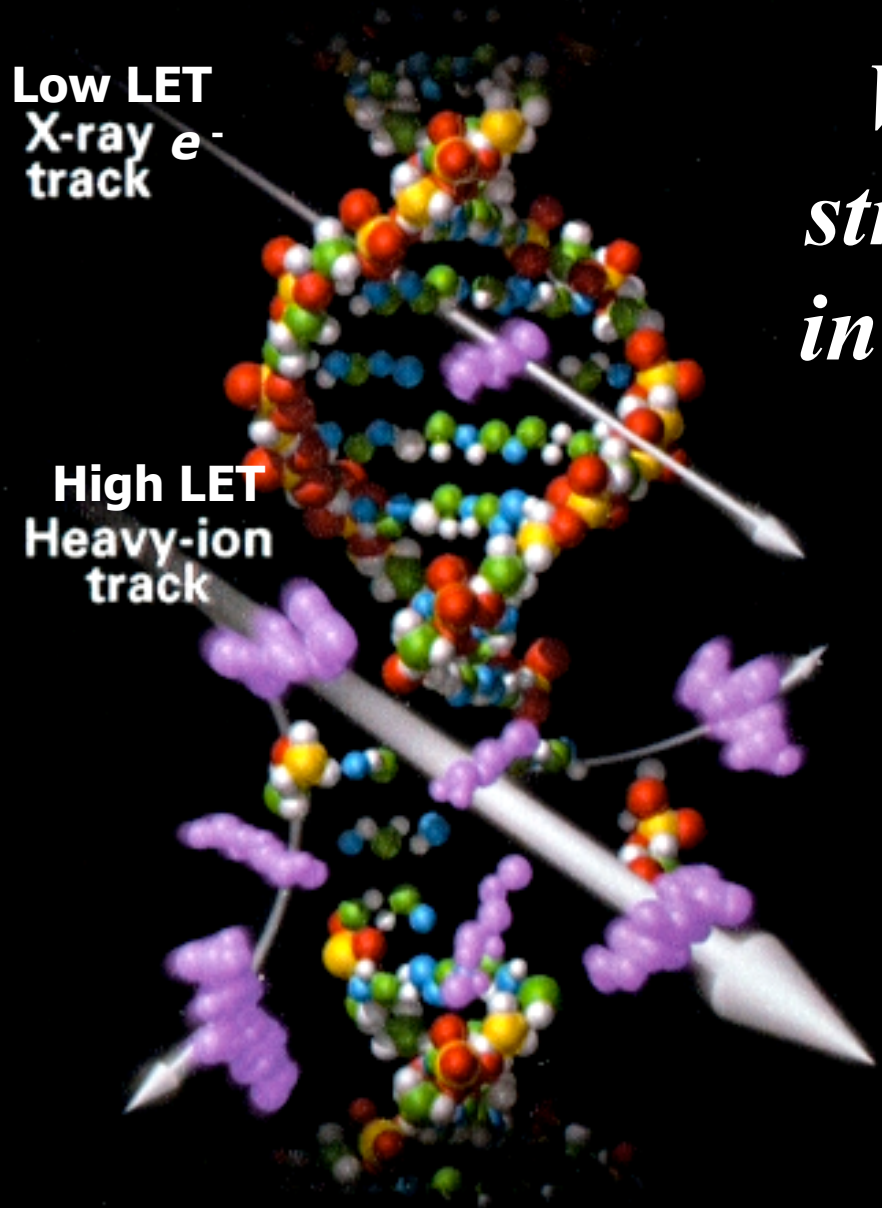


Variation in DNA double strand break repair capacity in diploid human fibroblasts after low doses of IR

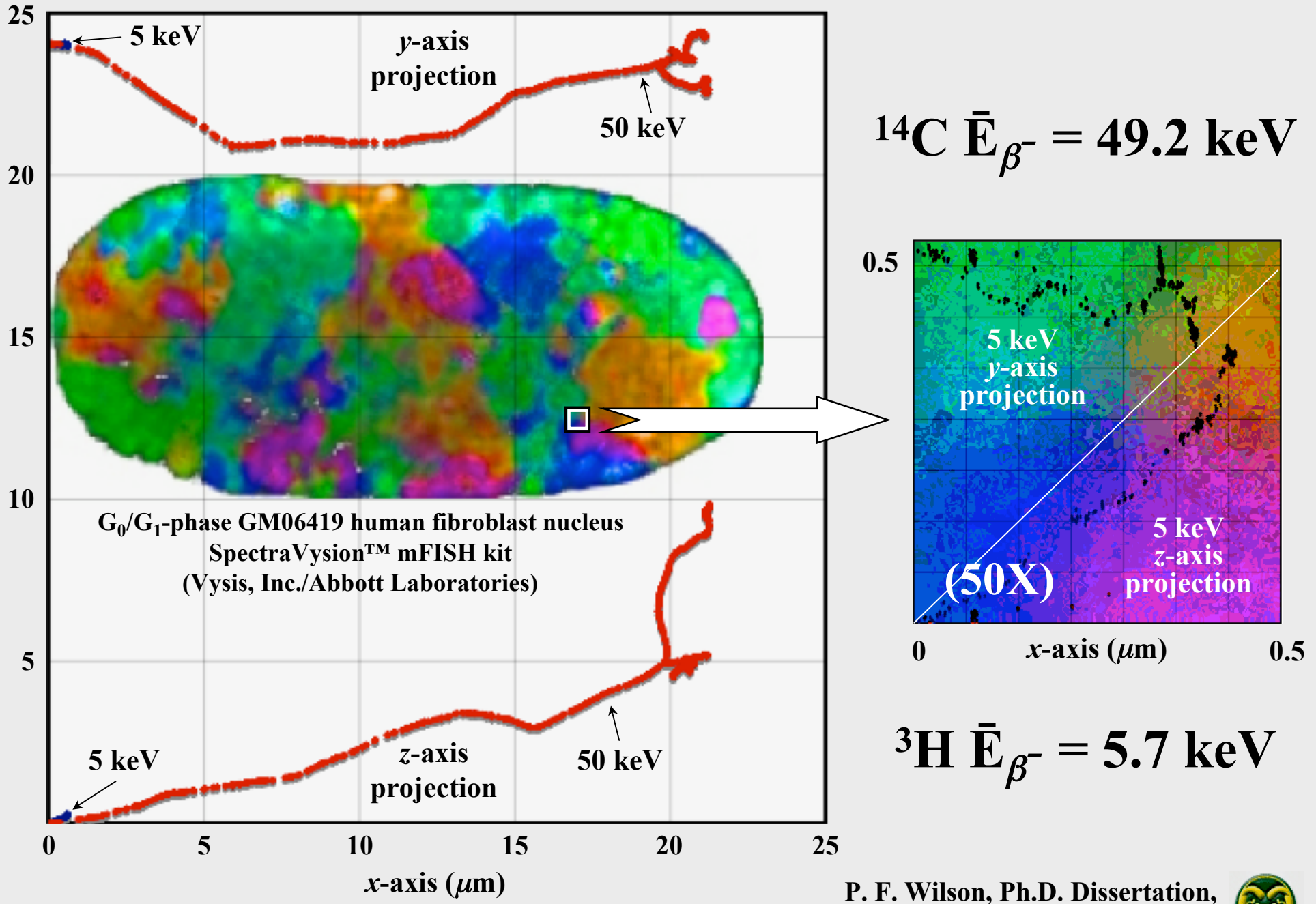
Paul Wilson, Salustra Urbin, Peter Nham, Cindy Thomas, John Hinz, Irene Jones, and Larry Thompson

Biosciences and Biotechnology Division
Chemistry, Material and Life Sciences Directorate
Lawrence Livermore National Laboratory
Livermore, California, USA, 94551-0808

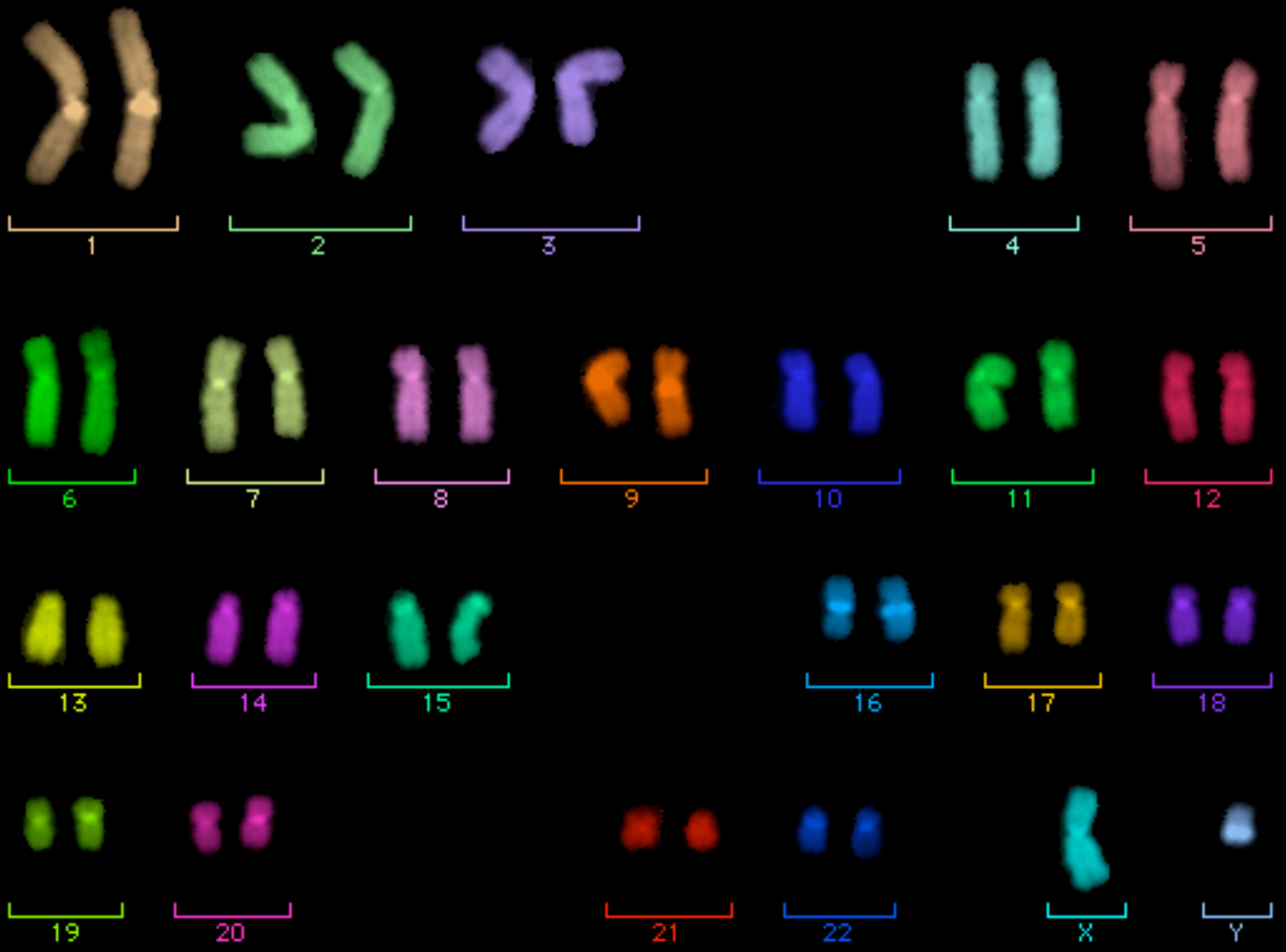
UCRL-PRES-228180

Introduction

- Inefficiently and incorrectly repaired DSBs are the primary cause of IR-induced cancer at low doses.
  - Chromosome aberrations with tumorigenic potential
- Does the efficiency of DSB repair after low doses of IR vary substantially among different individuals, and if so, how much?
  - Coriell Cell Repositories
- Does the efficiency of DSB repair after low doses of IR vary substantially with the dose delivered, and if so, how much?



