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

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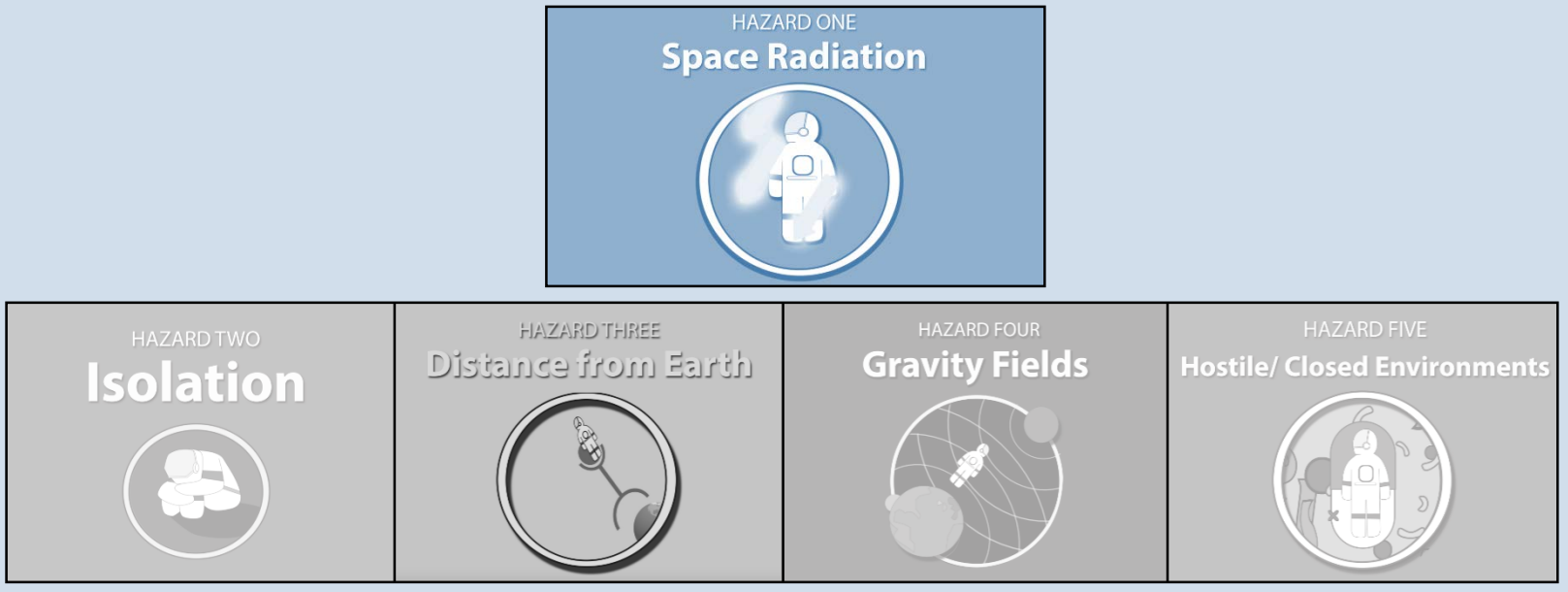
NASA Ames Research Center, Space Biosciences Branch

Human Research Program HRP #NNJ16HP24I

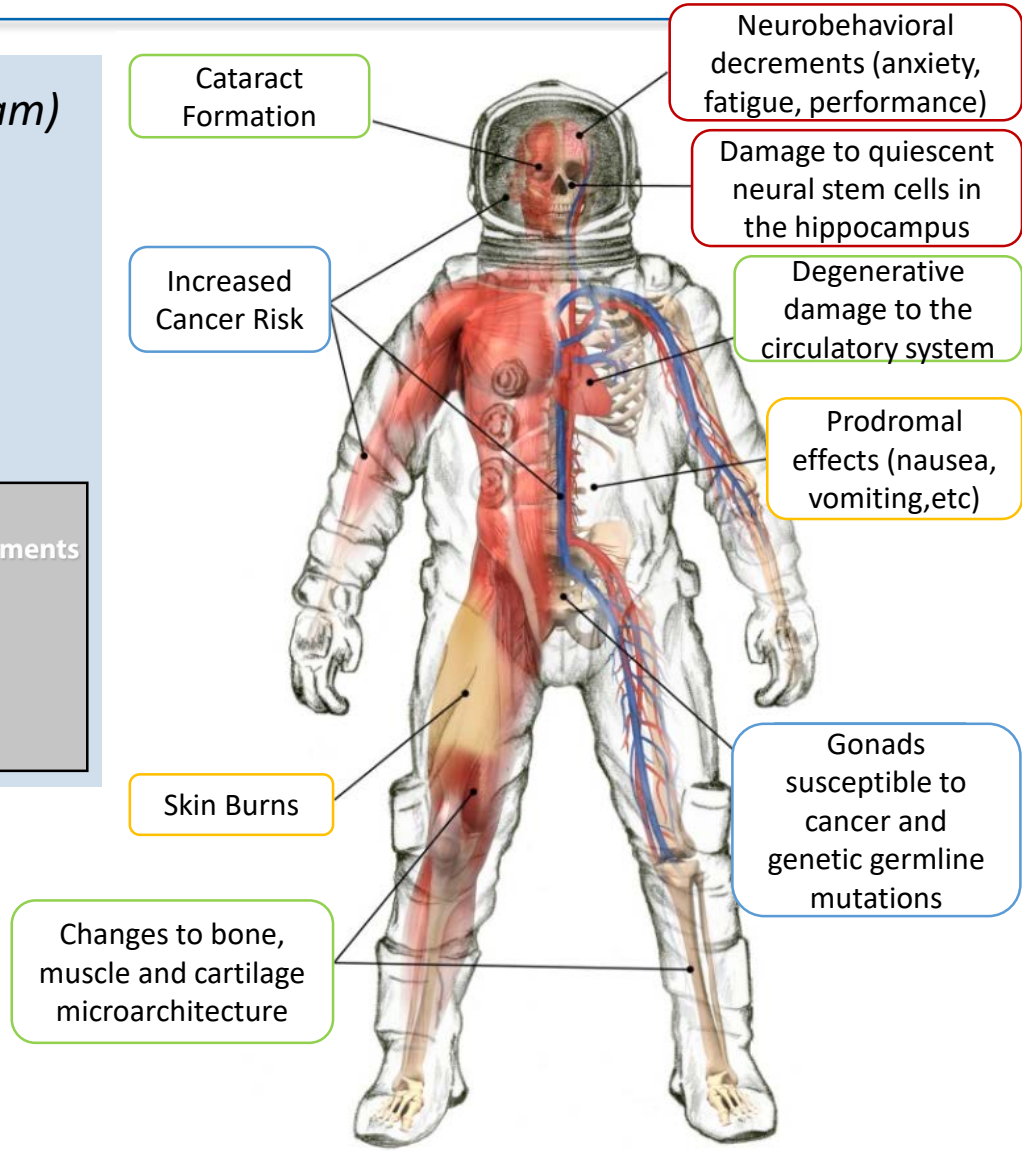
Space Radiation Risks

One of the 5 Hazards of Human Spaceflight *(NASA's Human Research Program)*



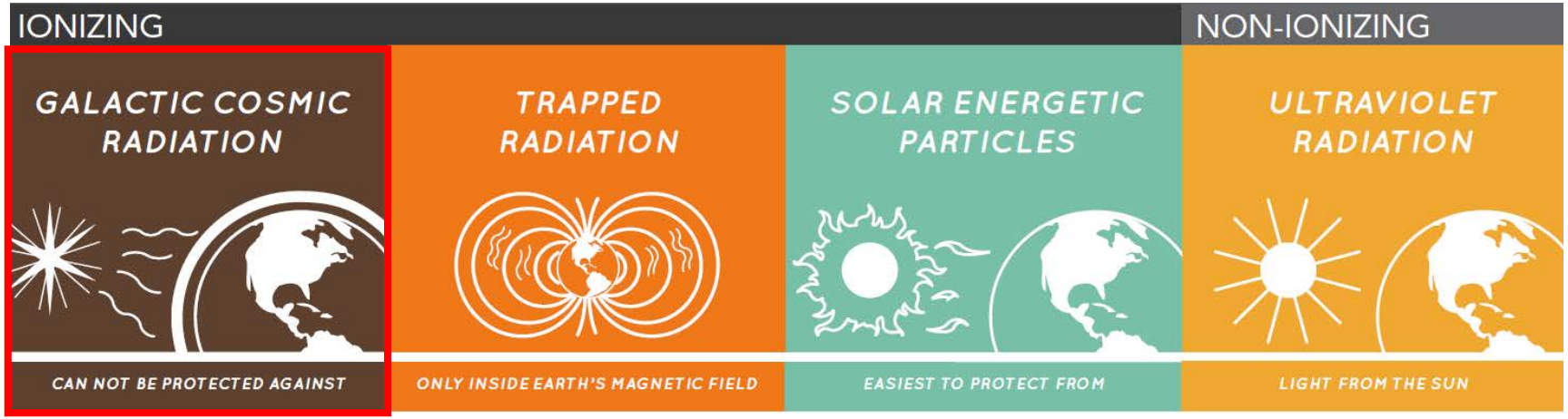
Health Risks from Space Radiation:

