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Multitasking Ubiquitin Ligases in the DNA Damage Response

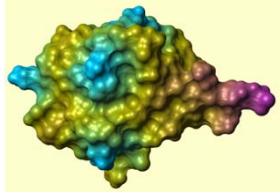
Promega tour, Karolinska Institutet, March 8, 2013

Nico Dantuma
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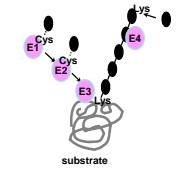
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Ubiquitin


Ubiquitin



The ubiquitin relay




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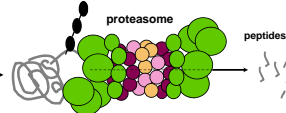
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The ubiquitin-proteasome system


Ubiquitylation



Degradation



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Different forms of ubiquitin chains

ubiquitin

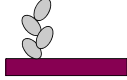
1 K K K K K K 76

6 11 27 29 33 48 63

mono-ubiquitination




poly-ubiquitination

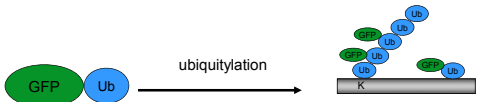


monoubiquitination	→	membrane trafficking, DNA repair, transcriptional regulation signalling (rare?)
Lys 6 polyubiquitination	→	proteasomal degradation (rare?)
Lys 29 polyubiquitination	→	proteasomal degradation
Lys 48 polyubiquitination	→	DNA repair, signalling
Lys 63 polyubiquitination	→	


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GFP-tagged ubiquitin

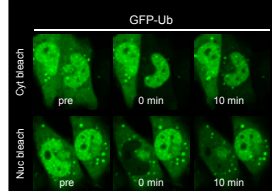


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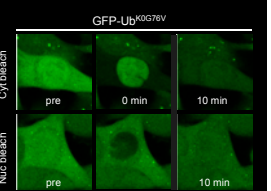
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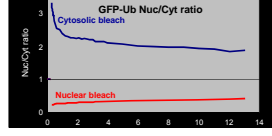
Dynamics of GFP-tagged ubiquitin

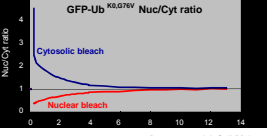
GFP-Ub



GFP-Ub^{K6376V}







Dantuma et al J. Cell Biol. 2006 Nico Dantuma March 8, 2013

Response of GFP-Ub in nucleus

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Accumulation of GFP-tagged ubiquitin over long time

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GFP-ubiquitin accumulation in bleached spot

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DNA damage-induced ubiquitylation of histone H2A

Nico Dantuma
October 8, 2010

H2A is also ubiquitylated in response to double strand breaks

Cell 2007
RNF8 Transduces the DNA-Damage Signal via Histone Ubiquitylation and Checkpoint Protein Assembly
 Michael S. Hamstra, Robert Grant, Isaac Mankin, Kay Miao, Xiaochun Yu, Michael B. Yaffe, and Andre Chen

Science 2007
Orchestration of the DNA-Damage Response by the RNF8 Ubiquitin Ligase
 Nathan K. Avkin, Steve Chapman, Shoshiko Nakata, Jukka Yankis, Richard Chikara, Khabib D. Seeman, Yoshitaka Furuta, Miquel Morales, Jan Wilmanns, Timothy B. Rossmore, Lucienne Palmiter, Douglas F. Jackson, David Dundee

Cell 2009
RNF168 Binds and Amplifies Ubiquitin Conjugates on Damaged Chromosomes to Allow Accumulation of Repair Proteins
 Christoph Helm, Yulia Kovalenko, Michael Bacher, Jennifer Pagan-Morales, Dorina Henkel-Lara, Roger Phipps, and Wolfgang Hopmann

Cell 2007
RNF8 Ubiquitylates Histones at DNA Double-Strand Breaks and Promotes Assembly of Repair Proteins
 Wen-Ming Wang, Steven Bekker-Jensen, Heesoo Fung, Frank Miska, Jan Bernik, Claudia Lukas, and Jan Lukas

