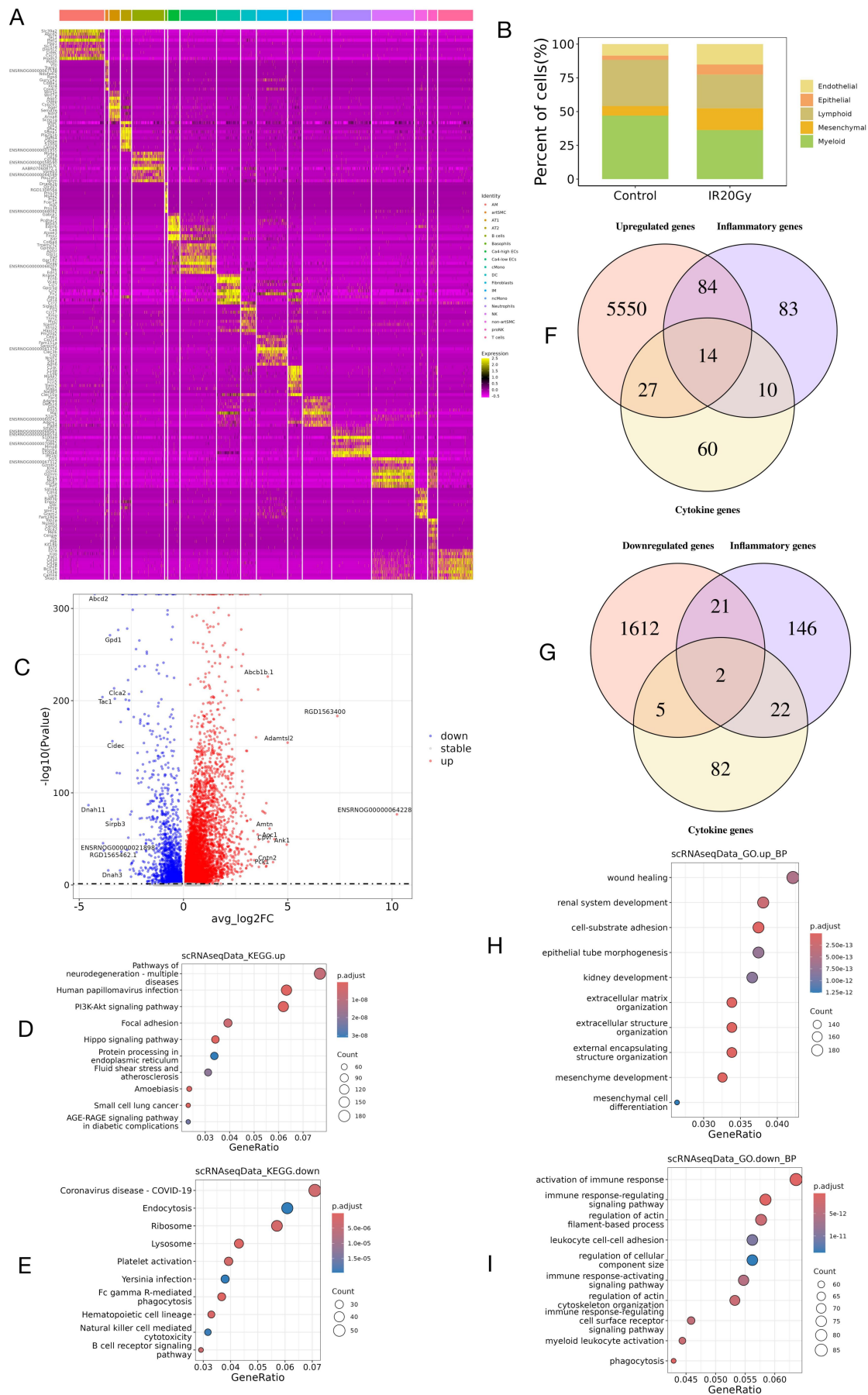


## **SUPPLEMENTAL DATA**

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

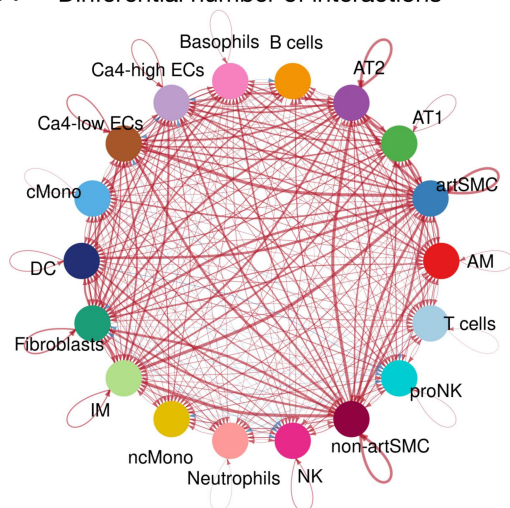




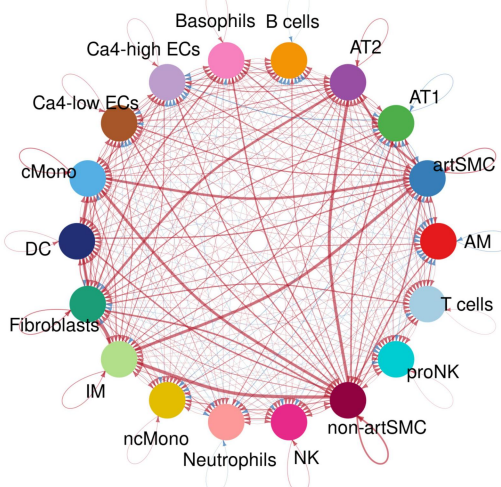
**Figure S1. Cellular and molecular characterization of cells after IR.** (A) Heat map shows the expression of 18 cell types top ten markers. (B) Dynamics in cell proportions of the 5 cell populations across the control and IR20Gy group. (C) The volcano plot shows the upregulated and downregulated genes 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) Venn 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 the IR20Gy 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 of overlapping 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.



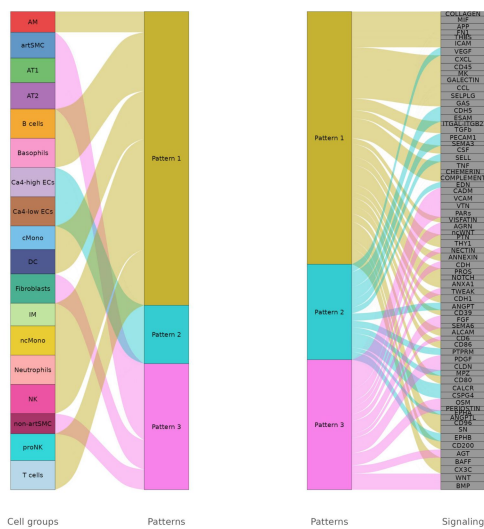
## A Differential number of interactions



