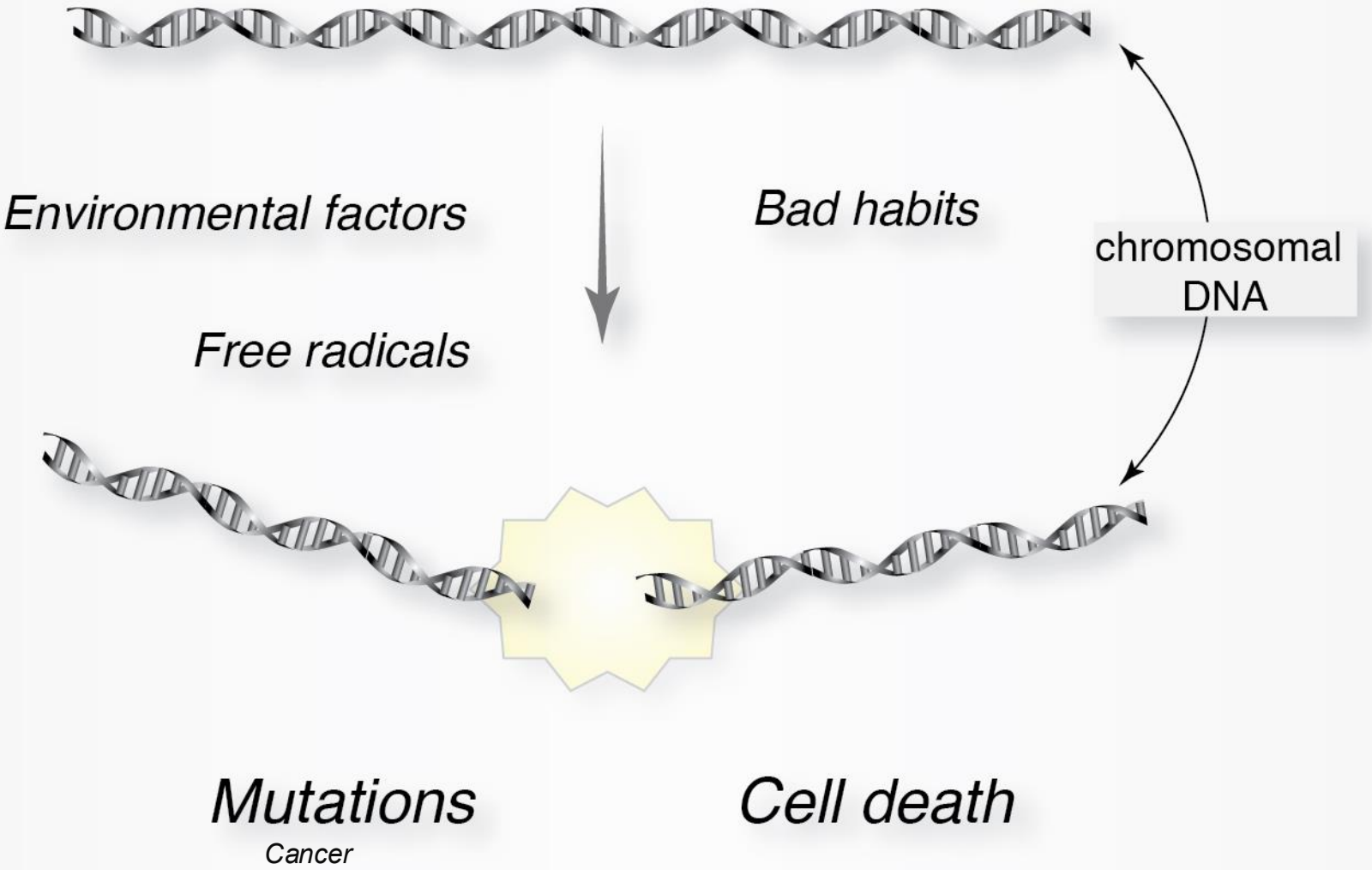
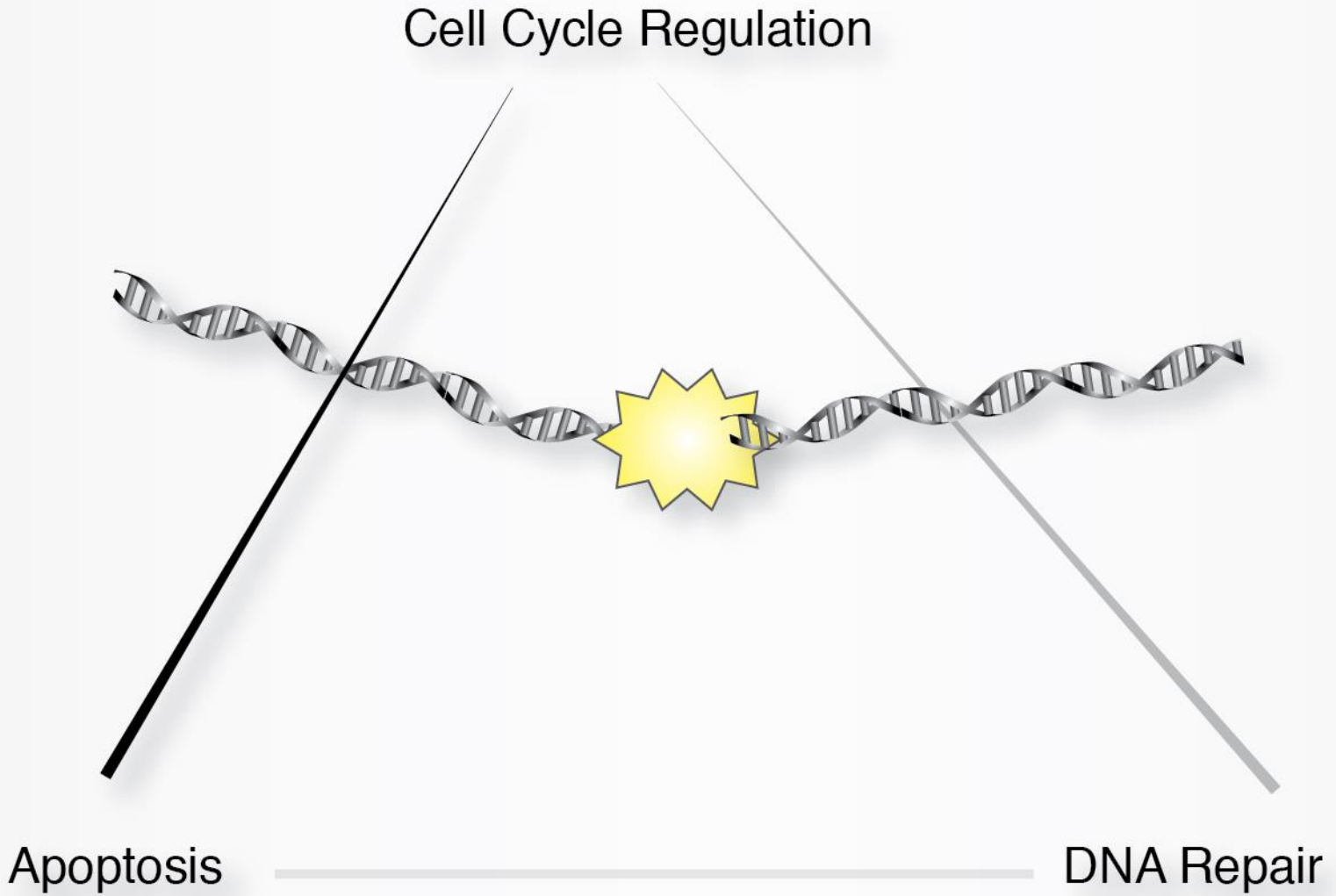
DNA damage is part of everyday life

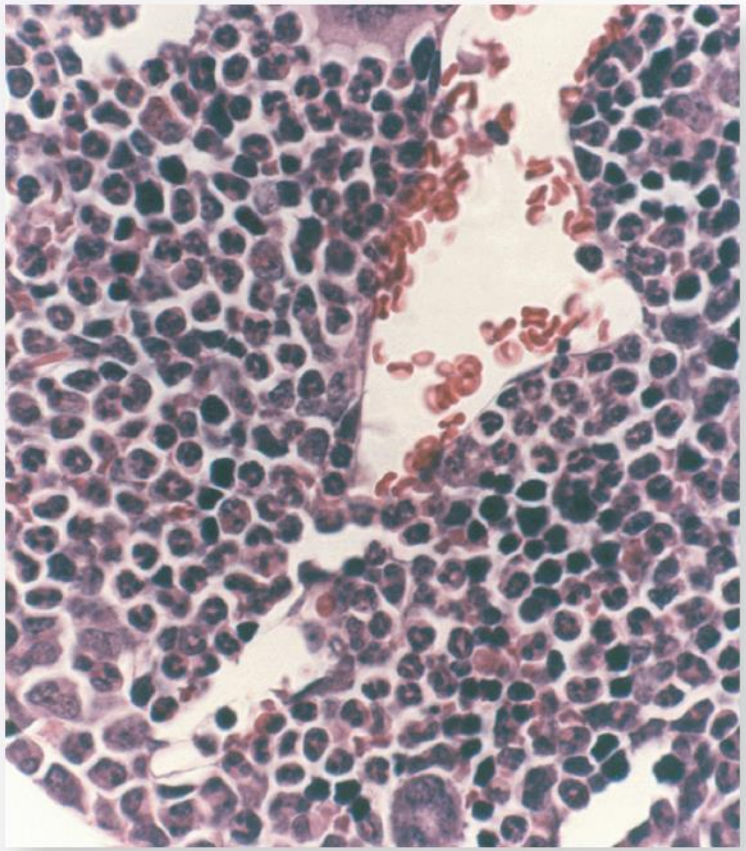


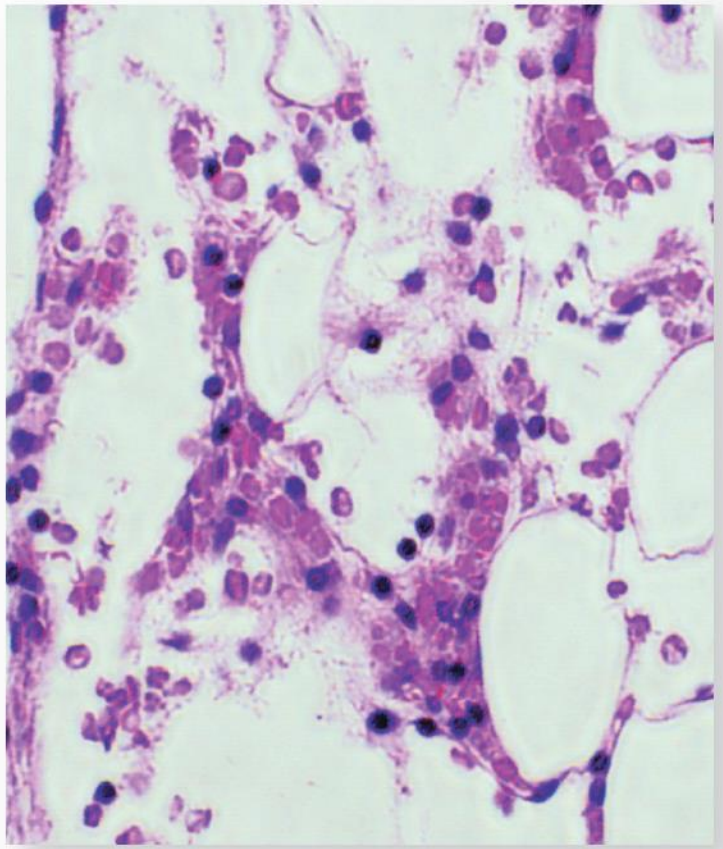
The DNA Damage Response Network



DDR Normal

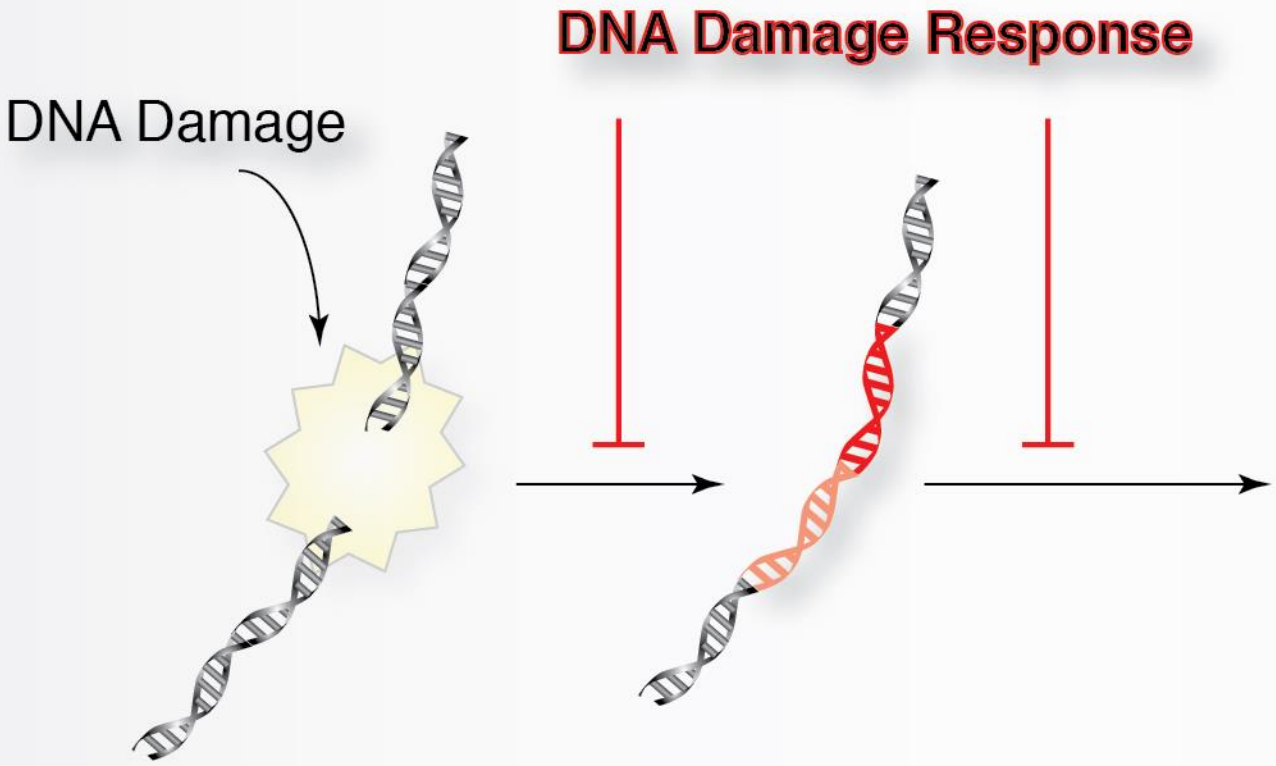
DDR Defective



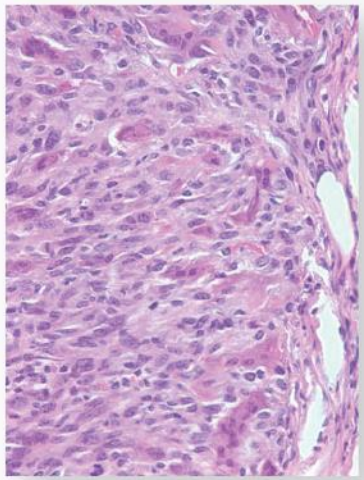


bone marrow

DNA Damage Response Inhibits Carcinogenesis induced by DNA damage



- Cell Death
- Mutation or Rearrangement
- Changes in Gene Expression

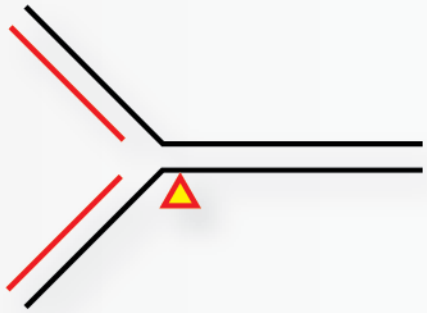


Malignancy

Table 1. DNA Lesions Generated by Endogenous and Exogenous DNA Damage

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day	
Depurination	AP site	10000[a]	
Cytosine deamination	Base transition	100–500[a]	
SAM-induced methylation	3meA	600[a]	
	7meG	4000[a]	
	O^6meG	10–30[b]	
Oxidation	8oxoG	400–1500[c]	
Exogenous DNA Damage	**Dose Exposure (mSv)**	**DNA Lesions Generated**	**Estimated Number Lesions/Cell**
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

DNA replication stress



RPA

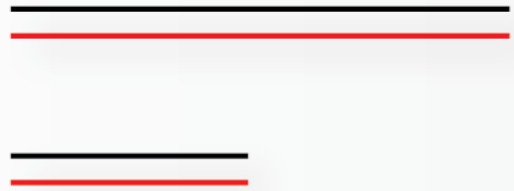
↓

ATR

↓

- *Arrest*
- *Genome integrity*
- *Viability*

DNA damage



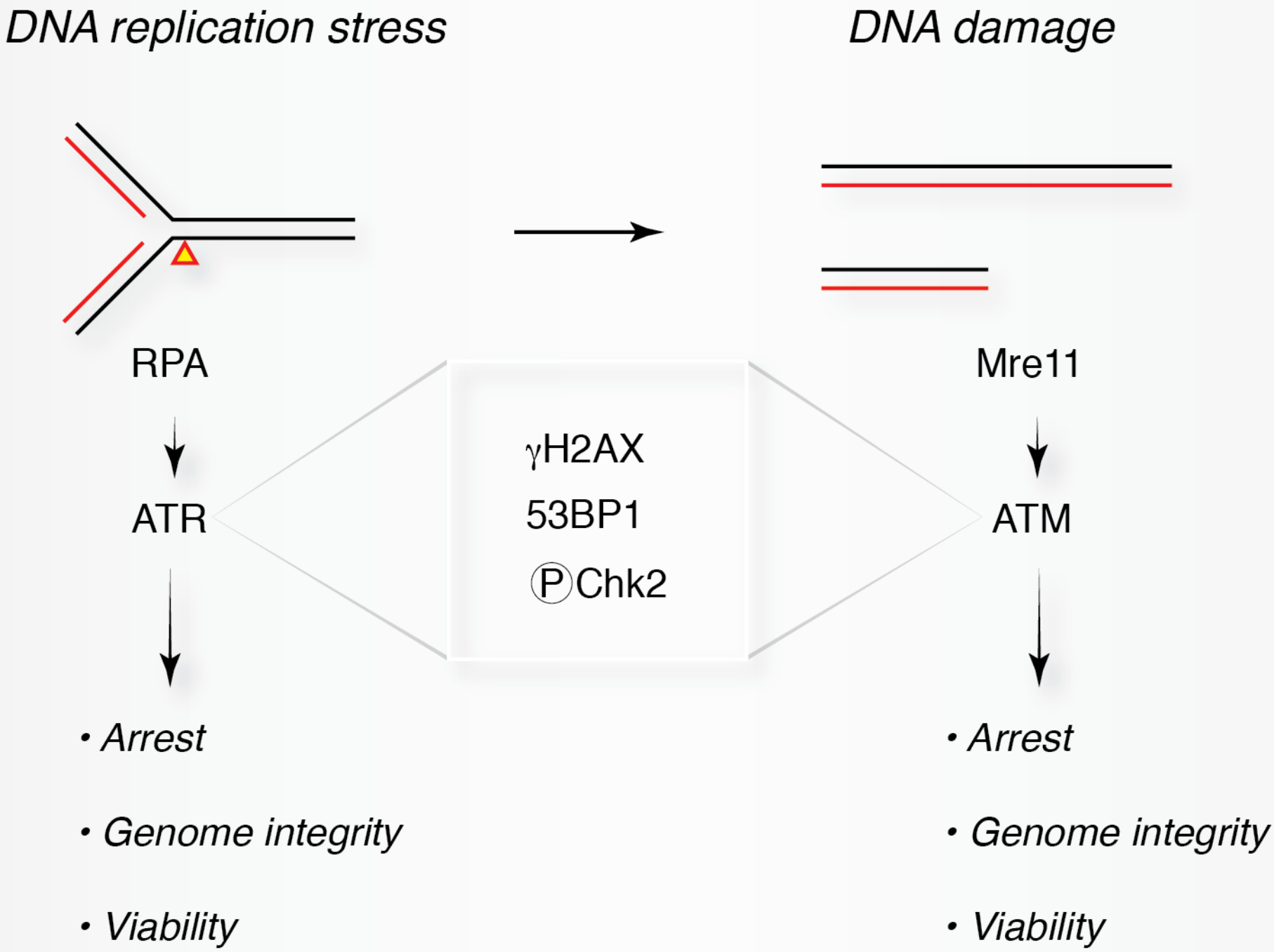
Mre11

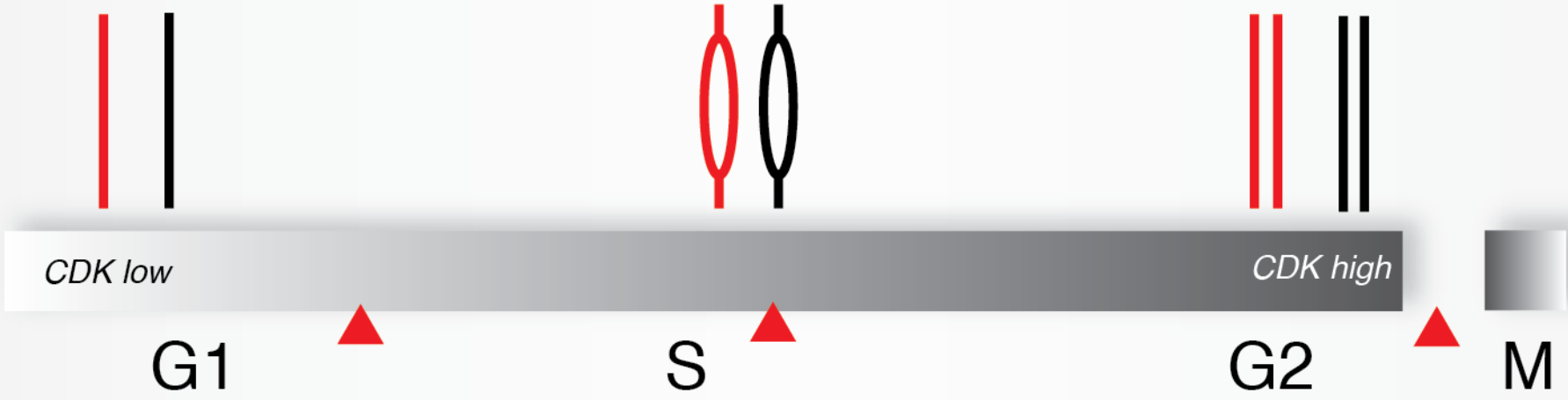
↓

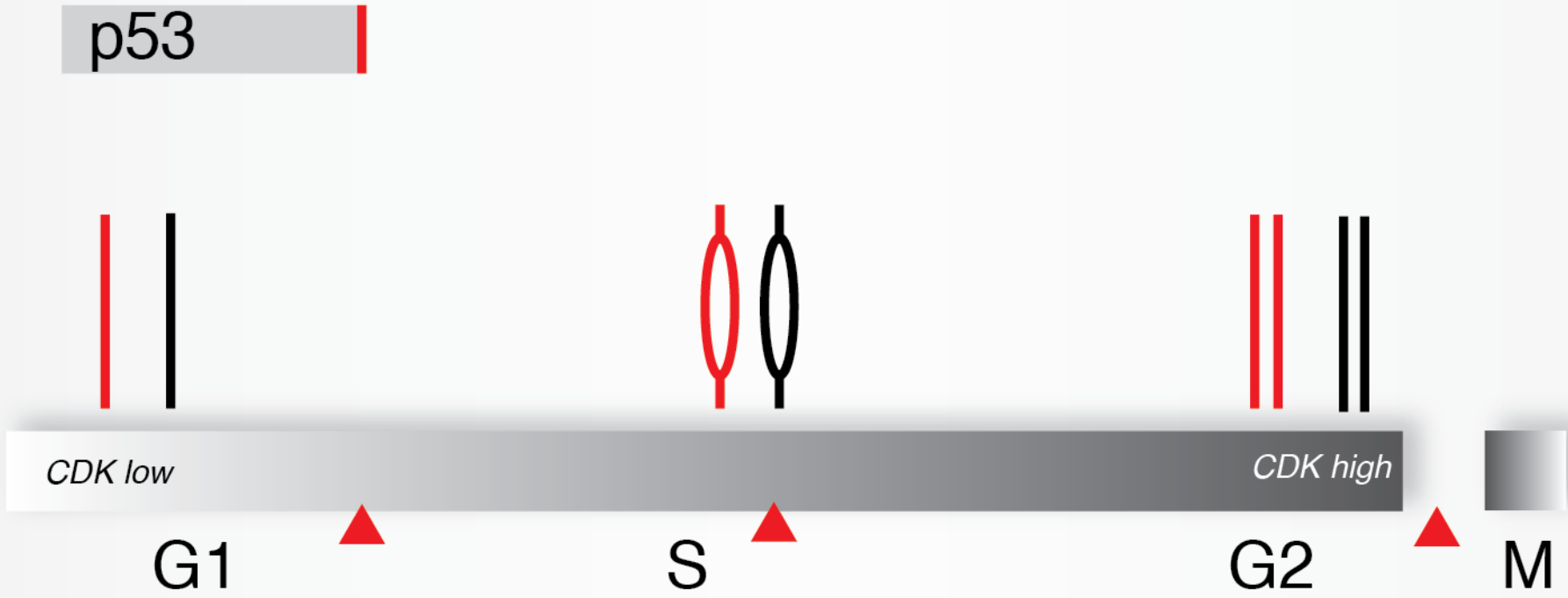
ATM

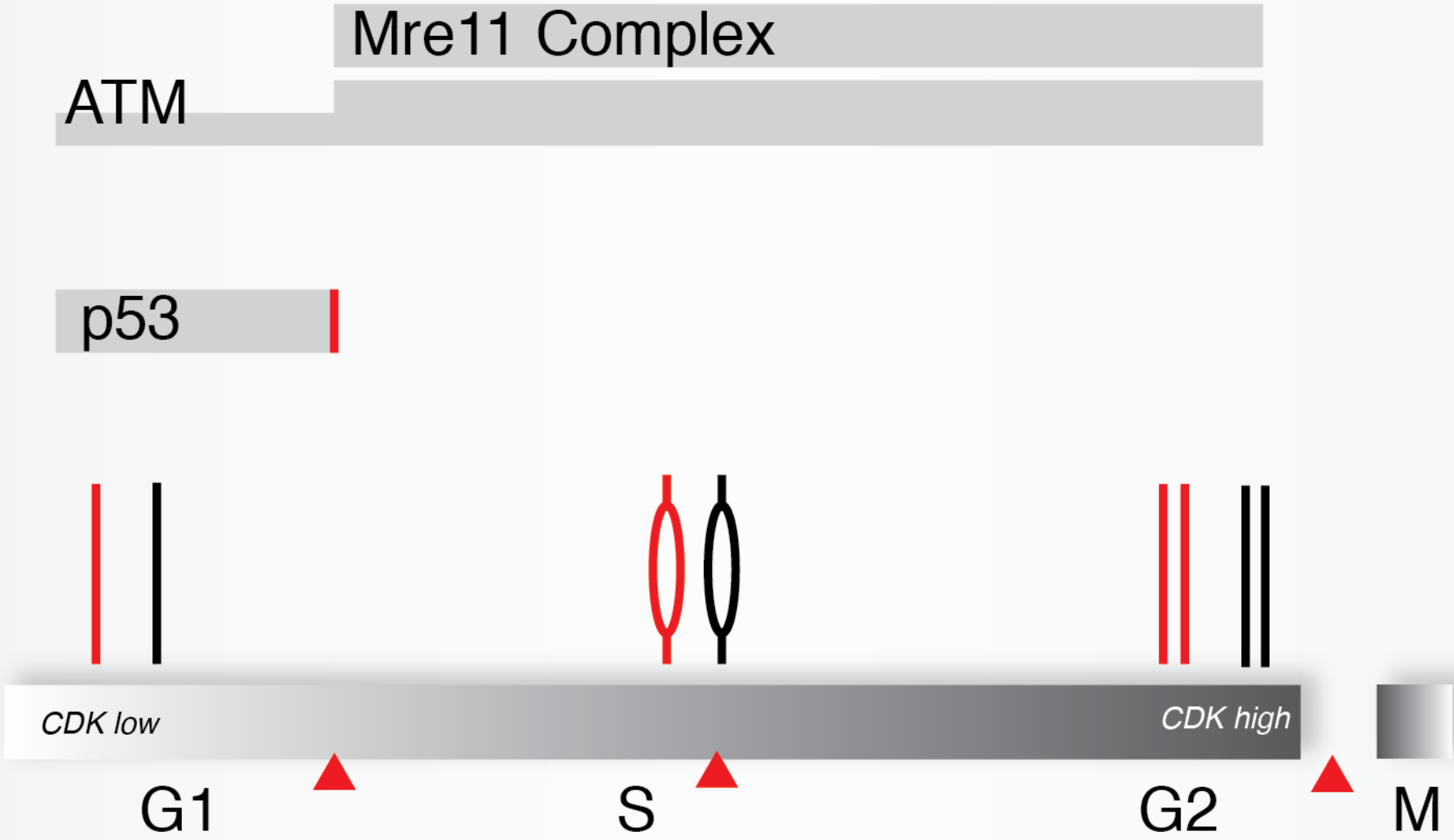
↓

- *Arrest*
- *Genome integrity*
- *Viability*

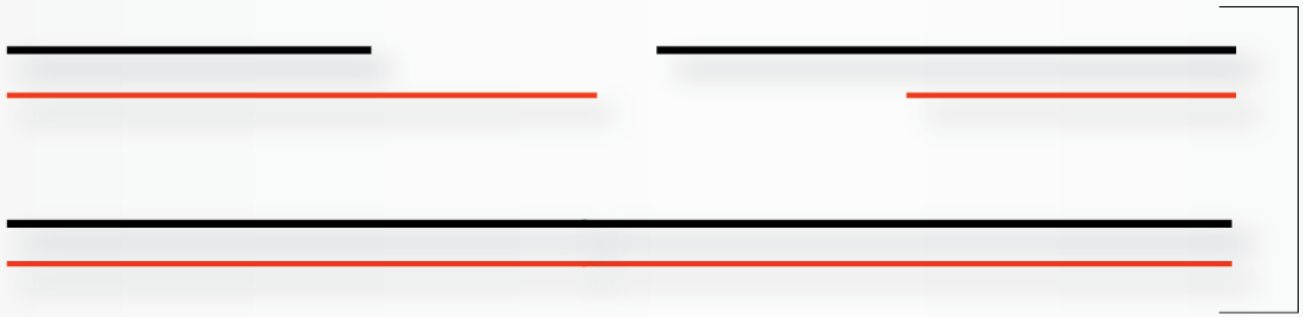

DNA replication stress
DNA damage
RPA
Mre11
γH2AX
53BP1
ⓅChk2
ATR
ATM
• Arrest
• Genome integrity
• Viability
• Arrest
• Genome integrity
• Viability


CDK low
CDK high
G1
S
G2
M


p53
CDK low
CDK high
G1
S
G2
M


Mre11 Complex
ATM
p53
CDK low
CDK high
G1
S
G2
M

Where do DNA double strand breaks come from?



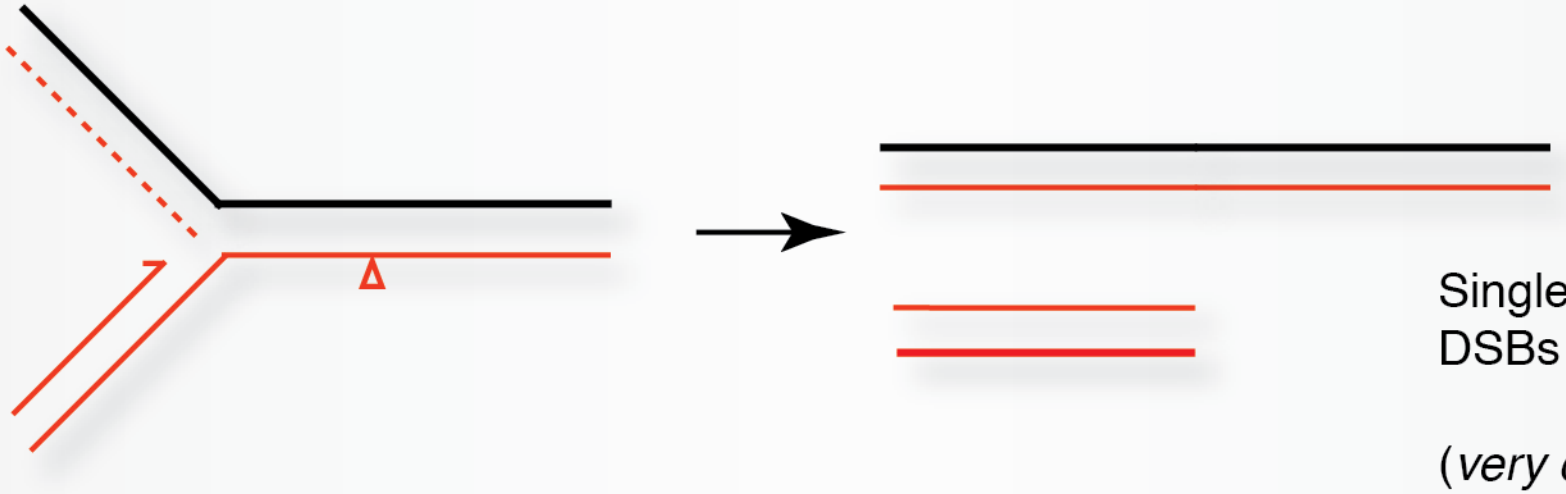
Meiosis
(*common*)

IR
(*uncommon!*)

Where do DNA double strand breaks come from?

Meiosis
(*common*)

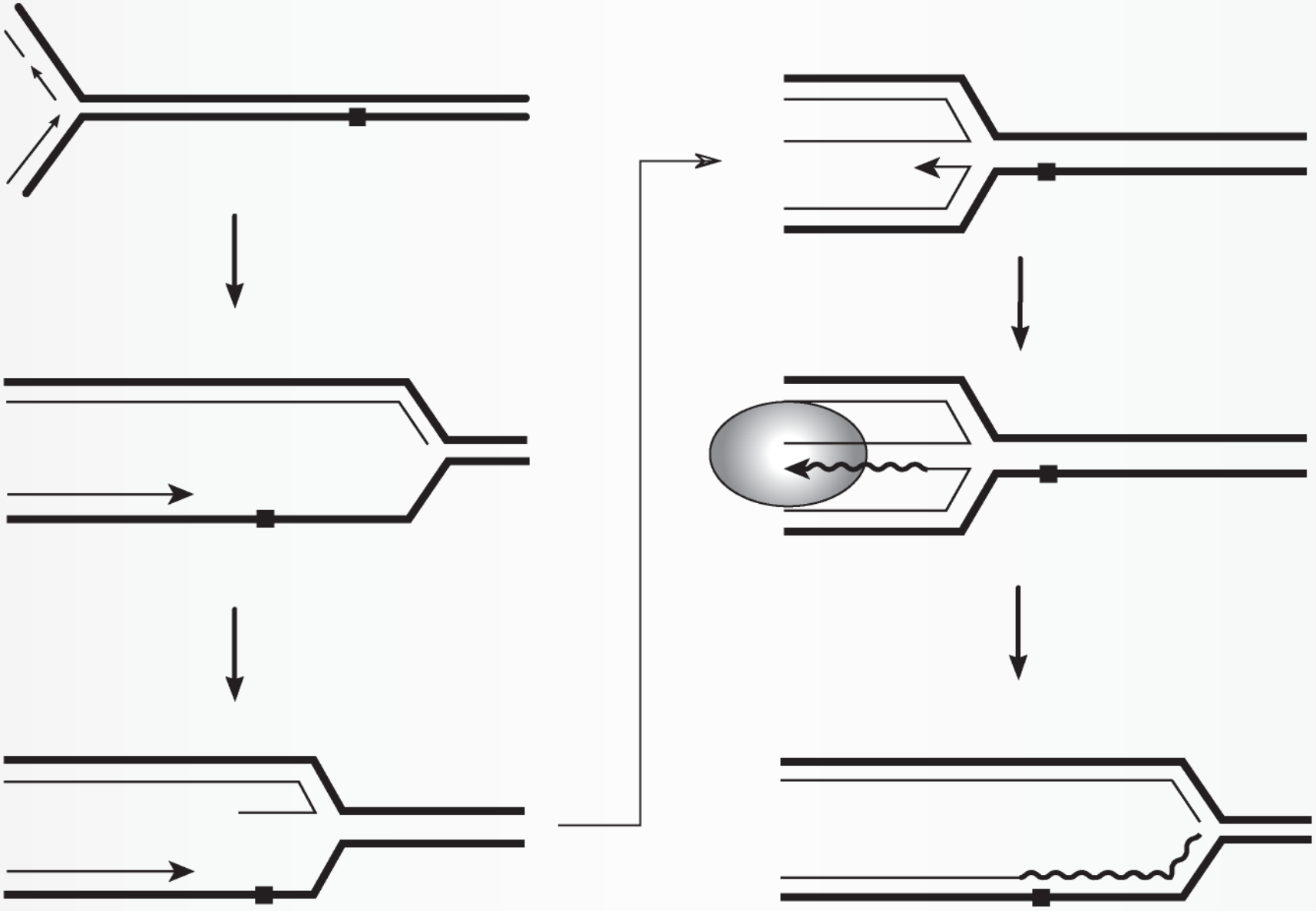
IR
(*uncommon!*)

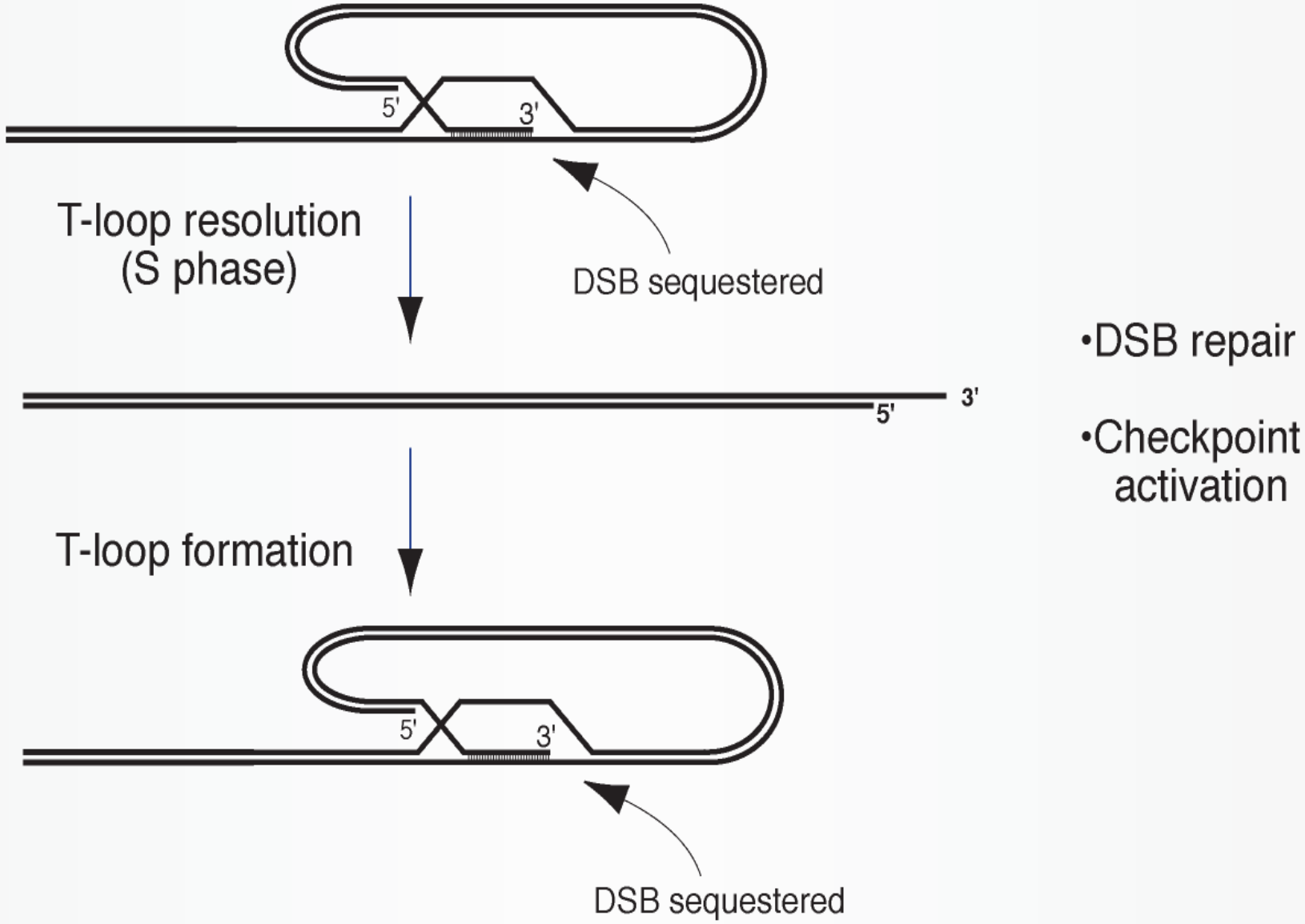


Single-ended
DSBs

(*very common!*)

