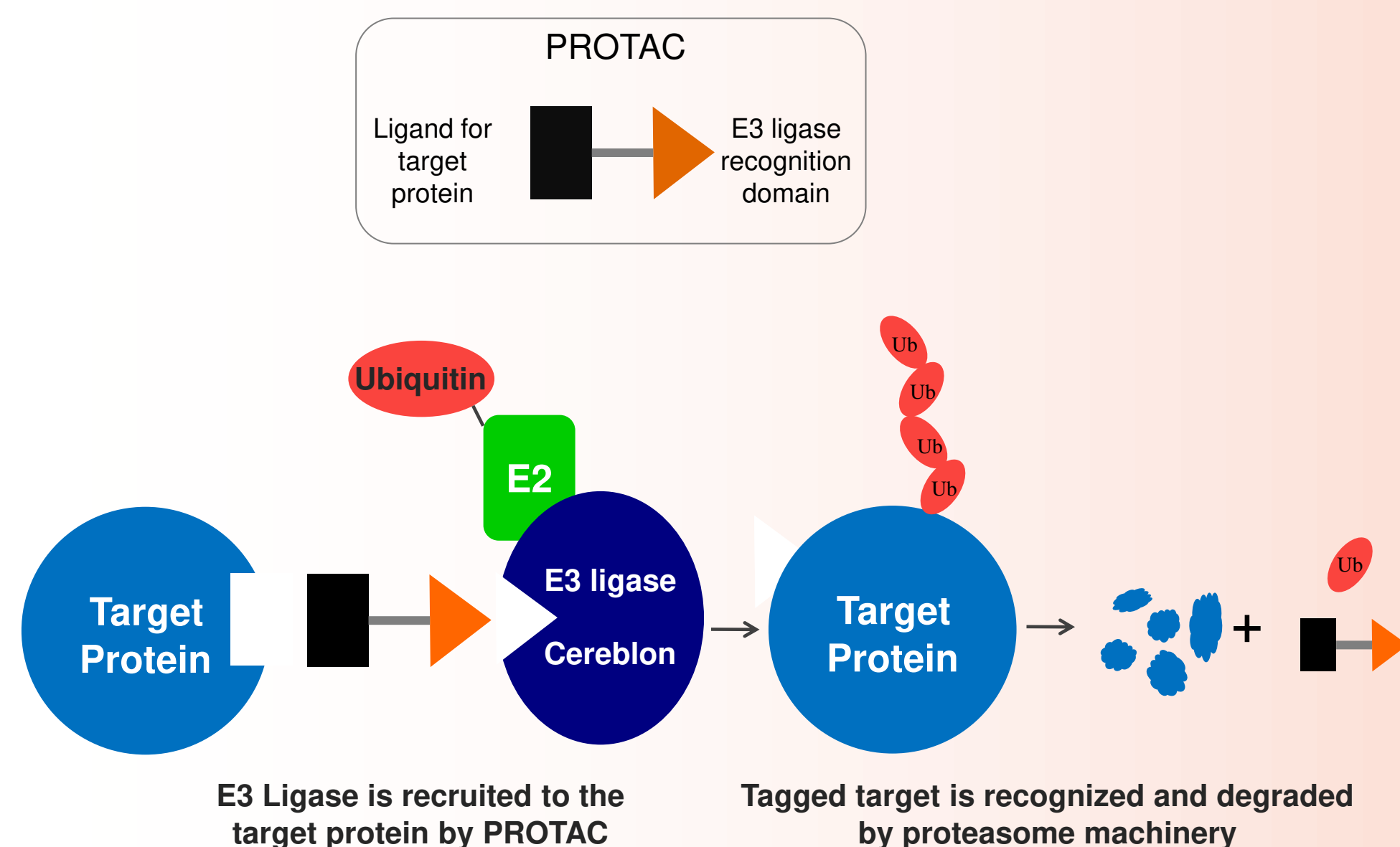


## Abstract:

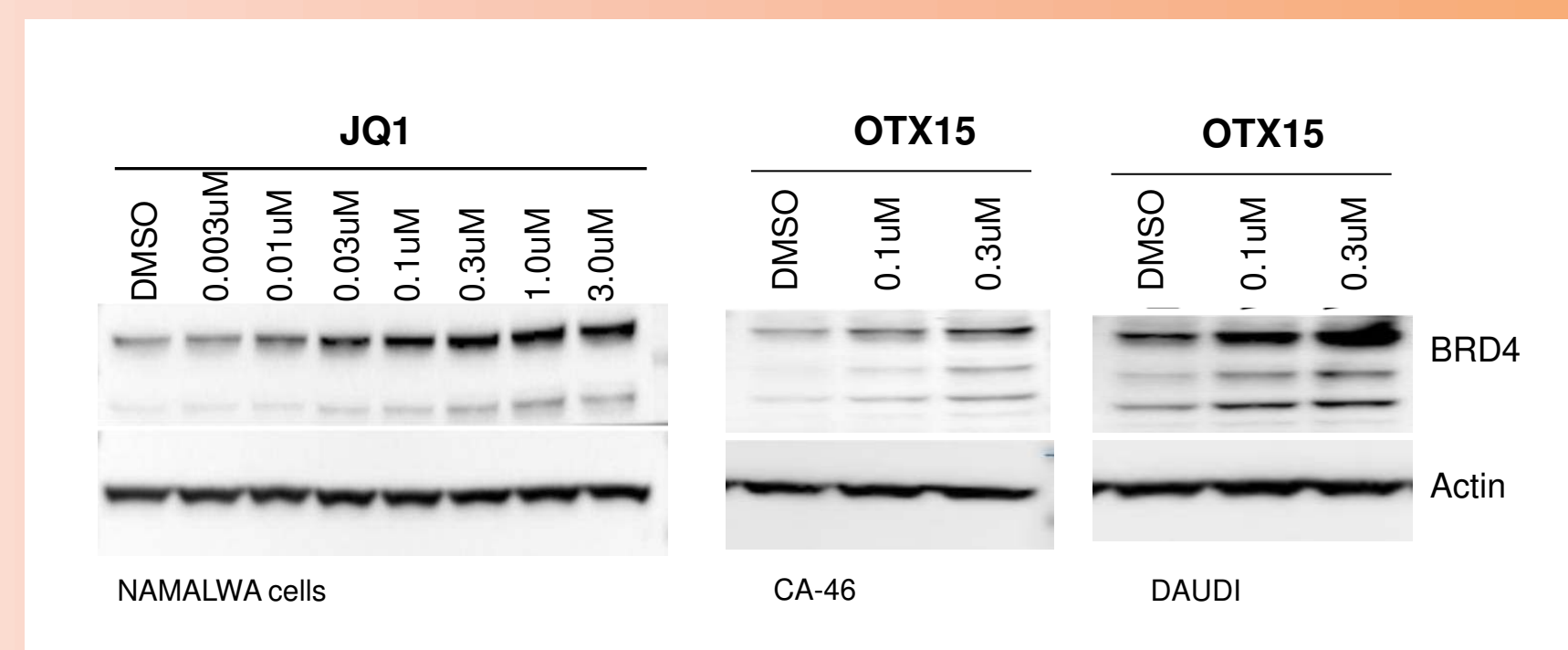
- BRD4, a member of the bromodomain and extraterminal domain (BET) family, has emerged as an attractive oncology target.
- BET bromodomain inhibitors have shown promising effects in certain preclinical settings, particularly in c-MYC driven hematological malignancies.
- Interestingly, we found that BRD4 inhibitors lead to a rapid and robust accumulation of BRD4, which may partially account for their moderate suppression of c-MYC and inhibition of cell proliferation.
- We designed a Proteolysis Targeting Chimera (PROTAC) compound, containing a BRD4 binding moiety and an E3 ubiquitin ligase cereblon ligand.
- BRD4 PROTAC leads to fast and efficient degradation of BRD4.
- BRD4 PROTAC provides more pronounced and longer-lasting effect in suppressing c-MYC levels than small molecule BRD4 inhibitors.
- BRD4 PROTAC is more effective in inhibiting Burkitt Lymphoma cell proliferation and inducing apoptosis compared to BRD4 inhibitors.
- A cereblon-based PROTAC provides a better and more efficient strategy in targeting BRD4 than traditional small molecule inhibitors.

