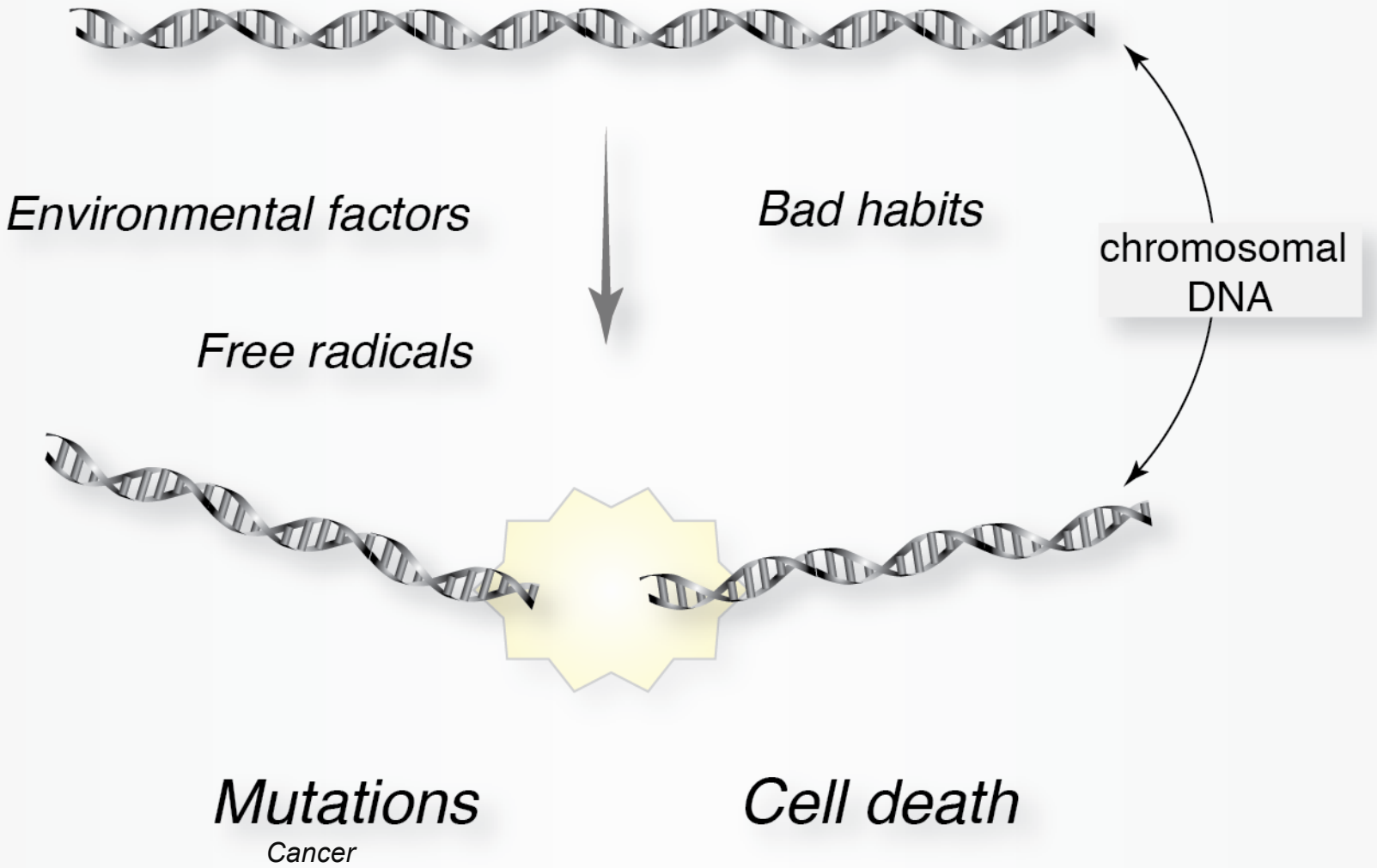
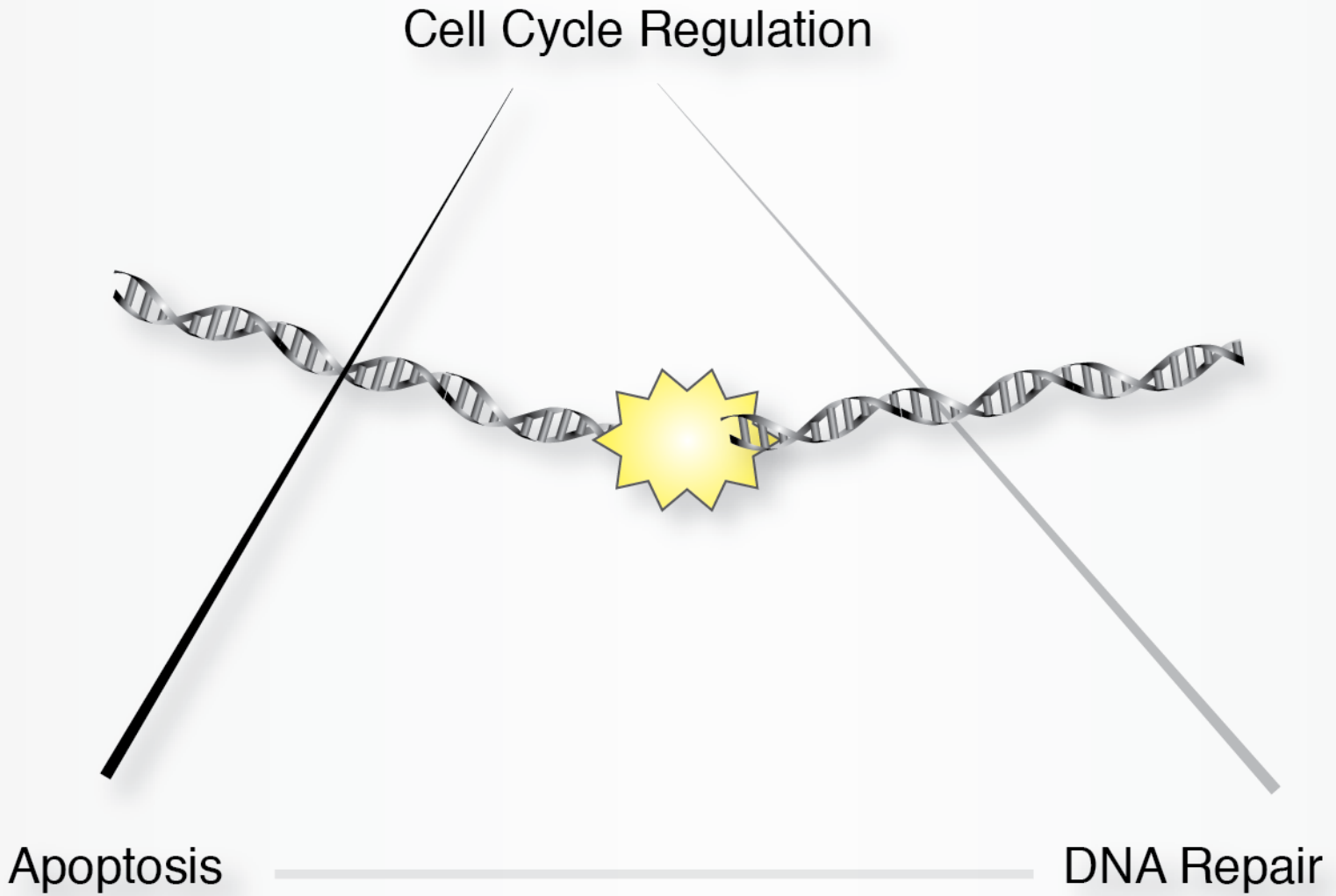
DNA damage is part of everyday life



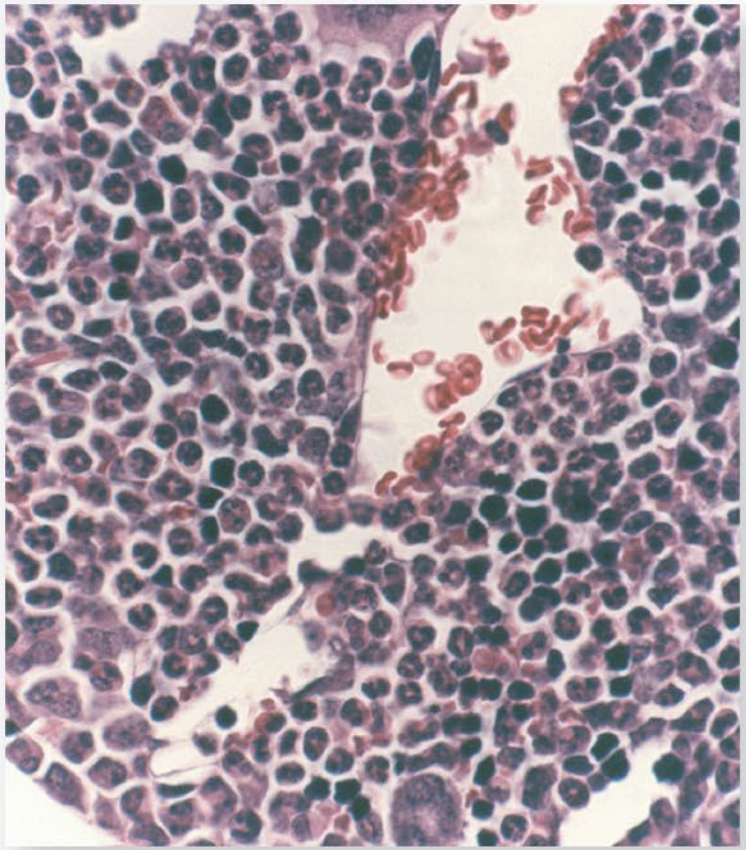
The DNA Damage Response Network

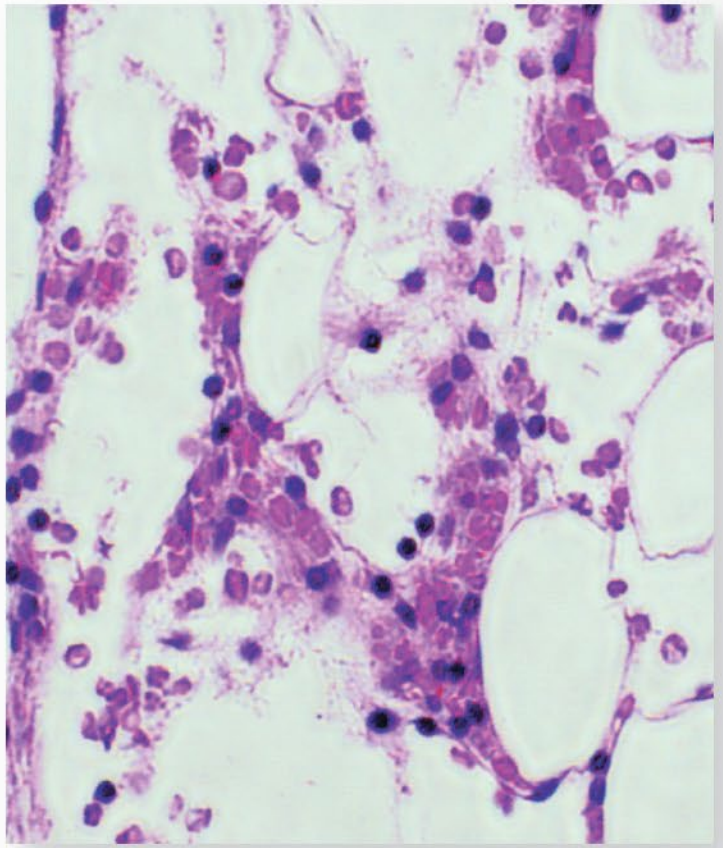




DDR Normal

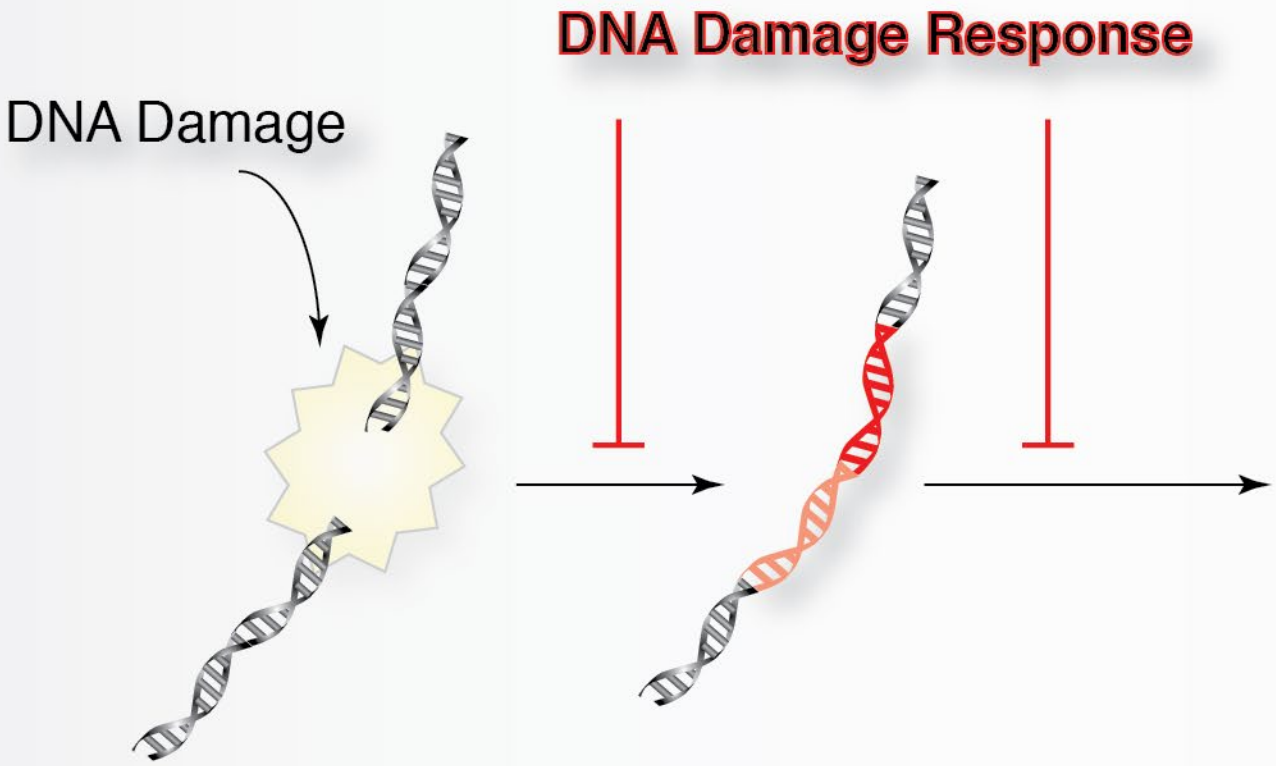
DDR Defective



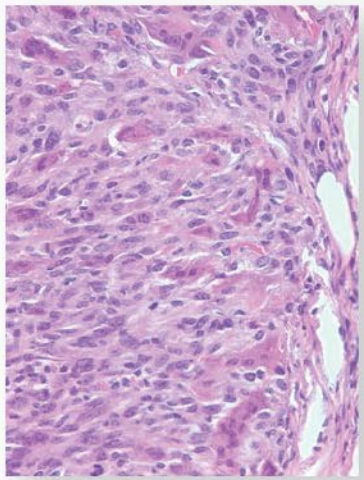


bone marrow

DNA Damage Response Inhibits Carcinogenesis induced by DNA damage



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

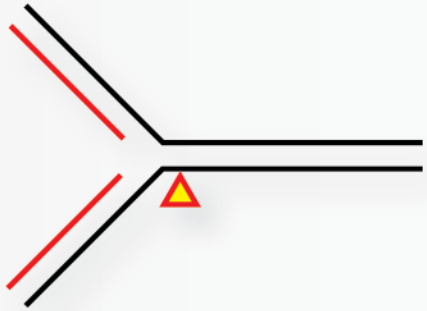


Malignancy

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

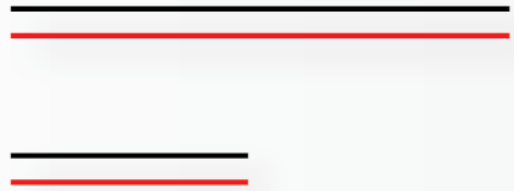
↓

ATR

↓

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

DNA damage



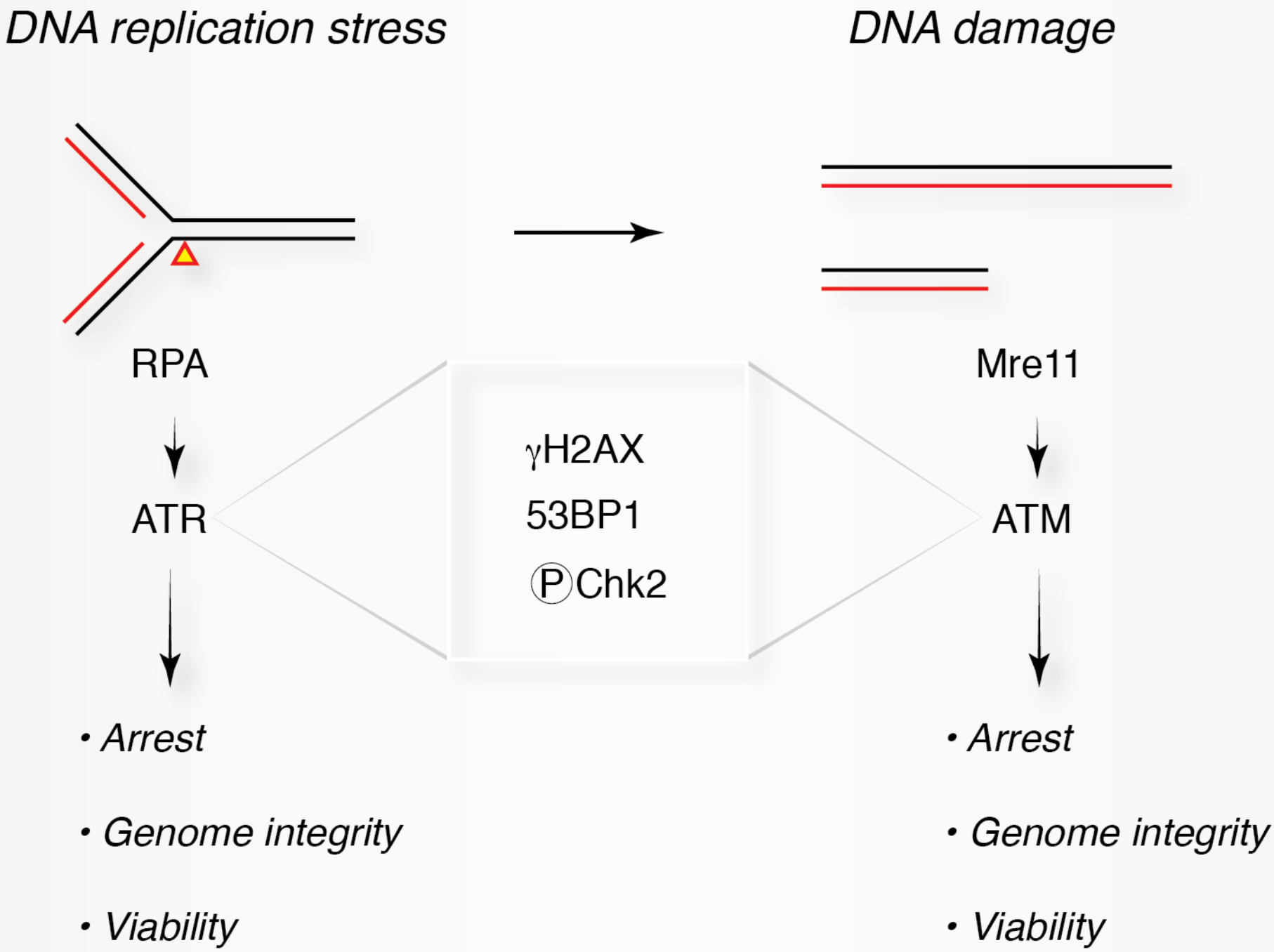
Mre11

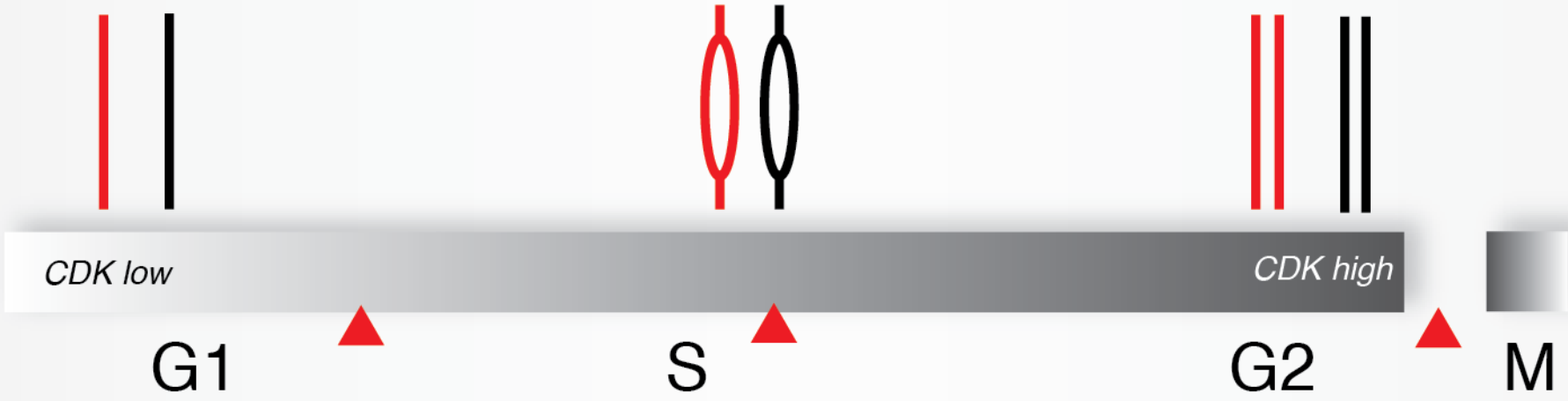
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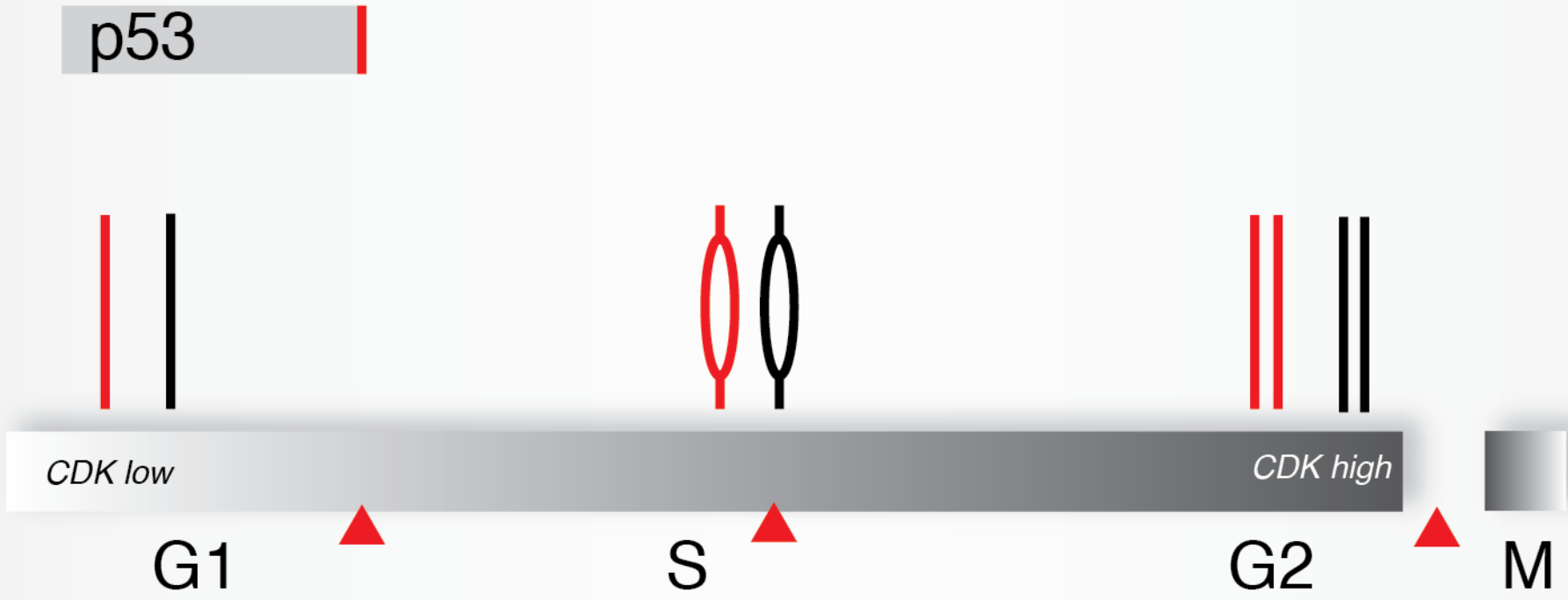
ATM

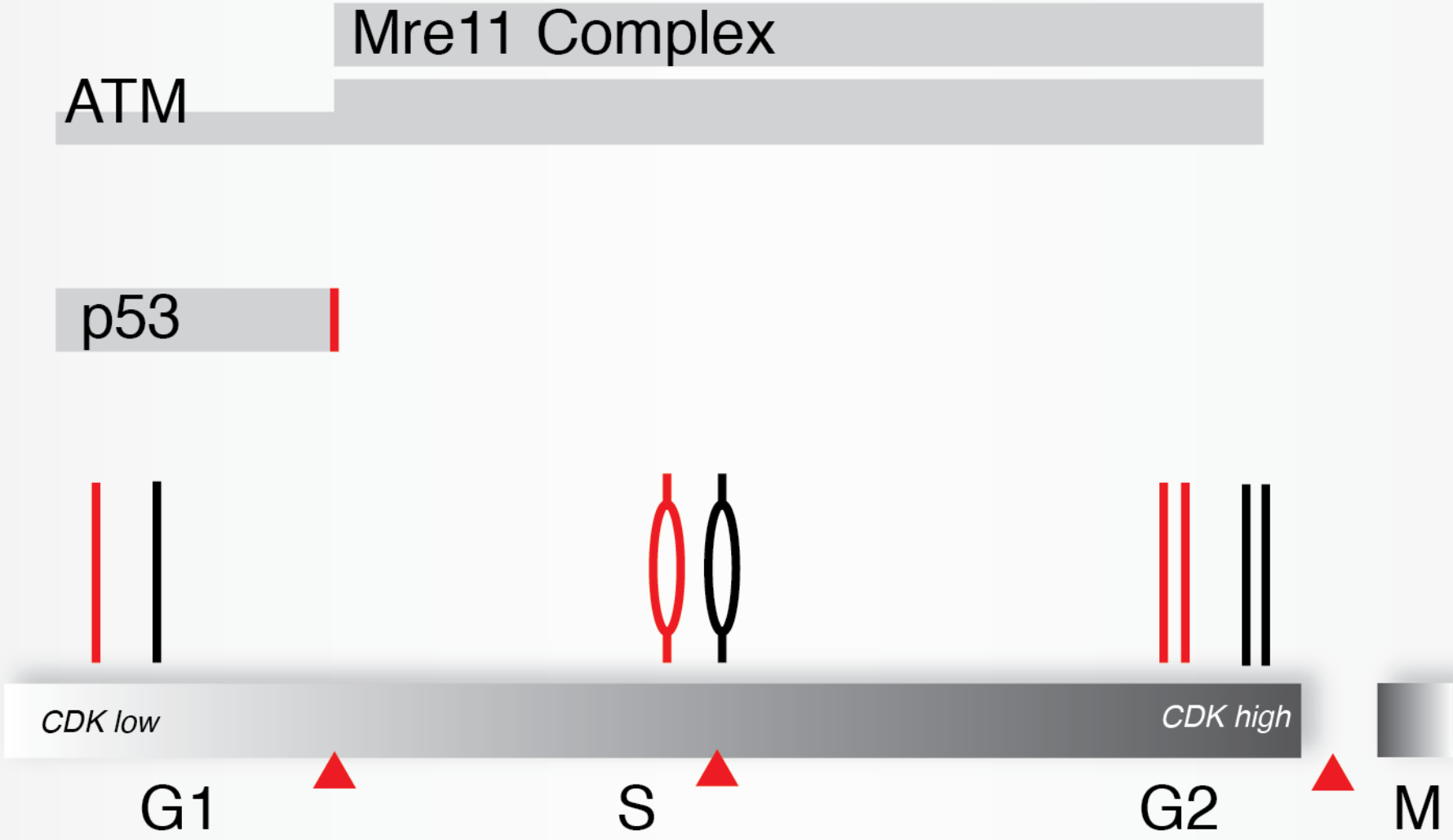
↓

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


DNA replication stress
DNA damage
RPA
ATR
Mre11
ATM
γH2AX
53BP1
ⓅChk2
• 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?

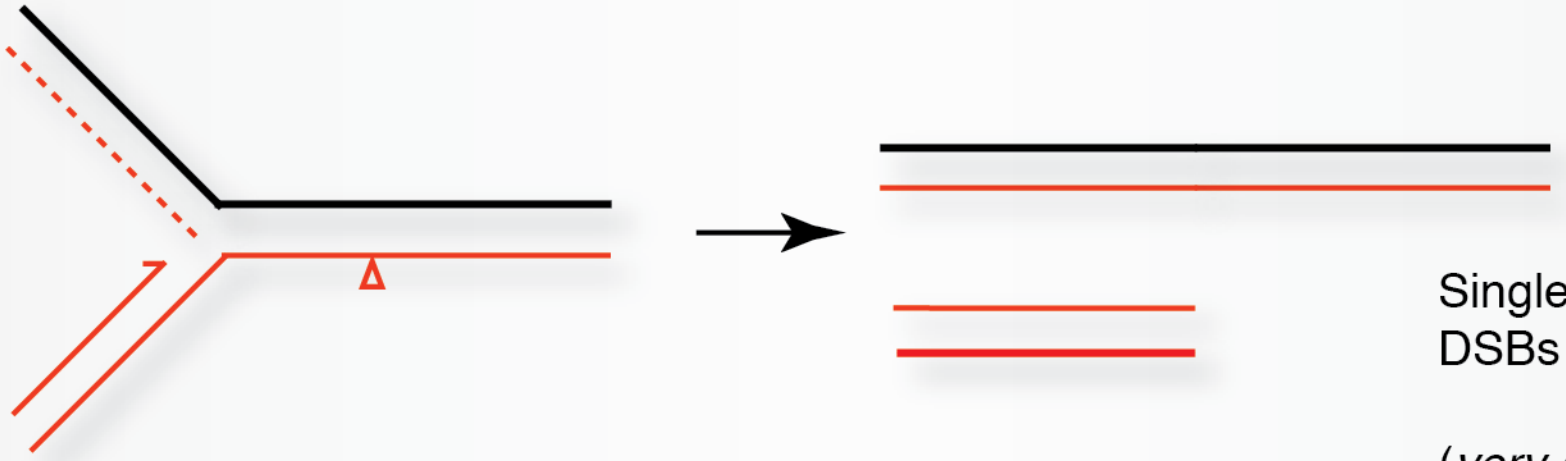


Where do DNA double strand breaks come from?



Meiosis
(*common*)

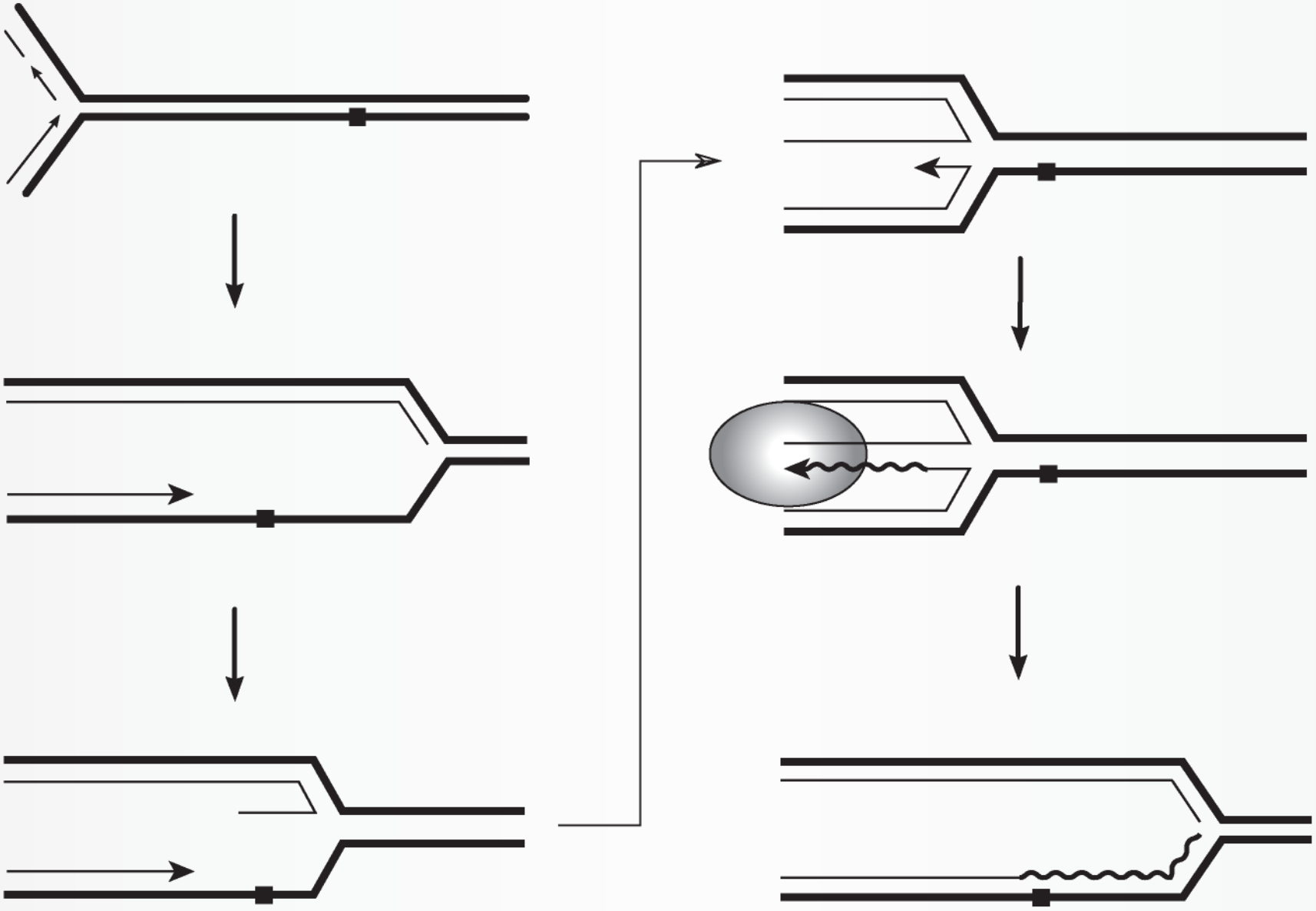
IR
(*uncommon!*)

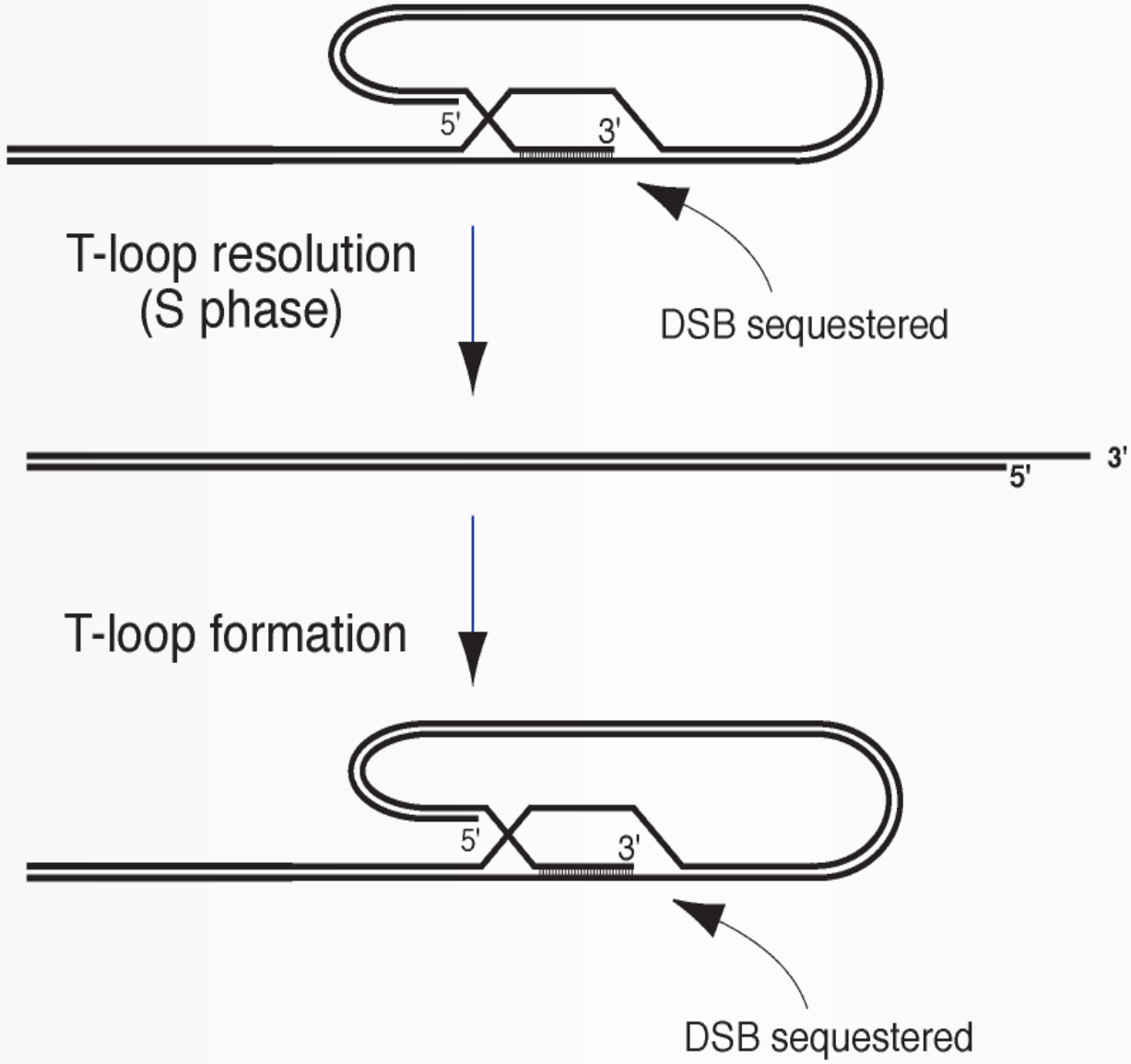


Single-ended
DSBs

(*very common!*)

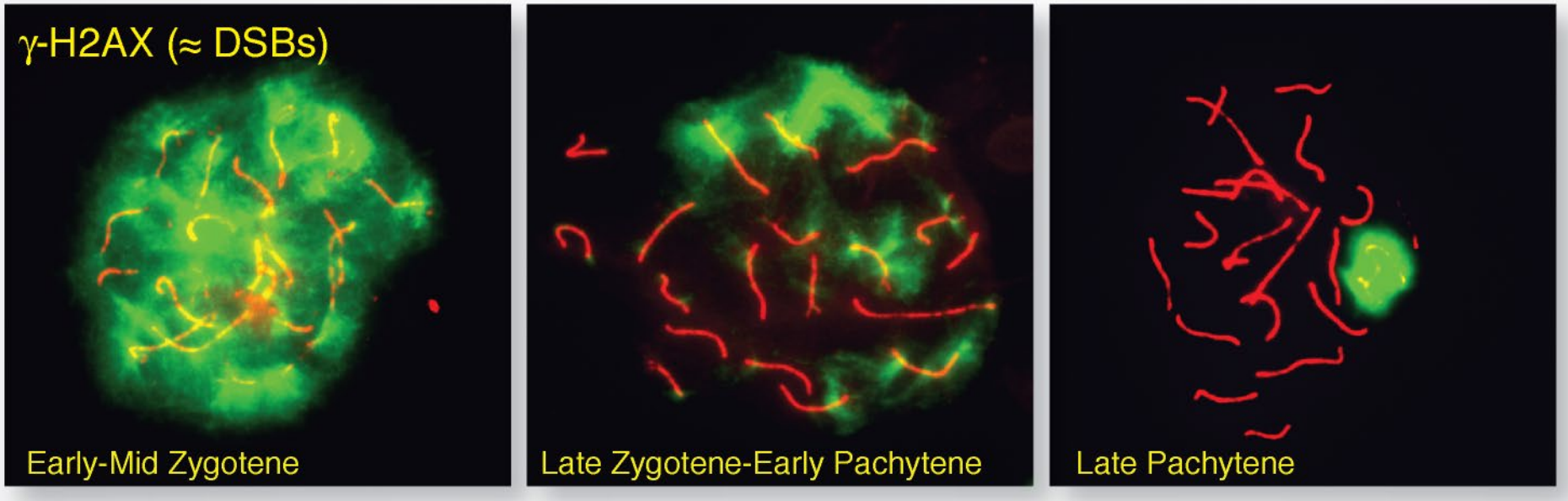
Repair + Replication Restart

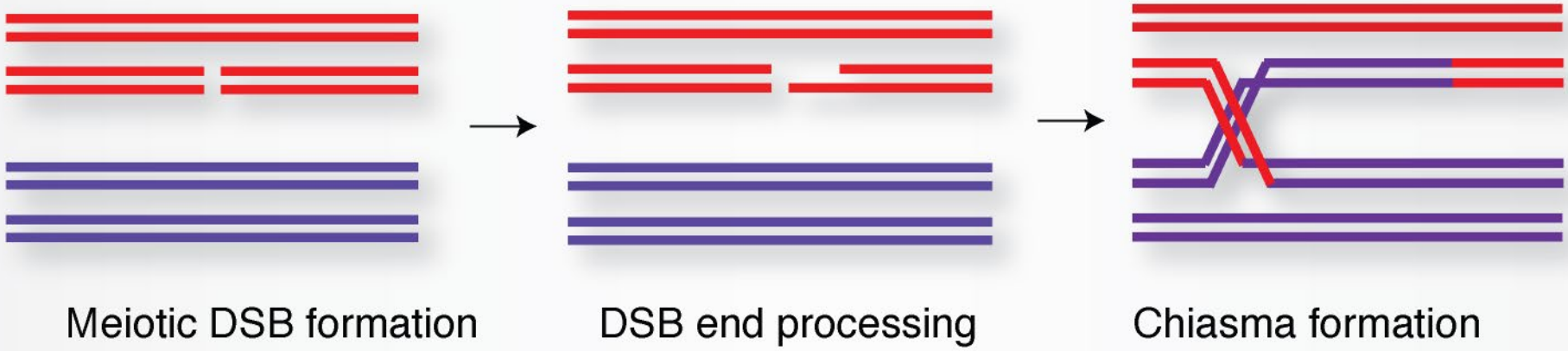




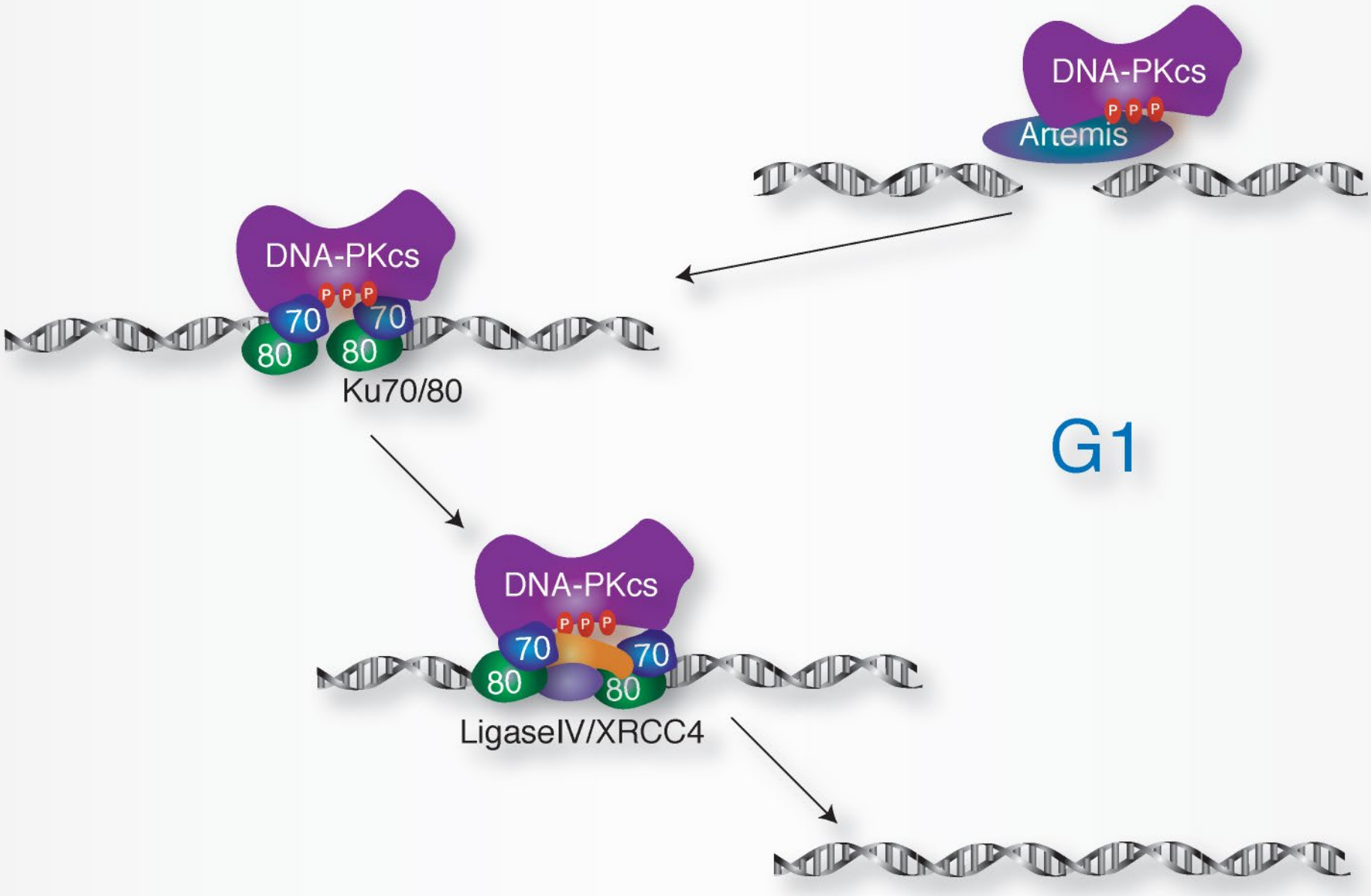
- DSB repair
- Checkpoint activation

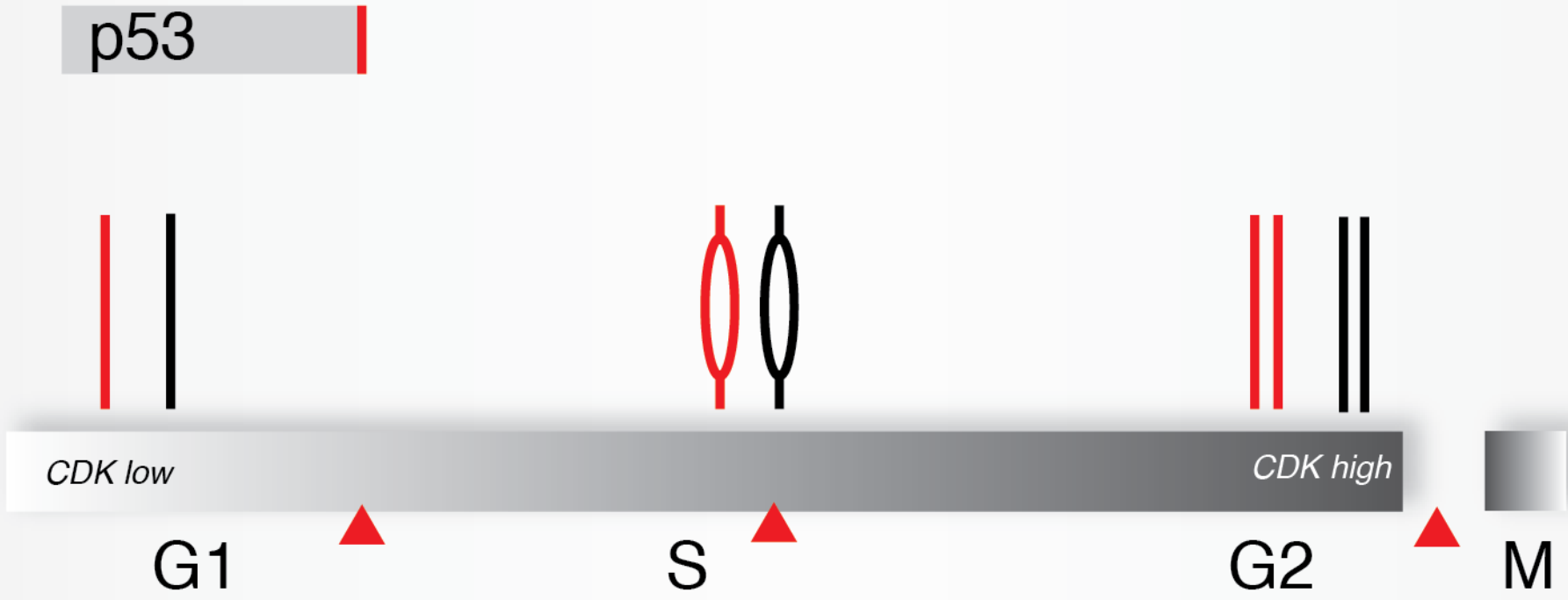
DSBs are induced to initiate meiotic recombination

