Development of Radiation Countermeasures for Acute Radiation Syndrome: Current Status of Biomarker Identification and Validation

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The speaker reports no conflicts of interest.

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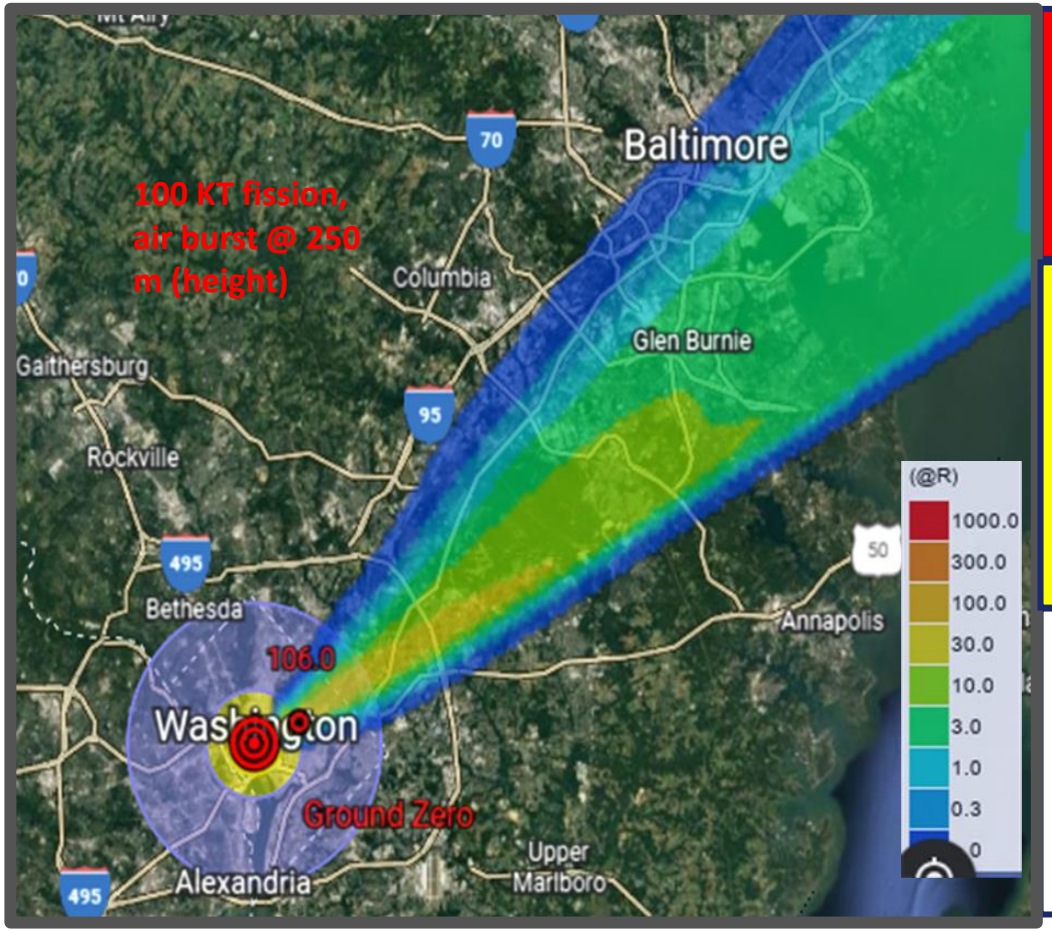
RADIATION COUNTERMEASURES
Instantaneous Exposure at Detonation



Severe Injuries (~1.5 miles)

- Rescue efforts in this range not likely to be effective
- MCM not likely to increase operational abilities
- MCM not helpful to exposed victims

Moderate Injuries (~2.5 miles)

- Rescue efforts in this range would be effective and enhanced with MCM
- MCM would improve outcomes in this range

Limited to Minor Injuries (~6.5 miles)

- Rescue efforts in this range would be effective
- MCM would vastly increase operational time
- MCM would improve outcomes in this range

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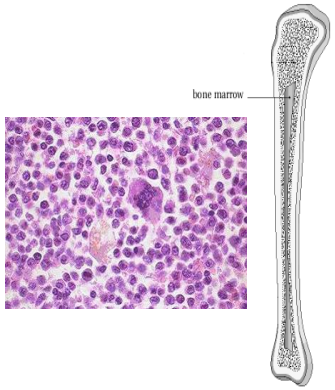
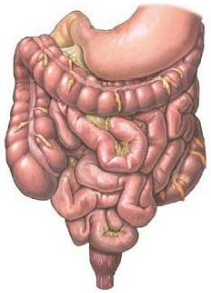
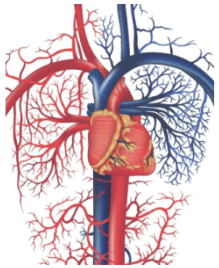
ARS's MAJOR CLINICAL SUBSYNDROMES

INCREASING INJURY SEVERITY







Central nervous system

Cardiovascular

Gastrointestinal

Hematopoietic

Subclinical

<1 Gy

2 - 6 Gy

6 - 10 Gy

>10 Gy

Neurovascular syndrome

SURVIVAL DECLINES

LATENCY PERIOD DECREASES

Acute, delayed, late and chronic effects

Also pulmonary and cutaneous

INCREASING EXPOSURE (DOSE) LEVELS

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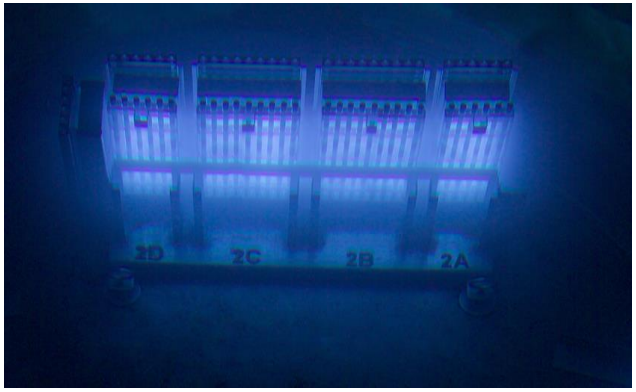
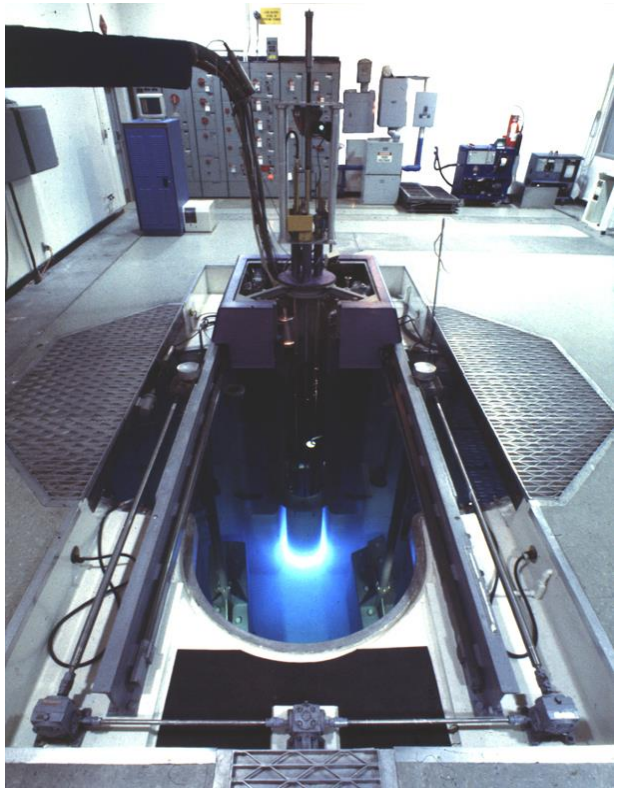
Radiation Sources for Studies

Cobalt-60
Gamma- Irradiator



TRIGA Reactor



SARRP

Low Level Cobalt-60
Panoramic Irradiator

Linear Accelerator

Biomarkers

Radiation exposure dose assessment

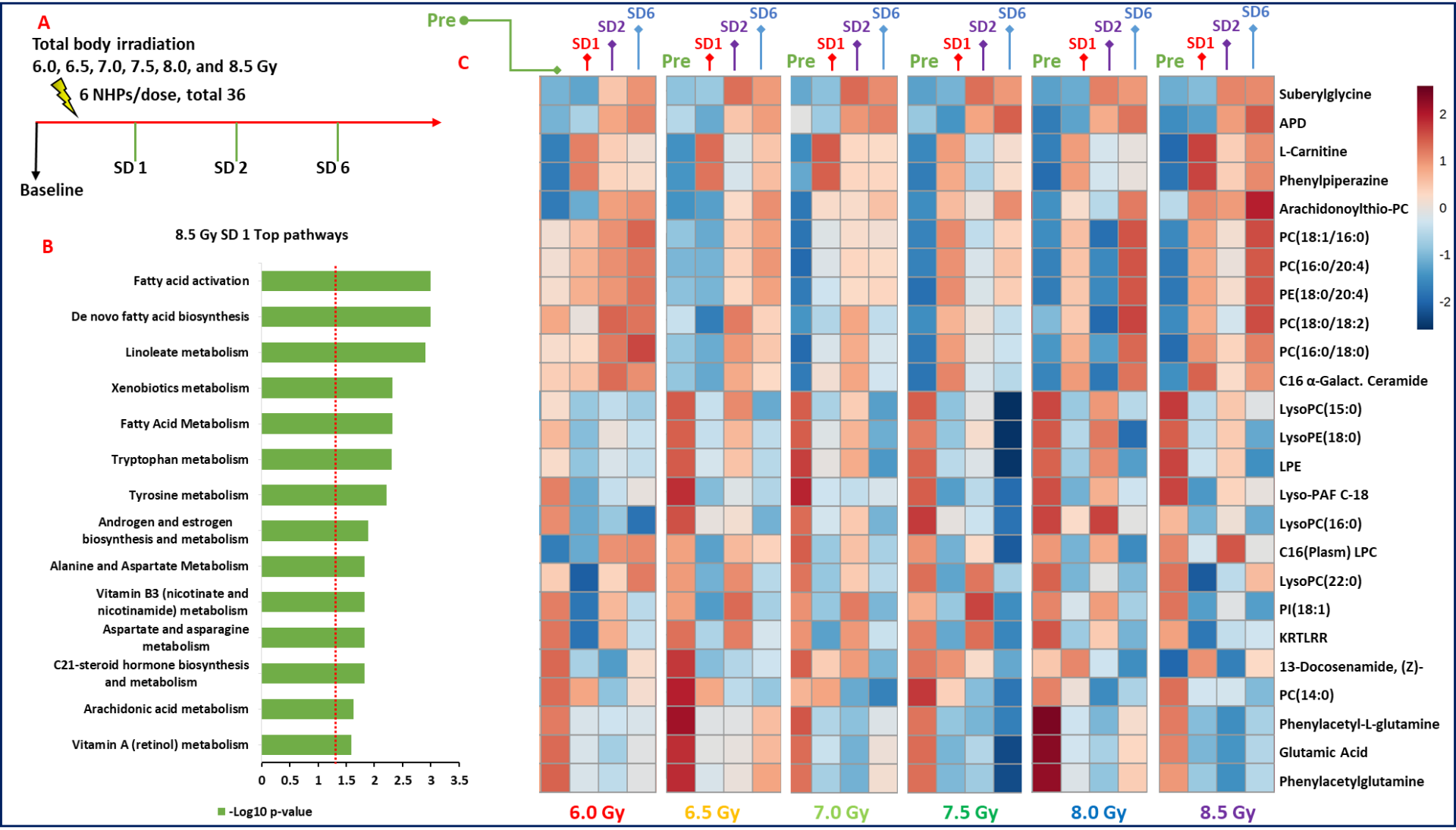
FDA "Animal Rule": Development of countermeasures where human clinical trials are neither ethical nor feasible

MCM dose conversion from animal models to human

Countermeasure efficacy biomarkers:

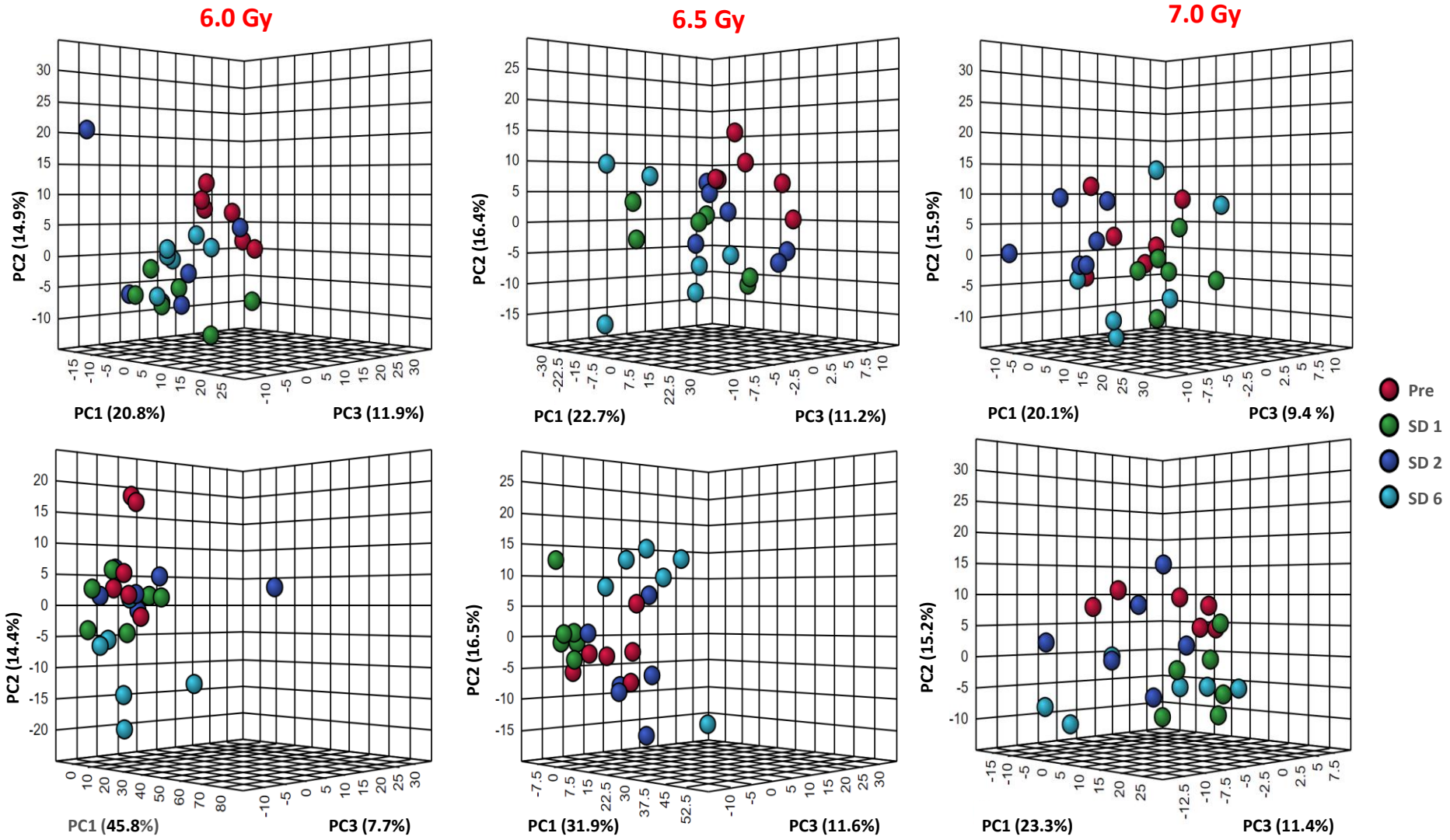
1. **Induction should depend on the drug's mechanism of action**
2. **Induced under irradiated and unirradiated conditions**
3. **Should express over a range of doses and correlate with survival**
4. **Responsive should be across multiple species.**
5. **Should be quantifiable by using readily available assays, in samples obtainable by relatively non-invasive procedures**

Study Scheme for omics study and dysregulated pathway/metabolites



Int J Rad Oncol Biol Phys 114:310-320 2022

Exposure to radiation induces strong dysregulation in metabolomic and lipidomic profiles in NHPs.



