

Ubiquitin & NF- κ B activation

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Overview

Basic Principles

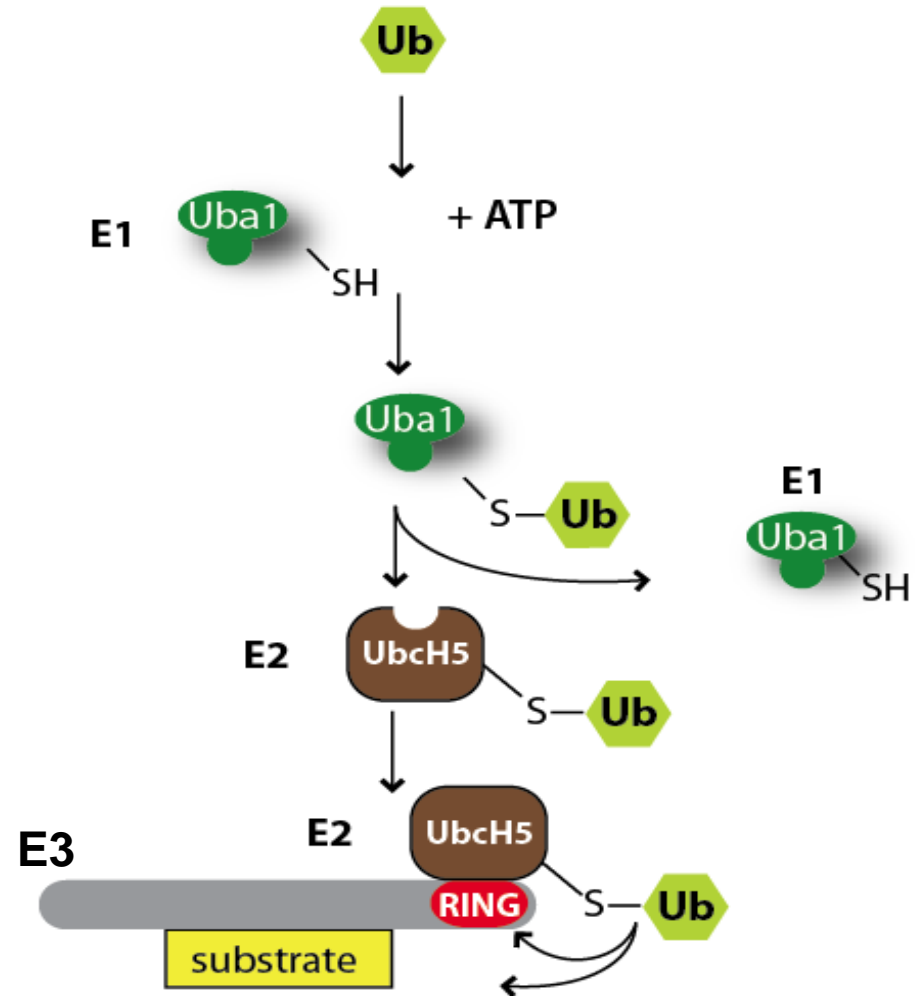
- Ubiquitin
- NF- κ B

Novel research

- Role of linear ubiquitylation in NF- κ B signalling and inflammation

Ubiquitin

- Post-translational modifications (PTM) of proteins include: Phosphorylation, glycosylation, acetylation, etc ... and ubiquitylation
- Ubiquitin is a small (8 kDa) protein abundantly present in cells
- **Ubiquitylation:** the covalent attachment of ubiquitin to Lysine (K) residues of target proteins (substrate)



Ubiquitylation: 3 step process

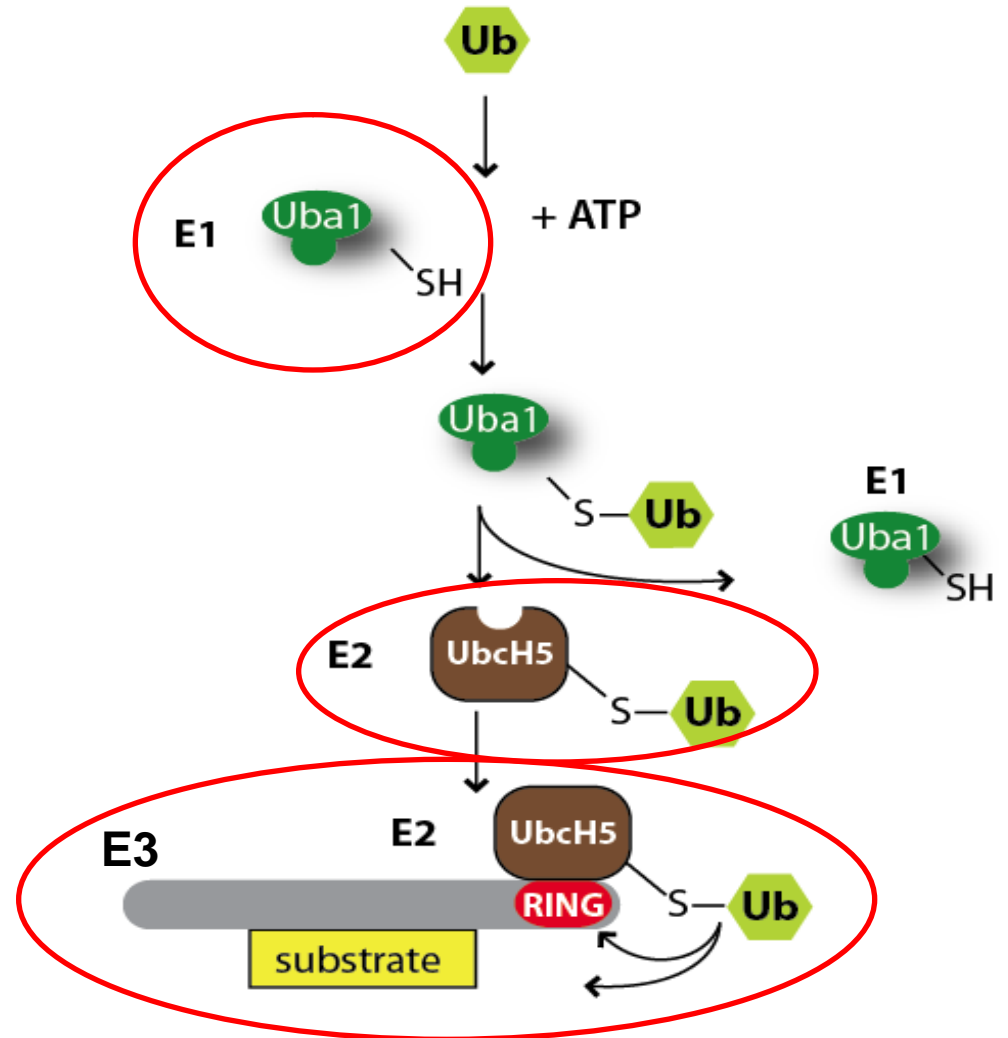
E1 : Ubiquitin activating enzyme
(ATP-dependent)

E2 : Ubiquitin-conjugating enzyme

Ubiquitin molecule is transferred to E2,
which can bind to E3

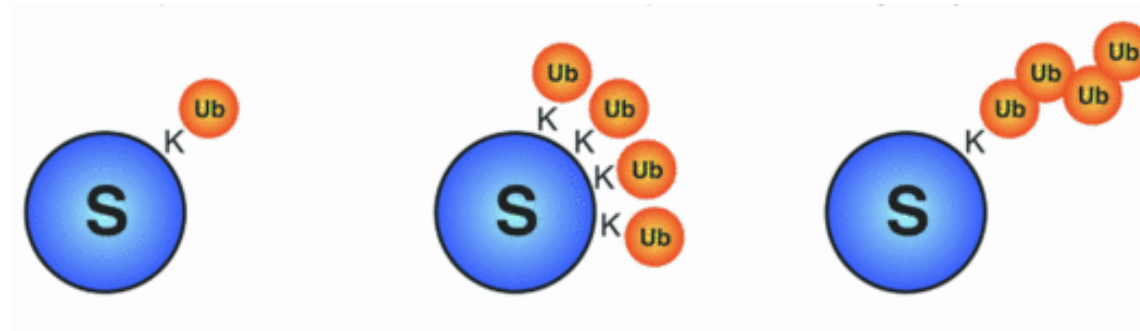
E3 : Ubiquitin ligase

E3 enzymes bind to substrates (target
protein) and mediate the transfer of ubiquitin
from E2 to substrate



Ubiquitylation (ubiquitination)

