CIP for protein localization control

2025/06/12 YOUHI HWANG

Contents

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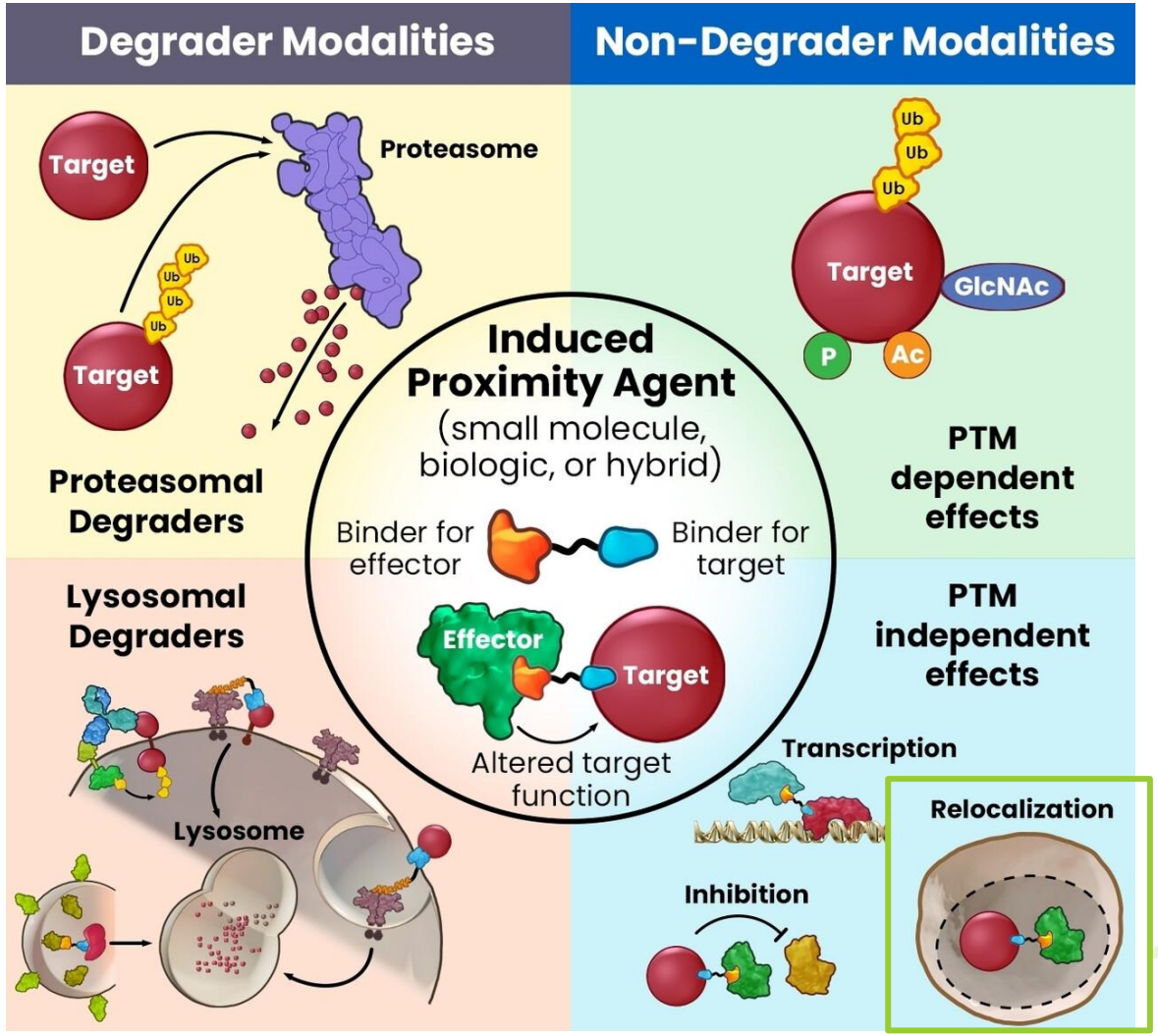
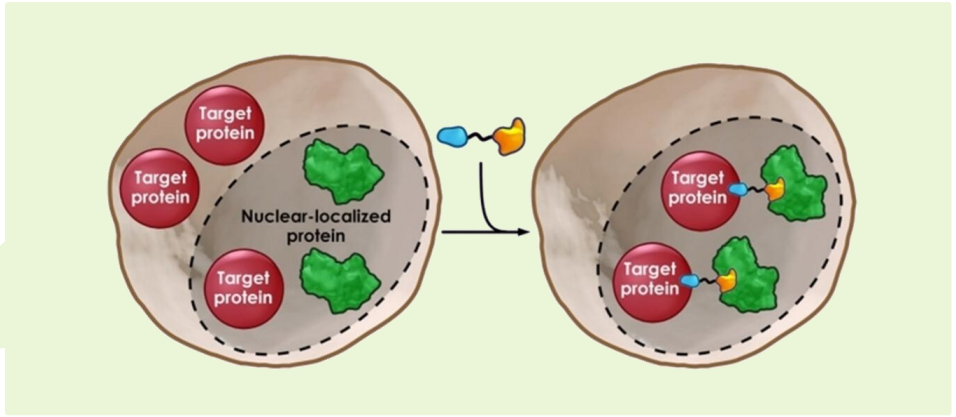
Chemical inducers of proximity (CIP)

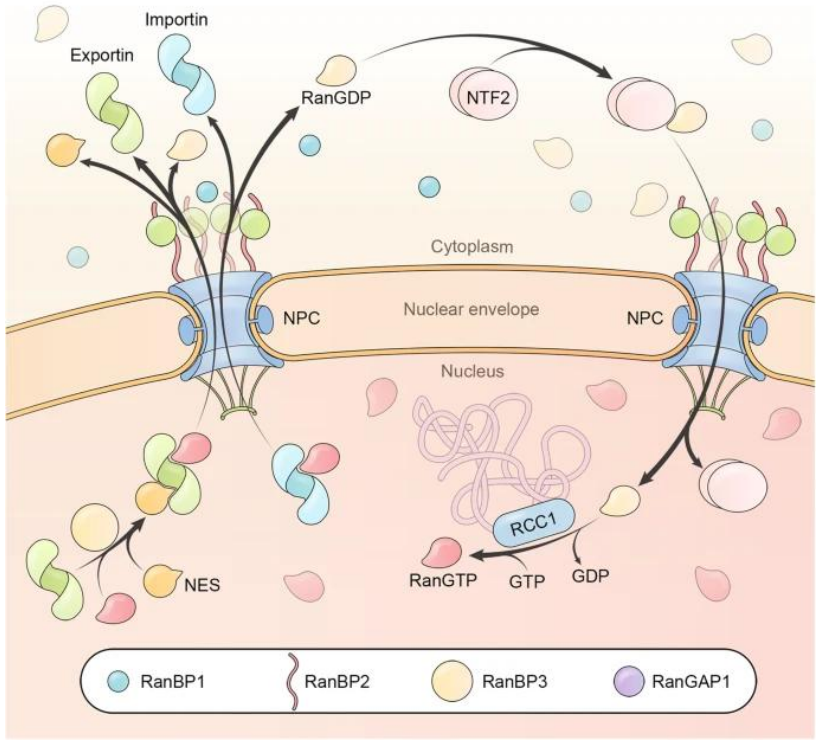


CIP : a small molecule that brings two substrates into proximity

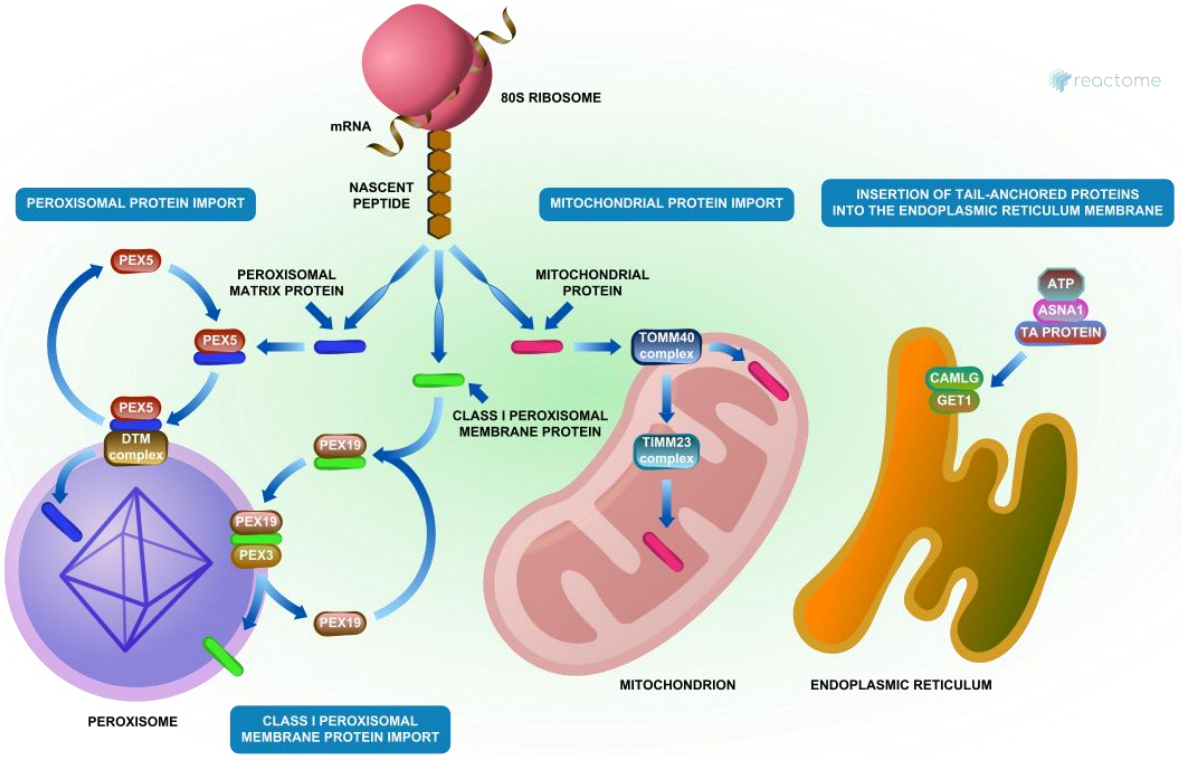
CIP has been shown to regulate subcellular localization(today's topic).



