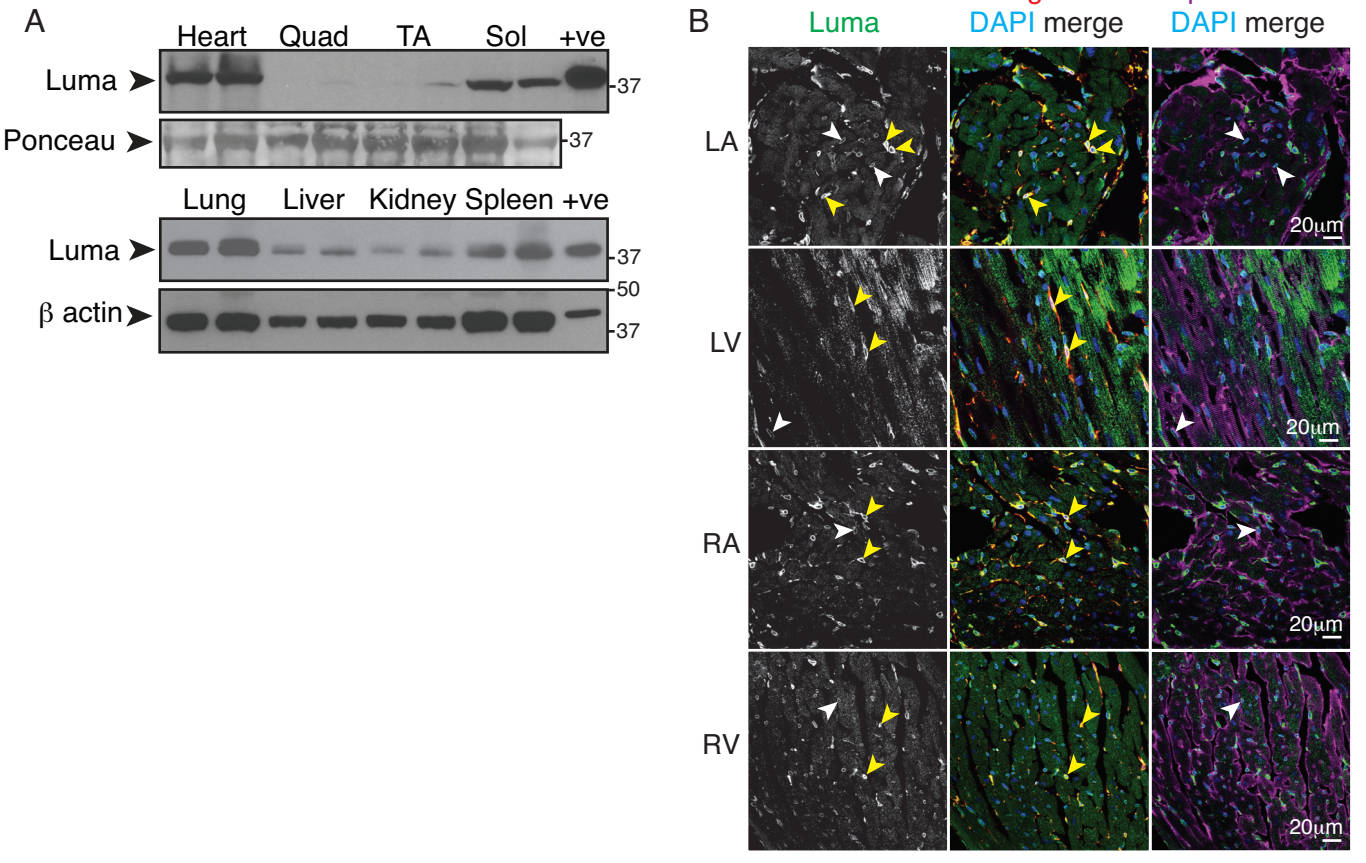
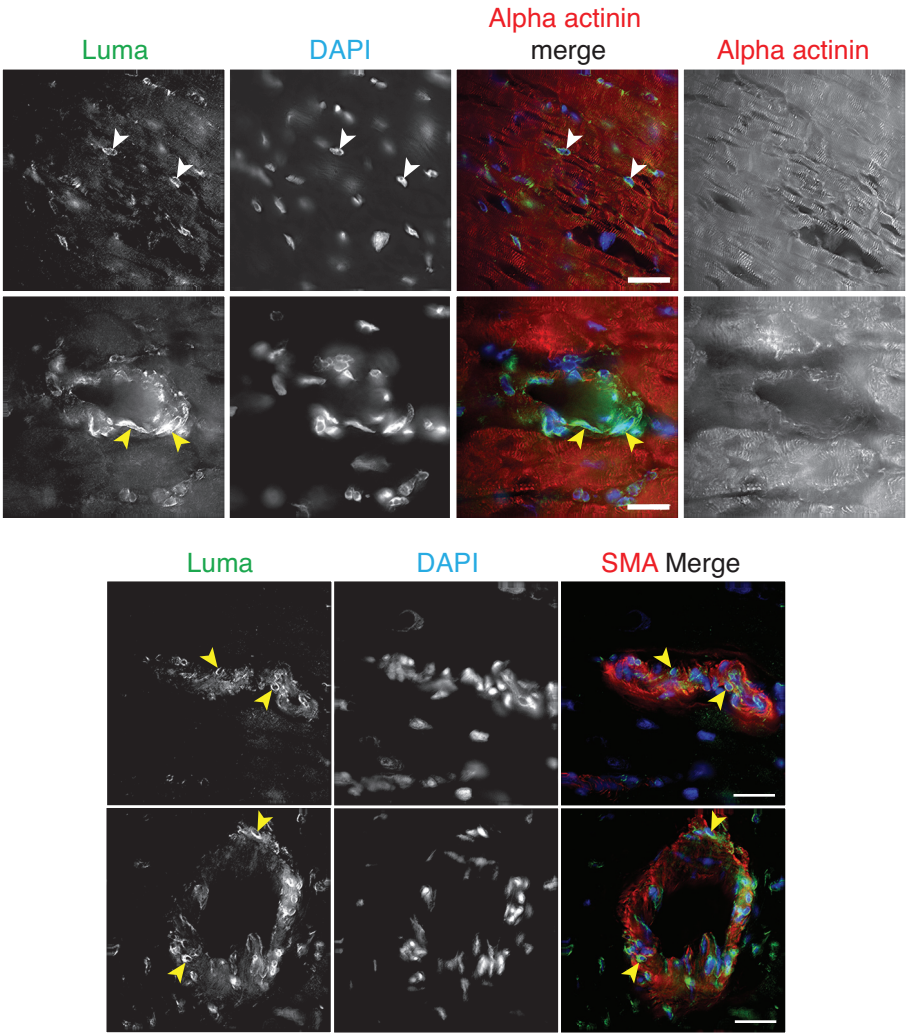
Supplementary Figure 1



