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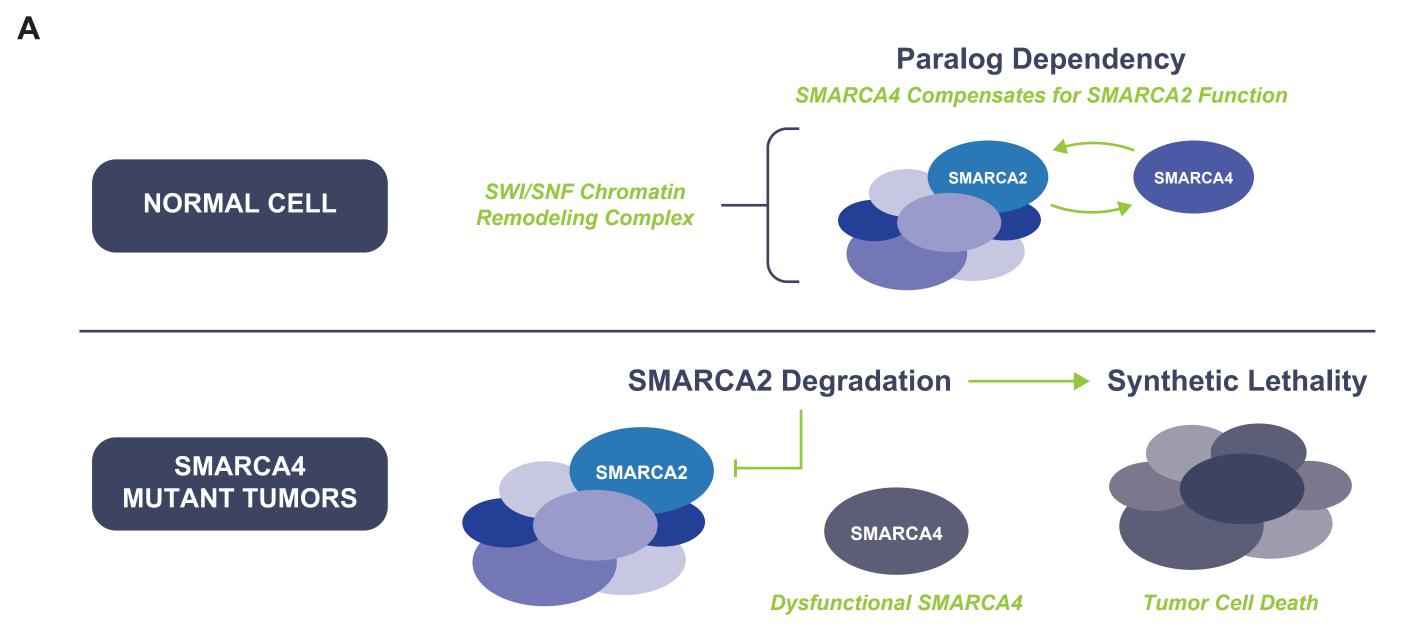
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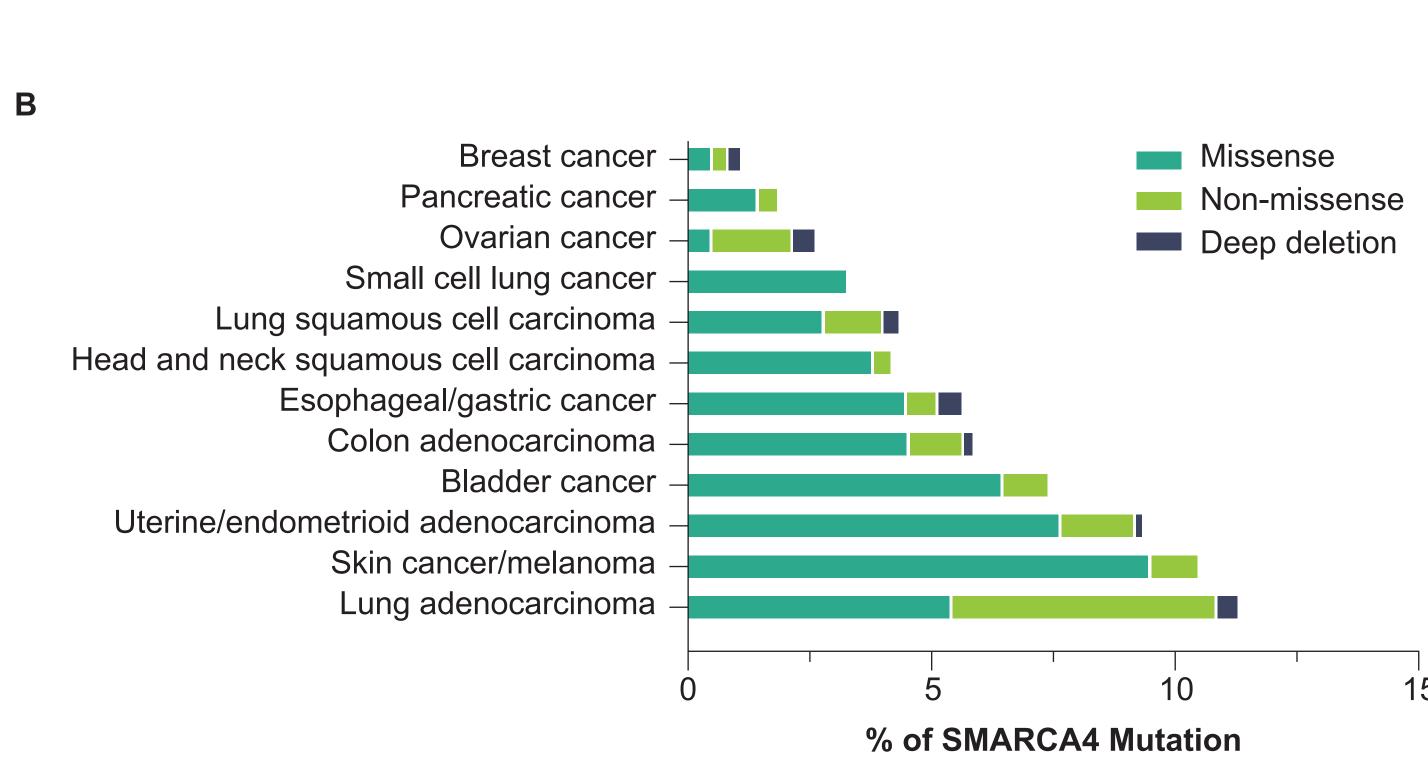
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Background

