# Modeling and countering the effects of cosmic radiation using bioengineered human tissues

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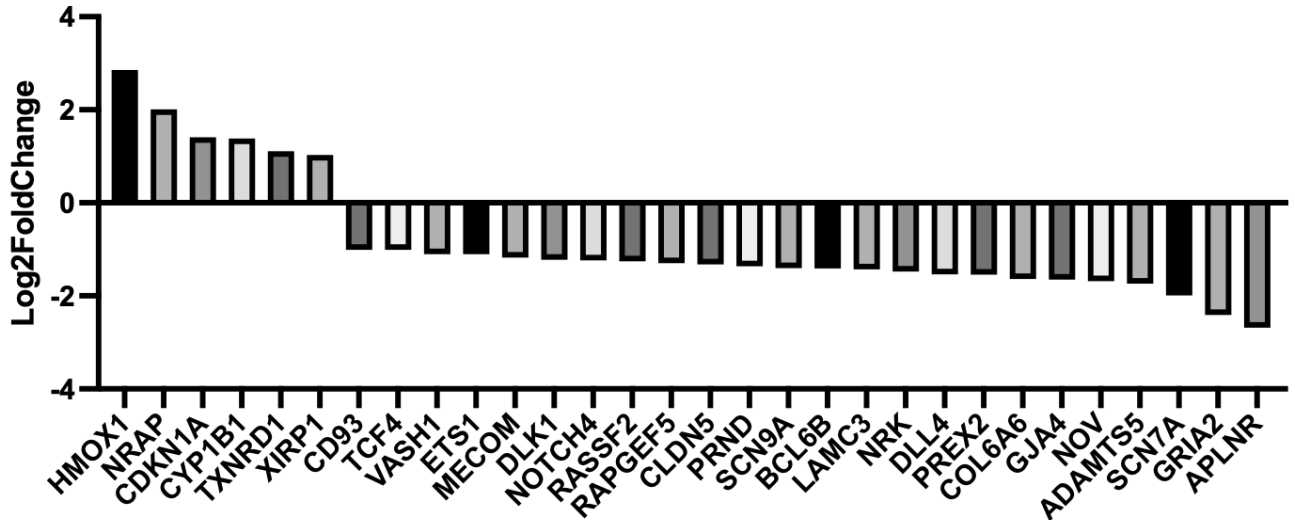
Supplemental Figures
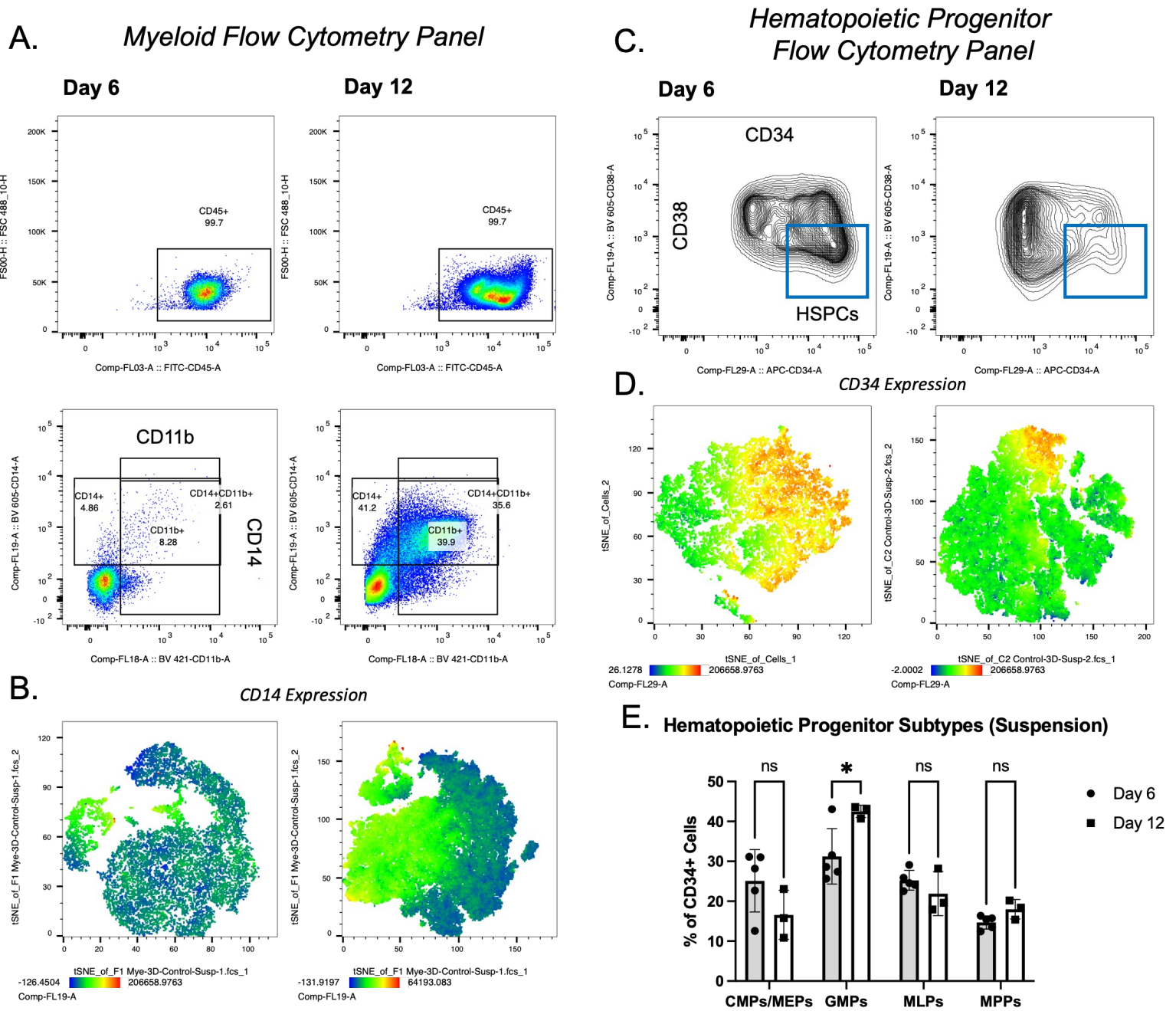
Supplementary Figure 1. Sample flow cytometry gating for suspension cells in engineered bone marrow (eBM). Myeloid cell panel. (A-B) Shifting cell populations over time (A) and (B) dimensionality reduction for CD14. **Hematopoietic cell panel.** (C-D) Shifting cell populations over time (C) and (D) dimensionality reduction for CD34). (E) Subpopulations of hematopoietic progenitors described in **Figure 2**.
