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Mining the CRBN Target Space Redefines Rules for Molecular Glue- induced Neosubstrate Recognition

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Authors

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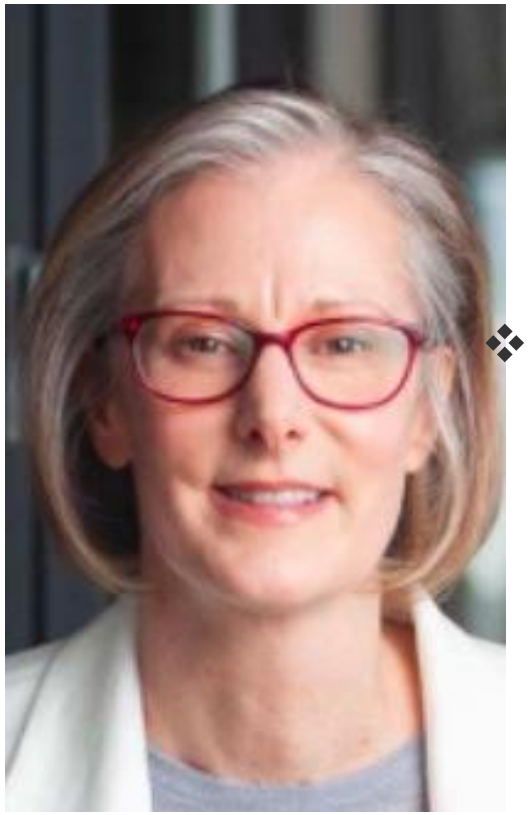
Monte Rosa Therapeutics



Sharon Townson

CSO

- Application of structural /computational biology in molecule therapeutics

John Castle

Chief Data and Information Officer

- Applications of genomics, bioinformatics and immunomics for the discovery of new targets

Background

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- **Targeting PPIs** represents a powerful therapeutic strategy.

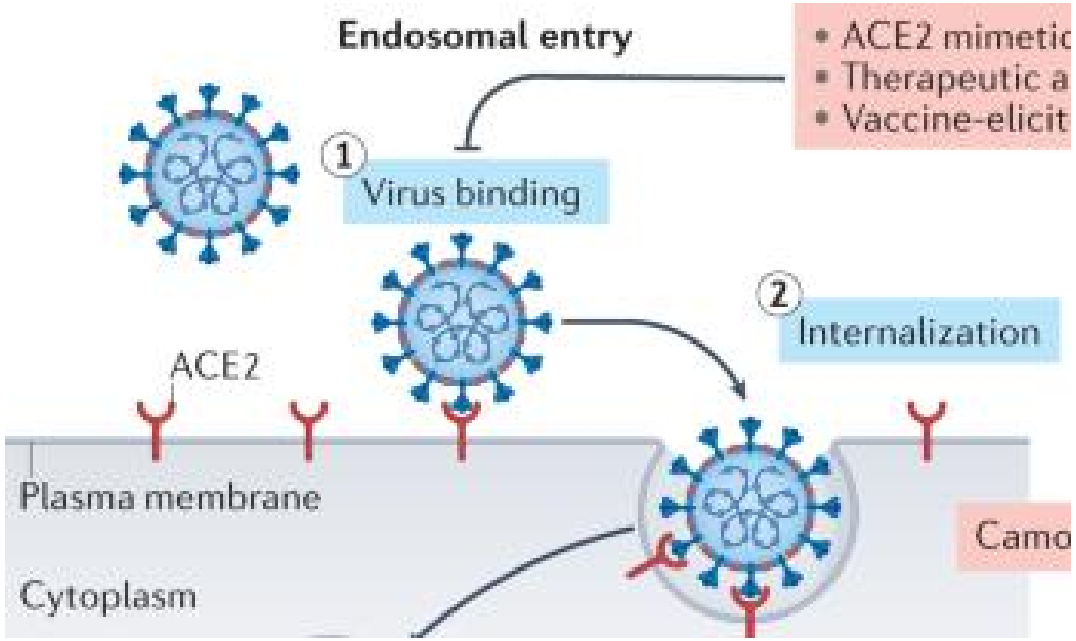

Fig1. PPI in SARS-CoV-2 entry pathway

- Molecular glues can either **stabilize endogenous PPIs** or induce **non-native ones**

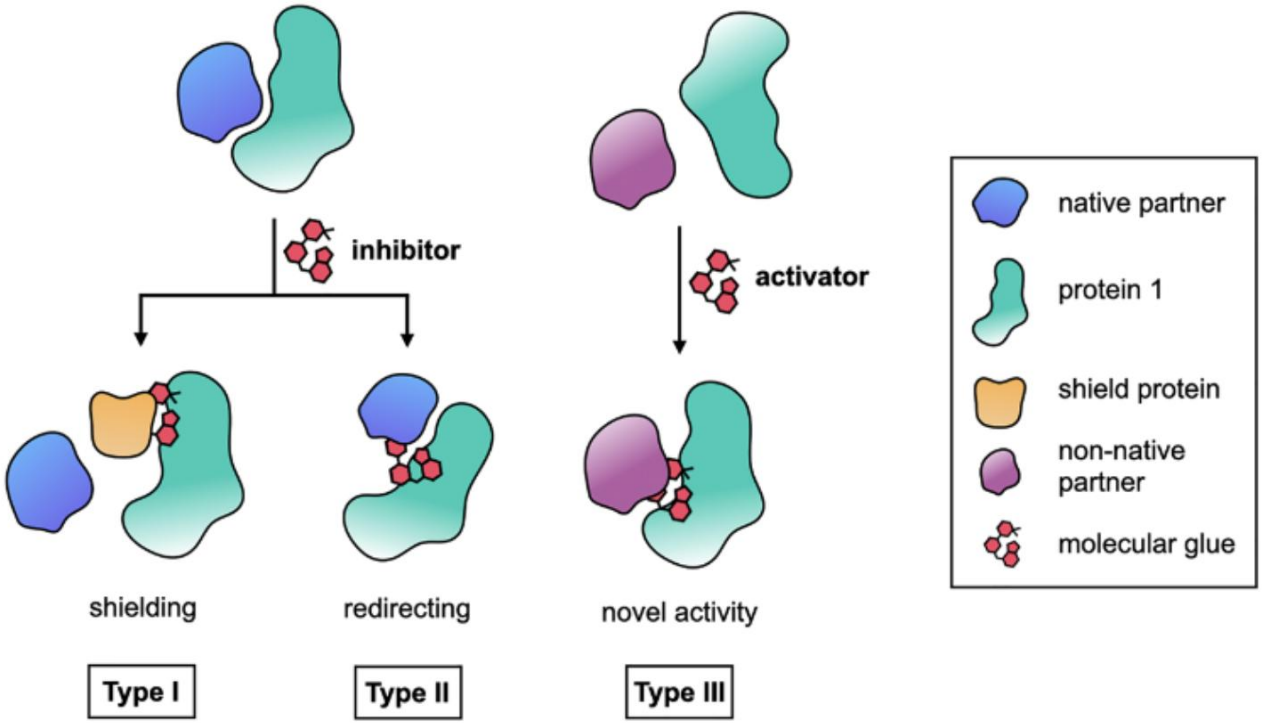

Fig2. Molecular glue classification system based on its mechanism of action

Jeffrey A. Dewey,Clémence Delalande .et al. Molecular Glue Discovery: Current and Future Approaches

Background

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- *"Molecular glue degraders(MGD) can induce the proximity between ubiquitin ligases and target protein to induce the degradation of target protein"*

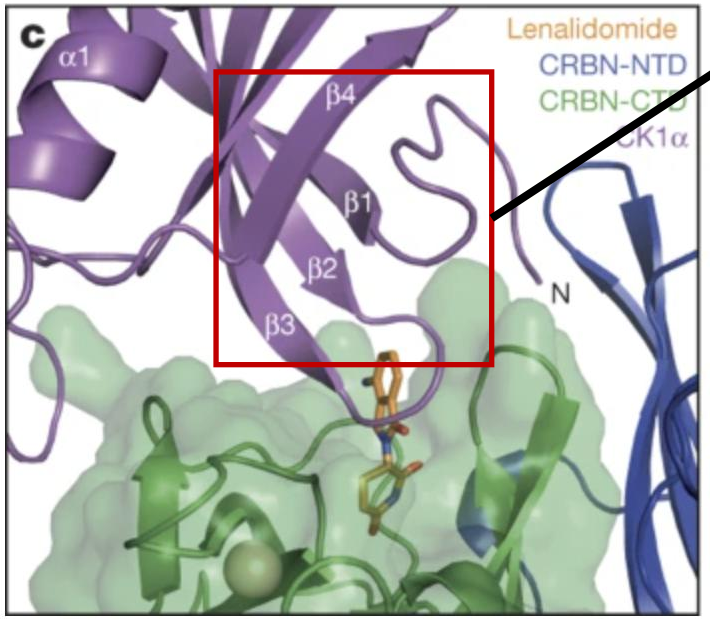

Fig1. A β-hairpin loop of CK1α binds CRBN on top of its lenalidomide-binding pocket

- These **β-hairpin G-loops** on the target proteins define surface features to make it can stabilize **protein-protein interactions with CRBN**

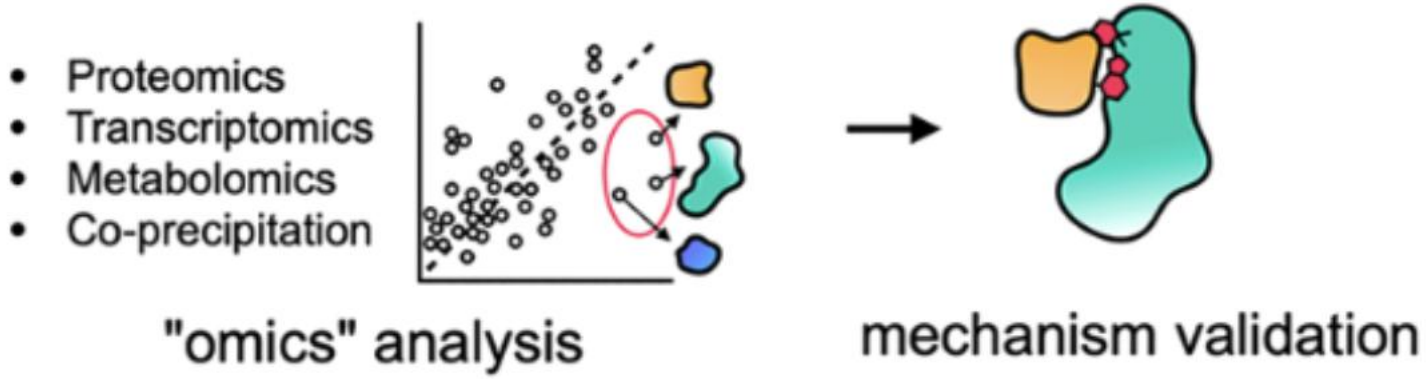

Fig2. Using computational method to make prediction for the target of Molecular Glues

- Knowing this motif, we can predict more potential targets of this CRBN/MGD to **broaden the target space of CRBN**

G. Petzold, E. S. Fischer, N. H. Thoma, Structural basis of lenalidomide-induced CK1α degradation by the CRL4^{CRBN} ubiquitin ligase