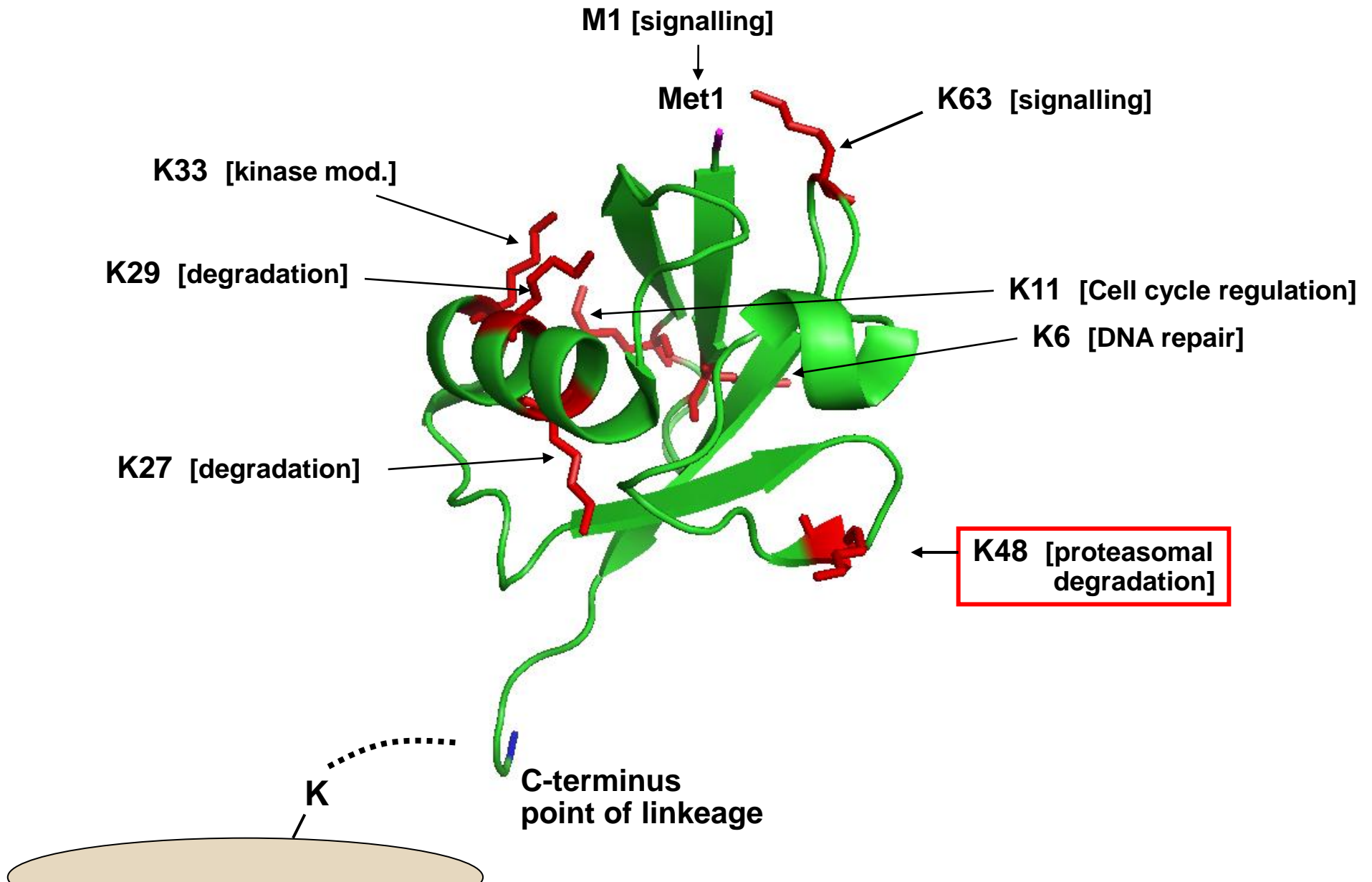
Multi-mono-ubiquitylation



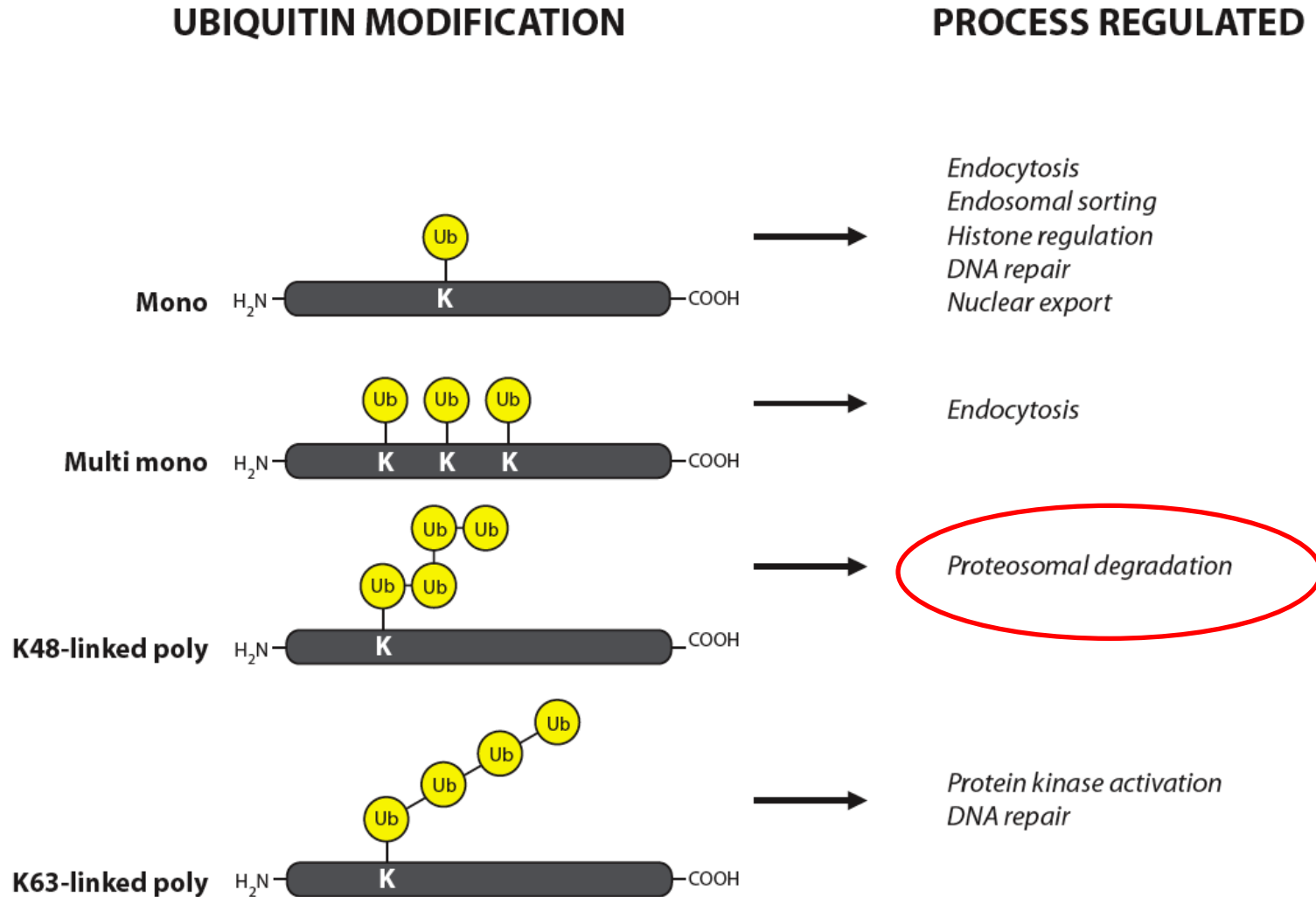
Mono-ubiquitylation

poly-ubiquitylation

Ubiquitin carries 7 internal Lysines

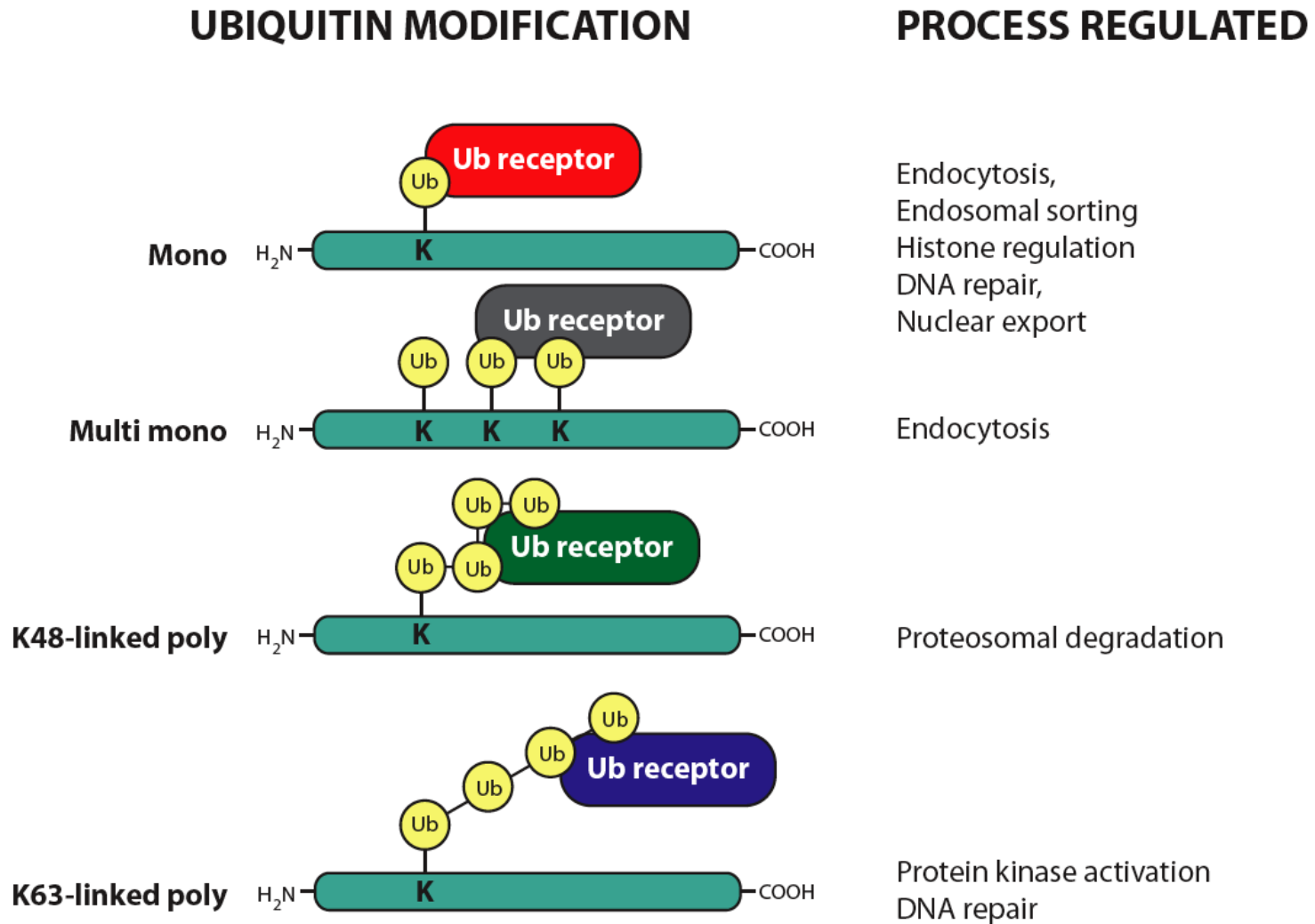


Ubiquitin function



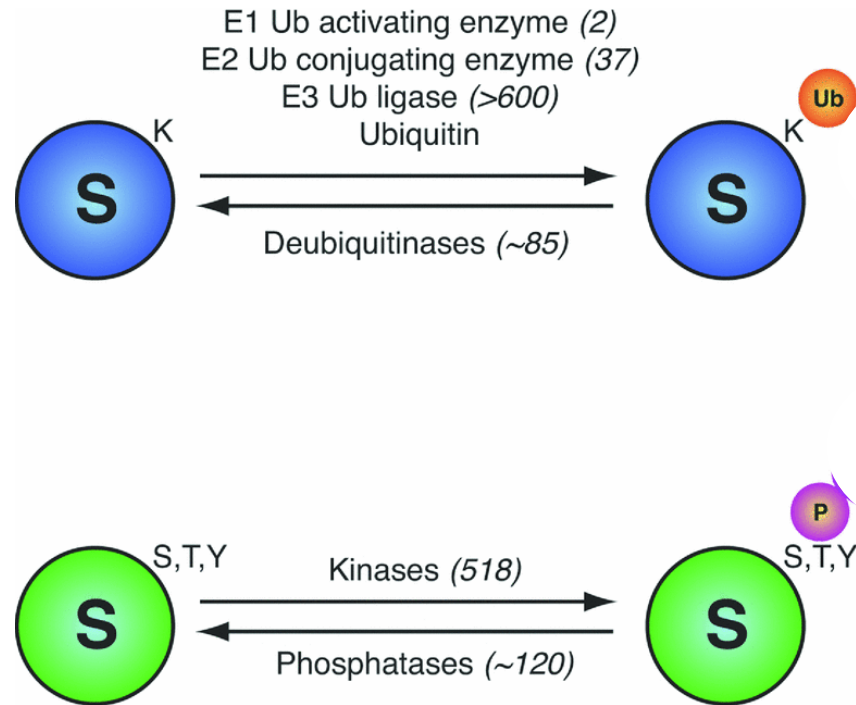
(Adapted from Hoeller et al. Nature Reviews Cancer 2006)

Ubiquitin binding domains (UBD).

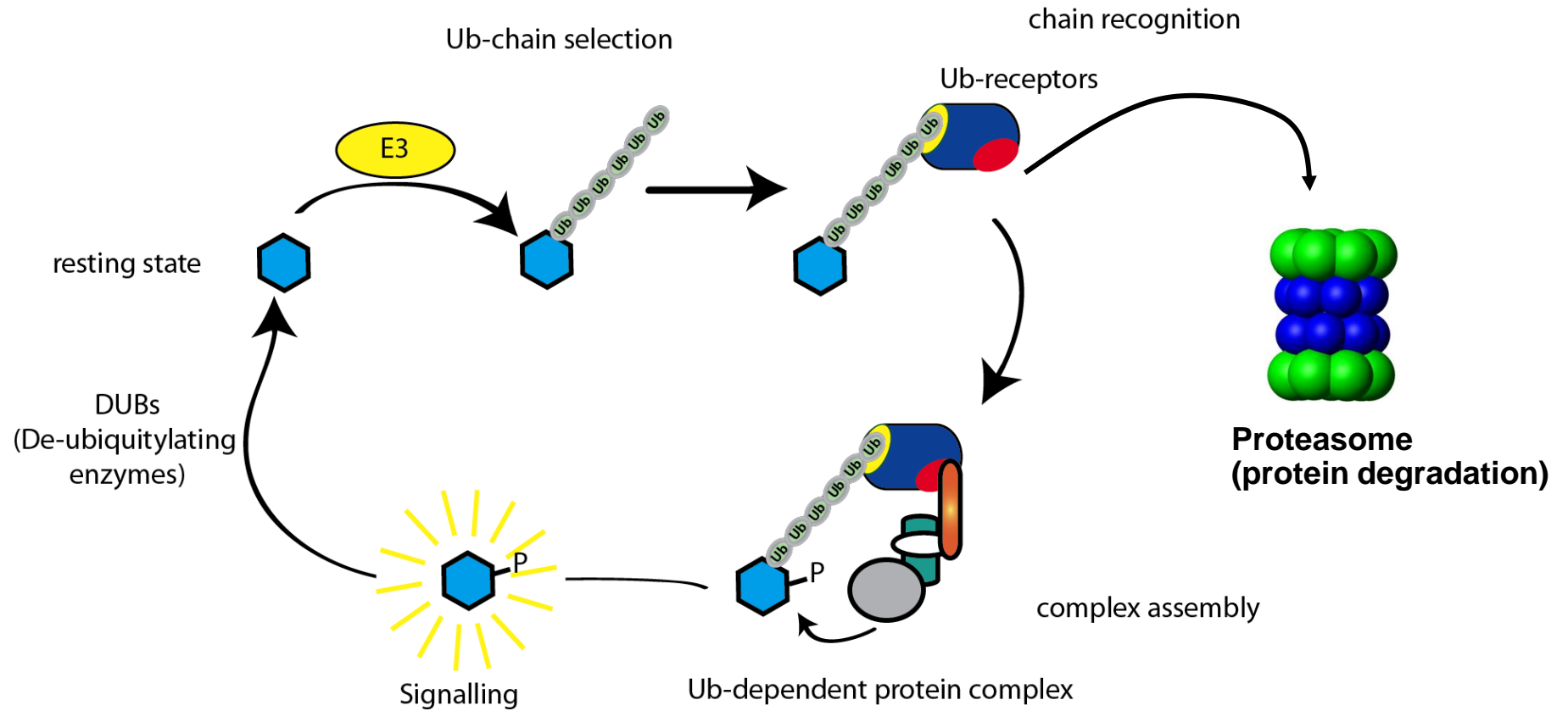


(Adapted from Hoeller et al. Nature Reviews Cancer 2006)

De-ubiquitylating enzymes (DUB)

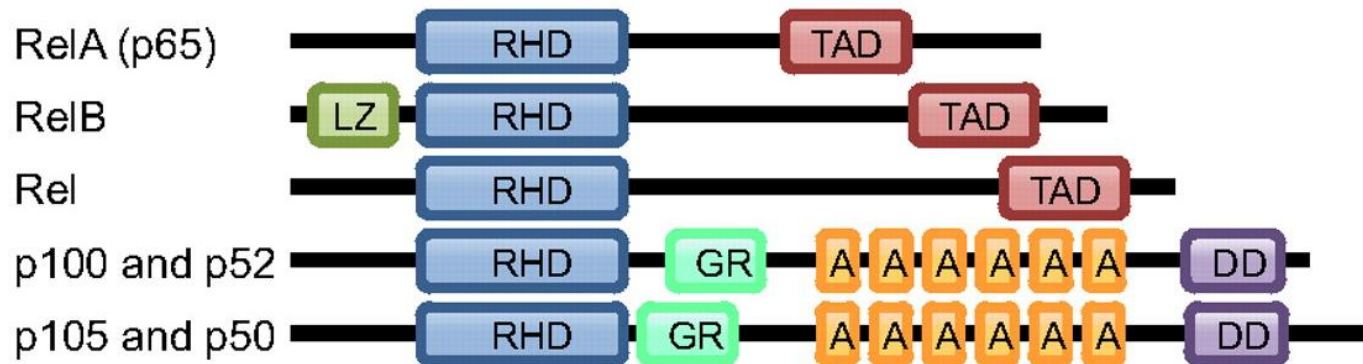


Ubiquitin signalling



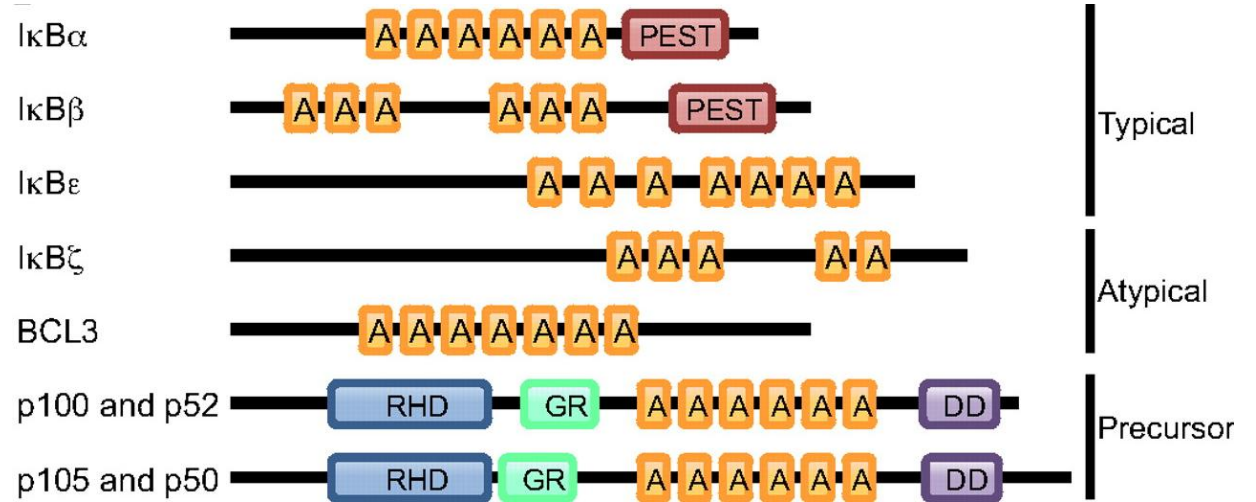
NF- κ B

- Nuclear-Factor (NF)- κ B: family of transcription factors
- Important in the regulation of genes involved in cell survival, cell growth and inflammation.



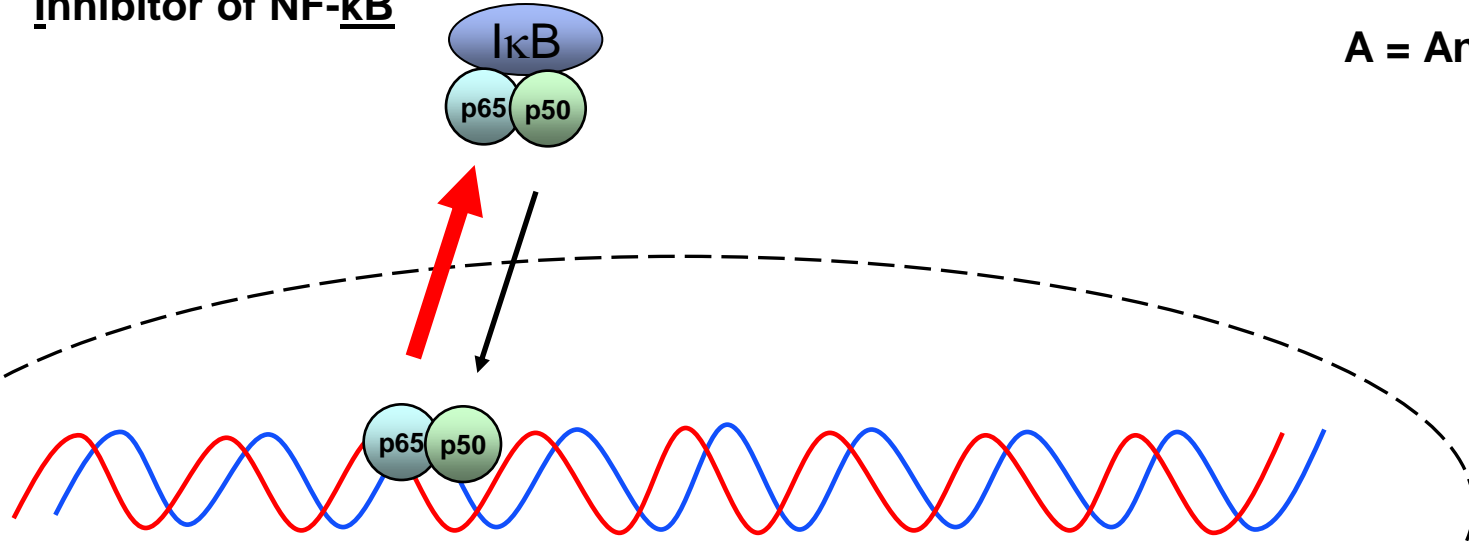
- RHD = Rel homology domain
- Function as hetero/homo-dimers

I κ B proteins inhibit NF- κ B activation by sequestering NF- κ B dimers in the cytoplasm



A = Ankyrin repeats

Inhibitor of NF- κ B

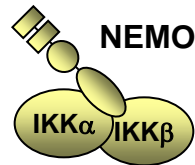


NF- κ B activation

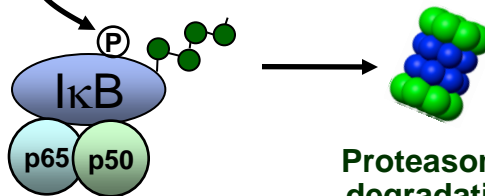
Upstream signals

Part II :
How is IKK activated?

