Table S1. Mouse gender and body mass data. Separate sets of mice were used for experiments related to histology/immunohistochemistry and quantitative PCR. Groups within these categories are presented as months post-total body irradiation (TBI), and individual data for gender and body mass (grams) is given for all age-matched non-irradiated (NI) and TBI mice.

Histology/IHC				**qPCR**				
pTBI(mo)	Gender	NI (g)	TBI (g)	pTBI(mo)	Gender	NI (g)	Gender	TBI (g)
4	M	36.4	28.8	7	M	27.2	M	36.6
4	M	37.0	22.9	7	M	35.7	M	28.5
4	F	26.6	22.9	7	M	34.5	F	28.0
4	F	23.7	25.8	7	M	24.8	F	26.5
6	M	33.3	30.2	7	F	23.0	F	28.1
6	M	38.4	27.2	7	F	29.1	F	25.6
6	F	29.2	28.5	13	M	37.1	M	27.8
6	F	23.6	24.3	13	M	38.1	M	41.6
13	M	47.5	35.9	13	M	55.8	M	33.2
13	M	41.4	33.0	13	F	30.2	F	27.4
13	F	31.2	30.0	13	F	28.4	F	24.8
13	F	30.4	29.6	13	F	41.3	F	26.2
18	M	42.8	29.0	22	M	35.4	M	22.4
18	M	37.9	29.2	22	M	40.0	M	26.5
18	M	45.2	31.6	22	M	34.1	M	28.8
18	M	44.8	32.0	22	M	46.5	M	16.1
18	M	38.4	27.6	22	F	42.6	M	22.6
18	F	26.9	33.3	22	F	38.5	NA	NA
18	F	NA	19.0					

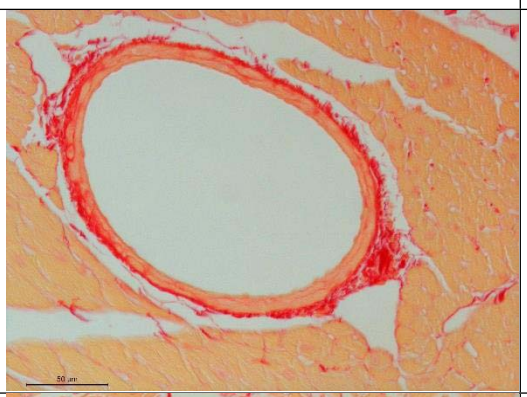
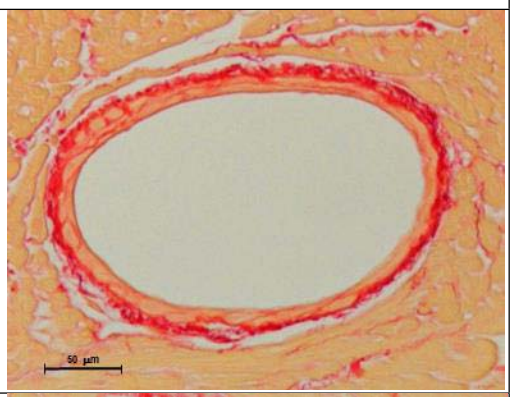
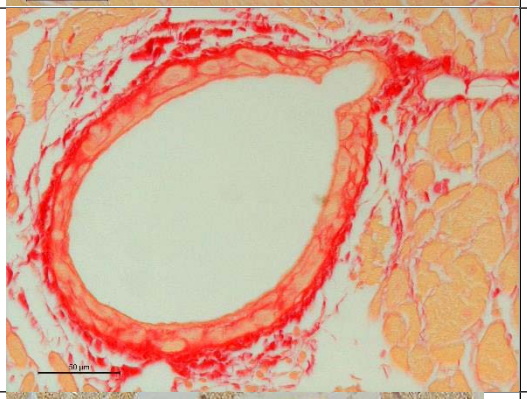
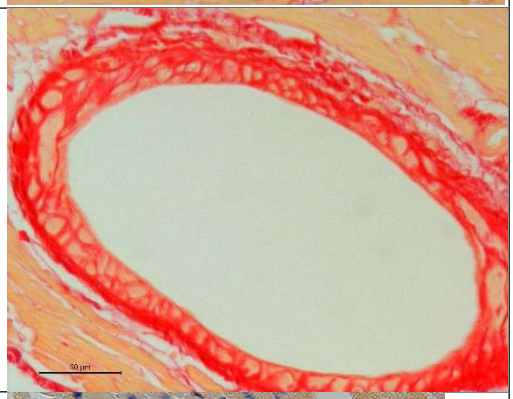
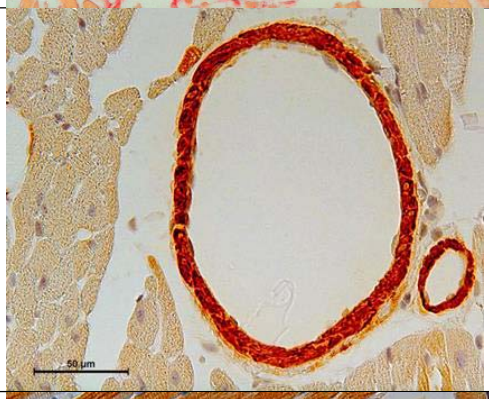
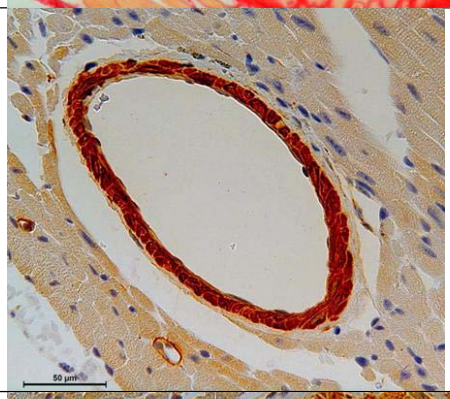
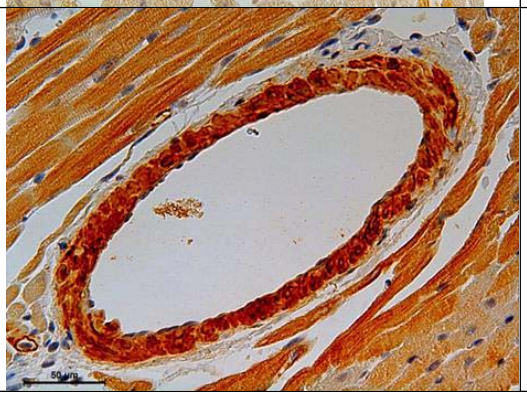
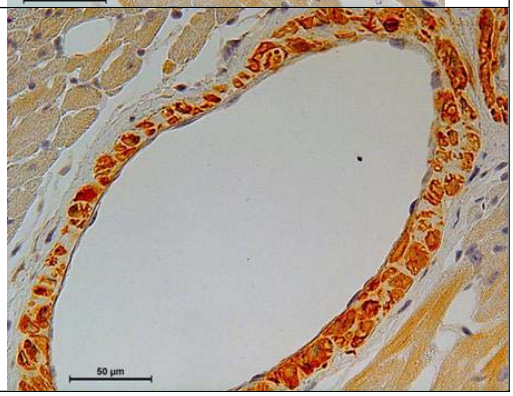
	Non-irradiated Control	Total Body Irradiated
4 mo, 8.72 Gy		
18 mo, 8.53 Gy		
4 mo, 8.53 Gy		
18mo 8.53 Gy		