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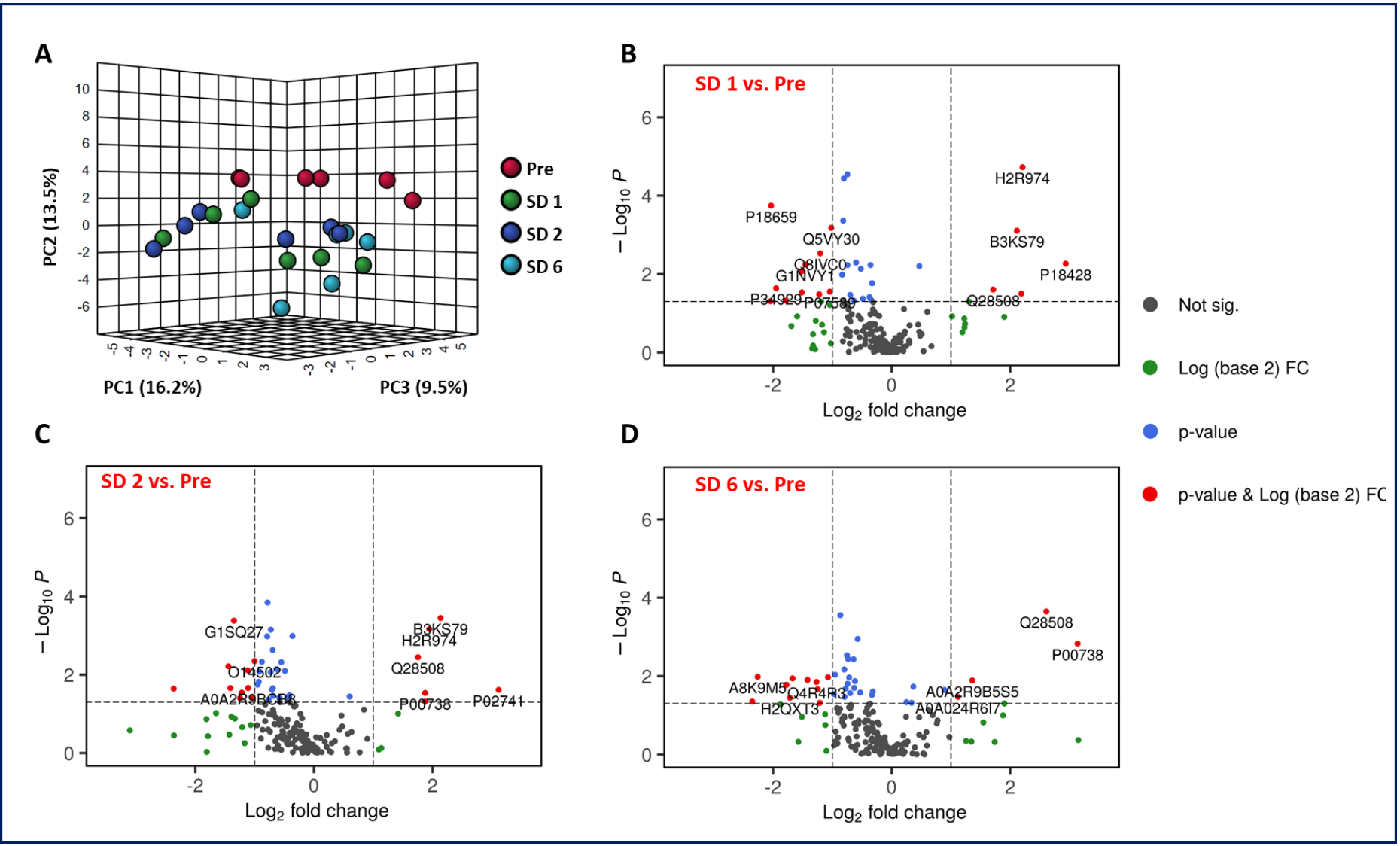
PCA comparing chages of the proteomic profies - 8.5 Gy



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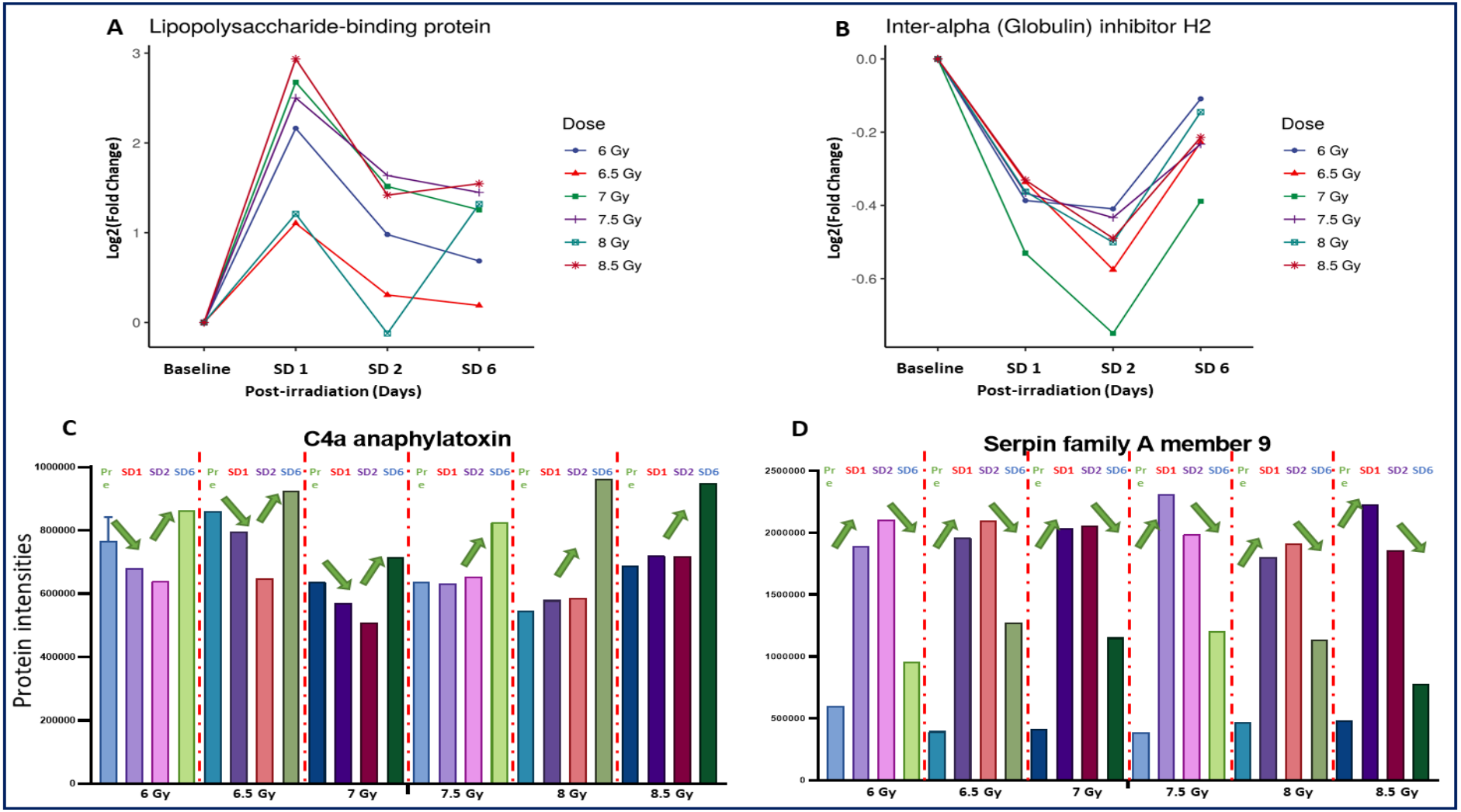
Temporal patterns of protein abundance for all radiation doses as a function of time for a subset of proteins



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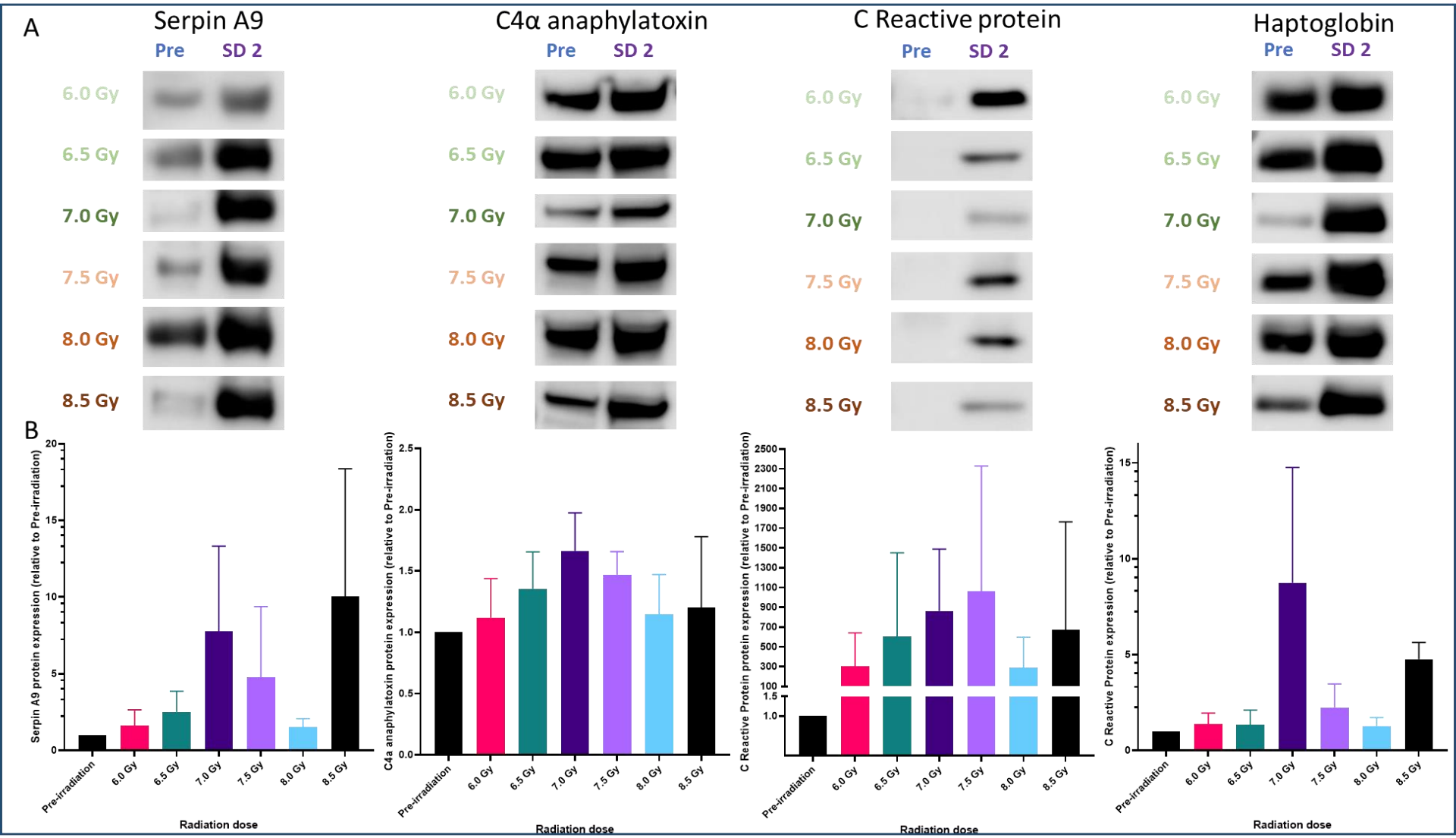
Validation of discovery proteomics data for a select group of proteins using western blot analysis



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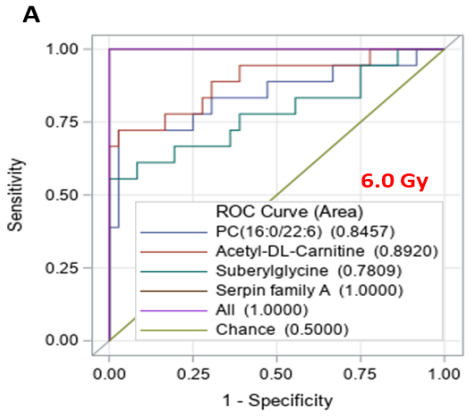
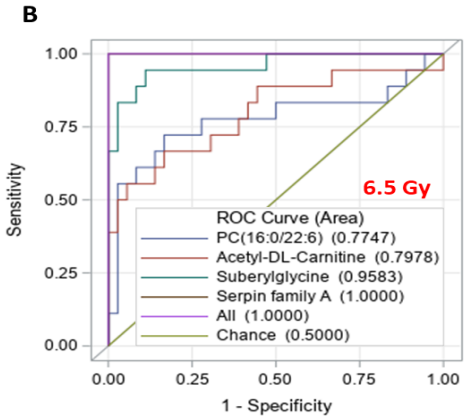
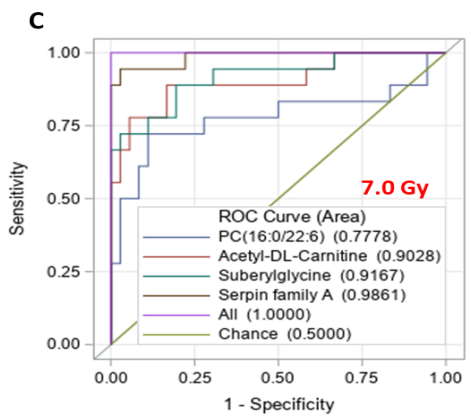
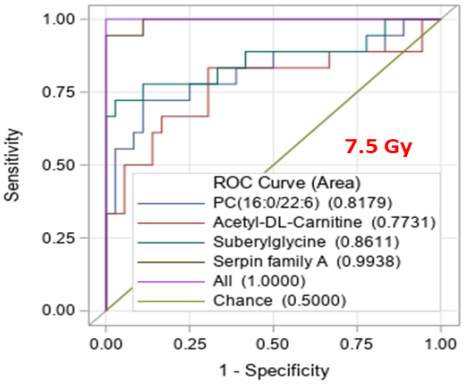
Development of a 4-analyte multi-omics panel for assessment of radiation exposure in NHPs

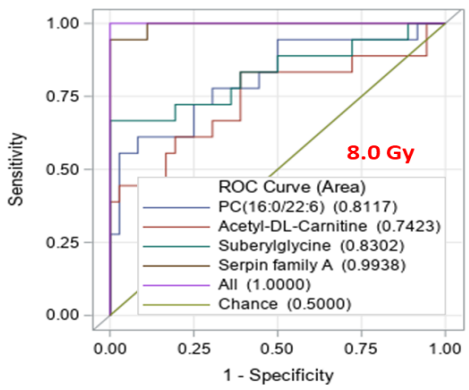


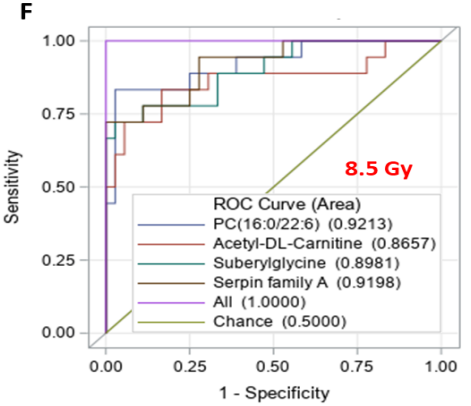




	6.0 Gy				6.5 Gy				7.0 Gy			
Name	P-value	FDR	Fold Change	log2(FC)	P-value	FDR	Fold Change	log2(FC)	P-value	FDR	Fold Change	log2(FC)
Serpin Family A member 9	3.89E-16	9.88E-14	3.61	1.85	1.17E-17	2.98E-15	3.89	1.96	6.24E-17	1.59E-14	3.82	1.93
Acetyl-DL-Carnitine	7.72E-14	9.80E-12	2.41	1.27	2.99E-13	2.54E-11	2.42	1.28	3.52E-15	4.47E-13	2.51	1.33
PC(16:0/22:6)	1.11E-10	3.97E-09	3.99	2.00	2.23E-04	8.19E-04	2.82	1.49	2.01E-04	7.35E-04	2.72	1.44
Suberylglycine	8.36E-08	6.85E-07	4.78	2.26	2.80E-13	2.54E-11	5.88	2.56	3.25E-12	1.65E-10	6.28	2.65







	7.5 Gy				8.0 Gy				8.5 Gy			
Name	p.value	FDR	Fold Change	log2(FC)	p.value	FDR	Fold Change	log2(FC)	p.value	FDR	Fold Change	log2(FC)
Serpin Family A member 9	6.10E-18	1.55E-15	4.00	2.00	3.47E-17	8.81E-15	3.53	1.82	1.54E-11	2.79E-10	3.54	1.82
Acetyl-DL-Carnitine	6.01E-04	1.64E-03	2.12	1.09	3.32E-04	9.91E-04	2.47	1.31	6.63E-07	2.63E-06	3.00	1.58
PC(16:0/22:6)	4.46E-06	2.70E-05	2.47	1.31	7.07E-06	3.92E-05	2.30	1.20	1.77E-10	2.36E-09	3.46	1.79
Suberylglycine	1.05E-10	3.34E-09	6.01	2.59	2.74E-10	7.73E-09	5.42	2.44	7.07E-12	1.63E-10	5.67	2.50

