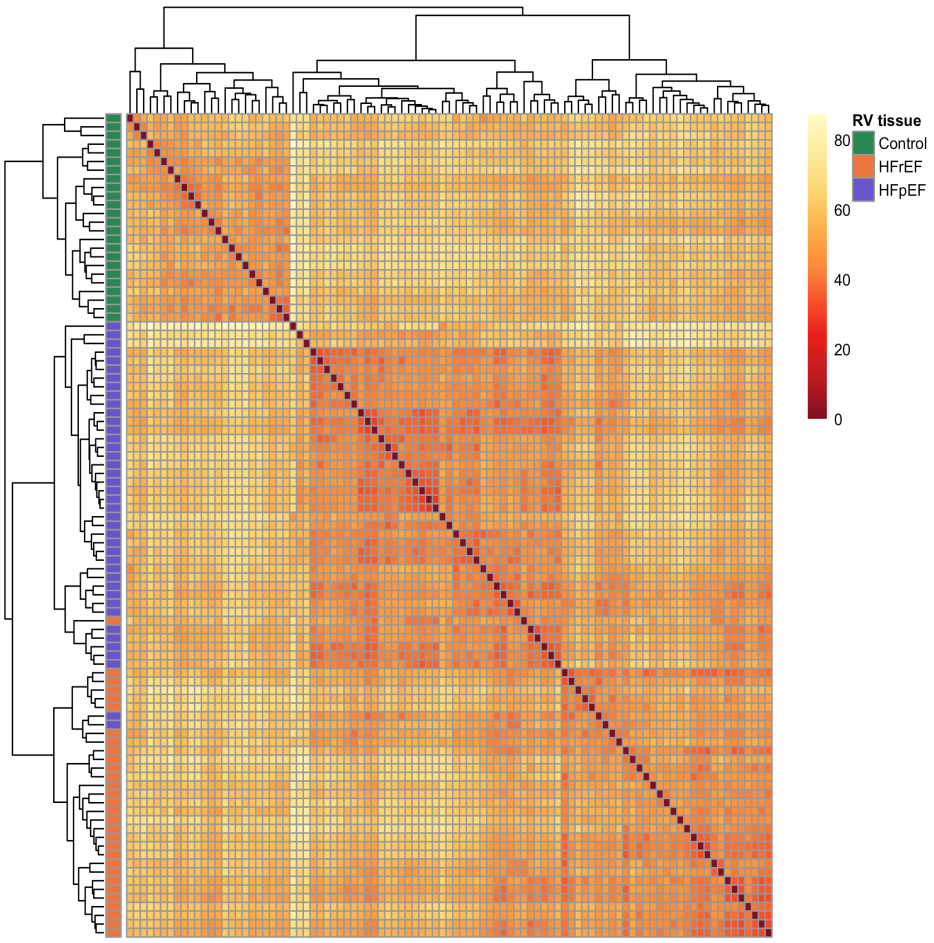
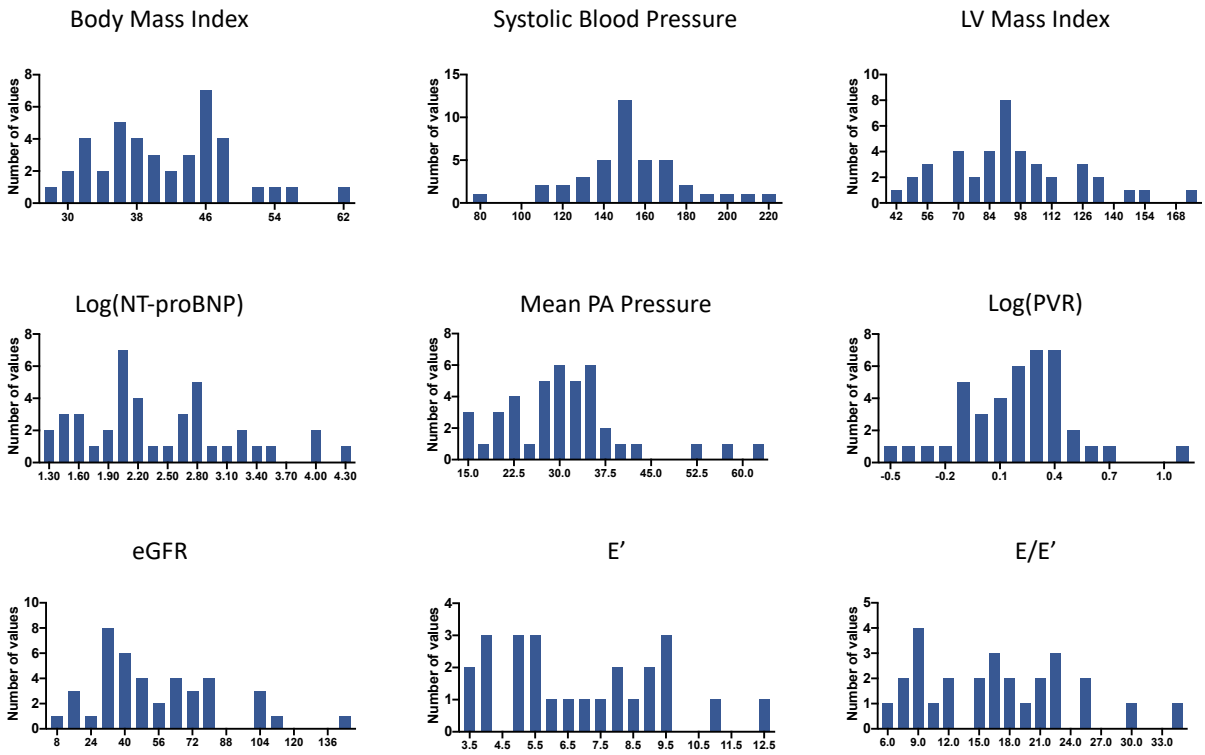
SUPPLEMENTAL MATERIAL