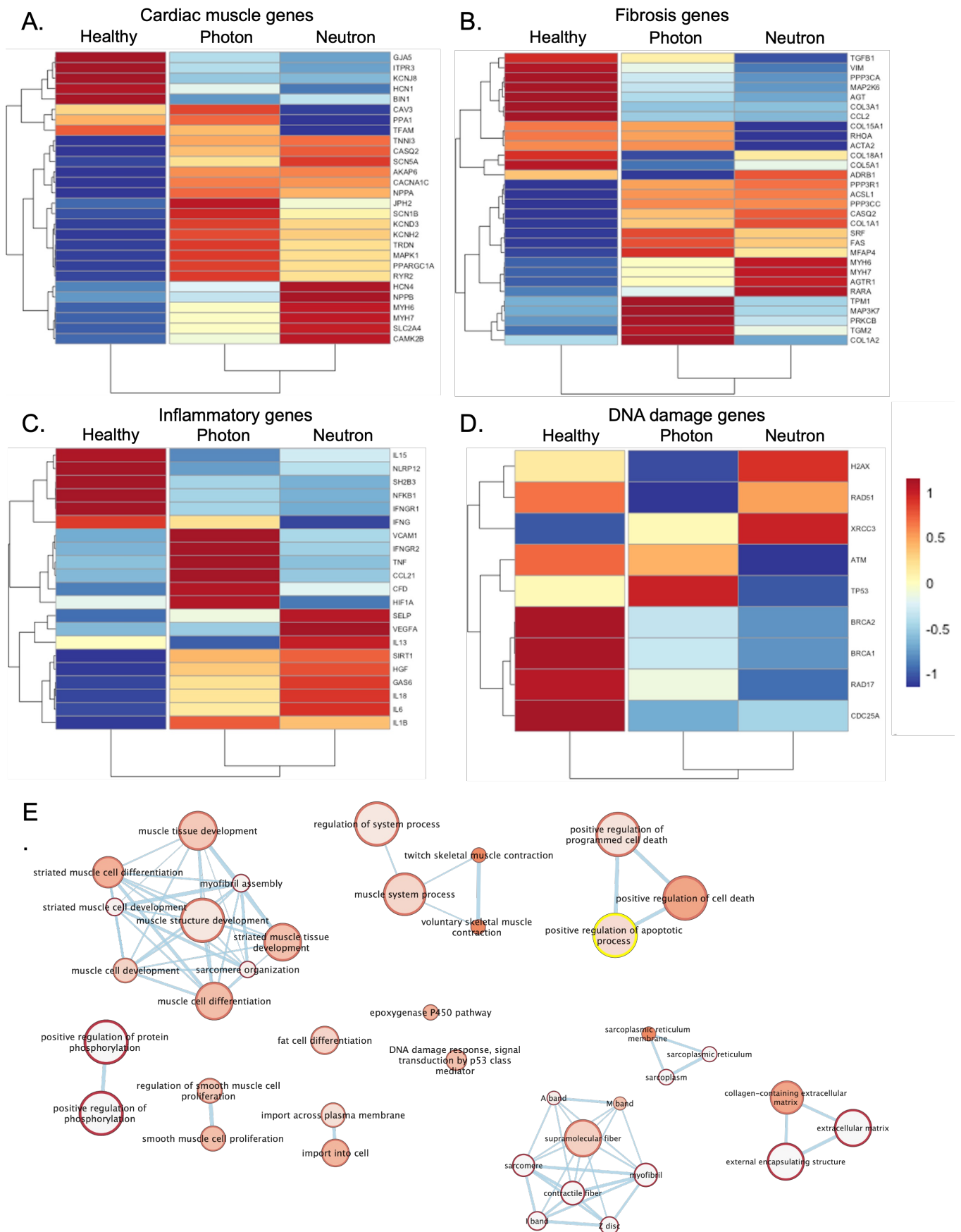
Supplementary Figure 2. Transcriptomic changes to engineered cardiac tissueds (eCTs) in response to radiation (by key phenotypic genes). (A) Cardiac-specific genes (B) Fibrosis genes (c) Inflammatory genes (D) DNA damage/repair genes. (E) Network analysis using Cytoscape of differentially-expressed genes between neutron irradiated and control eCTs.
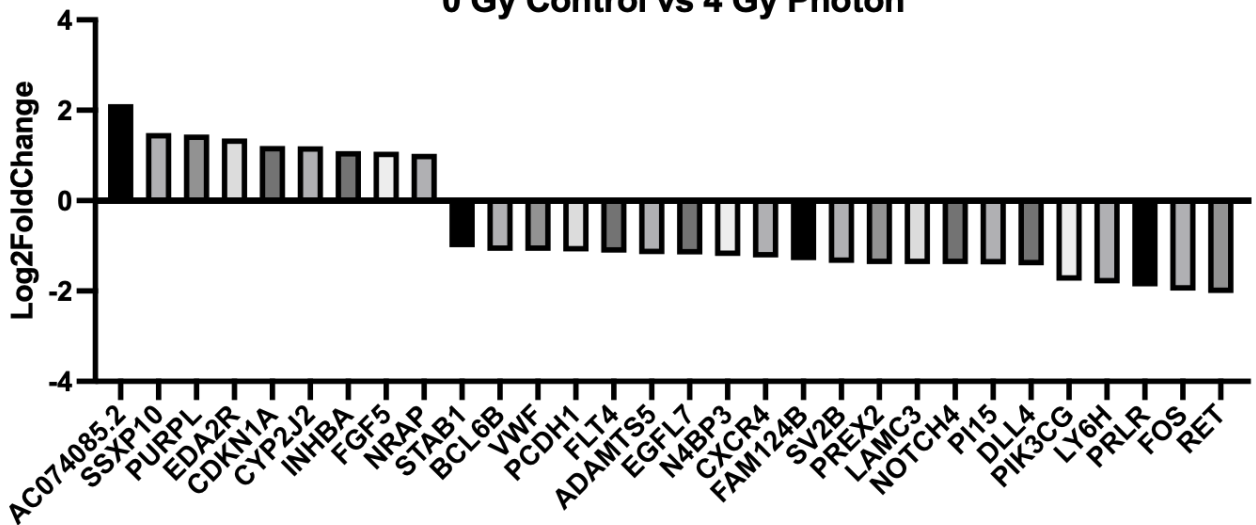
Supplementary Figure 3. Bulk gene expression changes in irradiated engineered cardiac tissues (eCTs) 3-weeks post-radiation. (A) Differentially expressed genes by fold change for (A) neutron and (B) photon radiation, compared to the control eCTs.