## Degrader Technology

### PROTAC: PROteolysis Targeting Chimera

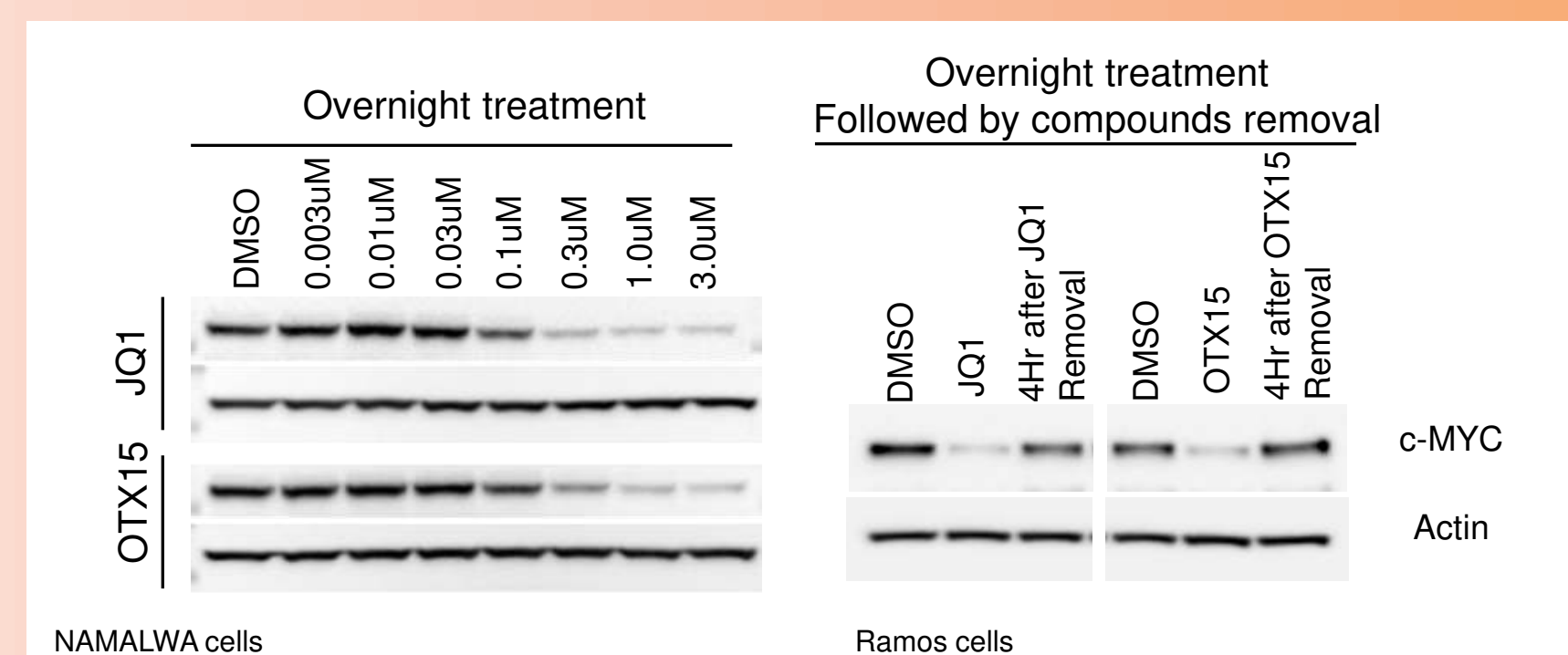


## BRD4 inhibitors lead to fast