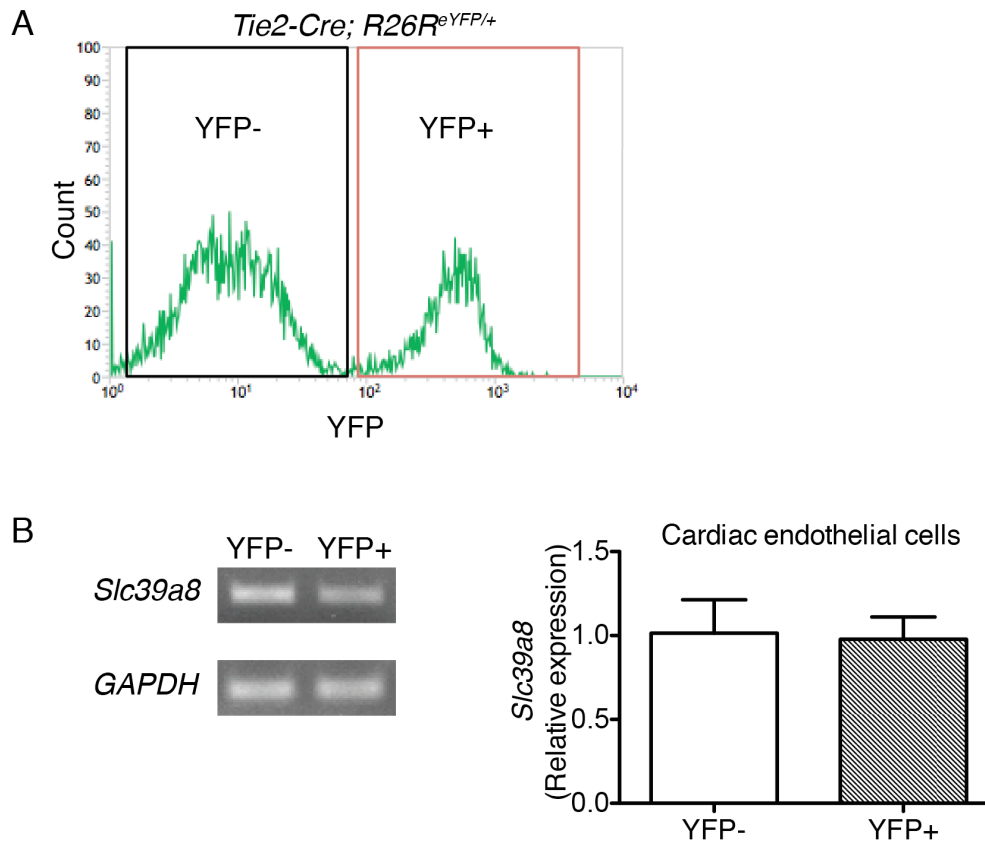
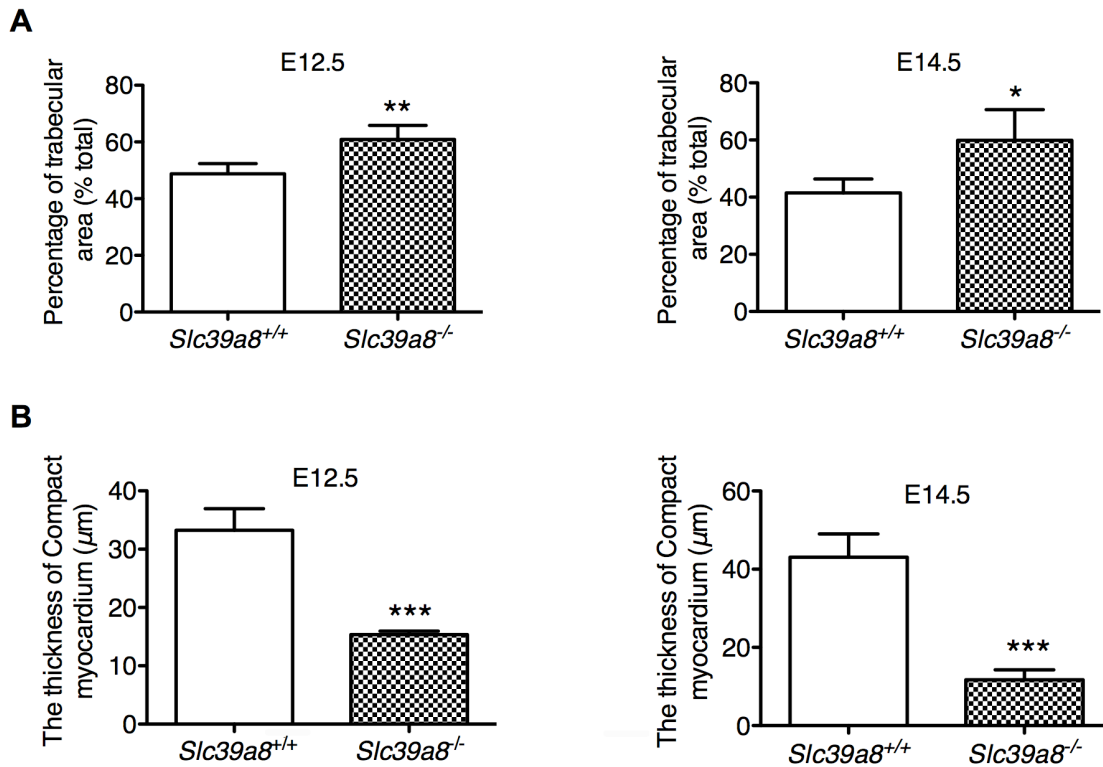


