Persistent Sex-, Dose-, Dose Rate-, and Time-Dependent Plasma and Saliva EV miRNA Cargo after Ionizing Radiation Injury, Supporting EVs as Biomarkers and Mediators of ARS/DEARE

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UF College of Medicine
UNIVERSITY of FLORIDA

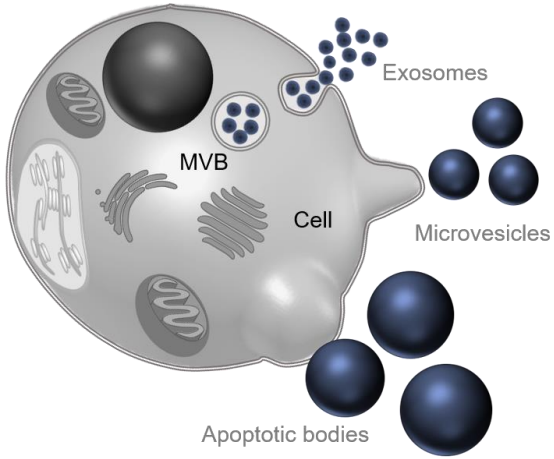
No relevant commercial relationships to disclose.

Objectives

- **Explain** how plasma EV-miRNA cargo changes after ionizing radiation (IR) injury as a function of sex, dose, dose rate, and time after exposure.

- **Describe** how persistent plasma EV-miRNA changes 14 days after radiation exposure can serve as longitudinal biomarkers for IR exposure.

- **Compare** plasma and salivary EV-miRNA signatures and evaluate salivary EVs a non-invasive way to perform EV profiling.

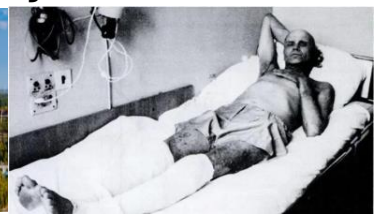


Radiation Exposure / Injury

- Radiation accidents and nuclear threats continue to be a hazard of the 21st century
- **Acute Radiation Syndrome** (**ARS**) occurs after sudden, full-body exposure to high levels of **ionizing radiation (IR)** in a short period of time.
- After the initial immune dysfunction and shock, chronic injuries can arise and develop over time, known as the **Delayed Effects of Acute Radiation Exposure** (**DEARE**).
  → persistent **infection susceptibility**

- Concern about possible use of nuclear weapons or radiation to harm citizens and service members
  - stealth nuclear devices *in public locations*

Chernobyl 1986 → **Tohoku 2011**

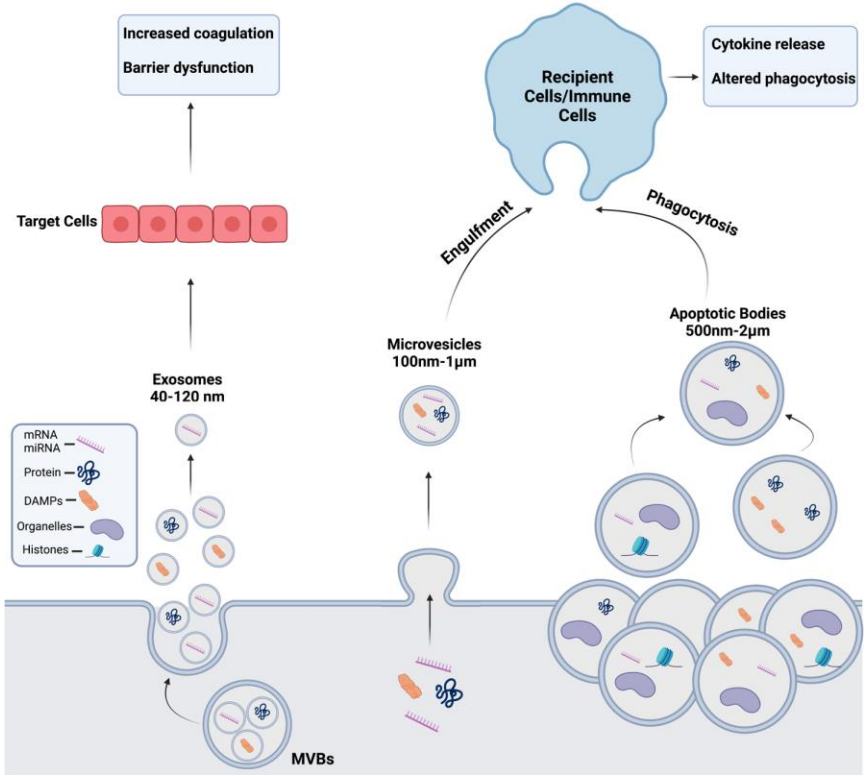
Radiation Exposure / Injury

Unmet needs:

- Lack of biomarkers to identify radiation exposure
- Lack of radiation medical countermeasures (**MCM**) that can act as mitigators of radiation-induced injury (and concurrent polytrauma).