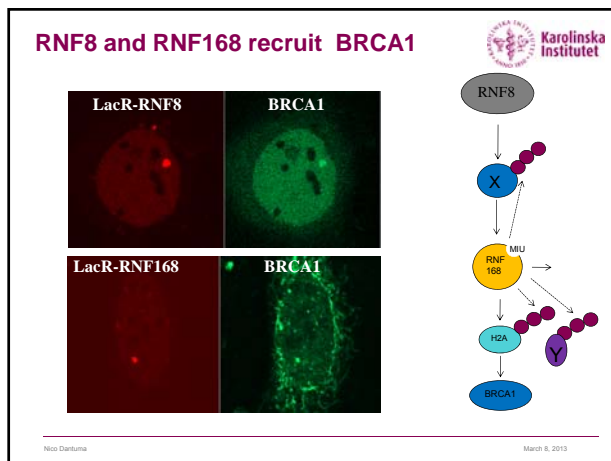
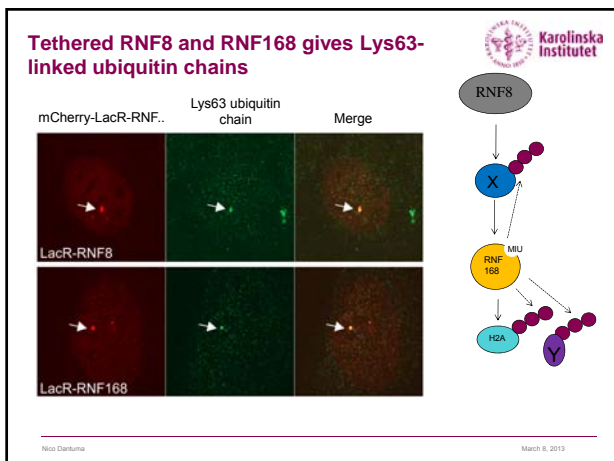
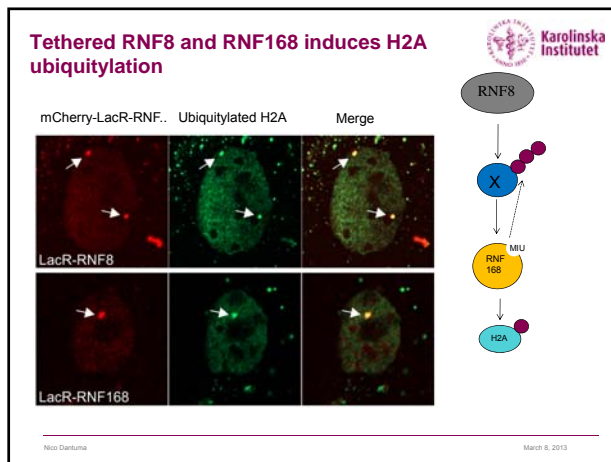
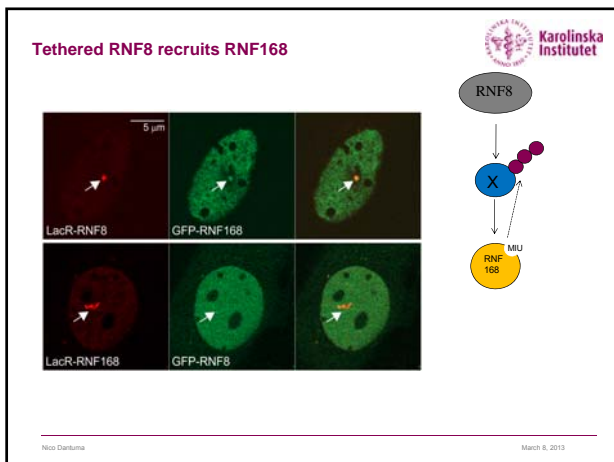
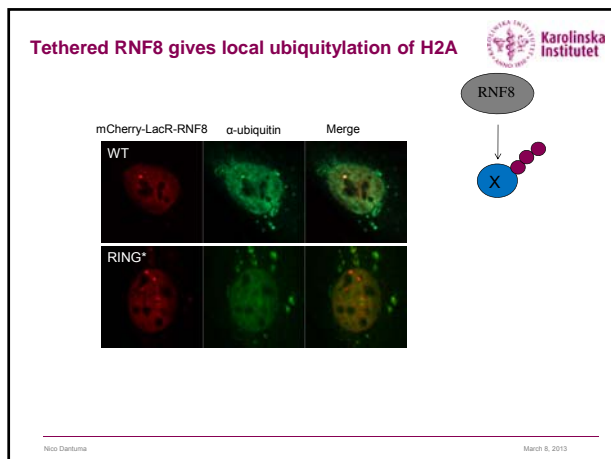
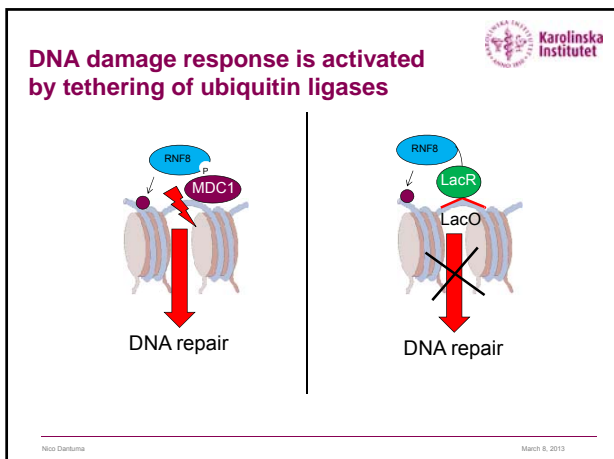
PNAS 2007
Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brc1/Brc36 complex in response to DNA damage
 Wen-Ming Wang and Stephen J. Elledge