- SWItch/Sucrose Non-Fermentable (SWI/SNF) complexes play an important role in controlling gene expression by remodeling chromatin.¹
- ➤ SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 2 (SMARCA2) (Brahma homolog [BRM]) and SMARCA4 (Brahma-related gene 1 [BRG1]) are the core catalytic subunits of the SWI/SNF complexes (Fig. 1A).¹
- ► SMARCA4 has been shown to be mutated in multiple cancers, including 10 to 12% of non-small cell lung cancer¹ (NSCLC; Fig. 1B) and SMARCA4-deficient cancer cells can become highly dependent on SMARCA2 for their survival.²
- ► Therefore, targeting SMARCA2 in SMARCA4-deleted cancers using selective SMARCA2 degraders induces synthetic lethality while sparing SMARCA4 wild-type (WT) normal cells (Fig. 1A).¹

Figure 1. SMARCA4 and SMARCA2 Regulate Chromatin Accessibility and Gene Expression





(A) Model of SMARCA2 degradation—induced synthetic lethality in SMARCA4-deleted cancers.
 (B) Percentage of SMARCA4 mutation in different types of cancer.³ Non-missense mutation includes nonsense, splice-site, and frame-shift deletion mutations.

Objectives

► To identify and characterize a highly potent and selective SMARCA2 protein degrader (PRT3789), which is designed to specifically inhibit SMARCA4-deficient human cancers.

