

# PREDICTING CANCER RISK FROM IONIZING RADIATION

*Sylvain V. Costes, Ph.D.*

**Senior Research Scientist**

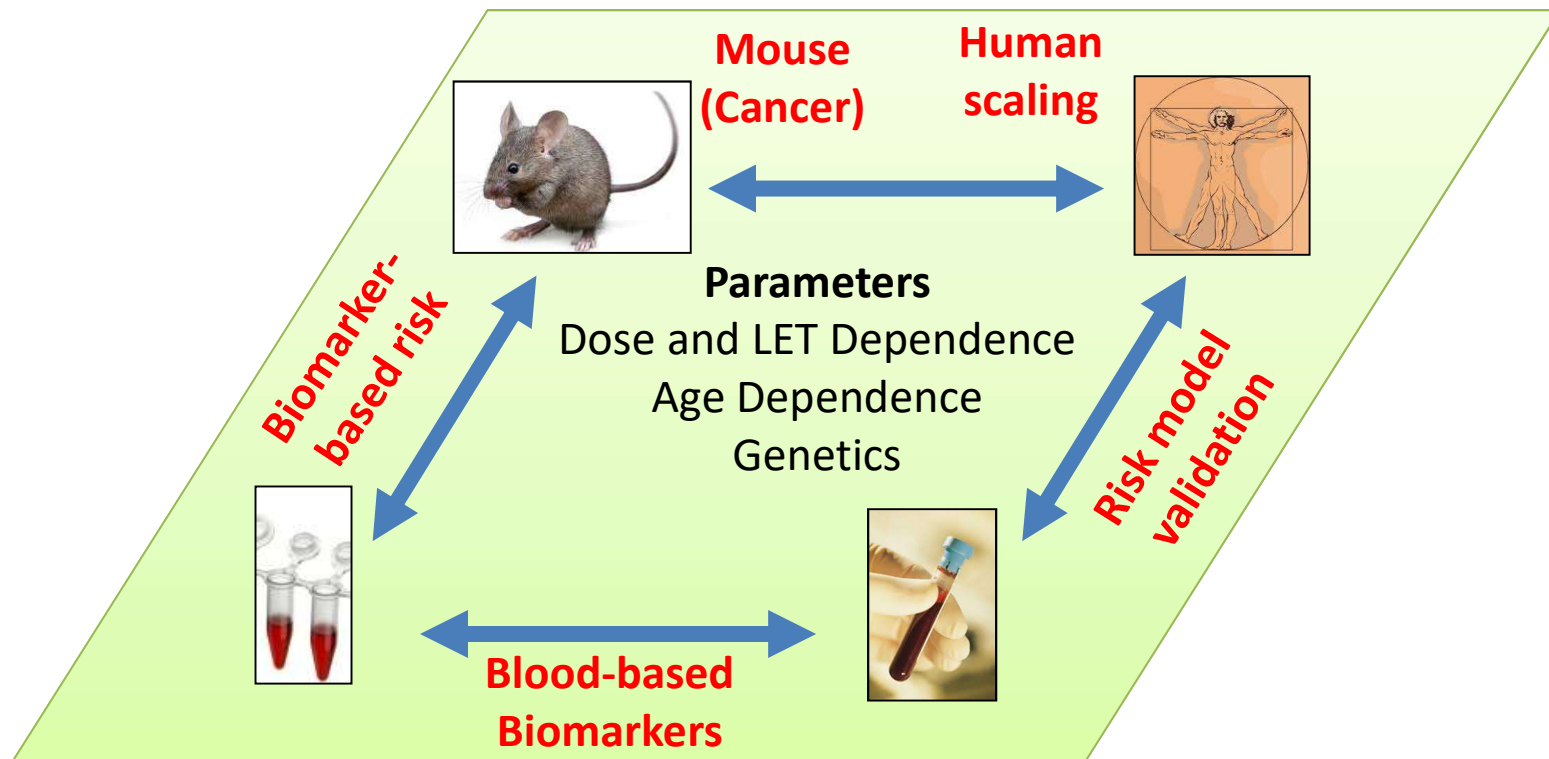
Space Biosciences Research Branch (SCR)

NASA Ames Research Center

**Goal:** establish a computer model that will estimate individualized risk for astronauts based on an array of phenotypic and genetic information.

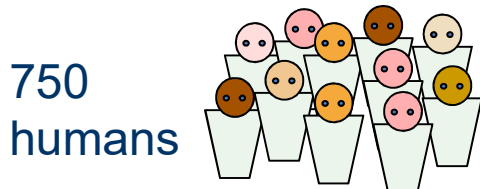
**Potential outcome:** Individualized risk assessment for Astronauts

# A scalable approach: from mice to human



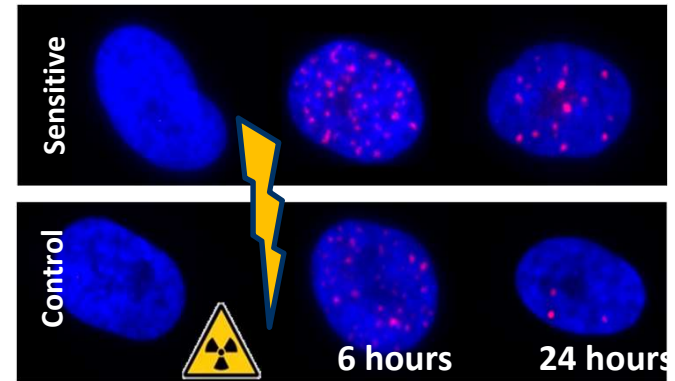
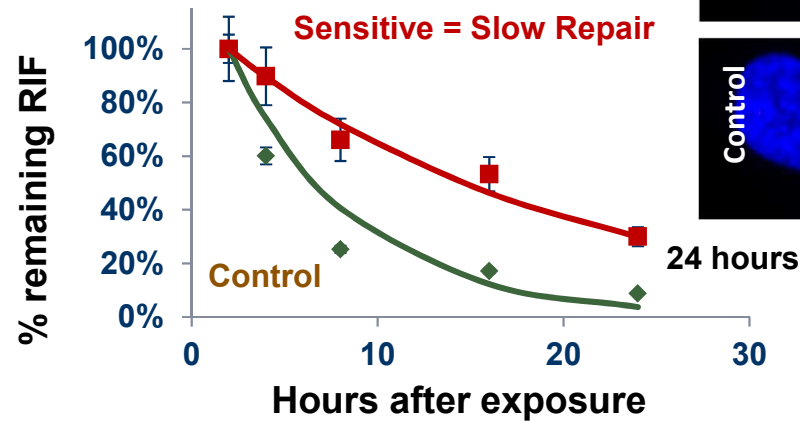
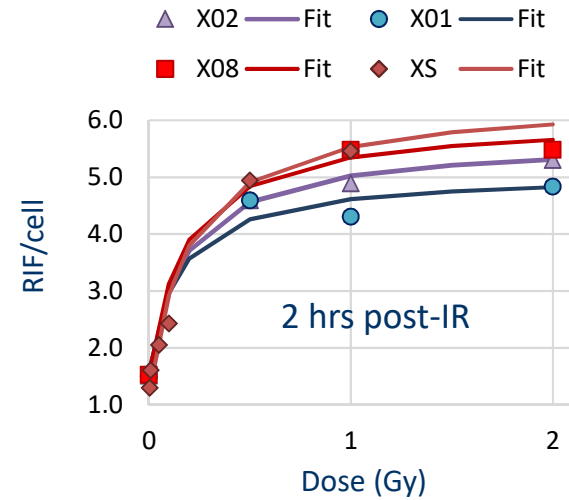
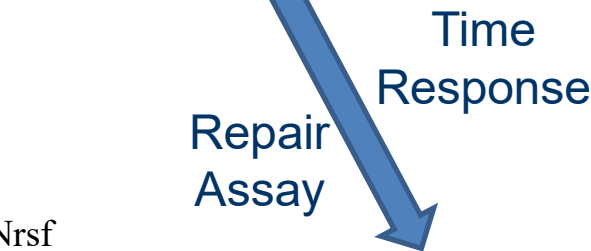
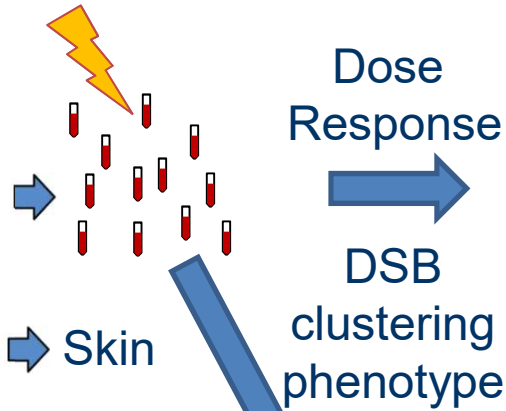
Low dose DOE parallelogram approach adapted for blood assay

# The DNA damage phenotypes



5 Ref Strains with known cancer

1. B6C3F
2. BALB/cByJ
3. C57BL/6J
4. CBA/CaJ
5. C3H/HeMsNrsf





# DSB clustering based on repair domain

OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

Image-Based Modeling Reveals Dynamic Redistribution of DNA Damage into Nuclear Sub-Domains

2007

Sylvain V. Costes<sup>1\*</sup>, Artem Ponomarev<sup>2,3</sup>, James L. Chen<sup>1</sup>, David Nguyen<sup>1</sup>, Francis A. Cucinotta<sup>2</sup>, Mary Helen Barcellos-Hoff<sup>1</sup>

**Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells**

2012

Teresa Neumaier<sup>a</sup>, Joel Swenson<sup>b,c</sup>, Christopher Pham<sup>d</sup>, Aris Polyzos<sup>d</sup>, Alvin T. Lo<sup>d</sup>, PoAn Yang<sup>d</sup>, Jane Dyball<sup>d</sup>, Aroumougame Asaithamby<sup>e</sup>, David J. Chen<sup>e</sup>, Mina J. Bissell<sup>d,1</sup>, Stefan Thalhammer<sup>a</sup>, and Sylvain V. Costes<sup>d,1</sup>

PNAS | January 10, 2012 | vol. 109 | no. 2 | 443–448

PNAS

Proceedings of the National Academy of Sciences of the United States of America

Combinatorial DNA Damage Pairing Model Based on X-Ray-Induced Foci Predicts the Dose and LET Dependence of Cell Death in Human Breast Cells

