Targeted Protein Degradation (TPD): Advancement and Applications

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20.10.2020

Why investigate TPD?

Research tool

CRISPR-Cas9/ RNAi :
a. Days or weeks are required when knockout or knockdown.
b. Genetic manipulation generally affects all protein copies.
c. Protein depletion is indirect and dependent on the inherent turnover of the protein.
d. May have enough time to activate compensatory mechanisms, which may mask phenotypes
……

TPD:
a. Depletes the target proteins in minutes to hours.
b. Targeting proteins directly allow certain protein variants to be selectively degraded.
c. Reversible, more fine-grained control.

Why investigate TPD?

Therapeutic strategy

Traditional drugs:
Limited by the requirements of specific measurable functions of the target protein and accessible binding sites whose occupancy directly influences their function.

Gene therapy:
They are large biomolecules, the gene therapy reagents are difficult to deliver, especially for neurological disease

TPD:
Provides powerful tools to degrade undruggable targets.

Why investigate TPD?

e.g. Proteinopathies

Proteins structurally abnormal, lose their function.
Toxic in some way, disrupt the function of cells, tissues and organs of the body.

Approach for these diseases treatment:
Lowering the levels of disease-causing proteins.

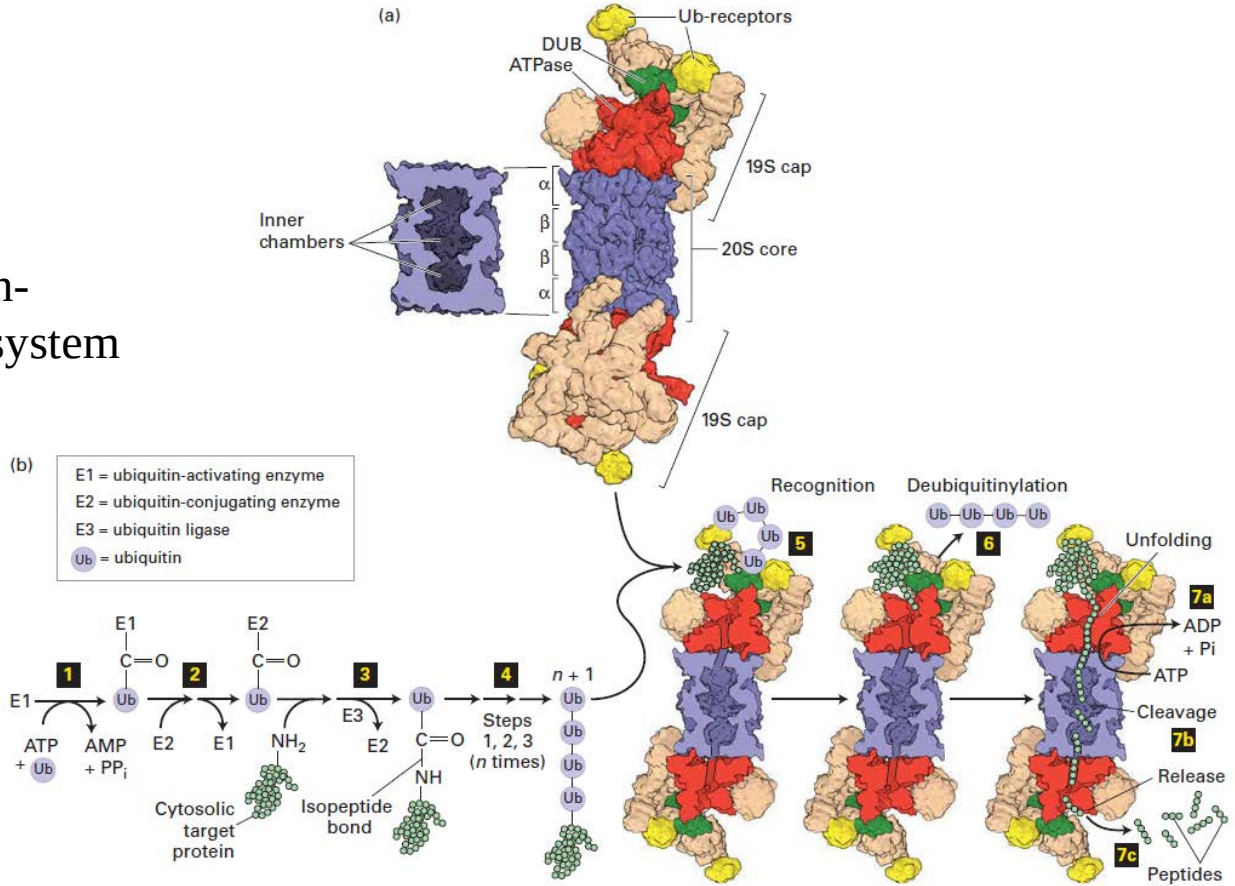
Challenging:
How to specifically eliminate the abnormal protein but do not disturb the wild-type one.

--Achieved by TPD

How is TPD implemented?

Mechanism of TPD—Degradation pathway

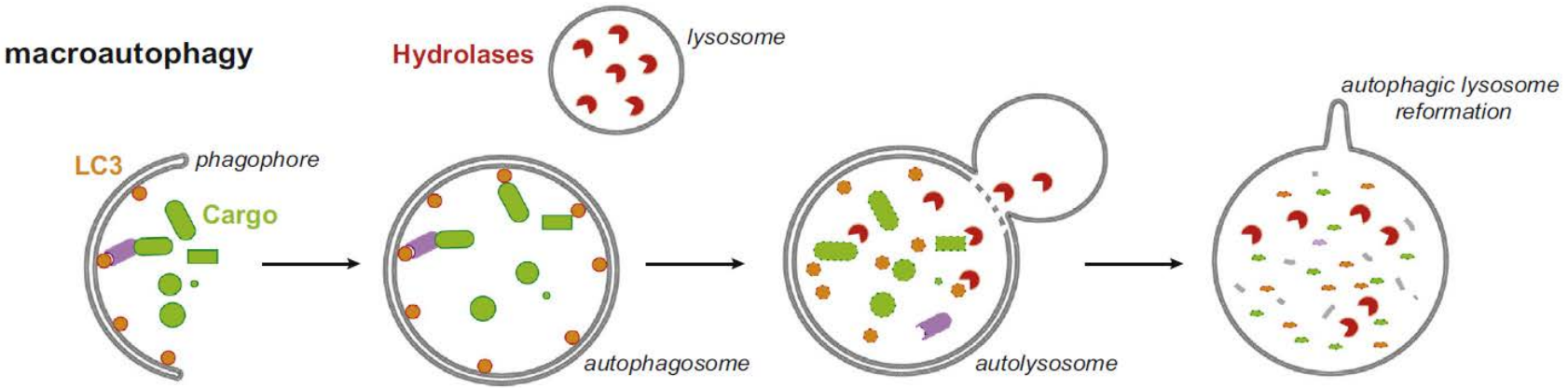
The ubiquitin-proteasome system (UPS）



Lodish, H. Freeman and Company, 2016

Mechanism of TPD—Degradation pathway

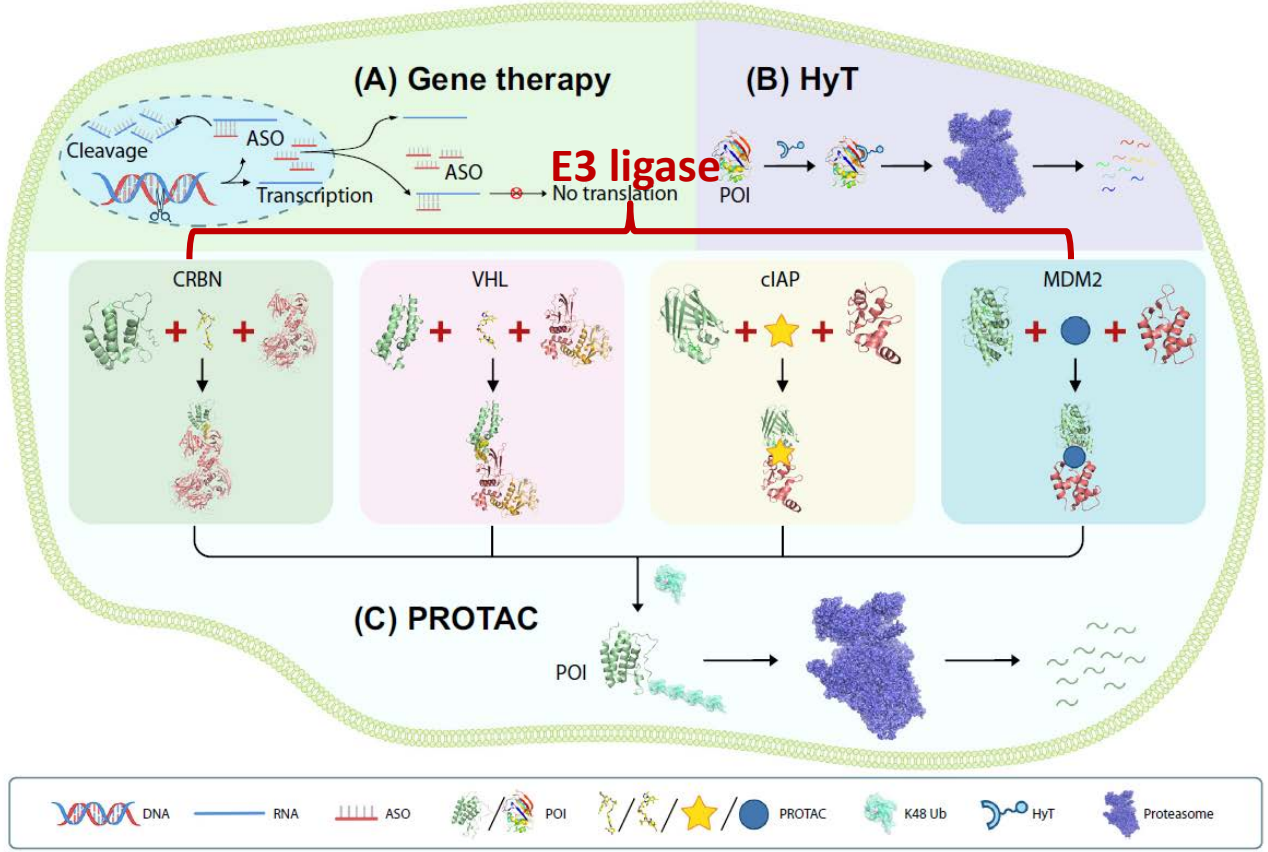
The Autophagy Lysosomal Pathway (ALP)



LC3: The autophagosome protein microtubule-associated protein 1A/1B light chain 3

Bingol B,. Mol Cell Neurosci, 2018

How is the substrate recruited—
Small Molecule-Induced Selective Protein Degradation



