SUPPLEMENTAL MATERIAL

Top-Down Quantitative Proteomics Identified Phosphorylation of Cardiac Troponin I as a Candidate Biomarker for Chronic Heart Failure

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Running Title: Top-down proteomics for cardiac biomarker discovery

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Supplemental Tables

Table S1. Clinical characteristics of postmortem human heart samples. NOR, controls with normal cardiac function; HYP, mild hypertrophy; SHD, severe hypertrophy/dilation; CHF, congestive heart failure; CAD, coronary artery disease; LV, left ventricle; RV, right ventricle; AA, African American; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, Ca^{2+} channel blocker. (-) indicates data not available.

Subject#	Age	Sex	Race	Heart Defect(s)
NOR1	43	M	White	None
NOR2	86	F	White	None
NOR3	52	M	White	None
NOR4	58	F	White	None
NOR5	65	F	White	None
NOR6	21	M	AA	None
NOR7	66	F	White	None
HYP1	47	F	White	LV hypertrophy
HYP2	71	F	White	Mild LV&RV hypertrophy
HYP3	53	M	White	LV hypertrophy,
HYP4	68	M	White	LV hypertrophy
HYP5	49	F	White	Moderate hypertrophy
SHD1	20	M	White	Severe LV hypertrophy& dilation, RV hypertrophy
SHD2	61	F	Hispanic	Arrythmia, severe dilation
SHD3	65	M	White	Severe hypertrophy
SHD4	71	M	White	Severe hypertrophy &dilation
CHF1	39	M	White	CHF, severe CAD
CHF2	66	M	White	Severe CAD, CHF
CHF3	55	M	White	Severe CAD & CHF
CHF4	85	F	White	CHF, Mild CAD,
CHF5	85	M	White	Severe CAD,CHF
CHF6	54	F	White	Severe CHF

Subject #	Medication									
	ACE - Inhibitor	ARB Agents	Amiod-arone	β-blocker	CCB	Digoxin	Diu-retic	Nitrate	Spirono -lactone	Warf -arin
HYP1	n	n	n	y	y	n	y	n	n	n
HYP2	n	y	n	n	n	n	n	n	n	n
HYP3	n	n	n	n	n	n	y	n	n	n
HYP4	n	n	n	n	n	y	y	n	n	n

HYP5	n	n	n	n	n	n	y	n	n	n
SHD1	n	n	n	y	n	n	y	n	n	n
SHD2	-	-	-	-	-	-	-	-	-	-
SHD3	y	n	n	n	n	n	n	n	n	n
SHD4	y	n	n	y	n	n	y	n	n	n
CHF1	n	n	n	y	n	n	y	y	n	n
CHF2	n	n	n	y	n	y	y	y	n	n
CHF3	y	n	n	y	n	n	y	n	n	n
CHF4	n	n	n	n	n	n	n	n	n	n
CHF5	n	n	n	n	n	n	n	n	n	n
CHF6	n	n	n	n	n	n	y	n	n	n

Table S2. Clinical characteristics of donor and end-stage failing hearts from transplant surgical operations. DOR, donor hearts with normal cardiac function but deemed unacceptable for transplants; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy. [a], explanted tissues during operations; OCT, orthotopic cardiac transplantation; VAD, ventricular assist device. LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers; (-) indicates data not available.

Subject #	Surgical operation[a]	Gender	Age	LVEF	Fractional shortening	Hypertension
DOR1	Donor	M	46	-	-	-
DOR2	Donor	F	59	-	-	-
DOR3	Donor	M	-	-	-	-
DOR4	Donor	F	14	-	-	-
ICM1	OCT	M	52	10%	-	y
ICM2	VAD	M	65	10%	-	n
ICM3	VAD	M	54	15%	4-29%	y
ICM4	VAD	M	67	30%	9-29%	y
ICM5	VAD	M	61	30%	-	n
ICM6	OCT	M	63	25%	28-29%	n
DCM1	VAD	M	56	10%	7-29%	n
DCM2	VAD	M	64	25%	-	y
DCM3	OCT	M	-	-	-	-
DCM4	VAD	M	-	-	-	-

Subject #	Medication in mg/day								
	ACE Inhibitor	ARB Agents	Amiod-arone	β-blocker	Digoxin	Diuretic	Nitrate	Aldosterone antagonist	Warf-arin
ICM1	5	0	200	12.5	0	160	0	25	0
ICM2	30	0	0	25	62.5	0	0	12.5	0
ICM3	0	25	0	0	0	80	0	25	5
ICM4	0	80	320	50	0.25	40	0	0	2.5
ICM5	0	0	0	25	0	790	90	50	2.5
ICM6	0	0	0	0	0	160	0	0	7.5
DCM1	30	0	0	100	0.25	320	0	50	4
DCM2	0	0	0	25	0.125	160	30	0	7.5
DCM3	-	-	-	-	-	-	-	-	-
DCM4	-	-	-	-	-	-	-	-	-

Table S3. ECD fragment list and ion assignments of mono-phosphorylated cTnI ($_{p}$cTnI) from normal heart samples in support of Fig. 5A and S3a. $c/z^{\bullet}$ ions containing mono-phosphorylation were labeled as "$_{p}$" . Please note that c ions counts from the N-terminus and $z^{\bullet}$ counts from the C-terminus. E.g., c_{21} covers the first 21 amino acids from the N-terminus (residues 1-21) and $z^{\bullet}_{21}$ covers the first 21 amino acids from the C-terminus (resides 189-209). Ser22 was identified as the phosphorylation site for the following reasons: (1) No phosphorylated product ions were detected for smaller c ions before Ser22 (c_9 - c_{21}). (2) The detection of mono-phosphorylated c_{22} ($_{p}c_{22}$) clearly identified the phosphorylation of Ser22, and the absence of un-phosphorylated c_{22} indicated the nearly exclusive phosphorylation occupancy at Ser22 in $_{p}$cTnI. (3) All the larger c ions (c_{23} – c_{208}) were present only as mono-phosphorylated forms, supporting the phosphorylation site assignment at Ser22. This is also consistent with the fact that all the $z^{\bullet}$ ions ($z^{\bullet}_{3}$- $z^{\bullet}_{186}$) which do not cover Ser22 were detected in their un-phosphorylated form and $z^{\bullet}$ ions ($z^{\bullet}_{208}$) including Ser22 was detected in its mono-phosphorylated form only.

Observed most abundant M/Z	Charge state	Experimental monoisotopic mass (Da)	Fragment assignment	Calculated monoisotopic mass (Da)	Error (ppm)
890.397	1	889.389	c_{9}	889.389	0.3
769.372	2	1536.730	c_{15}	1536.728	1.4
853.419	2	1704.824	c_{17}	1704.818	3.7
909.954	2	1817.894	c_{18}	1817.902	-4.5
659.343	3	1974.004	c_{19}	1974.003	0.5
711.377	3	2130.106	c_{20}	2130.104	0.8
572.810	4	2286.206	c_{21}	2286.205	0.3
819.078	3	2453.211	$_{p}c_{22}$	2453.204	2.9
848.087	3	2540.238	$_{p}c_{23}$	2540.236	0.9
664.825	4	2654.272	$_{p}c_{24}$	2654.279	-2.6
940.460	3	2817.347	$_{p}c_{25}$	2817.342	1.7
744.619	4	2973.446	$_{p}c_{26}$	2973.443	0.9
282.723	12	3379.597	$_{p}c_{30}$	3379.628	-9.2
722.550	5	3605.706	$_{p}c_{32}$	3605.724	-5.0
937.212	4	3742.783	$_{p}c_{33}$	3742.783	0.1
764.169	5	3813.809	$_{p}c_{34}$	3813.820	-2.9
679.674	6	4069.993	$_{p}c_{36}$	4070.010	-4.1
701.027	6	4198.102	$_{p}c_{37}$	4198.105	-0.7
715.531	6	4285.142	$_{p}c_{38}$	4285.137	1.1
736.878	6	4413.216	$_{p}c_{39}$	4413.232	-3.6