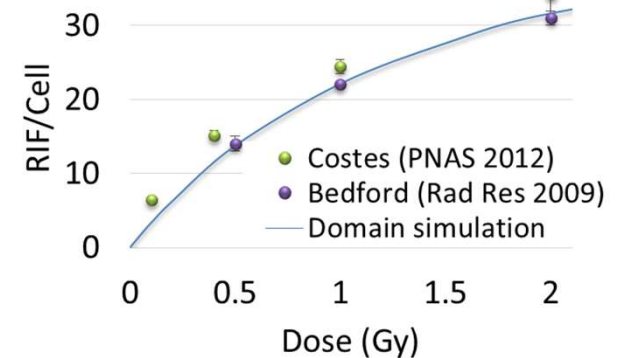
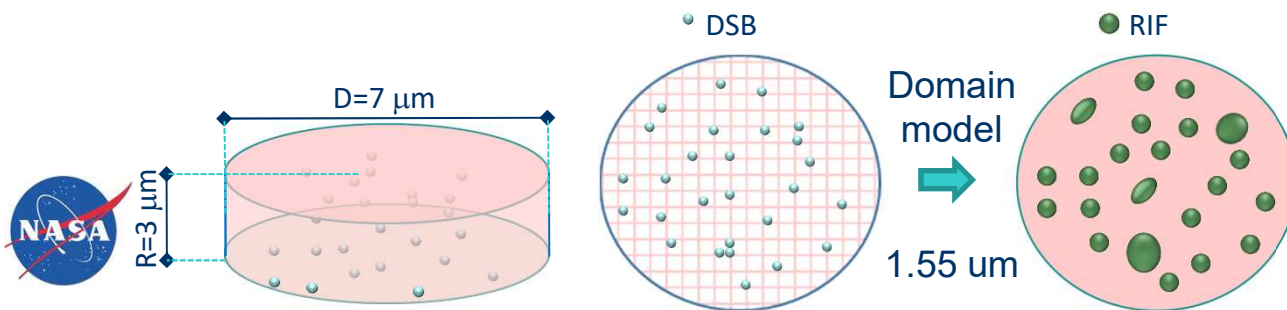
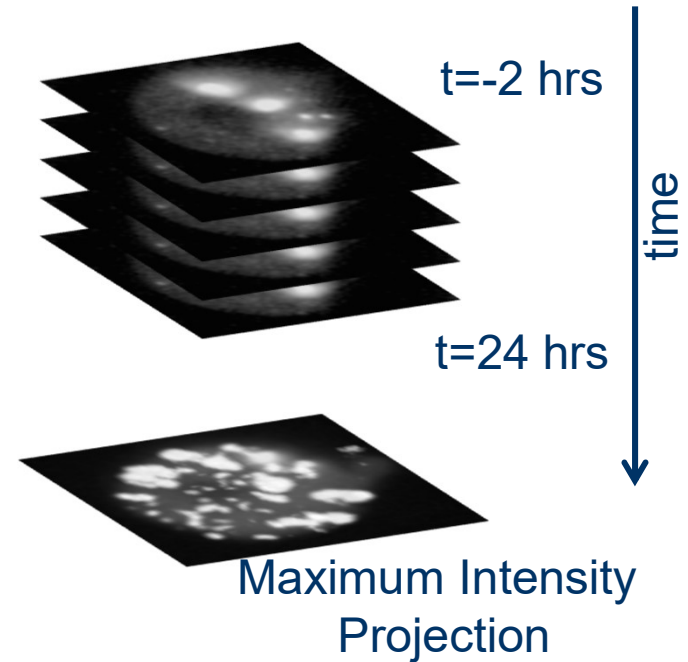
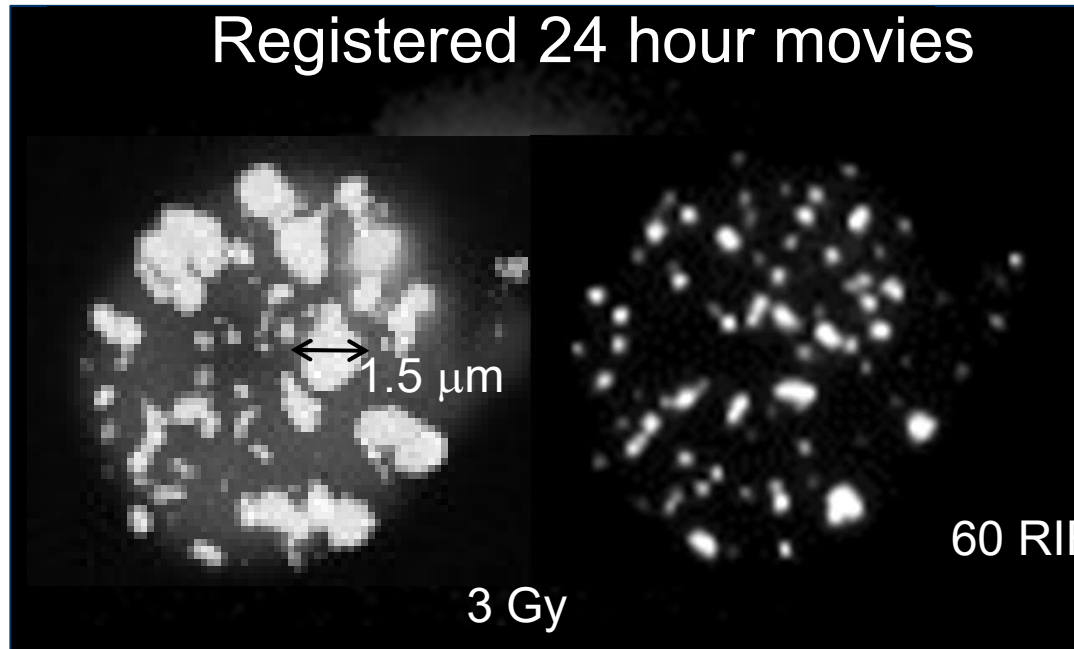
Rad Res 2014

Nikhil Vadhavkar,<sup>a</sup> Christopher Pham,<sup>b</sup> Walter Georgescu,<sup>c</sup> Thomas Deschamps,<sup>c</sup> Anne-Catherine Heuskin,<sup>d</sup> Jonathan Tang<sup>c</sup> and Sylvain V. Costes<sup>c,1</sup>



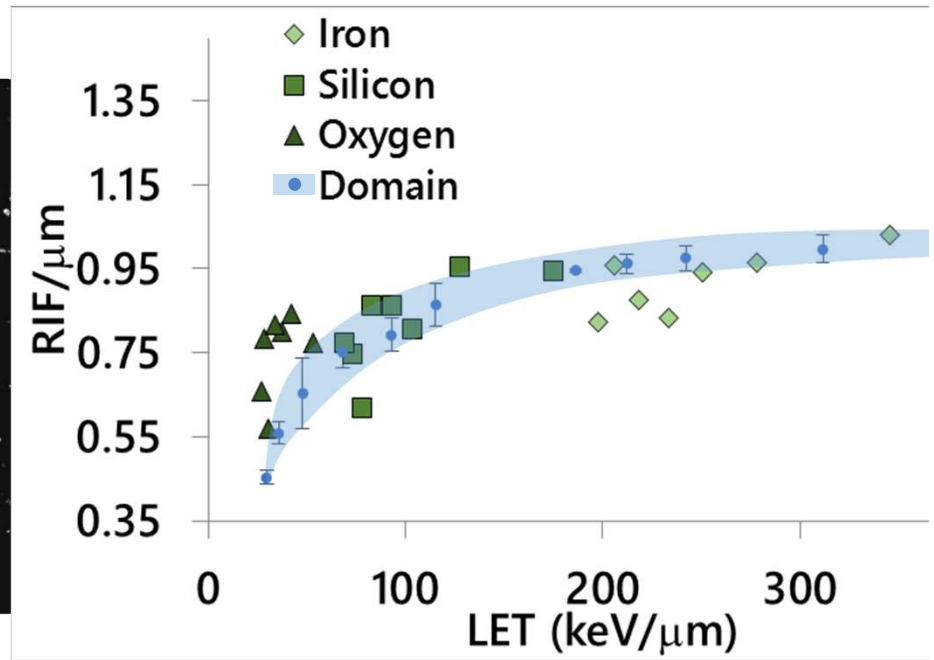
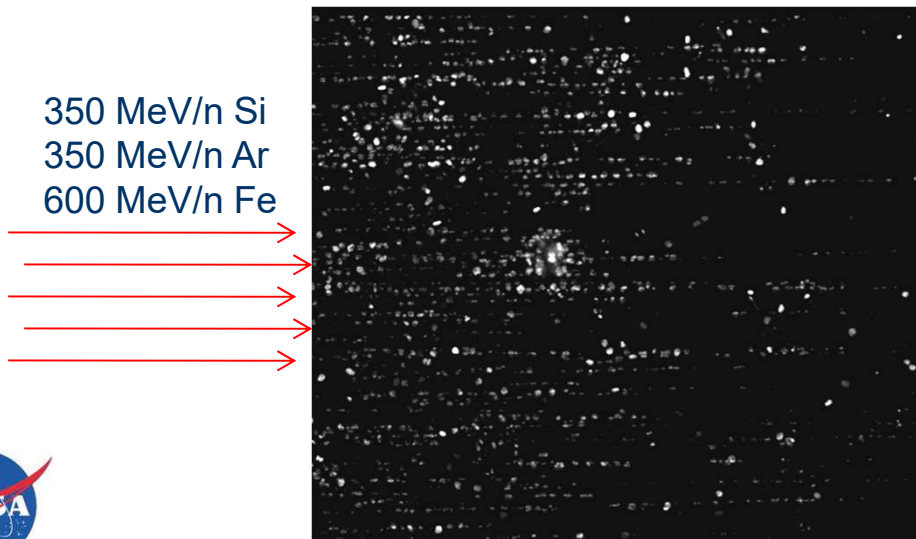
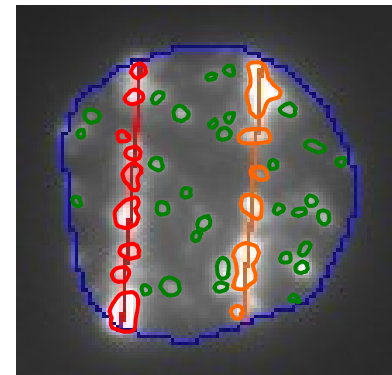
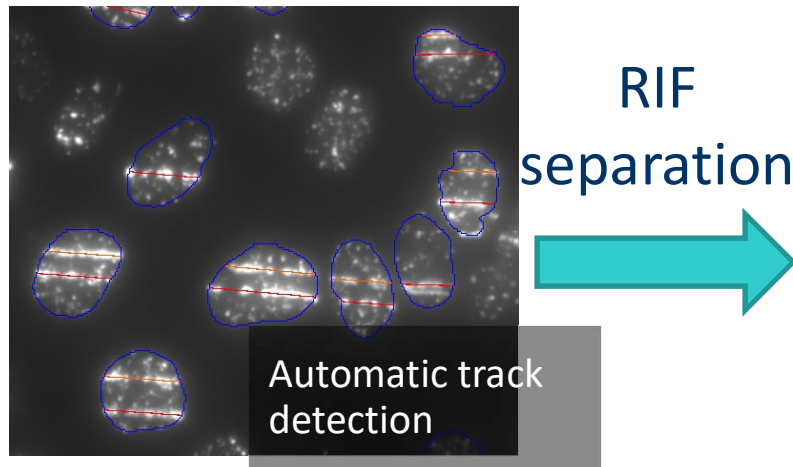
*Georgescu et al., PLoS One* 2015

