DSB

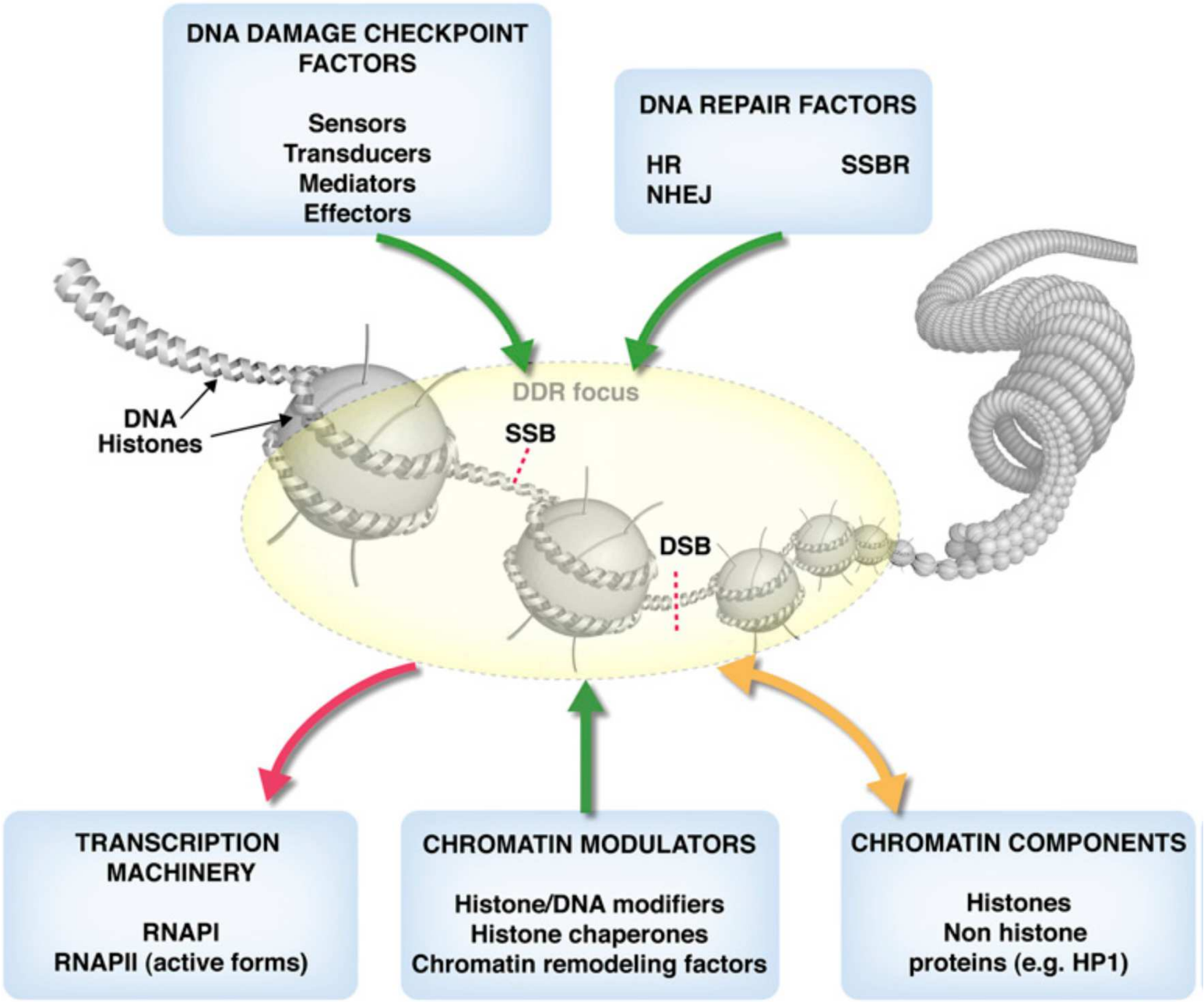
Double-Strand Breaks

causate da

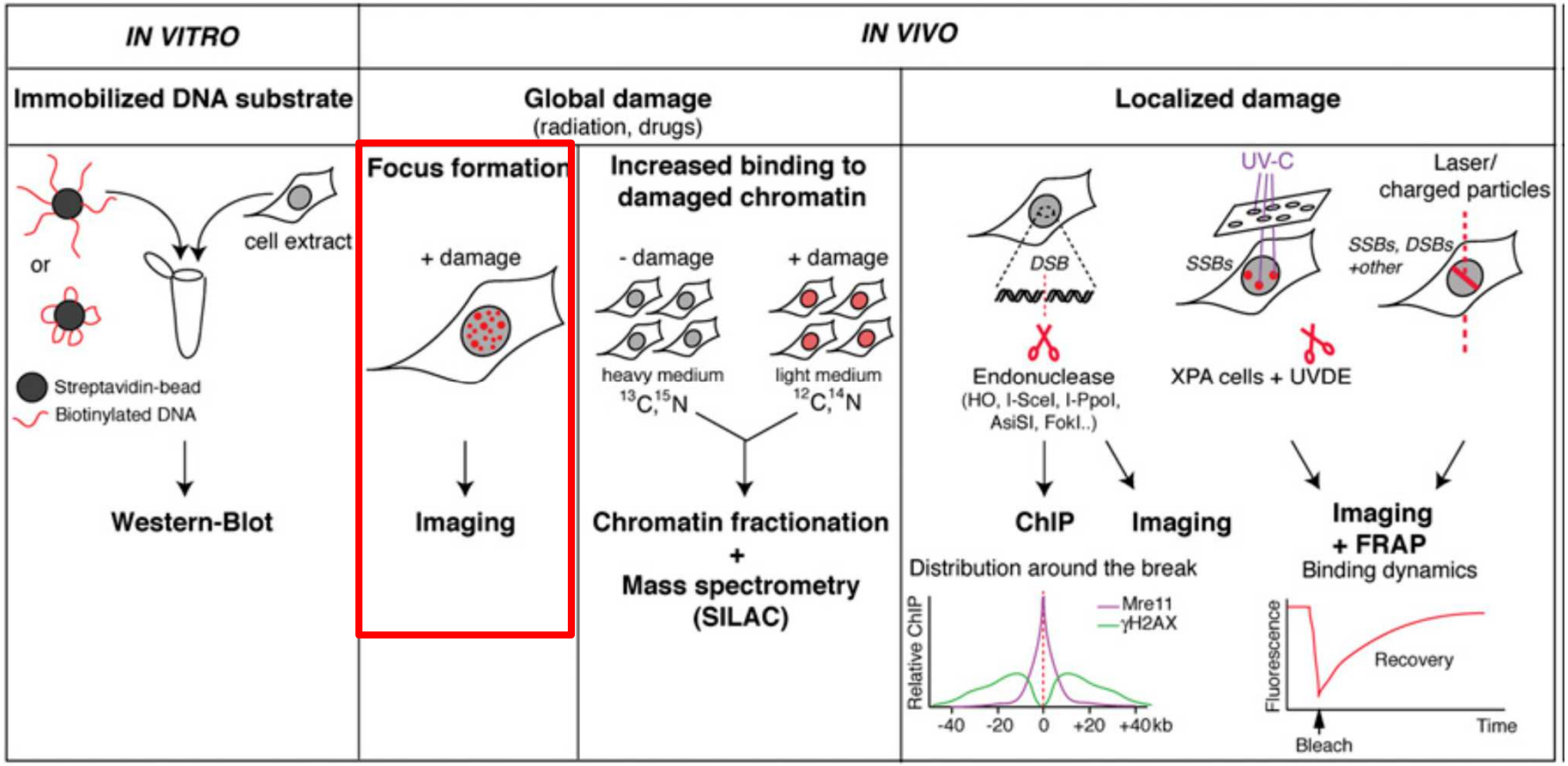
radiazioni

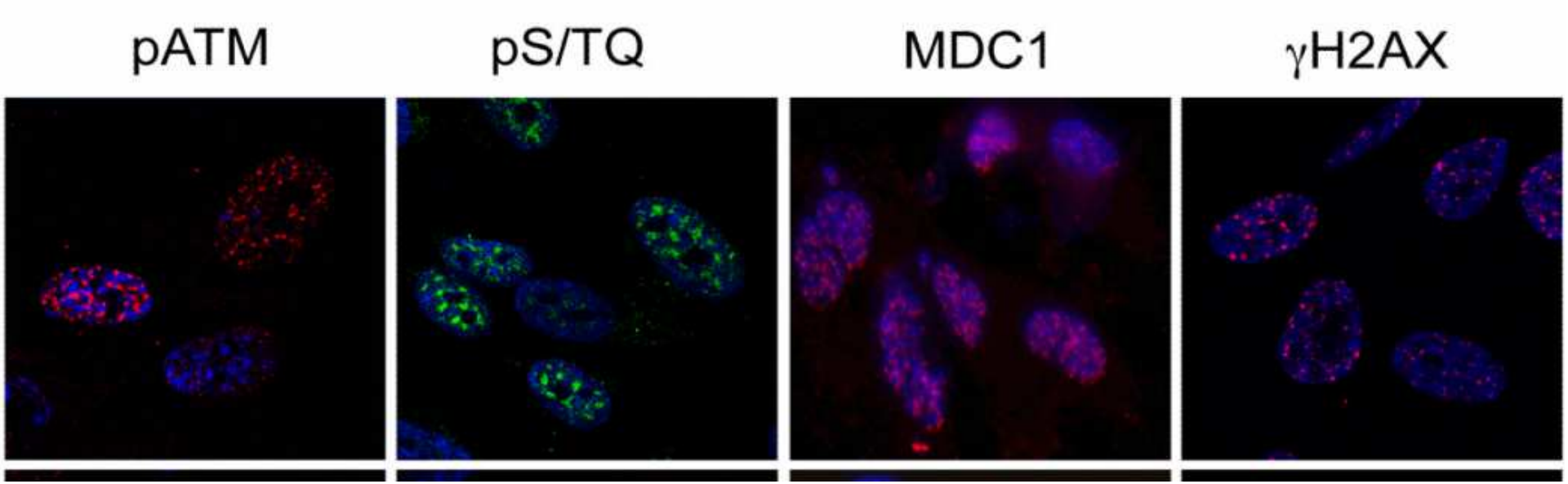
stress ossidativo

farmaci


DNA DAMAGE CHECKPOINT FACTORS
Sensors
Transducers
Mediators
Effectors
DNA REPAIR FACTORS
HR
SSBR
NHEJ
DNA
Histones
DDR focus
SSB
DSB
TRANSCRIPTION MACHINERY
RNAPI
RNAPII (active forms)
CHROMATIN MODULATORS
Histone/DNA modifiers
Histone chaperones
Chromatin remodeling factors
CHROMATIN COMPONENTS
Histones
Non histone
proteins (e.g. HP1)