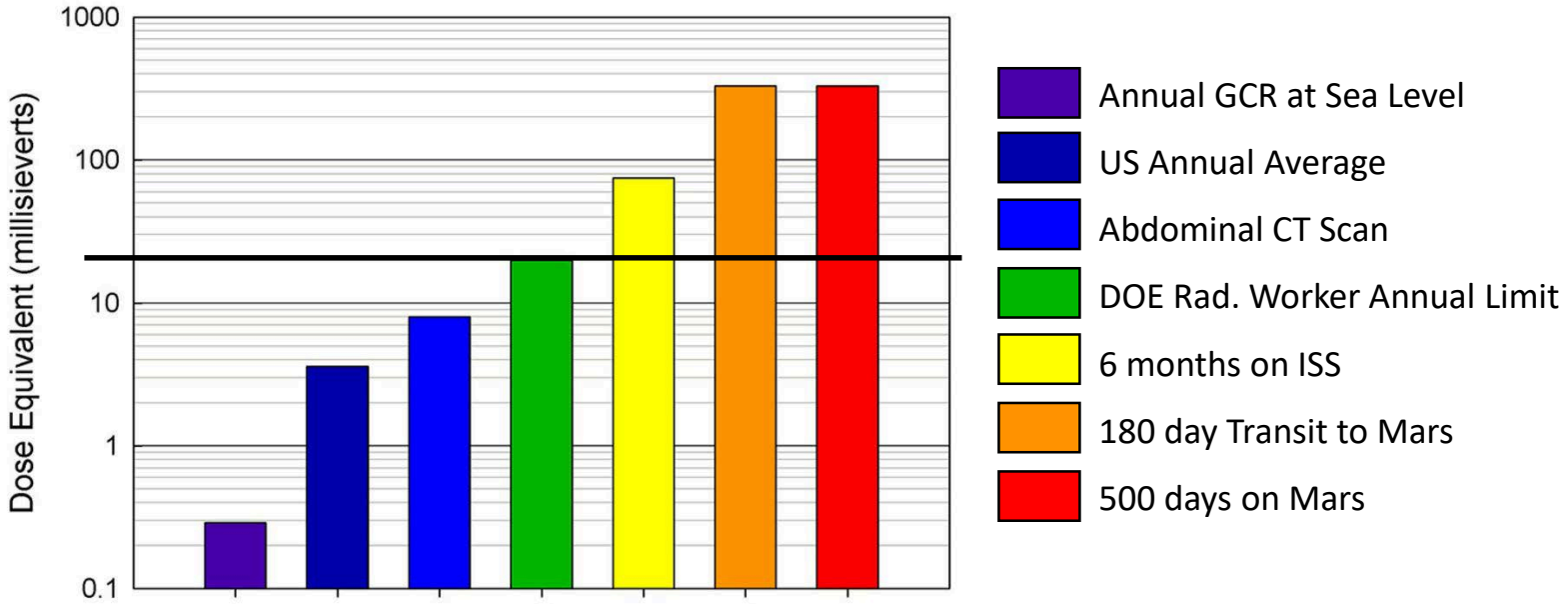
- Increased risks of cancer
- Acute and late central nervous system effects
- Degenerative tissue effects
- Acute radiation risks



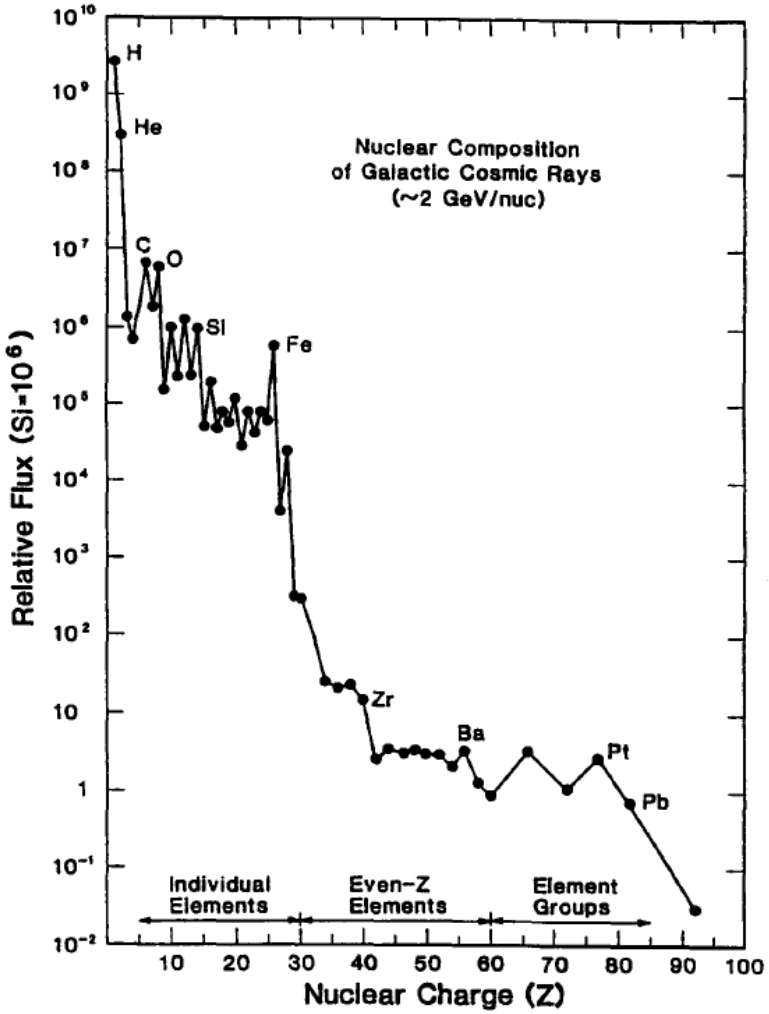
Chancellor et al., Life, 2014

Hazardous Components of Space Radiation



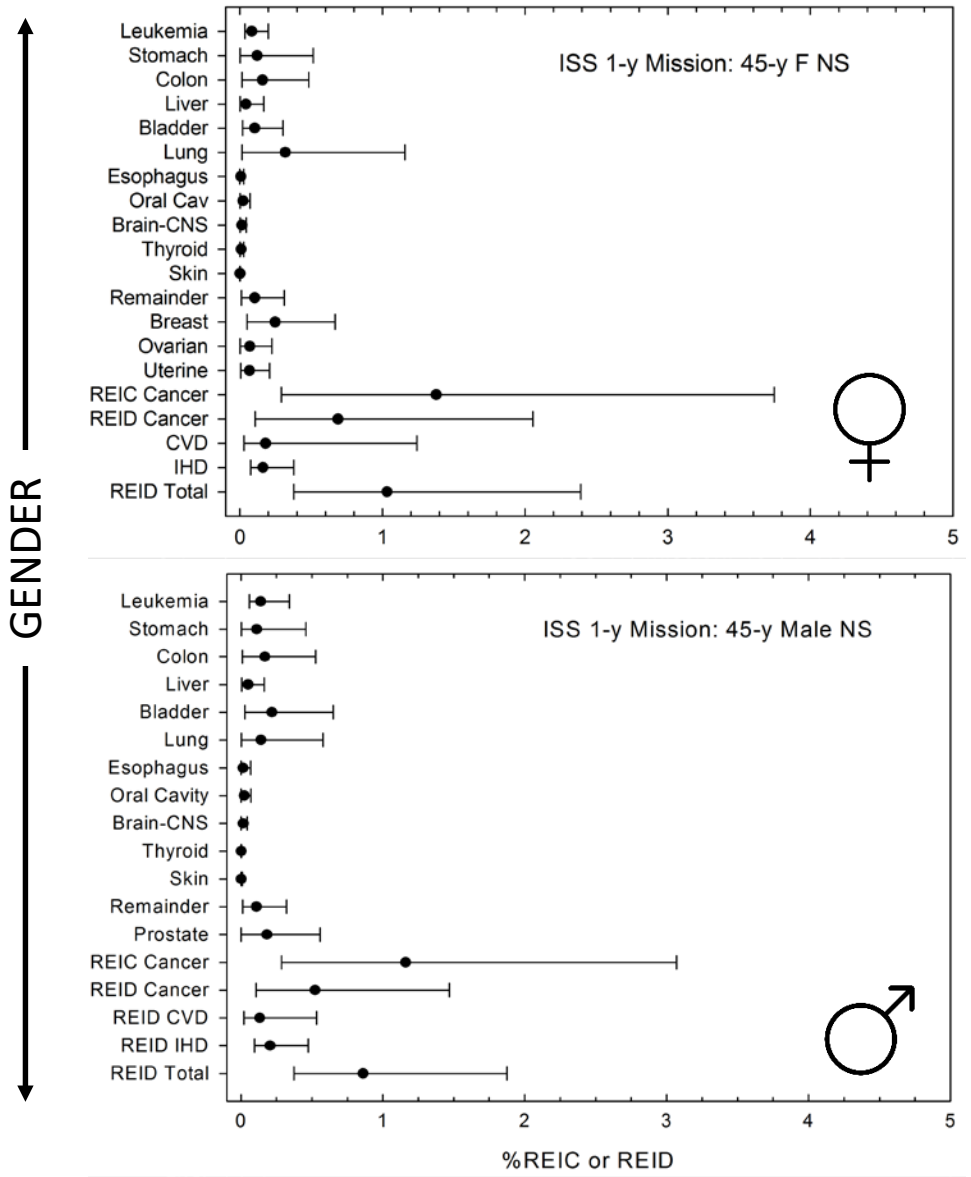


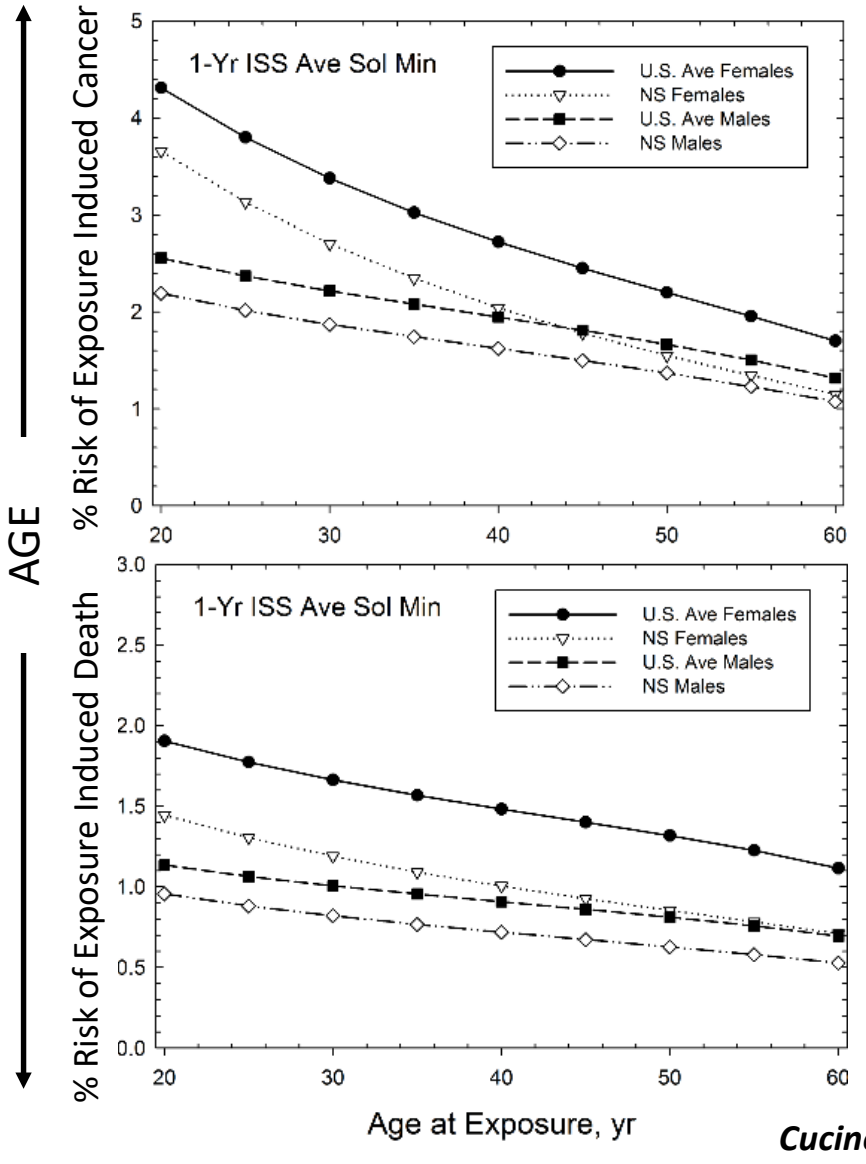
Hassler et al., Science, 2014



Mewaldt, Adv. Space Res., 1994

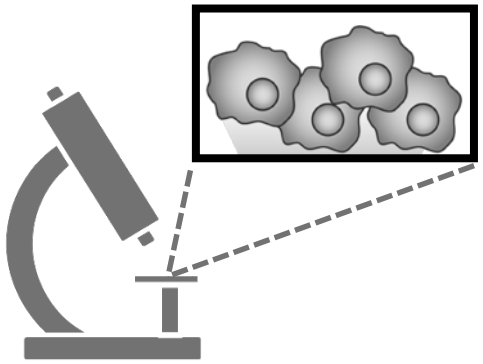
Predicting Individual Radiation Sensitivity