# Registration algorithms and maximum projection in time reveals nuclear repair sub-domains



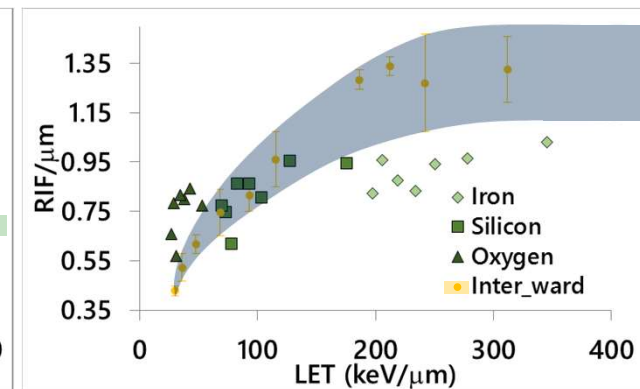
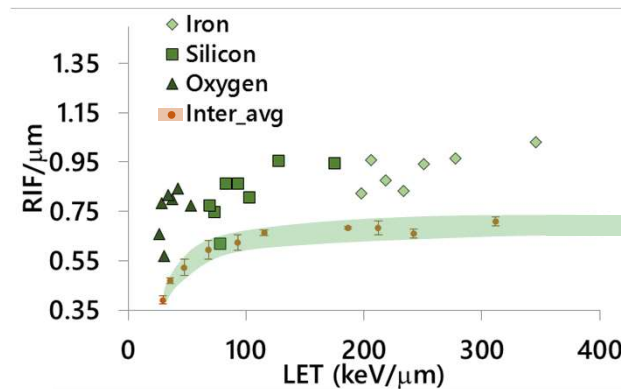
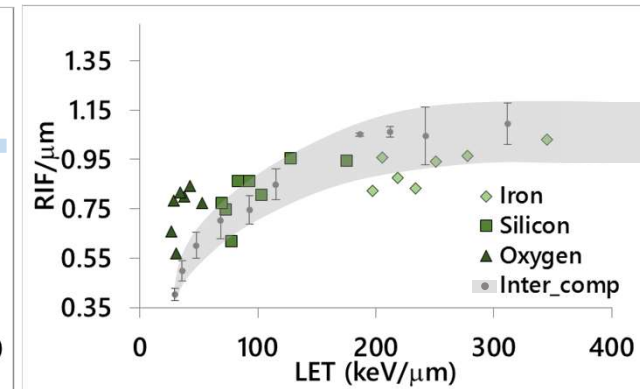
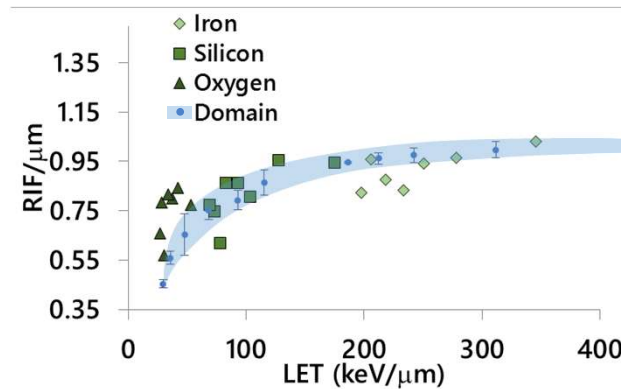
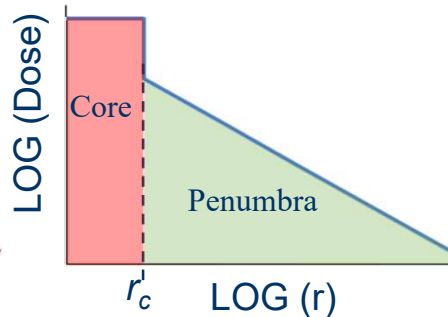
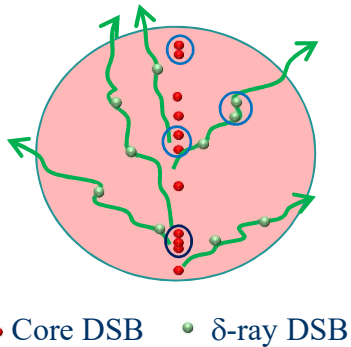
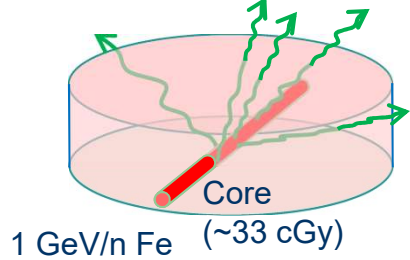


# Local dose effect is demonstrated with high LET tracks



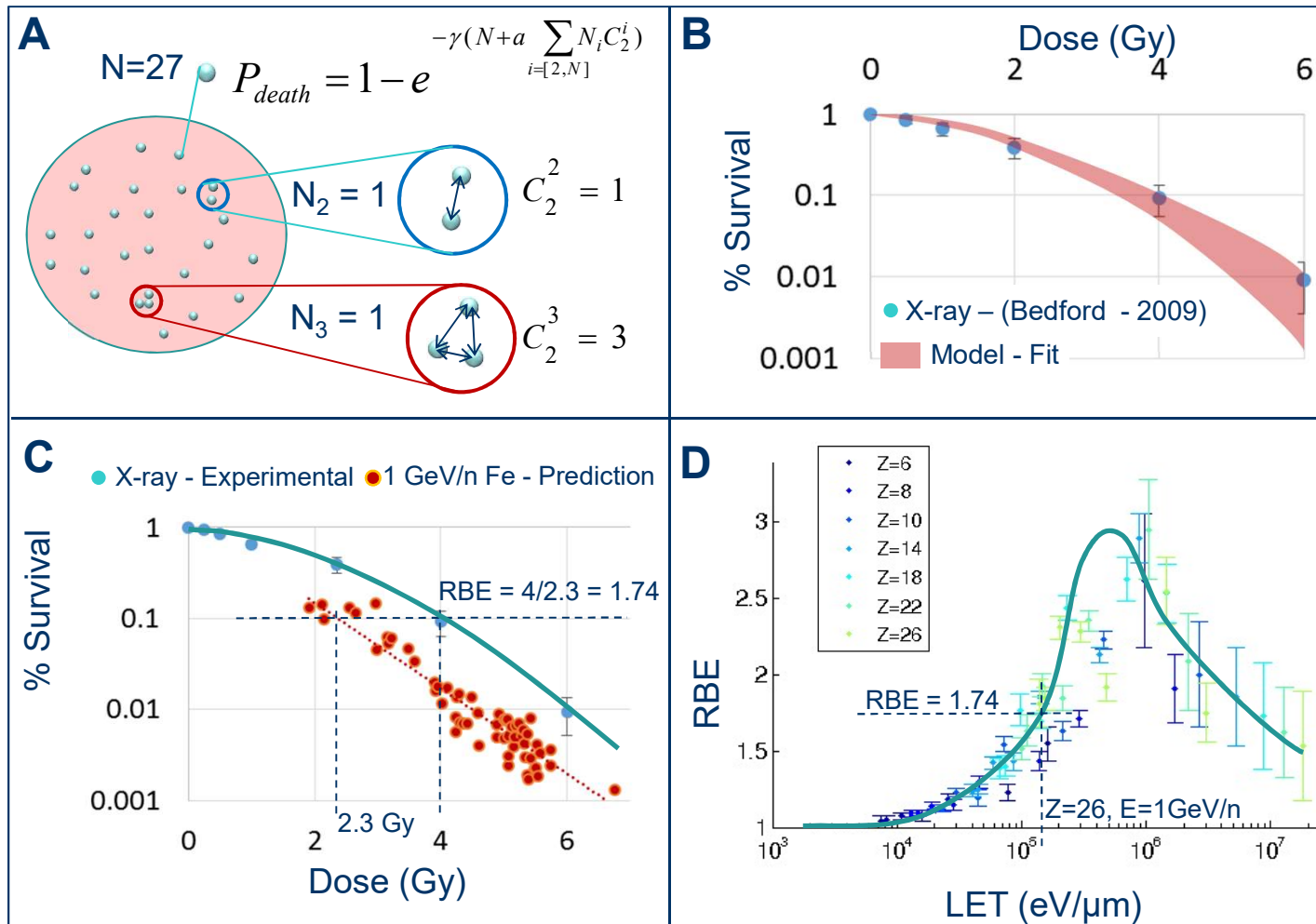
# Prediction of RIF yield along high-LET tracks

Delta-rays (~33 cGy)



Poisson process simulation of high-LET ions. Applies clustering metric from X-ray simulations to predict high-LET RIF yield.

# MC model of DSB clustering lead to higher death at higher dose or LET





# Experimental Design

## Constants

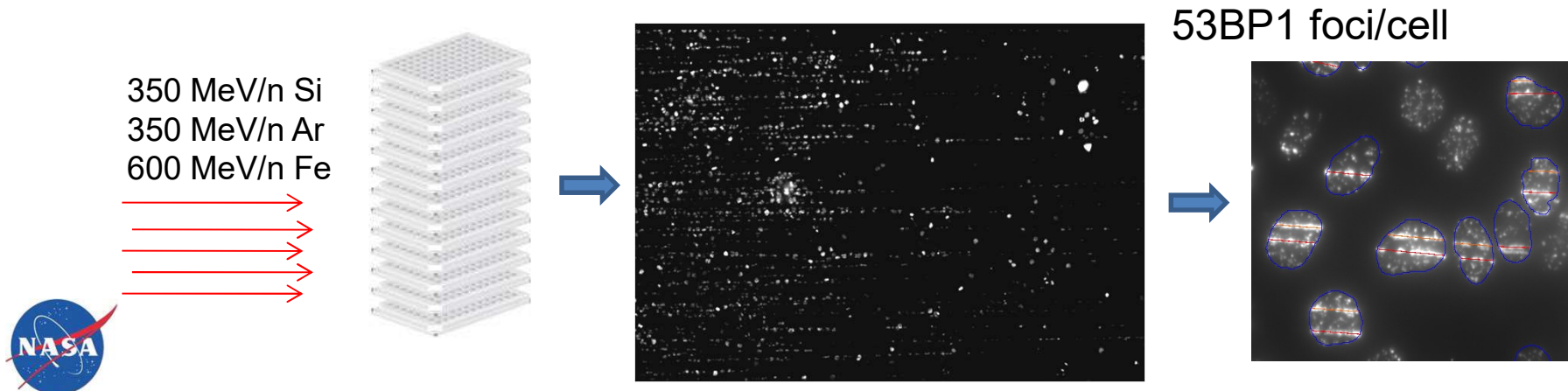
- Strains of animals
- Human PBMC (Year 2 and 3)

## Variables

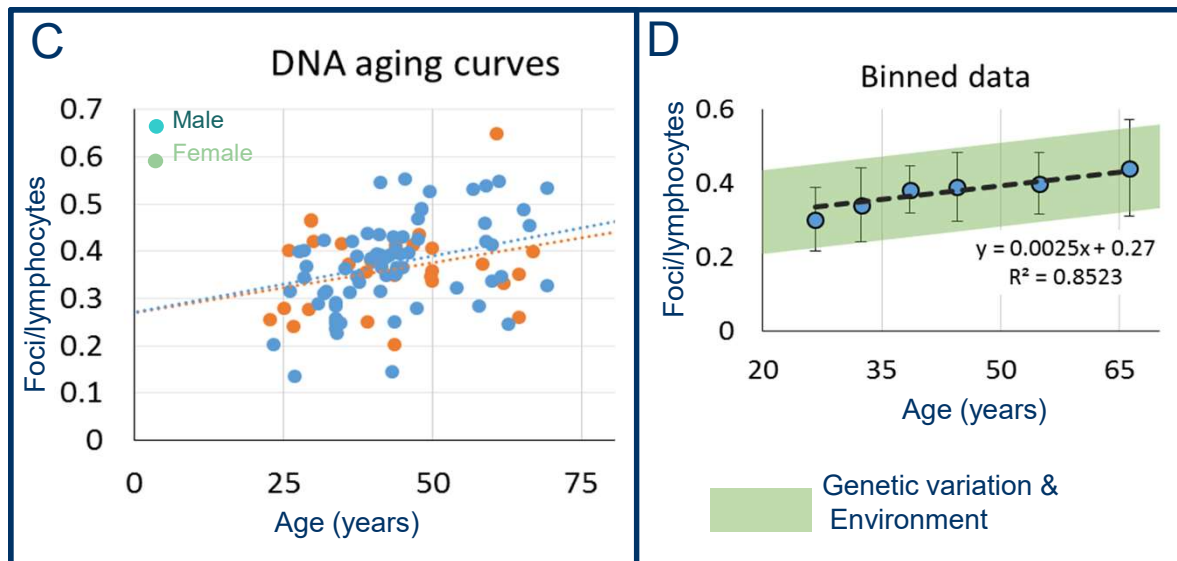
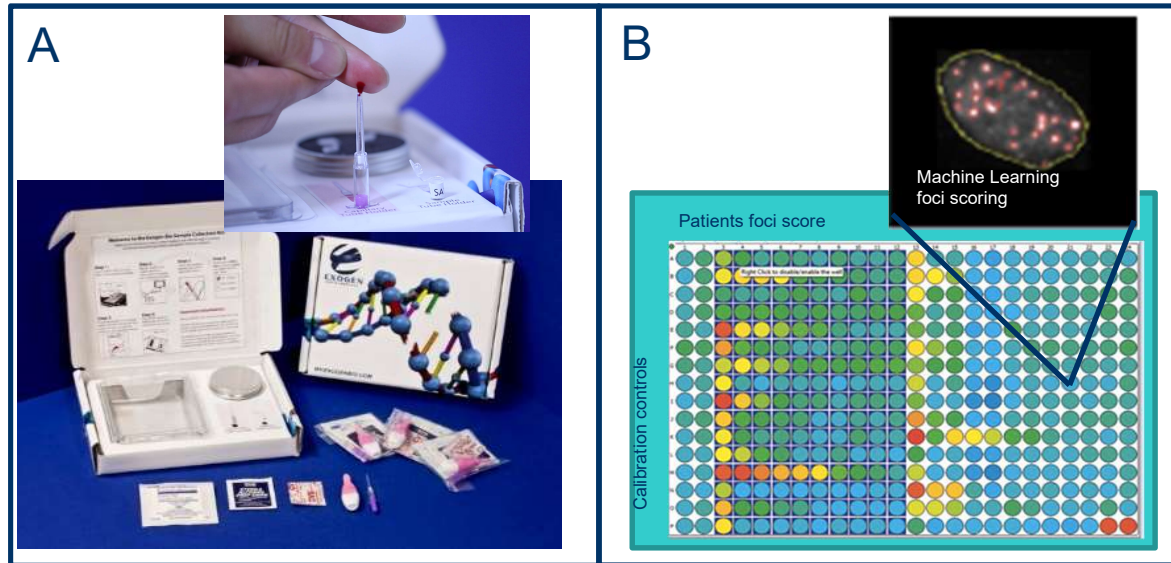
- LET
- Dose
- Time post-IR

