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

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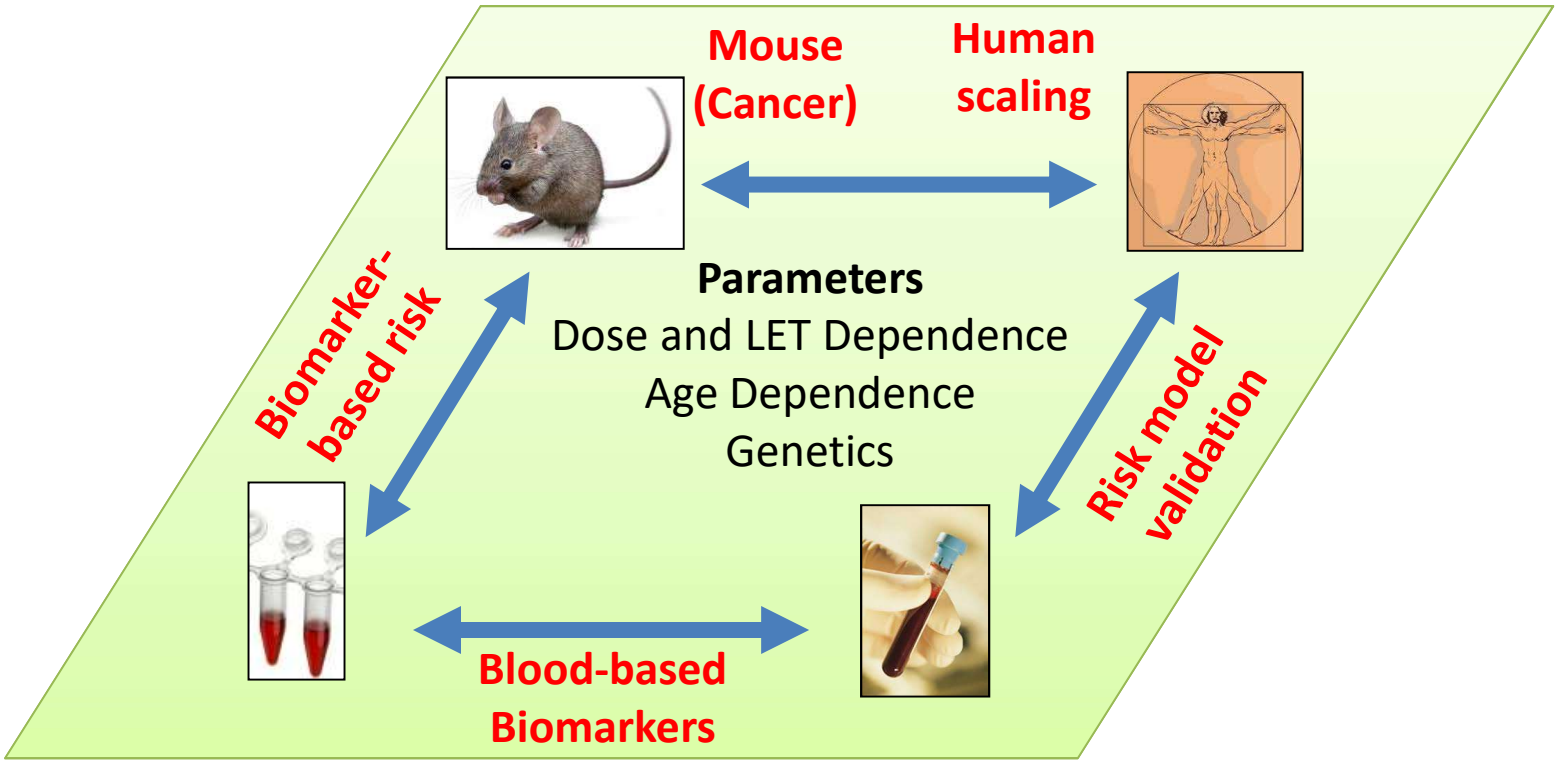
Goal: establish a computer model that will estimate individualized risk for astronauts based on an array of phenotypic and genetic information.

Potential outcome: Individualized risk assessment for Astronauts

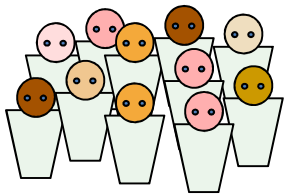
A scalable approach: from mice to human



Low dose DOE parallelogram approach adapted for blood assay

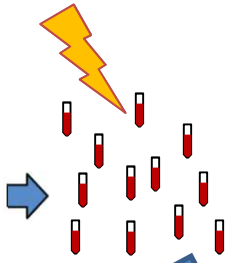
The DNA damage phenotypes

750 humans



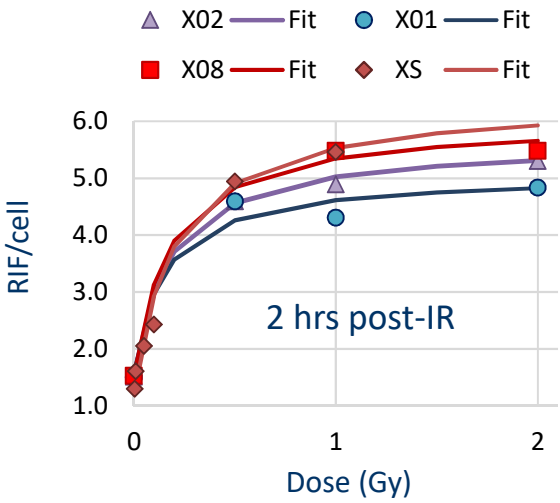
10 CC Strains

Skin



Dose Response

DSB clustering phenotype

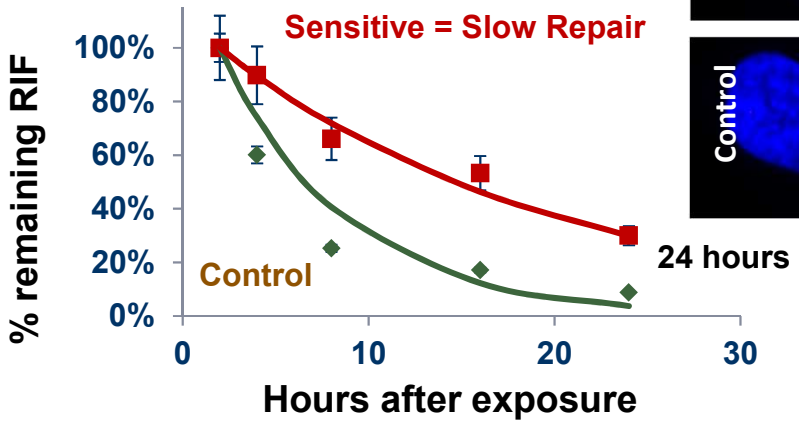


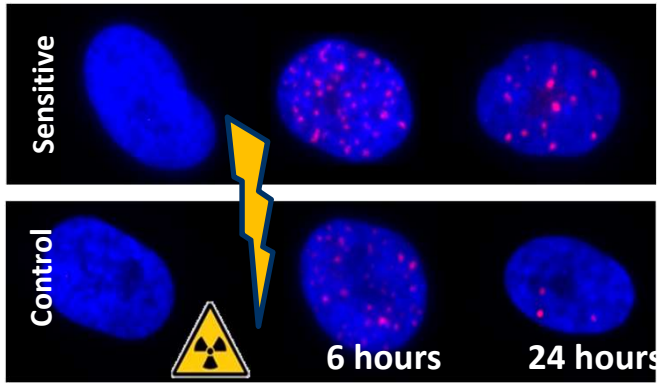
Time Response

Repair Assay

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. Chen[1], David Nguyen[1], Francis A. Cucinotta[2], Mary Helen Barcellos-Hoff[1]

2012

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

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

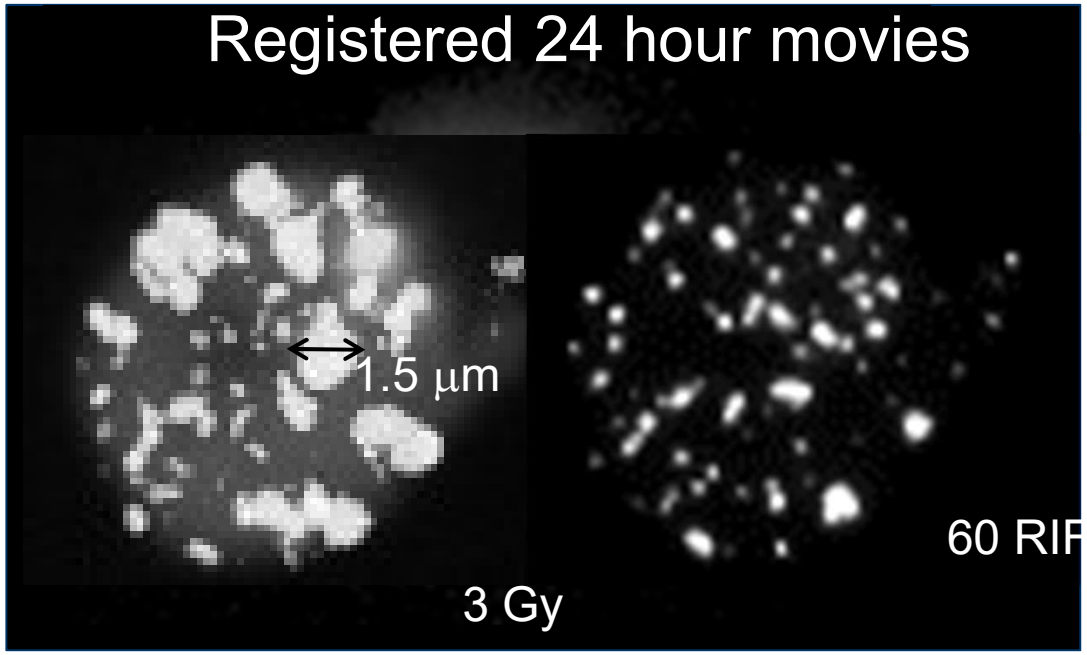
Combinatorial DNA Damage Pairing Model Based on X-Ray-Induced Foci Predicts the Dose and LET Dependence of Cell Death in Human Breast Cells

