Supplemental Information

Quantitative phosphoproteomics using acetone-based peptide labeling: Method evaluation and application to a cardiac ischemia/reperfusion model.

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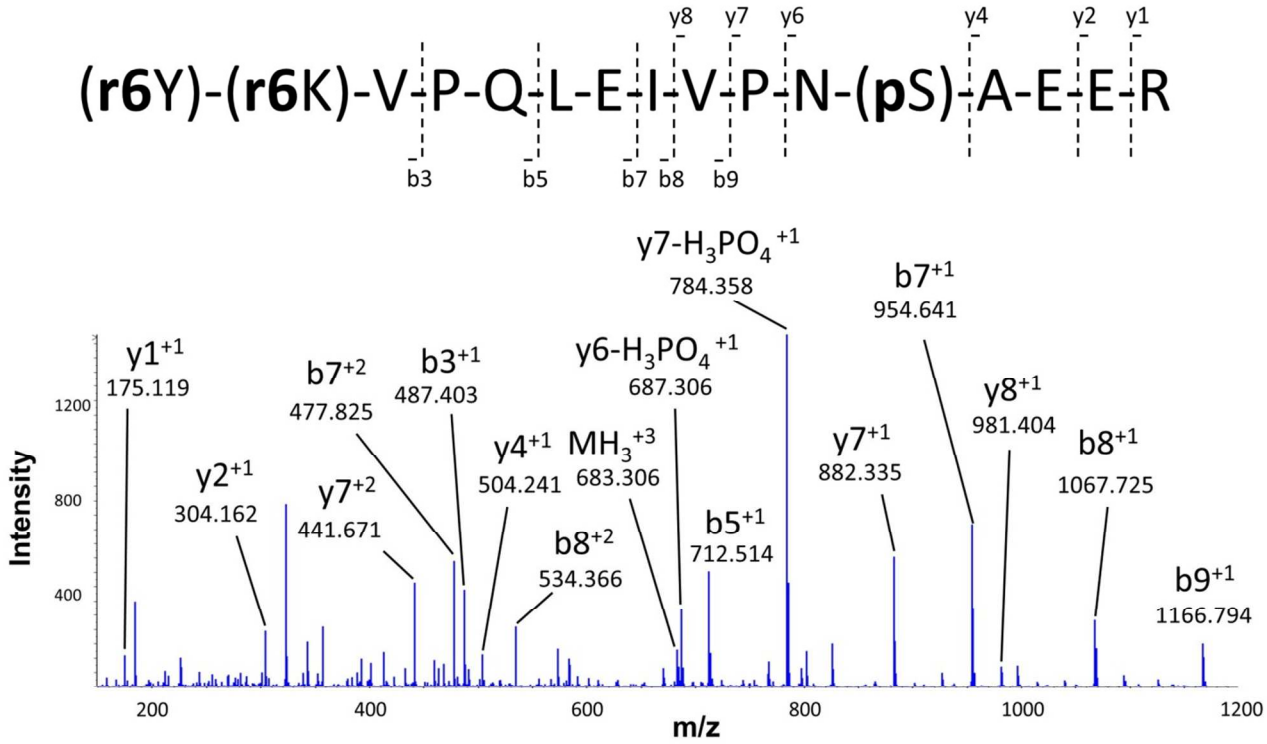
Supplemental Table 1 Summary of proteins with higher degree of phosphorylation in LMW FGF2 "expressed" cardiac tissue extracts and the confidently identified and quantified phosphopeptides. MS/MS profiles are illustrated in supplementary document and the references for figures are also included. Quantitative figures representing average MS-profiles and XIC profiles for each phosphopeptide/phosphosite are illustrated in both main document and supplementary document. Respective references for these figures are also included in the table. For each phosphopeptide pair, quantification value represented as average $\log_2[H/L]^*_{Pair}$ from five biologically distinct pairwise comparisons is also indicated with the standard error (± SE).* Normalized to internal standard—fully labeled phosphopeptide representing bovine α-casein. ‡Could not distinguish the specific site, thus same value was assigned for either sites.