## Measurements

- 53BP1 foci detection as DNA double strand break marker
- Repair Kinetic parameters
  - (power function)
- Foci saturation (Asymptotic fit)
- Foci Background
- 800 cells/condition

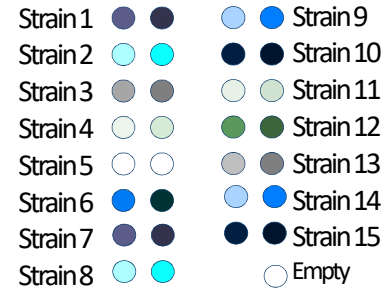
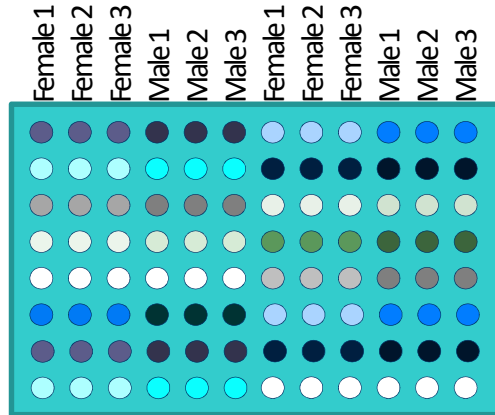


# DNA DSB assay – Collaboration with industry



# Experimental Design

- Mouse skin cells exposed ex-vivo
- Duplicate plate per condition



15 strains  
X 3 males  
X 3 females

Radiation

BNL

LBNL

High-LET Control

1.1 Particles/100  $\mu\text{m}^2$

3 Particles/100  $\mu\text{m}^2$

X ray control

X ray 0.1 Gy

X ray 1 Gy

X ray 4 Gy

4

8

24

48

X2 ions

# of 96 well plates (53BP1)

12+8

12

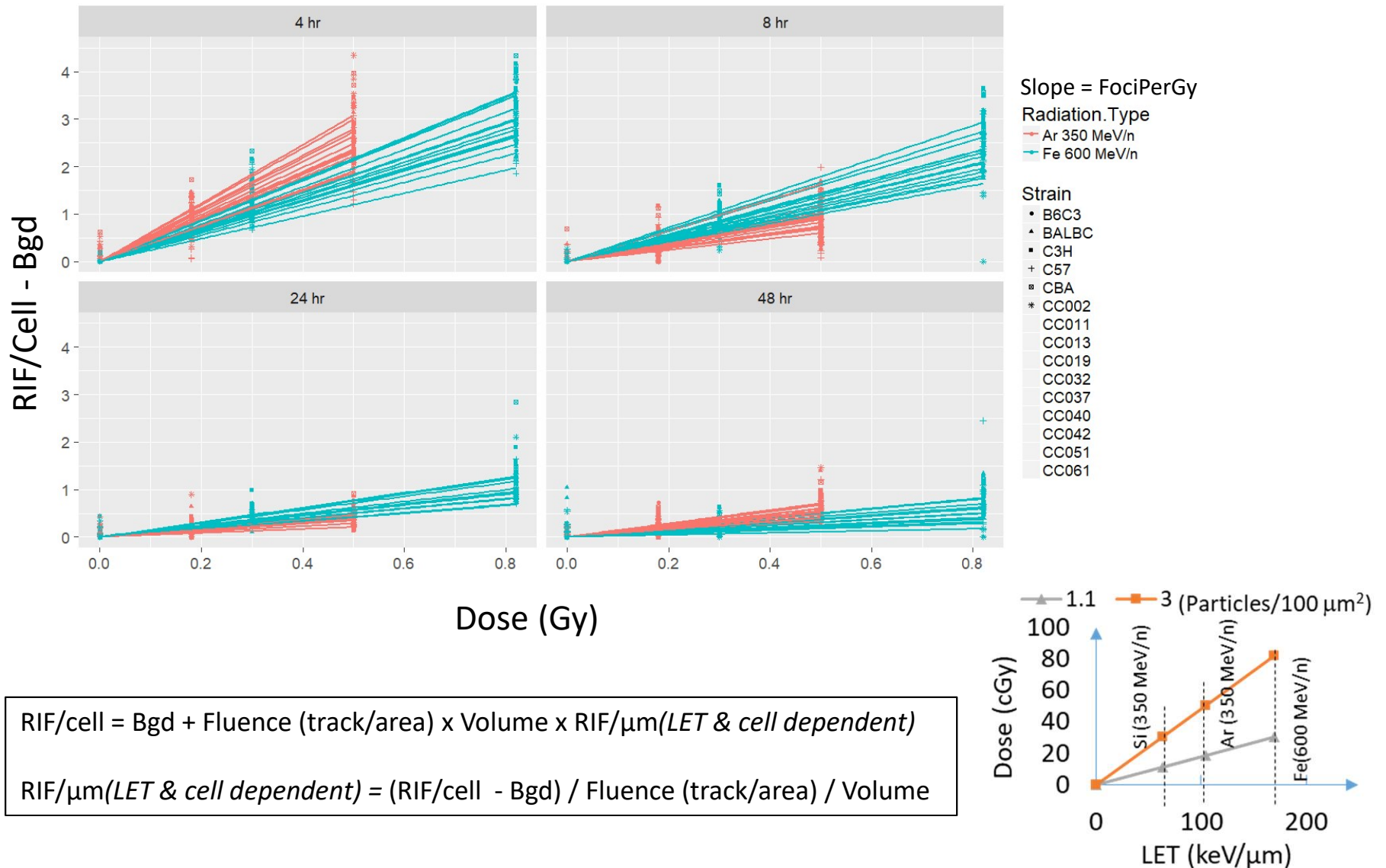
12+8

12+8

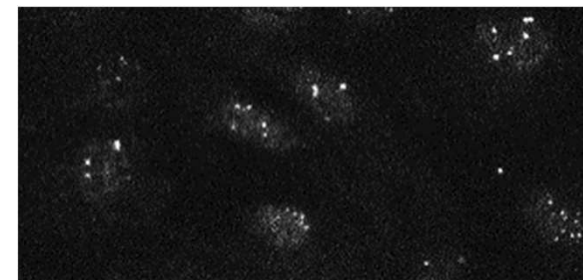
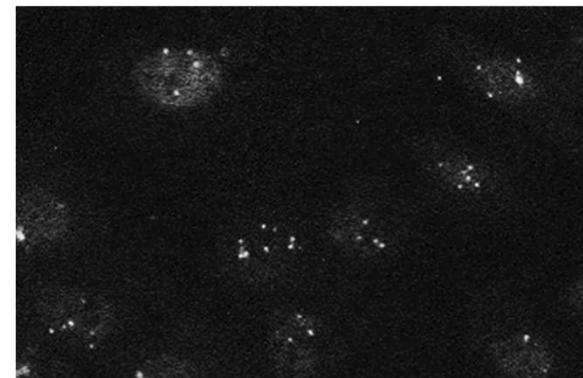
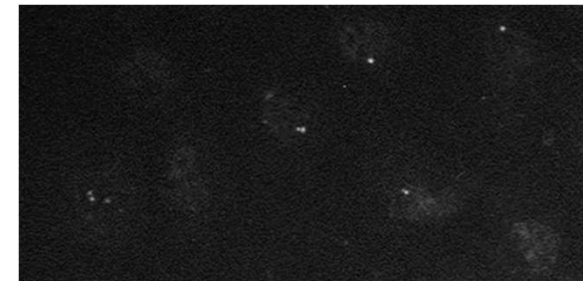
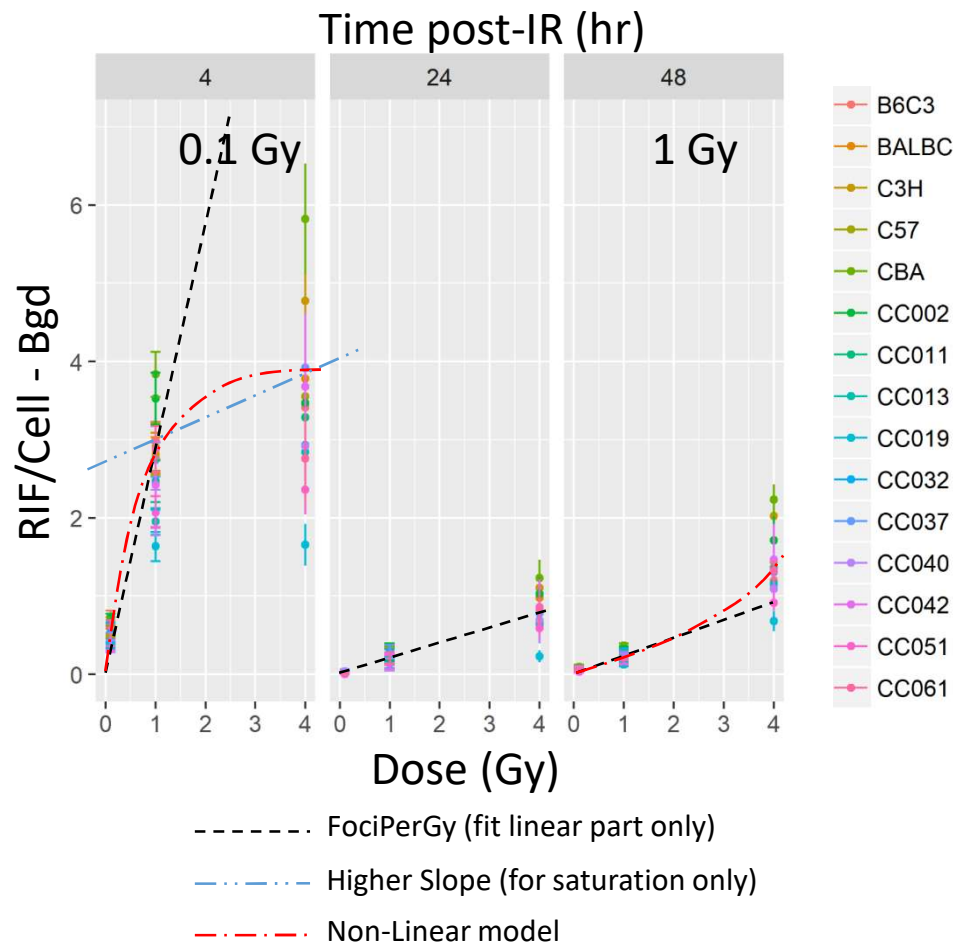
72 plates

