Table S1. Supplement to Materials and Methods.

Table includes buffer recipes, antibodies for western blot, antibodies for immunofluorescence microscopy, and primer sequences.

| Method                  | Solution                      | Composition                                                                 |
|-------------------------|-------------------------------|-------------------------------------------------------------------------------|
| Myofiber Mechanics      | pCa 9.0 Relaxing Buffer       | in mM: 7 EGTA, 100 BES, 0.017 CaCl2, 5.491 MgCl2, 5 DTT, 15 creatine phosphate, and 4.655 ATP; pH adjusted to 7.0 with KOH and ionic strength set to 180 with K-propionate |
| Myofiber Mechanics      | pCa4.5 Activating Buffer      | in mM: 7 EGTA, 100 BES, 7.005 CaCl2, 5.294 MgCl2, 5 DTT, 15 creatine phosphate, and 4.724 ATP; pH adjusted to 7.0 with KOH and ionic strength set to 180 with K-propionate |
| Western Blot            | Tissue Lysis Buffer           | in mM: 50 Tris, 150 NaCl, 10 NaF, 5 EGTA, 5 EDTA, 10 NaF, 1 PMSF, 1 sodium orthovanadate, 12 sodium deoxycholate, with 0.5% (w/v) Triton X-100, 1% (w/v) SDS, and protease inhibitor (Roche, 11873580001) |
| Western Blot            | Blocking Buffer               | in mM: 20 Tris, 500 NaCl, 0.05% (v/v) Tween-20, with 5% (w/v) nonfat dry milk |
| Microtubule Assay       | Microtubule Stabilization Buffer | in mM: 25 Na4P2O7, 10 Na2HPO4, 0.5 EGTA, 0.5 MgSO4, 0.5 GTP, 1.0 ATP, and with (% v/v), 50% glycerol, 5% DMSO, 1% NP40 alternative (Millipore, 492018), and with protease/phosphatase inhibitor (Pierce, A32961) |
| Immunofluorescence      | Permeabilization buffer       | in mM: 20 Tris, 500 NaCl, with 0.2% v/v TritonX-100                           |
| Immunofluorescence      | Blocking buffer               | in mM: 20 Tris, 500 NaCl, with 0.02% (v/v) TritonX-100, 2% (w/v) BSA, and 10% (v/v) goat serum |
Electrophysiology  | Internal Solution  | in mM: 10 NaCl, 90 aspartic acid, 70 CsOH, 10 EGTA, 20 CsCl, 10 HEPES, and pH adjusted to 7.35 using CsOH
---|---|---
Electrophysiology  | Extracellular Solution  | in mM: 145 NaCl, 4.5 KCl, 10 HEPES, 1 MgCl2, 1.5 CaCl2, and pH adjusted to 7.35 using CsOH

Cell Culture  | Cardiomyocyte Media  | DMEM/F12 media supplemented with 5% horse serum, 10 mM HEPES, 1% ITS, 15 μg/mL Gentamycin, and 100 μM BRDU

