

Supplementary Information

Phosphomimetic cardiac myosin-binding protein C partially rescues a cardiomyopathy phenotype in murine engineered heart tissue

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Suppl. Table S1: Primer sequences for site-directed mutagenesis

Primer	Sequence (5' to 3')
<i>Mybpc3</i> S282D F	GAGCAGGTCGGAGAACCGATGACAGCCATGAAGATG
<i>Mybpc3</i> S282D R	CATCTTCATGGCTGTCATCGGTTCTCCGACCTGCTC

Suppl. Table S2: Primer sequences for RT-PCR and RT-qPCR with SYBR-Green

Primer	Sequence (5' to 3')
FLAG F	GGATTACAAGGATGACGACGA
<i>Gapdh</i> F	ATTCAACGGCACAGTCAAG
<i>Gapdh</i> R	TGGCTCCACCCTTCAAGT
<i>Mybpc3 ex.1</i> F	CACCCCTGGTGTGACTGTTCTCAA
<i>Mybpc3 ex.2</i> R	GTCATCAGGGCTCGCATC
<i>Mybpc3 ex.2</i> R	CTGACCGCTCCGTCTCAG
<i>Mybpc3 ex.4</i> F	TCTTTCTGATGCGACCACAG
<i>Mybpc3 ex.9</i> R	TCCAGAGTCCCAGCATCTTC

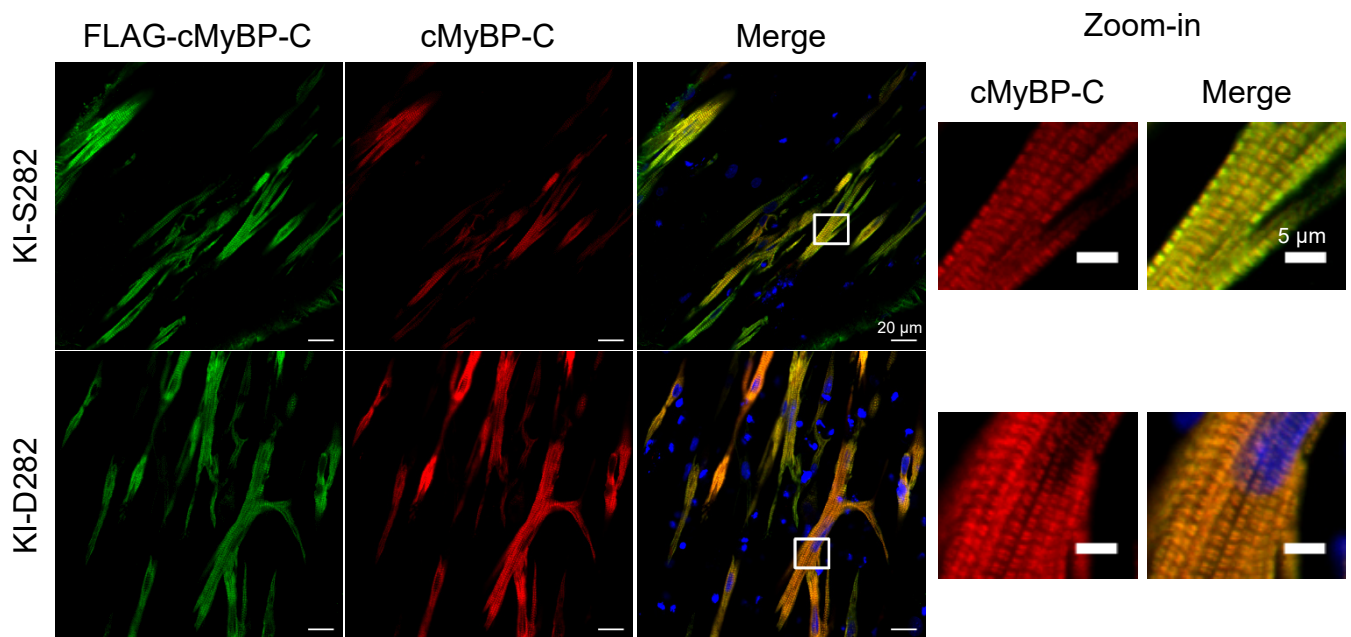
Suppl. Table S3: Primers and probes sequences for RT-qPCR

