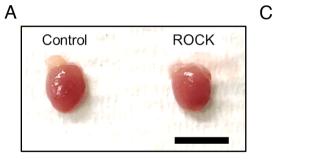
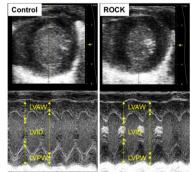
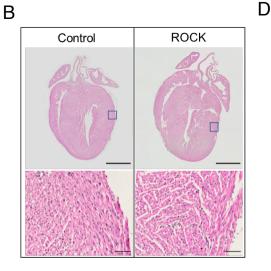


Fig. S1. Tamoxifen administration has no effect on actomyosin contractility. Western blot and quantitative analysis of pMLC2 and MLC2 expression in heart tissue lysates from WT C57BL/6J P7 mice with (n=4) and without (n=4) tamoxifen treatment. ns, non-significant, by two-tailed unpaired Student's t-test. Error bars represent S.E.M.







	Control	ROCK	
	Control	RUCK	р
n	10	10	N/A
Gender (M/F)	7/3	8/2	N/A
Age	P7	P7	N/A
Heart rate (bpm)	536.147 ± 11.777	537.974 ± 8.354	p>0.05
LVID;s (mm)	0.924 ± 0.035	0.864 ± 0.034	p>0.05
LVID;d (mm)	1.933 ± 0.046	1.864 ± 0.047	p>0.05
LV vol;s (mm)	1.687 ± 0.167	1.645 ± 0.150	p>0.05
LV vol;d (mm)	11.732 ± 0.690	11.245 ± 0.539	p>0.05
EF (%)	85.876 ± 0.794	85.541 ± 1.026	p>0.05
FS (%)	52.579 ± 0.960	52.230 ± 1.280	p>0.05
LVPW;d (mm)	0.518 ± 0.025	0.587 ± 0.034	p>0.05
LVPW;s (mm)	0.915 ± 0.019	0.975 ± 0.028	p>0.05
LVAW;d (mm)	0.571 ± 0.015	0.567 ± 0.016	p>0.05
LVAW;s (mm)	1.031 ± 0.024	1.034 ± 0.016	p>0.05

Values are mean ± SEM; n, number of mice. LVID, left ventricular internal dimension; LV vol, left ventricular volume; EF, ejection fraction; FS, fraction shortening; LVPW, left ventricular posterior wall; LVAW, left ventricular anterior wall; d, diastole; s, systole, p values were determined by 2-tailed student's t test.

Fig. S2. Histological and cardiac function analysis of ROCK2:ER P7 hearts.

(A) Representative whole mount images of heart from control and ROCK P7 mice. Scale bar: 5 mm. (B) Representative H&E staining of heart sections from control and ROCK P7 mice. *Upper panel* shows the whole section images, scale bar: 1 mm. *Lower panel* shows higher magnification images of the boxed areas, scale bar: 50 μ m. (C) M-mode two-dimensional echocardiography displaying cross section view of LV chamber. (D) Echocardiographic assessment of chamber dimensions and cardiac function in control (n=10) and ROCK (n=10) mice at P7. Data represent Mean \pm S.E.M.. p value was analyzed by two tailed unpaired Student's t-test and shown in the table.

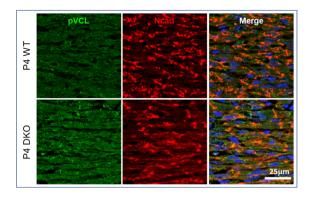


Fig. S3. Increased VCL Y822 phosphorylation correlates with aberrant N-cadherin expression in α E-/ α T-catenin double knockout (DKO) heart. Representative immunofluorescence images of heart sections from control and α E-/ α T-catenin DKO P4 mice co-stained for pVCL-Y822 (green) and N-cadherin (red). Similar to ROCK-activated hearts, pVCL appeared primarily diffuse throughout the cytosol.