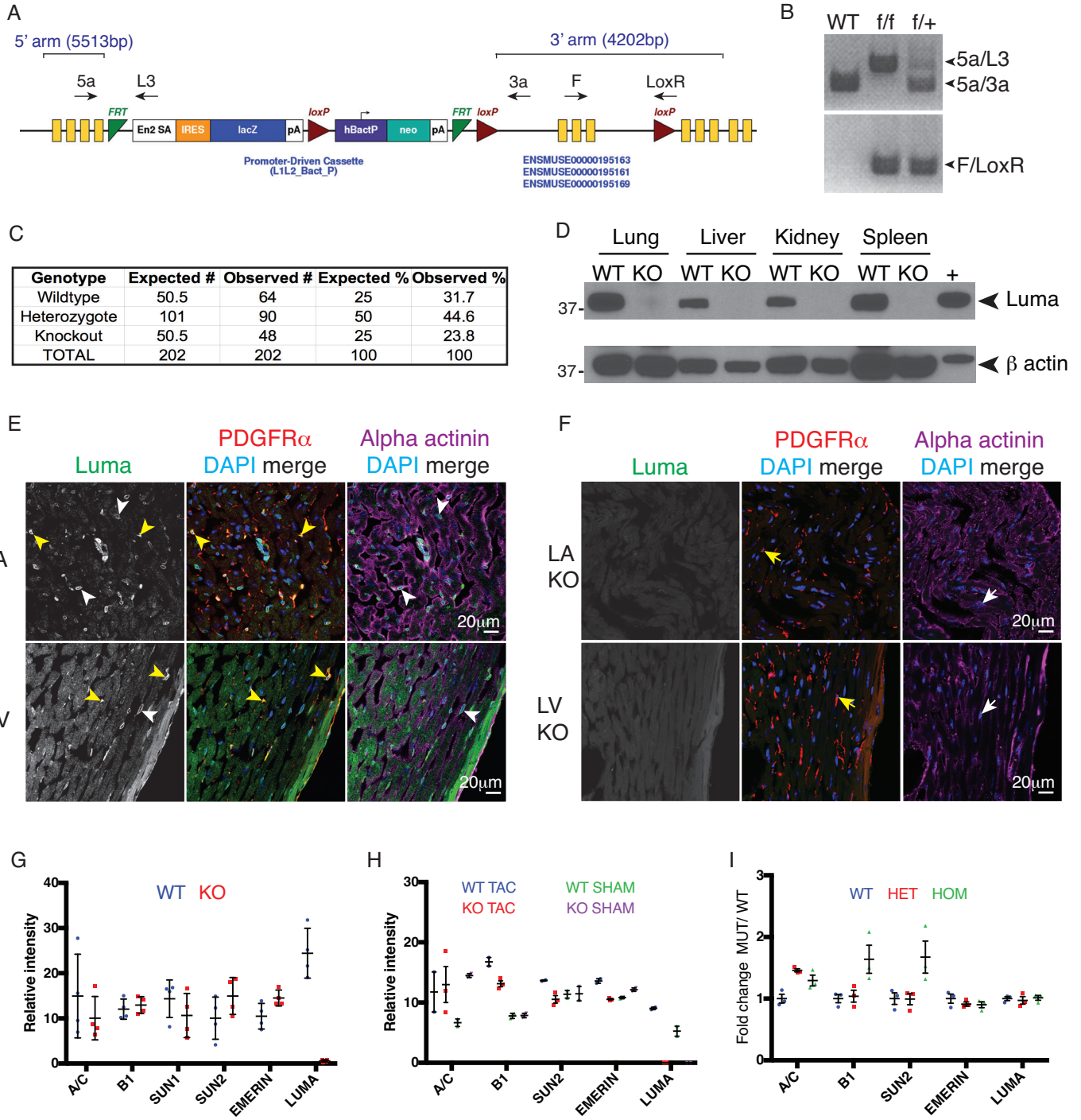
Supplementary Figure 1: Luma is ubiquitously expressed and is found in different regions of adult mouse hearts. (A) Western blot analysis showing tissue distribution of Luma in wildtype adult mouse tissues at 88w. Quad, quadriceps; TA, tibialis anterior; Sol, soleus; +ve, COS7 cells transiently expressing a HA-conjugated version of Luma. Arrowheads show the predicted MW of the indicated proteins. MW is denoted in kDa. (n=2/ genotype). **(B)** Immunofluorescence analysis of adult hearts isolated from Collagen 1a1-GFP expressing mice. Sections were stained using antibodies against Luma (green) and alpha actinin to mark cardiomyocytes (magenta). Collagen 1a1-GFP was used to mark fibroblasts (red). DNA is stained with DAPI (blue). Note that Luma was expressed in all fibroblasts (yellow arrowheads), and some cardiomyocytes (white arrowheads) throughout the heart. Left Atrium, Ventricle (LA, LV), Right Atrium, Ventricle (RA, RV). Images are representative of 3 mouse hearts per genotype.

