# DDRNAs

# DNA DAMAGE RESPONSE RNAs

LETTER

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# Site-specific DICER and DROSHA RNA products control the DNA-damage response

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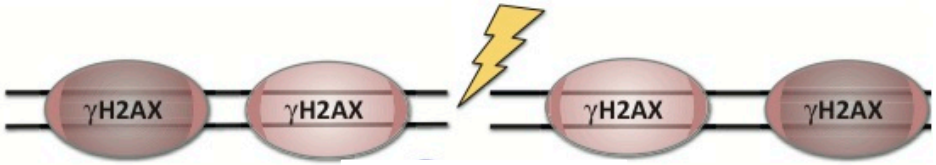
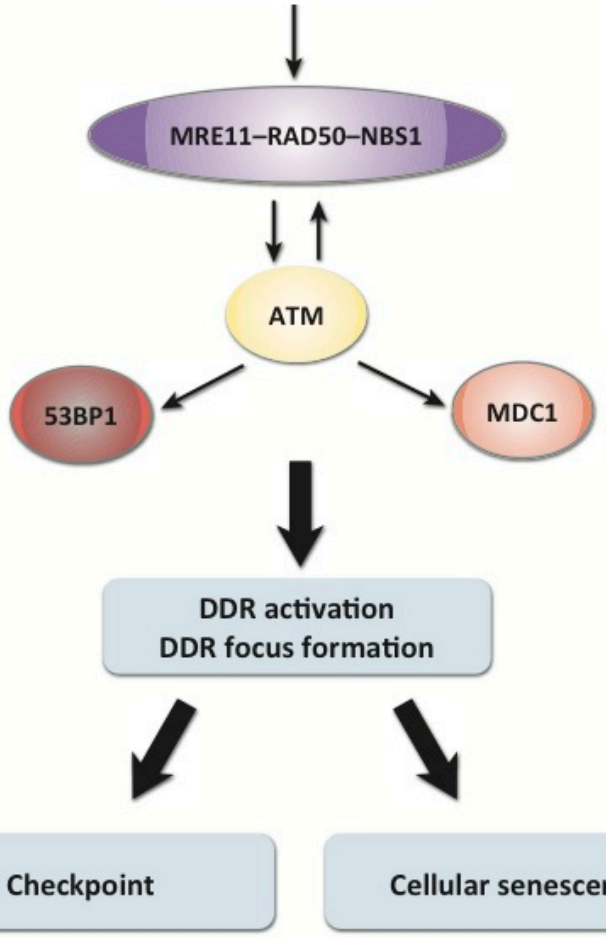
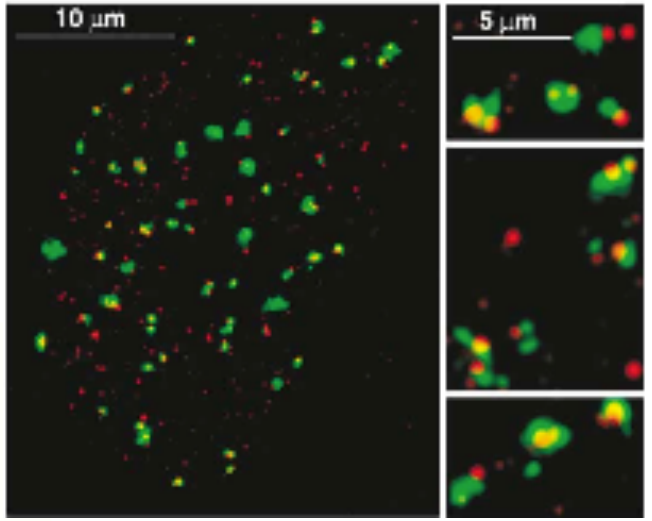
# The DNA damage response revisited



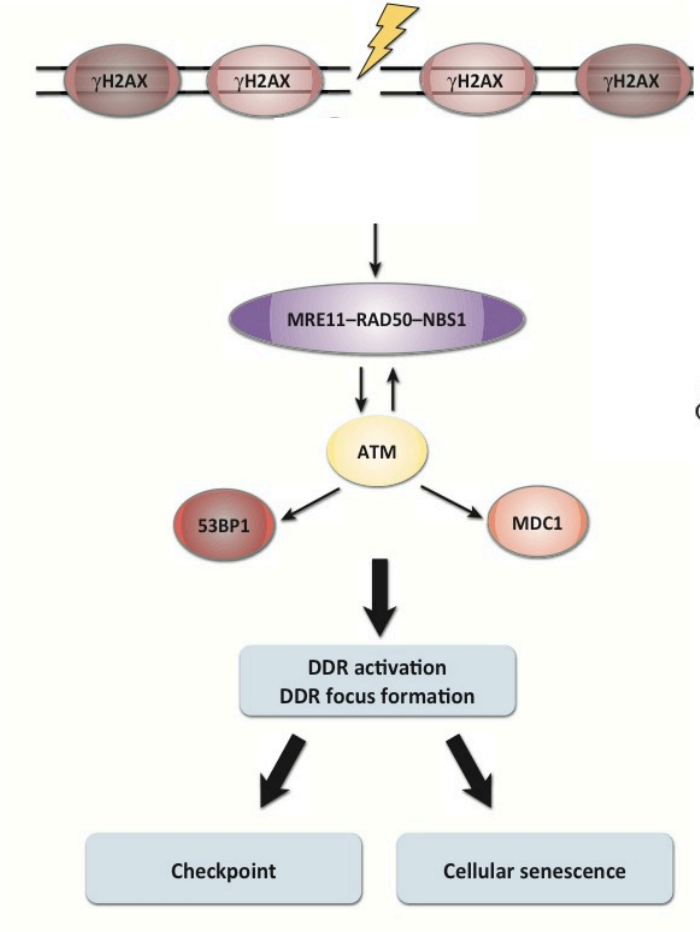



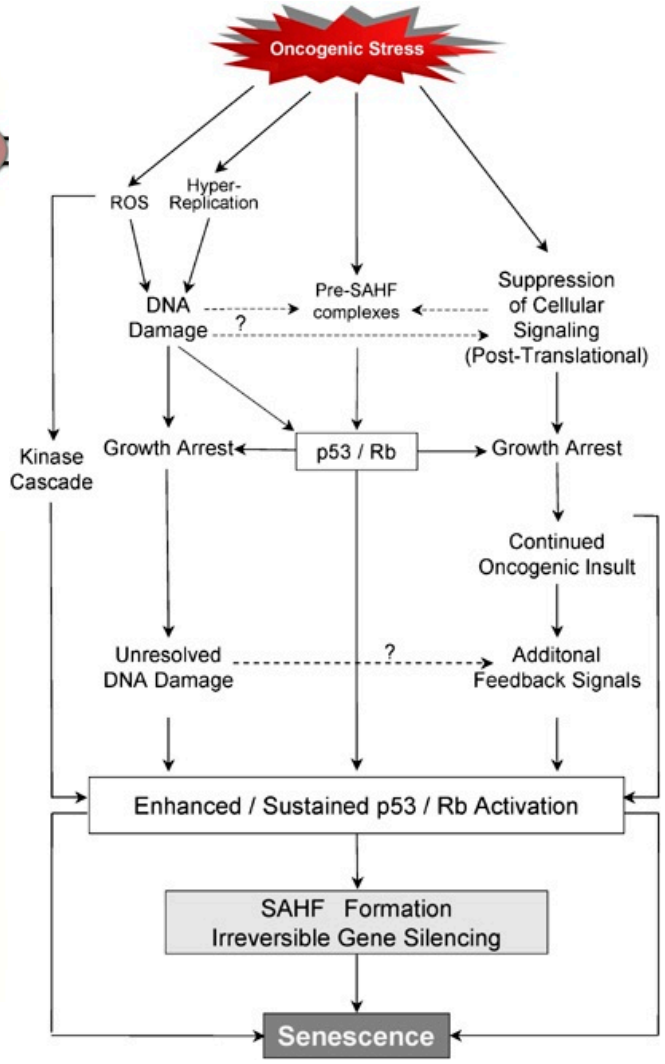

**DNA Damage Foci**




**γH2AX**
53BP1
MRE11
P-ATM

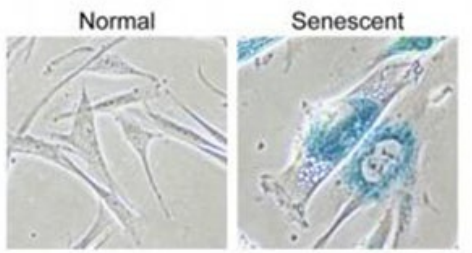
# Model system for persistent DNA damage: ONCOGENE INDUCED SENESCENCE