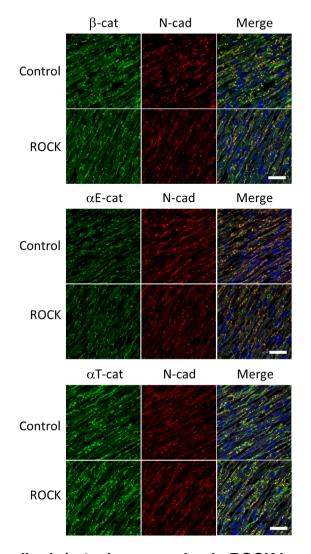


Fig. S4. N-cadherin/catenin expression in ROCK hearts.

Representative immunofluorescence images of P7 heart sections from control and ROCK mice co-stained with β -cat (green)/N-cad (red), α E-cat (green)/N-cad (red), and α T-cat (green)/N-cad (red). Scale bar: 25 μ m.

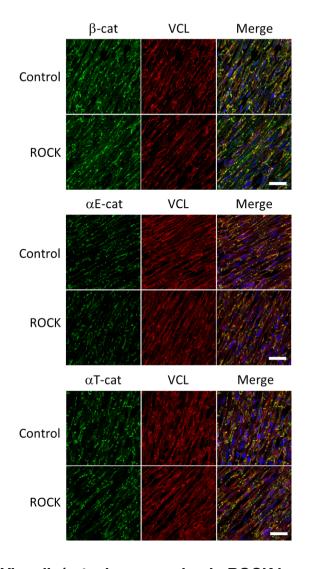


Fig. S5. Vinculin/catenin expression in ROCK hearts. Representative immunofluorescence images of P7 heart sections from control and ROCK co-stained with β -cat (green)/VCL (red), α E-cat (green)/VCL (red), and α T-cat (green)/VCL (red). Scale bar: 25 μ m.

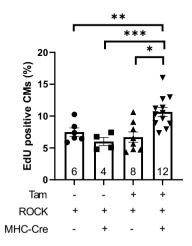


Fig. S6. No increase in cell cycle activity in different control hearts. Quantitative analysis of immunofluorescent images of heart sections from ROCK and ROCK; MHC-Cre P7 mice with or without tamoxifen treatment co-stained with EdU/α-actinin. ns, non-significant, *, P<0.05, **, P<0.01 ***, P<0.001 by one-way ANOVA with Tukey's multiple comparisons. Error bars represent S.E.M..

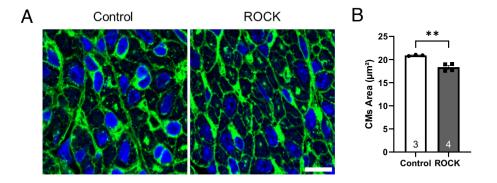


Fig. S7. Reduced CM cross sectional area in ROCK hearts. (A)

Representative images of WGA (green) staining of heart sections from control and ROCK P7 mice. (B) Quantitated data from images showing average CM cross sectional area of control (n=3) and ROCK (n=4) hearts at P7. A minimum of 1500 cells from 6 different fields (40X) per heart were analyzed. ** P<0.01 by two tailed unpaired Student's t-test. Error bars represent S.E.M. Scale bar: 10 μm .

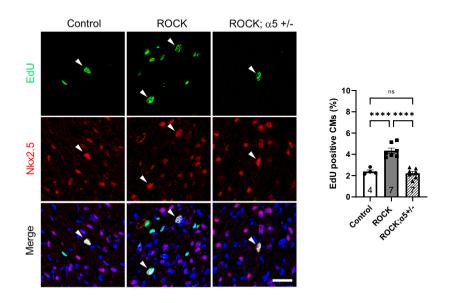


Fig. S8. α**5 integrin is required for increased proliferation in ROCK hearts.** Representative immunofluorescence images and quantification of heart sections from control (n=4), ROCK (n=7) and ROCK; α5 +/- (n=7) P7 mice co-stained with EdU (green)/Nkx2.5 (red). Arrowheads indicate EdU +, Nkx2.5 + nuclei. ns, non-significant, ****, P<0.0001 by one-way ANOVA with Tukey's multiple comparisons. Error bars represent S.E.M.. Scale bar: 25 μm.