METODI


IN VITRO
IN VIVO
Immobilized DNA substrate
Global damage
(radiation, drugs)
Localized damage
Focus formation
Increased binding to
damaged chromatin
cell extract
or
Streptavidin-bead
Biotinylated DNA
+ damage
- damage
+ damage
heavy medium
$^{13}C,^{15}N$
light medium
$^{12}C,^{14}N$
DSB
UV-C
SSBs
Laser/
charged particles
SSBs, DSBs
+other
Endonuclease
(HO, I-SceI, I-PpoI,
AsiSI, FokI..)
XPA cells + UVDE
Western-Blot
Imaging
Chromatin fractionation
+
Mass spectrometry
(SILAC)
ChIP
Imaging
Imaging
+ FRAP
Binding dynamics
Distribution around the break
Relative ChIP
Mre11
γH2AX
-40
-20
0
+20
+40kb
Fluorescence
Recovery
Time
Bleach

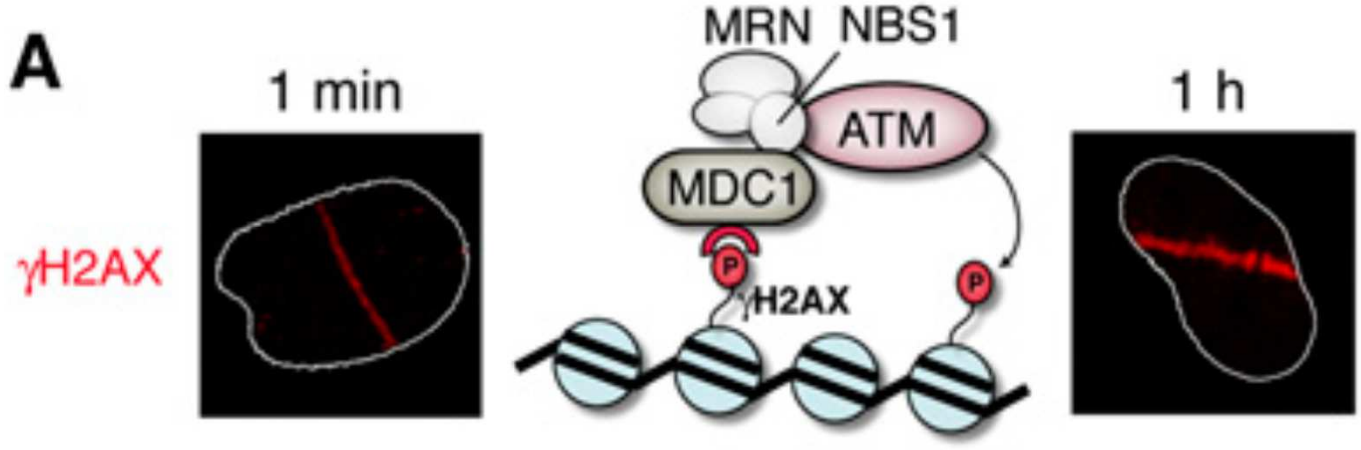


DDR foci formation in irradiated (2 Gy) cells
fixed 2 h later

IRIF IRradiation Induced Focus

DDR signal spreading

Laser micro-irradiation



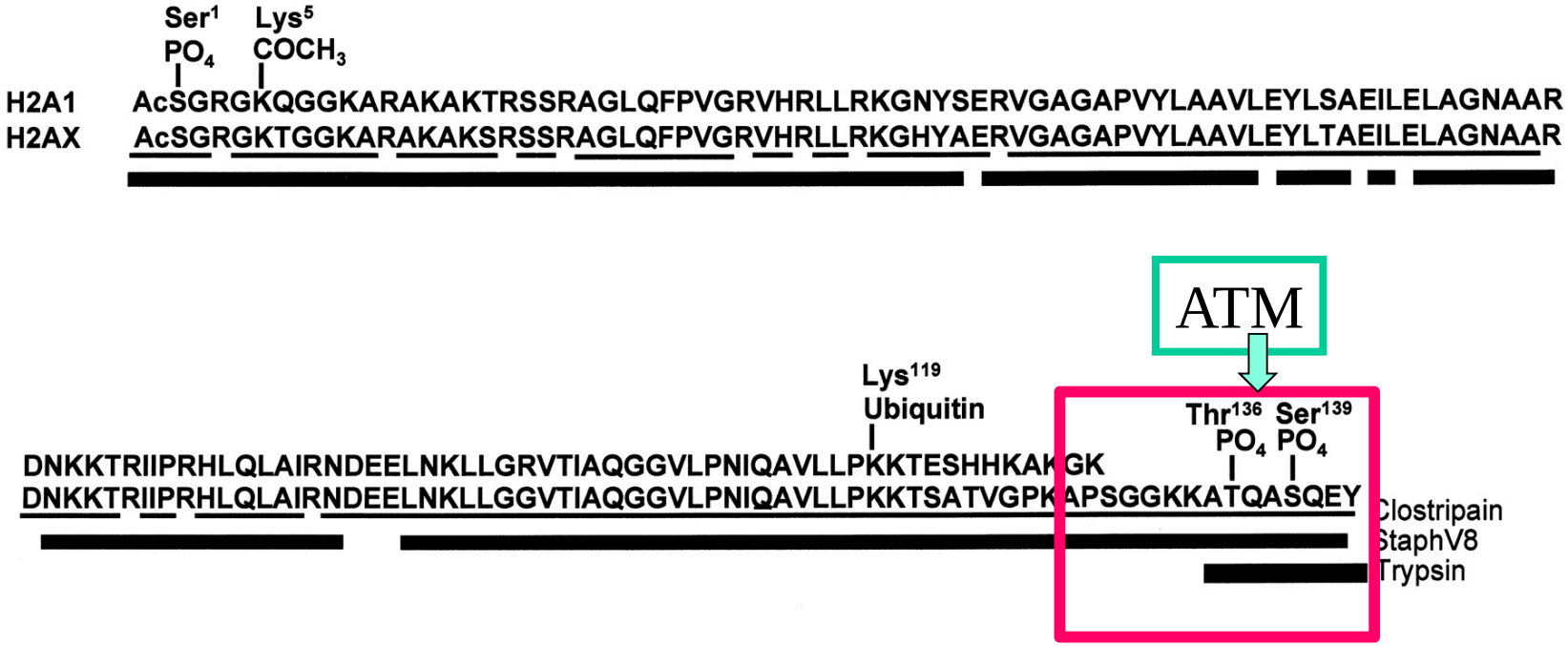
DDR proteins initially accumulate at DSB sites and then spread at distance via a positive feedback loop involving MDC1, which binds gH2AX, the MRN complex, and ATM kinase, which phosphorylates additional H2AX molecules further away from the break site.

MODIFICAZIONE ISTONI

- Eukaryotes have several histone variants, which, as a result of their altered amino-acid composition, can affect both the structure of individual nucleosomes and the ability of nucleosomes to form higher order chromatin structure

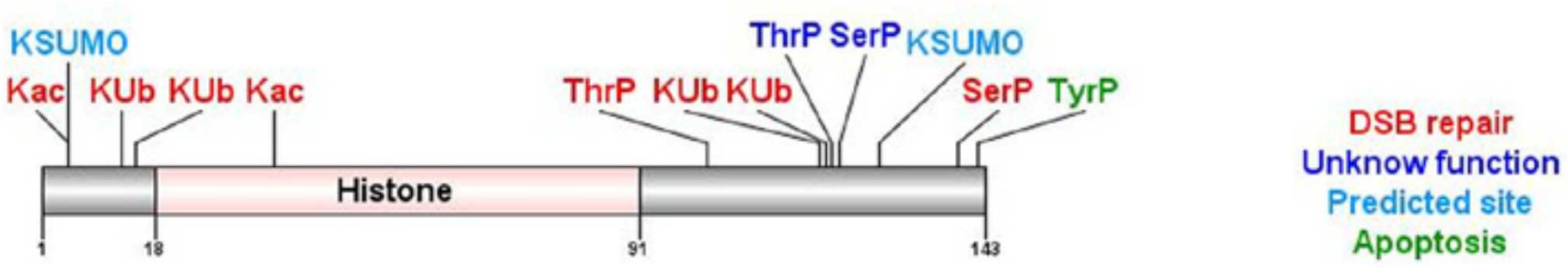
- The earliest and most robust modification induced by DSB is phosphorylation of the histone H2A variant H2AX on its extended C-terminal tail.
- Within seconds, phosphorylated H2AX (known as γ-H2AX) spreads over a region spanning thousands to millions of bases surrounding a DSB

Sequences of H2A1, H2AX, and recombinant H2AX constructs.



Emmy P. Rogakou et al. J. Biol. Chem. 1998;273:5858-5868

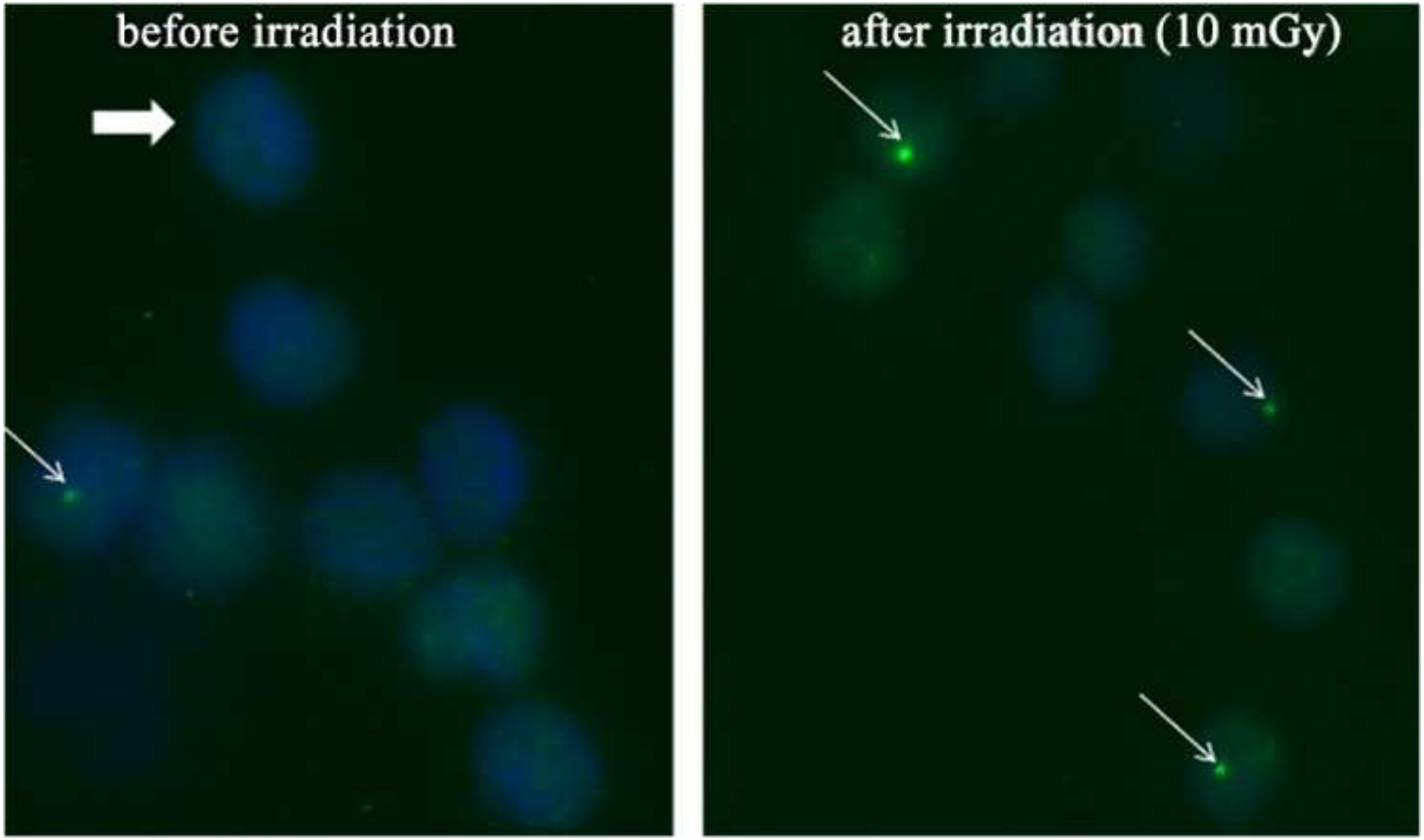
jbc



H2AX protein domain and
the multiple regulatory PTMs

The determination of radiation exposure in diagnostic and interventional radiology