Gene acronym	Full name	Primers/Probe	Sequence (5' to 3')
<i>Gnas</i>	<i>Guanine nucleotide-binding protein, alpha stimulating</i>	F	CAAGGCTCTGTGGGAGGAT
		R	CGAAGCAGGTCCTGGTCACT
		Probe	FAM-GCTGATTGACTGTGCCAGTACTTCCT-TAMRA
<i>Mybpc3</i>	<i>Myosin-binding protein C, cardiac</i>	WT/Mut1 F	GTGTCTACCAAGGACAAATTTGACA
		WT/Mut1 R	CCAGGTCTCCAGAACCAATG
		WT Probe	FAM-AACCTCACTGTCCATGAG-MGB
		Mut1 Probe	VIC-CTCACTGTCCATAAGG-MGB
		Mutant 2/3 F	TGGACCTGAGCAGCAAAGTG
		Mutant 2/3 R	GGTCCAGGTCTCCAGAACCA
		Mutant 2/3 Probe	FAM-CCAGCAAGAGGCCA-MGB

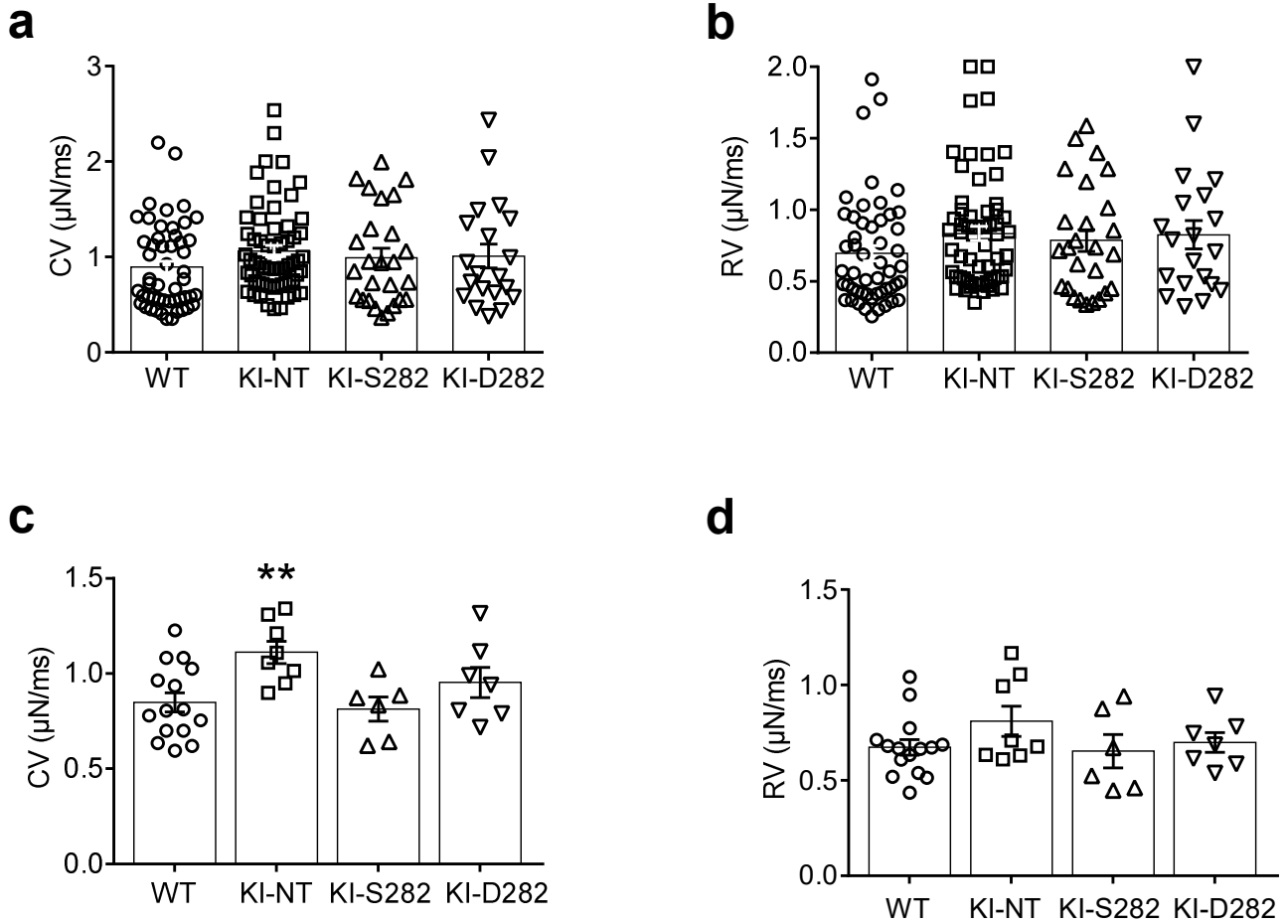
Suppl. Table S4: Gene acronym, full name and expression level in WT, KI-NT, KI-S282 and KI-D282 EHTs