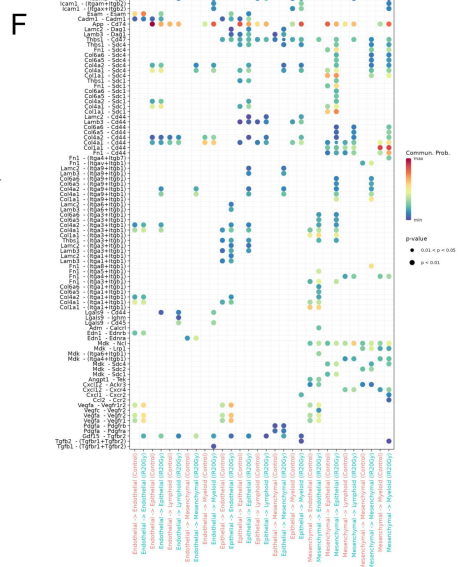
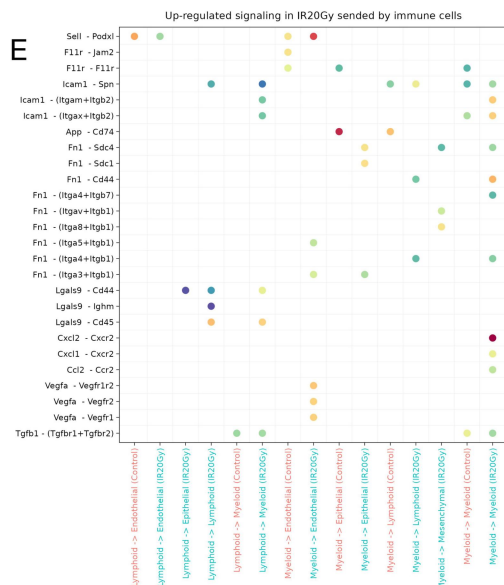
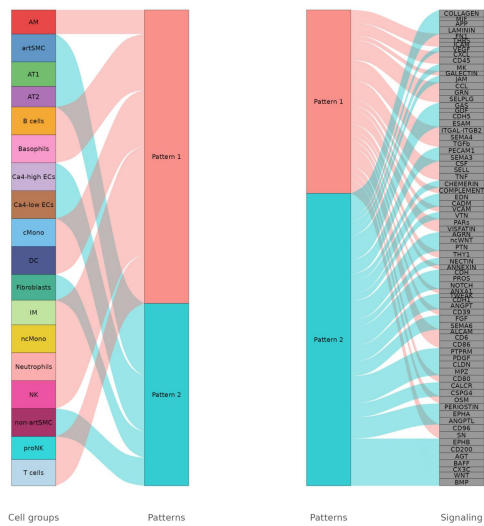
## B Differential interactions strength