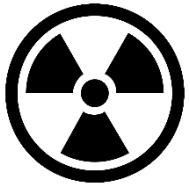


Cucinotta, Plos One, 2014

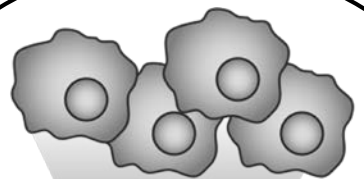
Investigation in a cohort of 780 human donors



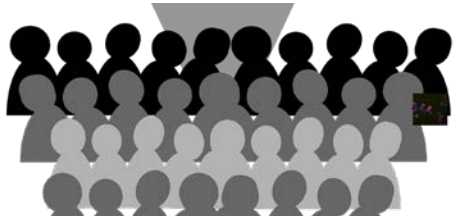
Baseline Quantification of DNA Damage
53BP1+ immunostaining
High-throughput imaging



Irradiation at Brookhaven National Laboratory
Gamma, ^{28}Si (350 MeV/n), ^{40}Ar (350 MeV/n), ^{56}Fe (600 MeV/n)

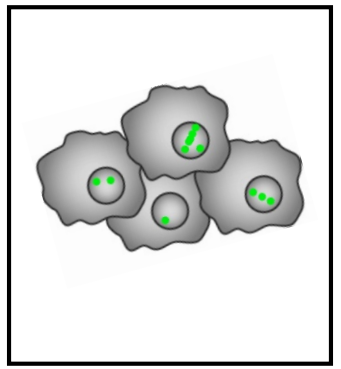


Peripheral Blood Mononuclear Cells (PBMCs)

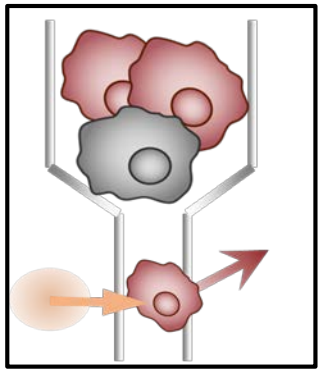


780 healthy subjects

4h & 24h post-irradiation



Quantification of DNA Damage

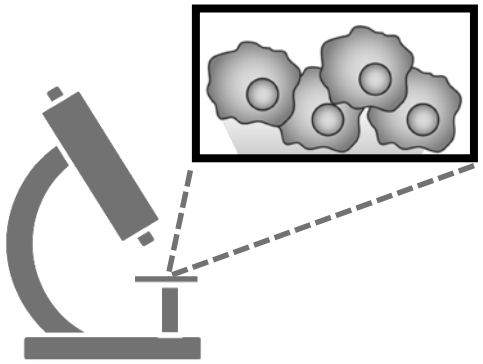


Quantification of Cell Death and Oxidative Stress

Whole Genome Sequencing
NovaSeq 6000, GeneLab

Oklahoma Blood Institute
European Descent, 18-75 years old, 50/50 males/females

Preliminary Results



Baseline Quantification of DNA Damage

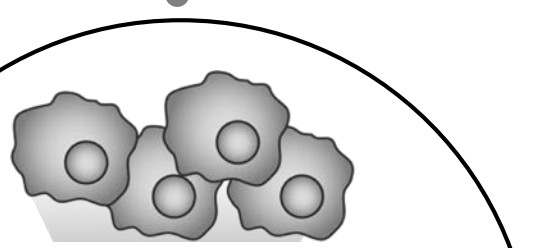
53BP1+ immunostaining

High-throughput imaging

Quantified for 557 individuals out of 780 (70%)

Whole Genome Sequencing

NovaSeq 6000, GeneLab



Peripheral Blood Mononuclear Cells (PBMCs)

780 healthy subjects

Oklahoma Blood Institute

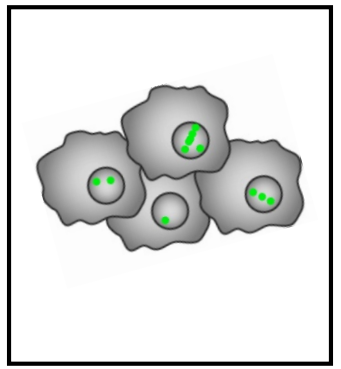
European Descent, 18-75 years old, 50/50 males/females

Irradiation at Brookhaven National Laboratory

Gamma, ^{28}Si (350 MeV/n), ^{40}Ar (350 MeV/n), ^{56}Fe (600 MeV/n)

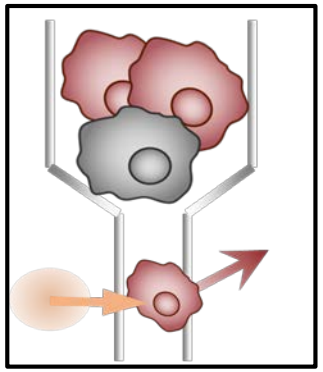
Irradiated 6 sets out of 8 (75%)

4h & 24h post-irradiation



Quantification of DNA Damage

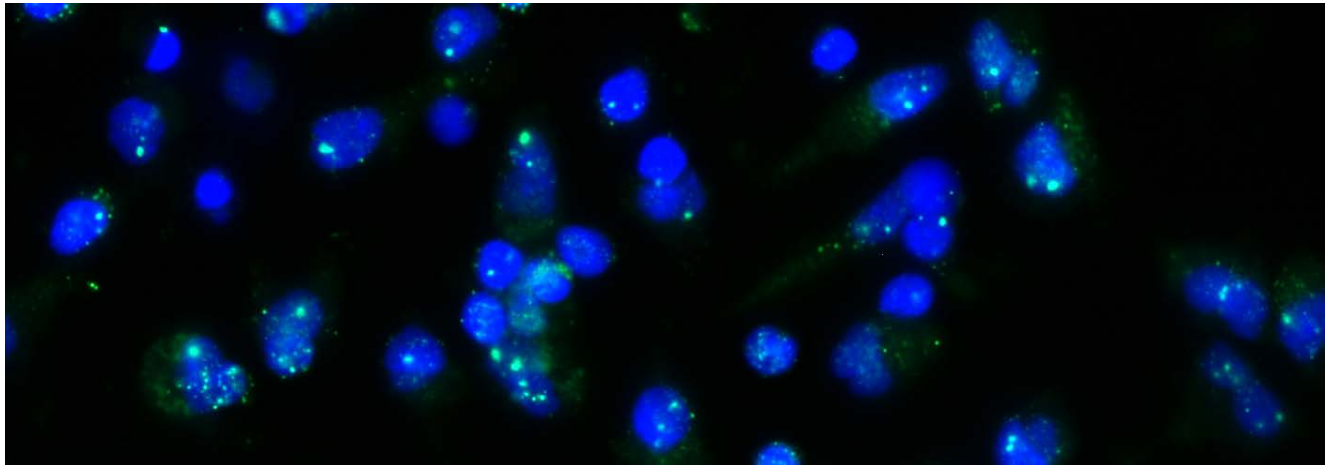
Analyzed 2 sets out of 8 (25%)



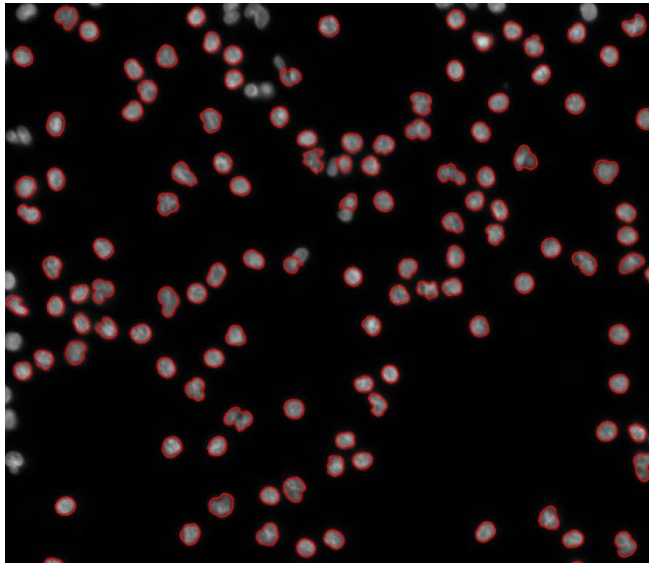
Quantification of Cell Death and Oxidative Stress

Analyzed 2 sets at time point 24h & 4 sets at time point 4h

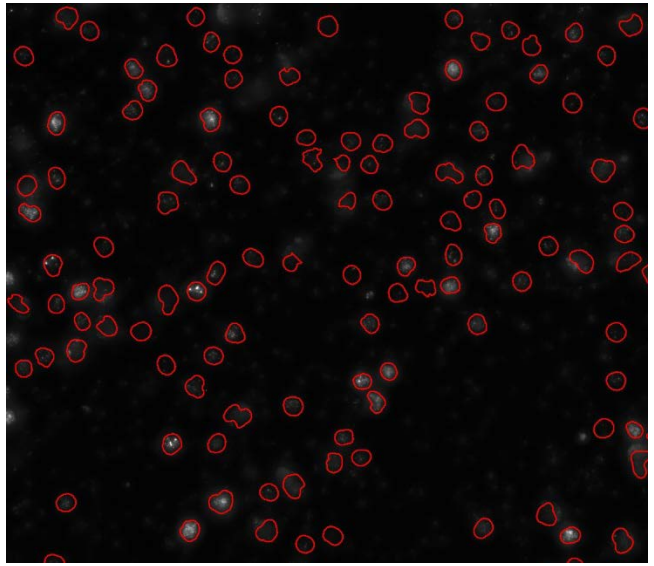
Individual Variability in Baseline Level of DNA Damage