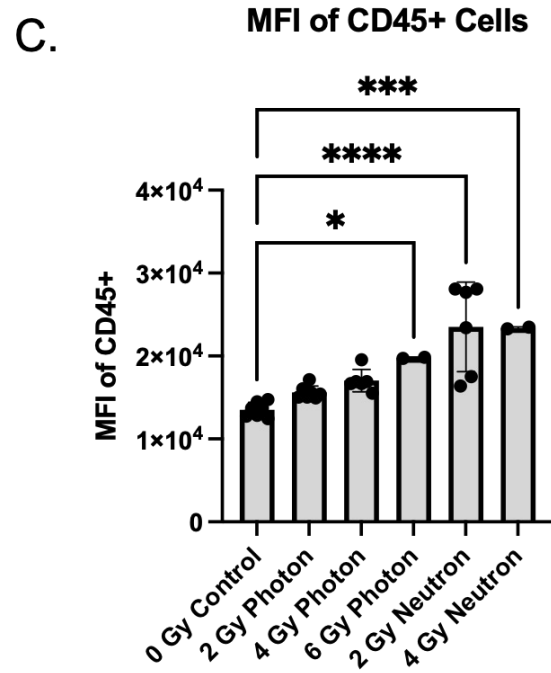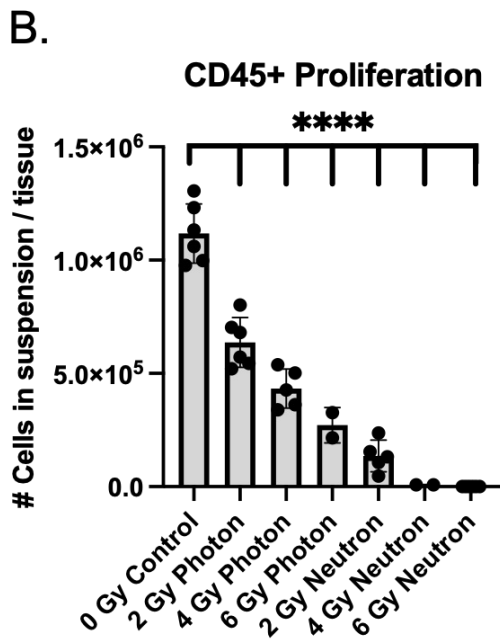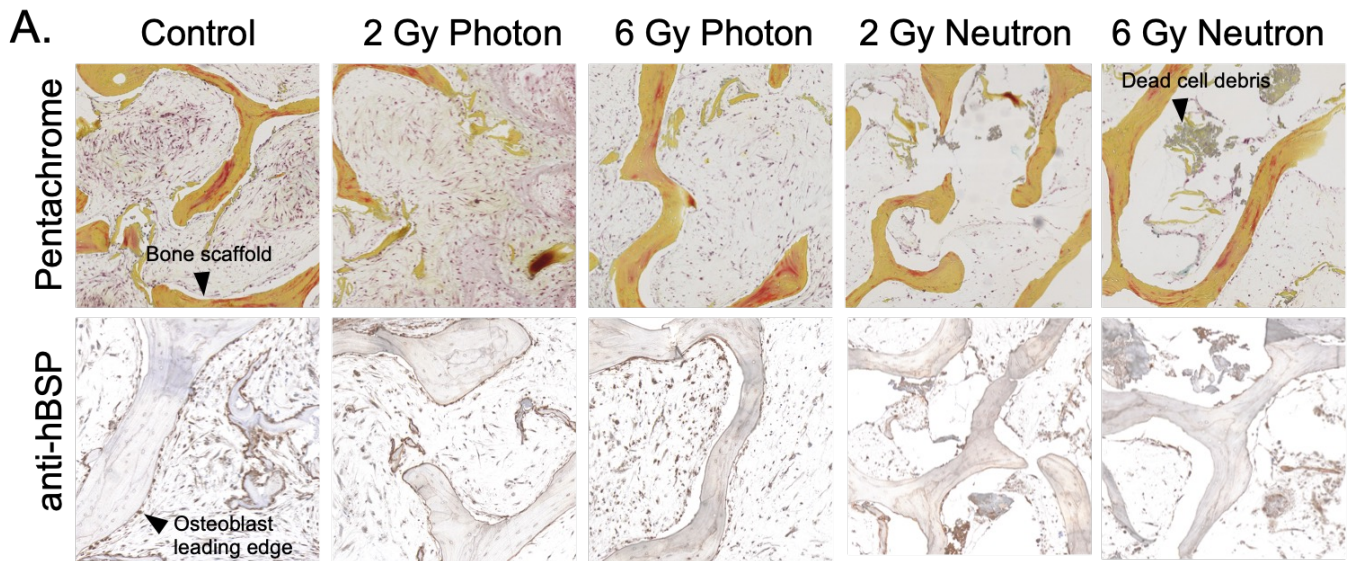
Supplementary Figure 4. Dose-dependent effects of radiation on engineered bone marrow (eBM) tissues and hematopoietic cells 2 weeks after radiation exposure. (A) Photon and neutron radiation decreased the intensity of pentachrome (top) and bone sialoprotein (BSP, bottom) staining in a dose-dependent manner. (B) Decreasing CD45+ cell production, and (C) increasing CD45+ cells median fluorescence intensity (MFI) with increasing radiation dose over two weeks. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05
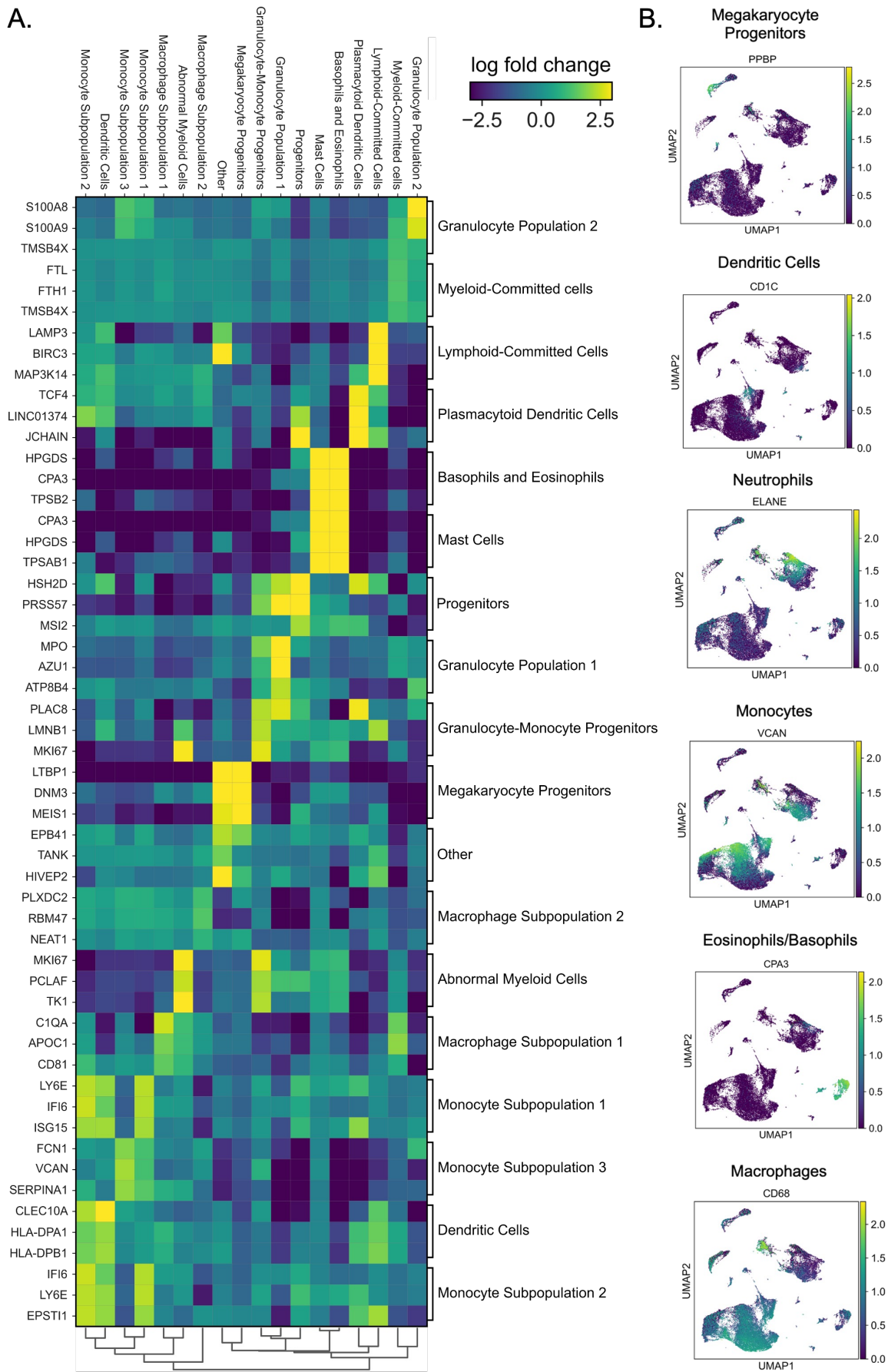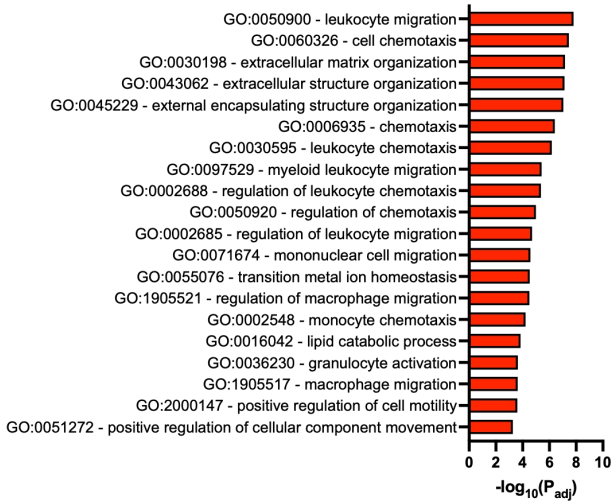
