SUPPLEMENTAL MATERIAL

Supplemental methods and results:

Comorbidity definitions: Hypertension and diabetes mellitus were defined per the physician's clinical diagnoses. Coronary artery disease was defined as history of angina, ≥50% angiographic stenosis of one or more coronary arteries, myocardial infarction or coronary revascularization. We assessed creatinine level closest to death and estimated GFR (MDRD) as measurements of chronic kidney disease. Creatinine was available on 80% of subjects (182 of 228): 88% of control (92 of 104) and 73% of HFpEF (90 of 124).

Sensitivity analysis for quantification of microvascular density using no minimum stain area: Total vascular density (97% vessels < 314 um) defined using exclusion of stain areas < 10 um^2 or no exclusion was assessed. When no minimal stain area was used, values for MVD in controls (2214 (2037-2439) vessels/mm^2) and HFpEF (2055 (1756-2353) vessels/mm^2) were higher than when stain areas < 10 um^2 were excluded (controls (1368 (1193-1512) vessels/mm^2) and HFpEF (1035 (849-1231) vessels/mm^2) but group differences (HFpEF vs controls) were similar ($p<0.001$) for both.

Comparison of the autopsy cohort to observational studies: We provide comparison of clinical characteristics of these subjects to those of our previously published Olmsted HFpEF (defined as Framingham validated HF + EF≥ 50%)

community cohort [1] (now updated with additional enrollees, total n = 554) in **Supplemental Table 1.** Briefly, our autopsy cohort was about 4 years younger and had a slightly higher prevalence of clinically diagnosed CAD.

When we restricted the analysis to autopsy patients with EF≥50%, the autopsy cohort was still slightly younger but there were no other differences.

Supplemental Table 1: Comparison of clinical characteristics of autopsy HFpEF to community HFpEF

	Community HFpEF	**Autopsy HFpEF (EF≥40%)**	**Autopsy HFpEF (EF≥50%)**
N	554	124	97
Sex, men	238 (43%)	55 (44%)	38 (39%)
Age, years	79 (72-86)	75 (66-83)*	75 (64-84)*
Hypertension	479 (85%)	98 (79%)	77 (79%)
Diabetes mellitus	196(35%)	52 (42%)	43 (44%)
Coronary artery disease	311 (55%)	81 (65%)*	59 (61%)
Ejection fraction, %	61 (56-65)	56 (50-62)†	60 (55-65)
Body surface area, m^2	1.93 (1.73-2.16)	1.94 (1.72-2.12)	1.94 (1.74-2.10)
Body mass index, kg/m^2	28.6 (24.7-34.5)	28.0 (23.7-34.5)	28.3 (24.4-34.9)

*P<0.05 vs community HFpEF: †NA for EF comparison due to differences in study inclusion criteria

Cause of death in controls and HFpEF: The immediate cause of death as defined by the autopsy report differed by group with more HFpEF patients dying of HF and other cardiovascular causes than in controls.

Supplemental Table 2: Cause of death (immediate) in control and HFpEF

	Control	HFpEF
N	104	124
HF death	0 (0%)	28 (22.6%)
Non HF cardiovascular death	13 (12.5%)*	42 (33.9%)
Non cardiovascular death	91 (87.5%)	54 (43.5%)

* all vascular or pulmonary embolism

Correlation of echocardiographic data and MVD and fibrosis: Echocardiographic parameters were not consistently available on all subjects, particularly LA volume and E/e' ratio which were introduced only in later years of the study period. Several more consistently available parameters such as E/A ratio and deceleration time do not always bear a linear relationship with filling pressures/diastolic dysfunction due to the pseudonormalization phase of the diastolic dysfunction progression. Recognizing the limitations noted, we obtained all available echocardiographic data and utilized the last echo data available (closest to death) for each variable.