The Criteria and Workflow for identifying the Motif

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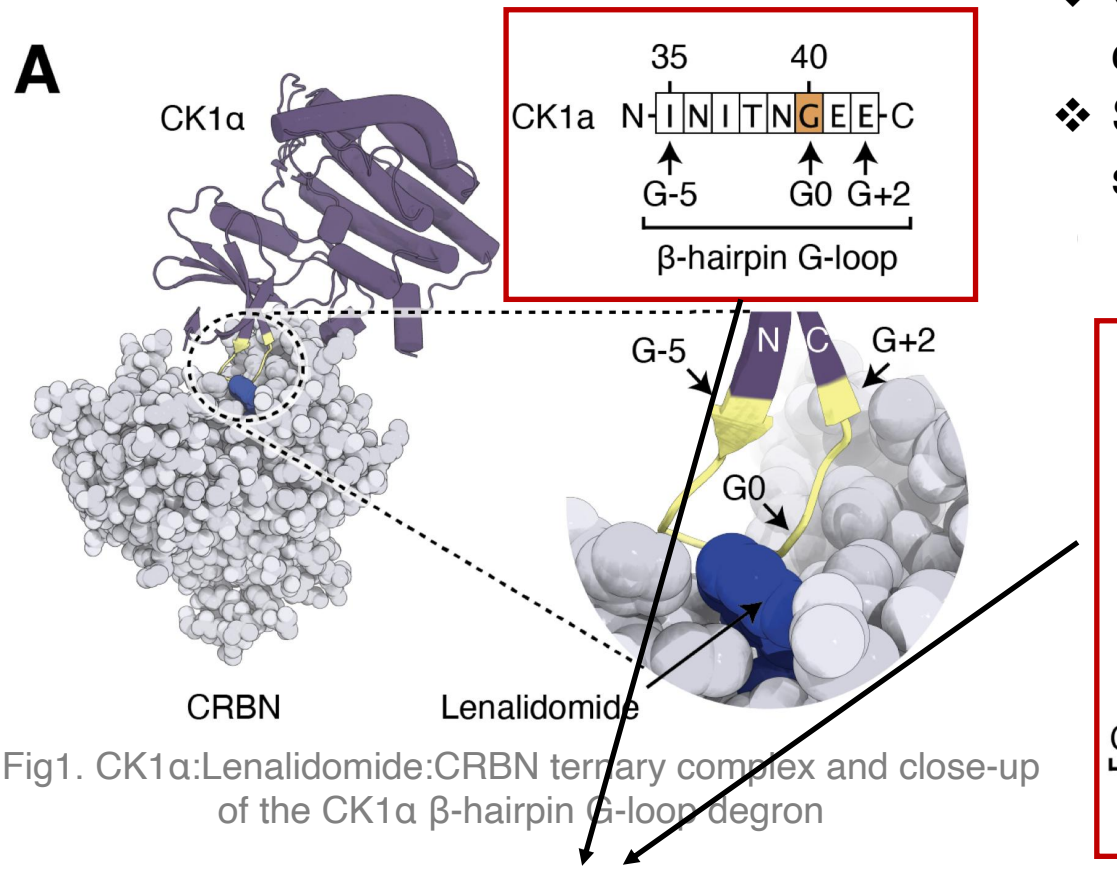

Fig1. CK1α:Lenalidomide:CRBN ternary complex and close-up of the CK1α β-hairpin G-loop degron

- The structure of β-hairpin G-loops is defined by an **8-residue G-loop**

- Choose **CK1α β-hairpin G-loops around glycine 40** as query
- Selected **from CRBN:lenlidomide:CK1α ternary complex** structure

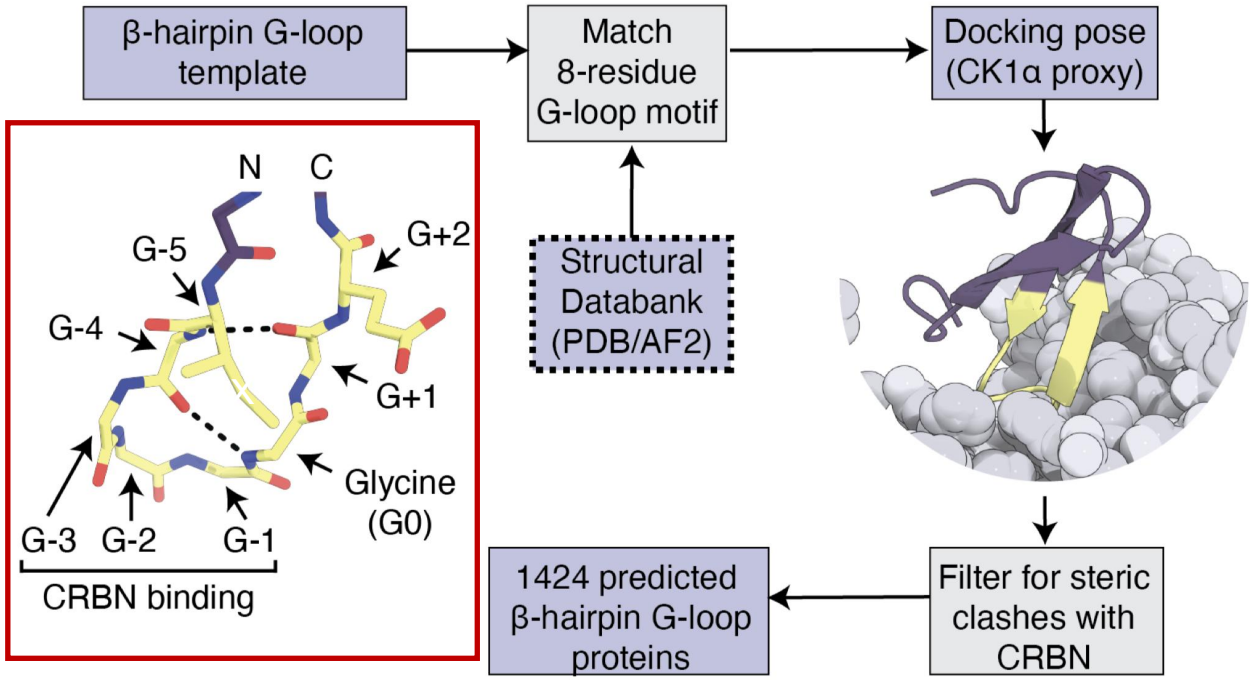

Fig2. Definition of the β-hairpin G-loop motif and schematic of the computational workflow.

Predicted target distributed in different protein classes and structural domains

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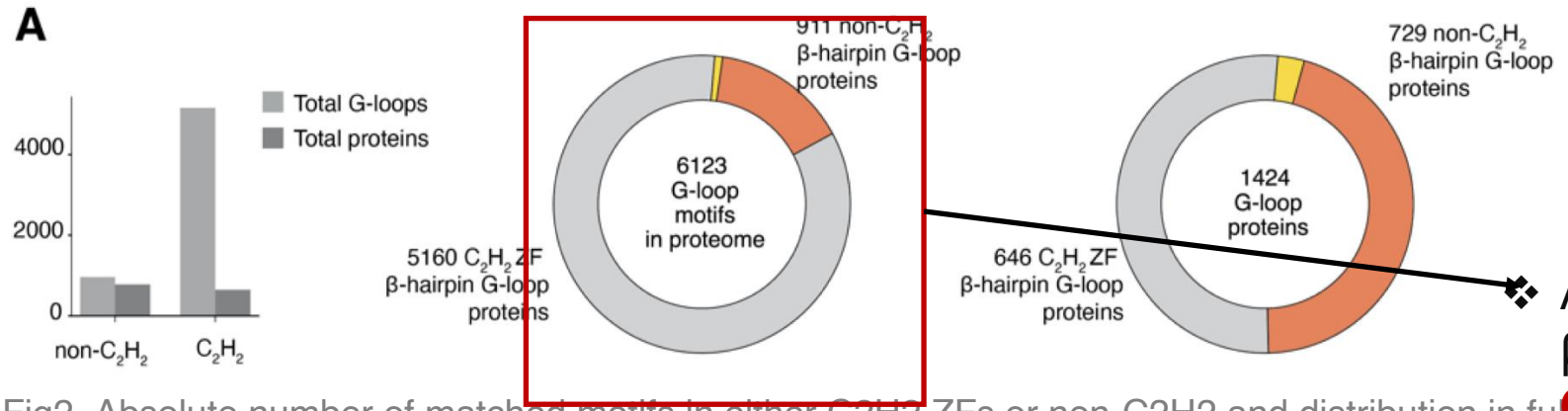

Fig2. Absolute number of matched motifs in either C2H2 ZFs or non-C2H2,and distribution in full length proteins. Mined motifs matching the β- hairpin G-loop query in the human

"Recovered all previously reported β-hairpin G-loop targets"

- A significant majority (84%) of the identified β-hairpin G-loop motifs are **found within C2H2ZF proteins**

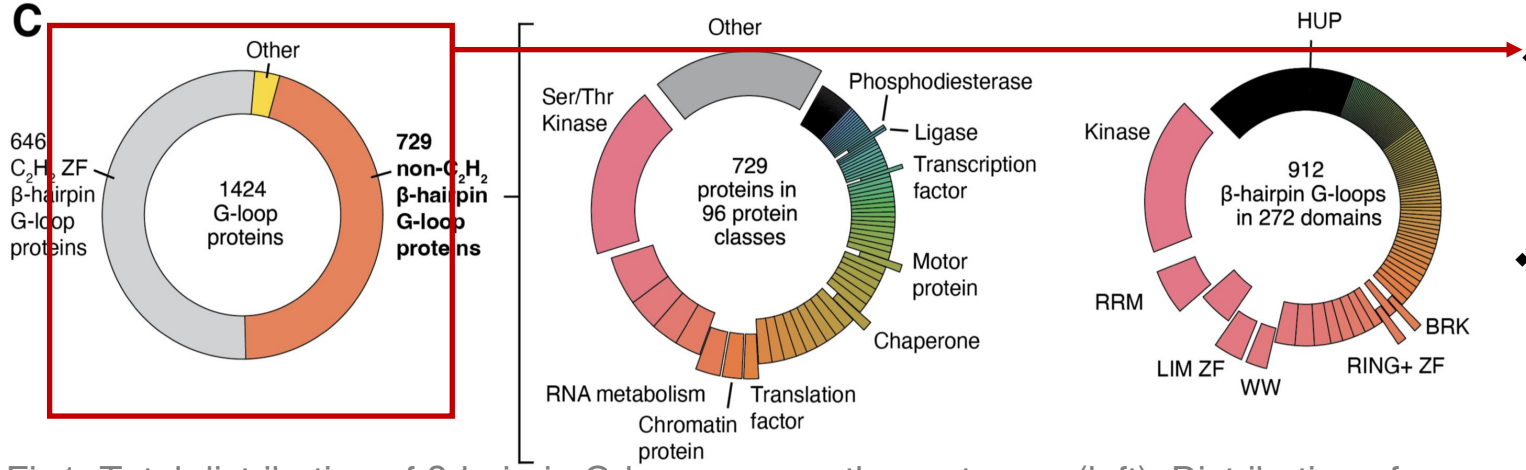

Fig1. Total distribution of β-hairpin G-loops across the proteome (left). Distribution of non-C2H2 β- hairpin G-loops across protein classes (center) and structural domains (right)

- 6,123 Motifs Distributing in **1,424 human Proteins** were identified
- Identified proteins belongs to **96 classes and 912 structural domains**

Validation of predicted targets

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"Having established a computational map of the CRBN-compatible β-hairpin G-loop proteome, we next sought to ***validate compound-dependent CRBN recruitment of predicted targets*** *using proximity-ligation experiments"*