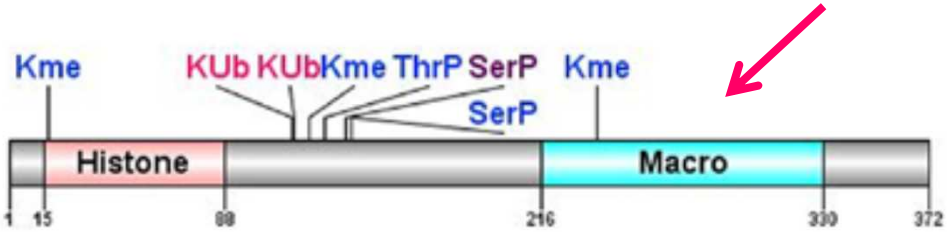
- γ-H2AX immunofluorescence microscopy is a reliable and sensitive method for the quantification of radiation induced DNA double-strand breaks (DSB) in blood lymphocytes.
- The detectable amount of these DNA damages correlates well with the dose received.



Microscopic image of γ-H2AX foci in human blood lymphocytes before and after irradiation with 10 mGy

specific γ-H2AX antibody (Anti-H2A.X-Phosphorylated (Ser 139)

macro domain
a lysine (K) rich H1-like linker region that
includes a random coil with no similarity to histones

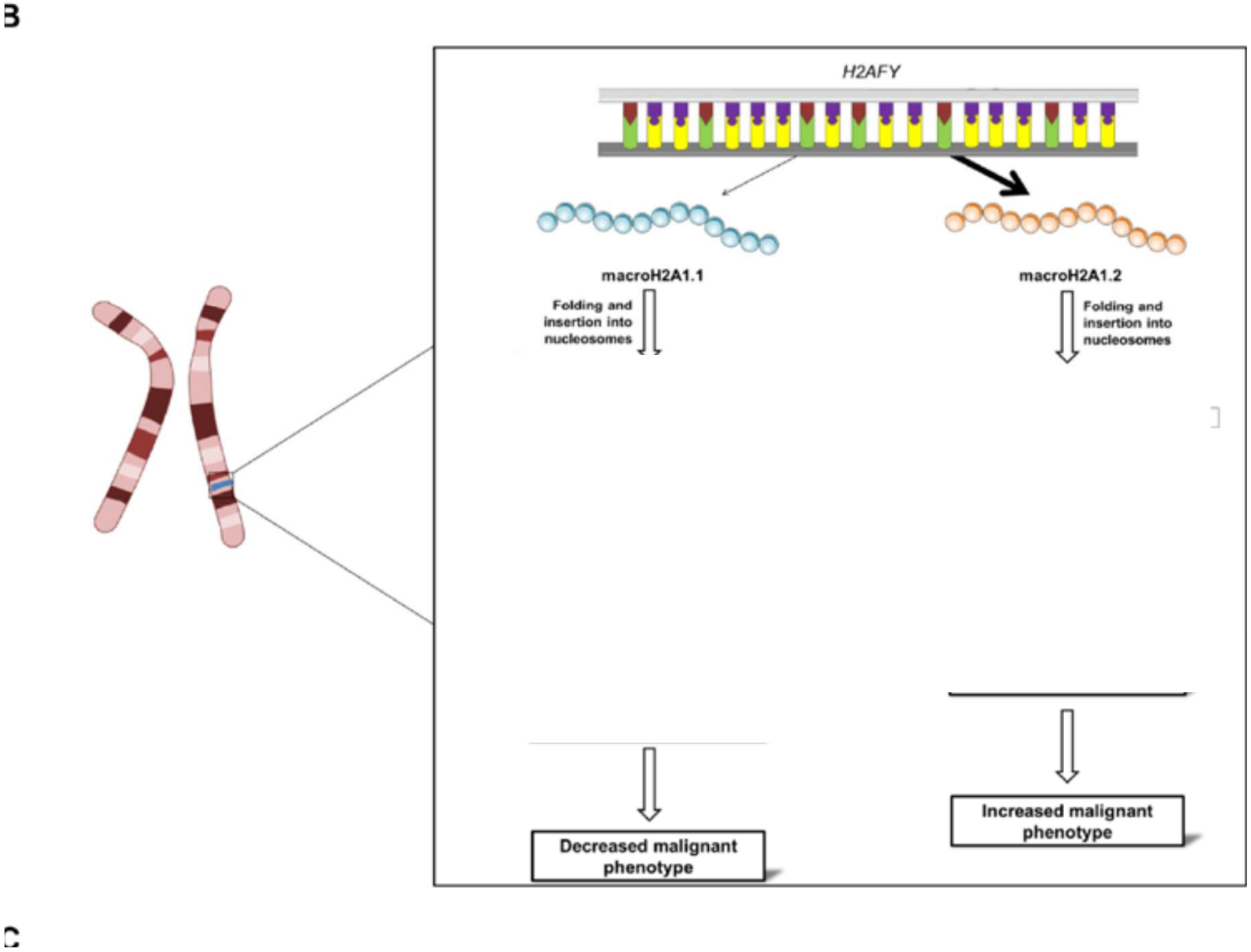


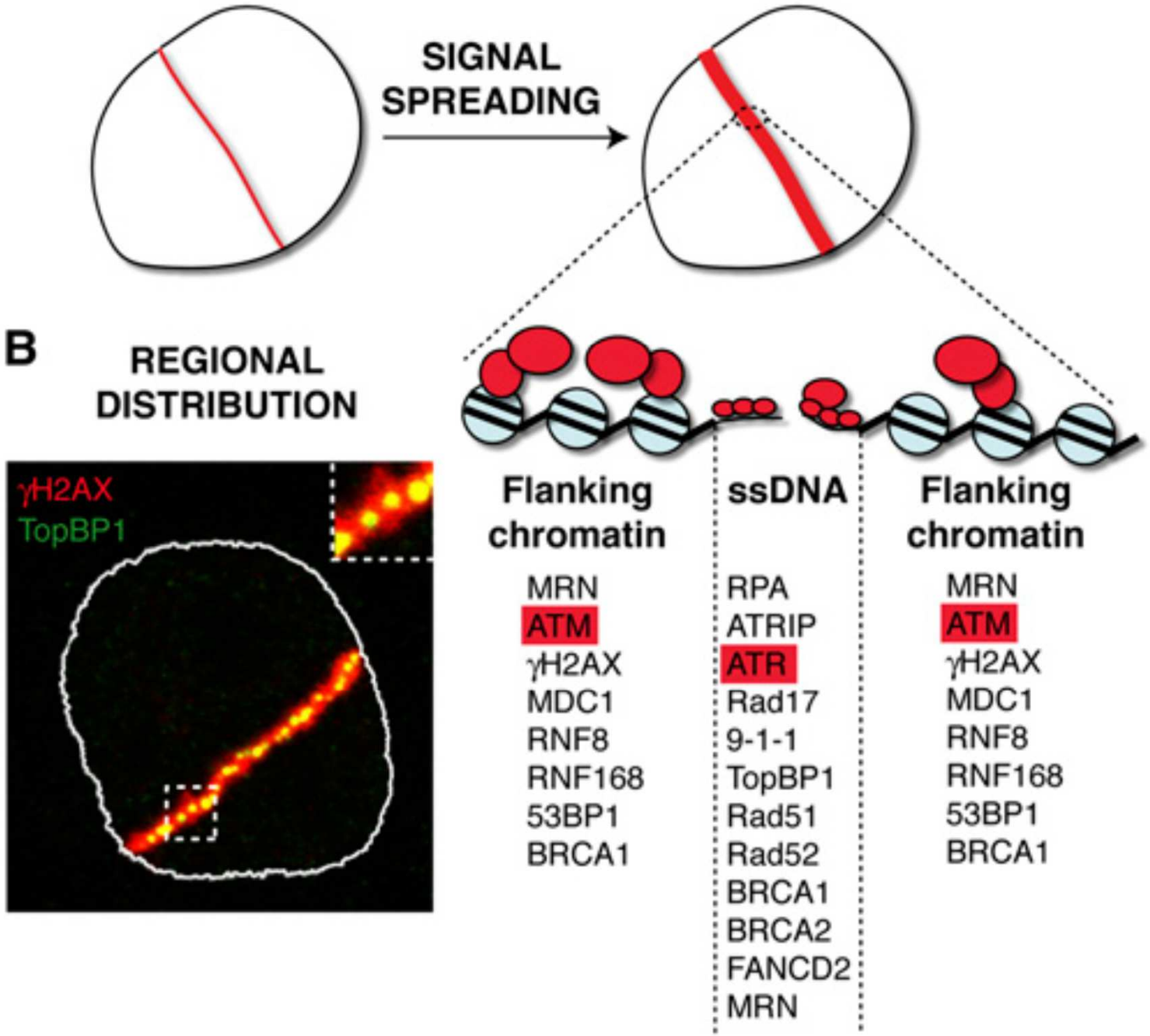
Cell cycle regulation
Unknown function
X chromosome inactivation

