Dried Plum as a candidate radiomitigant

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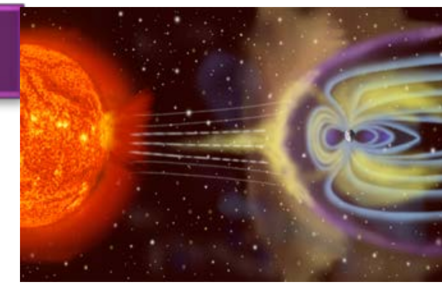
Space Biosciences
NASA AMES RESEARCH CENTER

Problem: Spaceflight conditions lead to bone loss

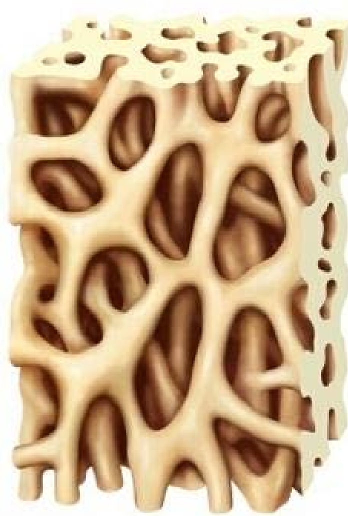
Microgravity

Space conditions



Space Radiation

results



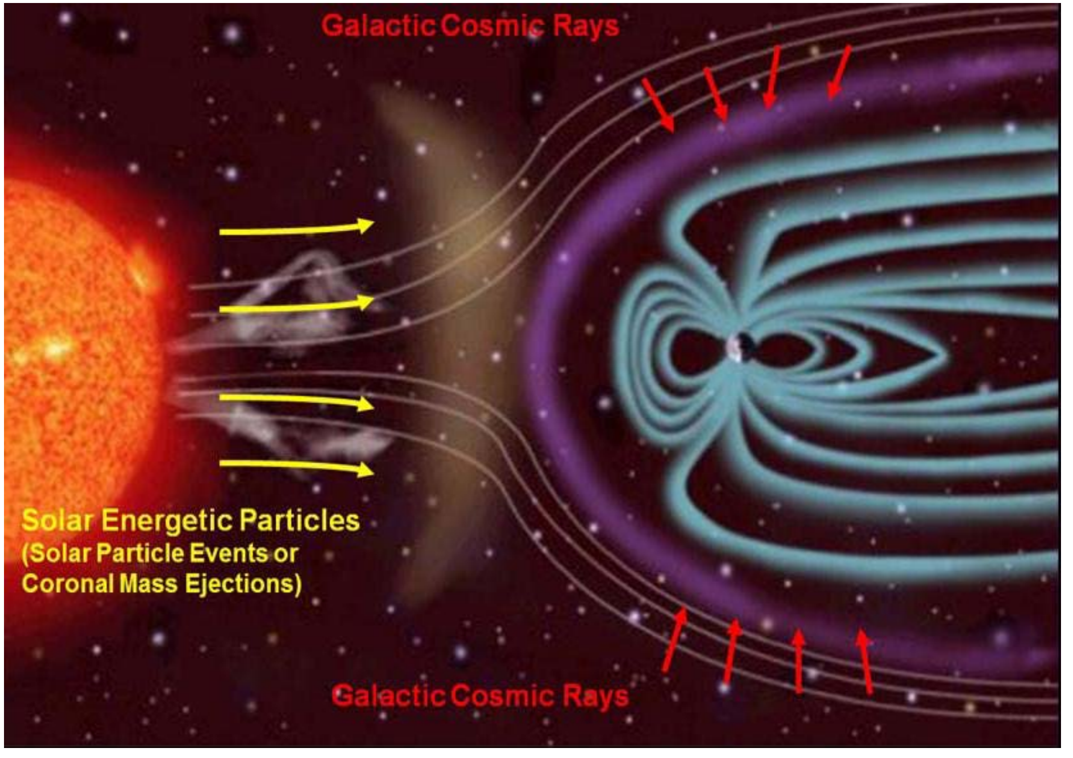
Healthy bone

Osteoporotic bone

Space Radiation

Space radiation sources are:

- Galactic Cosmic Rays (GCR)
- Solar Particle Events (SPE)
- protons and electrons trapped in the Earth's magnetic field

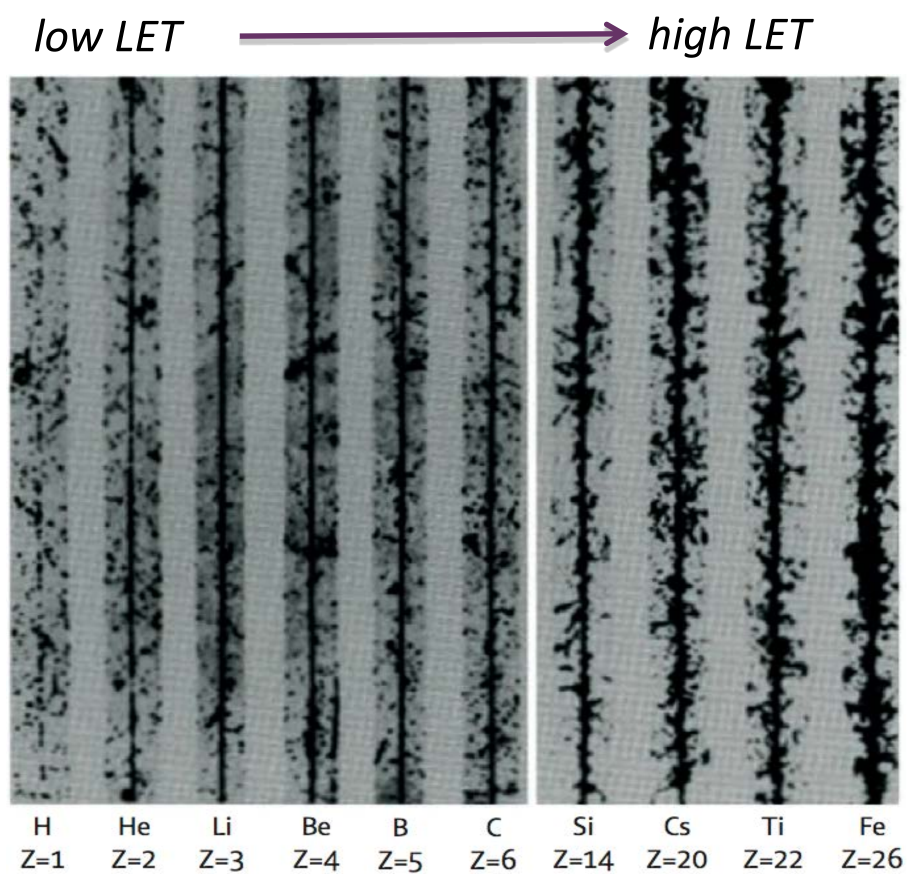


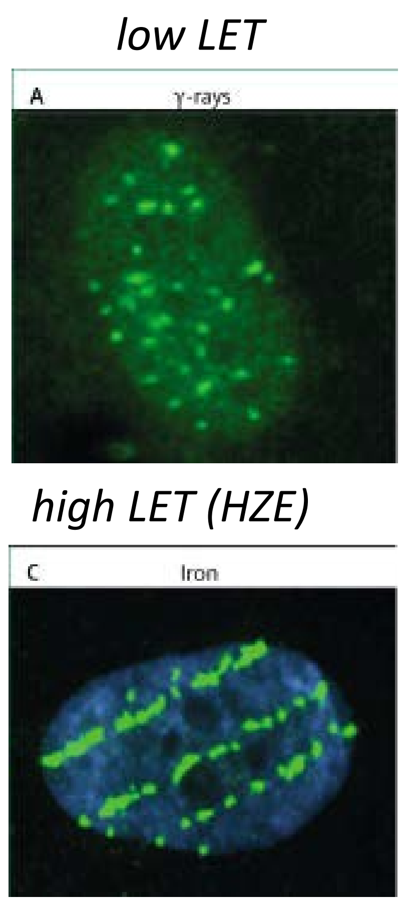
Durante and Cucinotta 2011
Norbury et al. 2016

Space Radiation

Space radiation is composed of a range of ions

- High-Z High Energy Ions (HZE)/ high Linear Energy Transfer (LET)
- low LET e.g. Protons ^{1}H



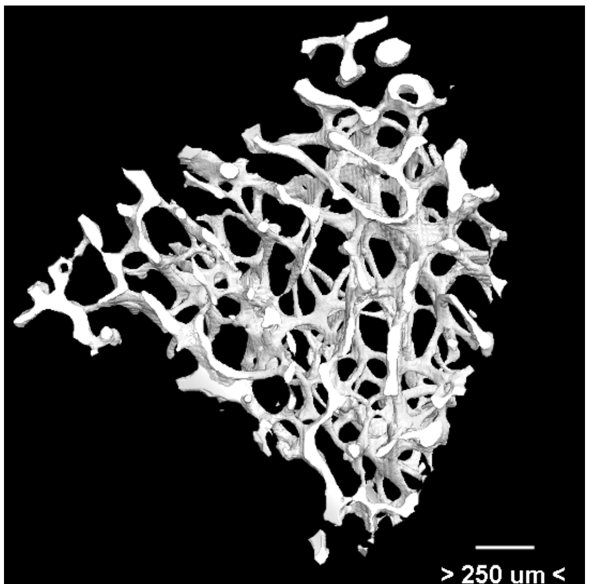


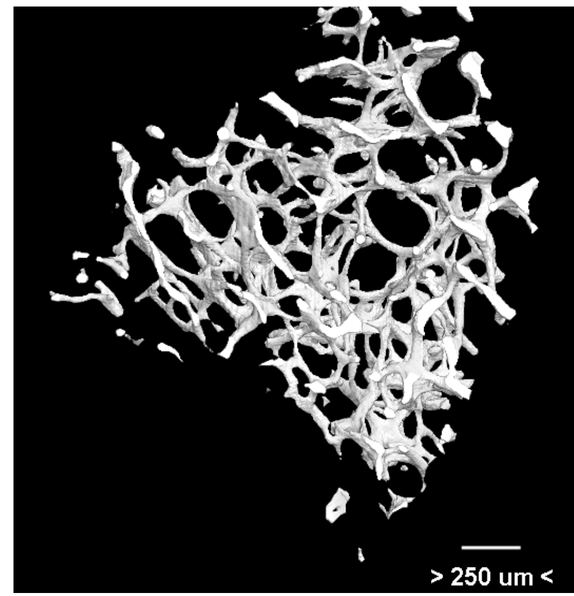
Cucinotta, Durante,2006
Norbury et al. 2016

Radiation induces cancellous bone loss

^{137}Cs (Gamma) ^{1}H (Protons); (100-200 cGy)

HZE (^{56}Fe) Iron; (50-100 cGy)





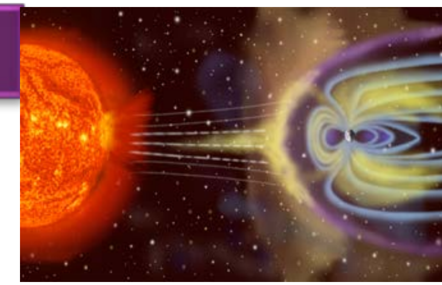
- ↓ Bone Volume/Total Volume
- ↓ Trabecular number

Schreurs et al, 2016
Alwood, Tran, Schreurs et al. 2017

Problem: Spaceflight conditions lead to bone loss

Space conditions

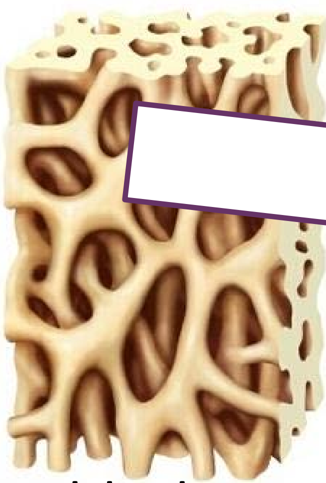
Microgravity



Space Radiation

results

Preventable??



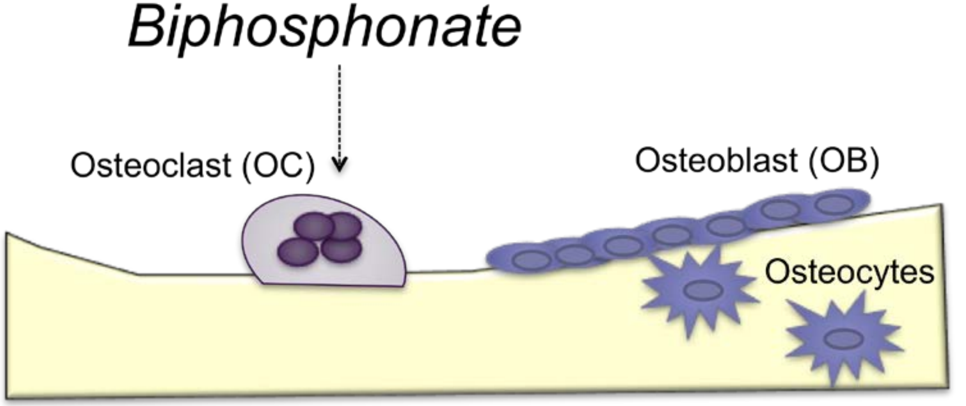
Healthy bone

Osteoporotic bone

Current methods for bone loss prevention

- **Resistive exercises** that mimic mechanical loading (i.e. ARED)

- **Bisphosphonate** prevents BMD decrements (bone mineral density), but has adverse effects. Also only acts on the bone-resorptive osteoclasts.