ASO: Antisense oligonuleotides

HyT: Hydrophobic tagging

POI: Protein of interest

CEBN: Cereblon

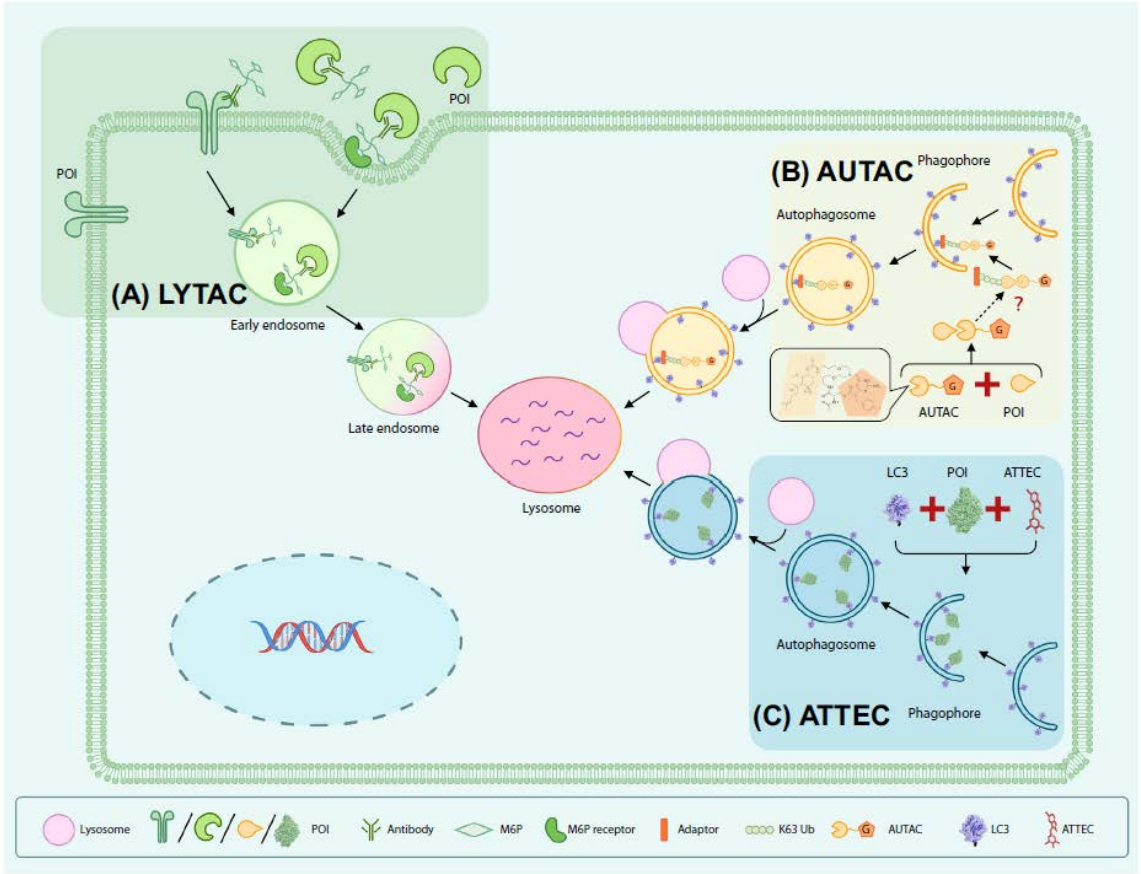
VHL: Von Hippel Lindau

cIAP: Celluler inhibitor of apoptosis protein

PROTAC: Proteolysis-targeting chimera

Li, Z., Nature, 2019

How is the substrate recruited—
Emerging New Concepts of Small Molecule-Induced Protein Degradation



LYTAC: Lysosome targeting chimera

AUTAC: The autophagy-targeting chimera

ATTEC: Autophagosome-tethering compound

M6P: mannose-6-phosphate

Li, Z., Nature, 2019

RESEARCH ARTICLE

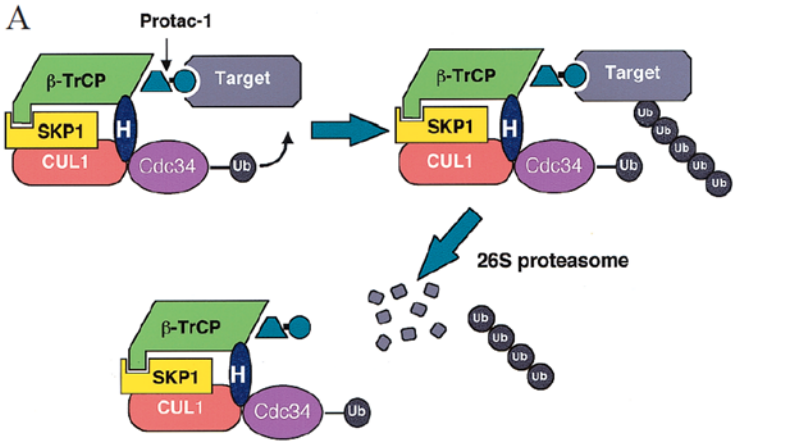
Protacs: Chimeric molecules that target proteins to the Skp1–Cullin–F box complex for ubiquitination and degradation

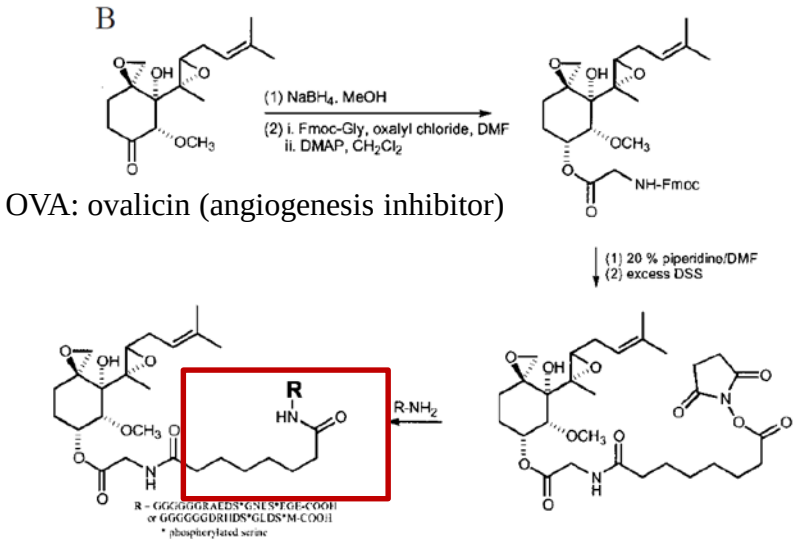
Kathleen M. Sakamoto, Kyung B. Kim, Akiko Kumagai, Frank Mercurio, Craig M. Crews, and Raymond J. Deshaies

PNAS July 17, 2001 98 (15) 8554-8559; https://doi.org/10.1073/pnas.141230798

Communicated by Alexander Varshavsky, California Institute of Technology, Pasadena, CA (received for review March 29, 2001)

The first example of using chimeric molecules to redirect the specificity of a ubiquitin ligase toward a target protein of interest





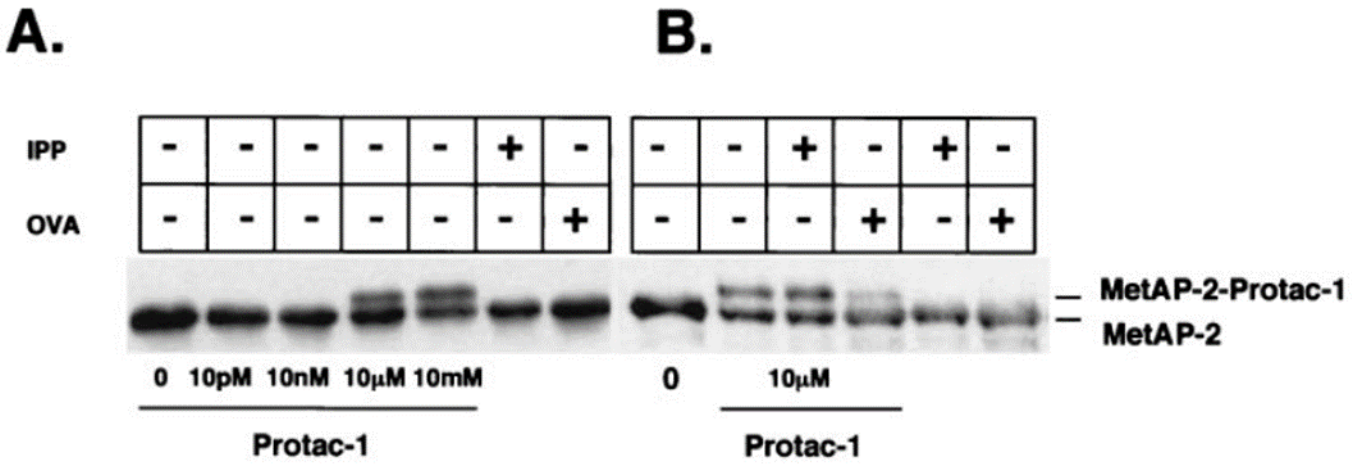
OVA: ovalicin (angiogenesis inhibitor)

IPP: IkBa phosphopeptide

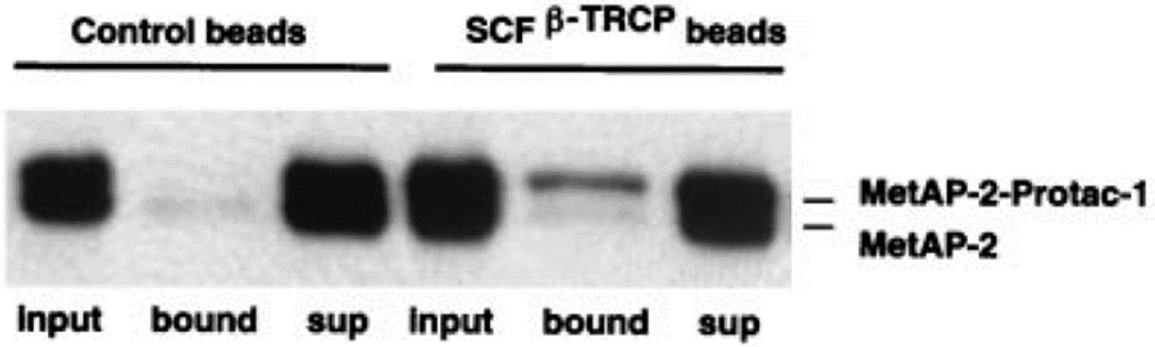
(A) Protac-1 targets methionine aminopeptidase-2 (MetAP-2) to Skp1-Cullin-F box complex containing Hrt1 (SCF)