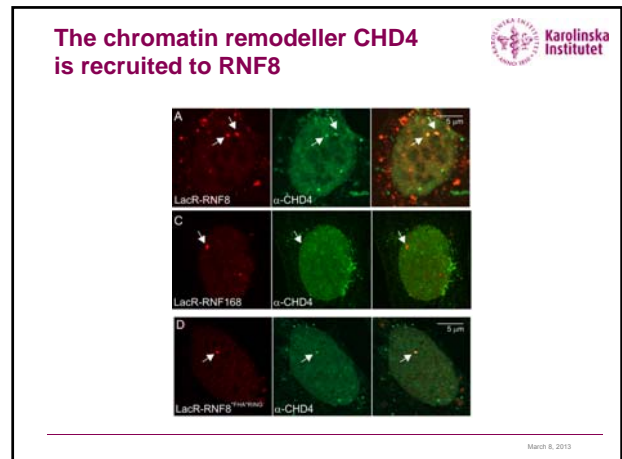
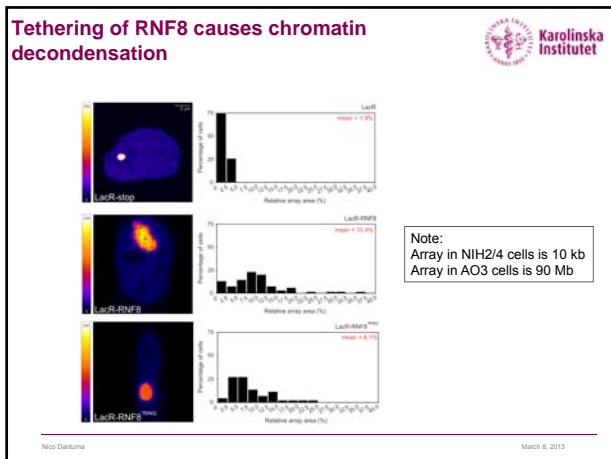
Cell 2009
The RIDDLE Syndrome Protein Mediates a Ubiquitin-Dependent Signaling Cascade at Sites of DNA Damage
 David B. Brown, Benjamin Pardo, Andy Thomson, Michael A. Di Meola, Heather R. Kelly, Edward S. Miller, Benjamin Schmitt, James Hester, Roger Chen, Megan Reynolds, Carl Hocker, and Christopher Alan Hudgins