Use 3 different known molecular glue to validate…

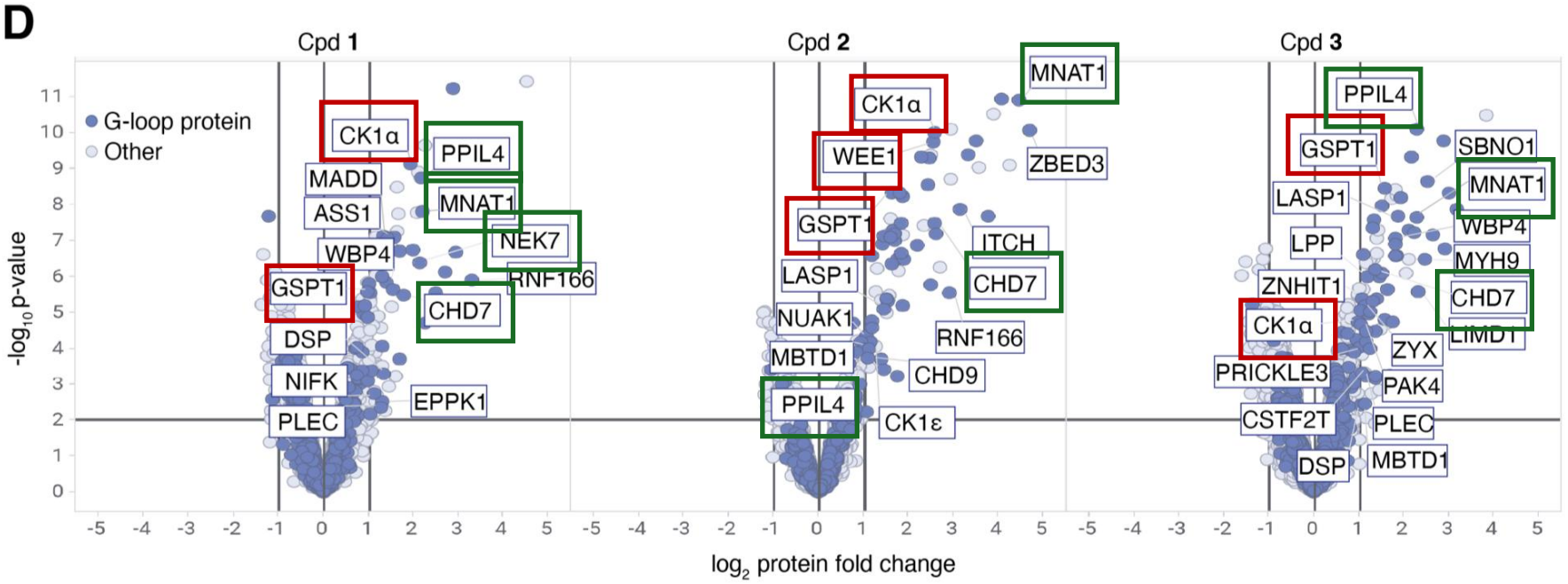

Fig1. Volcano plots of TurboID experiments in CAL51 cells

- Experiment validation showed an enrichment of **some known targets**
- Experiment also identified **novel β-hairpin G-loop proteins**

NanoBRET and TR-FRET further validate among the Targets

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- *Although the proximity-ligation experiment validated a broad range of targets, for avoiding the indirect biotinylation of targets, we need to validate the direct target engagement*

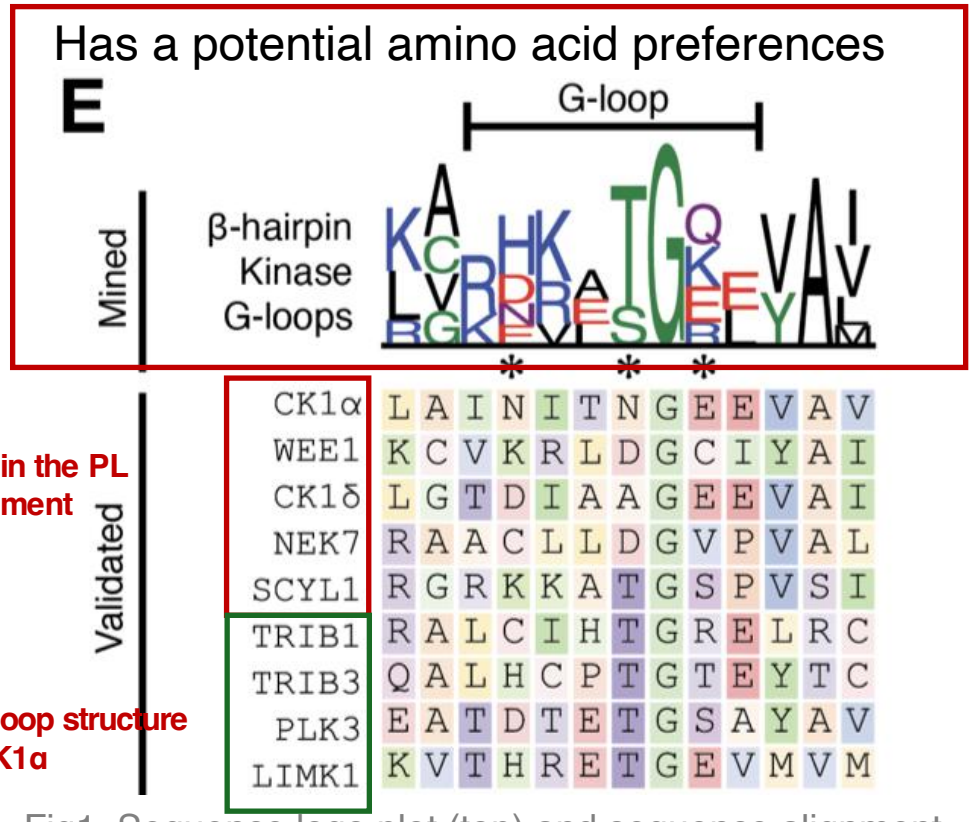

Fig1. Sequence logo plot (top) and sequence alignment of β-hairpin G-loops in kinases(bottom)

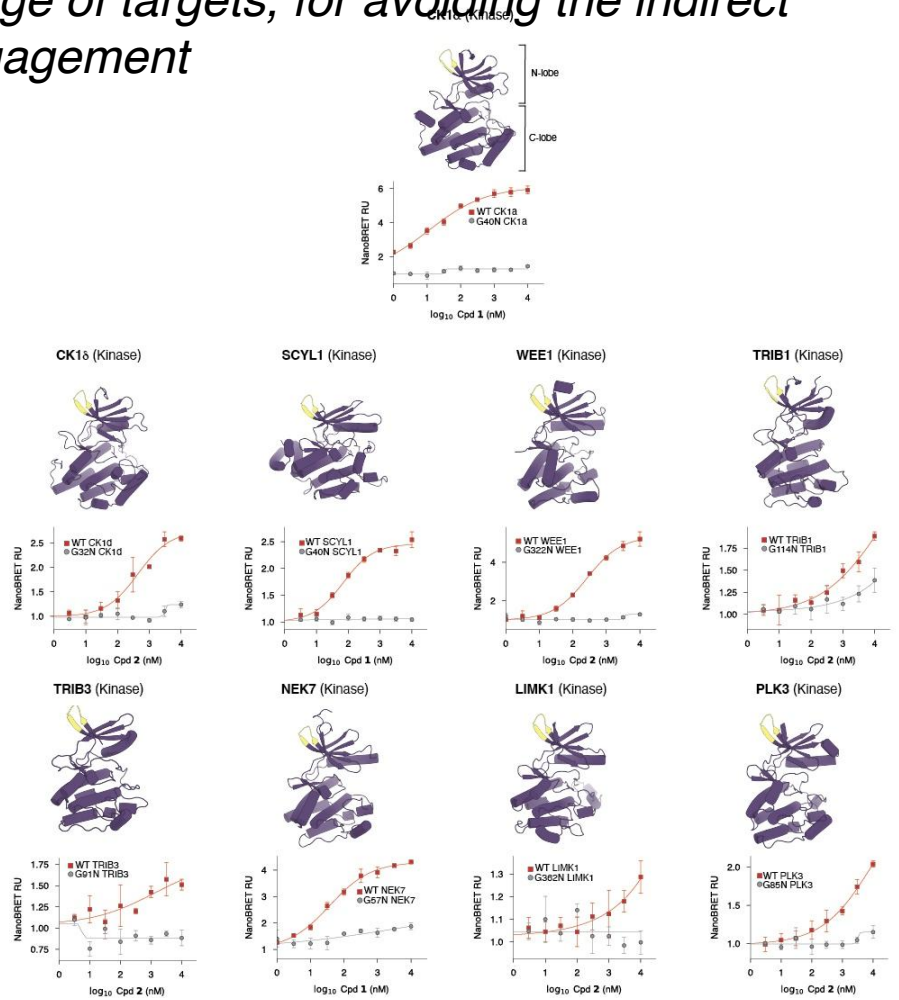

Fig2. NanoBRET validation of β-hairpin G-loops

There is a dependence of G-loop in the CRBN-engagement

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"G-loop glycine to asparagine mutation that is predicted to prevent binding of other G-loop targets"

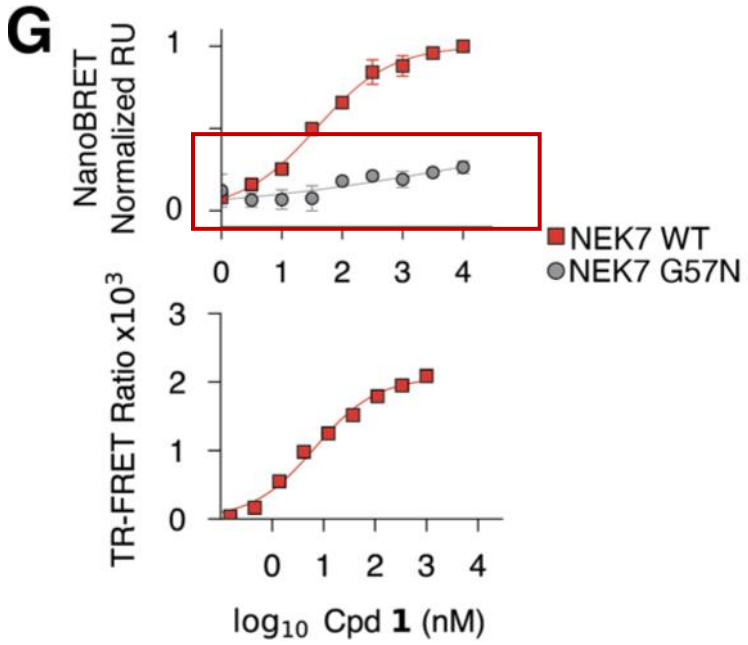

Fig1. Ternary complex formation between CRBN and wild-type or mutant NEK7

- Glycine to asparagine mutations within the identified targets ablated the NanoBRET signal

