SUPPORTING INFORMATION

Proteomic evaluation of the acute radiation syndrome of the gastrointestinal tract in a murine total-body irradiation model.

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Retinoid analysis.

Retinoid levels were determined by liquid chromatography-multistage tandem mass spectrometry (LC-MRM^3) which is an LC-MS/MS method utilizing two distinct fragmentation events for enhanced selectivity (Jones et al. 2015). Preparation of mouse intestinal tissue included flushing out contents with PBS. The jejunum and ileum were isolated from the small intestine and snap frozen with liquid nitrogen and stored at -80 °C until extraction. Tissues were homogenized in saline and extraction of retinoids was performed under yellow lights using a two-step liquid-liquid extraction that has been described in detail previously using 4,4-dimethyl-RA as an internal standard (Kane et al. 2005; Kane et al. 2008b; Kane and Napoli 2010; Jones et al. 2015). Levels of RA were measured using a Shimadzu Prominence UFLC XR liquid chromatography system (Shimadzu, Columbia, MD) coupled to an AB Sciex 5500 QTRAP hybrid triple quadrupole mass spectrometer (AB Sciex, Framingham, MA) using atmospheric pressure chemical ionization (APCI) operated in positive ion mode as previously described (Jones et al. 2015). Retinol and RE were quantified via HPLC-UV according to previously published methodology (Kane et al. 2008a; Kane and Napoli 2010). Retinoid levels were measured using n=5 per condition except for control (0) which is an average of n=15 un-irradiated control mice euthanized at day -6 (n=5), day 0 (n=5), or day 6 (n=5). The statistical analysis relied on the unpaired Student's *t*-test between groups and the data was represented as means ± standard deviation. Retinoid data are shown in **Supplemental Fig. 11** and **Supplemental Fig. 12**.

References.

Jones JW, Pierzchalski K, Yu J, Kane MA. Use of fast HPLC multiple reaction monitoring cubed for endogenous retinoic acid quantification in complex matrices. Anal Chem 87: 3222-30; 2015.

Kane MA, Chen N, Sparks S, Napoli JL. Quantification of endogenous retinoic acid in limited biological samples by LC/MS/MS. Biochem J 388: 363-9; 2005.

Kane MA, Folias AE, Napoli JL. HPLC/UV quantitation of retinal, retinol, and retinyl esters in serum and tissues. Anal Biochem 378: 71-9; 2008a.

Kane MA, Folias AE, Wang C, Napoli JL. Quantitative profiling of endogenous retinoic acid in vivo and in vitro by tandem mass spectrometry. Anal Chem 80: 1702-8; 2008b.

Kane MA, Napoli JL. Quantification of endogenous retinoids. Methods Mol Biol 652: 1-54; 2010.

Supplemental Figures

Supplementary Figure 1. Expression of proteins most changed after radiation. Minimum FC > 10 of expression for at least one condition and FDR adjusted ANOVA $p < 0.01$. Data shown as a function of dose on a given day after dose. Data shown as a function of time after dose at a given radiation dose is shown in **Fig. 1**.

Supplementary Figure 2. Canonical pathways altered by radiation. (2a) Canonical pathways altered after radiation where criteria for pathway changes was activation z-score > 2 for at least one condition and Fisher's exact test $p < 0.01$. **(2b)** Protein changes associated with the top three pathways altered by radiation. Minimum FC > 2 for at least one condition with a FDR corrected ANOVA $p < 0.05$. Data shown as a function of dose on a given day after dose. Data shown as a function of time after dose at a given radiation dose is shown in **Fig. 2.**

Supplementary Figure 3. Upstream regulators altered by radiation. Criteria for transcription regulators was absolute activation z-score > 2 for at least one condition and a Fisher's exact test $p < 0.01$**.** Data shown as a function of dose on a given day after dose. Data shown as a function of time after dose at a given radiation dose is shown in **Fig. 3.**

Supplemental Figure 4. Venn diagram of differential protein expression showing the effect of day after 8 Gy irradiation.

Supplemental Figure 5. Venn diagram of differential protein expression showing the effect of day after 10 Gy irradiation.

Supplemental Figure 6. Venn diagram of differential protein expression showing the effect of day after 12 Gy irradiation.

Supplemental Figure 7. Venn diagram of differential protein expression showing the effect of day after 14 Gy irradiation.

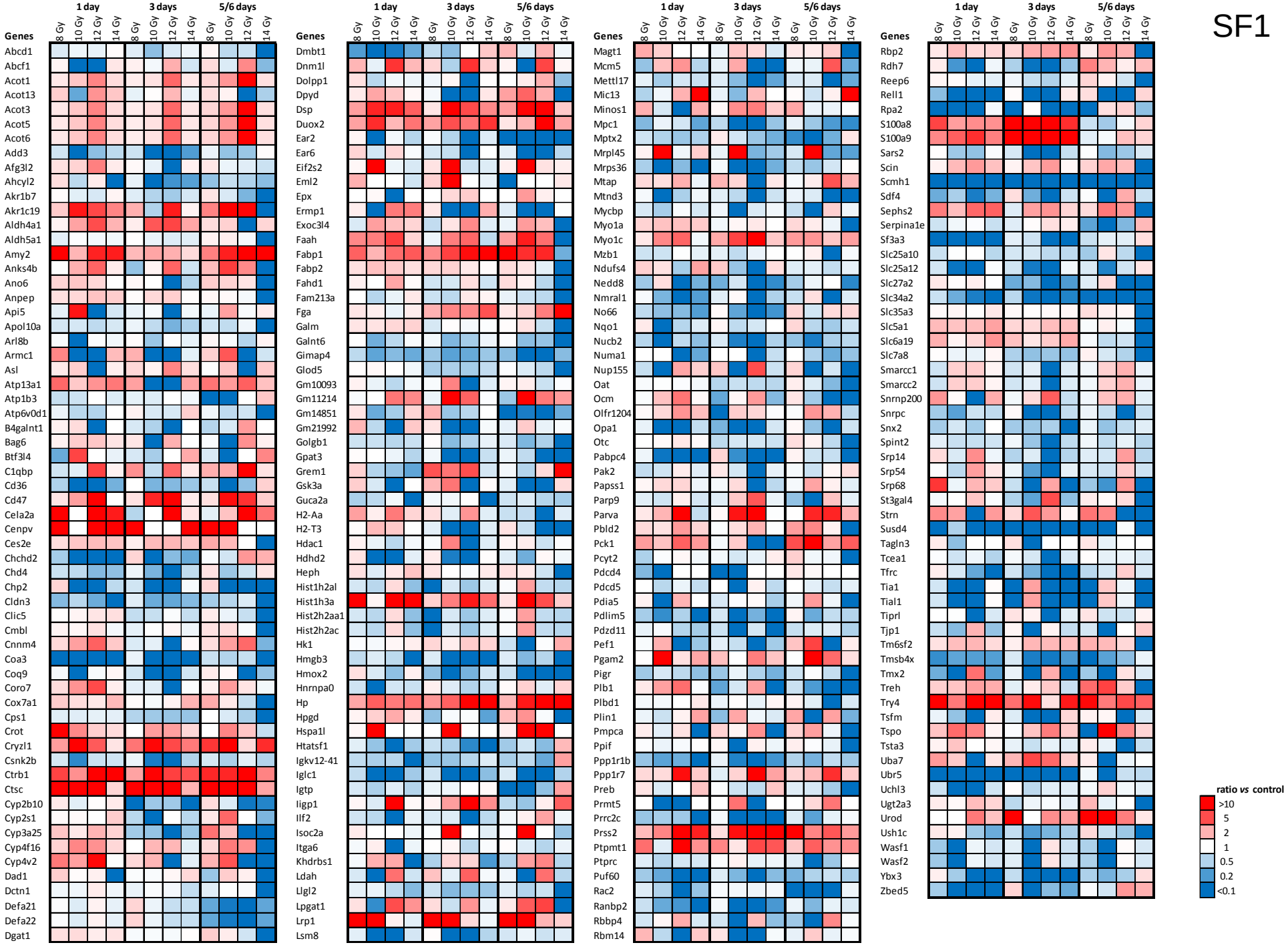
Supplemental Figure 8. Venn diagram of differential protein expression showing the effect of dose on day 1 after irradiation.

