

The use of *SureFire*[®] *Ultra*[™] assays to demonstrate PROTAC mediated degradation and the effects on signaling pathways

1. Overview

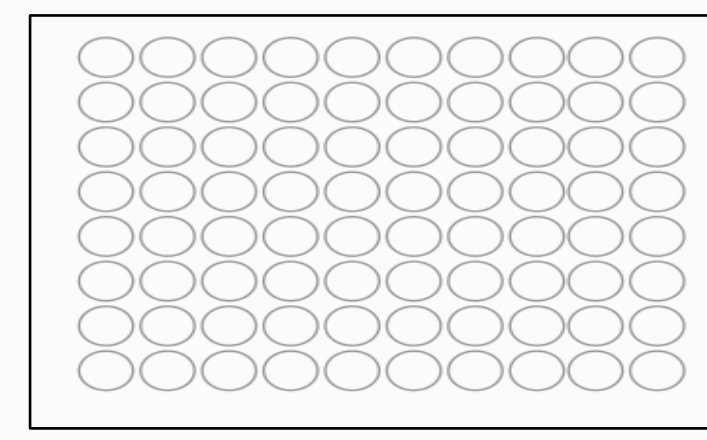
SureFire Ultra is a rapid, homogenous bead-based assay with exceptional sensitivity and enhanced signal windows for kinase screening programs. Endogenous cellular phosphorylation can be measured in all types of cellular extracts.

PROTAC compounds are gaining attention as a strategy to target disease related protein for degradation in a manner that is specific, with fewer side effects and with the ability to target proteins previously considered to be undruggable.

A broad range of Total assays provide a simple method to assess PROTAC degradation in a cellular context as well as analysis of downstream signaling pathways.

2. Methods

Cell treatment with PROTAC

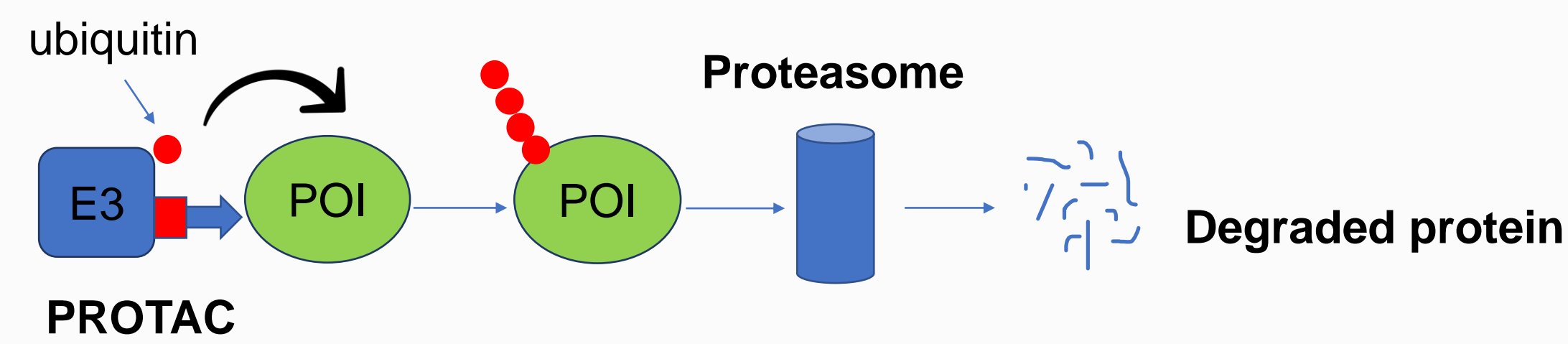
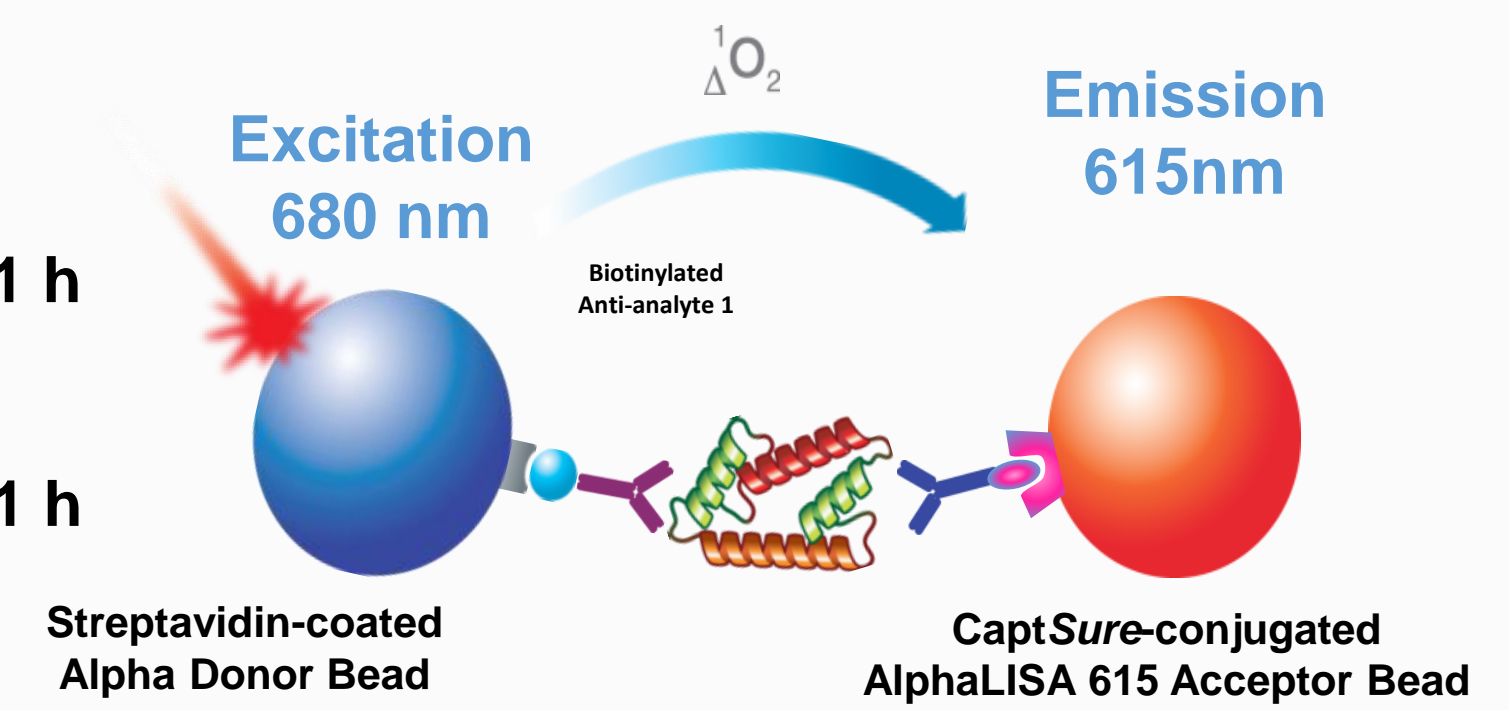


