New Tools for Targeted Protein Degradation

Technical Journal Club

Daniel Heinzer

March 13th 2018

Current methods for modulation of protein concentrations

Nucleic Acid-based agents:

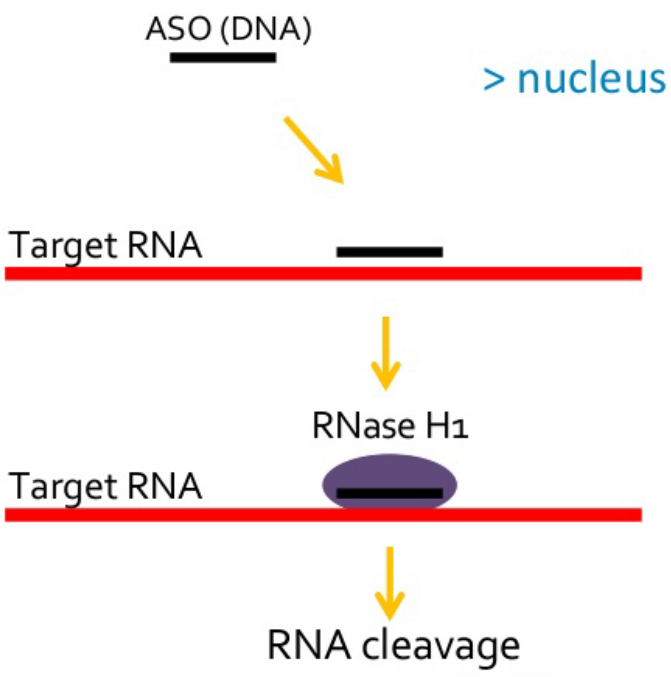
- Antisense Oligonucleotides (ASO)
- RNA interference (siRNA, miRNA, shRNA)
- CRISPR/Cas9

Ligand-based agents

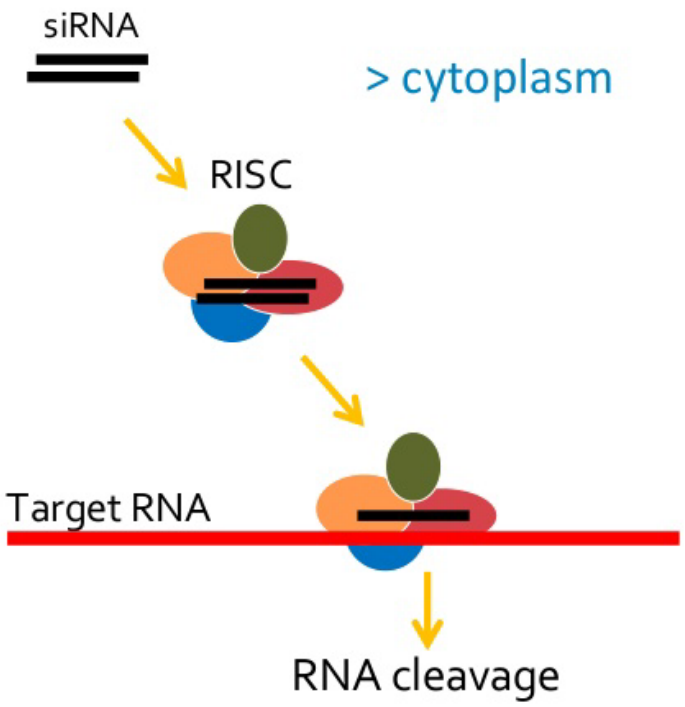
Antibody-based degradation

Modulation of protein concentration: Nucleic Acid-based agents

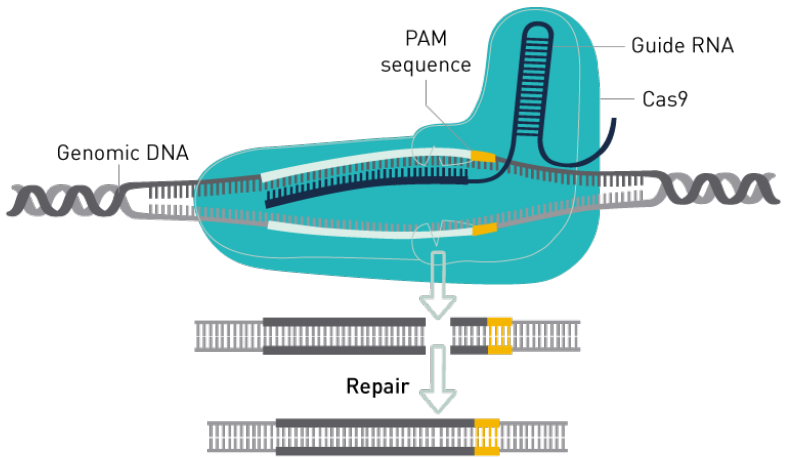
Antisense oligonucleotides (ASOs)



RNA interference (RNAi)

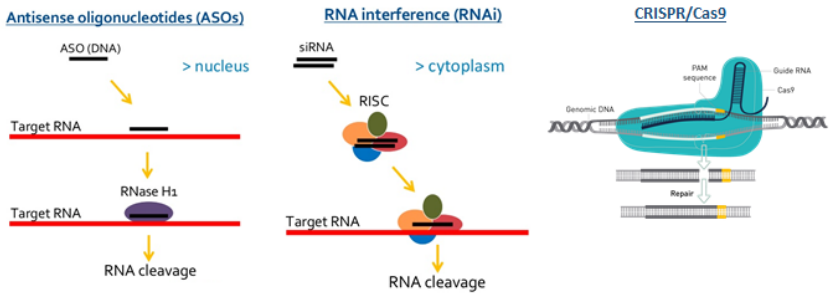


CRISPR/Cas9



Drawbacks of Nucleic Acid-based agents

Drawbacks



- **Delayed effect**
  - Time for compensatory mechanisms to kick in
  - Possible secondary, non specific defects can accumulate over time
- **Problematic for proteins with low turnover**
- **Off-target effects, especially for RNAi**
- **RNAi for some cell types unfeasible**
  - e.g. primary macrophages due to nucleotide sensing machinery

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Modulation of protein concentration: Ligand-based agents

1. **HSP90 inhibitors (overactive in cancer cells)**

   Targeting of HSP90 ATP-binding domain leading to the *degradation of HSP90 client proteins*

   Extremly unspecific regulation of protein concentration via HSP90 ligand

2. **Selective estrogen receptor degraders (SERDs) and selective androgen receptor degraders (SARDs)**

   Compounds were originally developed as modulators of protein function, but were subsequently serendipitously discovered to be *protein degraders*

   Can specifically downregulate hormone receptors by inducing structural changes that lead to increased surface hydrophobicity and subsequent degradation

Modulation of protein concentration: Ligand-based agents

3. Hydrophobic tagging (HyT)

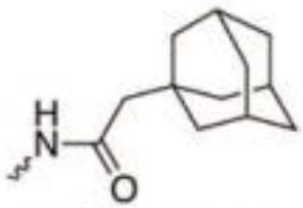
bifunctional molecules with hydrophobic tag

Two propose

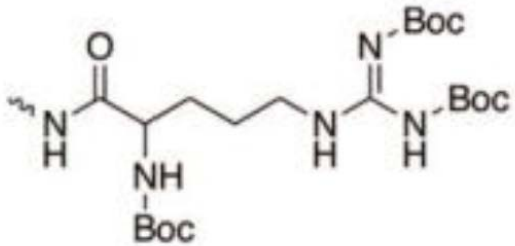
-HyT destabi st, thereby recruiting e es to the unfolded pro

or

-chaperones ectly and mediate prot

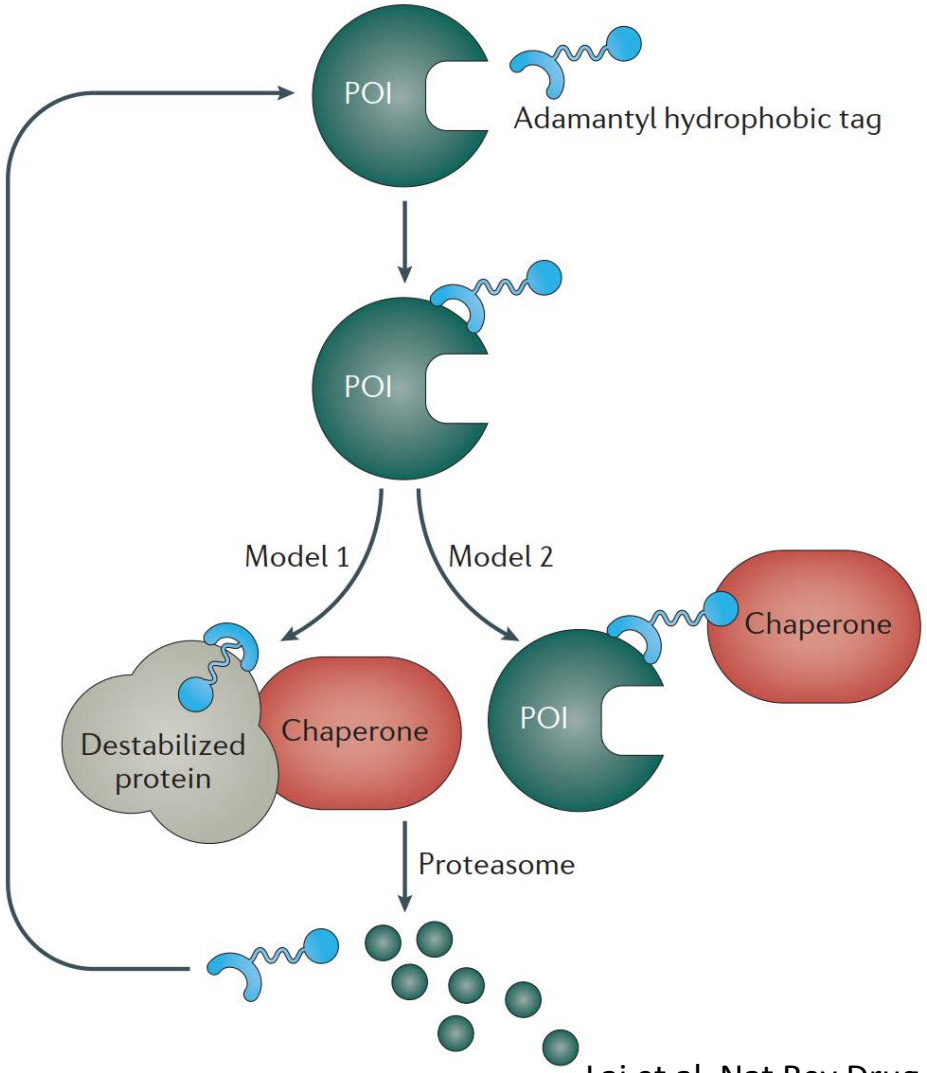


Adamantane (HyT)[111]



Arg-Boc$_3$ (HyT)[108]