IKK (I κ B Kinase)
complex



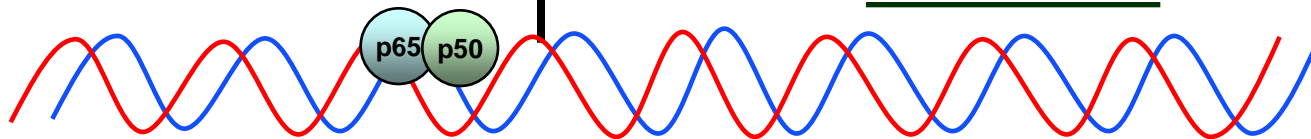
K48-linked poly-ubiquitylation



Proteasomal
degradation

pro-survival

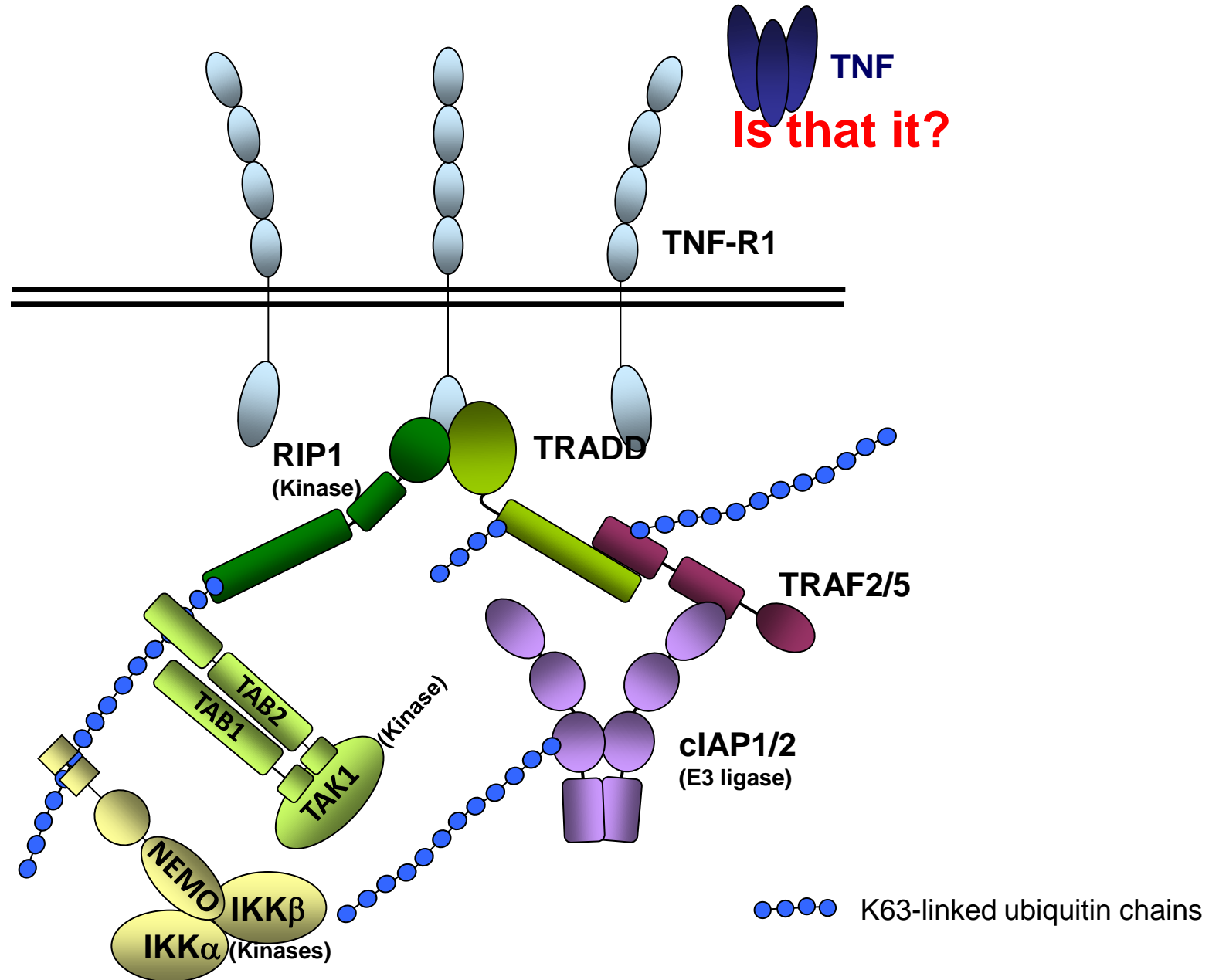
pro-inflammatory



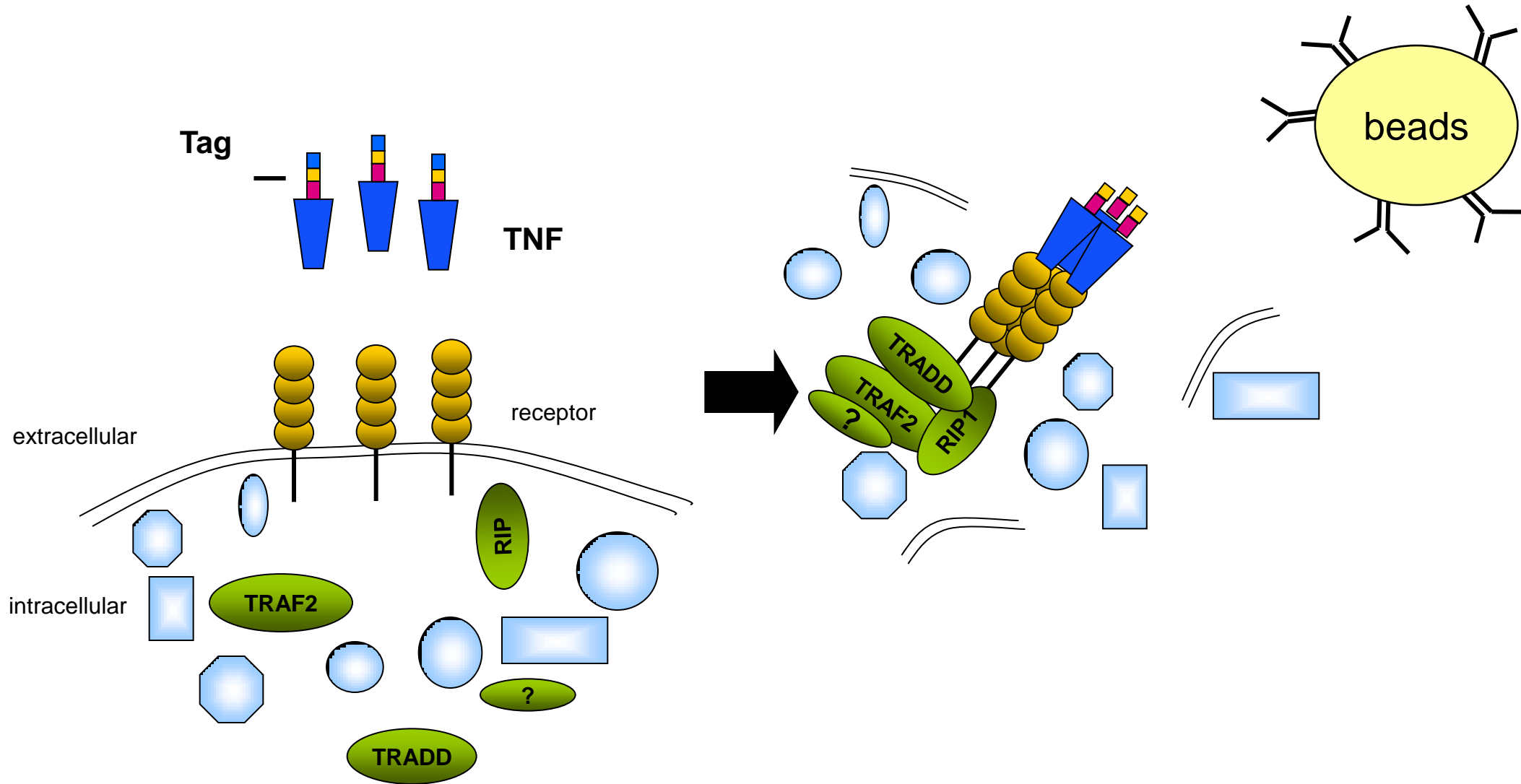
TNF-R1

- TNF (tumour necrosis factor) is a cytokine that binds to TNF-Receptor I (TNF-R1)
- TNF-R1 belongs to a large family of plasma-membrane-receptors that can activate **NF- κ B as well as cell death**, depending on the cellular context
- TNF-R1 signalling is implicated in cancer, innate immunity and inflammation
- TNF-R1 signalling is a model for **ubiquitin-mediated NF- κ B activation**

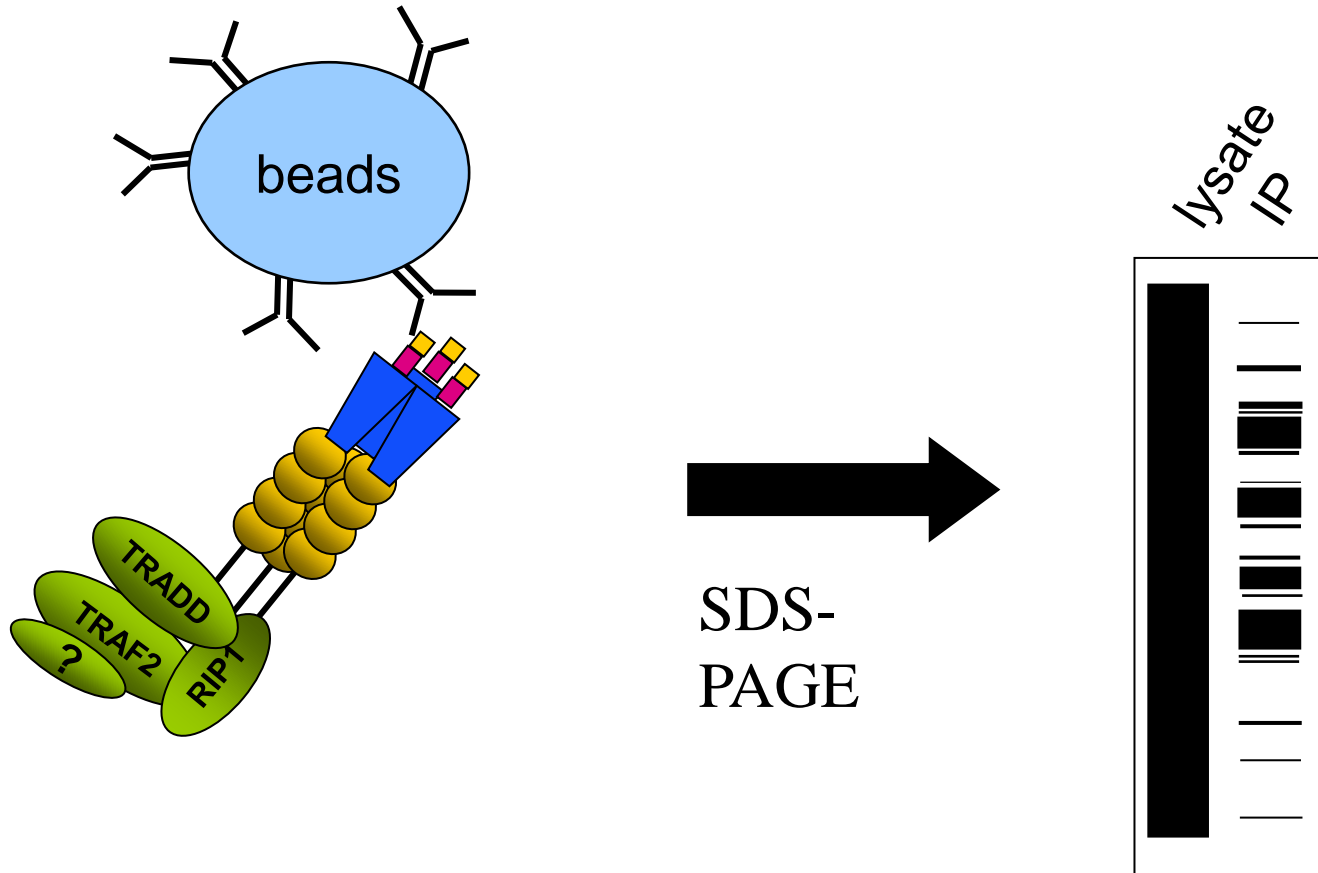
Composition of the TNF-receptor signalling complex (TNF-RSC)



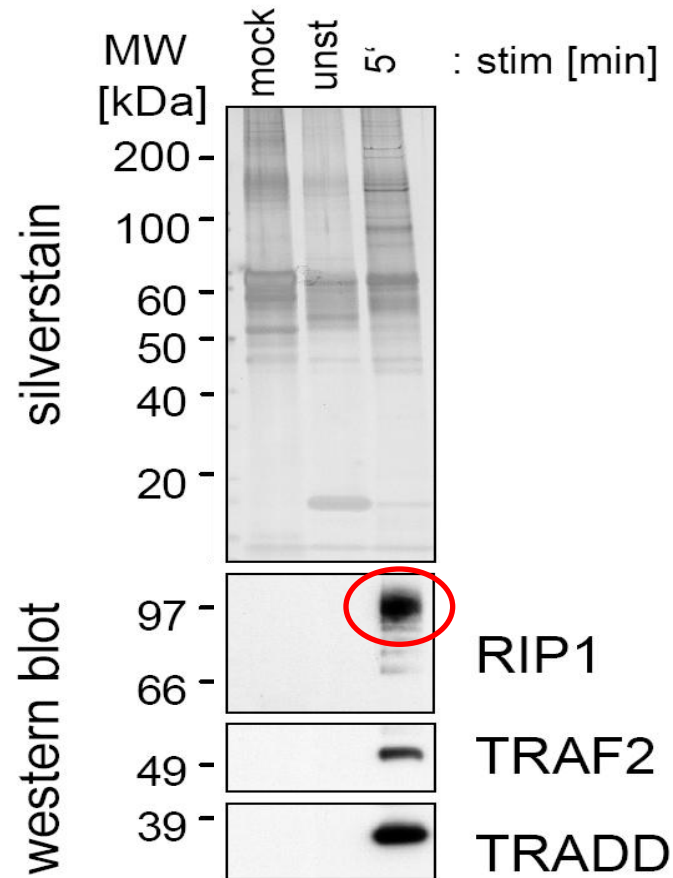
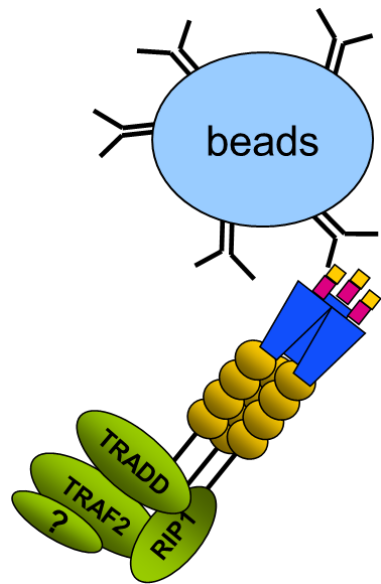
Purification of TNF-Receptor Signalling Complex (TNF-RSC) by Immunoprecipitation (IP)



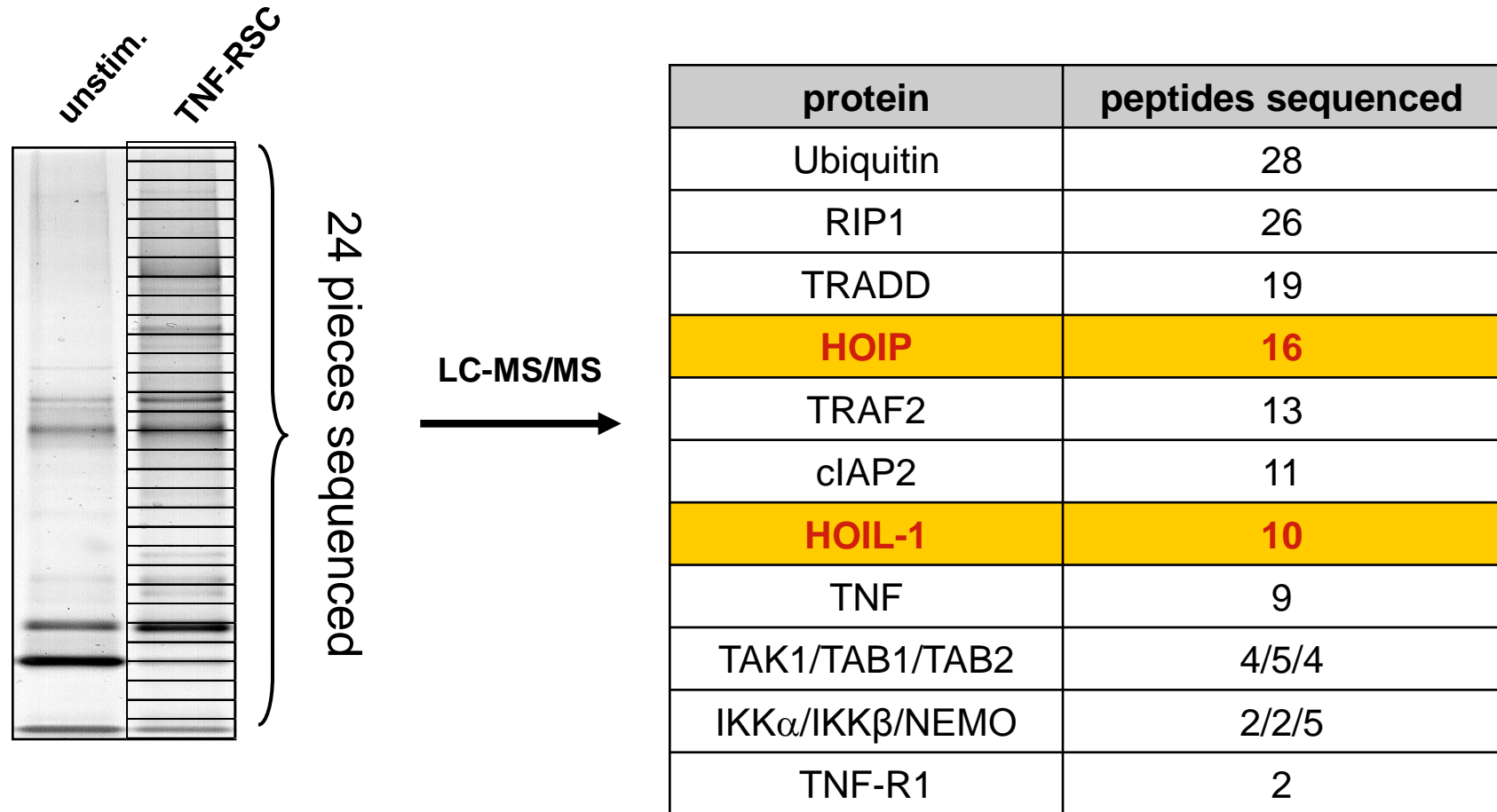
Purification of TNF-Receptor Signalling Complex (TNF-RSC) by Immunoprecipitation (IP)