Supplemental materials



Supplemental Figure 1 *Slc39a8* is expressed in cardiac endothelial cells. (A)

Fluorescence-activated cell sorting (FACS) showed a clear separation of YFP- and YFP+ cell populations of E12.5 *Tie2-Cre; R26R^{eYFP/+}* hearts. (B) *Slc39a8* expression in YFP- and YFP+ population was analyzed by both regular RT-PCR (CT numbers for *GAPDH* and *Slc39a8* are 22 and 28 respectively) and qRT-PCR (n=3 per group).



Supplemental Figure 2 Quantification of trabecular area and the thickness of

compact myocardium in *Slc39a8*^{-/-} and *Slc39a8*^{+/+} hearts. H&E pictures of both

Slc39a8^{-/-} and *Slc39a8*^{+/+} hearts at similar anatomical levels were chosen for the

quantification using ImageJ. (A) Quantification of the percentage of ventricular

trabecular area over total ventricular myocardial area in *Slc39a8*^{+/+} and *Slc39a8*^{-/-} hearts

at E12.5 and E14.5. n=4 for E12.5 and n=3 for E14.5 per genotype. **P*<0.05. ***P*<0.01.

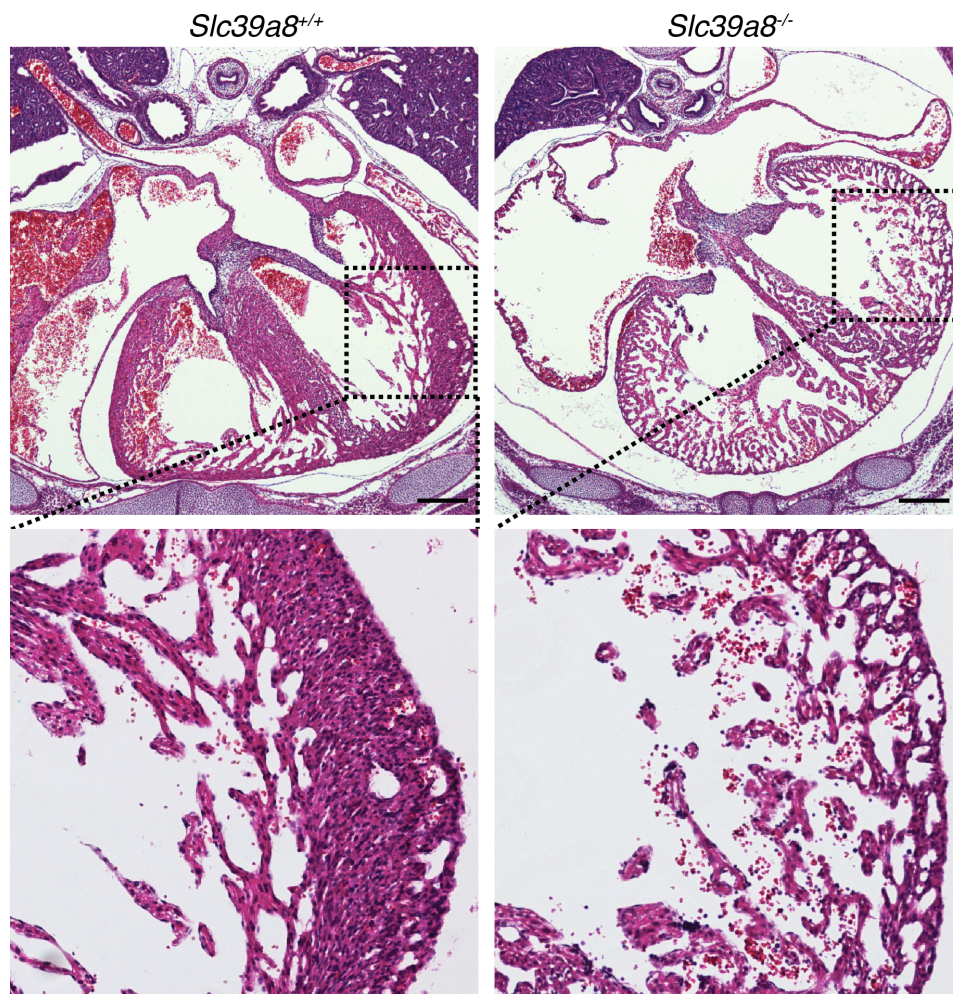
Note: there were no significant differences in total ventricular areas between *Slc39a8*^{+/+}