Protein/ Phosphosite	Phosphopeptide(s)	MS/MS profile	Quantitative Figure	Average $\log_2[H/L]^*_{Pair}$ ± SE (n=5)
Microtubule-associated protein tau isoform a / Ser-191, Ser-393	**184**-SGYSSPG(**pS**)PGTPGSR-**198** **385**-SPVVSGDT(**pS**)PR-**395**	Sup.Fig.2 Sup.Fig.3	Sup.Fig.17 Sup.Fig.18	0.76 ± 0.10 1.49 ± 0.29
Gap junction alpha-1 protein / Ser-297	**288**-LVTGDRNNS(**pS**)CR-**299**	Sup.Fig.4	Sup.Fig.19	1.39 ± 0.39
Septin-2 a / Ser-218	**210**-IYHLPDAE(**pS**)DEDEDFKEQTR-**229**	Sup.Fig.5	Sup.Fig.20	0.89 ± 0.24
Heat shock protein beta-6 / Ser-157	**123-** LPPGVDPAAVTSALSPEGVLSIQATPASAQA QLP(**pS**)PPAAK-**162**	Sup.Fig.6	Sup.Fig.21	1.42 ± 0.24
Myosin light chain kinase, smooth muscle / Ser-1469	**1437-** AVNVYGTSEPSQESELTAVGEKPEEPKDEV EV(**pS**)DDDEKEPEVDYR-**1481**	Sup.Fig.7	Sup.Fig.22	0.96 ± 0.29
Myosin regulatory light chain 2, ventricular/cardiac muscle isoform/ Ser-14 or Ser-15	**8**-KRIEGG(**pS**)SNVFSMFEQTQIQEFK-**30** **10**-IEGGS(**pS**)NVFSMFEQTQIQEFK-**30**	Sup.Fig.8 Sup.Fig.9	Sup.Fig.23 Sup.Fig.24	1.14 ± 0.51 1.16 ± 0.59
Myosin-binding protein C, cardiac-type / Thr-281; Ser-273; Ser-282 or Ser-284	**281**-(pT)SDSHEDAGTLDFSSLLK-**298** **271**-RT(**pS**)LAGAGR-**279** **280**-RT(**pS**)DSHEDAGTLDFSSLLK-**299** **280**-RTSD(**pS**)HEDAGTLDFSSLLK-**299**	Sup.Fig.10 Sup.Fig.11 Sup.Fig.12 Sup.Fig.13	Sup.Fig.25 Sup.Fig.26 Fig.2C Fig.2C	1.35 ± 0.38 2.12 ± 0.51 1.38 ± 0.38* 1.38 ± 0.38*
Cytochrome b-c1 complex subunit 1, mitochondrial precursor / Ser-212	**210**-RL(**pS**)RTDLTDYLNR-**222**	Sup.Fig.14	Sup.Fig.27	1.02 ± 0.43
2-Oxoisovalerate dehydrogenase subunit alpha, mitochondrial / Ser-338	**334**-IGHH(**pS**)TSDDSSAYR-**347**	Sup.Fig.15	Sup.Fig.28	1.23 ± 0.46
Myoglobin / Thr-68	**65**-HGC(**pT**)VLTALGTILK-**78**	Sup.Fig.16	Sup.Fig.29	1.92 ± 0.93

