SUPPLEMENTAL MATERIAL

Methods

Cardiac volumes and function: Cardiac-gated, steady-state free precession scans were acquired along apical 2 and 4 chamber views with the following parameters: TE/TR =2.0/4.0ms, flip angle = 25°, FOV = 8x6 cm, matrix = 128x96, slice thickness = 2mm, 6 averages, 16 frames. Regions of interests bounded by the left ventricle inner walls were made in commercially-available software (Analyze 7.0, AnalyzeDirect, Overland Park, KS) at end diastole and end systole timepoints. Volumes and diameters were calculated using a modified Simpson's rule in MATLAB (Mathworks, Natick, MA).

Early-systolic and early-diastolic myocardial velocities*:* For a parametric imaging of time-dependent myocardial excursion and generation of contractile and relaxation force, we analyzed radial myocardial dynamic velocities in a subset of rats. For initial methodological optimization, we compared our data analysis algorithm with a study from Weytjens et al which measured mean radial systolic velocities by Doppler Echocardiogram in adult male rats[1]. Quantification of systolic and diastolic radial contraction and relaxation velocities was done using the freely available software Segment version 2.1 R5960 (*http://segment.heiberg.se*; ***Supplemental Figure 1***)[2]. The endocardial borders were first identified using short-axis cine images obtained at the level of mid-papillary muscles. Radial velocity vs. time axis were plotted over 16 time-increments of the cardiac cycle with a LV volume-curve as a reference to define the systole and diastole. Average radial velocity was measured using 4 different myocardial segments (septal, anterior, lateral and inferior), with mid-septum as a reference rotation point. Datasets were exported and quantitative analyses for the radial velocity (cm/s) were done at Early-systole (Es) and Early diastole (Ed), respectively.

Dynamic contrast imaging for myocardial extracellular matrix volume (T1 imaging): Prior studies have validated the relationship between dynamic contrast imaging and the extent of myocardial extracellular matrix, namely collagen[3, 4]. and Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) was employed with cardiac and respiratory-gated, spoiled gradient echo (SPGR) acquisitions were employed for DCE-scans with the following parameters: echo time = 2.2ms, flip angle=90°, matrix =128x128, FOV = 6.0x6.0 cm, 2mm slice thickness, 4 averages, which were adapted from previously reported study protocols[5]. Repetition time (TR) was controlled by cardiac rate, which was maintained at an R-R interval between 170 and 200ms. Single slices were acquired in the short axis view midline across papillary muscles. Following acquisition of three baseline SPGR images, gadolinium (III) diethylenetriaminepentacetate (Gd-DPTA, Magnevist®) was administered via tail vein at a dose of 0.3 mmol/kg body weight and gated-SPGR scans were acquired for up to 25 minutes after injection. A series of NMR tubes containing 1% agarose and increasing concentrations of CuSO4 (0-3mM) were included for signal intensity normalization (**Supplemental Figure 2**). Contrast agent washout rates were calculated as change in normalized signal intensity change per minute over the view of the LV myocardium.

Cardiac fibroblast culture, RNA isolation and Real-time PCR (RT-PCR):

Briefly, heart was extracted after sacrifice and rinsed with PBS solution. Heart was transferred to ice cold PBS in 10 cm petri dish placed on a chill plate, to wash away any residual blood and sliced into 1-mm fragments. These tissue fragments were digested with 0.1% of collagenase B, 2.4 U/ml of Dispase II solution and 2.5 mM of CaCl2 in HBSS buffer for 30 min at 37°C. A detailed cell isolation protocol has been reported previously[6].

Mac-$2^{+/+}$ and $Mac^{-/-}$ mice cardiac fibroblasts were exposed to 9Gy radiation and incubated for 72 hours. Non-radiated cells were used as controls. RNA was isolated with E.Z.N.A.® Total RNA Kit I (Omega Bio-tek) according to manufacturer's instructions. cDNA was generated (Verso cDNA Synthesis Kit) and matching quantities of mRNA were reverse transcribed (SsoAdvanced™ Universal SYBR® Green Supermix, Bio-Rad) to evaluate cycle threshold (CT). Mean CT was calculated from the average of two independent experiments. Expression levels relative to GAPDH were calculated using ▲CT method. Primer information is provided in ***data supplement Table 1***.

