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SUPPLEMENTAL DATA

Single-cell transcriptomic analysis of radiation-induced lung injury in rat model

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#Xing-Yuan Shi,You-Qing Zhu, and Chan-Jin Liang contributed equally to this work.

Full article is available at the following link: Single-cell transcriptomic analysis of radiation-induced lung injury in rat model

Table S1. Orthologous rat genes associated with inflammatory processes

1	*Abca1*
2	*Abi1*
3	*Acvr1b*
4	*Acvr2a*
5	*Adgre1*
6	*Adm*
7	*Adora2b*
8	*Adrm1*
9	*Ahr*
10	*Aplnr*
11	*Aqp9*
12	*Atp2a2*
13	*Atp2b1*
14	*Atp2c1*
15	*Axl*
16	*Bdkrb1*
17	*Best1*
18	*Bst2*
19	*Btg2*
20	*C3ar1*
21	*Calcrl*
22	*Ccl17*
23	*Ccl20*
24	*Ccl22*
25	*Ccl24*
26	*Ccl5*
27	*Ccl7*
28	*Ccr7*
29	*Ccrl2*

30	*Cd14*
31	*Cd40*
32	*Cd48*
33	*Cd69*
34	*Cd70*
35	*Cd82*
36	*Cdkn1a*
37	*Chst2*
38	*Clec5a*
39	*Cmklr1*
40	*Csf1*
41	*Csf3*
42	*Csf3r*
43	*Cx3cl1*
44	*Cxcl10*
45	*Cxcl11*
46	*Cxcl9*
47	*Cxcr6*
48	*Cybb*
49	*Dcbld2*
50	*Ebi3*
51	*Edn1*
52	*Eif2ak2*
53	*Emp3*
54	*Ereg*
55	*F3*
56	*Ffar2*
57	*Fpr1*
58	*Fzd5*
59	*Gabbr1*

60	*Gch1*
61	*Gna15*
62	*Gnai3*
63	*Gp1ba*
64	*Gpc3*
65	*Gpr132*
66	*Gpr183*
67	*Has2*
68	*Hbegf*
69	*Hif1a*
70	*Hpn*
71	*Icam1*
72	*Icam4*
73	*Ifitm1*
74	*Ifnar1*
75	*Ifngr2*
76	*Il10*
77	*Il10ra*
78	*Il12b*
79	*Il15*
80	*Il15ra*
81	*Il18*
82	*Il18r1*
83	*Il18rap*
84	*Il1a*
85	*Il1b*
86	*Il1r1*
87	*Il2rb*
88	*Il6*
89	*Il7r*

90	*Inhba*
91	*Irak2*
92	*Irf1*
93	*Irf7*
94	*Itga5*
95	*Itgb3*
96	*Itgb8*
97	*Kcna3*
98	*Kcnj2*
99	*Kcnmb2*
100	*Klf6*
101	*Lamp3*
102	*Lck*
103	*Lcp2*
104	*Ldlr*
105	*Lif*
106	*Lpar1*
107	*Lta*
108	*Ly6e*
109	*Lyn*
110	*Marco*
111	*Mefv*
112	*Mep1a*
113	*Met*
114	*Mmp14*
115	*Msr1*
116	*Mxd1*
117	*Myc*
118	*Nampt*
119	*Ndp*

120	*Nfkb1*
121	*Nfkbia*
122	*Nlrp3*
123	*Nmi*
124	*Nmur1*
125	*Nod2*
126	*Olr1*
127	*Osm*
128	*Osmr*
129	*P2rx4*
130	*P2rx7*
131	*P2ry2*
132	*Pcdh7*
133	*Pde4b*
134	*Pdpn*
135	*Pik3r5*
136	*Plaur*
137	*Prok2*
138	*Psen1*
139	*Ptafr*
140	*Ptger2*
141	*Ptger4*
142	*Ptgir*
143	*Ptpre*
144	*Pvr*
145	*Raf1*
146	*Rasgrp1*
147	*Rela*
148	*Rgs1*
149	*Rgs16*

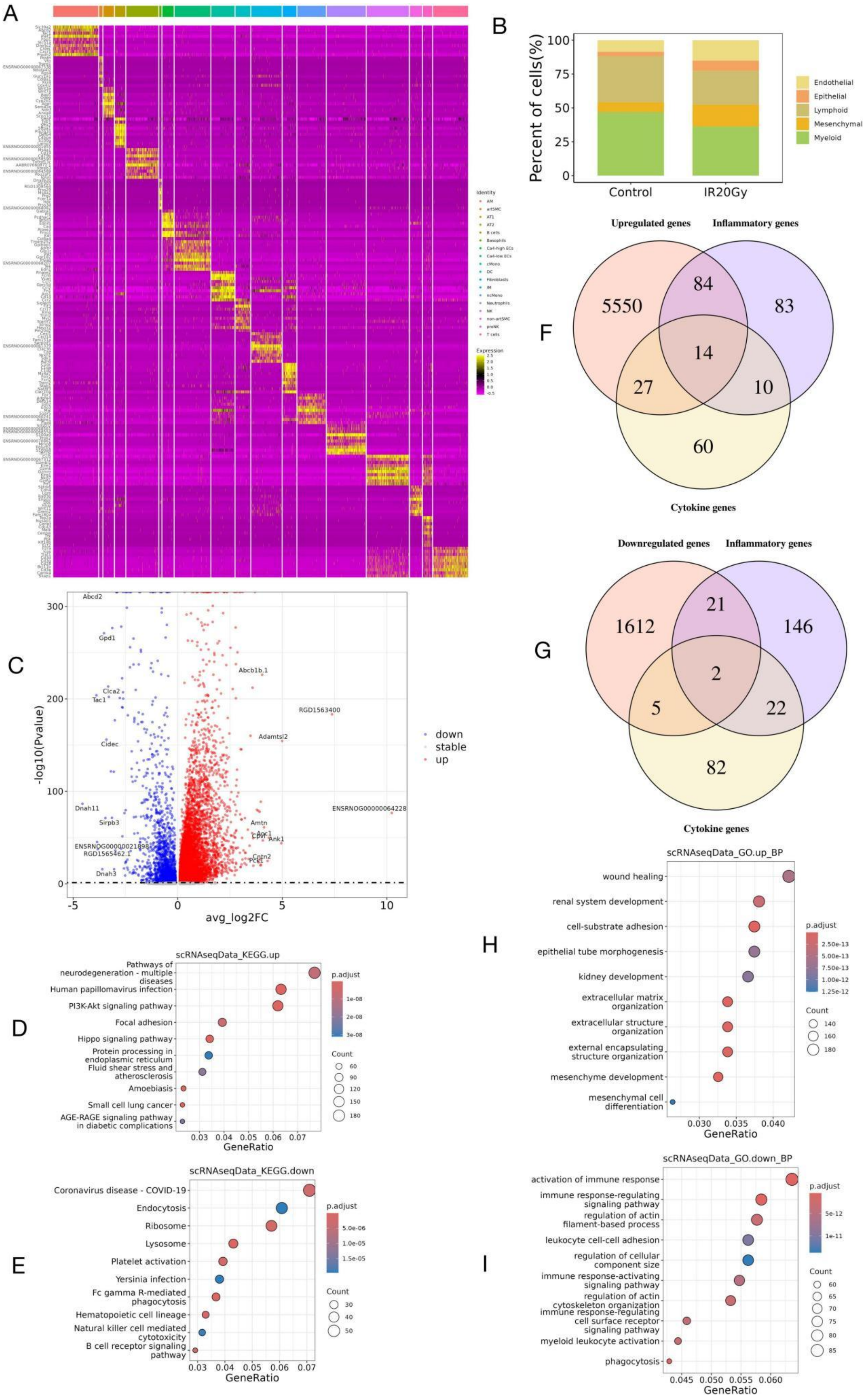
150	*Rhog*
151	*Ripk2*
152	*Rnf144b*
153	*Ros1*
154	*Rtp4*
155	*Scarf1*
156	*Scn1b*
157	*Sele*
158	*Selenos*
159	*Sell*
160	*Sema4d*
161	*Serpine1*
162	*Sgms2*
163	*Slamf1*
164	*Slc11a2*
165	*Slc1a2*
166	*Slc28a2*
167	*Slc31a1*
168	*Slc31a2*
169	*Slc4a4*
170	*Slc7a1*
171	*Slc7a2*
172	*Sphk1*
173	*Sri*
174	*Stab1*
175	*Tacr1*
176	*Tacr3*
177	*Tapbp*
178	*Timp1*
179	*Tlr1*