Chernobyl 1986 → **Tohoku 2011**

EVs are Key Mediators in “Cell to Cell” Communication



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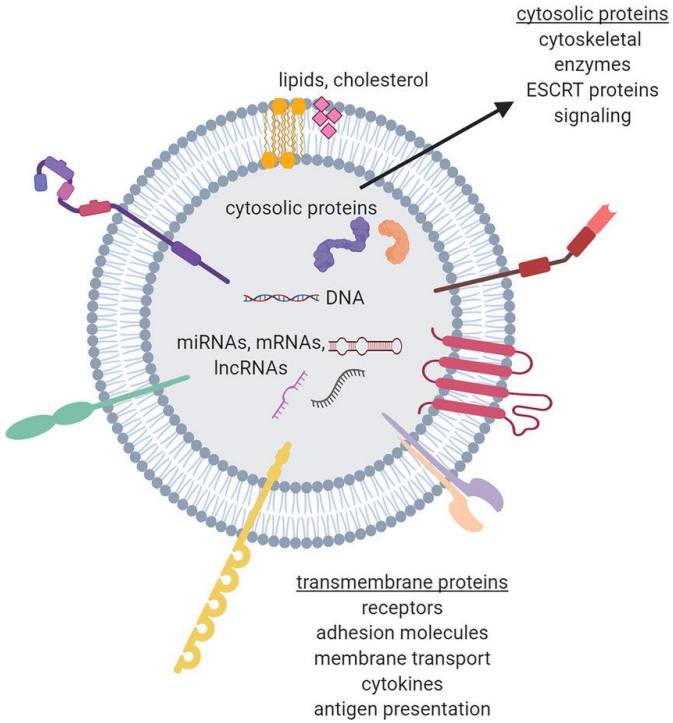
- Extracellular vesicles (**EVs**) are nano-sized vesicles encapsulated in a lipid bilayer released by all cells during physiological and pathological processes.
- Three major categories of EVs based on size:
  - exosomes (30 - 100 nm)
  - microvesicles (100 - 1000 nm)
  - apoptotic bodies (1000 - 5000 nm)
- Cell-cell communication *via* EVs occurs locally to neighboring cells and distally to various tissues and organs.
- All contribute to “cell to cell” communication which is regulated by the “cargo” within EVs.

EV Cargo is Diverse

- EVs contain molecular cargo:
  - Proteins and lipids
  - Nucleic acids (mRNA, lncRNA, miRNA)
  - miRNA can regulate many biological processes, including immune responses
- EVs are attractive as biomarkers, as their cargo can be indicators of cellular or tissue dysfunction / disease.



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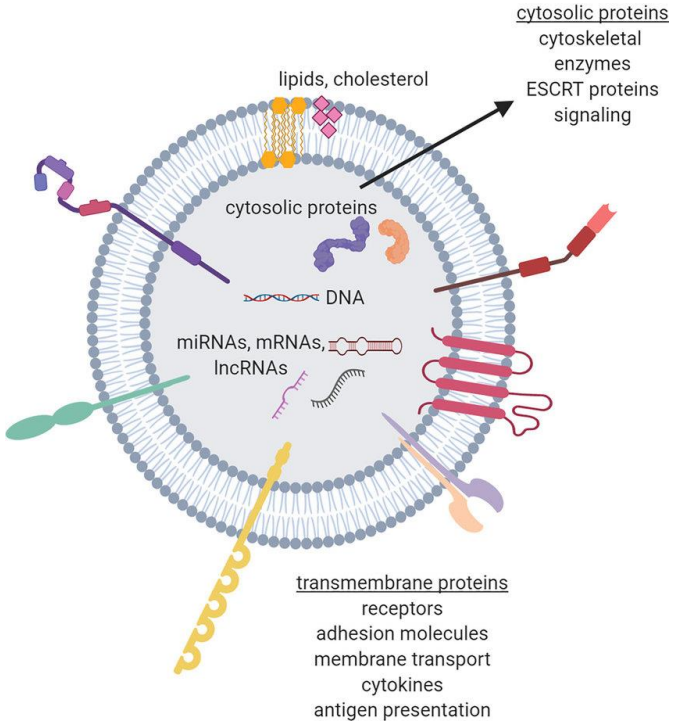
Hypothesis

We hypothesized that EVs may serve as longitudinal cell-free biomarkers of ionizing radiation injury



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CDMRP DEPARTMENT OF DEFENSE CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS

US ARMY Military Burn Research Program BA220315

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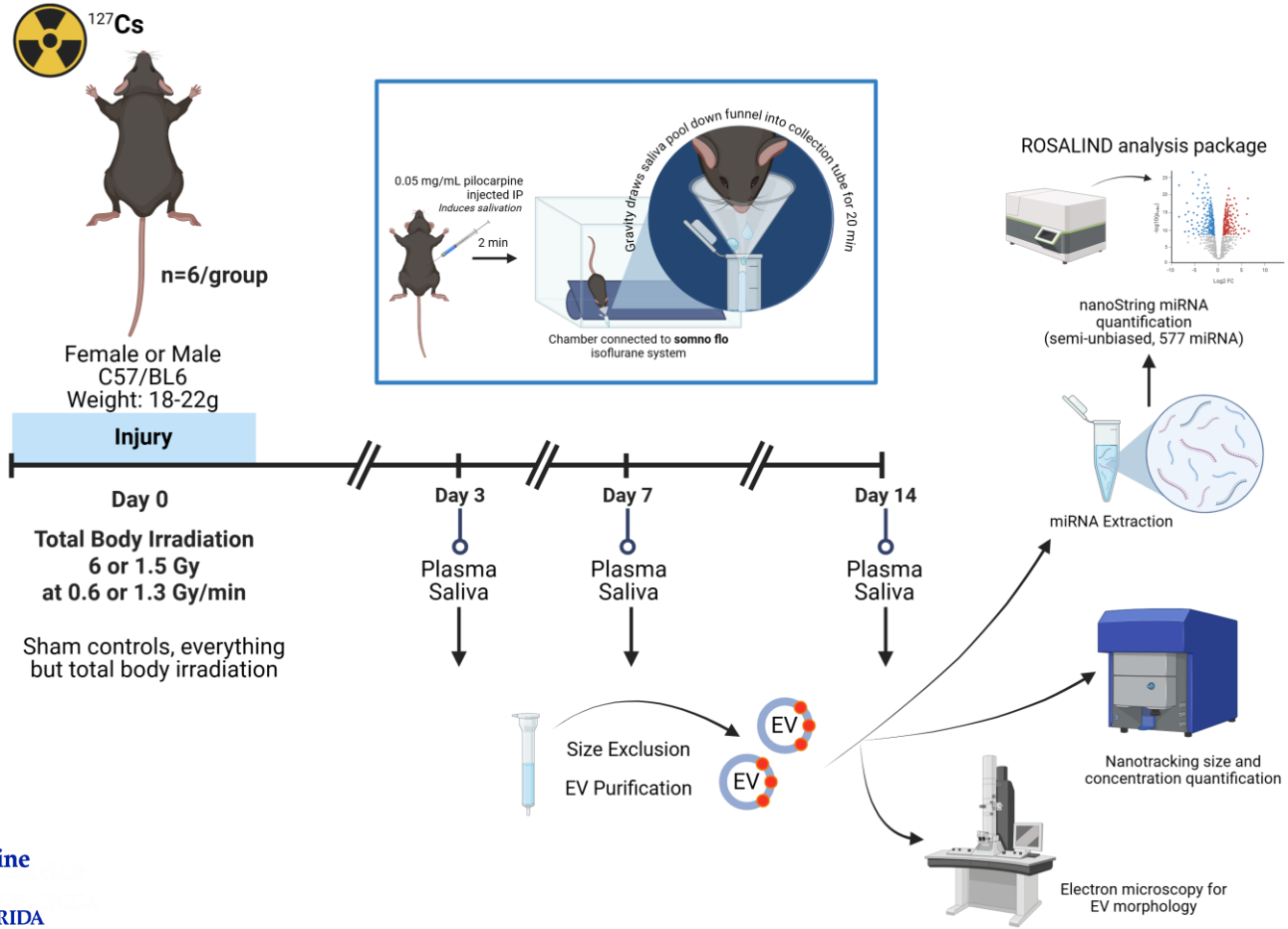
Experimental Design