<u>Modular: Pr d, as long as a ligand t entified</u>



Lai et al, Nat Rev Drug Discov., 2017

Modulation of protein concentration: Ligand-based agents

4. proteolysis-targeting chimaera (PROTAC)

bifunctional PROTAC molecules

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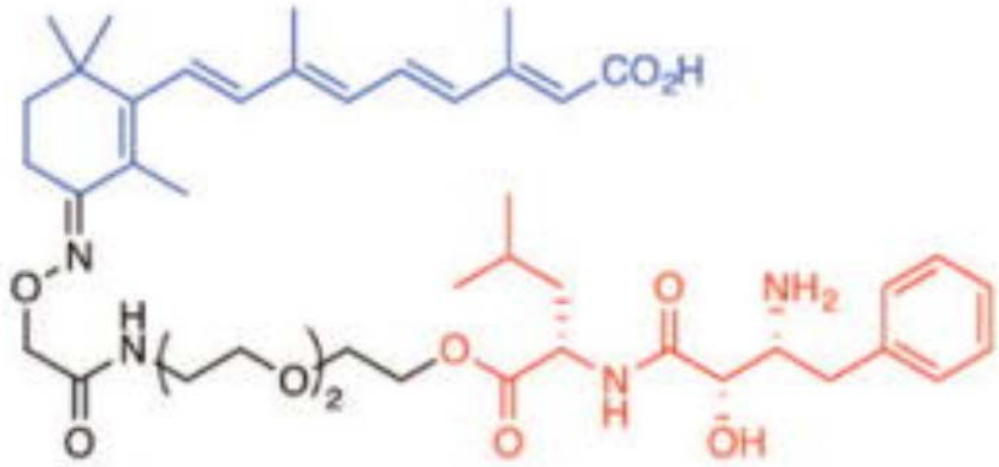
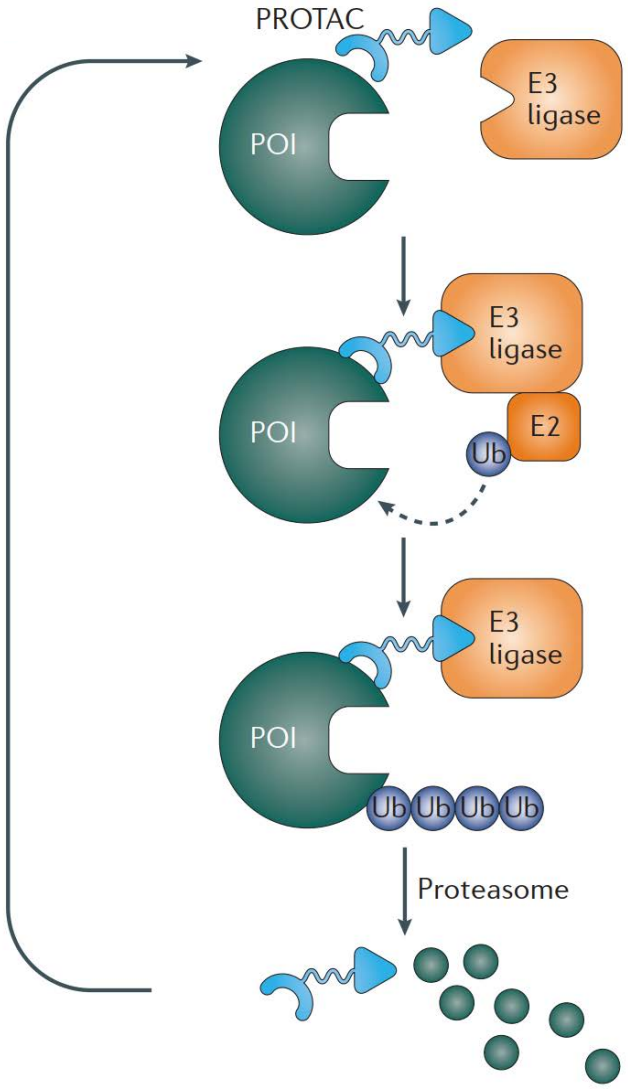


cIAP-based PROTAC
(CRABP-II-targeting)[139]

If

dissociate again and degrade another POI (substochimoetric)



Lai et al, Nat Rev Drug Discov., 2017

Example for PROTAC mediated protein degradation

Article

Cell Chemical Biology

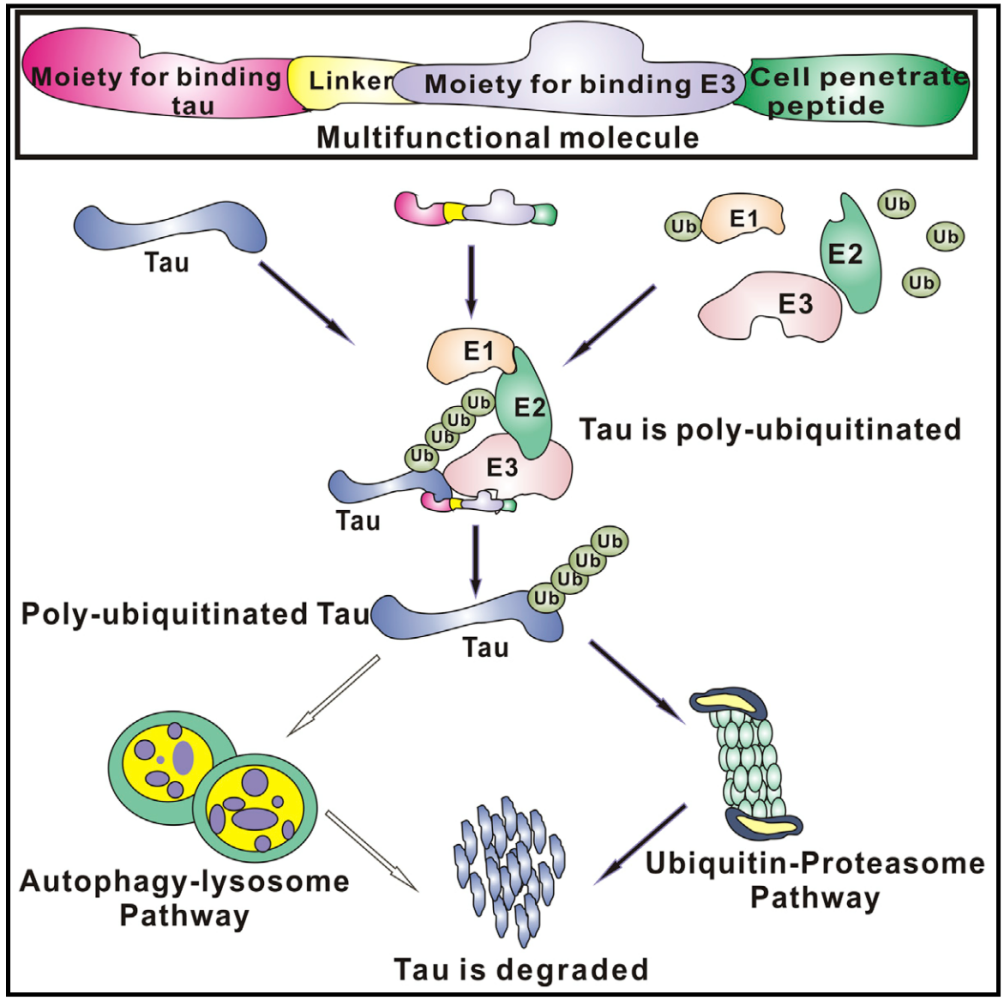
Specific Knockdown of Endogenous Tau Protein by Peptide-Directed Ubiquitin-Proteasome Degradation

Authors

Ting-Ting Chu, Na Gao,
Qian-Qian Li, ..., Yong-Xiang Chen,
Yu-Fen Zhao, Yan-Mei Li

Example for PROTAC mediated protein degradation

Graphical Abstract

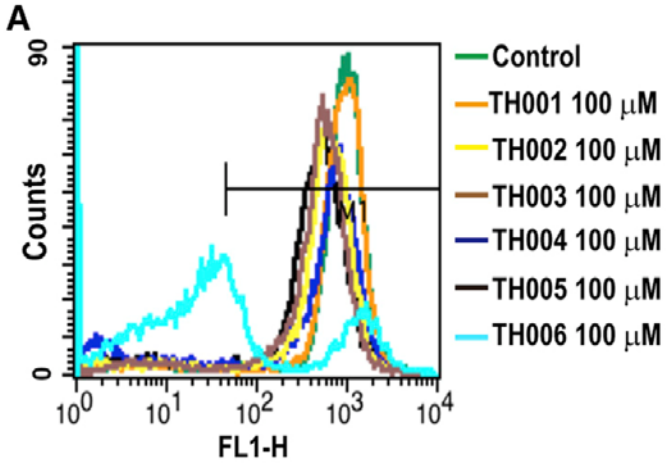


Six different multifunctional molecules consisting of:

- **Tau binding domain** (two different peptides from α- and β-tubulin known to interact with Tau)
- **Linker** (GSGS to increase flexibility)
- **Moiety for binding E3** (two different peptides based on the substrate of two E3 ligases)
- **Cell penetrating peptide** (Poly-D-Arginine)

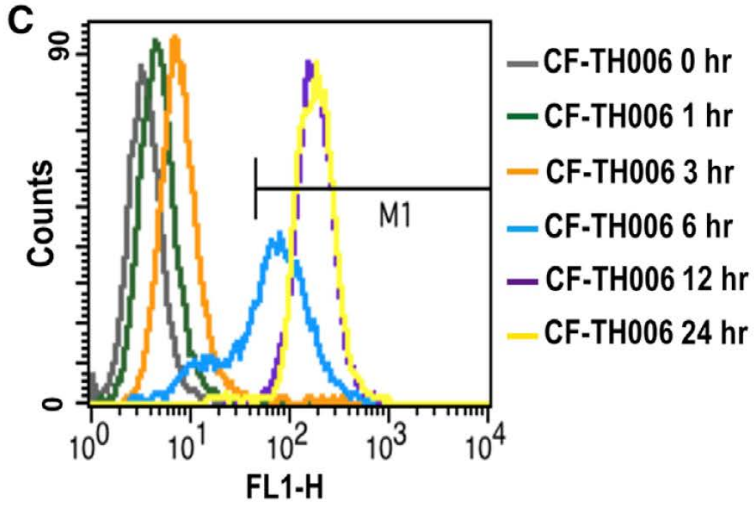
have been assessed with regards to their Tau degradation capabilites

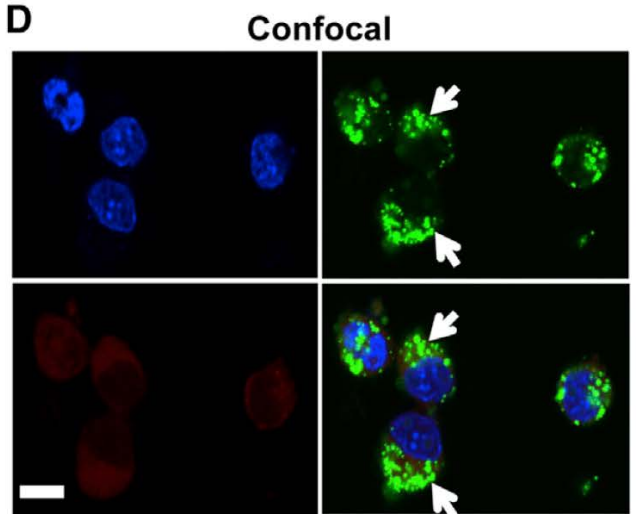
Example for PROTAC mediated protein degradation



Tau-EGFP expressing cells showed decrease in EGFP intensity after treatment with TH006

