J Clin Invest. 2018 Oct 1;128(10):4280-4296.
Ubiquitin-specific protease 7 sustains DNA damage response and promotes cervical carcinogenesis.

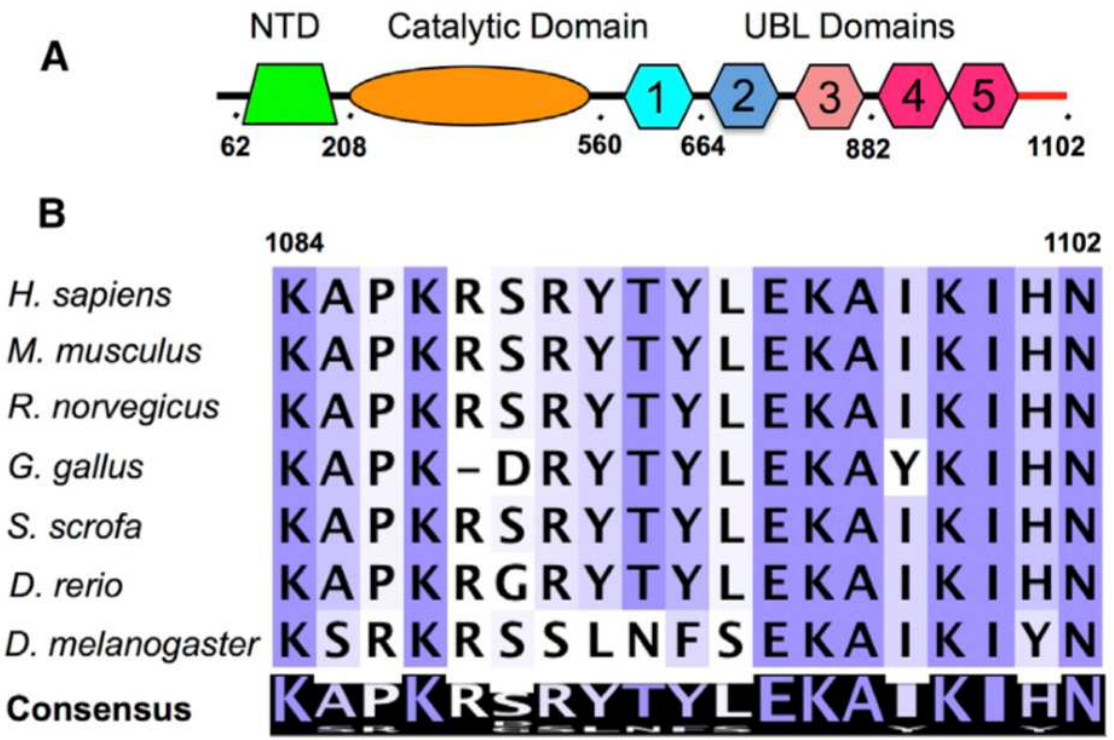
Ubiquitination -DeUbiquitination

- Ubiquitination is a central player in DSB repairs
- Ubiquitination is constantly opposed through the action of specific deubiquitinating enzymes (DUBs)
- Ubiquitin-specific proteases (USPs) comprise the largest subfamily of DUBs.
- USP7 is involved in multiple oncogenic pathways

USP7

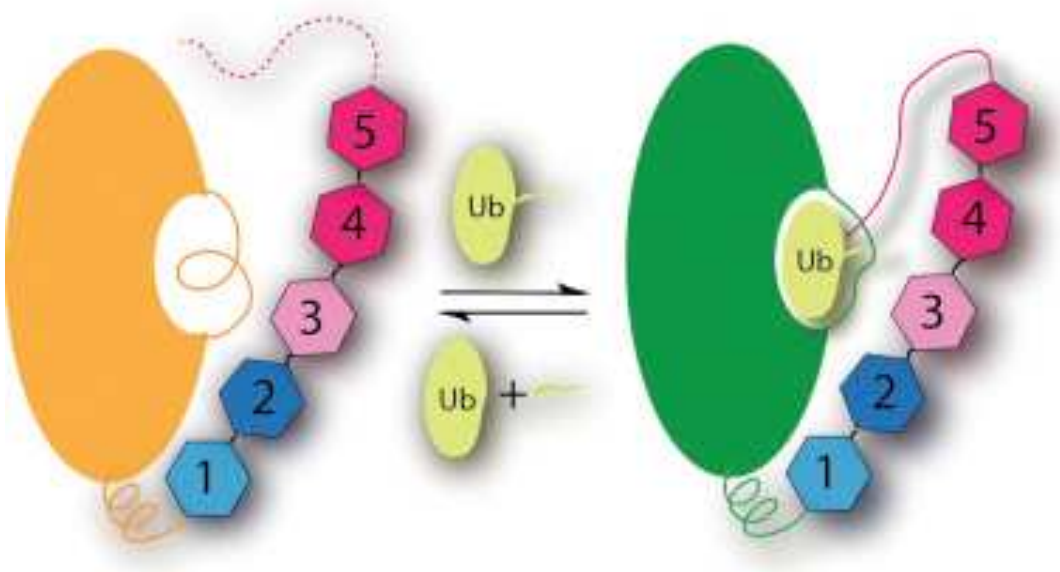
Substrate peptide binding

Specific protein interaction

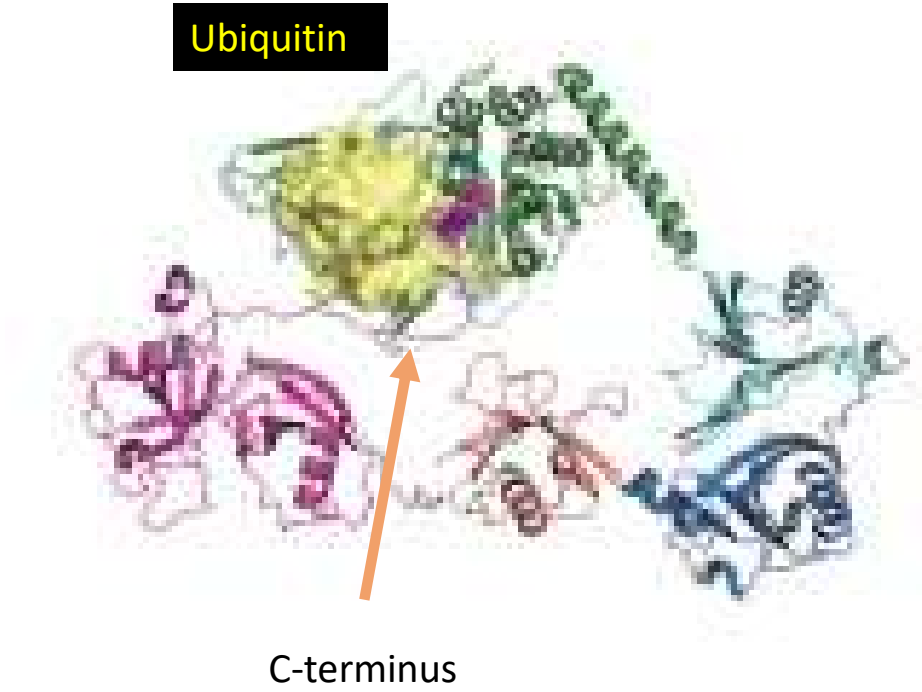


C-terminal residues: regulation of catalytic activity (50X!)