and *Slc39a8*^{-/-} hearts at either E12.5 (2.1±1.1 mm² vs. 1.8±0.7 mm²) or E14.5 (9.2±1.7

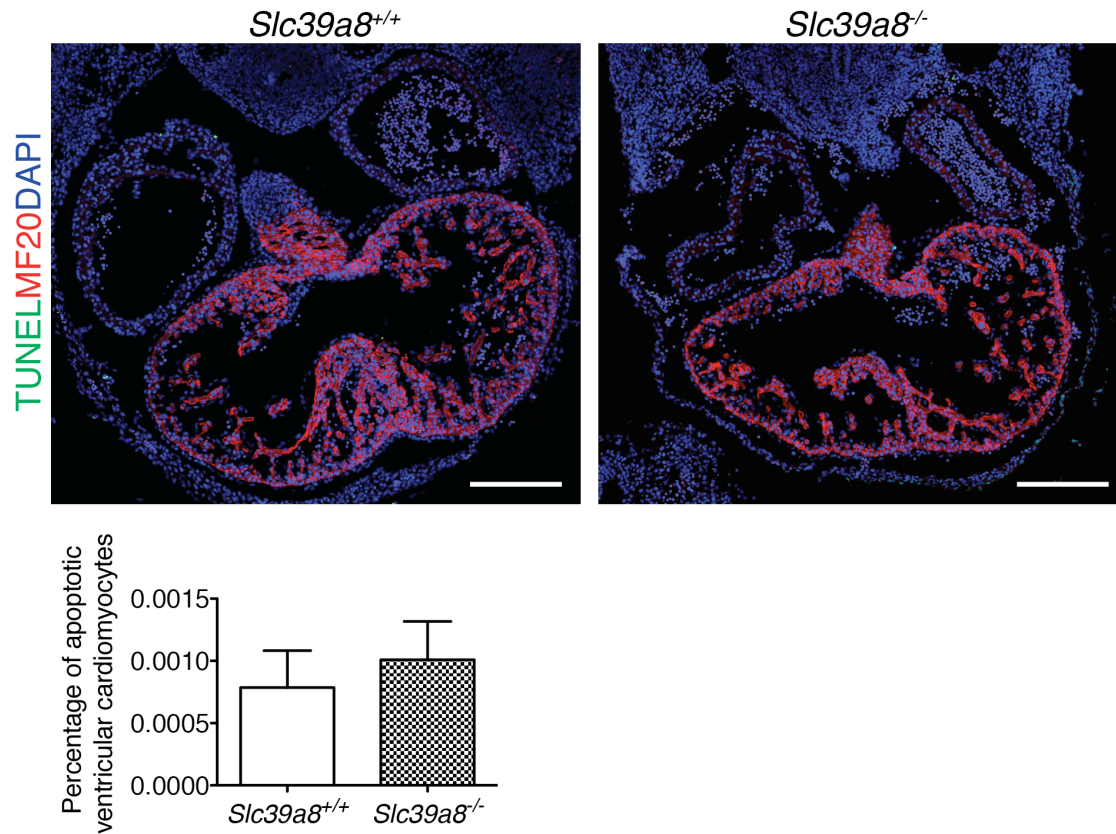
mm² vs. 8.7±0.09 mm²). (B) Quantification of the thickness of compact myocardium in