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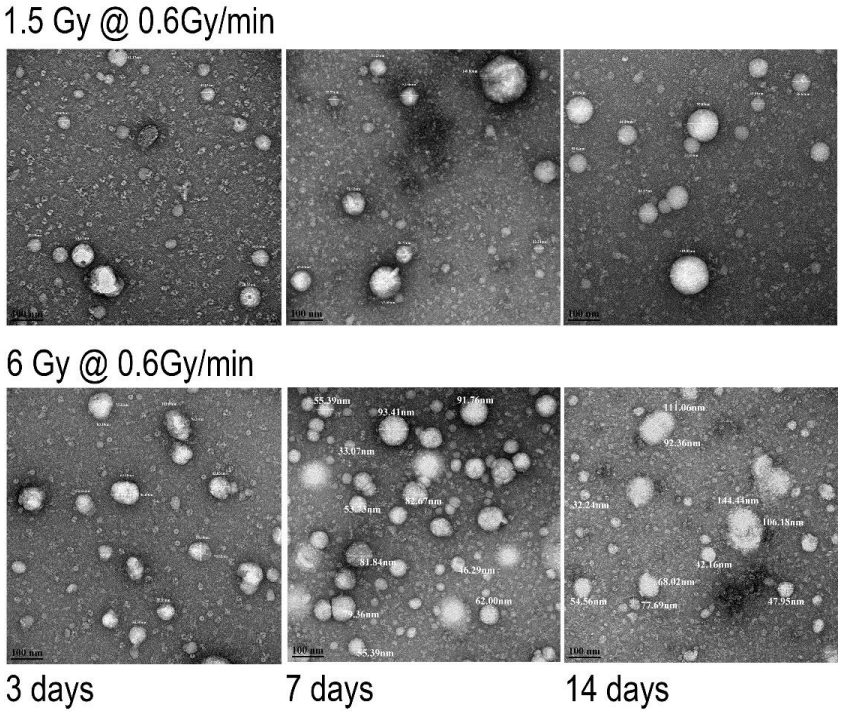
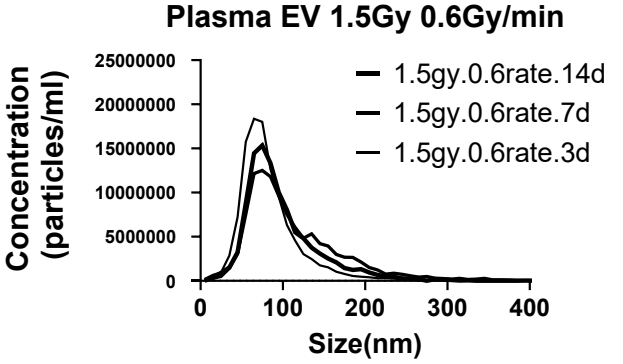
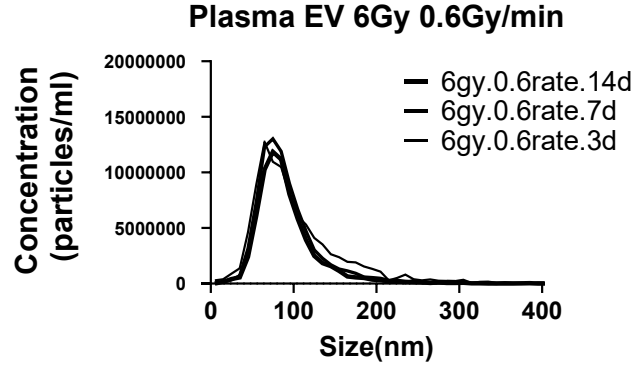
Results: Electron microscopy and nanotracking analysis confirmed plasma EV isolation







Morphology and size plots of **plasma** EV samples collected from 3, 7, and 14-**days** post injury, 1.5 Gy and 6 Gy **dose,** 0.6 Gy/min **dose rate**