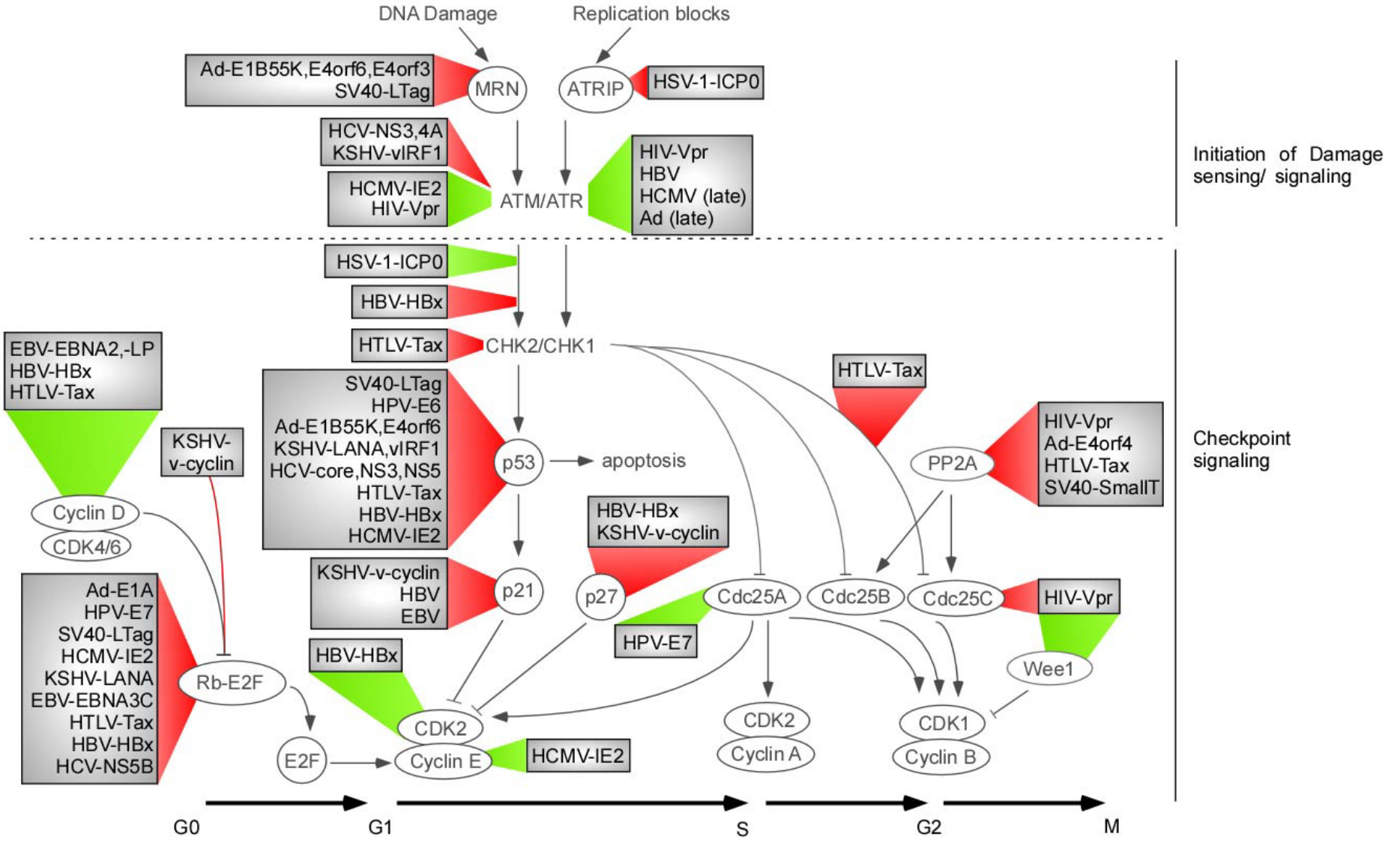




Antigen receptor gene assembly: DSBs in G1

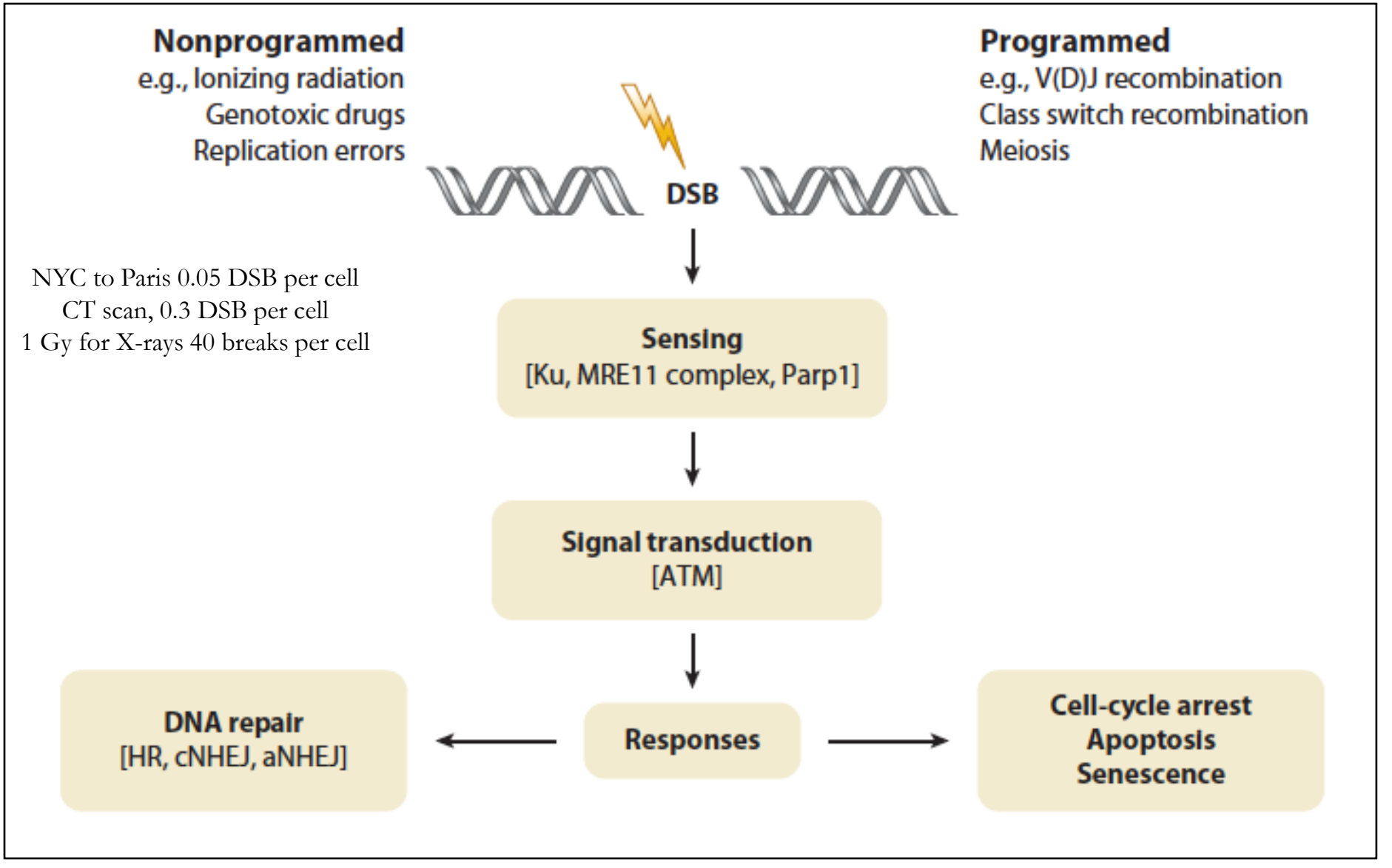



p53
CDK low
CDK high
G1
S
G2
M

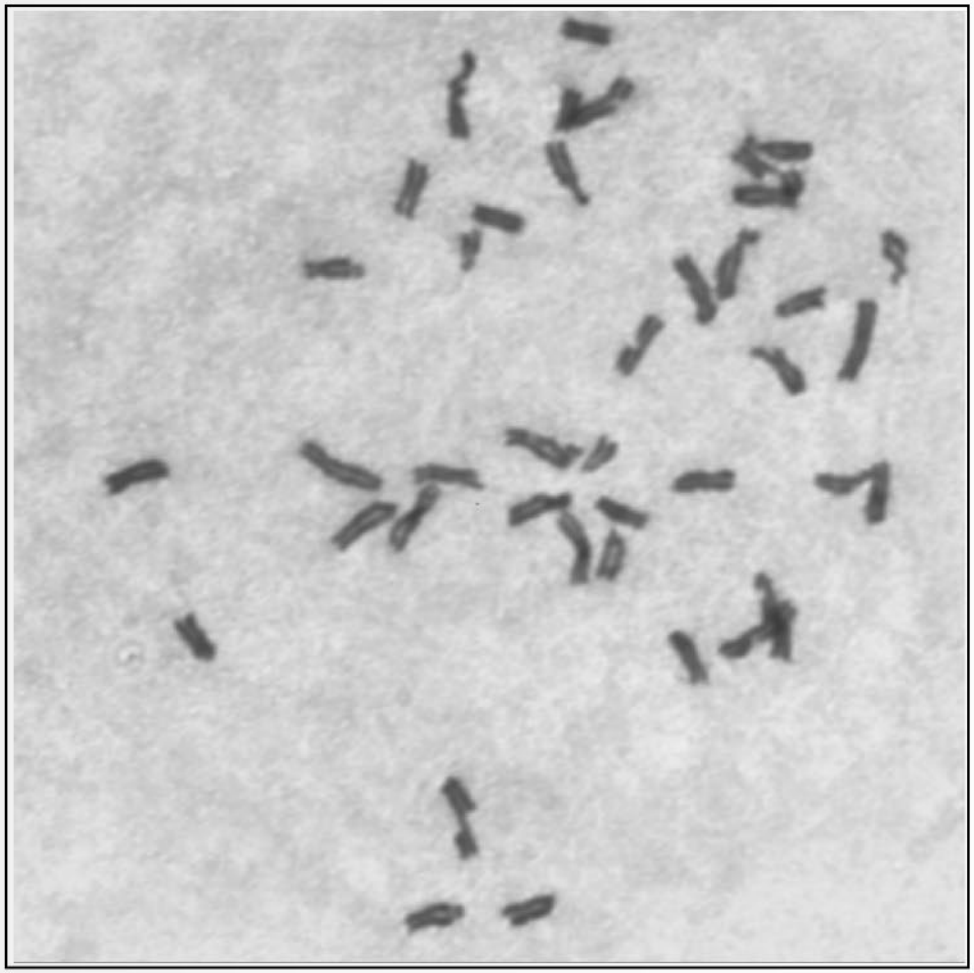


Chaurushiya & Weitzman 2009

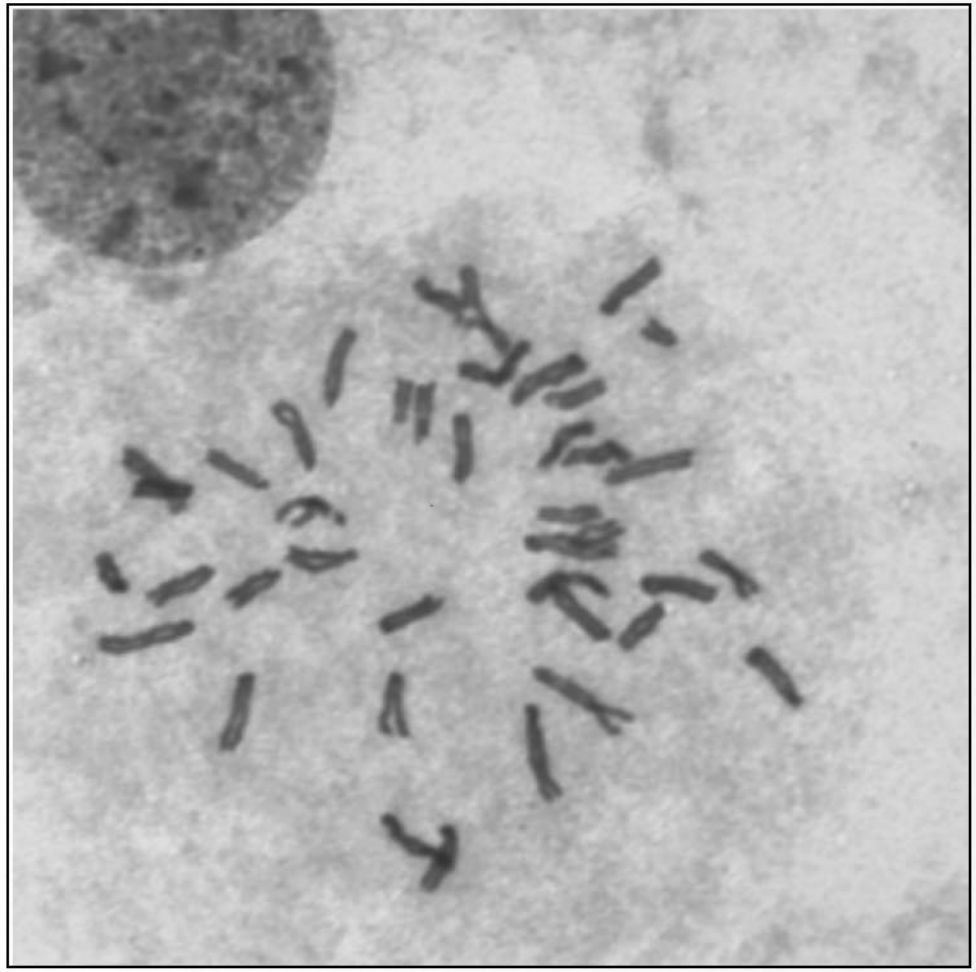
DNA double-strand break repair



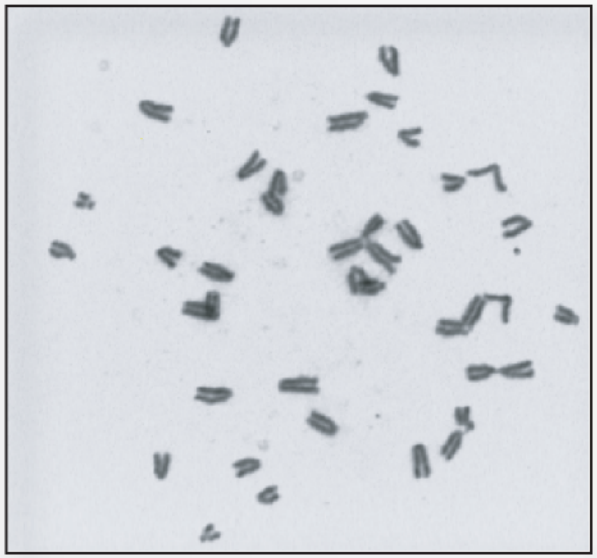
Deriano and Roth. Annu. Rev. Genet. 2013.



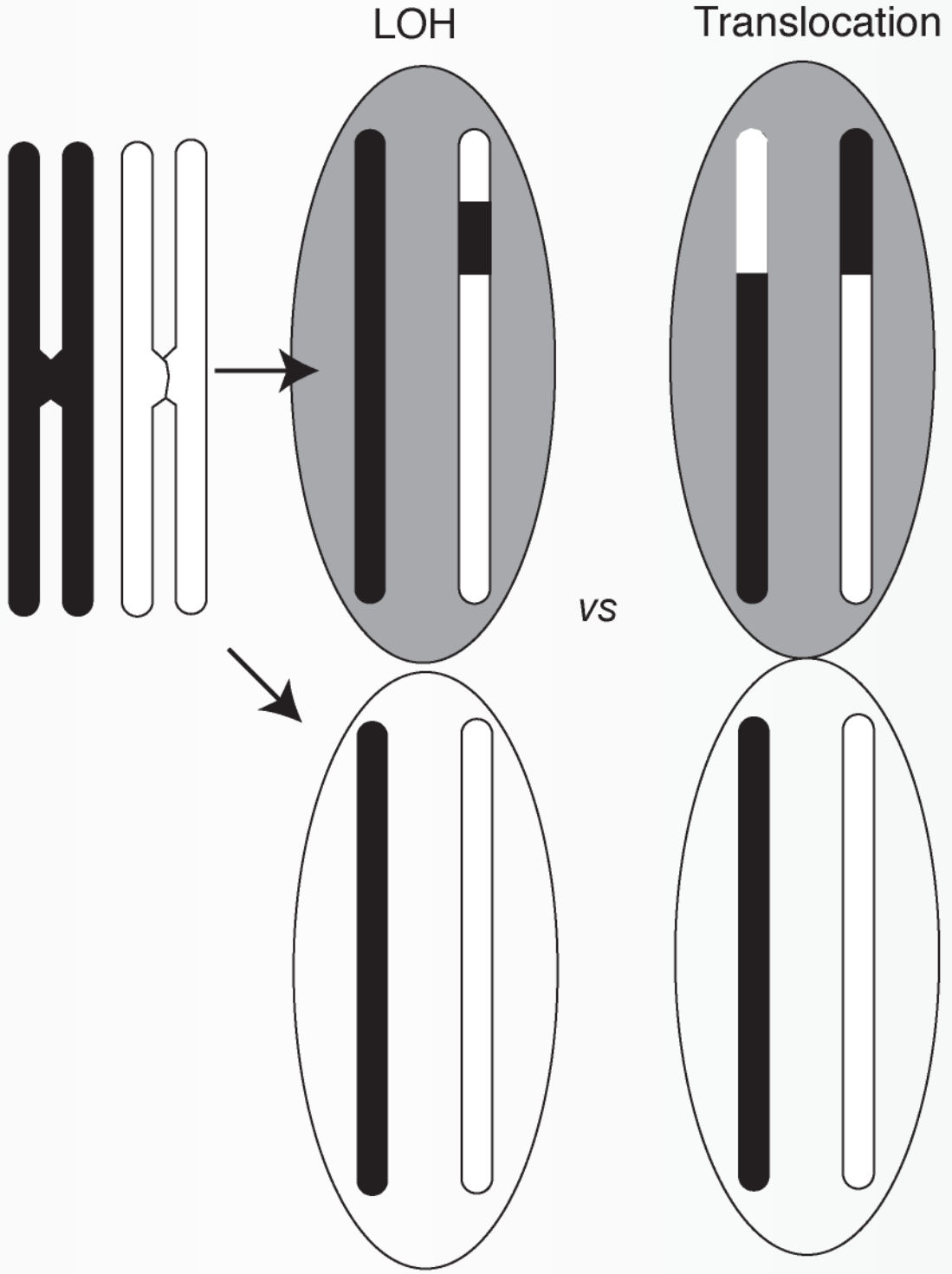
Breaks

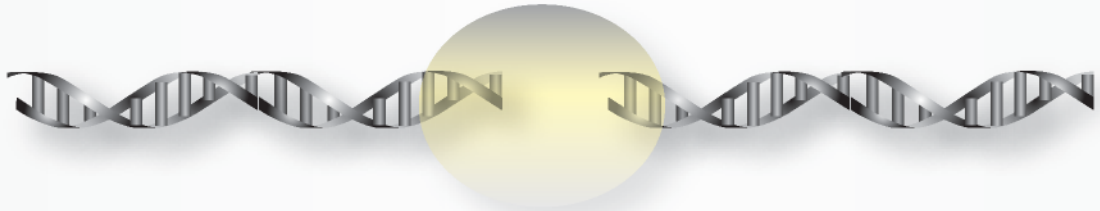


Rearrangements



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...

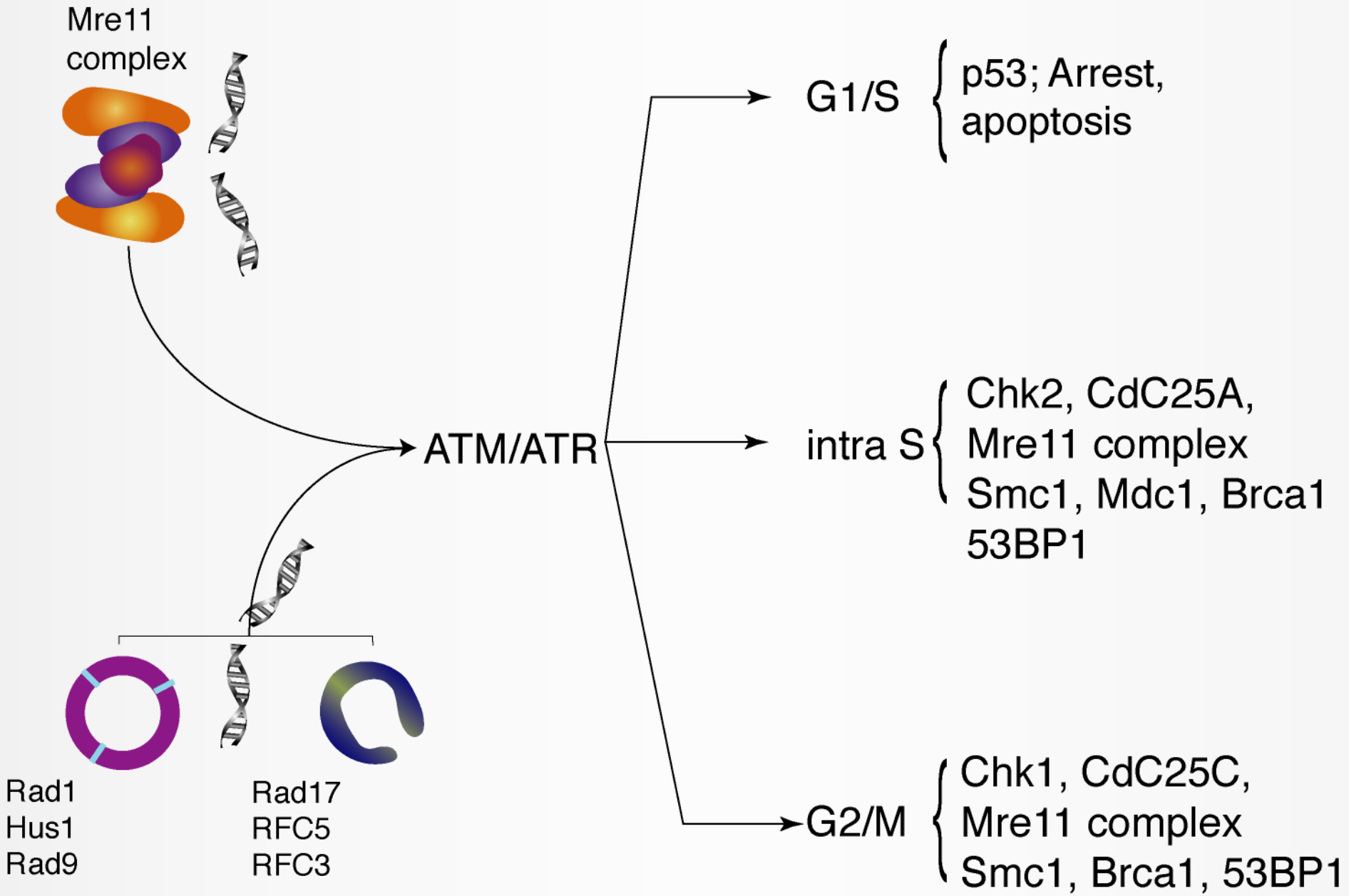
Signal amplification

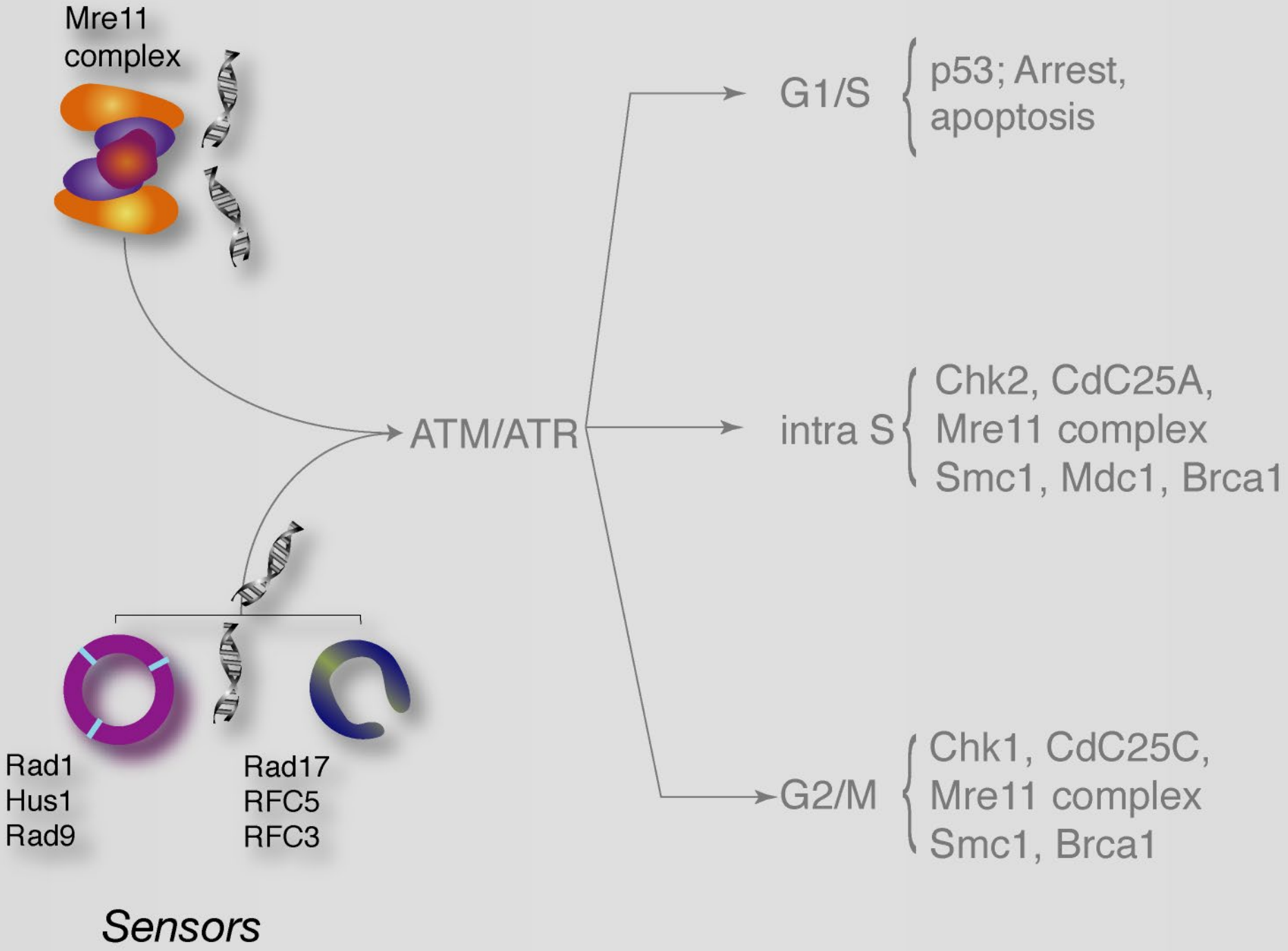
Transcription
Cell cycle arrest
Apoptosis

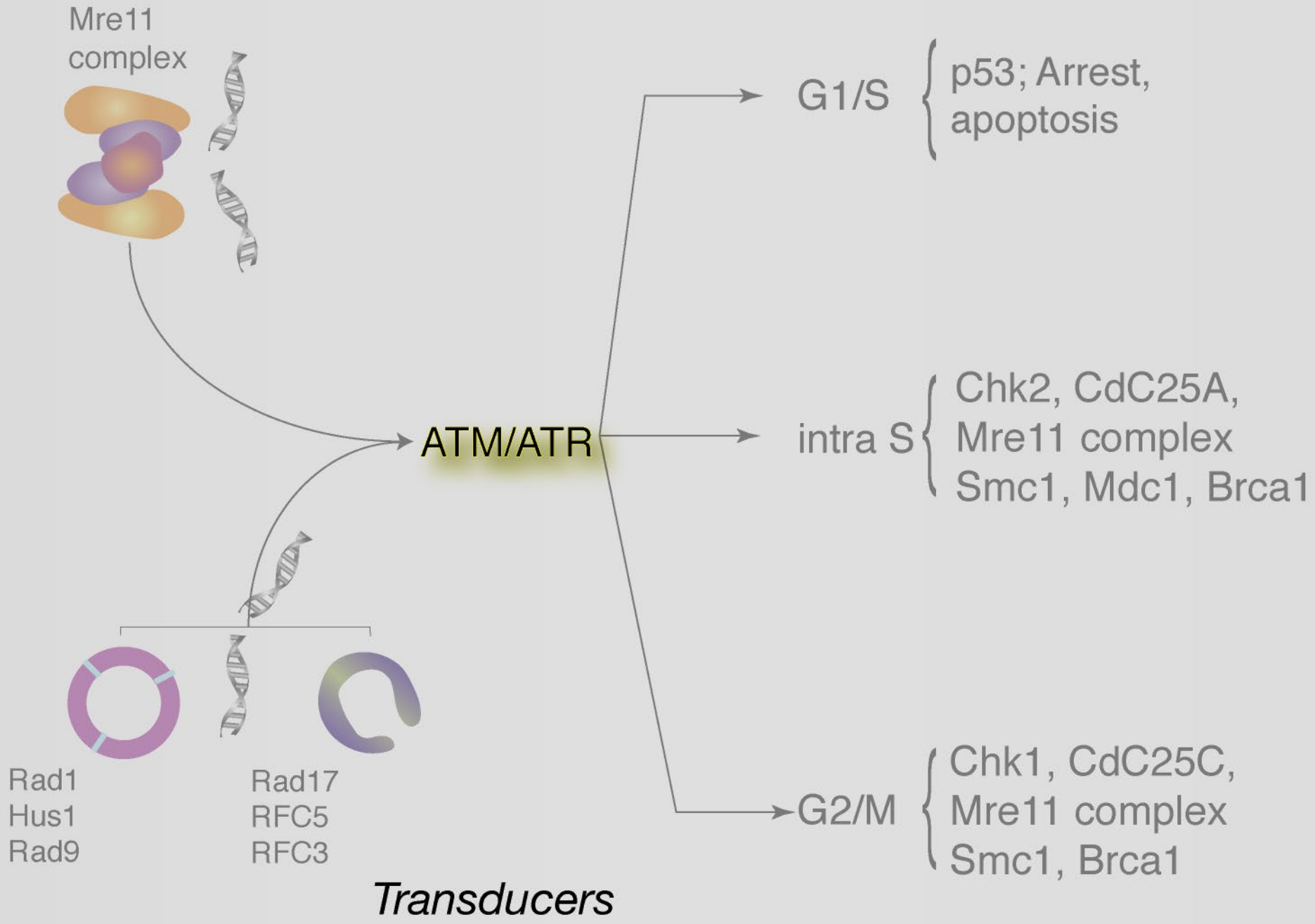
DNA repair*

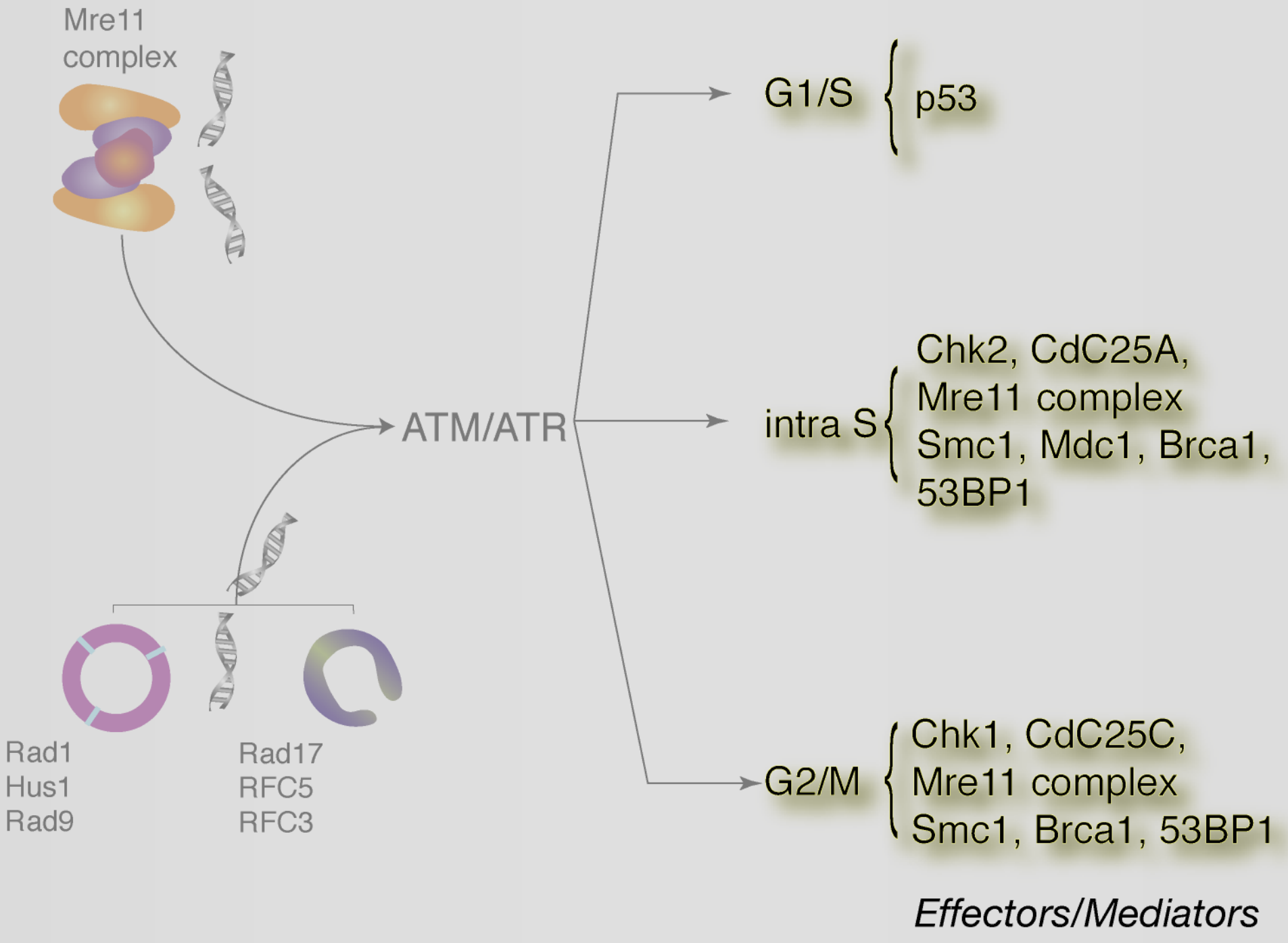
Suppression of Malignancy

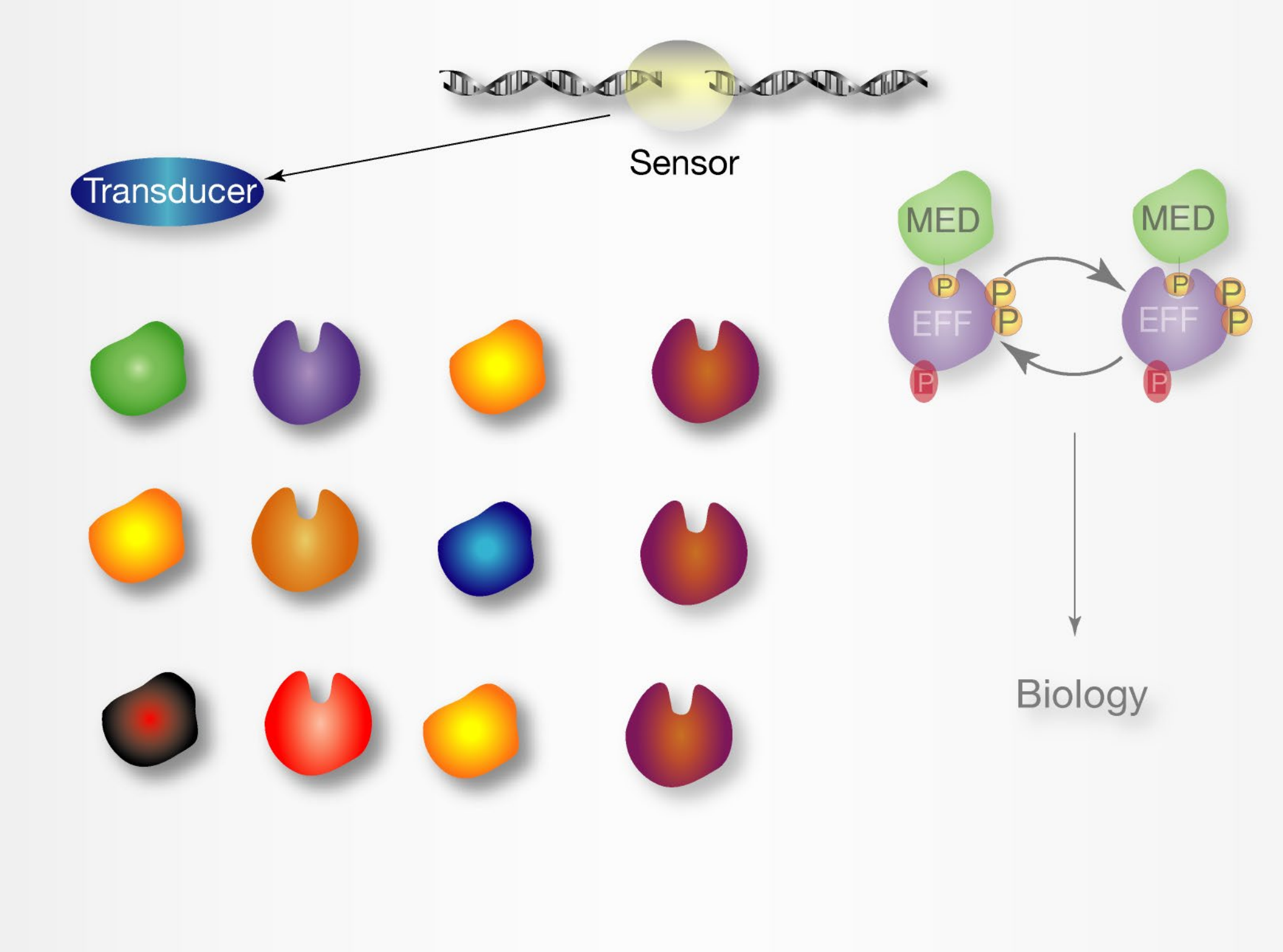
** incl. programmed gene rearrangement*

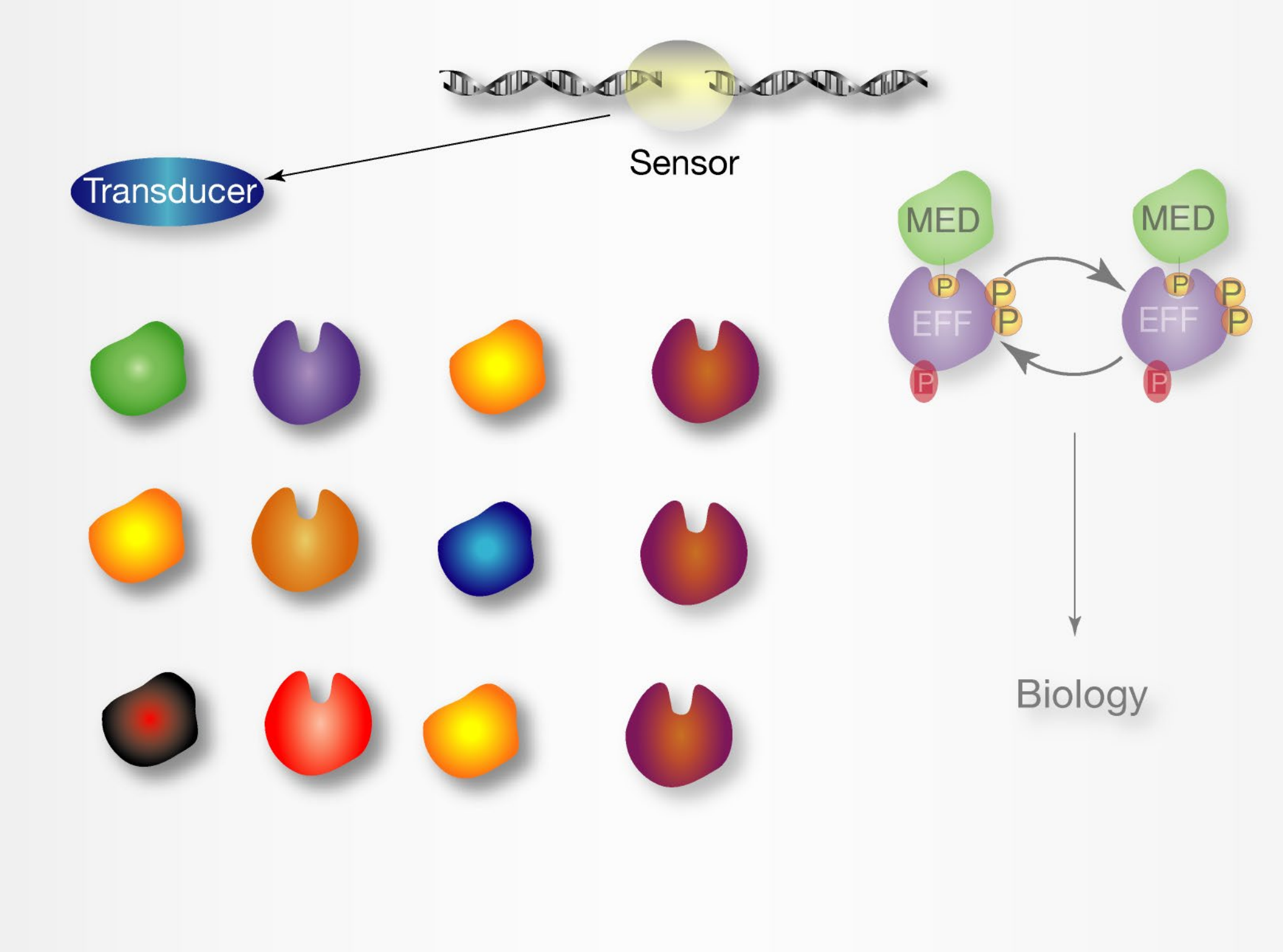

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
53BP1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1, 53BP1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