(B) The synthesis scheme for Protac-1

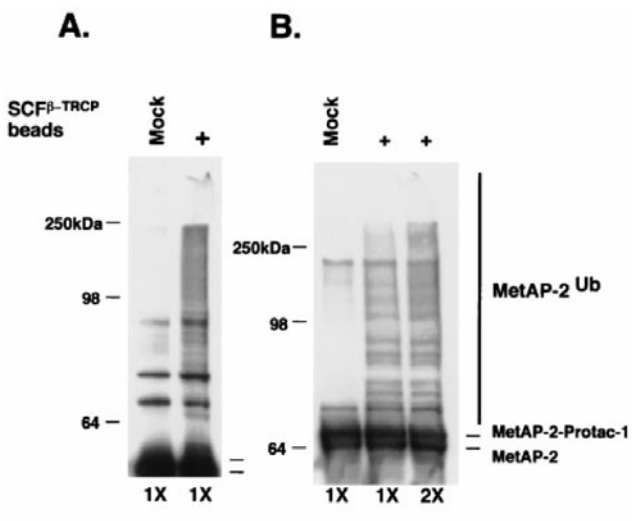
MetAP-2 Specifically Binds Protac-1 in Vitro



Protac-1 recruits MetAP-2 to SCFb-TRCP

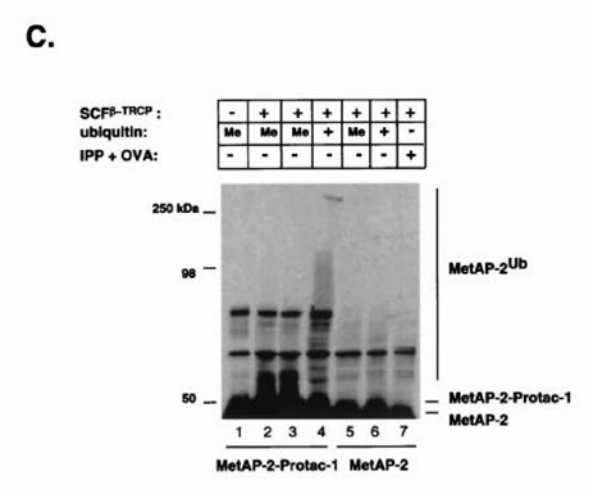


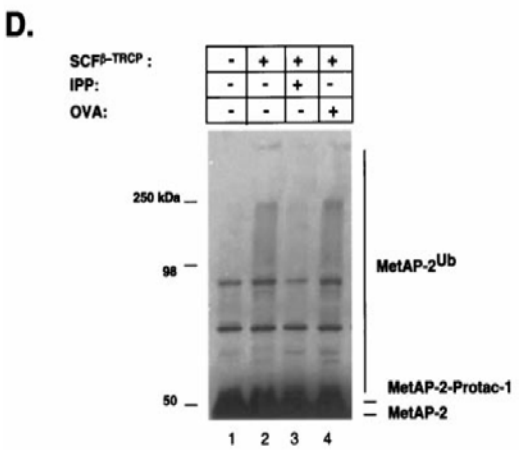
Protac mediates MetAP-2 ubiquitination by SCF.



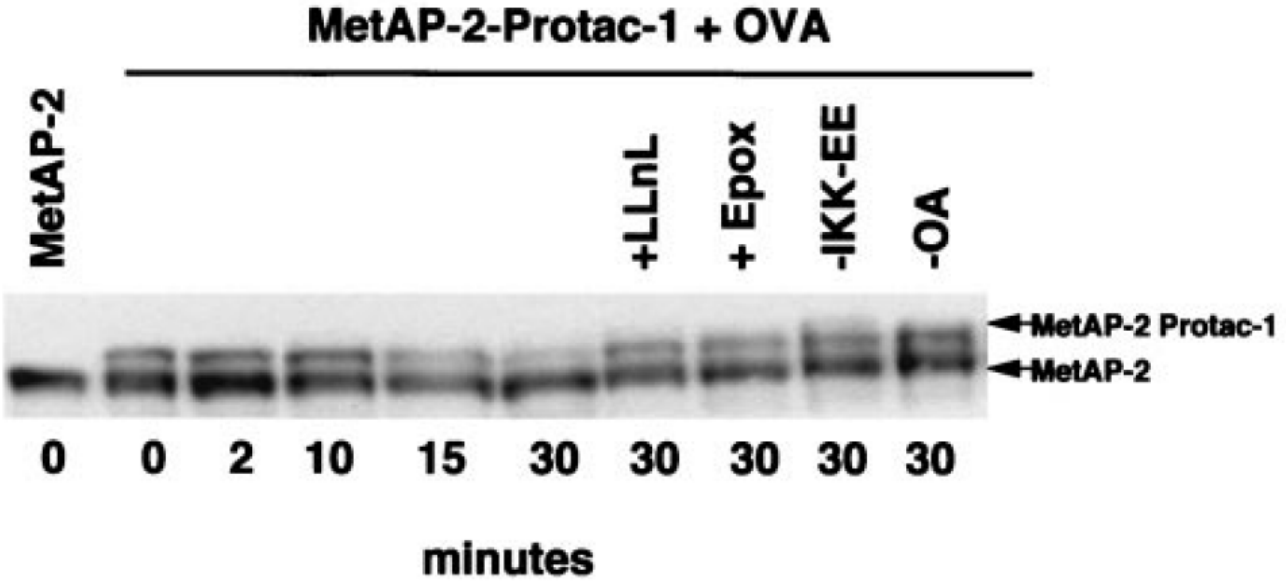
Autocatalyzed cleavage

Full length

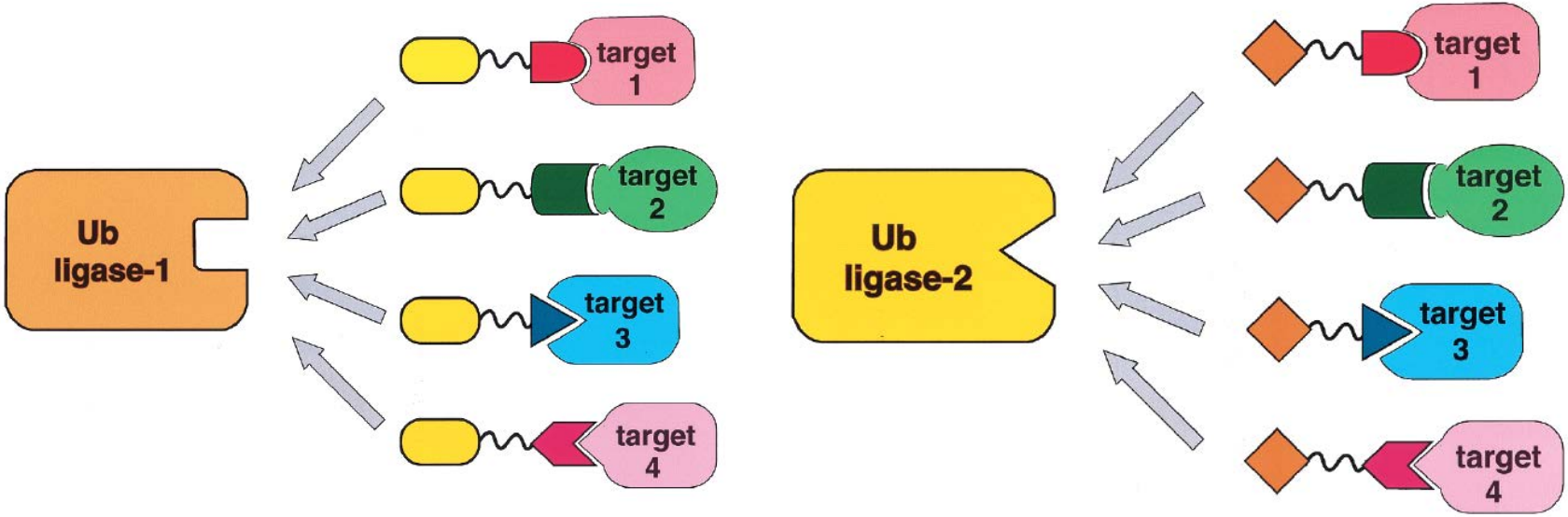




MetAP-2-Protac is degraded in Xenopus Egg extracts



General application principle of Protacs



Conclusions

Protacs may be useful research tools for manipulating the phenotype of cells by means of the targeted elimination of specific proteins.

A useful therapeutic agents for targeting the elimination of disease-promoting proteins.

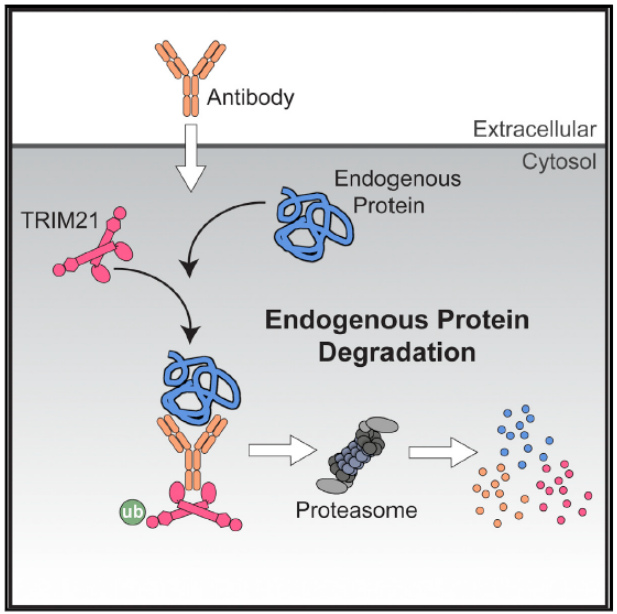
An obstacle to realizing these goals, however, is that the phosphopeptide containing Protac-1 described here is unlikely to penetrate cells.