and robust BRD4 accumulation

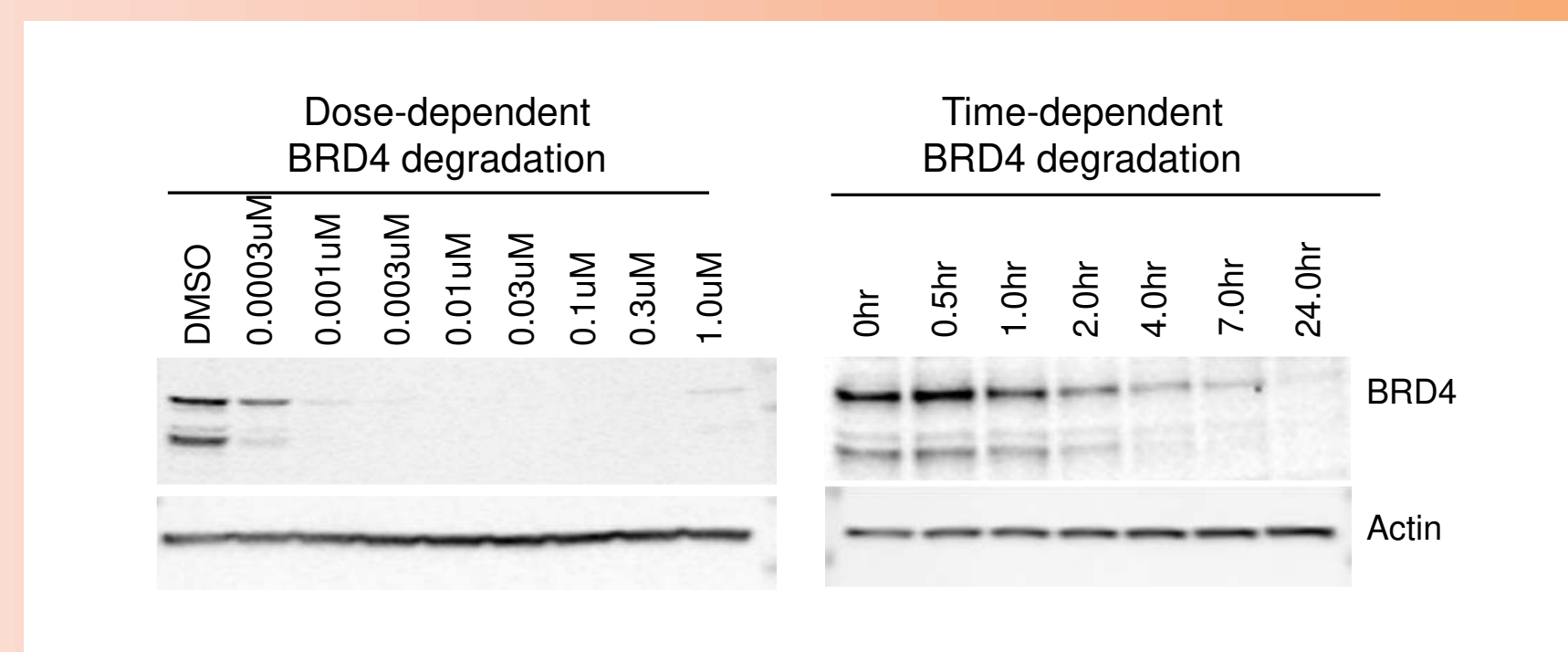


Various BL cell lines treated with small molecule inhibitors overnight