### Antibodies for Western Blot

| Antibody   | Source              | Product # | Dilution Ratio |
|------------|---------------------|-----------|----------------|
| acetyl-TUBA| Cell Signaling      | 12152S    | 1:1000         |
| ACTN2      | Sigma               | A7732     | 1:1000         |
| ATP2A2     | Thermo              | 2a7-a1    | 1:1000         |
| CDH2       | BD Transduction Labs| 610920    | 1:1000         |
| CTNNB1     | Sigma               | PLA0230   | 1:2000         |
| DES        | DSHB                | D3        | 1:50           |
| dTyr-TUBA  | Abcam               | ab48389   | 1:1000         |
| GAPDH      | Santa Cruz          | sc-25778  | 1:3000         |
| GJA1       | Abclonal            | A11752    | 1:1000         |
| GJA5       | Abclonal            | A11752    | 1:500          |
| ITGB1D     | Abcam               | ab8991    | 1:500          |
| JPH2       | Santa Cruz          | sc-51313  | 1:2000         |
| Protein | Supplier       | Catalog Number | Dilution |
|---------|----------------|----------------|----------|
| JUP     | Proteintech    | 11146-1-AP     | 1:1000   |
| KCNH2   | Alomone        | APC-062        | 1:500    |
| MAPRE1  | Sigma          | e3406          | 1:1000   |
| MAPRE1  | BD Transduction Labs | 610535 | 1:1000 |
| MLC1    | DSHB           | f310           | 1:500    |
| MYBPC3  | S. Sadayappan, PMID# 19237661 | custom | 1:1000 |
| p282-MYBPC3 | S. Sadayappan, PMID# 19237661 | custom | 1:1000 |
| S100A1  | Proteintech    | 16027-1-AP     | 1:500    |
| SCN5A   | Alomone        | 493-511        | 1:500    |
| SORBS1  | Aviva          | ARP51833_P050  | 1:500    |
| SORBS2  | Proteintech    | 24643-1-AP     | 1:1000   |
| SORBS2  | Sigma          | SAB4200183     | 1:1000   |
| TJP1    | Proteintech    | 21773-1-AP     | 1:500    |
| p22/23-TNNI3 | Sigma           | sab4504001    | 1:1000   |
| TNNI3   | Sigma          | WH0007137M4    | 1:1000   |
| TPM1    | DSHB           | CH1            | 1:50     |
| TUBA    | Cell Signaling | 3873S          | 1:1000   |
| TUBB    | DSHB           | E7             | 1:500    |
| VCL     | Cell Signaling | 13901          | 1:1000   |
| anti-mouse-HRP | Jackson Immunoresearch | 115-035-146 | 1:25,000 |
| anti-rabbit-HRP | Jackson Immunoresearch | 111-035-144 | 1:25,000 |
| anti-mouse-DyLight-680 | Invitrogen   | 35519          | 1:5000   |
| anti-rabbit-DyLight-800 | Invitrogen   | sa5-10036      | 1:5000   |
## Antibodies for Immunofluorescent Microscopy

| Antibody    | Source          | Product #     | Dilution/final concentration |
|-------------|-----------------|---------------|------------------------------|
| ACTN2       | sigma           | A7732         | 1:1000                       |
| ACTN2       | Sigma           | A7732         | 1:1000                       |
| GJA1        | Abclonal        | A11752        | 1:100                        |
| SORBS2      | Proteintech     | 24643-1-AP    | 1:500                        |
| SORBS2      | Sigma           | SAB4200183    | 1:500                        |
| VCL         | Cell Signaling  | 13901         | 1:500                        |
| WGA-af488   | Thermo Fisher Sci./Invitrogen-Life Techn. | W11261 | 10 µg/mL                     |
| Phalloidin-af568 | Thermo Fisher Sci./Invitrogen-Life Techn. | A12380 | ≈ 66 nM                     |
| anti-mouse-488 | Thermo Fisher Sci./Invitrogen-Life Techn. | A11017 | 1:1000                       |
| anti-mouse-568 | Thermo Fisher Sci./Invitrogen-Life Techn. | A11019 | 1:1000                       |
| anti-mouse-647 | Thermo Fisher Sci./Invitrogen-Life Techn. | A21237 | 1:1000                       |
| anti-rabbit-488 | Thermo Fisher Sci./Invitrogen-Life Techn. | A11070 | 1:1000                       |
| anti-rabbit-568 | Thermo Fisher Sci./Invitrogen-Life Techn. | A21069 | 1:1000                       |
| anti-rabbit-647 | Thermo Fisher Sci./Invitrogen-Life Techn. | A21246 | 1:1000                       |
| anti-rat-488 | Thermo Fisher Sci./Invitrogen-Life Techn. | A11006 | 1:1000                       |
| Reson   | Primer          | Sequence                                                                 |
|---------|-----------------|--------------------------------------------------------------------------|
| Genotyping | SORBS2gtF1     | GCAGCCATCGTCATGCTTTG          |
| Genotyping | SORBS2gtF2     | CACCTTAGGTCTAGGGATCGAGCTC     |
| Genotyping | aMHCgtF1       | CTAGCCCACACCAGAAATGACAGAC     |
| Genotyping | CREgtR1        | GAACCTCATCACTCGTTGCAATCGAC   |
| cloning  | mmSORBS2F       | AAAAAACCGCTAGCCATGAATACAGATAGCGGTGGGTGTCGCAAAC |
Table S2. Dataset description for Sorbs2 RNA dysregulation in heart failure. Information includes the accession number (Acc#), disease, sample number for control and disease groups, log2-fold-change value (l2fc) and -log10 p-value (-l10P). Blank rows correspond to the gaps in the Figure 1a plot.