Supplementary Figure 5. scRNAseq cell type classification of engineered bone marrow (eBM) blood cells. (A) Top genes for unique cell types. (B) Example marker genes for identifying known cell types.
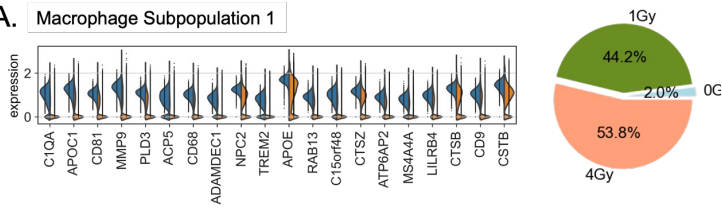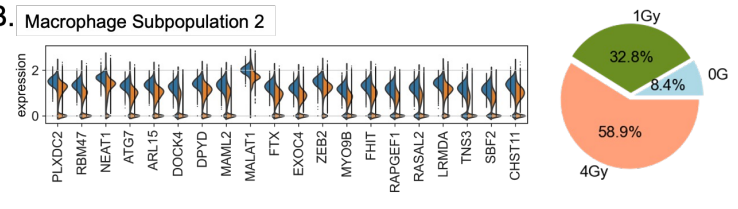
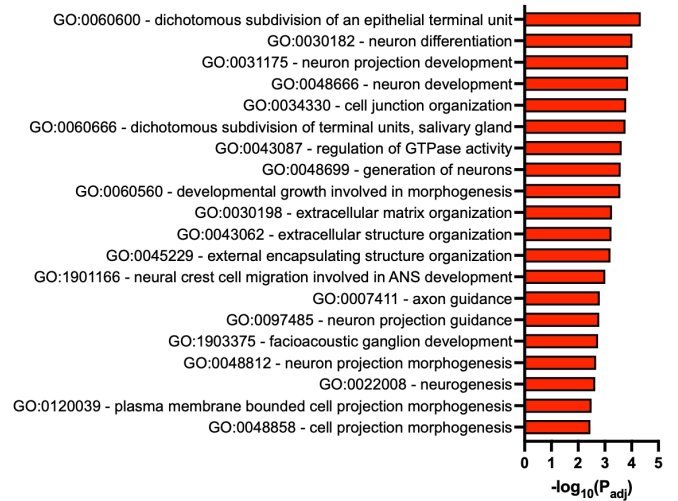**Supplementary Figure 6. Identification of unique cell populations emerging in engineered bone marrow (eBM) 3-weeks post-irradiation.** Data correspond to single-cell RNA sequencing shown from eBMs in **Figure 5.** (A-D) Most significant differentially-expressed genes and pathways implicated in (A) Macrophage subpopulation 1, (B) Macrophage subpopulation 2, (C) Monocyte subpopulation 3, and (D) Abnormal myeloid cells.
