Supplemental Material

Supplemental Figures

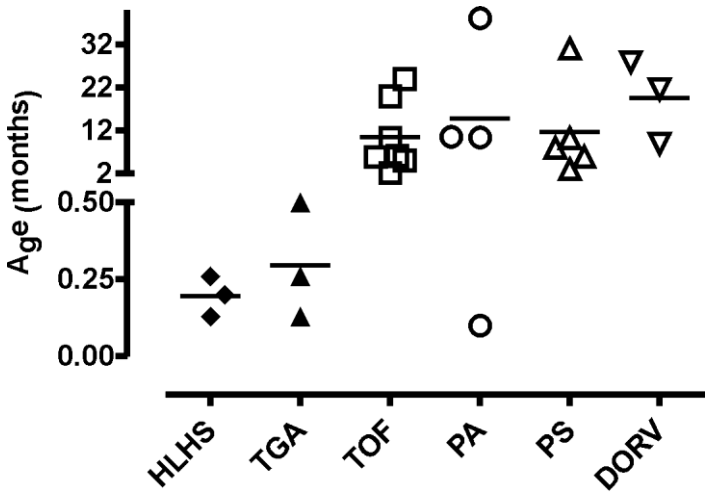

Figure S1. Relation between the age of the patients at the surgery and their clinical diagnosis. Patients with HLHS and TGA were operated at a significantly lower age whereas in the other patients the time of surgery did not depend on the diagnosis. Lines represent means. TOF, tetralogy of Fallot; PS, pulmonary stenosis; HLHS, hypoplastic left heart syndrome; DORV, double-outlet right ventricle; PA, pulmonary atresia; TGA, transposition of the great arteries. Exept for HLHS and TGA no significant differences were observed, indicating that the diagnosis does not affect timing of surgery.