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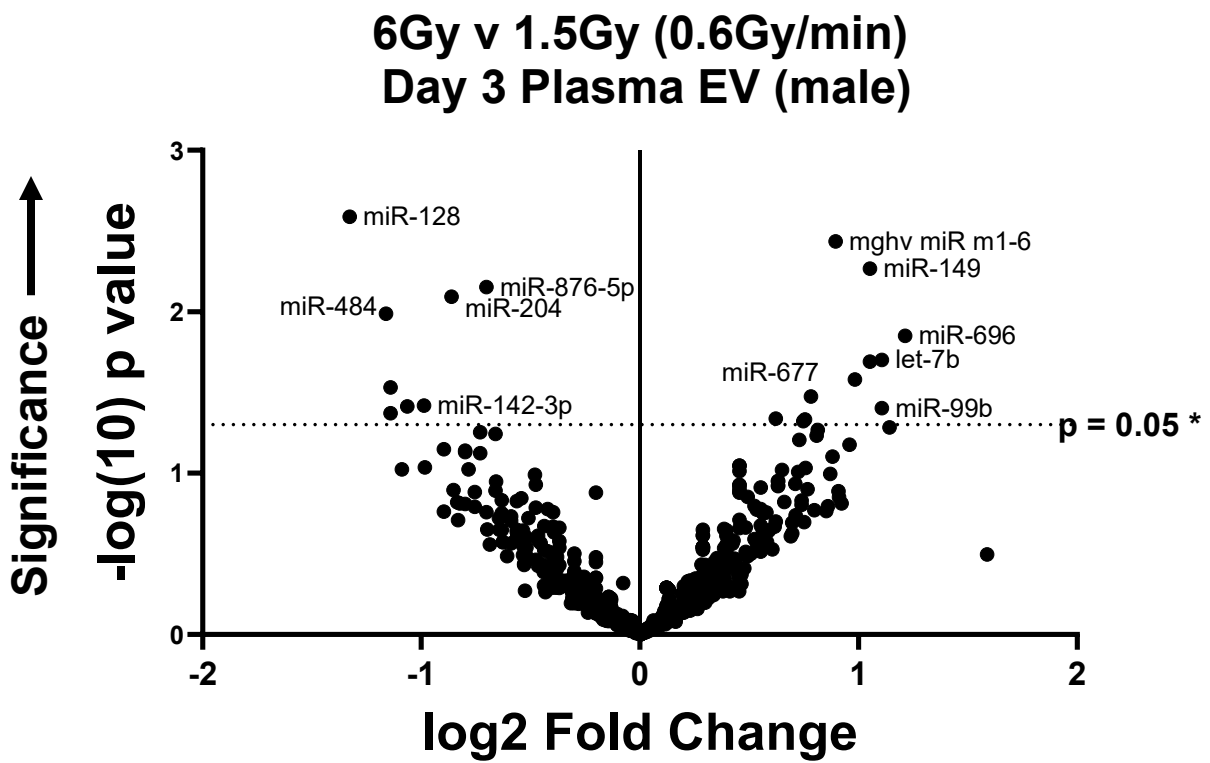
Results: EV miRNA cargo is dependent on IR dose

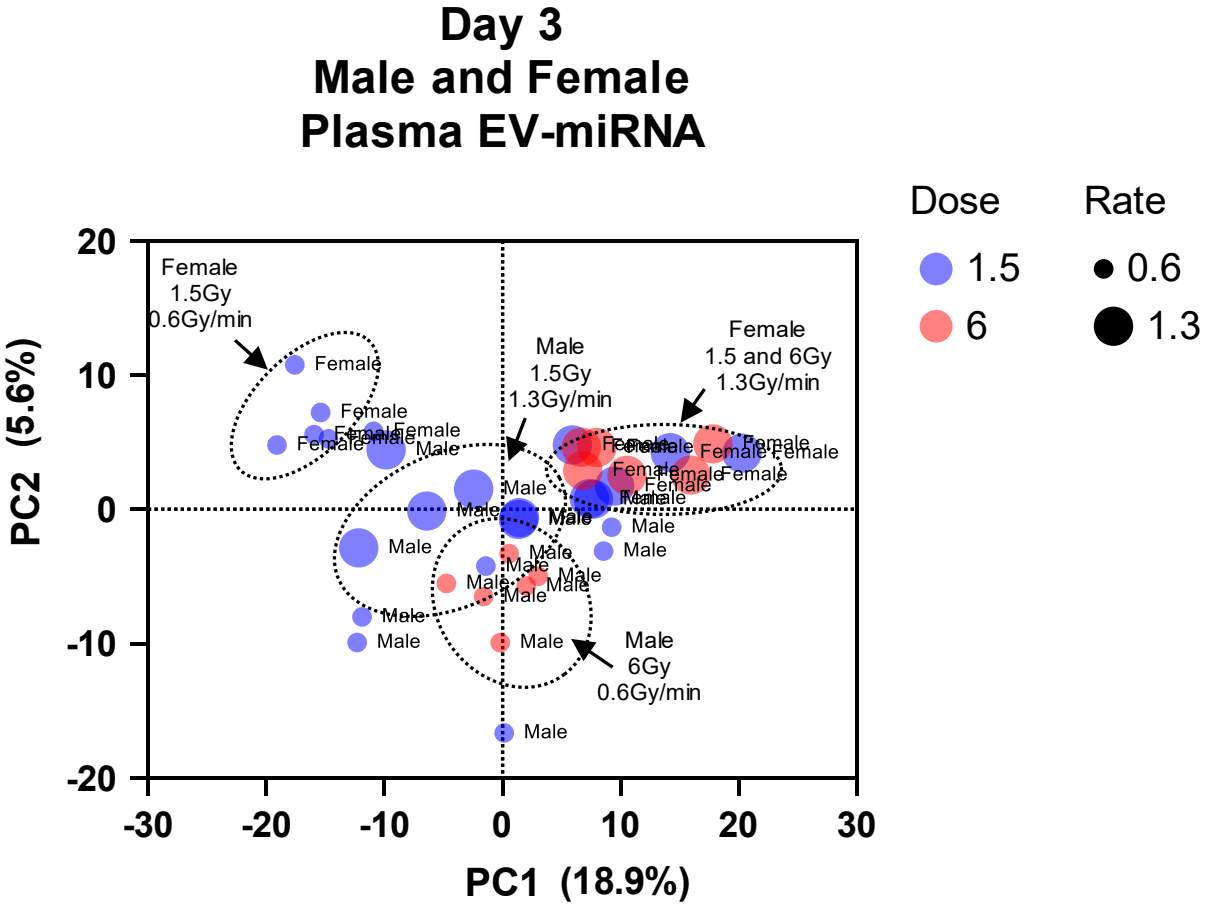
Comparison of EV miRNA cargo:

- **Sex** - Male
- 3-**days** post injury
- 0.6 Gy/min **dose rate**
- 6 Gy vs 1.5 Gy **dose**



Results: EV miRNA cargo clusters with IR dose, IR rate, sex and time-after-injury.