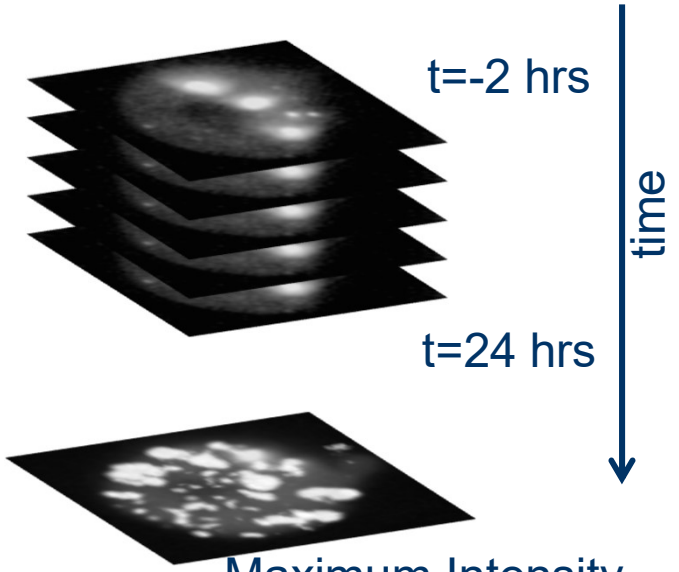
Rad Res 2014

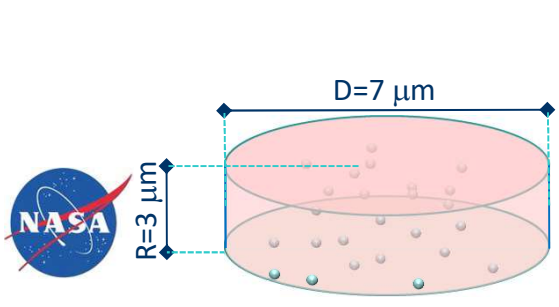
Nikhil Vadhavkar,[a] Christopher Pham,[b] Walter Georgescu,[c] Thomas Deschamps,[c] Anne-Catherine Heuskin,[d] Jonathan Tang[c] and Sylvain V. Costes[c,1]

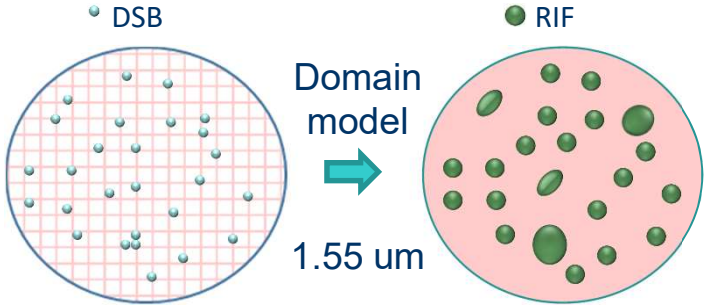
Georgescu et al., PLoS One **2015**

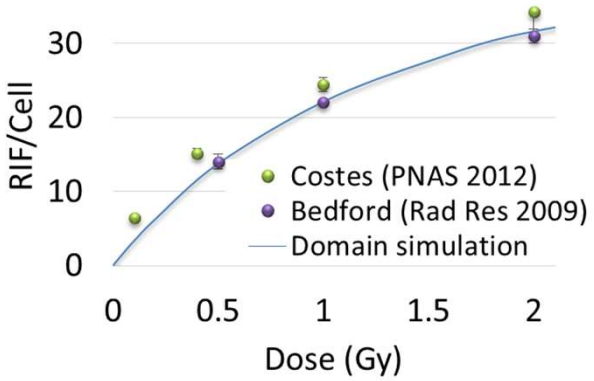
Registration algorithms and maximum projection in time reveals nuclear repair sub-domains



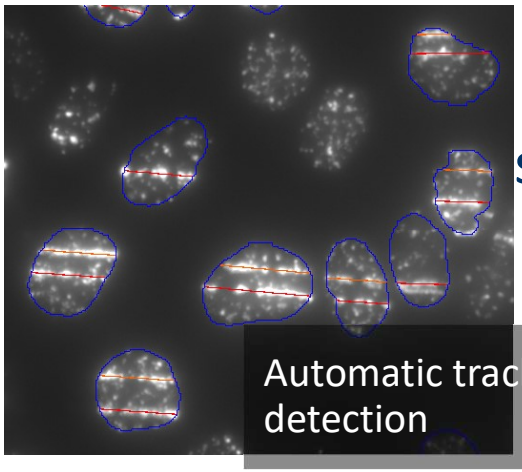




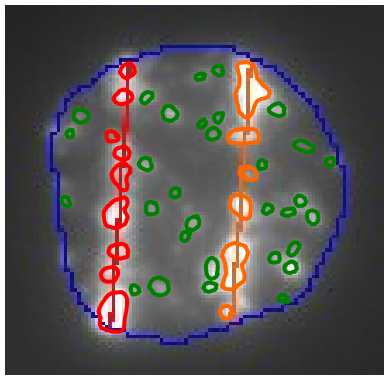




Local dose effect is demonstrated with high LET tracks



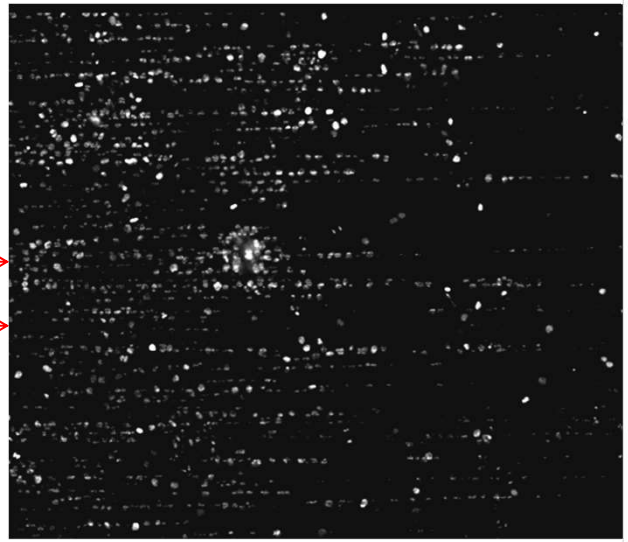
RIF separation

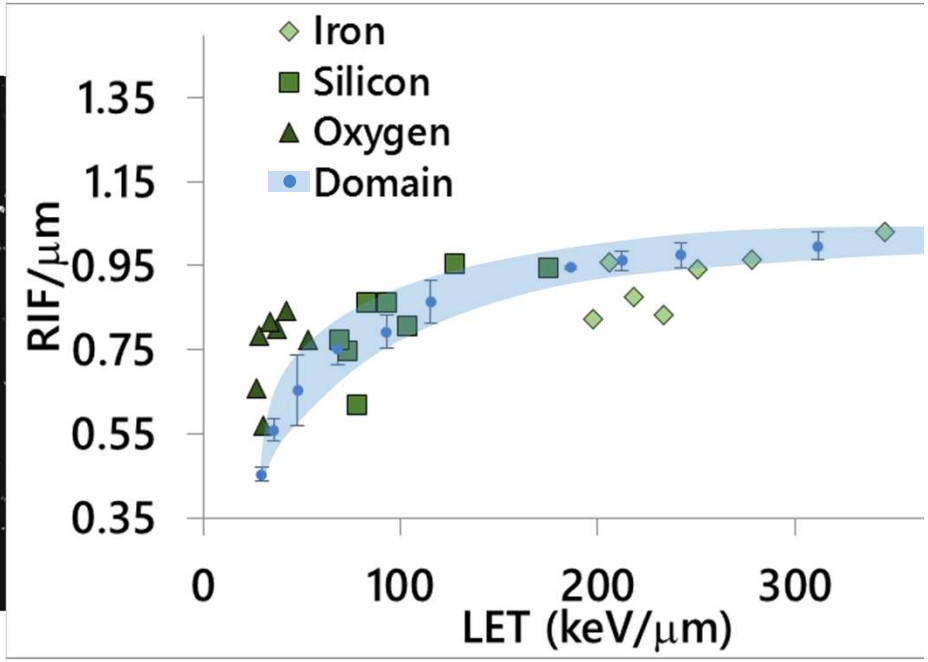


Low-LET component (delta-rays)