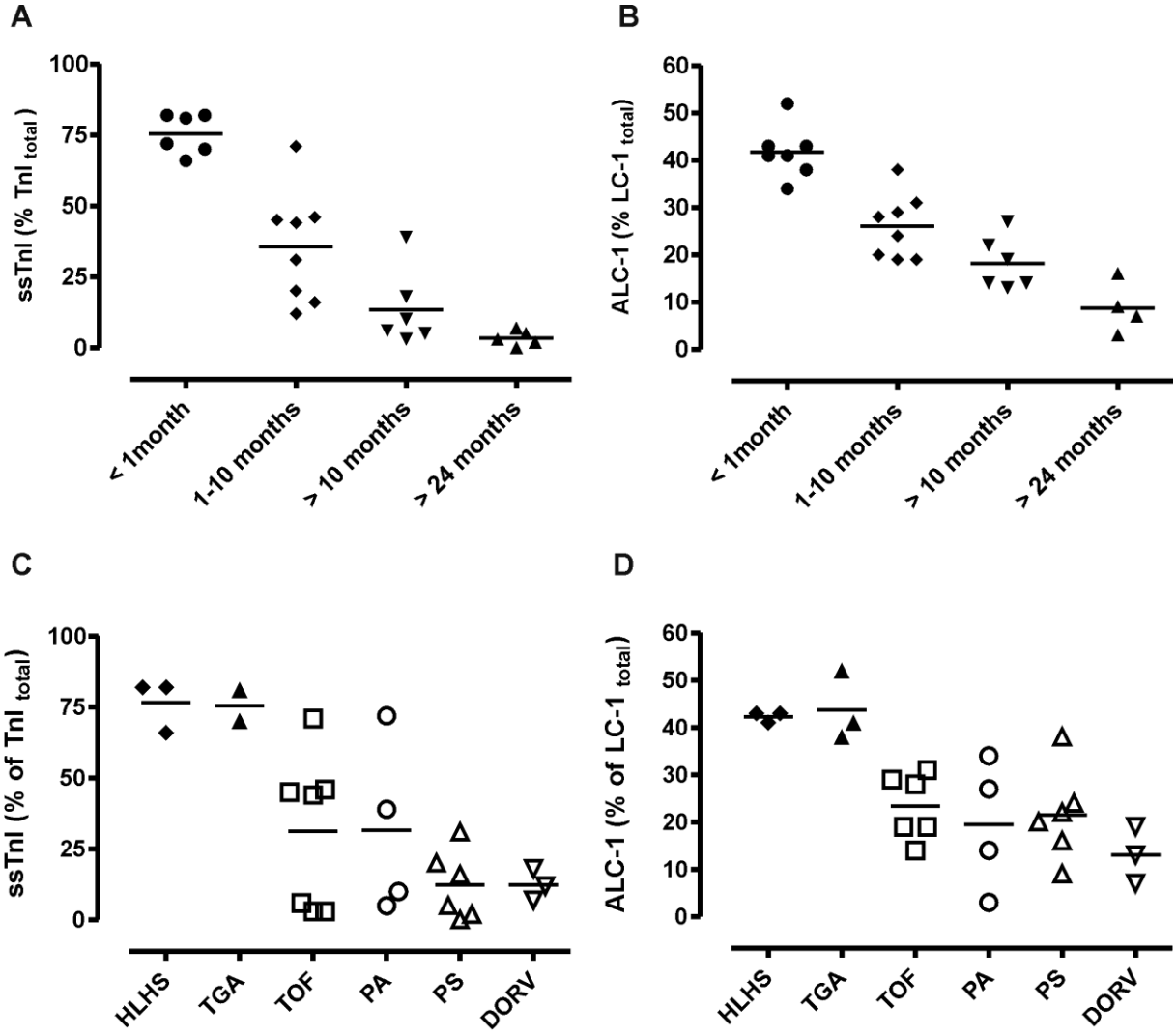

Figure S2. Dependence of ssTnI and ALC-1 expression versus age (A and B) and versus clinical diagnosis (C and D). Replot of expression levels of ALC-1 (A) as in Auckland et al. (1986) *Cardiovasc Res.* 20:828-36 as well as ssTnI (B) for different age groups. The decline in ALC-1 expression in our cohort is consistent with the published results. Patients with HLHS and TGA expressed significantly higher levels of the fetal ssTnI (C) and ALC-1 (D) isoforms than the other patients. There were no significant differences in these parameters between the other patients indicating that in these patients the diagnosis does not affect protein expression levels. The high variability in expression levels relates to the large age range at which repair surgery was performed; lines represent means. TOF, tetralogy of Fallot; PS, pulmonary stenosis; HLHS, hypoplastic left heart syndrome; DORV, double-outlet right ventricle; PA, pulmonary atresia; TGA, transposition of the great arteries.

