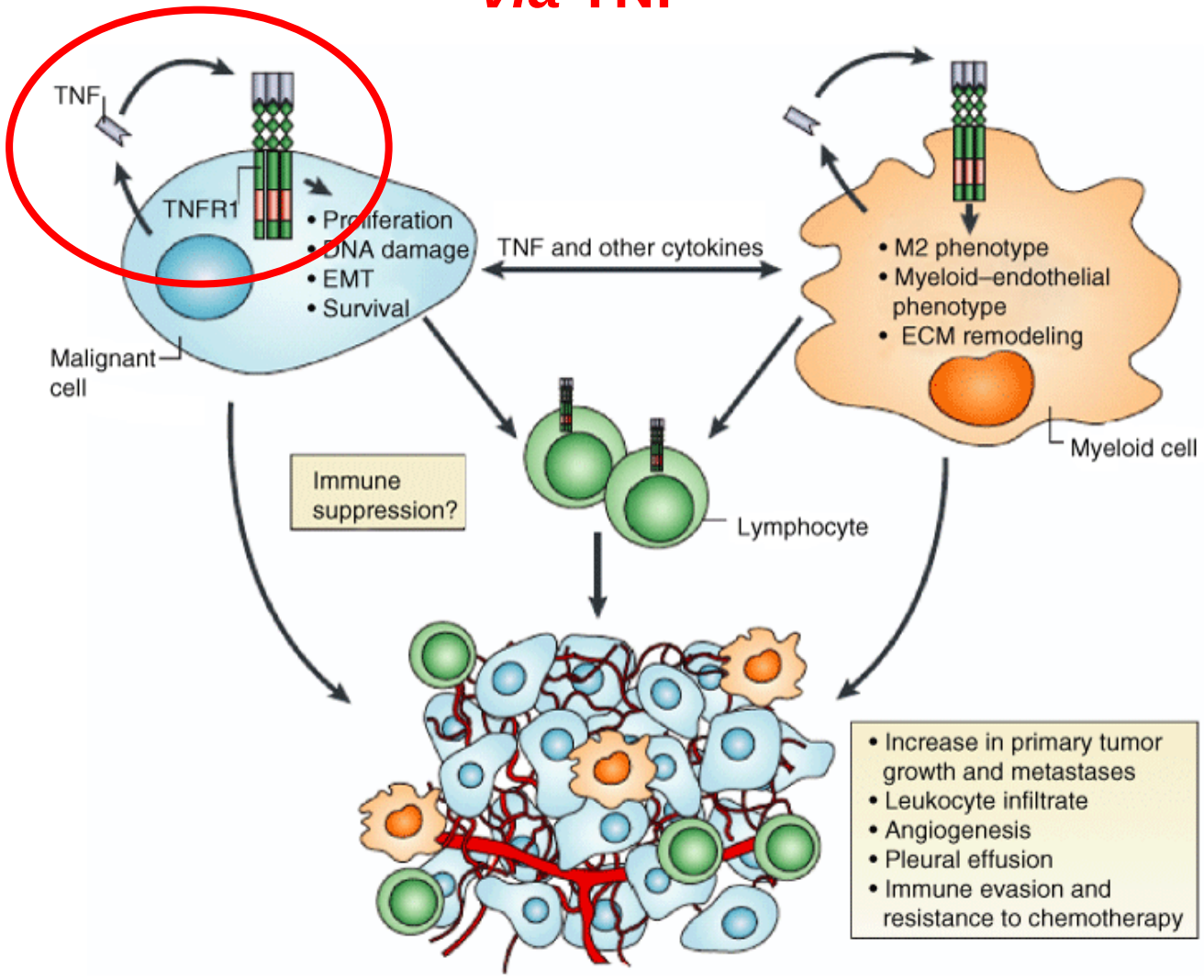
Imperial College London

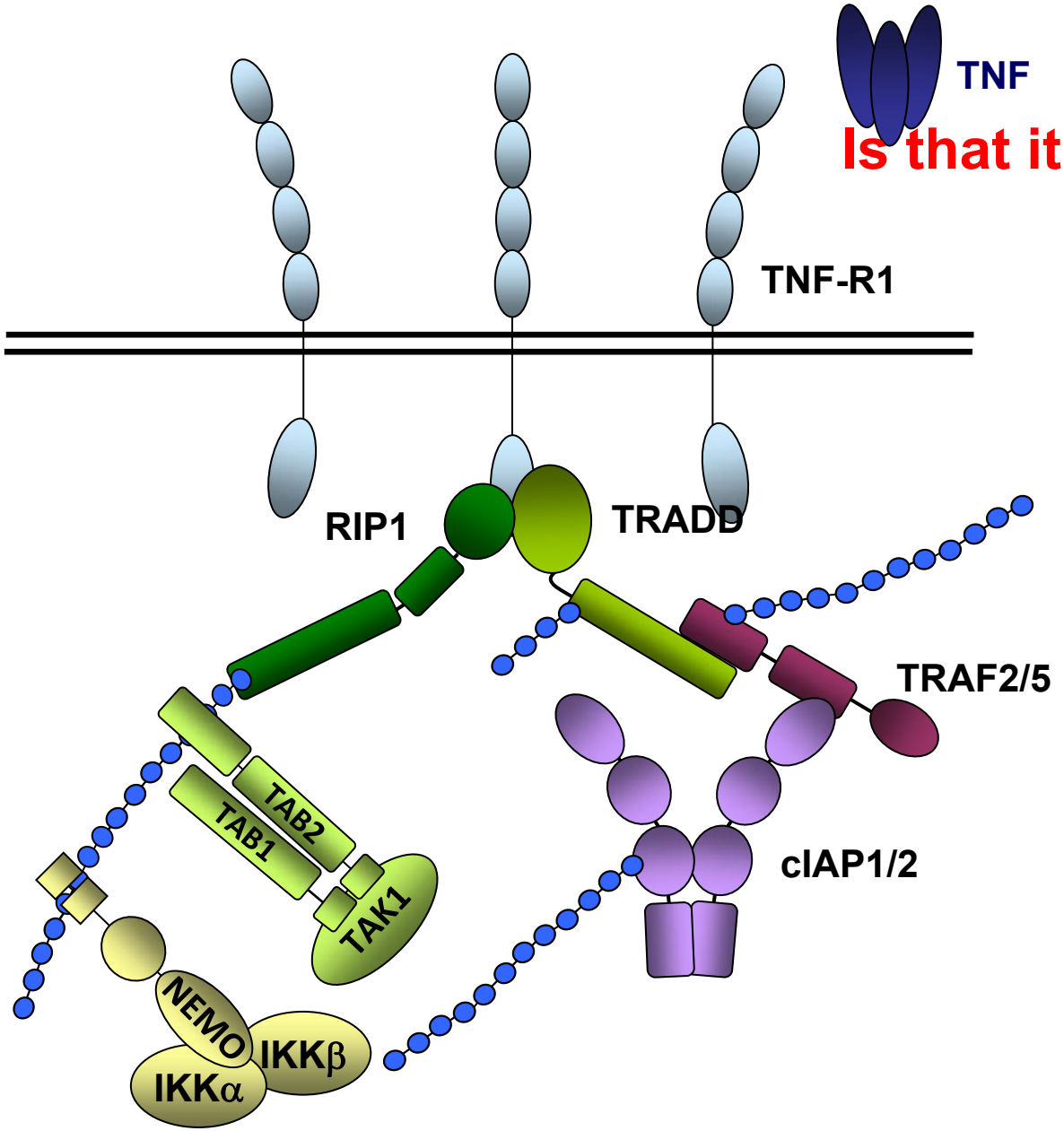
Ubiquitin in NF-κB activation

Henning Walczak
Tumour Immunology Unit
Division of Immunology and Inflammation

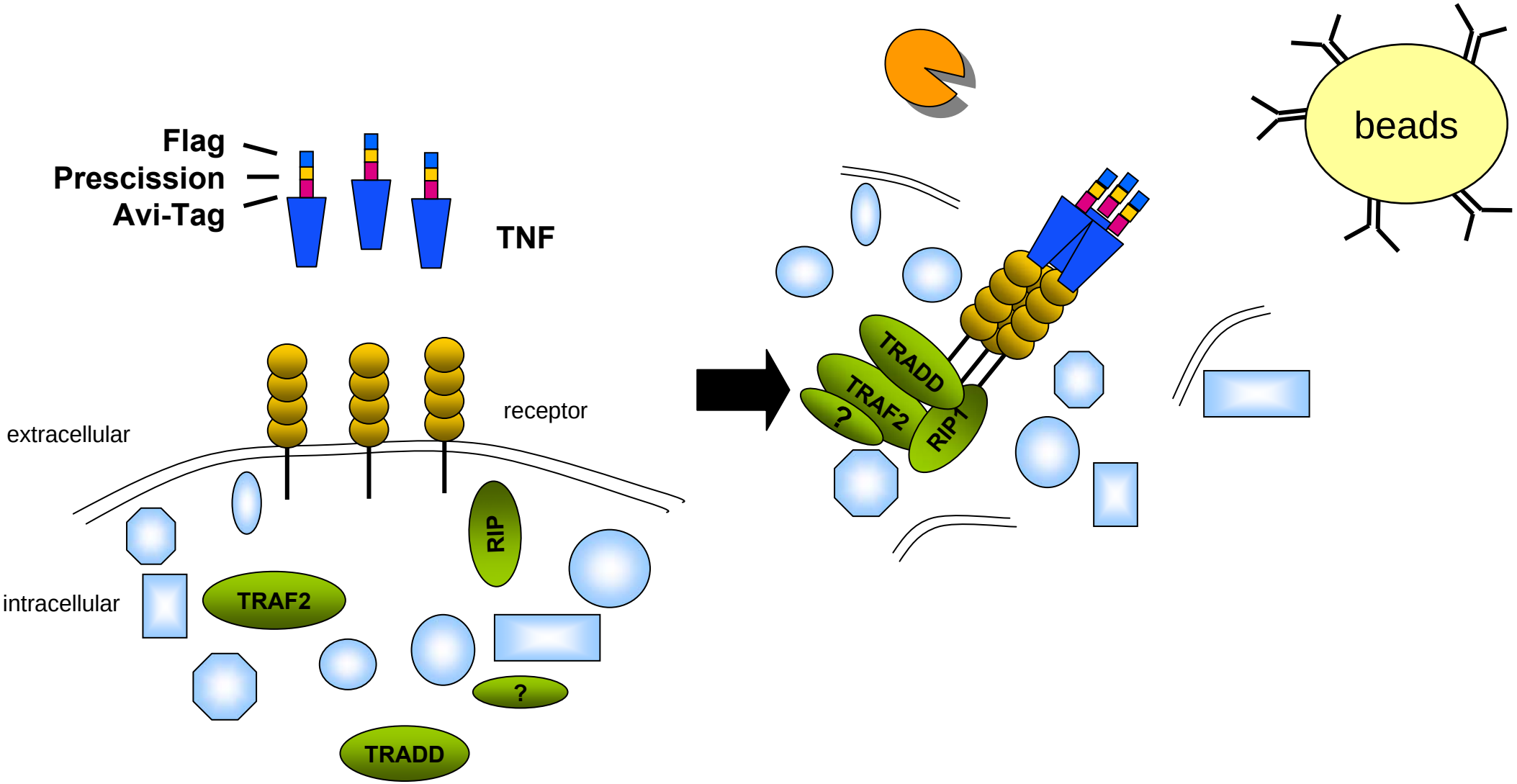
Inflammation and immune regulation are linked *via* TNF



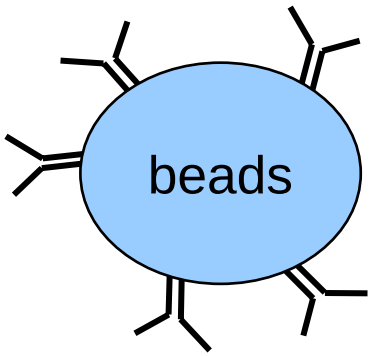
Composition of the TNF-receptor complex

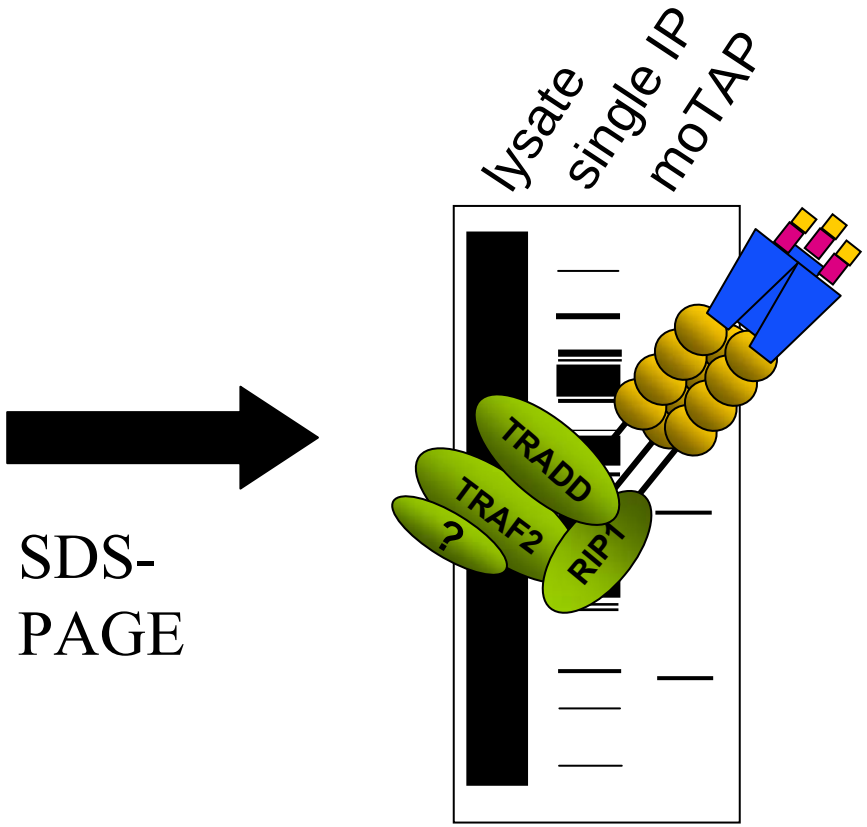


The moTAP technology

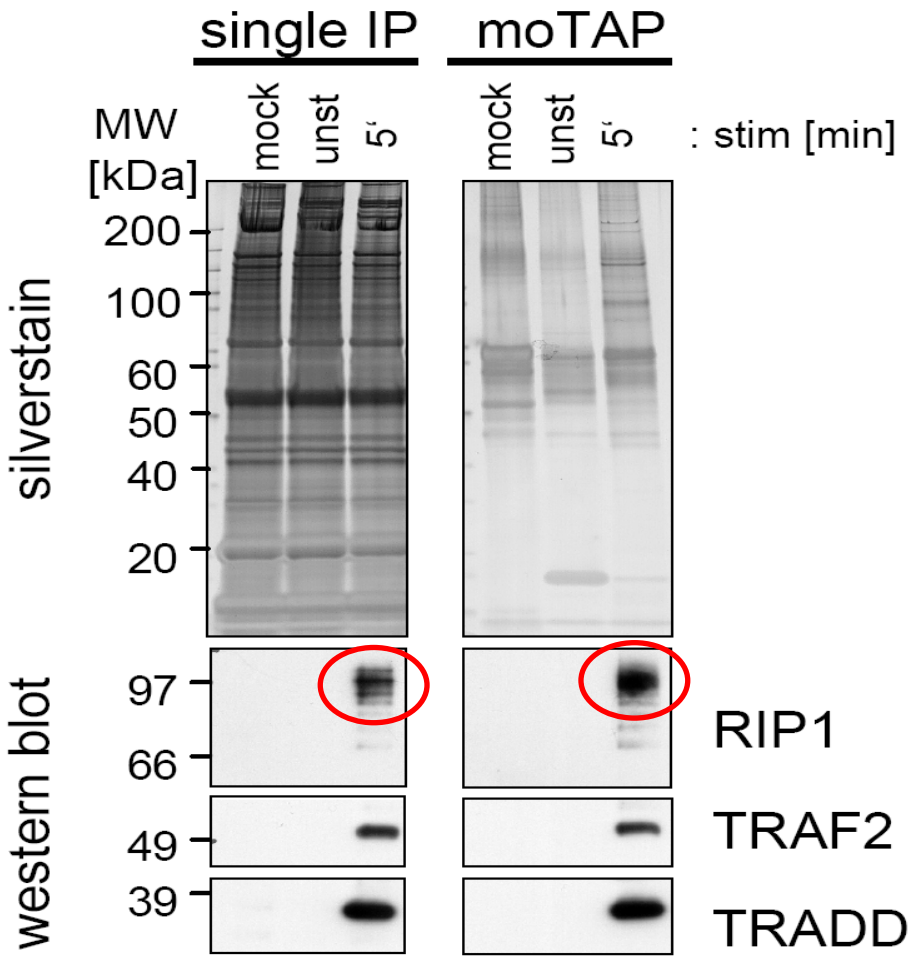


The moTAP technology





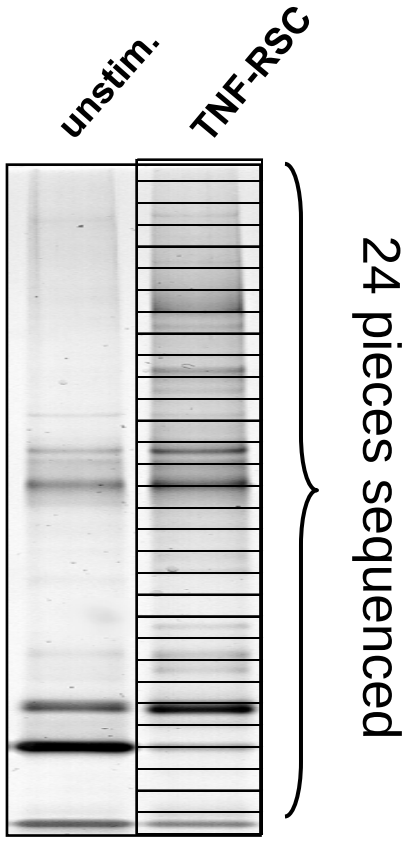
Increased specificity of the TNF-RSC isolation by moTAP



