

# **H2AX**

(unpublished data removed 2016-05-22)

**Ancient history from the last millenium**

**$\gamma$ -H2AX characterization**

**KO mouse**

**Senescence**

**Bystander effect**

**Dosimetry**

**Pre-clinical studies**

**Characterization of NCI60 panel**

**H2AX and the Epithelial Mesenchymal Transition**

# H2AX

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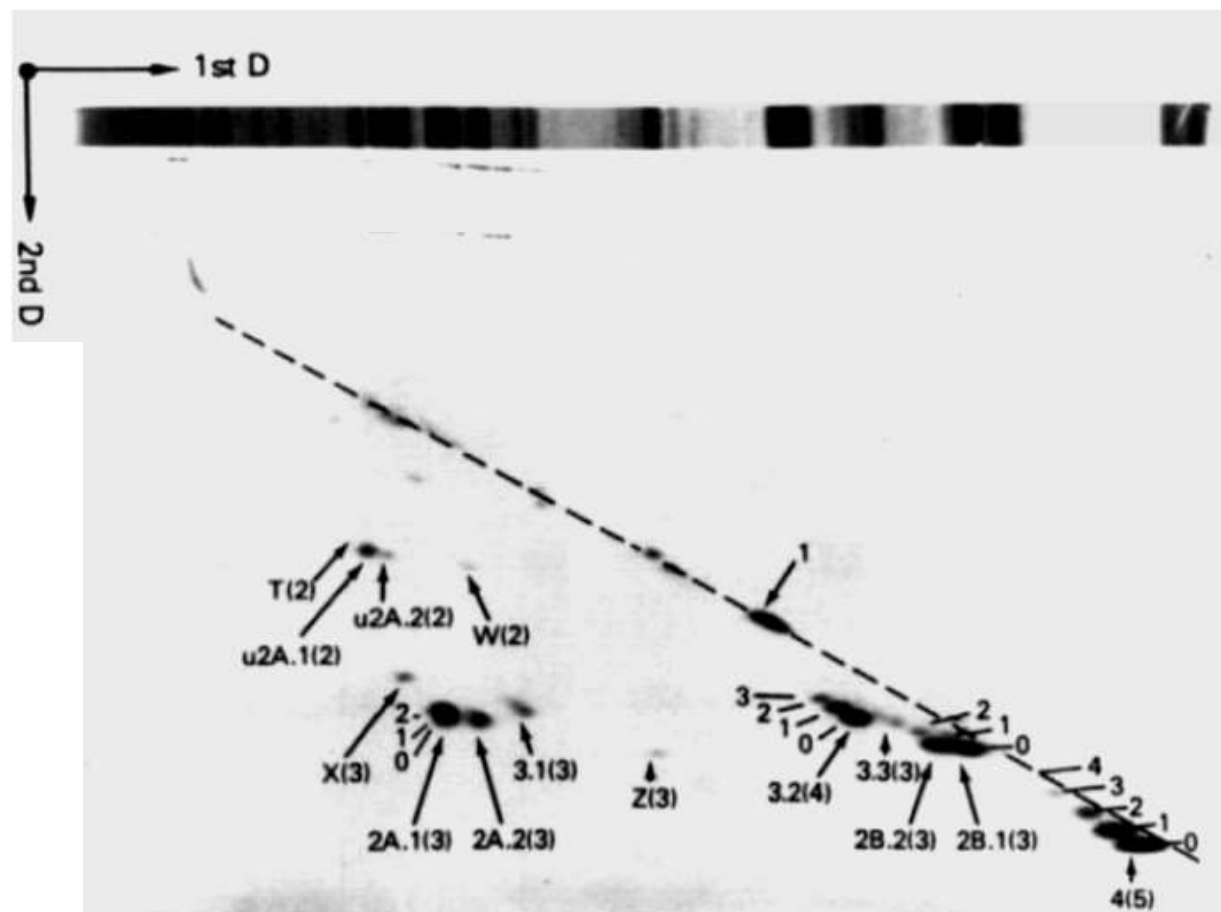
**Characterization of NCI60 panel**

**H2AX and the Epithelial Mesenchymal Transition**

# Histone 2A, a Heteromorphous Family of Eight Protein Species<sup>†</sup>

Michael H. P. West and William M. Bonner\*

*Biochemistry* 1980, 19, 3238-3245

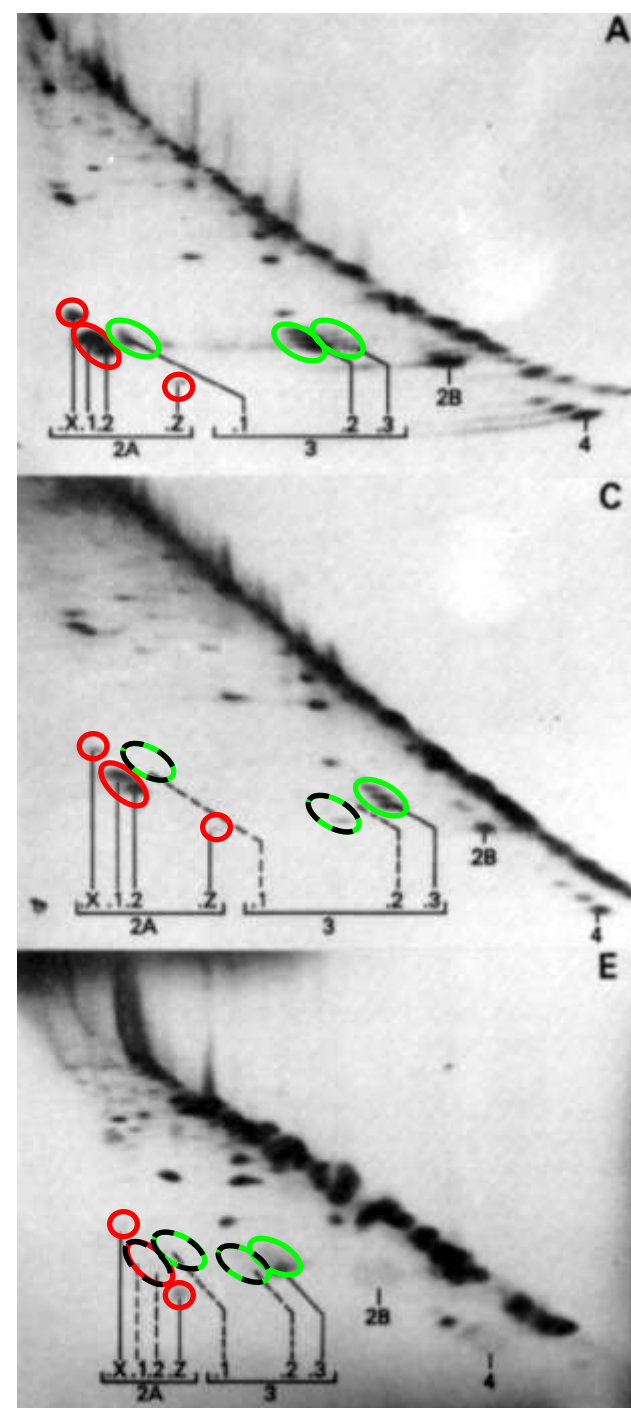


## Patterns of Histone Variant Synthesis Can Distinguish G0 from G1 Cells

Roy S. Wu,\* Shien Tsai† and William M. Bonner\*

\* Laboratory of Molecular Pharmacology

In S phase (top): All variants are being synthesized.  
In G1 (middle): H3.1 and H3.2 are turned off.  
In G0 H2A.1 and H2A.2 are also turned off.



**H2A.X, a histone isoprotein with a conserved C-terminal sequence, is encoded by a novel mRNA with both DNA replication type and polyA 3' processing signals**

Cecilia Mannironi, William M. Bonner\* and Christopher L. Hatch

## Nucleic Acids Research

### H2A.X-type

Human H2A.X	K K T S A T V G P K A P S G G <u>K K A T Q A S Q E Y</u> *
Sac. cere H2A.1	K K - S A - - - - - - - - - - <u>K A T K A S Q E L</u> *
Sac. cere H2A.2	K K - S A - - - - - - - - - - <u>K T A K A S Q E L</u> *
Aspergillus H2A	K K T P - - - - - - - - - - <u>K A G K G S Q E L</u> *
Tetrahymena H2A.1	K K T E - - - - - - - - - - S R - - <u>G Q A S Q D I</u> *
Schiz. pombe H2A.1	T K T S - - - - - - - - - - G R - T G <u>K P S Q E L</u> *
Schiz. pombe H2A.2	T K Q S - - - - - - - - - - G K - - G <u>K P S Q E L</u> *

## H2A.X, a histone isoprotein with a conserved C-terminal sequence, is encoded by a novel mRNA with both DNA replication type and polyA 3' processing signals

1 ACAGCAATTACACTGCGGGGGGCTCTGTTCTAGTGTTTGAGCCGTCGTGCTTCACC6GTACCTCGCTAGC

74 ATGTCGGGCGCGGCAAGACTGGCGGCAAGGCCCGCGCAAGGCCAAGTCGCGCTCGTCGCGCGCCGGCCTC  
(x) MET Ser Gly Arg Gly Lys Thr Gly Gly Lys Ala Arg Ala Lys Ala Lys Ser Arg Ser Ser Arg Ala Gly Leu  
(1) 1 5 Gln 10 15 Thr 20  
146 CAGTTCACASTGGGCGGTGTACACCGGTGCTGCGGAAGGGCCACTACGCCGAGCGCTTGGCGCGCGCGG  
(x) Gln Phe Pro Val Gly Arg Val His Arg Leu Leu Arg Lys Gly His Tyr Ala Glu Arg Val Gly Ala Gly Ala  
(1) 30 Ala 39 Ser 45  
218 CCAGTGTACCTGGCGGCACTGCTGGAGTACCTCACCCTGAGATCCTGGAGCTGGCGGGCAATGCGGCCCGC  
(x) Pro Val Tyr Leu Ala Ala Val Leu Glu Tyr Leu Thr Ala Glu Ile Leu Glu Leu Ala Gly Asn Ala Ala Arg  
(1) 50 60 70  
290 GACAACAAGAGACGCGAATCATCCCCGCCACTGCAAGTGGCCATCCGCAACGACGAGGAGCTCAACAAG  
(x) Asp Asn Lys Lys Thr Arg Ile Ile Pro Arg His Leu Gln Leu Ala Ile Arg Asn Asp Glu Glu Leu Asn Lys  
(1) 80 90  
362 CTGCTGGGCGCGTACGATCGCCAGGGAGGCGTCTGCCCAACATCCAGGCCGTGCTGCTGCCAAGAAG  
(x) Leu Leu Gly Gly Val Thr Ile Ala Glu Gly Gly Val Leu Pro Asn Ile Gln Ala Val Leu Leu Pro Lys Lys  
(1) 98 Arg 110  
434 ACCAGCCACCGTGGGCGAAGGCCCTCGGGCGCAAGAAGGCCACCCAGGCCCTCCAGGAGTACTAA  
(x) Thr Ser Ala Thr Val Gly Pro Lys Ala Pro Ser Gly Gly Lys Ala Thr Gln Ala Ser Gln Glu Tyr  
(1) 120 Glu Ser His His Lys Ala Val Glu Lys 130 140 142  
506 GAGGGCCCGCGCCGCGCCGCGCCCGCCAGCTCCCCATG **CACCACAAAGGCCCTTTAAGGCCACC** / CCG  
1 2  
578 **CCTCATGGAAGAGCTGAG** CCGCTTCAGACTGCGGGGCAAGCGGGCGCGGCTCCCTCCCTCCCTCCCTCC  
2 2  
650 CTCGCCCGCTTCGCCCGCCGGCTCGAGTCCCCGCCCGCCCGGCTCCCGTCCCGCACCGCTGCCCGCTC  
722 GGCCTCGGGCTGCCCTGTCCGCCSTCCGCCCTCGGTAAGGTTCCGGCTTCCGATGCGGCTTGGCGCT  
794 CTTCCGGGACCTCCGTGGCGGGAAGACCCGAGCTGCCGGGGGAGGCCGCGCGCGCCACCTGCCCGCC  
866 TCGGCTTCTGTACTCAGCCGCCCATCCGAGTGCCTAAGGGCTGCGGGGAGGCCGAGCACCTTCTGGA  
938 A5ACTTGGCTTCCGCTCTGACGAGGGCCGAGGTGGGCAATCCAGGCCGAGAGCCGGCGGCTTGAAGGTG  
1010 AGTGAGGCCCTCGGCAAGTGCAGCCGGGGTGTCTGGTACCCCCCGCGTGGTGTCTTAGCCAGGACTTTCA  
1082 GACGGCCGCTGGCGGGGAGGCTTTGGTGGGAGAGACGCGATCGCCGATTTCCGCTTGGCGCCCTTCTGCGG  
1154 CCGGGACCCAGGCCCTTCCATCAGCTCTCCCTCCATCTTCAATCAGGCTGCGCTGGGGCCGGGACGAA  
1226 GCACCTGGTAACAGGCACATCTTCTCCCGAGTGAAGTGCCTCTAGGAGGACATTTAGGGGAGGGCAGAGGC  
1298 CTGCAGTTTGGCTTACGCTGCGTATGTGGACAGCAAGAGTGTGTTTGGGAAACGCGACTGGCAGCCAGGC  
1370 CTGTCGGGCCCCGACGCGCCCATTTCCCTTCCAGCAAATCAACTCGGCAATCCAGCACCTAGATACC  
1442 AGCACAAGTCCGTTAATCCCTGTCTGGACTGAGCTCCGTTGGCTTCTGAAGTGAATTCTGCAGCTAACCC  
1514 TTCCAGACTAGAACCTTAGGCATGGGGAGTTTTAGATGACTAATT **TATTAAAGGA** TTGTTTTTTTTT  
3 3  
(1585 total bases)