Standard Workflow

Cell Lysate (10 µL)
+
Acceptor Mix (5 µL)
+
Donor Bead Mix (5 µL)

1 h
1 h

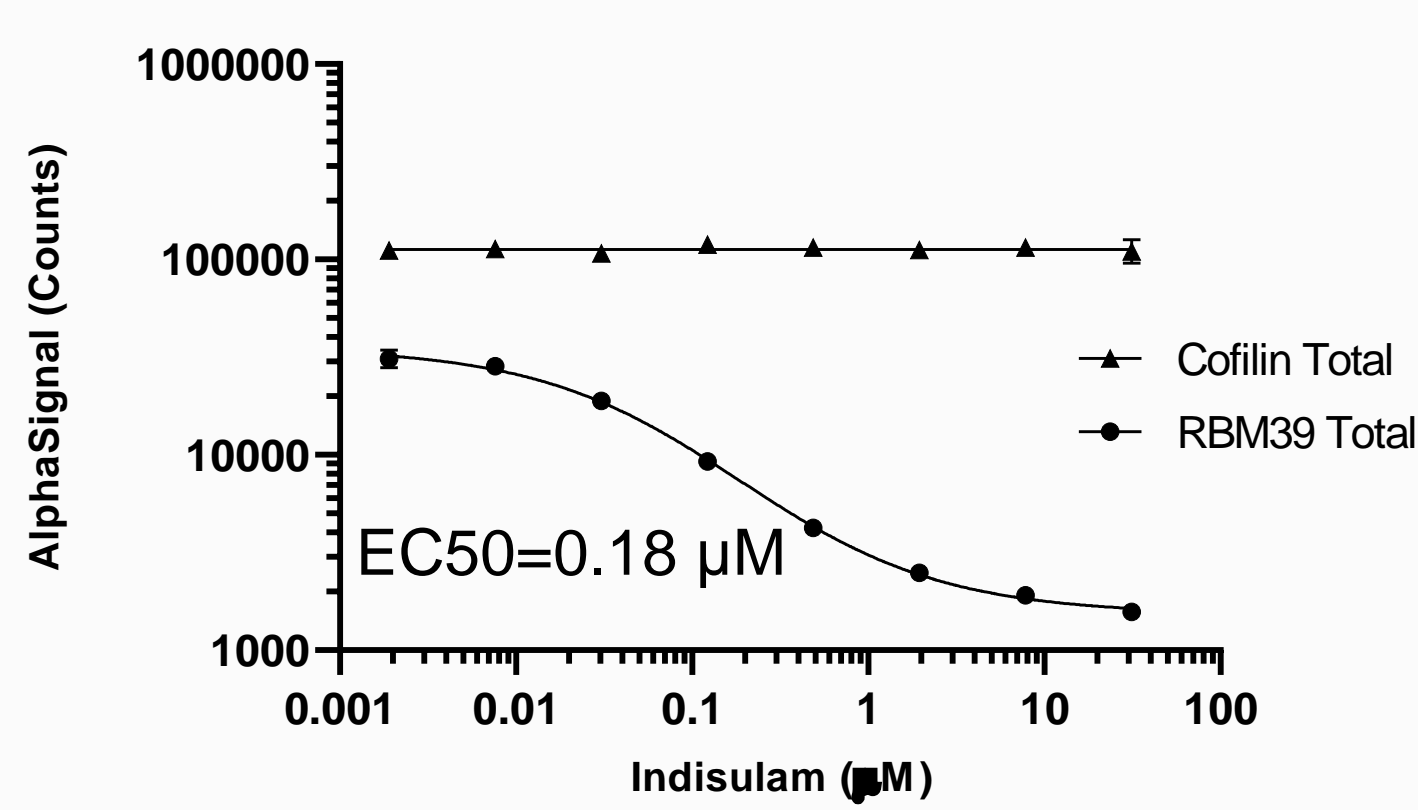
Alpha *SureFire Ultra* Assay



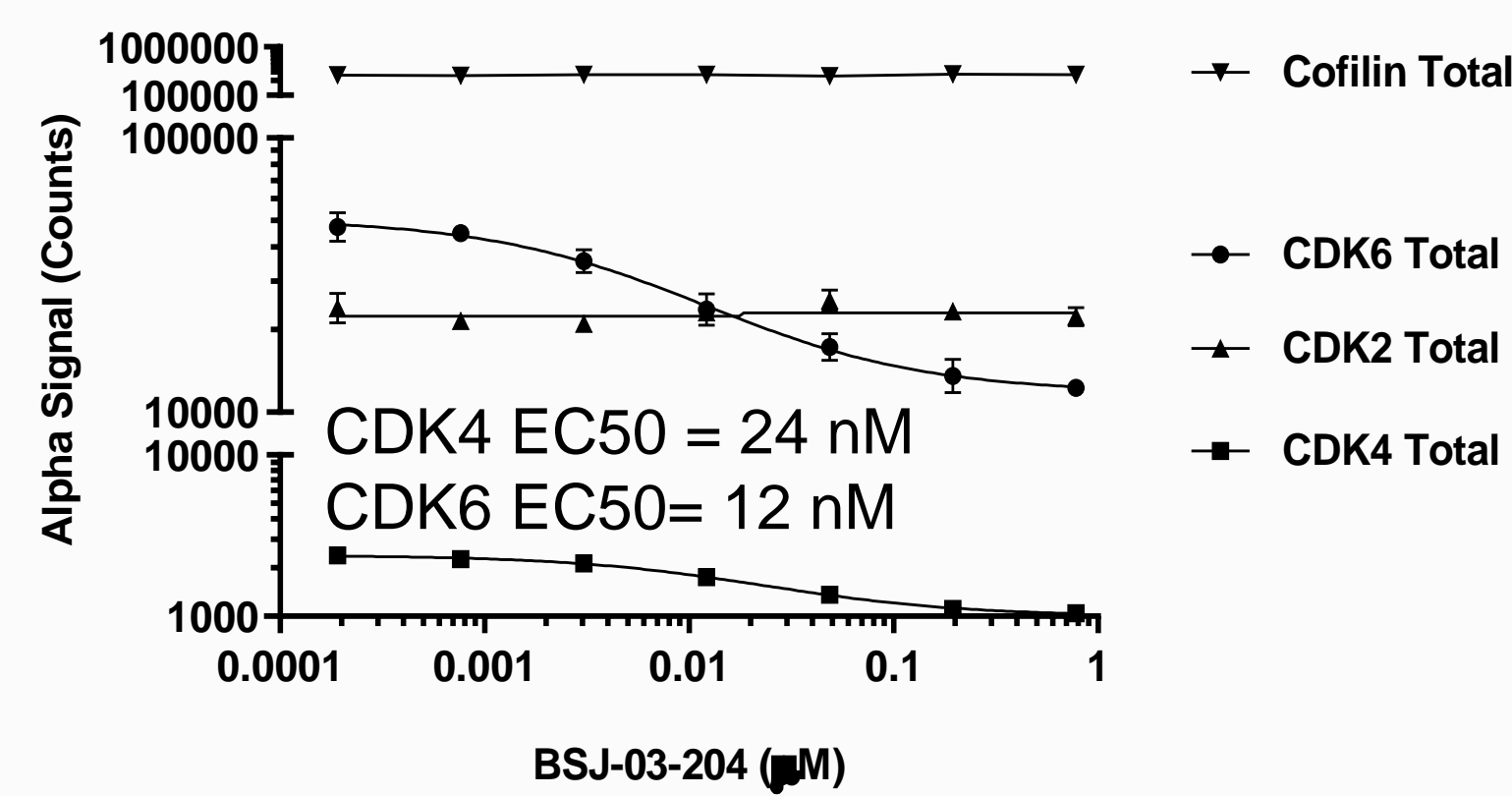
3. Results

3.1 Target specific PROTAC degradation

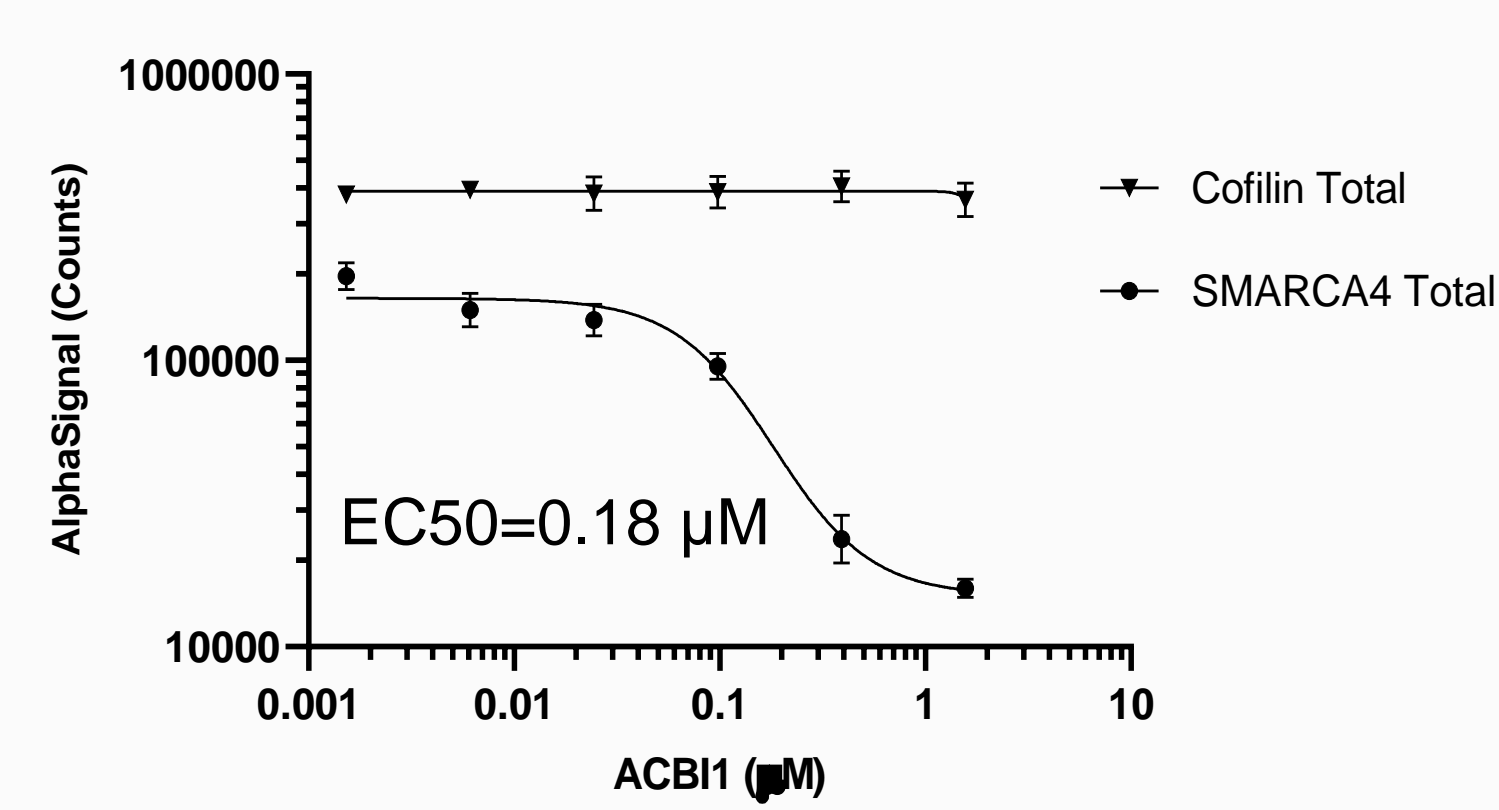
Degradation of RBM39



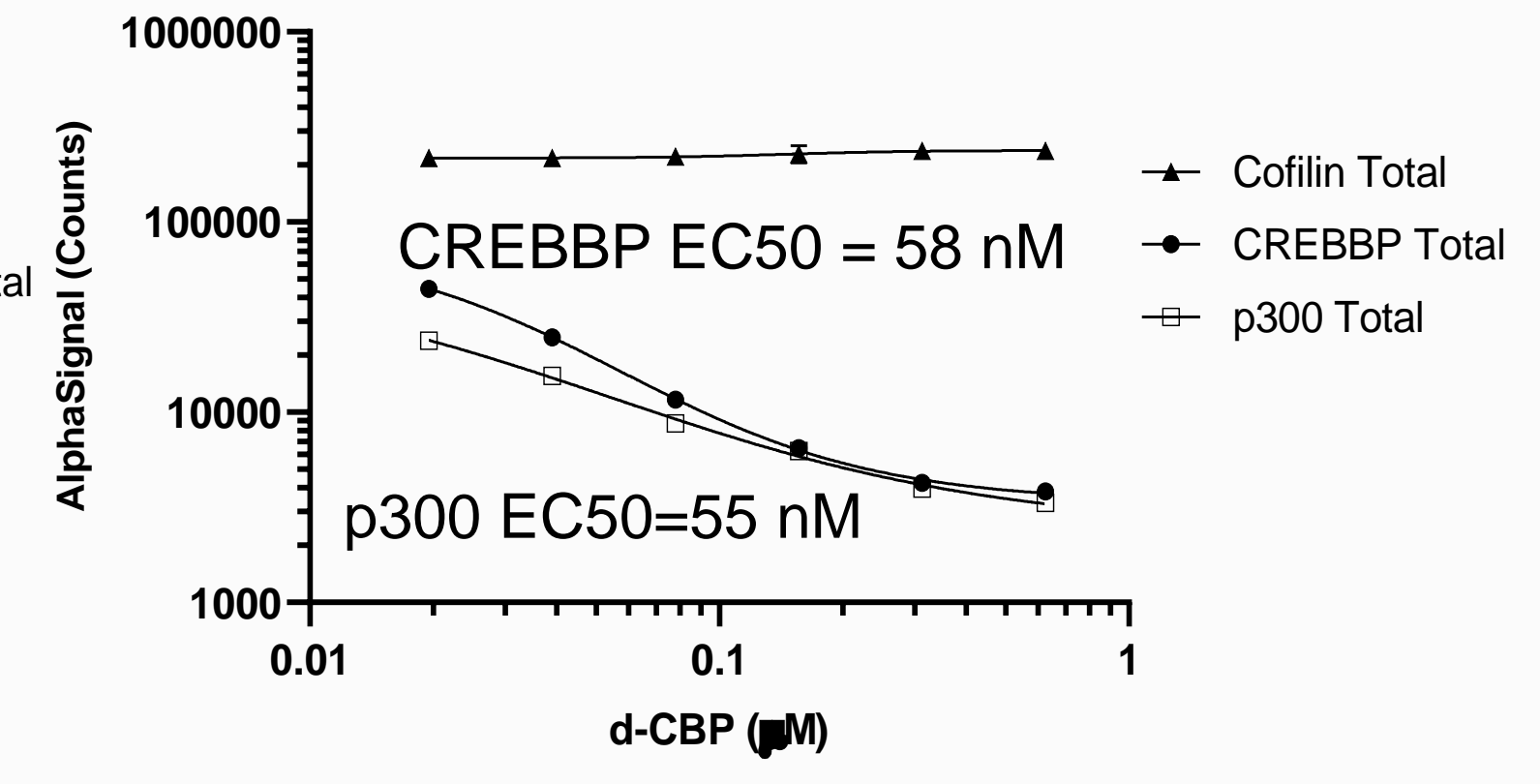
PROTAC degradation of CDK4/6



PROTAC degradation of SMARCA4



PROTAC degradation of CREBBP/p300



- Indisulam degradation of RBM39
- A549 cells treated for 8 hours
- 4,000 cells/datapoint – RBM39
- 800 cells/datapoint - Cofilin

- BSJ-03-204 degradation of CDK4/6
- SH-SY5Y cells treated for 6 hours
- 4,000 cells/datapoint – CDK2/4/6
- 800 cells/datapoint - Cofilin

- ACB1 degradation of SMARCA4
- SH-SY5Y cells treated for 4 hours
- 4,000 cells/datapoint – SMARCA4
- 800 cells/datapoint - Cofilin