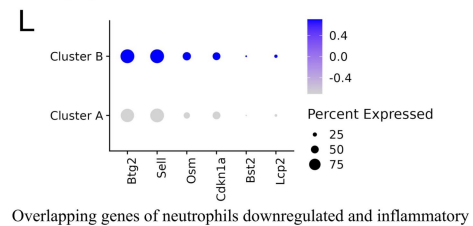
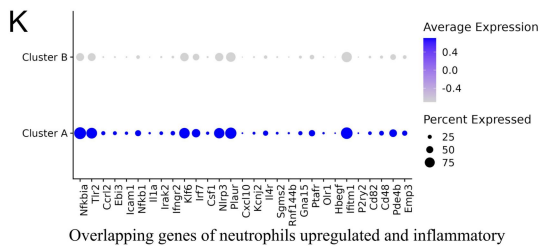
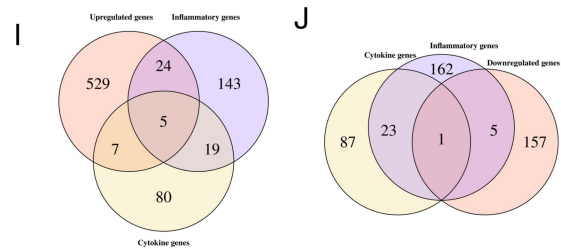
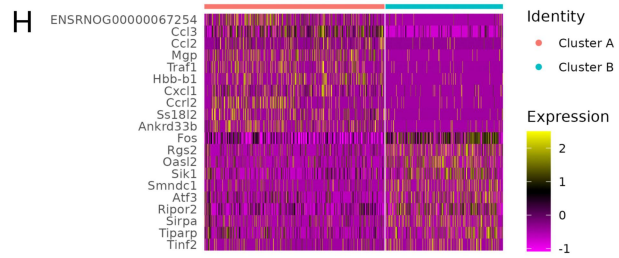
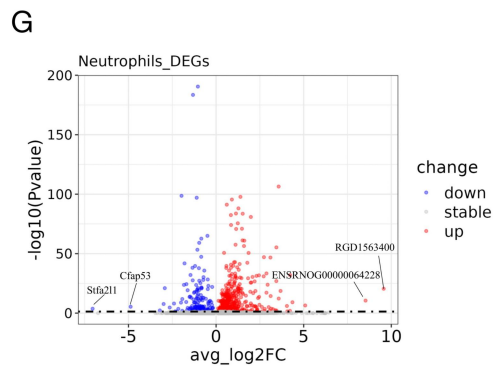
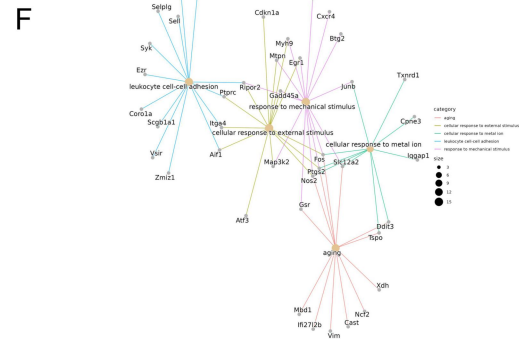
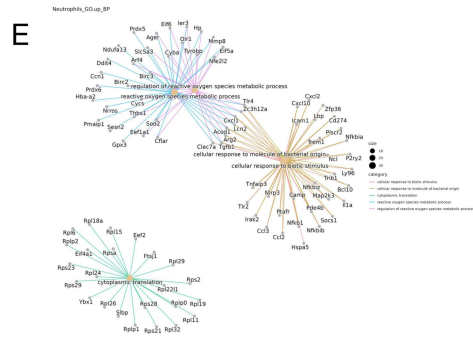
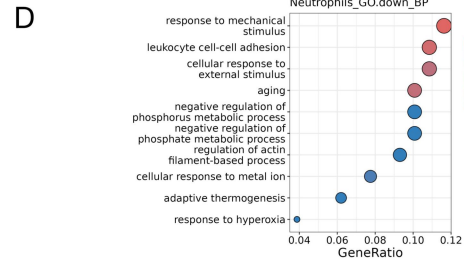
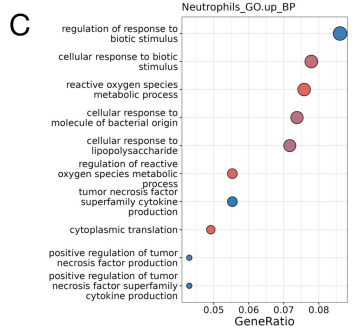
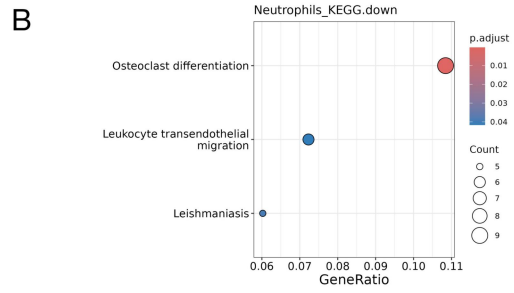
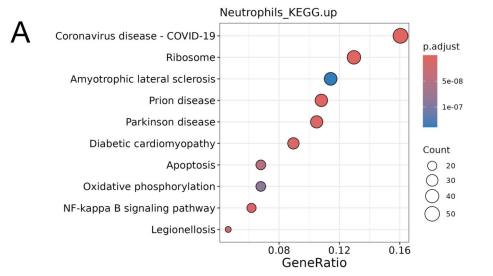
## C Incoming communication patterns of target cells