# H2AX

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KO mouse

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Bystander effect

Dosimetry

Pre-clinical studies

Characterization of NCI60 panel

H2AX and the Epithelial Mesenchymal Transition

# DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139\*

(Received for publication, July 25, 1997)

Emmy P. Rogakou, Duane R. Pilch, Ann H. Orr, Vessela S. Ivanova, and William M. Bonner‡

From the Laboratory of Molecular Pharmacology, Division of Basic Sciences, NCI, National Institutes of Health, Bethesda, Maryland 20892

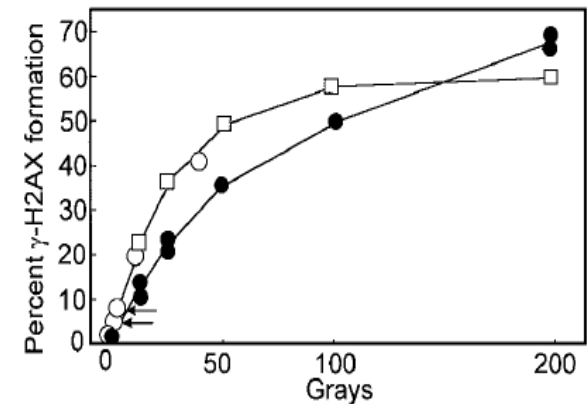
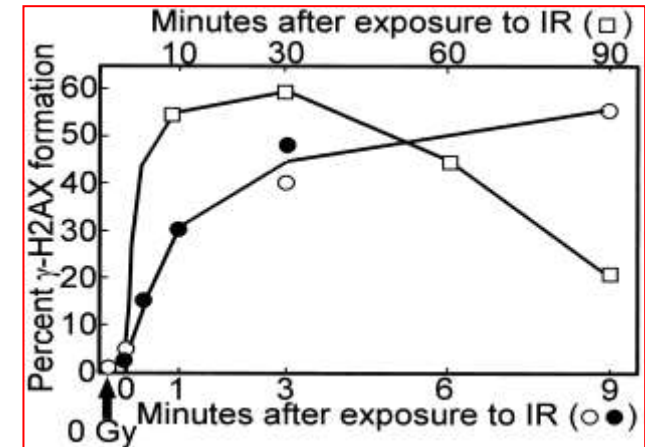
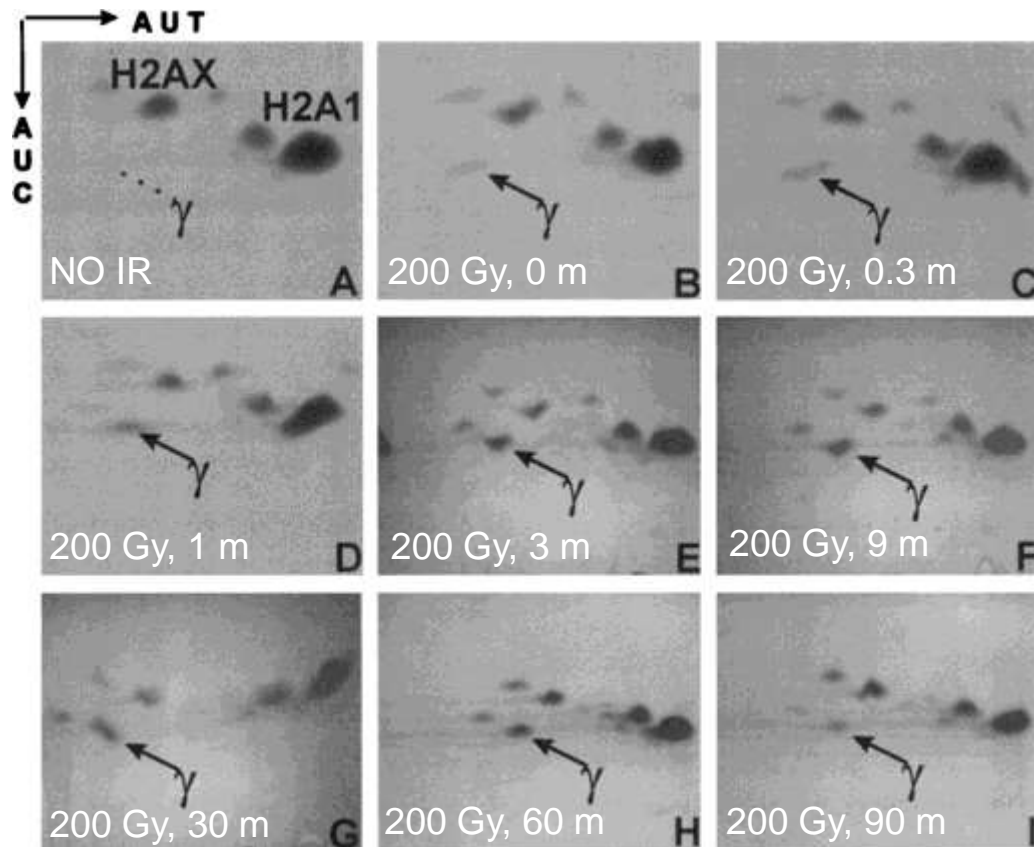




TABLE II

*Constant percentages, not numbers, of H2AX molecules are  $\gamma$ -modified per Gy*

The stained H2A2, H2A1, and H2AX species on two-dimensional gels were recorded as TIFF images and quantitated with ImageQuant software version 3.3. The  $\gamma$ -H2AX/H2AX ratio was determined 30 min after exposing the cell cultures to 25 Gy. The following conversion factors and assumptions were used. 1) The mammalian  $G_1$  genome contains  $6 \times 10^9$  bp of DNA, hence about  $30 \times 10^6$  nucleosomes (200 bp/nucleosome) and  $60 \times 10^6$  H2A molecules (2 molecules/nucleosome). 2) 25 Gy induces about 875 DNA double-stranded breaks per  $G_1$  genome. 3) H2AX is randomly distributed in the chromatin.

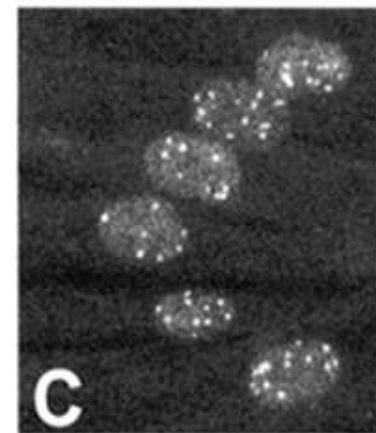
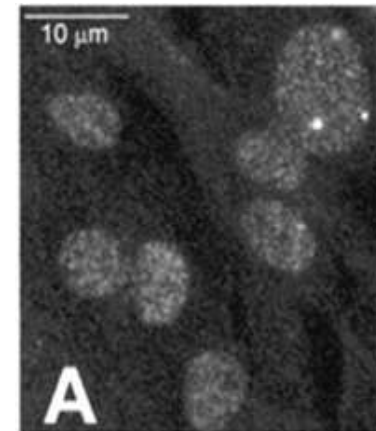
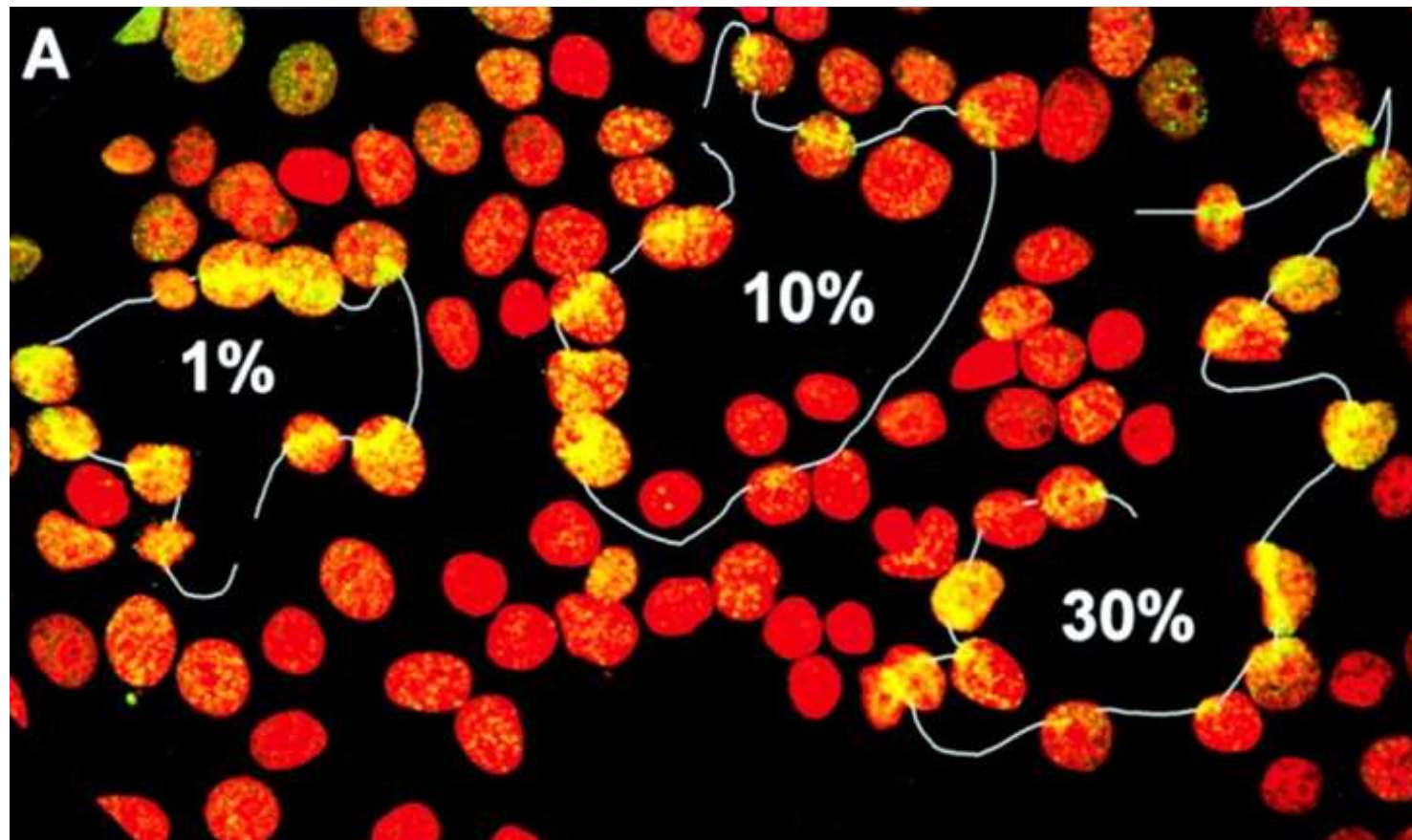
Cell type	H2AX/total H2A	$\gamma$ -H2AX/total H2AX	No. of H2AX/cell	No. of $\gamma$ -H2AX/cell	No. of $\gamma$ -H2AX/dsb	$\gamma$ -H2AX/dsb	bp of DNA/dsb
	%	%				%	
VA13	2.6	28	$1.6 \times 10^6$	$0.45 \times 10^6$	530	0.033	$2.0 \times 10^6$
HeLa	2.4	30	$1.4 \times 10^6$	$0.45 \times 10^6$	490	0.035	$2.1 \times 10^6$
IMR90	9.8	30	$5.9 \times 10^6$	$1.7 \times 10^6$	2100	0.035	$2.1 \times 10^6$
CHO	9.4	34	$5.6 \times 10^6$	$1.9 \times 10^6$	2240	0.040	$2.4 \times 10^6$
SF268	25	50	$15 \times 10^6$	$7.5 \times 10^6$	8800	0.059	$3.5 \times 10^6$

