



# Promotion of homologous recombination and genomic stability by **RAD51AP1**

Claudia Wiese, Ph.D.

Scientist

Life Sciences Division

Lawrence Berkeley National Laboratory

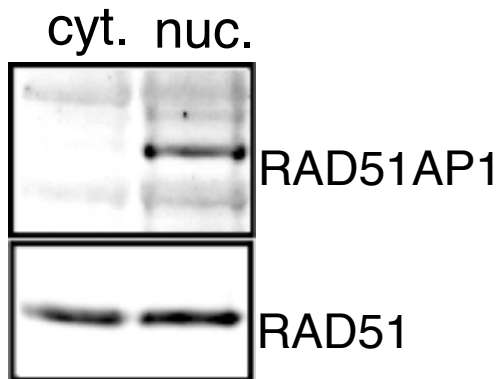
# **RAD51AP1: RAD51 Associated Protein 1**

**[aka: PIR51 (human) and RAB22 (mouse)]**

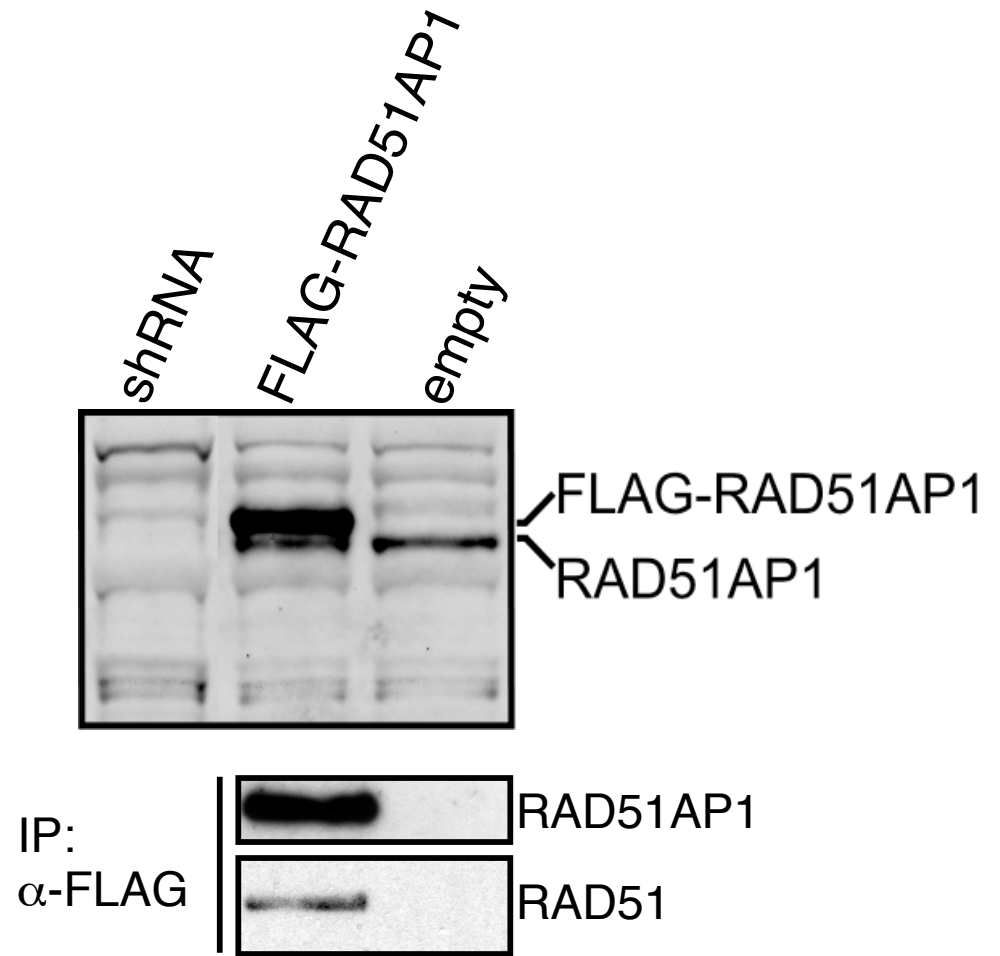
- **RAD51-interacting protein\***  
[Y2H library screens: C. Radding (PIR51), F. Alt (RAB22), both in 1997].
- **vertebrate-specific**
- **binds DNA and RNA**
- **upregulated:**    **hepatocellular carcinoma (Song *et al.*, 2004)**  
                          **aggressive lymphoma (Henson *et al.*, 2006)**  
                          **cholangiocarcinoma (Obama *et al.*, 2008)**
- **function *in vivo* previously unknown**

\* no sequence homology with RAD51

**RAD51AP1: is localized to the nucleus and associates with RAD51 (IP)**

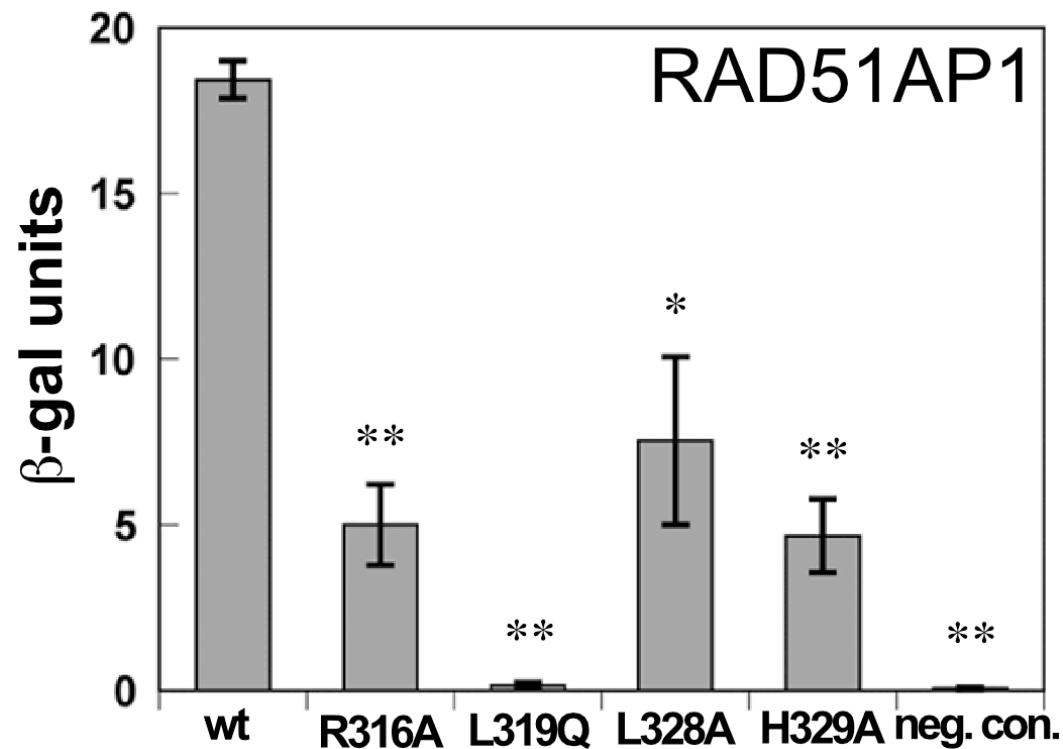
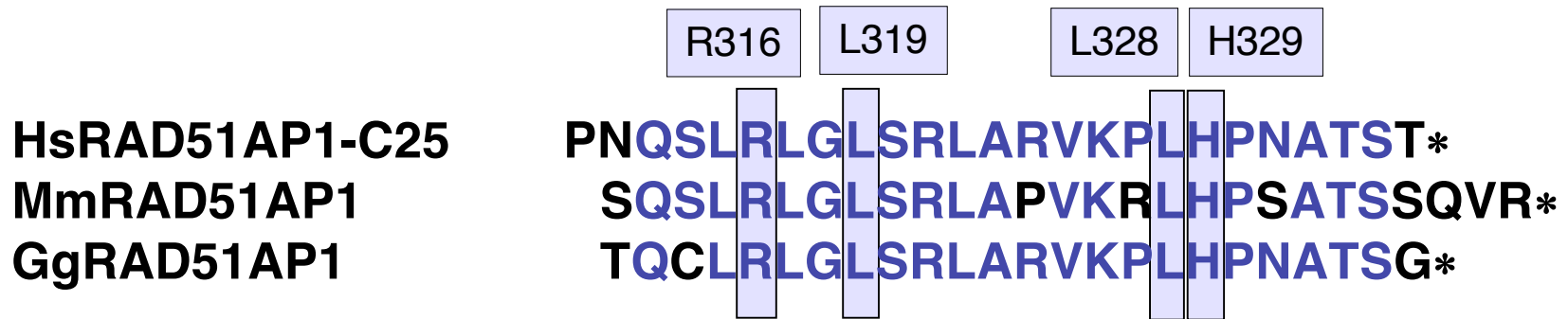