=> TH006 has been chosen for further investigations

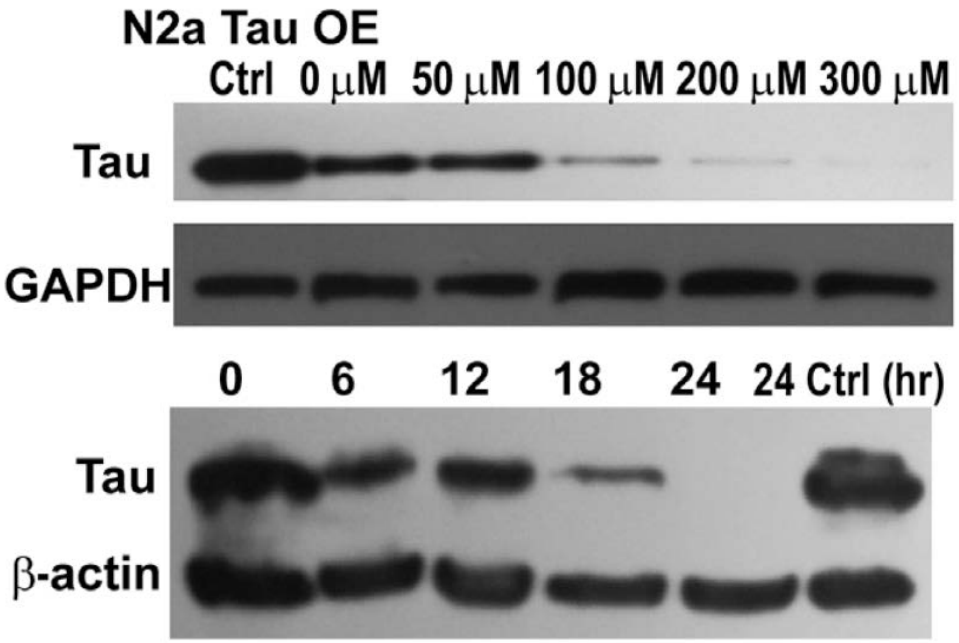




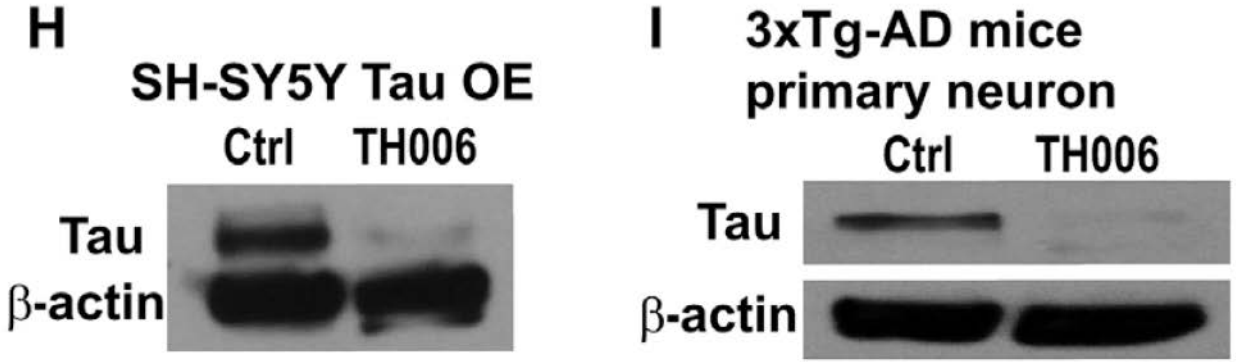
CF-TH006 can enter into wild type N2a cells and colocalizes with Tau

Green: CF-TH006 (TH006 labeled by 5(6)-carboxyfluorescein CF)
Red: Tau
Blue: Nucleus

Example for PROTAC mediated protein degradation

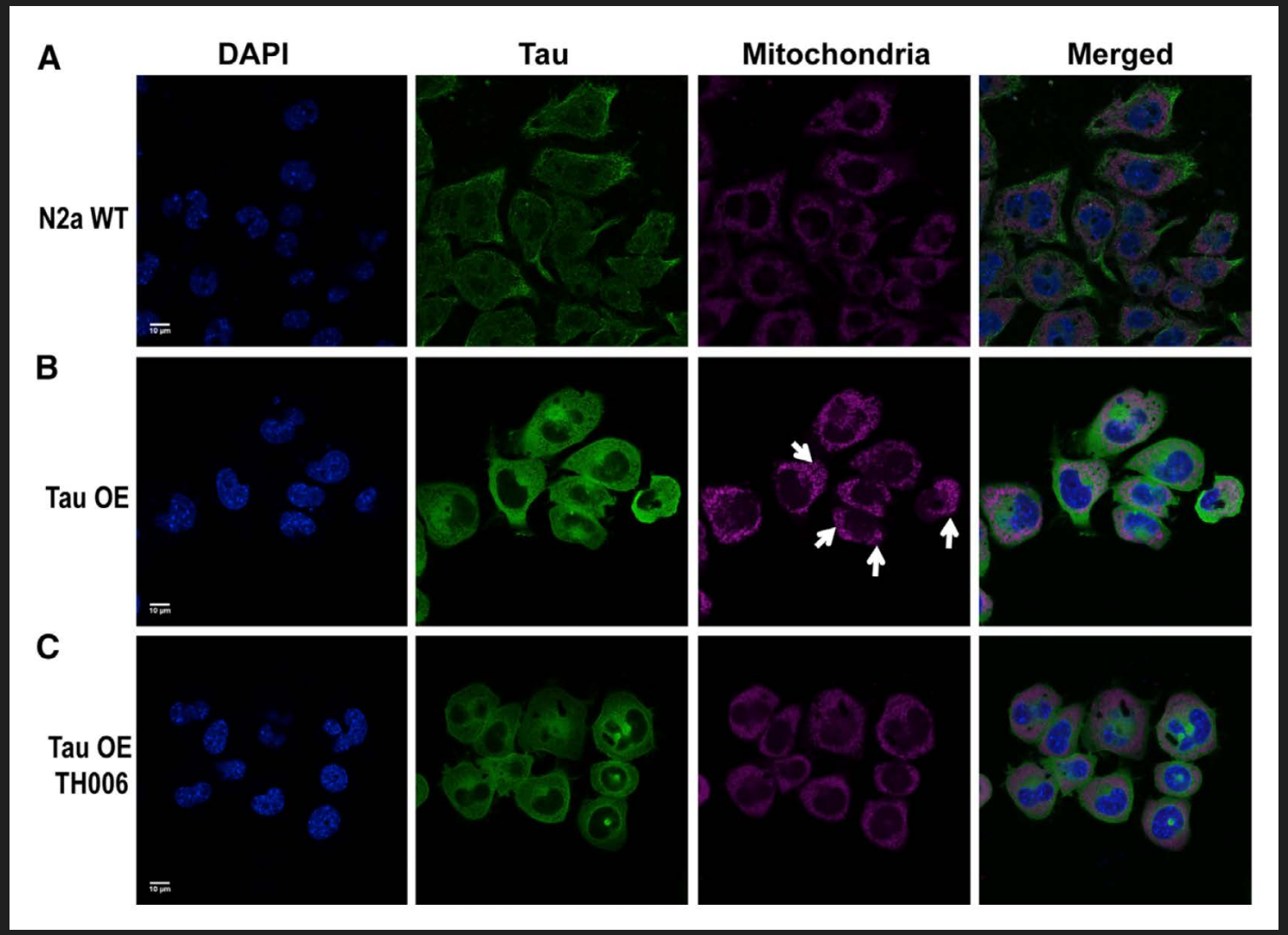


Tau overexpressing N2a cells showed decrease in Tau levels with increasing amount of and prolonged exposure to TH006



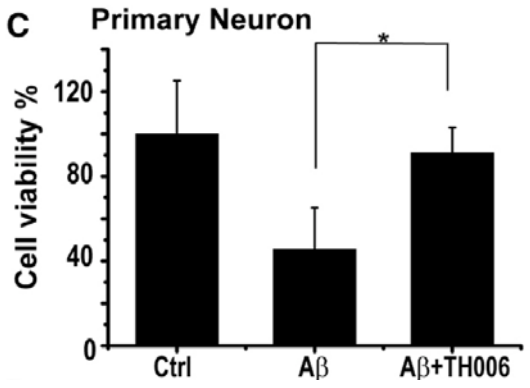
TH006 was able to reduce Tau levels in SH-SY5Y Tau overexpressing cells and primary neuron cells derived from 3xTg-AD mice

Example for PROTAC mediated protein degradation



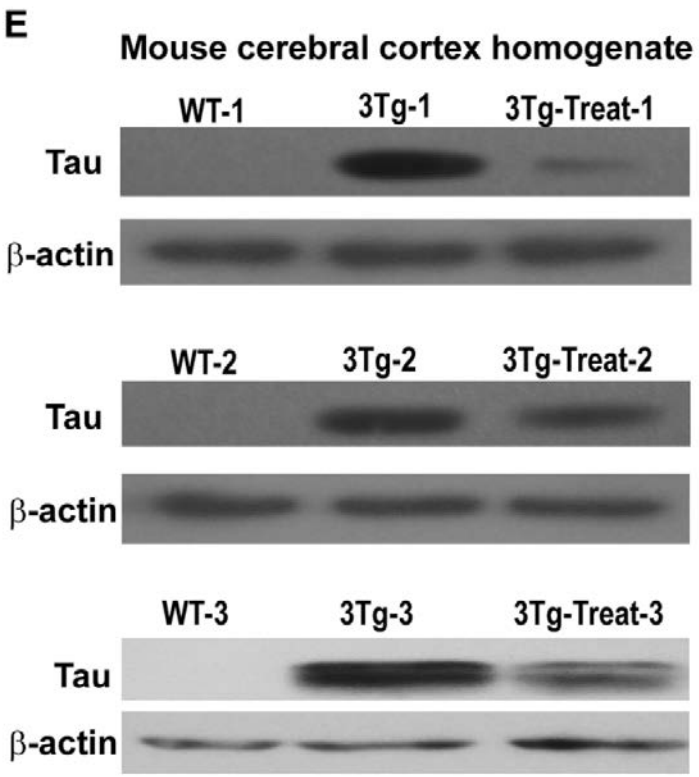
TH006 was able to reduce Tau levels in N2a Tau overexpressing cells and could restore the even distribution of the mitochondria

Example for PROTAC mediated protein degradation



Aβ42 induced neurotoxicity in cultured primary hippocampal neurons could be reduced by treatment with TH006

Intranasal administration combined with intravenous injection of TH006 for 10 days promoted degradation of Tau in cerebral cortex regions of 3xTg-AD mice



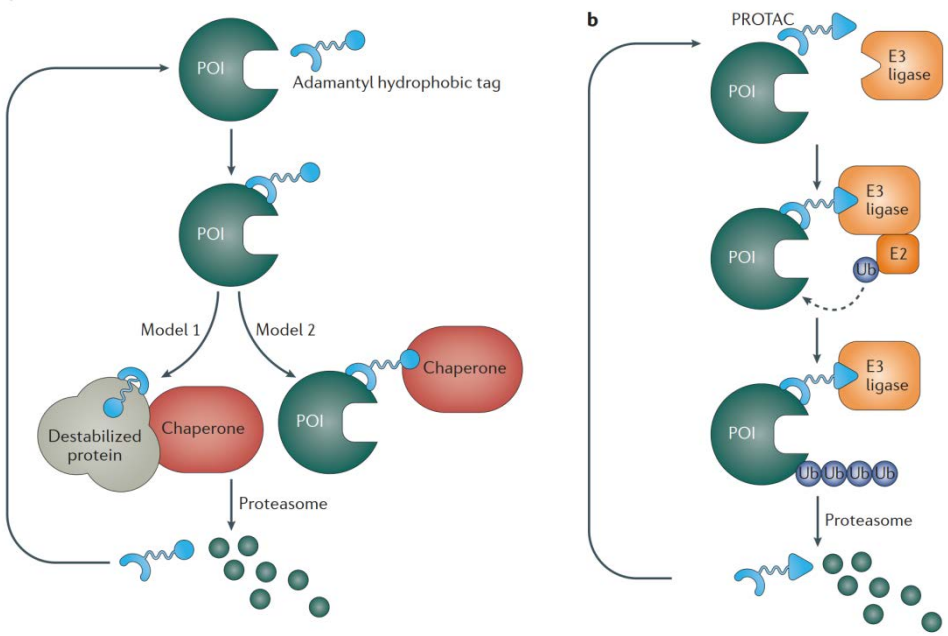
Summary Paper 1

- Specific knockdown of Tau protein by peptide-directed ubiquitin proteasome degradation
- Description of *in vivo* targeted induced protein degradation by a PROTAC
- Paving the way for a new approaches with synthetic molecules which might facilitate the development of effective therapeutics for AD.