# Megabase Chromatin Domains Involved in DNA Double-Strand Breaks In Vivo

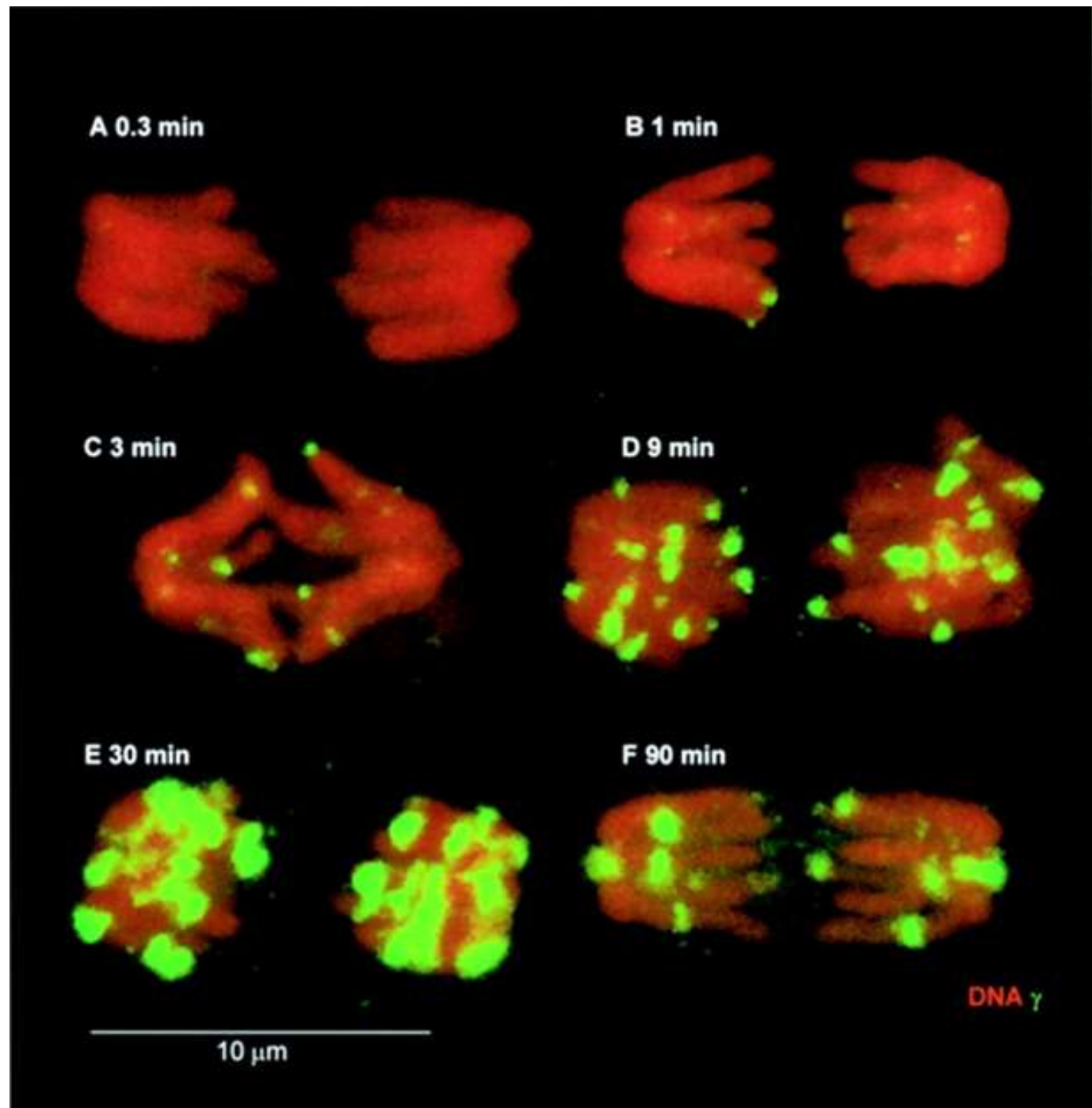
Emmy P. Rogakou, Chye Boon, Christophe Redon, and William M. Bonner

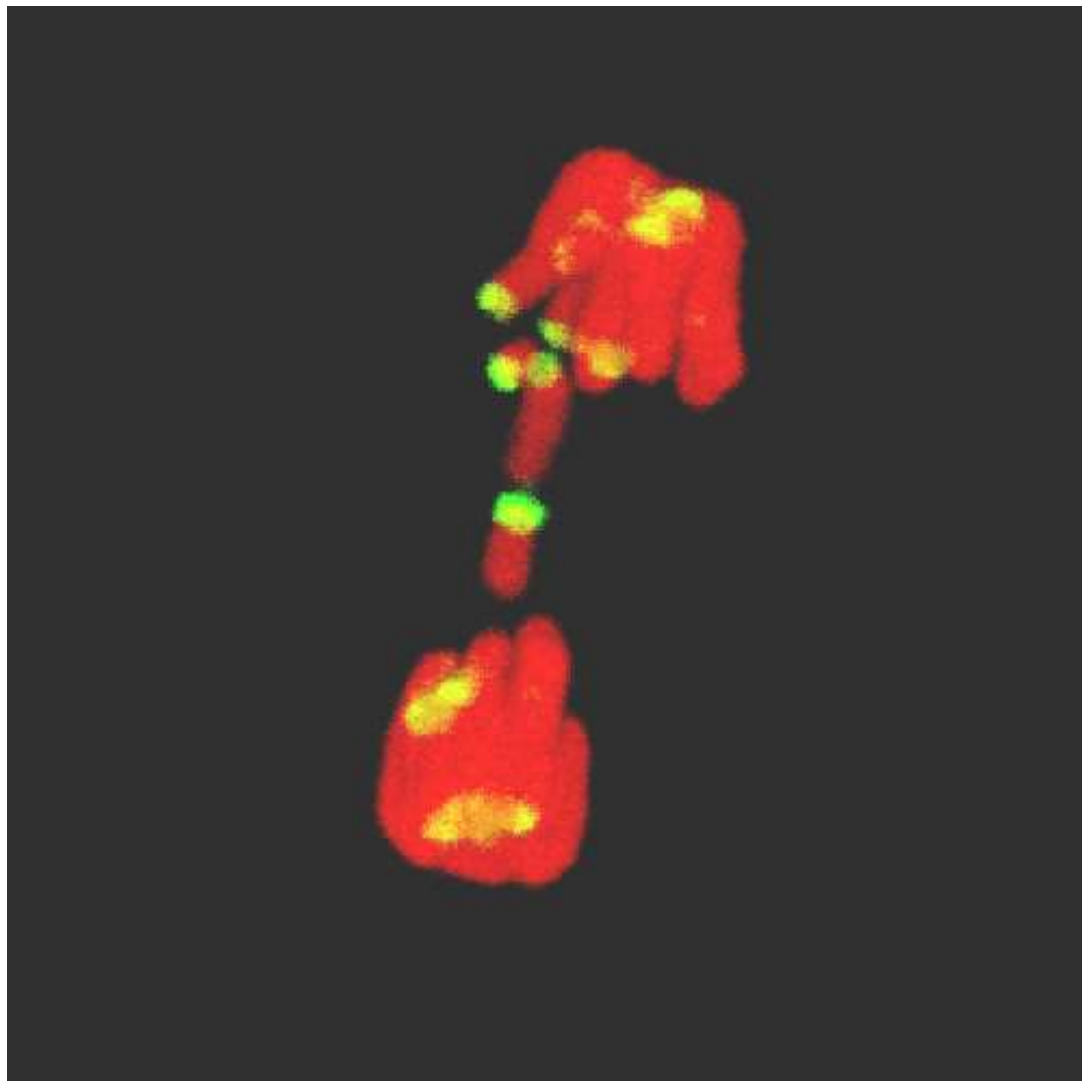
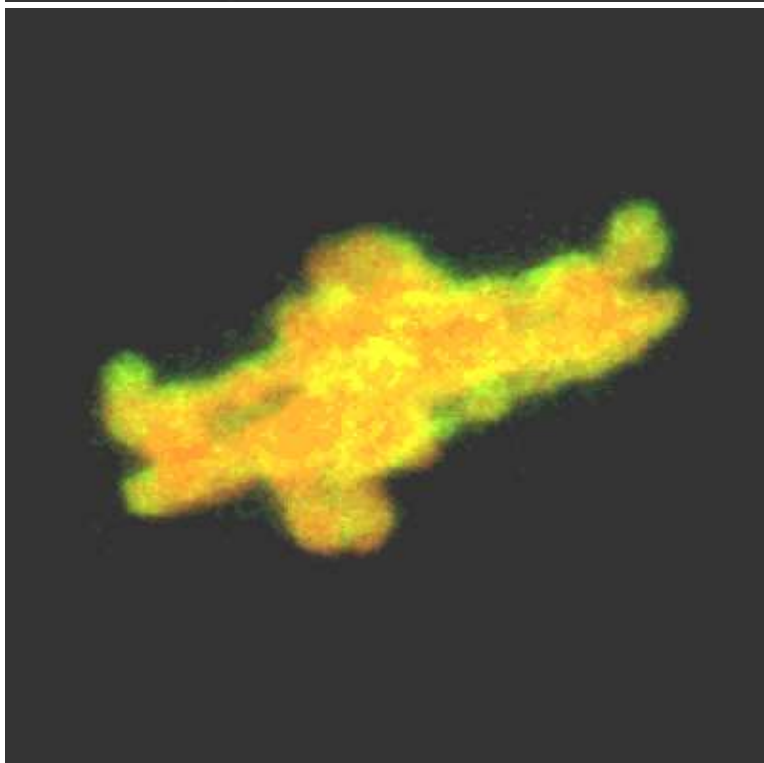
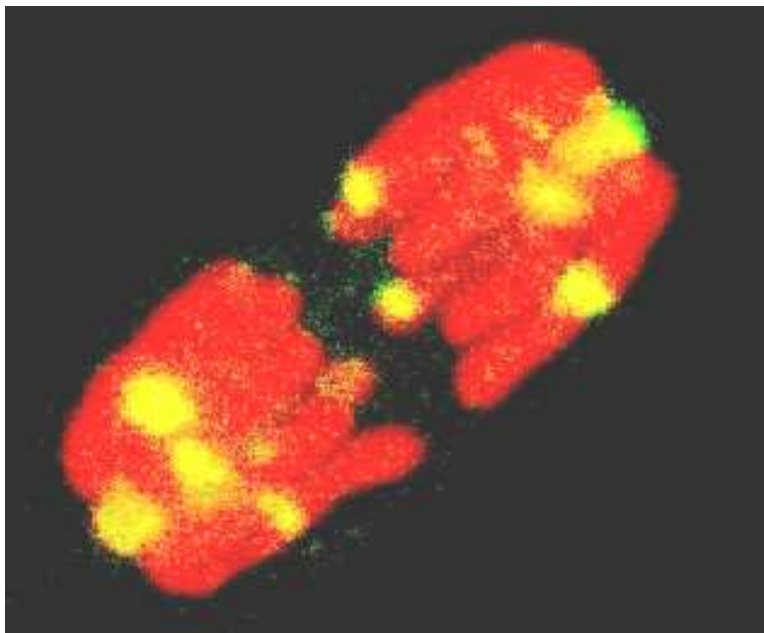
Laboratory of Molecular Pharmacology, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

*The Journal of Cell Biology*, Volume 146, Number 5, September 6, 1999 905–915

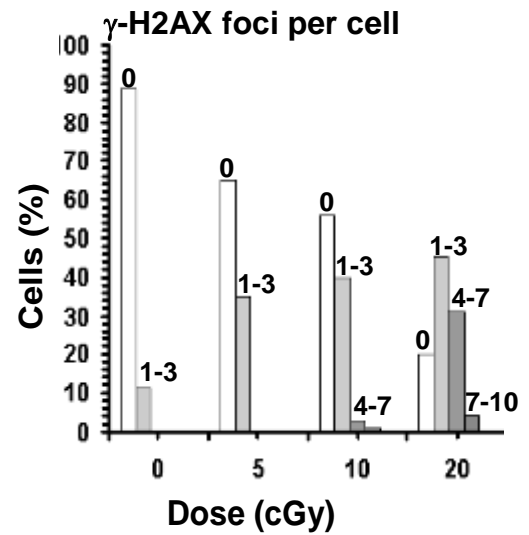
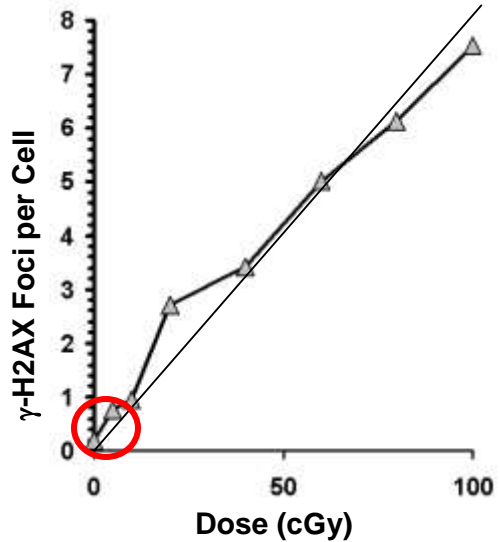
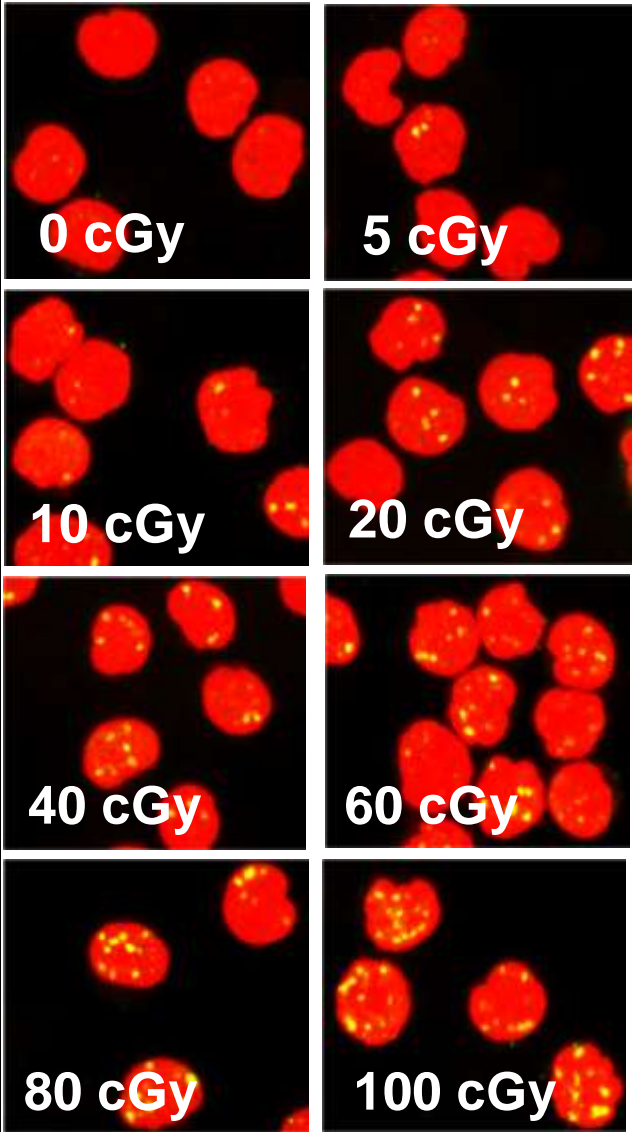


