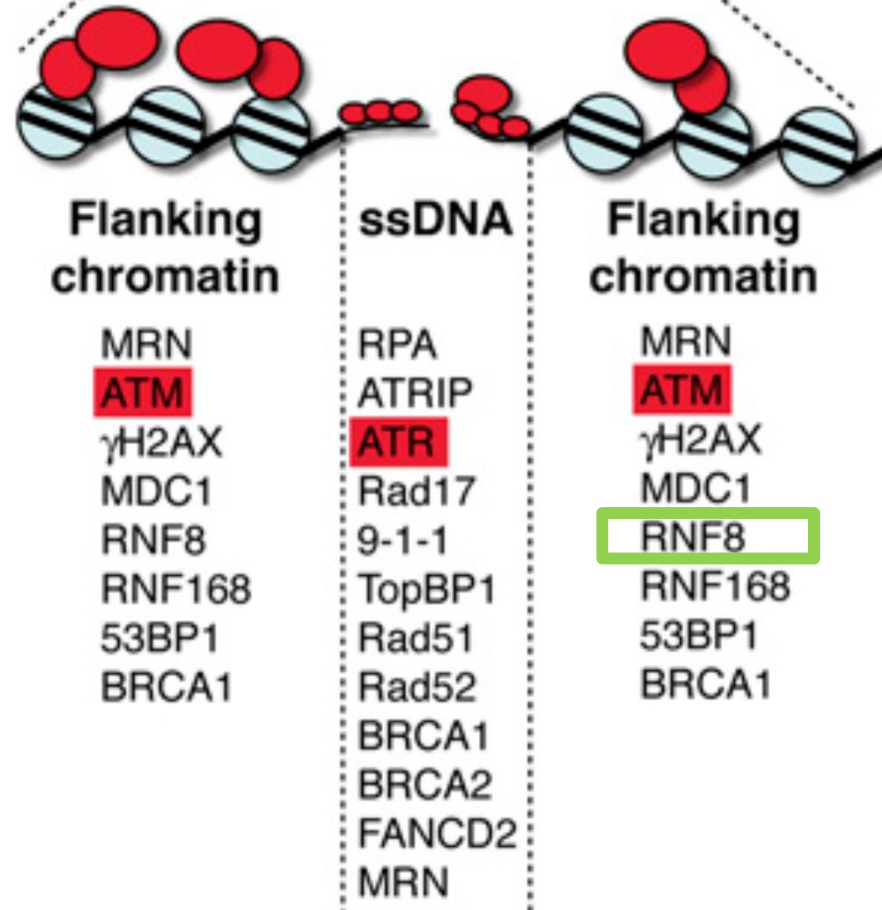
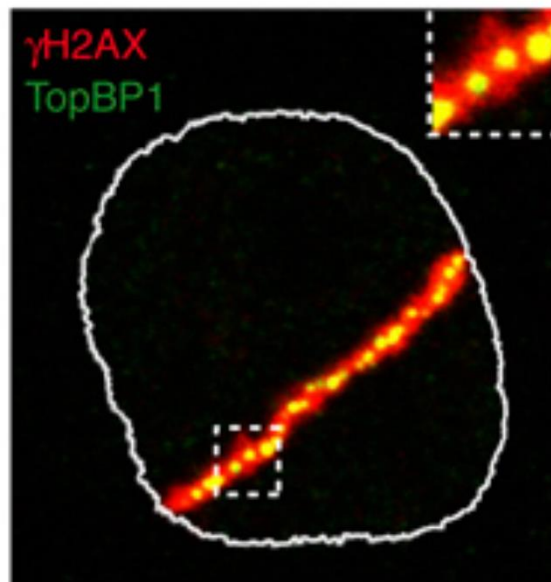


B REGIONAL DISTRIBUTION

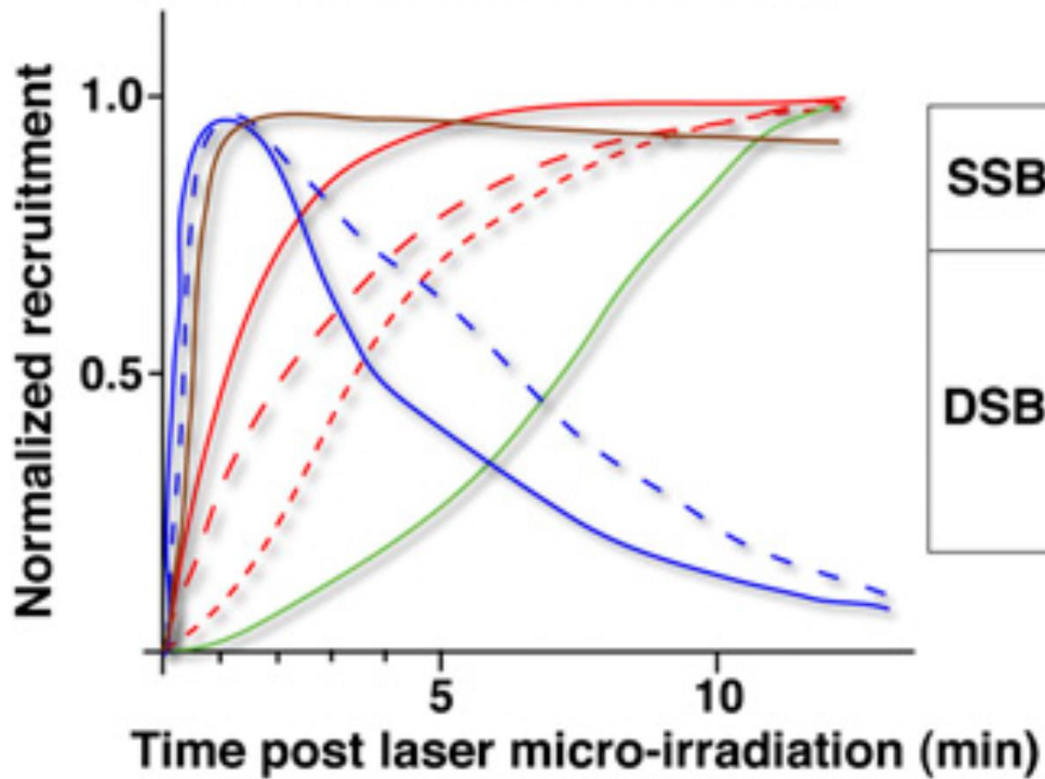


RNF8

Temporal regulation of DDR protein accumulation at DNA breaks

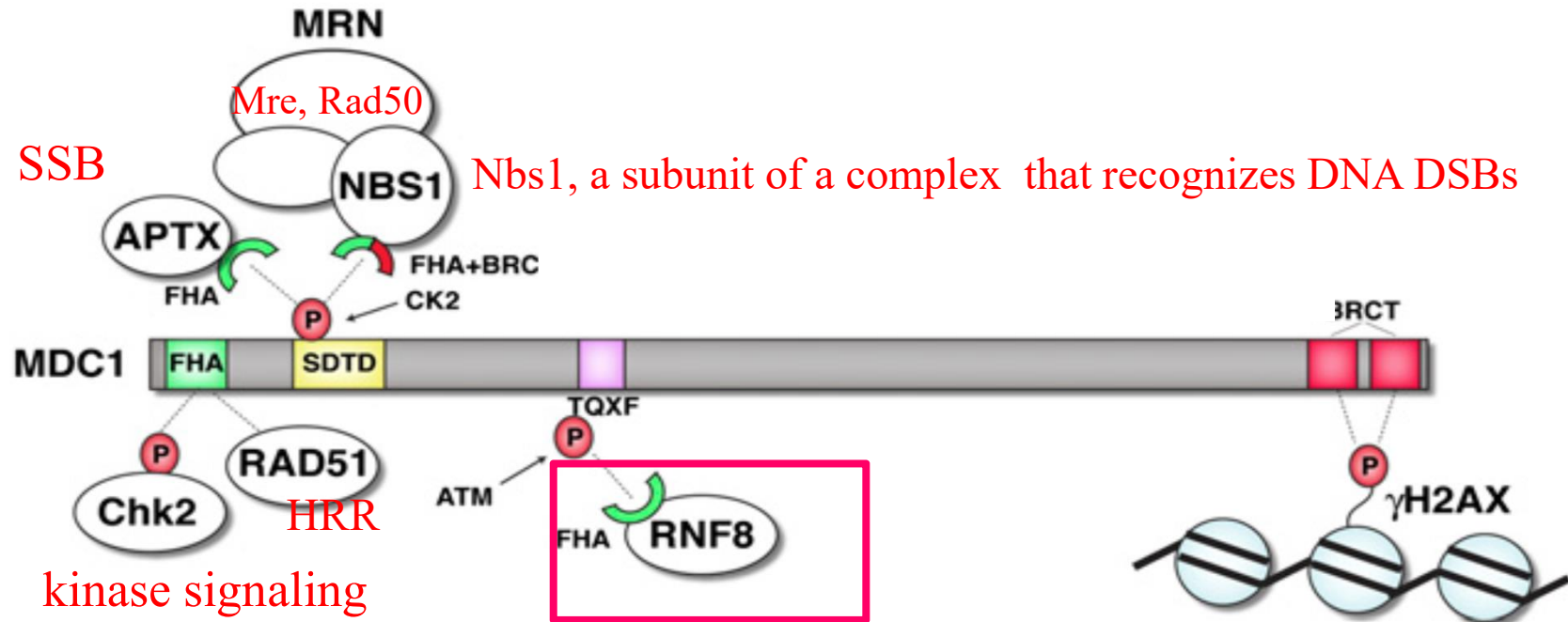
A

RECRUITMENT KINETICS

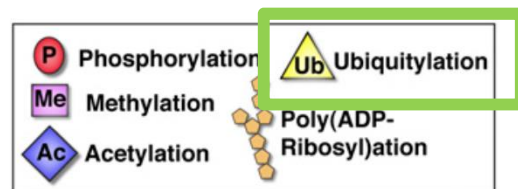
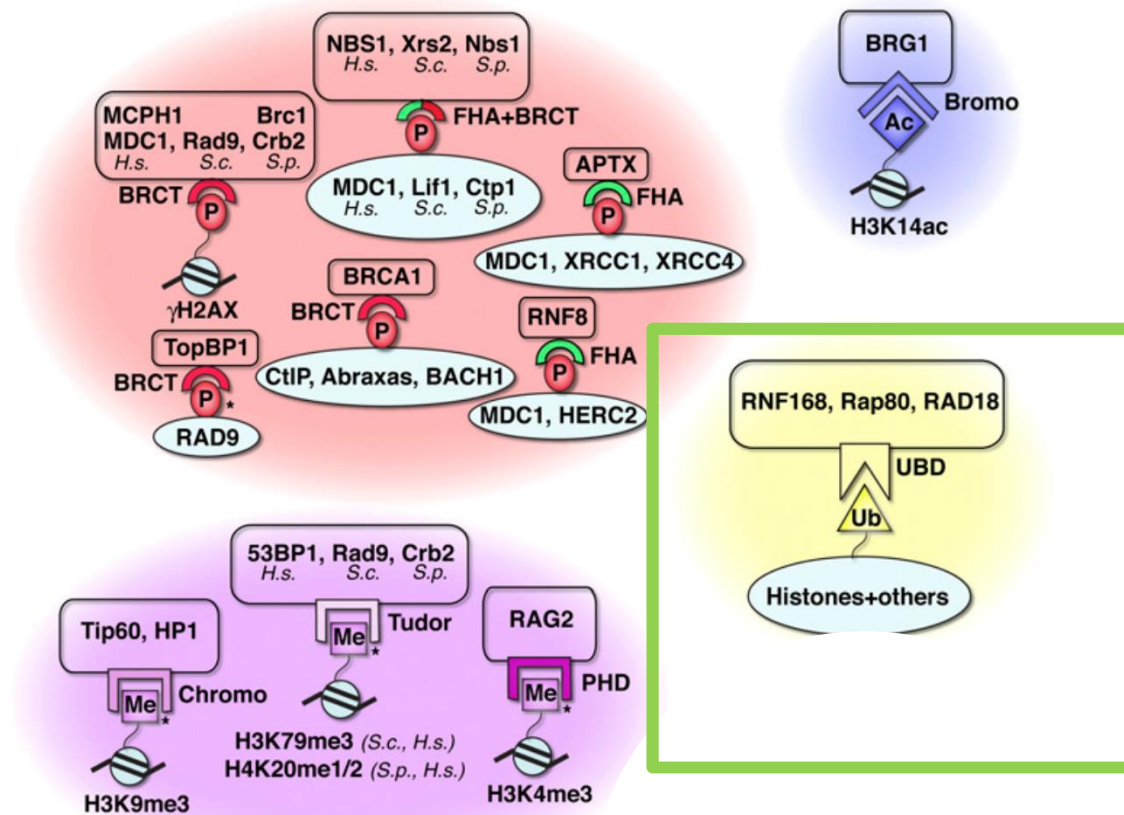