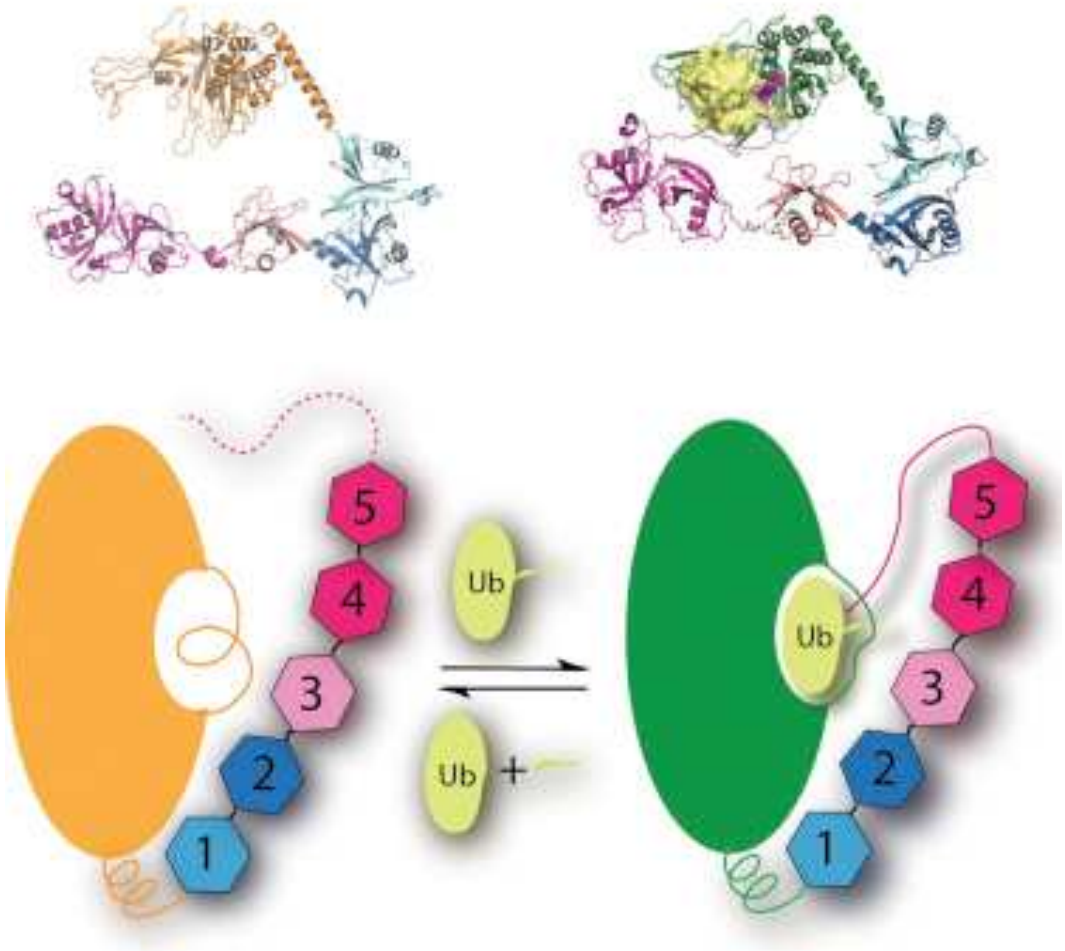
Modello di attivazione di USP7



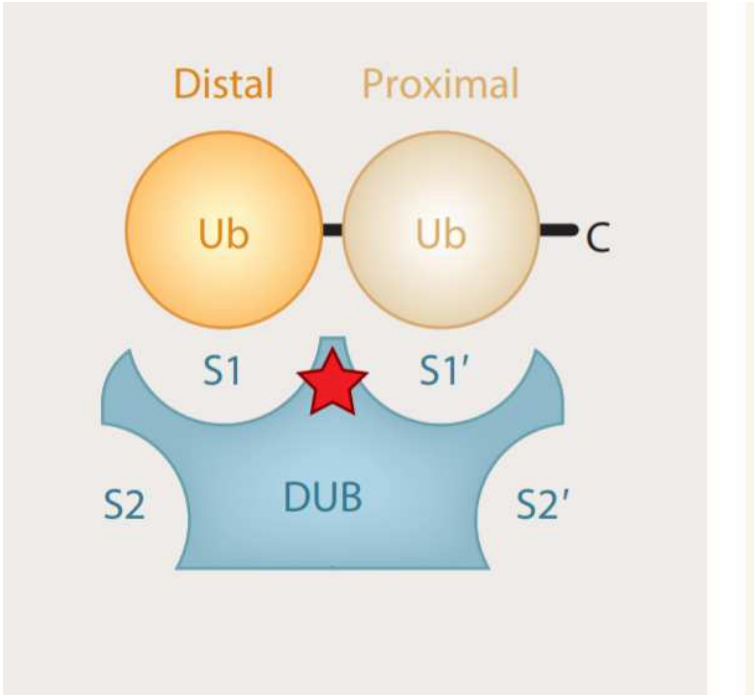
Modello di attivazione di USP7

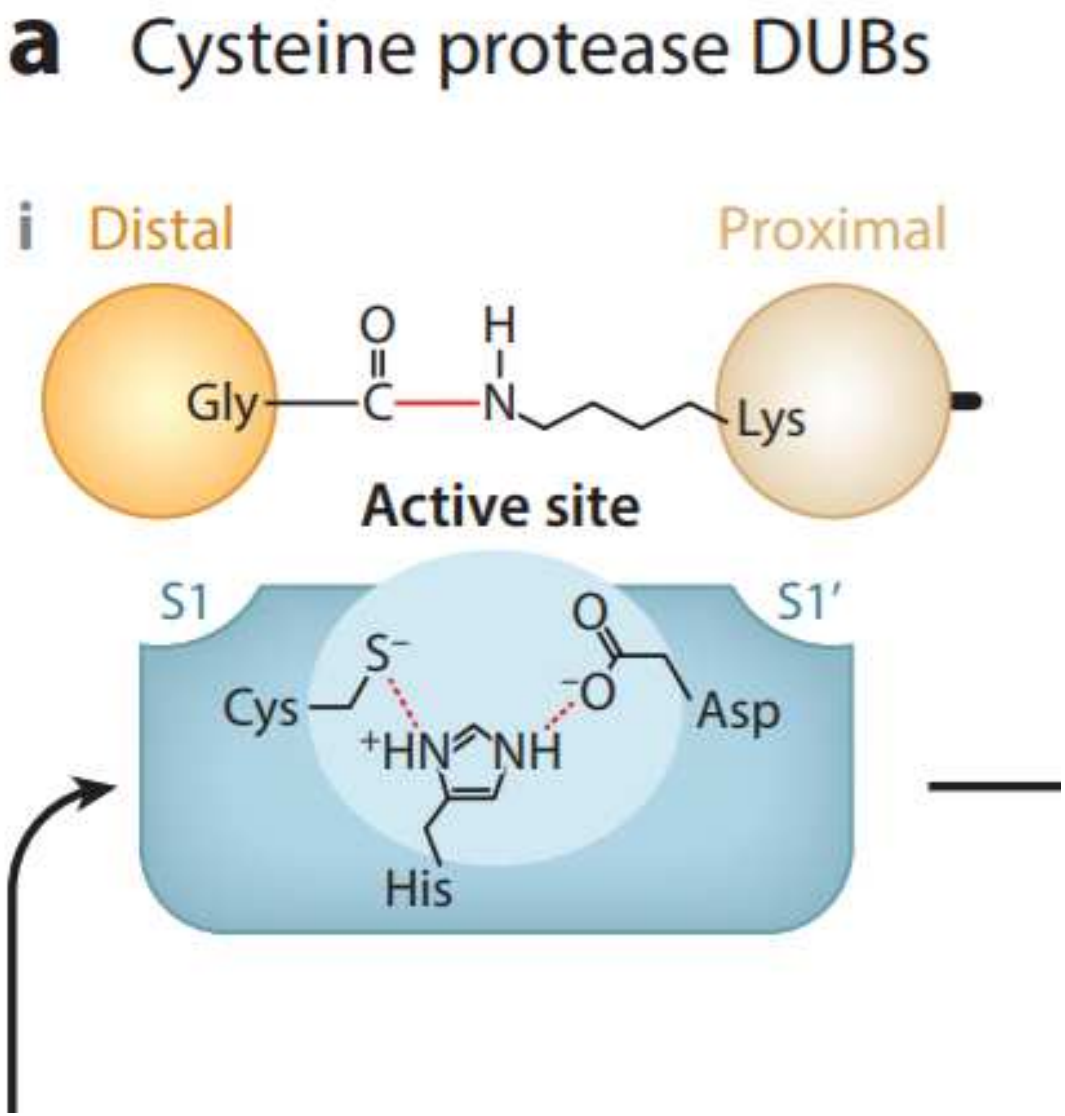


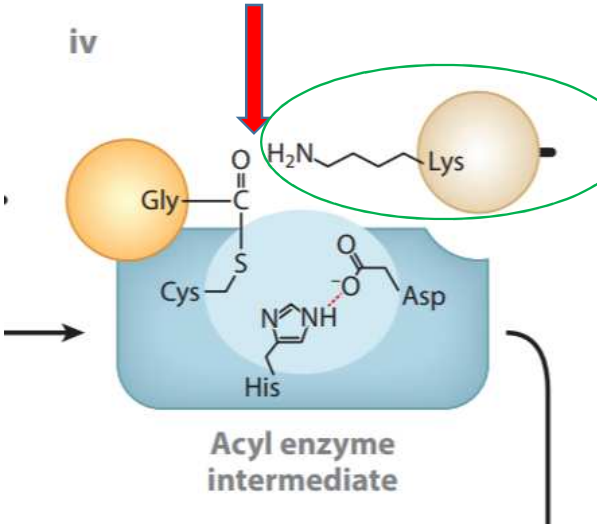
Modello di attivazione di USP7

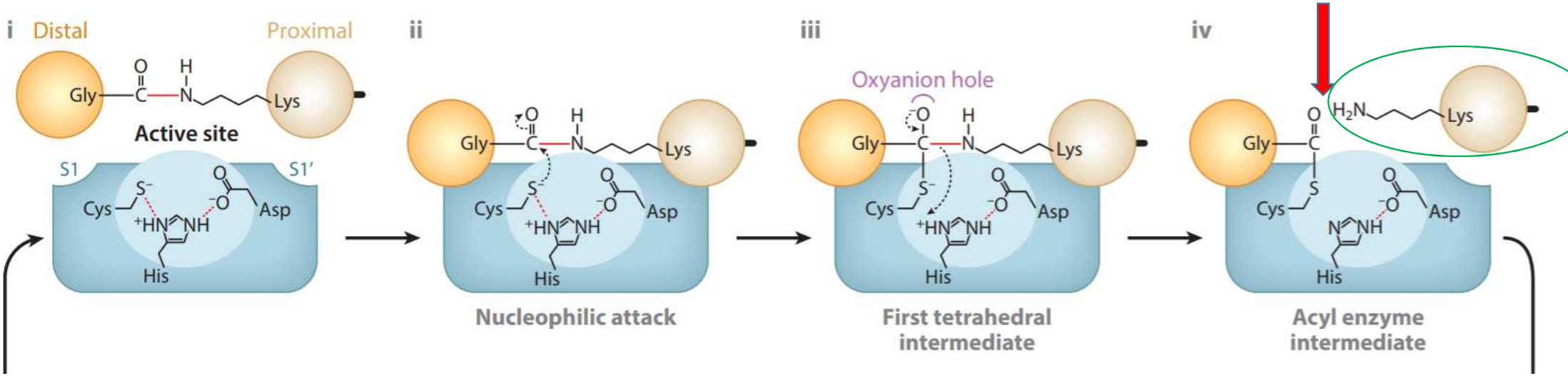


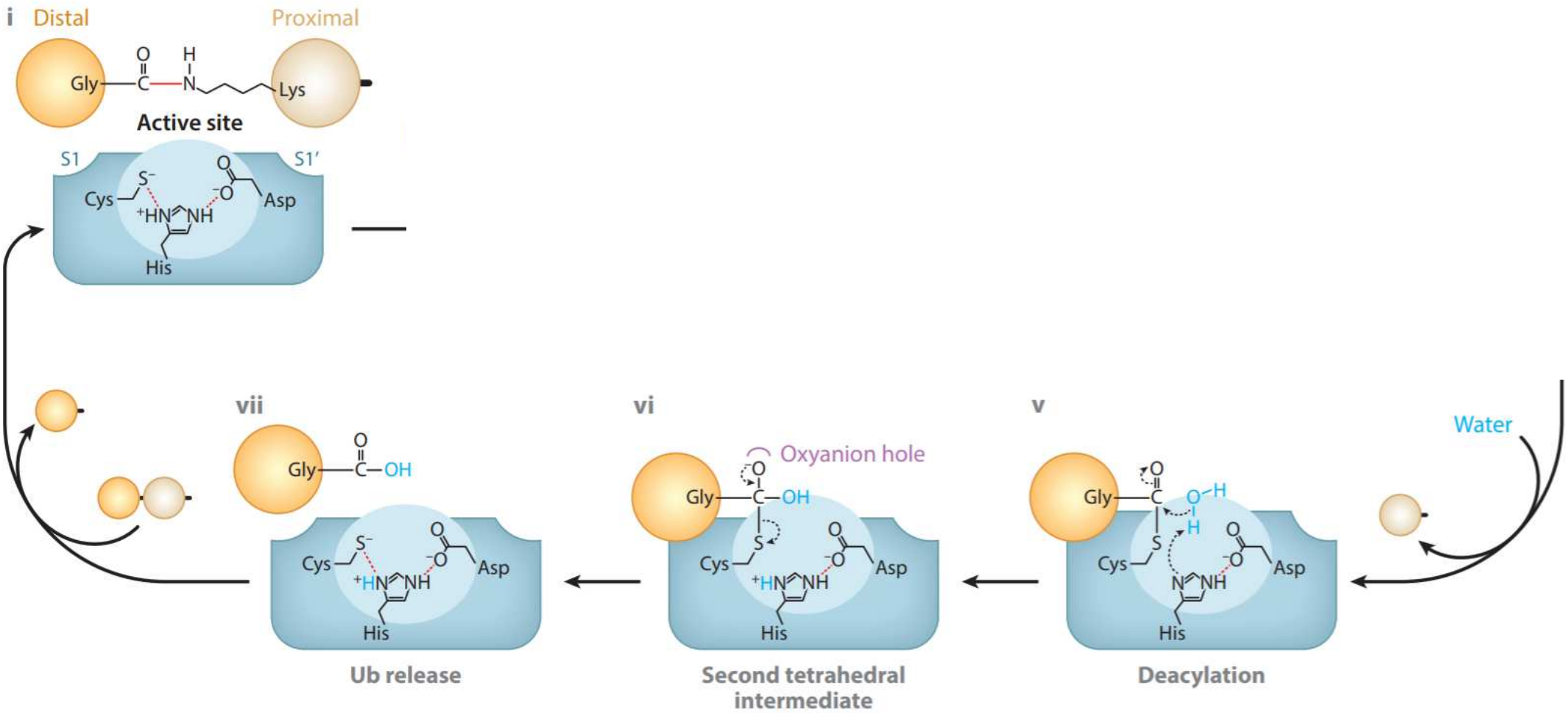
Deubiquitinasi (DUBs) meccanismo



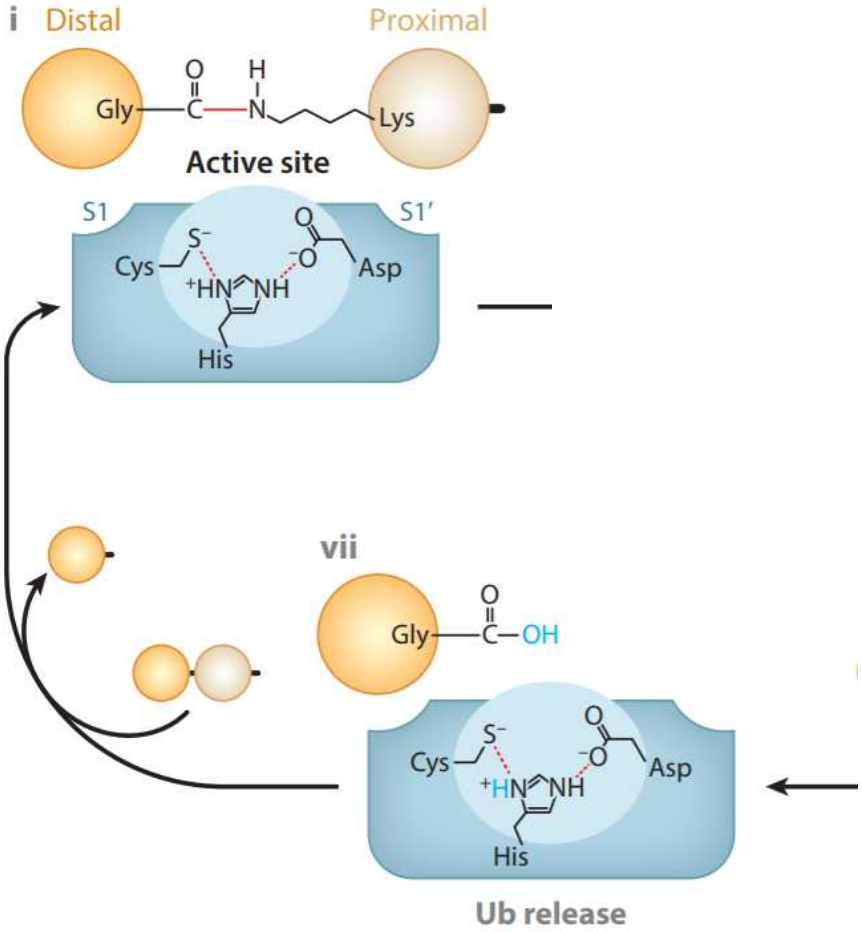

a Cysteine protease DUBs
i
Distal
Proximal
O
H
Gly
C
N
Lys
Active site
S1
S1′
S⁻
O
Cys
⁻O
Asp
⁺HN
NH
His


iv
Gly
O
C
S
Cys
H_2N
Lys
O
O
Asp
N
NH
His
Acyl enzyme
intermediate


a Cysteine protease DUBs
i Distal
Proximal
Gly
Lys
Active site
S1
S1′
Cys
Asp
His