180	*Tlr2*
181	*Tlr3*
182	*Tnfaip6*
183	*Tnfrsf1b*
184	*Tnfrsf9*
185	*Tnfsf10*
186	*Tnfsf15*
187	*Tnfsf9*
188	*Tpbg*
189	*Vip*
190	*Icoslg*
191	*Il4r*

Table S2. T-test results of cytokine scores across 18 cell types

Celltype	t_results	Mean in group Control	Mean in group IR20Gy	p值
AM	t = -12.778, df = 297.3, p-value < 2.2e-16	-0.008659967	0.053528692	****
artSMC	t = 0.31734, df = 123.35, p-value = 0.7515	-0.05934343	-0.06122003	ns
AT1	t = 2.36, df = 136.91, p-value = 0.01969	-0.04096486	-0.04925046	*
AT2	t = -12.874, df = 632.25, p-value < 2.2e-16	-0.05248481	-0.01815936	****
B_cells	t = -4.1721, df = 1099.5, p-value = 3.255e-05	-0.07451257	-0.067212	****
Basophils	t = -1.2533, df = 82.589, p-value = 0.2136	-0.000522102	0.010895054	ns
Ca4_high_ECs	t = -5.5999, df = 682.45, p-value = 3.111e-08	-0.08174042	-0.06721135	****
Ca4_low_ECs	t = -12.392, df = 1676.2, p-value < 2.2e-16	-0.06399606	-0.04483439	****
cMono	t = -32.57, df = 1415.9, p-value < 2.2e-16	-0.01479567	0.07672557	****
DC	t = -5.6892, df = 900.41, p-value = 1.725e-08	-0.07829924	-0.05767231	****
Fibroblasts	t = -1.9233, df = 1133.4, p-value = 0.05469	-0.03900779	-0.03520612	ns
IM	t = -14.936, df = 545.65, p-value < 2.2e-16	0.03924923	0.11841488	****
ncMono	t = -12.334, df = 860.91, p-value < 2.2e-16	-0.05848296	-0.03564636	****
Neutrophils	t = -17.153, df = 2380.6, p-value < 2.2e-16	0.02732937	0.06187343	****
NK	t = -7.7912, df = 2502.6, p-value = 9.647e-15	-0.005116842	0.006858141	****
non_artSMC	t = 1.408, df = 689.65, p-value = 0.1596	-0.03524705	-0.03895056	ns
proNK	t = -3.9008, df = 469.37, p-value = 0.0001099	-0.04730814	-0.02676025	***

T_cells	t = -5.1264, df = 1215.2, p-value = 3.434e-07	-0.06432242	-0.05652421	****
tips:p<0.05 *,p<0.01 **,p<0.001 ***,p<0.0001 ****				


A
Identity
AM
artSMC
AT1
AT2
B cells
Basophils
Ca4-high ECs
Ca4-low ECs
cMono
DC
Fibroblasts
IM
ncMono
Neutrophils
NK
non-artSMC
proNK
T cells
Expression
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
B
Percent of cells(%)
100
75
50
25
0
Control
IR20Gy
Endothelial
Epithelial
Lymphoid
Mesenchymal
Myeloid
C
-log10(Pvalue)
300
200
100
0
avg_log2FC
-5
0
5
10
Abcd2
Gpd1
Clca2
Tac1
Cidec
Dnah11
Sirpb3
RGD1565462.1
Dnah3
Abcb1b.1
RGD1563400
Adamtsl2
ENSRNOG00000064228
Amtn
Ank1
down
stable
up
D
scRNAseqData_KEGG.up
Pathways of neurodegeneration - multiple diseases
Human papillomavirus infection
PI3K-Akt signaling pathway
Focal adhesion
Hippo signaling pathway
Protein processing in endoplasmic reticulum
Fluid shear stress and atherosclerosis
Amoebiasis
Small cell lung cancer
AGE-RAGE signaling pathway in diabetic complications
GeneRatio
0.03
0.04
0.05
0.06
0.07
p.adjust
1e-08
2e-08
3e-08
Count
60
90
120
150
180
E
scRNAseqData_KEGG.down
Coronavirus disease - COVID-19
Endocytosis
Ribosome
Lysosome
Platelet activation
Yersinia infection
Fc gamma R-mediated phagocytosis
Hematopoietic cell lineage
Natural killer cell mediated cytotoxicity
B cell receptor signaling pathway
GeneRatio
0.03
0.04
0.05
0.06
0.07
p.adjust
5.0e-06
1.0e-05
1.5e-05
Count
30
40
50
F
Upregulated genes
Inflammatory genes
Cytokine genes
5550
84
83
14
27
10
60
G
Downregulated genes
Inflammatory genes
Cytokine genes
1612
21
146
2
5
22
82
H
scRNAseqData_GO.up_BP
wound healing
renal system development
cell-substrate adhesion
epithelial tube morphogenesis
kidney development
extracellular matrix organization
extracellular structure organization
external encapsulating structure organization
mesenchyme development
mesenchymal cell differentiation
GeneRatio
0.030
0.035
0.040
p.adjust
2.50e-13
5.00e-13
7.50e-13
1.00e-12
1.25e-12
Count
140
160
180
I
scRNAseqData_GO.down_BP
activation of immune response
immune response-regulating signaling pathway
regulation of actin filament-based process
leukocyte cell-cell adhesion
regulation of cellular component size
immune response-activating signaling pathway
regulation of actin cytoskeleton organization
immune response-regulating cell surface receptor signaling pathway
myeloid leukocyte activation
phagocytosis
GeneRatio
0.045
0.050
0.055
0.060
p.adjust
5e-12
1e-11
Count
60
65
70
75
80
85

