SUPPLEMENTAL MATERIALS

SUPPLEMENTARY TABLES

Supplementary Table 1: Echocardiographic parameters demonstrating development of DCM in $PLN^{R9C/+}$ mice.

Age	N	LVEDD (mm)	P-value vs. 8wk	P-value vs. 18wk	FS (%)	P-value vs. 8wk	P-value vs. 18wk
8wk	5	3.2 ± 0.1			48 ± 4		
18wk	7	4.0 ± 0.2	1.5×10^{-6}		27 ± 4	9.6×10^{-5}	
20.5wk	8	4.8 ± 0.4	1.8×10^{-6}	1.5×10^{-4}	12 ± 4	2.0×10^{-7}	9.9×10^{-6}

Age is average age at echocardiography in $PLN^{R9C/+}$ mice. LVEDD, LV end-diastolic diameter; FS, fractional shortening.

Supplementary Table 2: Expression of cell type-specific marker genes demonstrates purity of non-myocyte and cardiomyocyte cell populations.

Cell Compartment	Gene	Lineage	Wild Type	$PLN^{R9C/+}$
Cardiomyocyte-specific genes in non-myocyte cell compartment	Actc1	Cardiomyocyte	1.9%	2.6%
	Myh6	Cardiomyocyte	2.2%	2.6%
	Myh7	Cardiomyocyte	2.0%	2.5%
	Tnnc1	Cardiomyocyte	1.6%	2.9%
	Tnni3	Cardiomyocyte	1.8%	2.8%
	Tnnt2	Cardiomyocyte	1.9%	2.7%
Non-myocyte-specific genes in myocyte cell compartment	Postn	Fibroblast	1.0%	0.4%
	Ddr2	Fibroblast	0.3%	0.8%
	Thy1	Fibroblast	0.4%	0.7%
	Col3a1	Fibroblast	0.9%	0.6%
	Vim	Mesenchymal	0.3%	0.6%
	Eng	Endothelial	1.8%	0.8%
	Tie1	Endothelial	3.5%	2.4%
	Cdh5	Endothelial	3.2%	1.6%
	Tagln2	Vascular SMC	0.7%	0.8%
	Myh11	Vascular SMC	3.9%	3.6%
	Hcls1	Hematopoietic	0.7%	1.0%

Actc1 – cardiac α-actin, Myh6 – α-myosin heavy chain, Myh7 – β-myosin heavy chain, Tnnc1 – cardiac troponin C, Tnni3 –

cardiac troponin I, Tnnt2 – cardiac troponin T, Postn – periostin, Ddr2 – discoidin domain receptor 2, Thy1 – thymus cell antigen 1θ, Col3a1 – collagen α-1(III), Vim – vimentin, Eng – endoglin, Tie1 – tyrosine-protein kinase receptor 1, Cdh5 – cadherin 5, Tagln2 – transgelin 2, Myh11 – myosin heavy chain 11, Hcls1 – hematopoietic lineage cell-specific protein

Supplementary Table 3: see attached excel file. All differentially expressed genes pre-DCM (sheet 1), with DCM (sheet 2) and with HF (sheet 3) in non-myocytes and cardiomyocytes (sheets 4-6, respectively). Normalized reads = reads per million library.

Supplementary Table 4: see attached excel file. All significantly enriched (FDR <0.01) IPA canonical pathways in non-myocytes pre-DCM (sheet 1), with DCM (sheet 2), and with HF (sheet 3), and in cardiomyocytes pre-DCM (sheet 4), with DCM (sheet 5), and with HF (sheet 6). FDR represents Benjamini-Hochberg adjusted p-value corrected for multiple hypothesis testing. Note, pathways with FDR <0.05 are shown for PreDCM cardiomyocytes given paucity of significant pathways at FDR <0.01.

Supplementary Table 5: see attached excel file. All significantly enriched (FDR <0.01) GO terms in non-myocytes pre-DCM (sheet 1), with DCM (sheet 2), and with HF (sheet 3), and in cardiomyocytes pre-DCM (sheet 4), with DCM (sheet 5), and with HF (sheet 6). FDR represents Bonferroni adjusted p-value corrected for multiple hypothesis testing. Note, GO terms with FDR <0.05 are shown for PreDCM cardiomyocytes given lack of GO terms with FDR <0.01.

