Online supplemental material

MUSCLE CREATINE KINASE DEFICIENCY TRIGGERS BOTH ACTIN DEPOLYMERIZATION AND DESMIN DISORGANIZATION BY ADVANCED GLYCATION END-PRODUCTS IN DILATED CARDIOMYOPATHY

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TABLES

Spot No[a]	Accession number[b]	Protein[b]	Function[c]	Score MS[d]	Sequence coverage[d]	Score MS/MS[d]	pI[d]	MW[b]	FC[e]	T-test[e]
UP-REGULATED										
30	P31001	Desmin	muscle adaptation	420	74%	217	5.21	53 465	2.88	4.4E-06
31	P31001	Desmin	muscle adaptation	153	20%	102	5.21	53 465	2.73 / 1.37*	4.0E-06 / 1.6E-03
29	P31001	Desmin	muscle adaptation	68	19%	40	5.21	53 465	2.56 / 1.41*	5.5E-06 / 1.2E-02
32	P31001	Desmin	muscle adaptation	527	74%	278	5.21	53 465	2.49 / 1.26*	1.2E-05 / 6.9E-03
22	P08238	Heat shock protein HSP 90-beta	protein folding	148	40%	53	4.97	82 373	2.31	5.2E-06
37	P50462	cysteine and glycine-rich protein 3 (Csrp3/ MLP)	cardiac muscle tissue development	66	67%	225	8.90	20 881	2.3	4.6E-05
36	Q00623	Apolipoprotein A-I	cholesterol transport	293	48%	162	5.64	30 569	2.21	3.6E-02
26	P09103	Protein disulfide-isomerase	cell redox homeostasis	193	42%	74	4.79	57 018	2.05	3.6E-04
27	P20152	Vimentin	intermediate filament-based process	190	30%	126	5.06	53 655	2	8.2E-05
35	P48036	Annexin A5	calcium signaling	332	43%	202	4.83	35 730	1.95	1.1E-04
43	Q9DCX2	ATP synthase subunit d. mitochondrial	ATP synthesis coupled proton transport	139	57%	62	5.52	18 738	1.93	3.6E-02
28	P68372	Tubulin beta-2C chain	microtubule-based process	123	59%	70	4.79	49 799	1.91	3.4E-02
41	P23927	Alpha-crystallin B chain	response to stress	53	10%	47	6.76	20 056	1.58*	1.9E-02
24	P63017	Heat shock cognate 71 kDa protein	protein folding	105	54%	298	5.37	70 827	1.58	1.6E-06
25	P63017	Heat shock cognate 71 kDa protein	protein folding	524	35%	298	5.37	70 827	1.53	1.4E-05
33	Q61598	Rab GDP dissociation inhibitor beta	protein transport	103	59%	61	5.93	50 505	1.52	1.8E-04
42	P23927	Alpha-crystallin B chain	response to stress	n.a.	10%	53	6.76	20 056	1.49*	2.7E-02
34	Q99L47	Hsc70-interacting protein (HIP)	protein folding	81	21%	62	5.19	41 629	1.49	3.9E-04
45	P05413	Fatty acid-binding protein. heart	fatty acid metabolic process	300	72%	170	6.29	14 849	1.32*	3.0E-02
DOWN-REGULATED										
5	P21550	Beta-enolase	glycolysis	705	82%	361	6.73	46 995	-1.3	3.5E-04
13	P05201	Aspartate aminotransferase. cytoplasmic	cellular amino acid metabolic process	376	62%	302	6.68	46 202	-1.31	4.5E-04
17	P42125	3.2-trans-enoyl-CoA isomerase. mitochondrial	fatty acid metabolic process	197	21%	130	8.87	32 058	-1.33	2.0E-02
15	O35459	Delta(3.5)-Delta(2.4)-dienoyl-CoA isomerase. mitochondrial	fatty acid metabolic process	134	31%	94	7.60	36 095	-1.37	1.2E-02
11	P45952	Medium-chain specific acyl-CoA dehydrogenase. mitochondrial	fatty acid metabolic process	95	27%	49	8.60	46 452	-1.38	2.8E-04
8	P50752	Troponin T. cardiac muscle	cardiac muscle contraction	148	30%	67	4.98	35 804	-1.43	2.3E-03
16	O35459	Delta(3.5)-Delta(2.4)-dienoyl-CoA isomerase. mitochondrial	fatty acid metabolic process	56	4%	56	7.60	36 095	-1.49	1.8E-03
18	P48787	Troponin I. cardiac muscle	cardiac muscle contraction	169	53%	85	9.57	24 244	-1.59	1.7E-03
9	P07310	Creatine kinase M-type	phosphocreatine metabolic process	325	13%	157	6.58	43 018	-2.2 / -1.47*	5.0E-06 / 5.3E-06
10	P07310	Creatine kinase M-type	phosphocreatine metabolic process	225	22%	51	6.58	43 018	-5.8 / -2.02*	1.3E-07 / 3.8E-05