High-LET component (core+high E electrons) R=0.4 μm

350 MeV/n Si
350 MeV/n Ar
600 MeV/n Fe





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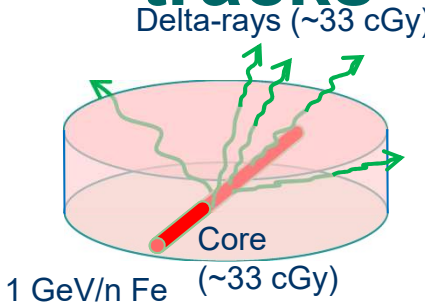
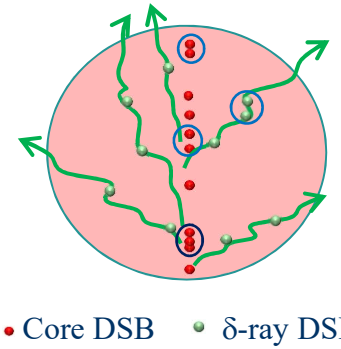
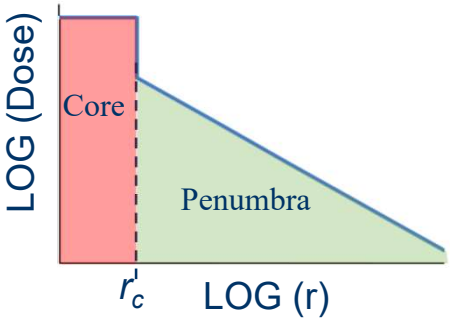
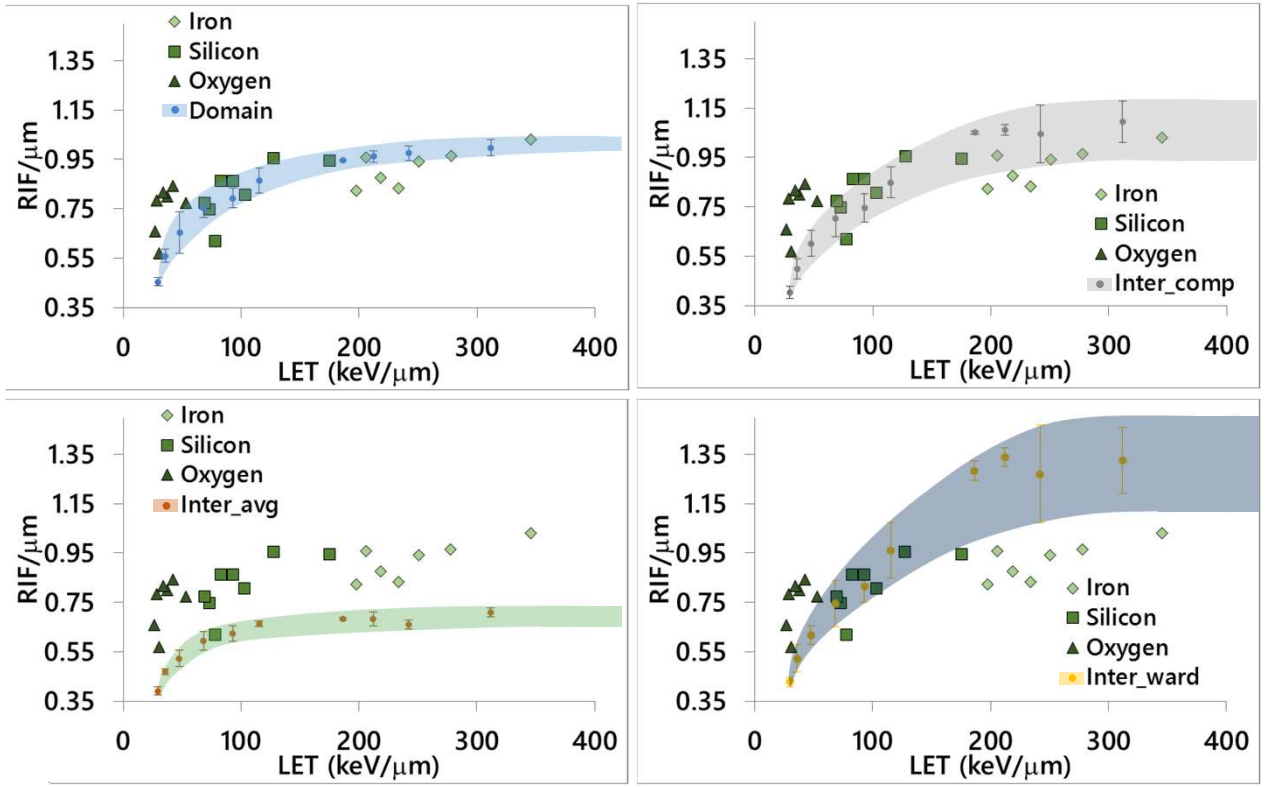
Prediction of RIF yield along high-LET tracks









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

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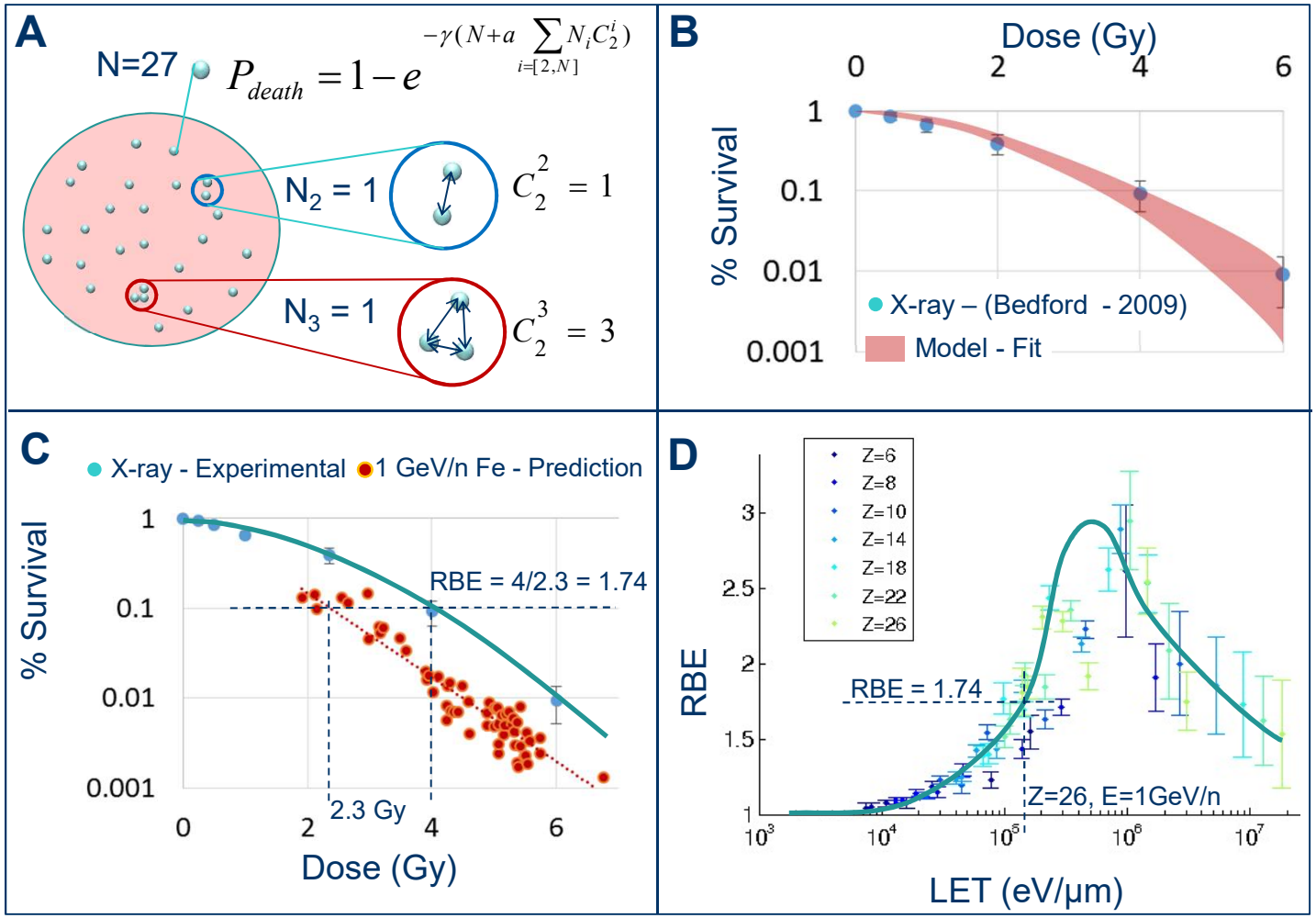
MC model of DSB clustering lead to higher death at higher dose or LET



Similar to LEM model from GSI

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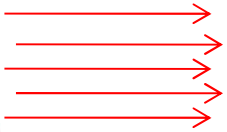
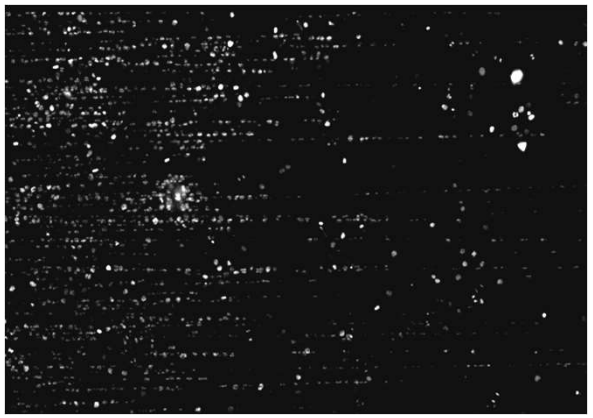
Particle of interest for NASA:
C, O, Ne, Si, Ar, Ti, Fe