HeLa cells

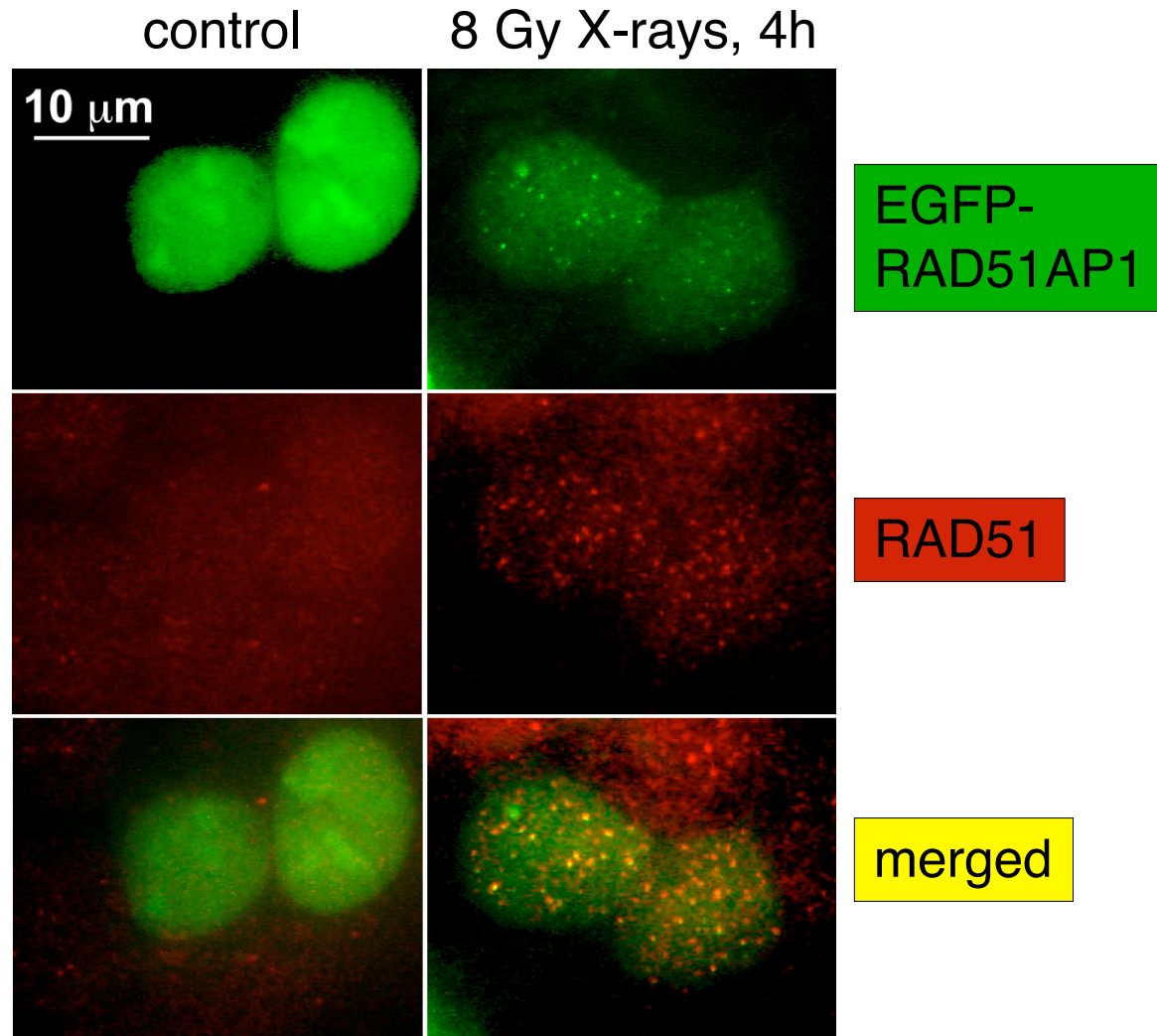


WTK1 cells

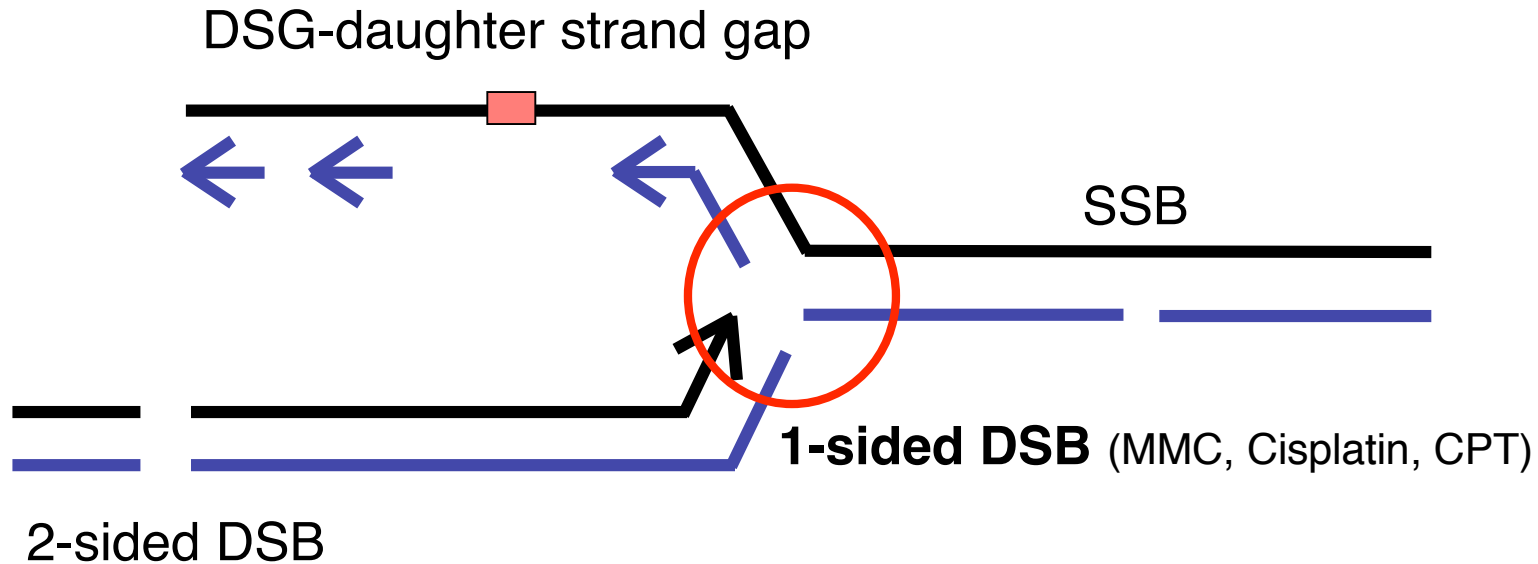
## Yeast 2-Hybrid system to test for loss of RAD51AP1-RAD51 interaction