- **Principal component analysis (PCA) was used to identify dominant miRNAs that drive sex/dose/dose-rate/time-after-injury group separation.**
- **Dose** produced the largest shifts in PCA space
- **Dose-rates** further modulated clustering, supporting sensitivity beyond total dose alone.



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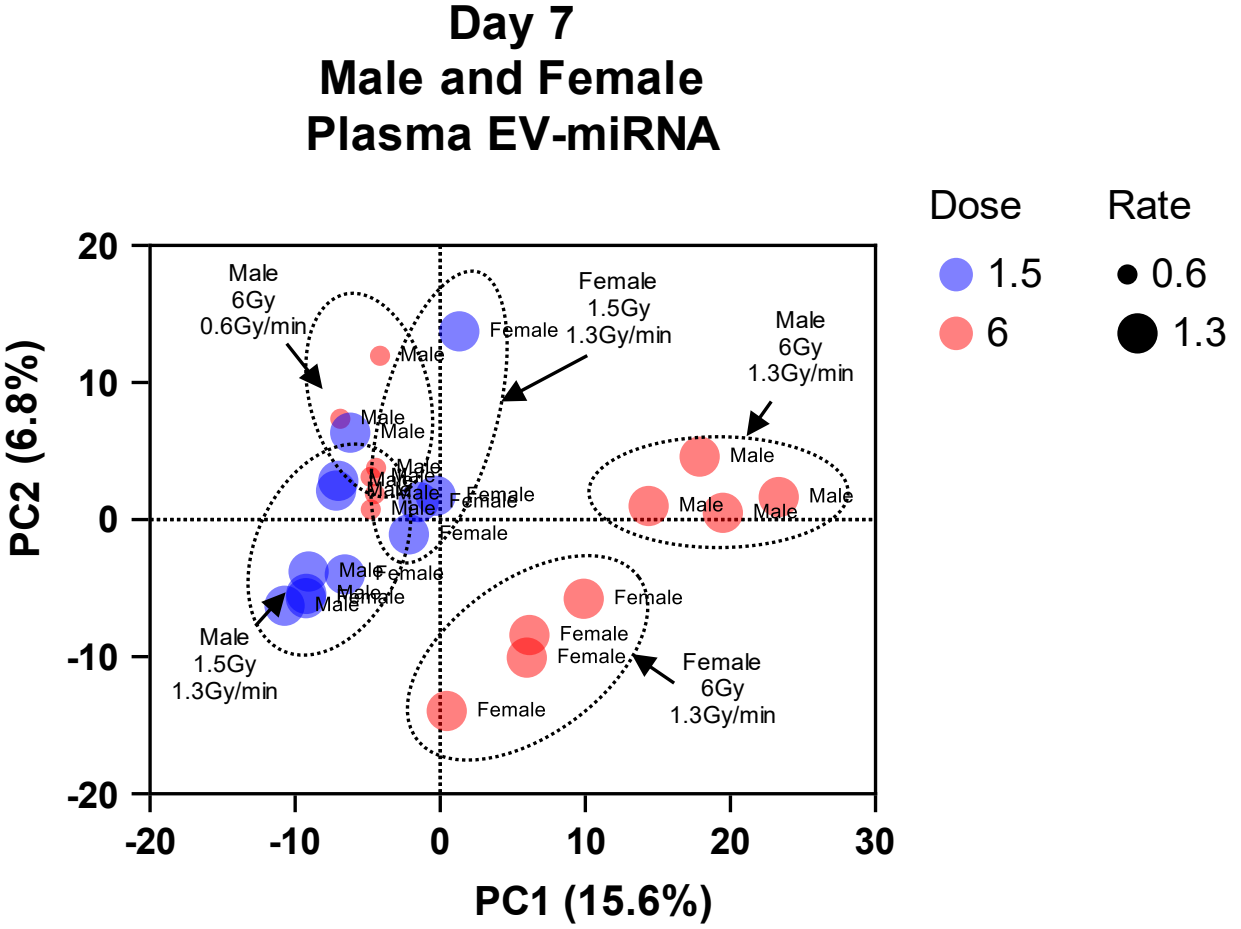