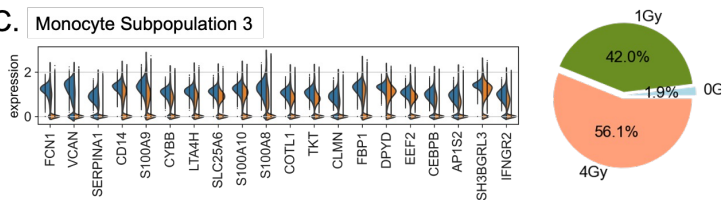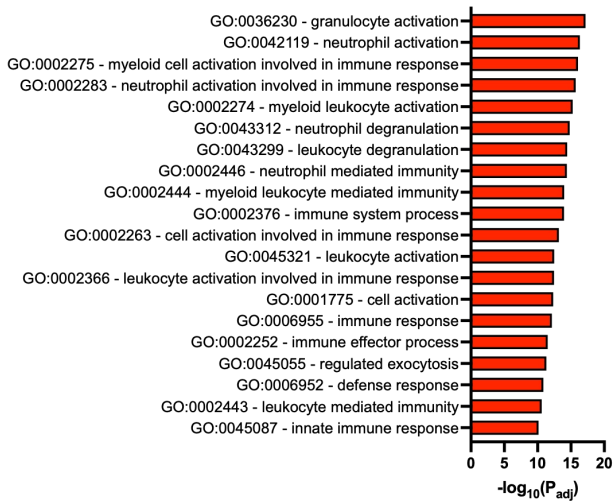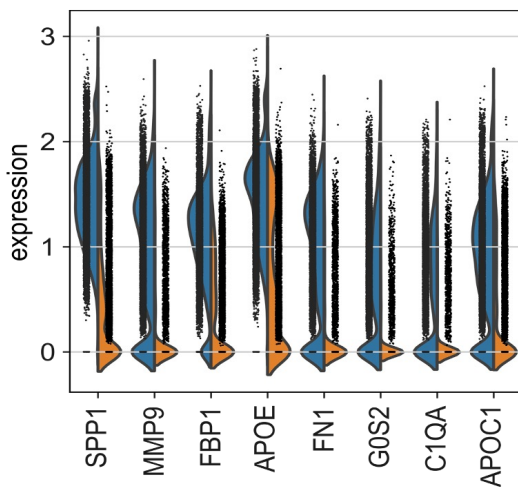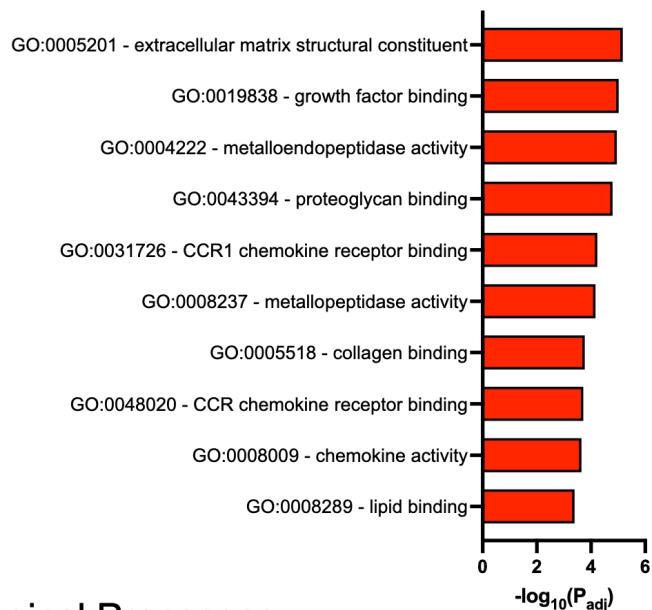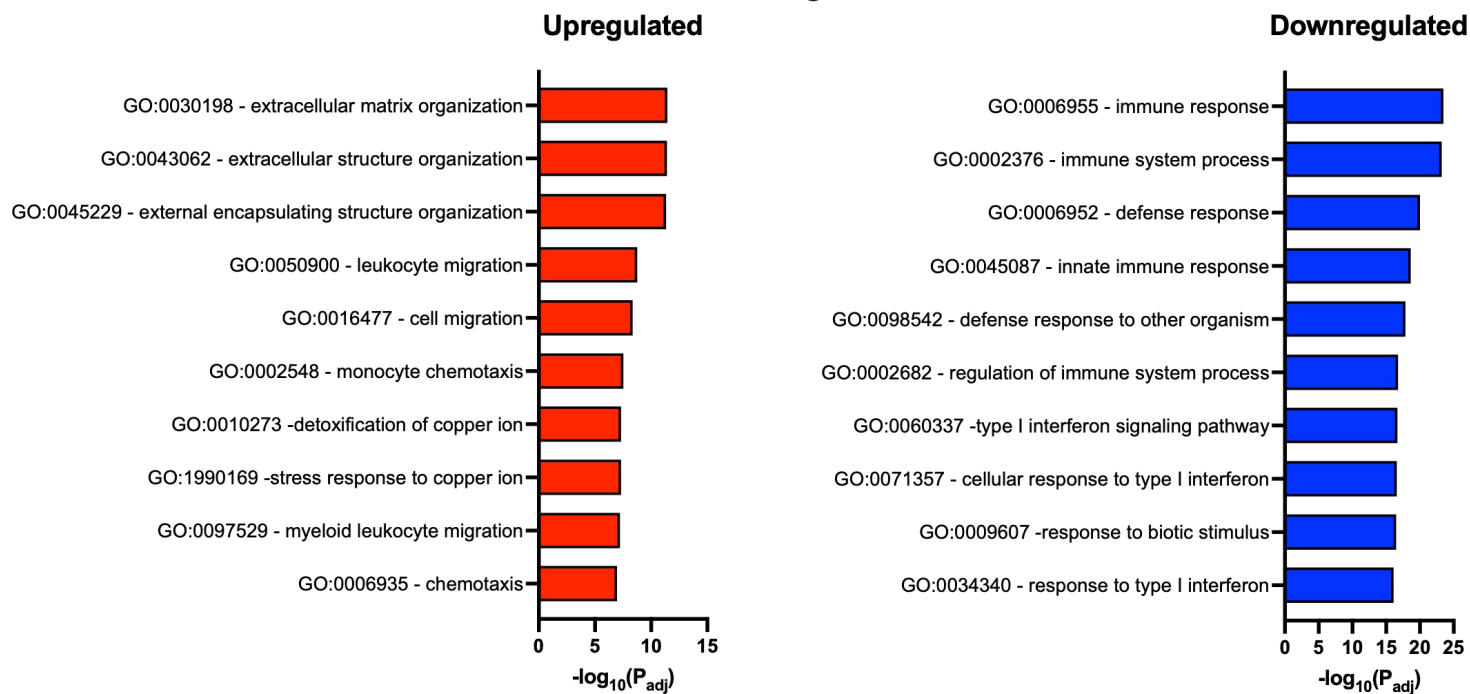
Supplementary Figure 7. Total gene expression changes in all engineered bone marrow (eBM) cells by single cell RNA sequencing in Figure 5. (A) Top significant, differentially-expressed genes in 1 Gy cells as compared to the 0 Gy healthy control. (B) Pathway analysis of significantly upregulated genes in the neutron-irradiated samples in GO molecular functions. (C) upregulated/downregulated genes in the neutron-irradiated samples in GO biological processes.