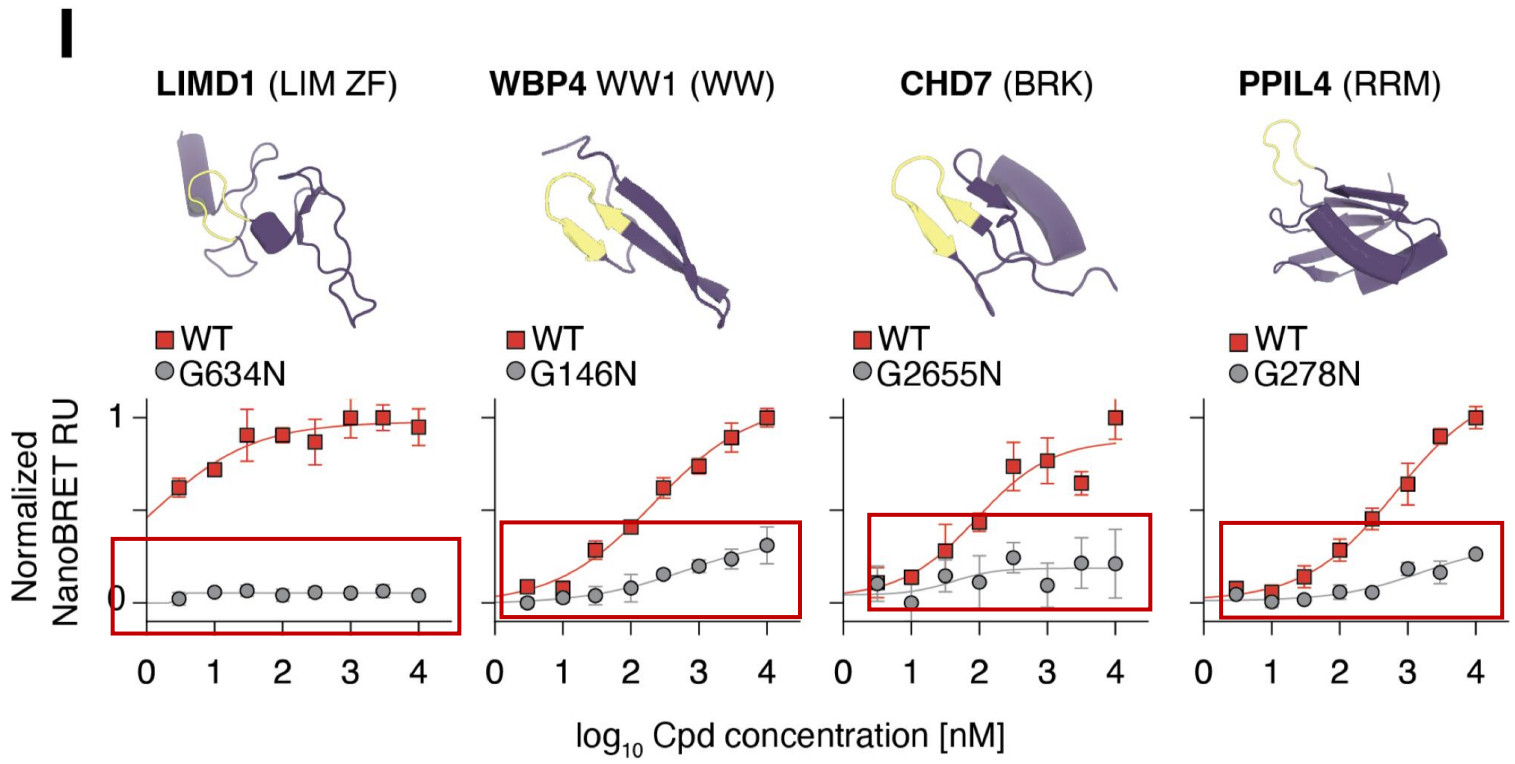

Fig2. Ternary complex formation between CRBN and β-hairpin G- loop proteins

β-hairpin G-loop is a Generalizable CRBN binding Motif

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- Select a set of proteins from other classes for further validation

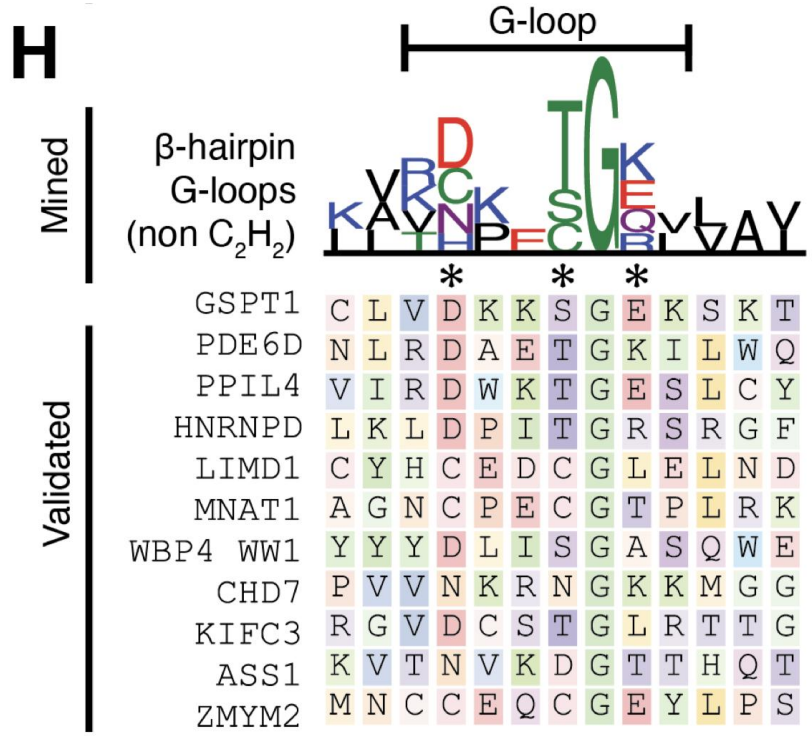

Fig1. Sequence logo plot (top) and sequence alignment of β-hairpin G-loops in selected proteins(bottom)

- **G-loop-dependent interactions with CRBN**

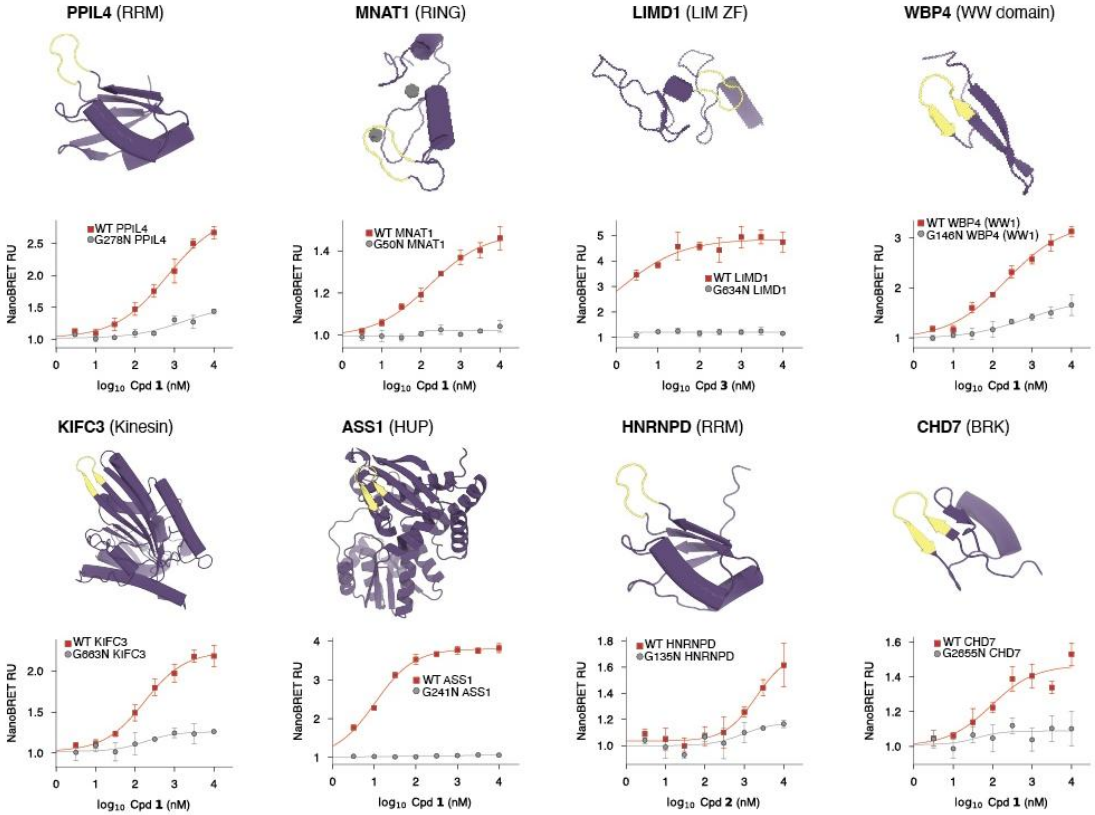

Fig2. NanoBRET validation of β-hairpin G-loops

Use a less stringent definition of G-loop to conduct mining

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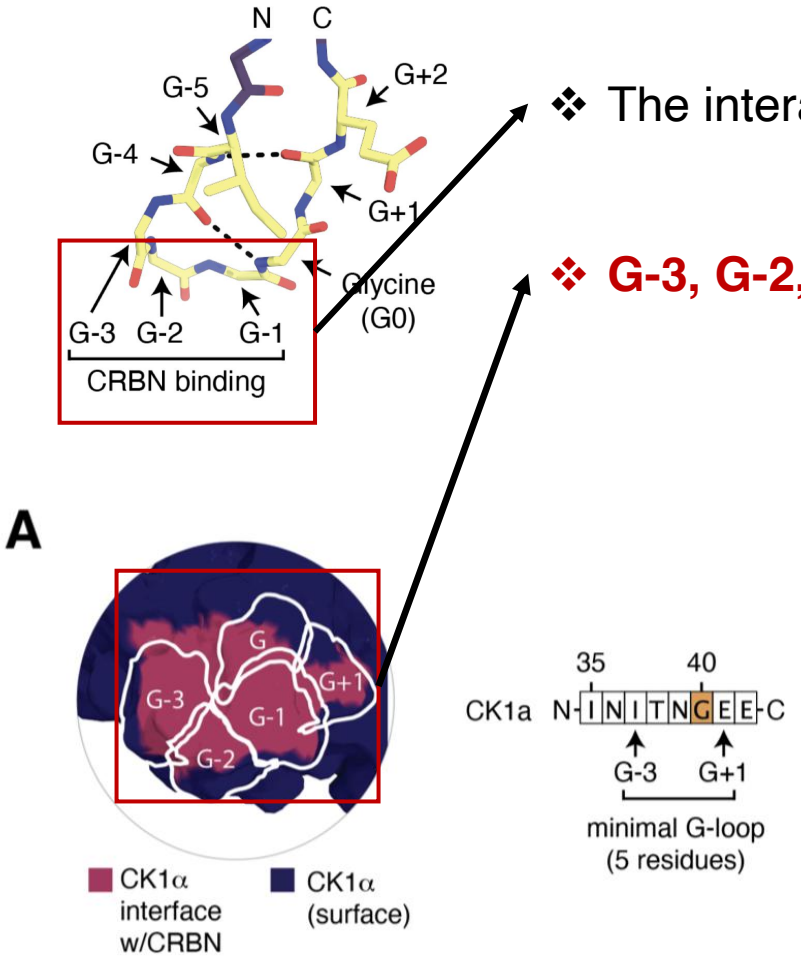

- The interaction between G-loop and CRBN largely affected **by G-3, G-2, G-1**
- **G-3, G-2, G-1, G, G+1** contributed the largest part of the interface

Fig1. CK1α β-hairpin G-loop surface with the region burried upon CRBN:Lenalidomide binding colored in red