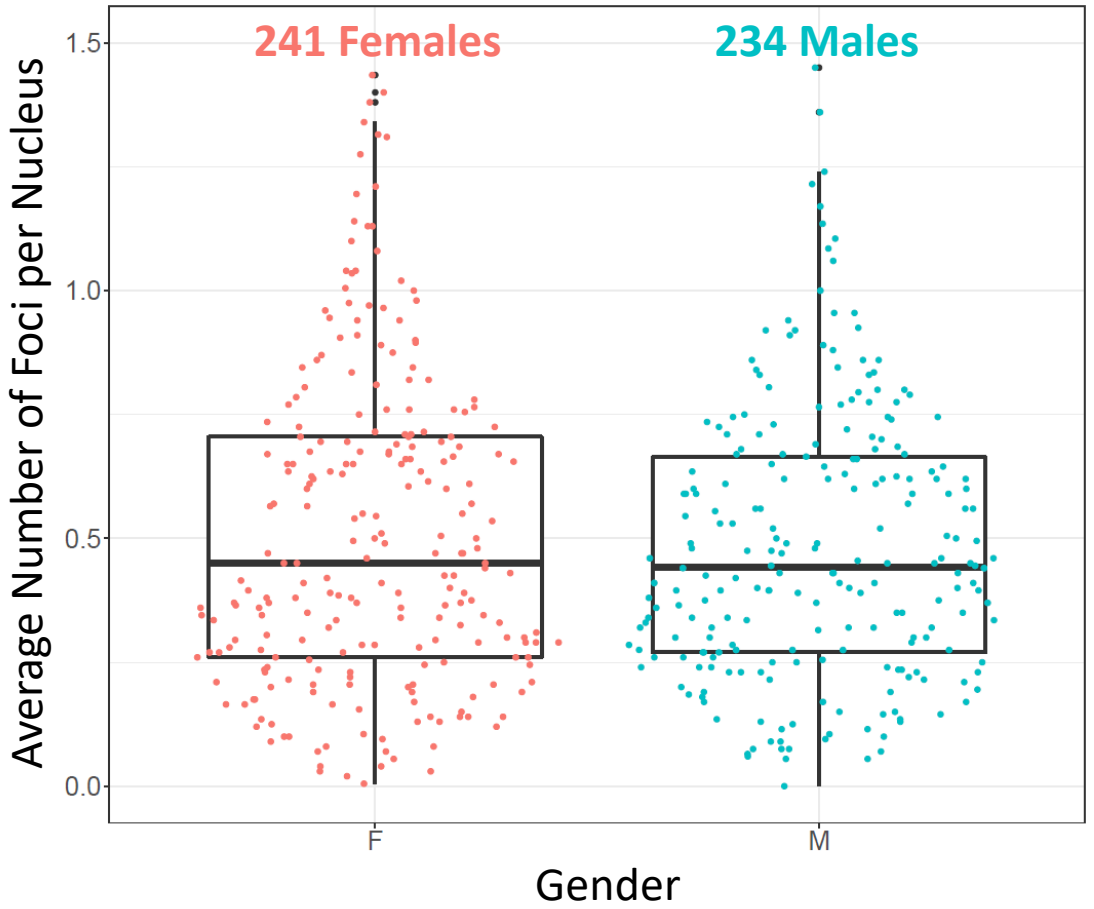
Nuclei Detection (DAPI)



Foci Detection (FITC)