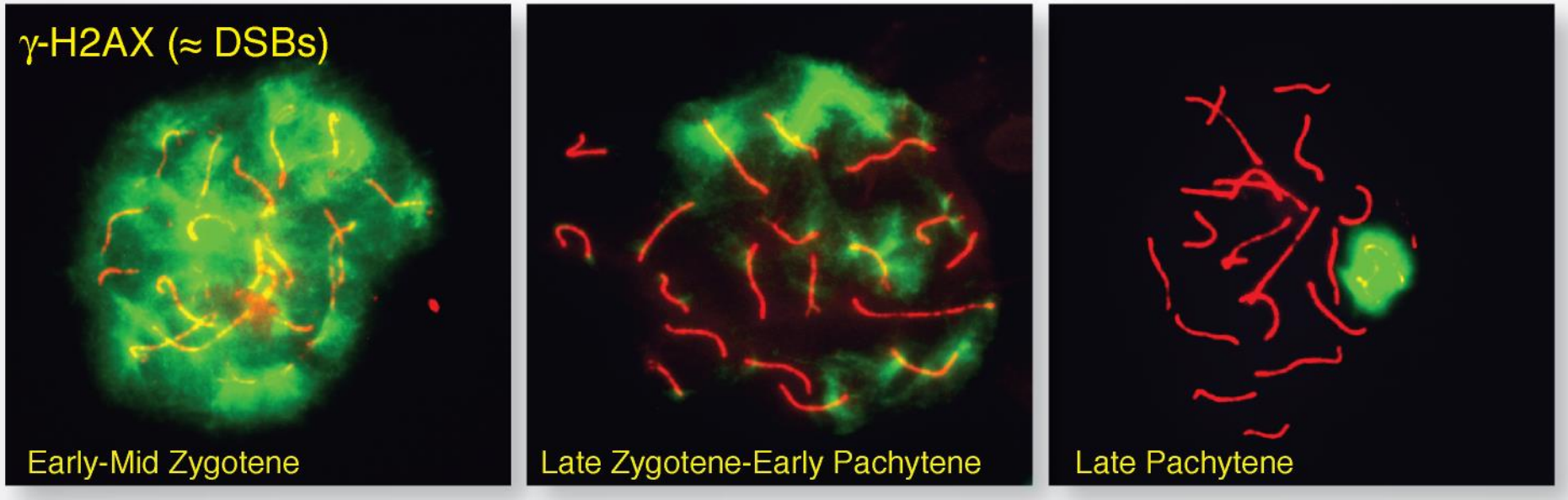
Repair + Replication Restart

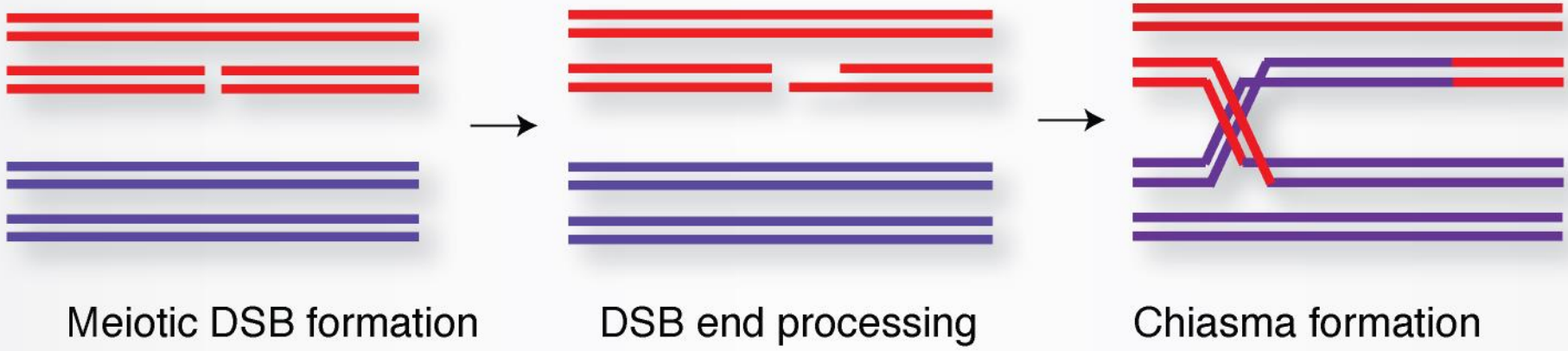




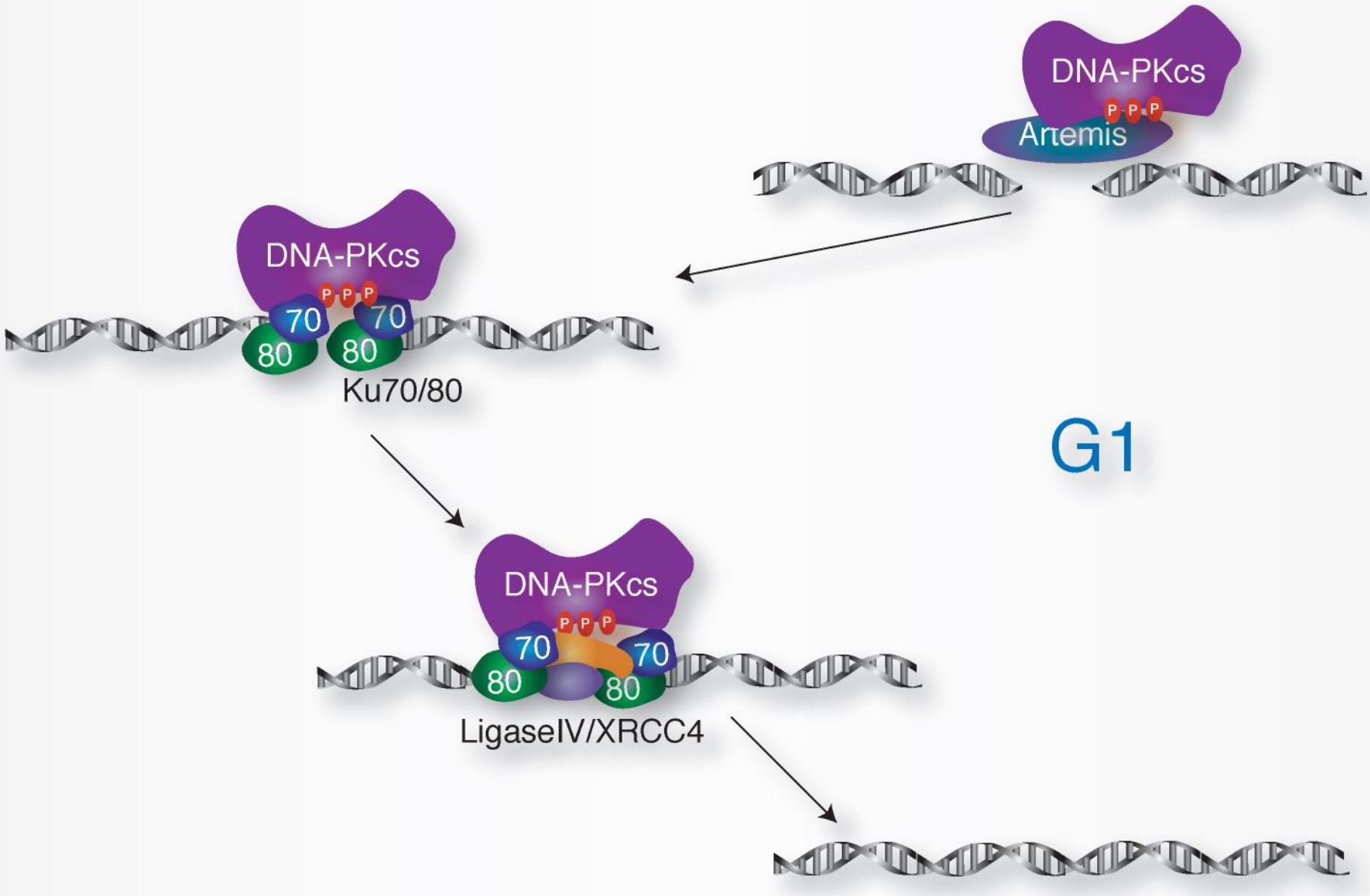
- DSB repair
- Checkpoint activation

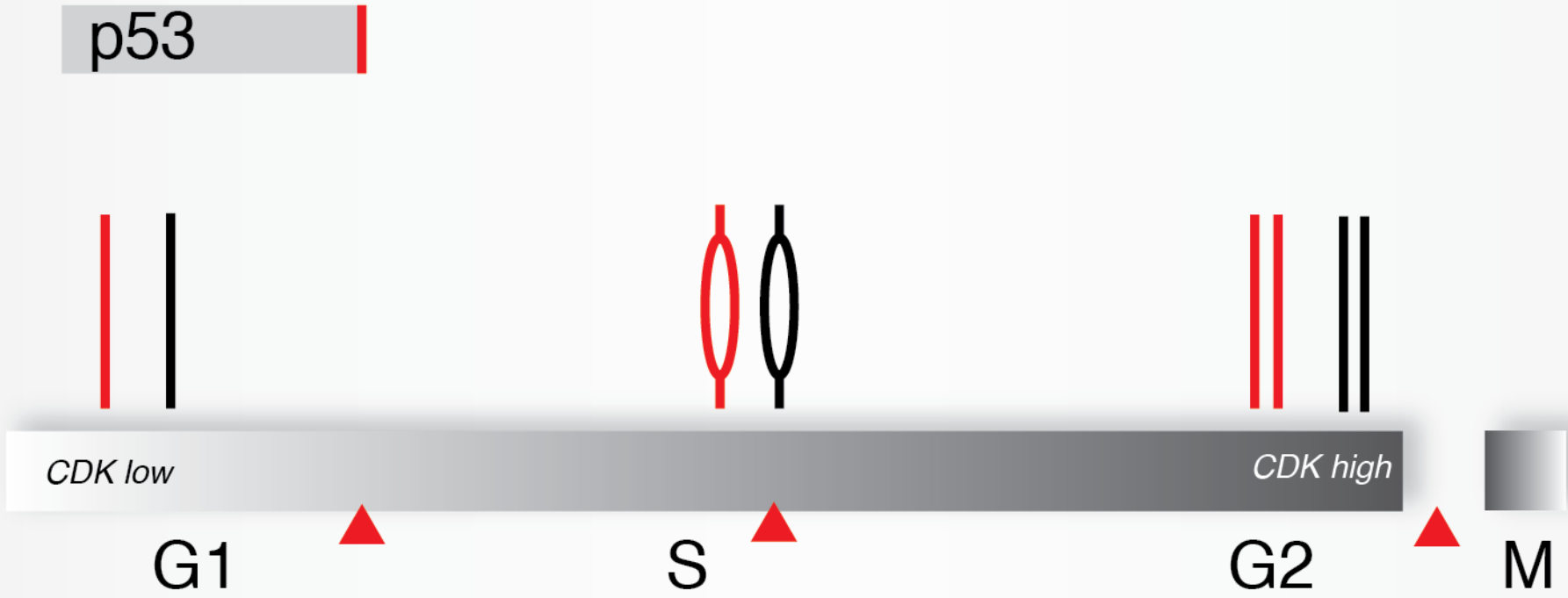
DSBs are induced to initiate meiotic recombination

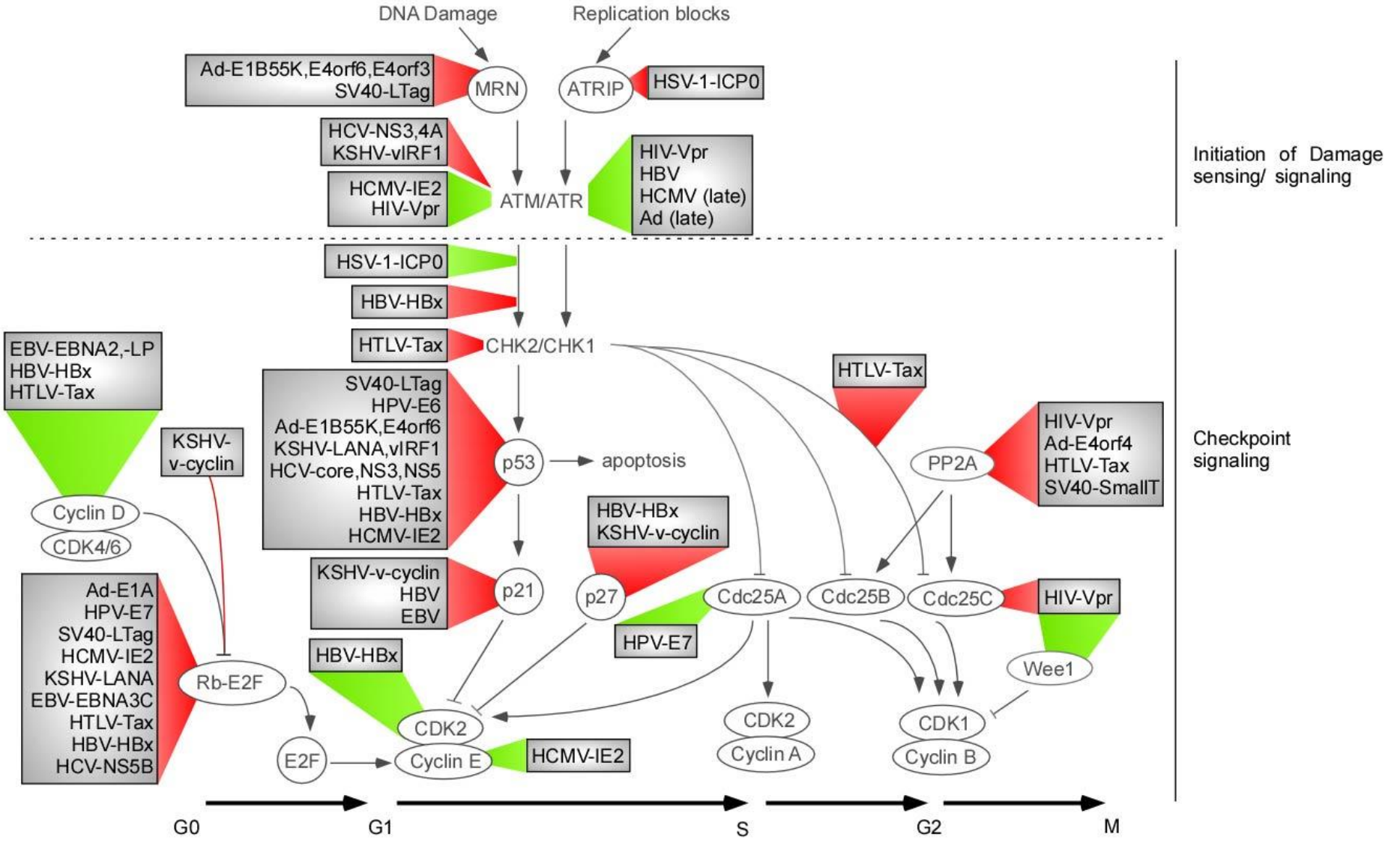




Antigen receptor gene assembly: DSBs in G1

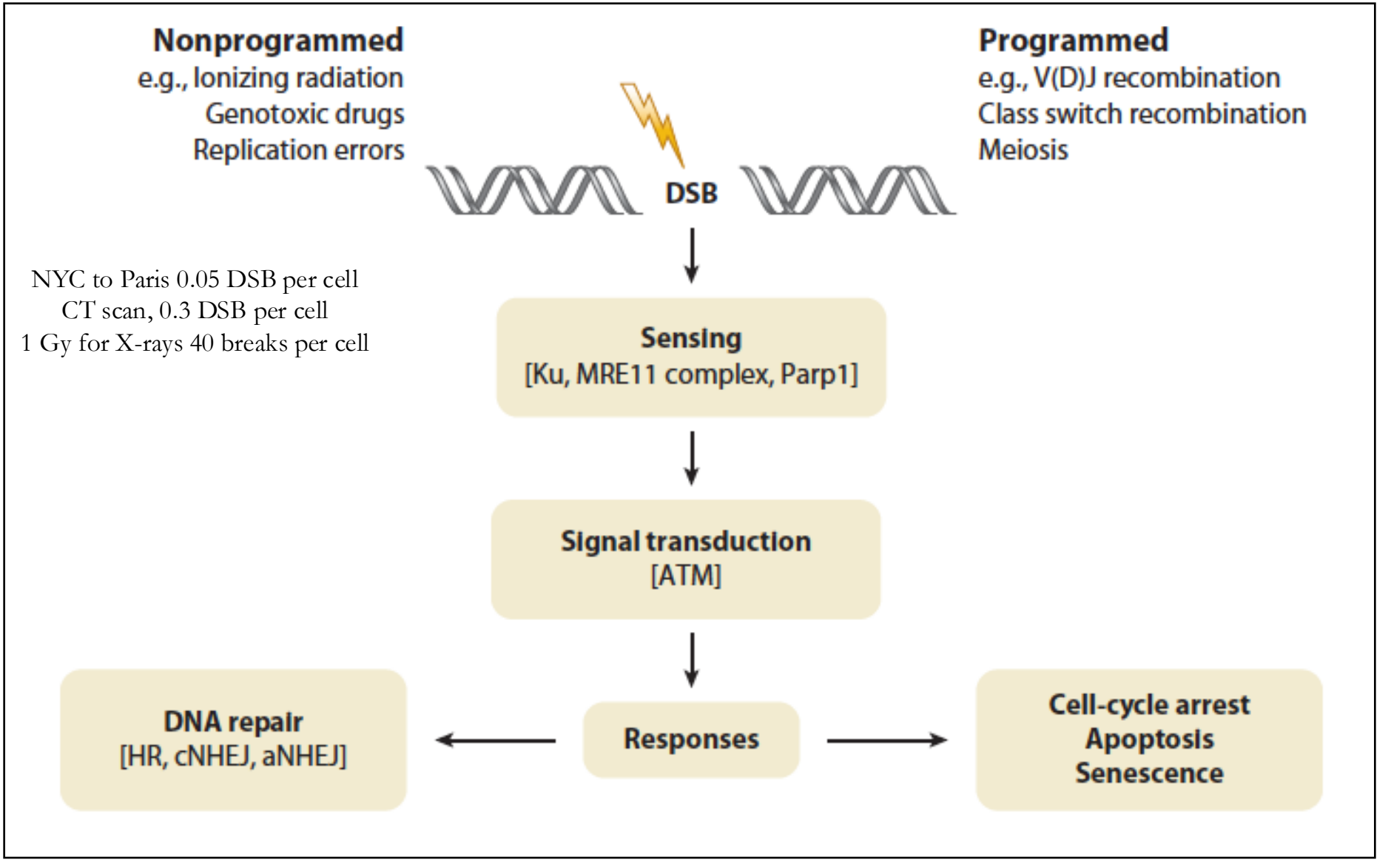



p53
CDK low
CDK high
G1
S
G2
M

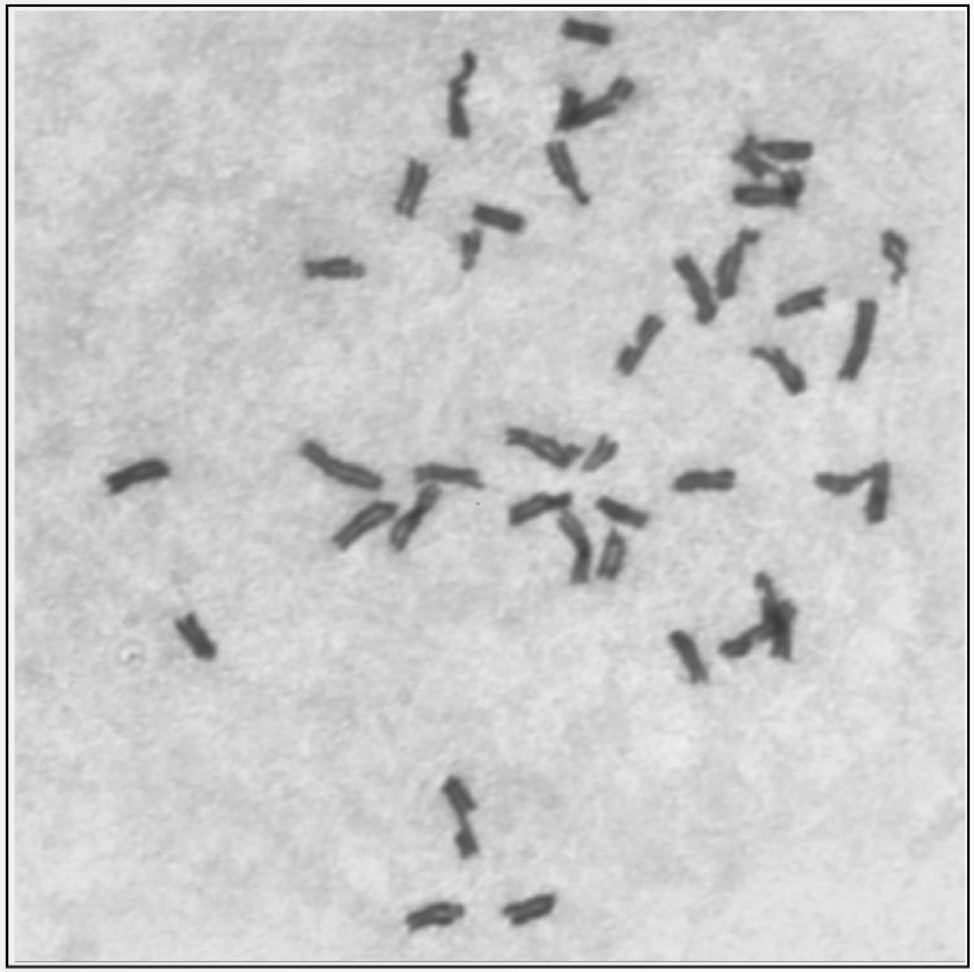


Chaurushiya & Weitzman 2009

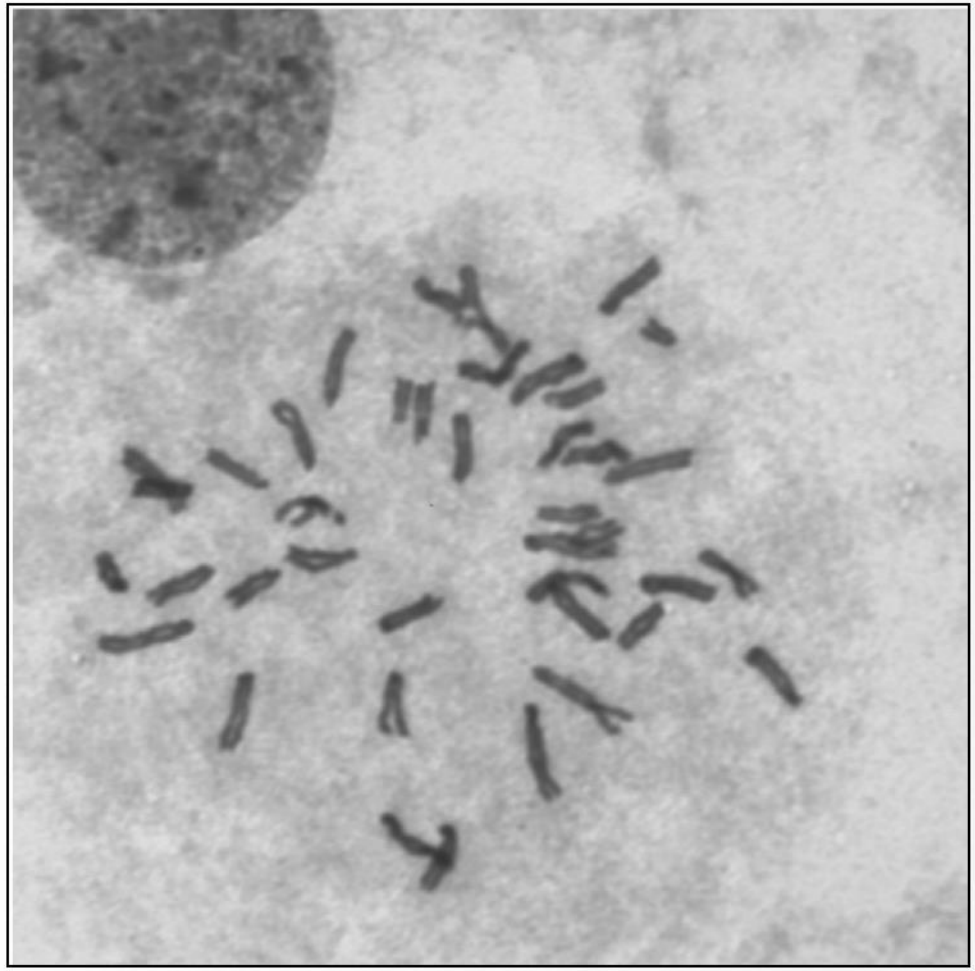
DNA double-strand break repair



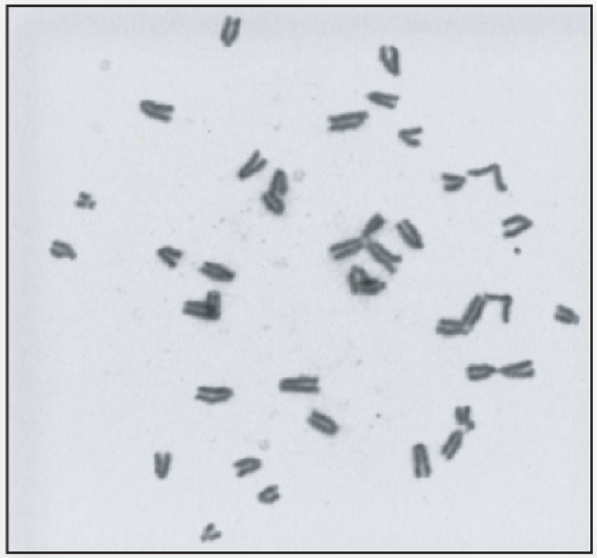
Deriano and Roth. Annu. Rev. Genet. 2013.



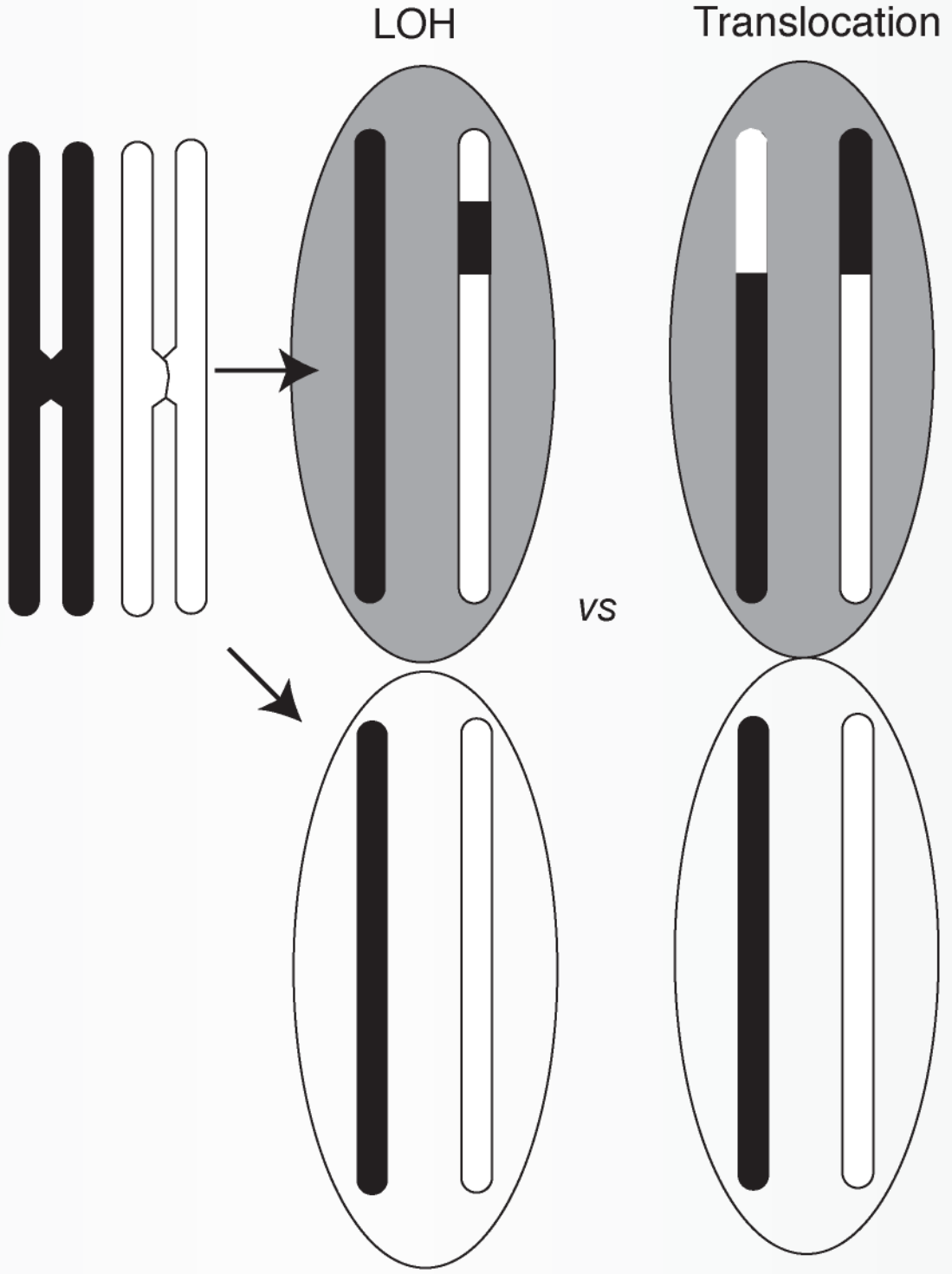
Breaks

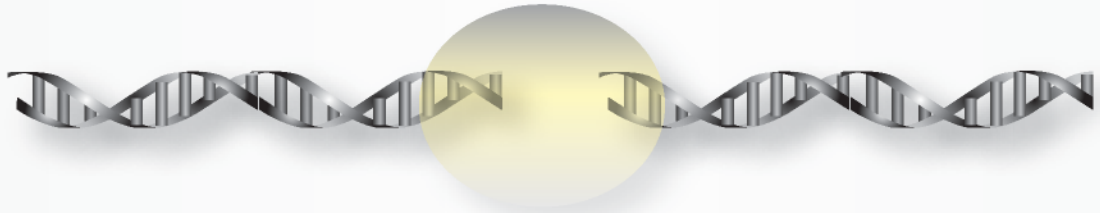


Rearrangements



Chromosome breakage and rearrangment





Detection

Mre11 complex, 9-1-1, Rad17...

Transduction

ATM, ATR, DNAPKcs

Effectors

Chk1, Chk2, p53, Mdm2...

Mediators

p53BP1, Mre11 complex, Mdc1, Claspin, TopBP1...

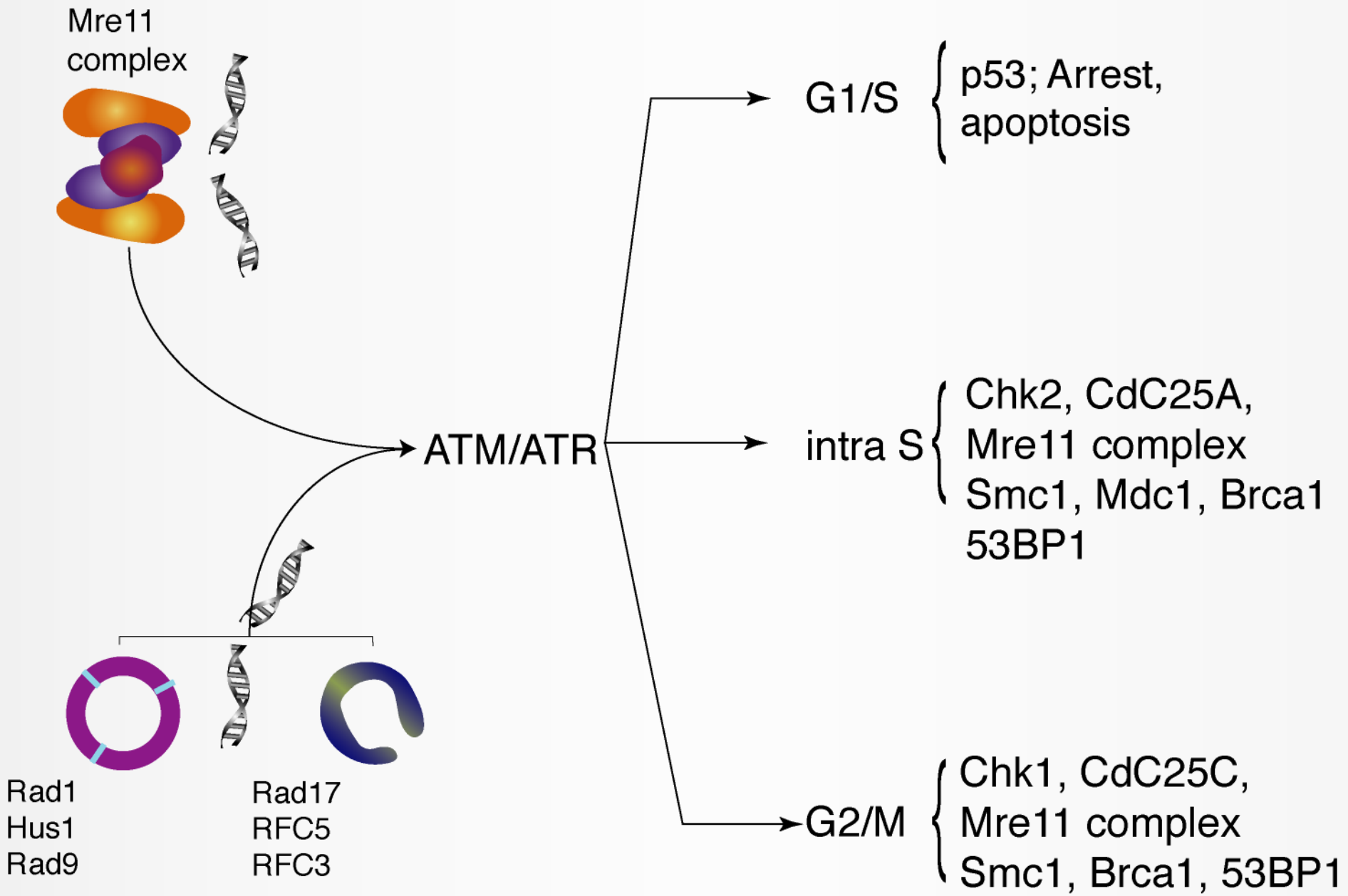
Signal amplification

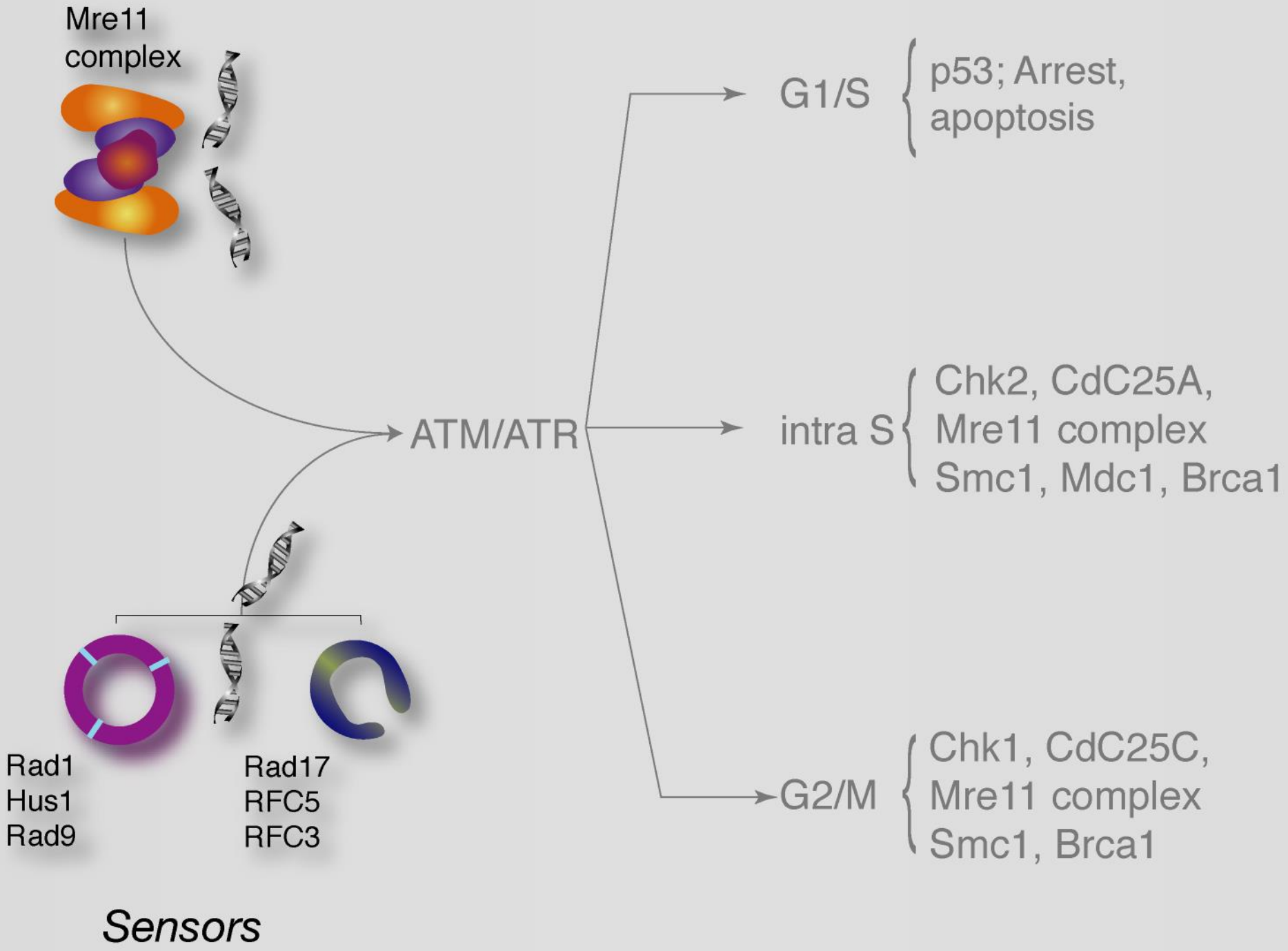
Transcription
Cell cycle arrest
Apoptosis