**Muntjac  
Chromosomes.  
Foci are  
apparent by 1  
min post IR.**

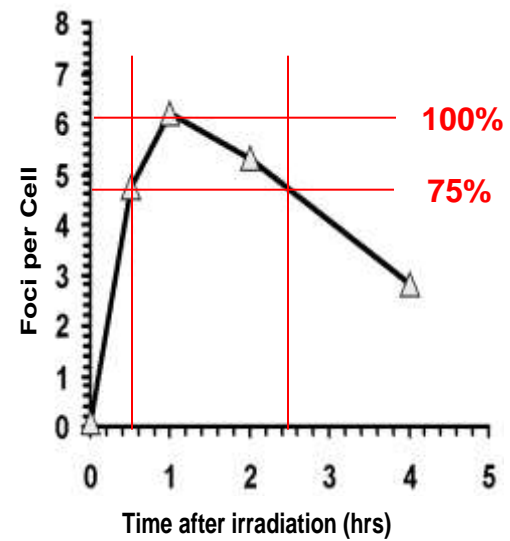




# Clinical: Human blood (NIH blood bank) irradiated in vitro



The assay is very sensitive, able to detect the effect of 5 cGy, and linear to >1 Gy.



Maximum sensitivity is from 0.5 to 2.5 hr post treatment.

# H2AX

Ancient history from the last millenium

$\gamma$ -H2AX characterization

**KO mouse**

Senescence

Bystander effect

Dosimetry

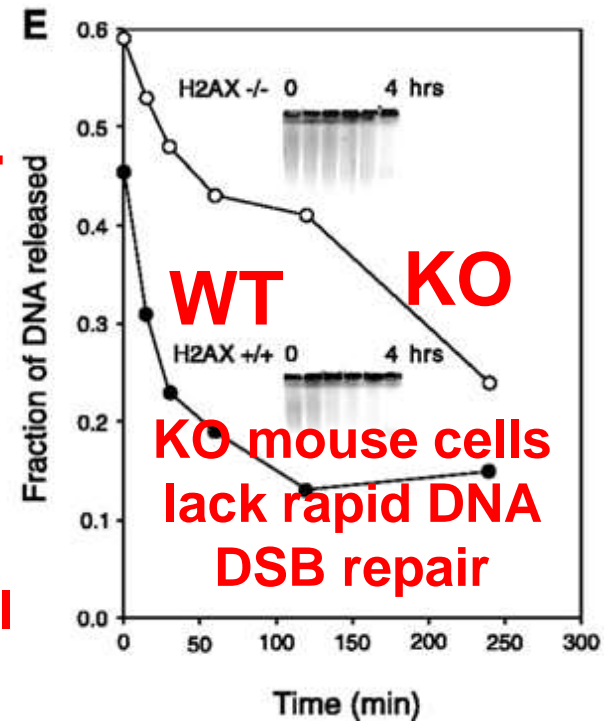
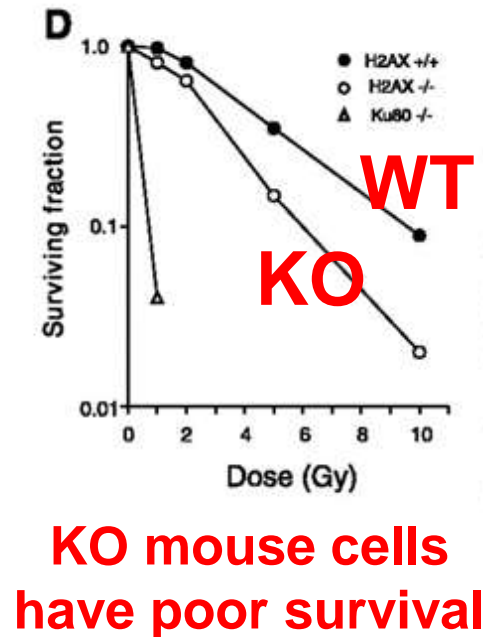
Pre-clinical studies

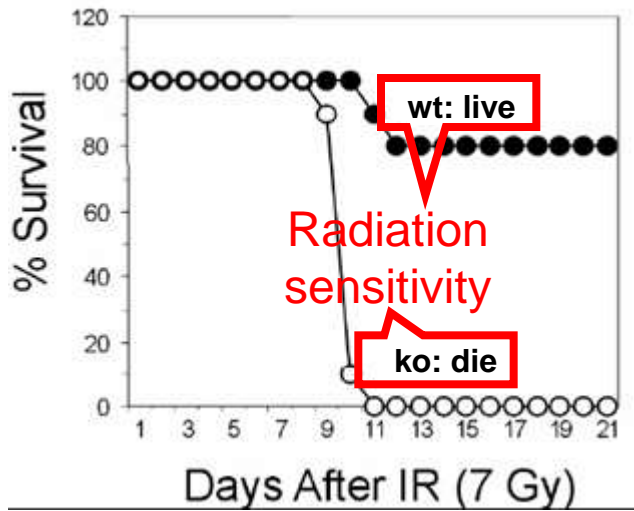
Characterization of NCI60 panel

H2AX and the Epithelial Mesenchymal Transition

Science. 2002 May 3;296(5569):922-7. Genomic instability in mice lacking histone H2AX. Celeste A(1), Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Olaru A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, Nussenzweig A.

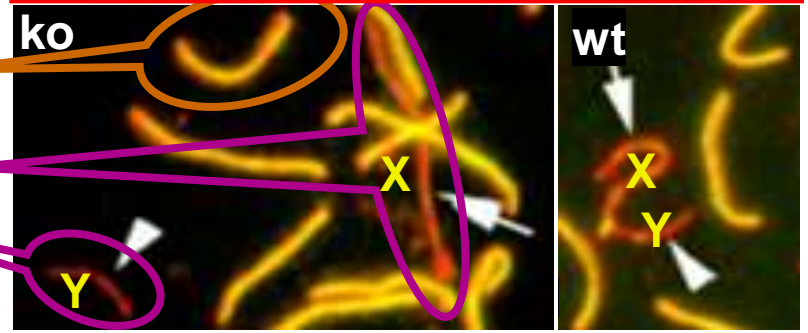
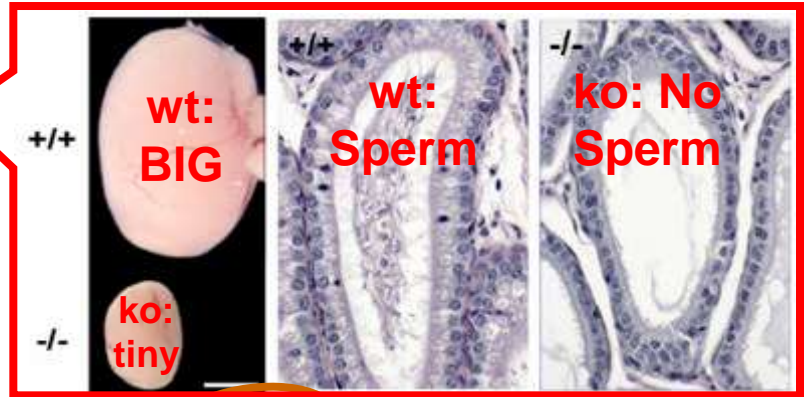
**H2AX<sup>-/-</sup>**  
immortalized  
MEFs exhibit poor  
survival and  
slower dsb repair.



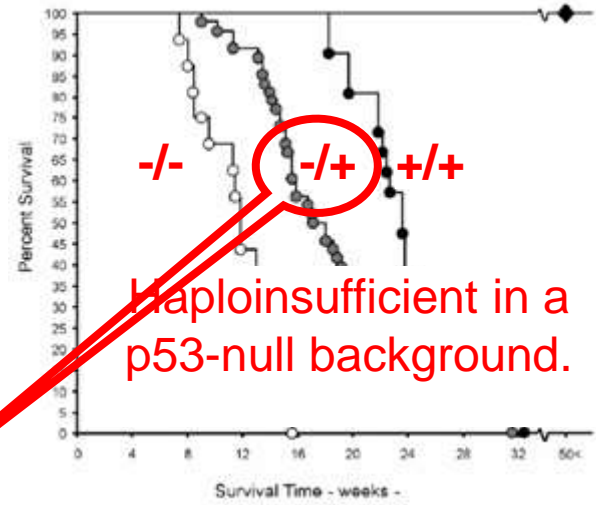
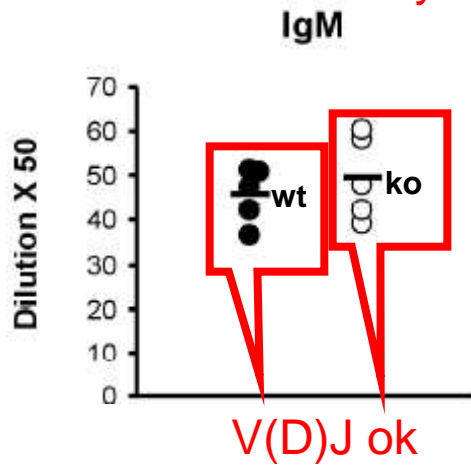


Male infertility.

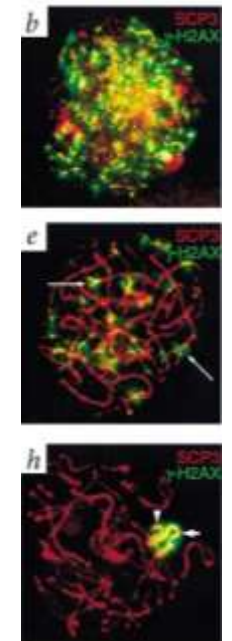
During meiosis, autosomes pair, but not the Y and X.



Defective Immune System Recombination

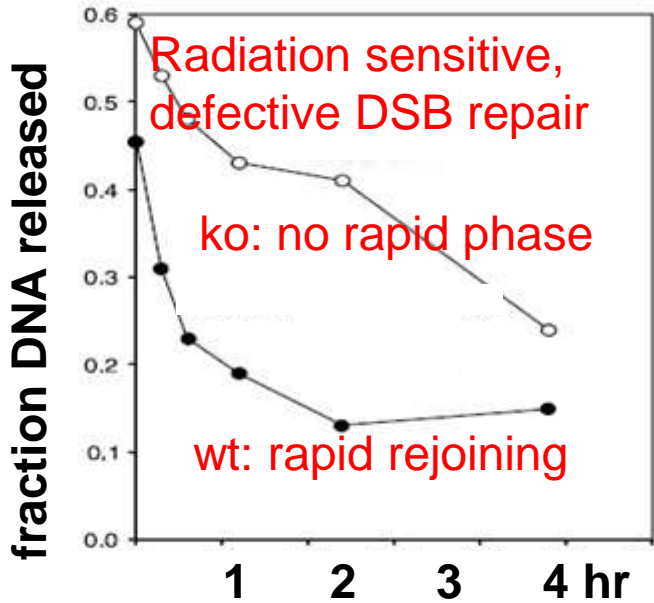


Haploinsufficient in a p53-null background.