	Gene acronym	Gene full name	WT	KI-NT	KI-S282	KI-D282
Hypertrophy	<i>Actc1</i>	<i>Actin, alpha, cardiac muscle 1</i>	1.00	2.03	1.32	1.49
	<i>Ctgf</i>	<i>Connective tissue growth factor</i>	1.00	1.39	1.22	1.21
	<i>Fhl1</i>	<i>Four-and-a-half-LIM-domains 1</i>	1.00	1.78	1.16	1.49
	<i>Meox</i>	<i>Mesenchyme homeobox 1</i>	1.00	2.16	2.21	2.13
	<i>Myh7</i>	<i>Myosin, heavy polypeptide 7, cardiac muscle, beta</i>	1.00	3.58	1.24	2.63
	<i>Nppb</i>	<i>Natriuretic peptide B</i>	1.00	1.94	0.65	1.04
	<i>Rcan1</i>	<i>Regulator of calcineurin 1</i>	1.00	1.63	1.05	1.30
Ca ²⁺ handling	<i>Cacna1c</i>	<i>Calcium channel, voltage-dependent, L type, alpha 1C subunit</i>	1.00	1.44	1.20	1.29
	<i>Cacna1g</i>	<i>Calcium channel, voltage-dependent, T type, alpha 1G subunit</i>	1.00	1.57	1.82	1.36
	<i>Gja1</i>	<i>Gap junction protein, alpha 1, 43kDa</i>	1.00	1.47	1.07	1.16
	<i>Pln</i>	<i>Phospholamban</i>	1.00	1.44	1.39	1.42
	<i>Ryr2</i>	<i>Ryanodine receptor 2, cardiac</i>	1.00	11.33	1.17	6.02
K ⁺ /Na ⁺ regulation	<i>Kcnb1</i>	<i>Potassium voltage gated channel, Shab-related subfamily, member 1</i>	1.00	1.36	1.46	1.40
	<i>Kcnp2</i>	<i>Kv channel-interacting protein 2</i>	1.00	3.55	0.78	2.17
	<i>Kcnj2</i>	<i>Potassium inwardly-rectifying channel, subfamily J, member 2</i>	1.00	nd	1.17	nd
	<i>Scn5a</i>	<i>Sodium channel, voltage-gated, type V, alpha</i>	1.00	1.39	1.10	1.54
Sarcomere	<i>Actn2</i>	<i>Actinin alpha 2</i>	1.00	1.47	1.30	1.32
	<i>Cryab</i>	<i>Crystallin, alpha B</i>	1.00	1.57	1.28	1.41
	<i>Csrp3</i>	<i>Cysteine and glycine-rich protein 3</i>	1.00	1.61	1.27	1.55
	<i>Des</i>	<i>Desmin</i>	1.00	2.53	1.07	1.42
	<i>Finc</i>	<i>FilaminC</i>	1.00	1.48	0.95	1.43
	<i>Ldb3</i>	<i>LIM domain binding 3</i>	1.00	1.69	1.36	1.46
	<i>Myl2</i>	<i>Myosin, light polypeptide 2, regulatory, cardiac</i>	1.00	1.71	1.75	1.76
	<i>Myl3</i>	<i>Myosin, light polypeptide 3, essential, cardiac</i>	1.00	1.69	1.48	1.54
	<i>Myoz2</i>	<i>Myozenin 2</i>	1.00	1.60	1.44	1.36
	<i>Mypn</i>	<i>Myopalladin</i>	1.00	2.18	1.45	1.20
	<i>Myzap</i>	<i>Myocardial zonula adherens protein</i>	1.00	2.05	1.34	1.24
	<i>Nexn</i>	<i>Nexilin</i>	1.00	1.52	1.28	1.49
	<i>Obscn</i>	<i>Obscurin</i>	1.00	1.53	1.17	1.77
	<i>Tcap</i>	<i>Telethonin</i>	1.00	1.34	1.87	1.41
	<i>Tnnc1</i>	<i>Troponin C, cardiac</i>	1.00	1.46	1.31	1.33
	<i>Ttn</i>	<i>Titin</i>	1.00	1.62	1.29	1.60

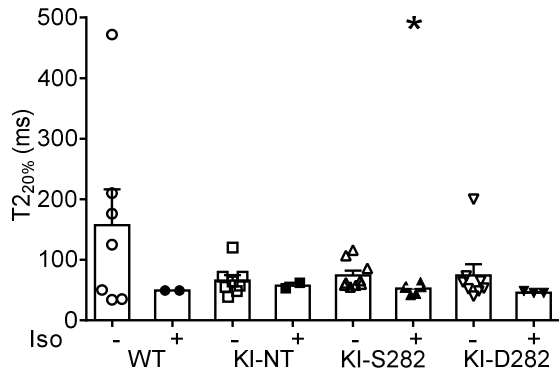
Values in KI-NT, KI-S282 and KI-D282 are given in fold-change over WT. nd: not-determined.



