Data S1. Supplementary materials and methods

Liquid chromatography (LC)-mass spectrometry (MS)/MS

   Heart tissue protein extraction, trypsin digestion and tandem mass tag (TMT) labeling. The apex of heart samples was ground in liquid nitrogen and then mixed with lysis buffer, followed by sonication three times on ice at a frequency of 20 kHz for 10 sec (Ningbo Scientz Biotechnology Co., Ltd.). The supernatant was collected after centrifugation with 12,000 x g at 4°C for 10 min and the protein concentration was measured using a BCA Protein assay kit (Beyotime Institute of Biotechnology). The protein solution was reduced with dithiothreitol and alkylated with iodoacetamide in the dark. The protein sample was diluted by adding 0.5 M tetraethylammonium bromide (TEAB). Finally, trypsin was added at a trypsin-to-protein mass ratio for the first digestion overnight, and repeated for a second 4 h-digestion. After trypsin digestion, the peptides were desalted with a Strata X C18 SPE column (Phenomenex) and vacuum-dried. The peptides were reconstituted in 0.5 M TEAB and processed following the manufacturer's instructions for the TMT kit (Thermo Fisher Scientific, Inc.).

High performance (HP)LC fractionation. High pH reverse-phase HPLC, using Agilent 300Extend C18 column (5-μm particles, 4.6-mm internal diameter, 250-mm length; Agilent Technologies, Inc.), was performed to fractionate the tryptic peptides into fractions. First, the peptides were isolated using a gradient of 8 to 32% acetonitrile (ACN, pH 9.0) to separate them into 60 fractions over 1 h. Then, the peptides were merged into 18 fractions and dried by vacuum freeze-drying.

LC-MS/MS analysis. The sample size was 2 μl. The tryptic peptides were dissolved in solvent A (0.1% formic acid in 2% ACN), directly loaded onto a home-made reversed-phase analytical column (15 cm length, 75 μm internal diameter, Thermo Fisher Scientific, Inc.) at 60°C and separated using an EASY-nLC 1000 UPLC system (Thermo Fisher Scientific, Inc.). The liquid gradient setting consisted of an increase from 9 to 25% solvent B (0.1% formic acid in 90% ACN) over 24 min, 25 to 36% over 30 min, and increasing to 36-80% over 32 min, then holding at 80% for the last 36 min. All of the aforementioned settings were maintained at a continuous flow rate of 350 nl/min.

   The peptides were subjected to a positive NSI source, which was followed by tandem MS/MS in Q Exactive™ Plus (Thermo Fisher Scientific, Inc.) coupled online to the ultra-performance LC. The electrospray voltage applied was 2.0 kV. Secondary fragments of the peptides were detected and analyzed using a high-resolution Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, Inc.). The scanning range of the primary mass spectrometry was set to 350-1800 m/z, and the scanning resolution was set to 700,000; the scanning range of the secondary mass spectrometry was set to a fixed starting point of 100 m/z, while the secondary scanning resolution was set to 17,500. The data acquisition mode used a data-dependent scanning program; that is, after the first-level scanning, the first 20 peptide parent ions with the highest signal strength were selected to enter the high-energy C-trap dissociation collision pool in turn to use 31% fragmentation energy for fragmentation, and the second-stage MS analysis was also carried out in turn. To improve the effective utilization of the MS, the automatic gain control was set to 5E4, the signal threshold was set to 10,000 ions/sec, the maximum injection time was set to 200 msec and the dynamic exclusion time for the tandem MS scanning was set to 30 sec to avoid repeated scanning of the parent ions. LC-MS/MS was conducted and analyzed by Jingjie PTM Biolab Co., Ltd.

Database search. Maxquant search engine (v.1.5.2.8, http://www.maxquant.org/) was used to process the MS/MS data results, and the tandem mass spectra were analyzed via the SwissProt Mouse database (version 201808, 16992 sequences, https://www.uniprot.org/) concatenated with the reverse decoy database to calculate the false positive rate (FDR) caused by random matching. In addition, common pollution databases (Jingjie PTM Biolabs, Inc.) were added to eliminate the influence of contaminating proteins in the identification results. Trypsin/P was regarded as the cleavage enzyme, allowing up to 2 missing cleavages, and the minimum length of the peptides was 7 amino acid residues. The mass error tolerance of the primary parent ion of the first search and main search were 20 and 5 ppm, respectively. The mass error tolerance of the secondary fragment ion was 0.02 dalton. The FDR was adjusted to <1% and the minimum score for the peptides was set to >40.