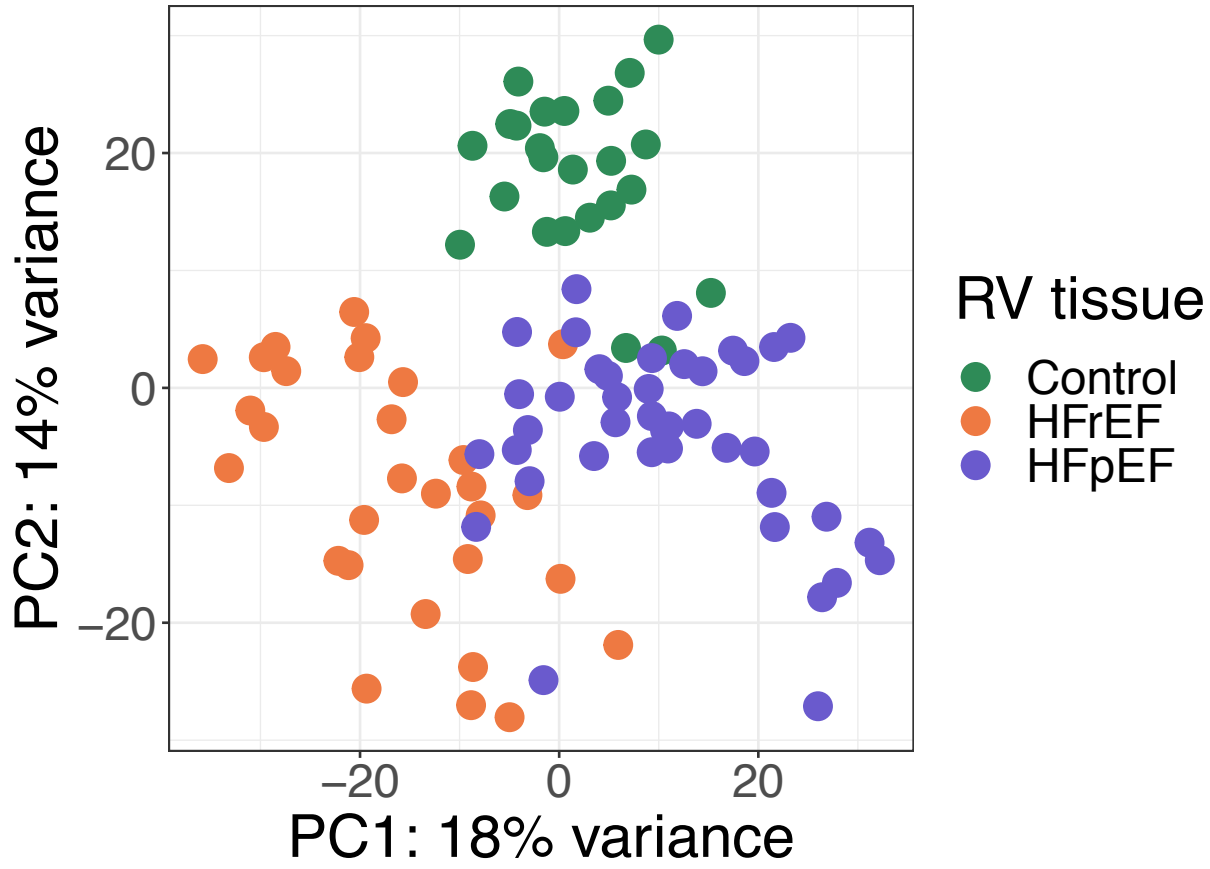
Hahn, Myocardial Gene Expression in HFpEF



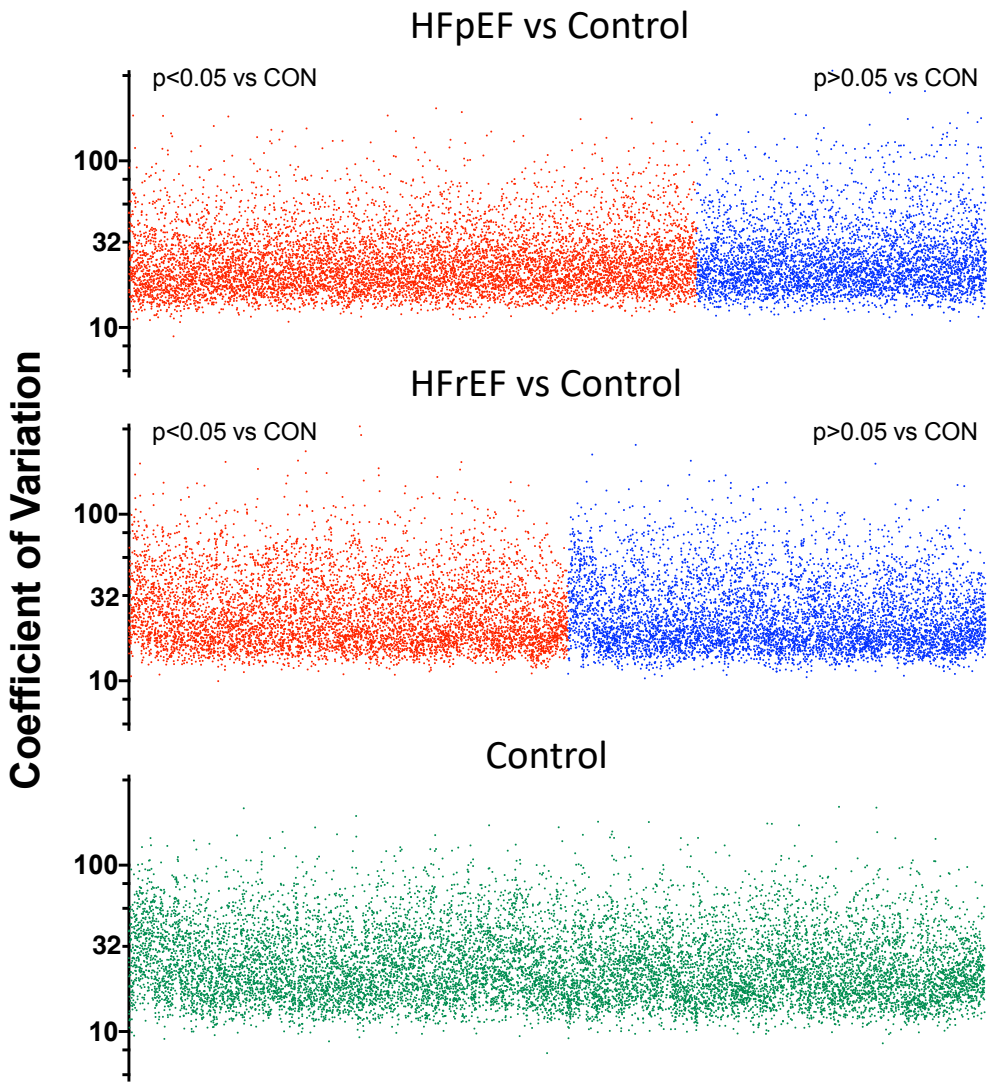
Supplemental Figure I. Correlation plot of all RVs samples demonstrates sample-sample correlation. The first three HFpEF samples (7709, 7546, 7715) do not correlate as strongly with the HFpEF group nor with each other, while 2 of the HFpEF samples cluster with HFrEF (7559, 7718).