Tables

Table 1 (Supplement). Primers used in the quantitative real time-PCR

Gene	Forward Primer 5′to 3′	Reverse Primer 5′to 3′	Length mRNA (bp)
GAPDH	ACCACAGTCCATGCCATCAC	AGCCTTGGCAGCACCAGTG	123
COL1A1	TGCCGTGACCTCAAGATGTG	CACAAGCGTGCTCTAGGTGA	462
COL3A1	GAGGAATGGGTGGCTATCCG	TTGCGTCCATCAAAGCCTCT	316
TGF-β1	ACTCCCGTGGCTTCTAGTG	GGACTGGCGAGCCTTAGTTT	145

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; COL3A1, collagen, type 3α1; COL1A1, collagen, type 1 α1; TGF-β1, transforming growth factor β1

Table 2 (Supplement). Comparison of LV dimensions and function in relation to cardiac ionizing radiation and Ac-SDKP therapy

Parameters	Control	Radiation	Radiation + Ac-SDKP
LVESD (mm)	6.0±0.2	5.7±0.2 (p=0.2 *vs.* con)	5.4±0.4 (p=0.3 *vs.* rad)
LVEDD (mm)	9.2±0.2	8.7±0.2 (p=0.1 *vs.* con)	8.3±0.3 (p=0.3 *vs.* rad)
FS (%)	34±2	34±1 (p=0.9 *vs.* con)	35±5 (p=0.6 *vs.* rad)
EF (%)	65±2	66±4 (p=0.6 *vs.* con)	67±5 (p=0.8 *vs.* rad)
Stroke volume (mm^3)	477±25	456±48 (p=0.7 *vs.* con)	346±46 (p=0.2 *vs.* rad)

LVESD, LV end-systolic diameter; LVEDD, LV end-diastolic diameter; FS, fractional shortening; EF, ejection fraction. Con, control; Rad, radiation. N = 4-6/per group.

Table 3 (Supplement). Effects of radiation and Ac-SDKP on complete blood counts and body weight

Parameters	Baseline	Radiation	Baseline	Radiation + Ac-SDKP
WBC (K/ul)	12±0.6	11±0.6	13±0.6	12±1.7
Neutrophils (%)	9±0.7	12±1.4	8±1.3	12±6
Lymphocytes (%)	86±0.7	83±1.6	89±1.2	83±6
Monocytes (%)	3±0.4	4±0.2*	2 ± 0.2	4±0.8
Hb (g/dL)	15±0.2	16±0.2	16± 0.6	15±0.3
Platelets (K/ul)	896±50	650±49*	782±34	617±92
Body weight (g)	374±10	390±10	372±12	400±14

WBC, white blood cells; Hb, hemoglobin; *, $p < 0.05$ *vs.* baseline. N = 5 per group.