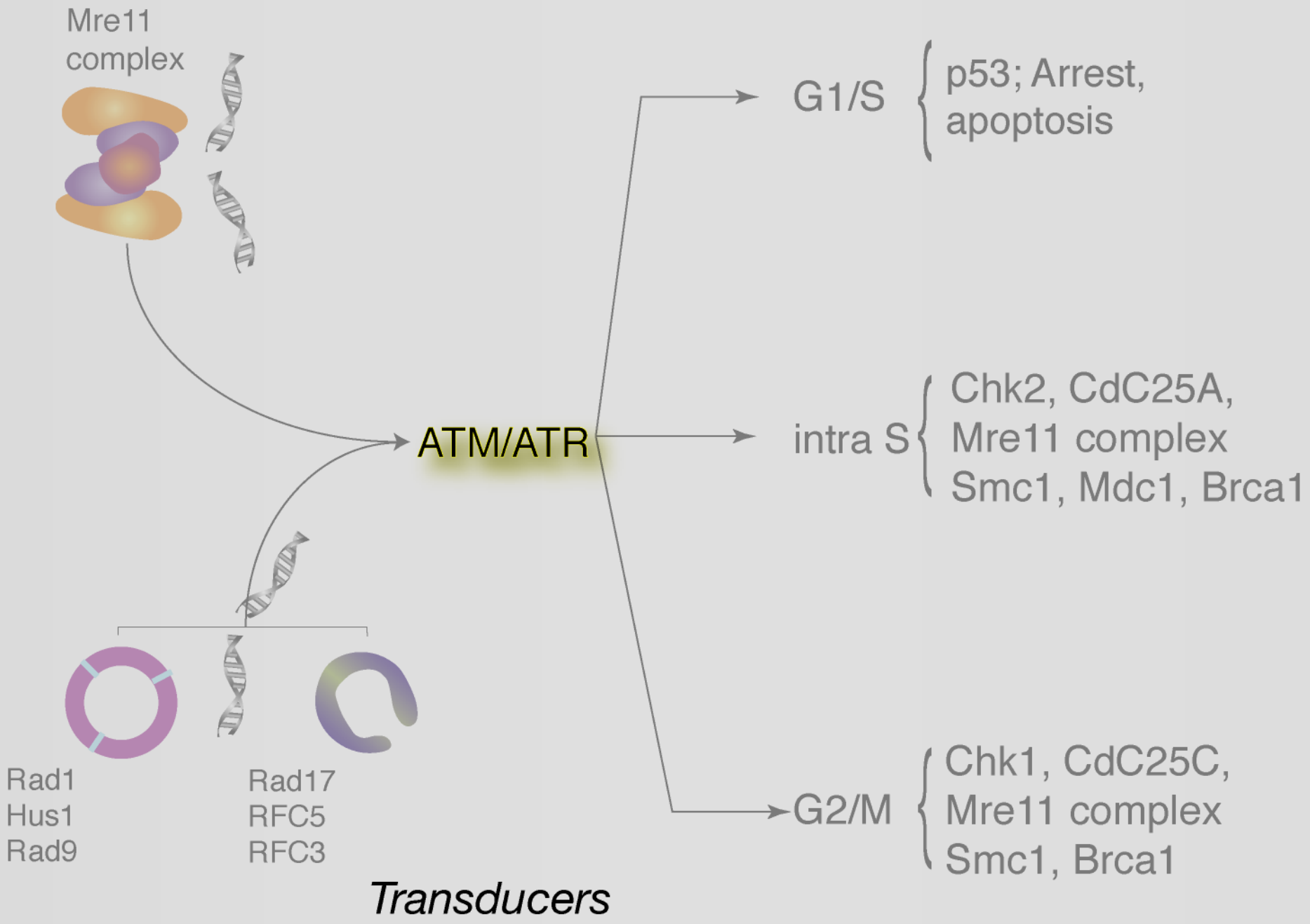
DNA repair*

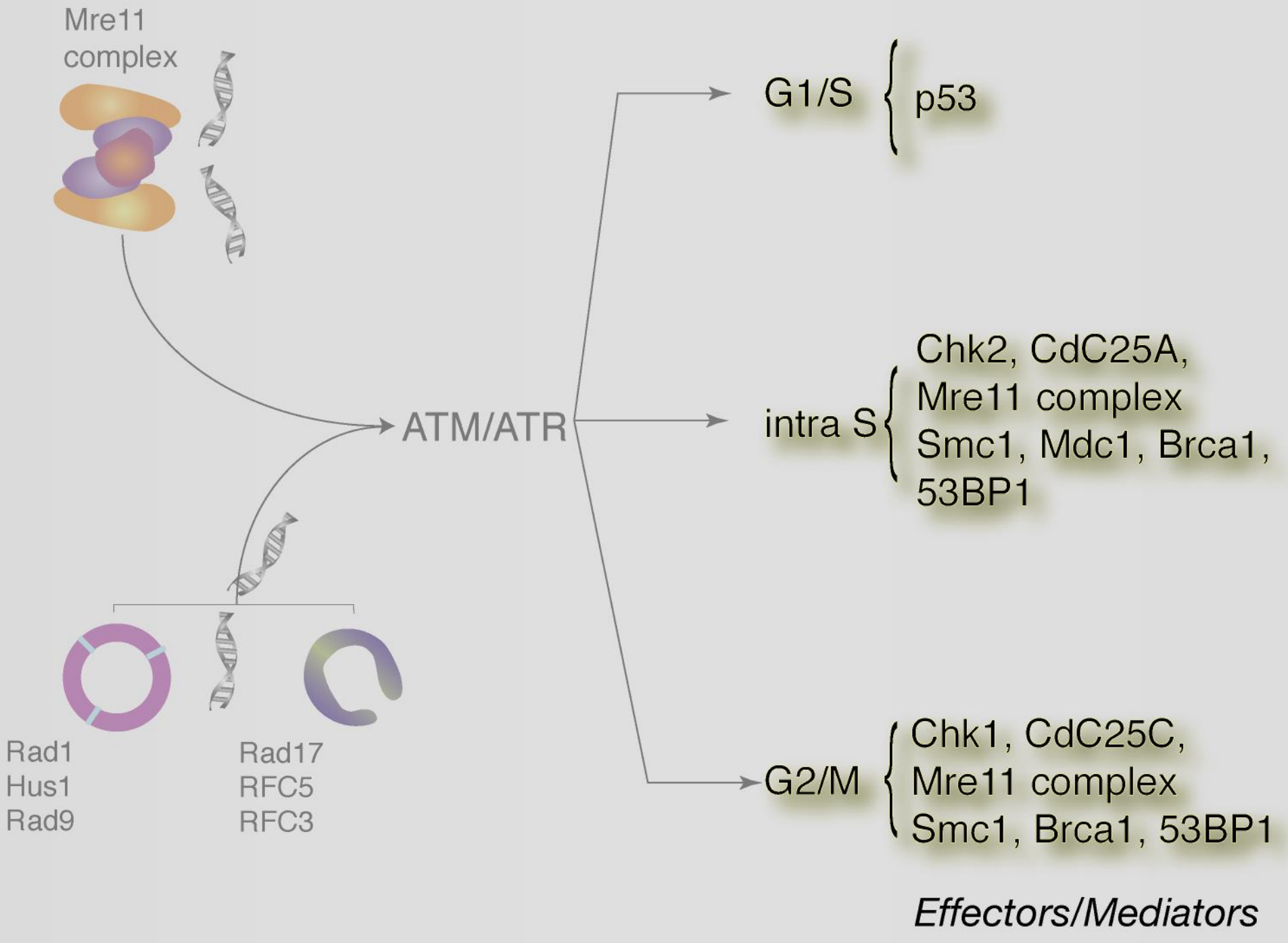
Suppression of Malignancy

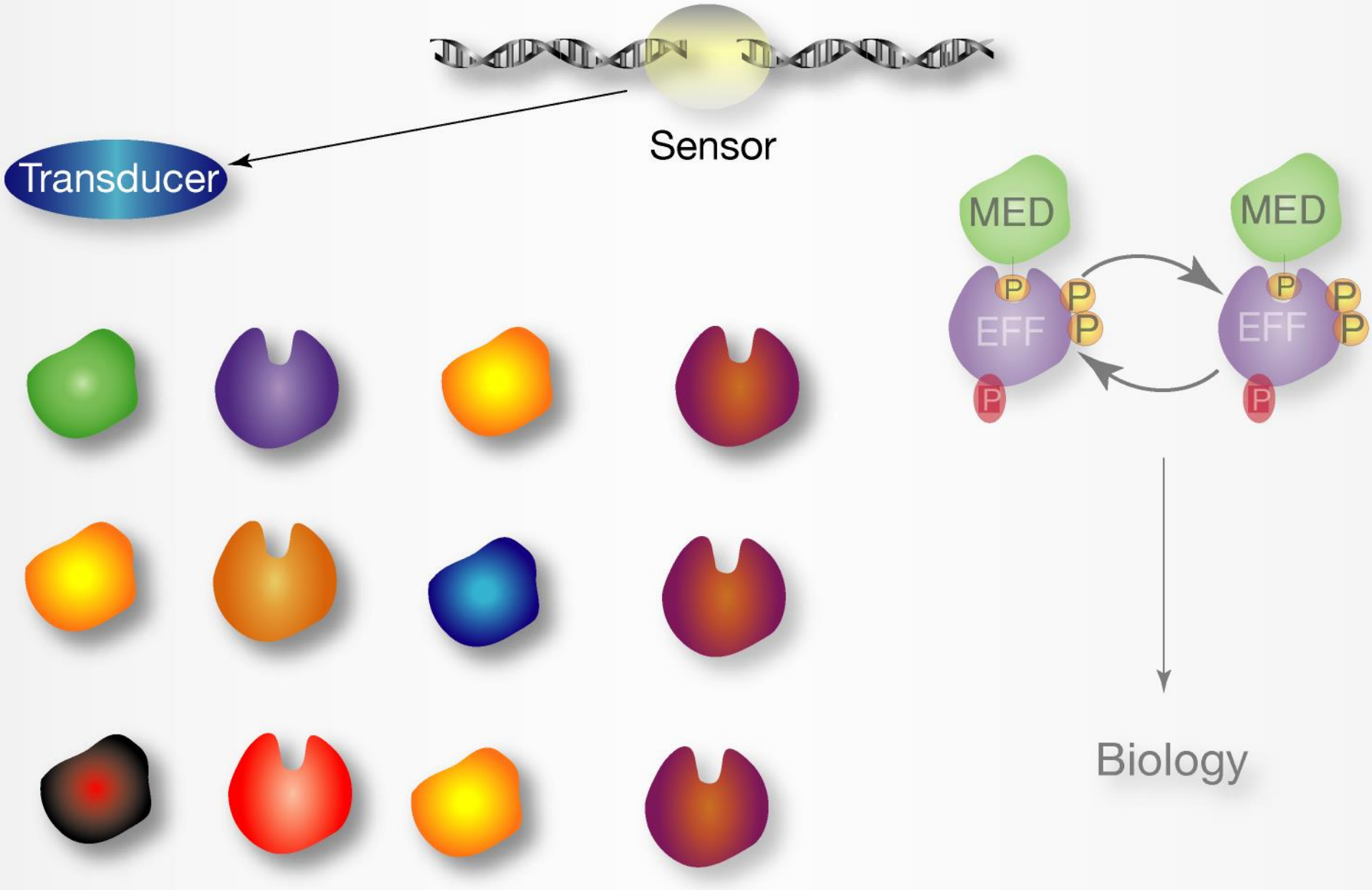
** incl. programmed gene rearrangement*

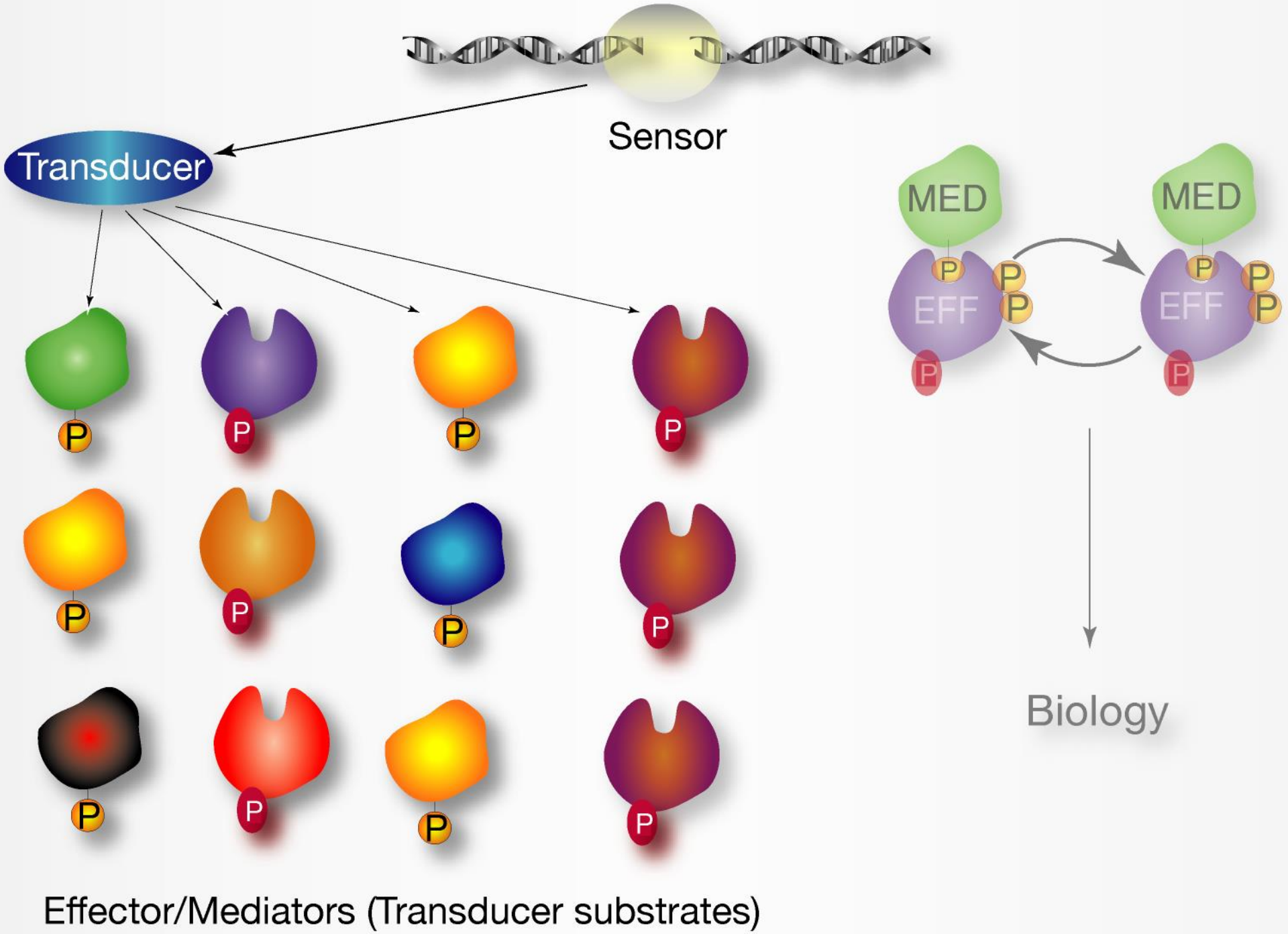

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
53BP1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1, 53BP1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

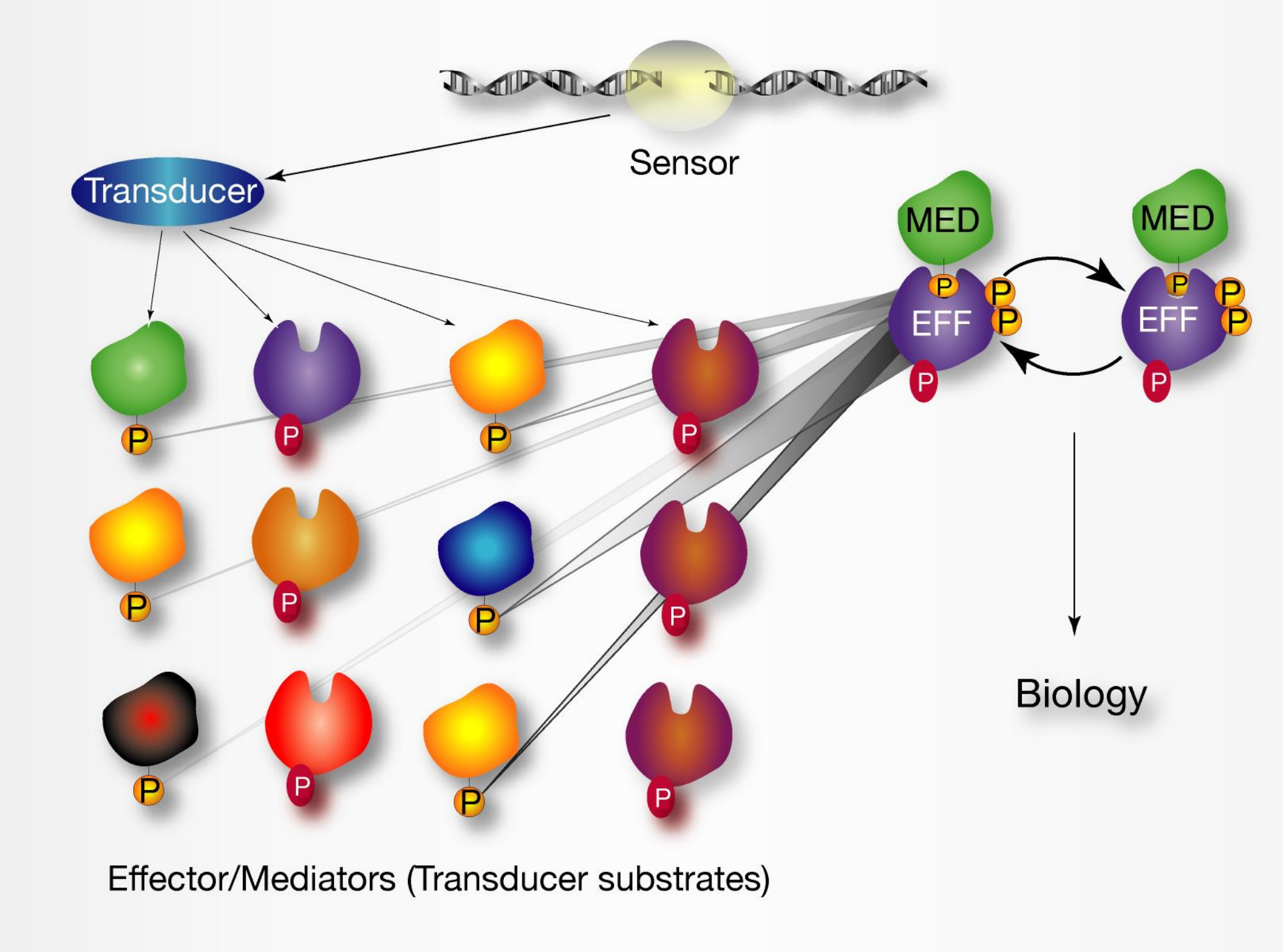

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Sensors

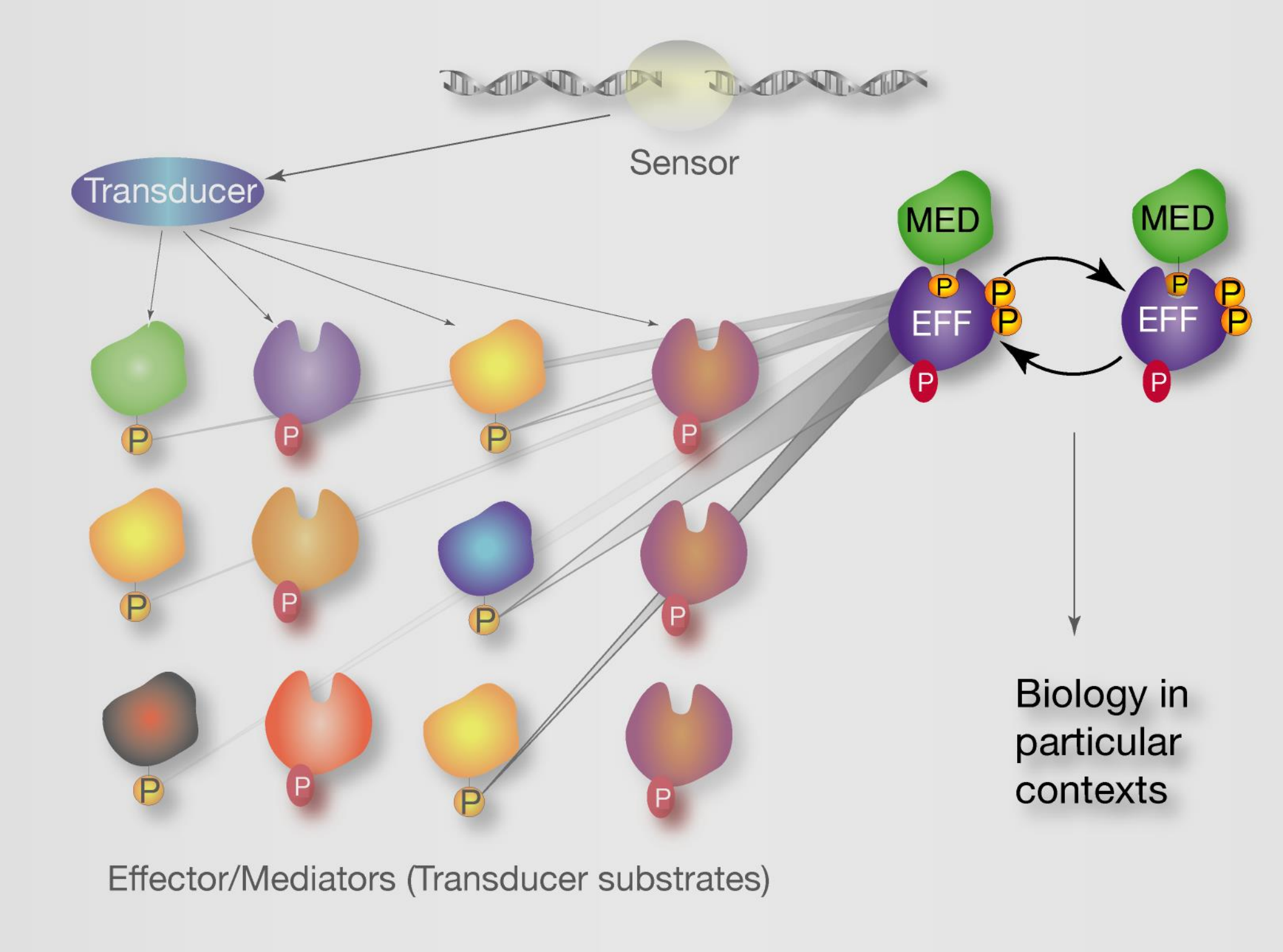

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Transducers

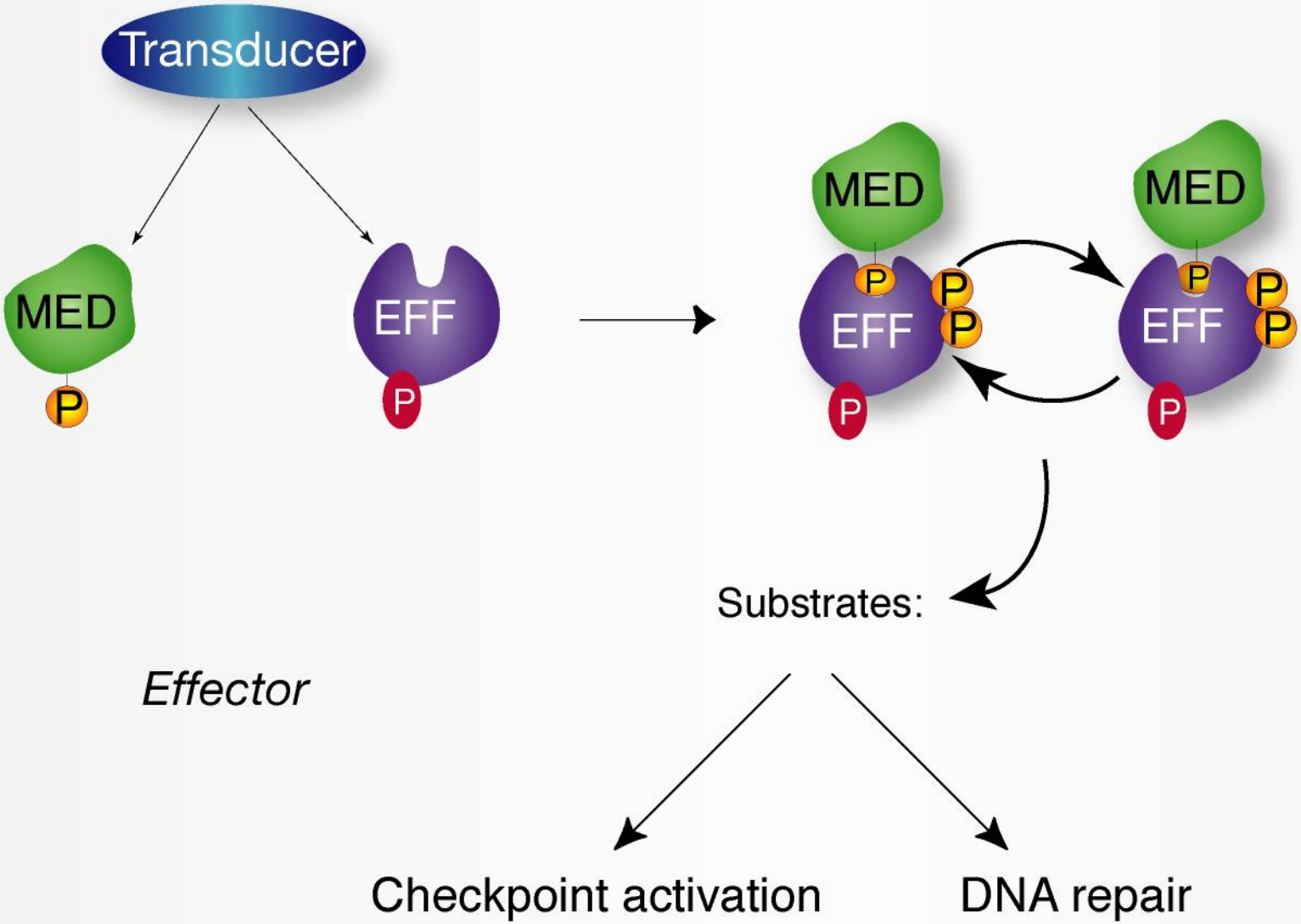

Mre11
complex
ATM/ATR
G1/S
p53
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1,
53BP1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1, 53BP1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Effectors/Mediators

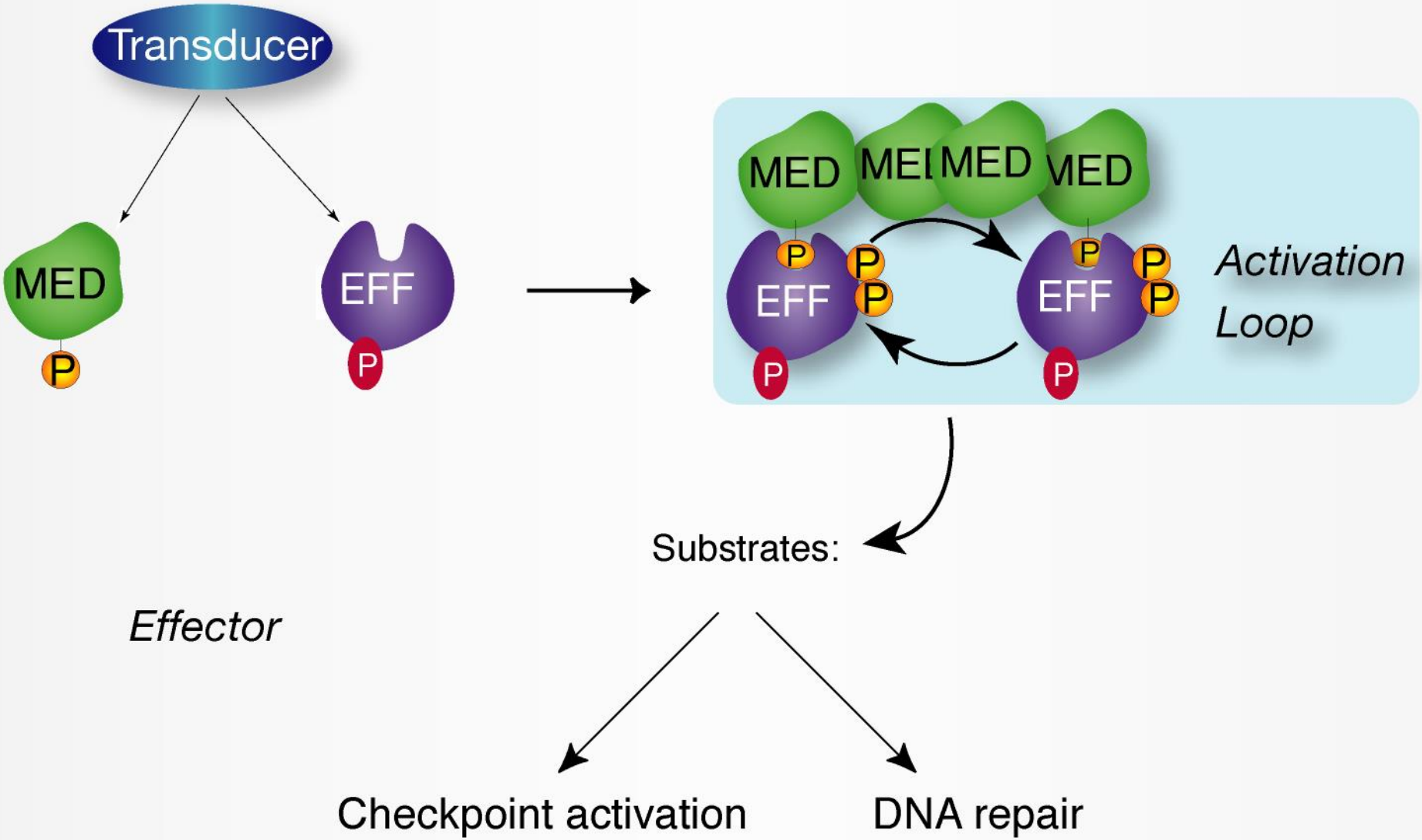

Sensor
Transducer
MED
MED
P
P
P
P
P
EFF
EFF
P
P
P
Biology

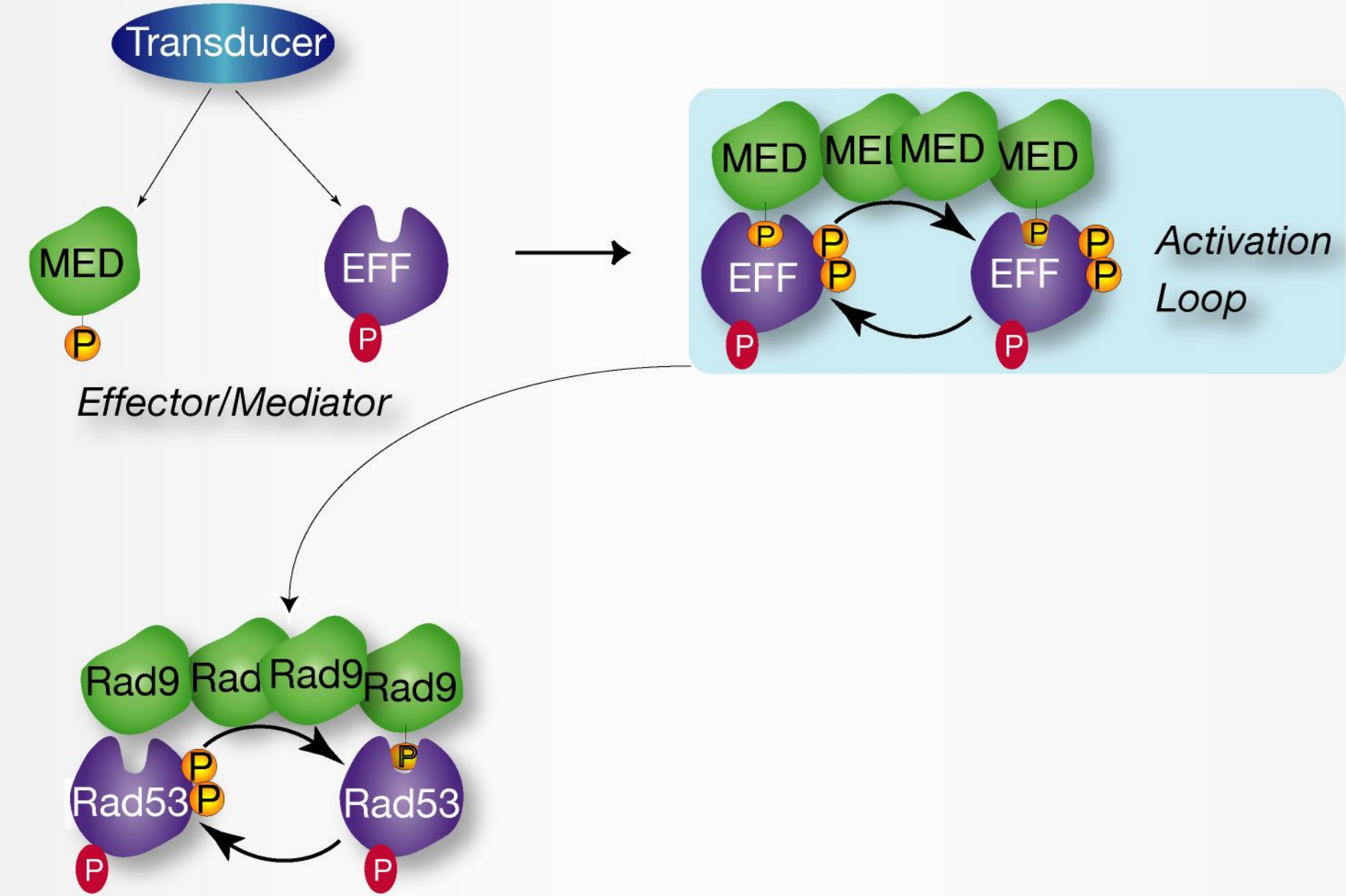

Sensor
Transducer
MED
EFF
P
MED
EFF
P
Biology
Effector/Mediators (Transducer substrates)

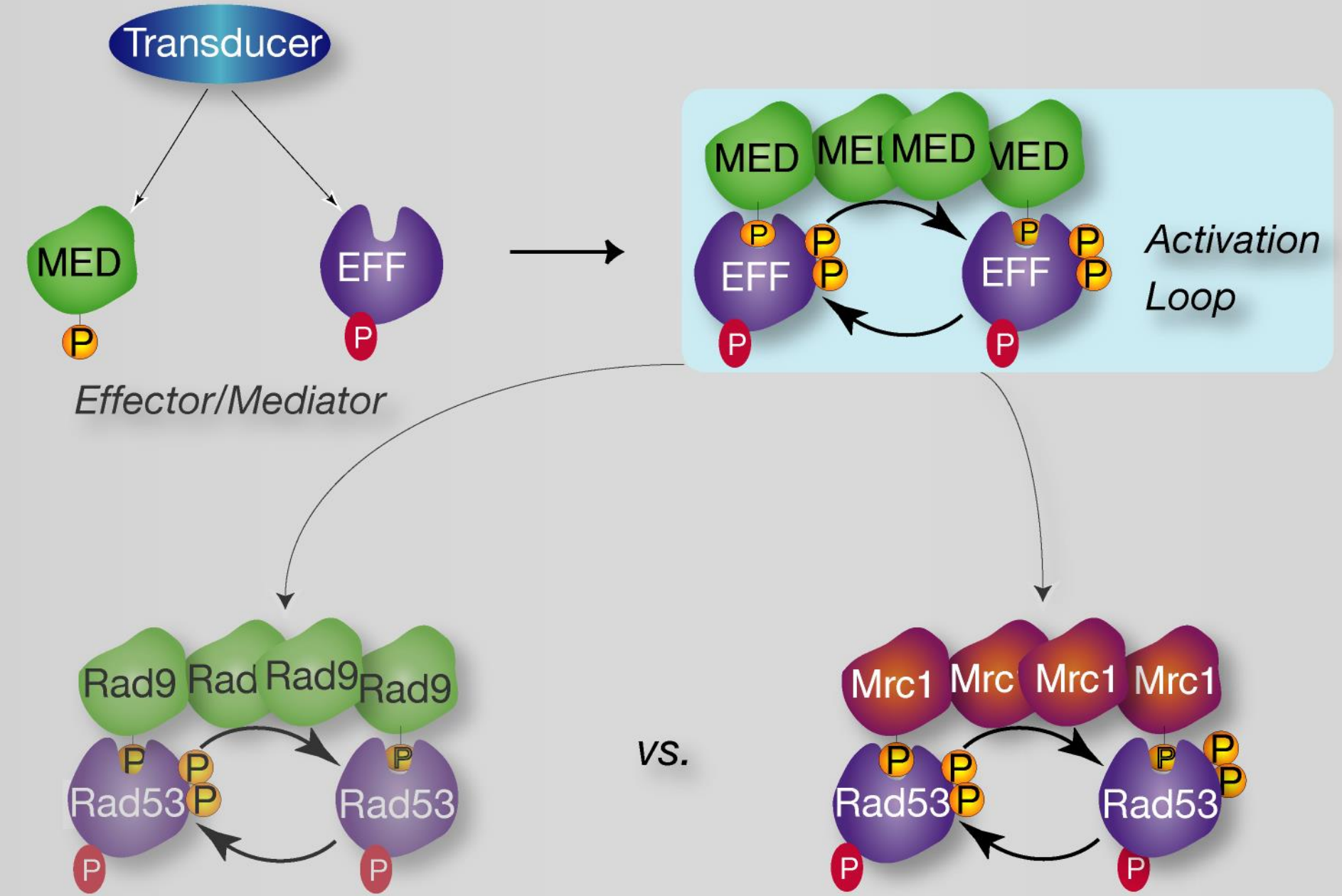

Sensor
Transducer
MED
MED
EFF
EFF
P
Biology
Effector/Mediators (Transducer substrates)

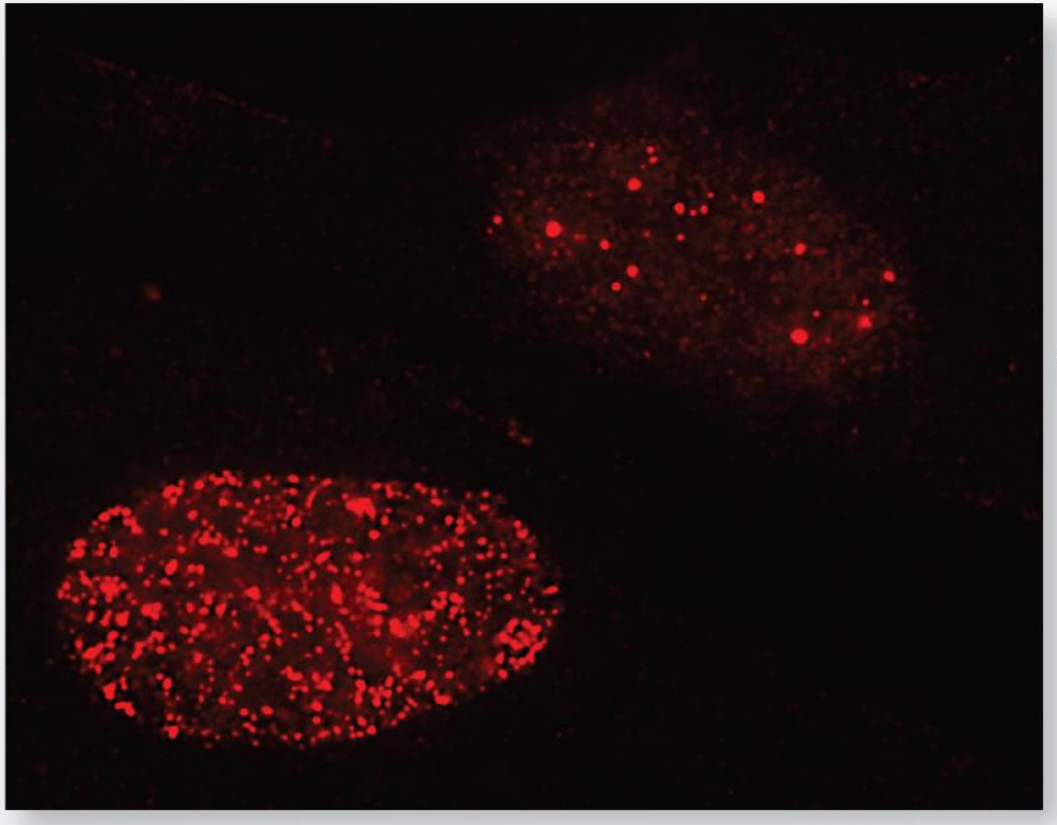

Sensor
Transducer
MED
EFF
MED
EFF
P
Biology in particular contexts
Effector/Mediators (Transducer substrates)


Transducer
MED
P
EFF
P
MED
P
EFF
P
P
MED
P
EFF
P
P
Substrates:
Effector
Checkpoint activation
DNA repair

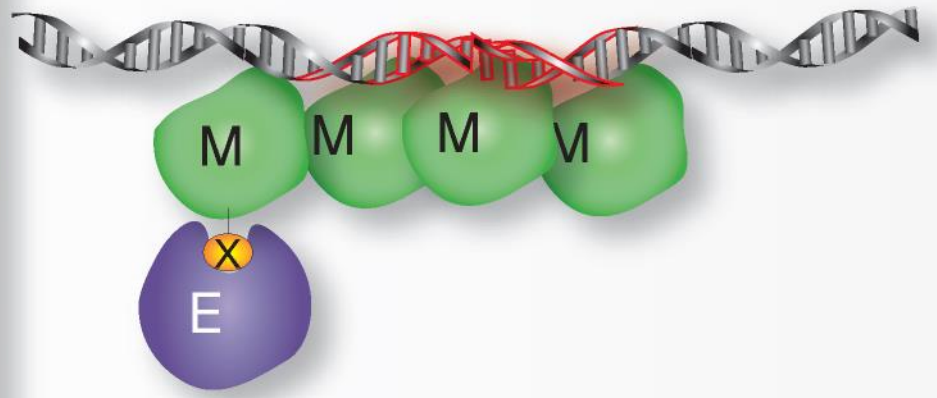

Transducer
MED
P
EFF
P
MED
MED
MED
EFF
P
P
P
EFF
P
P
P
P
Activation
Loop
Substrates:
Effector
Checkpoint activation
DNA repair


Transducer
MED
P
EFF
P
Effector/Mediator
MED
MED
MED
EFF
P
P
P
P
EFF
P
P
P
P
Activation
Loop
Rad9
Rad
Rad9
Rad9
Rad53
P
P
P
Rad53
P
P
DNA Damage
(e.g., telomere dysfunction)


Transducer
MED
EFF
P
Effector/Mediator
MED
MED
MED
EFF
EFF
Activation
Loop
Rad9
Rad9
Rad9
Rad53
Rad53
vs.
Mrc1
Mrc1
Mrc1
Rad53
Rad53
DNA Damage
(e.g., telomere dysfunction)
DNA Replication Stress
(e.g., HU exposure (dNTP depletion))

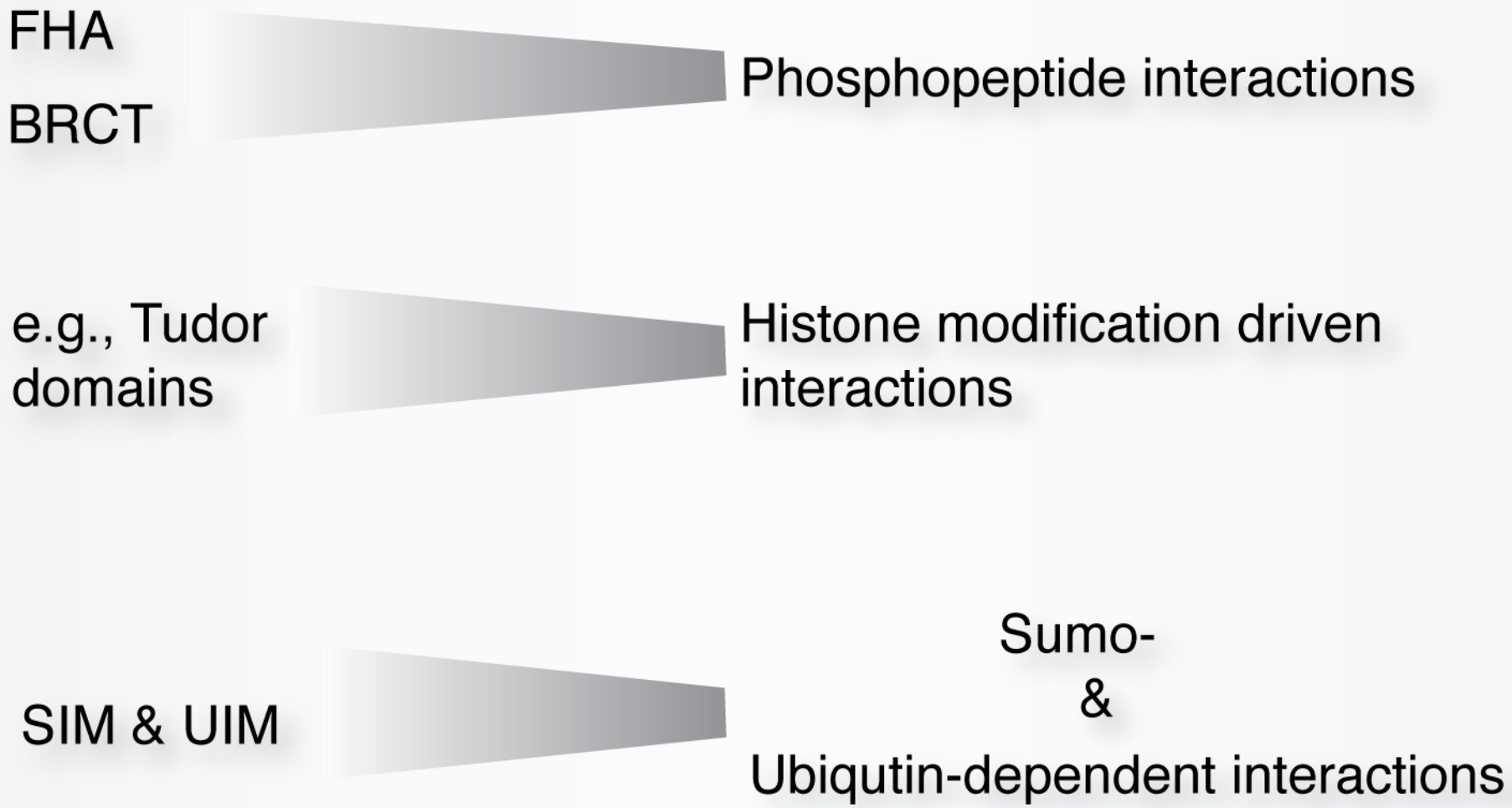


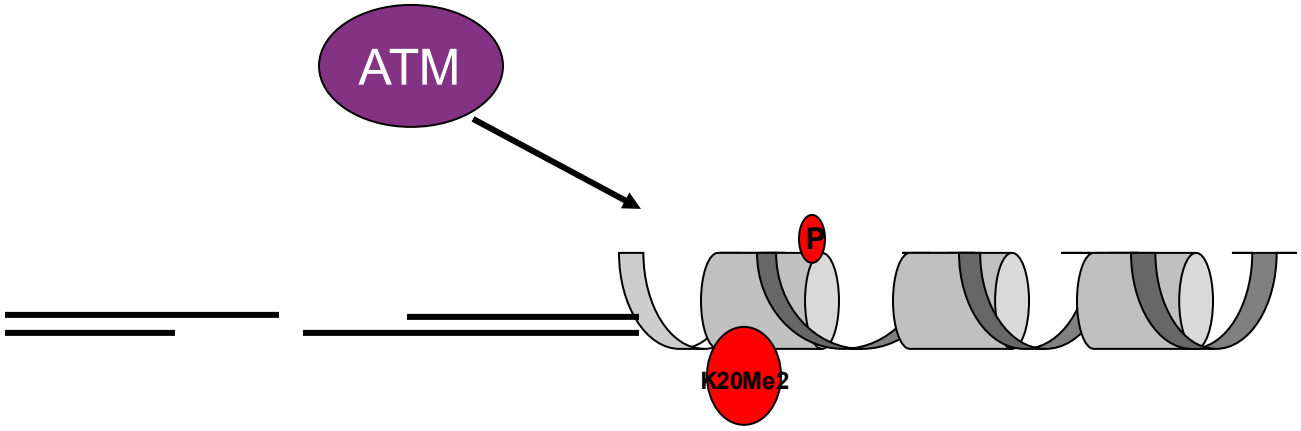
Oligomeric assemblies in the DNA damage response



- Other activities (e.g., ubiquitination. sumoylation etc...)
- DNA itself?

