

Variability in galactic cosmic radiation- induced DNA damage response in inbred mice is modulated by genetics

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Senior Research Scientist

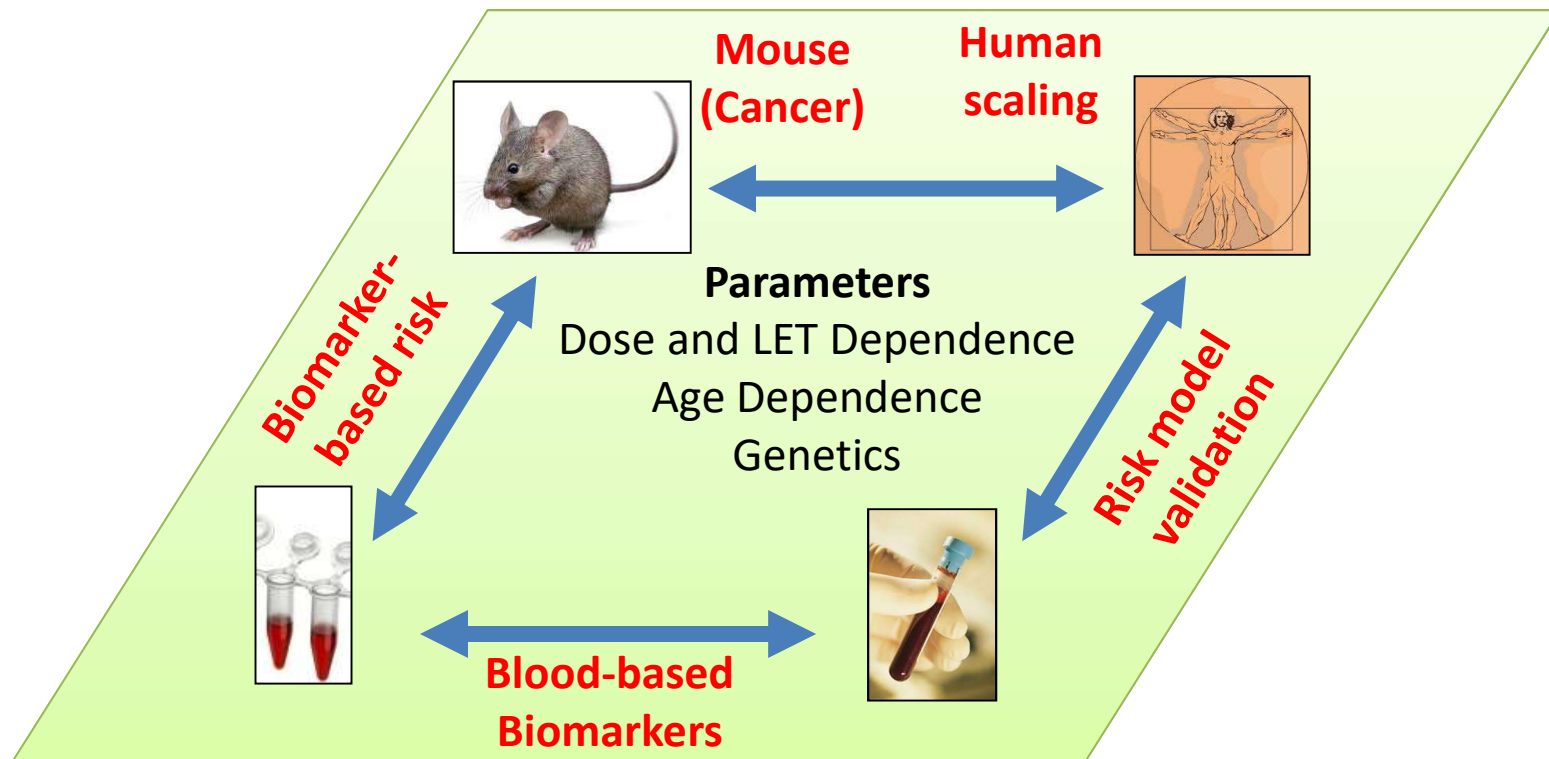
Space Biosciences Research Branch (SCR)

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.

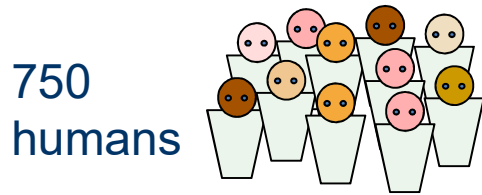
Potential outcome: Individualized risk assessment for Astronauts

A scalable approach: from mice to human



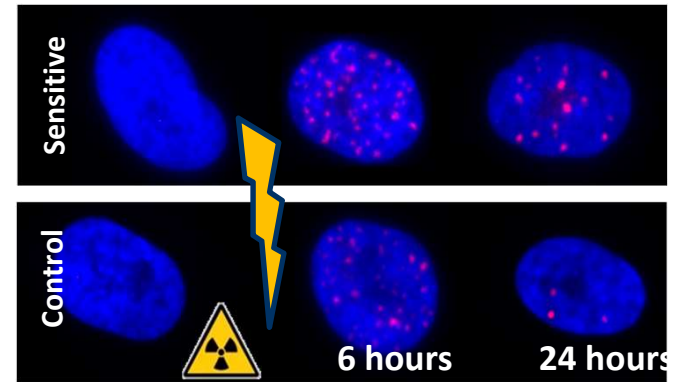
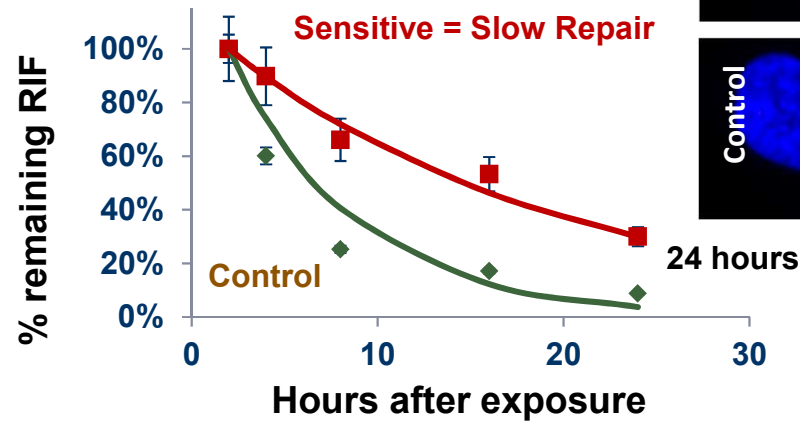
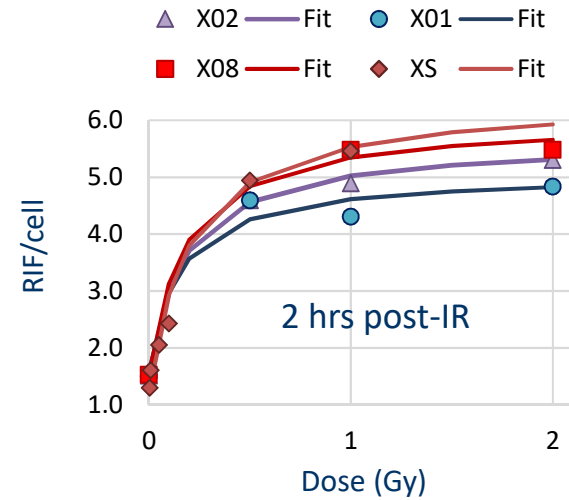
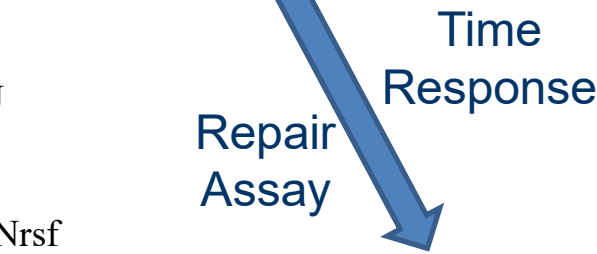
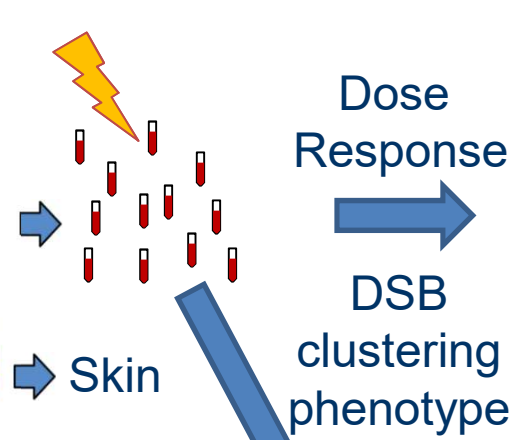
Low dose DOE parallelogram approach adapted for blood assay

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^{1*}, Artem Ponomarev^{2,3}, James L. Chen¹, David Nguyen¹, Francis A. Cucinotta²,
Mary Helen Barcellos-Hoff¹