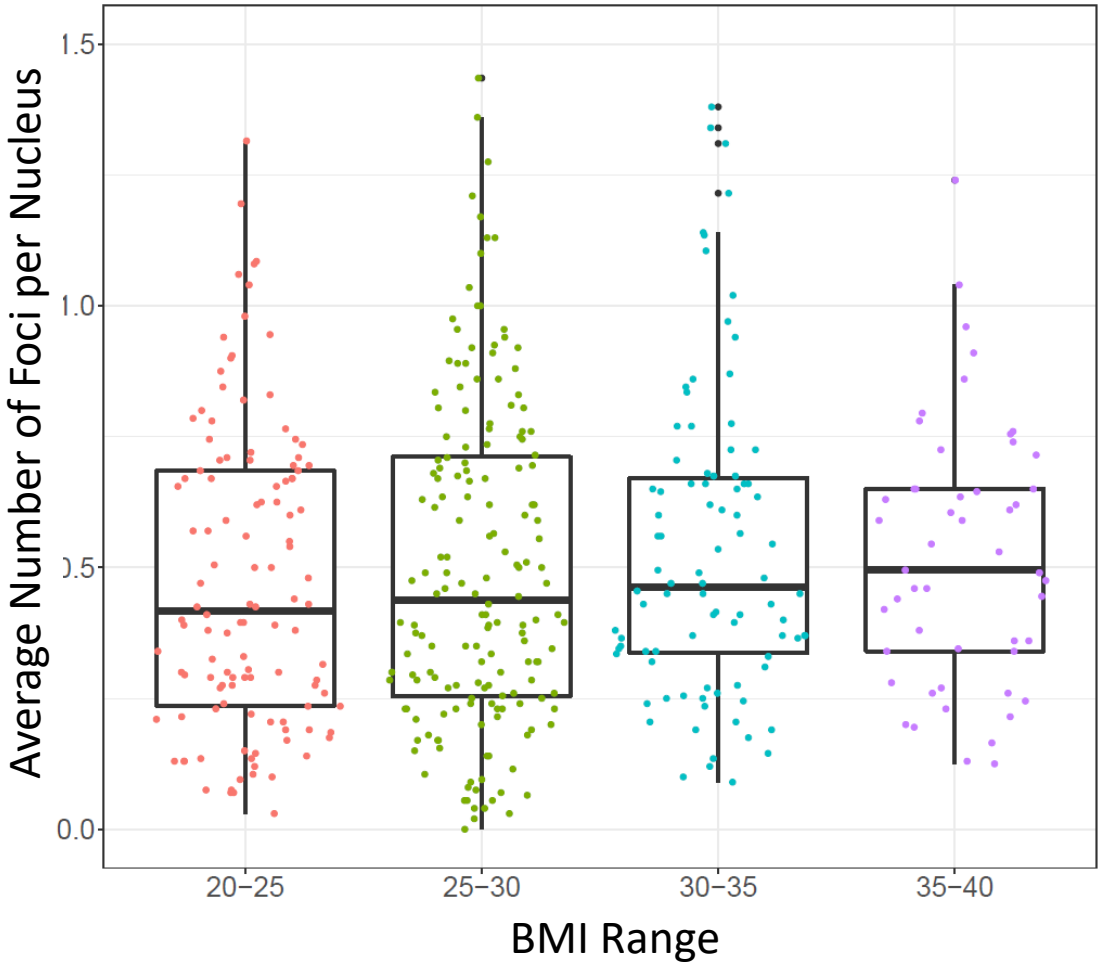


BY GENDER

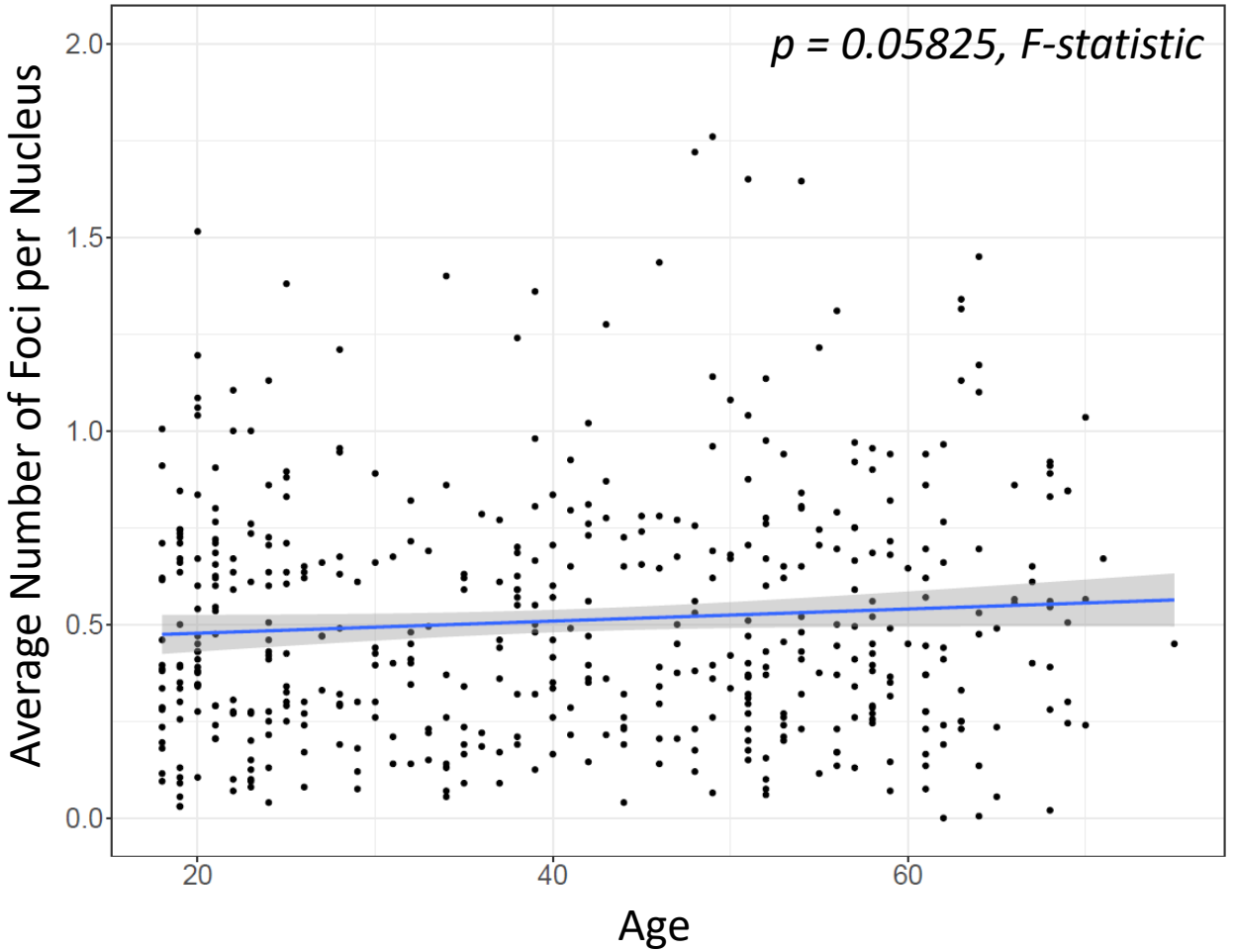


Influence of Demographic Variables on the Baseline Level of DNA Damage

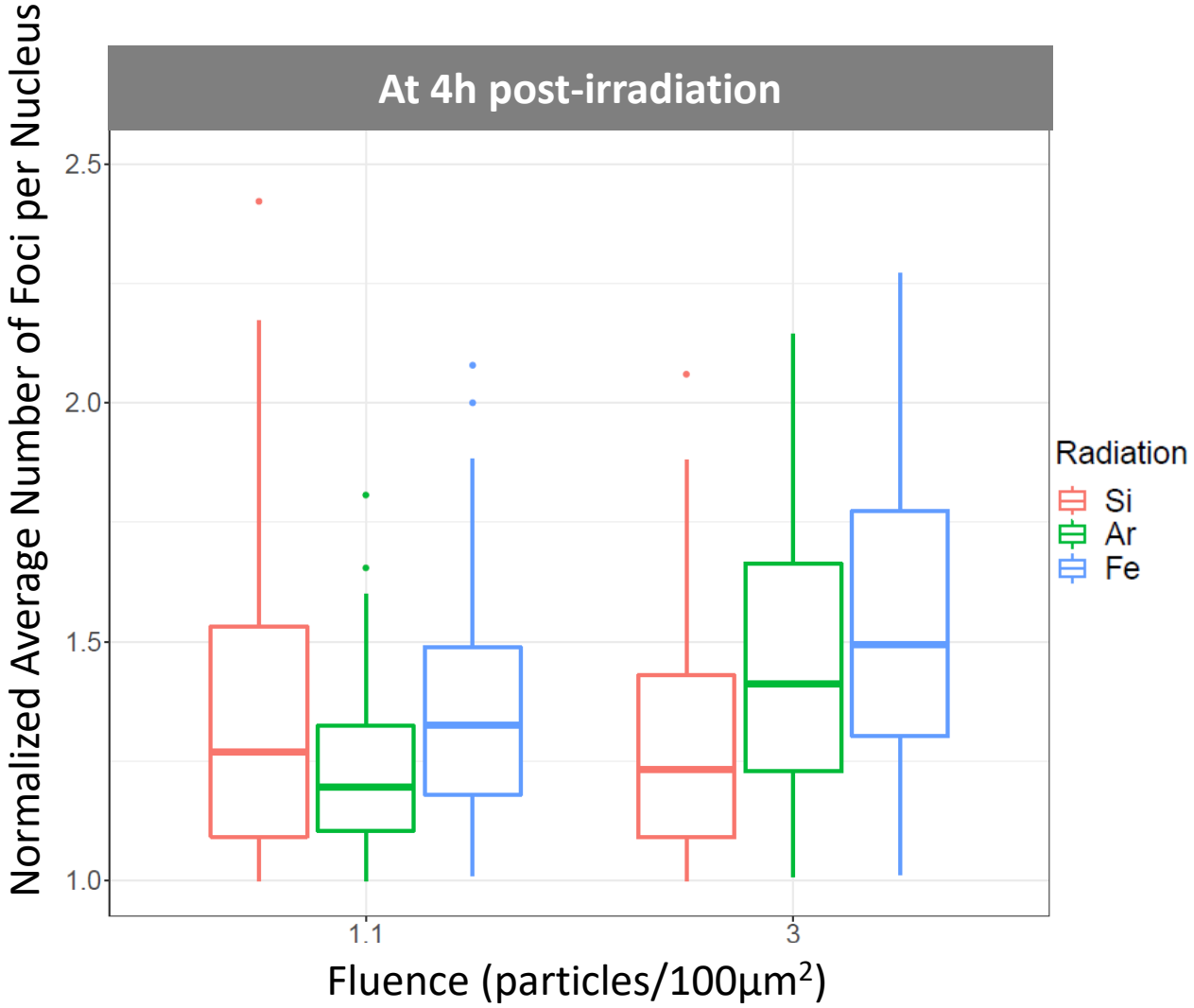
BODY MASS INDEX

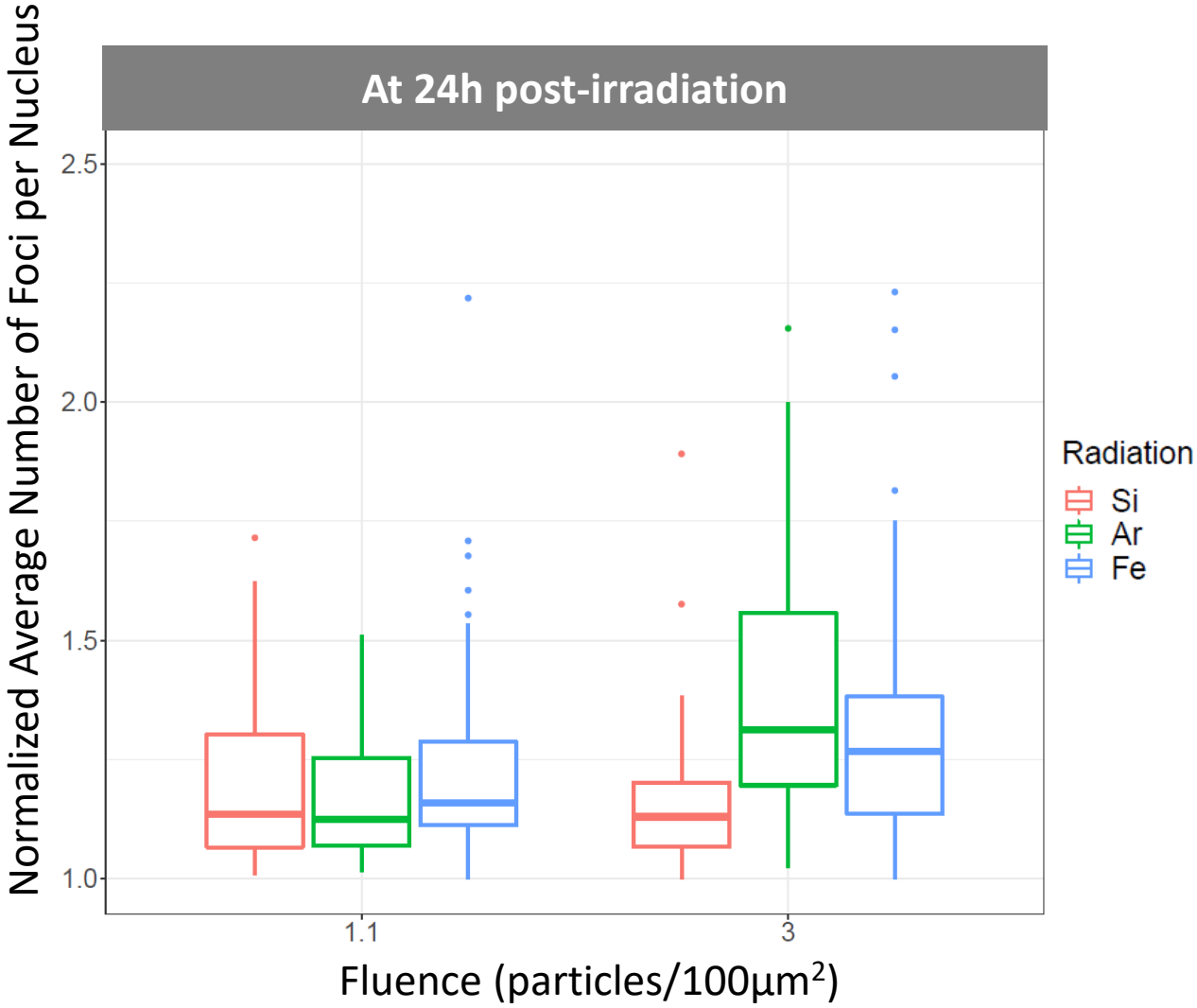


AGE



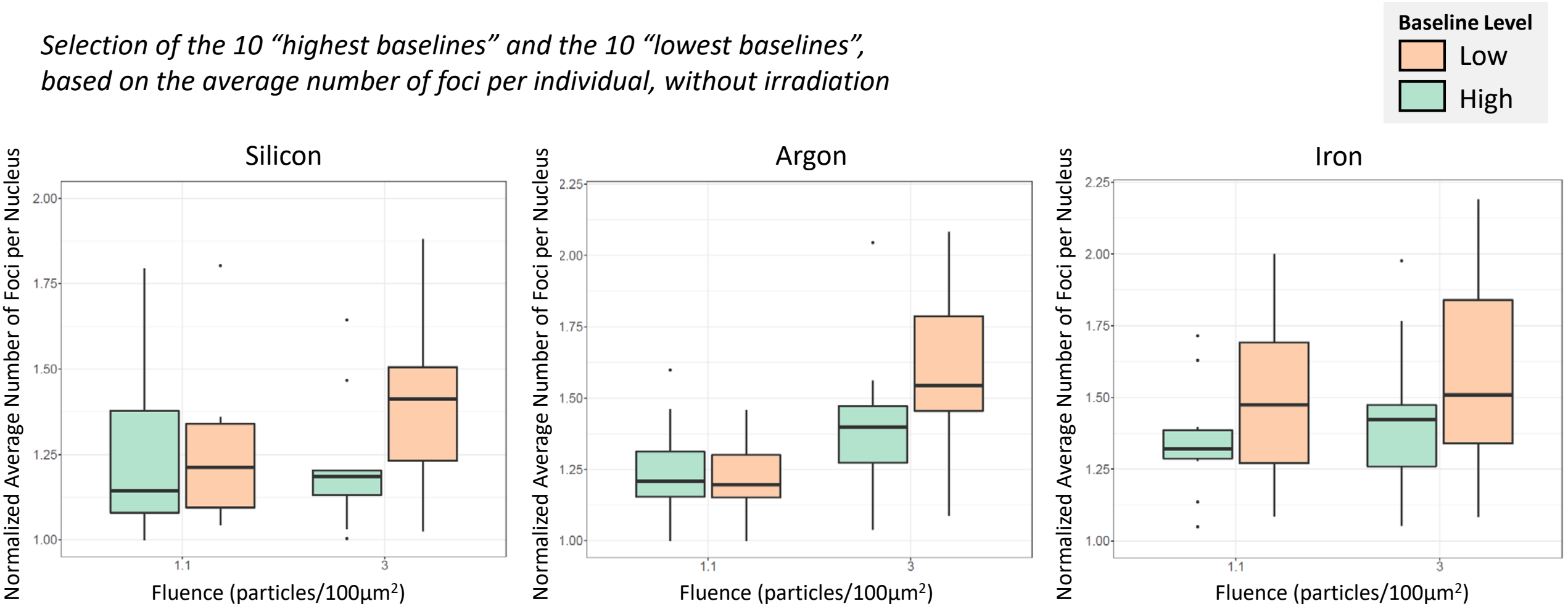
Radiation Response: DNA Damage