Proteine piattaforma

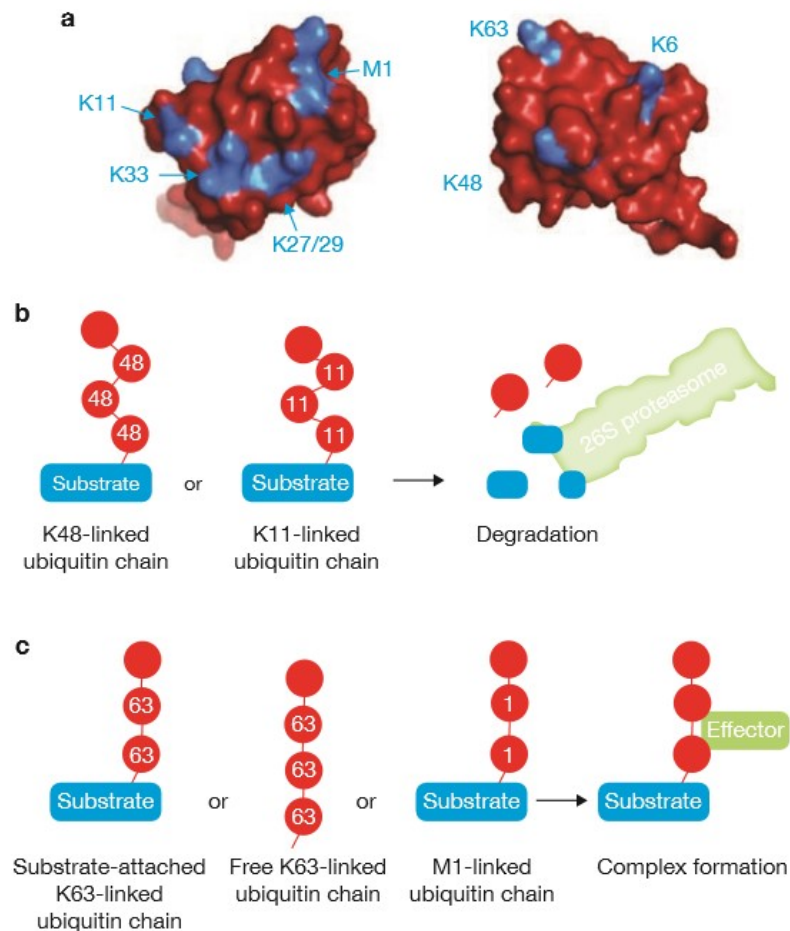
Damage signaling



Specialized binding modules for recognition of post-translational modifications (PTMs) at DNA breaks.



Ubiquitina e poliubiquitina struttura e funzioni alternative

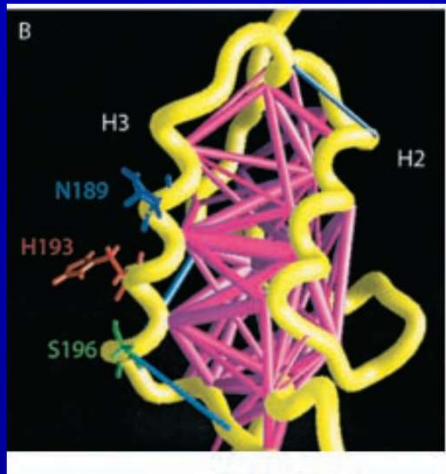


Domain architecture of RNF8



Forkhead associated(FHA) domain
bind phosphothreonine-bearing epitopes
interaction with ATM-phosphorylated MDC1.

Ubiquitin ligase activity

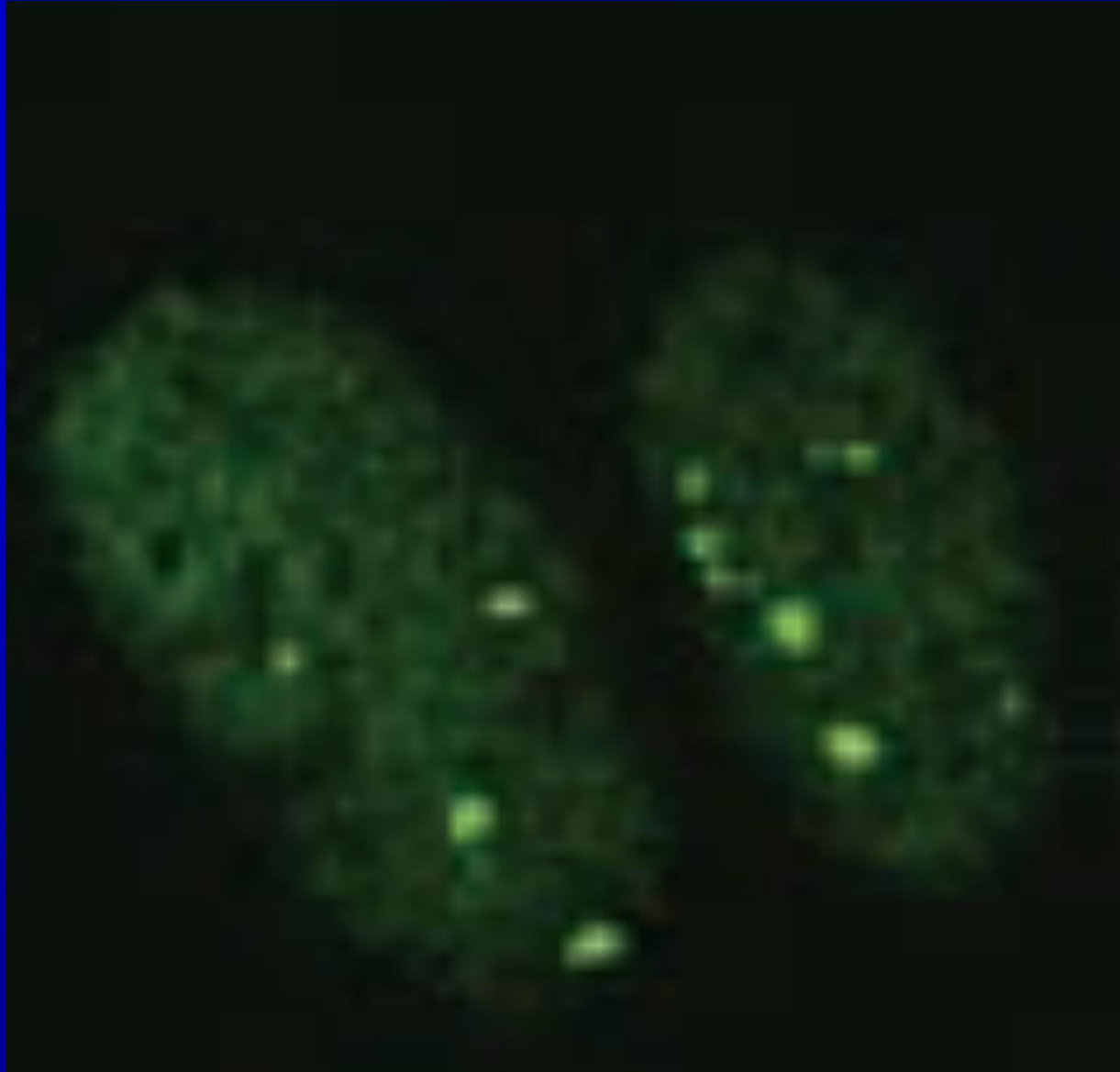


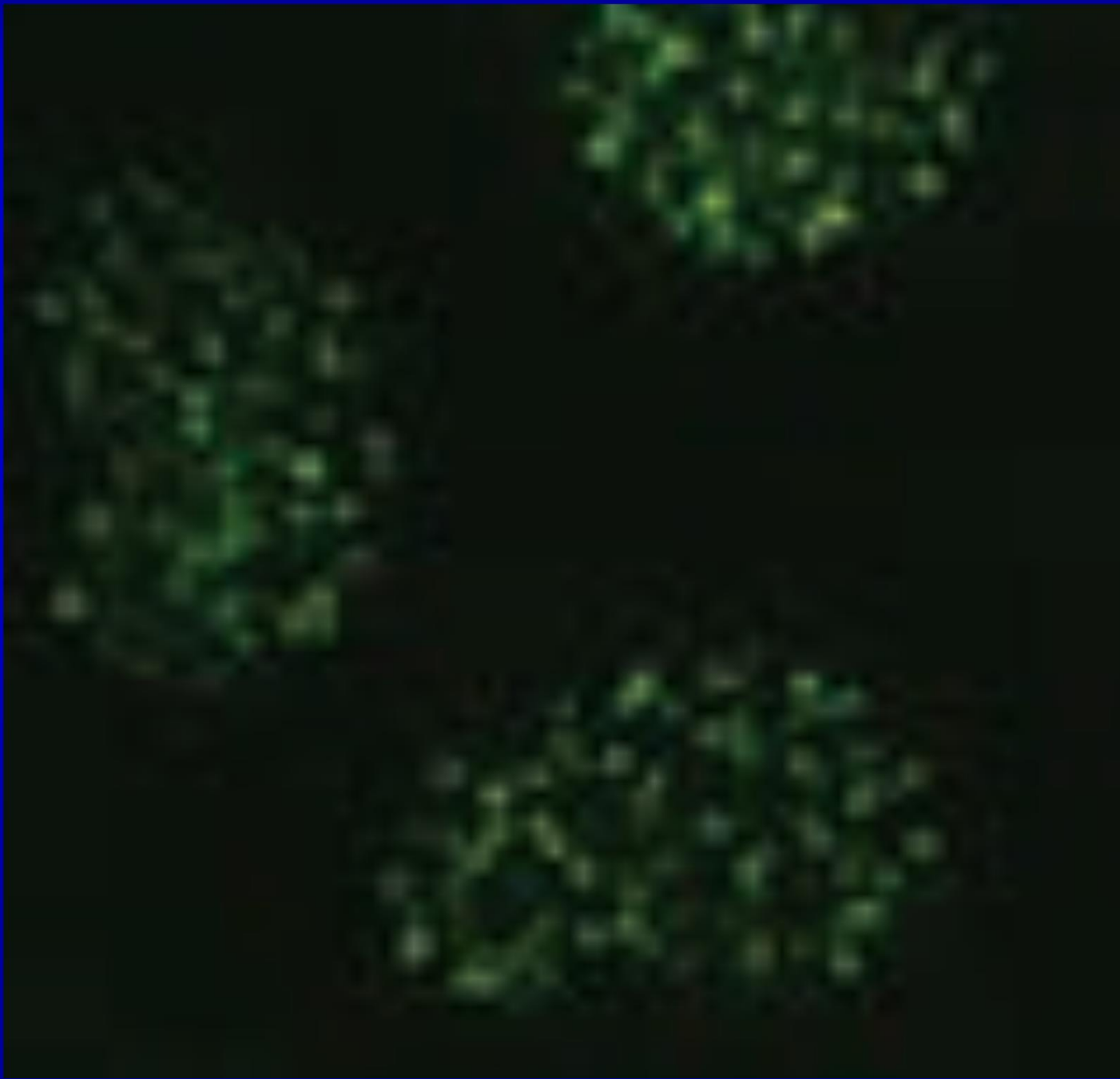
Forkhead domain

- FHA-(R42A) and
- RING finger (C406s) mutants.