Protein localization



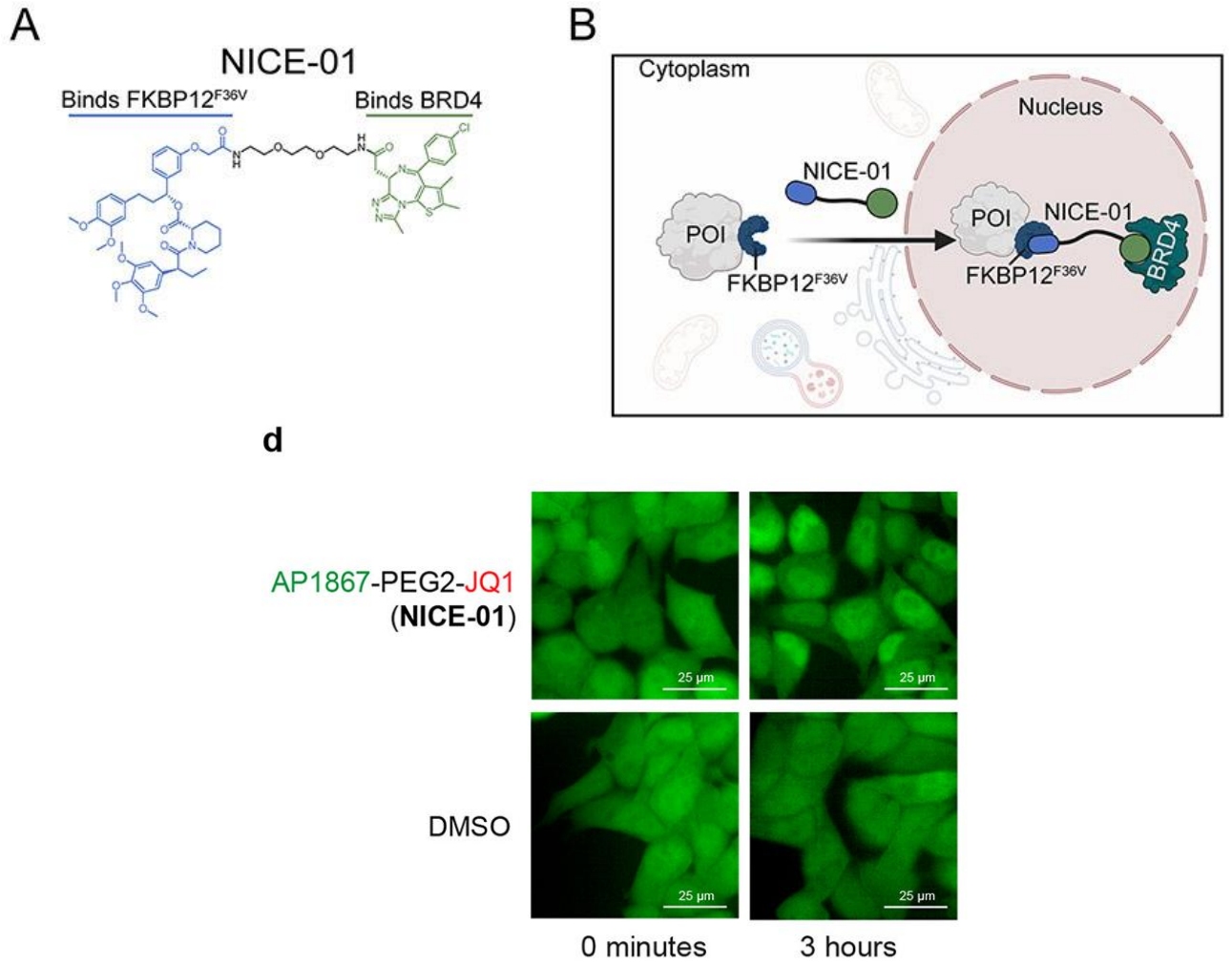
Yang, Y., Guo, L., Chen, L. *et al. Sig Transduct Target Ther* **8**, 425 (2023).



Fabregat A, et al. Reactome diagram viewer: data structures and strategies to boost performance. Bioinformatics (Oxford, England). 2018 Apr;34(7) 1208-1214.

- **Subcellular protein localization regulates protein function.**
- **Signaling sequences(ex:NLS) decides location.**
- **Shuttle or anchor protein has a role of protein localization.**

Protein localization control by CIP

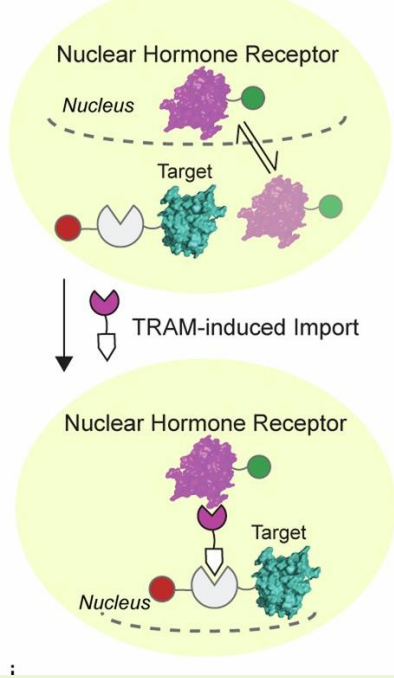


- ✓ **Bifunctional small molecules capable of inducing protein translocation from the cytosol to the nucleus**
- ◆ **Functional consequences of such relocalization remains elusive**
- ◆ **Directly modulating unligandable and endogenous targets is difficult**

Stuart L. Schreiber, et al.
Journal of the American Chemical Society **2023** *145* (48), 26028-26037

Today's topics

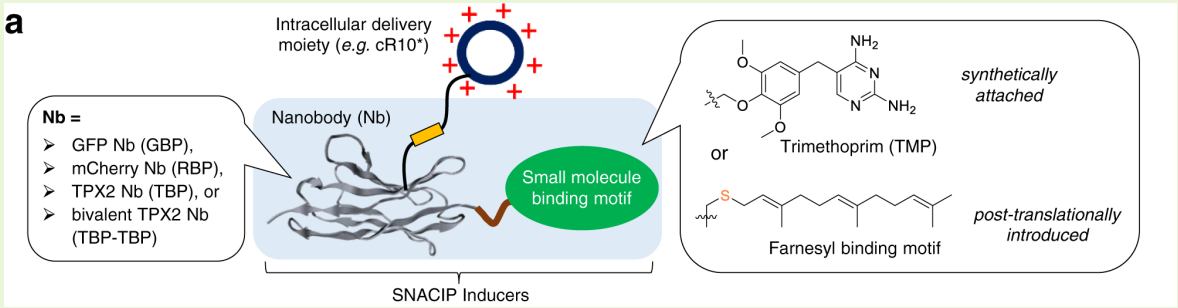
targeted relocalization-activating molecules (TRAMs)



Coupling misplaced proteins to cellular shuttles, relocating them to their proper places and correcting diseased phenotypes

Ng, C.S.C., Liu, A., Cui, B. *et al. Nature* **633**, 941–951 (2024)

small molecule-nanobody conjugate inducers of proximity (SNACIPs)



- **Additions to currently existing sets of CIP molecules**
- **Directly modulating unligandable and endogenous targets**

Sun, X., Zhou, C., Xia, S. *et al. Nat Commun* **14**, 1635 (2023)

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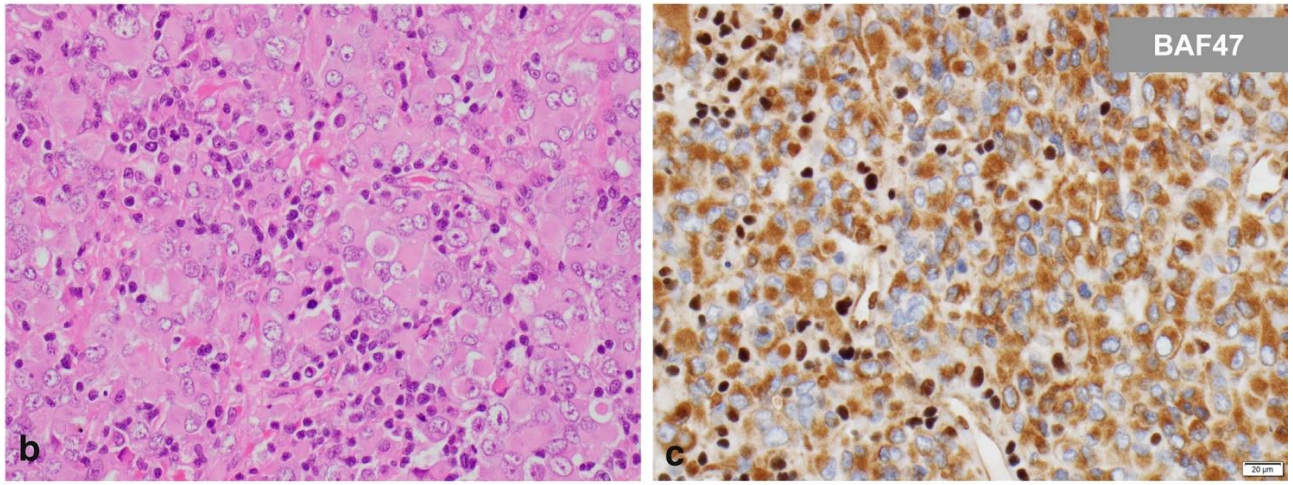
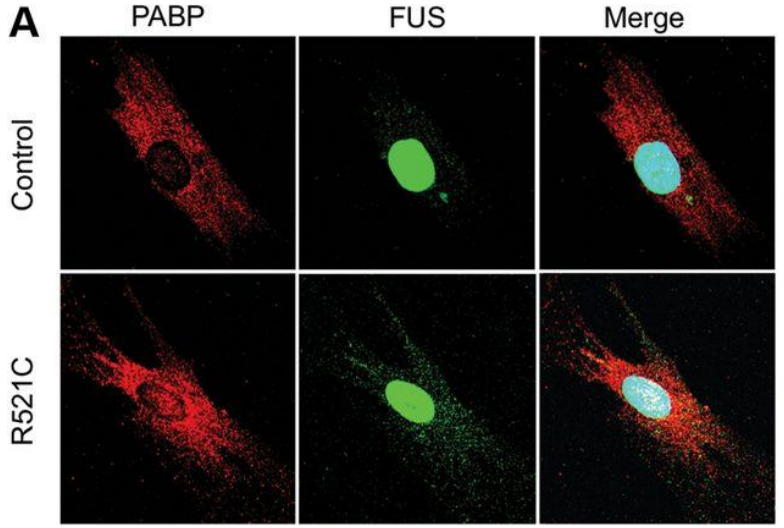
Protein mislocalization

Cytoplasmic SMARCB1 staining in ATRT



Pathak, R., Zin, F., Thomas, C. *et al. Acta Neuropathol* **142**, 361–374 (2021).