| Acc#          | Disease          | Control N | Disease N | l2fc  | -l10P  |
|--------------|------------------|-----------|-----------|-------|--------|
| GSE36961     | HCM              | 39        | 106       | 0.827 | 31.157 |
| GSE29819     | ARVC             | 6         | 6         | 0.466 | 3.623  |
| GSE57345     | Idiopathic       | 139       | 84        | 0.414 | 26.111 |
| EGAS00001002454 | DCM            | 113      | 149       | 0.290 | 25.738 |
| GSE3586      | DCM              | 15        | 13        | 1.009 | 7.438  |
| GSE5406      | Idiopathic       | 16        | 86        | 0.677 | 5.300  |
| GSE1145      | Idiopathic       | 11        | 15        | 1.454 | 5.126  |
| GSE79962     | HF               | 11        | 9         | 0.416 | 3.876  |
| GSE46224     | HF               | 8         | 8         | 0.650 | 2.988  |
| GSE53081     | Idiopathic       | 4         | 8         | 0.362 | 1.166  |
| GSE48166     | Ischemic         | 139       | 96        | 0.254 | 6.767  |
| GSE1145      | Ischemic         | 11        | 11        | 1.595 | 3.699  |
| GSE5406      | Ischemic         | 16        | 108       | 0.463 | 2.062  |
| GSE53081     | Ischemic         | 4         | 8         | 0.533 | 2.023  |
| GSE16499     | Ischemic         | 15        | 15        | 0.264 | 0.903  |
| GSE48166     | Ischemic         | 15        | 15        | 0.372 | 0.210  |
| GSE13874     | genetic HF       | 4         | 4         | 1.565 | 5.903  |
| GSE68518     | genetic HF       | 6         | 6         | 0.554 | 4.559  |
| GSE36074     | TAC/HF (decompensated) | 5   | 7         | 0.596 | 5.712  |
| GSE58455     | TAC              | 4         | 4         | 0.557 | 4.413  |
| GSE36074     | TAC/NF (compensated) | 5  | 7         | 0.368 | 3.285  |
| GSE4648      | MI               | 10        | 12        | 0.884 | 2.526  |
| GSE107569    | TAC              | 6         | 6         | 0.786 | 2.151  |
| GSE35350     | TAC              | 4         | 3         | 0.670 | 2.025  |
| GSE5500      | TAC              | 4         | 6         | 0.384 | 1.290  |
| GSE5996      | cells Strech     | 2         | 2         | 0.602 | 5.648  |
| GSE5996      | cells PE         | 2         | 2         | 0.587 | 3.358  |
| GSE73896     | cells-PE         | 4         | 4         | 0.524 | 2.770  |
Table S3. Human heart failure patient demographics.

| Description / Measurement | Nonfailing | Ischemic HF |
|---------------------------|------------|-------------|
| Date of explant           | 8/21/2013  | 7/25/2014   |
| SampleID                  | 1480       | 1631        |
| Tissue Source             | HF         | HF          |
| Cause of Heart Failure     | Isch       | Isch        |
| Heart Weight              | 402        | 307         |
| % of LV/ht                | 61         | 61          |
| Height (cm)               | 170.2      | 175.3       |
| LVESD_1                   | 2.3        | 2.6         |
| Ech Date n/a              | 11/8/2012  | 7/23/2014   |
| Ech Date                 | 4/1/2020   | 10/10/2014  |
| Heart Weight              | 34         | 2.3         |
| % of LV/ht                | 54         | 61          |
| Height (cm)               | 170        | 175         |
| LVESD_1                   | 2.3        | 2.6         |
| Ech Date n/a              | 11/8/2012  | 7/23/2014   |
| Ech Date                 | 4/1/2020   | 10/10/2014  |