Communication in the DNA damage response

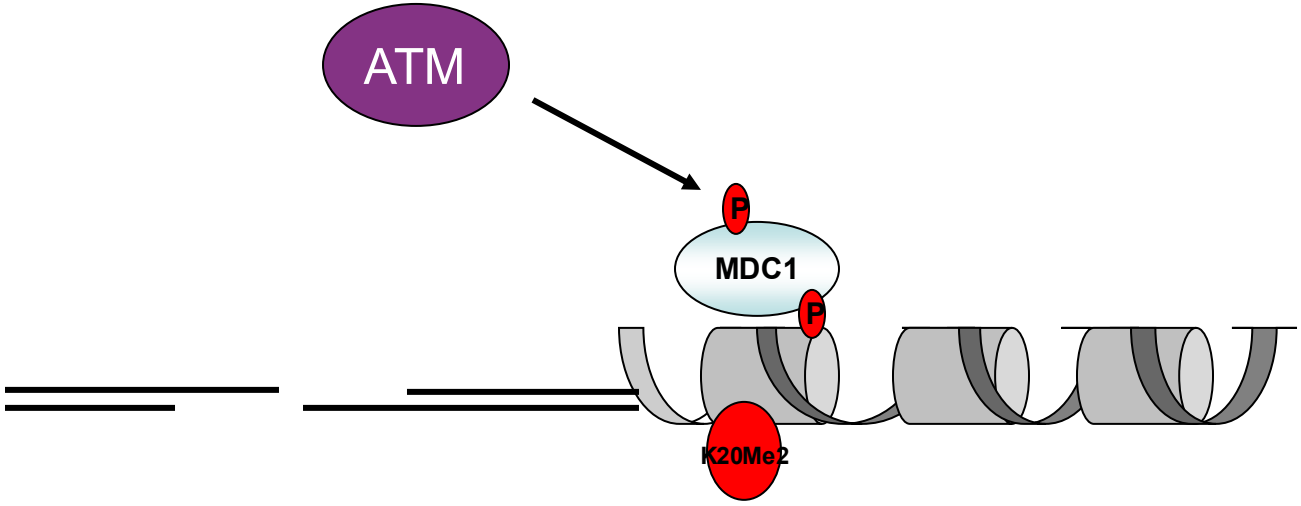




Constitutive mark

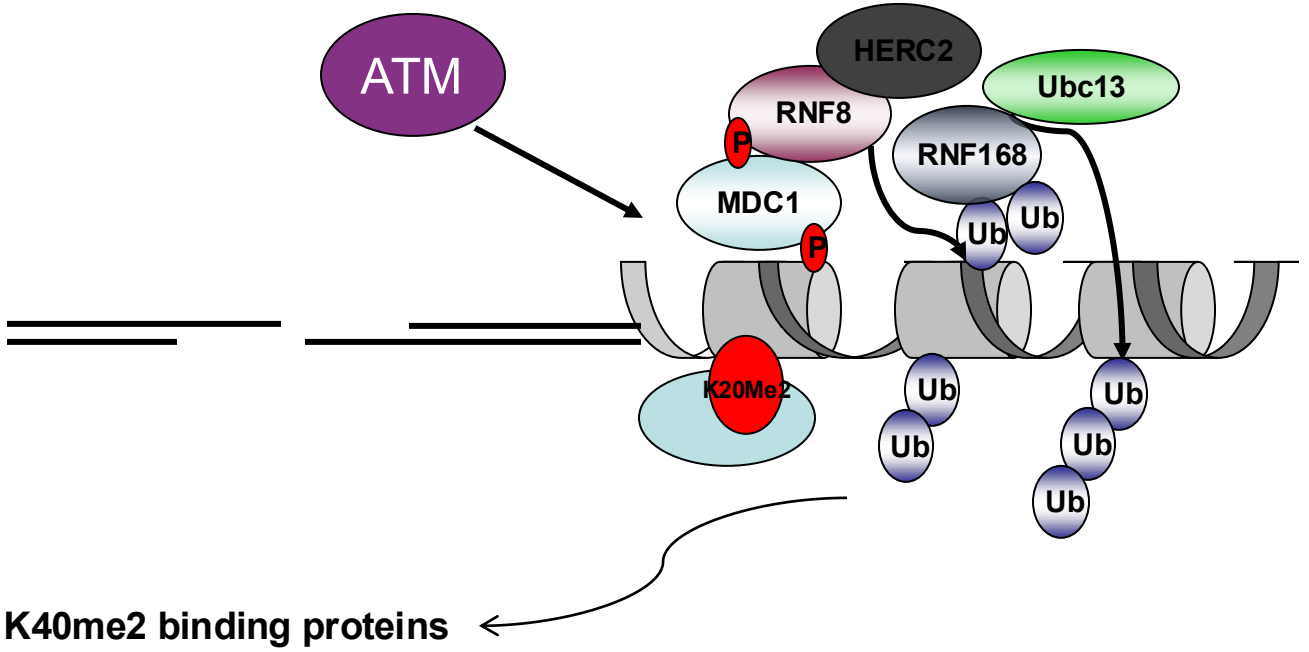
Kolas et al Science 2007
Huen et al. Cell 2007
Doil et al. Science 2007
Stewart et al. Cell 2009
Mailand et al. Cell 2009
Wang et al. 2009
Galanty et al. Nature 2009
Morris et al. Nature 2009

Sobhian et al Science 2007
Kim et al. Science 2007
Wang et al. Science 2007
Shao et al. Genes Dev 2009
Sy et al. Genes Dev 2009
Wang et al Genes Dev 2009
Nikkila et al. Oncogene 2009
Solyom et al. Sci Tranl med 2012



Kolas et al Science 2007
Huen et al. Cell 2007
Doil et al. Science 2007
Stewart et al. Cell 2009
Mailand et al. Cell 2009
Wang et al. 2009
Galanty et al. Nature 2009
Morris et al. Nature 2009

Sobhian et al Science 2007
Kim et al. Science 2007
Wang et al. Science 2007
Shao et al. Genes Dev 2009
Sy et al. Genes Dev 2009
Wang et al Genes Dev 2009
Nikkila et al. Oncogene 2009
Solyom et al. Sci Tranl med 2012



Kolas et al Science 2007
Huen et al. Cell 2007
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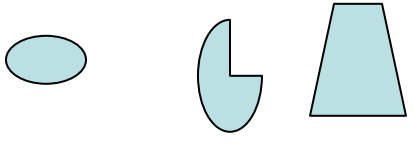
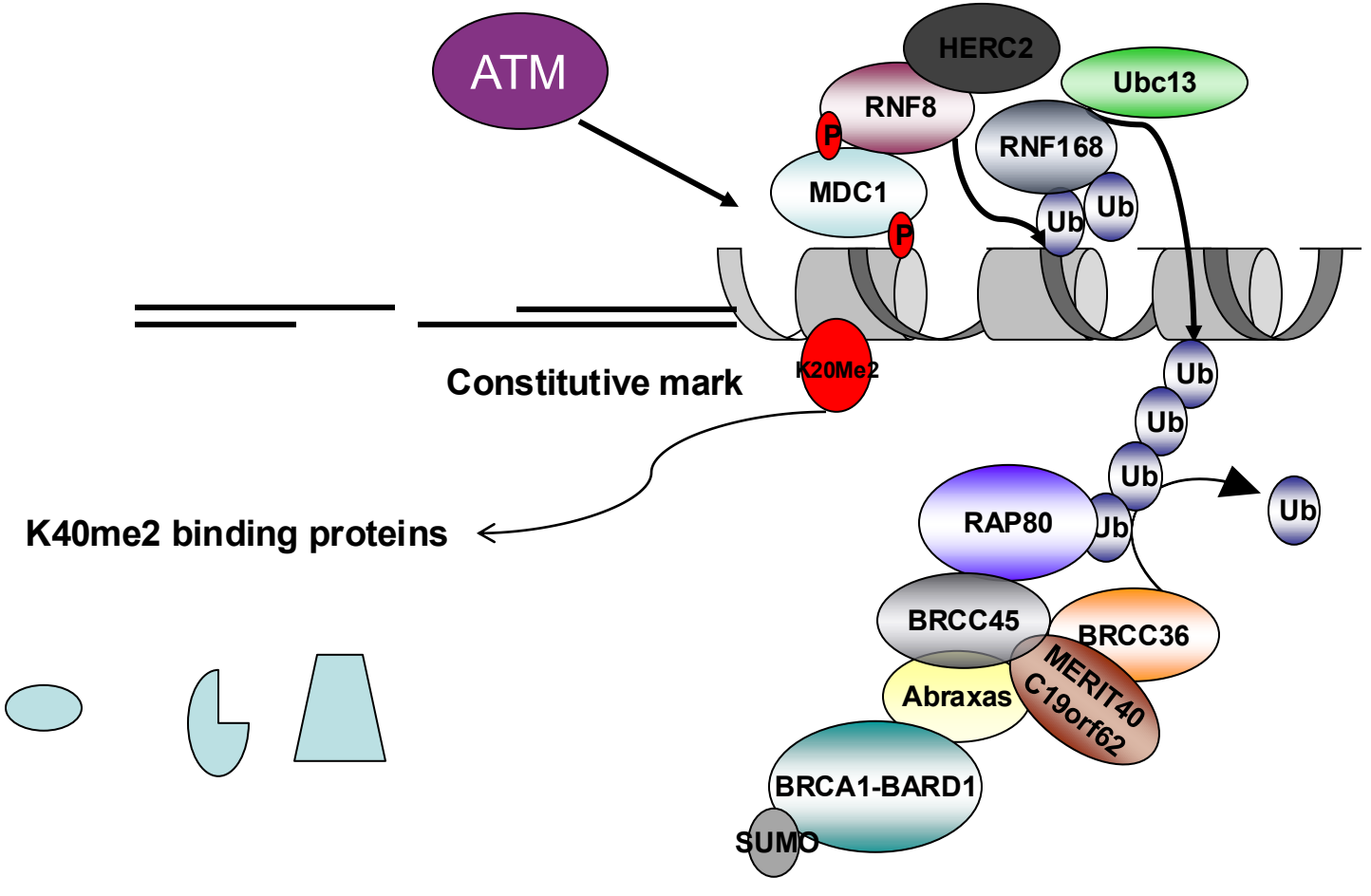
Stewart et al. Cell 2009
Mailand et al. Cell 2009
Wang et al. 2009
Galanty et al. Nature 2009
Morris et al. Nature 2009

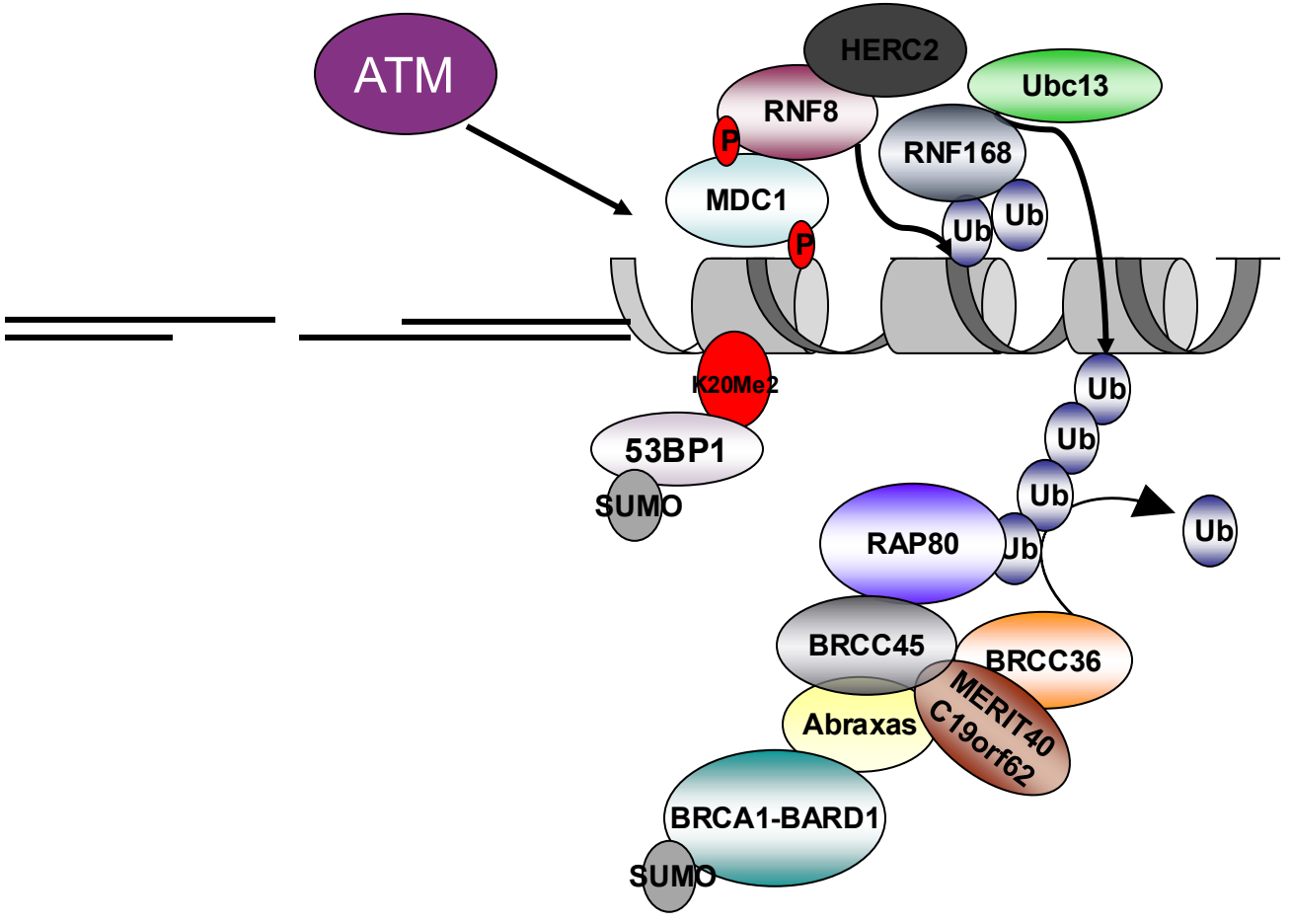


Sobhian et al Science 2007
Kim et al. Science 2007
Wang et al. Science 2007
Shao et al. Genes Dev 2009
Sy et al. Genes Dev 2009
Wang et al Genes Dev 2009
Nikkila et al. Oncogene 2009
Solyom et al. Sci Tranl med 2012



Kolas et al Science 2007
Huen et al. Cell 2007
Doil et al. Science 2007
Stewart et al. Cell 2009
Mailand et al. Cell 2009
Wang et al. 2009
Galanty et al. Nature 2009
Morris et al. Nature 2009

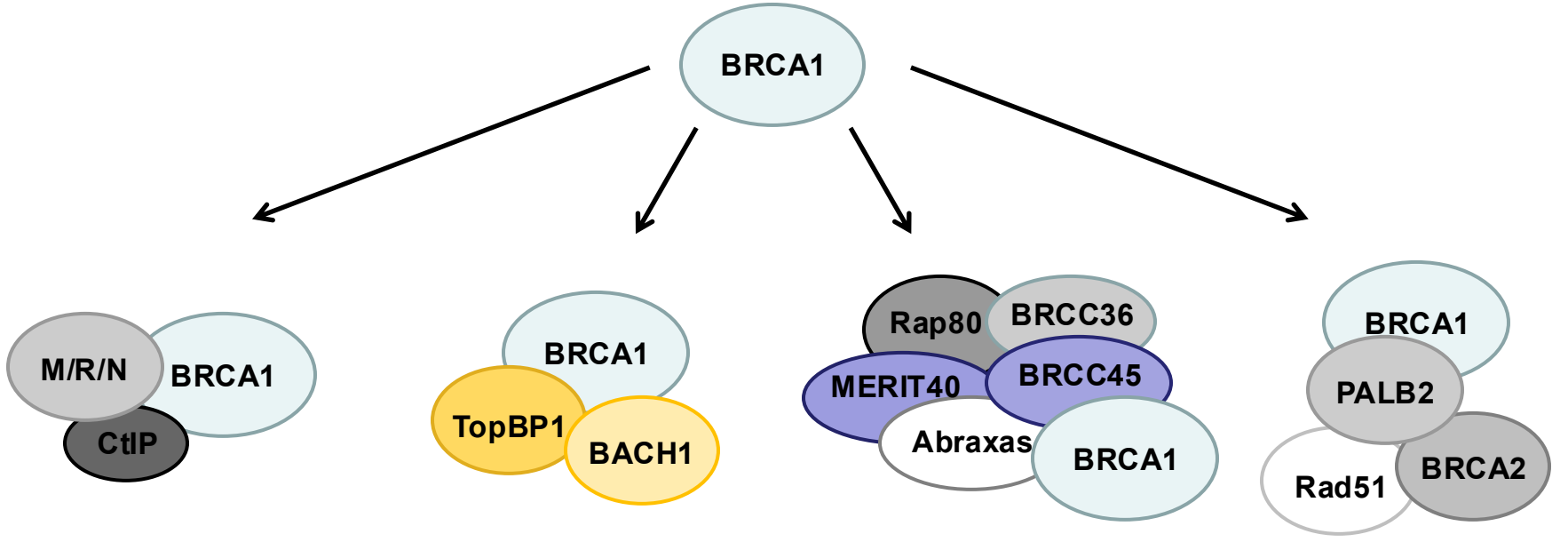
Sobhian et al Science 2007
Kim et al. Science 2007
Wang et al. Science 2007
Shao et al. Genes Dev 2009
Sy et al. Genes Dev 2009
Wang et al Genes Dev 2009
Nikkila et al. Oncogene 2009
Solyom et al. Sci Tranl med 2012



Kolas et al Science 2007
Huen et al. Cell 2007
Doil et al. Science 2007
Stewart et al. Cell 2009
Mailand et al. Cell 2009
Wang et al. 2009
Galanty et al. Nature 2009
Morris et al. Nature 2009

Sobhian et al Science 2007
Kim et al. Science 2007
Wang et al. Science 2007
Shao et al. Genes Dev 2009
Sy et al. Genes Dev 2009
Wang et al Genes Dev 2009
Nikkila et al. Oncogene 2009
Solyom et al. Sci Tranl med 2012

The BRCA1 Tumor Suppressor Network



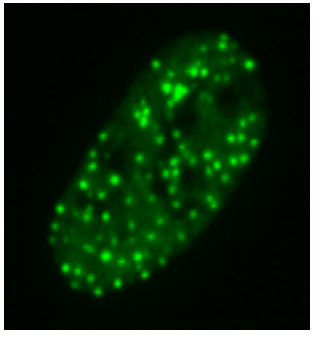
End resection
ATR/CHK1 activation
Homlog Recombin

ATL-D Syndrome
Sporadic CA

S phase repair
Homolog Recombin at ICL

Fanconi Anemia
Breast CA

K63-Ub Chromatin targeting
G2 checkpoint
DSB repair

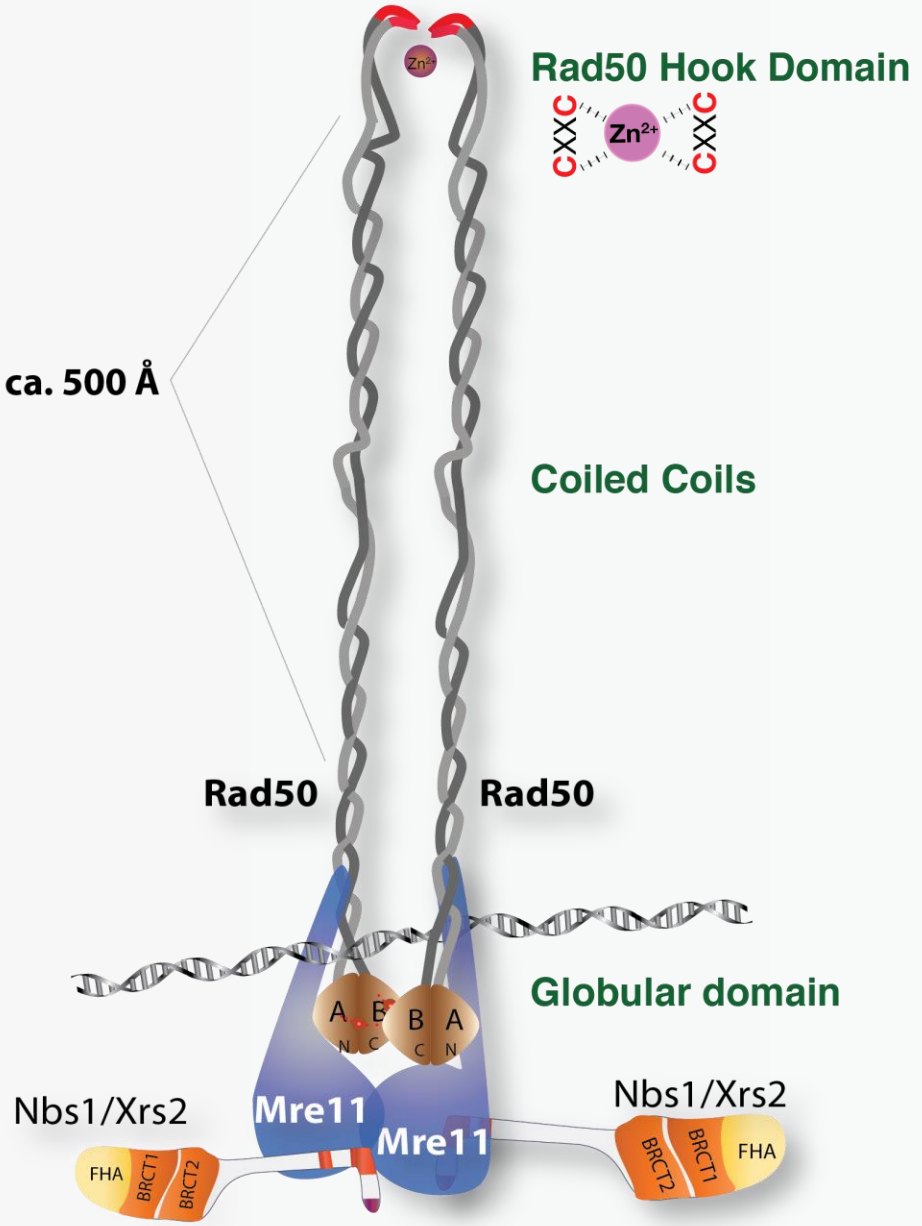


Rad51 Strand invasion
Homolog Recombin

Fanconi Anemia
Breast CA
Prostate CA
Pancreatic CA

What is a sensor?

Mre11 complex's Architecture and Functions

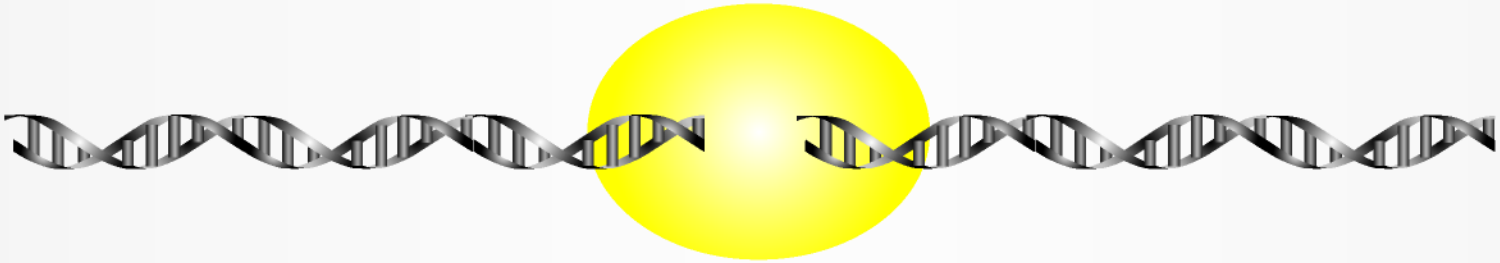


1° Sensor of DNA damage

Required for ATM activation

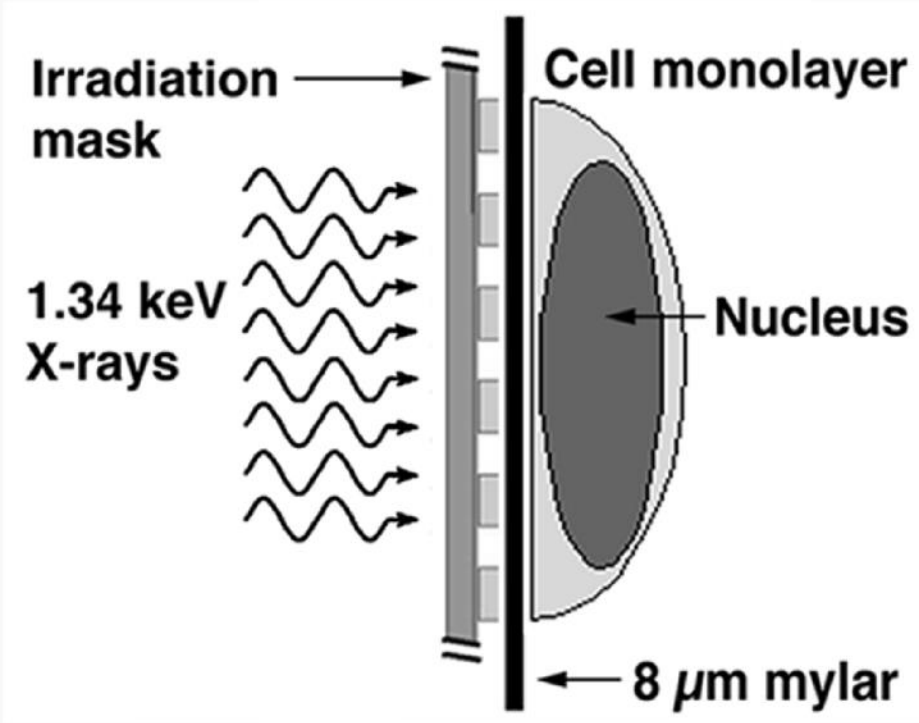
Required for DNA DSB repair

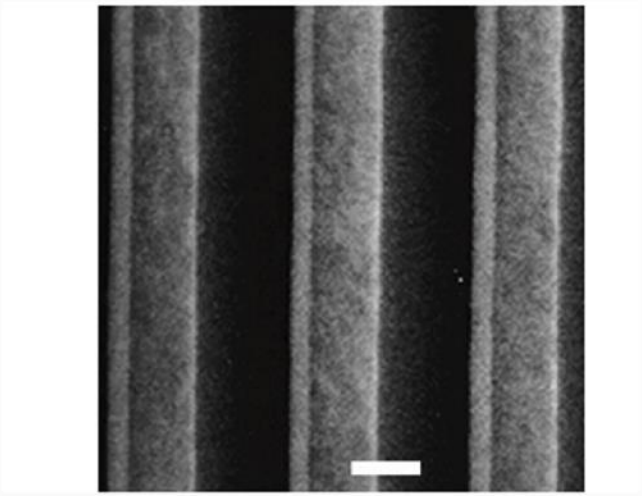
**Essential for viability*
(in higher eukaryotes)

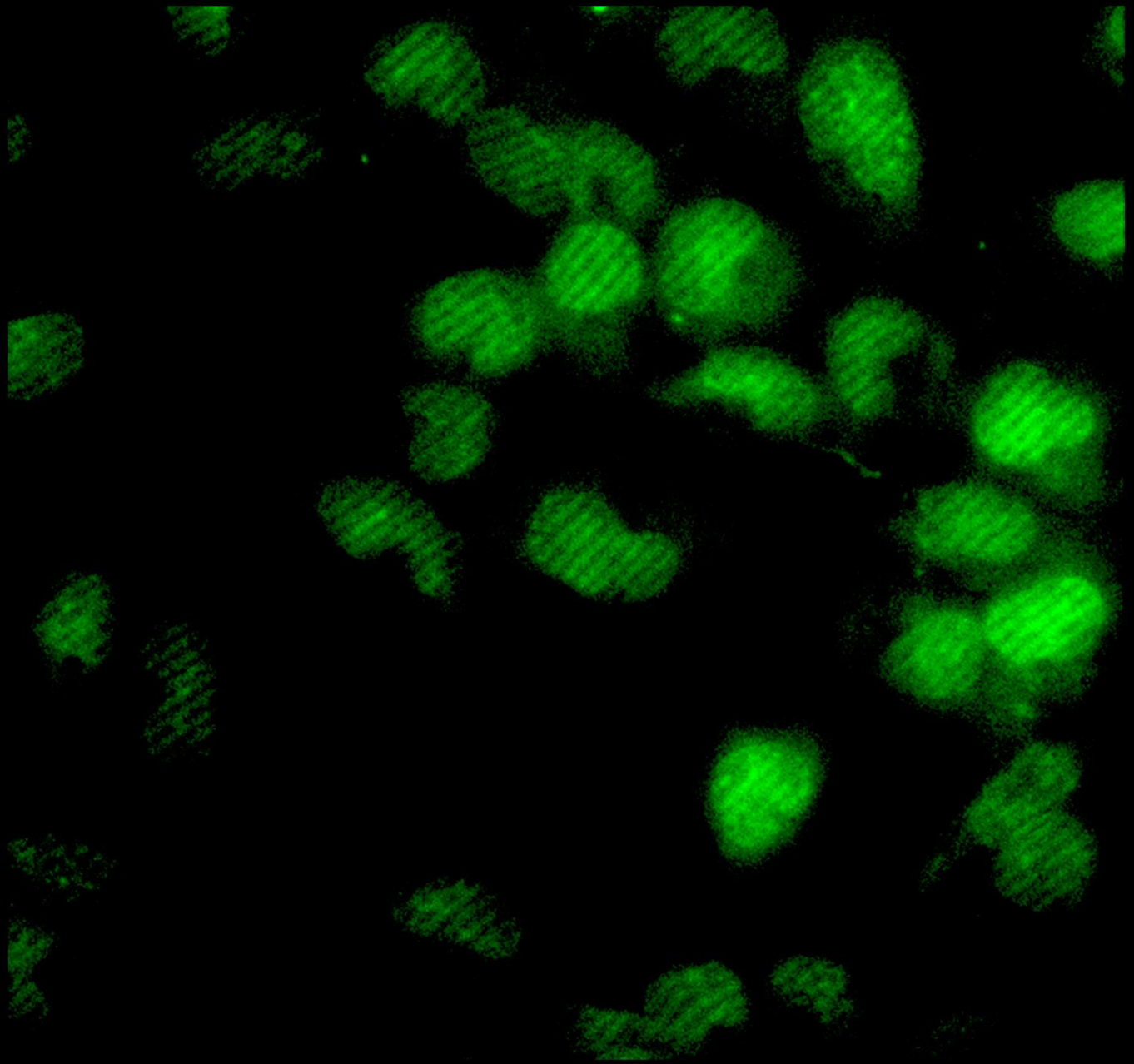


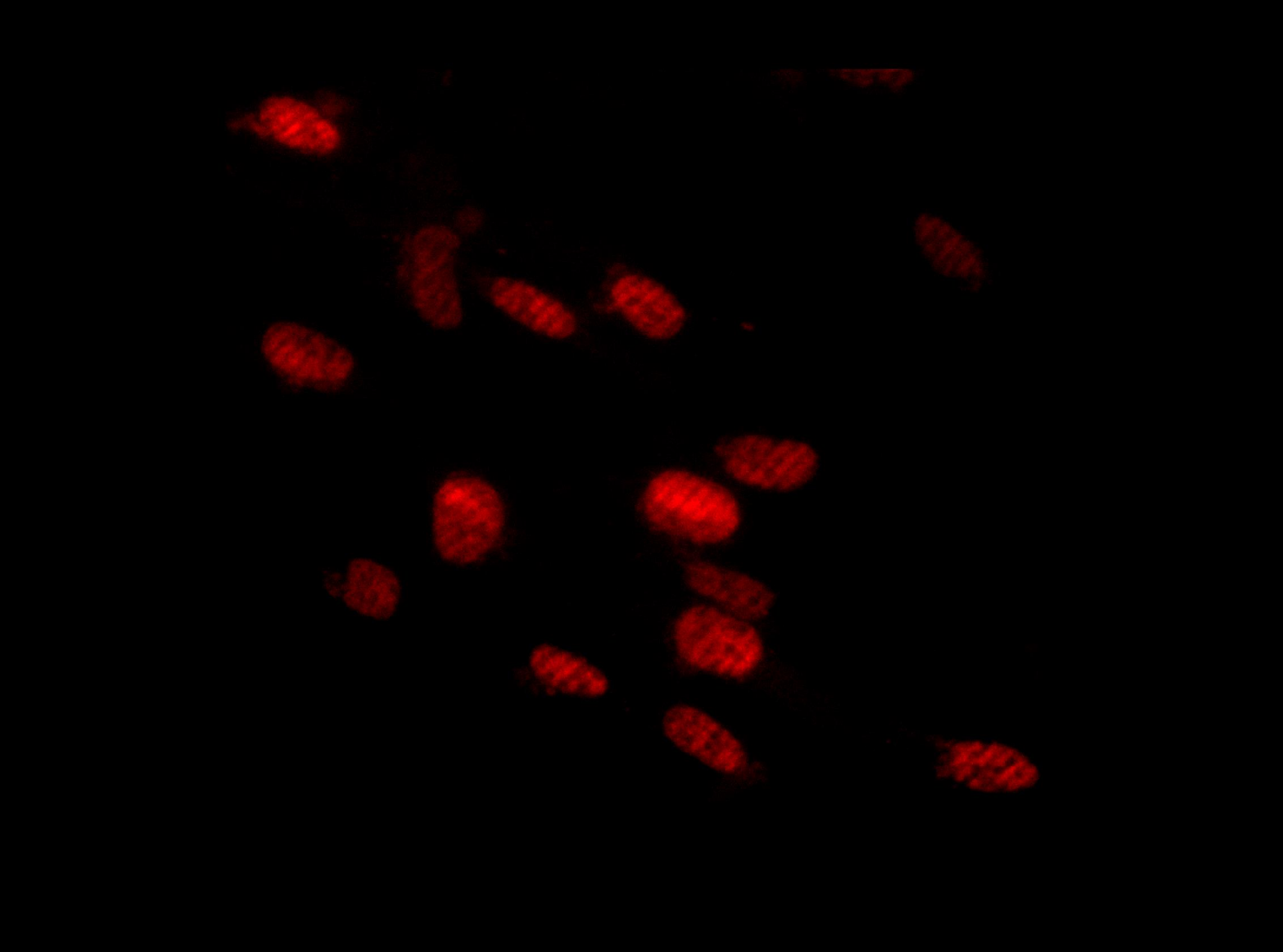
DNA damage sensors

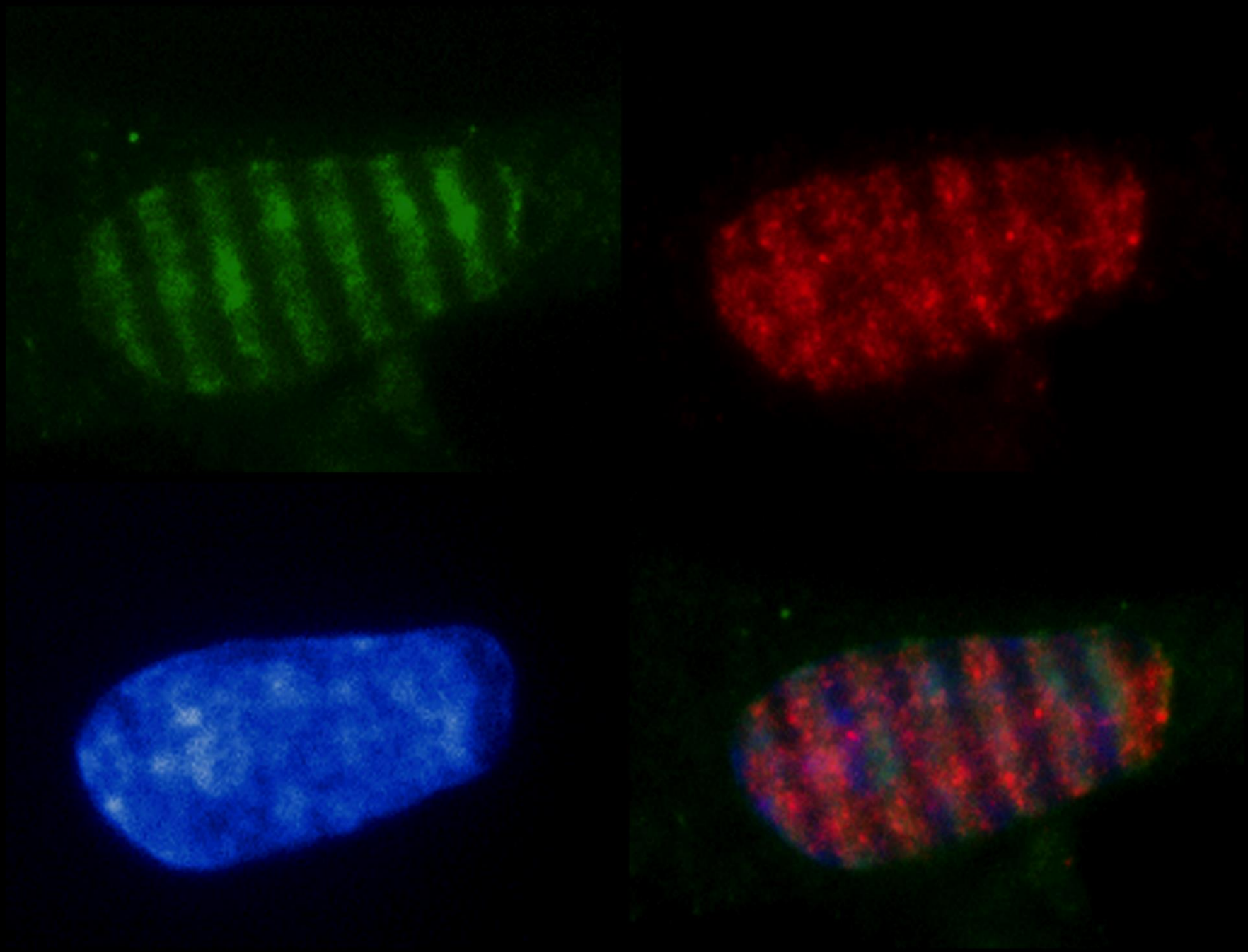
- Avidity for altered DNA structures (e.g., DNA breaks)


Irradiation
mask
1.34 keV
X-rays
Cell monolayer
Nucleus
8 μm mylar



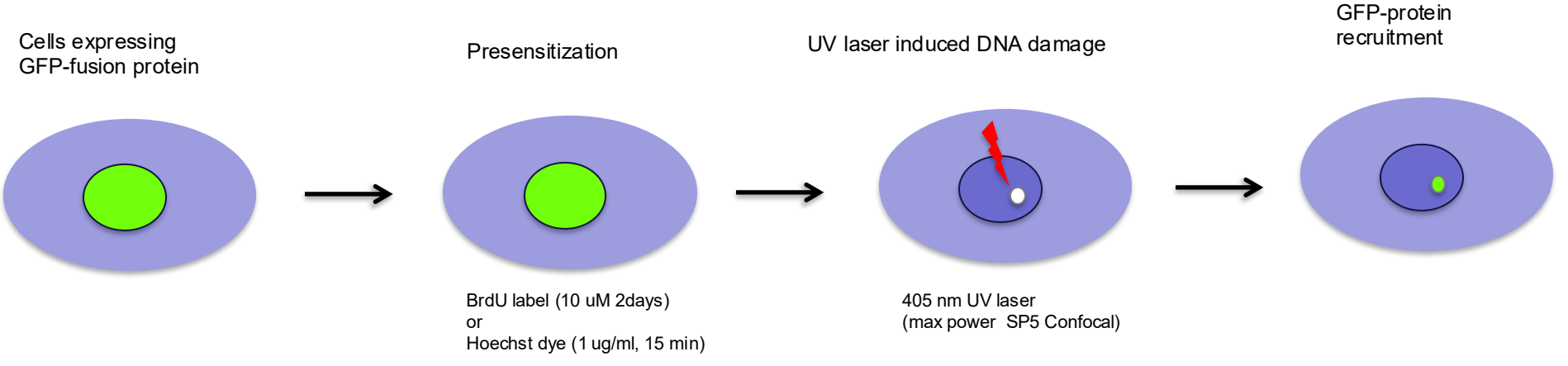


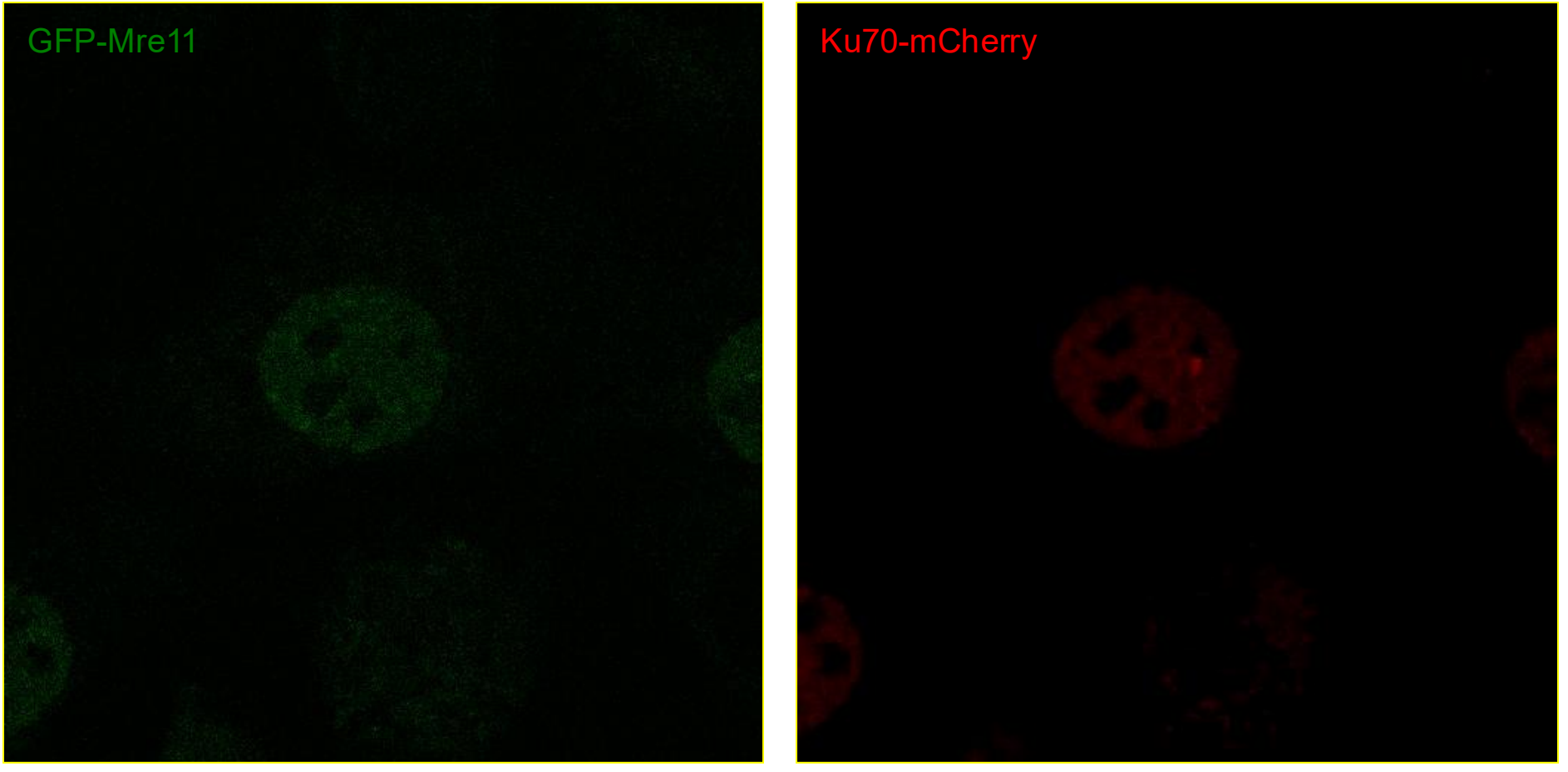


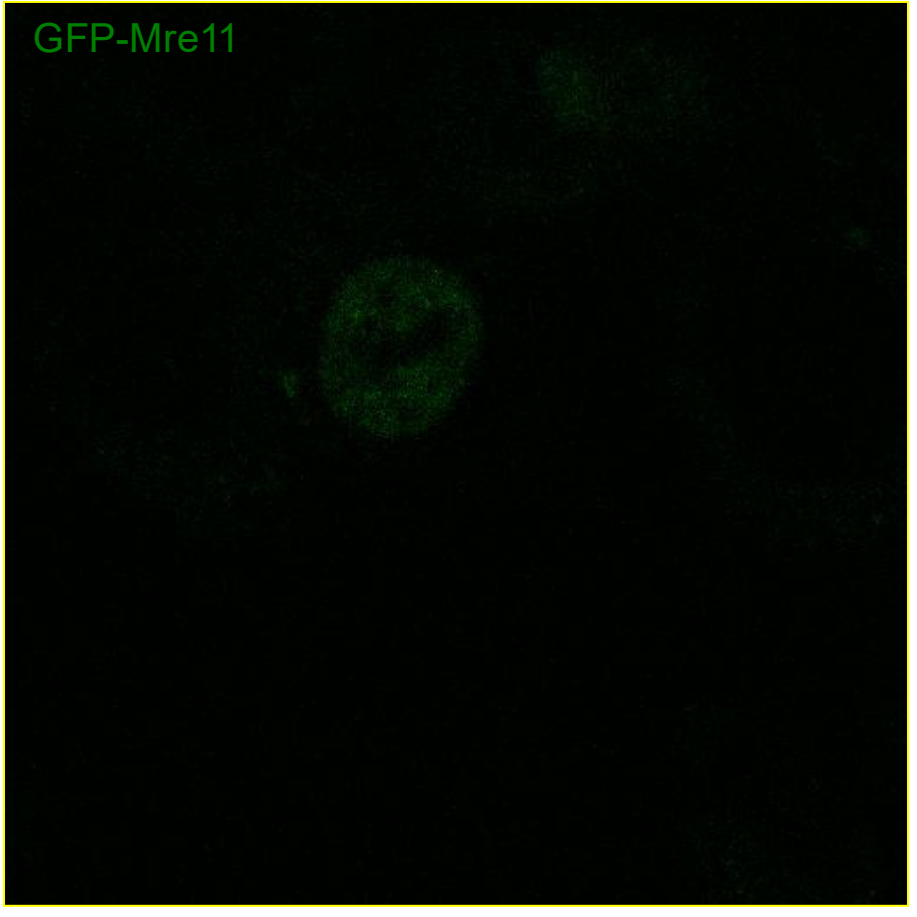


Does Nob1 localize at DNA damage site?