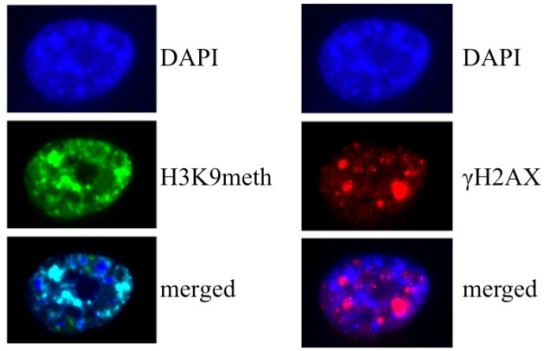



## Senescence markers

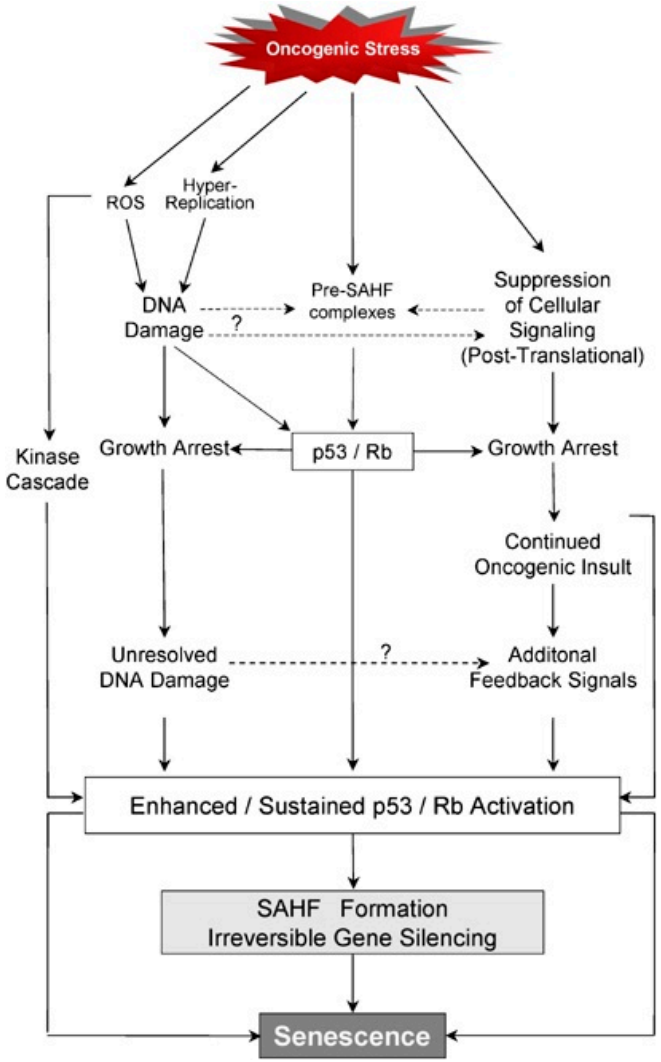
**Beta galactosidase positve cells**
**Big foci of heterochromatin**
**That colocalize with gH2AX**




**Expression of oncogenes mediates increased DNA damage load**
**= tumorsuppressor mechanism**
**→Additional mutations required to escape from tumorsuppression**
**→Cancer formation**

# LOSS OF DICER/DROSHA REDUCES ONCOGENE INDUCES SENESCENCE

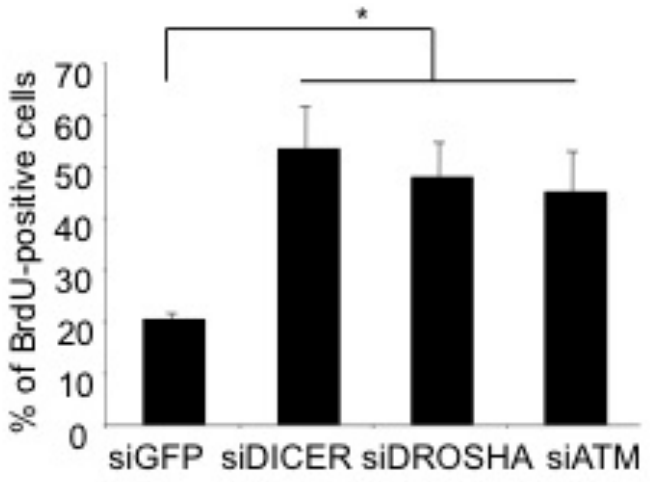



BJ cells retroviraly transduced with a vector Encoding a Ras cDNA containing an ocogenic mutation = **H-RasV12**

**=Oncogene induced senescent cells ("OIS cells")**

**H-RasV12 drives excessive proliferation → Accumulation of DNA damagage → Senescence → SAHF**

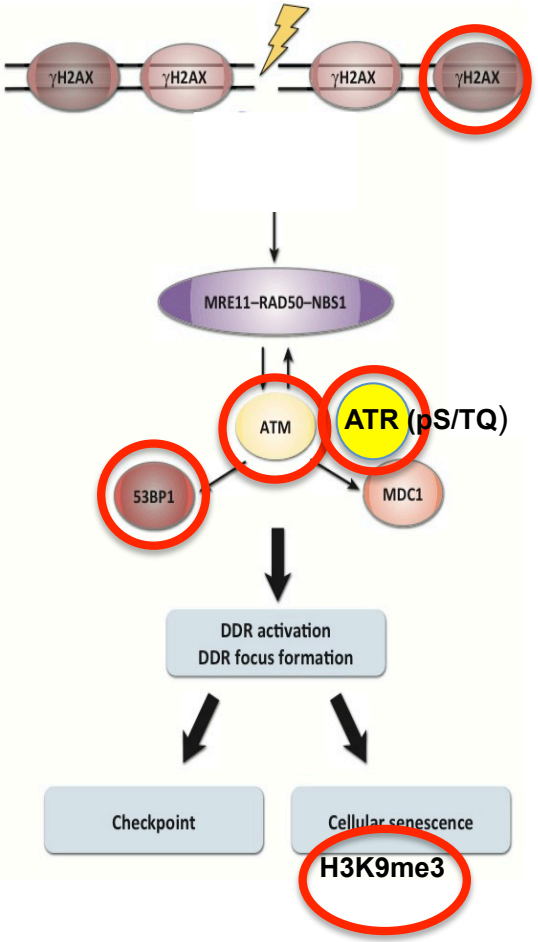
OBSERVATION: Knock-down of DICER and DROSHA or ATM increases cell proliferation markers



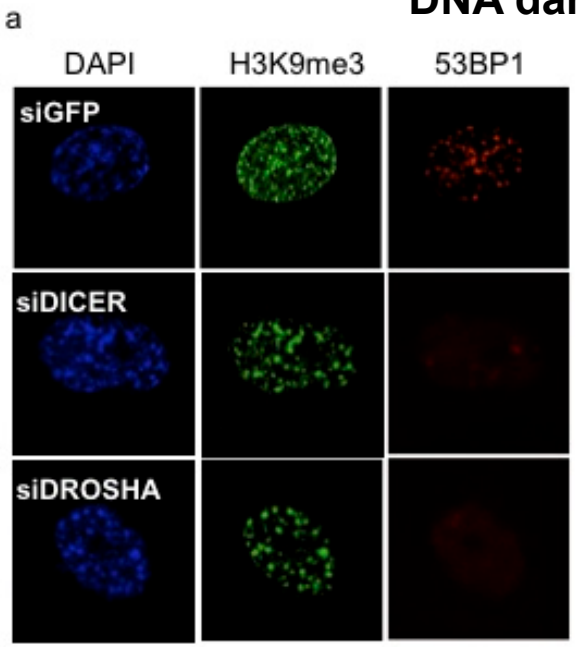

BrdU is incorporated in S-Phase and can be detected

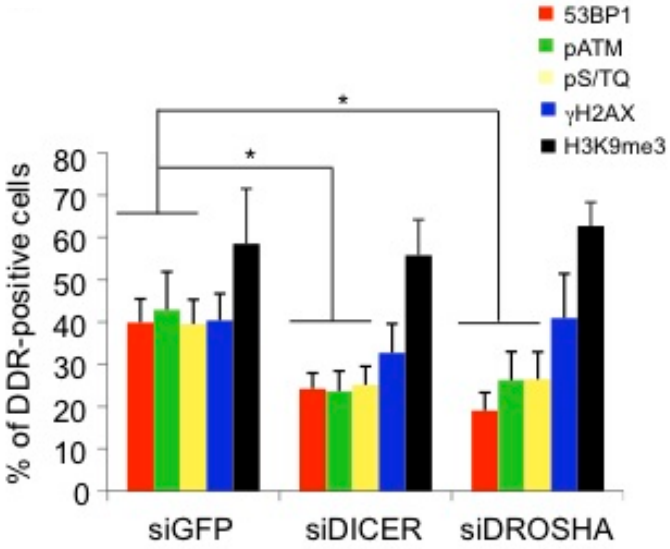
Using an antibody (IF); more BrdU+ cells = more proliferation

