Supplemental Figure Legends

Figure S1. Measurement of left ventricular ejection fraction (by echocardiography), systolic blood pressure (by invasive catheterization), cardiac output (by invasive catheterization), and left atrial diameter in mice subjected to 0, 2, 5, or 12 weeks of L-NAME in water and high fat diet. Representative M-mode images are also presented for mice at baseline vs 12 weeks of L-NAME and high fat diet.

Figure S2. Weight gain in mice after 2, 5, and 12 weeks of L-NAME in water and high fat diet compared to mice given normal water and a calorie neutral low fat diet. Weight gain is presented as a percent change from starting weight at the beginning of the study.

Figure S3. Western blot and quantification of relative density of bands for CD68 expression in whole lung lysate of mice given L-NAME in water and high fat diet for 2, 5, and 12 weeks.

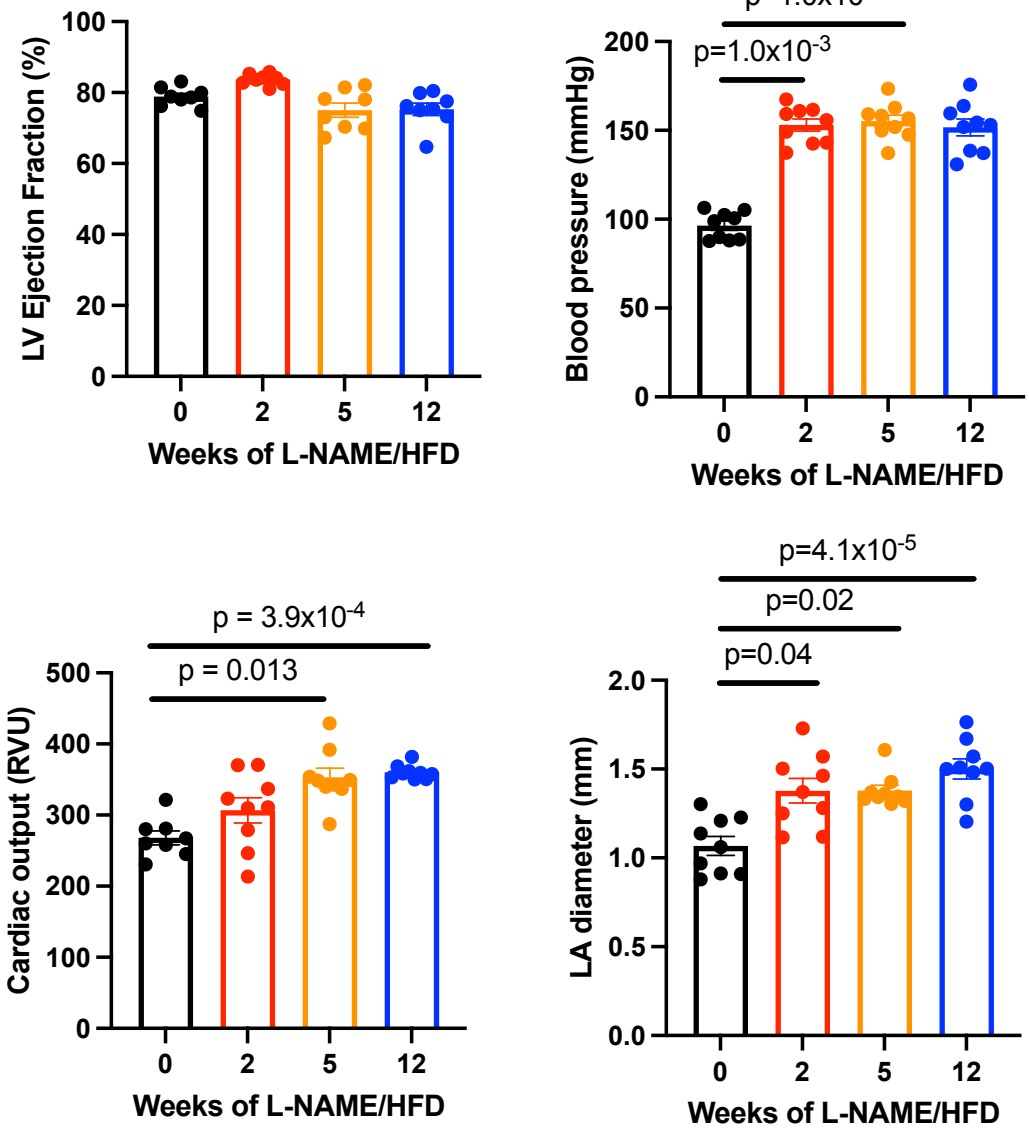
Figure S4. Measurement of right ventricular systolic pressure, right ventricular end-diastolic pressure, Fulton index (ratio of right ventricular mass to left ventricular and septal mass), and IL1β levels by ELISA in whole lung lysate of 6 month old db/db mice.