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

2012

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

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

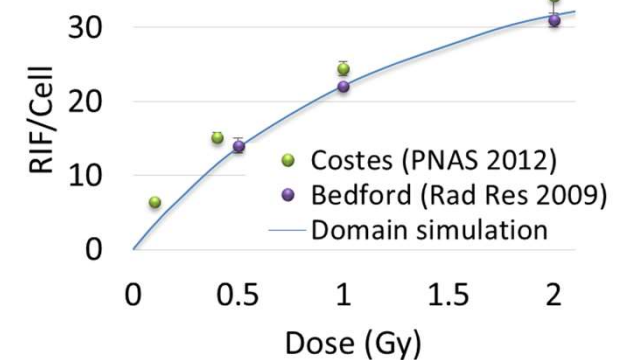
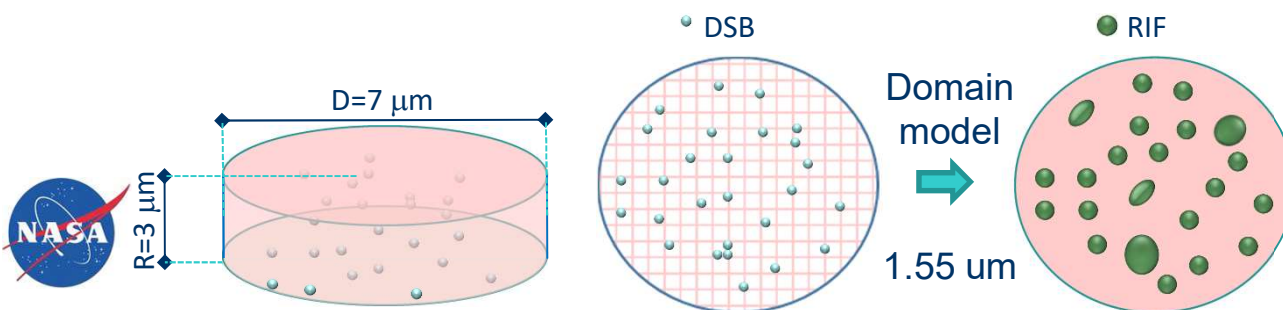
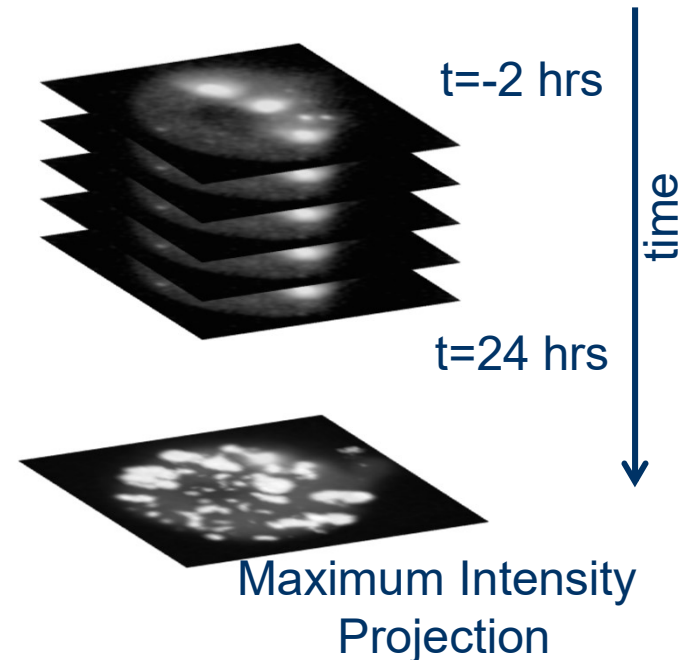
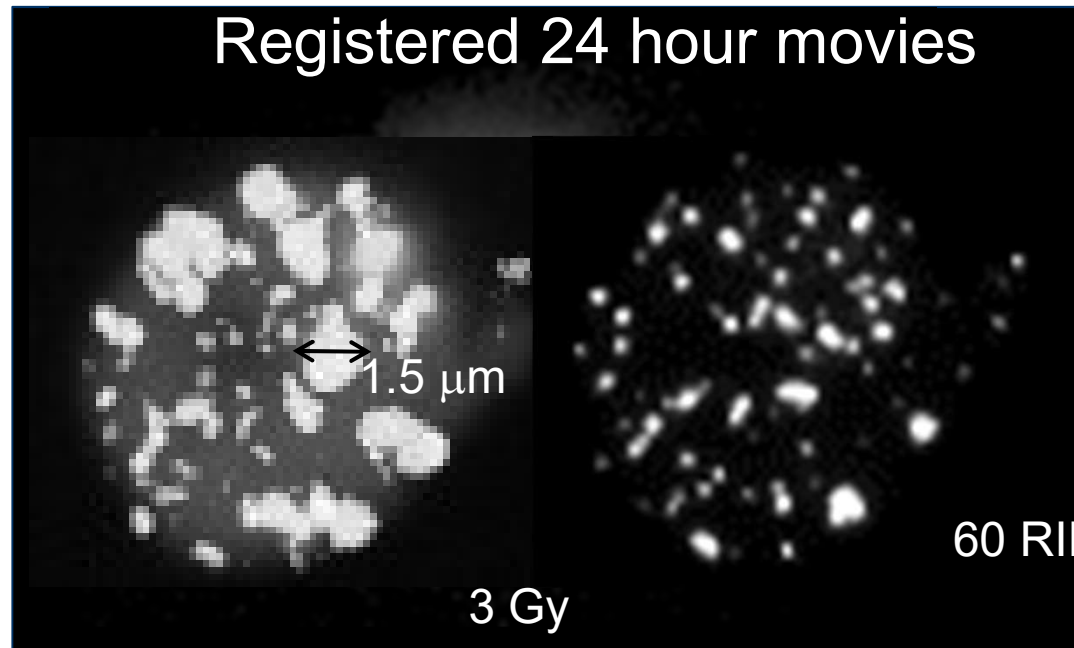
Rad Res 2014

Nikhil Vadhavkar,^a Christopher Pham,^b Walter Georgescu,^c Thomas Deschamps,^c Anne-Catherine Heuskin,^d
Jonathan Tang^c and Sylvain V. Costes^{c,1}

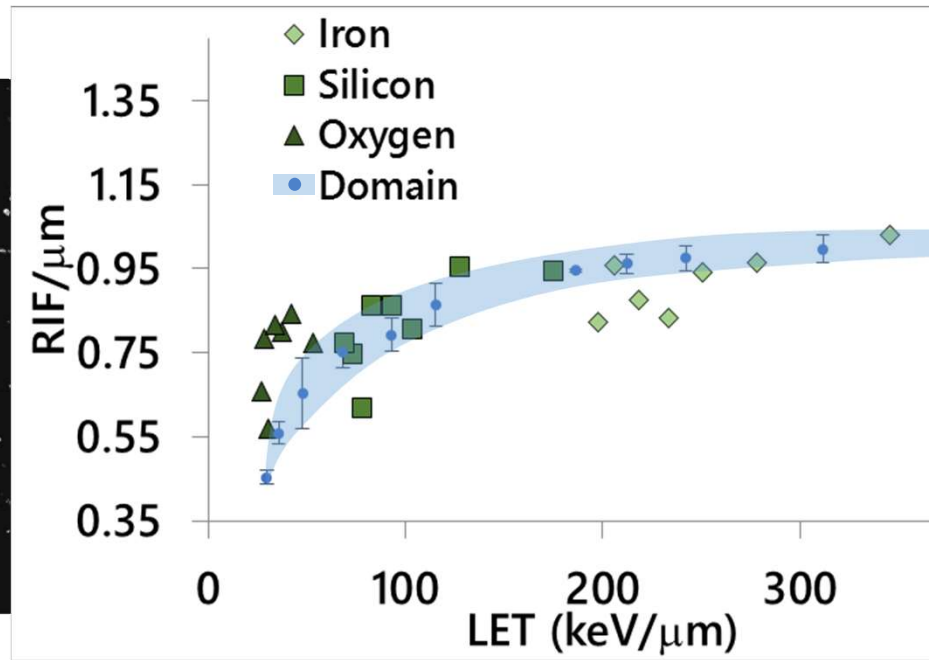
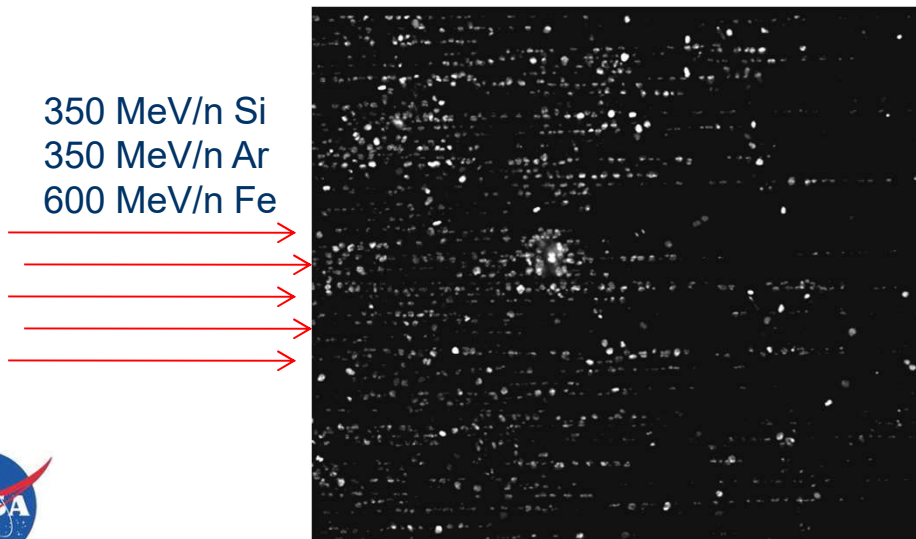
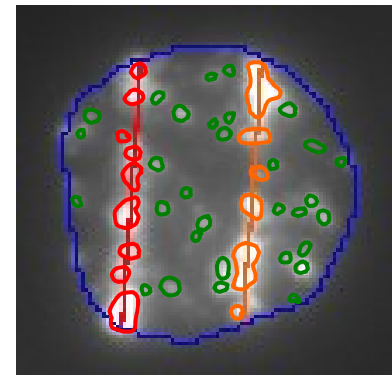
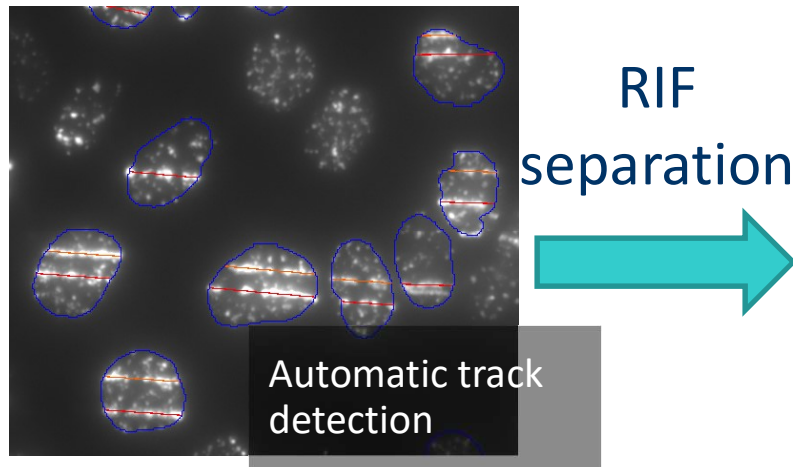