## D Outgoing communication patterns of secreting cells



**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 based 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.

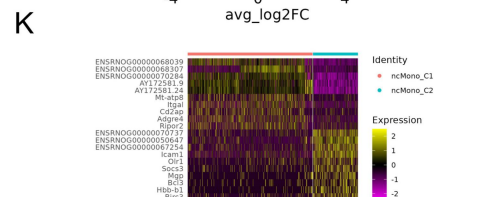
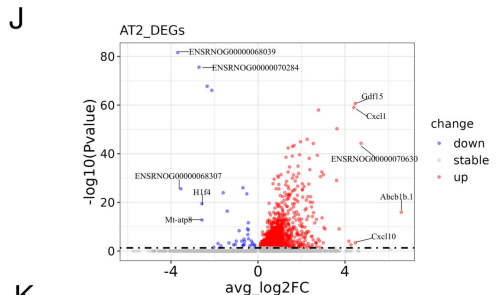
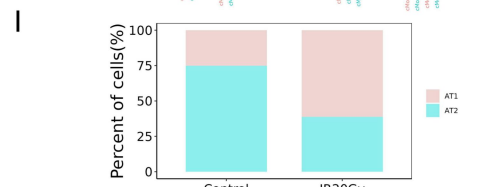
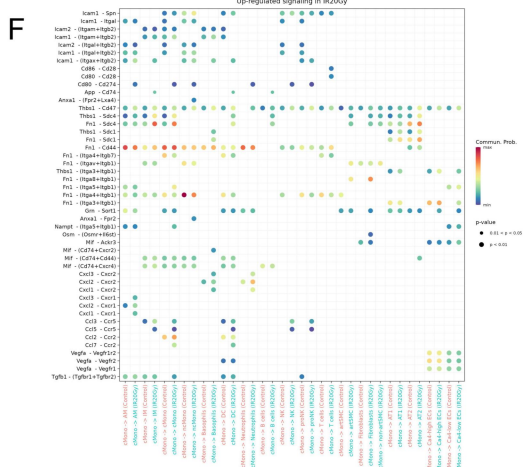
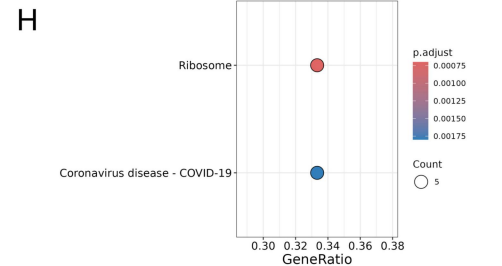


**Figure S3. Cellular and molecular characterization of neutrophil cells.** (A-B) KEGG enrichment analysis of the neutrophil differentially expressed genes in IR20Gy compared to the control. (A) Upregulated, (B) Downregulated. (C-D) GO terms enriched by the neutrophil differentially expressed genes in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



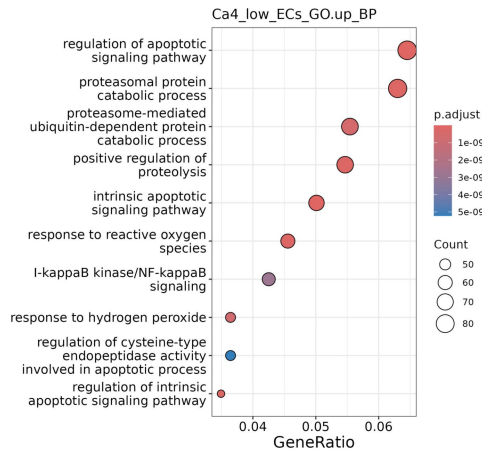
**Figure S4. Cellular and molecular characterization of macrophage cells. (A-E)** Cellular and molecular characterization of alveolar macrophages (AM). **(A)** Heatmap showing the top ten differentially expressed genes of the five subcelltypes of AM. **(B-C)** KEGG pathways enriched by the differentially 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.



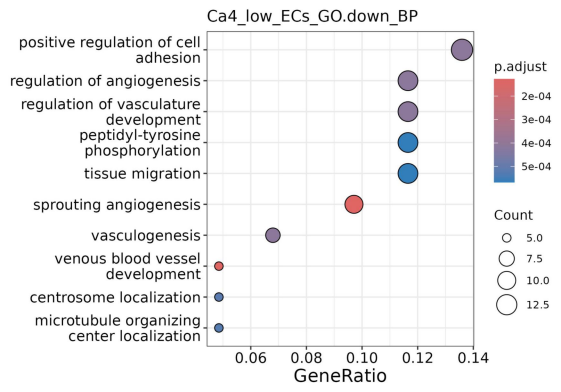


**Figure S5. Cellular and molecular characterization of monocytes and epithelials. (A-E)** Cellular and molecular characterization of cMono. **(A)** Heatmap showing the top ten differentially expressed genes of the five subcelltypes of cMono. **(B)** The volcano plot shows the differential genes of cMono 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