ii
Nucleophilic attack
iii
Oxyanion hole
First tetrahedral intermediate
iv
H_2N
Acyl enzyme intermediate


a Cysteine protease DUBs
i
Distal
Proximal
Gly
Lys
Active site
S1
S1′
Cys
Asp
His
Water
v
Deacylation
vi
Oxyanion hole
Second tetrahedral intermediate
vii
Ub release

a Cysteine protease DUBs



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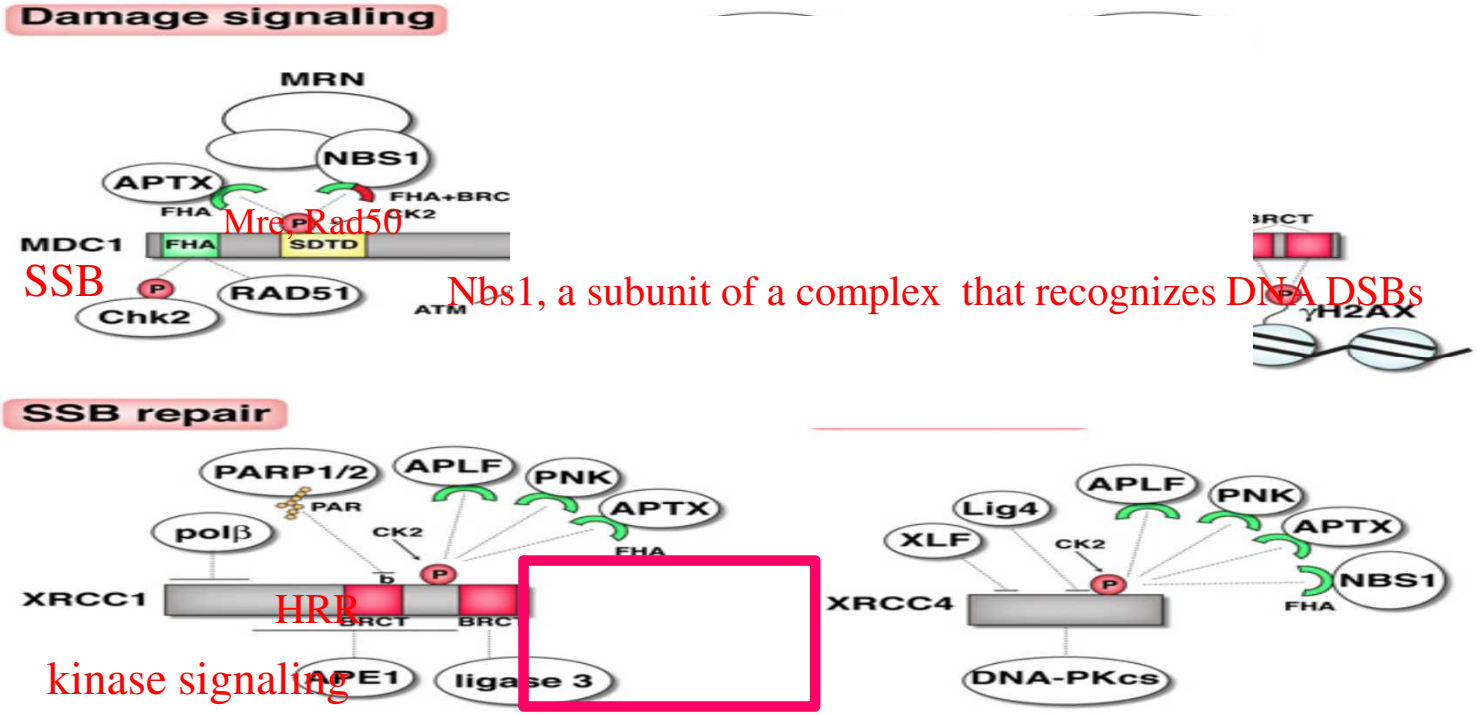
- Ubiquitination is constantly opposed through the action of specific deubiquitinating enzymes (DUBs)
- Ubiquitin-specific proteases (USPs) comprise the largest subfamily of DUBs.
- USP7 is involved in multiple oncogenic pathways
- USP7 has been reported to target a panel of DDR proteins