Haas, Emmerich et al., *Molecular Cell*, 2009

Identification of three possible novel components of the native TNF-RSC

isolation of the native TNF-RSC by modified tandem affinity purification

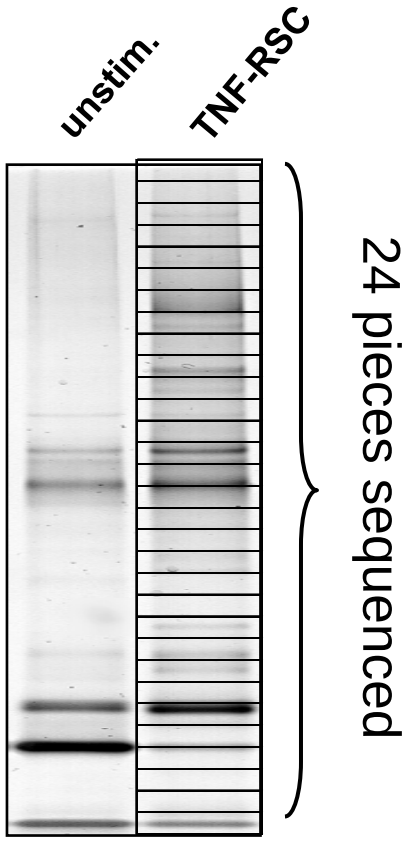


LC-MS/MS

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
IKKα/IKKβ/NEMO	2/2/5
TNF-R1	2

Two of them were HOIL-1 and HOIP

isolation of the native TNF-RSC by modified tandem affinity purification



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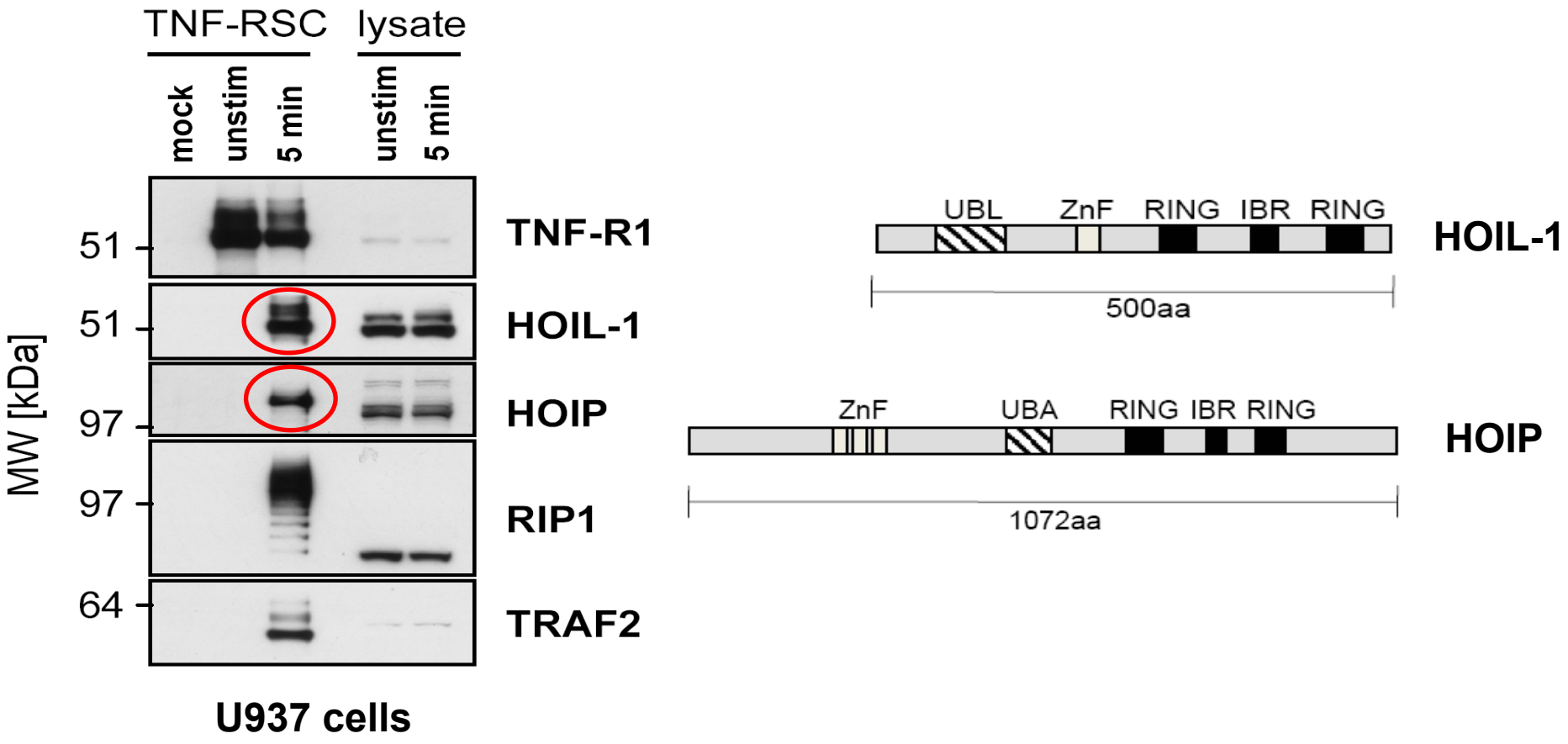
HOIL-1 and HOIP are recruited to the TNF-RSC in a stimulation-dependent manner



Haas, Emmerich et al., *Molecular Cell*, 2009

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

Kirisako et al. (EMBO 2006):

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

Tokunaga et al. (Nat Cell Biol 2009):

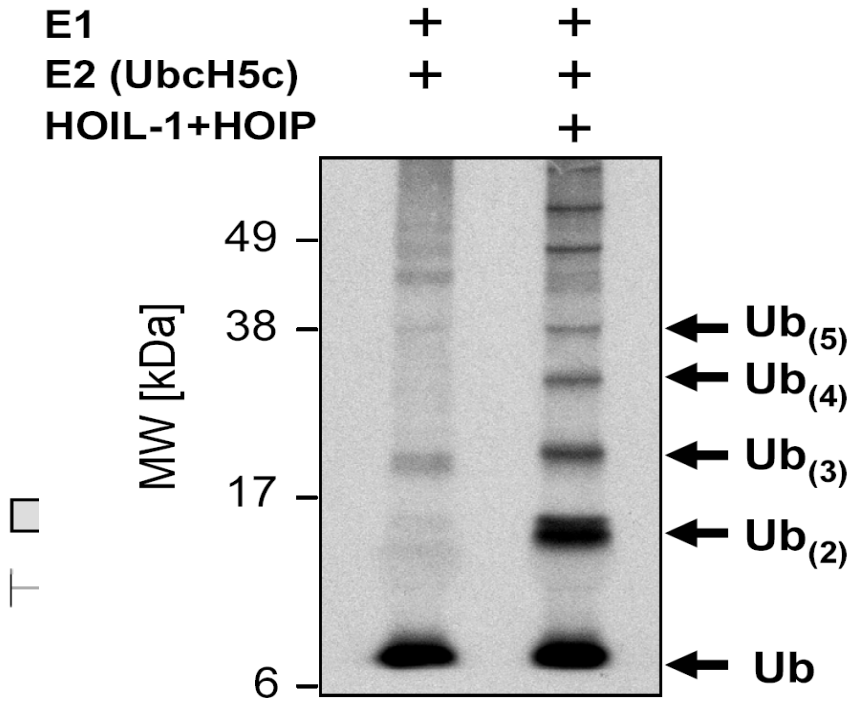
- LUBAC acts as an E3 for NEMO

Lo et al. (Mol Cell 2009)
Rahigi, Ikeda et al. (Cell 2009)

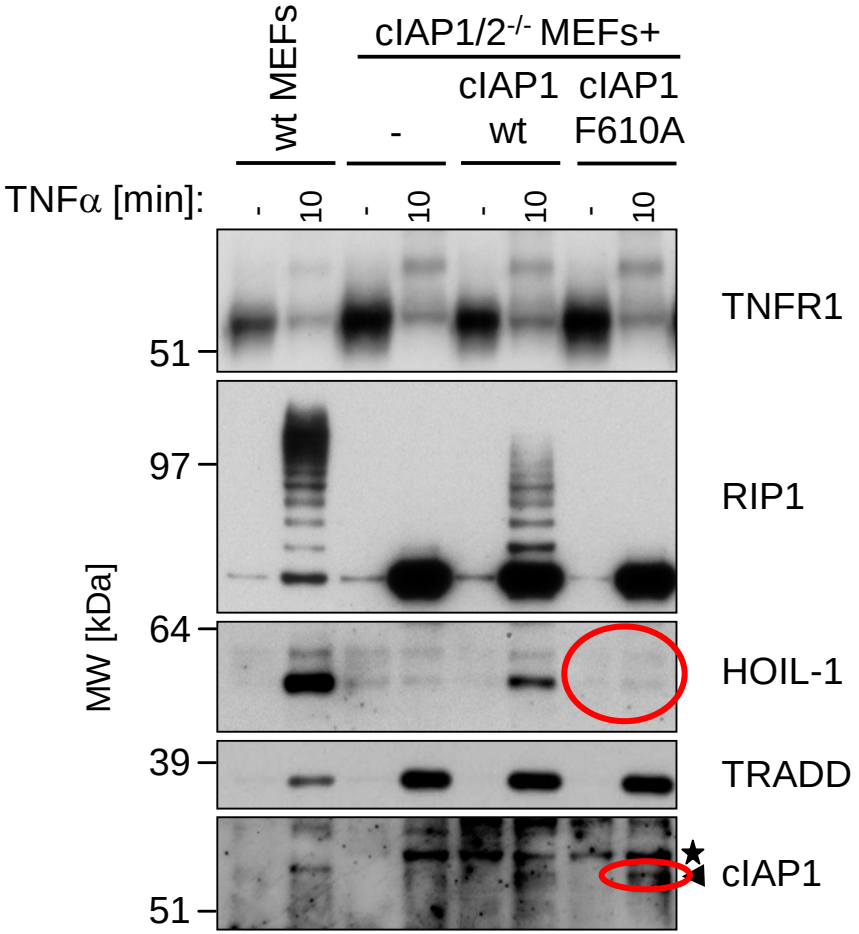
- NEMO binds more efficiently to linear ubiquitin than to K63-linked ubiquitin

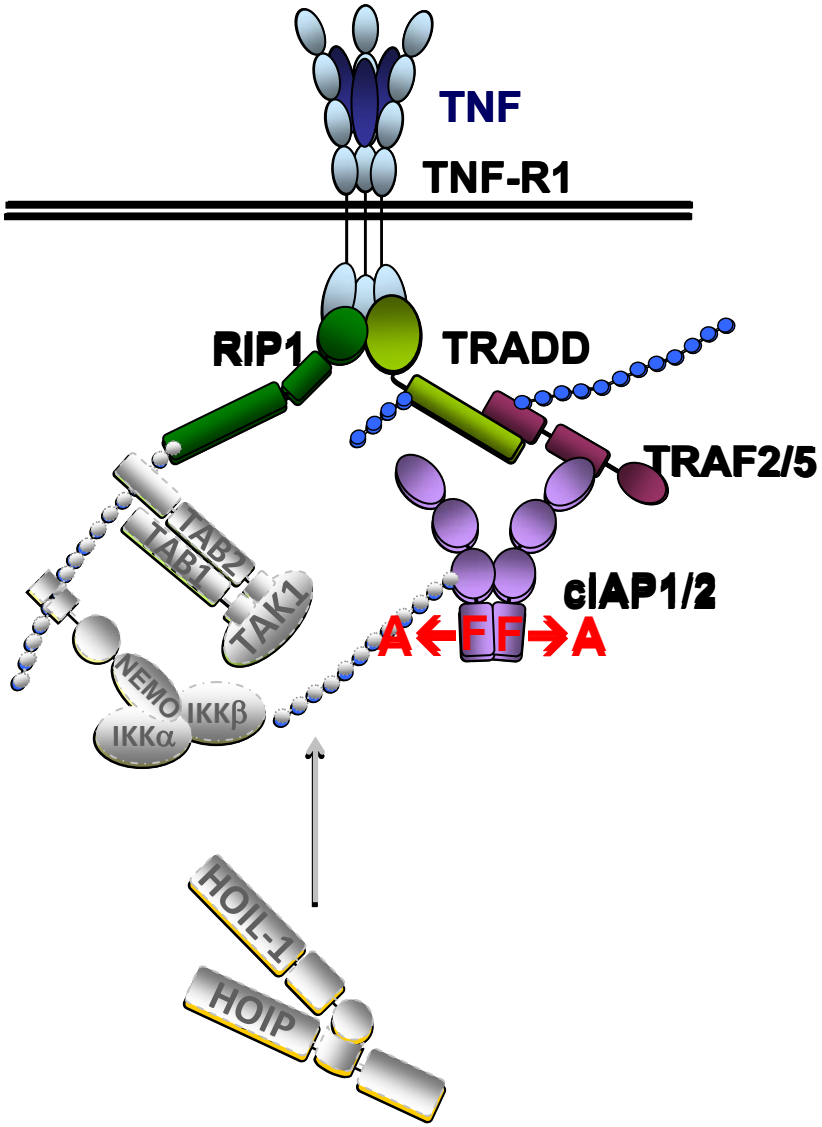
Haas, Emmerich et al. (Mol Cell 2009)

- LUBAC is recruited to the TNF-RSC in a TRADD/TRAF2/cIAP-dependent manner and stabilises the complex
- LUBAC is required for efficient TNF-induced NF-κB and JNK activation and inhibits induction of cell death by TNF



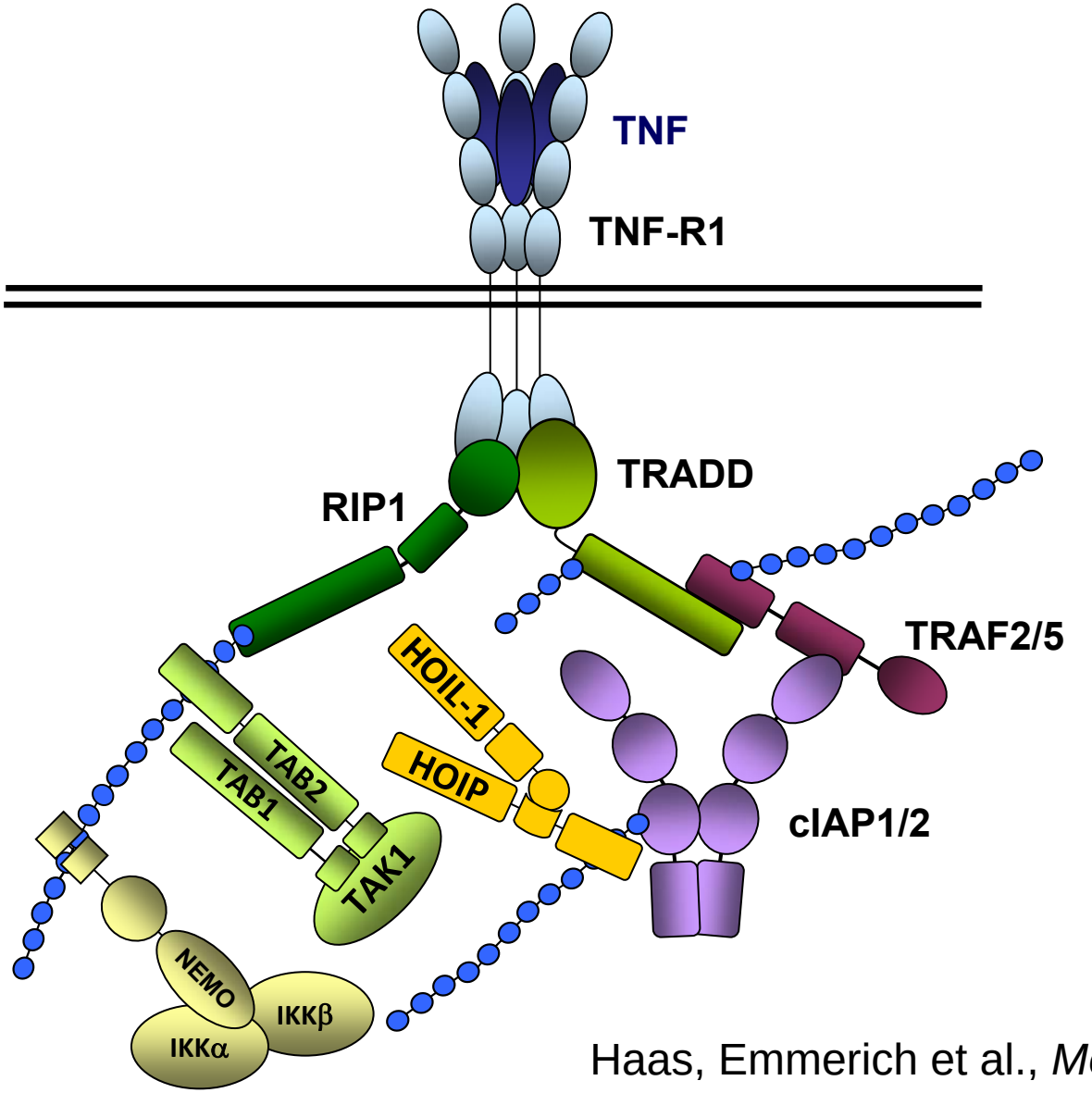
HOIL-1 recruitment depends on the catalytic activity of cIAP1/2





Haas, Emmerich et al., *Molecular Cell*, 2009

HOIL-1 and HOIP are recruited to the TNF-RSC by cIAP-generated Ubiquitin chains via the TRADD-TRAF2-cIAP signalling axis



