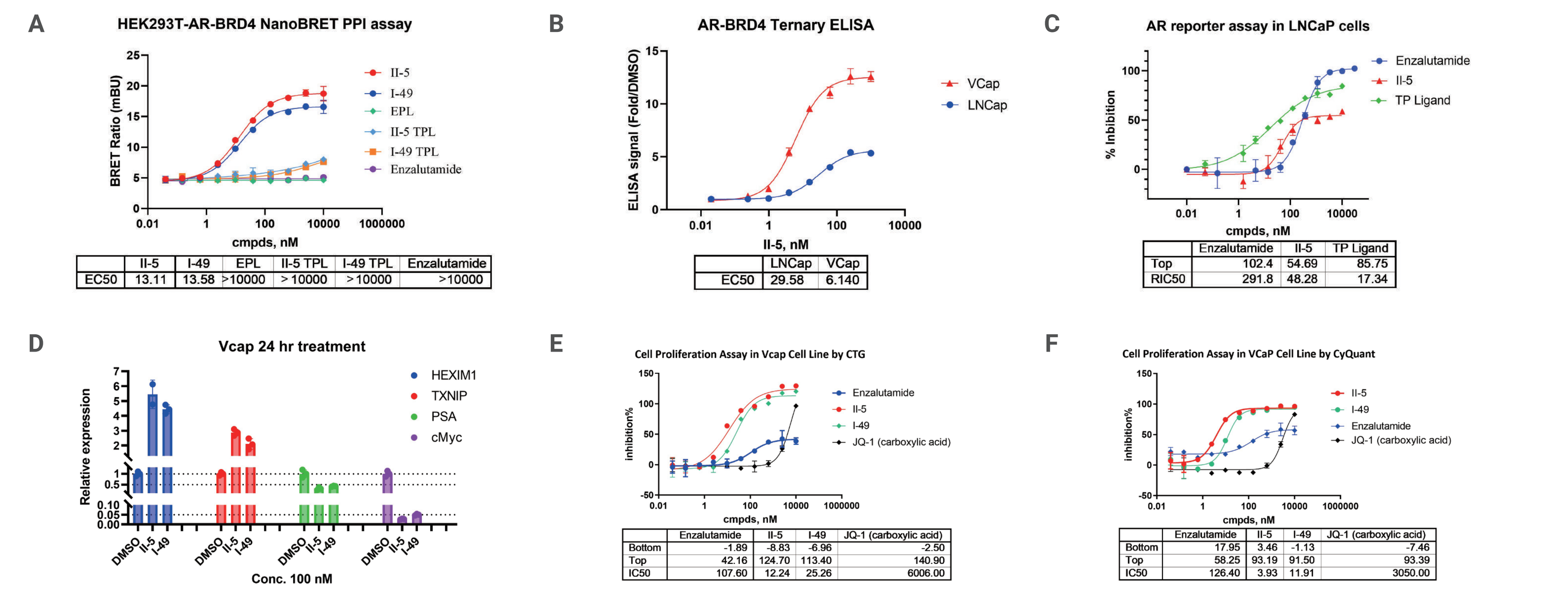


Figure 3. RIPTAC Hold-to-Kill model illustrated by compound II-5. (A) II-5 and I-49 induce ternary complex formation via nanoBRET in HEK293T cells.(B) II-5 induces endogenous ternary complexes in VCaP and LNCaP cells.(C) II-5 shows weaker AR reporter inhibition than its AR ligand.(D) II-5 strongly inhibits BRD4 signaling (c-Myc) but weakly inhibits AR signaling (PSA).(E, F) In AR-high VCaP cells, RIPTAC is more potent than Enzalutamide and JQ-1 (carboxylic acid), as shown by CTG (ATP) and CyQuant (DNA) assays.