# LOSS OF DICER/DROSHA REDUCES DNA DAMAGE SIGNALLING IN OIS CELLS

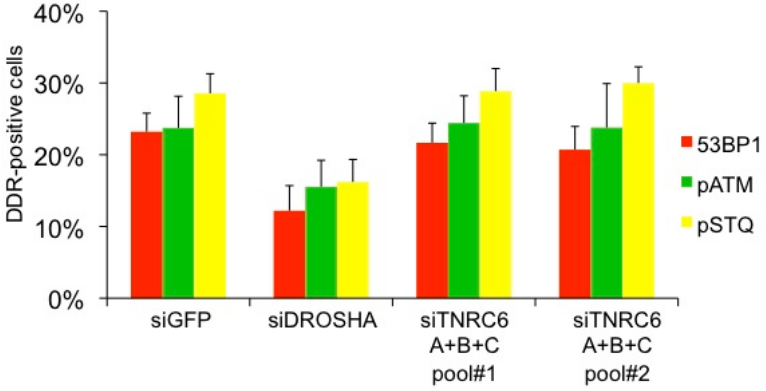



## siRNA/miRNA pathways are important for DNA damage response



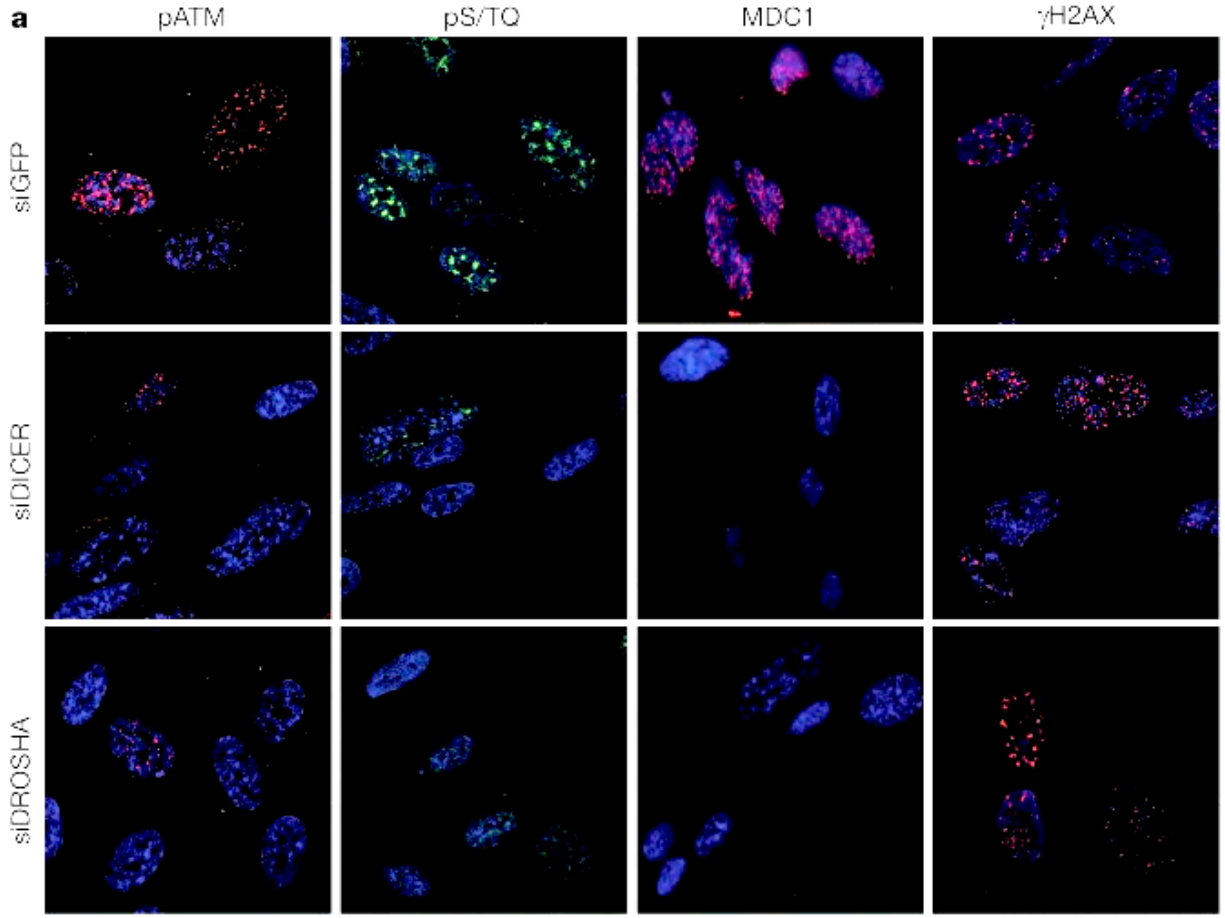




KNOCK DOWN OF GENES IMPORTANT FOR miRNA FUNCTION (TNRC6A,B,C; translational repression) **do not impact on DNA damage response**

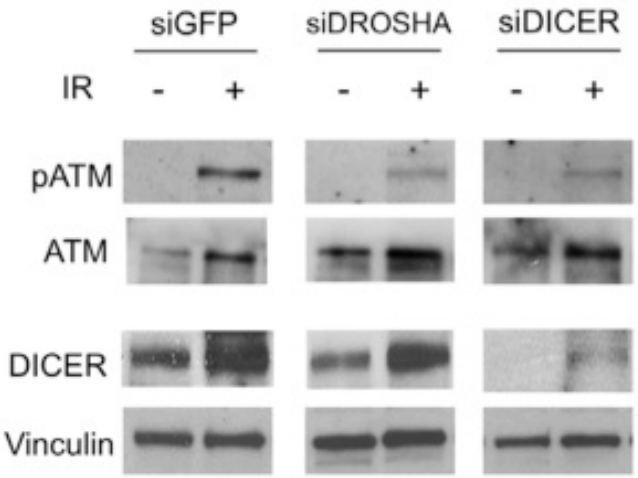


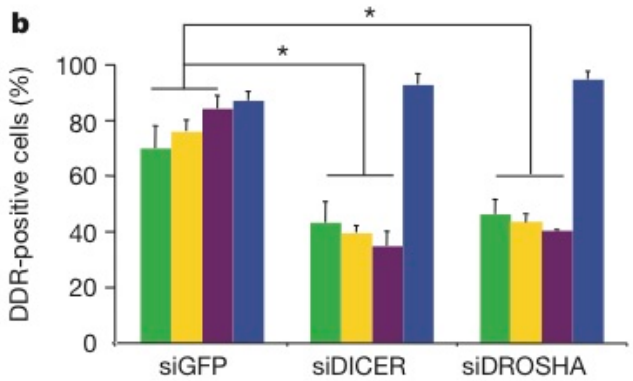

**siRNA PATHWAYS ARE INVOLVED IN THE CONTROL OF DNA DAMAGE RESPONSE**

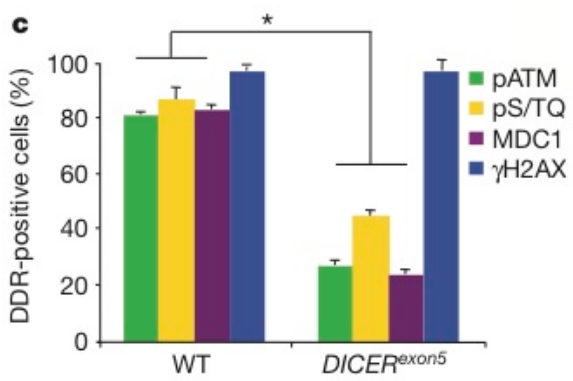
# ANOTHER MODEL: GAMMA IRRADIATION OF NORMAL FIBROBLASTS




**Knock-down of DICER and DROSHA Impairs the activation of a DNA damage response In gamma irradiated cells**



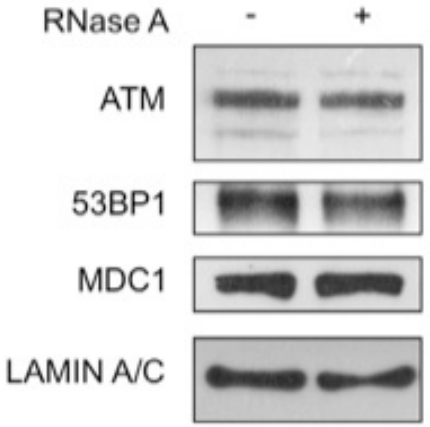
# IS RNA REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?