For Table S3, the descriptions and measurements include:
- **Date of explant**: 8/21/2013, 7/25/2014
- **SampleID**: 1480, 1631
- **Tissue Source**: HF, HF
- **Cause of Heart Failure**: Isch, Isch
- **Heart Weight**: 402, 307
- **% of LV/ht**: 61, 61
- **Height (cm)**: 170.2, 175.3
- **LVESD_1**: 2.3, 2.6
- **Ech Date n/a**: 11/8/2012, 7/23/2014
- **Ech Date**: 4/1/2020, 10/10/2014

The table continued with similar entries for other measurements such as gender, age, race, and more.
### C57BL/6J vs caCMKII Data

| Description / Measurement | Control | C57BL/6J vs caCMKII | caCMKII |
|---------------------------|---------|---------------------|--------|
| **Sham**                  |         |                     |        |
| Body weight (BW) (6wks)    | 23.22   | 18.26               | 22.16  |
| Date of sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Body weight (BW) (8wks)    | 20.86   | 19.62               | 20.77  |
| Date of Sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Date of Surgery (6wks)     | 6/17/15 | 6/17/15             | 6/17/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |
| Date of Injection (3wks)   | 5/27/15 | 5/27/15             | 5/27/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |

### Table S4. Mice heart failure model phenotyping data.

| Description / Measurement | Control | C57BL/6J vs caCMKII | caCMKII |
|---------------------------|---------|---------------------|--------|
| **Sham**                  |         |                     |        |
| Body weight (BW) (3wks)    | 12.89   | 7.71                | 9.9    |
| Date of sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Body weight (BW) (8wks)    | 20.86   | 19.62               | 20.77  |
| Date of Sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Date of Surgery (6wks)     | 6/17/15 | 6/17/15             | 6/17/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |
| Date of Injection (3wks)   | 5/27/15 | 5/27/15             | 5/27/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |

### Table S4. Mice heart failure model phenotyping data.

| Description / Measurement | Control | C57BL/6J vs caCMKII | caCMKII |
|---------------------------|---------|---------------------|--------|
| **Sham**                  |         |                     |        |
| Body weight (BW) (6wks)    | 23.22   | 18.26               | 22.16  |
| Date of sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Body weight (BW) (8wks)    | 20.86   | 19.62               | 20.77  |
| Date of Sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Date of Surgery (6wks)     | 6/17/15 | 6/17/15             | 6/17/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |
| Date of Injection (3wks)   | 5/27/15 | 5/27/15             | 5/27/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |

### Table S4. Mice heart failure model phenotyping data.

| Description / Measurement | Control | C57BL/6J vs caCMKII | caCMKII |
|---------------------------|---------|---------------------|--------|
| **Sham**                  |         |                     |        |
| Body weight (BW) (6wks)    | 23.22   | 18.26               | 22.16  |
| Date of sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Body weight (BW) (8wks)    | 20.86   | 19.62               | 20.77  |
| Date of Sacrifice (16wks)  | 8/4/15  | 8/4/15              | 8/4/15 |
| Date of Surgery (6wks)     | 6/17/15 | 6/17/15             | 6/17/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |
| Date of Injection (3wks)   | 5/27/15 | 5/27/15             | 5/27/15|
| Body weight (BW) (16wks)   | 25.35   | 26.83               | 26.94  |
Table S5. Summary statistics for cardiomyocyte-specific Sorbs2 knockout mice. Table supplements data plotted in Figure 4. Mean values were binned on weeks on age for WT and Sorbs2-cKO mice. P-values indicate difference between WT and cKO curves analyzed using 2-way ANOVA with Sidak’s multiple comparisons testing the interaction between age and genotype.