# RIF/Cell vs dose for high-LET always linear

(Reflects more track than DSB – See Costes et al, Rad Res 2006)

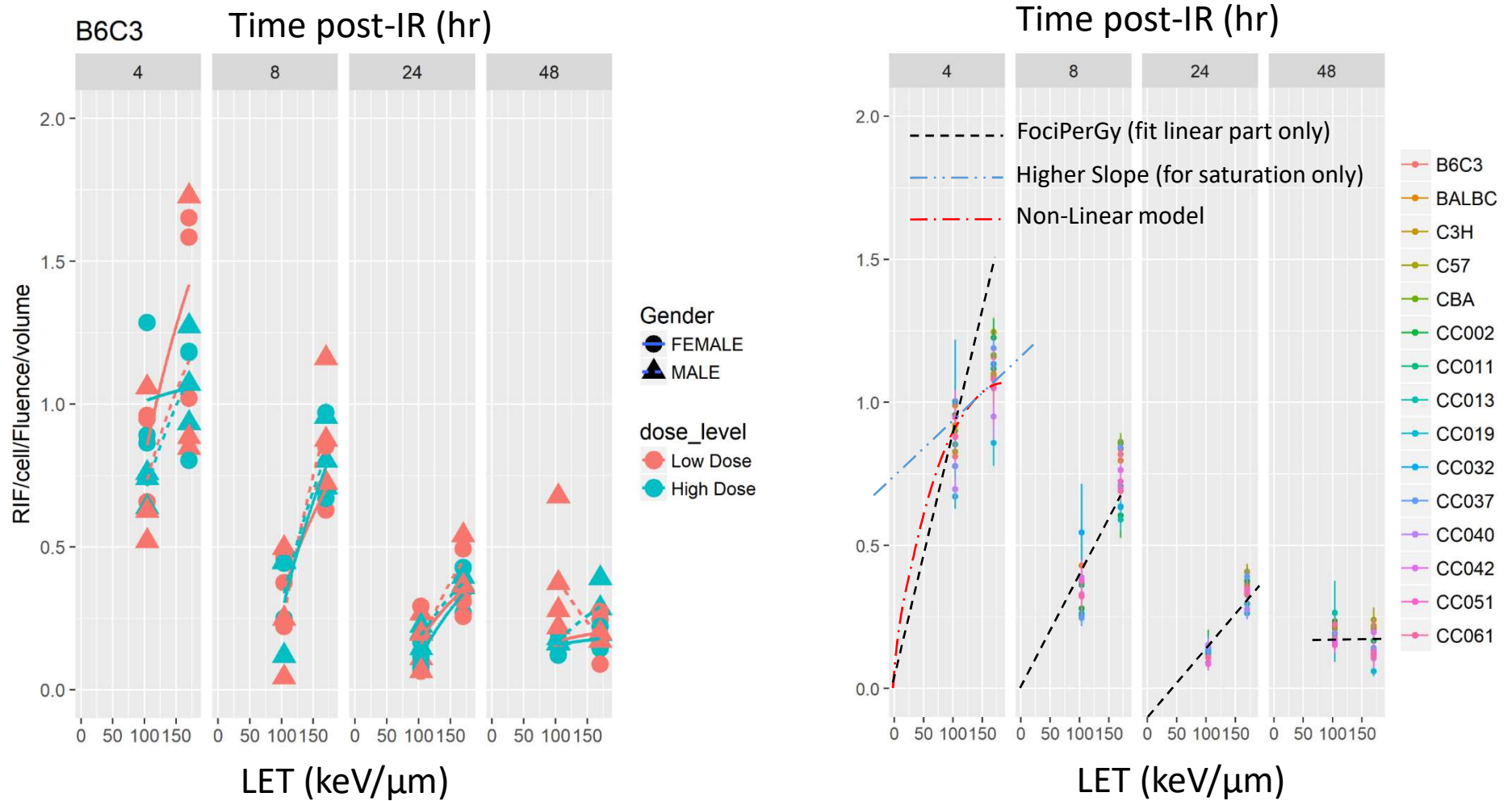


# Low-LET dose dependence is a function of time post-IR



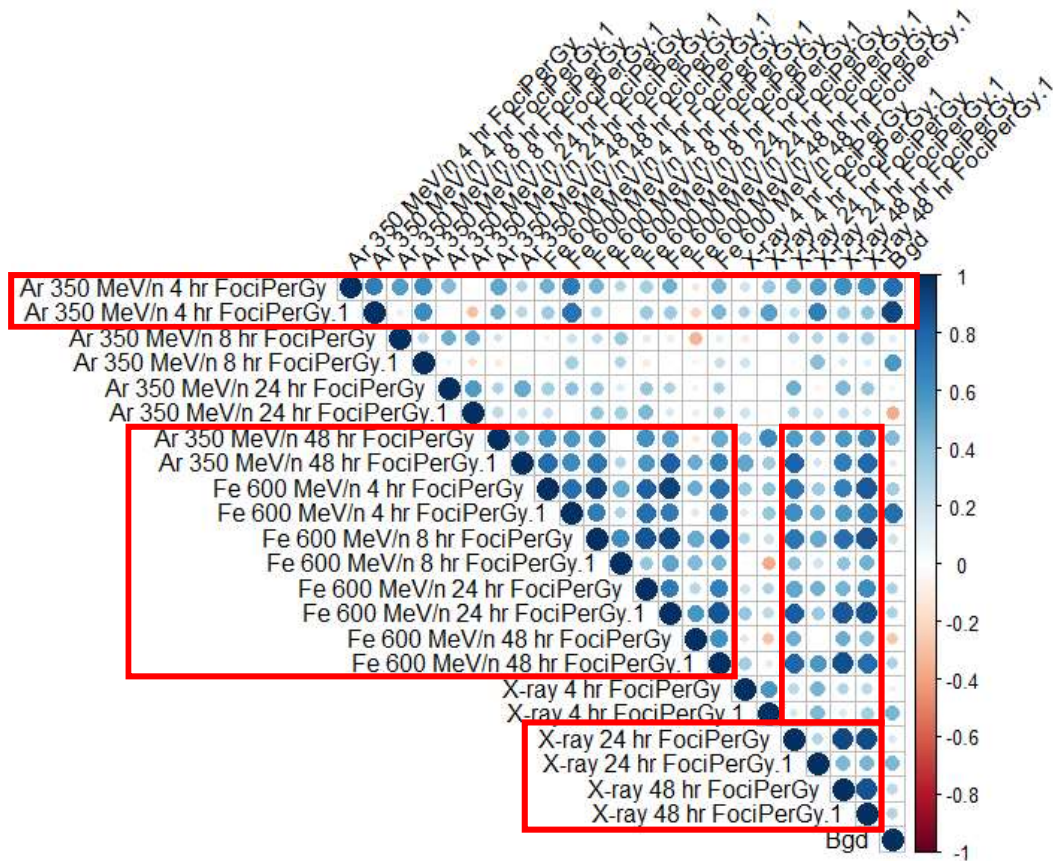


# RIF/ $\mu\text{m}$ dependence saturates for increasing LET at early time point

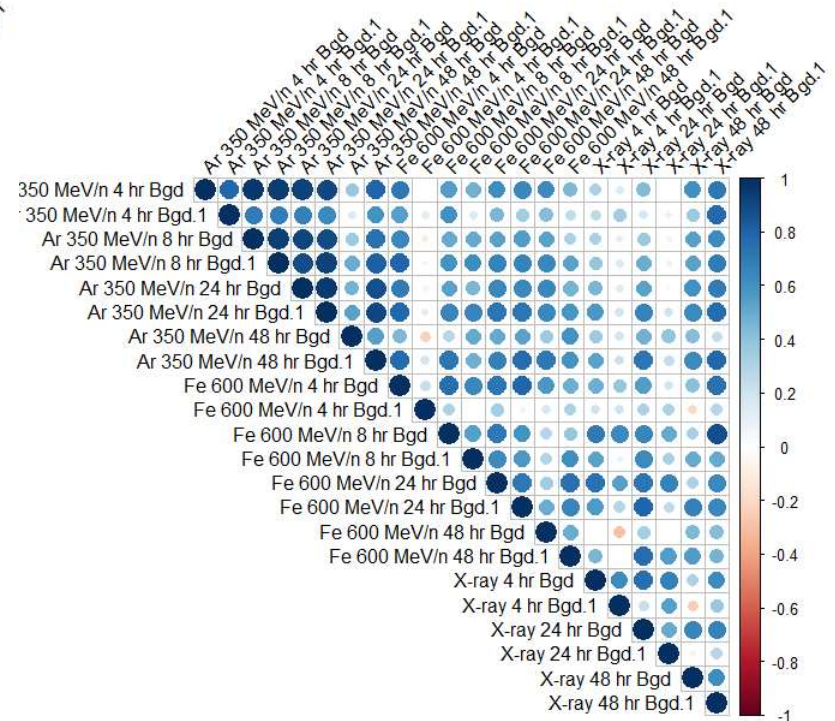


# Correlation Graph – Slope and Background

Strong correlation across 15 strains of mice between low and high LET

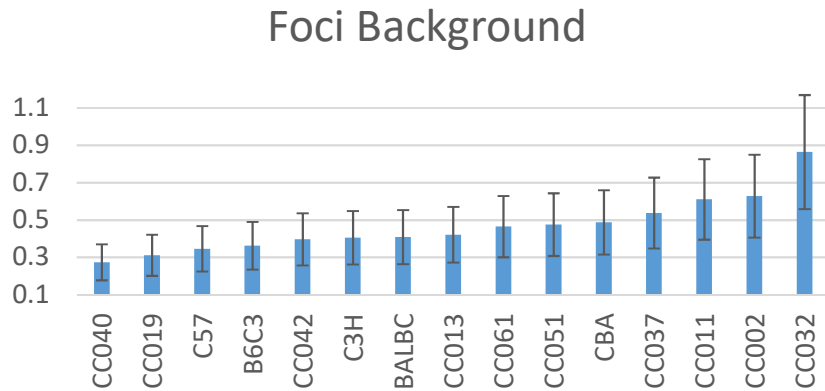


RIF/Gy  
(Slope)