## BRD4 inhibitors lead to inefficient and transient suppression on c-MYC

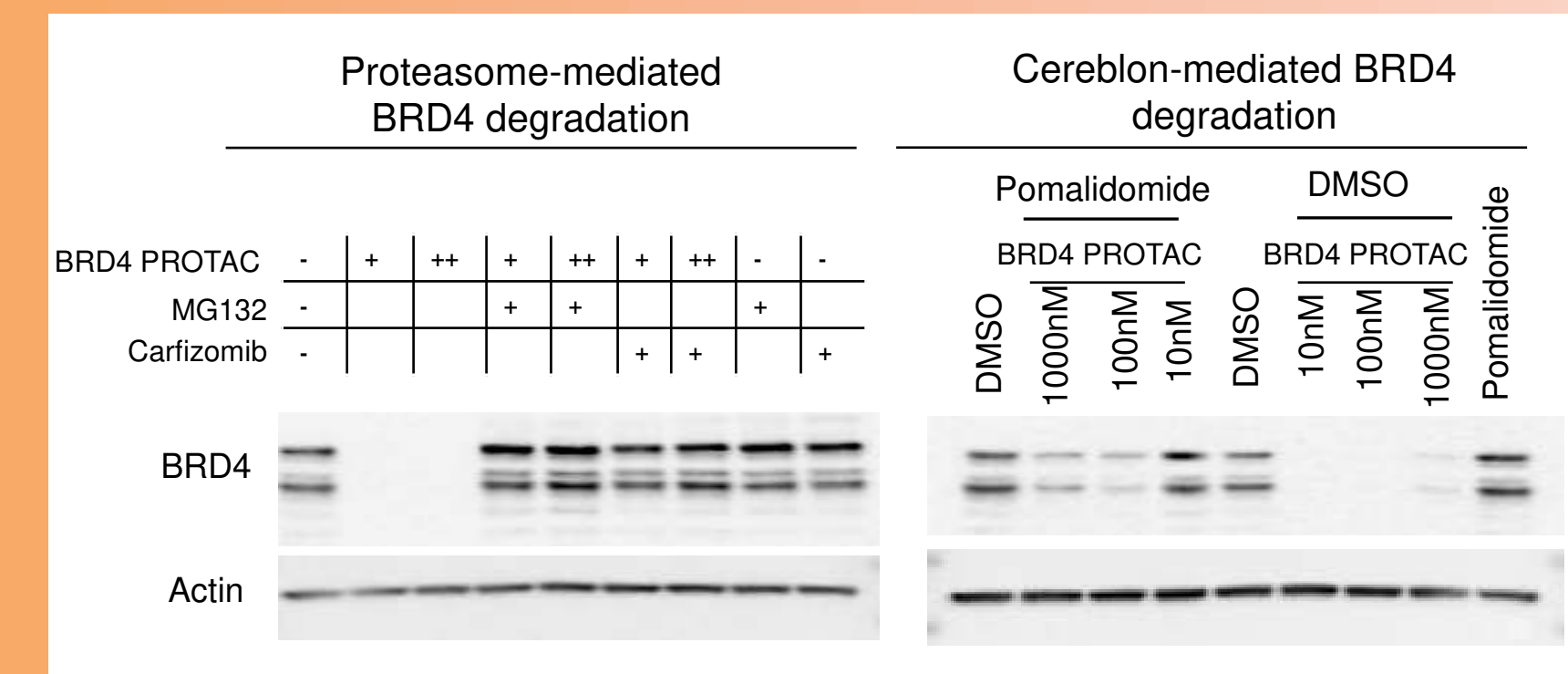