UV laser microirradiation (live cell imaging)



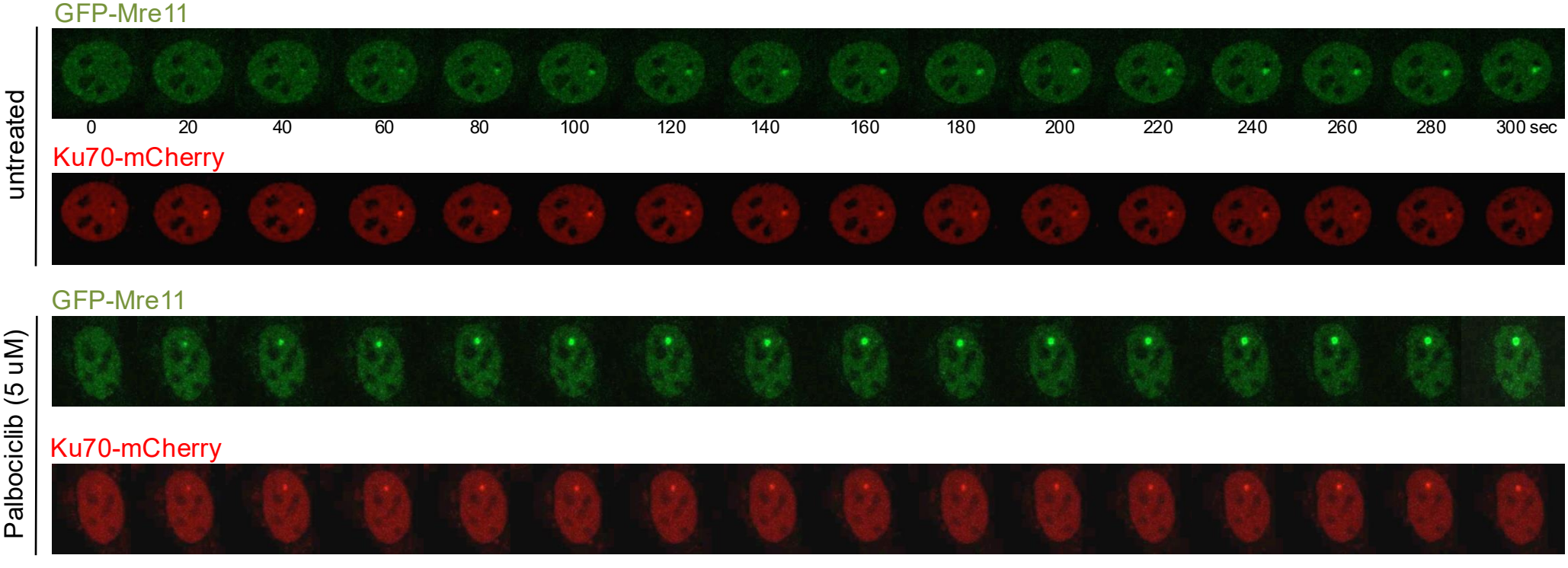

GFP-Mre11
Ku70-mCherry
5 min



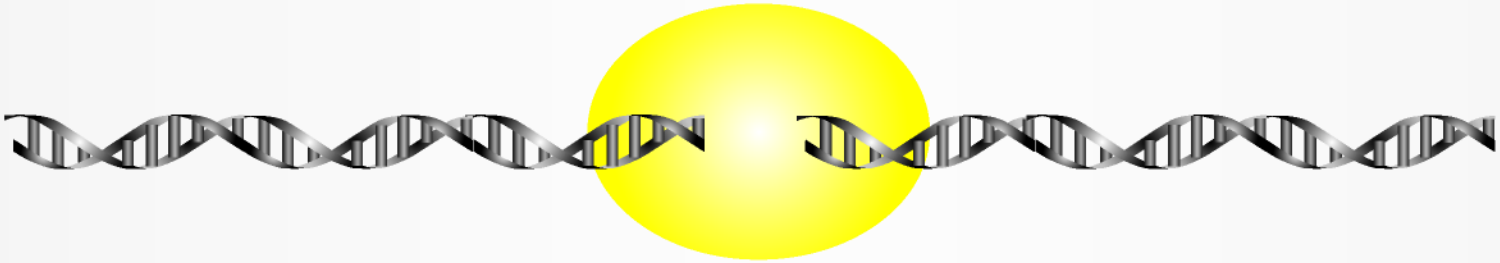
5 min

CDK4 inhibitor increases/speeds up Mre11 foci but not Ku foci.

Increased/fast Mre11 foci may not due to delayed DNA damage repair.



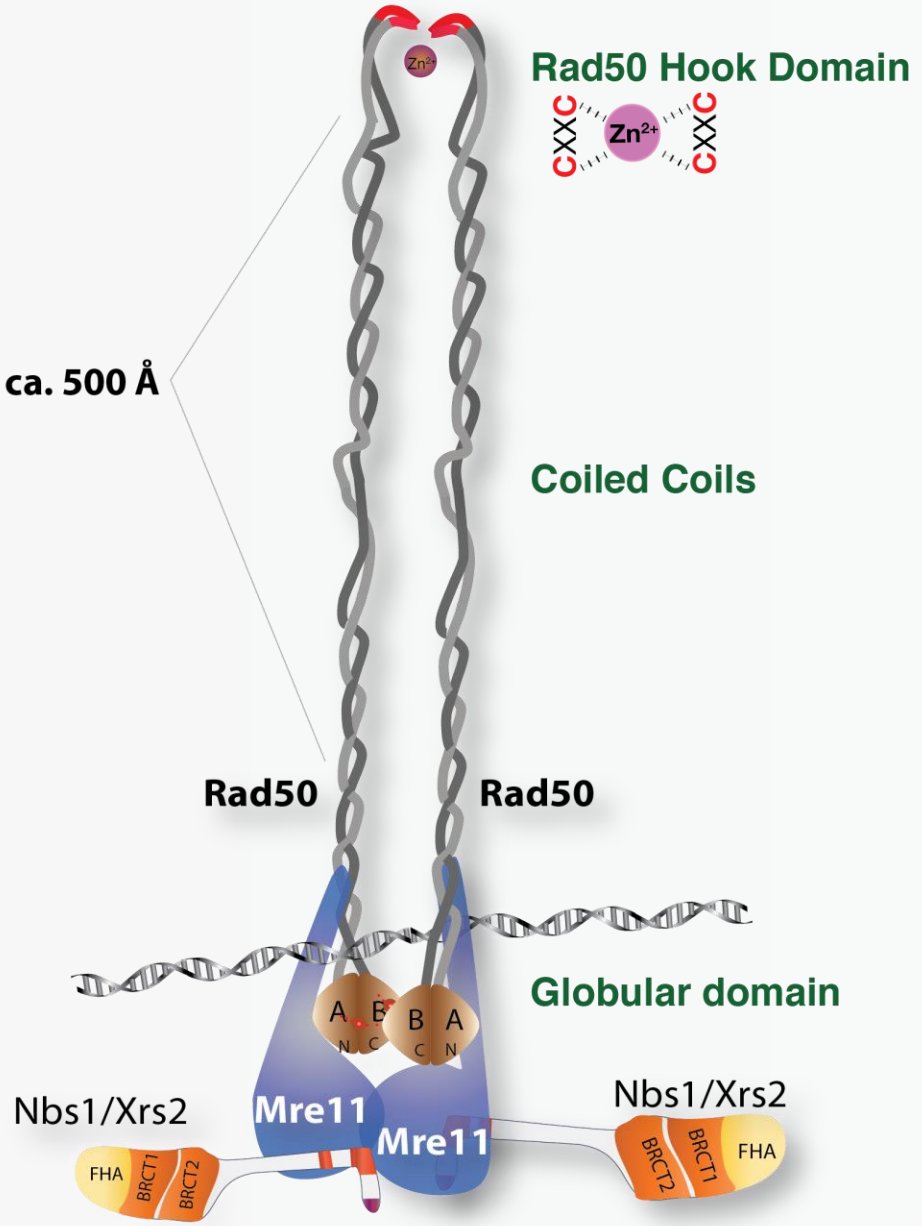
will repeat



DNA damage sensors

- Avidity for altered DNA structures (e.g., DNA breaks)
- Deficiency blocks activation of downstream functions

Mre11 complex's Architecture and Functions



1° Sensor of DNA damage

Required for ATM activation

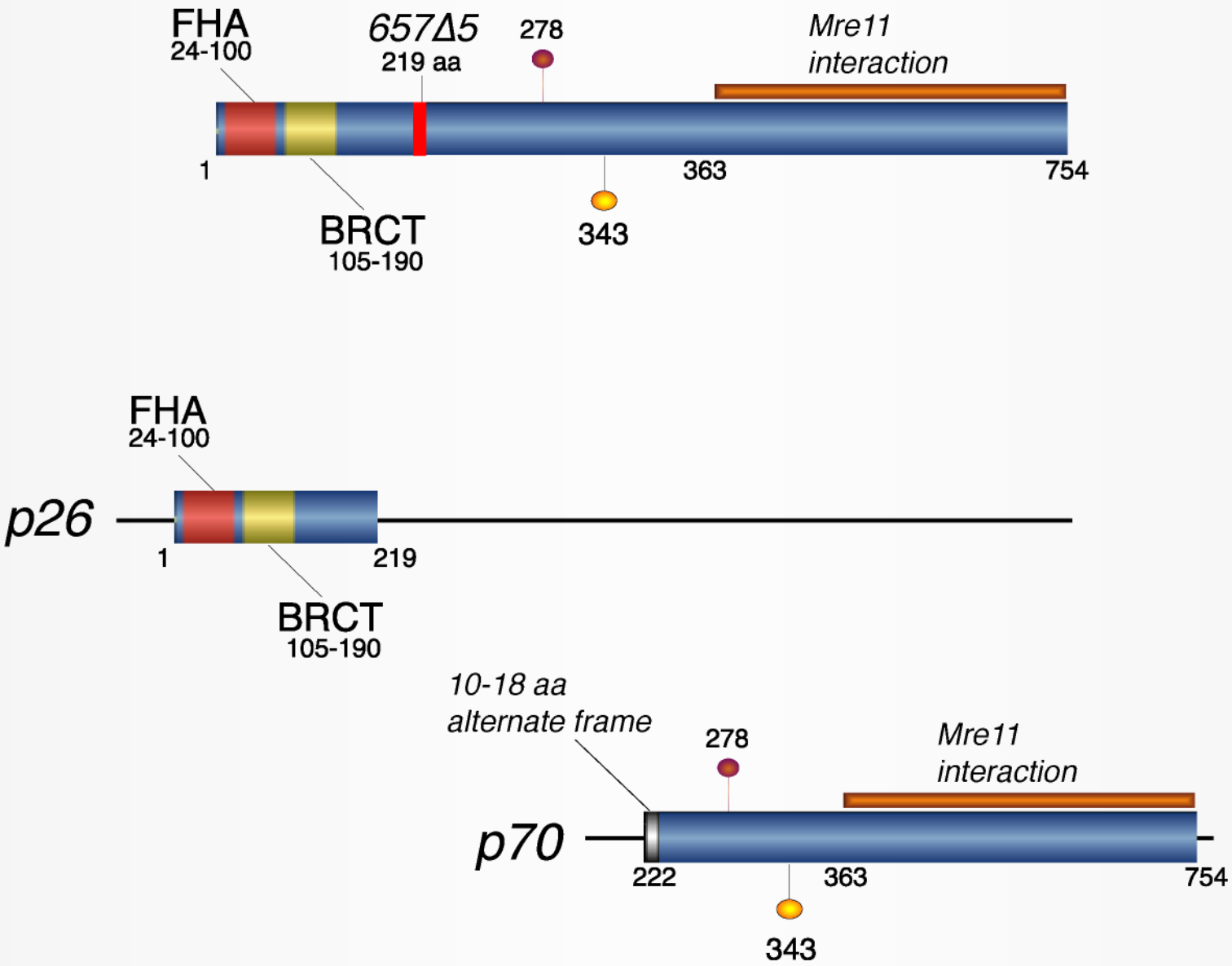
Required for DNA DSB repair

**Essential for viability*
(in higher eukaryotes)

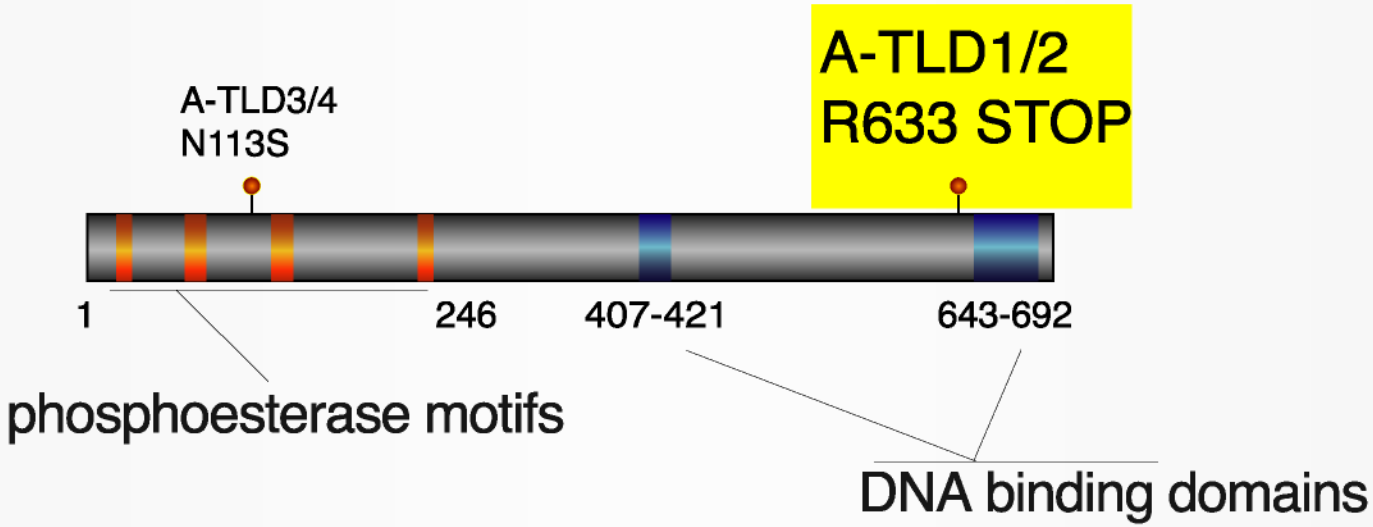
Inherited Mre11 complex mutations: Nijmegen breakage syndrome & the ataxia-telangiectasia like disorder

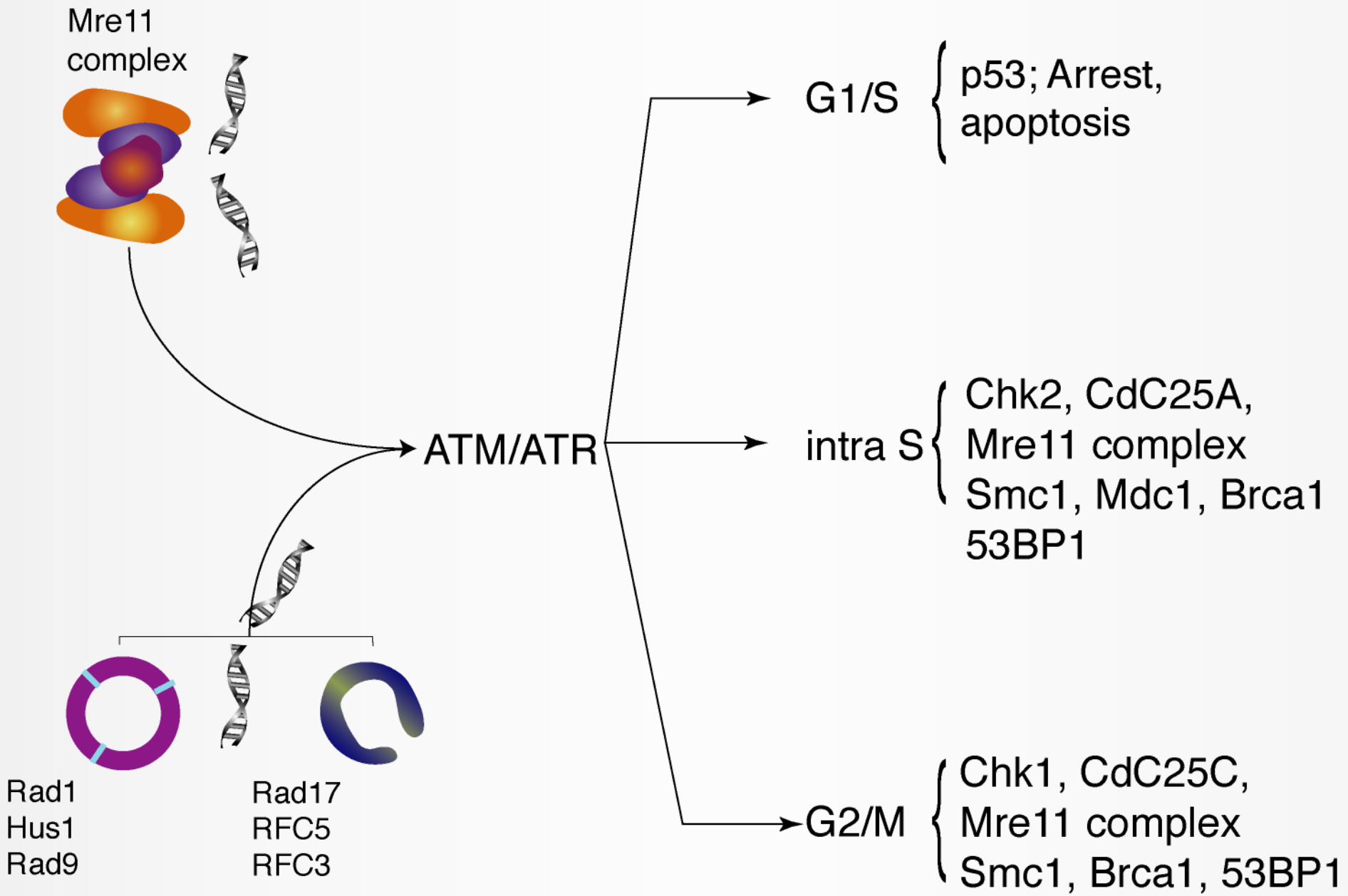
- *Nbs1* and *Mre11* mutations respectively
- Hypersensitivity to clastogens
- Chromosome fragility
- Intra-S phase checkpoint defect
- G2/M checkpoint defect
- Attenuated ATM activity

Two products of the common NBS allele

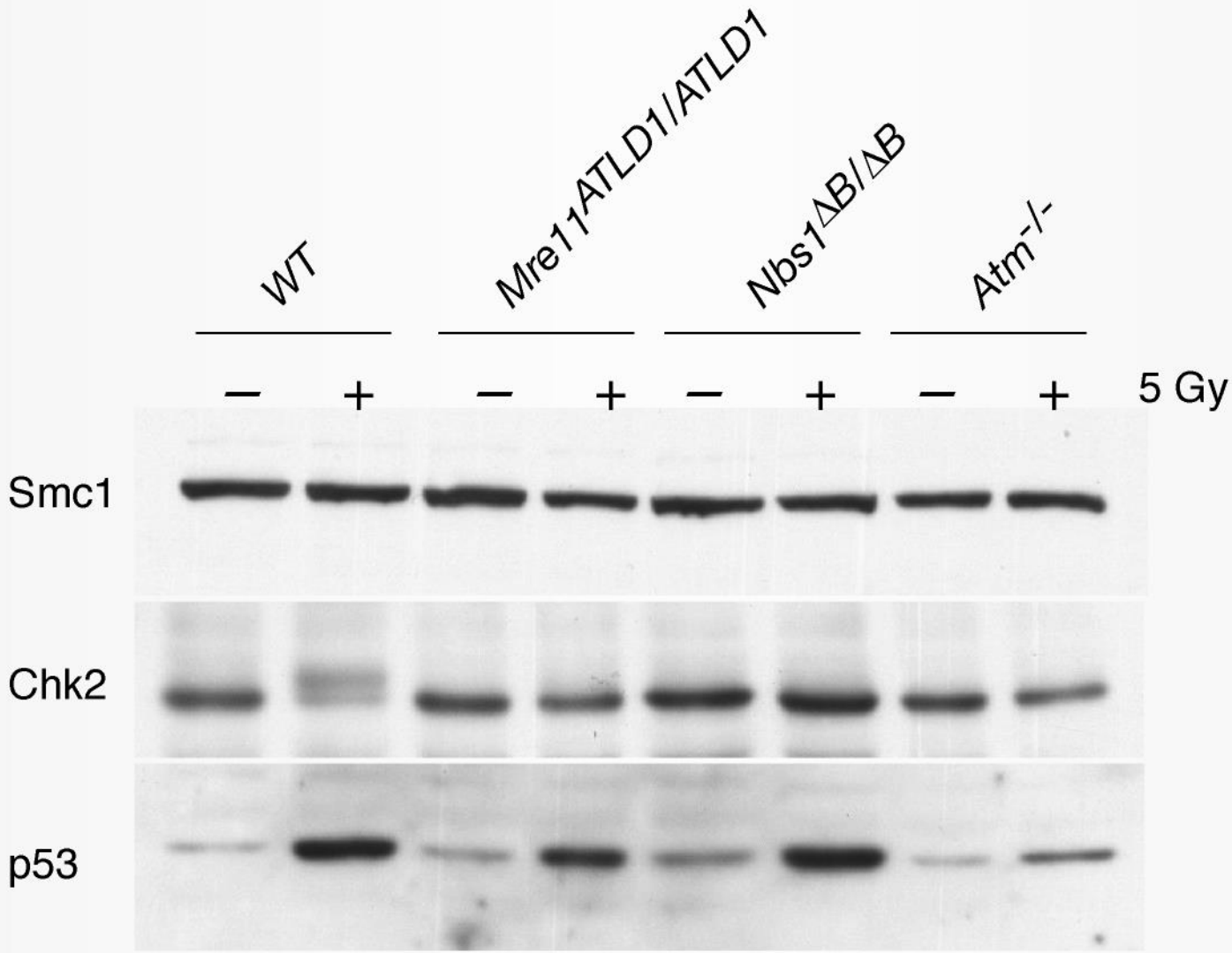


Mre11

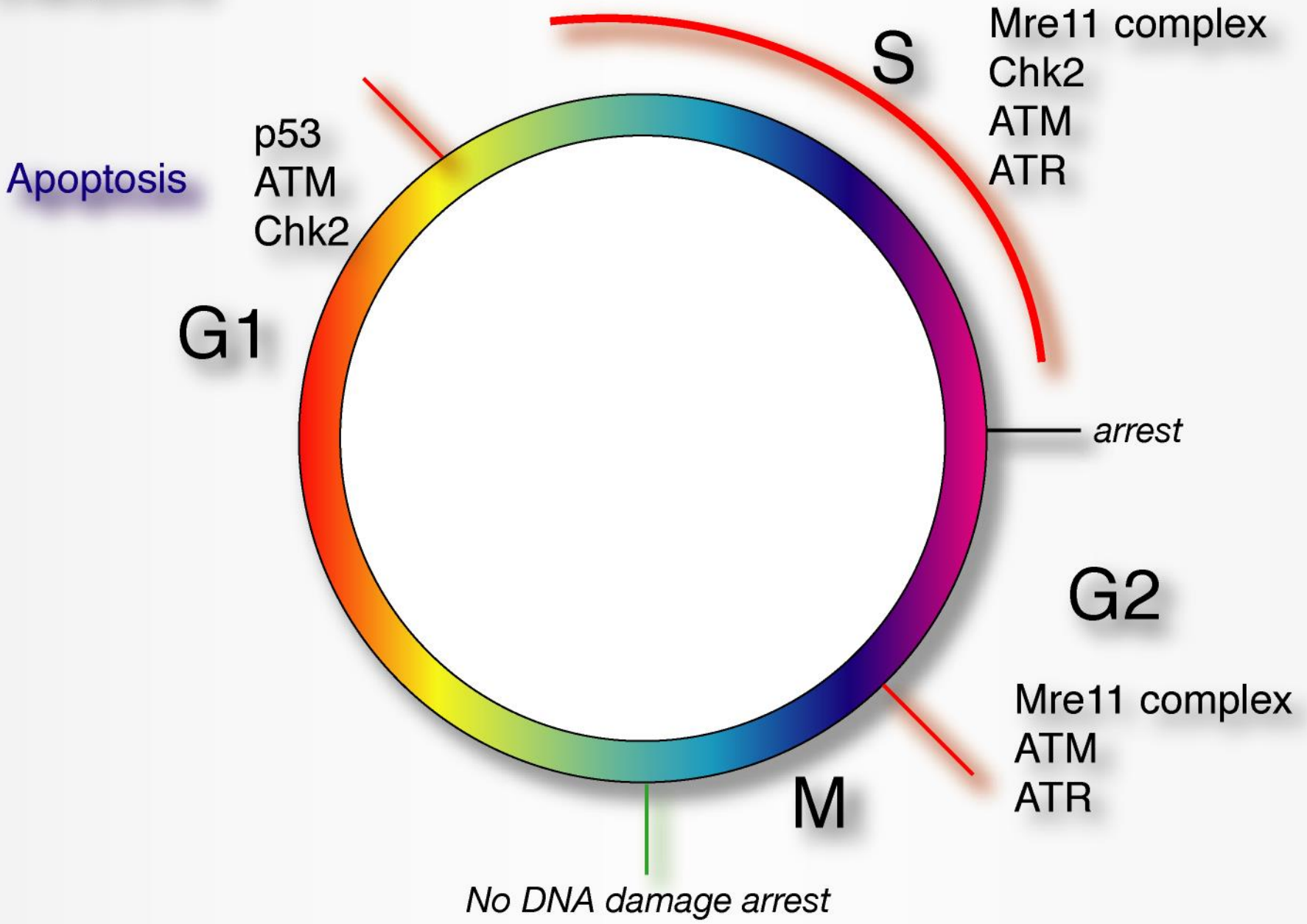

A-TLD3/4
N113S
A-TLD1/2
R633 STOP
1
246
407-421
643-692
phosphoesterase motifs
DNA binding domains

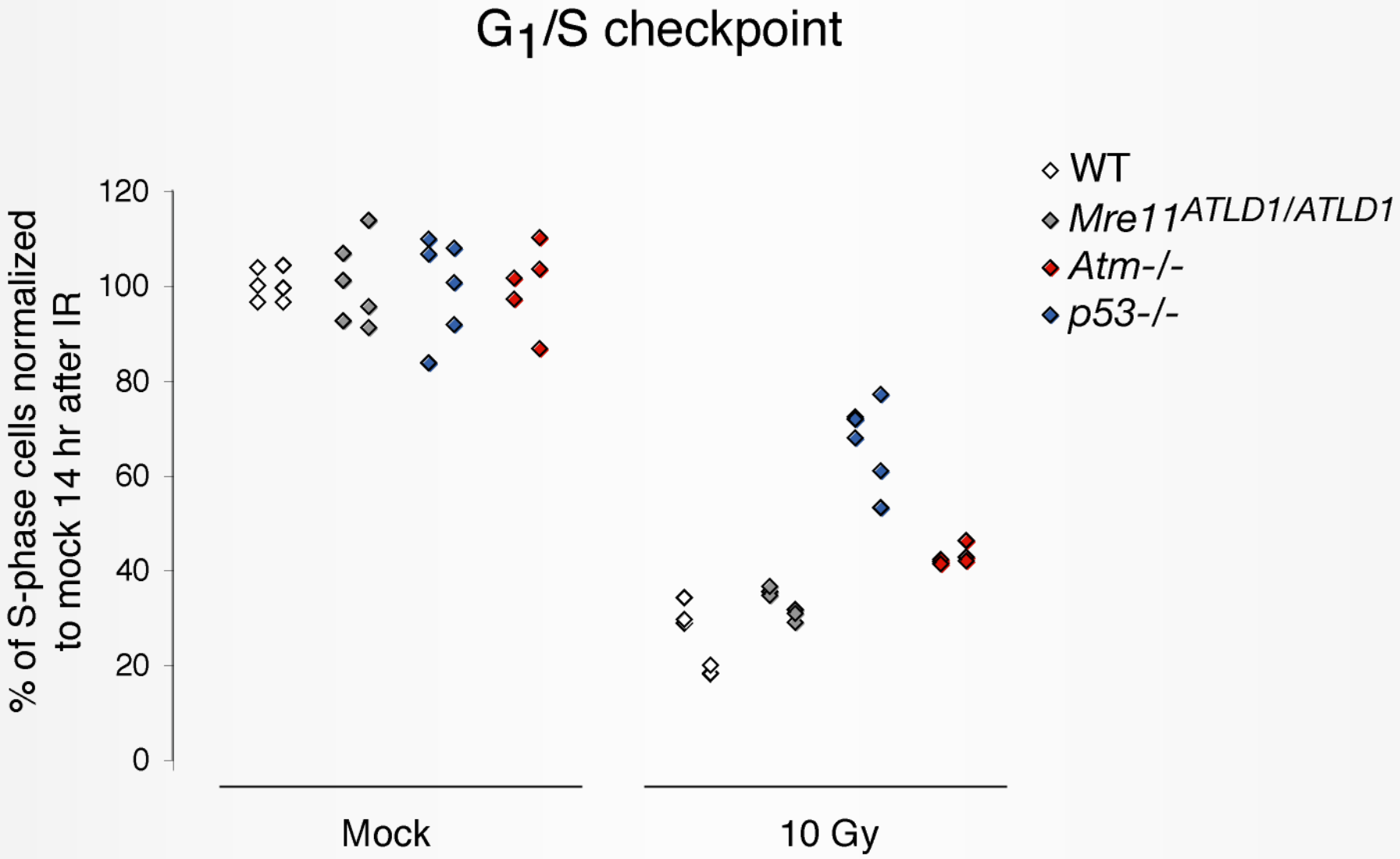

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
53BP1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1, 53BP1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

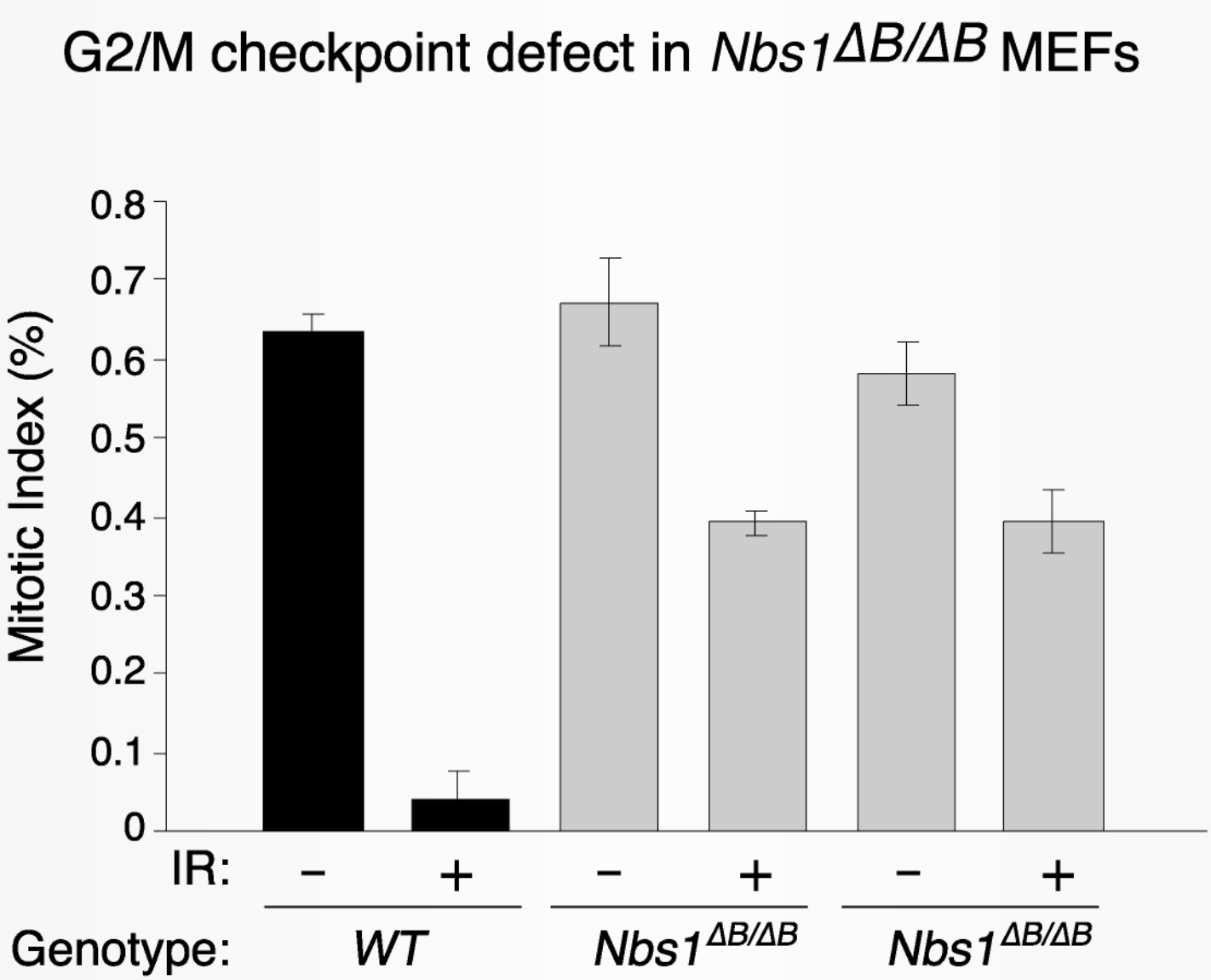
ATM targets in thymocytes



DNA damage dependent checkpoints

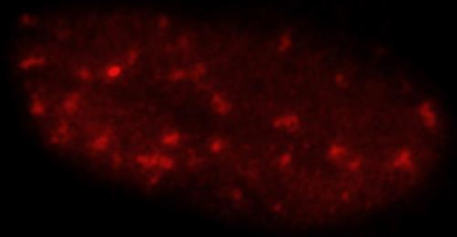



G1/S checkpoint
% of S-phase cells normalized to mock 14 hr after IR
120
100
80
60
40
20
0
Mock
10 Gy
WT
Mre11ATLD1/ATLD1
Atm-/-
p53-/-

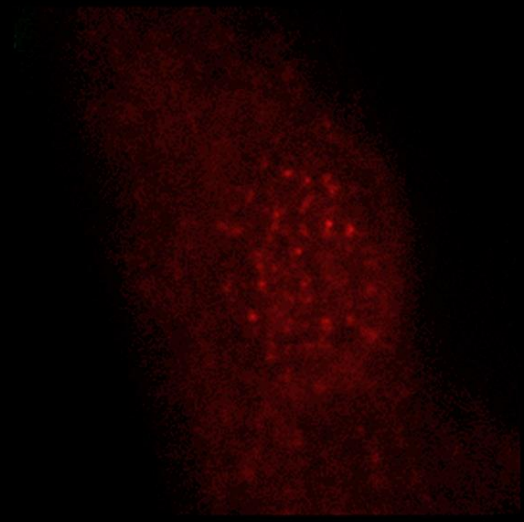

G2/M checkpoint defect in Nbs1ΔB/ΔB MEFs
Mitotic Index (%)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IR:
–
+
–
+
–
+
Genotype:
WT
Nbs1ΔB/ΔB
Nbs1ΔB/ΔB