P. F. Wilson, Ph.D. Dissertation,
Colorado State University (2006).



Coriell Apparently Normal Fibroblast Strain AG01521

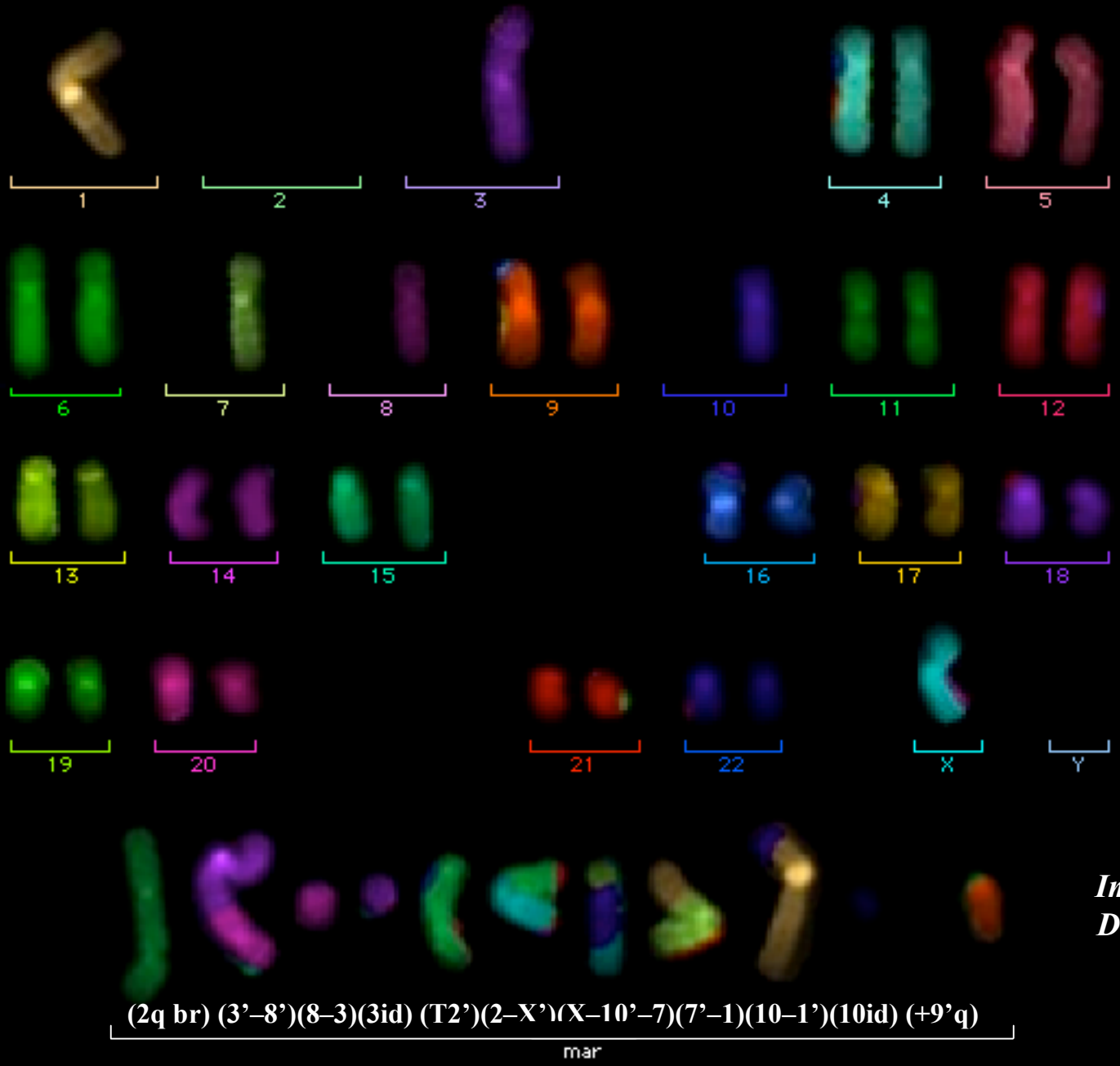
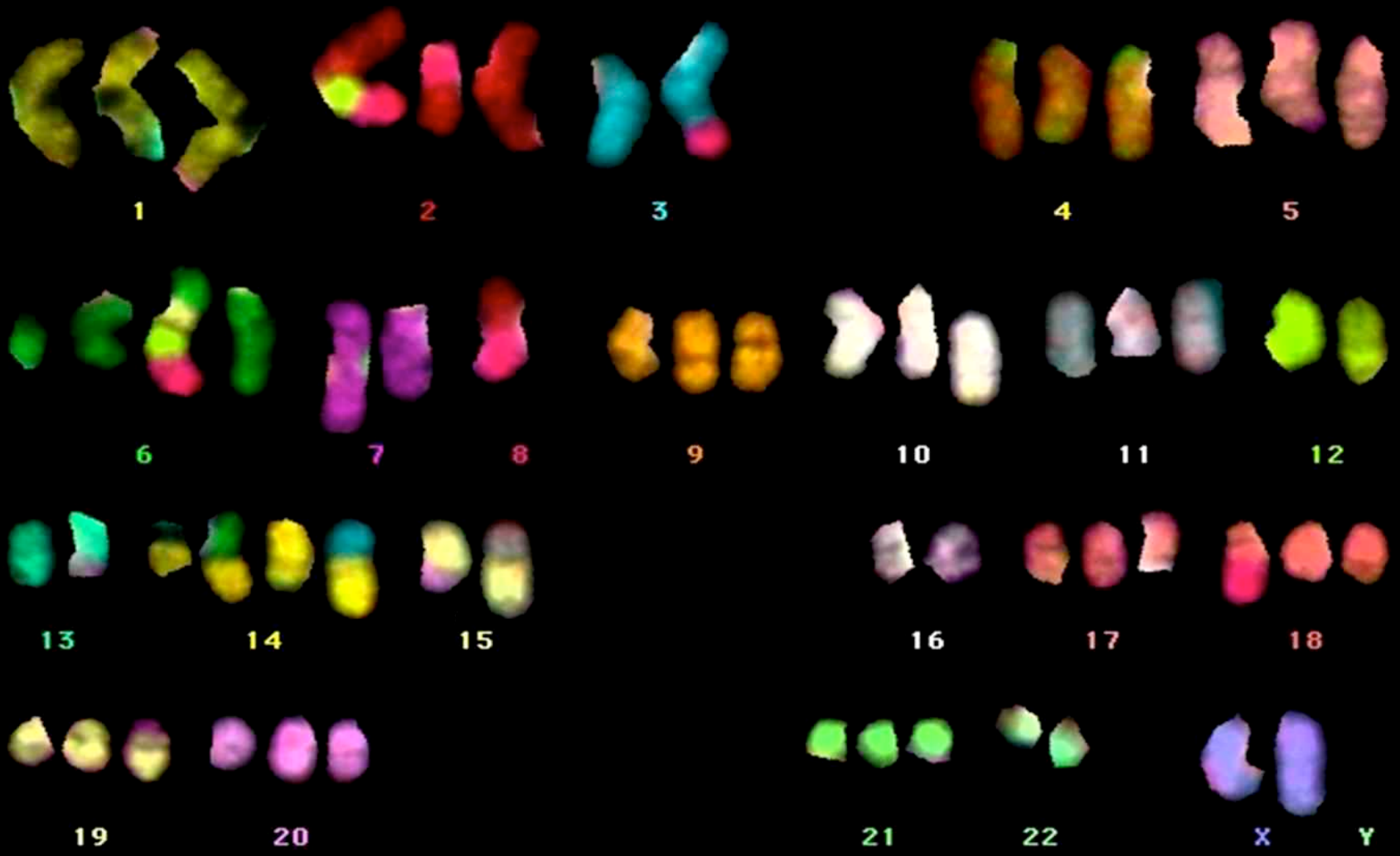


Image courtesy of Dr. Larry Dugan, CSU, LLNL

Coriell NBS Fibroblast Strain GM07166 (100 cGy)

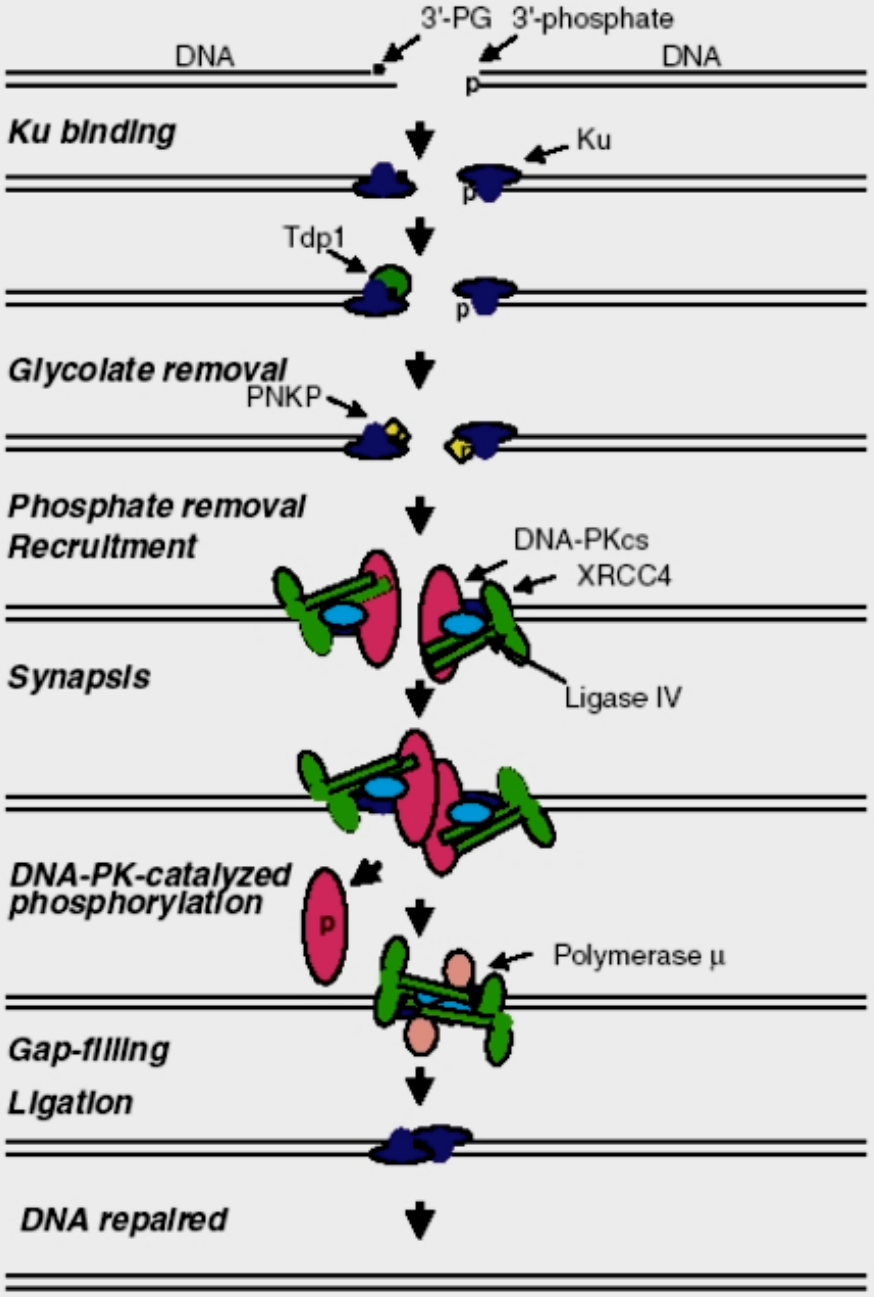


Breast Cancer Cell Line MDA-MB-231, Clone I

Watson, *et al.*, *Cancer Therapy* **2**, 167–172 (2004).