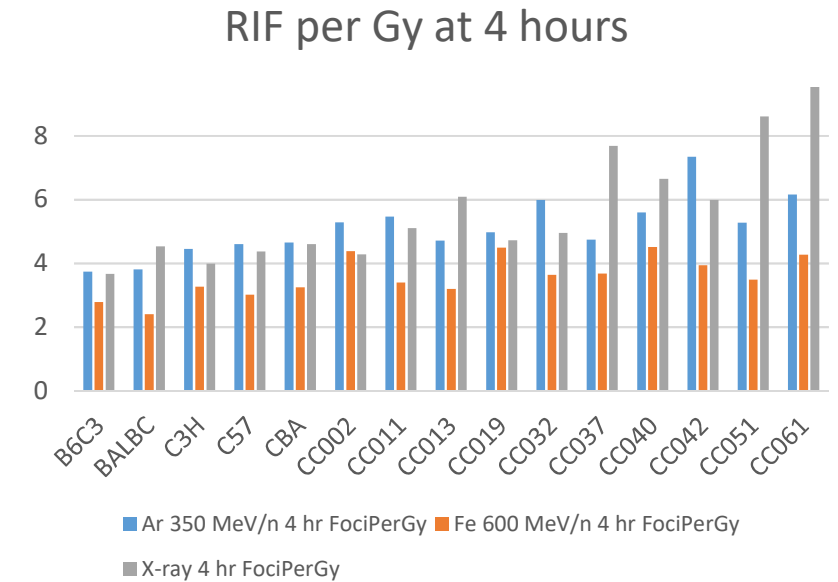
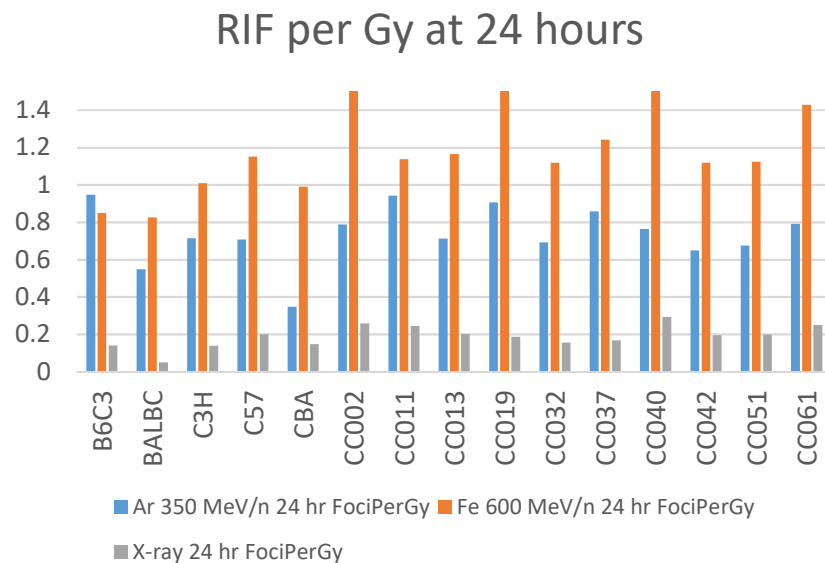


Background Foci  
(Slope intercept)

# DSB detection sensitivity is driven by genetic



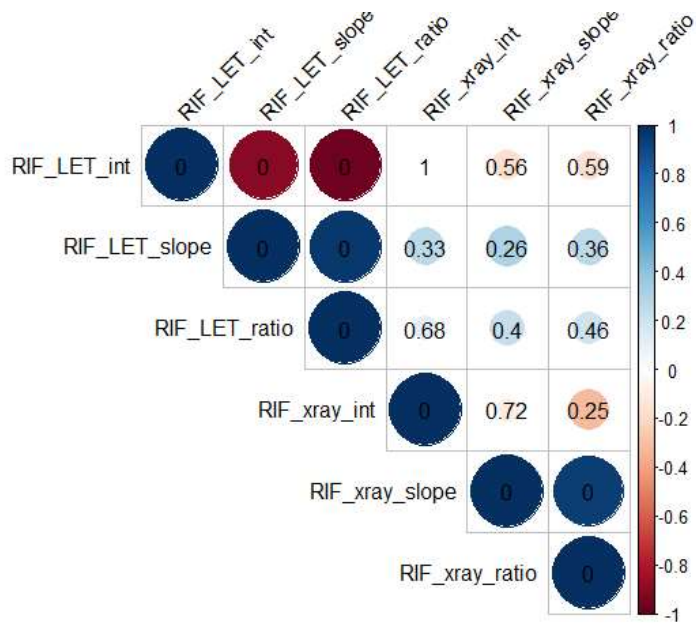
- Different ranking for background



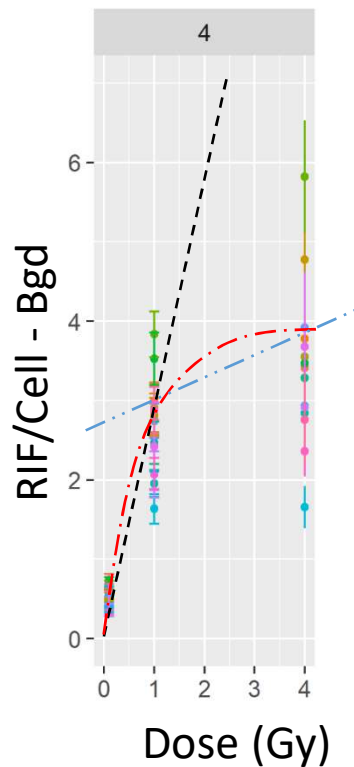
- The higher the LET, the more clustering
- Reference mice show lower detection

- Ranking persists at 24 hrs
- Higher LET have steeper slopes (more remaining damage)

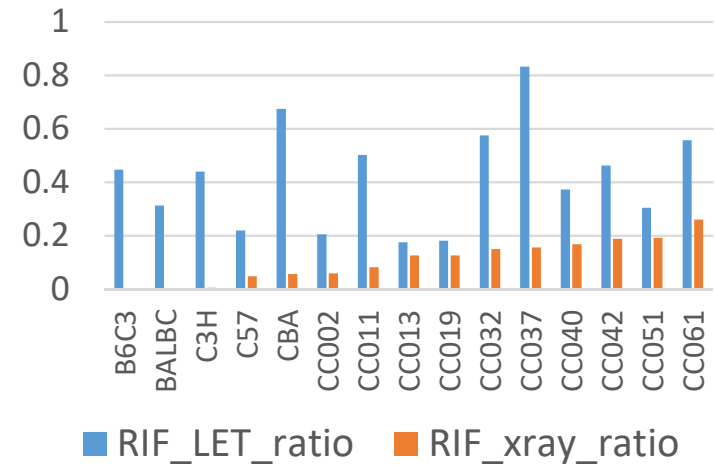
# Saturation parameters



Time post-IR (hr)



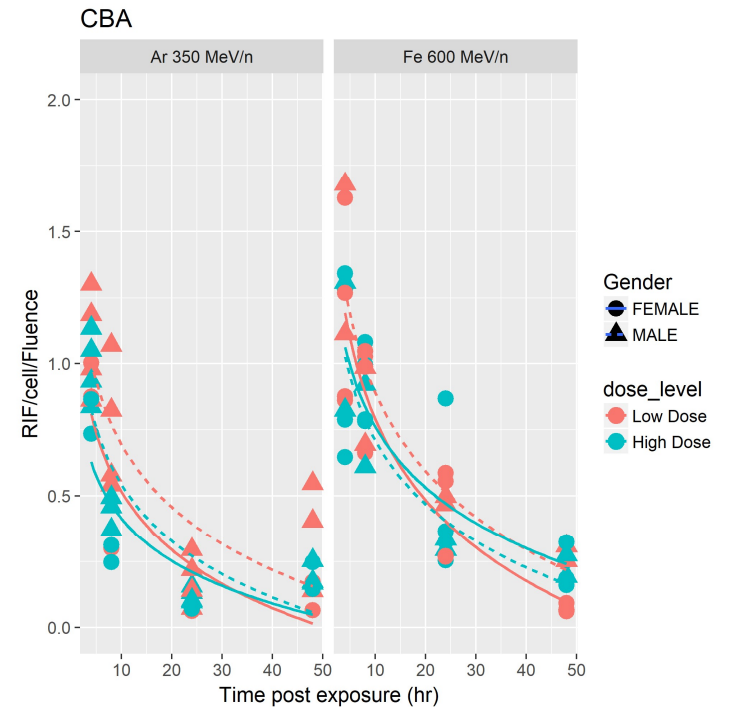
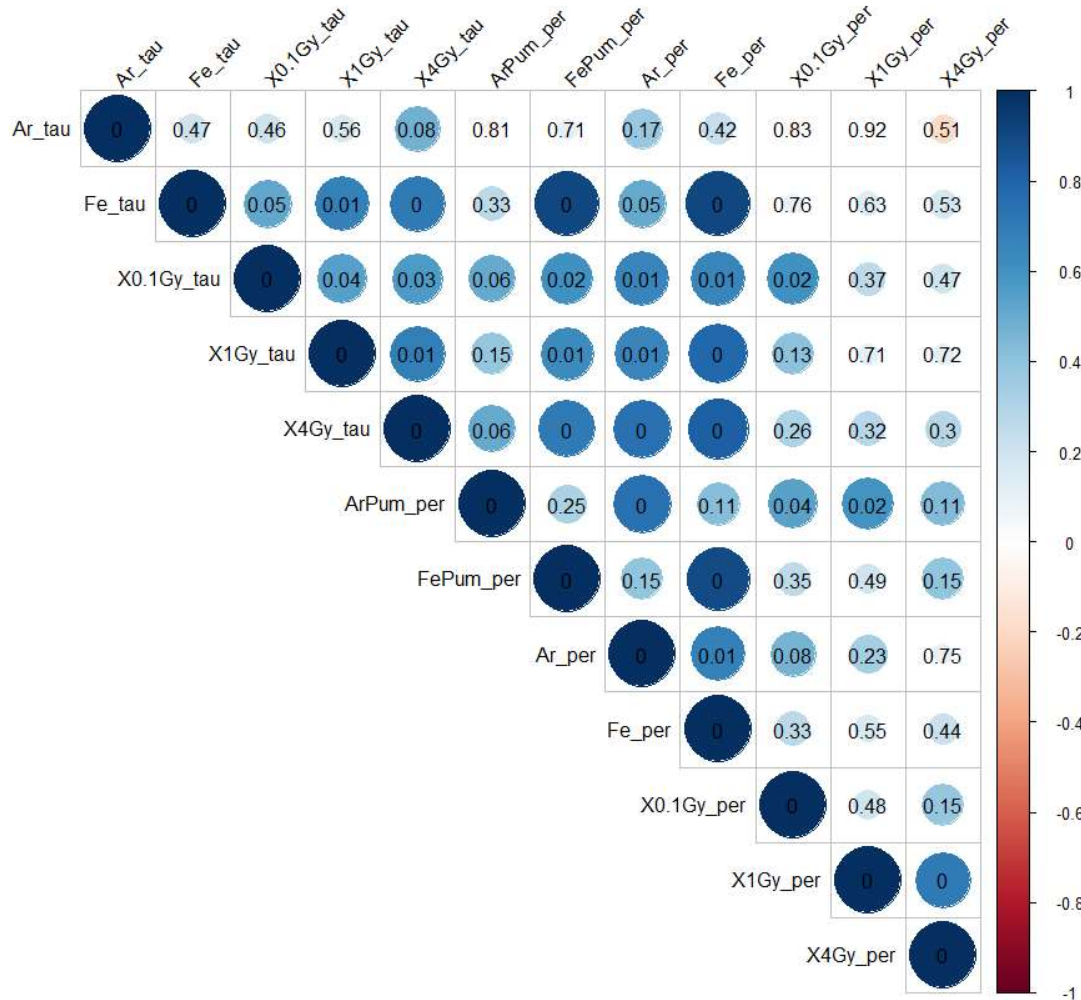
Saturation: Slope Ratio



\* Note: For 15 pairs, R must be  $\geq 0.52$  for p-value to be less than 0.05



# Kinetic parameters

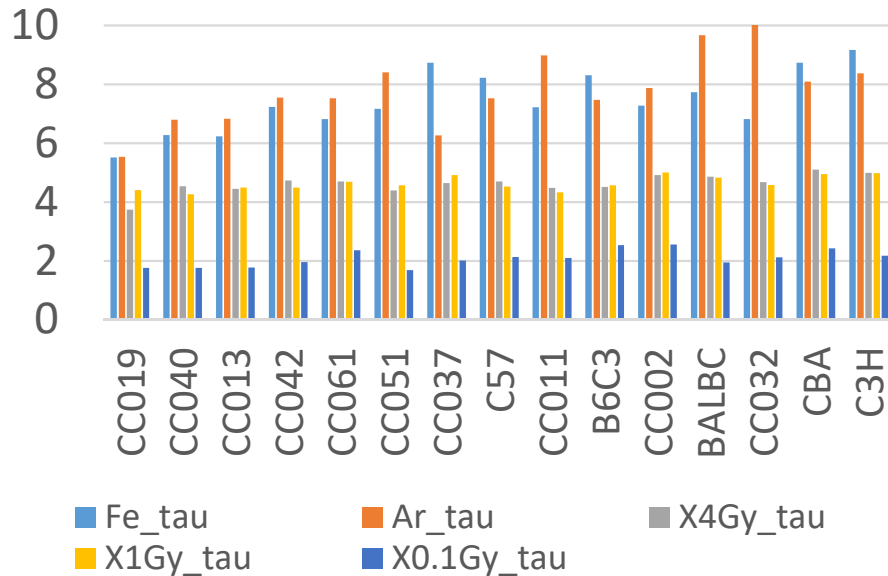


- Persistent RIF at 24 and 48 hours (RIF/um noted FePum\_Per for Fe, RIF/cell noted Fe\_per)
- Exponential fit:  $RIF/um = RIF/um(0).EXP(-t/tau)$
- $RIF/um(0) = LET(keV/um)/80$  for LET, and  $RIF(0) = 35.Dose(Gy)$  for X-ray
- Excluding 4 hr, 4 Gy for X-ray