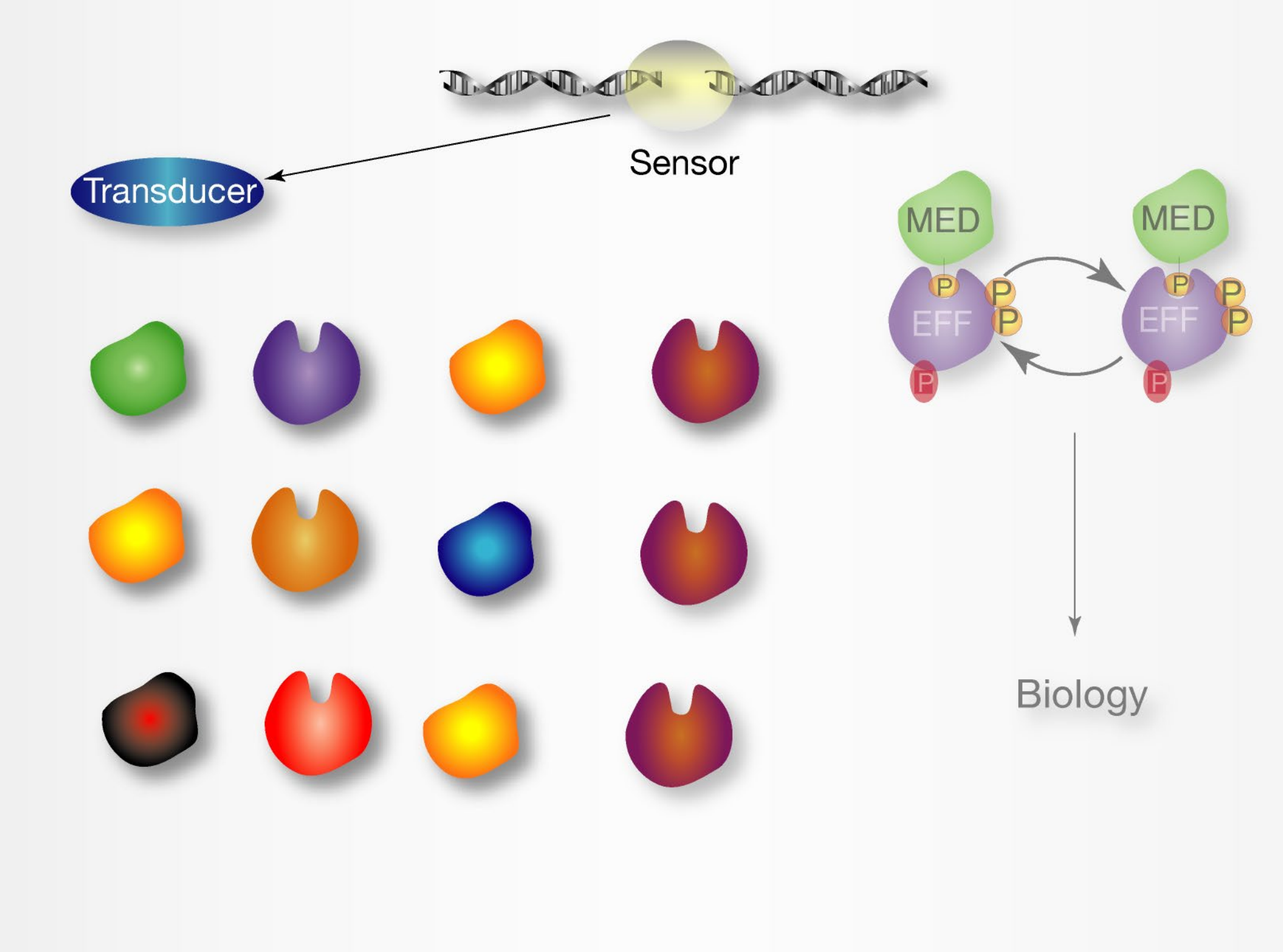

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Sensors

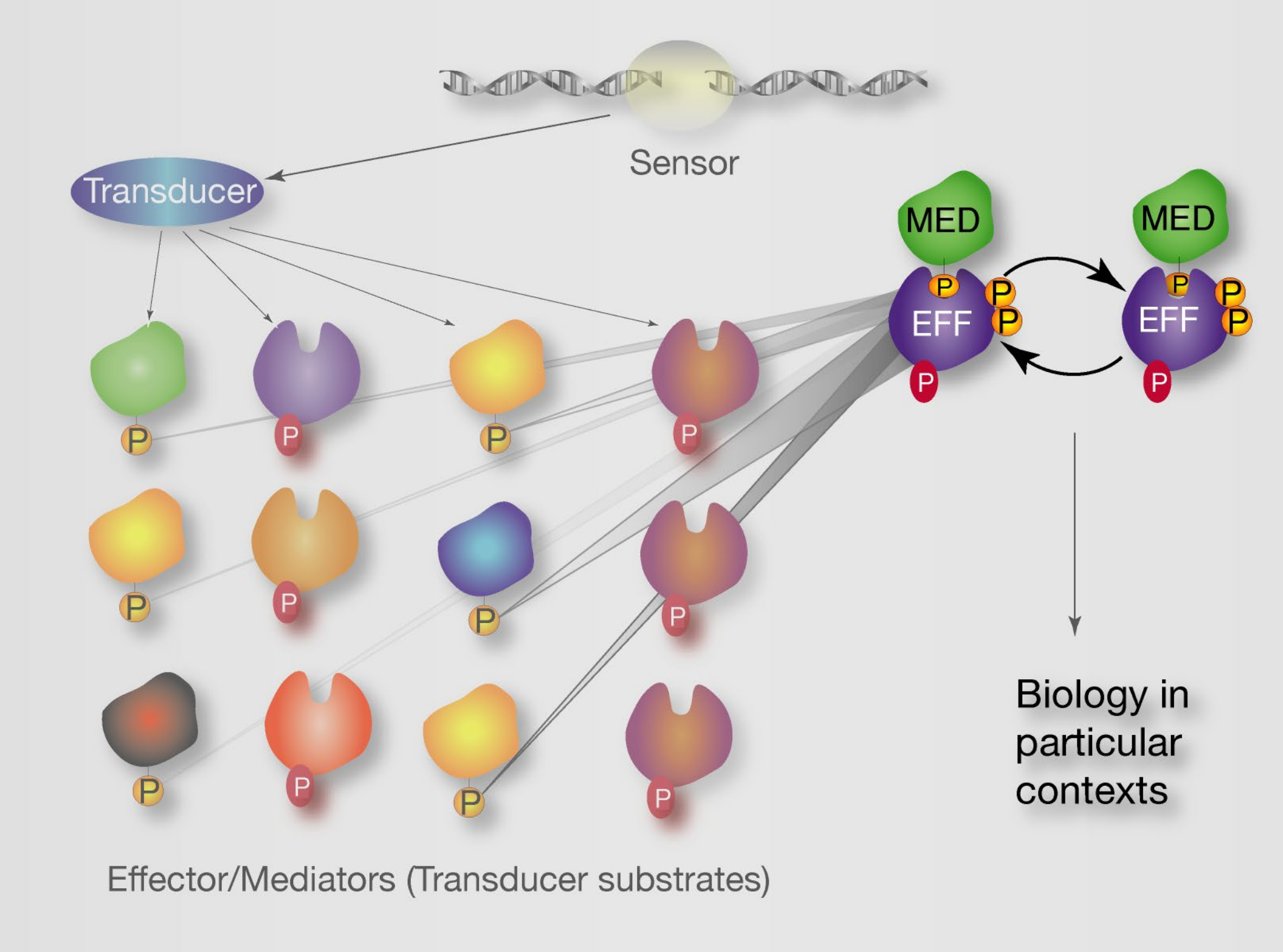

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Transducers

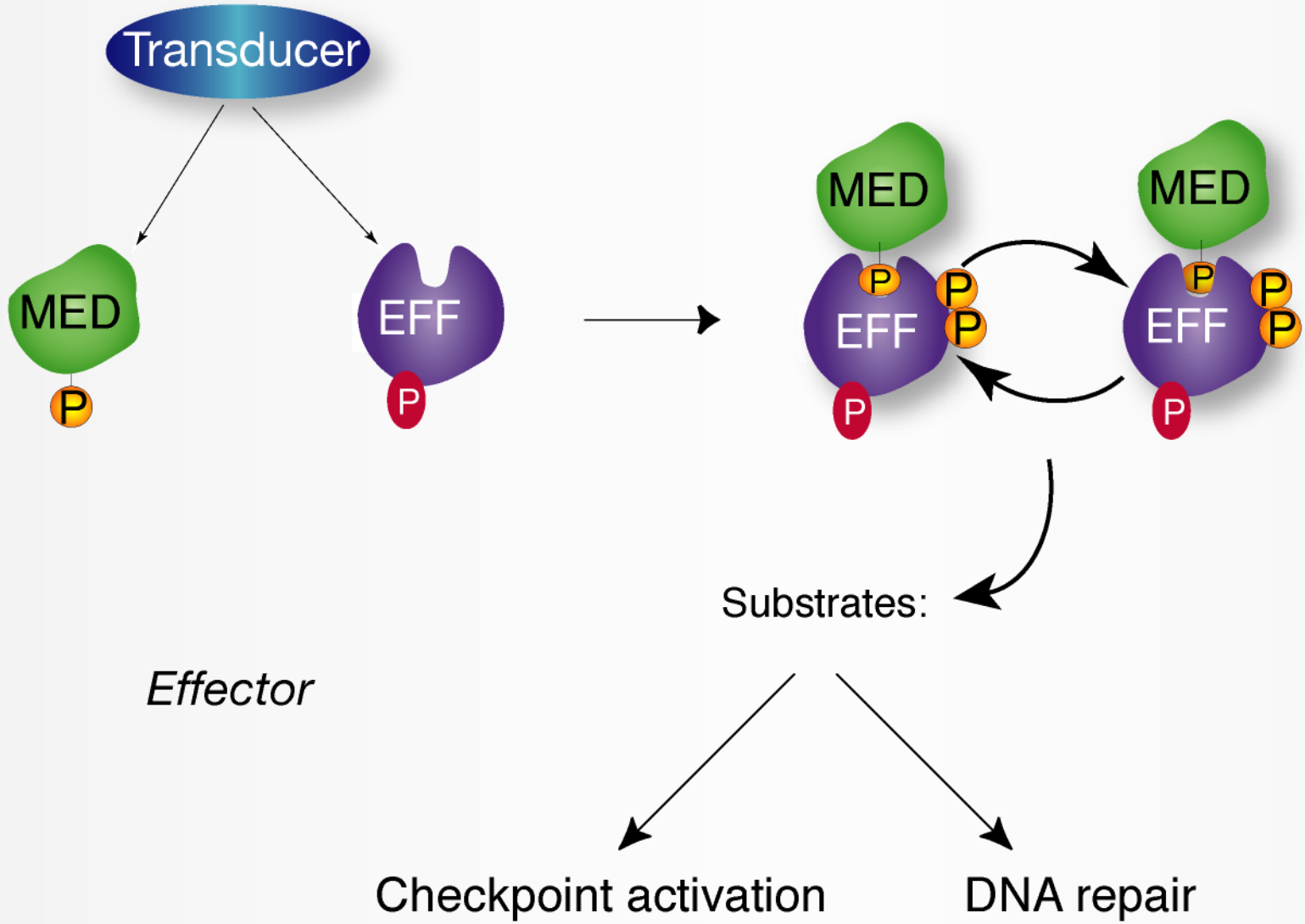

Mre11
complex
ATM/ATR
G1/S
p53
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1,
53BP1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1, 53BP1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3
Effectors/Mediators

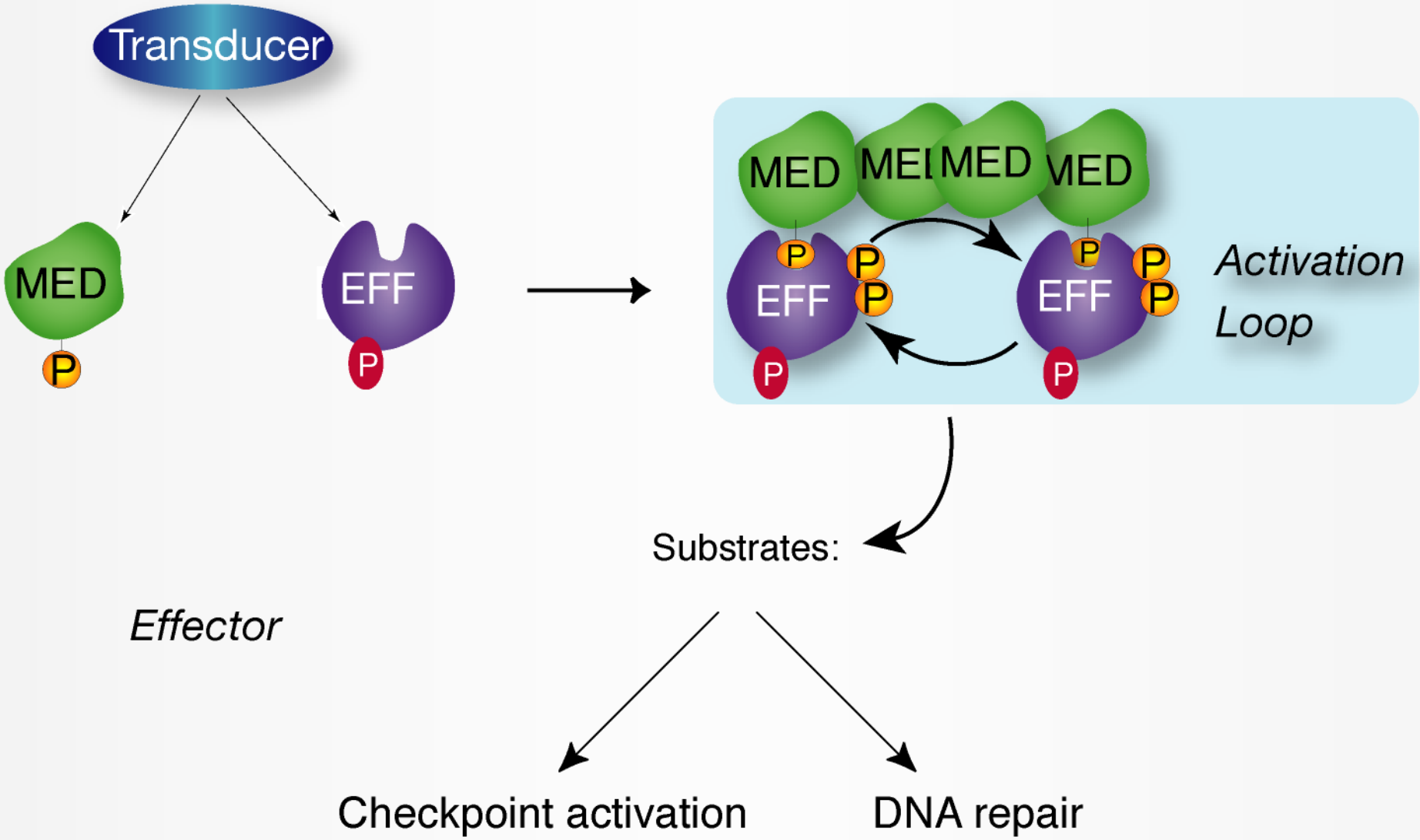

Sensor
Transducer
MED
MED
P
P
P
P
EFF
EFF
P
P
P
P
Biology

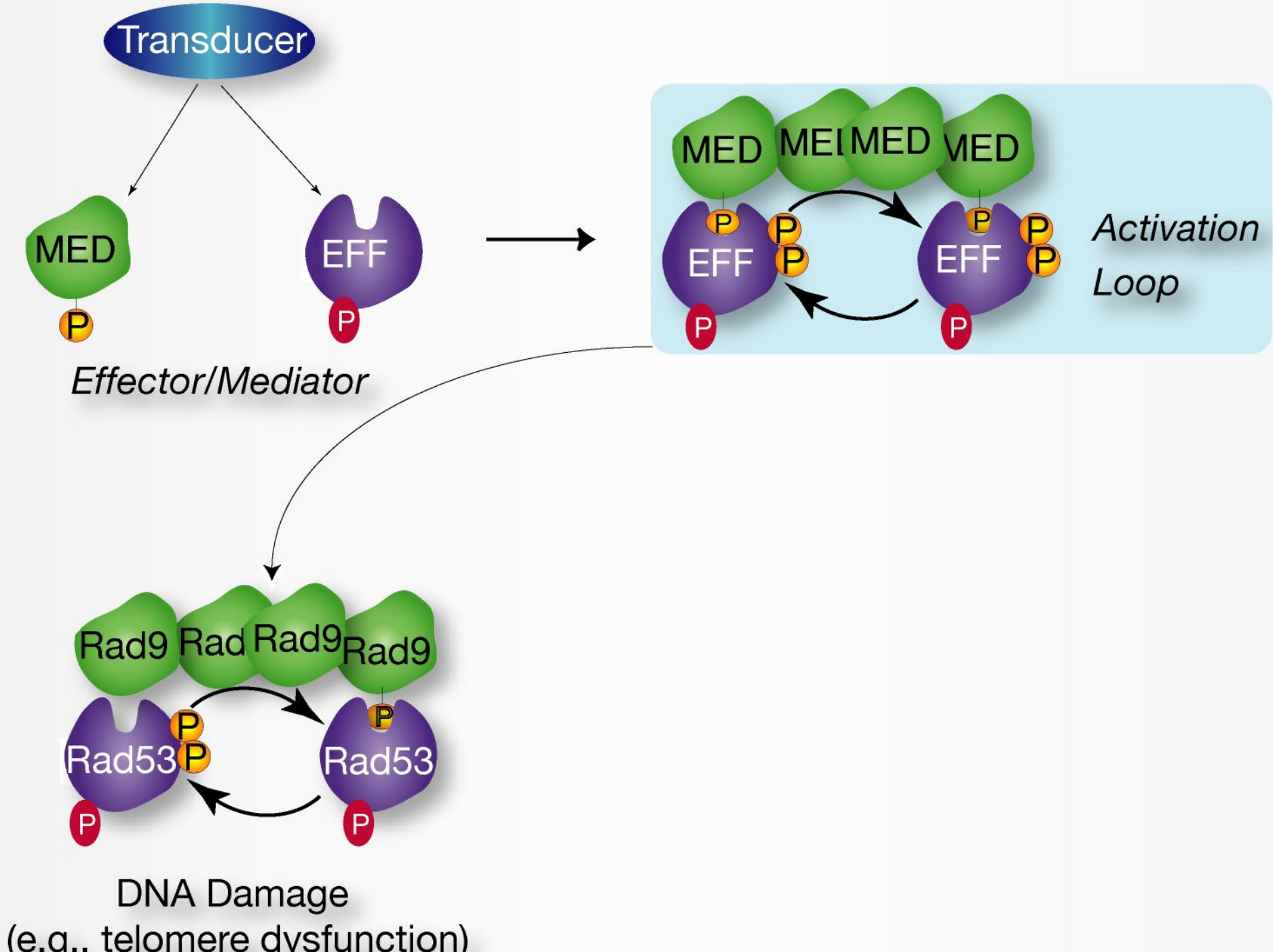

Sensor
Transducer
MED
MED
P
P
P
P
EFF
EFF
P
P
P
P
Biology

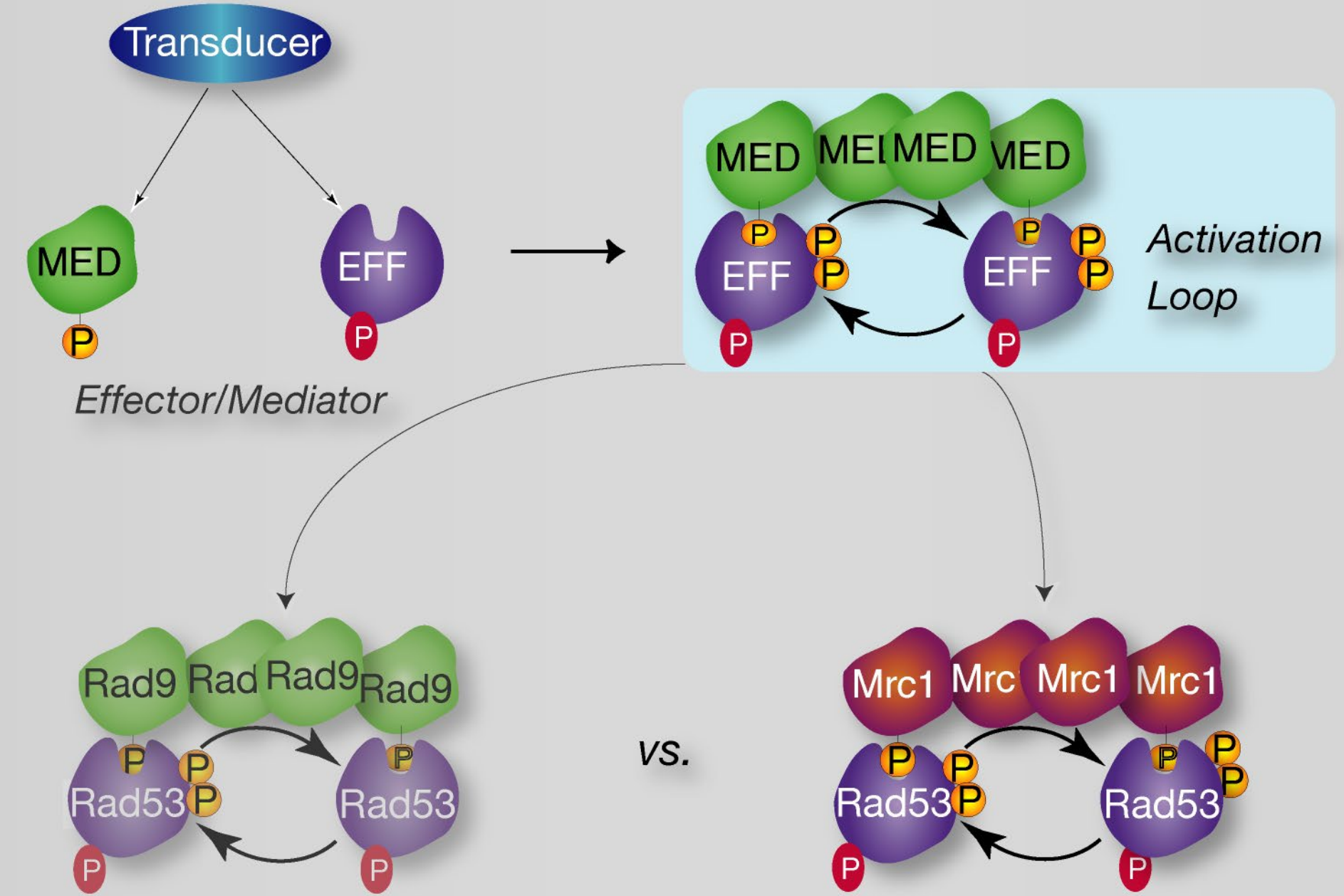

Sensor
Transducer
MED
MED
P
P
P
P
EFF
EFF
P
P
P
P
Biology

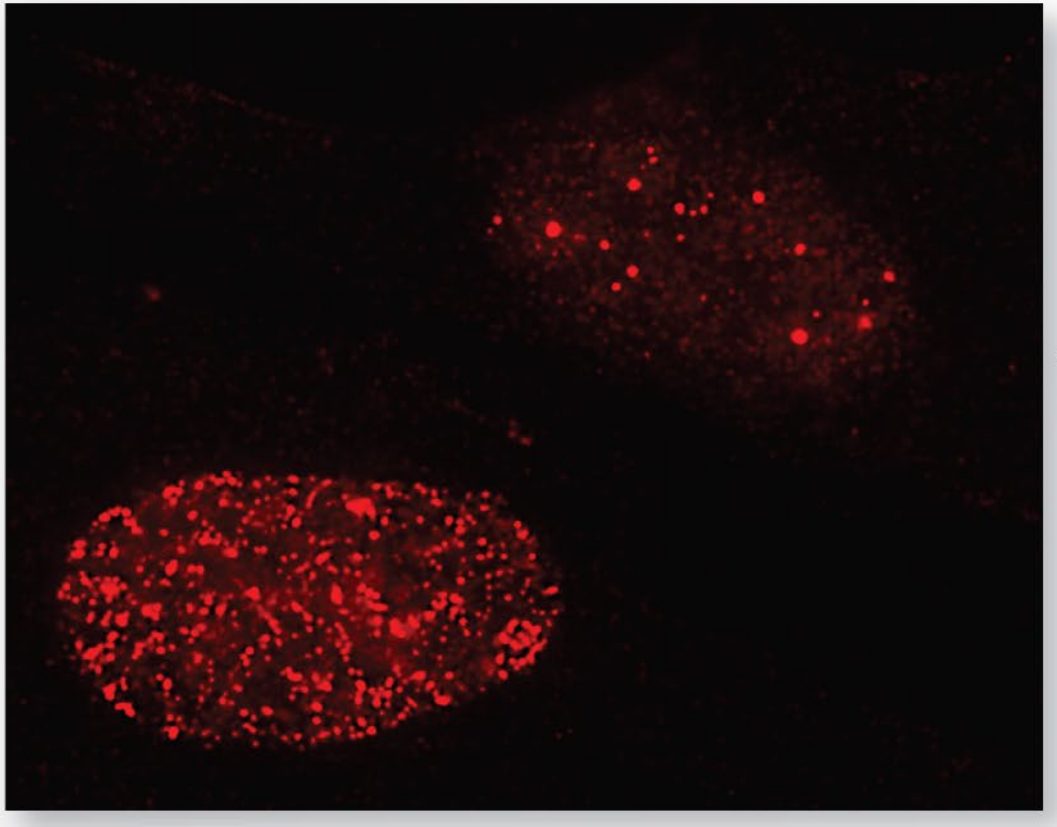

Sensor
Transducer
MED
EFF
MED
EFF
P
Biology in particular contexts
Effector/Mediators (Transducer substrates)


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

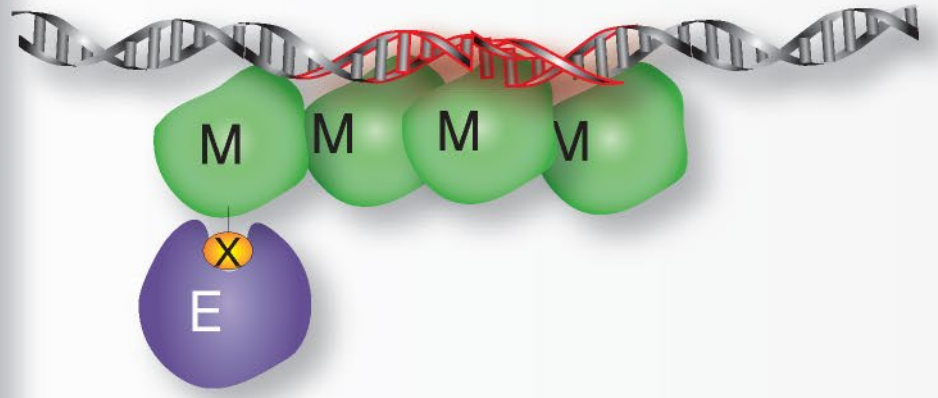

Transducer
MED
P
EFF
P
MED
MED
MED
EFF
P
P
P
EFF
P
P
P
Activation
Loop
Substrates:
Effector
Checkpoint activation
DNA repair


Transducer
MED
P
EFF
P
Effector/Mediator
MED
MED
MED
EFF
EFF
Activation
Loop
Rad9
Rad
Rad9
Rad9
Rad53
Rad53
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

FHA

BRCT



Phosphopeptide interactions

e.g., Tudor domains



Histone modification driven interactions

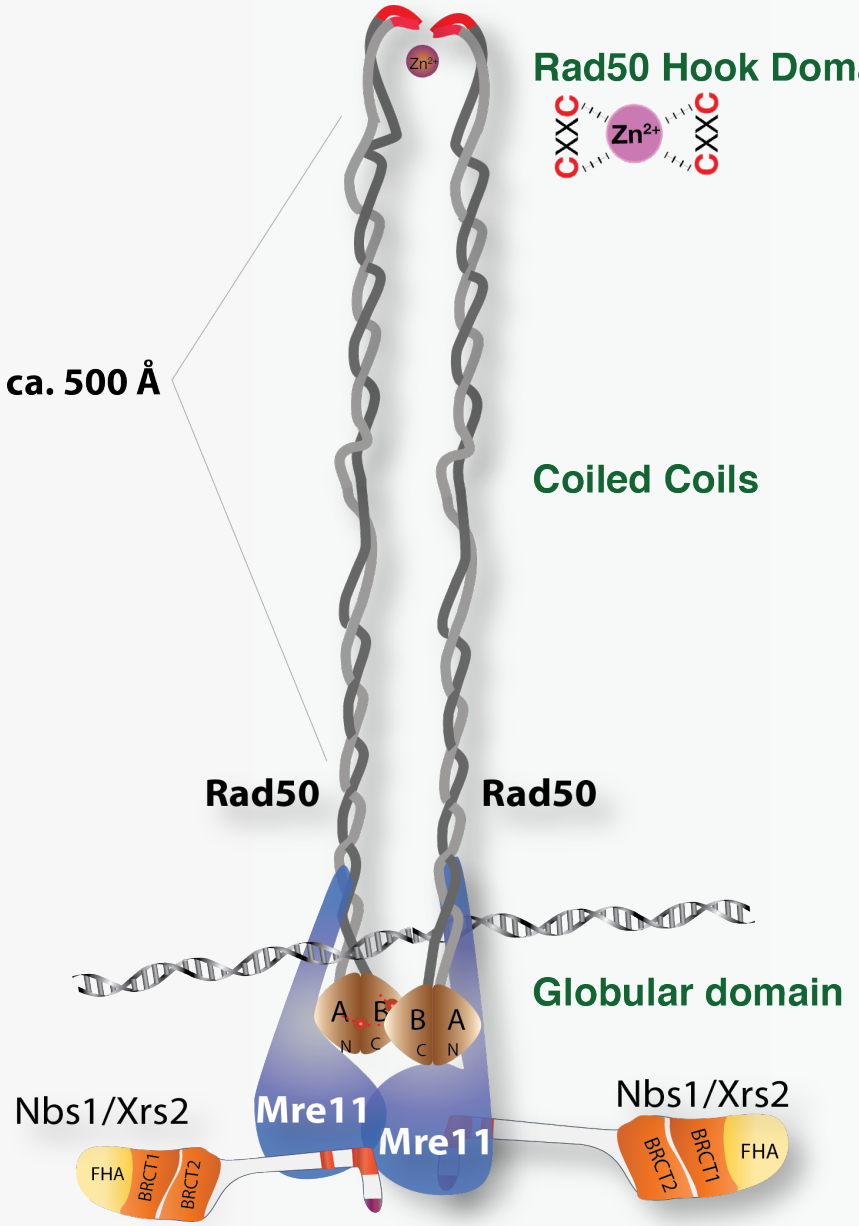
SIM & UIM



Sumo-
&
Ubiqutin-dependent interactions

What is a sensor?

Mre11 complex's Architecture and Functions

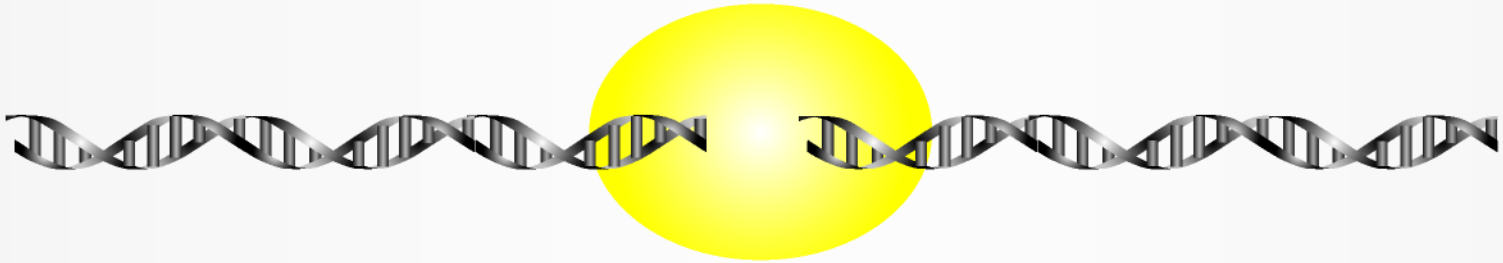


1° Sensor of DNA damage

Required for ATM activation

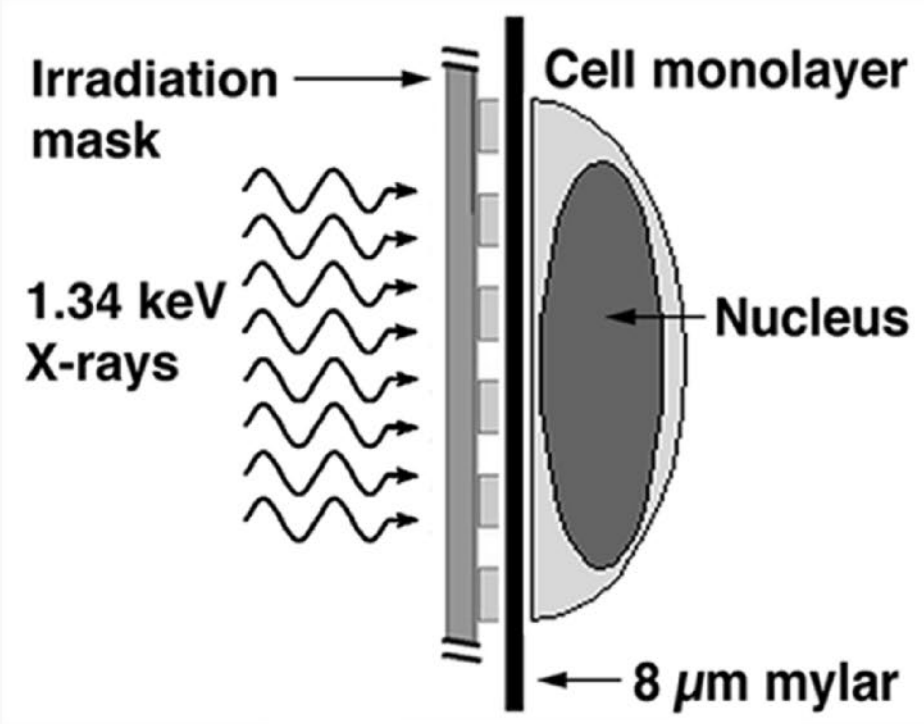
Required for DNA DSB repair

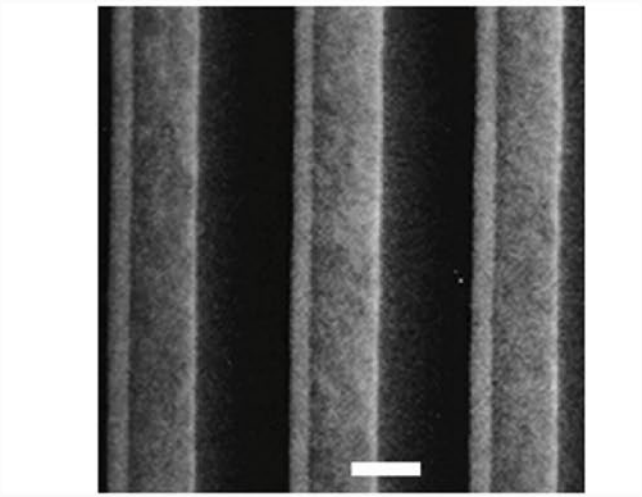
Essential for viability
(in higher eukaryotes)

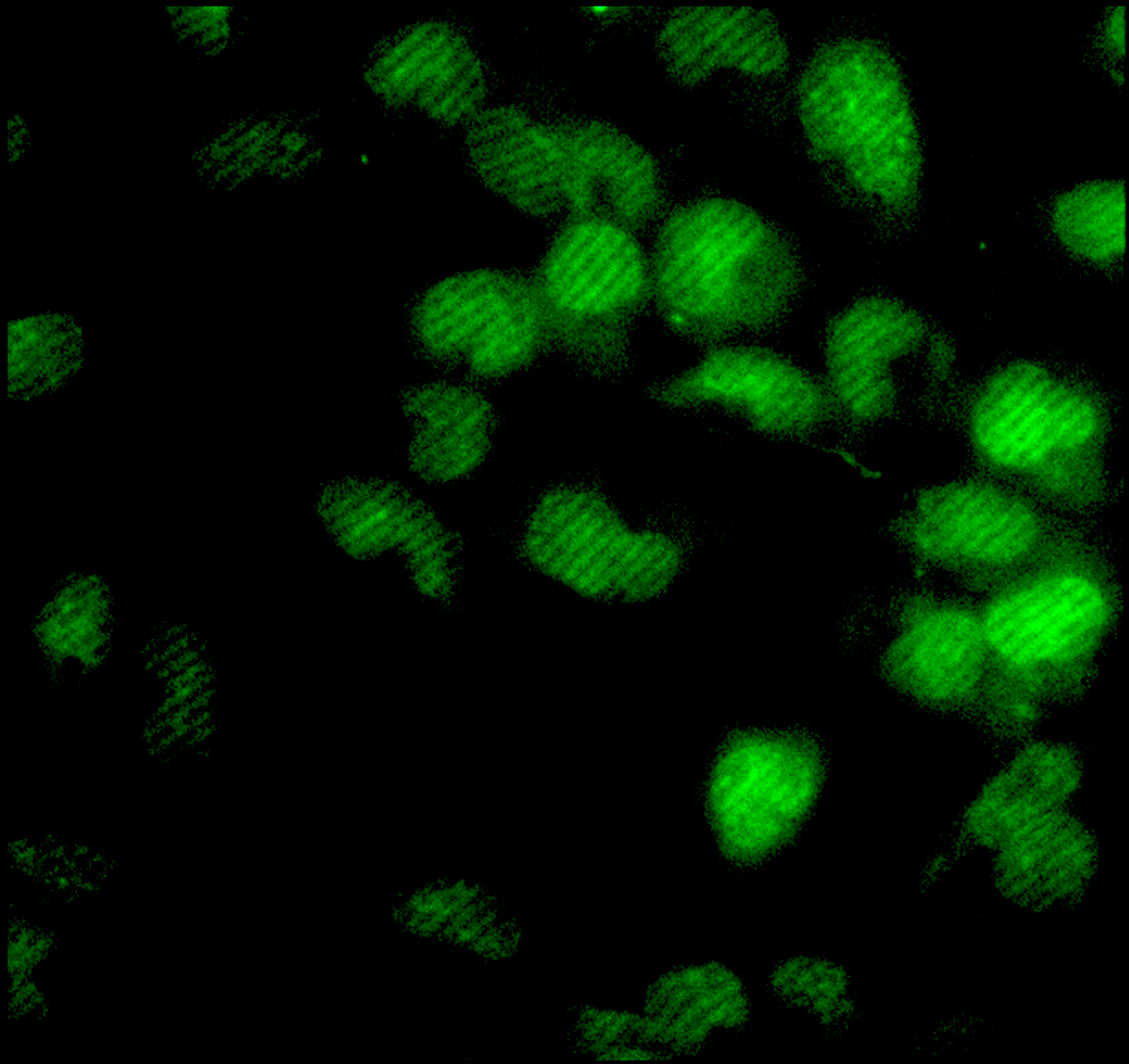


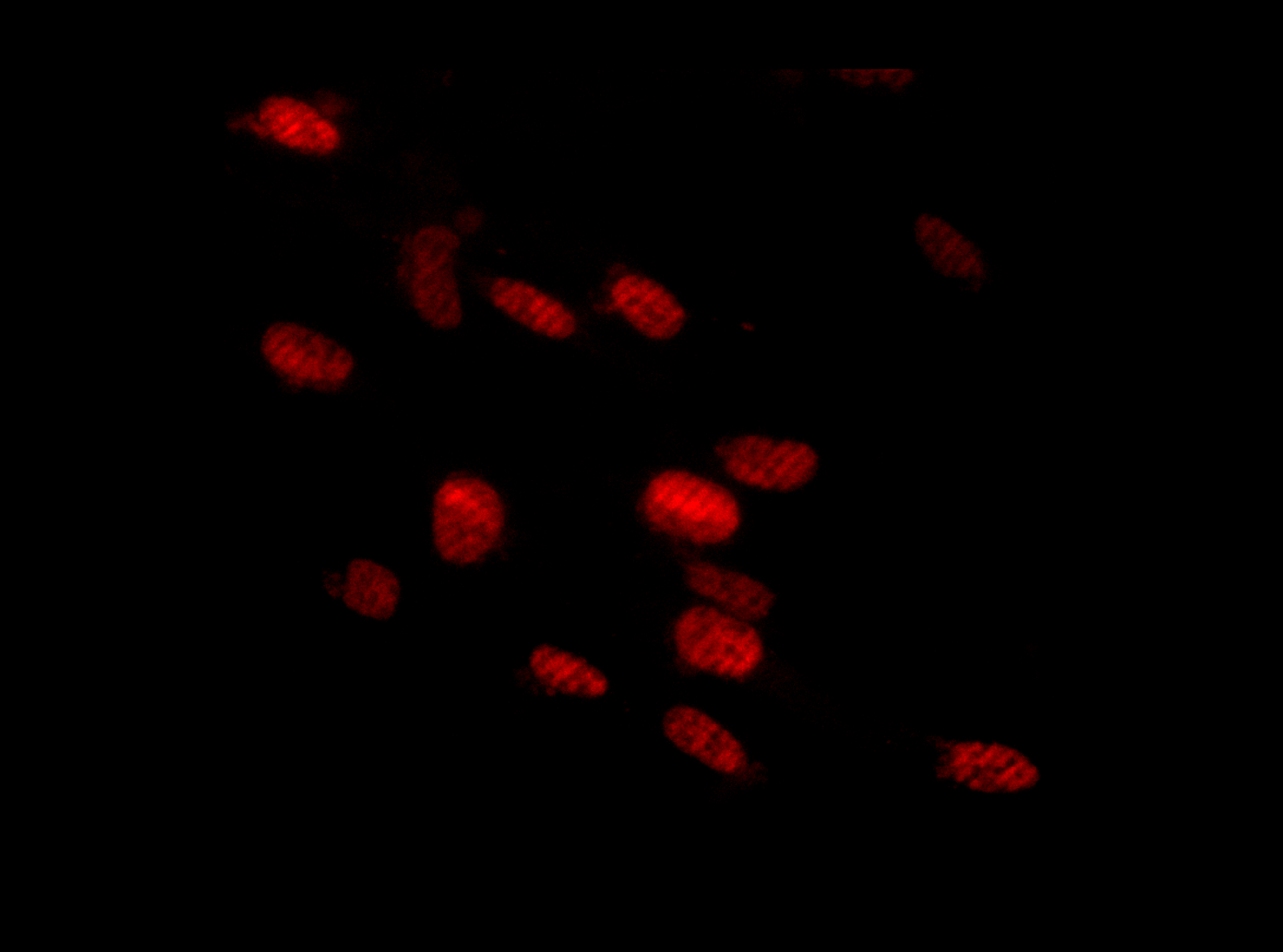
DNA damage sensors

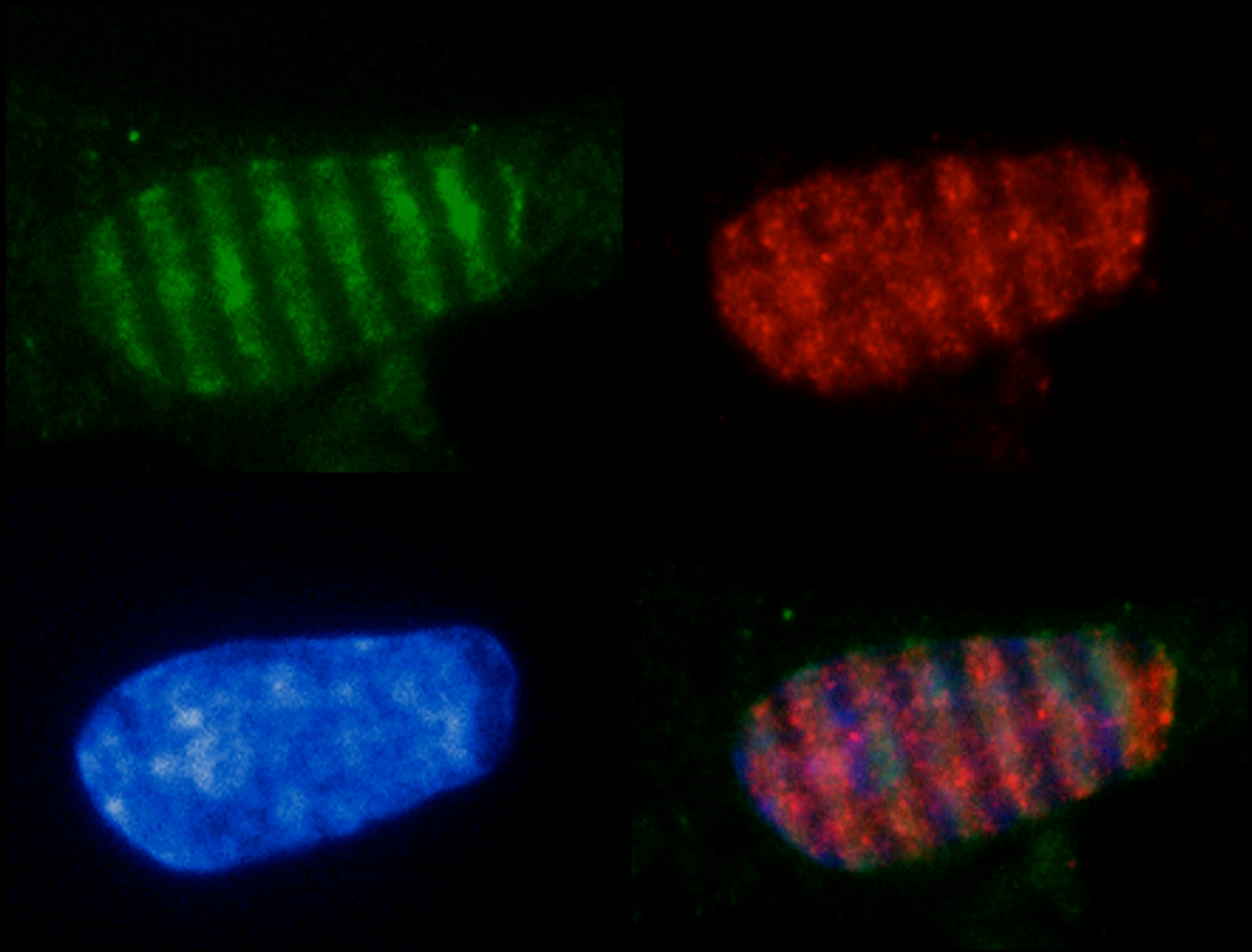
- Avidity for altered DNA structures (e.g., DNA breaks)


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



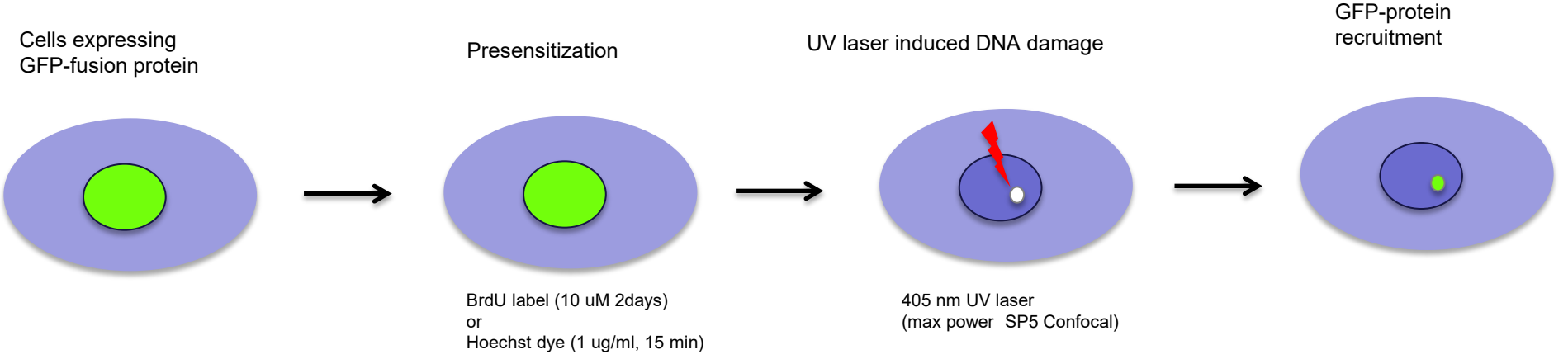


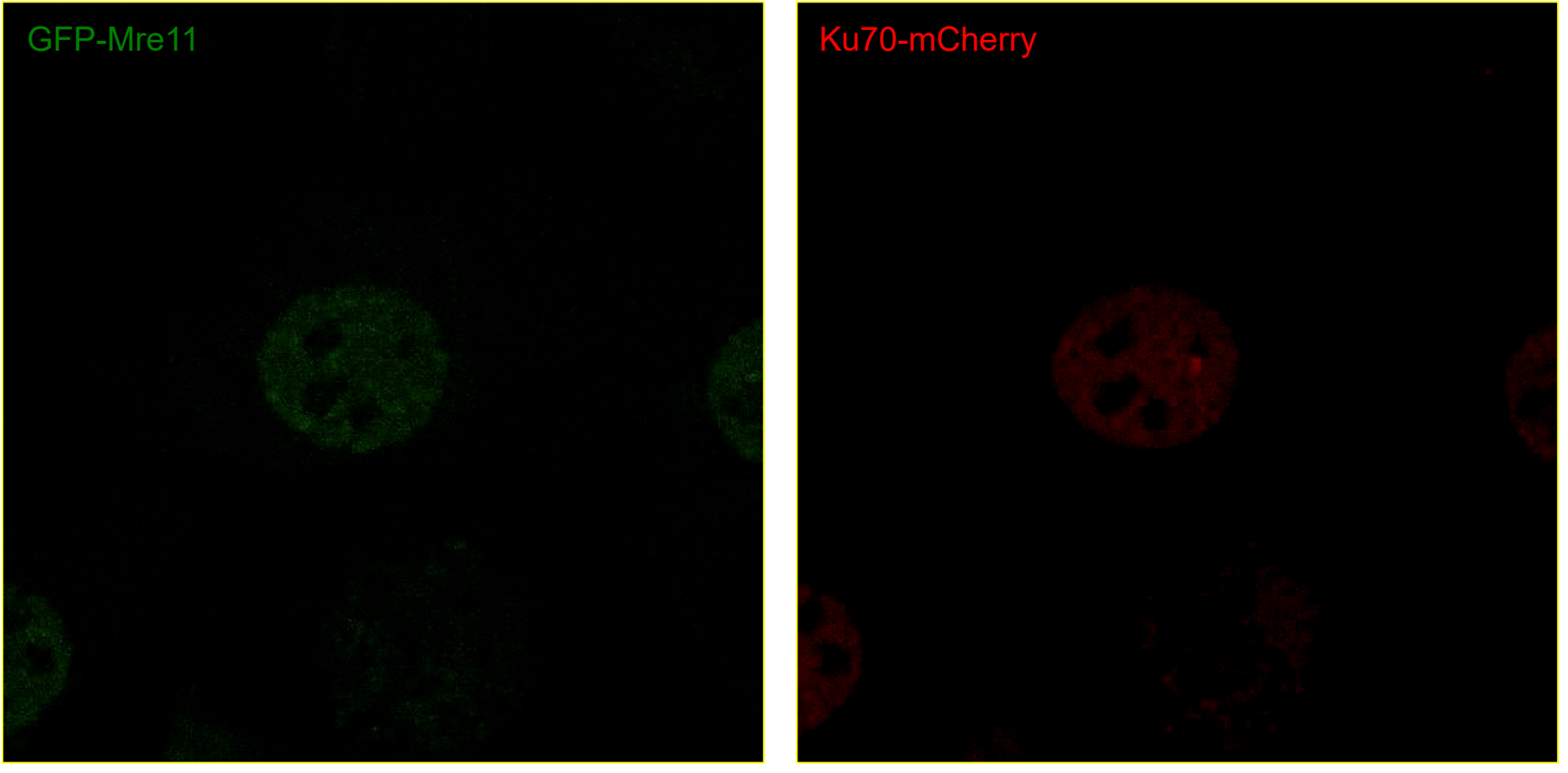




Does Nob1 localize at DNA damage site?