Experimental Design

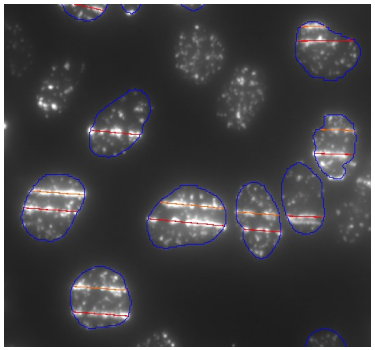
Constants	Variables	Measurements
• Strains of animals • Human PBMC (Year 2 and 3)	• LET • Dose • Time post-IR	• 53BP1 foci detection as DNA double strand break marker • Repair Kinetic parameters • (power function) • Foci saturation (Asymptotic fit) • Foci Background • 800 cells/condition

350 MeV/n Si
350 MeV/n Ar
600 MeV/n Fe

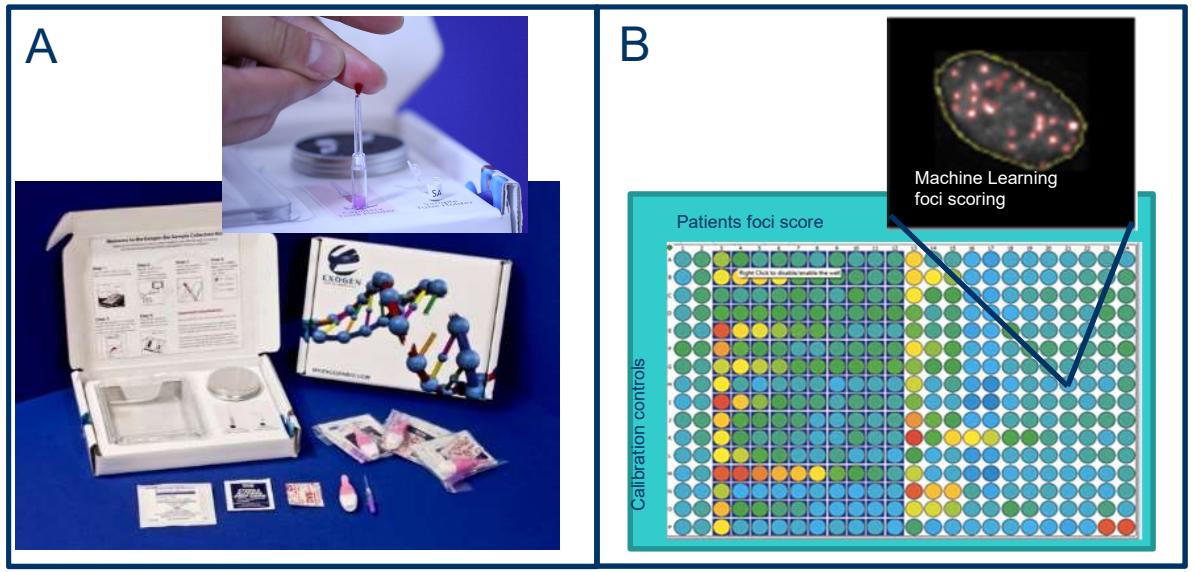


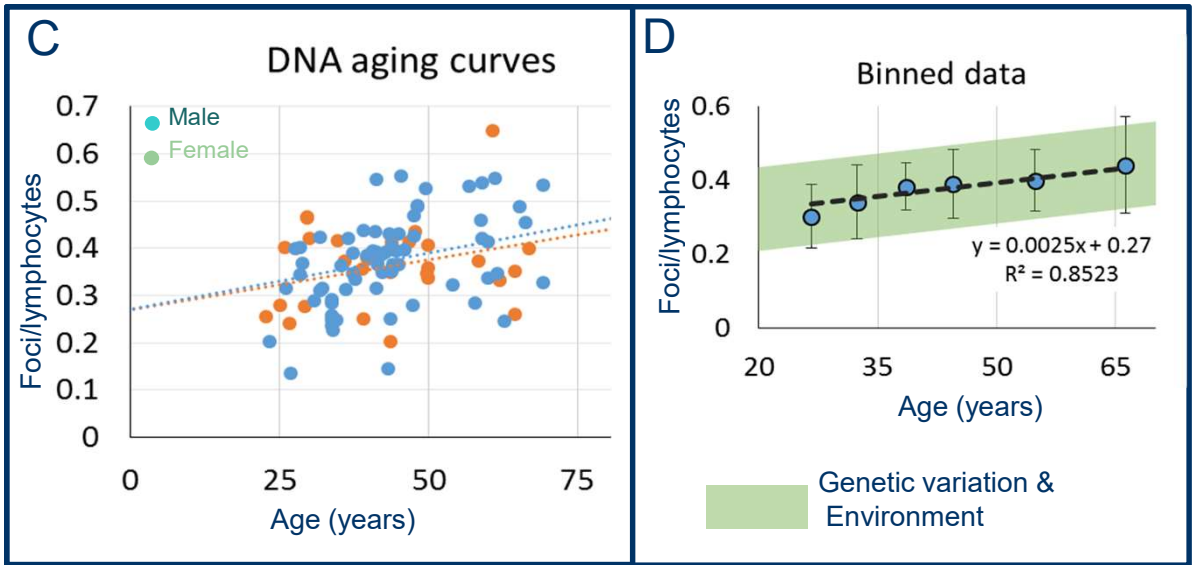


53BP1 foci/cell



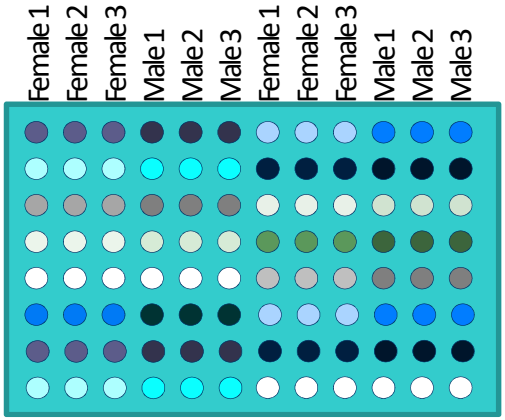
DNA DSB assay – Collaboration with industry





Experimental Design

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



Strain 1
Strain 2
Strain 3
Strain 4
Strain 5
Strain 6
Strain 7
Strain 8
Strain 9
Strain 10
Strain 11
Strain 12
Strain 13
Strain 14
Strain 15
Empty