Slc39a8^{+/+} and *Slc39a8*^{-/-} hearts at E12.5 and E14.5. n=4 per genotype at E12.5; n=4

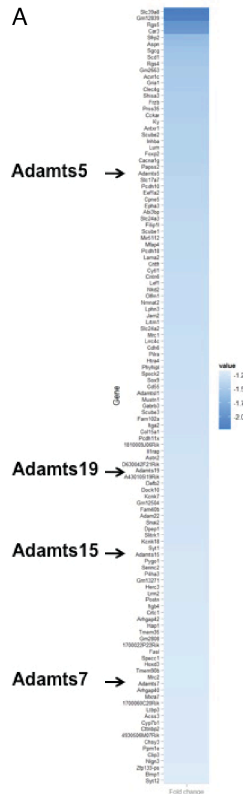
per genotype at E14.5. ****P*<0.001.



Supplemental Figure 3 Severe cardiac LVNC phenotype in E16.5 *Slc39a8*^{-/-} hearts.
H&E staining of E16.5 *Slc39a8*^{+/+} and *Slc39a8*^{-/-} hearts. Compared to the littermate wild type control, the E16.5 *Slc39a8*^{-/-} heart exhibited remarkable hypertrabeculation and thin ventricular compact myocardium phenotype. Scale bars, 250 μ m.



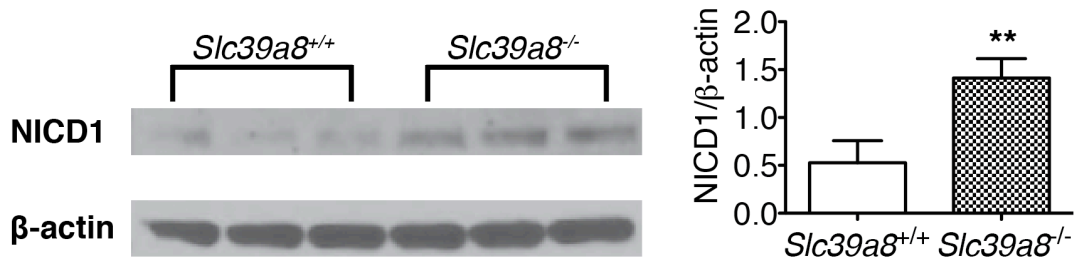
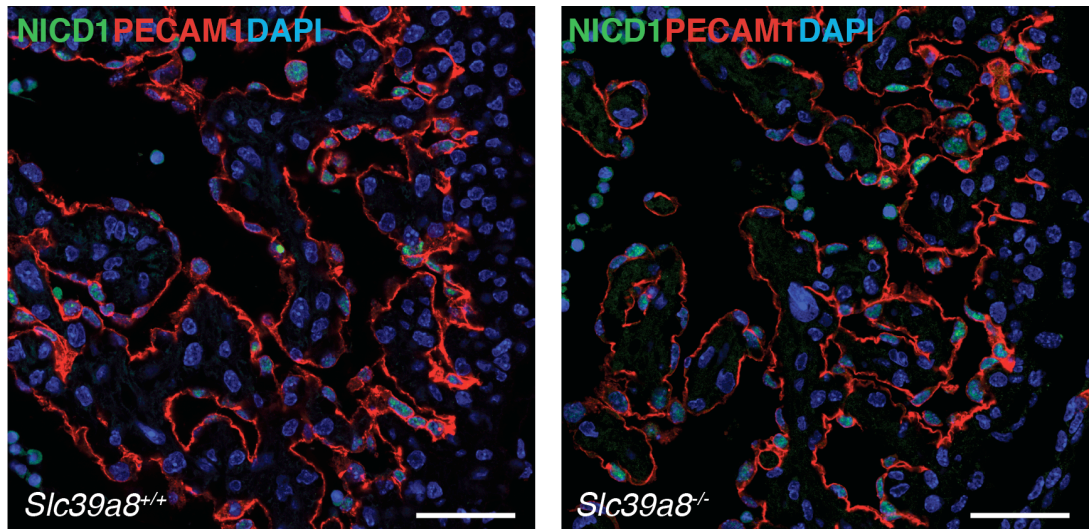
Supplemental Figure 4 Apoptosis in E12.5 *Slc39a8*^{+/+} and *Slc39a8*^{-/-} hearts. TUNEL staining showed that the percentage of apoptotic ventricular cardiomyocytes was not different between *Slc39a8*^{+/+} and *Slc39a8*^{-/-} hearts at E12.5 ($P=0.3058$). $n=5$ for *Slc39a8*^{+/+} hearts; $n=4$ for *Slc39a8*^{-/-} hearts. Scale bars, 250 μm .