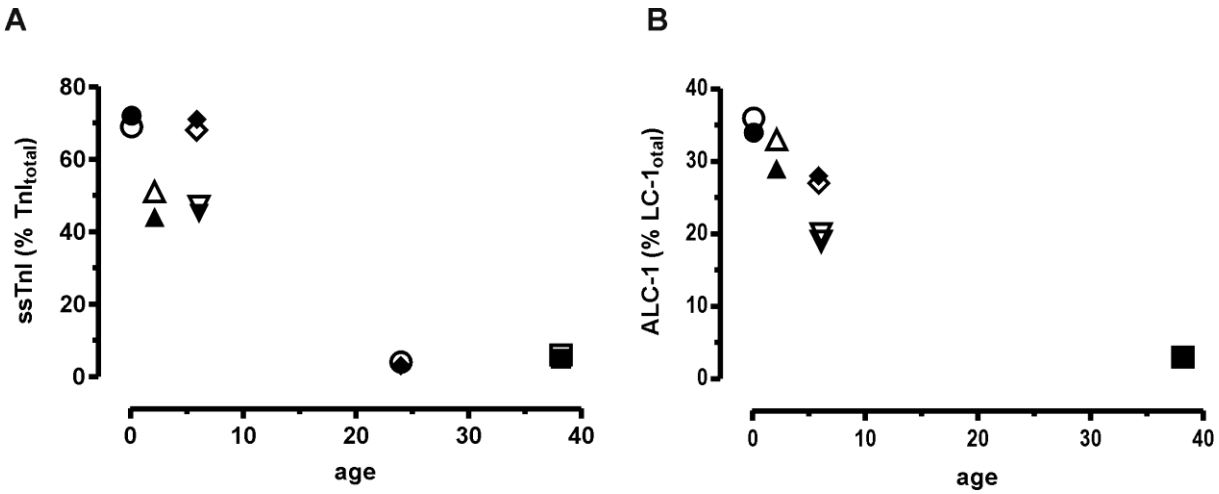

Figure S3. Expression levels of ssTnI (A) and ALC-1 (B) in tissues from the infundibulum and the moderator band from the RV taken from the same hearts. Open symbols. infundibulum; closed symbols, moderator band; circles, PA; squares, TOF. Each symbol represents one measurement.