Drawback of Ligand-based agents

Drawback

- **Only applicable to proteins with known and specific ligands**



Current methods for modulation of protein concentrations

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- CRISPR/Cas9

Ligand-based agents

Antibody-based degradation

New approach for targeted protein degradation

Resource

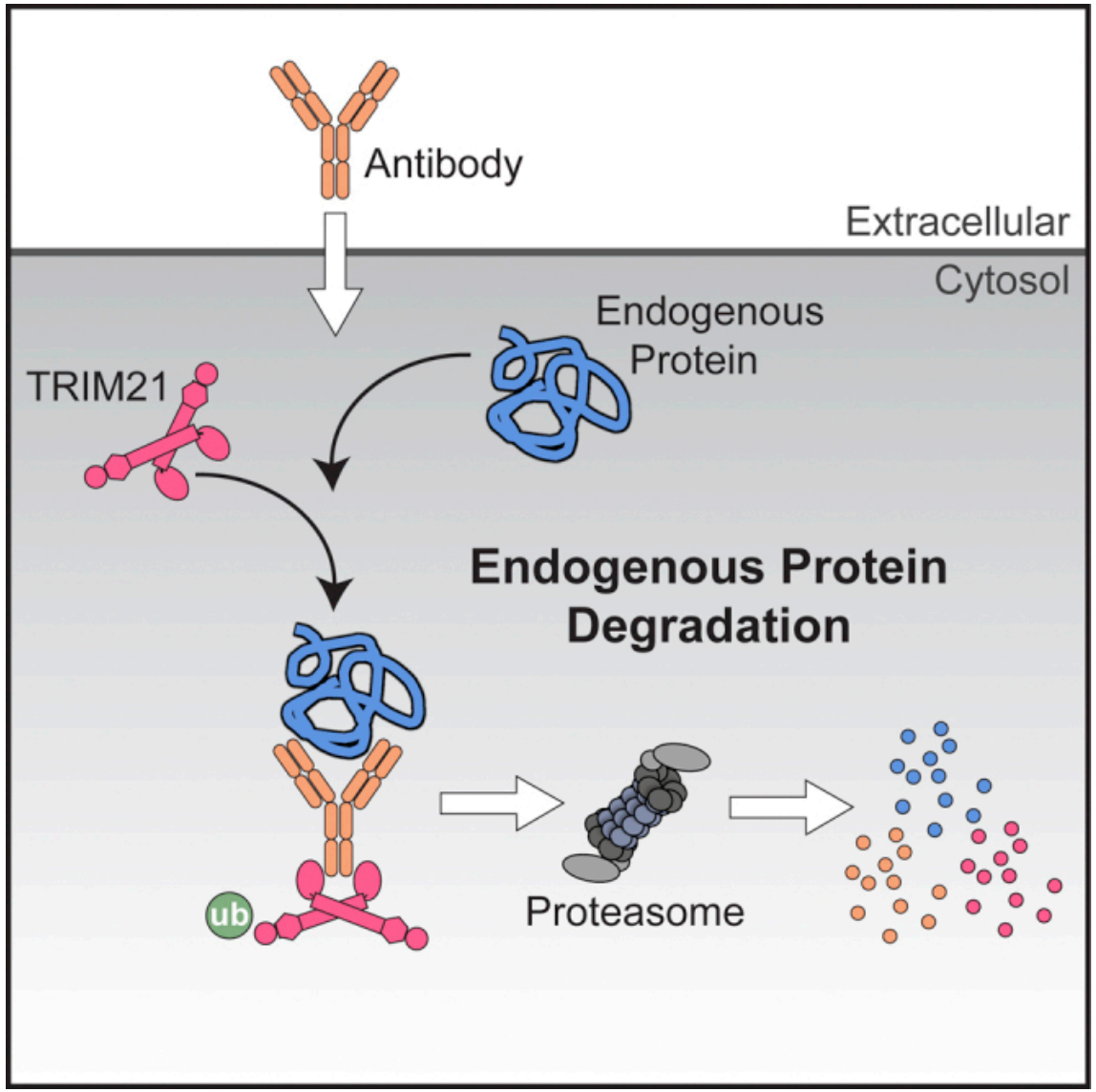
Cell

A Method for the Acute and Rapid Degradation of Endogenous Proteins

Authors

Dean Clift, William A. McEwan, Larisa I. Labzin, Vera Konieczny, Binyam Mogessie, Leo C. James, Melina Schuh

Trim-Away



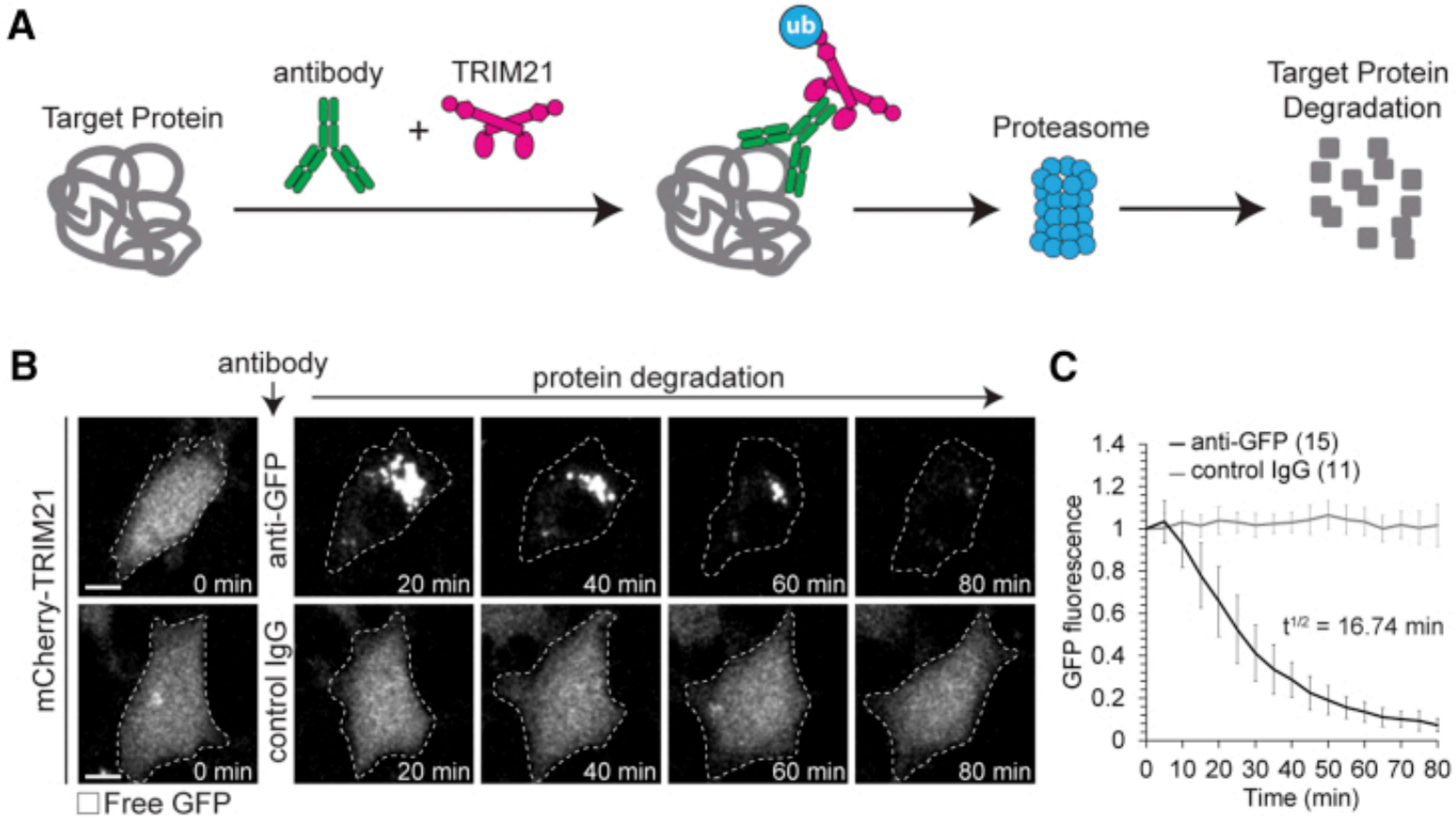
Trim-away harnesses the cellular protein degradation machinery to remove unmodified native proteins within minutes of application:

- Post-translational protein depletion method based on protein targeting by **antibodies**
- **TRIM21**, an E3 ubiquitin ligase binds with high affinity to the Fc domain of antibodies

TRIM21

- **TRIM21** is an cytosolic IgG receptor in innate immunity and acts as a E3 ubiquitin-protein ligase
- Here, TRIM21 was repurposed to establish a method to degrade endogenous proteins

https://www.mybiosource.com/products.php?keywords=SSA1+Ro52&type=Recombinant+Protein



Principle of Protein Degradation by Trim-Away

Schematic of the approach

NIH 3T3 cells overexpressing mCherry-TRIM21 (not shown) and free GFP (greys) were microinjected with anti-GFP antibody or control IgG

Principle of Protein Degradation by Trim-Away

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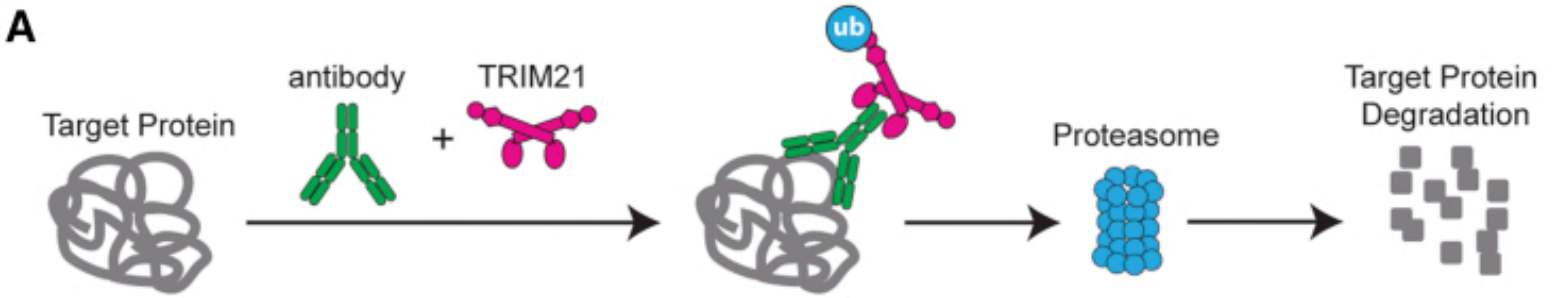
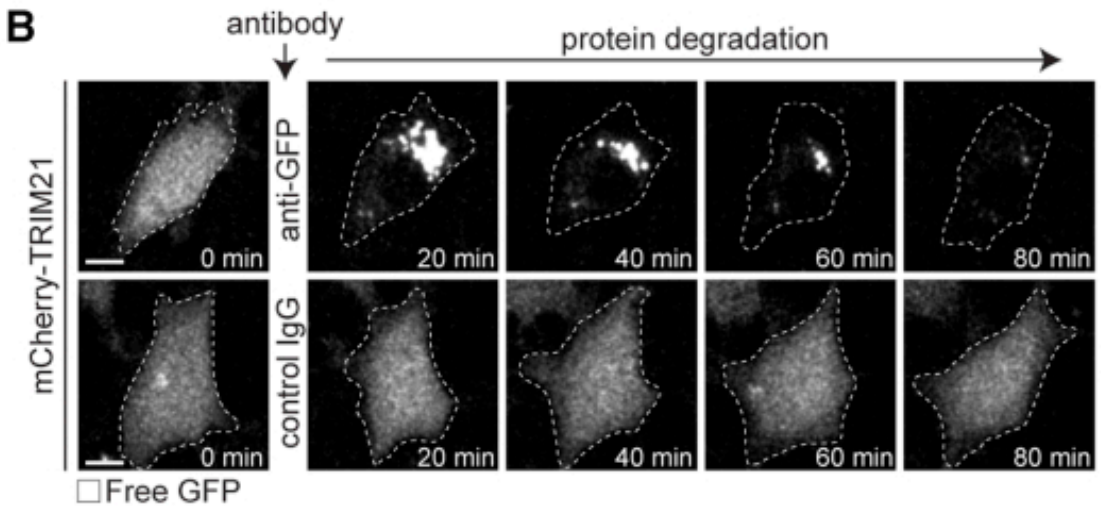
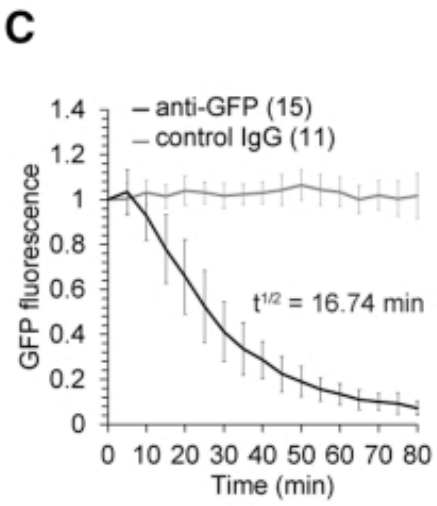
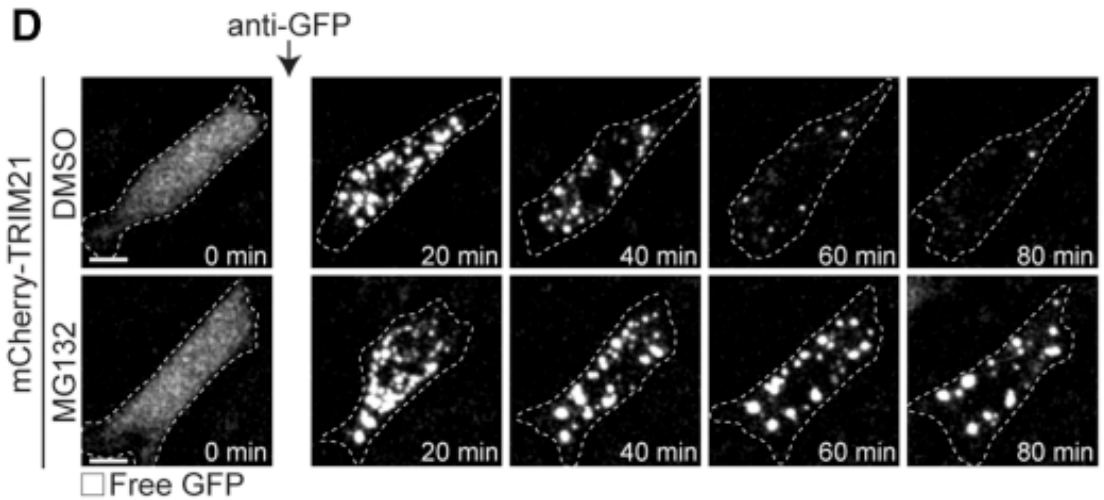