Supplemental Figure 9. Venn diagram of differential protein expression showing the effect of dose on day 3 after irradiation.

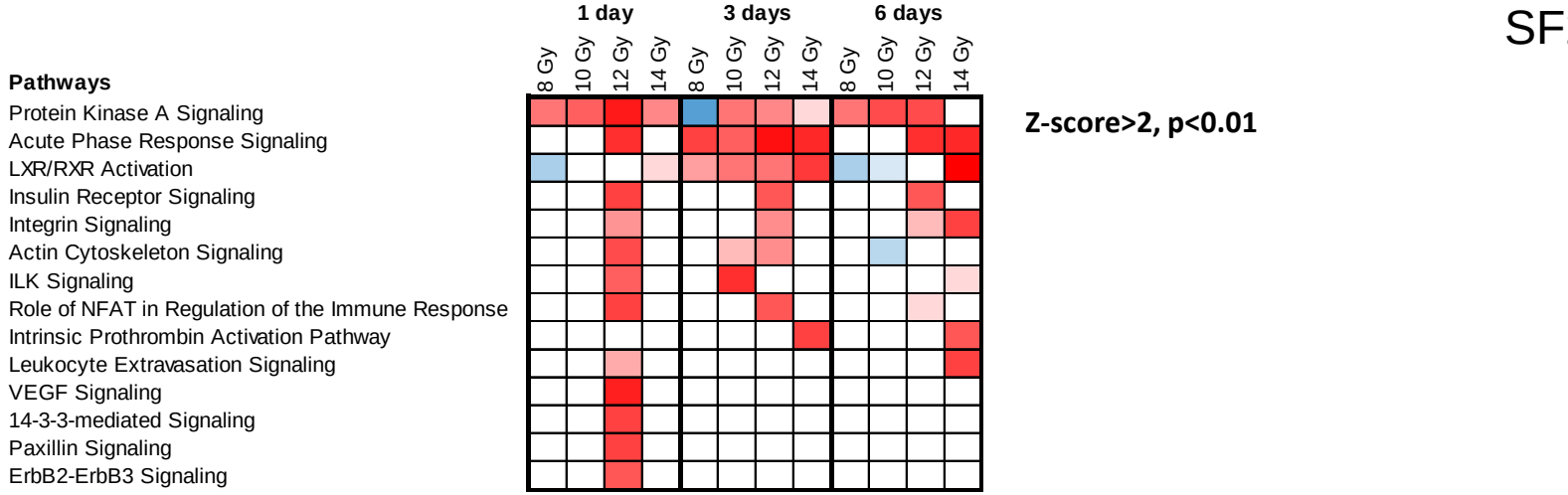
Supplemental Figure 10. Venn diagram of differential protein expression showing the effect of dose on day 6 after irradiation.

Supplemental Figure 11. Retinoid quantification in small intestine (jejunum) after TBI radiation. Radiation doses and times after radiation dose are notated. (a.) RA, (b.) ROL, (c.) RE. Retinoid levels were measured using n=5 per condition except for control (0) which is an average of n=15 un-irradiated control mice euthanized at day -6 (n=5), day 0 (n=5), or day 6 (n=5). The statistical analysis relied on the unpaired Student's *t*-test between groups and the Data is mean ± standard deviation, * $p<0.05$ using student's *t*-test between groups as compared to control (0). Data shown as a function of time after dose at a given radiation dose. Data shown as a function of dose on a given day after dose is shown in **Supplementary Fig. 12**. RA=retinoic acid, ROL = retinol, RE=total retinyl esters

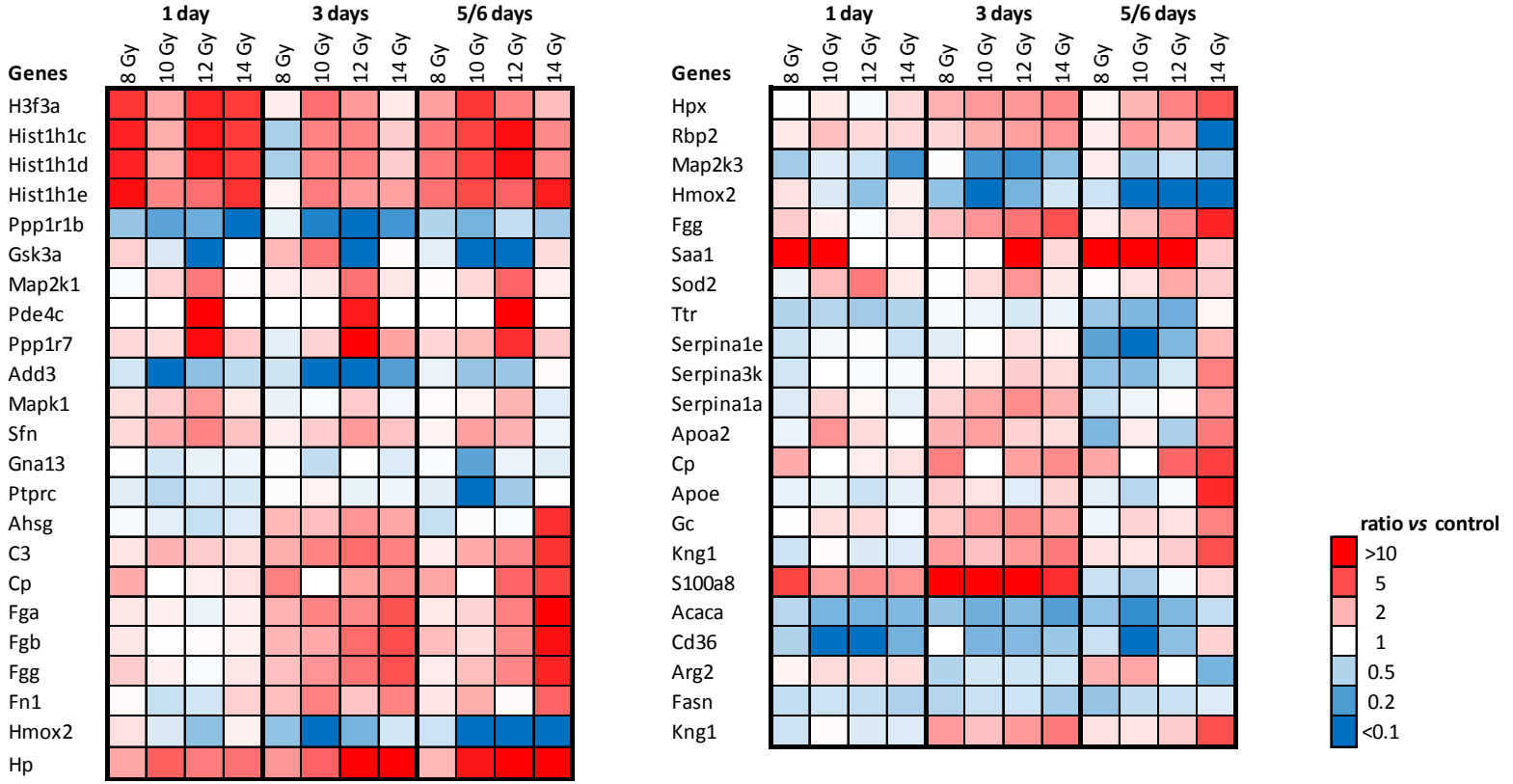
Supplemental Figure 12. Retinoid quantification in small intestine (jejunum) after TBI radiation. Radiation doses and times after radiation dose are notated. (a.) RA, (b.) ROL, (c.) RE. Retinoid levels were measured using n=5 per condition except for control (0) which is an average of n=15 un-irradiated control mice euthanized at day -6 (n=5), day 0 (n=5), or day 6 (n=5). The statistical analysis relied on the unpaired Student's *t*-test between groups and the Data is mean ± standard deviation, *$p<0.05$ using student's *t*-test between groups as compared to control (0). Data shown as a function of dose on a given day after dose. Data shown as a function of time after dose at a given radiation dose is shown in **Supplemental Fig. 11.** RA=retinoic acid, ROL = retinol, RE=total retinyl esters