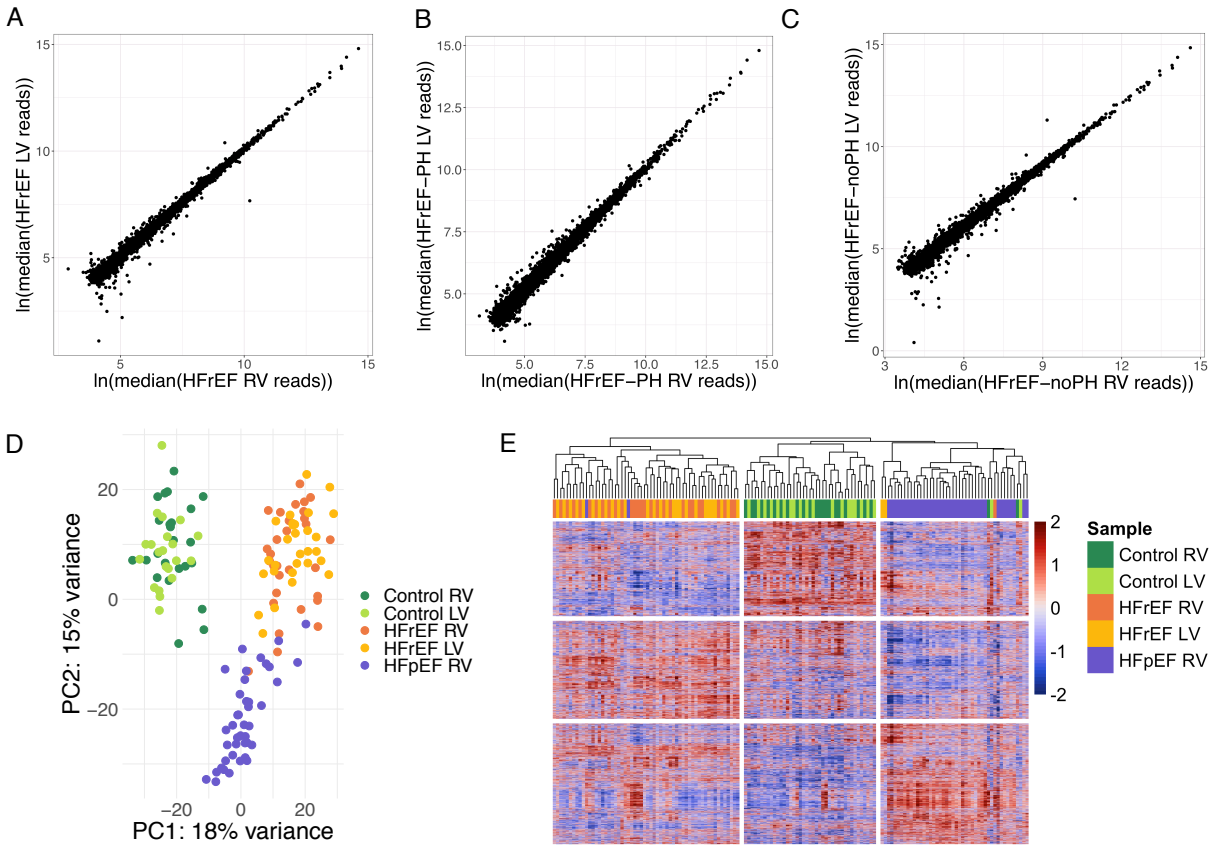
Supplemental Figure II. Frequency distribution histograms of clinical features and hemodynamic parameters of the HFpEF cohort reveals substantial heterogeneity of the patient group. Most all patients were obese though BMI was distributed over a broad range. Most had systolic hypertension, with about 50% exhibiting abnormal LV mass. Though mean pulmonary artery (PA) pressure was generally above normal range, this was almost always associated with a rise in downstream pulmonary capillary wedge pressure so that pulmonary vascular resistance (PVR) was not abnormal for the majority of patients. Estimated glomerular filtration rate (eGFR), E' mitral annular tissue velocity during E-wave, and E/E' (ratio of early diastolic filling rate to tissue velocity) displayed substantial variability among the patients. Log_{10} transformed values displayed for NT-proBNP and Pulmonary Vascular Resistance.



Supplemental Figure III. Principal component analysis using sex-adjusted gene counts for Control (green), Heart Failure with Reduced Ejection Fraction (HFrEF, orange), and Heart Failure with Preserved Ejection Fraction (HFpEF, purple).

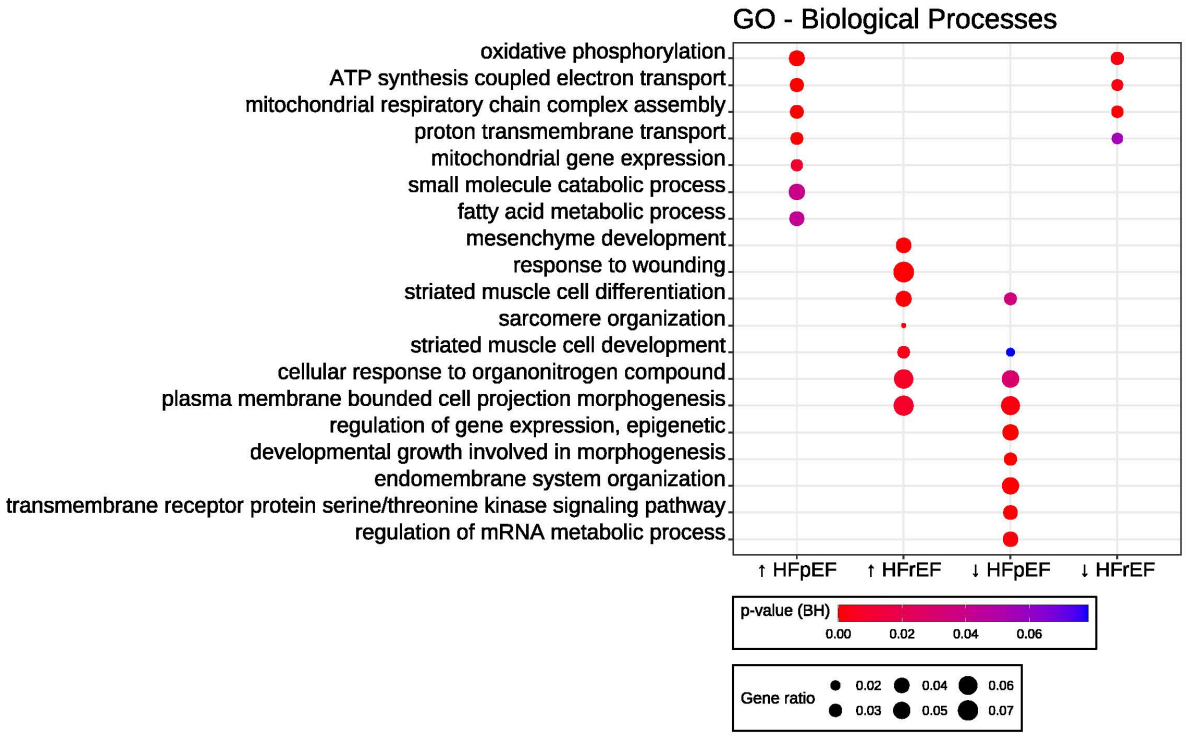


Supplemental Figure IV. Coefficient of variation of individual patient myocardial gene expression for all 13,000 genes identified by RNAseq. The vast majority of genes displayed a COV of <32% and this was similar for all patient groups. For HFpEF and HFrEF, this was negligibly different for those genes significantly different from control (adjusted $p<0.05$) and those that were not. Lowest P value (far left of plot) is 10^{-103} for HFpEF and 10^{-86} for HFrEF). COV% median (15th-85th) for HFpEF: 23% (17%-38%), for HFrEF: 22% (16%-42%).