it remains an open question as to how USP7 contributes to the response to and repair of DSBs, especially at the initial step of the DDR.

MDC1



Mre Rad50

Nbs1, a subunit of a complex that recognizes DNA DSBs

SSB

HRR

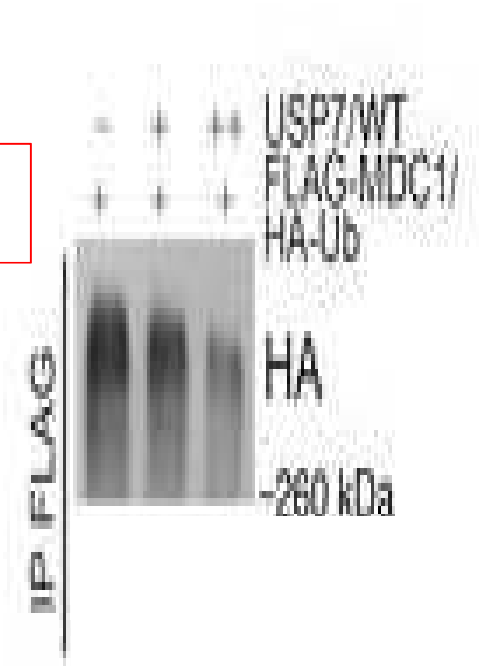
kinase signaling

- MDC1 is a ubiquitinated protein
- USP7 promotes the stabilization of MDC1

Does USP7 function to deubiquitinate MDC1?

In vitro deubiquitination assays

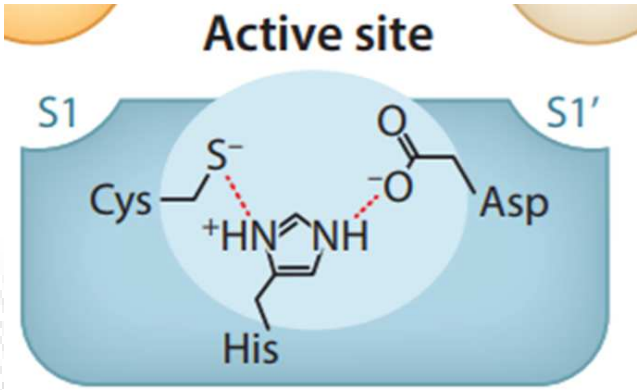
HA-Ub–conjugated MDC1 purified from HeLa cells



USP7 deubiquitinates MDC1

USP7/WT was capable of deubiquitinating MDC1

In vitro deubiquitination assays



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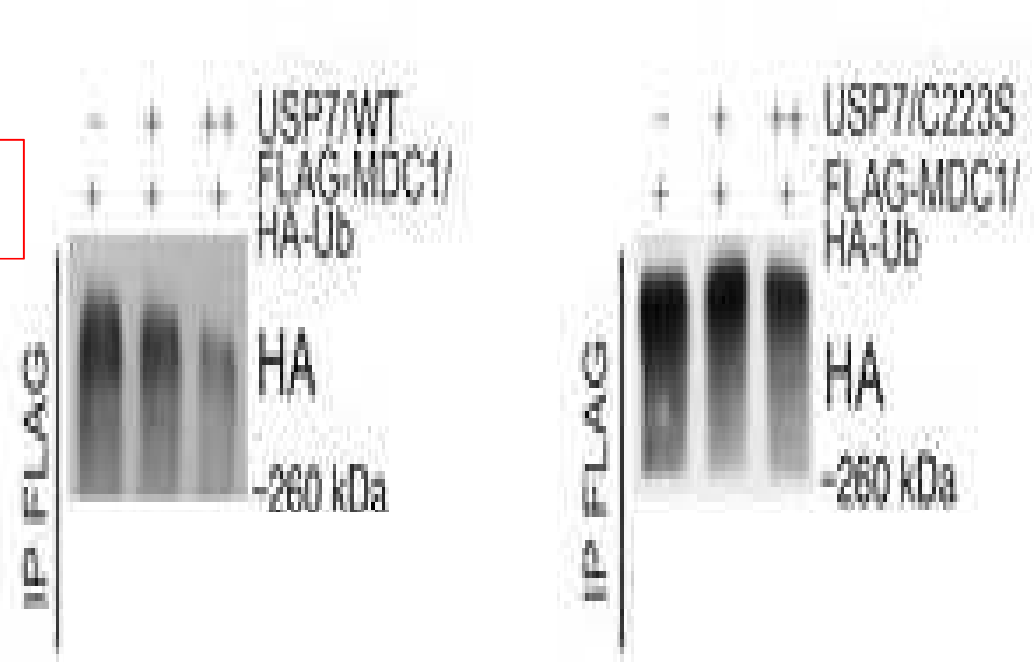


USP7 deubiquitinates MDC1

USP7/WT was capable of deubiquitinating MDC1, whereas USP7/C223S was not

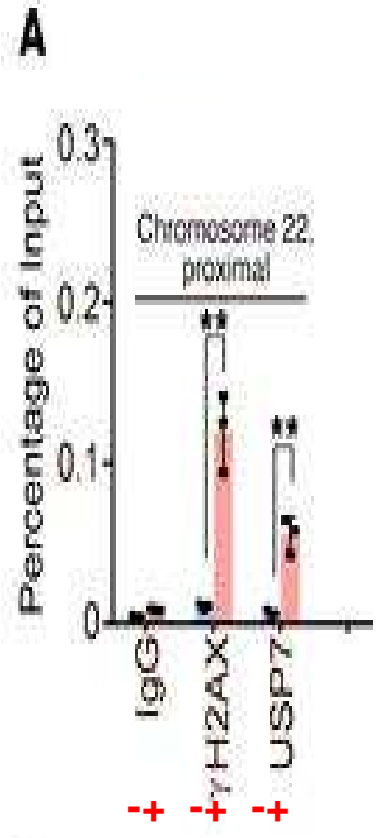
Is USP7 recruited to DSBs?

endonuclease AsiSI-based system

endogenous sequence–specific DSBs could be generated in the presence of 4-hydroxyl- tamoxifen (4-OHT)

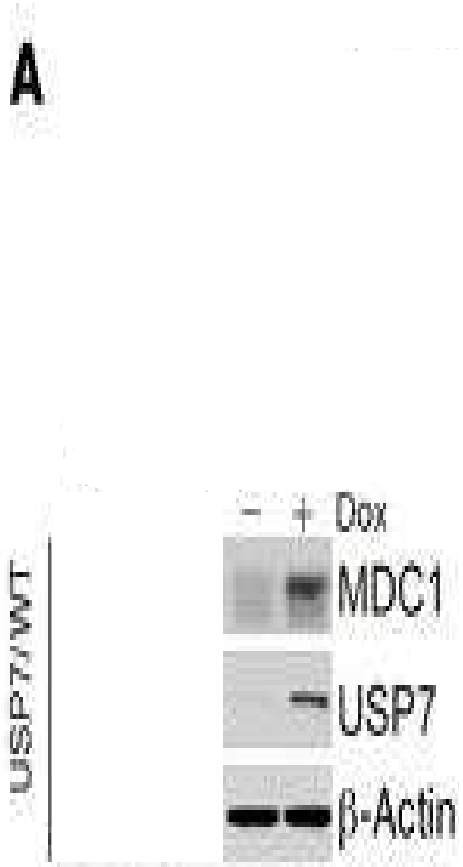
USP7 recruitment around sites of DSBs

qChIP analysis of USP7 recruitment around sites of DSBs.



endonuclease AsiSI-based system,
endogenous sequence–specific DSBs (chr22) generated in the presence of 4-hydroxyl- tamoxifen (4-OHT) +