Mre11 complex mislocalization in NBS and A-TLD

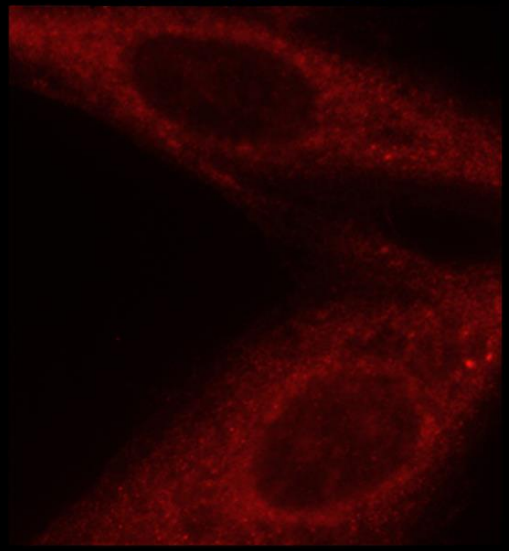
WT/Nbs1

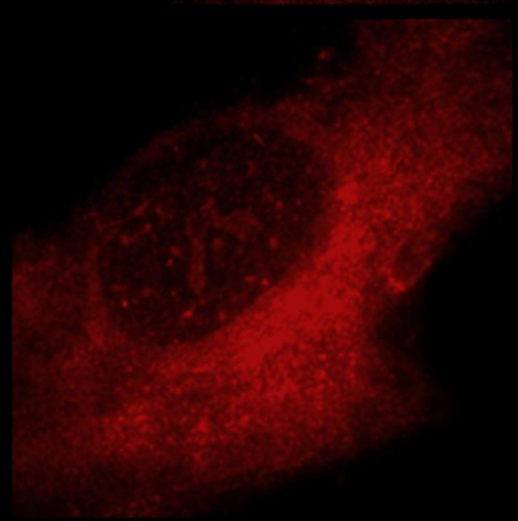


A-TLD/Nbs1

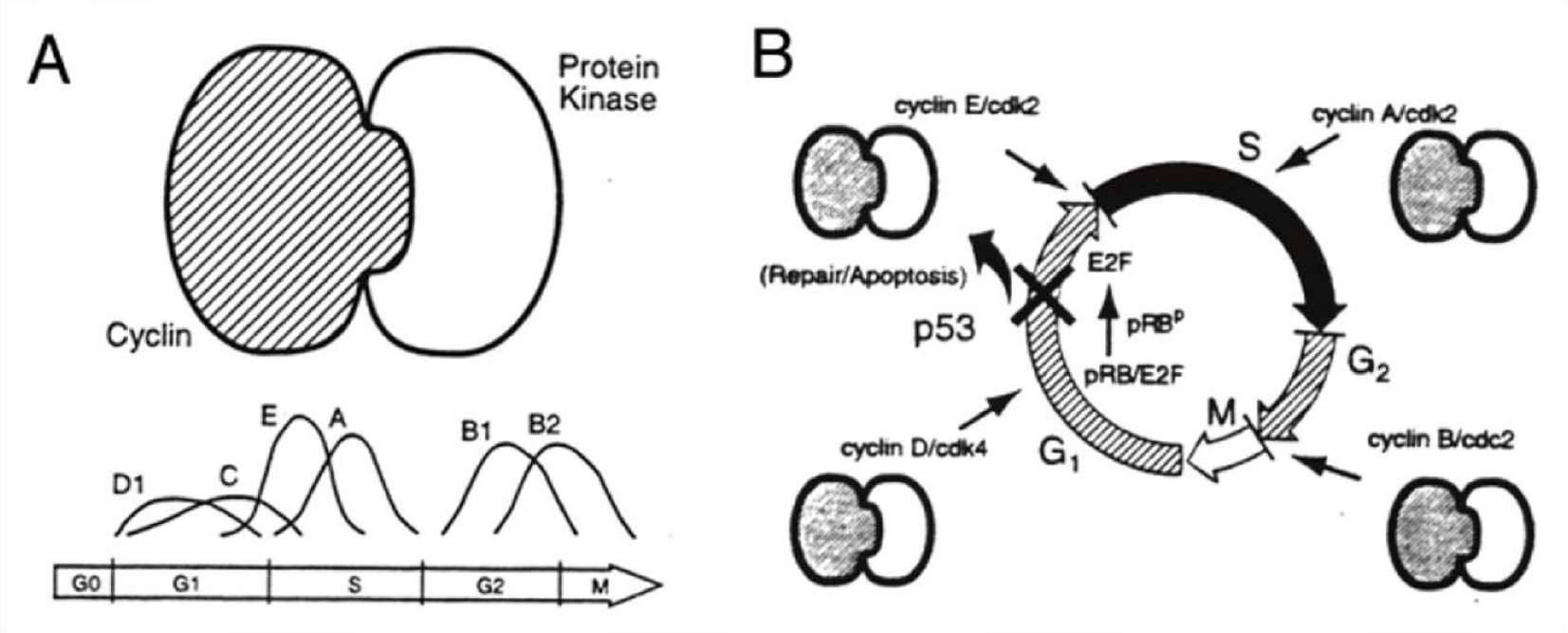


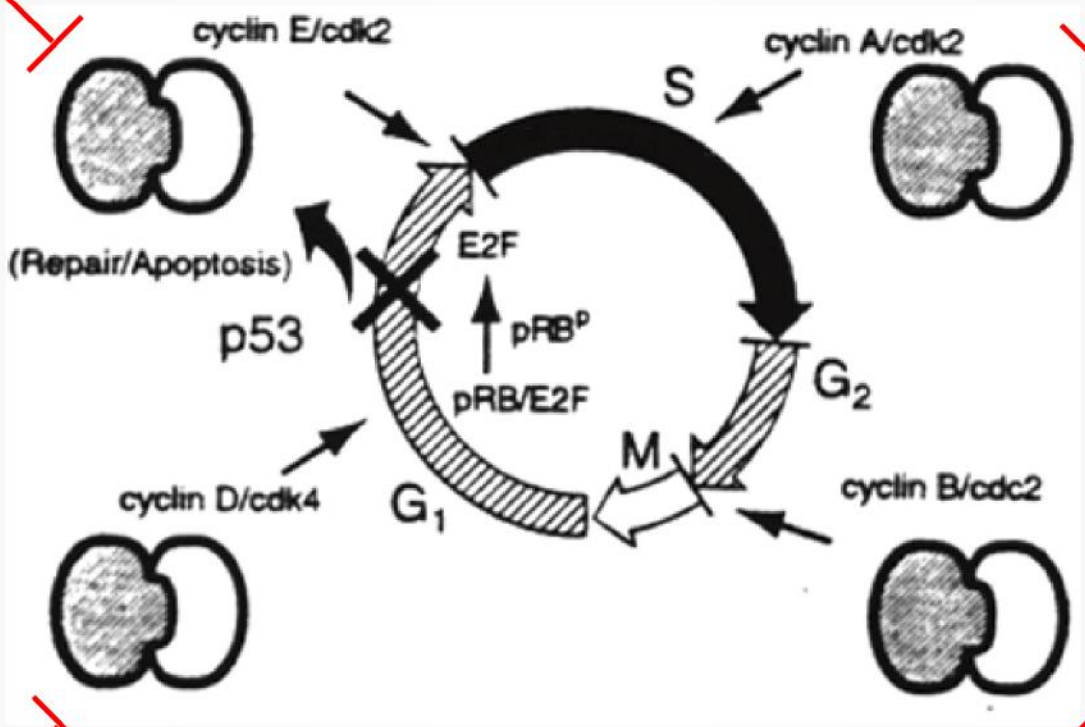
NBS/Mre11

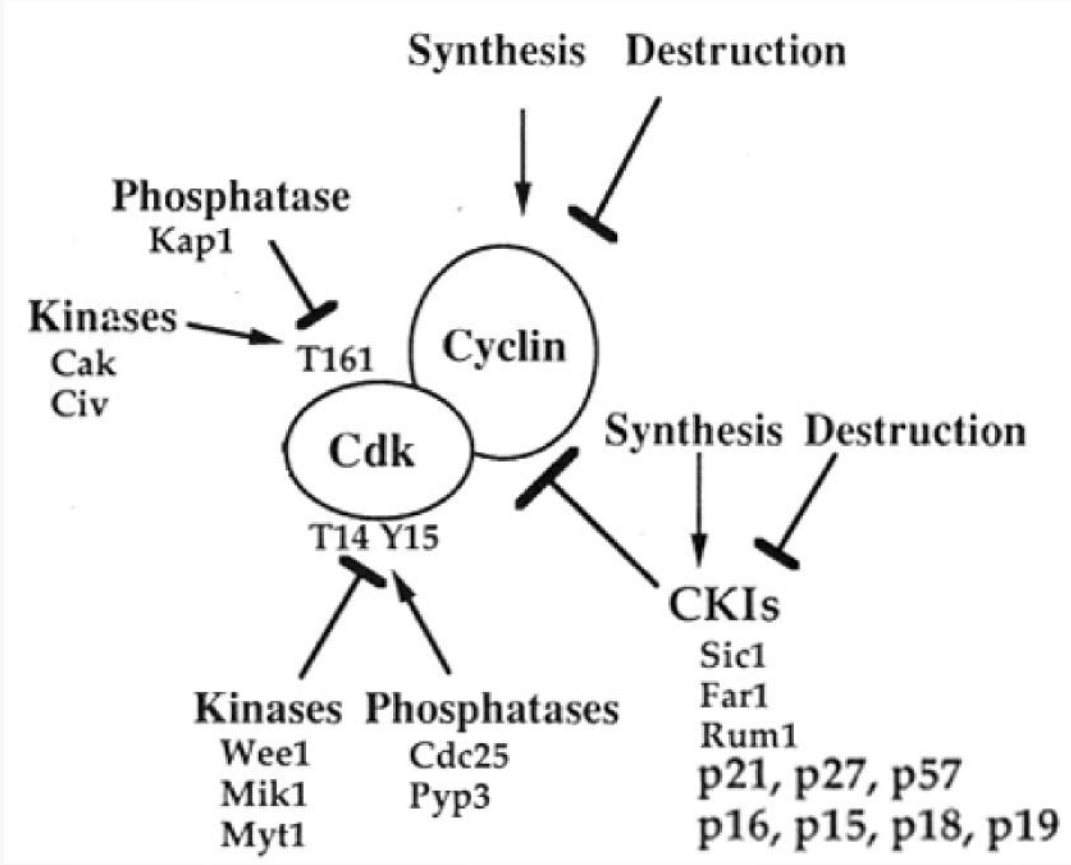


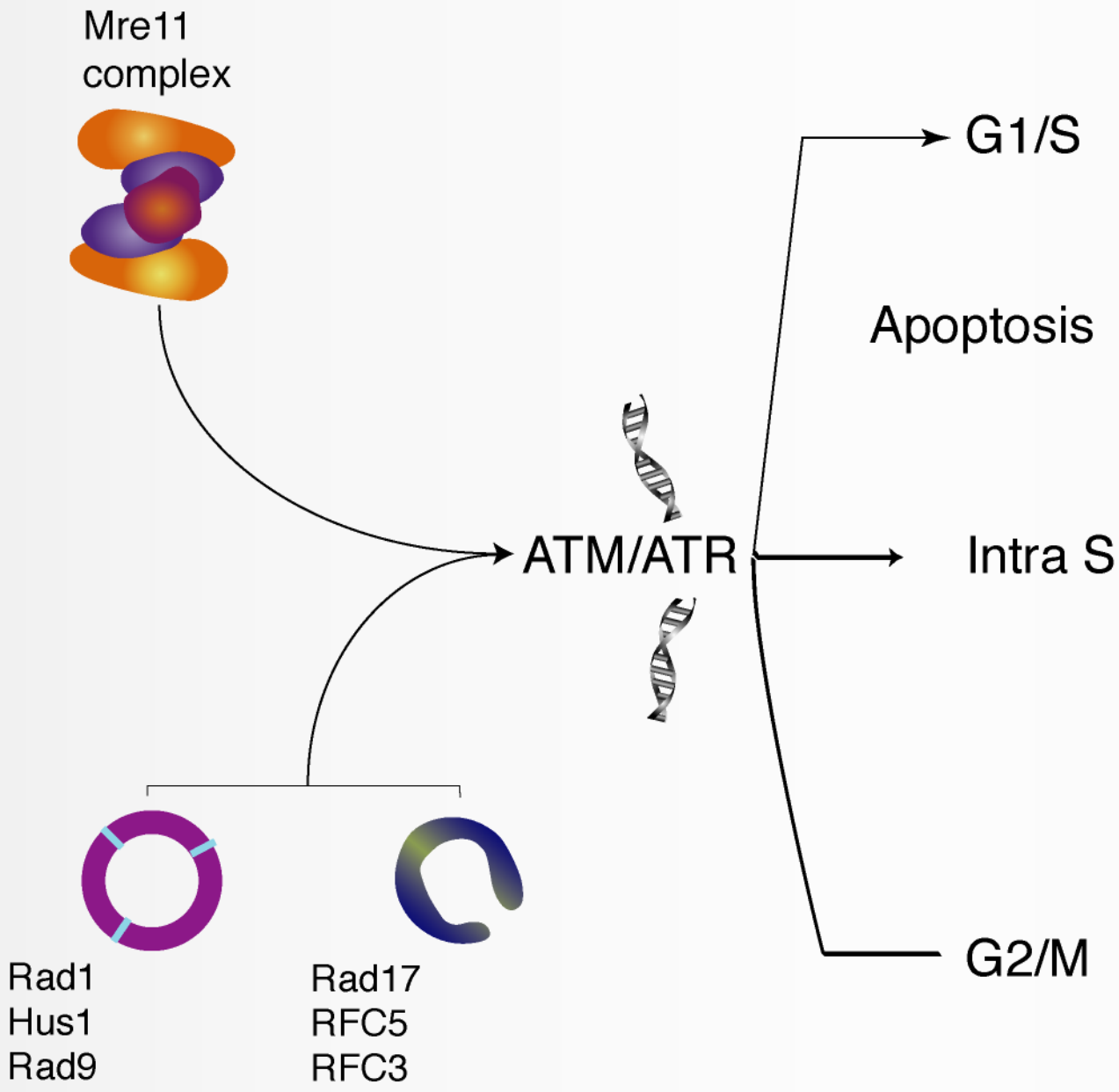


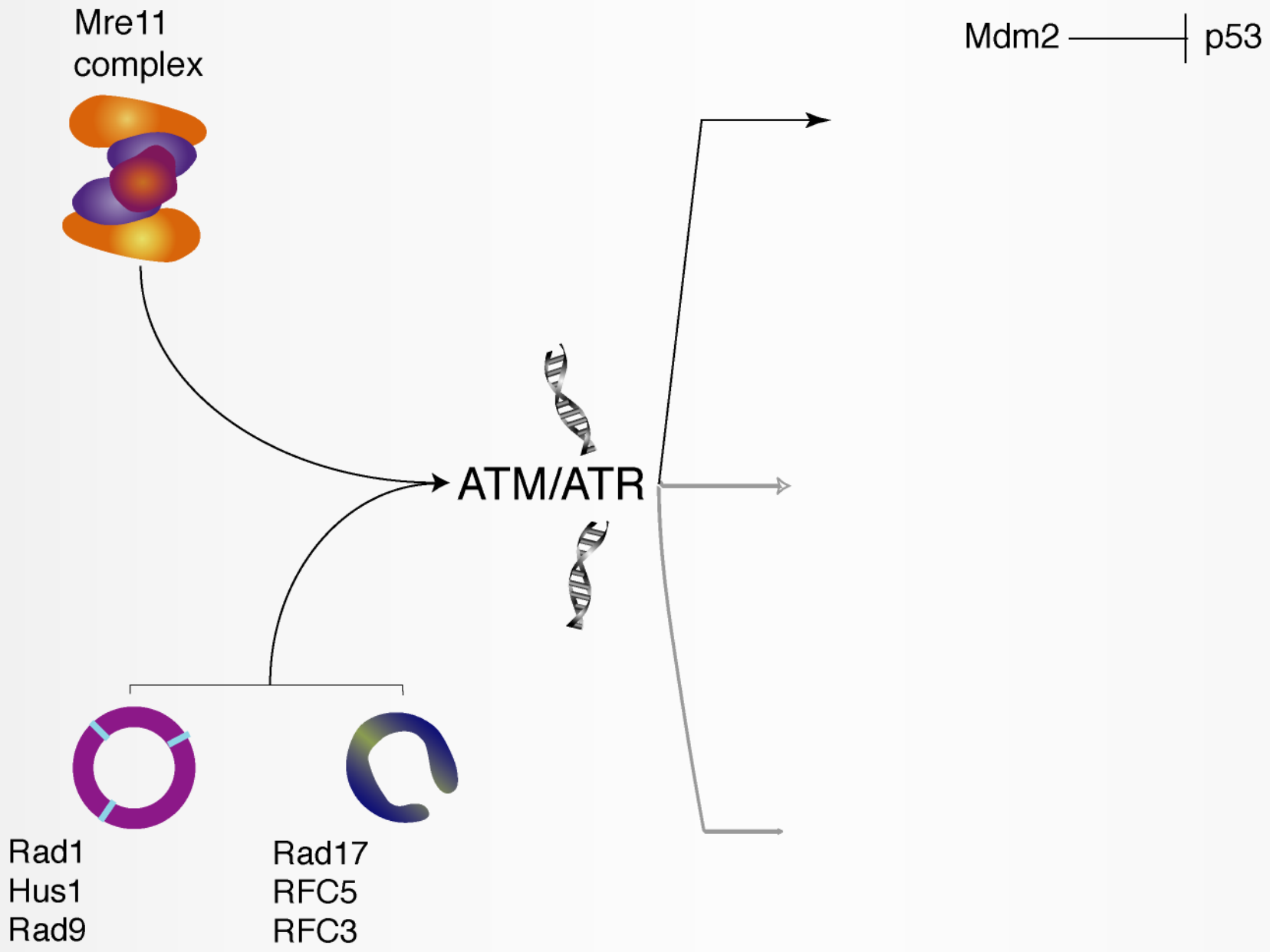
Where is this all going?

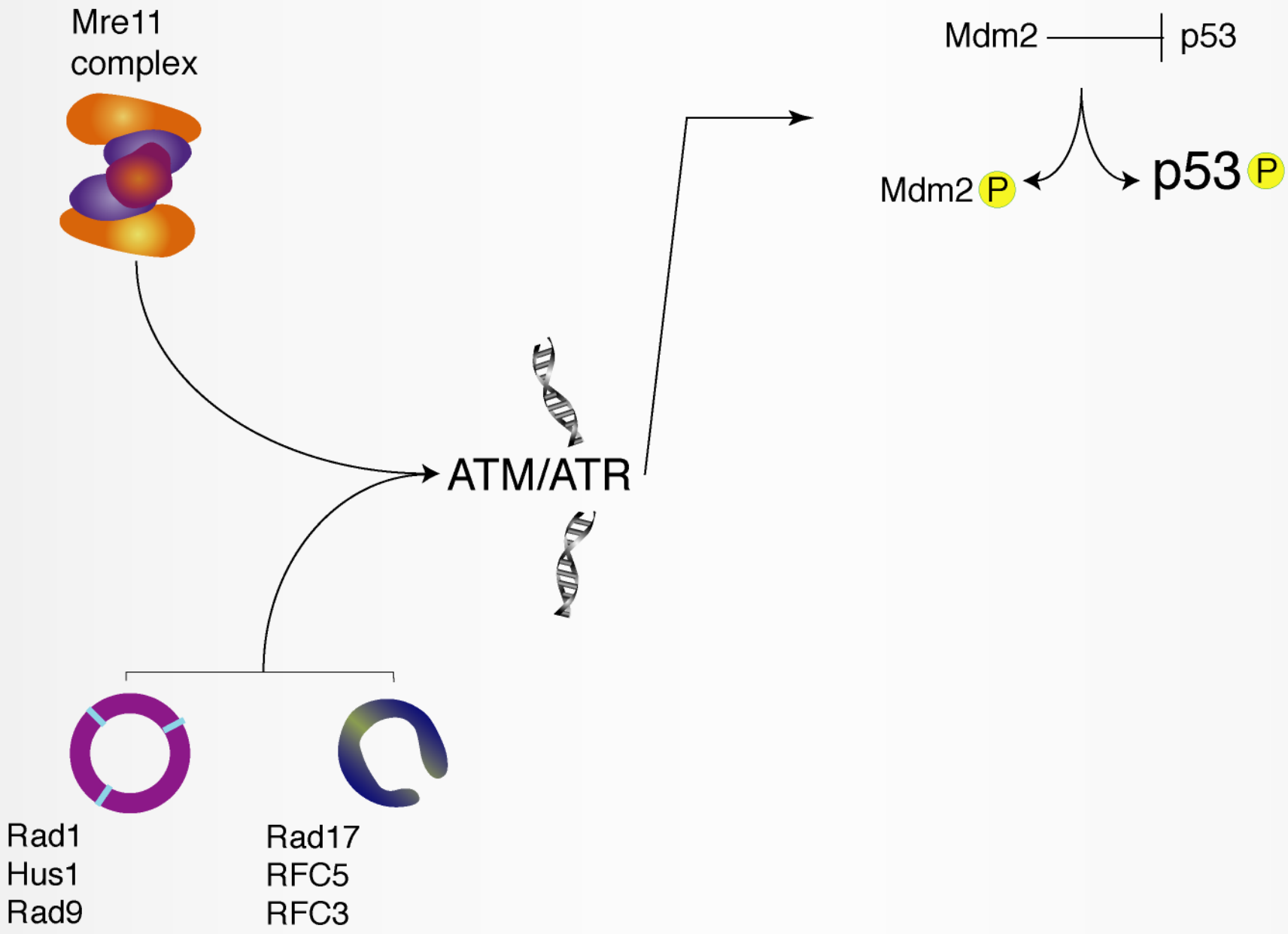

A
Protein Kinase
Cyclin
E
A
B1
B2
D1
C
G0
G1
S
G2
M
B
cyclin E/cdk2
cyclin A/cdk2
S
(Repair/Apoptosis)
E2F
p53
pRB^p
pRB/E2F
G2
M
cyclin D/cdk4
G1
cyclin B/cdc2

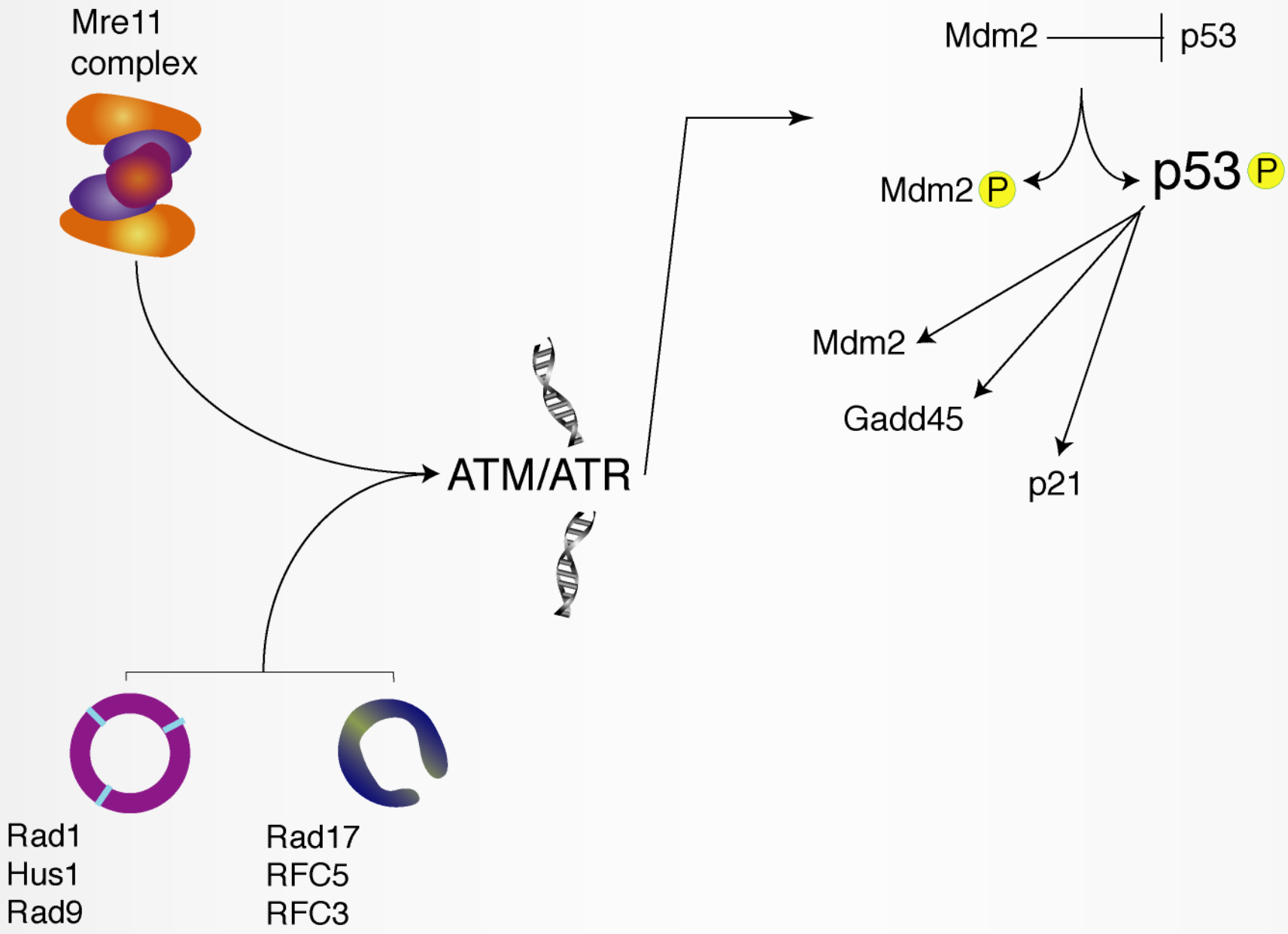

cyclin E/cdk2
cyclin A/cdk2
S
(Repair/Apoptosis)
E2F
p53
pRB^p
pRB/E2F
G2
M
cyclin D/cdk4
G1
cyclin B/cdc2

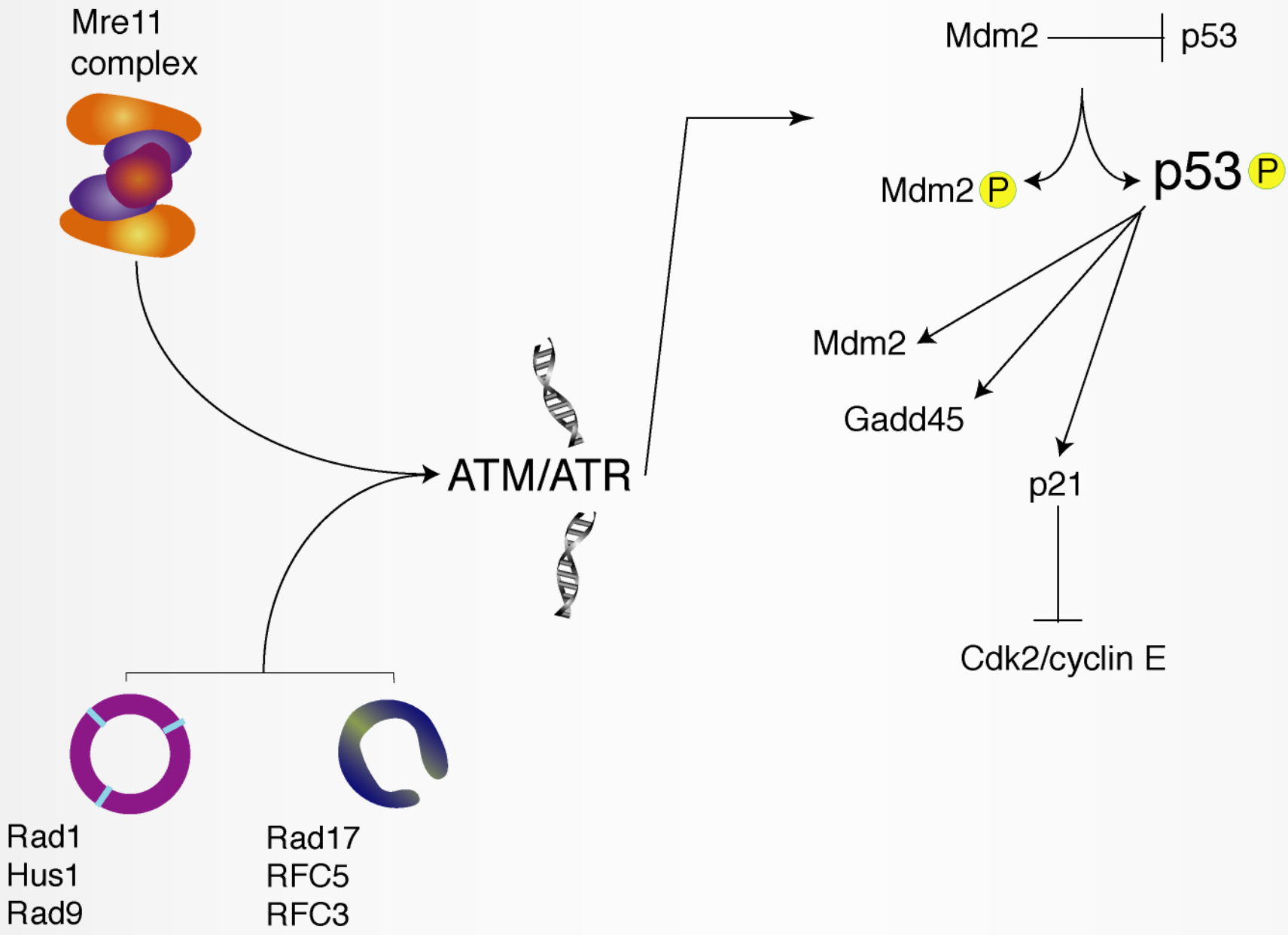

Synthesis
Destruction
Phosphatase
Kap1
Kinases
Cak
Civ
T161
Cyclin
Cdk
T14 Y15
Synthesis
Destruction
CKIs
Sic1
Far1
Rum1
p21, p27, p57
p16, p15, p18, p19
Kinases
Wee1
Mik1
Myt1
Phosphatases
Cdc25
Pyp3

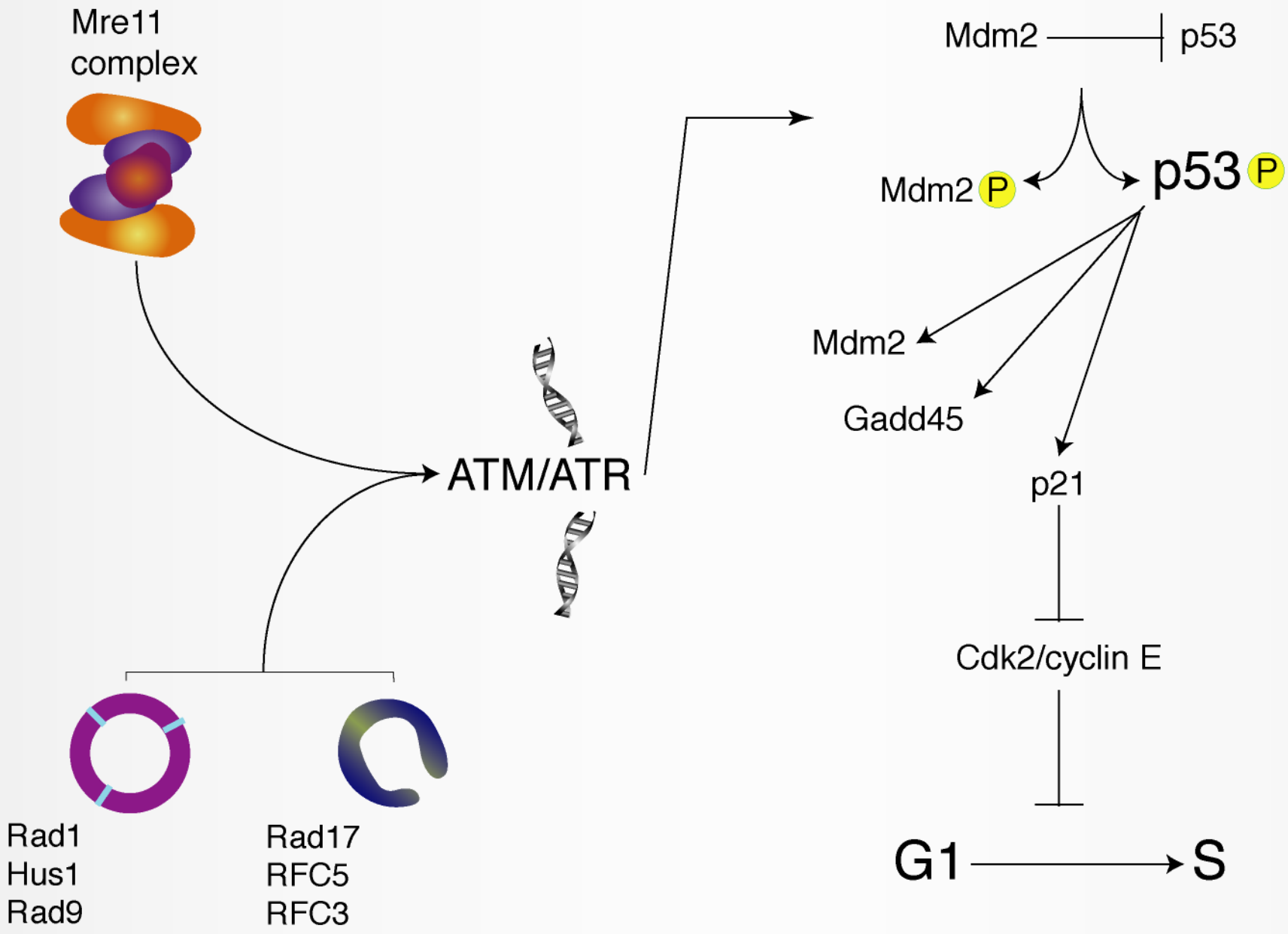

Mre11
complex
G1/S
Apoptosis
ATM/ATR
Intra S
G2/M
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

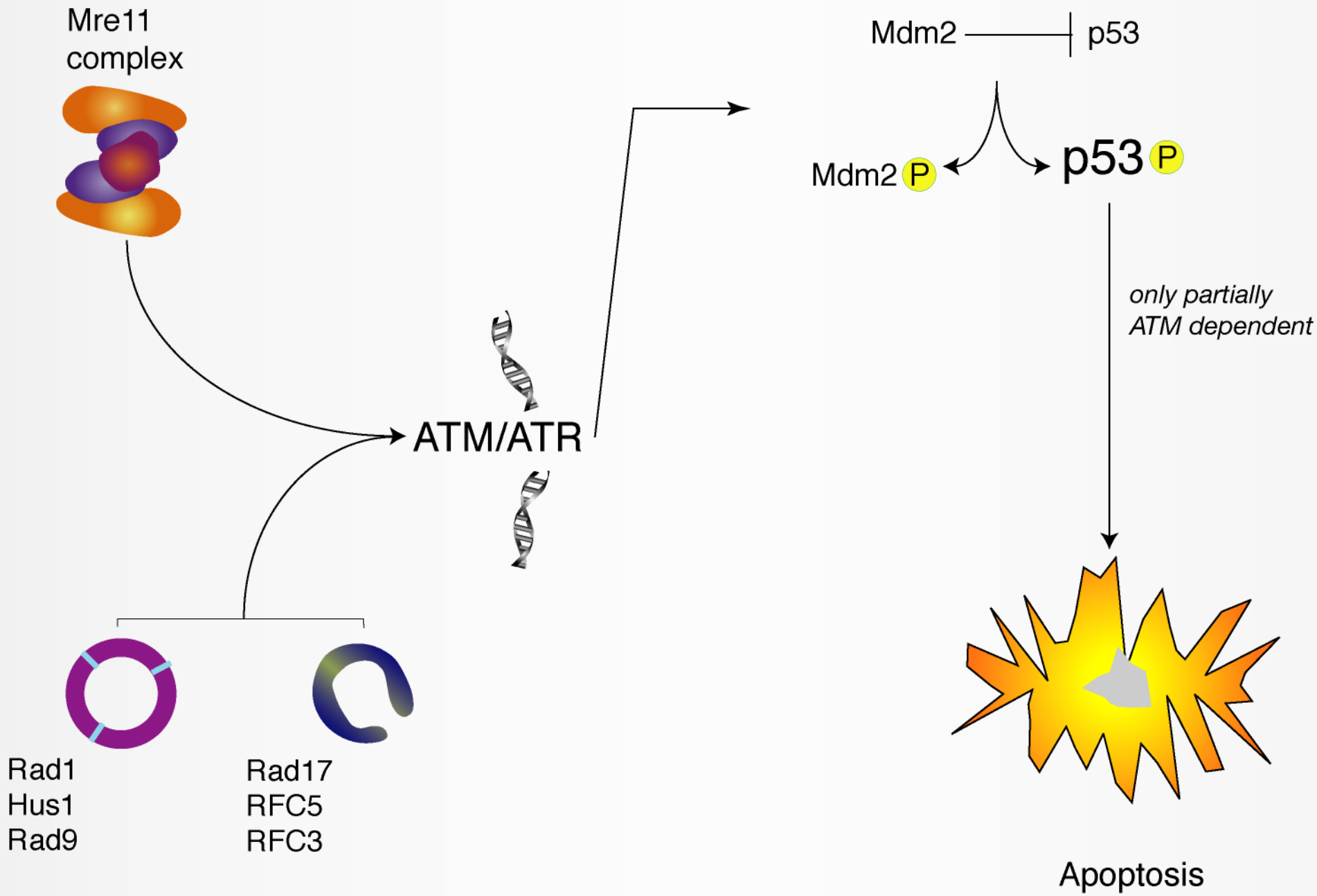

Mre11
complex
Mdm2
p53
ATM/ATR
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

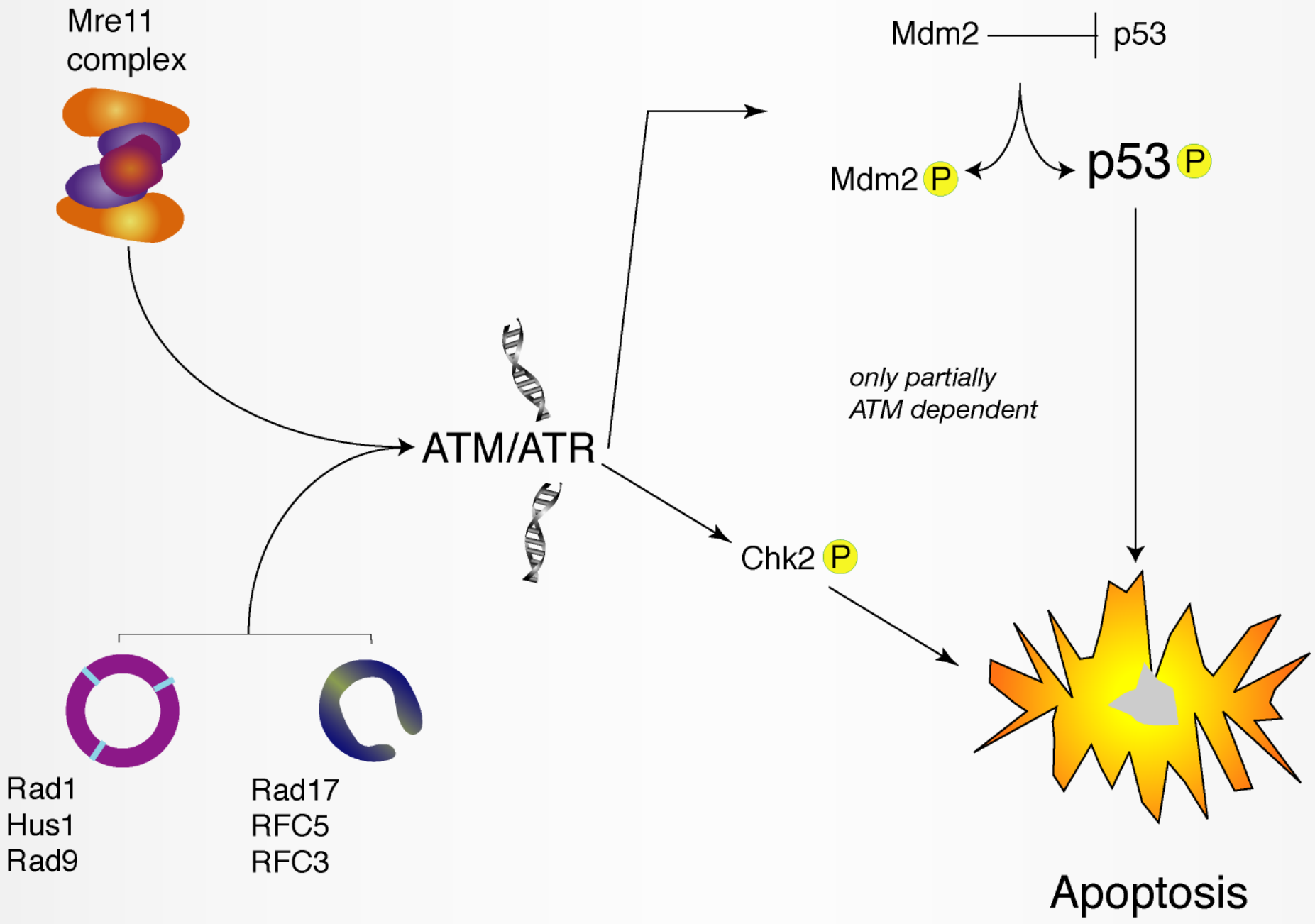

Mre11
complex
Mdm2
p53
Mdm2 P
p53 P
ATM/ATR
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

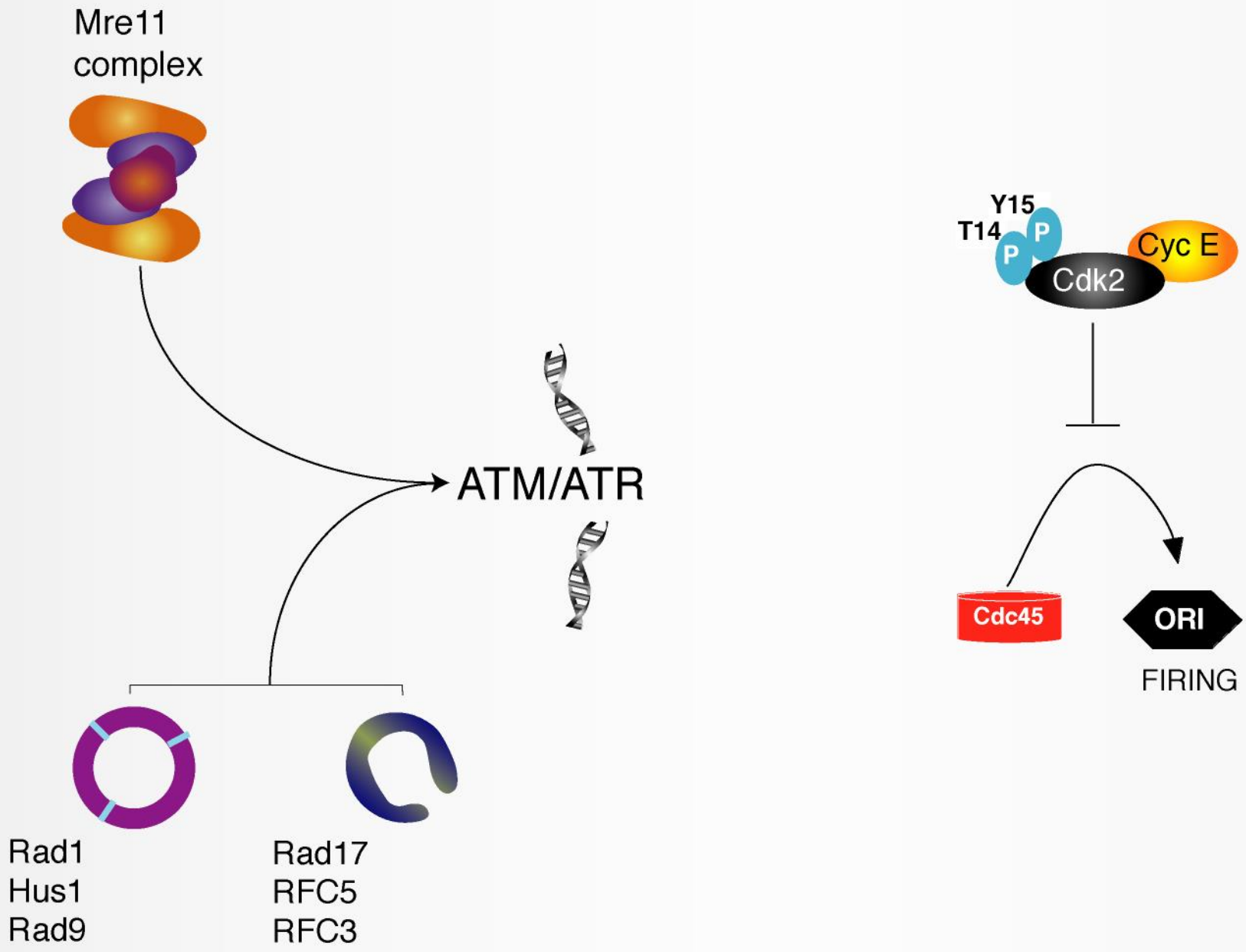

Mre11
complex
Mdm2
p53
Mdm2 P
p53 P
Mdm2
Gadd45
p21
ATM/ATR
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

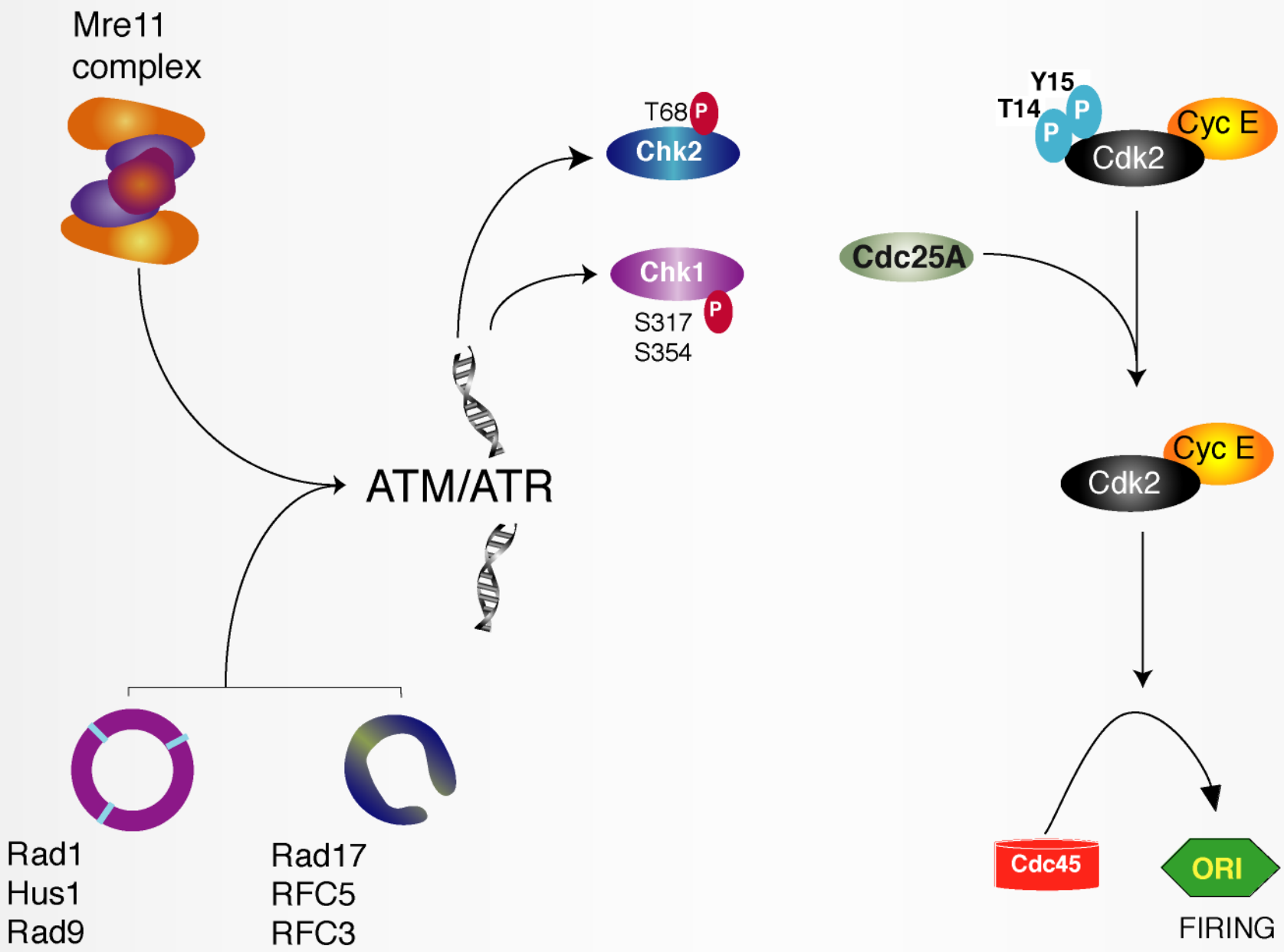

Mre11
complex
Mdm2
p53
Mdm2 P
p53 P
Mdm2
Gadd45
p21
ATM/ATR
Cdk2/cyclin E
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