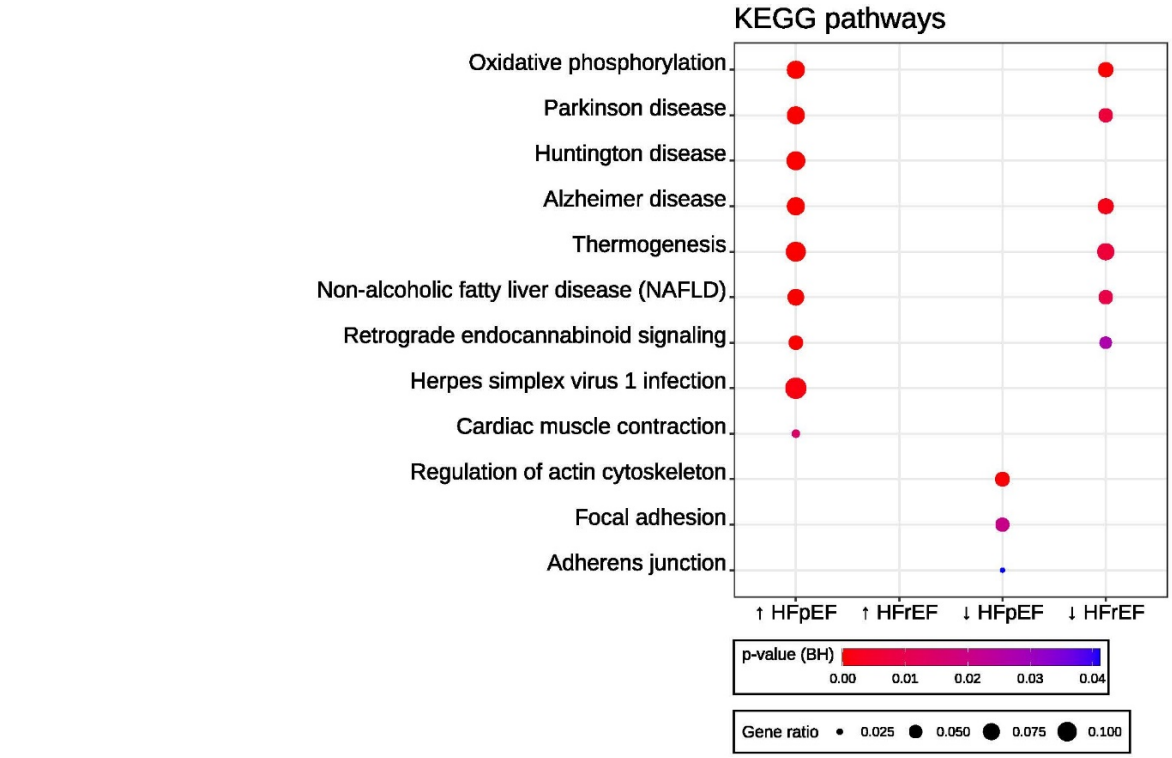


Supplemental Figure V. Right and left ventricular septum have similar transcriptomic profile. Natural log-transformed median reads for each of the ~13,000 genes were plotted as LV versus RV in **A) HFrEF, B) HFrEF** with PVR≥3wu ("HFrEF-PH"), and **C) HFrEF** with PVR<3wu ("HFrEF-noPH"). **D)** Principal component analysis and **E)** Hierarchical clustering displayed as a heatmap demonstrate RV and LV cluster together within disease groups for CON (green), HFpEF (purple), and HFrEF (orange), with minimal overlap between HFrEF and HFpEF.

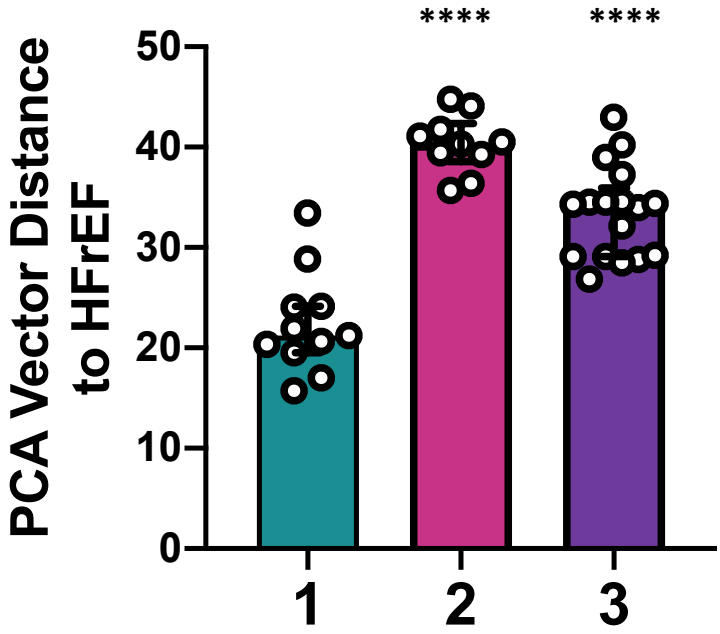
A)


GO - Biological Processes
oxidative phosphorylation
ATP synthesis coupled electron transport
mitochondrial respiratory chain complex assembly
proton transmembrane transport
mitochondrial gene expression
small molecule catabolic process
fatty acid metabolic process
mesenchyme development
response to wounding
striated muscle cell differentiation
sarcomere organization
striated muscle cell development
cellular response to organonitrogen compound
plasma membrane bounded cell projection morphogenesis
regulation of gene expression, epigenetic
developmental growth involved in morphogenesis
endomembrane system organization
transmembrane receptor protein serine/threonine kinase signaling pathway
regulation of mRNA metabolic process
↑ HFpEF
↑ HFrEF
↓ HFpEF
↓ HFrEF
p-value (BH)
0.00
0.02
0.04
0.06
Gene ratio
0.02
0.04
0.06
0.03
0.05
0.07

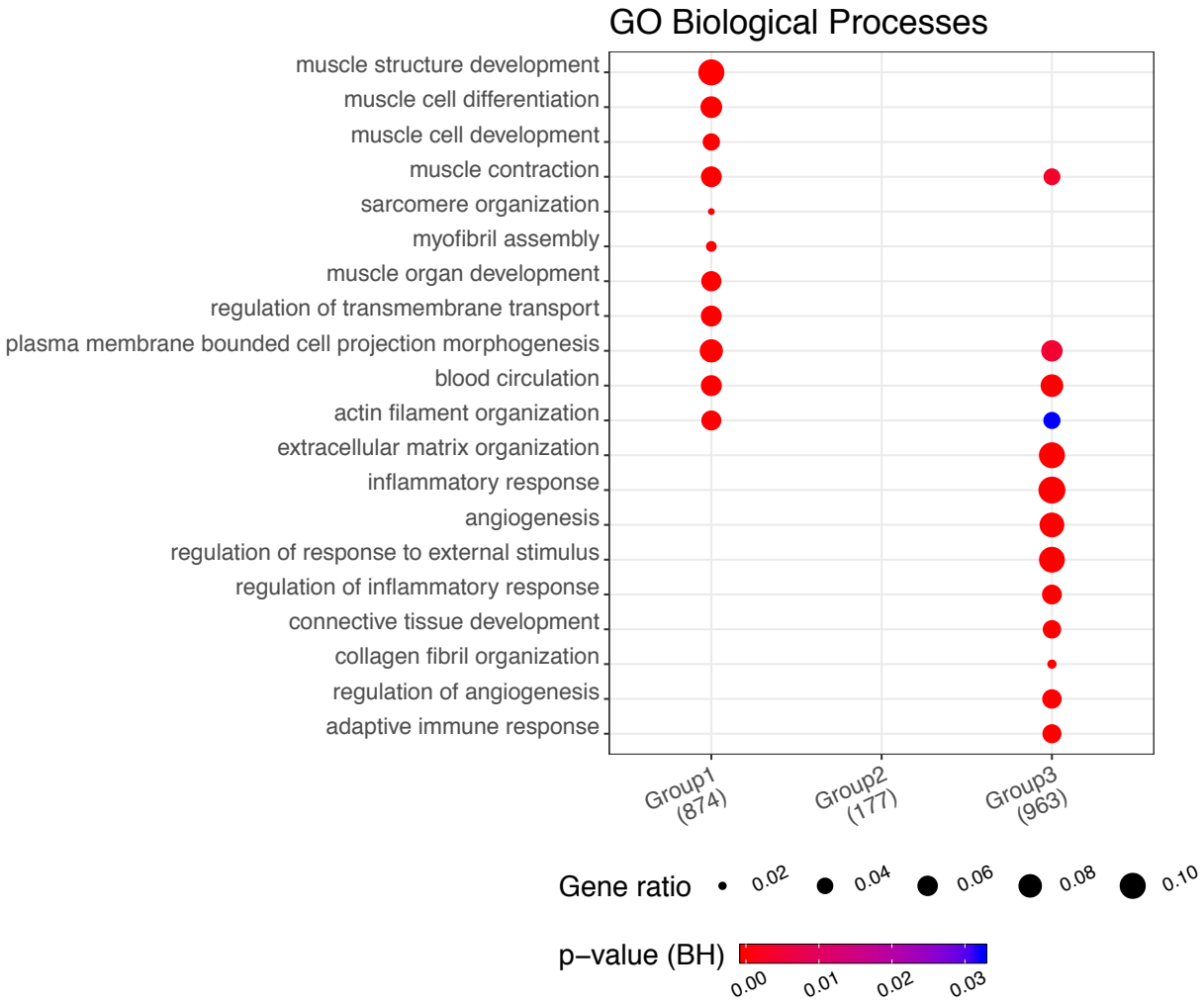
B)