A. MS/MS Spectra for Phosphopeptides with Statistically Confident Quantitation

1. alpha-S1-casein precursor [Bos taurus]: Internal Standard

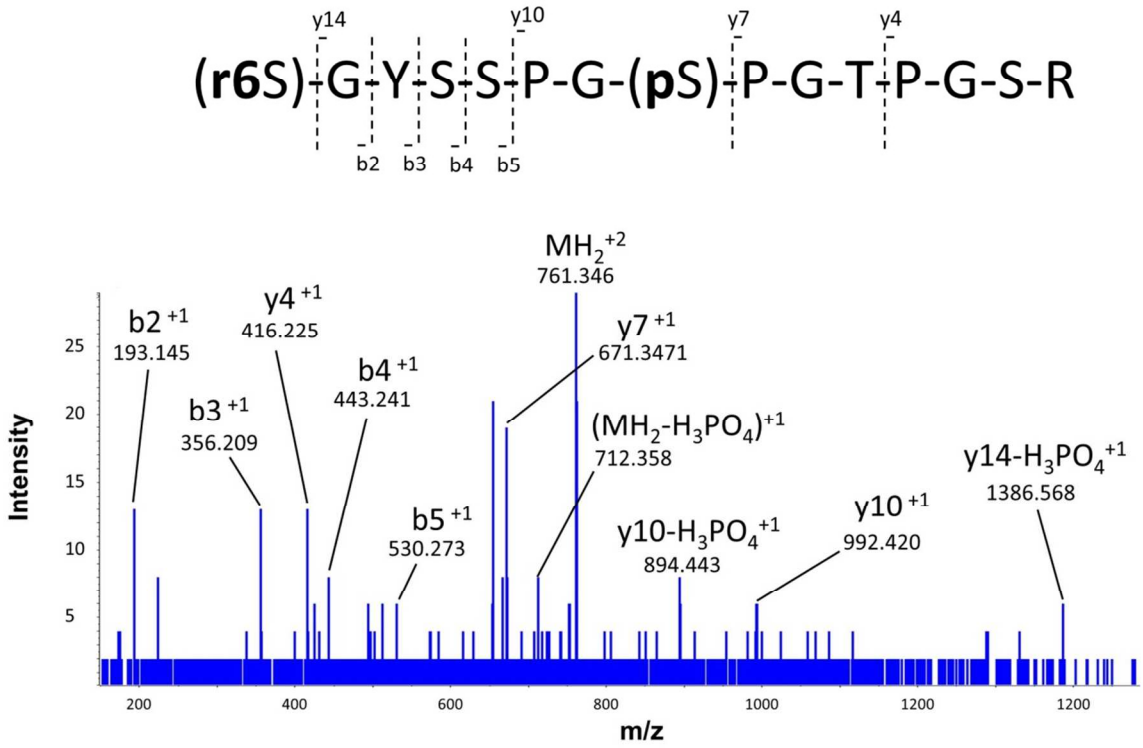
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 1: MS/MS fragmentation spectrum obtained for the precursor ion at m/z 683.3787 Da (+3), that represents 104-YKVPQLEIVPN(**pS**)AEER-119. The N-terminus and the lysine residue @2 of the peptide are d6-RABA-tagged. The N-terminus, the primary amine groups of lysine residues @2 of the peptide are d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