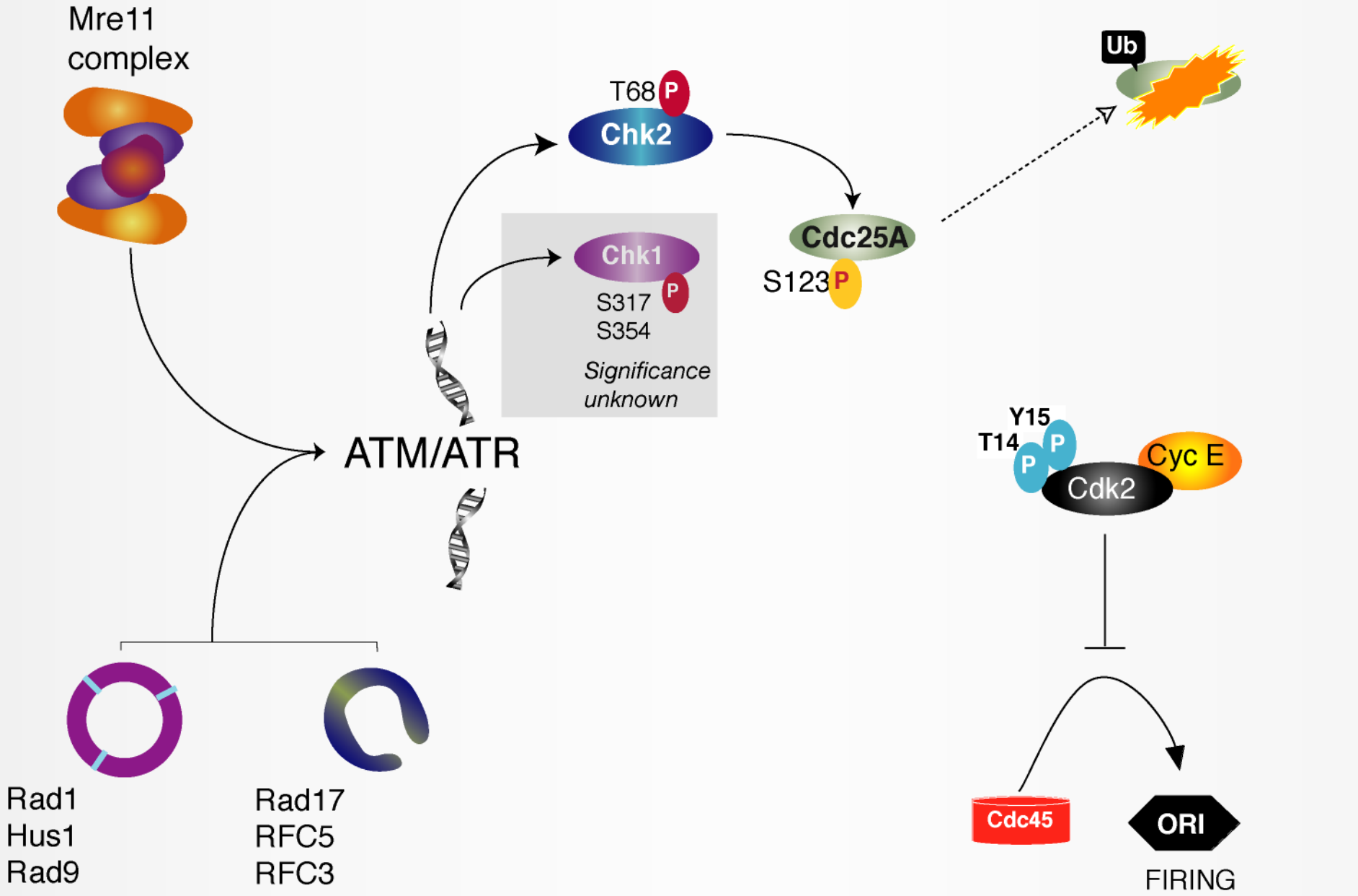

Mre11
complex
Mdm2
p53
Mdm2 P
p53 P
Mdm2
Gadd45
p21
ATM/ATR
Cdk2/cyclin E
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
G1
S


Mre11
complex
Mdm2
p53
Mdm2 P
p53 P
only partially
ATM dependent
ATM/ATR
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Apoptosis

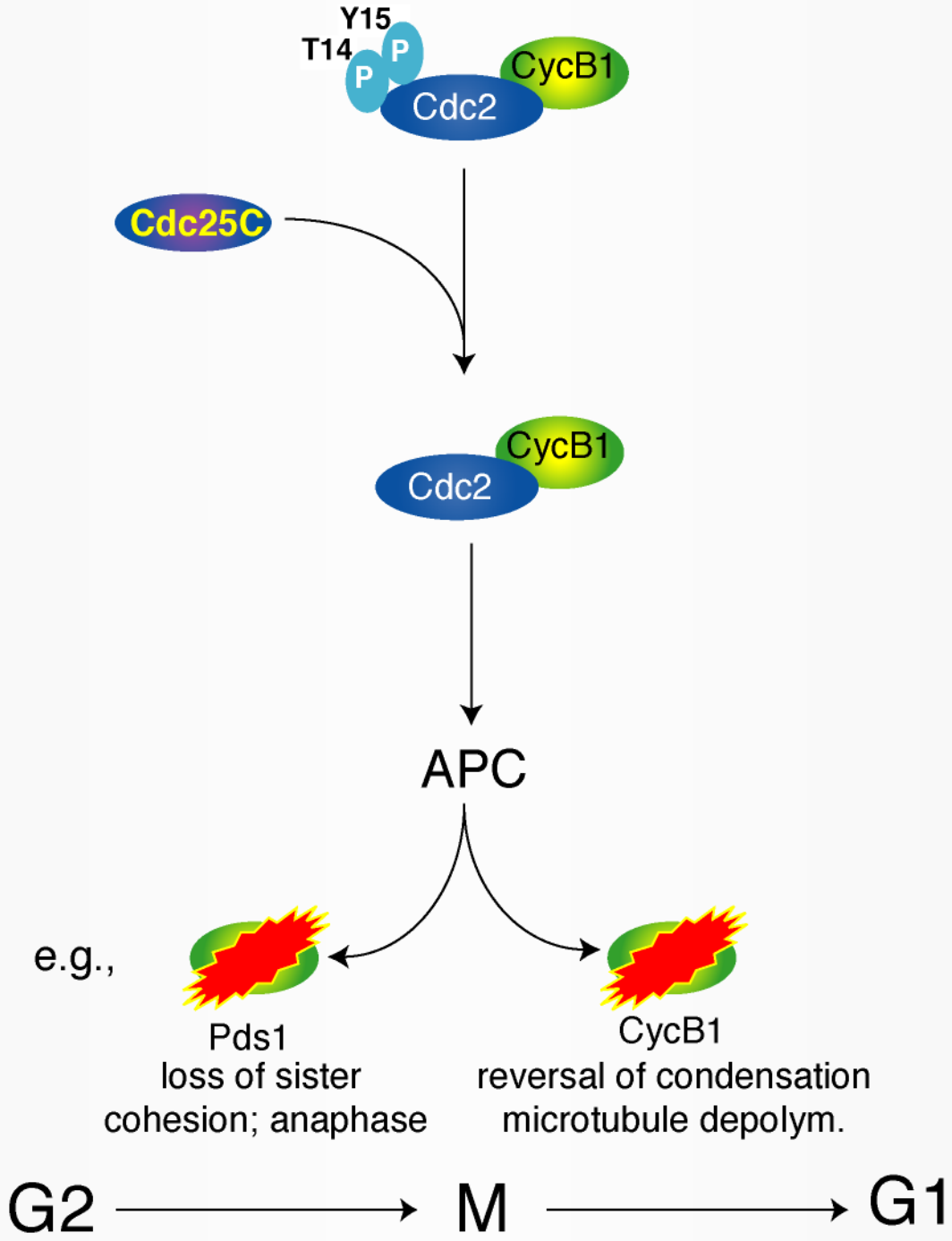

Mre11
complex
Mdm2
p53
Mdm2 P
p53 P
only partially
ATM dependent
ATM/ATR
Chk2 P
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Apoptosis

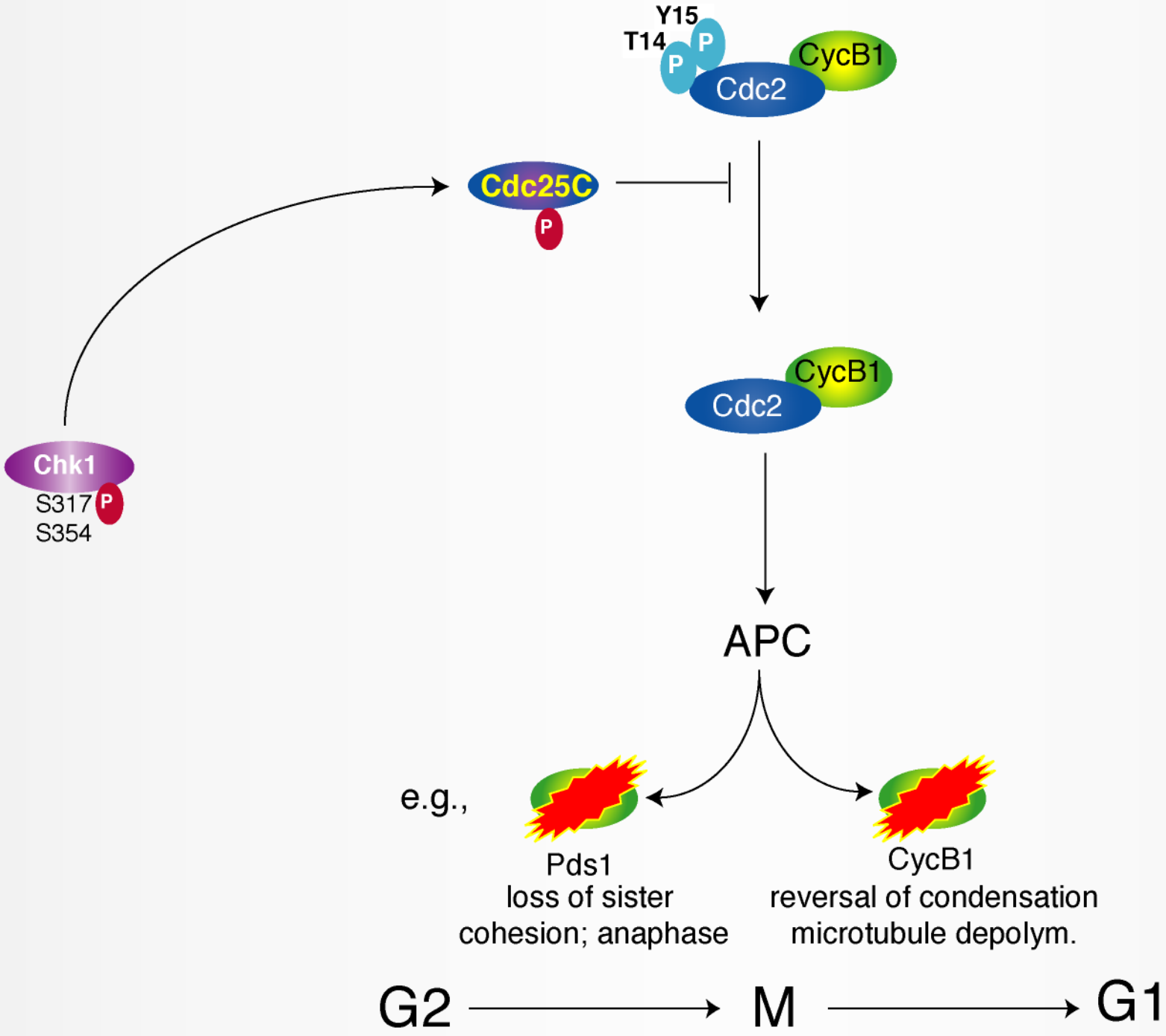

Mre11
complex
ATM/ATR
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Y15
T14
P
P
Cyc E
Cdk2
Cdc45
ORI
FIRING

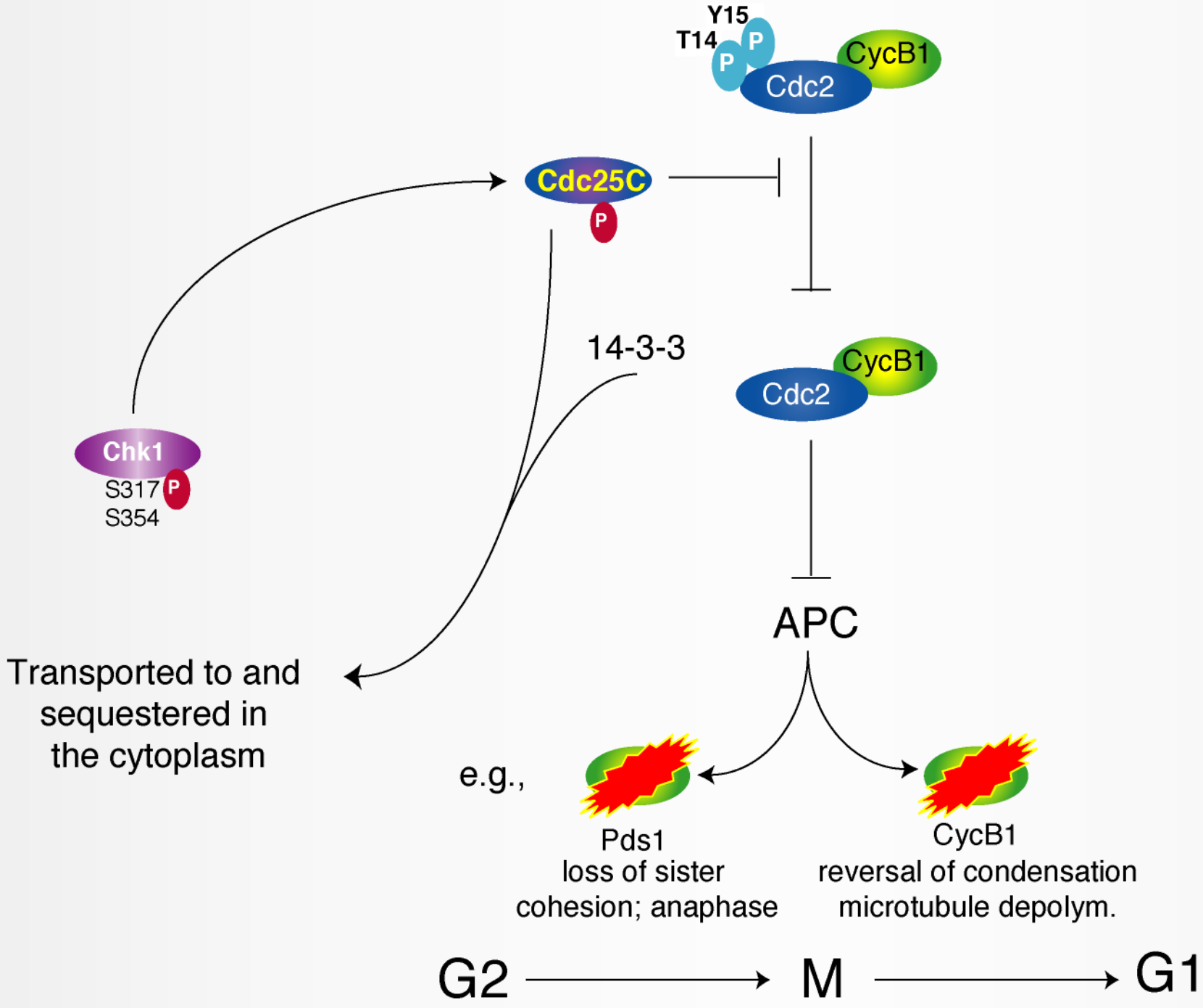

Mre11
complex
T68
P
Chk2
Chk1
S317
S354
P
ATM/ATR
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Y15
T14
P
P
Cyc E
Cdk2
Cdc25A
Cyc E
Cdk2
Cdc45
ORI
FIRING

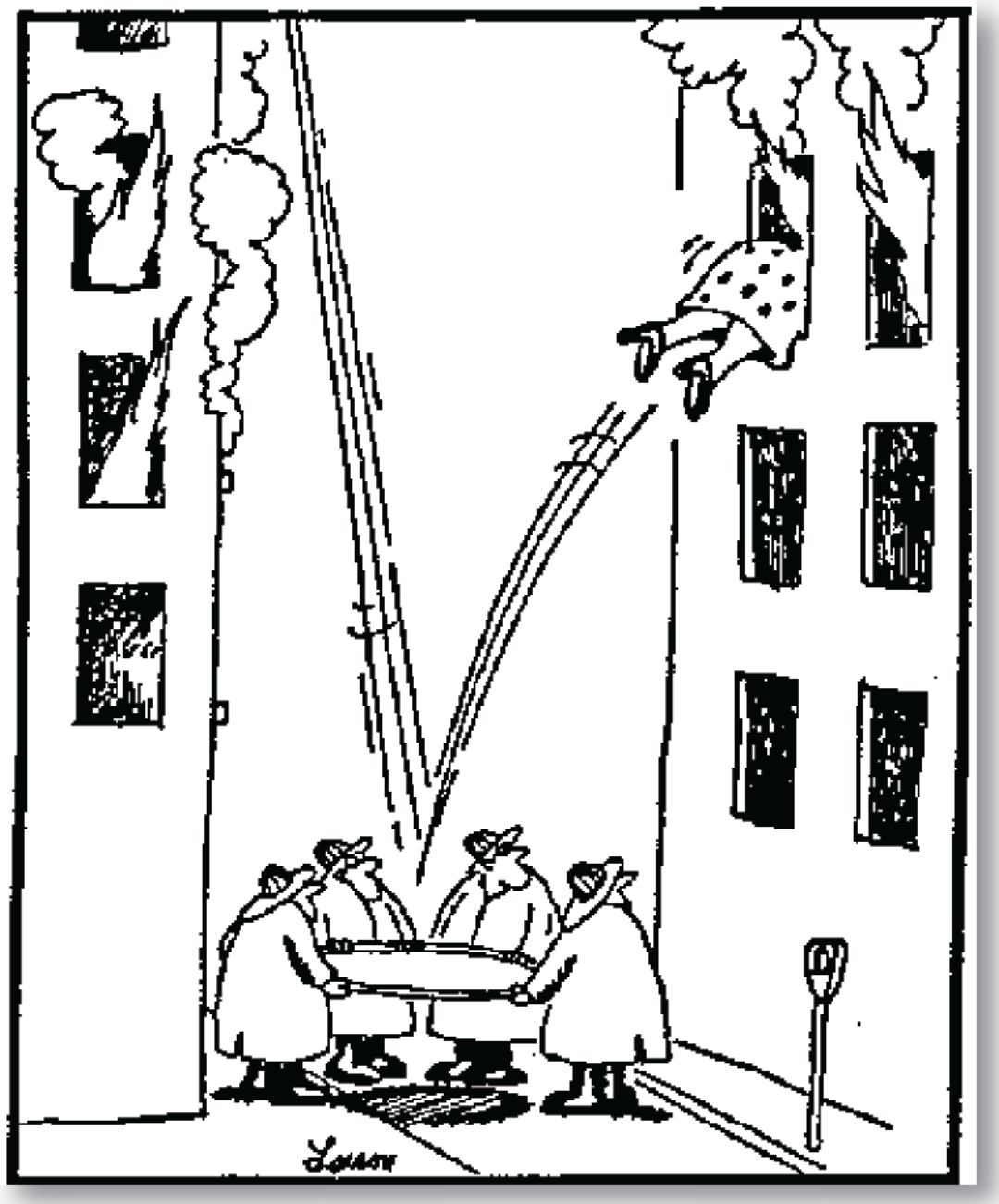


Intra S phase checkpoint

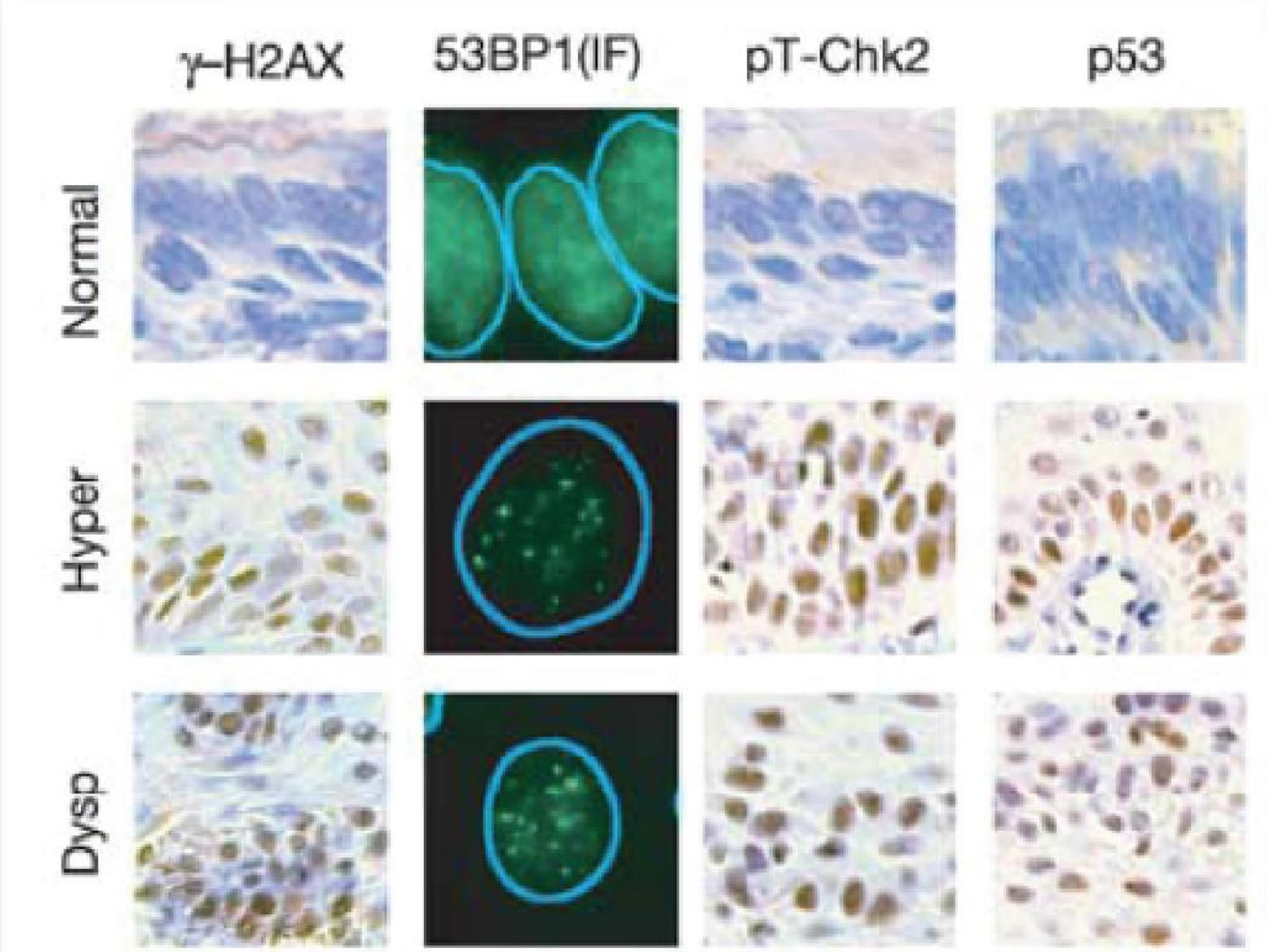

Y15
T14
P
P
Cdc2
CycB1
Cdc25C
Cdc2
CycB1
APC
e.g.,
Pds1
loss of sister
cohesion; anaphase
CycB1
reversal of condensation
microtubule depolym.
G2
M
G1


Y15
T14
P
P
Cdc2
CycB1
Cdc25C
P
Chk1
S317
S354
P
Cdc2
CycB1
APC
e.g.,
Pds1
loss of sister
cohesion; anaphase
CycB1
reversal of condensation
microtubule depolym.
G2
M
G1


Y15
T14
P
P
Cdc2
CycB1
Cdc25C
P
14-3-3
CycB1
Cdc2
Chk1
S317
P
S354
APC
Transported to and
sequestered in
the cytoplasm
e.g.,
Pds1
loss of sister
cohesion; anaphase
CycB1
reversal of condensation
microtubule depolym.
G2
M
G1

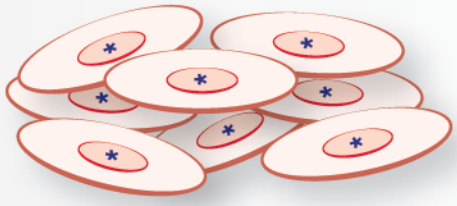


Indices of DDR activation in preneoplastic lesions



Gorgoulis VG, et. al. Nature (2005)

DDR Activation Upon Oncogene Expression

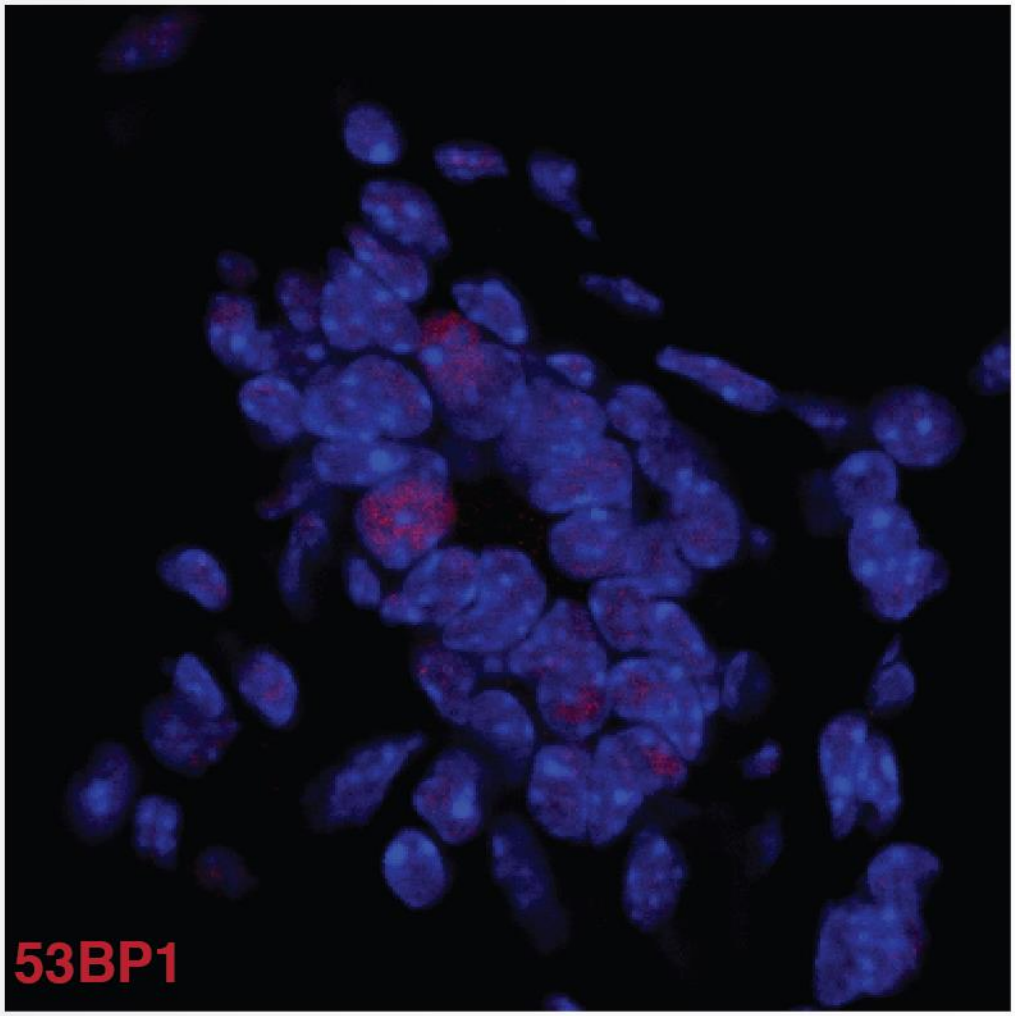


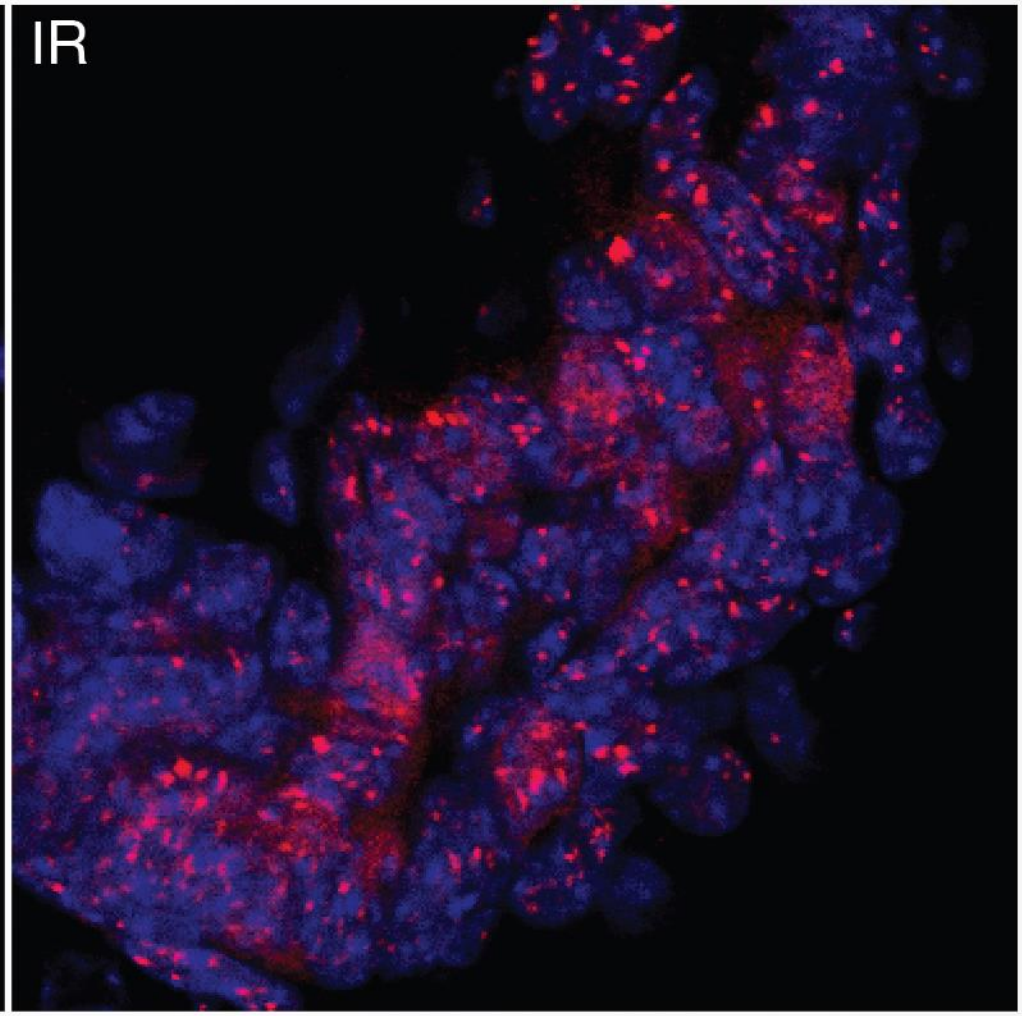
DDR

Transformation
(soft agar colony growth and nude mouse subcutaneous tumor growth)

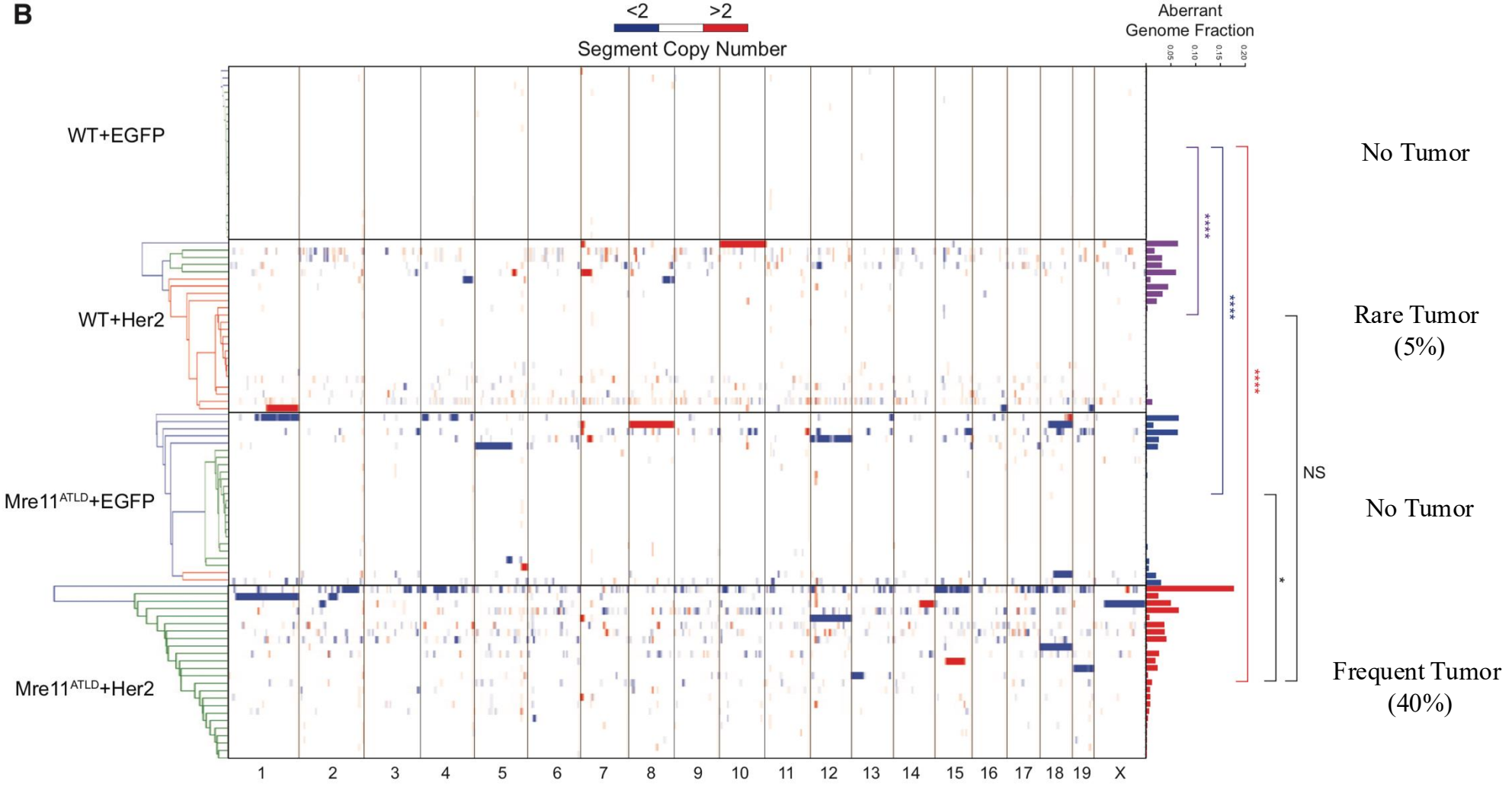
Bartkova J, et al. Nature (2006)
Di Micco R, et al. Nature (2006)

IR-induced 53BP1 foci in mammary epithelium

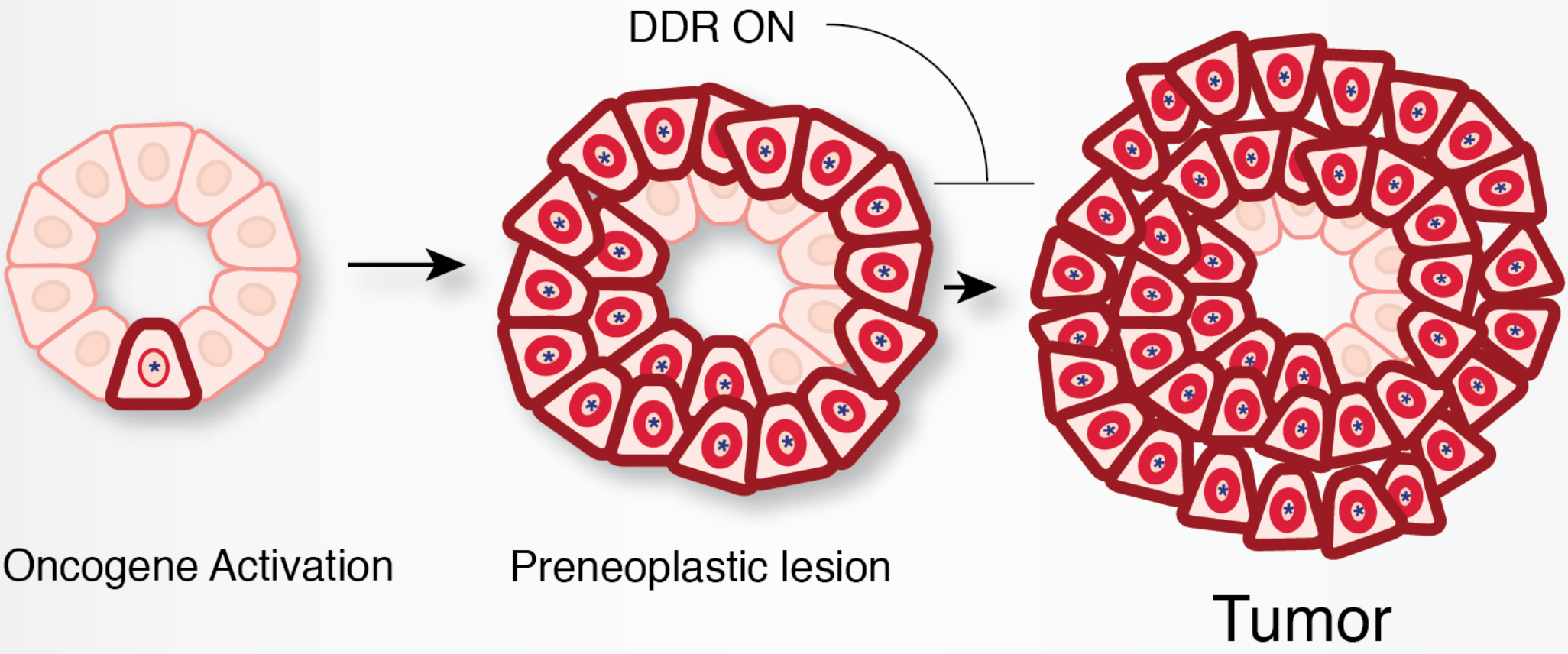


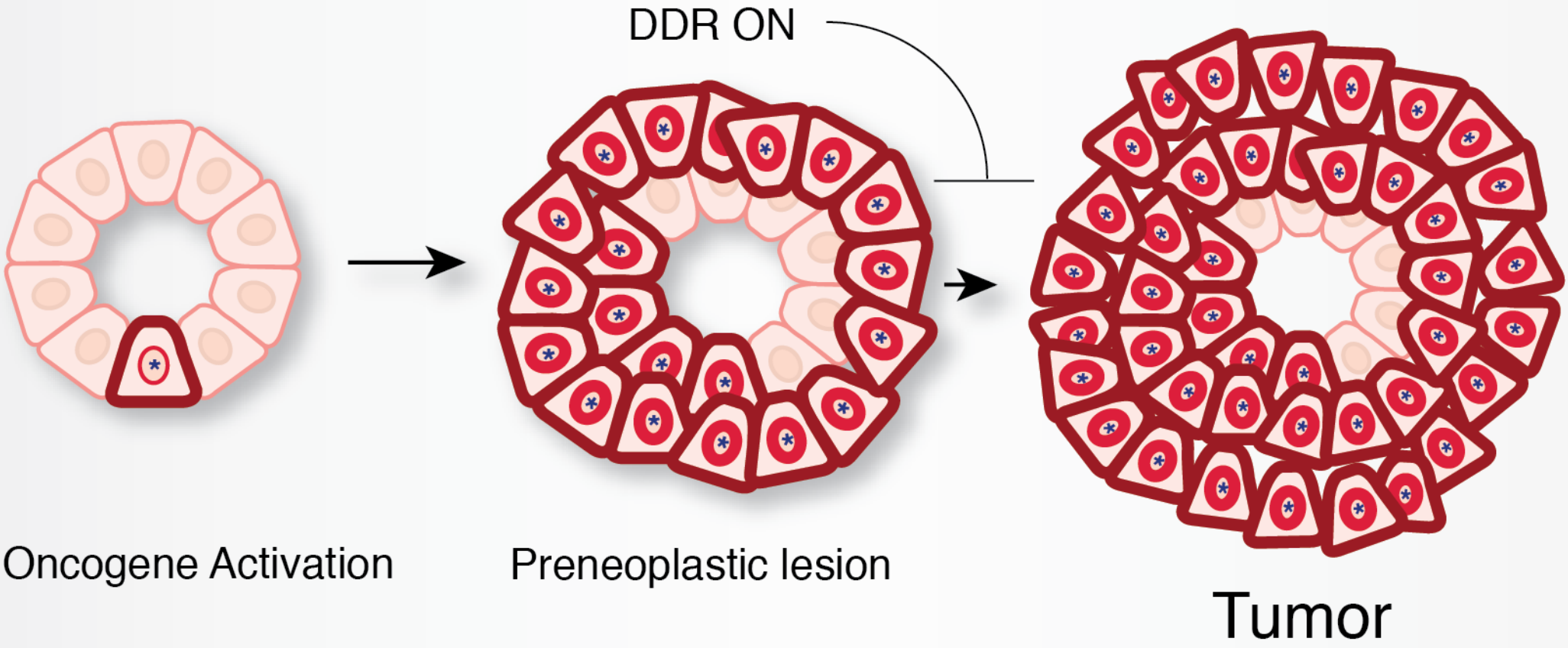


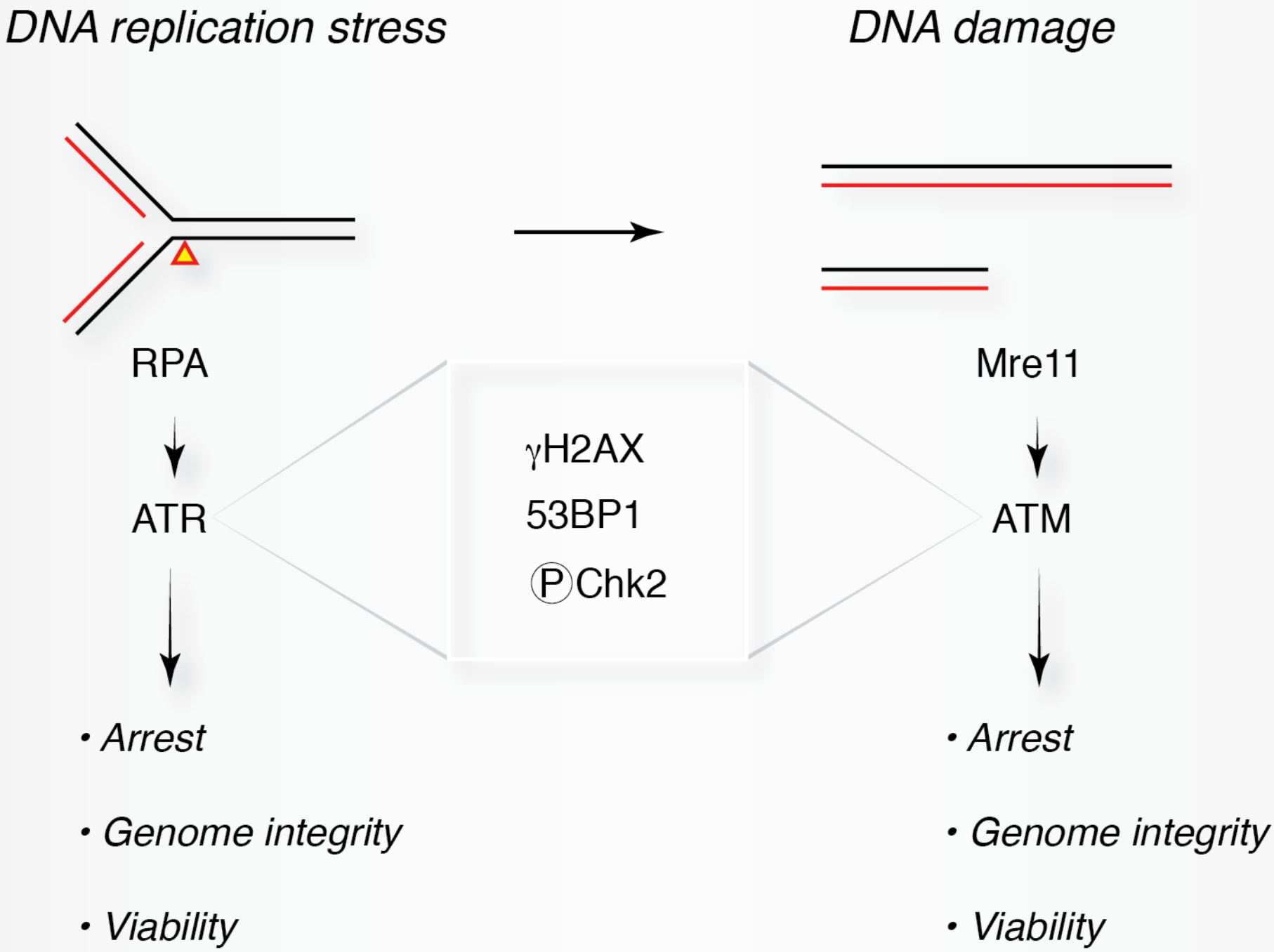
CNVs form scDNA-Seq



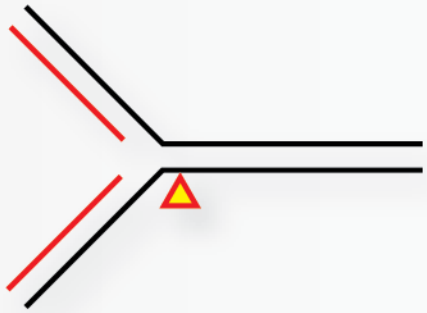
Modified from Fagan-Solis, K. D. *et al*. *Cell Rep*. 2020