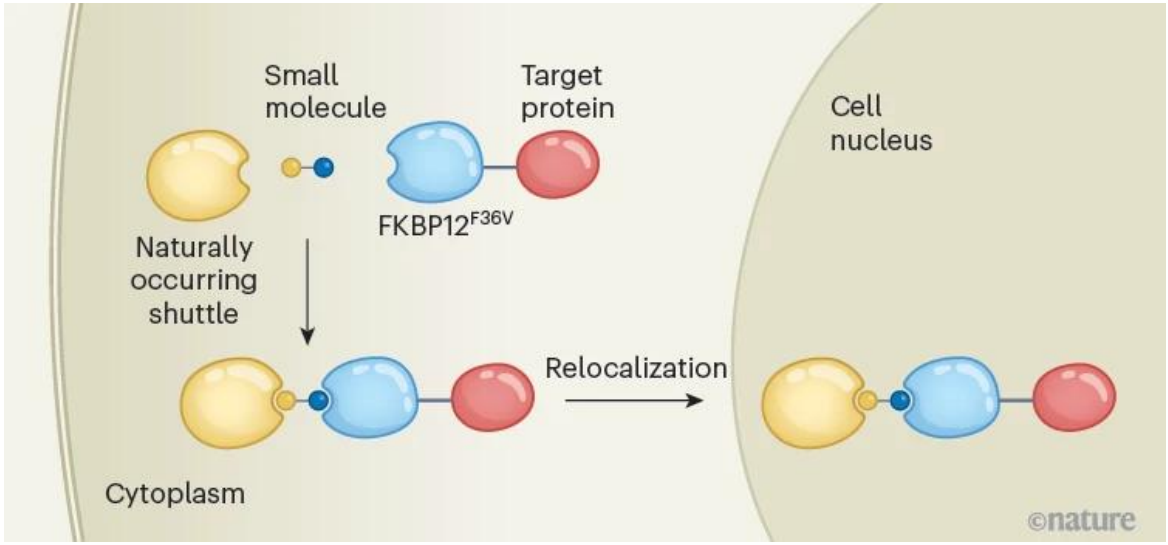
FUS co-localizes with stress granules in fibroblasts from ALS patients with FUS mutations

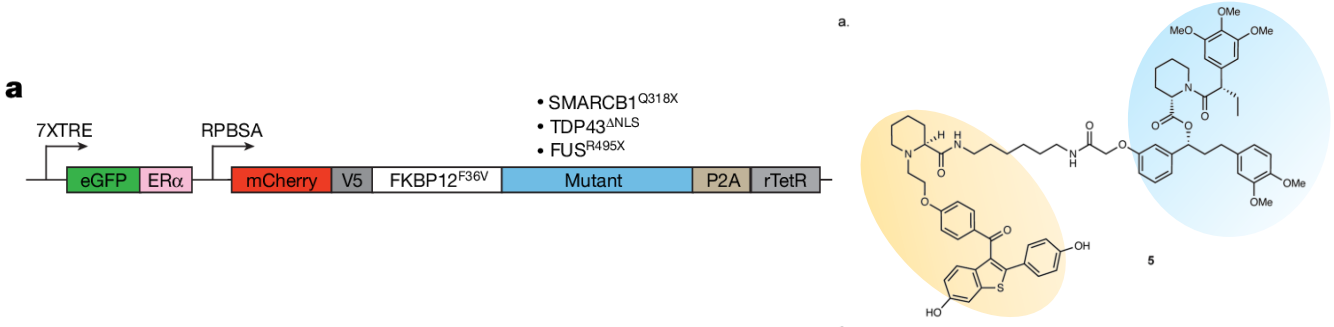


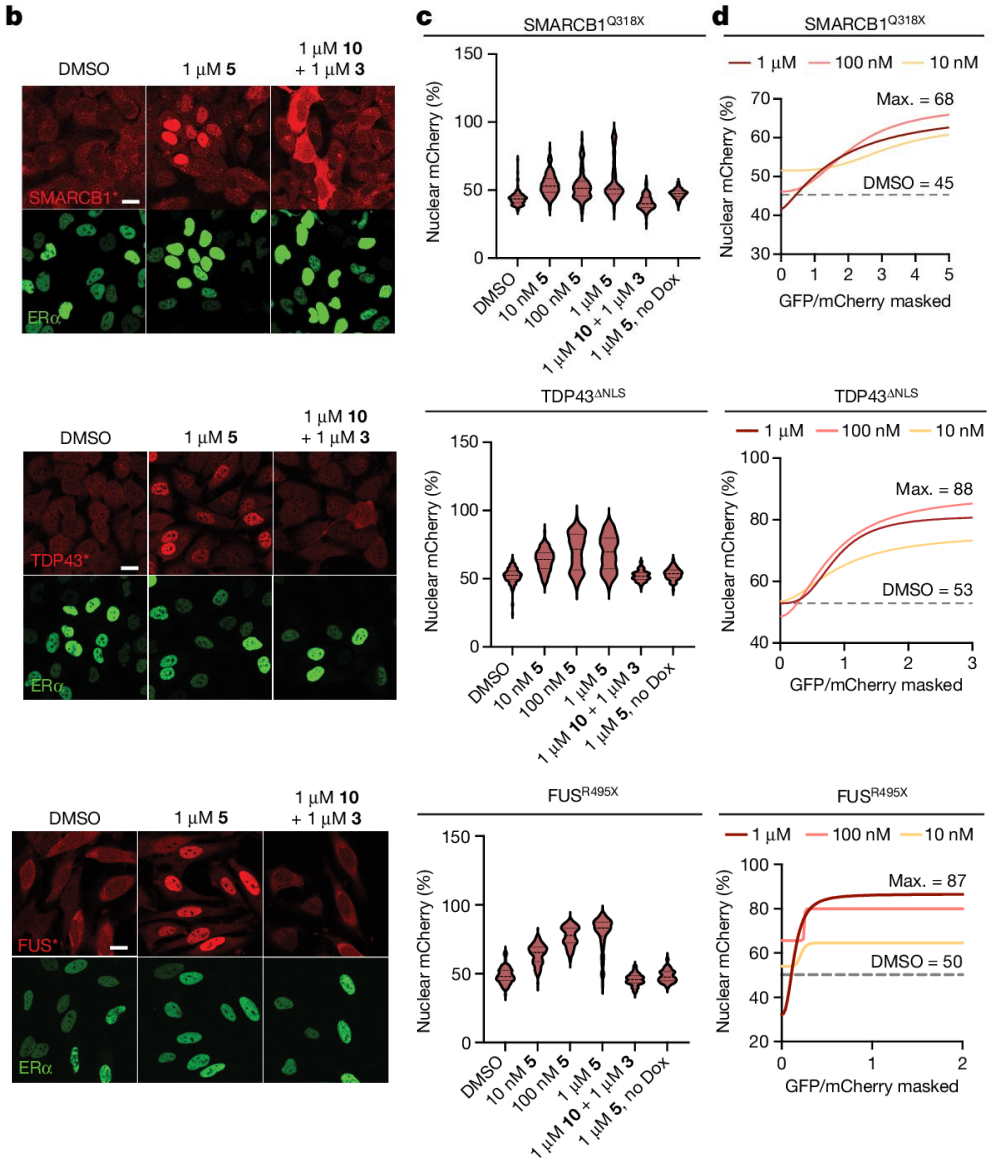
Caroline Vance, Emma L. Scotter et al, Shaw *Human Molecular Genetics*, Volume 22, Issue 13, 1 July 2013, Pages 2676–2688

Aberrant trafficking and localization of proteins underlies numerous diseases.
→regulating target protein location could expand the range of therapeutic options.