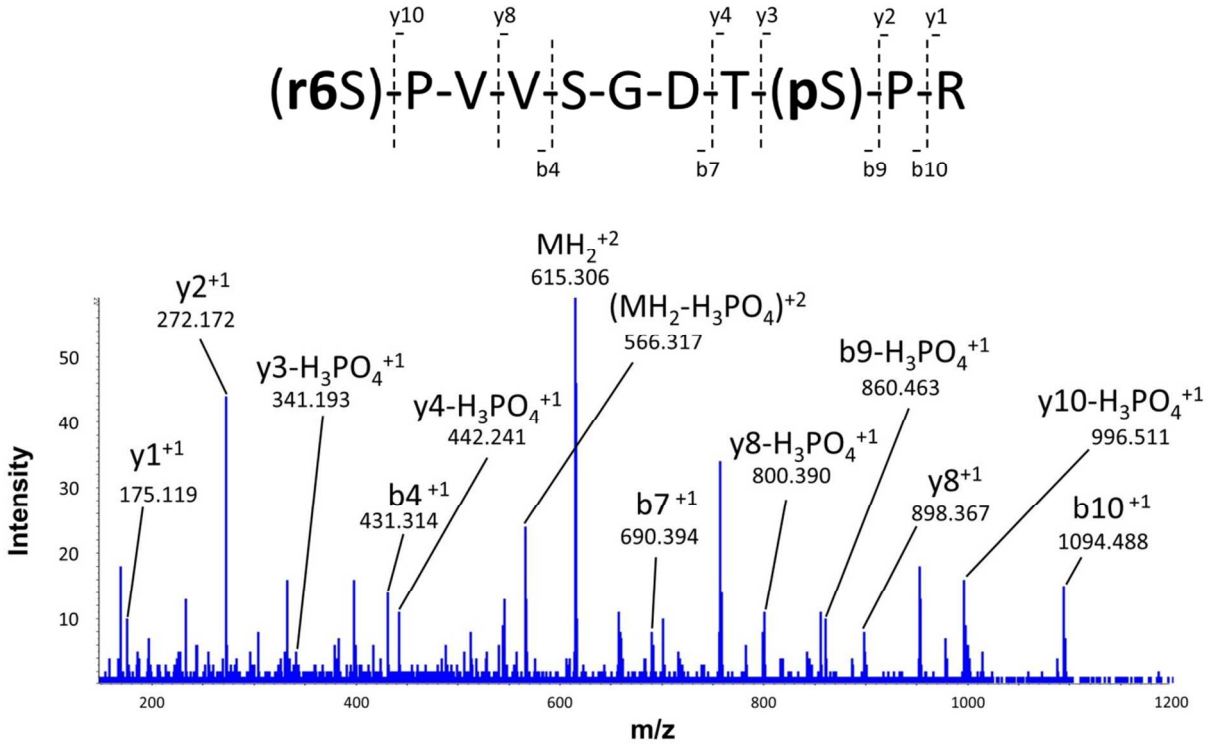
2. Microtubule-associated protein tau isoform a [Mus musculus]/ Ser-191.

[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 3: MS/MS fragmentation spectrum obtained for the precursor ion at m/z 761.3481 Da (+2), that represents 184-SGYSSPG(**pS**)PGTPGSR-198. The N-terminus of the peptide is d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

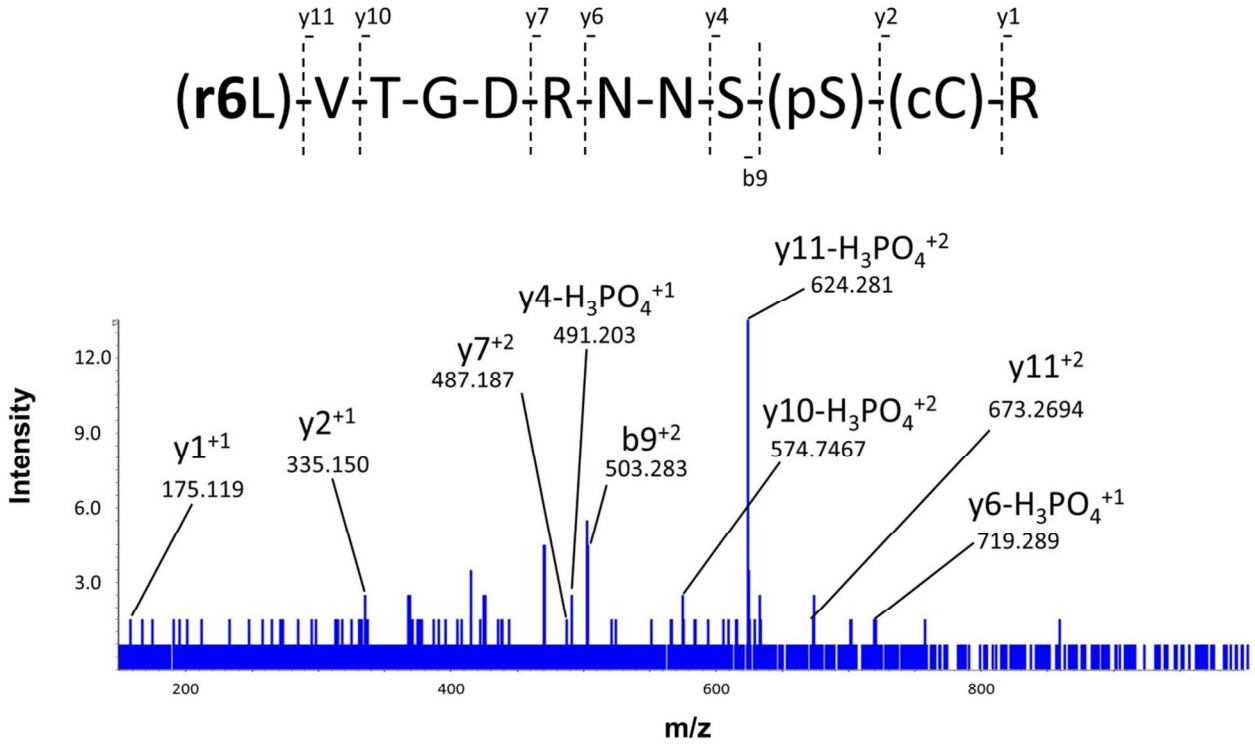
3. Microtubule-associated protein tau isoform a [Mus musculus]/ Ser-393.
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 4: MS/MS fragmentation spectrum obtained for the precursor ion at m/z 615.304 (+2), that represents 385-SPVVSGDT(**pS**)PR-395. The N-terminus of the peptide is d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