Georgescu et al., PLoS One 2015

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

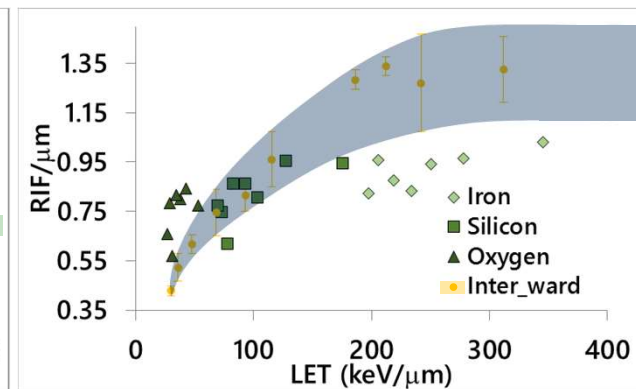
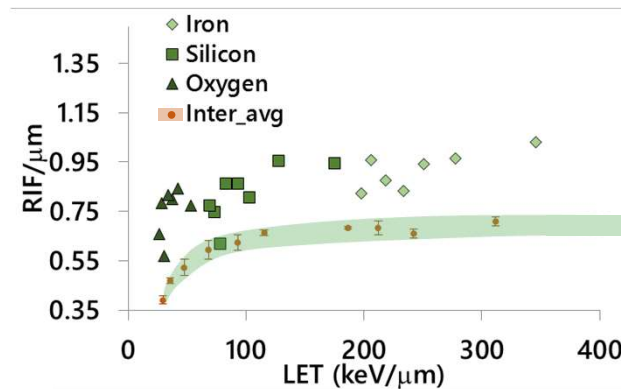
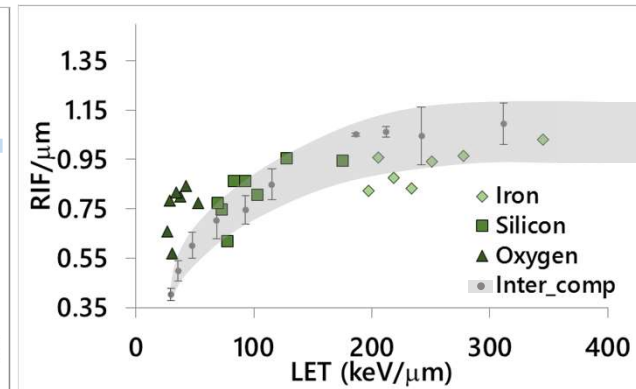
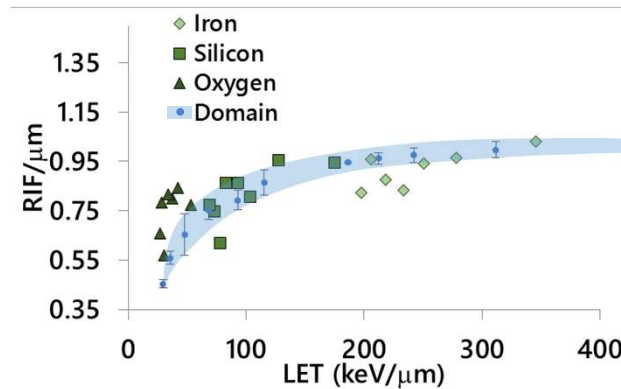
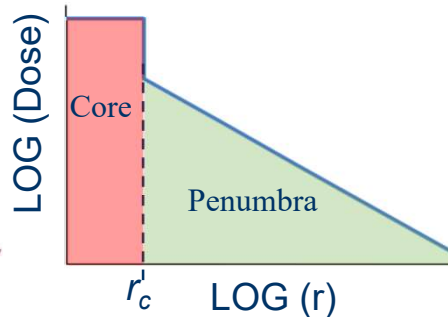
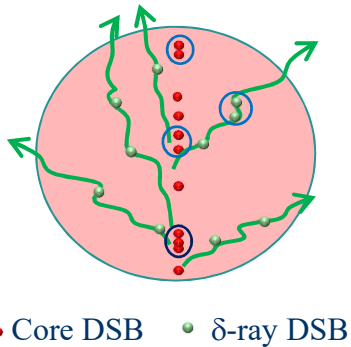
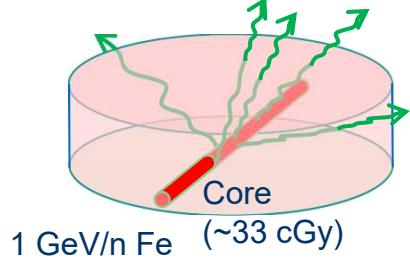


Local dose effect is demonstrated with high LET tracks



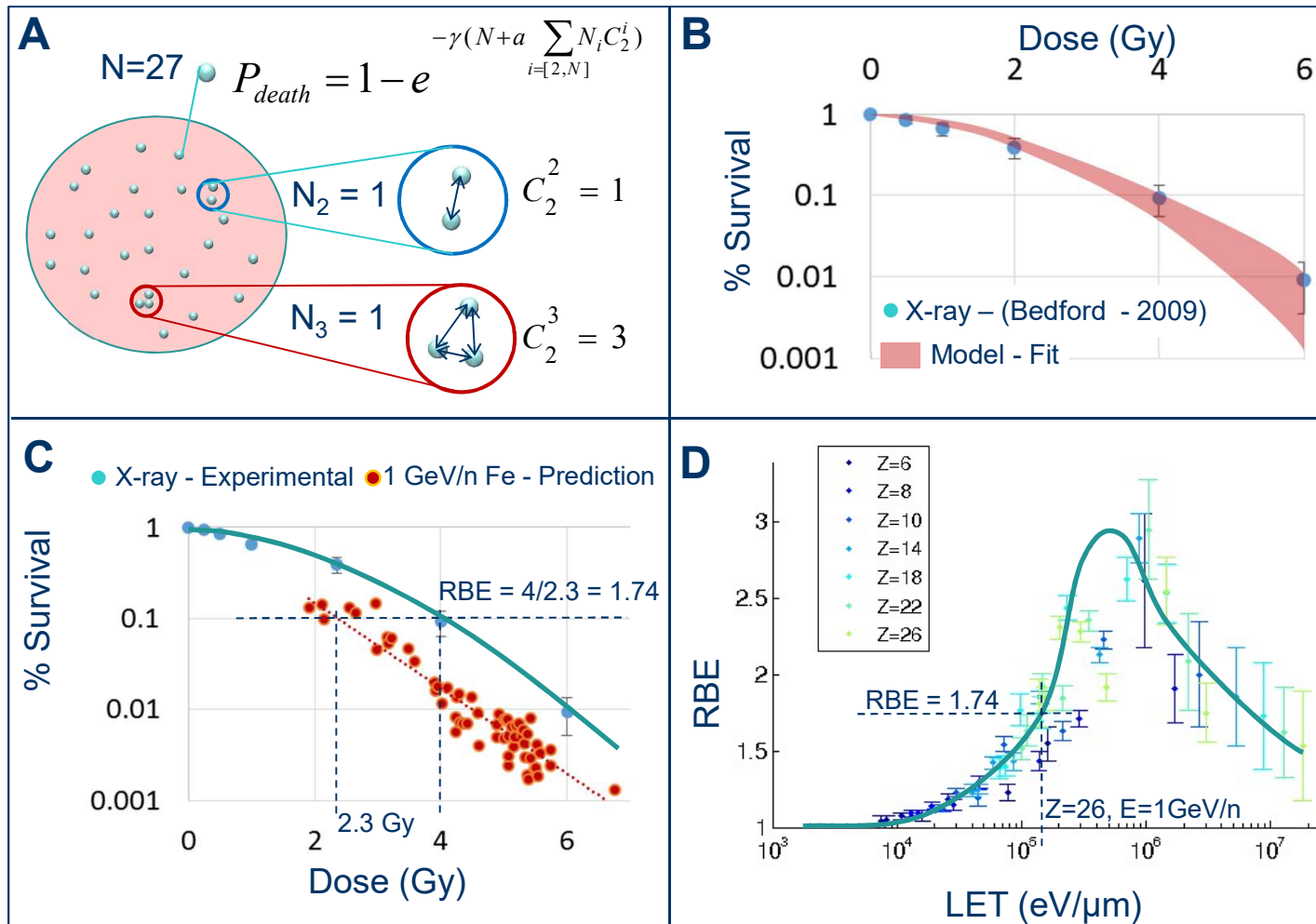
Prediction of RIF yield along high-LET tracks