Table S1: Differentially expressed proteins in the dilated heart after SRF inactivation.
Ranked by fold change (1.3 ≤ FC ≤ -1.3)
(a) Spot no. refers to Fig. 1
(b) SwissProt accession number, protein name and molecular weight (MW).
(c) Biological function indicated by Gene Ontology database.
(d) MASCOT scores for MS and MS/MS profiles following MALDI-TOF and TOF/TOF analyses. Mascot calculated isoelectric point (pI)
(e) Decyder fold change (FC) expressed as an average ratio of mutant over control spot volume and Decyder T-test p-val (p≤0.05). All FC value are given for the gels analyzed at day 45 after SRF inactivation except for some spots also identified at day 25 (asterisk).

Spot No[(a)]	Accession number[(b)]	Protein[(b)]	Function[(c)]	Score MS[(d)]	Sequence coverage[(d)]	pI[(d)]	MW[(b)]	FC[(e)]	T-test[(e)]
40	Q9R0Y5	Adenylate kinase isoenzyme 1	ATP metabolic process	219	51%	5.67	21 526	2.14	1.00E-01
38	Q9DCX2	ATP synthase subunit d, mitochondrial	ATP synthesis coupled proton transport	181	59%	5.52	18 738	2.05	7.70E-02
44	Q9DCX2	ATP synthase subunit d, mitochondrial	ATP synthesis coupled proton transport	168	93%	5.52	18 738	2.00	1.30E-01
39	Q9DCX2	ATP synthase subunit d, mitochondrial	ATP synthesis coupled proton transport	94	49%	5.52	18 738	1.91	2.20E-02
20	Q64727	Vinculin	cell adhesion	63	41%	5.77	116 64 4	1.60	2.70E-04
21	Q64727	Vinculin	cell adhesion	111	35%	5.77	116 64 4	1.58	2.50E-04
23	P63017	Heat shock cognate 71 kDa protein	protein folding	132	38%	5.37	70 827	1.57	2.10E-04
4	P56480	ATP synthase subunit beta, mitochondrial	ATP biosynthetic process	581	71%	5.19	56 265	-1.03	6.70E-01
19	P09542	Myosin light chain 3	skeletal muscle tissue development	292	75%	5.03	22 407	-1.13	1.90E-02
7	P68033	Actin, alpha cardiac muscle 1	cardiac muscle contraction	210	22%	5.23	41 992	-1.23/ -1.15*	4.7E-02/ 8.5 E-03
1	Q99KI0	Aconitate hydratase, mitochondrial	metabolic process	125	32%	8.08	85 410	-1.20	9.30E-03
14	P58771	Tropomyosin alpha-1 chain	cardiac muscle contraction	53	41%	4.69	32 661	-1.22	1.50E-01
12	Q0KK56	Protein FAM184B	unknown	83	65%	5.59	107534	-1.31	2.30E-03
6	Q9Z2I9	Succinyl-CoA ligase [ADP-forming] subunit beta, mitonchondrial	tricarboxylic acid cycle	68	36%	6.57	50 082	-1.33	3.70E-04
2	P41216	Long-chain-fatty-acid--CoA ligase 1	fatty acid metabolic process	105	26%	6.81	77 873	-1.36	7.20E-04
3	Q9D0K2	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1 mitochondrial	ketone body catabolic process	98	46%	8.73	55 953	-1.46	4.60E-05

Table S2: Additional cardiac proteins identified by MS only.
Ranked by average ratio. Spots numbers refer to spots circled in black in Fig. 1
(a) Spot no. refers to Fig. 1
(b) SwissProt accession number, protein name and molecular weight (MW).
(c) Biological function indicated by Gene Ontology database.
(d) MASCOT scores for MS and MS/MS profiles following MALDI-TOF and TOF/TOF analyses. Mascot calculated isoelectric point (pI)
(e) Decyder fold change (FC) expressed as an average ratio of mutant over control spot volume and Decyder T-test p-val. All FC value are given for the gels analyzed at day 45 after SRF inactivation except for some spots also identified at day 25 (asterisk).