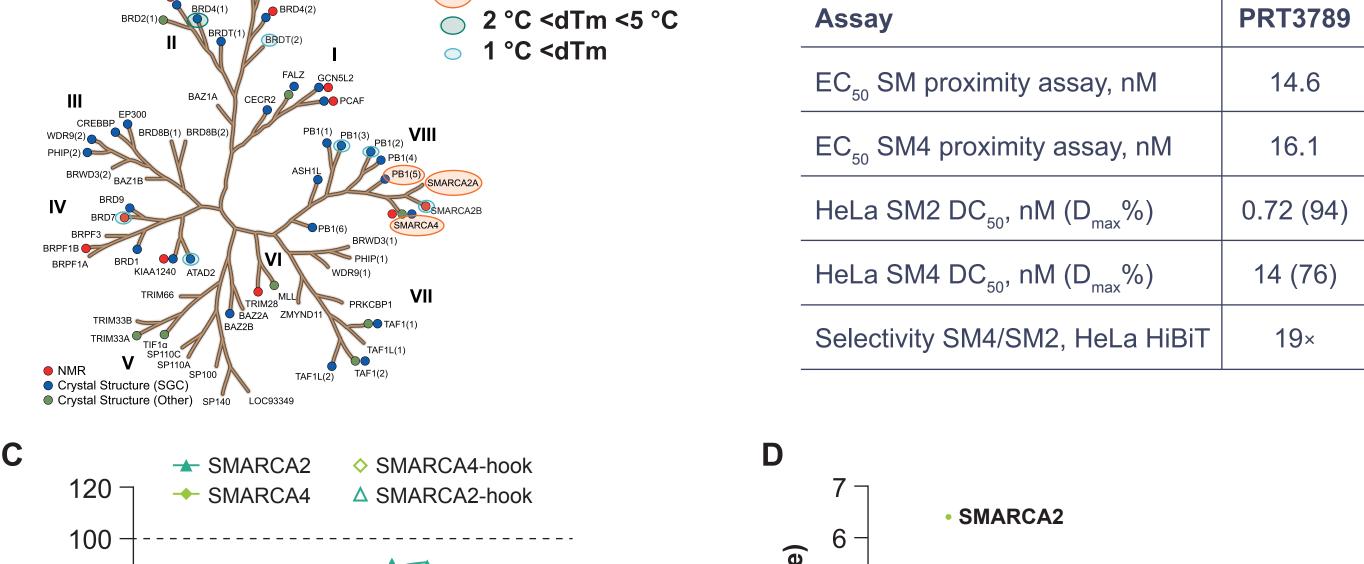
Key Findings

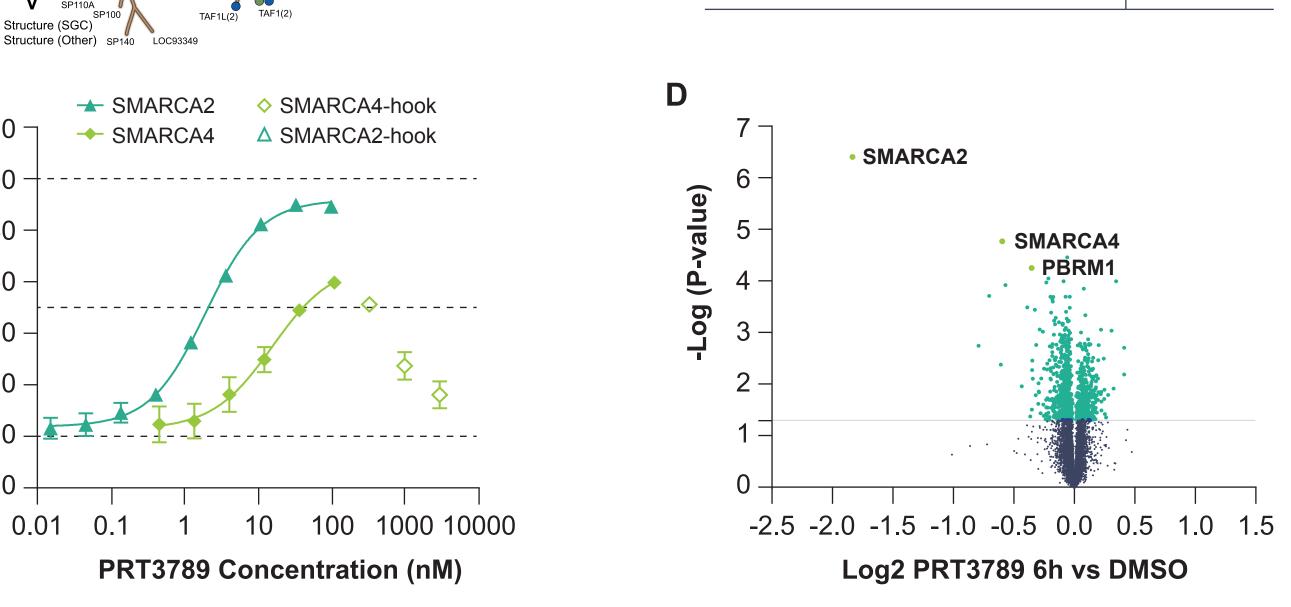
Our potent and selective SMARCA2-targeted degrader (PRT3789) shows favorable pharmacokinetic properties and induces strong synthetic lethality in SMARCA4-deleted cancers in vitro, cell line—derived xenograft (CDX) models in vivo, and patient-derived xenograft (PDX) models ex vivo.