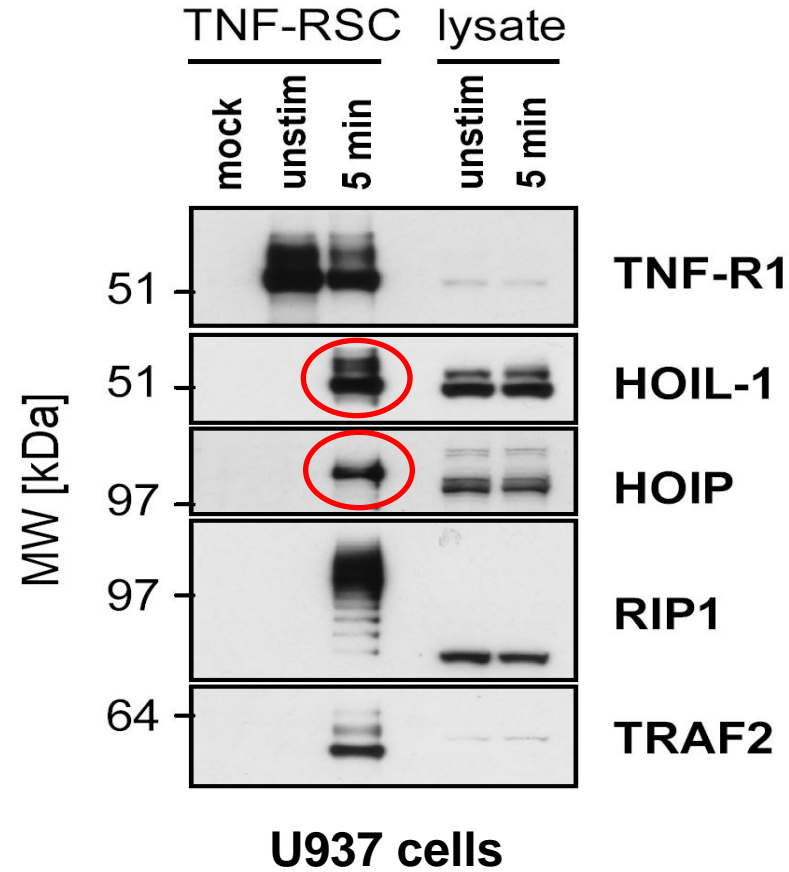
Purification of TNF-Receptor Signalling Complex (TNF-RSC) by Immunoprecipitation (IP)



Mass spectrometric identification of all so far known and two new proteins of the TNF-RSC



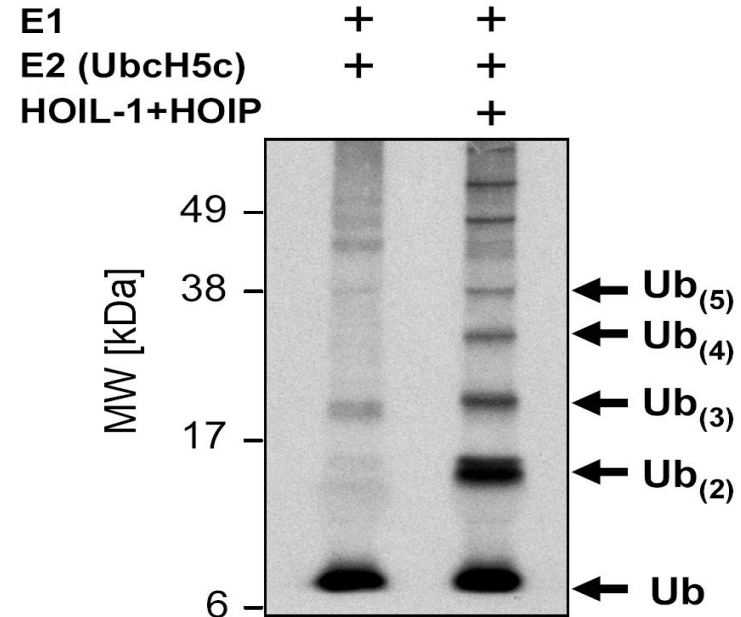
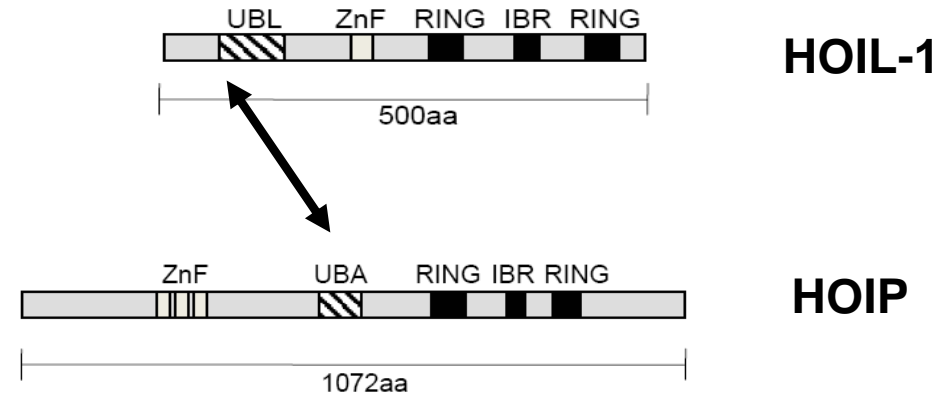
HOIL-1 and HOIP are recruited to the TNF-RSC in a stimulation-dependent manner



HOIL-1 and HOIP interact and form an E3-ligase complex

Kirisako et al. (EMBO 2006):

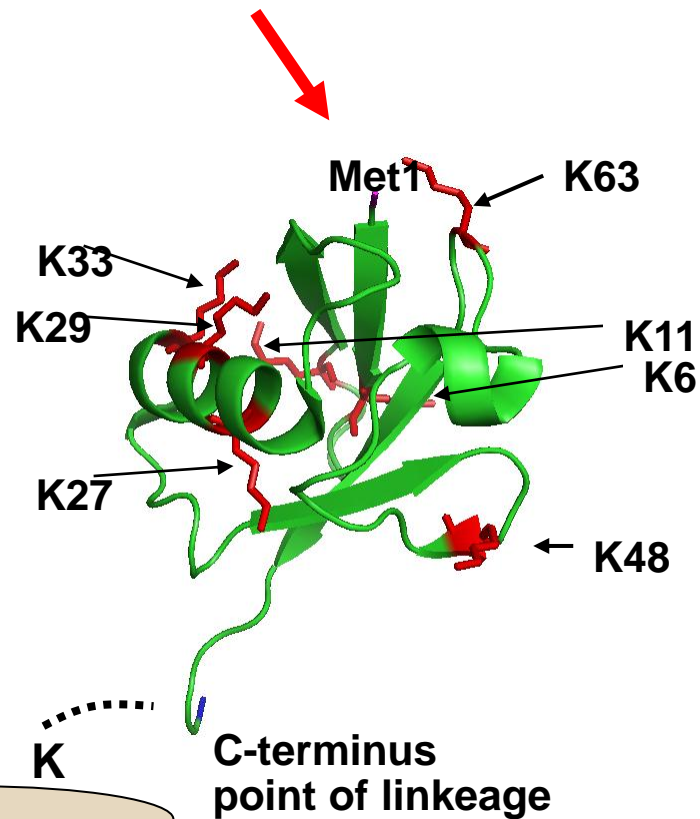
- HOIL-1 and HOIP interact
- HOIL-1 and HOIP form a linear ubiquitin assembly complex (LUBAC) = E3 ligase complex



Linear ubiquitylation

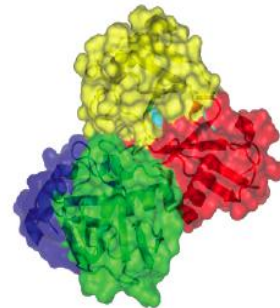
Linear ubiquitylation: M1-linked chains (head-to-tail)

Poly-ubiquitin chains linked through the N-terminal Methionine of ubiquitin instead of an internal lysine.

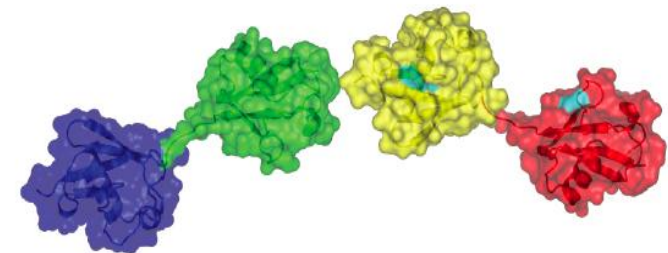


LUBAC is only E3 ligase that can make linear ubiquitin chains!

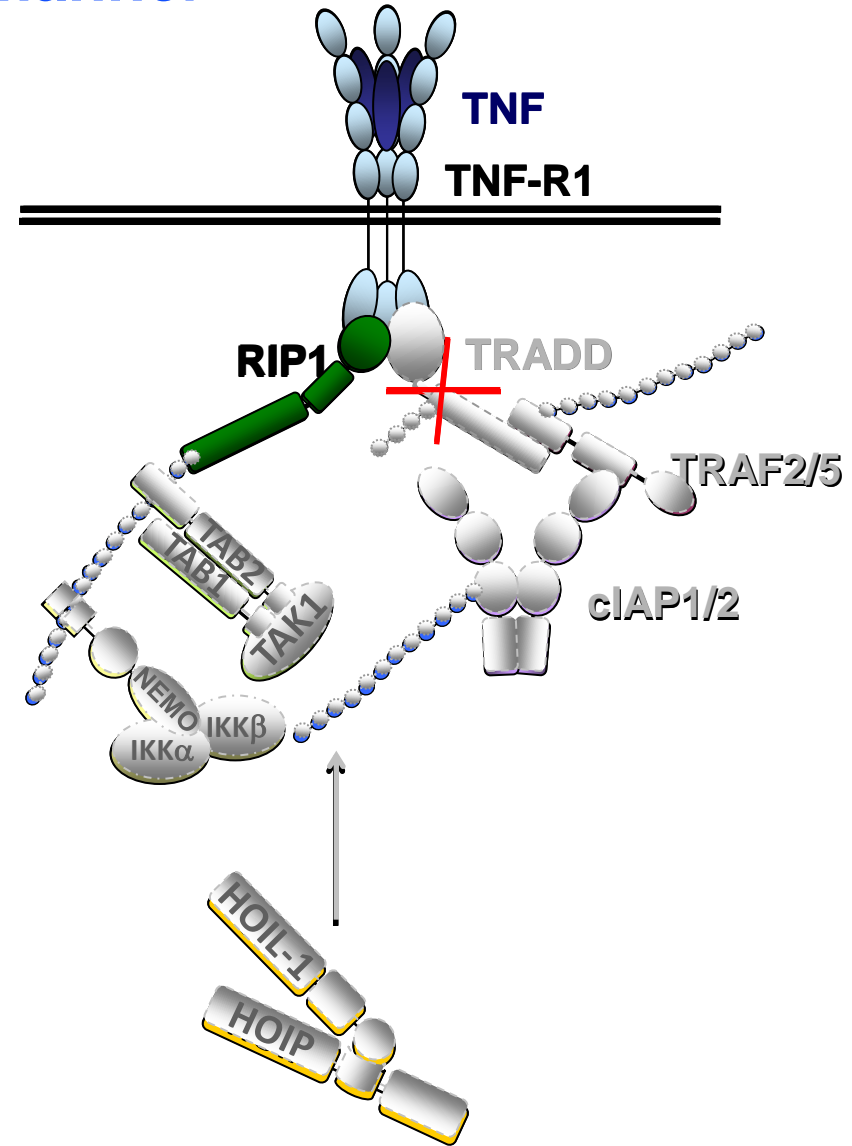
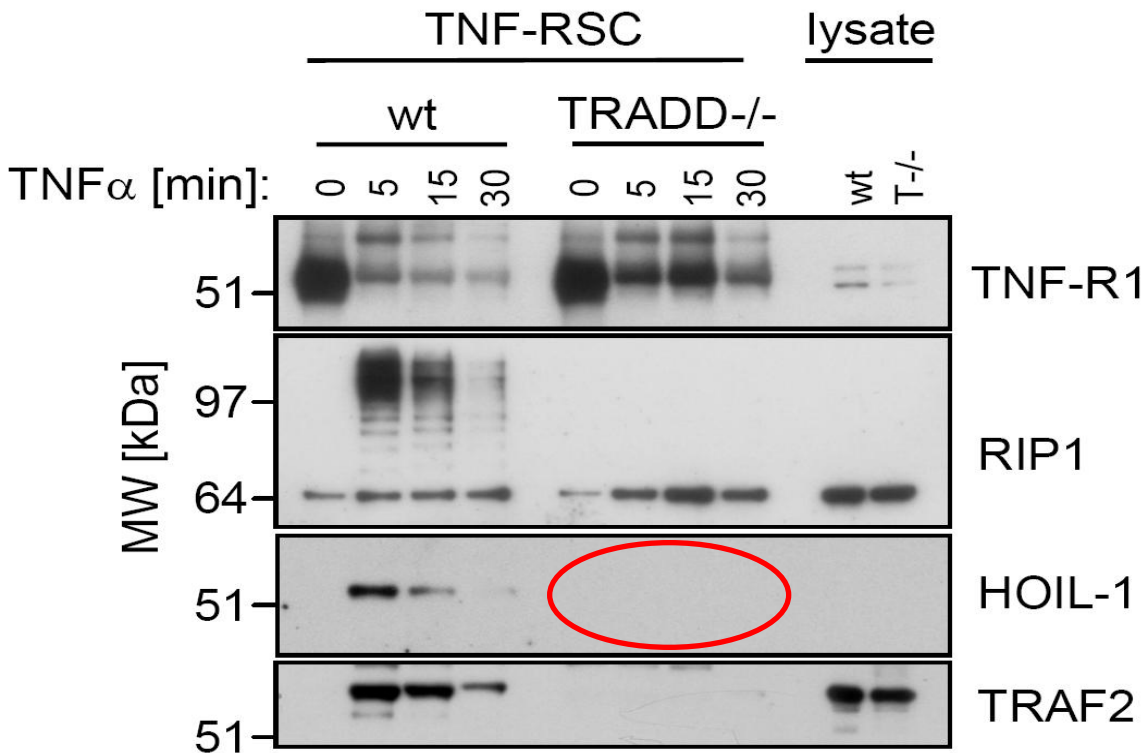
48-linked tetra-Ubiquitin



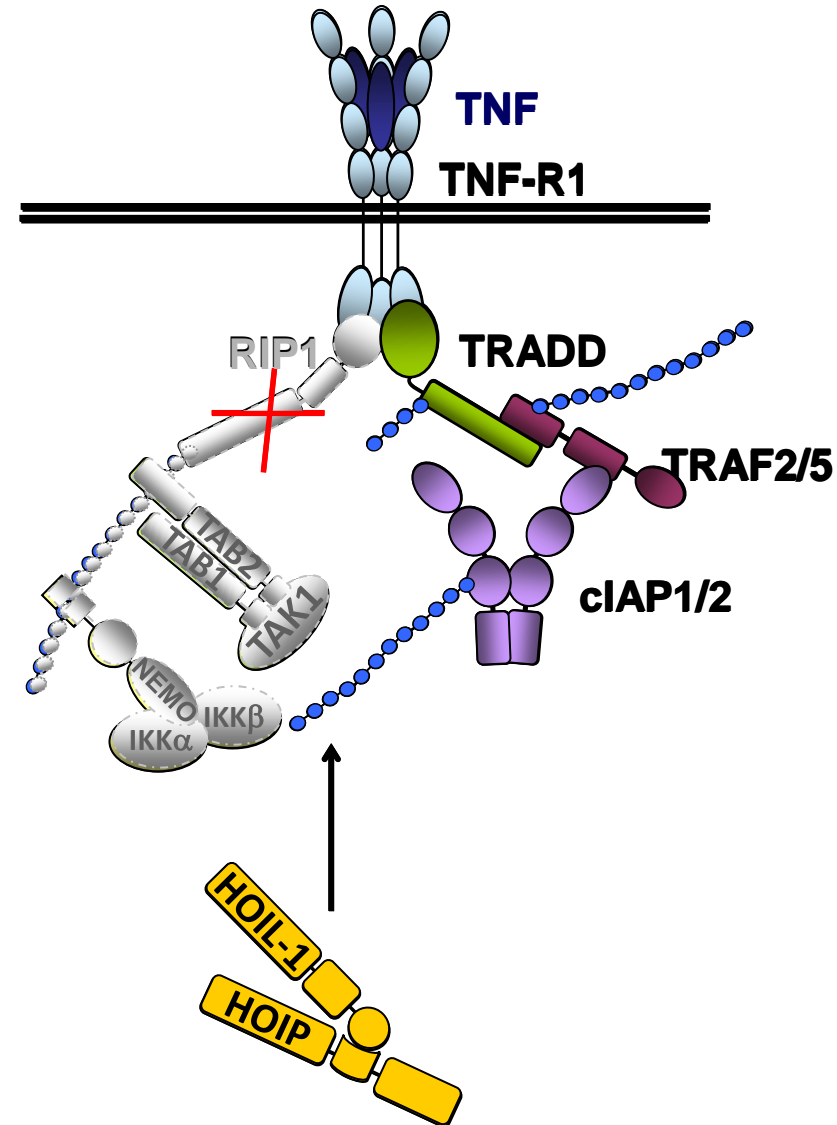
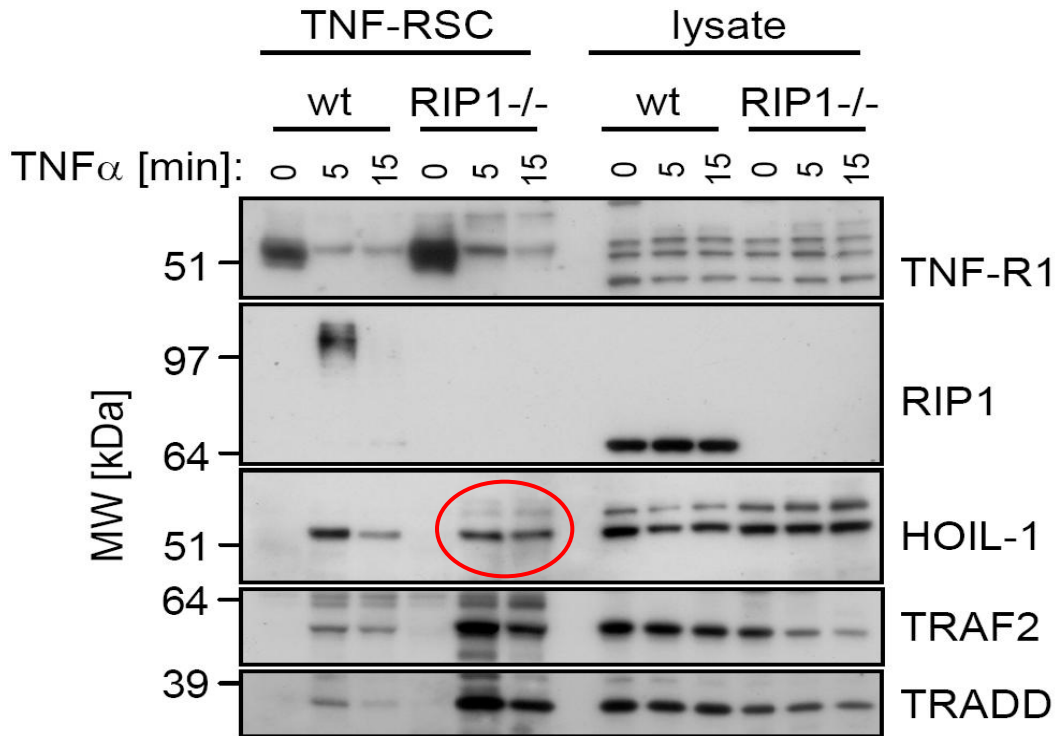
linear tetra-Ubiquitin