USP7 deubiquitination stabilizes MDC1

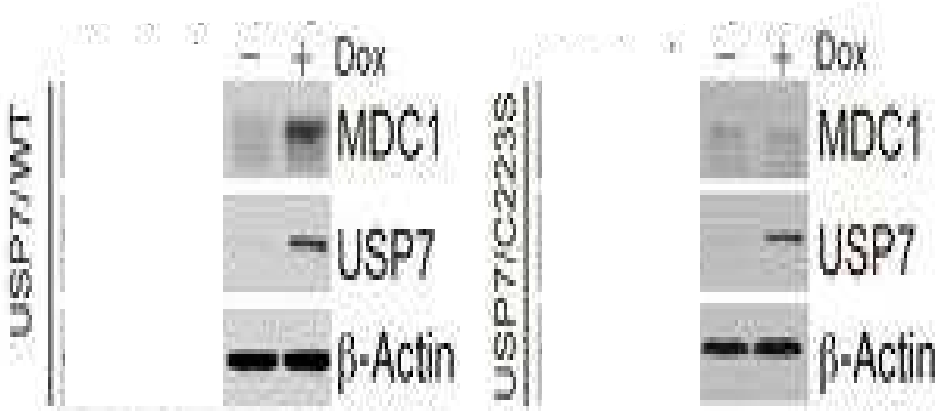


inducible expression +/-
of FLAG-USP7/WT

USP7 deubiquitination stabilizes MDC1

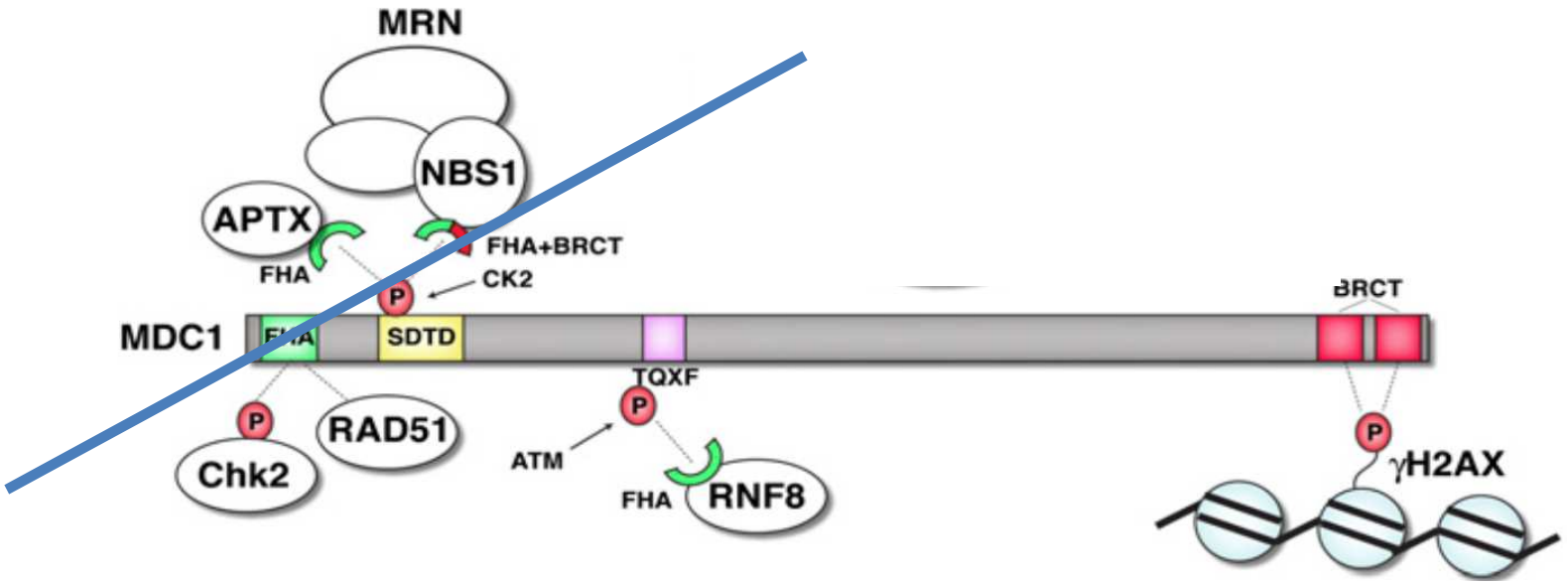
Dox-inducible expression +/-
of FLAG-USP7/WT
or FLAG-USP7/C223S

A



catalytically inactive mutant of USP7
(USP7/C223S)

depletion of USP7 impaired the engagement of the MRN-MDC1 complex and the consequent recruitment of the downstream factors p53-binding protein 1 (53BP1) and breast cancer protein 1 (BRCA1) at DNA lesions



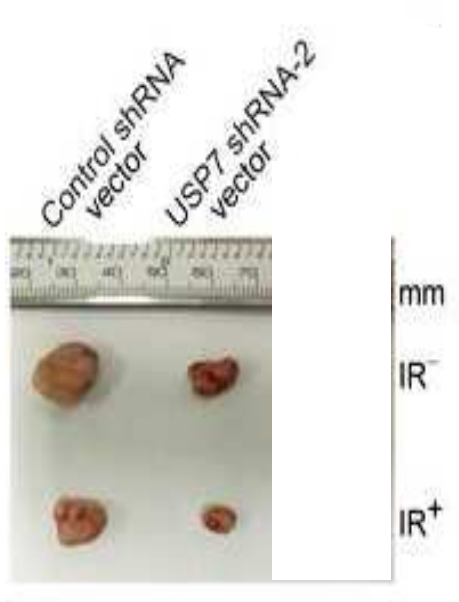
DSB repair and Cervical cancers

Deregulation of the key players involved in DSB repair is believed to play an important role in the development and progression of cervical cancer,

Cervical cancers are the fourth most common cause of malignancy and deaths from cancer in women worldwide

1) tumors stably expressing shRNAs were transplanted into athymic mice (n = 12)

2) half of the mice in each group were subjected to 10 Gy x-ray IR (**IR+**) 1 week after tumor transplantation.



Tumor volumes were measured weekly, and tumors were harvested and weighed when mice were sacrificed

J Clin Invest DOI: 10.1172/JCI120518

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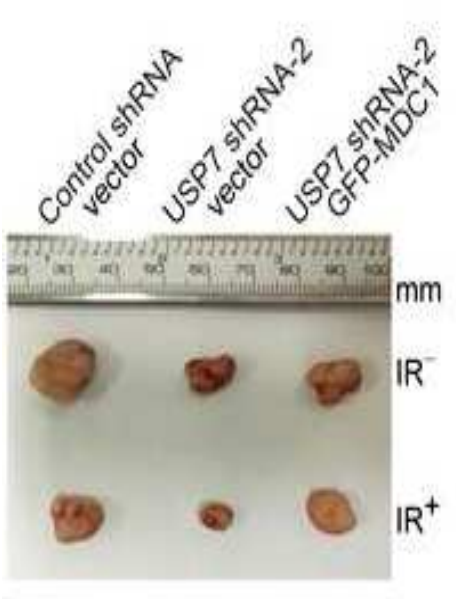

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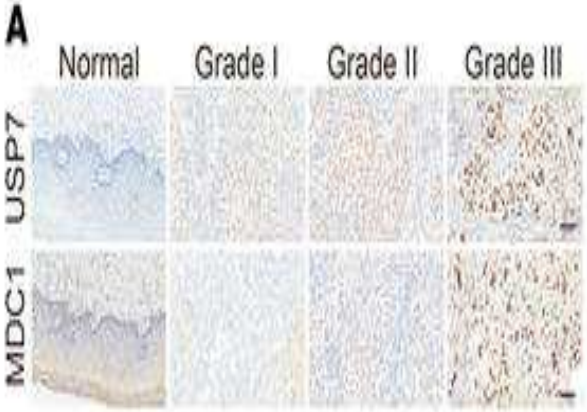
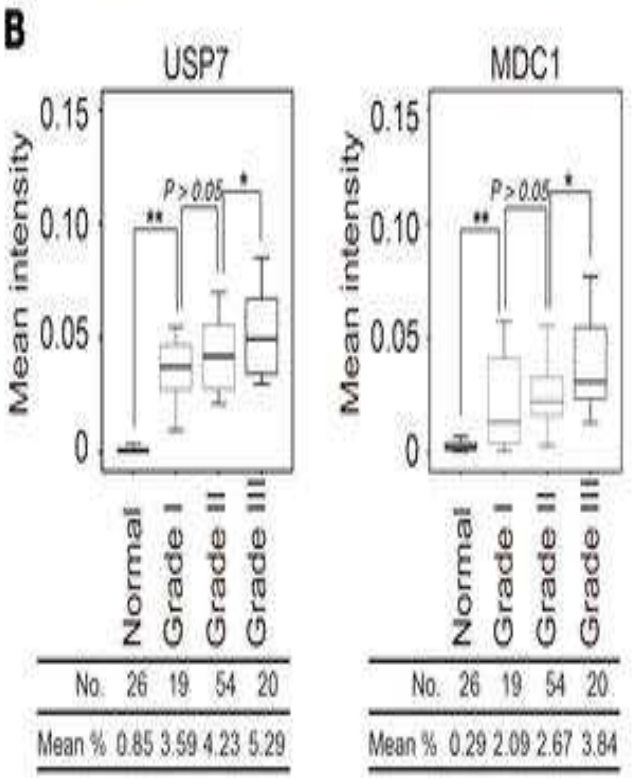
USP7 is implicated in cervical carcinogenesis