Figure S5. Flow diagram of inclusion and selection criteria for identification of patients with heart failure with preserved ejection fraction and pulmonary hypertension who underwent clinically indicated autopsy at Vanderbilt University Medical Center.

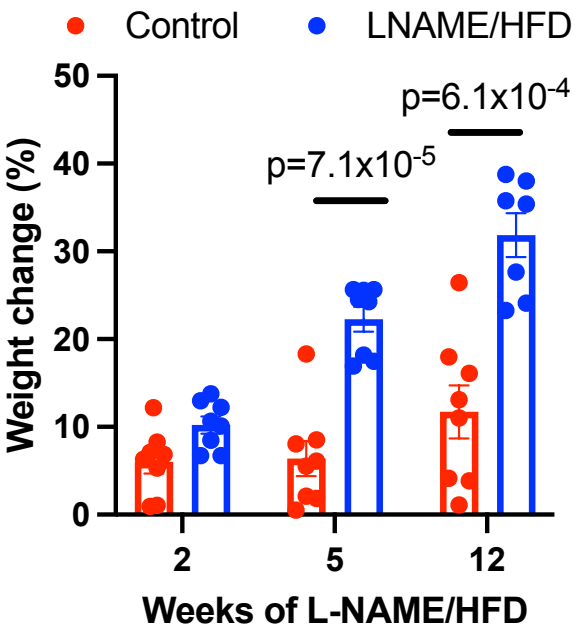
Figure S6. (Top) Bar plot depiction of individual samples analyzed using single cell RNA sequencing technology and proportions of identified cell types. (Bottom) Expression pattern for identified cell types.

Figure S7. Transcript expression of select monocyte/macrophage-related transcripts by bulk RNA sequencing in mice treated with 5 weeks of L-NAME in water and high fat diet compared to normal water and low fat diet controls.

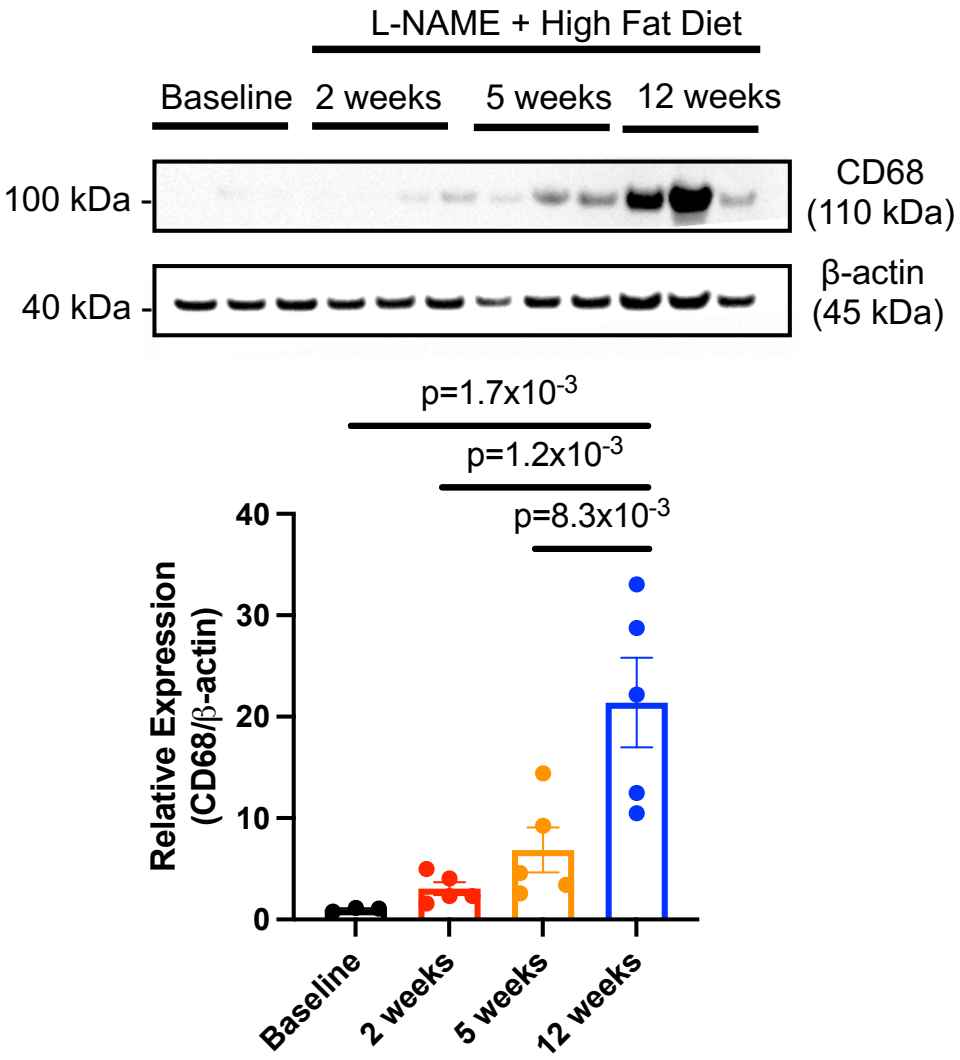
Figure S8. Western blot for CD68 expression in whole lung lysate of mice treated with L-NAME and high fat diet and either F4/80 (macrophage) neutralizing antibody or isotype control antibody. Measurement of weight gain, right ventricular systolic pressure, right ventricular end-diastolic pressure, and Fulton index (right ventricular mass to left ventricular and septal mass ratio in mice treated with L-NAME/HFD and either isotype control or F4/80 neutralizing antibody. Representative images and quantification demonstrating muscularization of small vessels in mice treated with isotype control antibody compared to mice treated with F4/80 neutralizing antibody.