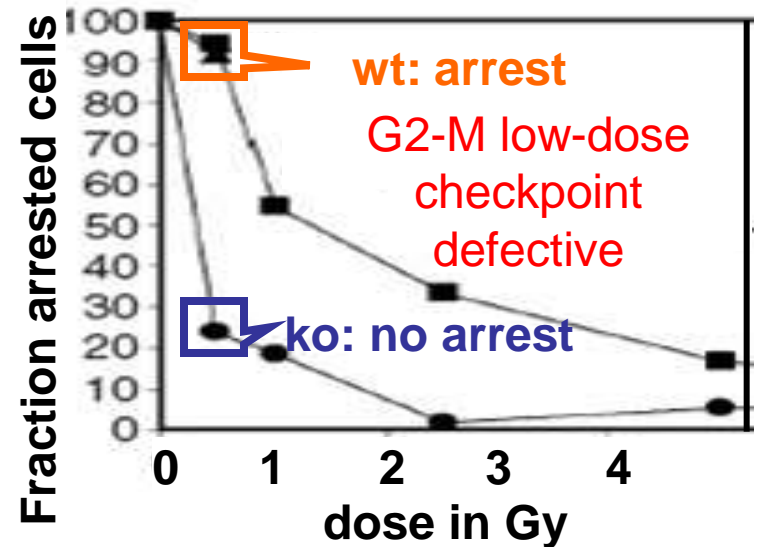
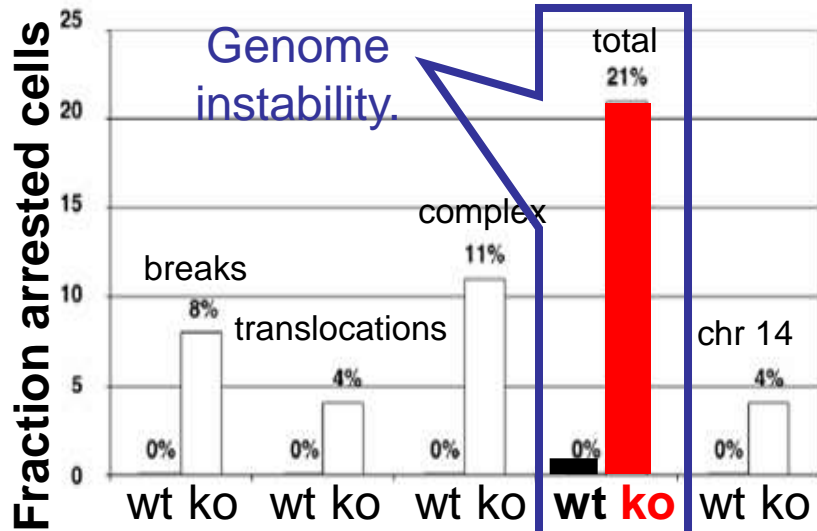
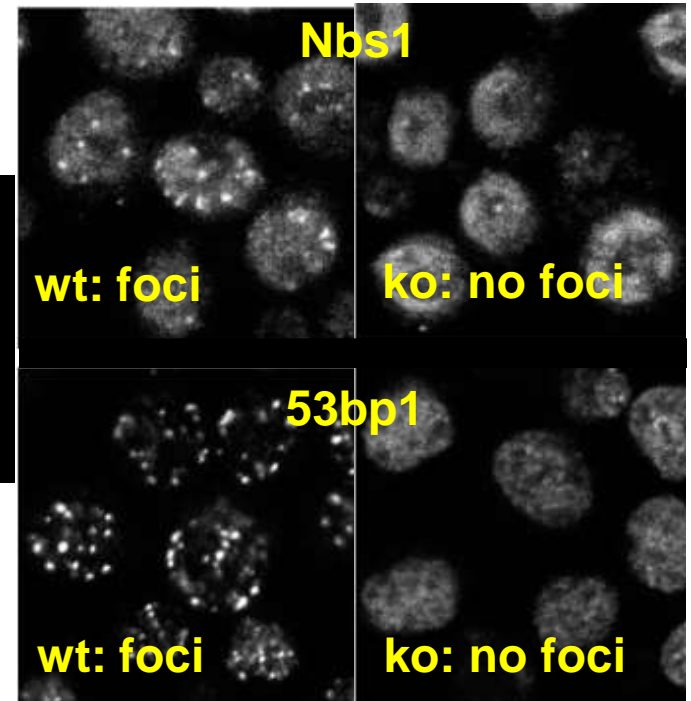


Deaths in p53-null H2AX haploids primarily due to thymic lymphomas





No mobilization of DSB-repair proteins to foci after IR.



# H2AX

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$\gamma$ -H2AX characterization

KO mouse

**Senescence**

Bystander effect

Dosimetry

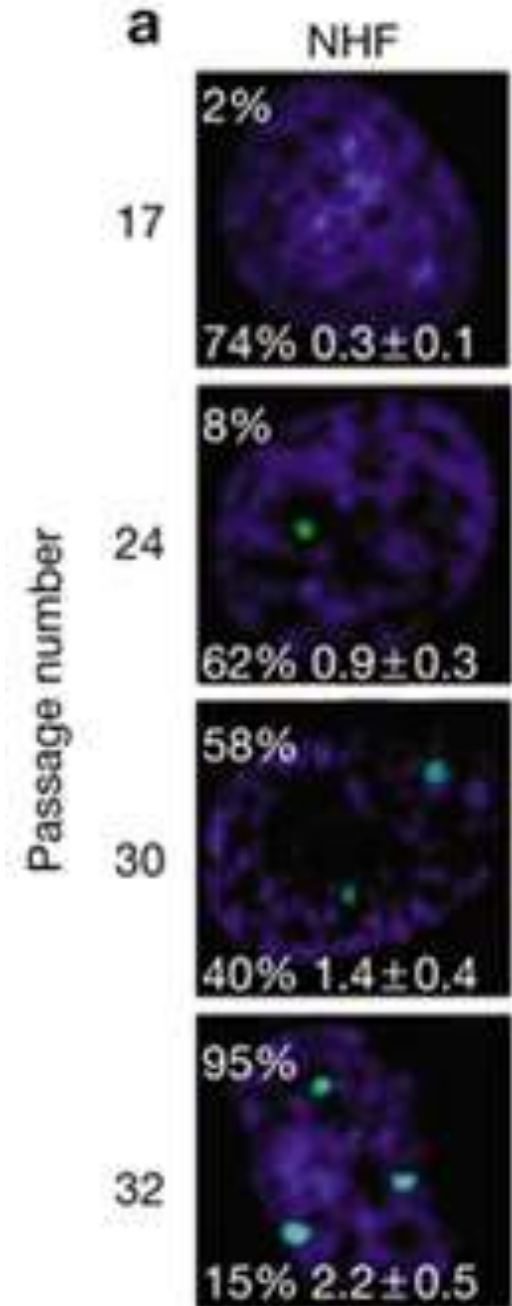
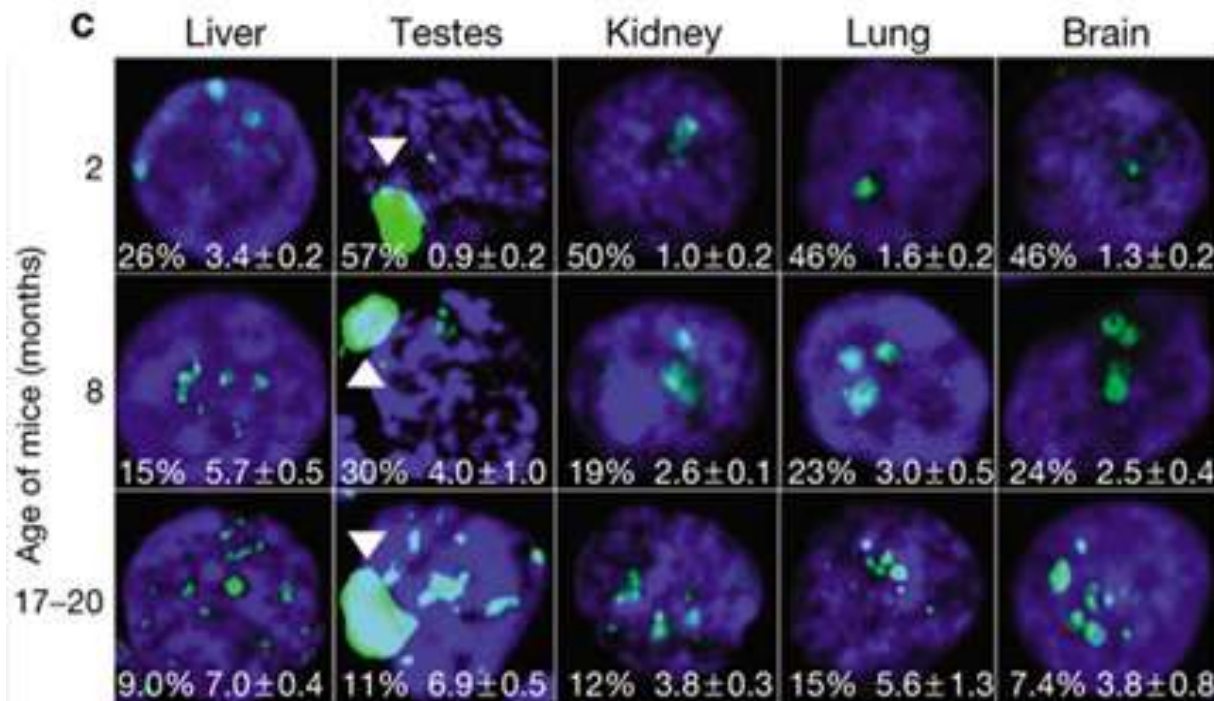
Pre-clinical studies

Characterization of NCI60 panel

H2AX and the Epithelial Mesenchymal Transition

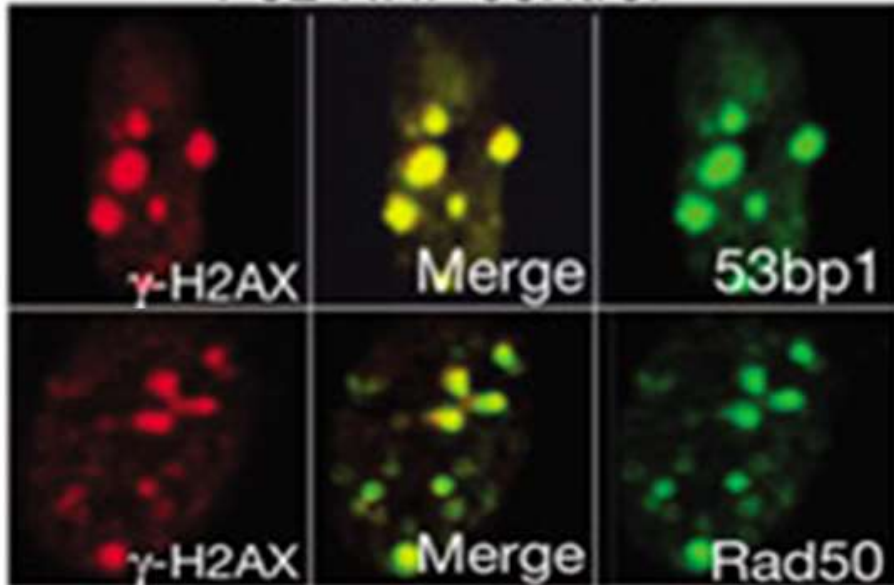
Senescing human cells and ageing mice accumulate DNA lesions with unreparable double-strand breaks. Sedelnikova OA1, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, Barrett JC. Nat Cell Biol. 2004 Feb;6(2):168-70.

As NHFs and mouse tissues age, the percentage of foci-free cells decreases and the average foci per cell values increase.

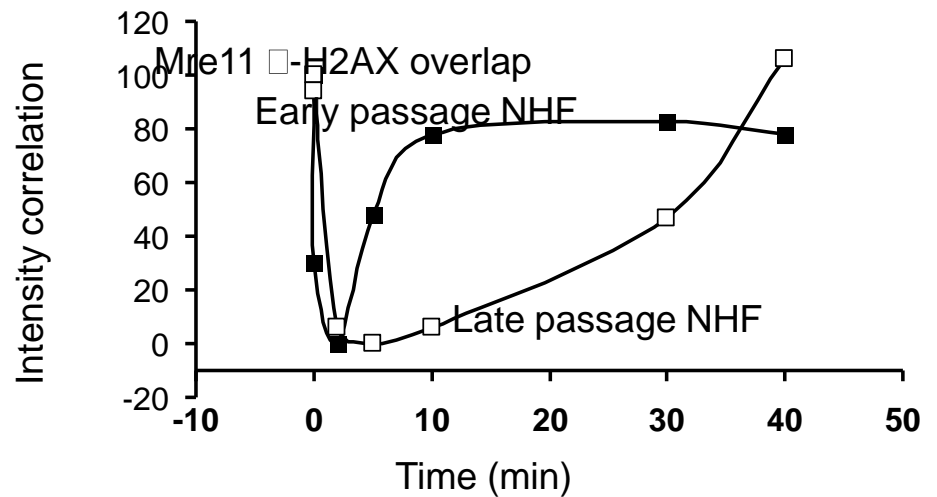
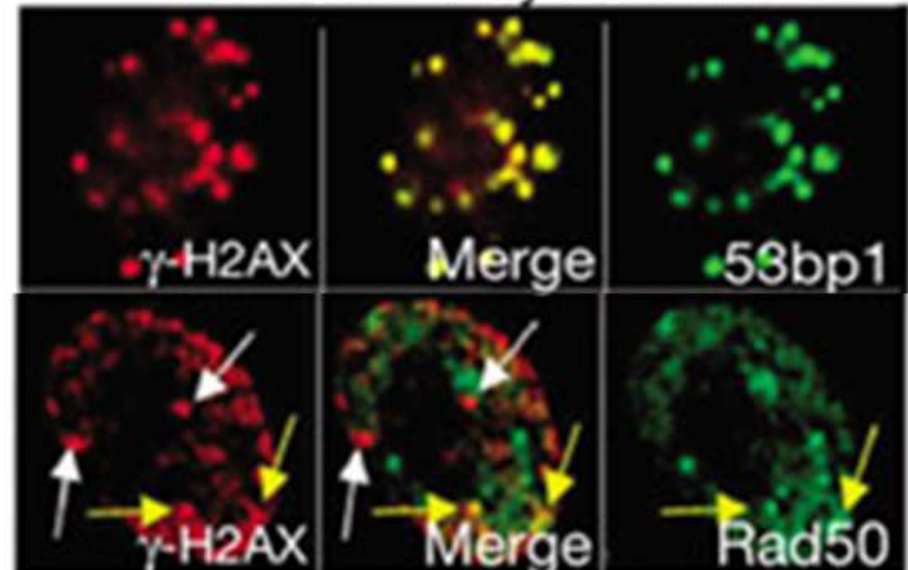


In senescent P32 NHF cultures, proteins are recruited more slowly to foci.  
 Yellow arrows, gamma-foci overlapping Rad50.  
 White arrows, gamma-foci lacking Rad50.