Radiation Response for Extreme Baselines

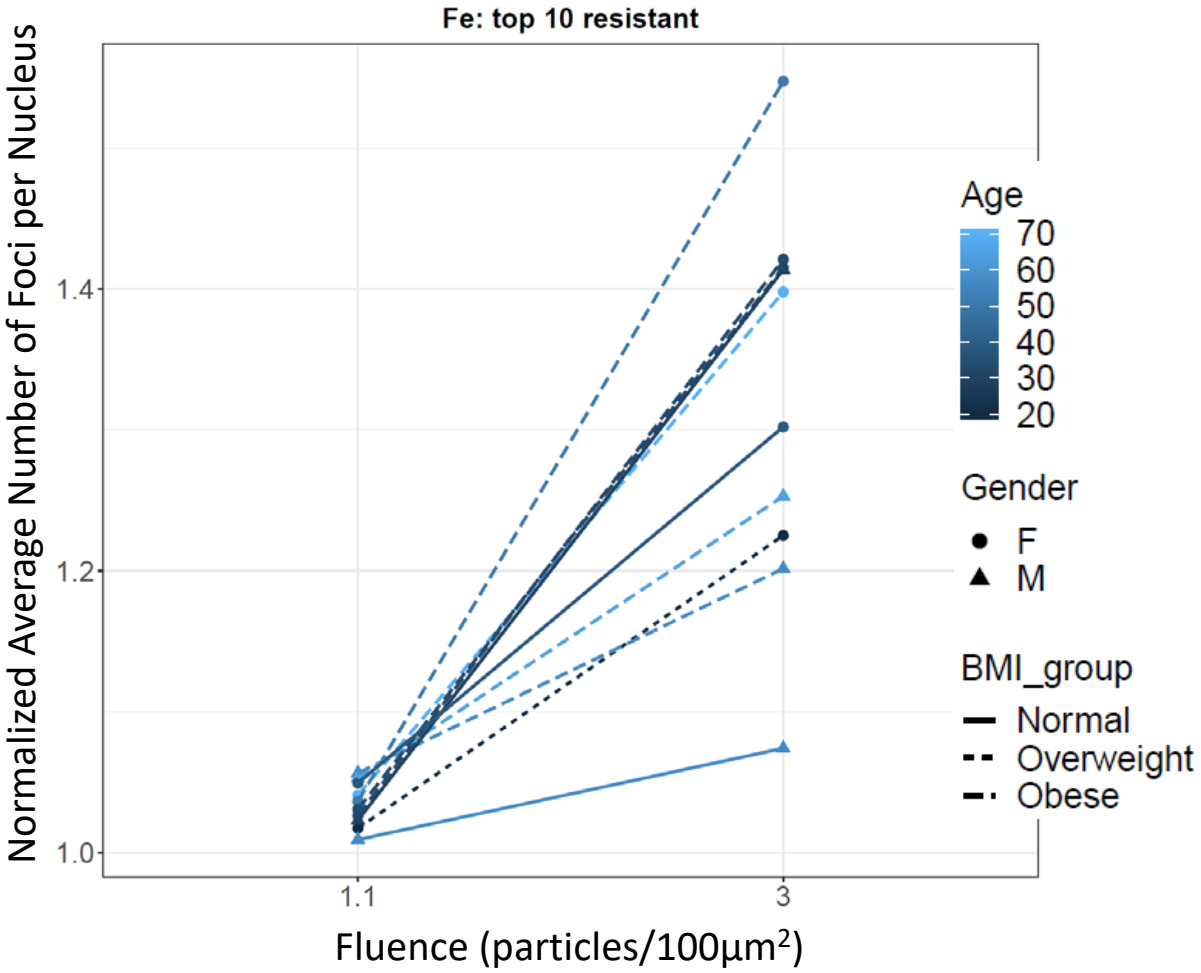
Selection of the 10 "highest baselines" and the 10 "lowest baselines", based on the average number of foci per individual, without irradiation

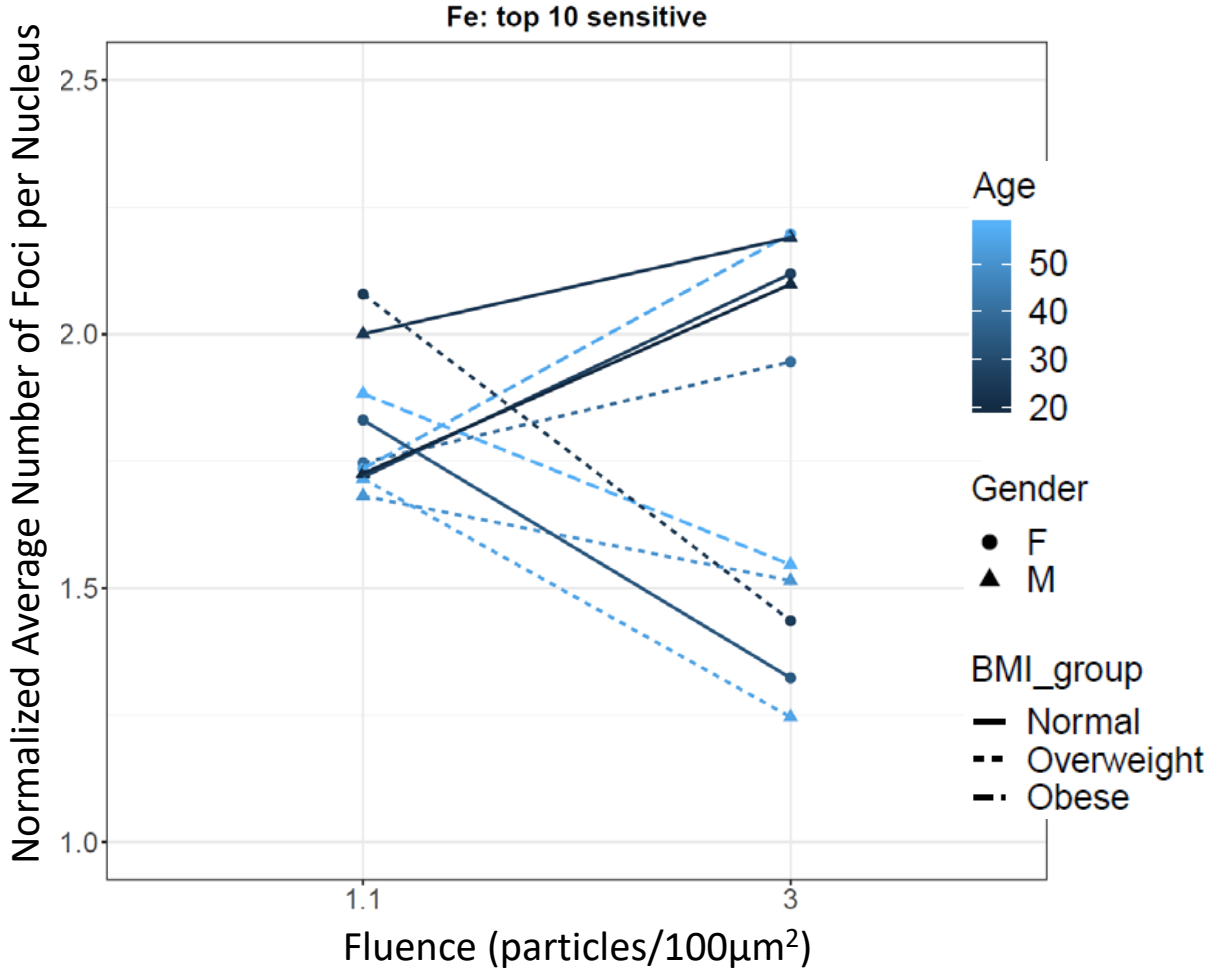


- Individuals with low number of foci at baseline seem to be more responsive to radiation