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

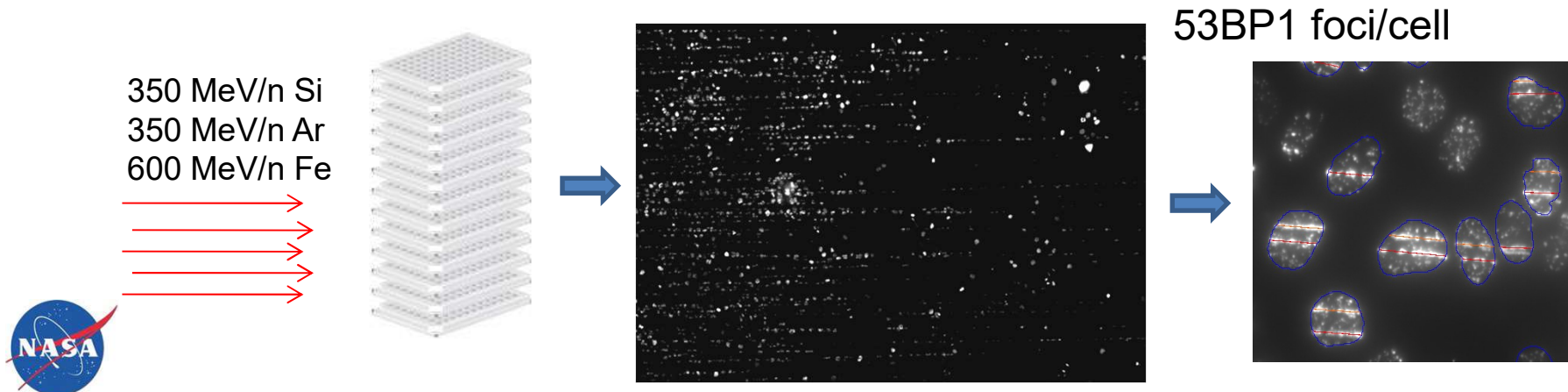
- Strains of animals
- Human PBMC (Year 2 and 3)

Variables

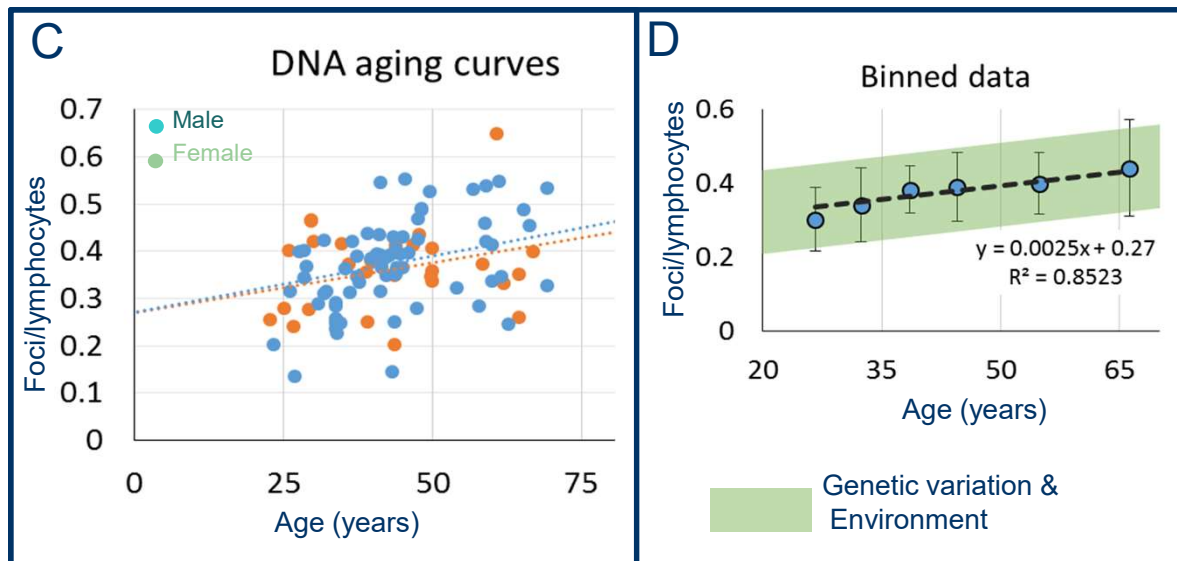
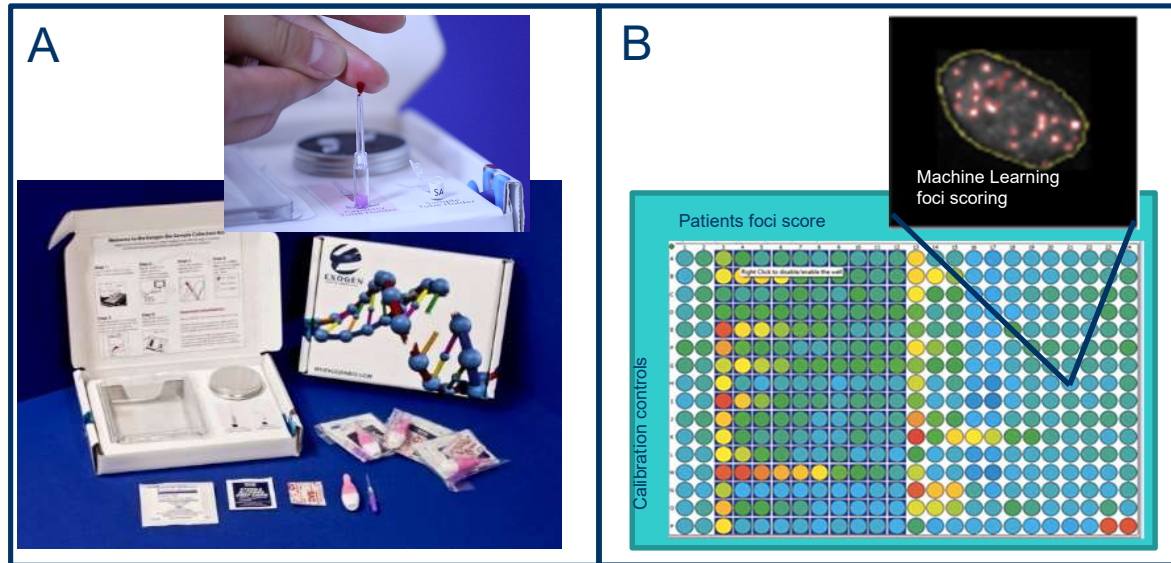
- LET
- Dose
- Time post-IR

Measurements

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

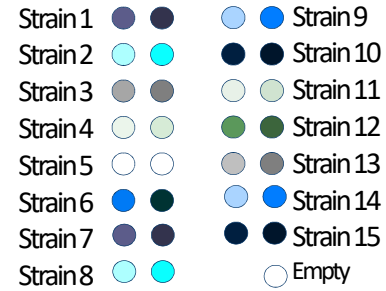
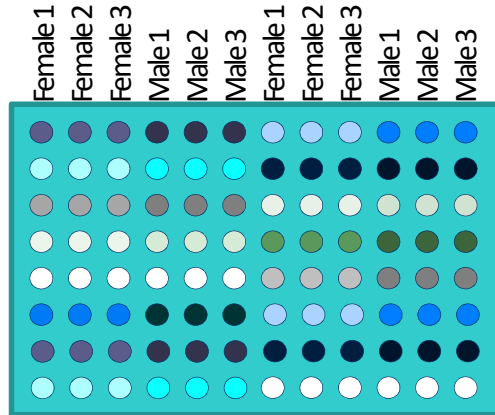