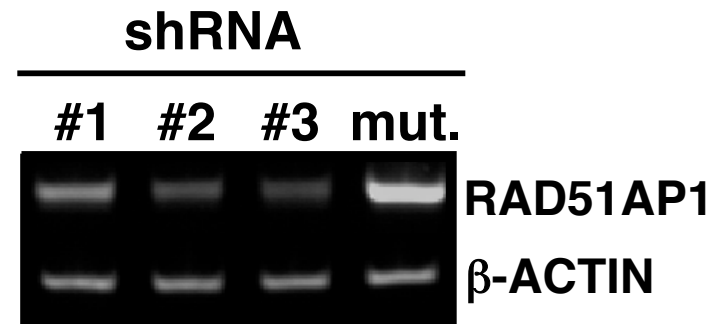
**EGFP-RAD51AP1 forms foci after X-rays:  
partial co-localization with RAD51 (U2OS cells)**



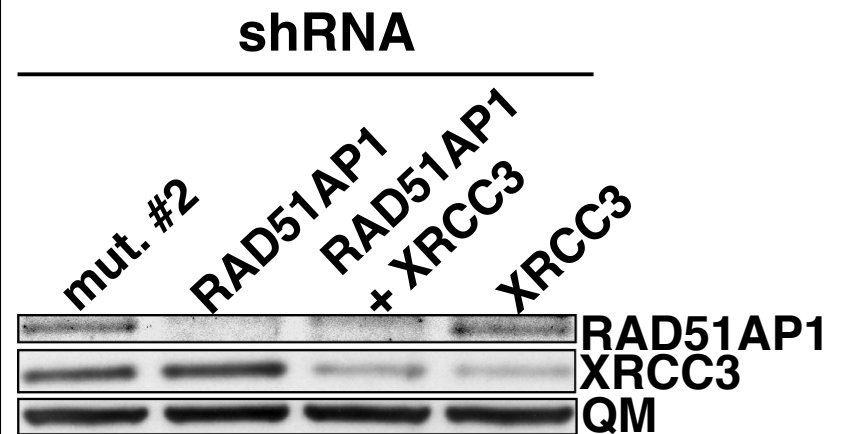
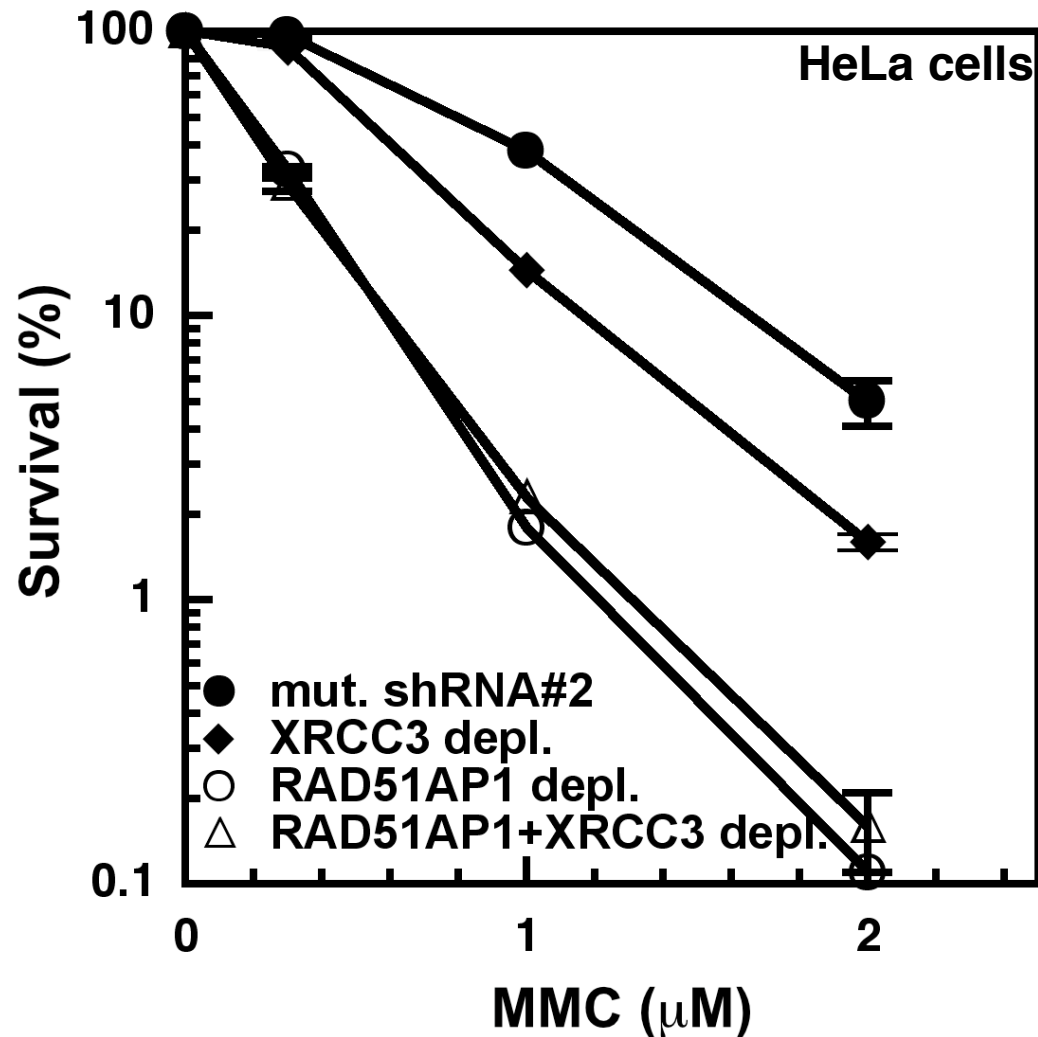
**DNA repair by homologous recombination is particularly important for the repair of damaged replication forks**