Figure S1. A mouse RIHD model. (A) 3D image of radiation field. (B) Representative dose volume histogram plot of heart, lung and spinal cord. (C) Horizontal section, (D) sagittal section and (E) coronal section of RIHD mouse model. RIHD, radiation-induced heart damage.
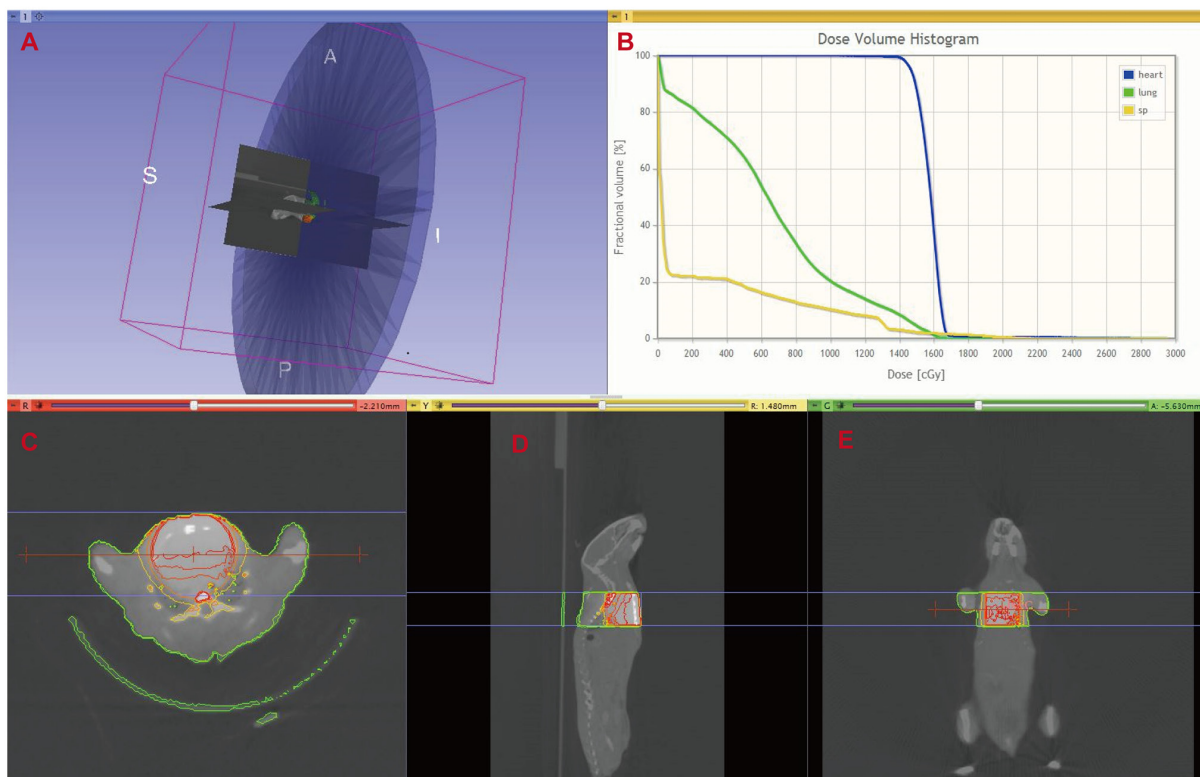
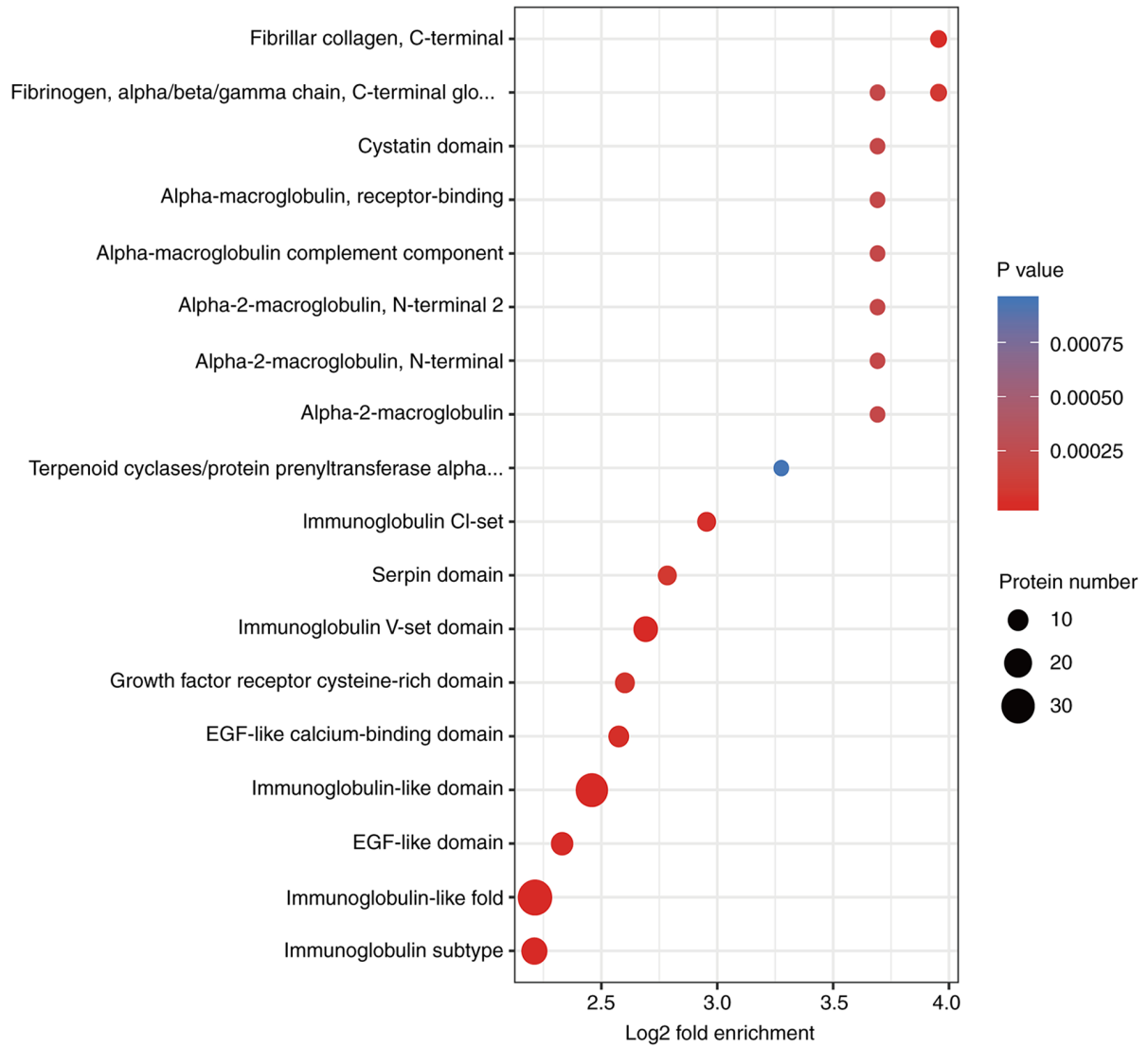Figure S2. Protein domain enrichment bubble plot of the DEPs.