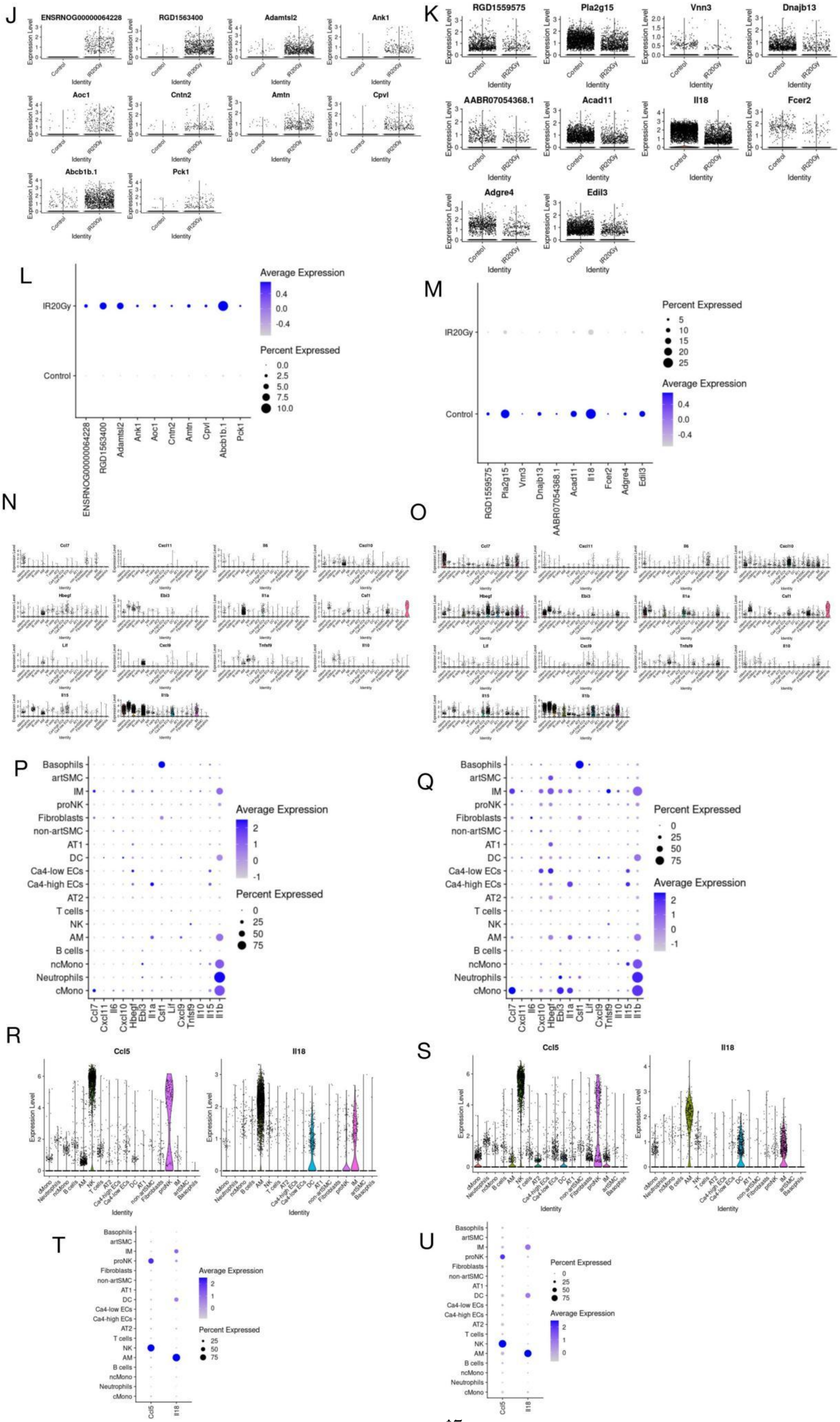

J
ENSRNOG00000064228
RGD1563400
Adamtsl2
Ank1
Aoc1
Cntn2
Amtn
Cpvl
Abcb1b.1
Pck1
Expression Level
Identity
Control
IR20Gy
K
RGD1559575
Pla2g15
Vnn3
Dnajb13
AABR07054368.1
Acad11
Il18
Fcer2
Adgre4
Edil3
L
Average Expression
0.4
0.0
-0.4
Percent Expressed
0.0
2.5
5.0
7.5
10.0
M
Percent Expressed
5
10
15
20
25
Average Expression
0.4
0.0
-0.4
N
O
Ccl7
Cxcl11
Il6
Cxcl10
Hbegf
Ebi3
Il1a
Csf1
Lif
Cxcl9
Tnfsf9
Il10
Il15
Il1b
P
Q
Basophils
artSMC
IM
proNK
Fibroblasts
non-artSMC
AT1
DC
Ca4-low ECs
Ca4-high ECs
AT2
T cells
NK
AM
B cells
ncMono
Neutrophils
cMono
Average Expression
2
1
0
-1
Percent Expressed
0
25
50
75
R
S
Ccl5
Il18
T
U
Ccl5
Il18
Average Expression
2
1
0
Percent Expressed
25
50
75

Figure S1. Cellular and molecular characterization of cells after IR. (A) Heat map shows the expression of 18 cell types top ten markers. **(B)** Dynamics in cell proportions of the 5 cellsupolutions across the control and IR20Gy group. **(C)** The volcano plot shows the upregulated and downregulated genes in the IR20Gy compared to the control group. The genes marked in black font are the top ten genes that are upregulated or downregulated. **(D-E)** KEGG enriched by upregulated and downregulated genes respectively. **(F-G)** Wayne diagram shows overlapping genes of upregulated or downregulated genes, inflammatory response, and cytokines genes respectively. **(H-I)** GO terms enriched by upregulated and downregulated genes in IR20Gy compared to control based on biological process (BP) enrichment analysis. **D,F,H.** Upregulated (IR20Gy compared to the control group); **E,G,I.** Downregulated. **(J)** Violin plot shows the expression level of top ten upregulated genes in the IR20Gy and control group. **(K)** Violin plot shows the expression level of top ten downregulated genes in theIR20Gy and control group. **(L)** Dot plot shows the expression level of top ten upregulated genes in the IR20Gy and control group. **(M)** Dot plot shows the expression level of top ten downregulated genes in the IR20Gy and control group. **(N)** Violin plot shows the expression level of overlapping genes that are upregulated, inflammatory, and cytokines genes in each cell population in the control group. **(O)** Violin plot shows the expression level of overlapping genes that are upregulated, inflammatory, and cytokines genes in each cell population in the IR20Gy group. **(P)** Dot plot shows the expression level of overlapping genes that are upregulated, inflammatory, and cytokines genes in each cell population in the control group. **(Q)** Dot plot shows the expression level of overlapping genes that are upregulated, inflammatory, and cytokines genes in each cell population in the IR20Gy group. **(R)** Violin plot shows the expression level ofoverlapping genes that are downregulated, inflammatory, and cytokines genes in each cell population in the control group.

(S) Violin plot shows the expression level of overlapping genes that are downregulated, inflammatory, and cytokines genes in each cell population in the IR20Gy group. **(T)** Dot plot shows the expression level of overlapping genes that are downregulated, inflammatory, and cytokines genes in each cell population in the control group. **(U)** Dot plot shows the expression level of overlapping genes that are downregulated, inflammatory, and cytokines genes in each cell population in the IR20Gy group. KEGG: Kyoto Encyclopedia of Genes and Genomes; IR20Gy: Ionizing radiation exposure at a dose of 20 Gray; GO: Gene ontology.

