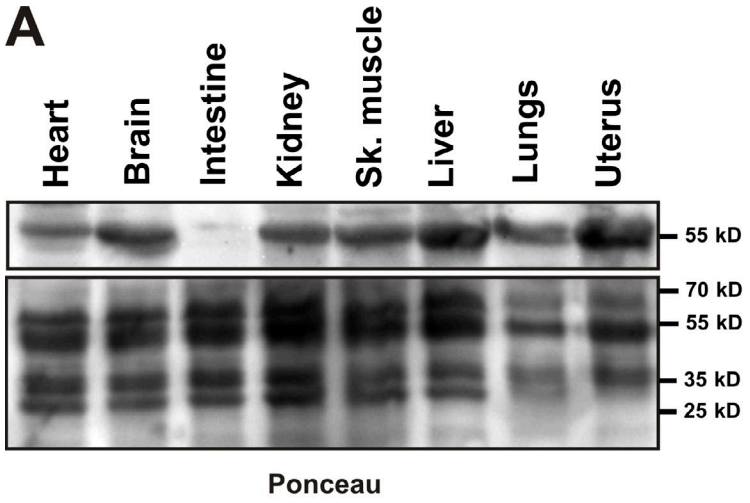
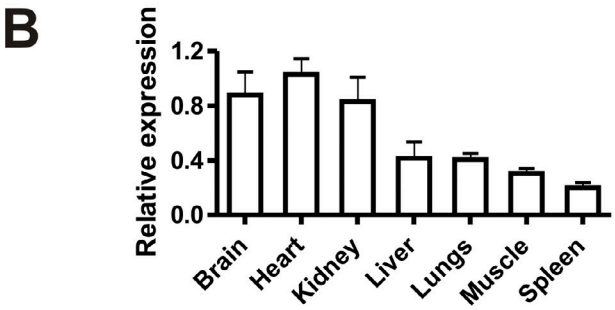
Rangrez et al., http://www.jcb.org/cgi/content/full/jcb.201303052/DC1