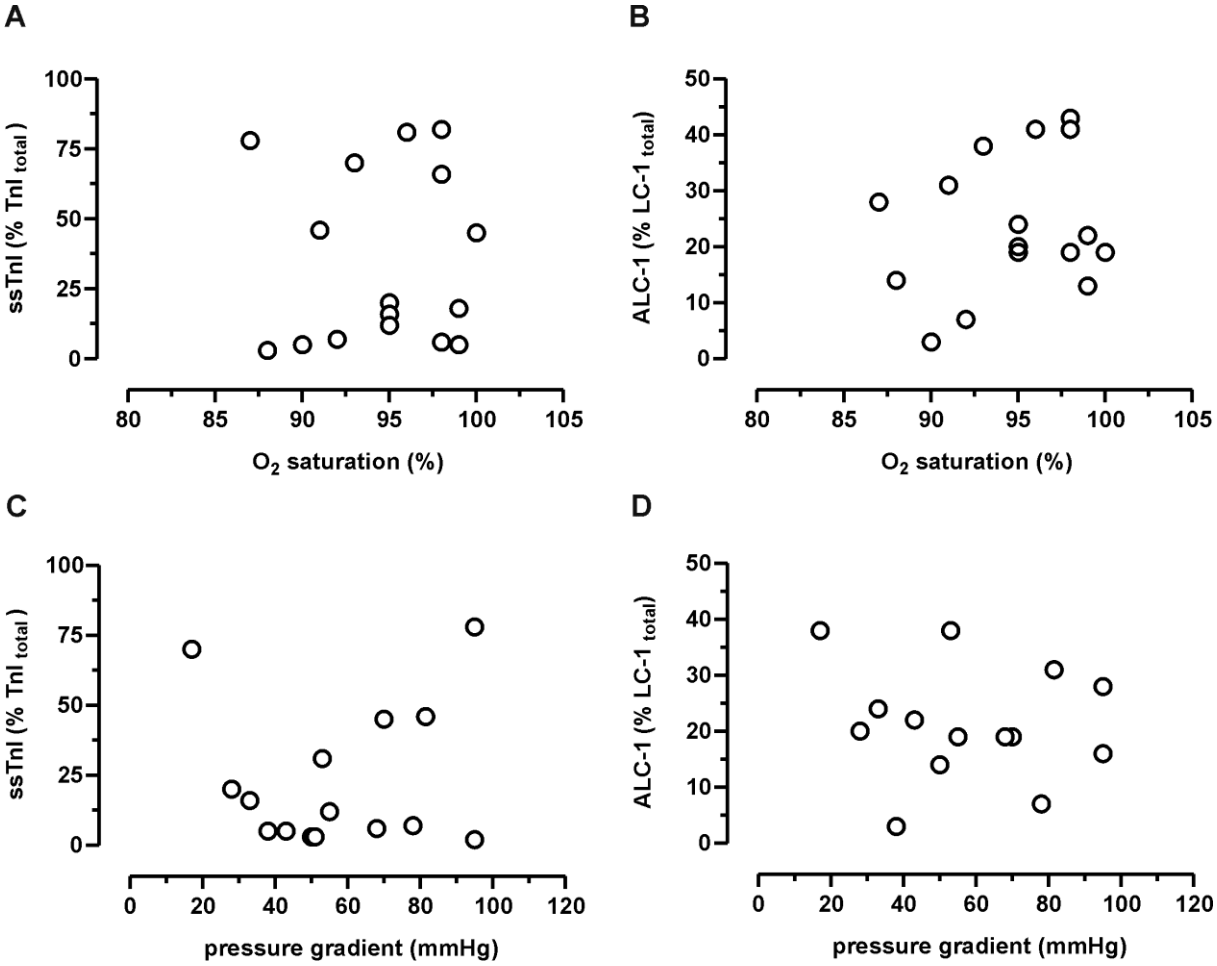

Figure S4. The expression levels of ALC-1 and ssTnI neither correlate with the oxygen saturation (A, B) nor the pressure gradient along the right ventricular outflow tract (C, D). The pressure gradient and the O_2 saturation were assessed before repair surgery.

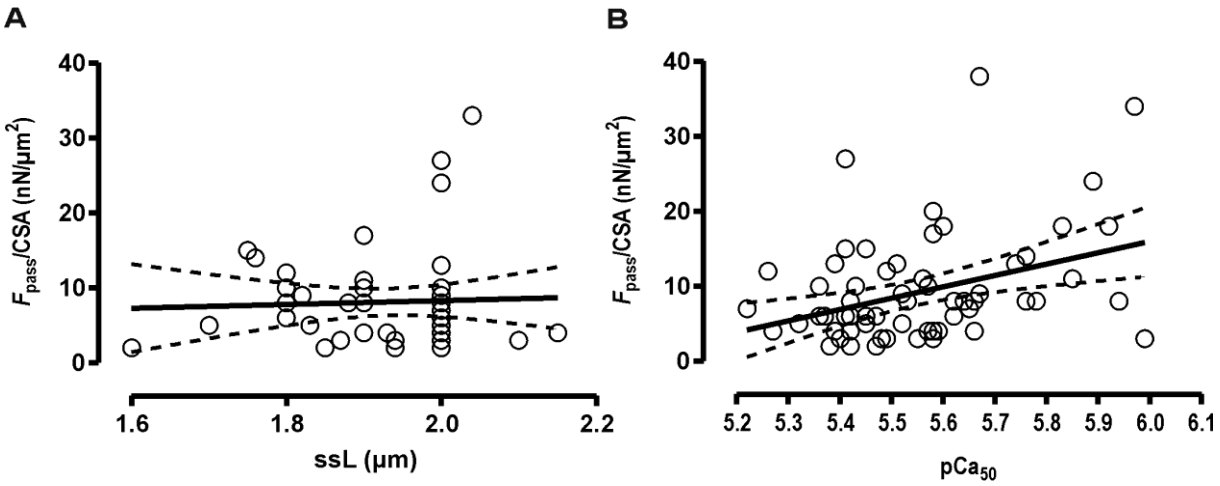

Figure S5. Interdependence of passive tension, slack sarcomere length (A), and Ca^{2+}-sensitivity (B) for individual myofibrillar bundles. While there was no effect of slack sarcomere length on passive tension (P=0.766), passive tension positively correlated with Ca^{2+}-sensitivity of force generation (r^2=0.13, P=0.003). Solid and dotted lines represent linear correlations and 95% confidence limits. Each data point represents determination from one myofibrillar bundle.