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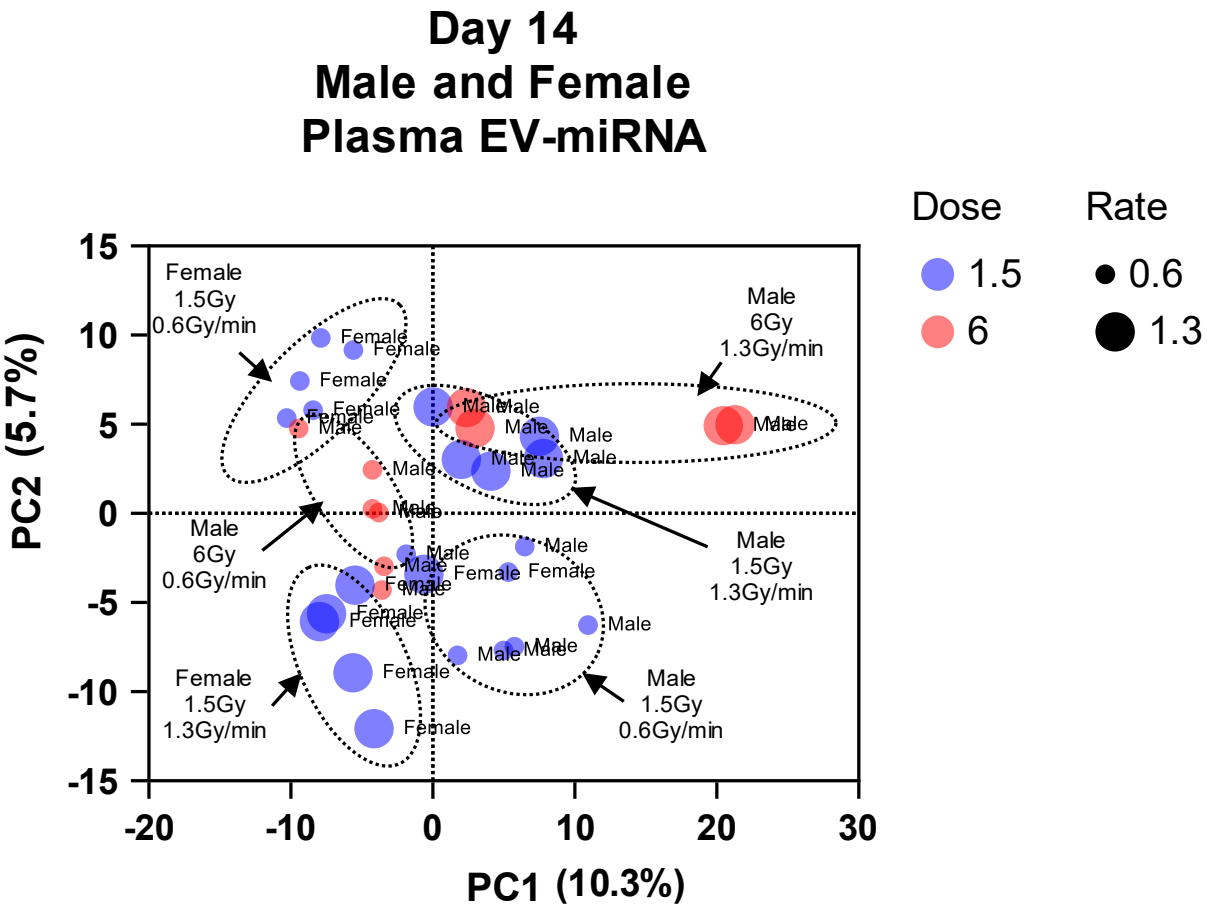
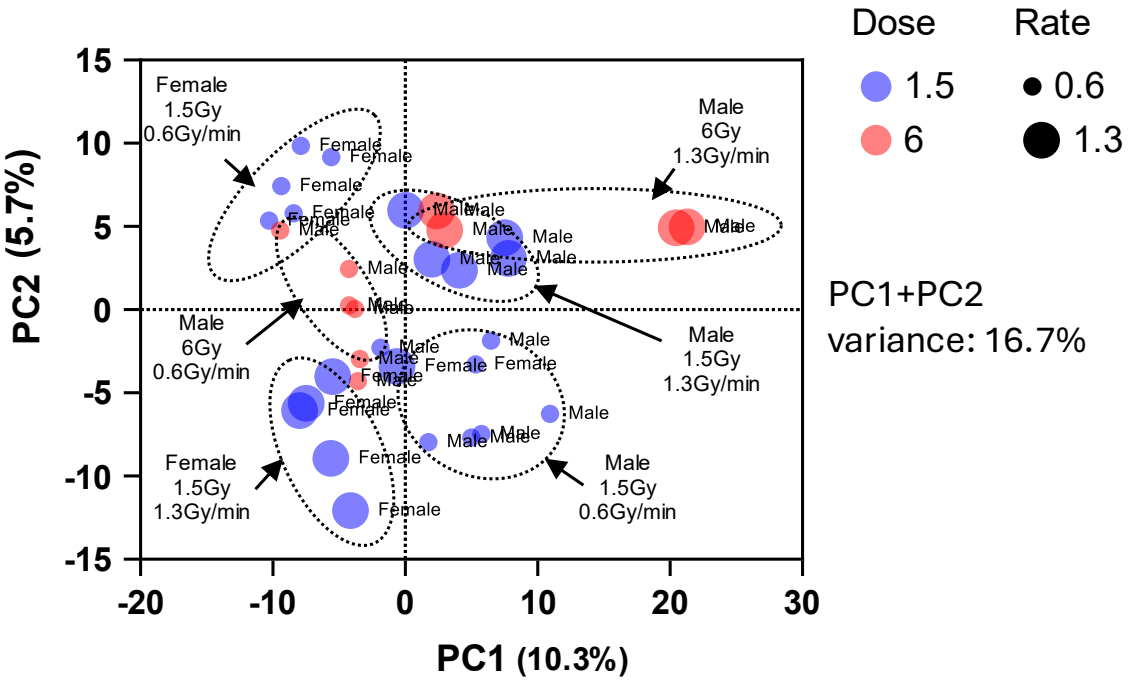


Results: EV miRNA cargo clusters with IR dose, IR rate, sex and time-after-injury.

- For **both sexes**, conserved high-loading plasma drivers included:
  - let-7a
  - miR-155
  - miR-34c
  - miR-543
  - miR-153
  - miR-700
  - miR-24
  - miR-43-3p
  - miR-1905