Figure S1. Representative images of coronary artery cross-sections stained with picrosirius red (top 4 panels) and α-smooth muscle actin (bottom 4 panels) from total body irradiated (TBI) mice at 4 and 18 mo post-TBI and age-matched non-irradiated (NI) controls. The peri-arterial picrosirius red staining increased with time in both NI and TBI mice, whereas medial picrosirius red staining increased only in the irradiated mice. Immunohistochemical staining of coronary artery cross-sections with α-smooth muscle actin demonstrated gaps between adjacent vascular smooth cells in mice at 18 mo post-TBI that were not observed at earlier time points. These results suggest that late effects of TBI include medial expansion due to collagen deposition. All images were acquired at 400x, and the scale bar represents 50 μm.

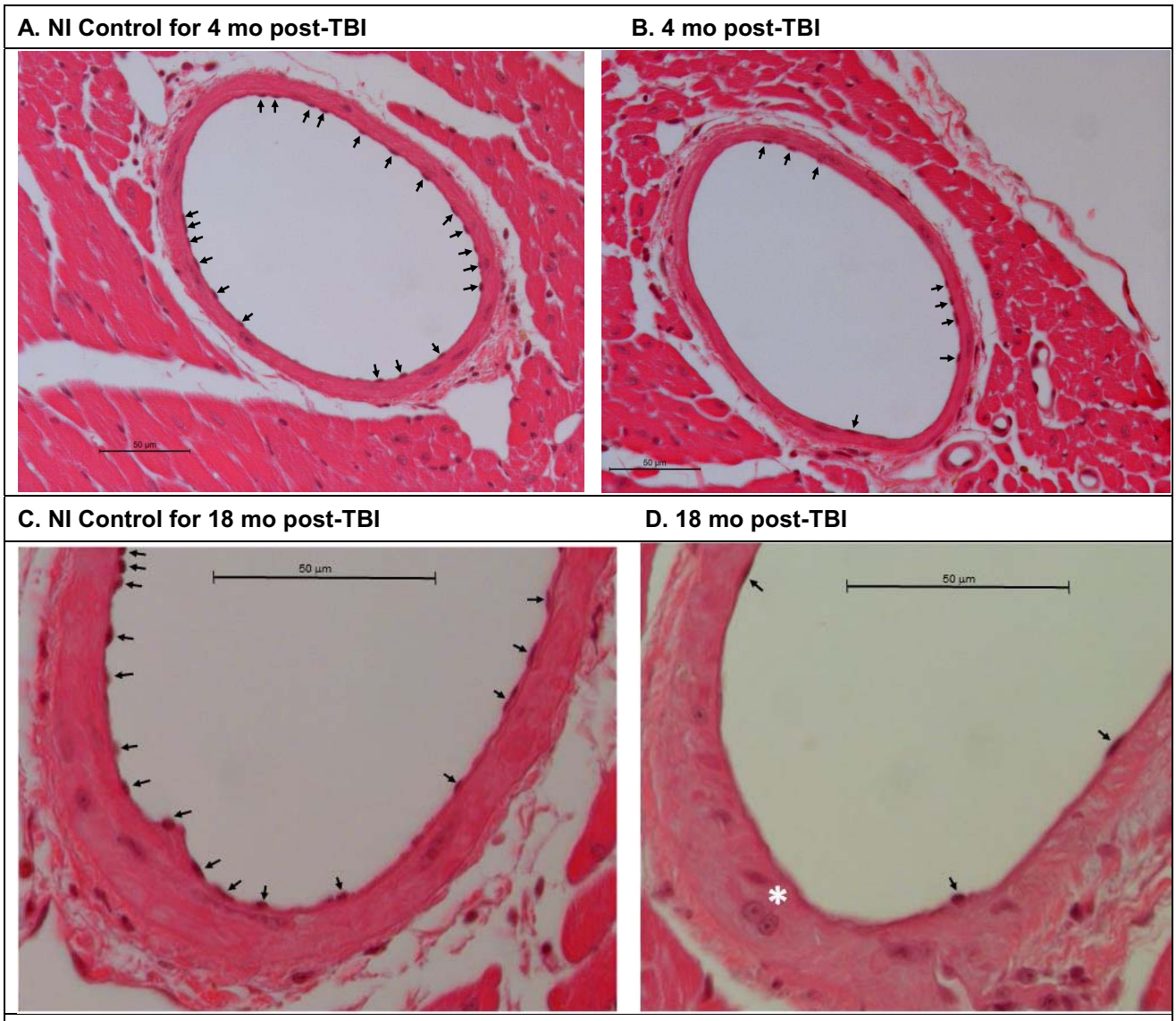


Figure S2. Representative images (400x) of left coronary artery cross-sections stained with H&E from total body irradiated (TBI) mice at 4 and 18 mo post-TBI and age-matched non-irradiated (NI) controls. Arrows indicate the location of intimal nuclei which were reduced in number in the TBI mice at all ages. There was no apparent decrease in the number of medial cell nuclei. The intimal cell nuclei were all consistent with an endothelial phenotype. Some of the medial cells were binucleate (see asterisk), an indication of senescence.

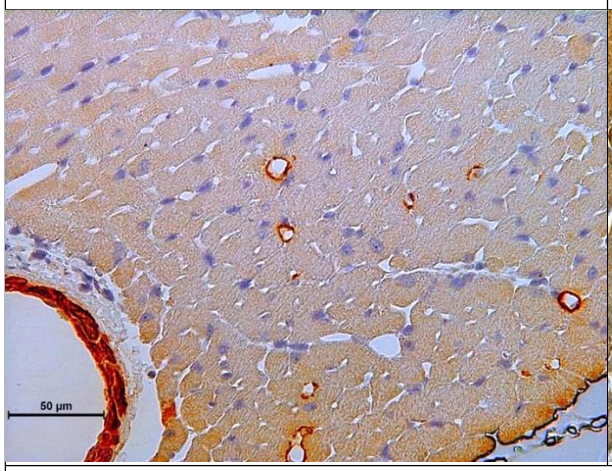
Non-irradiated Control	Total Body Irradiated
	

Figure S3. Representative images (400x) of α-actin positive arterioles in age-matched non-irradiated and total body irradiated (TBI) myocardium at 13 mo post-TBI. Images were acquired from formalin-fixed paraffin embedded sections of heart reacted with an antibody to α-smooth muscle cell actin as described in methods and used to determine arteriolar density. Arteriole numbers per unit area in the left ventricle of TBI mice decreased significantly at 13 mo compared to NI controls, and similar results were obtained at 18 mo post-TBI.