P32 NHF control



P32 NHF 1 Gy 30 min



Are the “unreparable”  $\gamma$ -foci at defective telomeres?

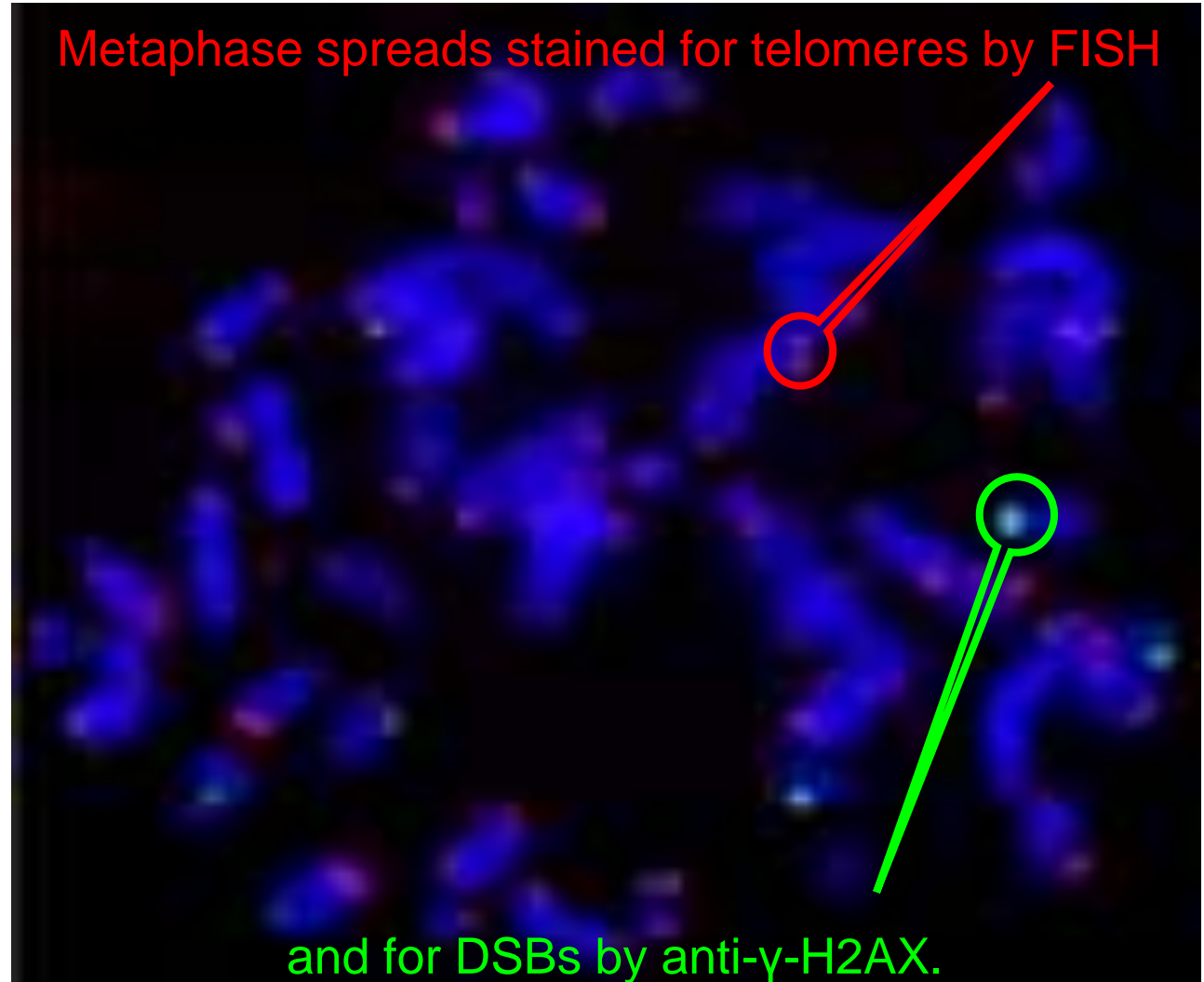
Humans: Yes, about 2/3 rds.

Wt Mice: No

TEL KO Mice:

4<sup>th</sup> gen:

Yes, about 2/3rds



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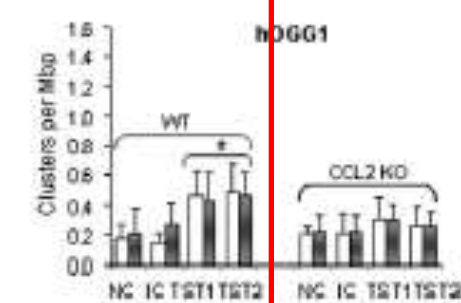
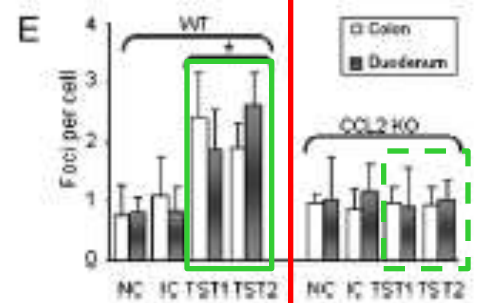
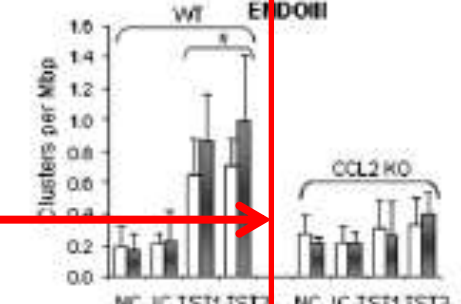
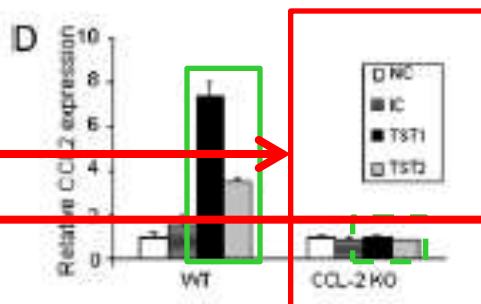
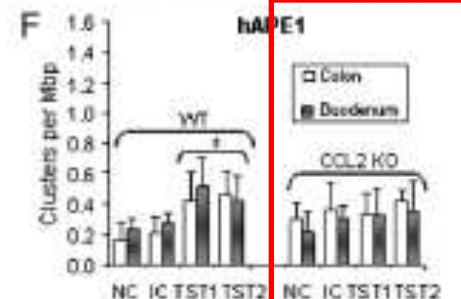
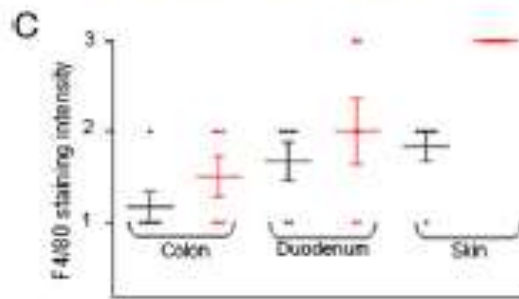
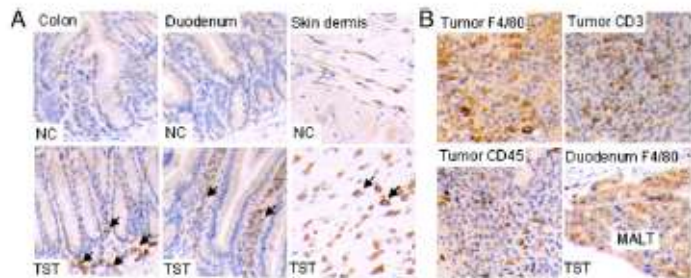
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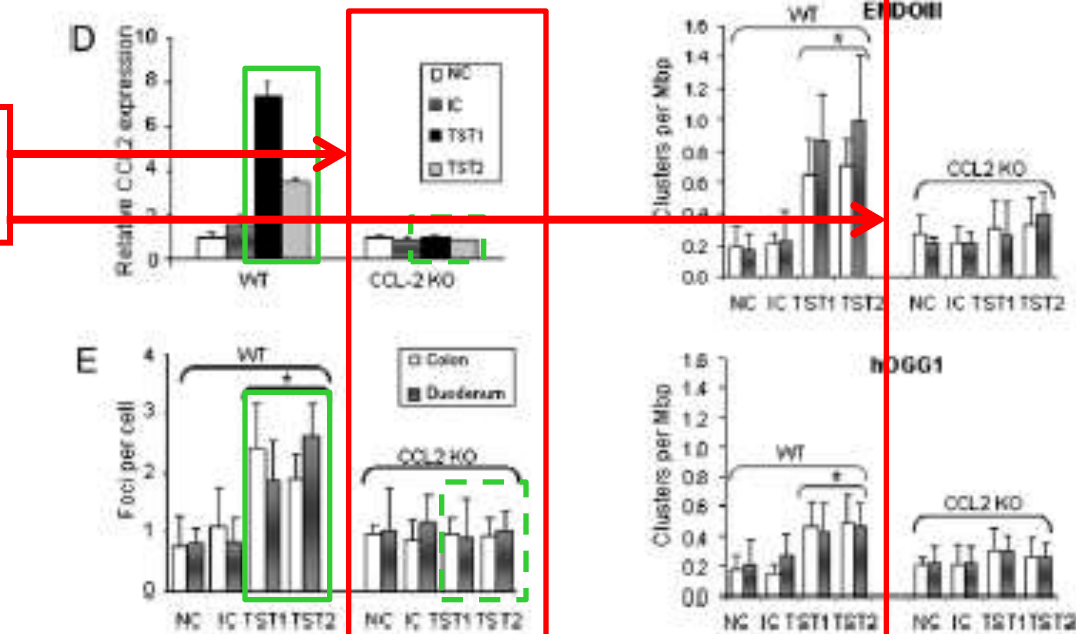
# Tumors induce complex DNA damage in distant proliferative tissues in vivo

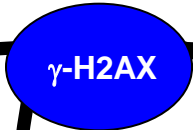
Christophe E. Redon<sup>a</sup>, Jennifer S. Dickey<sup>a</sup>, Asako J. Nakamura<sup>a</sup>, Irina G. Kareva<sup>a</sup>, Dieter Naf<sup>a,1</sup>, Somaira Newsheen<sup>c</sup>, Thomas B. Kryston<sup>c</sup>, William M. Bonner<sup>a</sup>, Alexandros G. Georgakilas<sup>c</sup>, and Olga A. Sedelnikova<sup>a,2</sup>

17992–17997 | PNAS | October 19, 2010 | vol. 107 | no. 42



CCL2 KO mouse did not exhibit distant DNA damage





*Basic Research*

DNA repair pathways  
Radiation Biology  
Drug development

γ-H2AX and other histone post-translational modifications in the clinic

Christophe E. Redon<sup>1</sup>, Urbain Weyemi<sup>1</sup>, Palak R. Parekh<sup>1</sup>, Dejun Huang<sup>1,2</sup>, Allison S. Burrell<sup>1,3</sup>, William M. Bonner<sup>1\*</sup>

*Animal Studies*

Drug development  
Radiation biodosimetry



*Translational Studies*

Diagnostics  
Irradiation procedures/treatments  
Drug development (chemotherapy)  
Biodosimetry (in vivo effects)  
Screenings  
Protocol improvement  
Clinical Trials (0, I, II, III)