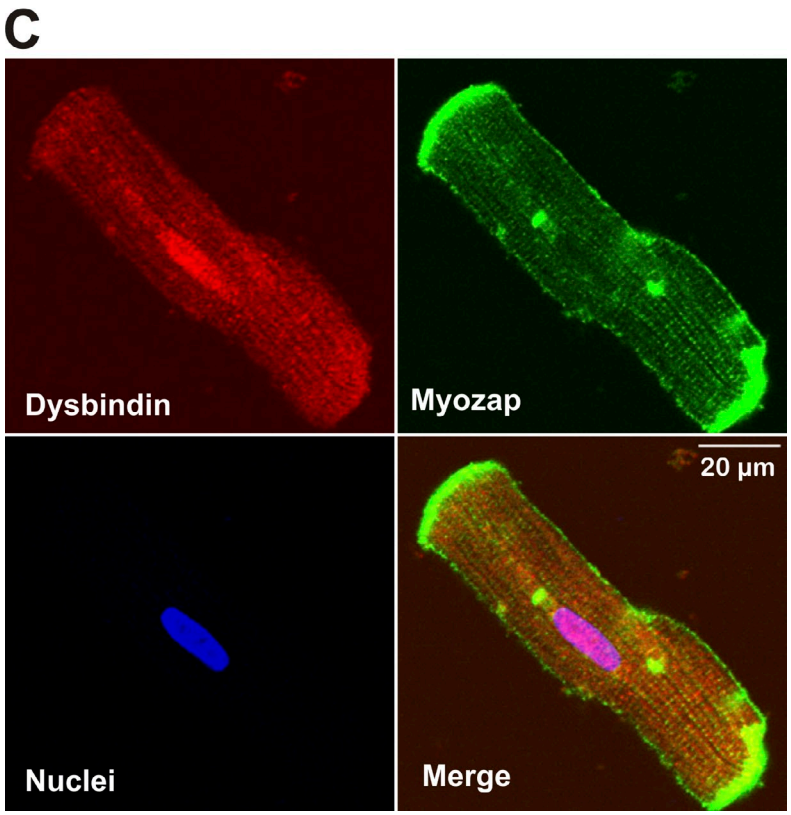


Figure S1. **Tissue distribution and subcellular localization of Dysbindin.** (A) Representative immunoblot indicating the expression levels of Dysbindin in various mouse tissues. (B) Expression levels of Dysbindin in various mouse tissues determined by qRT-PCR (n = 5). Error bars show means ± SEM. (C) Coimmunostaining of Dysbindin with Myozap in ARVCMs. Nuclei were stained with DAPI, and the immunofluorescence images were captured in a confocal microscope (LSM 510).