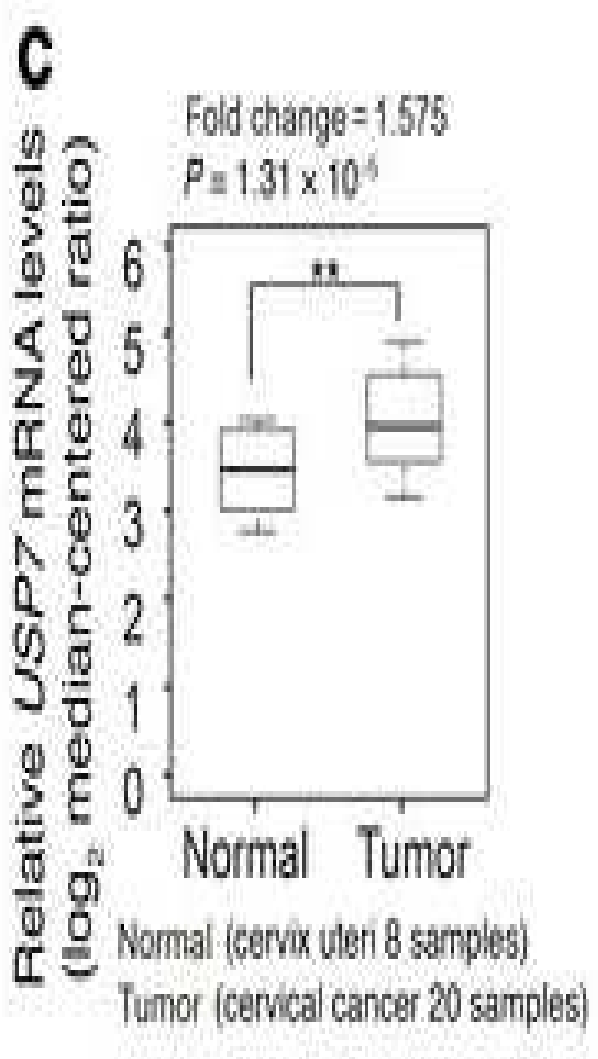
IHC

Human tissues containing cervical carcinoma with different grades

Tumor-adjacent cervical samples (Normal)



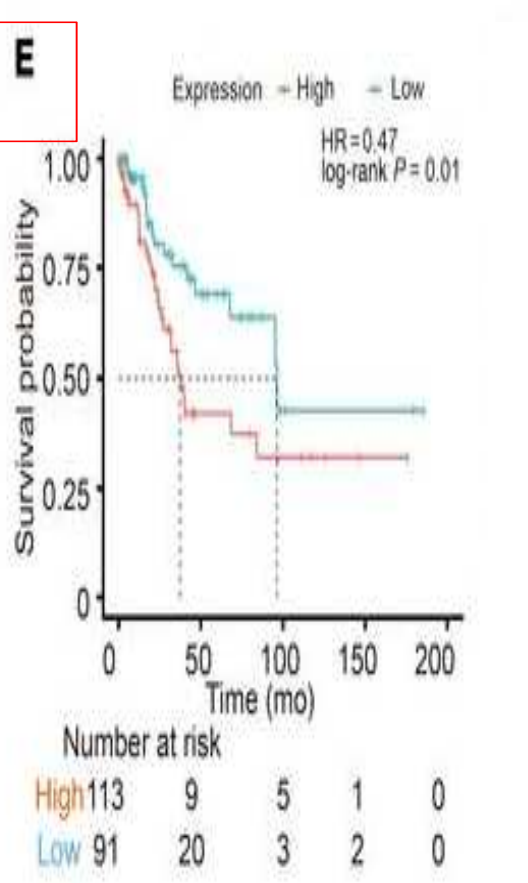
expression of USP7 mRNA is lower in normal human cervical tissues than cervical carcinoma samples



USP7 was overexpressed in cervical cancer, and the level of its expression positively correlated with that of MDC1

USP7 is implicated in cervical carcinogenesis and patient survival

expression levels of USP7 mRNA



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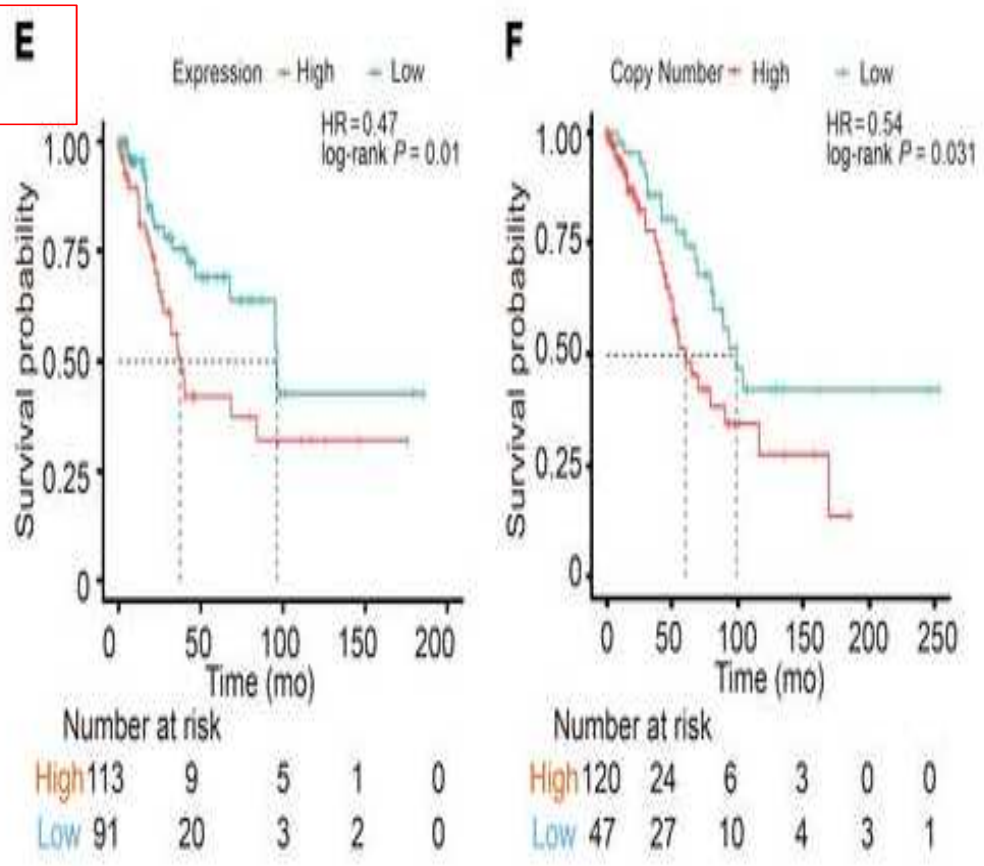
expression levels of USP7 mRNA



gene copy number of USP7

USP7 was overexpressed in cervical cancer, and the level of its expression positively correlated with that of MDC1 …

and worse survival rates for patients with cervical cancer

worse survival rates for patients with cervical cancer

Interpretazione

1) USP7-mediated MDC1 stabilization promoted cervical cancer cell survival and conferred resistance to genotoxic insults.

USP7 as a potential therapeutic target for cancer?

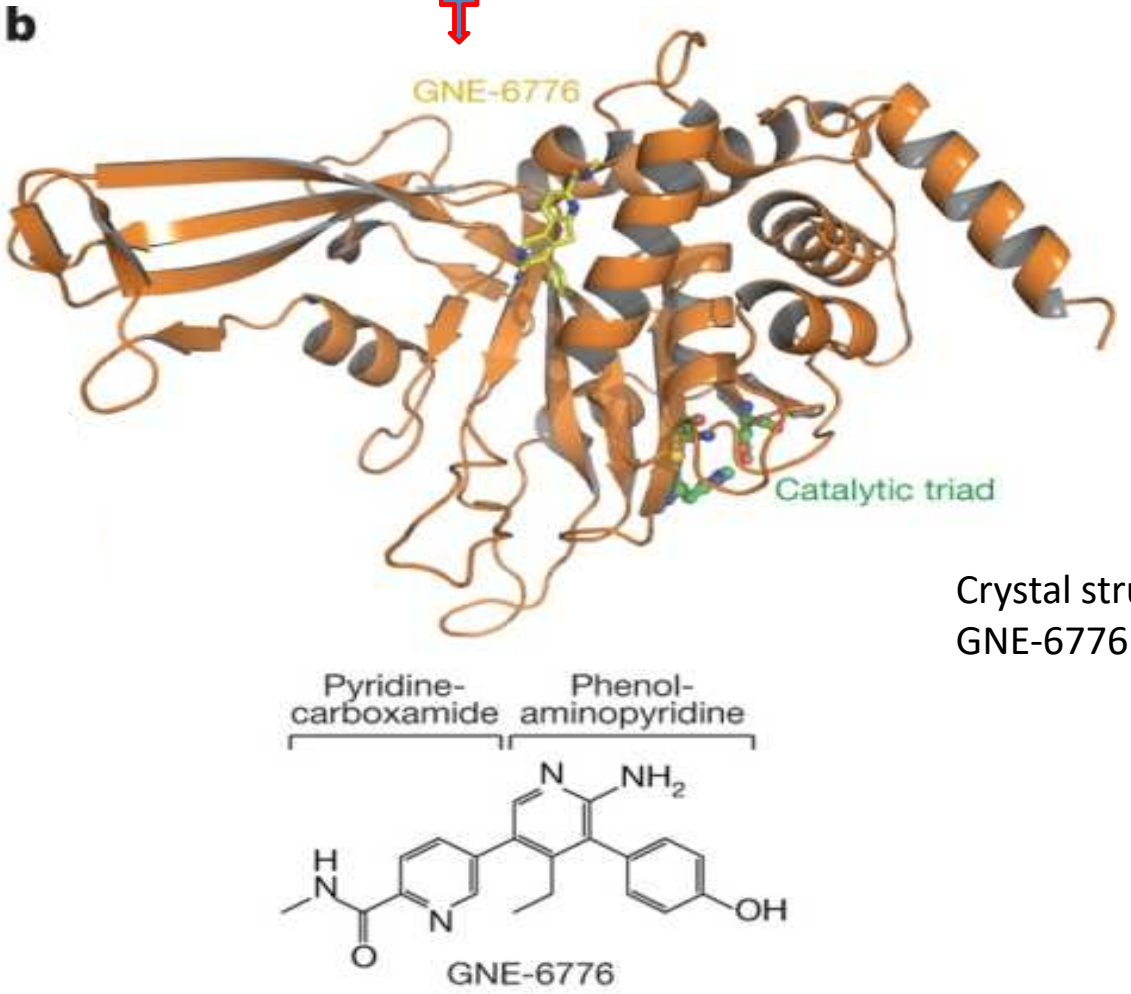
Nature. 2017;550(7677):534-538

USP7 small-molecule inhibitors interfere with ubiquitin binding

- These compounds attenuate ubiquitin binding and thus inhibit USP7 deubiquitinase activity

