H2AX

(unpublished data removed 2016-05-22)

Ancient history from the last millenium
γ-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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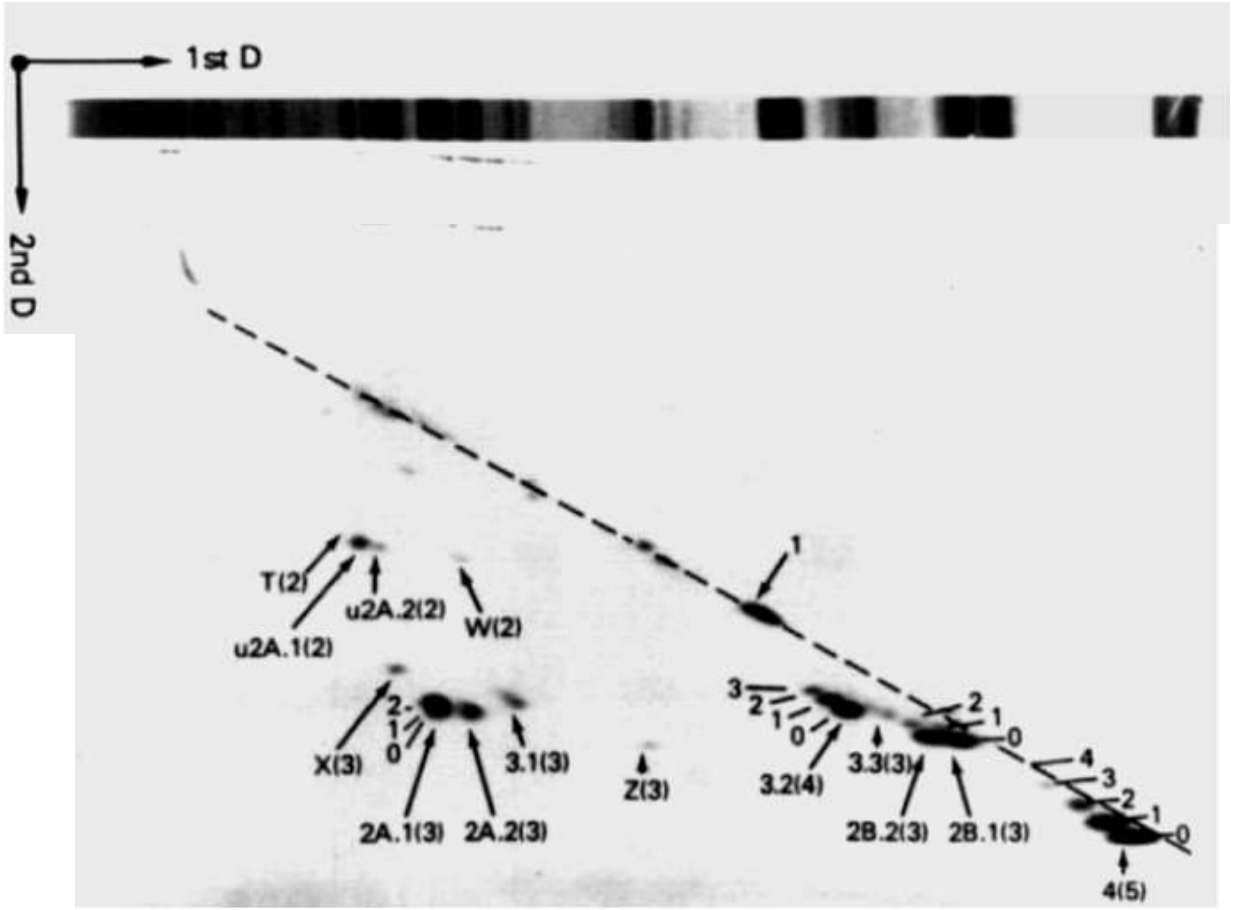
H2AX and the Epithelial Mesenchymal Transition

Histone 2A, a Heteromorphous Family of Eight Protein Species†

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

Biochemistry **1980**, *19*, 3238–3245

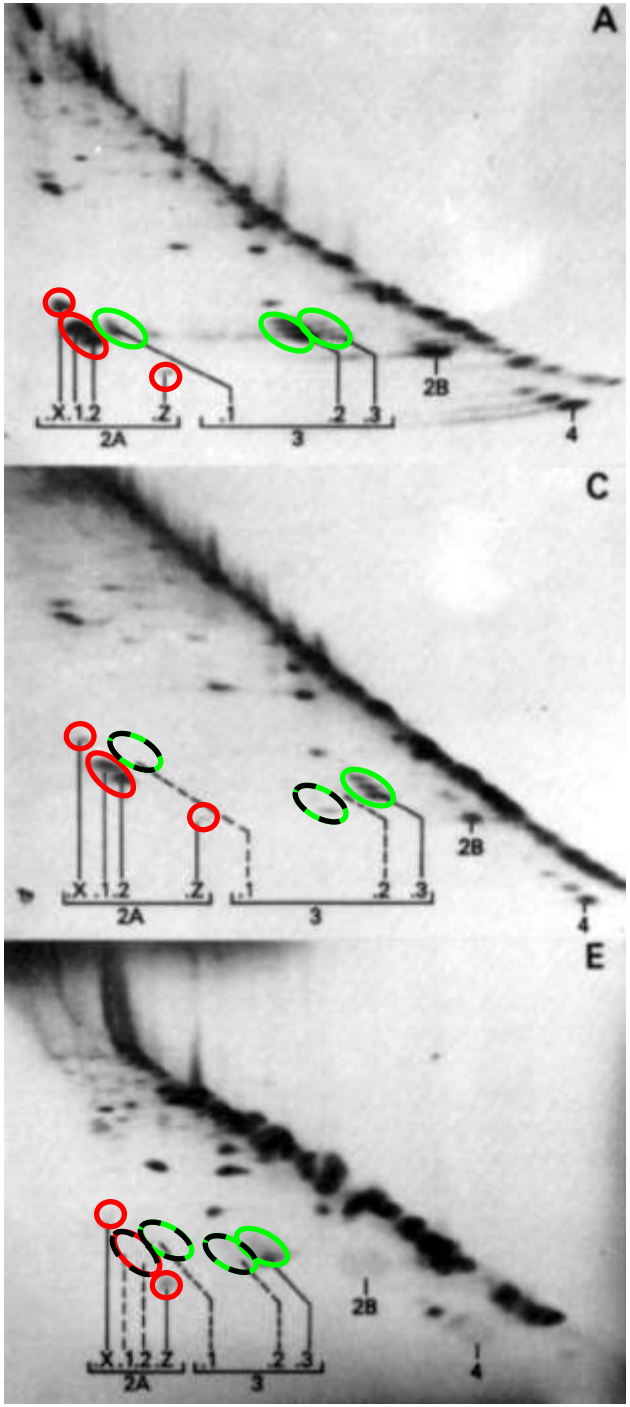


Cell, Vol. 31, 367–374, December 1982 (Part 1), Copyright © 1982 by MIT

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.



Volume 17 Number 22 1989 Nucleic Acids Research

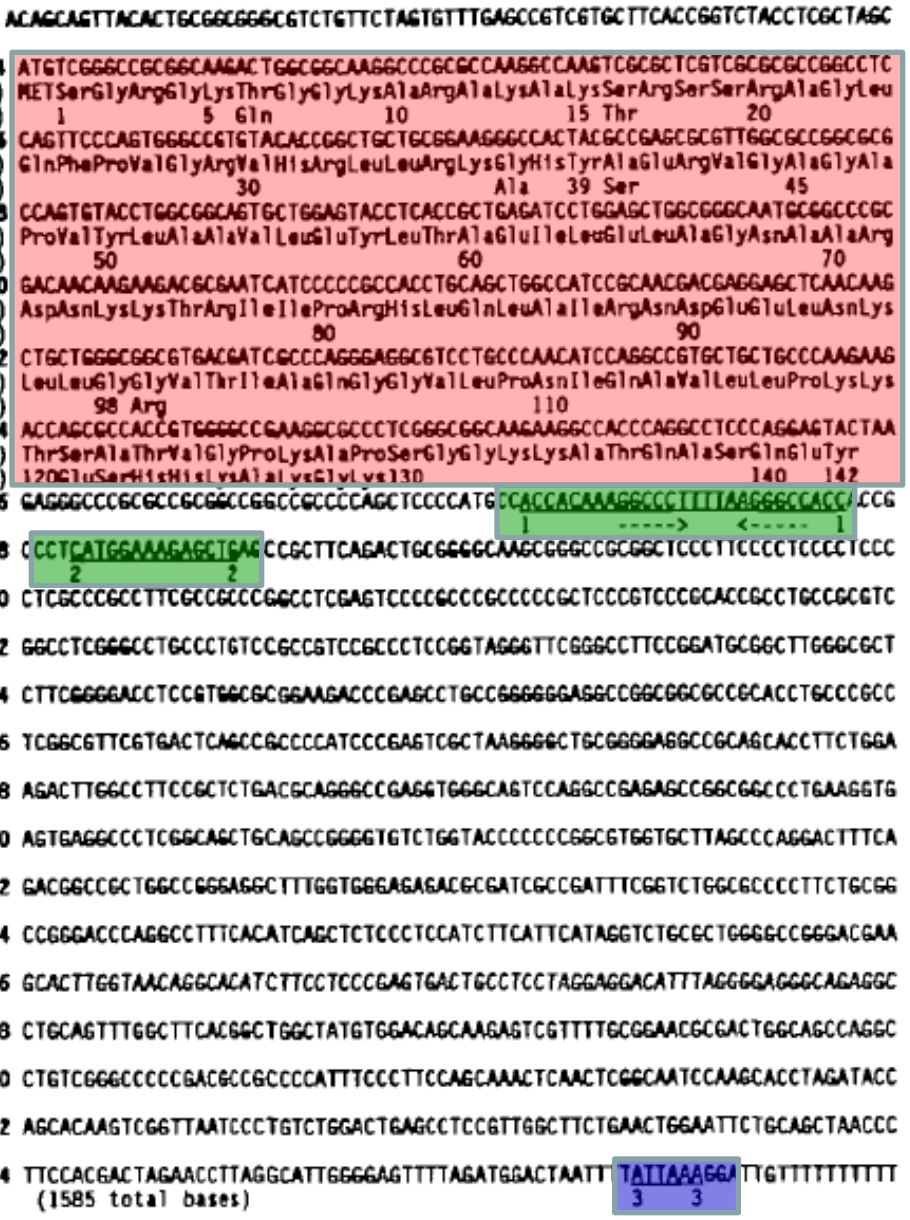
H2A.X. a histone isoprotein with a conserved C-terminal sequence, is encoded by a novel mRNA with both DNA replication type and polyA 3' processing signals

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 K K A T Q A S Q E Y*
Sac. cere H2A.1	K K - S A - - - - - - - - - - - K A T K A S Q E L*
Sac. cere H2A.2	K K - S A - - - - - - - - - - - K T A K A S Q E L*
Aspergillus H2A	K K T P - - - - - - - - - - - - K A G K G S Q E L*
Tetrahymena H2A.1	K K T E - - - - - - - - - - S R - - G Q A S Q D I*
Schiz. pombe H2A.1	T K T S - - - - - - - - - - G R - T G K P S Q E L*
Schiz. pombe H2A.2	T K Q S - - - - - - - - - - G K - - G K P S Q E L*

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



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THE JOURNAL OF BIOLOGICAL CHEMISTRY