### Ejection Fraction

| Bin (weeks) | WT (mean) | cKO (mean) | pval     |
|-------------|-----------|------------|----------|
| 10-20       | 0.793     | 0.765      | 0.362    |
| 20-30       | 0.883     | 0.847      | 0.067    |
| 30-40       | 0.798     | 0.687      | 0.035    |
| 40-50       | 0.832     | 0.596      | 9.48E-09 |
| 50-60       | 0.840     | 0.419      | 7.23E-21 |

### Indexed EDV

| Bin (weeks) | WT (mean) | cKO (mean) | pval     |
|-------------|-----------|------------|----------|
| 10-20       | 0.365     | 0.377      | 0.735    |
| 20-30       | 0.374     | 0.418      | 0.301    |
| 30-40       | 0.422     | 0.483      | 0.120    |
| 40-50       | 0.369     | 0.495      | 3.07E-04 |
| 50-60       | 0.389     | 0.734      | 9.54E-11 |

### LV Mass

| Bin (weeks) | WT (mean) | cKO (mean) | pval     |
|-------------|-----------|------------|----------|
| 10-20       | 60.067    | 59.129     | 0.826    |
| 20-30       | 71.044    | 69.945     | 0.795    |
| 30-40       | 95.392    | 96.634     | 0.763    |
| 40-50       | 82.019    | 85.332     | 0.421    |
| 50-60       | 78.739    | 95.106     | 0.001    |

### LV Thickness

| Bin (weeks) | WT (mean) | cKO (mean) | pval     |
|-------------|-----------|------------|----------|
| 10-20       | 0.756     | 0.760      | 0.911    |
| 20-30       | 0.772     | 0.746      | 0.638    |
| 30-40       | 0.841     | 0.794      | 0.131    |
| 40-50       | 0.832     | 0.862      | 0.238    |
| 50-60       | 0.841     | 0.777      | 0.018    |

### Atria / BW

| Bin (weeks) | WT (mean) | cKO (mean) | pval     |
|-------------|-----------|------------|----------|
| 10-20       | 0.270     | 0.397      | 0.094    |
| 20-30       | 0.328     | 0.427      | 0.138    |
| 30-40       | 0.218     | 0.335      | 1.03E-04 |
| 40-50       | 0.256     | 0.496      | 4.29E-05 |
| 50-60       | 0.208     | 0.982      | 1.87E-08 |
| 60-70       | 0.319     | 1.058      | 0.022    |

### Ventricle / BW

| Bin (weeks) | WT (mean) | cKO (mean) | pval     |
|-------------|-----------|------------|----------|
| 10-20       | 3.758     | 3.965      | 0.560    |
| 20-30       | 3.654     | 3.955      | 0.448    |
| 30-40       | 3.354     | 3.164      | 0.219    |
| 40-50       | 3.372     | 3.502      | 0.515    |
| 50-60       | 3.377     | 4.298      | 1.74E-05 |
| 60-70       | 3.252     | 3.623      | 0.377    |
Supplemental Figure Legends:

**Figure S1. Transcriptional dysregulation of Sorbs2 in heart failure.**

(A-C) Human Sorbs2 transcript expression analysis of RNA sequencing data acquired from SRA archive PRJNA477855 remapped using the Kallisto/Sleuth pipeline. Sorbs2 transcript numbers are listed on X-axis in panel C. Black bars are high-confidence protein-coding transcripts with complete CDS, grey bars are transcripts with incomplete CDS, white bars are non-coding transcripts. Sample sizes are N=14 (Nonfailing), N=13 (Ischemic Cardiomyopathy, ISCH), N=37 (Dilated Cardiomyopathy, DCM), * p<0.05, compared to nonfailing. (A) Differential expression (Log2-fold-change) of transcripts in dilated cardiomyopathy. (B) Differential expression of Sorbs2 transcripts in Ischemic cardiomyopathy. (C) Average expression of Sorbs2 transcripts in non-failing hearts, sorted from most to least abundant with a lower cutoff of TPM>1, (represents 18 of 65 transcripts). (D) Left axis, RNA and ribosome reads, and on right axis translational efficiency (TE) plotted from public ribosomal profiling data in nonfailing and DCM human hearts from Van Heesch, et. al.¹⁷ Both RNA and Ribosome reads are significantly upregulated in DCM hearts, suggesting that cardiac Sorbs2 expression at baseline and disease is primarily under transcriptional rather than translational regulation (i.e., TE = 1 and is not significantly different in DCM).