Results

Figure 2. PRT3789 SMARCA2 Degrader Displays Excellent SMARCA2 Degradation Selectivity and Potency

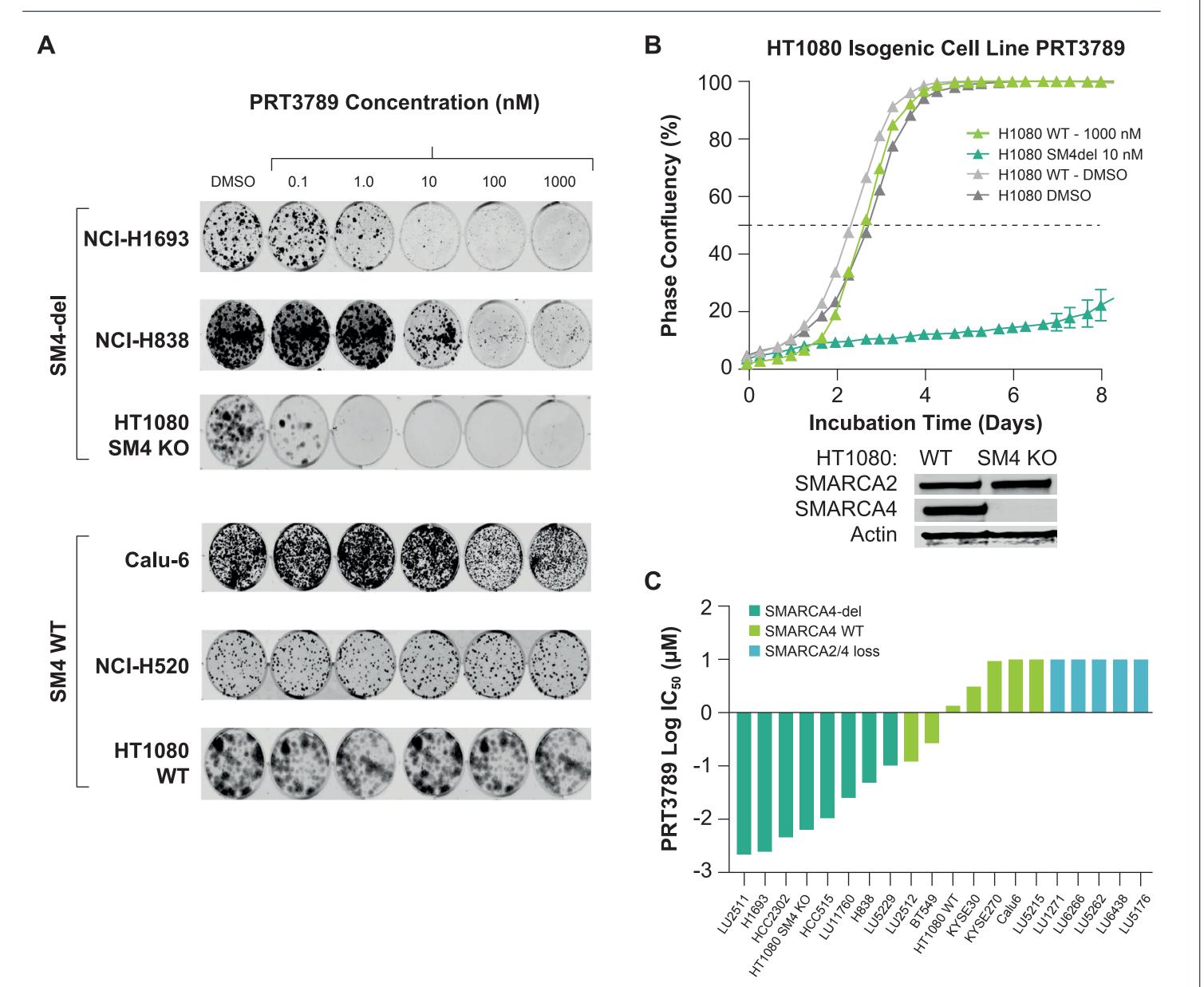
dTm >5 °C



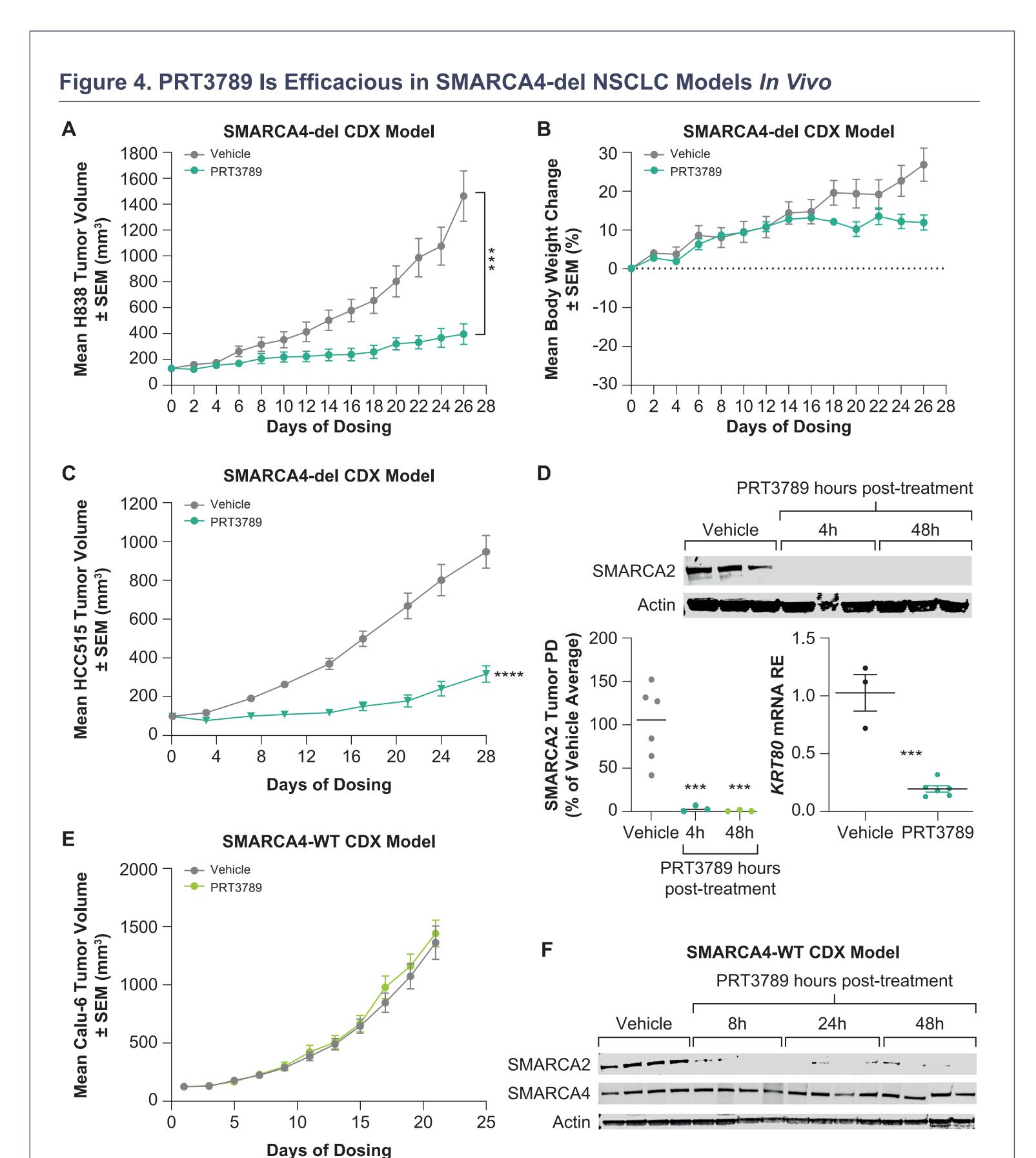


(A) DSF shows PRT3789 is selective to bromodomain family of proteins. Image modified with permission from Filippakopoulos P, et al. Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell.* 2012;149:214-231. Licensed under http://creativecommons.org/licenses/by/4.0/ for CC BY. (B) Proximity TR-FRET assays revealed that PRT3789 displays similar binding kinetics to SMARCA2 and SMARCA4. (B and C) PRT3789 demonstrates excellent SMARCA2 potency and selectivity over SMARCA4, as demonstrated by Hela HiBit assay. (D) Global proteomics shows PRT3789 to be highly selective against SMARCA2. DC₅₀, the concentration where 50% of the protein has been degraded; D_{max}%, maximal level of degradation; DMSO, dimethylsulfoxide; DSF, differential scanning fluorimetry; dTm, melting temperature; EC₅₀, half-maximal effective concentration; PBRM1, protein polybromo-1; SM2, SMARCA2; SM4, SMARCA4; TR-FRET, time-resolved fluorescence resonance energy transfer.