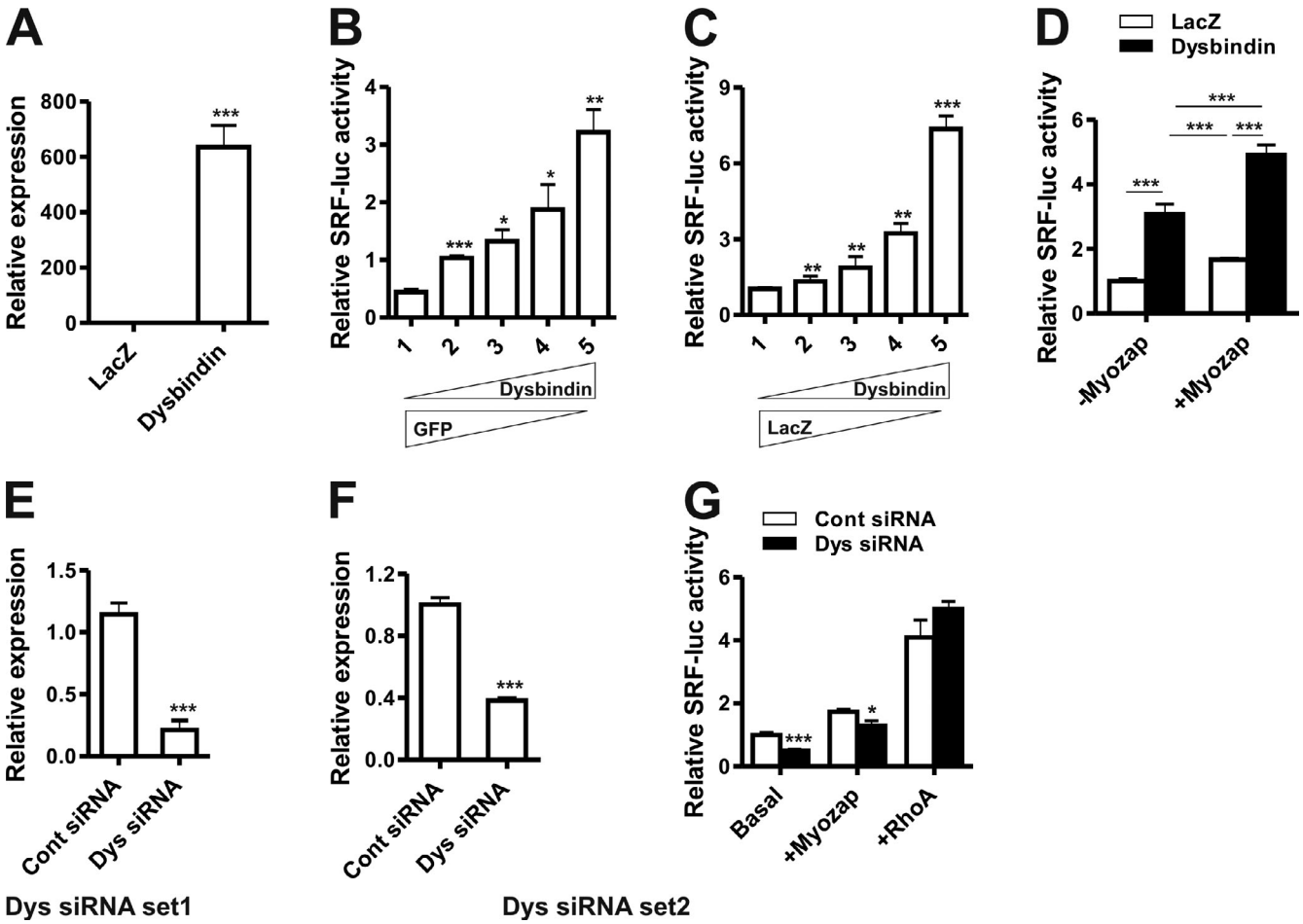


Figure S2. **Effect of Dysbindin overexpression and knockdown on SRF signaling in Cardiomyocytes.** (A) Dysbindin expression was determined in NRVCMs infected with Ad-LacZ as a control or Ad-Dysbindin for overexpression of Dysbindin. *n* = 6. (B) Dysbindin-mediated dose-dependent activation of SRF signaling was studied using increased infectious units of Ad-Dysbindin virus (5/10/15/50 ifu). Ad-GFP was used to maintain the equal load of virus in all sets of experiments. *n* = 6. (C) Dysbindin-mediated dose-dependent activation of SRF signaling same as in Fig. 2 B, except Ad-LacZ was used as a control virus. *n* = 6. (D) Effect of Myozap and Dysbindin on Luciferase activity determined by SRF-RE firefly luciferase reporter assay in C2C12 cells. Plasmids encoding Dysbindin, Myozap, and Renilla luciferase were transfected into C2C12 cells stably expressing SRF-RE reporter firefly luciferase. Empty vector was used as a negative control. Data shown are means of two independent experiments performed in hexaplicates. (E and F) Down-regulation of Dysbindin expression was confirmed in Dysbindin siRNA–transfected compared with the control siRNA–transfected NRVCMs by qRT-PCR using two independent siRNAs obtained from two different providers: siRNA denoted as set 1 is obtained from Santa Cruz Biotechnology, Inc. (E), and siRNA denoted set 2 is obtained from Ambion (F). (G) Effect of siRNA-mediated Dysbindin knockdown in the presence or absence of Myozap and RhoA on luciferase activity determined by SRF-RE firefly luciferase reporter assay in NRVCMs. Various adenoviruses expressing Myozap, RhoA, SRF-RE reporter based firefly luciferase (Ad-SRF-luc), and Renilla luciferase (Ad-Renilla, control) were used. Adenovirus expressing β-galactosidase (Ad-LacZ) was used as a control or to maintain the equal quantity of infected virus. Virus loads used were 20 ifu Ad-Myozap, 20 ifu Ad-RhoA, 10 ifu Ad-SRF-luciferase, and 10 ifu Ad-Renilla in different combinations as shown in the figure. *n* = 6. Statistical significance was determined using two-tailed Student's *t* test or by two-way ANOVA. Dys, Dysbindin; Cont, control. Error bars show means ± SEM. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$.