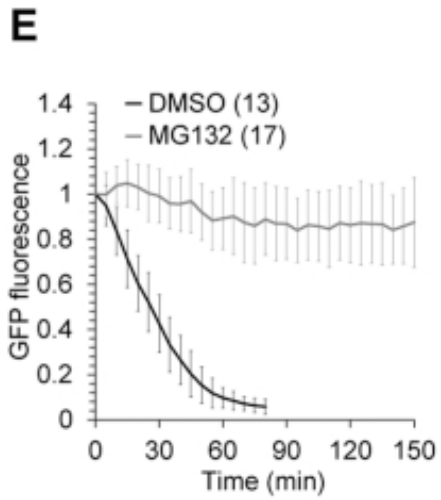




NIH 3T3 cells overexpressing mCherry-TRIM21 (not shown) and free GFP (greys) were microinjected with anti-GFP antibody or control IgG

or





treated with DMSO or MG132 and microinjected with anti-GFP antibody

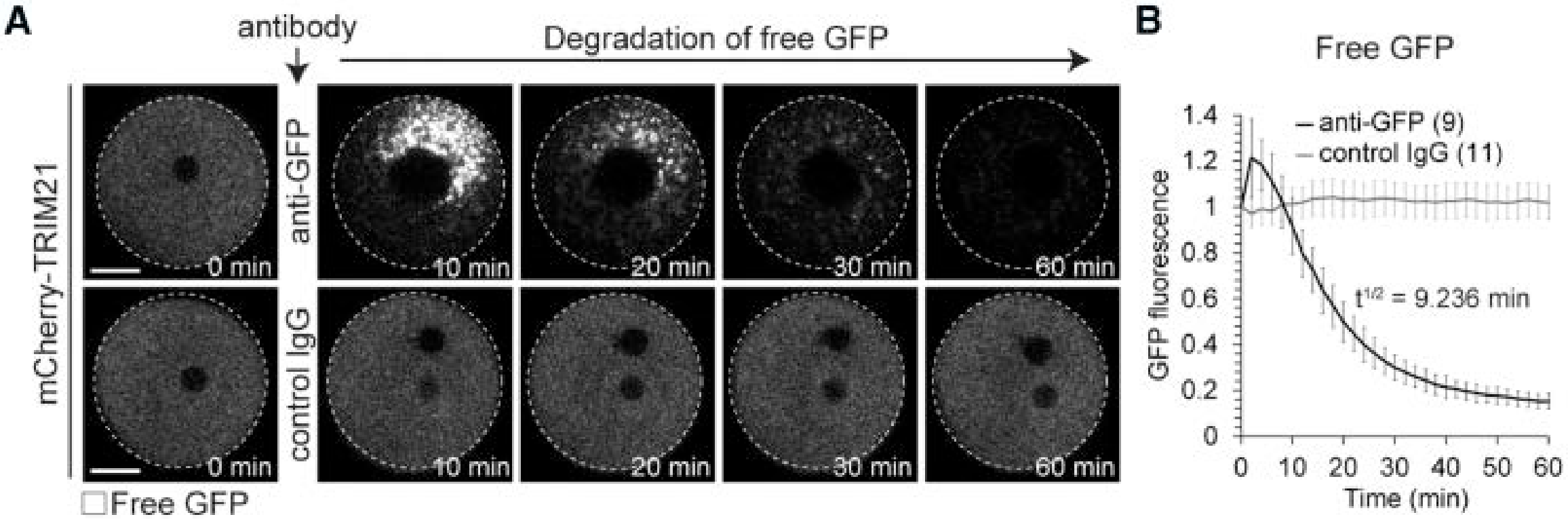
Fast degradation of GFP upon microinjection of anti-GFP antibody in Trim21 overexpressing cells using the ubiquitin-proteasome pathway

Protein Degradation in Primary Cells

Trim21 overexpressing mammalian oocytes were used, as they are transcriptionally silent, which precludes protein disruption by direct genome editing

Also, RNAi is inefficient due to large amount of stored proteins

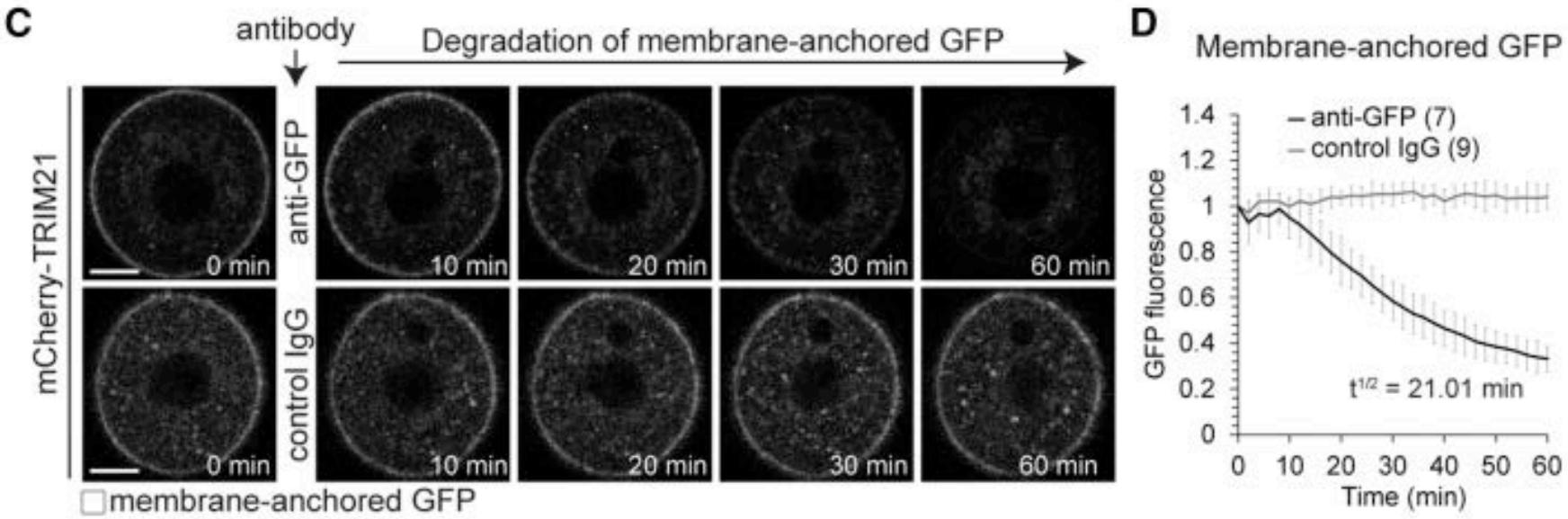
Anti-GFP antibody leads to fast degradation in Trim21 overexpressing oocytes



Trim-Away can target diverse cellular substrates

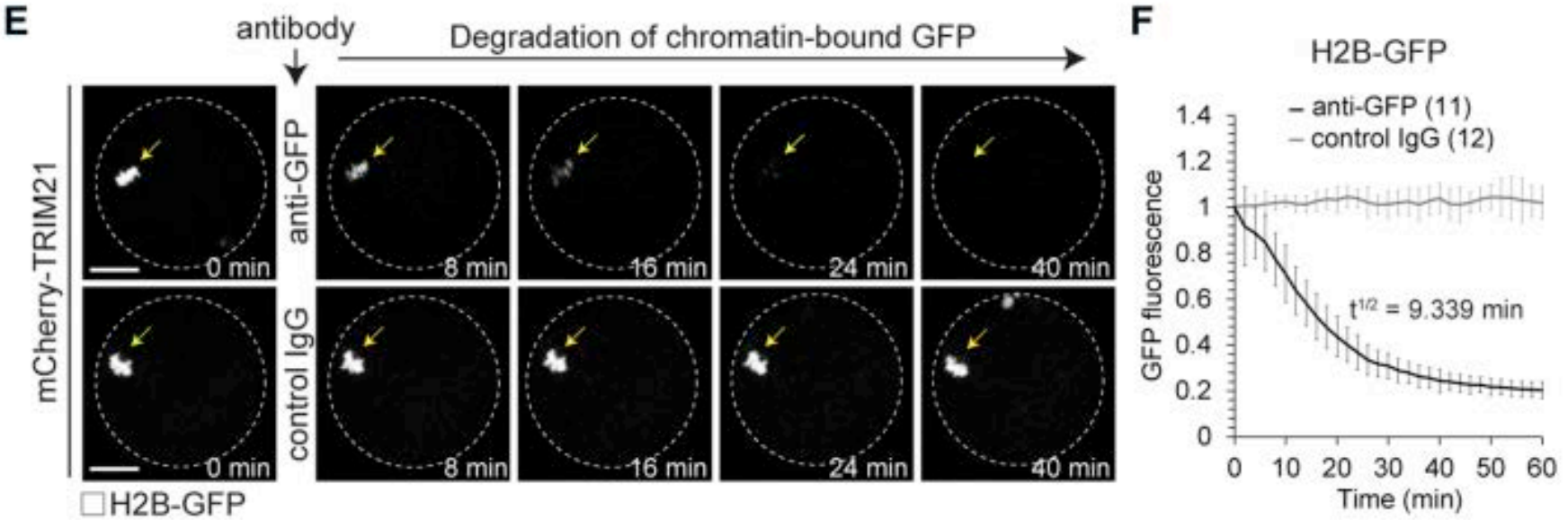
GFP was localized to different regions of the cell

GFP with N-terminal N-myristoyl and S-palmitoyl motif => membrane anchor



GFP fused to the histone H2B (in metaphase 2)