*Environmental Studies*

Chemical Genotoxicity Assessment  
Occupational studies



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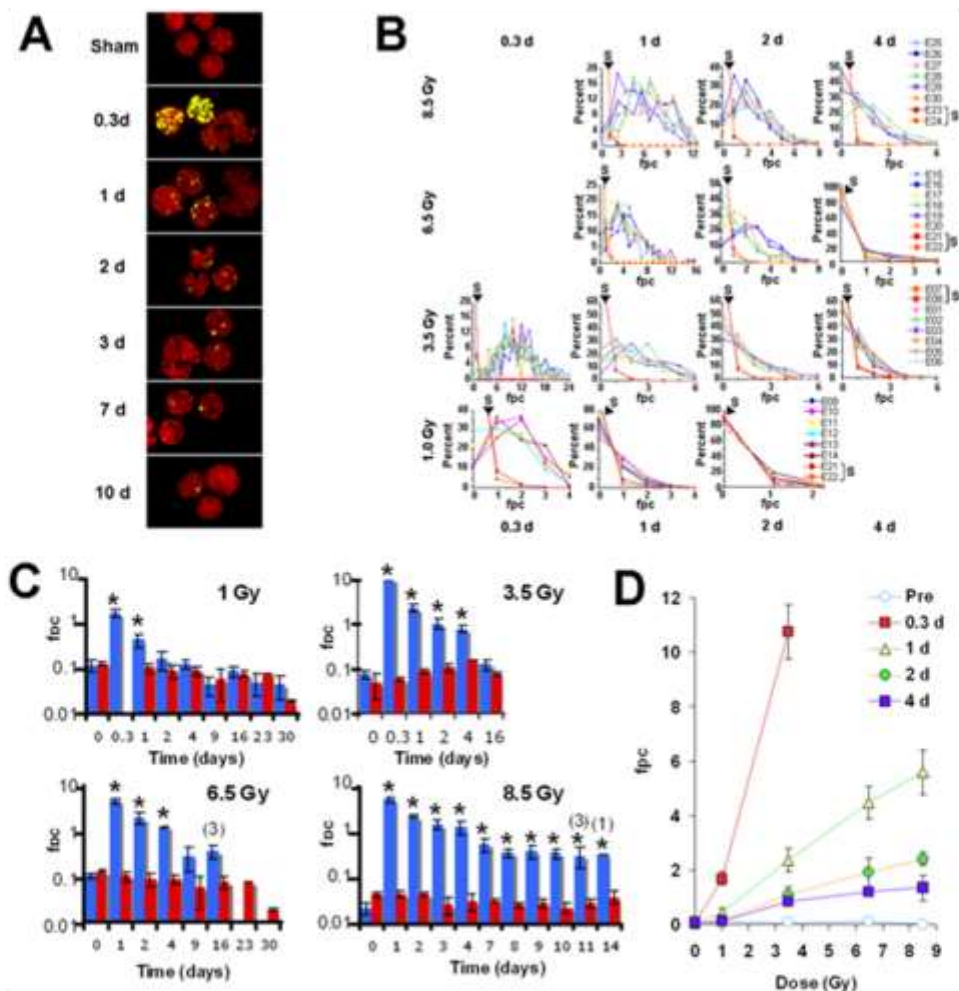
**Dosimetry**

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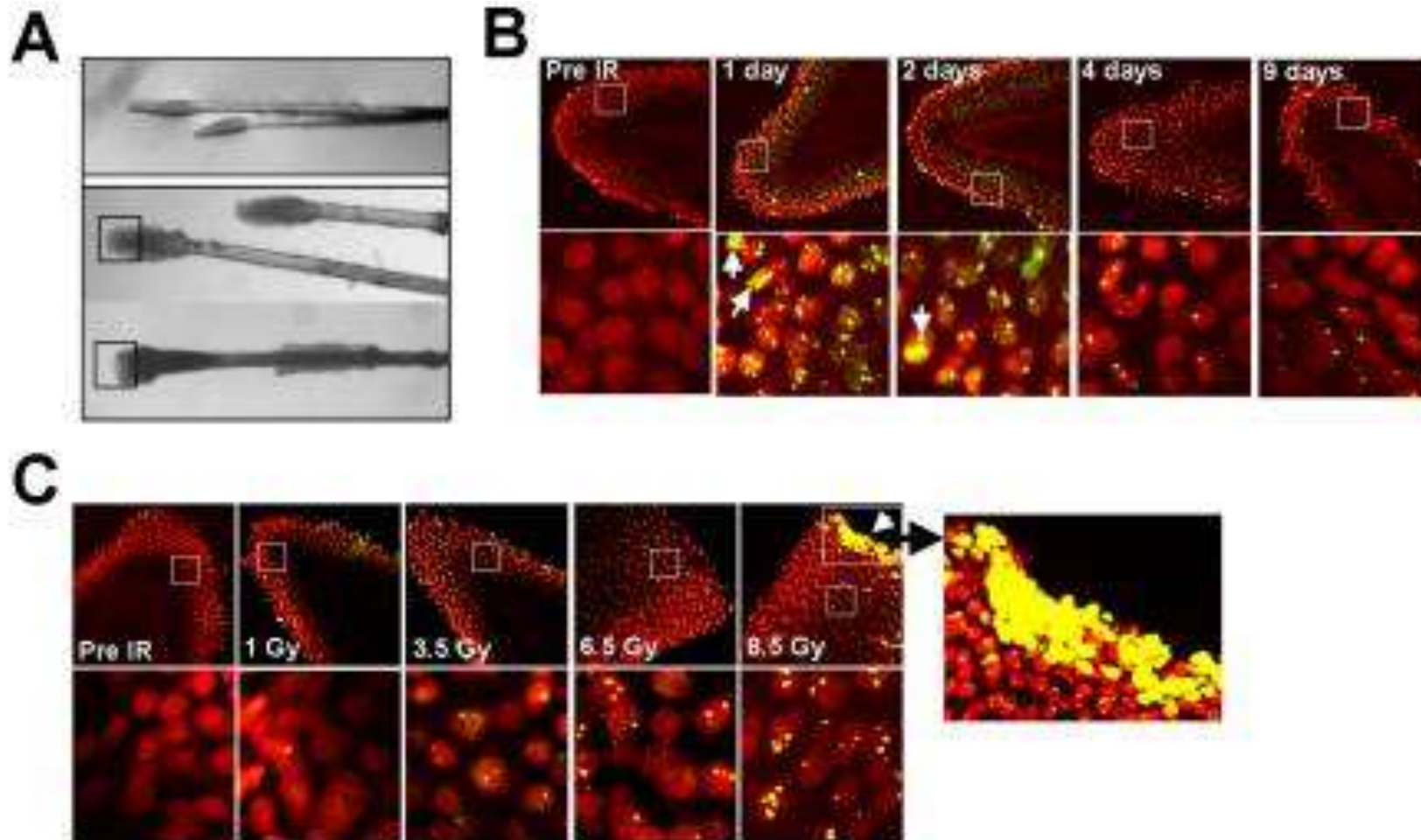
H2AX and the Epithelial Mesenchymal Transition

Figure 2. Kinetics for  $\gamma$ -H2AX foci loss in macaque lymphocytes after total body irradiation.



Redon CE, Nakamura AJ, Gouliava K, Rahman A, Blakely WF, et al. (2010) The Use of Gamma-H2AX as a Biosimeter for Total-Body Radiation Exposure in Non-Human Primates. PLoS ONE 5(11): e15544. doi:10.1371/journal.pone.0015544  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0015544>

Kinetics for  $\gamma$ -H2AX foci in macaque plucked hairs after total-body irradiation.



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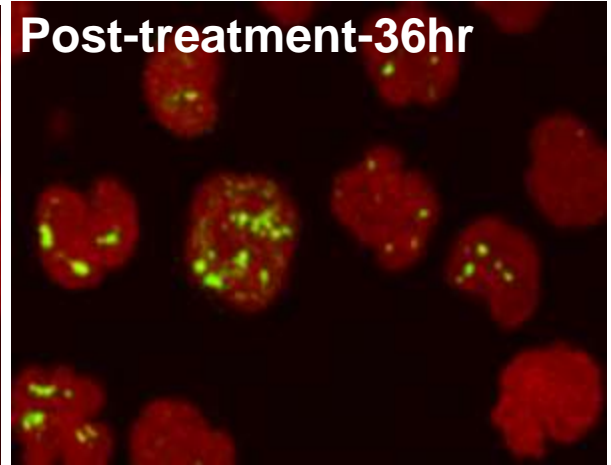
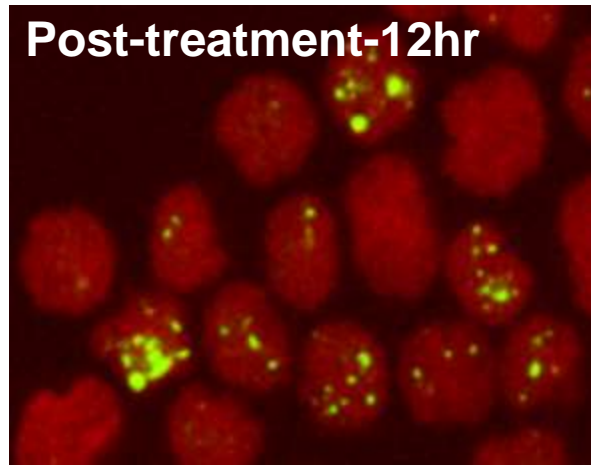
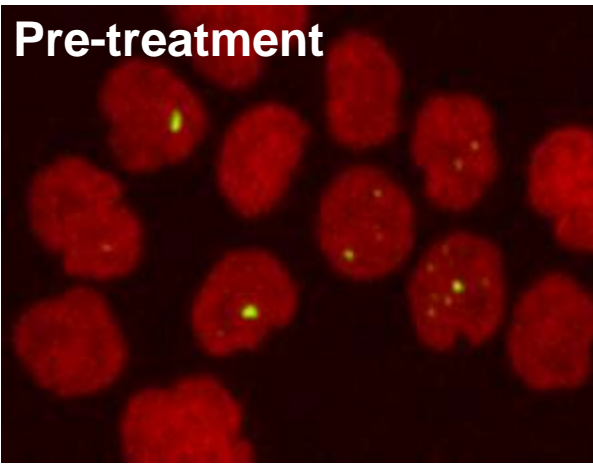
Bystander effect

Dosimetry

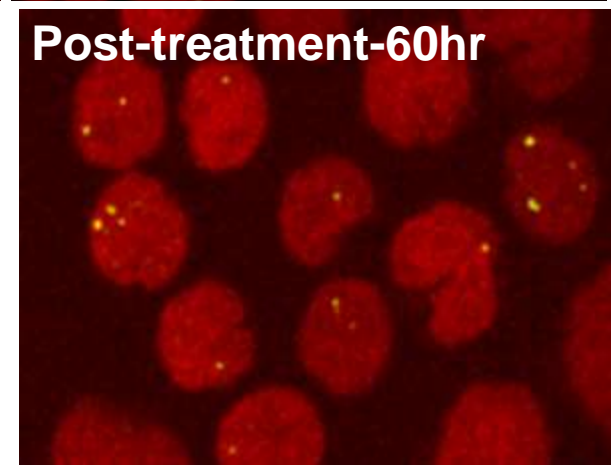
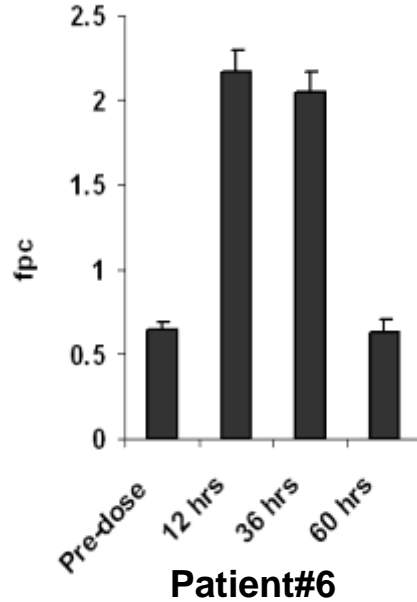
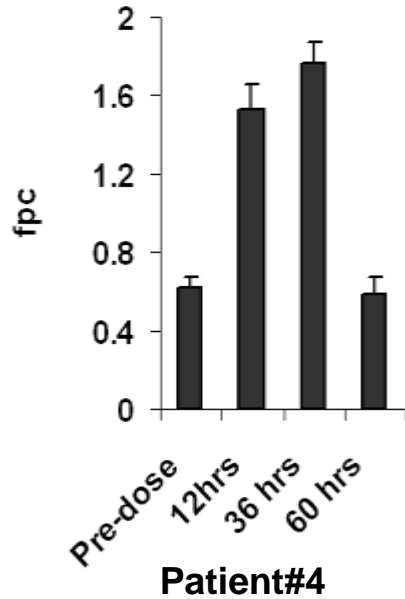
**Pre-clinical studies**

Characterization of NCI60 panel

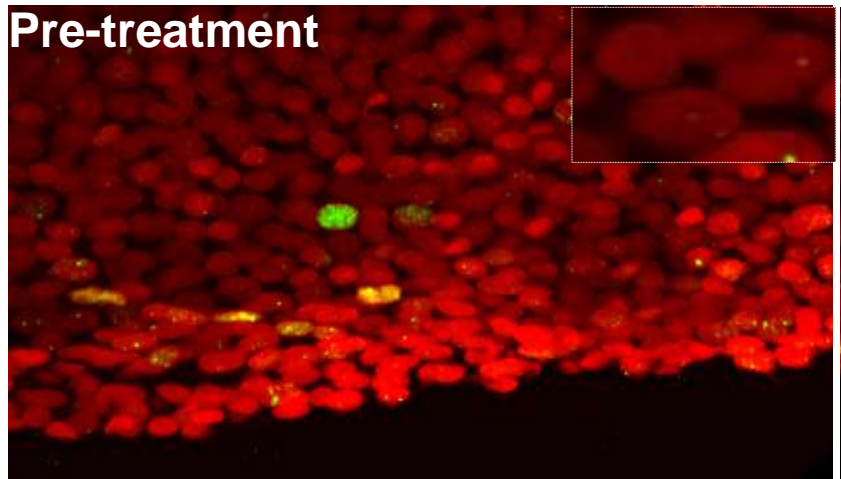
H2AX and the Epithelial Mesenchymal Transition