Resource

Cell

A Method for the Acute and Rapid Degradation of Endogenous Proteins

Graphical Abstract



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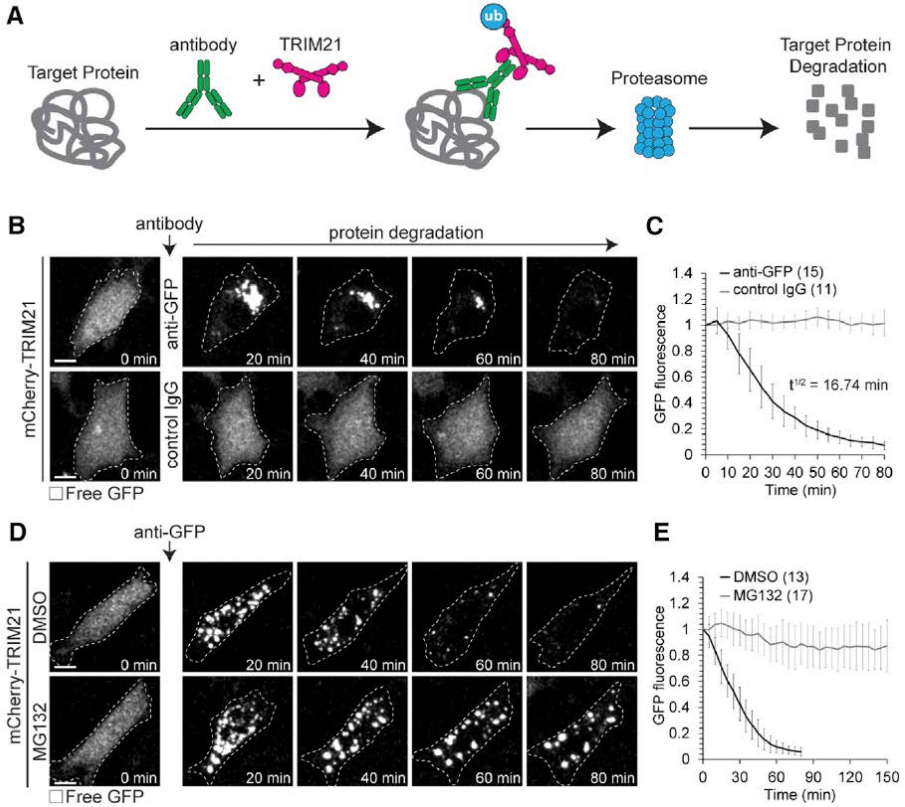
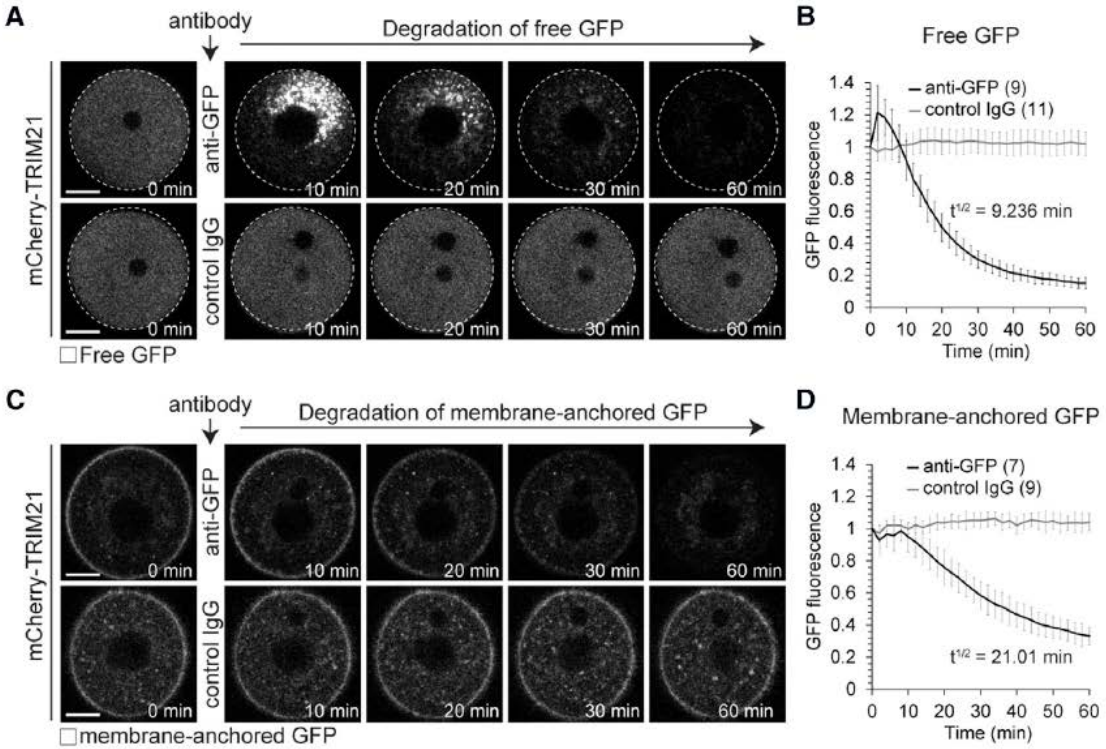
Acute Degradation of Proteins by Trim-Away



NIH 3T3 cells

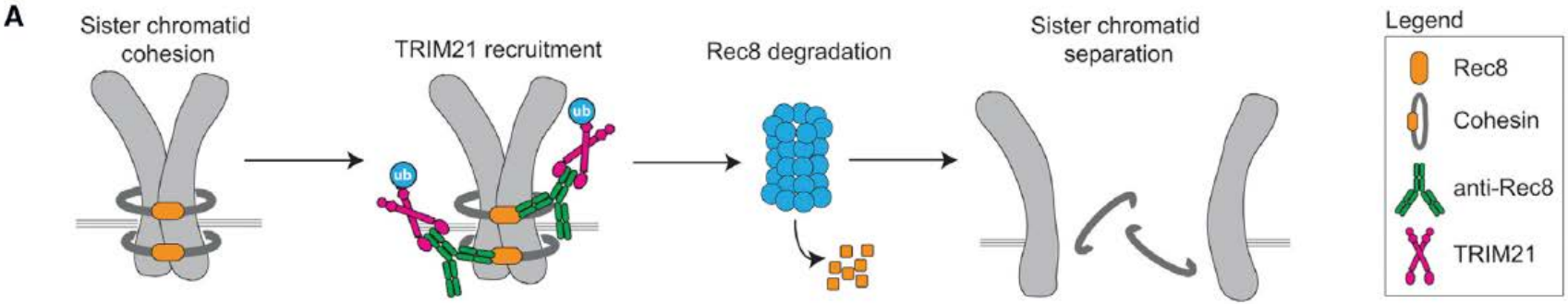
Trim-Away Degrades Diverse Cellular Substrates

Mammalian oocytes—post-mitotic, transcriptionally silent



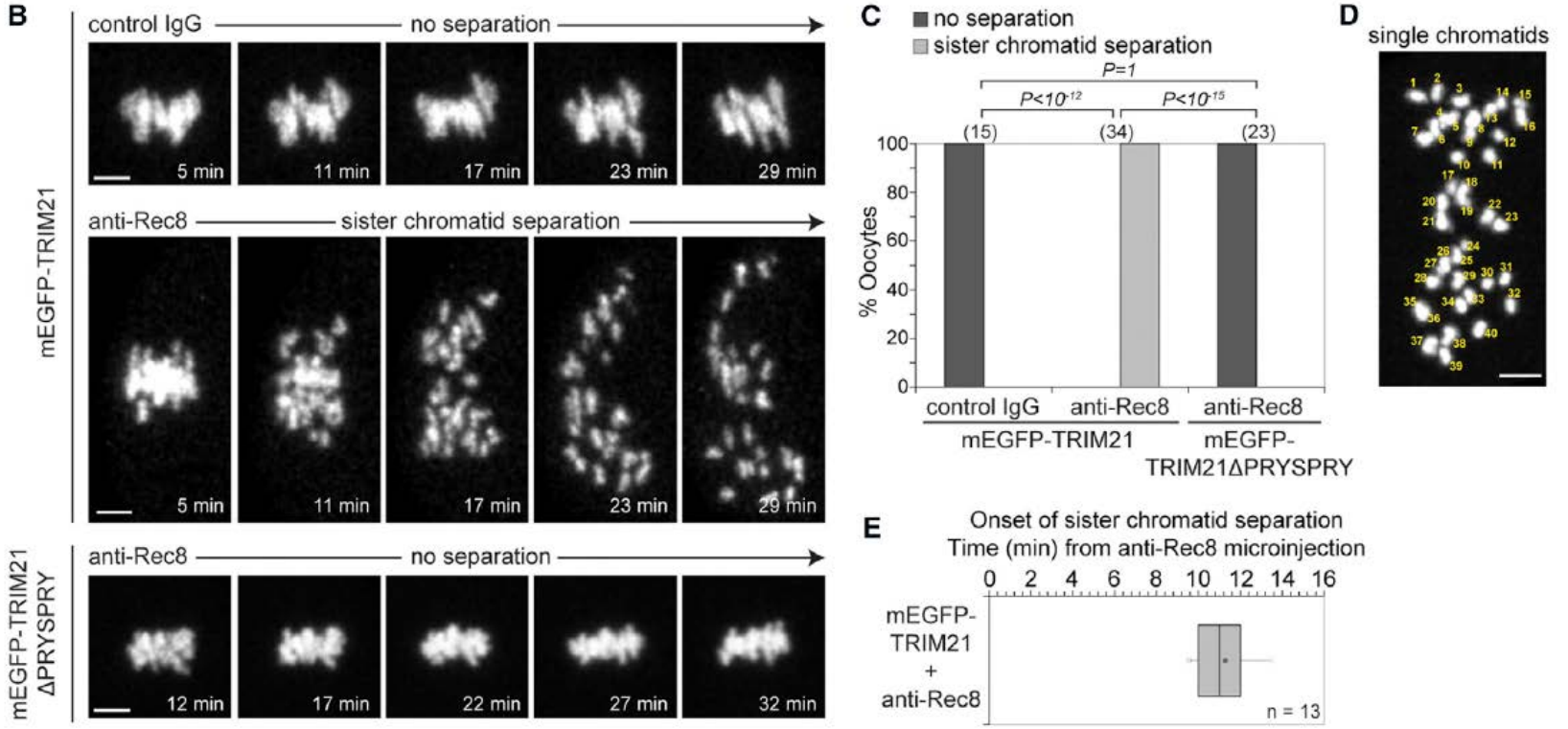
Also histone H2B and nuclear localization signal (NLS), data not show