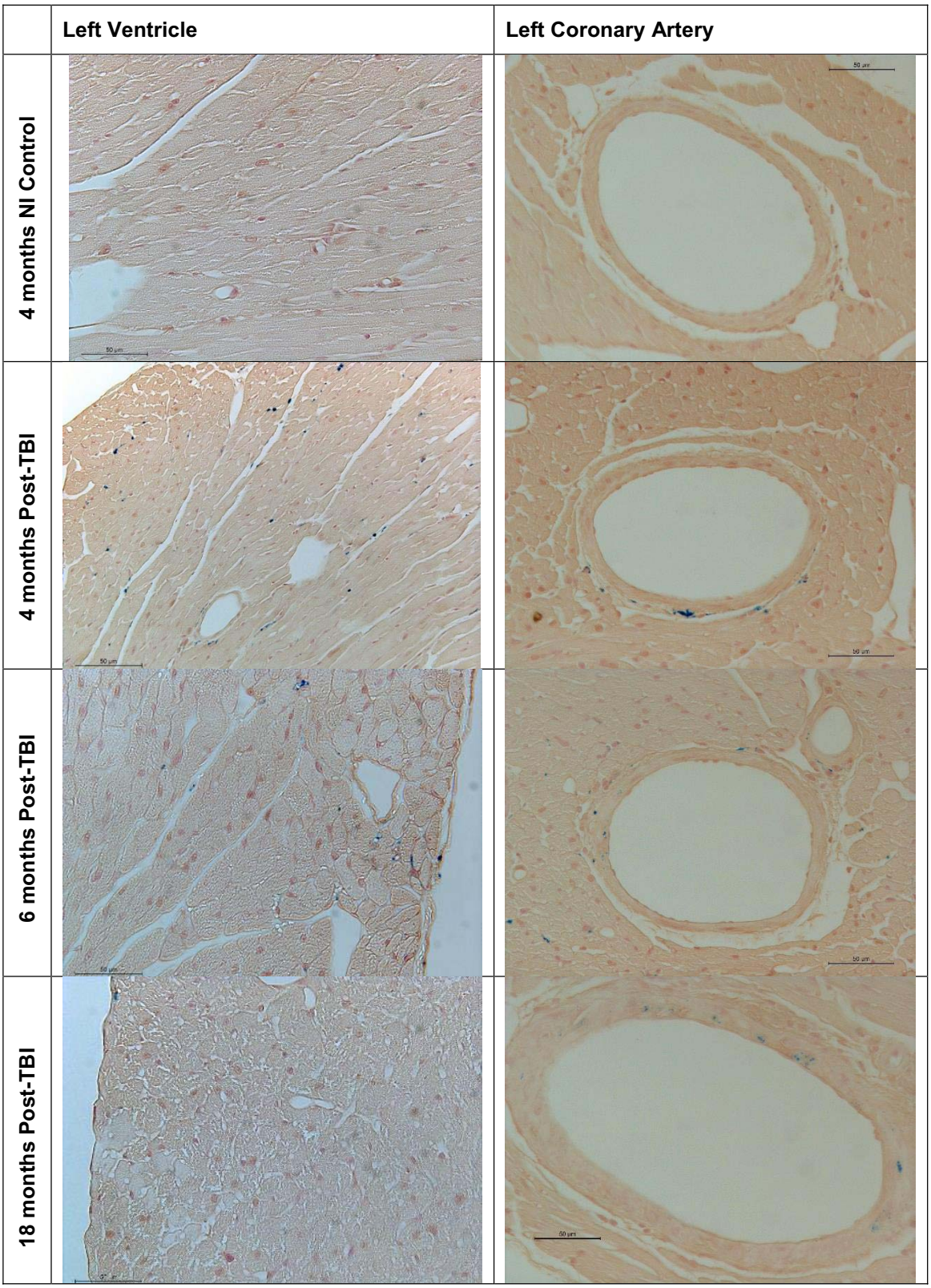

Left Ventricle
Left Coronary Artery
4 months NI Control
4 months Post-TBI
6 months Post-TBI
18 months Post-TBI

Figure S4. Representative images of left ventricle and coronary artery cross-sections stained with Perls' Prussian blue from total body irradiated (TBI) mice at 4 mo post-TBI with age-matched non-irradiated (NI) controls, and additional TBI tissues at 6 and 18 mo. All images were acquired at 400x. Perls' positive regions (hemosiderin deposits) were observed in the myocardium and coronary arteries of TBI mice. Sites of deposition included the myocardium and epicardium and, in arteries, the peri-arterial space, medial layer and intima.