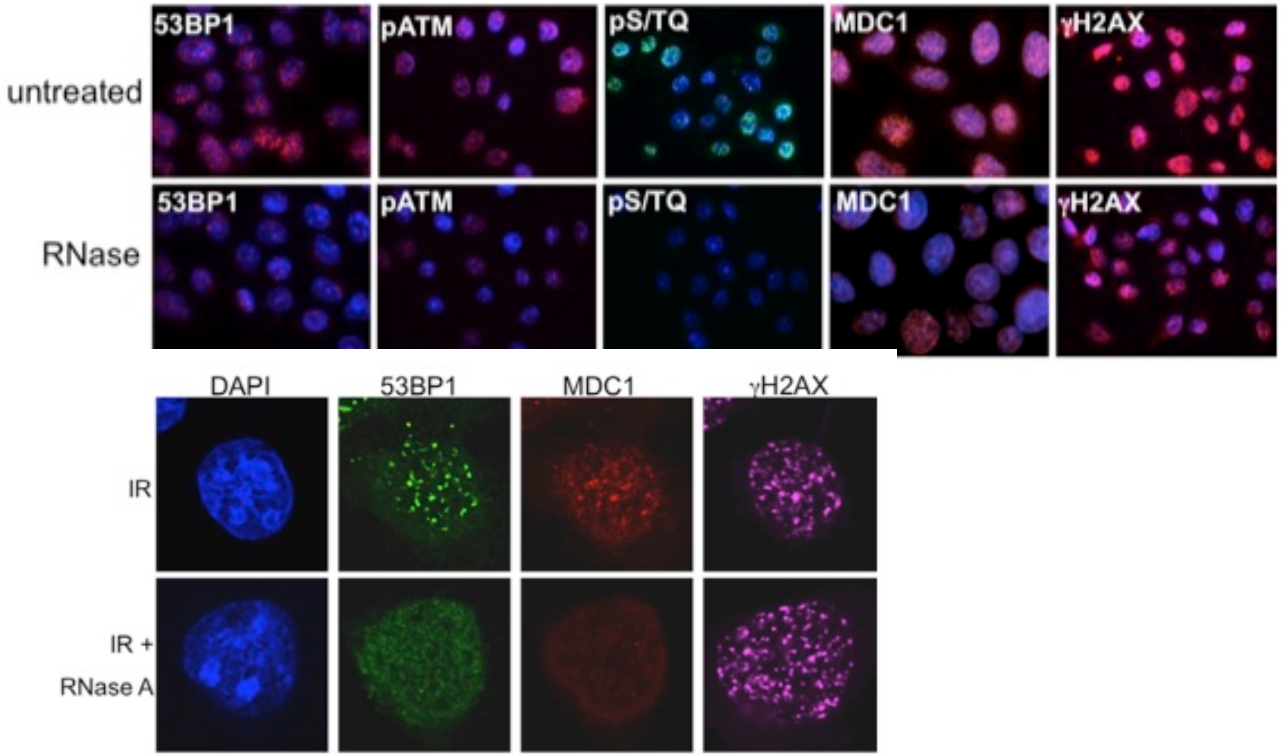
**Model**
**Heterochromatin**

Permeabilize Cells (detergent) → Treat with RNase → Heterochromatin Proteins are lost

**Model**
**DNA damage**

HeLa cells + Gamma irradiation+ permeabilize → Treat with RNAse → Check protein Markers for DNA damage



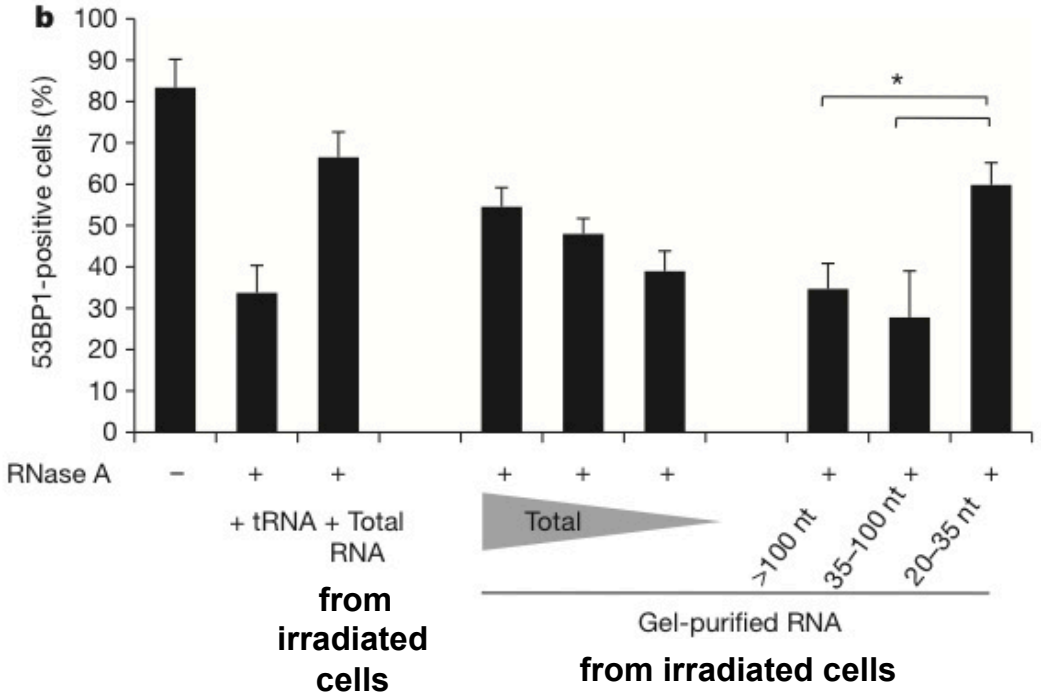

**RNase treatments reduces the amount of DNA damage markers after gamma irradiation**

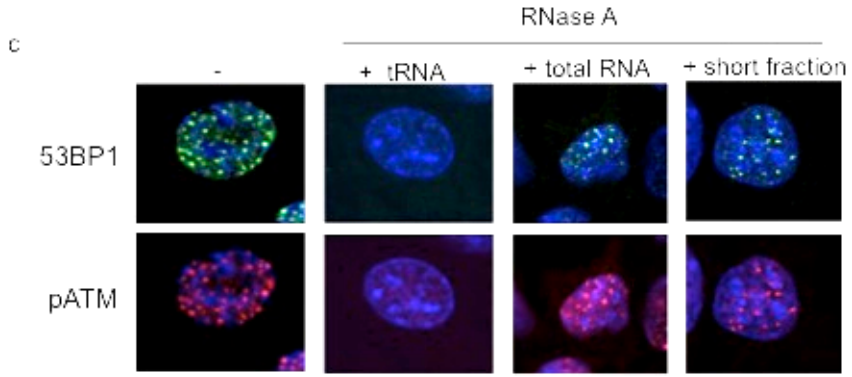
# WHAT KIND OF RNA IS REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?

**Model DNA damage**

HeLa cells + Gamma irradiation+ permeabilize → Treat with RNAse → → Check protein Markers for DNA damage

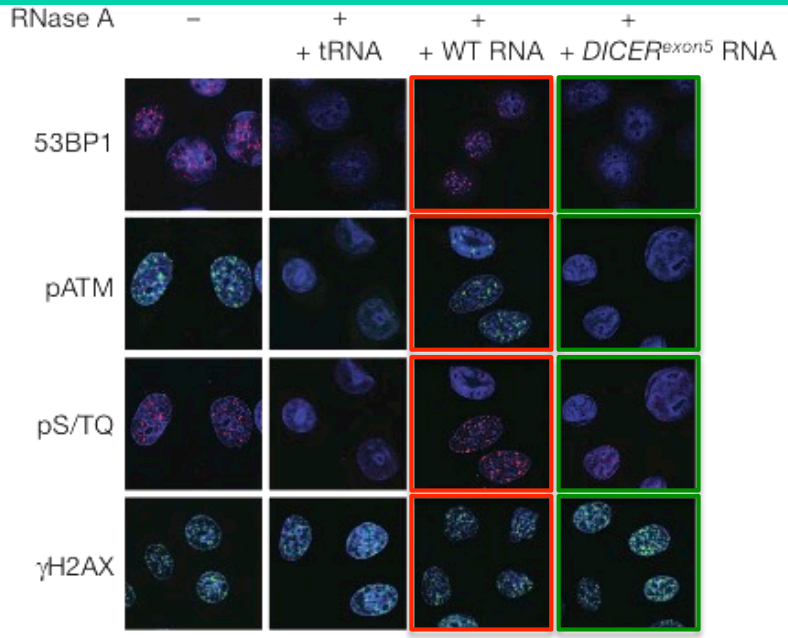
→ Add different kinds of RNA → Check protein Markers for DNA damage