Spaceflight induced bone loss: possible mechanisms of action

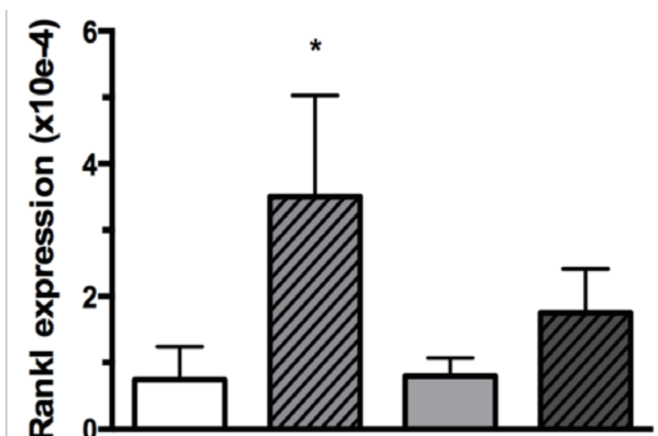
Simulated Spaceflight: Hindlimb Unloaded (ug) and Irradiation

↓

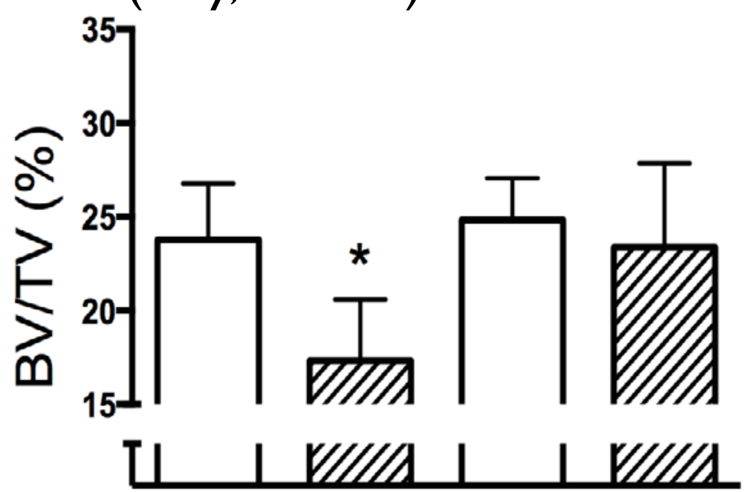
- ↑ bone resorption via osteoclasts
- ↓ bone formation via osteoblasts
- ↑ oxidative stress and damage
- ↑ bone loss

Dried plum diet protects from bone loss caused by ionizing radiation

- Based on hypothesis, selected multiple antioxidants and/or anti-inflammatory diets and drugs
- Diet composed of Dried Plums (DP) was the only effective one
- Prevents radiation-induced increase in markers of osteoclasts (*Rankl*, *Mcp1*), inflammation (*Tnf-a*) and oxidative stress (*Nrf2*)
- Prevents bone loss due to radiation: low LET such as gamma (2Gy, ^{137}Cs), and mix of protons and Iron (1Gy, ^{1}H ^{56}Fe)



Tx:	CD	CD	DP	DP
IR:	-	+	-	+



Tx:	CD	CD	DP	DP
IR:	-	+	-	+

Schreurs et al, 2016

Possible mechanisms of action

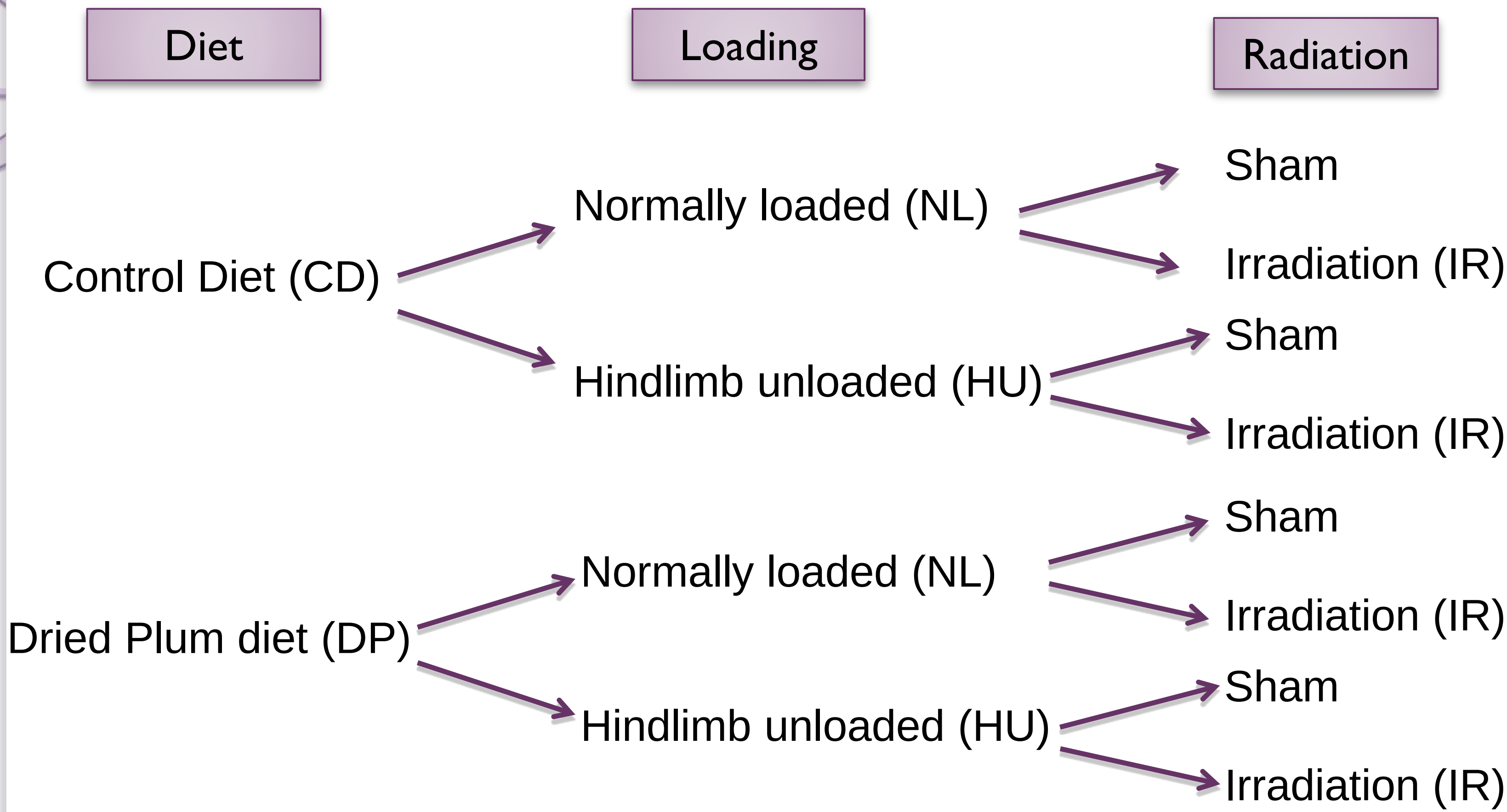
simulated spaceflight: HU (ug) and IR

↓

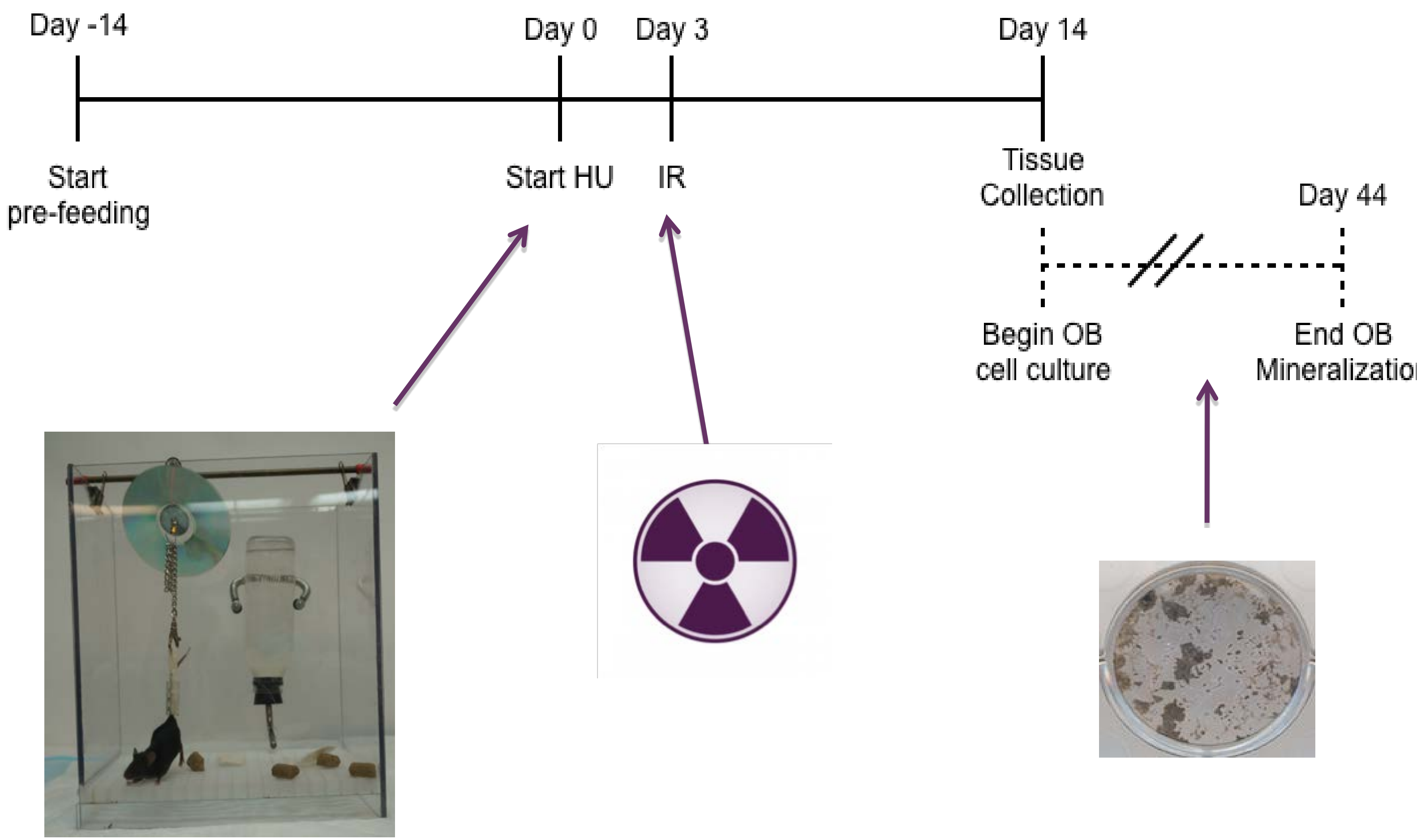
- ↑ bone resorption via osteoclasts
- ↓ bone formation via osteoblasts
- ↑ oxidative stress and damage
- ↑ bone loss (due to IR)

DP acts on → bone resorption via osteoclasts; bone loss (due to IR)