Supplementary Figure 2



Supplementary Figure 2: Luma is expressed in the same cell types in human and mouse myocardium. Immunofluorescence analysis of normal human myocardium stained with antibodies raised against Luma (green), DAPI (blue), and alpha actinin (red) (top panel), or smooth muscle actin (SMA, red) (bottom panel). Note that Luma is predominantly expressed in cells in between myocytes (white arrowheads) and smooth muscle cells (yellow arrowheads). Scale bars, 20 μm. Images are representative of 3 sections from a human heart.

Supplementary Figure 3



Genotype	Expected #	Observed #	Expected %	Observed %
Wildtype	50.5	64	25	31.7
Heterozygote	101	90	50	44.6
Knockout	50.5	48	25	23.8
TOTAL	202	202	100	100

Supplementary Figure 3: Generation of Luma knockout mice. (A) Targeting construct used to generate Luma knockout mice. Green and red triangles denote FRT and LoxP sites, respectively. Yellow rectangles denote exons; arrows indicate locations of genotyping primers used. **(B)** Genotyping of wildtype (WT, +) and floxed (f) mice by PCR analysis using primers noted in (A). **(C)** Table of Mendelian ratios from crossing heterozygote knockout mice. **(D)** Western blot analysis of Luma in tissues from wildtype (WT) and Luma knockout (KO) mice. + denotes lysate from cells overexpressing HA-tagged Luma used as a positive control. Beta actin serves as a loading control. Arrowheads show the predicted MW of the indicated proteins. MW is denoted in kDa. **(E, F)** Immunofluorescence analysis of **(E)** wildtype and **(F)** Luma KO hearts. Sections were stained with antibodies directed against Luma (green), PDGFR alpha (PDGFRα) to mark fibroblasts (red), and alpha actinin to mark cardiomyocytes (magenta). DAPI, blue. Note that Luma was expressed in both fibroblasts and cardiomyocytes (yellow and white arrowheads, respectively) and Luma staining was abolished in Luma KO hearts (arrows). LA, left atrium; LV, left ventricle. Images are representative of 3 mouse hearts per genotype. (**G, H, I**) Quantification of Western blots from Figures 3A, 4H, 6I respectively.