However, not degradable when located in an intact nucleus

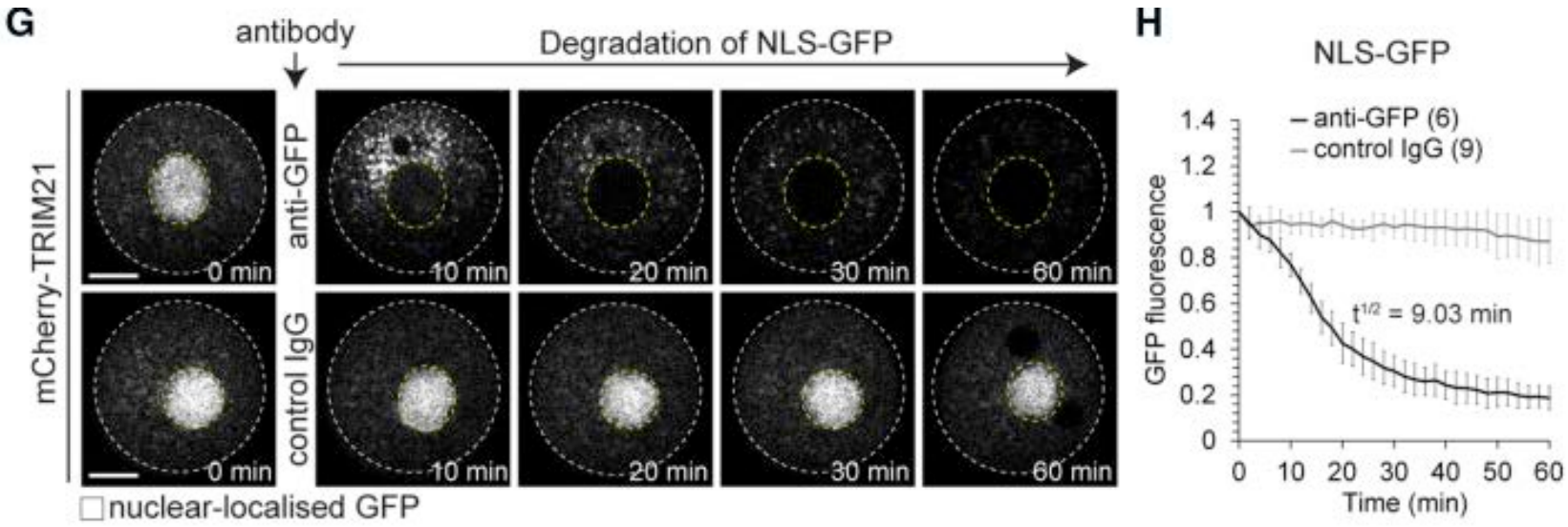


Trim-Away can target diverse cellular substrates

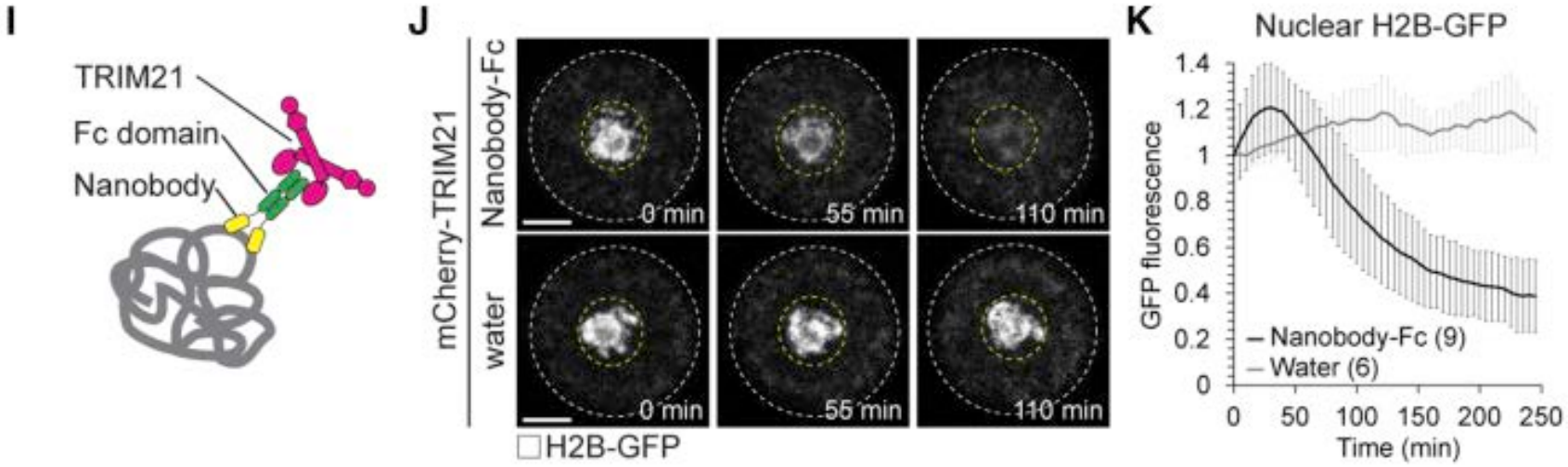
GFP was localized to different regions of the cell

GFP with a nuclear localization sequence

Degrades in the Cytoplasm

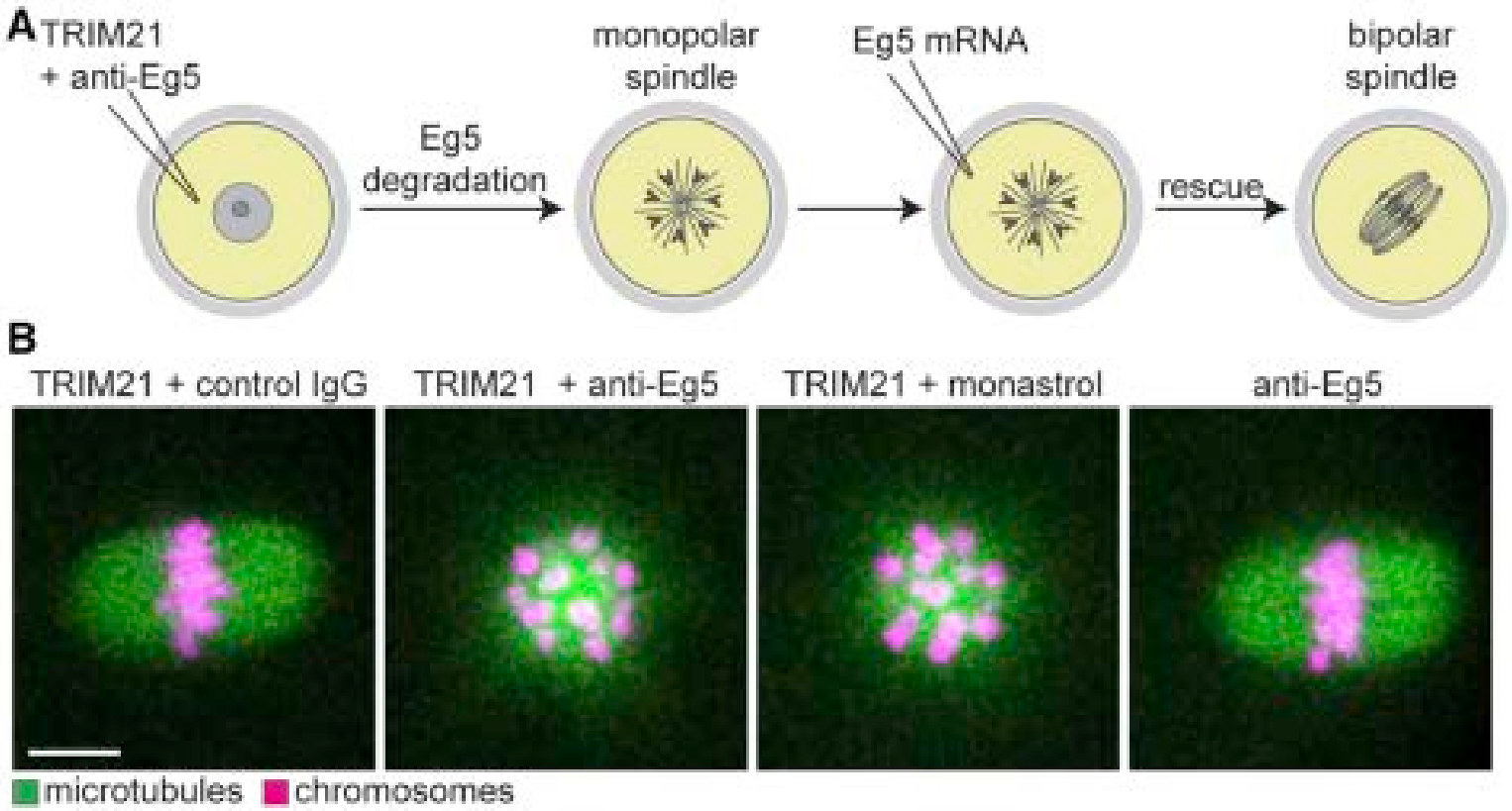


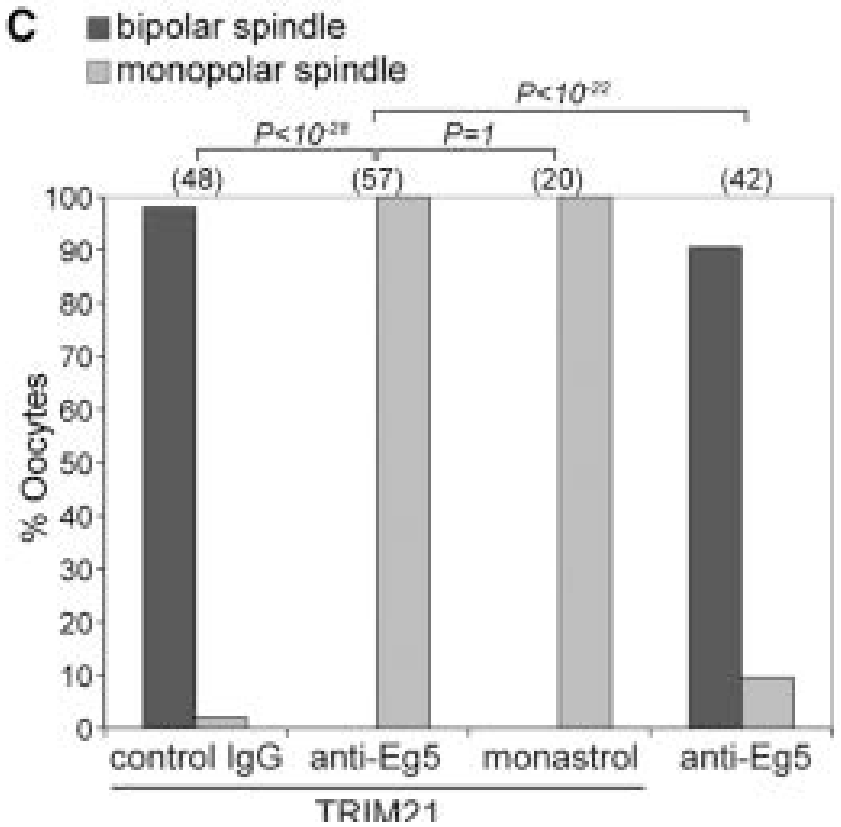
GFP-H2B within nucleus: **accessible via Fc-fused nanobodies**



Resuce experiment to confirm Trim-Away specificity

Targeting of Eg5, a microtubule associated protein required for spindle assembly during mitosis and meiosis