Supplemental Table 3: Echocardiographic characteristics in HFpEF patients

	Median (25^{th}-75^{th})	N with data (MVD)	r	P for correlation with MVD	N with data (fibrosis)	r	P for correlation with fibrosis
LV dimension, mm	50 (46-54)	115 (93%)	-0.16	0.08	117 (94%)	0.02	0.83
LV mass, gram	219 (181-268)	78 (63%)	-0.29	0.01	71 (57%)	0.04	0.77
Mitral E/e'	24 (16-38)	30 (24%)	-0.42	0.02	29 (23%)	0.35	0.06

Supplemental Table 4: Clinical and autopsy characteristics in HFpEF and HFrEF

	HFpEF (EF ≥ 40%)	HFrEF (EF<40%)	P value
N	124	27	
Clinical Characteristics			
Sex, men	44%	16 (56%)	0.39
Age at death, years	78 (66-83)	81 (73-86)	0.28
Age at HF event, years	75 (66-83)	79 (68-85)	0.23
Hypertension	79%	67%	0.21
Diabetes mellitus	42%	30%	0.28
Coronary artery disease diagnosis	65%	74%	0.50
Ejection fraction, %	56 (50-62)	26 (25-30)	NA
Autopsy - Gross pathology			
Body surface area, m^2	1.9 (1.7-2.1)	1.7 (1.5-2.2)	0.36
Body mass index, kg/m^2	28.0 (23.7-34.5)	24.3 (17.8-35.3)	0.20
Heart weight, gram	538 (440-659)	510 (420-722)	0.96
% expected heart weight	169 (144-202)	178 (149-219)	0.44
Left ventricular hypertrophy	74%	85%	0.32
Right ventricular hypertrophy	50%	43%	0.64
Left ventricular dilation	37%	65%	0.01
Right ventricular dilation	48%	55%	0.63
Atrial dilation	52%	76%	0.06
Infarct (old)	42%	57%	0.25
infarct (acute)	11%	10%	1.00
Fibrosis	25%	50%	0.03
Autopsy - Microscopic pathology			

Hypertrophy	31%	41%	0.42
Infarct	20%	47%	0.03
Fibrosis	58%	59%	1.00
CAD summary score	12 (8-14)	13 (7-15)	0.65

Abbreviations: HF, heart failure; CAD, coronary artery disease

Comparisons are by Wilcoxon Kruskal-Wallis test for continuous variables and Fischer's exact test for discrete variables**.**

Supplemental Figure legends

Figure 1. Quantification of collagen volume fraction using whole field digital microscopy.

Figure 2. Quantification of microvascular density using whole field digital microscopy and automated analysis. In A, the CD31 stained tissue is shown. In B, the automated vessel detection is shown. In C, the vessel size classification is shown. Vessels with an area between 10 μm^2 and 78.5 μm^2 are considered capillaries (yellow). Vessels with an area between 78.5 um^2 and 314 μm^2 are defined as small pre-capillary arterioles (orange). Vessels with an area greater than 314 μm^2 are defined as larger vessels (not microvessels) and are shown as red.

Figure 3. Regions of interest for analysis of MVD by region. The full-thickness LV sections were divided into the sub-endocardial, mid-mural, sub-epicardial and papillary regions.

Figure 4. Impact of tissue planes on MVD assessment. This figure shows processed CD 31 sections where our vessel size definitions are used to identify capillaries (yellow), pre-capillary arterioles (orange) and larger intra-myocardial arteries (red). Top panel, circumferentially oriented myofibers (mid-mural area) are shown and the longitudinally oriented vessels are coded as larger vessels (orange and red) with fewer cross sectional cut microvessels being coded as capillaries (yellow). Bottom panel, a section from the longitudinally oriented myofibers (as would be present in the sub-endocardial