ICEbio RIPTAC platform

PHASE 1: THE"HOLD'
(Complex Formation)

Biochemical Assays
- TP & EP Binary Afinity (SPR/TR-FRET)
- Ternary Cooperativity & Kinetics
- Protein complex Structure (Cryo-EM/X-ray)
- Computational Modeling

Cellular Engagement
- TP-EP Ternary Formation (NanoBRET/HTRF)
- Target Protein (TP) Engagement
- Effector Protein (EP) Engagement

PHASE 2: THE "KILL"
(Functional & Phenotypic)

MoA Validation
- EP Sequestration / Pathway Blockade (WB)
- Target Gene Expression (qPCR/Reporter)
- Apoptosis & Cell Cycle (Caspase-3/pRB)

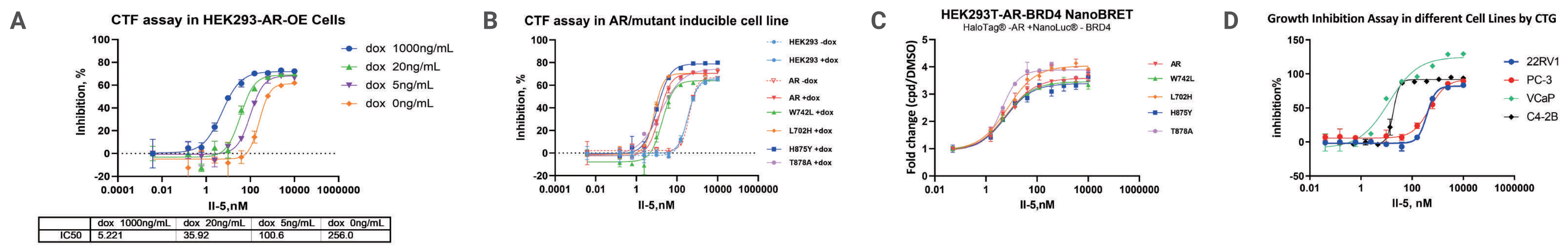
Selective Viability (CTG)
- TP-High (Cancer) vs TP-Low (Normal) Cells
- Inducible TP / Isogenic Mutant Cells
- Resistance Cell Line Panel

PHASE 3:TRANSLATION
(in vivo Models)

ADME & PKIPD
- In vitro / In vivo ADME Profiling
- PK/PD Modeling

In vivo Efficacy (CDXIPDX)
- Tumor Growth Inhibition(TGI)
- In vivo Ternary Formation Biomarkers
- PD Markers (Tumor WB/qPCR & Plasma)

II-5 Activity Linked to AR Expression with Favorable Treatment Window



E

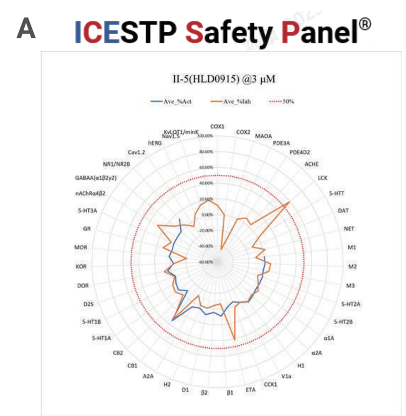
Prostate Cancer Panel	AR expression	R GI50, nM			A GI50, nM		
Cell Line	RNA level	II-5	Enzalutamide	ARV-766	II-5	Enzalutamide	ARV-766
PC-3	0.00	594.74	>10000	>10000	779.39	>10000	>10000
DU145	0.20	851.33	>10000	>10000	810.05	>10000	>10000
Incap C42B	high	18.360	660.32	854.02	19.58	>10000	3124.23
Lncap Clone FGC	63.8	9.33	540.84	611.75	9.80	980.43	954.01
22RV1	100.6(Mostly ARV7)	265.22	>10000	>10000	372.24	>10000	>10000
NCEH660	0.00	855.70	>10000	2177.30	698.98	>10000	>10000
LAPC4	medium	317.74	>10000	1835.97	145.74	>10000	>10000
MDA Pca 2b	33	332.01	>10000	>10000	356.21	>10000	>10000
Vcap	469.1	12.2473	107.514297	No data	8.9489	>10000	No data