Trim-Away Is Suitable to Degrade Long-Lived Proteins Acutely

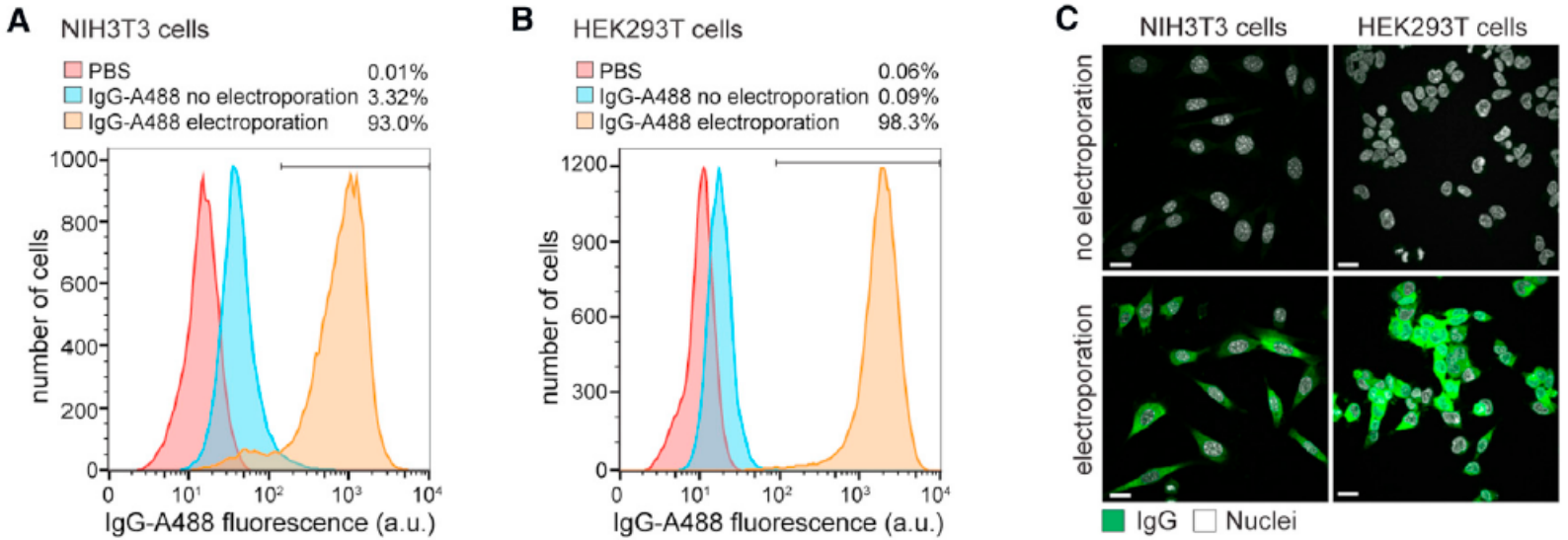


Rec8 is part of the cohesin protein complex that mediates sister chromatid cohesion in oocytes from birth until ovulation. Rec8 does not turnover but remains stably associated with chromosomes for months in mice and possibly decades in humans.

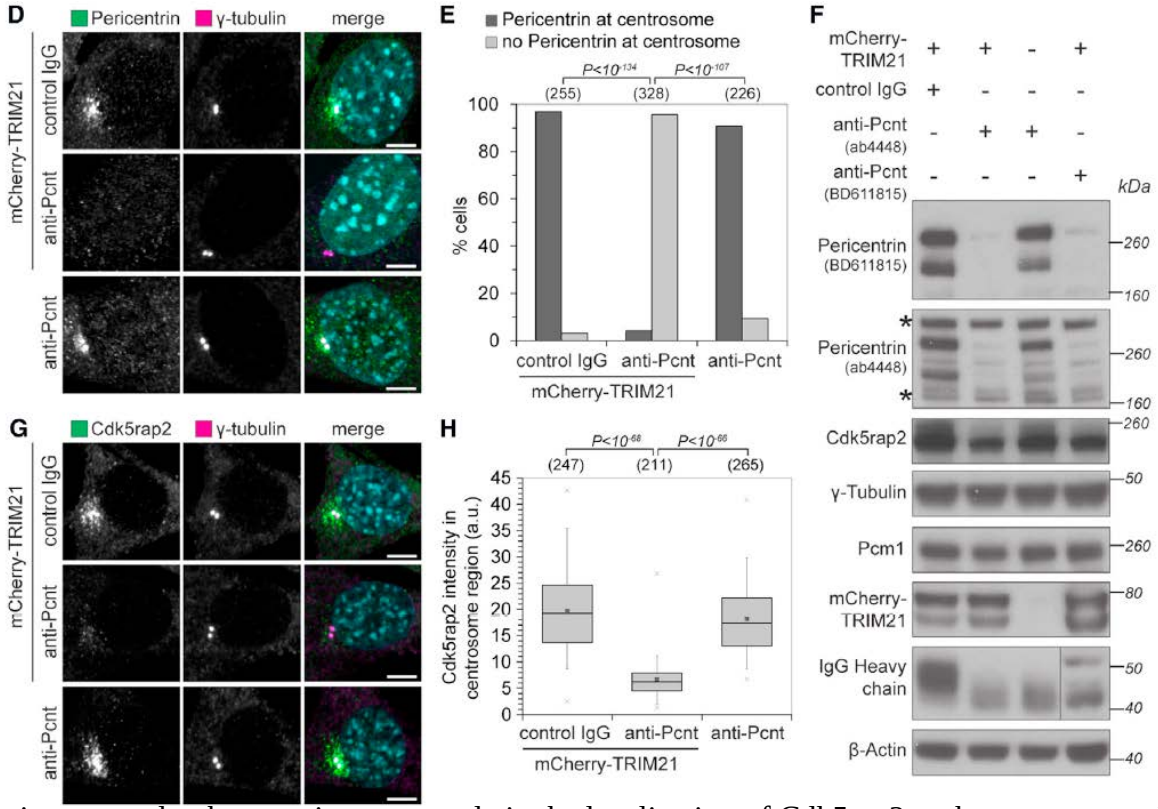
Trim-Away Is Suitable to Degrade Long-Lived Proteins Acutely



Trim-Away using antibody electroporation is compatible with quantitative analysis of cellular phenotypes

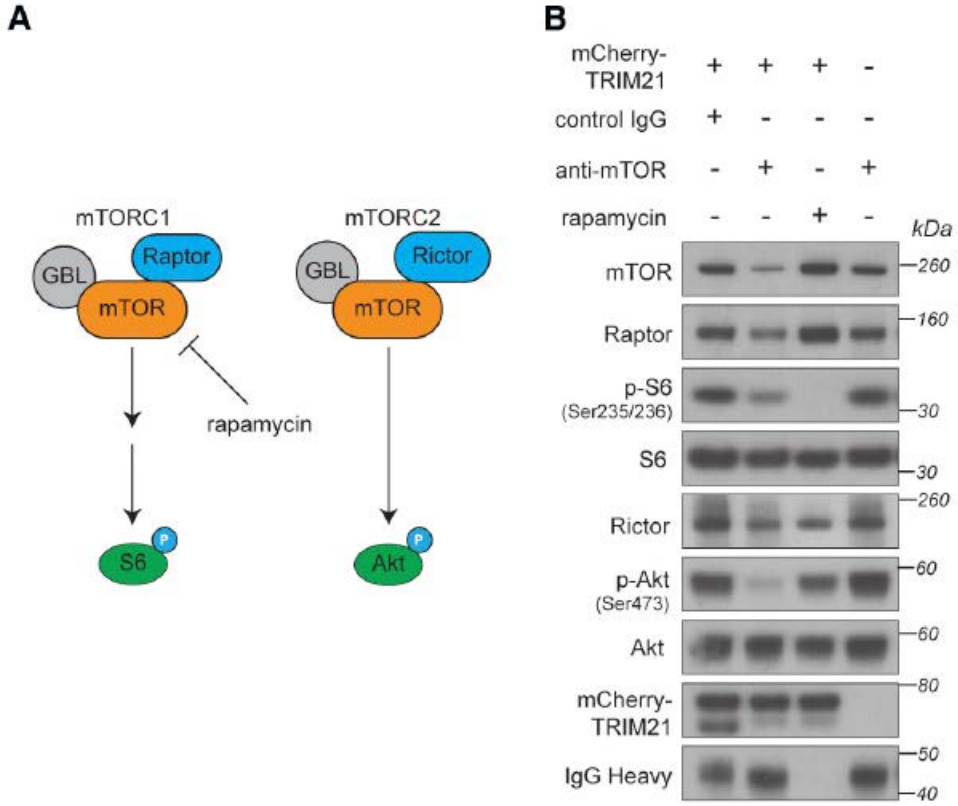


Trim-Away using antibody electroporation is compatible with quantitative analysis of cellular phenotypes



Pericentrin is proposed to have an important role in the localization of Cdk5rap2 to the centrosome. RNAi/gene knockout are impossible to distinguish between a role for pericentrin in Cdk5rap2 recruitment during the course of the centrosome cycle or in maintenance of Cdk5rap2 at the centrosome.

Selective Trim-Away of Signaling Pathway Components



Advantages

First, it allows protein function to be studied in non-dividing primary cells where DNA- and RNA-targeting methods are not suitable

Second, it allows the functional analysis of long-lived proteins that are resistant to current knockdown methods that rely on protein turnover.

Third, removal of essential endogenous proteins can now be achieved without the introduction of protein modifications such as degrons.

Fourth, the remarkable speed of Trim-Away means that phenotypes can be observed immediately following degradation of the endogenous protein at any stage of a particular biological process.

Finally, aberrant protein expression or activation is a hallmark of many human diseases such as neurodegeneration and cancer.

Paper-3

Article | Published: 30 October 2019

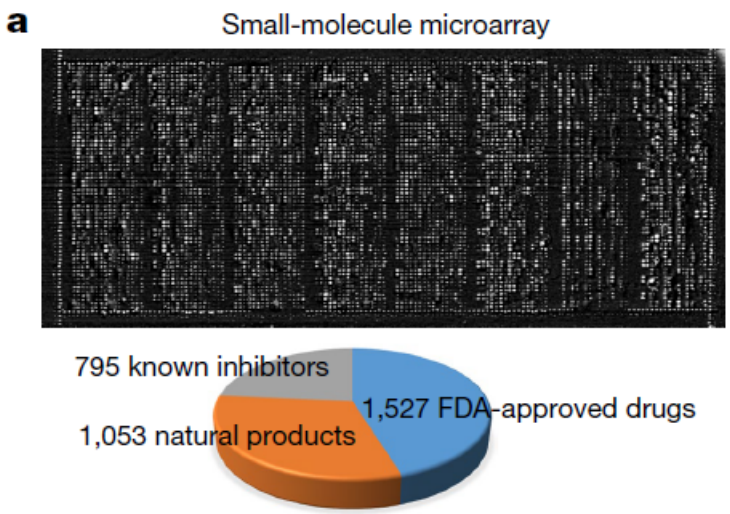
Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds

Zhaoyang Li, Cen Wang, Ziying Wang, Chenggang Zhu, Jie Li, Tian Sha, Lixiang Ma, Chao Gao, Yi Yang, Yimin Sun, Jian Wang, Xiaoli Sun, Chenqi Lu, Marian Difiglia, Yanai Mei, Chen Ding, Shouqing Luo, Yongjun Dang, Yu Ding ✉, Yiyan Fei ✉ & Boxun Lu ✉

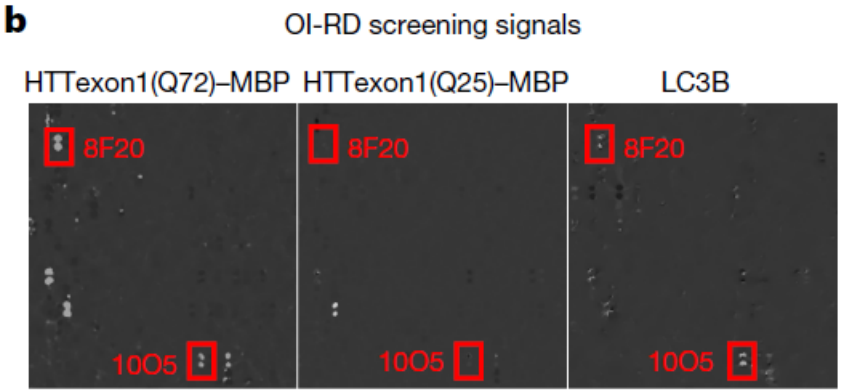
Nature **575**, 203–209(2019) | Cite this article