Logistic regression model

Near 100% efficacy of prediction across all doses until 6 d post-irradiation

PC (16:0/22:6), Acetyl-carnitine, Suberylglycine Serpin Family A9

Int J Rad Oncol Biol Phys (in press) 2022

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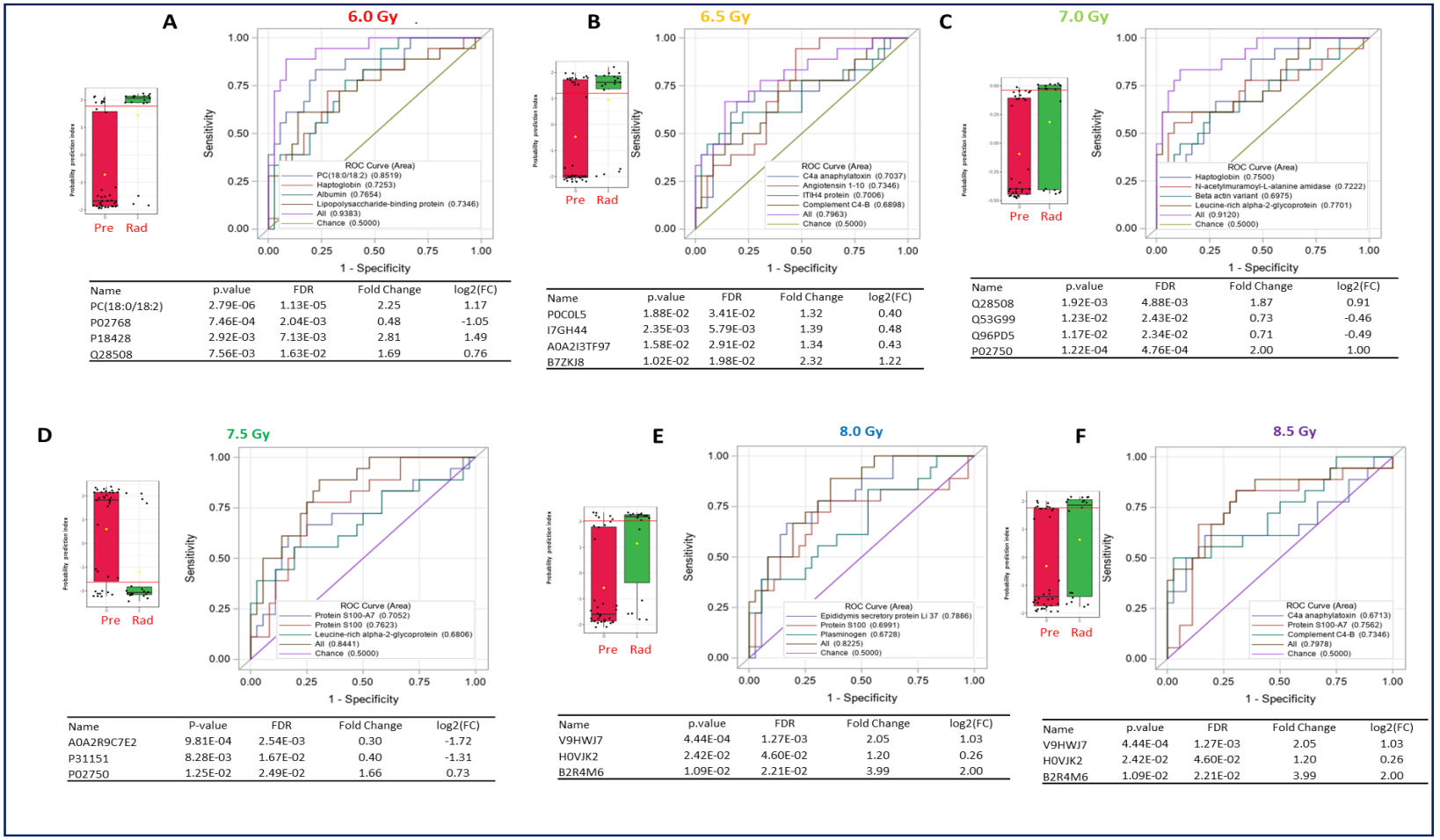
A dose-specific multi-omics biomarker panel to predict the extent of exposure to gamma radiation



Name	p.value	FDR	Fold Change	log2(FC)
PC(18:0/18:2)	2.79E-06	1.13E-05	2.25	1.17
P02768	7.46E-04	2.04E-03	0.48	-1.05
P18428	2.92E-03	7.13E-03	2.81	1.49
Q28508	7.56E-03	1.63E-02	1.69	0.76

Name	p.value	FDR	Fold Change	log2(FC)
P0C0L5	1.88E-02	3.41E-02	1.32	0.40
I7GH44	2.35E-03	5.79E-03	1.39	0.48
A0A2I3TF97	1.58E-02	2.91E-02	1.34	0.43
B7ZKJ8	1.02E-02	1.98E-02	2.32	1.22

Name	p.value	FDR	Fold Change	log2(FC)
Q28508	1.92E-03	4.88E-03	1.87	0.91
Q53G99	1.23E-02	2.43E-02	0.73	-0.46
Q96PD5	1.17E-02	2.34E-02	0.71	-0.49
P02750	1.22E-04	4.76E-04	2.00	1.00

Name	P-value	FDR	Fold Change	log2(FC)
A0A2R9C7E2	9.81E-04	2.54E-03	0.30	-1.72
P31151	8.28E-03	1.67E-02	0.40	-1.31
P02750	1.25E-02	2.49E-02	1.66	0.73

Name	p.value	FDR	Fold Change	log2(FC)
V9HWJ7	4.44E-04	1.27E-03	2.05	1.03
H0VJK2	2.42E-02	4.60E-02	1.20	0.26
B2R4M6	1.09E-02	2.21E-02	3.99	2.00

Name	p.value	FDR	Fold Change	log2(FC)
V9HWJ7	4.44E-04	1.27E-03	2.05	1.03
H0VJK2	2.42E-02	4.60E-02	1.20	0.26
B2R4M6	1.09E-02	2.21E-02	3.99	2.00

Index panel based on Logistic Regression model

and

PPI

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US FDA approved countermeasures for ARS

- **Radiomitigators for H-ARS approved by US FDA**
  - **G-CSF/Neupogen/filgrastim: March 2015**
  - **PEGylated G-CSF/Neulasta/Pegfilgrastim: November 2015**
  - **GM-CSF/Leukine/Sargramostim: March 2018**
  - **TPO – Nplate/Romiplostim (A synthetic TPO agonist): January 2021**
    - **All are repurposed and radiomitigators**
    - **None as radioprotector**
    - **Neupogen/Neulasta effective in NHPs only when used with full supportive care**
    - **May not be useful during mass casualty scenario**
    - **Side effects**

Radiation countermeasures

- **Medical countermeasures are being developed following US FDA Animal Rule.**
- **AFRRI has all possible radiation sources under one roof.**
- **We conduct studies from Discovery of MCM (with corporate partners) to phase III equivalent large animal studies.**
- **We use various animal models from mice to nonhuman primates.**
- **Large number of agents are under development: Small molecules, Biologicals, Cellular products**

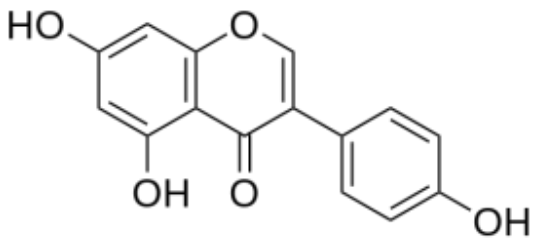
Promising Radioprotectors under Advanced Development

Identified and Patented at AFRRI

- **Genistein - Soy isoflavone**
- **Gamma-tocotrienol – Vitamin E component**

- **Being developed in collaboration with corporate partners**
- **Currently studied in NHPs**

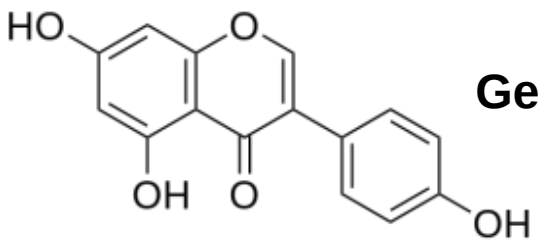
BIO 300 Background



Genistein

- **Active ingredient (genistein) discovered as a potent radioprotectant by AFRRI**
- **DOD was issued patents for use against lethal radiation and subsequently granted an exclusive worldwide license to Humanetics Corporation**
- **BIO 300 is in advanced development under 4 open INDs**
- **FDA Fast Track and Orphan Drug designations for H-ARS**
- **DOD has provided significant funding to Humanetics for development activities for ARS**
- **Significant work continues at AFRRI**

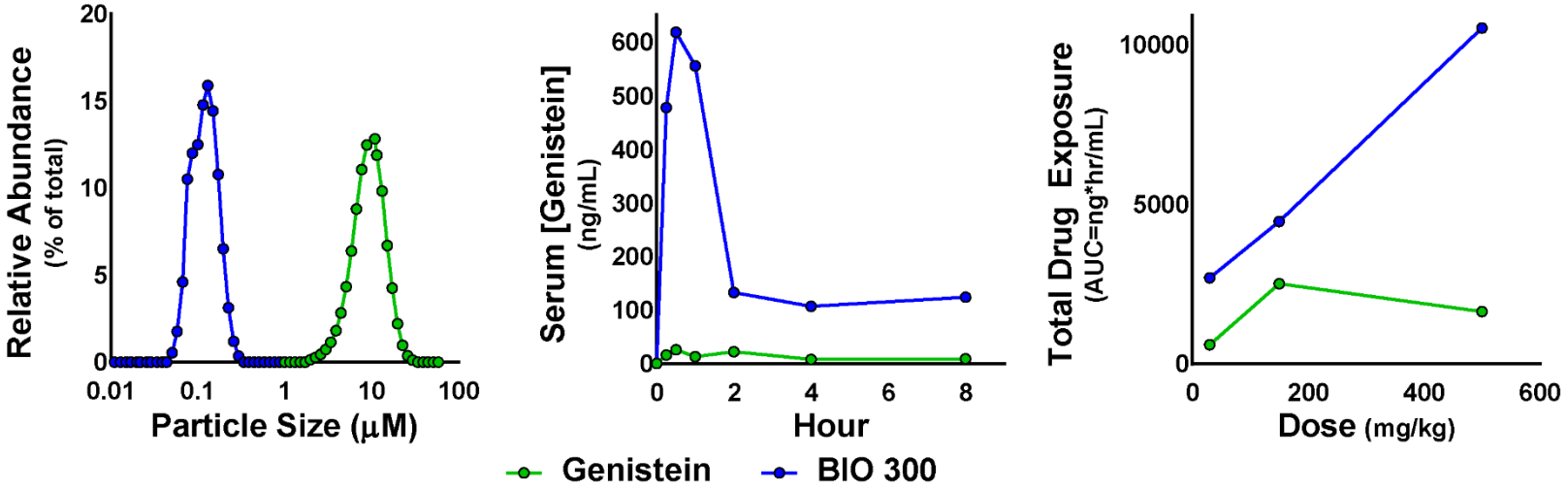
BIO 300 Key Attributes



Genistein

- **Proven oral efficacy as radioprotectant**
- **Two oral and one intraparenteral formulations in development**
- **Domestic GMP manufacturing established**
- **Shelf stable with no special handling needs**
- **Minimum 2-year shelf life**
- **Robust safety profile demonstrated in human trials**
- **Can be used “immediately” under IND Contingency Protocol (DoD) - Use will be considered a clinical study and will help support future EUA and FDA approval**

BIO 300: Nanoparticle Suspension Increases Bioavailability and Dose Linearity



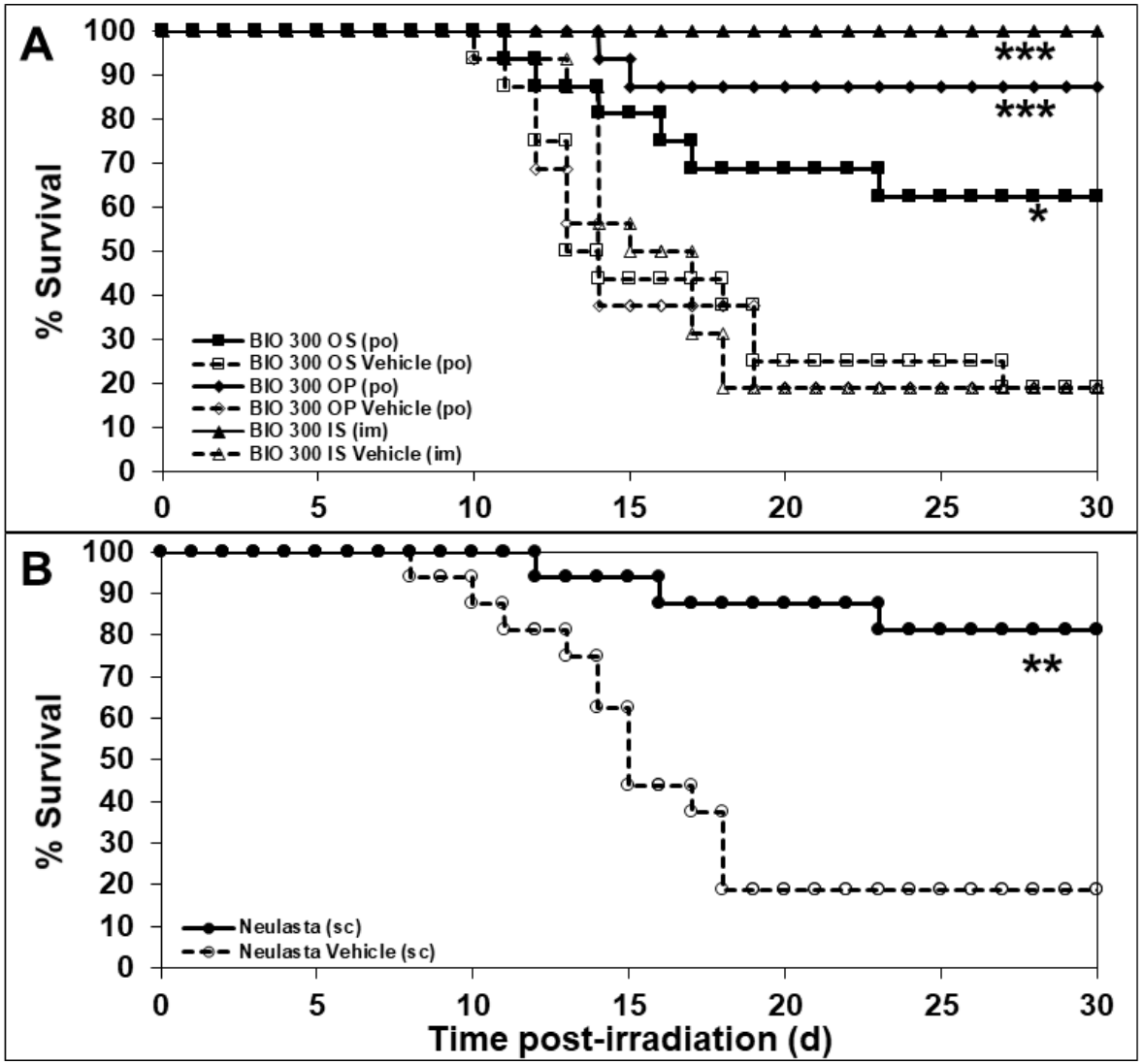
Murine Efficacy Study

Three formulations:

- Oral suspension (BIO 300 OS)
- Oral powder (BIO 300 OP)
- Injectable suspension (BIO 300 IS)

- **BIO 300 all formulations – 200 mg/kg/dose**
- **Neulasta 300 µg/kg, single dose, +24 h**
- **Radiation: 9.2 Gy (0.6 Gy/min), $LD_{70/30}$**

30-day survival of CD2F1 mice administered BIO 300 IS, OP, OS, or Neulasta



Int J Radiat Biol. 98:958-967, 2022

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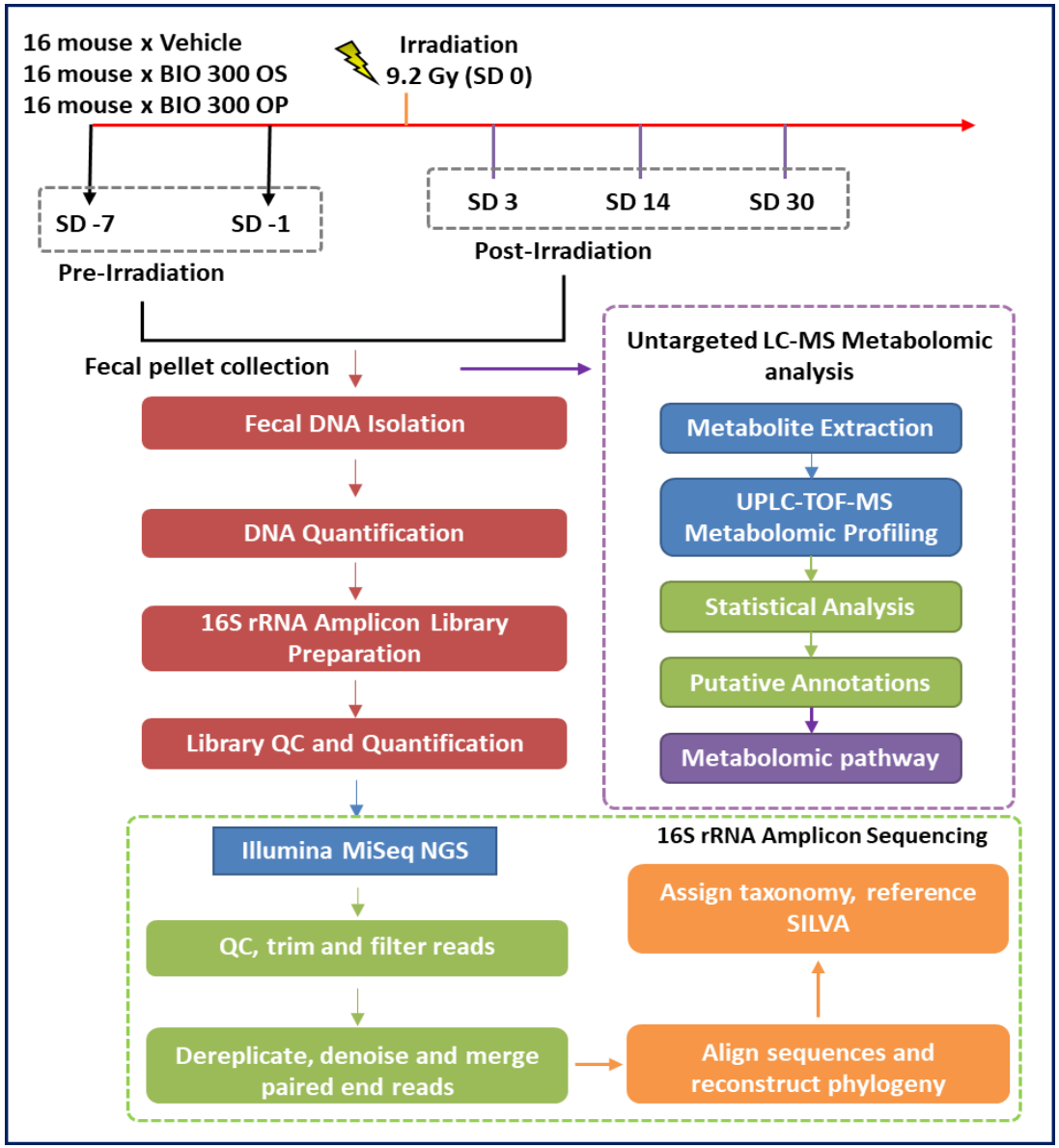
Microbiome Study: Experimental design BIO 300