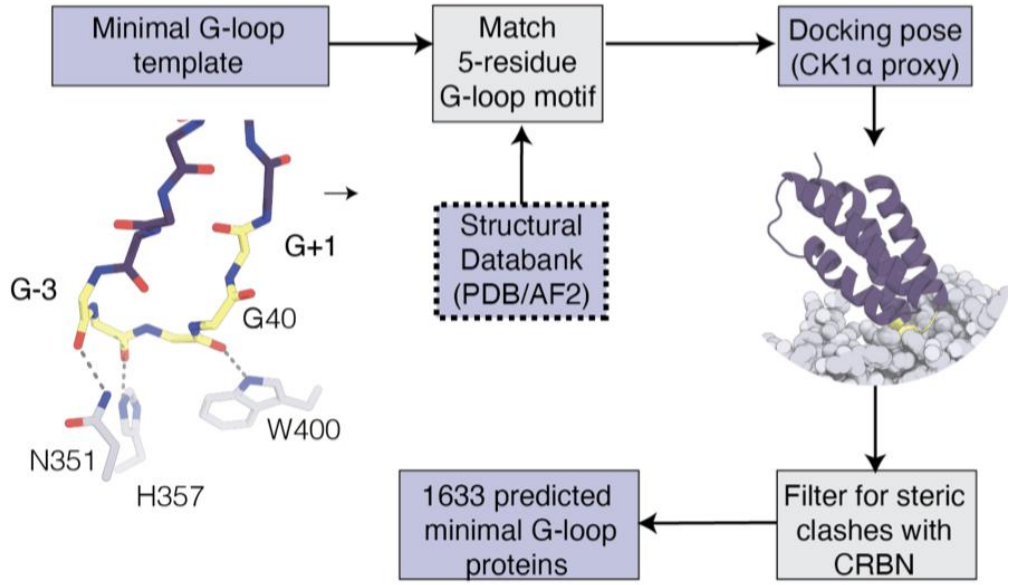

Fig2. Definition of the minimal G-loop and the corresponding computational workflow

A new class of Targets found with stringent definition

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- Identified more proteins, recovering **all validated targets**
- This approach reveal 217 helical G-loop motifs in **184 proteins**

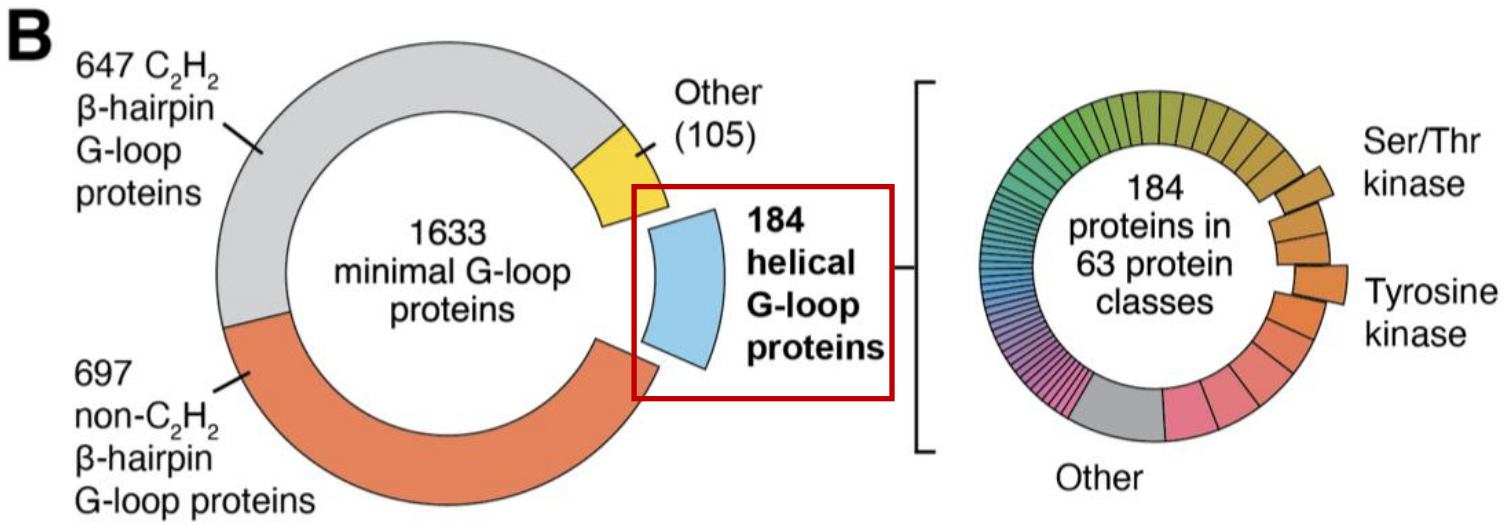

Fig1. Distribution of the minimal G-loop target space

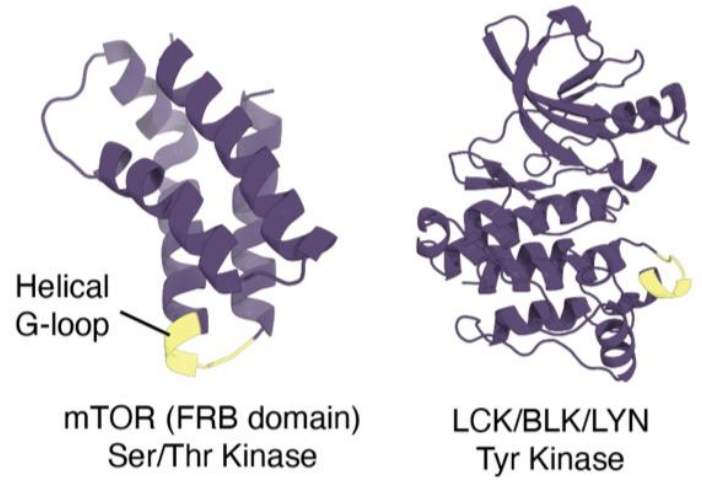

Fig2. Models of two domains predicted to carry a helical G-loop motifs.

- There are two classical and well-established targets contain such helical motifs

The helical G-loop motif can form a complex with target through CULT domain of CRBN

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- Nano-BRET validation confirmed the complex formation between **mTOR-FRB domain, CRBN and Cpd1**
- And this formation is also has a **dependence on residue Glycine**

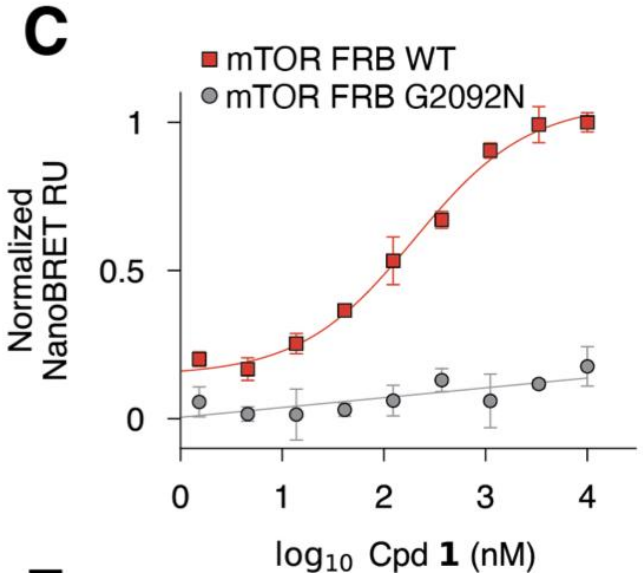
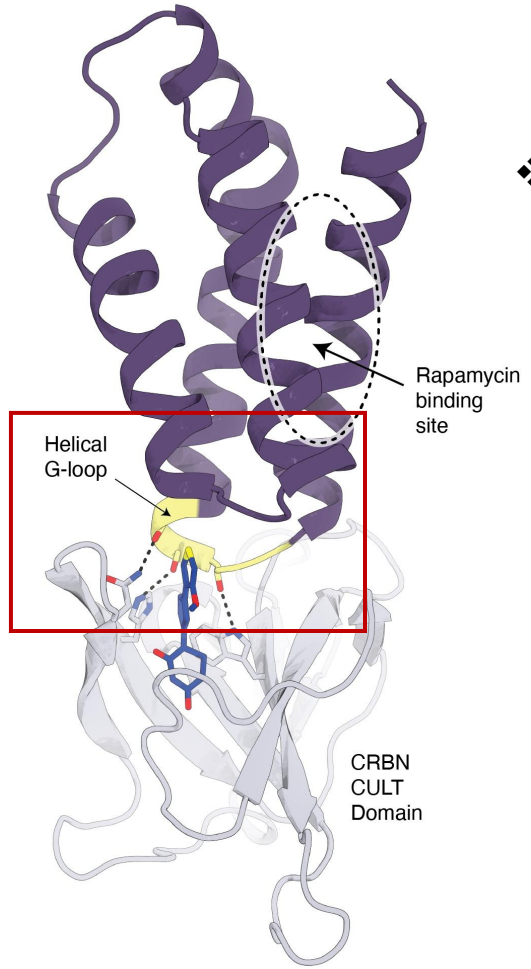


Fig1. Distribution of the minimal G-loop target space



- The helical G-loop engage CRBN through its **C-terminal CULT domain**

Fig2. Ternary complex structure of CRBN, mTOR-FRB and Cpd1

mTOR-FRB can form a more Compact interface than CK1 α

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"The backbone geometry of the G-4 residue differs from β-hairpin G-loops due to ***changes in torsion angles*** *"*

Expose of the side chain

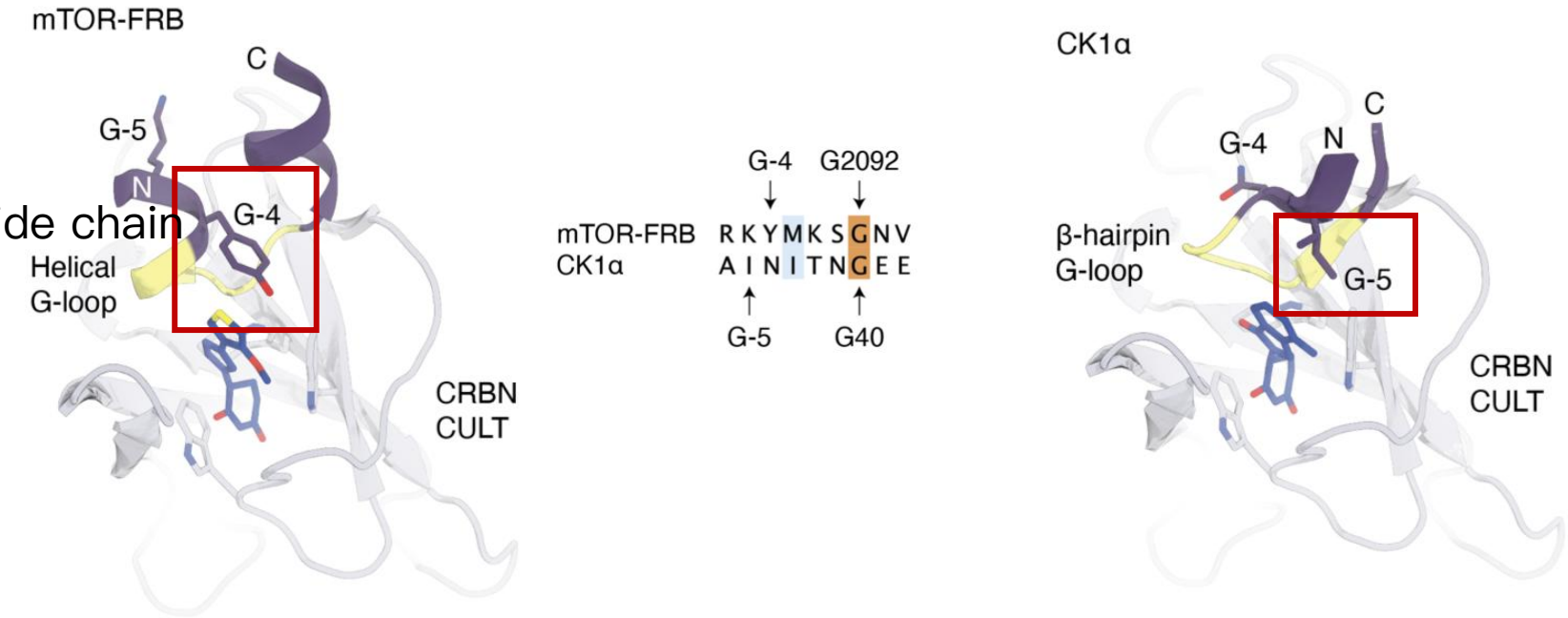

Fig1. Comparison of the mTOR-FRB helical G-loop (left) and the CK1α β-hairpin G-loop (right)