Structural domains and postranslational modifications identified on macroH2A.1

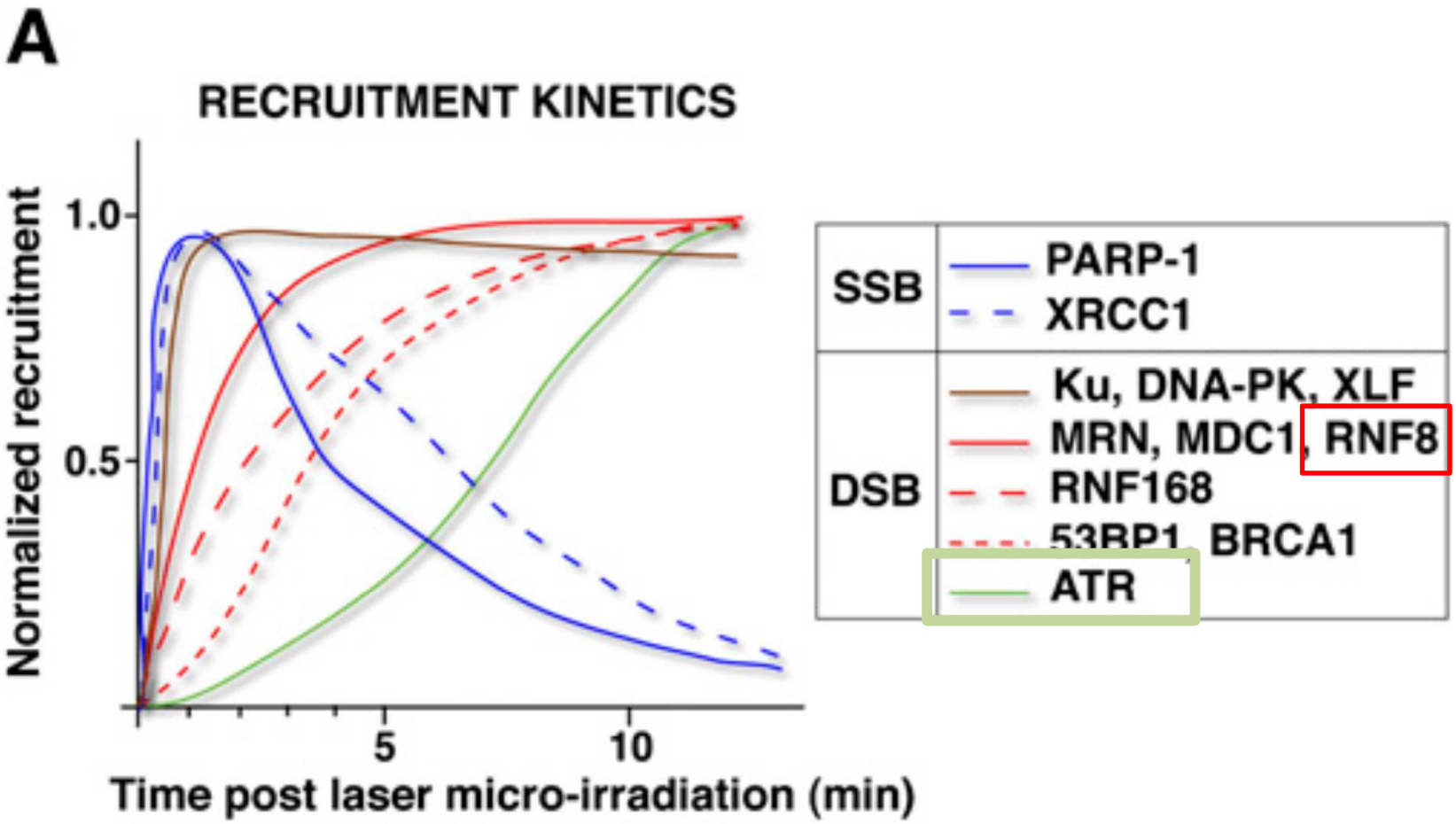
macroH2A.1

Alternative splicing of macroH2A




SIGNAL
SPREADING
B
REGIONAL
DISTRIBUTION
γH2AX
TopBP1
Flanking chromatin
MRN
ATM
γH2AX
MDC1
RNF8
RNF168
53BP1
BRCA1
ssDNA
RPA
ATRIP
ATR
Rad17
9-1-1
TopBP1
Rad51
Rad52
BRCA1
BRCA2
FANCD2
MRN
Flanking chromatin
MRN
ATM
γH2AX
MDC1
RNF8
RNF168
53BP1
BRCA1

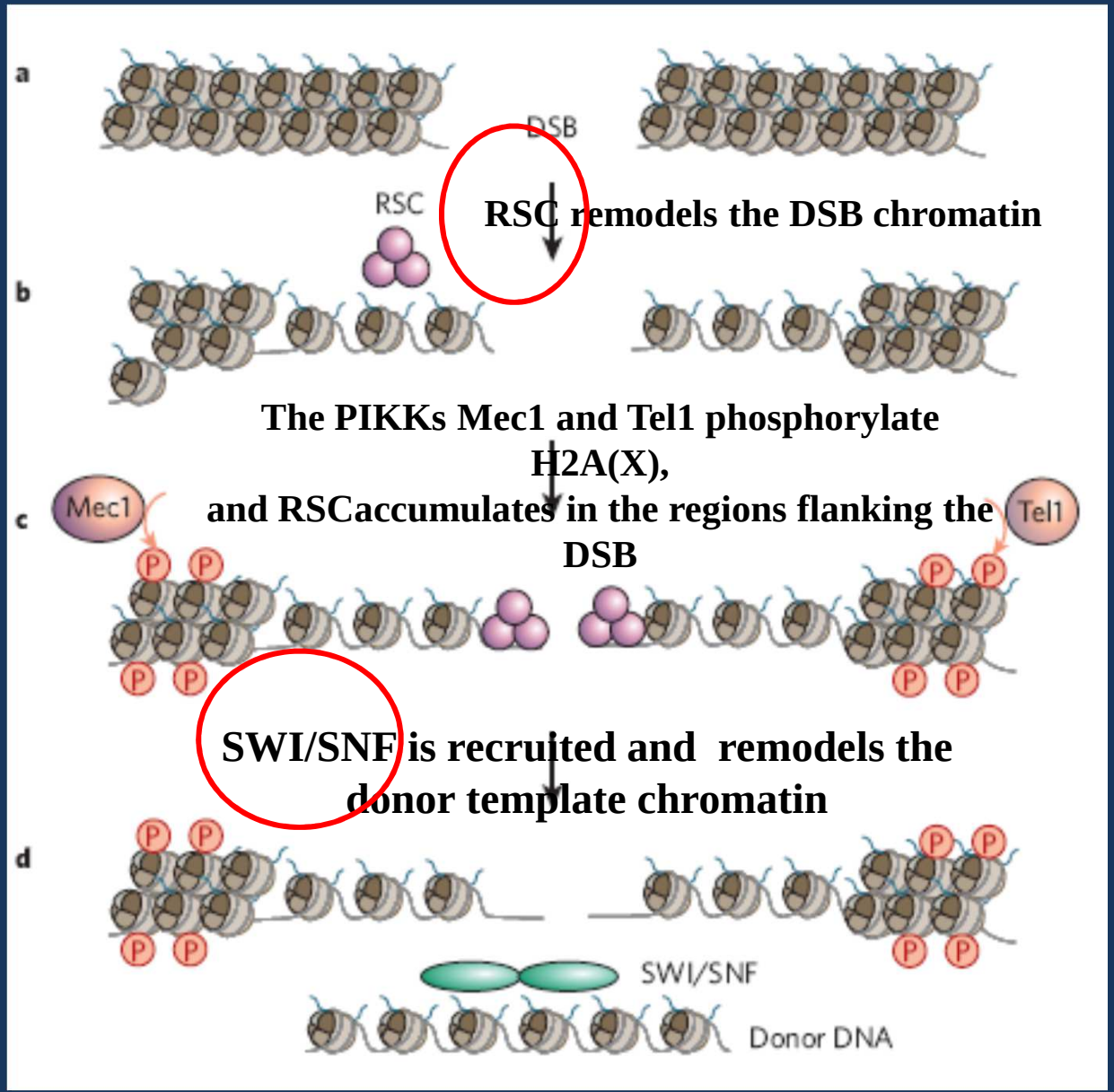
Temporal regulation of DDR protein accumulation at DNA breaks



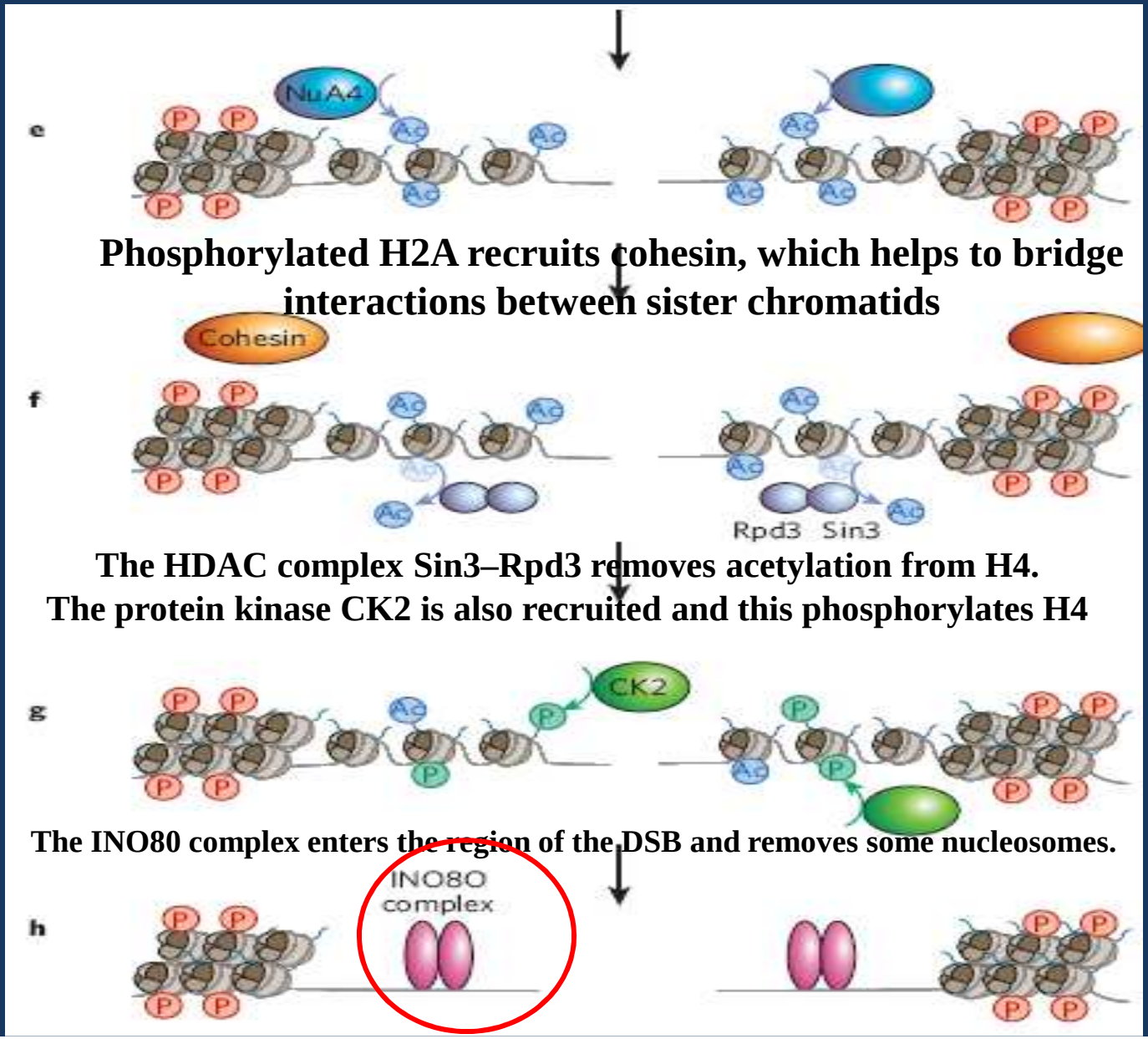
DSB
e CROMATINA

- Higher-order chromatin packaging is a barrier to the detection and repair of DNA damage
- **DSBs induce a local decrease in the density of the chromatin fibre, in addition to altering the position of nucleosomes**
- DSBs also elicit post-translational modifications on the protruding histone tails