Animal Microbiome 3:71,2021

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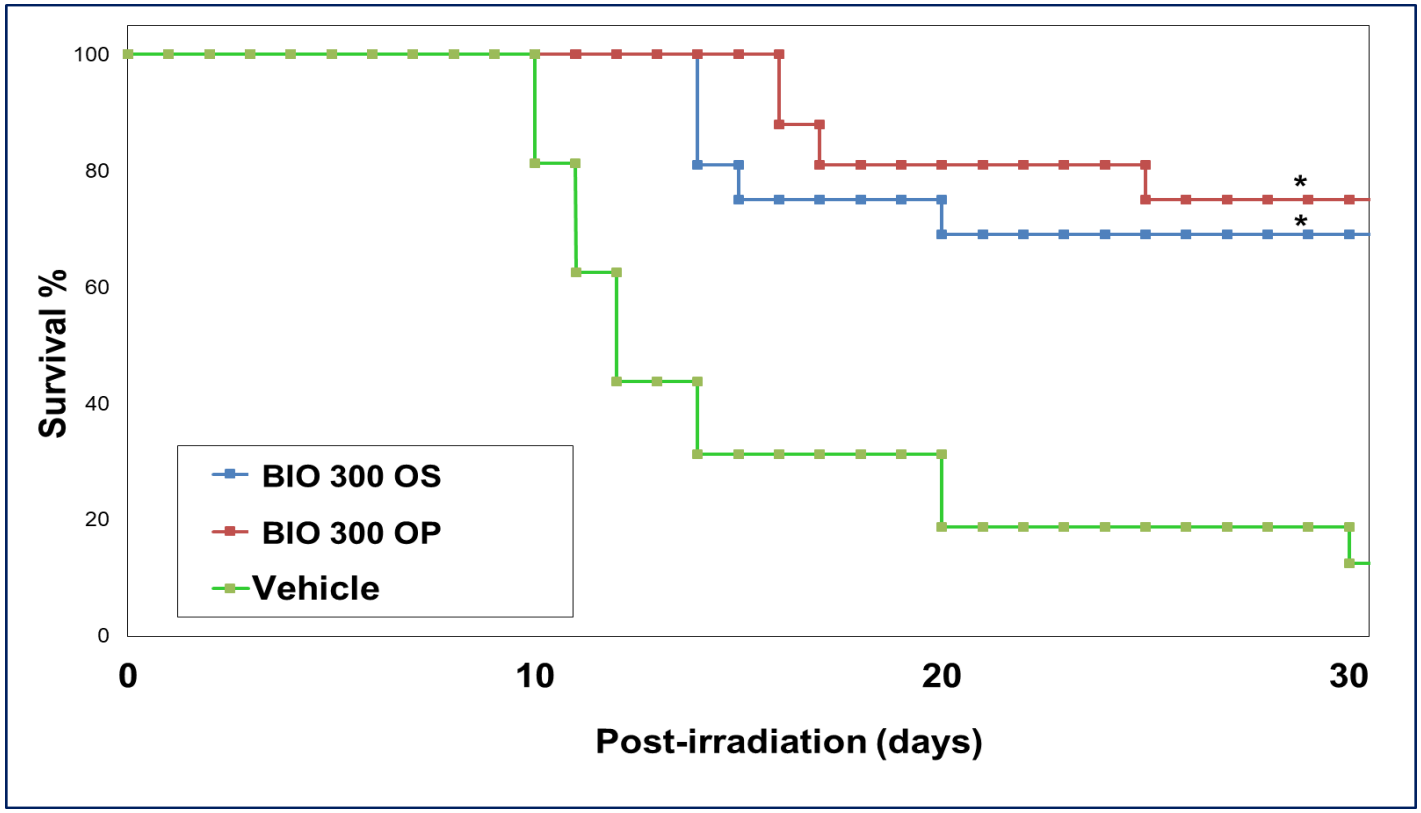
Monitoring survival 30d post-irradiation of three groups of mice



Animal Microbiome 3:71,2021

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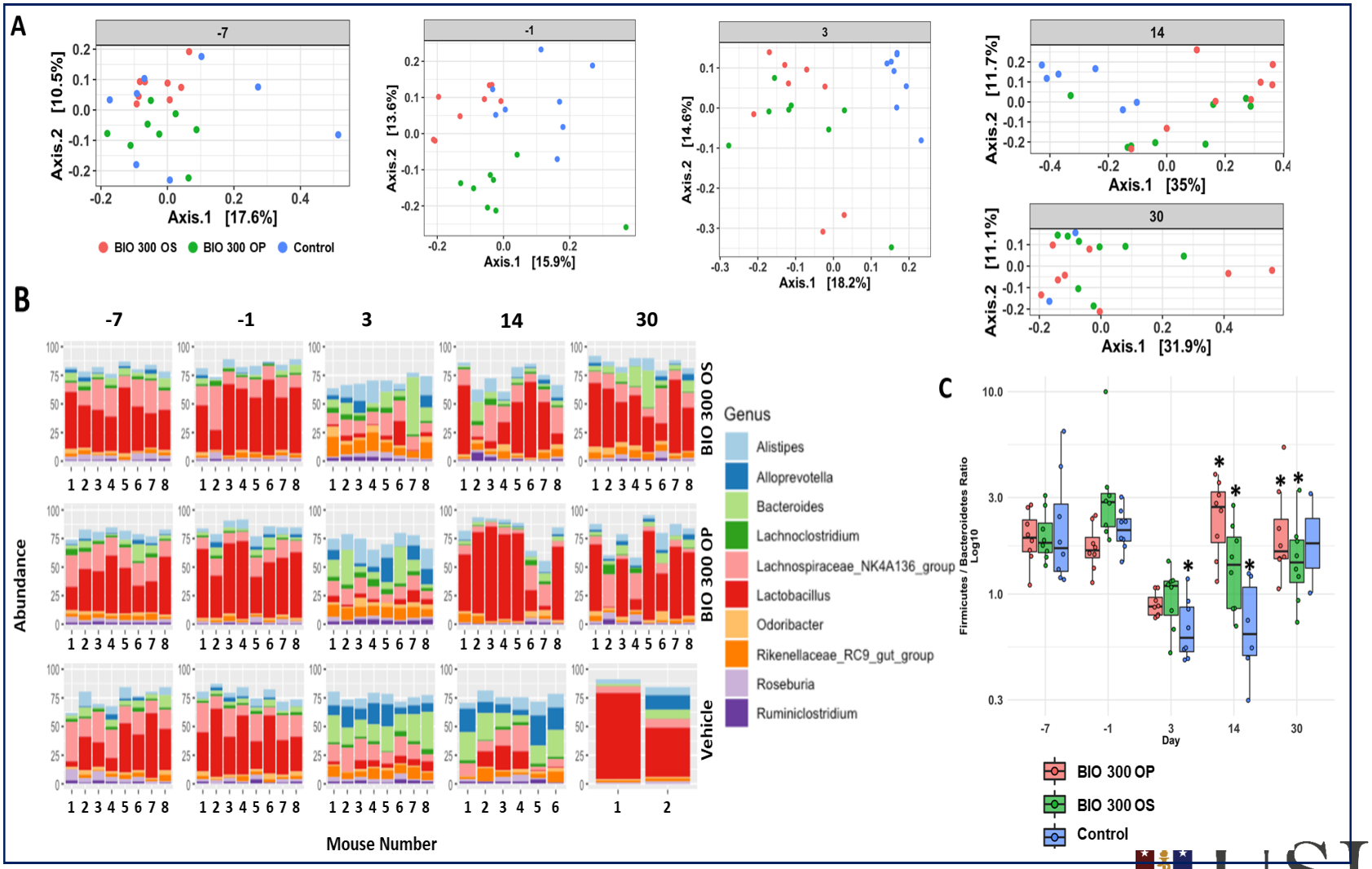
Alterations in radiation-induced microbiome diversity were alleviated by BIO 300 by day 14



Animal Microbiome 3:71,2021

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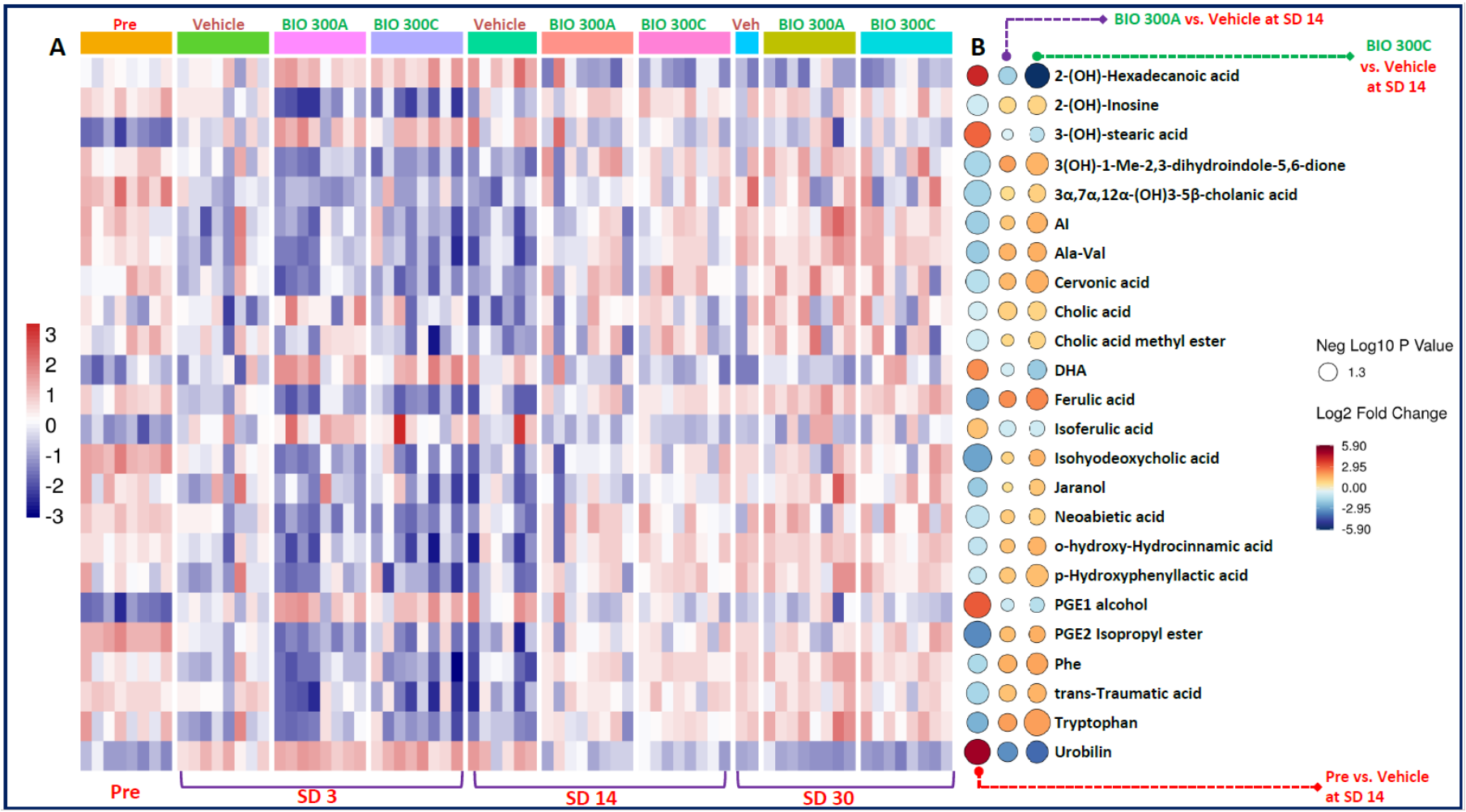
Prophylaxis treatment with BIO 300 restores metabolic abundance dysregulated by irradiation



Animal Microbiome 3:71, 2021

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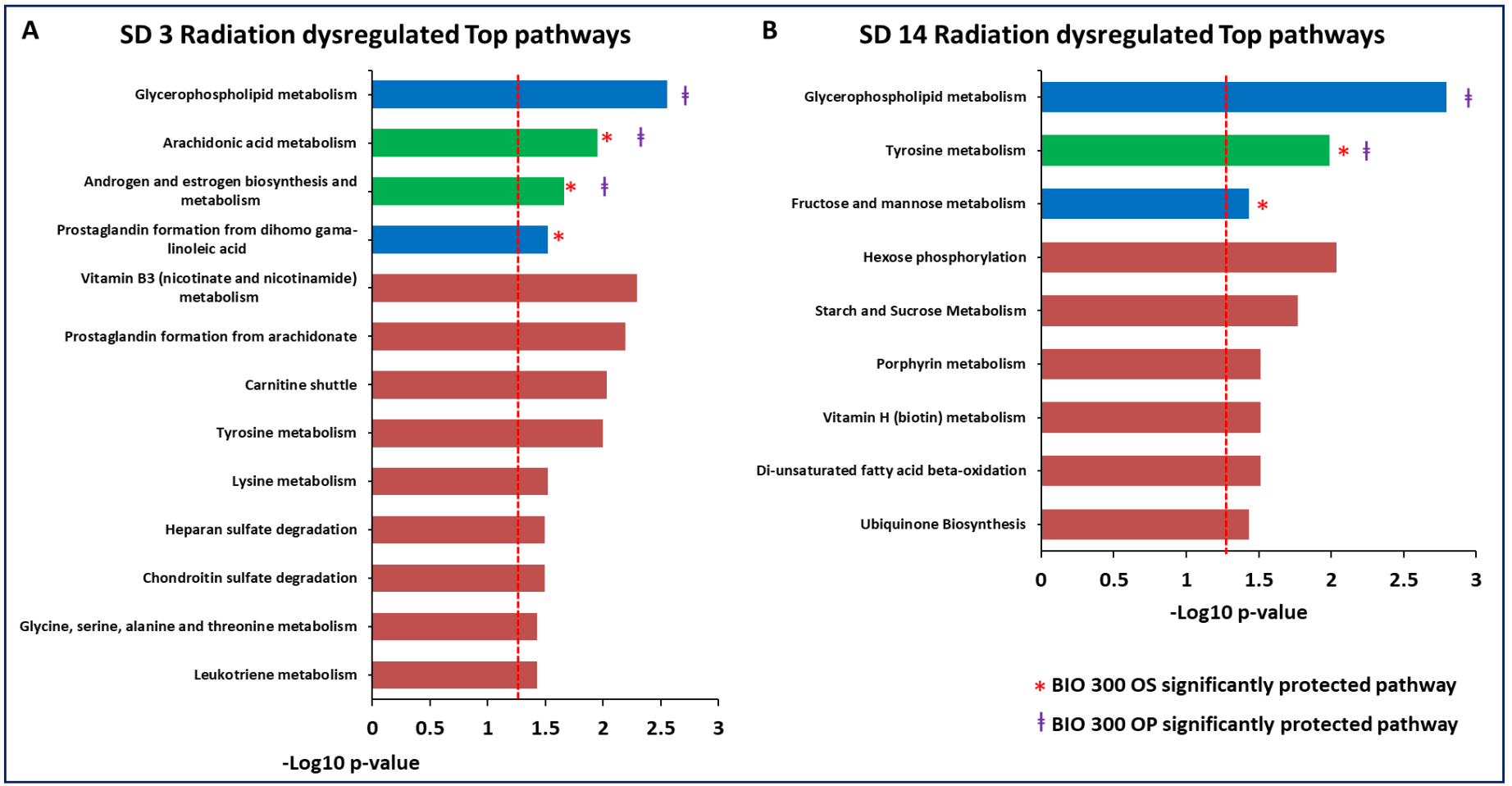
Pathway analysis showing significantly dysregulated pathway perturbations at day 3 and 14