## BRD4 PROTAC causes rapid and potent BRD4 degradation



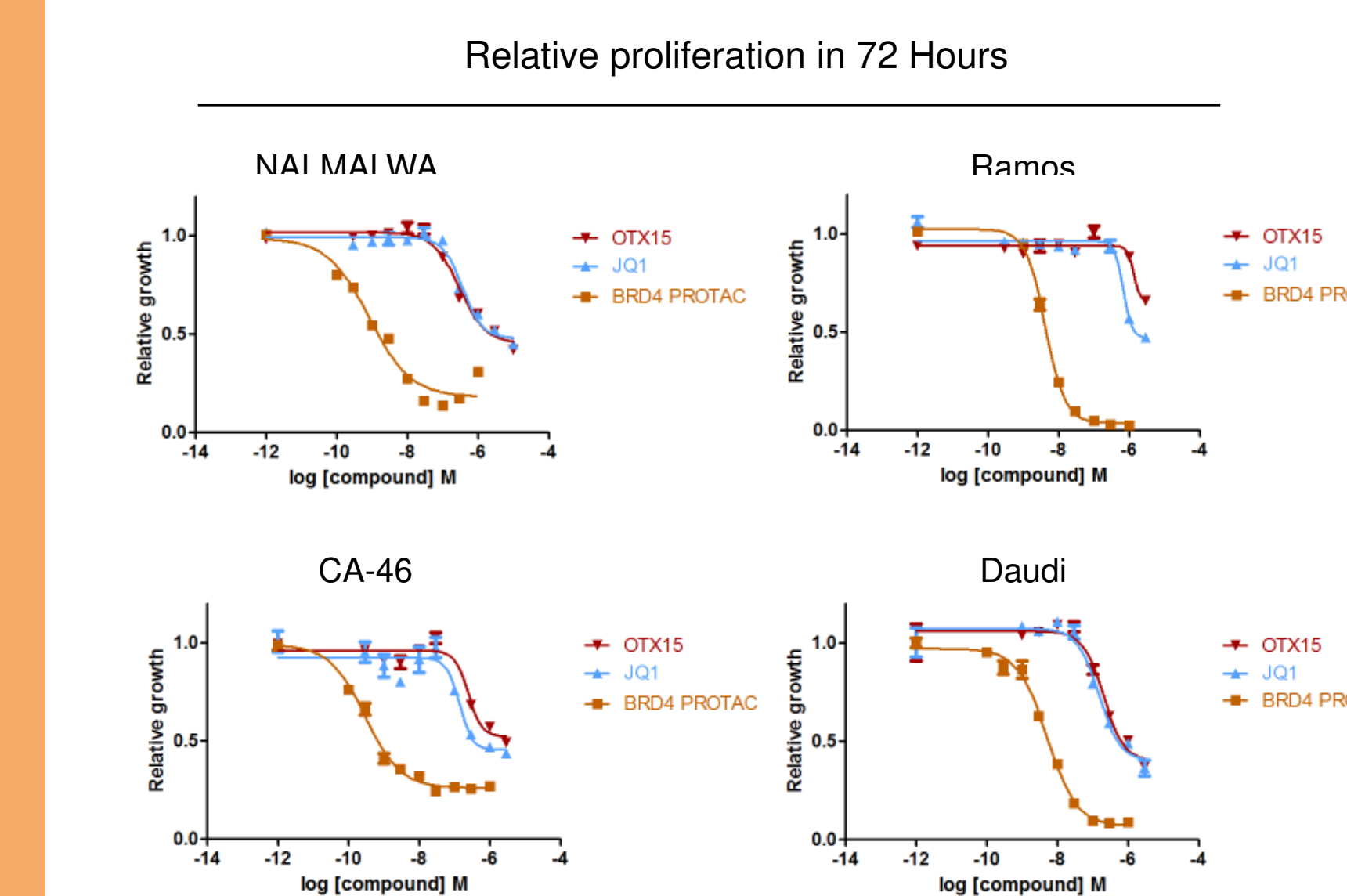
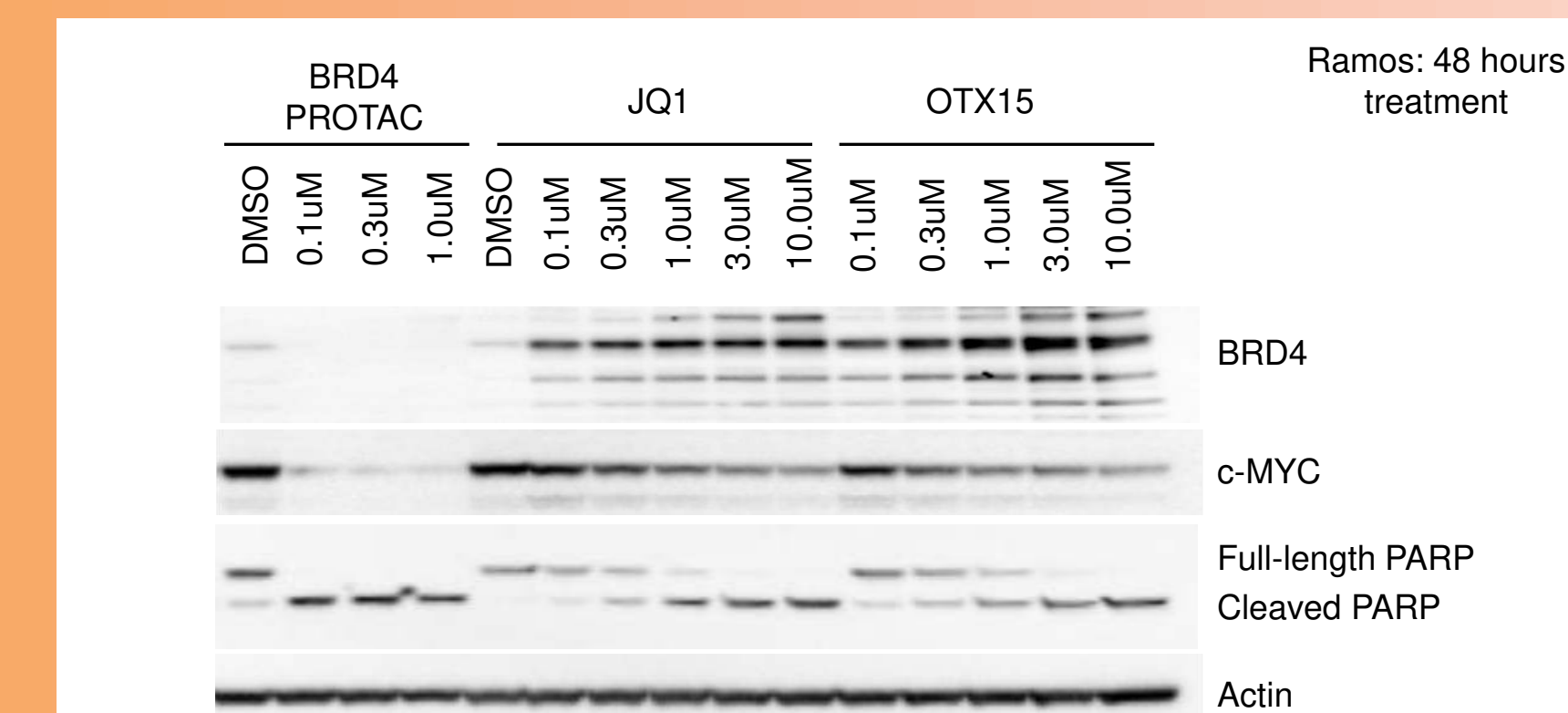
NAMALWA cells