Figure 3. PRT3789 Selectively Inhibits SMARCA4-Mutated Lung Cancer Proliferation *In Vitro* and *Ex Vivo*

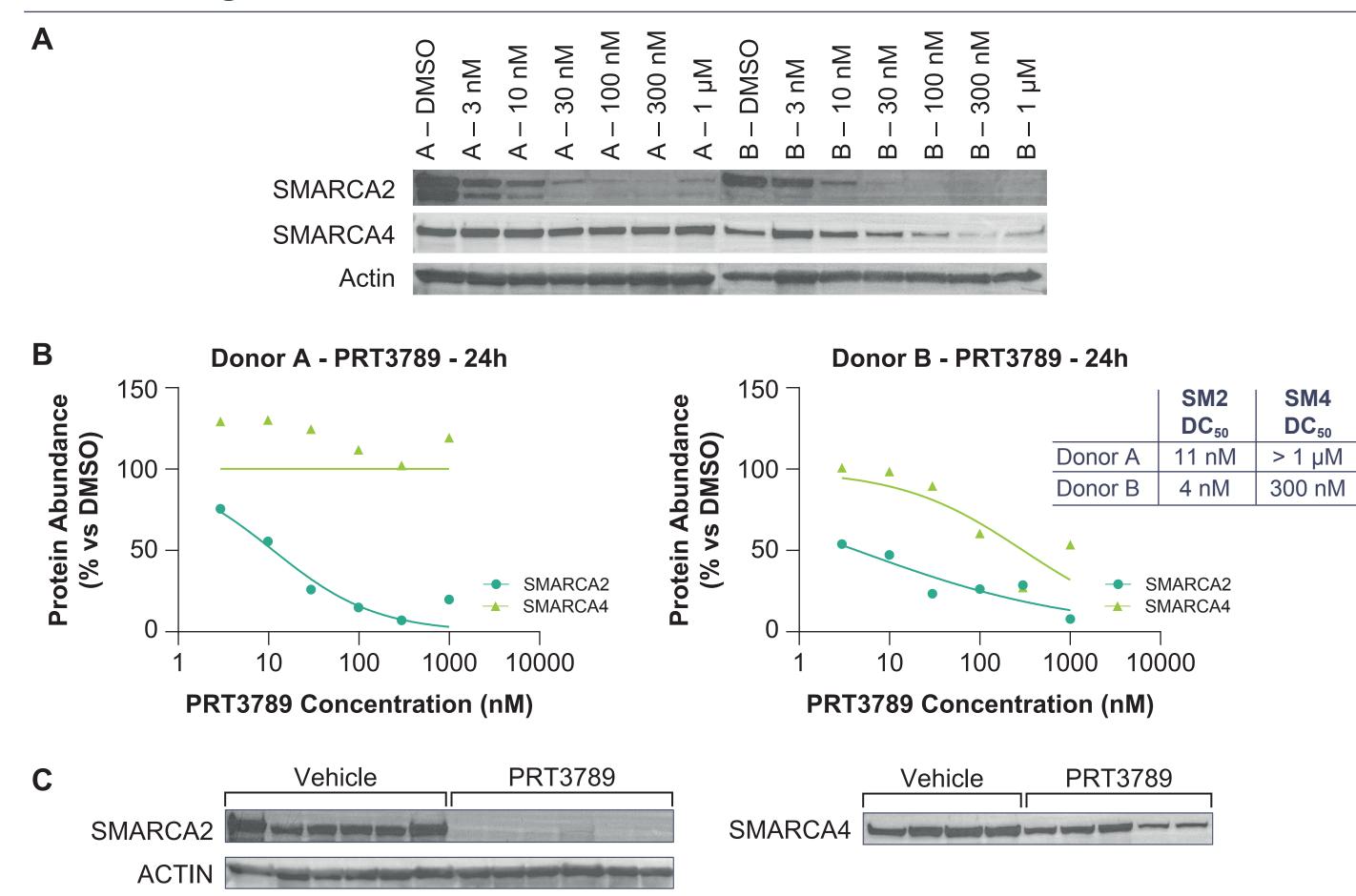


(A) PRT3789 inhibits proliferation of SMARCA4-del/KO cancer cell lines, but not SMARCA4 WT cancer cell lines as demonstrated by clonogenic assay and (B) Incucyte proliferation assay using isogenic SMARCA4-del and WT HT1080 cells. (C) SMARCA4-del cancer cell lines and *ex vivo* (PDX) models are more sensitive to PRT3789 versus WT and SMARCA2/4 dual loss models. Cell lines *in vitro*: 7-day CTG in 2D culture. PDX *ex vivo*: 7-day CTG in 3D culture (gIC₅₀). CTG, CellTiter-Glo®; del, deletion; gIC₅₀, half-maximal concentration that inhibits cell growth; IC₅₀, half-maximal inhibitory concentration; KO, knockout.