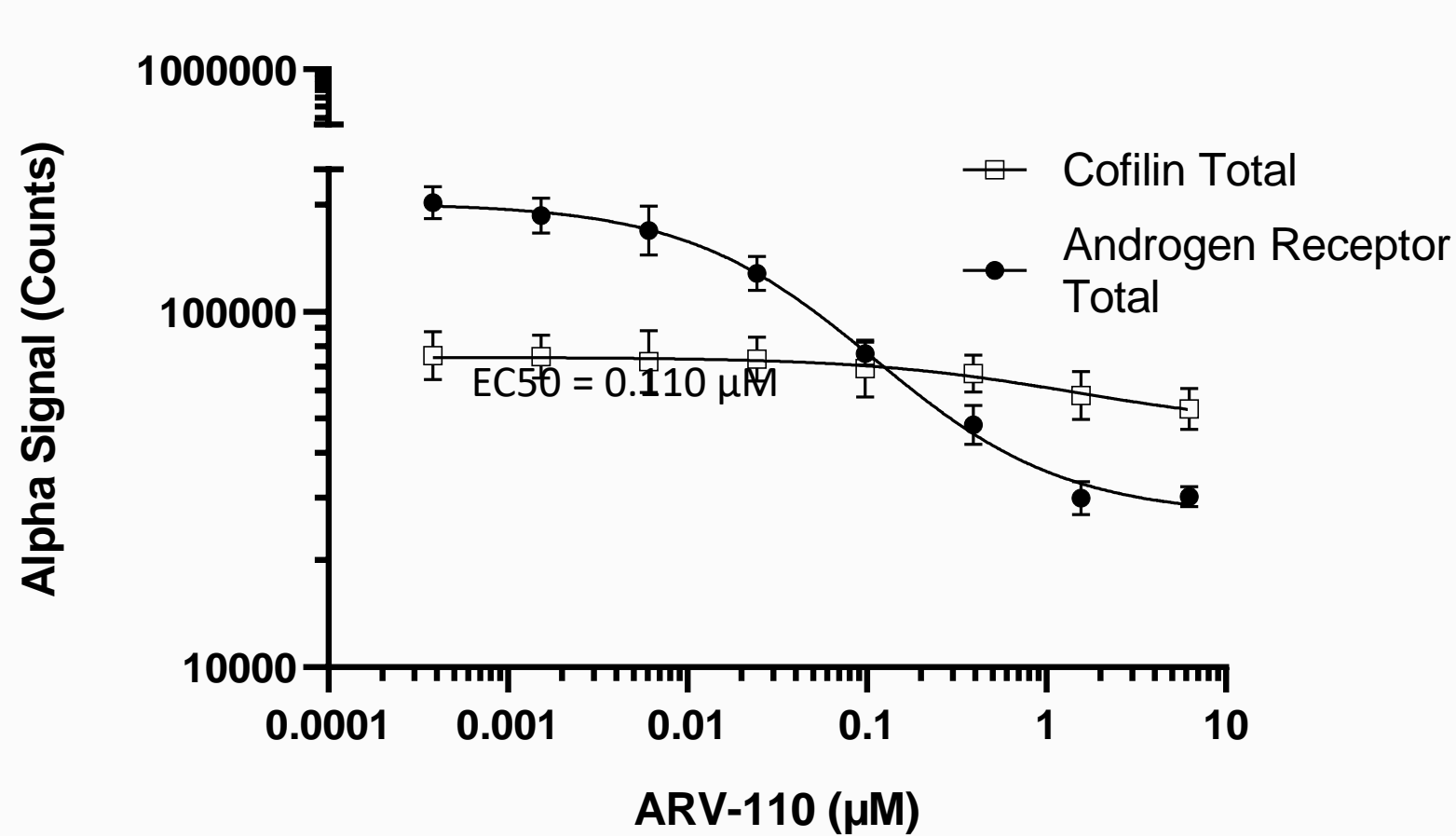
- d-CBP degradation of CREBBP/p300
- HeLa cells treated for 2 hours
- 4,000 cells/datapoint – CREBBP/p300
- 800 cells/datapoint - Cofilin

Specific target degradation

No degradation of close family members or unrelated proteins

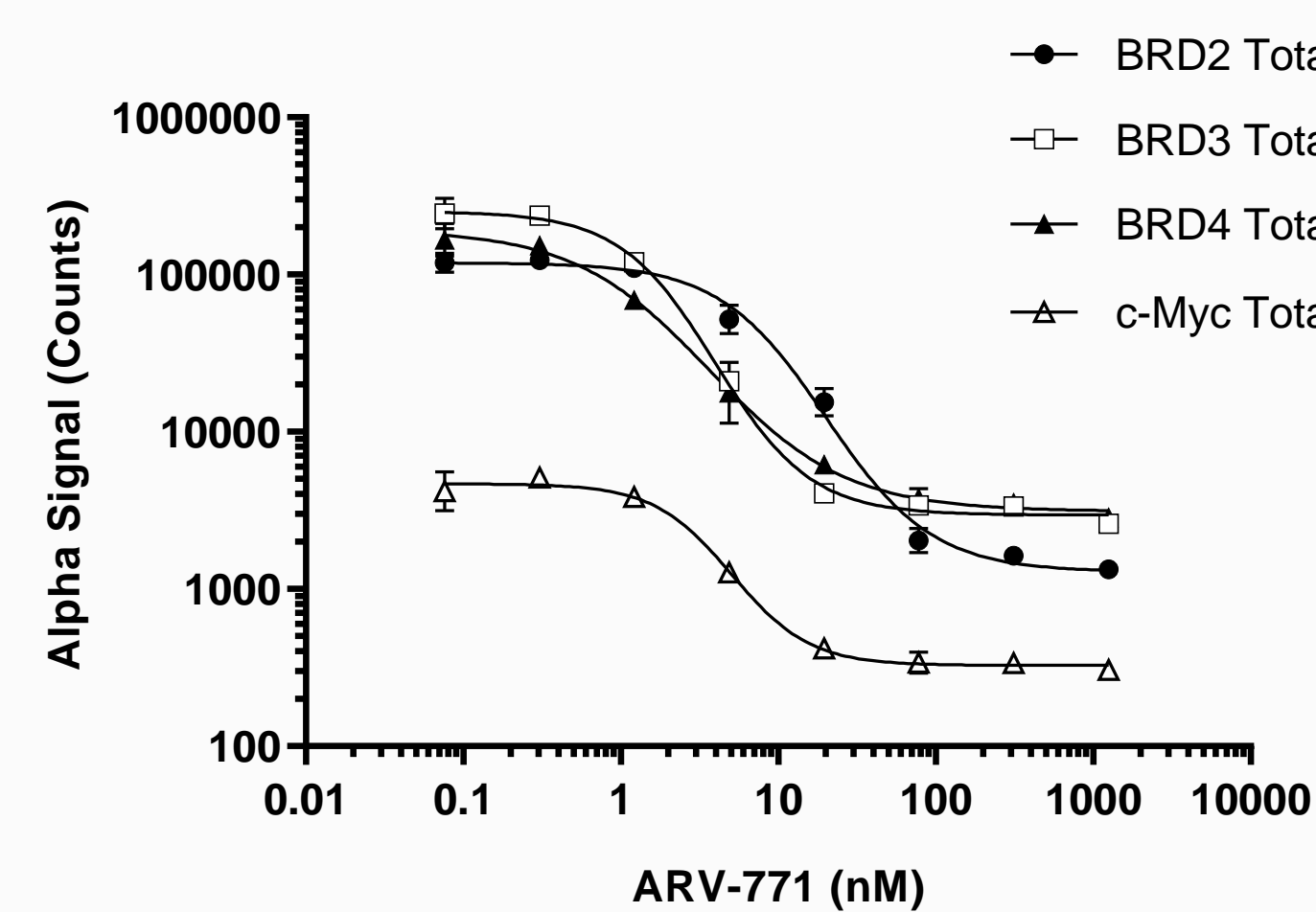
3.2 BET family degradation and down regulation of Androgen Receptor signalling¹