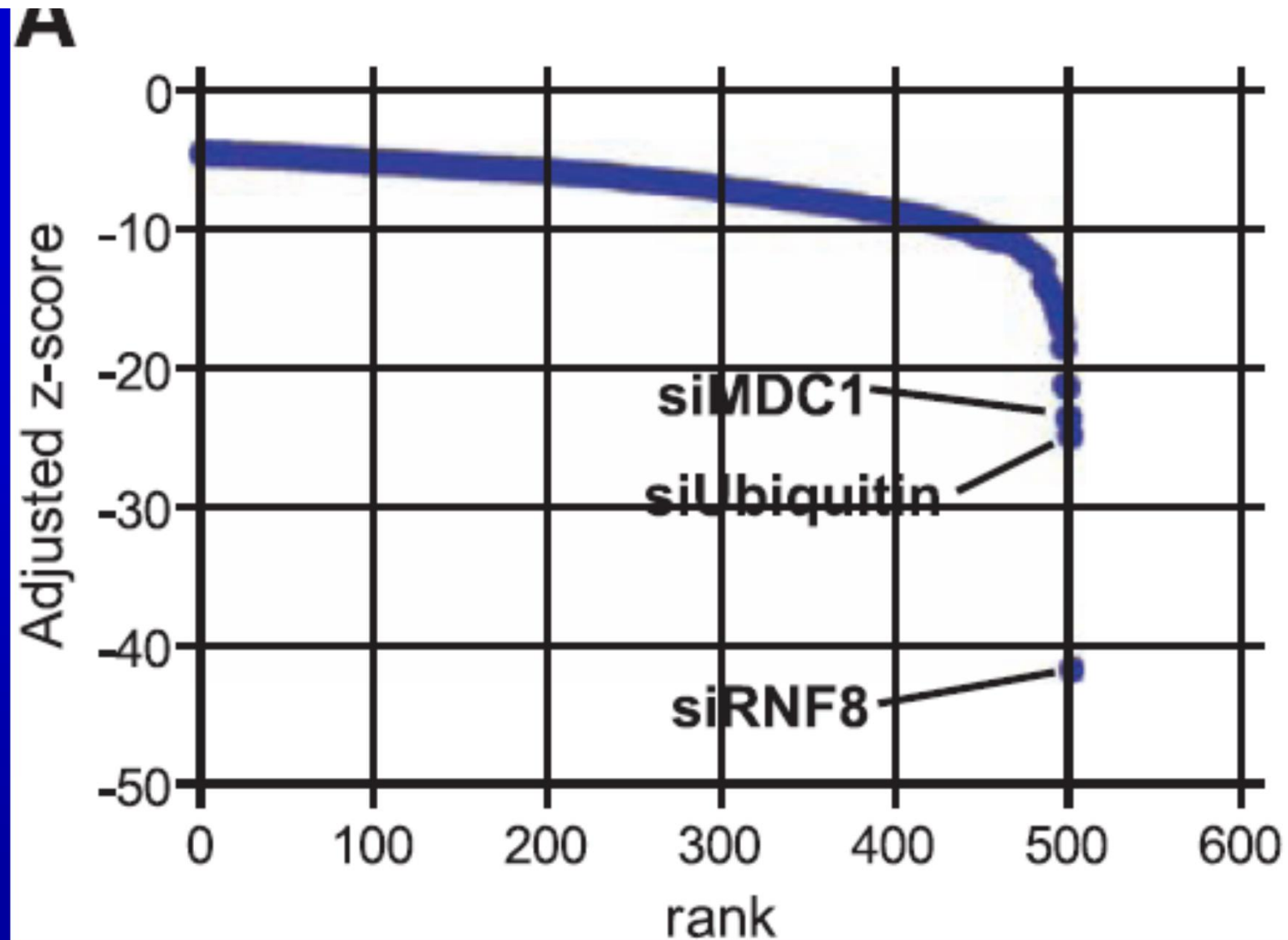
Orchestration of the DNA-Damage Response by the RNF8 Ubiquitin Ligase (Nadine Science Feb2008)

- Cells respond to DSBs by recruiting the DNA-damage mediator protein MDC1, the p53-binding protein 1 (53BP1) to sites of damaged DNA.
- 53BP1 is an established player- important role in modulating chromatin structure surrounding the break site- in the cellular response to DNA damage and is a canonical component of ionizing-radiation induced foci (IRIF)



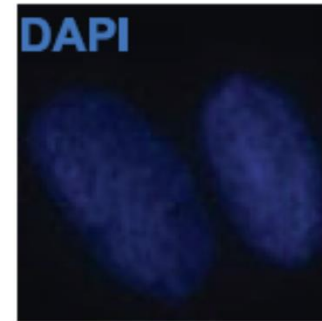
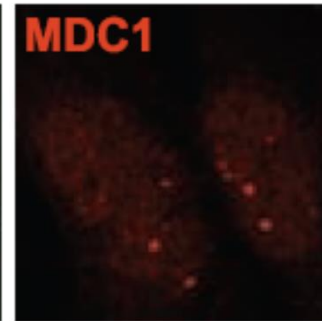
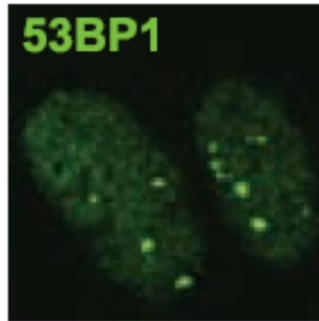


Ranking by z score of 500 siRNAs giving the least 53BP1 foci from a siRNA screen

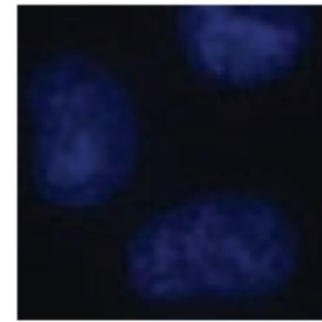
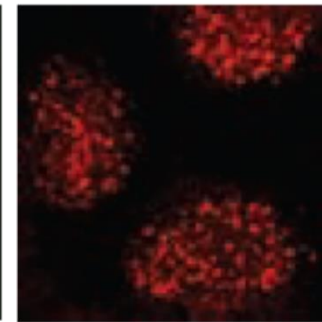
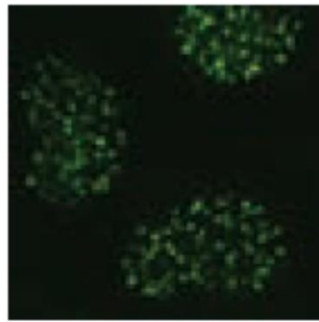


B

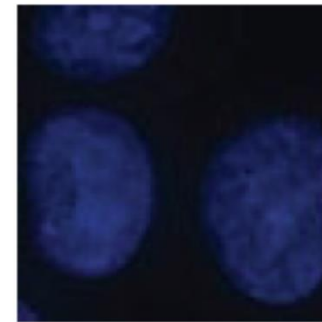
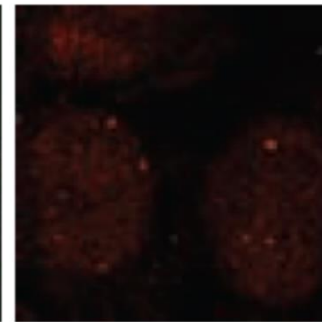
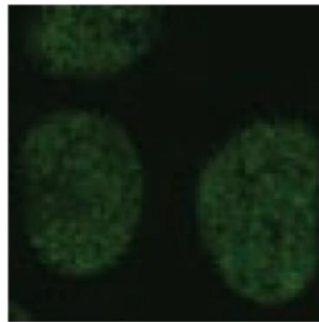
siCTRL
No IR



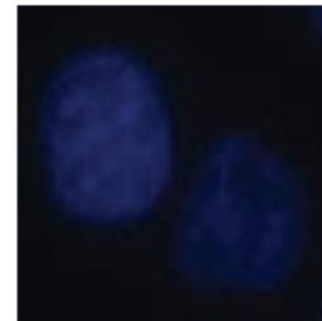
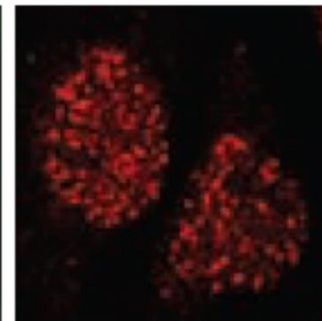
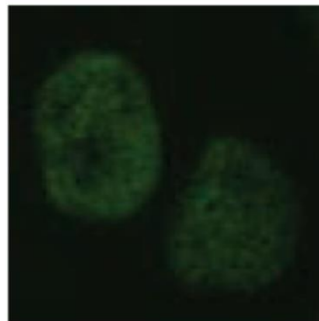
siCTRL
10Gy