Mislocalized mutant protein relocalization



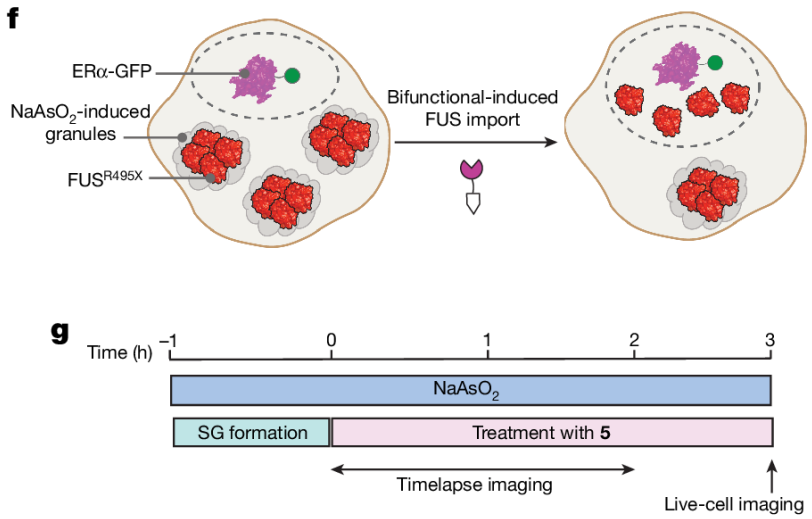


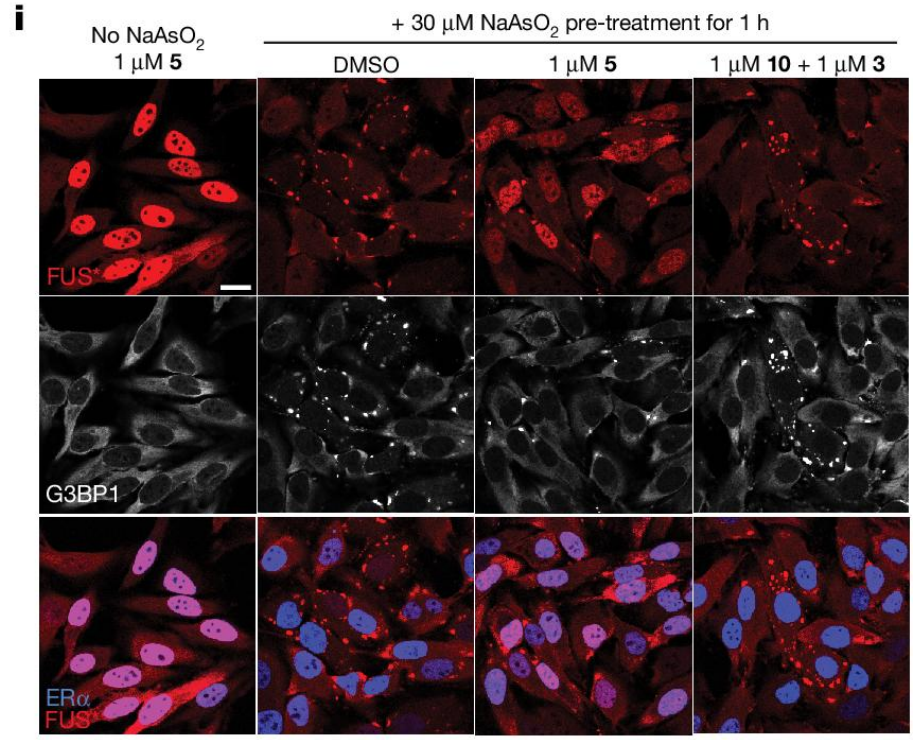


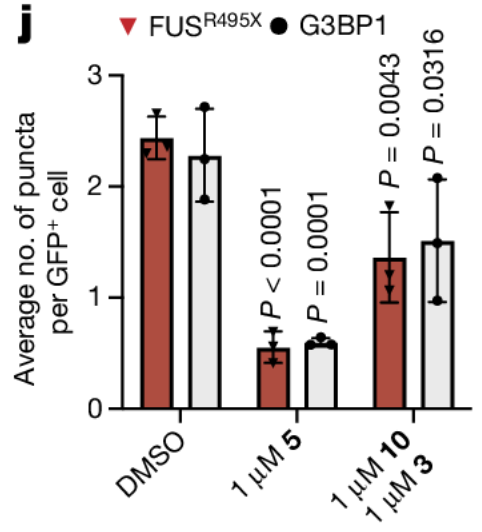
✓ **Coupling ERα(nuclear hormone receptor) to each mutant proteins can relocalize them to nuclei from cytoplasm.**

Ng, C.S.C., Liu, A., Cui, B. *et al. Nature* **633**, 941–951 (2024)

Mislocalized mutant protein relocalization







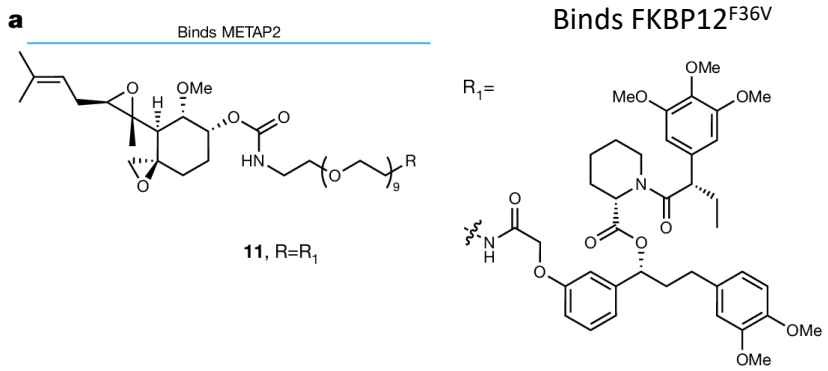
Ng, C.S.C., Liu, A., Cui, B. *et al. Nature* **633**, 941–951 (2024)

- ✓ **TRAM 5 can move FUS^{R495X} into nuclei and out of stress granules.**
- ✓ **TRAM treatment can reduce FUS^{R495X}-positive and G3BP1-positive granules**

Endogenous protein relocalization

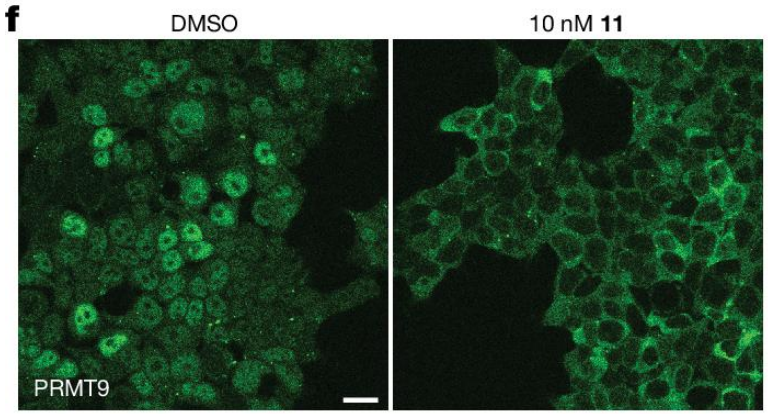
△The examination of targeted relocalization of endogenous proteins as a therapeutic approach is limited.

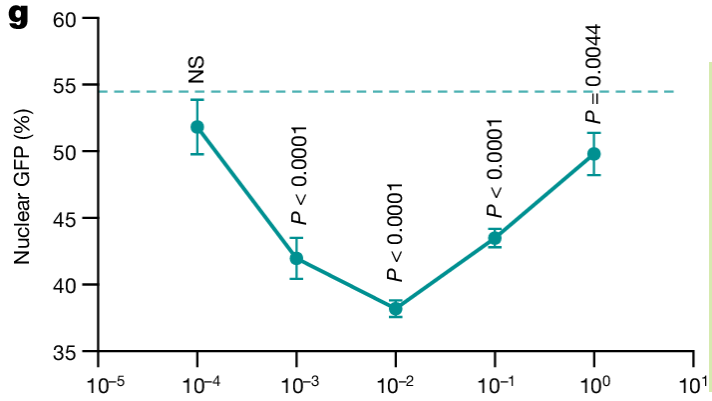
→Using CRISPR–Cas9 tagging and inserting a GFP–FKBP12^{F36V} cassette onto target proteins



METAP2 : general **shuttle protein** that are expressed at high levels in most cell types, selected as nuclear export shuttle

PRMT9 : a nuclear-enhanced protein explored as a **target** in acute myeloid leukaemia





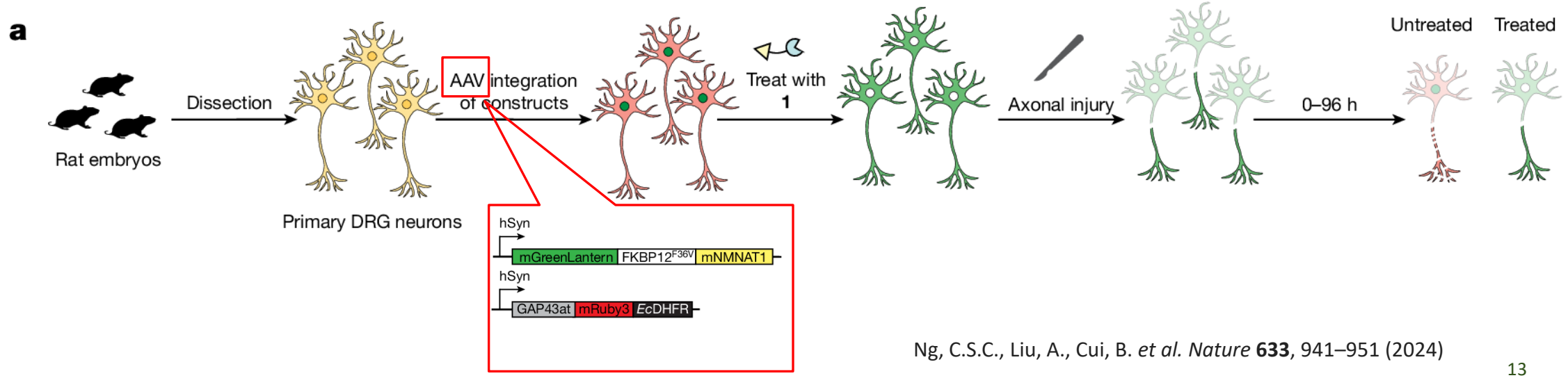
✓ **Treatment with TRAM 11 which engages endogenous METAP2 as an export shuttle could exclude nuclear PRMT9.**

Ng, C.S.C., Liu, A., Cui, B. *et al. Nature* **633**, 941–951 (2024)

Protein relocalization to drive a gain-of-function phenotype

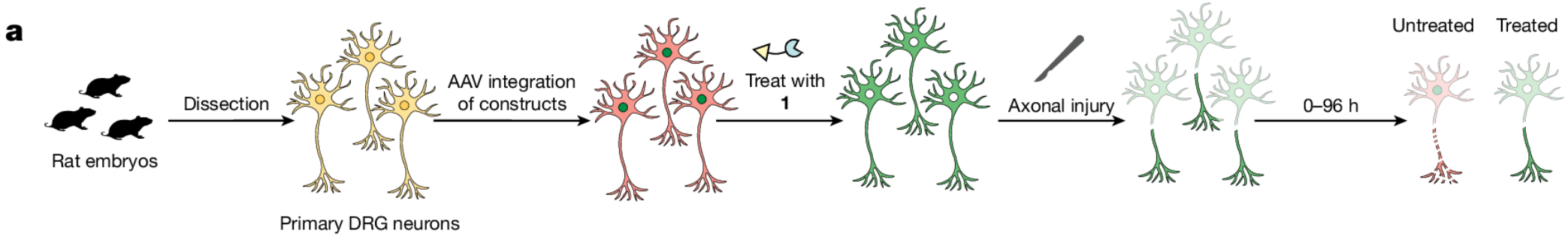
- Mice bearing the WldS(mutant protein consisting of mouse NMNAT1 (mNMNAT1) fused to a truncated N-terminal region of UBE4B) mutation have shown increased resistance to neuropathies and ALS.
- The ability for small amounts of WldS to traffic to the axon is crucial for its protective function against axonal injury.