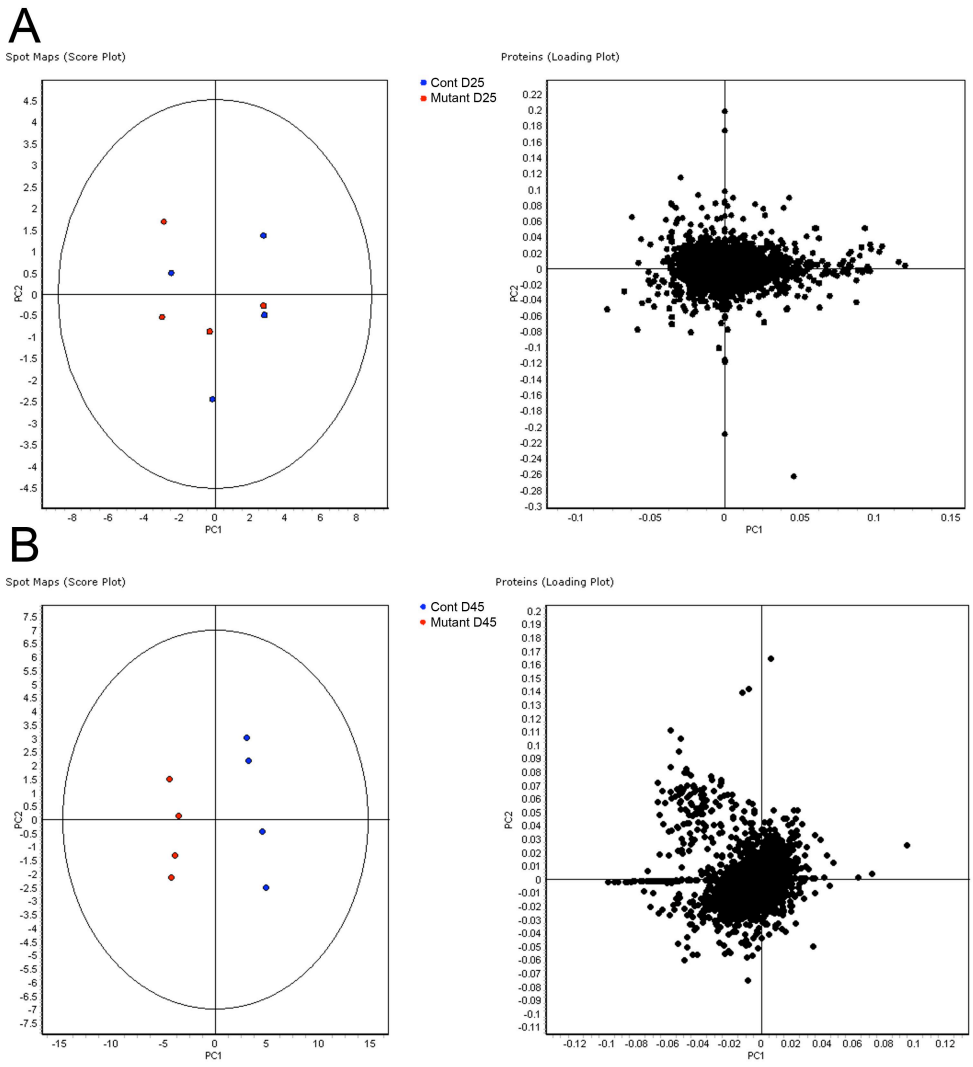

Fig. S1: Principal component analysis (PCA) of spot maps versus all proteins. (A) PCA at day 25 with a dataset of 1907 spots. Left panel: spots maps from different experimental group are not separated. Right panel: loading plot of the 1907 spots. (B) PCA at day 45 with a dataset of 2185 spots. Spot maps are separated following experimental groups based on principal component vector 1 (PC1). Therefore, there are major differences between the proteomes of control and mutant hearts at this stage.