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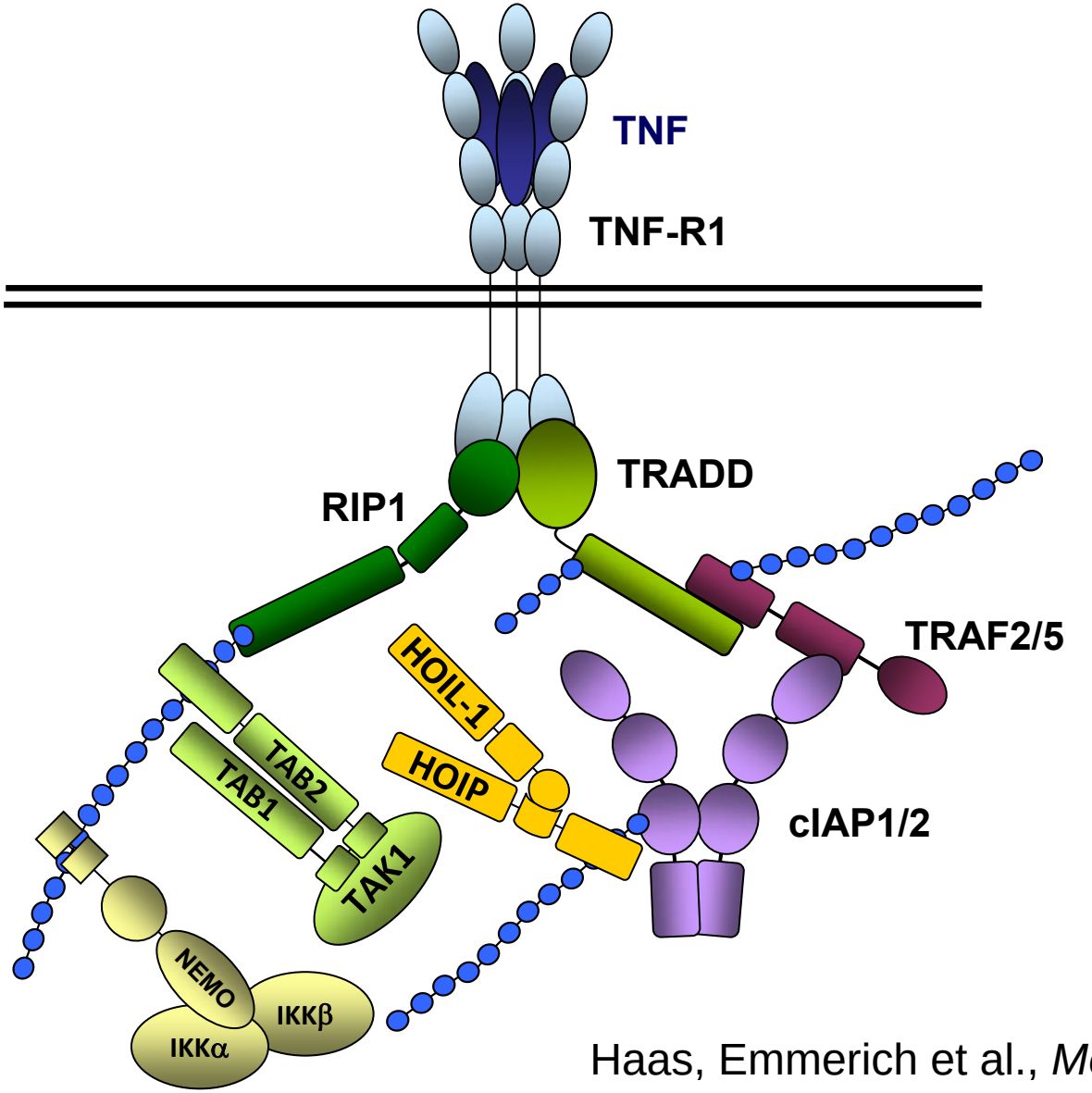
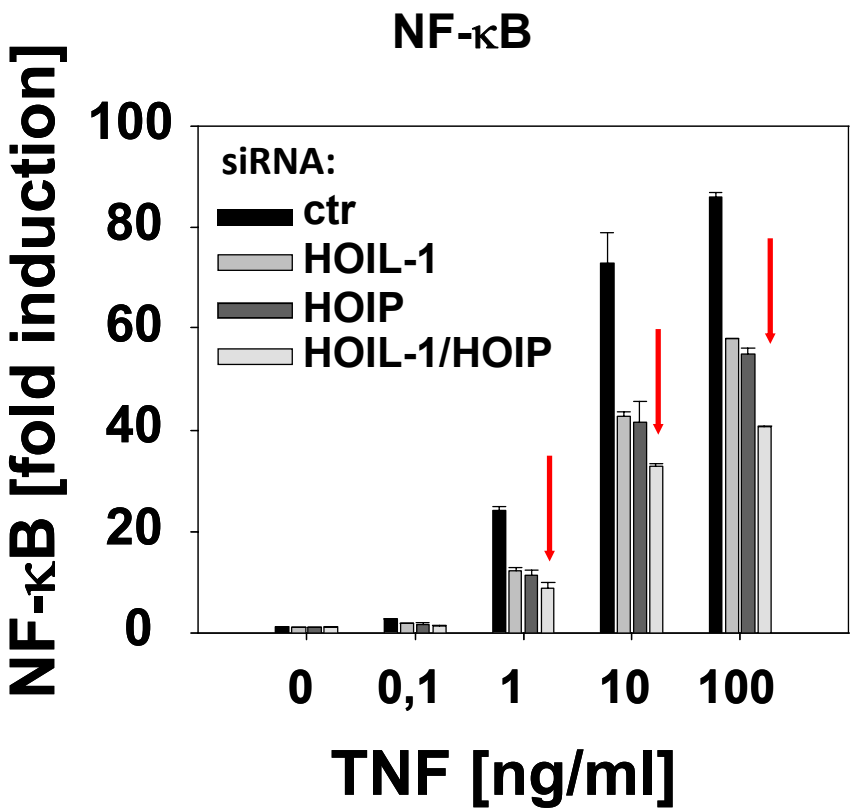
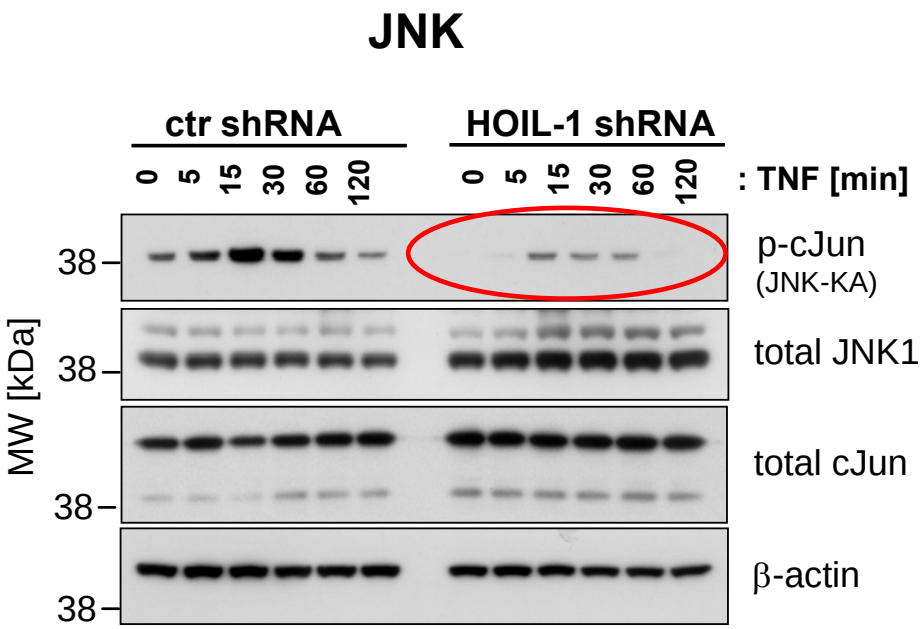


Haas, Emmerich et al., *Molecular Cell*, 2009

HOIL-1/HOIP knock-down reduces TNF-induced NF-κB and JNK activation

HEK293 cells

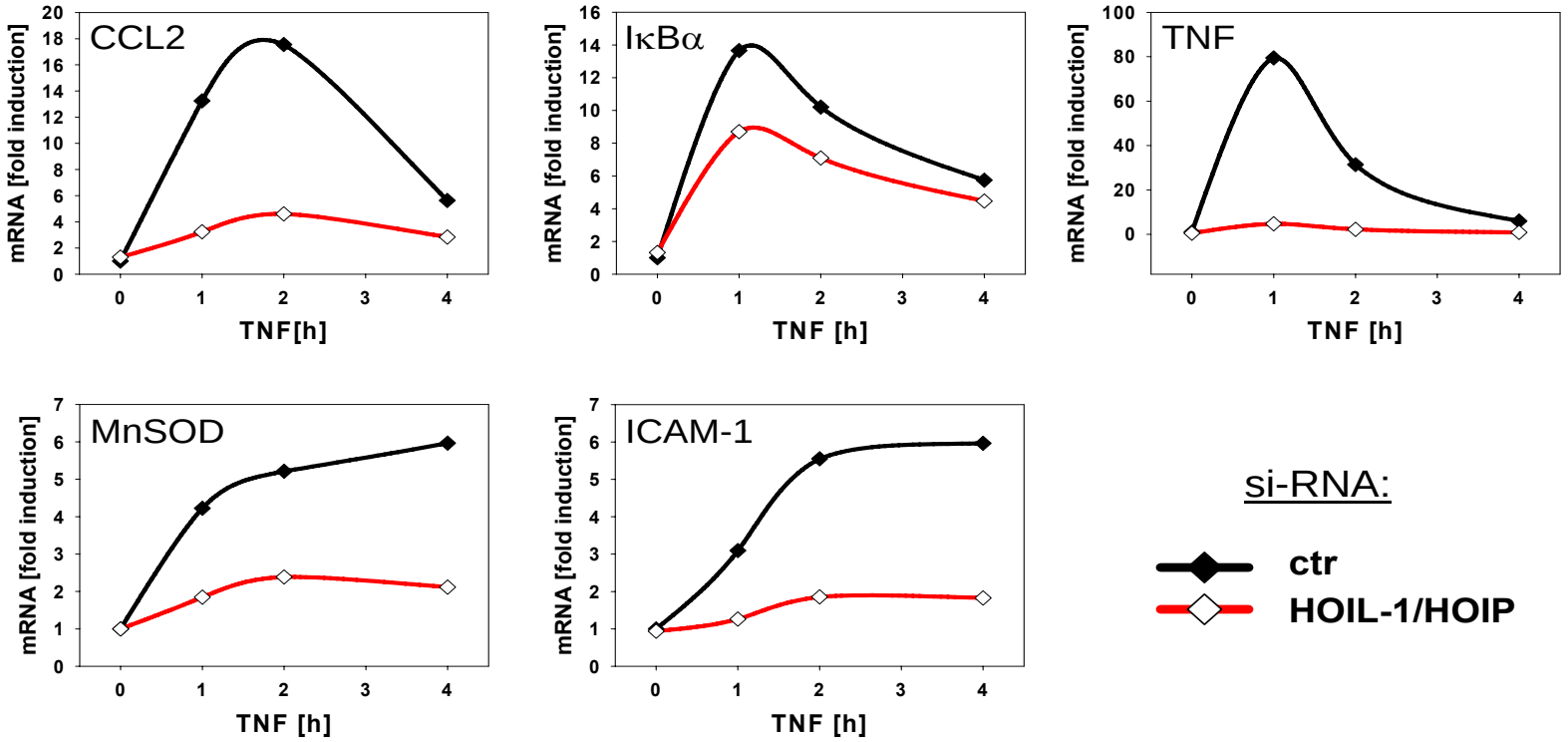




Haas, Emmerich et al., *Molecular Cell*, 2009

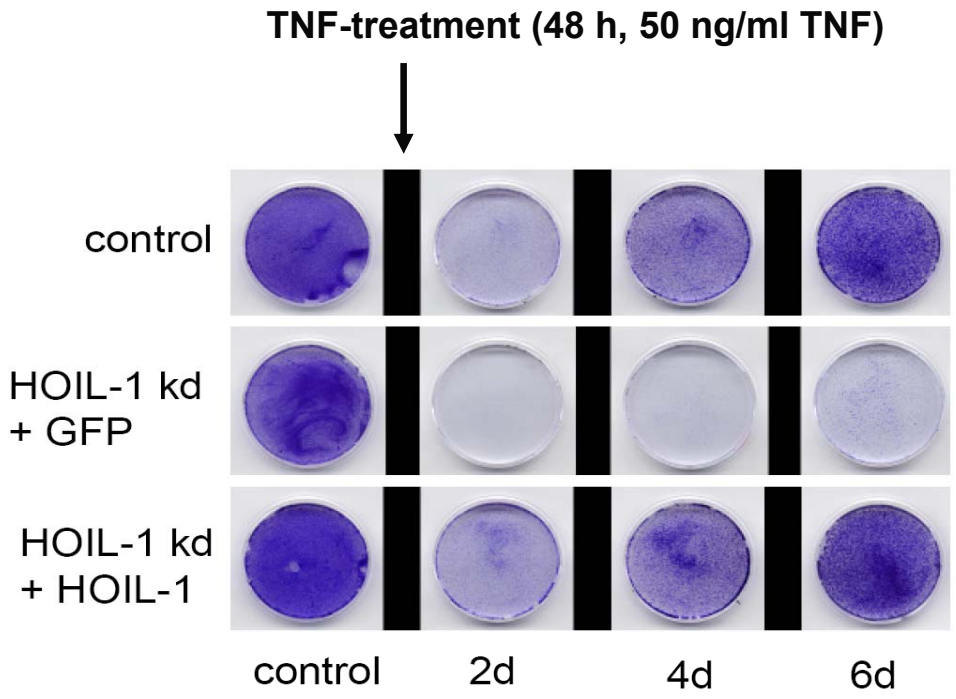
HOIL-1 and HOIP are crucial regulators for a number of TNF-dependent genes

HeLa cells



Haas, Emmerich et al., *Molecular Cell*, 2009

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

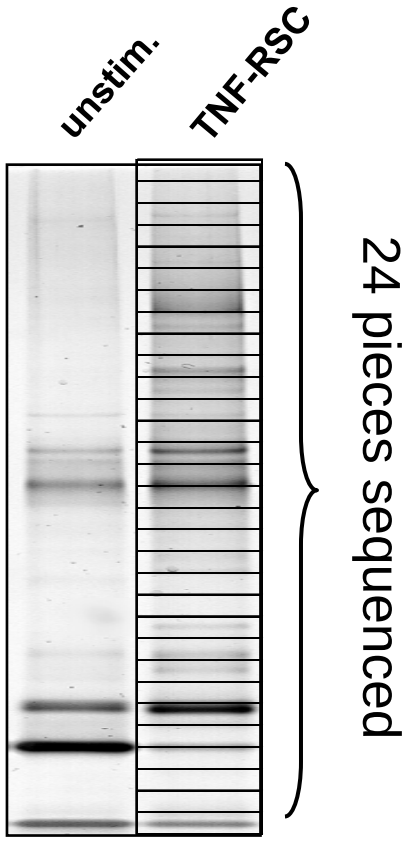


MCF-7 cells

Haas, Emmerich et al., *Molecular Cell*, 2009

Sharpin, a possible third novel component of the native TNF-RSC

isolation of the native TNF-RSC by modified tandem affinity purification



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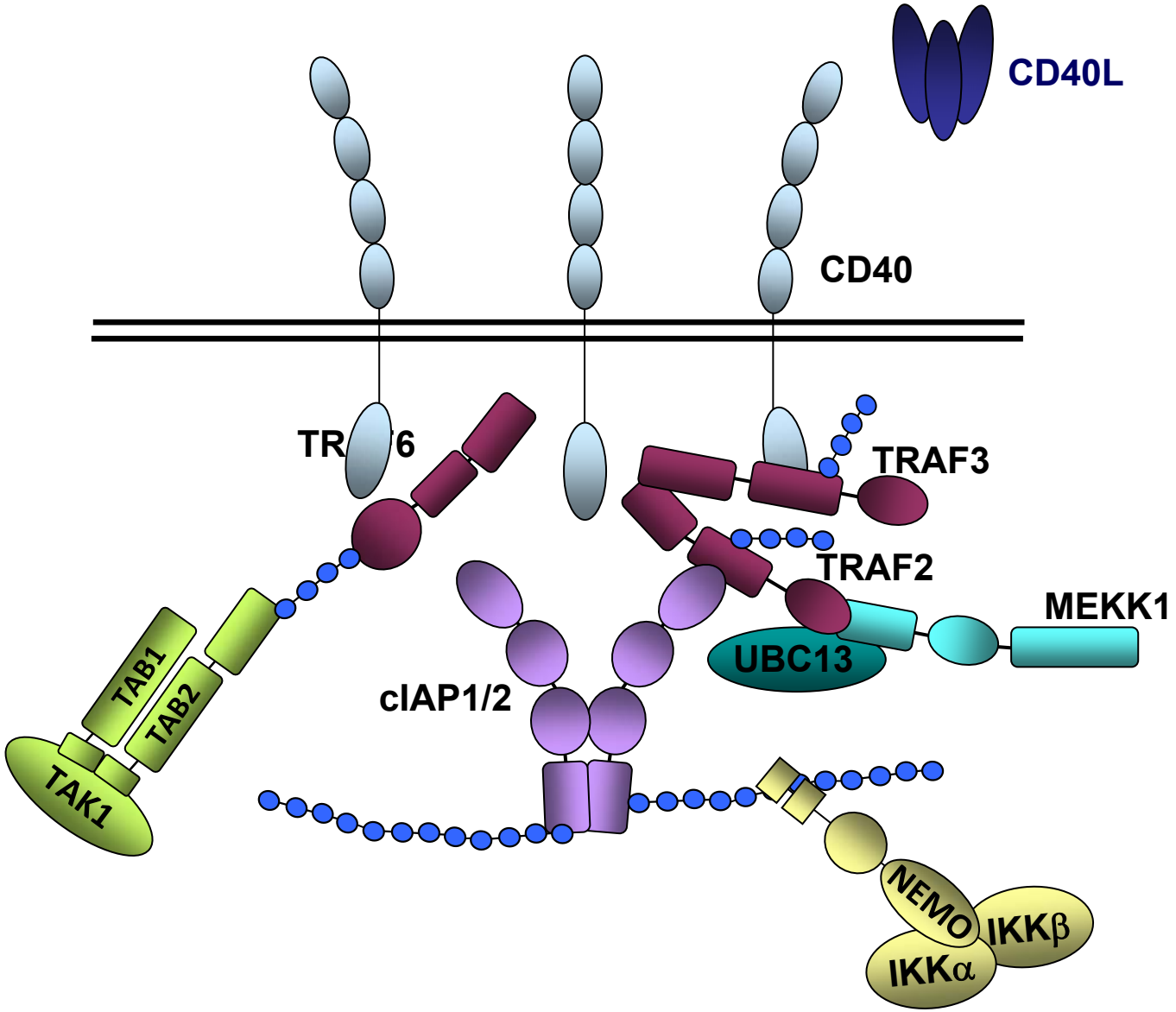
TNF-R1	2