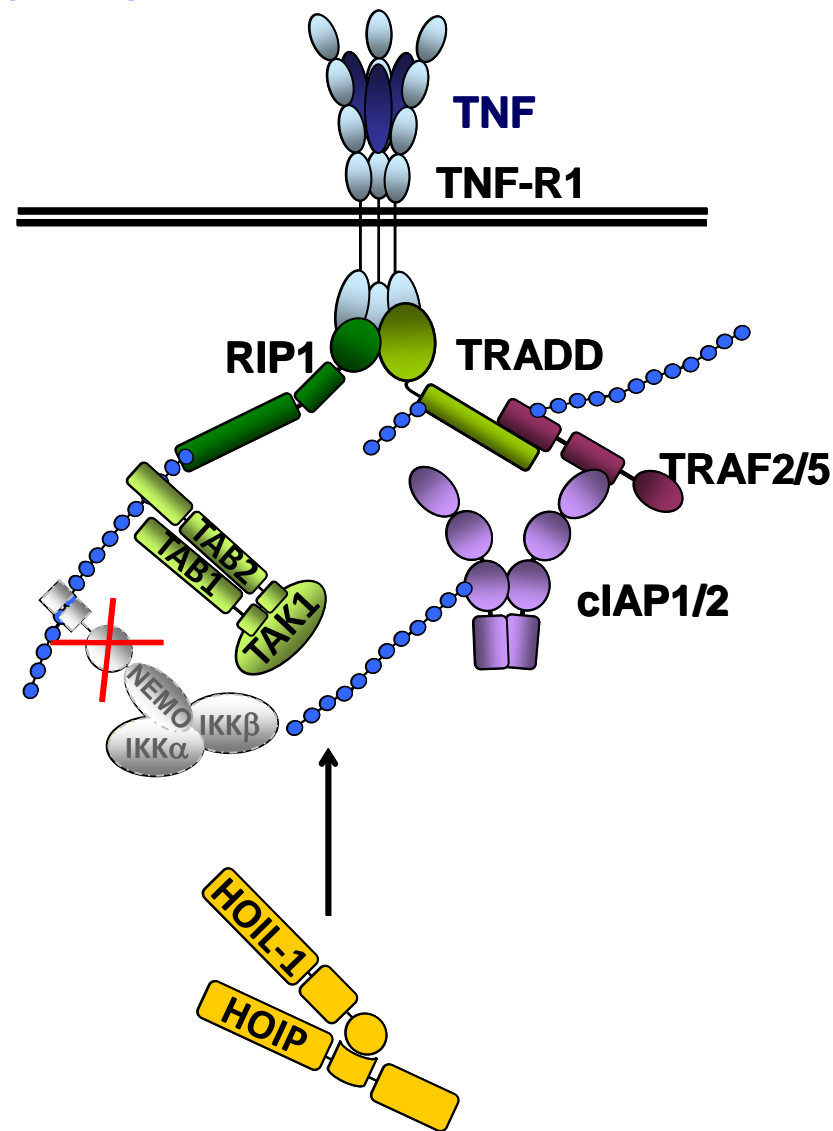
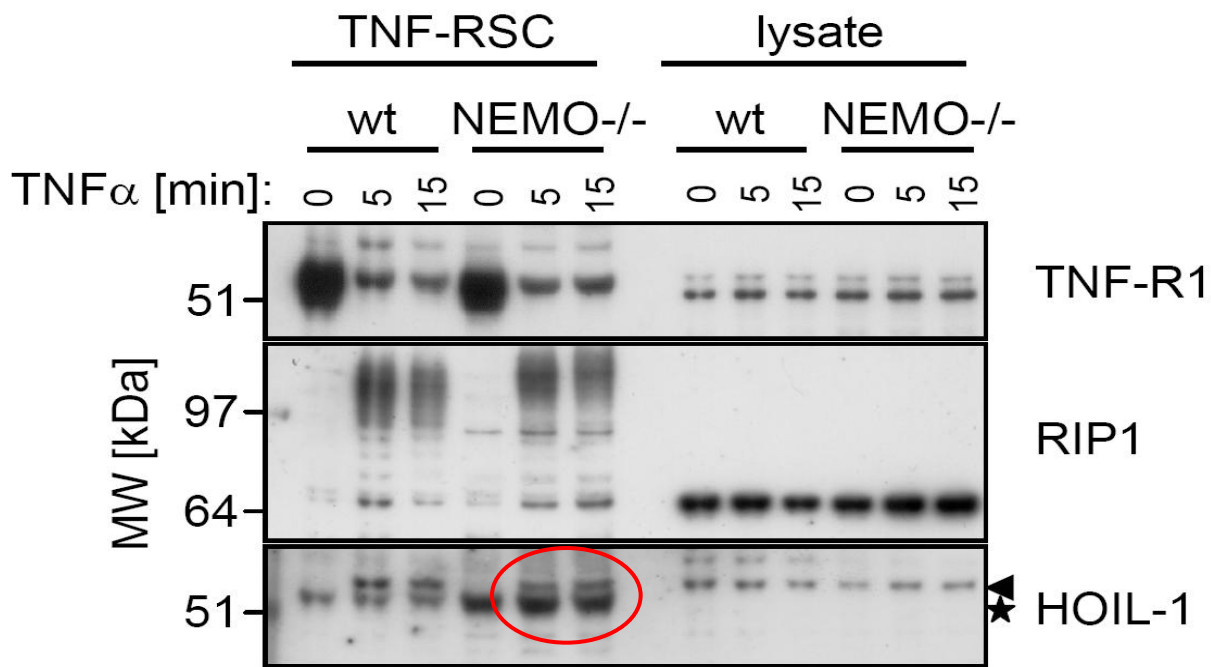
HOIL-1 is recruited to the TNF-RSC in a TRADD-dependent manner



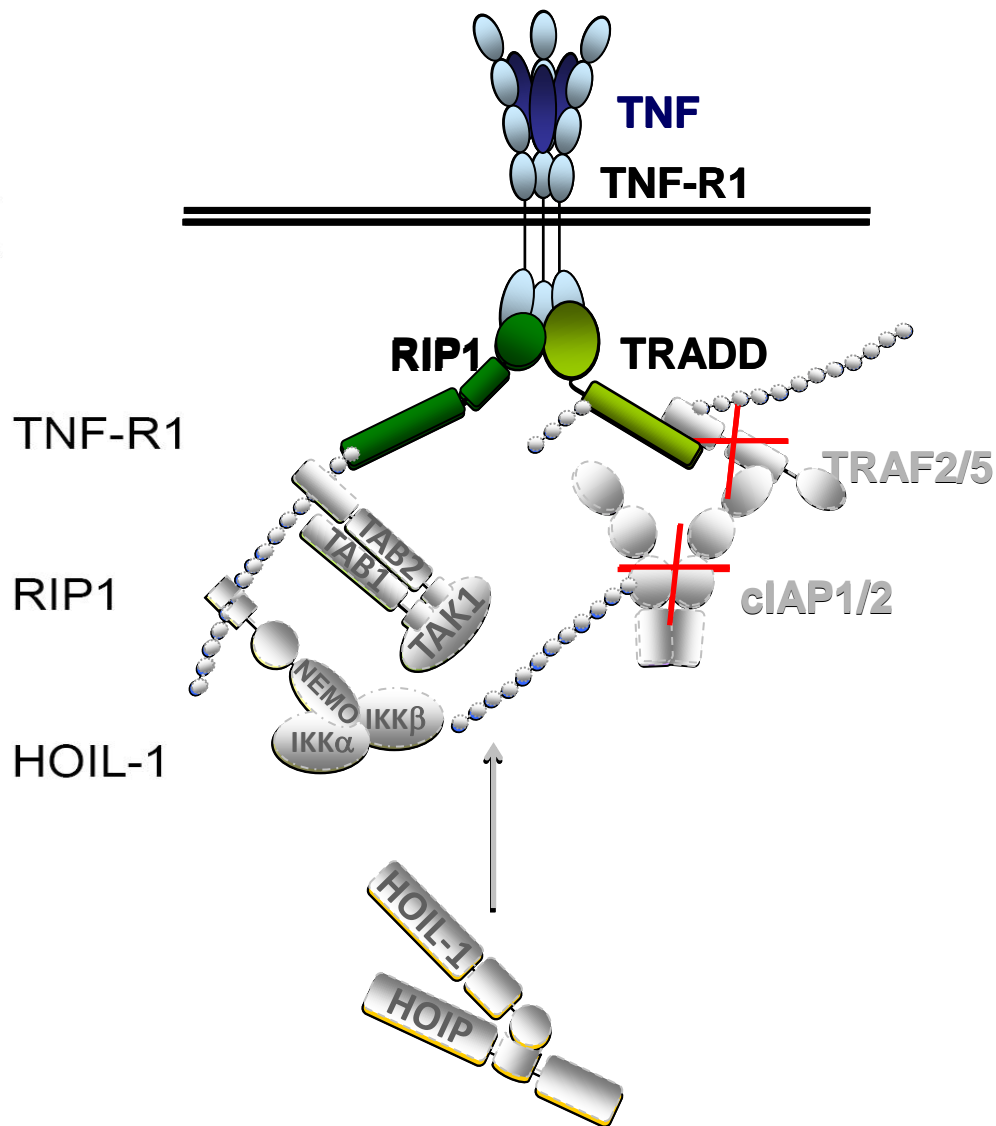
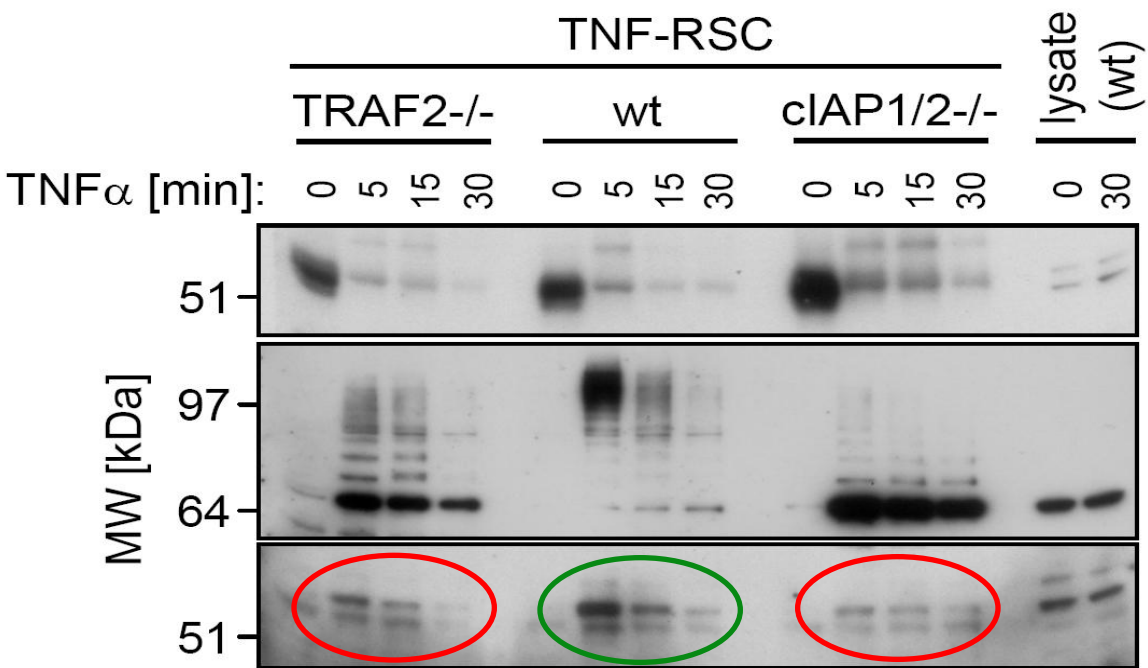
HOIL-1 is recruited to the TNF-RSC in a RIP1-independent manner



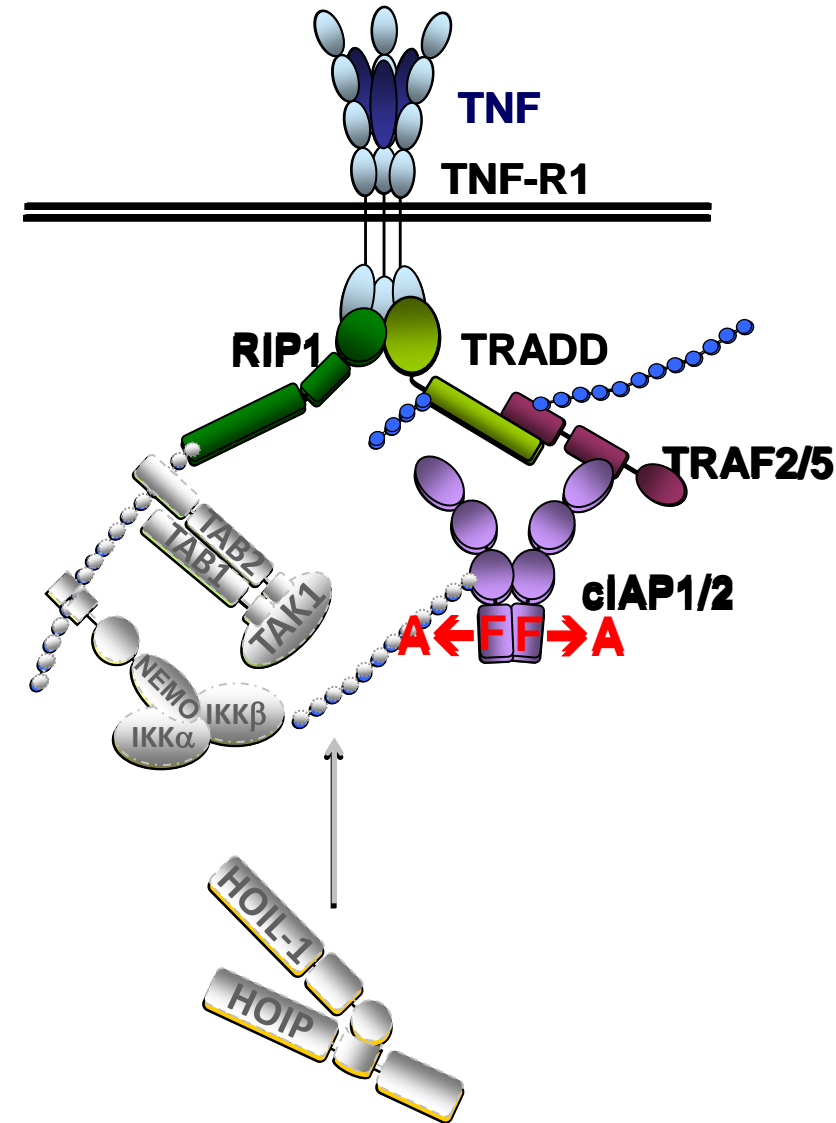
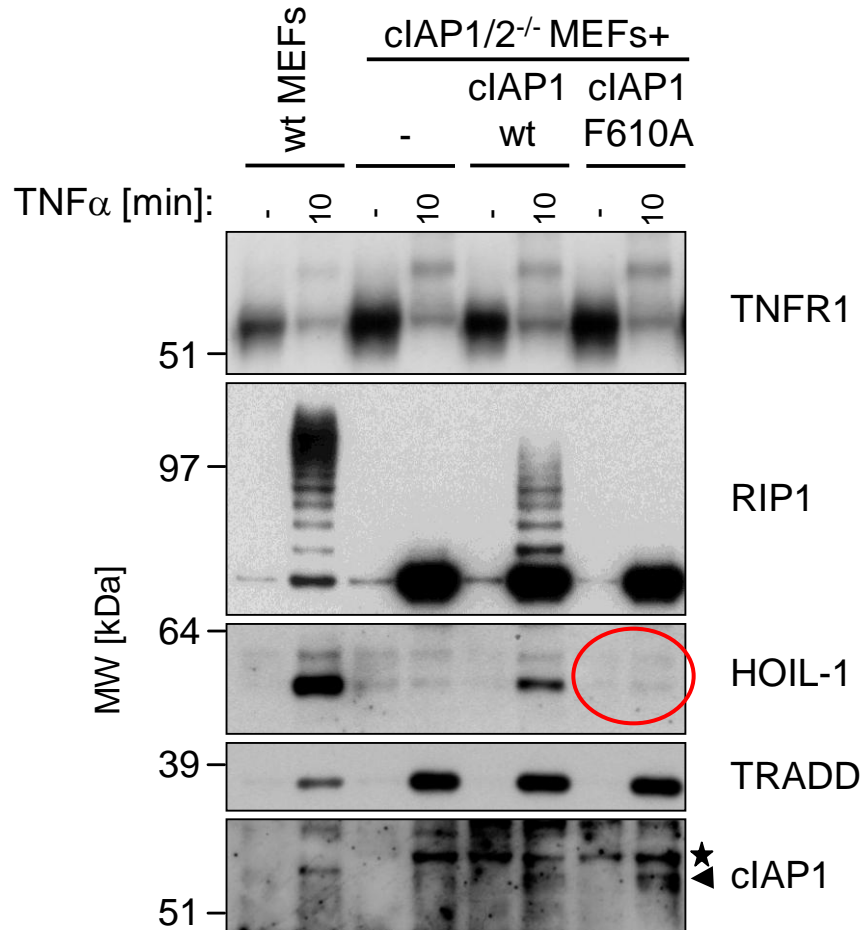
HOIL-1 is recruited to the TNF-RSC in a NEMO-independent manner