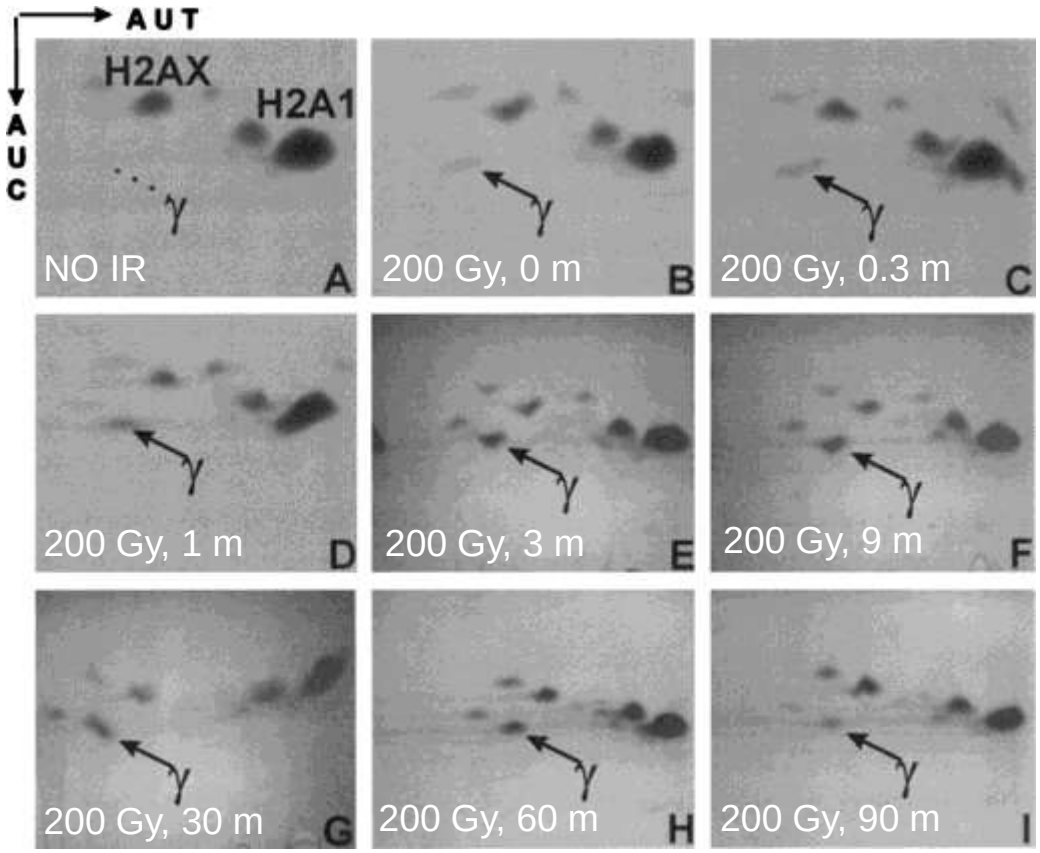
Vol. 273, No. 10, Issue of March 6, pp. 5858–5868, 1998
Printed in U.S.A.

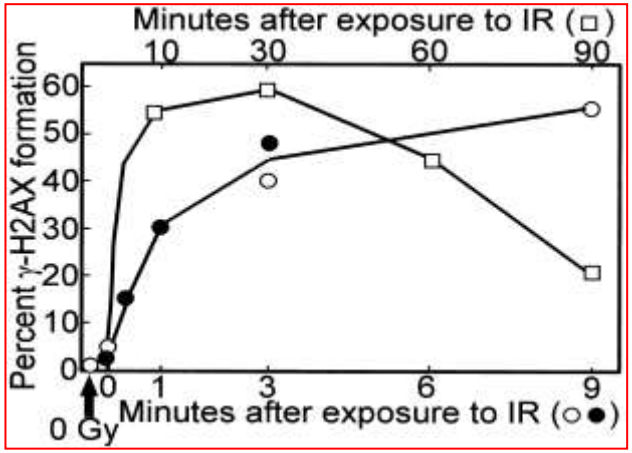
DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139*

(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





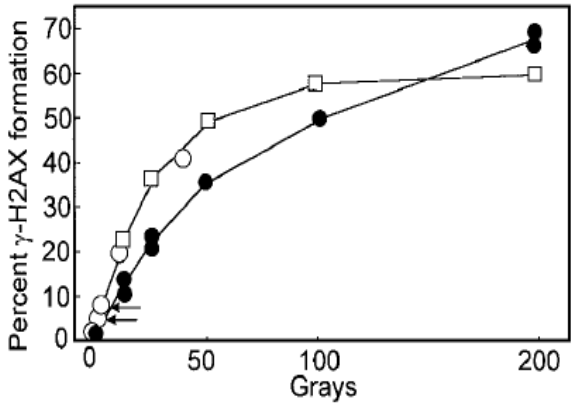


TABLE II

Constant percentages, not numbers, of H2AX molecules are γ-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 γ-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 6×10^9 bp of DNA, hence about 30×10^6 nucleosomes (200 bp/nucleosome) and 60×10^6 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.

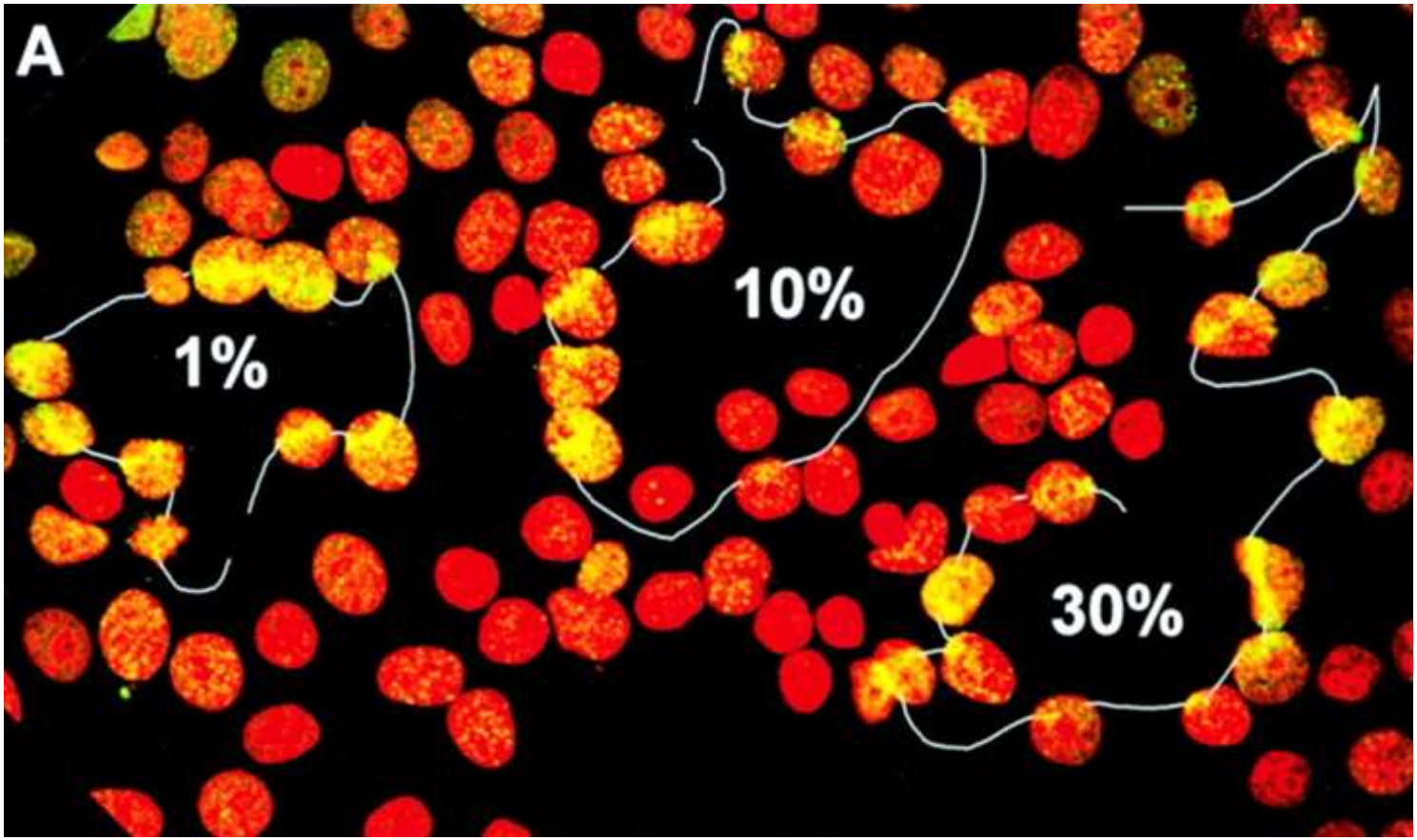
Cell type	H2AX/total H2A	γ-H2AX/total H2AX	No. of H2AX/cell	No. of γ-H2AX/cell	No. of γ-H2AX/dsb	γ-H2AX/dsb	bp of DNA/dsb
	%	%				%	
VA13	2.6	28	1.6×10^6	0.45×10^6	530	0.033	2.0×10^6
HeLa	2.4	30	1.4×10^6	0.45×10^6	490	0.035	2.1×10^6
IMR90	9.8	30	5.9×10^6	1.7×10^6	2100	0.035	2.1×10^6
CHO	9.4	34	5.6×10^6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

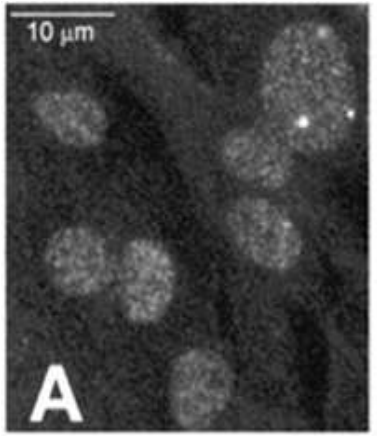
Megabase Chromatin Domains Involved in DNA Double-Strand Breaks In Vivo

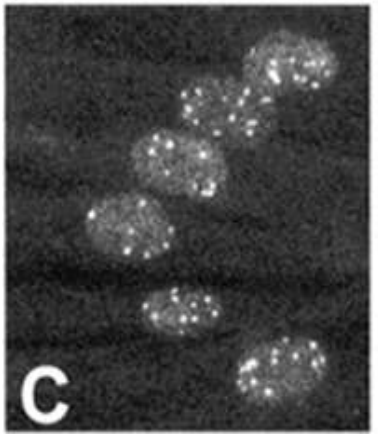
Emmy P. Rogakou, Chye Boon, Christophe Redon, and William M. Bonner

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

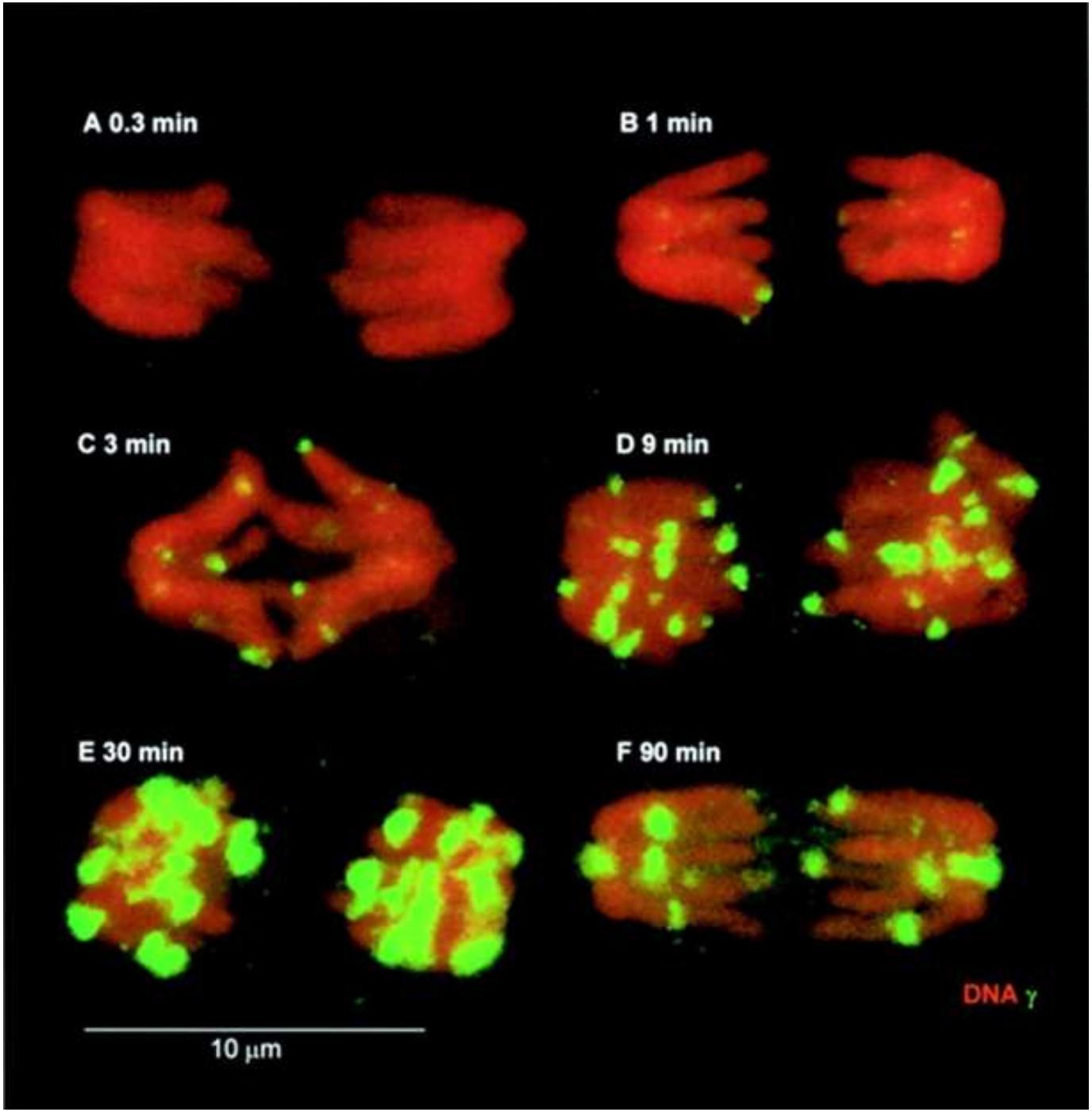
The Journal of Cell Biology, Volume 146, Number 5, September 6, 1999 905–915