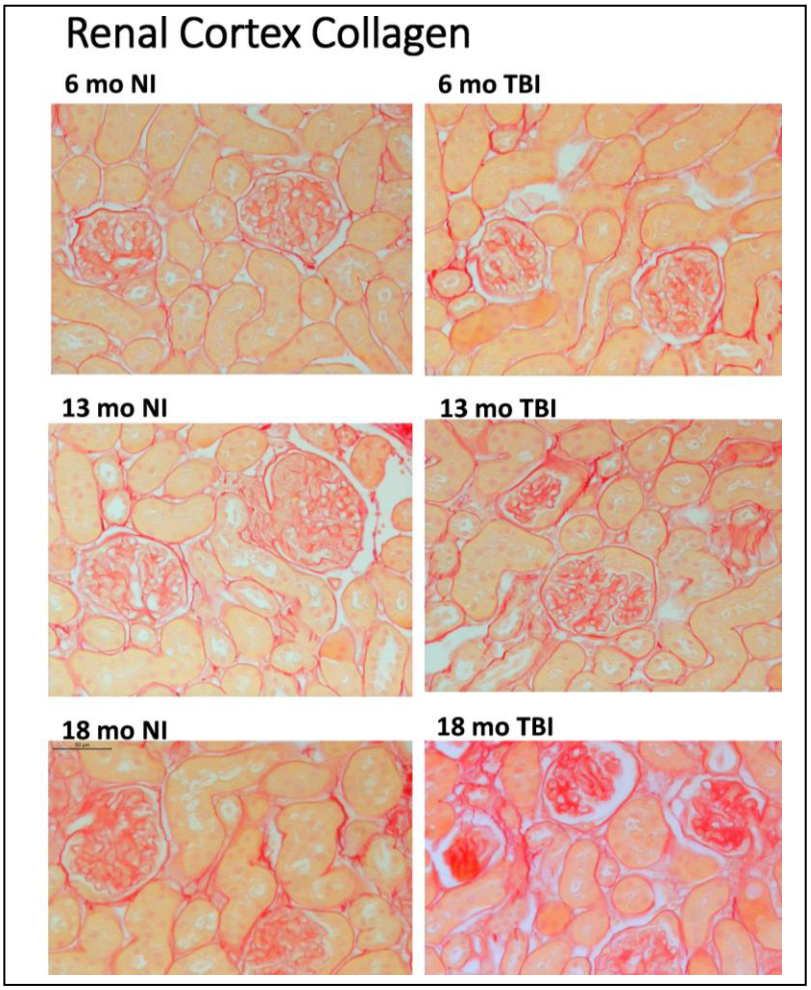


Figure S5. Representative images of the renal cortex stained with picrosirius red in total body irradiated (TBI) mice at 6, 13, and 18 mo post-TBI and age-matched non-irradiated (NI) controls. All images were acquired at 400x; 50 μm scale bar (18 mo NI) applies to all images. Increased picrosirius red staining in the renal cortex was observed first in the glomeruli of TBI mice and later (18 mo) in the interstitium, especially in regions of tubular atrophy.