Suppl. Fig. 1 Immunofluorescence images of KI EHTs transduced with AAV6 encoding wild-type cMyBP-C (S282) or phosphomimetic cMyBP-C (D282) KI EHTs were transduced at MOI of 1,000 vg/cell. After fixation EHTs were co-stained with antibodies directed against the FLAG-tag for exogenous cMyBP-C (green) and total cMyBP-C (red). Nuclei were stained with DRAQ5 (blue). Scale bars 20 μm. Higher magnifications (zoom-in) images are shown on the right side. Scale bars 5 μm.



Suppl. Fig. 2 Contraction velocity (CV) and relaxation velocity (RV) measured on the day of highest force development [a]-[b] Contraction and relaxation velocities under spontaneous contraction. Numbers of EHTs/batches: WT 51/5; KI-NT 61/7; KI-S282 26/5; KI-D282 20/5. **[c]-[d]** Contraction and relaxation velocities calculated under electrical stimulation. WT n = 15; KI-NT n = 8; KI-S282 n = 6; KI-D282 n = 7. Data are expressed as mean \pm SEM. ** $P < 0.01$ vs. WT, one-way ANOVA plus Dunnett's multiple comparisons test.



Suppl. Fig. 3 Effect of isoprenaline on relaxation time ($T_{20\%}$) under electrical stimulation. EHTs of all groups were treated 15 min with 100 nM isoprenaline at submaximal external $[Ca^{2+}]$ in Tyrode's solution and paced at 6 Hz. $T_{20\%}$ was decreased in WT, S282 (* $P < 0.05$ Student's t-test) and D282 EHTs.

Full agarose gels. Red rectangles show the selected part, red arrow shows displayed bands in the figures