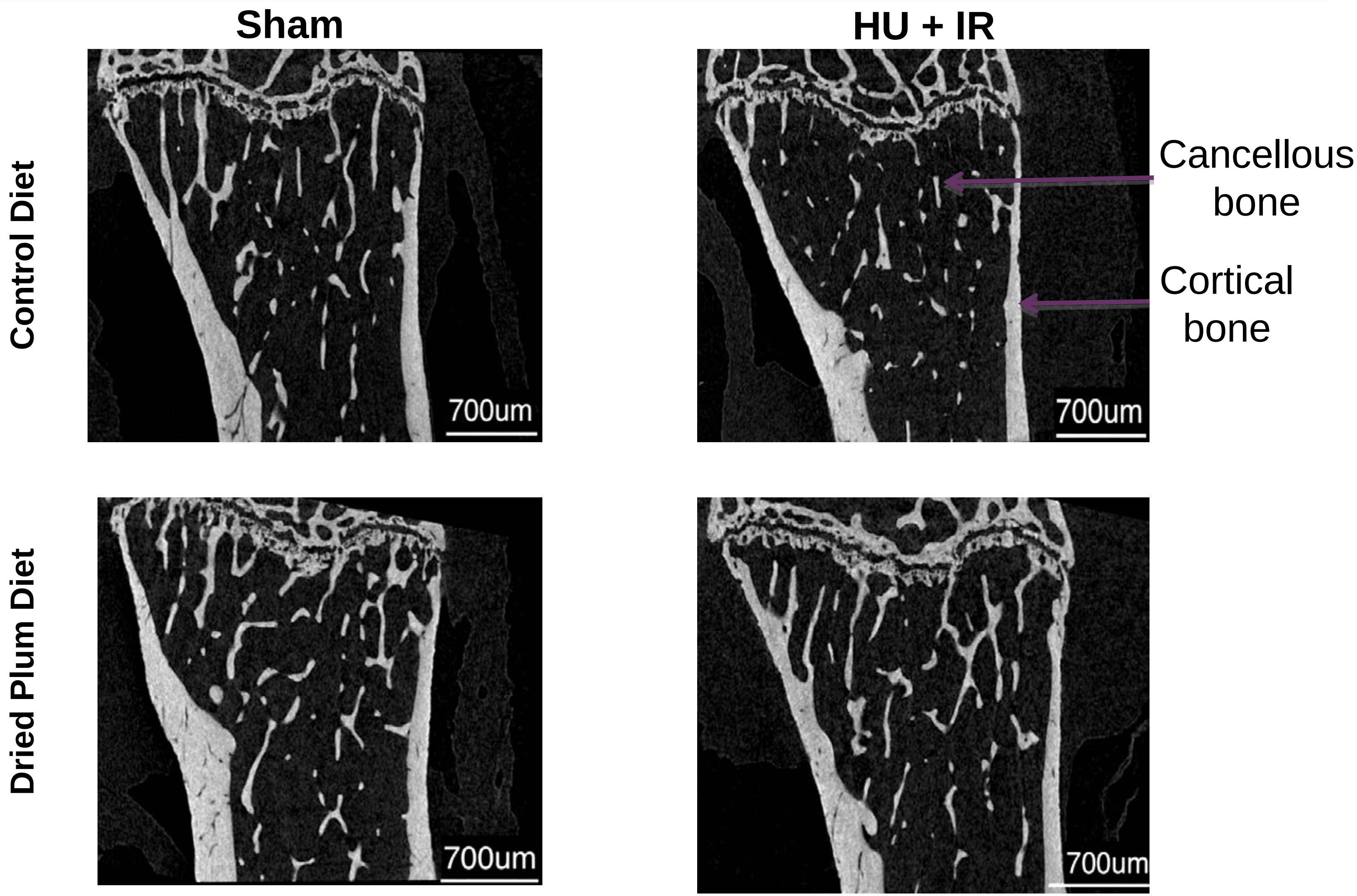
Experiment design



Experiment design

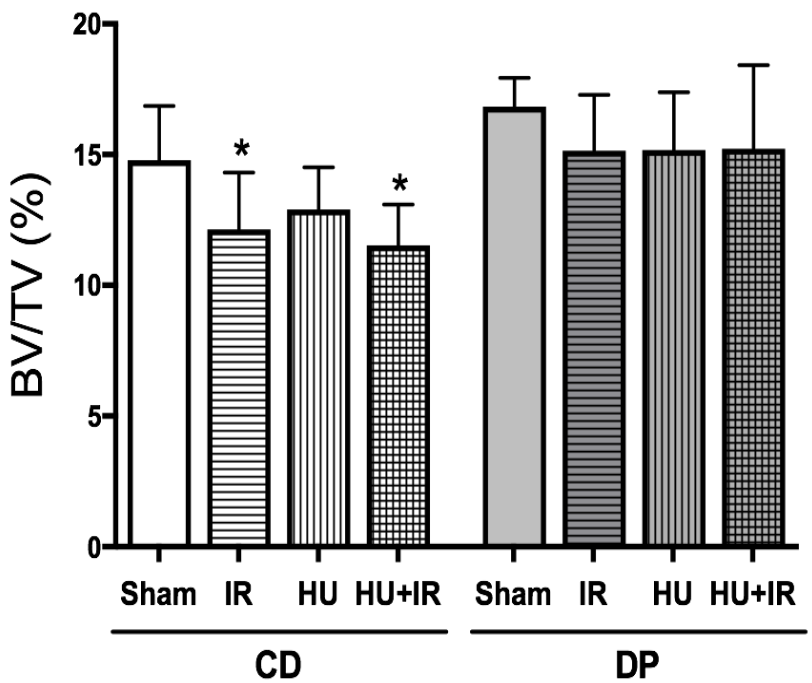


microCT images of the tibia after exposure to simulated weightlessness and ionizing radiation

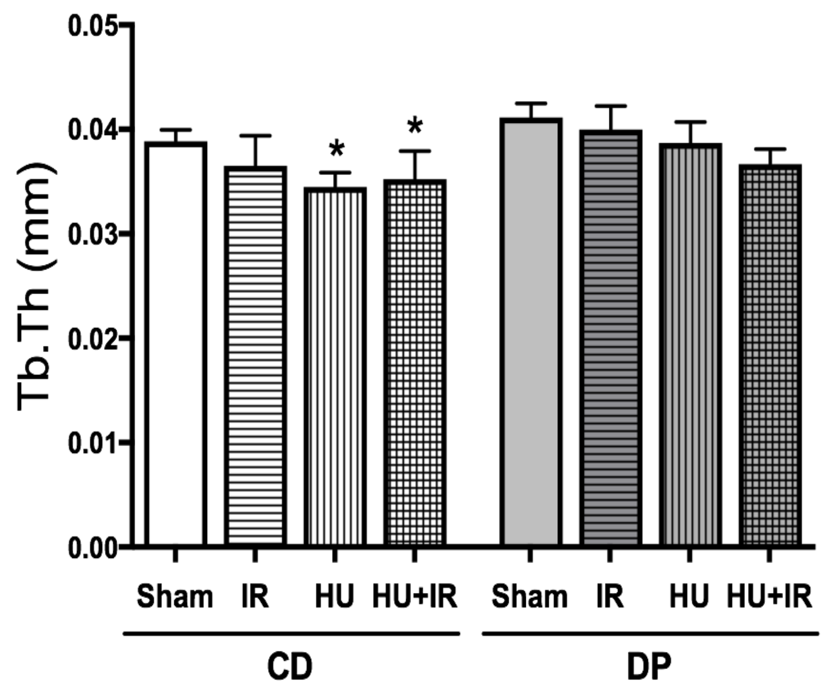


Dried Plum diet prevents cancellous bone loss in long bone (tibia)

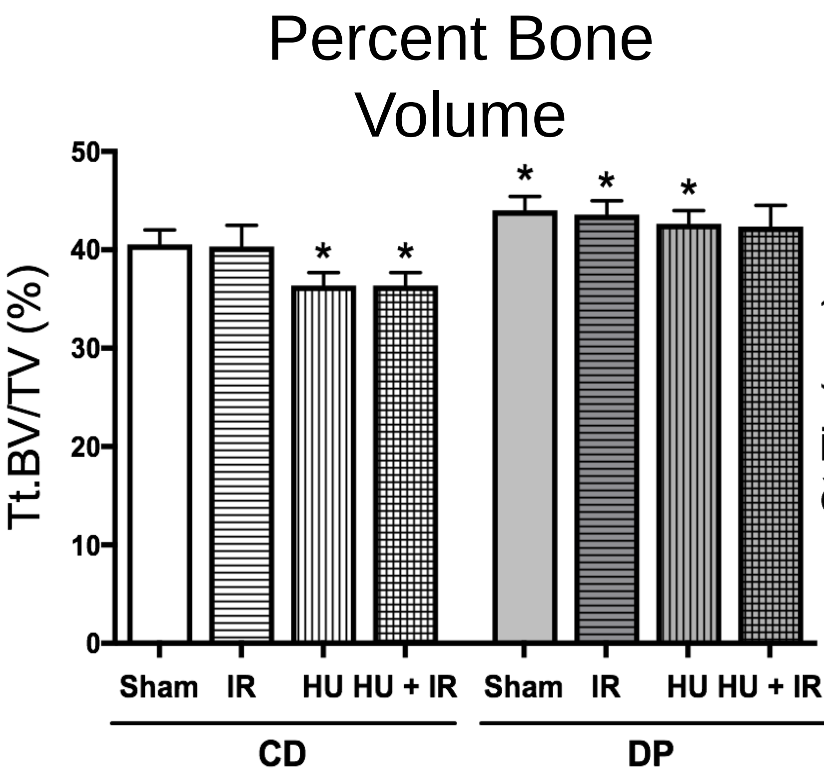
Percent Bone Volume

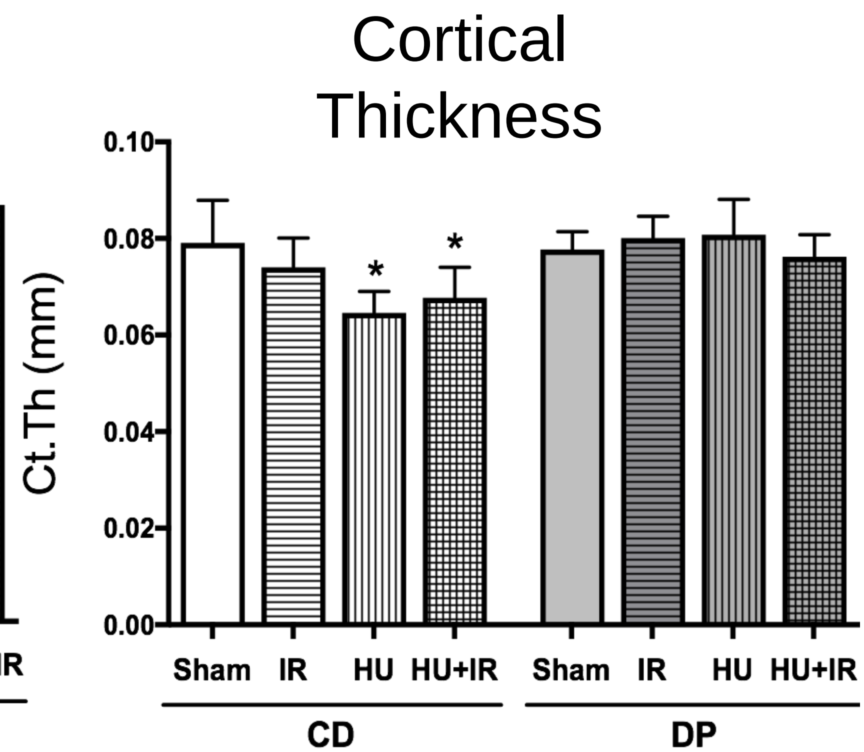


Trabecular Thickness

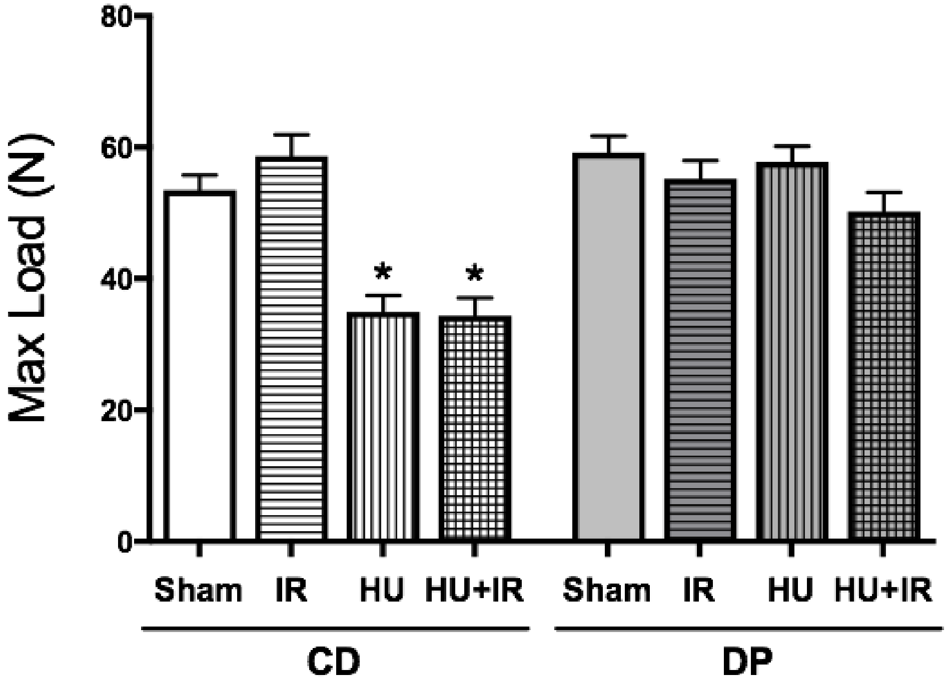


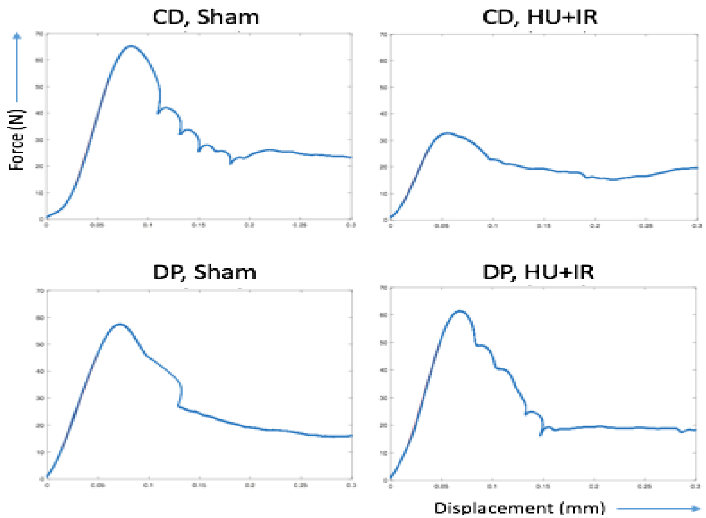
Dried Plum diet prevents cancellous and cortical bone loss in axial bone (vertebrae)