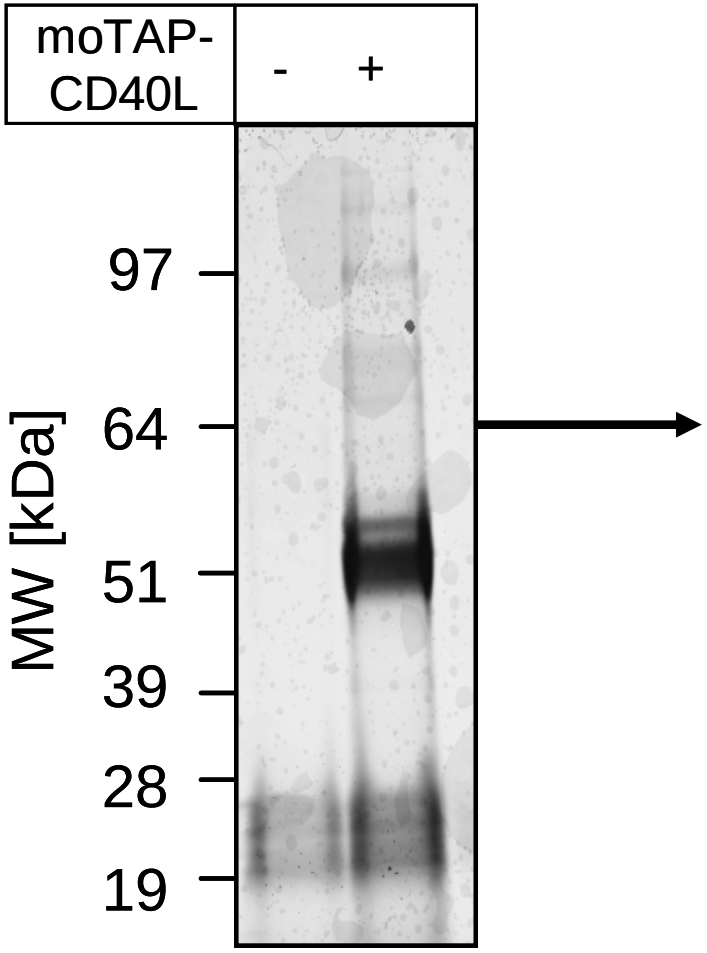
Gerlach, Cordier et al., *Nature,* 2011

Composition of the CD40-receptor complex



Gerlach, Cordier et al., *Nature,* 2011

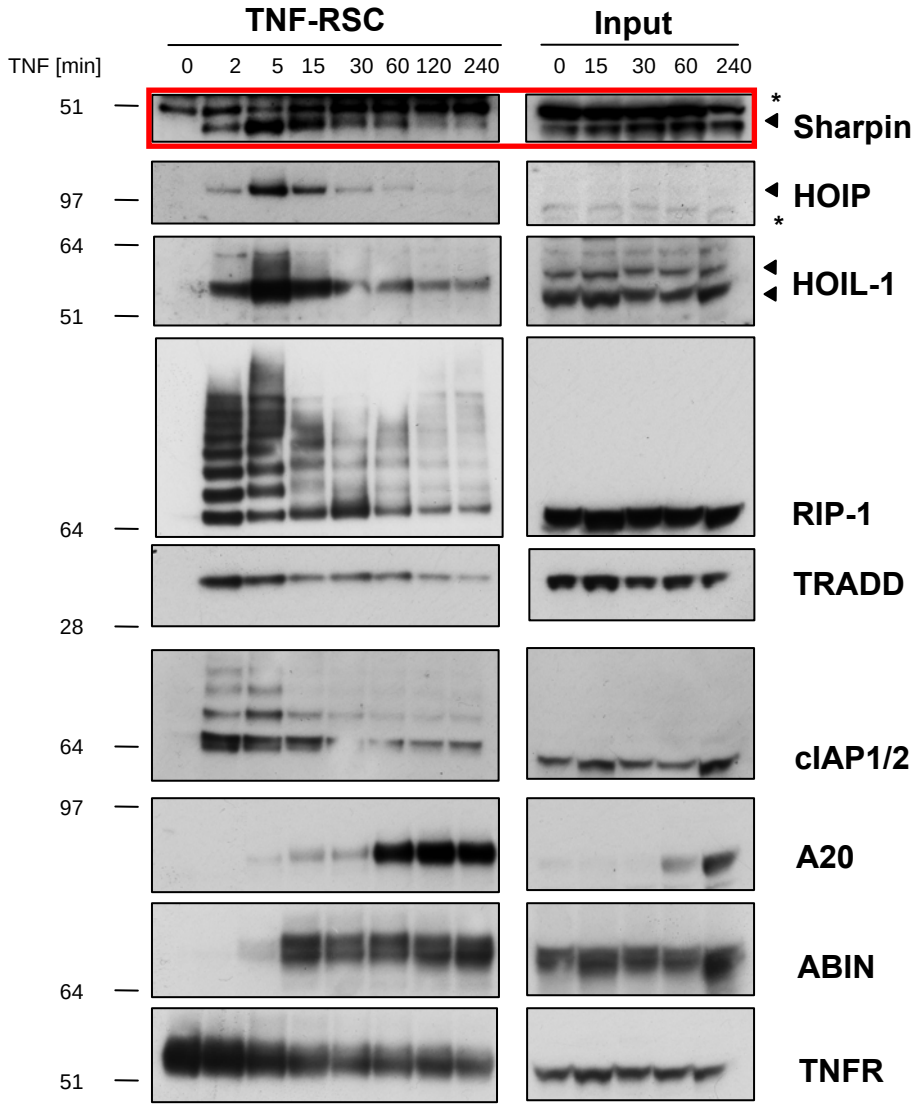
HOIL-1, HOIP and Sharpin are also possible novel components of the native CD40-RSC



protein	peptides sequenced
TRAF-2	17
cIAP1	15
HOIP	**13**
CD40	12
cIAP-2	11
CD40L	7
HOIL-1	**7**
Ubiquitin	6
Sharpin	**2**

Gerlach, Cordier et al., *Nature,* 2011

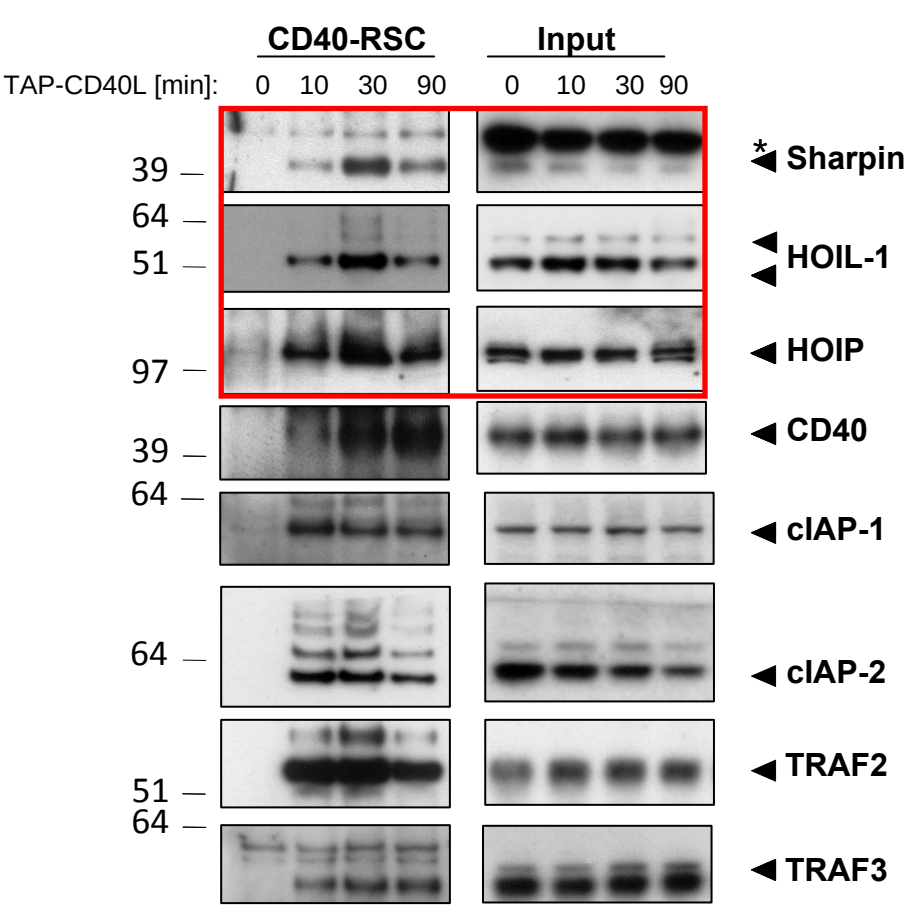
Sharpin, HOIP and HOIL-1 are recruited to the TNF-RSC with similar kinetics



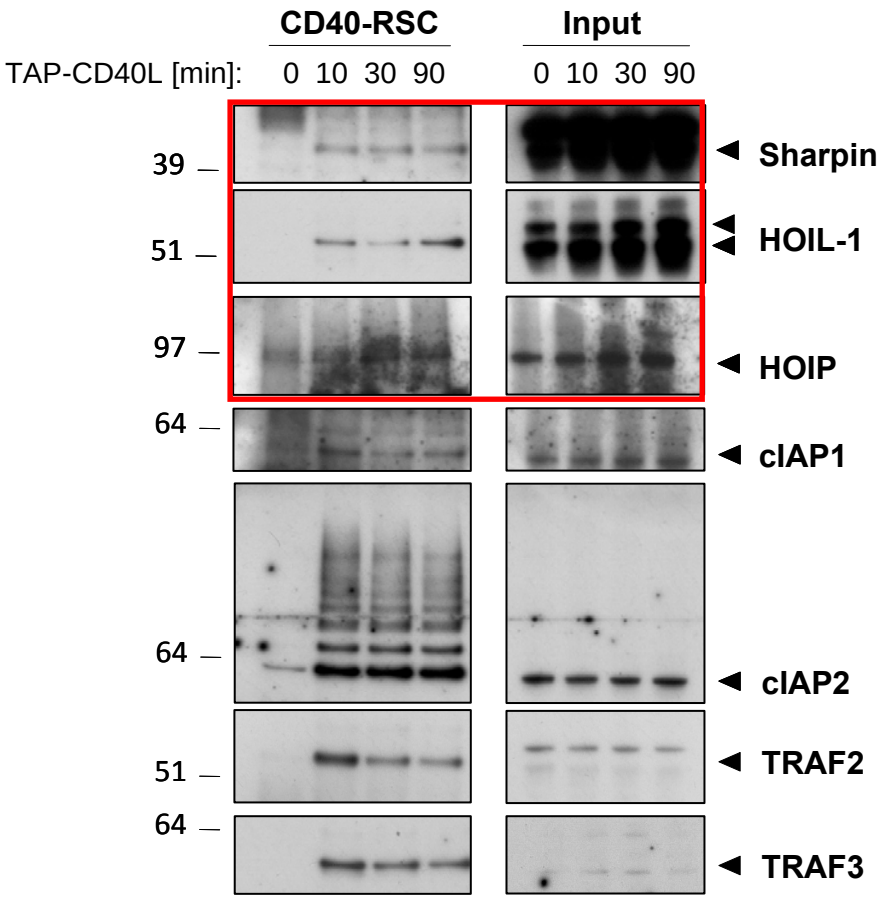
HeLa cells

Gerlach, Cordier et al., *Nature,* 2011

Sharpin, HOIP and HOIL-1 are recruited to the CD40-RSC in the human B cell line Raji and in primary human B cells



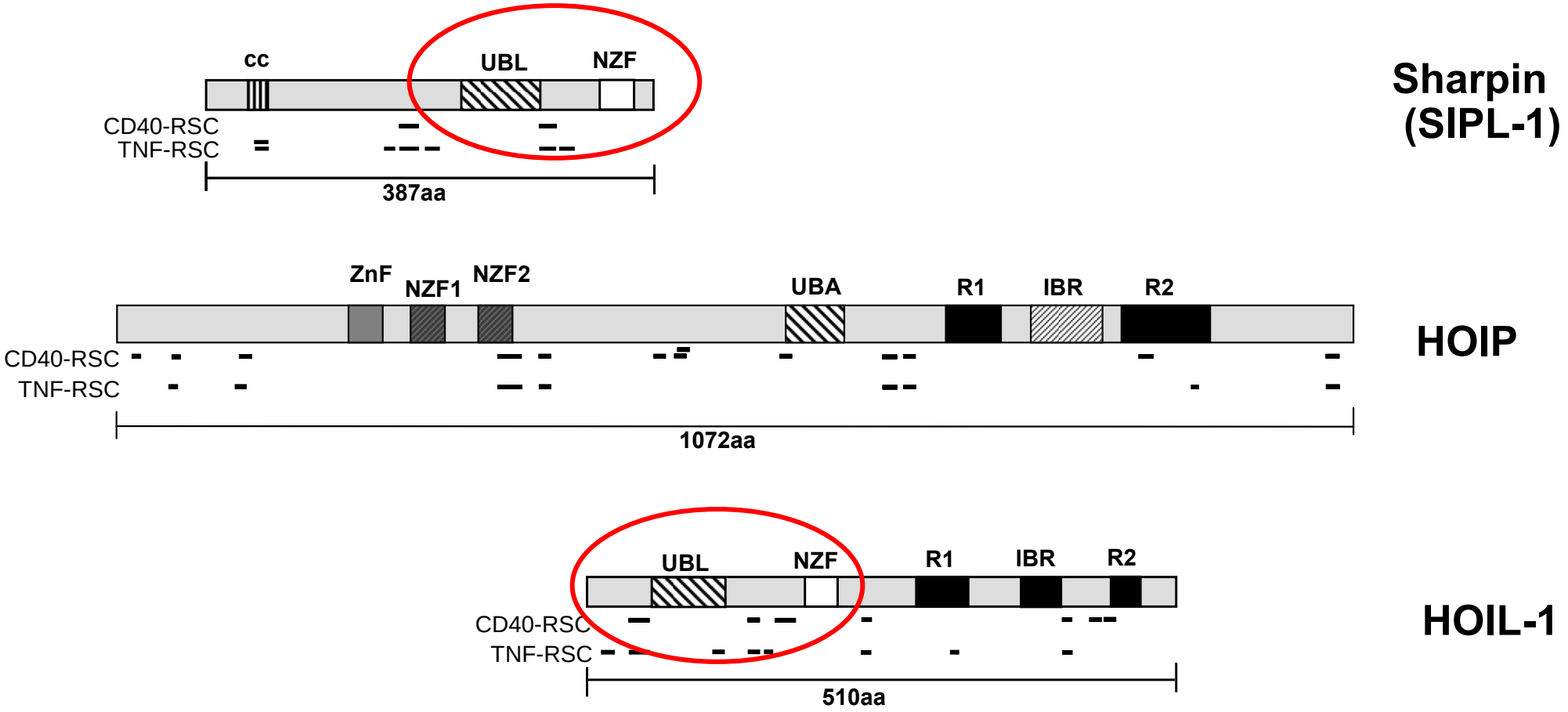
Raji cells



Primary human B cells

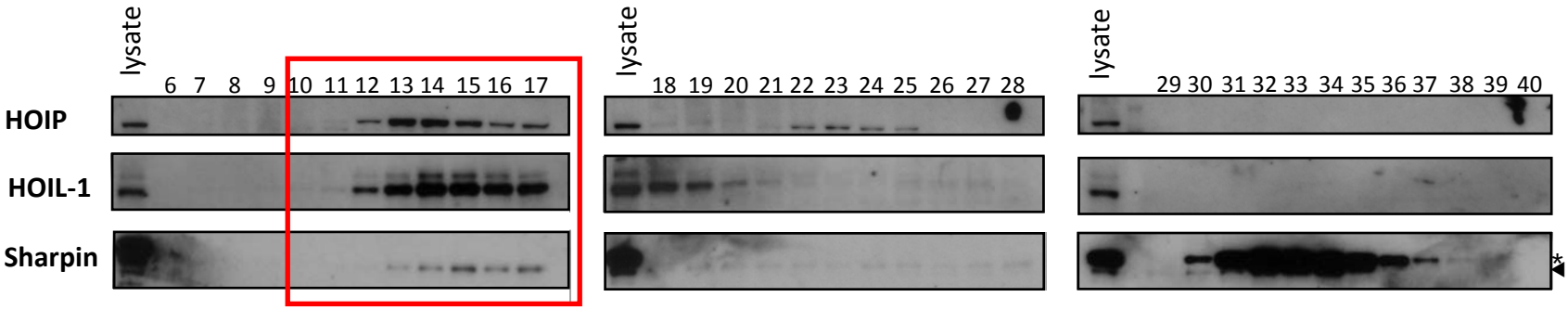
Gerlach, Cordier et al., *Nature,* 2011

Sharpin is homologous to HOIL-1

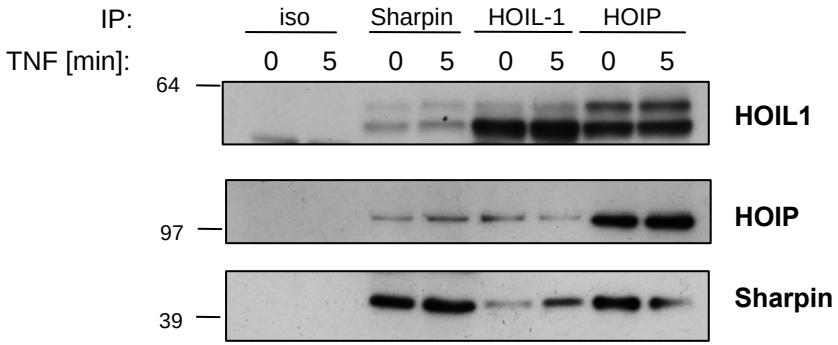


Gerlach, Cordier et al., *Nature,* 2011

Endogenous Sharpin, HOIP and HOIL-1 form part of a pre-formed cytoplasmic high-molecular weight complex and can be co-immunoprecipitated with each other in a stimulation-independent manner