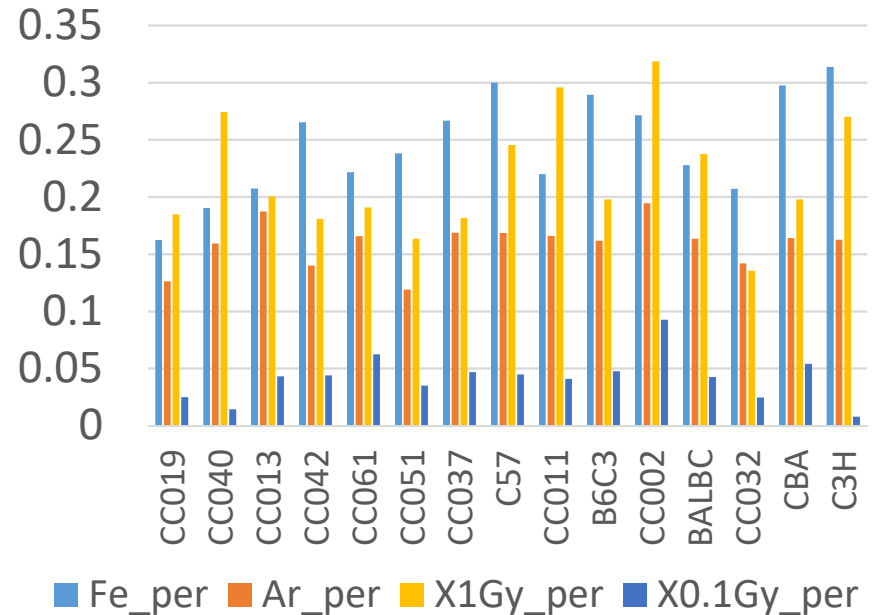


# Repair kinetics and Persistent Rf

Repair kinetic (in hours)



Persistent (RIF/um or RIF/cell)



Lethal dose for X-ray exposure (Gy)

Strain	LD <sub>50.30</sub> +/- S.E. (Gy)	
	Males	Females
C3HeB/J	6.76 +/- .11	6.89 +/- .7
CBA/J	6.56 +/- .9	6.89 +/- .8
C57BL/6J	6.47 +/- .15	6.70 +/- .6
BALB/cJ	5.70	5.85 +/- .12

Source: Storer (unpublished data)

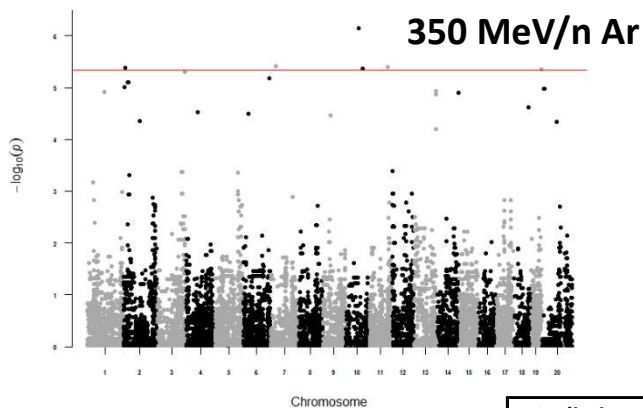
<http://www.informatics.jax.org/greenbook/chapters/chapter22.shtml>

All mice 3 to 4 months of age at time of irradiation.

		DNA damage in fibroblasts after X ray exposure	
		0.1 Gy	1 Gy
T-cell fraction	0.1 Gy	0.607	0.011
	1 Gy	0.276	0.053
B-cell fraction	0.1 Gy	0.38	0.097
	1 Gy	0.098	0.291

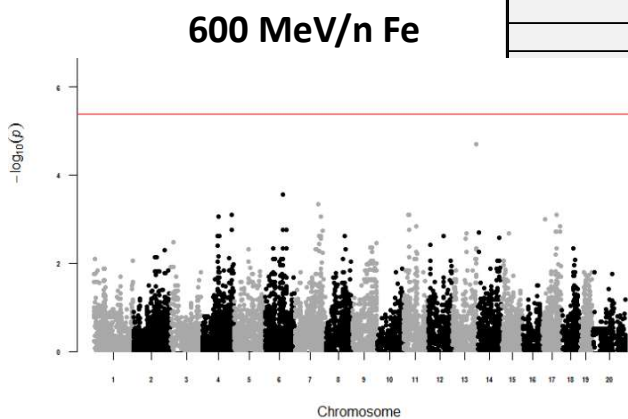
Link to in-vivo (whole blood response in CC mice – Mao/Snijders LBL Low Dose DOE)

# GWA results for RIF/cell/Gy at 48 hours post exposure to high and low LET radiation



Trait	CHR	Peak SNP	Position	p value	MAF	Linkage Disequilibriumblock (LD)	No. of genes
350 MeV/n Ar	10	UNC18214722	71976067	$7.22 \times 10^{-7}$	0.333	71805029 - 83277275	209
	10	JAX00021248	96091724	$4.24 \times 10^{-6}$	0.2857	96070217 - 96887381	2
	11	UNC20271233	102605060	$4.03 \times 10^{-6}$	0.2857	101434884 - 103096965	49
	19	JAX00481854	60388505	$4.5 \times 10^{-6}$	0.2857	60321655 - 61168855	15
	2	UNC2559552	10174335	$4.2 \times 10^{-6}$	0.1429	10174335 - 10388678	2
	3	UNC6555279	152286637	$4.9 \times 10^{-6}$	0.2857	152132653 - 152805055	11
	7	UNC12559788	30462618	$3.9 \times 10^{-6}$	0.2857	30384655 - 30509079	7

Trait	CHR	Peak SNP	Position	p value	MAF	Linkage Disequilibriumblock (LD)	No. of genes
600 MeV/n Fe	10	UNC18214722	71976067	$7.22 \times 10^{-7}$	0.333	71805029 - 83277275	209
	13	UNC23495258	119997660	$1.5 \times 10^{-8}$	0.2667	119997660 - 120160384	1
		UNC23497196	120160384	$9.5 \times 10^{-8}$	0.07143	119997660 - 120160385	0



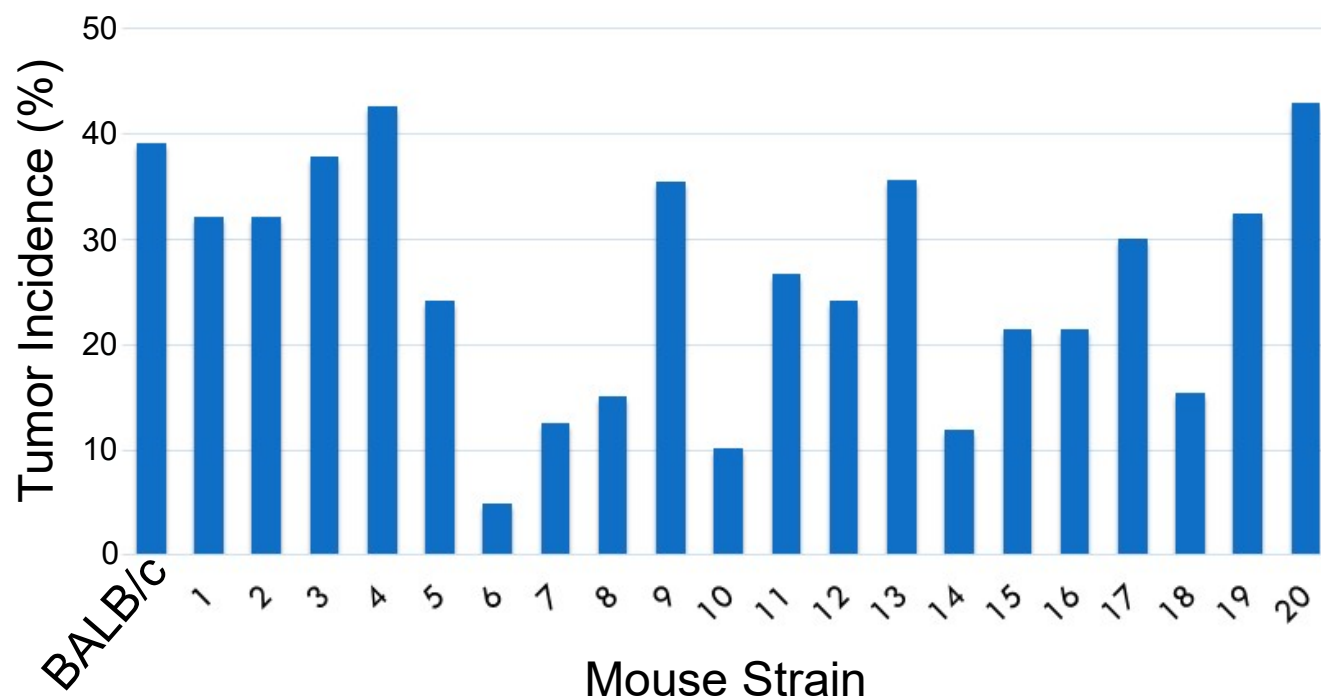
Radiation	CHR	SNP	P value	Gene Symbol	Function
350 MeV/n Ar	10	UNC18214722	$7.22 \times 10^{-7}$	Smcarb1	tumor suppressor, relieves repressive chromatin structures
				Mcm3ap	essential for the initiation of DNA replication
				Sumo3	Sumoylation - DNA replication and repair
				Dnmt3l	DNA Methyltransferase - genomic integrity, DNA Repair
				Rrp1	Ribosomal RNA Processing - DNA repair
				Cdc34	Cell Division Cycle 34 - initiation of DNA replication
				Polr2e	RNA Polymerase II - transcription of DNA
				Stk11	Serine/Threonine Kinase 11 - tumor suppressor
				Mum1	DNA damage response pathway, recruited to the vicinity of DNA breaks by TP53BP1
				Pias4	DNA Double-Strand Break Repair
				Fzr1	Required for the G2 DNA damage checkpoint after DNA damage
				Nfic	DNA-binding proteins - activates transcription and replication
				Sirt6	GIN, aging and DNA Double-Strand Break Repair
				Tdg	Removes thymine moieties from G/T mismatches [base-excision repair (BER)]
	10	JAX00021248	$4.24 \times 10^{-6}$	Btg1	DNA-damage inducible, anti proliferative
	11	UNC20271233	$4.03 \times 10^{-6}$	Atxn7l3	Chromatin organization, transcription
				Hdac5	Alters chromosome structure - transcriptional regulation
				Brca1	DNA damage sensor, maintains genomic stability, tumor suppressor
				Rdm1	DNA double-strand breaks and homologous recombination