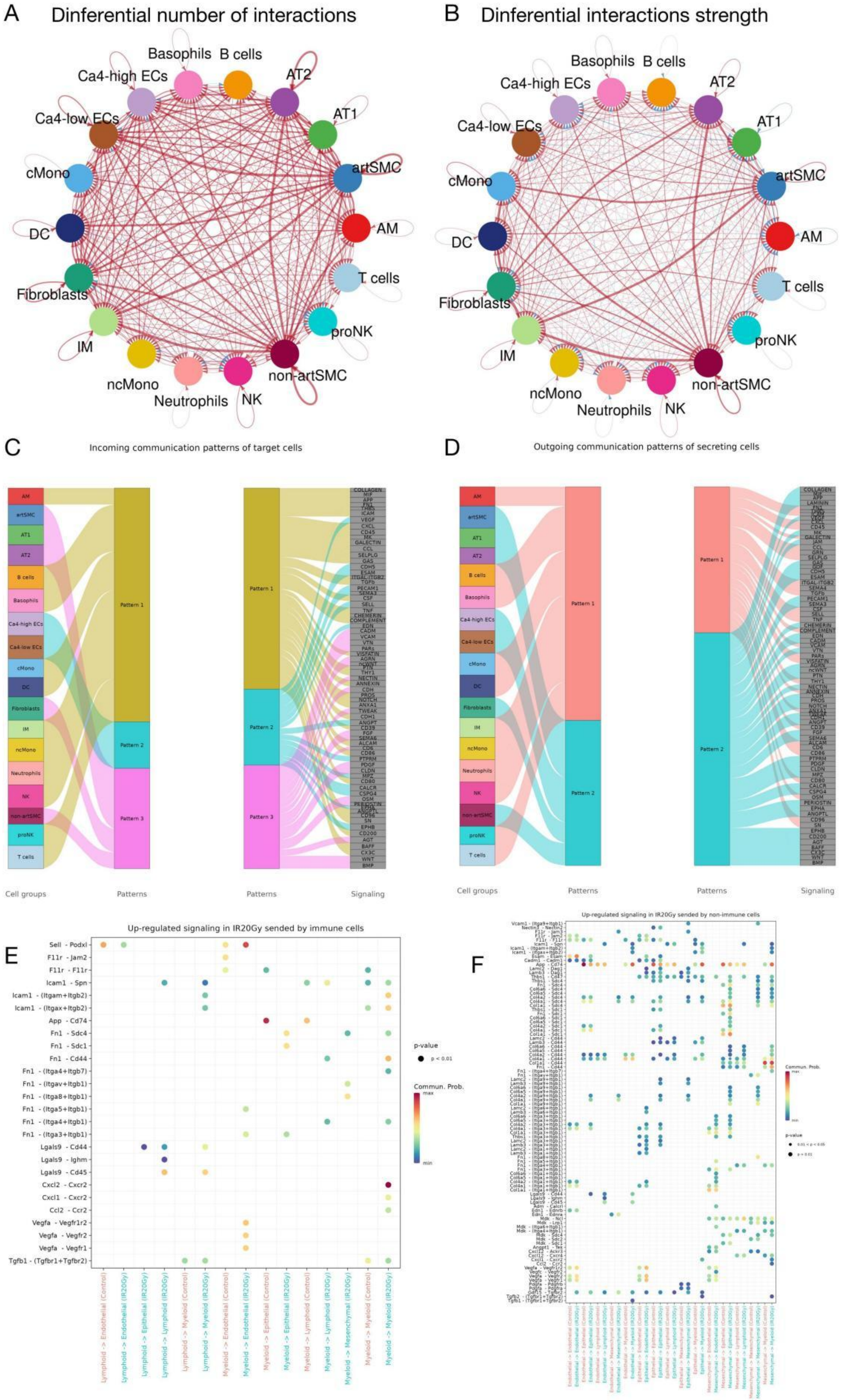
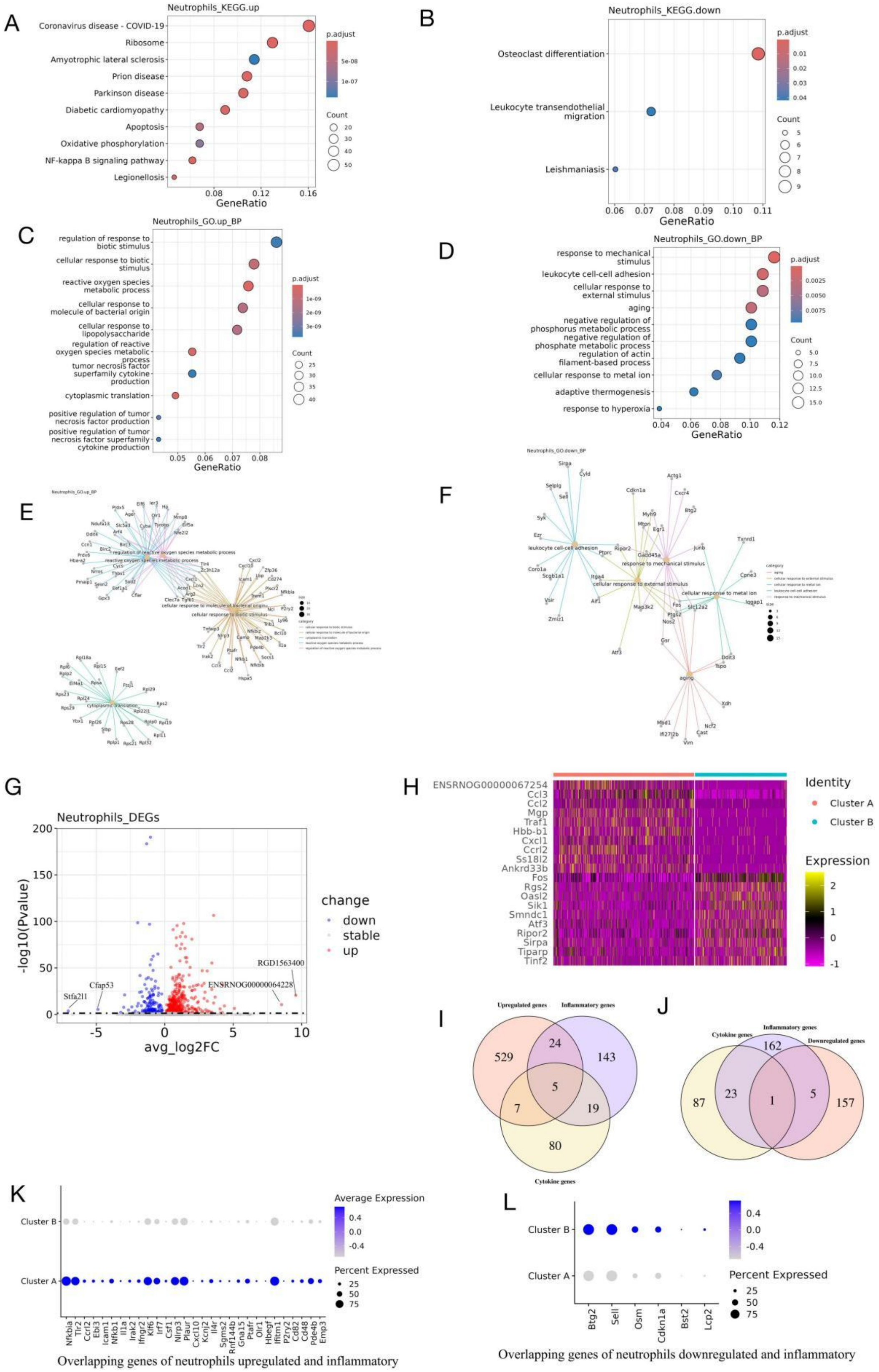

A
Dinferential number of interactions
B
Dinferential interactions strength
Basophils
B cells
AT2
AT1
Ca4-high ECs
Ca4-low ECs
cMono
artSMC
DC
AM
T cells
Fibroblasts
proNK
IM
ncMono
Neutrophils
NK
non-artSMC
C
Incoming communication patterns of target cells
D
Outgoing communication patterns of secreting cells
Pattern 1
Pattern 2
Pattern 3
Cell groups
Patterns
Signaling
E
Up-regulated signaling in IR20Gy sended by immune cells
Sell - Podxl
F11r - Jam2
F11r - F11r
Icam1 - Spn
Icam1 - (Itgam+Itgb2)
Icam1 - (Itgax+Itgb2)
App - Cd74
Fn1 - Sdc4
Fn1 - Sdc1
Fn1 - Cd44
Fn1 - (Itga4+Itgb7)
Fn1 - (Itgav+Itgb1)
Fn1 - (Itga8+Itgb1)
Fn1 - (Itga5+Itgb1)
Fn1 - (Itga4+Itgb1)
Fn1 - (Itga3+Itgb1)
Lgals9 - Cd44
Lgals9 - Ighm
Lgals9 - Cd45
Cxcl2 - Cxcr2
Cxcl1 - Cxcr2
Ccl2 - Ccr2
Vegfa - Vegfr1r2
Vegfa - Vegfr2
Vegfa - Vegfr1
Tgfb1 - (Tgfbr1+Tgfbr2)
p-value
p < 0.01
Commun. Prob.
max
min
Lymphoid -> Endothelial (Control)
Lymphoid -> Endothelial (IR20Gy)
Lymphoid -> Epithelial (IR20Gy)
Lymphoid -> Lymphoid (IR20Gy)
Lymphoid -> Myeloid (Control)
Lymphoid -> Myeloid (IR20Gy)
Myeloid -> Endothelial (Control)
Myeloid -> Endothelial (IR20Gy)
Myeloid -> Epithelial (Control)
Myeloid -> Epithelial (IR20Gy)
Myeloid -> Lymphoid (Control)
Myeloid -> Lymphoid (IR20Gy)
Myeloid -> Mesenchymal (IR20Gy)
Myeloid -> Myeloid (Control)
Myeloid -> Myeloid (IR20Gy)
F
Up-regulated signaling in IR20Gy sended by non-immune cells
Commun. Prob.
p-value

Figure S2. Cell-cell interaction changes in various lung cell populations after IR. **(A-B)** The circle graph illustrates alterations in the quantity and intensity of cell-cell interactions among the 18 cell populations in IR20Gy as compared to the control group. In this representation, red denotes an increase in the number or strength of interactions, while blue indicates the opposite. **(C-D)** The communication patterns of cells releasing signals, both outgoing and incoming, reveal the alignment between the inferred latent patterns, cell groups, and signaling pathways. **(C)** Incoming. **(D)** Outgoing. **(E-F)** Comparison of the significant ligand-receptor pairs between control and IR20Gy group base on the upregulated genes. Dot color reflects communication probabilities and dot size represents computed p-values. **(E)** Upregulated signaling in IR20Gy

sent by immune cells. **(F)** Upregulated signaling in IR20Gy sent by non-immune cells. IR20Gy: Ionizing radiation exposure at a dose of 20 Gray.



Overlapping genes of neutrophils upregulated and inflammatory

Overlapping genes of neutrophils downregulated and inflammatory

Figure S3. Cellular and molecular characterization of neutrophil cells. (A-B) KEGG enrichment analysis of the neutrophil differentially expressed genes in IR20Gy compared to the control. **(A)** Upregulated, **(B)** Downregulated. **(C-D)** GO terms enriched by the neutrophil differentially expressed genes in IR20Gy compared to the control. **(C)** Upregulated,

(D) Downregulated. **(E-F)** The related genes of top 5 significant GO terms enriched by the neutrophil differentially expressed genes in IR20Gy compared to the control. **(E)** Upregulated. **(F)** Downregulated. **(G)** The volcano plot shows the upregulated and downregulated genes of neutrophil in the IR20Gy compared to the control group. **(H)** Heatmap showing the top ten differentially expressed genes between cluster A and cluster B. **(I-J)** Wayne diagram shows the overlapping genes of the neutrophil differentially expressed genes, inflammatory response genes and cytokine genes. **(I)** Upregulated (*Ebi3*, *Il1a, Csf1, Cxcl10,Hbegf*). **(J)** Downregulated (*Osm*). **(K-L)** Dot plot shows the expression of the overlapping genes of the neutrophil differentially expressed genes and inflammatory response genes in the two subpopulations of the neutrophils. **(K)** Upregulated. **(L)** Downregulated. KEGG: Kyoto Encyclopedia of Genes and Genomes; IR20Gy:

Ionizing radiation exposure at a dose of 20 Gray; GO: Gene ontology.