SF1
1 day
3 days
5/6 days
8 Gy
10 Gy
12 Gy
14 Gy
Genes
Abcd1
Abcf1
Acot1
Acot13
Acot3
Acot5
Acot6
Add3
Afg3l2
Ahcyl2
Akr1b7
Akr1c19
Aldh4a1
Aldh5a1
Amy2
Anks4b
Ano6
Anpep
Api5
Apol10a
Arl8b
Armc1
Asl
Atp13a1
Atp1b3
Atp6v0d1
B4galnt1
Bag6
Btf3l4
C1qbp
Cd36
Cd47
Cela2a
Cenpv
Ces2e
Chchd2
Chd4
Chp2
Cldn3
Clic5
Cmbl
Cnnm4
Coa3
Coq9
Coro7
Cox7a1
Cps1
Crot
Cryzl1
Csnk2b
Ctrb1
Ctsc
Cyp2b10
Cyp2s1
Cyp3a25
Cyp4f16
Cyp4v2
Dad1
Dctn1
Defa21
Defa22
Dgat1
Dmbt1
Dnm1l
Dolpp1
Dpyd
Dsp
Duox2
Ear2
Ear6
Eif2s2
Eml2
Epx
Ermp1
Exoc3l4
Faah
Fabp1
Fabp2
Fahd1
Fam213a
Fga
Galm
Galnt6
Gimap4
Glod5
Gm10093
Gm11214
Gm14851
Gm21992
Golgb1
Gpat3
Grem1
Gsk3a
Guca2a
H2-Aa
H2-T3
Hdac1
Hdhd2
Heph
Hist1h2al
Hist1h3a
Hist2h2aa1
Hist2h2ac
Hk1
Hmgb3
Hmox2
Hnrnpa0
Hp
Hpgd
Hspa1l
Htatsf1
Igkv12-41
Iglc1
Igtp
Iigp1
Ilf2
Isoc2a
Itga6
Khdrbs1
Ldah
Llgl2
Lpgat1
Lrp1
Lsm8
Magt1
Mcm5
Mettl17
Mic13
Minos1
Mpc1
Mptx2
Mrpl45
Mrps36
Mtap
Mtnd3
Mycbp
Myo1a
Myo1c
Mzb1
Ndufs4
Nedd8
Nmral1
No66
Nqo1
Nucb2
Numa1
Nup155
Oat
Ocm
Olfr1204
Opa1
Otc
Pabpc4
Pak2
Papss1
Parp9
Parva
Pbld2
Pck1
Pcyt2
Pdcd4
Pdcd5
Pdia5
Pdlim5
Pdzd11
Pef1
Pgam2
Pigr
Plb1
Plbd1
Plin1
Pmpca
Ppif
Ppp1r1b
Ppp1r7
Preb
Prmt5
Prrc2c
Prss2
Ptpmt1
Ptprc
Puf60
Rac2
Ranbp2
Rbbp4
Rbm14
Rbp2
Rdh7
Reep6
Rell1
Rpa2
S100a8
S100a9
Sars2
Scin
Scmh1
Sdf4
Sephs2
Serpina1e
Sf3a3
Slc25a10
Slc25a12
Slc27a2
Slc34a2
Slc35a3
Slc5a1
Slc6a19
Slc7a8
Smarcc1
Smarcc2
Snrnp200
Snrpc
Snx2
Spint2
Srp14
Srp54
Srp68
St3gal4
Strn
Susd4
Tagln3
Tcea1
Tfrc
Tia1
Tial1
Tiprl
Tjp1
Tm6sf2
Tmsb4x
Tmx2
Treh
Try4
Tsfm
Tspo
Tsta3
Uba7
Ubr5
Uchl3
Ugt2a3
Urod
Ush1c
Wasf1
Wasf2
Ybx3
Zbed5
ratio vs control
>10
5
2
1
0.5
0.2
<0.1

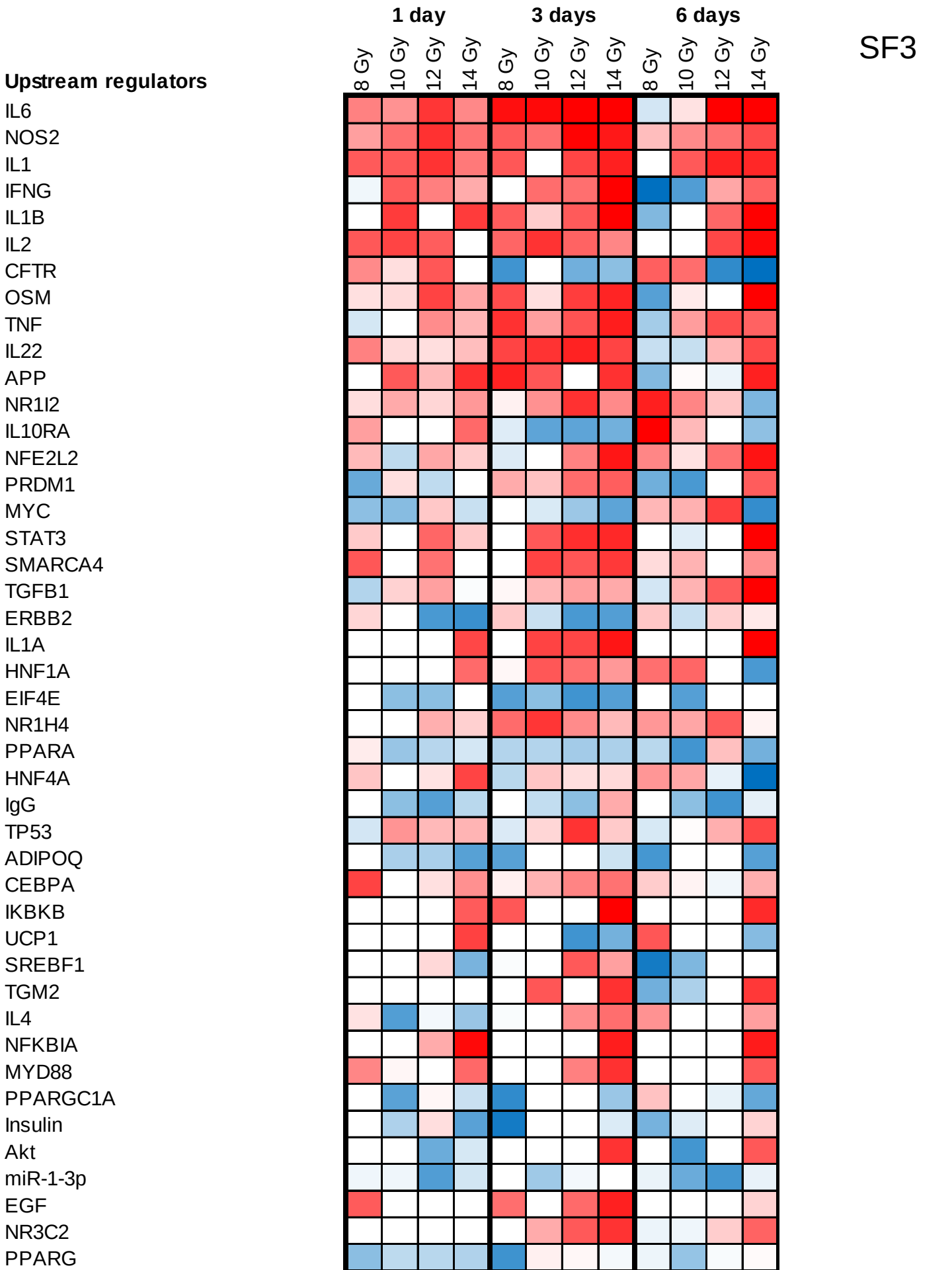
a


Pathways
Protein Kinase A Signaling
Acute Phase Response Signaling
LXR/RXR Activation
Insulin Receptor Signaling
Integrin Signaling
Actin Cytoskeleton Signaling
ILK Signaling
Role of NFAT in Regulation of the Immune Response
Intrinsic Prothrombin Activation Pathway
Leukocyte Extravasation Signaling
VEGF Signaling
14-3-3-mediated Signaling
Paxillin Signaling
ErbB2-ErbB3 Signaling
1 day
3 days
6 days
8 Gy
10 Gy
12 Gy
14 Gy
Z-score>2, p<0.01

b


Genes
H3f3a
Hist1h1c
Hist1h1d
Hist1h1e
Ppp1r1b
Gsk3a
Map2k1
Pde4c
Ppp1r7
Add3
Mapk1
Sfn
Gna13
Ptprc
Ahsg
C3
Cp
Fga
Fgb
Fgg
Fn1
Hmox2
Hp
1 day
3 days
5/6 days
8 Gy
10 Gy
12 Gy
14 Gy
Genes
Hpx
Rbp2
Map2k3
Hmox2
Fgg
Saa1
Sod2
Ttr
Serpina1e
Serpina3k
Serpina1a
Apoa2
Cp
Apoe
Gc
Kng1
S100a8
Acaca
Cd36
Arg2
Fasn
Kng1
ratio vs control
>10
5
2
1
0.5
0.2
<0.1