**Figure S2. Sorbs2 genetic loci in human and mice**

(A) Sorbs2 genetic locus in humans. Vertical pink lines denote exon junctions. Green boxes denote the location of annotated protein domains, as well as the location of a cardiac-specific exon. Black boxes denote the alignment of three different SORBS2 clones published related to cardiac research. Blue (Ribosome) and Red (RNA) tracks show sequencing data from human hearts (adapted from Van Heesch, et.al ¹⁷). Protein coding transcript tracks list those with complete CDS annotations (Gencode Basic, 8 of 65 putative transcripts). To the right is transcript abundance in nonfailing human hearts based on RNA sequencing data described in Figure S1C. Abbreviations SoHo = Sorbin Homology Domain, SH3 = SRC Homology 3 Domain, ZnF-C2H2 = C2H2-type Zinc Finger (RNA-binding domain). Note this RNA-binding domain is encoded by Addgene plasmid #74514, which was used to show Sorbs2-RNA binding activity of cardiac ion channels ²⁹, however it is located in an exon that is not expressed in human heart tissue, or present in two clones obtained from heart cDNA, including “our clone”, which was obtained by RT-PCR amplification of the cardiac-specific Sorbs2 isoform from mouse heart cDNA. (B) Sorbs2 genetic locus in mice, with a similar track layout. Note the red box indicating the obligate exon 12 with flanking loxP sites. These mice were crossed to αMHC-CRE expressing line to generate cardiomyocyte-specific Sorbs2 knockout mice (Sorbs2-cKO). The two tracks of black peaks represent Ribosome and RNA sequencing data specifically from cardiomyocytes, remapped from SRA-PRJNA484227 ¹⁰. Bar graph on right shows TPM expression of Sorbs2 protein coding transcripts (Gencode Basic, 11 of 37 putative transcripts) in mouse hearts from RNAseq data. ZnF-C2H2 represents the location of the defined RNA-binding domain in transcripts that are not abundant in hearts or cardiomyocytes. The cardiac-specific Sorbs2 exon remains unannotated in mouse reference transcriptomes yet is clearly present in RNA and Ribosome sequencing data.
Figure S3. Cardiomyocyte-specific Sorbs2 knockout mice.

(A) Western blot showing expression of Sorbs2, in WT and cKO heart samples from female mice at ~12 months age using an independent antibody (Sigma mouse monoclonal). Also shown on left side is a positive control (Sorbs2 plasmid cloned from mouse heart cDNA expressed in HT1080 cells), as a confirmation of on-target antibody binding. (B) Western blot of Sorbs1 expression from WT and cKO heart tissues does not show compensatory changes in Sorbs1 expression following cardiomyocyte loss of Sorbs2. Gapdh shown as loading control for panels A and B. (C) Representative Sorbs2 immunofluorescence (green) in heart sections from wildtype (WT) mice, co-localized with N-Cadherin (CDH2, red) at the intercalated disc (ICD). Also note Sorbs2 reactivity in the coronary artery on the right picture.

Figure S4. Cardiomyocyte-specific Sorbs2 knockout mice develop age-related systolic dysfunction, cardiac remodeling, and premature death.