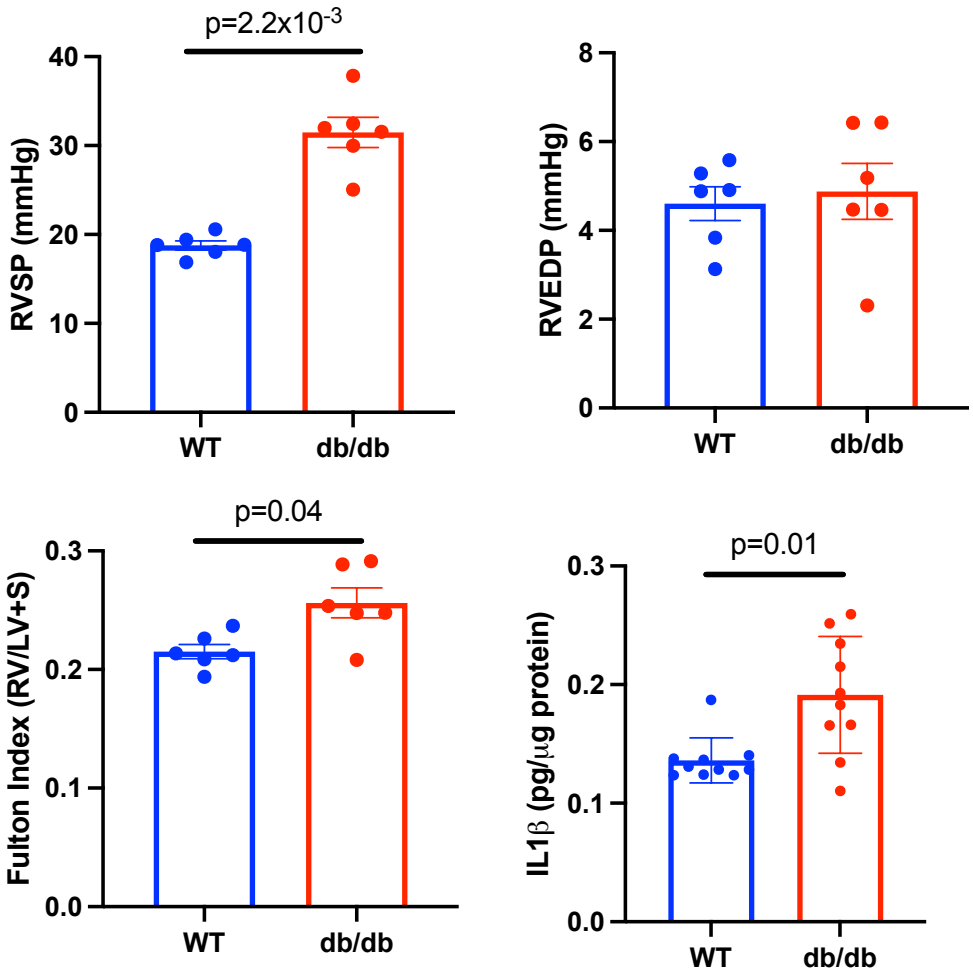
Supplementary Figure S1



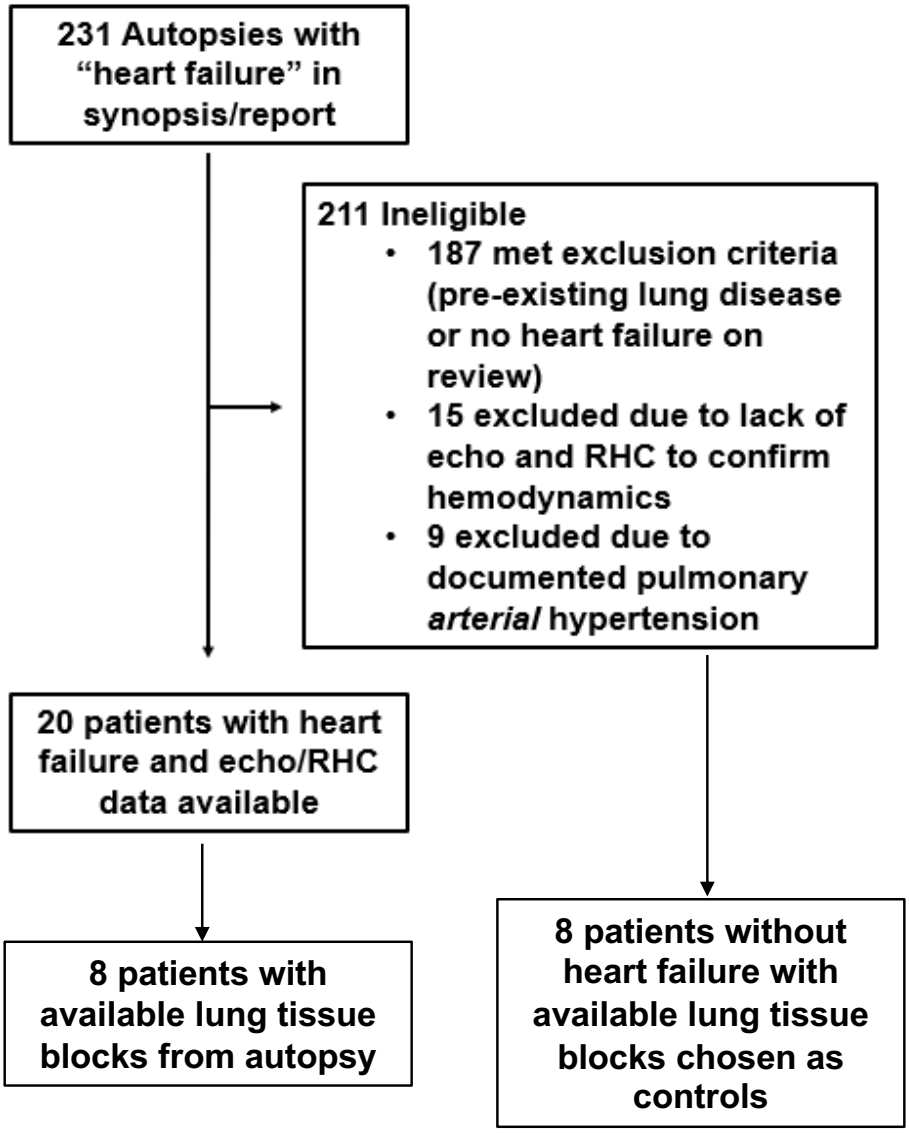
Supplementary Figure S2



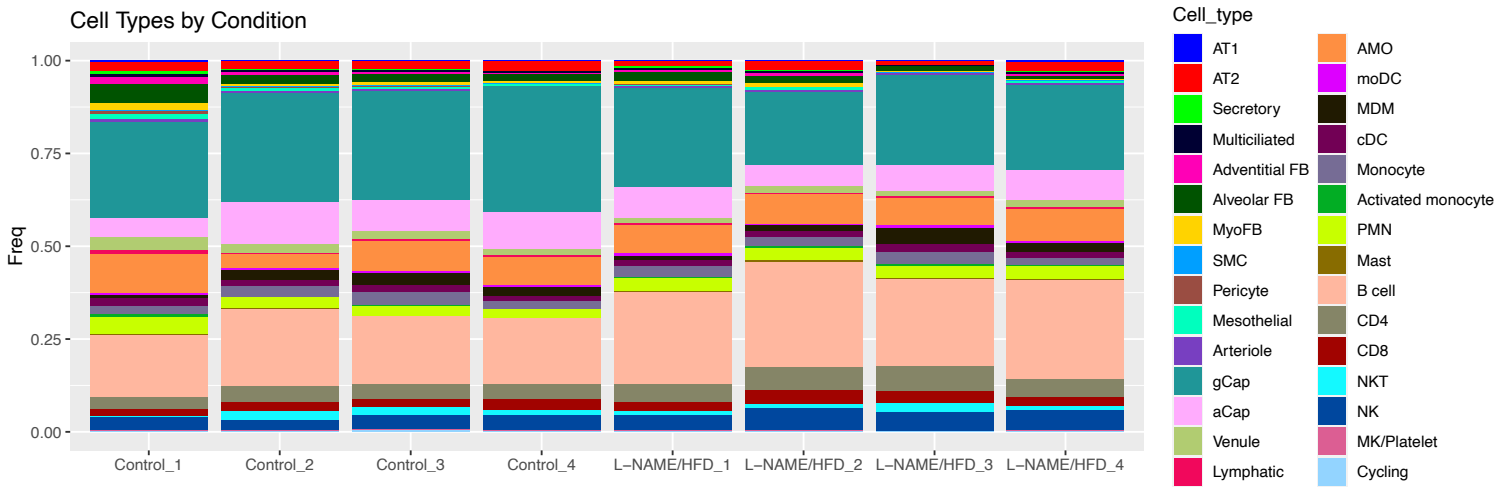
Supplementary Figure S3

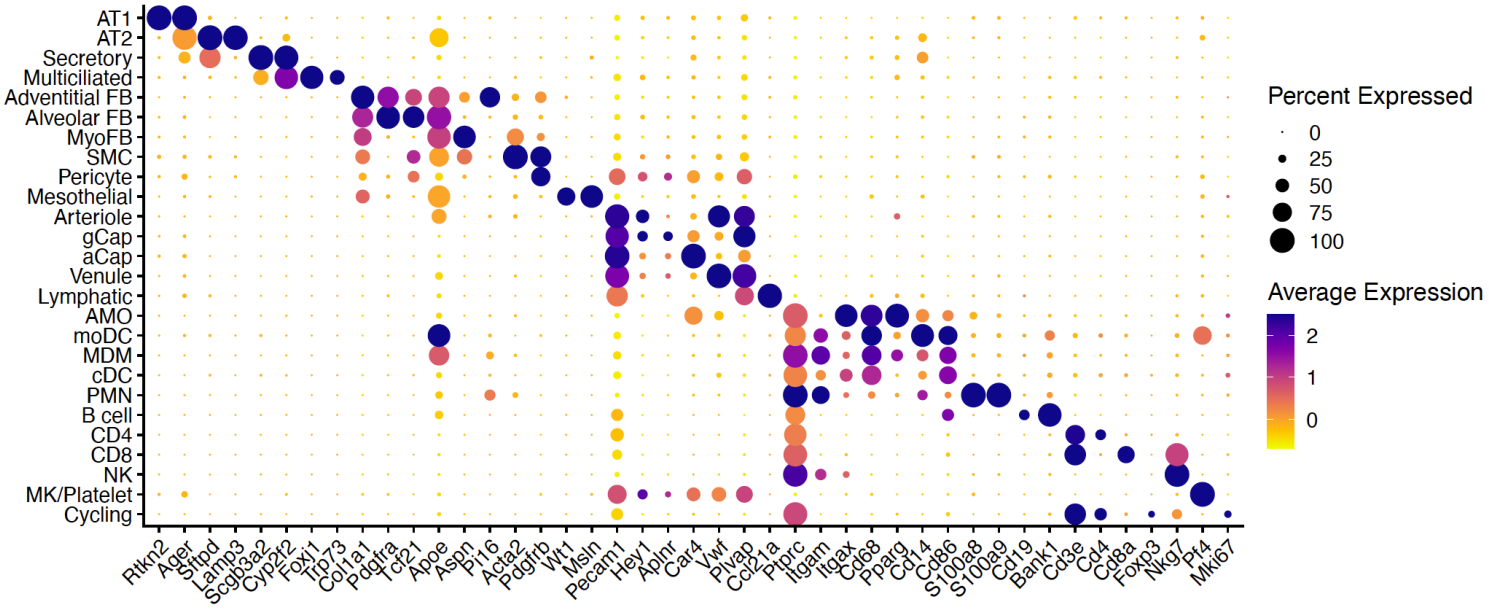


Supplementary Figure S4

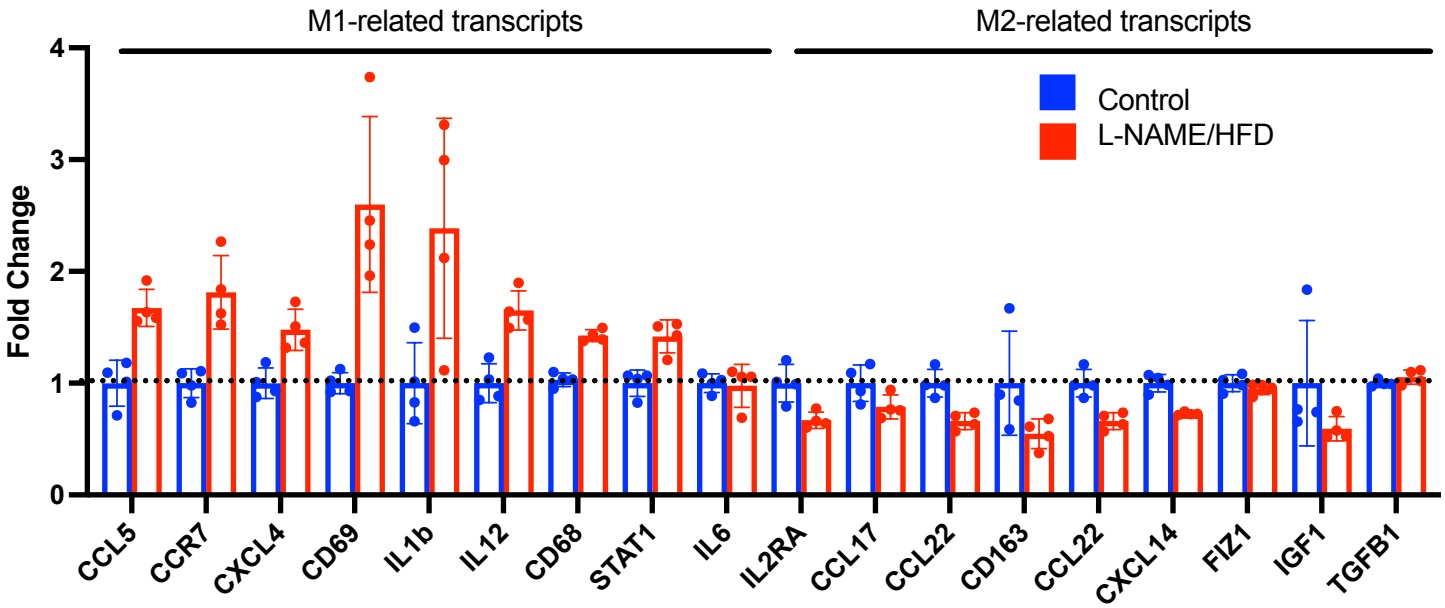


Supplementary Figure S5

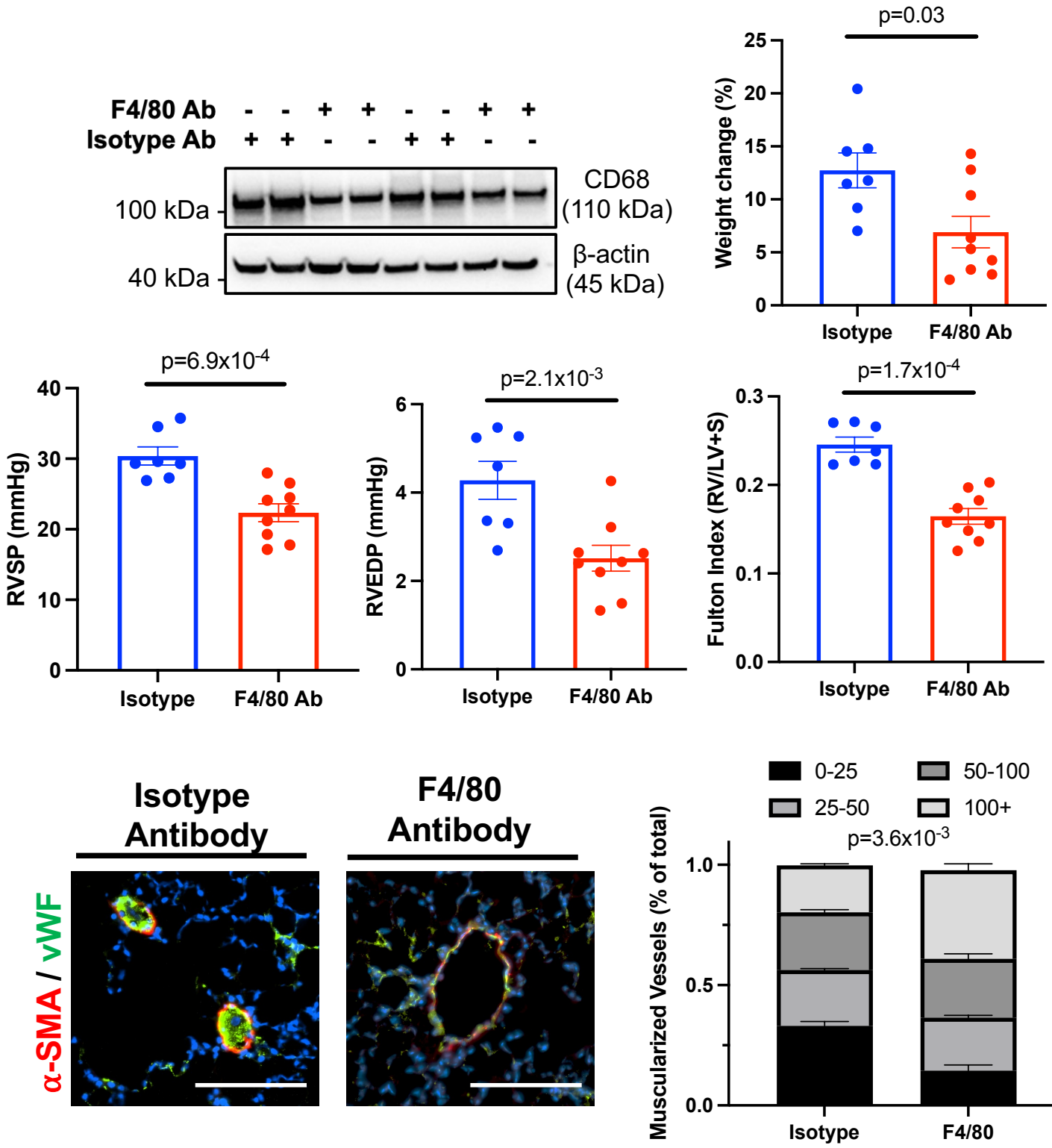




Supplementary Figure S6



Supplementary Figure S7



Supplementary Figure S8

Table S1. Over-represented gene ontologies in lungs of mice treated with L-NAME and high fat diet compared to control water and diet at 5 weeks.							
GO	Description	Size of Ontology	Overlapping Genes	Expected Overlap	Enrichment Ratio	P-value	FDR p-value