Figure 4. RIPTAC treatment window across AR expression contexts. (A) Dox-induced AR expression causes up to a 50-fold IC50 shift under II-5, with dox dose dependent shift.(B) II-5 shows superior activity against AR mutants.(C) II-5 induces strong ternary complex in AR mutants.(D) II-5 is potent in AR-high VCaP and C4-2B cells, but less effective in flAR-low PC-3 and 22RV1.(E) Cell panel data show AR expression positively correlates with II-5 efficacy. II-5 also outperforms Enzalutamide and ARV-766.

II-5: Safe Profile with In Vivo PD Modulation Through Ternary Complex

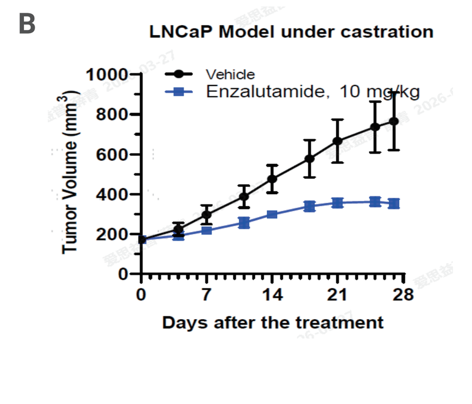
A

ICESTP Safety Panel®



Targets	ICESTP SAFETYPANEL® 44	ICESTP SAFETYPANEL® 77	ICESTP SAFETYPANEL® PLUS	ICESTP SAFETYPANEL® DrugAbuse	ICESTP SAFETYPANEL® CNS Core
Target No.	44	77	108	55	70
Total Functional Assays	74	116	162	101	123
Turn around time	10-15 Business Days Weekly	20-25 Business Days Weekly	20-25 Business Days Weekly	20-30 Business Days Monthly	20-30 Business Days Monthly
Data	dose–response			single-point	
application	Small molecule/Peptide			CNS drugs	

- All Functional assay
- Agonist plus antagonist modes
- Dose responsive curve



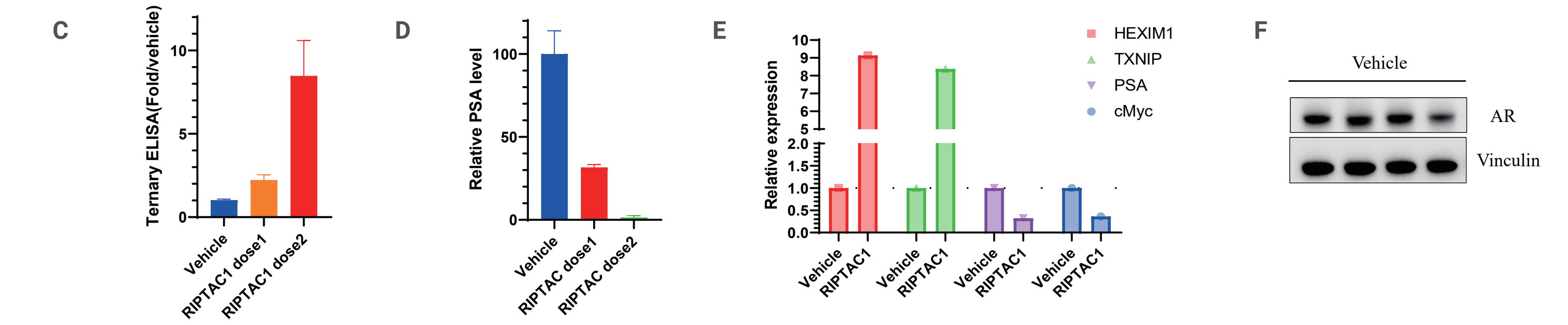


Figure 5. Safety and in vivo evaluation of RIPTAC. (A) Safety panel evaluation of II-5 shows overall favorable profile, with minor off-target activity at 3 μM toward LCK (validation on-going).(B) LNCaP and other CDX models are available at ICEbio.(C) RIPTAC treatment induces AR–BRD4 ternary complex formation in CDX models with dose-responsive effects.(D) RIPTAC triggers PC marker PSA reduction in CDX models.(E) qPCR detection of PD markers confirms AR and BRD4 target responses.(F) CDX WB.