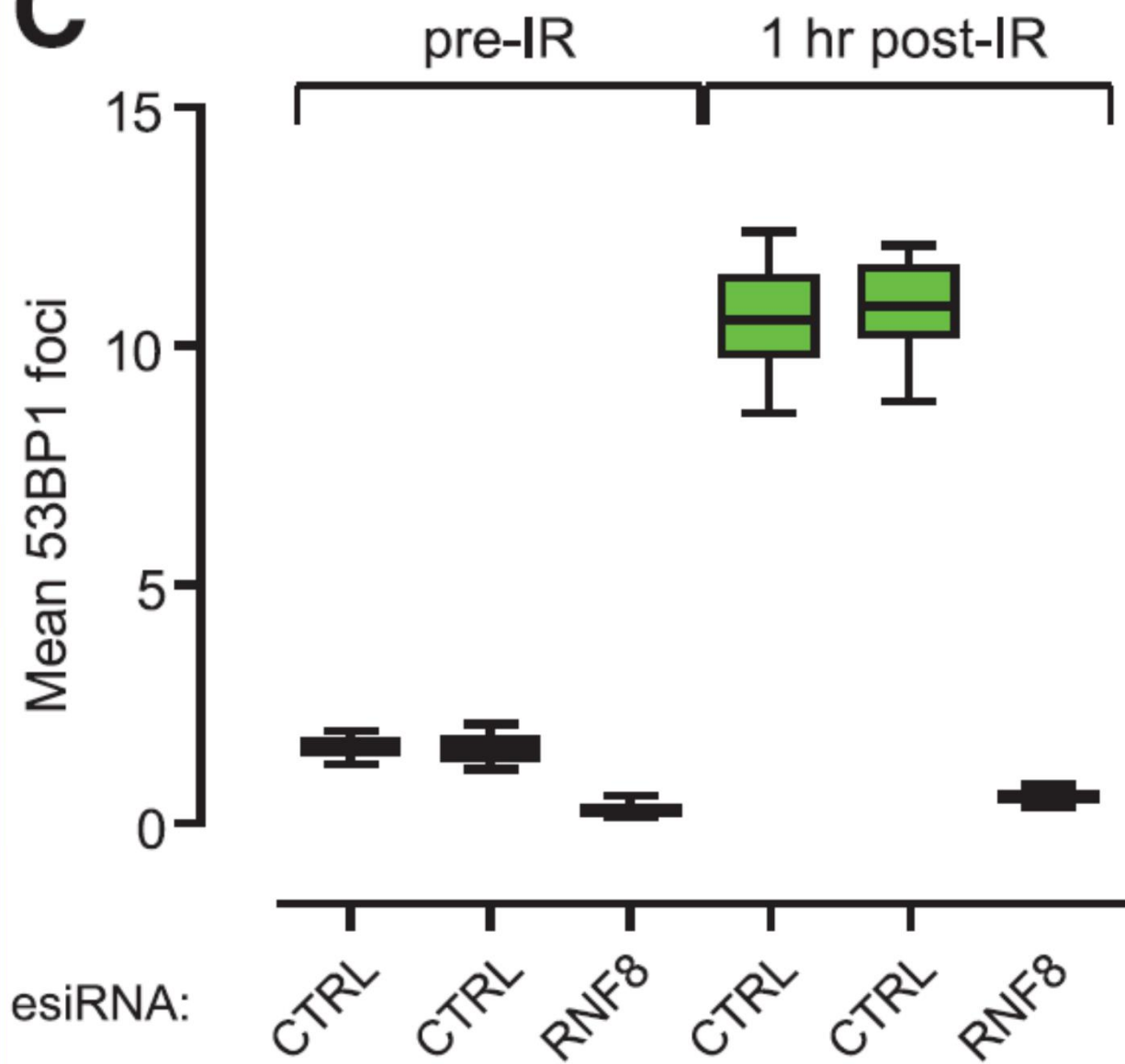


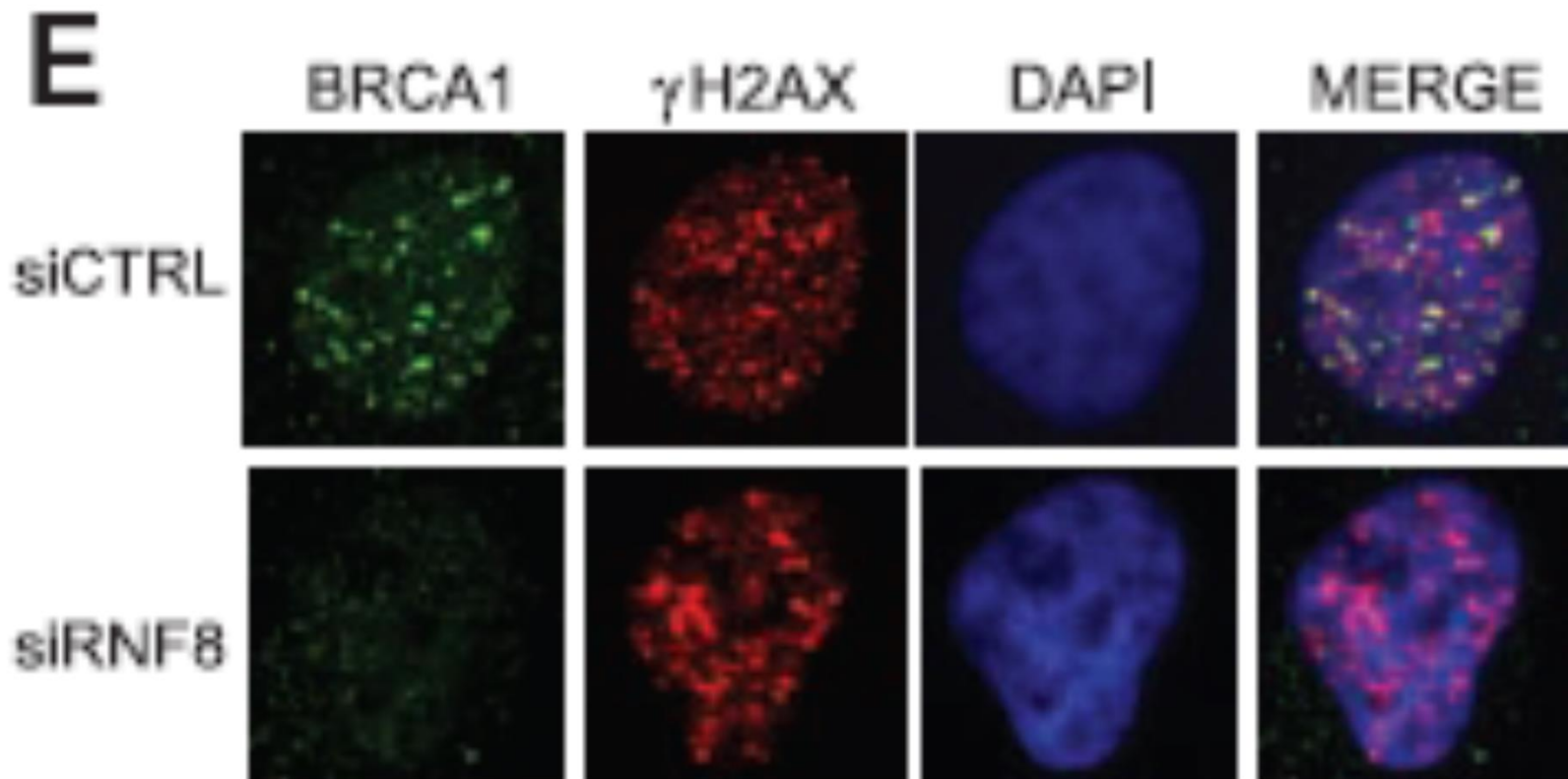
siRNF8
No IR



siRNF8
10Gy



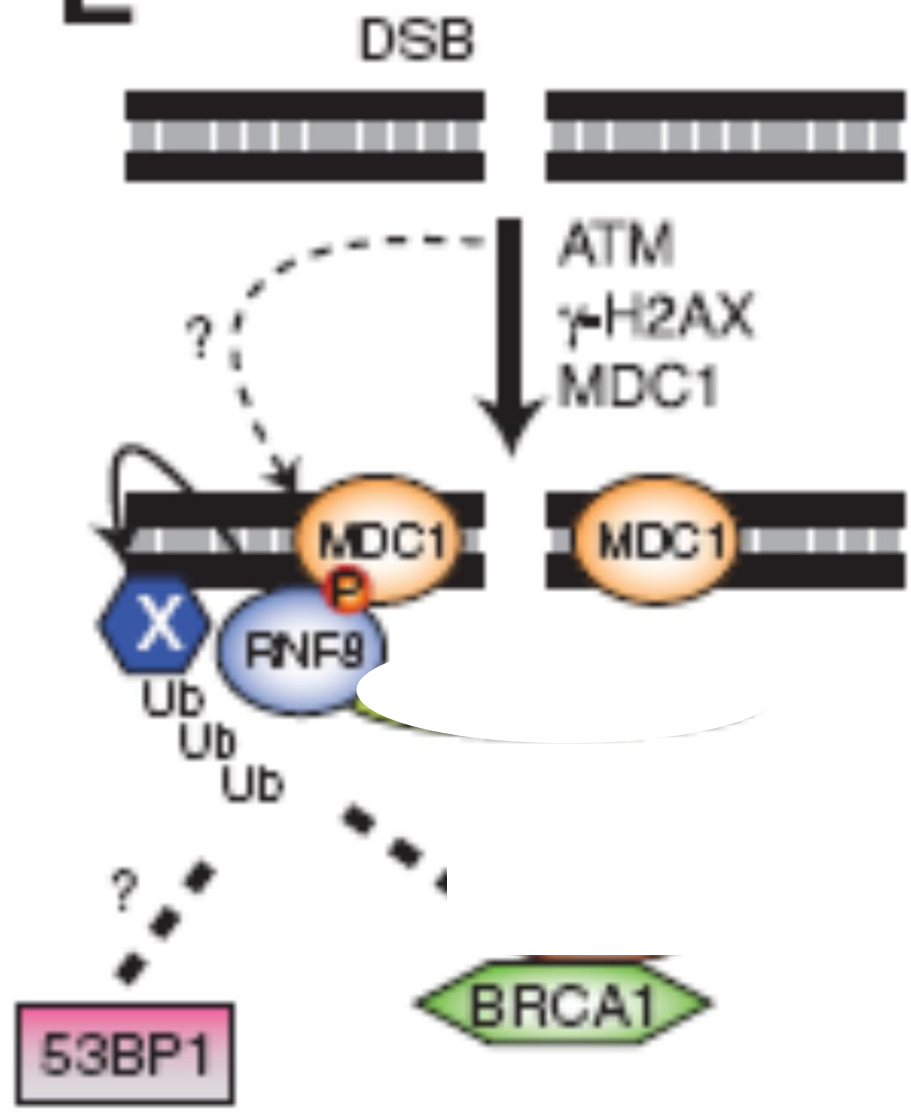
C



Irradiated (10 Gy) HeLa cells transfected with the indicated siRNAs were stained with antibodies to γ H2AX, BRCA1

- Together with the E2-conjugating enzyme UBC13, RNF8 catalyzes histone H2AX Lys 63-linked ubiquitination in response to DSBs.
- Ubiquitinated histone H2AX tethers DNA repair factors including RNF8, p53-binding protein 1 (53BP1), and BRCA1 to the DSB sites, thereby initiating homologous recombination (HR)

E



Orchestration of the DNA-Damage Response by the RNF8 Ubiquitin Ligase (Nadine Science Feb2008)

- Cells respond to DSBs by recruiting the DNA-damage mediator protein MDC1, the p53-binding protein 1 (53BP1), and the breast cancer susceptibility protein BRCA1 to sites of damaged DNA.
- **The ubiquitin ligase RNF8 mediates ubiquitin conjugation and 53BP1 and BRCA1 focal accumulation at sites of DNA lesions.**

MDC1 recruits RNF8 through phosphodependent interactions between the RNF8 forkhead-associated domain and motifs in MDC1 that are phosphorylated by the DNA-damage activated protein kinase ataxia telangiectasia mutated (ATM).

Depletion of the E2 enzyme UBC13 impairs 53BP1 recruitment to sites of damage, which suggests that it cooperates with RNF8.

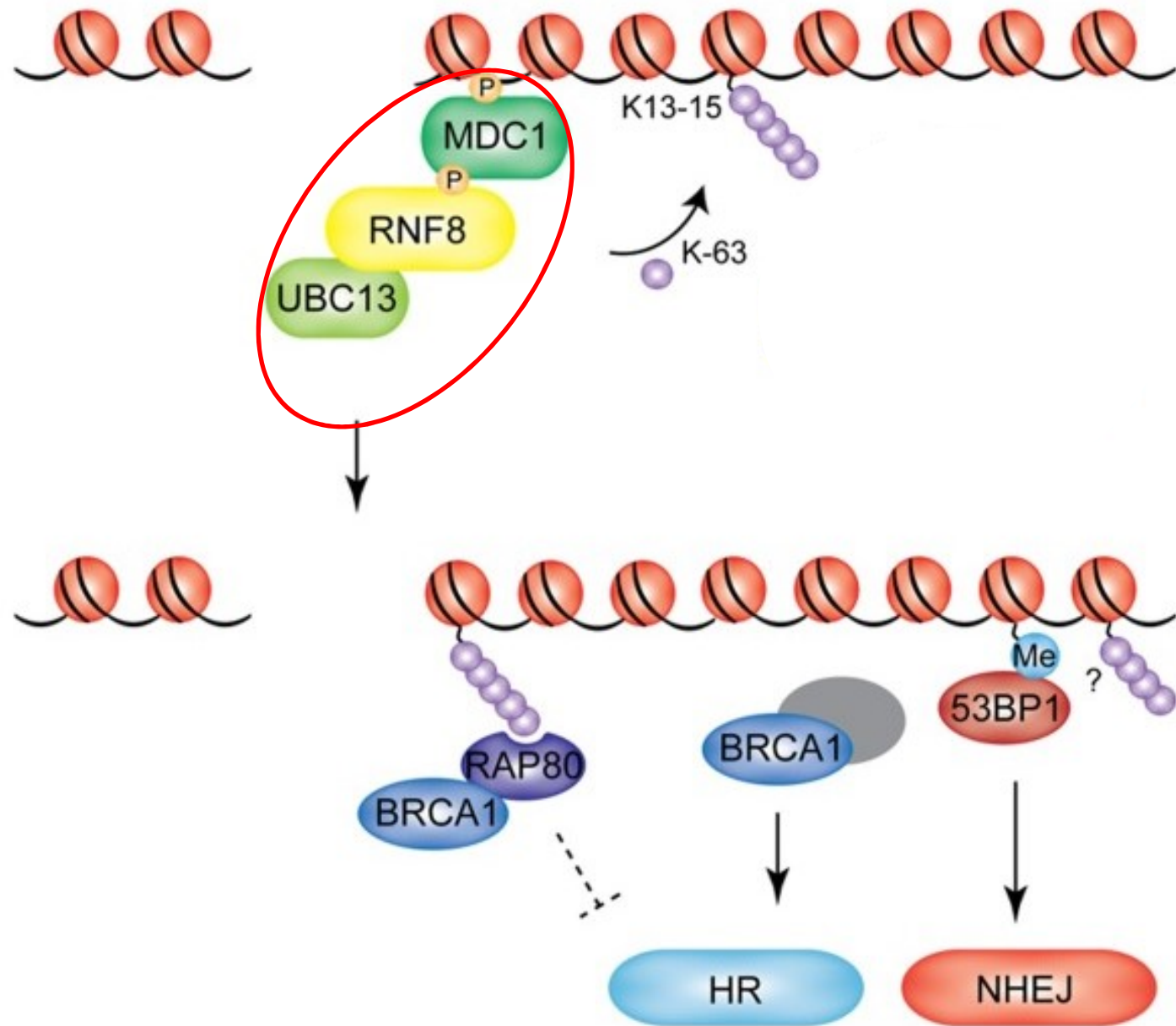
RNF8 promotes the G2/M DNA damage checkpoint and resistance to ionizing radiation.

the DNA-damage response is orchestrated by ATM-dependent phosphorylation of MDC1 and RNF8-mediated ubiquitination.