B Functional annotation of top down-regulated genes in E12.5 *Slc39a8*^{-/-} hearts

Term	Gene count	P value
Proteinaceous extracellular matrix	17	2.0E-8
Extracellular matrix	17	3.6E-8
Calcium ion binding	24	3.7E-8
Cell adhesion	19	8.1E-7
Biological adhesion	19	8.3E-6
Ion binding	53	1.4E-6
Metal ion binding	52	1.9E-6
Cation binding	52	2.5E-6
Extracellular region part	23	3.3E-5
Metallopeptidase activity	9	7.6E-5

Supplemental Figure 5 Microarray and functional annotation analysis on E12.5 *Slc39a8*^{+/+} and *Slc39a8*^{-/-} hearts. (A) Microarray demonstrated that *Adamts* family members were down-regulated in *Slc39a8*^{-/-} hearts compared to *Slc39a8*^{+/+} hearts at E12.5. n=3 for each genotype. (B) Functional annotation of top down-regulated genes using DAVID in E12.5 *Slc39a8*^{-/-} hearts demonstrated that extracellular matrix and metallopeptidase are among the top down-regulated gene categories.



Supplemental Figure 6 *Slc39a8* deletion leads to elevated Notch signaling activity.

IF staining and western blot showed increased NICD1 in E12.5 *Slc39a8*^{-/-} hearts compared to *Slc39a8*^{+/+} hearts. ** $P < 0.01$. Scale bars, 50 μ m.

Supplemental Table I Genotype distribution of *Slc39a8* progeny (mating: *Slc39a8*^{+/-} x *Slc39a8*^{+/-})

Stage	Total number of progeny examined	Number of <i>Slc39a8</i> ^{-/-} mice (%)	<i>P</i> value (Chi-square test)
E12.5	93	20 (21.51%)	0.4709
E14.5	244	49 (20.08%)	0.07
E16.5	45	5 (11.11%)	0.0314
Weaning	104	0	2.4E-9

P value was calculated using Chi-square test which compares the observed genotype distribution and the expected Mendelian ratio (25%).

Supplemental Table II Primers for real-time PCR analysis

Gene	Sequence
<i>Slc39a8</i> -forward	CAACGCAAAGCCCAGTCTTT
<i>Slc39a8</i> -reverse	GCGTTTGAGAAAAGAGTCCCAA
<i>Gapdh</i> -forward	TGTGTCCGTCGTGGATCTGA
<i>Gapdh</i> -reverse	CCTGCTTCACCACCTTCTTGAT
<i>Adamts1</i> -forward	CTCTCACCTTCGGAATTTCTG
<i>Adamts1</i> -reverse	GGAGCCACATAAATCCTGTCTG
<i>Adamts5</i> -forward	CGACCCTCAAGAACTTTTGC
<i>Adamts5</i> -reverse	CGTCATGAGAAAGGCCAAGT
<i>Adamts7</i> -forward	TCATGAACATGGTGGCTGGACTCT
<i>Adamts7</i> -reverse	AGTCTCTTCGGCATGGTGTGTGAT

<i>Adamts15</i> -forward	TCTACACCTGACGCCAGATG
<i>Adamts15</i> -reverse	TCACATACCCGGAATAGAAGCA
<i>Adamts19</i> -forward	CCAGATGCCTCCTGCTTTTAC
<i>Adamts19</i> -reverse	GGTGCGGGTGACCTATGAT
<i>Bmp10</i> -forward	ACCAAGCTGAGGACACCGGAAGG
<i>Bmp10</i> -reverse	CTTCGTGGGCACACAGCAGGCTTT
<i>ADAMTS1</i> -forward	CAAAGGCATTGGCTACTTCTTC
<i>ADAMTS1</i> -reverse	TACACACTGTCCTTGACACACAG
GAPDH-forward	GCACCGTCAAGGCTGAGAAC
GAPDH-reverse	TGGTGAAGACGCCAGTGGA