4. Gap junction alpha-1 protein [Mus musculus]/Ser-297

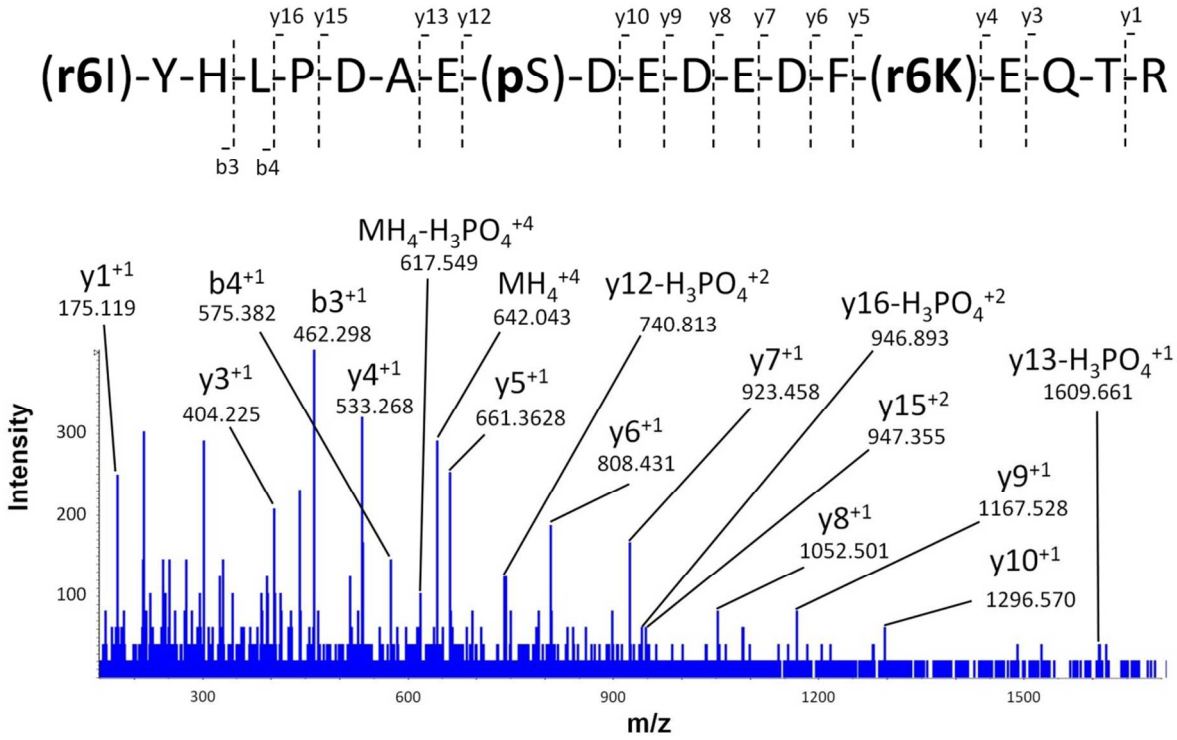
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 5: MS/MS fragmentation spectrum obtained for the precursor ion at m/z : 502.9007 Da (+3), that represents 288-LVTGDRNNS(**pS**)CR-299. The N-terminus of the peptide is d6-RABA-tagged. The cystine residue @11 is carbamidomethylated. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