DDR ON
Oncogene Activation
Preneoplastic lesion
Tumor


DDR ON
Oncogene Activation
Preneoplastic lesion
Tumor
Proliferative signals
Re-replication
Collisions
ROS
Metabolic derangements


DNA replication stress
DNA damage
RPA
Mre11
γH2AX
53BP1
ⓅChk2
ATR
ATM
• Arrest
• Genome integrity
• Viability
• Arrest
• Genome integrity
• Viability

Oncogene activation



RPA

ATR

γH2AX

53PB1

ⓅChk2

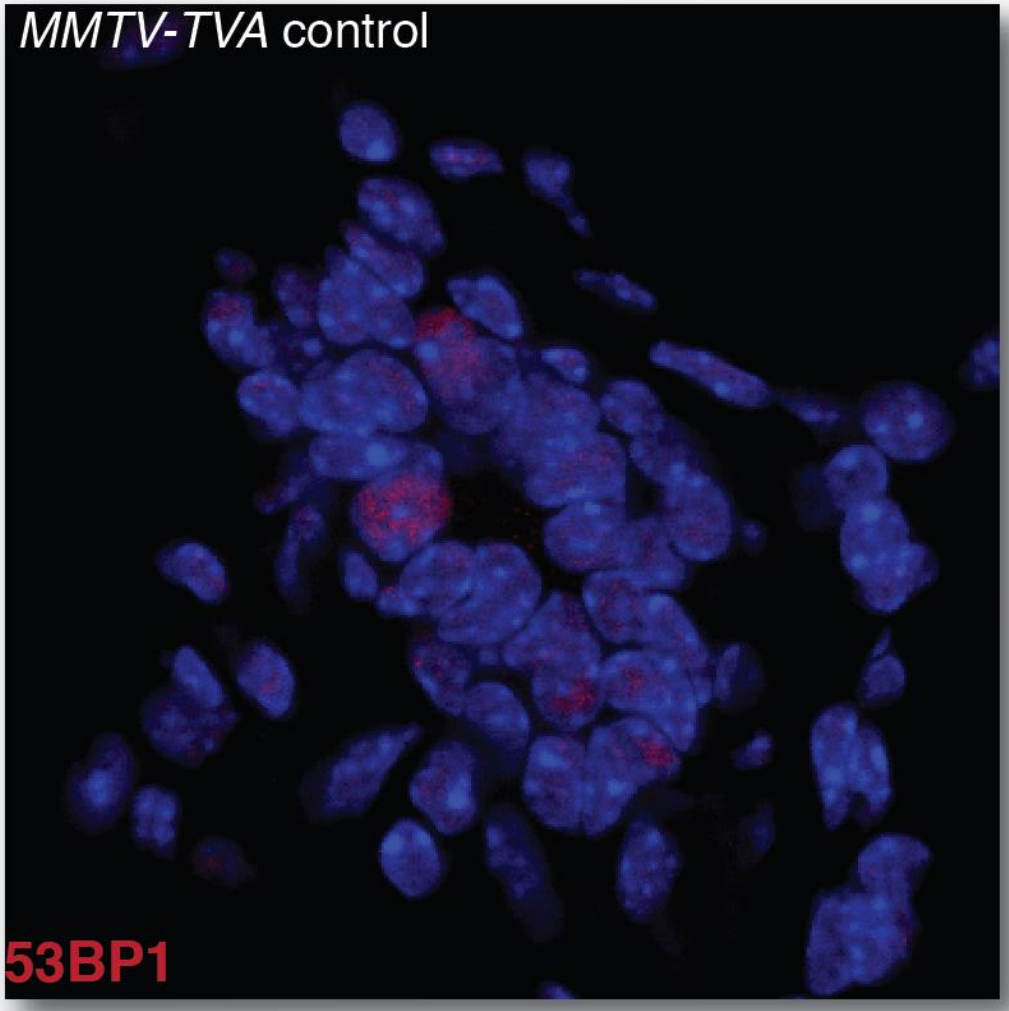
~~Mre11~~

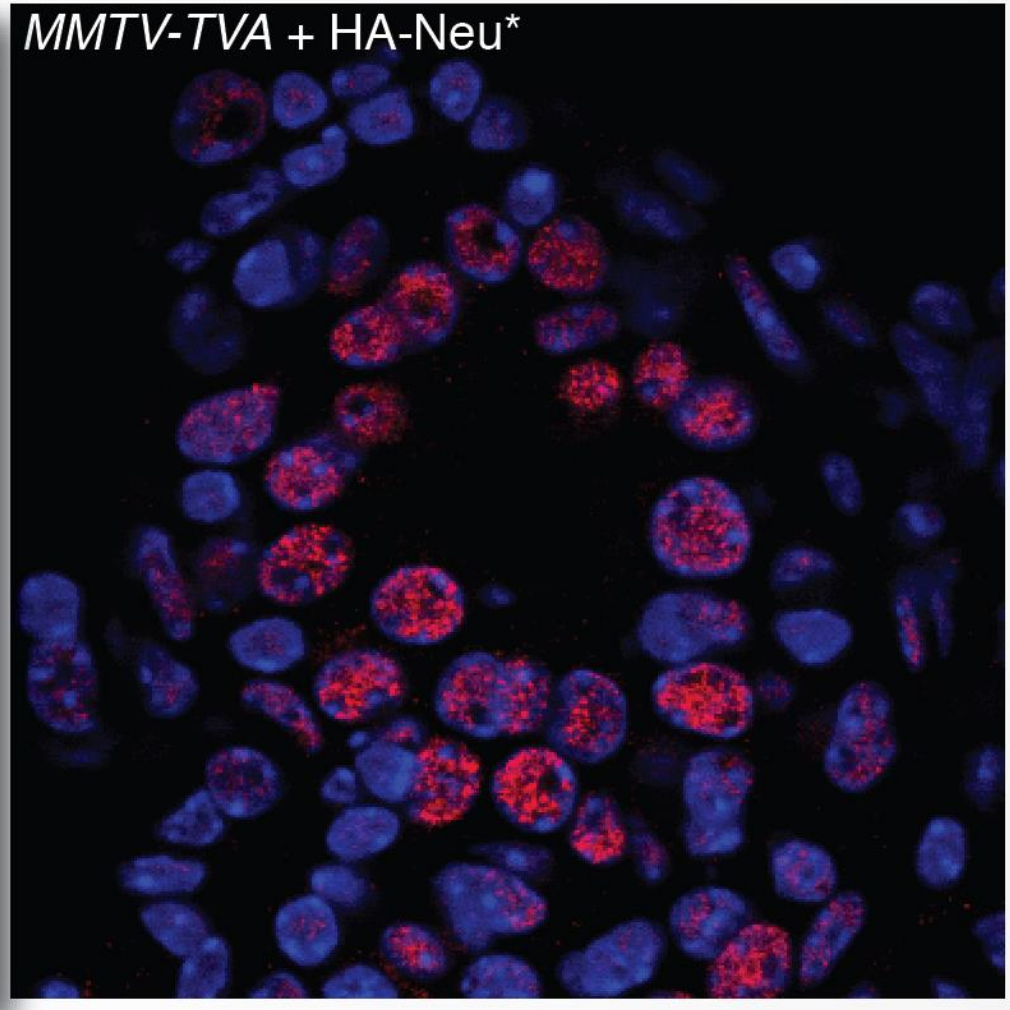
ATM

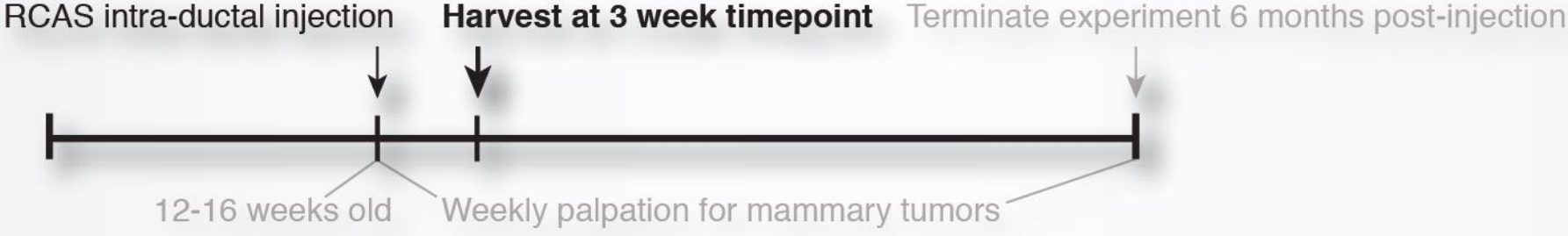
- *Arrest*
- *Genome integrity*
- *Viability*

- *Hyperplasia*
- *Genome instability*
- *Malignancy*

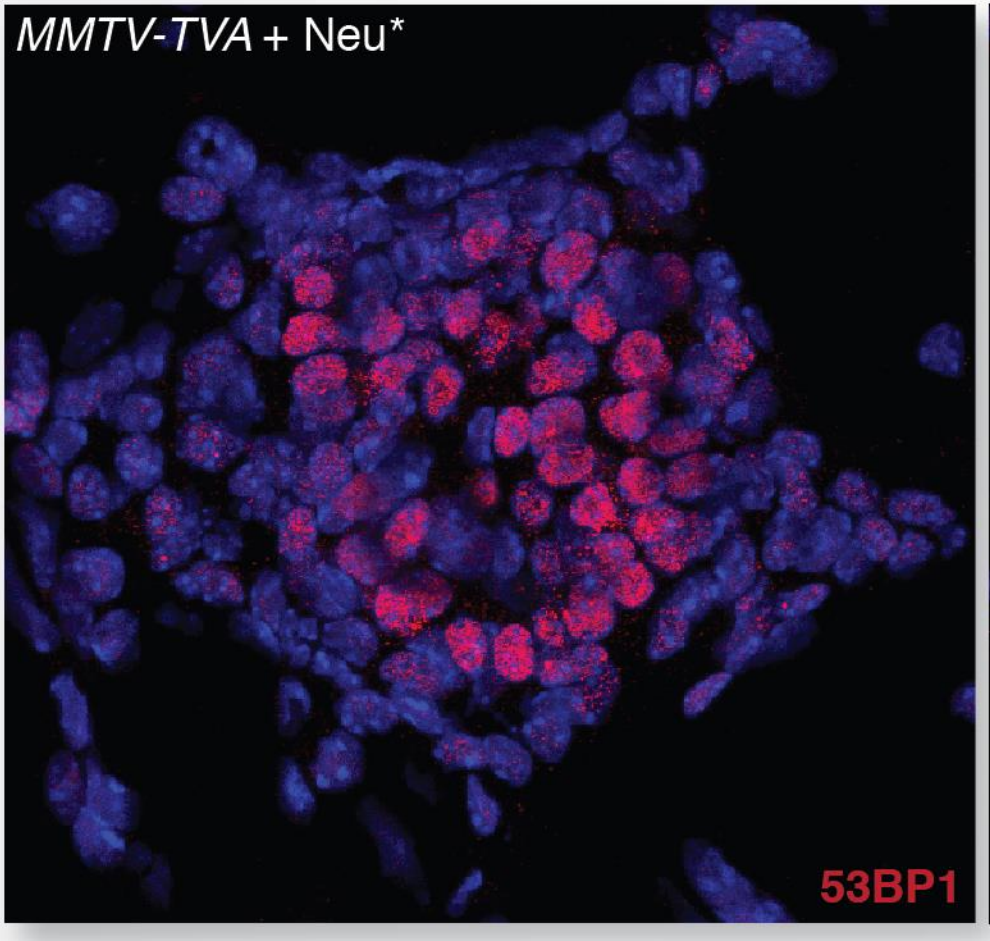
Oncogene-induced 53BP1 accumulation

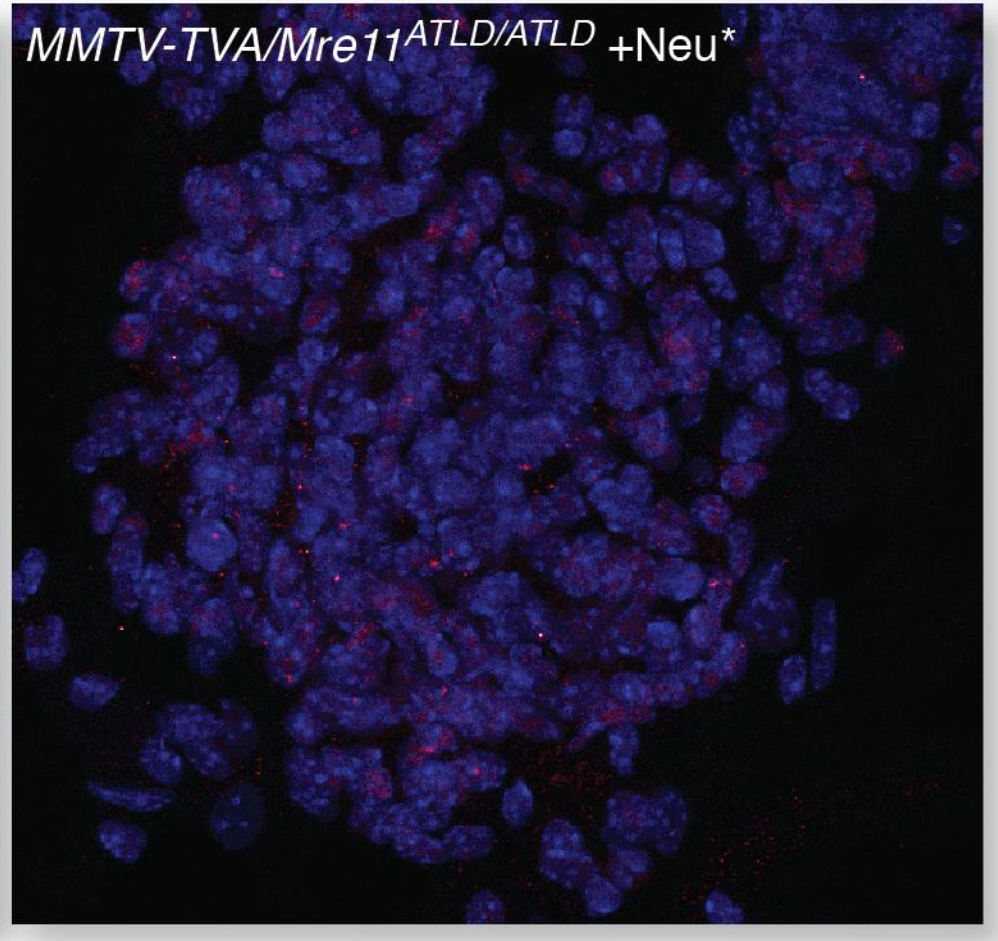


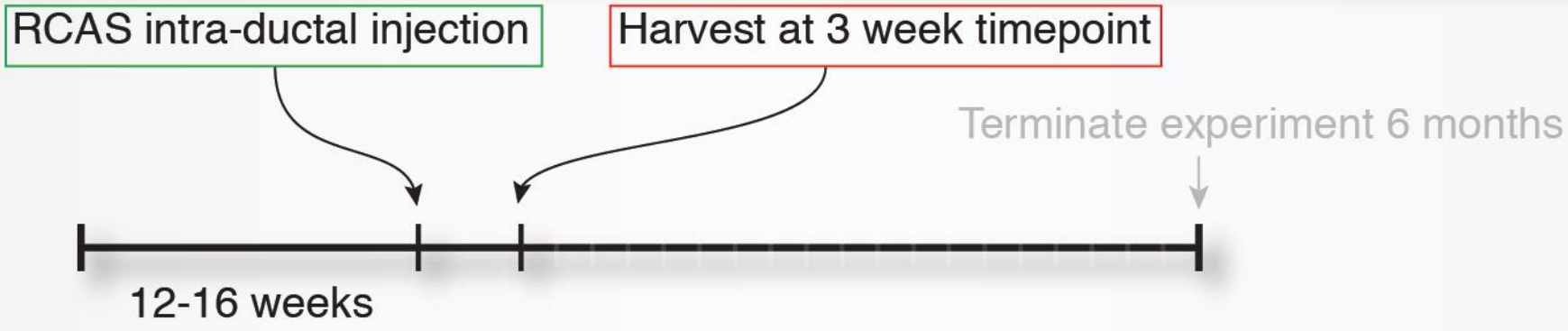




Mre11$^{ATLD1/ATLD1}$: reduction in oncogene induced DDR



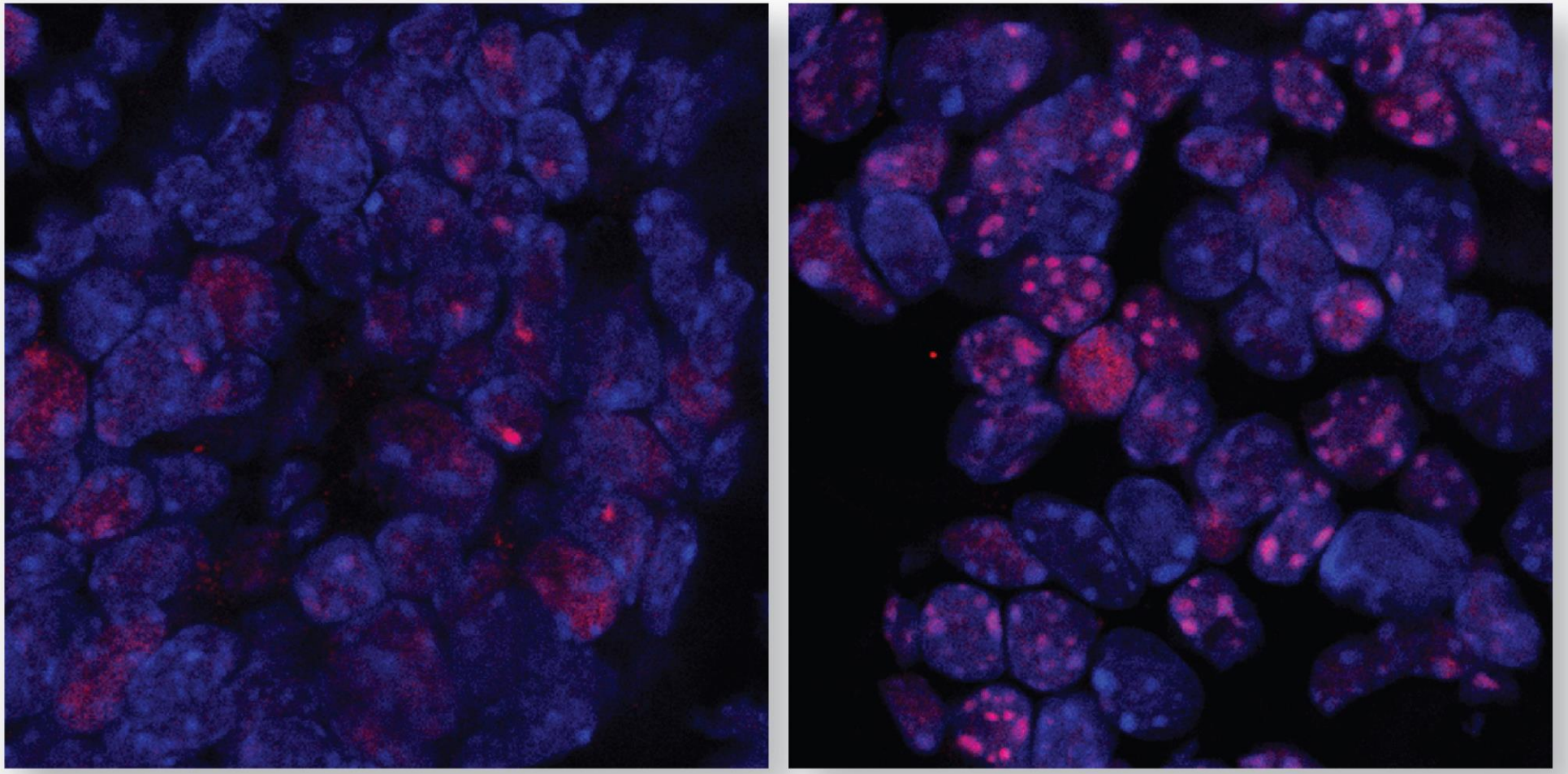




Oncogene induced heterochromatic changes

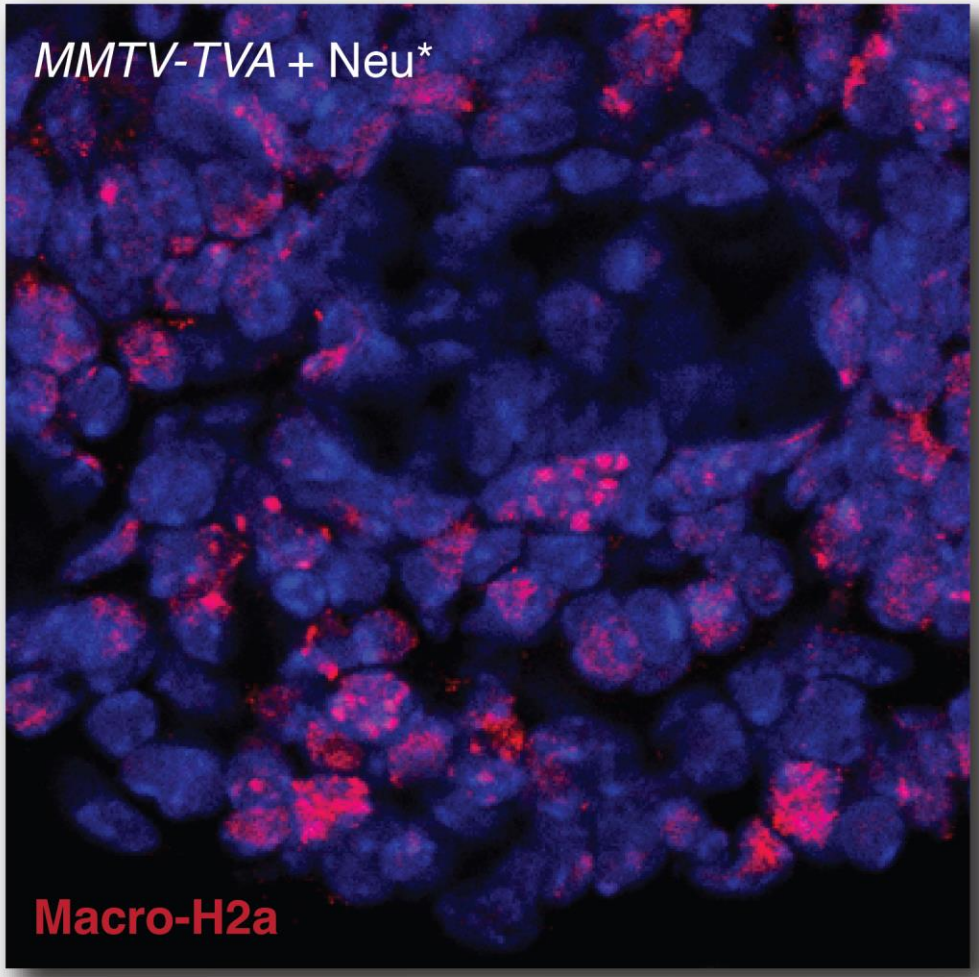
MMTV-TVA

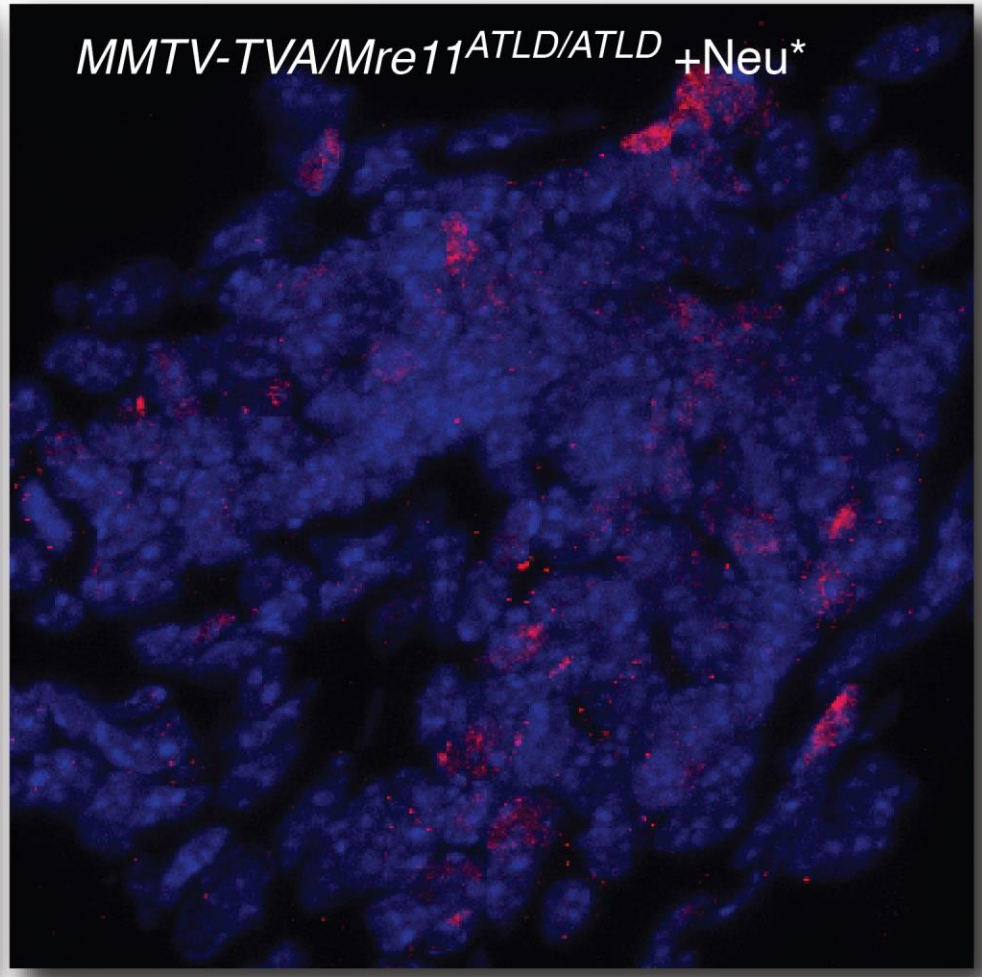
MMTV-TVA + Neu*

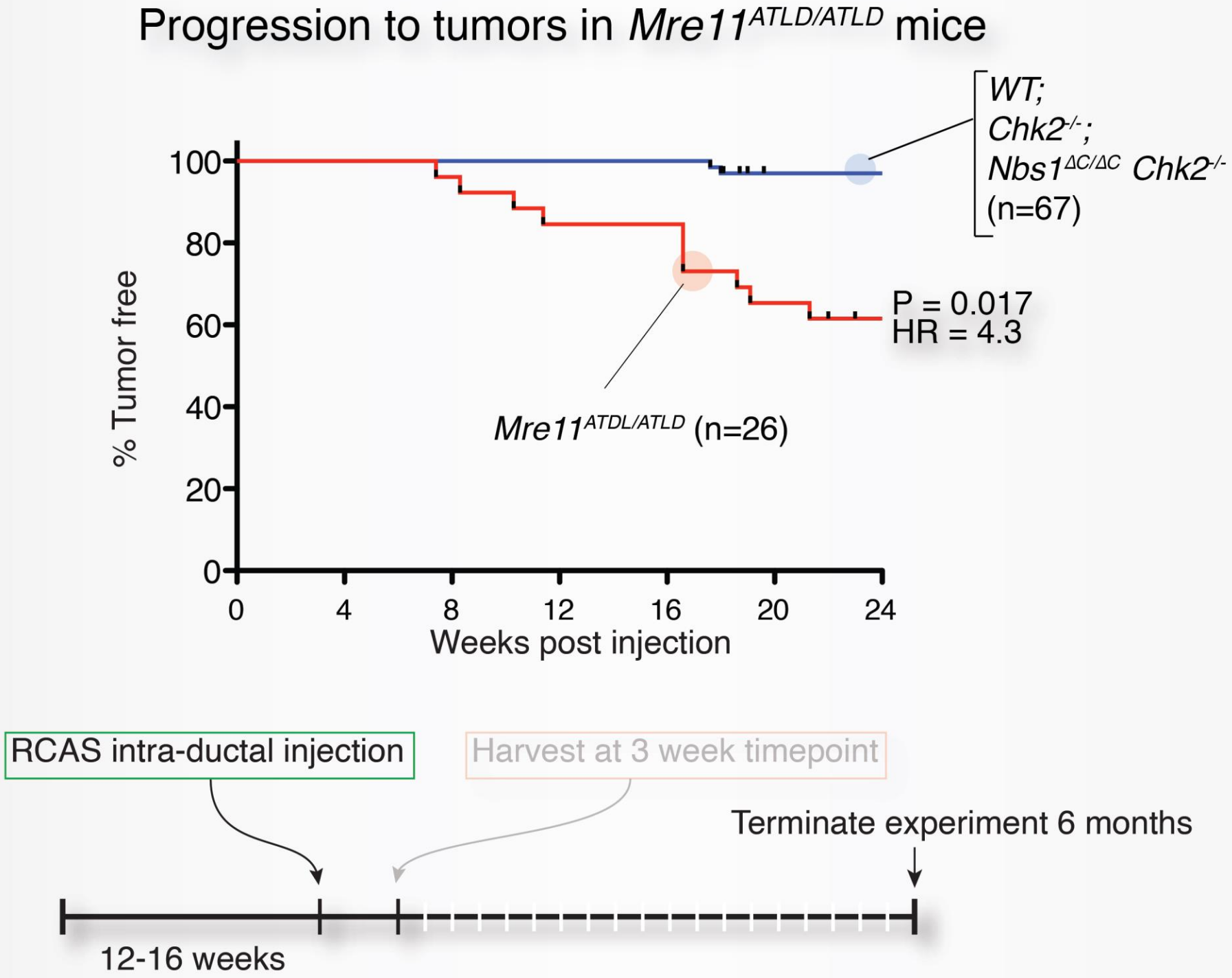


macroH2A

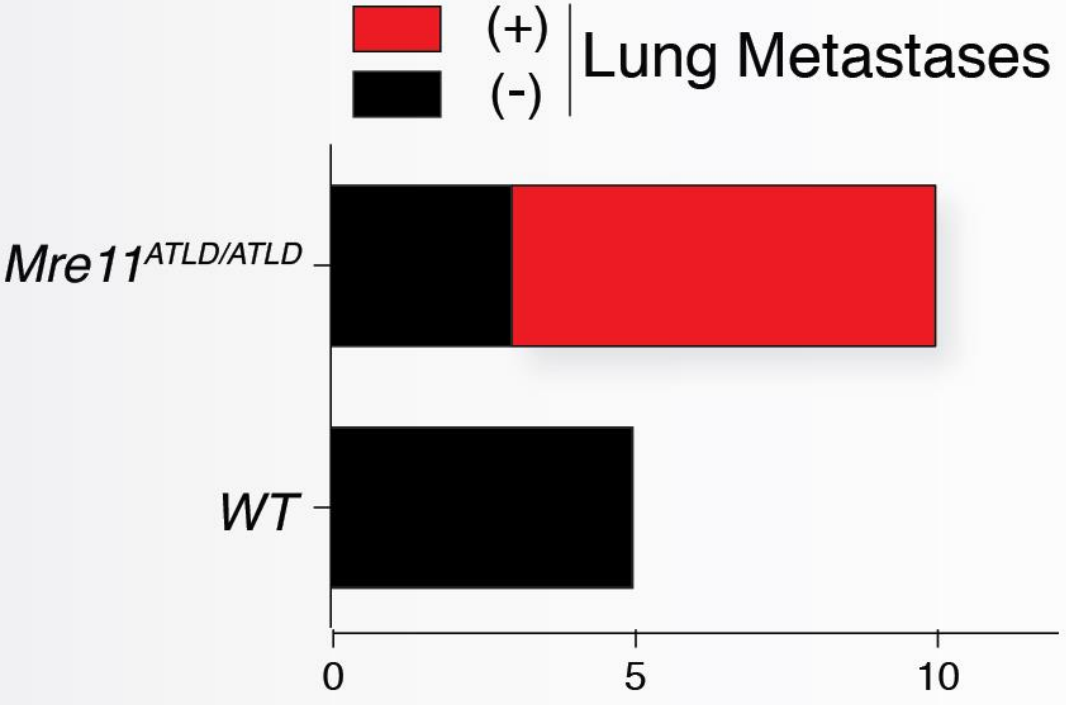
Reduction in heterochromatic changes in *Mre11*$^{ATLD1/ATLD1}$

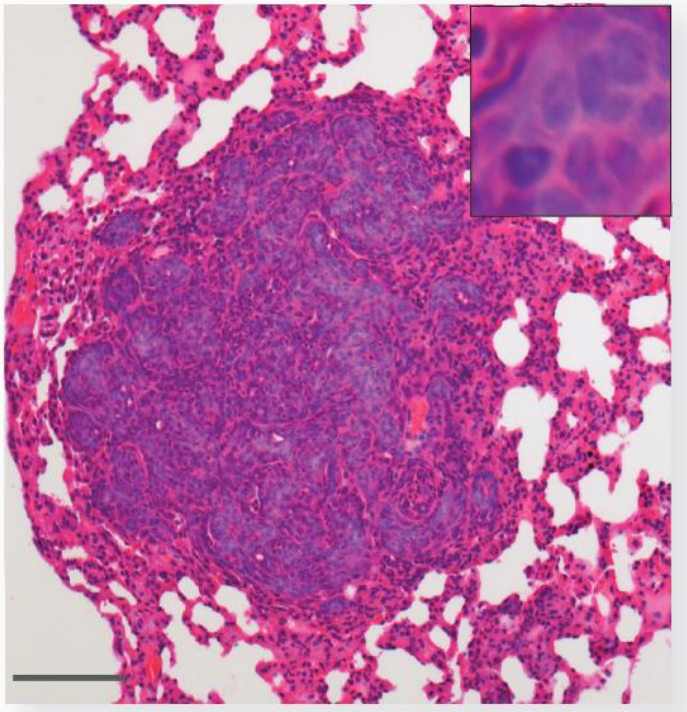


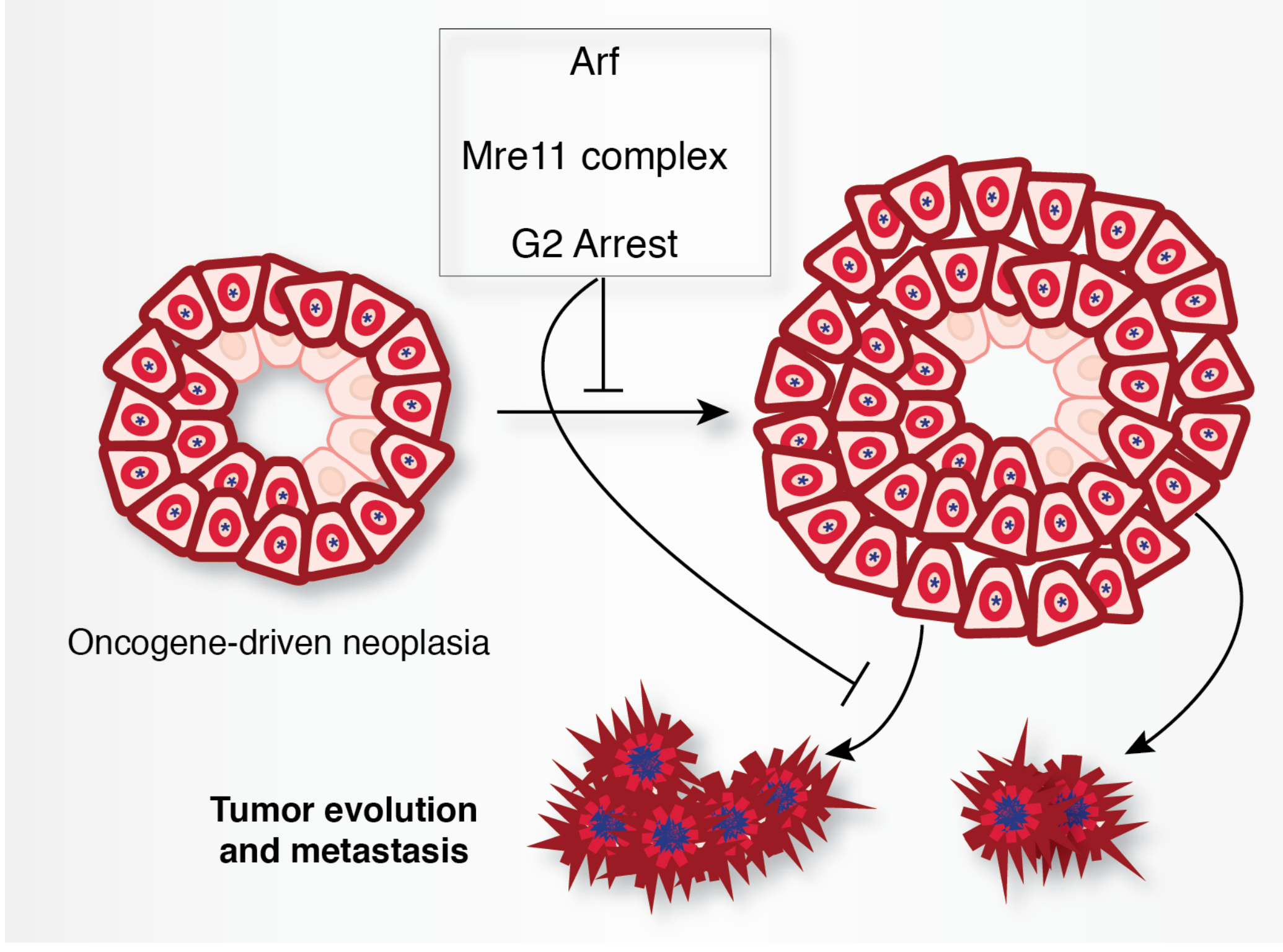



Progression to tumors in Mre11ATLD/ATLD mice
WT;
Chk2-/-;
Nbs1ΔC/ΔC Chk2-/-
(n=67)
P = 0.017
HR = 4.3
Mre11ATDL/ATLD (n=26)
% Tumor free
100
80
60
40
20
0
0
4
8
12
16
20
24
Weeks post injection
RCAS intra-ductal injection
Harvest at 3 week timepoint
Terminate experiment 6 months
12-16 weeks

Mre11$^{ATLD/ATLD}$ Tumors are Metastatic






Arf
Mre11 complex
G2 Arrest
Oncogene-driven neoplasia
Tumor evolution
and metastasis

THE END