SF3


1 day
3 days
6 days
8 Gy
10 Gy
12 Gy
14 Gy
Upstream regulators
IL6
NOS2
IL1
IFNG
IL1B
IL2
CFTR
OSM
TNF
IL22
APP
NR1I2
IL10RA
NFE2L2
PRDM1
MYC
STAT3
SMARCA4
TGFB1
ERBB2
IL1A
HNF1A
EIF4E
NR1H4
PPARA
HNF4A
IgG
TP53
ADIPOQ
CEBPA
IKBKB
UCP1
SREBF1
TGM2
IL4
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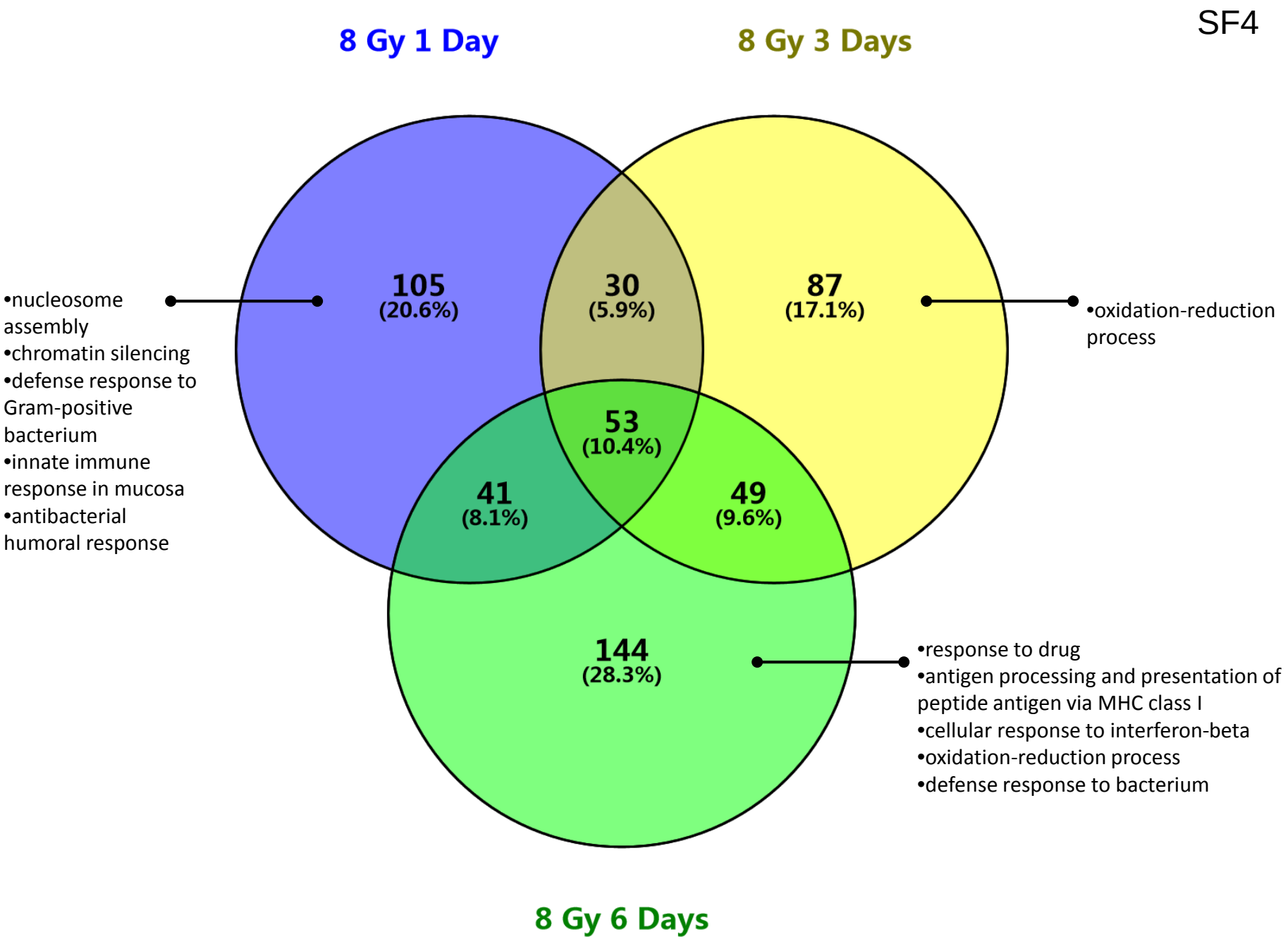
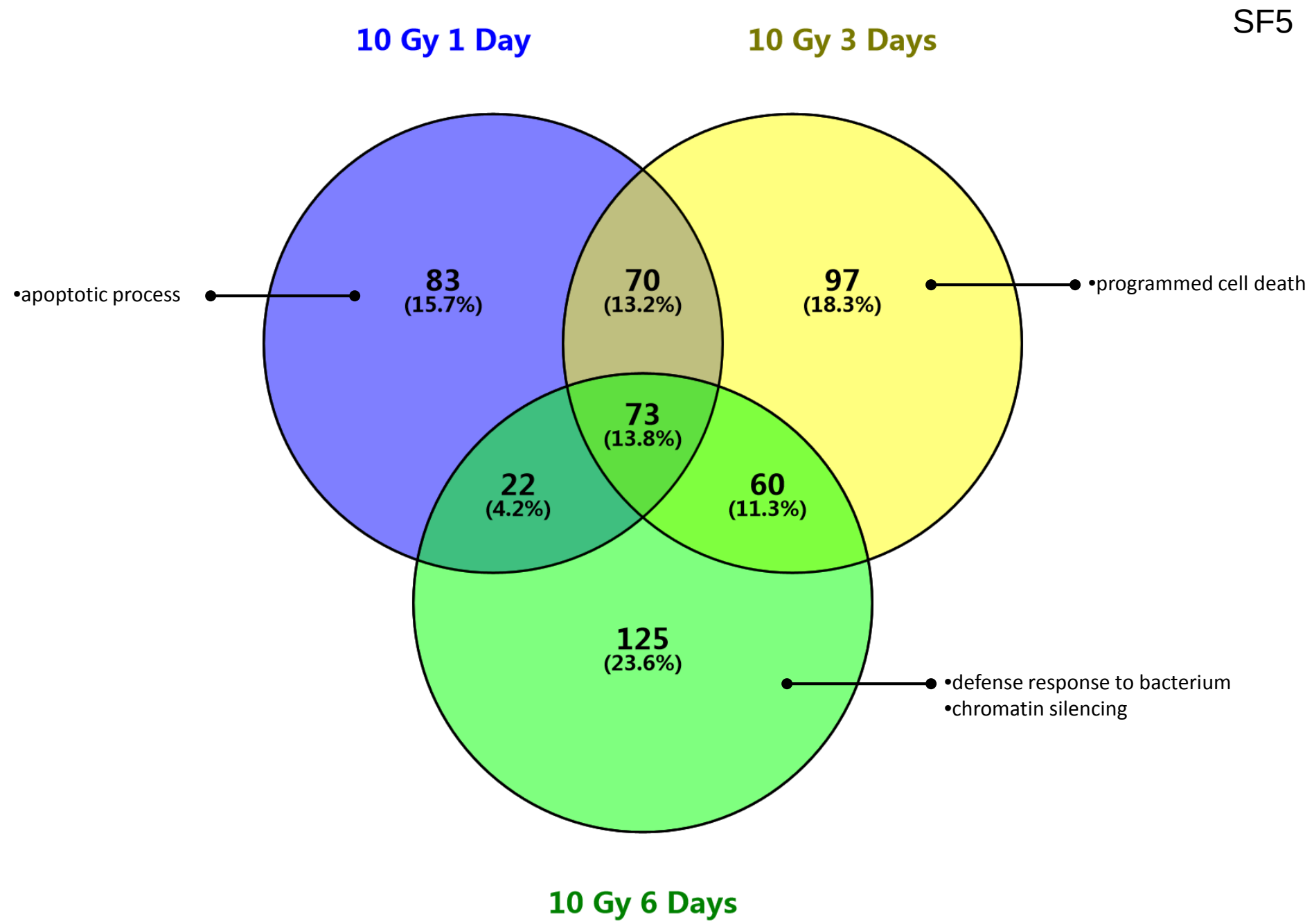
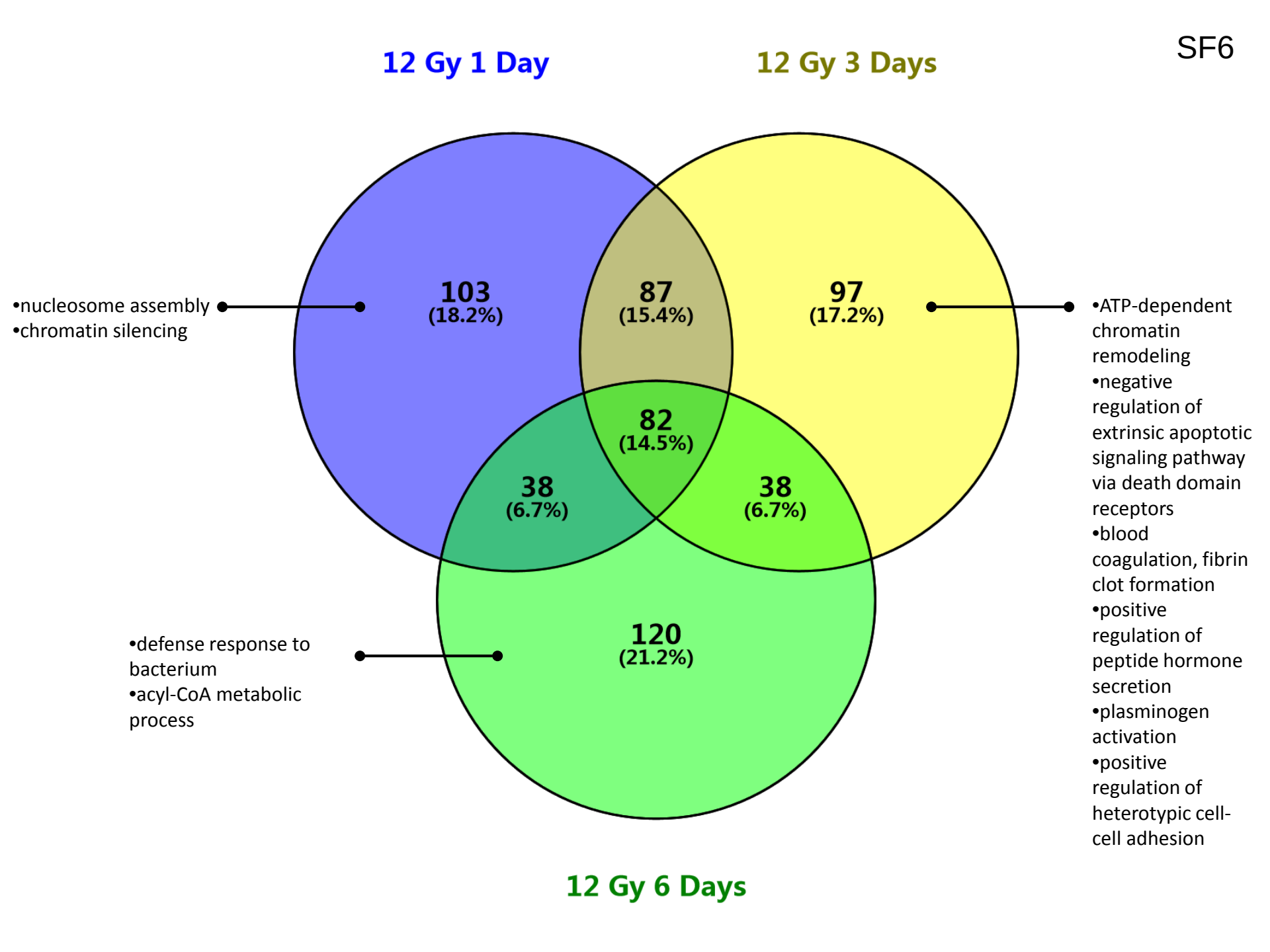
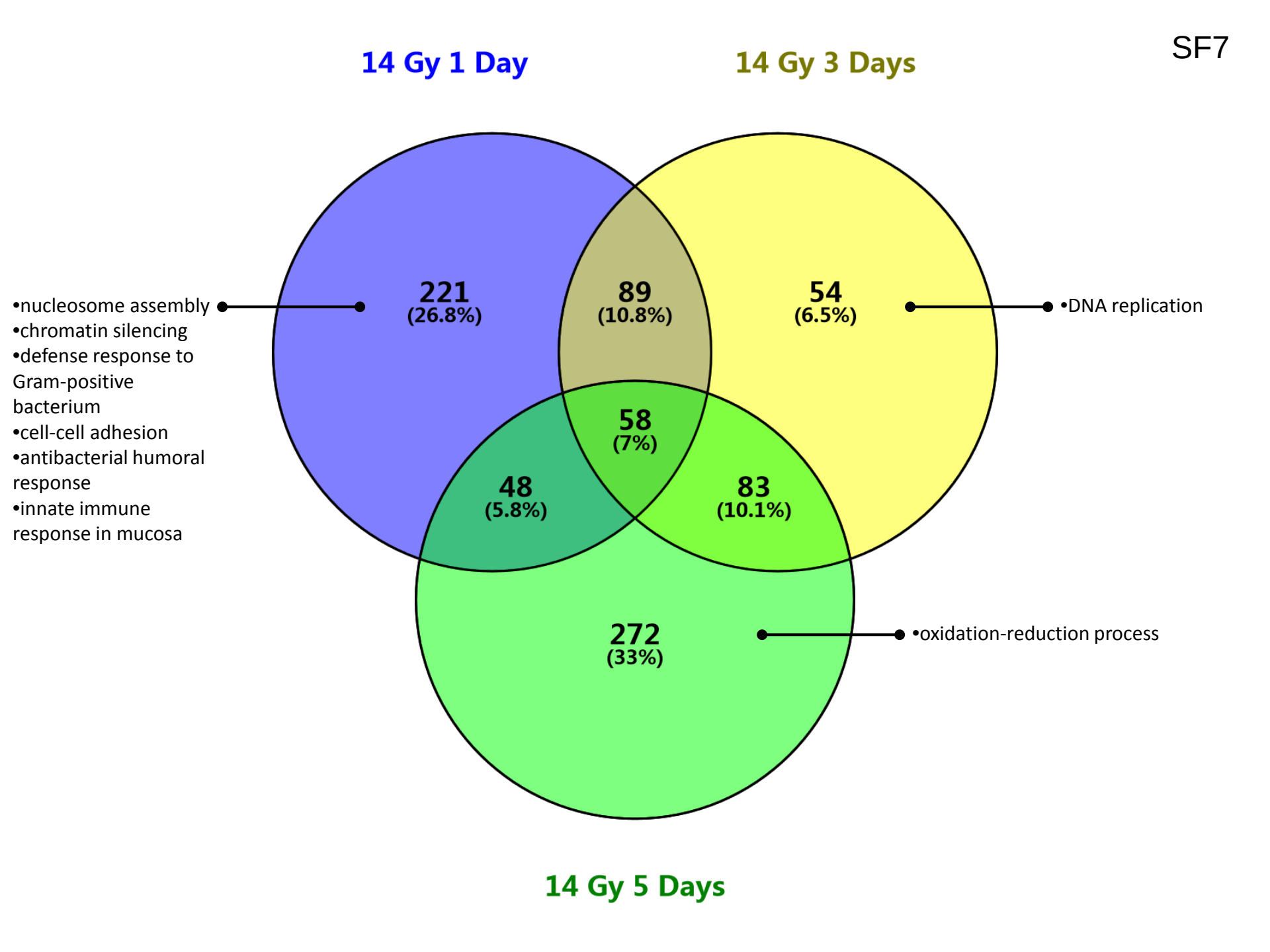
MYD88
PPARGC1A
Insulin
Akt
miR-1-3p
EGF
NR3C2
PPARG