5. Septin-2 a [Mus musculus]/Ser-218

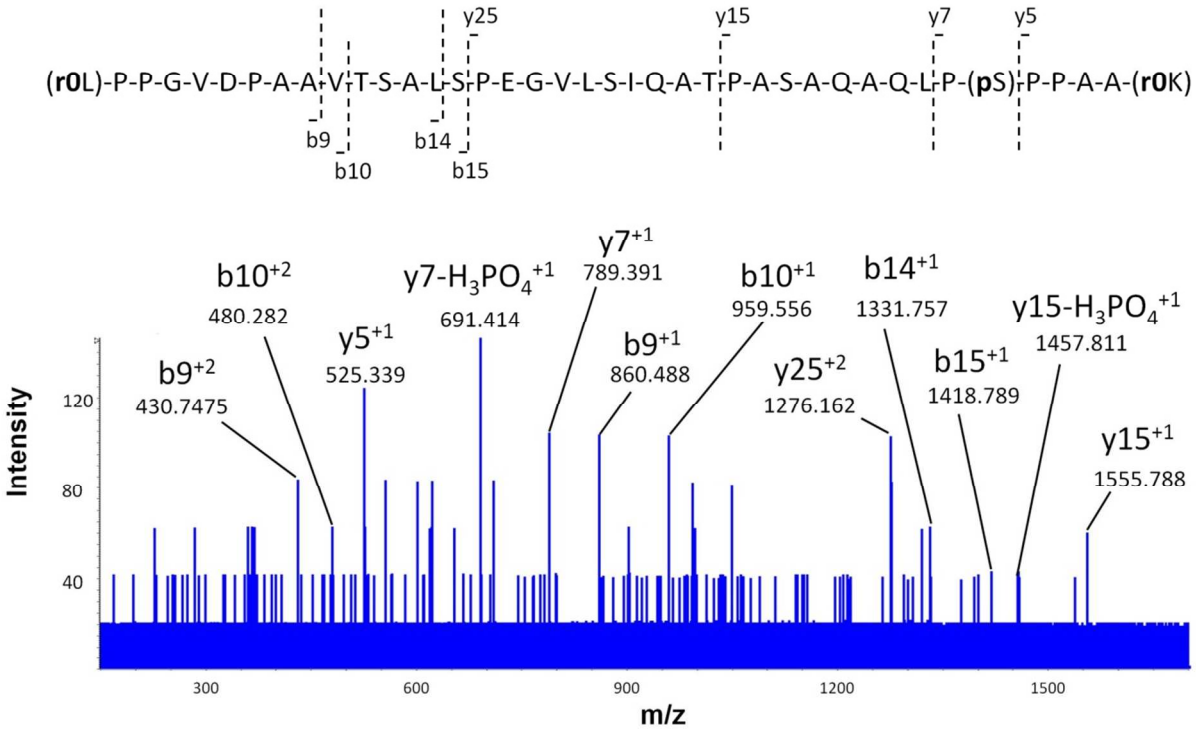
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 6: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 642.038 Da (+4), that represents 210-IYHLPDAE(**pS**)DEDEDFKEQTR-229. The N-terminus of the peptide and the primary amine group of lysine residue @16 of the peptide are d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