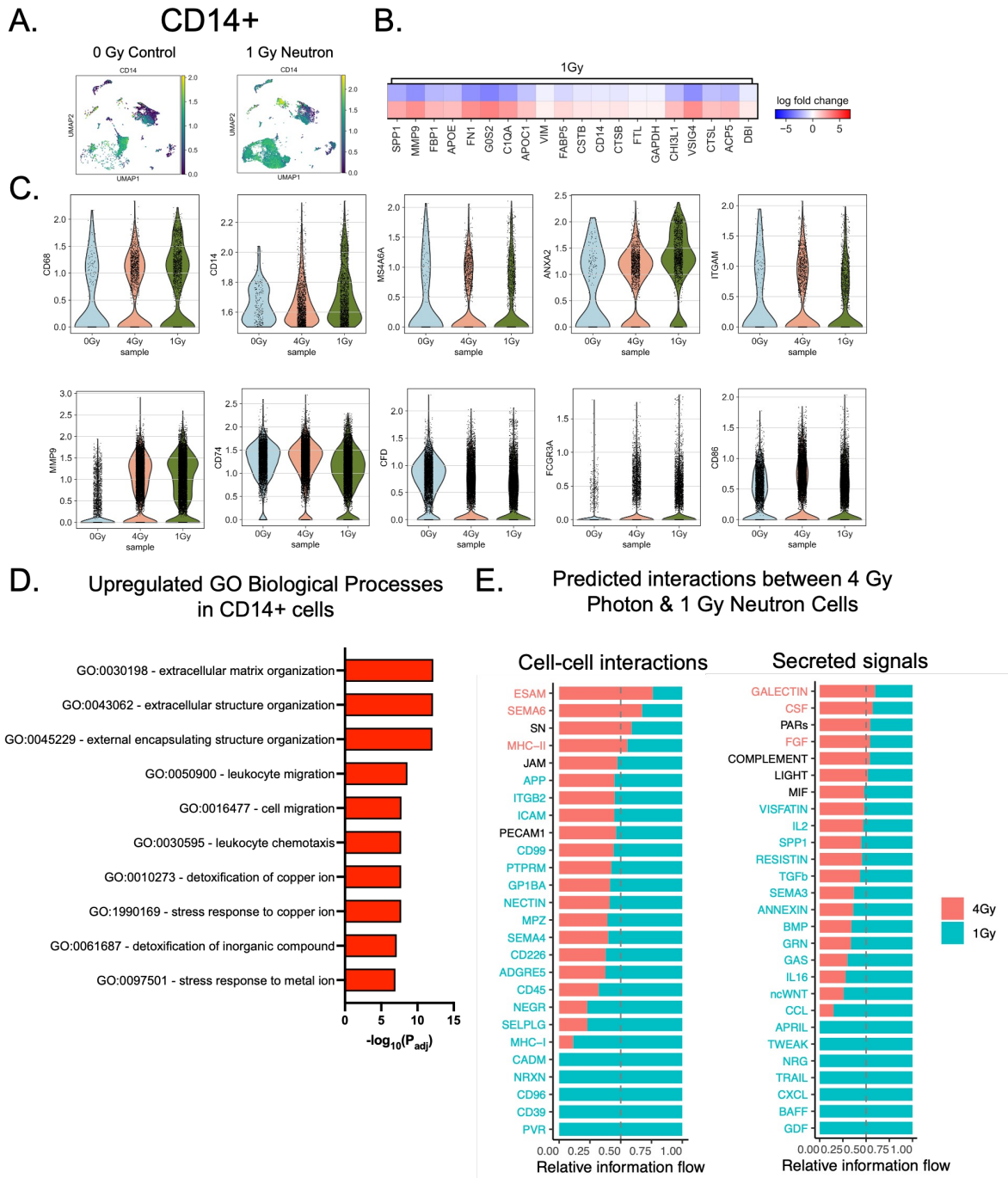
Supplementary Figure 8. Differential expression of isolated CD14+ cells from engineered bone marrow (eBM) single-cell RNA sequencing in response to radiation. (A) UMAP visualization of CD14+ myeloid cells. (B) Differential gene expression in neutron irradiated (1 Gy) tissues relatively to controls. (C) Example violin plots of key myeloid genes. (D) GO pathway analysis of top biological processes. (E) Comparative analysis of