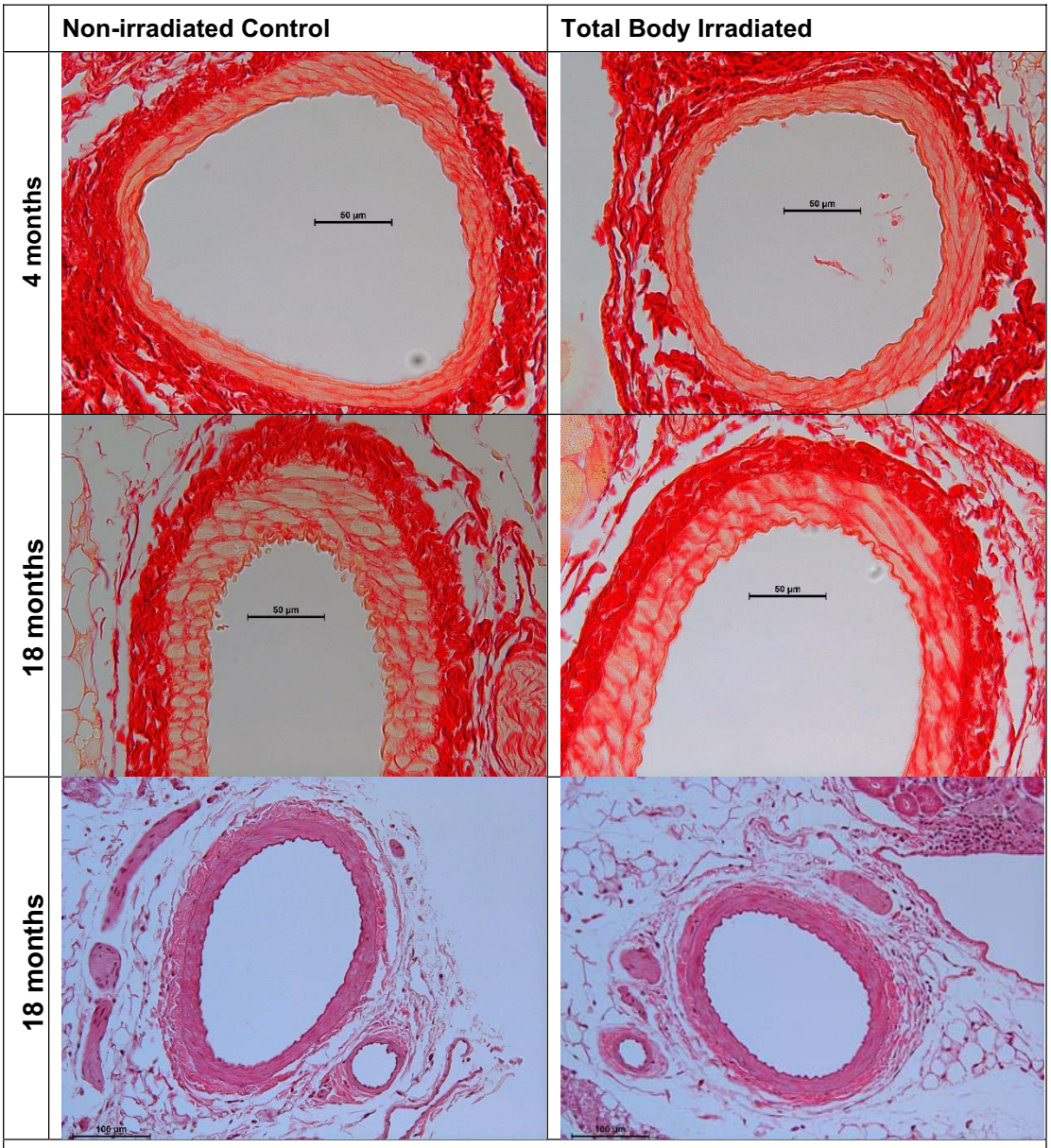


Figure S6. Representative images of picrosirius red (top 4 panels: 400x) and H&E stained cross-sections (bottom 2 panels) of renal arteries from total body irradiated (TBI) and age-matched non-irradiated (NI) control mice. Fewer renal arteries were available for analysis than coronary arteries as not all kidney sections were obtained at the location which contained these arteries. In the picrosirius red stained sections, some renal arteries from TBI mice appeared to have more collagen in the