Figures

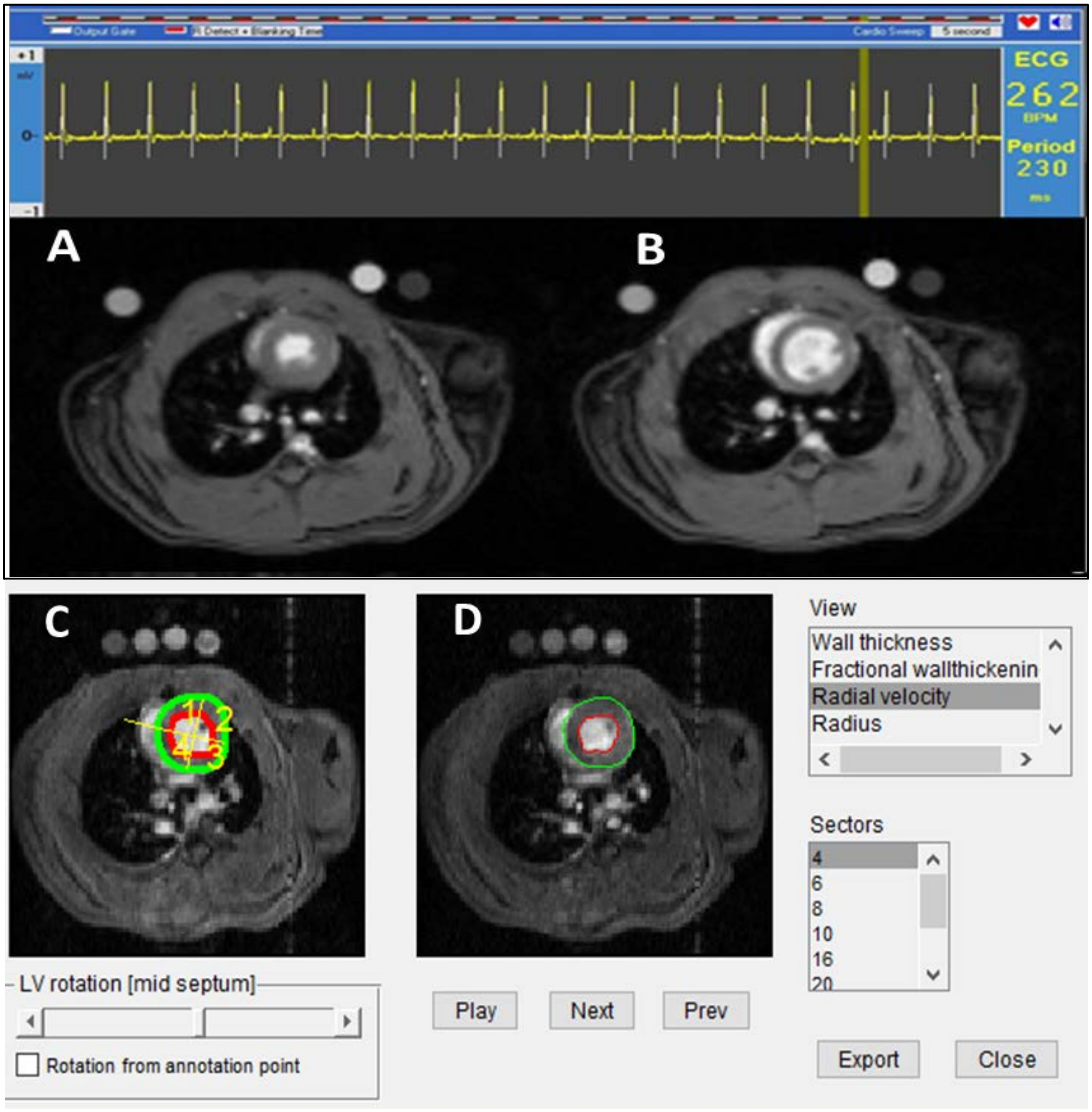

Figure 1 (Supplement). The ECG- and respiratory gated cardiac MRI to evaluate cardiac volumes and velocities. Panel A and B represent end-systolic and end-diastolic frames. The cumulative volumetric analysis at multiple ECG-gated stacked images is used for the calculation of ejection fraction. Panel C and D show the representative short-axis images used for the calculation of early-systolic and early-diastolic myocardial radial velocities. Heart Rate (HR)= 262BPM.