cell-cell interactions and secreted signals in 4 Gy Photon versus 1 Gy Neutron eBM tissues.
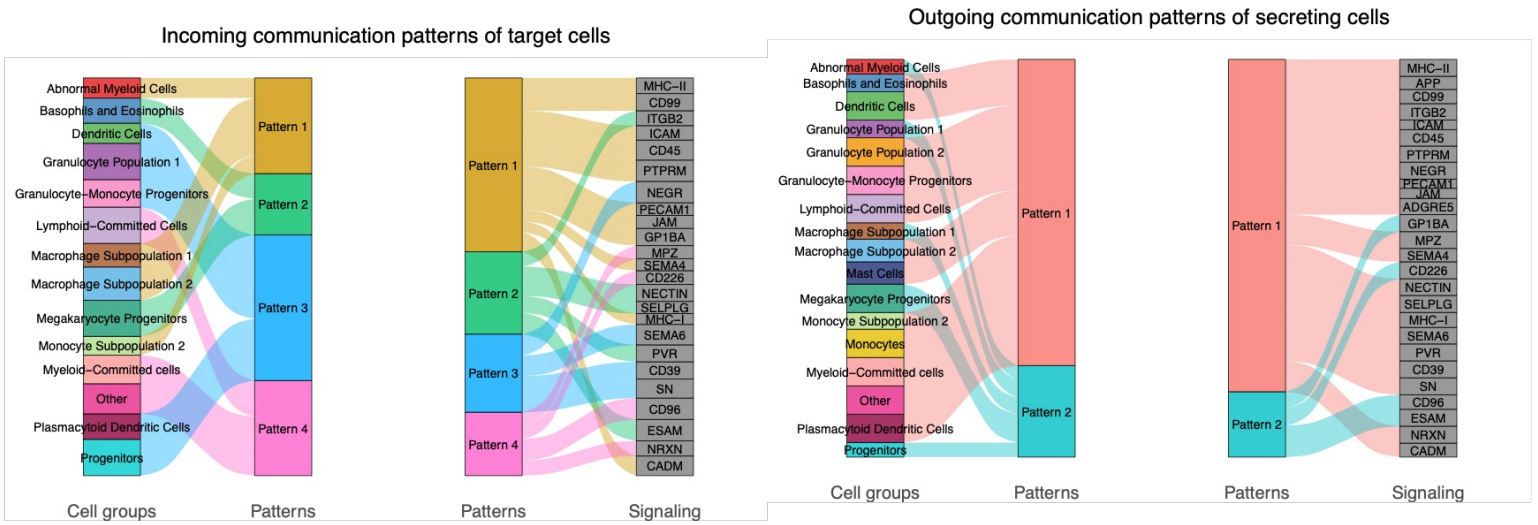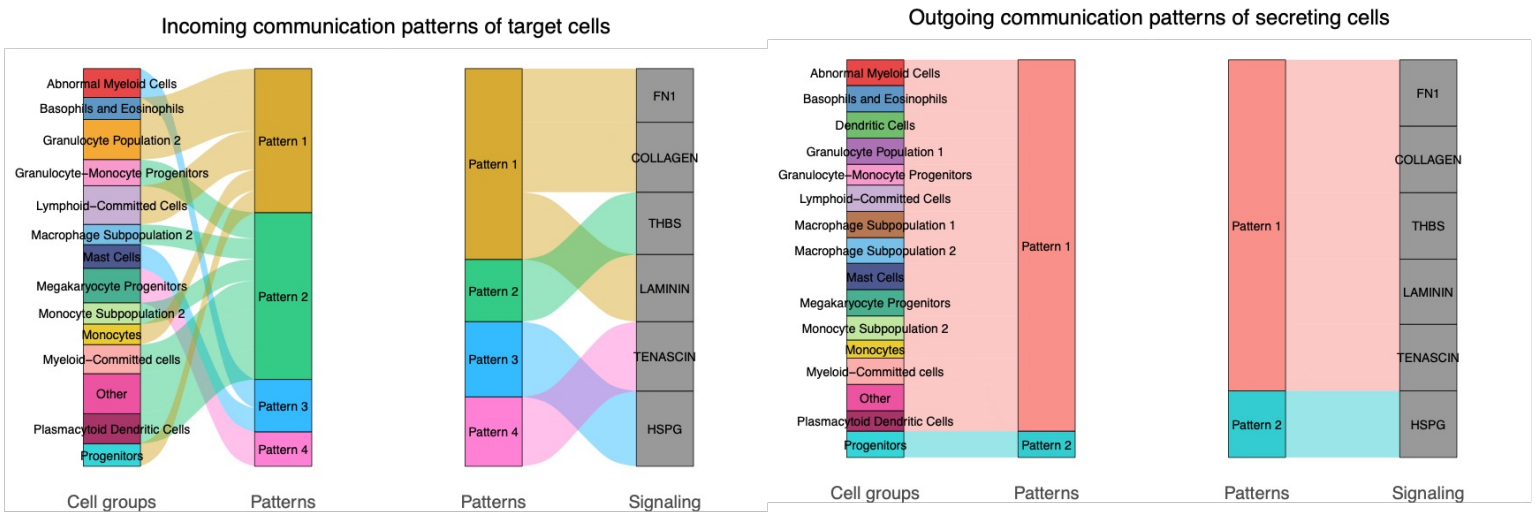
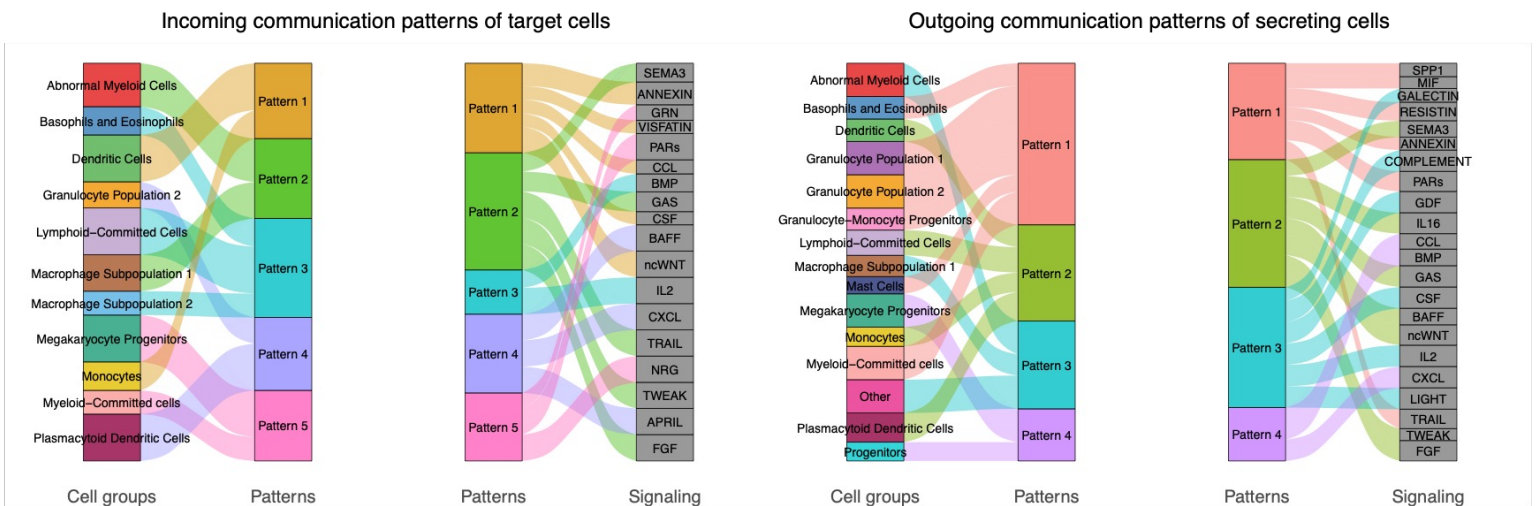**Supplementary Figure 9**. **Computationally-derived interactions within the 1Gy Neutron-irradiated engineered bone marrow (eBM) blood cells via CellChat.** (A-C) Inferred incoming and outgoing (A) cell-cell, (B) cell-ECM, and (C) cell-secretome interactions.