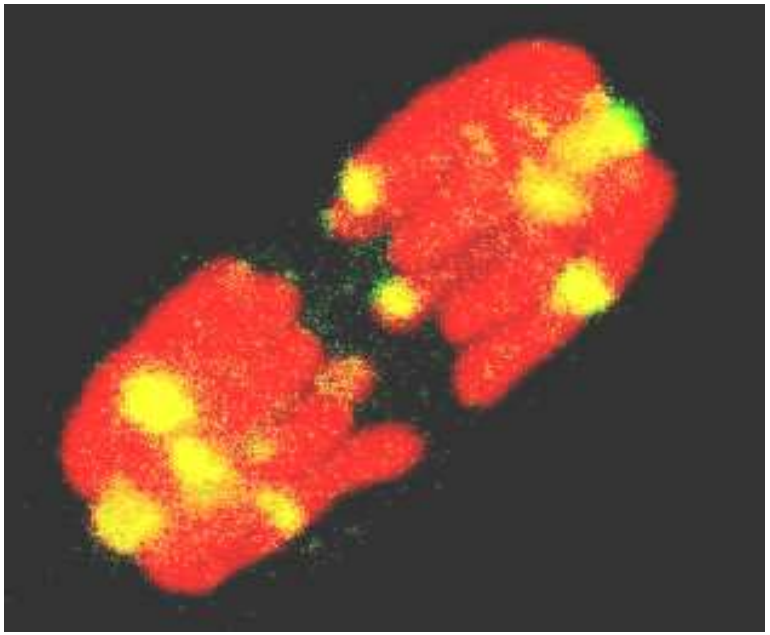


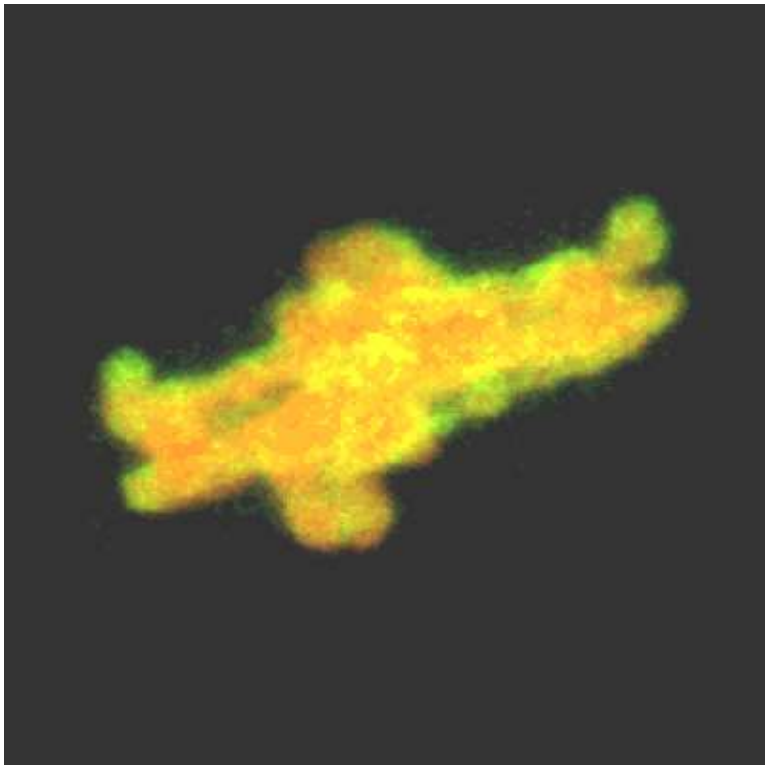


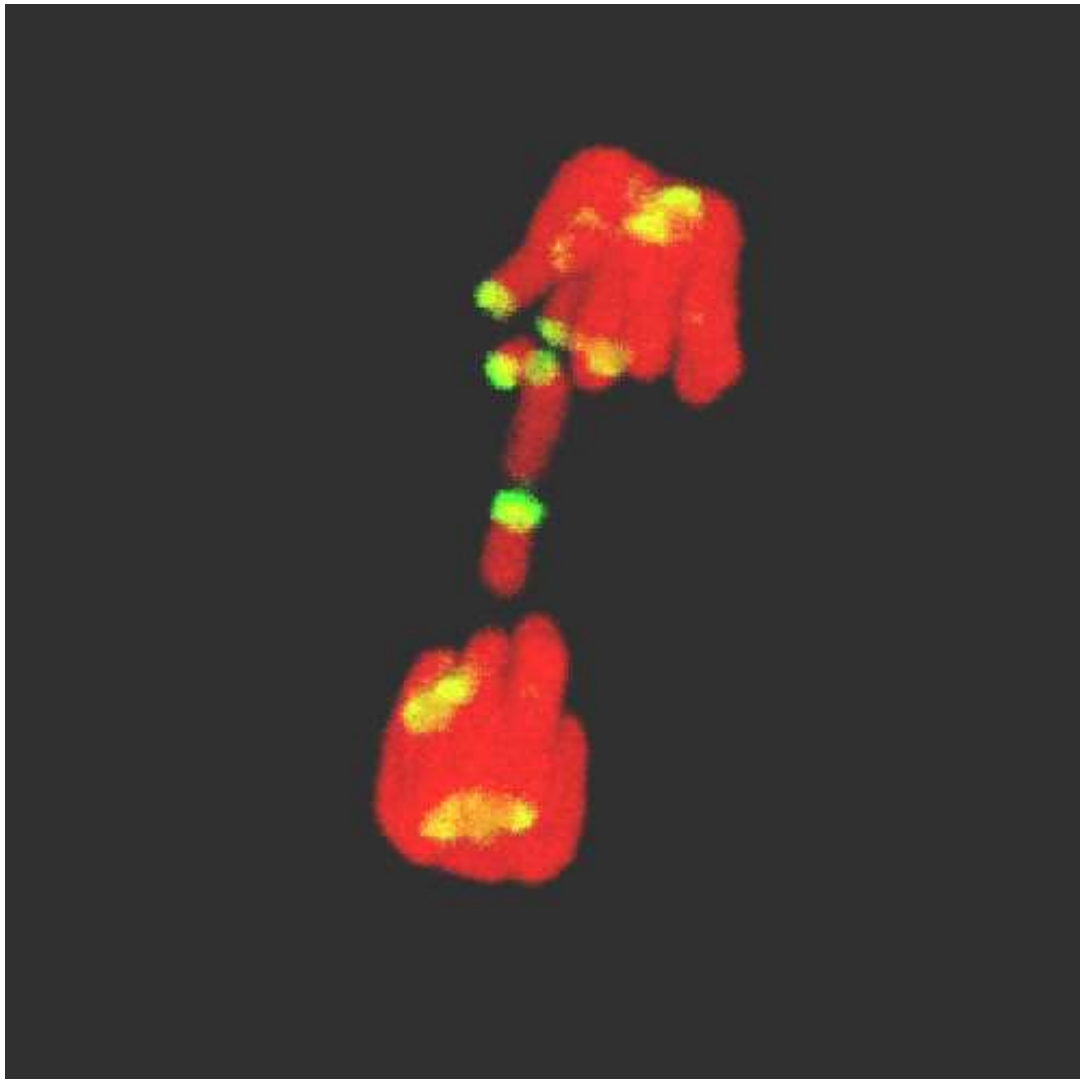


Muntjac Chromosomes. Foci are apparent by 1 min post IR.

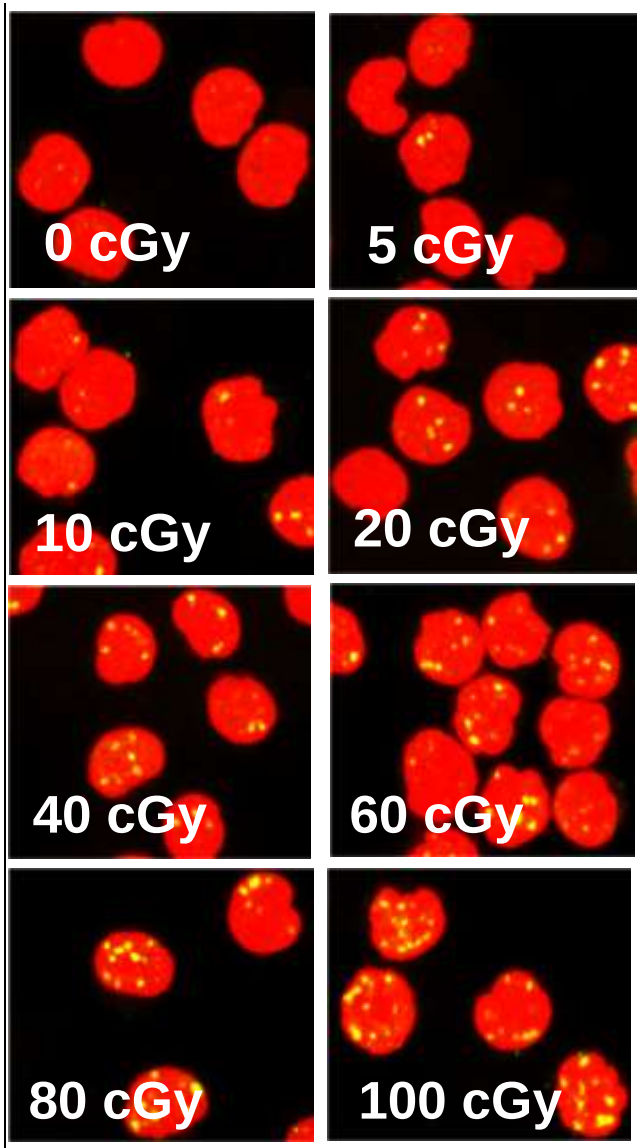


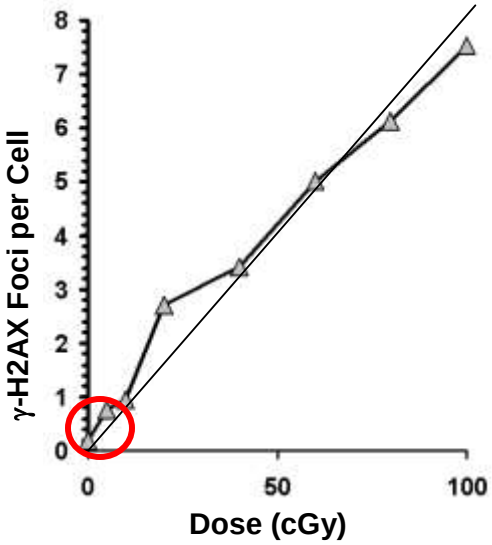


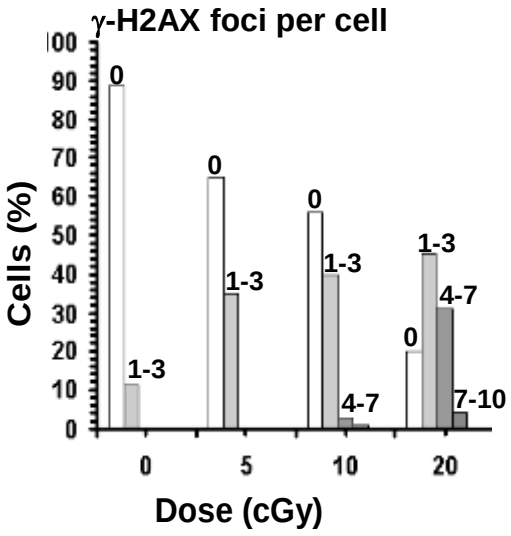




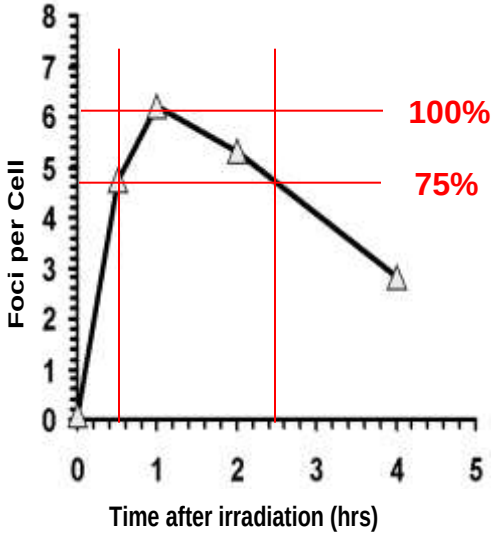
Clinical: Human blood (NIH blood bank) irradiated in vitro