Animal Microbiome 3:71,2021

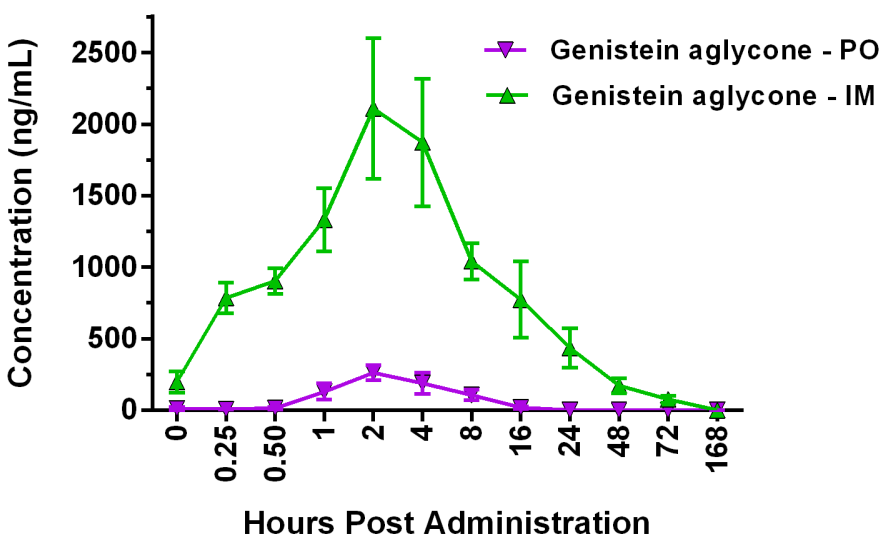
Pharmacokinetic Studies in NHPs

***IM* dosing = 50 mg/kg BIO 300 IS**

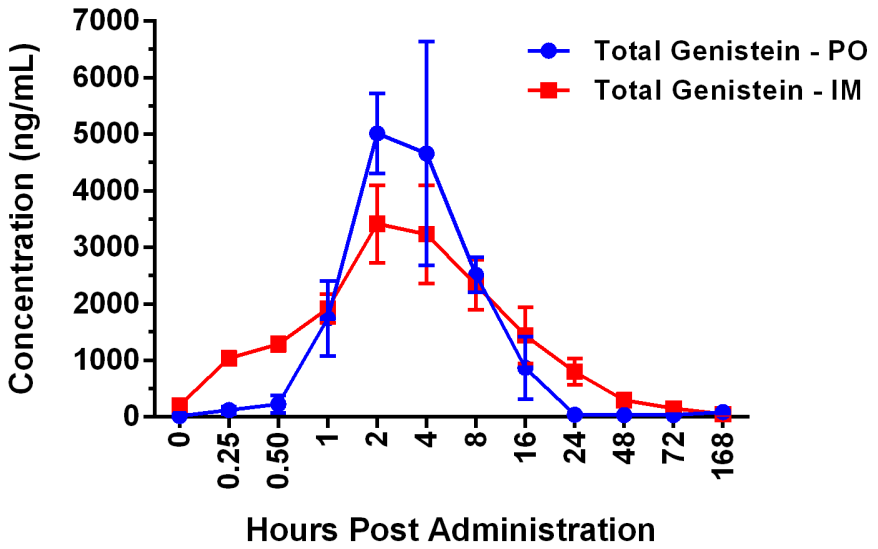
***PO* dosing = 100 mg/kg BIO 300 OS**

Pharmacokinetic analysis of BIO 300: Active Versus Total Drug Comparison

Non-glucuronidated (Active Drug)



Total Drug



- Both routes of administration achieve blood levels to activate therapeutic target
- IM administration results in higher blood levels of active drug
- IM administration leads to blood levels resulting in sustained activation of target

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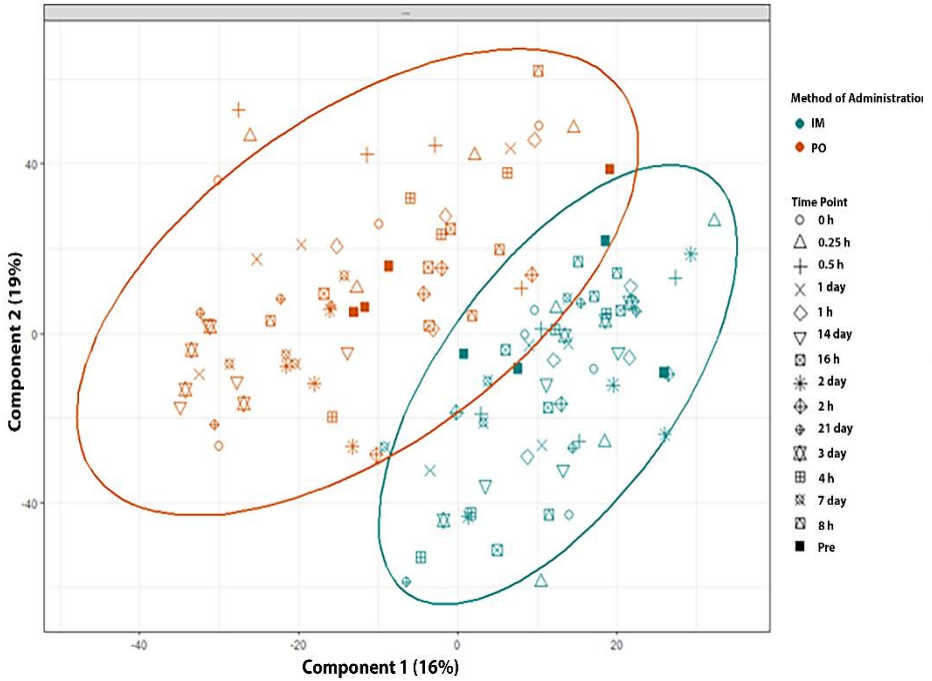
Metabolomics Studies in NHPs

***IM* dosing = 50 mg/kg BIO 300 IS**

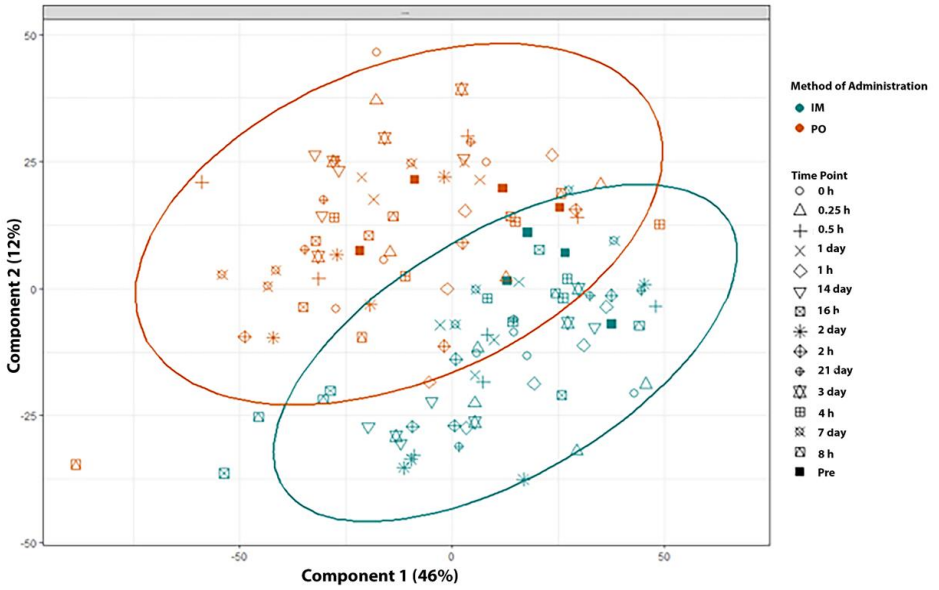
***PO* dosing = 100 mg/kg BIO 300 OS**

Partial Least Square-Discriminant Analysis (PLS-DA) to determine longitudinal changes in metabolomic profiles

A



B



ESI positive mode – Protonated molecule

15 time points – Human data base

ESI negative mode – Deprotonated molecules

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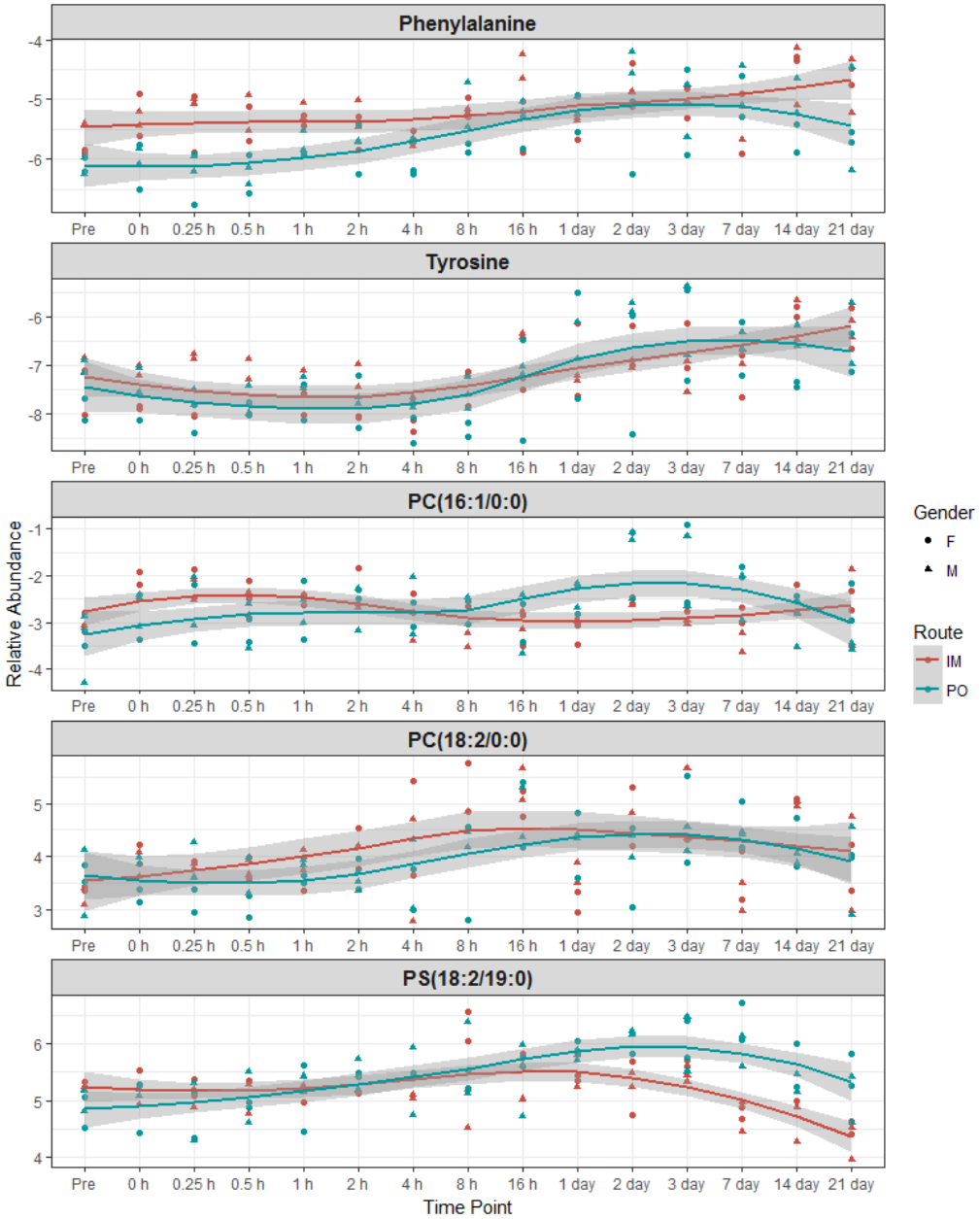
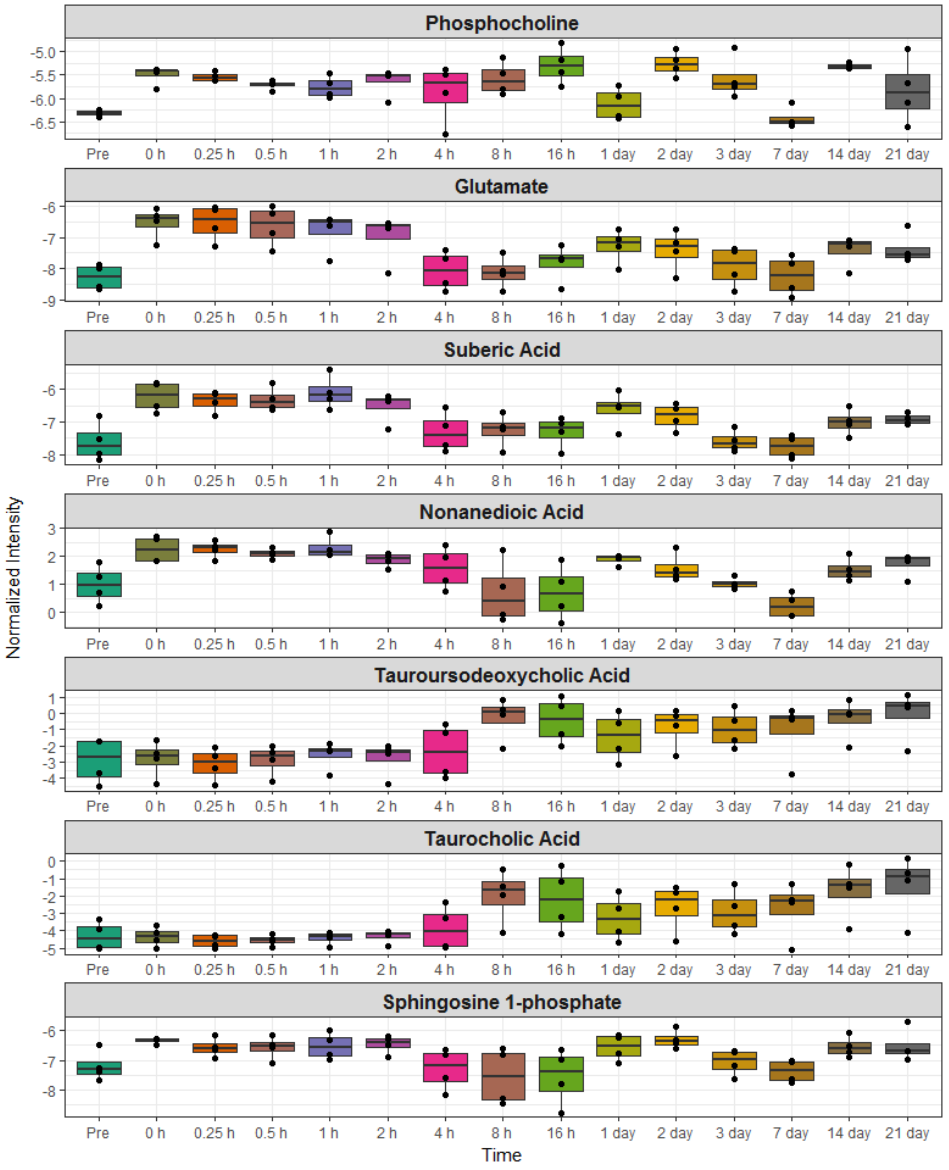
ANOVA analyses help delineate metabolites showing common trend in both routes of administration

Changes during 2 - 7 days



Box and whisker plot: Time dependent transient changes in serum metabolite profiles that are unique to *im* administration of BIO 300 in NHPs



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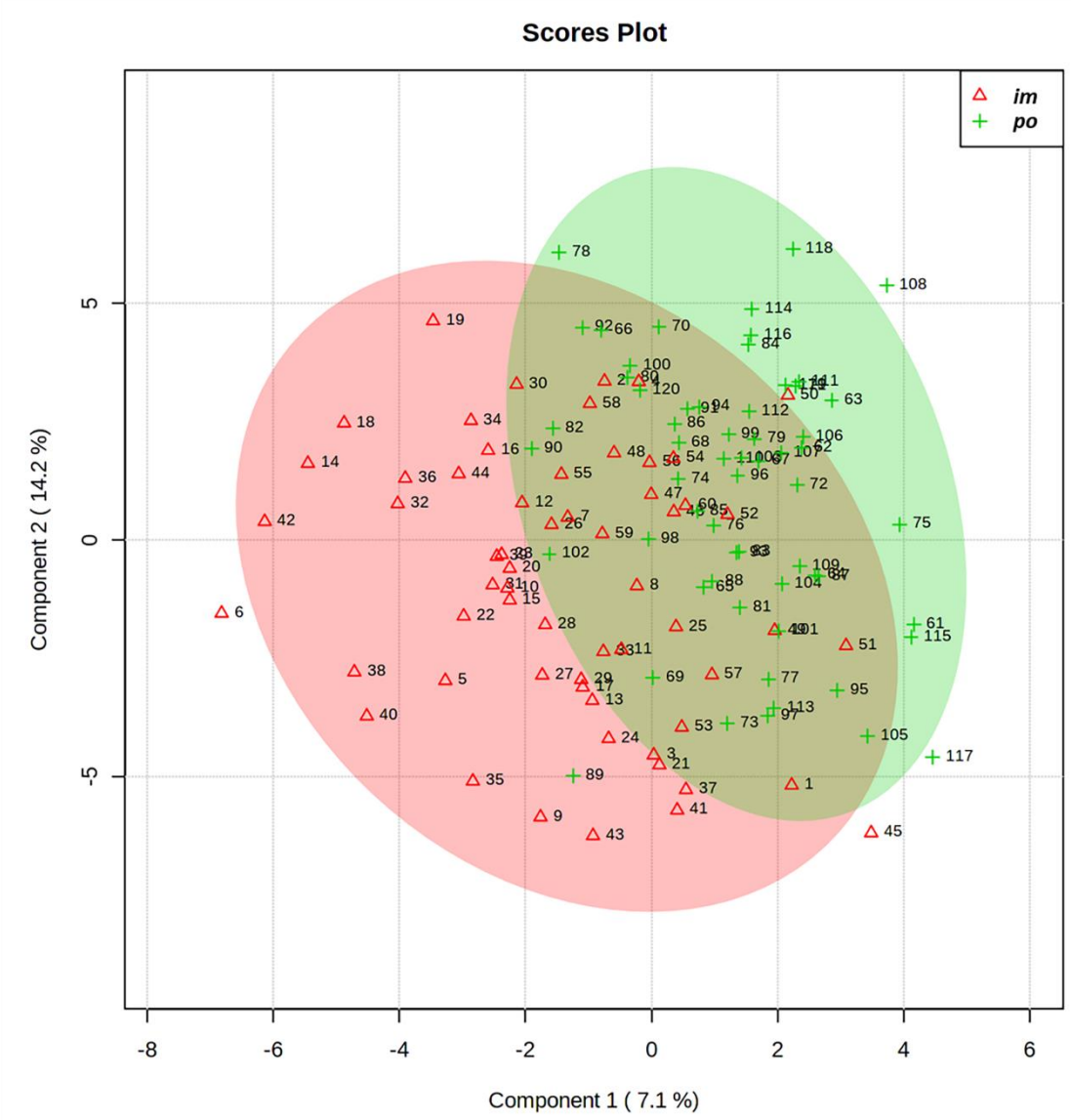
Proteomic Study in NHPs