KEGG pathways
Oxidative phosphorylation
Parkinson disease
Huntington disease
Alzheimer disease
Thermogenesis
Non-alcoholic fatty liver disease (NAFLD)
Retrograde endocannabinoid signaling
Herpes simplex virus 1 infection
Cardiac muscle contraction
Regulation of actin cytoskeleton
Focal adhesion
Adherens junction
↑ HFpEF
↑ HFrEF
↓ HFpEF
↓ HFrEF
p-value (BH)
0.00
0.01
0.02
0.03
0.04
Gene ratio
0.025
0.050
0.075
0.100

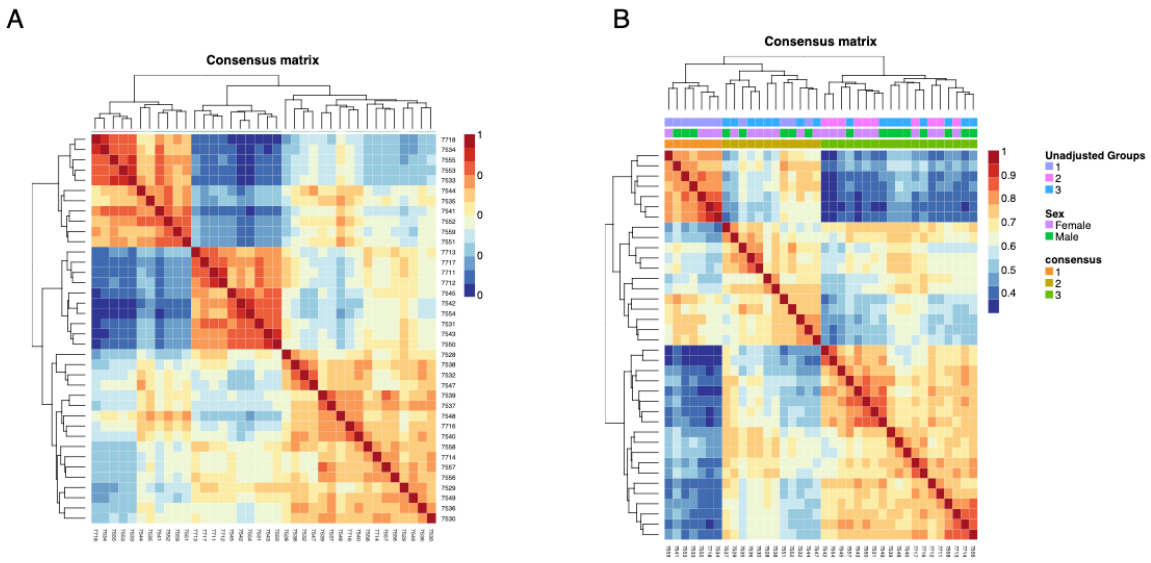
Supplemental Figure VI. A) Curated GO biological process list demonstrating distinctive pathways engaged by HFpEF versus HFrEF. Circle size reflects gene ratio - proportion of differentially expressed genes in a pathway versus all differentially expressed genes that map to GO genes; Color coding reflects Fisher's exact P value after Benjamini-Hochberg (BH) adjustment for multiple comparisons. **B)** KEGG pathway analysis demonstrating distinctive pathways engaged in HFpEF versus HFrEF.



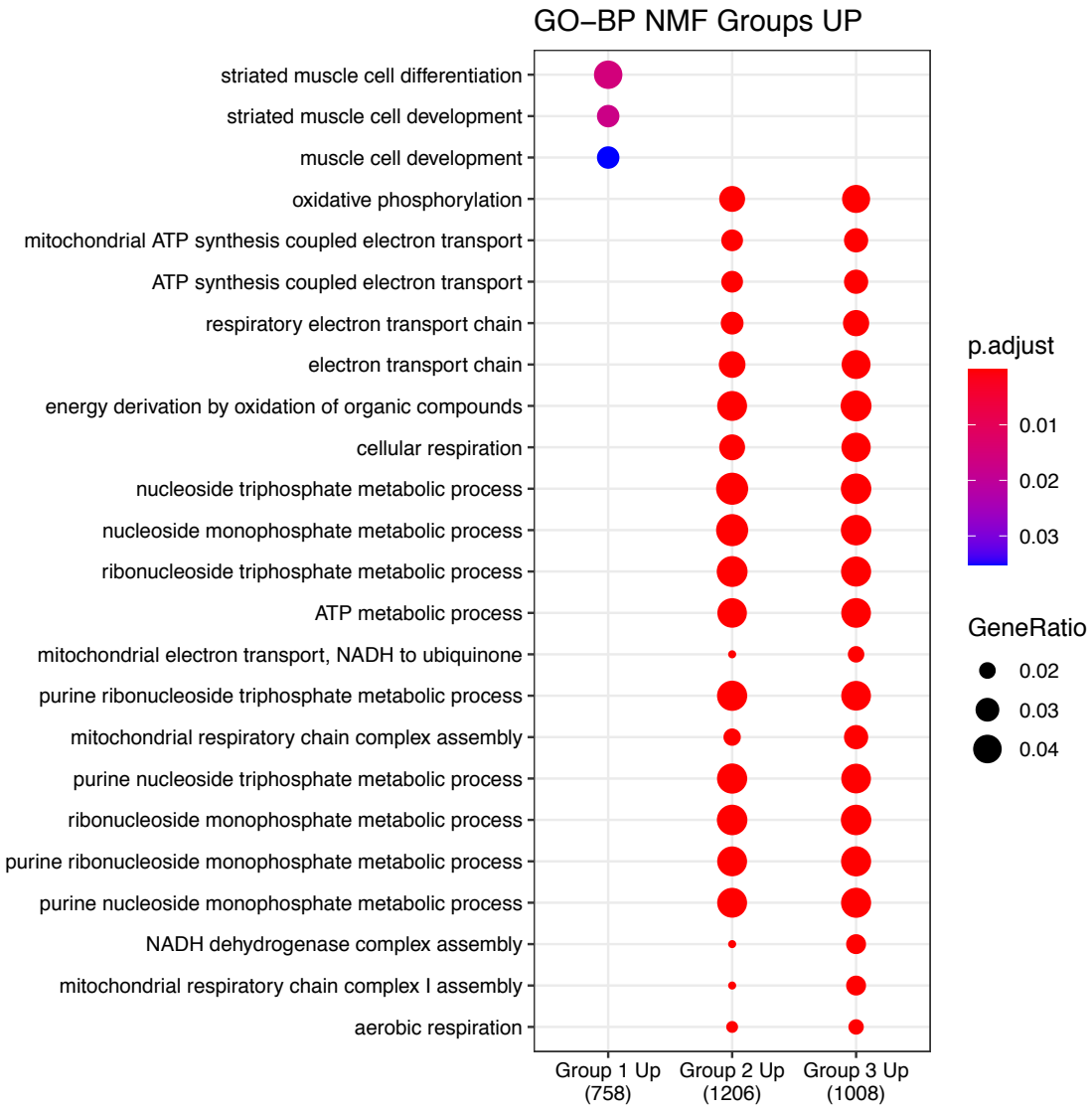
Supplemental Figure VII. Relative proximity of HFpEF sub-groups to HFrEF based on vector length between center-of-mass for HFrEF and each respective PCA (x,y) coordinate for each HFpEF patient. **** $p \leq 0.0001$ vs Group 1, One-way Analysis of Variance with post-hoc Tukey test for multiple comparisons.