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

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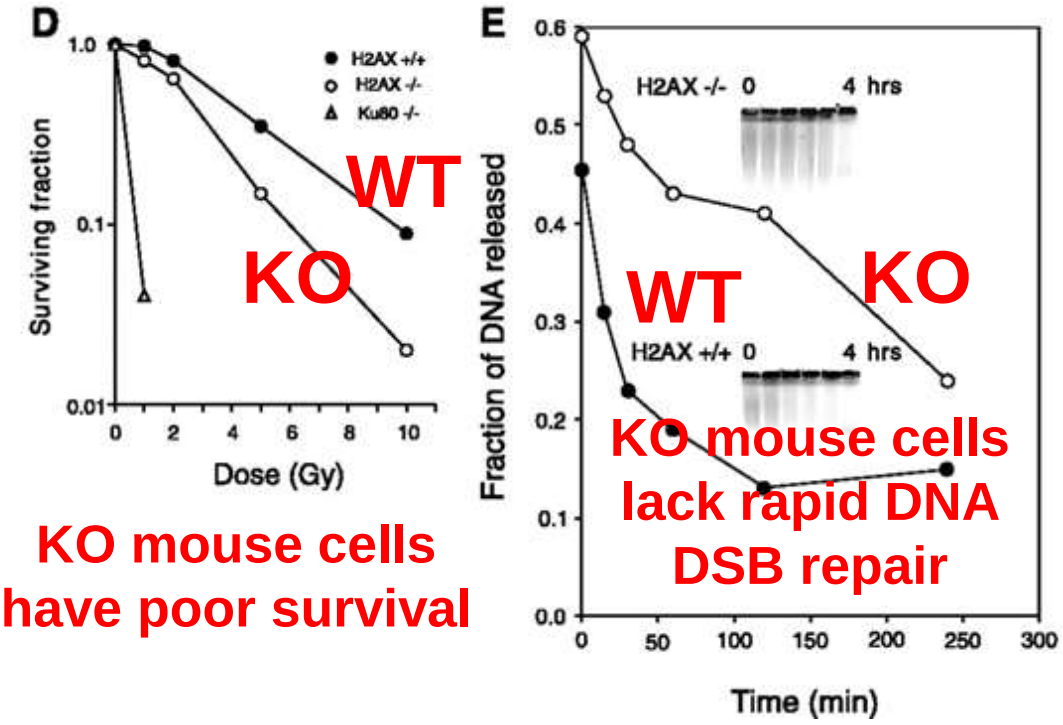
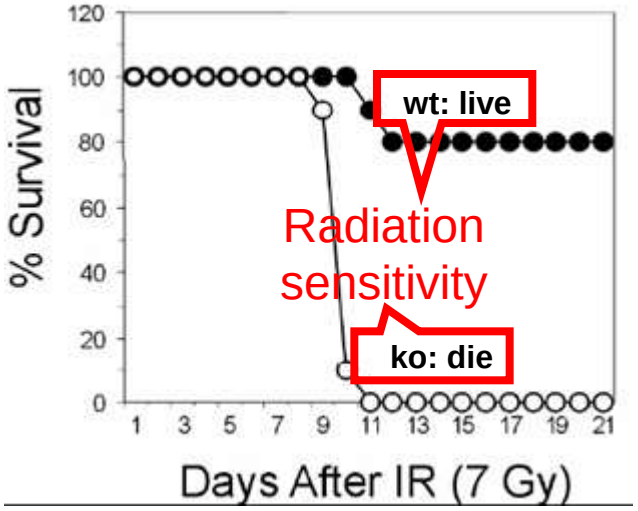
H2AX and the Epithelial Mesenchymal Transition

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.

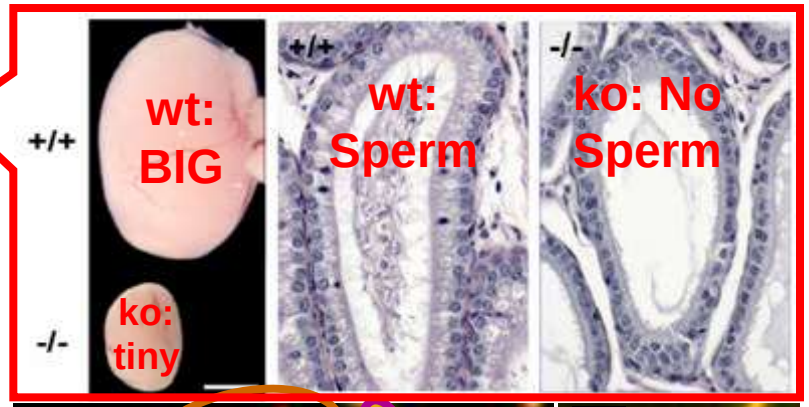
H2AX−/− immortalized MEFs exhibit poor survival and slower dsb repair.



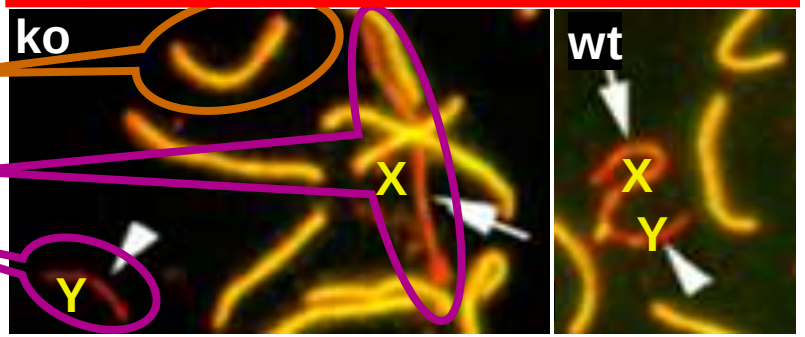


Radiation sensitivity

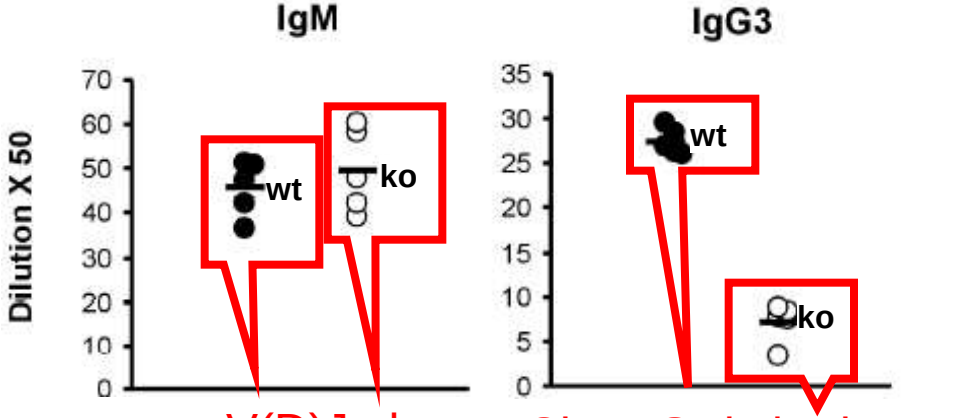
Male infertility.



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

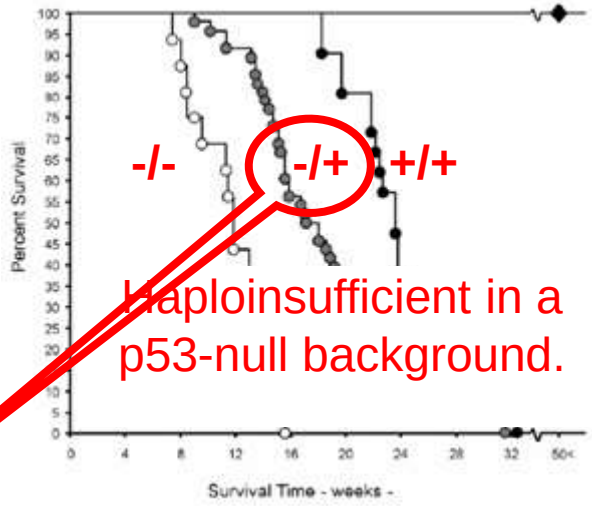


Defective Immune System Recombination

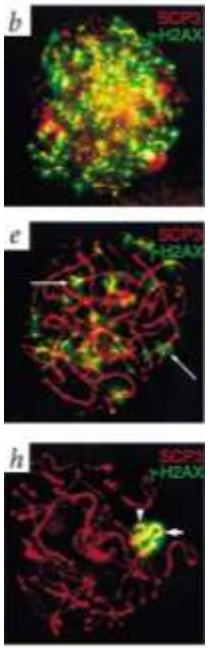


V(D)J ok

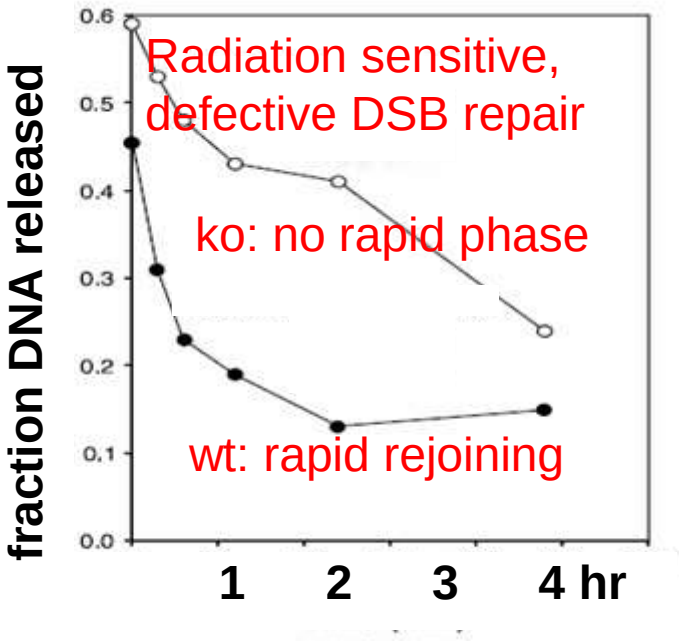
Class Switch absent



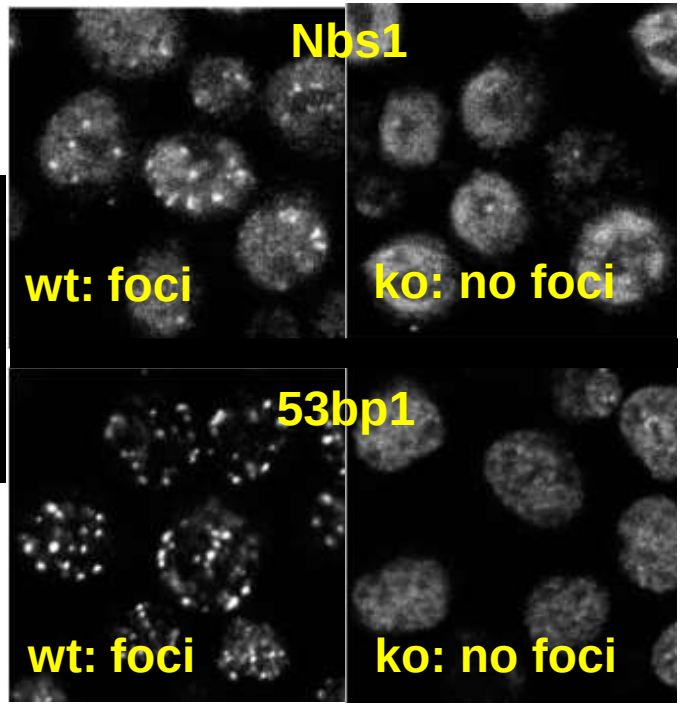
Haploinsufficient in a p53-null background.

