Mechanistic characterization of selective monovalent direct degraders of SMARCA2

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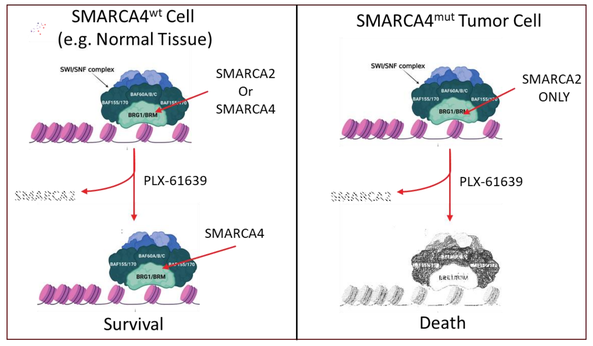
Abstract

SMARCA2 and SMARCA4 are essential, redundant, catalytic subunits of the multi-subunit BRG1/BRM-associated factor (BAF) complex. This complex regulates gene expression and DNA repair through chromatin remodeling activity. The paralogues SMARCA2 and SMARCA4 bind acetylated histones through their highly conserved bromodomains and alter DNA-histone contacts using ATP-dependent helicase activity. Recently, mutations in specific components of the BAF complex have been identified. Of great interest are loss of function mutations in SMARCA4, found in 5-7% of all human cancers and occur with high prevalence (~10%) in non-small cell lung cancer (NSCLC), making these SMARCA4-deficient tumor cells highly dependent on SMARCA2 for viability. This synthetic lethal relationship may provide a unique opportunity for SMARCA2-selective degraders to block the growth of SMARCA4 deficient tumors, while sparing normal tissues.

To enable degrader discovery, we focused our efforts on a monovalent direct degrader approach, where small molecules are designed to bind the target protein and induce its degradation through the recruitment of an E3 ligase complex. Initial screening hits from distinct chemical series were optimized to produce potent and selective SMARCA2 degraders. Co-treatment with a proteasome inhibitor confirmed that degradation was mediated via the ubiquitin proteasome system (UPS), and a UPS-focused CRISPR screen identified the E3 ligase responsible for compound-induced degradation of SMARCA2. Cell based protein-protein interaction (PPI) studies advanced our understanding of compound-mediated E3 ligase recruitment and provided mechanistic insights into the observed selectivity for SMARCA2 over SMARCA4. Ternary complex dynamics were further investigated *in vitro* via surface plasmon resonance (SPR) studies. Using a combination of co-immunoprecipitation, mass spectrometry, and point mutant studies, specific amino acids that are required for compound-induced PPI and subsequent degradation of SMARCA2 were identified. Collectively, these results highlight the discovery and characterization of novel small molecule monovalent direct degraders of SMARCA2. The detailed description of the degradation mechanism presented here provides critical insights into an efficient approach to eliminating pathogenic proteins through targeted protein degradation.



Plexium's approach to monovalent direct degrader discovery

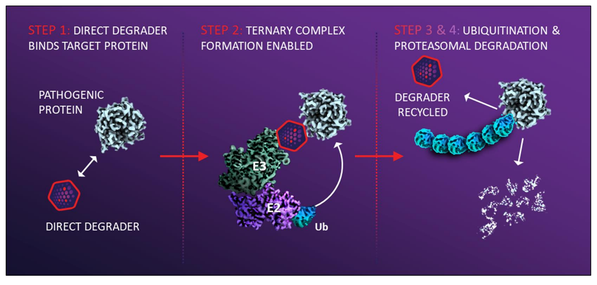
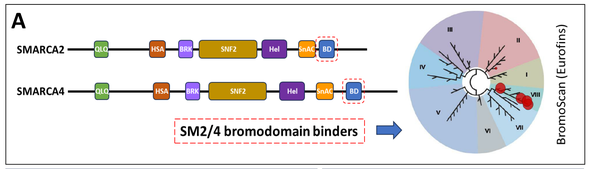


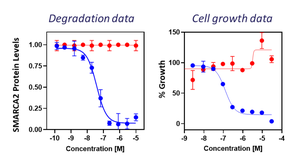
Figure 1: Overview of the Plexium Direct Degrader design strategy. Small molecules are designed to bind to the protein-of-interest (POI) and elicit its degradation through induced interactions with an E3 ligase complex. Prospective degraders can be designed in an E3-agnostic manner and screened for activity in cell-based assays which expose compounds to the repertoire of cellular E3 ligases.