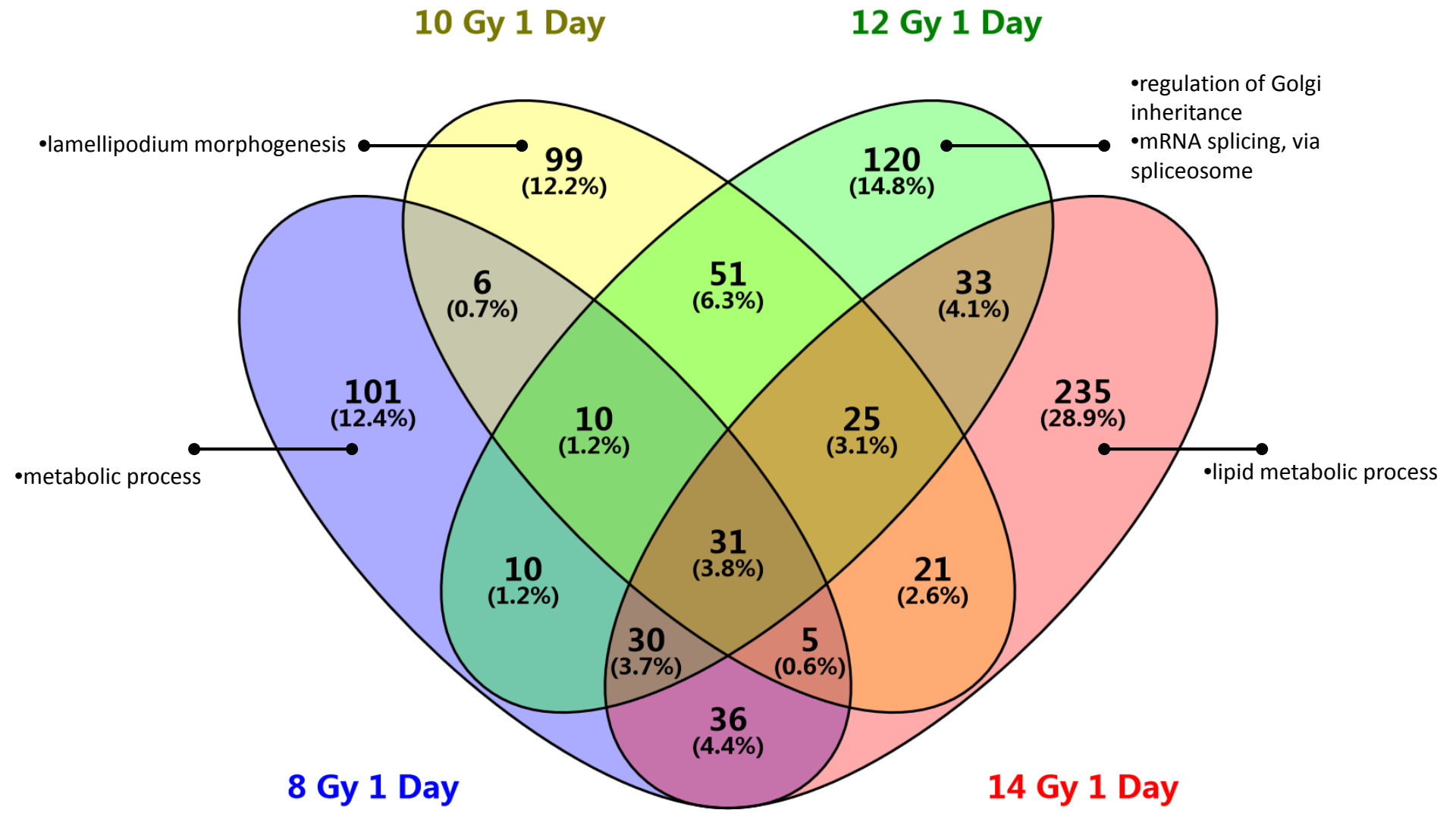

8 Gy 1 Day
8 Gy 3 Days
8 Gy 6 Days
105
(20.6%)
30
(5.9%)
87
(17.1%)
53
(10.4%)
41
(8.1%)
49
(9.6%)
144
(28.3%)
•nucleosome assembly
•chromatin silencing
•defense response to Gram-positive bacterium
•innate immune response in mucosa
•antibacterial humoral response
•oxidation-reduction process
•response to drug
•antigen processing and presentation of peptide antigen via MHC class I
•cellular response to interferon-beta
•oxidation-reduction process
•defense response to bacterium