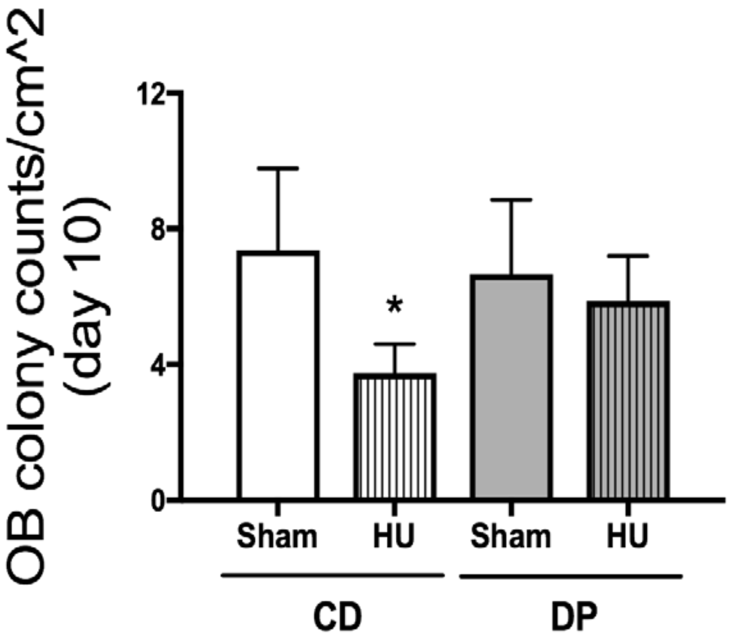


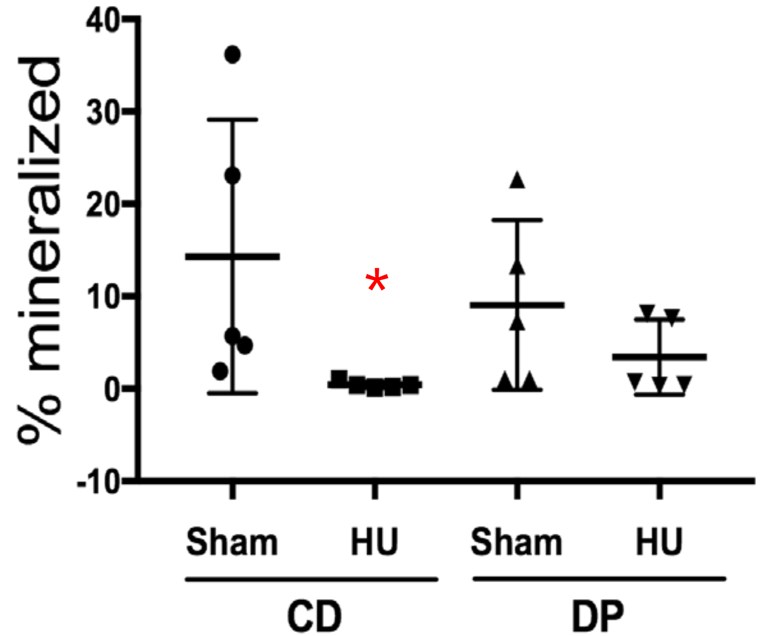
Dried Plum diet prevents bone strength decrease in axial bone (vertebrae)

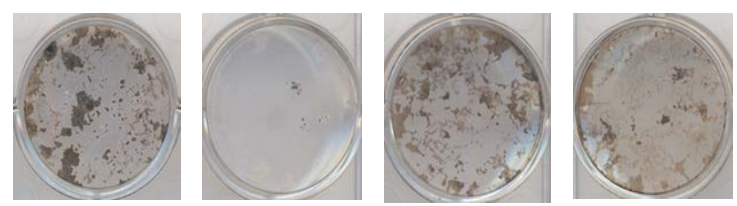




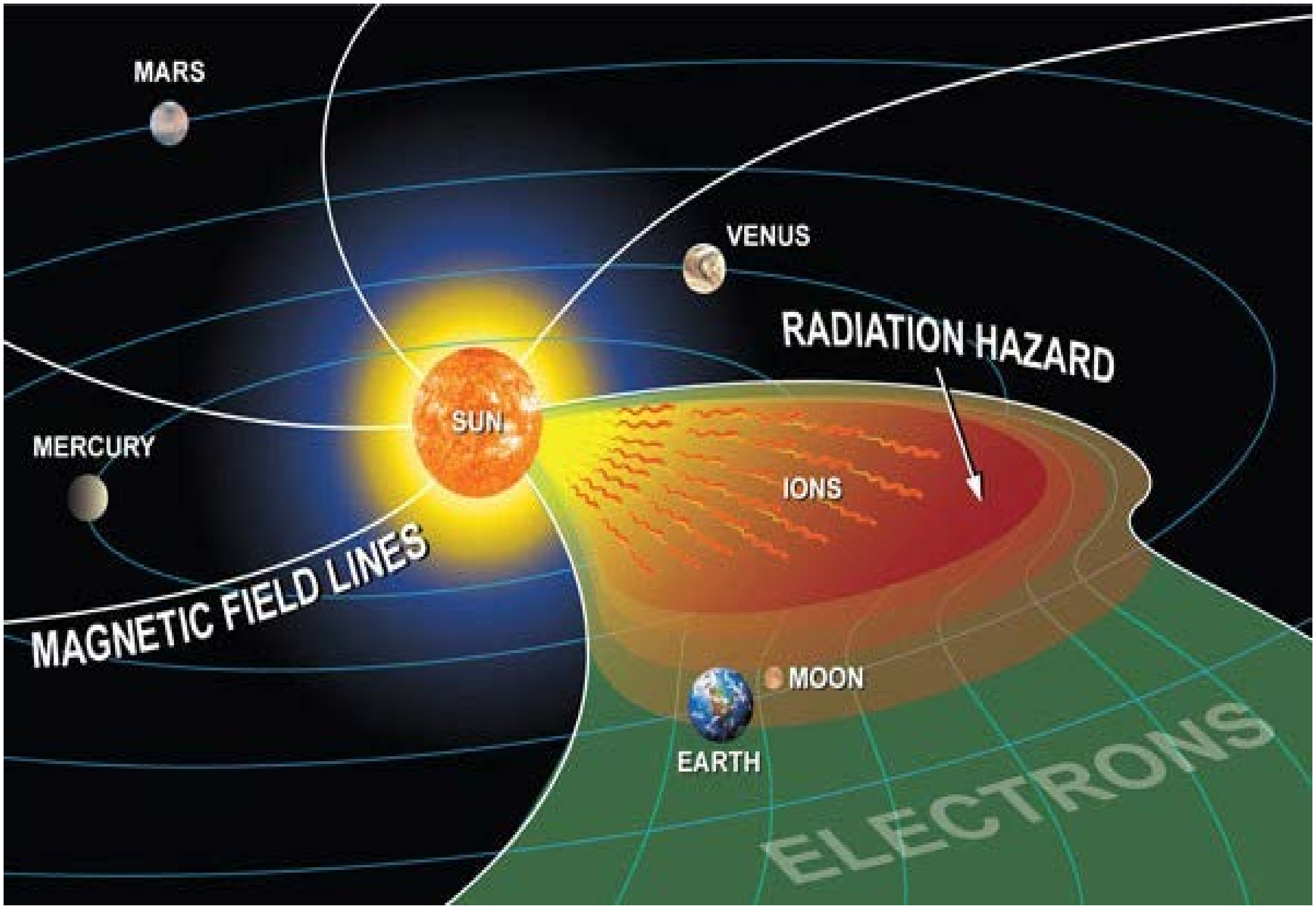
Dried Plum diet protects osteoblast progenitors





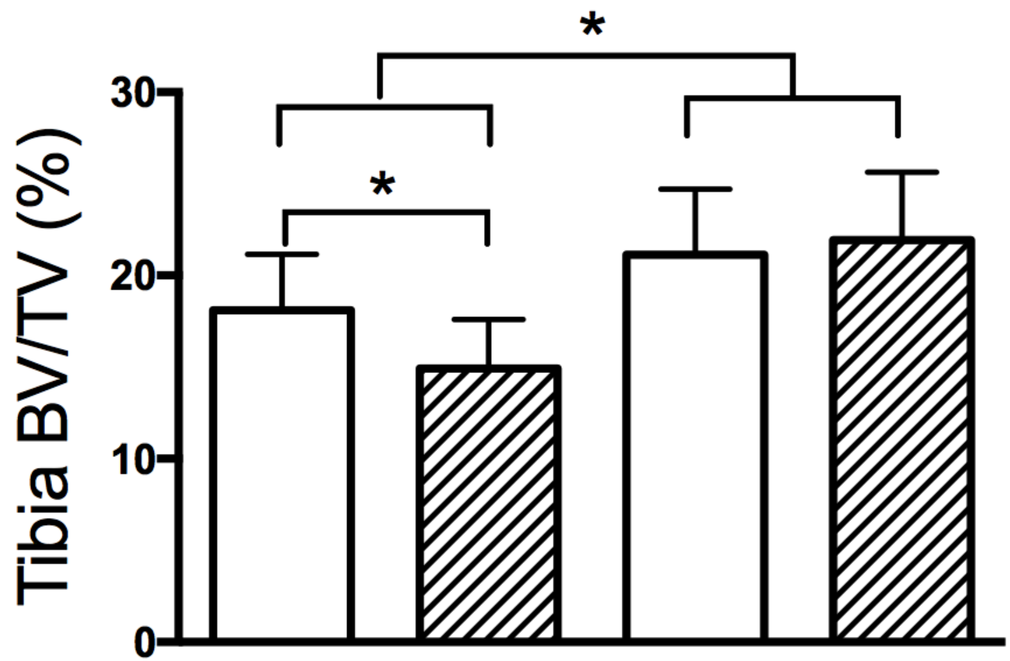


Does DP protect from high dose of HZE particles? Does it protect the osteoprogenitors?



DP prevents from HZE induced cancellous bone loss (1Gy ^{56}Fe)

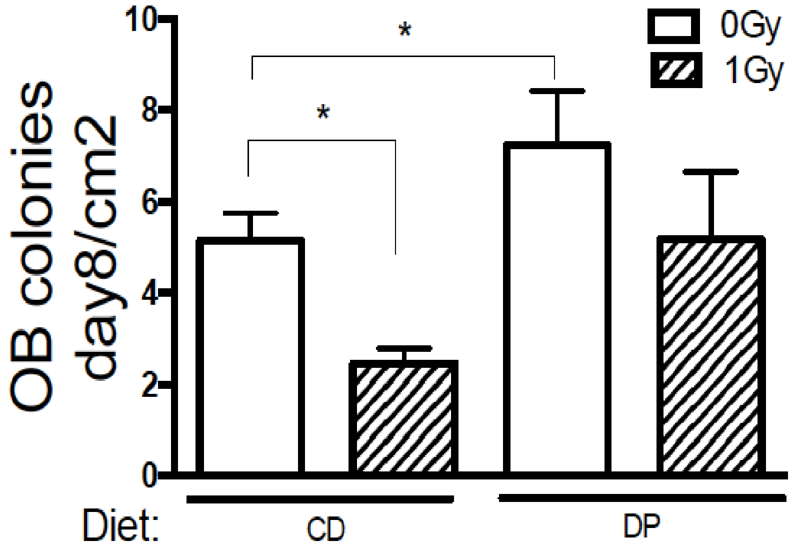
HZE
day 30

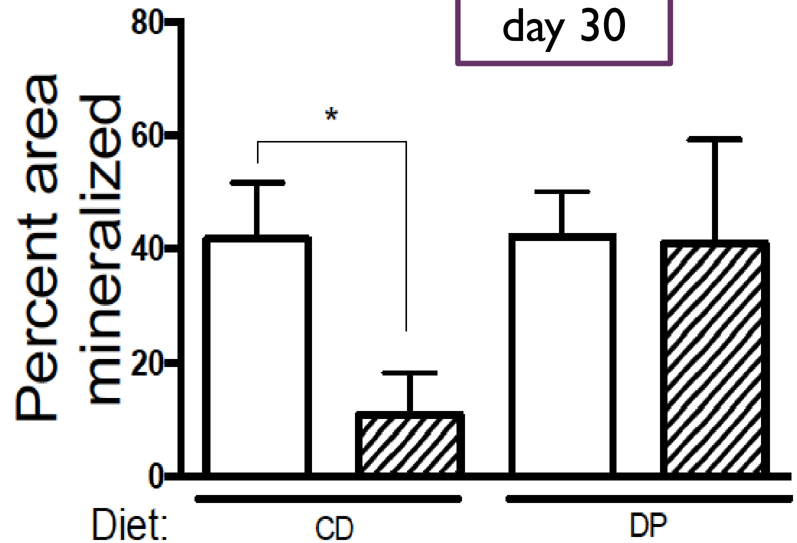


Tx:	CD	CD	DP	DP
IR:	-	+	-	+

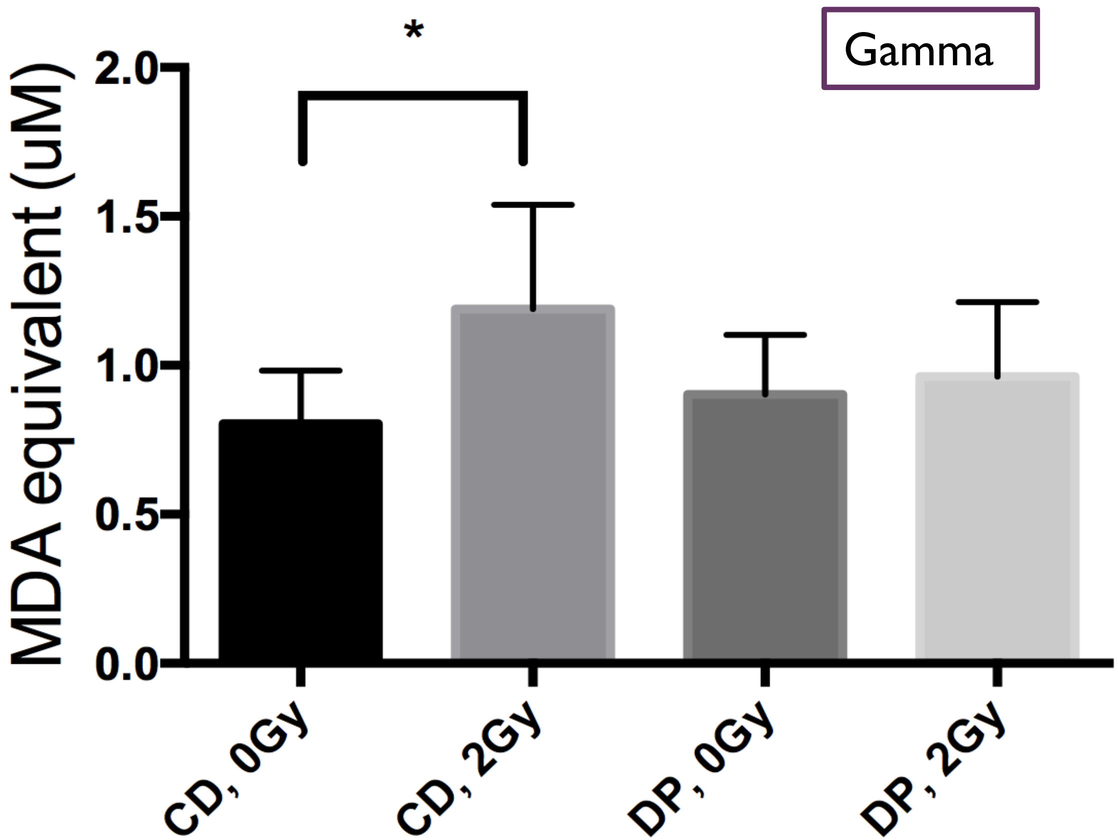
Dried Plum protects marrow-derived osteoblast progenitors in mice exposed to HZE