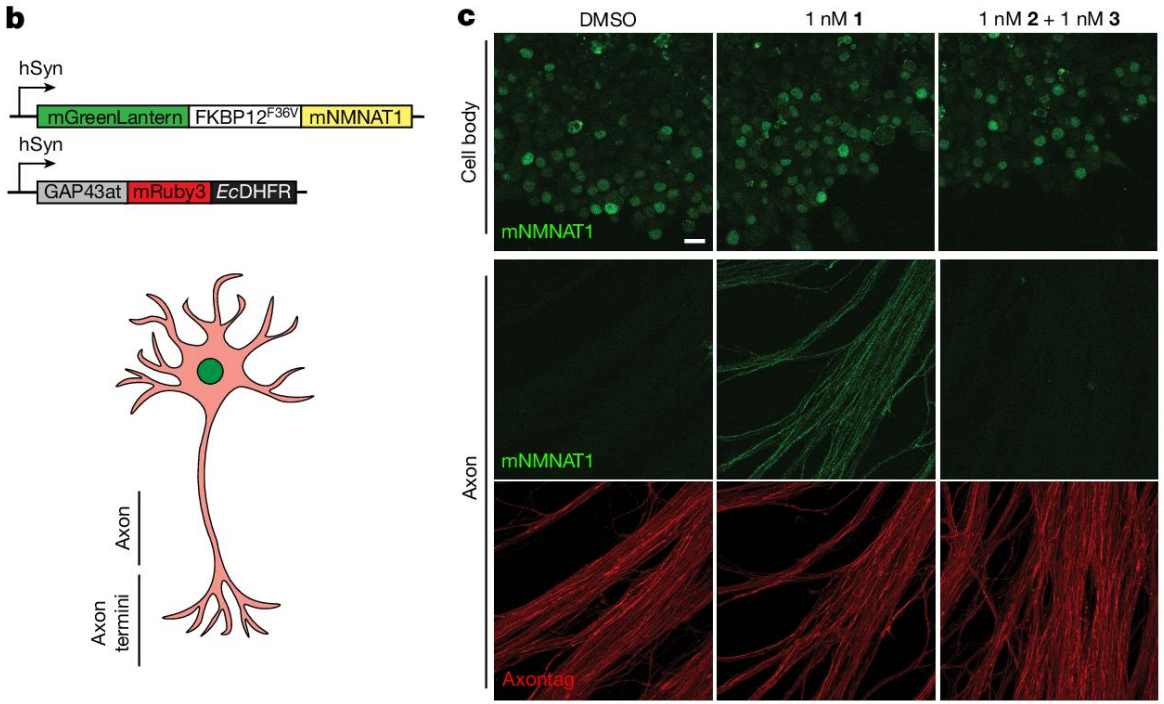
Hypothesis : Small-molecule-mediated transport of NMNAT1 from the nucleus down the axon might serve a similar function to WldS

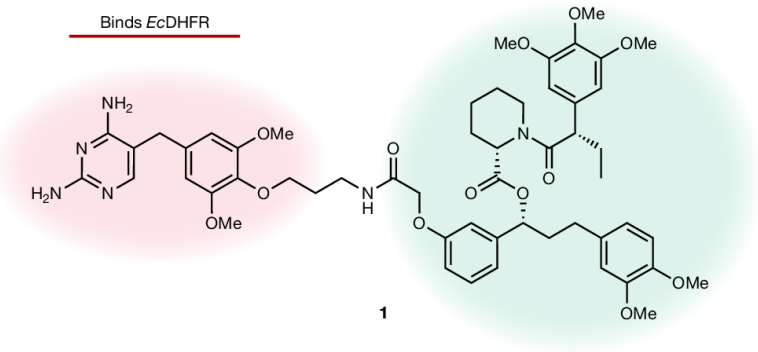


Ng, C.S.C., Liu, A., Cui, B. *et al. Nature* **633**, 941–951 (2024)

Protein relocalization to drive a gain-of-function phenotype



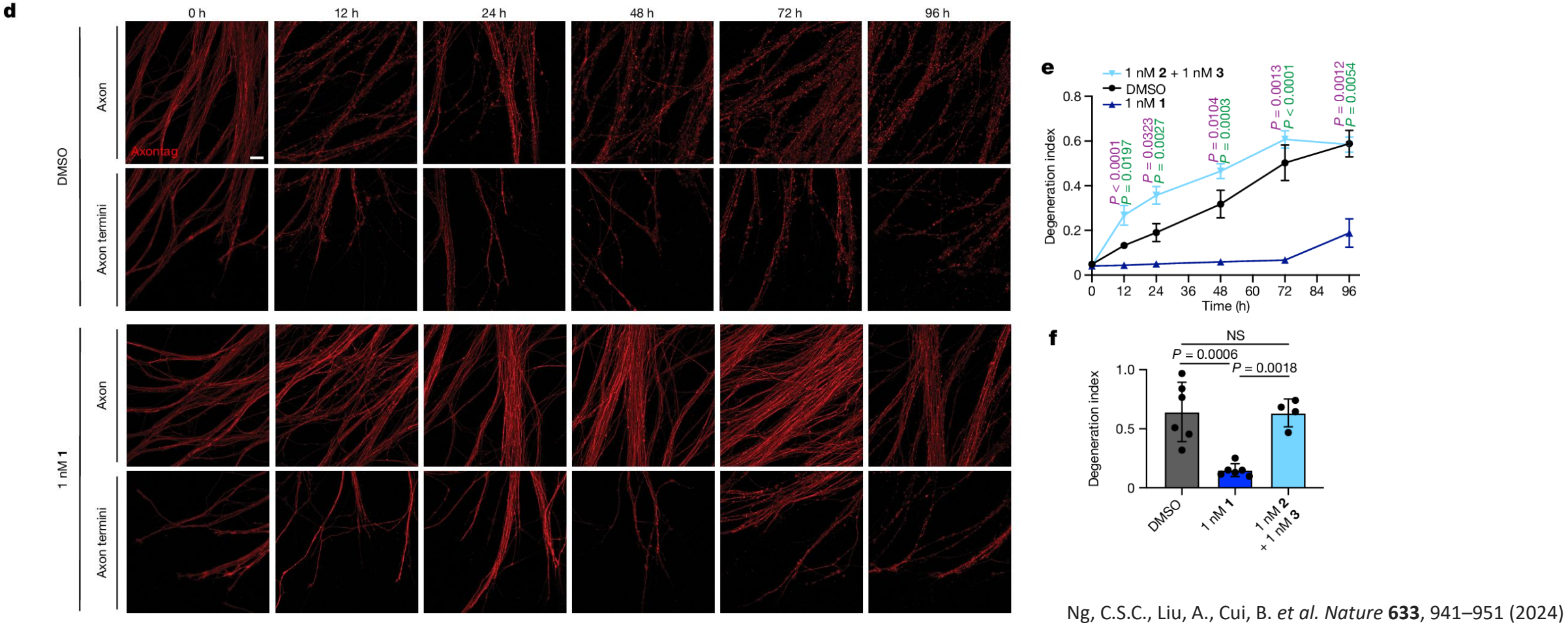




✓ **Clear redistribution of mNMNAT1 down axons was observed.**

Ng, C.S.C., Liu, A., Cui, B. *et al. Nature* **633**, 941–951 (2024)

Protein relocalization to drive a gain-of-function phenotype



Ng, C.S.C., Liu, A., Cui, B. *et al. Nature* **633**, 941–951 (2024)

- ✓ **NMNAT1 relocalization slows axonal degeneration**
- ✓ **Targeted relocalization of NMNAT1 can protect against axon injury**

Short summary

TRAMs coupling shuttles and targets can advance approaches for therapeutic modulation of cellular physiology

- TRAMs can correct diseased phenotypes that result directly from protein mislocalization

  TRAM-mediated relocalization of FUS^{R495X} to the nucleus from the cytoplasm correlated with a reduction in the number of stress granules in a model of cellular stress.