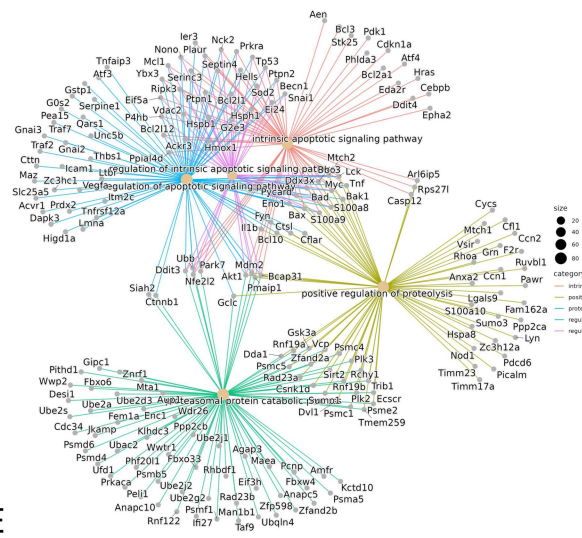


B



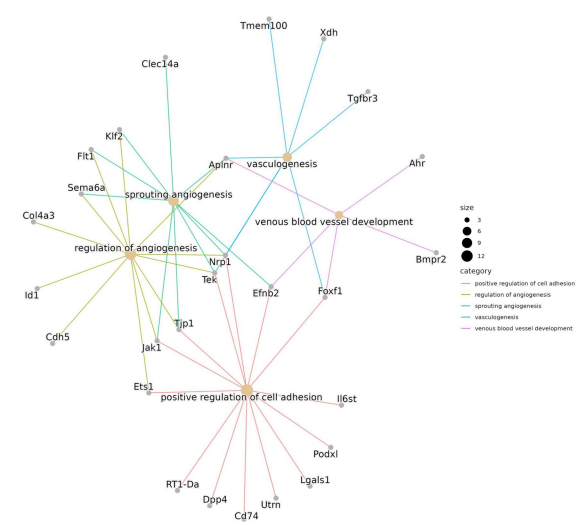
C

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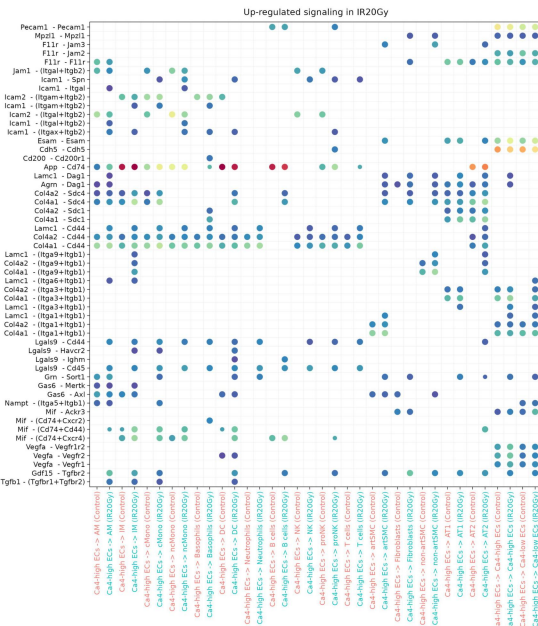