Characterization of SMARCA2 direct degraders



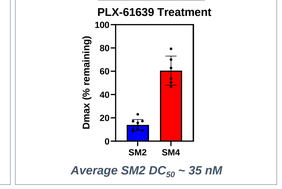
SMARCA2 profile in SK-MEL-5

Cpd	Kd (nM)	DC_{50} (nM)	EC_{50} (nM)
PLX-61639	2	40	36
Non-degrader	2	>10,000	>10,000

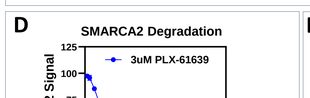


Bromodomain Binding

Target	K_D (nM)
SMARCA2	1
SMARCA4	1



Average SM2 DC_{50} ~ 35 nM



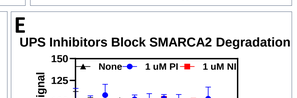
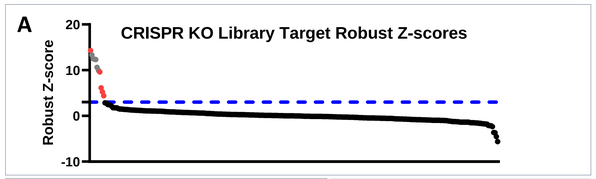


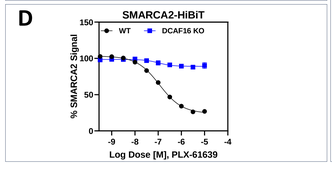
Figure 2: **A.** SMARCA2 and its homologue SMARCA4 possess highly similar protein domain structures. Potent and selective bromodomain (BD) binders (as shown in the BROMO*scan* chart (Eurofins)) serve as the starting point for the degrader discovery program. **B.** SK-MEL-5 cells were treated with PLX-61639 vs a BD-binding non-degrader. Anti-proliferative activity was observed only with PLX-61639. **C.** PLX-61639 demonstrates degradation selectivity despite having similar BD-binding affinity. Maximum degradation achieved in a panel of $SM4^{def}$ and $SM4^{wt}$ tumor cell lines, as monitored by immunofluorescence (IF). **D.** SMARCA2-HiBiT HeLa cells were treated with 3uM PLX-61639 over the course of 48 hrs. SMARCA2 signal is measured using HiBiT luciferase. **E.** Degradation is proteasome and neddylation dependent. HiBiT cells were pre-incubated +/- proteasome inhibitor (PI; 1μM bortezomib) or neddylation inhibitor (NI; 1μM Pevonedistat) for 1hr, followed by 6hr co-incubation with PLX-61639.

PLX-61639 requires DCAF16 to degrade SMARCA2



Gene	Robust Z-score
RBX1	18.30
COPS5	14.32
DDB1	9.61
NEDD8	6.52
DCAF16	6.13
CUL4B	5.26
CAND1	4.41

Subunits and cofactors of DCAF16 CRL



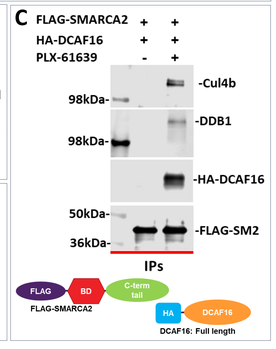


Figure 3: **A.** CRISPR knockout (KO) screening data utilizing a UPS focused sgRNA library (Synthego). SMARCA2 degradation was assessed by IF after CRISPR KO and subsequent treatment with PLX-61639 (3μM for 18hr). Hits were identified using robust Z-score calculations (0.675*(Xi-median)/MAD). Red dots = library plate screening hits; gray dots = negative control wells. **B.** Table showing $CUL4B^{DCAF16}$ CRL subunits and related cofactors identified in CRISPR screen. **C.** FLAG-SMARCA2 immunoprecipitation (IP) demonstrates PLX-61639-induced interactions with HA-tagged DCAF16 and co-purification of selected endogenous components of $CRL4B^{DCAF16}$. Schematics of proteins used for IP are shown. **D.** SMARCA2 degradation is not observed in SMARCA2-HiBiT HeLa $DCAF16^{-/-}$ cells. Clonal knockout cells were generated using Synthego's DCAF16 sgRNA. Wildtype and knockout cells were treated for 24hr with PLX-61639.