Example for autophagosome-tethering compound strategy

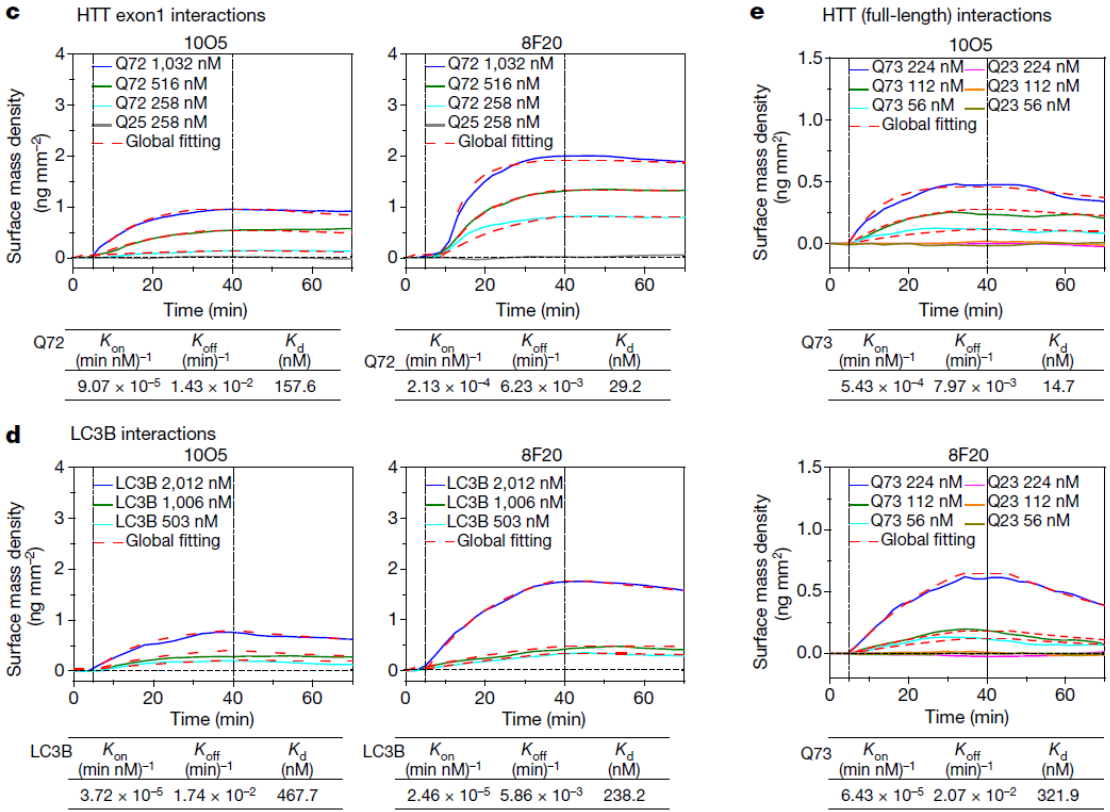
Identification of mHTT–LC3 linker compounds



nucleophile-isocyanate reaction

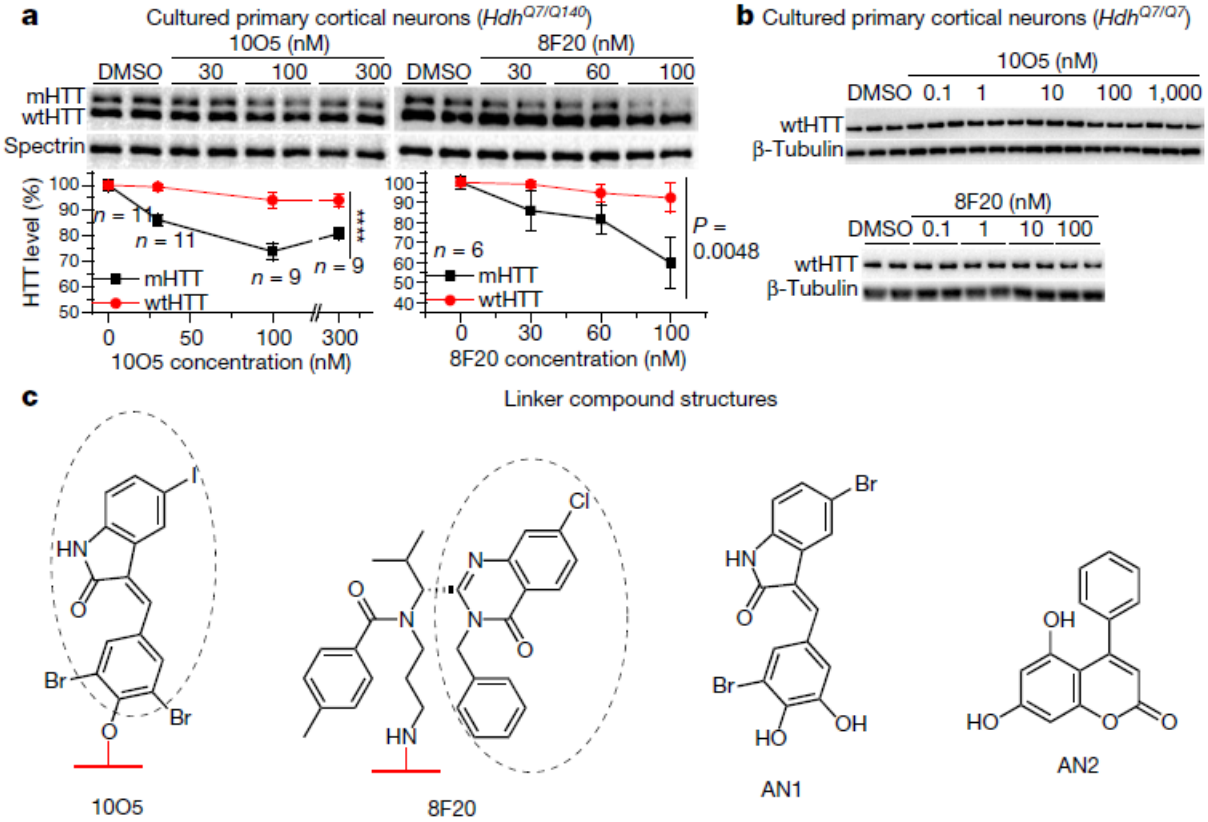


OI-RD: scanning oblique-incidence reflectivity difference

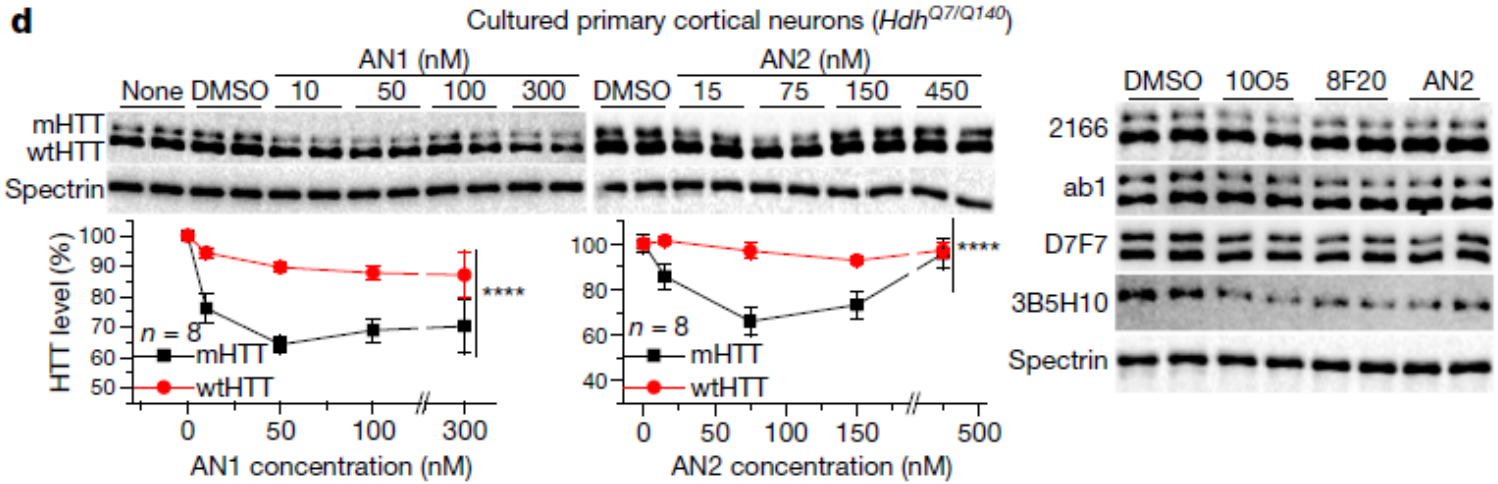


on and off rates (K_{on} and K_{off},respectively), dissociation constants (K_d)