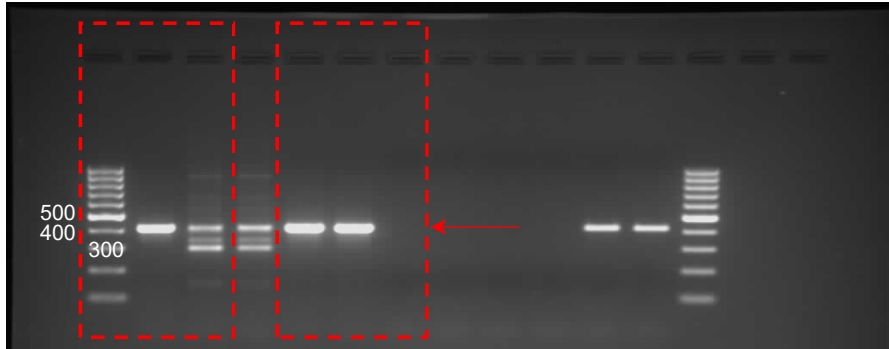


Fig. 1a upper panel

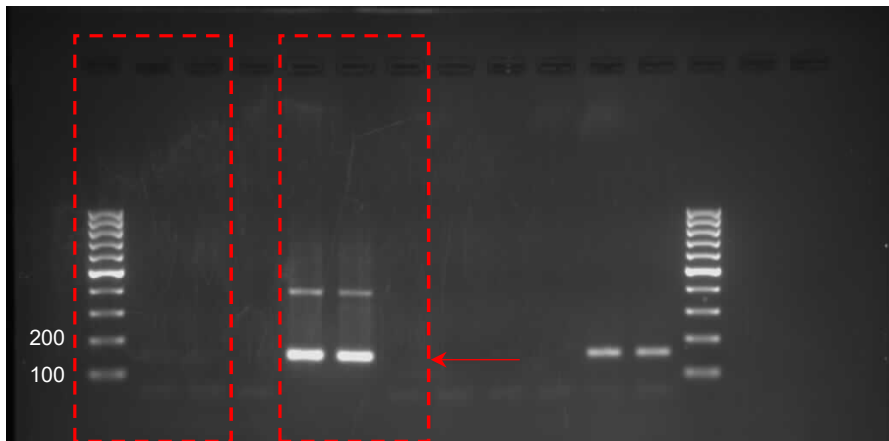


Fig. 1a middle panel

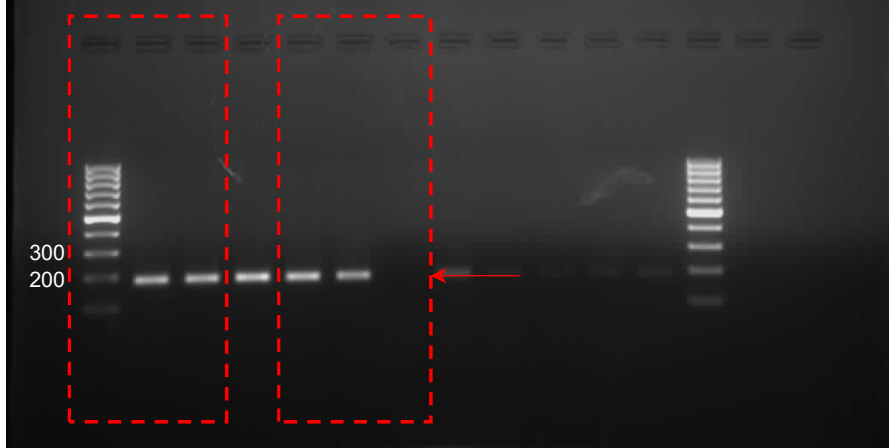


Fig. 1a bottom panel

Full Western blot membranes. Red rectangles show the selected part, red arrow shows displayed bands in the figures

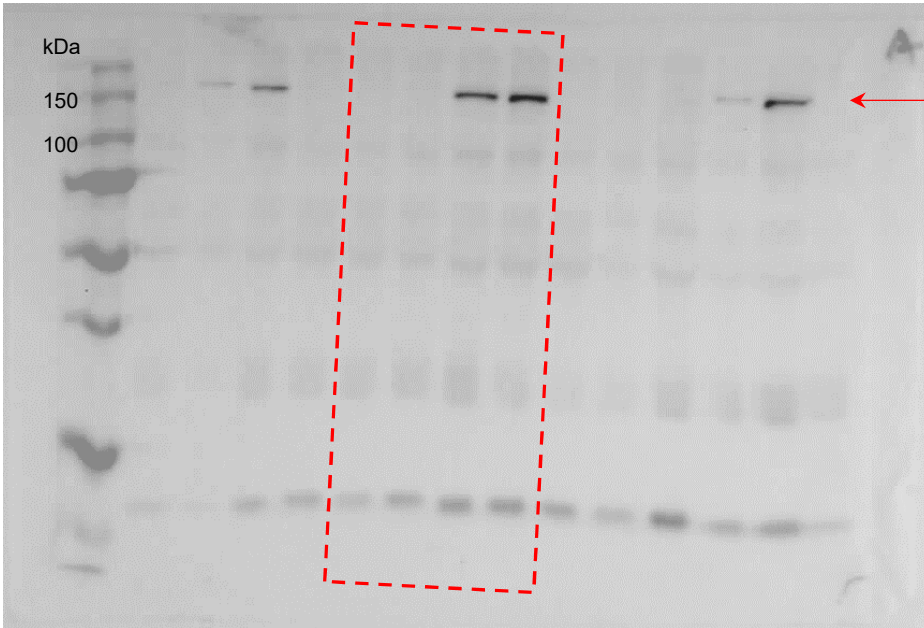


Fig.1g FLAG

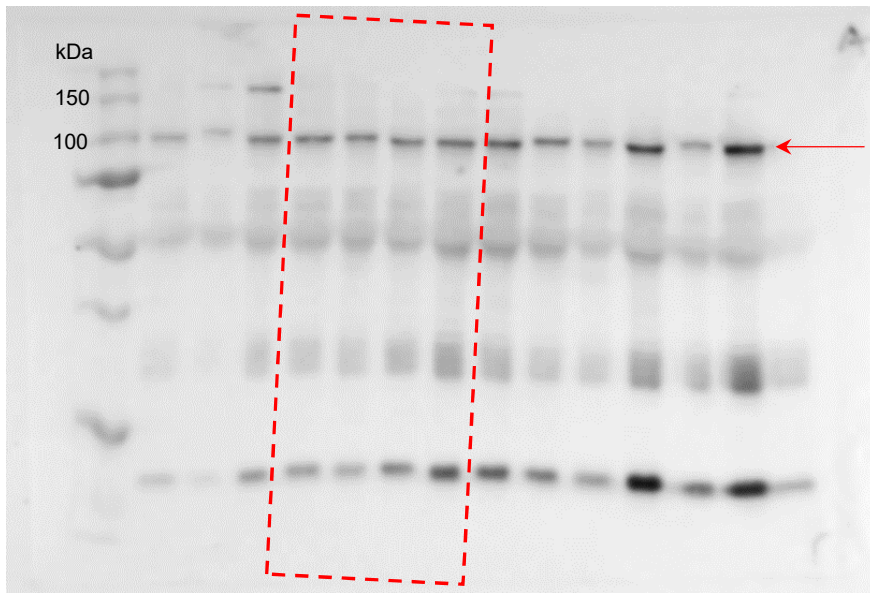


Fig.1g α -Actinin

Full Western blot membranes. Red rectangles show the selected part, red arrow shows displayed bands in the figures