adventitia and media at later times post-TBI; but there was significant variation within and between mice of both the NI and TBI groups. As shown in the H&E stained sections (bottom 2 panels), no striking differences were observed in intimal cell nuclear number ~~as observed in the heart~~ for either large arteries or arterioles, contrary to what was observed in the heart.

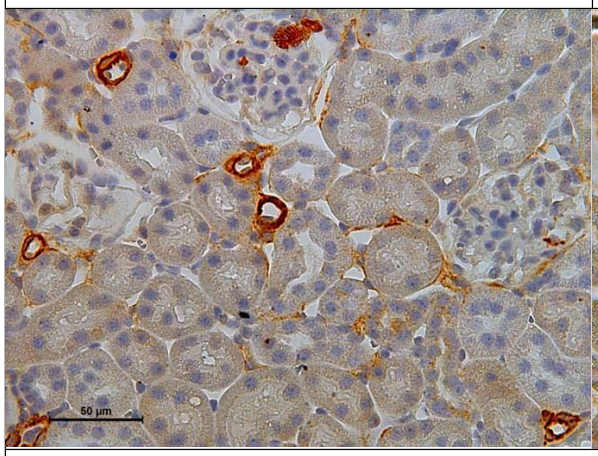
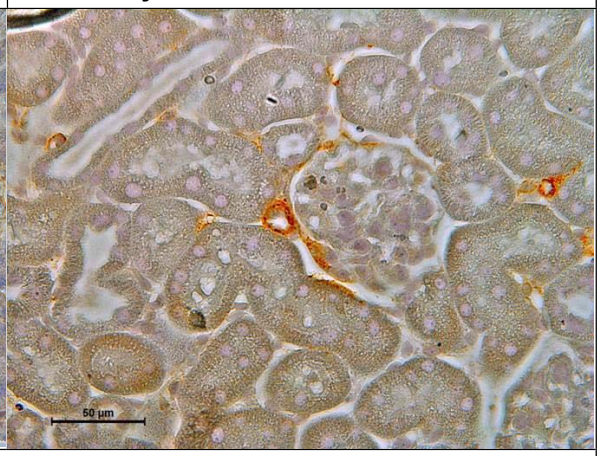
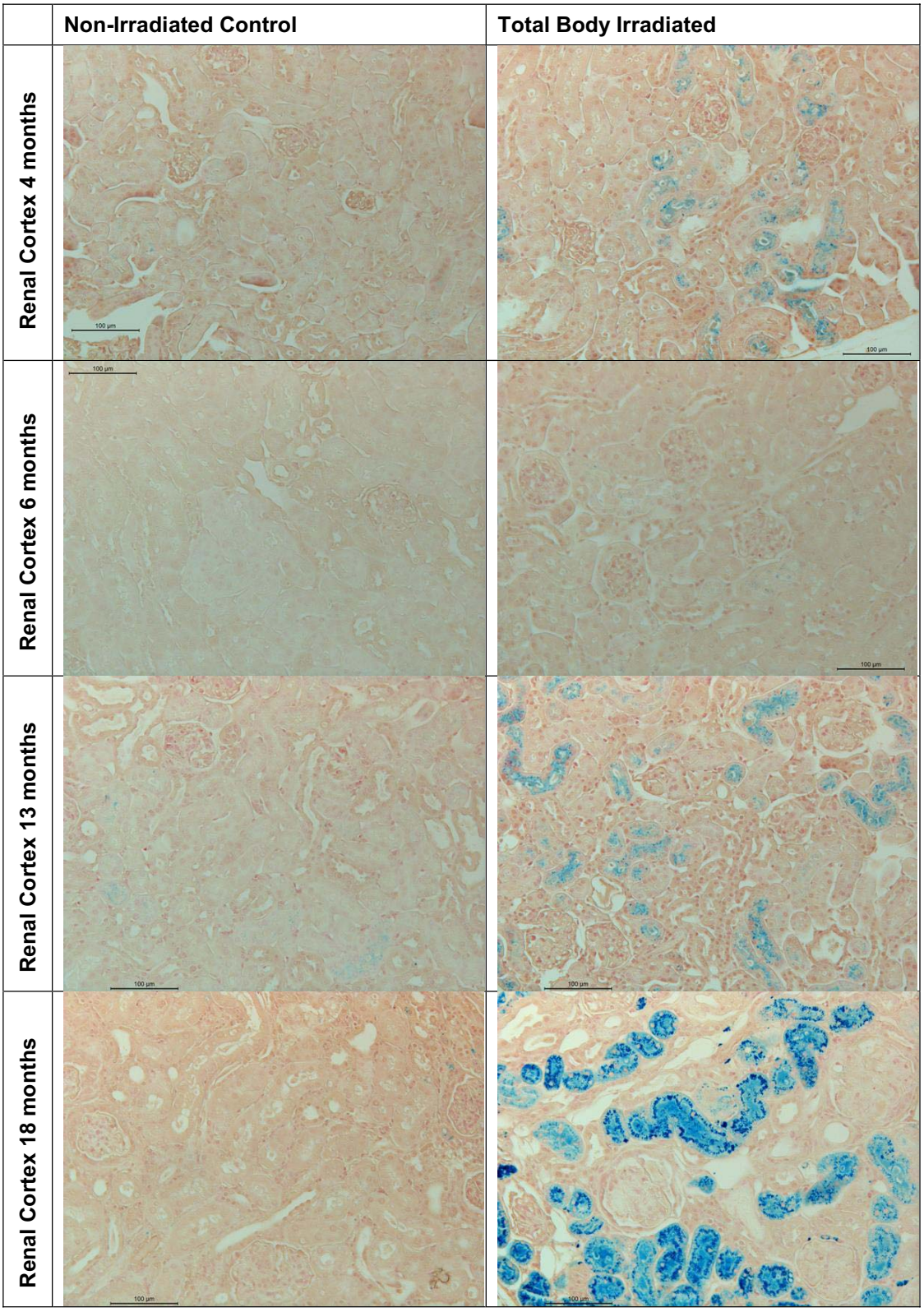
Non-irradiated Control	Total body Irradiated
	