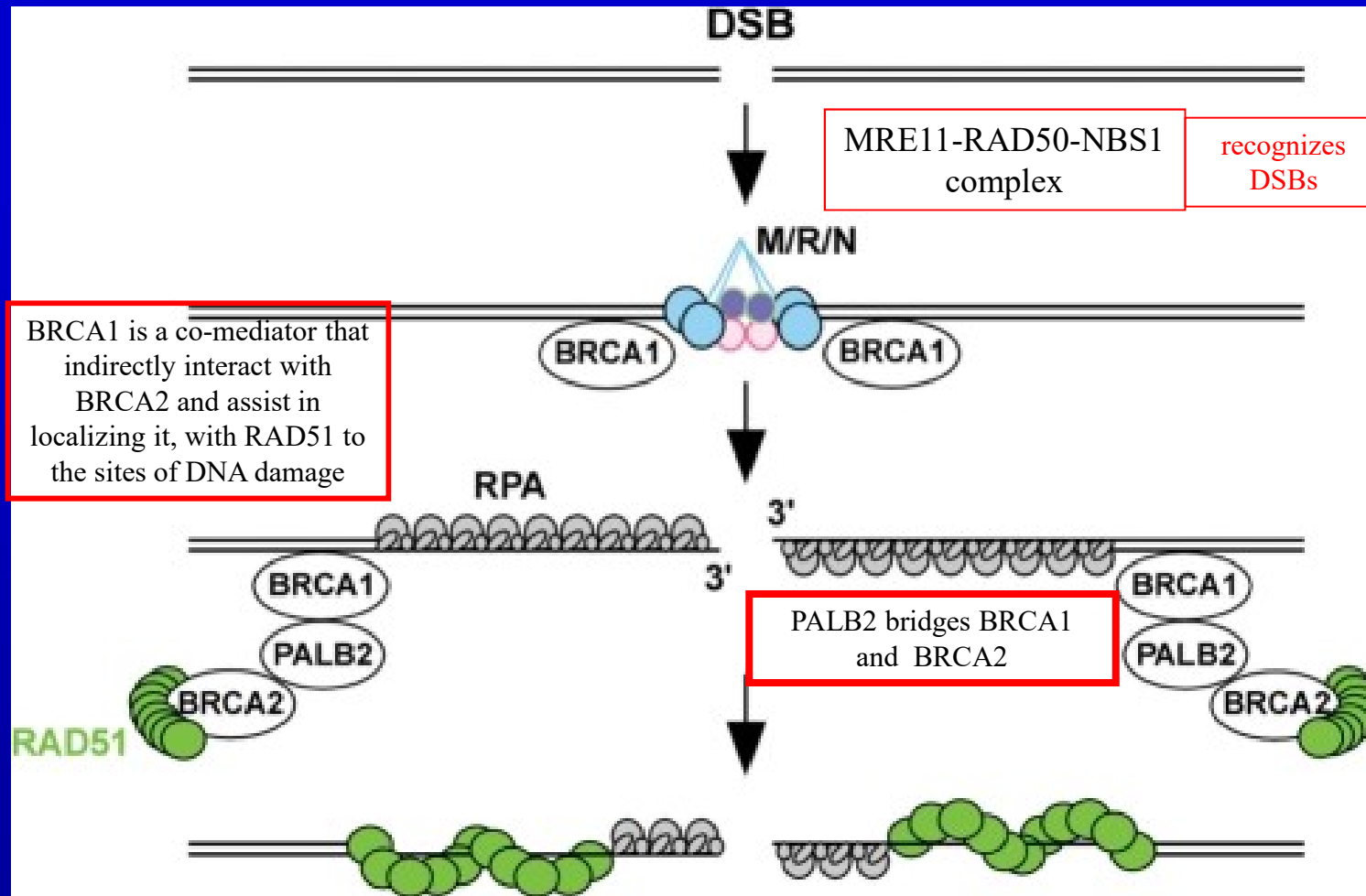
- It is possible to interfere with ubiquitin-dependent recruitment of DDR factors by expressing proteins containing ubiquitin binding domains (UBDs) that bind to lysine 63-linked polyubiquitin chains.

RNF8 e RNF168

- The rapid ubiquitination of chromatin surrounding DNA double-stranded breaks (DSB) drives the formation of large structures called ionizing radiation-induced foci (IRIF), comprising many DNA damage response (DDR) proteins.
- This process is regulated by RNF8 and RNF168 ubiquitin ligases and is thought to be necessary for DNA repair and activation of signaling pathways involved in regulating cell cycle checkpoints.

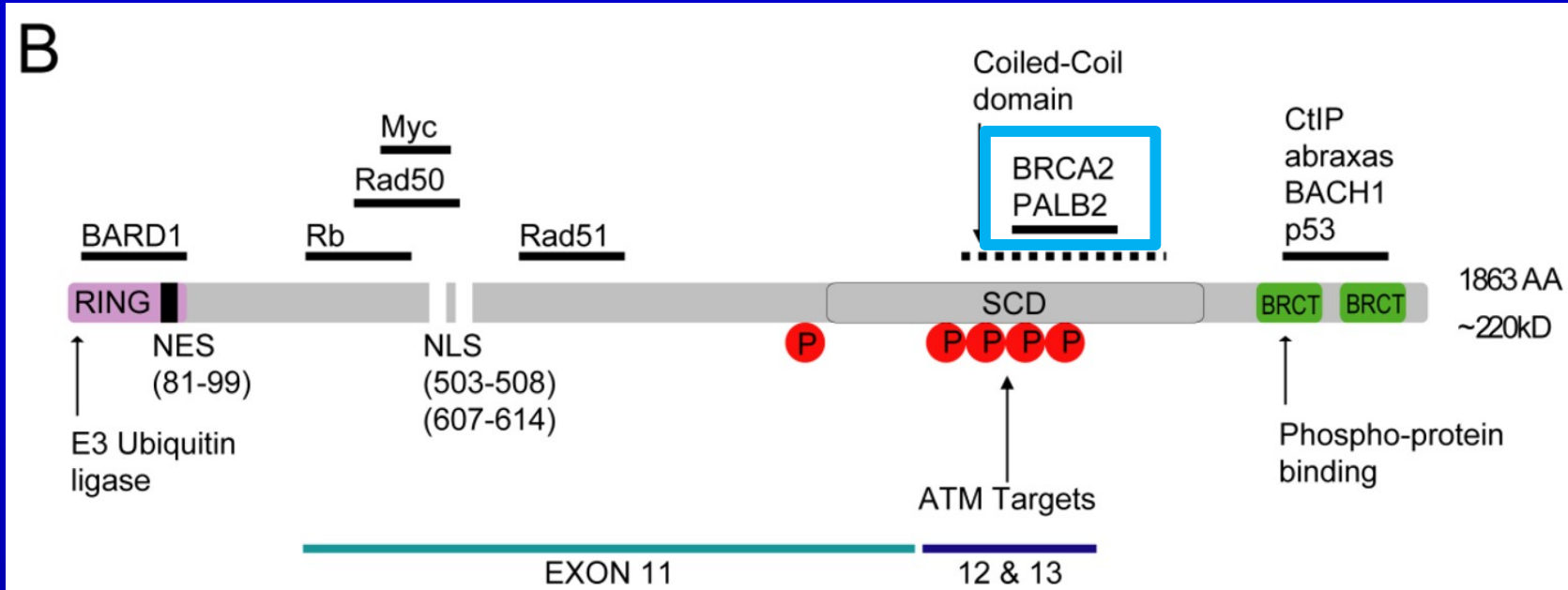


BRCA1/BRCA2



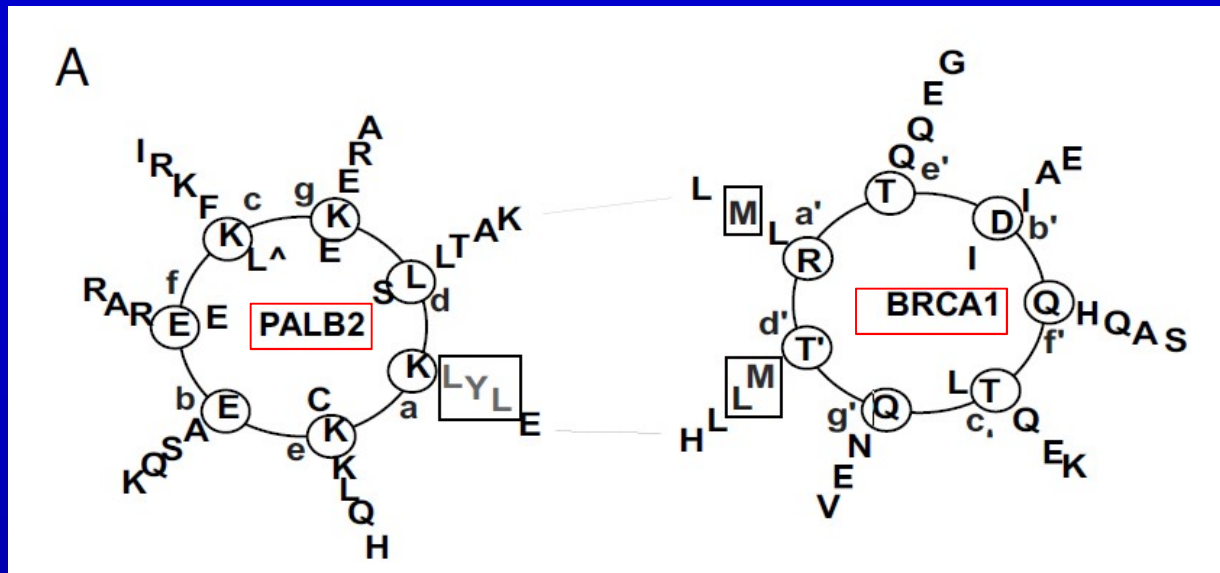
Human BRCA2 is a mediator that interacts directly with approximately eight RAD51 molecules and transports them to the site of ss-DNA bound by RPA

BRCA1 is implicated in multiple cellular functions



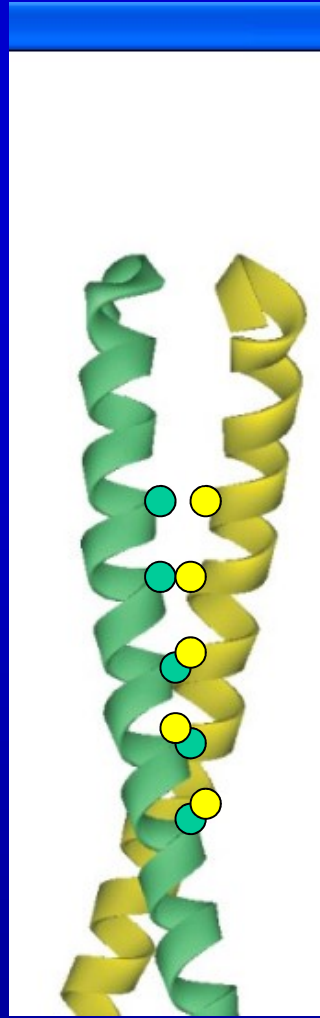
PALB2 binds directly to BRCA1 and serves as the molecular scaffold in the formation of the BRCA1-PALB2-BRCA2 complex.

regions required for the BRCA1-PALB2 interaction.

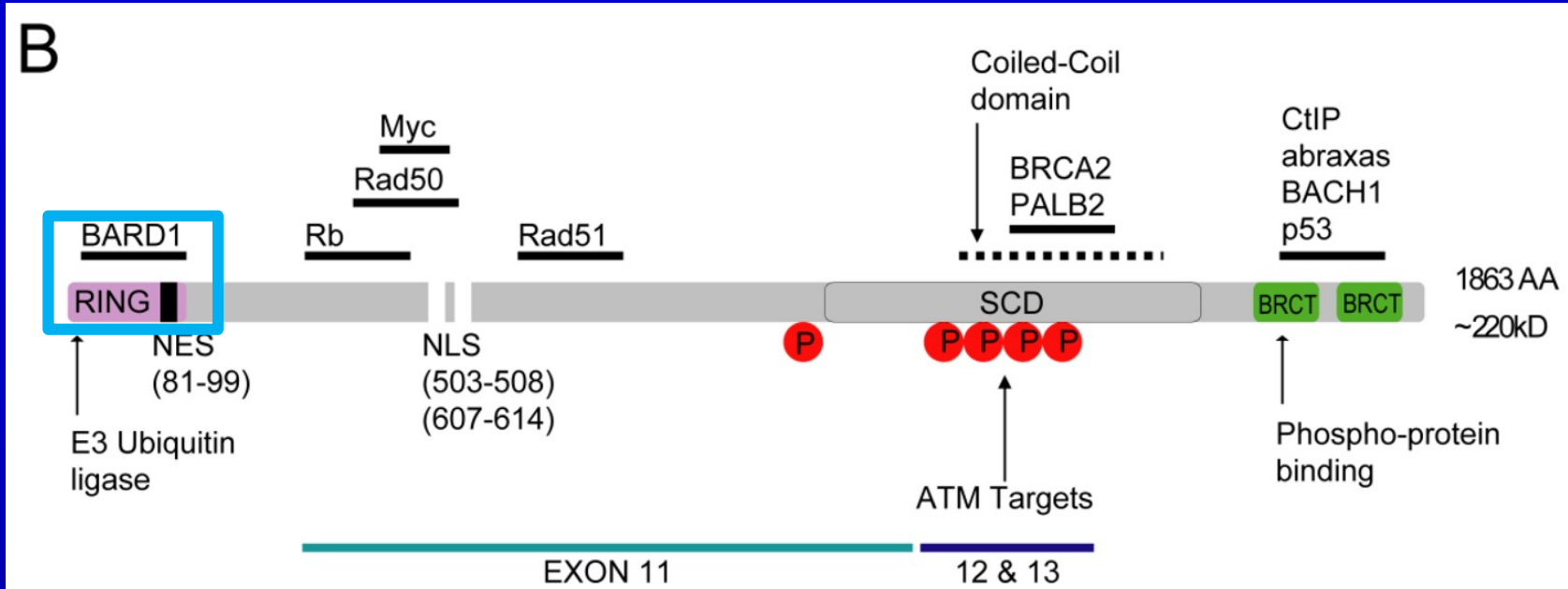


(A) Graphical projection of association between PALB2 (residues 9 – 42) and BRCA1 (residues 1393–1424) coiled-coil domains.