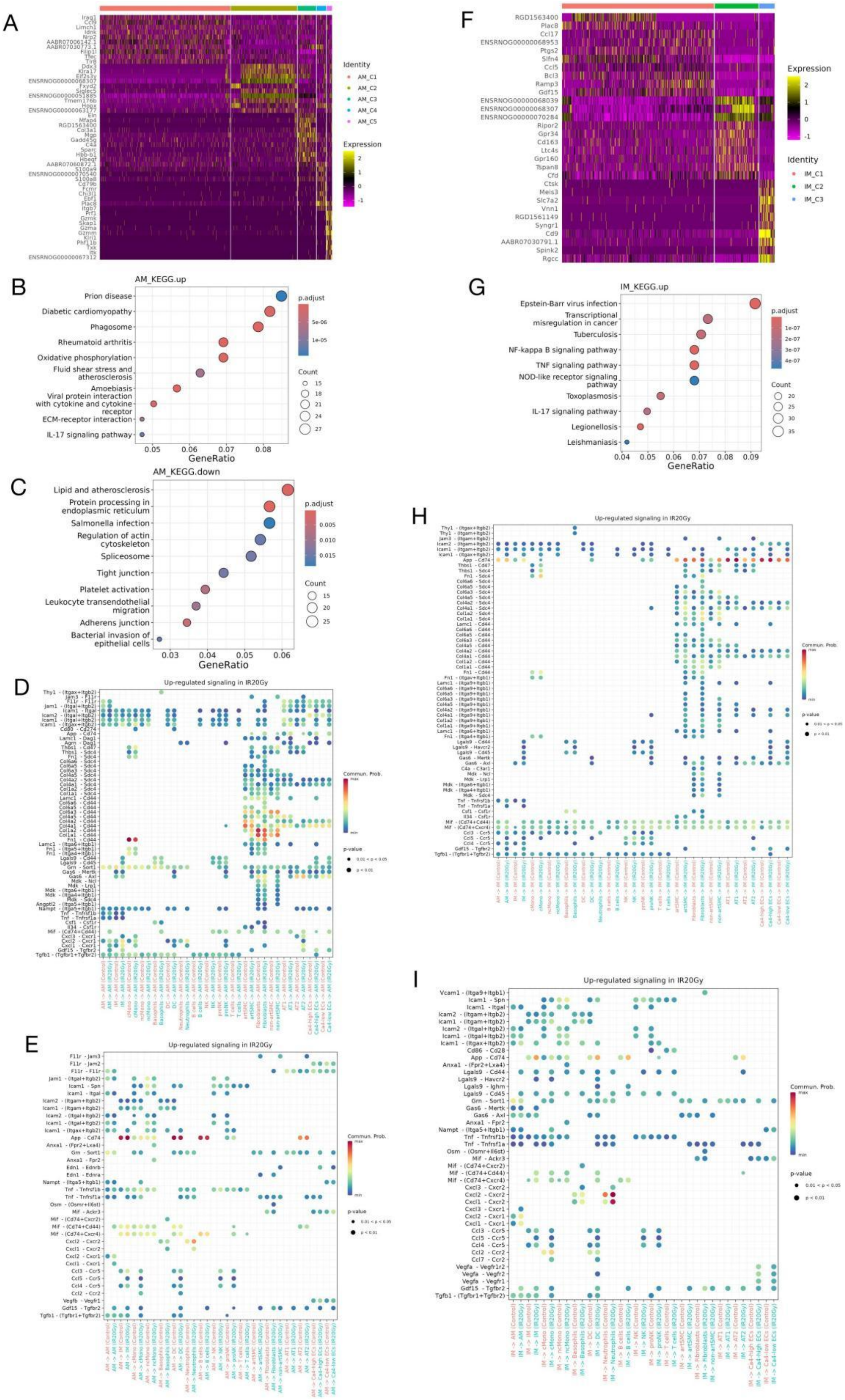

A
Identity
AM_C1
AM_C2
AM_C3
AM_C4
AM_C5
Expression
2
1
0
-1
F
RGD1563400
Plac8
Ccl17
ENSRNOG00000068953
Ptgs2
Slfn4
Ccl5
Bcl3
Ramp3
Gdf15
ENSRNOG00000068039
ENSRNOG00000068307
ENSRNOG00000070284
Ripor2
Gpr34
Cd163
Ltc4s
Gpr160
Tspan8
Cfd
Ctsk
Meis3
Slc7a2
Vnn1
RGD1561149
Syngr1
Cd9
AABR07030791.1
Spink2
Rgcc
Expression
2
1
0
-1
Identity
IM_C1
IM_C2
IM_C3
B
AM_KEGG.up
Prion disease
Diabetic cardiomyopathy
Phagosome
Rheumatoid arthritis
Oxidative phosphorylation
Fluid shear stress and atherosclerosis
Amoebiasis
Viral protein interaction with cytokine and cytokine receptor
ECM-receptor interaction
IL-17 signaling pathway
0.05
0.06
0.07
0.08
GeneRatio
p.adjust
5e-06
1e-05
Count
15
18
21
24
27
G
IM_KEGG.up
Epstein-Barr virus infection
Transcriptional misregulation in cancer
Tuberculosis
NF-kappa B signaling pathway
TNF signaling pathway
NOD-like receptor signaling pathway
Toxoplasmosis
IL-17 signaling pathway
Legionellosis
Leishmaniasis
0.04
0.05
0.06
0.07
0.08
0.09
GeneRatio
p.adjust
1e-07
2e-07
3e-07
4e-07
Count
20
25
30
35
C
AM_KEGG.down
Lipid and atherosclerosis
Protein processing in endoplasmic reticulum
Salmonella infection
Regulation of actin cytoskeleton
Spliceosome
Tight junction
Platelet activation
Leukocyte transendothelial migration
Adherens junction
Bacterial invasion of epithelial cells
0.03
0.04
0.05
0.06
GeneRatio
p.adjust
0.005
0.010
0.015
Count
15
20
25
D
Up-regulated signaling in IR20Gy
Commun. Prob.
max
min
p-value
0.01 < p < 0.05
p < 0.01
E
Up-regulated signaling in IR20Gy
Commun. Prob.
max
min
p-value
0.01 < p < 0.05
p < 0.01
H
Up-regulated signaling in IR20Gy
Commun. Prob.
max
min
p-value
0.01 < p < 0.05
p < 0.01
I
Up-regulated signaling in IR20Gy
Vcam1 - (Itga9+Itgb1)
Icam1 - Spn
Icam1 - Itgal
Icam2 - (Itgam+Itgb2)
Icam1 - (Itgam+Itgb2)
Icam2 - (Itgal+Itgb2)
Icam1 - (Itgal+Itgb2)
Icam1 - (Itgax+Itgb2)
Cd86 - Cd28
App - Cd74
Anxa1 - (Fpr2+Lxa4)
Lgals9 - Cd44
Lgals9 - Havcr2
Lgals9 - Ighm
Lgals9 - Cd45
Grn - Sort1
Gas6 - Mertk
Gas6 - Axl
Anxa1 - Fpr2
Nampt - (Itga5+Itgb1)
Tnf - Tnfrsf1b
Tnf - Tnfrsf1a
Osm - (Osmr+Il6st)
Mif - Ackr3
Mif - (Cd74+Cxcr2)
Mif - (Cd74+Cd44)
Mif - (Cd74+Cxcr4)
Cxcl3 - Cxcr2
Cxcl2 - Cxcr2
Cxcl1 - Cxcr2
Cxcl3 - Cxcr1
Cxcl2 - Cxcr1
Cxcl1 - Cxcr1
Ccl3 - Ccr5
Ccl5 - Ccr5
Ccl4 - Ccr5
Ccl2 - Ccr2
Ccl7 - Ccr2
Vegfa - Vegfr1r2
Vegfa - Vegfr2
Vegfa - Vegfr1
Gdf15 - Tgfbr2
Tgfb1 - (Tgfbr1+Tgfbr2)
Commun. Prob.
max
min
p-value
0.01 < p < 0.05
p < 0.01

Figure S4. Cellular and molecular characterization of macrophage cells. (A-E) Cellular and molecular characterization of alveolar macrophages (AM). **(A)** Heatmap showing the top ten differentially expressed genes of the five subcelltypes of AM. **(B-C)** KEGG pathways enriched by the differentially expressed genes of AM in IR20Gy compared to the control. **(B)** Upregulated, **(C)** Downregulated. **(D)** Upregulated signaling in IR20Gy received by AM. **(E)** Upregulated signaling in IR20Gy sent by AM. **(F-I)** Cellular and molecular characterization of interstitial macrophages (IM). **(F)** Heatmap showing the top ten differentially expressed genes of the three clusters of IM. **(G)** KEGG enriched by the upregulated genes of IM in IR20Gy compared to the control (No significant KEGG pathway was enriched by the downregulated gene). **(H)** Upregulated signaling in IR20Gy received by IM. **(I)** Upregulated signaling in IR20Gy sent by IM. KEGG: Kyoto Encyclopedia of Genes and Genomes; IR20Gy: Ionizing radiation exposure at a dose of 20 Gray; IM: Interstitial macrophages; AM: Alveolar macrophages.