DNA DSB assay – Collaboration with industry



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 μm^2

3 Particles/100 μm^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

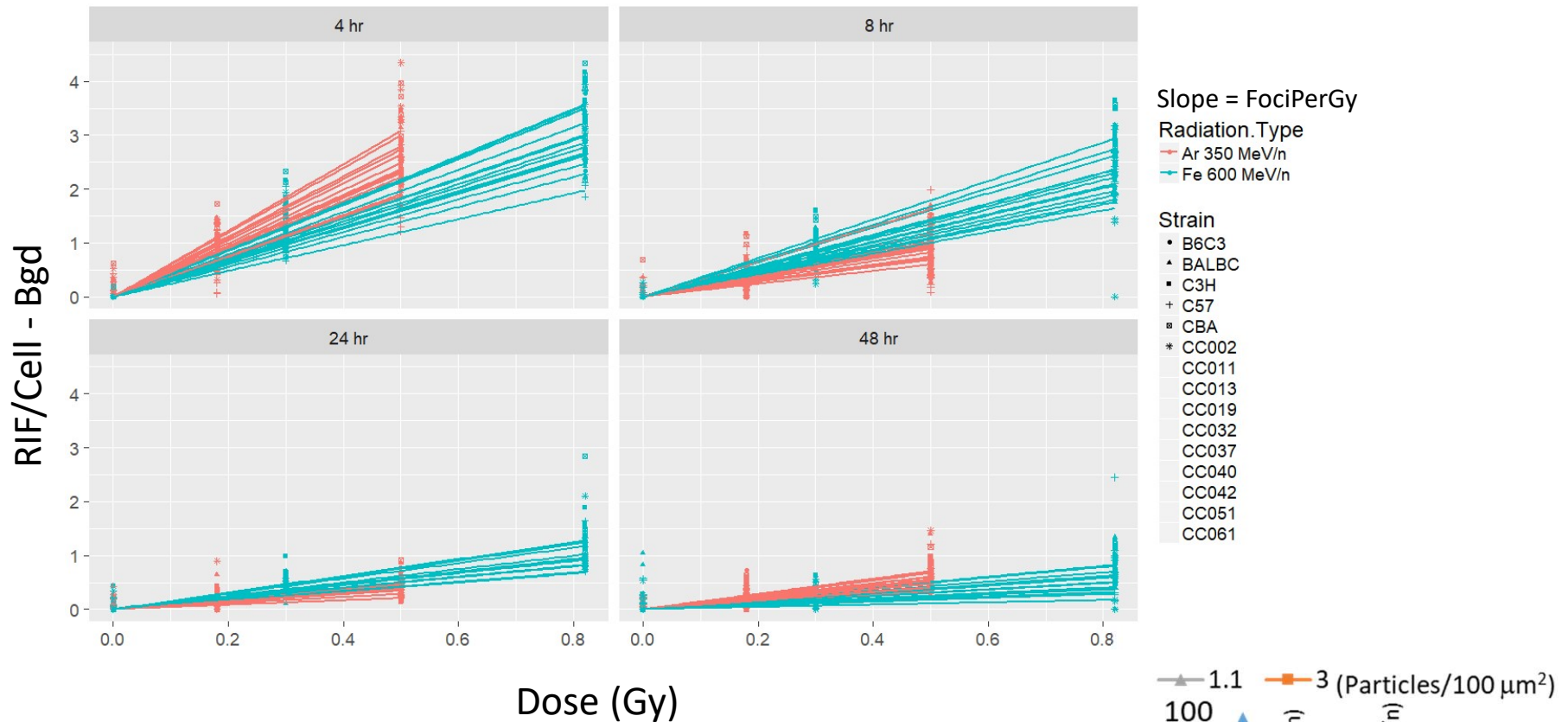
12+8

12+8

72 plates

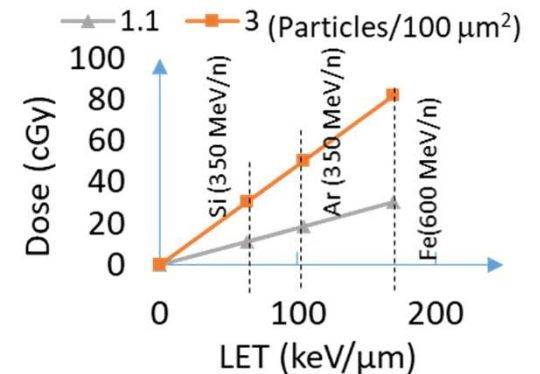
RIF/Cell vs dose for high-LET always linear

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

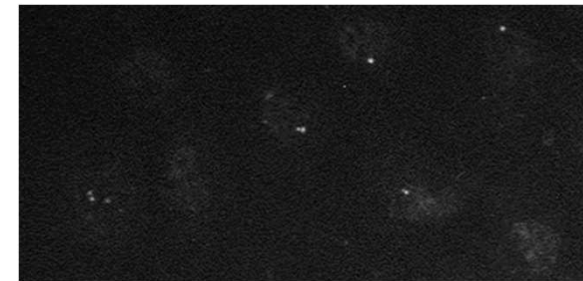
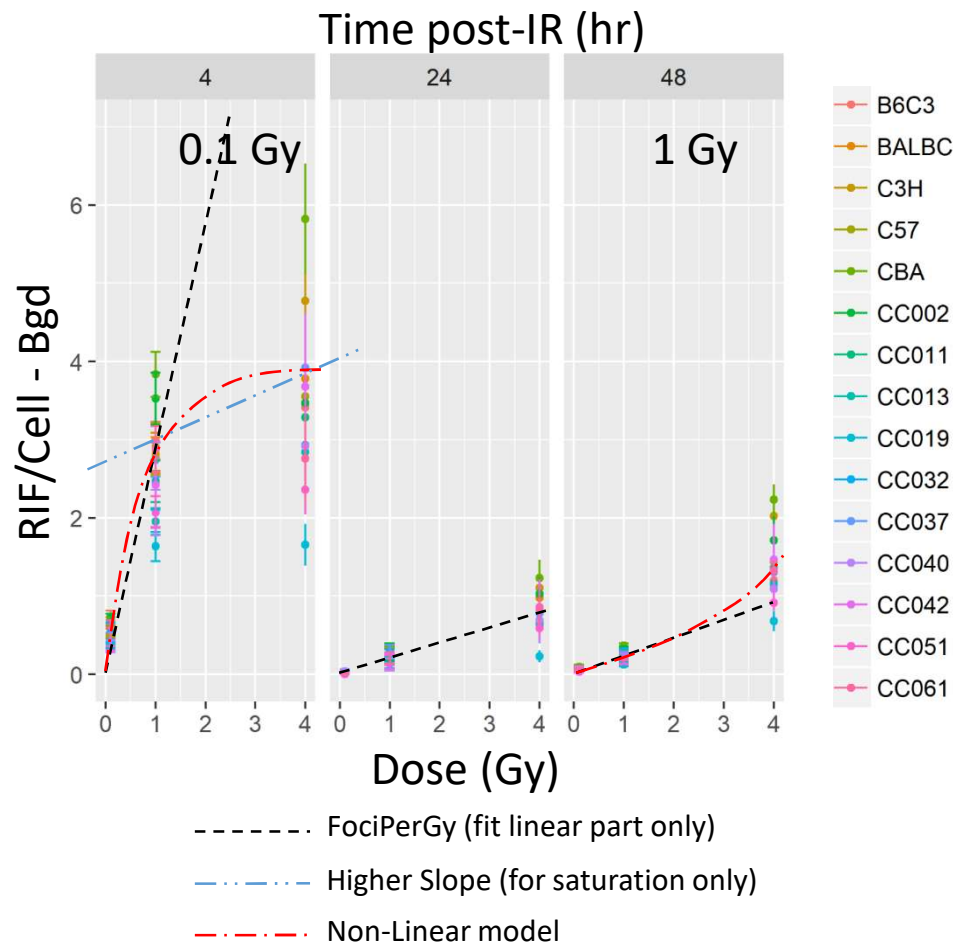


$$\text{RIF/cell} = \text{Bgd} + \text{Fluence (track/area)} \times \text{Volume} \times \text{RIF}/\mu\text{m}(\text{LET \& cell dependent})$$

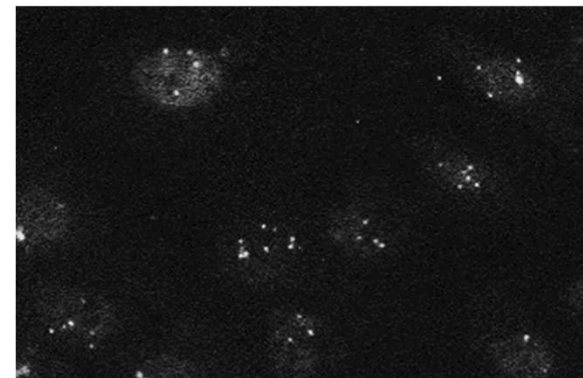
$$\text{RIF}/\mu\text{m}(\text{LET \& cell dependent}) = (\text{RIF/cell} - \text{Bgd}) / \text{Fluence (track/area)} / \text{Volume}$$



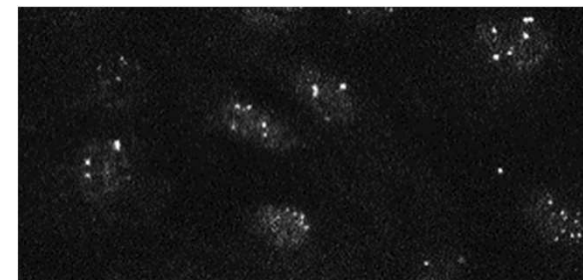
Low-LET dose dependence is a function of time post-IR