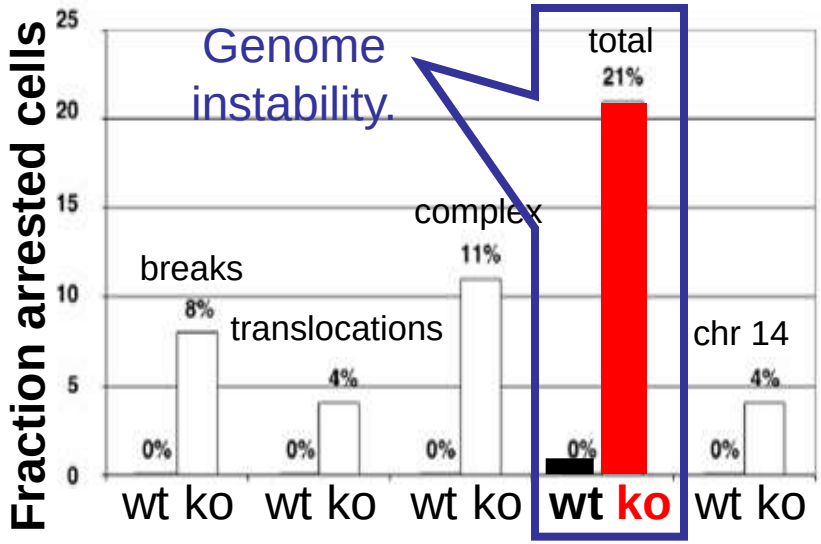


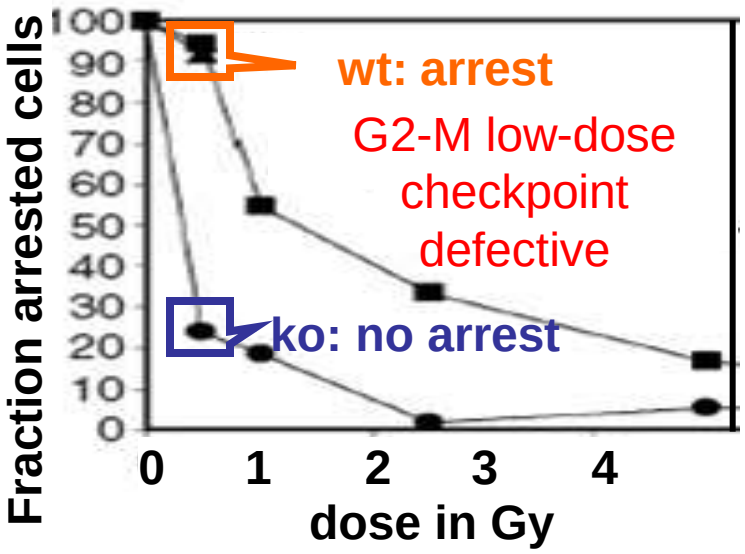
Deaths in p53-null H2AX haploids primarily due to thymic lymphomas



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







H2AX

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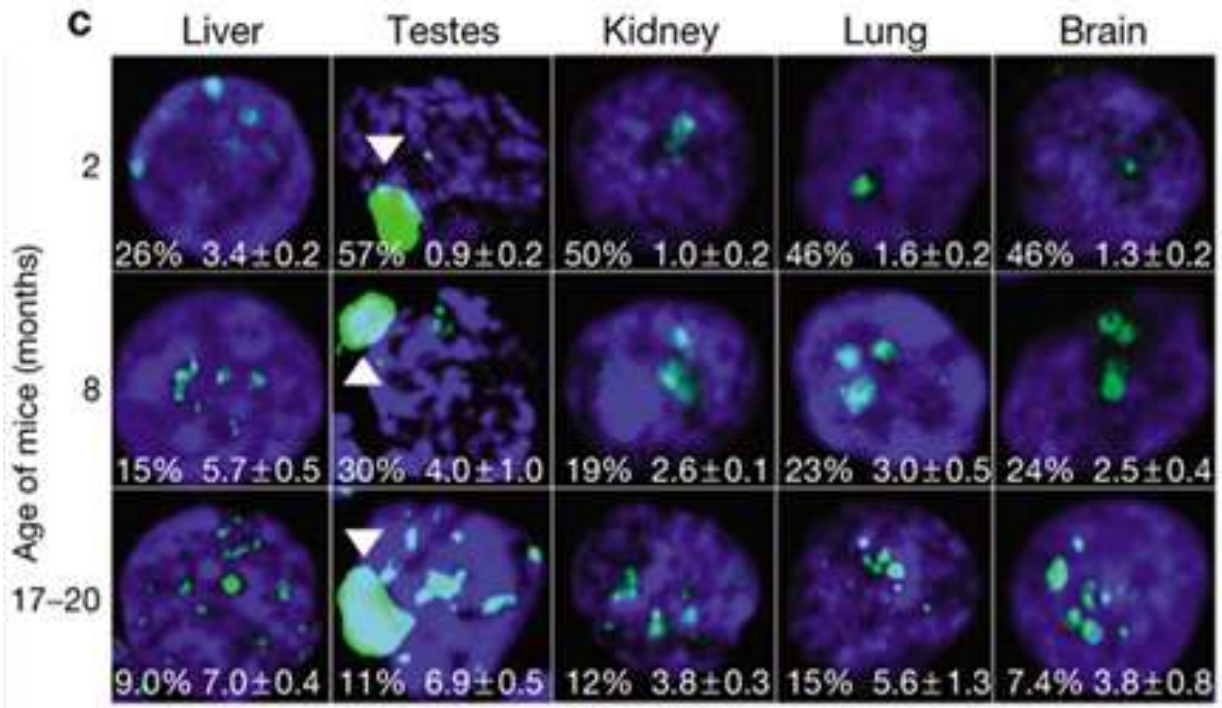
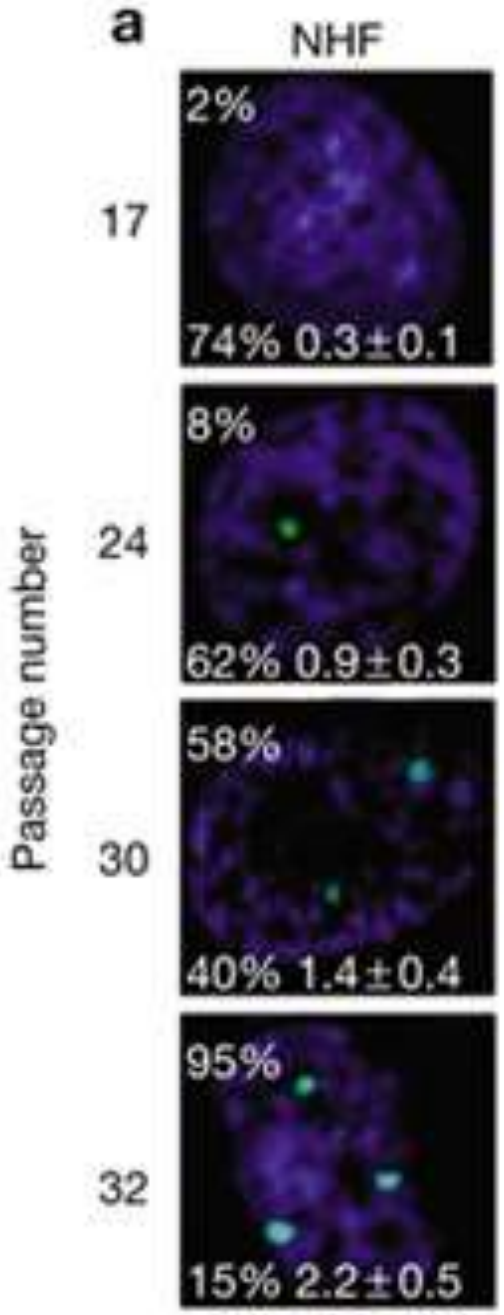
H2AX and the Epithelial Mesenchymal Transition

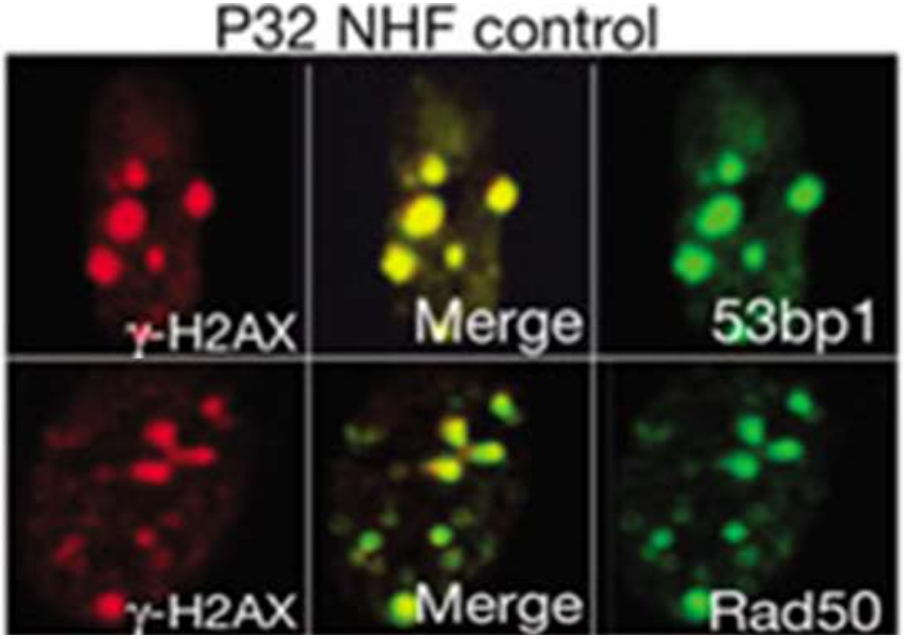
Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Sedelnikova OA1, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, Barrett JC. Nat Cell Biol. 2004 Feb;6(2):168-70.

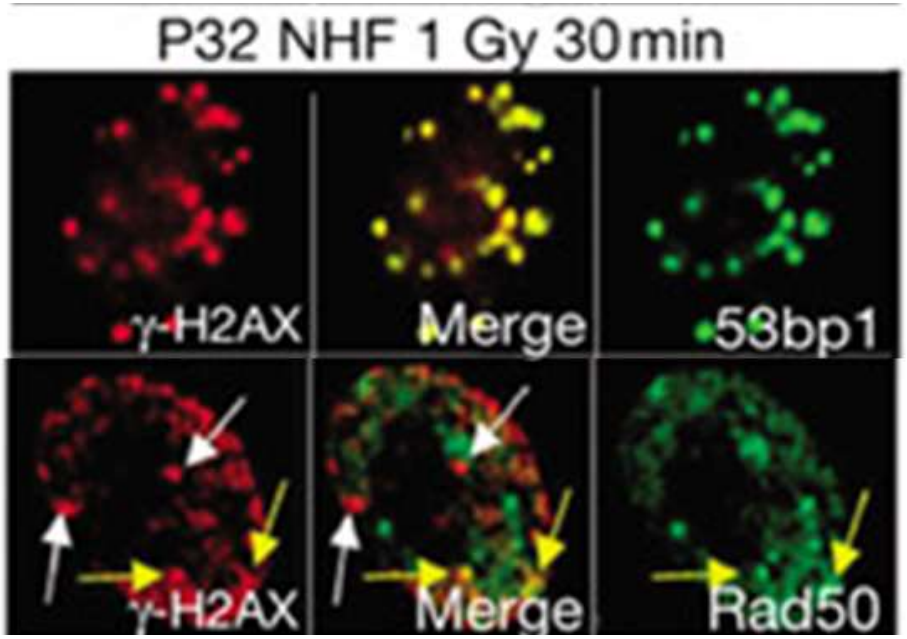
As NHFs and mouse tissues age, the percentage of foci-free cells decreases and the average foci per cell values increase.