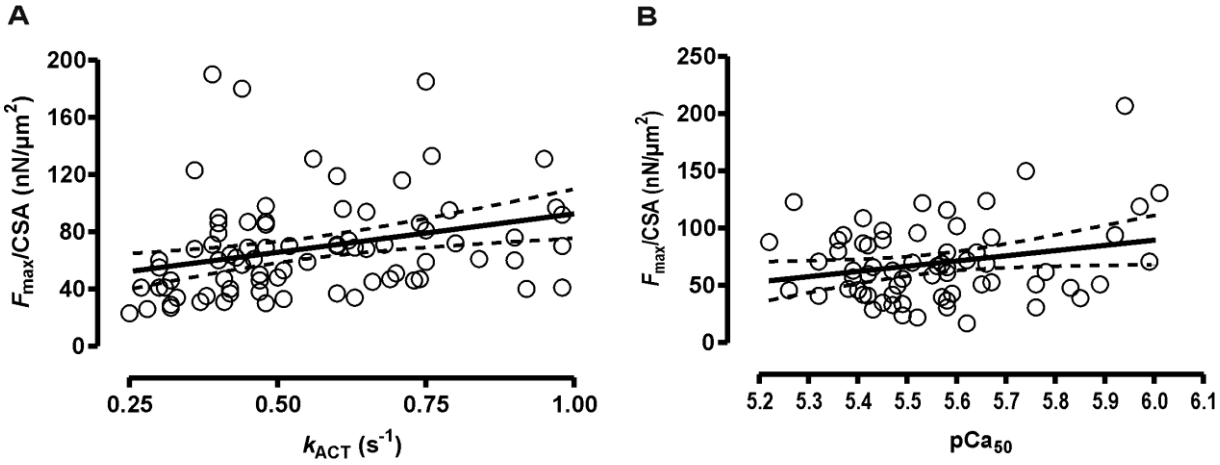

Figure S6. Relation between maximal force per cross-sectional area (F_{max}/CSA) and k_{ACT} (A), or pCa_{50} (B), respectively. The maximal tension correlated significantly with the Ca^{2+}-induced contraction kinetics (r^2=0.11, P=0.0025) and with the Ca^{2+}-sensitivity of force (r^2=0.06, P=0.044). The rate constant k_{ACT} reflects the sum of apparent rate constants by which the cross-bridges enter and leave the force-generating state; i.e., $k_{ACT} = f_{app} + g_{app}$. Furthermore, F_{max} is proportional to $f_{app}/(f_{app}+g_{app})$. Because F_{max} is positively and not negatively correlated with k_{ACT} , increased maximal force relates to an increase in f_{app} rather than to a decrease in g_{app}. Of note, k_{ACT} would also increase if g_{app} is increased. However, this would result in a decreased F_{max}. Thus, increased isometric force generation and contraction kinetics at high ALC1-expression levels can be explained by an increased f_{app}, i.e. by the increased probability of cross-bridges to form force-generating interactions with actin. Solid and dotted lines represent linear correlations and 95% confidence limits. Each data point represents determination from one myofibrillar bundle.

Supplemental Tables

Table S1 Clinical characteristics of patients.

Patient	**Age, months**	**Diagnosis**	**Palliation**	**Medication**	**SpO_2, %**	**G, mmHg**
1	2.16	TOF with predominant valvar PS , microdeletion 22q11	Pulmonary valvuloplasty	–		
2	5.1	TOF		beta blocker	91	81.5
3	5.9	TOF, severely underdeveloped PA system		beta blocker	87	95
4	6.1	TOF,		beta blocker	100	70
5	10.36	TOF with complete AVSD, trisomy 21	AP shunt	ACE, furosemide, ASS, beta blocker	85–90	50
6	20	Residual RVOTO after TOF correction, microdeletion 22q11		–	98	60–75
7	24	TOF	BT shunt	–	85–90	51
8	0.1	PA/ IVS	Pulmonary valvuloplasty	–		
9	10.46	PA with VSD, TGA, severely hypoplastic (rPA & lPA)	AP shunt, BD	–		
10	10.63	PA with VSD	AP shunt	–		

11	38.2	PA with VSD		–	90	35–40
12	3	Severe supravalvar PS, VSD	RV-PA conduit, VSD closure	spironolactone, HCT		53
13	5.9	RVOTO with VSD		spironolactone, furosemide	95	25–30
14	8	AVSD, moderate RVOTO, large secundum ASD, trisomy 21		beta blocker	95	30–35
15	10.23	HCM with pronounced LVH, RVH, Infundibular and valvar PS secundum ASD	Primary ASD closure, Patch reconstruction of RVOTO	–	99	40–45
16	31	Common arterial trunk post-correction; severe RV-PA conduit stenosis, microdeletion 22q11	Patch reconstruction of RVOTO, conduit replacement	–		90–100
17	127	PA with VSD, RV – PA conduit stenosis, severe PV insufficiency	RV-PA conduit replacement, VSD closure	–		45–55
18/1	9	DORV with valvar PS	Patch reconstruction of RVOTO, VSD closure	beta blocker	93–97	50–60
18/2	28	Subpulmonary stenosis after DORV correction		beta blocker	92	75–80
19	21.6	DORV, microdeletion of Chr 6	PAB	furosemide	98–99	
20	0.13	HLHS		PG	95–100	