(A) Representative serial m-mode echocardiographic images from one WT and one Sorbs2-cKO mice. The callout indicates the ejection fraction (EF) for that mouse calculated using the Endocardial and Epicardial traces (see Methods section 2.3). At three months old (first row) both WT and cKO mice show normal wall motion, wall thickness, and chamber dimensions in systole and diastole. At 10 months (second row) Sorbs2-cKO heart shows reduced wall motion, increased chamber dimension, and decreased EF. At 12 months old, (third row) Sorbs2-cKO hearts show significant LV systolic dysfunction with a big chamber, thin walls, and poor wall motion. (B) Time course of echocardiography from WT and Sorbs2-cKO hearts indicates trending to increased RV thickness in cKO hearts, coincident with emerging HF. (C) Postmortem gravimetric analyses of heart chamber mass in ~48-week male mice, normalized to bodyweight (BW). The left atrium (LA) is significantly enlarged in Sorbs2-cKO hearts compared to control, whereas, the right atrium (RA), the left ventricle plus septum (LV+S) and the right ventricle (RV) are not different. Dots show individual hearts, N=6 per group, with mean +/- SEM. Significance from t-test, * p<0.05, ns = not significant. (D) Postmortem gravimetric analysis of atrial mass normalized to either body weight (BW) or ventricular mass in mice 12-24 weeks of age. Dots show individual hearts with mean +/- SEM. Significance from t-test, * p<0.05, **p<0.01. (E) Male ratio of postmortem LA/RA mass over time (cKO regression, r²=0.596, p=2e-4). This indicates increases in LA mass rather than RA or bi-atrial mass are driving the significant increase in total atrial mass shown in Figure 3D. (F) Male correlation of atrial to ventricle size in WT and cKO mice hearts (cKO regression, r²=0.479, p<1e-4).

Figure S5. Cardiomyocyte-specific Sorbs2 knockout mice have severe contractile dysfunction.

(A) Datapoints represent the group mean +/- SEM change in heart rate from baseline through increasing dobutamine (DOB) concentrations (N=7 mice per group), and a black trendline denotes a linear regression fit from baseline through the highest dose (12 ng/g/min). No difference was found. These data show that Sorbs2-cKO hearts are unable to increase cardiac contractility in response to DOB despite having equivalent increases in heart rate. (B) Final 30 second average per dose for dP/dT Min. Dots show individual mice with mean +/- SEM,
statistics acquired using one-way ANOVA with Sidak posthoc test comparing selected groups (each comparison shown on plot), * p<0.05, ns = not significant. (C-F) Waterfall plots of EKG intervals (30 seconds stacked back to front) in WT and Sorbs2-cKO mice. (C) WT hearts have normal sinus rhythm with regular R-R, P-P, and P-R intervals. (D) cKO hearts also show normal sinus rhythm and intervals, despite obvious bifid P-waves, increased P duration, and QRS duration. (E-F) WT and cKO hearts after DOB challenge, both have expected increase in heart rate, indicated by reduced R-R interval, and maintain sinus rhythm.

**Figure S6. Cardiomyocyte-specific Sorbs2 knockout mice have dysregulated cytoskeletal protein expression not ICD.**

(A-D) Heatmaps reprint from Figure 6 alongside digitized western blot images that show analysis of protein expression from WT and Sorbs2-cKO cardiac lysates at 3, 6, and 12 months of age. Each box represents the mean integrated intensity, normalized to loading control and expressed relative to 3-month WT; n=4 mice per group. Significant differences (p<0.05) denoted with (*, black for down-regulated and white for up-regulated) overlay on the heatmap, were acquired using t-test comparing cKO to WT at each age. (Abbreviations: CS = Cell Signaling, AB = Abclonal). Heatmaps and blots are organized by (A) intercalated disc (ICD) proteins, (B) ion channels and calcium handling proteins, (C) sarcomere proteins, and (D) structural cytoskeletal proteins.