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Recruitment BRCA1 and 53BP1

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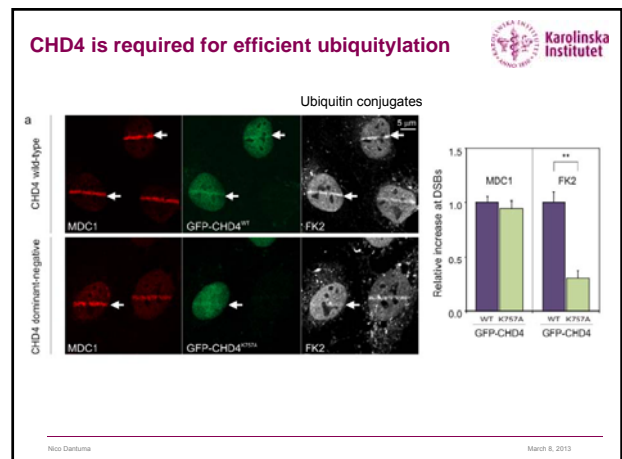
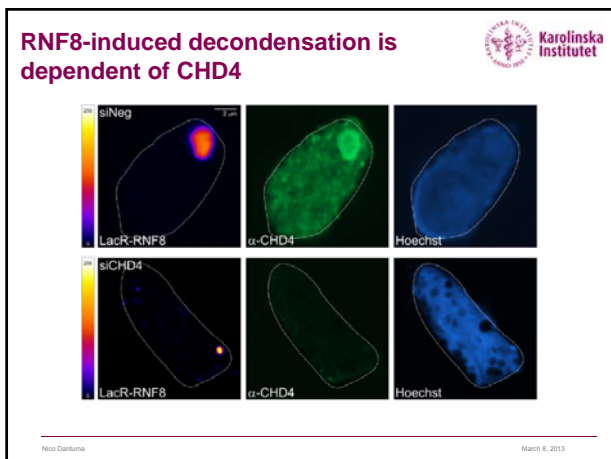
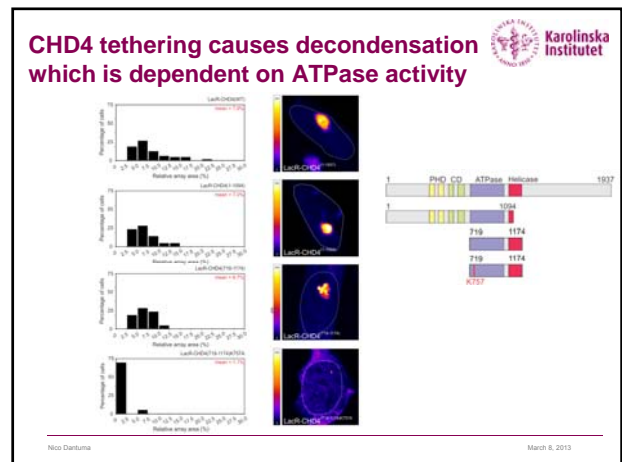