PLX-61639 induced ternary complexes correlate with target degradation

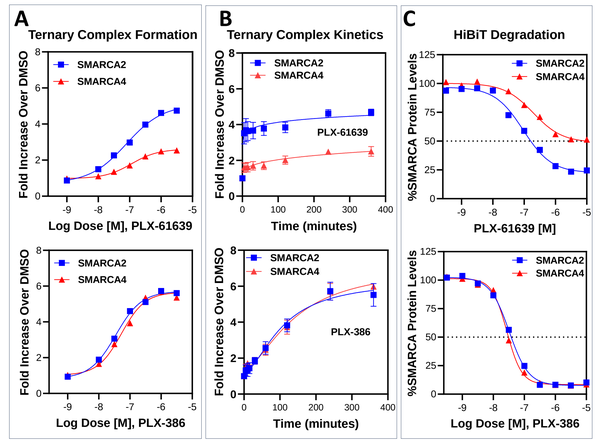


Figure 4: A. Ligand induced SMARCA2/SMARCA4:DCAF16 ternary complex formation. **B.** Complex formation kinetics. **C.** SMARCA2/SMARCA4-HiBiT degradation. Top graphs in **A.**, **B.**, and **C.** profile PLX-61639, demonstrating SM2>>SM4 ternary complex formation, relative complex formation kinetics, and SM2>>SM4 degradation. Bottom graphs profile a potent and non-selective degrader, PLX-386, which promotes SM2=SM4 complex formation with similar kinetics. This results in SM2=SM4 degradation.

Degradation of SMARCA2 is mediated by covalent engagement of DCAF16

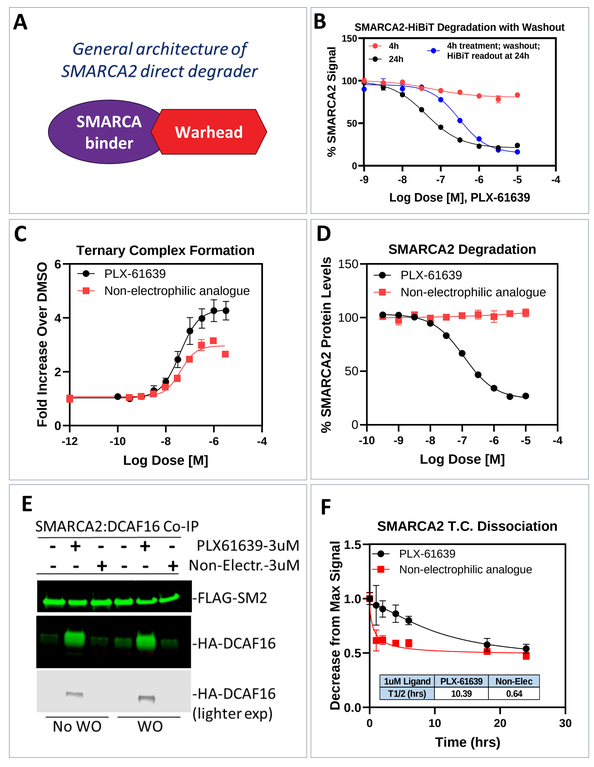


Figure 5: **A.** Schematic of SMARCA2 direct degrader including SMARCA2 binder and reactive warhead. **B.** SMARCA2-HiBiT cells were treated with PLX-61639 for 4hr and 24hr, as well as a 4hr treatment followed by ligand washout and reading of the plate at 24hr. Full depth of SMARCA2 degradation is achieved even after ligand washout, suggestive of a covalent MOA. **C.** Non-electrophilic analogue of PLX-61639 has activity in the SMARCA2:DCAF16 ternary complex assay, but complexes are non-productive (see D.). **D**. SMARCA2-HiBiT degradation assay comparing PLX-61639 and its non-electrophilic analogue. **E.** Co-IP of FLAG-SMARCA2 and HA-DCAF16. Proteins were transiently expressed in HEK293T cells. Following Bortezomib pretreatment (0.1uM, 1 hr), cells were treated with indicated ligands for an additional 4 hrs. DCAF16 co-purifies with SMARCA2 in samples treated with PLX-61639. DCAF16:SMARCA2 interactions persist even after PLX-61639 removal via washout (WO). **F.** Washout kinetic studies demonstrate that SMARCA2:DCAF16 ternary complexes are much more stable with PLX-61639 vs the non-electrophilic analogue.