0.1 Gy

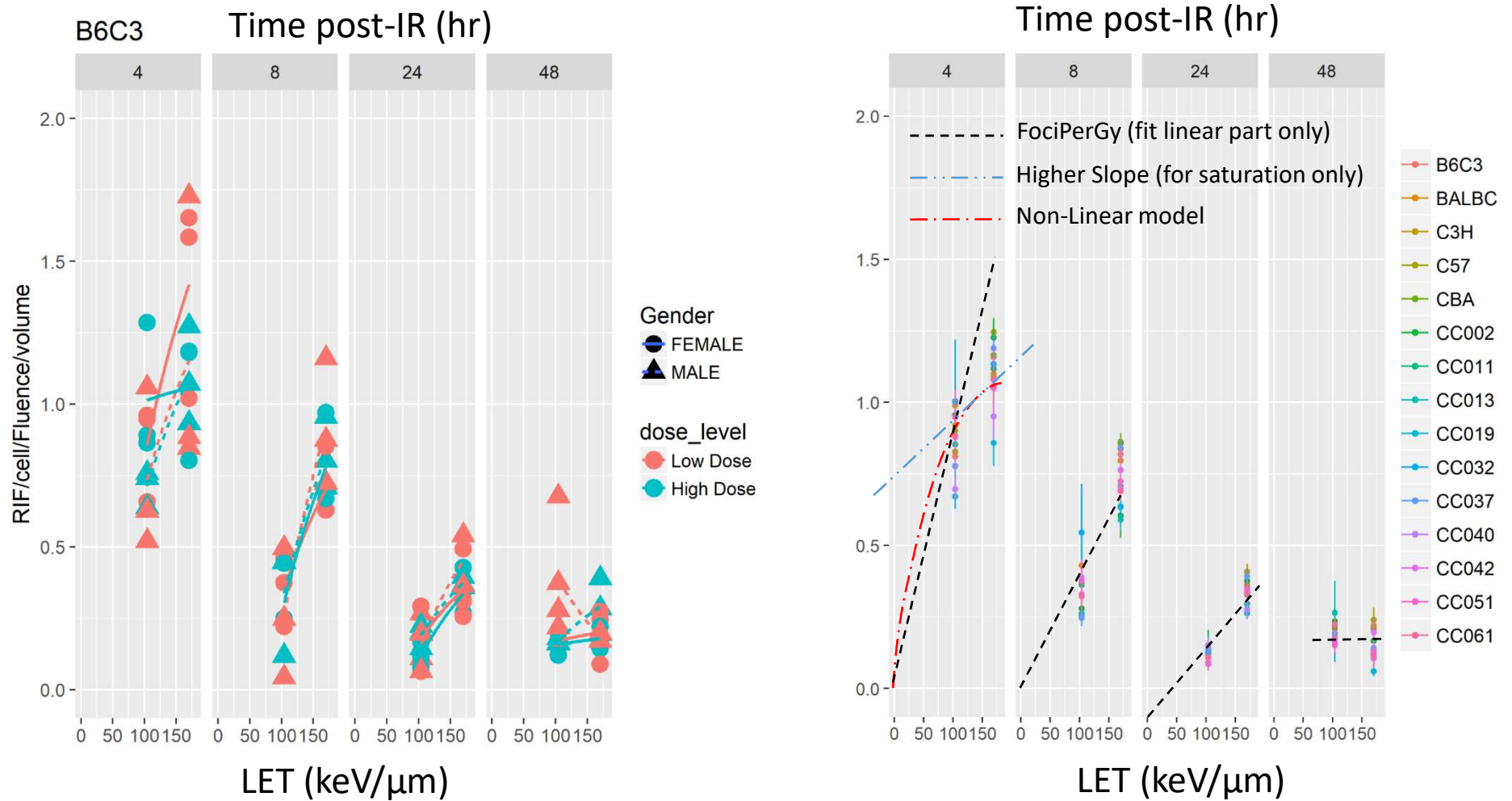


1 Gy



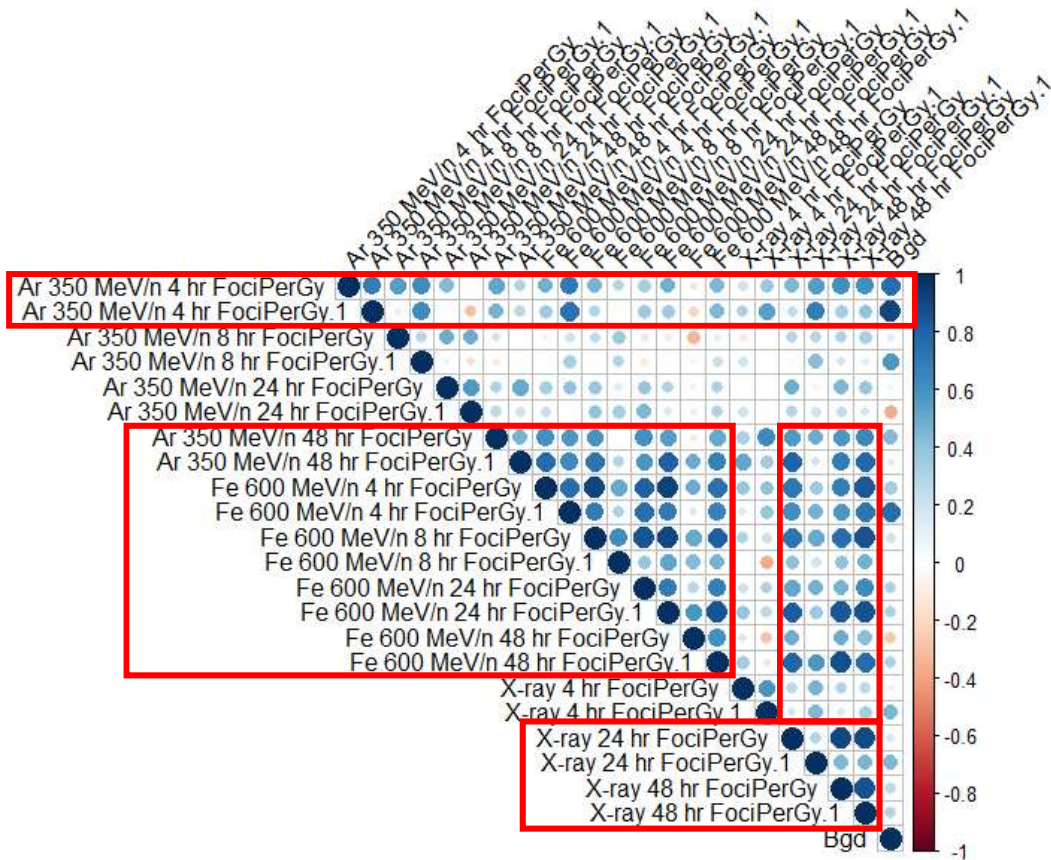
4 Gy

RIF/ μm dependence saturates for increasing LET at early time point

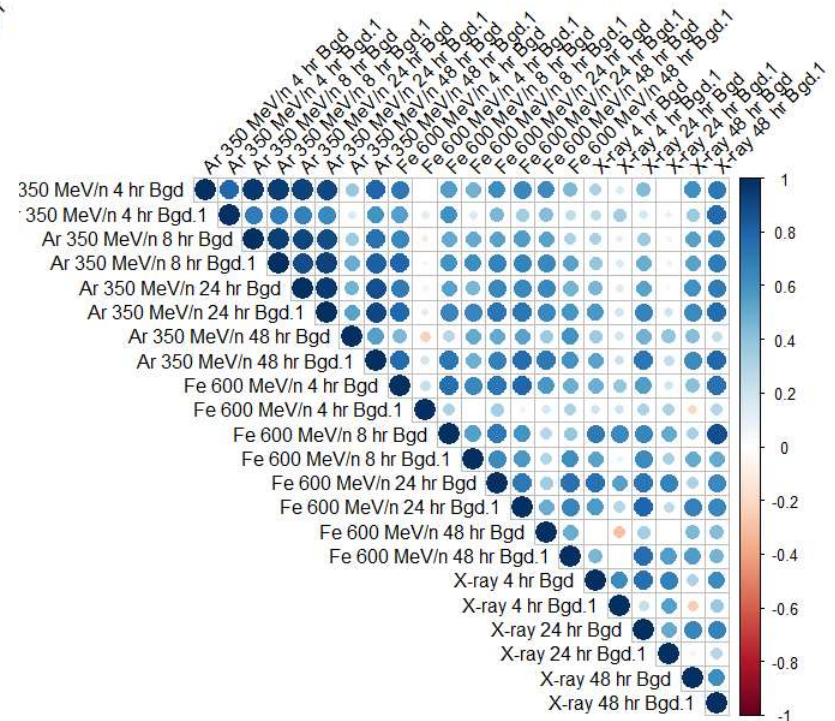


Correlation Graph – Slope and Background

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

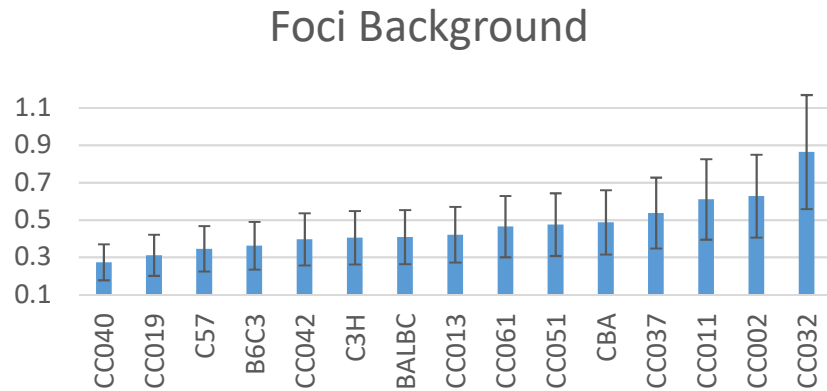


RIF/Gy
(Slope)