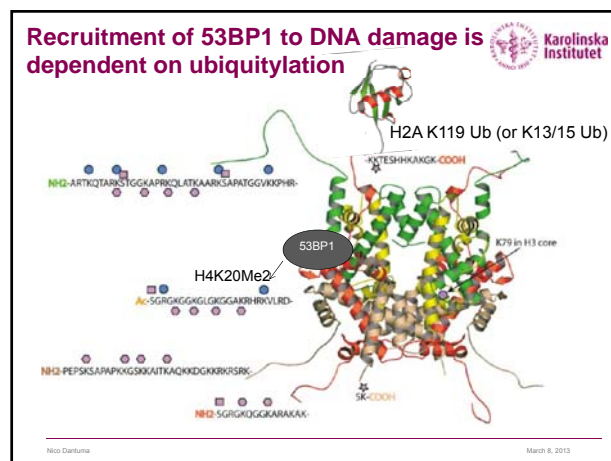
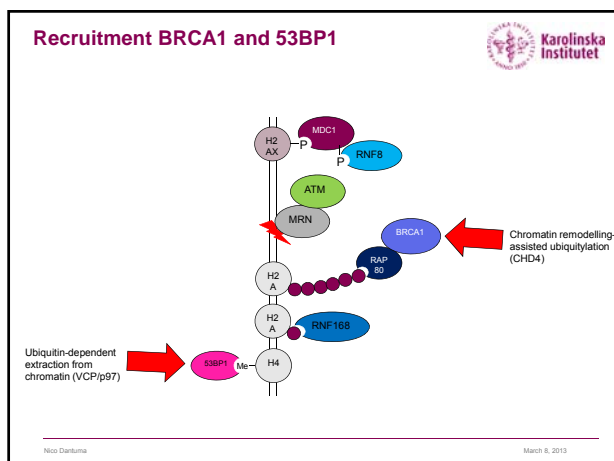
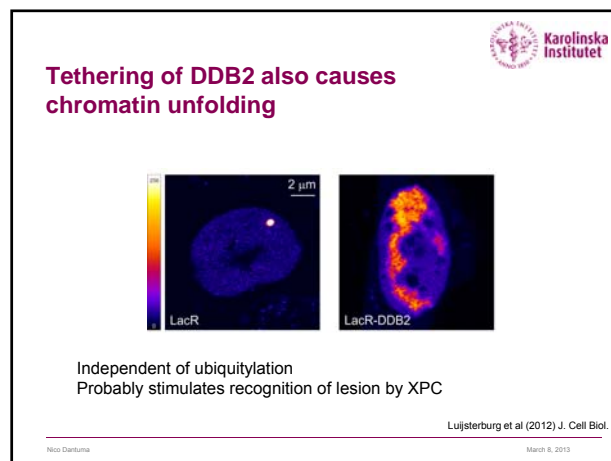
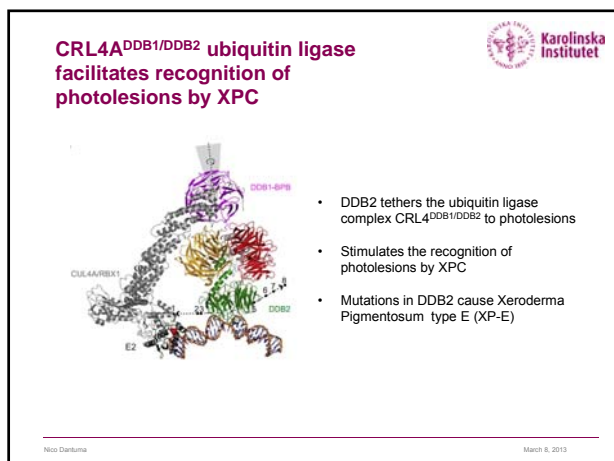
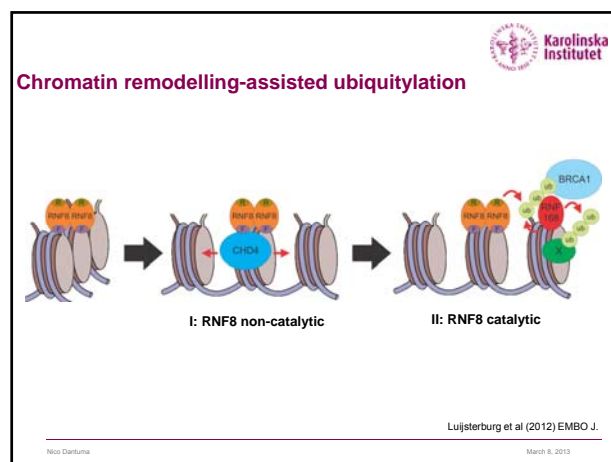
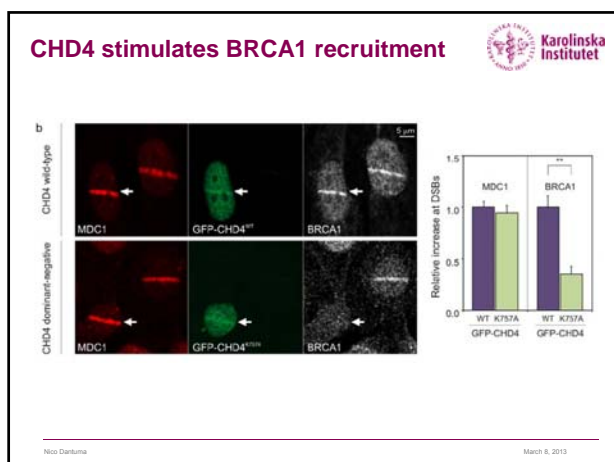


CHD4 is part of NuRD complex

- ATPase domain (SNF2 family)
- In NuRD complex: CHD4, histone deacetylates HDAC1 and 2 and histone demethylase LSD1
- Linked to repression and heterochromatin formation

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RNF8 recruits the ubiquitin-selective AAA-ATPase VCP/p97/Cdc48

- Dependent on ubiquitin ligase activity
- Specific for RNF8

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Valosin-containing protein (VCP)p97/Cdc48

Huytin et al 2003 J. Struc. Biol.

