De Lange et al., http://www.jgp.org/cgi/content/full/jgp.201311018/DC1

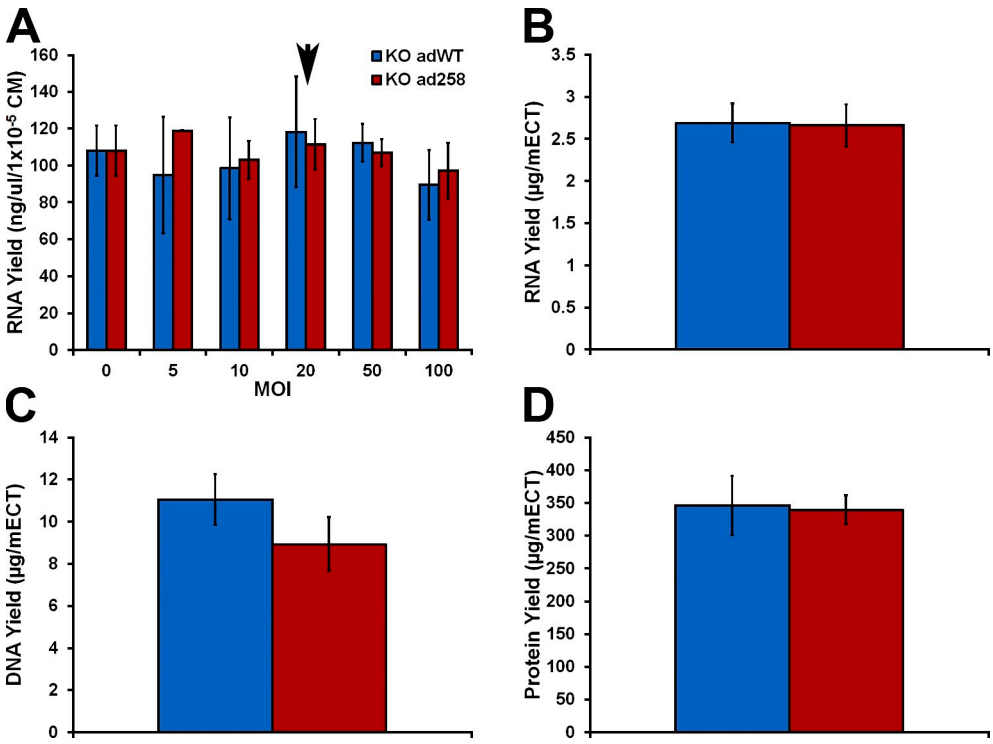

Figure S1. Effect of WT and E258K cMyBP-C expression on cardiomyocytes survival. (A) Effect of adenoviral transduction on cardiac cell viability in neonatal mouse cMyBP-C$^{-/-}$ cardiac cells in monolayer culture, transduced with adWT (blue bars) and ad258 (red bars) at MOIs of 0, 5, 10, 20, 50, and 100 as assessed by total RNA yield. (B–D) The effect of adenoviral transduction on cardiac cell viability in mECT as assessed by total RNA content (B), DNA content (C), and total protein content (D) in KO adWT (blue filled bars) and KO ad258 mECT (red bars). The arrow in A indicates an MOI = 20 used to transduce mECT. *, $P < 0.05$ (one-way ANOVA with a Tukey's post-hoc test). $n = 3$ for the monolayer experiment (A); $n \geq 4$ for mECT experiments (B–D). Error bars indicate SEM.