Figure S7. Representative images (400x) of α-actin positive arterioles in age-matched non-irradiated and total body irradiated (TBI) renal cortex at 18 mo. Images were acquired from formalin-fixed paraffin embedded sections of kidney reacted with an antibody to α-smooth muscle cell actin as described in methods and used to determine arteriolar density. A decrease in arteriole number per unit area was significant only at 18 mo post-TBI.


Non-Irradiated Control
Total Body Irradiated
Renal Cortex 4 months
Renal Cortex 6 months
Renal Cortex 13 months
Renal Cortex 18 months
100 µm

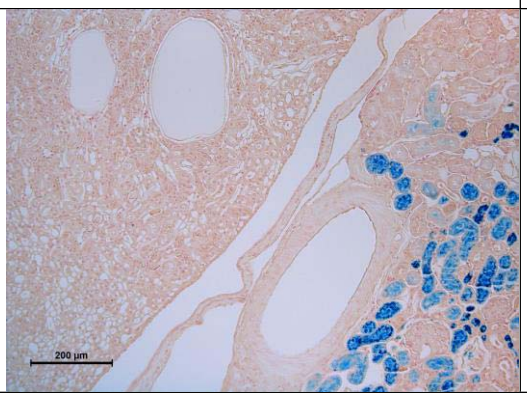
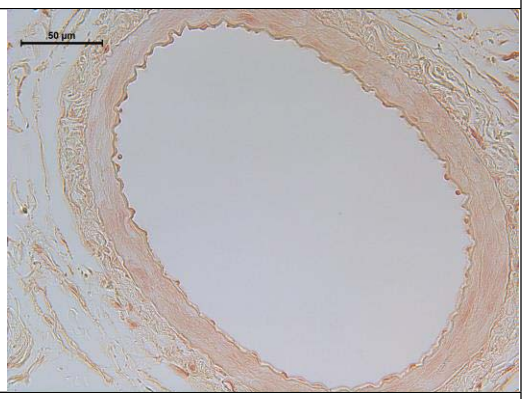
	Total Body Irradiated	Total Body Irradiated
Renal Artery 18 months		