# Colorado State University – Collaboration with Dr. Weil



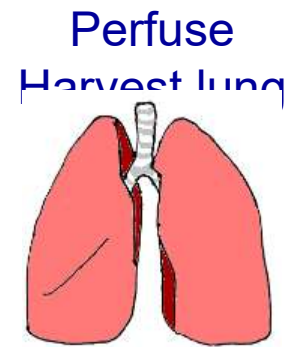
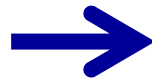
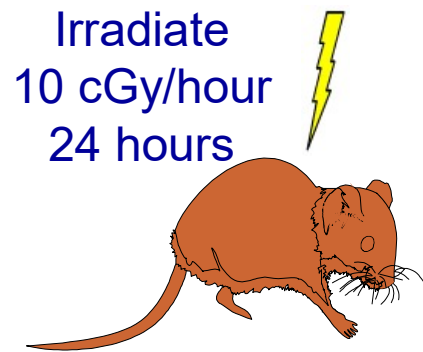
Donasian Ochola, DVM, PhD

## Radiogenic Lung Cancer in Recombinant Congenic Mice

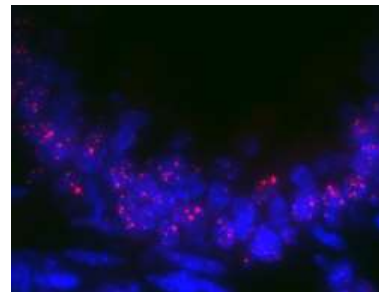


From: *Szymanska et al.*, 1999

# Low Dose Rate $\gamma$ -H2AX Assay



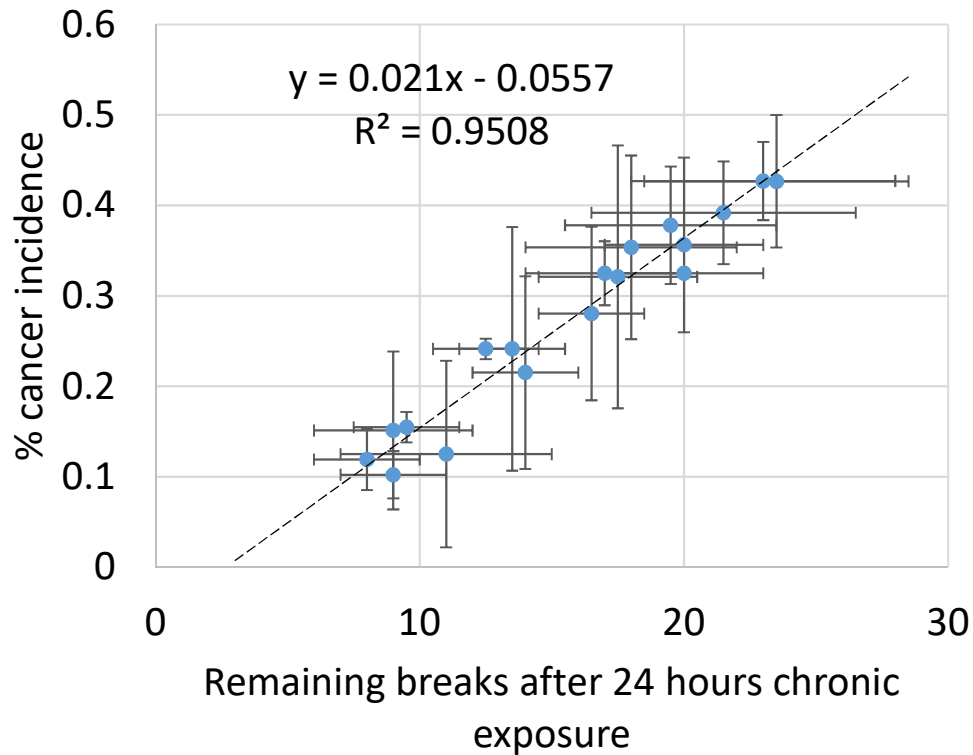
Immunodetection of  $\gamma$ -H2AX foci



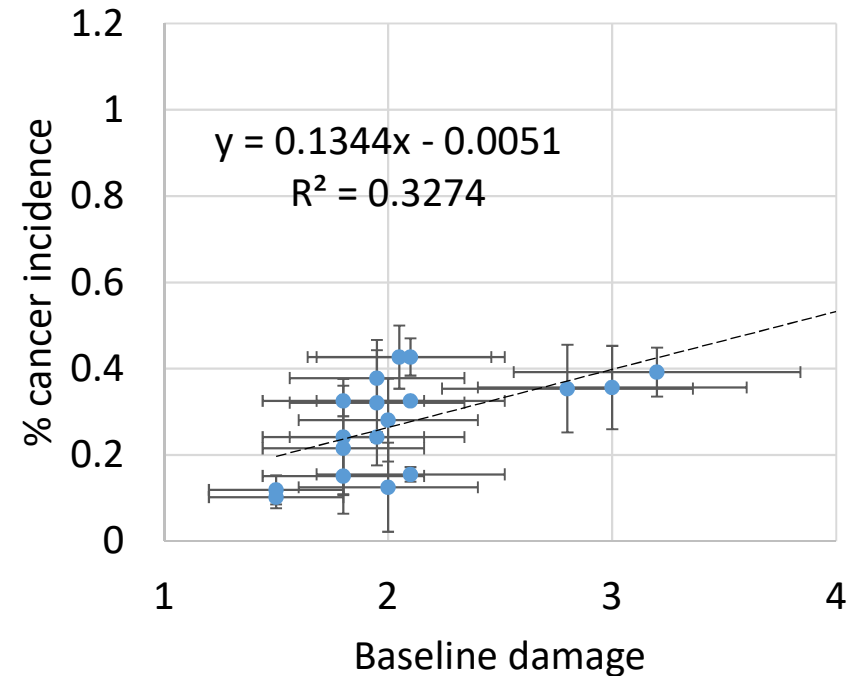


# Repair efficiency correlates with incidence

Lung Cancer - Life Study - 1600 mice -  
18 Strains



Lung Cancer - Life Study - 1600  
mice - 18 Strains

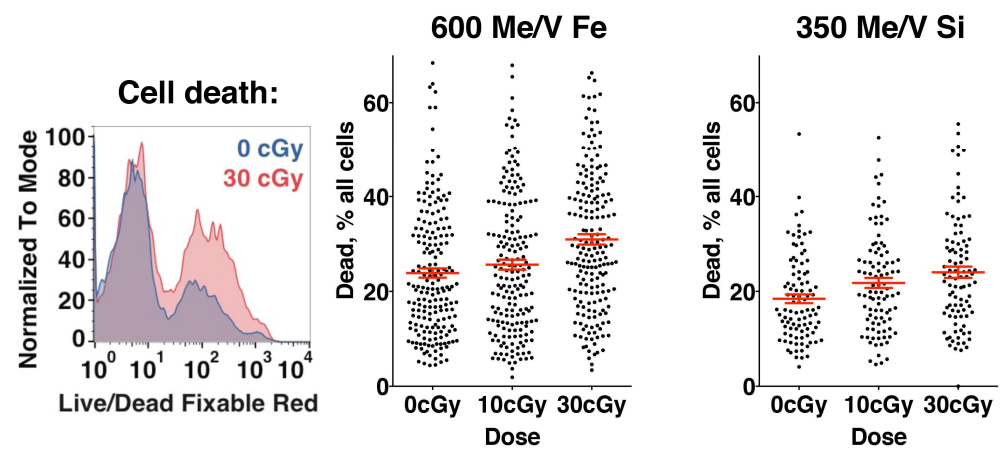
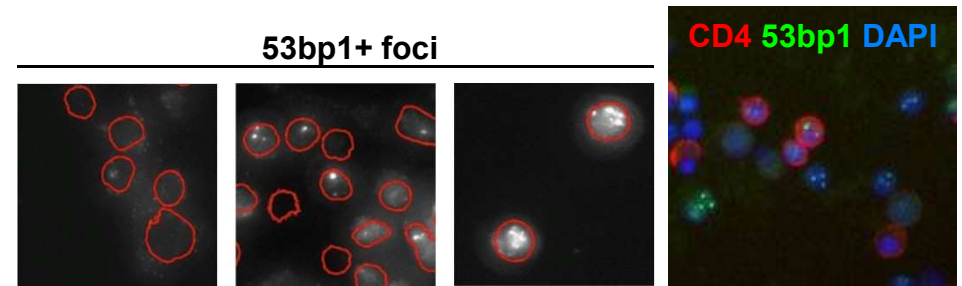
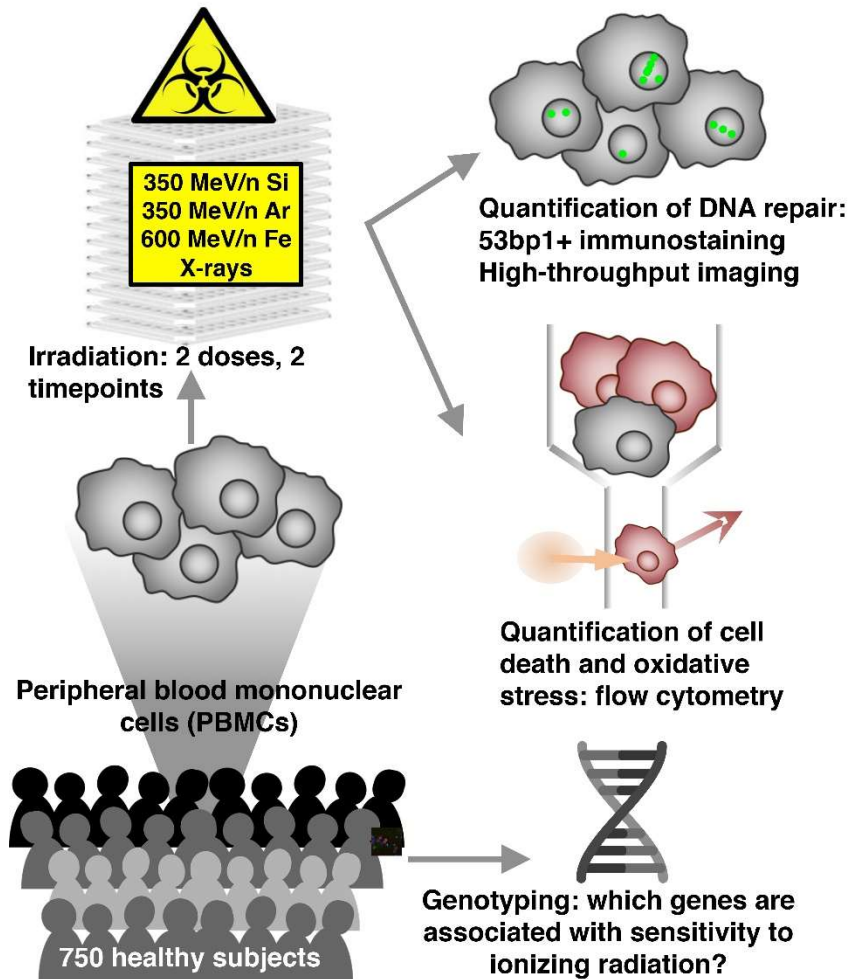


- Similar results with PBMC irradiated in vivo
- Low-dose computerized tomography lung cancer screening reduces lung cancer mortality, but has a high potential for over-diagnosis
- Restrict screening to those actually at risk

# Using DNA damage to investigate the individual variability of human sensitivity to ionizing radiation



Egle Cekanaviciute, Poster # PS8-12. Wednesday 5:15pm.





# THANK YOU

## **COLLABORATORS:**

**LBL:** Gary Karpen, Jian-Hua Mao, Antoine Snijders

**NASA:** Steve Blattmig, Artem Ponomarev, Ianik Plante

**US:** Mike Weil, Mary Helen Barcellos-Hoff

**INSERM-FRANCE:** François Paris

**Exogen Biotechnology Inc.:** Jonathan Tang

**FUNDING: NASA HRP, DOE Low dose program**