705.366	7	4927.497	$_{p}\boldsymbol{c}_{44}$	4927.518	-4.3
723.669	7	5055.628	$_{p}\boldsymbol{c}_{45}$	5055.613	2.9
862.951	6	5168.681	$_{p}\boldsymbol{c}_{46}$	5168.697	-3.1
663.474	8	5296.750	$_{p}\boldsymbol{c}_{47}$	5296.755	-1.1
677.614	8	5409.851	$_{p}\boldsymbol{c}_{48}$	5409.840	2.1
693.626	8	5537.938	$_{p}\boldsymbol{c}_{49}$	5537.934	0.6
807.002	7	5638.964	$_{p}\boldsymbol{c}_{50}$	5638.982	-3.2
720.388	8	5752.043	$_{p}\boldsymbol{c}_{51}$	5752.066	-4.0
734.524	8	5865.124	$_{p}\boldsymbol{c}_{52}$	5865.150	-4.5
748.660	8	5978.216	$_{p}\boldsymbol{c}_{53}$	5978.234	-3.0
235.980	26	6106.304	$_{p}\boldsymbol{c}_{54}$	6106.293	1.8
787.682	8	6290.376	$_{p}\boldsymbol{c}_{56}$	6290.414	-6.0
1071.254	6	6418.475	$_{p}\boldsymbol{c}_{57}$	6418.509	-5.3
728.735	9	6546.540	$_{p}\boldsymbol{c}_{58}$	6546.568	-4.2
743.184	9	6675.582	$_{p}\boldsymbol{c}_{59}$	6675.610	-4.3
755.748	9	6788.672	$_{p}\boldsymbol{c}_{60}$	6788.694	-3.3
989.847	7	6917.874	$_{p}\boldsymbol{c}_{61}$	6917.737	19.8
787.431	9	7073.809	$_{p}\boldsymbol{c}_{62}$	7073.838	-4.1
801.768	9	7202.844	$_{p}\boldsymbol{c}_{63}$	7202.881	-5.0
809.666	9	7273.923	$_{p}\boldsymbol{c}_{64}$	7273.918	0.7
823.996	9	7402.892	$_{p}\boldsymbol{c}_{65}$	7402.960	-9.2
943.003	8	7531.980	$_{p}\boldsymbol{c}_{66}$	7532.003	-3.0
855.682	9	7688.086	$_{p}\boldsymbol{c}_{67}$	7688.104	-2.3
785.827	10	7844.179	$_{p}\boldsymbol{c}_{68}$	7844.205	-3.3
791.528	10	7901.191	$_{p}\boldsymbol{c}_{69}$	7901.227	-4.6
893.832	9	8030.413	$_{p}\boldsymbol{c}_{70}$	8030.269	17.9
908.041	9	8158.294	$_{p}\boldsymbol{c}_{71}$	8158.364	-8.6
762.503	11	8371.418	$_{p}\boldsymbol{c}_{73}$	8371.487	-8.2
768.974	11	8442.622	$_{p}\boldsymbol{c}_{74}$	8442.524	11.6
730.062	12	8743.655	$_{p}\boldsymbol{c}_{77}$	8743.688	-3.7
1272.968	7	8899.731	$_{p}\boldsymbol{c}_{78}$	8899.789	-6.5
1423.434	12	17059.101	$_{p}\boldsymbol{c}_{148}$	17059.204	-6.0
1397.901	13	18147.608	$_{p}\boldsymbol{c}_{159}$	18147.704	-5.3

1473.579	13	19131.287	${}_{p}c_{168}$	19131.244	2.3
1435.630	15	21506.473	${}_{p}c_{188}$	21506.511	-1.7
915.684	26	23766.578	${}_{p}c_{207}$	23766.700	-5.2
957.436	25	23895.697	${}_{p}c_{208}$	23895.743	-1.9
406.258	1	405.251	$z^{\bullet}_{3}$	405.250	0.8
494.236	1	493.228	$z^{\bullet}_{4}$	493.230	-3.3
622.330	1	621.322	$z^{\bullet}_{5}$	621.325	-4.0
750.424	1	749.417	$z^{\bullet}_{6}$	749.420	-3.8
453.768	2	905.521	$z^{\bullet}_{7}$	905.521	0.2
482.278	2	962.542	$z^{\bullet}_{8}$	962.542	-0.1
546.800	2	1091.585	$z^{\bullet}_{9}$	1091.585	0.3
612.320	2	1222.626	$z^{\bullet}_{10}$	1222.625	0.2
640.829	2	1279.643	$z^{\bullet}_{11}$	1279.647	-3.3
725.885	2	1449.755	$z^{\bullet}_{13}$	1449.752	1.9
776.404	2	1550.793	$z^{\bullet}_{14}$	1550.800	-4.4
833.917	2	1665.820	$z^{\bullet}_{15}$	1665.827	-4.3
675.025	3	2021.052	$z^{\bullet}_{18}$	2021.049	1.3
1132.601	2	2262.188	$z^{\bullet}_{19}$	2262.182	2.7
827.428	3	2478.258	$z^{\bullet}_{21}$	2478.256	0.6
879.460	3	2634.351	$z^{\bullet}_{23}$	2634.346	1.7
922.472	3	2763.394	$z^{\bullet}_{24}$	2763.389	1.7
731.128	4	2919.477	$z^{\bullet}_{25}$	2919.490	-4.5
759.642	4	3033.533	$z^{\bullet}_{26}$	3033.533	0.1
1069.223	3	3203.648	$z^{\bullet}_{27}$	3203.638	2.9
823.926	4	3290.674	$z^{\bullet}_{28}$	3290.670	1.2
685.352	5	3419.709	$z^{\bullet}_{29}$	3419.713	-1.1
1213.598	3	3635.776	$z^{\bullet}_{31}$	3635.788	-3.3
974.732	4	3892.900	$z^{\bullet}_{33}$	3892.925	-6.6
1006.759	4	4020.992	$z^{\bullet}_{34}$	4021.020	-7.0
1063.551	4	4248.171	$z^{\bullet}_{36}$	4248.147	5.6
730.717	6	4376.246	$z^{\bullet}_{37}$	4376.242	1.0

899.273	5	4489.328	$z^{\bullet}_{38}$	4489.326	0.5
899.273	5	4489.323	$z^{\bullet}_{38}$	4489.326	-0.7
810.259	6	4853.507	$z^{\bullet}_{41}$	4853.523	-3.4
829.273	6	4966.585	$z^{\bullet}_{42}$	4966.607	-4.6
867.295	6	5194.721	$z^{\bullet}_{44}$	5194.718	0.6
1057.954	5	5281.756	$z^{\bullet}_{45}$	5281.750	1.0
1083.761	5	5410.753	$z^{\bullet}_{46}$	5410.793	-7.4
1123.584	5	5609.942	$z^{\bullet}_{48}$	5609.925	3.0
962.509	6	5765.997	$z^{\bullet}_{49}$	5766.026	-5.0
974.348	6	5837.027	$z^{\bullet}_{50}$	5837.063	-6.3
859.609	7	6007.207	$z^{\bullet}_{52}$	6007.169	6.4
875.758	7	6120.250	$z^{\bullet}_{53}$	6120.253	-0.5
549.795	12	6581.454	$z^{\bullet}_{57}$	6581.429	3.7
1733.392	4	6925.527	$z^{\bullet}_{61}$	6925.563	-5.2
1043.568	7	7293.915	$z^{\bullet}_{64}$	7293.816	13.6
952.254	8	7605.977	$z^{\bullet}_{66}$	7606.018	-5.4
703.108	11	7719.108	$z^{\bullet}_{67}$	7719.102	0.8
870.354	9	7820.090	$z^{\bullet}_{68}$	7820.150	-7.7
1154.902	7	8073.257	$z^{\bullet}_{70}$	8073.304	-5.9
836.347	10	8348.418	$z^{\bullet}_{72}$	8348.467	-5.9
854.862	10	8533.536	$z^{\bullet}_{74}$	8533.584	-5.6
801.711	11	8802.817	$z^{\bullet}_{76}$	8802.769	5.4
812.170	11	8917.753	$z^{\bullet}_{77}$	8917.796	-4.8
878.661	11	9648.143	$z^{\bullet}_{83}$	9648.234	-9.4
906.580	12	10860.845	$z^{\bullet}_{94}$	10860.905	-5.5
904.413	13	11737.281	$z^{\bullet}_{101}$	11737.303	-1.8
1713.912	7	11981.279	$z^{\bullet}_{103}$	11981.372	-7.8
1533.679	14	21444.373	$z^{\bullet}_{186}$	21444.531	-7.4
1405.102	17	23854.560	$p z^{\bullet}_{208}$	23854.693	-5.6

Table S4. ECD fragment list and ion assignments of mono-phosphorylated cTnI ($_p$cTnI) from hypertrophy (HYP) heart samples in support of Fig. 5B and S3b. $c/z^{\bullet}$ ions containing mono-phosphorylation were labeled as "$_p$". Ser22 was identified as the phosphorylation site for the similar reasons as described in Table S3.