- TRAMs coupling METAP2 as endogenous shuttles can redistribute endogenous proteins by means of endogenous knock-in of binding domains

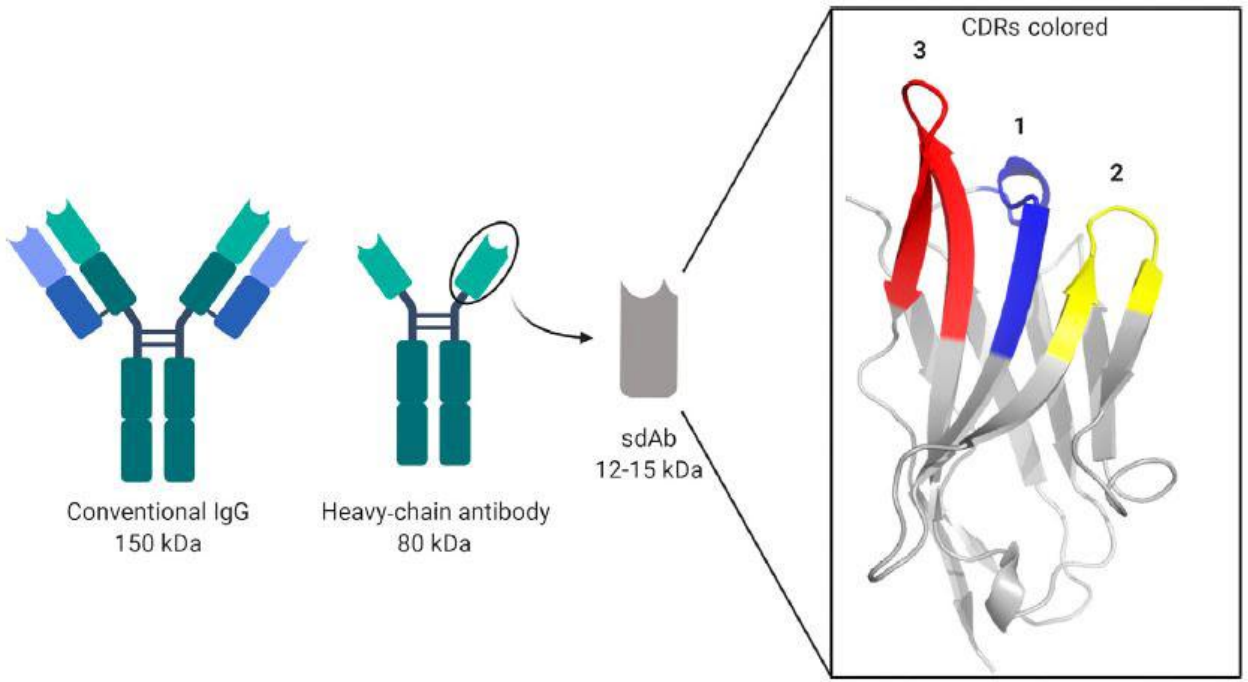
- TRAMs can impart beneficial function through protein relocation.

  Small-molecule-mediated redistribution of **NMNAT1** from nuclei to axons in primary neurons was able to slow axonal degeneration and pharmacologically mimic the genetic WldS gain-of-function phenotype

Contents

Nanobody

Nanobody : single-chain V_{HH} antibody fragment with a substantially reduced size (~15 kD) than traditional antibodies (~150 kD).



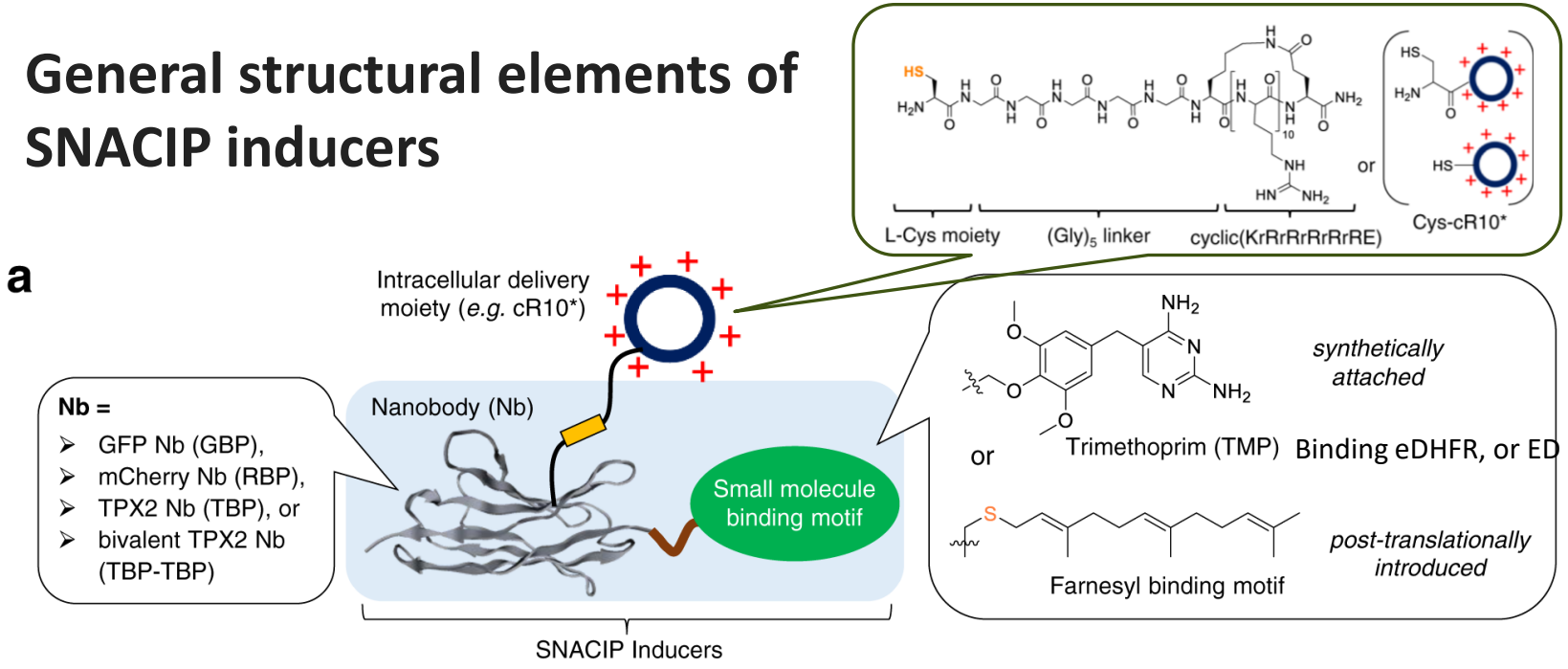
Czajka, Timothy F. et al. Trends in Microbiology, Volume 29, Issue 3, 195 - 203

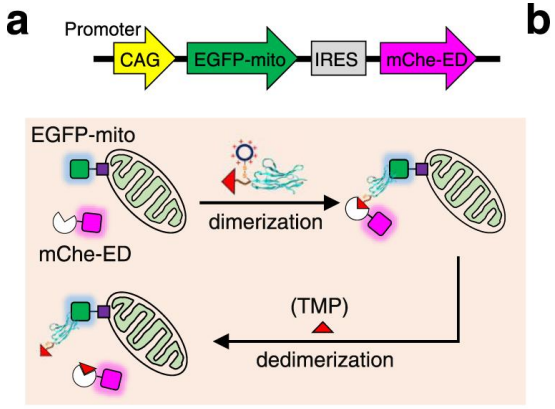
- ✓ high specificities
- ✓ nanomolar level high affinities towards their binding partners.

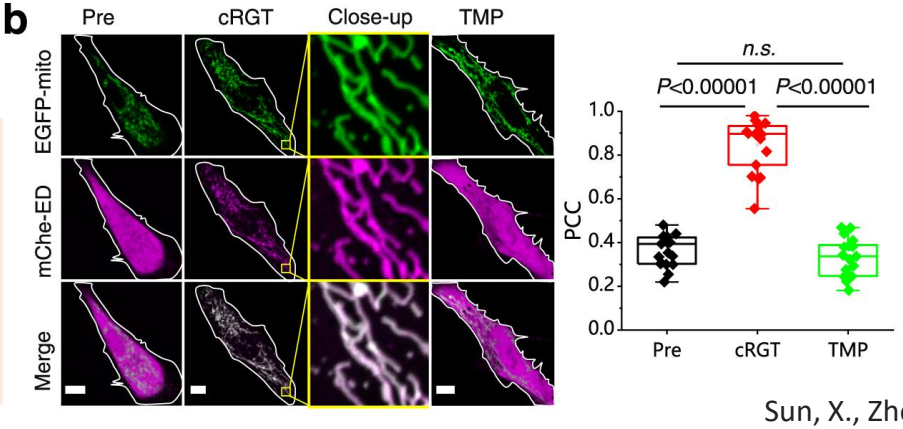
→Nanobody has the potential as a proximity-inducing module.

CIP using nanobody: SNACIPs

General structural elements of SNACIP inducers





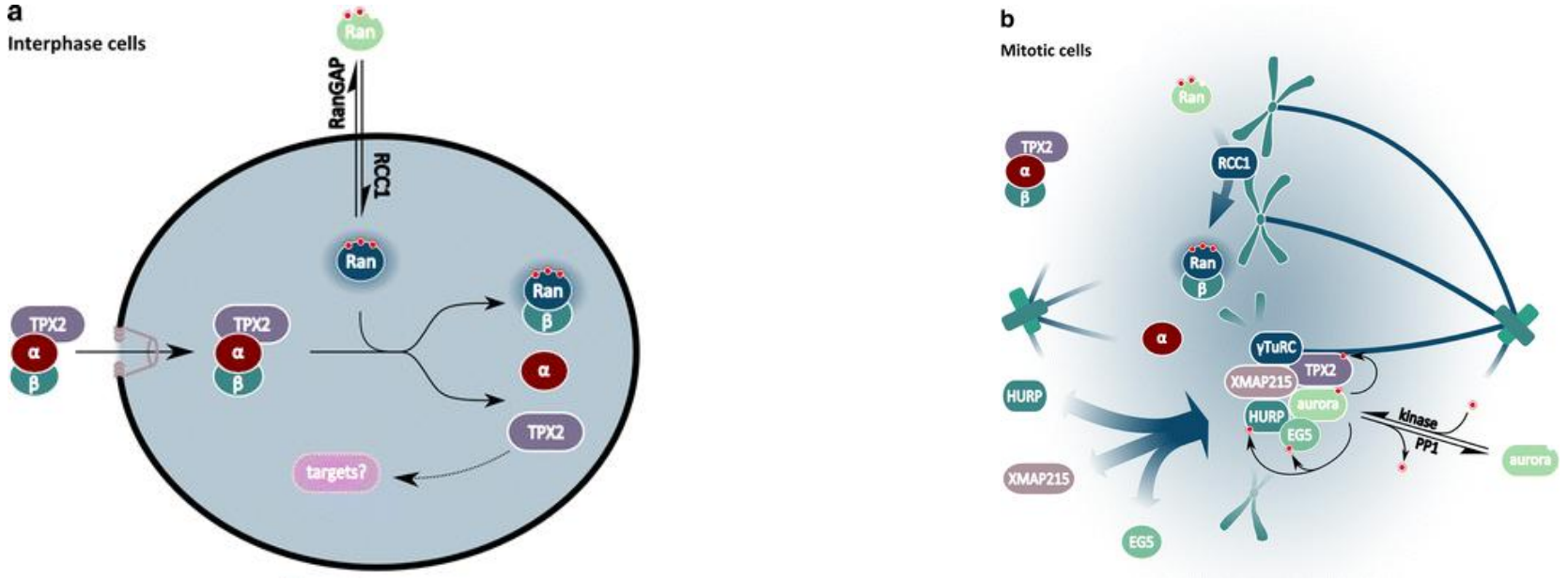


cRGT(= cR10*-SS-GBP-TMP) **directed mCherry-eDHFR to EGFP-mito at the mitochondria inside living cells.**