- AAA-ATPase
- Binds ubiquitin
- Critical for proteasomal degradation of ER substrates (ERAD)
- Unfoldase/segregase activity

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Kinetics of VCP recruitment

Nico Dantuma November 15, 2012 33

VCP/p97 is required for efficient recruitment of 53BP1

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Hypothesis

VCP's involvement explain the need for ubiquitin in 53BP1 recruitment.

But how does VCP facilitate binding of 53BP1?

We know that VCP segregates proteins

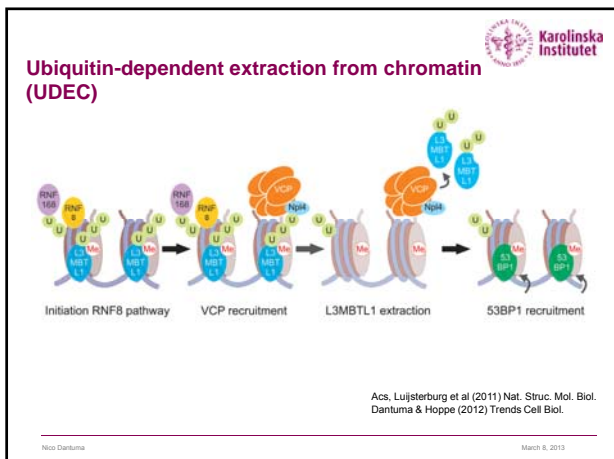
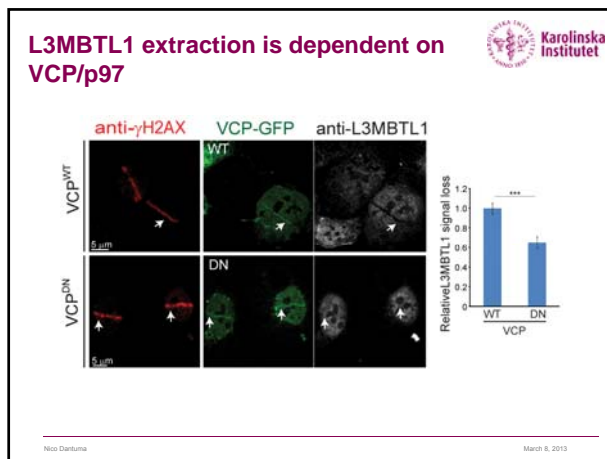
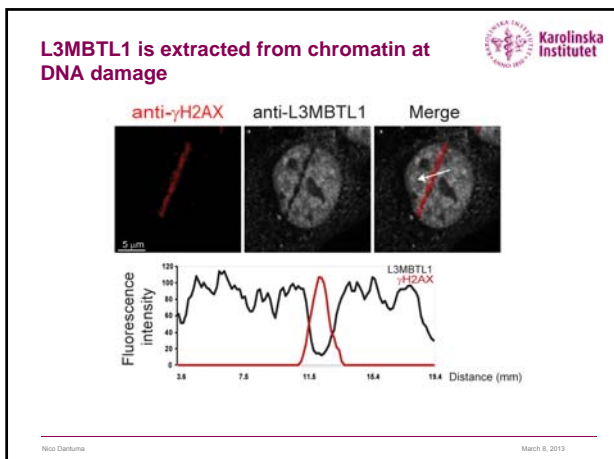
Hypothesis: VCP facilitates binding of 53BP1 to H4K20me2 by extracting a protein that occupies the same histone mark.

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53BP1 and L3MBTL1 bind to same H4K20me2

From Min et al (2007) Nat. Struc. Mol. Biol.

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Conclusions

- RNF8 recruits CHD4 independent of its ubiquitin ligase activity
- CHD4 decondensates chromatin and stimulates ubiquitylation
- RNF8 recruits VCP/p97 dependent of its ubiquitin ligase activity
- VCP/p97 extracts L3MBTL1 and facilitates 53BP1 recruitment

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