**Figure S7. Cardiomyocyte-specific Sorbs2 knockout mice have normal localization of cytoskeletal and ICD proteins.**

(A-B) Representative immunofluorescent microscope images of heart tissue oriented with longitudinal myofibers from (A) WT and (B) Sorbs2-cKO mice. Inset pictures show staining with (1) wheat germ agglutinin (WGA) in green, (2) Sorbs2 in white, (3) Ctnnb1 in red, and (4) a color overlay. A blinded review of images from WT and cKO mice for Ctnnb1 localization and several other cytoskeletal and ICD proteins (C-F) were indistinguishable from each other, (n=4 images per mouse and n=4 mice per group). These include (C) phalloidin staining actin fibers in red, (D) alpha actinin 2 (Actn2) staining Z-disk and sarcomeres in red, (E) vinculin (Vcl) staining ICDs and costameres in white, and (F) connexin 43 (Gja1) staining ICDs and vasculature in white. Representative WT images shown on the top row with cKO images on the bottom row.

**Figure S8. Sorbs2 regulation of intracellular calcium and Scn5a activity in vitro.**

(A) Western blot analysis of Sorbs2 protein levels in NRCMs transiently transfected with control or Sorbs2 siRNAs for ~48 hours. TUBB shows consistent loading. (B) Intracellular calcium signal intensity from Fura-2AM loaded NRCMs. Data were acquired in 4 independent experiments, 2 plates per experiment per condition with ~ 40-50 cells analyzed per plate. Dots represent individual cells with mean +/- SEM, statistics are acquired from T-test, **** p<0.0001, ns=not significant. (C-D) Overexpression or knock down of Sorbs2 did not significantly alter sodium current-voltage relationships derived from electrophysiology experiments in transiently transfected NRCMs. (E-F) Peak current density is also not significantly different. Data shown are mean SEM, with individual cells plotted in (D, E), N = 5-7 cells per treatment, statistics are acquired from T-test, ns = not significant.
Supp. Figure 3

A  SORBS2

| Plasmid | WT | SORBS2-cKO |
|---------|----|------------|
| GAPDH   |    |            |

B  SORBS1

| Plasmid | WT | SORBS2-cKO |
|---------|----|------------|
| GAPDH   |    |            |

C

- SORBS2
- Overlay
- CDH2
- Overlay
Supp. Figure 5

A) Delta Heart Rate (BPM) vs. Dobutamine (ng/g/min)

B) dP/dT Min (mmHg/sec) vs. WT and cKO

C) SORBS2-WT

D) SORBS2-cKO

E) SORBS2-WT

F) SORBS2-cKO

Baseline vs. DOB, 12 ng/g/min
Supp. Figure 6

**A. ICD Proteins**

|          | 3 Month | 6 Month | 12 Month |
|----------|---------|---------|----------|
| WT       | ⊗       | ⊗       | ⊗        |
| cKO      | ⊗       | ⊗       | ⊗        |

**B. Ion Channels / Calcium Handling**

|          | 3 Month | 6 Month | 12 Month |
|----------|---------|---------|----------|
| WT       | ⊗       | ⊗       | ⊗        |
| cKO      | ⊗       | ⊗       | ⊗        |

**C. Sarcomere Proteins**

|          | 3 Month | 6 Month | 12 Month |
|----------|---------|---------|----------|
| WT       | ⊗       | ⊗       | ⊗        |
| cKO      | ⊗       | ⊗       | ⊗        |

**D. Structural Cytoskeletal Proteins**

|          | 0.5     | 1.0     | 1.5     | 2.0     | 2.5     | 3.0     | 3.5     | 4+       |
|----------|---------|---------|---------|---------|---------|---------|---------|----------|

**Relative Intensity**

- 0.5
- 1.0
- 1.5
- 2.0
- 2.5
- 3.0
- 3.5
- 4+
Supp. Figure 8

A

siControl siSORBS2

SORBS2

TUBB

B

Fura-2 AM (340/380nM)

siControl siSORBS2

C

Current Density (pA/pF)

Membrane Potential (mV)

GFP (n=7) SORBS2 (n=5)

D

Current Density (pA/pF)

Membrane Potential (mV)

GFP (n=7) siSORBS2 (n=5)

E

Peak Current Density (pA/pF)

GFP SORBS2

F

Peak Current Density (pA/pF)

siControl siSORBS2

✱✱✱✱