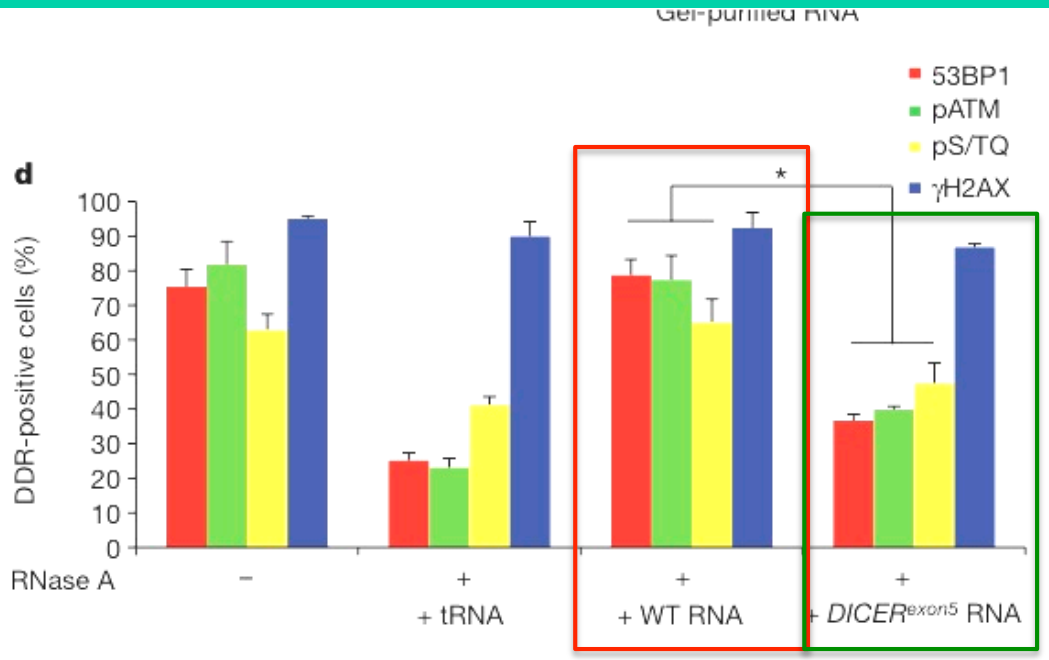

**A short RNA fraction (20-35 nt) rescues DNA damage response after RNAse treatment**
**= POTENTIAL DICER/DROSHA PRODUCTS**

# WHAT KIND OF RNA IS REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?




**RNA From Irradiated Dicer wt Cells = Small RNAs present**

**RNA From Irradiated Dicer KO Cells = NO small RNAs**



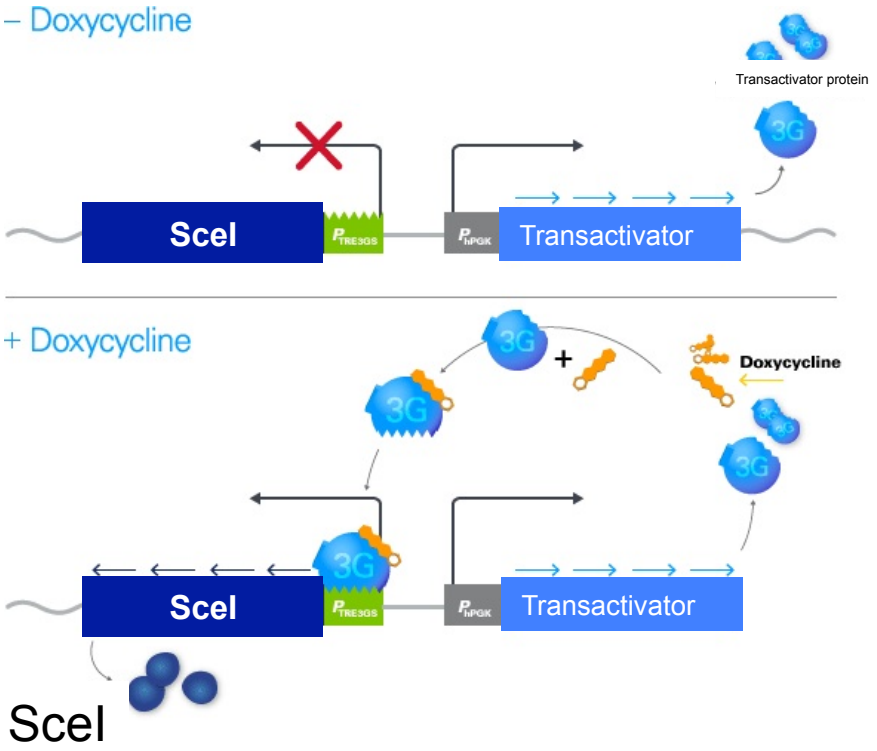

Total RNA from Dicer null cells cannot rescue defects of DNA damage foci formation after RNAse treatment

**→Dicer has a critical role in DNA damage response**

**CAN SMALL RNAs = DDRNAs (DNA DAMAGE RESPONSE RNAs) ACT DURING DNA DAMAGE RESPONSE AT A DEFINED SITE IN THE GENOME**

# A MODEL SYSTEM TO STUDY THE KINETICS OF DNA DAMAGE

**Cell line:**
Contains
1. An inducible transactivator
2. the restriction enzyme SceI under the control of a inducible promoter
3. A SceI site between Lac Repressor DNA sequences
4. The Lac Repressor that binds DNA seqeuences around the SceI sites




SceI

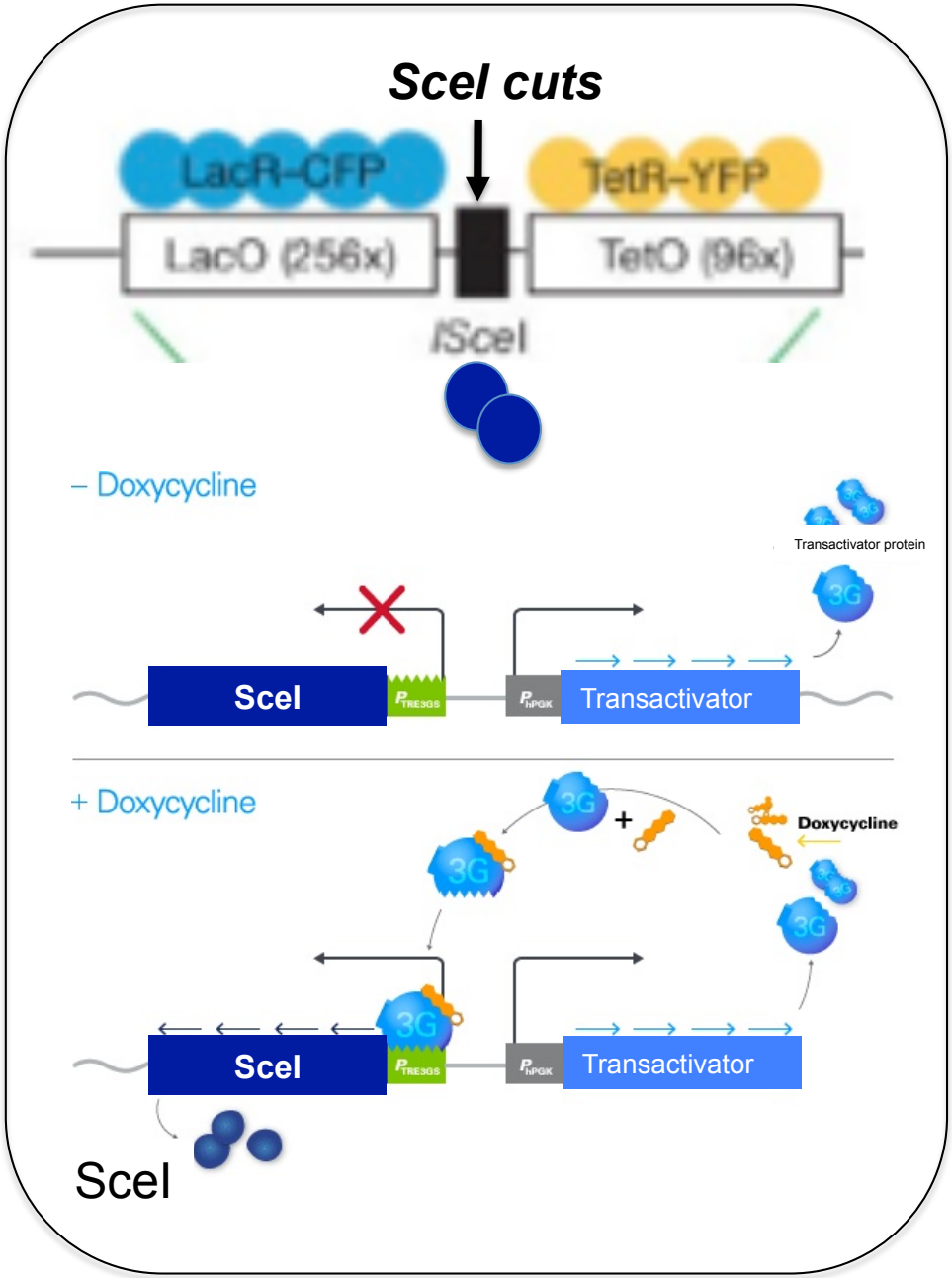
5′...TAGGGATAACAGGGTAAT...3′
3′...ATCCCTATTGTCCCATTA...5′