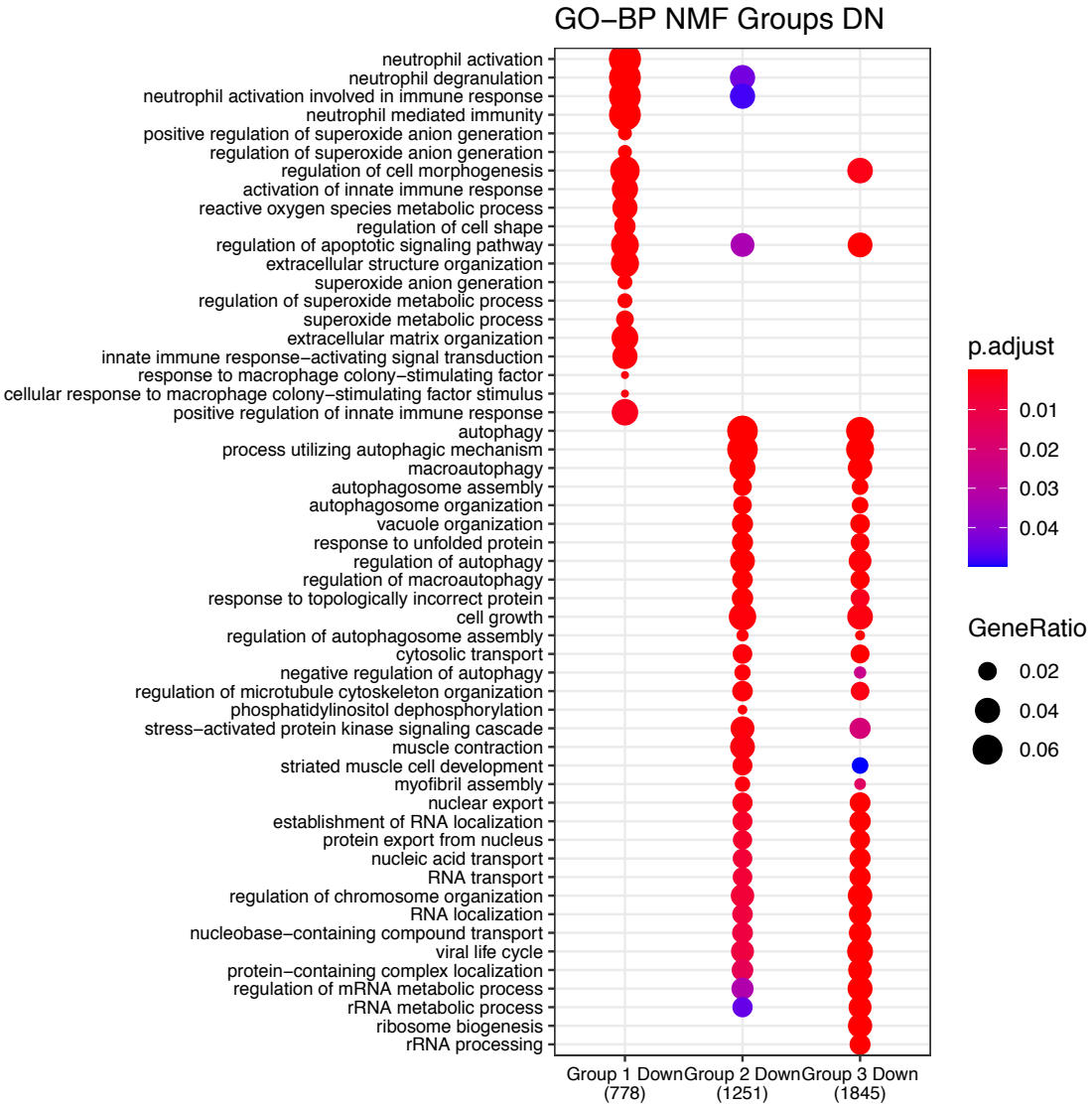
Supplemental Figure VIII. Gene Ontology Biological Processes of genes identified in HFpEF subgroups determined by Non-negative matrix factorization. Circle size reflects gene ratio - proportion of differentially expressed genes in a pathway versus all differentially expressed genes; Color coding reflects Fisher's exact P value after Benjamini-Hochberg (BH) adjustment for multiple comparisons.



Supplemental Figure IX. Non-negative matrix factorization (NMF) identifies 3 subgroups of Heart Failure with Preserved Ejection Fraction (HFpEF). A) Patient-patient correlation plot demonstrates no difference in the unadjusted NMF groups after removing genes differentially expressed between HFpEF-men and HFpEF-women. B) Adjustment for sex led to more sex-balanced subgroups of HFpEF, but did not change inherent clinical characteristics for each group **(Supplemental Table IV).**



Supplemental Figure X. Gene Ontology Biological Processes of differentially expressed genes identified in HFpEF subgroups, upregulated compared to control. Differentially expressed genes shared across all three groups were removed from the pathway enrichment gene list. Circle size reflects gene ratio - proportion of differentially expressed genes in a pathway versus all differentially expressed genes; Color coding reflects Fisher's exact P value after Benjamini-Hochberg (BH) adjustment for multiple comparisons.



Supplemental Figure XI. Gene Ontology Biological Processes of differentially expressed genes identified in HFpEF subgroups, downregulated compared to control. Differentially expressed genes shared across all three groups were removed from the pathway enrichment gene list. Circle size reflects gene ratio - proportion of differentially expressed genes in a pathway versus all differentially expressed genes; Color coding reflects Fisher's exact P value after Benjamini-Hochberg (BH) adjustment for multiple comparisons.

Supplemental Table I. Additional Clinical Characteristics of HFpEF Study group (n=41).

Parameter	HFpEF (N=41)
HF Hospitalization past 12 mos, n (%)	29 (71%)
NYHA Class	
II, n (%)	14 (34%)
III, n (%)	26 (63%)
IV, n (%)	1 (2%)
Symptoms of Heart Failure	
Dyspnea, n (%)	33 (80%)
Orthopnea, n (%)	19 (46%)
Paroxysmal Nocturnal Dyspnea, n (%)	9 (22%)
Edema, n (%)	28 (68%)
Exertional Intolerance, n (%)	36 (88%)
Medications	
Aldosterone antagonist, n (%)	13 (32%)
Furosemide-Equivalent Dose Per Day, mg	80 (40-160)
Past Medical History	
Obstructive Sleep Apnea, n (%)	24 (59%)
COPD or Asthma, n (%)	15 (37%)
Systolic Blood Pressure, mmHg	141 (130-169)
Diastolic Blood Pressure, mmHg	74 (65-80)
Echocardiography	

IVS, cm	1.4 (1.2-1.6)
LA Diameter, cm	4.2 (3.5-4.6)
LV mass, g	224 (185-260)
Septal e' velocity, cm/s	6.5 (5.0-9.1)
E/e'	17 (10-21)
Laboratory values	
Hemoglobin, g/dL	12.4 (11.3-13.2)
Sodium, mmol/L	141 (139-142)
Creatinine, mg/dL	1.3 (1.1-2.0)
Troponin I, ng/mL	0 (0-0)
NTproBNP, pg/mL	169 (94-614)
Invasive Hemodynamics	
PADP, mmHg	22 (18-25)
CO, L/min	5.9 (4.9-7.0)
Stroke Volume, mL/beat	80 (71-91)
Stroke Volume, mL/beat/m^2	37 (31-39)
SVRI	2982 (2339-3343)
SVR, dynes/s/cm^{-5}	1123 (795-1326)
PVR, wu	1.7 (1.0-2.3)
RV Ea = PASP/SV, mmHg/mL	0.6 (0.4-0.7)
Clinical Histology	
Myocyte Hypertrophy	
None, n (%)	4 (10%)

Mild or patchy, n (%)	13 (32%)
Moderate, n (%)	22 (54%)
Severe, n (%)	2 (5%)
Myocardial Fibrosis	
Mild or patchy, n (%)	25 (61%)
Moderate, n (%)	12 (29%)
Severe, n (%)	4 (10%)

Data are reported as median (25^{th}-75^{th} percentile) or N (%). HF, Heart Failure; NYHA, New York Heart Association; COPD, chronic obstructive pulmonary disease; IVS, intraventricular septal thickness; LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PADP, pulmonary artery diastolic pressure; CO, cardiac output; SVRI, systemic vascular resistance index; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; RV Ea, right ventricular effective arterial elastance; PASP, pulmonary artery systolic pressure; SV, stroke volume.