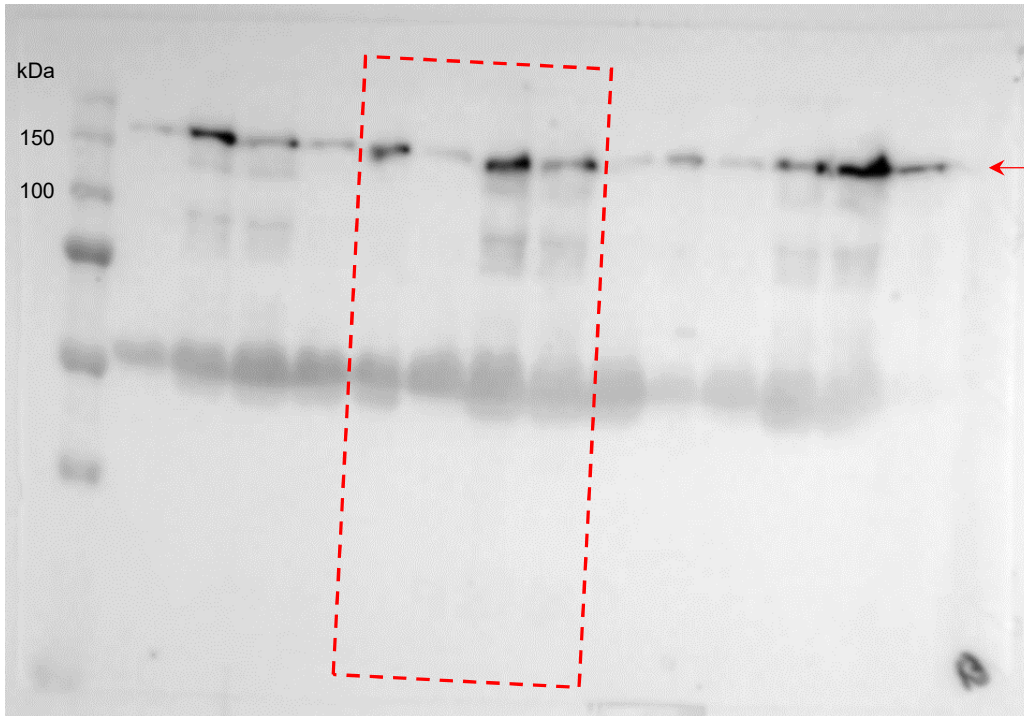


Fig.1g cMyBP-C

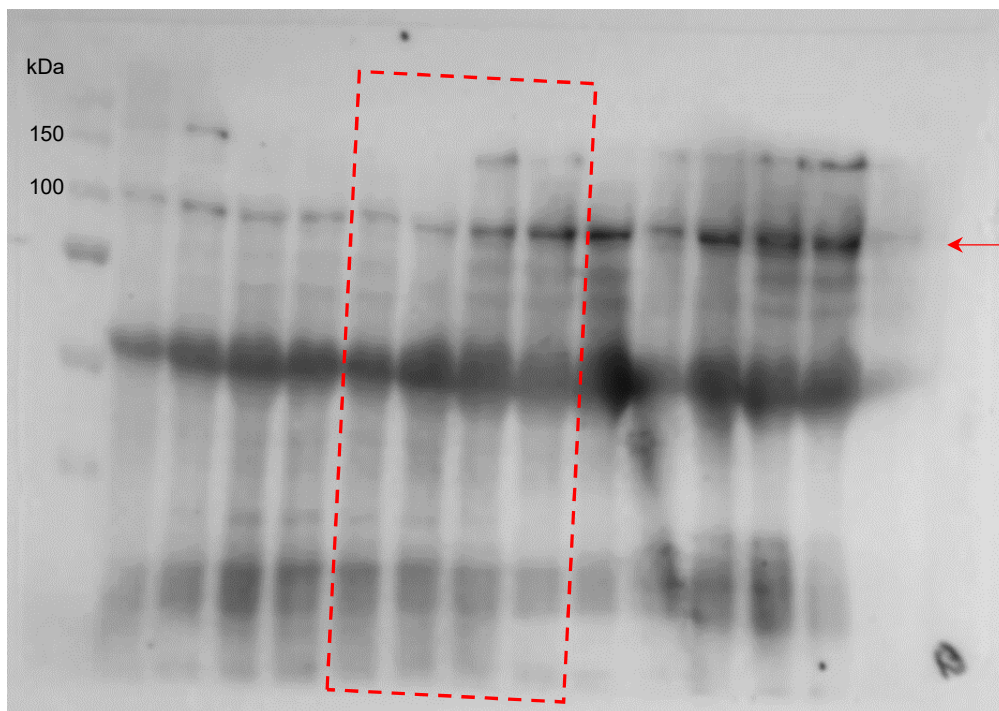


Fig.1g α -Actinin

Full Western blot membranes. Red rectangles show the selected part, red arrow shows displayed bands in the figures