Figure S3. Electron micrographs of the cardiac mitochondria from 3-month group after 16 Gy radiation. Left magnification, x3,000; Middle magnification, x6,000; Right magnification, x20,000.
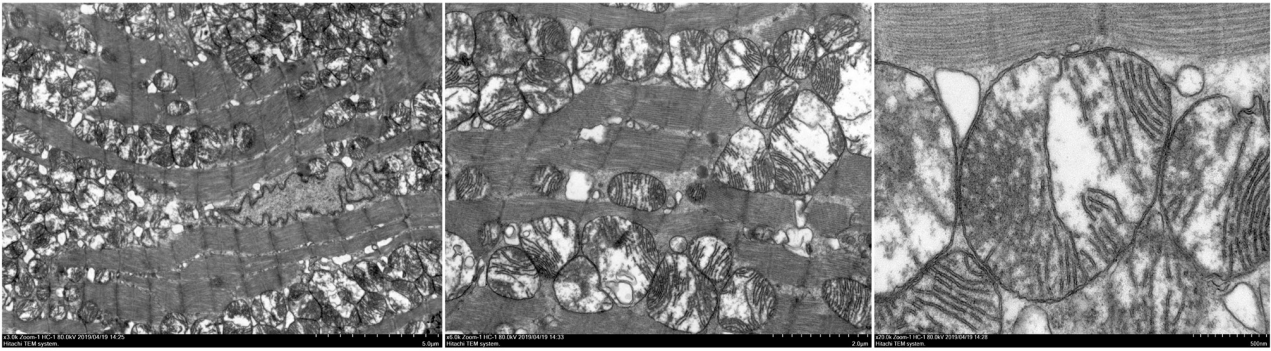
Table SI. Gene Ontology enrichment of differentially expressed proteins in cluster 1.