Sample	Composite Karyotype
MDA-MB231 (I)	**(38-99).** 1x3, 2x1, der(2)t(2;12;8), der(2)t(8;2)x2, 3x1, der(3)t(3;8), 4x4[3], 4x3[3], 4x2[2] der(4)t(4;9), 5x2, der(5)t(5;7), 6x2, der(6), der(6)t(6;14), der(6)t(6;19;12;8), 7x2, der(8)t(18;8), 9x3, 10x3, 11x3, 12x2, 13x1, der(13)t(16;13), 14x2, der(14), der(11)t(11;15), der(15)t(20;15), 16x2, 17x3, 18x2, der(?)t(18;5), 19x2[4], 19x3[4], der(19)t(19;7), 20x2, 21x3, 22x1, Xx2 **[cp 8].**

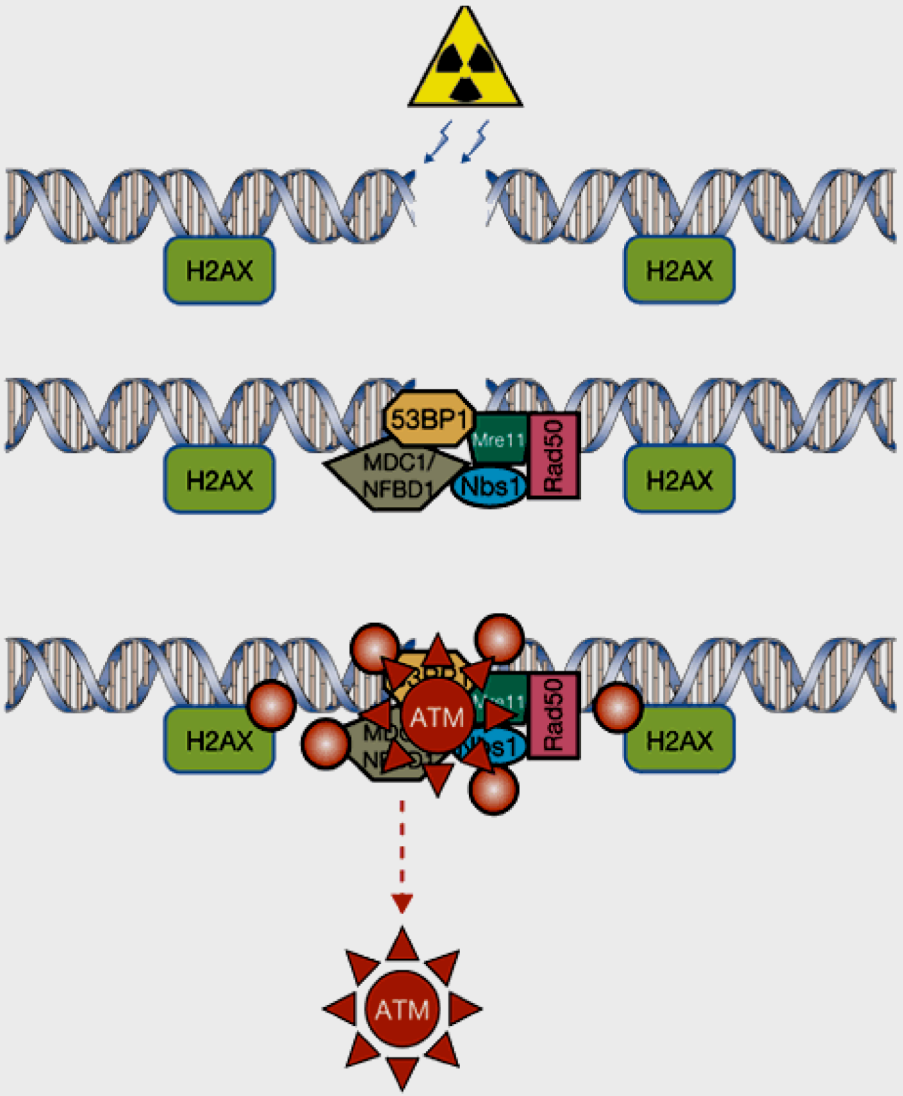
Non-Homologous End-Joining (NHEJ)



Valerie and Povirk, *Oncogene* **22**, 5792–5812 (2003).

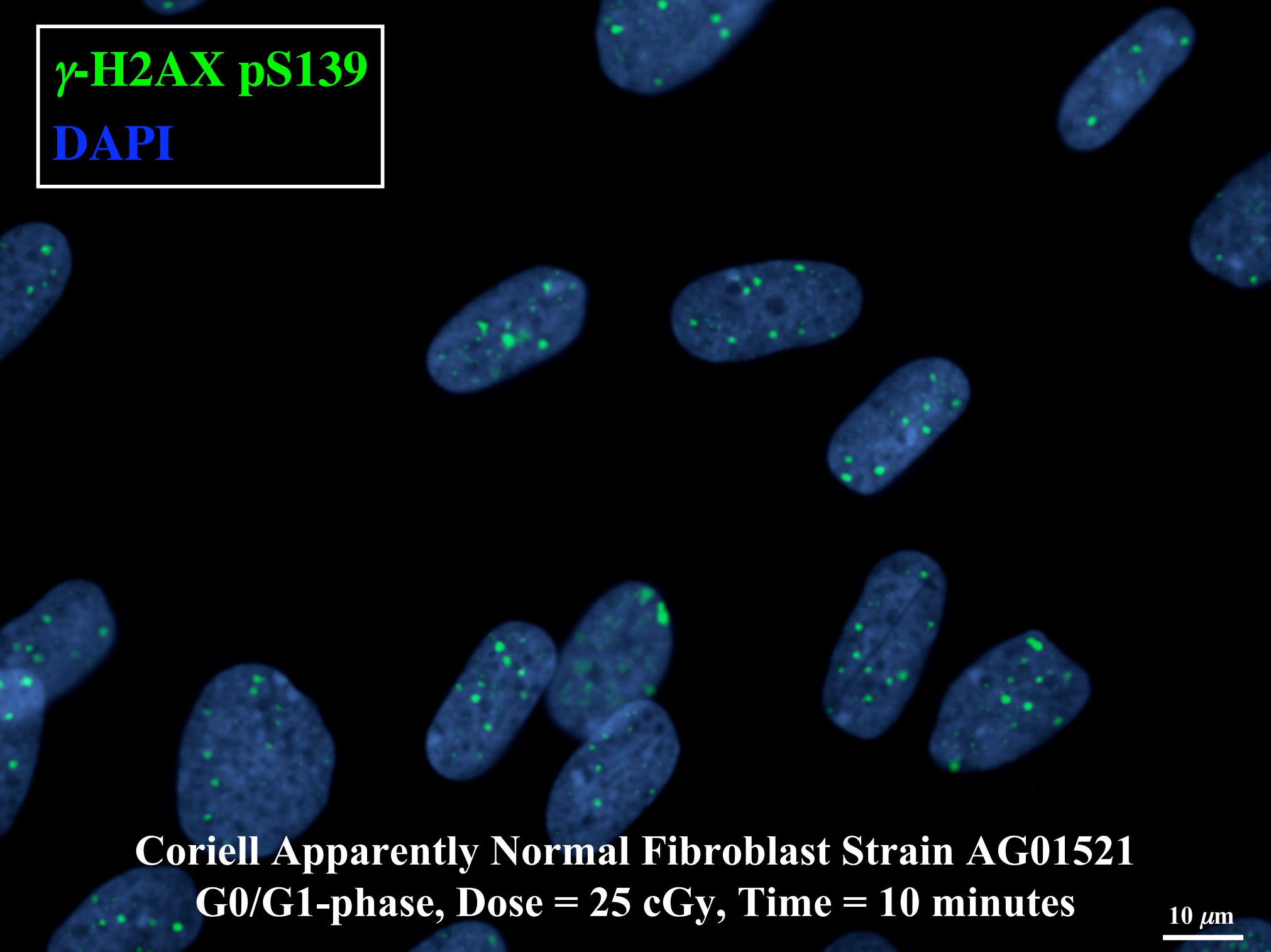
- Binding of MRN complex, Ku70/80, 53BP1, MDC1
- Clean-up terminal nucleotides to 5'-PO_4 and 3'-OH
  - APE1, TDP1, PNK, Artemis
- Recruitment of DNA-PKcs
  - XRCC4/Ligase IV
- Synapsis and annealing
  - Direct or microhomology-driven
- DNA-PK autophosphorylation and release
- Gap-filling by DNA Pol μ/λ
- Ligation by XRCC4/Ligase IV