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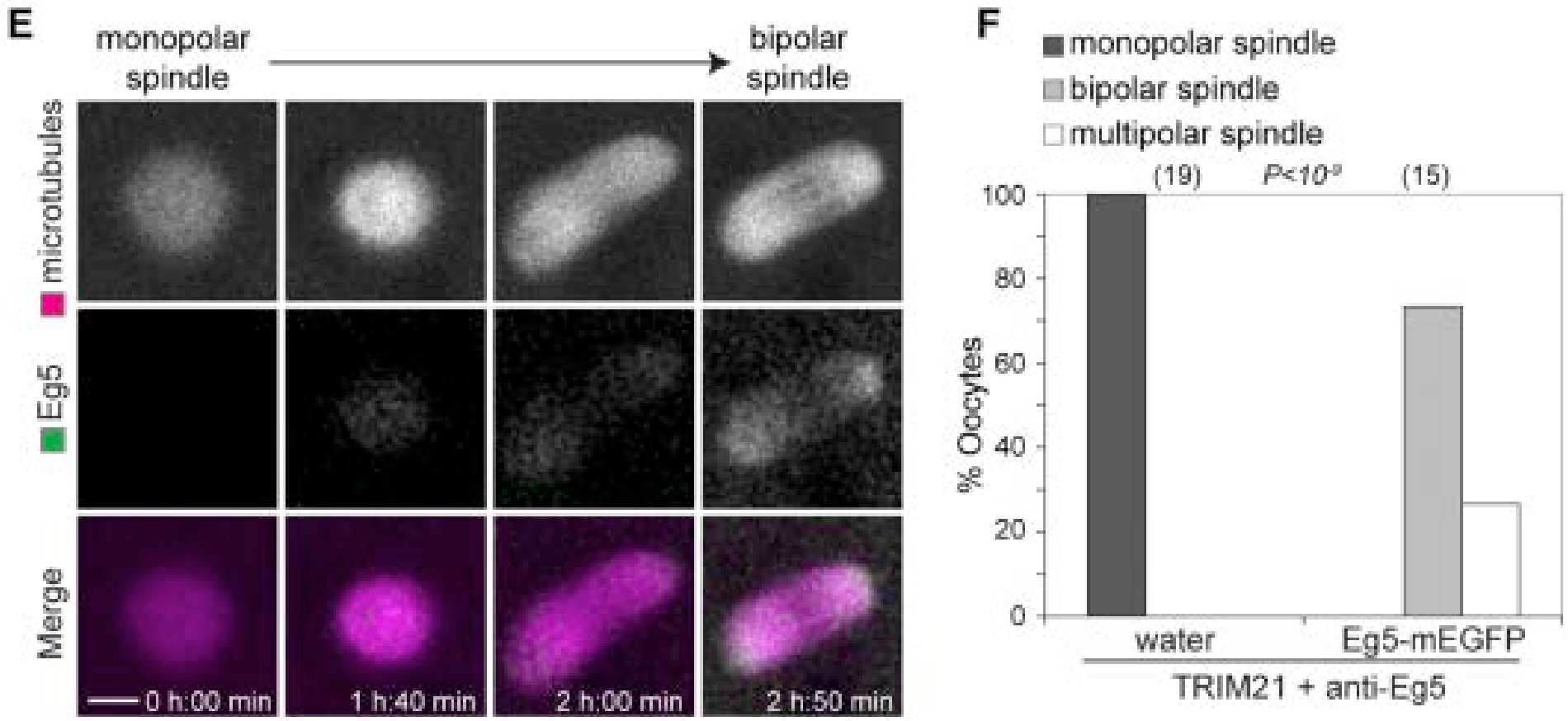
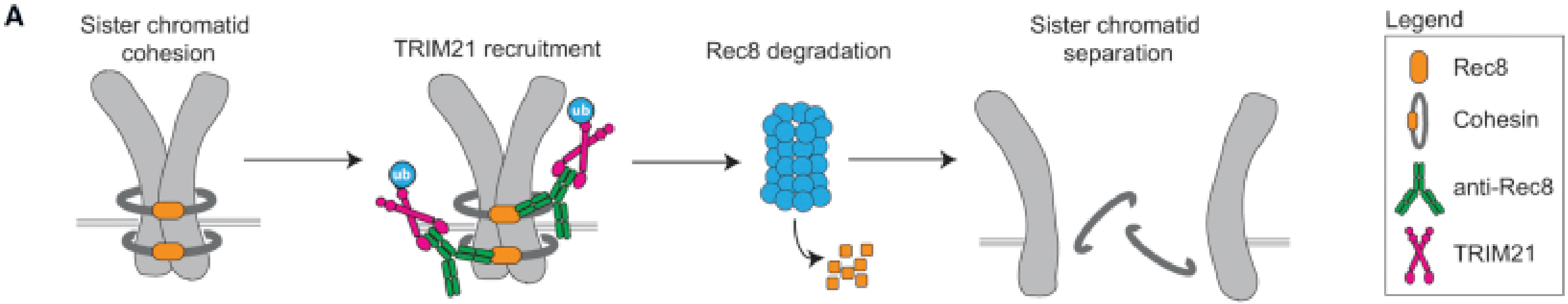


Introduction of excess Eg5 mRNA could rescue bipolar spindle formation

Trim-Away is suitable to degrade long-lived proteins acutely

Lack of protein turnover does not allow depletion by RNAi. Knockout models of long-lived proteins mostly lethal. Rec8 is part of the cohesin protein complex that mediates sister chromatid cohesion in oocytes from birth until ovulation (no turn-over!).



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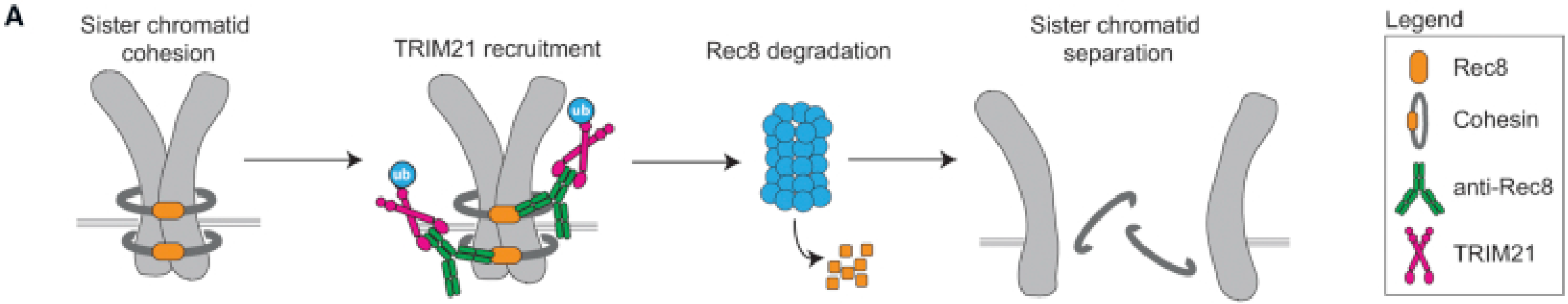
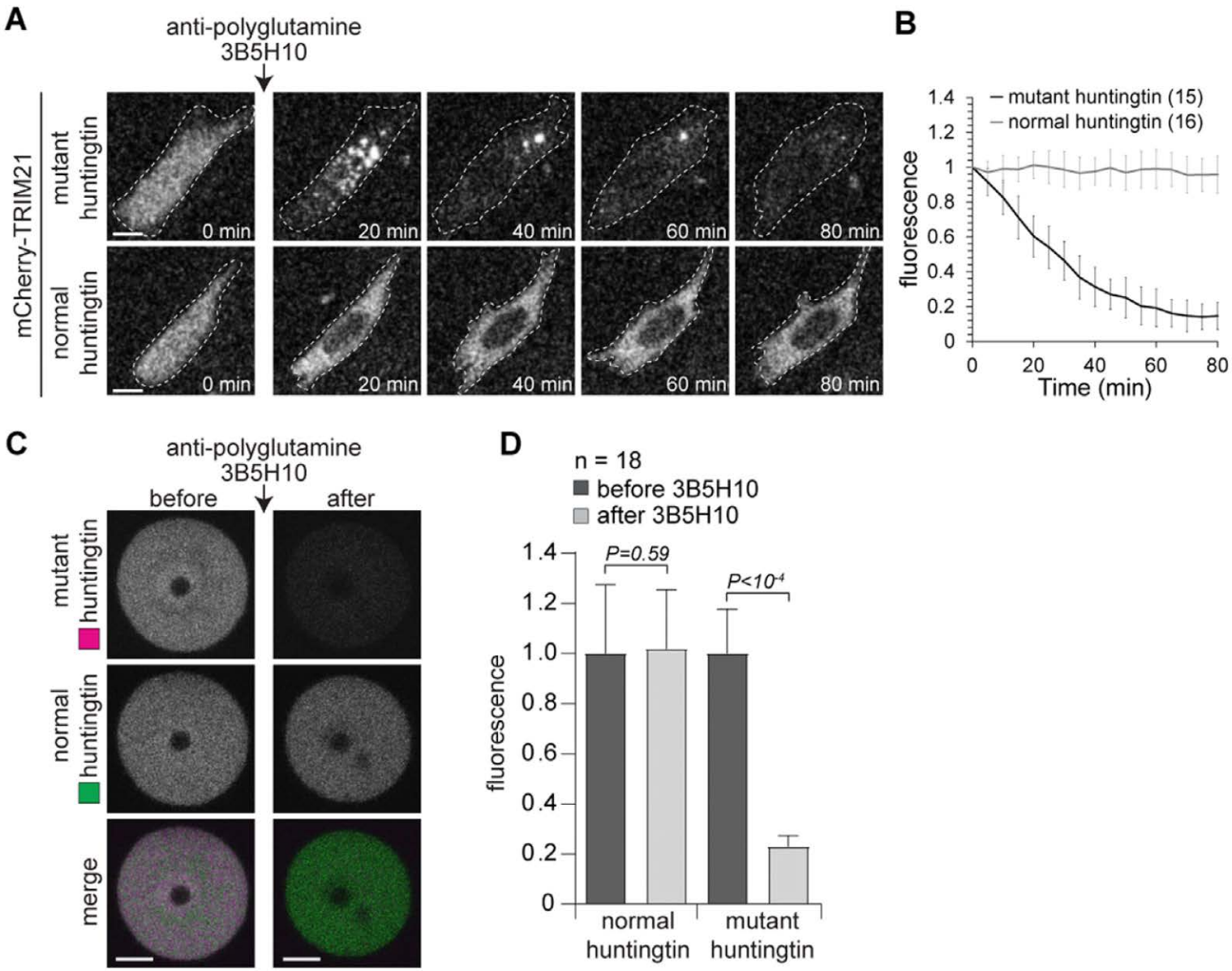


Sister chromatids began to separate on average just 8 min after anti-Rec8 antibody into mEGFP-Trim21 overexpressing eggs.

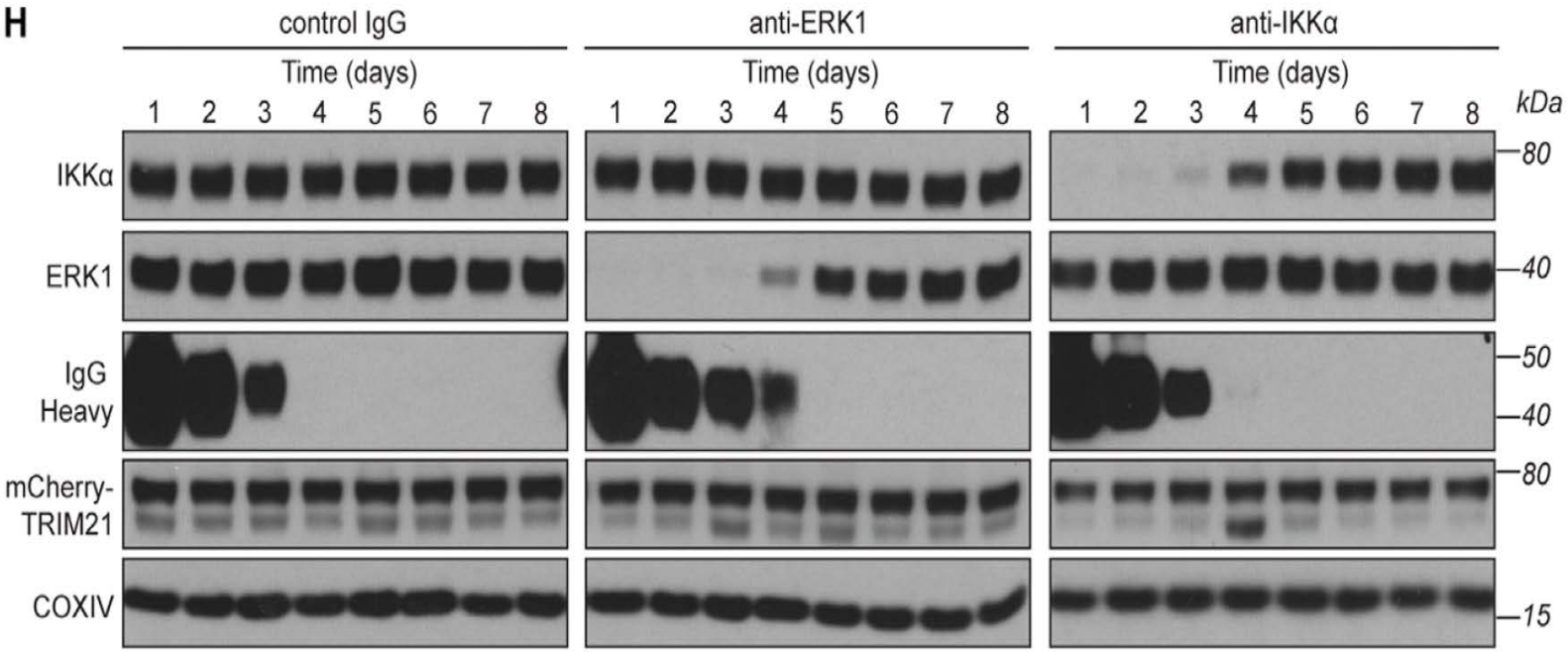
=> Trim-Away can degrade very long-lived proteins with unprecedented speed.