D

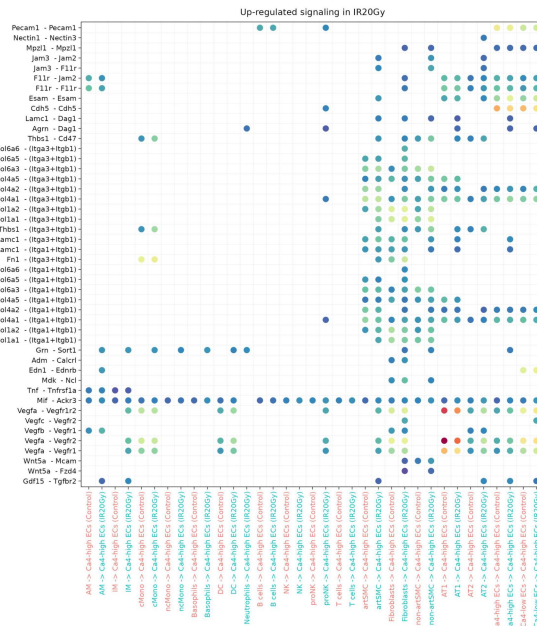
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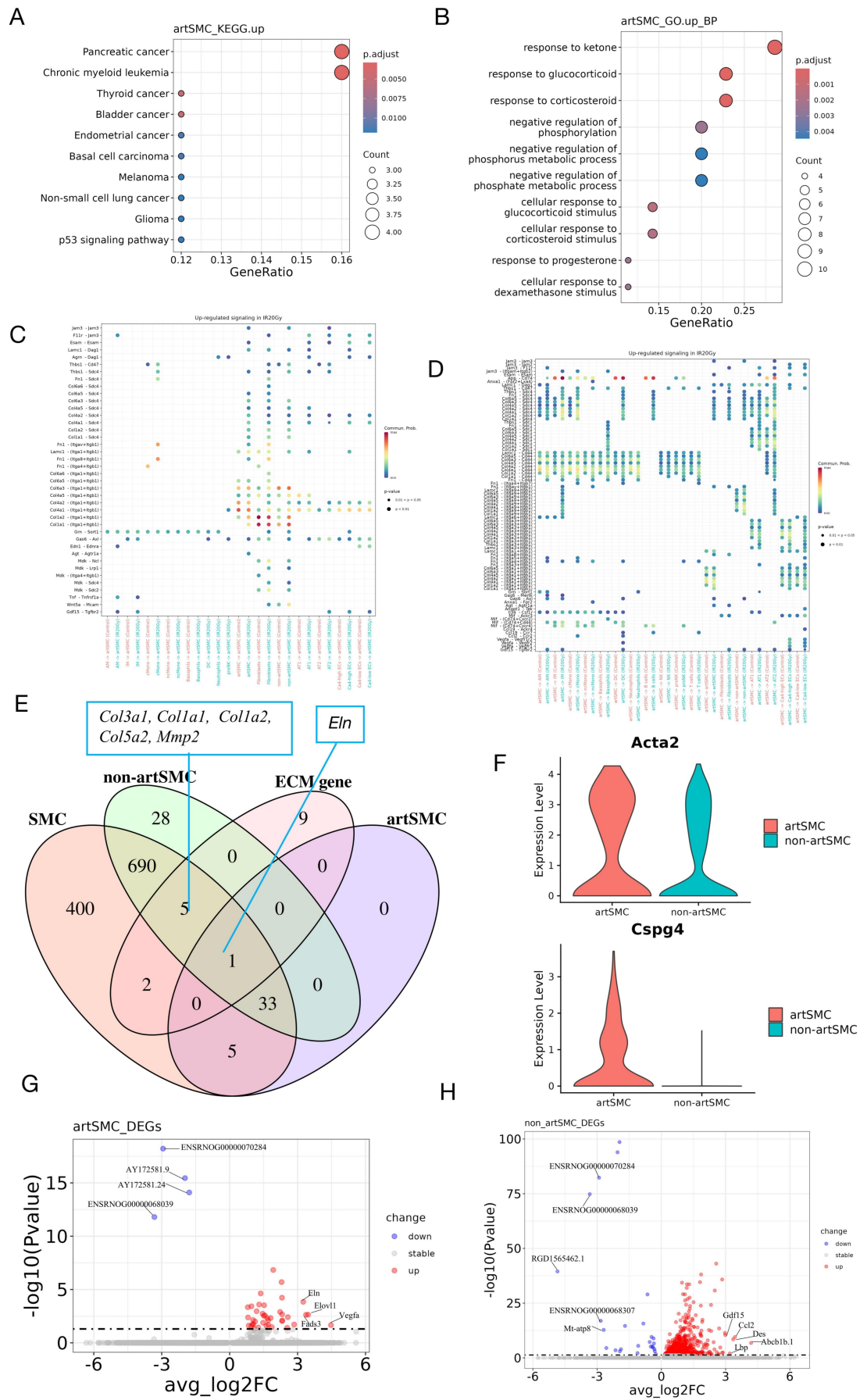
E



F



**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.



**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.