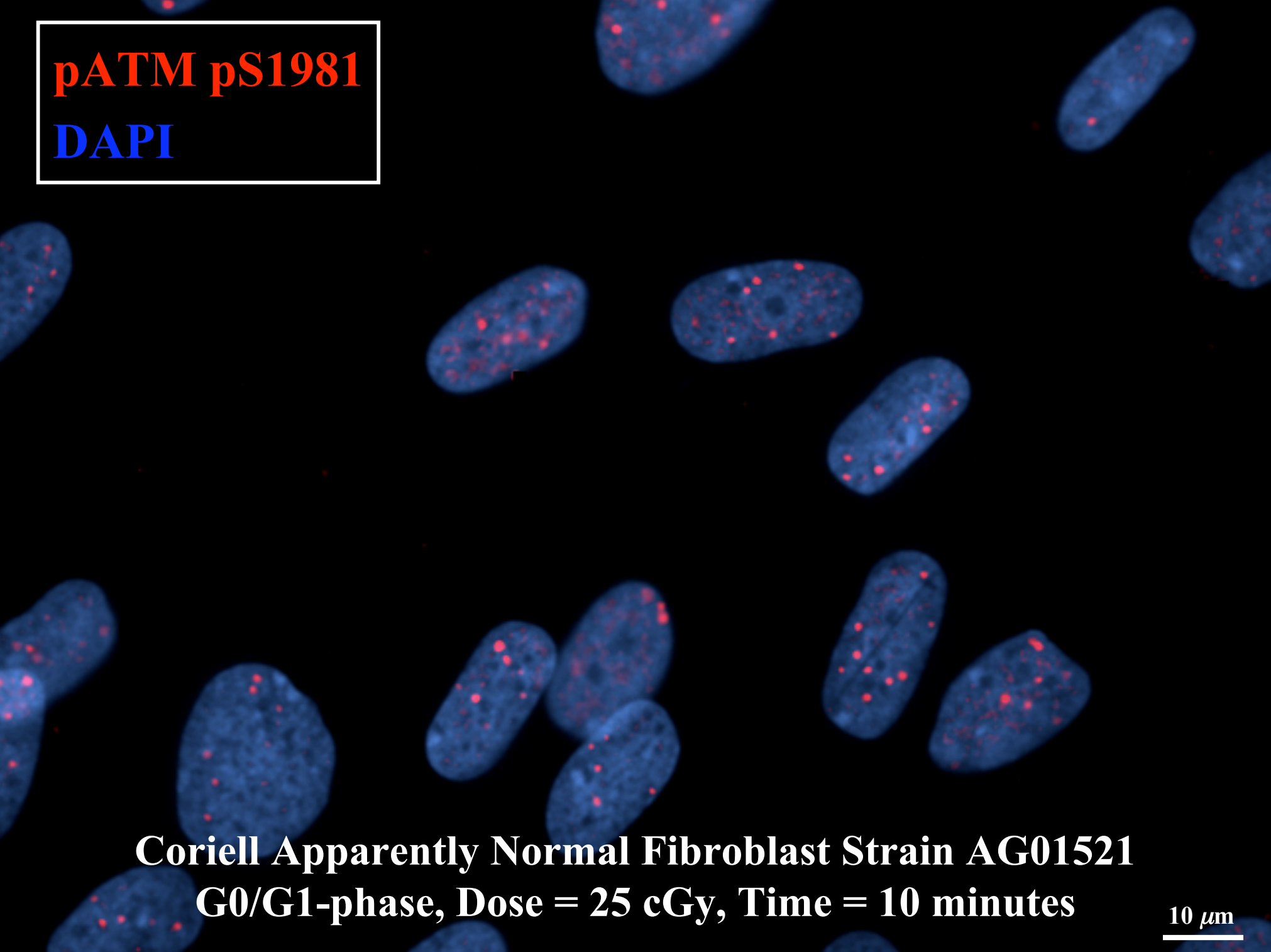
Initial Sensing of IR-induced DSBs

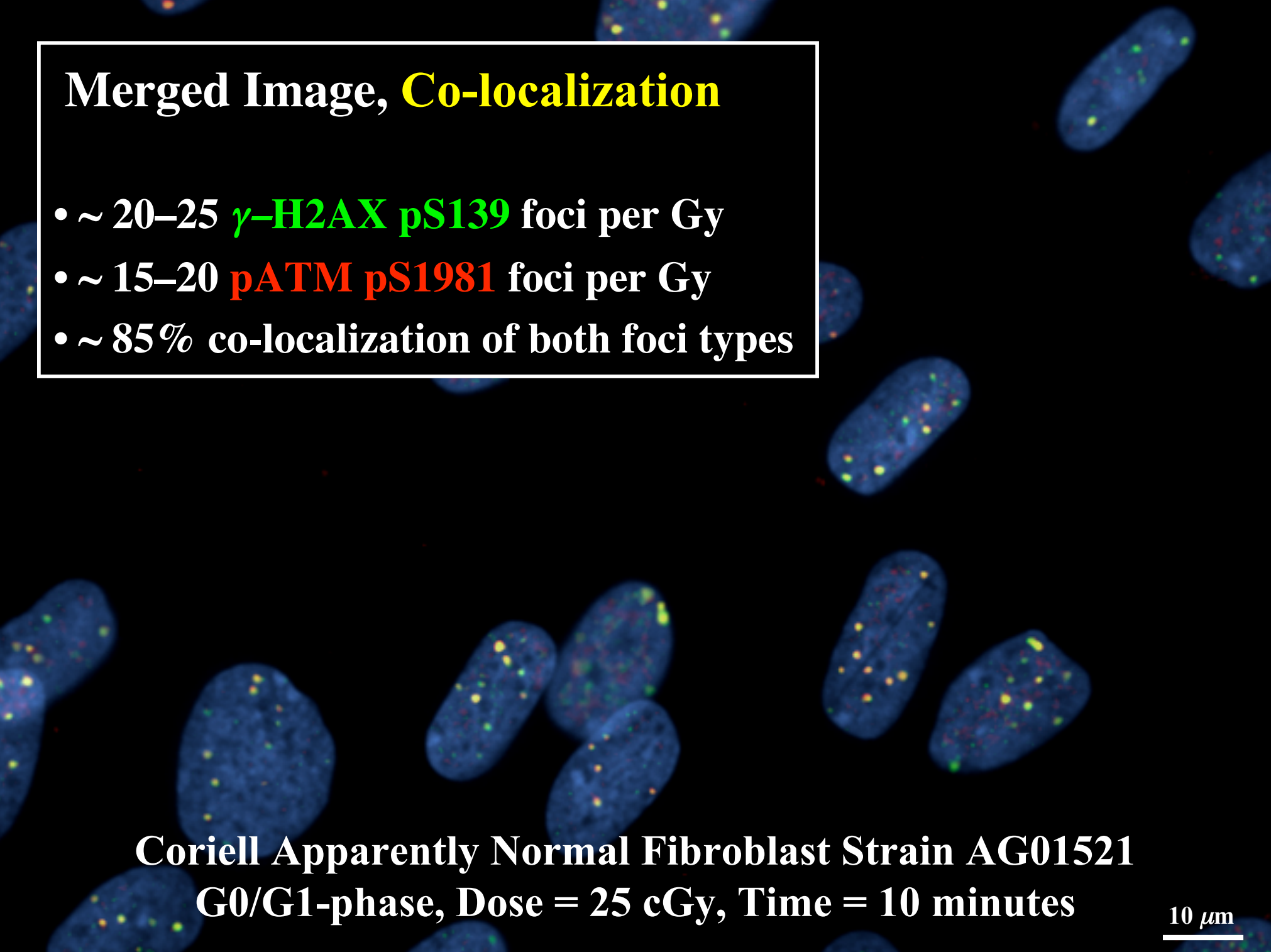


Shiloh and Lehmann, *Nature Cell Biology* **6**, 923–8 (2004).

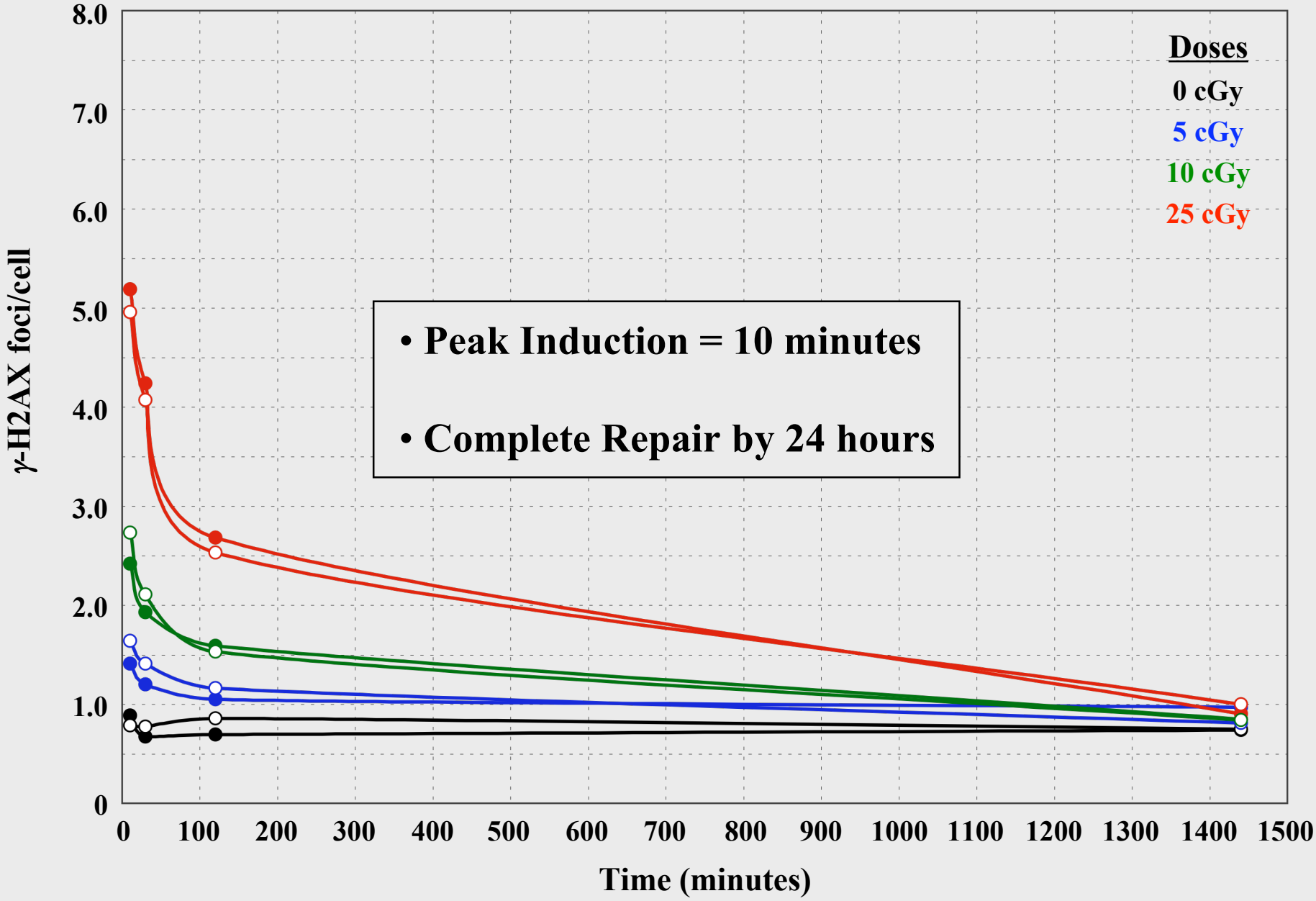
- Histone variant **H2AX**
- Mre11/Rad50/**Nbs1** complex, 53BP1, MDC1, Ku70/80…
- Recruitment and activation of **ATM** (and DNA-PK, ATR)
- Activation of DSB repair, cell cycle checkpoints, cellular stress responses, apoptosis/ senescence pathways…
- **Form nuclear foci at DSBs**


γ-H2AX pS139
DAPI
Coriell Apparently Normal Fibroblast Strain AG01521
G0/G1-phase, Dose = 25 cGy, Time = 10 minutes
10 μm

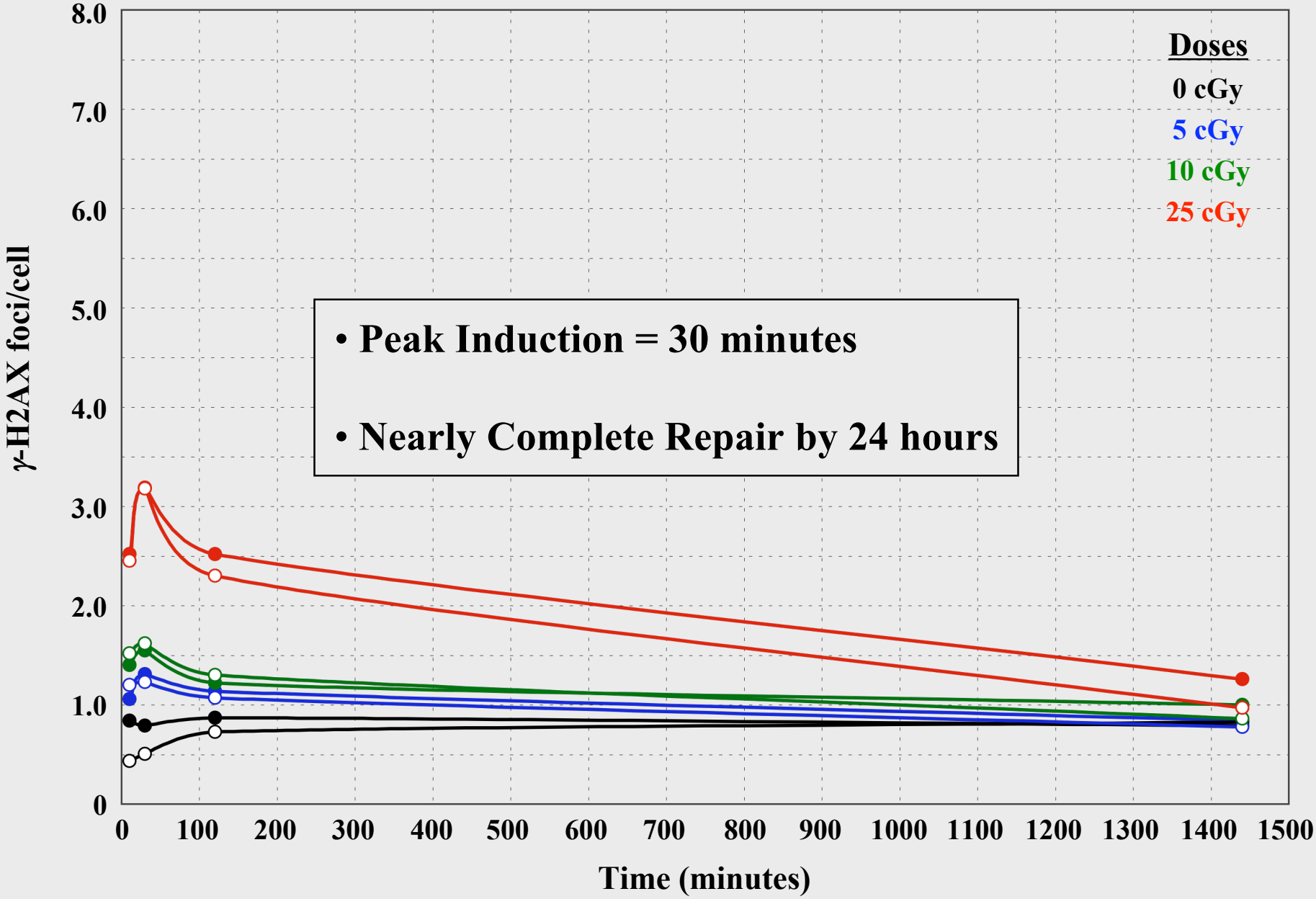

pATM pS1981
DAPI
Coriell Apparently Normal Fibroblast Strain AG01521
G0/G1-phase, Dose = 25 cGy, Time = 10 minutes
10 μm