**Functional loss of *RAD51AP1*:**

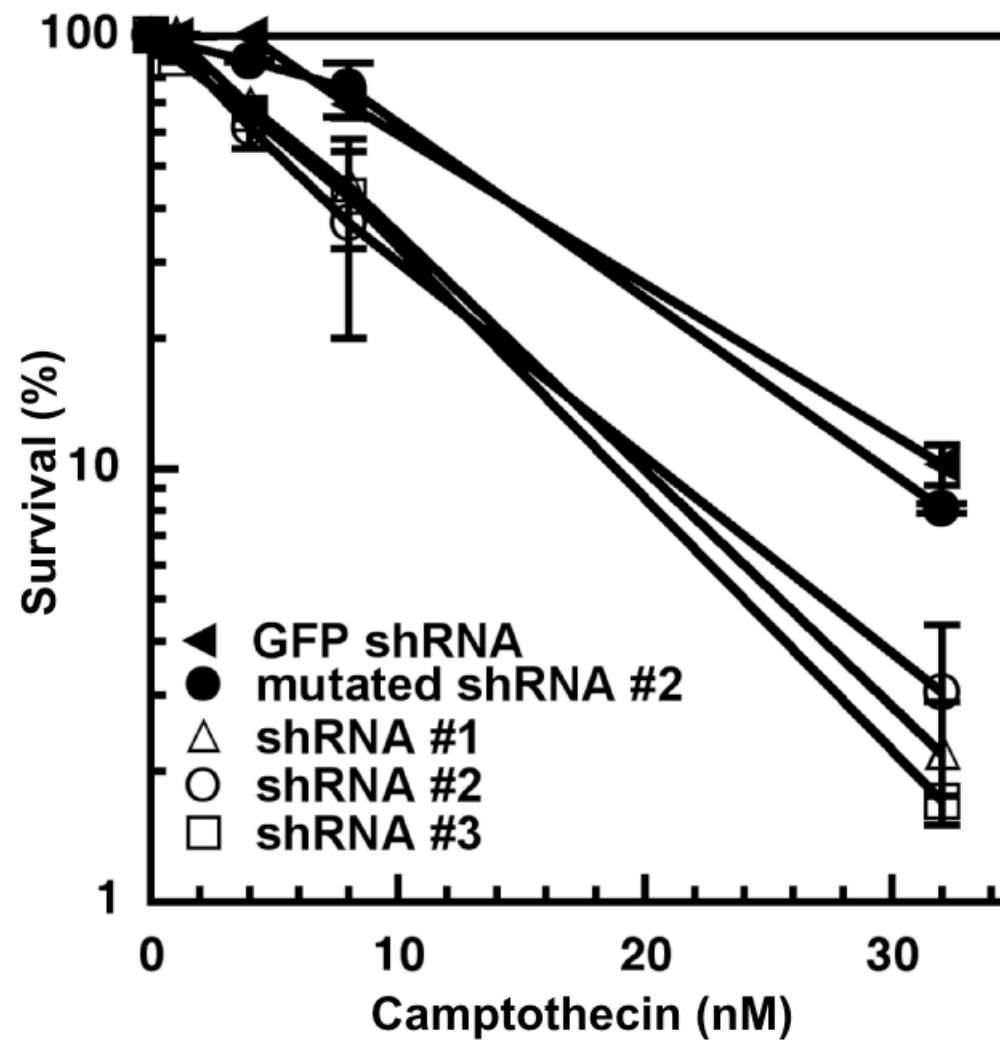


# RAD51AP1 and XRCC3 are within the same epistasis group



C. Wiese, E. Dray, T. Groesser, J. San Filippo, I. Shi, D.W. Collins, M.-S. Tsai, G. J. Williams, B. Rydberg, P. Sung & D. Schild (2007). *Mol Cell*, 28, 482-490.

## RAD51AP1 knockdown: sensitizes HeLa cells to Camptothecin





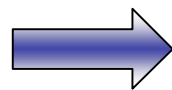
## **RAD51AP1: Rad51 Associated Protein 1**

**[aka: PIR51 (human) and RAB22 (mouse)]**

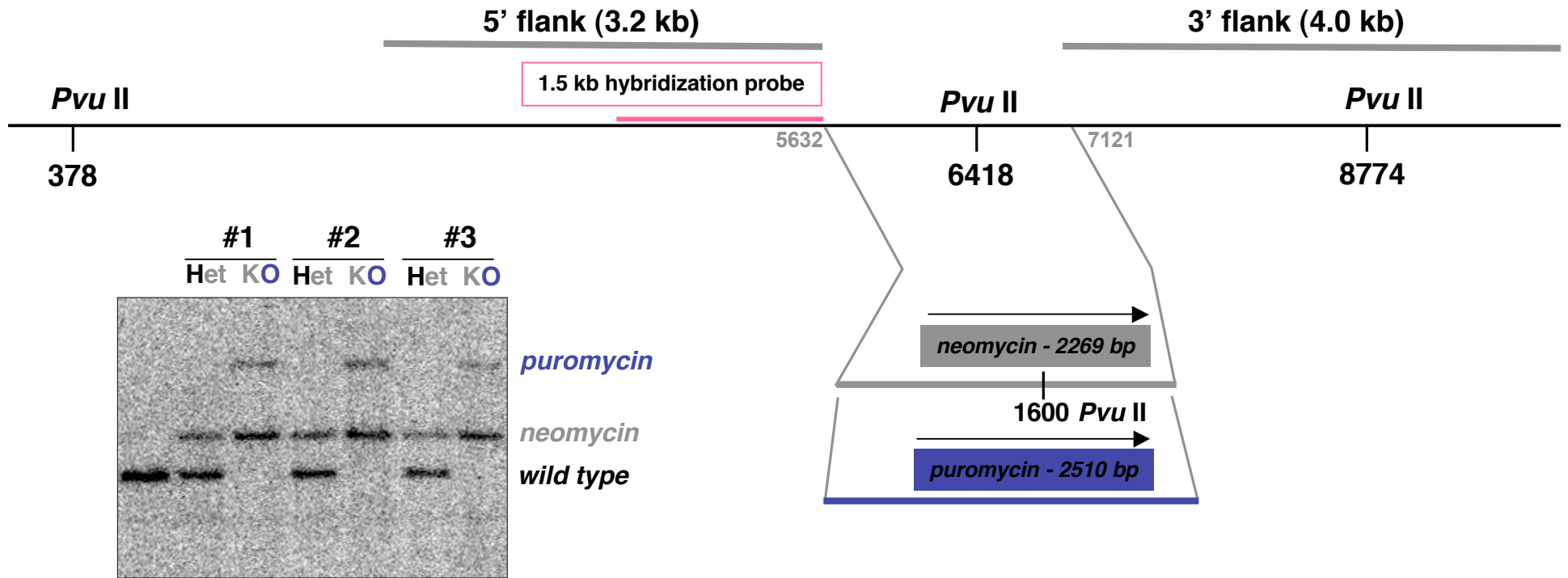
➤ **RAD51-interacting protein\***

**[Y2H library screens: C. Radding (PIR51), F. Alt (RAB22), both in 1997].**

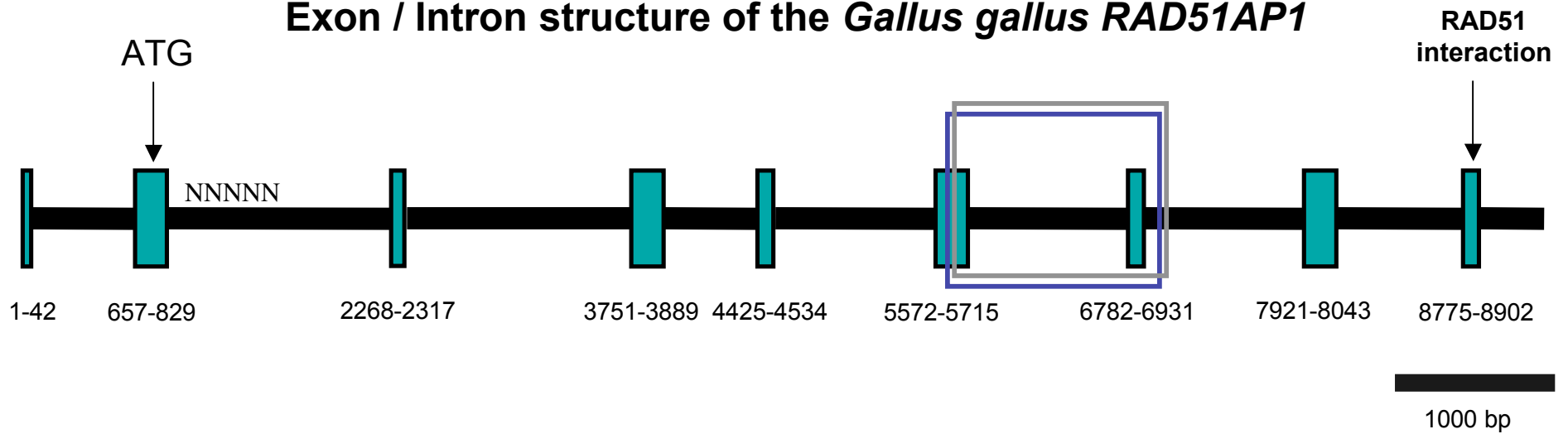
➤ **vertebrate-specific:           no orthologues in yeast or flies**