Validation of Generalizability of helical G-loop

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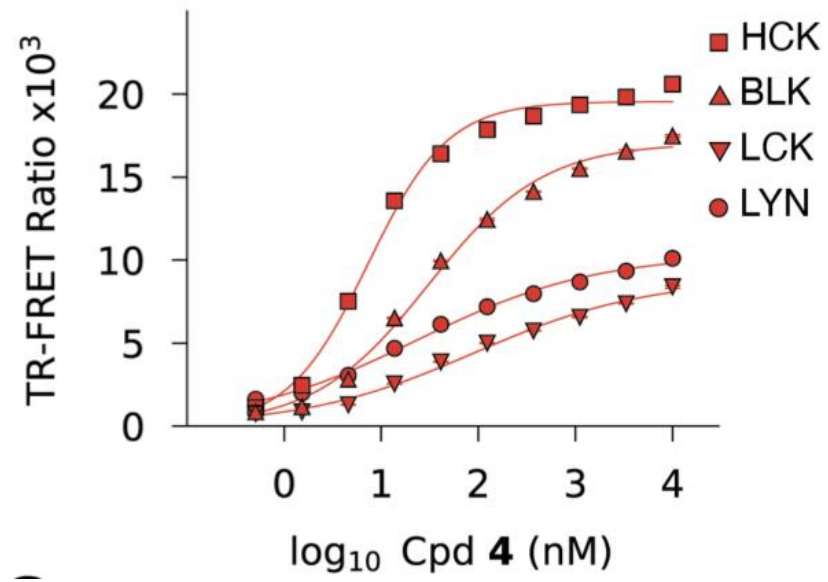

Fig1. TR-FRET validation of ternary complex formation

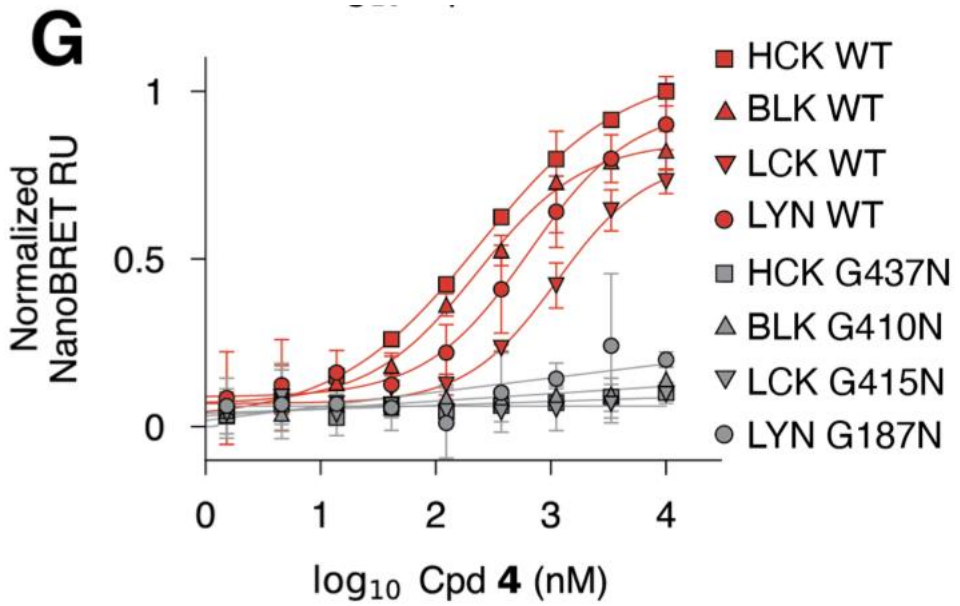

Fig2. NanoBRET validation of ternary complex formation

❖ This analysis uncover a **structurally differentiated helical G-loop motif** present in 184 proteins that extends neosubstrate recognition by CRBN beyond β-hairpin α-turns.

Only several targets showed a degradation after adding MGD

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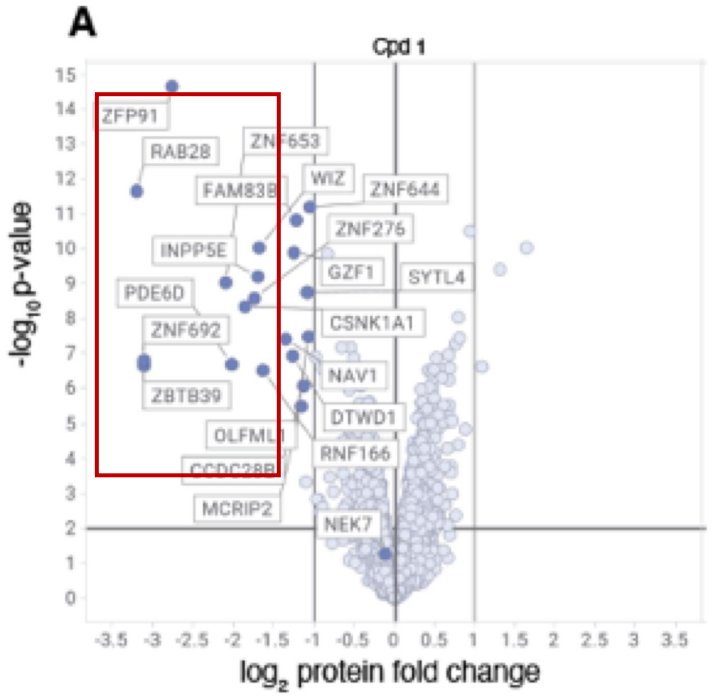

Fig2. Degradation proteomics in CAL51 cells exposed to 10 µM of Cpd 1

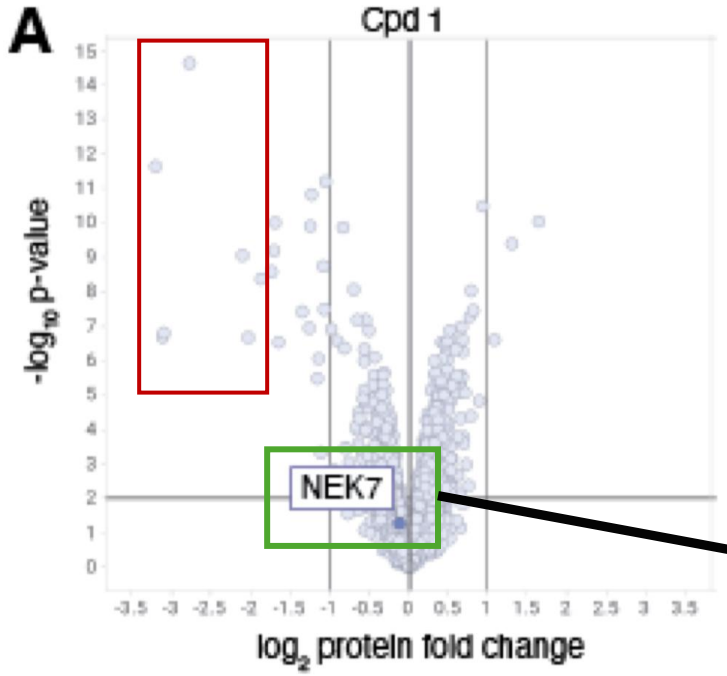

Fig1. Global TMT proteomics in CAL51 cells, DMSO/MGD

*"It has been shown that weak target recruitment can be converted into degradation through **differentiated chemistry**"*

Whether differentiated MGD lead to degradation in these cases?

Use this to screen

CRBN recruitment can be improved by MGDs

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- Cpd 5 induced a **higher formation of complex** between NEK7, CRBN and Cpd5

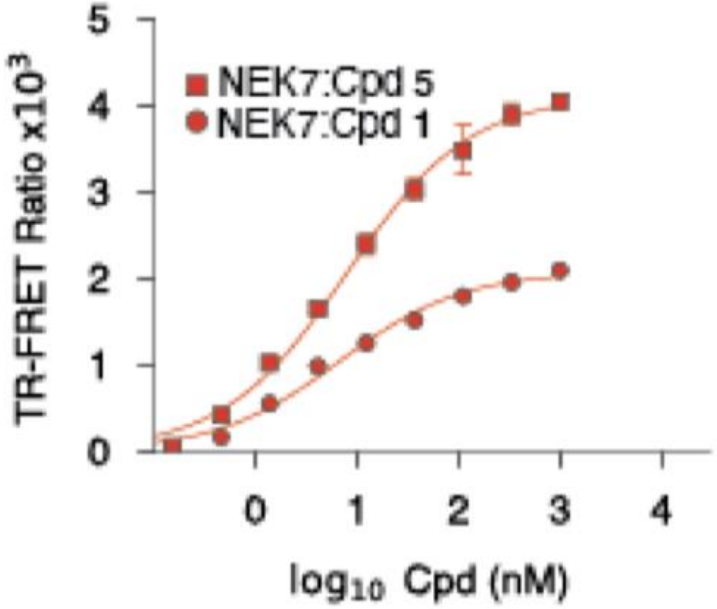

Fig1. TR-FRET validation of compound-dependent NEK7 binding to CRBN.

- Treatment of CAL51 cells with Cpd 5 led to **potent NEK7 degradation**

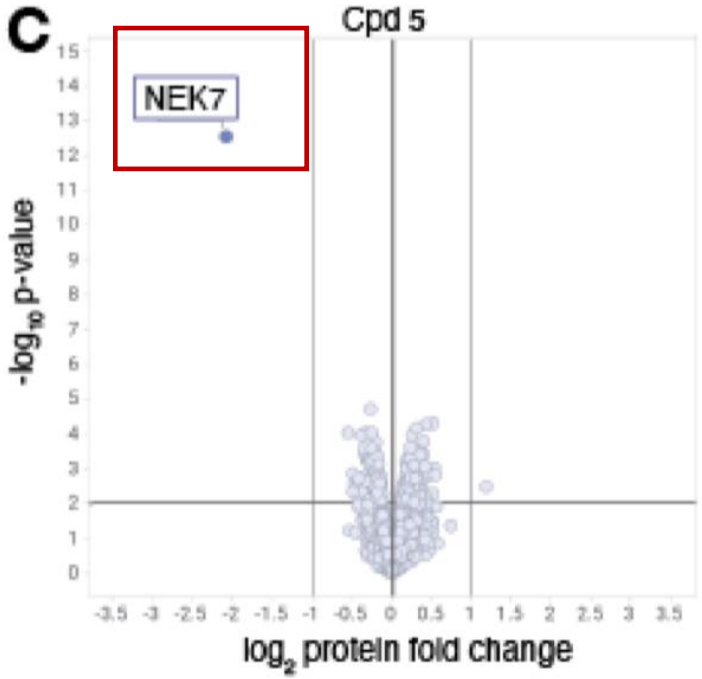

Fig2. Global TMT proteomics in CAL51 cells, DMSO/MGD

Expended PPI interface is critical for ternary Complex formation

- This extension of NEK7 seem to **extend the PPI interface** with CRBN

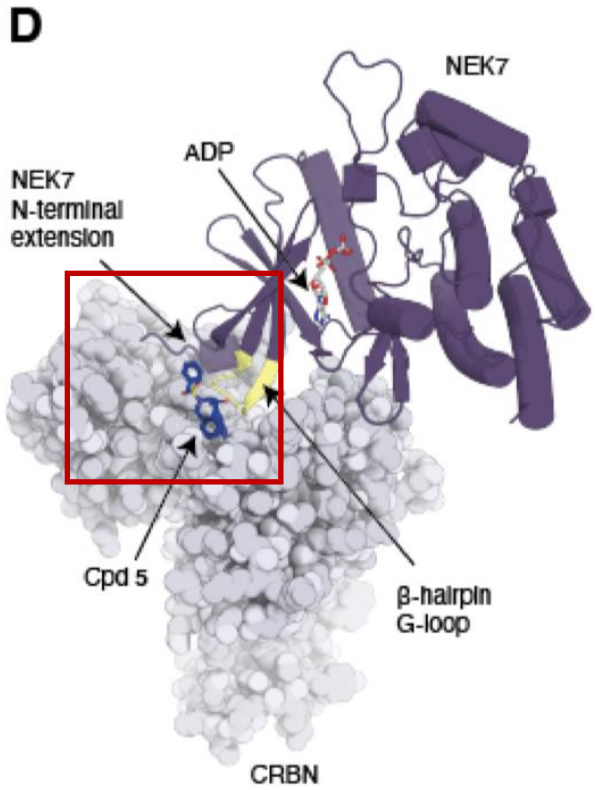

Fig1. NEK7:CRBN:Cpd. 5 ternary complex structure.