- **These miRNA regulate inflammatory and stress/DNA-damage-linked regulation.**



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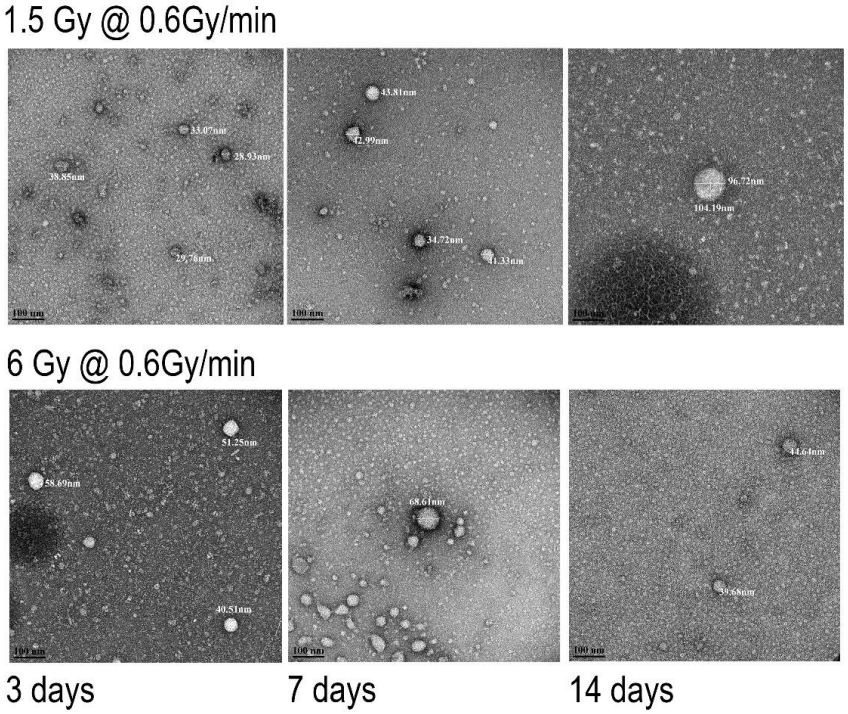

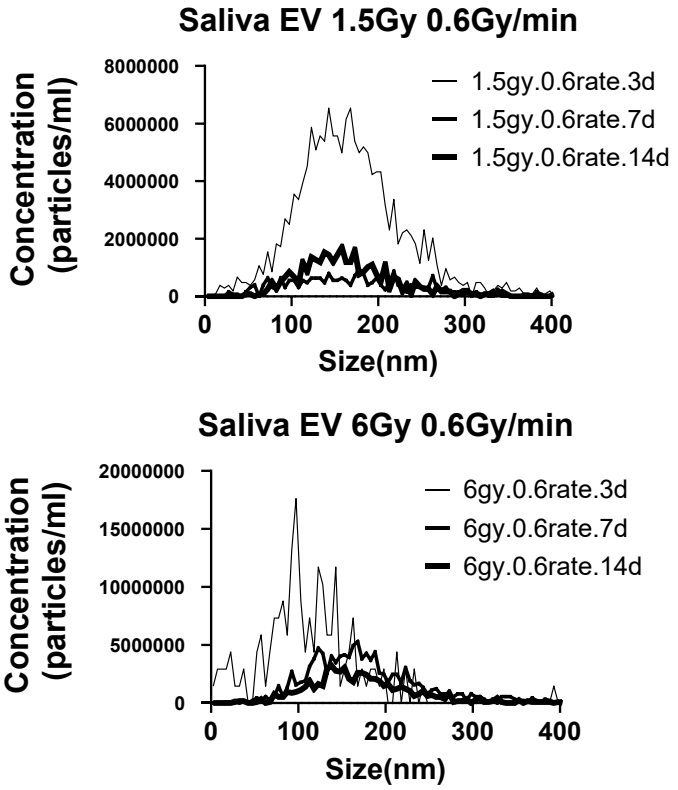

Morphology and size plots of **saliva** EV samples collected from 3, 7 and 14-**days** post injury, 1.5Gy and 6 Gy **dose,** 0.6Gy/min **dose rate**