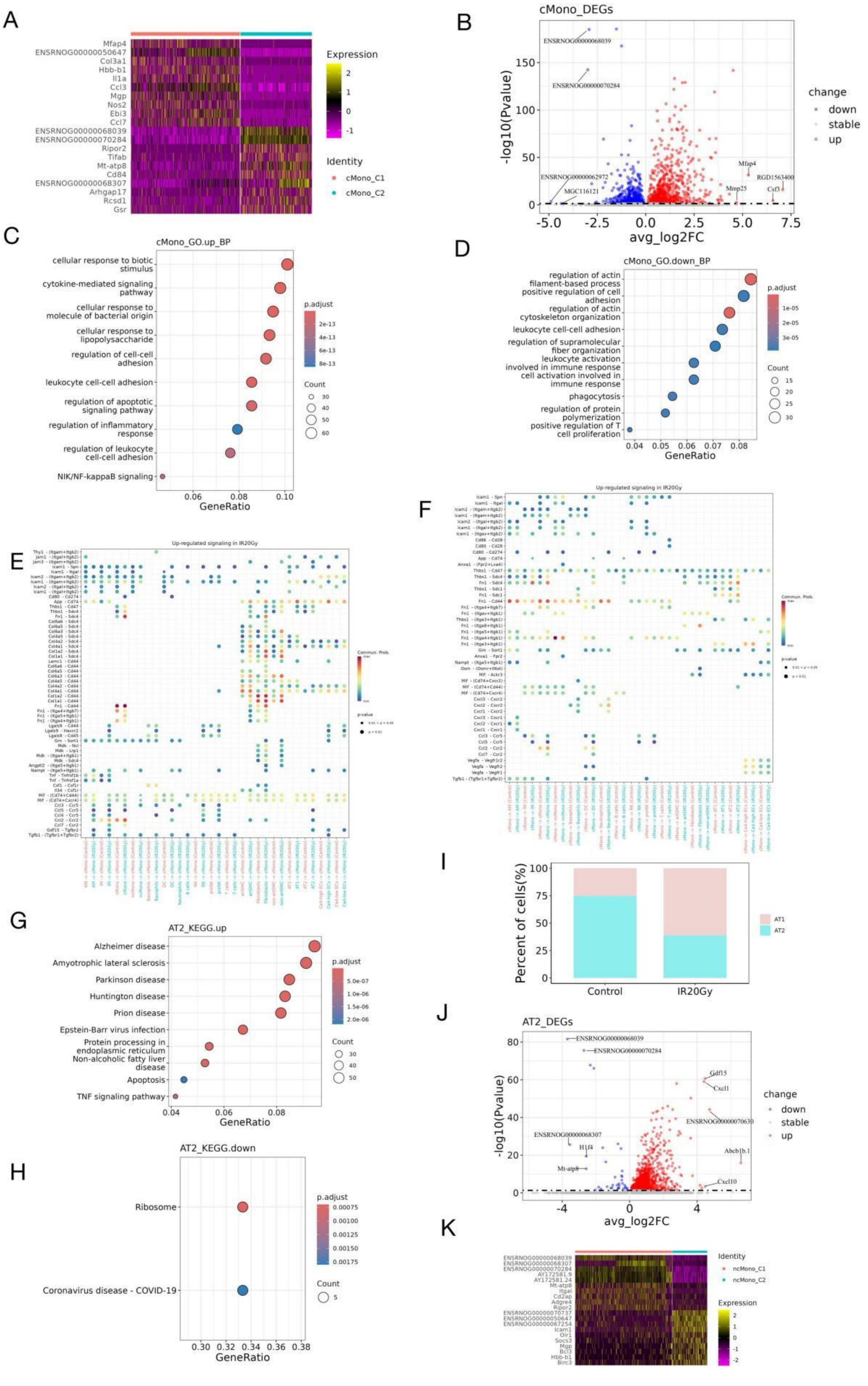

A
Mfap4
ENSRNOG00000050647
Col3a1
Hbb-b1
Il1a
Ccl3
Mgp
Nos2
Ebi3
Ccl7
ENSRNOG00000068039
ENSRNOG00000070284
Ripor2
Tifab
Mt-atp8
Cd84
ENSRNOG00000068307
Arhgap17
Rcsd1
Gsr
Expression
2
1
0
-1
Identity
cMono_C1
cMono_C2
B
cMono_DEGs
ENSRNOG00000068039
ENSRNOG00000070284
ENSRNOG00000062972
MGC116121
Mfap4
RGD1563400
Mmp25
Cxl3
-log10(Pvalue)
150
100
50
0
-5.0
-2.5
0.0
2.5
5.0
7.5
avg_log2FC
change
down
stable
up
C
cMono_GO.up_BP
cellular response to biotic stimulus
cytokine-mediated signaling pathway
cellular response to molecule of bacterial origin
cellular response to lipopolysaccharide
regulation of cell-cell adhesion
leukocyte cell-cell adhesion
regulation of apoptotic signaling pathway
regulation of inflammatory response
regulation of leukocyte cell-cell adhesion
NIK/NF-kappaB signaling
0.06
0.08
0.10
GeneRatio
p.adjust
2e-13
4e-13
6e-13
8e-13
Count
30
40
50
60
D
cMono_GO.down_BP
regulation of actin filament-based process
positive regulation of cell adhesion
regulation of actin cytoskeleton organization
leukocyte cell-cell adhesion
regulation of supramolecular fiber organization
leukocyte activation involved in immune response
cell activation involved in immune response
phagocytosis
regulation of protein polymerization
positive regulation of T cell proliferation
0.04
0.05
0.06
0.07
0.08
GeneRatio
p.adjust
1e-05
2e-05
3e-05
Count
15
20
25
30
E
Up-regulated signaling in IR20Gy
F
Up-regulated signaling in IR20Gy
G
AT2_KEGG.up
Alzheimer disease
Amyotrophic lateral sclerosis
Parkinson disease
Huntington disease
Prion disease
Epstein-Barr virus infection
Protein processing in endoplasmic reticulum
Non-alcoholic fatty liver disease
Apoptosis
TNF signaling pathway
0.04
0.06
0.08
GeneRatio
p.adjust
5.0e-07
1.0e-06
1.5e-06
2.0e-06
Count
30
40
50
H
AT2_KEGG.down
Ribosome
Coronavirus disease - COVID-19
0.30
0.32
0.34
0.36
0.38
GeneRatio
p.adjust
0.00075
0.00100
0.00125
0.00150
0.00175
Count
5
I
Percent of cells(%)
100
75
50
25
0
Control
IR20Gy
AT1
AT2
J
AT2_DEGs
ENSRNOG00000068039
ENSRNOG00000070284
Gdf15
Cxcl1
ENSRNOG00000070630
ENSRNOG00000068307
H1f4
Mt-atp8
Abcb1b.1
Cxcl10
-log10(Pvalue)
80
60
40
20
0
-4
0
4
avg_log2FC
change
down
stable
up
K
ENSRNOG00000068039
ENSRNOG00000068307
ENSRNOG00000070284
AY172581.9
AY172581.24
Mt-atp8
Itgal
Cd2ap
Adgre4
Ripor2
ENSRNOG00000070737
ENSRNOG00000050647
ENSRNOG00000067254
Icam1
Olr1
Socs3
Mgp
Bcl3
Hbb-b1
Birc3
Identity
ncMono_C1
ncMono_C2
Expression
2
1
0
-1
-2

Figure S5. Cellular and molecular characterization of monocytes and epithelials. **(A-E)** Cellular and molecular characterization of cMono. **(A)** Heatmap showing the top ten differentially expressed genes of the five subcelltypes of cMono. **(B)** The volcano plot shows the differential genes of cMono in the IR20Gy compared to the control group. **(C-D)** GO terms enriched by the differentially expressed genes of cMono in IR20Gy groups compared to the control. **(C)** Upregulated, **(D)** Downregulated. **(E)** Upregulated signaling in IR20Gy received by cMono. **(F)** Upregulated signaling in IR20Gy sent by cMono. **(G-J)** Cellular and molecular characterization of epithelials. **(G-H)** KEGG enriched by the upregulated and downregulated genes of AT2 in IR20Gy compared to the control. **(G)** Upregulated. **(H)** Downregulated.