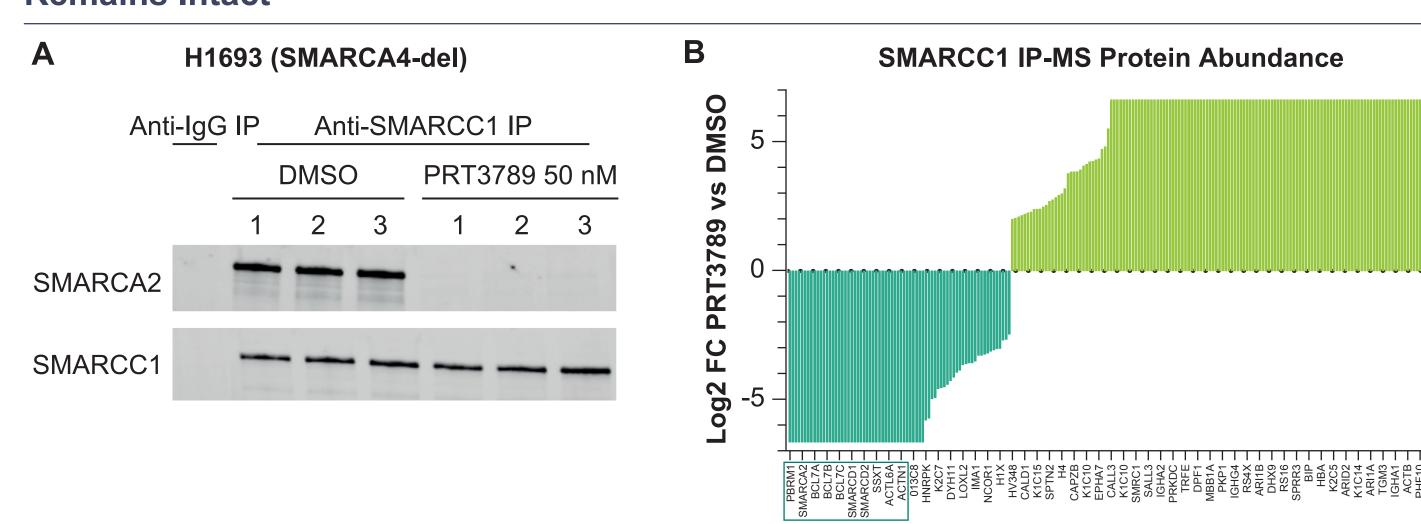
(A-C) PRT3789 significantly inhibits growth of SMARCA4-del NSCLC CDX models at well-tolerated doses. (D) Single-dose PRT3789 treatment completely suppresses SMARCA2 protein levels and KRT80 mRNA levels for 48 hours *in vivo*. (E) SMARCA4-WT CDX model is unaffected by PRT3789 treatment. (F) PRT3789 selectivity is demonstrated *in vivo* in a Calu-6 SMARCA4-WT CDX model. ***P<0.001, ****P<0.0001 versus vehicle (two-tailed unpaired t test). PD, pharmacodynamic; RE, relative expression.

Figure 5. PD Assay – *Ex Vivo* and *In Vivo* PRT3789 Treatment Demonstrated Robust Selective Degradation of SMARCA2 in PBMCs