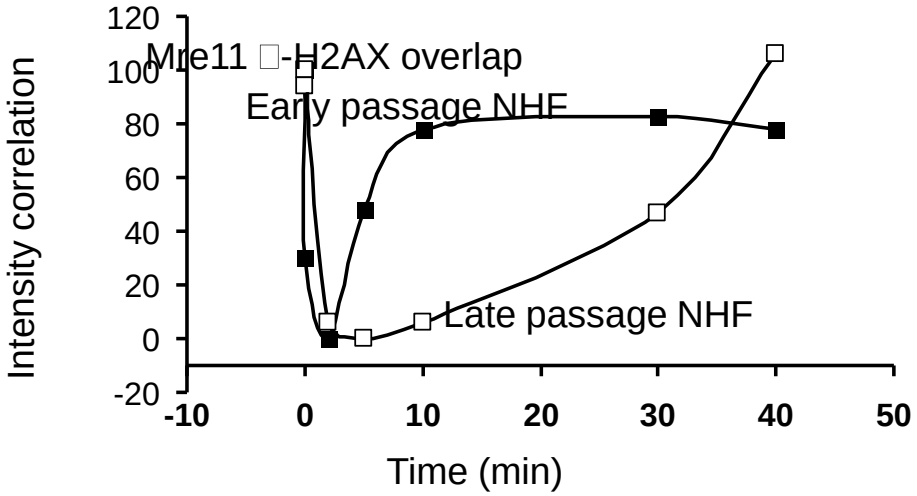




In senescent P32 NHF cultures, proteins are resruited more slowly to foci.
Yellow arrows, gamma-foci overlapping Rad50.
White arrows, gamma-foci lacking Rad50.

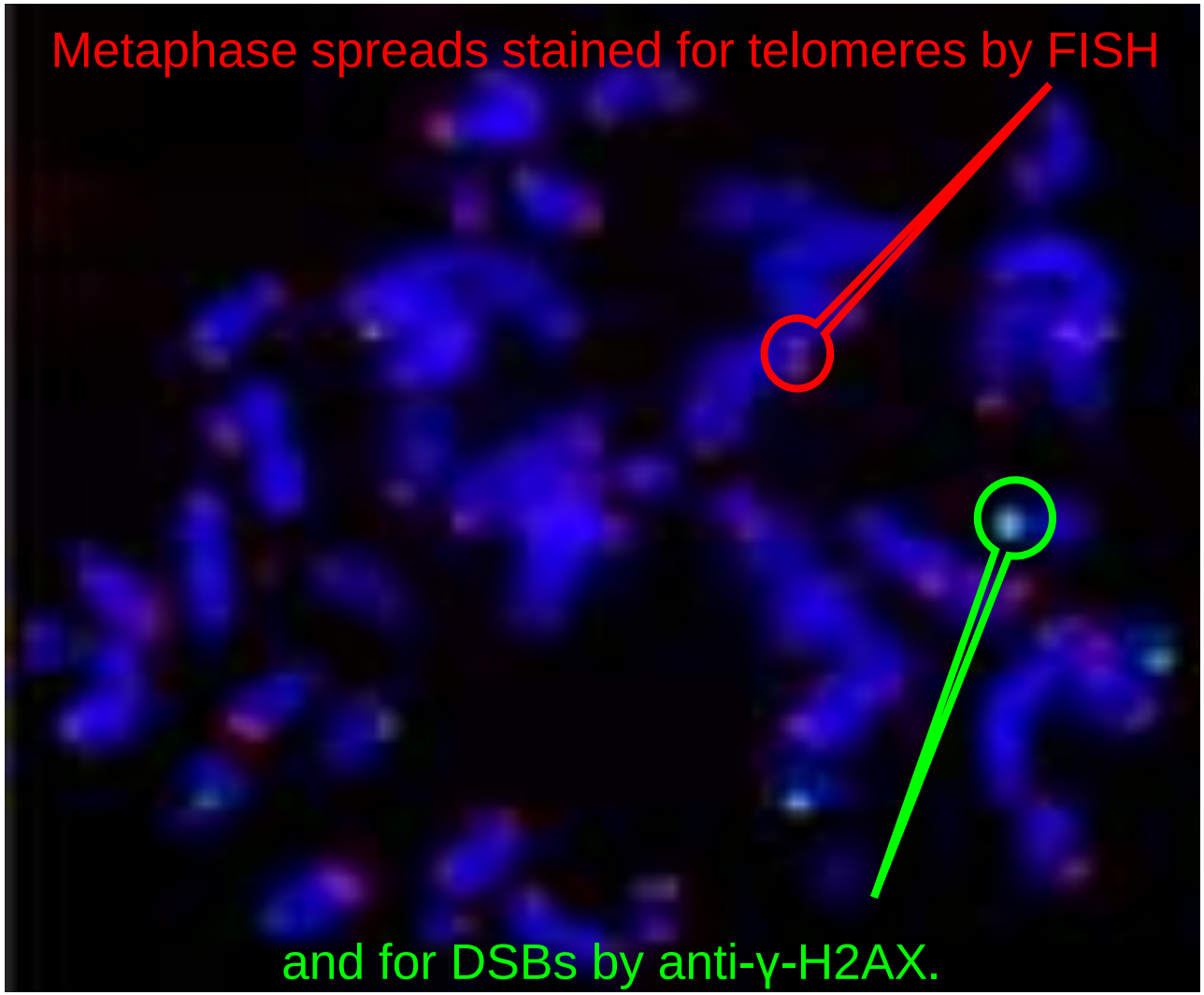






Are the “unrepairable” γ-foci at defective telomeres?
Humans: Yes, about 2/3 rds.
Wt Mice: No

TEL KO Mice:
4th gen:
Yes, about 2/3rds



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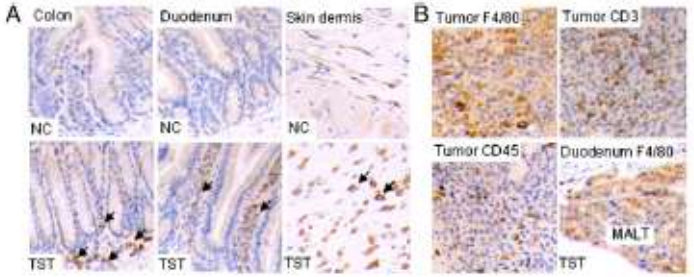
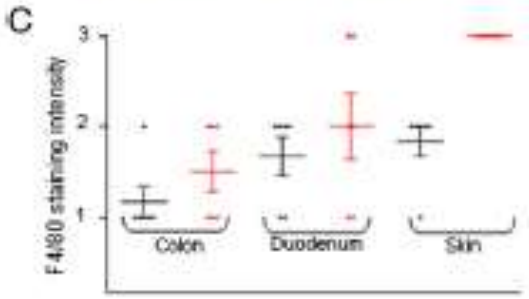
Tumors induce complex DNA damage in distant proliferative tissues in vivo

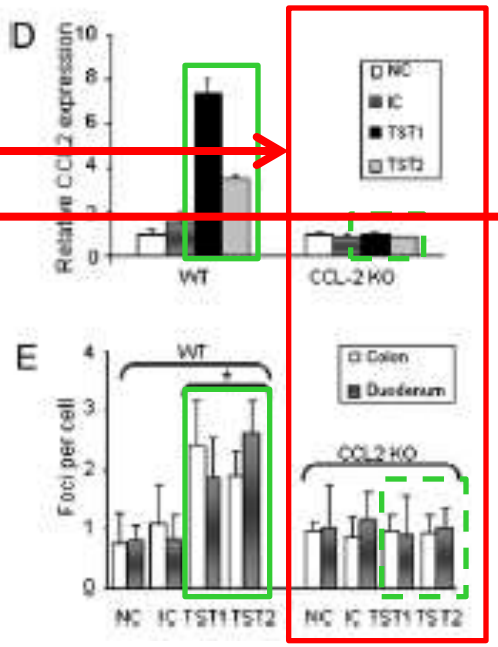
Christophe E. Redon[a], Jennifer S. Dickey[a], Asako J. Nakamura[a], Irina G. Kareva[a], Dieter Naf[b,1], Somaira Nowsheen[c], Thomas B. Kryston[c], William M. Bonner[a], Alexandros G. Georgakilas[c], and Olga A. Sedelnikova[a,2]

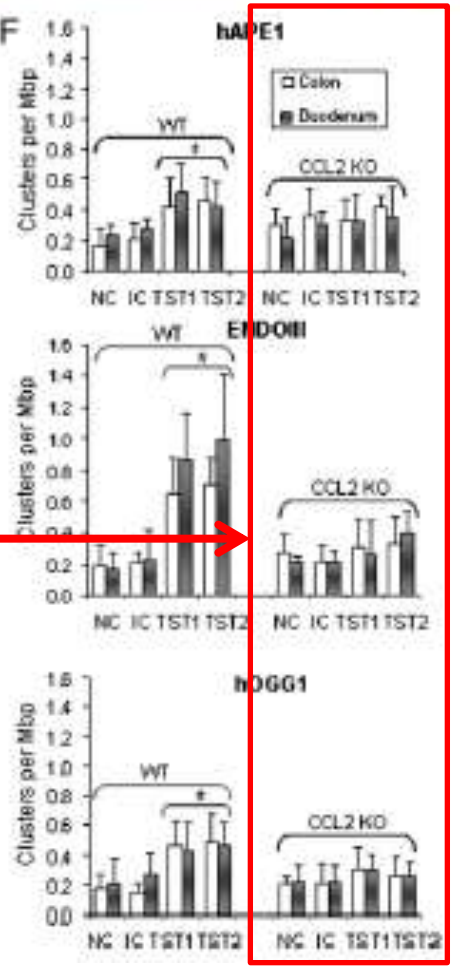
17992–17997 | PNAS | October 19, 2010 | vol. 107 | no. 42





CCL2 KO mouse did not exhibit distant DNA damage





γ-H2AX

DNA repair pathways

Radiation Biology

Drug development

Basic Research

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

Christophe E. Redon[1], Urbain Weyemi[1], Palak R. Parekh[1], Dejun Huang[1,2], Allison S. Burrell[1,3], William M. Bonner[1]*

Drug development

Radiation biodosimetry

Animal Studies

Diagnostics

Irradiation procedures/treatments

Drug development (chemotherapy)

Translational Studies

Biodosimetry (in vivo effects)

Screenings

Protocol improvement

Clinical Trials (0, I, II, III)