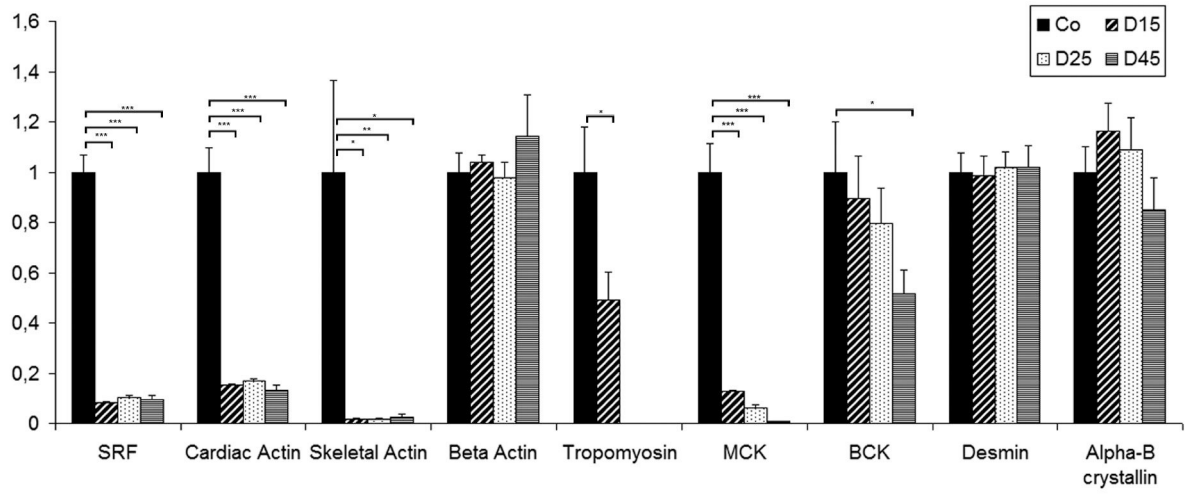

Fig. S2: Q-PCR Analysis for gene expression in the SRF^{HKO}
Messenger RNA expression was analyzed by RT-qPCR in controls and SRF mutants at 15, 25 and 45 days extracts HPRT as a control gene for normalization.
*: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.05$

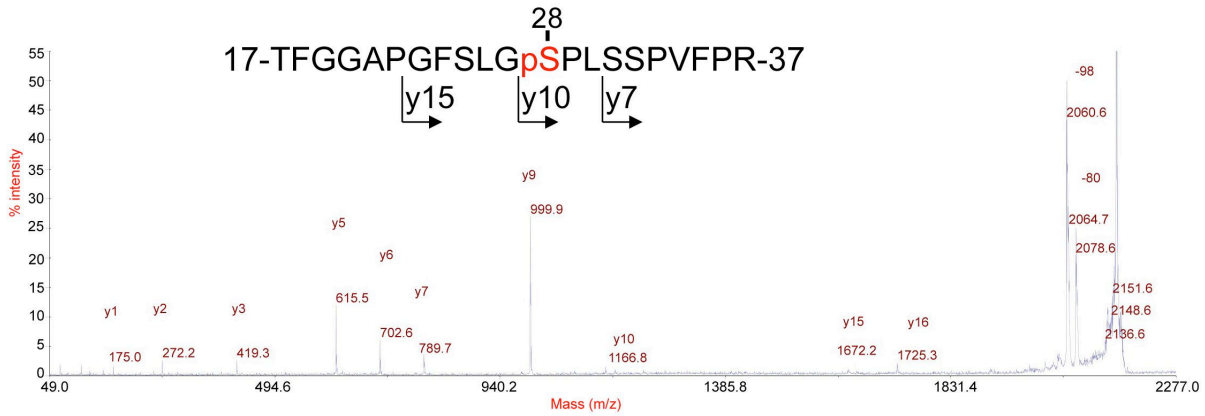

Fig. S3: MS/MS spectrum of the 2158.O8 ion showing the phosphorylation of serine 28. The spectrum is annotated for the y-ions series, which fit with a single phosphate group added to serine 28. Note the 2 peaks at 2060.06 and 2078.6 with a typical loss of the phosphate group (-98 or -80).