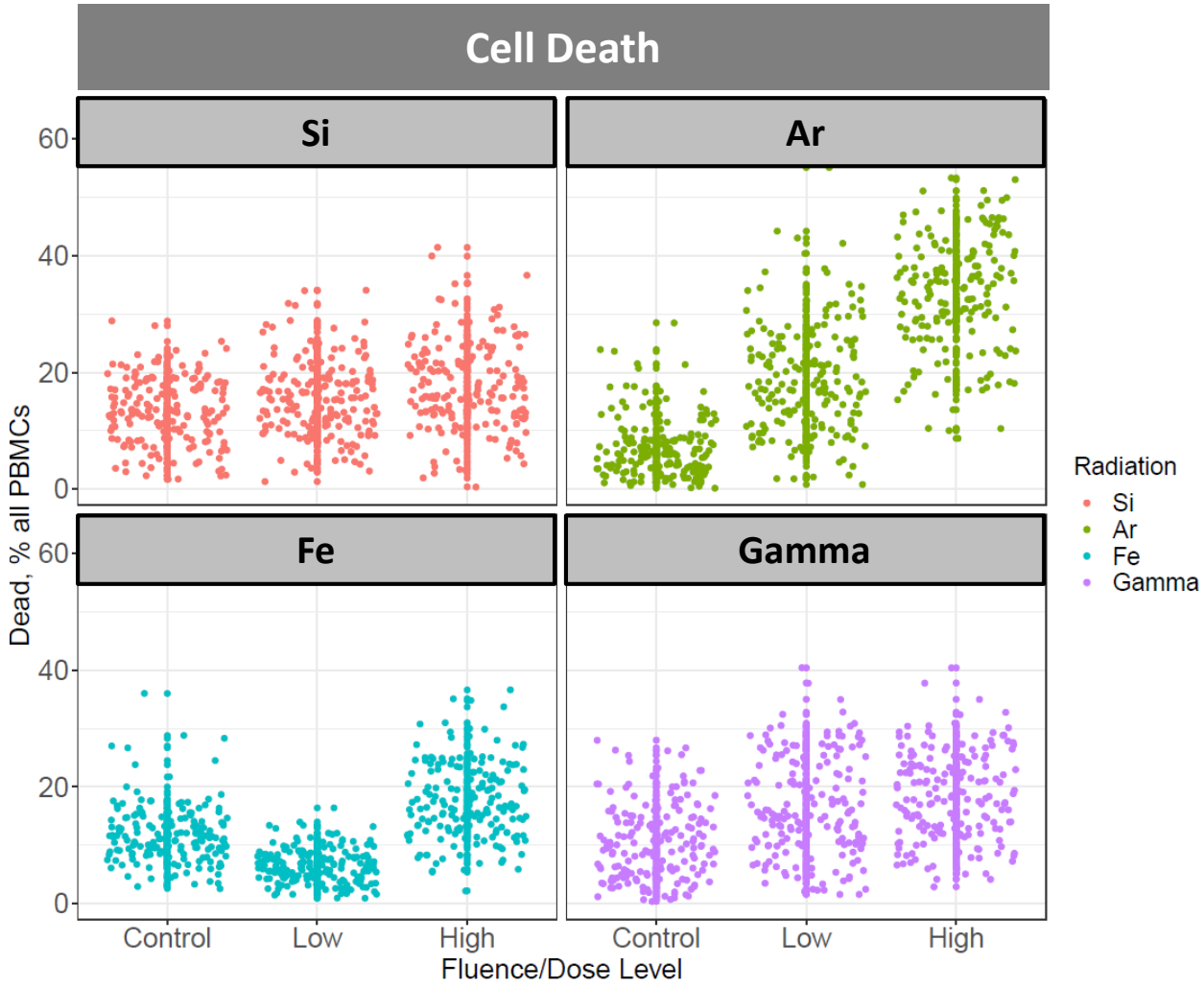
Identifying Extreme Responders

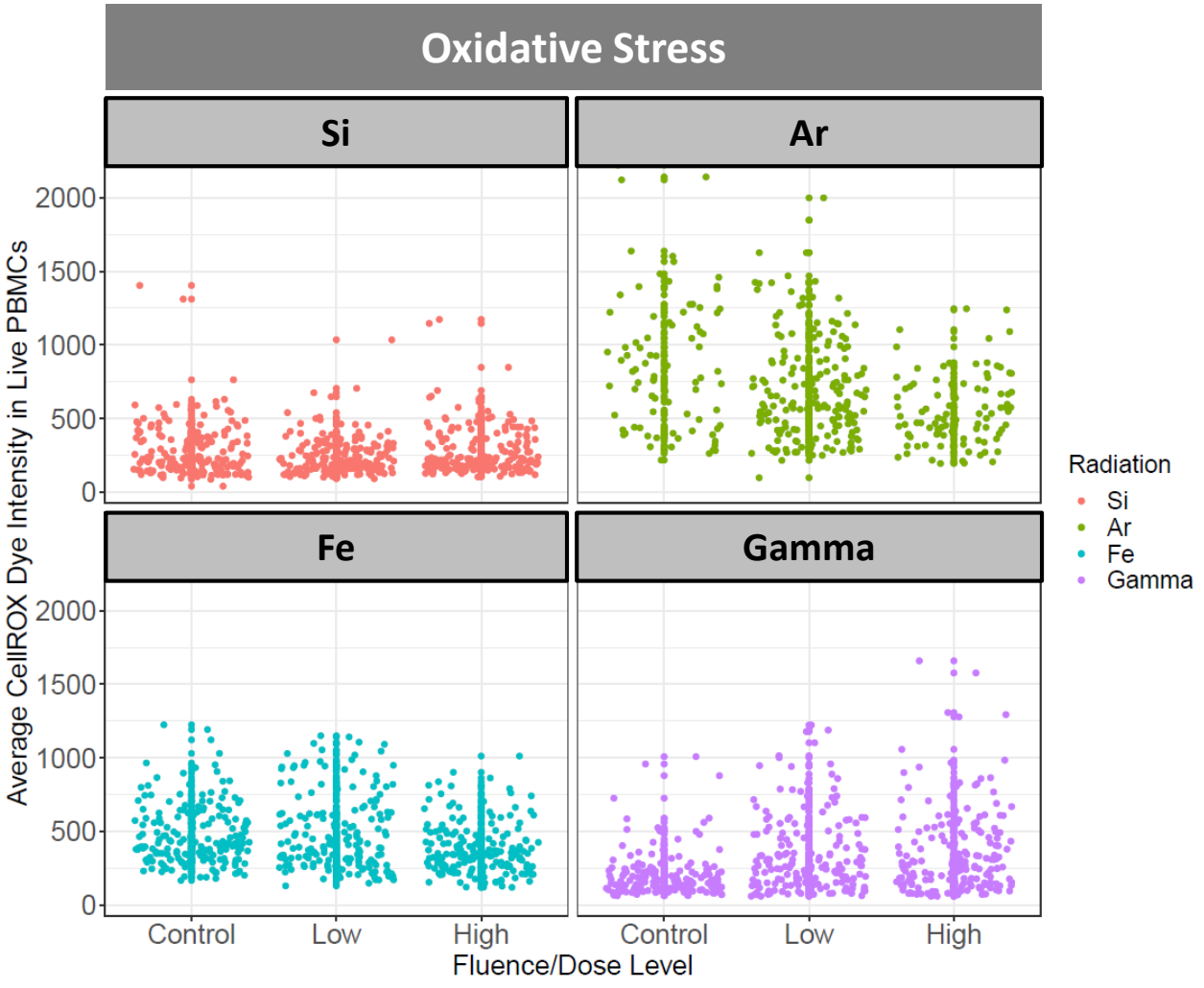
Selection of the 10 "highest responders" and the 10 "lowest responders", based on the level of DNA damage at Fluence 1





Additional Radiation Response Phenotypes





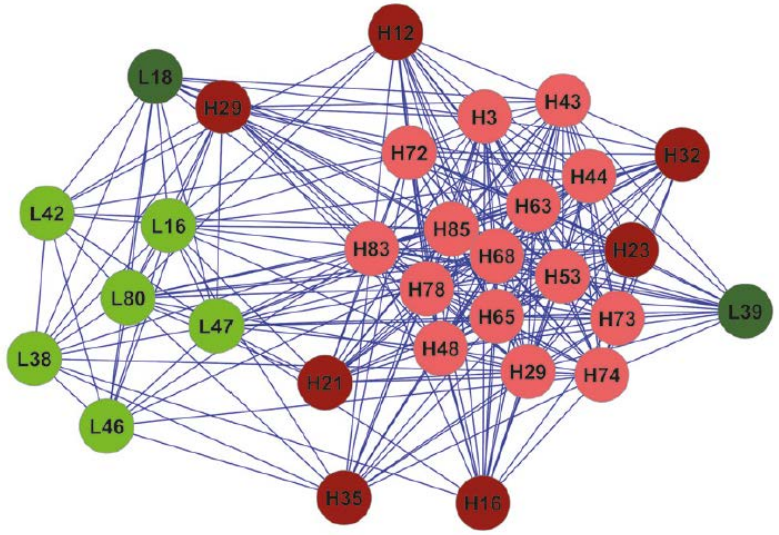
Perspectives: Systems Biology Analysis

- *Node size encoding for the "response score"*
- *Length of connecting edges encoding for the distance between subject signatures*

Multiple Potential Predictors:
Genotype
Secreted Factors
Immune Phenotype

Systems Biology Approach

Multiple Outputs of Radiation Sensitivity:
DNA damage
Cell death
Oxidative Stress



[1]Marchetti et al., Hindawi, Exploring the Limitations of Peripheral Blood Transcriptional Biomarkers in Predicting Influenza Vaccine Responsiveness, 2017
The Microsoft Research-University of Trento Center for Computational and Systems Biology (COSBI)

Identification of relevant genes of radiation sensitivity for:
- Discovery of relevant pathways for radiation countermeasures and biomarkers
- Personalized radioprotective approaches for astronauts
- Improved treatment planning in radiotherapy

Radiation Biophysics Lab at NASA Ames Research Center:

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Sherina Malkani, Alejandra Lopez-Macha, Vanesa Gomez Gonzalez

Brookhaven National Laboratory

Adam Rusek & Peter Guida

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- **Exogen Biotechnology Inc.:** Jonathan Tang