## Confirmation of proteasome mediated, cereblon-based mechanism in driving BRD4 degradation by BRD4 PROTAC

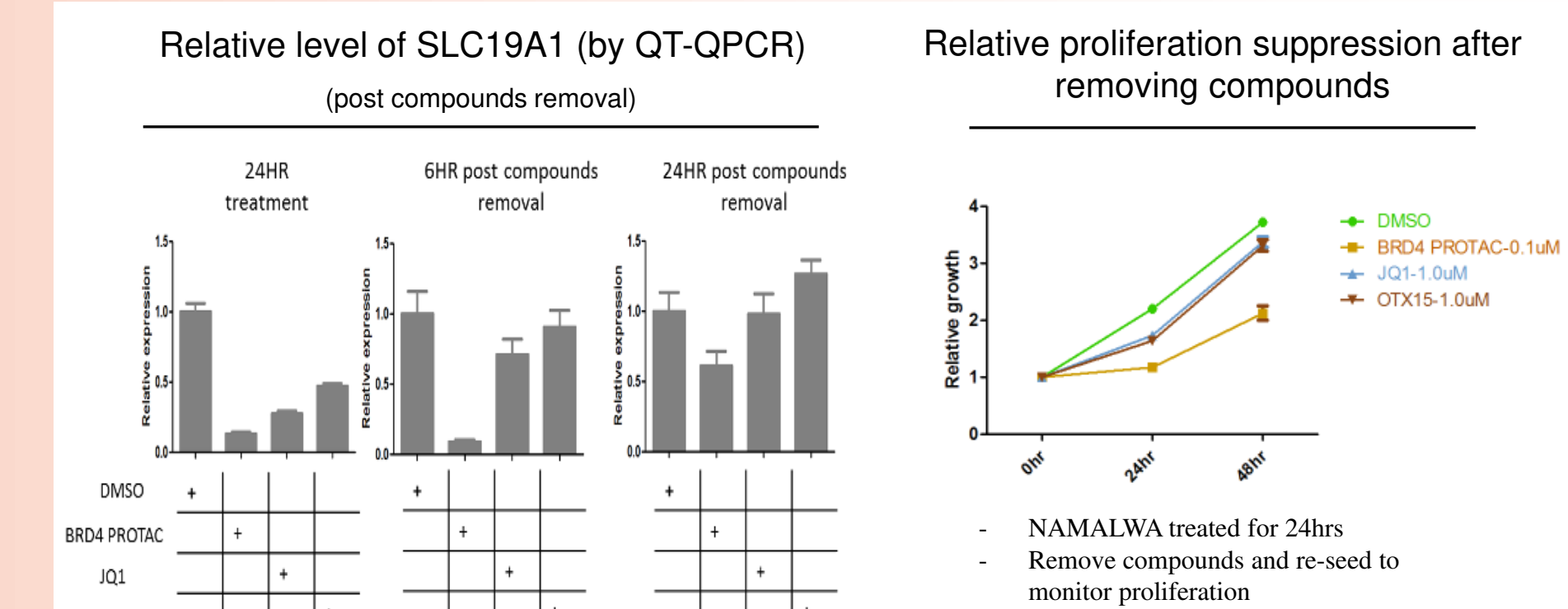
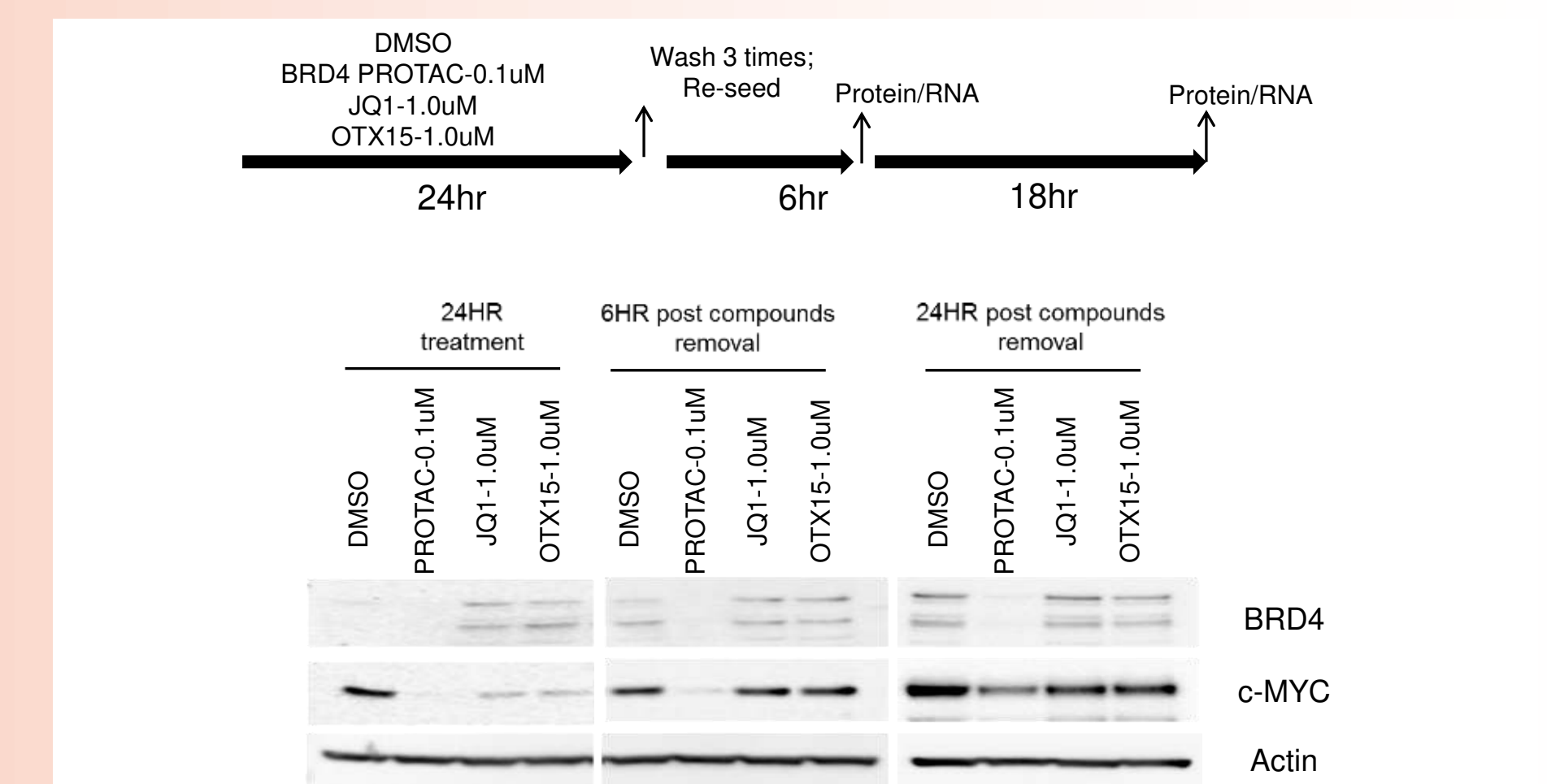


NAMALWA cells, + (10nM), ++ (100nM), MG132 (5uM), Carfizomib (5uM), Pomalidomide (10uM)

## BRD4 PROTAC leads to more significant c-MYC suppression, proliferation inhibition and apoptosis induction than small molecule inhibitors



## BRD4 PROTAC provides sustained effects on BRD4 degradation, c-MYC and its downstream signal suppression and proliferation inhibition



## Summary

- We successfully developed a BRD4 PROTAC that degrades BRD4 potently and rapidly.
- BRD4 PROTAC causes more significant suppression of c-MYC, more dramatic effect on proliferation inhibition and apoptosis induction compared to small molecule inhibitors.
- BRD4 PROTAC has long-lasting effect on repressing downstream signaling and proliferation.
- Targeted degradation of BRD4 by PROTAC provides a better and more effective strategy in targeting BRD4 than traditional small molecule inhibitors.
- Our study also demonstrates that PROTAC platform, by actively recruiting E3 ligase to target pathological protein for degradation, is a promising strategy in targeting the "undruggable" proteins by traditional approaches.