(I) The cell proportions changes of AT1 and AT2 in epithelial cells. **(J)** The volcano plot shows the differentially expressed genes of AT2 in the IR20Gy compared to the control group. **(K)** Heatmap showing the top ten differentially expressed genes of the two clusters of non-classical monocytes (ncMono). IR20Gy: Ionizing radiation exposure at a dose of 20 Gray; cMono: Classical monocytes; KEGG: Kyoto Encyclopedia of Genes and Genomes.

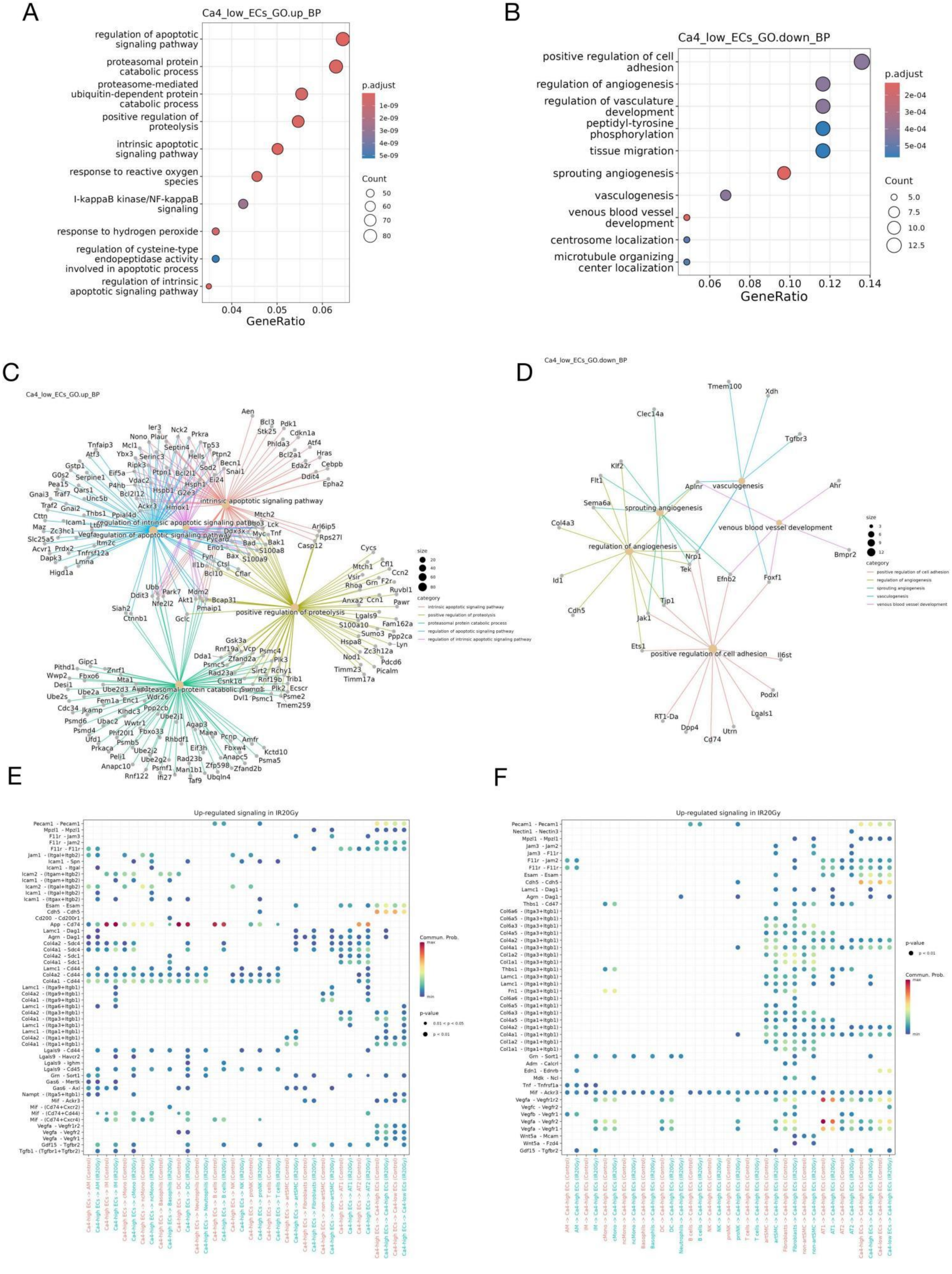
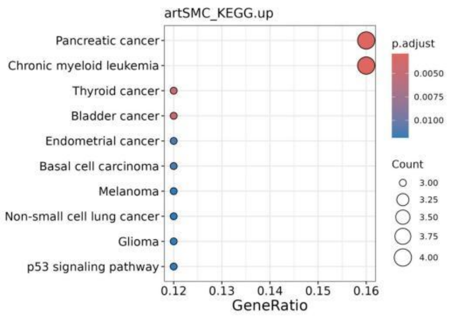
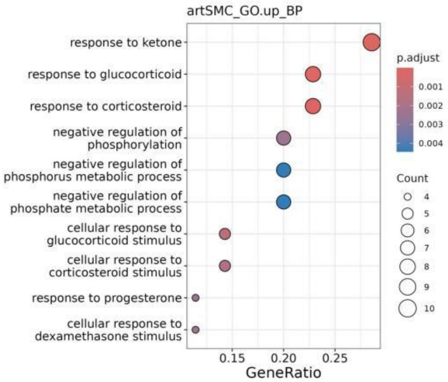

A
Ca4_low_ECs_GO.up_BP
regulation of apoptotic signaling pathway
proteasomal protein catabolic process
proteasome-mediated ubiquitin-dependent protein catabolic process
positive regulation of proteolysis
intrinsic apoptotic signaling pathway
response to reactive oxygen species
I-kappaB kinase/NF-kappaB signaling
response to hydrogen peroxide
regulation of cysteine-type endopeptidase activity involved in apoptotic process
regulation of intrinsic apoptotic signaling pathway
0.04
0.05
0.06
GeneRatio
p.adjust
1e-09
2e-09
3e-09
4e-09
5e-09
Count
50
60
70
80
B
Ca4_low_ECs_GO.down_BP
positive regulation of cell adhesion
regulation of angiogenesis
regulation of vasculature development
peptidyl-tyrosine phosphorylation
tissue migration
sprouting angiogenesis
vasculogenesis
venous blood vessel development
centrosome localization
microtubule organizing center localization
0.06
0.08
0.10
0.12
0.14
GeneRatio
p.adjust
2e-04
3e-04
4e-04
5e-04
Count
5.0
7.5
10.0
12.5
C
Ca4_low_ECs_GO.up_BP
intrinsic apoptotic signaling pathway
regulation of intrinsic apoptotic signaling pathway
regulation of apoptotic signaling pathway
positive regulation of proteolysis
proteasomal protein catabolic process
size
20
40
60
80
category
intrinsic apoptotic signaling pathway
positive regulation of proteolysis
proteasomal protein catabolic process
regulation of apoptotic signaling pathway
regulation of intrinsic apoptotic signaling pathway
D
Ca4_low_ECs_GO.down_BP
Tmem100
Xdh
Clec14a
Tgfbr3
Klf2
Flt1
Aplnr
vasculogenesis
Ahr
Sema6a
sprouting angiogenesis
Col4a3
venous blood vessel development
regulation of angiogenesis
Bmpr2
Nrp1
Tek
Id1
Efnb2
Foxf1
Tjp1
Cdh5
Jak1
Ets1
positive regulation of cell adhesion
Il6st
Podxl
Lgals1
RT1-Da
Dpp4
Utrn
Cd74
size
3
6
9
12
category
positive regulation of cell adhesion
regulation of angiogenesis
sprouting angiogenesis
vasculogenesis
venous blood vessel development
E
Up-regulated signaling in IR20Gy
Commun. Prob.
max
min
p-value
0.01 < p < 0.05
p < 0.01
F
Up-regulated signaling in IR20Gy
p-value
p < 0.01
Commun. Prob.
max
min

Figure S6. Cellular and molecular characterization of endothelial cells. (A-B) GO terms enriched by the Ca4-low ECs differentially expressed genes in IR20Gy compared to the control. **(A)** Upregulated. **(B)** Downregulated. **(C-D)** The related genes of the GO terms enriched by the Ca4-low ECs differentially expressed genes in IR20Gy compared to the control. **(C)** Upregulated. **(D)** Downregulated. **(E)** Upregulated signaling in IR20Gy sent by Ca4-high ECs. **(F)** Upregulated signaling in IR20Gy received by Ca4-high ECs. IR20Gy: Ionizing radiation exposure at a dose of 20 Gray; GO: Gene ontology; ECs: Epithelial cells.