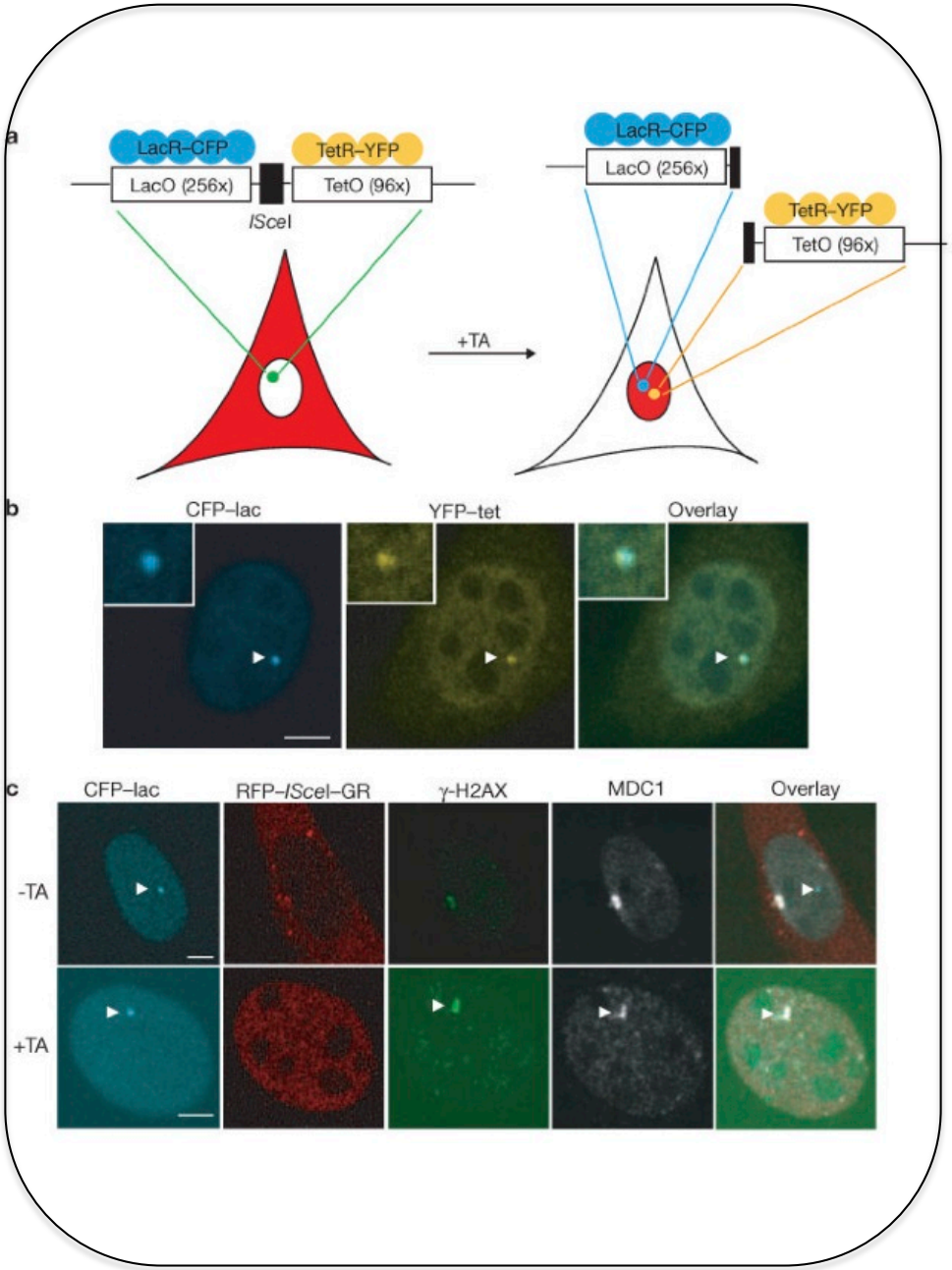
**SceI is a restiction enzyme That does not cut in the human genome**

***Inducing SceI expression does not cut genomic DNA in human cells!***

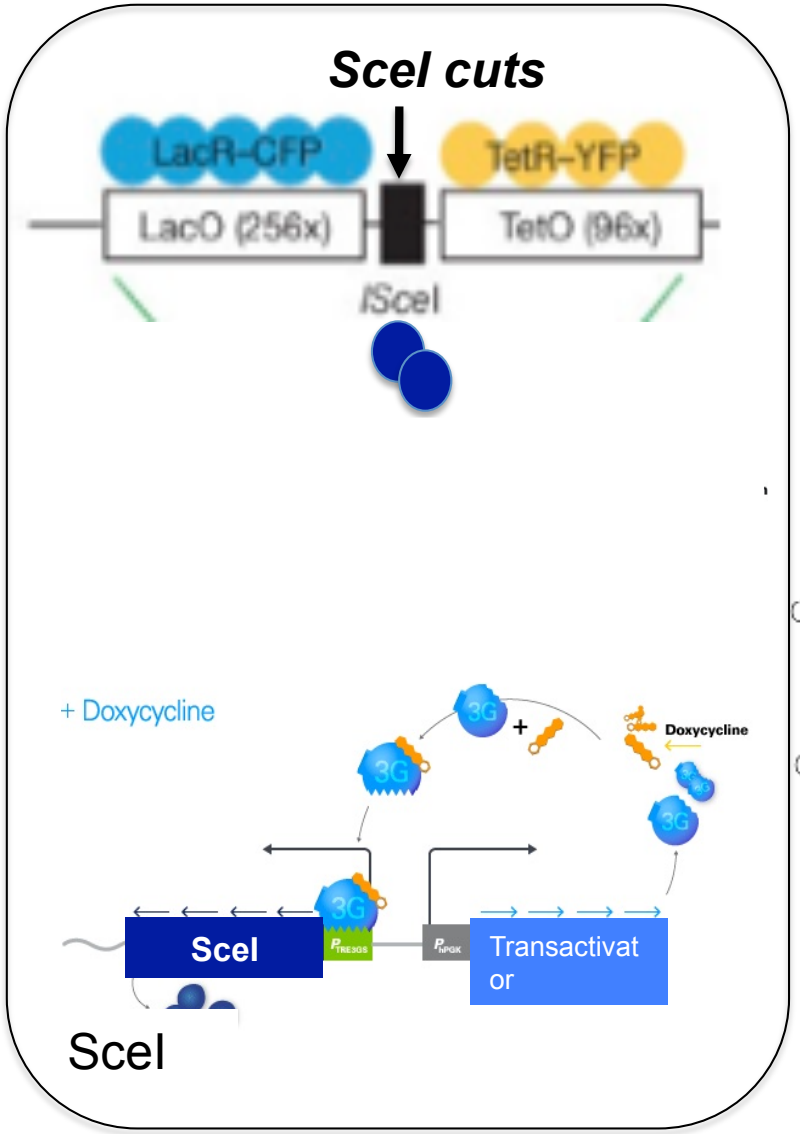
***LETS INTRODUCE A SceI SITE AND MARK THE SceI SITE USING SEQEUNCES BOUND BY THE Lac REPRESSOR***