15 strains
X 3 males
X 3 females

4 **8** **24** **48**

Radiation

BNL

- **High-LET Control**
- **1.1 Particles/100 µm²**
- **3 Particles/100 µm²**

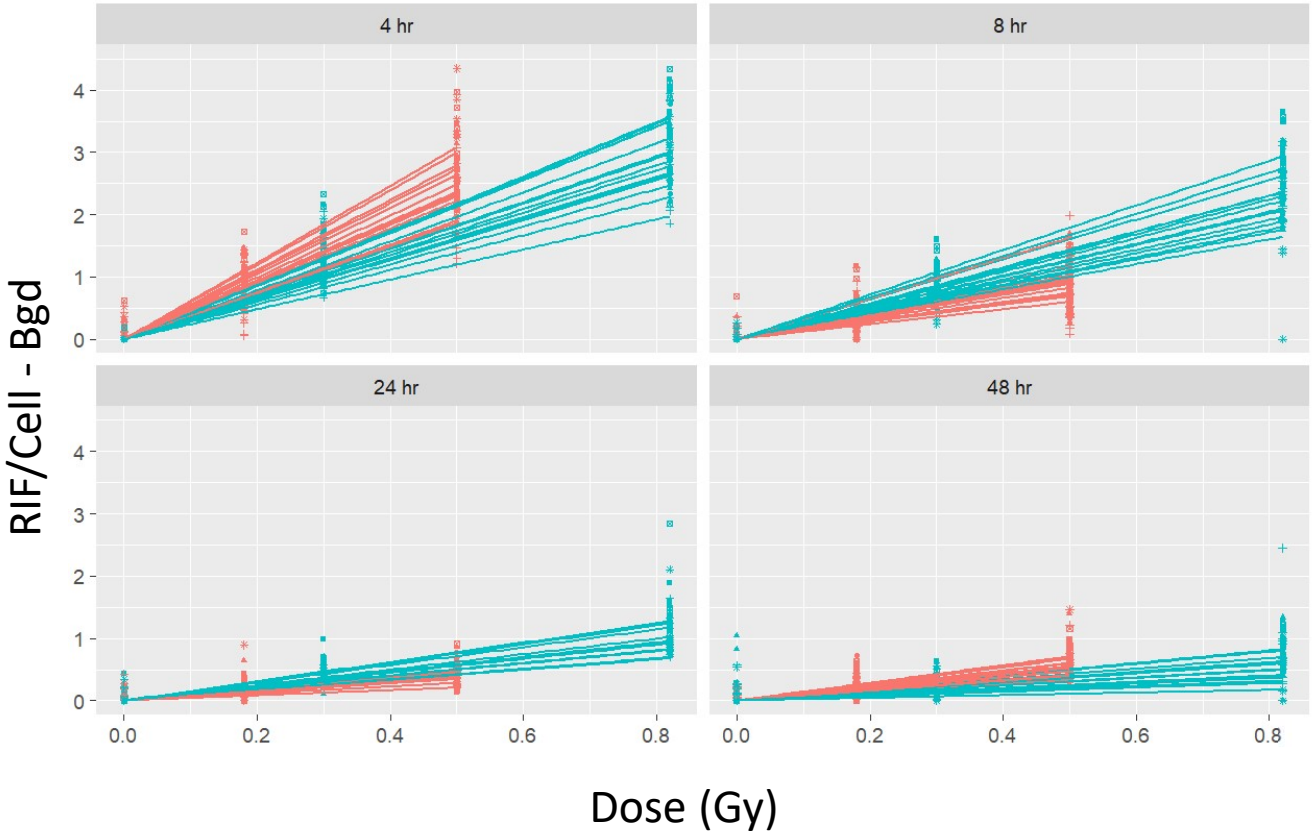
X2 ions

LBNL

- **X ray control**
- **X ray 0.1 Gy**
- **X ray 1 Gy**
- **X ray 4 Gy**

# of 96 well plates (53BP1)	12+8	12	12+8	12+8	72 plates

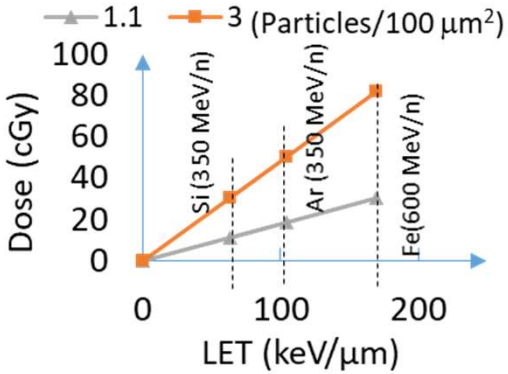
RIF/Cell vs dose for high-LET always linear
(Reflects more track than DSB – See Costes el al, Rad Res 2006)



Slope = FociPerGy

Radiation.Type
- Ar 350 MeV/n
- Fe 600 MeV/n

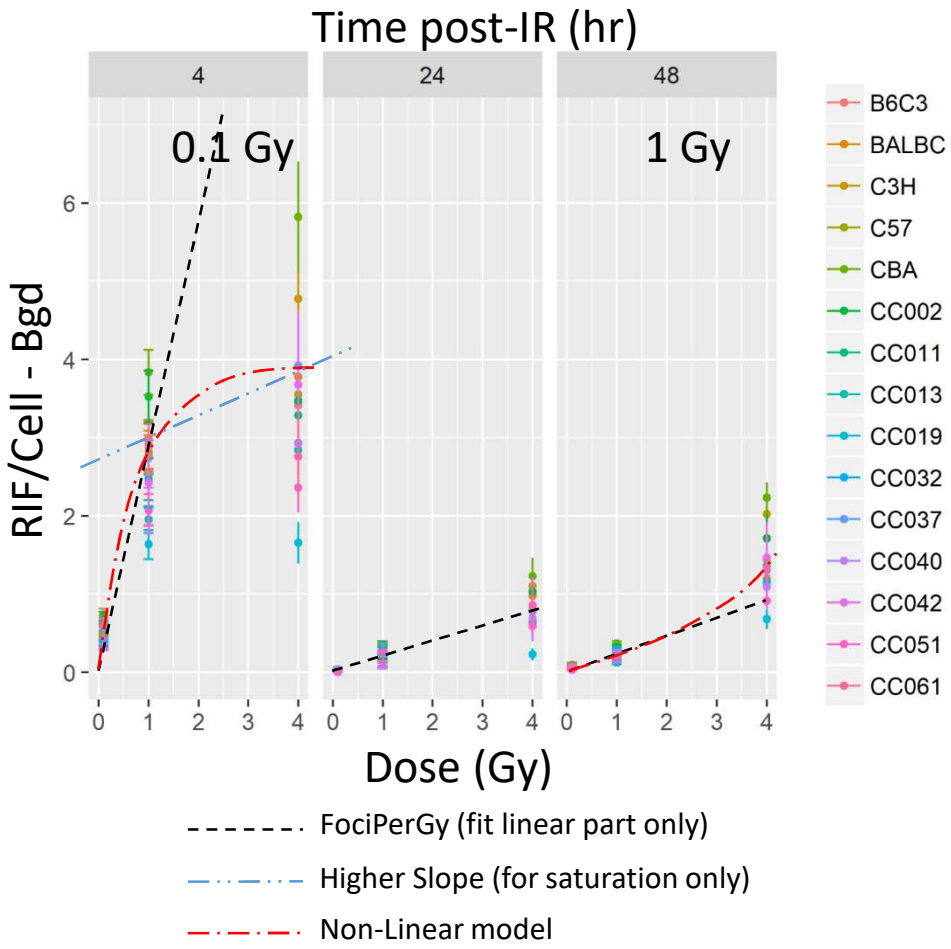
Strain
- B6C3
- BALBC
- C3H
- C57
- CBA
- CC002
- CC011
- CC013
- CC019
- CC032
- CC037
- CC040
- CC042
- CC051
- CC061

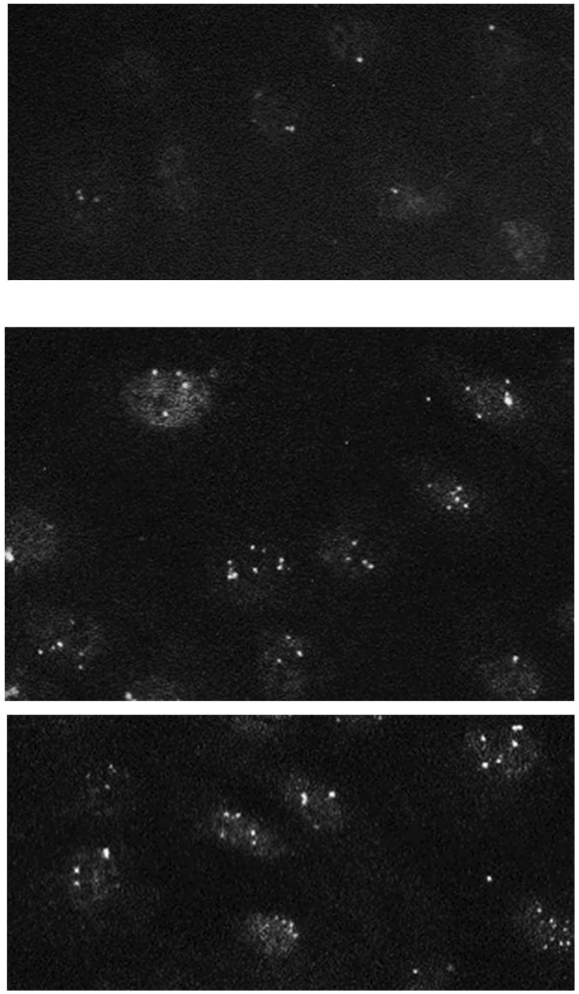


RIF/cell = Bgd + Fluence (track/area) x Volume x RIF/µm*(LET & cell dependent)*

RIF/µm*(LET & cell dependent)* = (RIF/cell - Bgd) / Fluence (track/area) / Volume