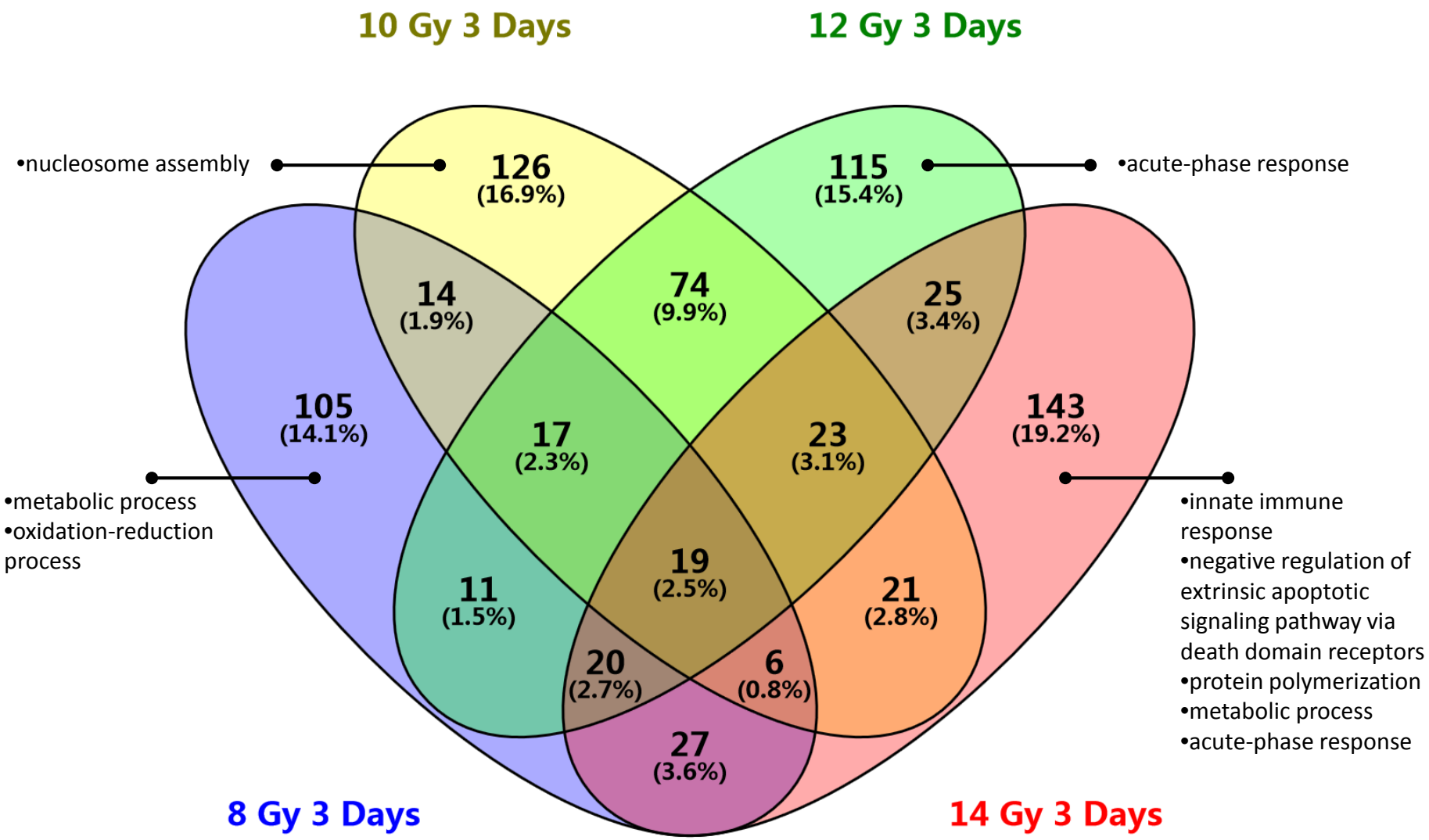

10 Gy 1 Day
10 Gy 3 Days
10 Gy 6 Days
83
(15.7%)
70
(13.2%)
97
(18.3%)
73
(13.8%)
22
(4.2%)
60
(11.3%)
125
(23.6%)
•apoptotic process
•programmed cell death
•defense response to bacterium
•chromatin silencing

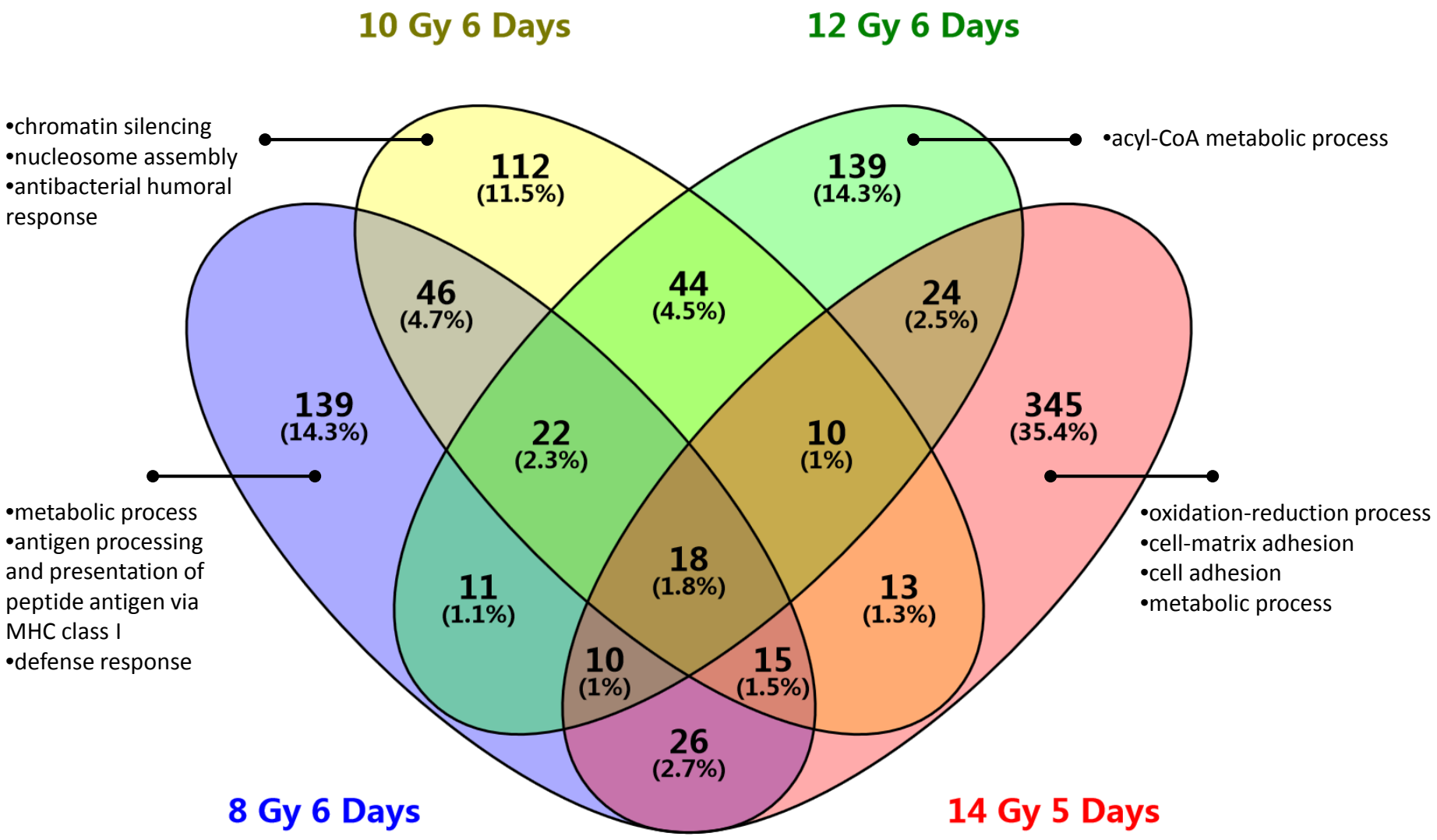
SF6


12 Gy 1 Day
12 Gy 3 Days
12 Gy 6 Days
103
(18.2%)
87
(15.4%)
97
(17.2%)
82
(14.5%)
38
(6.7%)
38
(6.7%)
120
(21.2%)
•nucleosome assembly
•chromatin silencing
•ATP-dependent chromatin remodeling
•negative regulation of extrinsic apoptotic signaling pathway via death domain receptors
•blood coagulation, fibrin clot formation
•positive regulation of peptide hormone secretion
•plasminogen activation
•positive regulation of heterotypic cell-cell adhesion
•defense response to bacterium
•acyl-CoA metabolic process


14 Gy 1 Day
14 Gy 3 Days
14 Gy 5 Days
221
(26.8%)
89
(10.8%)
54
(6.5%)
58
(7%)
48
(5.8%)
83
(10.1%)
272
(33%)
•nucleosome assembly
•chromatin silencing
•defense response to Gram-positive bacterium
•cell-cell adhesion
•antibacterial humoral response
•innate immune response in mucosa
•DNA replication
•oxidation-reduction process

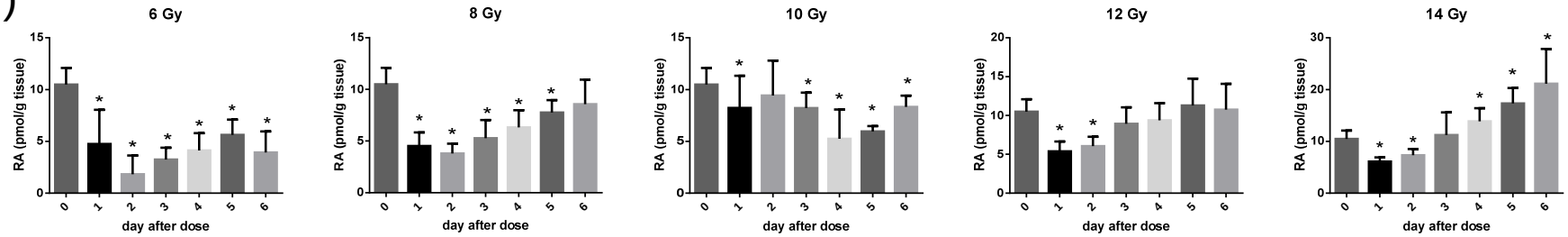

10 Gy 1 Day
12 Gy 1 Day
•lamellipodium morphogenesis
•regulation of Golgi inheritance
•mRNA splicing, via spliceosome
99
(12.2%)
120
(14.8%)
6
(0.7%)
51
(6.3%)
33
(4.1%)
101
(12.4%)
10
(1.2%)
25
(3.1%)
235
(28.9%)
•metabolic process
•lipid metabolic process
10
(1.2%)
31
(3.8%)
21
(2.6%)
30
(3.7%)
5
(0.6%)
36
(4.4%)
8 Gy 1 Day
14 Gy 1 Day