Chromating remodelling and DSBs



Chromating remodelling and DSBs



chromatin remodeler family

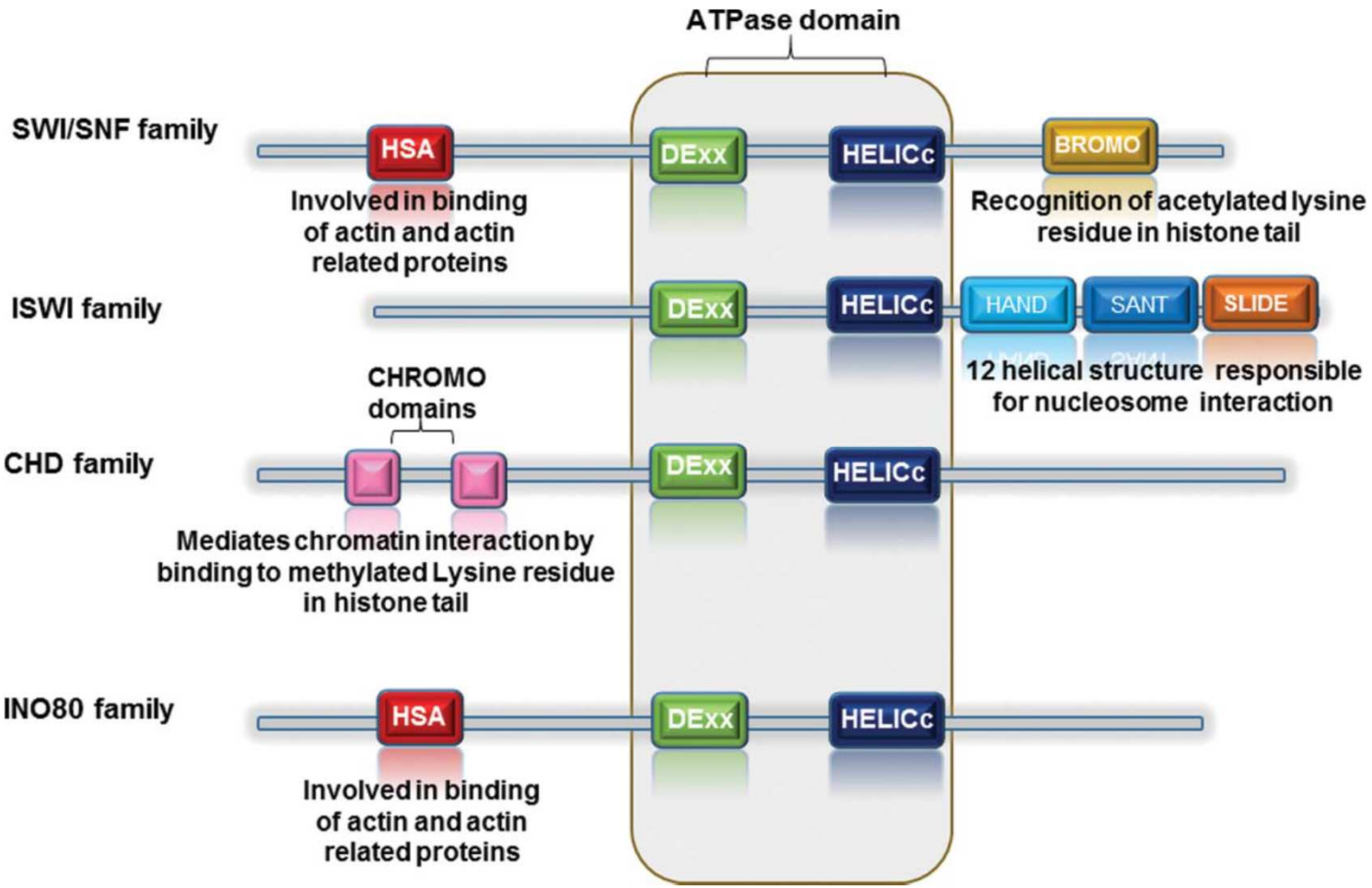


Table 1. Function of chromatin remodelers and associated diseases.

	Chromatin Remodeller		Species	Interacting partner/partners	Function	Disease
SWI/SNF (switching defective) Family	Complex	Swi2/Snf2	Yeast		Transcriptional activation / repression	Coffin-Siris syndrome and Nicolaides-Baraitser syndrome, congenital heart disease, cardiac hypertrophy, malignant rhabdoid tumors, such as choroid plexus carcinoma, medulloblastoma
		BAP (Brahma Associated Protein)	*Drosophila*			
		PBAP (Polybromo-associated BAP)				
		BAF (BRG1- associated factors)	Human			
		PBAF (Polybromo-associated BAF)	*Drosophila*			
ISWI (imitation switch) family	Complex	NURF			Nucleosome spacing, DNA damage repair, transcriptional repression.	William'S syndrome, Melanotic tumour, anencephaly
		ACF				
		CHRAC				
		ISW1	Yeast			
		ISW2				
		NURF	Human			
		ACF				
		CHRAC				
		NoRC				
		RSF				
		WICH				
CHD (Chromodomain-Helicase-DNA binding) family	Subfamily 1			SSRP1 protein H3K4me	ATPase activity and relocates nucleosomes. HDAC activity.	Prostate cancer, Hereditary diffuse gastric cancer (HDGC), Ehlers-Danlos syndrome
		Chd2		A+T-rich DNA	Helicase activity	Lennox-Gastaut Syndrome, epileptic encephalopathies, autism
	Subfamily2	Chd3		H3K36, HDAC1, HDA2, ATR, TRIM27	HDAC activity	Dermatomyositis, Hodgkin's lymphoma
		Chd4		HDAC1, HDAC2, TRIM28	DNA dependent ATPase activity, Epigenetic transcriptional repression	Dermatomyositis
		Chd5		Unmodified Histone, H3K27me3,	Expressed in neuronal cells, forms nucleosome remodeling and deacetylation complex	Neuroblastoma
	Subfamily3	Chd6		RNA Polymerase II, NRF2, NQO1	Transcriptional activation, Role in redox homeostasis	Pitt- Hopkin syndrome
		Chd7		Chromatin	Development of neural crest cells	CHARGE syndrome
		Chd8		CTCF, Duplin	Transcriptional repressor, developmental regulation	Autism spectrum disorder (ASD)
		Chd9		PPAR1α, CBAf1, osteocalcin, myosin	Transcriptional and developmental regulation, Nuclear receptor activation	osteogenic differentiation
INO80 (inositol requiring 80)	Complex	INO 80		transcription factor YY1, Rvb1, Rvb2, NFR B, Arp4,Arp5, Arp8	DNA helicase activity, DNA repair and replication	Aortic hypoplasia, premature atherosclerosis, Immunoglobulin class-switch recombination defects (CSR-D)
		Swr 1		Arp4, Arp 6, Swc2, Rvb1, Rvb2 H2AZ, H2B		

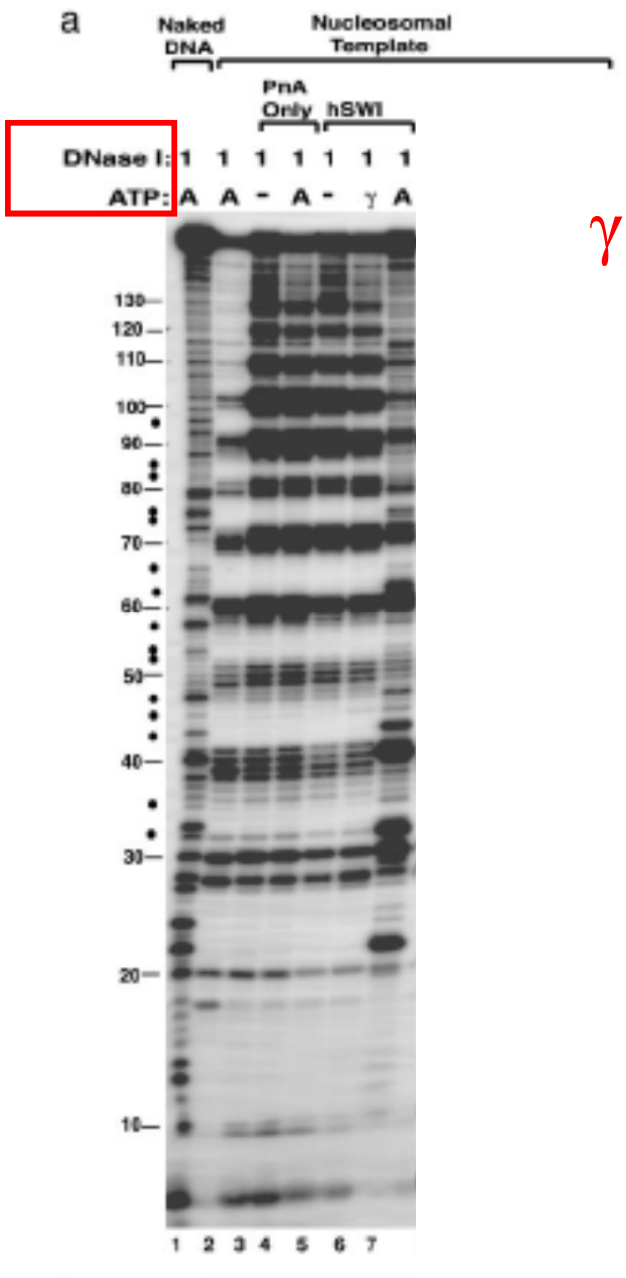
RSC

complex RSC (remodels the structure of chromatin)

ATP-dependent chromatin-remodelling

RSC can mediate nucleosome sliding, alter histoneDNA contacts and remove histones from DNA.