Observed most abundant M/Z	Charge State	Observed monoisotopic mass (Da)	Fragment assignment	Calculated monoisotopic mass (Da)	Error (ppm)
890.391	1	889.384	c_{9}	889.389	-5.7
1116.485	1	1115.478	c_{11}	1115.484	-6.0
769.372	2	1536.730	c_{15}	1536.728	1.4
853.418	2	1704.821	c_{17}	1704.818	1.6
909.960	2	1817.906	c_{18}	1817.902	1.9
988.516	2	1974.006	c_{19}	1974.003	1.4
711.378	3	2130.107	c_{20}	2130.104	1.5
763.411	3	2286.208	c_{21}	2286.205	1.3
819.077	3	2453.207	$_pc_{22}$	2453.204	1.4
848.088	3	2540.238	$_pc_{23}$	2540.236	1.1
664.824	4	2654.263	$_pc_{24}$	2654.279	-5.8
940.459	3	2817.352	$_pc_{25}$	2817.342	3.4
744.620	4	2973.448	$_pc_{26}$	2973.443	1.8
677.331	5	3379.617	$_pc_{30}$	3379.628	-3.3
722.554	5	3605.730	$_pc_{32}$	3605.724	1.7
749.967	5	3742.795	$_pc_{33}$	3742.783	3.3
764.174	5	3813.823	$_pc_{34}$	3813.820	1.0
658.324	6	3941.892	$_pc_{35}$	3941.915	-5.6
679.673	6	4069.986	$_pc_{36}$	4070.010	-5.9
841.031	5	4198.113	$_pc_{37}$	4198.105	2.1
715.527	6	4285.112	$_pc_{38}$	4285.137	-5.8
736.877	6	4413.212	$_pc_{39}$	4413.232	-4.4
670.488	7	4684.360	$_pc_{42}$	4684.385	-5.3
682.921	7	4771.386	$_pc_{43}$	4771.417	-6.4
705.369	7	4927.527	$_pc_{44}$	4927.518	1.9
844.107	6	5055.587	$_pc_{45}$	5055.613	-5.2
739.824	7	5168.707	$_pc_{46}$	5168.697	2.0

1060.964	5	5296.766	$_pc_{47}$	5296.755	2.0
774.272	7	5409.846	$_pc_{48}$	5409.840	1.2
792.566	7	5537.902	$_pc_{49}$	5537.934	-5.8
706.258	8	5639.001	$_pc_{50}$	5638.982	3.3
720.394	8	5752.087	$_pc_{51}$	5752.066	3.7
734.523	8	5865.121	$_pc_{52}$	5865.150	-5.0
748.658	8	5978.199	$_pc_{53}$	5978.234	-6.0
692.380	9	6219.327	$_pc_{55}$	6219.377	-8.0
714.509	9	6418.516	$_pc_{57}$	6418.509	1.1
728.734	9	6546.534	$_pc_{58}$	6546.568	-5.1
743.189	9	6675.620	$_pc_{59}$	6675.610	1.5
1699.173	4	6788.653	$_pc_{60}$	6788.694	-6.1
693.178	10	6917.699	$_pc_{61}$	6917.737	-5.4
787.436	9	7073.852	$_pc_{62}$	7073.838	2.0
809.661	9	7273.879	$_pc_{64}$	7273.918	-5.3
823.998	9	7402.957	$_pc_{65}$	7402.960	-0.5
1077.583	7	7531.991	$_pc_{66}$	7532.003	-1.5
989.162	8	7901.242	$_pc_{69}$	7901.227	1.9
817.345	10	8158.367	$_pc_{71}$	8158.364	0.4
857.069	10	8555.614	$_pc_{75}$	8555.608	0.7
796.340	11	8743.642	$_pc_{77}$	8743.688	-5.3
751.654	12	9002.791	$_pc_{79}$	9002.798	-0.7
800.101	12	9583.119	$_pc_{84}$	9583.120	-0.1
674.697	15	10099.331	$_pc_{90}$	10099.390	-5.8
1001.879	12	12003.450	$_pc_{106}$	12003.395	4.6
876.359	26	23619.482	$_pc_{206}$	23619.632	-6.4
259.188	1	258.180	$z^\bullet{}_2$	258.182	-5.3
387.282	1	386.275	$z^\bullet{}_3$	386.277	-5.5
494.237	1	493.230	$z^\bullet{}_4$	493.230	0.5
622.333	1	621.326	$z^\bullet{}_5$	621.325	1.3
750.428	1	749.421	$z^\bullet{}_6$	749.420	1.4
453.768	2	905.522	$z^\bullet{}_7$	905.521	0.9
482.279	2	962.543	$z^\bullet{}_8$	962.542	1.0

546.800	2	1091.586	$z^{\bullet}_{9}$	1091.585	1.2
612.321	2	1222.627	$z^{\bullet}_{10}$	1222.625	1.3
640.832	2	1279.650	$z^{\bullet}_{11}$	1279.647	2.2
652.346	2	1302.677	$z^{\bullet}_{12}$	1302.684	-5.6
740.890	2	1479.765	$z^{\bullet}_{13}$	1479.763	1.6
765.908	2	1529.802	$z^{\bullet}_{14}$	1529.811	-5.8
833.922	2	1665.830	$z^{\bullet}_{15}$	1665.827	2.0
605.673	3	1813.997	$z^{\bullet}_{16}$	1814.007	-5.5
668.033	3	2000.076	$z^{\bullet}_{17}$	2000.086	-5.3
1059.059	2	2115.101	$z^{\bullet}_{18}$	2115.113	-5.9
727.059	3	2177.154	$z^{\bullet}_{19}$	2177.150	1.8
789.086	3	2363.232	$z^{\bullet}_{20}$	2363.229	1.1
1240.639	2	2478.262	$z^{\bullet}_{21}$	2478.256	2.4
846.436	3	2535.283	$z^{\bullet}_{22}$	2535.278	2.1
879.458	3	2634.350	$z^{\bullet}_{23}$	2634.346	1.3
934.480	3	2799.416	$z^{\bullet}_{24}$	2799.432	-5.9
977.179	3	2927.511	$z^{\bullet}_{25}$	2927.527	-5.7
1012.522	3	3033.536	$z^{\bullet}_{26}$	3033.533	1.0
1055.537	3	3162.580	$z^{\bullet}_{27}$	3162.575	1.3
1098.235	3	3290.679	$z^{\bullet}_{28}$	3290.670	2.6
1141.583	3	3419.719	$z^{\bullet}_{29}$	3419.713	1.7
1178.263	3	3529.764	$z^{\bullet}_{30}$	3529.782	-5.1
1213.608	3	3635.803	$z^{\bullet}_{31}$	3635.788	4.1
1256.623	3	3764.843	$z^{\bullet}_{32}$	3764.830	3.3
972.754	4	3884.983	$z^{\bullet}_{33}$	3885.004	-5.5
1004.779	4	4013.077	$z^{\bullet}_{34}$	4013.099	-5.4
1033.048	4	4126.159	$z^{\bullet}_{35}$	4126.183	-5.9
1063.548	4	4248.156	$z^{\bullet}_{36}$	4248.147	2.0
1095.571	4	4376.254	$z^{\bullet}_{37}$	4376.242	2.7
1123.845	4	4489.344	$z^{\bullet}_{38}$	4489.326	4.0
1158.108	4	4626.371	$z^{\bullet}_{39}$	4626.385	-3.1