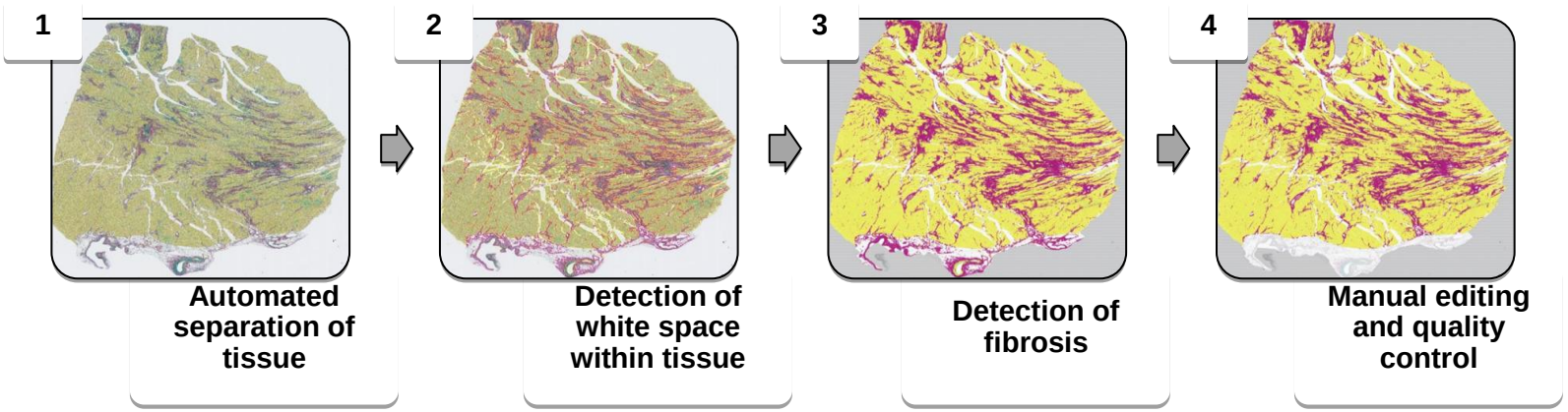
and sub-epicardial regions) have more microvessels cut in cross section and most are coded as capillaries (yellow).

Figure 5. MVD and Fibrosis in HFpEF and Control subjects without or with hypertension. Microvascular density (A) and percent fibrosis (B) are compared among controls without (n=71) and with (n=32) a history of hypertension and among HFpEF patients without (n=25) and with (n=97) a history of hypertension. Data are displayed as Tukey box plots (box: median, 75^{th}, and 25^{th} percentiles; whiskers: highest value within 75^{th} percentile plus 1.5*IQR and lowest value within the 25^{th} percentile minus 1.5*IQR, symbols show outliers if present). Statistical comparisons by Wilcoxon test for multiple comparisons.

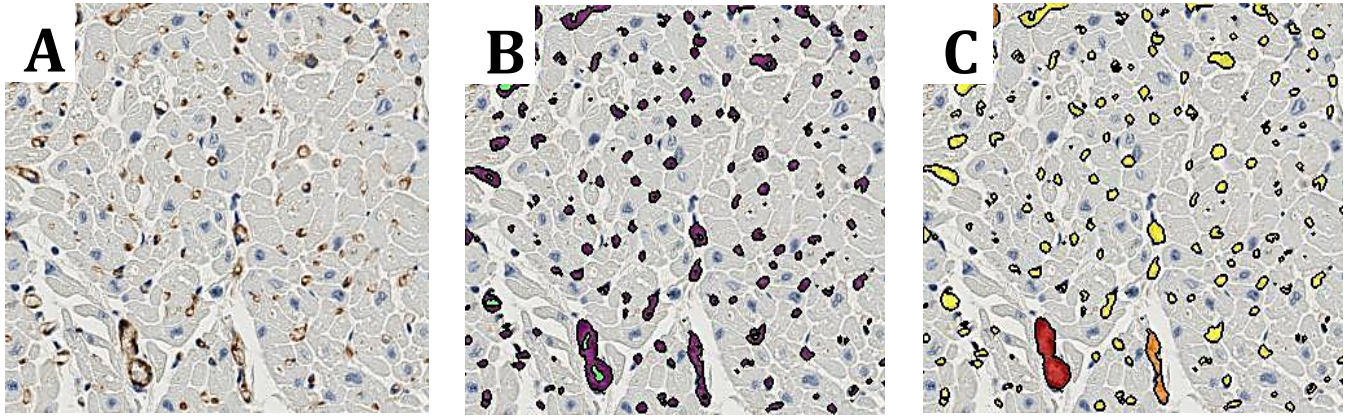
Figure 6. Association between microvessel density and echocardiographic LV hypertrophy (LV mass) and diastolic function (E/e') in HFpEF.

Figure 7. Association between microvessel density and LV fibrosis and QRS duration and QTc interval in HFpEF.