Positions of the heptad repeat (positions a to g) were predicted by the Coil program
Boxed residues were experimentally demonstrated to be responsible for the hetero-oligomeric interaction between PALB2 and BRCA1.



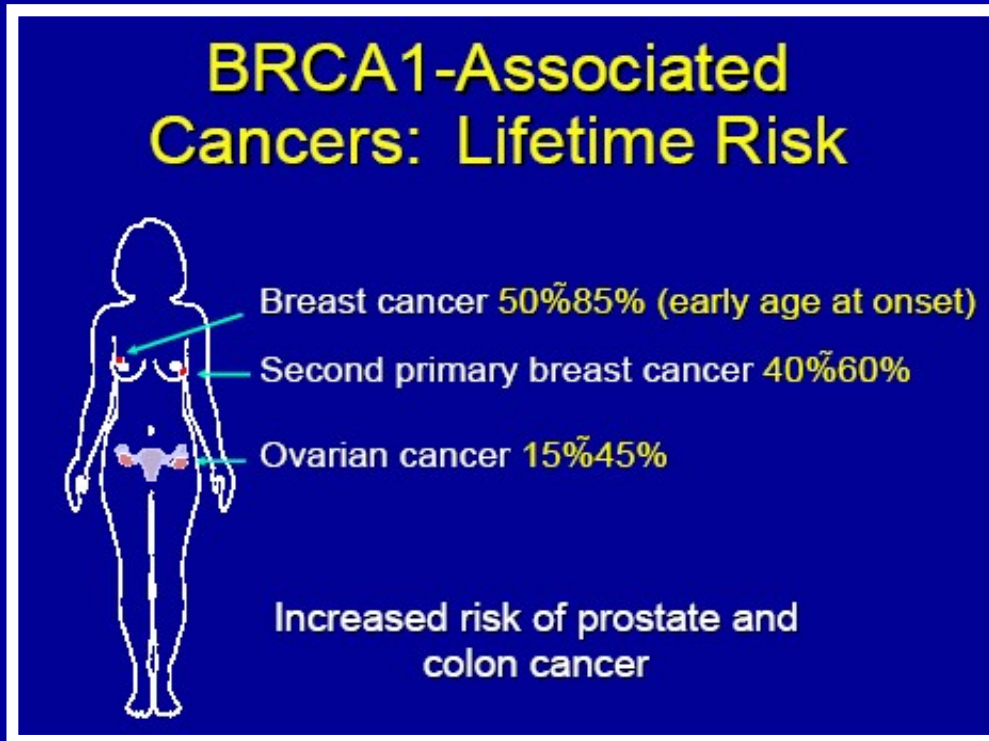
BRCA1 is implicated in multiple cellular functions



BRCA 1

- An E3 ubiquitin ligase mediates the transfer of activated ubiquitin from an E2 ubiquitin-conjugating enzyme to its substrate lysine residues.
- BRCA1 has the ability to direct the synthesis of specific polyubiquitin chain linkages, depending on the E2 bound to its RING.

Mutazioni in BRCA 1 o 2 → inattivazione meccanismo HRR
→ predisposizione allo sviluppo di **Carcinoma mammario ereditario**, con insorgenza precoce tumore seno e ovaie



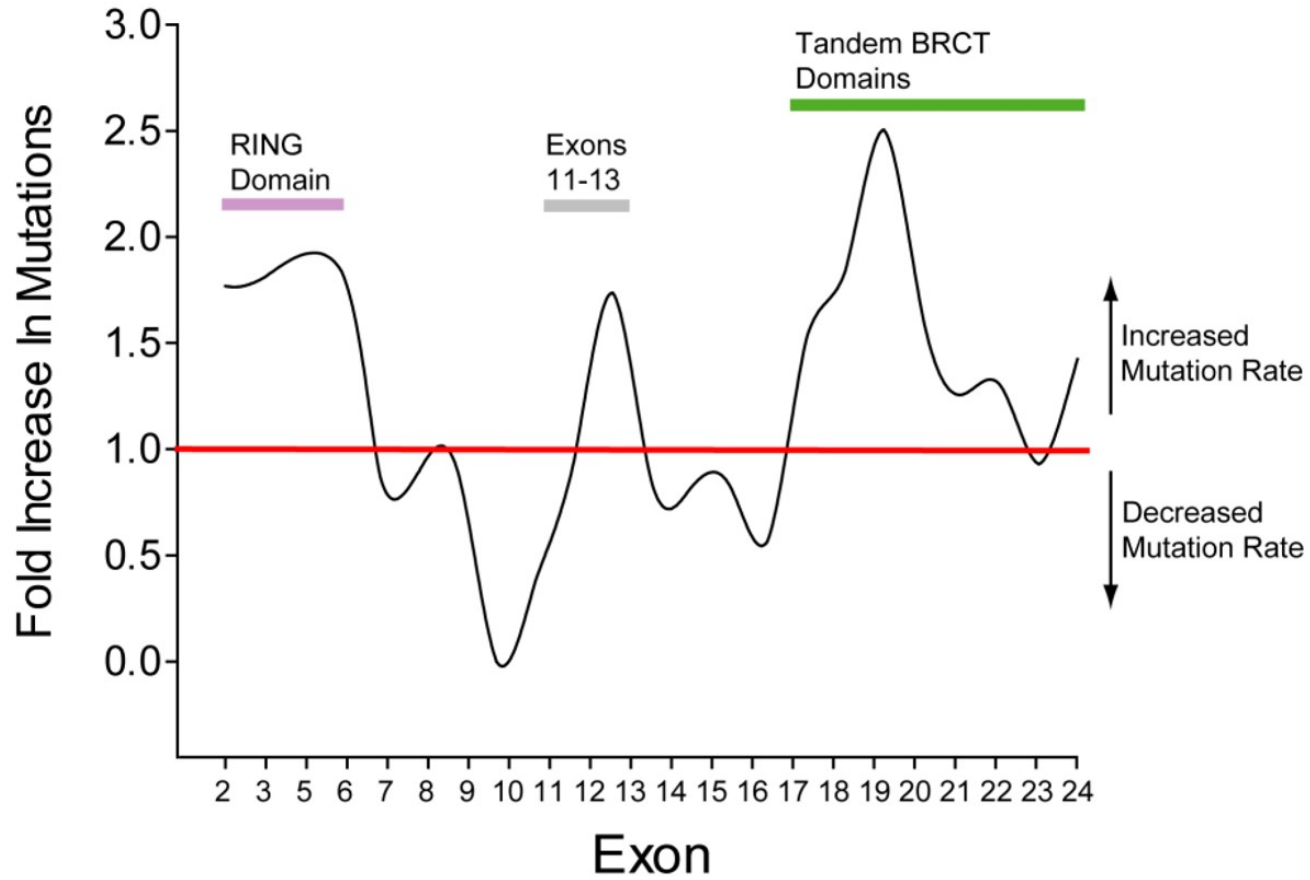
BRCA 1: 50% mutazioni tumore mammario familiare

BRCA 2: 35% mutazioni tumore mammario familiare

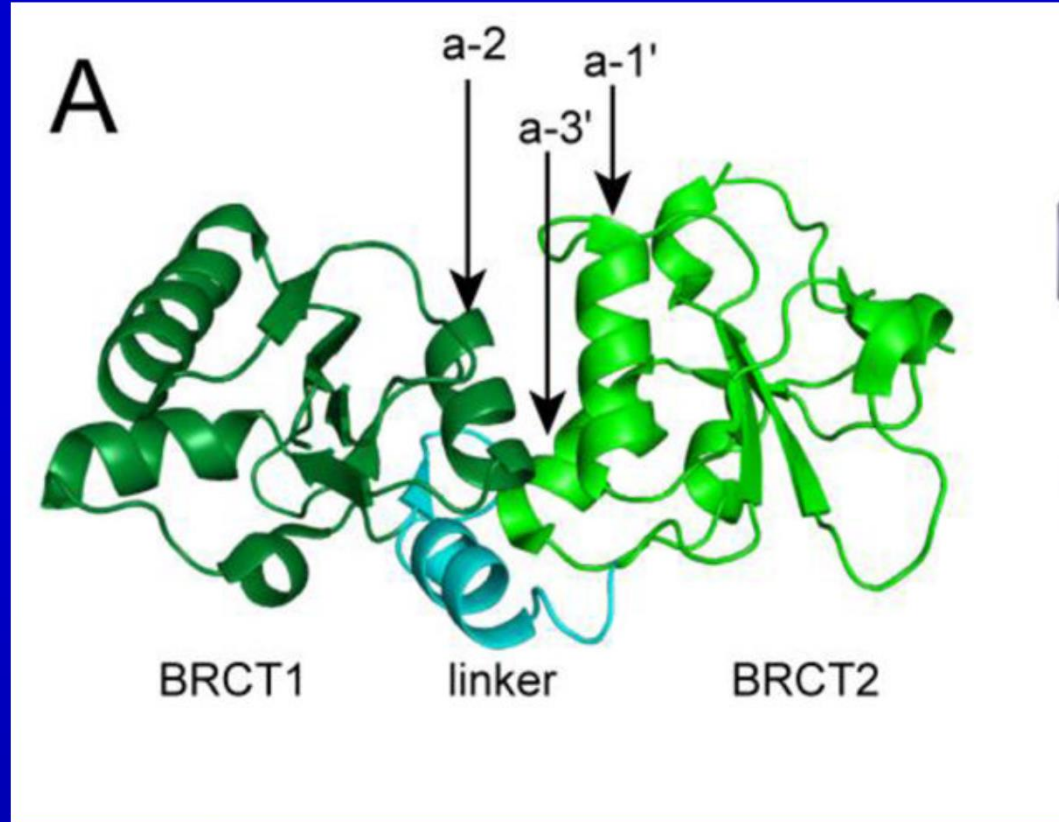
Eredità di un allele mutante → predisposizione al tumore, che insorge solo quando la seconda copia del gene è persa o mutata (**perdita di eterozigotità**)