1175.866	4	4697.436	$z^{\bullet}_{40}$	4697.422	3.0
810.263	6	4853.534	$z^{\bullet}_{41}$	4853.523	2.1
985.123	5	4918.582	$z^{\bullet}_{42}$	4918.607	-5.1
1011.133	5	5047.609	$z^{\bullet}_{43}$	5047.650	-8.1
875.967	6	5246.751	$z^{\bullet}_{45}$	5246.782	-6.0
901.984	6	5402.861	$z^{\bullet}_{46}$	5402.883	-4.1
1096.387	5	5473.895	$z^{\bullet}_{47}$	5473.920	-4.5
1123.596	5	5609.926	$z^{\bullet}_{48}$	5609.925	0.1
807.723	7	5643.992	$z^{\bullet}_{49}$	5644.026	-6.0
835.305	7	5837.077	$z^{\bullet}_{50}$	5837.063	2.3
1002.708	6	6007.198	$z^{\bullet}_{52}$	6007.169	4.9
1016.044	6	6087.215	$z^{\bullet}_{53}$	6087.246	-5.0
801.923	8	6404.291	$z^{\bullet}_{56}$	6404.350	-9.3
941.783	7	6581.434	$z^{\bullet}_{57}$	6581.429	0.6
939.064	7	6562.387	$z^{\bullet}_{58}$	6562.420	-5.0
1129.599	6	6767.531	$z^{\bullet}_{59}$	6767.493	5.6
1155.943	6	6925.617	$z^{\bullet}_{61}$	6925.563	7.8
906.865	8	7242.836	$z^{\bullet}_{63}$	7242.875	-5.5
1043.552	7	7293.838	$z^{\bullet}_{64}$	7293.816	3.0
1088.157	7	7606.046	$z^{\bullet}_{66}$	7606.018	3.6
858.133	9	7710.139	$z^{\bullet}_{67}$	7710.161	-2.9
1010.549	8	8073.321	$z^{\bullet}_{70}$	8073.304	2.1
758.417	11	8326.500	$z^{\bullet}_{72}$	8326.542	-5.0
845.466	10	8439.596	$z^{\bullet}_{73}$	8439.626	-3.6
949.741	9	8533.594	$z^{\bullet}_{74}$	8533.584	1.2
792.525	11	8701.679	$z^{\bullet}_{75}$	8701.721	-4.9
979.650	9	8802.774	$z^{\bullet}_{76}$	8802.769	0.6
775.178	12	9285.039	$z^{\bullet}_{80}$	9285.091	-5.6
878.667	11	9648.252	$z^{\bullet}_{83}$	9648.234	1.9
831.378	14	11618.168	$z^{\bullet}_{100}$	11618.229	-5.3
857.329	14	11981.489	$z^{\bullet}_{103}$	11981.372	9.8

Table S5. ECD fragment list and ion assignments of bis-phosphorylated cTnI ($_{pp}$cTnI) from normal heart samples in support of Fig. 5C and S3c. ***c*/*z***$^{\bullet}$ ions containing mono-, or bis-phosphorylation were labeled as "$_{p}$", or "$_{pp}$" respectively). Ser22 and Ser23 were identified as the phosphorylation sites for the similar reasons as described in Table S3. $\boldsymbol{c}_{22}$ was detected in its mono-phosphorylated form ($_{p}\boldsymbol{c}_{22}$) since it only contains one phosphorylation site (Ser22) whereas $\boldsymbol{c}_{23}$ and larger ***c*** ions was detected in their bis-phosphorylated froms (i.e. $_{pp}\boldsymbol{c}_{23}$) because they contain two phosphorylation sites, Ser22 and Ser23). The fact that neither un- nor mono-phosphorylated $\boldsymbol{c}_{23}$ and $\boldsymbol{c}_{24}$ ($\boldsymbol{c}_{23}$ and c_{24}, $_{p}\boldsymbol{c}_{23}$ and $_{p}\boldsymbol{c}_{24}$) were observed suggests that Ser22/23 are the only two phosphorylation sites. Conversely, if other sites were phosphorylated (in case of positional isomers), one would expect to see un- and mono-phosphorylated $\boldsymbol{c}_{23}$ and $\boldsymbol{c}_{24}$.

Observed most abundant M/Z	Charge State	Experimental monoisotopic mass (Da)	Fragment assignment	Calculated monoisotopic mass (Da)	Error (ppm)
890.396	1	889.389	$\boldsymbol{c}_{9}$	889.389	0.1
1116.488	1	1115.481	$\boldsymbol{c}_{11}$	1115.484	-3.2
769.371	2	1536.727	$\boldsymbol{c}_{15}$	1536.728	-0.6
853.416	2	1704.817	$\boldsymbol{c}_{17}$	1704.818	-0.3
659.343	3	1974.002	$\boldsymbol{c}_{19}$	1974.003	-0.7
711.376	3	2130.103	$\boldsymbol{c}_{20}$	2130.104	-0.5
572.807	4	2286.197	$\boldsymbol{c}_{21}$	2286.205	-3.4
819.076	3	2453.203	$_{p}\boldsymbol{c}_{22}$	2453.204	-0.4
874.739	3	2620.194	$_{pp}\boldsymbol{c}_{23}$	2620.202	-3.0
912.753	3	2734.235	$_{pp}\boldsymbol{c}_{24}$	2734.245	-3.8
967.107	3	2897.298	$_{pp}\boldsymbol{c}_{25}$	2897.308	-3.5
764.607	4	3053.397	$_{pp}\boldsymbol{c}_{26}$	3053.409	-4.2
866.404	4	3459.584	$_{pp}\boldsymbol{c}_{30}$	3459.595	-3.0
765.954	5	3822.733	$_{pp}\boldsymbol{c}_{33}$	3822.749	-4.1
714.351	6	4278.056	$_{pp}\boldsymbol{c}_{37}$	4278.071	-3.5
1153.072	4	4606.254	$_{pp}\boldsymbol{c}_{40}$	4606.282	-6.0
783.566	6	4693.348	$_{pp}\boldsymbol{c}_{41}$	4693.314	7.2
836.086	6	5007.459	$_{pp}\boldsymbol{c}_{44}$	5007.484	-5.0
857.434	6	5135.564	$_{pp}\boldsymbol{c}_{45}$	5135.579	-2.9
876.281	6	5248.640	$_{pp}\boldsymbol{c}_{46}$	5248.663	-4.4
1076.947	5	5376.687	$_{pp}\boldsymbol{c}_{47}$	5376.722	-6.5
703.618	8	5617.881	$_{pp}\boldsymbol{c}_{49}$	5617.901	-3.5

818.282	7	5718.919	$_{pp}c_{50}$	5718.948	-5.2
737.731	9	6626.508	$_{pp}c_{58}$	6626.534	-3.9
845.951	8	6755.547	$_{pp}c_{59}$	6755.577	-4.3
920.739	8	7353.848	$_{pp}c_{64}$	7353.884	-4.9
936.870	8	7482.895	$_{pp}c_{65}$	7482.927	-4.2
1088.999	7	7611.951	$_{pp}c_{66}$	7611.969	-2.4
888.246	9	7981.160	$_{pp}c_{69}$	7981.193	-4.2
916.705	9	8238.268	$_{pp}c_{71}$	8238.330	-7.6
803.609	11	8823.607	$_{pp}c_{77}$	8823.654	-5.3
827.074	11	9082.727	$_{pp}c_{79}$	9082.764	-4.1
967.913	10	9663.046	$_{pp}c_{84}$	9663.086	-4.2
794.428	13	10308.463	$_{pp}c_{91}$	10308.398	6.3
1304.463	8	10421.632	$_{pp}c_{92}$	10421.482	14.4
930.878	13	12083.315	$_{pp}c_{106}$	12083.361	-3.8
1028.893	12	12327.614	$_{pp}c_{108}$	12327.430	14.9
868.465	15	13003.850	$_{pp}c_{113}$	13003.748	7.8
822.304	16	13132.740	$_{pp}c_{114}$	13132.791	-3.9
1017.295	13	13203.758	$_{pp}c_{115}$	13203.828	-5.3
1129.342	12	13531.997	$_{pp}c_{118}$	13532.039	-3.1
869.463	16	13887.272	$_{pp}c_{121}$	13887.261	0.8
845.005	20	16869.947	$_{pp}c_{146}$	16869.985	-2.3
849.424	22	18654.145	$_{pp}c_{163}$	18653.940	11.0
859.278	27	23159.292	$_{pp}c_{202}$	23159.212	3.4
857.817	28	23975.647	$_{pp}c_{208}$	23975.709	-2.6
494.237	1	493.229	$z^{\bullet}_{4}$	493.230	-0.9
622.332	1	621.324	$z^{\bullet}_{5}$	621.325	-0.6
750.427	1	749.420	$z^{\bullet}_{6}$	749.420	-0.3
906.528	1	905.520	$z^{\bullet}_{7}$	905.521	-0.5
482.276	2	962.539	$z^{\bullet}_{8}$	962.542	-3.6
1092.589	1	1091.583	$z^{\bullet}_{9}$	1091.585	-2.2
612.320	2	1222.625	$z^{\bullet}_{10}$	1222.625	-0.5
640.830	2	1279.646	$z^{\bullet}_{11}$	1279.647	-0.7