GO:0002682	regulation of immune system process	1230	32	9.853483715	3.247582371	2.45E-09	2.20E-05
GO:0002684	positive regulation of immune system process	879	26	7.041635923	3.692323813	7.45E-09	3.36E-05
GO:0002252	immune effector process	678	22	5.431432487	4.050496817	2.41E-08	7.24E-05
GO:0080134	regulation of response to stress	1227	30	9.829450827	3.052052503	3.45E-08	7.78E-05
GO:0050776	regulation of immune response	705	22	5.647728471	3.895371407	4.80E-08	8.66E-05
GO:0001775	cell activation	893	24	7.153789396	3.354865327	1.81E-07	2.16E-04
GO:0050865	regulation of cell activation	521	18	4.173711395	4.312708354	2.01E-07	2.16E-04
GO:0031347	regulation of defense response	580	19	4.646358174	4.089224138	2.05E-07	2.16E-04
GO:0042592	homeostatic process	1809	36	14.49183093	2.484158157	2.15E-07	2.16E-04
GO:0002697	regulation of immune effector process	373	15	2.988088964	5.019930859	3.33E-07	3.00E-04
GO:0002443	leukocyte mediated immunity	345	14	2.763782017	5.065522502	7.52E-07	5.90E-04
GO:0050778	positive regulation of immune response	572	18	4.582270475	3.928183658	7.85E-07	5.90E-04
GO:0032101	regulation of response to external stimulus	708	20	5.671761358	3.526241451	9.92E-07	6.88E-04
GO:0045321	leukocyte activation	784	21	6.280594498	3.343632519	1.22E-06	7.49E-04
GO:0006955	immune response	1293	28	10.35817434	2.703179061	1.25E-06	7.49E-04
GO:0022407	regulation of cell-cell adhesion	369	14	2.956045114	4.736057624	1.66E-06	8.94E-04