Broadening Induced Proximity: New RIPTAC Pairs and TCIPs

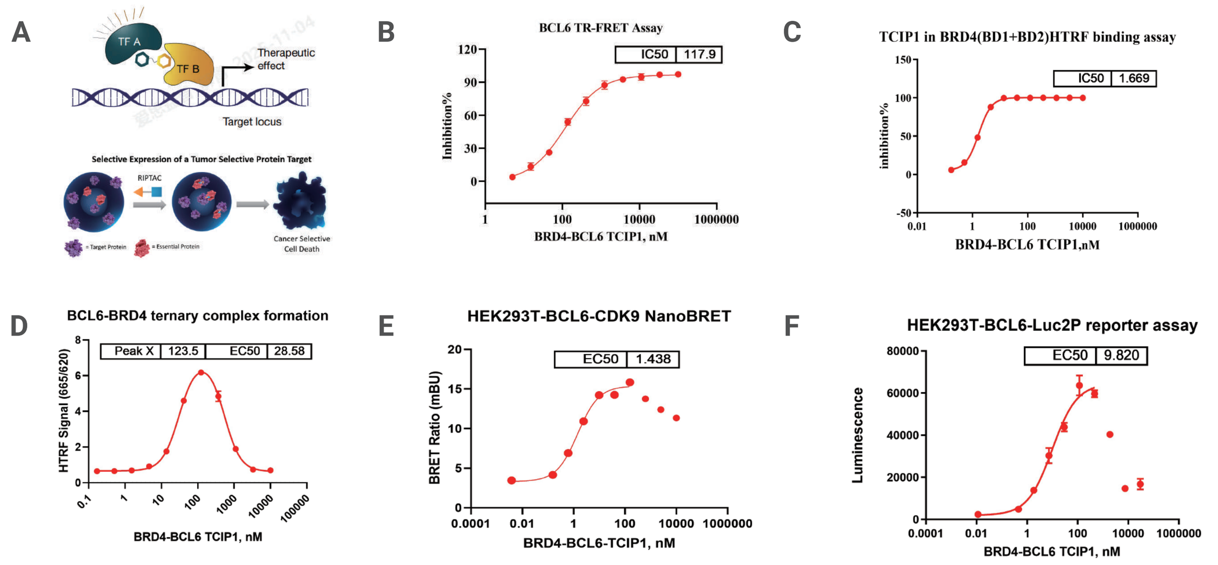


Figure 6. RIPTAC and TCIP expands the induced proximity landscape. (A) Illustration of TCIP and RIPTAC working models (adapted from refs. 2, 4).(B) BCL6 binary HTRF assay of TCIP1.(C) BRD4 binary HTRF assay of TCIP1.(D) BCL6–BRD4 ternary HTRF assay of TCIP1.(E) NanoBRET assay shows BCL6–BRD4 ternary complex.(F) BCL6 reporter assay aligns with ternary results.

Summary

ICE Biosciences' RIPTAC platform integrates biochemical screening, computational docking, and multi-level cellular and in vivo assays to connect target engagement with therapeutic efficacy. By combining ternary complex prediction, kinetic modeling, and validation across biochemical, cellular, and tumor tissue contexts, the platform establishes mechanistic links between AR expression and selective cancer cell inhibition. Supported by our extensive expertise in targeted protein degradation (TPD), the modular design enables rapid migration to new target pairs. This flexibility, together with translational profiling and safety evaluation, empowers domestic and international partners to accelerate first-in-class drug discovery programs and expand the induced proximity landscape with clinically meaningful insights.

Reference

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