740.888	2	1479.762	$z^{\bullet}_{13}$	1479.763	-0.6
776.407	2	1550.799	$z^{\bullet}_{14}$	1550.800	-0.4
833.920	2	1665.826	$z^{\bullet}_{15}$	1665.827	-0.4
890.460	2	1778.906	$z^{\bullet}_{16}$	1778.911	-2.7
947.986	2	1892.953	$z^{\bullet}_{17}$	1892.954	-0.7
675.022	3	2021.043	$z^{\bullet}_{18}$	2021.049	-3.1
727.058	3	2177.149	$z^{\bullet}_{19}$	2177.150	-0.3
789.084	3	2363.228	$z^{\bullet}_{20}$	2363.229	-0.7
1240.637	2	2478.256	$z^{\bullet}_{21}$	2478.256	-0.2
1269.146	2	2535.279	$z^{\bullet}_{22}$	2535.278	0.5
1318.679	2	2634.345	$z^{\bullet}_{23}$	2634.346	-0.6
1383.203	2	2763.388	$z^{\bullet}_{24}$	2763.389	-0.3
974.505	3	2919.486	$z^{\bullet}_{25}$	2919.490	-1.3
1012.520	3	3033.531	$z^{\bullet}_{26}$	3033.533	-0.6
1055.533	3	3162.575	$z^{\bullet}_{27}$	3162.575	-0.1
1098.232	3	3290.670	$z^{\bullet}_{28}$	3290.670	-0.1
1141.580	3	3419.715	$z^{\bullet}_{29}$	3419.713	0.5
881.698	4	3520.755	$z^{\bullet}_{30}$	3520.761	-1.5
1213.605	3	3635.790	$z^{\bullet}_{31}$	3635.788	0.6
754.371	5	3764.817	$z^{\bullet}_{32}$	3764.830	-3.5
1375.039	3	4120.093	$z^{\bullet}_{35}$	4120.088	1.0
1417.727	3	4248.151	$z^{\bullet}_{36}$	4248.147	0.8
876.653	5	4376.225	$z^{\bullet}_{37}$	4376.242	-3.9
1123.841	4	4489.328	$z^{\bullet}_{38}$	4489.326	0.4
772.402	6	4626.367	$z^{\bullet}_{39}$	4626.385	-4.0
940.886	5	4697.398	$z^{\bullet}_{40}$	4697.422	-5.2
694.795	7	4853.510	$z^{\bullet}_{41}$	4853.523	-2.8
1243.412	4	4966.586	$z^{\bullet}_{42}$	4966.607	-4.3
1272.167	4	5081.633	$z^{\bullet}_{43}$	5081.634	-0.3
1300.439	4	5194.715	$z^{\bullet}_{44}$	5194.718	-0.7
1322.198	4	5281.759	$z^{\bullet}_{45}$	5281.750	1.6

1354.458	4	5410.812	$z^{\bullet}_{46}$	5410.793	3.5
693.748	8	5538.920	$z^{\bullet}_{47}$	5538.888	5.9
1404.240	4	5609.928	$z^{\bullet}_{48}$	5609.925	0.6
825.151	7	5766.001	$z^{\bullet}_{49}$	5766.026	-4.4
1461.025	4	5837.063	$z^{\bullet}_{50}$	5837.063	0.0
738.141	8	5894.065	$z^{\bullet}_{51}$	5894.085	-3.3
1203.043	5	6007.156	$z^{\bullet}_{52}$	6007.169	-2.2
1021.547	6	6120.233	$z^{\bullet}_{53}$	6120.253	-3.2
885.903	7	6191.265	$z^{\bullet}_{54}$	6191.290	-4.0
1054.733	6	6319.350	$z^{\bullet}_{55}$	6319.348	0.2
1291.685	5	6450.390	$z^{\bullet}_{56}$	6450.389	0.2
941.784	7	6581.432	$z^{\bullet}_{57}$	6581.429	0.3
951.925	7	6652.419	$z^{\bullet}_{58}$	6652.467	-7.1
1355.307	5	6767.491	$z^{\bullet}_{59}$	6767.493	-0.3
761.287	9	6838.513	$z^{\bullet}_{60}$	6838.531	-2.5
1386.922	5	6925.563	$z^{\bullet}_{61}$	6925.563	0.0
1029.402	7	7194.753	$z^{\bullet}_{63}$	7194.748	0.7
1217.310	6	7293.803	$z^{\bullet}_{64}$	7293.816	-1.8
1243.329	6	7449.871	$z^{\bullet}_{65}$	7449.917	-6.2
952.260	8	7606.019	$z^{\bullet}_{66}$	7606.018	0.1
773.316	10	7719.080	$z^{\bullet}_{67}$	7719.102	-2.9
1305.034	6	7820.156	$z^{\bullet}_{68}$	7820.150	0.7
1154.909	7	8073.303	$z^{\bullet}_{70}$	8073.304	-0.1
929.172	9	8348.471	$z^{\bullet}_{72}$	8348.467	0.5
949.739	9	8533.574	$z^{\bullet}_{74}$	8533.584	-1.2
870.477	10	8689.686	$z^{\bullet}_{75}$	8689.685	0.2
1101.980	8	8802.739	$z^{\bullet}_{76}$	8802.769	-3.4
1275.694	7	8917.795	$z^{\bullet}_{77}$	8917.796	-0.2
756.828	12	9064.838	$z^{\bullet}_{78}$	9064.864	-2.9
966.427	10	9648.250	$z^{\bullet}_{83}$	9648.234	1.7
988.903	11	10860.805	$z^{\bullet}_{94}$	10860.905	-9.3

842.384	13	10931.883	$z^{\bullet}_{95}$	10931.942	-5.5
738.804	15	11060.939	$z^{\bullet}_{96}$	11060.985	-4.1
1162.537	10	11608.248	$z^{\bullet}_{100}$	11608.260	-1.1
1320.274	9	11866.306	$z^{\bullet}_{102}$	11866.345	-3.3
1333.051	9	11981.393	$z^{\bullet}_{103}$	11981.372	1.8
1222.563	10	12208.547	$z^{\bullet}_{105}$	12208.536	1.0
1234.067	10	12323.584	$z^{\bullet}_{106}$	12323.563	1.7
1145.165	11	12578.722	$z^{\bullet}_{108}$	12578.732	-0.8
822.441	17	13956.366	$z^{\bullet}_{120}$	13956.414	-3.5
1154.162	13	14981.990	$z^{\bullet}_{130}$	14981.969	1.4
1037.996	16	16581.805	$z^{\bullet}_{144}$	16581.807	-0.1
1025.655	22	22528.221	$_{pp}z^{\bullet}_{194}$	22528.005	9.6
998.902	24	23934.561	$_{pp}z^{\bullet}_{208}$	23934.659	-4.1

Supplemental Figures

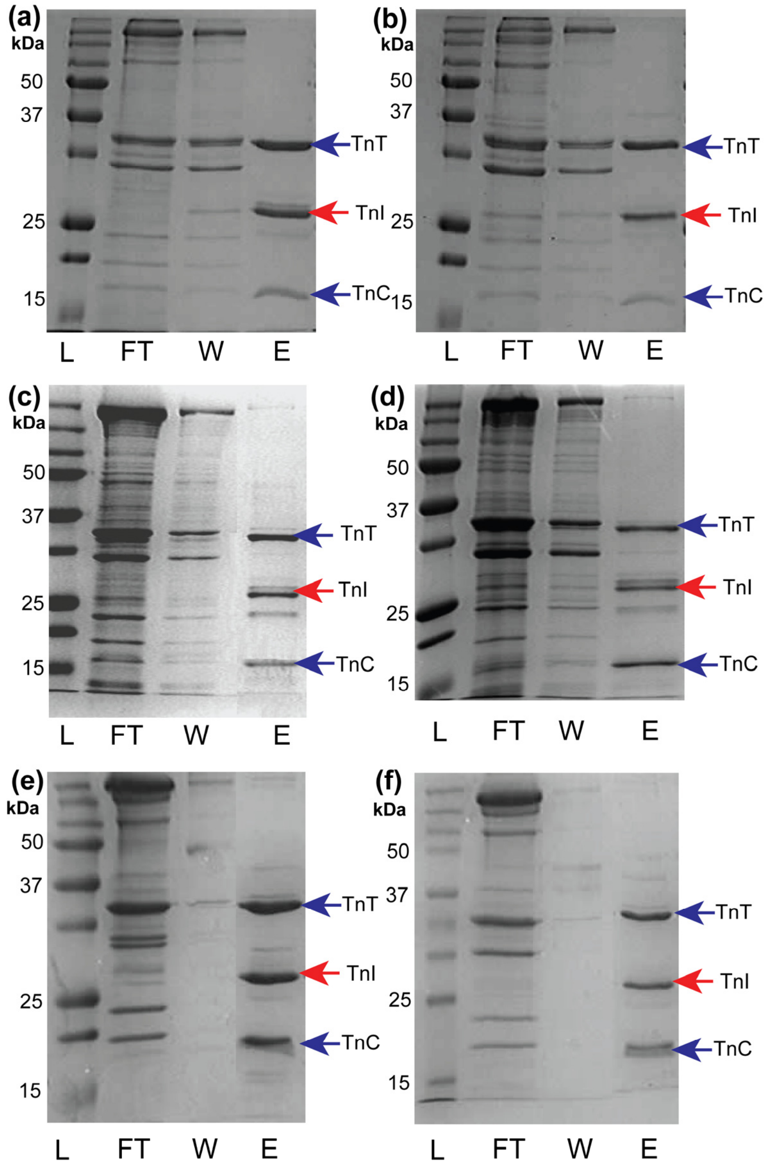


Fig. S1. Representative SDS-PAGE analysis of immunoaffinity purification of cTn from postmortem (a-d) and transplant (e-f) human myocardial tissues. (a) NOR1; (b) HYP3; (c) SHD3; (d) CHF1; (e) DOR4; (f) DCM4. L stands for molecular markers; FT, flow through; W, wash; E, affinity elution with 0.1 M glycine solution.