UV laser microirradiation (live cell imaging)




GFP-Mre11
Ku70-mCherry
5 min

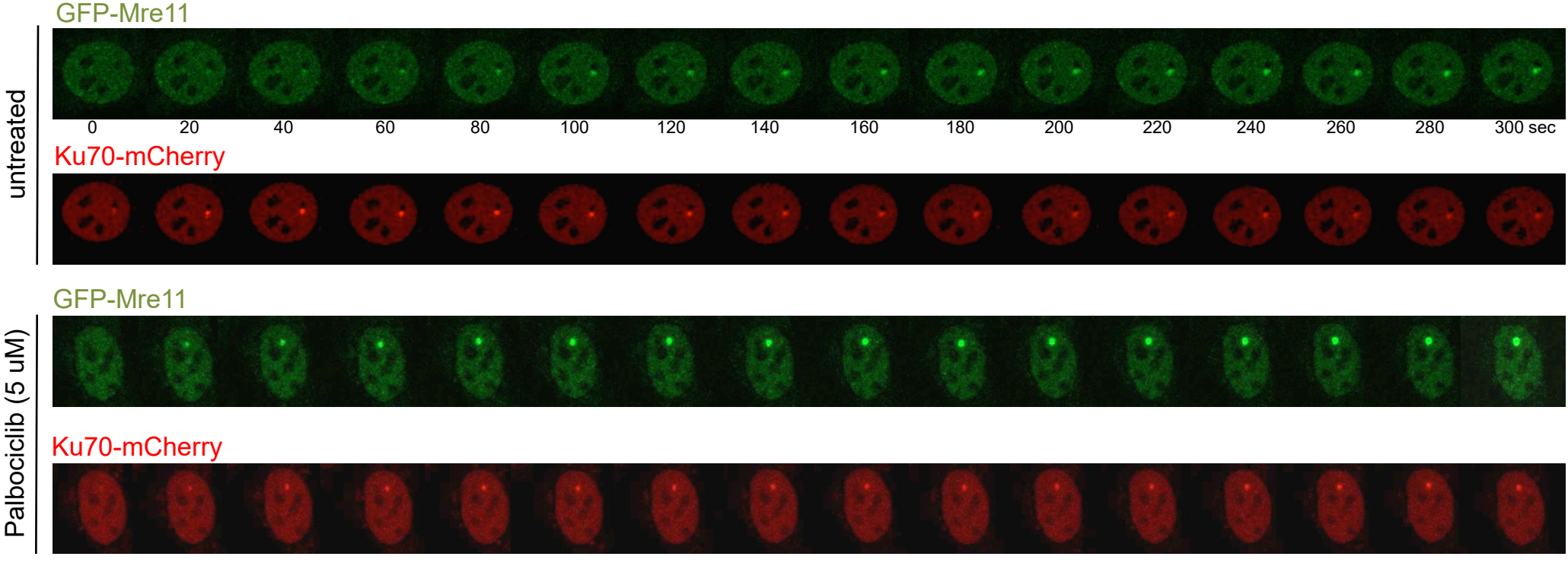


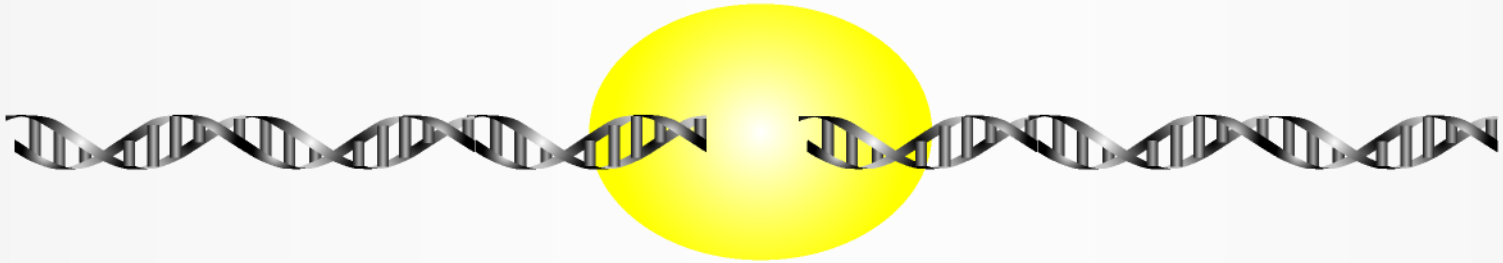


5 min

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

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

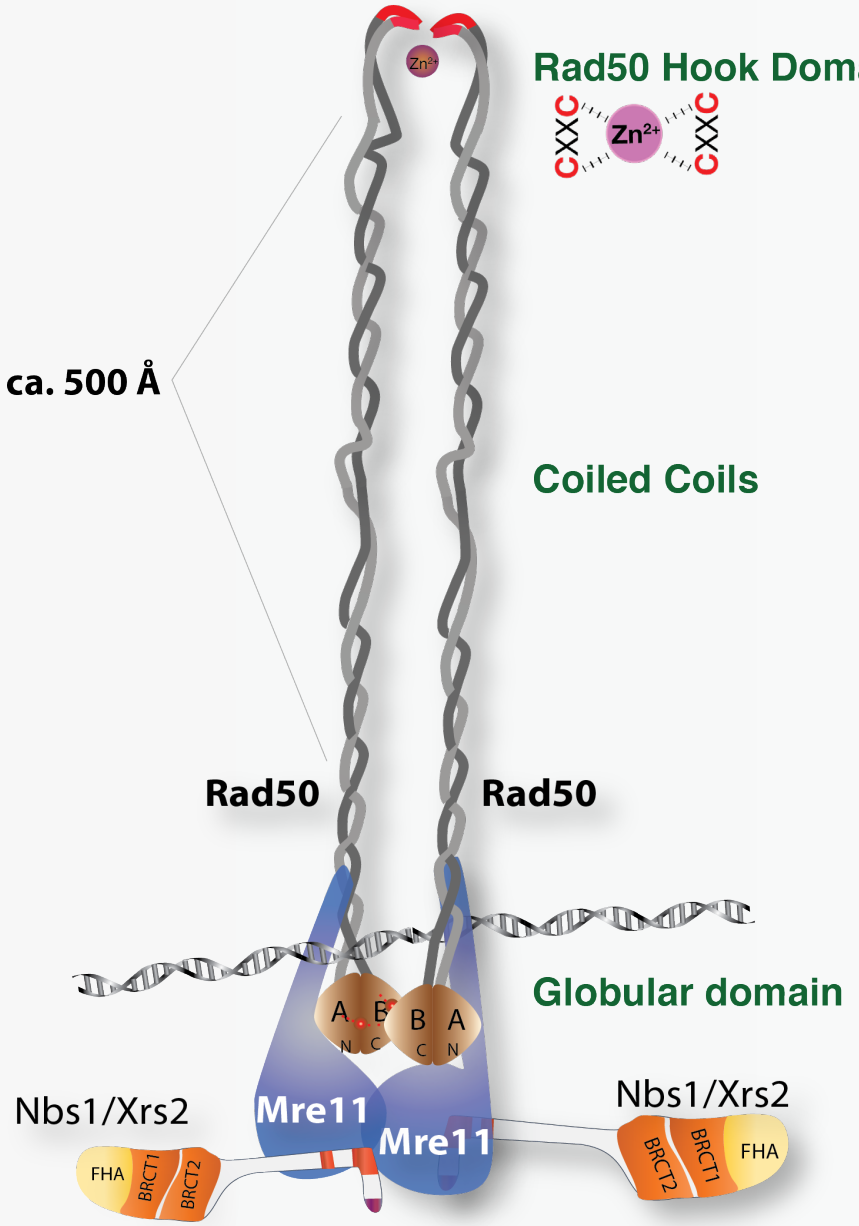




DNA damage sensors

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

Mre11 complex's Architecture and Functions



1° Sensor of DNA damage

Required for ATM activation

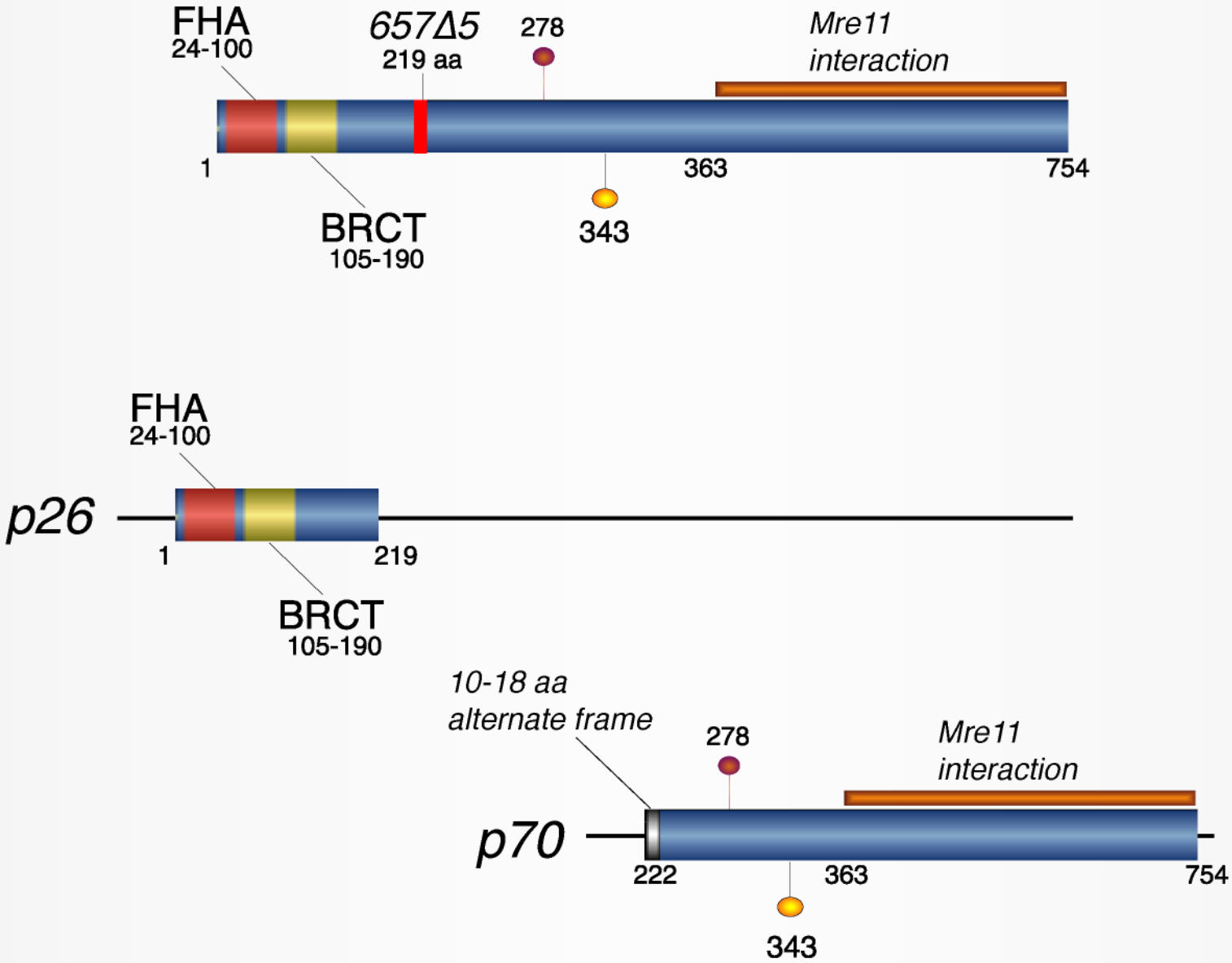
Required for DNA DSB repair

**Essential for viability*
(in higher eukaryotes)

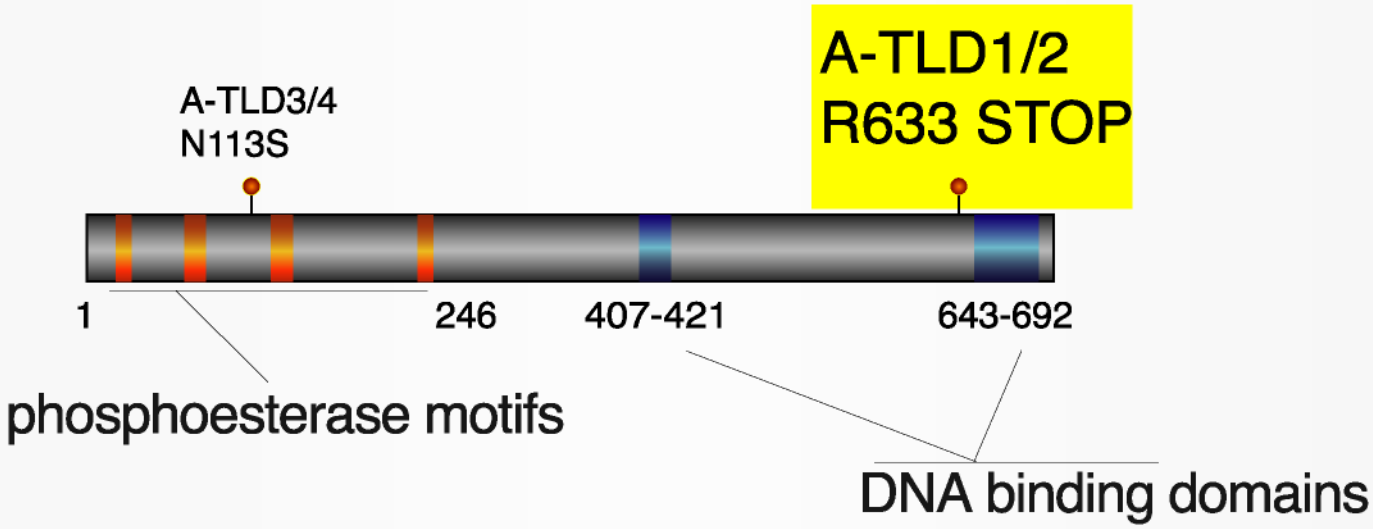
Inherited Mre11 complex mutations: Nijmegen breakage syndrome & the ataxia-telangiectasia like disorder

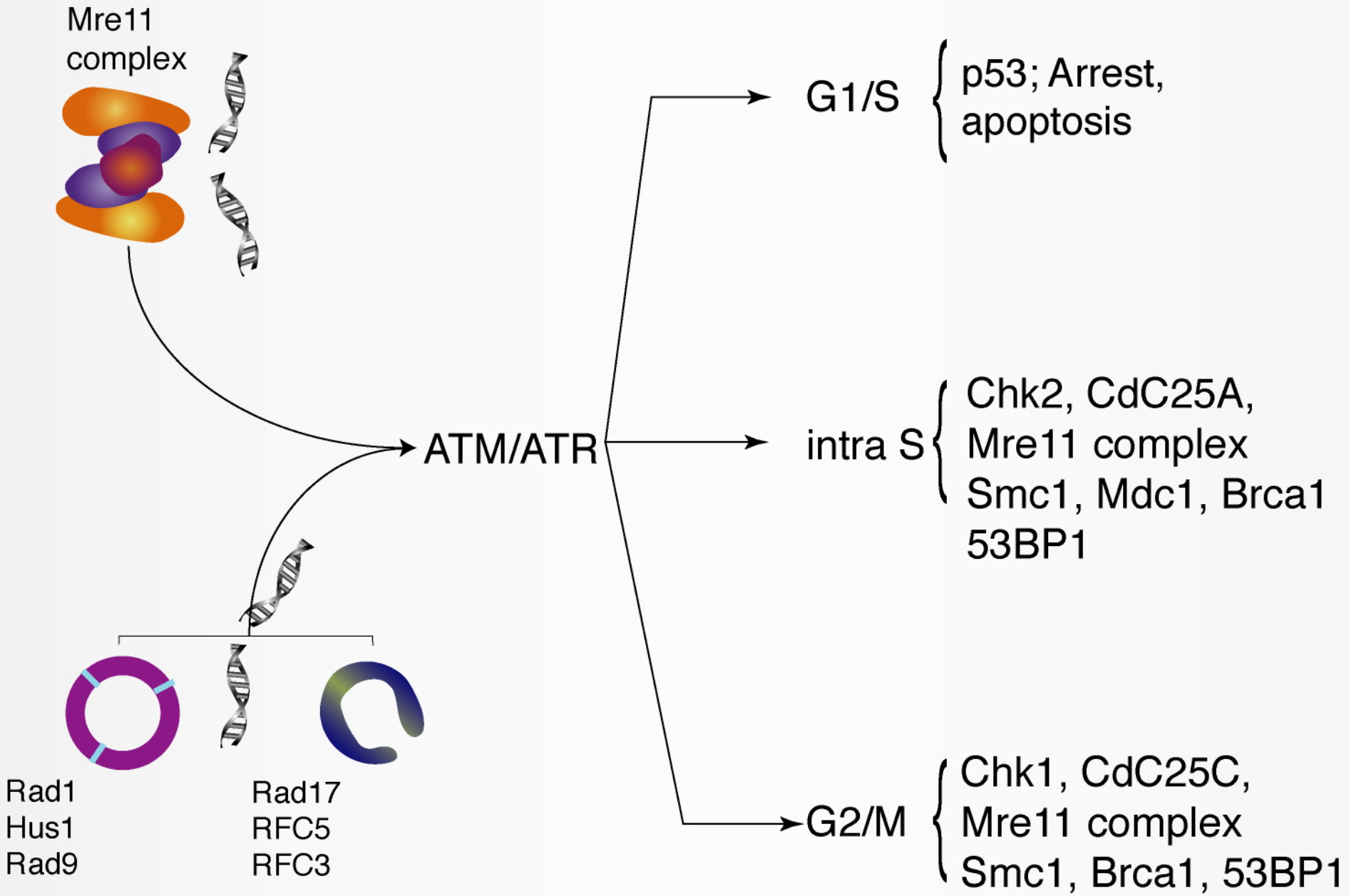
- *Nbs1* and *Mre11* mutations respectively
- Hypersensitivity to clastogens
- Chromosome fragility
- Intra-S phase checkpoint defect
- G2/M checkpoint defect
- Attenuated ATM activity

Two products of the common NBS allele

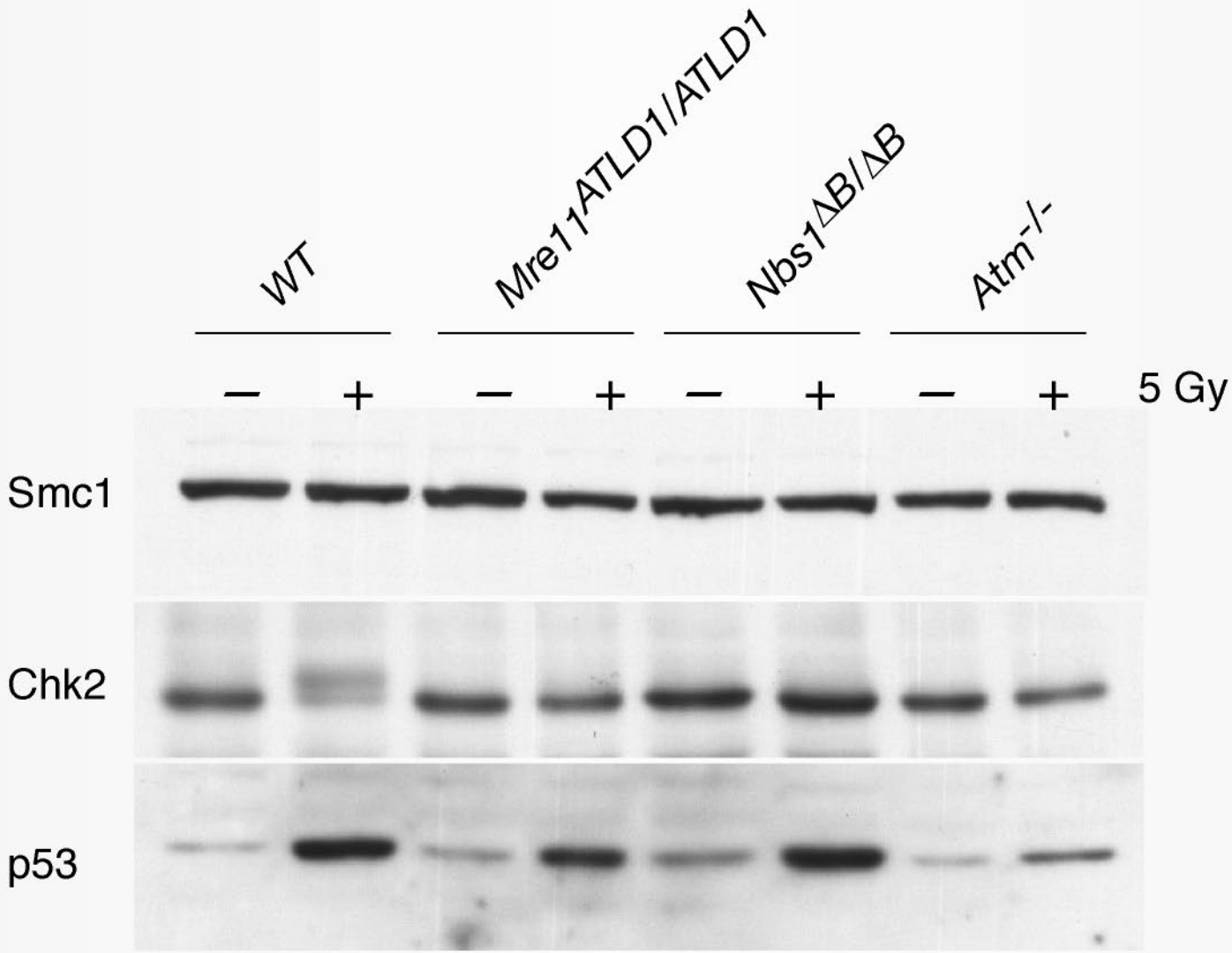


Mre11

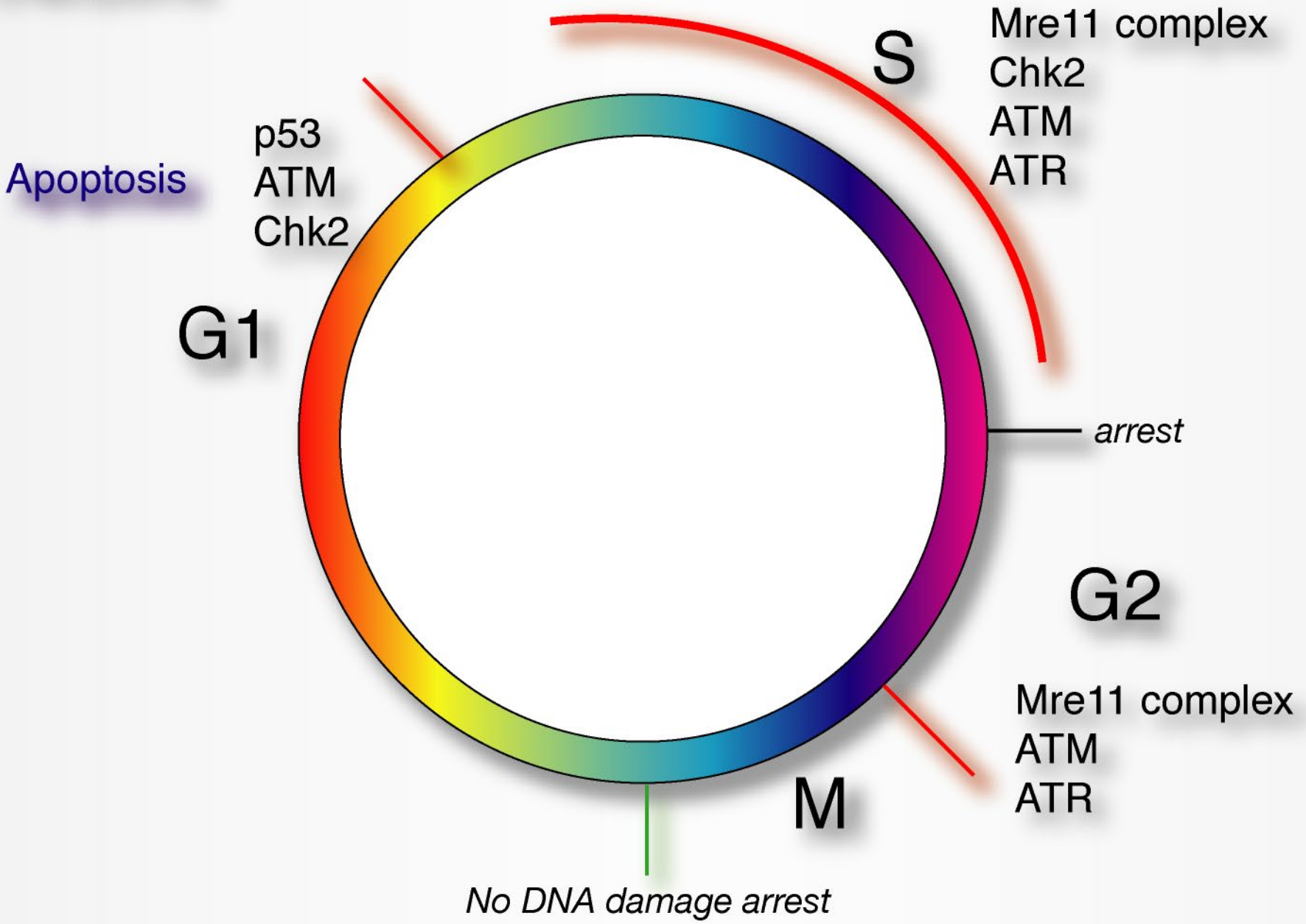

A-TLD3/4
N113S
A-TLD1/2
R633 STOP
1
246
407-421
643-692
phosphoesterase motifs
DNA binding domains

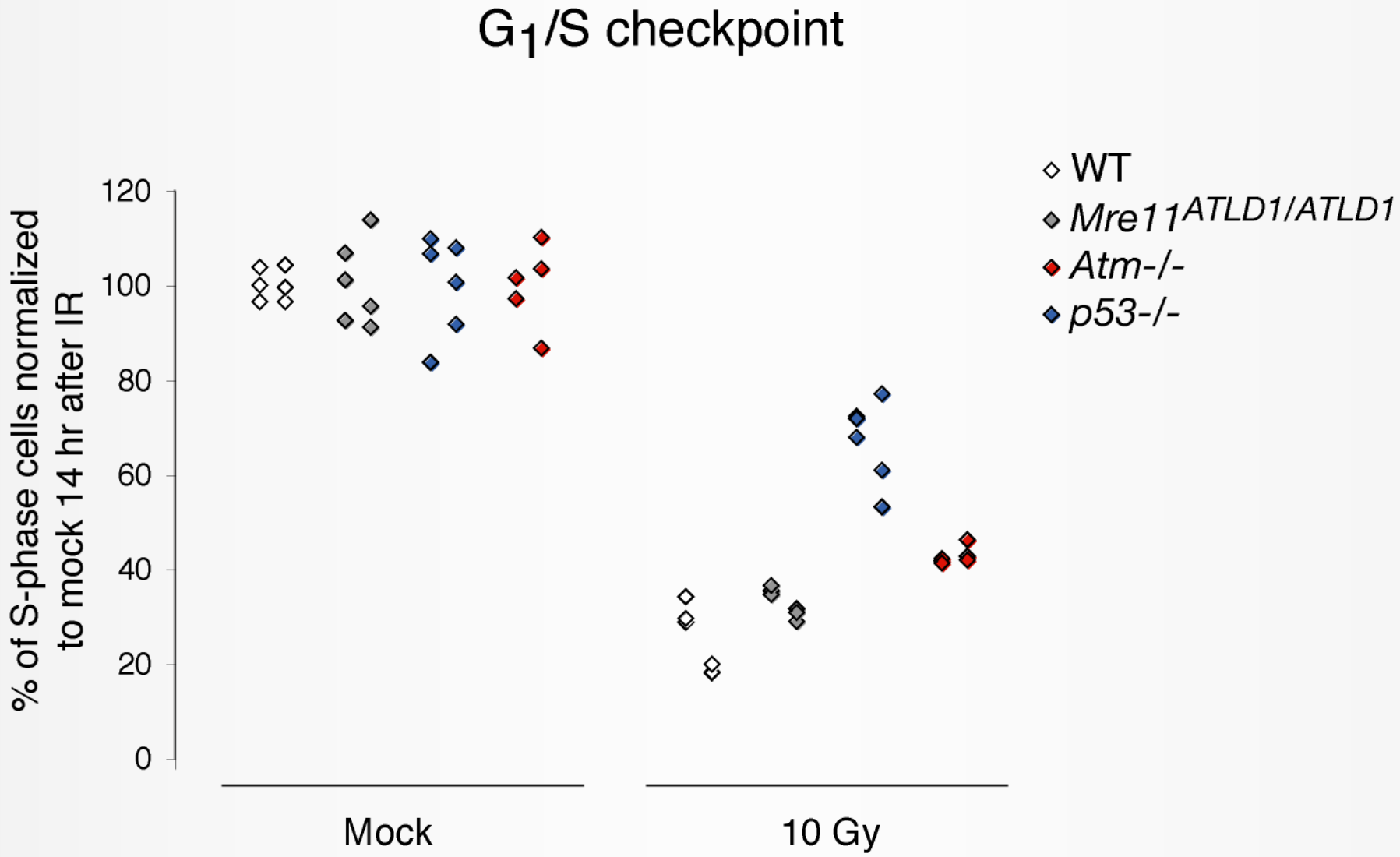

Mre11
complex
ATM/ATR
G1/S
p53; Arrest,
apoptosis
intra S
Chk2, CdC25A,
Mre11 complex
Smc1, Mdc1, Brca1
53BP1
G2/M
Chk1, CdC25C,
Mre11 complex
Smc1, Brca1, 53BP1
Rad1
Hus1
Rad9
Rad17
RFC5
RFC3

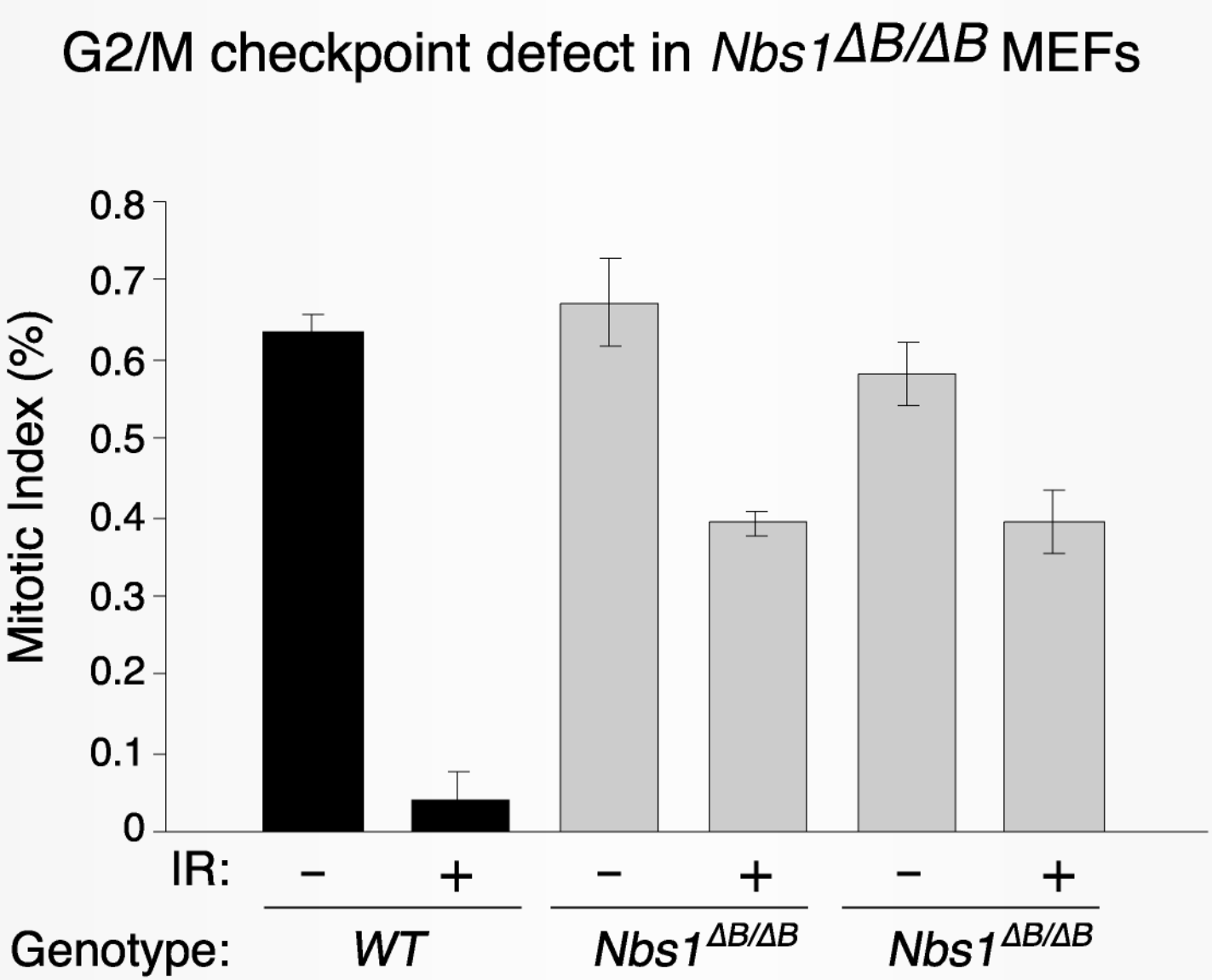
ATM targets in thymocytes



DNA damage dependent checkpoints

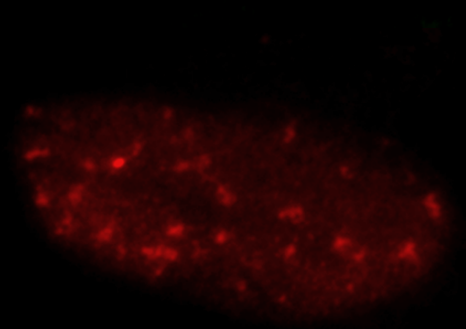



G1/S checkpoint
% of S-phase cells normalized to mock 14 hr after IR
120
100
80
60
40
20
0
Mock
10 Gy
WT
Mre11ATLD1/ATLD1
Atm-/-
p53-/-

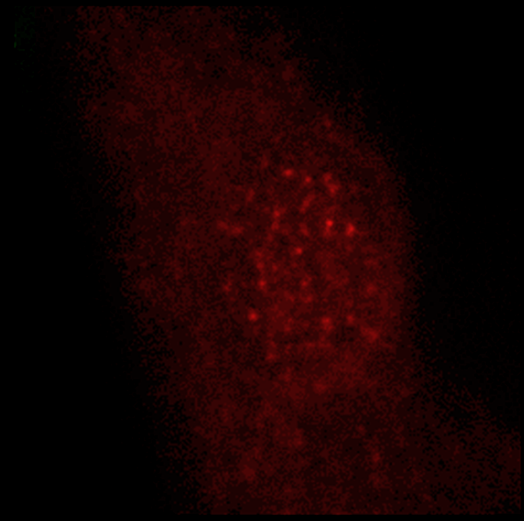

G2/M checkpoint defect in Nbs1ΔB/ΔB MEFs
Mitotic Index (%)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IR:
–
+
–
+
–
+
Genotype:
WT
Nbs1ΔB/ΔB
Nbs1ΔB/ΔB

Mre11 complex mislocalization in NBS and A-TLD

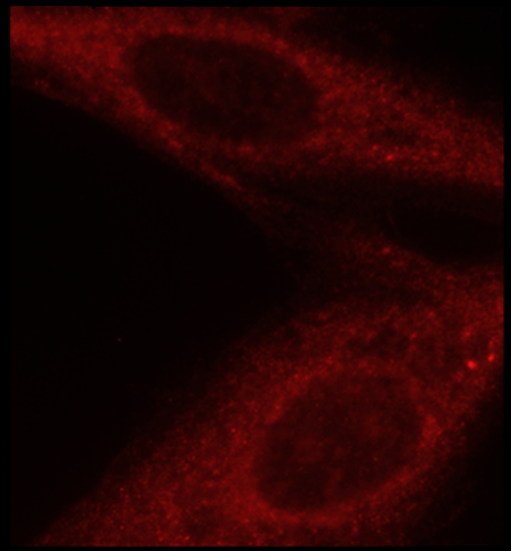
WT/Nbs1

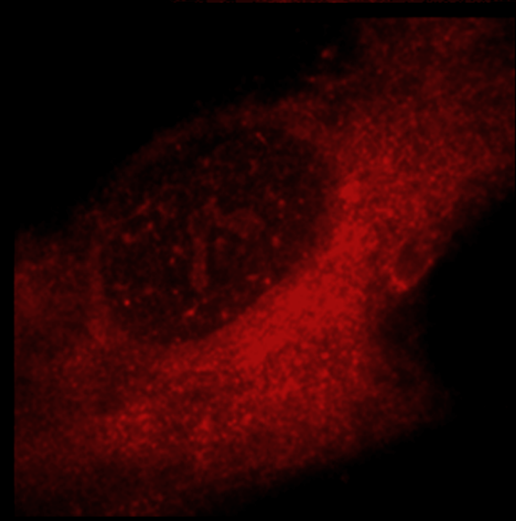


A-TLD/Nbs1



NBS/Mre11



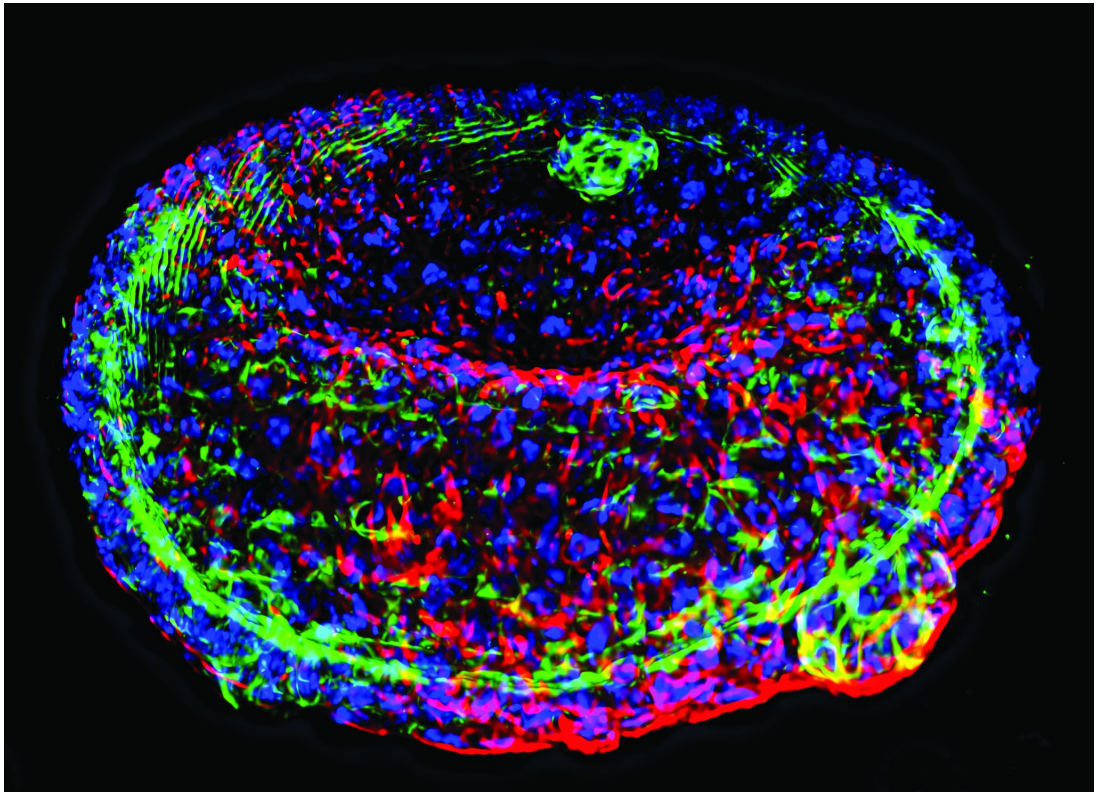


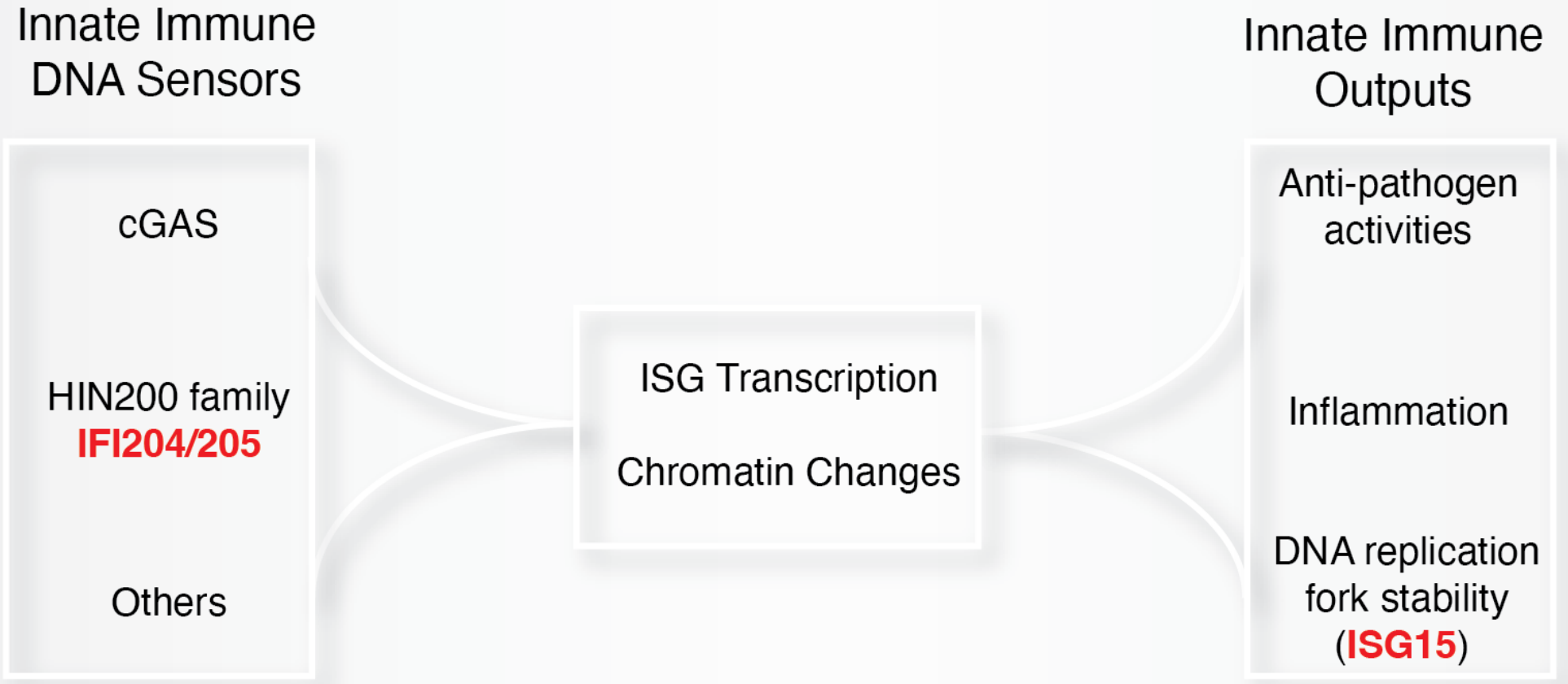
THE END OF THE INTRO

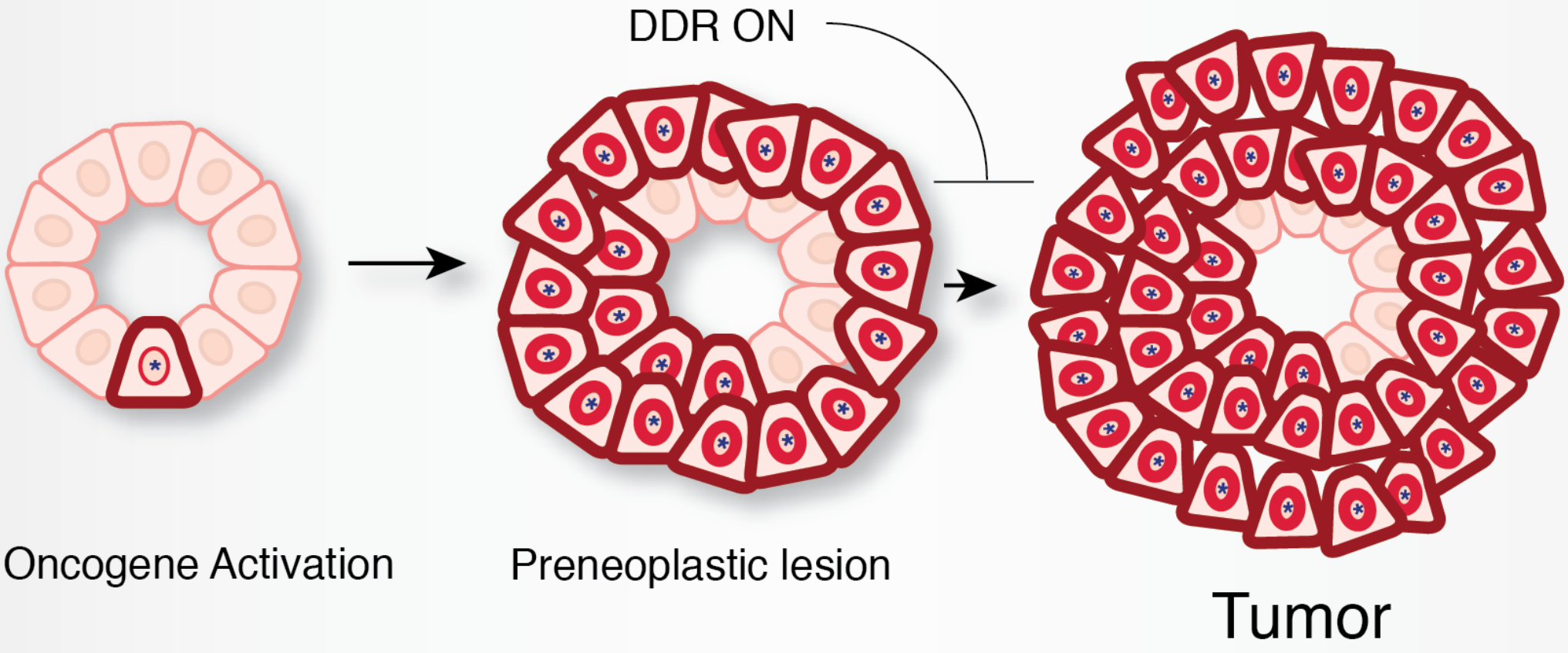
Almost

About innate immunity……

Genome Instability, Innate Immune Signaling, Oncogene Activation

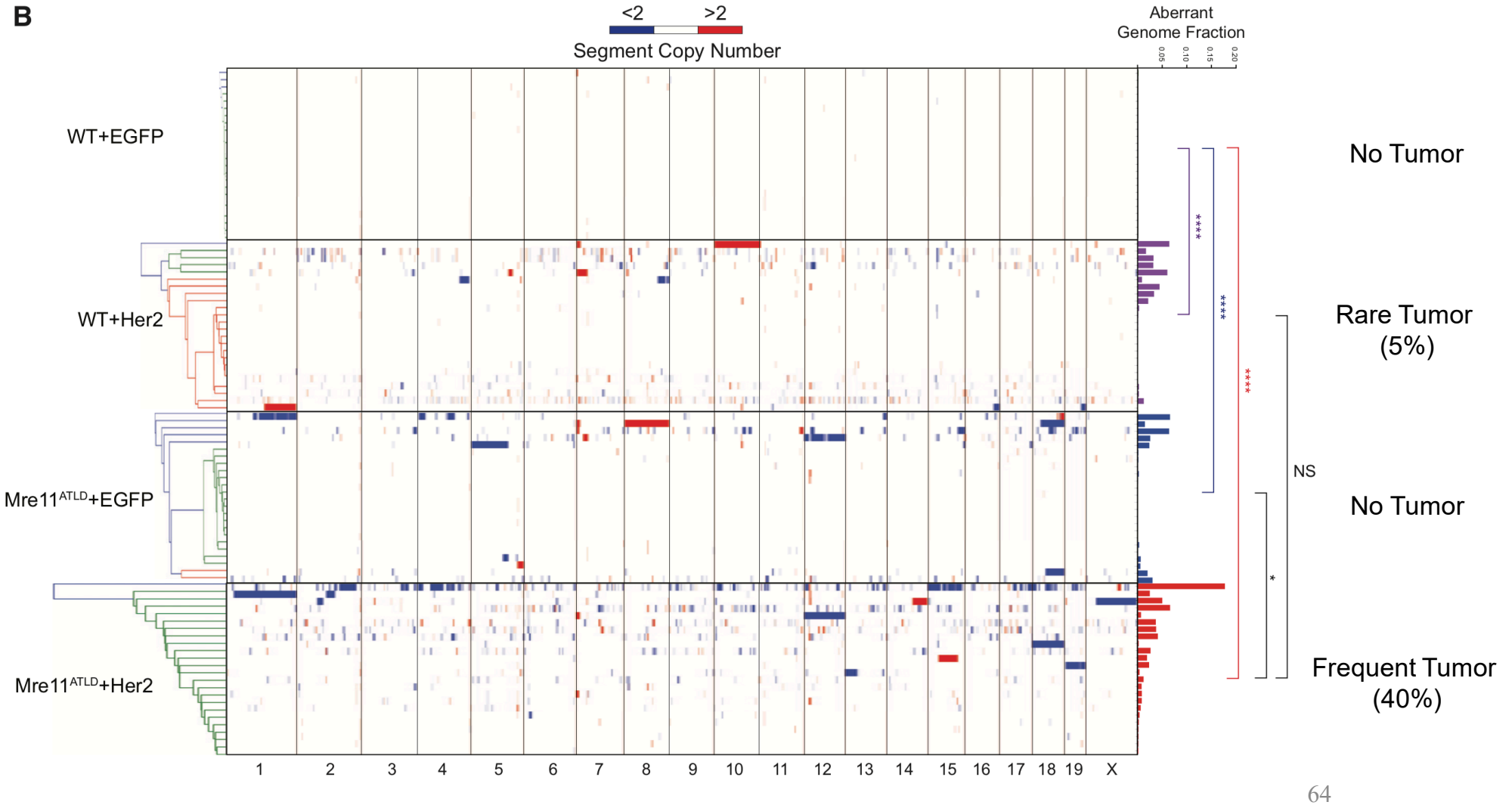



Innate Immune DNA Sensors
cGAS
HIN200 family
IFI204/205
Others
ISG Transcription
Chromatin Changes
Innate Immune Outputs
Anti-pathogen activities
Inflammation
DNA replication fork stability (ISG15)