PROTAC degradation of AR

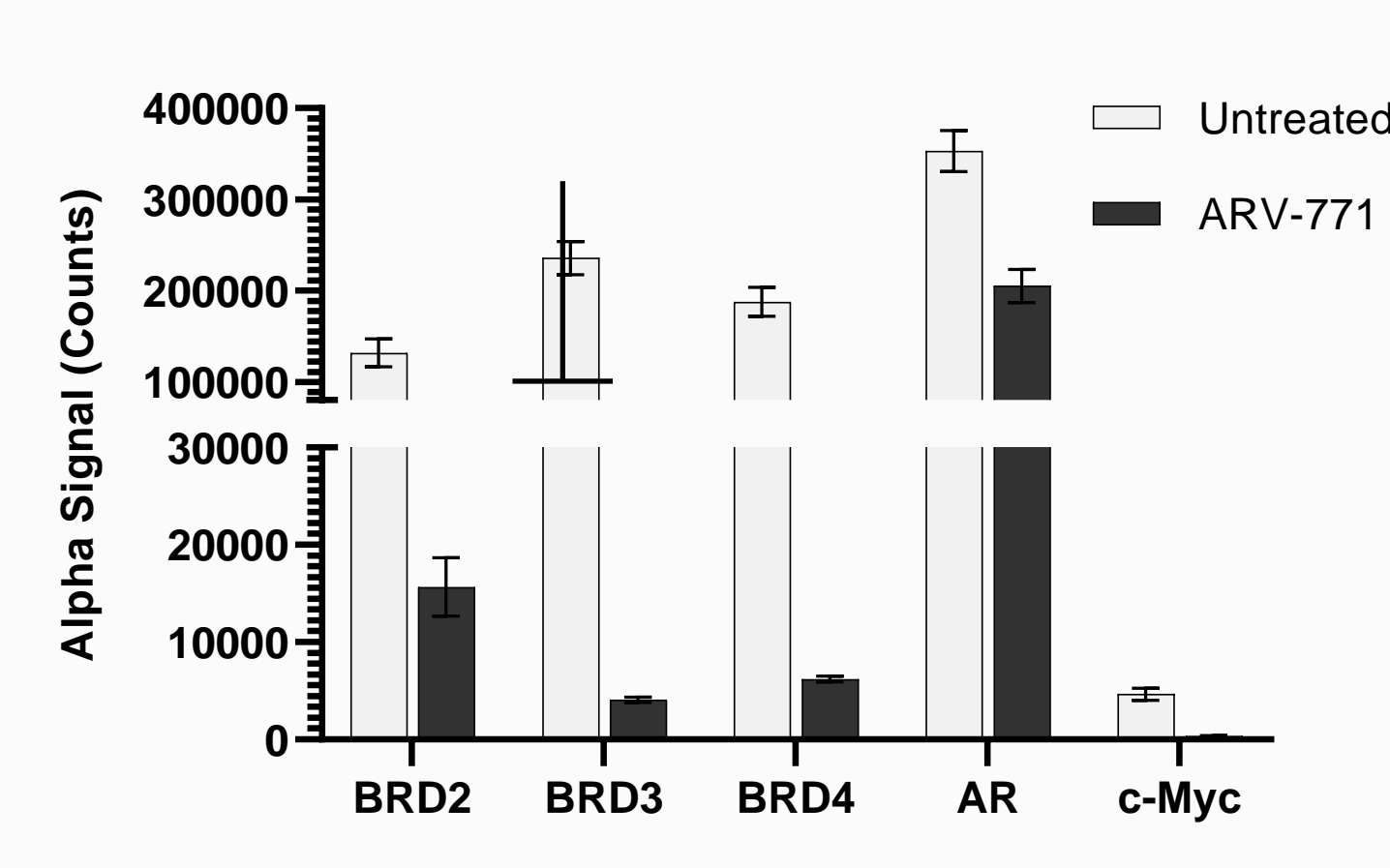


- ARV-110 degradation of AR
- LNCaP cells treated for 8 hours
- 4,000 cells/datapoint – AR
- 800 cells/datapoint - Cofilin