- These compounds induce tumour cell death and enhance cytotoxicity with chemotherapeutic agents

USP7 inhibitor binding to USP7



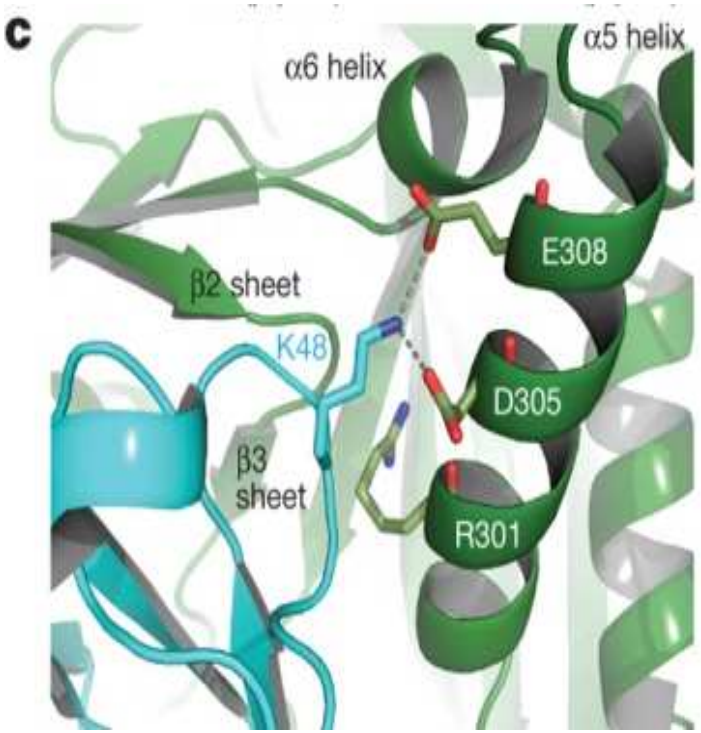
Crystal structure of USP7 catalytic domain in complex with GNE-6776

nature

L Kategaya *et al. Nature* 1–5 (2017) doi:10.1038/nature24006

USP7 inhibitors compete with ubiquitin binding to USP7

USP7 catalytic domain (green)
complexed with ubiquitin (cyan)



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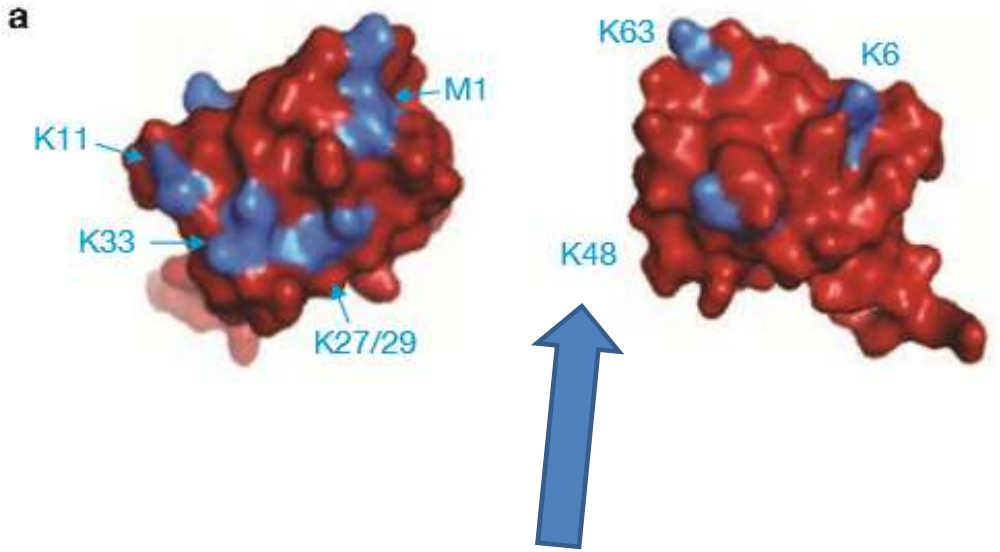
nature

Ubiquitina e poliubiquitina
struttura e funzioni alternative

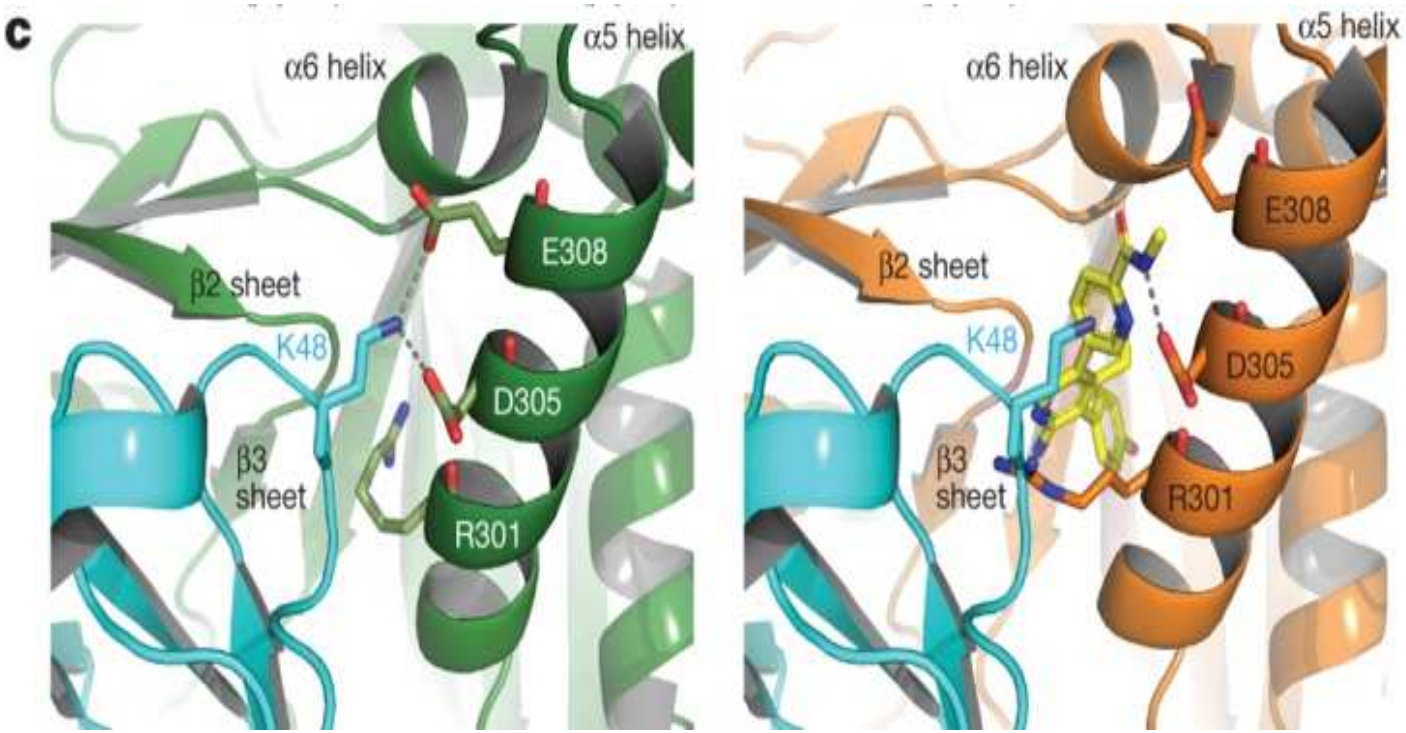


eight potential attachment sites for chain formation

USP7 inhibitors compete with ubiquitin binding to USP7

USP7 catalytic domain (green) complexed with ubiquitin (cyan)

structure of USP7 (orange cartoon) and GNE-6776)yellow sticks) with ubiquitin (cyan)