HZE
day 30



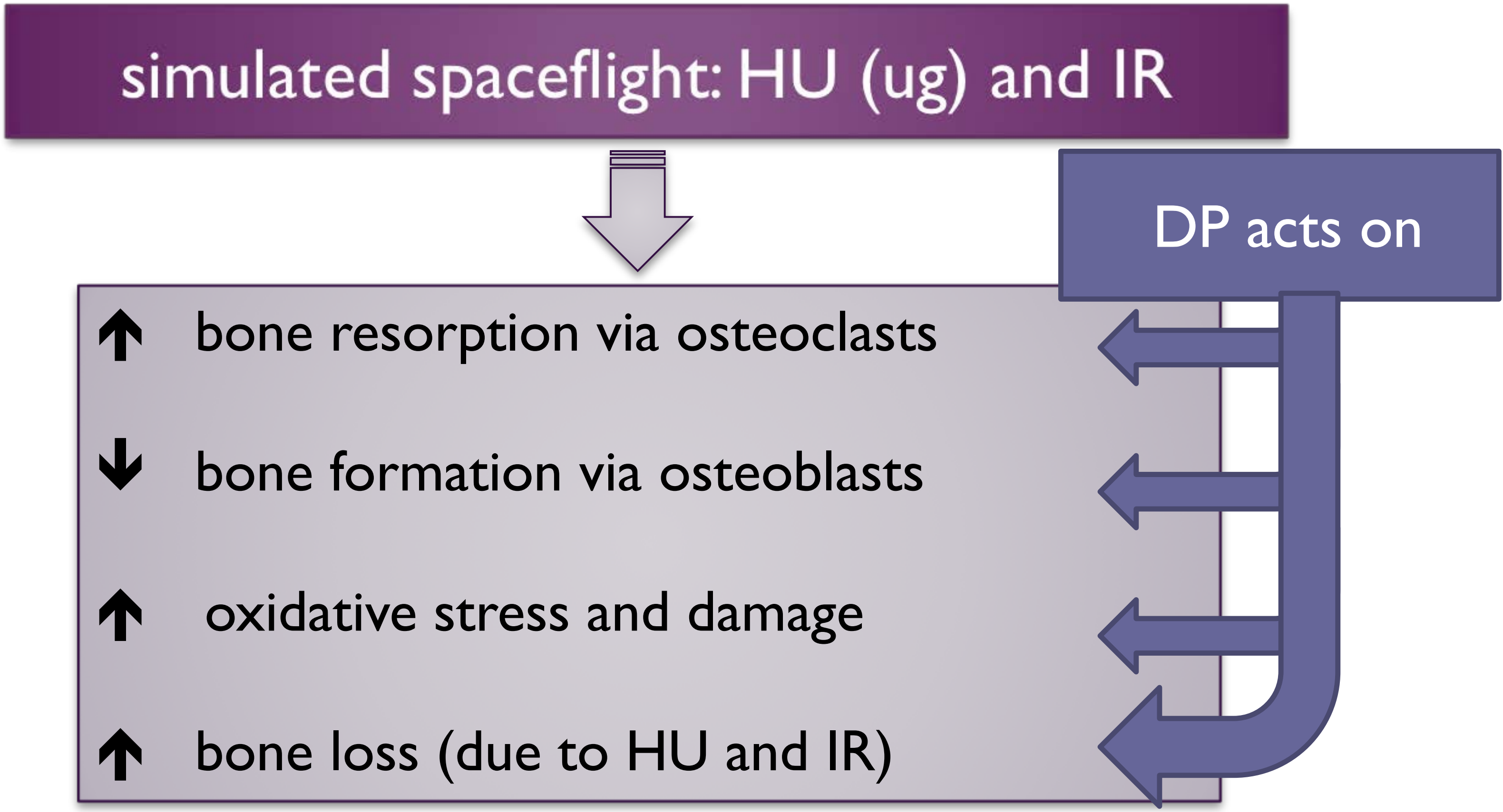


A broader, radiation countermeasure for multiple tissues?



Dietary DP prevents radiation-induced elevation in systemic marker of oxidative stress (serum TBARS)

DP: Possible mechanisms of actions



Dried Plum as potential spaceflight countermeasure

★*DP diet prevents bone decrements due to simulated spaceflight:*

- ***Cancellous and cortical bone loss prevented***
- ***Bone quality and strength***

★*DP diet fully prevents radiation-induced bone loss from low-LET and high-LET radiation:*

- ***Relevance for both spaceflight and possibly radiotherapy patients***

Dried Plum as potential spaceflight countermeasure

★ *DP protects marrow-derived osteoprogenitors:*

- ***possible relevance to mesenchymal stem cells***

★ *Mechanisms of DP :*

- ***mitigating early increase in pro-osteoclast cytokines***
- ***protecting the bone-forming osteoblasts***
- ***reduces oxidative stress and inflammation***
- ***A multi-tissue solution?***

Questions that we may be able to address using RNAseq data

1. What gene networks are responsive to DP?
2. What do these gene networks tell us about the possible mode of action DP?

3. What gene networks are responsive to ionizing radiation exposure? (Regardless of any observed microstructural changes)

4. Does DP prefeeding “prime” bone to defend against IR exposure?

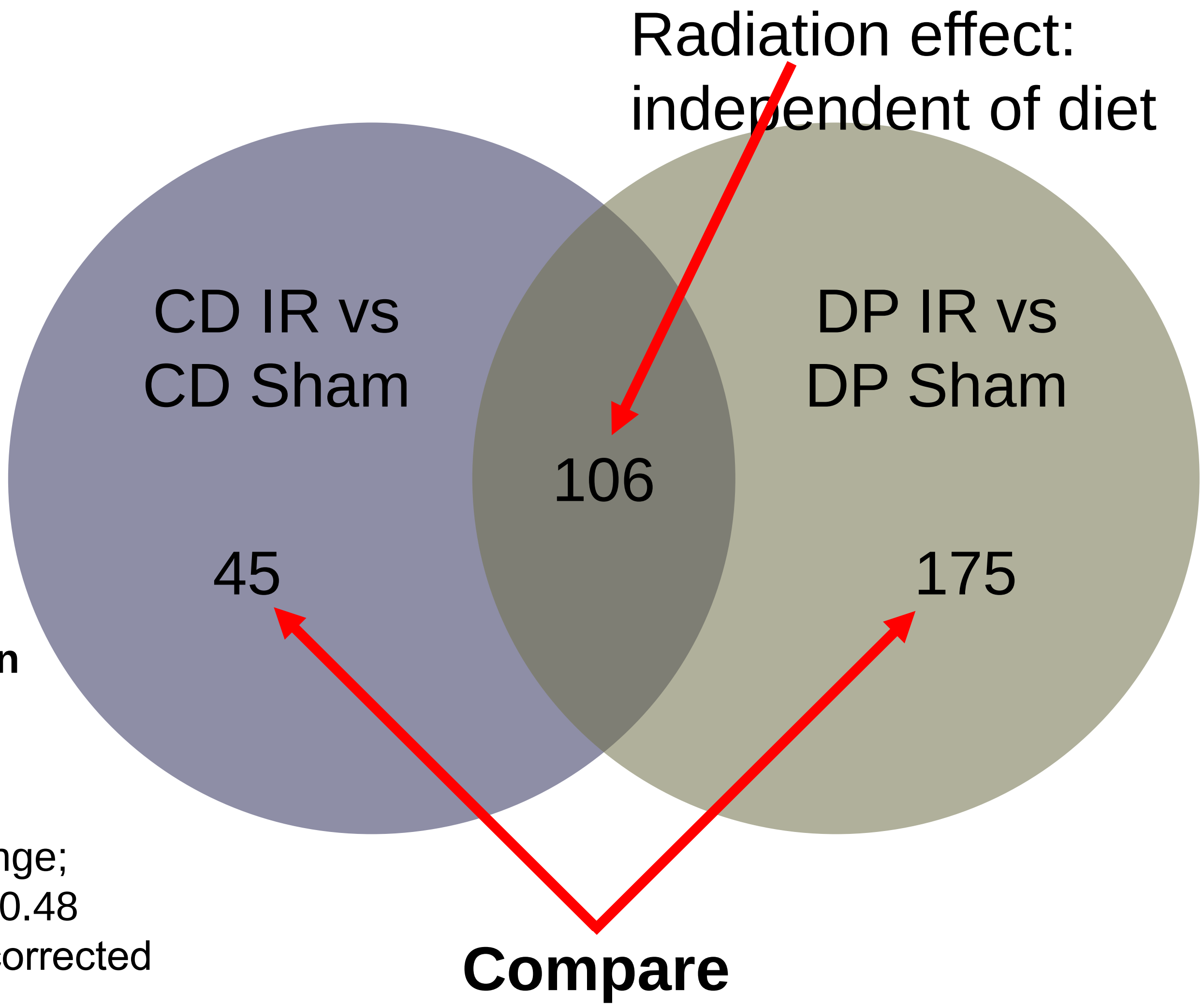
What are the DP-specific responses to radiation?

Unraveling DP diet-induced responses to ionizing radiation

Downregulated genes

CD IR vs CD Sham: 151

DP IR vs DP Sham: 281



Over representation analysis using WEBGESTALT

Cut-off: 1.4 fold change;
Hence, Fold log 2 = 0.48
$p \leq 0.05$ Benjamini corrected

DP-specific responses to IR: downregulated gene networks

KEGG Pathways

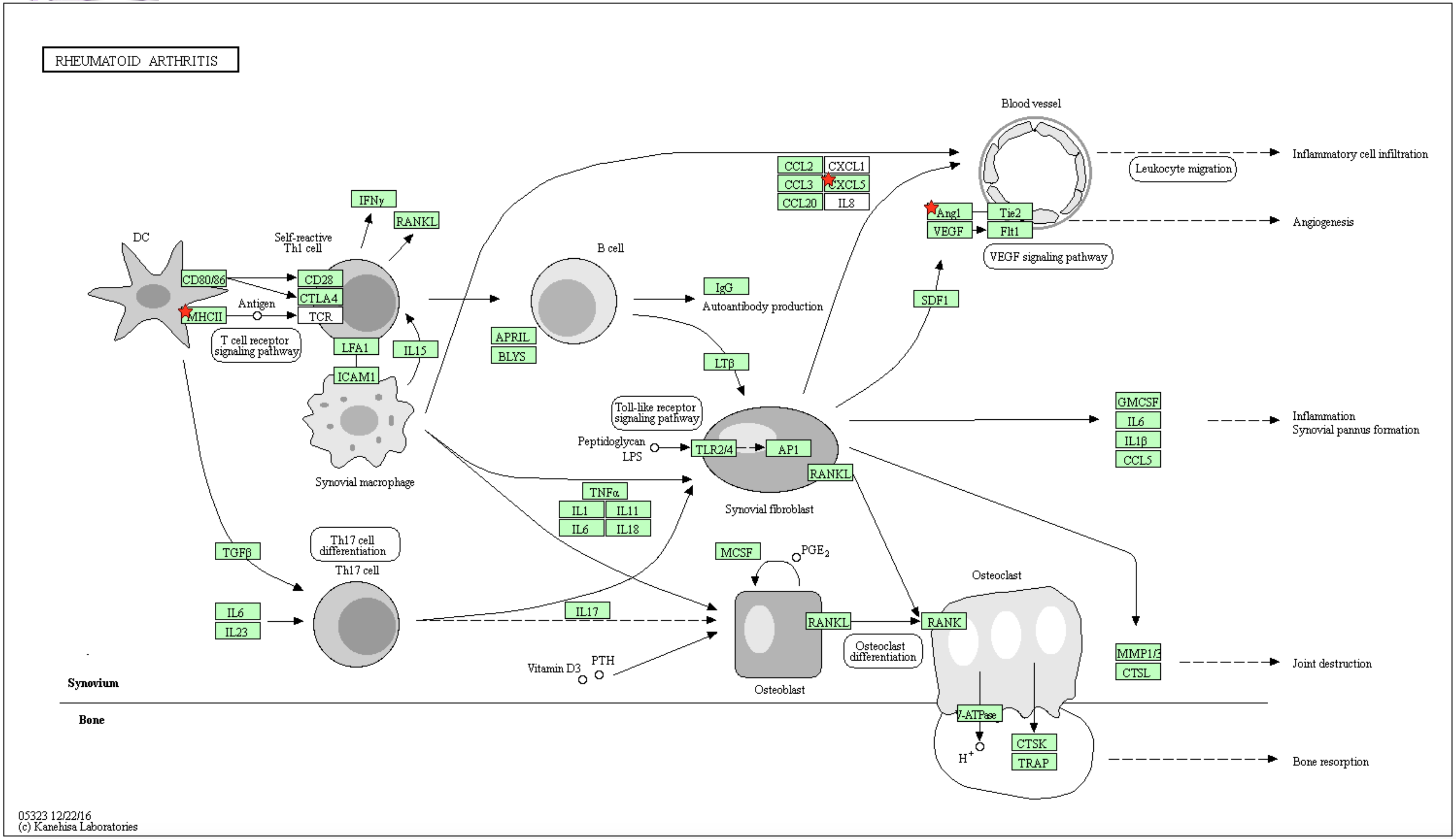
Description	# of genes	FDR
Hematopoietic cell lineage - Mus musculus (mouse)	10	1.11E-05
Asthma - Mus musculus (mouse)	6	2.21E-05
Platelet activation - Mus musculus (mouse)	10	4.35E-05
Staphylococcus aureus infection - Mus musculus (mouse)	7	6.40E-05
Antigen processing and presentation - Mus musculus (mouse)	8	0.00021852
Intestinal immune network for IgA production - Mus musculus (mouse)	6	0.00021852
Toxoplasmosis - Mus musculus (mouse)	8	0.00076871
Rheumatoid arthritis - Mus musculus (mouse)	7	0.00076871
Leishmaniasis - Mus musculus (mouse)	6	0.00179921
Th17 cell differentiation - Mus musculus (mouse)	7	0.00218071
Tuberculosis - Mus musculus (mouse)	9	0.00218071
Phagosome - Mus musculus (mouse)	9	0.00244633
Systemic lupus erythematosus - Mus musculus (mouse)	8	0.00244633
Th1 and Th2 cell differentiation - Mus musculus (mouse)	6	0.00541676
Cell adhesion molecules (CAMs) - Mus musculus (mouse)	8	0.0061156
Inflammatory bowel disease (IBD) - Mus musculus (mouse)	5	0.0061156
Allograft rejection - Mus musculus (mouse)	5	0.0084218
Graft-versus-host disease - Mus musculus (mouse)	5	0.00854804
Type I diabetes mellitus - Mus musculus (mouse)	5	0.01140061
Autoimmune thyroid disease - Mus musculus (mouse)	5	0.01876814
Influenza A - Mus musculus (mouse)	7	0.02477853
Complement and coagulation cascades - Mus musculus (mouse)	5	0.02624234
Viral myocarditis - Mus musculus (mouse)	5	0.02640207