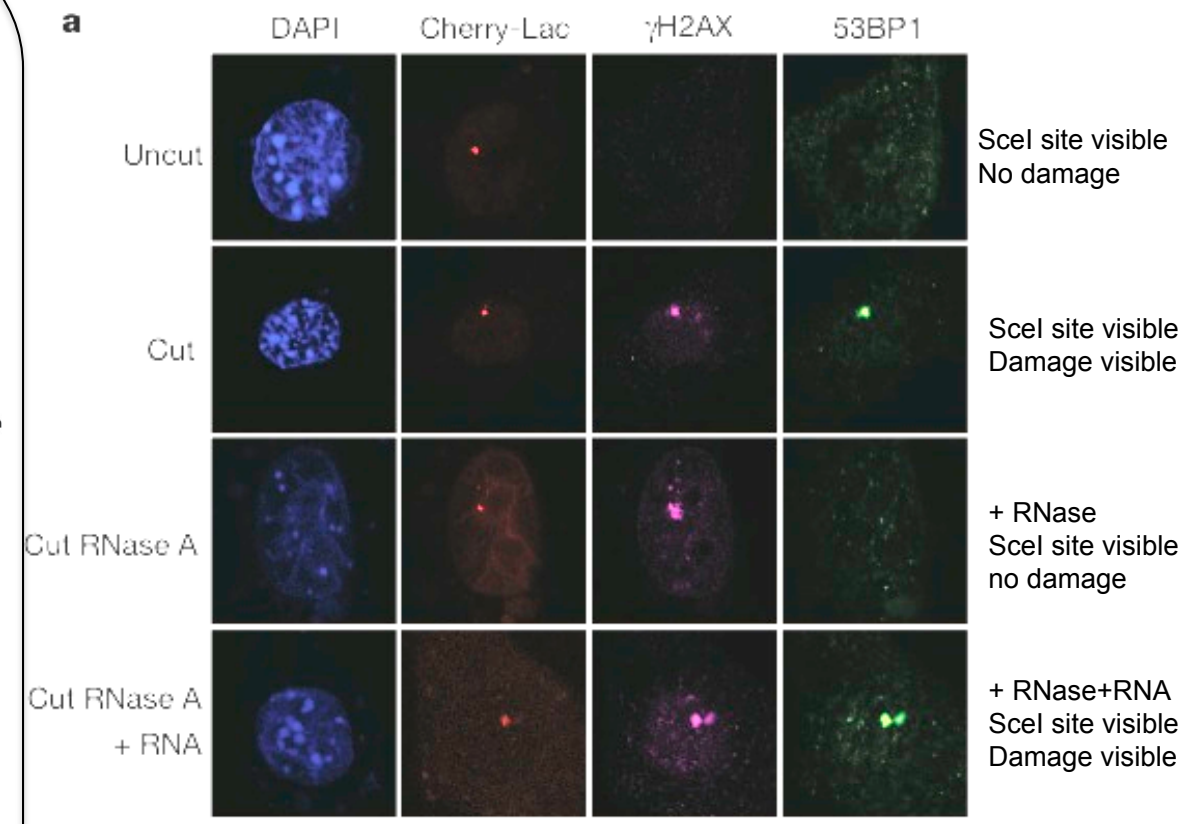
# A MODEL SYSTEM TO STUDY THE KINETICS OF DNA DAMAGE



# DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE

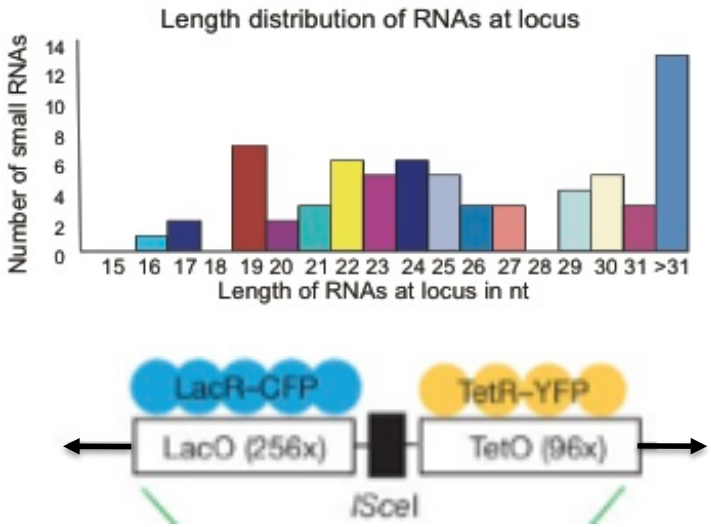



**→ RNA was prepared from cells that have cut the SceI site**
**→ RNA from cells that do not have the SceI site but express SceI cannot rescue!!!!!**

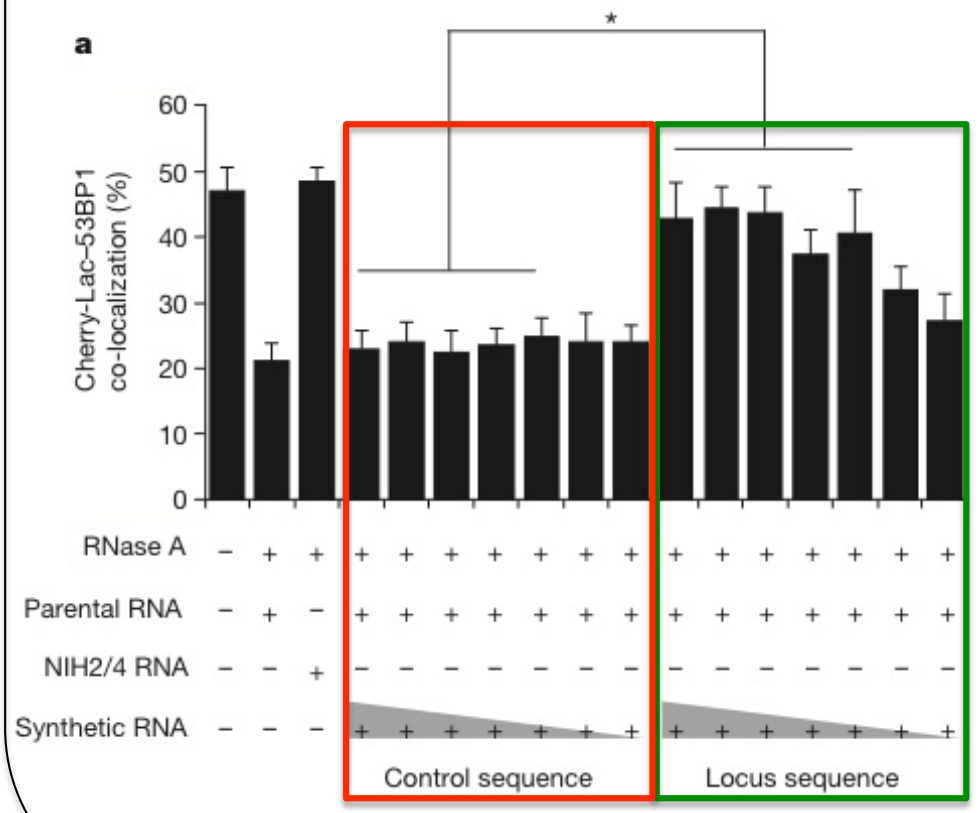
# DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE - EVIDENCE 1

**Take SceI cells:**
**-induce cutting**
**- prepare RNA**
**-make RNASeq**
**detect small RNAs from locations around the SceI sites**



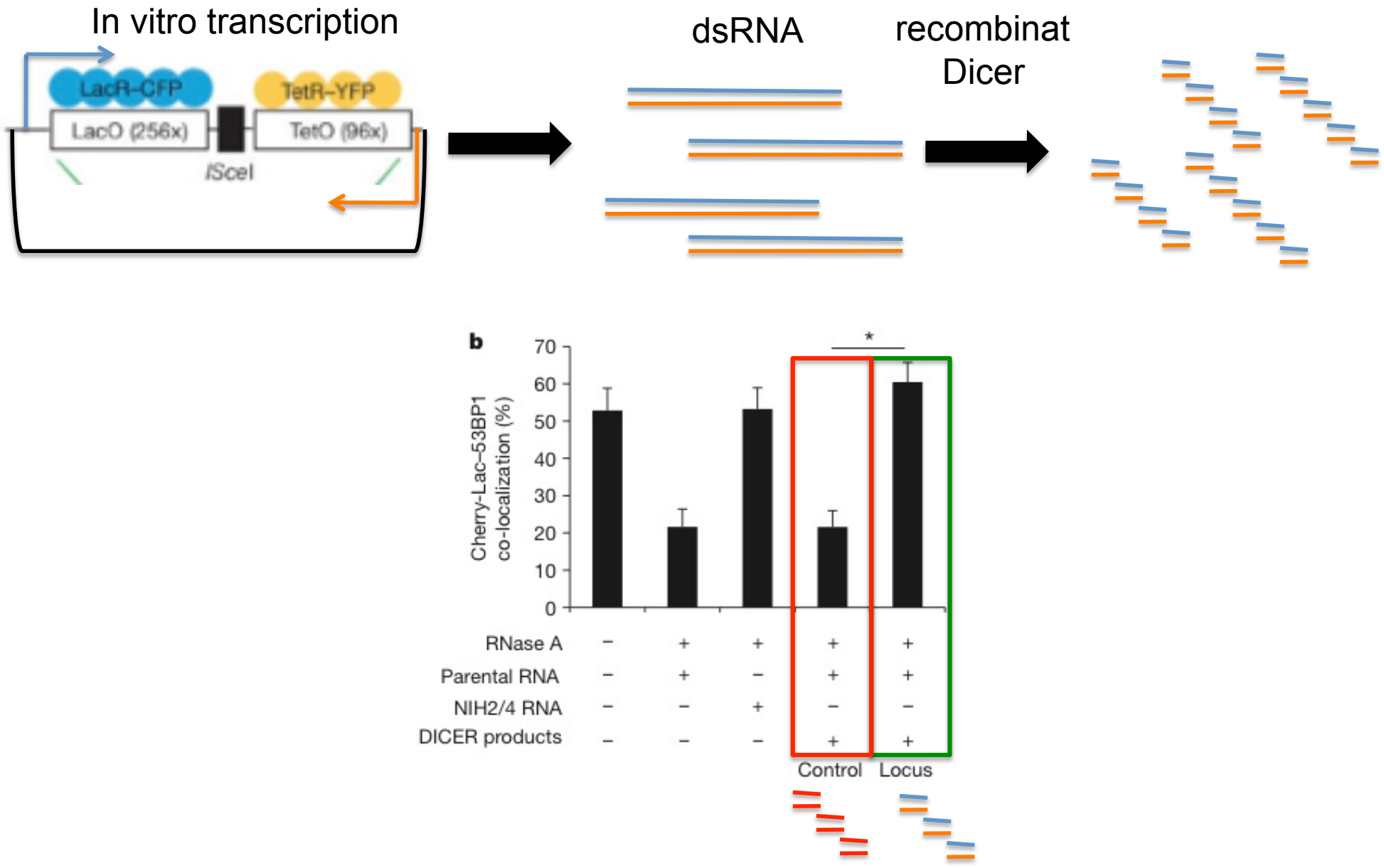

**!!!!!Small RNAs (16-31) arising from regions around the SceI site can be identified !!!!!**

**Small RNAs with sequences that resemble the region around the SceI site can rescue the formation of DNA damage foci at the SceI site**