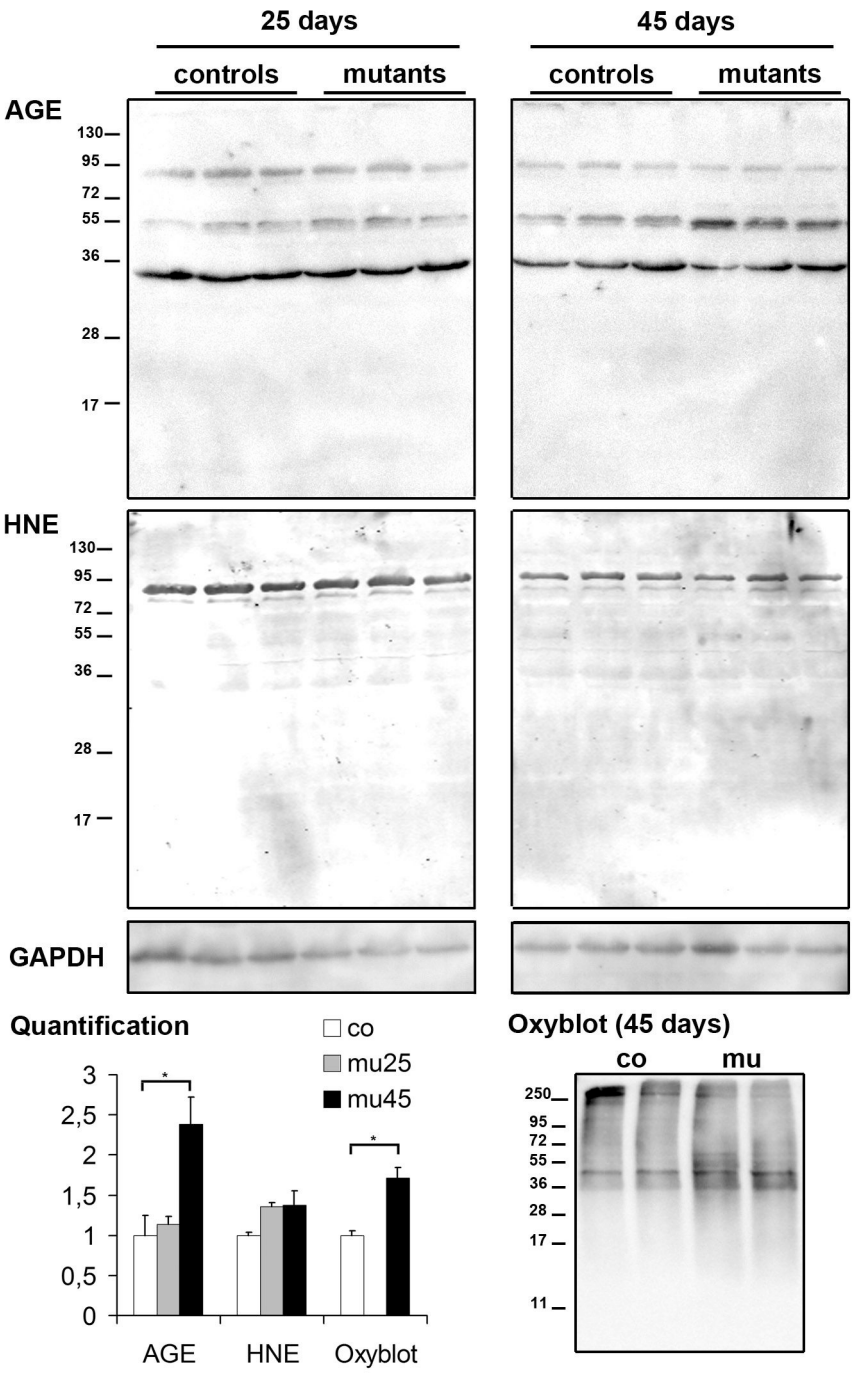

Fig. S4: Analysis of post-translational modifications associated with oxidative stress. 1D western blot with anti-AGE (Advanced Glycation End-product), anti-HNE (4-hydroxy-2-nonenal lipid peroxidation product) and Oxyblot protein oxidation detection revealing oxidized protein. Quantification of AGE western blot is shown at 25 and 45 days after SRF inactivation.