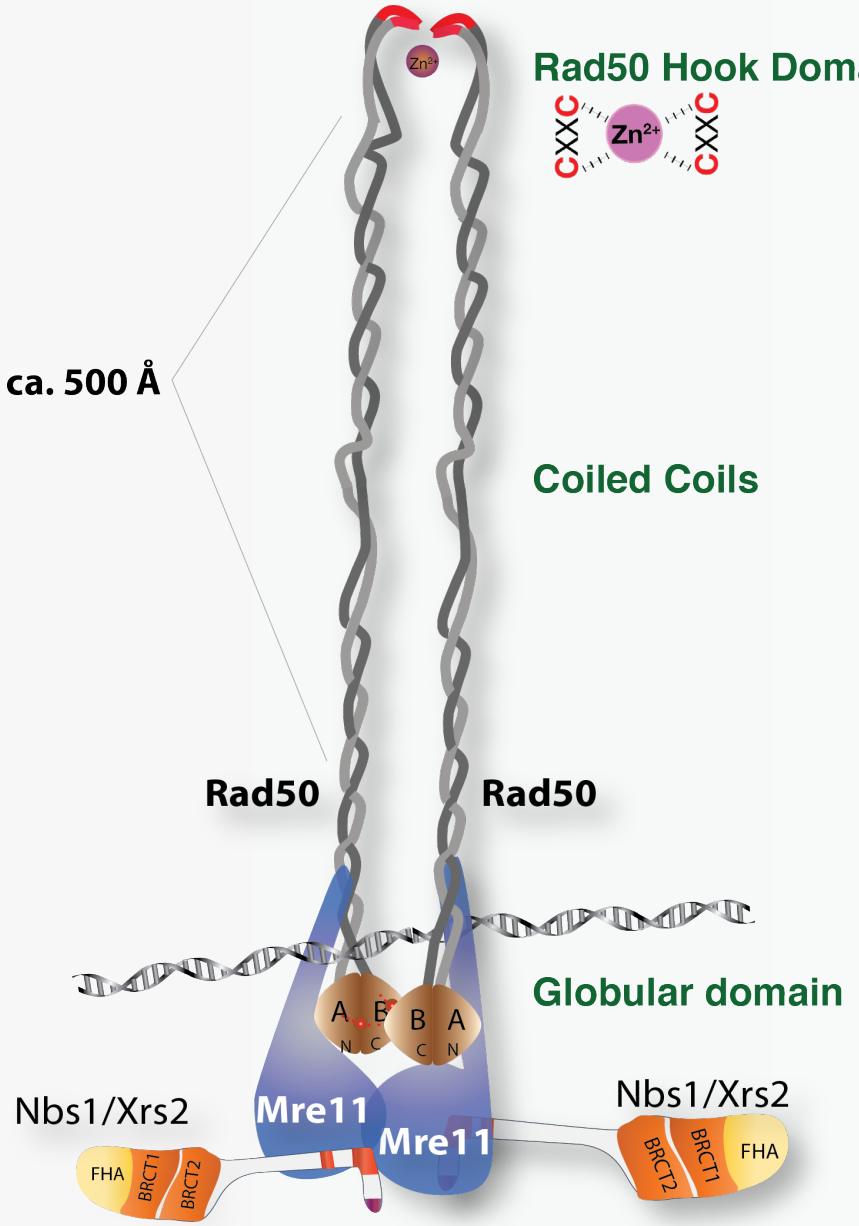
Proliferative signals
Re-replication
Collisions
ROS
Metabolic derangements

CNVs from scDNA-Seq



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

Mre11 complex's Architecture and Functions



1° Sensor of DNA damage

Required for ATM activation

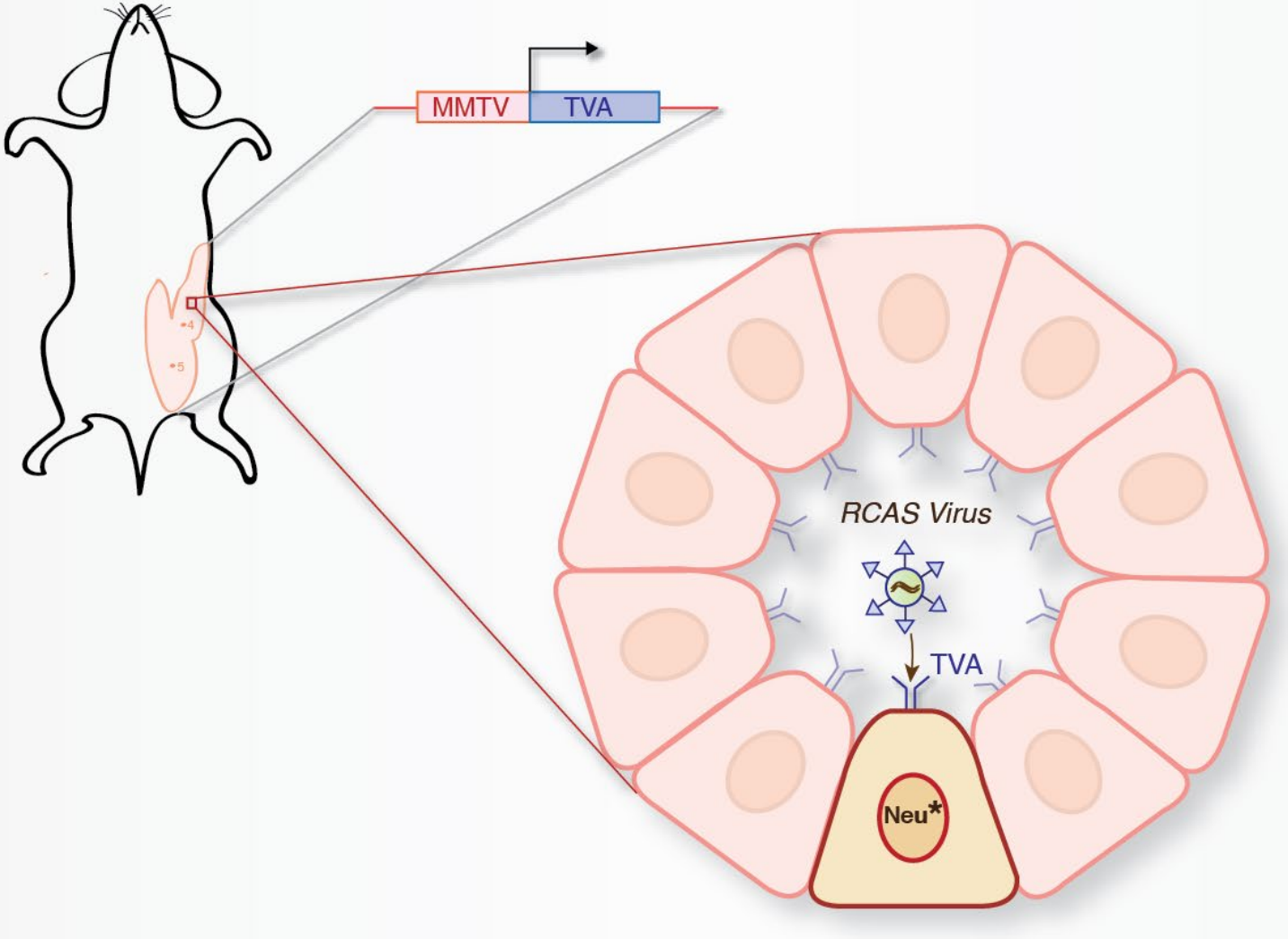
Required for DNA DSB repair

Essential for viability
(in higher eukaryotes)

The Mre11^{ATLD1} allele: Phenotypic features you need to know:

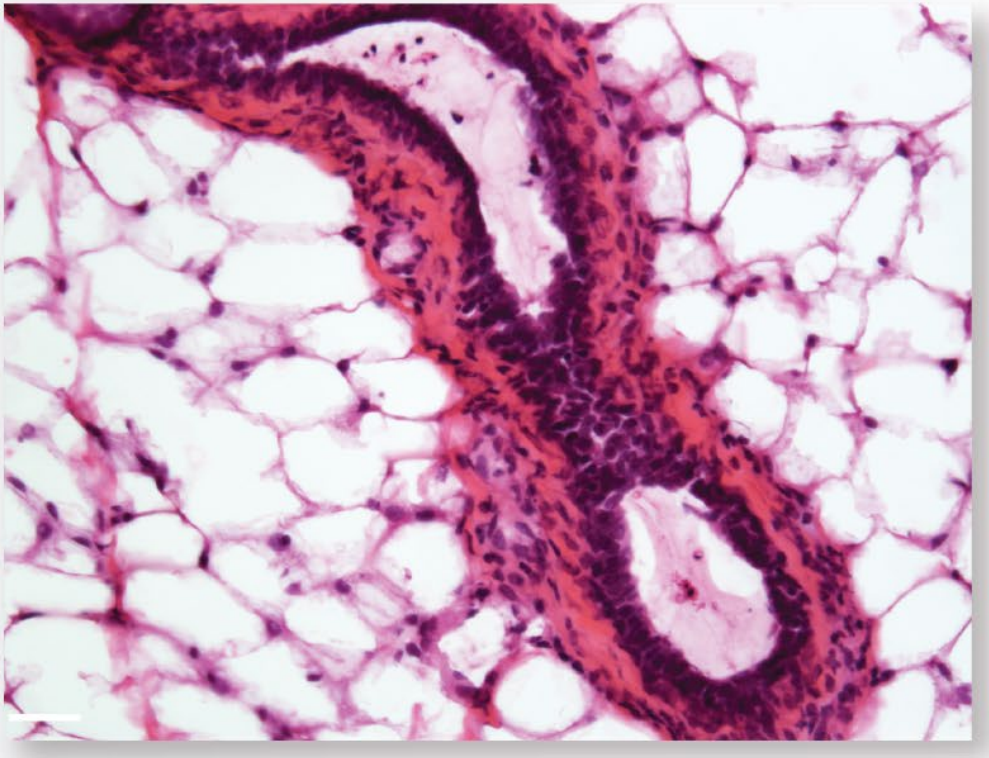
- High level of chromatid breakage, indicative of DNA replication defects
- Defects in DNA repair
- Defects in DNA damage signaling

Model for oncogene dependent breast cancer

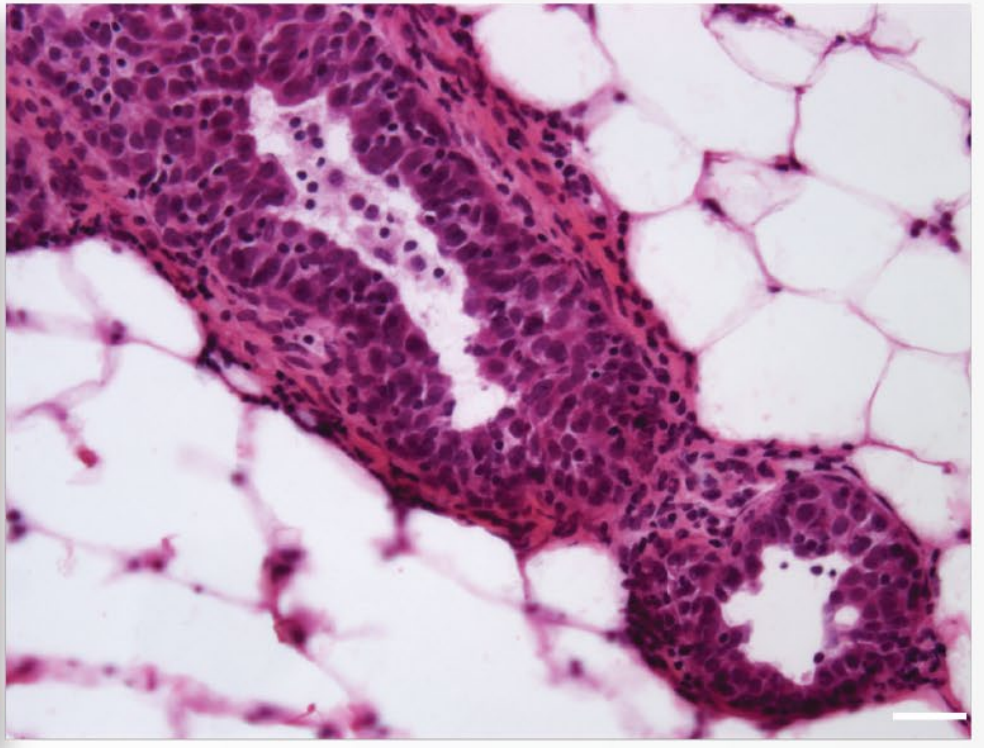


Three Week Timepoint

MMTV-TVA + RCAS-ß-Actin

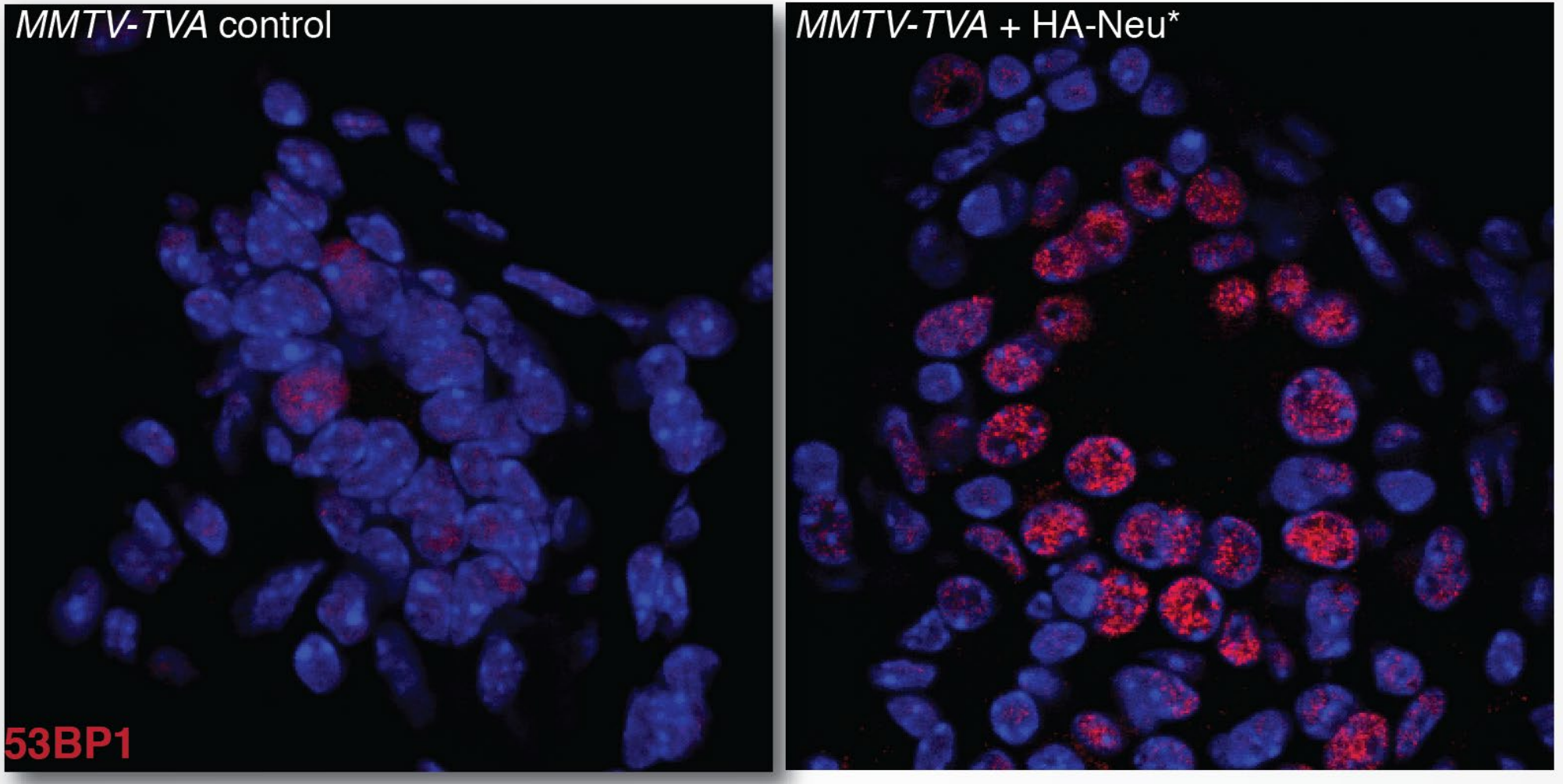


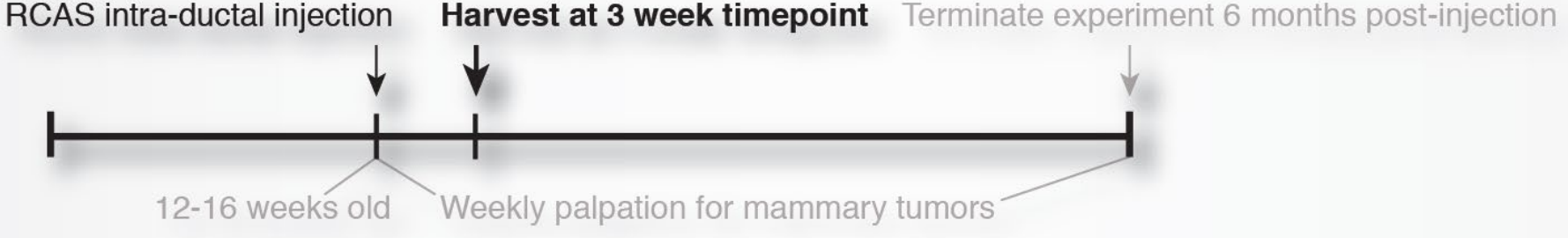
MMTV-TVA + RCAS-HA-Neu*





Oncogene-induced 53BP1 accumulation

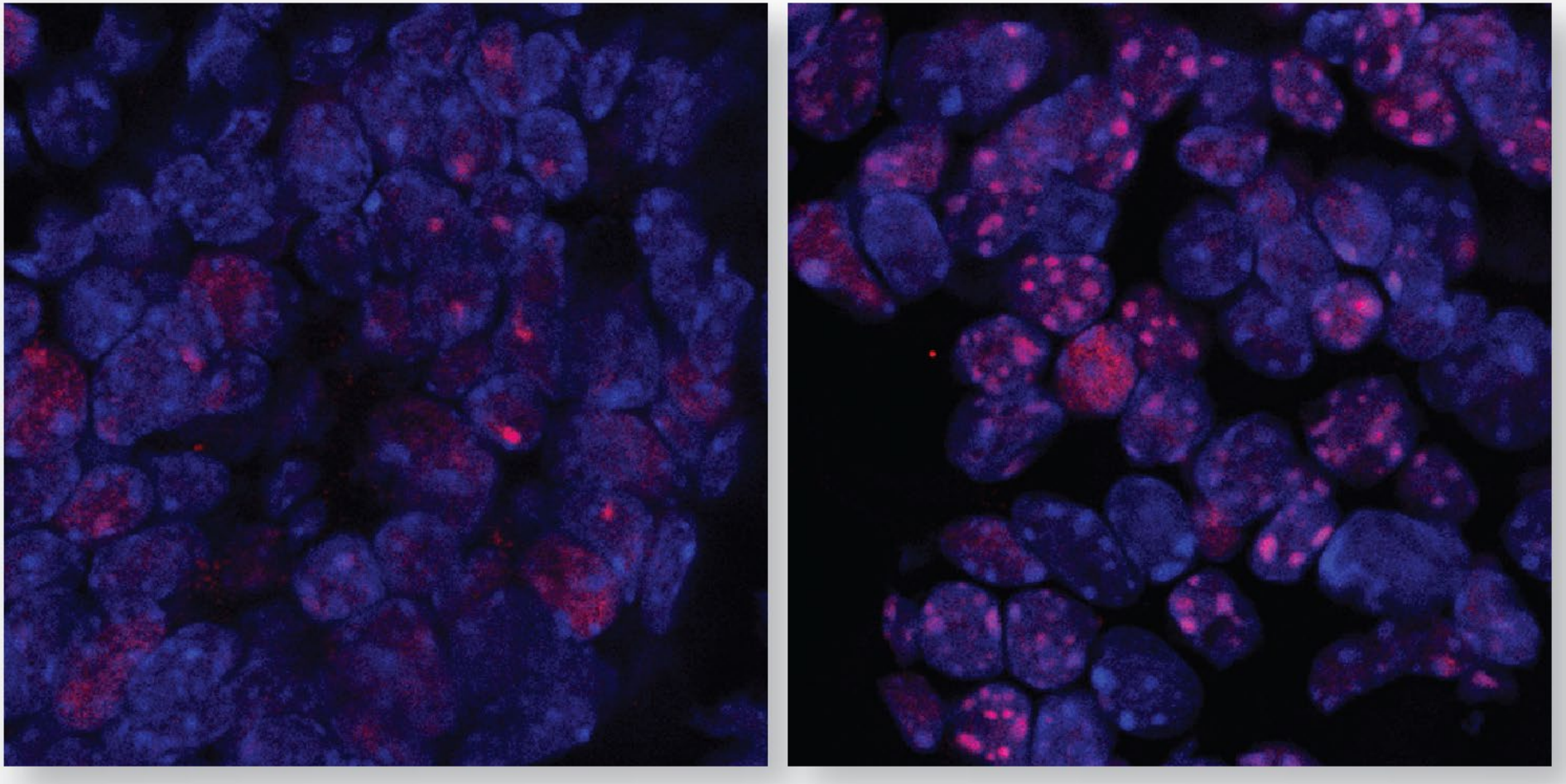




Oncogene induced heterochromatic changes

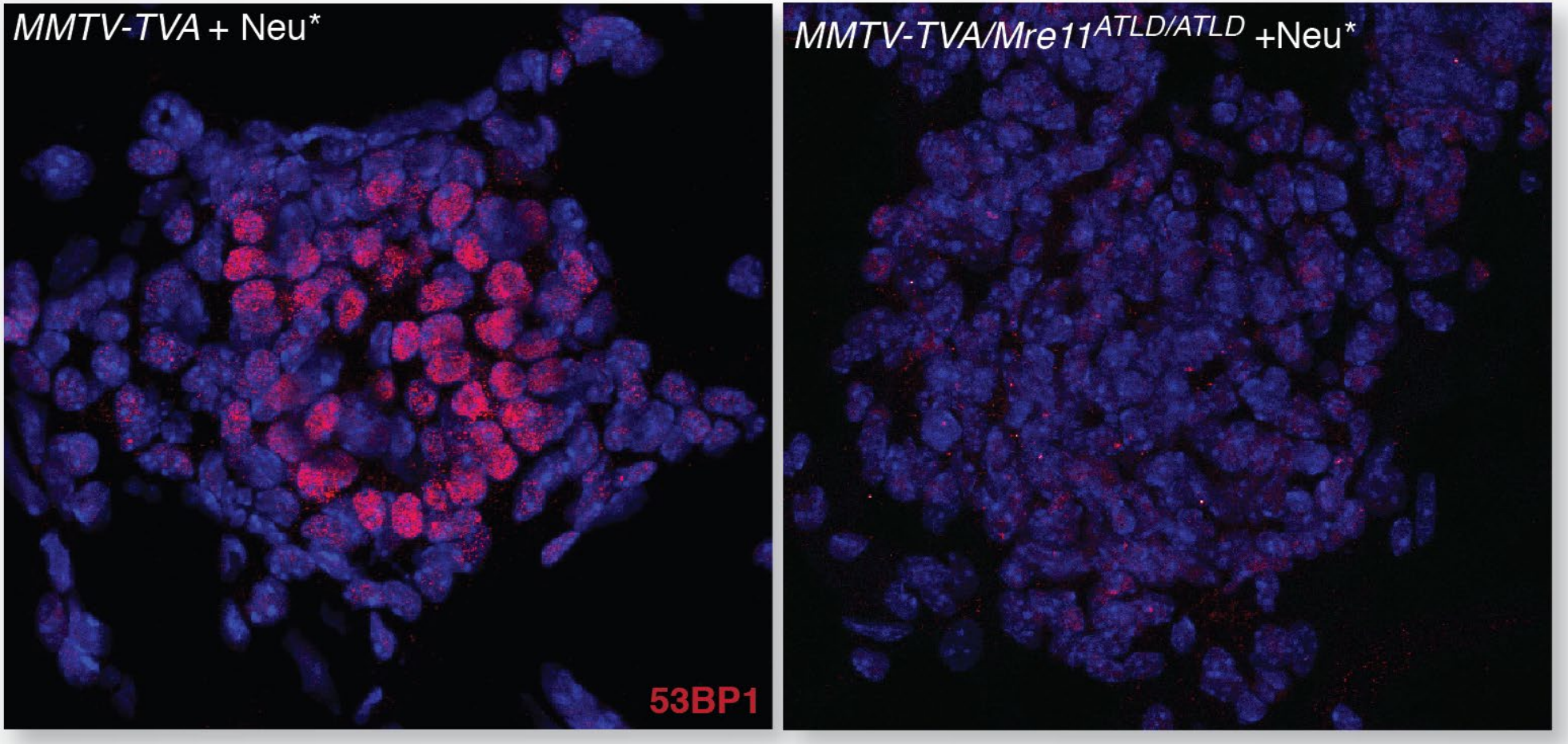
MMTV-TVA

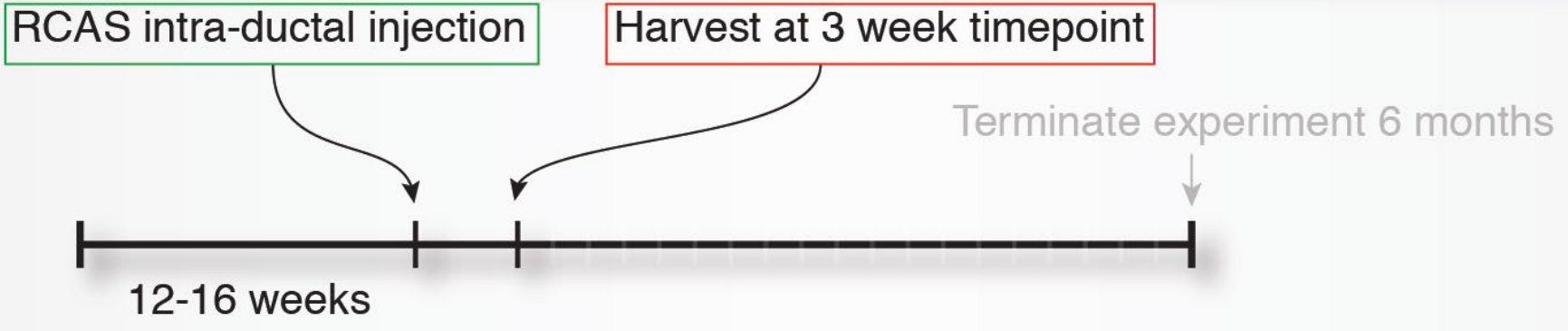
MMTV-TVA + Neu*



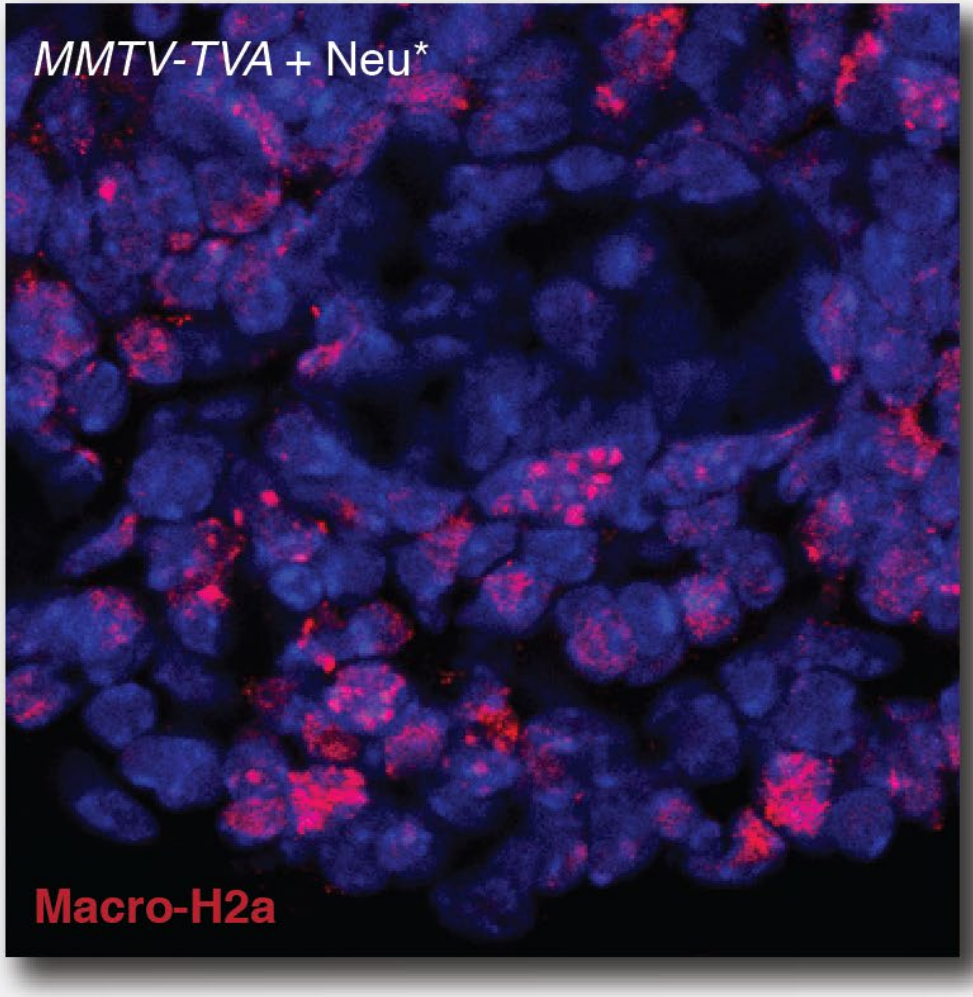
macroH2A

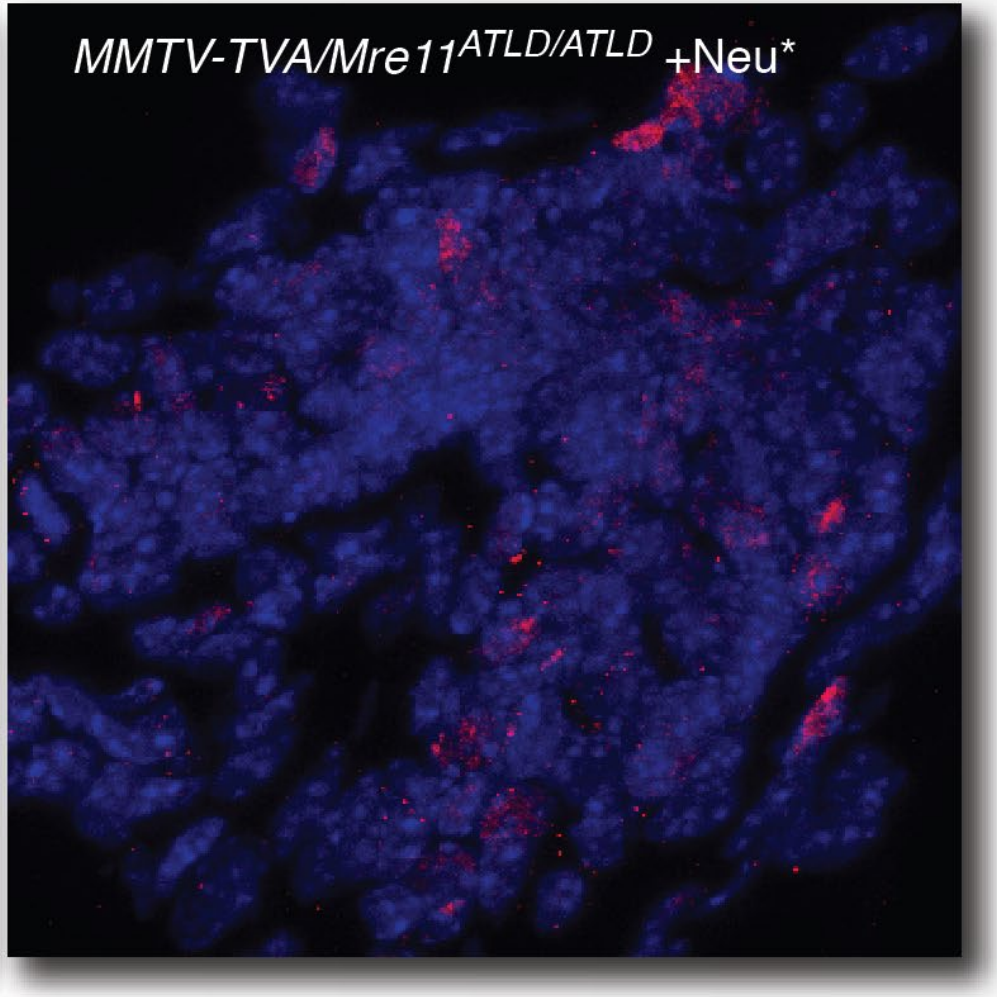
Mre11$^{ATLD1/ATLD1}$: reduction in oncogene induced DDR

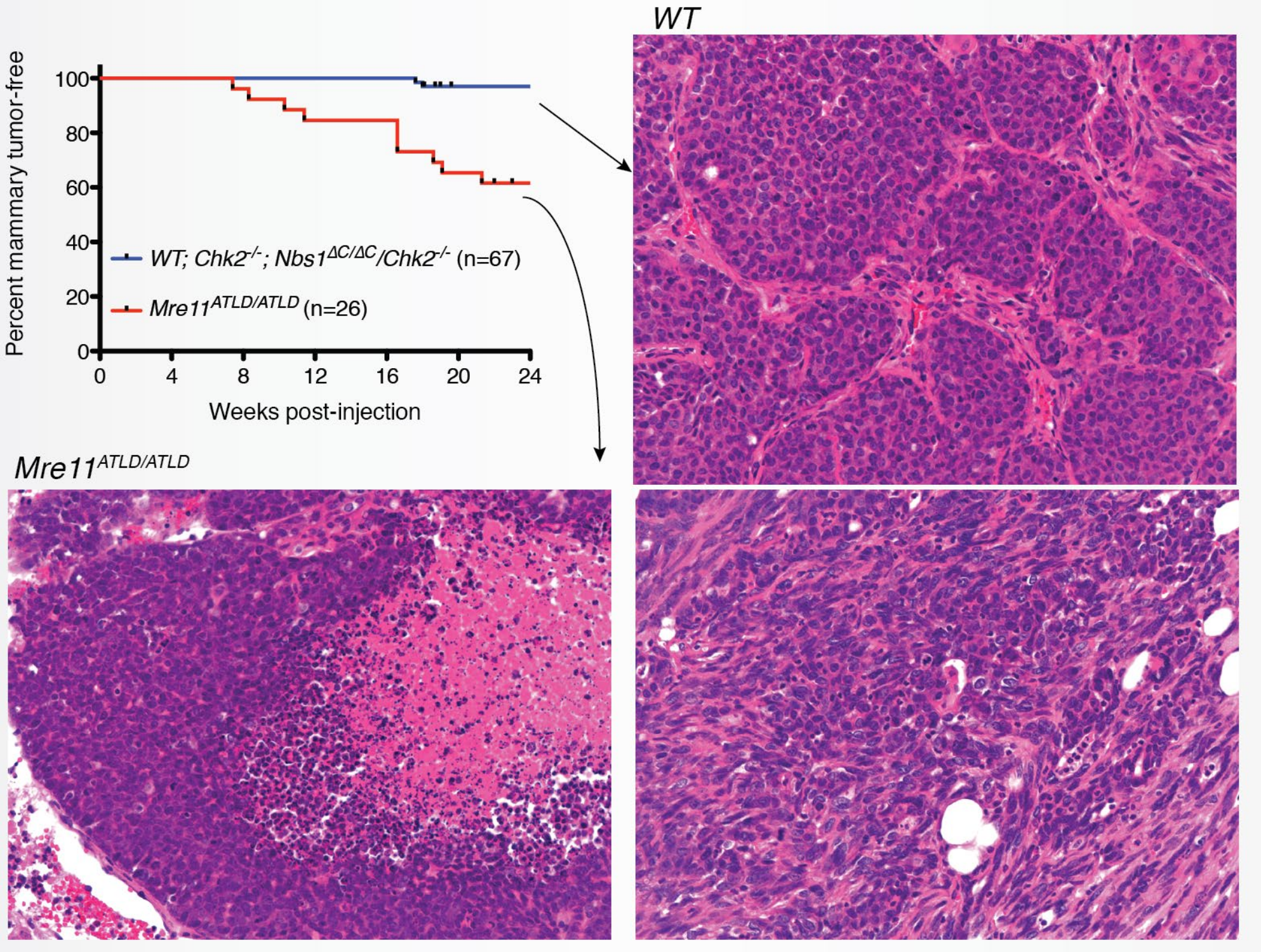




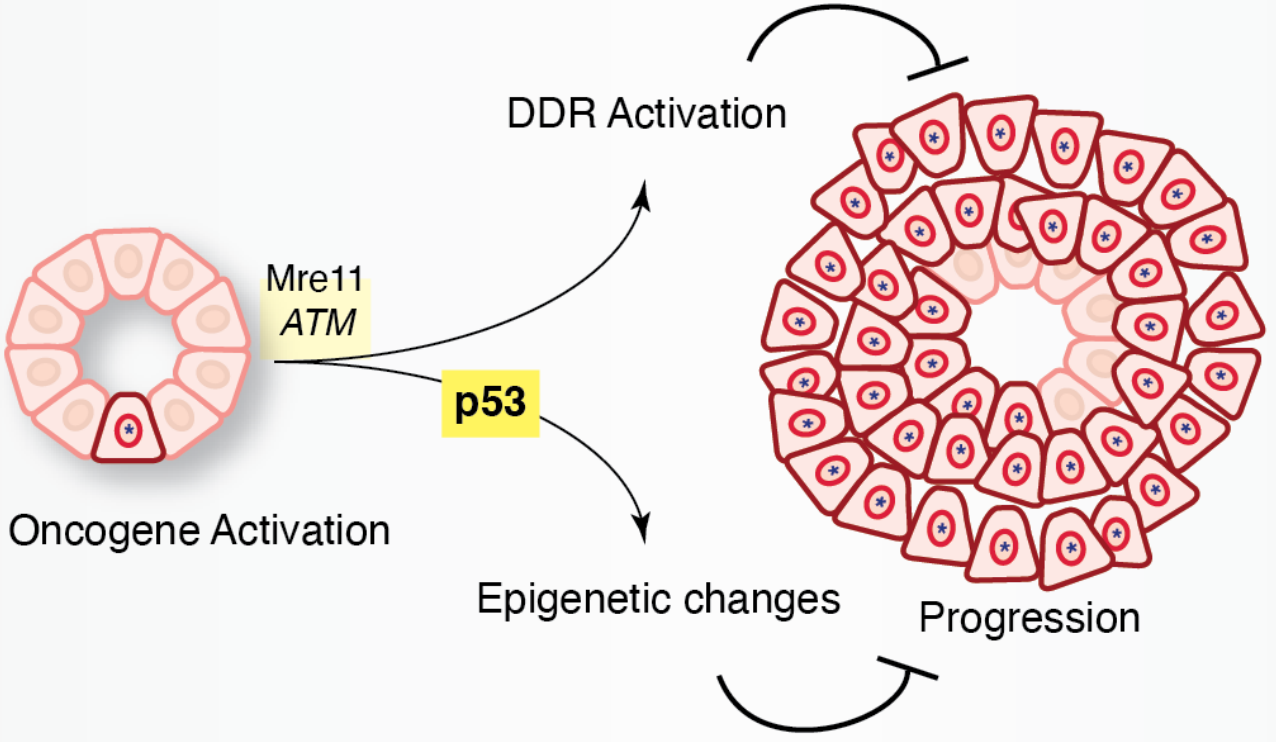
Reduction in heterochromatic changes in *Mre11*$^{ATLD1/ATLD1}$



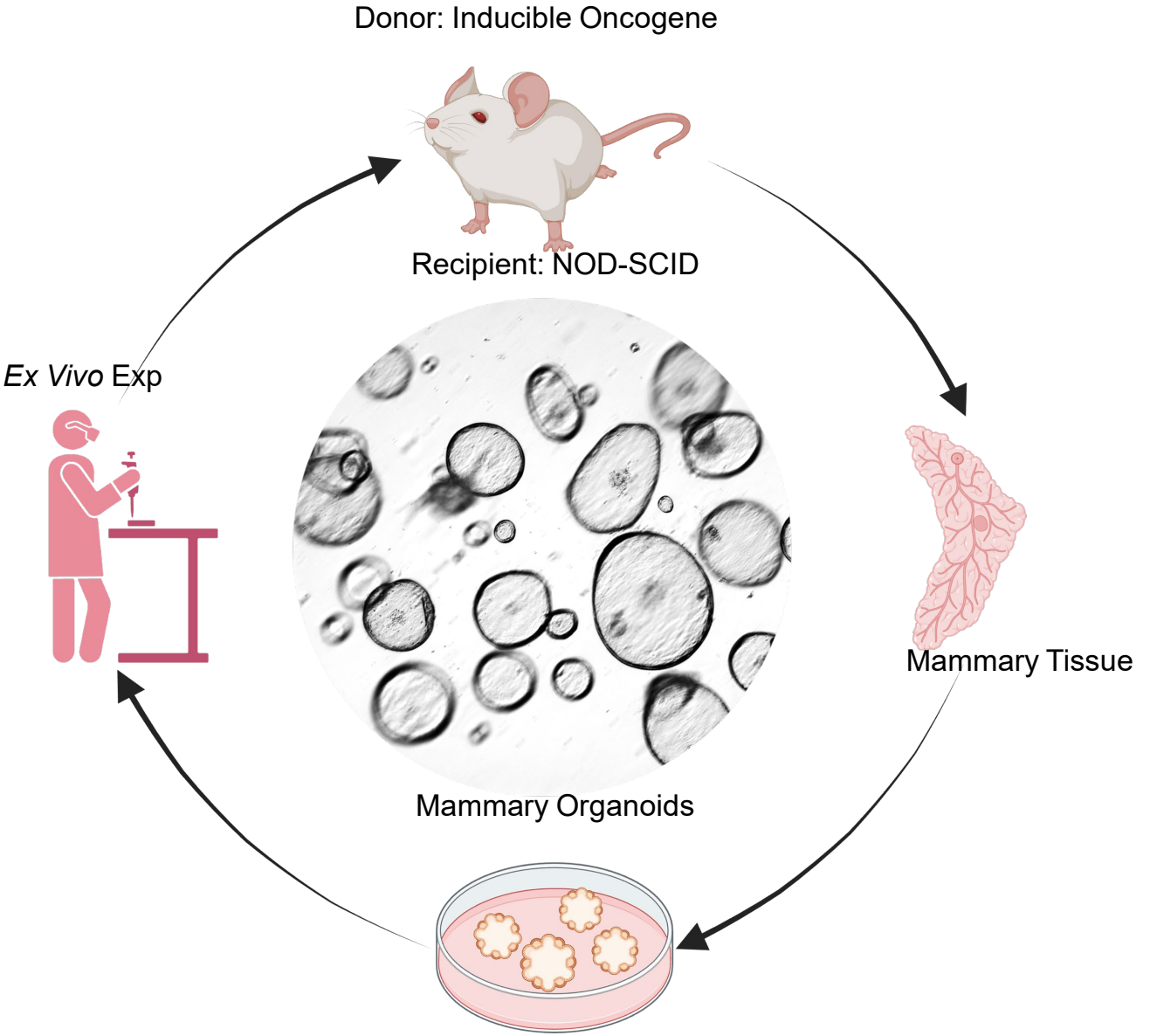


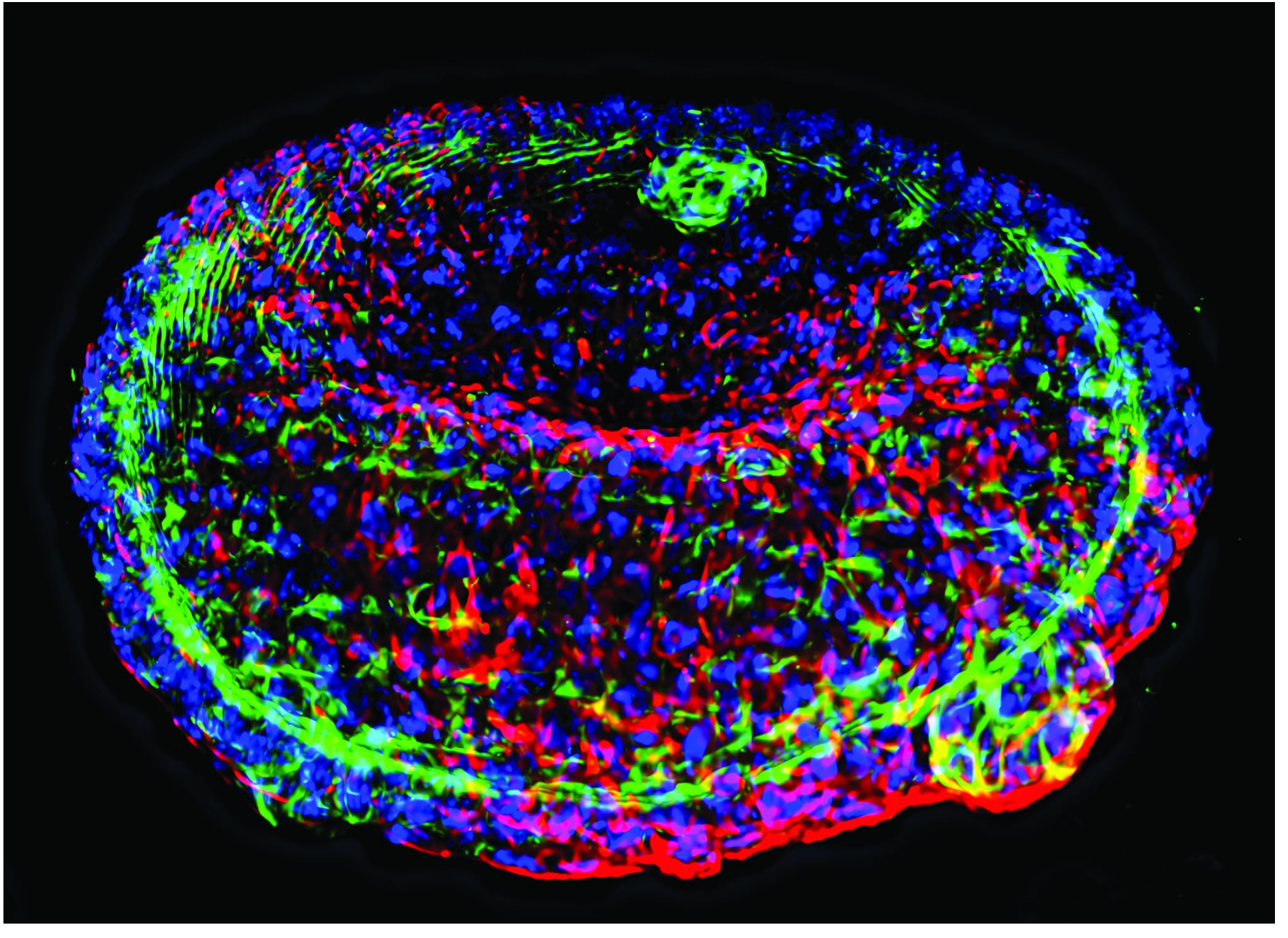

Percent mammary tumor-free
100
80
60
40
20
0
0
4
8
12
16
20
24
Weeks post-injection
WT; Chk2-/-; Nbs1ΔC/ΔC/Chk2-/- (n=67)
Mre11ATLD/ATLD (n=26)
WT
Mre11ATLD/ATLD