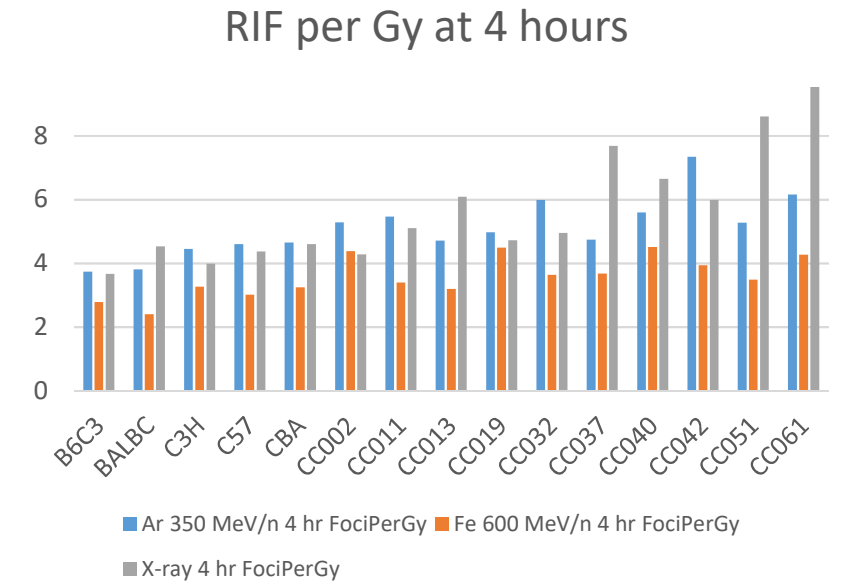
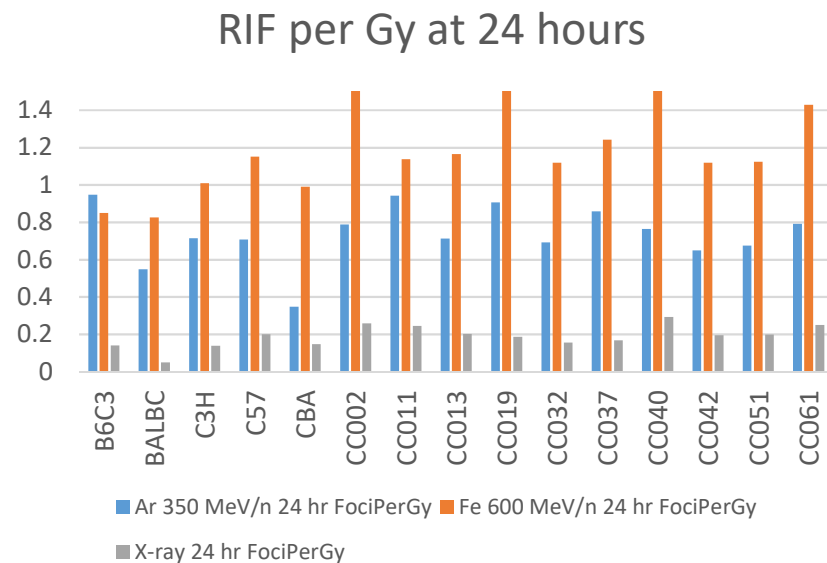


Background Foci
(Slope intercept)

DSB detection sensitivity is driven by genetic



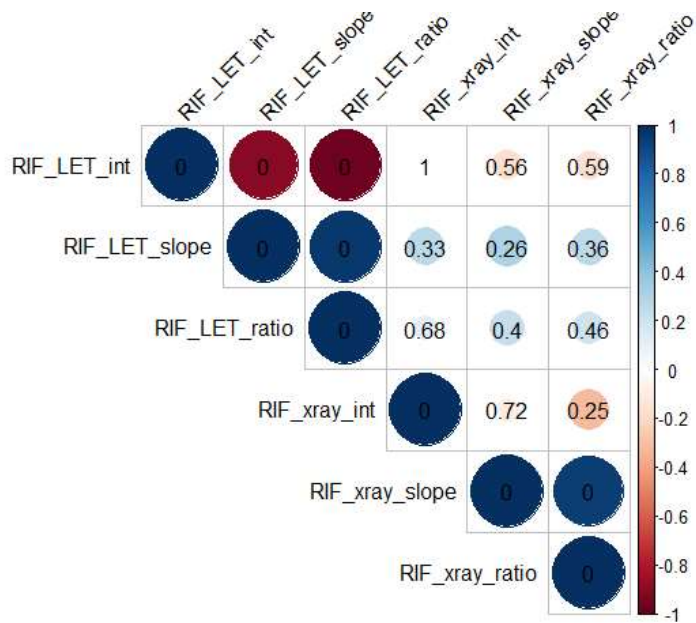
- Different ranking for background



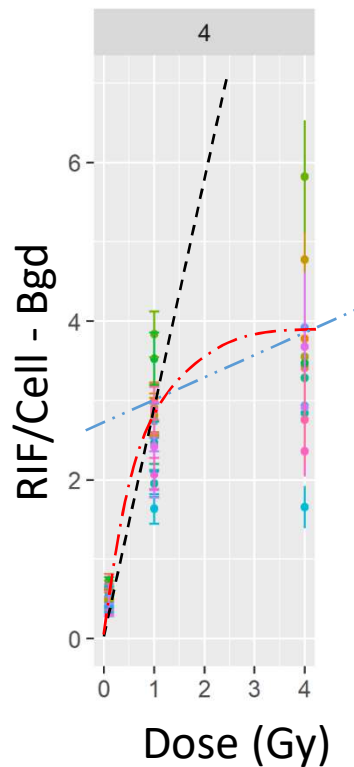
- The higher the LET, the more clustering
- Reference mice show lower detection

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

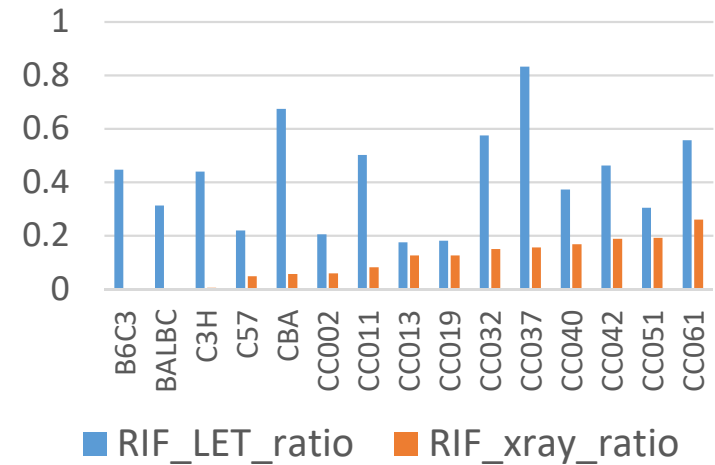
Saturation parameters



Time post-IR (hr)

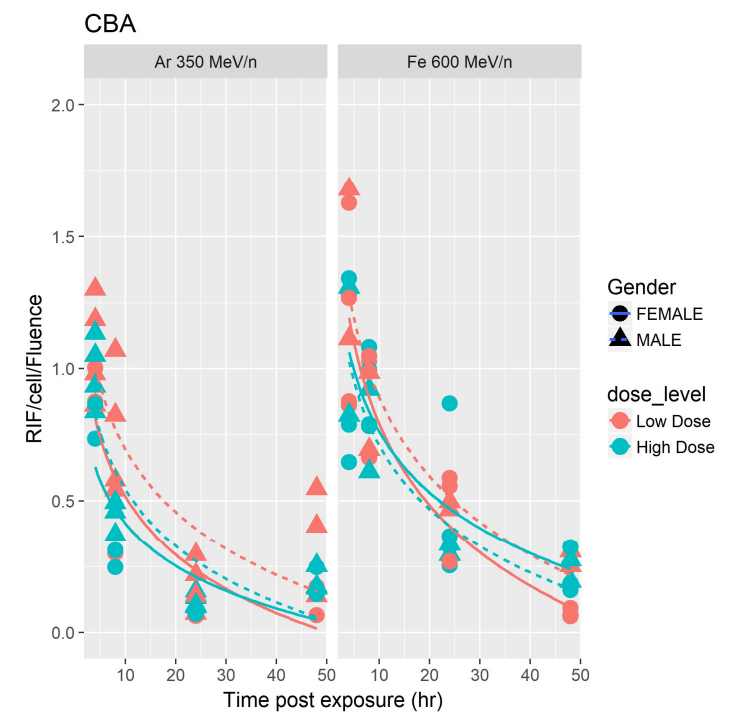
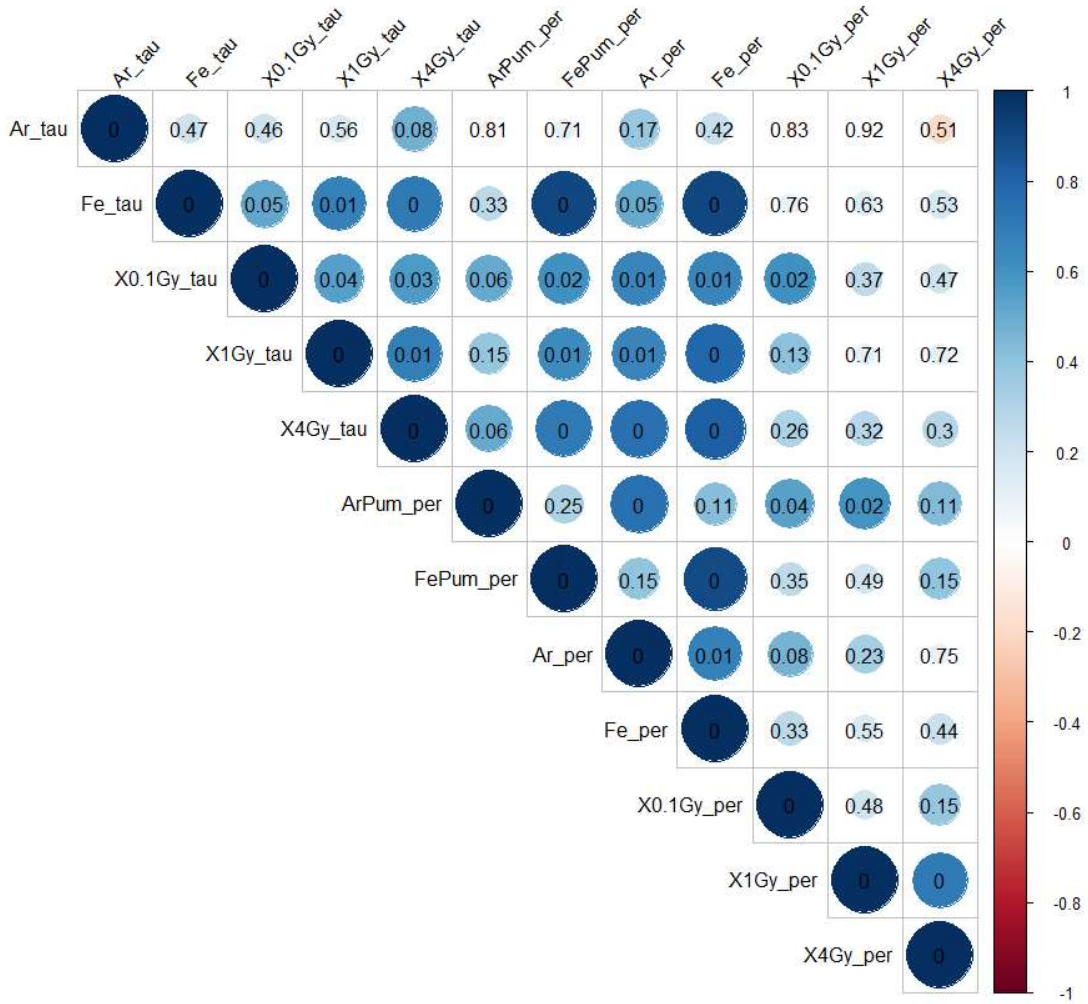