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



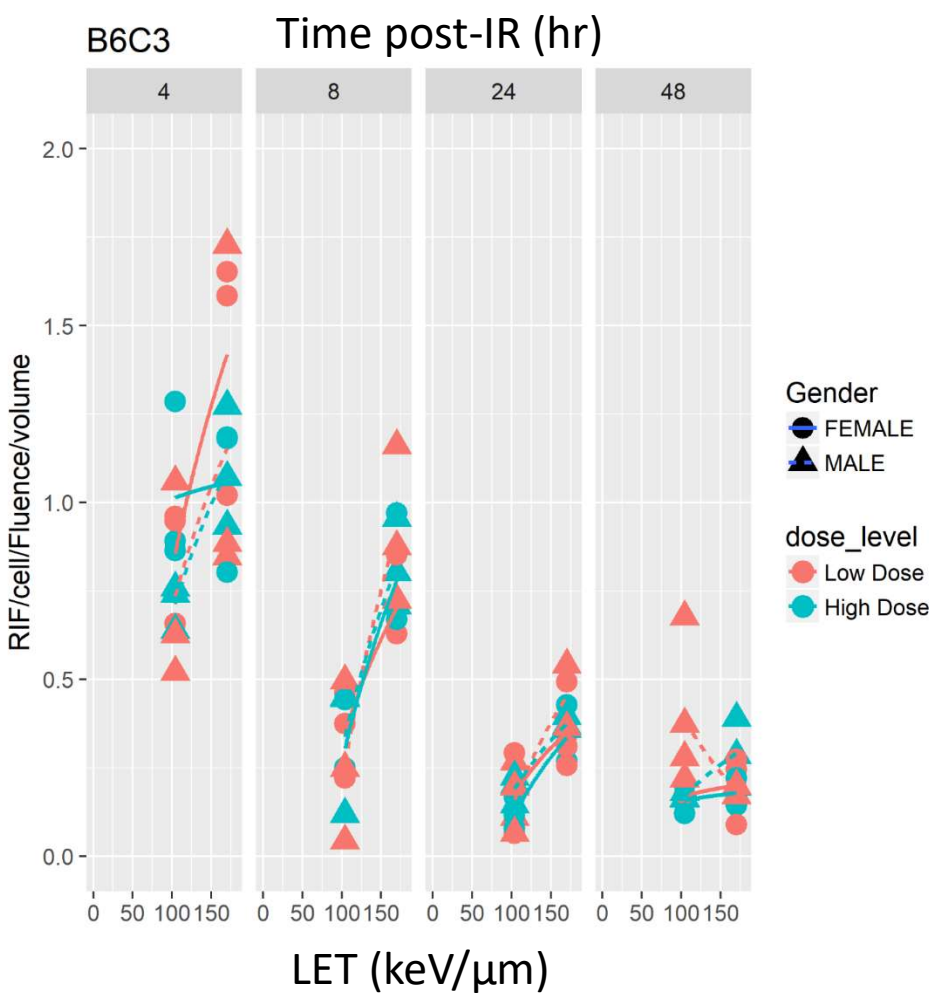


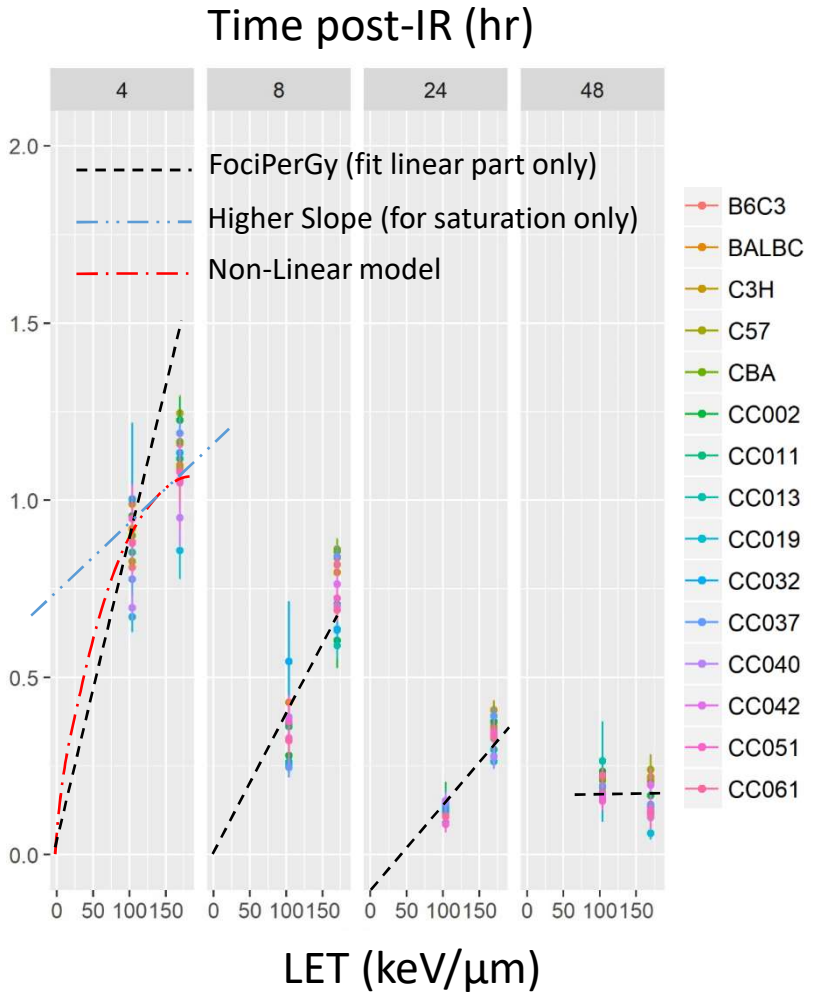
0.1 Gy

1 Gy

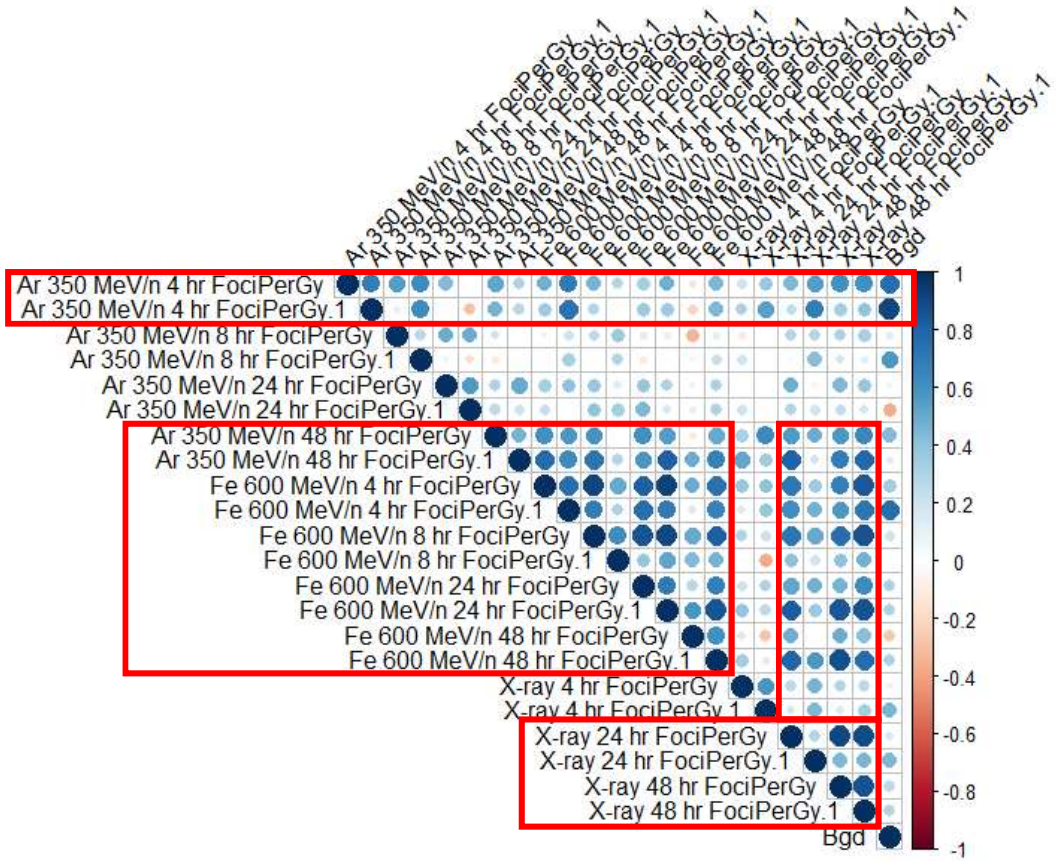
4 Gy

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

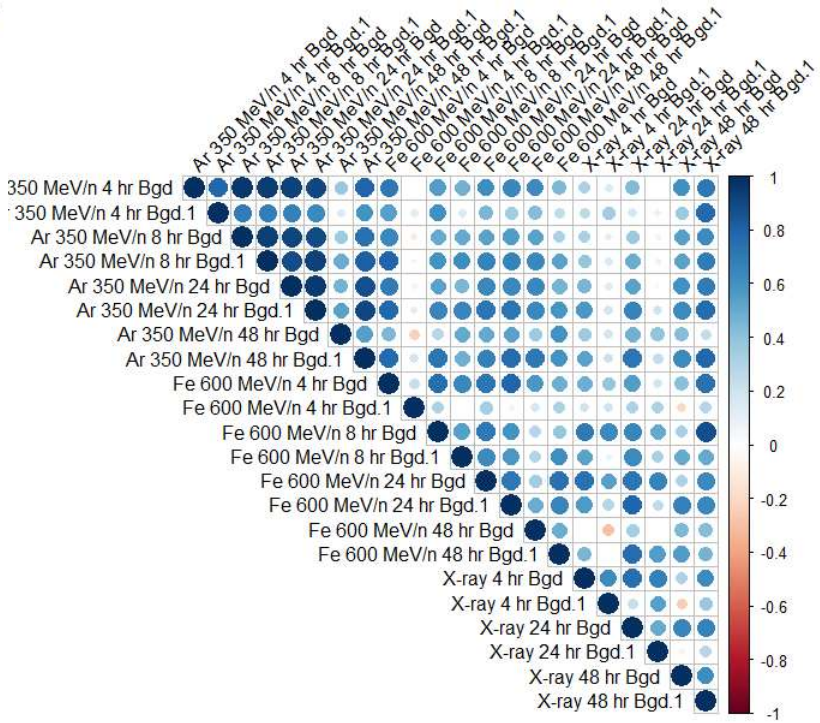




Correlation Graph – Slope and Background
Strong correlation across 15 strains of mice between low and high LET

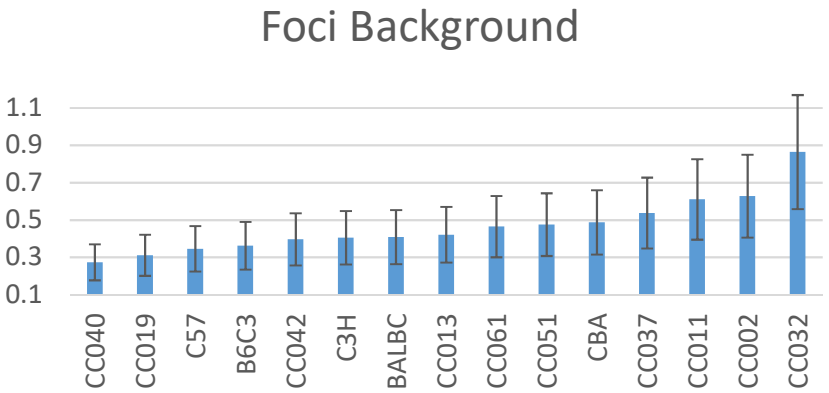


RIF/Gy
(Slope)