Supplemental Figure 1

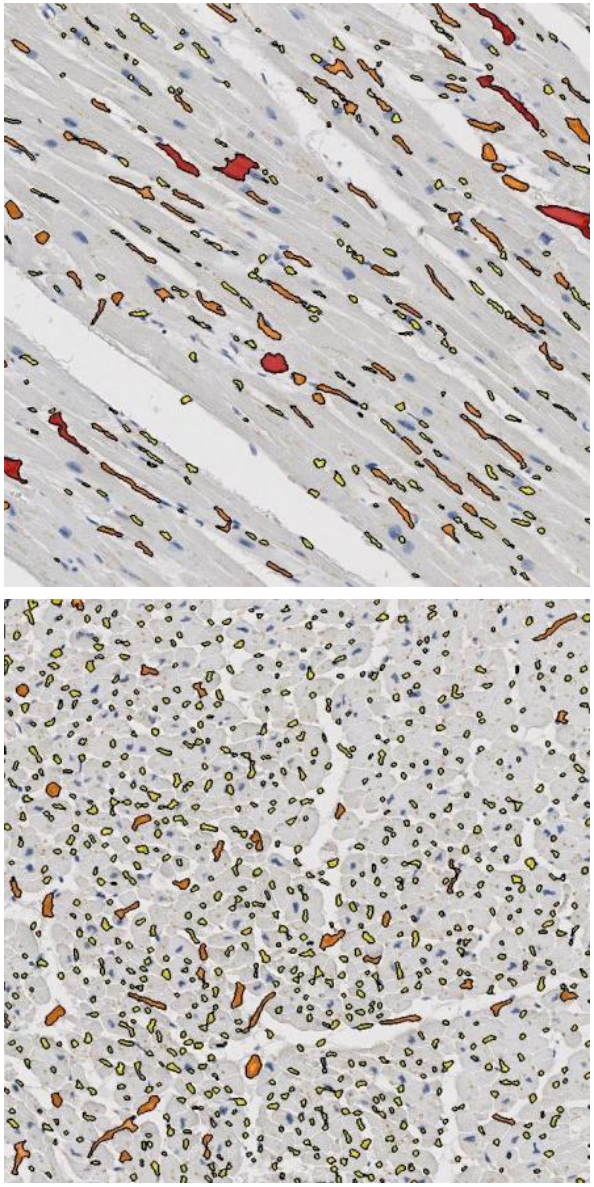


Supplemental Figure 2

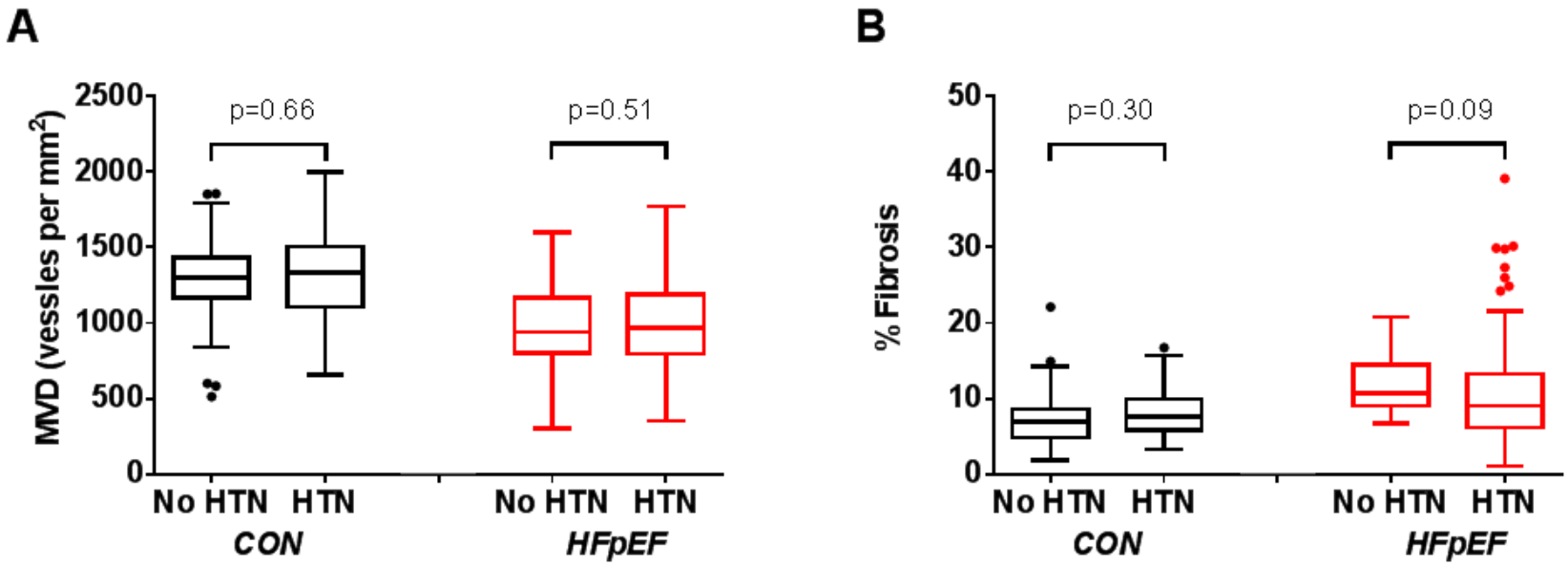


Supplemental Figure 3

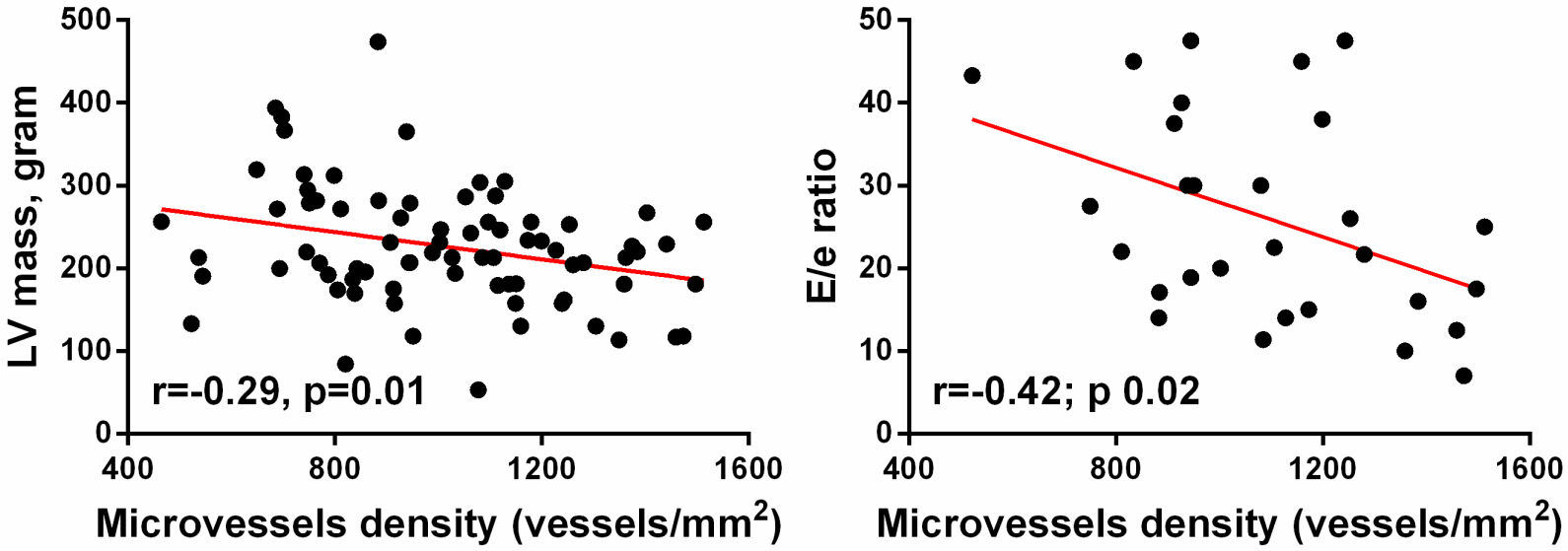