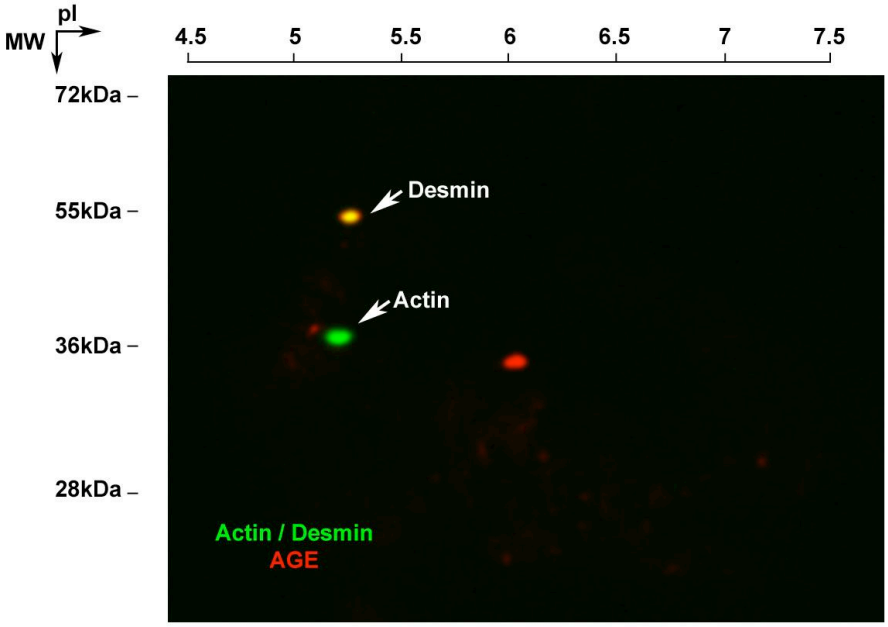

Fig. S5: 2D Western blot for desmin, sarcomeric actin and AGE antibodies.

Cardiac protein extracts from SRF^{HKO} at 45 days were analyzed by 2D-PAGE electrophoresis and blotted on PVDF membranes. Membranes were incubated simultaneously with mouse monoclonal antibodies against desmin and sarcomeric actin and rabbit polyclonal anti-AGE antibody followed by revelation with IR fluorescent dye couple secondary antibodies agains mouse IgG (green) and rabbit IgG (red). Merge of green and red channel show the desmin spot in orange because it is co-labelled by anti-desmin and anti-AGE. Actin is not labelled by the anti-AGE antibody and appears in the green channel only. Additional spots in red highlight other unidentified glycated proteins.

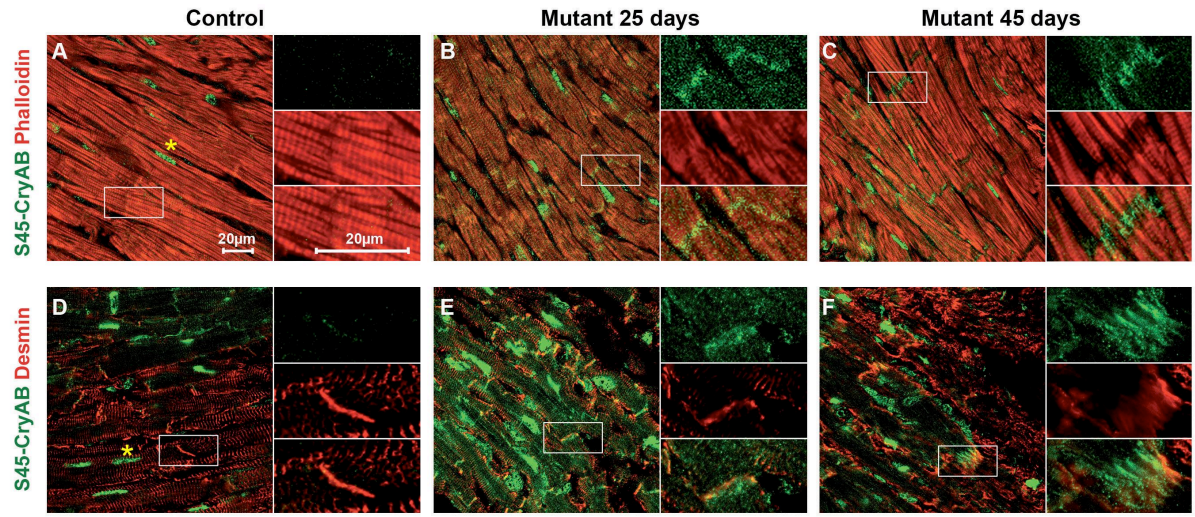

Fig. S6: Changes in phospho-S45 αB-crystallin and desmin localization in SRF^{HKO} cardiomyocytes. Immunofluorescent co-staining for phospho-S45 αB-crystallin and F-actin (phalloidin-TRITC) or desmin on heart tissue sections at D25 and D45. (A, D) control, (B, E) mutant 25 days, (C, F) mutant 45 days. Upper panel (A-C): anti-phospho-S45 αB-crystallin (green) and phalloidin-TRITC (red) Lower panel (D-F): anti-phospho-S45 αB-crystallin (green) and anti-Desmin (red). Insets: higher magnification of boxed area. *: nuclear staining with anti-phospho-S45 αB-crystallin. The presence of some forms of αB-crystallin in the nucleus has been reported by others **(1)**.

