



Supplementary Figure S1. Image guided irradiation of the rat heart. (A) X-ray image of rat, anesthetized and placed vertically in a plexiglas holder. (B) Collimator is attached to the X-ray tube and diaphragm is used as a landmark to guide the collimator to the heart. (C) Once the collimator is covering the heart only, the animal will be irradiated. (D) X-ray image of rat turned at 90° from (A). (D & E) The same strategy is used to identify and irradiate the heart.

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Supplementary Figure S2. HPLC analysis of reduced glutathione (GSH) and oxidized glutathione (GSSG) in left ventricles at 2 hours to 9 months after local heart irradiation in adult male Sprague-Dawley rats. A radiation-induced increase in GSSG levels caused a significant decrease in GSH/GSSG ratios from 2 hours up to 10 weeks after irradiation. GSH and GSSG levels were calculated as nmol per mg total protein. Data are presented as average \pm sem, n= 3-6. *p<0.05 compared to time-matched sham-irradiated control.



Supplementary Figure S3. Effects of radiation and TSB on mitochondrial membrane properties. (A) Swelling assays with mitochondria isolated from left ventricle at 2 weeks after irradiation and *ex vivo* exposed to doxorubicin. Swelling is expressed as mitochondrial optical density at 540 nm (OD540nm), relative to OD540nm immediately before the start of the assay. Doxorubicin (DOX, 5 μ M) caused significant swelling in mitochondria isolated from irradiated hearts only. Swelling was inhibited by the mPTP inhibitor cyclosporin A (CsA), indicating that swelling was due to enhanced mPTP opening. (B) Mitochondrial membrane potential, as assessed by mitochondrial uptake of TMRM at 2 weeks and 28 weeks after irradiation. TMRM uptake is expressed as OD590nm relative to that of mitochondria isolated from sham-irradiated hearts. Average ± sem, n=5-6. *p<0.05 compared to sham-irradiated control, #p<0.05 compared to vehicle-pretreated irradiated.



Vehicle (Sham)Vehicle (21 Gy)TSB (21 Gy)Supplementary Figure S4. Effects of radiation and TSB on protein levels of
cleaved caspase 3. (A) Local heart irradiation caused an increase in left ventricular
cleaved caspase 3 levels at both 2 weeks and 28 weeks after irradiation. TSB-
pretreated groups were not significantly different from vehicle-pretreated sham
controls. (B) Representative Western-Blot image of cleaved caspase 3. Average ±

GAPDH

controls. (**B**) Representative Western-Blot image of cleaved caspase 3. Average ± sem, n=5-6. *p<0.05 compared to sham-irradiated control. #p<0.05 compared to vehicle-pretreated irradiated.



Supplementary Figure S5. Representative images of histological staining at 28 weeks after irradiation. (A) Left ventricular collagen deposition as indicated by Sirius Red + Fast Green. (B) Mast cells as indicated by Toluidine Blue with Eosin background staining. Mast cells are stained blue. 20X magnification, scale bar 100 µm.