21	0.2	HLHS	PG	98	
22	0.26	HLHS	PG, furosemide		
23	0.13	DILV, TGA			
24	0.26	DILV, TGA	PG, furosemide	93	13–20
25	0.5	DILV, TGA	–	96	

Abbreviations in alphabetical order: AP, aortopulmonary; AVI, aortic valve insufficiency; BT, Blalock–Taussig; DILV, double inlet left ventricle; DORV, double-outlet right ventricle; G, pre-surgery pressure gradient across right ventricular outflow tract; HCM, hypertrophic cardiomyopathy; HCT, Hydrochlorothiazide; HLHS, hypoplastic left heart syndrome; IVS, intact ventricular septum; LVH, left ventricular hypertrophy; PA, pulmonary atresia; PAB, pulmonary artery banding; PG, prostaglandin; PS, pulmonary stenosis; PV, pulmonary valve; RVH, right ventricular hypertrophy; RVOTO, right ventricular outflow tract obstruction; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect. 18/1 and 18/2 are samples from the same patient obtained 9 and 28 months after birth, respectively.

Table S2 Expression levels of sarcomeric protein isoforms in RV samples.

Patient	Diagnosis	Age (M)	% ssTnI	% ALC-1	LC-1/LC-2	TnT4	N2AB:N2B
1	TOF	2.16	44	29	1.11	nd	nd
2	TOF	5.1	46	31	1.14	−	nd
3	TOF	5.9	71	28	1.10	+	37:63
4	TOF	6.1	45	19	1.11	−	nd
5	TOF	10.36	3	14	1.03	+	38:62
6	TOF	20	6	19	1.04	−	nd
7	TOF	24	3	nd	nd	−	nd
8	PA	0.1	72	34	1.11	+	nd
9	PA	10.46	10	27	1.06	+	44:56
10	PA	10.63	39	14	0.93	nd	nd
11	PA	38.2	5	3	0.84	−	31:69
12	PS	3	31	38	0.84	+	nd
13	PS	5.9	20	20	1.07	nd	nd
14	PS	8	16	24	1.00	−	nd
15	PS	10.23	5	22	0.99	nd	42:58
16	PS	31	2	16	1.39	−	44:56
17	PS	127	0	9	0.99	−	nd
18a	DORV	9	12	19	0.84	nd	nd
18b	DORV	28	7	7	0.94	nd	nd
19	DORV	21.6	18	13	0.81	−	nd

20	HPLHS	0.13	82	43	0.93	+	nd
21	HPLHS	0.2	66	41	1.03	−	nd
22	HPLHS	0.26	82	43	0.99	+	nd
23	TGA	0.13	nd	52	0.93	−	nd
24	TGA	0.26	70	38	0.96	−	60:40
25	TGA	0.5	81	41	1.09	−	nd

TOF, tetralogy of Fallot; PS, pulmonary stenosis; HPLHS, hypoplastic left heart syndrome; DORV, double-outlet right ventricle; PA, pulmonary atresia; TGA, transposition of the great arteries. Age is given in months. (−) indicates that the fetal isoforms of TnT were not detectable, and nd indicates not done. Note that in patients 20 -25 no functional data could be determined.

Table S3 Summary of functional characterization.