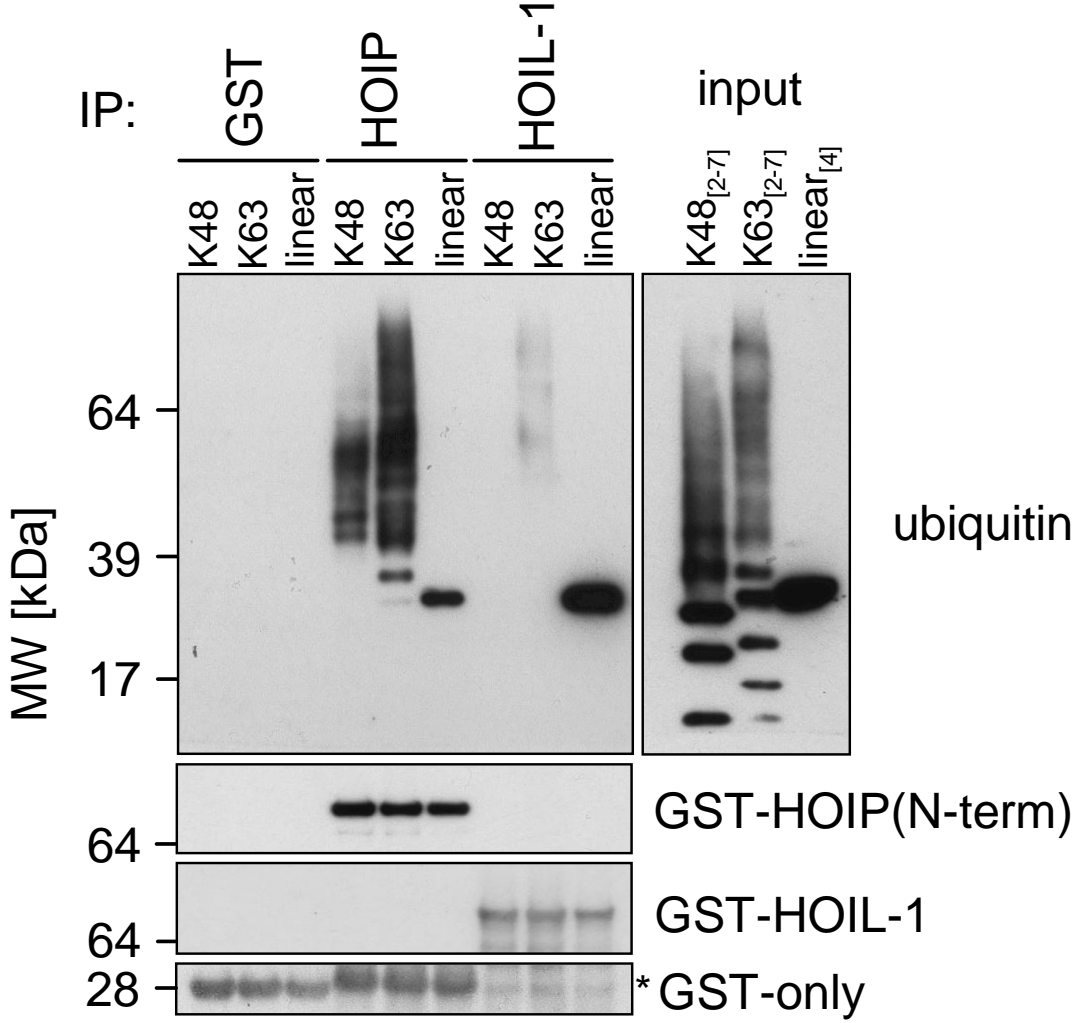
HOIL-1 is recruited to the TNF-RSC in a TRAF2/cIAP-dependent manner



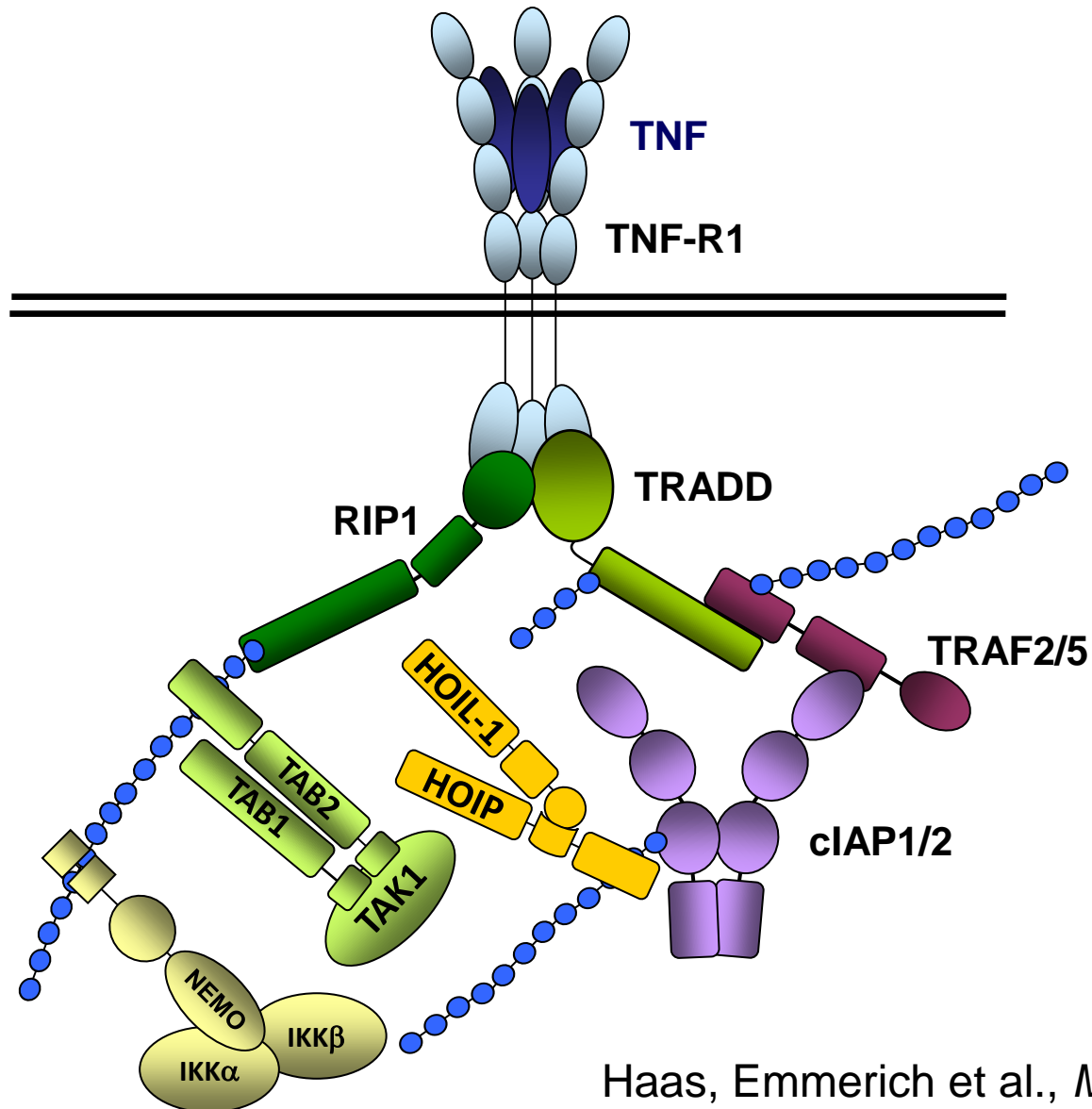
HOIL-1 recruitment depends on the catalytic activity of cIAP1/2



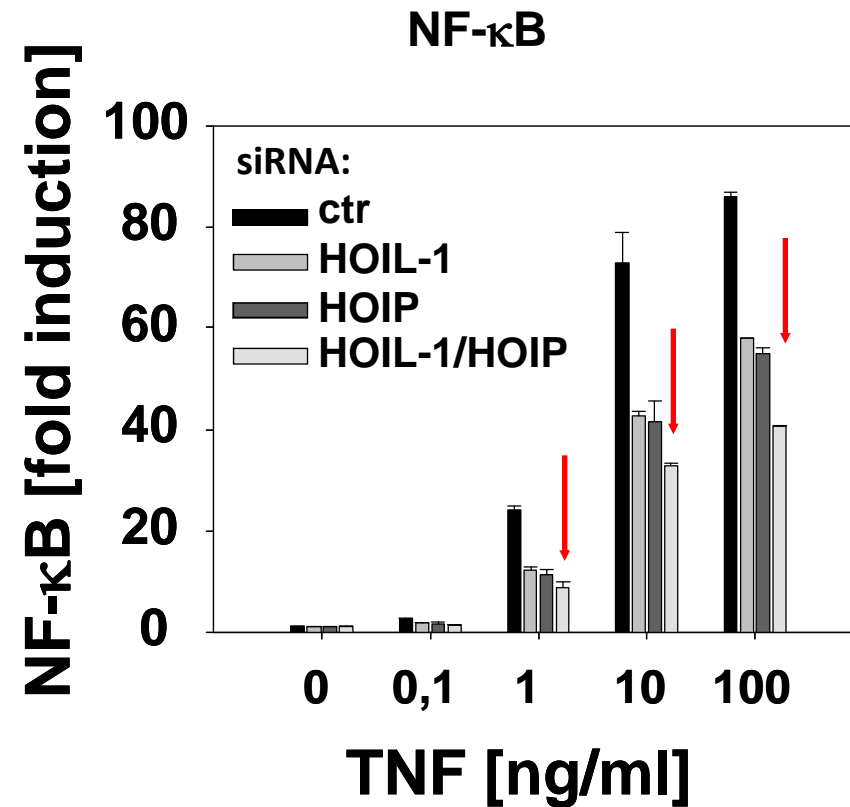
HOIL-1 and HOIP directly interact with specific polyubiquitin chains



LUBAC is recruited to the TNF-RSC by cIAP-generated Ubiquitin chains via the TRADD-TRAF2 cIAP signalling axis