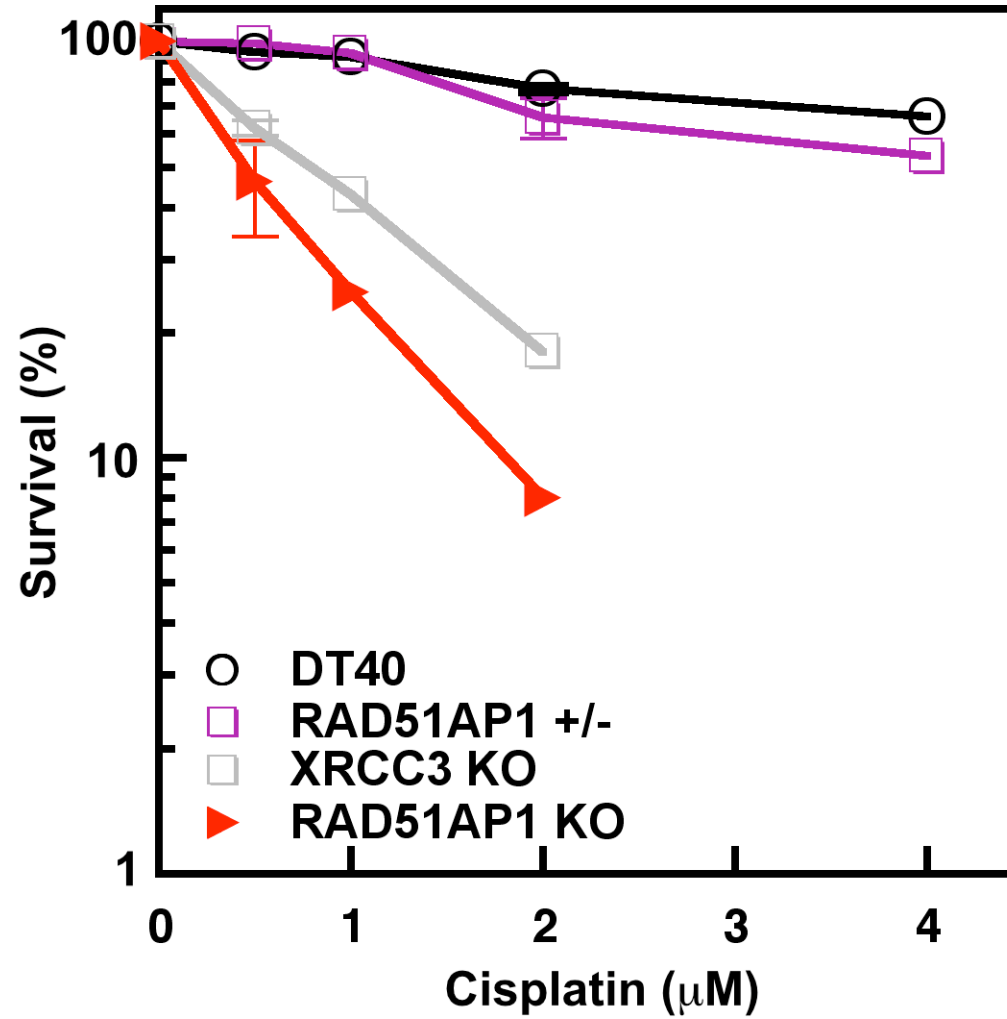
**chicken DT40 cell line**



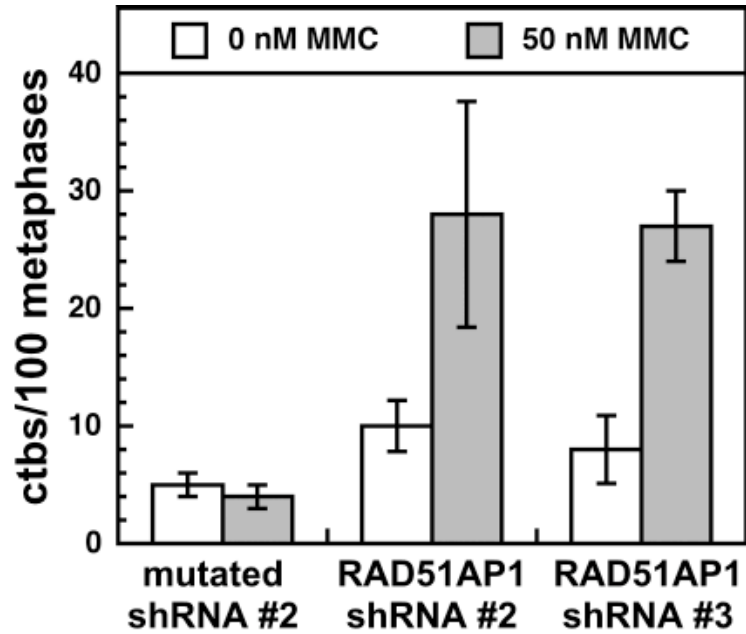
### Exon / Intron structure of the *Gallus gallus* RAD51AP1



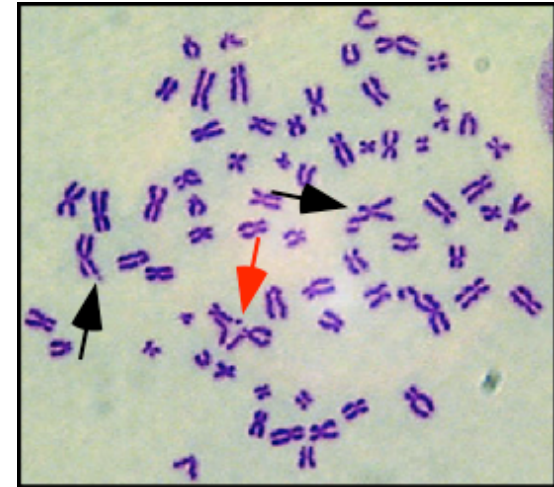
**A *rad51ap1*-deficient DT40 cell line is sensitive to Cisplatin**



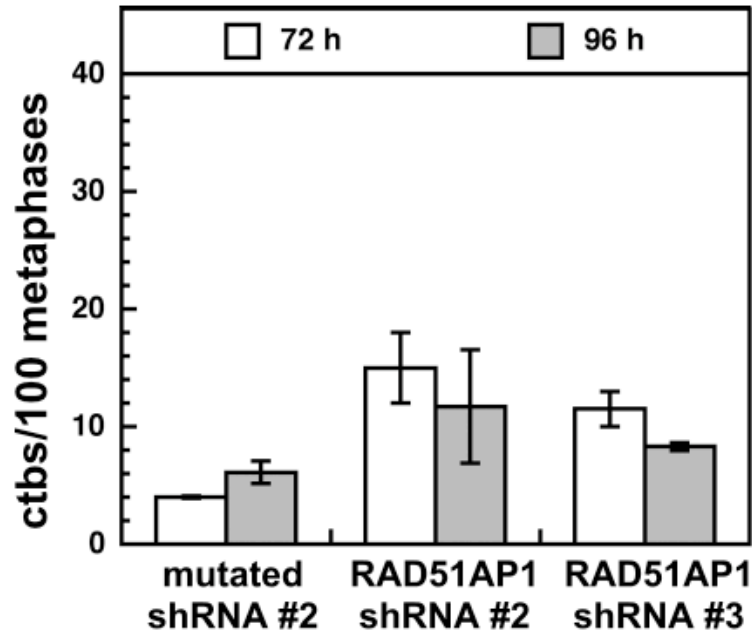
# RAD51AP1 knockdown: increases chromatid-type aberrations