BRCA MUTAZIONI ED INTERAZIONI

A

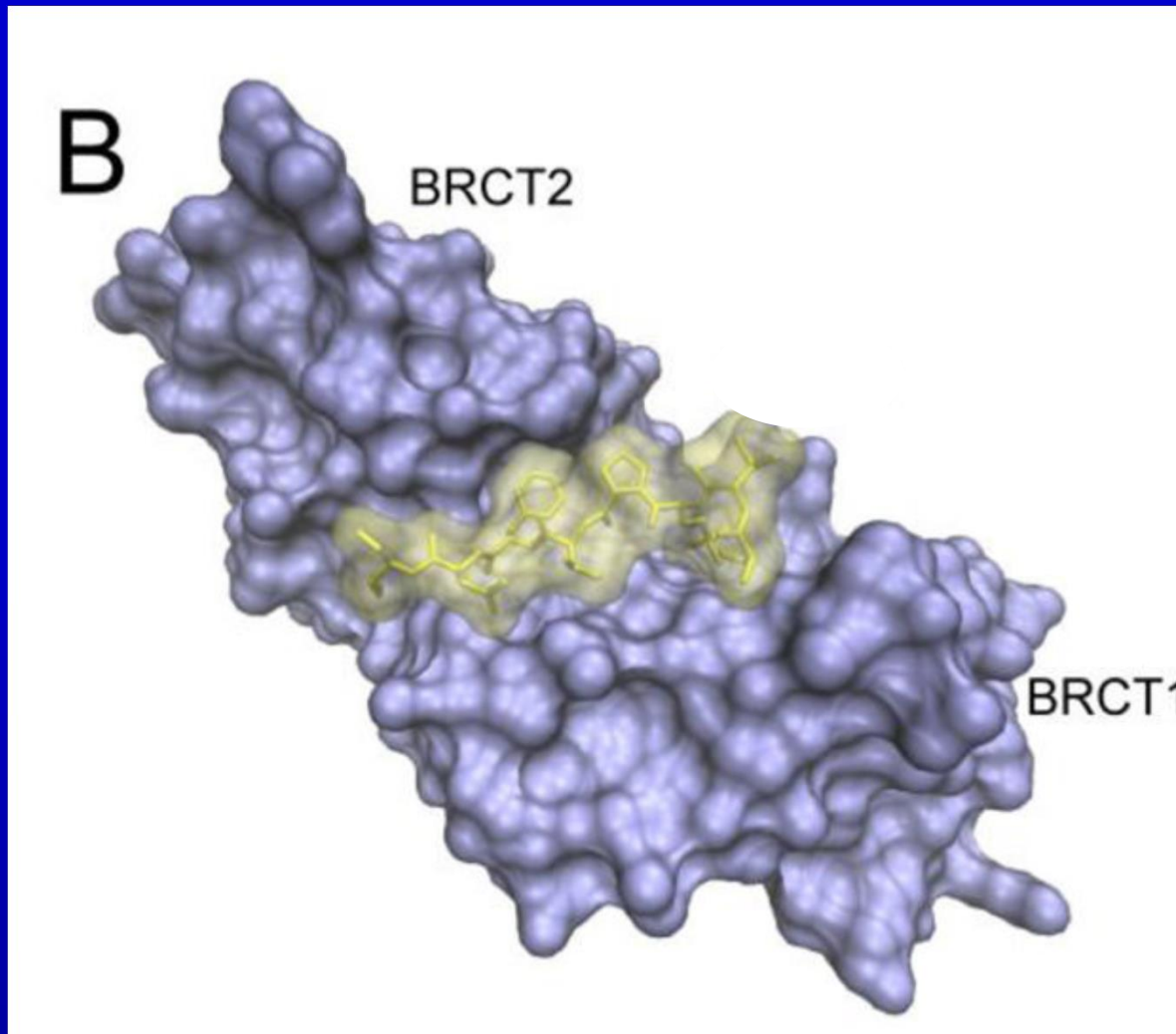


D



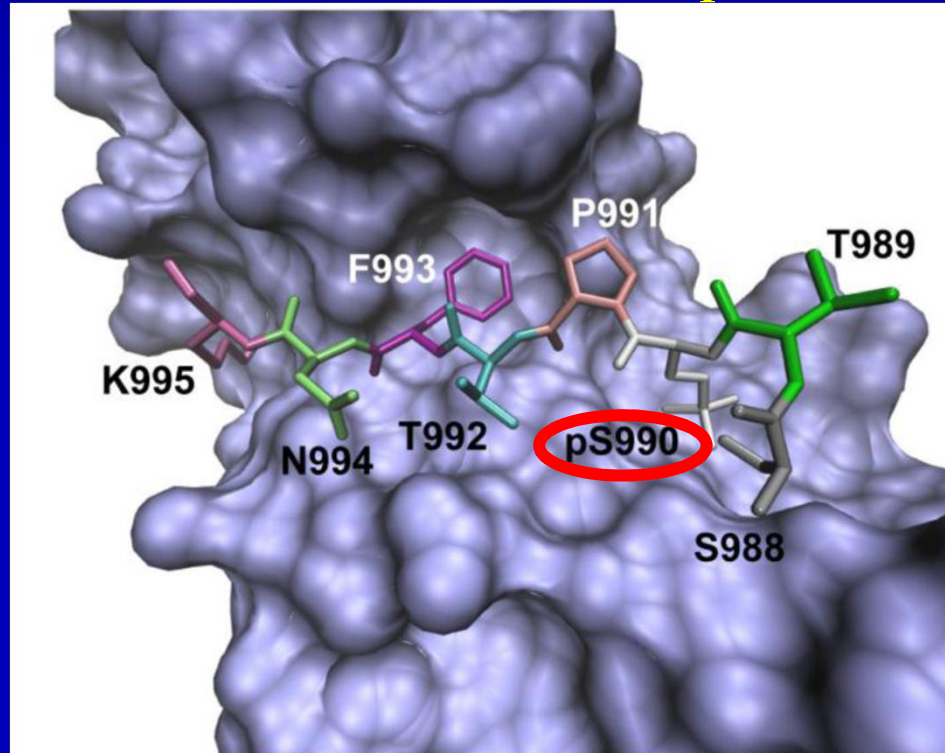
BRCT1 and BRCT2 pack together in a head-to-tail orientation and are connected by a linker helix.

Helix 2 from BRCT1 and helices 1 and 3 from BRCT2 form a hydrophobic core and stabilize the structure.



The cleft between BRCT1 and BRCT2 forms the binding pocket for proteins phosphorylated by ATM and ATR.

Il dominio BRCT di BRCA1 Riconoscimento fosfoproteine



T1

The consensus sequence for BRCA1 BRCT recognition of phosphoproteins (ATM) is 990pSer-X-X-Phe993.

BRCA1 BRCT/BACH1 binding pocket.

BRCA 1

- impairment of homologous repair in Brca1-deficient mouse embryonic stem cells
- increase in the frequency of NHEJ in Brca1-deficient cells

Riparazione per ricombinazione omologa (HRR)

Ripara le DSBs (Double-Strand Breaks) causate da radiazioni, stress ossidativo, farmaci

Replicazione e trascrizione vengono bloccate nel sito della DSB e le estremità esposte sono soggette a degradazione con perdita di materiale genetico → importanza HRR

HRR utilizza come stampo il cromatidio fratello → protezione dagli errori

HRR avviene in tarda fase S o in G₂, quando i cromatidi fratelli sono vicini

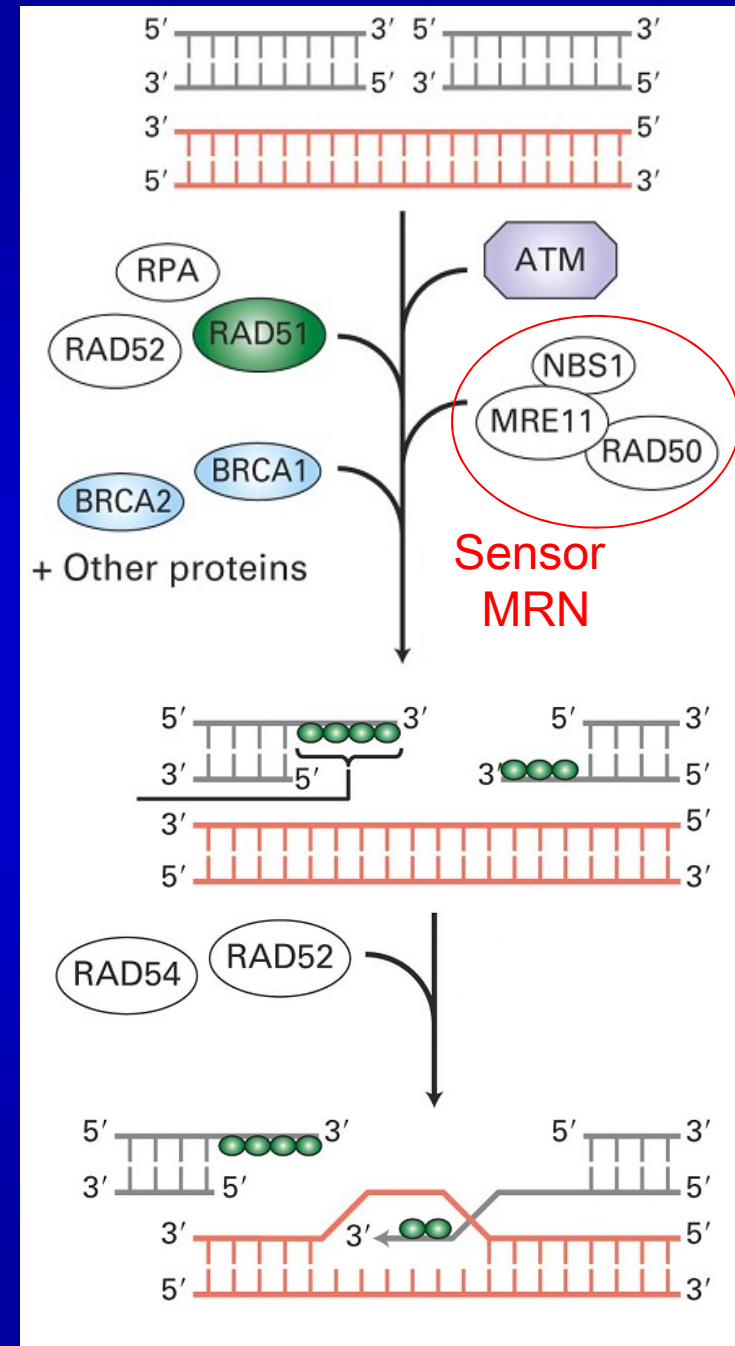
RPA

(ss DNA replication binding protein)

la polimerizzazione di **RAD51** sul 3' libero è **BRCA1/2**-dipendente



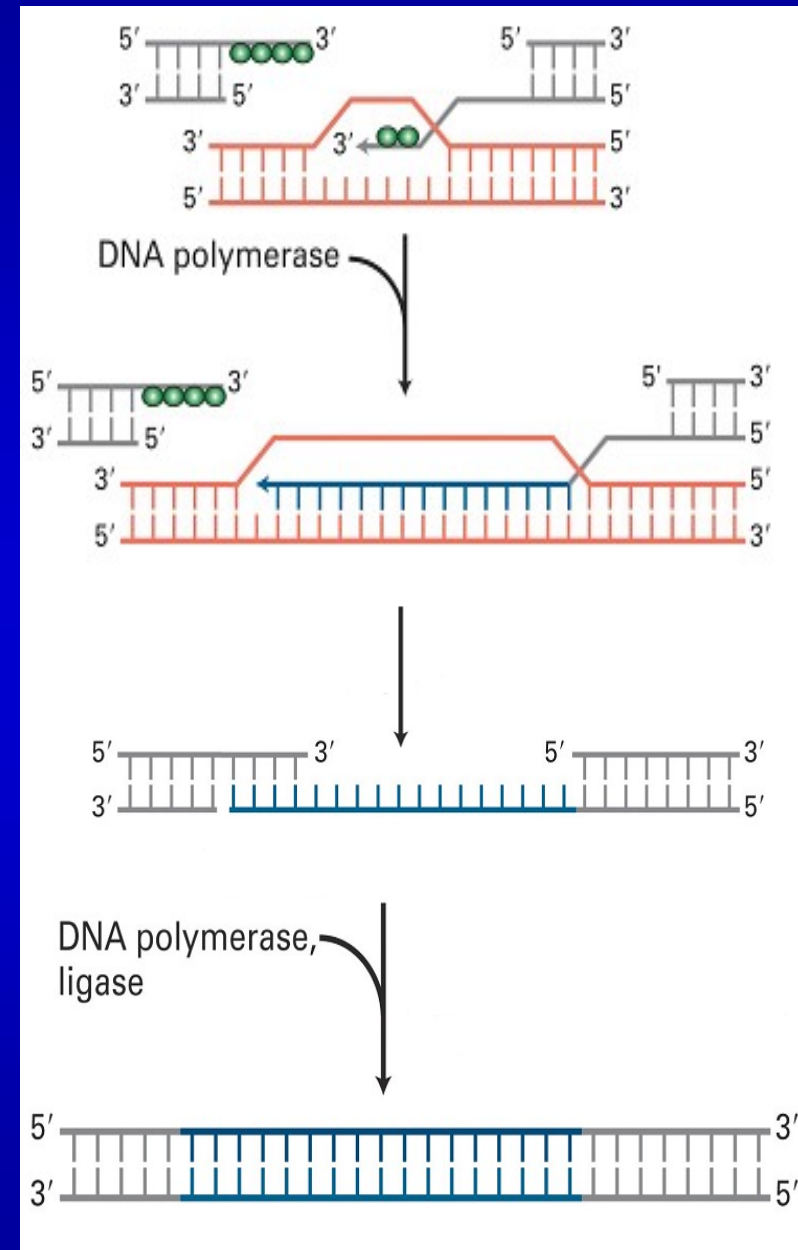
RAD51 (aiutata dall'elicasi **RAD54**) cerca la sequenza omologa sul cromatidio fratello e invade la doppia elica; le regioni 3' a singola elica si appaiano con quelle complementari