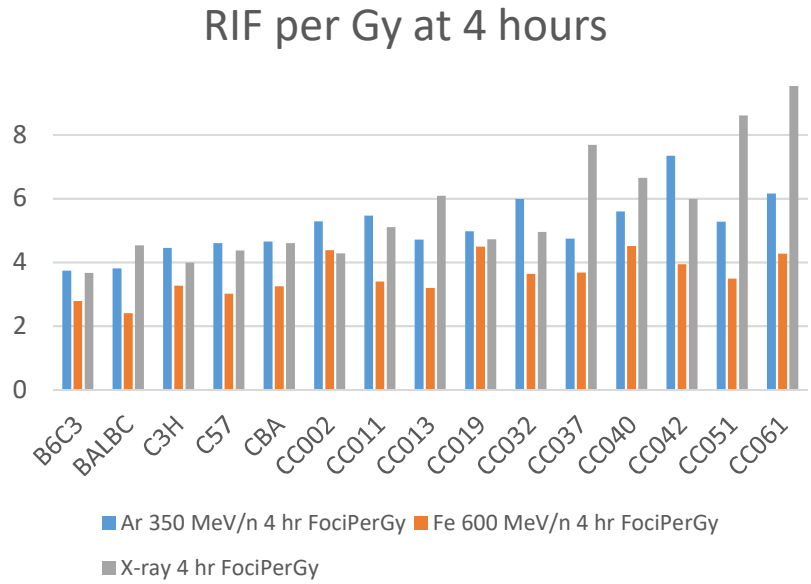


Background Foci
(Slope intercept)

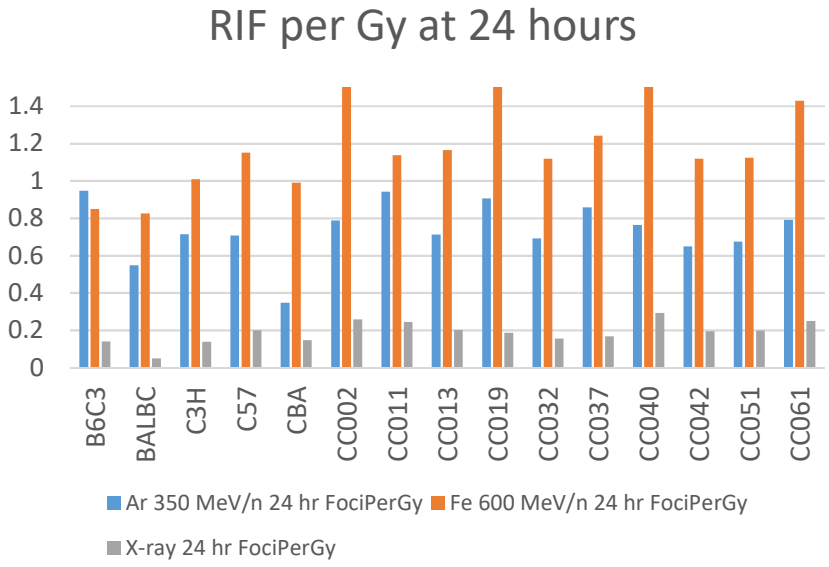
DSB detection sensitivity is driven by genetic



- Different ranking for background

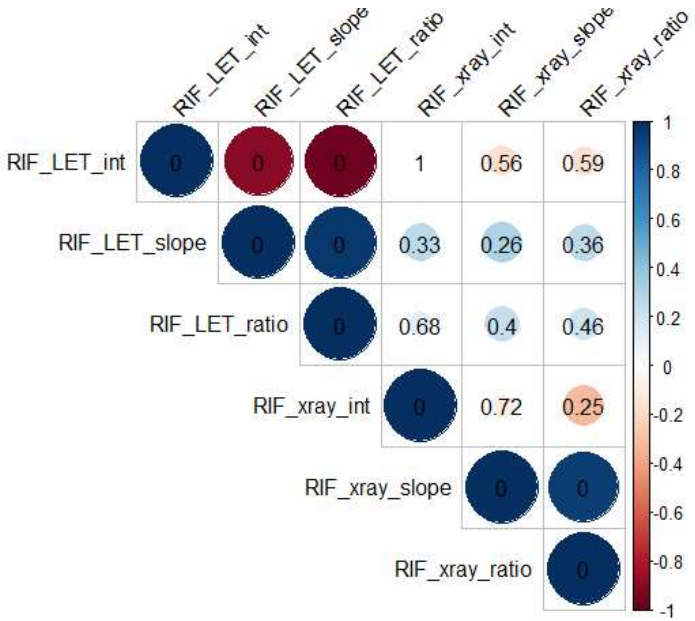


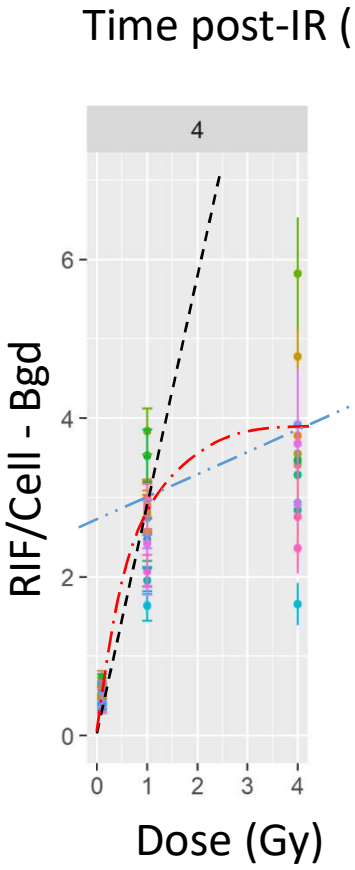
- The higher the LET, the more clustering
- Reference mice show lower detection

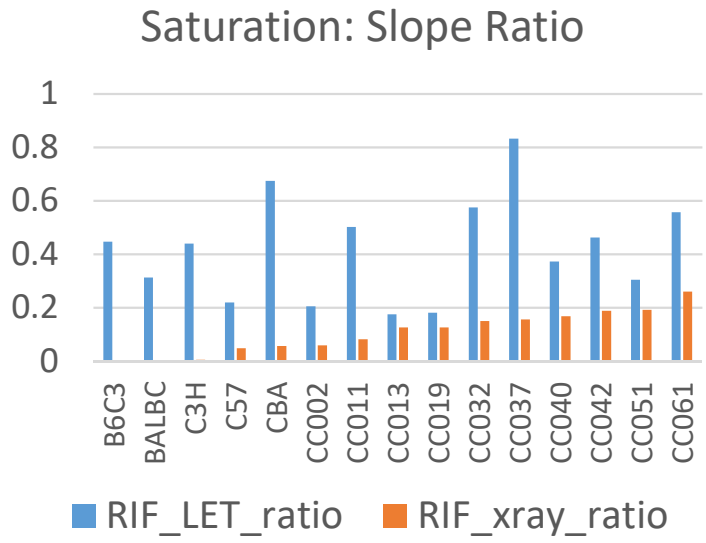


- Ranking persists at 24 hrs
- Higher LET have steeper slopes (more remaining damage)