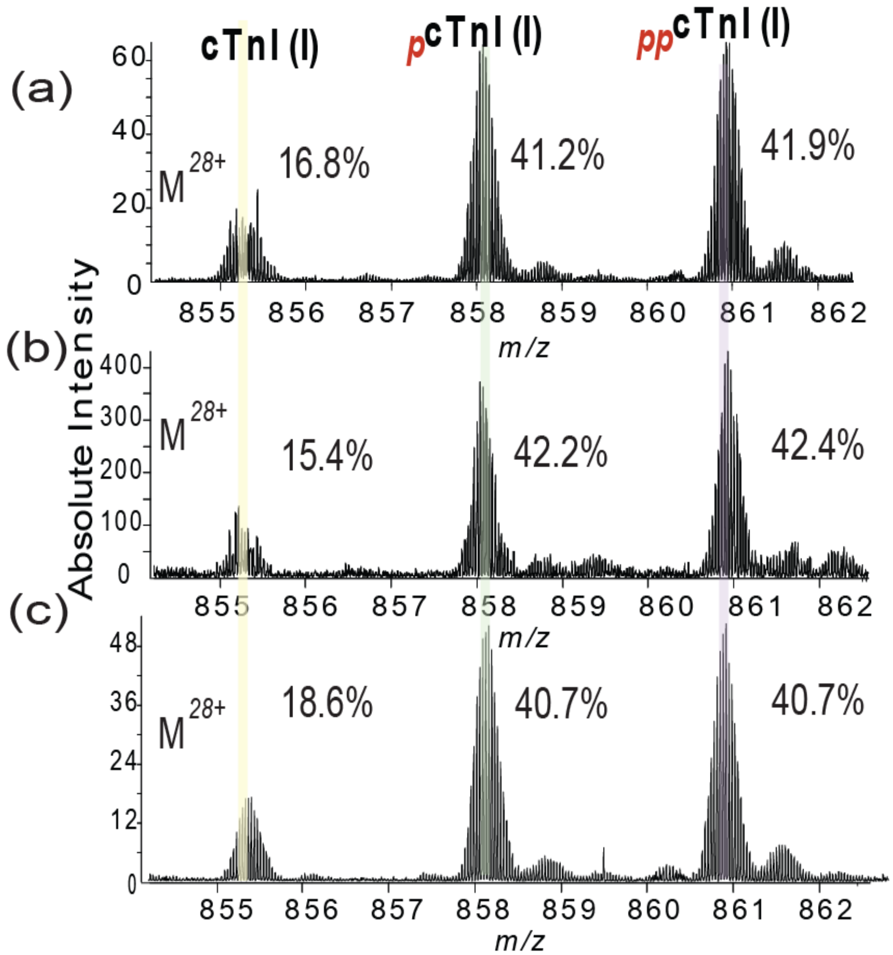


Fig. S2. Technical reproducibility of top-down quantitative proteomics analysis of cTnI phosphorylation. (a-c) three representative experimental repeated measurements (technical replicates) for one biological sample (NOR1). Subscript *p* stands for monophosphorylation and *pp* stands for bisphosphorylation.

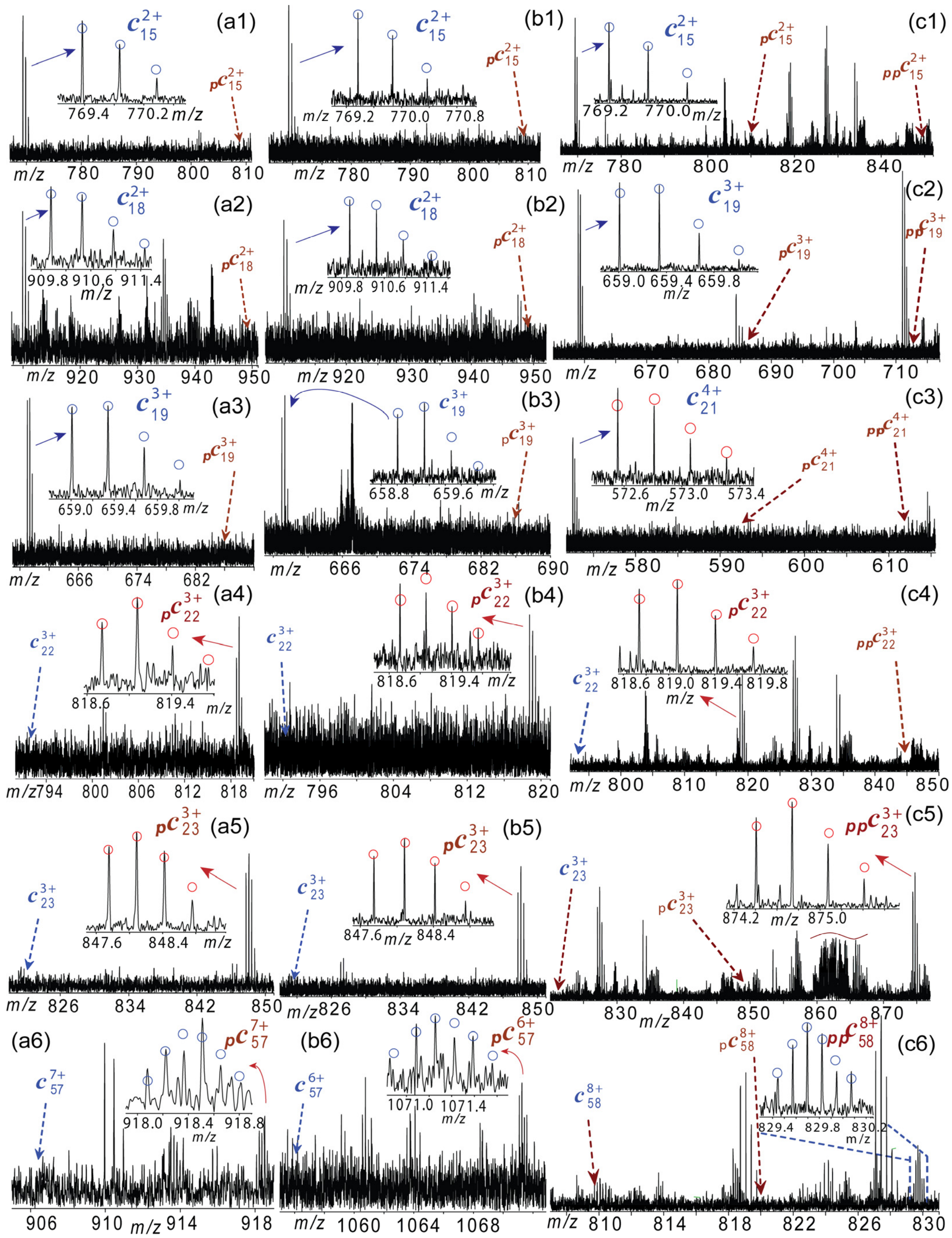


Fig. S3. Mapping phosphorylation site(s) in mono- and bis-phosphorylated cTnI purified from normal and diseased heart samples. (a1-6), key product ions from ECD fragmentation of mono-phosphorylated cTnI ($_{p}$cTnI) in a normal heart sample (NOR1); (b1-6), key product ions from ECD fragmentation of mono-phosphorylated cTnI ($_{p}$cTnI) in a hypertrophic heart sample (HYP2); (c1-6), key product ions from ECD fragmentation of bis-phosphorylated cTnI ($_{pp}$cTnI) in a normal heart sample (NOR1). ECD was not performed on the bis-phosphorylated cTnI in diseased heart samples due to its low abundance.