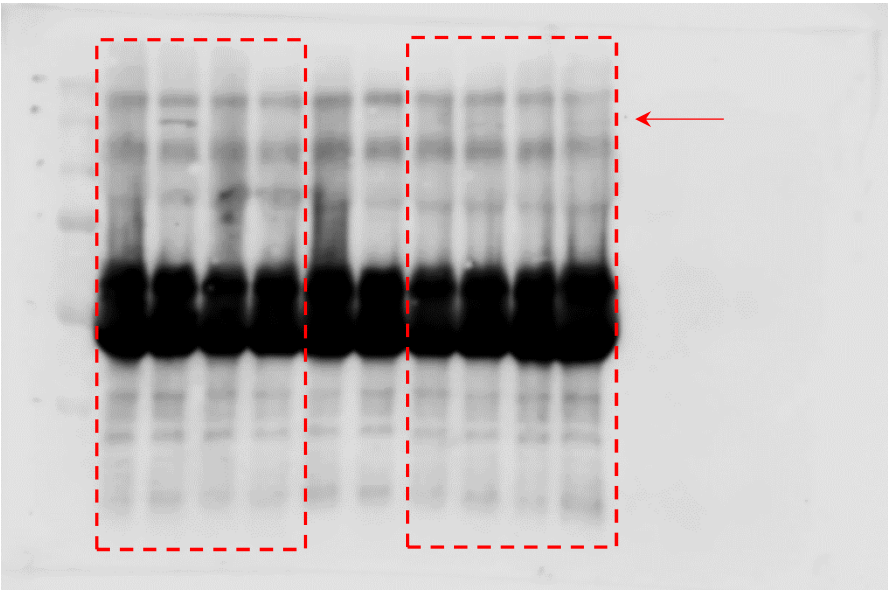


Fig.1i pSer-273 cMyBP-C

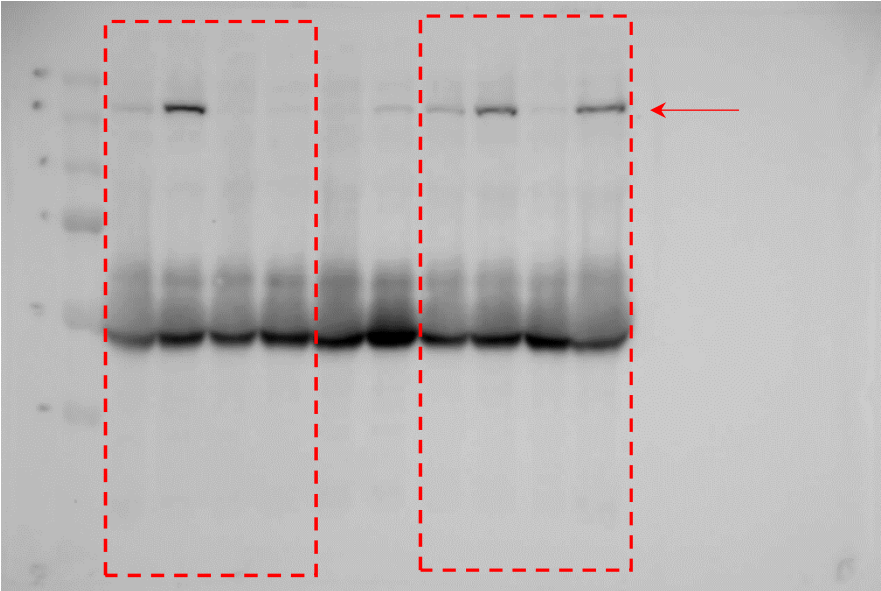


Fig.1i pSer-302 cMyBP-C

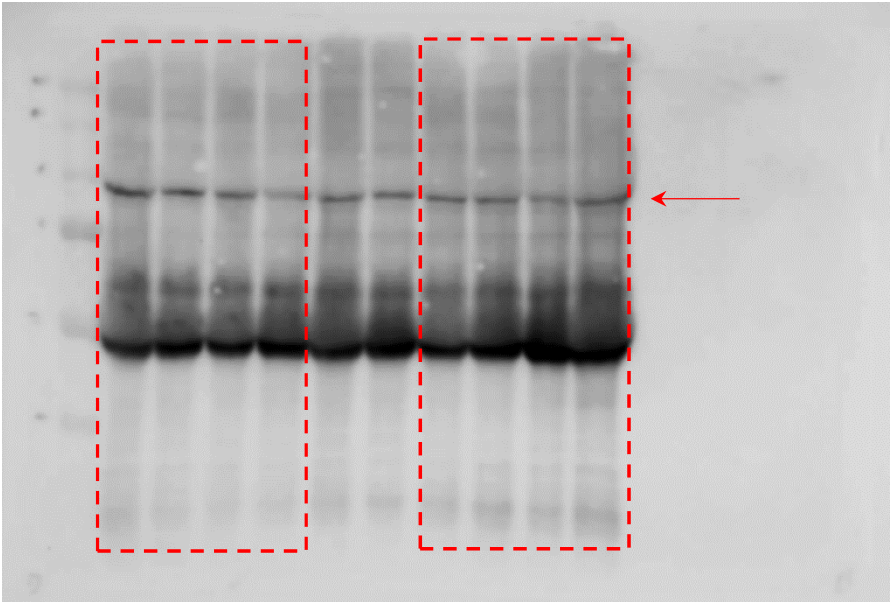


Fig.1i α -Actinin