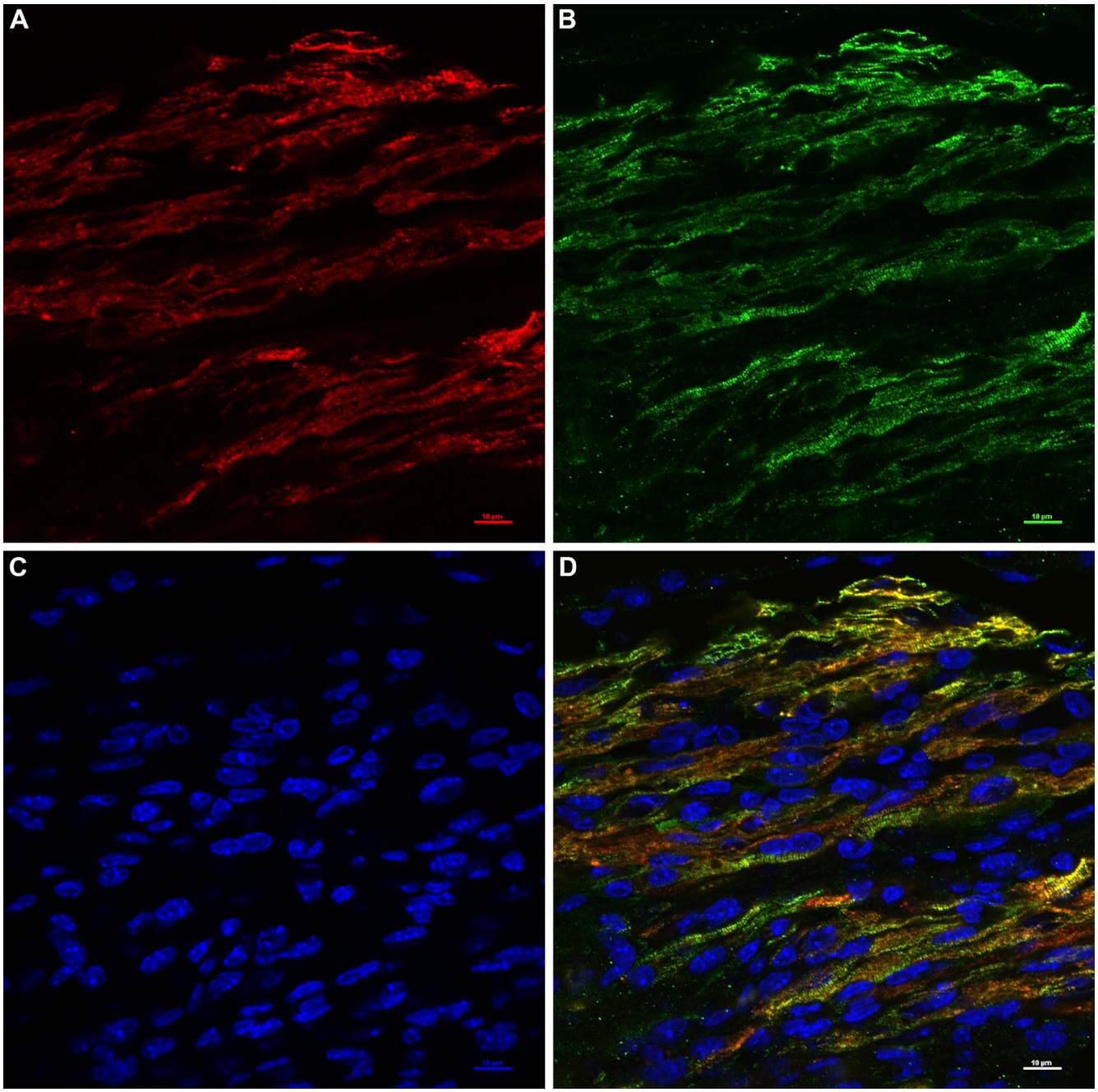

Figure S2. Human E258K cMyBP-C is efficiently incorporated into the sarcomere in KO ad258 mECT. Immunohistochemical analysis of a KO ad258 mECT. (A) α-Tropomyosin (red fluorescence). (B) cMyBP-C (green fluorescence). (C) Nuclei stained with DAPI (blue fluorescence). (D) An overlay of α-tropomyosin, cMyBP-C, and DAPI. Bars, 10 µm.