***IM* dosing = 50 mg/kg BIO 300 IS**

***PO* dosing = 100 mg/kg BIO 300 OS**

Principal Component analysis (PCA) plot for perturbation in proteins expression



Scientific Reports 10:19343, 2020

Rhesus macaque database

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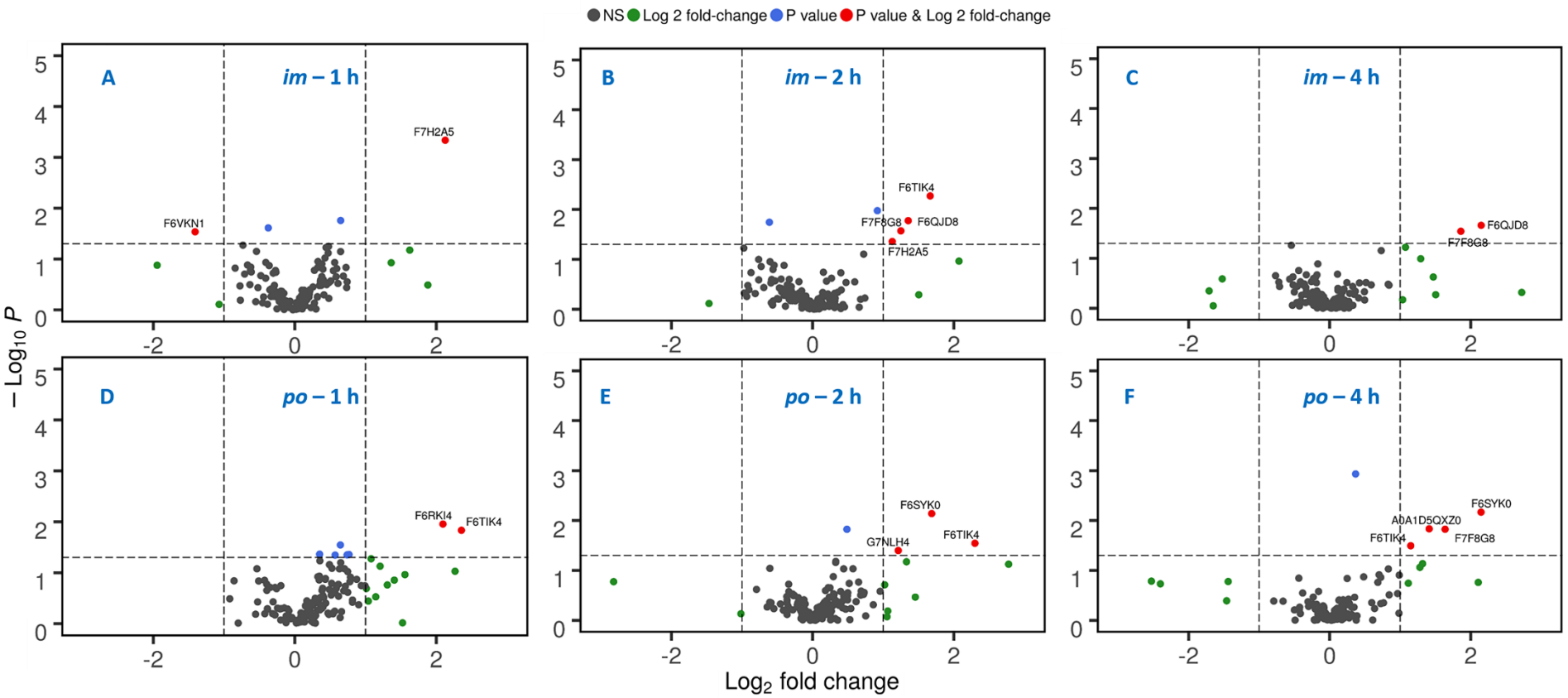
Volcano plots illustrating significantly dysregulated proteins - Rhesus macaque database



Scientific Reports 10:19343, 2020

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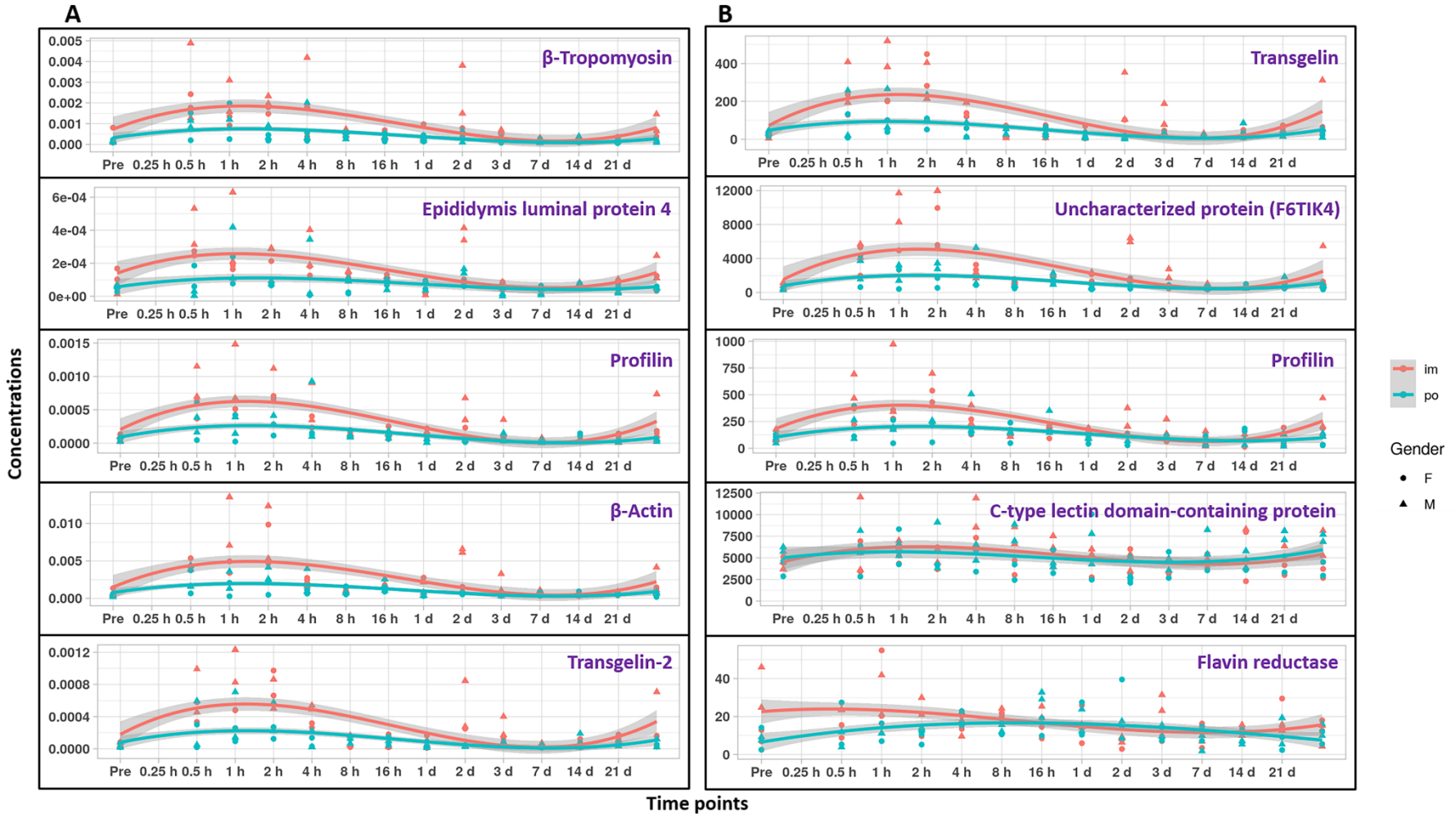
Trend lines of proteins that rapidly increase (30’ – 2 h) following *po* or *im* administration of BIO 300



Scientific Reports 10:19343, 2020

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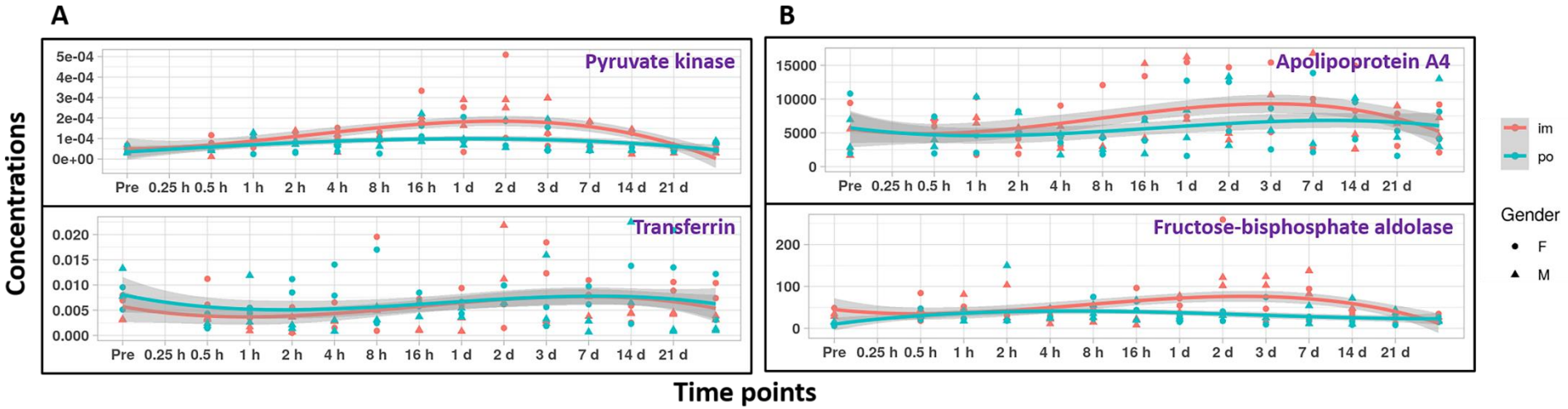
Expression for proteins after oral as well as *im* administration of BIO 300 – Progressive increase (2 - 4 h to 7 d)



Scientific Reports 10:19343, 2020

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Overall study design for metabolic study of BIO 300 OP in NHPs

Session 1 BIO 300 OP Dose 100 mg/kg (n = 4 NHPs)

Session 2 BIO 300 OP Dose 200 mg/kg (n = 4 NHPs)

Summary of BIO 300 Oral Powder single dose PK parameters in NHPs

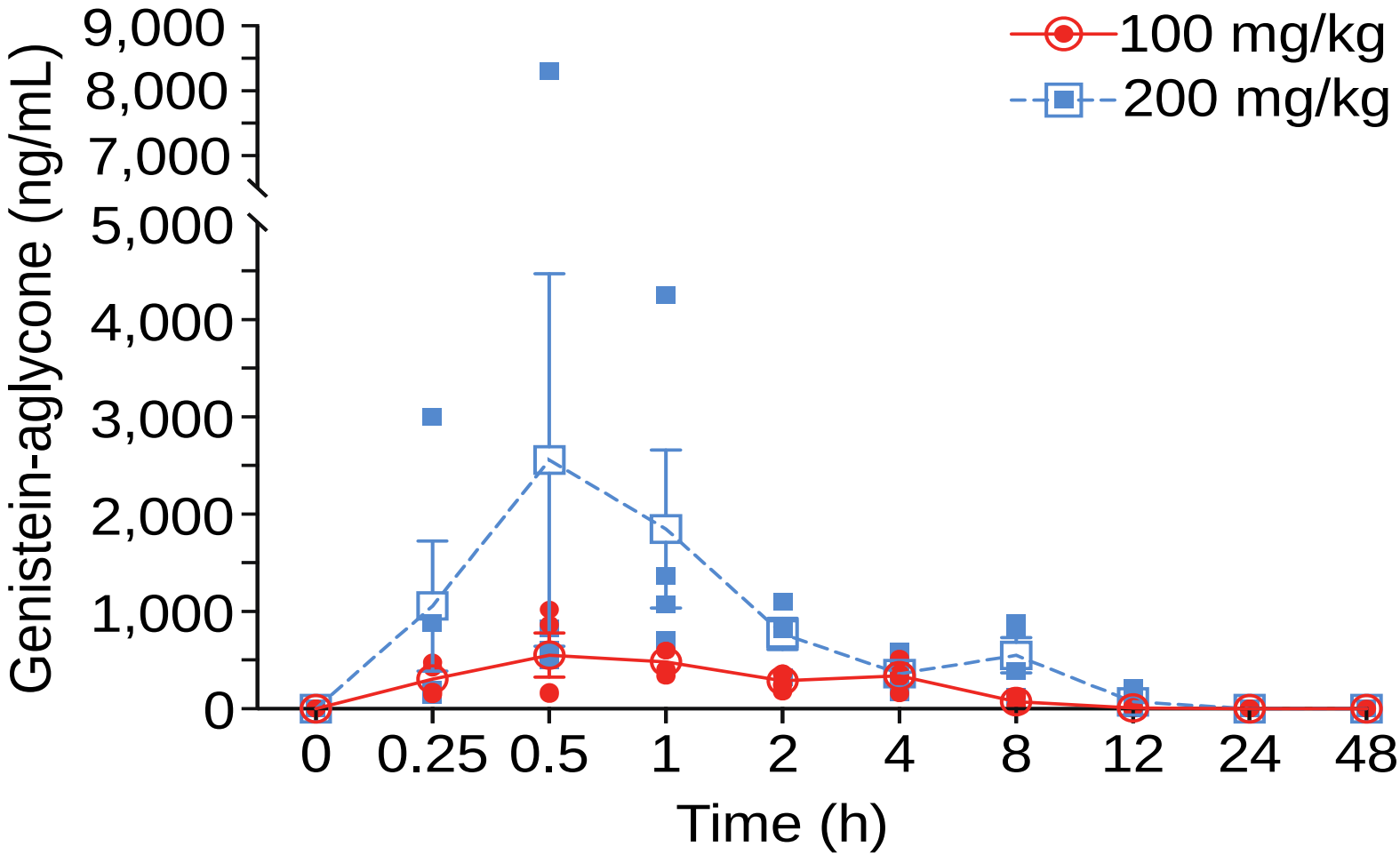
Session 1 BIO 300 OP Dose 100 mg/kg (n = 4 NHPs)
Session 2 BIO 300 OP Dose 200 mg/kg (n = 4 NHPs)

Total number of serum samples analyzed = 80

Dose	Animals	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-48} (ng.h/ml)	AUC_{0-inf} (ng.h/ml)	$T_{1/2}$ (h)
100 mg/kg	All	1.0±0.71	662.8±329.1	2481±822.2	2505±832.2	1.69±0.17
	Males	0.75±0.35	633.5±326.0	2201±1304	2214±1315	1.56±0.01
	Females	1.25±1.1	692.0±463.9	2761±120.9	2796±94.2	1.83±0.11
200 mg/kg	All	1.13±0.63	2867±3632	7645±3937	7649±3938	1.78±0.27
	Males	0.75±0.35	4504±5369	7026±6672	7030±6673	1.73±0.46
	Females	1.5±0.71	1230±183.8	8268±676.7	8268±678.2	1.84±0.01

Sci Rep 12:13475, 2022

PK analysis of BIO 300 OP (100 mg/kg and 200 mg/kg)



Sci Rep 12:13475, 2022

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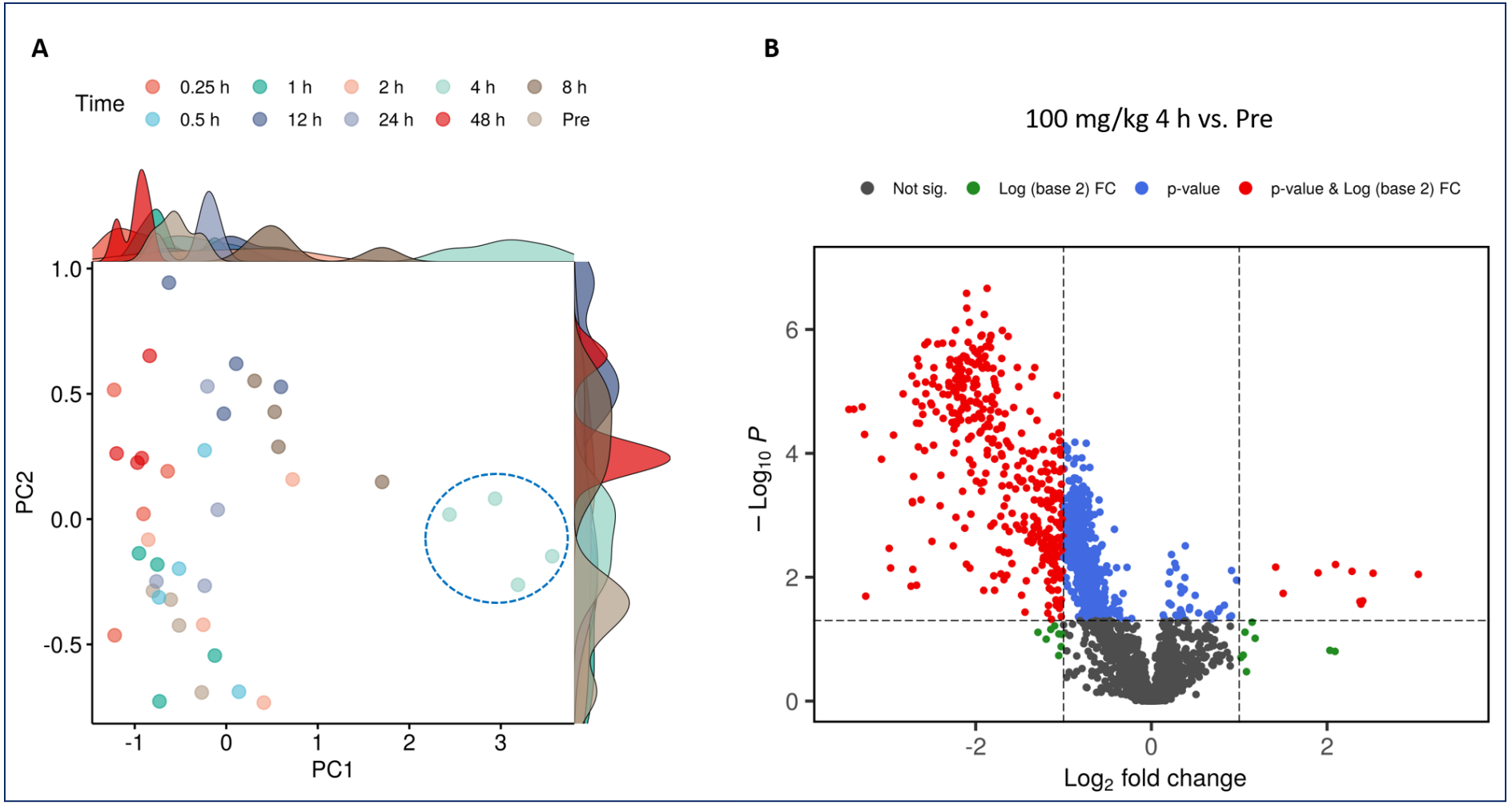
A 2D-PCA plot and Volcano plot