MMC

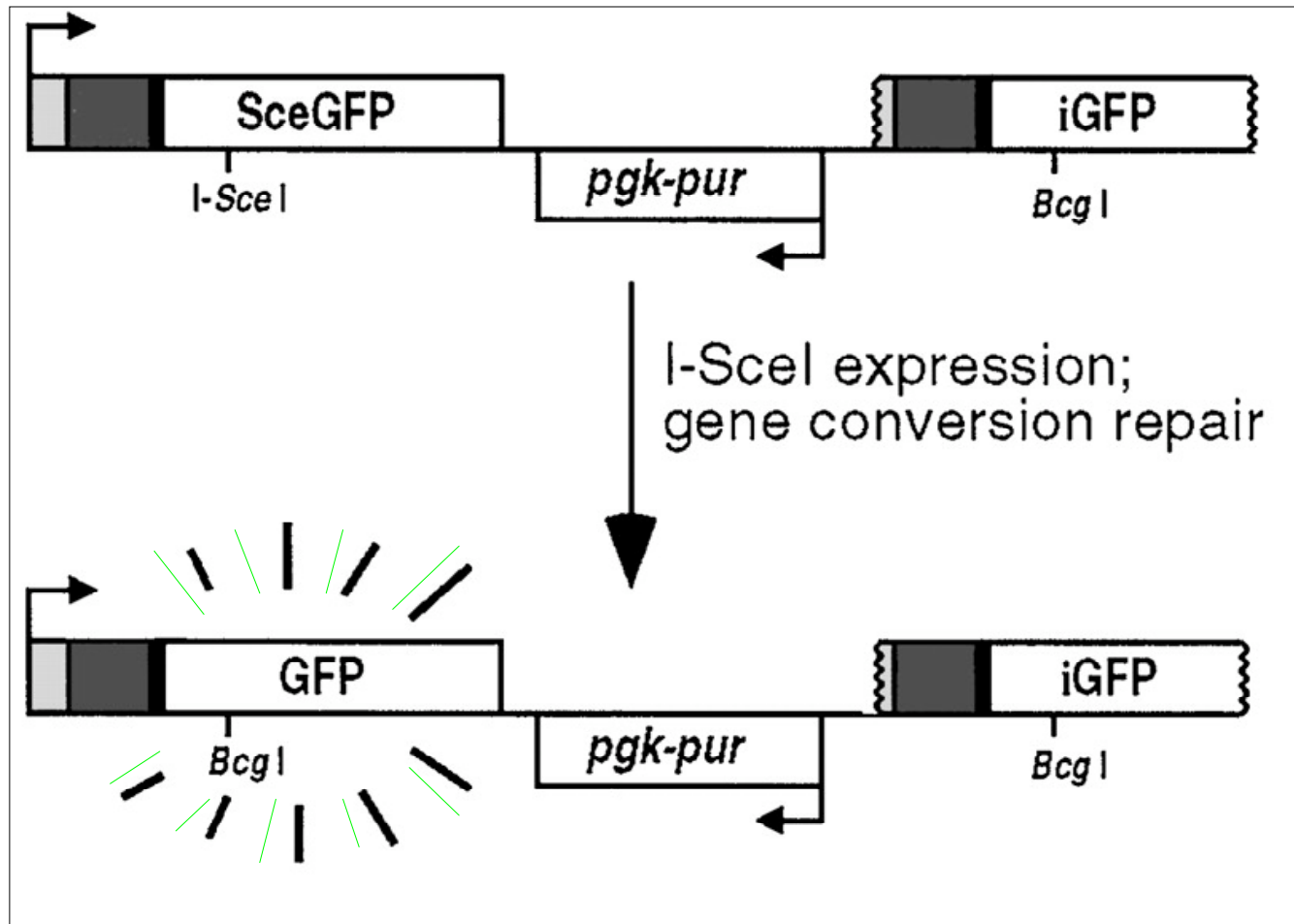


HeLa cells



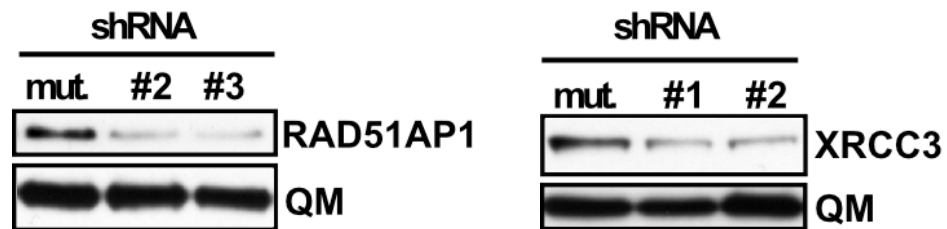
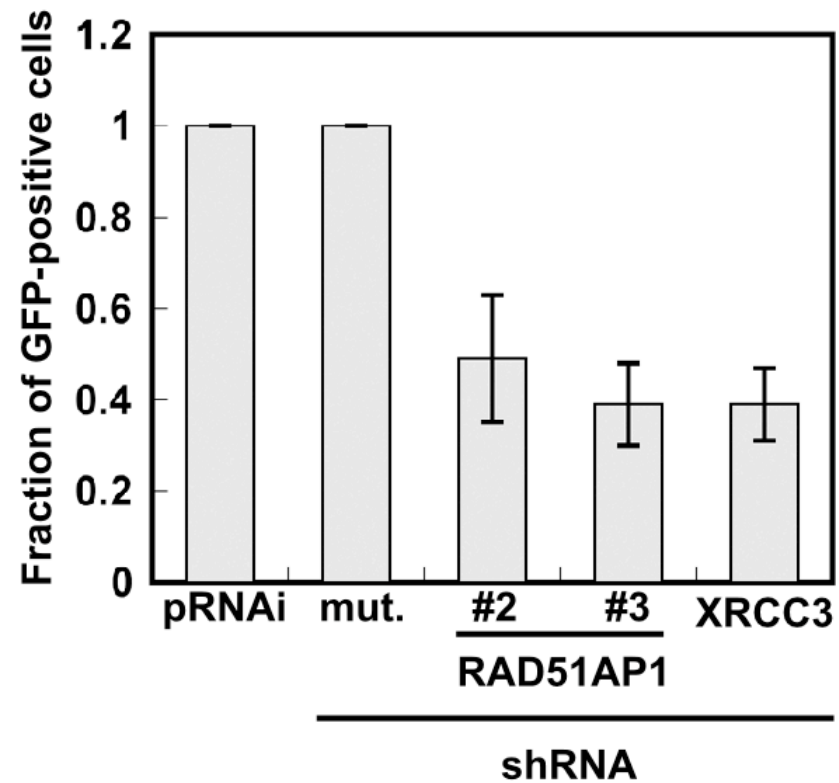
spontaneously