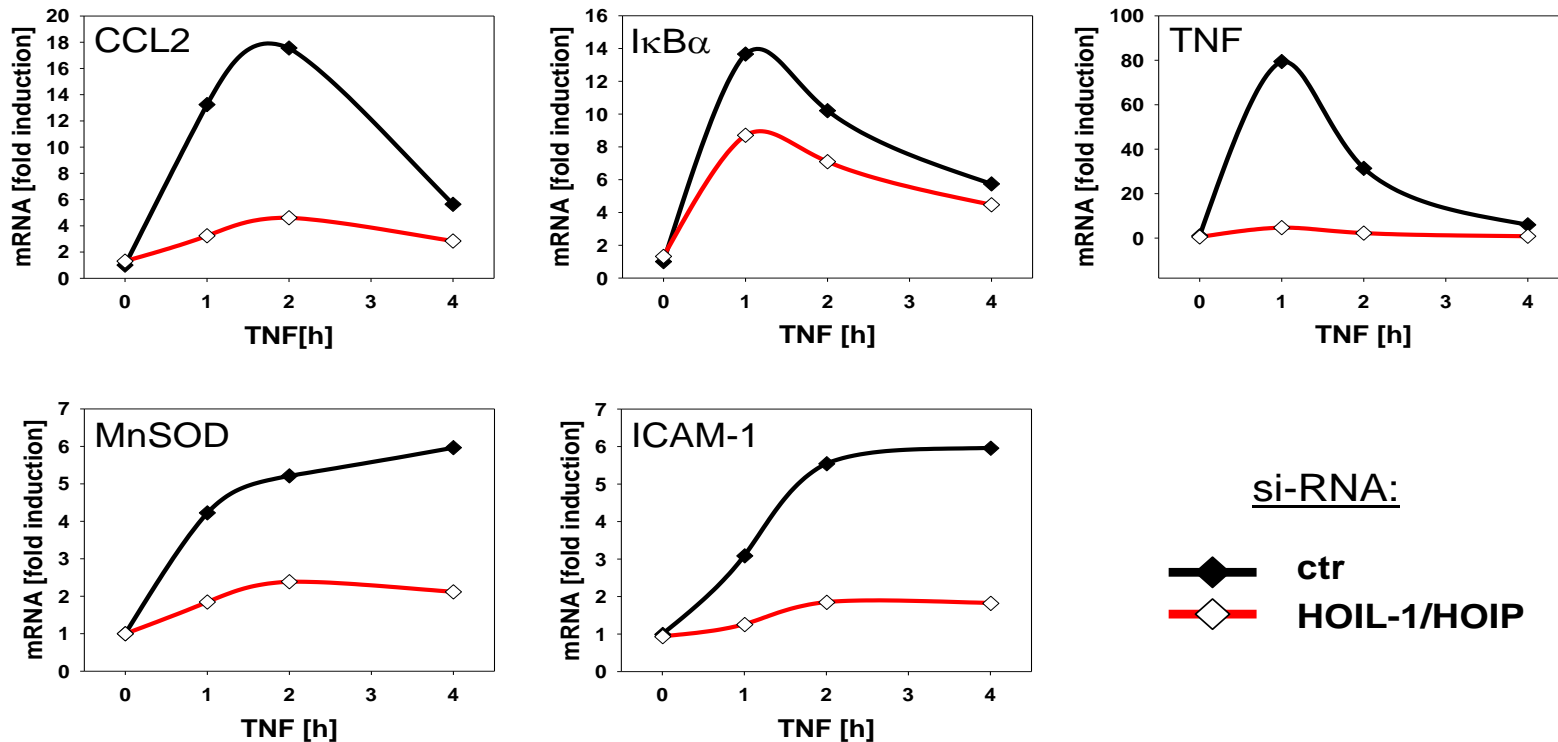


HOIL-1/HOIP knock-down reduces TNF-induced NF- κ B

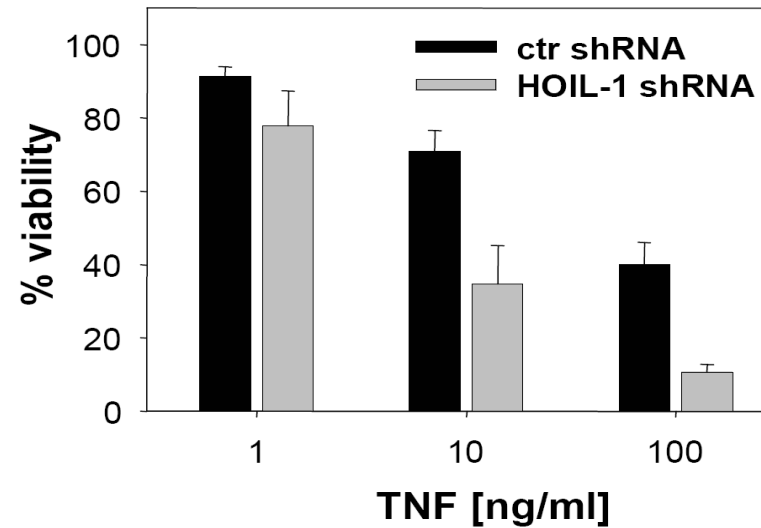


HOIL-1 and HOIP are crucial regulators for a number of TNF-dependent genes

HeLa cells

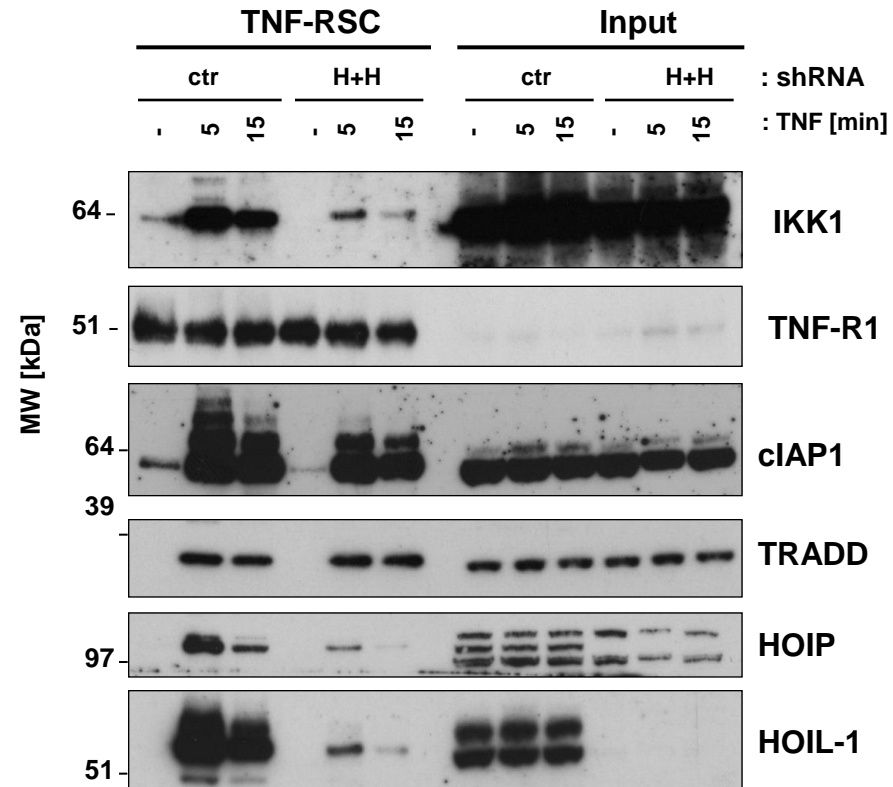


Absence of HOIL-1 renders cells more sensitive to TNF-induced apoptosis

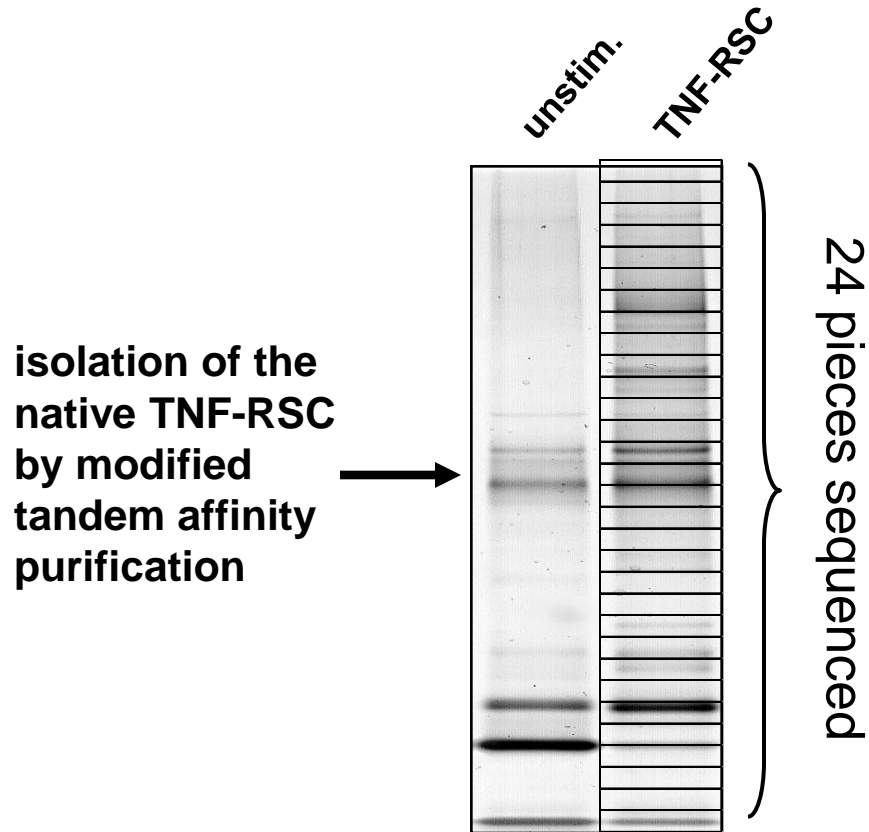


MCF-7 cells

LUBAC knockdown reduces recruitment/retention of TNF-RSC components to the complex



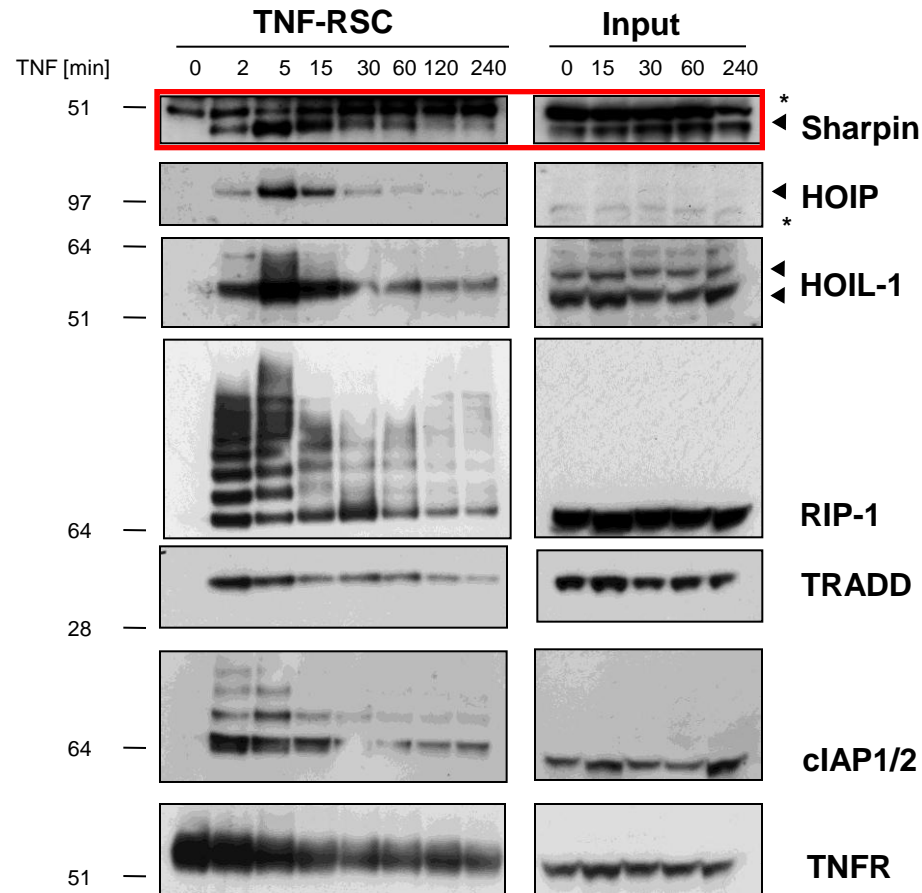
Sharpin is the third novel component of the native TNF-RSC besides HOIP and HOIL-1



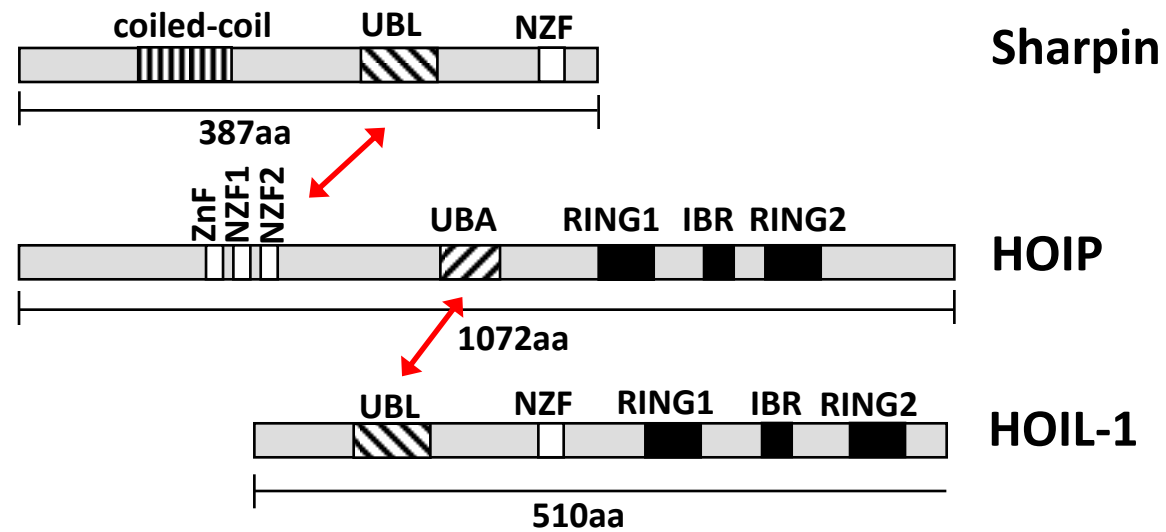
LC-MS/MS

protein	peptides sequenced
Ubiquitin	28
RIP1	26
TRADD	19
HOIP	16
TRAF2	13
cIAP2	11
HOIL-1	10
Sharpin	9
TNF	9
TAK1/TAB1/TAB2	4/5/4
IKK α /IKK β /NEMO	2/2/5
TNF-R1	2

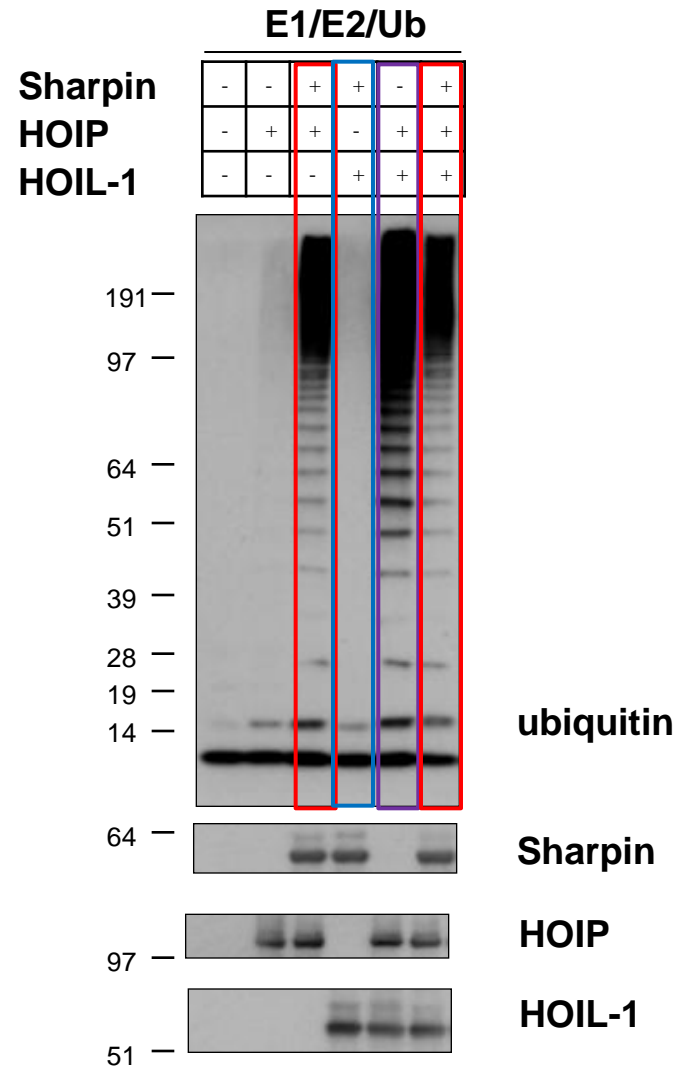
Sharpin, HOIP and HOIL-1 are recruited to the TNF-RSC with similar kinetics



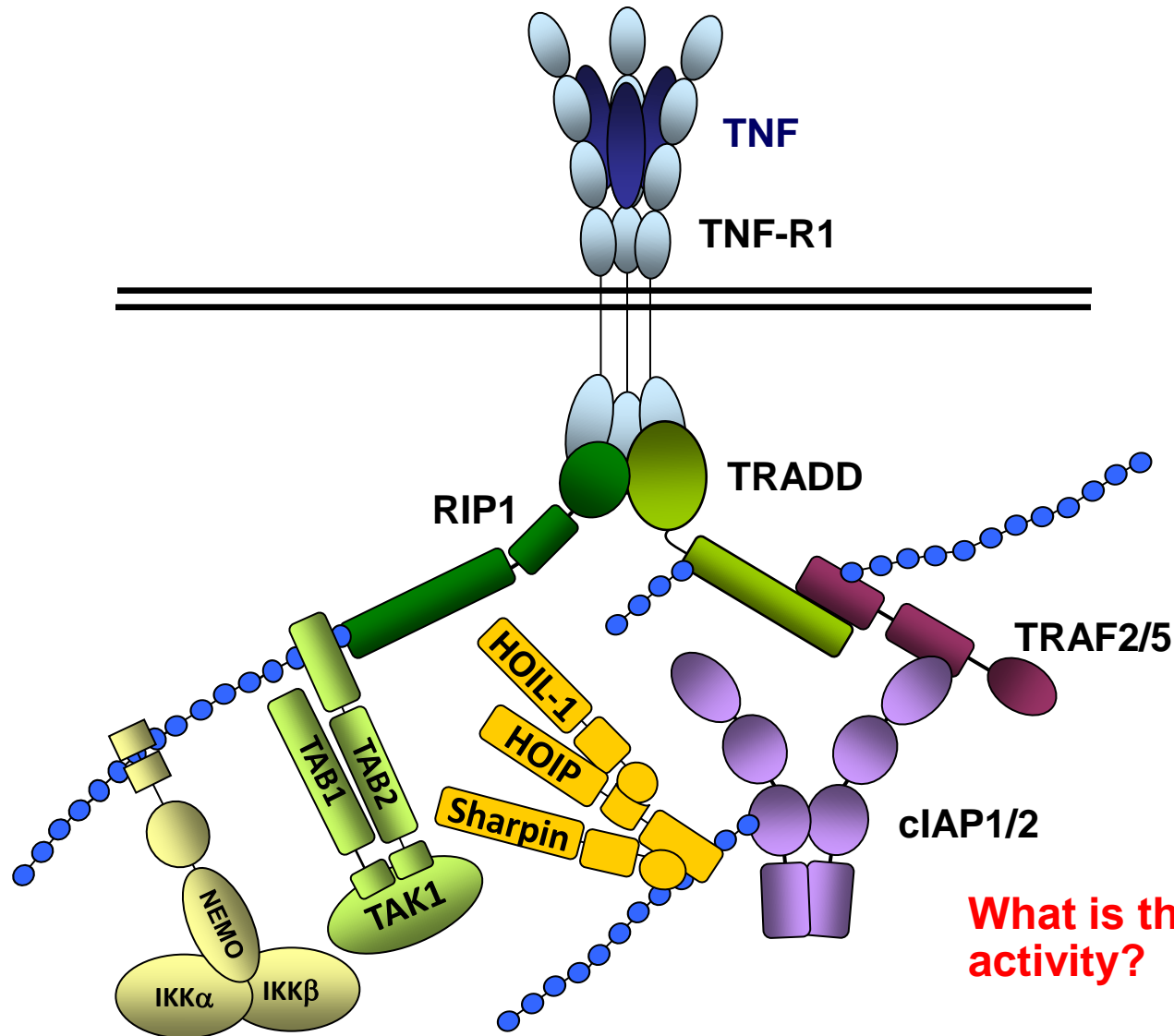
Sharpin, HOIL-1 and HOIP form a trimeric complex (LUBAC)



HOIP is also able to form linear ubiquitin chains in combination with Sharpin

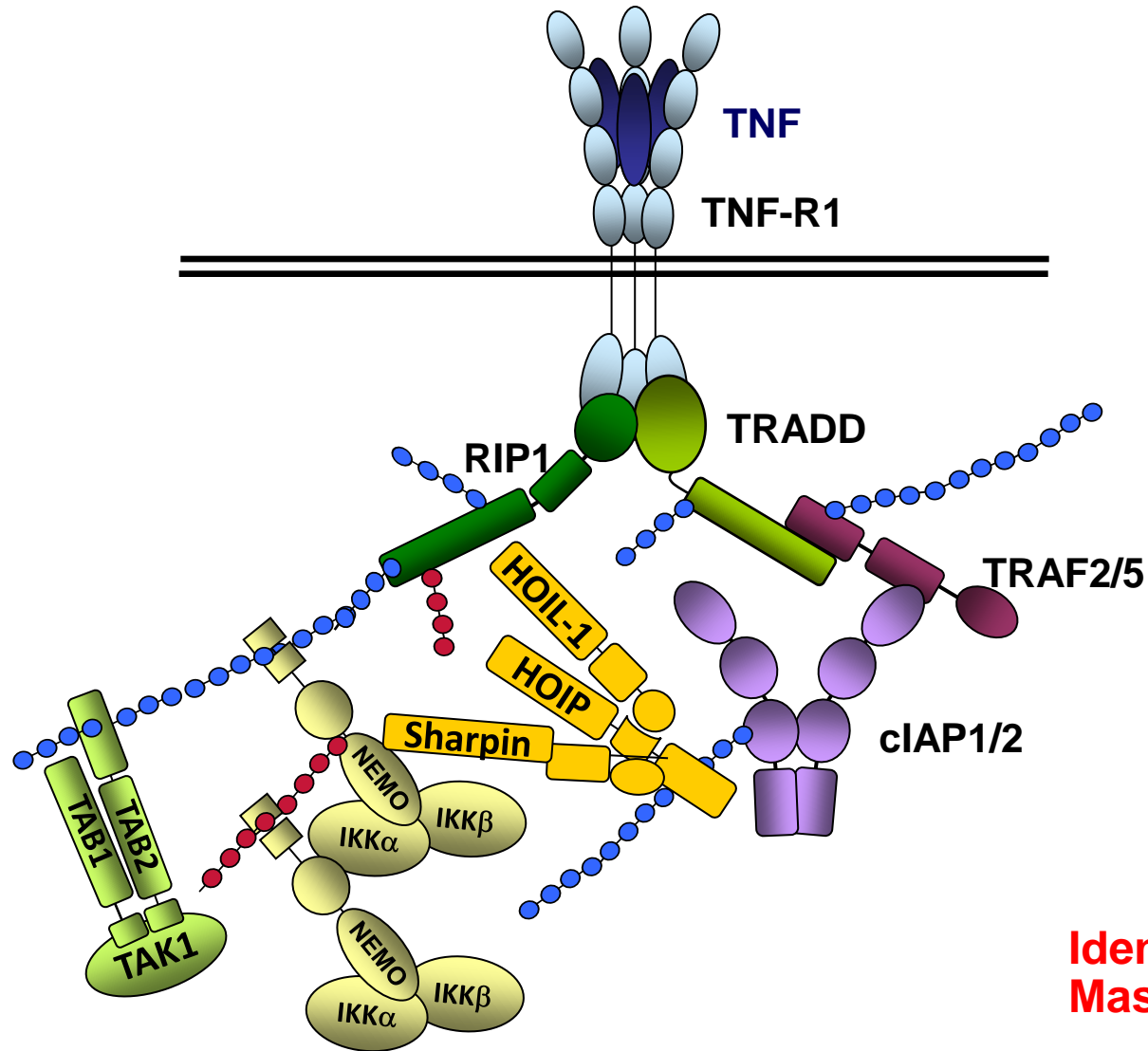


Recruitment of Sharpin, HOIP and HOIL-1 to the TNF-RSC depends on cIAP1/2 and on HOIP



What is the target of LUBAC's activity?