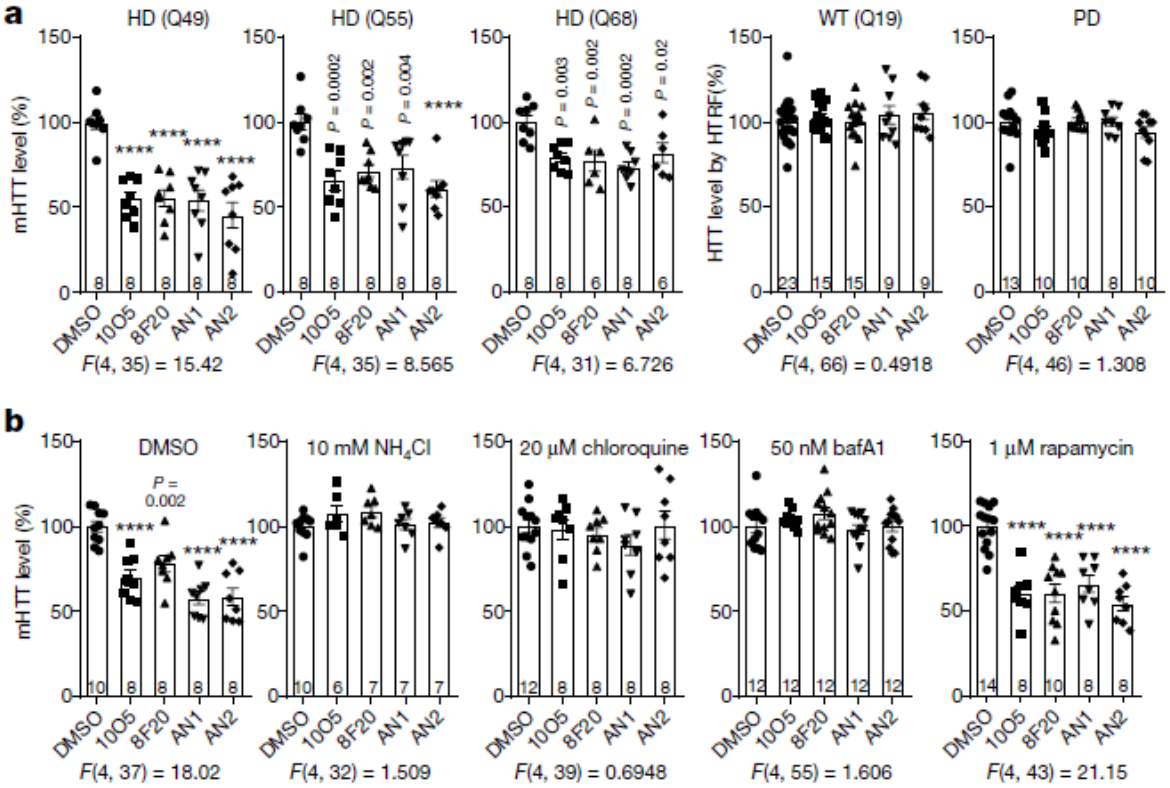
Linkers induced allele-selective mHTT lowering



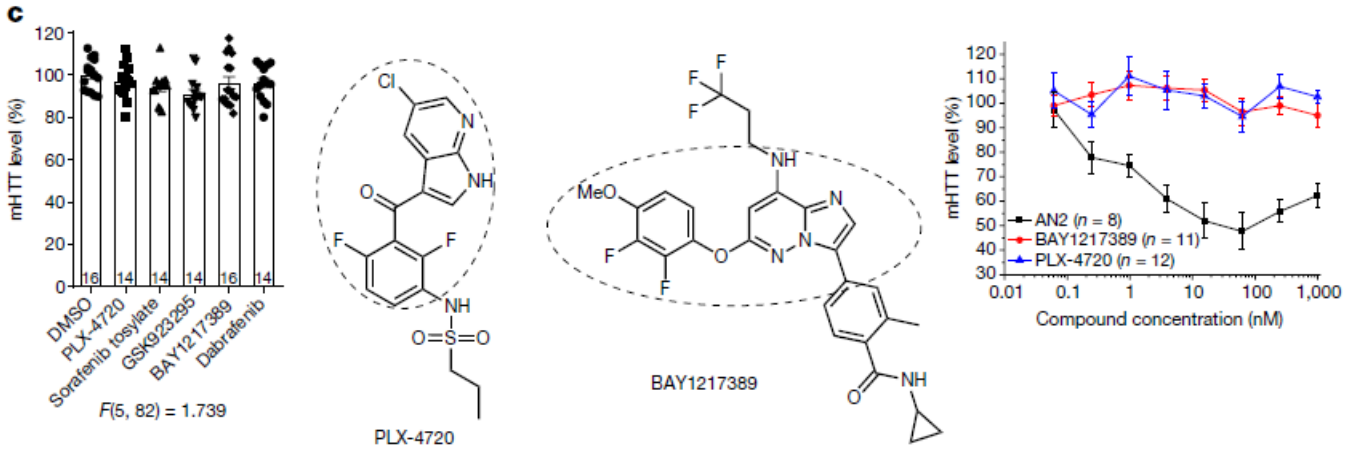
Similarities: contain an aryl ring connected to a lactam-based bicyclic structure with halogen-substituted aryl group.


d
Cultured primary cortical neurons (Hdh^Q7/Q140)
AN1 (nM)
None DMSO 10 50 100 300
AN2 (nM)
DMSO 15 75 150 450
mHTT
wtHTT
Spectrin
HTT level (%)
n = 8
mHTT
wtHTT
****
AN1 concentration (nM)
AN2 concentration (nM)
DMSO 10O5 8F20 AN2
2166
ab1
D7F7
3B5H10
Spectrin

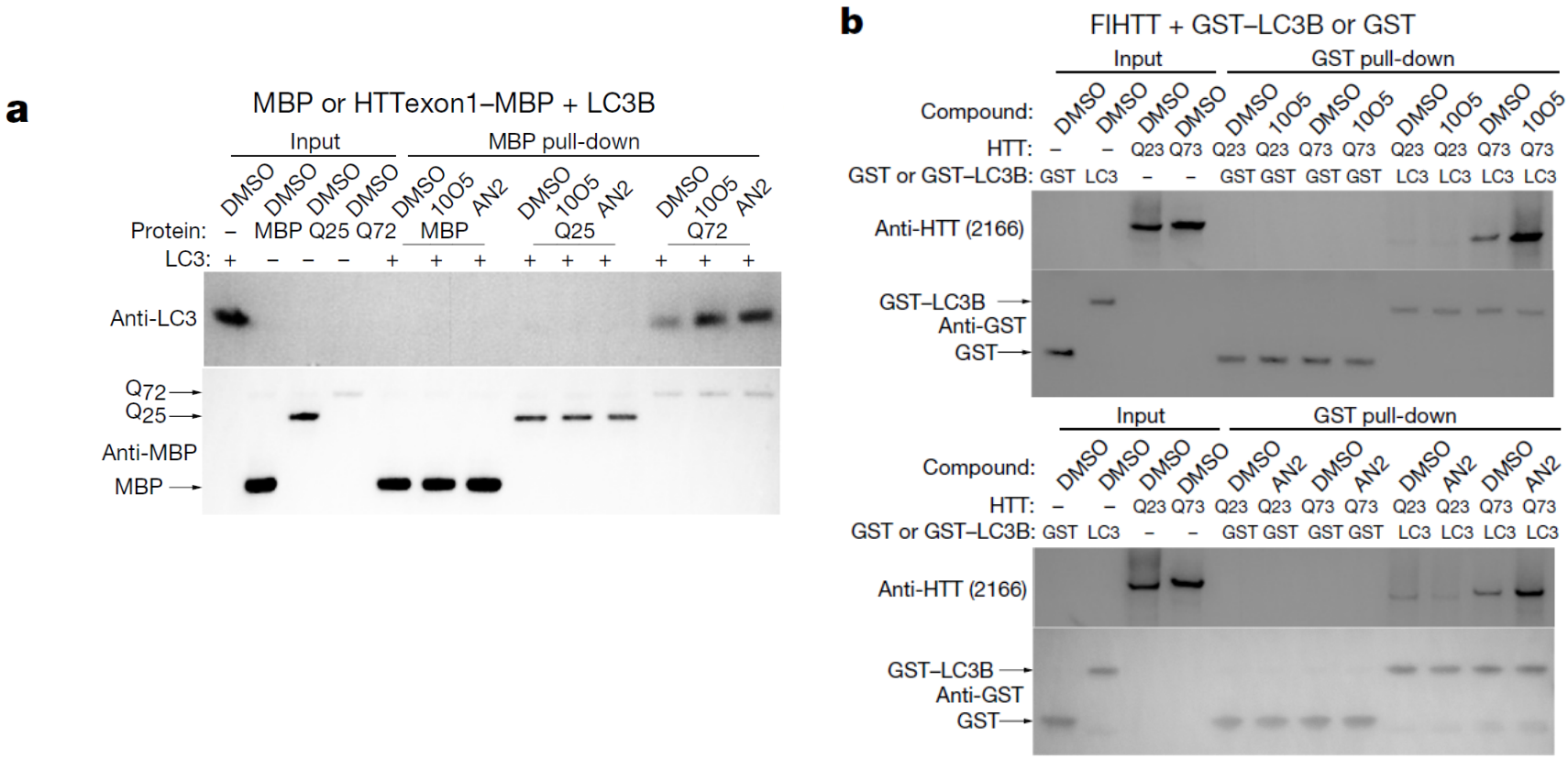
mHTT–LC3 linker compounds lower mHTT in fibroblasts from patients with HD