Full Western blot membranes. Red rectangles show the selected part, red arrow shows displayed bands in the figures

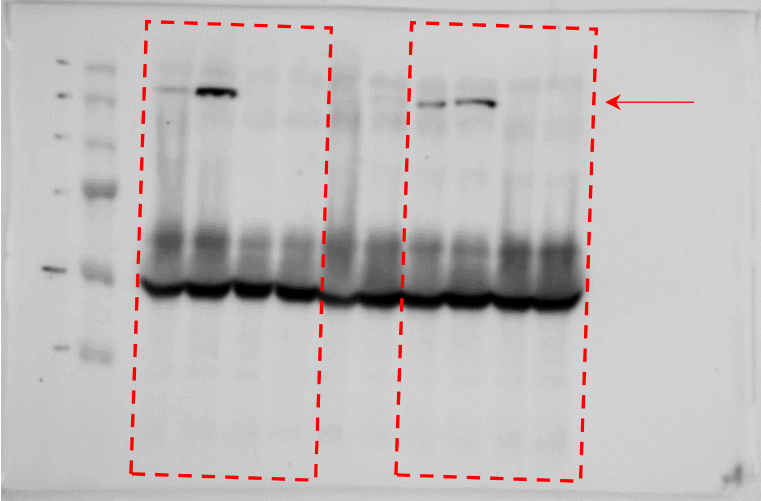


Fig.1i pSer-282 cMyBP-C

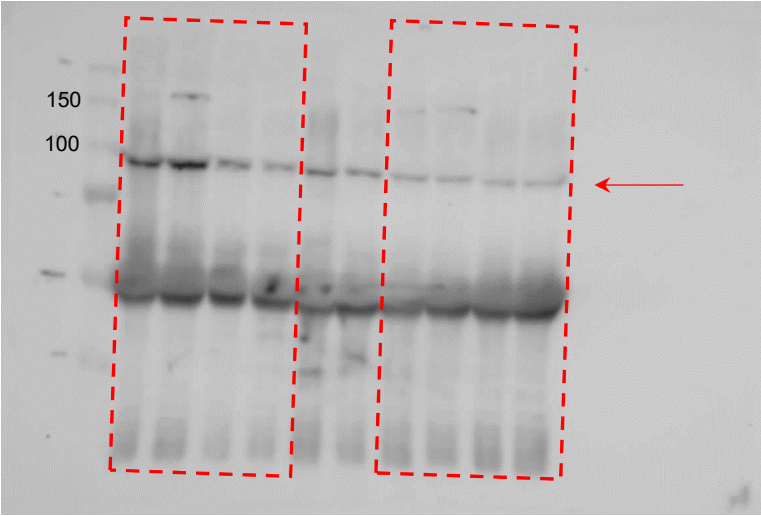


Fig.1i α -Actinin