## RAD51AP1 knockdown: reduces homology-directed repair in TK6 cells

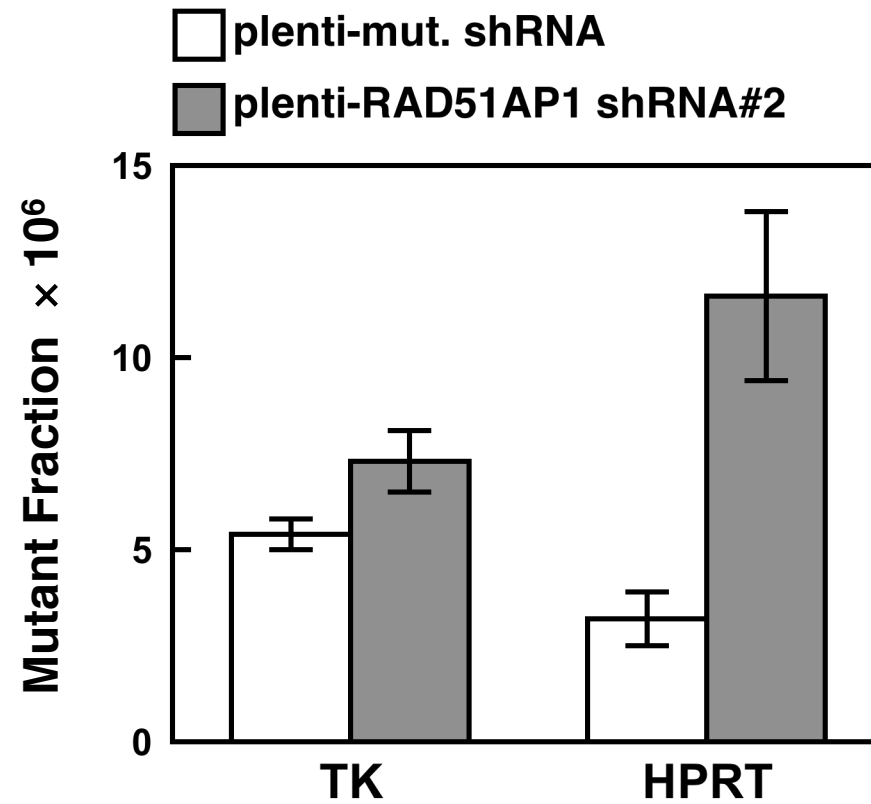
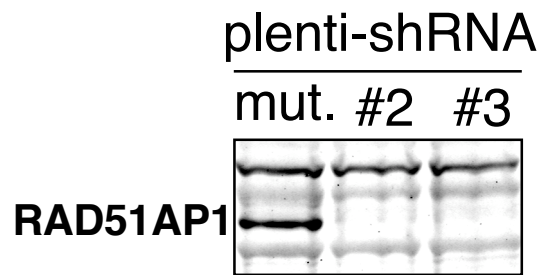


A.J. Pierce, R.D. Johnson, L.H. Thompson & M. Jasin (1999). *Genes Dev*, 13, 2633-8.

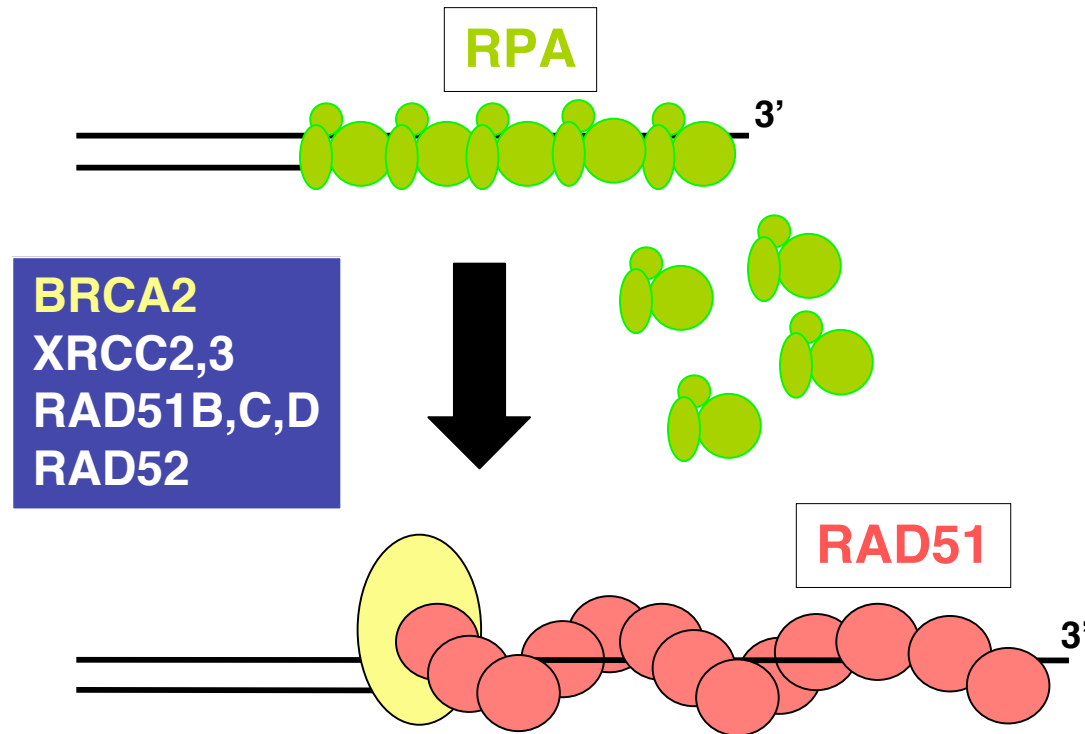
# RAD51AP1 knockdown: reduces homology-directed repair in TK6 cells



# RAD51AP1 knockdown promotes spontaneous mutagenesis in TK6 cells



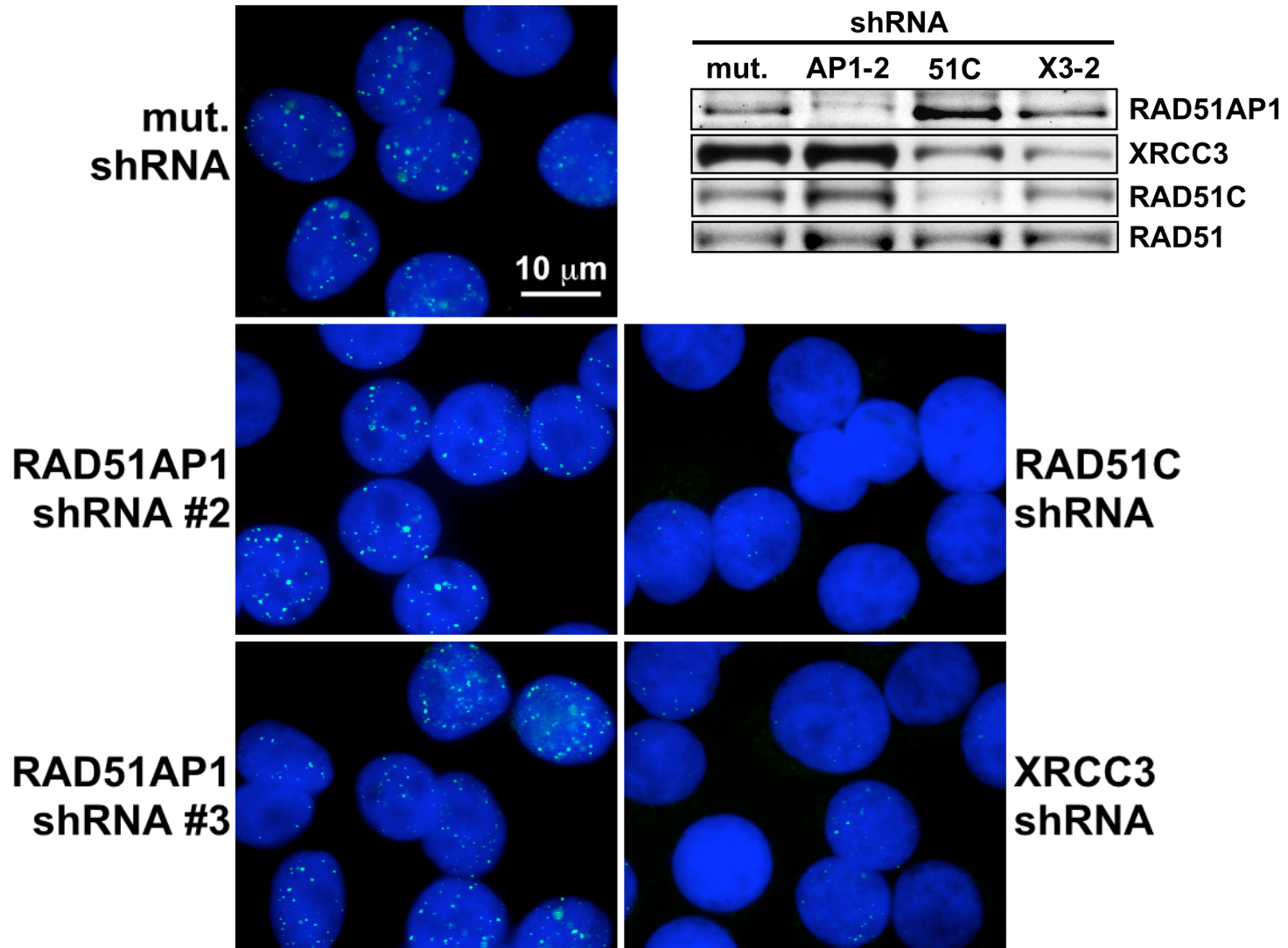
**RAD51-filament formation is a complicated process  
and requires recombination mediators**