Patient	Diagnosis	Age at surgery (months)	pCa_{50}	n_H	F_{max}/CSA (nN/µm^2)	F_{pass}/CSA (nN/µm^2)	k_{ACT} (s^{-1})	k_{TR} (s^{-1})	k_{LIN} (s^{-1})	t_{LIN} (s^{-1})	k_{REL} (s^{-1})
1	TOF	2.16	5.71 ± 0.05	2.42 ± 0.44	69 ± 4	9 ± 5	nd	0.37 ± 0.05	0.29 ± 0.04	0.25 ± 0.02	5.98 ± 0.21
2	TOF	5.1	5.56 ± 0.03	2.23 ± 0.17	100 ± 12	11 ± 3	0.70 ± 0.07	0.55 ± 0.03	0.47 ± 0.07	0.19 ± 0.01	3.85 ± 0.22
3	TOF	5.9	5.84 ± 0.05	1.16 ± 0.25	62 ± 16	11 ± 2	0.57 ± 0.09	0.53 ± 0.05	0.34 ± 0.03	0.26 ± 0.02	2.78 ± 0.19
4	TOF	6.1	5.52 ± 0.03	2.42 ± 0.19	64 ± 10	16 ± 4	0.43 ± 0.03	0.57 ± 0.07	0.18 ± 0.04	0.33 ± 0.05	1.67 ± 0.19
5	TOF	10.36	5.40 ± 0.05	2.20 ± 0.13	75 ± 13	5 ± 1	0.42 ± 0.06	0.36 ± 0.02	0.44 ± 0.07	0.21 ± 0.02	3.19 ± 0.34
6	TOF	20	5.41 ± 0.01	2.20 ± 0.20	62 ± 12	5 ± 1	0.41 ± 0.03	0.26 ± 0.03	0.46 ± 0.11	0.17 ± 0.02	2.97 ± 0.23
7	TOF	24	5.55 ± 0.07	1.65 ± 0.20	61 ± 3	5 ± 1	0.35 ± 0.02	0.27 ± 0.03	0.21 ± 0.09	0.26 ± 0.06	2.17 ± 0.30
8	PA	0.1	5.95 ± 0.03	1.50 ± 0.34	128 ± 27	16 ± 7	0.63 ± 0.07	0.62 ± 0.06	0.36 ± 0.08	0.36 ± 0.02	3.99 ± 0.39
9	PA	10.46	5.54 ± 0.03	2.03 ± 0.15	83 ± 11	5 ± 1	0.62 ± 0.08	0.61 ± 0.06	0.38 ± 0.03	0.24 ± 0.03	2.64 ± 0.29
11	PA	38.2	5.33 ± 0.05	1.88 ± 0.23	91 ± 6	6 ± 1	0.47 ± 0.04	0.37 ± 0.05	0.27 ± 0.09	0.27 ± 0.03	2.31 ± 0.29
14	PS	8	5.41 ± 0.04	1.54 ± 0.09	86 ± 10	14 ± 2	0.68 ± 0.07	0.51 ± 0.05	0.27 ± 0.07	0.30 ± 0.03	3.16 ± 0.34

15	PS	10.23	5.47 ± 0.03	1.90 ± 0.13	80 ± 9	7 ± 1	0.66 ± 0.03	0.54 ± 0.06	0.46 ± 0.04	0.13 ± 0.01	4.39 ± 0.30
17	PS	127	5.54 ± 0.03	4.69 ± 1.30	37 ± 6	4 ± 1	0.30 ± 0.04	0.29 ± 0.02	0.25 ± 0.02	0.32 ± 0.02	1.77 ± 0.06
19	DORV	216	5.67 ± 0.07	1.15 ± 0.20	89 ± 10	15 ± 3	0.71 ± 0.08	0.56 ± 0.09	0.41 ± 0.14	0.17 ± 0.02	2.91 ± 0.23

pCa_{50}, $-\log[Ca^{2+}]$ required for half-maximal activation; n_H, steepness of force-Ca^{2+} relation; F_{max}, active steady state force; F_{pass}, passive steady state force; CSA, cross-sectional area; k_{ACT} and k_{TR}, rate constant of Ca^{2+}-induced and mechanically-induced contraction, respectively; k_{LIN} (lasting for t_{LIN}) and k_{REL}, rate constant of initial, slow, linear and of the subsequent rapid exponential relaxation phase, respectively; TOF, tetralogy of Fallot; PS, pulmonary stenosis; HPLHS, hypoplastic left heart syndrome; DORV, double-outlet right ventricle; PA, pulmonary atresia; TGA, transposition of the great arteries; nd, not done. Values are means ± SEM of 2–12 myofibrils for each patient.