Figure S8. Representative images of kidney cross-sections stained with Perls' Prussian blue from total body irradiated (TBI) mice and their age-matched non-irradiated (NI) controls. Perl's staining was significantly elevated in the renal cortex of most irradiated mice at 4 mo post-TBI, was reduced to near NI values in most TBI mice at 6 mo (3 of 4), and then became elevated in 50% of the mice (5 of 10) at 13-18 mo pTBI. Perls' staining was observed primarily in the tubules of the superficial cortex region, but not all tubules within a region exhibited staining. Staining was also observed in some glomeruli at later time points. Perls' positive staining or deposits of hemosiderin were not observed in the renal arteries, even when the arteries were surrounded by tubules with hemosiderin.

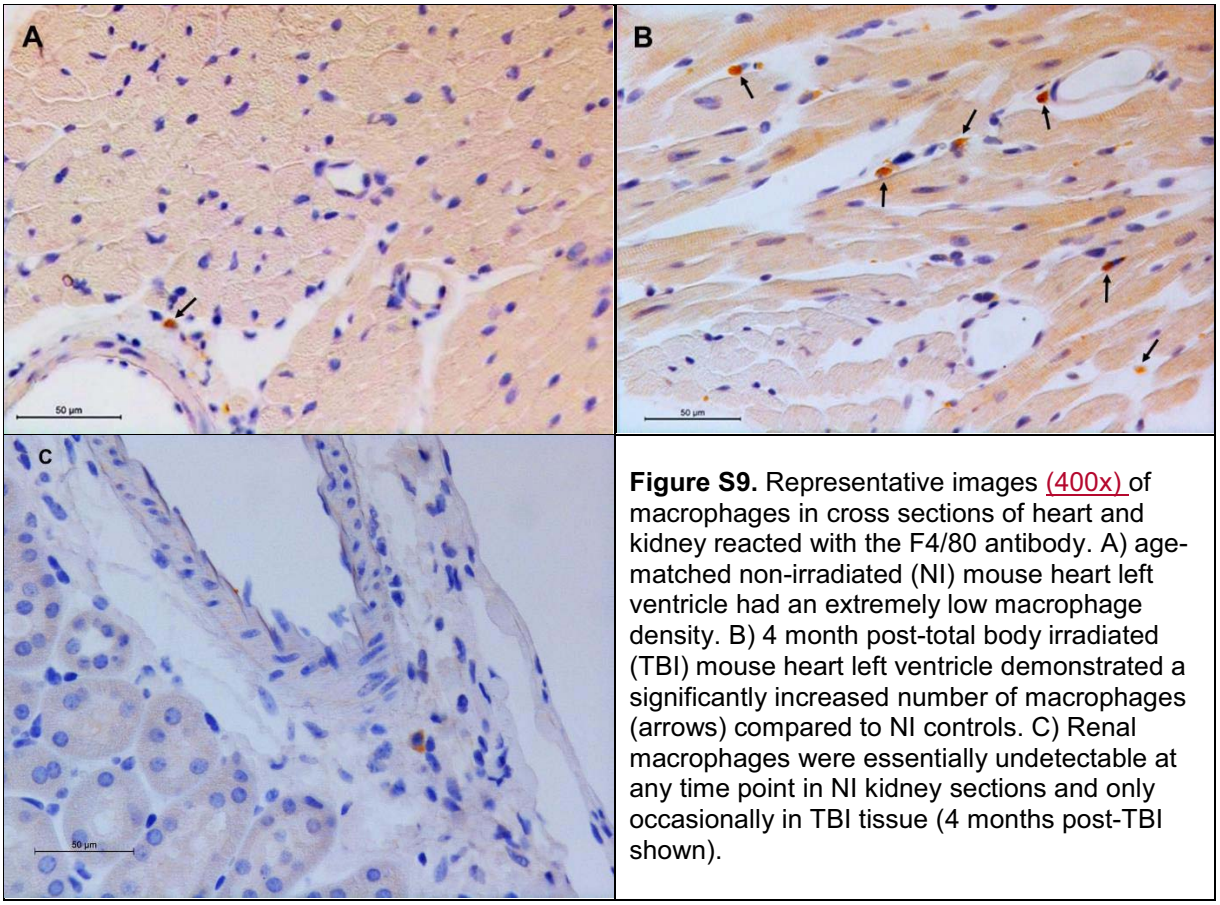


Figure S9. Representative images (400x) of macrophages in cross sections of heart and kidney reacted with the F4/80 antibody. A) age-matched non-irradiated (NI) mouse heart left ventricle had an extremely low macrophage density. B) 4 month post-total body irradiated (TBI) mouse heart left ventricle demonstrated a significantly increased number of macrophages (arrows) compared to NI controls. C) Renal macrophages were essentially undetectable at any time point in NI kidney sections and only occasionally in TBI tissue (4 months post-TBI shown).