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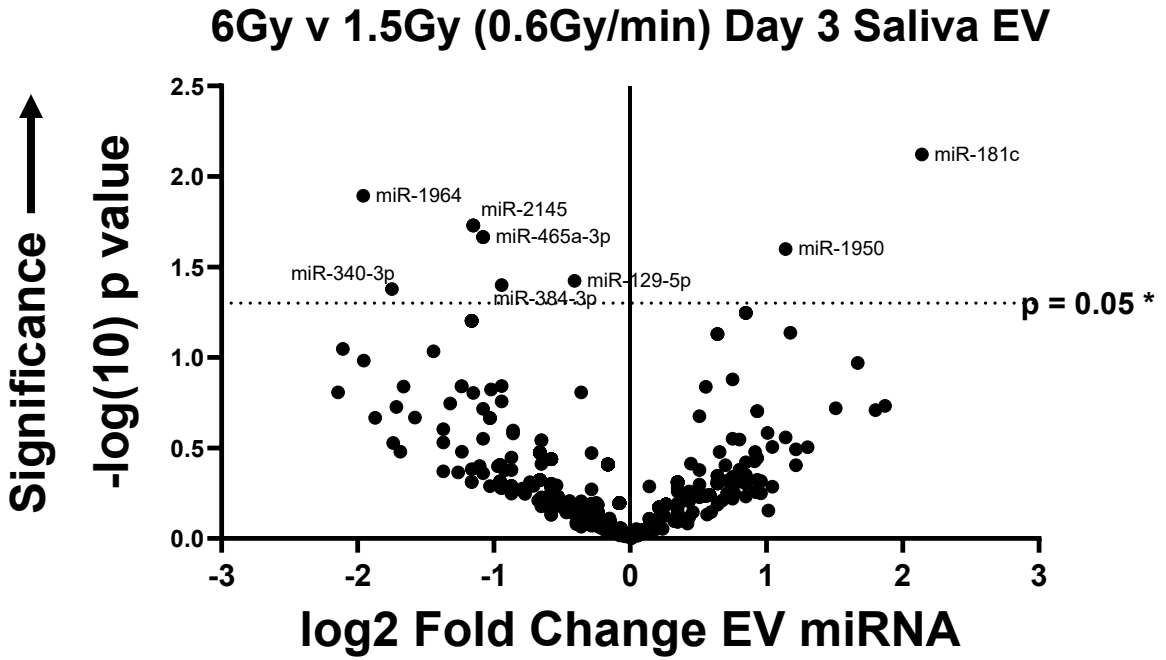
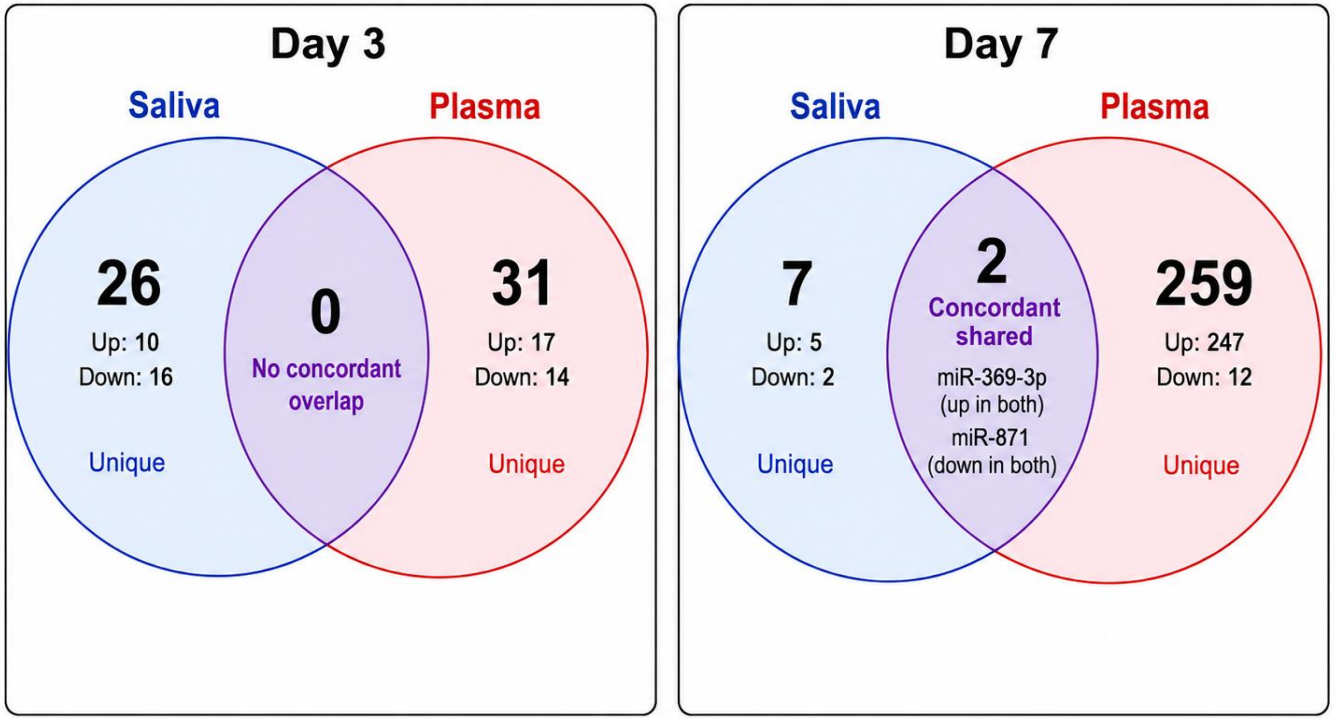
Results: Saliva EV miRNA cargo is also dependent on IR dose

Comparison of EV miRNA cargo at:

- 3-**days** post injury
- 0.6 Gy/min **dose rate**
- 6 Gy vs 1.5 Gy **dose**



Results: Only two miRNAs overlapped concordantly between saliva and plasma EV cargo and only at Day 7 post-injury



Saliva captures a limited subset of the broader plasma EV-miRNA response.

Conclusions

- **EV miRNA cargo provides a time-resolved readout of radiation exposure sensitive to IR dose and dose rate**
  - Data also indicate a sex-dependent response in miRNA cargo
- **Persistence of changes in EV-miRNA at 14-days post exposure supports the use of EVs as longitudinal biomarkers**
- **Saliva EVs have the potential to provide an early, non-invasive way to screen for IR exposures in a dose dependent manner.**
- Data not shown also indicate that these EVs drive downstream immune dysfunction after IR injury and thus, EVs are biomarkers with functional consequences.

Future Directions

We are seeking funding to develop an EV-based biosensor (plasma / saliva-based):

- Microfluidic EV enrichment → rapid miRNA detection for field use
- Validate in human / primate samples

UF UNIVERSITY of FLORIDA
J. Crayton Pruitt Family Department of Biomedical Engineering

Wake Forest University School of Medicine

We have utilized our murine combined burn and IR injury model to evaluate if concurrent burn injury alters EV cargo and utility of EVs as biomarkers

We have identified EVs that arise after radiation injury (and burn injury, and RCI) are *immunopathogenic,* and we are investigating medical countermeasures targeting these EV

- **UF Burn Lab** – PI Rob Maile, PhD
  - Madelyn Bucci, MS
  - Heaven Brandt
  - Ryan Ugarte
  - Joe Canestrari
  - Angel Charles, MD
  - Denise Hernandez
  - Micah Willis, PhD
  - Simon Reinhard *et al*
- **Wallet Lab** – PI Shannon Wallet, PhD
- Sepsis and Critical Illness Research Center (SCIRC)
  - Directors: Philip Efron, MD FACS FCCM, Rob Maile, PhD
    -Lyle L. Moldawer, PhD, Ricardo Ungaro, Alicia Mohr, MD, FACS, FCCM, *et al*

- UF Burn: Andrea Munden MD / Amalia Cochran MD / Audrey Currans MD / Janet Popp MSN, RN, CCRN / Richard Bush

QUESTIONS?

UF Burn Research Lab

NIH NIGMS R01 GM131124

NIH NIGMS R01 GM146134

NIH NIGMS F31 GM149109 (Willis)

DoD NDSEG Fellowship
US ARMY MBRP MB230028

US ARMY MBRP BA220315

Medical Student Research Program
NIH T35 HL007489 (Berceli / Maile)

Molecular Biology and Gene Therapy in Burns and Trauma
NIH T32 GM08431 (Efron)