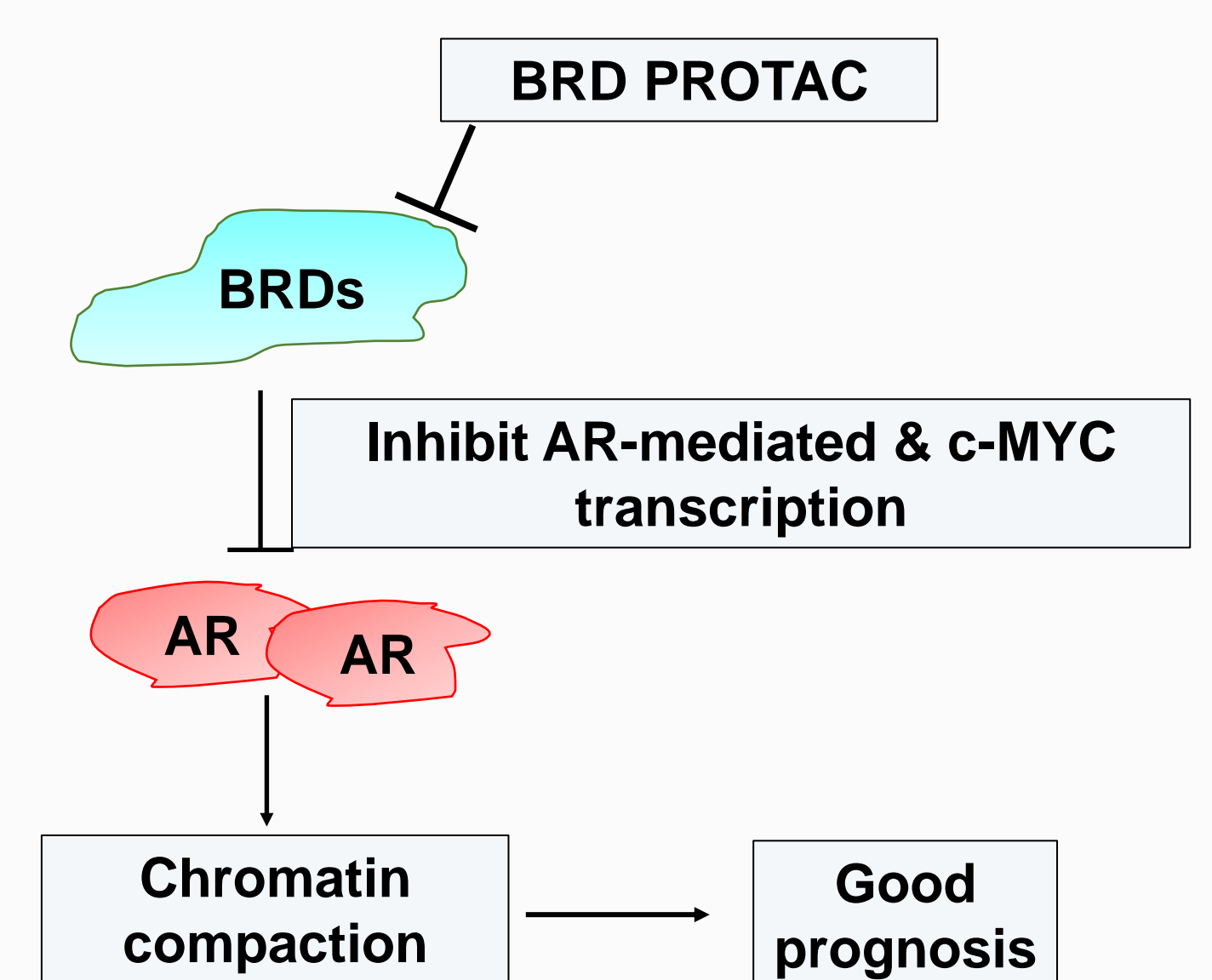
PROTAC degradation of BET family



Degradation of BET family (BRD 2,3,4)