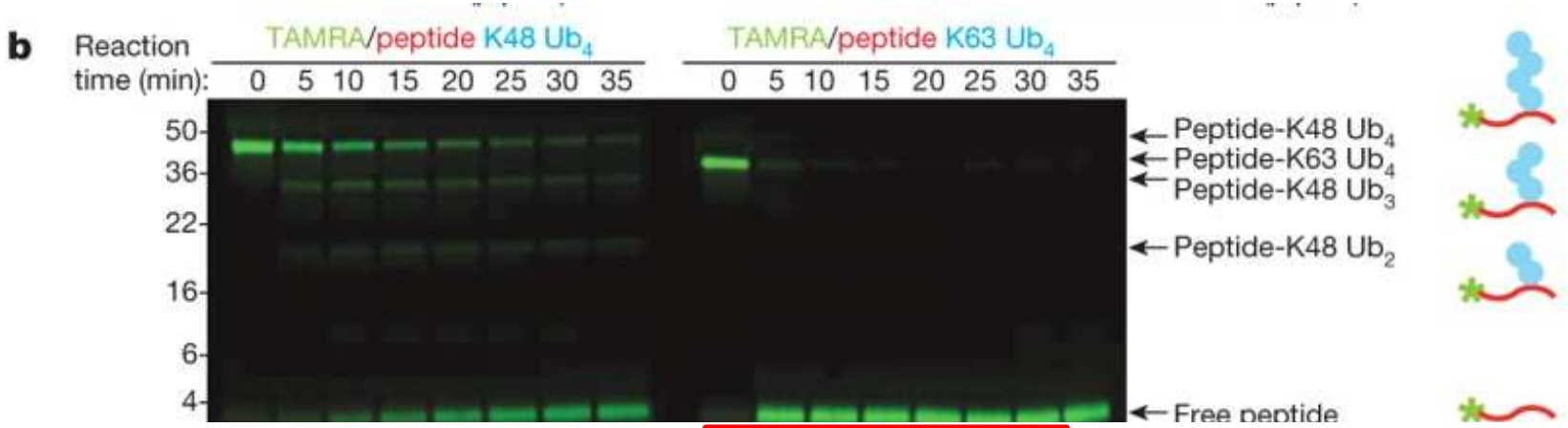


GNE-6776 steric blockade of USP7/ubiquitin-binding.

nature

L Kategaya *et al. Nature* 1–5 (2017) doi:10.1038/nature24006

USP7 preferentially binds and cleaves ubiquitin moieties with free K48 side chains



free K48 side chains

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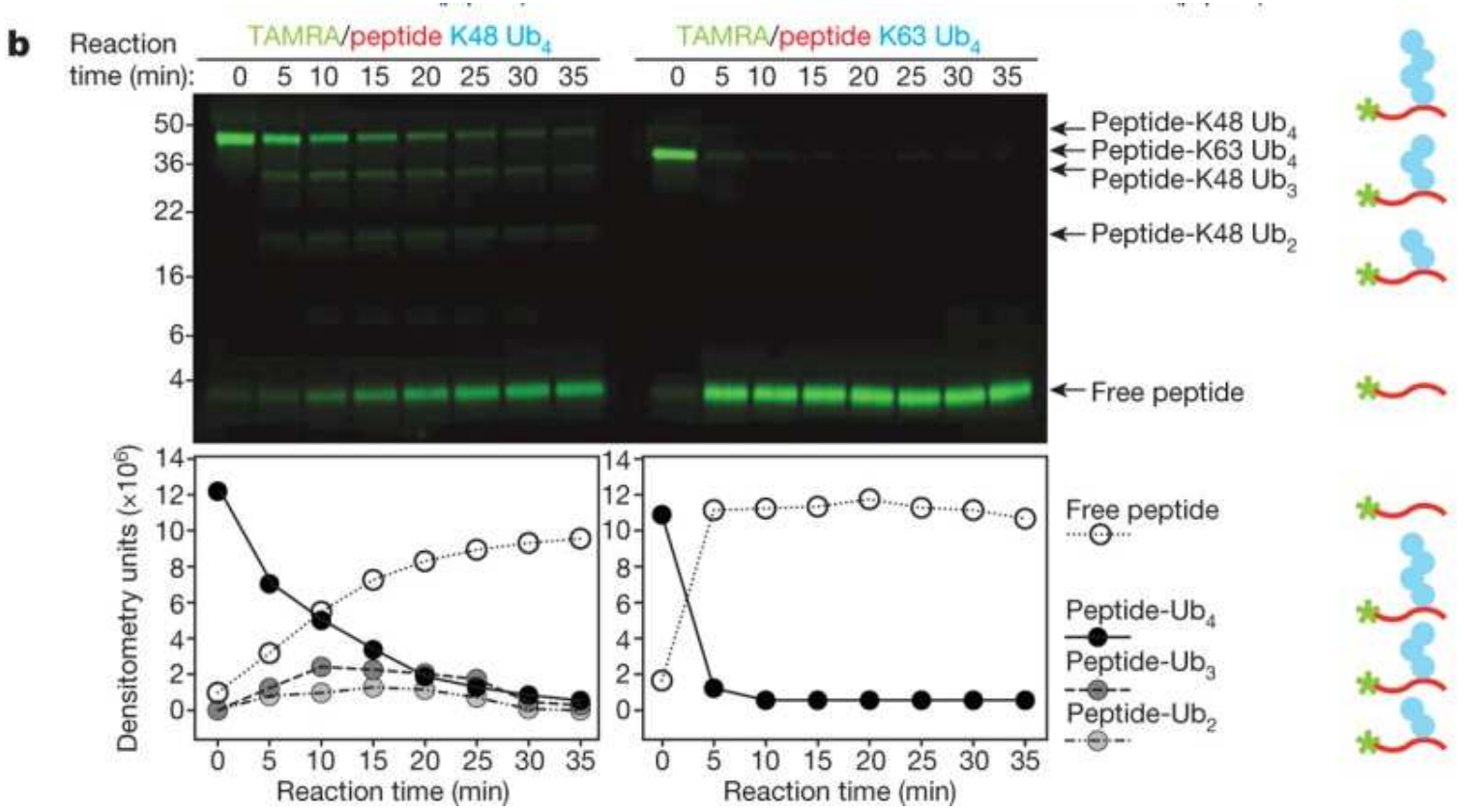

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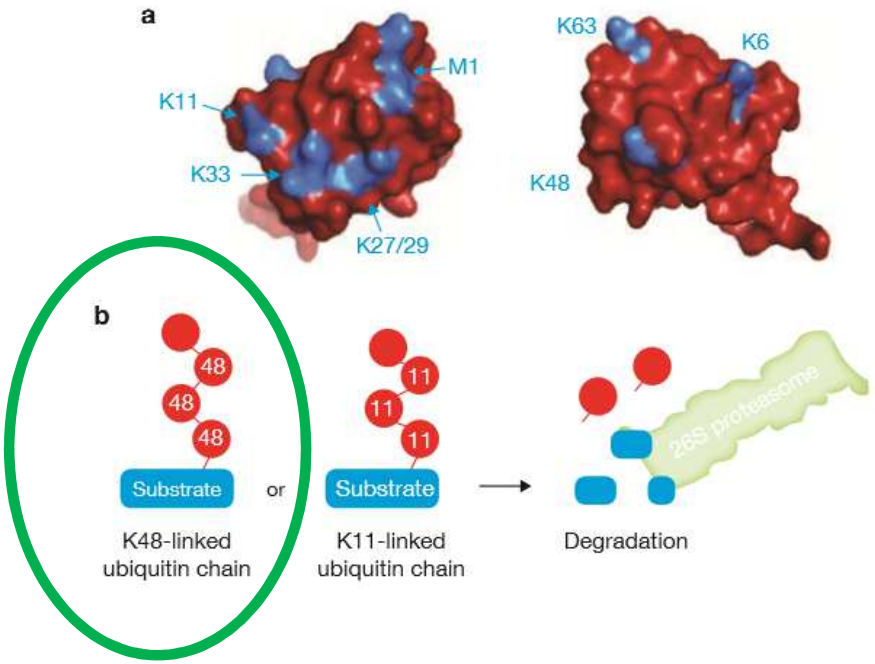
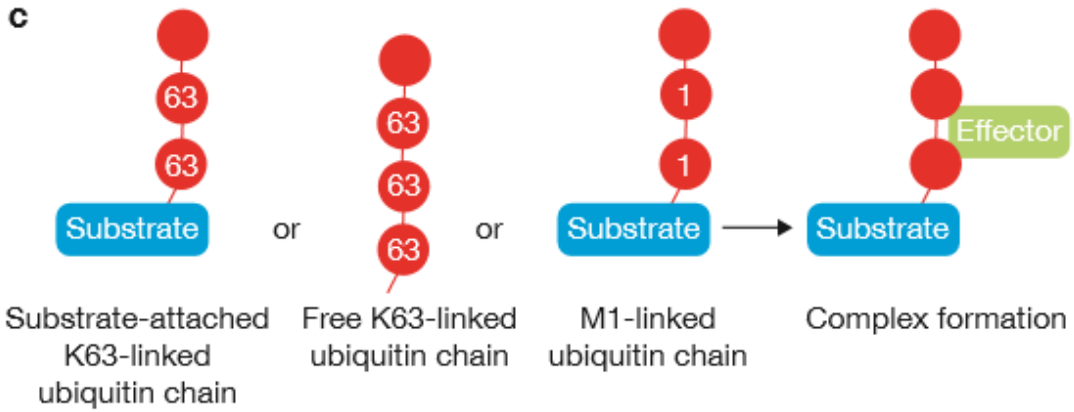
Ubiquitina e poliubiquitina
struttura e funzioni alternative



eight potential attachment sites for chain formation

Ubiquitina e poliubiquitina
struttura e funzioni alternative

K48 FREE!



GNE-6640 and GNE-6776 interact with acidic residues that mediate hydrogen-bond interactions with the ubiquitin Lys48 side chain, suggesting that USP7 preferentially interacts with and cleaves ubiquitin moieties that have free Lys48 side chains.