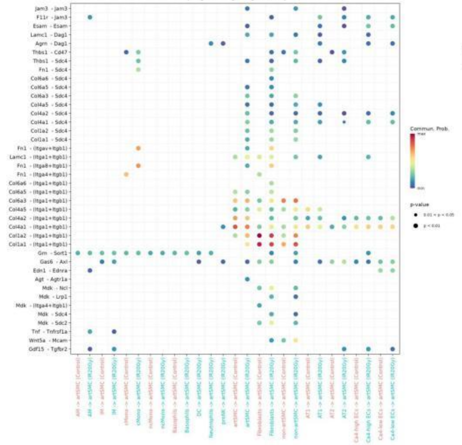
A


artSMC_KEGG.up
Pancreatic cancer
Chronic myeloid leukemia
Thyroid cancer
Bladder cancer
Endometrial cancer
Basal cell carcinoma
Melanoma
Non-small cell lung cancer
Glioma
p53 signaling pathway
0.12
0.13
0.14
0.15
0.16
GeneRatio
p.adjust
0.0050
0.0075
0.0100
Count
3.00
3.25
3.50
3.75
4.00

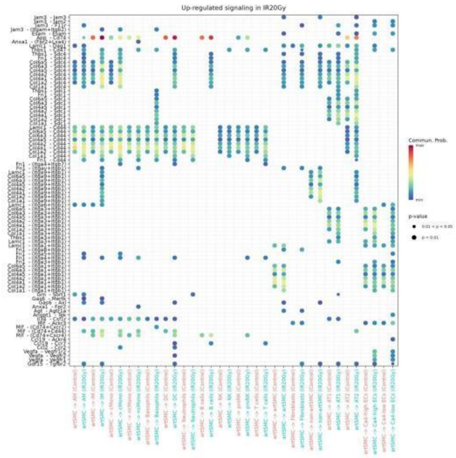
B


artSMC_GO.up_BP
response to ketone
response to glucocorticoid
response to corticosteroid
negative regulation of phosphorylation
negative regulation of phosphorus metabolic process
negative regulation of phosphate metabolic process
cellular response to glucocorticoid stimulus
cellular response to corticosteroid stimulus
response to progesterone
cellular response to dexamethasone stimulus
0.15
0.20
0.25
GeneRatio
p.adjust
0.001
0.002
0.003
0.004
Count
4
5
6
7
8
9
10

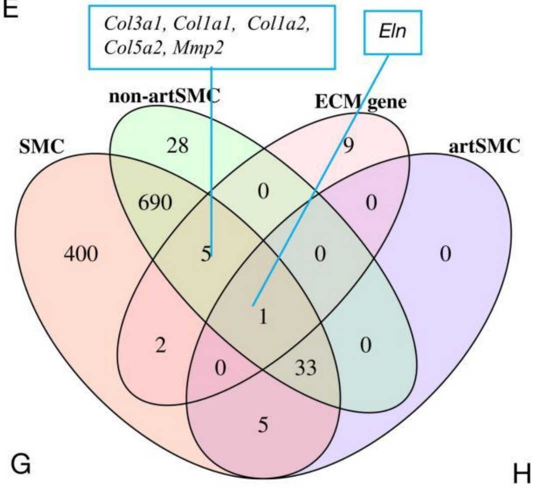
C


Up-regulated signaling in IR20Gy
Commun. Prob.
p-value

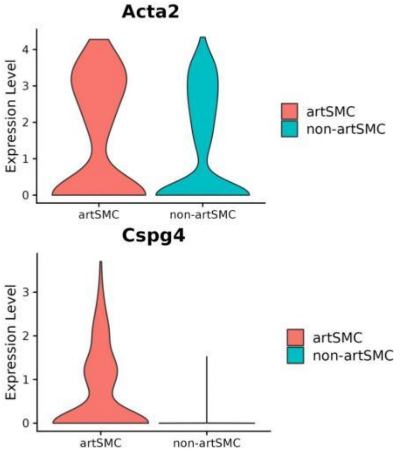
D


Up-regulated signaling in IR20Gy
Commun. Prob.
p-value

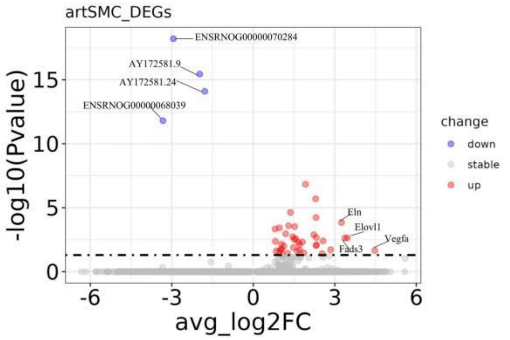
E


Col3a1, Col1a1, Col1a2, Col5a2, Mmp2
Eln
non-artSMC
ECM gene
SMC
artSMC
28
9
690
0
0
400
5
0
0
1
2
0
33
0
5

F


Acta2
Expression Level
4
3
2
1
0
artSMC
non-artSMC
artSMC
non-artSMC
Cspg4
Expression Level
3
2
1
0
artSMC
non-artSMC
artSMC
non-artSMC

G


artSMC_DEGs
ENSRNOG00000070284
AY172581.9
AY172581.24
ENSRNOG00000068039
Eln
Elovl1
Vegfa
Fads3
-log10(Pvalue)
15
10
5
0
-6
-3
0
3
6
avg_log2FC
change
down
stable
up

H

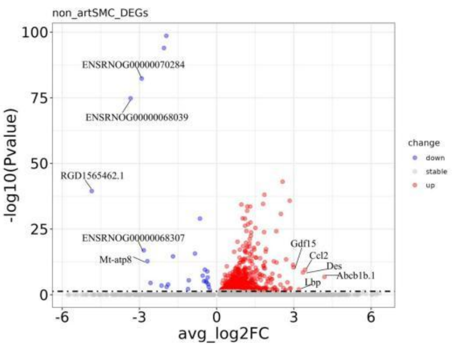

non_artSMC_DEGs
ENSRNOG00000070284
ENSRNOG00000068039
RGD1565462.1
ENSRNOG00000068307
Mt-atp8
Gdf15
Ccl2
Des
Abcb1b.1
Lbp
-log10(Pvalue)
100
75
50
25
0
-6
-3
0
3
6
avg_log2FC
change
down
stable
up

Figure S7. Cellular and molecular characterization of smooth muscle cells. (A) GO terms enriched by the artSMC upregulated genes in IR20Gy compared to the control. **(B)** KEGG enriched by the artSMC upregulated genes in IR20Gy compared to the control. **(C)** Upregulated signaling in IR20Gy received by artSMC. **(D)** Upregulated signaling in IR20Gy sent by artSMC. **(E)** Wayne diagram shows overlapping genes of SMC, non-artSMC, artSMC upregulated genes and ECM genes. **(F)** The expression of Acta2 and Cspg4 in the two subpopulations. **(G-H)** The volcano plot shows the artSMC and non-artSMC upregulated and downregulated genes in the IR20Gy compared to the control group. **(G)** artSMC. **(H)** non- artSMC. KEGG: Kyoto Encyclopedia of Genes and Genomes; IR20Gy: Ionizing radiation exposure at a dose of 20 Gray; GO: Gene ontology; artSCM: Arterial smooth muscle cells; non-artSCM: Non- arterial smooth muscle cells.