HeLa cells

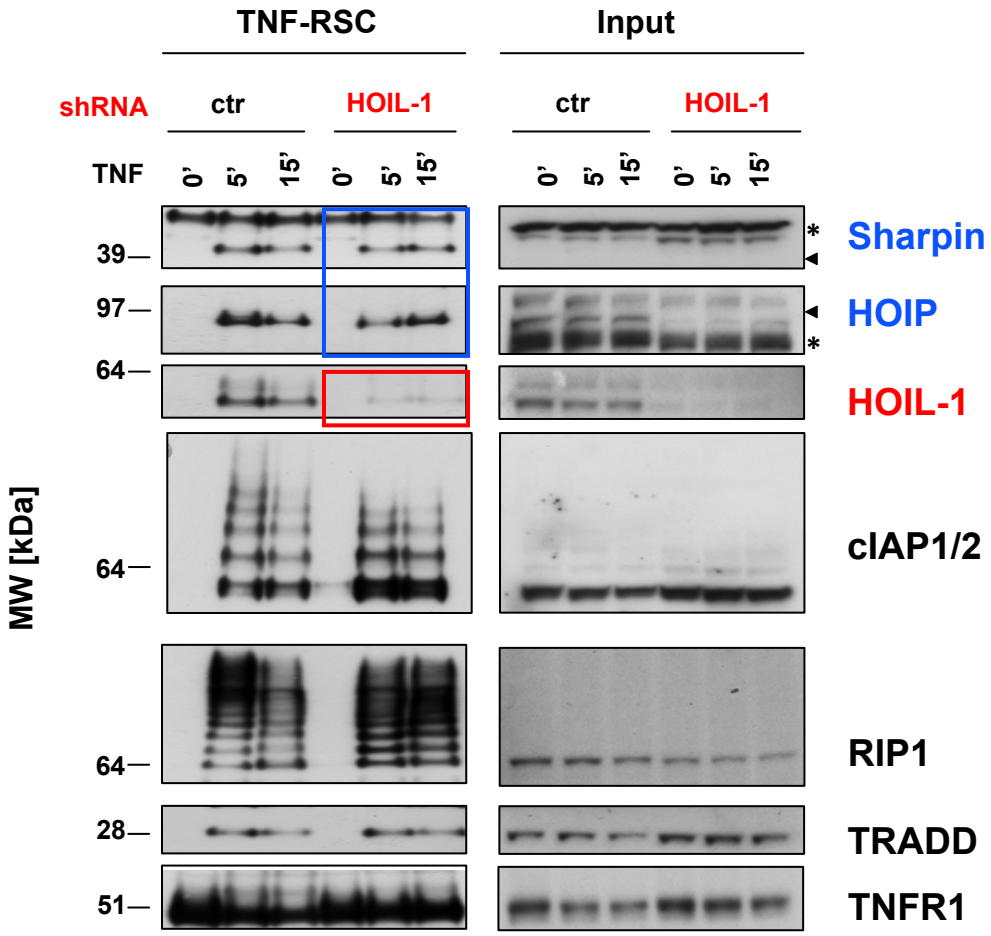


U937 cells

LUBAC is a tripartite complex

Gerlach, Cordier et al., *Nature,* 2011

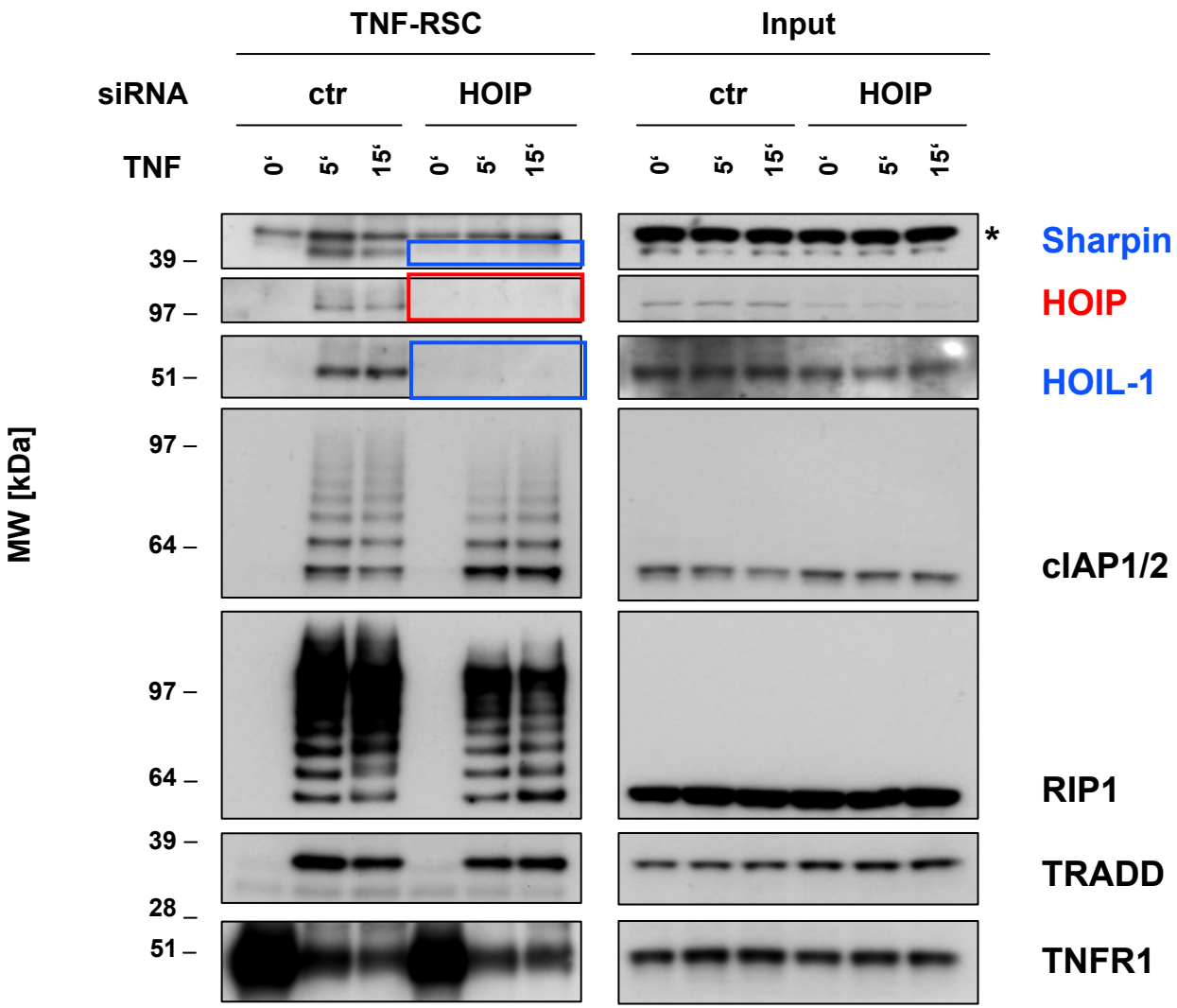
Sharpin and HOIP are still recruited to the TNF-RSC in HOIL-1 knockdown cells



HeLa cells

Gerlach, Cordier et al., *Nature,* 2011

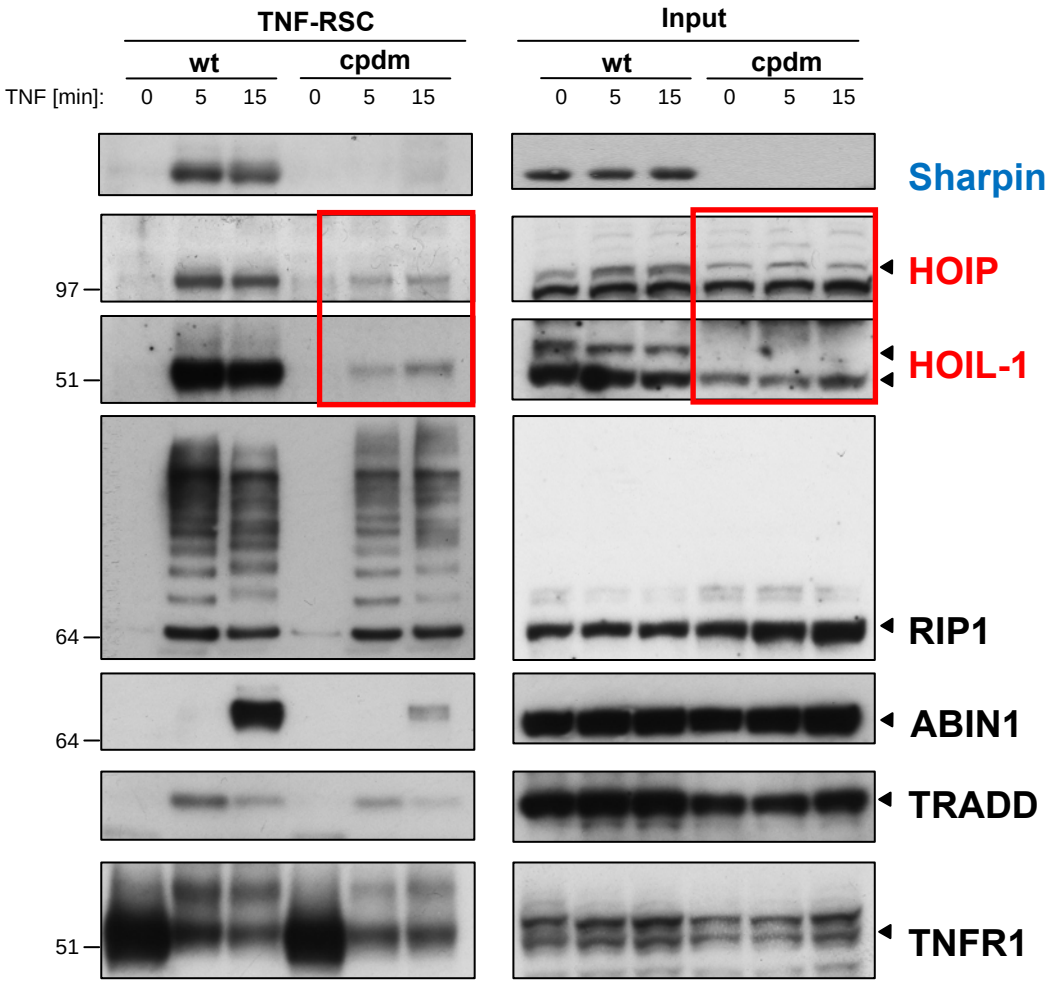
Knockdown of HOIP abolishes recruitment of Sharpin and HOIL-1 to the TNF-RSC



HeLa cells

Gerlach, Cordier et al., *Nature,* 2011

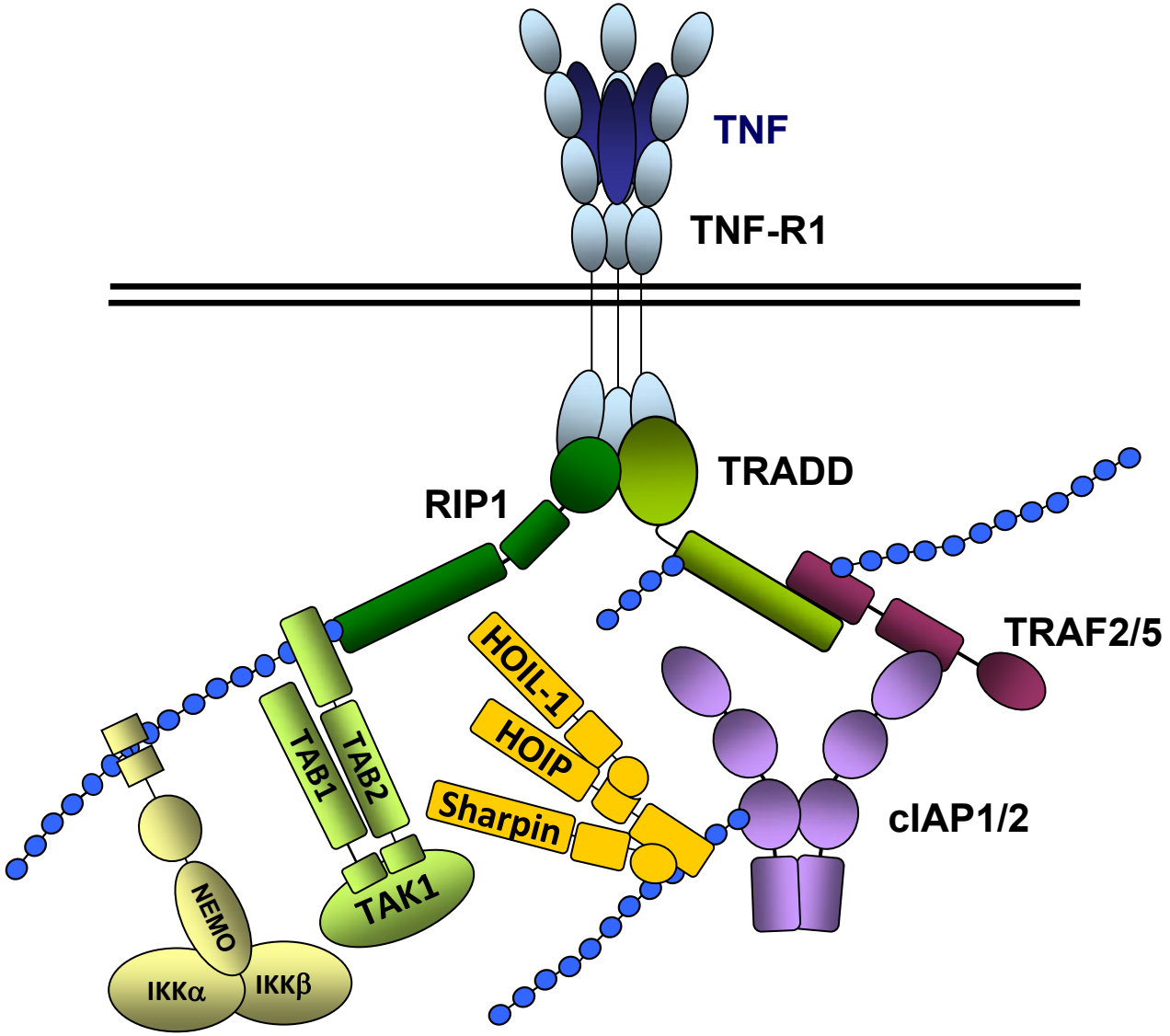
Expression and recruitment of HOIL-1 and HOIP to the TNF-RSC is attenuated in Sharpin-deficient *cpdm* MEFs



Murine embryonic fibroblasts (MEF)

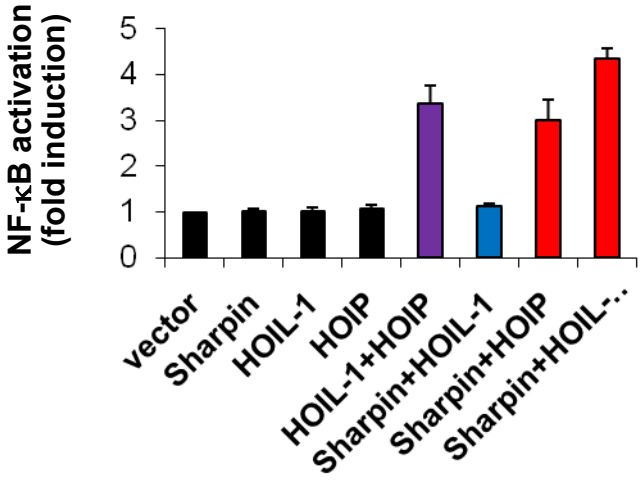
Gerlach, Cordier et al., *Nature,* 2011

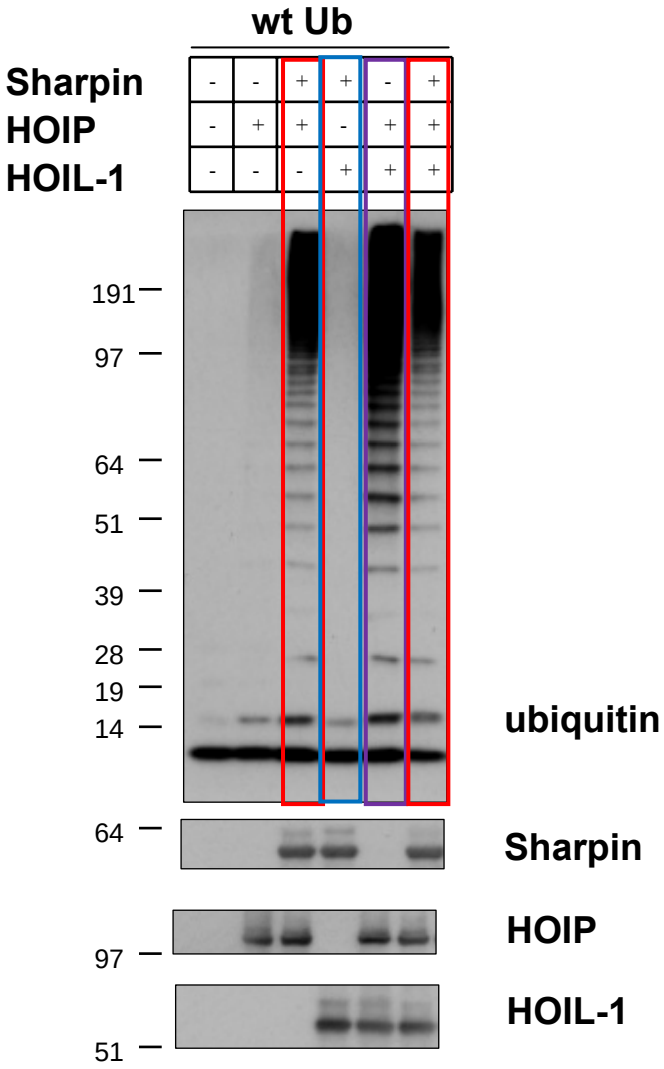
Recruitment of Sharpin, HOIP and HOIL-1 to the TNF-RSC depends on cIAP1/2 and on HOIP