PLX-61639 selectively modifies a single DCAF16 Cysteine

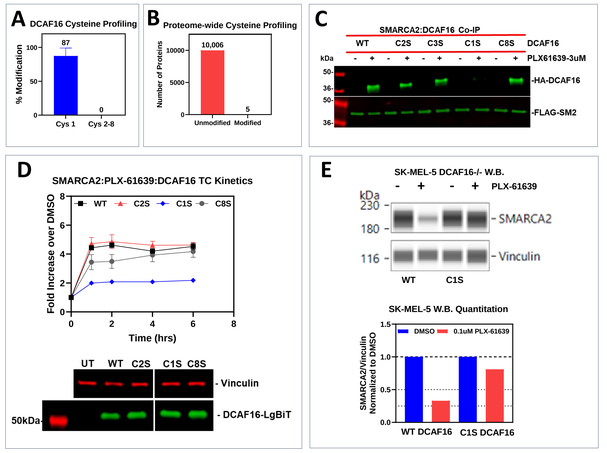


Figure 6: **A.** Mass Spec analysis of co-IP'ed DCAF16 shows covalent modification exclusively at a single cysteine (C1). **B.** Proteome-wide cysteine profiling of NCI-H1568 cells treated with 3μM PLX-61639 (4hr) shows only 5 out of 10,006 identified proteins with PLX-61639 modifications > 15%. **C.** Co-IP of FLAG-SMARCA2 and HA-DCAF16 (WT and Cys to Ser mutants). PLX-61639 promotes SMARCA2:DCAF16 interaction with WT and select DCAF16 Cys mutants except C1S. **D.** Graph of ternary complex formation kinetics using DCAF16 Cys mutants. C1S is impaired in activity. Western blot shows DCAF16 expression from constructs used in assay. Protein abundance was measured using an antibody directed to Lg-BiT. **E.** WT or C1S mutant DCAF16 was transiently expressed in SK-MEL-5 DCAF16 KO cells. Transfected cells were then treated with DMSO or PLX-61639 (0.1uM) for 24 hrs. WT DCAF16 supports SMARCA2 degradation. Graph shows quantitated Simple Western bands.

Catalytic mechanism promotes sustained degradation of SMARCA2

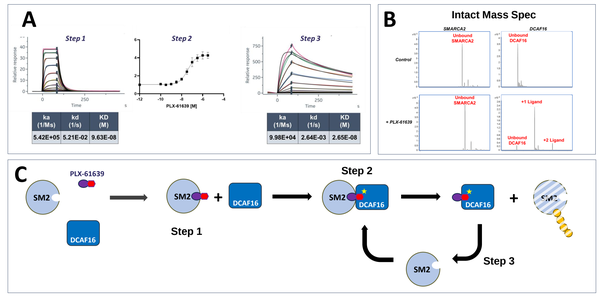
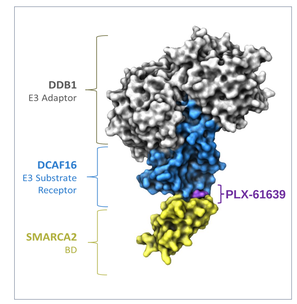


Figure 7: **A.** Overview of PLX-61639 MOA incorporating surface plasma resonance (SPR) data (Viva Biotech). **Step 1:** PLX-61639 binds to SMARCA2. Association (ka) and dissociation (kd) constants are noted. **Step 2:** SMARCA2:PLX-61639:DCAF16 ternary complexes form. Representative PPI data are shown. **Step 3:** DCAF16 bound with PLX-61639 interacts with SMARCA2, demonstrating rapid ka and slow kd. **B.** MS evaluation of recombinant proteins used in **A.** shows singular and selective Cys modification of DCAF16 upon incubation with SMARCA2 and PLX-61639. Covalent modification occurs primarily at C1. **C.** Schematic of PLX-61639-induced SMARCA2 degradation. PLX-61639 binds to SMARCA2 forming a binary complex that then engages with DCAF16. In the SMARCA2:PLX-61639:DCAF16 ternary complex, the reactive warhead of PLX-61639 is in proximity of DCAF16 C1, promoting covalent modification. SMARCA2 is then ubiquitinated by the $CRL4B^{DCAF16}$ ligase, followed by proteasomal degradation. Covalently modified DCAF16 can engage with another SMARCA2 molecule and promote additional rounds of degradation (catalytic cycle).

Summary and Model



- Plexium has developed a potent and selective monovalent direct degrader of SMARCA2 using its design platform
- SMARCA2 degradation relies on BD-binding and a covalent warhead
- A UPS focused CRISPR library screen identified $CRL4B^{DCAF16}$ as being required for PLX-61639 mediated SMARCA2 degradation
- MOA studies demonstrate PLX-61639-mediated SMARCA2:DCAF16 protein interactions and the requirement of covalent binding to DCAF16 C1 to support a stable ternary complex (model shown) and subsequent SMARCA2 degradation
- PLX-61639 is currently in IND-enabling studies