Saturation: Slope Ratio



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

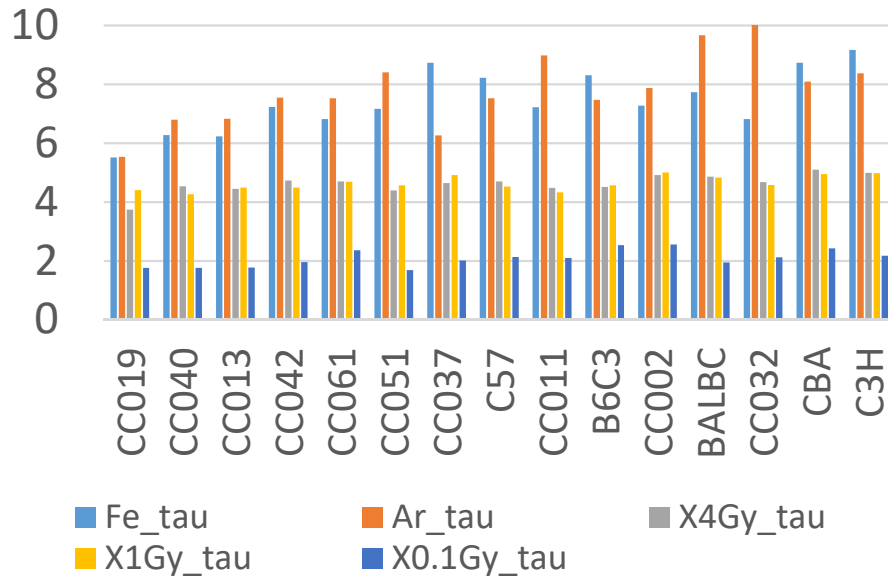
Kinetic parameters



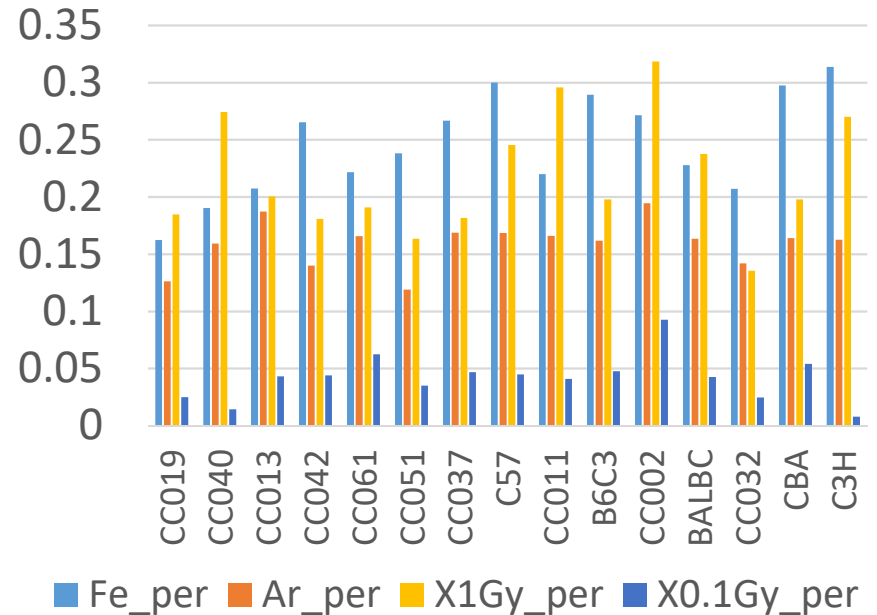
- Persistent RIF at 24 and 48 hours (RIF/um noted FePum_Per for Fe, RIF/cell noted Fe_per)
- Exponential fit: $RIF/um = RIF/um(0).EXP(-t/tau)$
- $RIF/um(0) = LET(keV/um)/80$ for LET, and $RIF(0) = 35.Dose(Gy)$ for X-ray
- Excluding 4 hr, 4 Gy for X-ray

Repair kinetics and Persistent Rf

Repair kinetic (in hours)



Persistent (RIF/um or RIF/cell)



Lethal dose for X-ray exposure (Gy)

Strain	LD _{50.30} +/- 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)

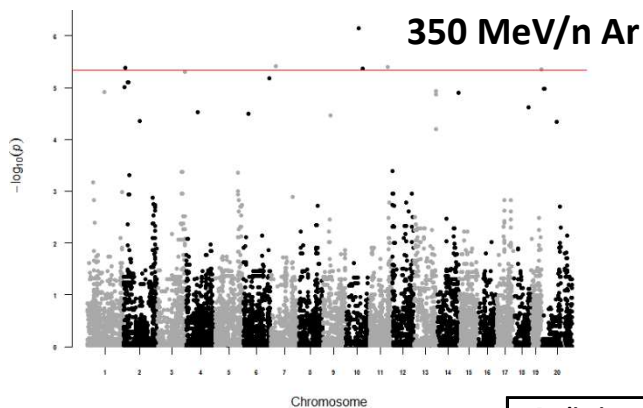
<http://www.informatics.jax.org/greenbook/chapters/chapter22.shtml>

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

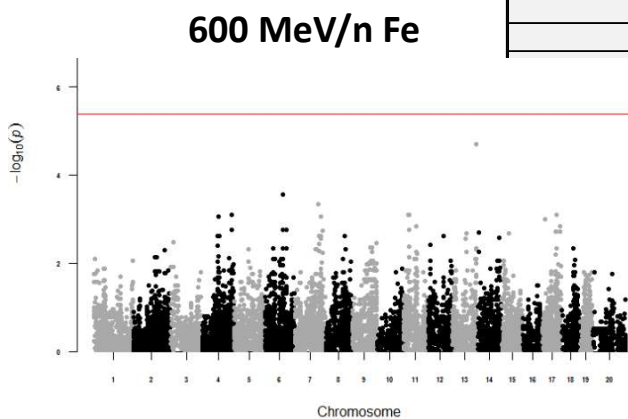
Link to in-vivo (whole blood response in CC mice – Mao/Snijders LBL Low Dose DOE)

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	7.22×10^{-7}	0.333	71805029 - 83277275	209
	10	JAX00021248	96091724	4.24×10^{-6}	0.2857	96070217 - 96887381	2
	11	UNC20271233	102605060	4.03×10^{-6}	0.2857	101434884 - 103096965	49
	19	JAX00481854	60388505	4.5×10^{-6}	0.2857	60321655 - 61168855	15
	2	UNC2559552	10174335	4.2×10^{-6}	0.1429	10174335 - 10388678	2
	3	UNC6555279	152286637	4.9×10^{-6}	0.2857	152132653 - 152805055	11
	7	UNC12559788	30462618	3.9×10^{-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	7.22×10^{-7}	0.333	71805029 - 83277275	209
	13	UNC23495258	119997660	1.5×10^{-8}	0.2667	119997660 - 120160384	1
		UNC23497196	120160384	9.5×10^{-8}	0.07143	119997660 - 120160385	0



Radiation	CHR	SNP	P value	Gene Symbol	Function
350 MeV/n Ar	10	UNC18214722	7.22×10^{-7}	Smarb1	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	4.24×10^{-6}	Btg1	DNA-damage inducible, anti proliferative
	11	UNC20271233	4.03×10^{-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



THANK YOU

COLLABORATORS:

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US: Mike Weil, Mary Helen Barcellos-Hoff

INSERM-FRANCE: François Paris

Exogen Biotechnology Inc.: Jonathan Tang

FUNDING: NASA HRP, DOE Low dose program