Gerlach, Cordier et al., *Nature,* 2011

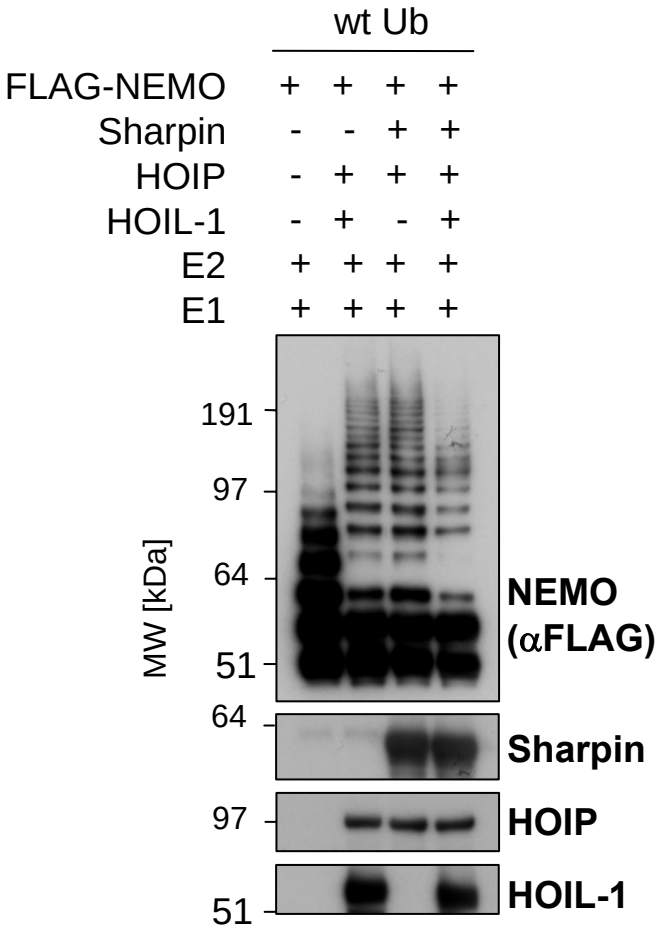
HOIP is also able to form linear ubiquitin chains in combination with Sharpin





Gerlach, Cordier et al., *Nature,* 2011

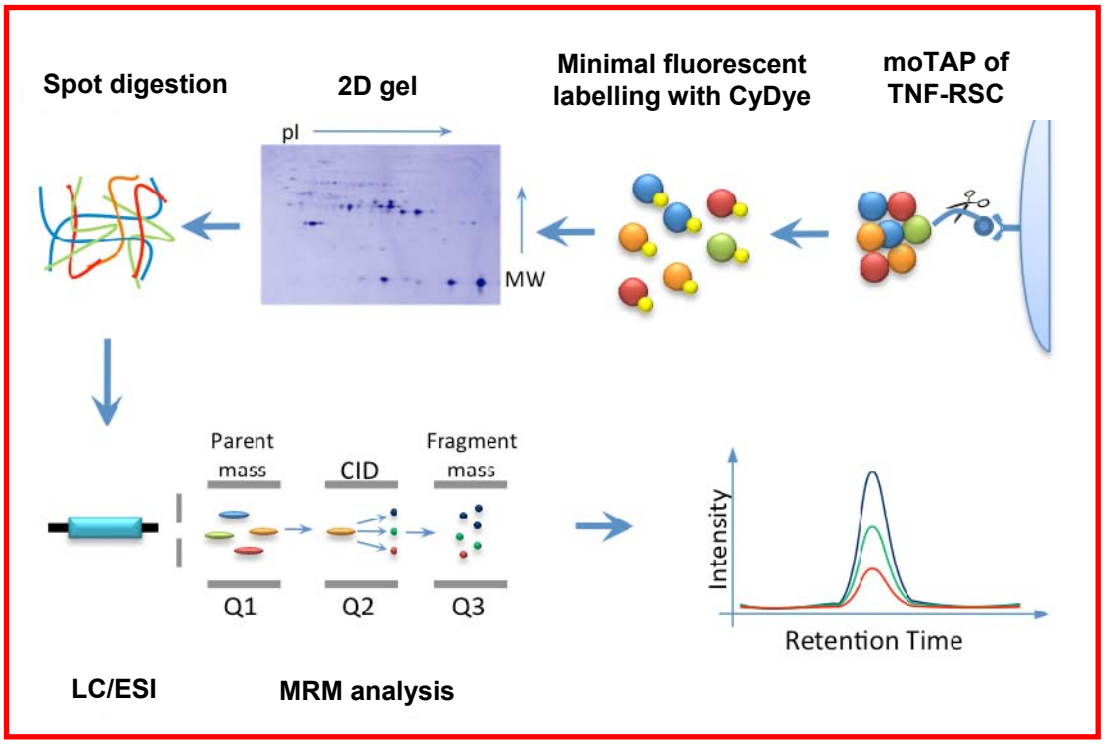
... and it does so on NEMO, at least *in vitro*



Gerlach, Cordier et al., *Nature,* 2011

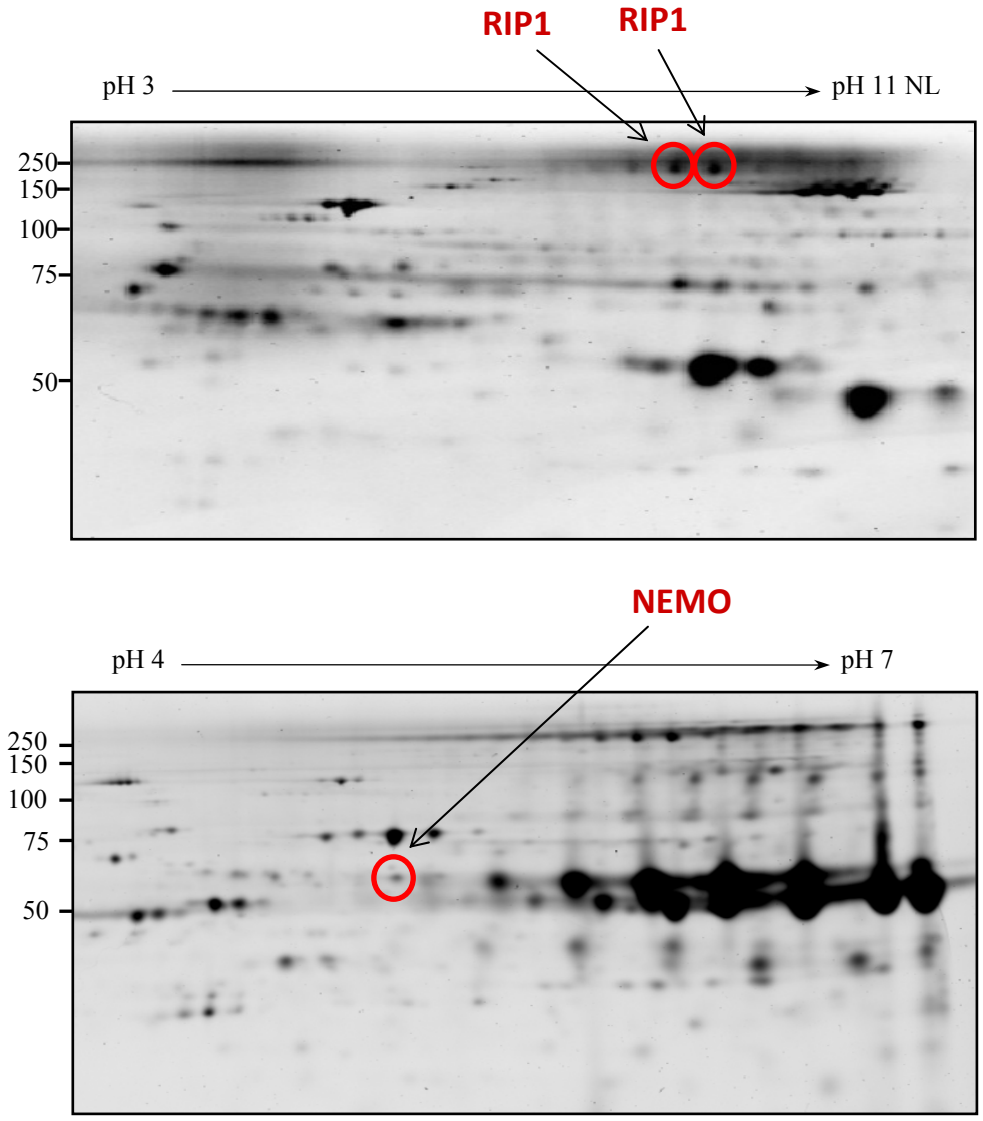
Nice, ... but does it also happen in the native TNF-RSC?

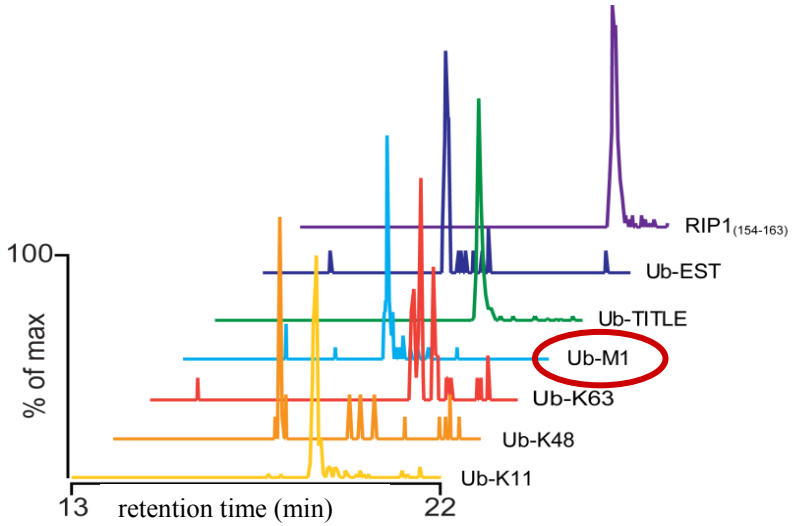
Combined moTAP-2D-MRM approach



Gerlach, Cordier et al., *Nature,* 2011

Endogenous RIP1 and NEMO are linearly ubiquitinated in the native TNF-RSC





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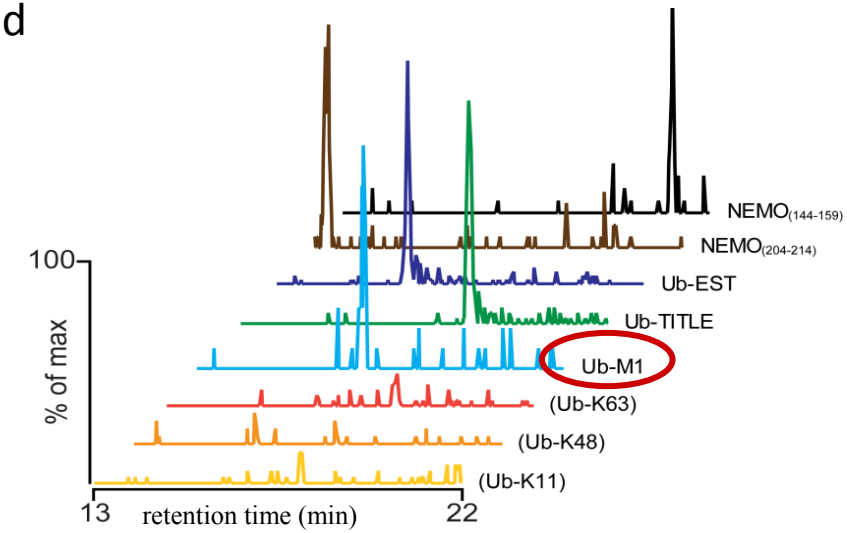
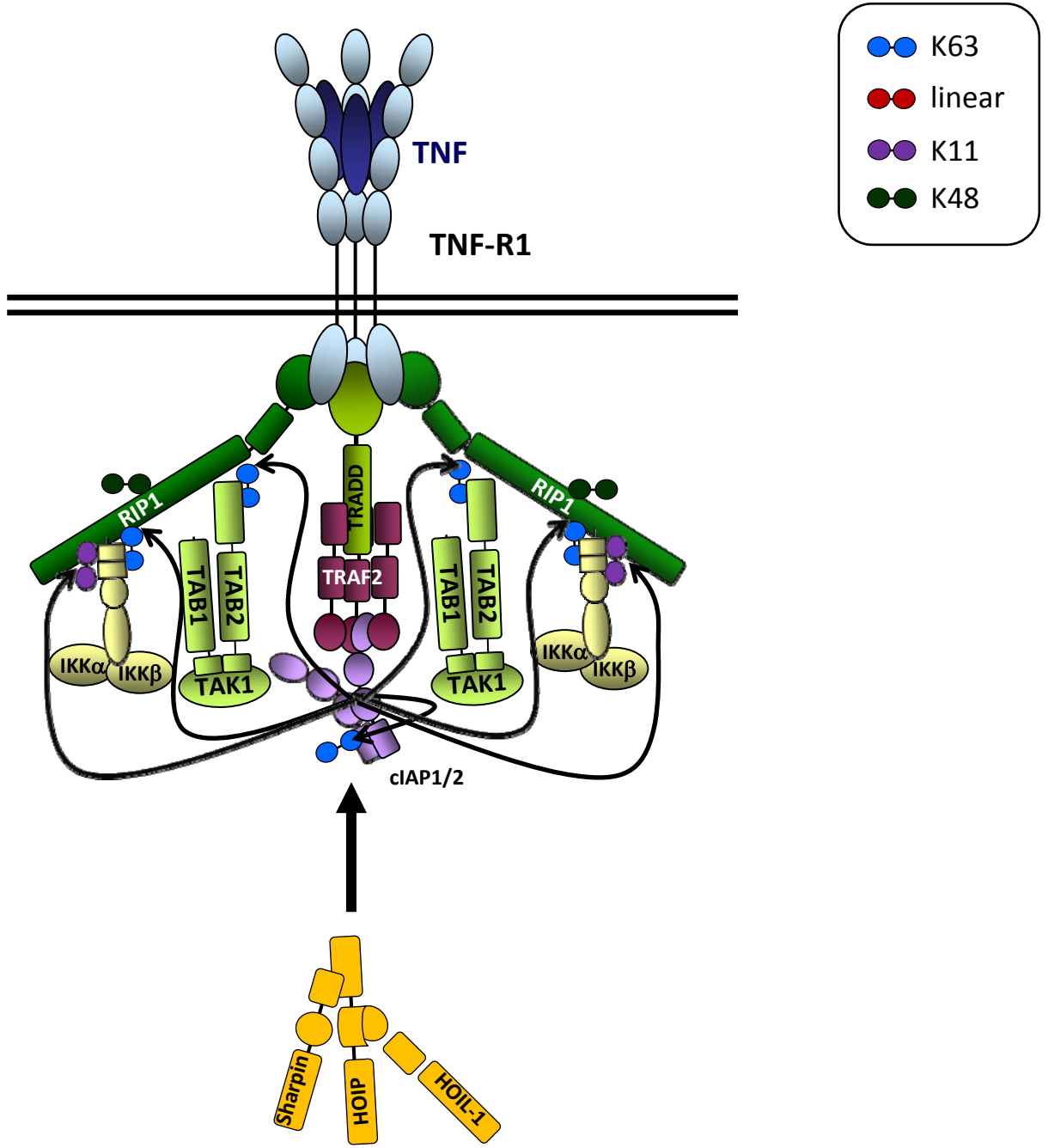