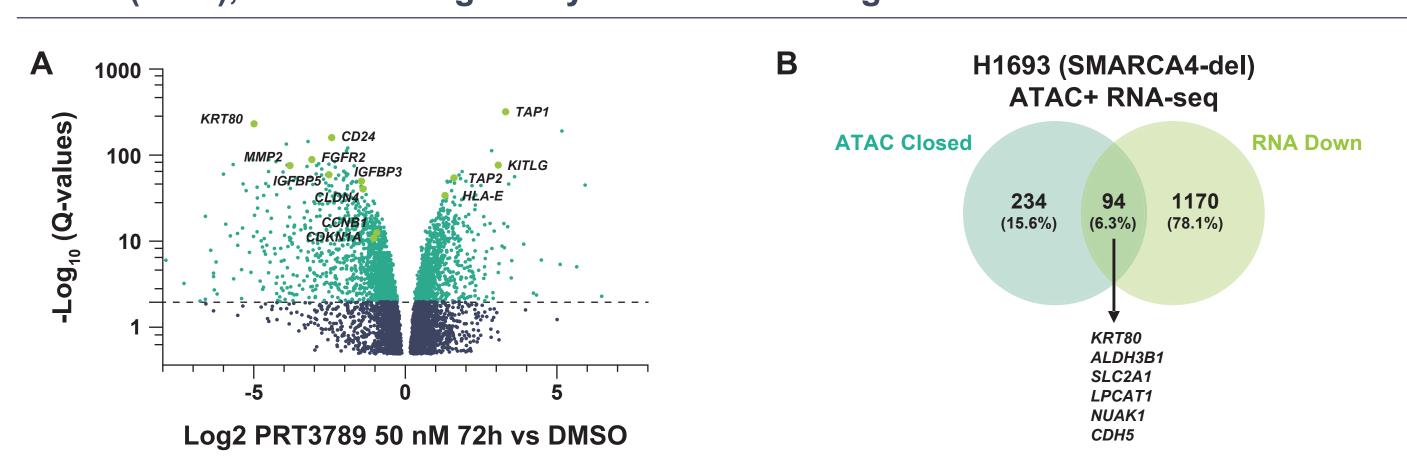
(A) Purified PBMCs from two healthy donors were cultured *ex vivo* for 24 hours in the presence of PRT3789, followed by Western blot (WB) analysis. (B) PRT3789 SMARCA2 and SMARCA4 DC₅₀s in donor PBMCs were determined by WB. (C) PRT3789 treatment leads to robust degradation of SMARCA2 protein in PBMCs in a 1-week rat study. Rats were dosed on days 1, 4, and 7 and PBMCs were collected on day 8. PBMCs, peripheral blood mononuclear cells.

Figure 6. PRT3789 Induces Dissociation of Actin-Related Protein (ARP) Module, SS18 and BCL7 Subunits From SWI/SNF Complex, While Core DNA Finger Complex Remains Intact



(A) Coimmunoprecipitation (Co-IP) of SMARCC1 followed by WB of SMARCA2 reveals complete degradation of SMARCA2 following PRT3789 treatment in the SMARCA4-del H1693 cell line. WB of SMARCC1 demonstrates successful SMARCC1 Co-IP and anti-immunoglobulin G (IgG) Co-IP demonstrates specificity of SMARCC1 Co-IP. (B) Immunoprecipitation-mass spectrometry data plotted as PRT3789 versus DMSO log2 fold change (FC) protein abundance (P<0.05, FC>2). Nonspecific proteins removed versus IgG immunoprecipitation. Subunits of the SWI/SNF complex depleted following PRT3789 treatment are outlined in a green box on the x-axis.

Figure 7. PRT3789 Regulates Chromatin Accessibility and Cell Migration, Extracellular Matrix (ECM), and Immunogenicity-Related Gene Signatures



(A) Volcano plots display Log2 (FC vs DMSO) gene expression and adjusted P value (Q value) in SMARCA4-del NCI-H1693 cells treated with PRT3789 for 72 hours. Global mRNA expression was analyzed by NGS (Illumina HiSeq). Key genes downregulated and upregulated by PRT3789 are highlighted. KRT80 and other oncogenic genes were downregulated at 72 hours (total 600 DEG). Cell migration, ECM modulation, and cell cycle—related gene signatures were downregulated. Antigen processing/presentation—related proteins (TAP/HLA subtypes) were upregulated (B) ATAC-seq (differentially closed >1.5 FC) integrated with RNA-seq (>1.5 FC) reveals a loss of chromatin accessibility and decreased expression of specific genes upon PRT3789 treatment. Genes listed are oncogenes and negative prognostic biomarkers relevant to lung cancer. DEG, differentially expressed genes (false discovery rate <0.01, FC >2); NGS, next-generation sequencing; TAP, transporter associated with antigen processing.

Conclusions

- Targeting SMARCA2 in SMARCA4-deficient cancers induces synthetic lethality in SMARCA4-mutant tumors, while sparing normal cells that express SMARCA4 protein.
 PRT3789 SMARCA2 protein degrader displays excellent SMARCA2 selectivity and potency
- and is efficacious at well-tolerated doses in SMARCA4-del NSCLC models *in vivo*.

 ➤ Robust selective SMARCA2 degradation can be detected in human PBMCs following
- PRT3789 treatment.

 DNA finger region of SWI/SNF complex remains intact following PRT3789 treatment in
- SMARCA4-del NSCLC.
 PRT3789 regulates chromatin accessibility and cell migration, ECM, and immunogenicity-related gene signatures.

Referen

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Disclosures

All authors are employees of Prelude Therapeutics, Inc. and may own stocks or shares.

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