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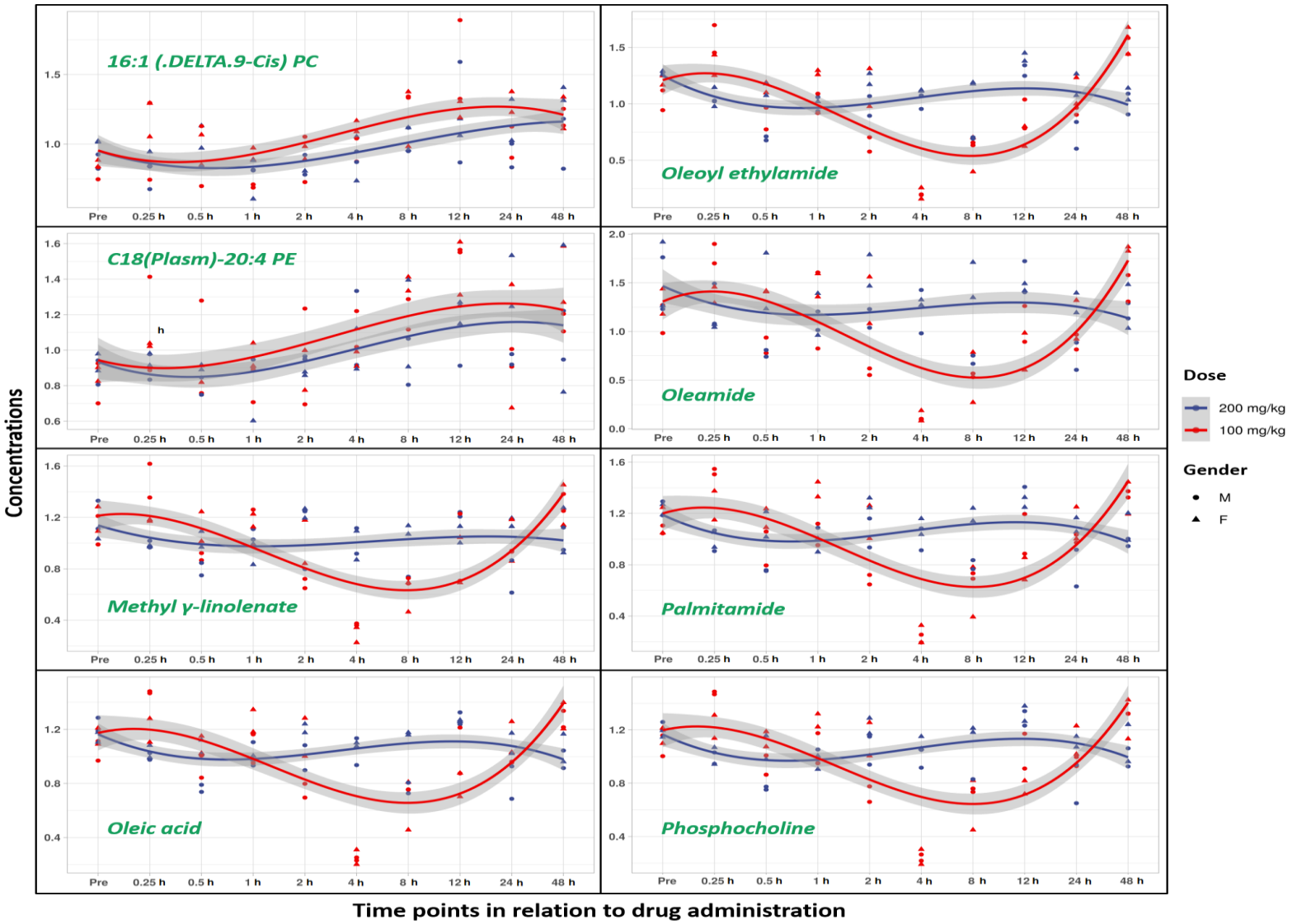
A trend line of a subset of metabolites/lipids



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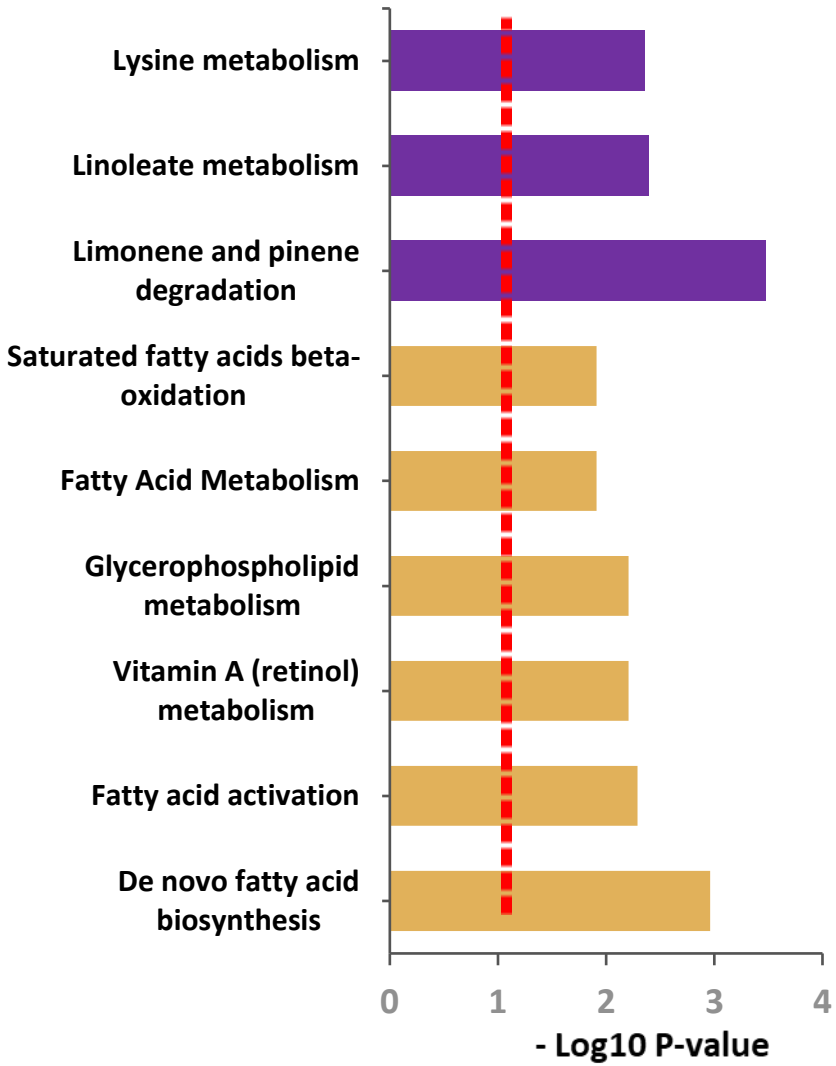
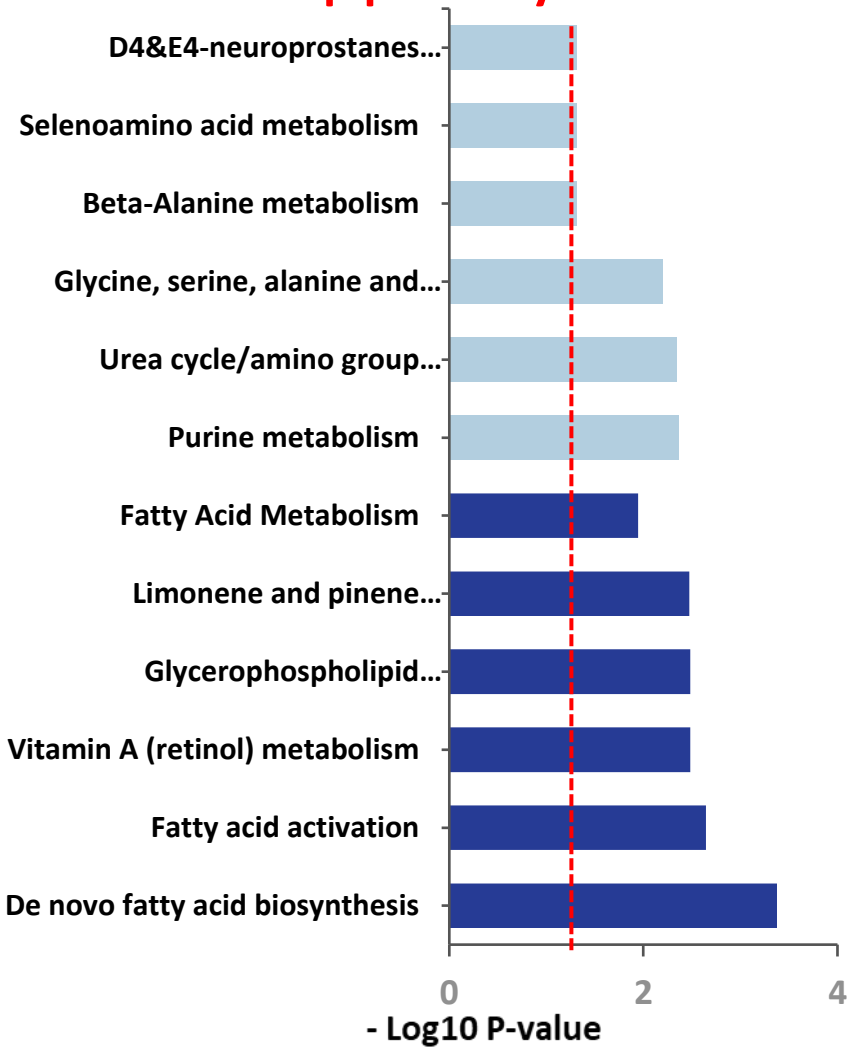
Pathway analysis at 4 and 8 h time points

Top pathways at 4 h



Top pathways at 8 h



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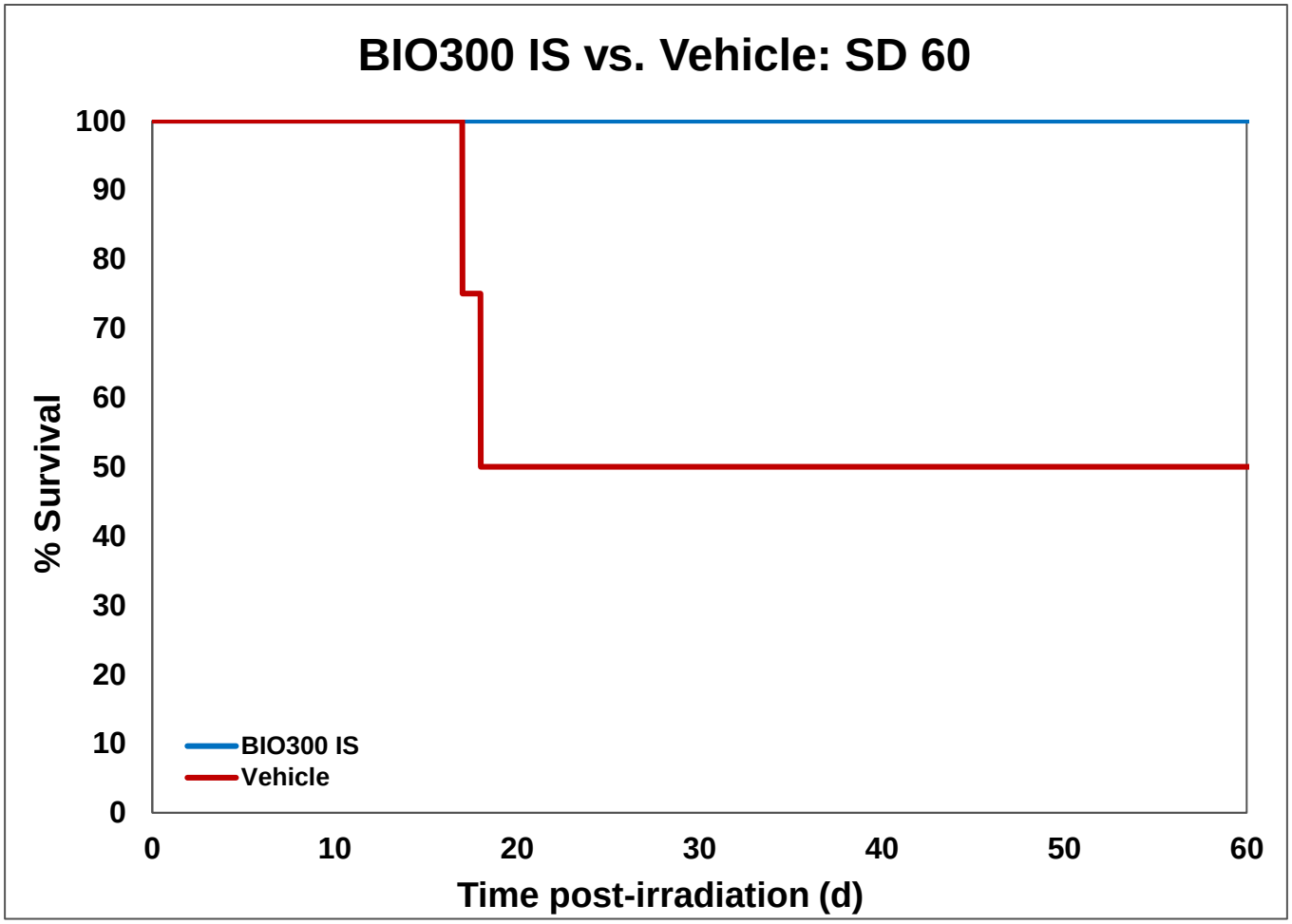
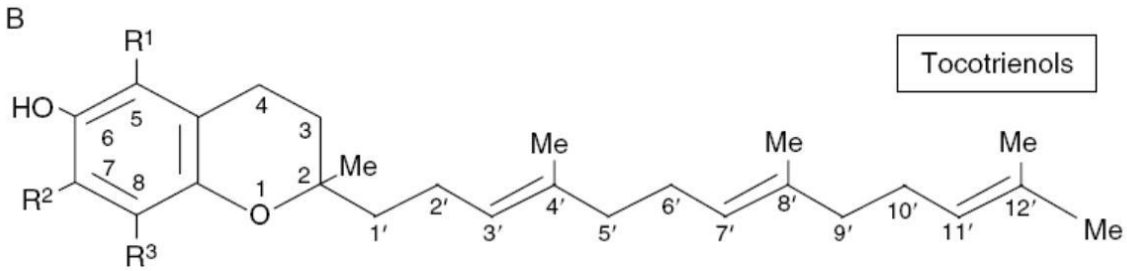
BIO 300 IS: Efficacy against Cobalt-60 gamma TBI in NHPs