Gerlach, Cordier et al., *Nature,* 2011


K63
linear
K11
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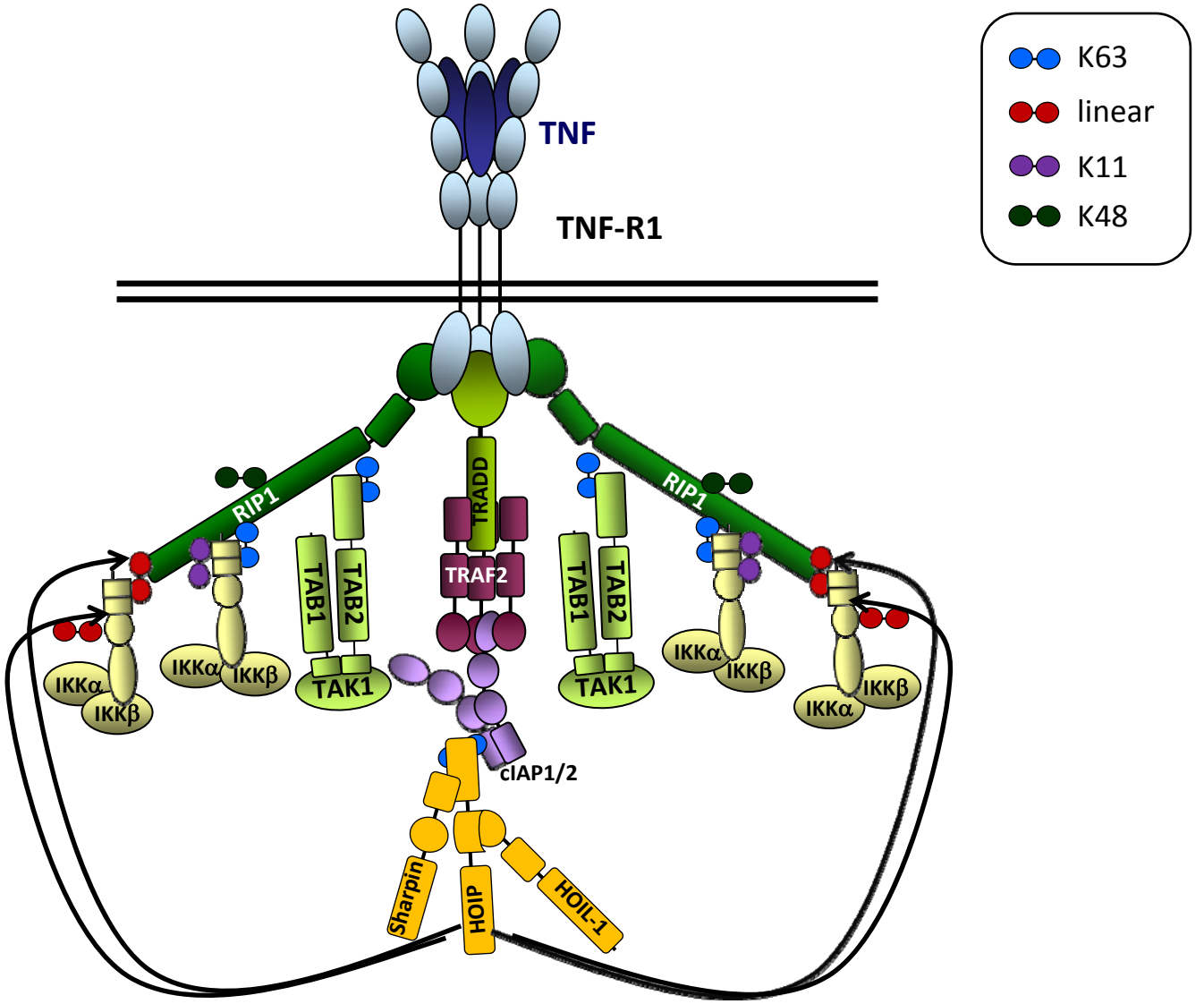
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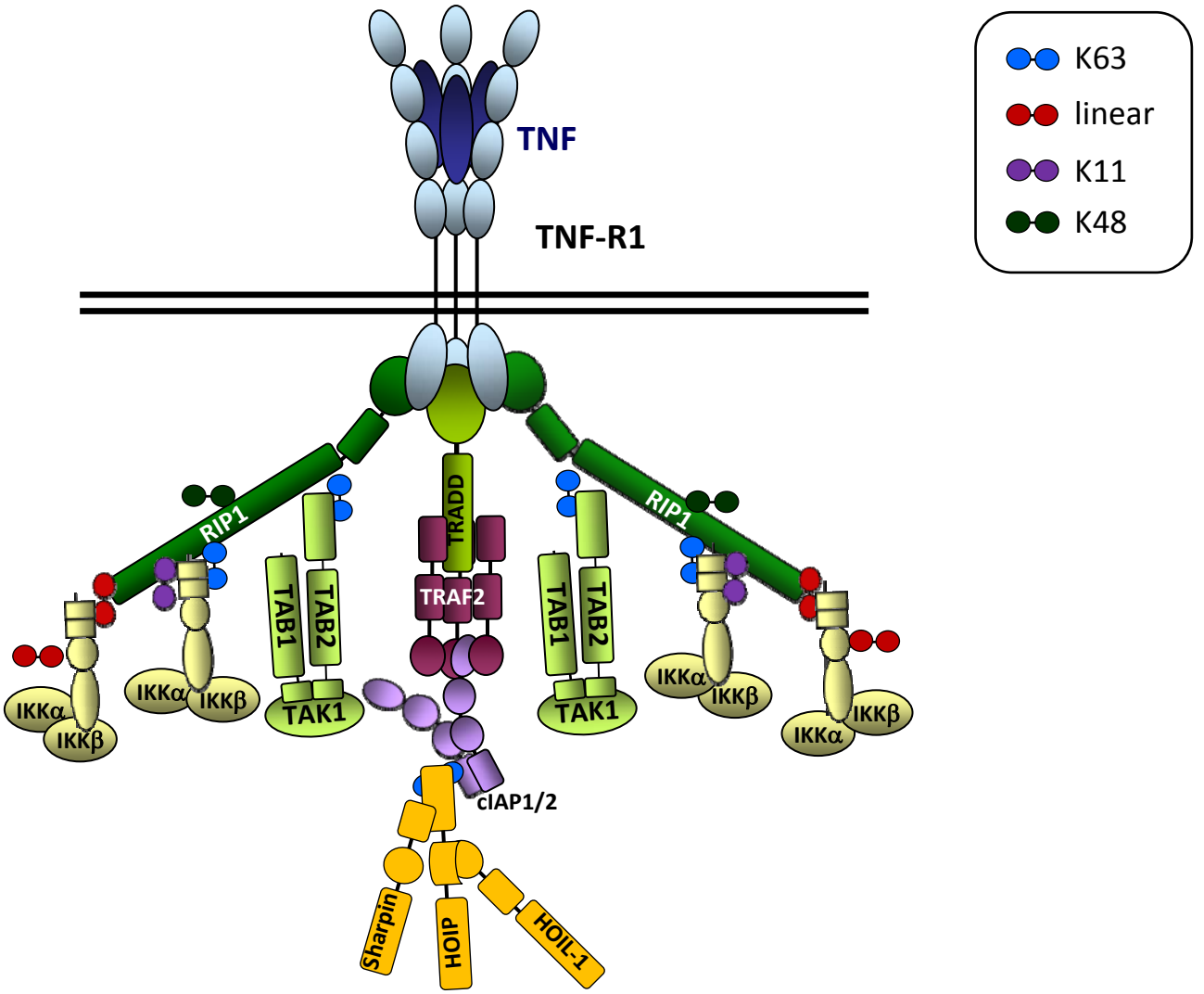
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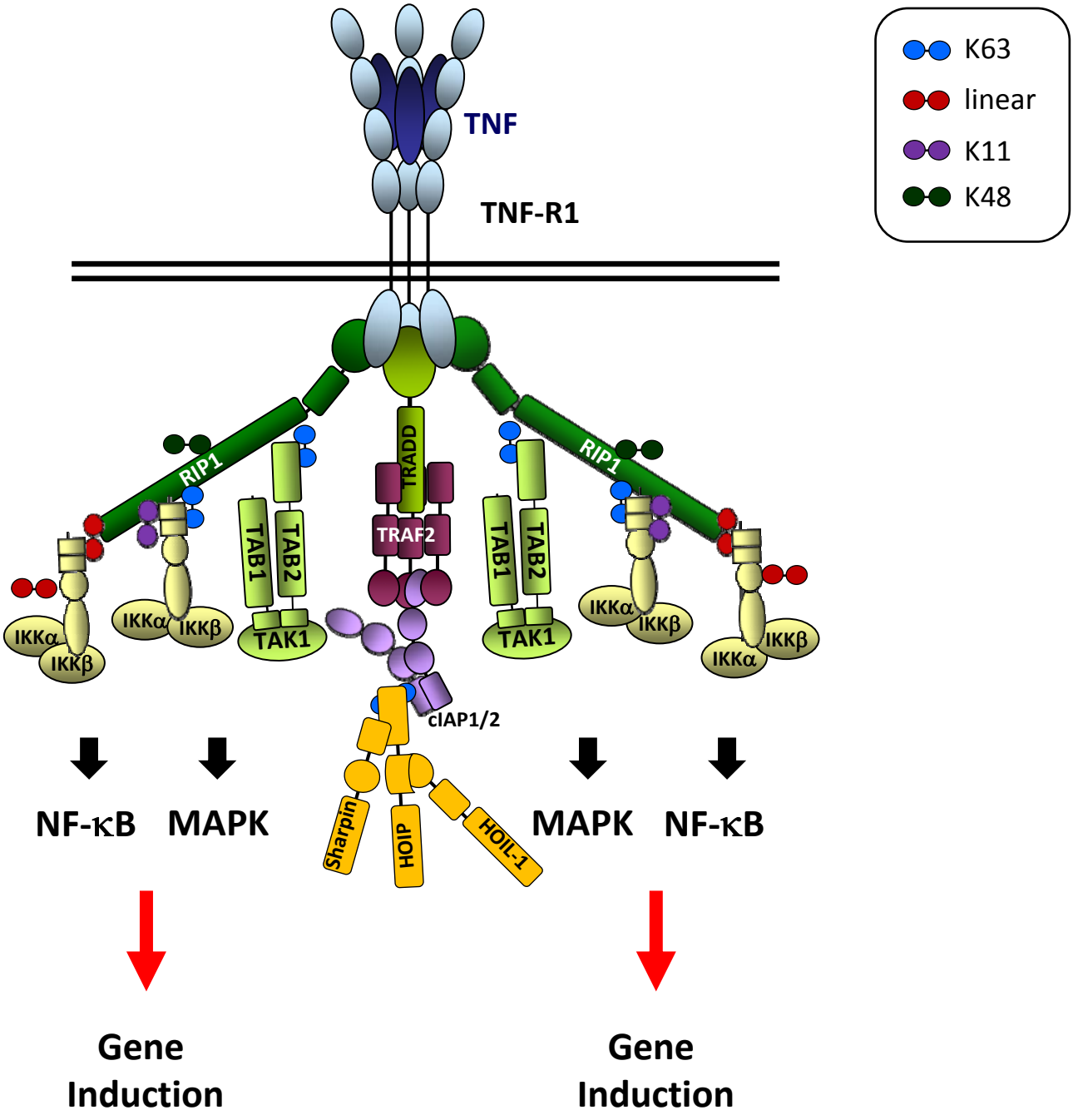
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IKKα
IKKβ
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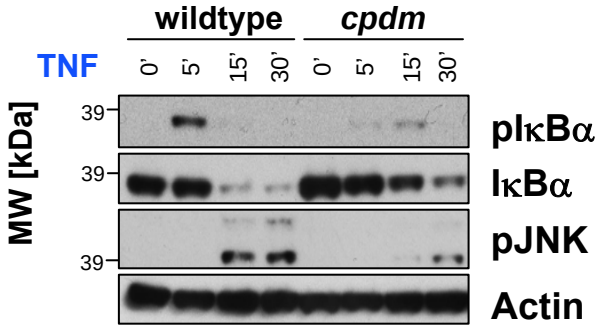
Sharpin
HOIP
HOIL-1
NF-κB
MAPK
MAPK
NF-κB
Gene Induction
Gene Induction

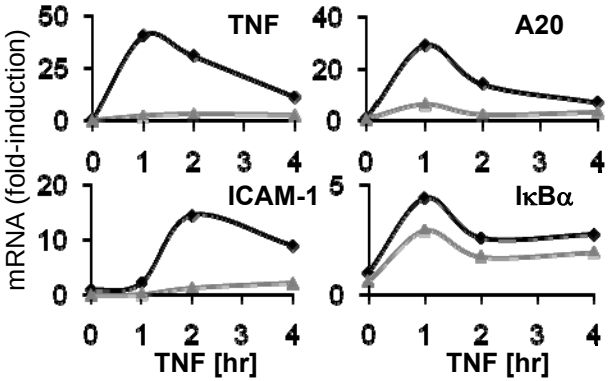
Phenotype of Sharpin mutant *cpdm* Mice

- Spontaneous base pair deletion in the Sharpin gene 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

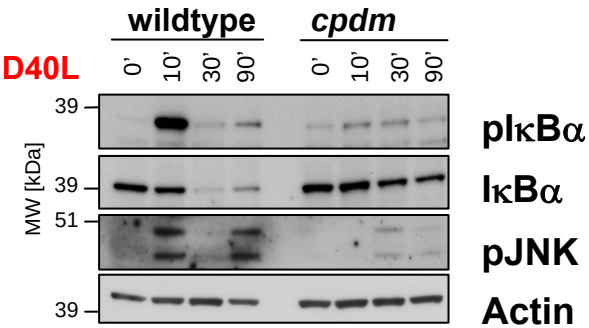
Sharpin is required for effective TNF -induced NF-κB and JNK activation

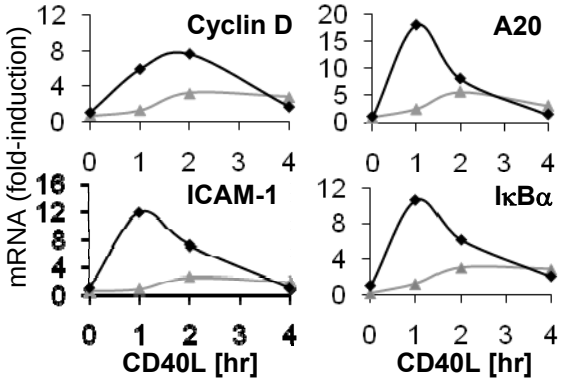
MEFs





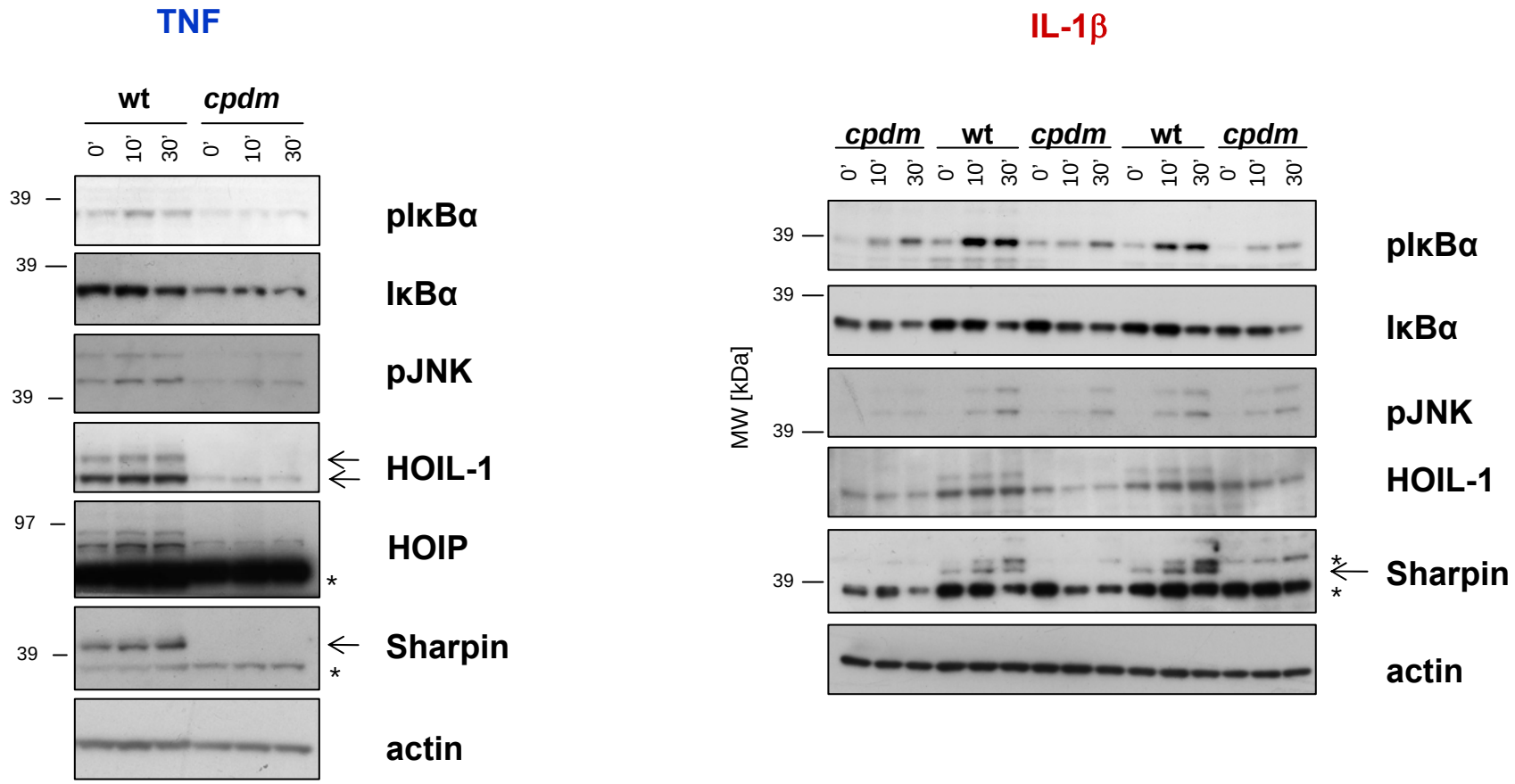
Primary splenic B cells





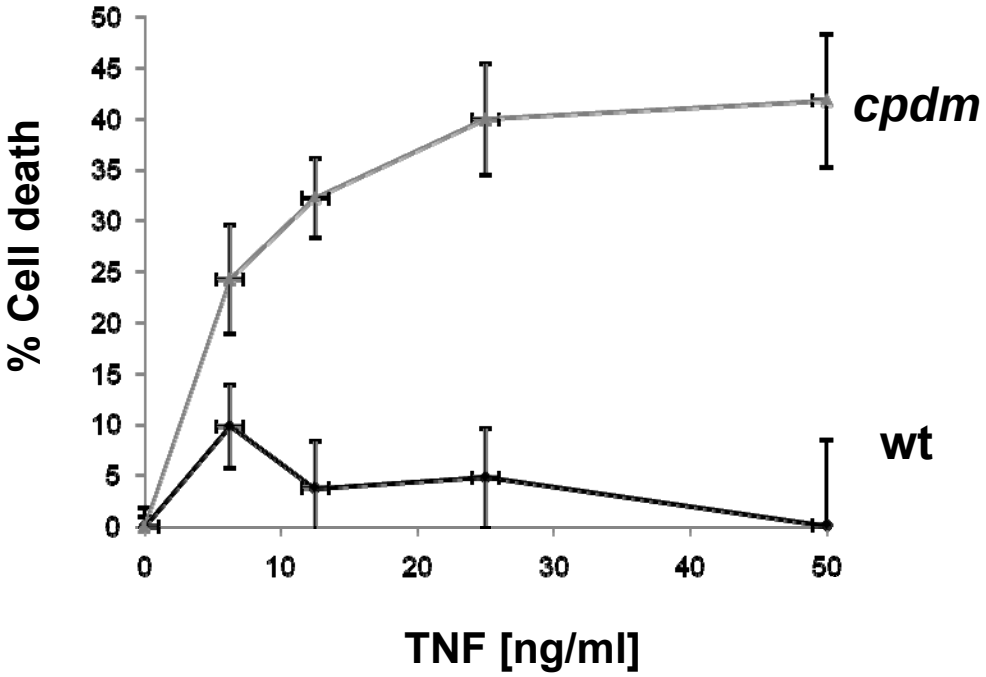
Gerlach, Cordier et al., *Nature,* 2011

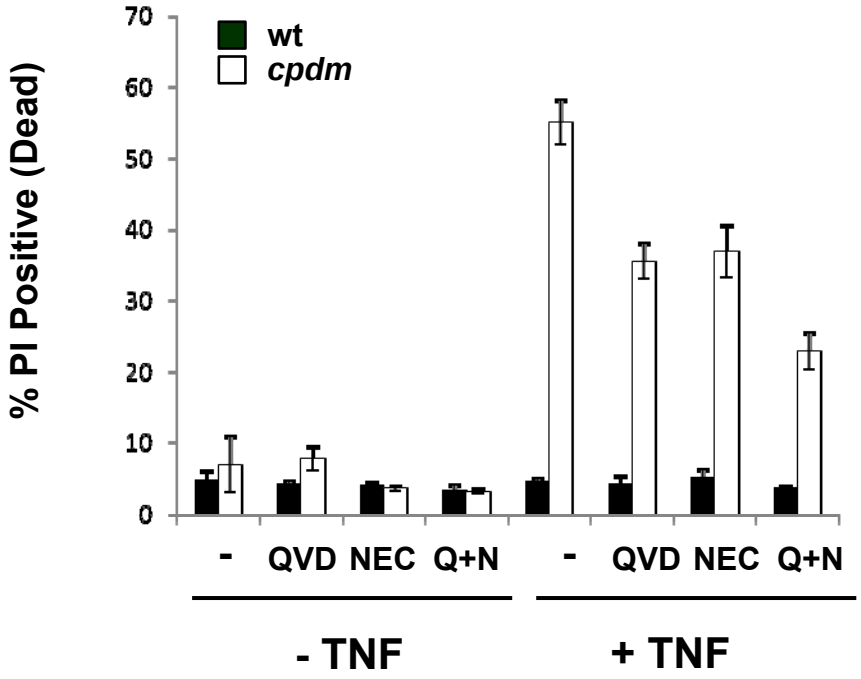
TNF and IL-1β-induced NF-κB activation is impaired in primary *cpdm*-derived keratinocytes cells