GO:0002694	regulation of leukocyte activation	482	16	3.861283862	4.143699498	1.69E-06	8.94E-04
GO:0002274	myeloid leukocyte activation	194	10	1.5541267	6.434481823	3.78E-06	0.001848402
GO:0002250	adaptive immune response	397	14	3.180352061	4.402028371	3.89E-06	0.001848402
GO:0051707	response to other organism	942	22	7.546326552	2.915325735	6.17E-06	0.002693107
GO:0043207	response to external biotic stimulus	945	22	7.570359439	2.906070732	6.49E-06	0.002693107
GO:0032102	negative regulation of response to external stimulus	305	12	2.443343523	4.911302847	6.57E-06	0.002693107
GO:0002764	immune response-regulating signaling pathway	310	12	2.483398335	4.832088285	7.75E-06	0.003036618
GO:0002449	lymphocyte mediated immunity	261	11	2.090861178	5.260990119	8.47E-06	0.003099585
GO:0002253	activation of immune response	368	13	2.948034152	4.409718249	8.59E-06	0.003099585
GO:0045088	regulation of innate immune response	267	11	2.138926953	5.142765622	1.05E-05	0.003440007
GO:0072676	lymphocyte migration	94	7	0.753030463	9.295772676	1.05E-05	0.003440007
GO:0009607	response to biotic stimulus	977	22	7.826710235	2.810887249	1.09E-05	0.003440007
GO:0012501	programmed cell death	1874	33	15.01254348	2.198161827	1.11E-05	0.003440007
GO:0030155	regulation of cell adhesion	639	17	5.119004954	3.320957911	1.49E-05	0.004488588

Table S2. Patient Characteristics of PH-HFpEF and Control Patients Who Underwent Autopsy		
	PH-HFpEF (n=8)	**Controls (n=8)**
Age (years)	63 ± 12	64 ± 9
Female Sex (n - %)	3 (37.5)	2 (25)
Comorbidities (n - %)		
Hypertension	7 (87.5)	6 (75)
Diabetes Mellitus	6 (75)	4 (50)
Obesity	6 (75)	4 (50)
Heart Failure	8 (100)	0 (0)
Atrial Fibrillation	3 (37.5)	0 (0)
Hyperlipidemia	7 (87.5)	4 (50)
LVEF (%)	64 ± 5	-
RA (mmHg)	13 ± 4	-
mPAP (mmHg)	44 ± 13	-
PAWP (mmHg)	25 ± 7	-
PVR (Wood Units)	4.4 ± 2.1	
Indirect Fick CO (L/min)	4.3 ± 0.4	-
Indirect Fick CI (L/min/m2)	2.3 ± 0.2	-

PH-HFpEF = pulmonary hypertension and heart failure with preserved ejection fraction
LVEF = left ventricular ejection fraction)
RA = right atrial pressure
mPAP = mean pulmonary arterial pressure
PAWP = pulmonary arterial wedge pressure
PVR = pulmonary vascular resistance