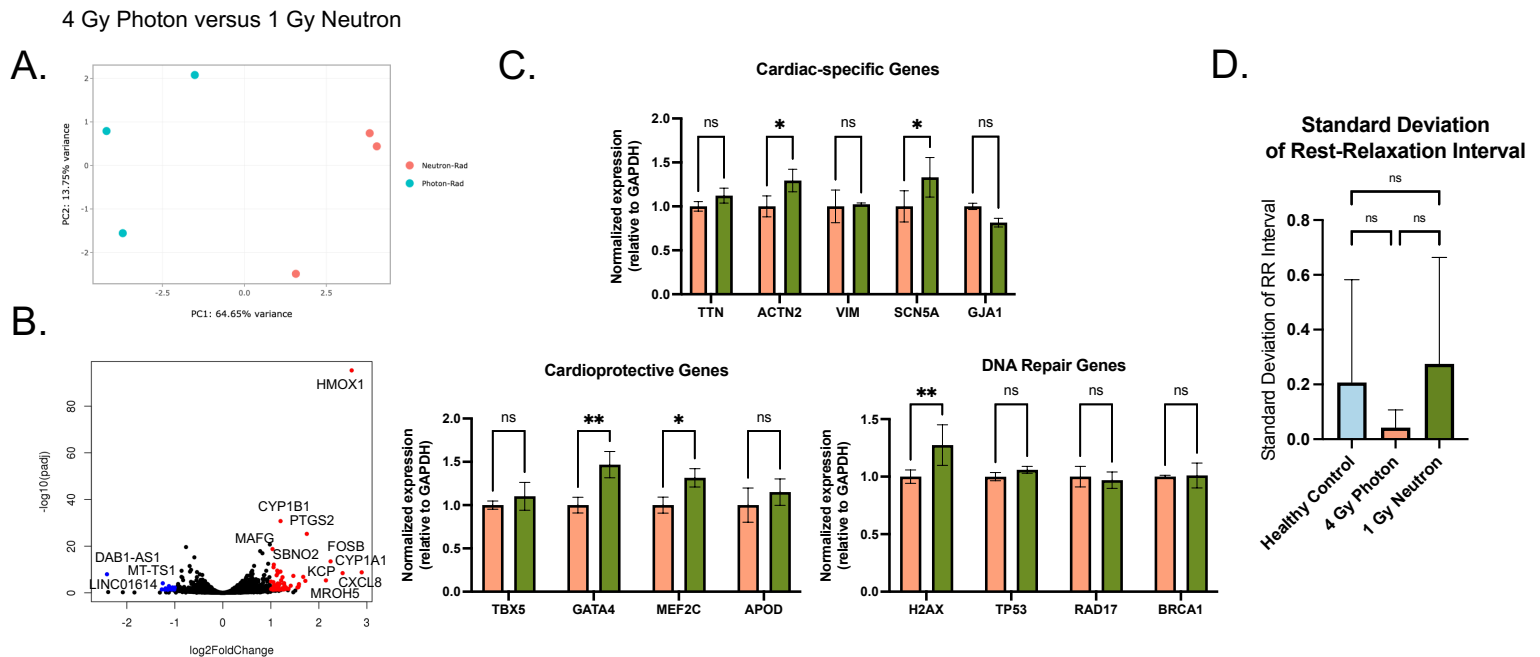
Supplementary Figure 10. Differentially expressed changes associated with engineered cardiac tissues (eCT) in response to 4 Gy photon vs 1 Gy neutron radiation. (A) Principle component analysis of eCT in response to photon and neutron radiation. (B) Differentially expressed genes between the two groups ($p_{adj}<0.05$, log2FC>1). (C) Individualized visualization of genes associated with cardiac function/maturation, cardioprotection, and DNA repair, shown with multiple, unpaired t-tests *p<0.05; **p<0.01. (D) From calculating the standard deviation of the rest-relaxation interval during cardiac muscle tissue spontaneous beating, no differences were observed to indicate presences of arrythmias (n = 2 - 4 tissues).
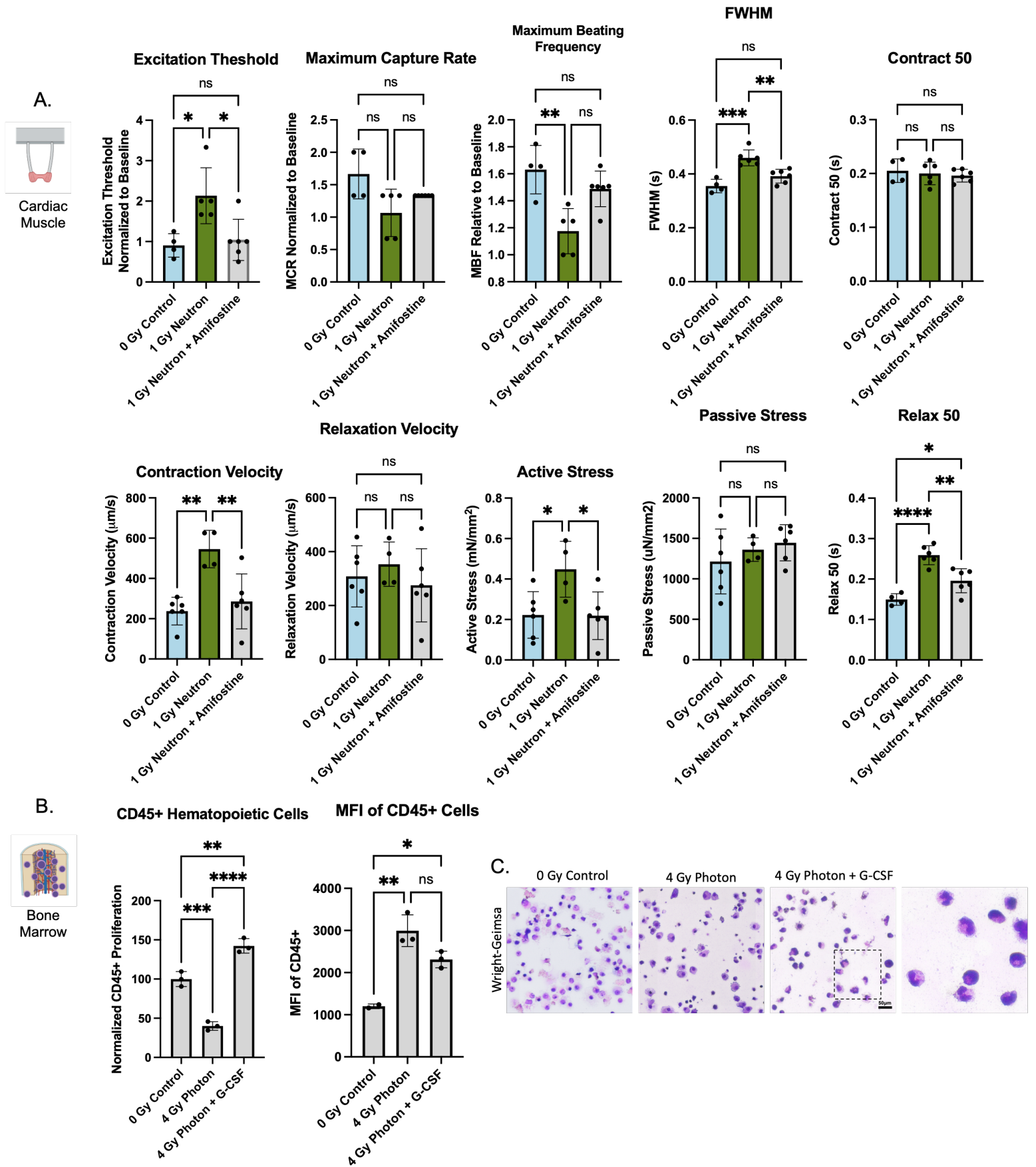
Supplementary Figure 11. Use of engineered human tissues for testing of therapeutic effects of radioprotective agents. (A) Pre-treatment of engineered cardiac tissues (eCT) with radioprotective drug Amifostine prior to acute exposures prevented abnormal hypertrophic functionality of neutron-irradiated tissues. (B) G-CSF stimulation increases CD45+ cell production by (C) increasing neutrophil production *in vitro*.