Gerlach, Cordier et al., *Nature,* 2011

Increased TNF-induced cell death in *cpdm* MEFs





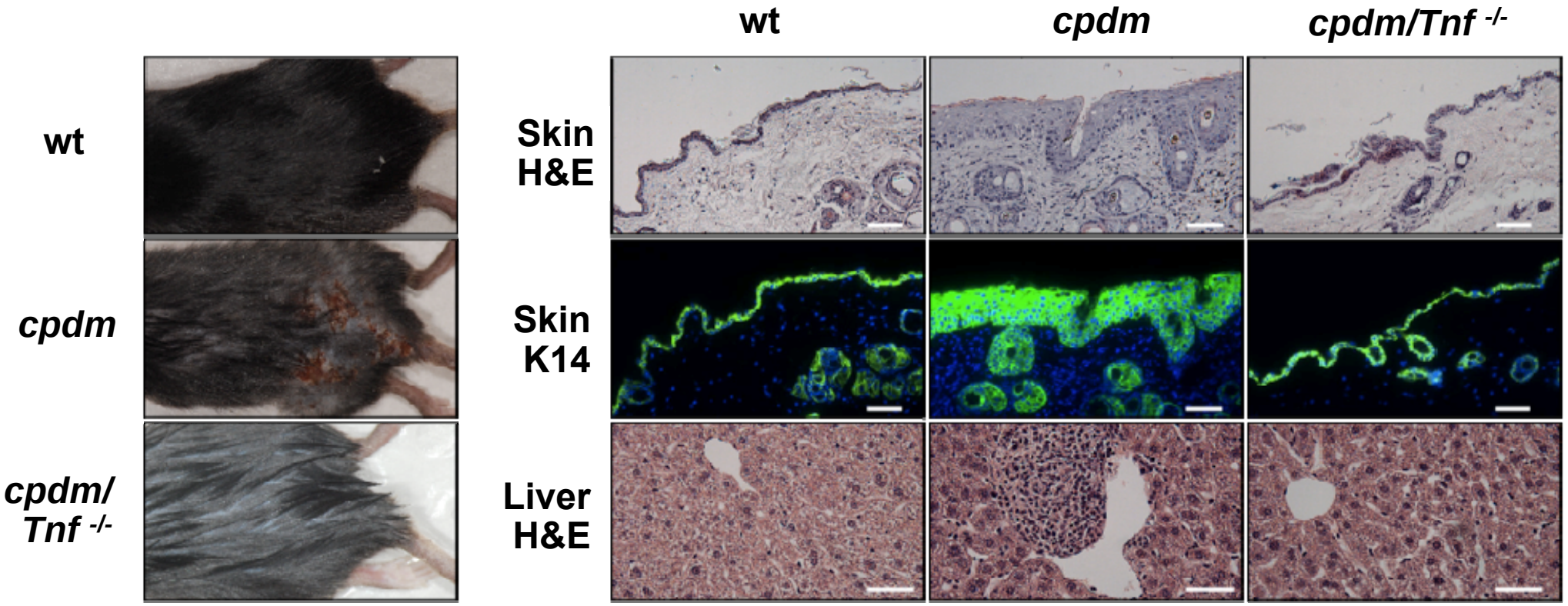
Loss of Sharpin results in a cell death-favouring dysregulation of TNF-induced signalling

Gerlach, Cordier et al., *Nature,* 2011

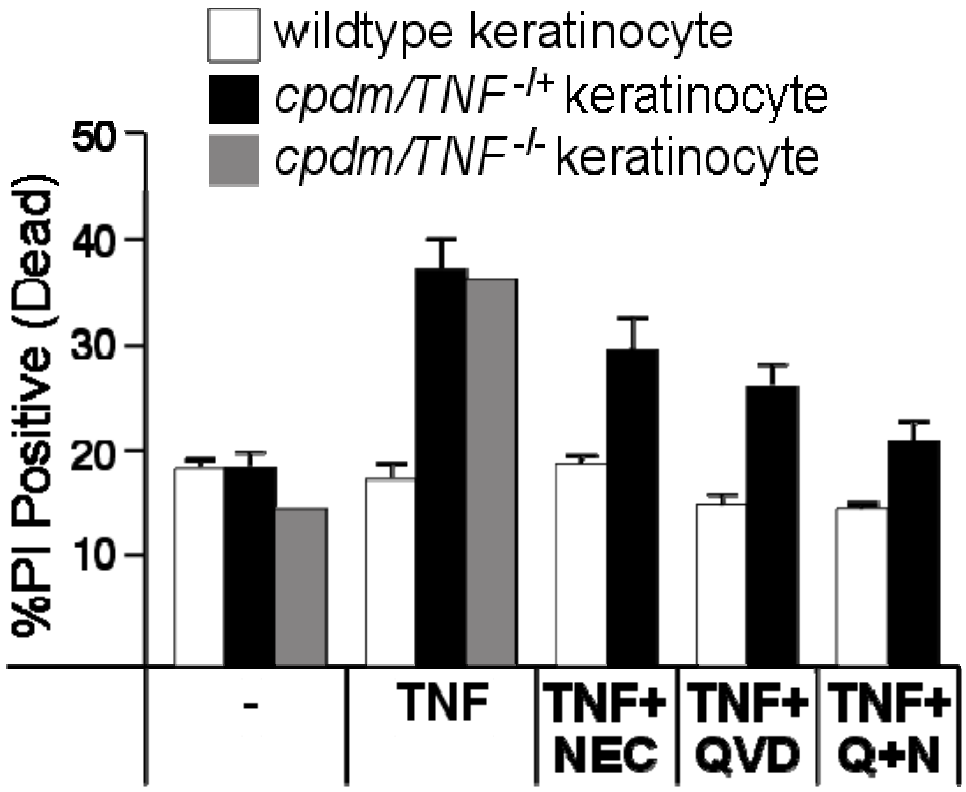
Phenotype of Sharpin mutant *cpdm* Mice

- 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

TNF deficiency corrects the inflammatory phenotype in *cpdm* mice

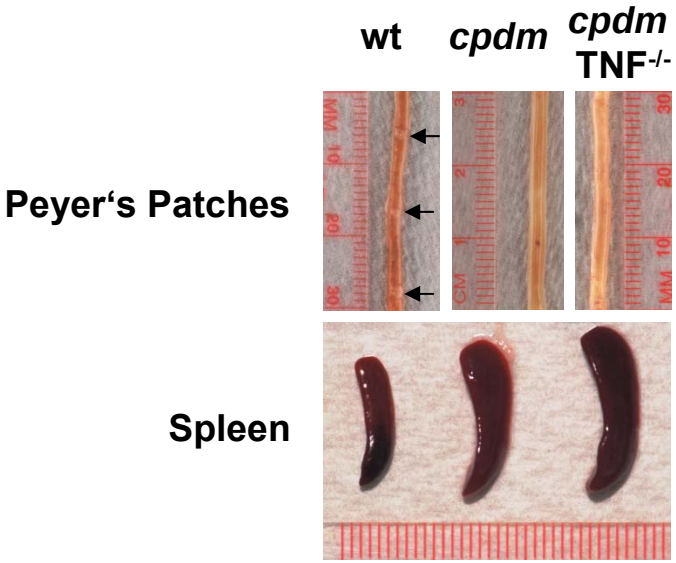


Gerlach, Cordier et al., *Nature,* 2011

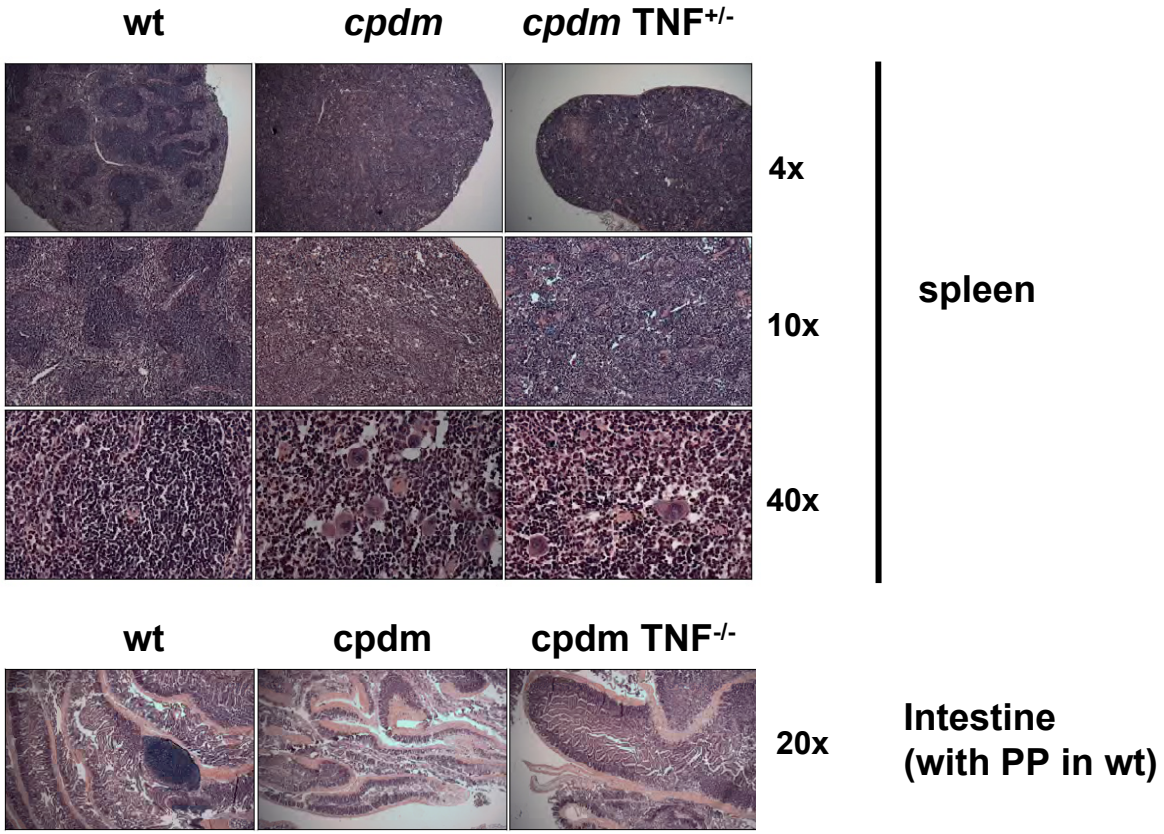


Gerlach, Cordier et al., *Nature,* 2011

Immunological alterations of *cpdm* mice are independent of TNF



	Spleen [g] weight	Peyer's Patches
Wt	0,08 0,075 0,08	8/20 cm 5/20 cm 7/30cm
cpdm	0,31 0,164 0,44	0/20 cm 0/20 cm 0/20 cm
cpdm TNF$^{-/-}$	0,23 0,124 0,19	0/17 cm 0/20 cm 0/40 cm



Gerlach, Cordier et al., *Nature,* 2011

Summary

- LUBAC is a new component of the TNF and CD40 receptor signalling complexes
- LUBAC recruitment to these complexes depends on cIAPs
- LUBAC deficiency attenuates gene induction by TNF, CD40L and IL-1β
- and renders cells more susceptible to TNF-induced cell death
- TNF deficiency corrects the inflammatory but not the immunological abnormalities observed in *cpdm* mice

Conclusions

- This identifies linear ubiquitination as a third type of ubiquitination required for physiological innate and adaptive immune signalling

=> A physiological role for linear ubiquitination

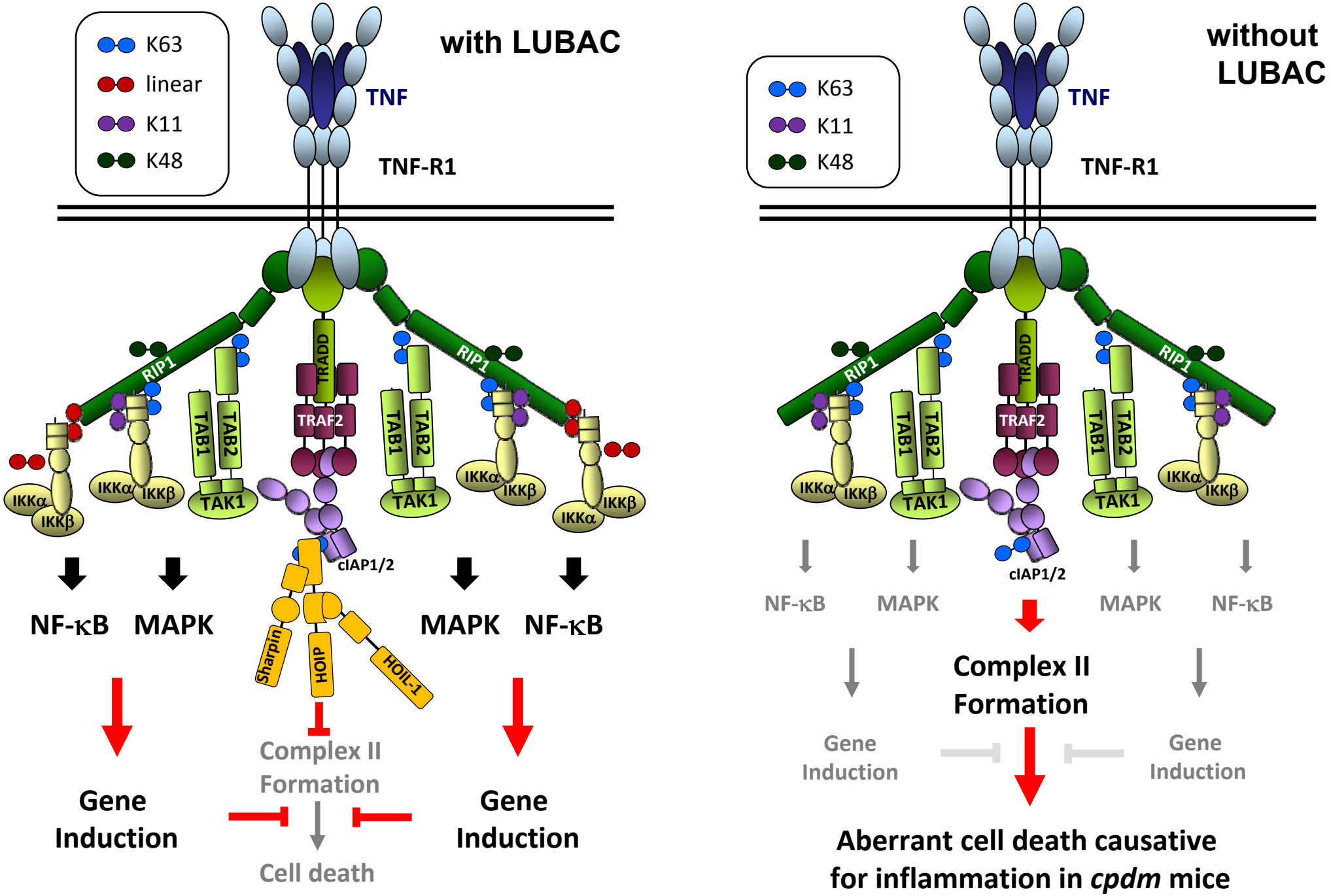

K63
linear
K11
K48
with LUBAC
TNF
TNF-R1
RIP1
TRADD
TRAF2
TAB1
TAB2
TAK1
IKKα
IKKβ
cIAP1/2
Sharpin
HOIP
HOIL-1
NF-κB
MAPK
MAPK
NF-κB
Gene Induction
Complex II Formation
Cell death
Gene Induction
without LUBAC
K63
K11
K48
TNF
TNF-R1
RIP1
TRADD
TRAF2
TAB1
TAB2
TAK1
IKKα
IKKβ
cIAP1/2
NF-κB
MAPK
MAPK
NF-κB
Gene Induction
Complex II Formation
Gene Induction
Aberrant cell death causative for inflammation in *cpdm* mice

Figure 7

Conclusions

- This identifies linear ubiquitination as a third type of ubiquitination required for innate and adaptive immune signalling

=> A physiological function for linear ubiquitination

- Linear ubiquitination balances the output of TNF signal transduction

- Lack of LUBAC activity causes a cell death-favouring dysregulation of TNF signalling responsible for inflammation

=> Identification of a new aetiology for autoimmunity that depends on pro-inflammatory cell death rather than pro-inflammatory gene induction

LUBAC activity plays a central role for signals emanating from the TNF-RSC