(1). Nuclear import of αB-crystallin is phosphorylation-dependant and hampered by hyperphosphorylation of the myopathy-related mutant R120G. den Engelsman J. et al. J. Biol. Chem. 2005; 280; 37139-48.

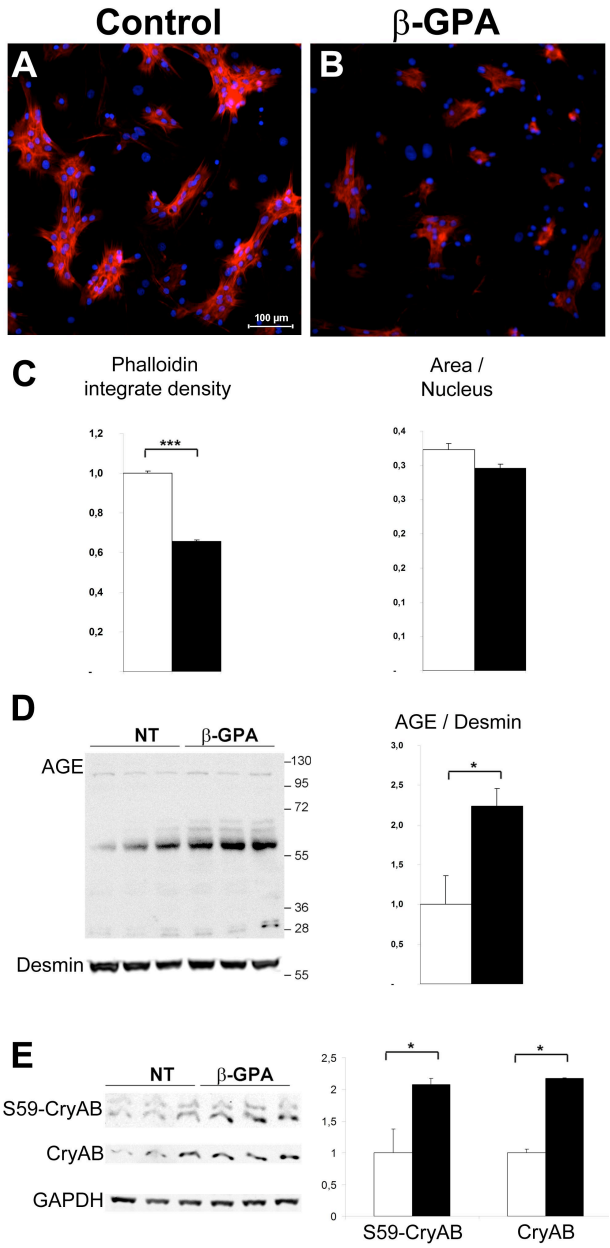

Fig. S7: Inhibition of creatine/phosphocreatine shuttle by the β-GPA competitor leads to actin depolymerization, αB-crystallin phosphorylation and AGE formation on desmin: Neonatal rat cardiomyocytes were isolated and cultured in presence or not of β-GPA (10 mM) from day 3 to 8 after plating. **(A-B)** Fluorescence staining for F-actin (phalloidin-TRITC) and nucleus (Hoechst). **(C)** Quantification of the phalloidin signal integrate density and cell area was measured on individual cardiomyocytes (N=300) and normalized by the number of nuclei. For the phalloidin signal integrate density, data are expressed as a fold change over mean control value. **(D)** Western-blot analysis of control and β-GPA treated cardiomyocytes with anti-AGE, -Desmin antibodies (left) and quantification of AGE/desmin ratio (right). **(E)** Western-blot analysis of control and β-GPA treated cardiomyocytes with anti-αB-crystallin (CryAB), -phospho-S59-CryAB and -GAPDH antibodies. Quantification of Western-blot (N=3 for each group). Band intensities are normalized on GAPDH as a loading control. Ratios are expressed as fold change over mean control value ± s.e.m. *: $p \leq 0.05$.