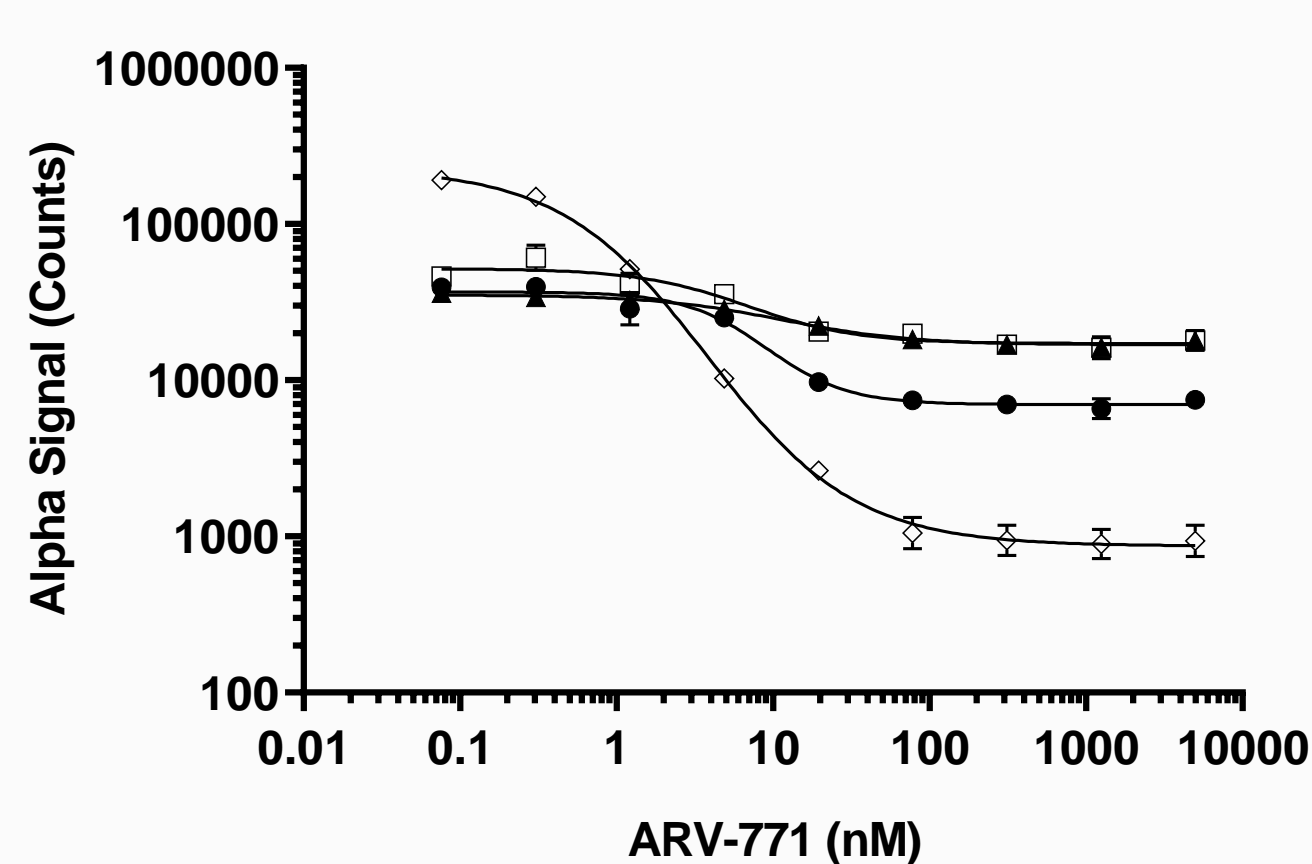


Suppression of c-Myc and reduced AR levels

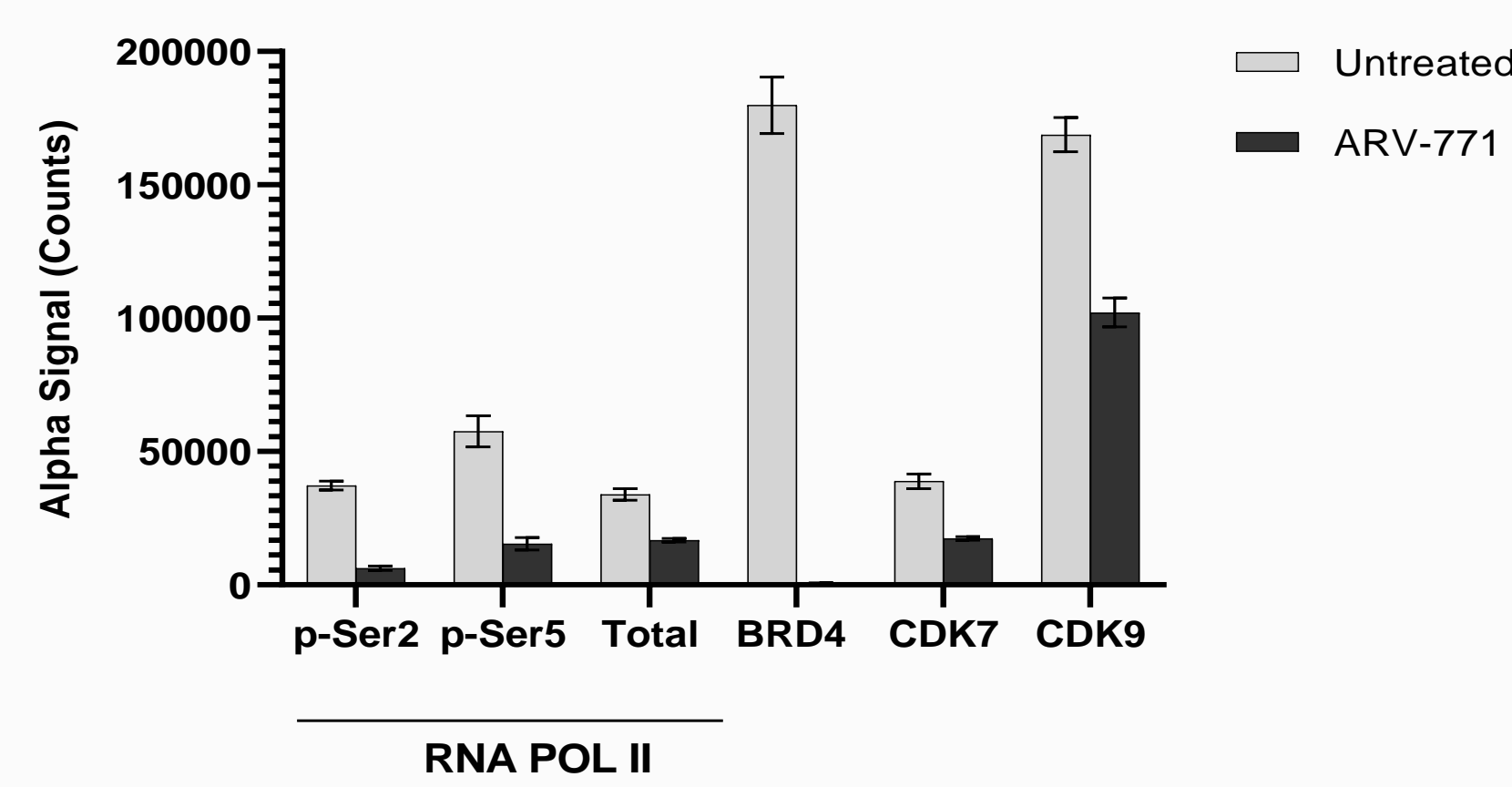


1. Raina K, et al. PROTAC-induced BET protein degradation as a therapy for castration resistant prostate cancer. PNAS, 2016.

3.3 Regulation of RNA polymerase and transcription by BRD4²

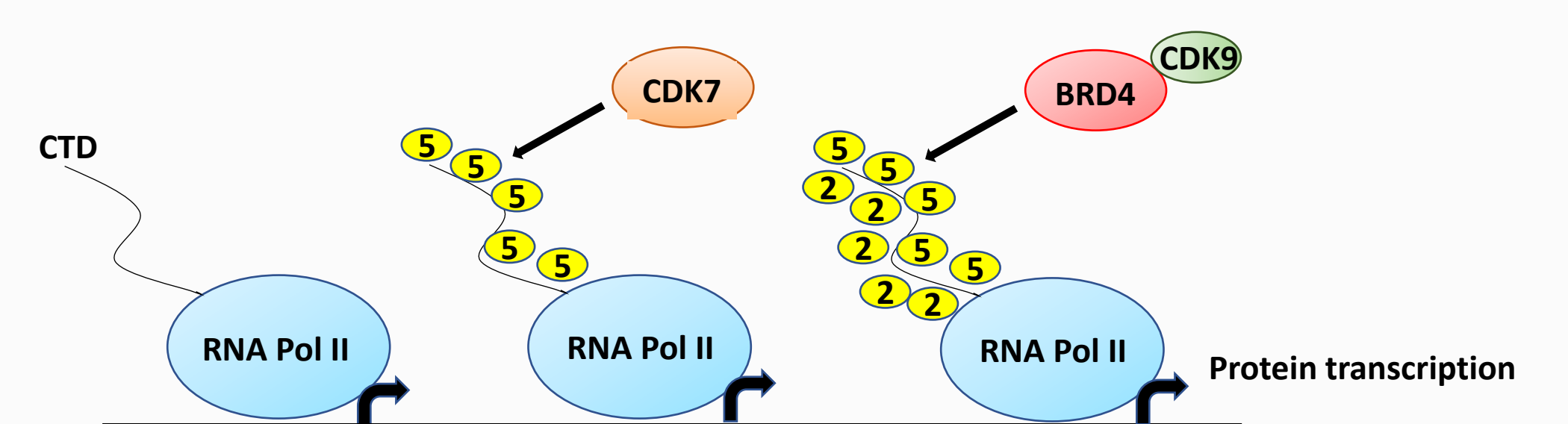


- ARV-771 degradation of BRD4
- LNCaP cells treated for 20 hours
- 4,000 cells/datapoint



Degradation of BRD4 and reduction of CDK7/9 (Pol II CTD kinases)

Subsequent regulation of RNA Pol II phosphorylation



2. Devaiah and Singer. Cross-talk among RNA Polymerase II Kinases modulates C-terminal domain phosphorylation. JBC, 2012

4. Conclusions

- *SureFire Ultra* assays can be used to demonstrate degradation of specific proteins using PROTAC compounds in a cellular system.
- The simple workflow and the broad range of well characterized assays allows for the parallel testing of downstream targets.
- This study highlights the effectiveness of *SureFire Ultra* platform for the detection and characterisation of PROTAC targets and demonstrates the utility of functional downstream pathway analysis.