10 Gy 3 Days
12 Gy 3 Days
•nucleosome assembly
126
(16.9%)
115
(15.4%)
•acute-phase response
14
(1.9%)
74
(9.9%)
25
(3.4%)
105
(14.1%)
17
(2.3%)
23
(3.1%)
143
(19.2%)
•metabolic process
•oxidation-reduction process
11
(1.5%)
19
(2.5%)
21
(2.8%)
•innate immune response
•negative regulation of extrinsic apoptotic signaling pathway via death domain receptors
•protein polymerization
•metabolic process
•acute-phase response
20
(2.7%)
6
(0.8%)
27
(3.6%)
8 Gy 3 Days
14 Gy 3 Days

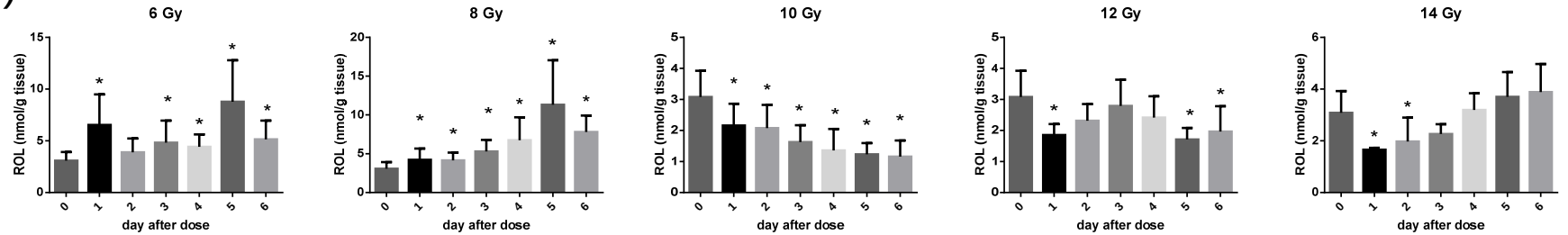

10 Gy 6 Days
12 Gy 6 Days
•chromatin silencing
•nucleosome assembly
•antibacterial humoral response
•acyl-CoA metabolic process
112
(11.5%)
139
(14.3%)
46
(4.7%)
44
(4.5%)
24
(2.5%)
139
(14.3%)
22
(2.3%)
10
(1%)
345
(35.4%)
•metabolic process
•antigen processing and presentation of peptide antigen via MHC class I
•defense response
•oxidation-reduction process
•cell-matrix adhesion
•cell adhesion
•metabolic process
18
(1.8%)
11
(1.1%)
13
(1.3%)
10
(1%)
15
(1.5%)
26
(2.7%)
8 Gy 6 Days
14 Gy 5 Days

SF11

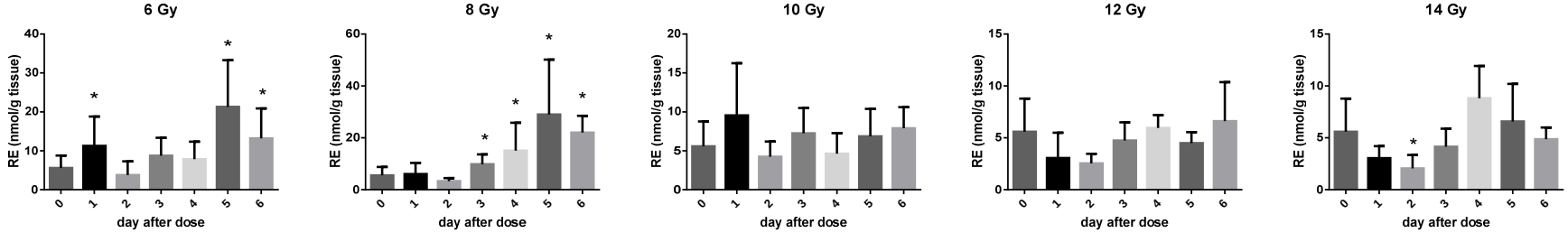
a.)


6 Gy
8 Gy
10 Gy
12 Gy
14 Gy
RA (pmol/g tissue)
day after dose

b.)

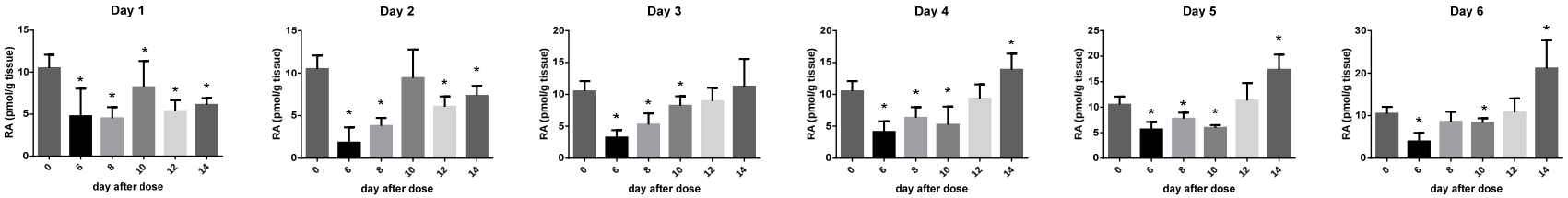

6 Gy
8 Gy
10 Gy
12 Gy
14 Gy
ROL (nmol/g tissue)
day after dose

c.)

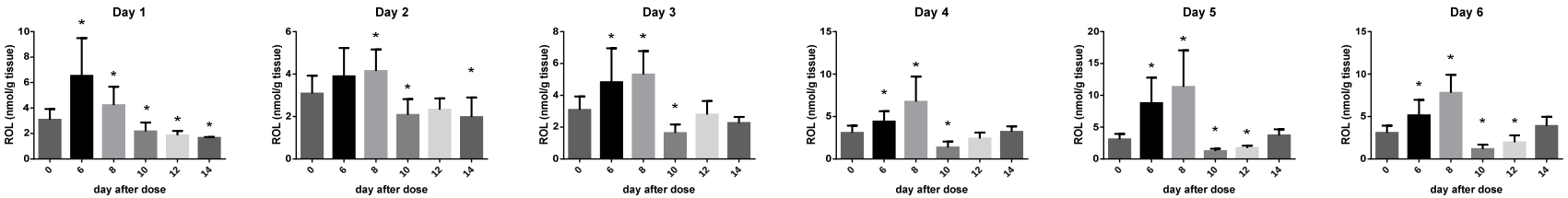

6 Gy
8 Gy
10 Gy
12 Gy
14 Gy
RE (nmol/g tissue)
day after dose

SF12

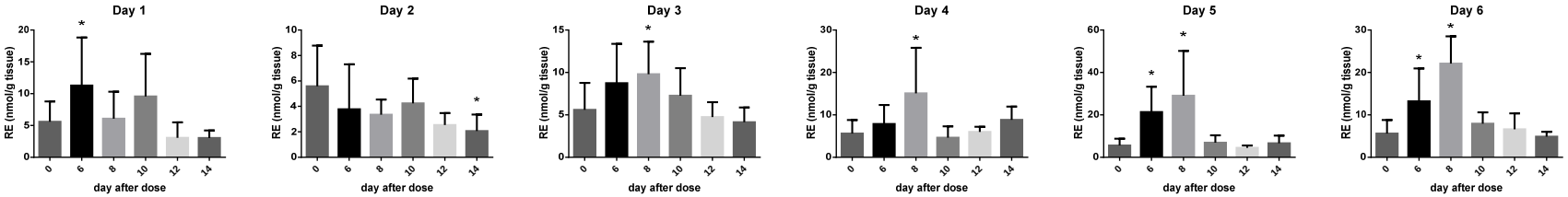
a.)


Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
RA (pmol/g tissue)
day after dose
0
6
8
10
12
14

b.)


Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
ROL (nmol/g tissue)
day after dose
0
6
8
10
12
14

c.)


Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
RE (nmol/g tissue)
day after dose
0
6
8
10
12
14