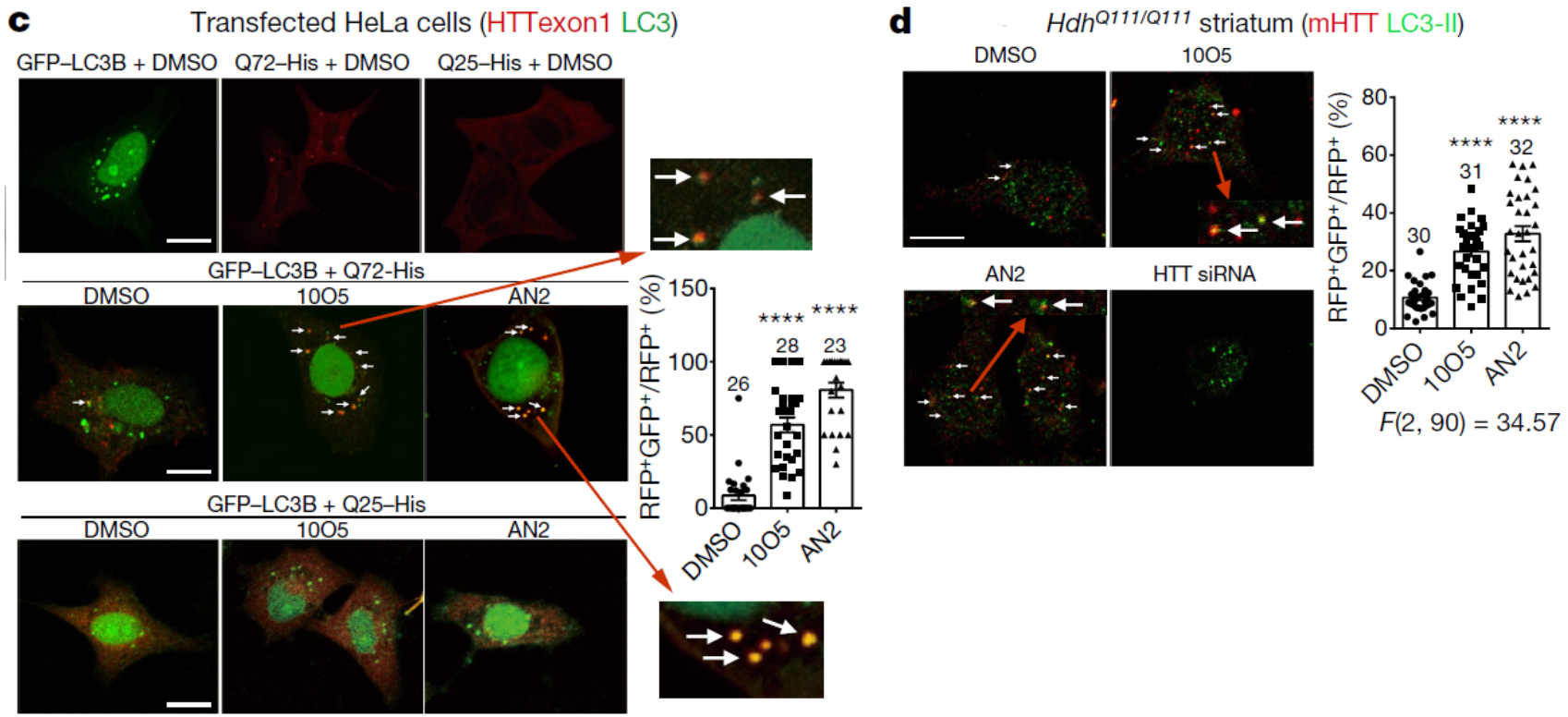


Using homologous time-resolved fluorescence (HTRF) assay

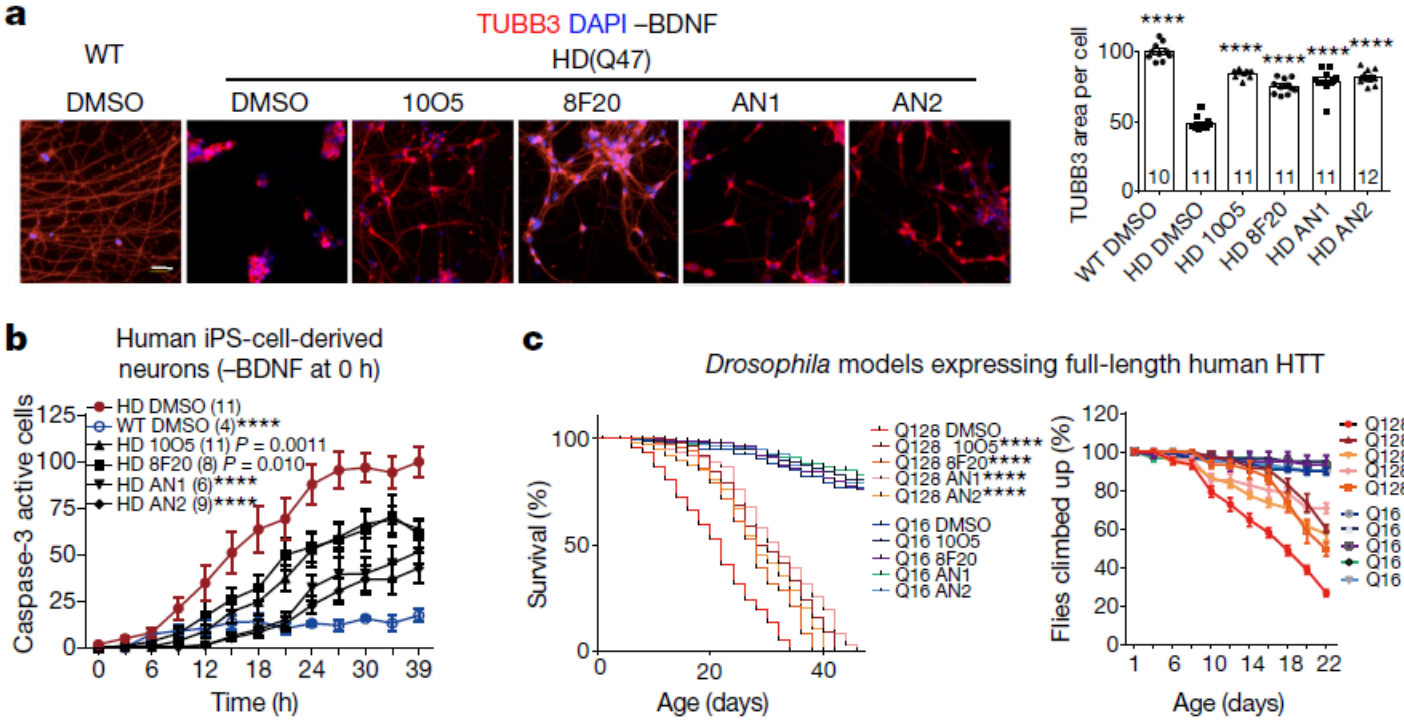

c
mHTT level (%)
120
100
80
60
40
20
0
16
14
14
14
16
14
DMSO
PLX-4720
Sorafenib tosylate
GSK923295
BAY1217389
Dabrafenib
F(5, 82) = 1.739
Cl
N
NH
O
F
F
NH
O=S=O
PLX-4720
F
F
NH
MeO
F
F
O
N
N
N
BAY1217389
O
NH
mHTT level (%)
120
110
100
90
80
70
60
50
40
30
AN2 (n = 8)
BAY1217389 (n = 11)
PLX-4720 (n = 12)
0.01
0.1
1
10
100
1,000
Compound concentration (nM)

Linkers tether mHTT to autophagosomes

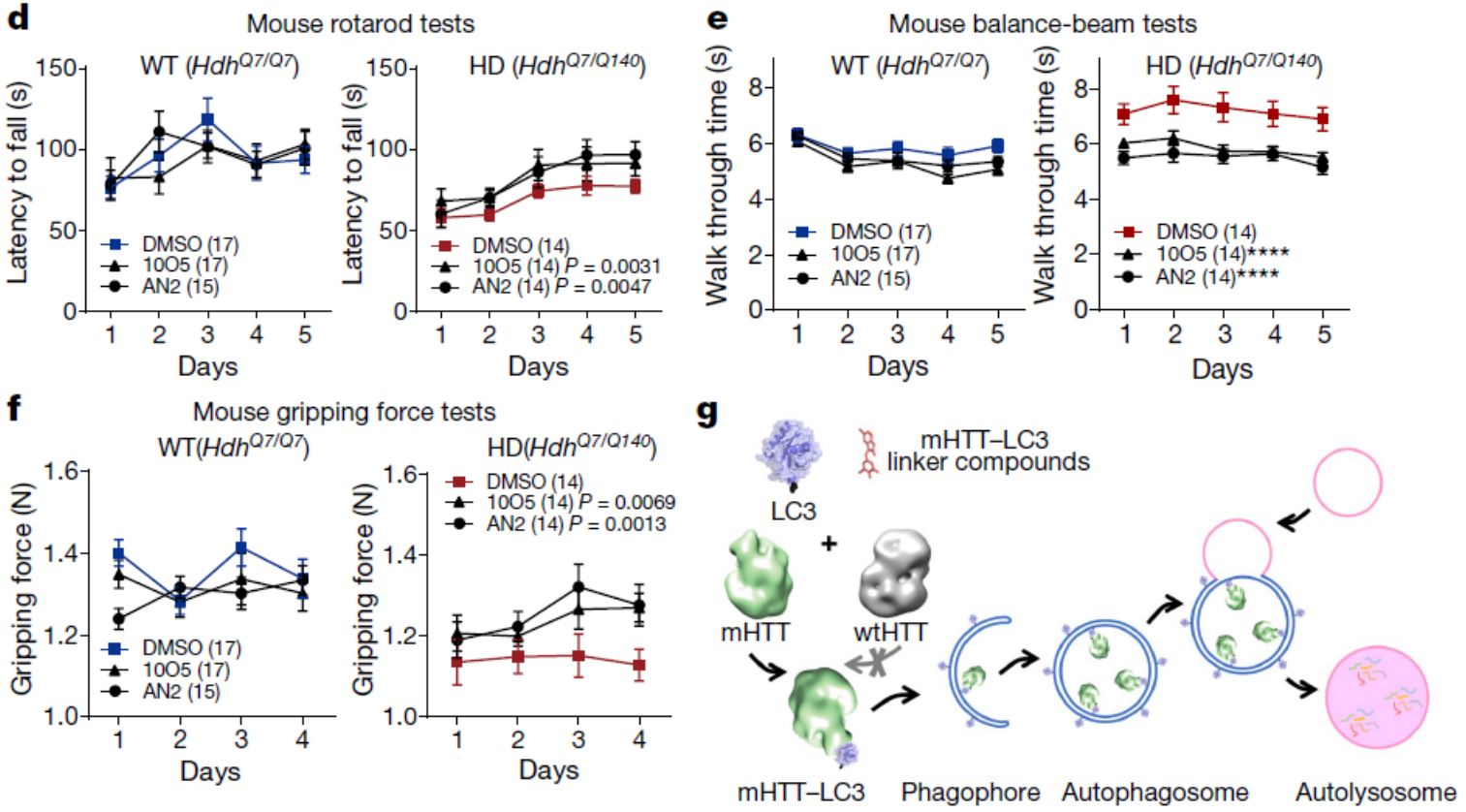



c
Transfected HeLa cells (HTTexon1 LC3)
GFP–LC3B + DMSO
Q72–His + DMSO
Q25–His + DMSO
GFP–LC3B + Q72-His
DMSO
10O5
AN2
GFP–LC3B + Q25–His
DMSO
10O5
AN2
RFP+GFP+/RFP+ (%)
150
100
50
0
26
28
23
****
****
DMSO
10O5
AN2
d
Hdh Q111/Q111 striatum (mHTT LC3-II)
DMSO
10O5
AN2
HTT siRNA
RFP+GFP+/RFP+ (%)
80
60
40
20
0
30
31
32
****
****
DMSO
10O5
AN2
F(2, 90) = 34.57

Linker compounds rescued HD-relevant phenotypes in cells



Linker compounds rescued HD-relevant phenotypes in vivo



Advantages and Limitations of PROTAC and Emerging Degrader Technologies

Degrader technology	Degradation pathway	Potential targets	Advantages	Limitations
PROTAC	Proteasome pathway	Intracellular proteins	Well established with structural information; clear mechanisms of action; relatively high selectivity; catalytic and sub-stoichiometric	E3-, ubiquitination-, and proteasome-dependent; generally undesirable pharmacokinetic profile; possible limitations of target spectrum
LYTAC	Endosome/lysosome pathway for degradation of glycosylated proteins	Extracellular proteins; transmembrane proteins	Applicable to extracellular and transmembrane proteins; independent of ubiquitination and proteasomal degradation	Large molecular weight and poor permeability; possible induction of immune response *in vivo*
AUTAC	Selective macroautophagy pathway	Intracellular proteins; damaged organelles associated with specific proteins	Potentially a broad target spectrum; proteasome-independent; demonstrated ability to degrade mitochondria	Lack of key information of mechanisms of action; dependent on K63 ubiquitination; possible influence on selective autophagy
ATTEC	Macroautophagy pathway	Intracellular proteins; non-protein autophagy substrates	Potentially a broad target spectrum; direct targeting to the degradation machinery; potentially effective in all cell types; low molecular weight	The LC3-bound chemical moieties need to be solved; lack of studies on designed chimeras

THANKS for you attention!