Sun, X., Zhou, C., Xia, S. *et al. Nat Commun* **14**, 1635 (2023)

SNACIP for modulating the endogenous unligandable protein

Target: TPX2 = intrinsically disordered protein and a key regulator in microtubule nucleation of spindle assembly overexpressing in many cancers



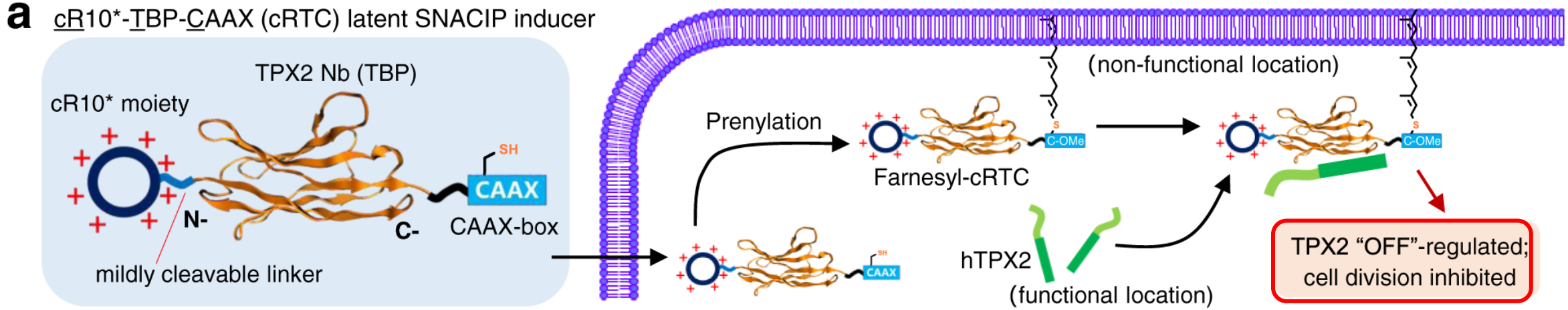
Neumayer, G., Belzil, C., Gruss, O.J. *et al.* , *Cell. Mol. Life Sci.* **71**, 3027–3047 (2014).

Positioning a molecule to the proximity of PM has been elegantly used by nature to deactivate cellular activities.

→**Hypothesis** : Bringing endogenous hTPX2 to the proximity of inner PM lipid bilayer could deactivate hTPX2 and subsequently inhibit cell proliferation**.**

SNACIP for modulating the endogenous unligandable protein

Design of a latent SNACIP inducer “cRTC” to control TPX2



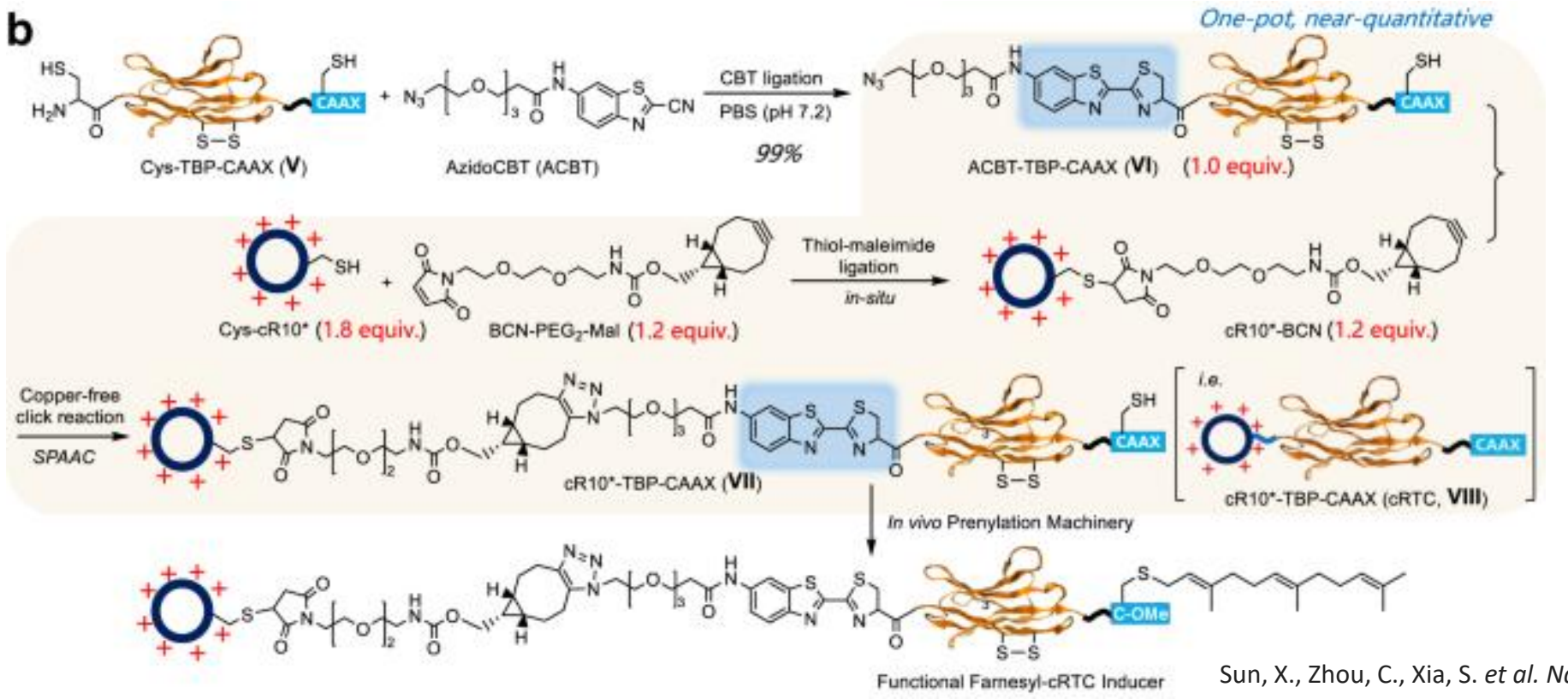
cR10*: an intracellular cleavable cyclic CPP, TPB : a human TPX2 (hTPX2) binding protein, CAAX-box : undergoing S-prenylation for binding with PM

1. cRTC is converted to a functional farnesyl-cRTC SNACIP
2. cRTC brings endogenous hTPX2 to the proximity of inner PM lipid bilayer
3. hTPX2 is recruited to the “rest”-PM position, deactivated, and subsequently inhibit cell proliferation

Sun, X., Zhou, C., Xia, S. *et al. Nat Commun* **14**, 1635 (2023)