Supplementary Figure 4

A

PAM Leu

Target sequence 5'...TTTGCCTTCTGTGTGGCCACTTTAC_TGACCCTGTTGACTGTGGCT...3'

Wildtype sequence 5'...TTTGCCTTCTGTGTGGCCACTTCCC_TGACCCTGTTGACTGTGGCT...3'

Ser

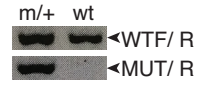
B

CrRNA: CTTCCCTGACCCTGTTGACT

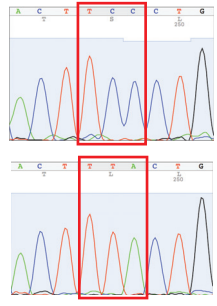
Donor oligo: TCC to TTA

5'-GGTTTCCTGTCTTCCGAGACCTTGTCAACATTGGCC
TGAAAGCCTTTGCCTTCTGTGTGGCCACTTTACTGACC
CTGTTGACTGTGGCTGCTGGCTGGCTCTTCTACCGAC
CGCTGTGGGCTGCTCTAATCGG-3'

C



D



E

Outcome	Exp #	Obs #	Exp %	Obs %
WT	39	47	25	31
M/+	77	72	50	47
M/M	39	35	25	23
TOTAL	154	154	100	100

Supplementary Figure 4: Luma S358L generation and S358L homozygous mutants were found at Mendelian ratios at weaning. (A) Sequence of the targeted region in the Luma gene.The Protospacer Adjacent Motif (PAM) is shown in blue. **(B)** CrRNA sequences used for targeting the Luma gene and the donor oligo used to replace nucleotides TCC (serine) with TTA (leucine). **(C)** Genotyping of mutant tails using S358L-specific primers. Note the presence of wildtype and mutant bands in the heterozygous mutant (m/+) compared to a single wildtype band in the wildtype mice (wt). **(D)** DNA sequencing confirming the successful mutation from TCC to TTA. **(E)** Table of Mendelian ratios at weaning after heterozygous crosses. Note that S358L homozygous mice were present at Mendelian ratios.

Supplementary Tables

Supplementary Table 1: Antibody table

Antibody	Source	Catalogue number
Luma	Abcam	EPR15378B
Sun1	Millipore	ABT285
Sun1	Abcam	ab103021
Sun2	Epitomics	EPR6557
Emerin	Santa Cruz	SC15378
Lamin A/C	Larry Gerace	n/a
Lamin B1	Larry Gerace	n/a
Lamin B2	Larry Gerace	n/a
Alpha actinin	Sigma	A7811
CD31	BD Biosciences	550274
Alpha smooth muscle actin	Abcam	ab5694
PDGFRa	R&D	AF1062
Beta actin	Sigma	A5441
GFP	Abcam	AB13970
GAPDH	Santa Cruz	SC32233

Supplementary Table 2: Primer sequences used for qRT-PCR analysis

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Col1α1	TCACCAAACTCAGAAGATGTAGGA	GACCAGGAGGACCAGGAAG
Col3α1	ACAGCAGTCCAACGTAGATGAAT	TCACAGATTATGTCATCGCAAAG
ANP	GATAGATGAAGGCAGGAAGCCGC	AGGATTGGAGCCCAGAGTGGACTAGG
BNP	TGTTTCTGCTTTTCCTTTATCTGTC	CTCCGACTTTTCTCTTATCAGCTC
MYH6	CTGCTGGAGAGGTTATTCCTCG	GGAAGAGTGAGCGGCGCATCAAGG
MYH7	TGCAAAGGCTCCAGGTCTGAGGGC	GCCAACACCAACCTGTCCAAGTTC
18S	GGAAGGGCACCACCAGGAGT	TGCAGCCCCGGACATCTAAG

Col1α1, collagen alpha 1 type I; *Col3α1*, collagen alpha1 type III; *ANP*, atrial natriuretic peptide; *BNP*, B-type natriuretic peptide; *MYH6*, alpha myosin heavy chain; MYH7, beta myosin heavy chain; *18S*, 18S ribosomal RNA.

Supplementary figure legends

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