Supplemental Table II. Clinical characteristics of three HFpEF groups derived by Non-negative Matrix Factorization				
	Group 1 (11)	Group 2 (10)	Group 3 (17)	P value
Self-identified Race/Ethnicity				0.34
African-American, n (%)	8 (73%)	7 (70%)	10 (59%)	
Caucasian, n (%)	2 (18%)	2 (20%)	7 (41%)	
Hispanic, n (%)	1 (9%)	0 (0%)	0 (0%)	
Middle Eastern, n (%)	0 (0%)	1 (10%)	0 (0%)	
Medications				
ACEi or ARB, n (%)	5 (45%)	9 (90%)	9 (53%)	0.081
Beta Blocker, n (%)	6 (55%)	6 (60%)	10 (59%)	>0.99
ALDO Antagonist, n (%)	1 (9%)*	6 (60%)	5 (29%)	0.057
Loop Diuretic, n (%)	11 (100%)	10 (100%)	17 (100%)	
CCB (dihydropiridine), n (%)	7 (64%)	7 (70%)	8 (47%)	0.49
Statin, n (%)	10 (91%)†	6 (60%)	7 (41%)	0.024
Symptoms of Heart Failure				
Dyspnea, n (%)	10 (91%)	9 (90%)	11 (65%)	0.2
Orthopnea, n (%)	7 (64%)	5 (50%)	6 (35%)	0.35
Edema, n (%)	8 (73%)	8 (80%)	11 (65%)	0.82
Exertional Intolerance, n (%)	10 (91%)	9 (90%)	15 (88%)	>0.99
Heart rate, bpm	88 (72, 94)	76 (69, 82)	74 (65, 86)	0.37

Interventricular Septum, mm	14.0 (13.0, 16.0)	13.0 (11.2, 14.0)	14.0 (12.0, 16.0)	0.38
LV posterior wall, mm	13.0 (11.0, 14.5)†	10.5 (10.0, 13.0)	11.0 (9.1, 13.0)	0.15
Cystatin C, mg/L	1.67 (1.45, 2.22)*	1.36 (0.95, 1.41)	1.30 (1.06, 1.85)	0.11
Invasive Hemodynamics				
Heart rate, bpm	74 (66, 82)	76 (66, 83)	71 (64, 75)	0.7
PADP, mmHg	23 (20, 28)	22 (16, 27)	21 (18, 25)	0.61
CO, L/min	7.00 (4.63, 8.12)	5.88 (4.70, 6.61)	5.90 (5.07, 6.23)	0.66
Stroke Volume Index, mL/m^2	37 (32, 43)	34 (30, 37)	37 (32, 39)	0.65
SVR, $dynes/s/cm^{-5}$	905 (617, 1740)	1089 (815, 1325)	1251 (1062, 1313)	0.66
RV Ea, mmHg/mL	0.65 (0.55, 0.83)	0.55 (0.49, 0.70)	0.51 (0.36, 0.61)	0.13
RA/PAWP ratio	0.58 (0.54, 0.63)	0.57 (0.51, 0.60)	0.60 (0.47, 0.67)	0.93
PA Pulsatility Index	2.75 (2.08, 5.70)†	1.71 (1.37, 2.62)	1.88 (1.33, 2.56)	0.082
Myocyte Hypertrophy, Clinical Histology				0.5
None, n (%)	0 (0%)	2 (20%)	2 (12%)	

Mild or patchy, n (%)	4 (36%)	3 (30%)	6 (35%)	
Moderate, n (%)	5 (45%)	5 (50%)	9 (53%)	
Severe, n (%)	2 (18%)	0 (0%)	0 (0%)	
Myocardial Fibrosis, Clinical Histology				0.77
Mild or patchy, n (%)	5 (45%)	7 (70%)	11 (65%)	
Moderate, n (%)	4 (36%)	2 (20%)	5 (29%)	
Severe, n (%)	2 (18%)	1 (10%)	1 (5.9%)	

Data presented as n (%) or median (25th-75th percentile). Fisher's exact test used for categorical variables. Kruskal-Wallis test used for continuous variables. *$p<0.05$ vs Group 2, †$p<0.05$ vs Group 3. HFpEF, heart failure with preserved ejection fraction; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ALDO, aldosterone; CCB, calcium channel blocker; PADP, pulmonary artery diastolic pressure; CO, cardiac output; SVR, systemic vascular resistance; RV Ea, pulmonary effective arterial elastance; PAWP, pulmonary artery wedge pressure; PA, pulmonary artery.

Supplemental Table III. Clinical characteristics of three HFpEF groups derived by Non-negative Matrix Factorization after adjustment for sex				
	Group 1 (7)	Group 2 (12)	Group 3 (19)	P value
Sex				>0.99
Female, n (%)	4 (57%)	7 (58%)	10 (53%)	
Male, n (%)	3 (43%)	5 (42%)	9 (47%)	
Self-identified Race/Ethnicity				0.093
African-American, n (%)	6 (86%)*	6 (50%)	13 (68%)	
Caucasian, n (%)	0 (0%)	6 (50%)	5 (26%)	
Hispanic, n (%)	1 (14%)	0 (0%)	0 (0%)	
Middle Eastern, n (%)	0 (0%)	0 (0%)	1 (5.3%)	
Age, years	62 (52, 72)	60 (57, 67)	66 (52, 68)	0.94
HF hospitalization prior 12mos, n (%)	6 (86%)	10 (83%)	11 (58%)	0.31
NYHA Class				0.83
II, n (%)	3 (43%)	5 (42%)	5 (26%)	
III, n (%)	4 (57%)	7 (58%)	13 (68%)	
IV, n (%)	0 (0%)	0 (0%)	1 (5.3%)	
Medications				
ACEi or ARB, n (%)	5 (71%)	6 (50%)	12 (63%)	0.74
Beta Blocker, n (%)	4 (57%)	8 (67%)	10 (53%)	0.83
ALDO Antagonist, n (%)	0 (0%)†	2 (17%)	10 (53%)	0.016