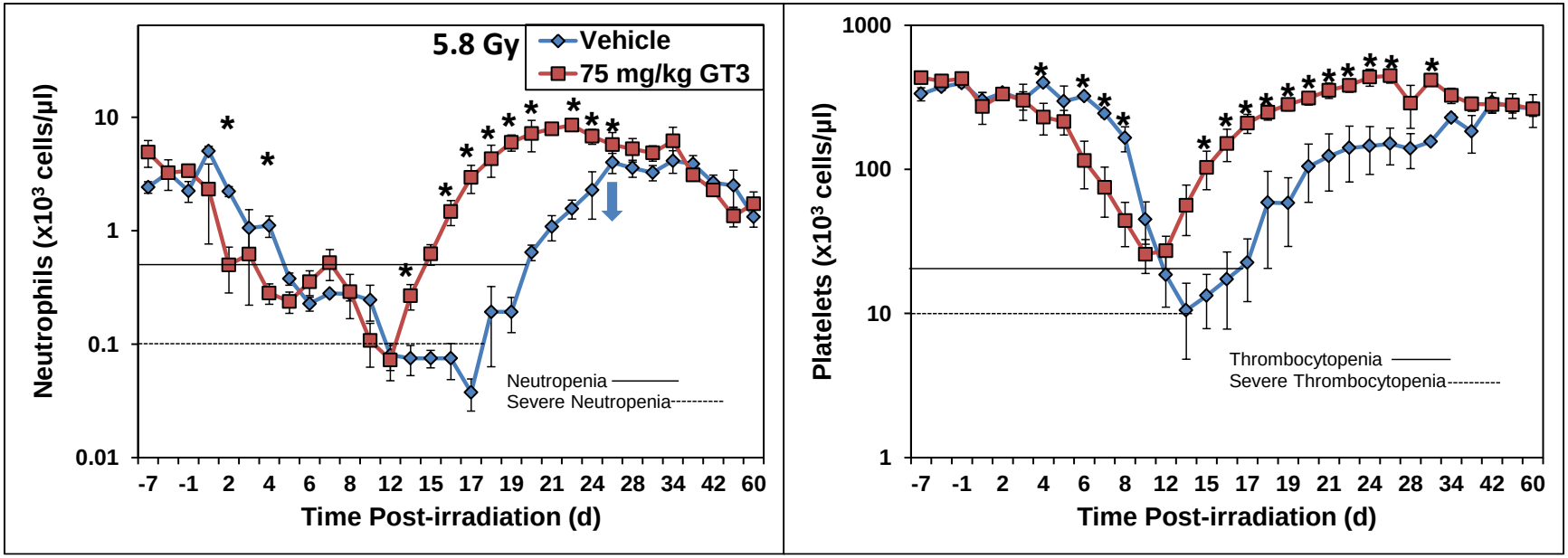


Gamma-tocotrienol



- **Naturally occurring isoform of vitamin E**
- **Tocotrienols are currently in several clinical trials**
- **Breast cancer, prostate cancer, and skin cancer**
- **Cholesterol lowering properties: Inhibition of Hydroxymethylglulateryl Coenzyme A reductase (HMGCR)**
- **Reduces type II diabetes**
- **Anti-inflammatory and potent antioxidant**

GT3-induced changes in neutrophils and platelets in irradiated NHPs (5.8 Gy)



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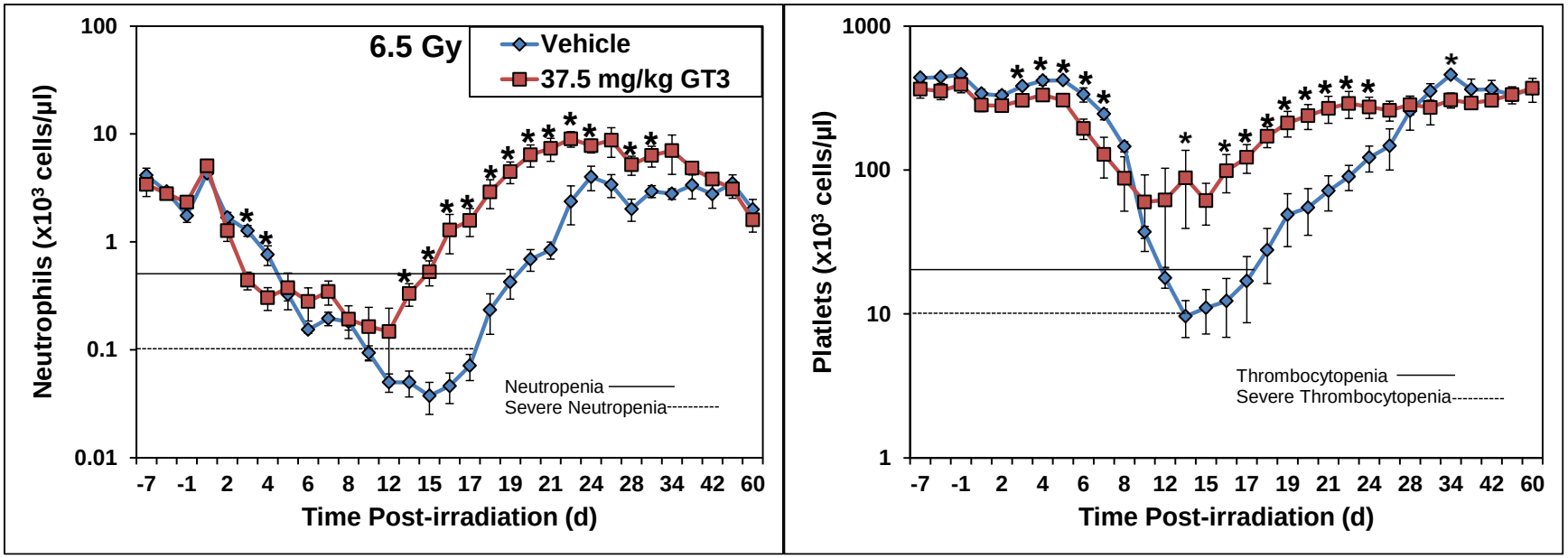
GT3-induced changes in neutrophils and platelets in irradiated NHPs (6.5 Gy)



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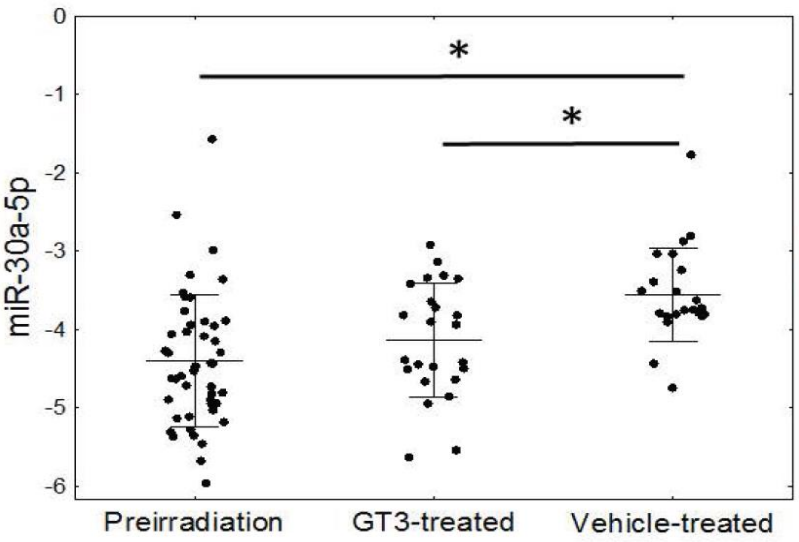
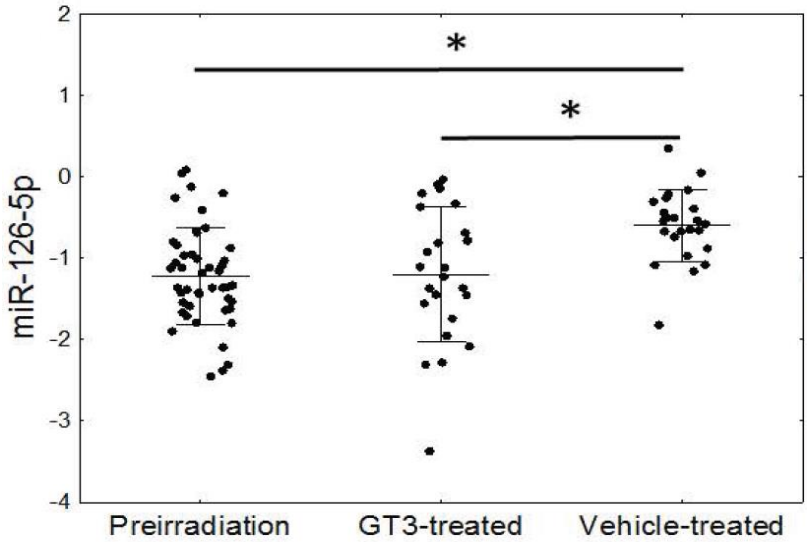
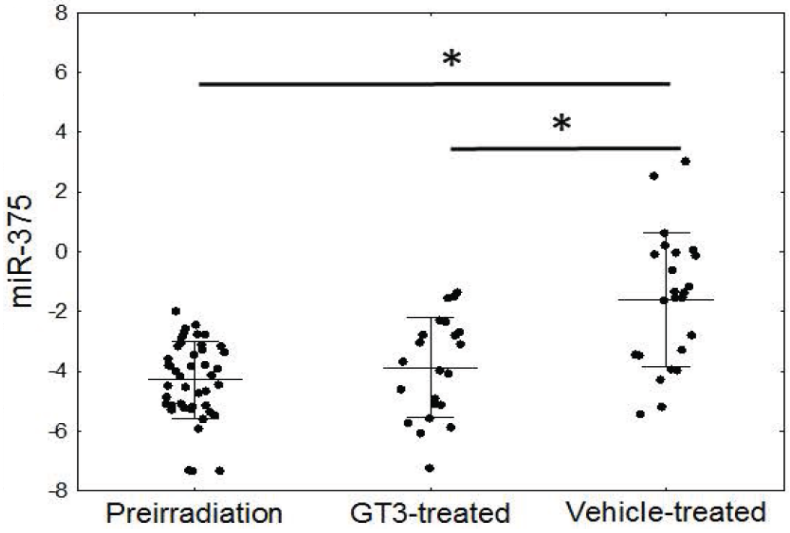
GT3 impact on radiation-dependent miRNA levels in NHPs







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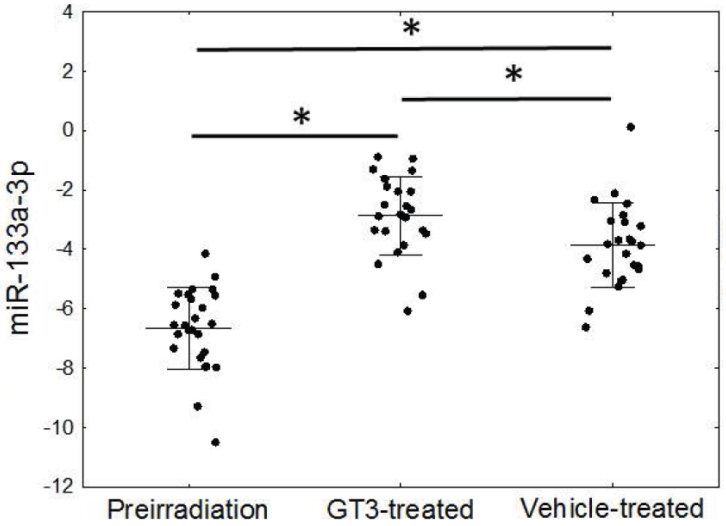
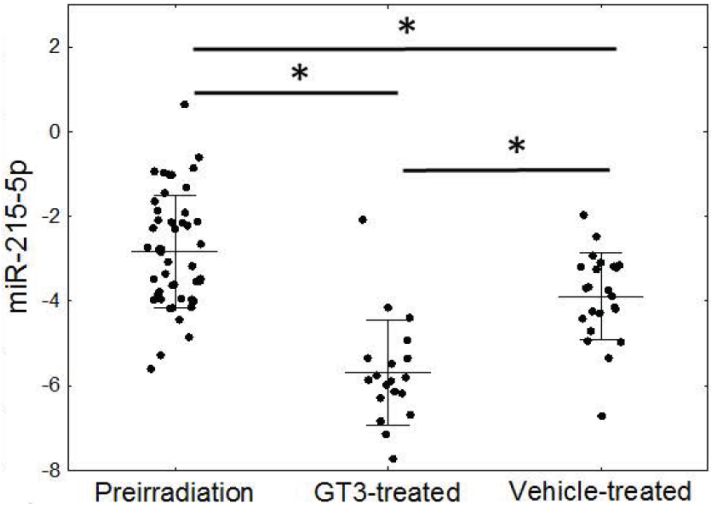
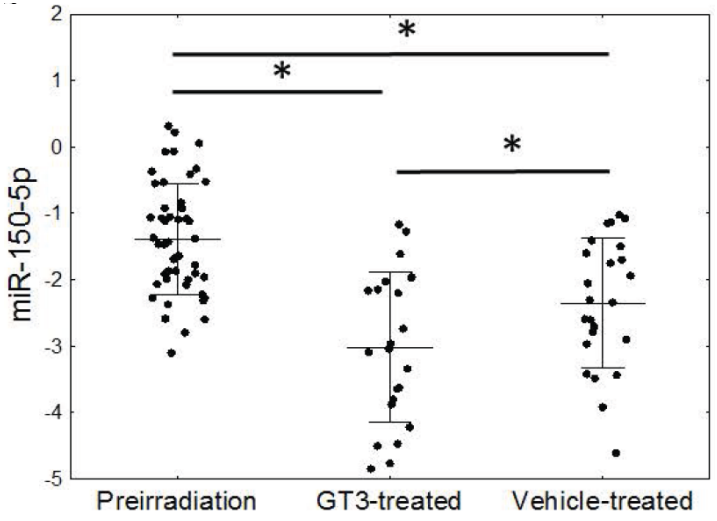
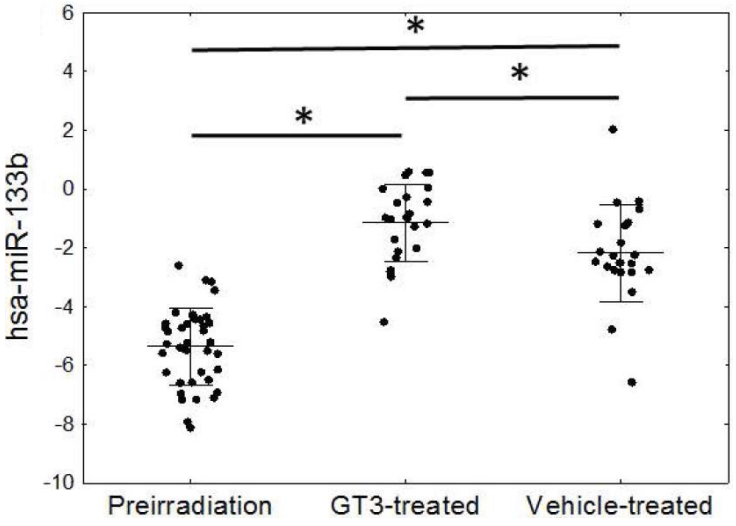
miRs - extreme radiation-induced change by GT3 in NHPs









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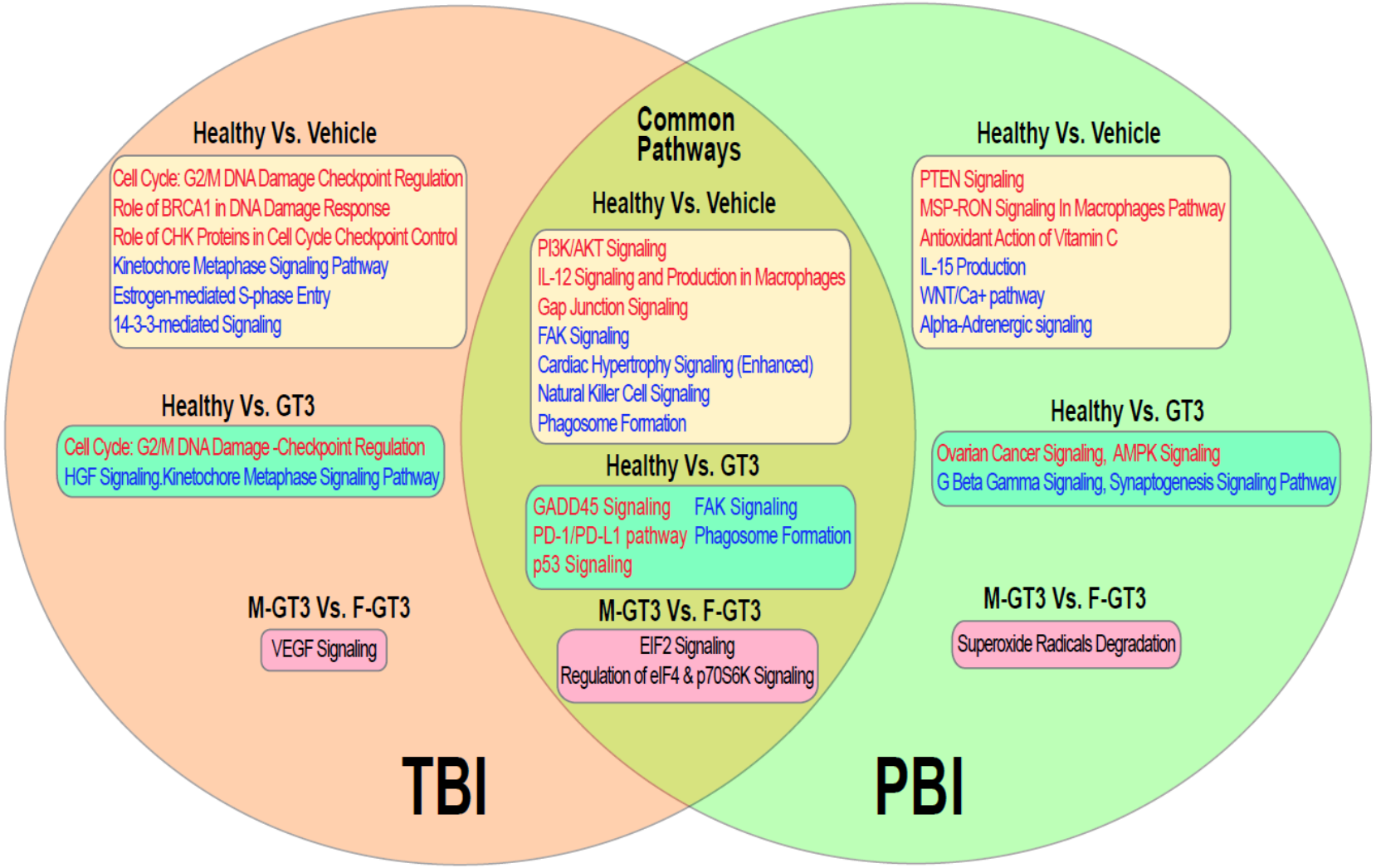
Common and unique pathways identified in different comparisons across TBI and PBI



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Questions ????

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