Trim-Away can be used to degrade specific protein variants selectively



An Antibody specific to the disease-causing variant of huntingtin could specifically degrade the mutated protein, whereas the nurmal protein was preserved

Antibody electroporation allows Trim-Away in bulk cell populations



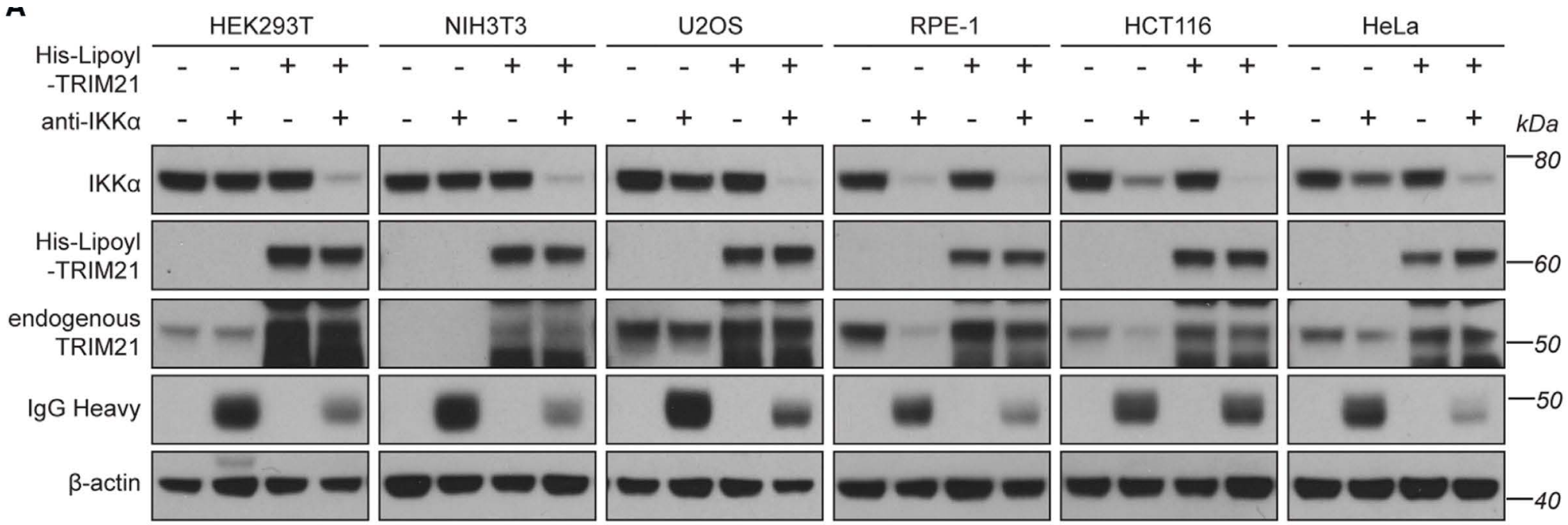
HEK293 T cells

Authors created an optimal electroporation protocol for bulk cells, without significant cell loss and normal continuation of the cell cycle.

Protocol was exploited to electroporate antibodies into cell lines overexpressing TRIM21

ERK1 and IKKa were depleted within 1–2 hr of antibody electroporation into TRIM21-overexpressing cells, with depletion lasting for 3–4 days

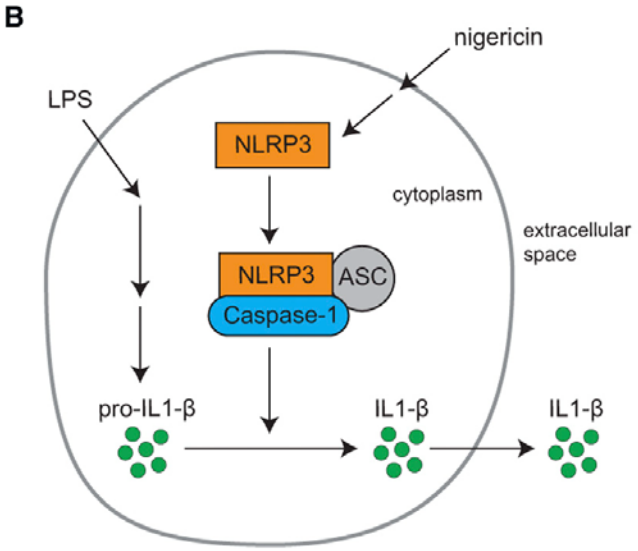
Co-electroporation of TRIM21 protein and antibody facilitate rapid protein degradation in unmodified cell lines

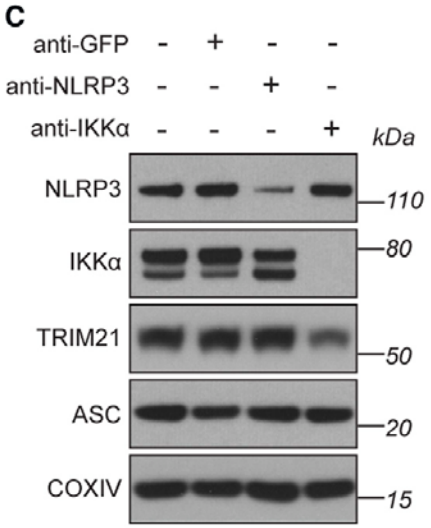


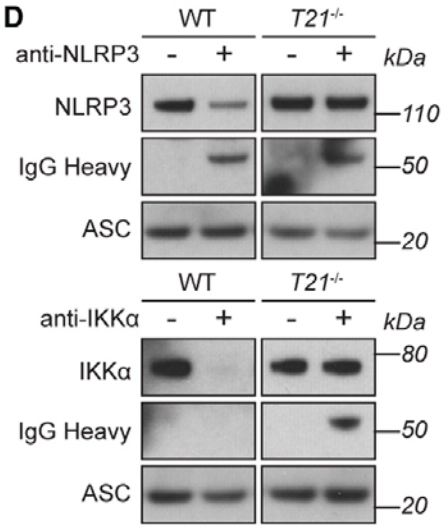
Co-electroporation of TRIM21 and antibody resulted in rapid protein degradation in every cell line assessed
For some of the cell lines, the endogenous expressed TRIM21 was sufficent to efficently donwregulate IKKa

Trim-Away by endogenous TRIM21 in human primary cells

Electroporation NLRP3 antibody into ex vivo human monocyte-derived macrophages (HMDMs), in which RNAi treatment is not possible

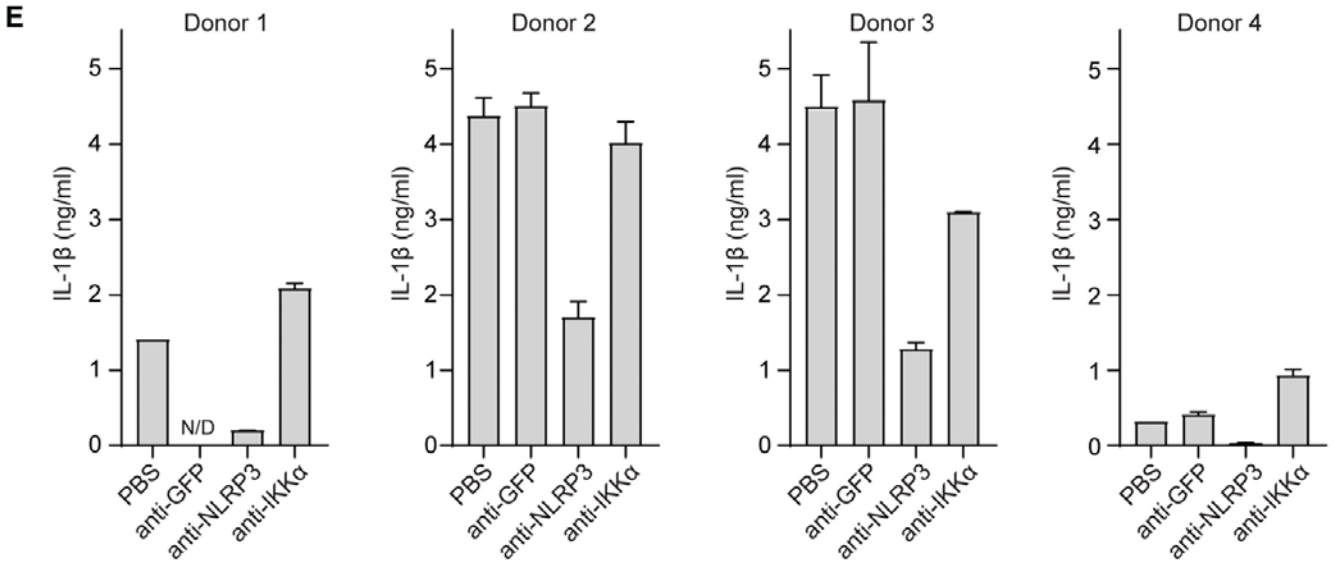






NLRP3 was targeted

NLRP3 is important for triggering of the inflammasome in response to diverse pro-inflammatory stimuli, as for example LPS



IL1-β response was measured after LPS stimulation. Trim-Away of NLRP3 reduced the amount of secreted IL1-β, showing that NLRP3 has a nonredundant and crucial role in inflammasome activation in primary human macrophages

Specificity of Trim-Away

- **Successful application of Trim-Away to 9 different endogenous proteins in 10 different cell types, without any prior modification of the target protein**

  Trim-Away is widely applicable and cell and substrate independent

- **Trim Away is highly specific, as shown in resuce experiments and by using different antibodies raised against different regions of the same protein**

  These approaches can be readily employed to confirm the specificity of Trim-Away phenotypes (non-specific antibodies should be avoided)

- **Trim-Away did not lead to the degradation of proteins in close spatial proximity of the target proteins**

  However, fate of multiprotein complexes may depend on the biology of the complex

Summary Paper 2

- Fast and efficient method to degrade endogenous proteins
- Specific (depending on the choice of antibody)
- Can be done with off-the-shelf reagents
- Bears high potential for further developments

- Not yet applied *in vivo*

Take home message

Although Nucleic Acid-based agents are still the most frequent used tools to modulate protein concentration, there are clear disadvantages.

Molecules targeting the protein of interest and leading to ubiquitin proteasome-mediated degradation can overcome some of these drawbacks by directly and specifically targeting the protein of interest. Identification of appropriate interaction partners of POIs remains an issue.

Antibody targeting acute protein degradation via TRIM21 opens new ways to degrade proteins in a fast and specific manner using off-the-shelf reagents.

Thanks for your attention