Saturation parameters

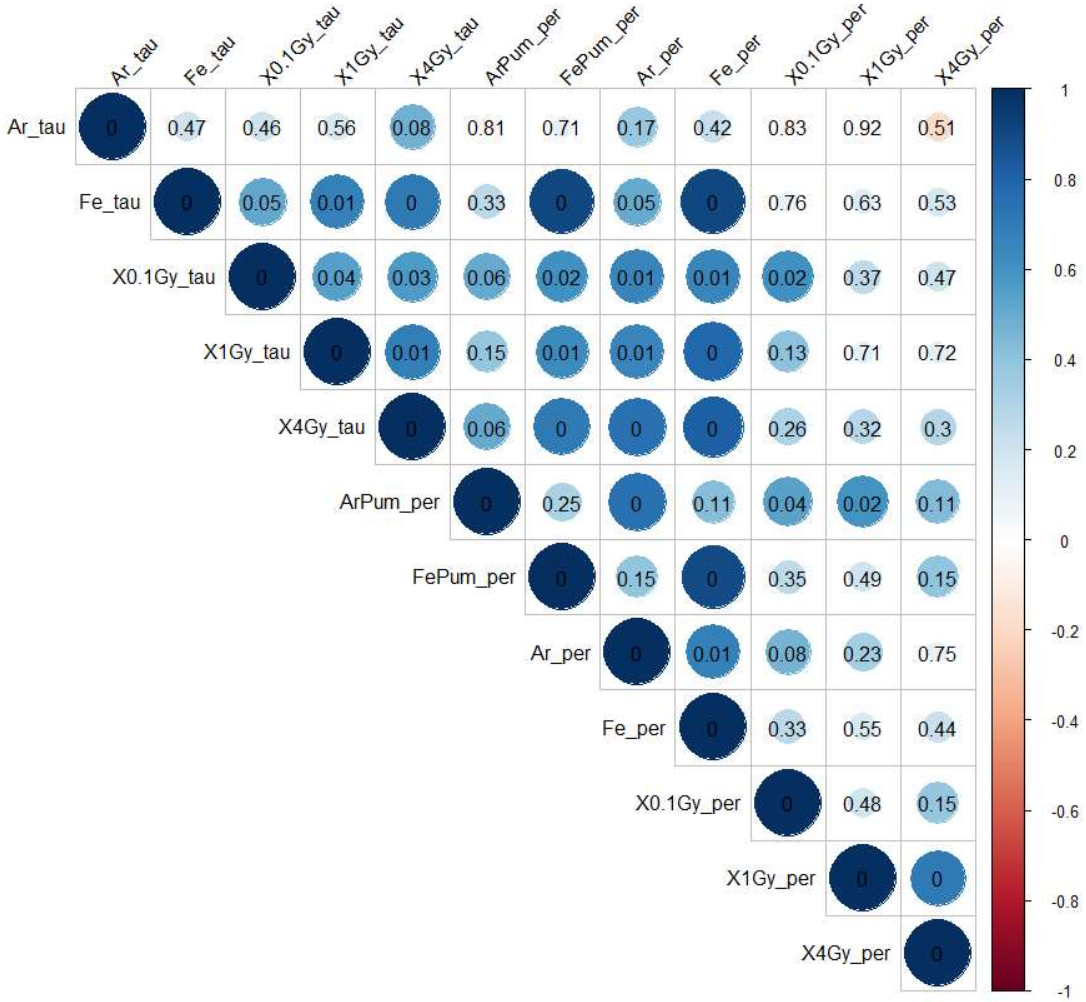


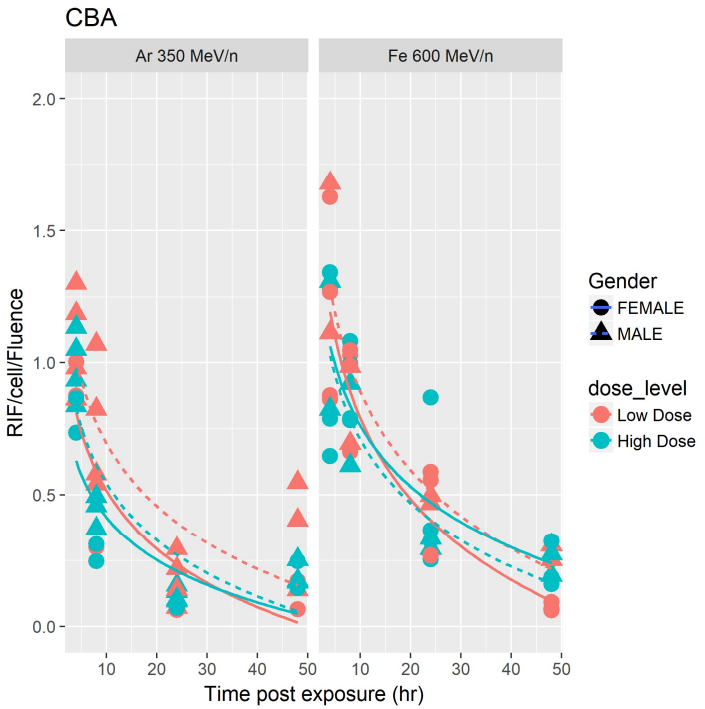




* Note: For 15 pairs, R must be >=0.52 for p-value to be less than 0.05

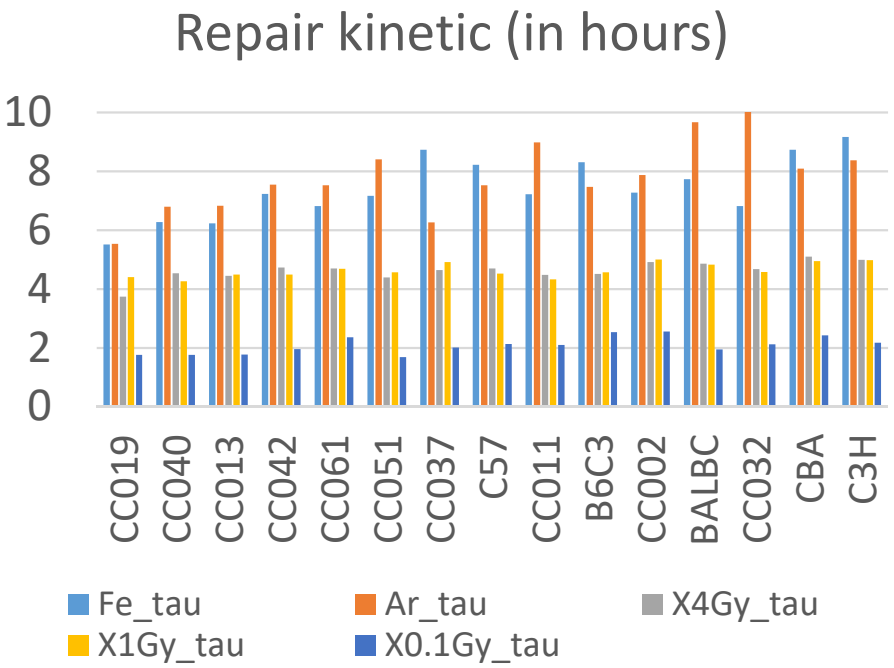
Kinetic parameters

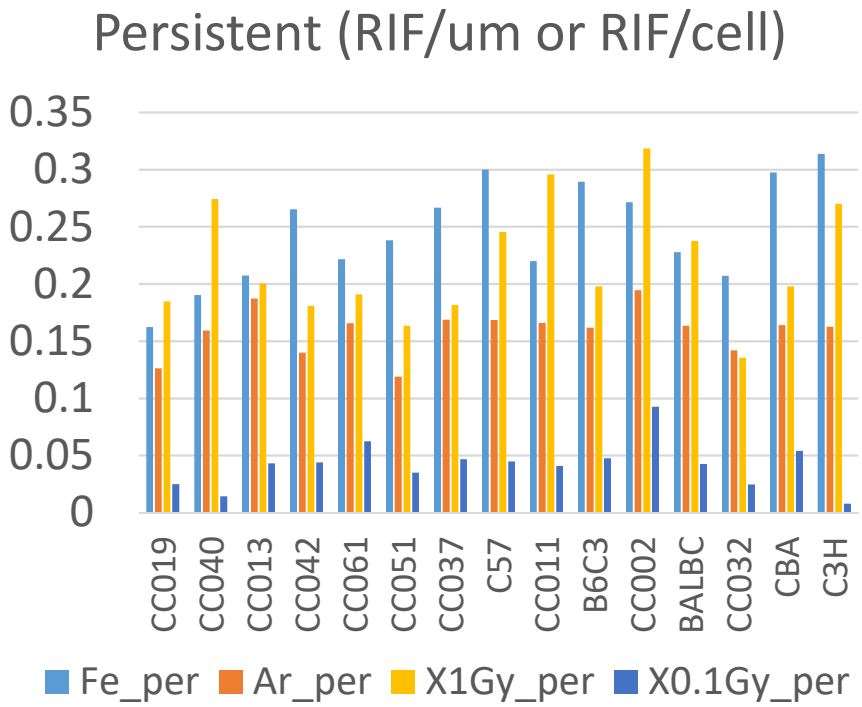




- Persistent RIF at 24 and 48 hours (RIF/um noted FePum_Per for Fe, RIF/cell noted Fe_per)
- Exponential fit: RIF/um = RIF/um(0).EXP(-t/tau)
- RIF/um(0) = LET(keV/um)/80 for LET, and RIF(0) = 35.Dose(Gy) for X-ray
- Excluding 4 hr, 4 Gy for X-ray

Repair kinetics and Persistent RIf





Lethal dose for X-ray exposure (Gy)

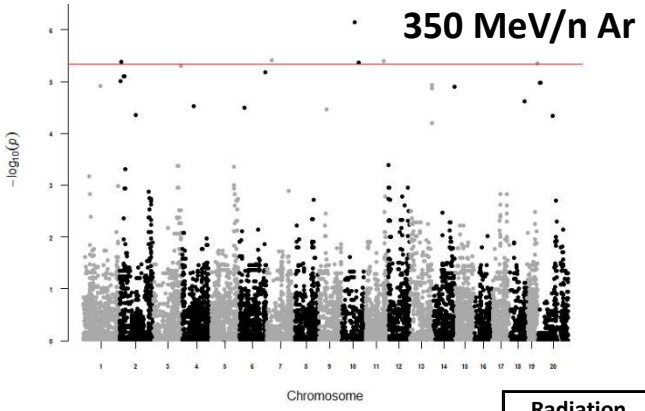
Strain	$LD_{50.30}$ +/- S.E. (Gy)	
	Males	Females
C3HeB/J	6.76 +/- .11	6.89 +/- .7
CBA/J	6.56 +/- .9	6.89 +/- .8
C57BL/6J	6.47 +/- .15	6.70 +/- .6
BALB/cJ	5.70	5.85 +/- .12

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

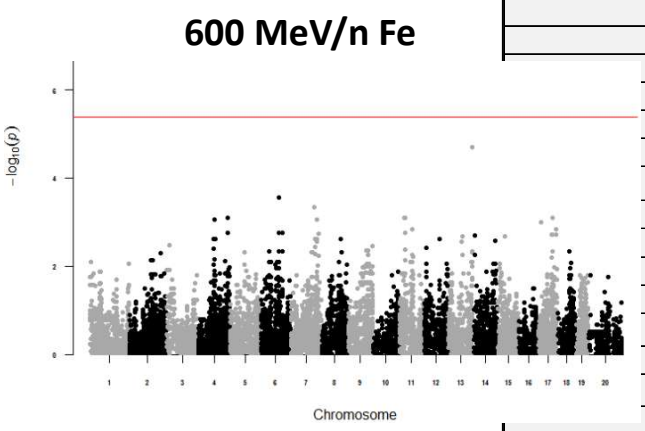
Link to in-vivo (whole blood response in CC mice – Mao/Snijders LBL Low Dose DOE)

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}	Smarcb1	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 supressor
				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

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