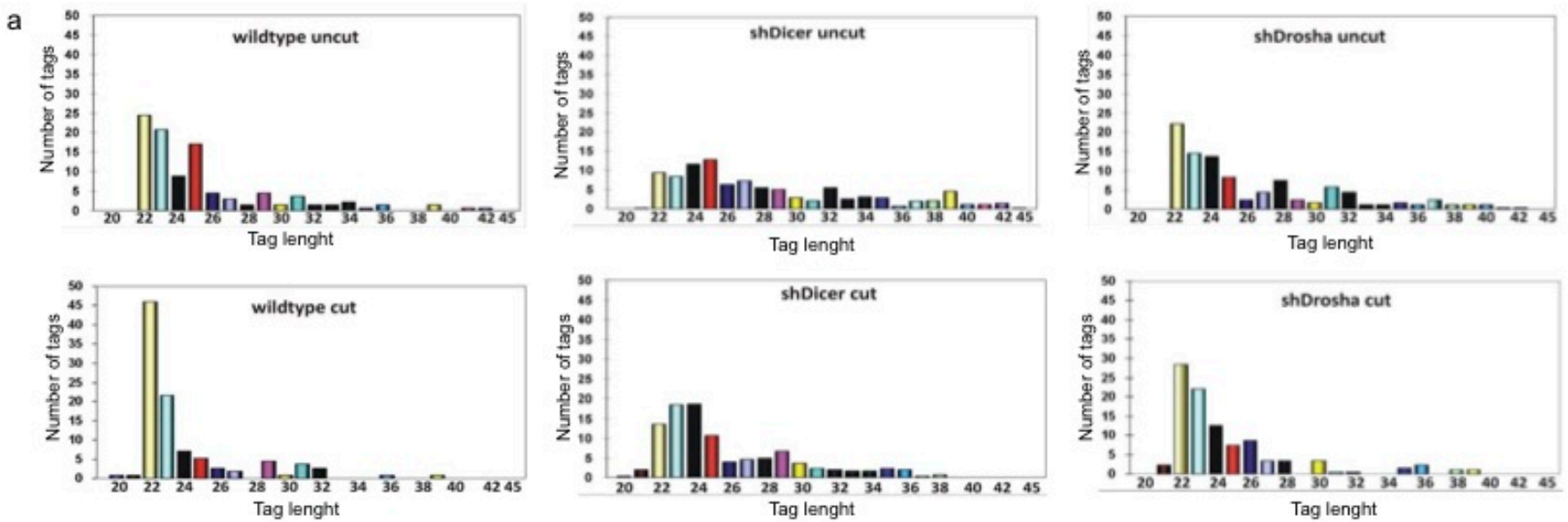

SceI region

# DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE - EVIDENCE 2



# DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE - EVIDENCE 2

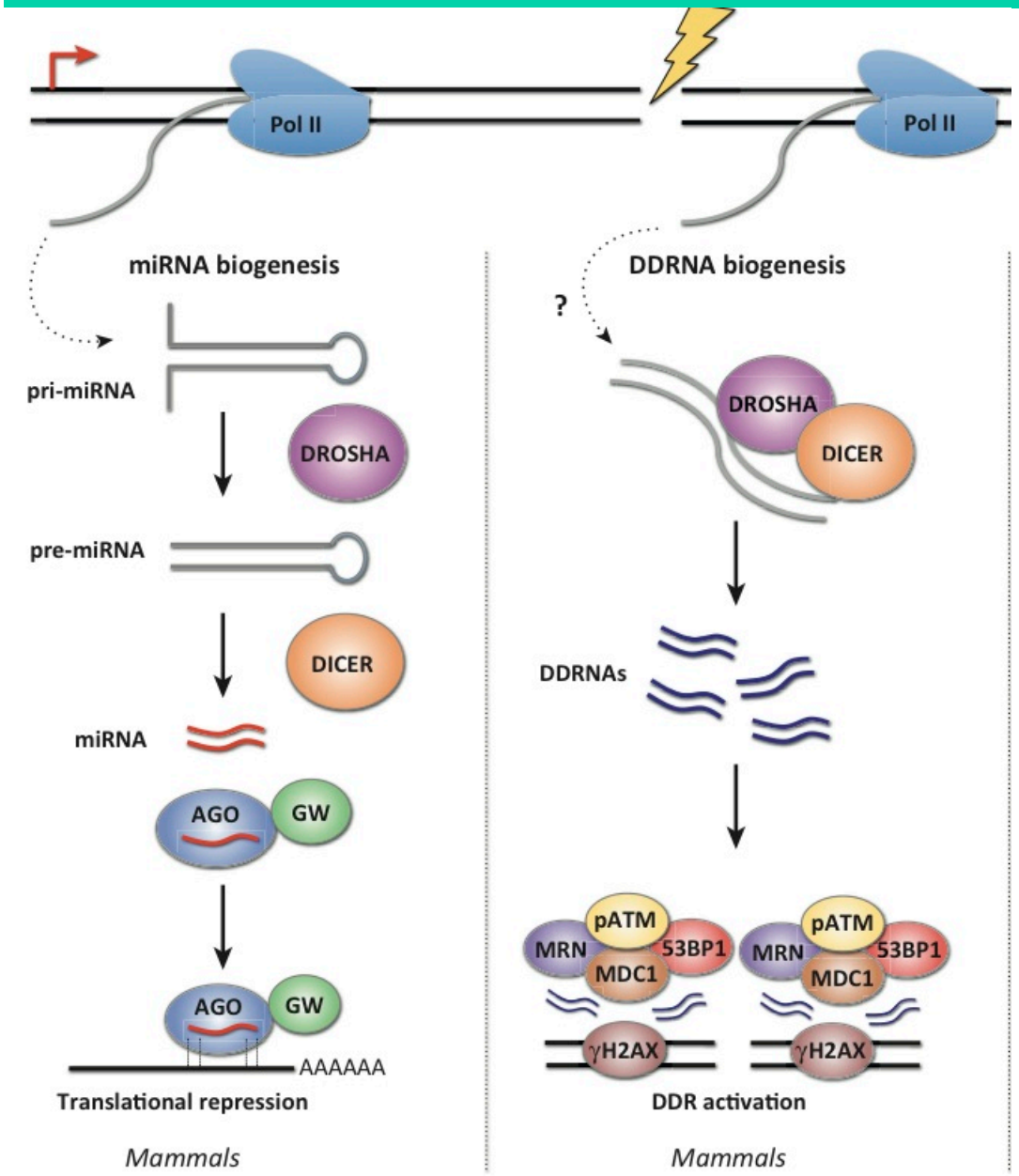
**Take SceI cells**
**- Knock down DICER or DROSHA:**
**-induce cutting**
**- prepare RNA**
**-make RNASeq**
**detect small RNAs from locations around the SceI sites**

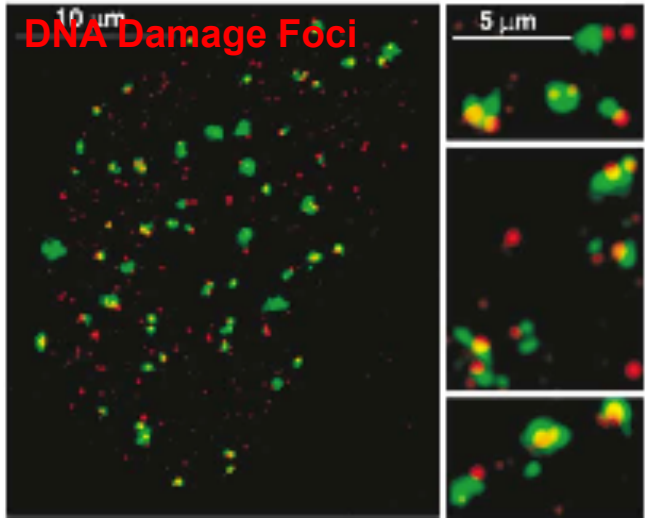



*DICER RNAi* *DROSHA RNAi*

**Small RNAs (16-31) arising from**
**regions around the SceI site is reduced in DICER/DROSHA knock-down cells**

# DNA DAMAGE RESPONSE RNAs (DDRNA) CONTROL THE DNA DAMAGE RESPONSE




**γH2AX**
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In summary, we demonstrate that different sources of DNA damage, including oncogenic stress, ionizing radiation and site-specific endonucleases, activate the DDR in a manner dependent on DDRNAs, which are DICER- and DROSHA-dependent RNA products with the sequence of the damaged site. DDRNAs control DDR foci formation and maintenance, checkpoint enforcement and cellular senescence in cultured human and mouse cells and in different cell types in living zebrafish larvae. They act differently from canonical miRNAs, as inferred by their demonstrated biological activity independent of other RNAs and of GW182-like proteins.