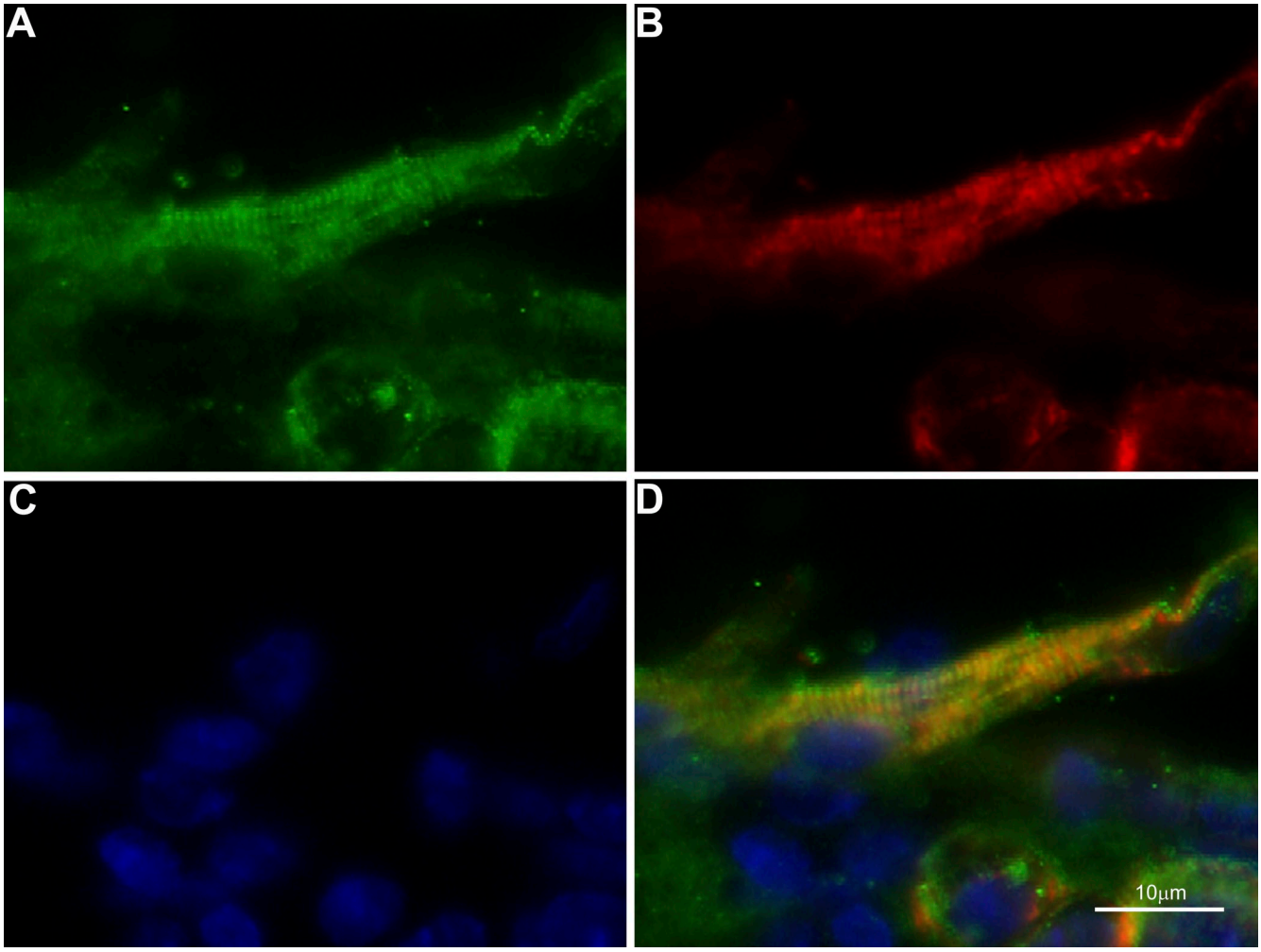

Figure S3. WT human cMyBP-C is efficiently incorporated into the sarcomere in KO adWT mECT. Immunohistochemical analysis of a KO adWT mECT. (A) cMyBP-C (green fluorescence). (B) Myc-tagged cMyBP-C (red fluorescence). (C) Nuclei stained with DAPI (blue fluorescence). (D) An overlay of cMyBP-C, Myc-tagged cMyBP-C, and DAPI.