DP-specific responses to IR: downregulated gene networks

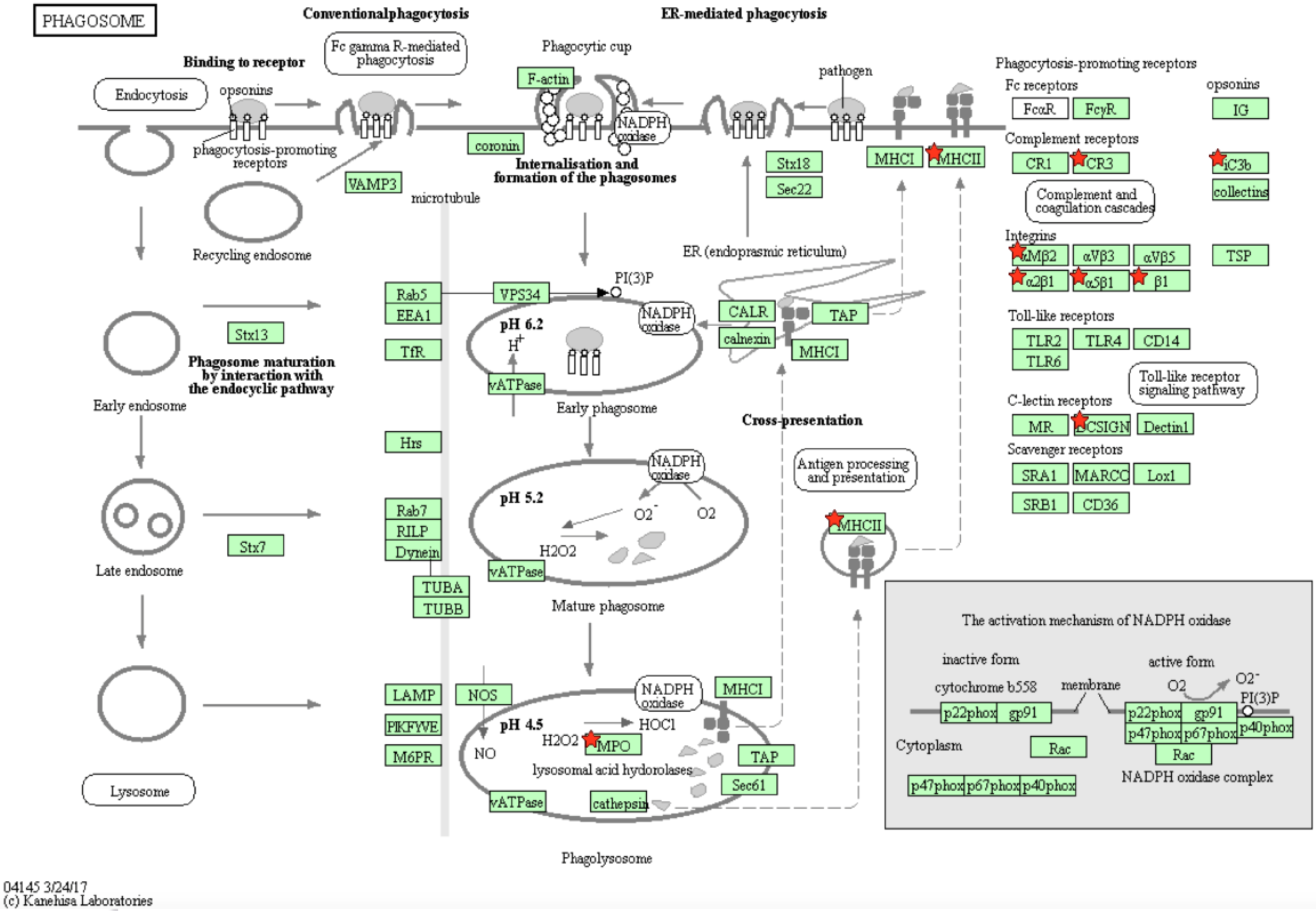
Reactome Pathways

Description	# of genes	FDR
Hemostasis	24	3.02E-08
Platelet activation, signaling and aggregation	14	9.87E-05
Platelet degranulation	9	0.00140932
Response to elevated platelet cytosolic Ca2+	9	0.00143111
Immune System	32	0.00372541
Common Pathway of Fibrin Clot Formation	4	0.01420829
Platelet homeostasis	5	0.02983486
Neutrophil degranulation	14	0.03487056
Innate Immune System	23	0.03487056

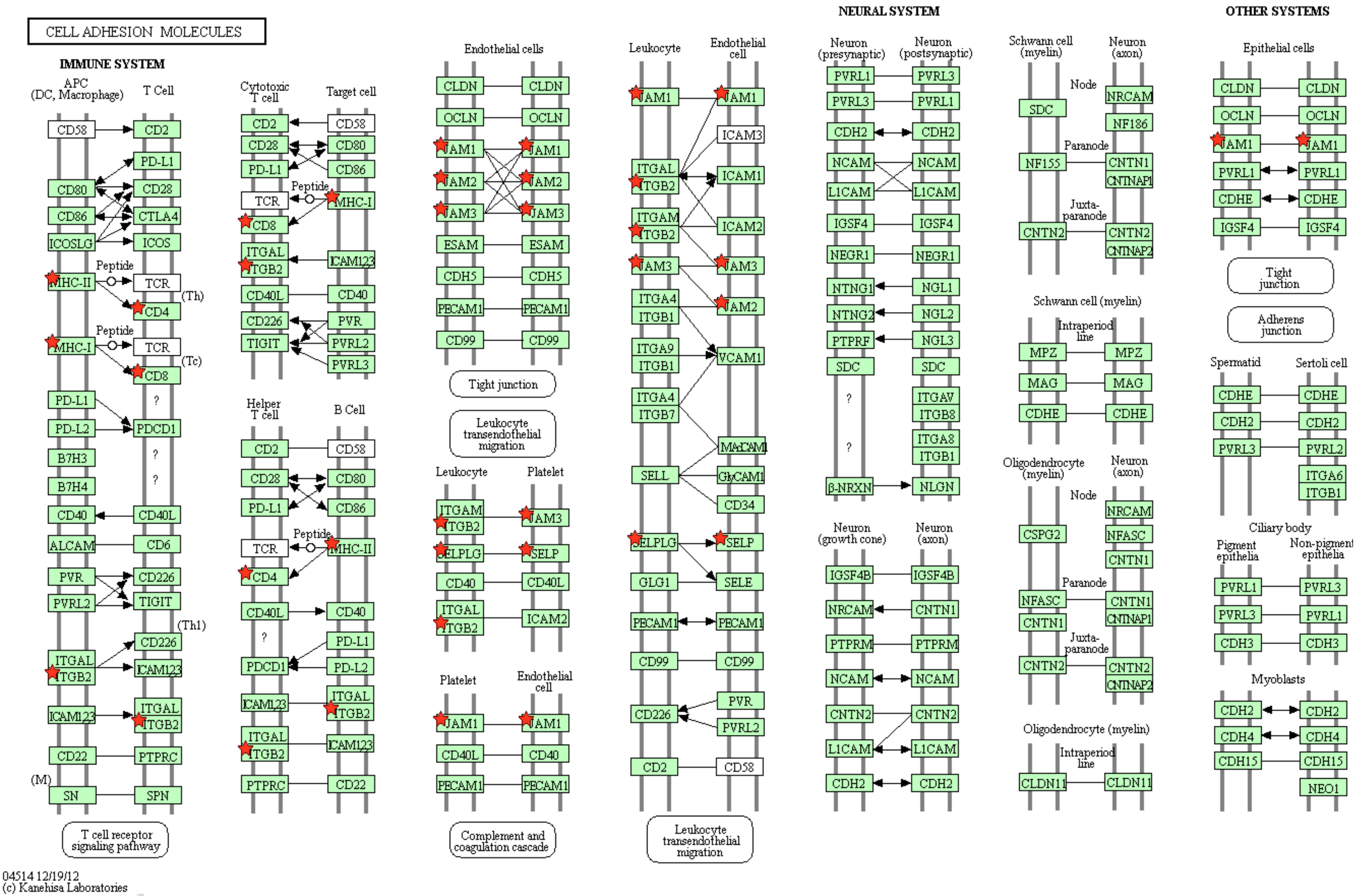
Rheumatoid arthritis-related genes are downregulated in bones of DP-fed animals exposed to IR



Genes associated with phagosome formation and lysosomal fusion are downregulated in bones of DP-fed animals exposed to IR