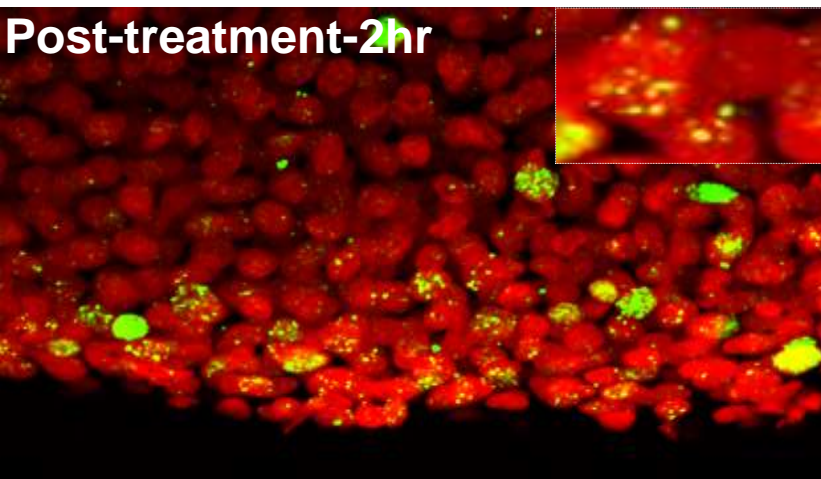
## Lymphocytes – Drug combination



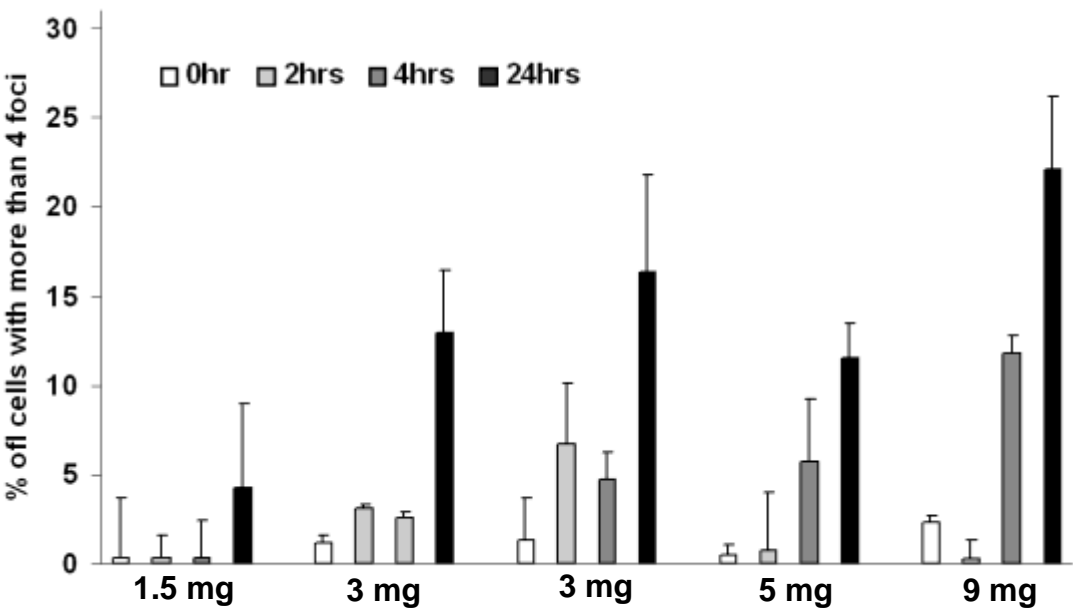
Pre-treatment



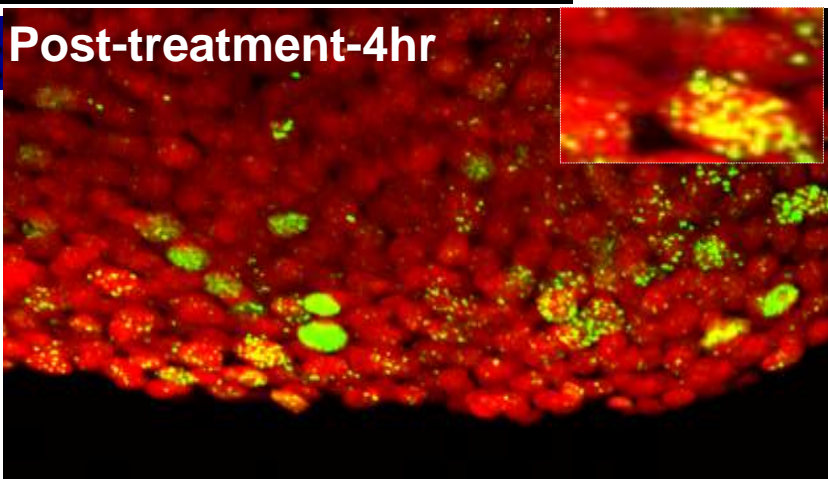
Post-treatment-2hr



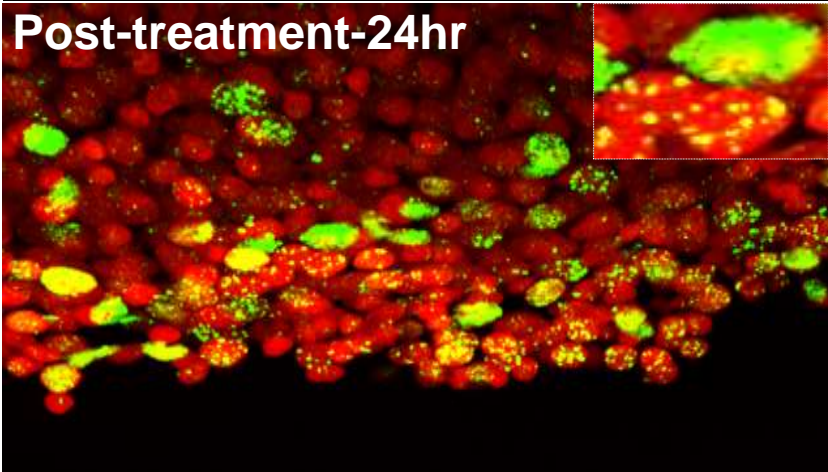
Plucked hairs - DNA alkylating agent



Post-treatment-4hr



Post-treatment-24hr



# H2AX

Ancient history from the last millenium

$\gamma$ -H2AX characterization

KO mouse

Senescence

Bystander effect

Dosimetry

Pre-clinical studies

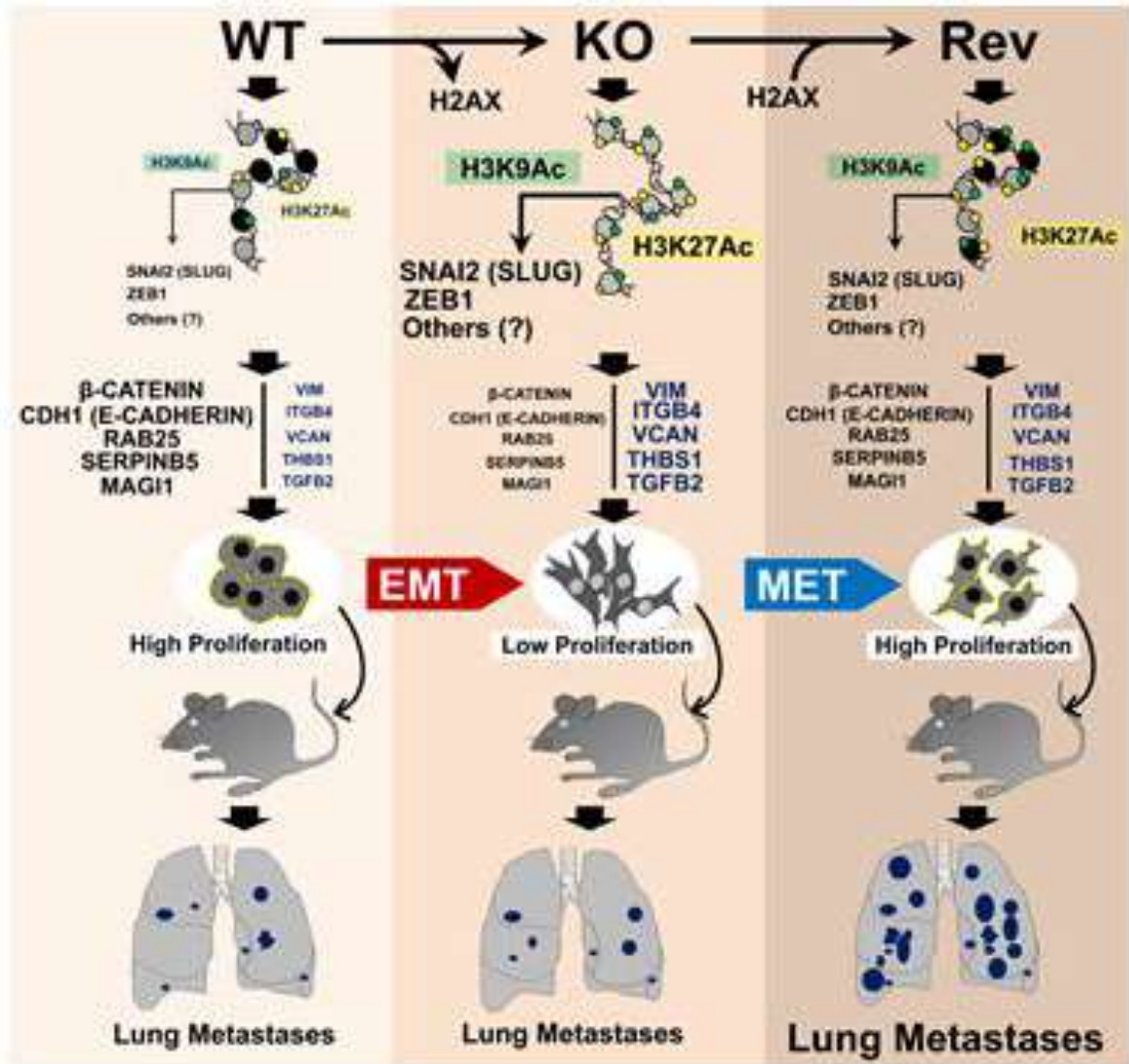
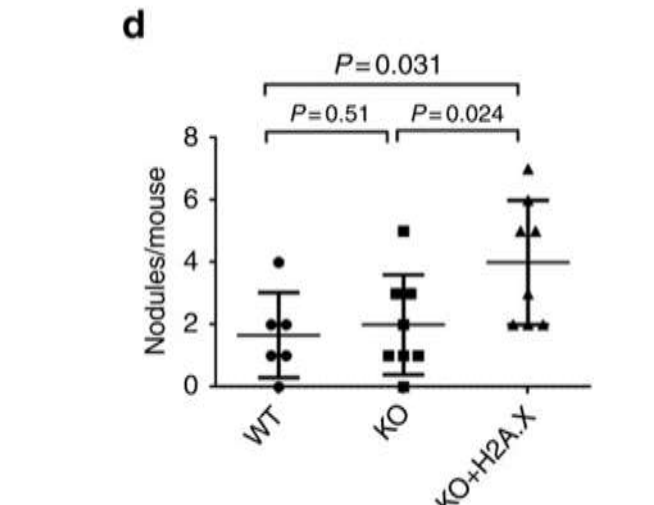
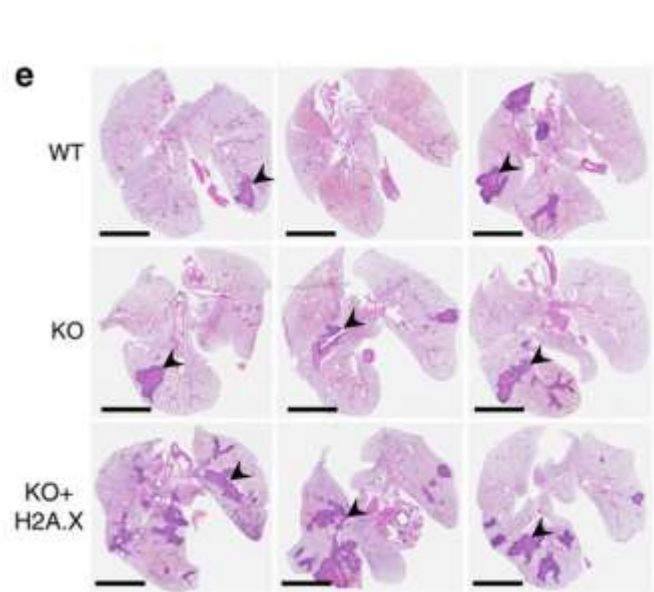
Characterization of NCI60 panel

**H2AX and the Epithelial Mesenchymal Transition**

**The histone variant H2A.X is a regulator of the epithelial-mesenchymal transition.**

Weyemi U, Redon CE, Choudhuri R, Aziz T, Maeda D, Boufraquech M, Parekh PR, Sethi TK, Kasoji M, Abrams N, Merchant A, Rajapakse VN, Bonner WM.

Nat Commun. 2016 Feb 15;7:10711. doi: 10.1038/ncomms10711.





## Collaborators

There have been a great many collaborators over the years. Here are the four that are here today.

In my group (present here today)

Emmy Rogakou: present at the discovery of gamma-H2AX and IR.  
University of Athens, Greece

Asako Nakamura: worked on foci structure.

Ibaraki University, Japan

Olga Sedelnikova: Developed senescence and bystander studies.

Peter Mac

Outside Collaborators (present here today)

Olga Kovalchik, Lethbridge University, Canada

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