Merged Image, Co-localization
• ~ 20–25 γ–H2AX pS139 foci per Gy
• ~ 15–20 pATM pS1981 foci per Gy
• ~ 85% co-localization of both foci types
Coriell Apparently Normal Fibroblast Strain AG01521
G0/G1-phase, Dose = 25 cGy, Time = 10 minutes
10 μm

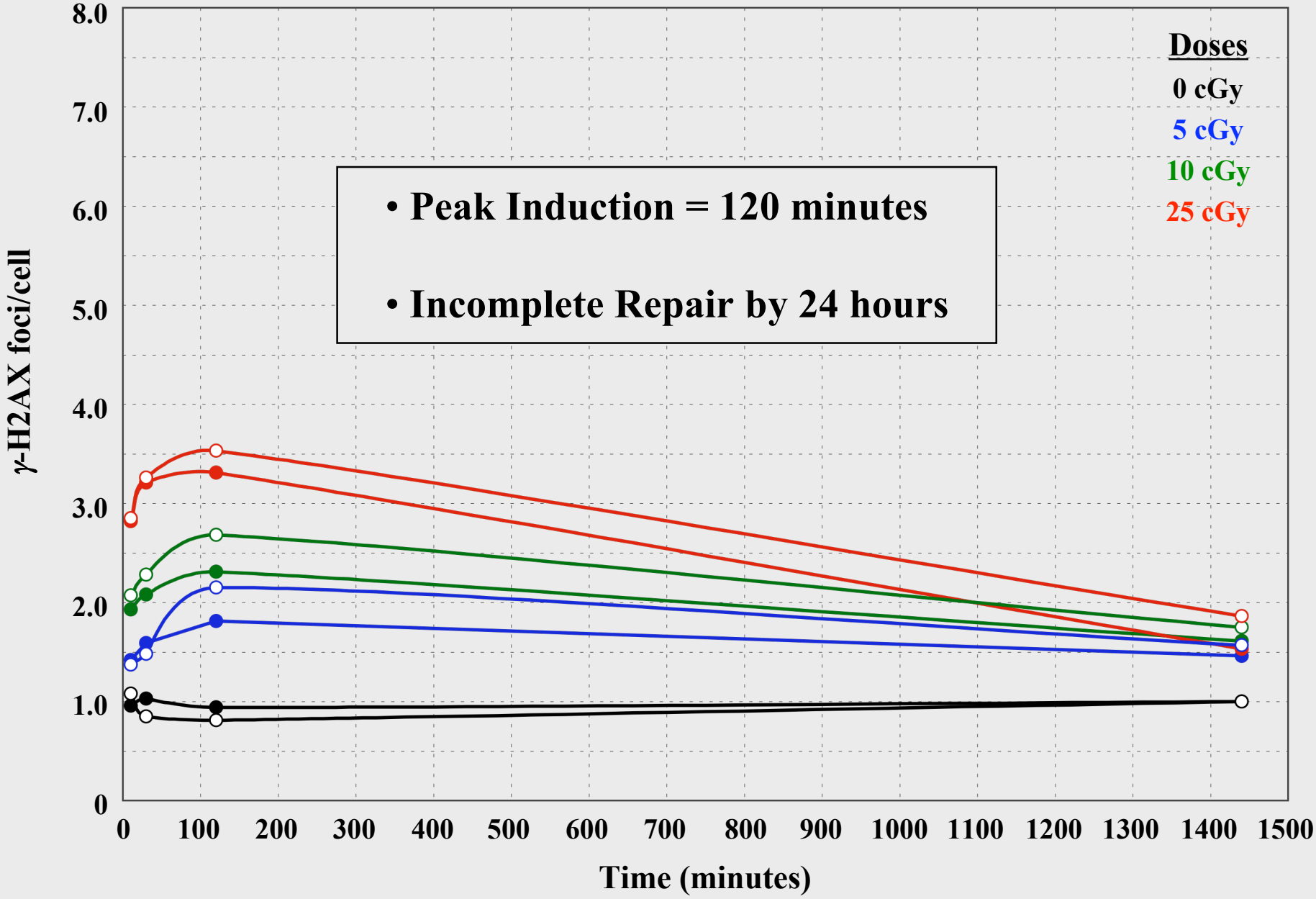
Coriell Apparently Normal Fibroblast Strain AG01522
Total γ-H2AX pS139 Foci Counts



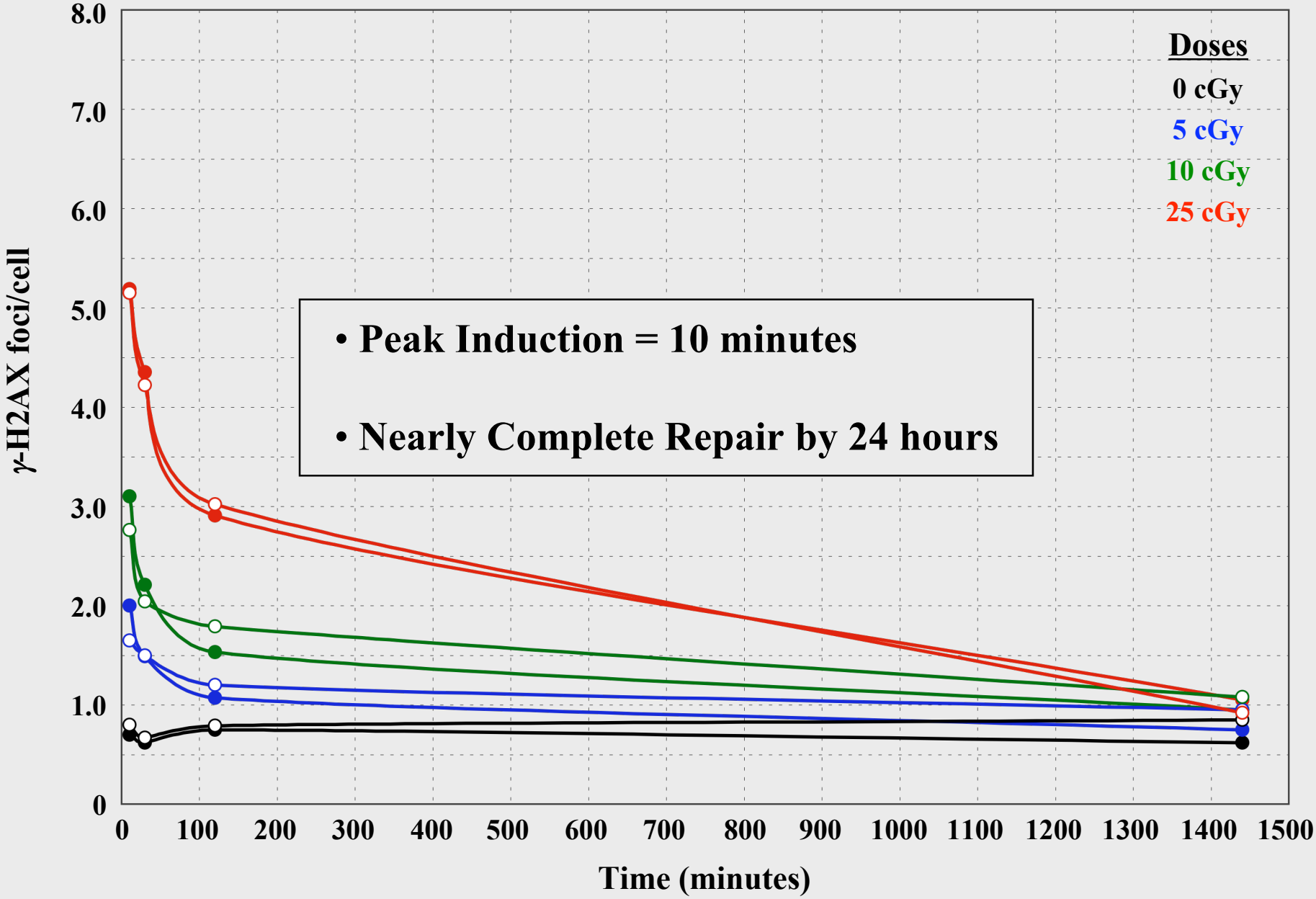
Coriell NBS Fibroblast Strain GM07166
Total γ-H2AX pS139 Foci Counts



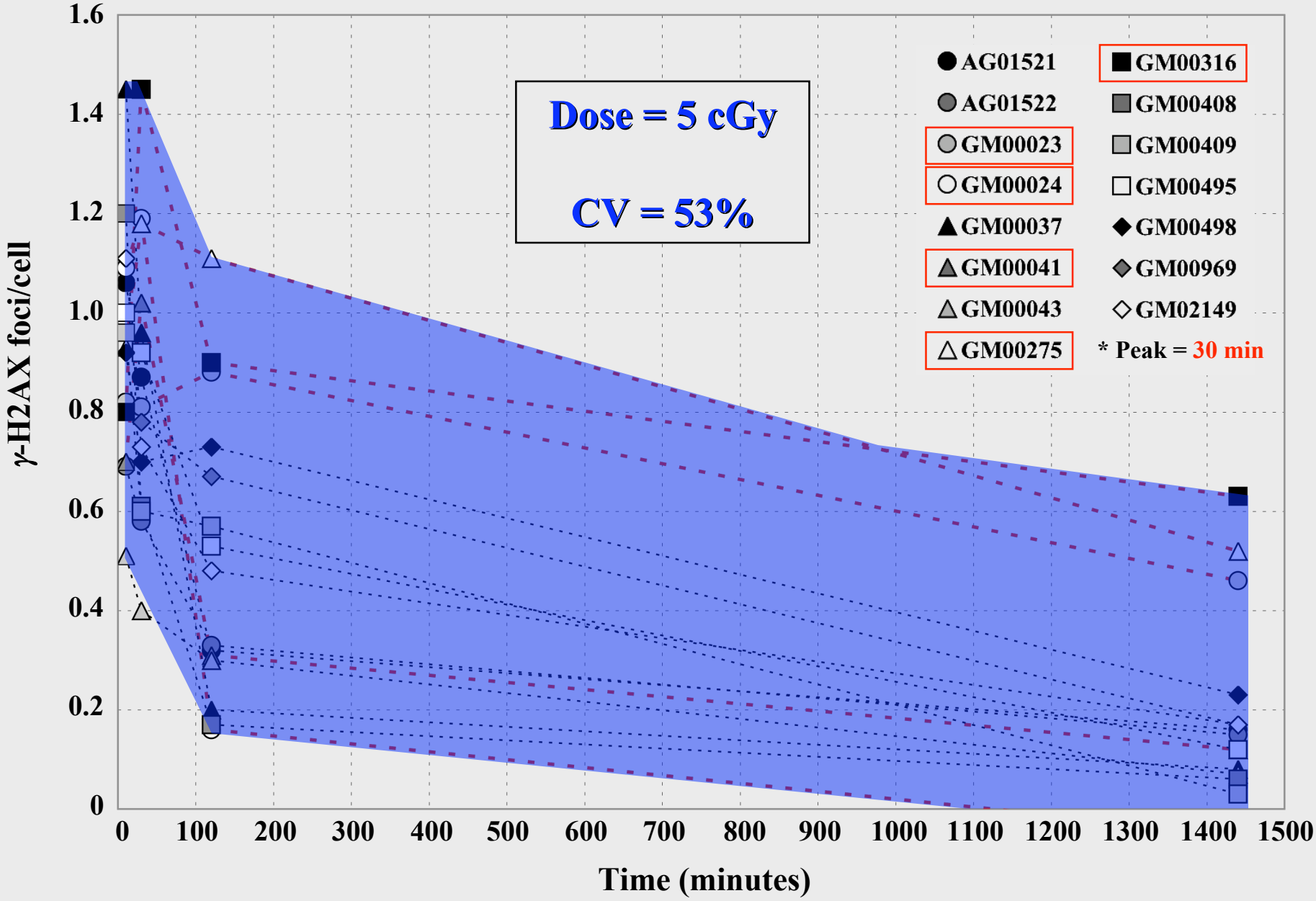
Coriell AT Fibroblast Strain GM02052
Total γ-H2AX pS139 Foci Counts