Cell adhesion genes are downregulated in bones of DP-fed animals exposed to IR

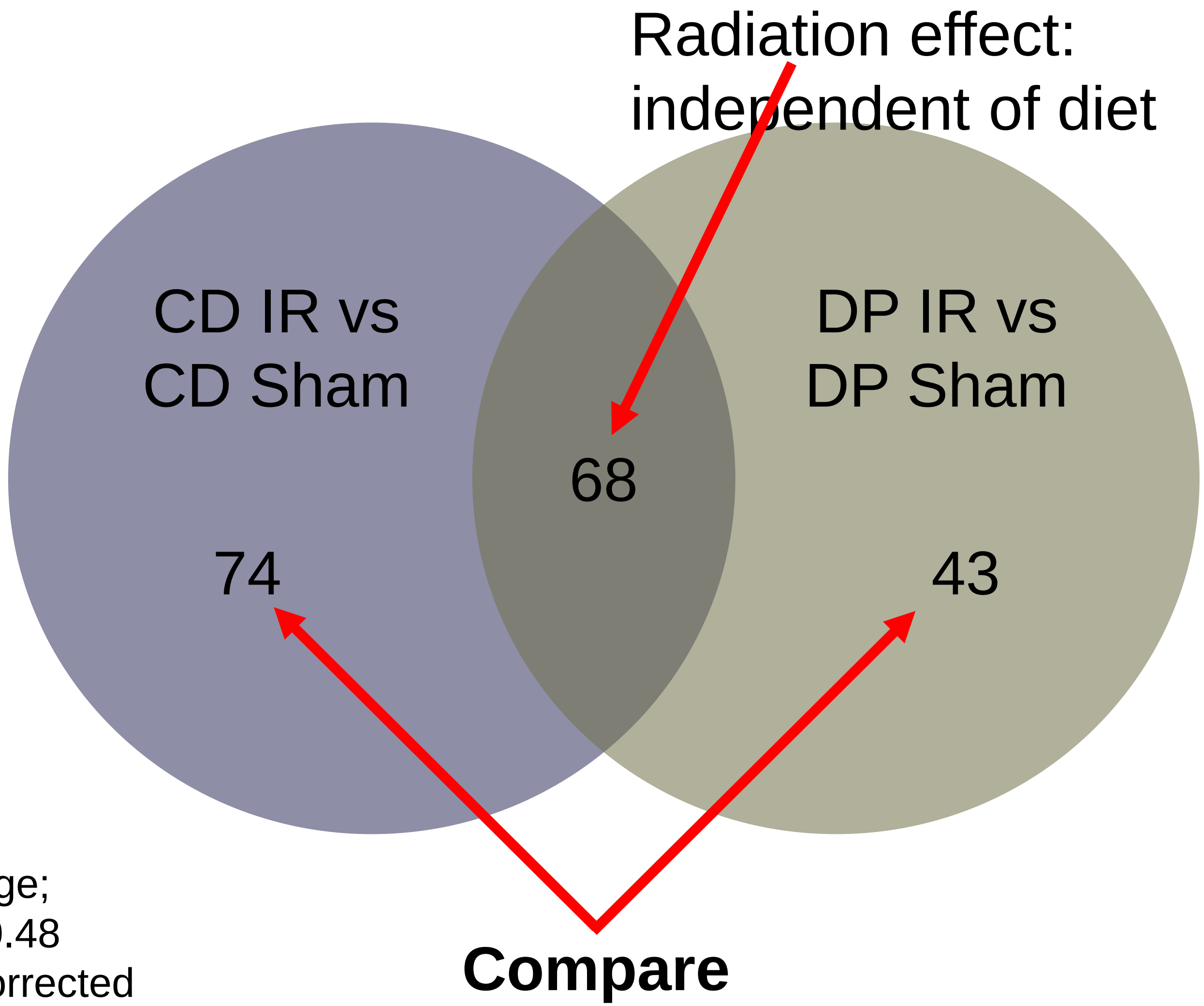


Unraveling DP diet-induced responses to ionizing radiation

Upregulated genes

CD IR vs CD Sham: 142

DP IR vs DP Sham: 111



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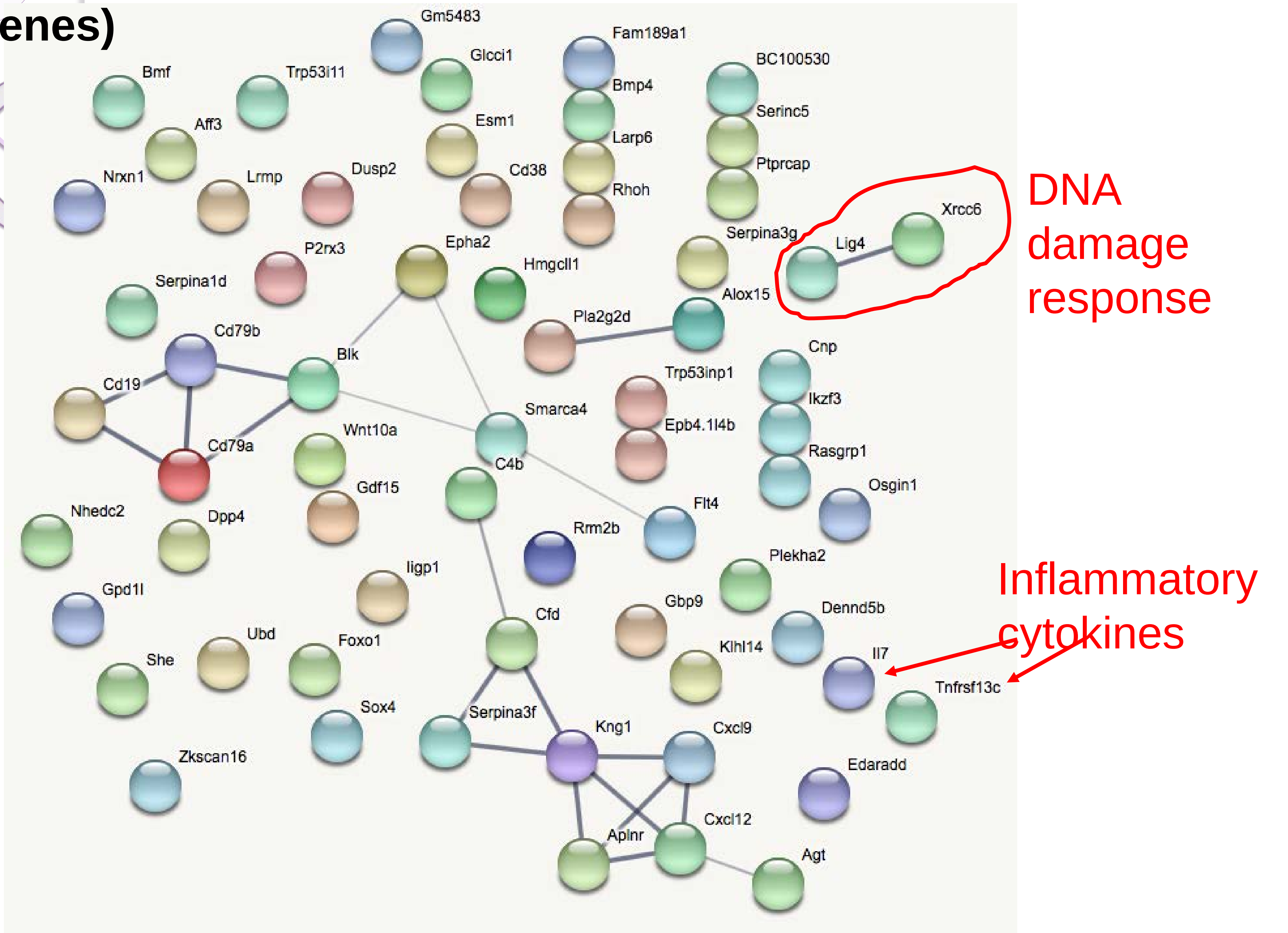
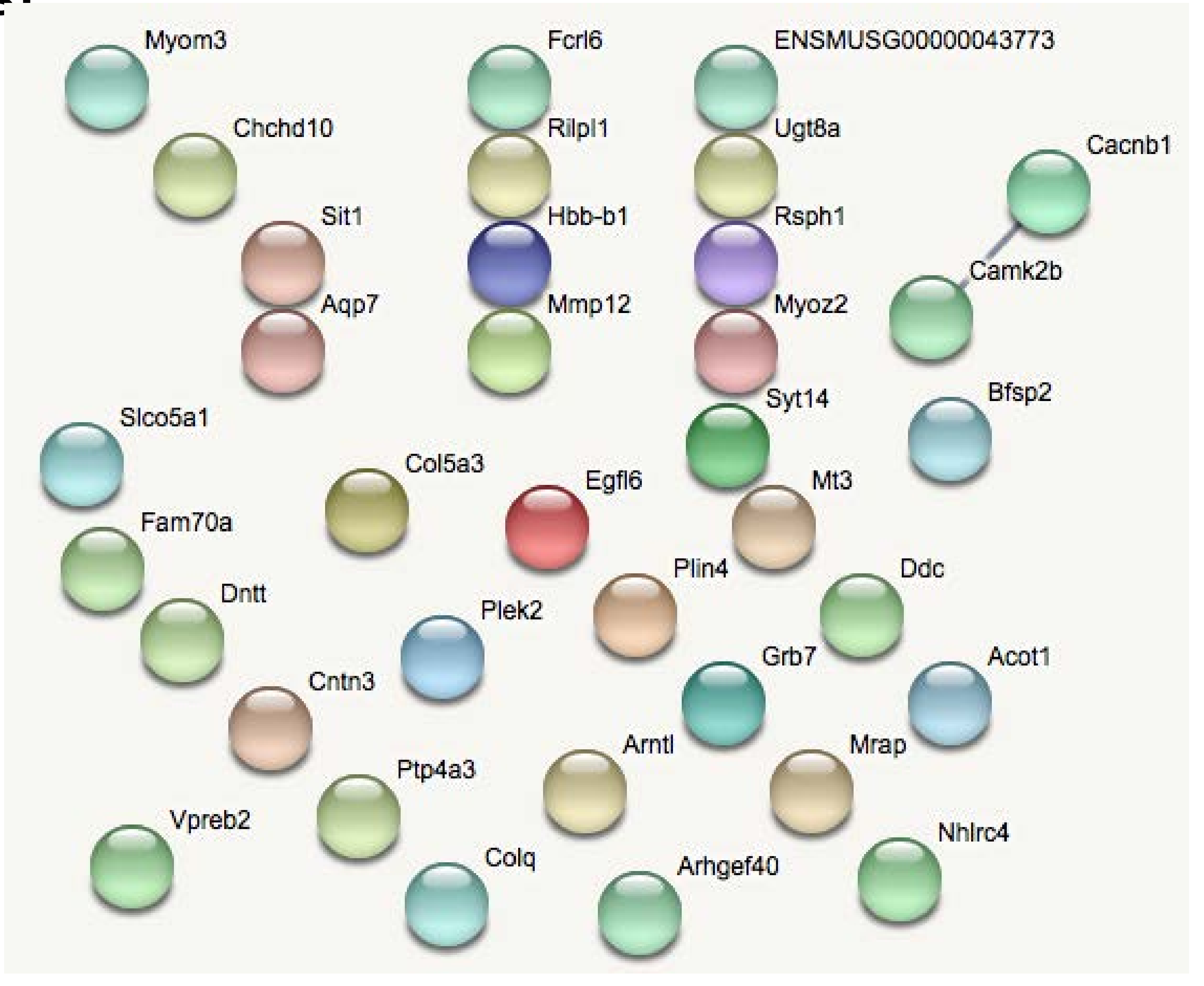
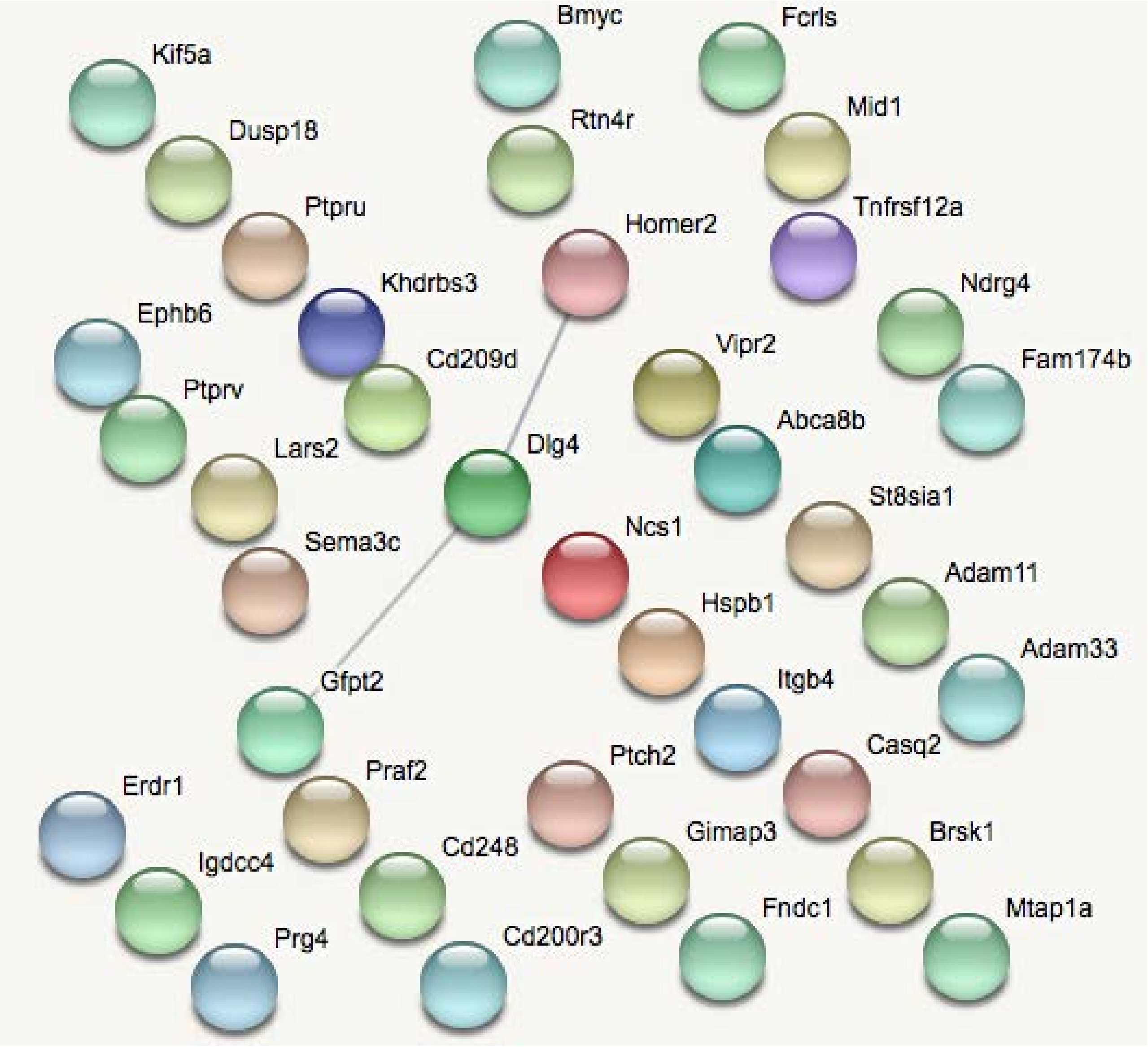
Upregulated genes in response to IR in CD group (all genes)