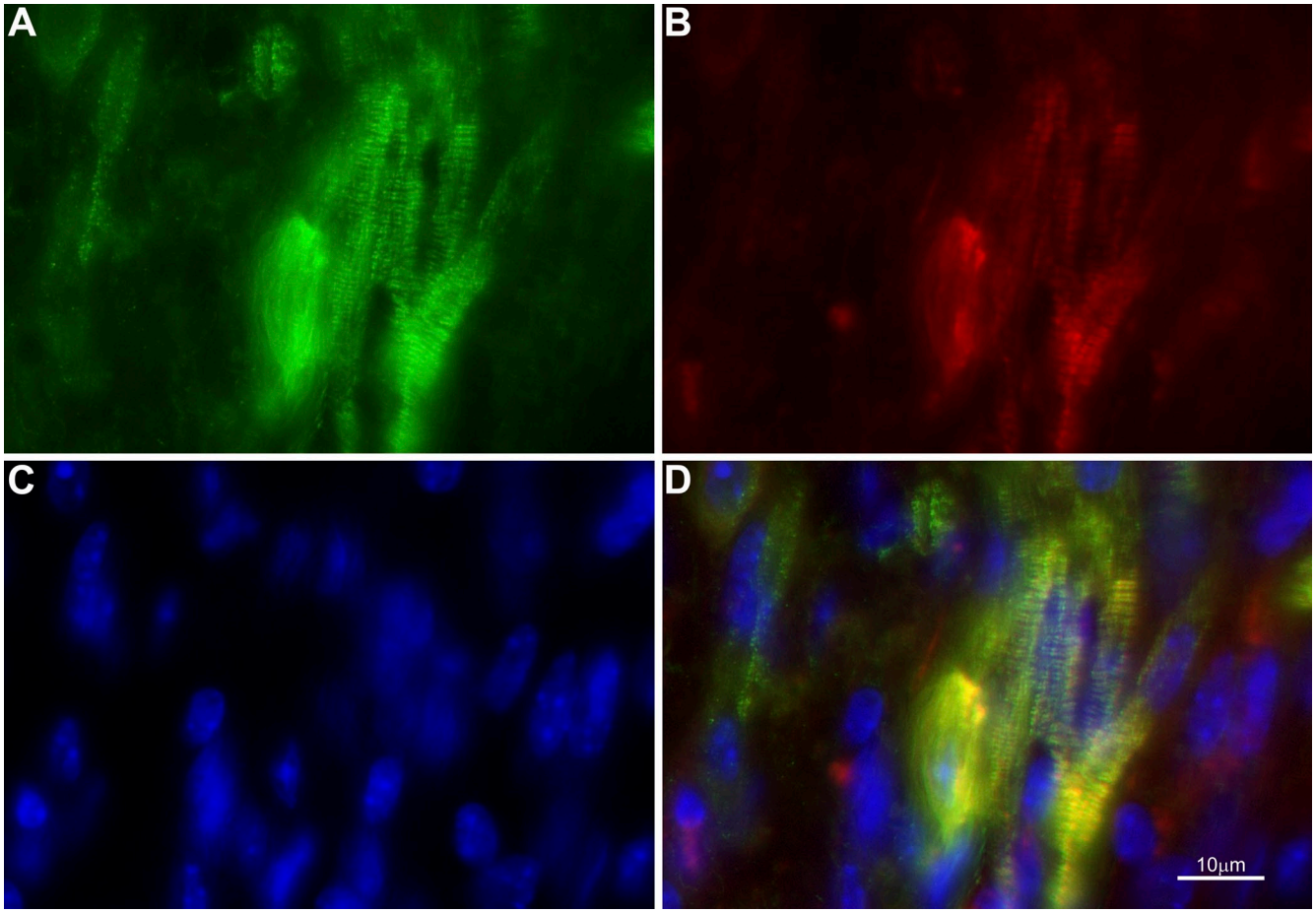

Figure S4. Human E258K cMyBP-C is efficiently incorporated into the sarcomere in KO ad258 mECT. Immunohistochemical analysis of a KO ad258 mECT. (A) E258K cMyBP-C (green fluorescence). (B) HA-tagged E258K cMyBP-C (red fluorescence). (C) Nuclei stained with DAPI (blue fluorescence). (D) An overlay of E258K cMyBP-C, HA-tagged E258K cMyBP-C, and DAPI.