The chromatin-remodelling activity of RSC is important for transcriptional regulation of genes that are involved in stress responses and cell-cycle progression



γ =Adenosine 5'-(gamma-thiotriphosphate)

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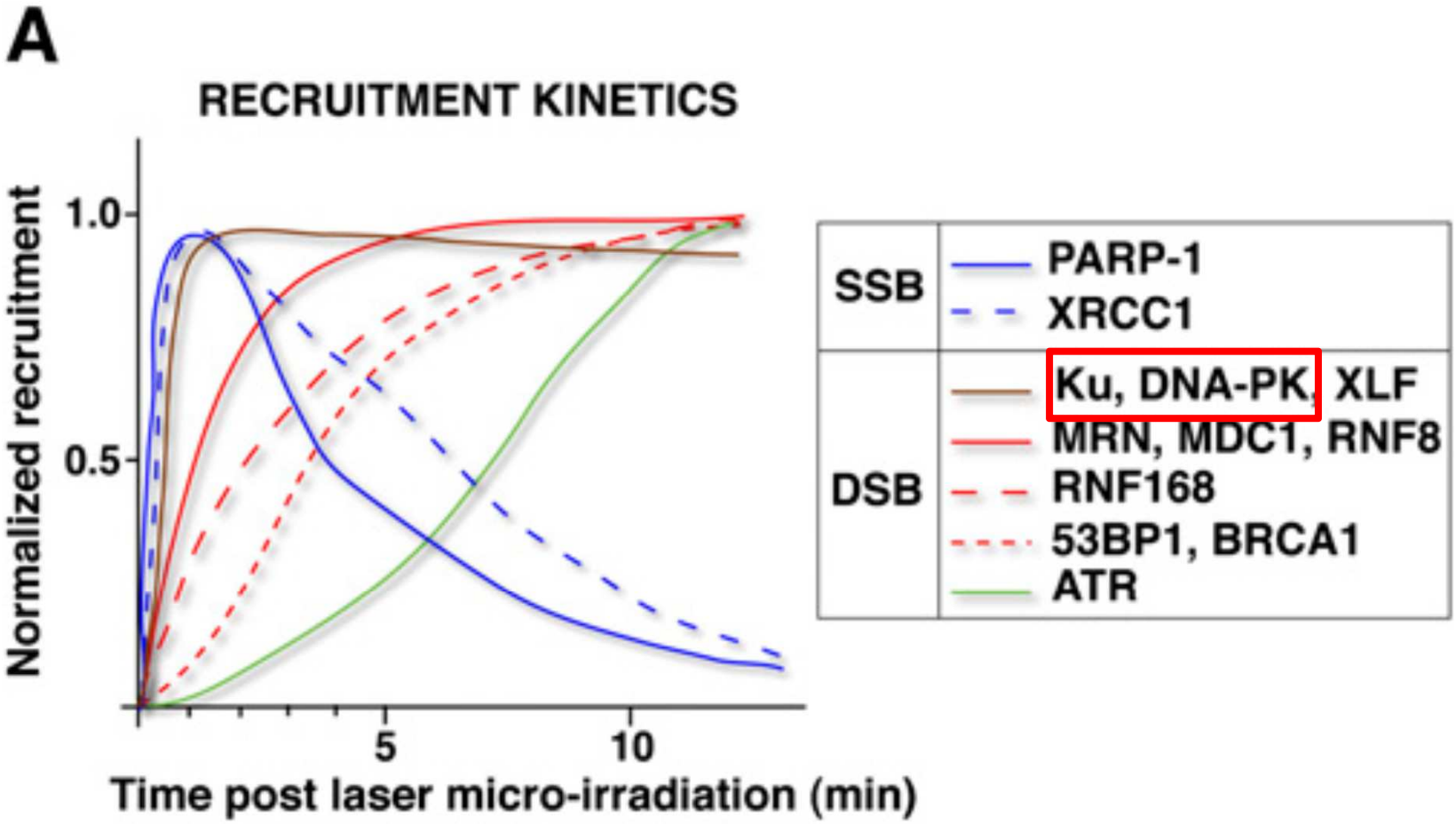
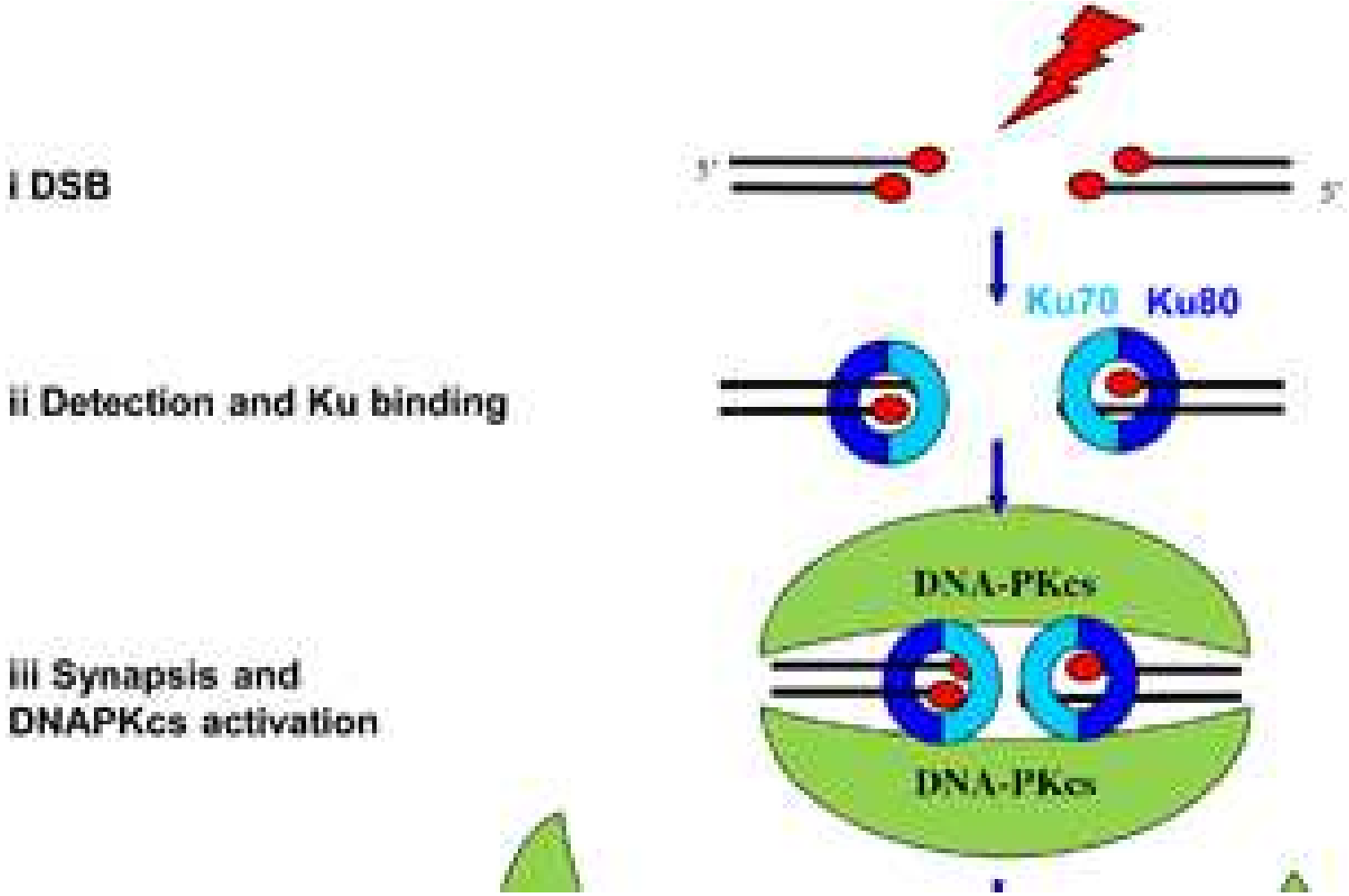
Temporal regulation of DDR protein accumulation at DNA breaks



Non-homologous end joining: Common interaction sites and exchange of multiple factors in the DNA repair process



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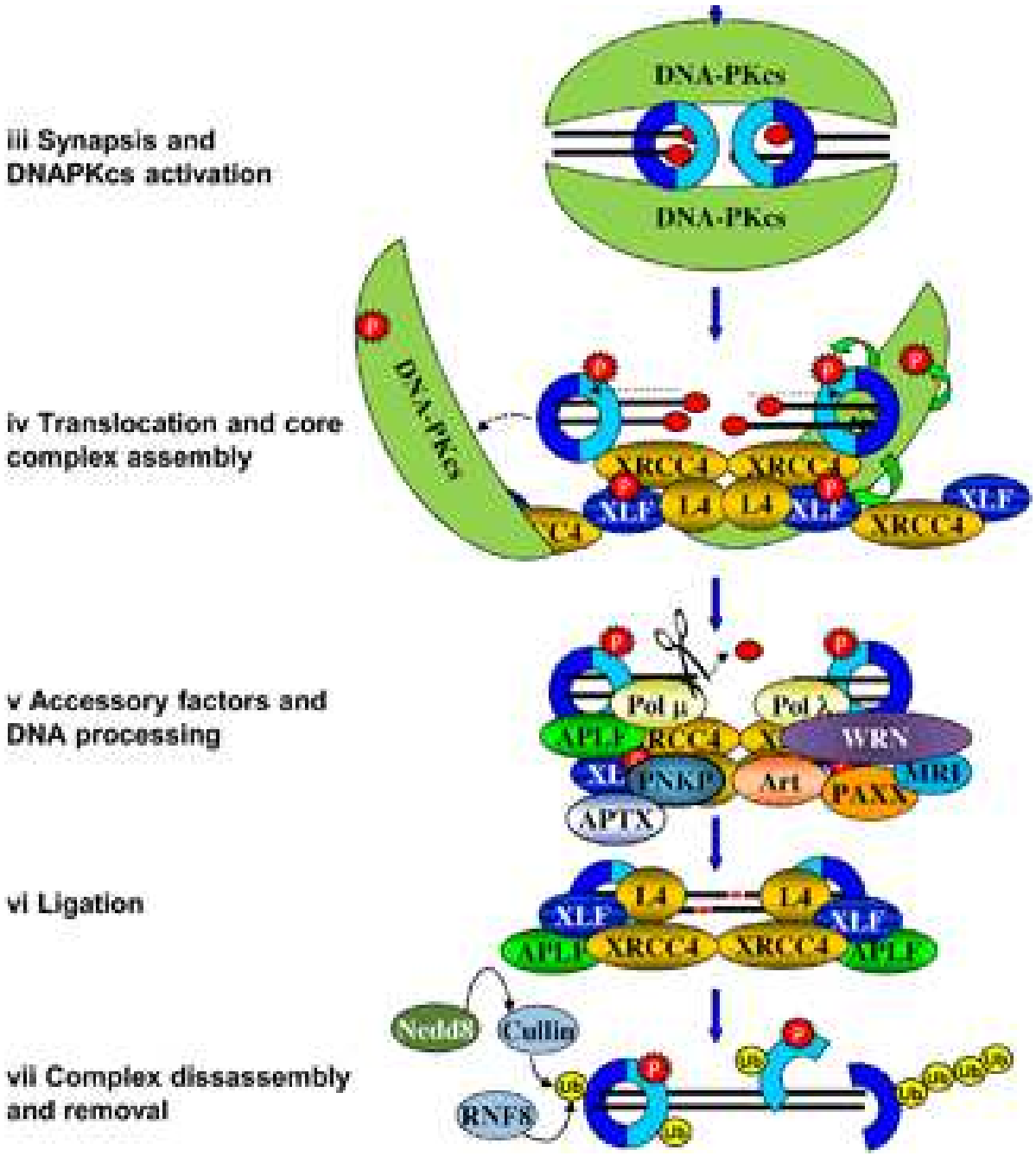