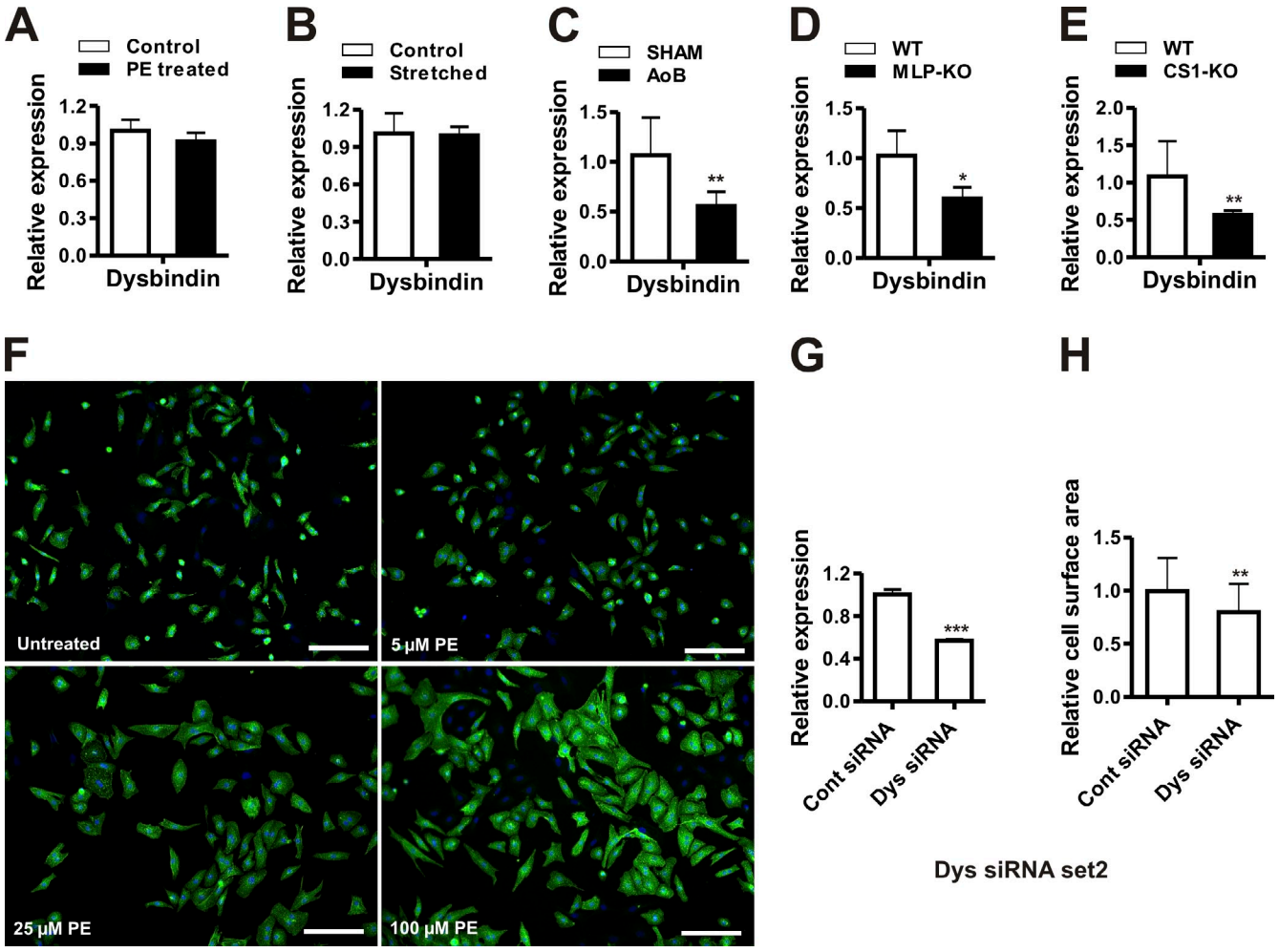


Figure S3. **Expression of Dysbindin in in vitro and in vivo models of hypertrophy and/or cardiomyopathy.** (A) Dysbindin expression was determined in NRVCMs treated with phenylephrine (PE) as a prohypertrophic agent compared with untreated NRVCMs. *n* = 6. (B) Mechanical stretch induced hypertrophic NRVCMs compared with unstretched control NRVCMs. *n* = 6. (C) Aortic banded (AoB) mice compared with sham-operated mice. *n* = 7 (sham)/9 (aortic banded). (D) Muscle LIM protein KO (MLP-KO) mice compared with their wild-type (WT) counterparts. *n* = 7 (muscle LIM protein KO)/9 (wild type). (E) Calsarcin-1 KO (CS1-KO) mice compared with the wild-type mice. *n* = 6. (F) Representative images showing the phenotypic effect of different PE doses. NRVCMs were cultured on coverslips in triplicates, treated with PE for 48 h, and immunostained with α-actinin. Nuclei were stained with DAPI. Bars, 50 µm. (G) Down-regulation of Dysbindin expression was confirmed in Dysbindin siRNA (Ambion)–transfected compared with the control siRNA–transfected NRVCMs by qRT-PCR. (H) NRVCMs were either transfected with control siRNA or with Dysbindin siRNA (Ambion). Images were captured on Keyence microscope, and cell surface area was measured using MacroCellCount analyzer as detailed in the Materials and methods. Statistical significance was determined using two-tailed Student's *t* test or by one/two-way ANOVA. Dys, Dysbindin; Cont, control. Error bars show means ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