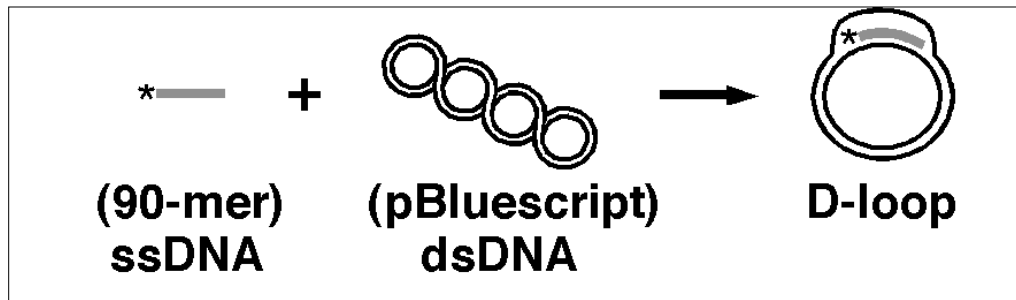
**Loss of recombination mediators: loss of RAD51 DNA repair foci**



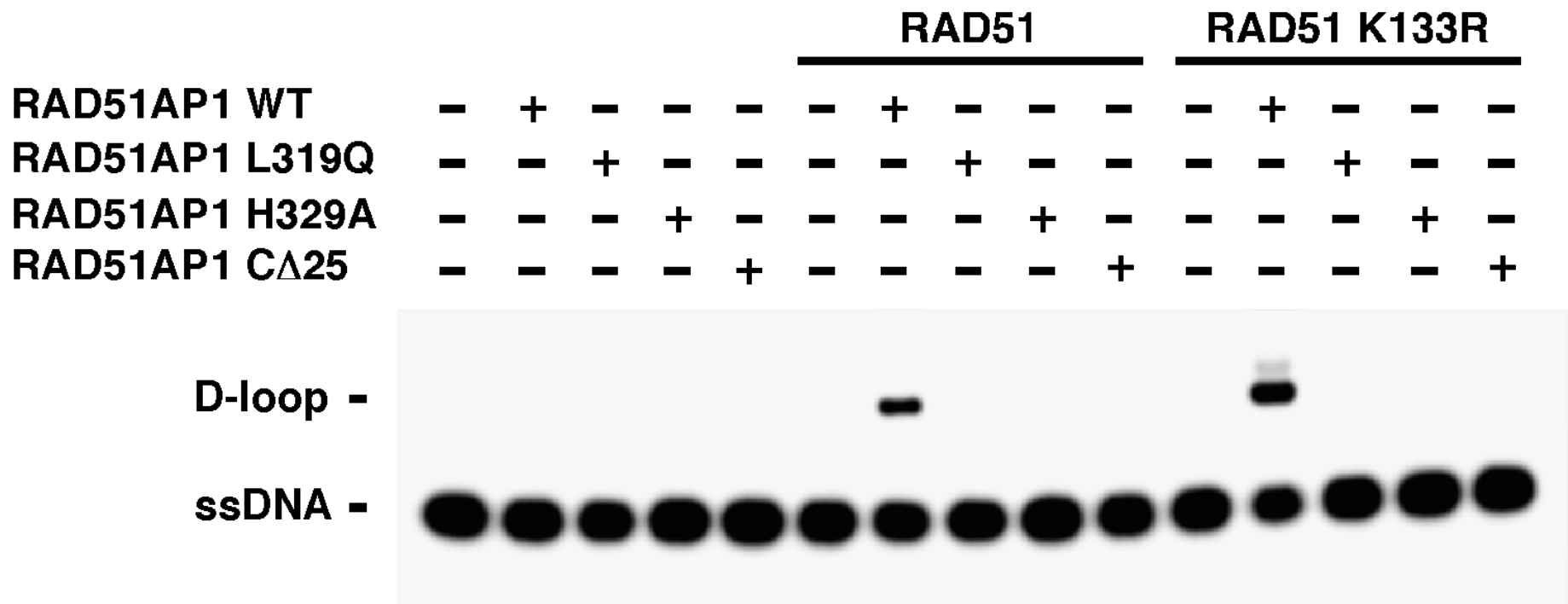
**RAD51AP1 knockdown: does not inhibit RAD51 foci formation  
(8 Gy X-rays, 8 h; HeLa cells)**



# RAD51AP1 stimulates the RAD51-mediated D-loop reaction



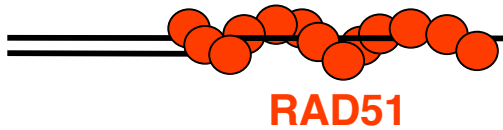
**Eloïse Dray**  
**Joseph San Filippo**  
**Idina Shi**  
**Patrick Sung**



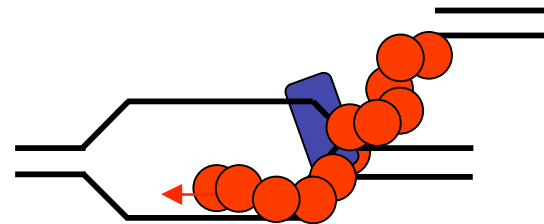
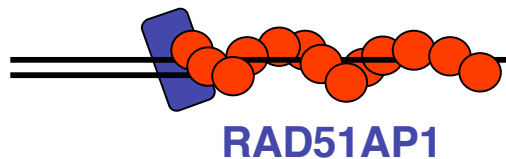
# Summary

- **RAD51AP1 and RAD51 interact:** Y2H, *in vitro*, *in vivo*
- **RAD51AP1-CTD:** facilitates the interaction with RAD51
- **Functional loss of RAD51AP1:** confers cellular sensitivity to genotoxic stress (S/S<sub>0</sub> ↓, ctbs ↑, MF ↑)
- **RAD51AP1 is an HR factor:** epistatic to the HR protein XRCC3  
required for homology-directed repair  
suppresses chromatid-type aberrations  
stimulates the DNA strand-pairing activity of RAD51  
(requires RAD51AP1-RAD51 interaction)

# Summary



RAD51AP1 functions **downstream** of presynaptic filament assembly:



- (1) RAD51AP1 knockdown does **not** inhibit the formation of RAD51 DNA repair foci.
- (2) RAD51AP1 stimulates the RAD51-mediated D-loop reaction under conditions wherein the RAD51 presynaptic filament is stable (**K133R**).

# Acknowledgements



## Yale University

**Patrick Sung**

**Eloïse Dray**

Joseph San Filippo

Idina Shi

## Dana-Farber

Oleg V. Kovalenko

## Lawrence Berkeley National Laboratory

**David Schild**

Torsten Größer

Bjorn Rydberg

Miaw-Sheue Tsai

Gareth W. Williams

David W. Collins

Katja Kratz

Sara B. Seidler

Faria Zafar

## Support

DOE-Low Dose

LBL-LDRD

NASA

NIH