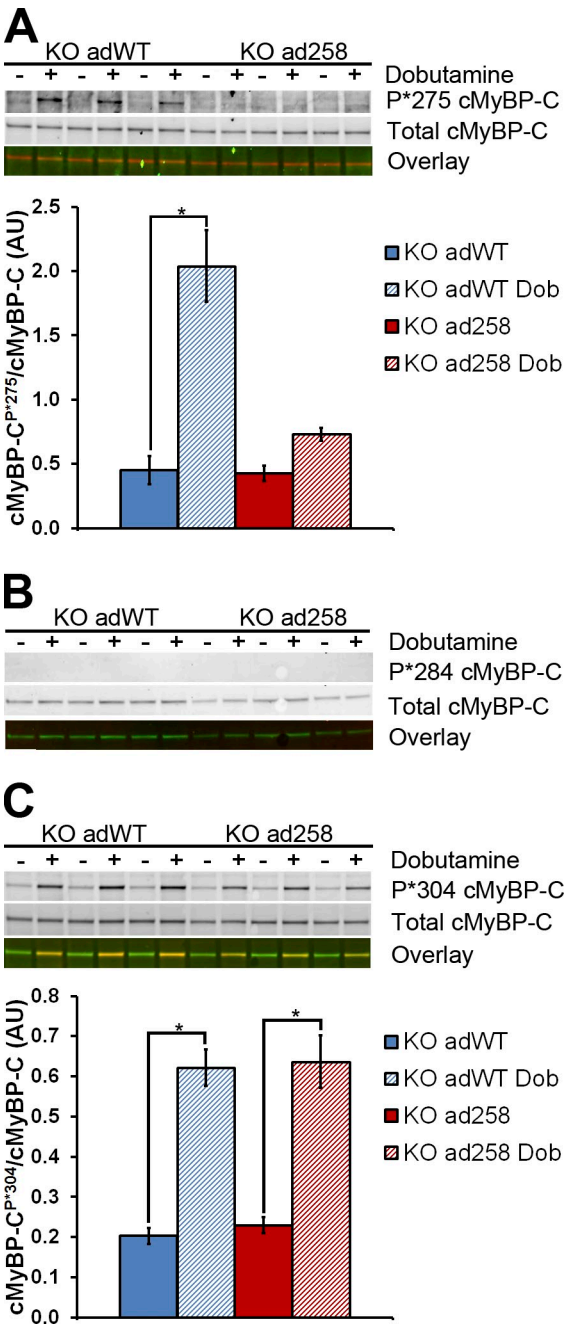

Figure S5. Effect of the E258K mutation on phosphorylation of cMyBP-C in 2D culture. (A–C) Phosphorylation of Ser275 (A), Ser284 (B), and Ser304 (C) in cMyBP-C$^{-/-}$ neonatal mouse cardiac cells transduced with either adWT or ad258 at an MOI = 20. In each panel the top Western blot rows show immunofluorescence after hybridization with the specific phospho-serine antibody and a goat anti–rabbit fluorescent secondary antibody, the middle rows show immunofluorescence after hybridization with a total cMyBP-C and a donkey anti–goat fluorescent secondary antibody, and the bottom rows show the dual fluorescence overlay, with the phospho- serine fluorescence in red and the total cMyBP-C fluorescence in green. +, cardiac cells treated with 5 µM dobutamine 5 min before cell harvest; −, cells not treated with dobutamine. The bar graphs show densitometric quantification of the Ser275 and Ser304 Western blots. *, $P < 0.05$ (one-way ANOVA with Tukey's post-hoc test; $n \geq 5$). Error bars indicate SEM.