Supplementary Table 6: see attached excel file. All significantly enriched (FDR <0.01) KEGG pathways in non-myocytes pre-DCM (sheet 1), with DCM (sheet 2), and with HF (sheet 3), and in cardiomyocytes pre-DCM (sheet 4), with DCM (sheet 5), and with HF (sheet 6). FDR represents Bonferroni adjusted p-value corrected for multiple hypothesis testing. Note, KEGG pathways with nominal p-value <0.05 are shown for PreDCM myocytes given lack of pathways with FDR <0.05.

Supplementary Table 7: see attached excel file. All significantly enriched IPA canonical pathways (FDR <0.01; sheet 1), GO terms (FDR <0.01; sheet 2), and KEGG pathways (FDR <0.01; sheet 3) in non-myocytes and cardiomyocytes (sheets 4-6 respectively) from MHC $^{R403Q/+}$ mice with HCM.

Supplementary Table 8: see attached excel file. Significantly enriched IPA canonical pathways (FDR <0.05) derived from non-myocyte (sheets 1 & 2) and cardiomyocyte (sheets 3 & 4) genes that display predominant differential expression in either DCM or HCM. Pathways were identified using genes that were (1) only differentially expressed in DCM or HCM, or (2) genes that were differentially expressed in both cardiomyopathies but with substantially greater fold change in either DCM or HCM. Pathways highlighted in red were common to both cardiomyopathies. No pathways were significantly enriched in HCM myocytes.

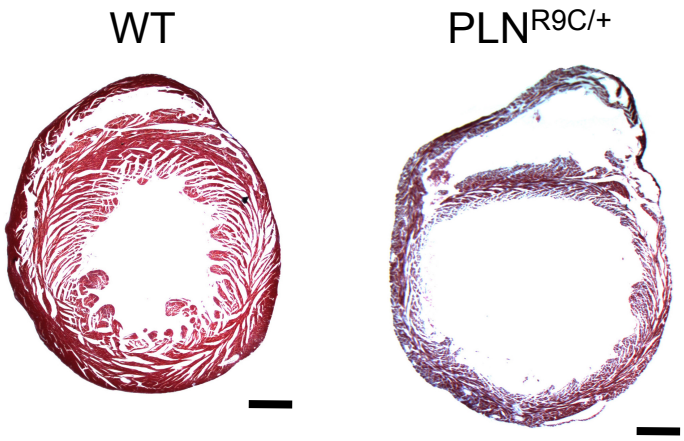
Supplementary Table 9: see attached excel file. Predicted upstream regulators (transcription factors and transcriptional coactivators, cytokines and growth factors, and kinases), that are common (sheet 1) or unique (sheets 2 & 3) to HCM and DCM non-myocytes (NM) and unique to HCM (sheet 4) and DCM (sheet 5) cardiomyocytes. Regulators were deemed significantly enriched if Z-score was $\geq \pm 2$ and p-value was <0.05.

Supplementary Table 10: see attached excel file. Expression levels of common cytokines in whole LV tissue with DCM and HCM, and age-matched wild type controls. Data expressed are normalized reads per million reads of the RNA-seq library.