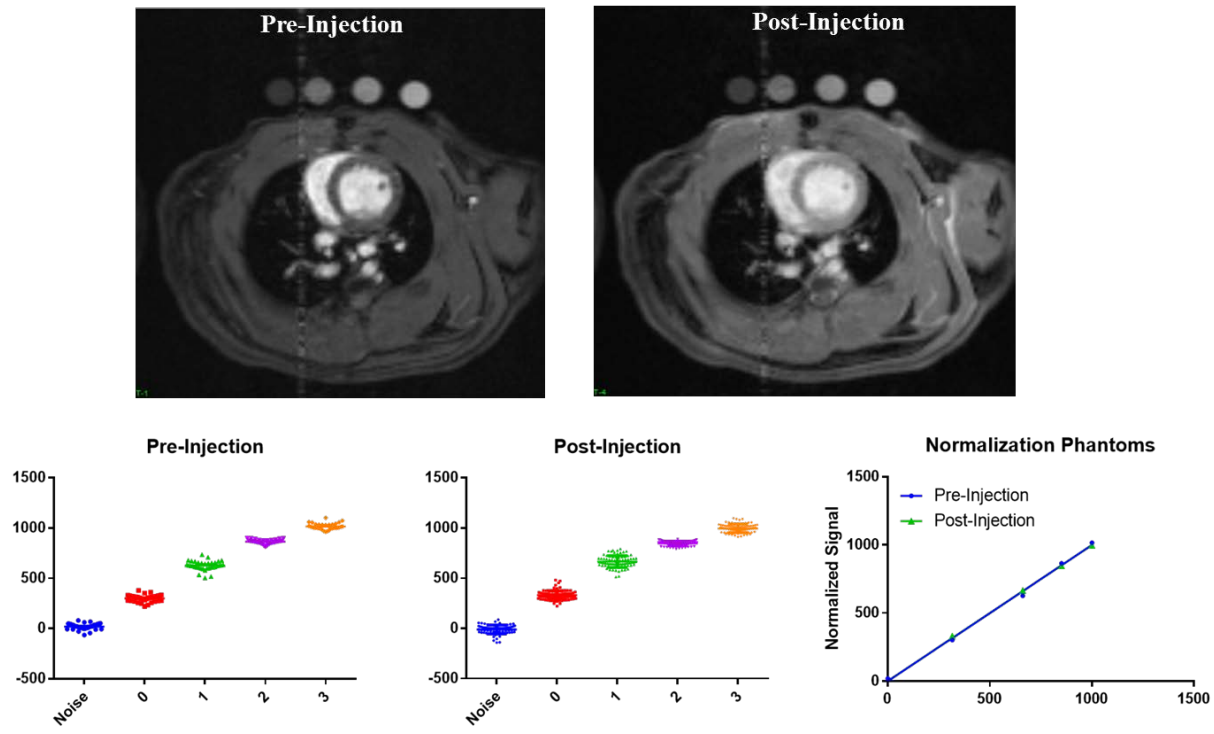

Figure 2 (Supplement). Signal optimization and quality assessment of MRI with normalization phantoms. The normalized pre- and post-gadolinium contrast signal shows strictly linear distribution. This is consistent with satisfactory normalization of background signal intensity and adequate nulling of the non-specific effects that can otherwise lead to MRI data artifacts.

References:

1. Weytjens C, Cosyns B, D'Hooge J, Gallez C, Droogmans S, Lahoute T, Franken P and Van Camp G. Doppler myocardial imaging in adult male rats: reference values and reproducibility of velocity and deformation parameters. *Eur J Echocardiogr*. 2006;7:411-7.

2. Heiberg E, Sjogren J, Ugander M, Carlsson M, Engblom H and Arheden H. Design and validation of Segment--freely available software for cardiovascular image analysis. *BMC Med Imaging*. 2010;10:1.

3. Zeng M, Qiao Y, Wen Z, Liu J, Xiao E, Tan C, Xie Y, An J, Zhang Z, Fan Z and Li D. The Association between Diffuse Myocardial Fibrosis on Cardiac Magnetic Resonance T1 Mapping and Myocardial Dysfunction in Diabetic Rabbits. *Sci Rep*. 2017;7:44937.

4. Messroghli DR, Nordmeyer S, Dietrich T, Dirsch O, Kaschina E, Savvatis K, D Oh-I, Klein C, Berger F and Kuehne T. Assessment of diffuse myocardial fibrosis in rats using small-animal Look-Locker inversion recovery T1 mapping. *Circ Cardiovasc Imaging*. 2011;4:636-40.

5. Schelbert EB, Testa SM, Meier CG, Ceyrolles WJ, Levenson JE, Blair AJ, Kellman P, Jones BL, Ludwig DR, Schwartzman D, Shroff SG and Wong TC. Myocardial extravascular extracellular volume fraction measurement by gadolinium cardiovascular magnetic resonance in humans: slow infusion versus bolus. *J Cardiovasc Magn Reson*. 2011;13:16.

6. Pfister O, Oikonomopoulos A, Sereti KI and Liao R. Isolation of resident cardiac progenitor cells by Hoechst 33342 staining. *Methods Mol Biol*. 2010;660:53-63.