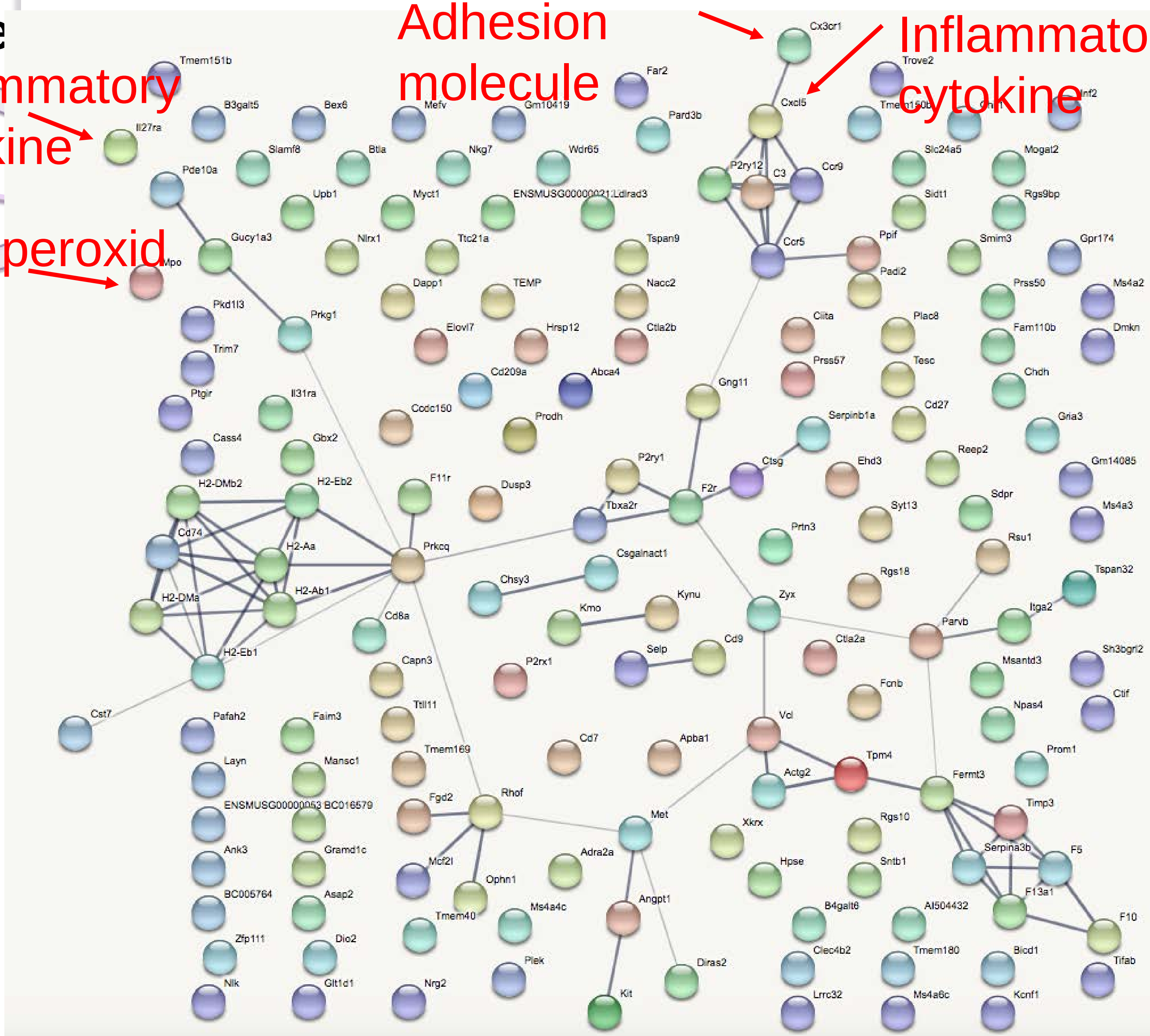
Upregulated genes in response to IR in DP group (all genes)



Downregulated genes in response to IR in CD group (all genes)



Downregulated genes in response to IR in DP group (all gene



Drugs that act on genes downregulated by IR in DP-treated mice (Top 20)

17: Drug [Display Chart] 157 input genes in category / 20813 annotations before applied cutoff / 22841 genes in category

	ID	Name	Source	pValue	FDR B&H	FDR B&Y	Bonferroni	Genes from Input	Genes in Annotation
1	CID009548826	T x A	Stitch	1.245E-9	2.590E-5	2.725E-4	2.590E-5	8	48
2	ctd:D002737	Chloroprene	CTD	2.793E-9	2.907E-5	3.058E-4	5.814E-5	31	1355
3	CID009854011	cangrelor	Stitch	2.517E-8	1.536E-4	1.616E-3	5.239E-4	6	27
4	CID000000899	N-acetylhexosamine	Stitch	2.953E-8	1.536E-4	1.616E-3	6.145E-4	17	472
5	CID000114873	thromboxane	Stitch	4.895E-8	1.925E-4	2.025E-3	1.019E-3	12	223
6	ctd:C022921	benzo(k)fluoranthene	CTD	5.638E-8	1.925E-4	2.025E-3	1.173E-3	19	620
7	CID000000191	arabinosyladenine	Stitch	6.507E-8	1.925E-4	2.025E-3	1.354E-3	21	762
8	ctd:C473384	lumiracoxib Cox-2 inhibitor NSAID	CTD	7.399E-8	1.925E-4	2.025E-3	1.540E-3	4	7
9	ctd:D016264	Dextran Sulfate	CTD	8.601E-8	1.989E-4	2.092E-3	1.790E-3	11	190
10	CID000005461	TXB2 (thromboxane B2	Stitch	1.284E-7	2.673E-4	2.812E-3	2.673E-3	9	118
11	CID006918456	prasugrel	Stitch	2.026E-7	3.833E-4	4.032E-3	4.216E-3	7	61
12	CID000121963	L-658,758	Stitch	2.635E-7	4.219E-4	4.439E-3	5.485E-3	4	9
13	ctd:C074619	bivalirudin	CTD	2.635E-7	4.219E-4	4.439E-3	5.485E-3	4	9
14	CID000160247	naphthol AS-D chloroacetate	Stitch	5.830E-7	8.667E-4	9.118E-3	1.213E-2	7	71
15	CID000002232	1dwc	Stitch	6.254E-7	8.678E-4	9.130E-3	1.302E-2	6	45
16	CID000004792	phorbol acetate myristate	Stitch	1.036E-6	1.185E-3	1.247E-2	2.157E-2	27	1400
17	CID000002499	enantio-PAF-C16	Stitch	1.048E-6	1.185E-3	1.247E-2	2.182E-2	11	244
18	2295 UP	Riluzole hydrochloride; Up 200; 14.8uM; MCF7; HT HG-U133A	Broad Institute CMAP Up	1.080E-6	1.185E-3	1.247E-2	2.248E-2	10	196
19	ctd:D004054	Diethylstilbestrol	CTD	1.082E-6	1.185E-3	1.247E-2	2.252E-2	26	1316
20	CID009543487	C4 m	Stitch	1.214E-6	1.234E-3	1.299E-2	2.526E-2	7	79

Drugs that act on genes upregulated by IR in DP-treated mice (Top 20)

Dietary supplement and drug given to patients with AD and senile dementia; increases lifespan of mice

17: Drug [Display Chart] 33 input genes in category / 6202 annotations before applied cutoff / 22841 genes in category

	ID	Name	Source	pValue	FDR B&H	FDR B&Y	Bonferroni	Genes from Input	Genes in Annotation
1	CID000171612	baciphelacin	Stitch	3.095E-6	1.372E-2	1.277E-1	1.919E-2	4	70
2	CID000157386	D-FMAUTP	Stitch	6.067E-6	1.372E-2	1.277E-1	3.763E-2	2	3
3	4268 UP	Meclofenoxate hydrochloride [3685-84-5]; Up 200; 13.6uM; PC3; HT HG-U133A	Broad Institute CMAP Up	7.809E-6	1.372E-2	1.277E-1	4.843E-2	5	192
4	2490 UP	Nifuroxazide [965-52-6]; Up 200; 14.6uM; HL60; HT HG-U133A	Broad Institute CMAP Up	8.847E-6	1.372E-2	1.277E-1	5.487E-2	5	197
5	ctd:C016517	indole-3-carbinol	CTD	2.507E-5	3.109E-2	2.895E-1	1.555E-1	9	1145
6	CID000503022	L-OddCTP	Stitch	4.232E-5	4.374E-2	4.072E-1	2.624E-1	2	7
7	ctd:C501413	tesaglitazar	CTD	7.478E-5	4.631E-2	4.311E-1	4.638E-1	6	504
8	CID000490401	FIAUTP	Stitch	9.043E-5	4.631E-2	4.311E-1	5.609E-1	2	10
9	ctd:C041786	palm oil	CTD	1.177E-4	4.631E-2	4.311E-1	7.298E-1	10	1742
10	4085 UP	Thioridazine hydrochloride [130-61-0]; Up 200; 9.8uM; PC3; HT HG-U133A	Broad Institute CMAP Up	1.250E-4	4.631E-2	4.311E-1	7.750E-1	4	179
11	CID000008095	EGEEA	Stitch	1.324E-4	4.631E-2	4.311E-1	8.211E-1	2	12
12	4070 UP	Methionine sulfoximine (L) [15985-39-4]; Up 200; 22.2uM; PC3; HT HG-U133A	Broad Institute CMAP Up	1.736E-4	4.631E-2	4.311E-1	1.000E0	4	195
13	3706 UP	Demeclocycline hydrochloride [64-73-3]; Up 200; 8uM; PC3; HT HG-U133A	Broad Institute CMAP Up	1.771E-4	4.631E-2	4.311E-1	1.000E0	4	196
14	4089 UP	Amyleine hydrochloride [532-59-2]; Up 200; 14.8uM; PC3; HT HG-U133A	Broad Institute CMAP Up	1.771E-4	4.631E-2	4.311E-1	1.000E0	4	196
15	5736 UP	Trioxsalen [3902-71-4]; Up 200; 17.6uM; PC3; HT HG-U133A	Broad Institute CMAP Up	1.771E-4	4.631E-2	4.311E-1	1.000E0	4	196
16	5949 UP	AG-013608 [351320-38-2]; Up 200; 10uM; PC3; HT HG-U133A	Broad Institute CMAP Up	1.806E-4	4.631E-2	4.311E-1	1.000E0	4	197
17	337 UP	felodipine; Up 200; 10uM; MCF7; HG-U133A	Broad Institute CMAP Up	1.806E-4	4.631E-2	4.311E-1	1.000E0	4	197
18	2129 UP	Retrorsine [480-54-6]; Up 200; 11.4uM; HL60; HT HG-U133A	Broad Institute CMAP Up	1.806E-4	4.631E-2	4.311E-1	1.000E0	4	197
19	3088 DN	Idazoxan hydrochloride [79944-56-2]; Down 200; 16.6uM; HL60; HT HG-U133A	Broad Institute CMAP Down	1.841E-4	4.631E-2	4.311E-1	1.000E0	4	198
20	2458 DN	Cefotiam hydrochloride; Down 200; 7.2uM; HL60; HT HG-U133A	Broad Institute CMAP Down	1.841E-4	4.631E-2	4.311E-1	1.000E0	4	198

Drugs that act on genes upregulated by IR in CD-treated mice (Top 20)

17: Drug [Display Chart] 63 input genes in category / 13457 annotations before applied cutoff / 22841 genes in category

	ID	Name	Source	pValue	FDR B&H	FDR B&Y	Bonferroni	Genes from Input	Genes in Annotation
1	ctd:D002737	Chloroprene	CTD	4.806E-13	6.468E-9	6.522E-8	6.468E-9	23	1355
2	ctd:C017096	n-butoxyethanol	CTD	5.519E-10	3.714E-6	3.745E-5	7.428E-6	14	587
3	CID000005292	AC1L1K12	Stitch	1.296E-8	5.812E-5	5.861E-4	1.744E-4	7	102
4	ctd:D004317	Doxorubicin (Chemotherapy drug)	CTD	3.324E-8	1.118E-4	1.128E-3	4.474E-4	20	1785
5	CID000022641	U-2112	Stitch	3.036E-7	7.885E-4	7.952E-3	4.085E-3	4	21
6	ctd:C008261	lead acetate	CTD	3.516E-7	7.885E-4	7.952E-3	4.731E-3	19	1862
7	ctd:D003520	Cyclophosphamide	CTD	8.802E-7	1.692E-3	1.706E-2	1.185E-2	11	617
8	CID003081759	DD-PCR	Stitch	2.020E-6	3.398E-3	3.427E-2	2.718E-2	5	75
9	CID000426387	NSC187724	Stitch	2.580E-6	3.604E-3	3.635E-2	3.472E-2	4	35
10	CID000003003	Betamethasone-d5	Stitch	2.678E-6	3.604E-3	3.635E-2	3.604E-2	15	1342
11	ctd:C006703	benzo(b)fluoranthene	CTD	3.647E-6	4.462E-3	4.499E-2	4.908E-2	16	1564
12	ctd:C031655	tauroursodeoxycholic acid	CTD	5.254E-6	5.163E-3	5.206E-2	7.071E-2	6	160
13	ctd:C030935	benz(a)anthracene	CTD	6.844E-6	5.163E-3	5.206E-2	9.210E-2	14	1264
14	CID005743054	DFC-28	Stitch	7.123E-6	5.163E-3	5.206E-2	9.585E-2	3	14
15	ctd:D015121	6-Ketoprostaglandin F1 alpha	CTD	7.123E-6	5.163E-3	5.206E-2	9.585E-2	3	14
16	CID000103535	AC1Q6PMP	Stitch	7.194E-6	5.163E-3	5.206E-2	9.681E-2	6	169
17	CID000002290	AC1L1DCL	Stitch	7.571E-6	5.163E-3	5.206E-2	1.019E-1	5	98
18	ctd:D002118	Calcium	CTD	8.213E-6	5.163E-3	5.206E-2	1.105E-1	9	495
19	ctd:D015122	6-Mercaptopurine	CTD	8.309E-6	5.163E-3	5.206E-2	1.118E-1	7	265
20	CID000004742	NSC272671	Stitch	8.500E-6	5.163E-3	5.206E-2	1.144E-1	6	174

Summary

Bones from animals pre-fed with DP displayed downregulation of pathways related to:

- Adhesion
- Inflammation (RA)
- phagosome maturation/lysosomal fusion pathways

DNA damage response genes were upregulated in CD-fed group that received IR; these genes not observed in DP-fed animals exposed to IR

DP appears to protect from IR-induced DNA damage, inflammation and pro-adhesion events in bone

Acknowledgements

Sonette Steczina
Moniece Lowe
Samantha Torres
Ons M'Saad
Bernard Halloran (UCSF)

Gabriella Loots (LLNL)
Aimy Sebastian (LLNL)
Nicholas Hum (LLNL)

Josh Alwood's lab
Eduarduo Almeida and Liz Blaber's Labs

BNL and NSRL teams

SLSTP program

Funding

HRP HHC Omnibus 15-15Omni2-0039 (PI: Schreurs)

NASA Postdoctoral Program – Space Biology

NSBRI Grant #MA02501 (PI: Globus)

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Questions?

Dried plum diet protects from bone loss caused by ionizing radiation

Bone loss caused by ionizing radiation is a potential health concern for radiotherapy patients, radiation workers and astronauts. In animal studies,...

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