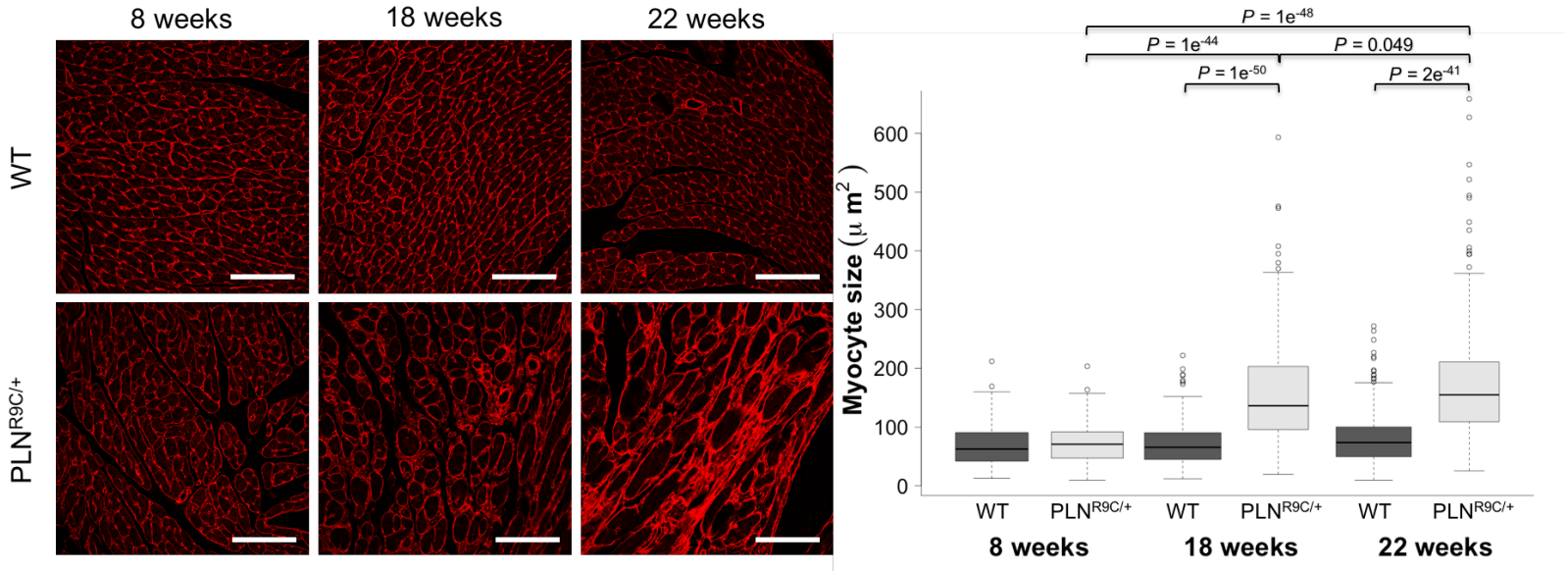
Supplementary Table 11: Q-PCR primers.

Gene	Forward Primer	Reverse Primer	Detection
Tgfb1	5' – GTGGAAATCAACGGGATCAG – 3'	5' – TTCTCTGTGGAGCTGAAGCA – 3'	SYBR green
Tgfb2	5' – CAGCAGTCATGCTCTTCAGC – 3'	5' – TCCTTGCATTCACACCTCCAG – 3'	SYBR green
Tgfb3	5' – GATGAGCACATAGCCAAGCA – 3'	5' – ATTGGGCTGAAAGGTGTGAC – 3'	SYBR green
Gdf15	5' – CCGAGAGGACTCGAACTCAG – 3'	5' – TTCAGGGGCCTAGTGATGTC – 3'	SYBR green
Polr3h	5' – ATGTTCGTGCTGGTGGAGAT – 3'	5' – GCGGAAATGGACTTTGGTGT – 3'	SYBR green
Ppara	ThermoFisher Scientific primer-probe set Mm00440939_m1		FAM
Ppargc1a	ThermoFisher Scientific primer-probe set Mm01208835_m1		FAM
Ppargc1b	ThermoFisher Scientific primer-probe set Mm00504730_m1		FAM
Tbx15	ThermoFisher Scientific primer-probe set Mm00447443_m1		FAM
Ndufs7	ThermoFisher Scientific primer-probe set Mm01144210_m1		FAM
Cox7a1	ThermoFisher Scientific primer-probe set Mm0438296_m1		FAM
Atp5a1	ThermoFisher Scientific primer-probe set Mm00431960_m1		FAM
Polr3h	ThermoFisher Scientific primer-probe set Mm00513483_m1		VIC