- Deletion of these N-terminal residues **reduce the NanoBRET signal**

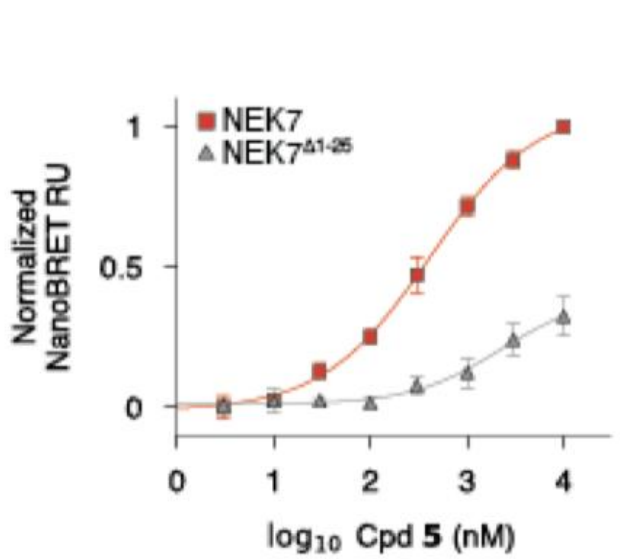

Fig2. NanoBRET validation of CRBN engagement

Interactions between CRBN and its Targets are malleable

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- Comparing the NEK7 ternary complex to the ternary complex of CK1α reveals **differences at the interface**

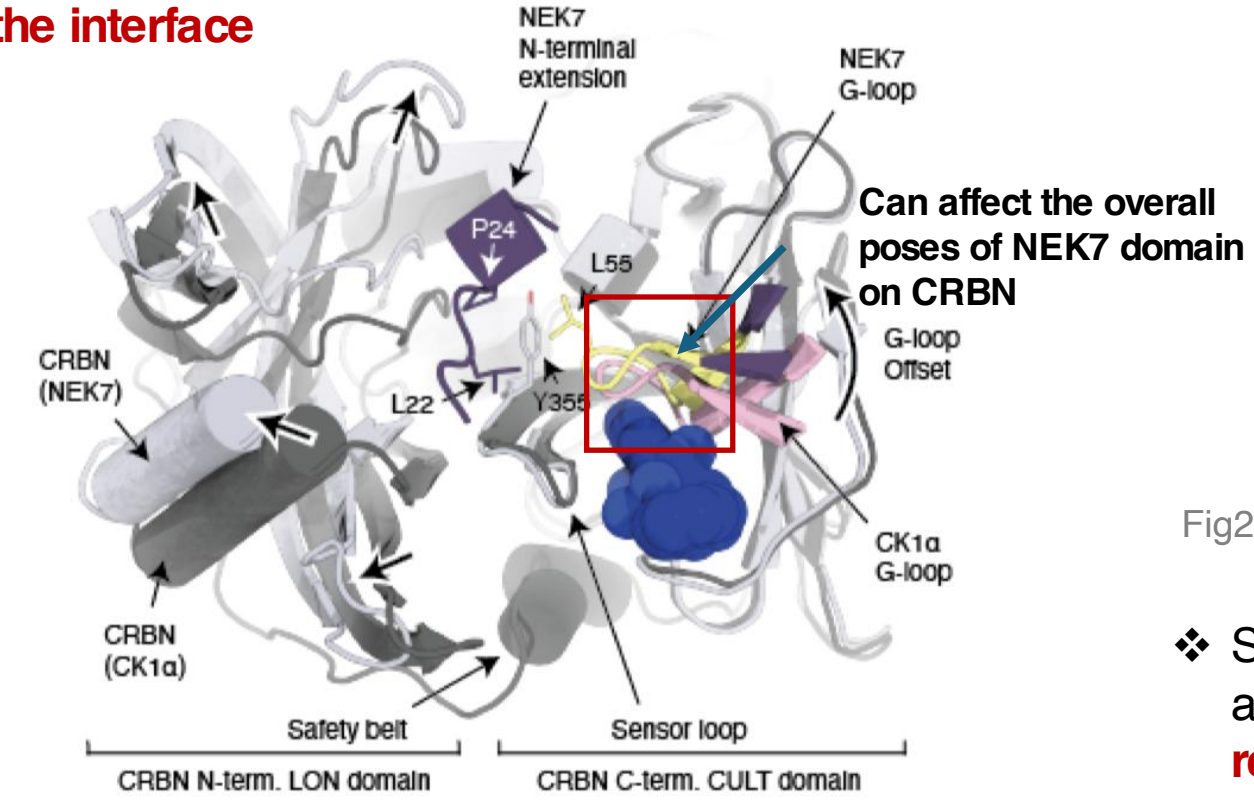

Fig1. Superposition of the NEK7:CRBN and CK1α:CRBN (PDB id: 5fqd) crystal structures

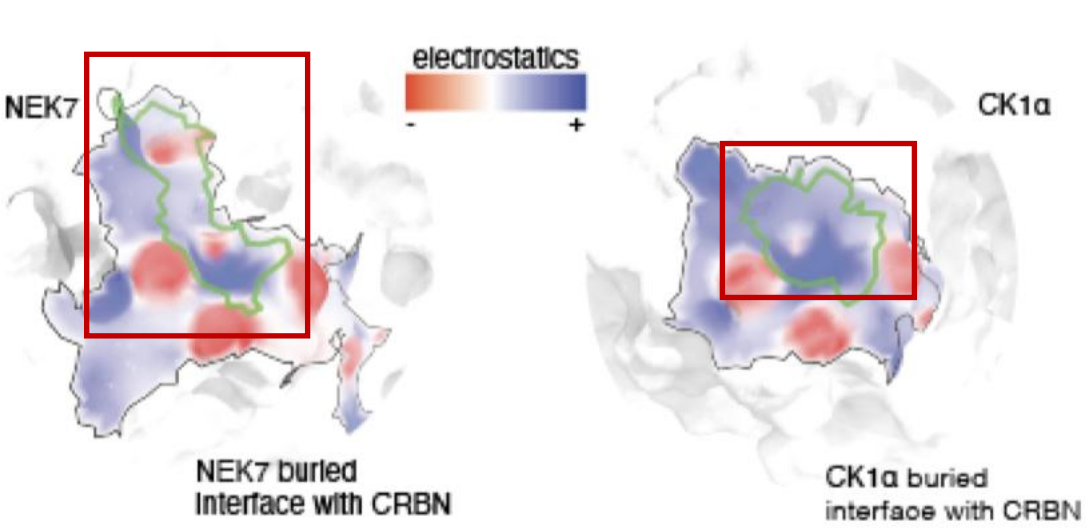

Fig2. Superposition of the NEK7:CRBN and CK1α:CRBN (PDB id: 5fqd) crystal structures

- Substantial differences in electrostatics and surface area coverage further prove diverging **interface requirements of both targets**

VAV1 SH3c has a large Similarity to GSPT1 despite the lack of Homology

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Other structural arrangements may exist that further depart from the G-loop paradigm, which can satisfy the interaction with CRBN

- Modified the surface matching algorithms to **detect surface similarity** to known CRBN binding motifs

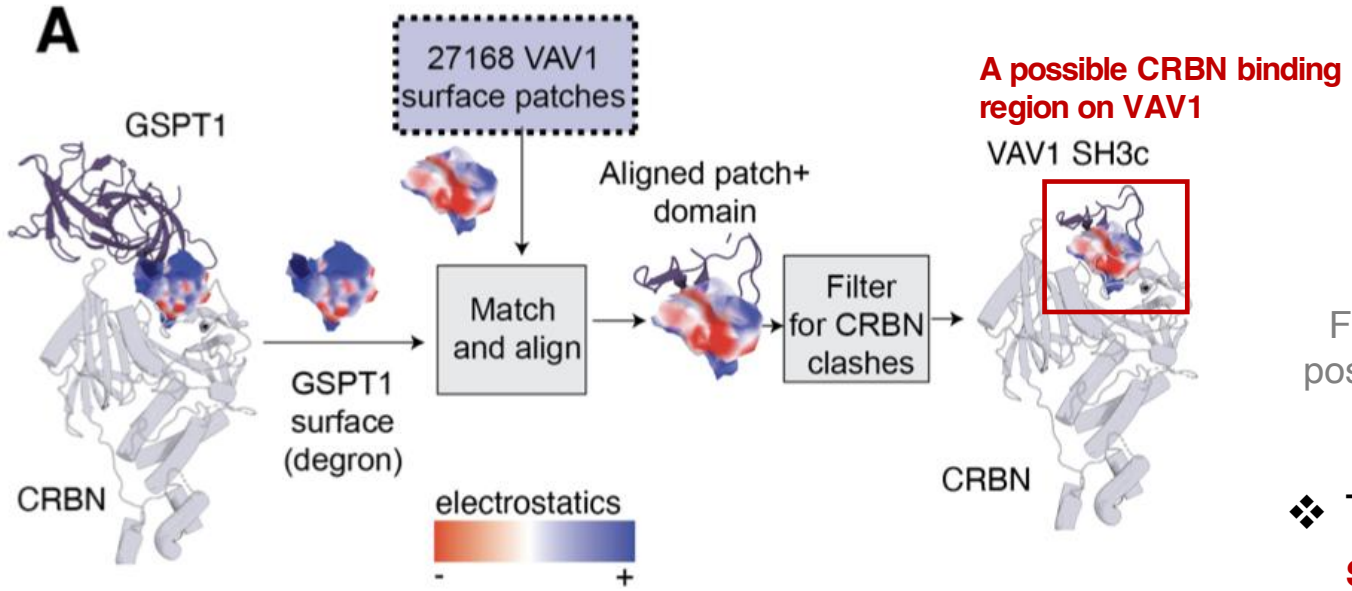

Fig1. Pipeline to match surface patches in VAV1 domains to known degron surfaces.