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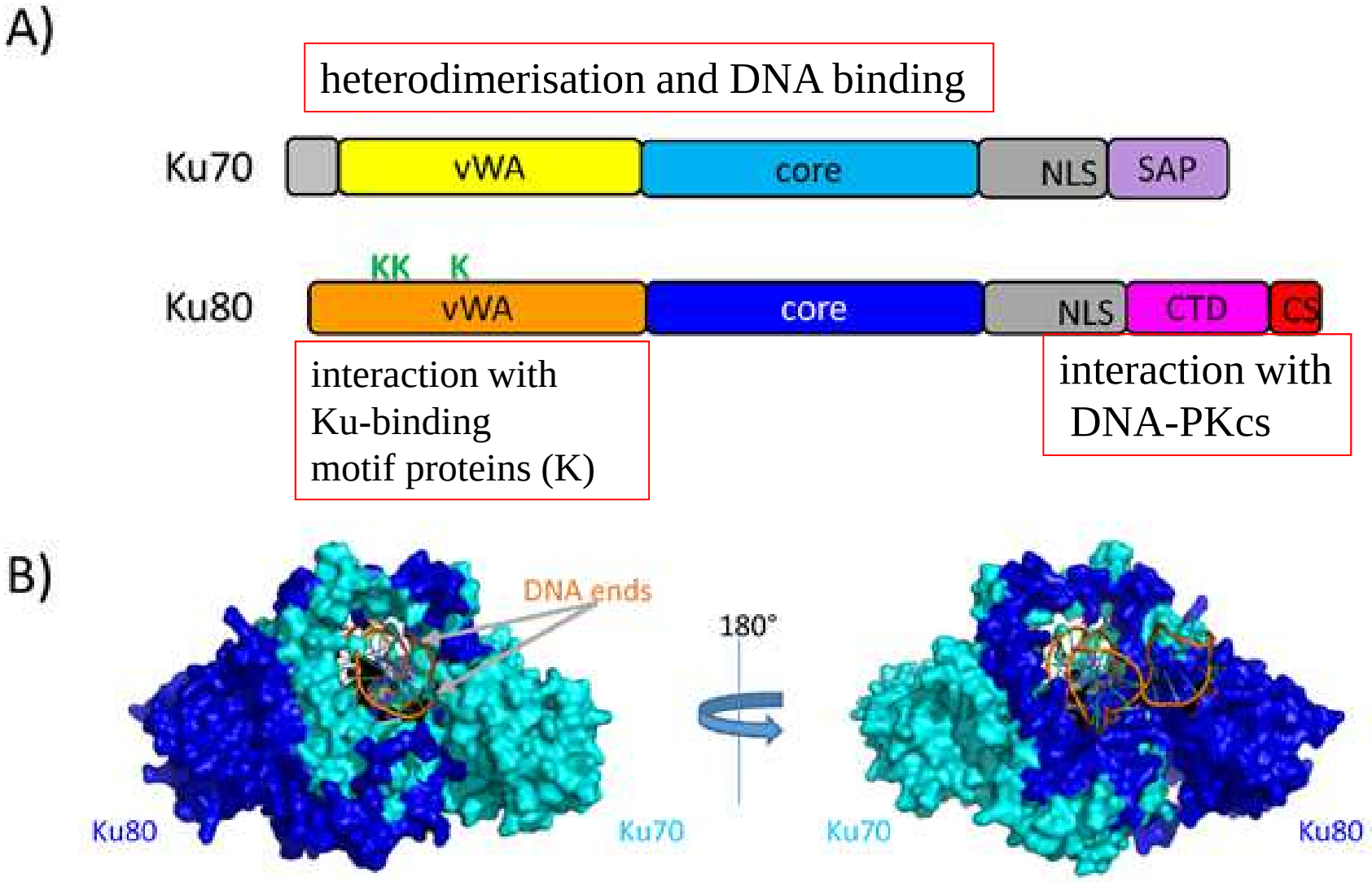
Ku proteins are central to DNA end recognition and recruitment of NHEJ factors



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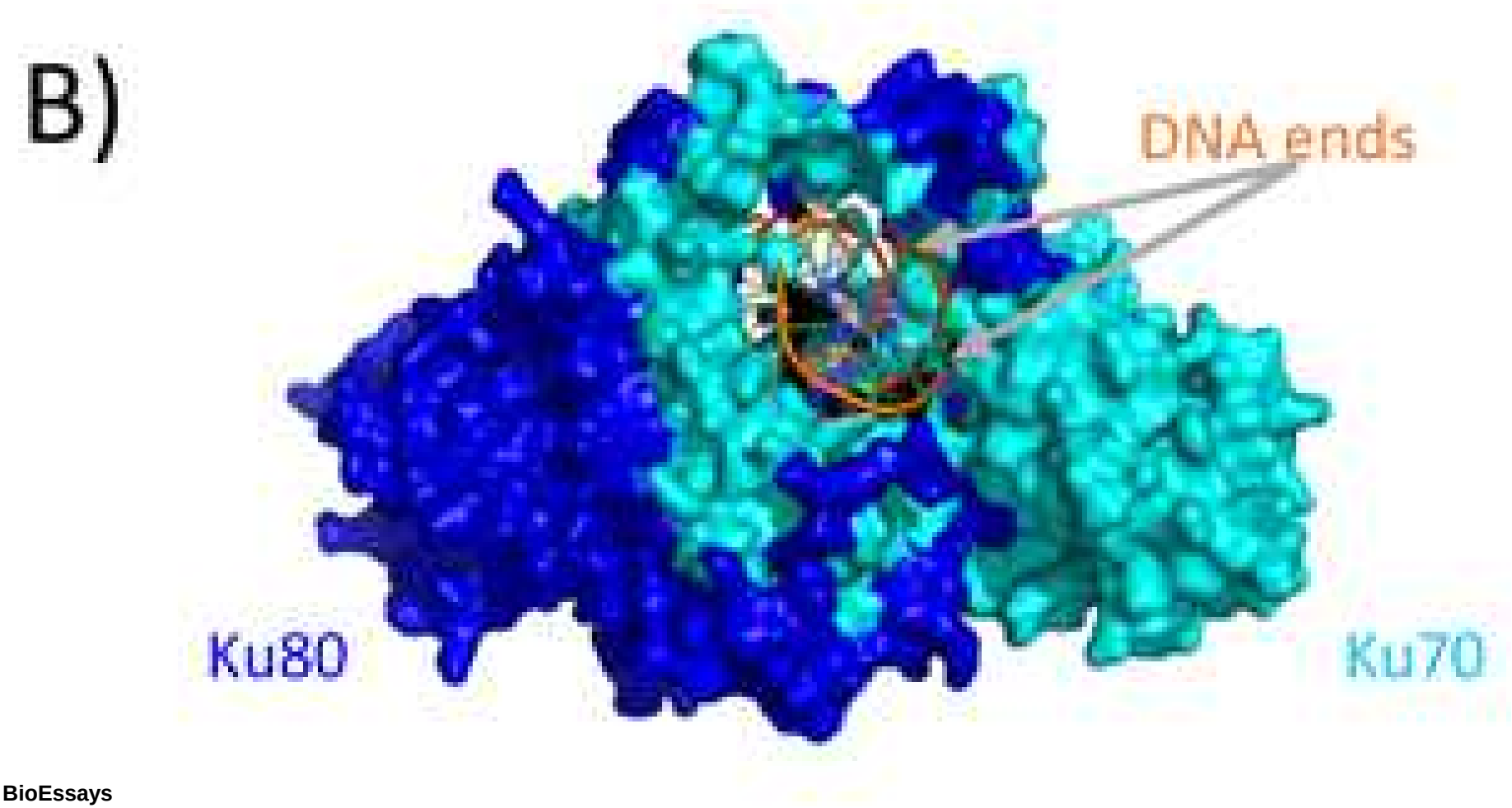

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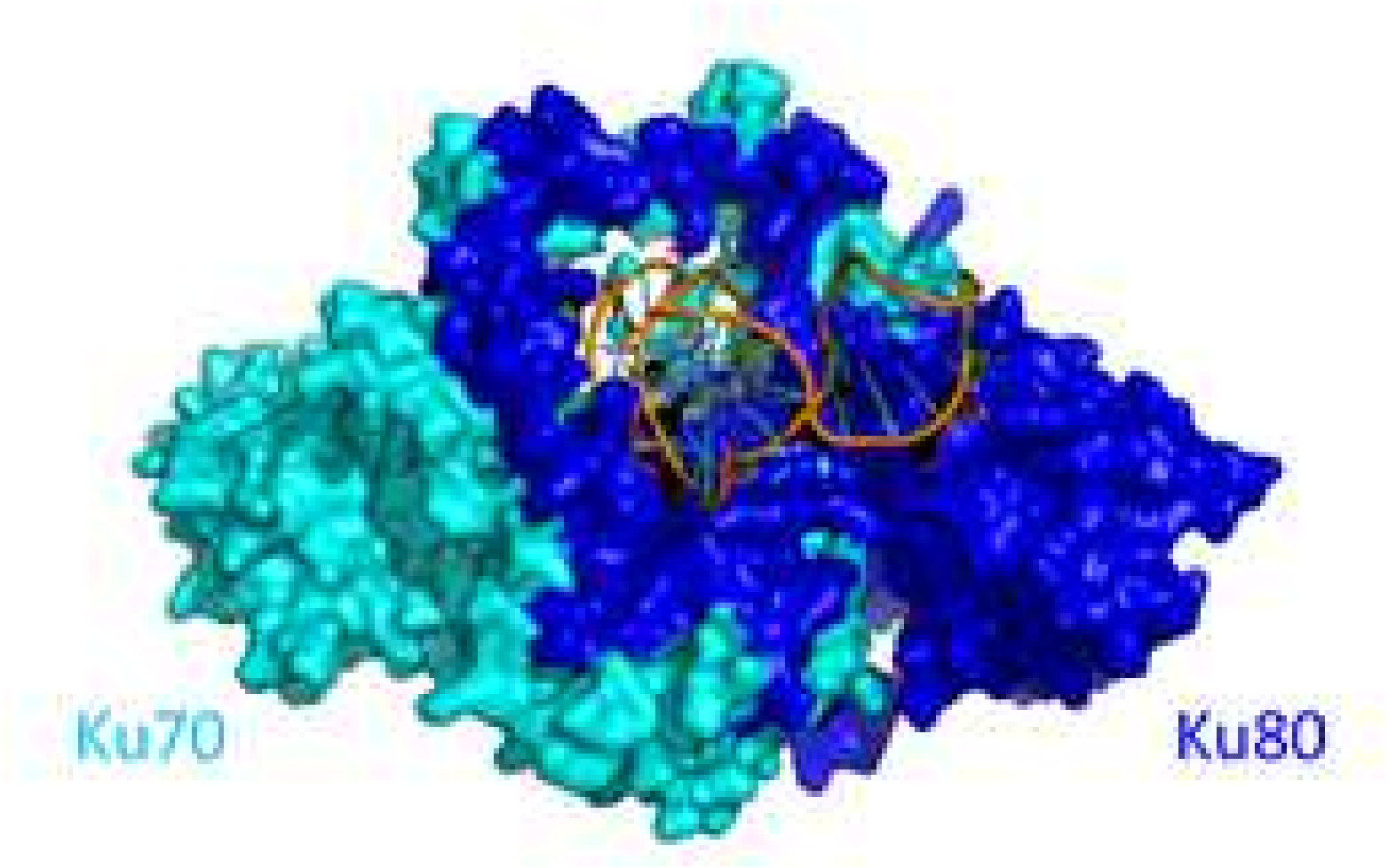