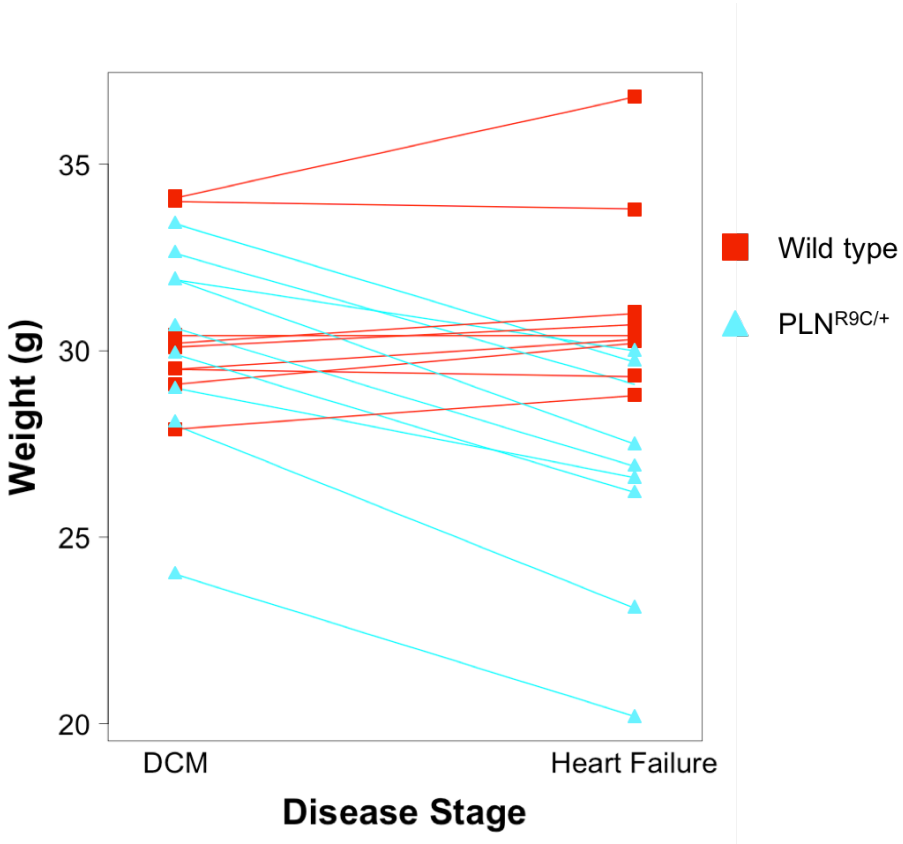
SUPPLEMENTARY FIGURES



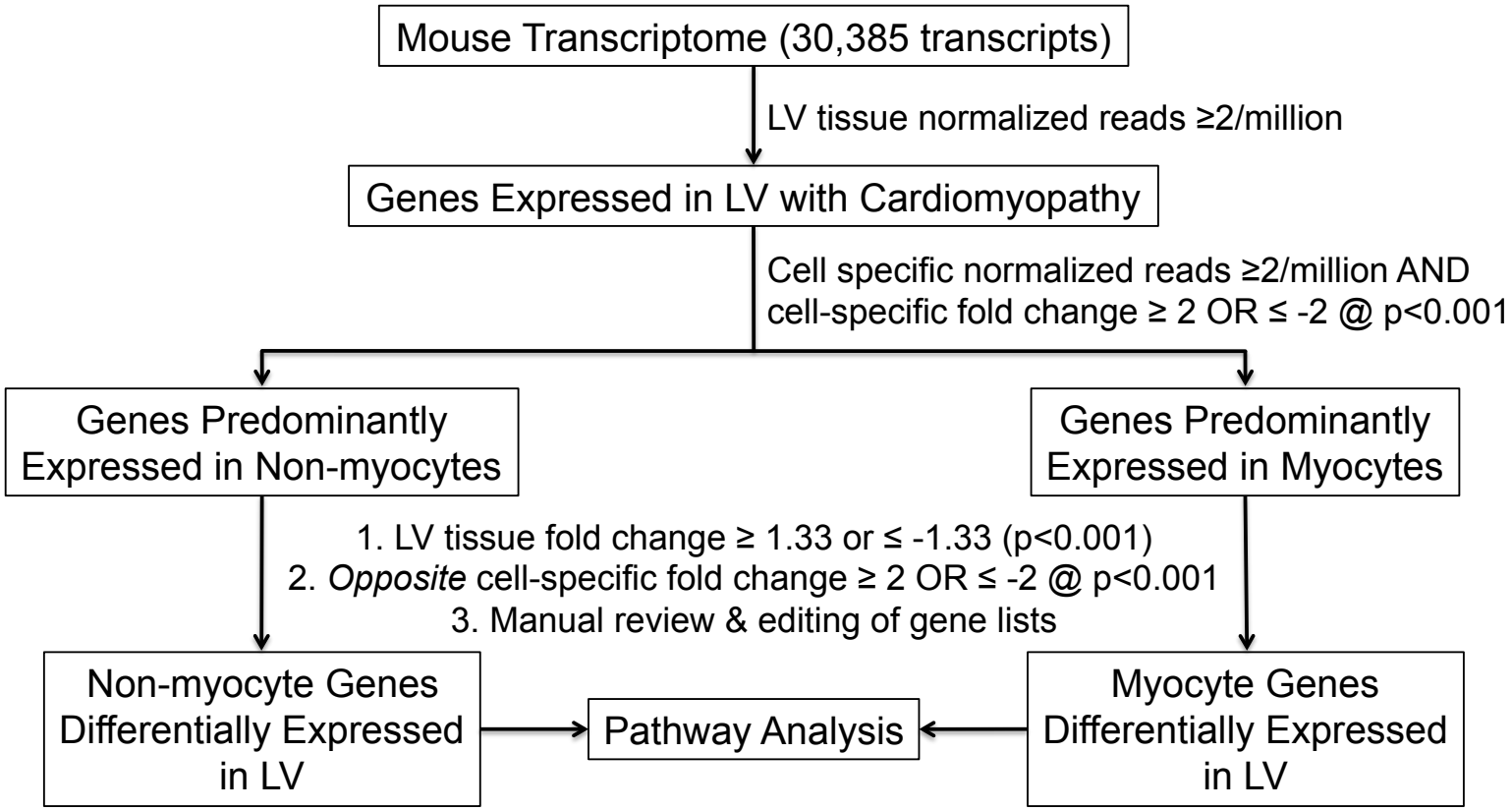
Supplementary Figure 1. PLN$^{R9C/+}$ mice exhibit biventricular dilatation and increased cardiac fibrosis. Heart cross-sections (scale bar=1mm) stained with Masson's trichrome at 22-weeks of age demonstrates profound biventricular dilatation and extensive biventricular fibrosis in PLN$^{R9C/+}$ mice compared to age-matched wild type control animals.



Supplementary Figure 2. PLN$^{R9C/+}$ mice develop cardiomyocyte hypertrophy with disease progression. Confocal microscopy (scale bar=75μm) and quantification of myocyte size at all stages from WGA-stained (red) LV sections. Data quantitated were individual LV slices at 10 levels (apex to base) from n=3 mice per group; *t*-test ± standard deviation.