Environmental Studies

Chemical Genotoxicity Assessment

Occupational studies

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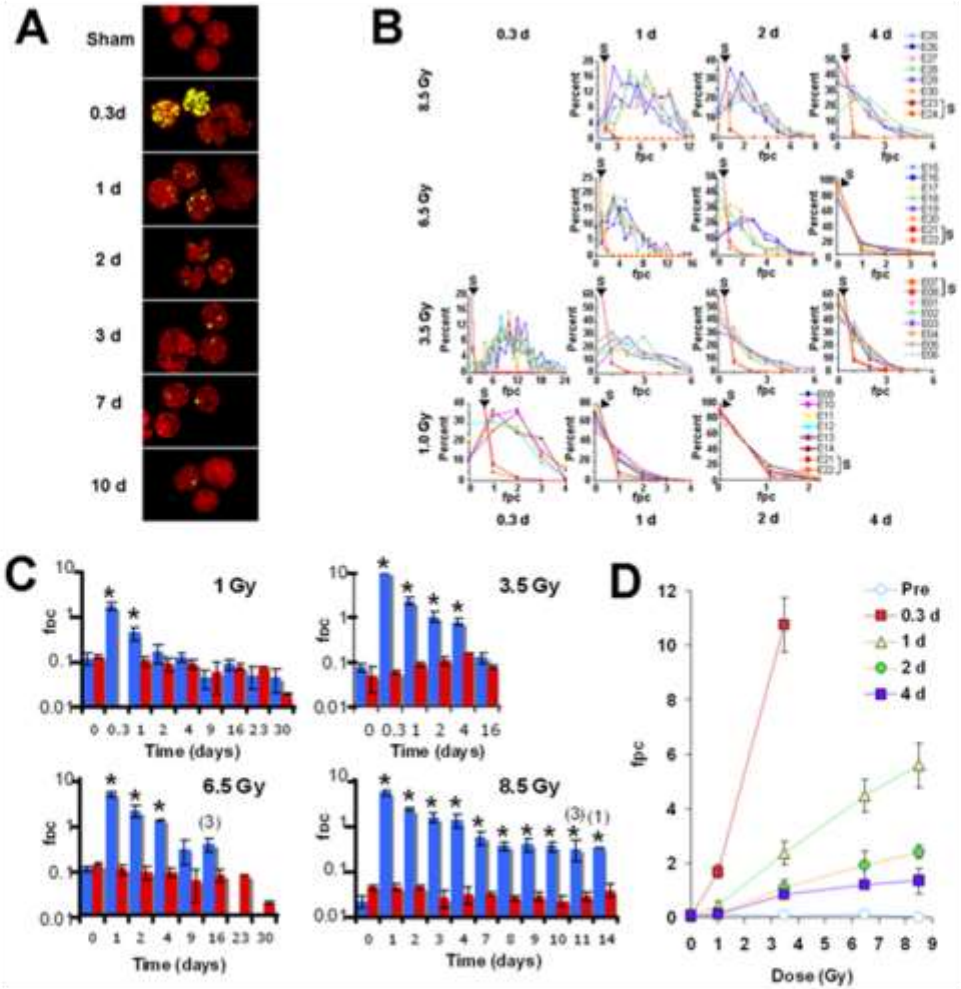
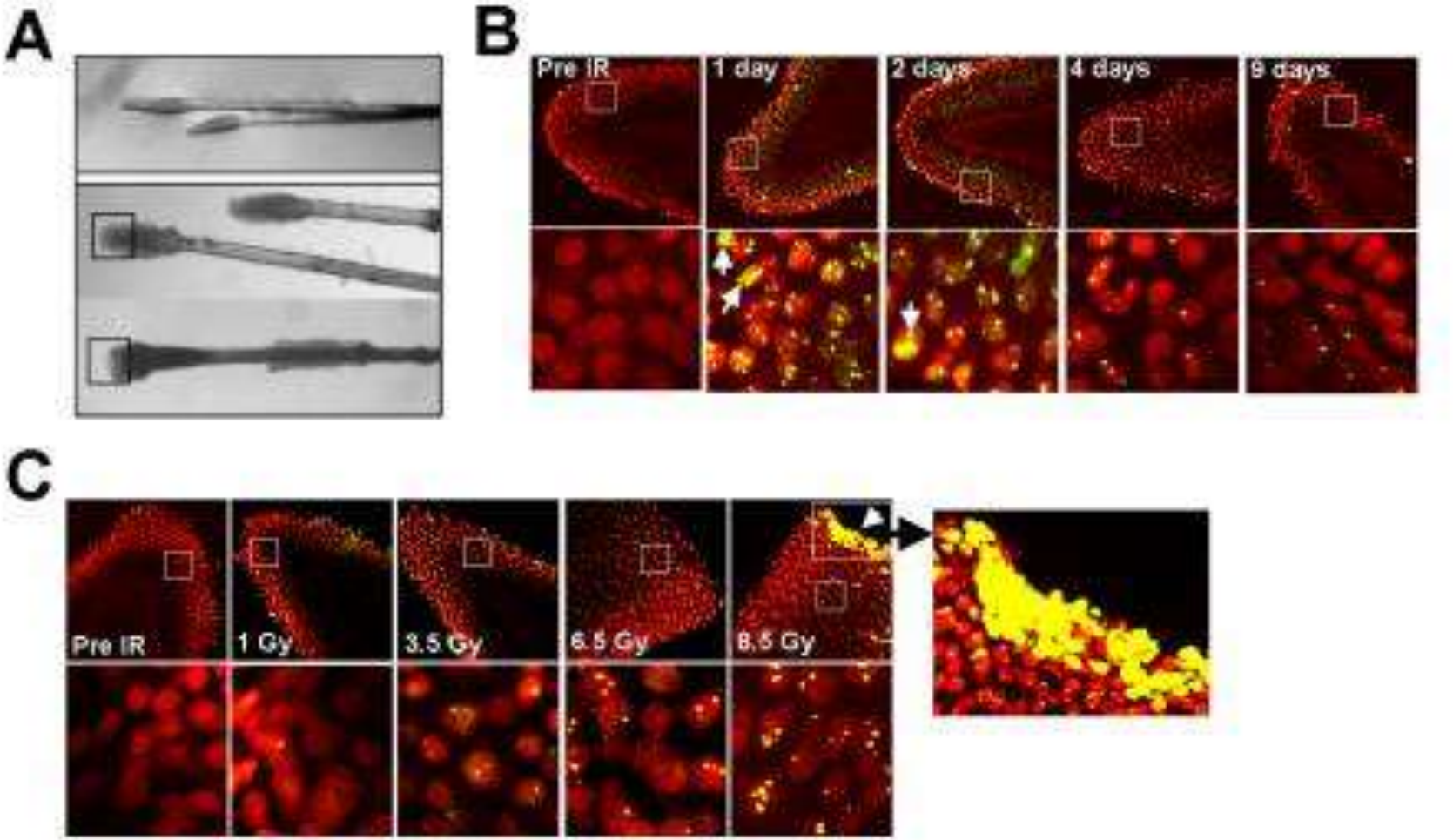
Figure 2. Kinetics for γ-H2AX foci loss in macaque lymphocytes after total body irradiation.



Redon CE, Nakamura AJ, Gouliaeva K, Rahman A, Blakely WF, et al. (2010) The Use of Gamma-H2AX as a Biodosimeter 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 γ-H2AX foci in macaque plucked hairs after total-body irradiation.



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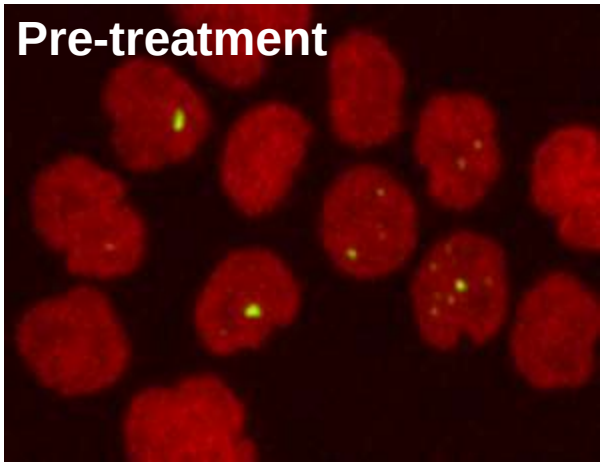
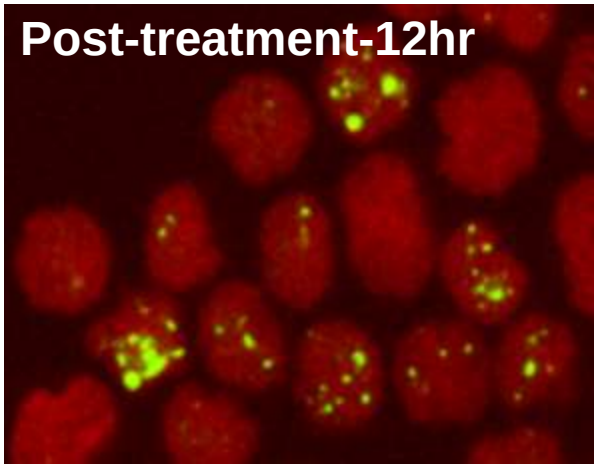
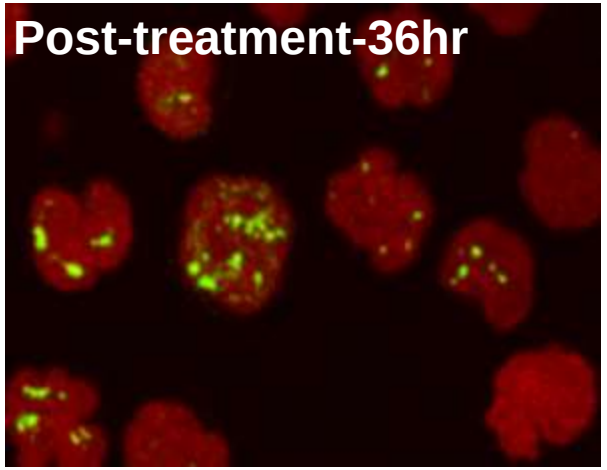
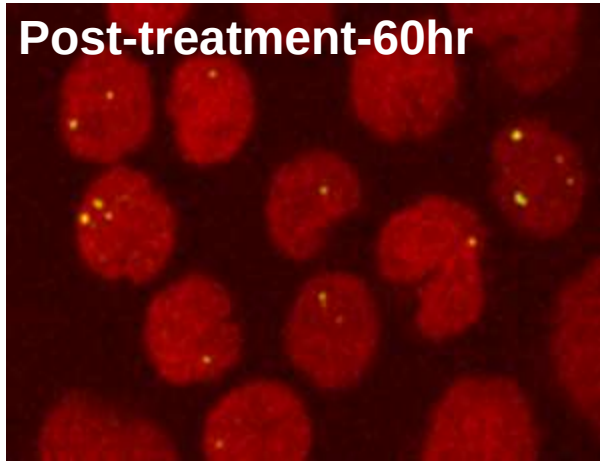
H2AX and the Epithelial Mesenchymal Transition