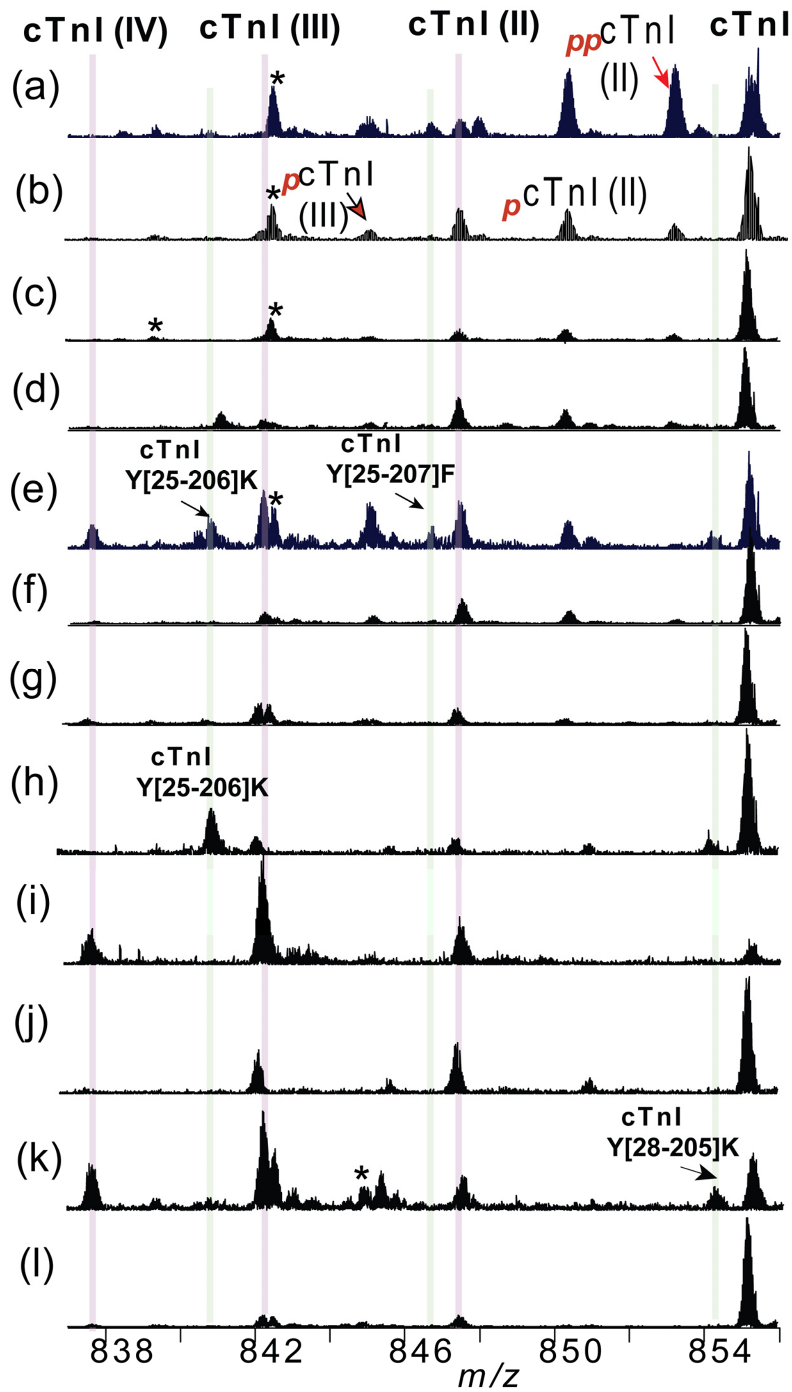


Fig. S4. Correlation between cTnI degradation level and heart disease conditions in postmortem samples. (a-c) NOR1-3; (d-f) HYP1-3; (g-i) SHD1-3; (j-l) CHF1-3. Only representative cases are shown. The three C-terminally trunctaed cTnI isoforms (II-IV) and three major degradation products are labeled and highlighted. Subscript *p* stands for mono-phosphorylation and *pp* stands for bis-phosphorylation. Asterisks represent the co-purified cTnT related products.

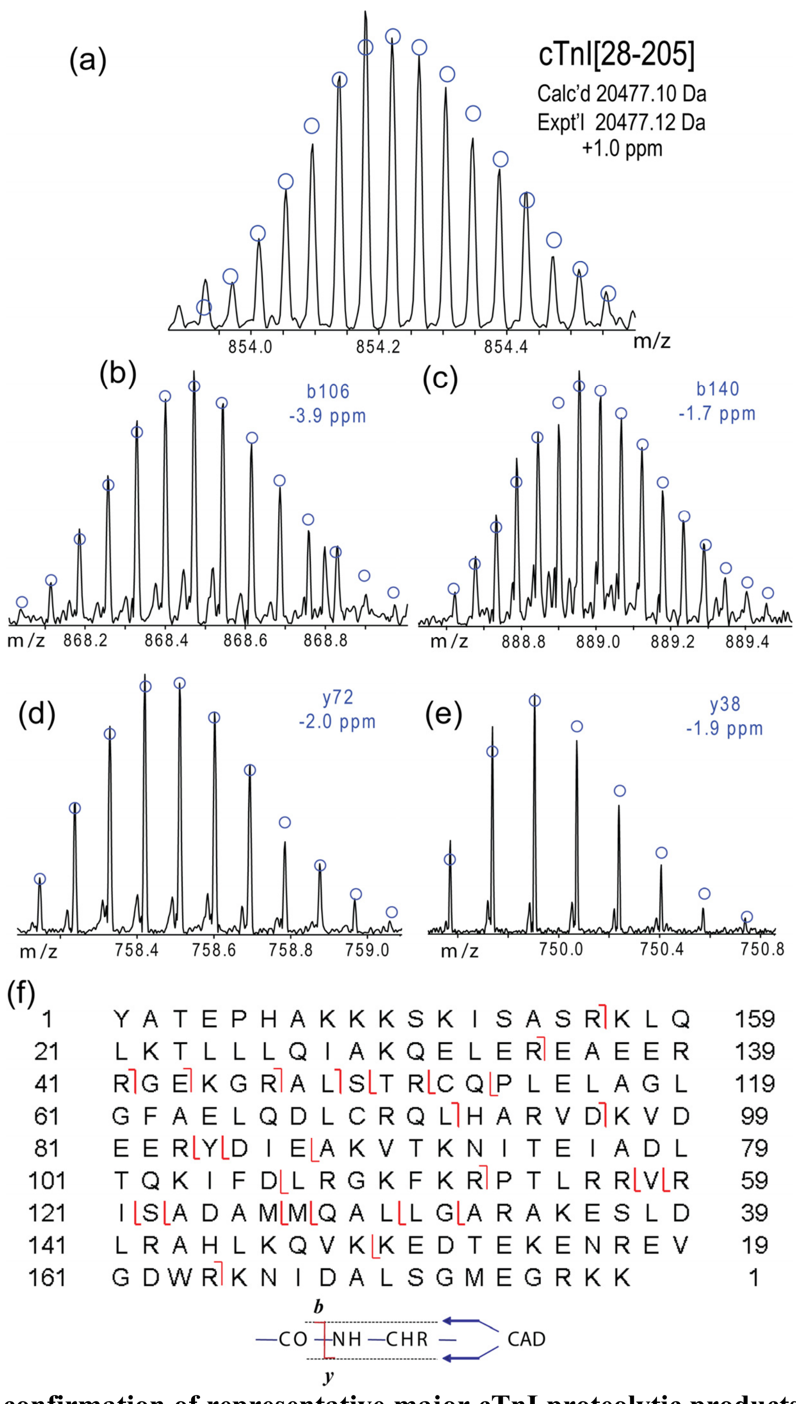


Fig. S5. Sequence confirmation of representative major cTnI proteolytic products by high accuracy mass measurements and MS/MS fragmentation. (a) High accuracy mass measurement of cTnI Y[28-205]K; (b)-(e) key product ions of cTnI Y[28-205]K; (f) fragmentation map of CAD experiment of cTnI Y[28-205]K.