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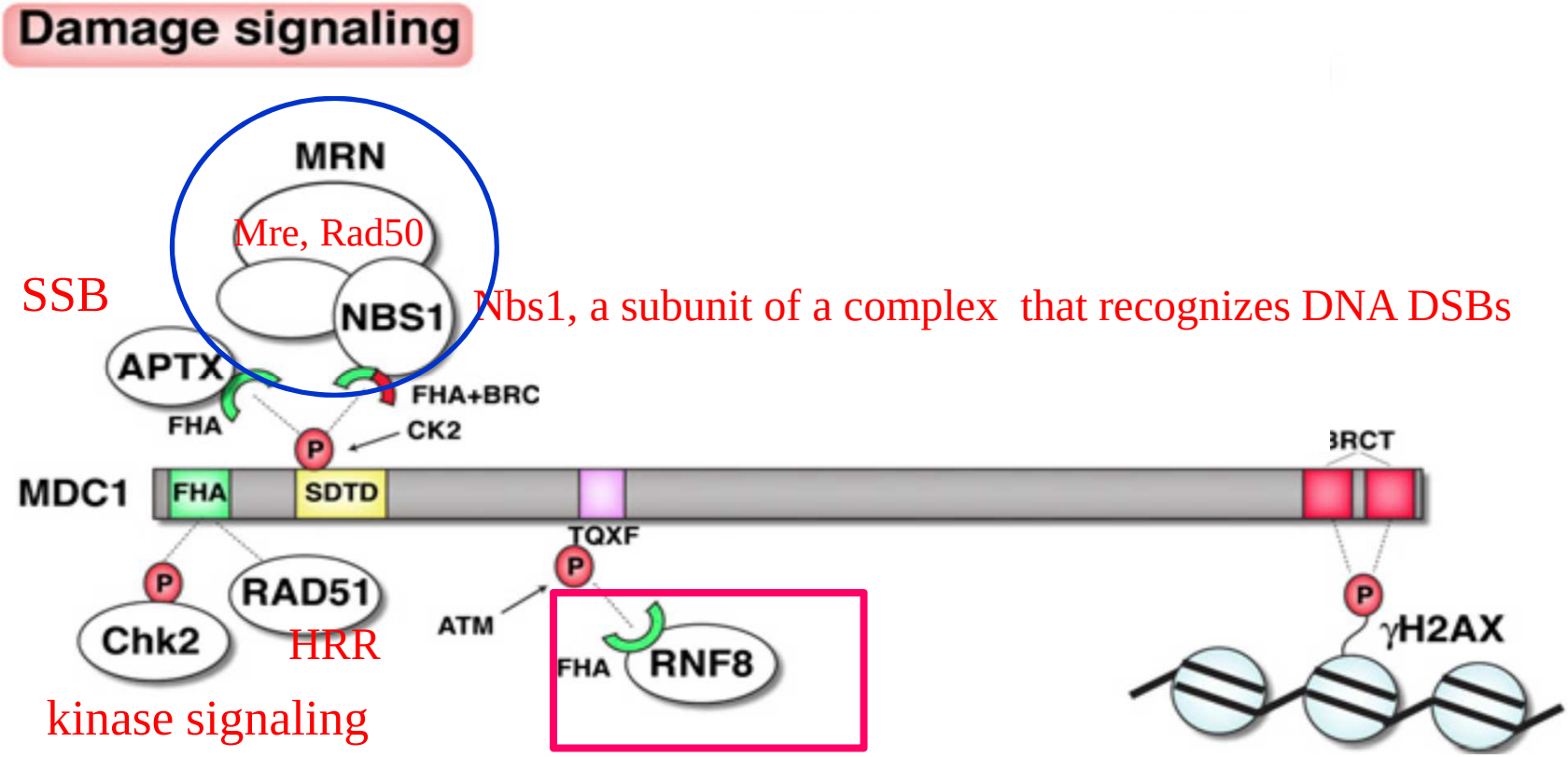
Proteine piattaforma



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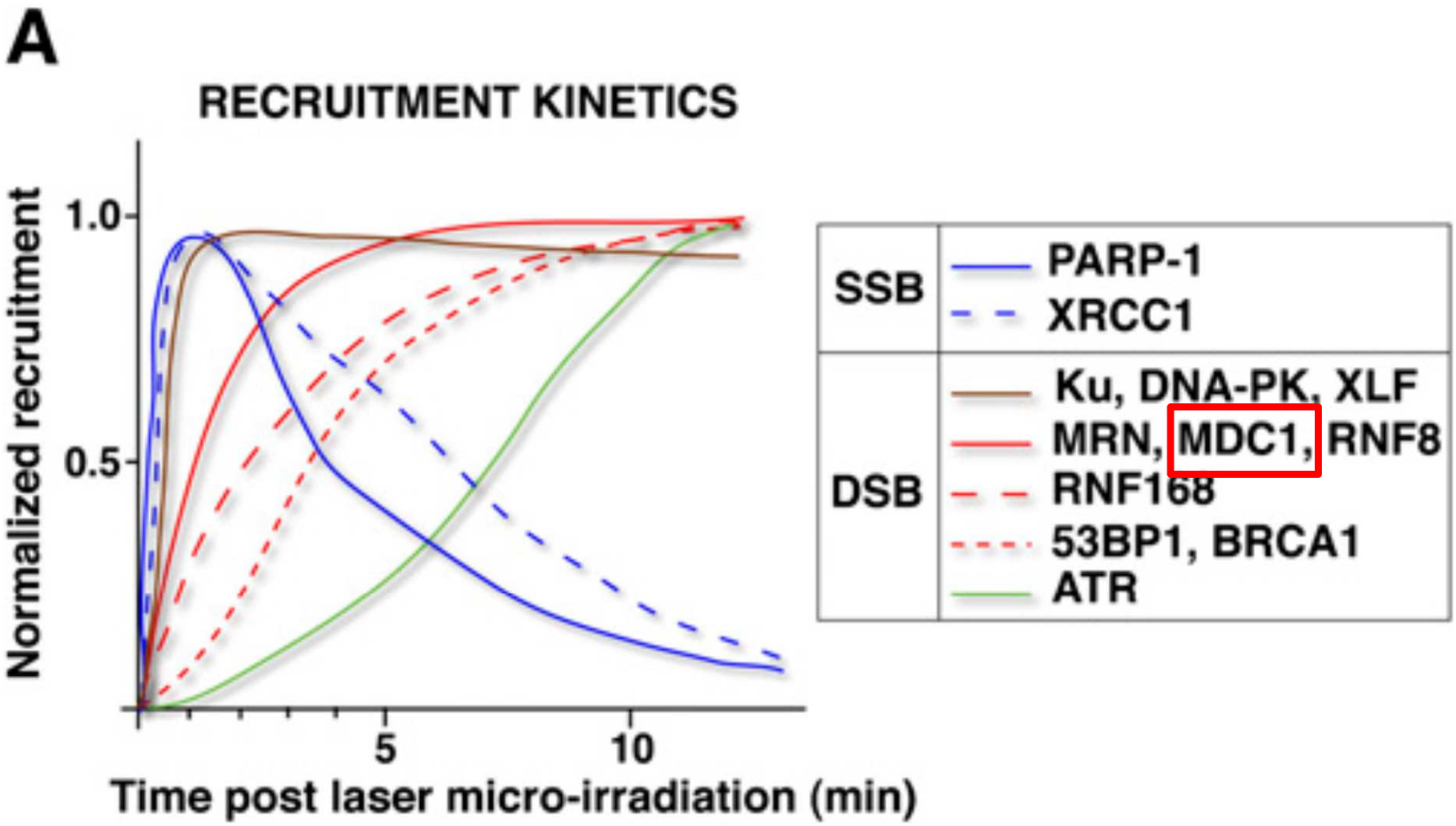

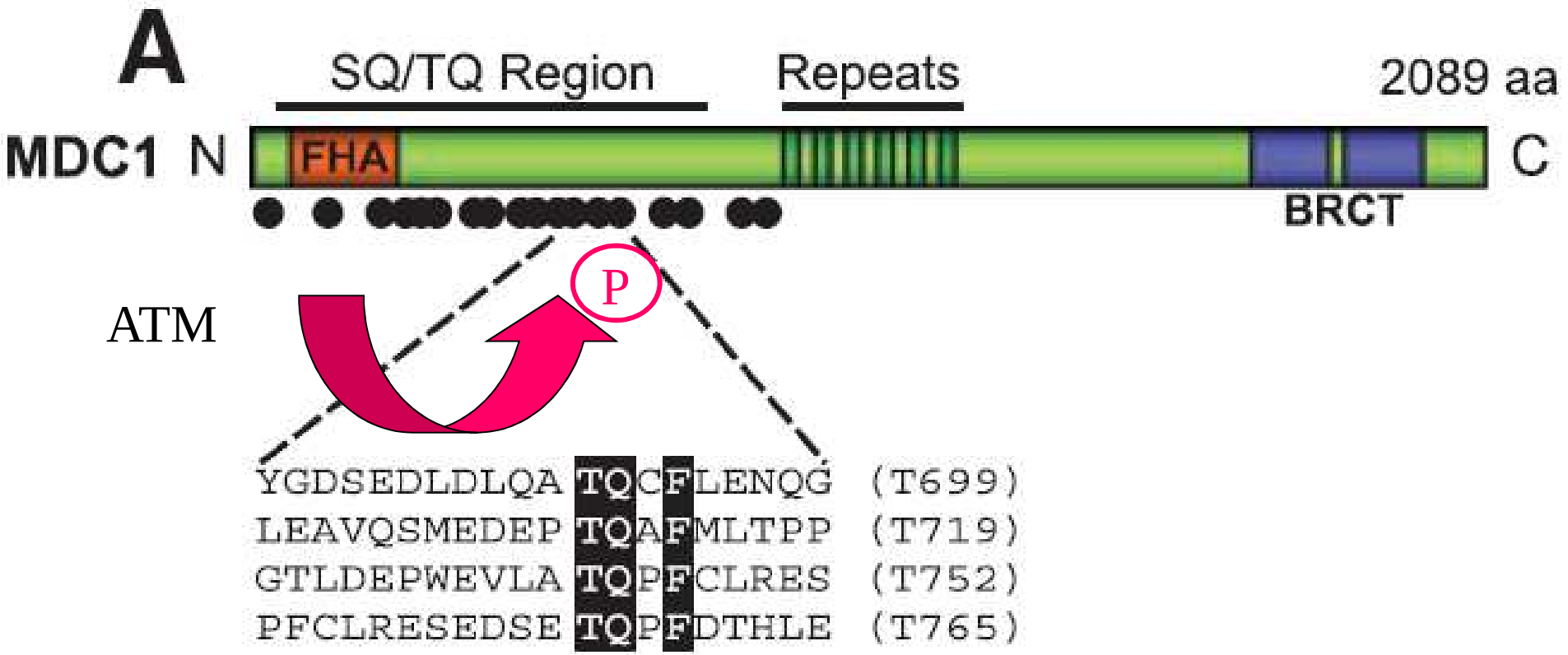

The MDC1 TQXF motifs are ATM targets required for 53BP1 IRIF. (A) Domain architecture of MDC1, with ATM consensus sites (dots).