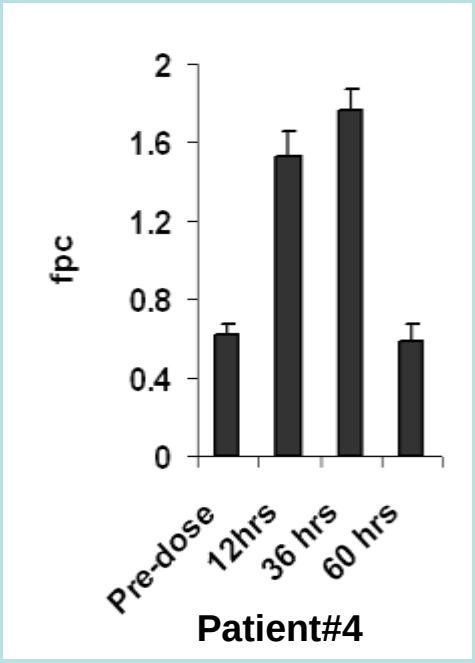


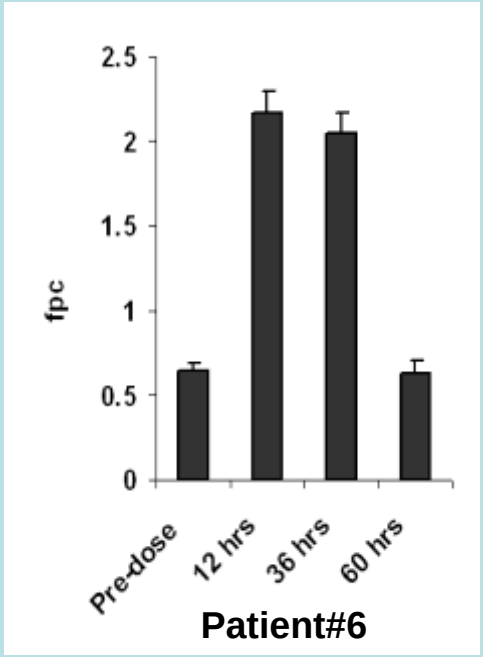


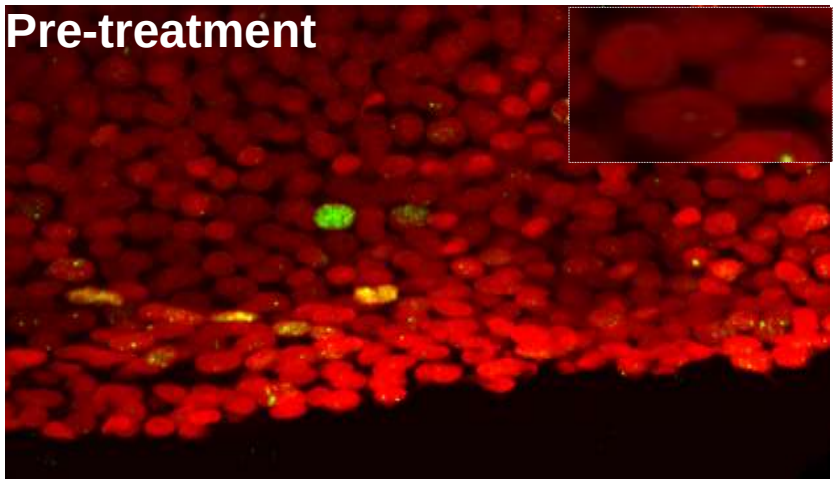


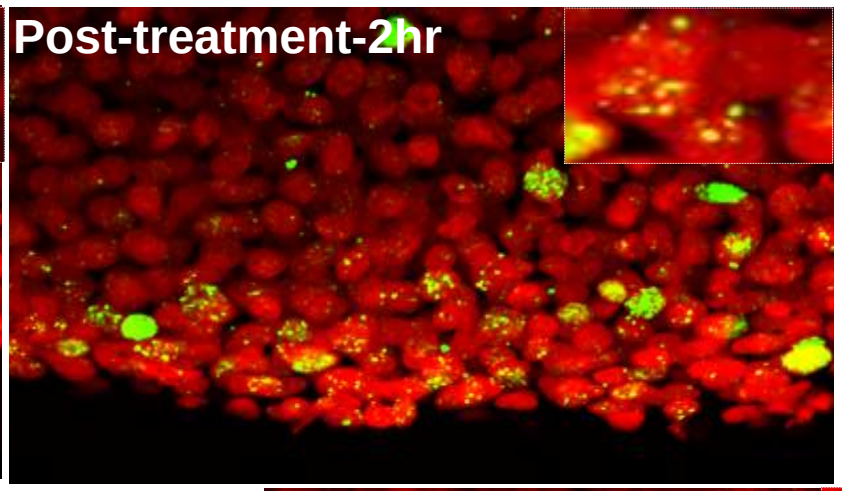


Lymphocytes – Drug combination

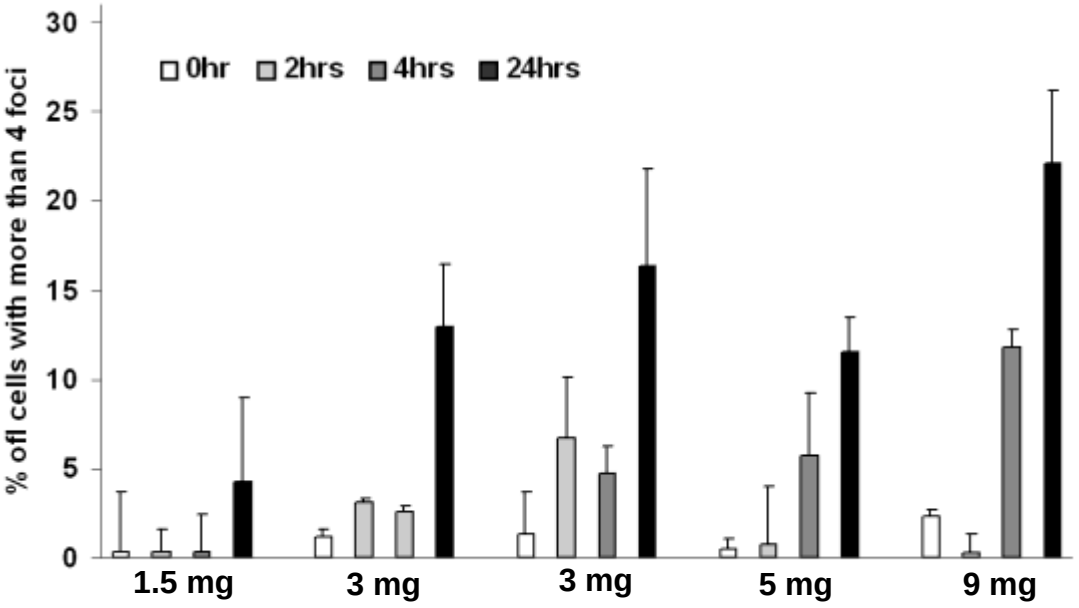


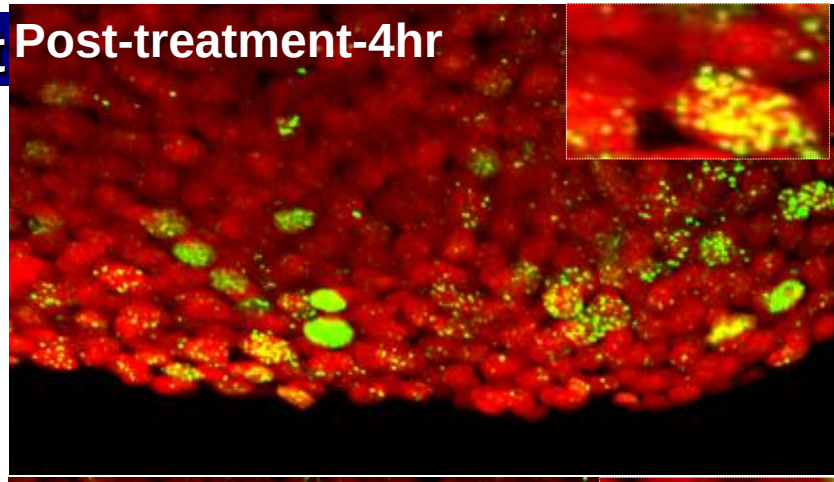


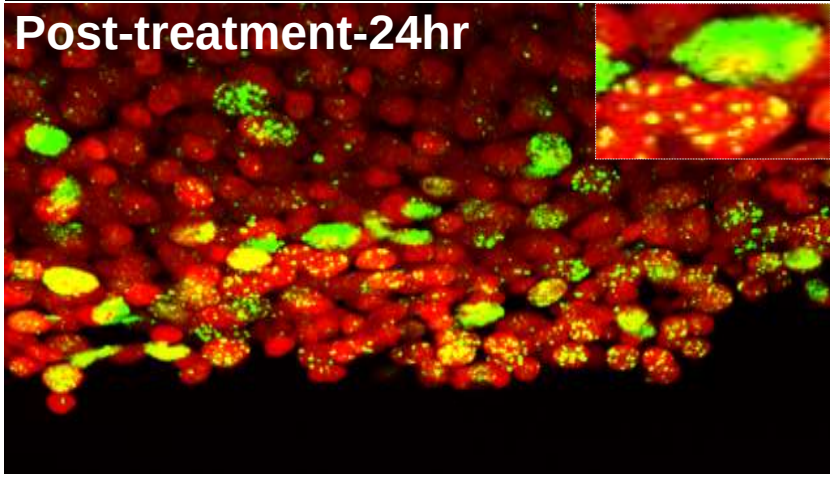




Plucked hairs - DNA alkylating agent



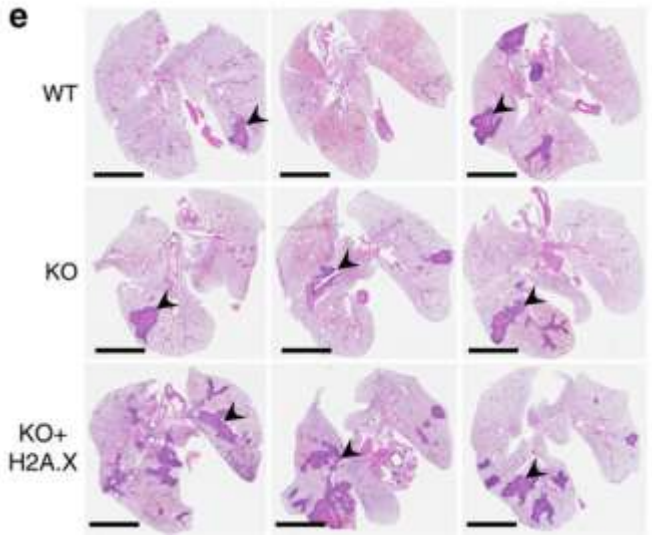


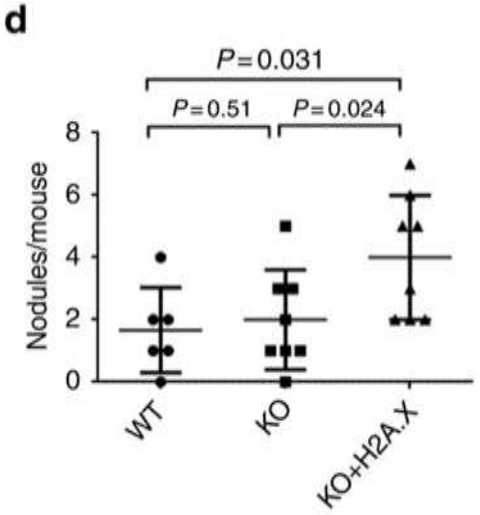


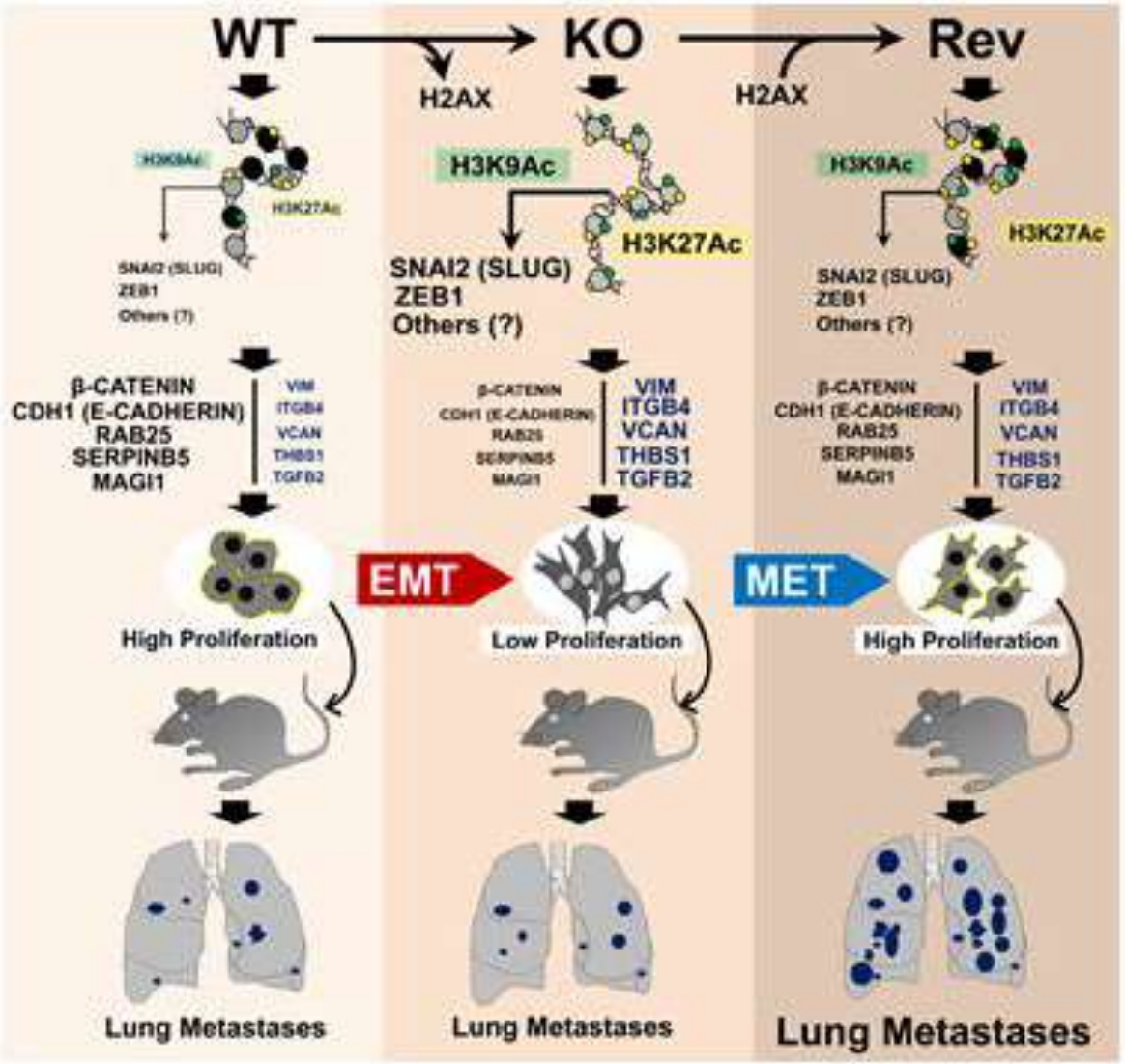
H2AX

Ancient history from the last millenium
γ-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, Boufraqech 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
.