Mre11-p53 Signaling Axis

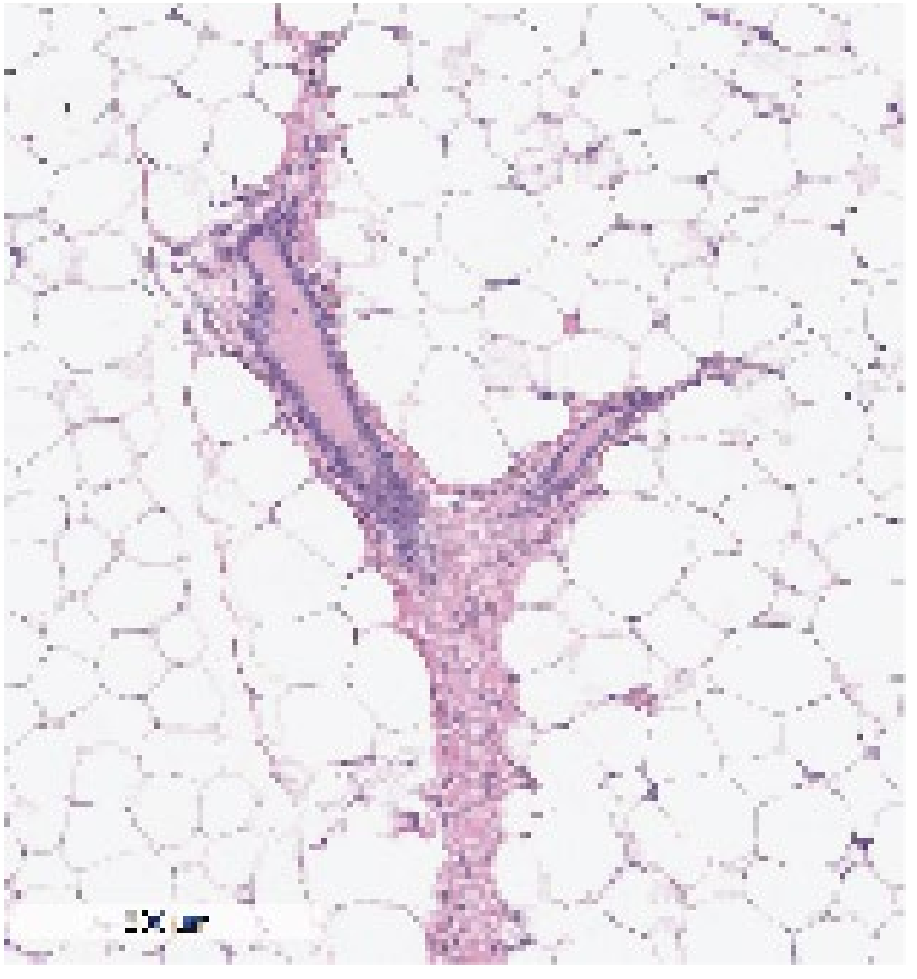


Mammary Organoid System

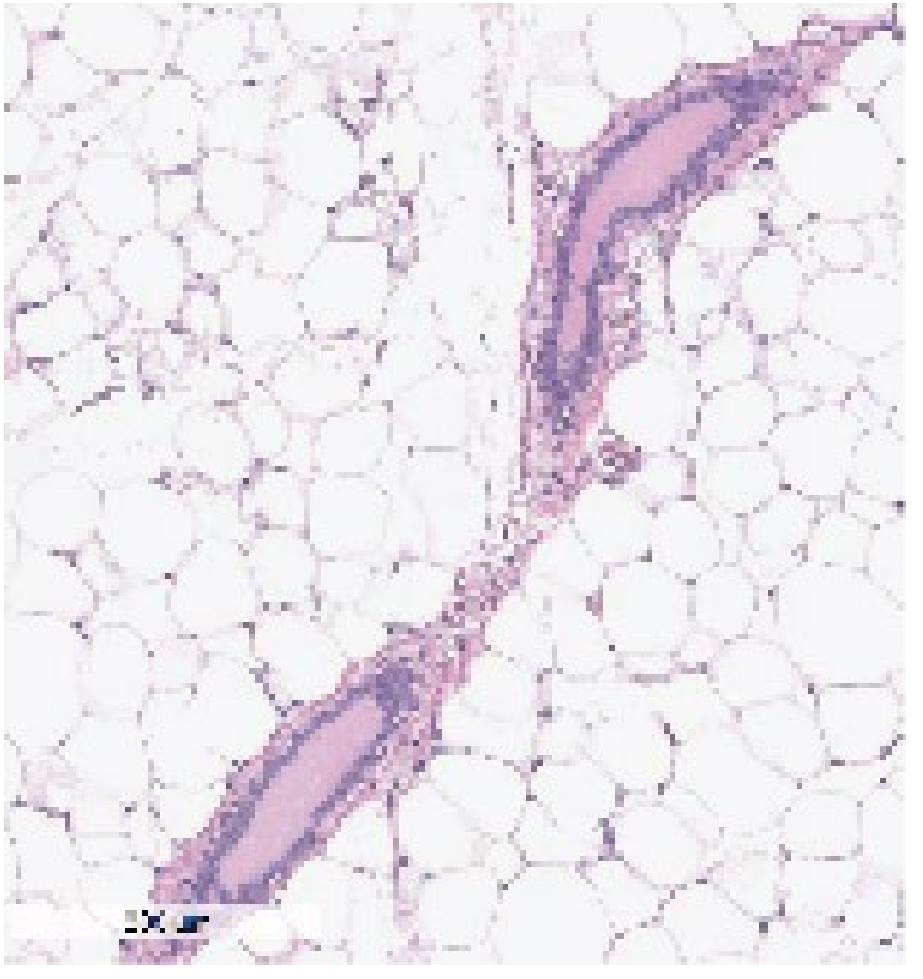




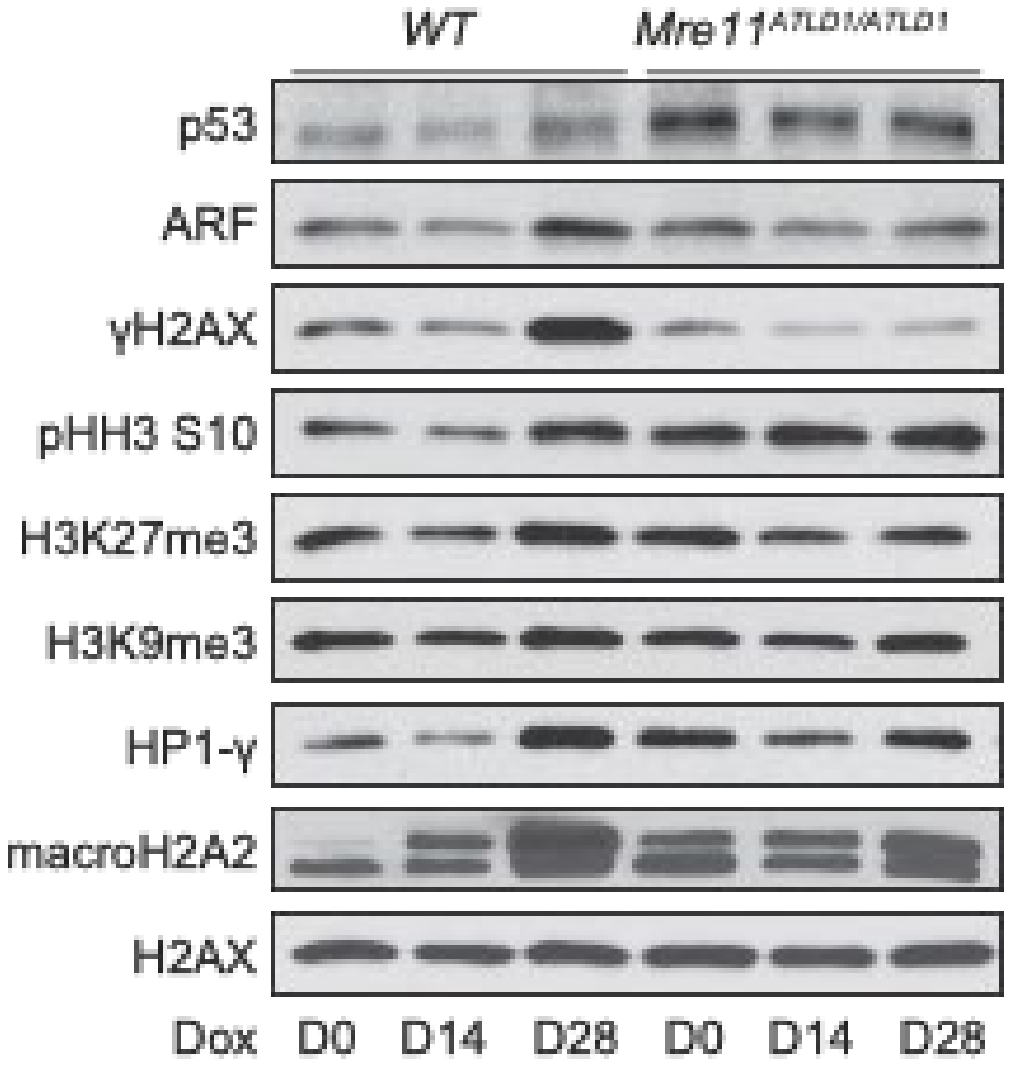
WT



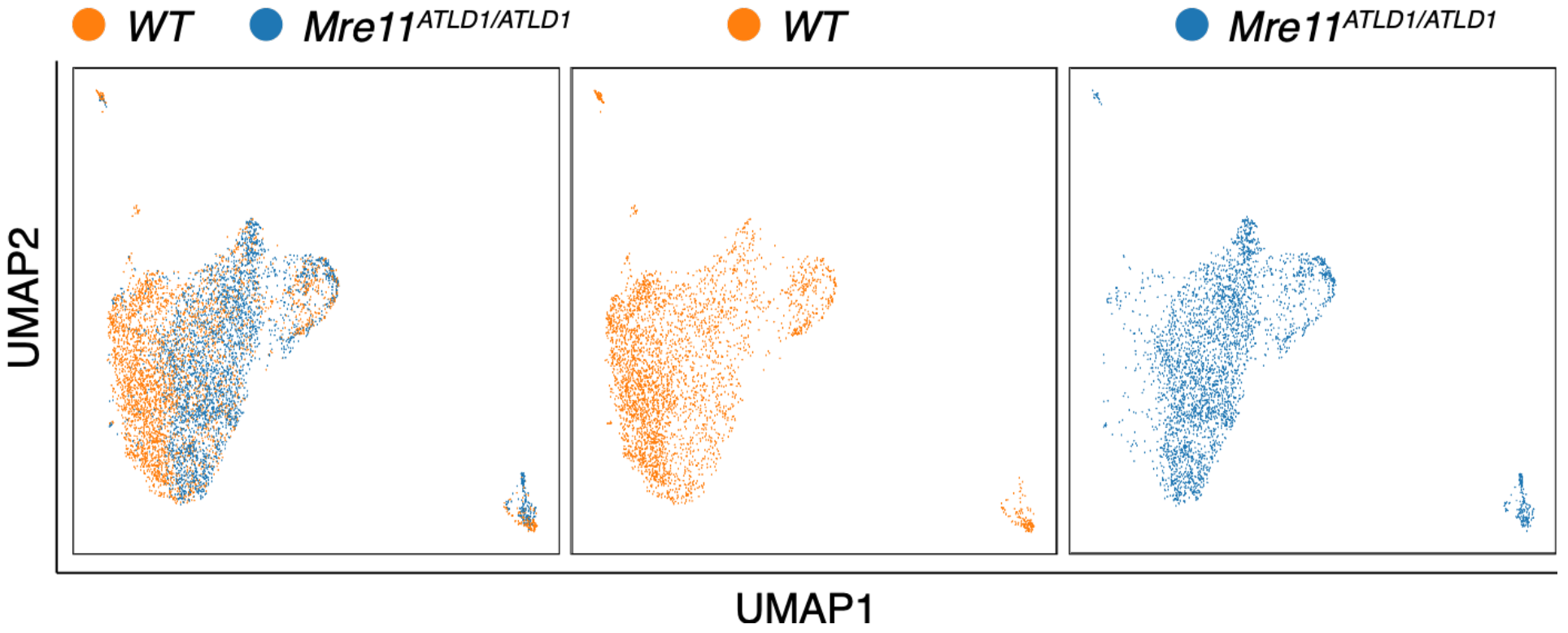
Mre11$^{ATLD1/ATLD1}$



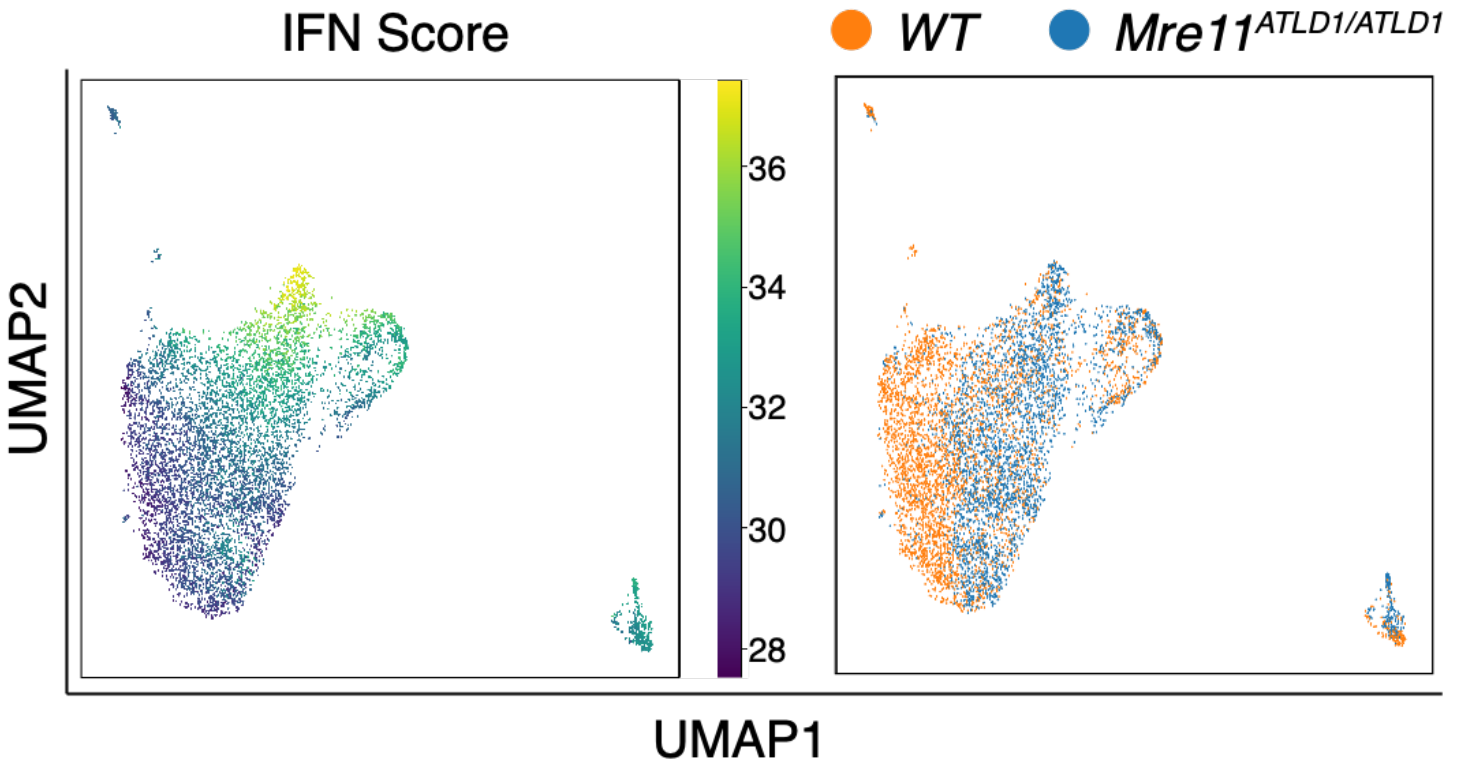
Organoid Response to Oncogene Activation



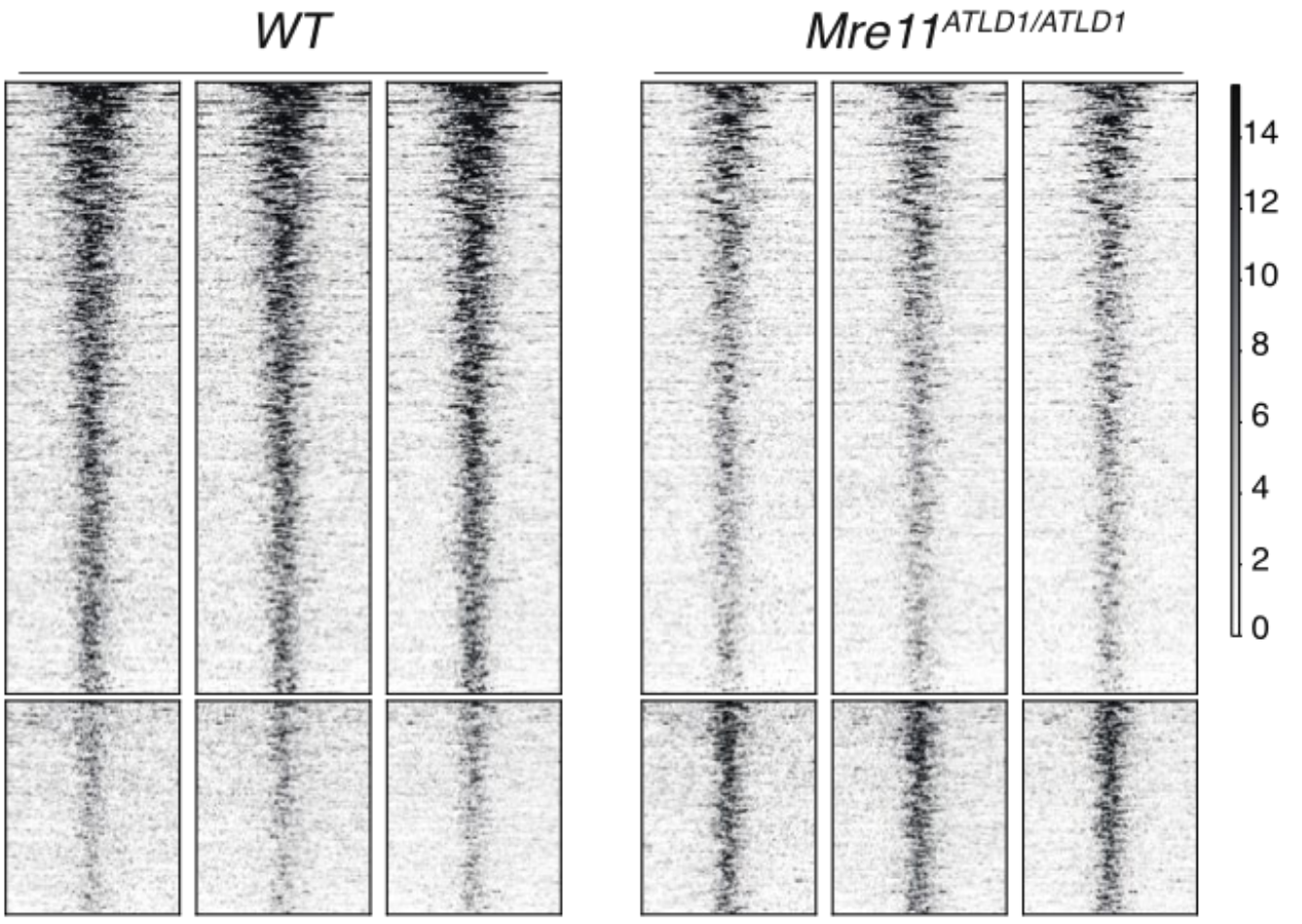
Mre11$^{ATLD1/ATLD1}$ Organoids are Slightly Different from *WT*



Interferon Signaling is More Active in *Mre11*$^{ATLD1/ATLD1}$



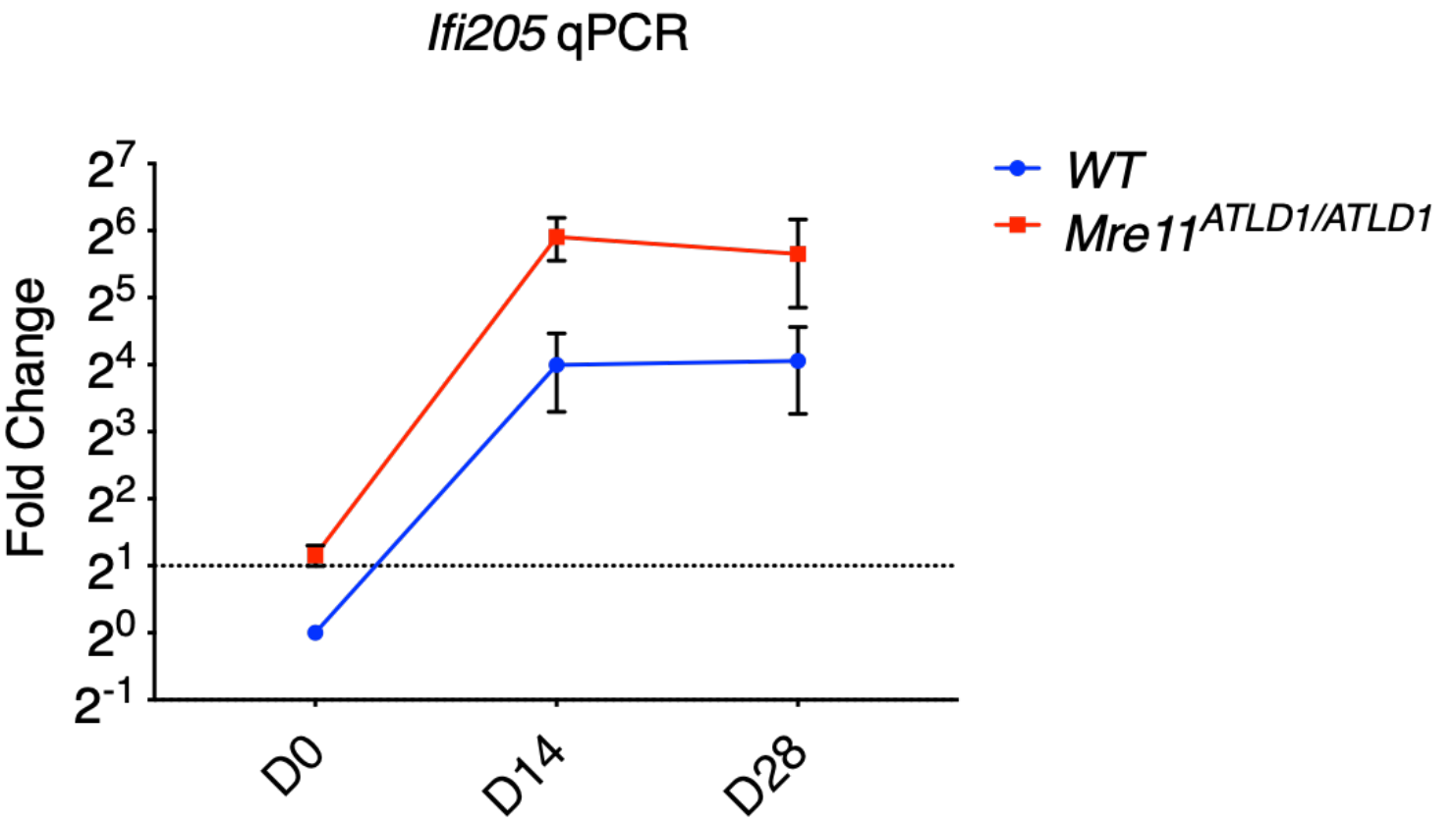
A Different Chromatin Status in *Mre11*$^{ATLD1/ATLD1}$



This is not due to cytoplasmic DNA nor does
It depend on cGAS…..

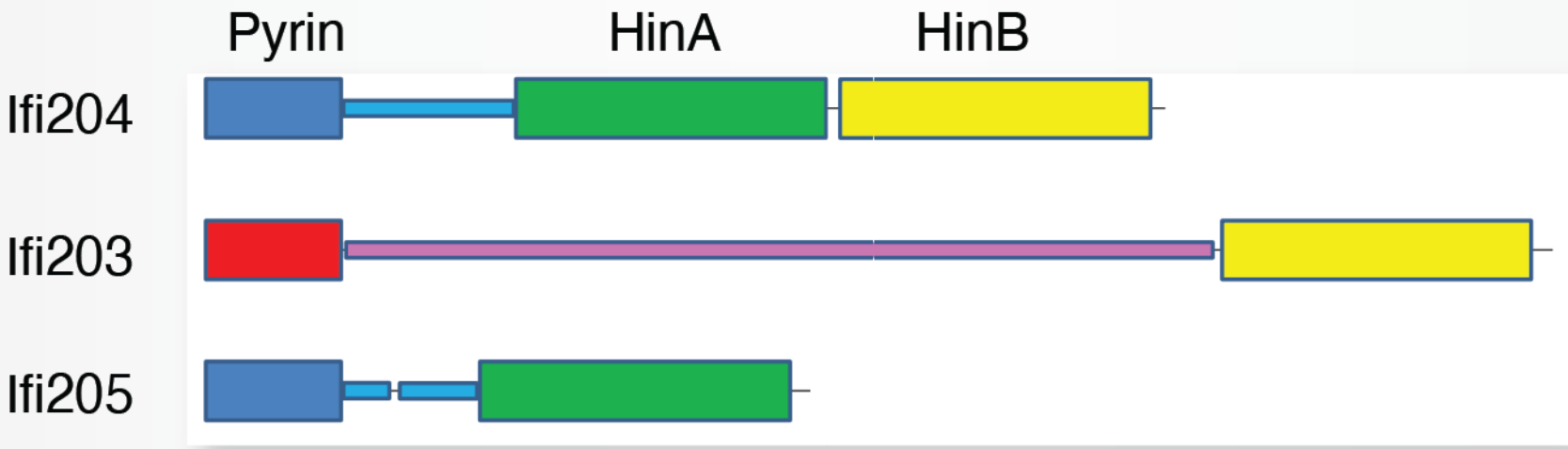
MSK Confidential — do not distribute

IFI205 is a Nuclear DNA Sensor



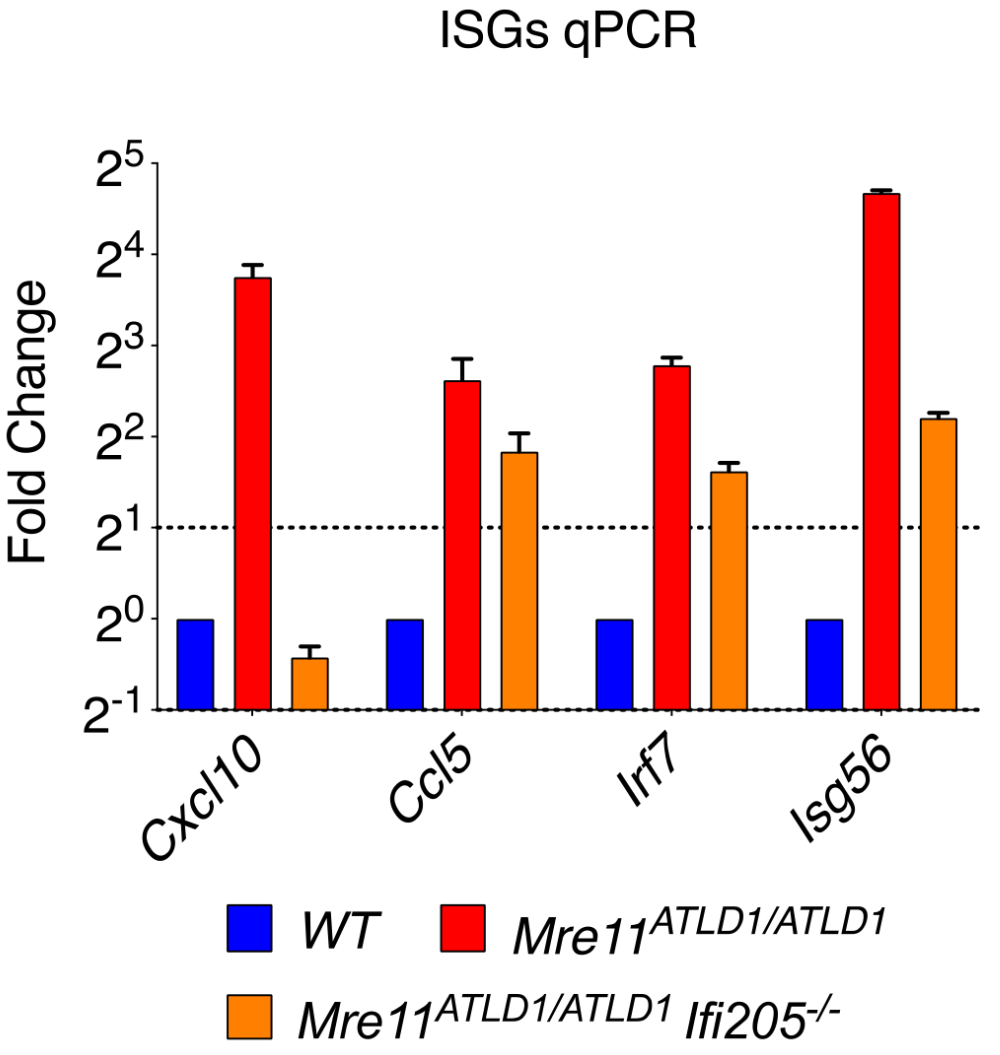
HIN200 Family Innate Immune Receptors

- Nuclear localization
- DSB DNA binding via HIN domains

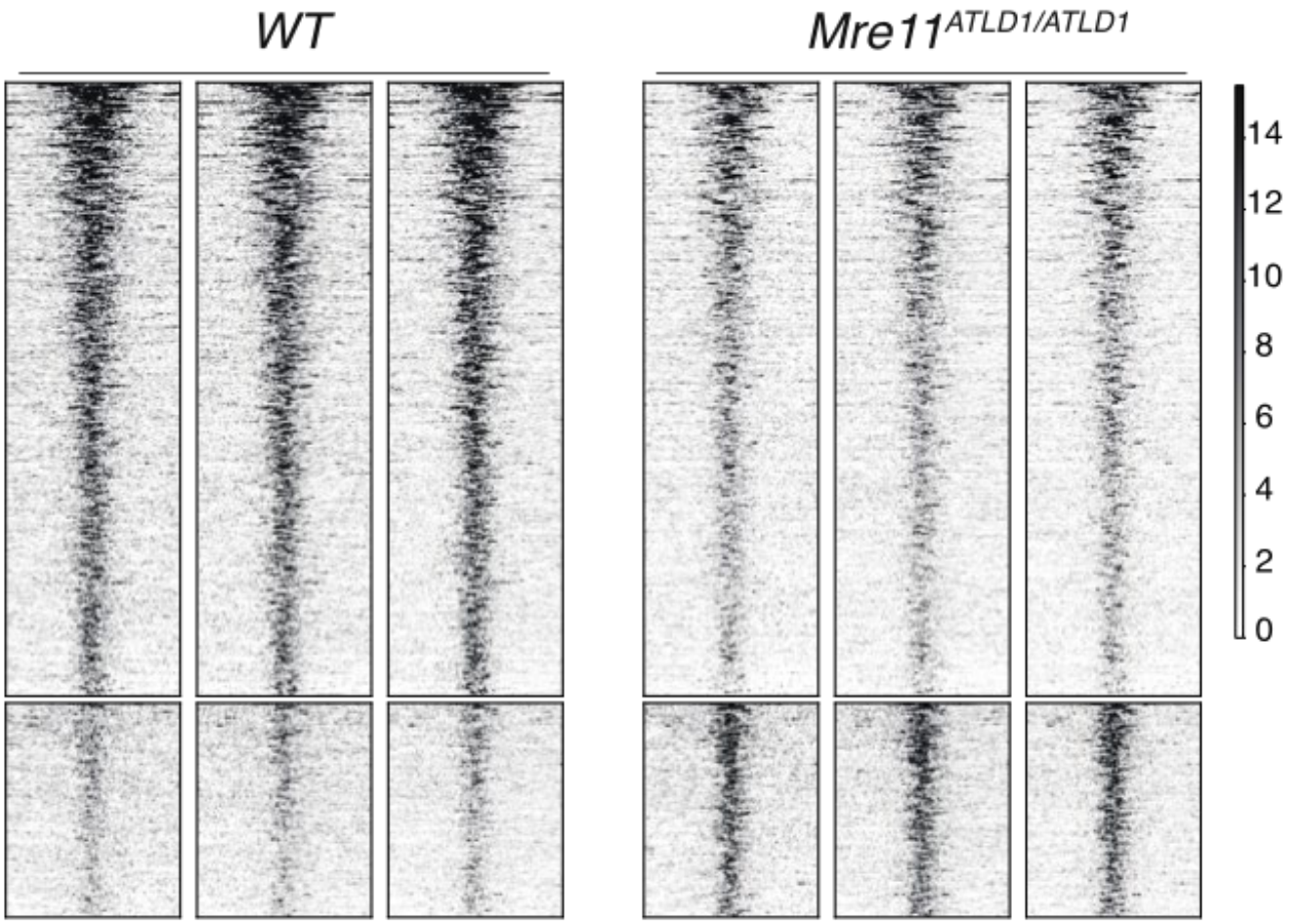


doi:10.1128/mBio.00944 -17 (2017).

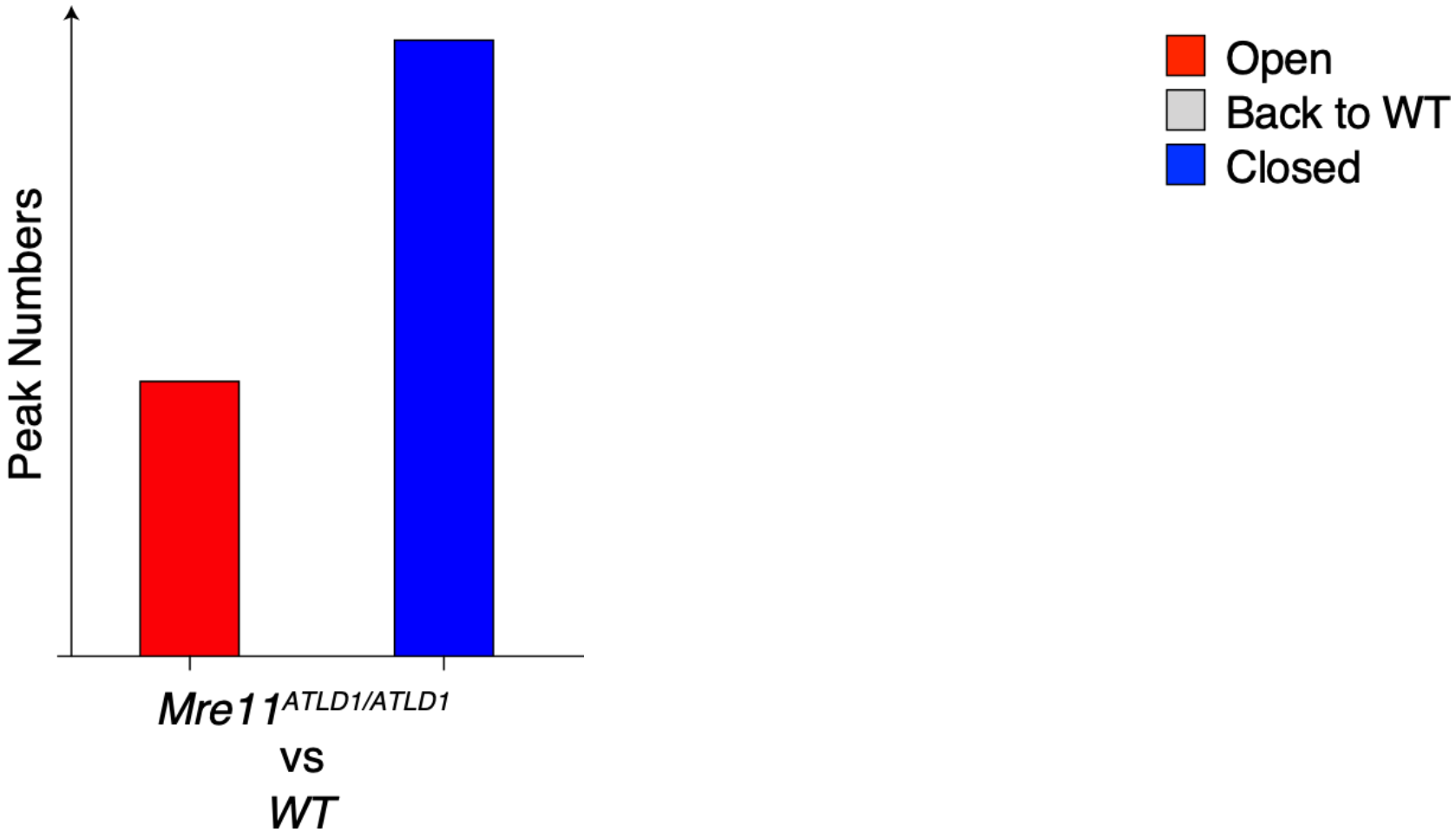
Ifi205 KO Inhibits Interferon Signaling



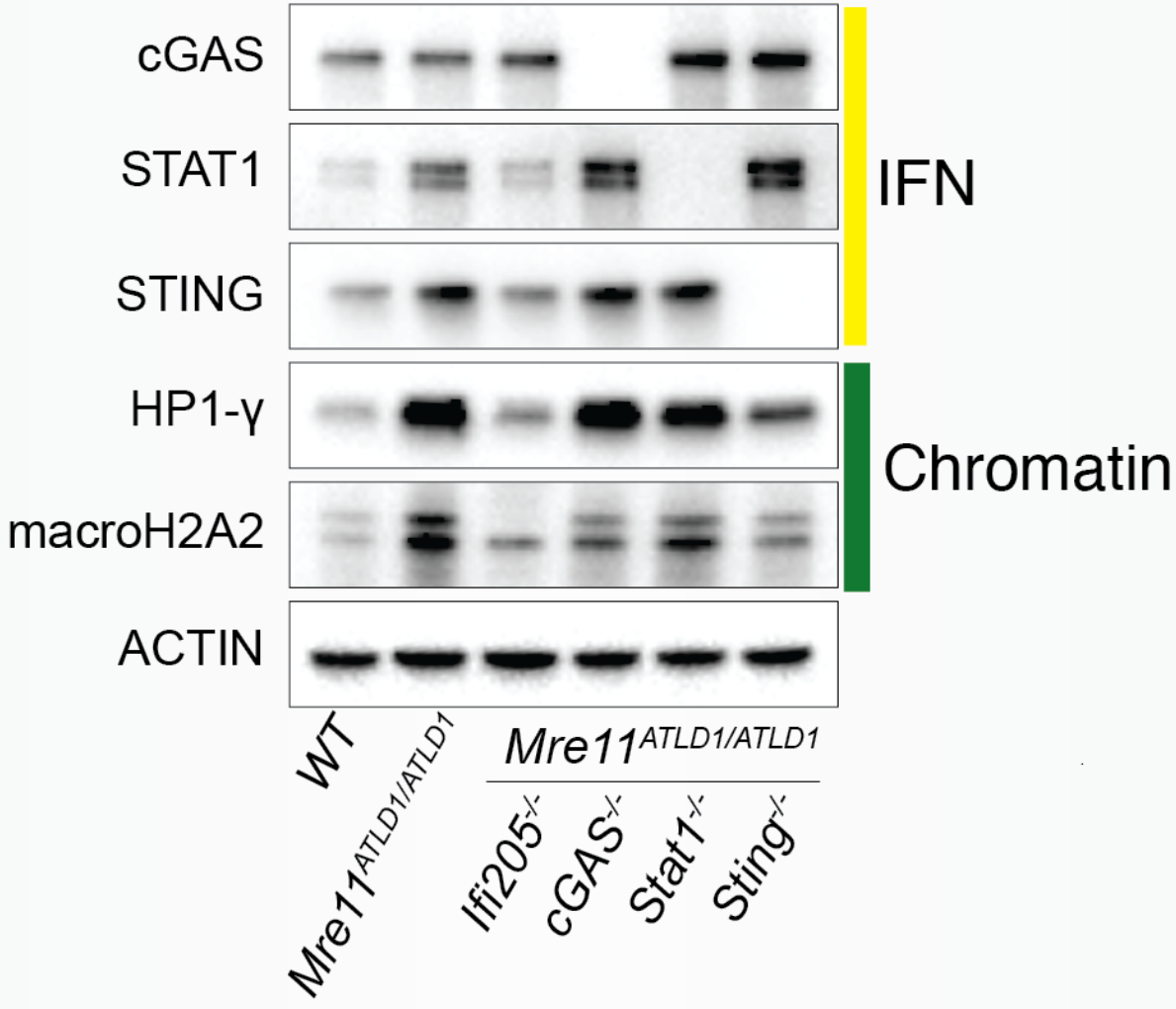
A Different Chromatin Status in *Mre11*$^{ATLD1/ATLD1}$



Mre11$^{ATLD1/ATLD1}$ *Ifi205*$^{-/-}$ Normalizes Chromatin Accessibility in *Mre11*$^{ATLD1/ATLD1}$



IFN Signaling and Chromatin Changes are IFI205 dependent



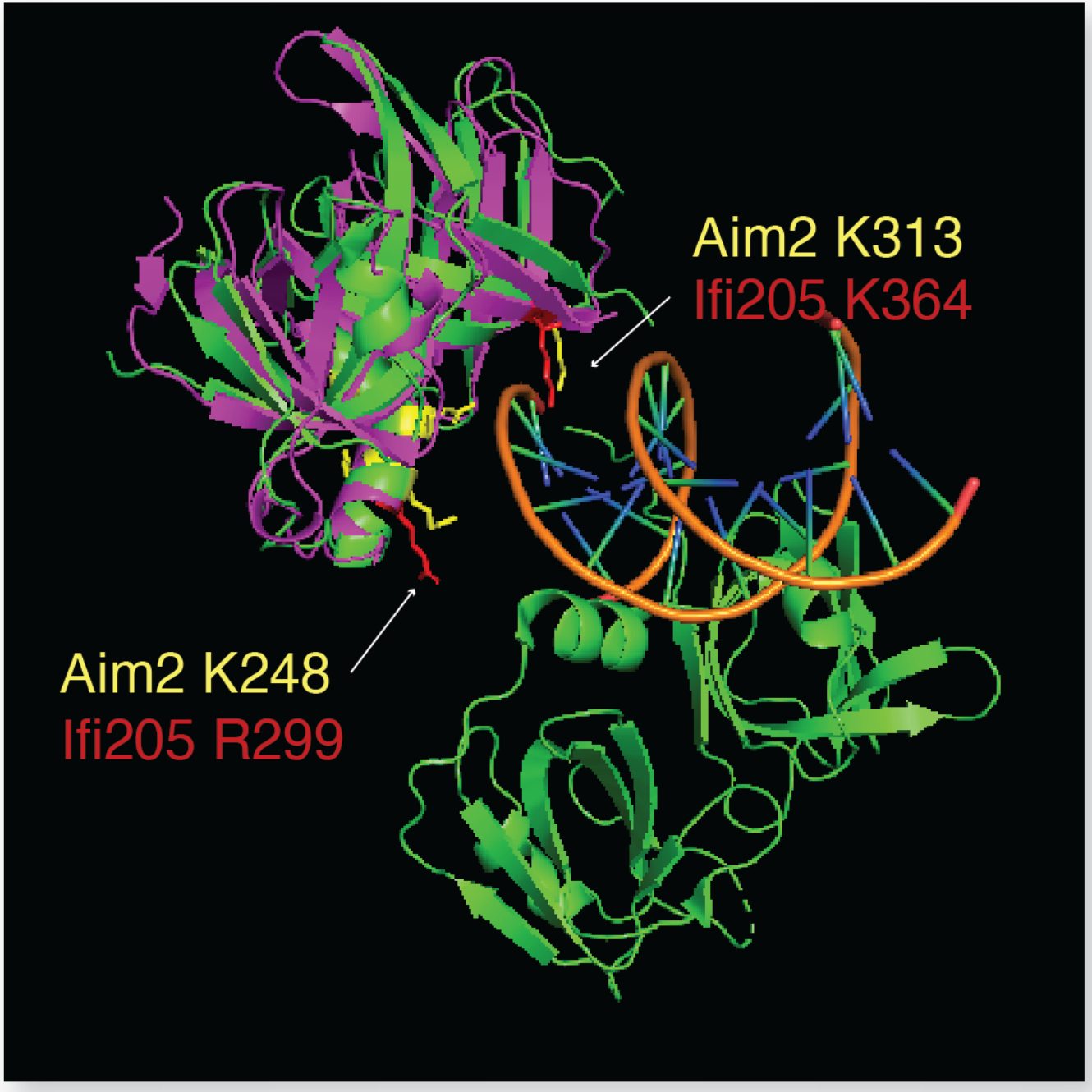
The Mre11^{ATLD1} allele: Phenotypic features you need to know:

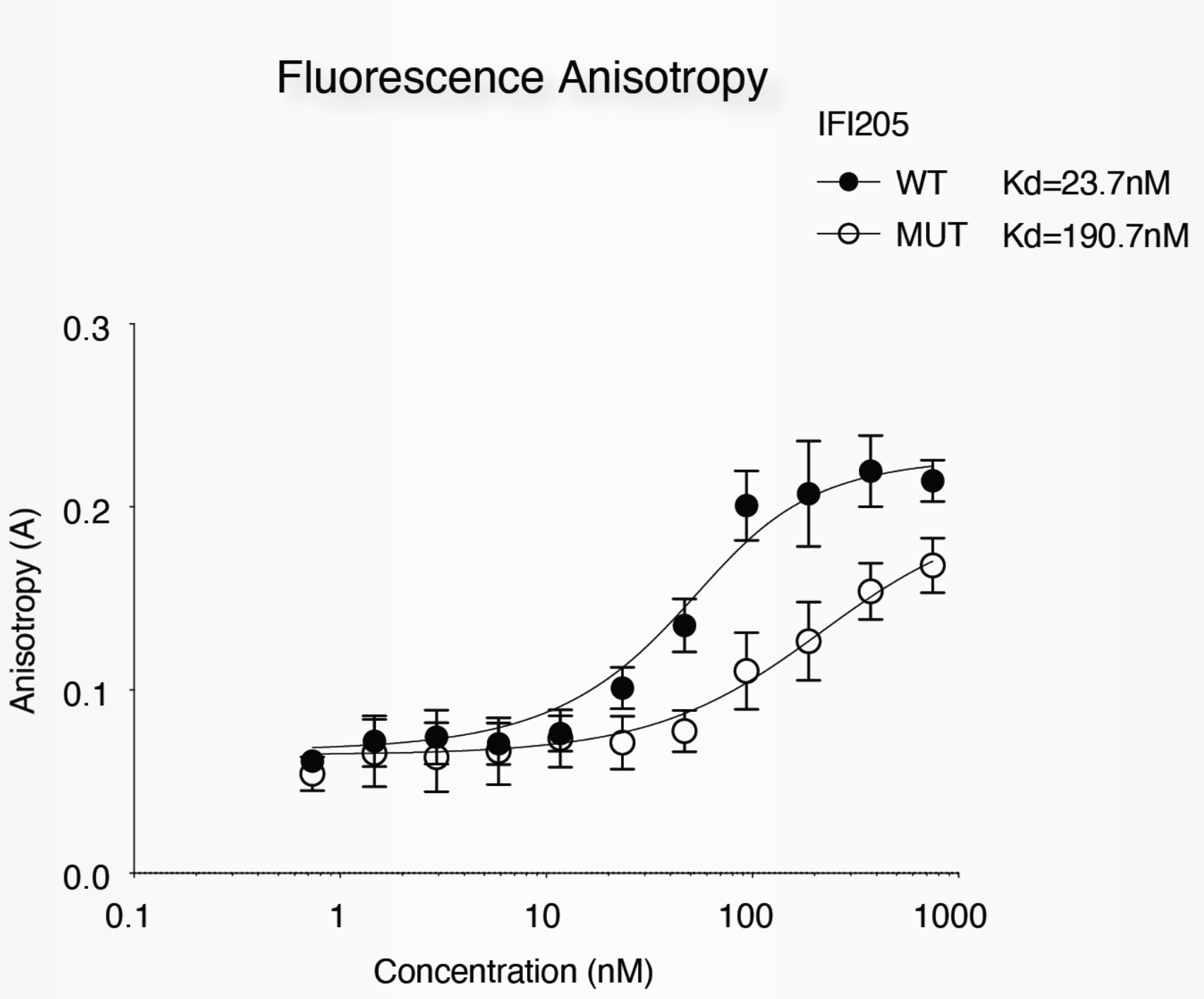
- High level of chromatid breakage, indicative of DNA replication defects
- Defects in DNA repair
- Defects in DNA damage signaling

Proposal:

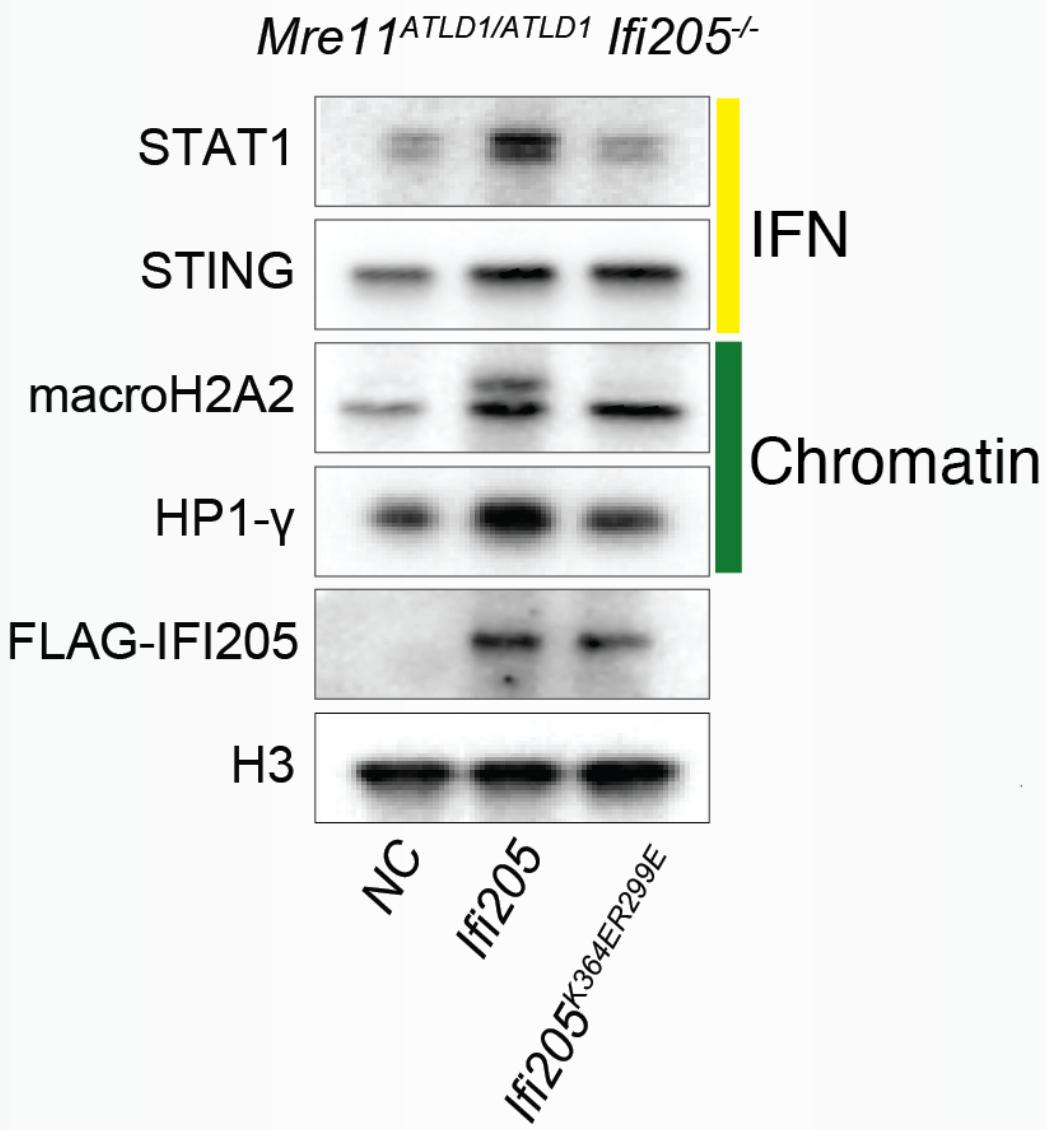
Aberrant DNA structures from Mre11 defects are engaged by IFI205 to induce ISG and attendant chromatin changes

IFI205 DNA Binding Mutant




Fluorescence Anisotropy
IFI205
WT Kd=23.7nM
MUT Kd=190.7nM
Anisotropy (A)
0.3
0.2
0.1
0.0
0.1
1
10
100
1000
Concentration (nM)

IFN Signaling and Chromatin Changes Depend on DNA Binding by IFI205

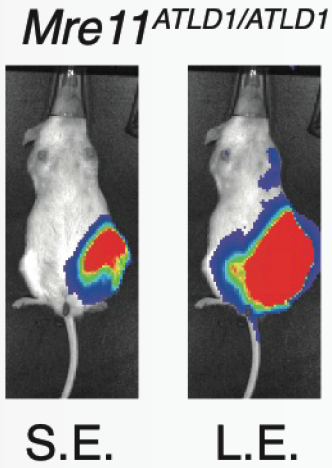


Organoids Implanted:

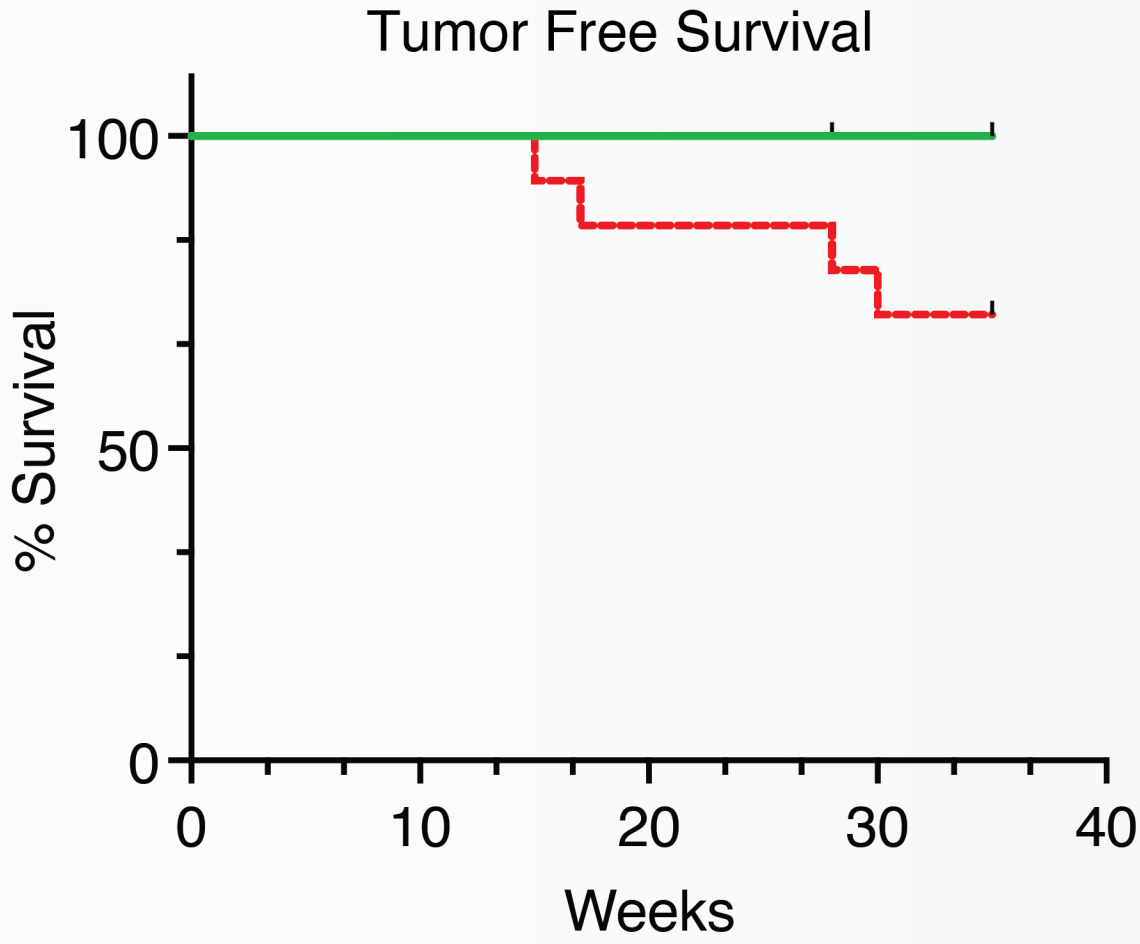
WT

Mre11$^{ATLD/ATLD}$

Mre11$^{ATLD/ATLD}$ IFI205$^{-/-}$



6.5m post dox induction

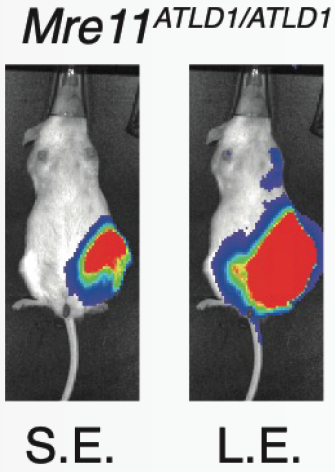


Organoids Implanted:

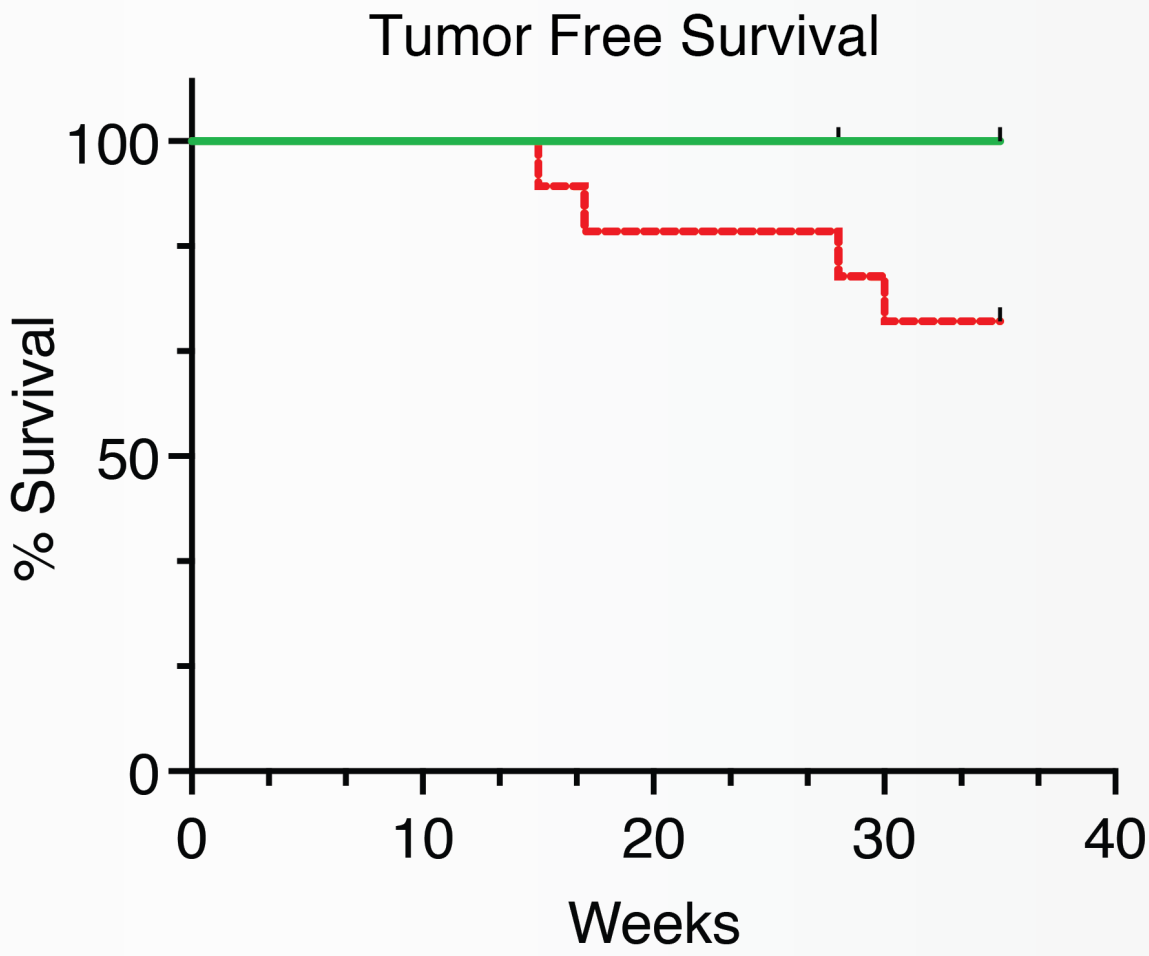
WT

Mre11$^{ATLD/ATLD}$

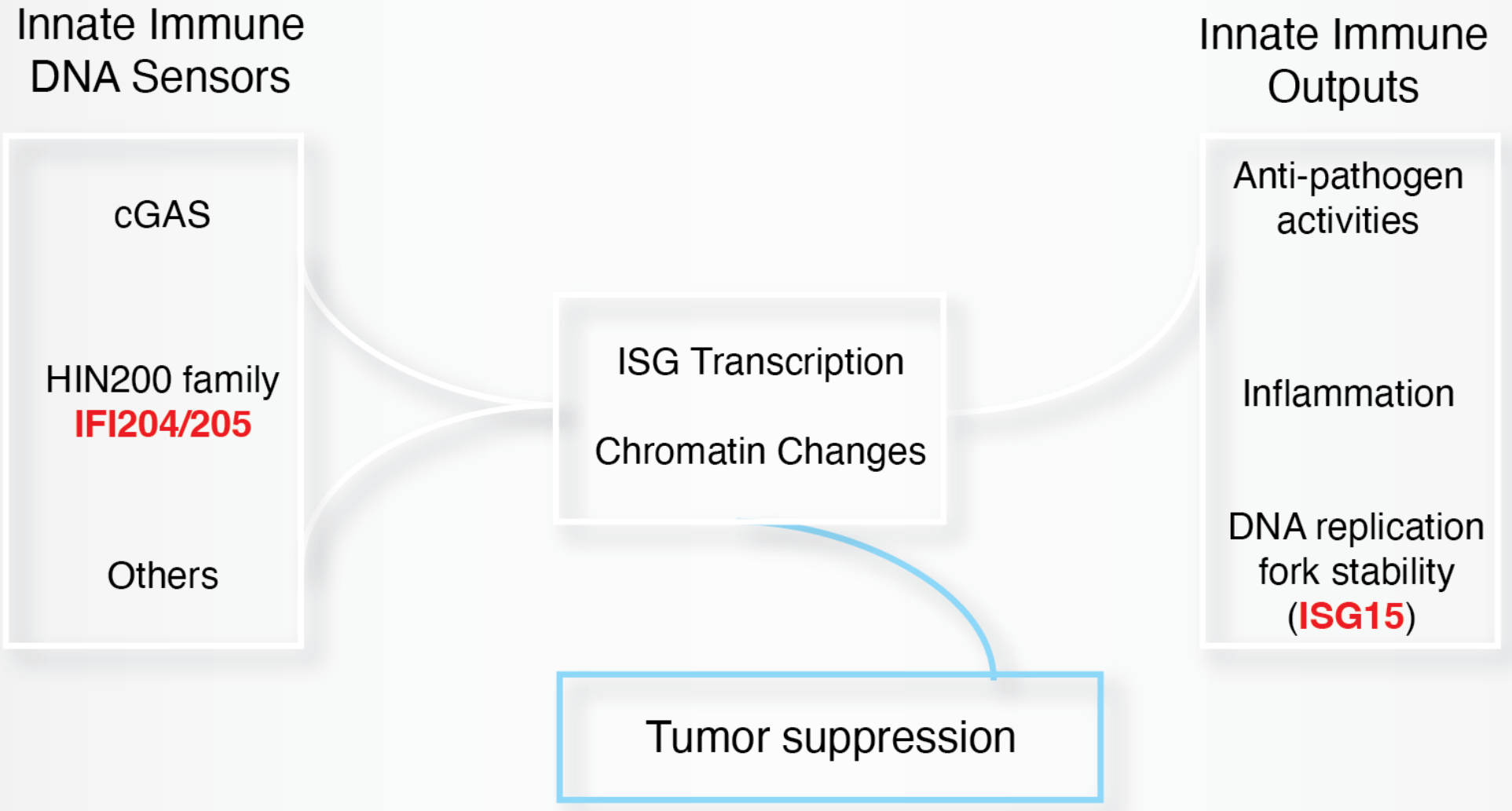
Mre11$^{ATLD/ATLD}$ IFI205$^{-/-}$



6.5m post dox induction

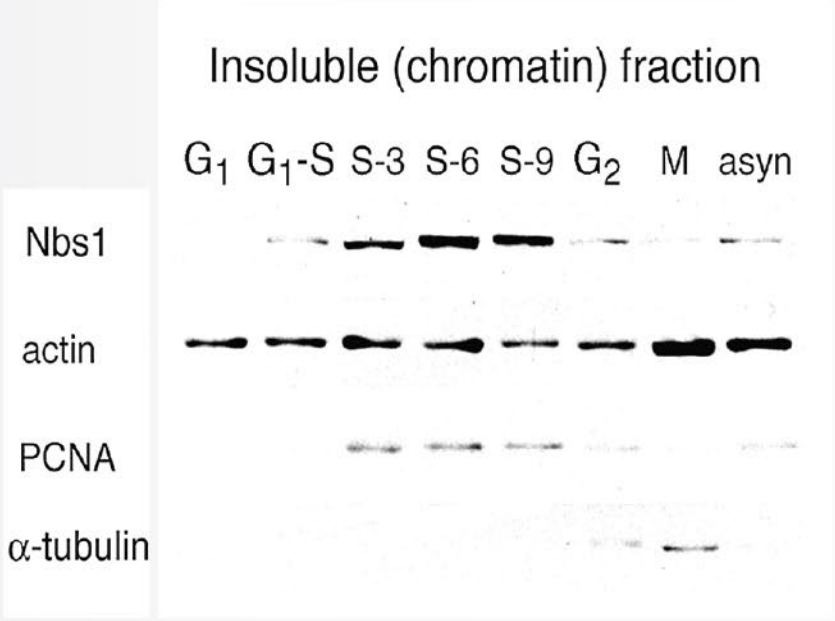


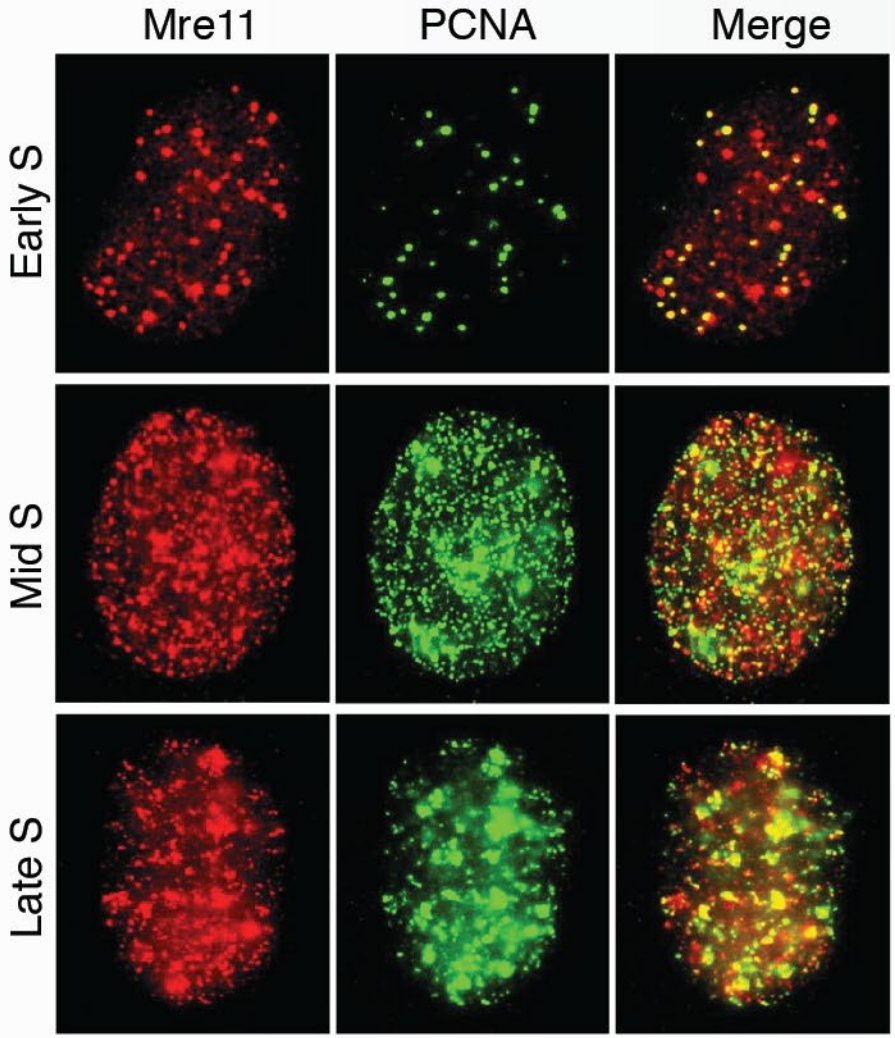
Today's Message



The Mre11 complex is unique among DNA repair machines

A Role for the Mre11 Complex During Unperturbed DNA Replication

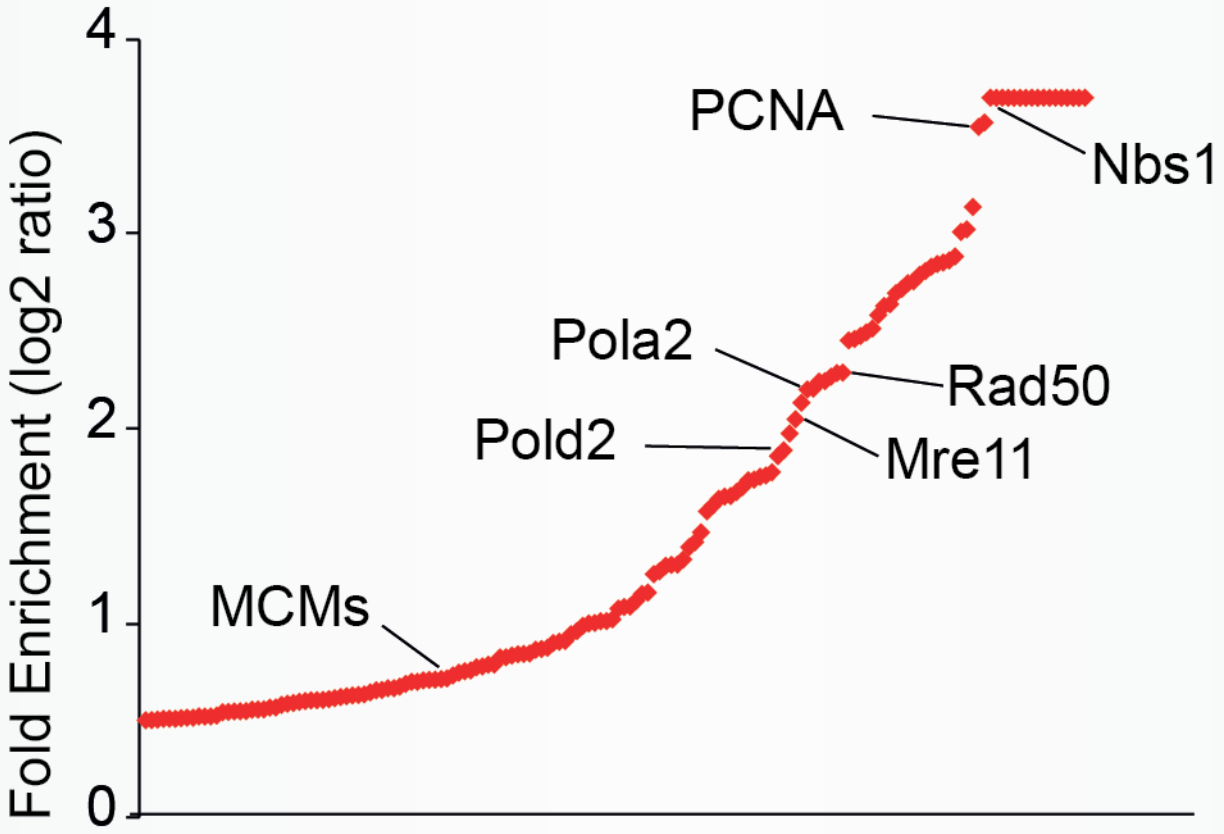




Mirzoeva and Petrini, 2003

Maser et al., 2001

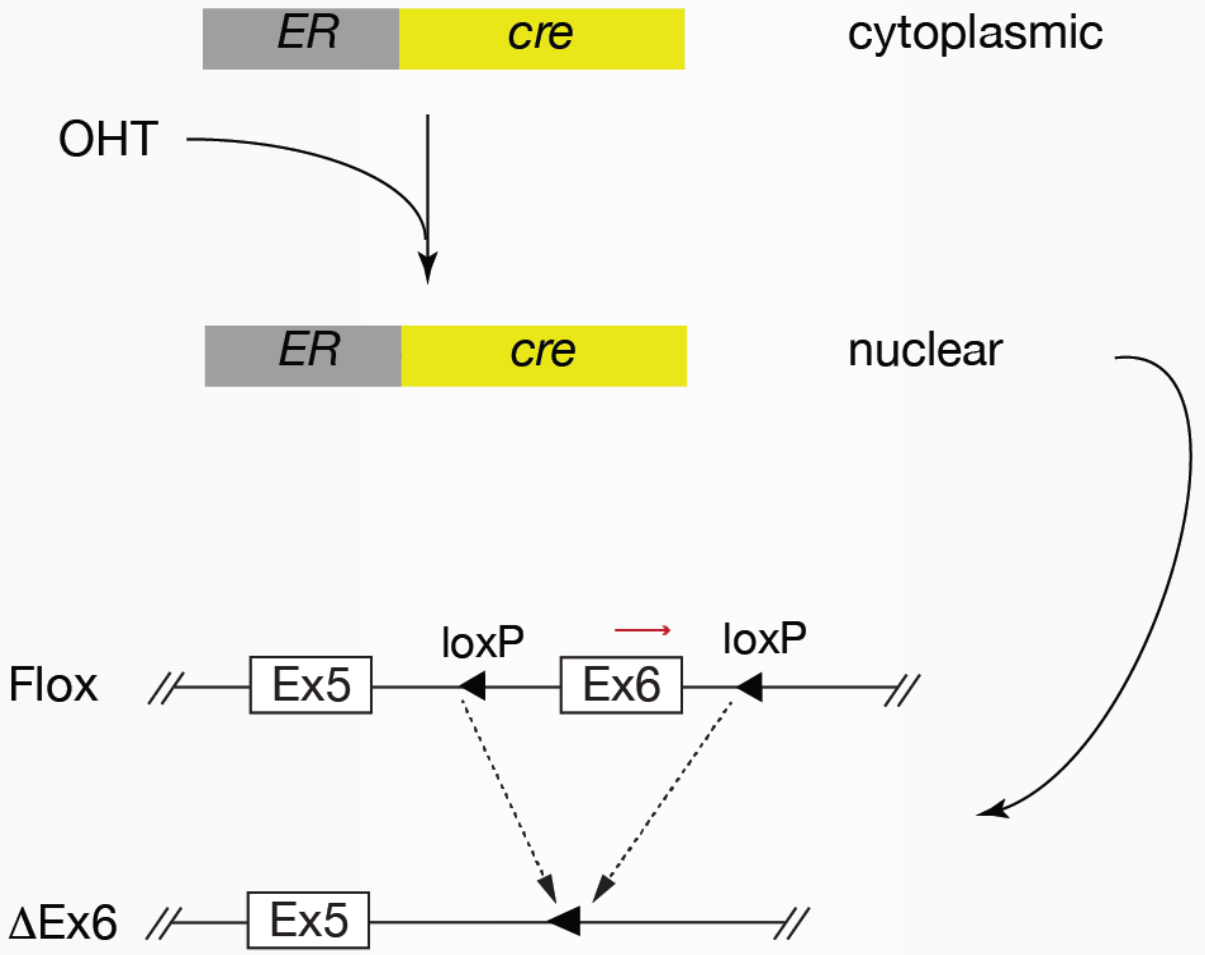
The Mre11 Complex is at the Replication Fork in Human Cells



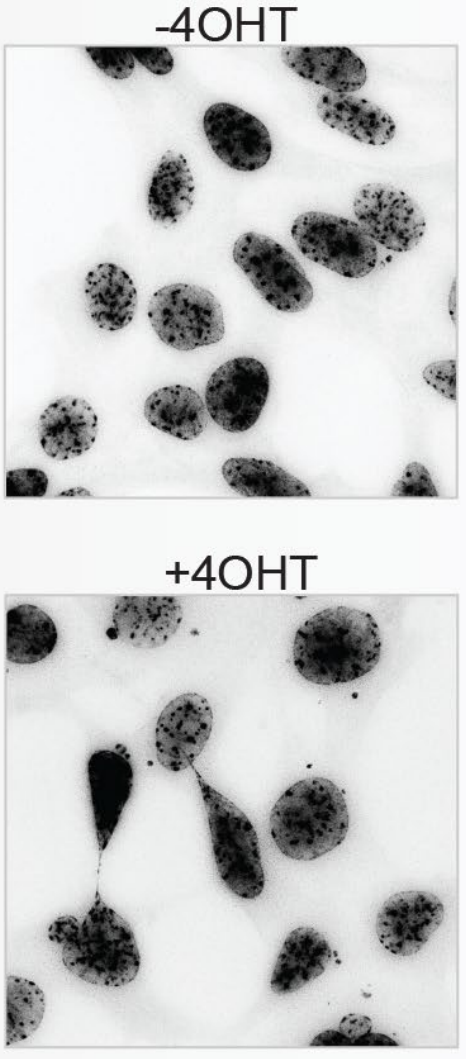
What is the Mre11 complex doing at the Fork?

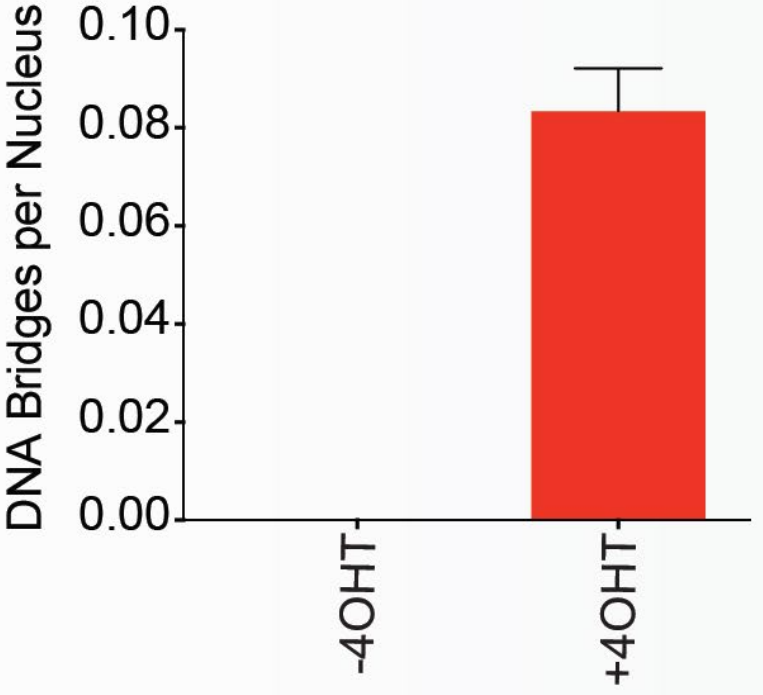
Cortez and Groth labs

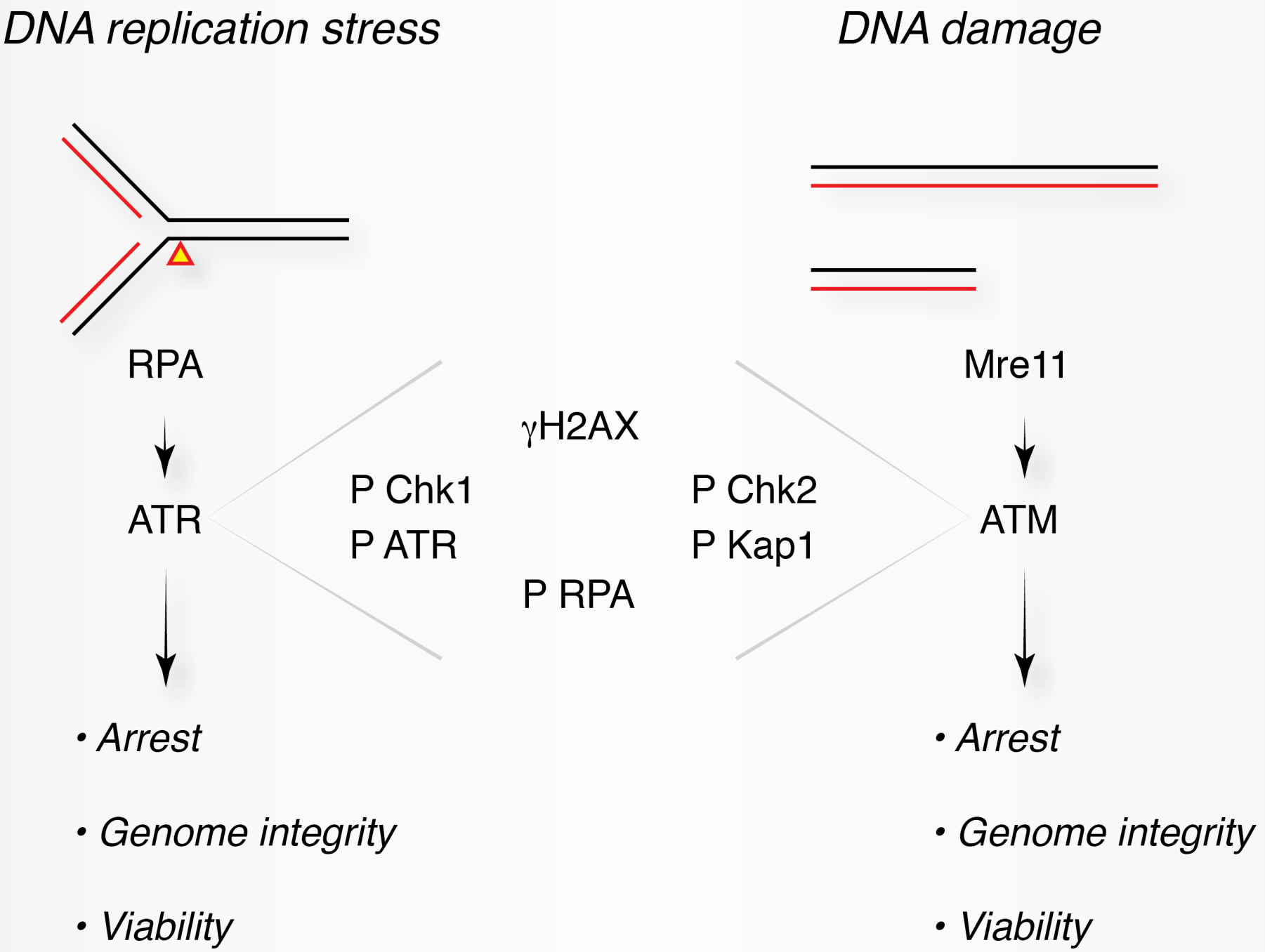
System for Acute Nbs1 Depletion



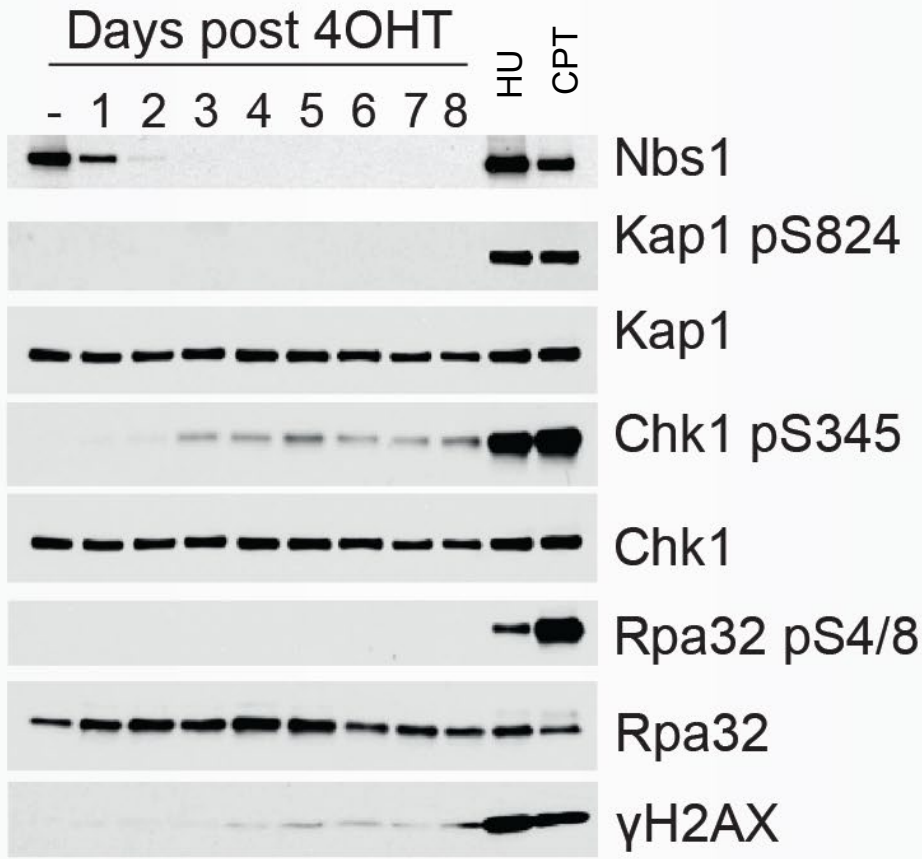
Nbs1 Depletion Causes DNA Breakage and Bridges






DNA replication stress
DNA damage
RPA
ATR
Mre11
ATM
γH2AX
P Chk1
P ATR
P RPA
P Chk2
P Kap1
• Arrest
• Genome integrity
• Viability
• Arrest
• Genome integrity
• Viability

Nbs1 Depletion and DNA Replication Stress



DNA Combing: Single Molecule Analysys of DNA replication

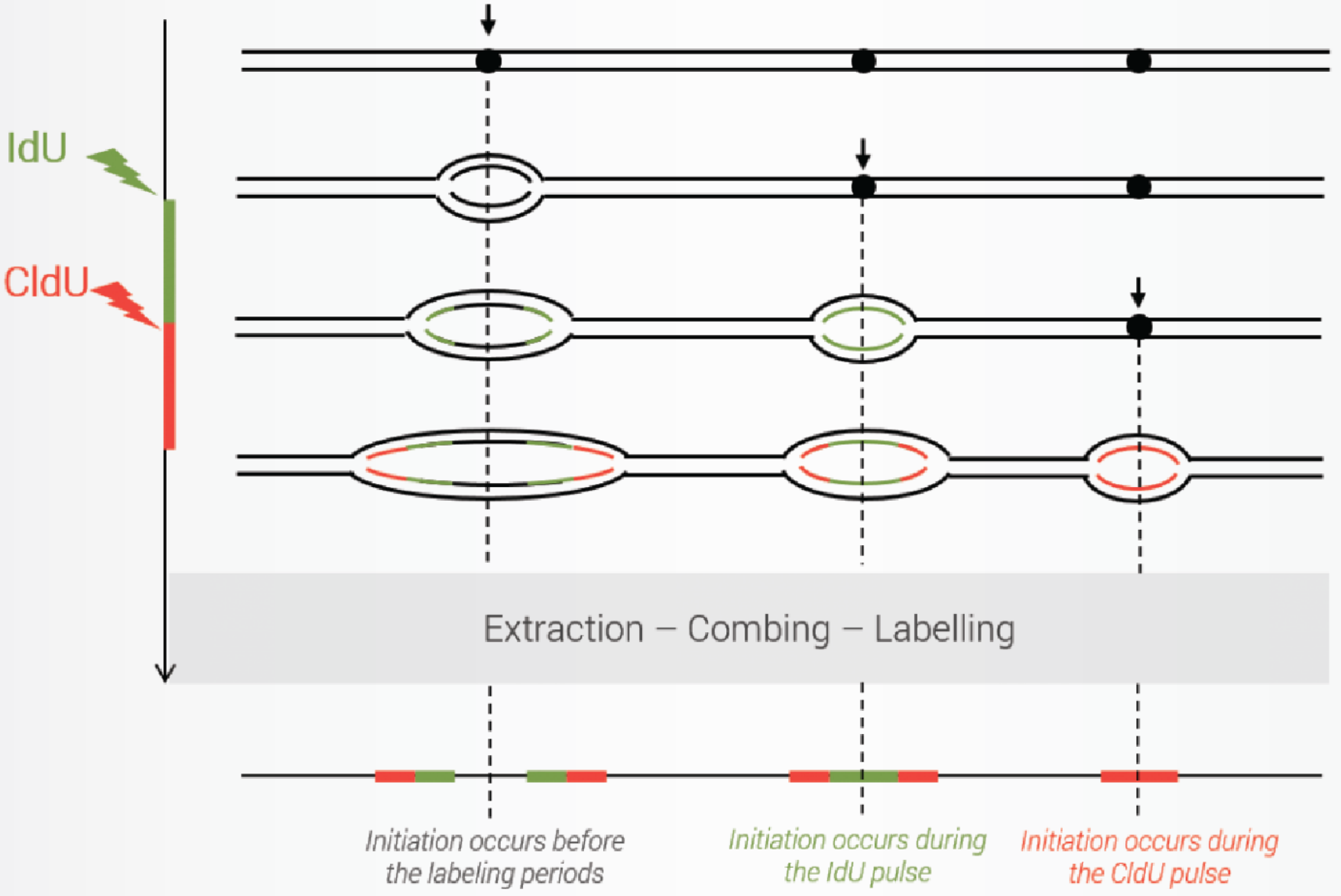
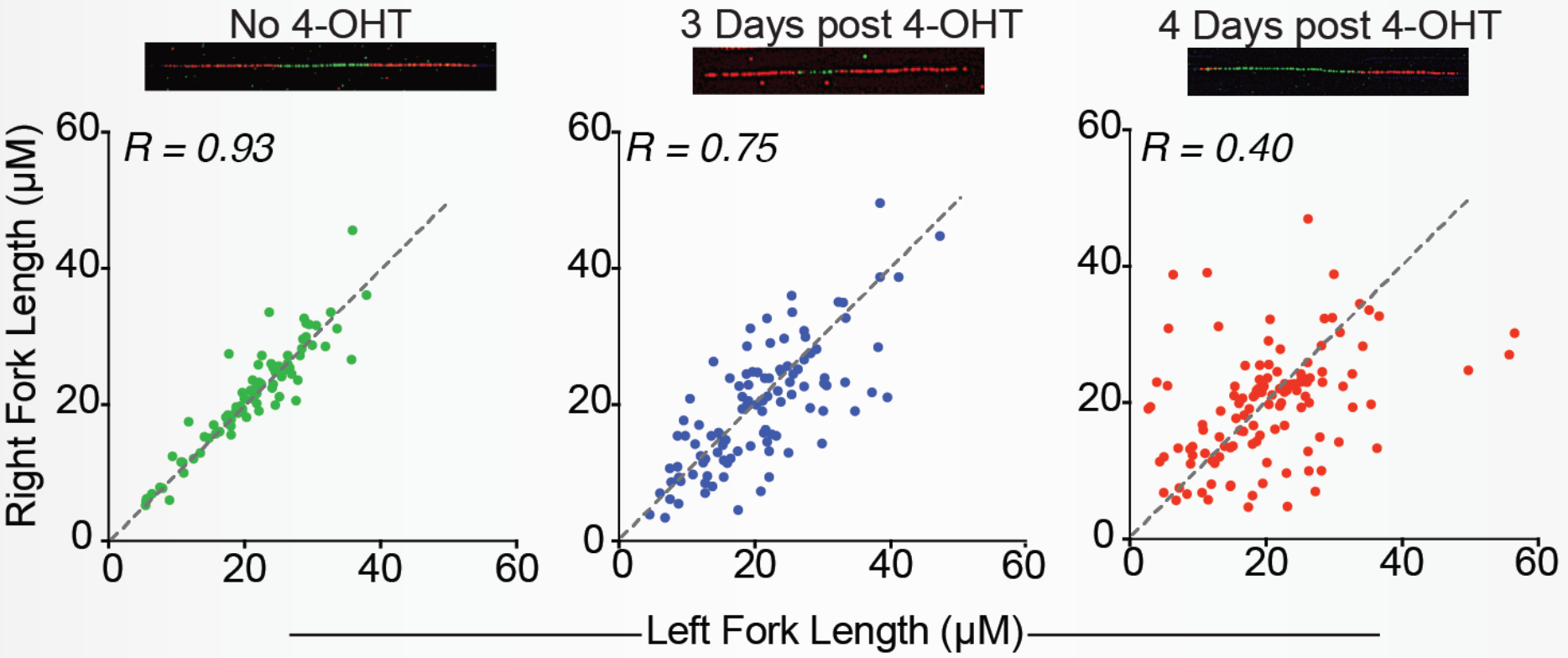