Loop Diuretic, n (%)	7 (100%)	12 (100%)	19 (100%)	>0.99
CCB (dihydropiridine), n (%)	5 (71%)	7 (58%)	10 (53%)	0.76
Statin, n (%)	7 (100%)††	8 (67%)	8 (42%)	0.021
Past Medical History				
Hypertension, n (%)	7 (100%)	12 (100%)	18 (95%)	>0.99
Diabetes, n (%)	7 (100%)	8 (67%)	10 (53%)	0.088
Coronary artery disease, n (%)	0 (0%)	0 (0%)	4 (21%)	0.16
Atrial fibrillation or flutter, n (%)	2 (29%)	4 (33%)	4 (21%)	0.8
Symptoms of Heart Failure				
Dyspnea, n (%)	6 (86%)	8 (67%)	16 (84%)	0.57
Orthopnea, n (%)	5 (71%)	5 (42%)	8 (42%)	0.4
Edema, n (%)	4 (57%)	10 (83%)	13 (68%)	0.36
Exertional Intolerance, n (%)	6 (86%)	10 (83%)	18 (95%)	0.49
Heart rate, bpm	90 (76, 96)	72 (64, 79)	77 (72, 86)	0.14
Systolic BP, mmHg	141 (138, 144)	160 (127, 176)	136 (125, 150)	0.25
Diastolic BP, mmHg	67 (65, 71)	74 (63, 79)	76 (66, 84)	0.6
BMI, kg/m^2	37 (33, 46)	45 (36, 47)	41 (38, 45)	0.61
LVEF, %	60.0 (55.0, 67.5)	65.0 (60.0, 70.0)	65.0 (60.0, 67.5)	0.66
LVEDD, cm	4.60 (4.20, 5.25)	5.10 (4.65, 5.62)††	4.20 (3.99, 4.75)	0.022
Interventricular Septum, mm	1.30 (1.15, 1.55)	1.45 (1.35, 1.68)	1.30 (1.10, 1.40)	0.27

LV posterior wall, mm	1.30 (0.99, 1.35)	1.20 (0.96, 1.42)	1.10 (1.00, 1.30)	0.86
LA Diameter, cm	3.80 (3.55, 4.75)	4.35 (4.10, 4.80)	4.20 (3.40, 4.45)	0.29
Sex-adjusted LV mass/height$^{1.7}$	113 (95, 130)	131 (113, 157)††	88 (74, 106)	0.004
LV mass index, g/m^2	111 (92, 128)	120 (88, 133)††	85 (61, 95)	0.015
eGFR, mL/min/1.73m^2	32 (24, 50)†	50 (37, 70)	49 (40, 91)	0.12
BUN, mg/dL	43 (32, 52)†	22 (18, 30)	21 (16, 26)	0.041
Creatinine, mg/dL	1.90 (1.45, 2.55)	1.35 (1.05, 2.00)	1.30 (0.95, 1.60)	0.16
Cystatin C, mg/L	2.18 (1.66, 2.27)††	1.39 (1.06, 1.75)	1.34 (1.06, 1.47)	0.037
NTproBNP, pg/mL	2,617 (807, 7,052)††	418 (120, 598)†	113 (38, 189)	0.007
Invasive Hemodynamics				
Heart rate, bpm	74 (66, 83)	67 (63, 75)	75 (67, 83)	0.26
RAP, mmHg	15.0 (12.5, 16.0)	11.5 (5.0, 12.2)	11.0 (8.5, 15.0)	0.19
PASP, mmHg	61 (54, 76)*,††	42 (33, 50)	44 (33, 50)	0.008
PADP, mmHg	24 (21, 36)	21 (16, 23)	22 (18, 25)	0.18
PAmean, mmHg	36 (33, 48)*,†	28 (24, 31)	29 (23, 34)	0.025
PAWP, mmHg	25 (20, 28)	19 (14, 22)	20 (14, 24)	0.14
CO, L/min	7.10 (4.54, 8.12)	5.86 (4.94, 7.09)	5.90 (4.70, 6.42)	0.57
CI, L/min/m^2	3.28 (2.28, 3.80)	2.48 (2.39, 3.01)	2.51 (2.25, 2.73)	0.47
PVR, wu	2.05 (1.57, 3.95)	1.53 (0.80, 2.80)	1.65 (1.25, 2.24)	0.36
PVR $\geq$ 3wu, n (%)	2 (29%)	3 (25%)†	0 (0%)	0.024
RVSWI, g.m/m^2/beat	9.7 (9.4, 14.6)†	7.8 (5.8, 11.2)	7.8 (5.5, 9.4)	0.035

Stroke Volume Index, mL/m^2	37 (30, 51)	38 (36, 40)†	34 (30, 37)	0.1
SVR, dynes/s/cm^{-5}	905 (508, 1,497)	1,164 (787, 1,558)	1,251 (907, 1,324)	0.56
RV Ea, mmHg/mL	0.65 (0.55, 1.14)	0.45 (0.35, 0.68)	0.59 (0.46, 0.70)	0.16
Trans-pulmonary gradient, mmHg	12.0 (11.5, 22.0)†	8.0 (5.8, 13.8)	8.0 (7.5, 11.5)	0.066
RA/PAWP ratio	0.60 (0.56, 0.63)	0.55 (0.42, 0.58)	0.60 (0.52, 0.62)	0.38
PA Pulsatility Index	2.62 (2.08, 2.84)	2.38 (1.22, 5.18)	1.88 (1.35, 2.53)	0.23
PA compliance, mL/mmHg	0.0029 (0.0016, 0.0039)	0.0043 (0.0023, 0.0060)	0.0036 (0.0032, 0.0047)	0.17
Myocyte Hypertrophy, Clinical Histology				0.76
None, n (%)	2 (29%)	4 (33%)	7 (37%)	
Mild or patchy, n (%)	4 (57%)	6 (50%)	9 (47%)	
Moderate, n (%)	0 (0%)	1 (8.3%)	3 (16%)	
Severe, n (%)	1 (14%)	1 (8.3%)	0 (0%)	
Myocardial Fibrosis, Clinical Histology				0.65
Mild or patchy, n (%)	3 (43%)	9 (75%)	11 (58%)	
Moderate, n (%)	3 (43%)	2 (17%)	6 (32%)	
Severe, n (%)	1 (14%)	1 (8.3%)	2 (11%)	
CD68 cells/mm^2	45 (34, 55)	48 (29, 100)	78 (43, 109)	0.23
% Fibrosis	7.2 (6.1, 7.5)	7.6 (3.6, 10.3)	7.3 (5.0, 11.2)	0.93

Data presented as n (%) or median (25th-75th percentile). Fisher's exact test used for categorical variables. Kruskal-Wallis test used for continuous variables. *$p<0.05$ vs Group 2,. †$p<0.05$ vs Group 3, ††$p\leq0.01$ vs Group 3 . HFpEF, heart failure with preserved ejection fraction; HF, heart failure; NYHA, New York Heart Association; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ALDO, aldosterone; CCB, calcium channel blocker; BP, blood pressure; BMI, body mass index; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter; LV, left ventricle; LA, left atrial; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; NTproBNP, N-terminal pro-B type natriuretic peptide; RAP, right atrial pressure; PASP, pulmonary artery systolic pressure; PADP, pulmonary artery diastolic pressure; PAmean, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; CO, cardiac output; CI, cardiac index; PVR, pulmonary vascular resistance; wu, Wood units; RVSWI, right ventricular stroke work index; SVR, systemic vascular resistance; RV Ea, pulmonary effective arterial elastance; RA, right atrium; PA, pulmonary artery; CD68, cluster of differentiation 68.

Supplemental Table IV. Weighted Gene Co-Expression Analysis Correlation of Gene Clusters with Clinical Characteristics		
Cluster	Clinical Characteristics	Gene Ontology Biological Processes
Pink	Lower RV and LV afterload (RV Ea, SVR), higher stroke volume (SVI) and cardiac index (CI)	Oxidative phosphorylation; Protein targeting to the endoplasmic reticulum
Green	Better renal function (eGFR)	Leukocyte activation involved in immune response; Neutrophil activation involved in immune response; Interleukin-1 beta production; Interferon-gamma-mediated signaling pathway; Regulation of cytoskeleton organization; Extracellular matrix organization
RV, Right Ventricle; LV, Left Ventricle; RV Ea, Right ventricle arterial elastance; SVR, systemic vascular resistance; SVI, stroke volume index; CI, cardiac index; eGFR, estimated glomerular filtration rate		