SNACIP for modulating the endogenous unligandable protein

Assembling cRTC in one-pot via tandem bioorthogonal ligations

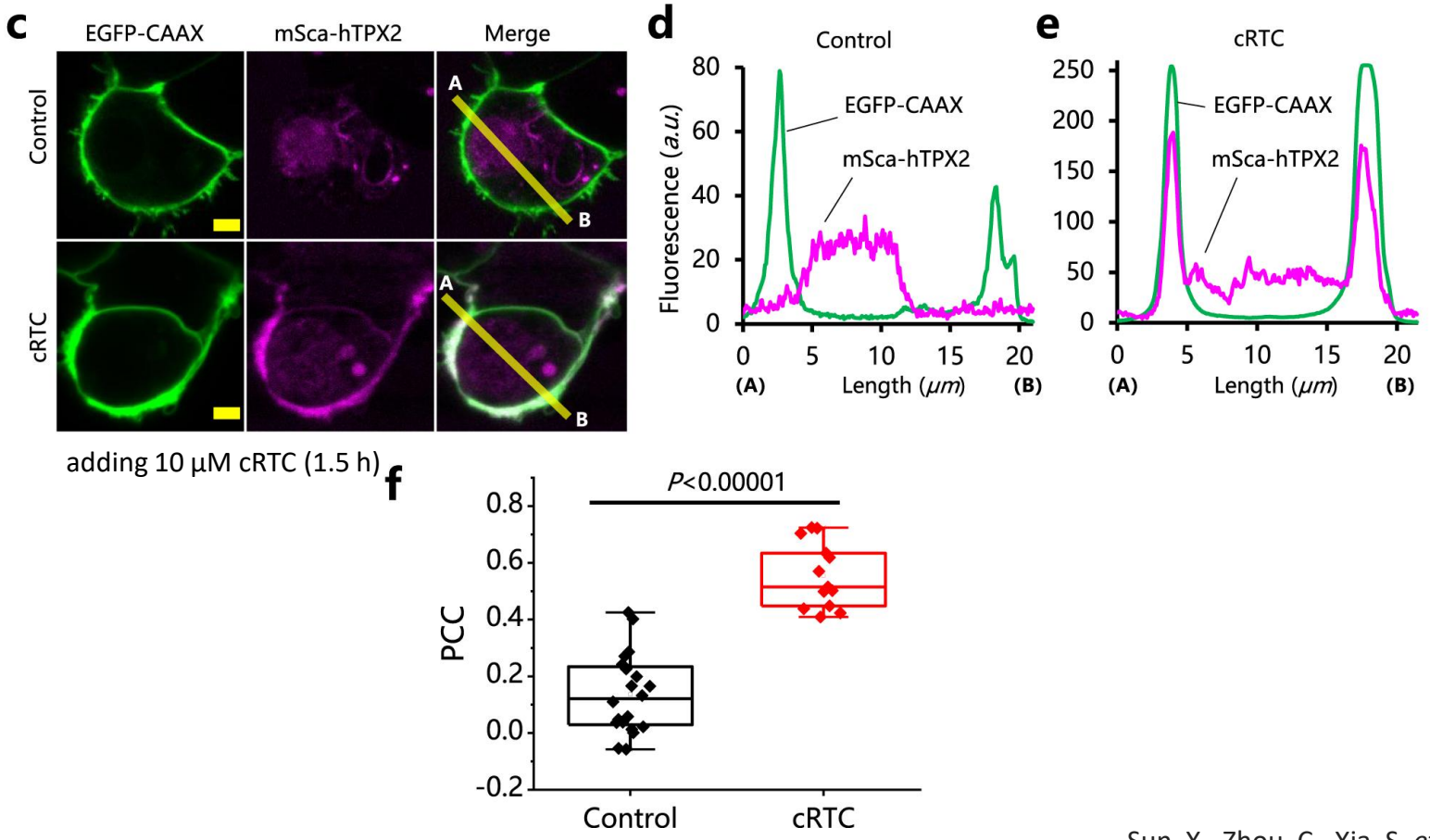


Sun, X., Zhou, C., Xia, S. *et al. Nat Commun* **14**, 1635 (2023)

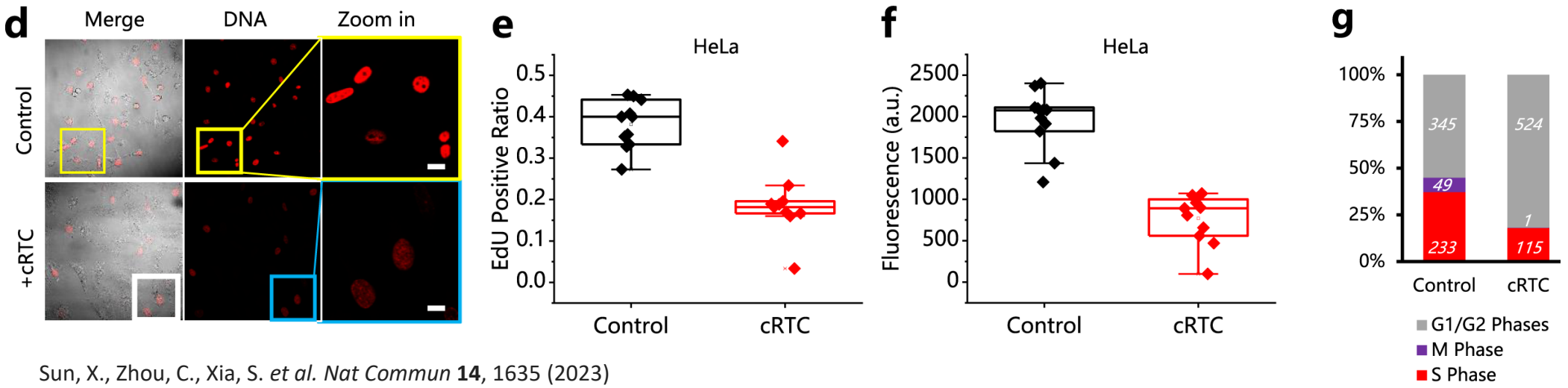
- The overall ligations rapidly assembled cRTC within 24 hours
- The free cysteine residue in the CAAX-box that is a requisite for prenylation was kept intact during the entire ligation course

SNACIP for modulating the endogenous unligandable protein

- ✓ **cRTC inducer clearly targeted cytosolic hTPX2 to the PM region in live HepG2 cells**



SNACIP for modulating the endogenous unligandable protein



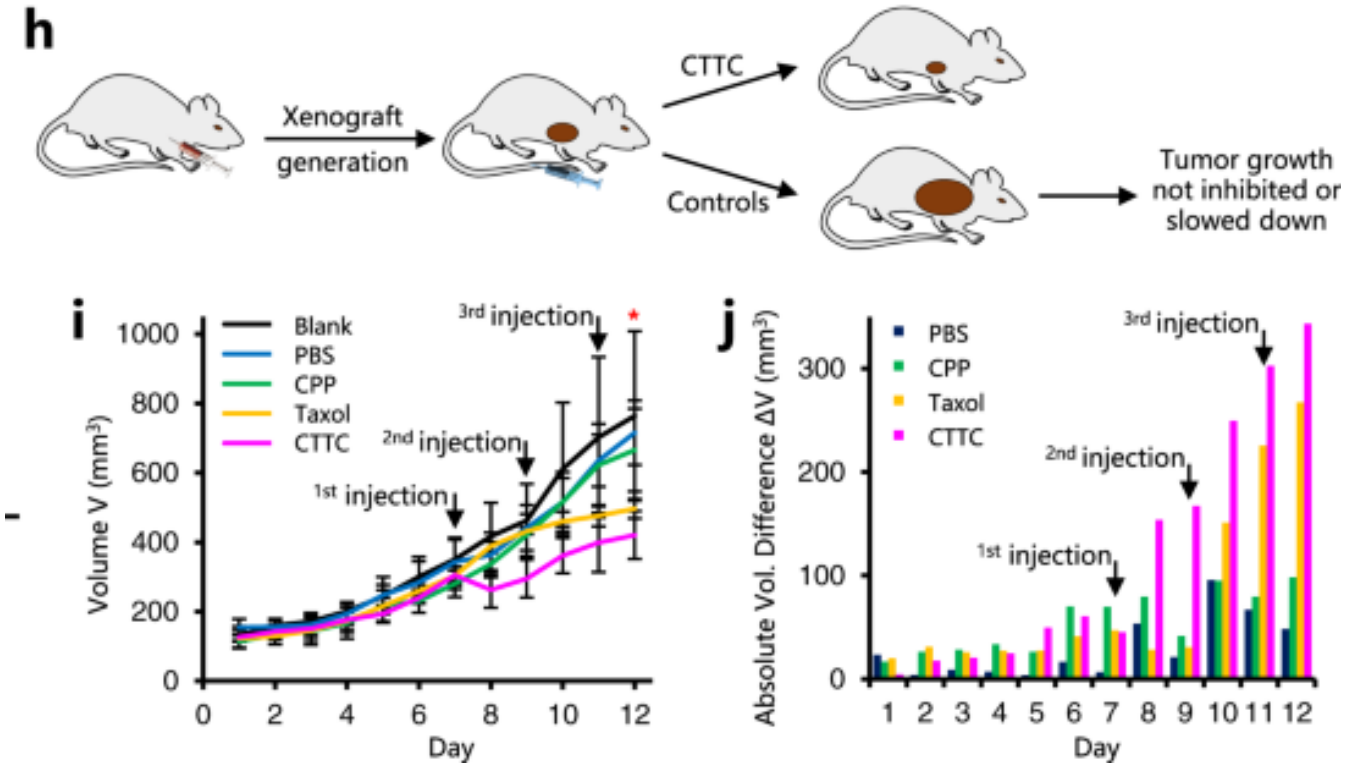
Sun, X., Zhou, C., Xia, S. *et al. Nat Commun* **14**, 1635 (2023)

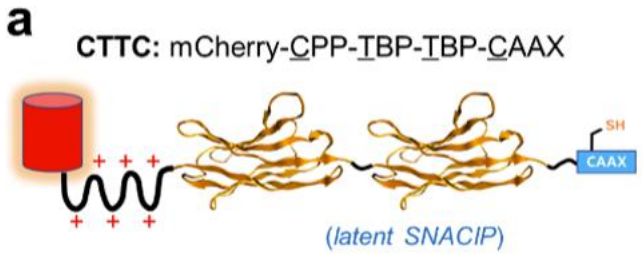
- ✓ Reduced proliferation activities were observed for cRTC-treated HeLa cells.
- ✓ S-phase ratio decreased significantly while M-phase was almost completely disappeared after cRTC treatment.

→TPX2 SNACIP inducers effectively inhibit cancer cell proliferation via blocking cell cycle progression to M-phase

SNACIP for modulating the endogenous unliganDable protein

TPX2 SNACIP inducers effectively suppress hepatocarcinoma cell tumor growth in vivo.





→highlighting the value of SNACIP in modulating endogenous undruggable targets for drug development.

Sun, X., Zhou, C., Xia, S. *et al. Nat Commun* **14**, 1635 (2023)

Short Summary

SNACIPs are valuable proximity inducers for regulating cellular functions.

- The presence of a nanobody binding module enables direct modulation of FP-fused proteins or endogenous targets.

- Latent-type SNACIPs including cRTC are functionally assembled inside living cells.

  Cancer cell proliferation is inhibited and tumor growth is suppressed in vivo through localizing hTPX to PM.
  →Demonstrating the value of SNACIP to modulate endogenous oncogenic unligandable targets for therapeutic intervention.

Contents

Summary

SNACIPs : Next generation proximity inducers

- ✓ Introducing nanobodies can control intracellular protein localization by modulating endogenous unligandable targets

TRAMs : Small molecules coupling the trafficking of a target protein to the trafficking of a shuttle protein

- ✓ Relocating mislocalized proteins to their proper places and correcting diseased phenotypes
- ✓ Protein relocalization to drive a gain-of-function phenotype

Remaining challenges

- The stoichiometric mode of action of TRAMs also requires higher expression of a shuttle protein compared with a target protein, which might limit the scope of potential targets for relocalization.

- As most warheads used for bifunctional molecule development are inhibitors, available ligands might not be suitable to fully realize the potential of TRAMs due to inhibition of effector functions.

→Developing non-inhibitory binders of target proteins is needed.

- Identification of appropriate targets and shuttles for a desired phenotype is still needed.