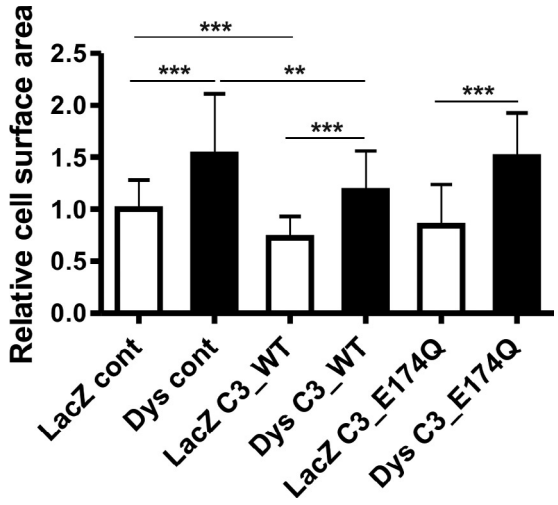


Figure S4. **Effect of C3 transferase and its point mutant E174Q on cell surface area.** C3 transferase and its point mutant (E174Q) were purified as recombinant GST fusion proteins in *E. coli* TG1 cells and used to study its effect on the cell surface area in the absence or presence of Dysbindin overexpression. NRVCMs were either infected with Ad-LacZ as a control or with Ad-Dysbindin. Images were captured on Keyence microscope, and cell surface area was measured using MacroCellCount analyzer as detailed in the Materials and methods. Statistical significance was determined using one-way ANOVA. Error bars show means ± SEM. **, $P < 0.01$; ***, $P < 0.001$.

Table S1. **Primers used for the cloning of full-length or different fragments of mouse Dysbindin**

Primer name	Sequence	Purpose
Dysbn_fw	5′-GCTGGCACCATGCTGGAGACCCTGCGC-3′	Full length
Dysbn_rv_MS[a]	5′-GCTGGGTCGCCTTAAATGTCCTGAGTTGA-3′	
Dysbn_fw	5′-GCTGGCACCATGCTGGAGACCCTGCGC-3′	Full length
Dysbn_rv_OS[b]	5′-GCTGGGTCGCCAATGTCCTGAGTTGAGTC-3′	
Dys-coil-fw	5′-GCTGGCACCATGTCTGCCCACTGGGAGAAG-3′	Coiled-coil domain
Dys-coil-rv-OS[b]	5′-GCTGGGTCGCCCAGCTTCAGTTGCTGGGT-3′	
Dys-dom-fw	5′-GCTGGCACCATGCAGAAGGCCCTGGAAATG-3′	Dysbindin domain
Dys-dom-rv-OS[b]	5′-GCTGGGTCGCCATCAGCAGTGTCCAGGTC-3′	
Dysbn_fw	5′-GCTGGCACCATGCTGGAGACCCTGCGC-3′	N terminus + coiled-coil domain
Dys-coil-rv-OS[b]	5′-GCTGGGTCGCCCAGCTTCAGTTGCTGGGT-3′	
Dys-dom-fw	5′-GCTGGCACCATGCAGAAGGCCCTGGAAATG-3′	Dysbindin domain + C terminus
Dysbn_rv_OS[b]	5′-GCTGGGTCGCCAATGTCCTGAGTTGAGTC-3′	

Dys, Dysbindin; fw, forward; rv, reverse.
[a]PCR amplicon is with stop codon to exclude C-terminal V5 tag from the expressed protein.
[b]PCR amplicon is without stop codon to include C-terminal V5 tag in the expressed protein.