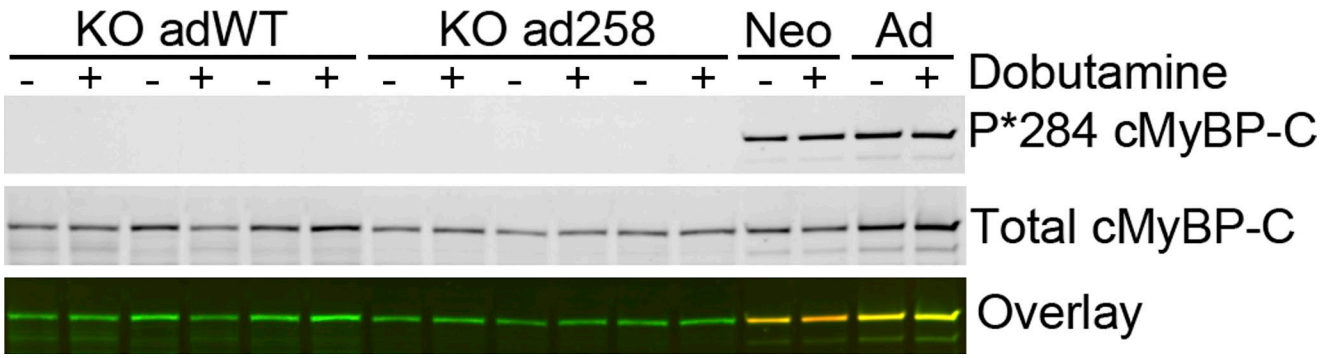

Figure S6. Phosphorylation of Ser284 in neonatal and adult hearts. Phosphorylation of Ser284 in KO adWT and KO ad258 mECT, as well as in neonatal (neo) and adult (Ad) hearts. The top Western blot row shows immunofluorescence after hybridization with the phospho-Ser284 antibody and a goat anti–rabbit fluorescent secondary antibody, the middle row shows immunofluorescence after hybridization with a total cMyBP-C and a mouse anti-goat fluorescent secondary antibody, and the bottom row shows the dual fluorescence overlay, with the phospho-Ser284 fluorescence in red and the total cMyBP-C fluorescence in green. +, samples treated with 5 µM dobutamine 5 min before cell harvest; −, cells not treated with dobutamine.

TABLE S1

Effect of the E258K mutation on twitch force production

Measure	Hz	adWT ($n = 11$)		ad258 ($n = 13$)			
		Value	SEM	Value	SEM	% change	P
TF (mN)	6	0.525	0.065	0.080	0.024	−84.8	<0.001[a]
	9	0.360	0.045	0.068	0.021	−81.1	<0.001[a]
CT_{100} (ms)	6	48.5	1.3	42.5	0.9	−12.4	0.001[a]
	9	46.7	0.9	41.0	0.9	−12.2	<0.001[a]
RT_{50} (ms)	6	37.7	1.8	28.2	0.9	−25.2	<0.001[a]
	9	32.2	0.7	24.7	0.6	−23.3	<0.001[a]
$RT_{50–90}$ (ms)	6	35.9	1.3	41.5	2.7	15.6	0.093
	9	26.0	0.4	29.3	1.2	12.7	0.021[a]

[a]$P < 0.05$ (Student's *t* test).

TABLE S2

Effect of the E258K mutation on cMyBP-C phosphorylation

Ratio	Type	KO adWT					KO ad258					P = KO adWT vs. KO ad258	
		Veh		Dob		P = Veh vs. Dob	Veh		Dob		P = Veh vs. Dob	Veh	Dob
		Value	SEM	Value	SEM		Value	SEM	Value	SEM			
$cMyBP\text{-}C_{P\text{-}S275}/cMyBP\text{-}C_{total}$	mECT	0.84	0.23	7.89	1.35	<0.001[a]	0.57	0.14	2.10	0.13	0.417	0.992	<0.001[a]
	2D	0.45	0.11	2.04	0.28	<0.001[a]	0.43	0.06	0.73	0.05	0.471	0.999	<0.001[a]
$cMyBP\text{-}C_{P\text{-}S284}/cMyBP\text{-}C_{total}$	mECT	ND	NA	ND	NA	NA	ND	NA	ND	NA	NA	NA	NA
	2D	ND	NA	ND	NA	NA	ND	NA	ND	NA	NA	NA	NA
$cMyBP\text{-}C_{P\text{-}S304}/cMyBP\text{-}C_{total}$	mECT	0.30	0.08	1.74	0.19	<0.001[a]	0.22	0.01	1.25	0.12	<0.001[a]	0.968	0.043
	2D	0.20	0.02	0.62	0.05	<0.001[a]	0.23	0.02	0.64	0.07	<0.001[a]	0.971	0.997

ND, not detected; NA, not applicable; Veh, vehicle; Dob, 5 µM dobutamine.
[a]$P < 0.05$ (one-way ANOVA with Tukey's correction for multiple comparisons).