Ontology	ID	Description	P-value	p.adjust	qvalue
BP	GO:0042730	Fibrinolysis	4.71×10^{-19}	1.72×10^{-16}	8.09×10^{-17}
BP	GO:0051346	Negative regulation of hydrolase activity	6.82×10^{-19}	1.72×10^{-16}	8.09×10^{-17}
BP	GO:0007596	Blood coagulation	6.99×10^{-19}	1.72×10^{-16}	8.09×10^{-17}
BP	GO:0007599	Hemostasis	8.18×10^{-19}	1.72×10^{-16}	8.09×10^{-17}
BP	GO:0050817	Coagulation	8.84×10^{-19}	1.72×10^{-16}	8.09×10^{-17}
CC	GO:0062023	Collagen-containing extracellular matrix	2.10×10^{-23}	1.16×10^{-21}	5.54×10^{-22}
CC	GO:0031012	Extracellular matrix	2.53×10^{-21}	6.95×10^{-20}	3.33×10^{-20}
CC	GO:0034364	High-density lipoprotein particle	9.55×10^{-11}	1.75×10^{-09}	8.38×10^{-10}
CC	GO:0034358	Plasma lipoprotein particle	7.82×10^{-10}	8.60×10^{-09}	4.11×10^{-09}
CC	GO:1990777	Lipoprotein particle	7.82×10^{-10}	8.60×10^{-09}	4.11×10^{-09}
MF	GO:0061134	Peptidase regulator activity	4.23×10^{-20}	4.02×10^{-18}	1.34×10^{-18}
MF	GO:0004866	Endopeptidase inhibitor activity	2.48×10^{-19}	9.53×10^{-18}	3.17×10^{-18}
MF	GO:0061135	Endopeptidase regulator activity	4.16×10^{-19}	9.53×10^{-18}	3.17×10^{-18}
MF	GO:0004857	Enzyme inhibitor activity	4.73×10^{-19}	9.53×10^{-18}	3.17×10^{-18}
MF	GO:0030414	Peptidase inhibitor activity	5.02×10^{-19}	9.53×10^{-18}	3.17×10^{-18}

BP, biological process; CC, cellular component; MF, molecular function.

Table SII. KEGG enrichment of differentially expressed proteins in cluster 1.

Analysis	ID	Description	P-value	p.adjust	qvalue
KEGG	mmu04610	Complement and coagulation cascades	9.40×10^{-26}	3.48×10^{-24}	2.27×10^{-24}
KEGG	mmu04979	Cholesterol metabolism	5.49×10^{-06}	1.02×10^{-04}	6.65×10^{-05}
KEGG	mmu04611	Platelet activation	2.15×10^{-04}	0.002	0.001
KEGG	mmu05150	Staphylococcus aureus infection	2.15×10^{-04}	0.002	0.001
KEGG	mmu04977	Vitamin digestion and absorption	0.002	0.012	0.008

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table SIII. Gene Ontology enrichment of differentially expressed proteins in cluster 2.

Ontology	ID	Description	P-value	p.adjust	qvalue
BP	GO:0030198	Extracellular matrix organization	5.22×10^{-18}	1.75×10^{-15}	8.68×10^{-16}
BP	GO:0030199	Collagen fibril organization	1.19×10^{-17}	2.00×10^{-15}	9.92×10^{-16}
BP	GO:0043062	Extracellular structure organization	2.78×10^{-17}	3.12×10^{-15}	1.54×10^{-15}
BP	GO:0060351	Cartilage development involved in endochondral bone morphogenesis	2.81×10^{-10}	2.36×10^{-08}	1.17×10^{-08}
BP	GO:0061448	Connective tissue development	6.85×10^{-10}	4.24×10^{-08}	2.10×10^{-08}
CC	GO:0062023	Collagen-containing extracellular matrix	1.98×10^{-23}	3.37×10^{-22}	1.25×10^{-22}
CC	GO:0031012	Extracellular matrix	1.05×10^{-21}	8.93×10^{-21}	3.32×10^{-21}
CC	GO:0005581	Collagen trimer	2.61×10^{-21}	1.48×10^{-20}	5.49×10^{-21}
CC	GO:0005583	Fibrillar collagen trimer	8.32×10^{-21}	2.83×10^{-20}	1.05×10^{-20}
CC	GO:0098643	Banded collagen fibril	8.32×10^{-21}	2.83×10^{-20}	1.05×10^{-20}
MF	GO:0005201	Extracellular matrix structural constituent	3.93×10^{-29}	1.02×10^{-27}	3.31×10^{-28}
MF	GO:0030020	Extracellular matrix structural constituent conferring tensile strength	8.88×10^{-22}	1.15×10^{-20}	3.74×10^{-21}
MF	GO:0048407	Platelet-derived growth factor binding	5.99×10^{-17}	5.19×10^{-16}	1.68×10^{-16}
MF	GO:0019838	Growth factor binding	7.75×10^{-10}	5.04×10^{-09}	1.63×10^{-09}
MF	GO:0043394	Proteoglycan binding	2.66×10^{-06}	1.39×10^{-05}	4.49×10^{-06}