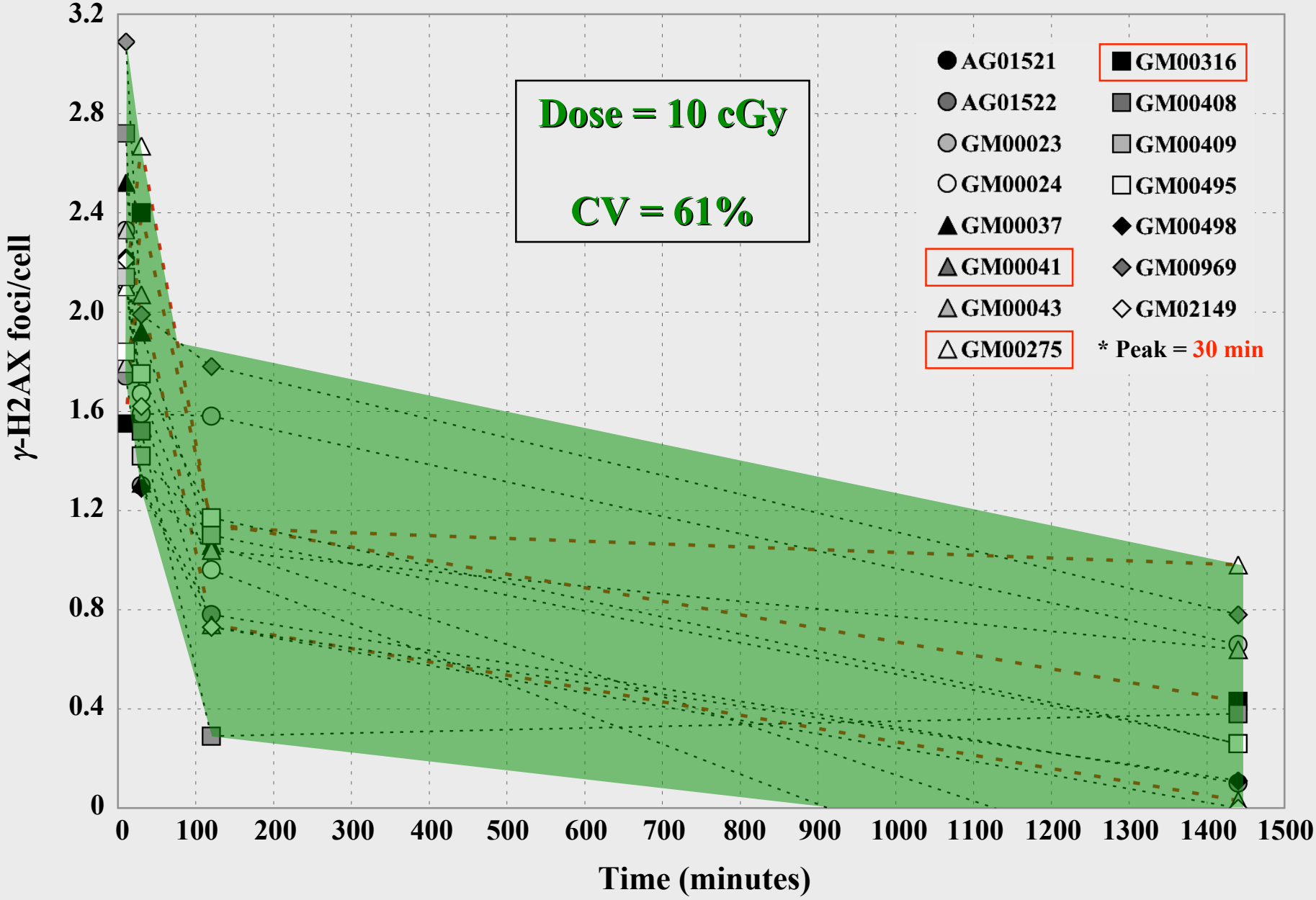
Coriell FA Group G Fibroblast Strain GM02361
Total γ-H2AX pS139 Foci Counts



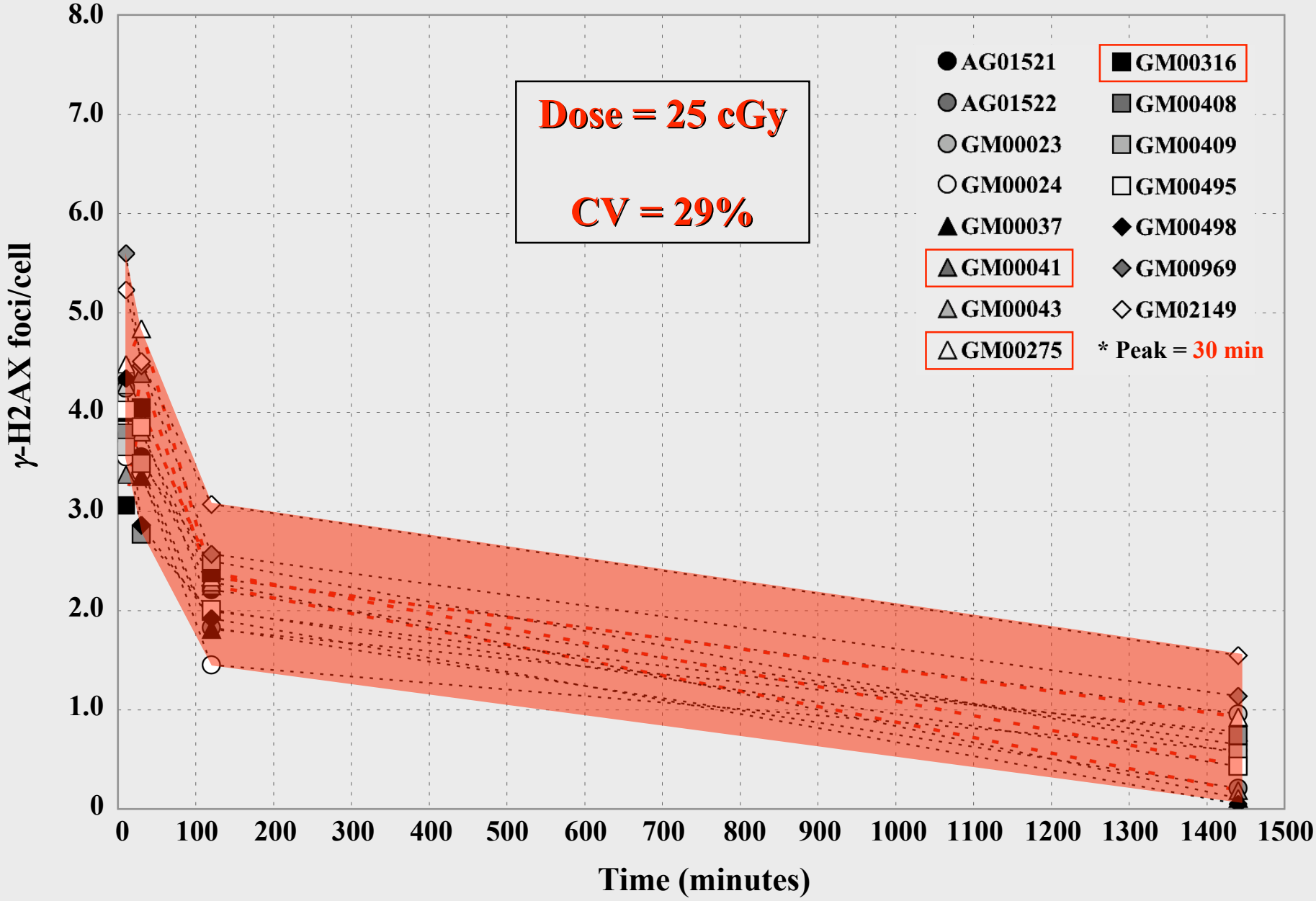
Coriell Apparently Normal Fibroblast Strains
Background-corrected γ-H2AX pS139 Foci Counts



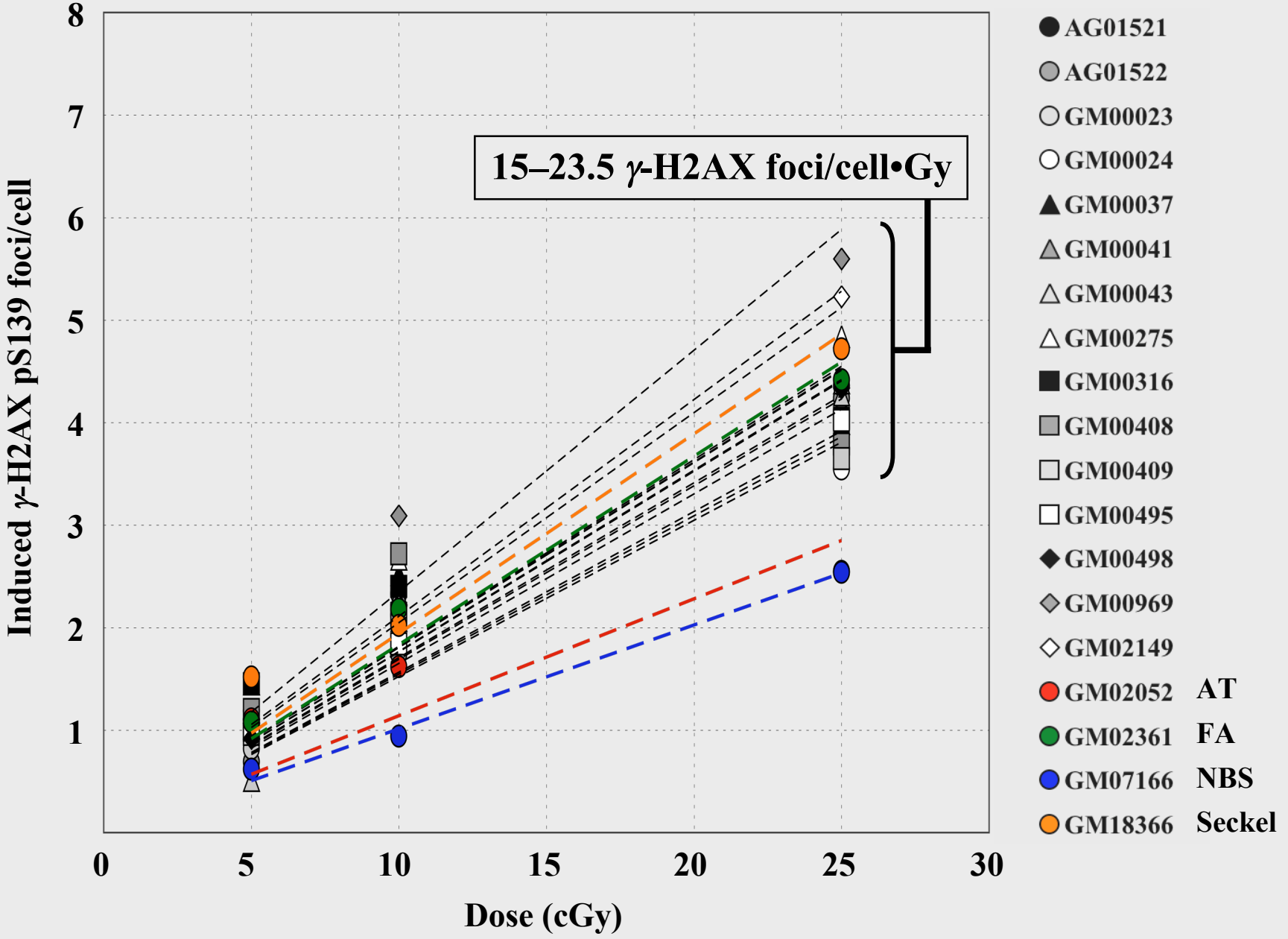
Coriell Apparently Normal Fibroblast Strains
Background-corrected γ-H2AX pS139 Foci Counts