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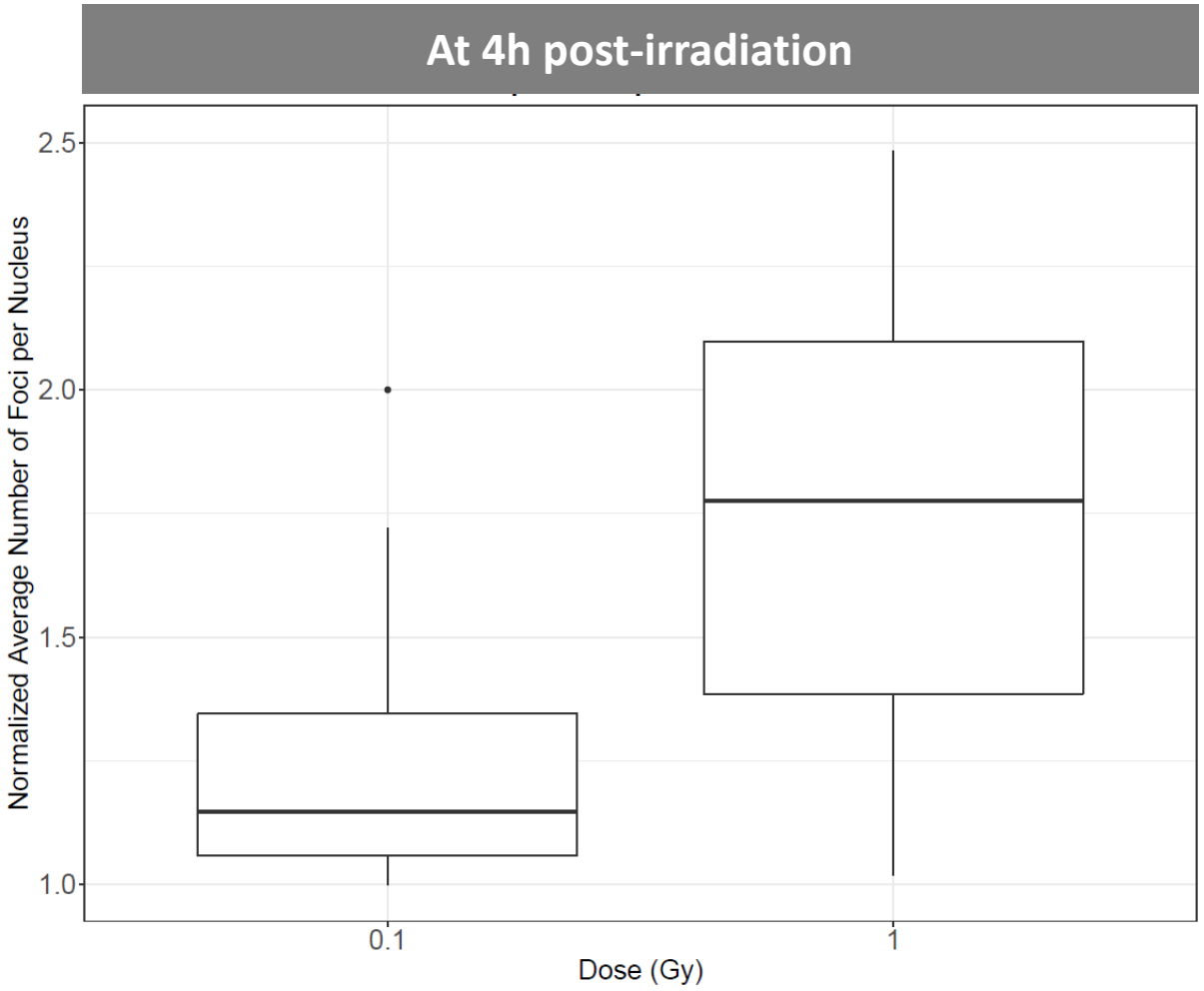
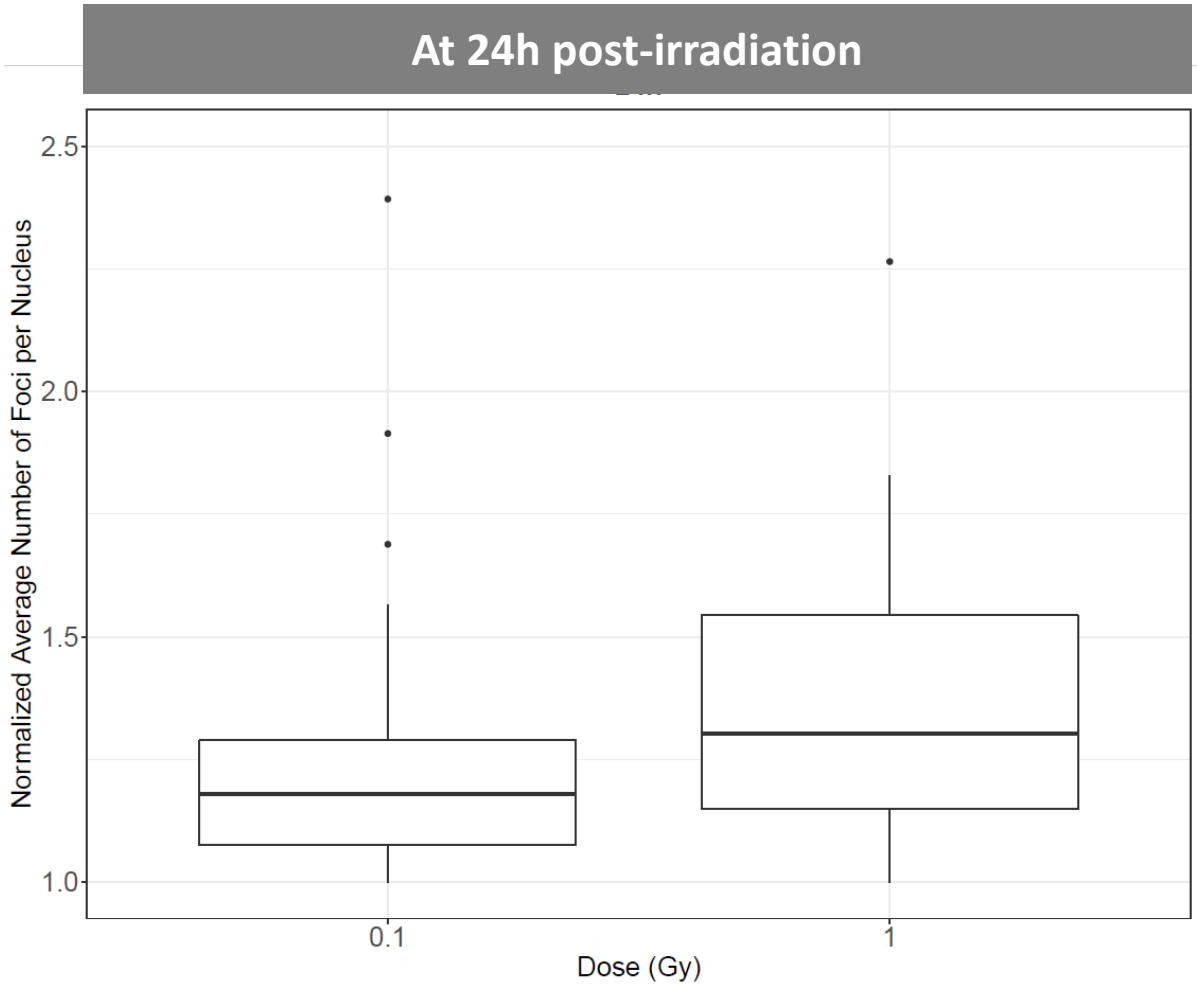
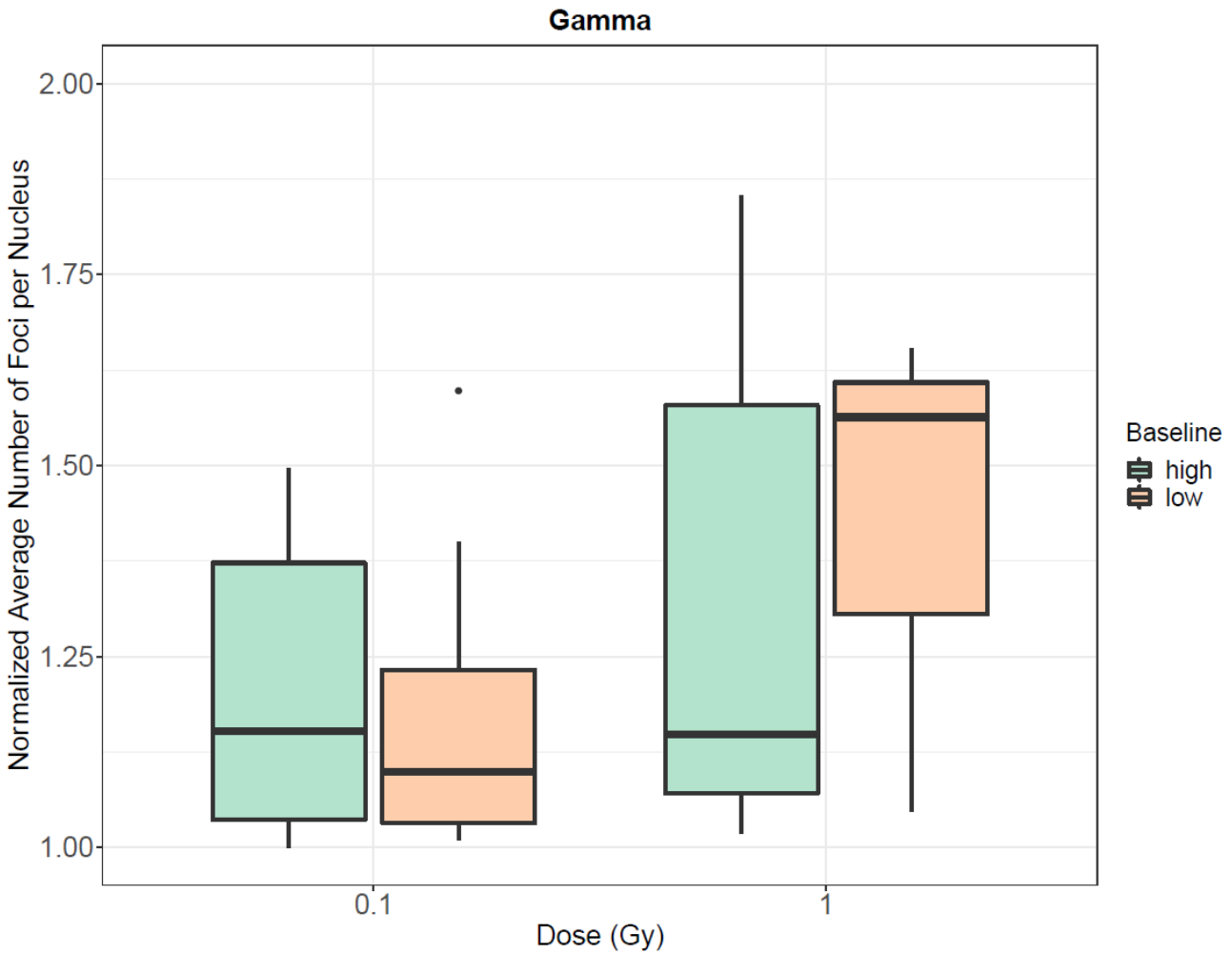
Radiation Response: DNA Damage for Gamma





Radiation Response for Extreme Baselines (gamma)



Radiat Res. 2019 Jul;192(1):1-12. doi: 10.1667/RR15338.1. Epub 2019 May 13.

Dose, LET and Strain Dependence of Radiation-Induced 53BP1 Foci in 15 Mouse Strains *Ex Vivo* Introducing Novel DNA Damage Metrics.

Penninckx S[1,2], Cekanaviciute E[3,4], Degorre C[5], Guiet E[1], Viger L[1], Lucas S[2], Costes SV[1,4].

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Abstract

We present a comprehensive comparative analysis on the repair of radiation-induced DNA damage *ex vivo* in 15 strains of mice, including 5 inbred reference strains and 10 collaborative-cross strains, of both sexes, totaling 5 million skin fibroblast cells imaged by three-dimensional highthroughput conventional microscopy. Non-immortalized primary skin fibroblasts derived from 76 mice were subjected to increasing doses of both low- and high-LET radiation (X rays; 350 MeV/n 40Ar; 600 MeV/n 56Fe), which are relevant to carcinogenesis and human space exploration. Automated image quantification of 53BP1 radiation-induced foci (RIF) formation and repair during the first 4-48 h postirradiation was performed as a function of dose and LET. Since multiple DNA double-strand breaks (DSBs) are induced in a dose- and LET-dependent manner, our data suggest that when DSBs are formed within the same discrete nuclear region, referred to as the "repair domain", novel mathematical formalisms used to report RIF allowed us to conclude that multiple DSBs can be present in single RIF. Specifically, we observed that the number of RIF per Gy was lower for higher X-ray doses or higher LET particles (i.e., 600 MeV/n ^{56}Fe), suggesting there are more DSBs per RIF when the local absorbed dose increases in the nucleus. The data also clearly show that with more DSBs per RIF, it becomes more difficult for cells to fully resolve RIF. All 15 strains showed the same dose and LET dependence, but strain differences were preserved under various experimental conditions, indicating that the number and sizes of repair domains are modulated by the genetic background of each strain.

	E (MeV/n)	LET (keV/um)	Fluence (/100um^2)	Dose in water (Gy)
Si	350	61.1	1.1	0.107536
Si	350	61.1	3	0.29328
Fe	600	173.8	1.1	0.305888
Fe	600	173.8	3	0.83424