NEMO and RIP1 are modified by LUBAC with linear-linked ubiquitin chains in the TNF-RSC



Identification of targets by
Mass-spectrometry

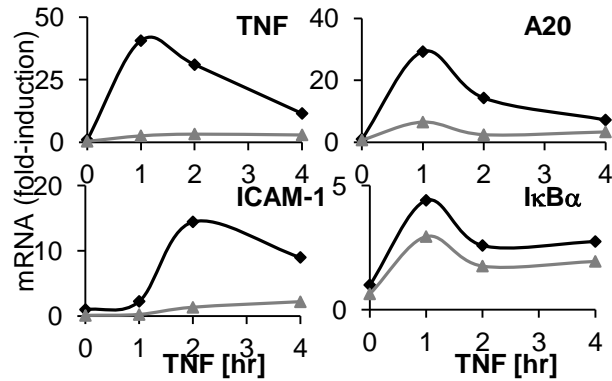
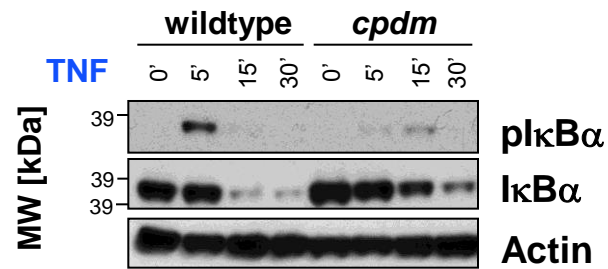
Phenotype of Sharpin mutant *cpdm* Mice

- Spontaneous base pair deletion leads to severe chronic proliferative dermatitis (***cpdm***)
- Multi-organ inflammation (apart from skin also affects liver, forestomach, oesophagus, etc.)
- Defective organisation of lymphoid tissue (lack of well-formed follicles, germinal centres and follicular DCs)
- absence of marginal zone in the spleen; absence of Peyer's patches
- **Increased cell death of keratinocytes**

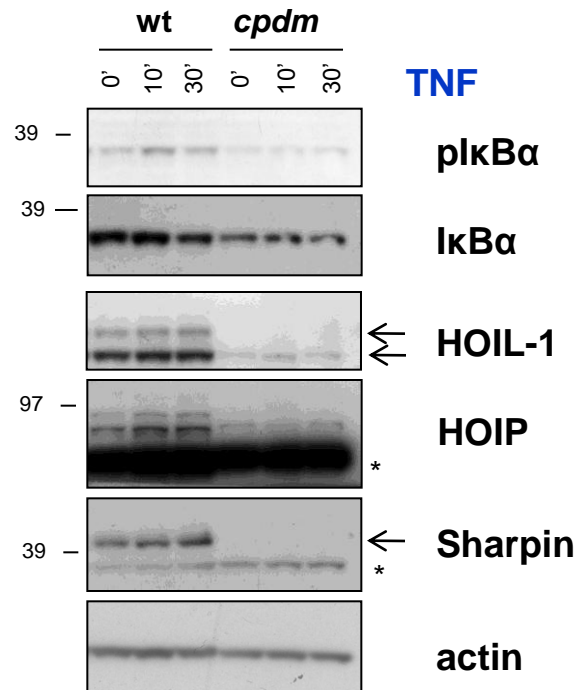


Sharpin is required for effective TNF-induced NF- κ B activation and, consequently, gene induction

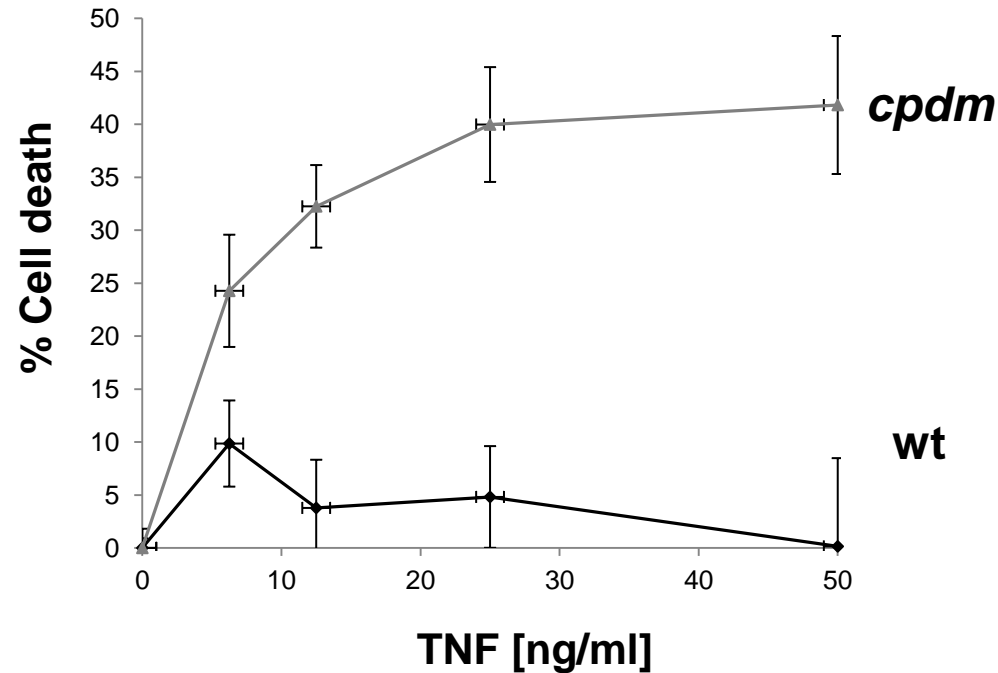
MEFs



TNF-induced NF- κ B activation is impaired in primary *cpdm*-derived keratinocytes cells



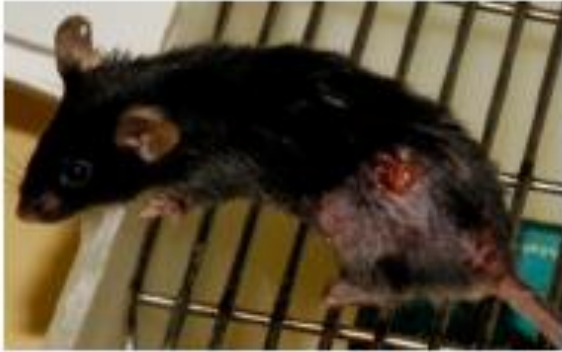
Increased TNF-induced cell death in *cpdm* MEFs



Loss of Sharpin results in a cell death-favouring dysregulation of TNF-induced signalling

TNF deficiency corrects the inflammatory phenotype in *cpdm* mice

cpdm TNF^{+/+}



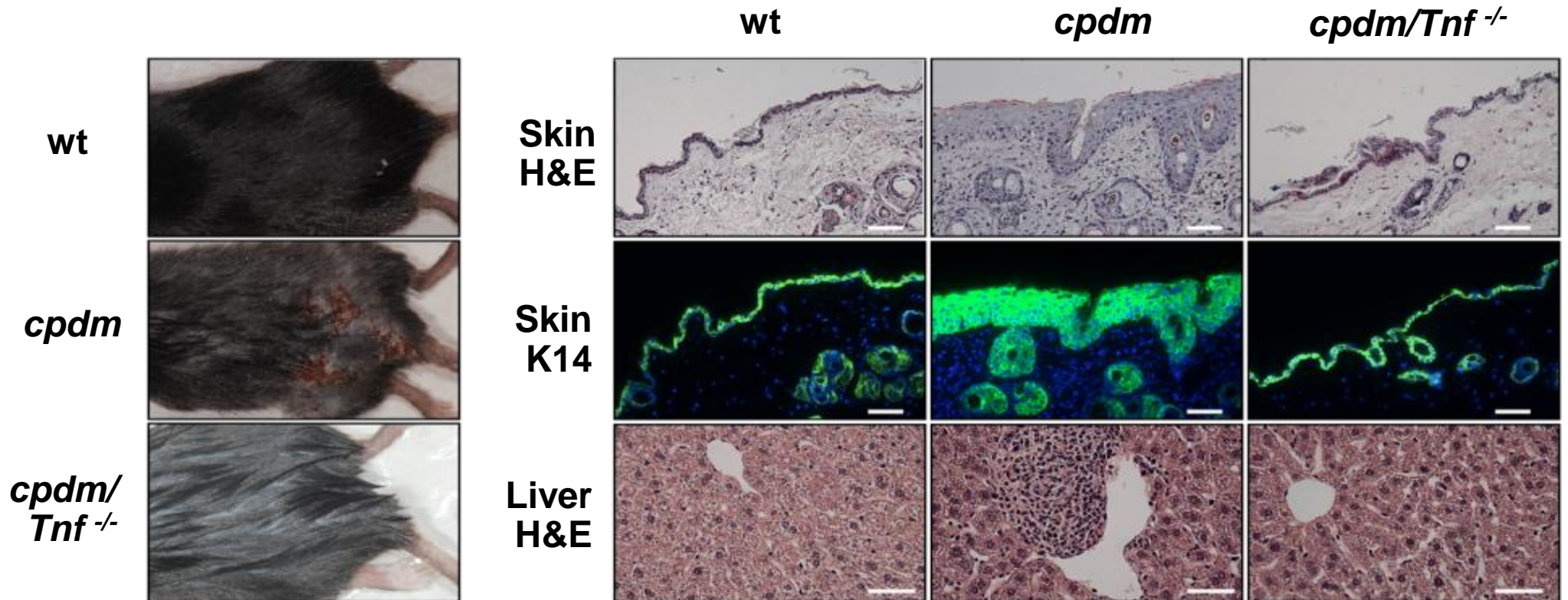
cpdm TNF^{-/-}



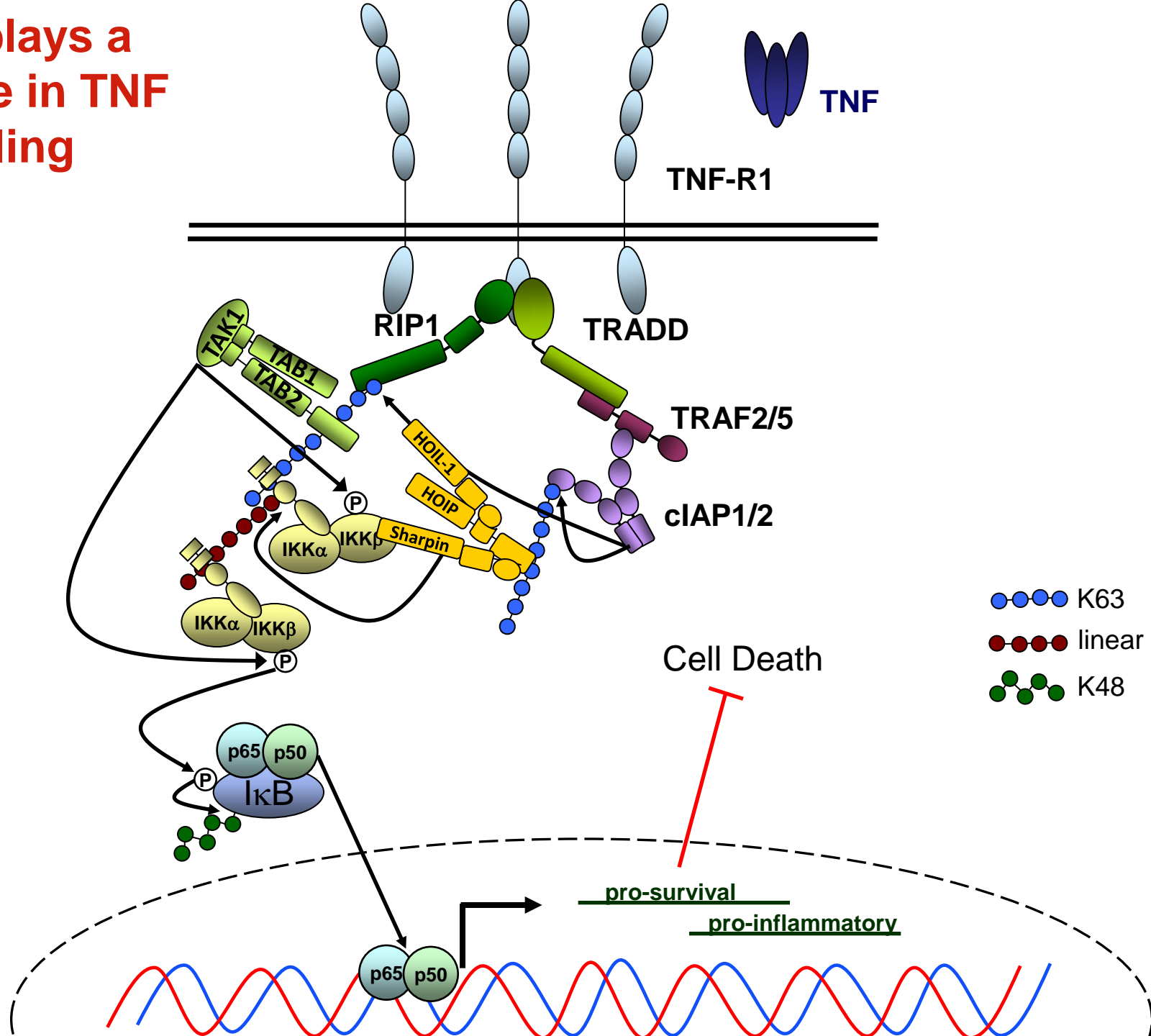
cpdm TNF^{+/-}



TNF deficiency corrects the inflammatory phenotype in *cpdm* mice



LUBAC plays a central role in TNF signalling



Summary

- Sharpin, HOIP and HOIL-1 form the Linear ubiquitin assembly complex (LUBAC) and are novel components of the TNF receptor signalling complex (TNF-RSC)
- Recruitment of Sharpin, HOIP and HOIL-1 to the TNF-RSC depends on cIAP1/2
- Linear ubiquitylation, mediated by LUBAC, enables efficient TNF-induced gene induction by NF- κ B and inhibition of cell death
- Lack of Sharpin causes a cell death-favouring dysregulation of TNF signalling and an inflammatory phenotype in mice (*cpdm*)
- *cpdm* MEFs and primary keratinocytes are susceptible to TNF-induced cell death
- TNF deficiency corrects the inflammatory abnormalities observed in *cpdm* mice

Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency

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We report the clinical description and molecular dissection of a new fatal human inherited disorder characterized by chronic autoinflammation, invasive bacterial infections and muscular amylopectinosis. Patients from two kindreds carried biallelic loss-of-expression and loss-of-function mutations in *HOIL1* (*RBCK1*), a component of the linear ubiquitination chain assembly complex (LUBAC). These mutations resulted in impairment of LUBAC stability. NF- κ B activation in response to interleukin 1 β (IL-1 β) was compromised in the patients' fibroblasts. By contrast, the patients' mononuclear leukocytes, particularly monocytes, were hyper-responsive to IL-1 β . The consequences of human HOIL-1 and LUBAC deficiencies for IL-1 β responses thus differed between cell types, consistent with the unique association of autoinflammation and immunodeficiency in these patients. These data suggest that LUBAC regulates NF- κ B-dependent IL-1 β responses differently in different cell types.

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Learning Objectives

You will be able to:

- describe the general mechanisms of ubiquitylation
- describe the general mechanisms of NF- κ B signalling
- describe the TNF-R1 signalling pathway
- explain the role of LUBAC and linear ubiquitylation in TNF-R1 signalling
- understand the biological consequences of loss of linear ubiquitylation