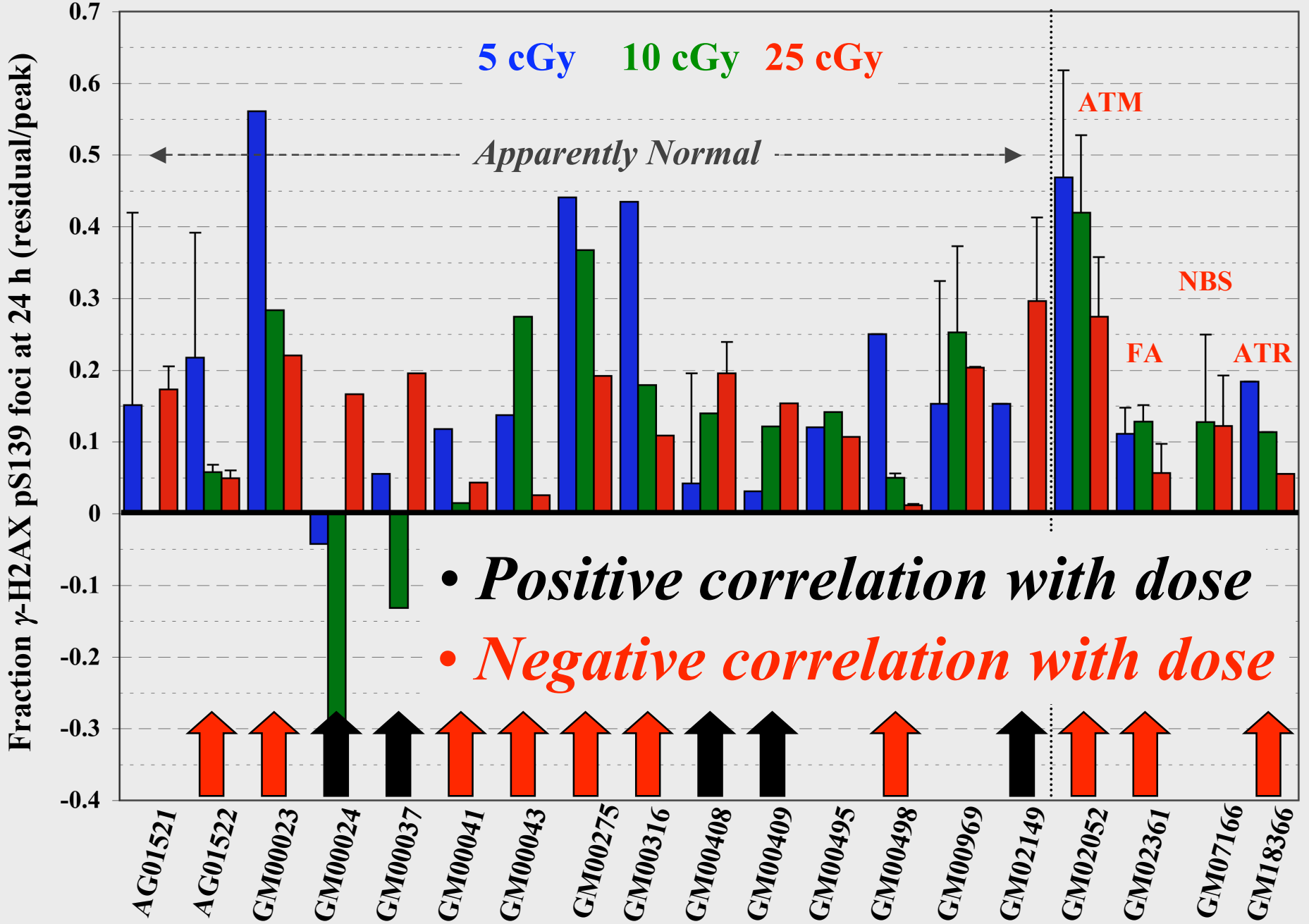
Coriell Apparently Normal Fibroblast Strains
Background-corrected γ-H2AX pS139 Foci Counts



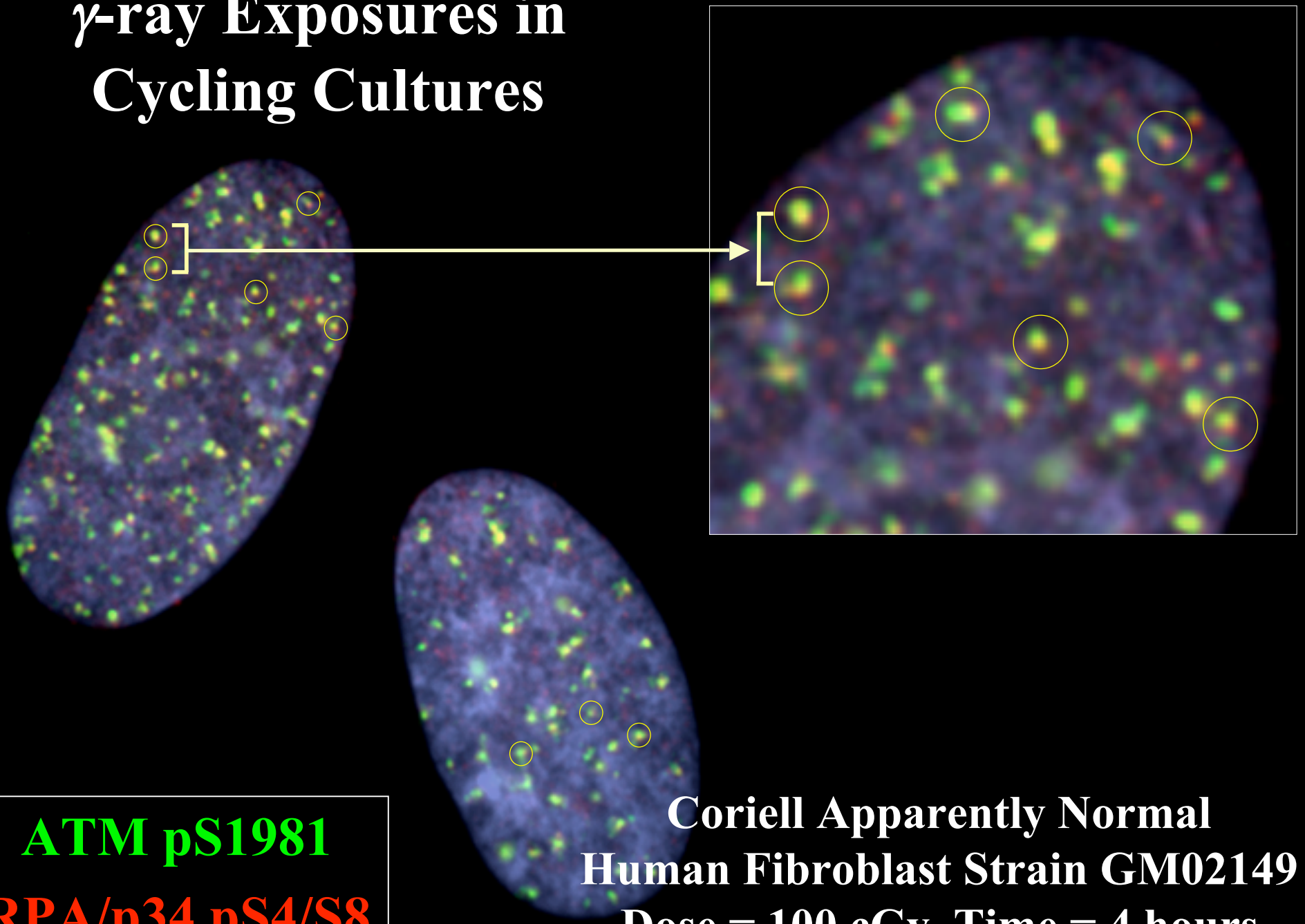
γ-H2AX pS139 Foci Dose Response



γ-H2AX pS139 Foci Remaining at 24 h



γ-ray Exposures in Cycling Cultures



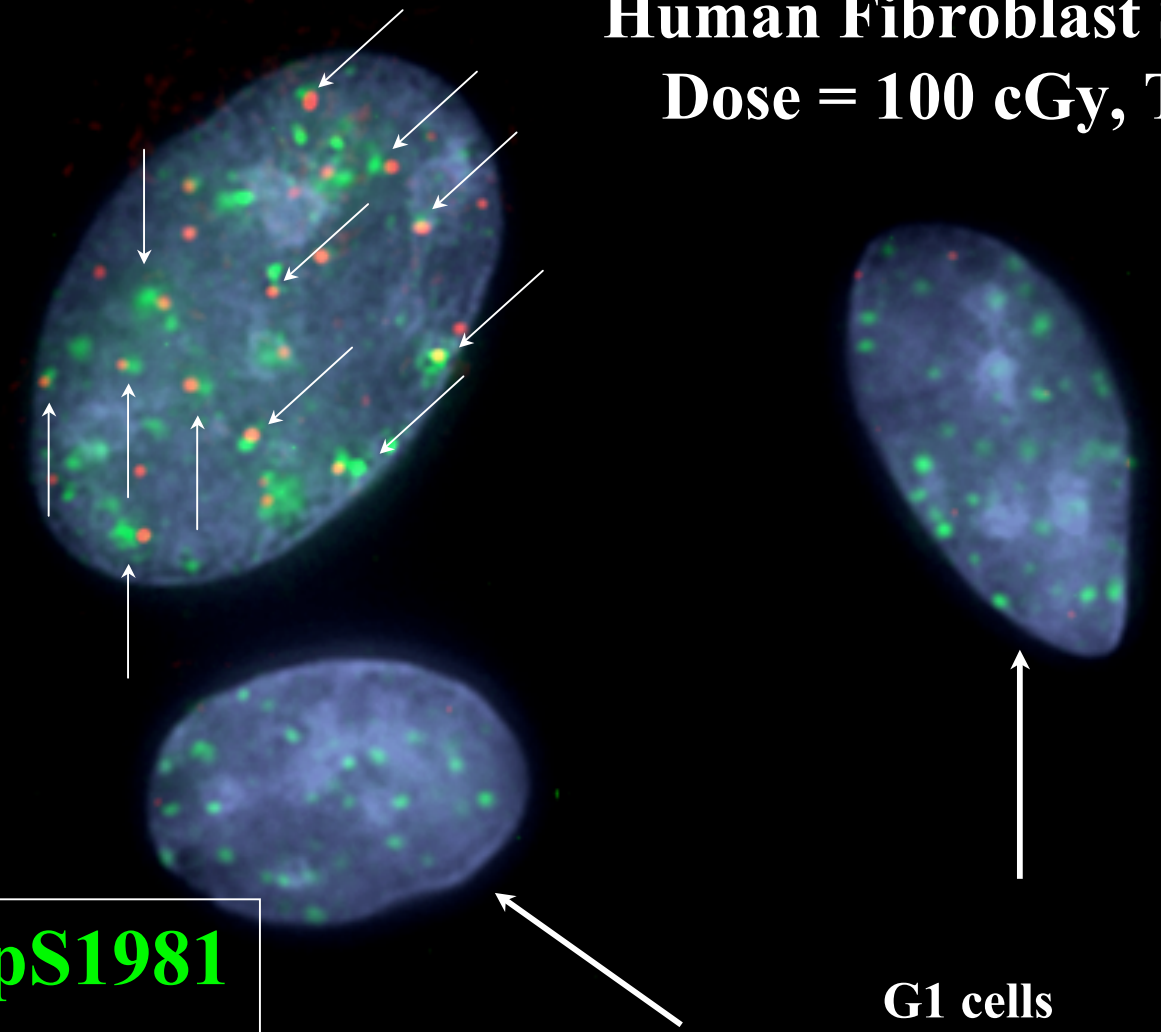
ATM pS1981

RPA/p34 pS4/S8

Coriell Apparently Normal
Human Fibroblast Strain GM02149
Dose = 100 cGy, Time = 4 hours