BP, biological process; CC, cellular component; MF, molecular function.

Table SIV. KEGG enrichment of differentially expressed proteins in cluster 2.

Ontology	ID	Description	P-value	p.adjust	qvalue
KEGG	mmu04974	Protein digestion and absorption	2.77×10^{-15}	3.87×10^{-14}	5.82×10^{-15}
KEGG	mmu04512	ECM-receptor interaction	1.01×10^{-07}	7.09×10^{-07}	1.07×10^{-07}
KEGG	mmu04510	Focal adhesion	6.18×10^{-06}	2.88×10^{-05}	4.33×10^{-06}
KEGG	mmu05146	Amoebiasis	1.29×10^{-05}	4.53×10^{-05}	6.81×10^{-06}
KEGG	mmu04151	PI3K-Akt signaling pathway	1.02×10^{-04}	2.50×10^{-04}	3.76×10^{-05}

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table SV. Gene Ontology enrichment of differentially expressed proteins in hub genes.

Ontology	ID	Description	P-value	p.adjust	qvalue
BP	GO:0010466	Negative regulation of peptidase activity	2.45×10^{-10}	6.02×10^{-08}	2.45×10^{-08}
BP	GO:0045861	Negative regulation of proteolysis	2.21×10^{-09}	2.72×10^{-07}	1.11×10^{-07}
BP	GO:0051346	Negative regulation of hydrolase activity	5.15×10^{-09}	3.62×10^{-07}	1.47×10^{-07}
BP	GO:0052547	Regulation of peptidase activity	5.88×10^{-09}	3.62×10^{-07}	1.47×10^{-07}
BP	GO:0010951	Negative regulation of endopeptidase activity	6.77×10^{-07}	3.33×10^{-05}	1.35×10^{-05}
CC	GO:0062023	Collagen-containing extracellular matrix	1.06×10^{-05}	1.59×10^{-04}	7.79×10^{-05}
CC	GO:0031012	Extracellular matrix	3.18×10^{-05}	2.38×10^{-04}	1.17×10^{-04}
CC	GO:0030670	Phagocytic vesicle membrane	0.005	0.022	0.011
CC	GO:0005767	Secondary lysosome	0.006	0.022	0.011
CC	GO:0030666	Endocytic vesicle membrane	0.014	0.041	0.020
MF	GO:0004867	Serine-type endopeptidase inhibitor activity	3.57×10^{-12}	1.00×10^{-10}	4.14×10^{-11}
MF	GO:0004866	Endopeptidase inhibitor activity	9.10×10^{-11}	8.79×10^{-10}	3.63×10^{-10}
MF	GO:0061135	Endopeptidase regulator activity	1.15×10^{-10}	8.79×10^{-10}	3.63×10^{-10}
MF	GO:0030414	Peptidase inhibitor activity	1.26×10^{-10}	8.79×10^{-10}	3.63×10^{-10}
MF	GO:0061134	Peptidase regulator activity	2.93×10^{-10}	1.64×10^{-09}	6.79×10^{-10}

BP, biological process; CC, cellular component; MF, molecular function.

Table SVI. KEGG enrichment of differentially expressed proteins in hub genes.

Ontology	ID	Description	BgRatio	P-value	p.adjust	qvalue
KEGG	mmu04610	Complement and coagulation cascades	93/8910	4.37×10^{-06}	1.31×10^{-05}	4.60×10^{-06}
KEGG	mmu04918	Thyroid hormone synthesis	74/8910	0.033	0.049	0.017
KEGG	mmu05150	Staphylococcus aureus infection	124/8910	0.055	0.055	0.019

KEGG, Kyoto Encyclopedia of Genes and Genomes.