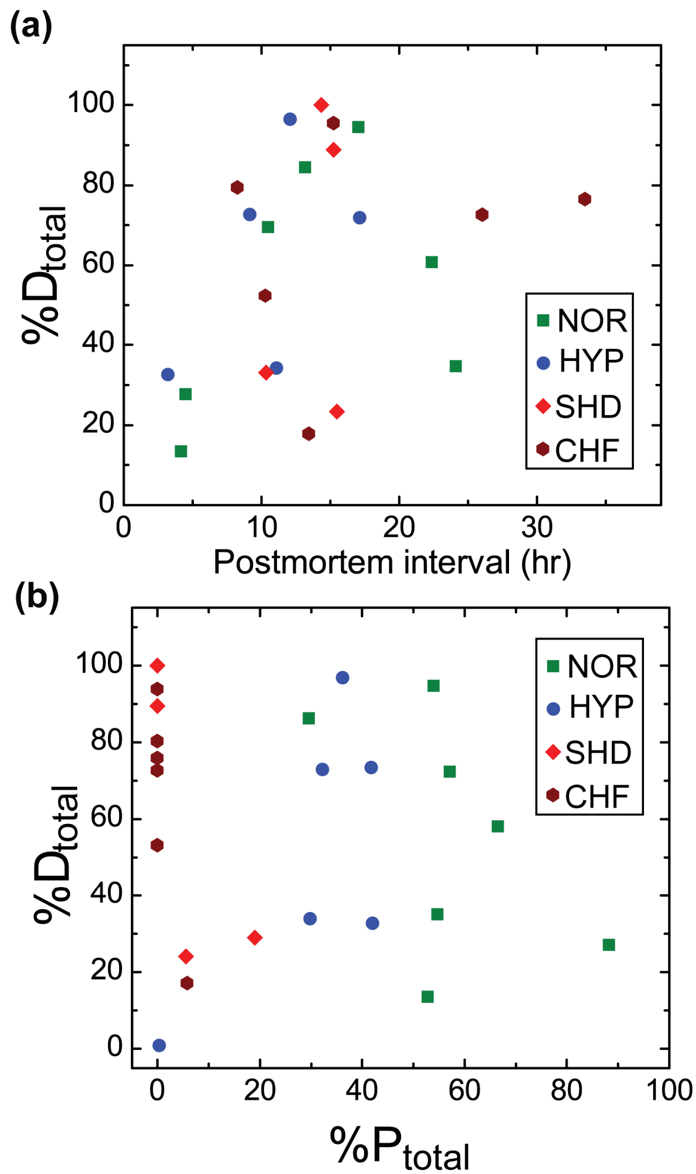
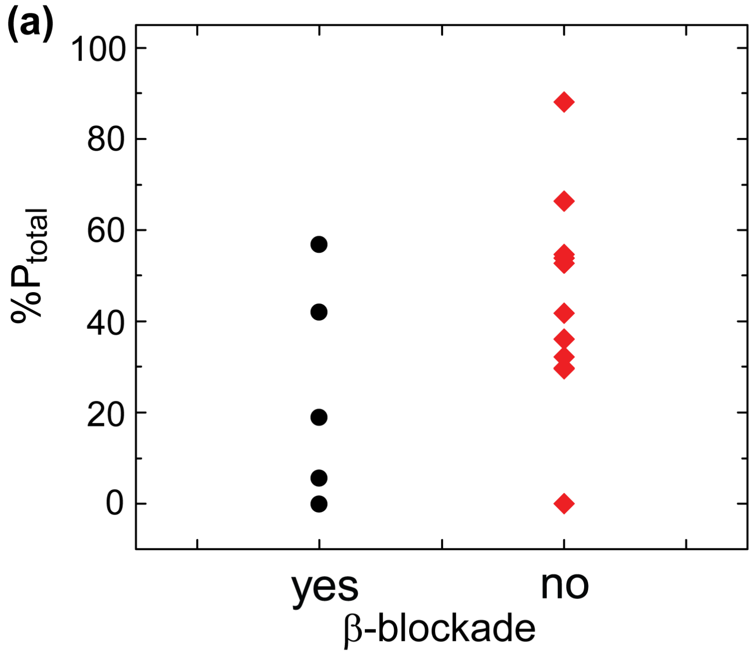


Fig. S6. Correlations between (a) cTnI degradation levels and postmortem interval and (b) cTnI degradation and phosphorylation level. No significant correlation was observed between these parameters.



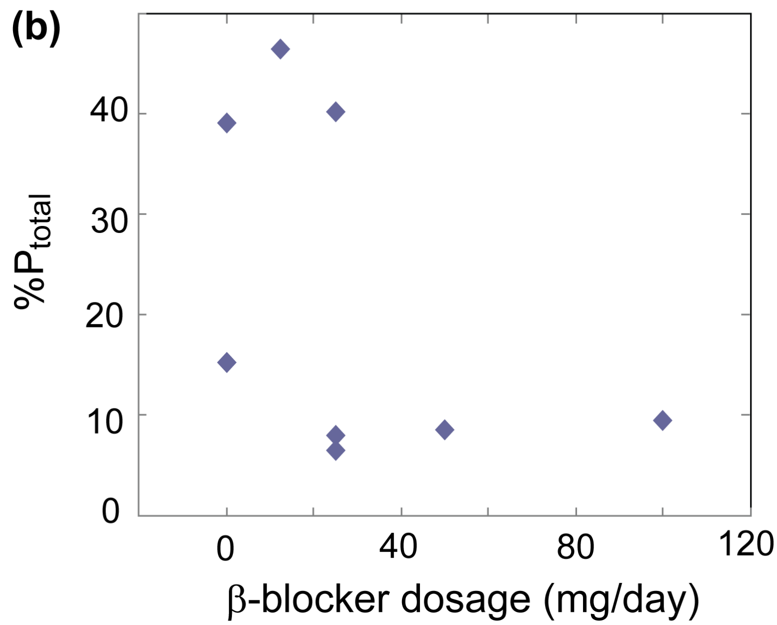


Fig. S7. Correlation between β-blockade and cTnI phosphorylation. (a) From post-mortem hearts including both control and diseased groups; (b) from transplant hearts of ICM/DCM. Detailed clinical characteristics are listed in Table S1 and S2. %P_{total}, total phosphorylated cTnI percentage over the entire cTnI populations. No significant correlation was observed between these parameters.

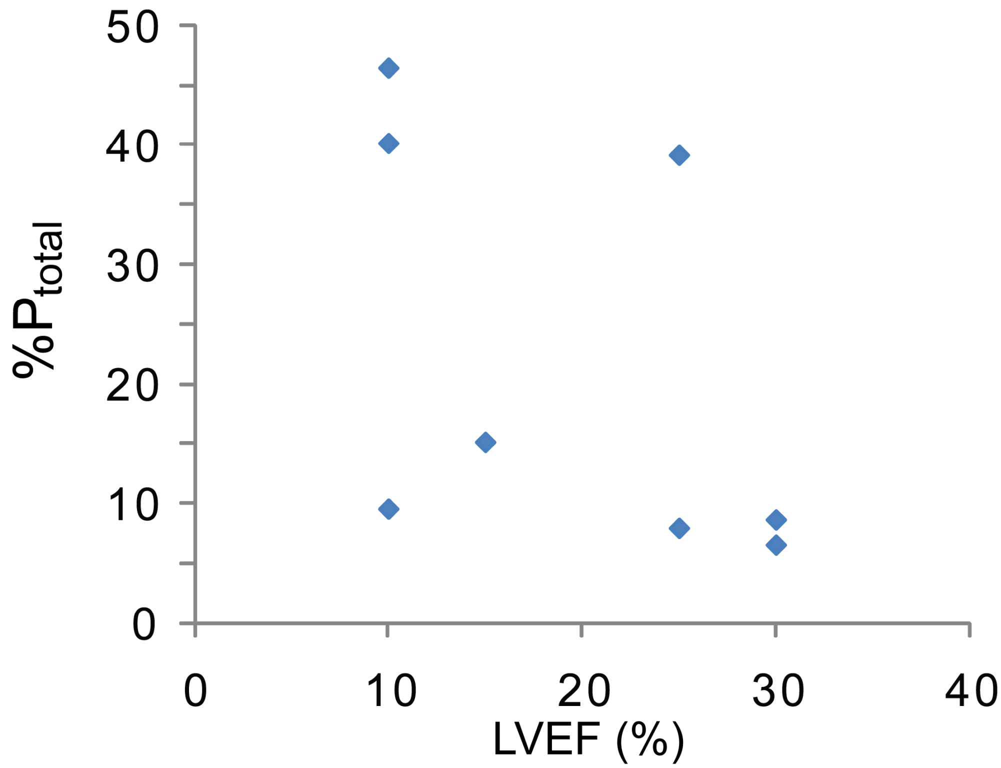


Fig. S8. Correlation between left ventricular ejection fraction (LVEF) and cTnI phosphorylation. Samples were from ICM/DCM group (see Table S2 for detailed clinical characteristics). No significant correlation was observed between these parameters.