γ-ray Exposures in Cycling Cultures

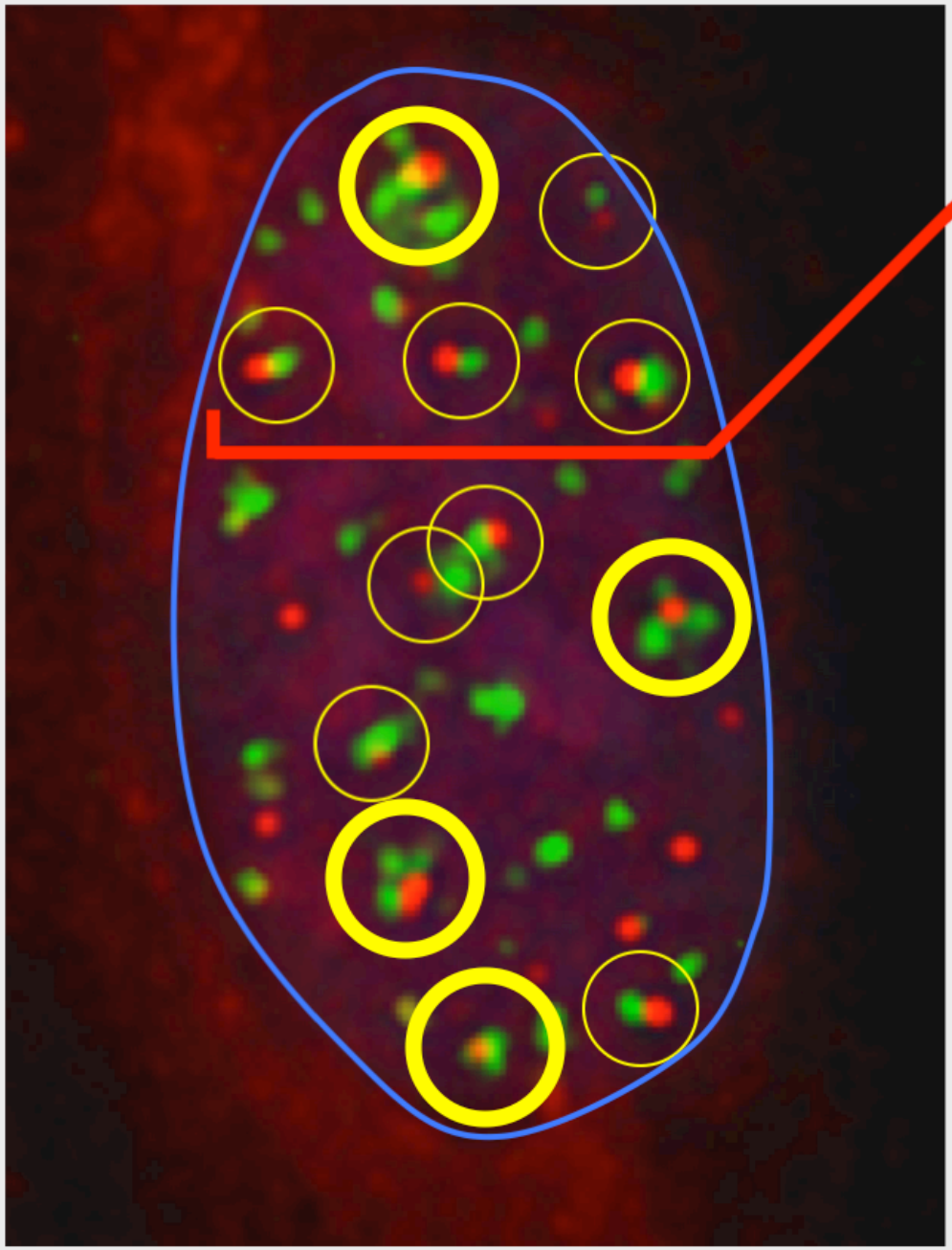
Coriell Apparently Normal
Human Fibroblast Strain GM02149
Dose = 100 cGy, Time = 4 hours

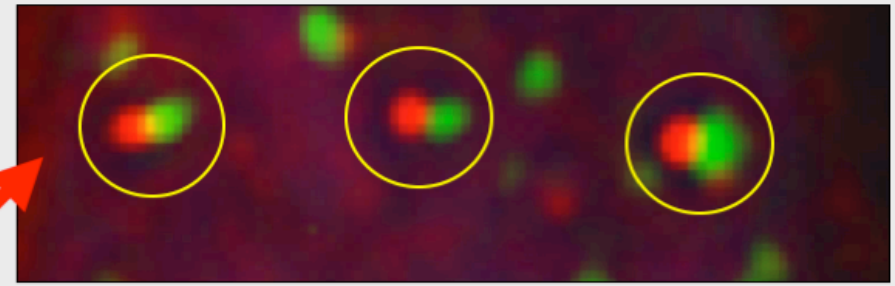


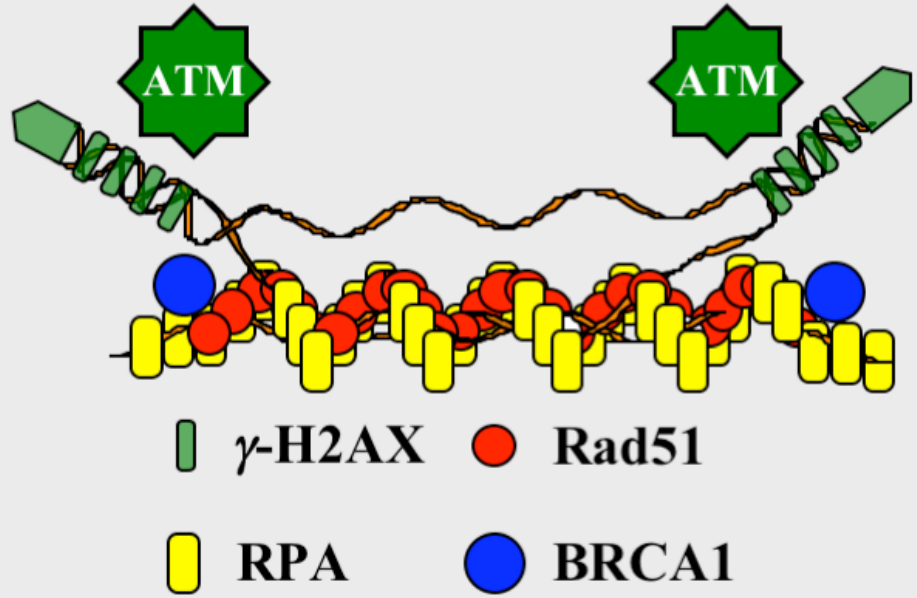
ATM pS1981
Rad51

G1 cells
No Rad51 foci

Closely-localizing (*vs.* Co-localizing) Foci







Clustering of multiple DSBs in Rad51-mediated repair centers

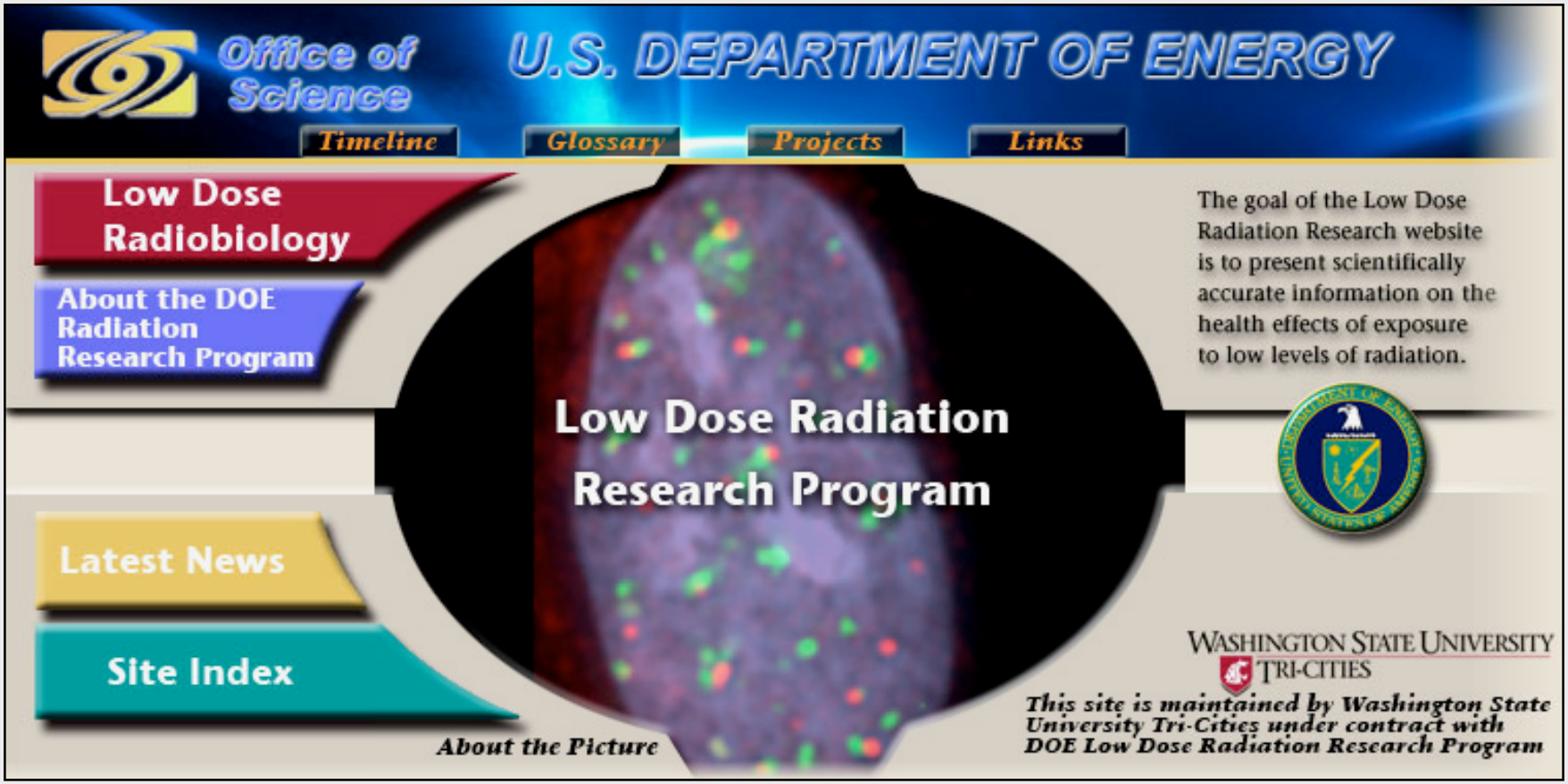
Conclusions to Date

- Precise assay of DSB induction and repair after low IR doses is achieved using **co-localizing foci markers**.
- Normal cell lines vary…
  - **5-fold** or more in the level of **spontaneous** γ-H2AX foci.
  - **>20-fold** in the fraction of **residual** induced foci at 24 hours.
  - Overall, more than the DNA repair mutants…
- Hint of **increasing repair efficiency** in the low dose region of 5 to 25 cGy.
  - Failure of radiosensitive cells to register low dose-induced DSBs above background endogenous levels?
  - Adaptive response, low dose hyper-radiosensitivity…

Future Directions

- Long-term outcome of residual γ-H2AX foci
  = residual chromosome breaks, sites of misrepair?
- Outcome of foci induced throughout cell cycle
  - Insight into spatio-temporal dynamics of HRR – closely-localizing foci
- Role of other DNA repair pathways
  - HRR – Generation of 1° human isogenic HRR^{-} cell line
  - Fanconi Anemia protein pathway
- Genetic determinants of variation in normal individuals
  - High-risk SNP/CNPs in critical DNA signaling and repair, cell cycle checkpoint proteins, *etc*.

This work was performed under the auspices of the U.S. Department of Energy by the University of California and the Lawrence Livermore National Laboratory under contract W-7405-Eng-48, and sponsored by grants from the U.S. DOE Low Dose Radiation Research Program.



www.lowdose.energy.gov