Supplemental Figure 4



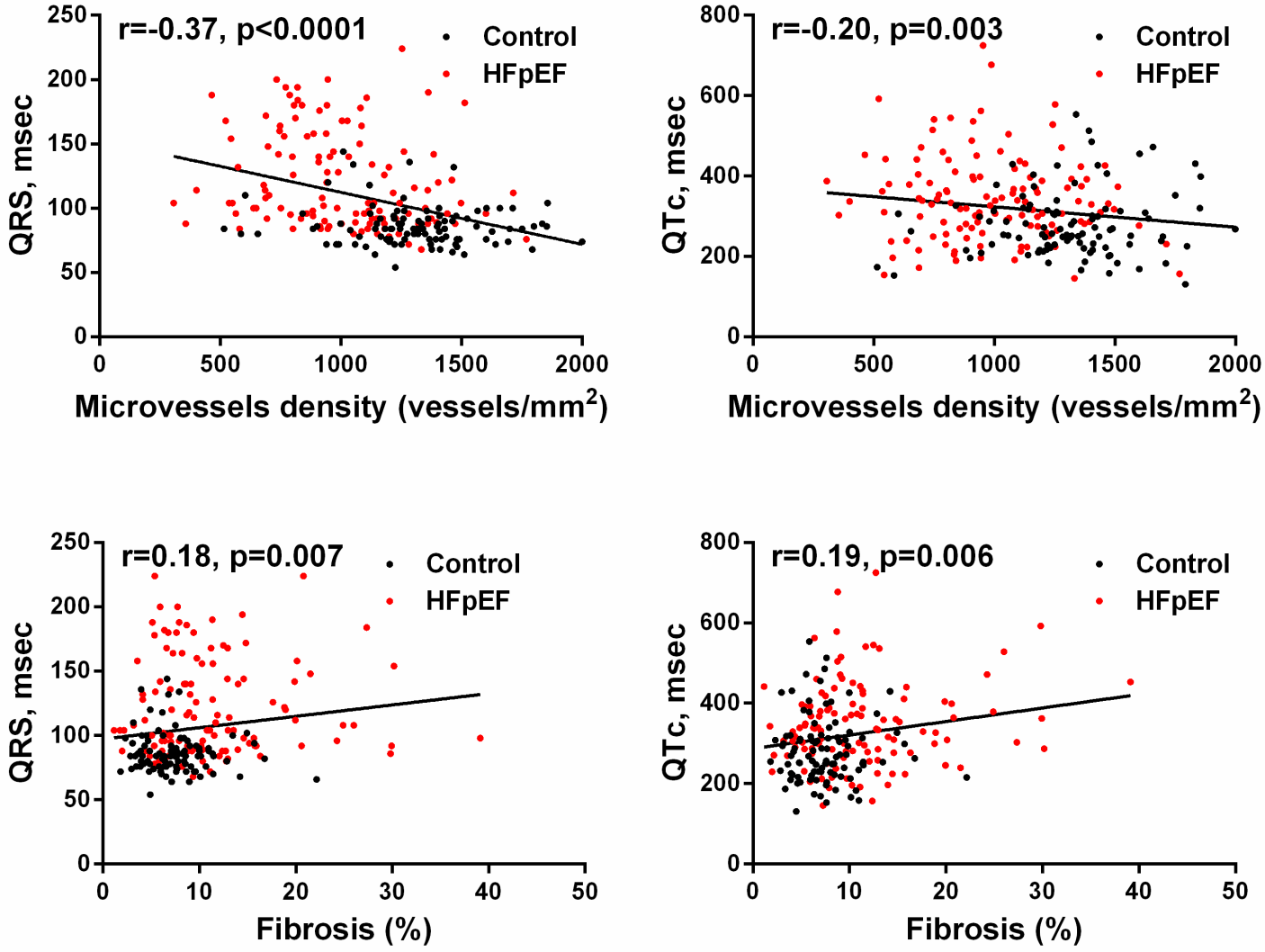
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental materials references

1. Mohammed SF, Borlaug BA, Roger VL, Mirzoyev SA, Rodeheffer RJ, Chirinos JA, Redfield MM. Comorbidity and ventricular and vascular structure and function in heart failure with preserved ejection fraction: A community based study. *Circ Heart Fail*. 2012