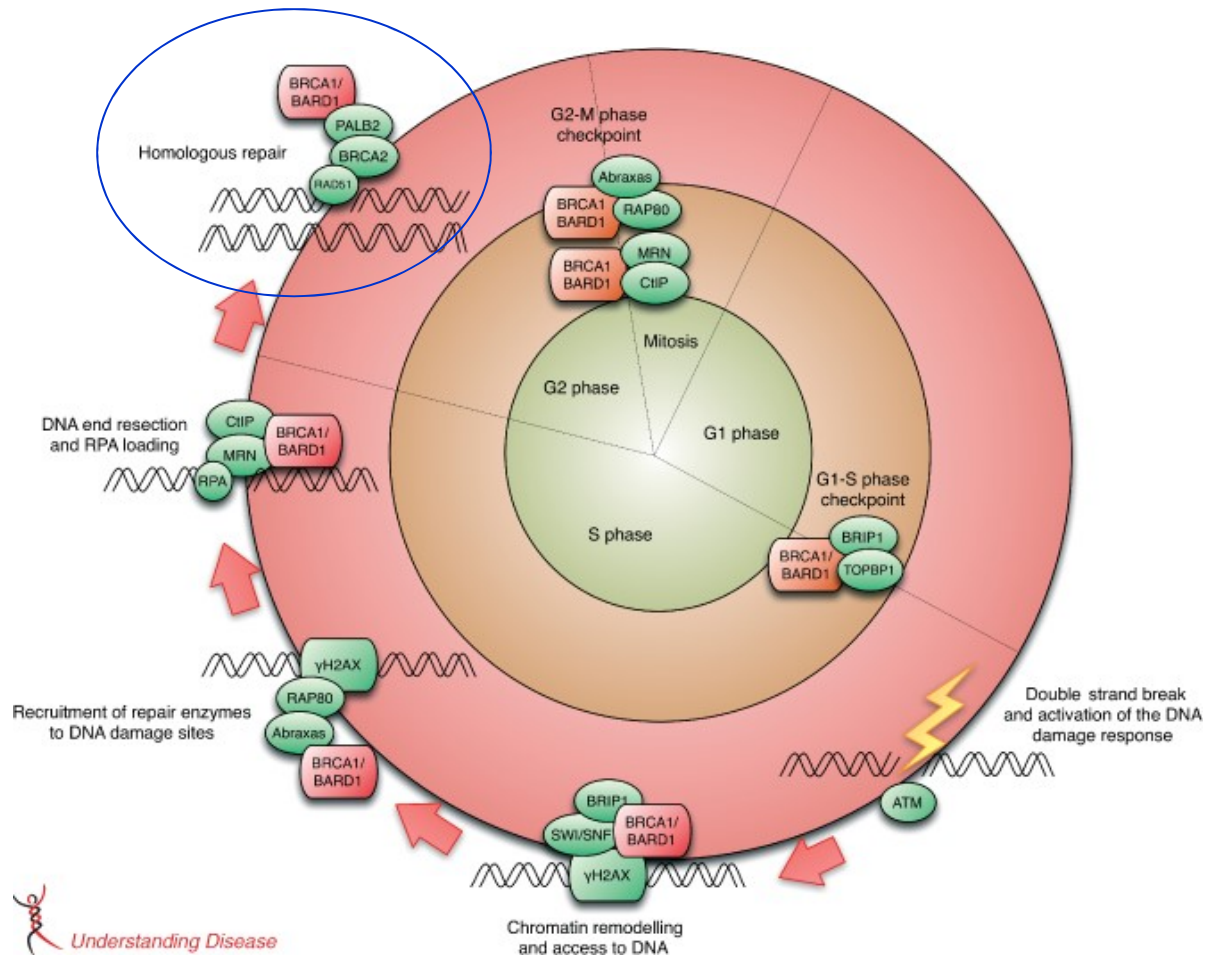
La DNA polimerasi allunga l'estremità 3' libera (in cui si trova la nucleoproteina RAD51) utilizzando come stampo il cromatidio omologo non danneggiato



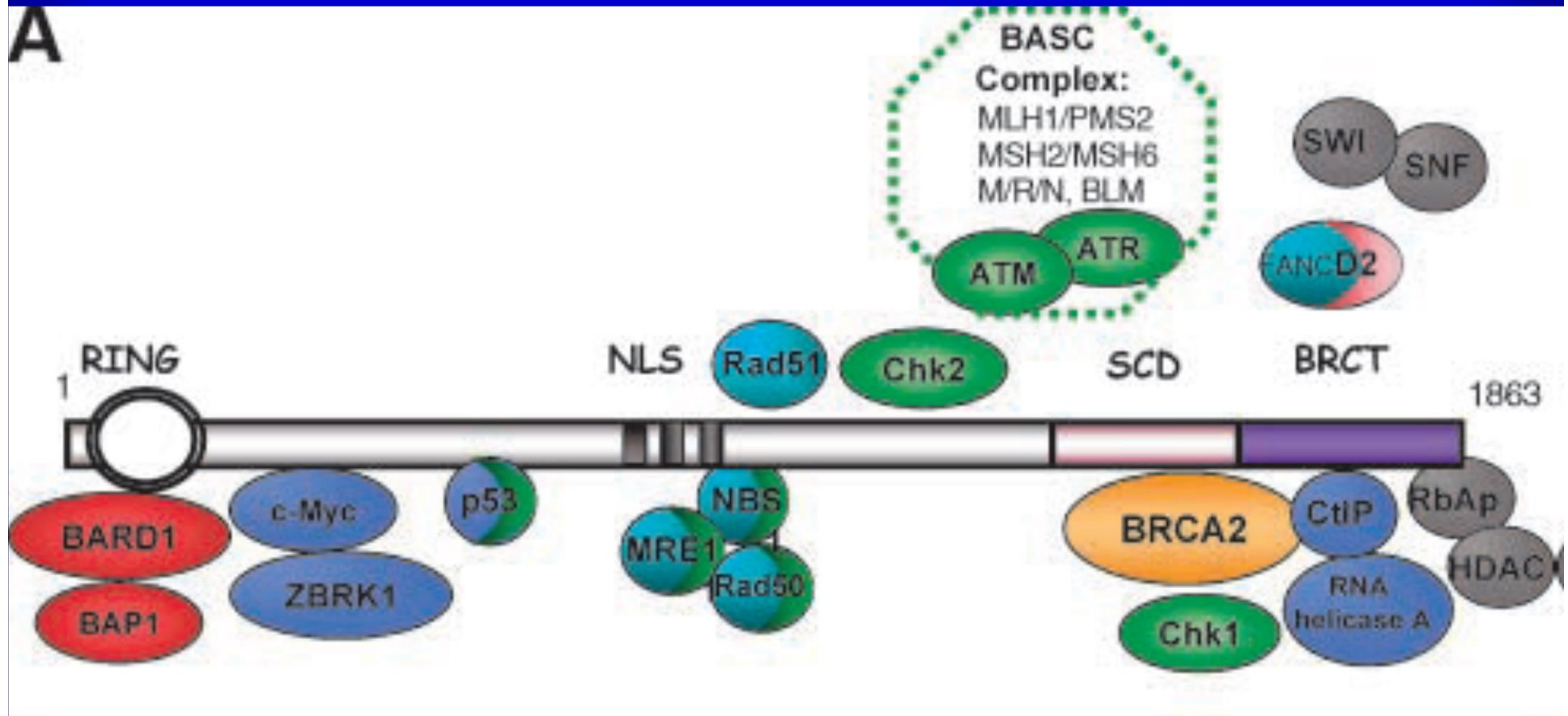
L'altra regione 3' a singola elica attorno alla zona danneggiata si appaia all'elica ormai corretta e gli eventuali gap sono riempiti da polimerasi e ligasi



In Brief: *BRCA1* and *BRCA2*



BRCA1 is implicated in multiple cellular functions

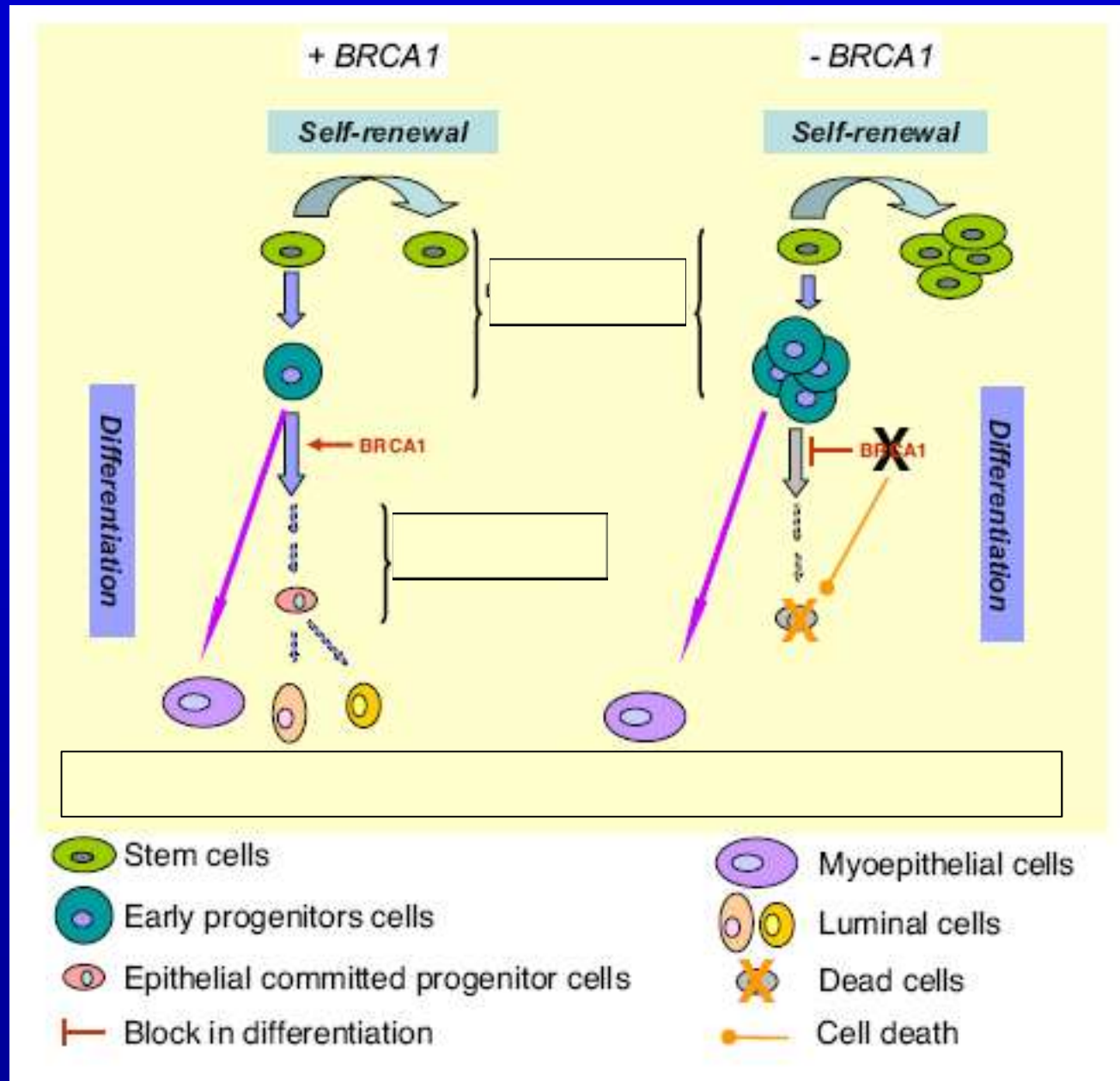


Red, ubiquitylation; **Blue, transcription**; Green, cell cycle checkpoint control; Light blue, DNA repair; Gray, chromatin

BRCA1

Differenziamento cellulare

BRCA1 regulates human mammary stem/progenitor cell fate



PNAS
February 5, 2008

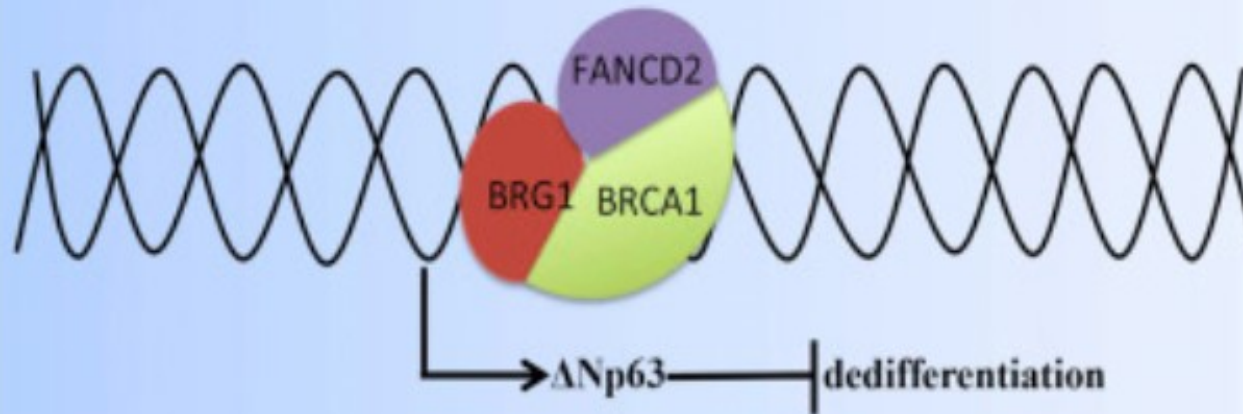
BRCA1 is necessary for the maintenance of mammary epithelial cell differentiation

-

Interstrand crosslink repair stabilizes mammary epithelial cell differentiation

Depleting BRCA1 or FANCD2 (Fanconi anemia [FA] proteins) or BRG1, a mSWI/SNF subunit, caused aberrant differentiation

Mammary epithelial progenitor cells with a stable genome



Mammary progenitor cells with interstrand DNA crosslinks