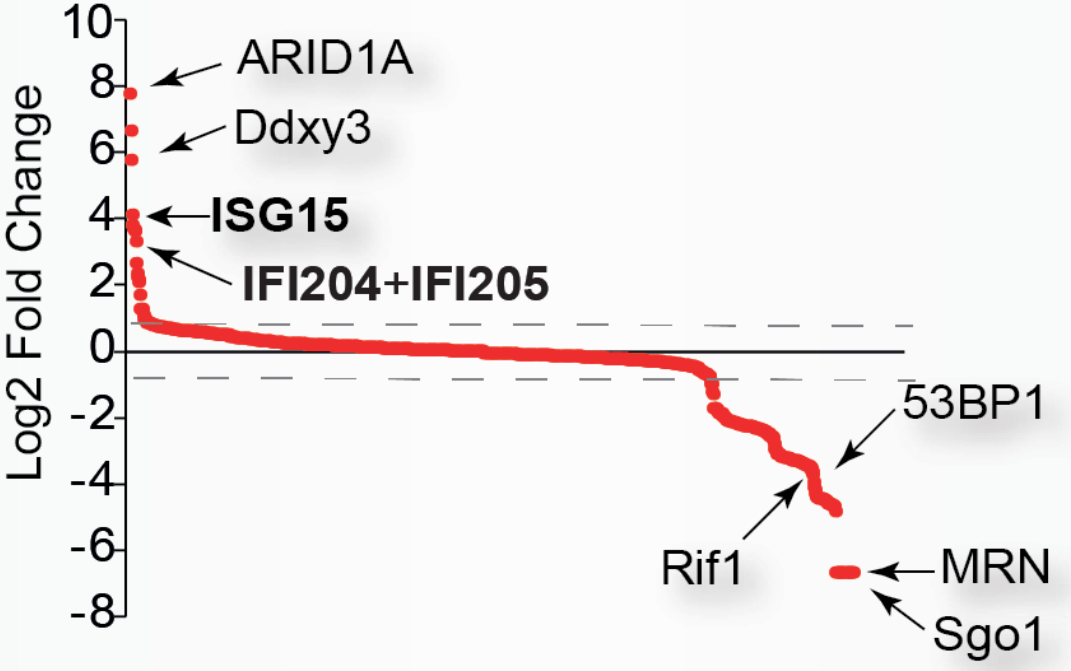
Image borrowed from Genome Vision website

Nbs1 Depletion and Replication Fork Asymmetry

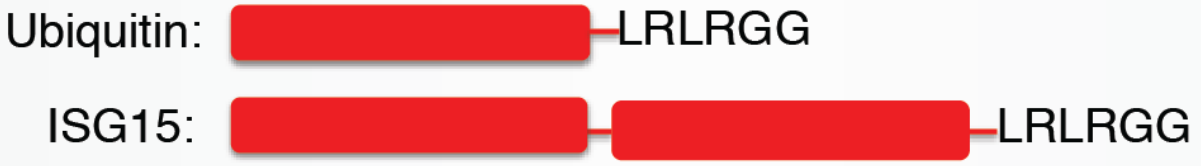


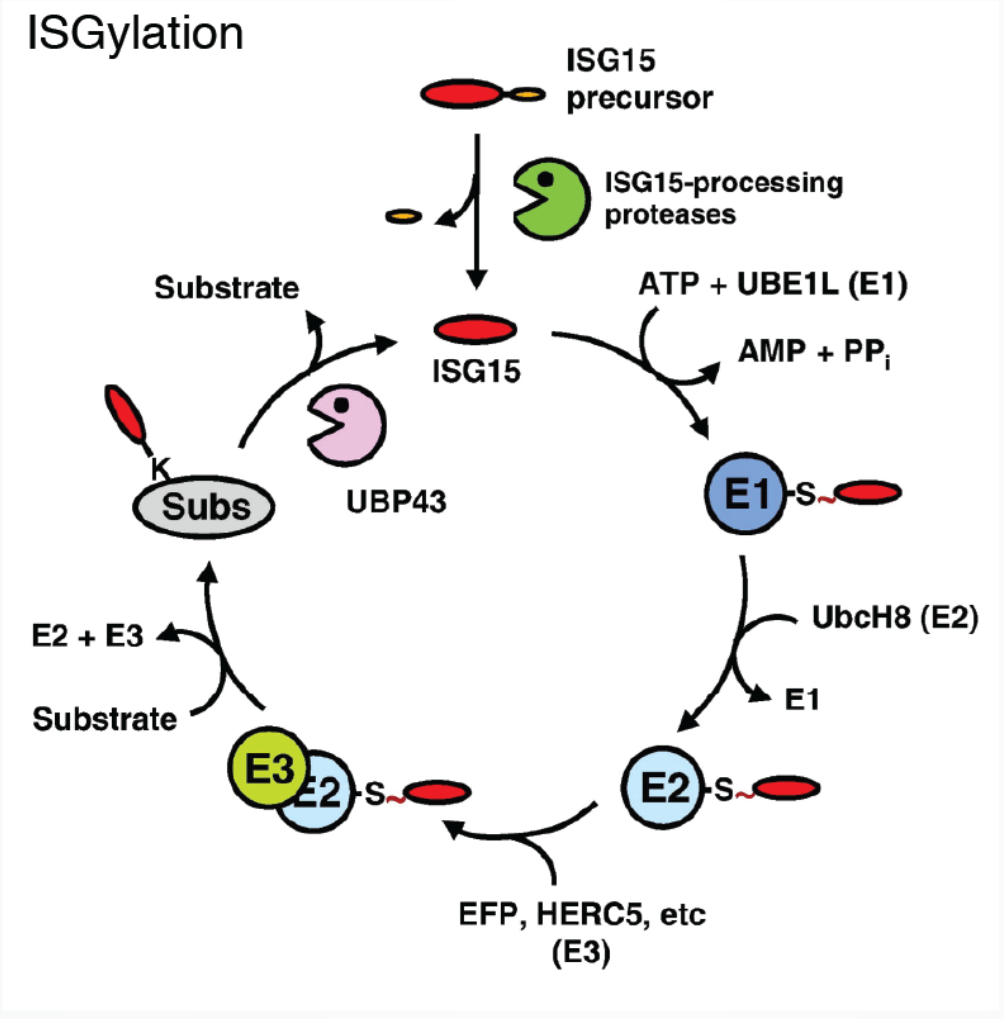
At the Fork (or not) in Nbs1 Depleted Cells





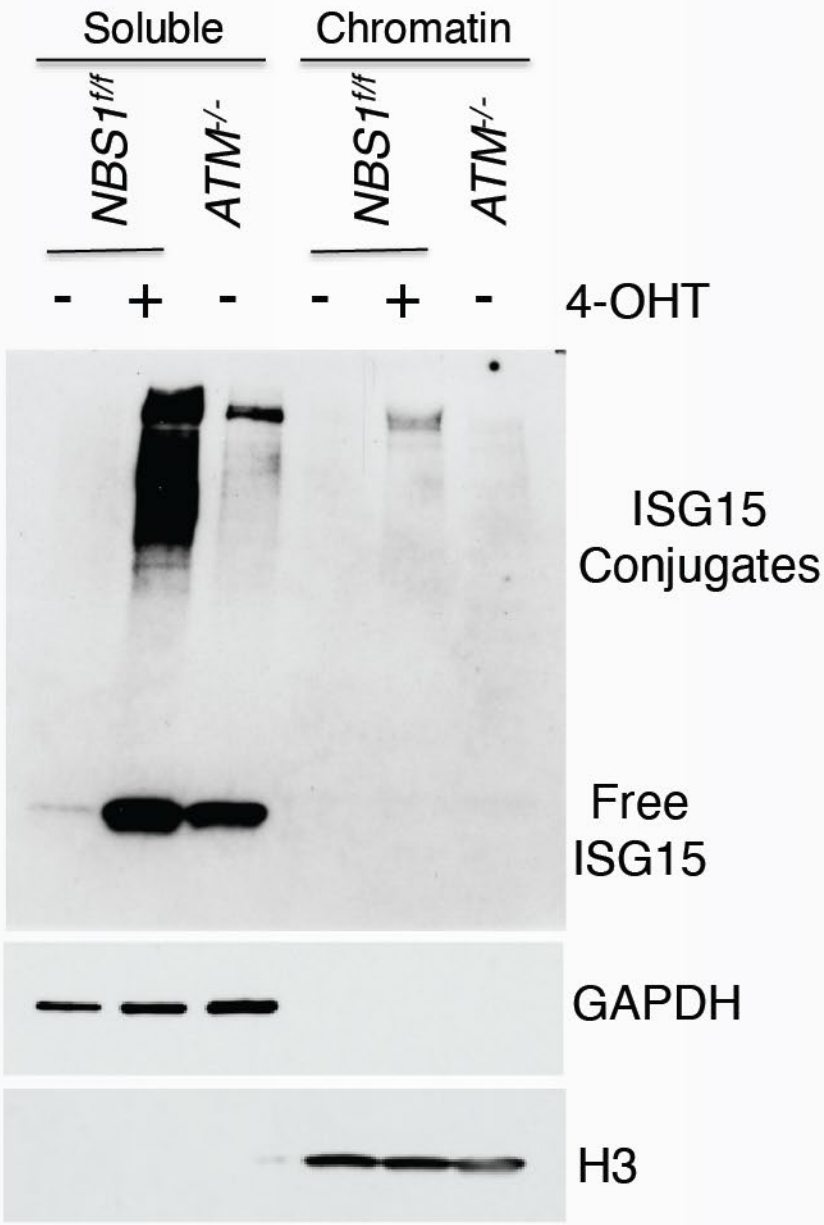
ISG15 (Interferon Stimulated Gene 15)





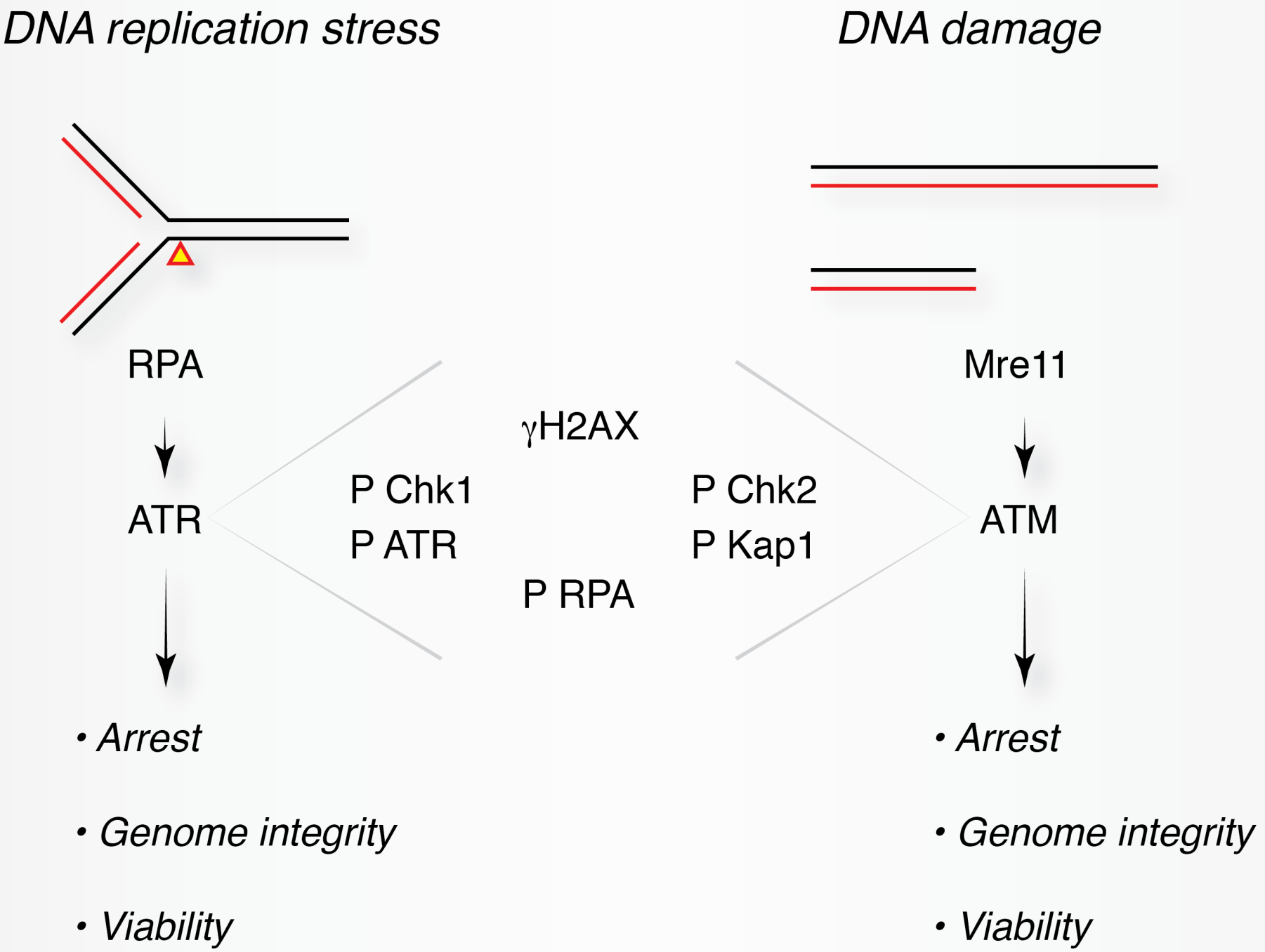
Jeon et al., 2010

Nbs1 Depletion Induces ISGylation on Chromatin

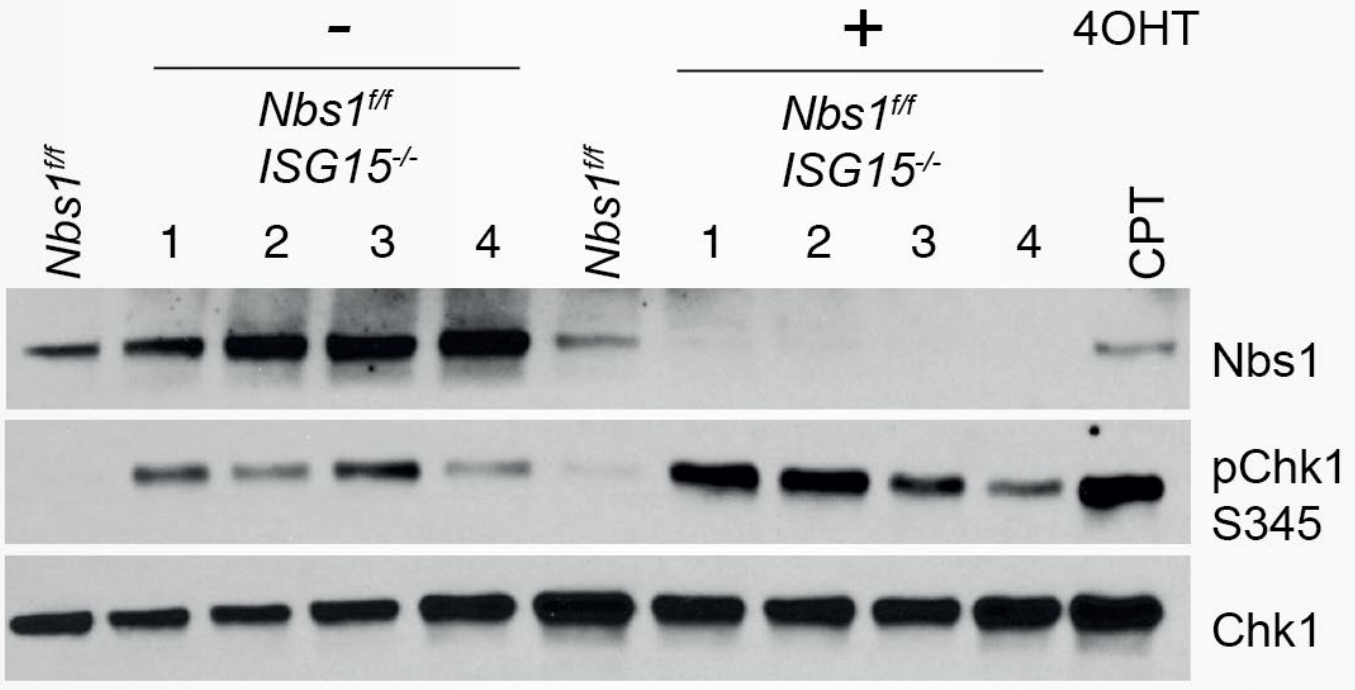


Does this mean anything?

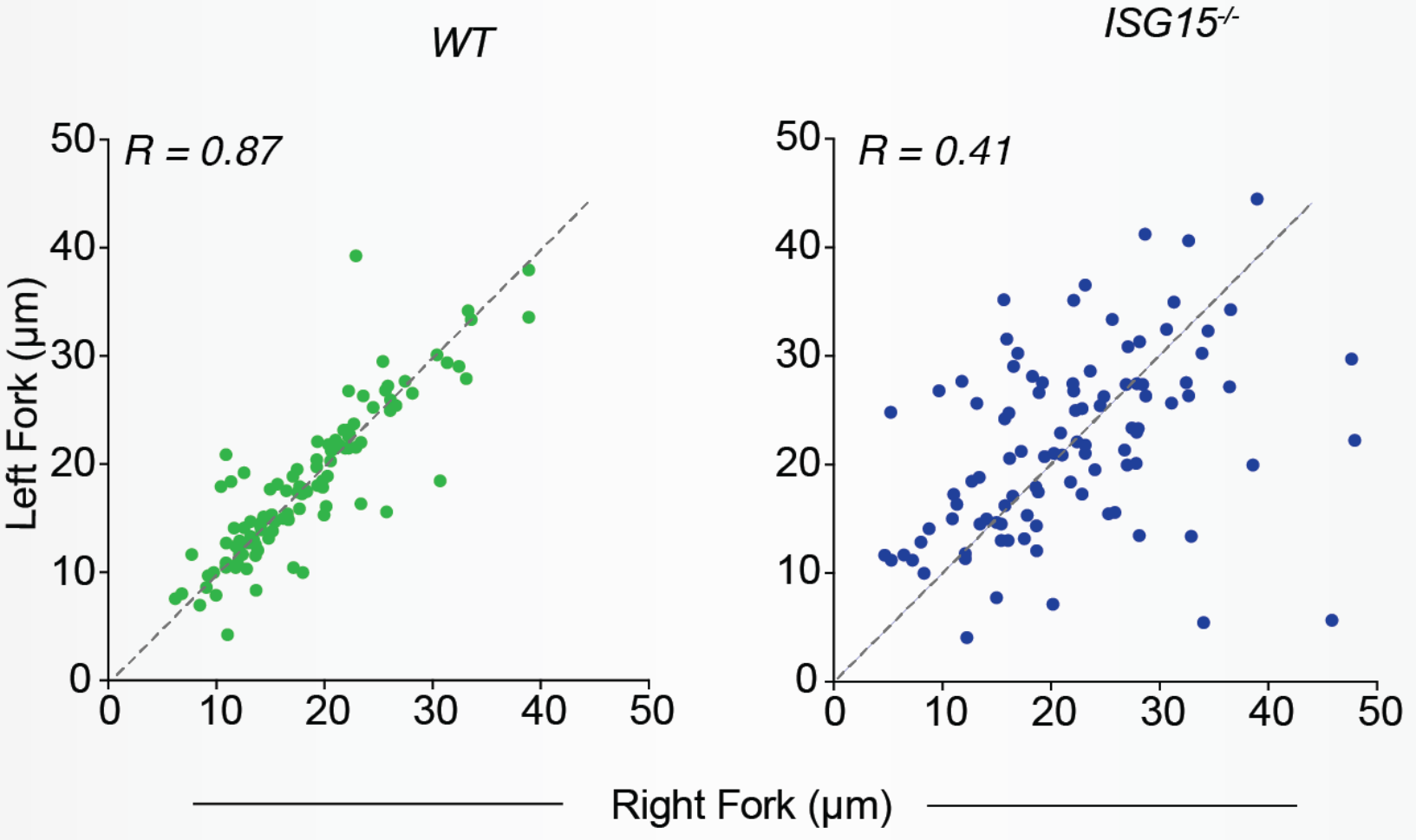
We made the ISG15 KO...


DNA replication stress
DNA damage
RPA
ATR
Mre11
ATM
γH2AX
P Chk1
P ATR
P RPA
P Chk2
P Kap1
• Arrest
• Genome integrity
• Viability
• Arrest
• Genome integrity
• Viability

Chronic Chk1 Activation in ISG15 KO Cells

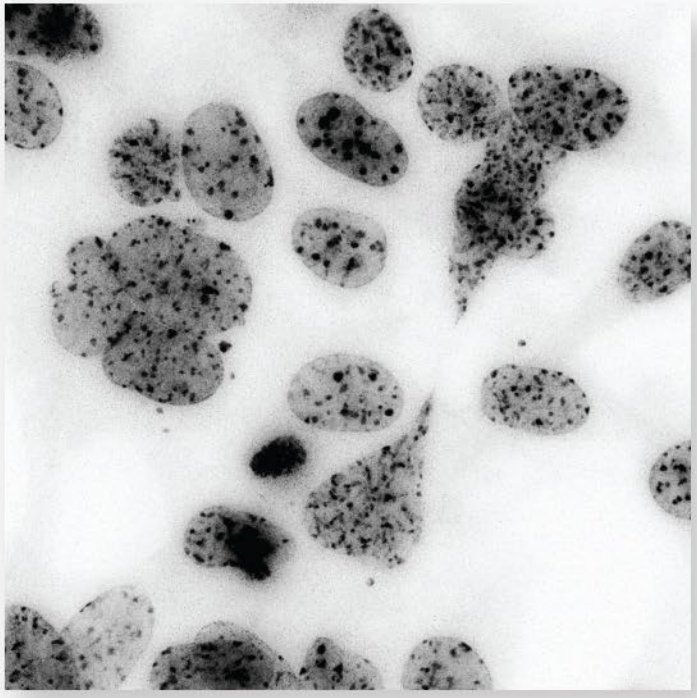


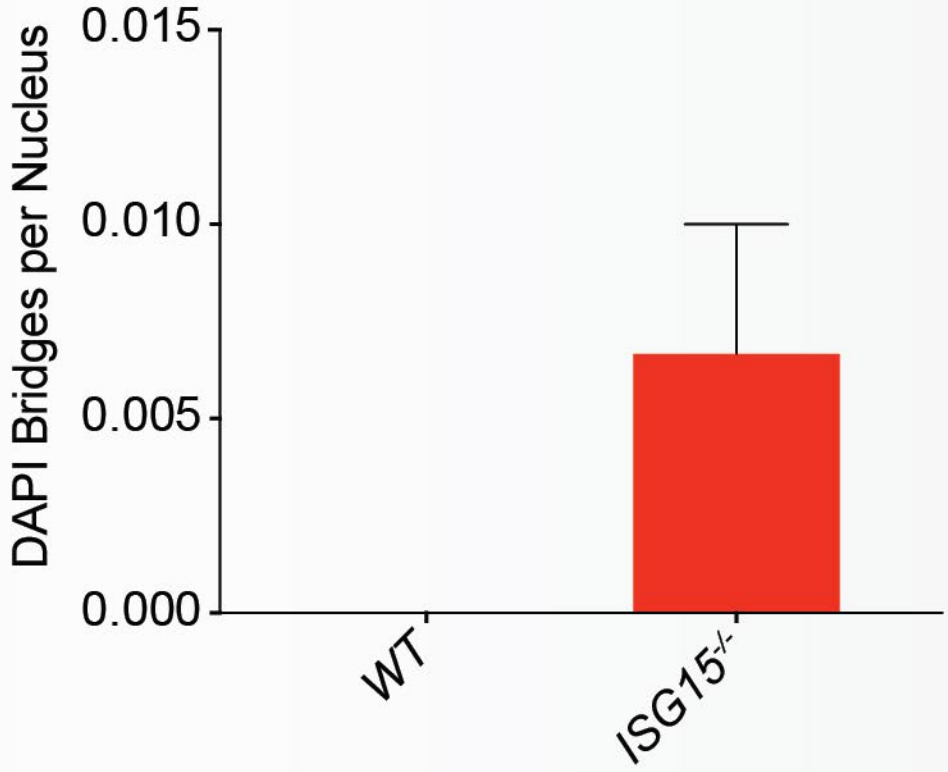
Replication Fork Asymmetry in ISG15 KO Cells



ISG15 Deficiency and DNA Bridges

ISG15$^{-/-}$

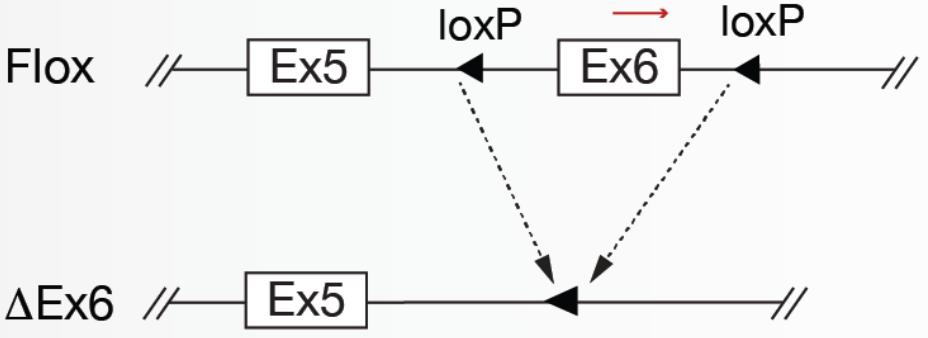




What does ISGylation do at the fork?

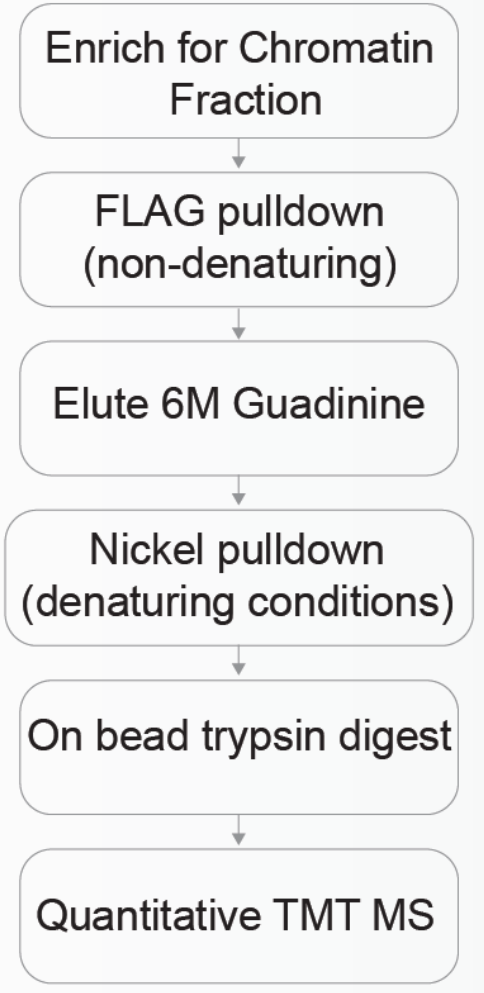
Evidence for two things.....

System for Purification of ISGylated Proteins

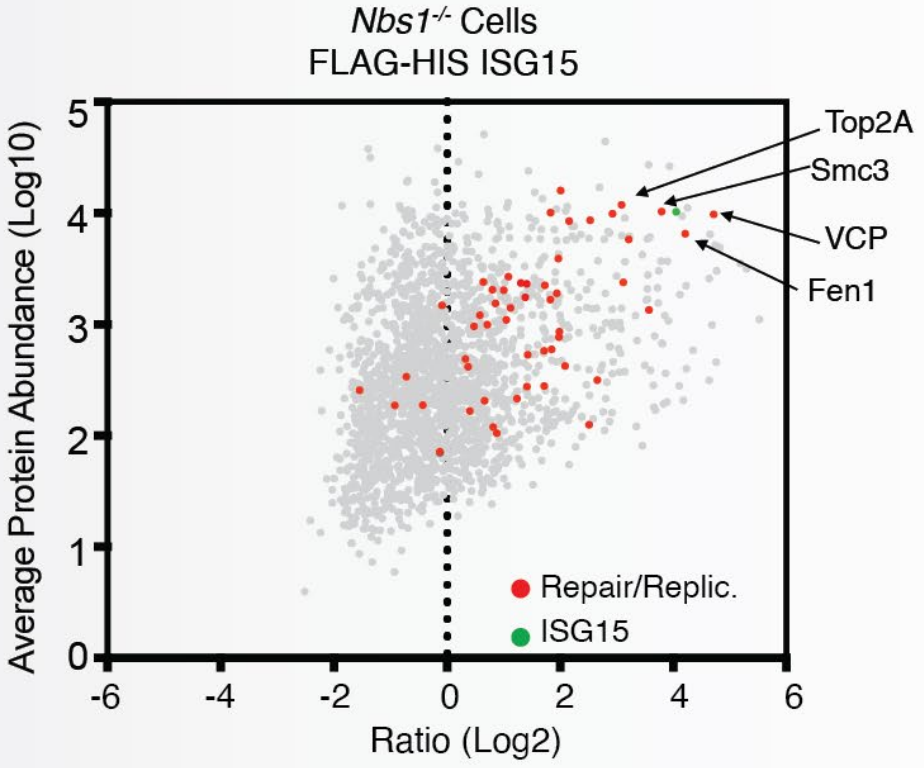


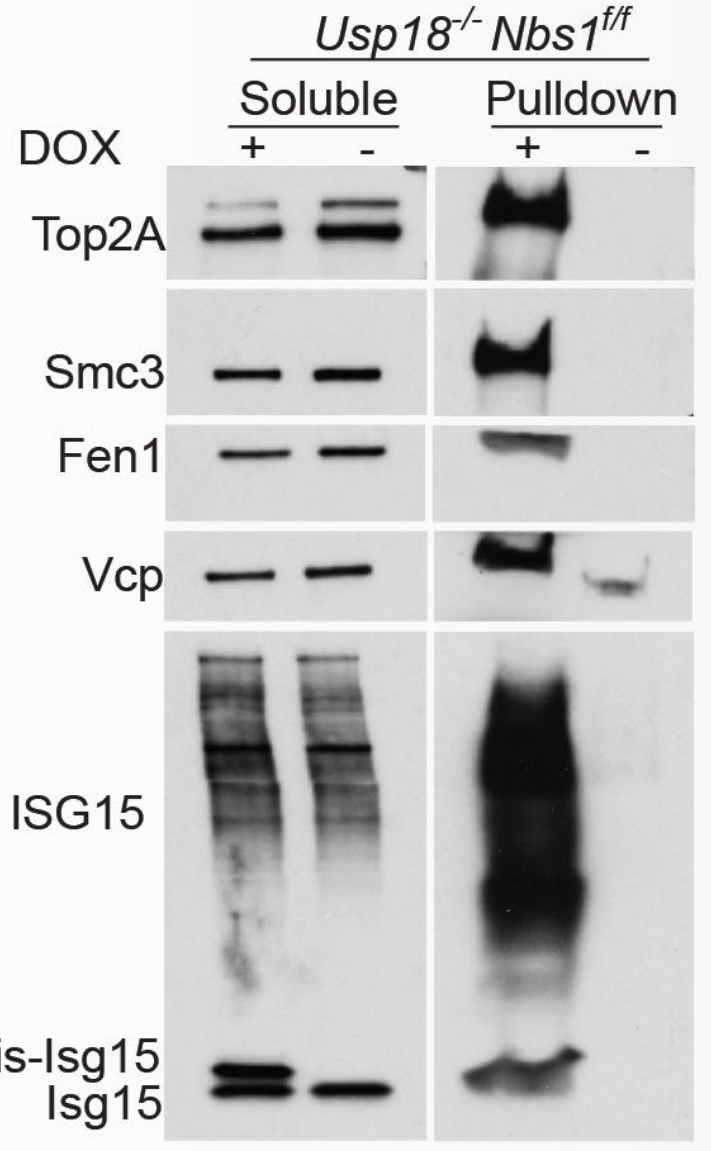
Endogenous *ISG15* Locus





Proteins Relevant to DNA Repair and Replication are ISGylated

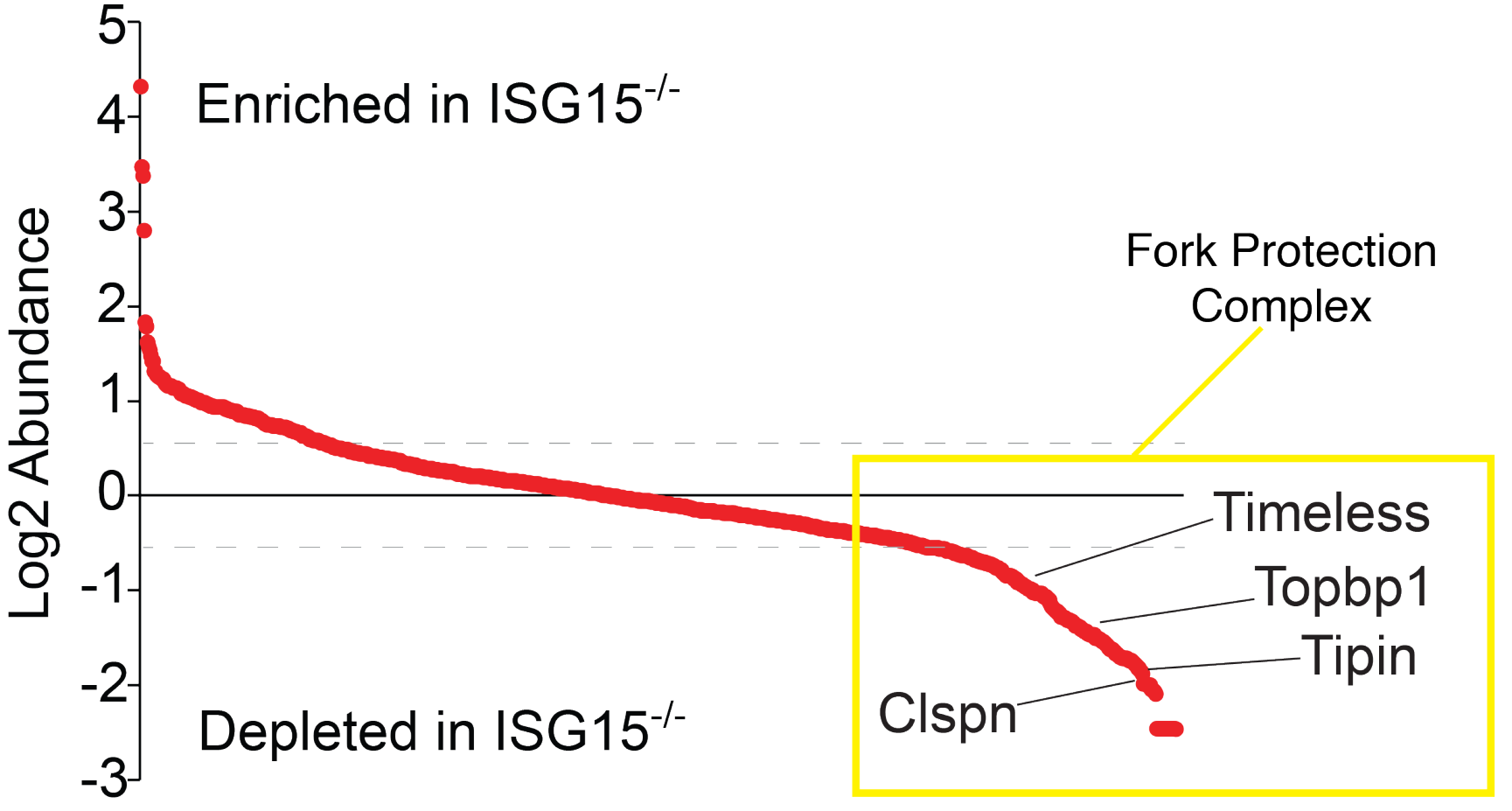


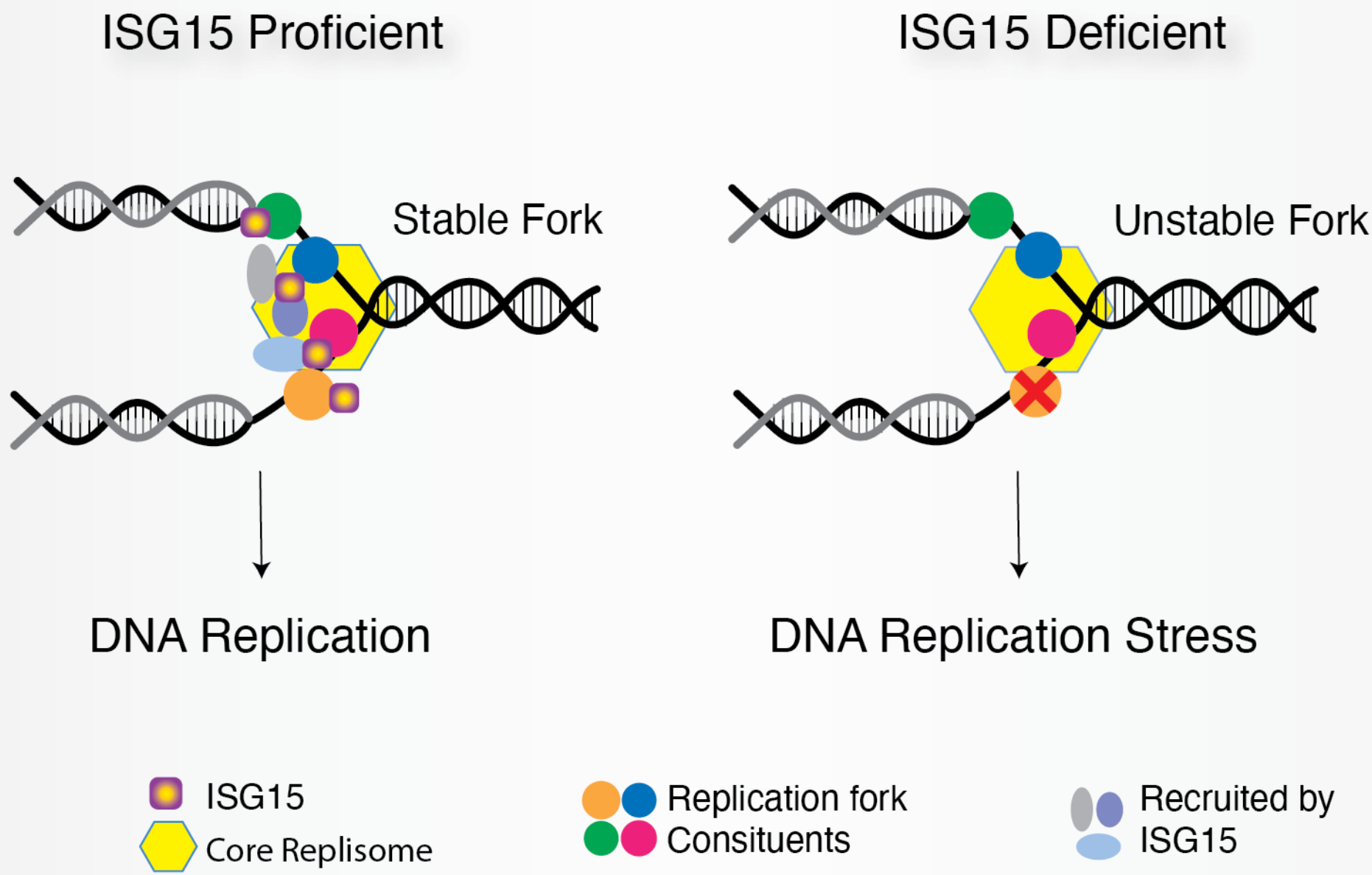


Dox inducible 6HIS-ISG15

Last iPond...which replication fork constituents are ISG15 dependent?

Occupancy of Fork Protection Complex is Diminished in ISG15KO

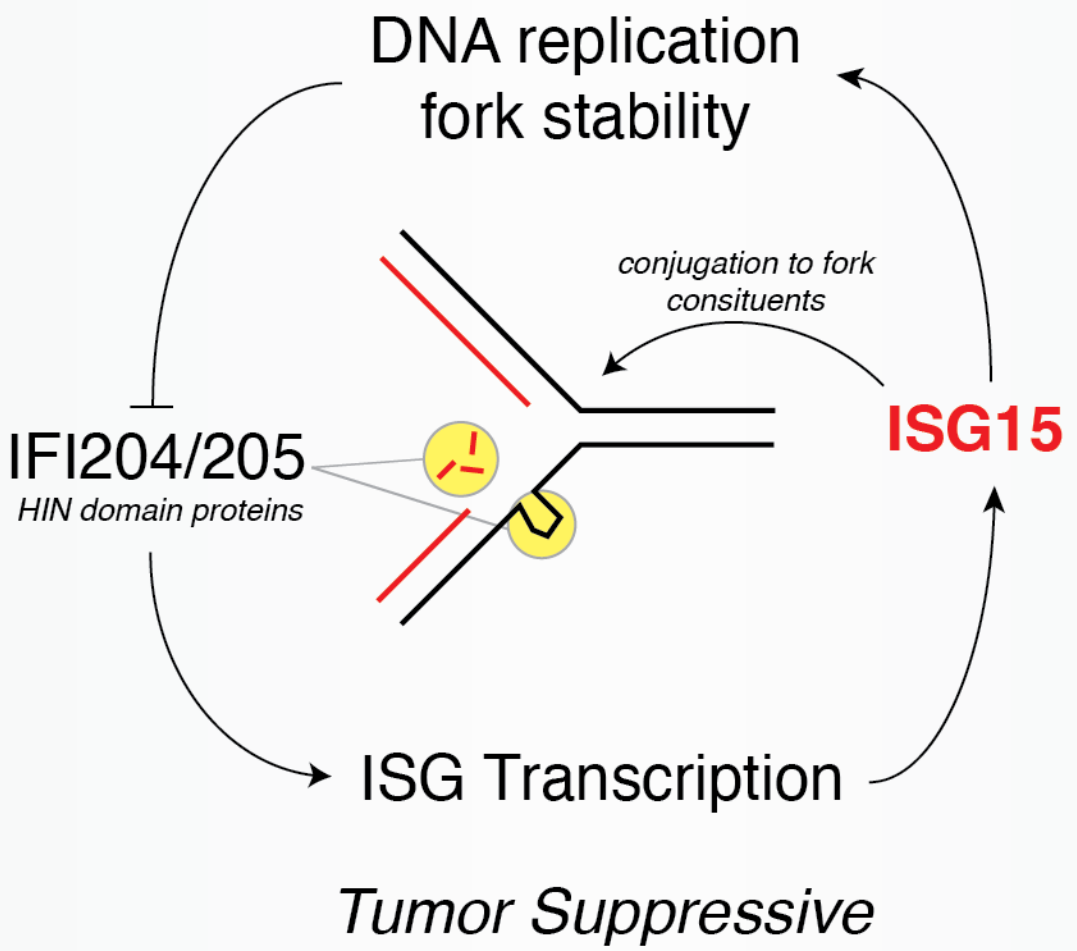



ISG15 Proficient
ISG15 Deficient
Stable Fork
Unstable Fork
DNA Replication
DNA Replication Stress
ISG15
Core Replisome
Replication fork
Consituents
Recruited by
ISG15

Speculative Takeaway:

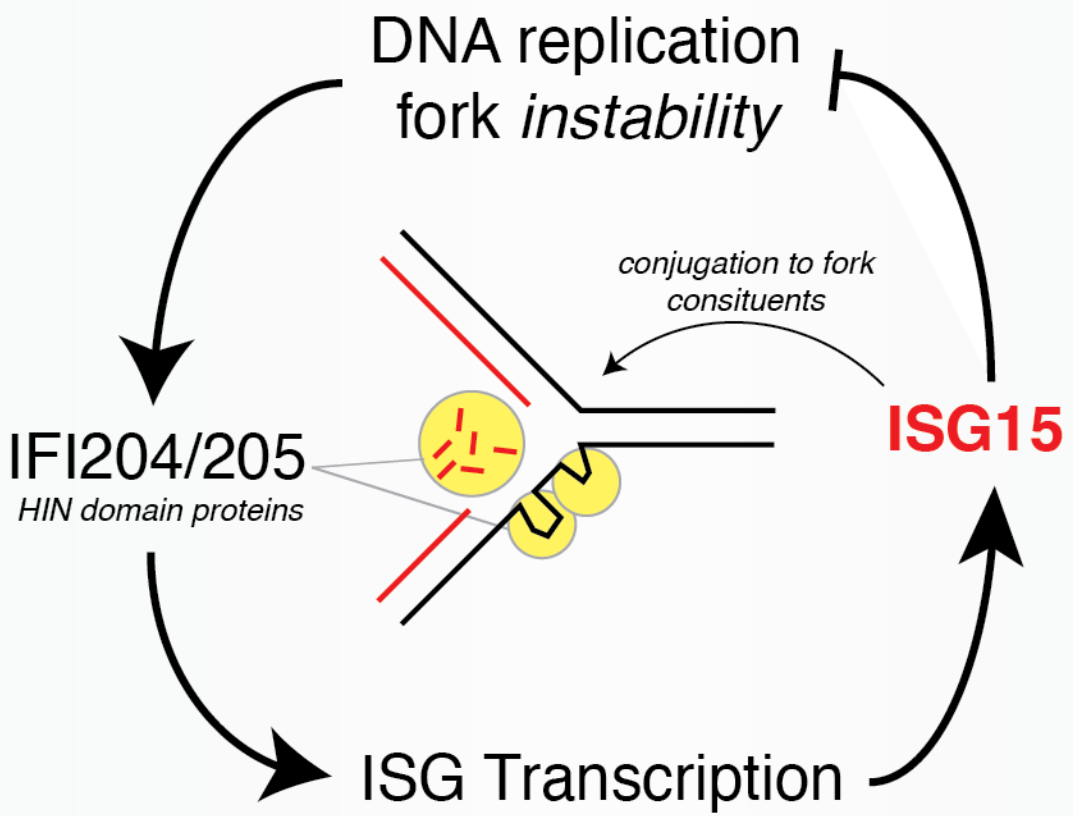
Engagement of DNA of *nuclear* extrachromosomal DNA may be a mechanism of replication fork surveillance.

Today's Message



Tumor Suppressive

Today's Message



Tumor Permissive

Thanks to…

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Sawyers Lab
Wouter Karthaus

Lowe Lab
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Francisco Sanchez-Rivera

Reis-Filho Lab
Sasi Arunachalam (Former Member)
David Brown
Pier Selenica

Leslie Lab
Han Yuan (Former Member)
Hyunwoo Cho (Former Member)
Alli Pine

Integrated Genomics Operation Core
Neeman Mohibullah
Cassidy Cobbs

We have room for more.
Lots to do!