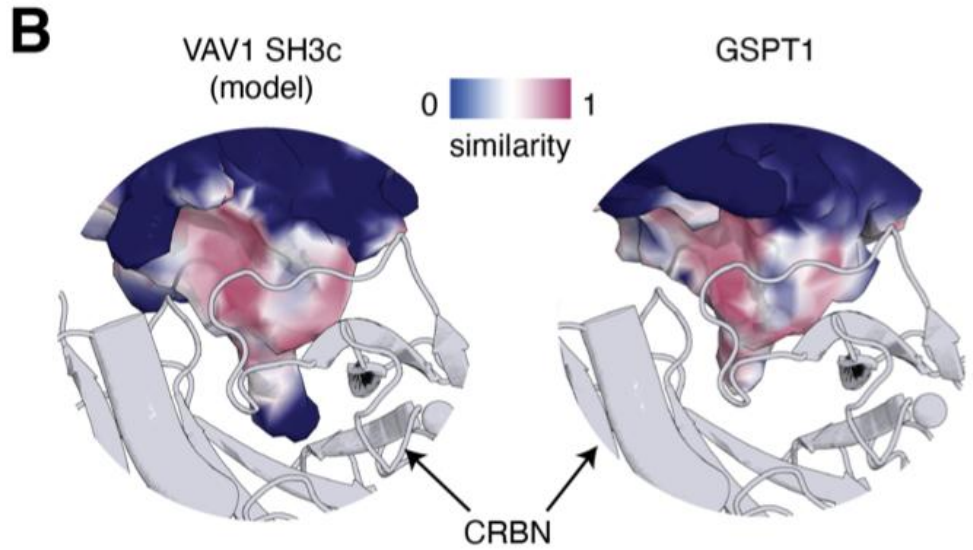

Fig2. Left: Surface similarity between the predicted VAV1 SH3c pose and GSPT1 Right: Coloring of the GSPT1 surface according to surface similarity to VAV1 SH3c in the model pose

- The second surface patch of VAV1 SH3c showed **striking similarities** to the GSPT1 surface

VAV1 form the ternary complex in a RT-loop dependent manner

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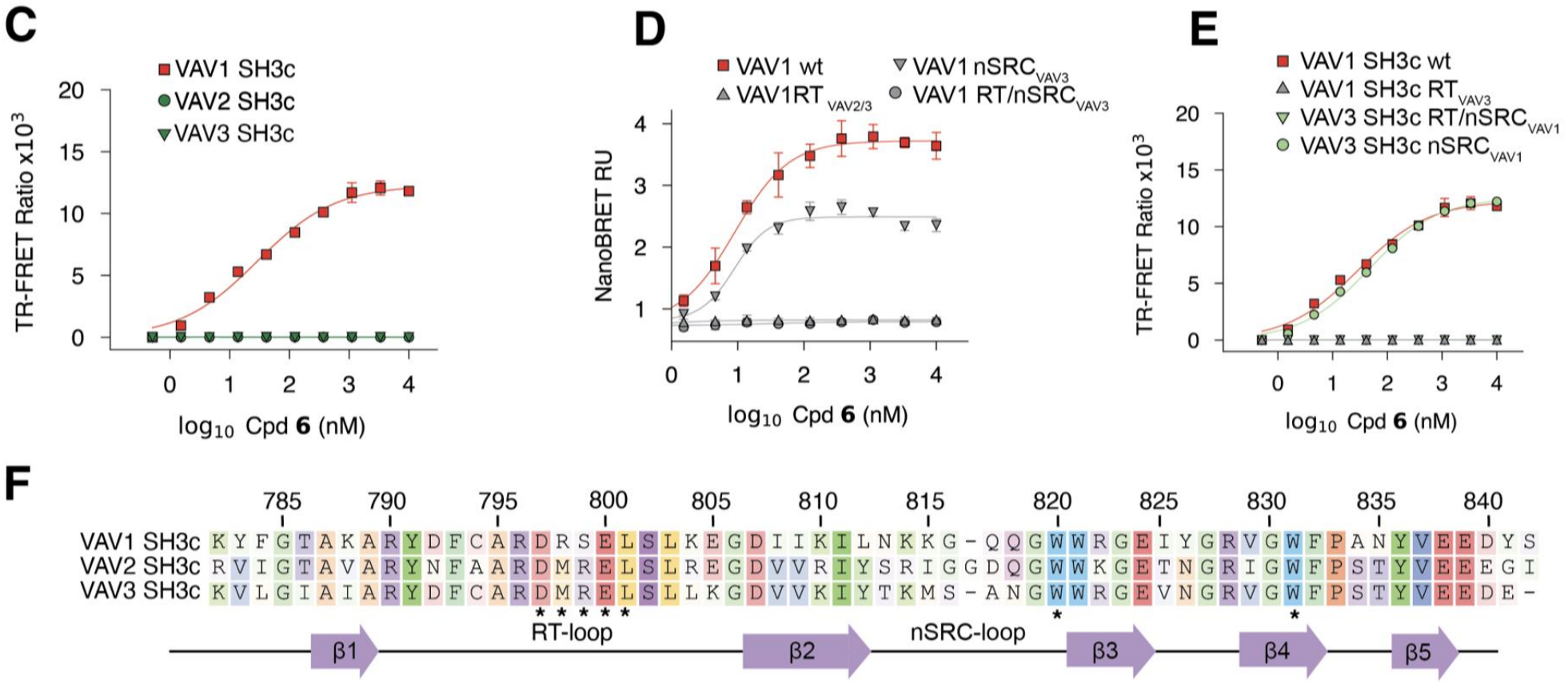
- TR-FRET identified Cpd. 6 as a potent inducer of ternary complex formation
- Recruitment of full length VAV1 to CRBN through Cpd. **6** could be confirmed in cells using NanoBRET



Proteins lacking G-loops can engage CRBN through surface complementarity

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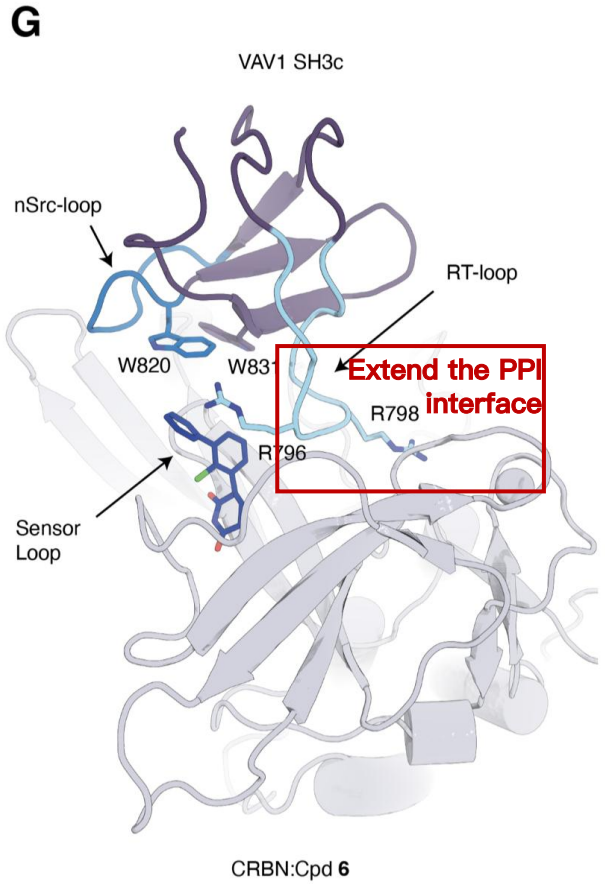

Fig1. VAV1:Cpd. 6:CRBN ternary complex structure.

- VAV1 **also forms hydrogen bond interactions** with the three critical CRBN residues

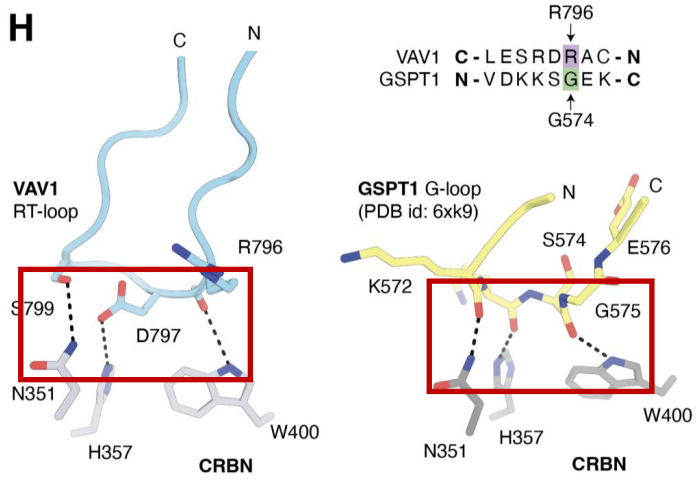

Fig2. Comparison of hydrogen bond interactions between CRBN and the VAV1 RT-loop and the GSPT1 G-loop

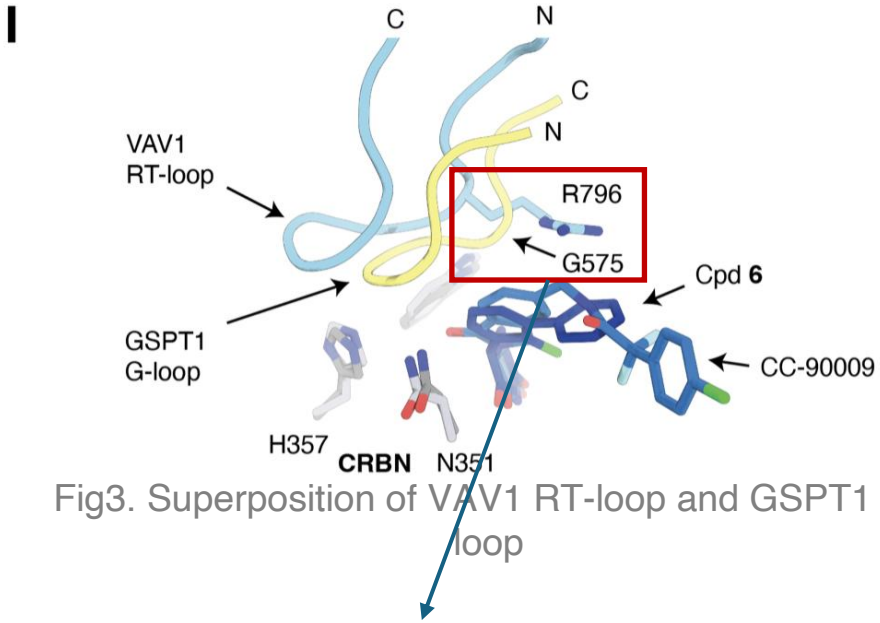

Fig3. Superposition of VAV1 RT-loop and GSPT1 G loop

- VAV1 engages two CRBN residues through the **side chains in the RT-loop** instead of backbone interaction of G-loop

Conclusion

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Conclusion

- **A proteome-wide map of the β-hairpin G-loop target space**
  - set the definition of β-hairpin G-loop motif and conduct the prediction
  - Validating the prediction
  - Test the dependence of the G-loop in CRBN engagement
- **CRBN engagement through helical G-loop motifs**
  - Use a less stringent definition of G-loop to conduct mining
  - mTOR-FRB(which has a helical G-loop motif) can form a more Compact interface than CK1 α
  - Validation of Generalizability of helical G-loop
- **NEK7 binds CRBN through a differentiated PPI interface**
  - CRBN recruitment can be improved by differentiated MGDs
  - Expended PPI interface is critical for ternary Complex formation of NEK7
  - Interactions between CRBN and its Targets are malleable
- **VAV1 engages CRBN through molecular surface mimicry of a known degron**
  - Interactions between CRBN and its Targets are malleable
  - VAV1 form the ternary complex in a RT-loop dependent manner
  - Proteins lacking G-loops can engage CRBN through surface complementarity

Questions

1. What kind of Challenges has been solved by this paper?

2. What are the potential therapeutic implications of expanding the CRBN target space beyond the β-hairpin G-loop paradigm?