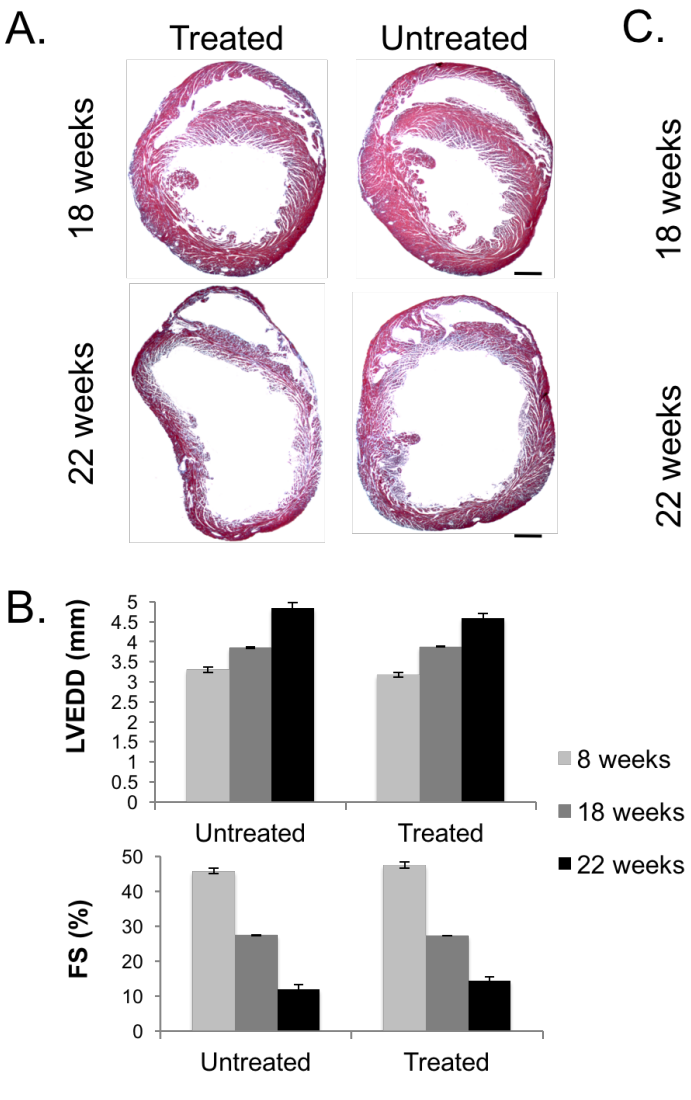


Supplementary Figure 3. PLN$^{R9C/+}$ mice develop pre-terminal cachexia. PLN$^{R9C/+}$ or wild type mice at 18-weeks-of-age (DCM stage) have similar body weight (30.1±2.7g vs. 30.5±2.0g, p=0.75) but with progression to HF, body weight falls significantly in PLN$^{R9C/+}$ mice (26.6±3.0g vs. 31.3±2.4g, p=0.003). PLN$^{R9C/+}$ mice were weighed between 21- and 25-weeks-of-age when objective evidence of overt HF was present; wild type mice were weighed at ~22-weeks-of-age.


Mouse Transcriptome (30,385 transcripts)
LV tissue normalized reads ≥2/million
Genes Expressed in LV with Cardiomyopathy
Cell specific normalized reads ≥2/million AND
cell-specific fold change ≥ 2 OR ≤ -2 @ p<0.001
Genes Predominantly Expressed in Non-myocytes
Genes Predominantly Expressed in Myocytes
1. LV tissue fold change ≥ 1.33 or ≤ -1.33 (p<0.001)
2. Opposite cell-specific fold change ≥ 2 OR ≤ -2 @ p<0.001
3. Manual review & editing of gene lists
Non-myocyte Genes Differentially Expressed in LV
Pathway Analysis
Myocyte Genes Differentially Expressed in LV

Supplementary Figure 4 (Above). Bioinformatics pipeline for analysis of RNA-seq gene data. Of all transcripts in mm9, genes were identified as being expressed in LV tissue if read depth was ≥2 reads/million reads in the RNA-seq library. Genes expressed in the heart were defined as predominantly expressed in non-myocytes or myocytes if read depth from isolated cell fractions was ≥2 reads/million reads in the RNA-seq library AND expression was ≥2 fold higher in non-myocyte or myocyte cells. These cell-specific gene lists were then edited to remove genes where differential expression was driven by changes in the opposite cell type and final lists were edited manually to ensure proper parsing of genes. Final gene lists included non-myocyte genes differentially expressed in whole LV tissue and myocyte genes differentially expressed in whole LV tissue. These gene lists were subjected to pathway analysis.

Supplementary Figure 5 (Below). Losartan does not prevent the onset of DCM in PLN$^{R9C/+}$ mice. (A) Masson trichrome (MT)-stained heart cross-sections demonstrate marked biventricular dilatation and fibrosis (scale bar=1mm). **(B)** LV end diastolic diameter (LVEDD) and fractional shortening (FS) by echocardiography demonstrate progressive dilatation and systolic dysfunction irrespective of treatment group (P=NS for all age-matched comparisons; n=4-5 mice per treatment group). **(C)** Light microscope images of MT-stained LV tissue demonstrates marked fibrosis. (20x magnification; scale bar=100μm). **(D)** Quantification of fibrotic area from LV at DCM stage (18 weeks) in treated and untreated animals (n=3).


A.
Treated
Untreated
18 weeks
22 weeks
B.
LVEDD (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Untreated
Treated
FS (%)
50
40
30
20
10
0
Untreated
Treated
8 weeks
18 weeks
22 weeks
C.
Treated
Untreated
18 weeks
22 weeks
D.
P = 1e-27
P = 1e-21
NS
Fibrotic Area (%)
10
8
6
4
2
0
WT
Untreated 18wks
Treated 18wks