6. Heat shock protein beta-6 [Mus musculus]/Ser-157

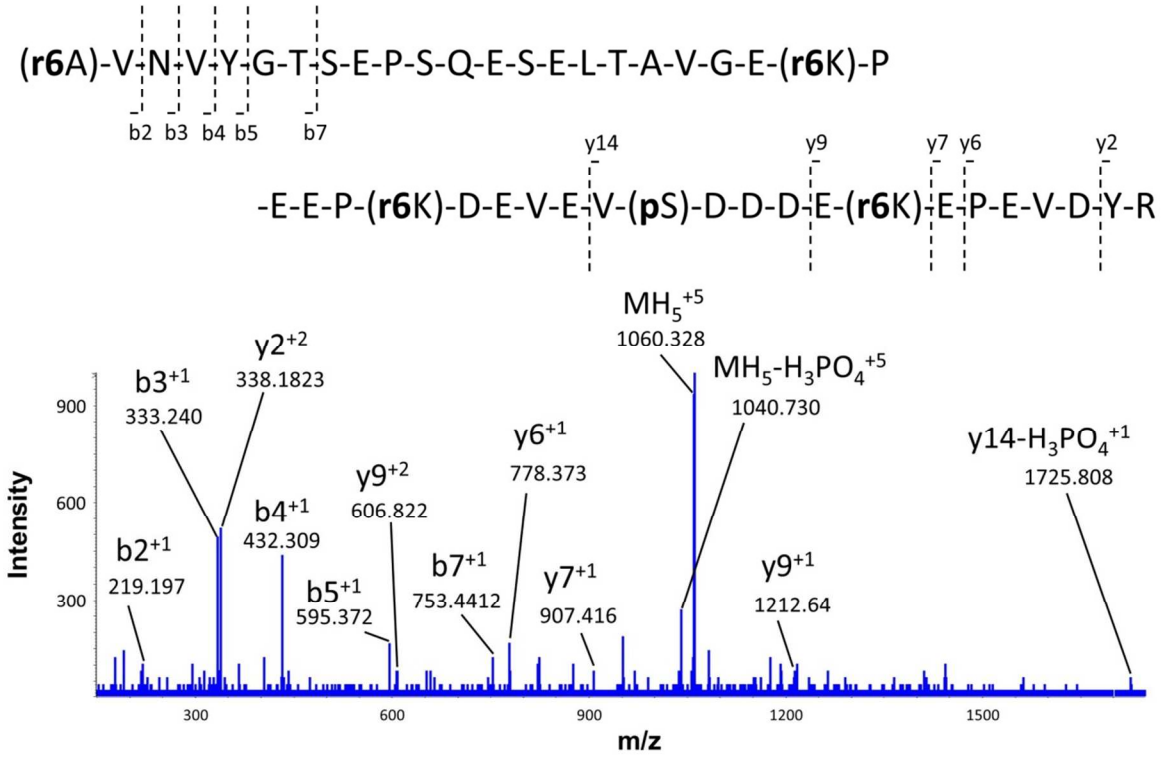
[r0: RABA:d(0)-tag; **p:** phosphorylation**]**



Supplementary Figure 7: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 993.0339 Da (+4), that represents 123-LPPGVDPAAVTSALSPEGVLSIQATPASAQAQLP(**pS**)PPAAK-162.The N-terminus of the peptide and the primary amine group of lysine residue @40 of the peptide are d0-RABA-tagged. Note: each d0-RABA tagging adds 42 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

7. Myosin light chain kinase, smooth muscle [Mus musculus]/Ser-1469

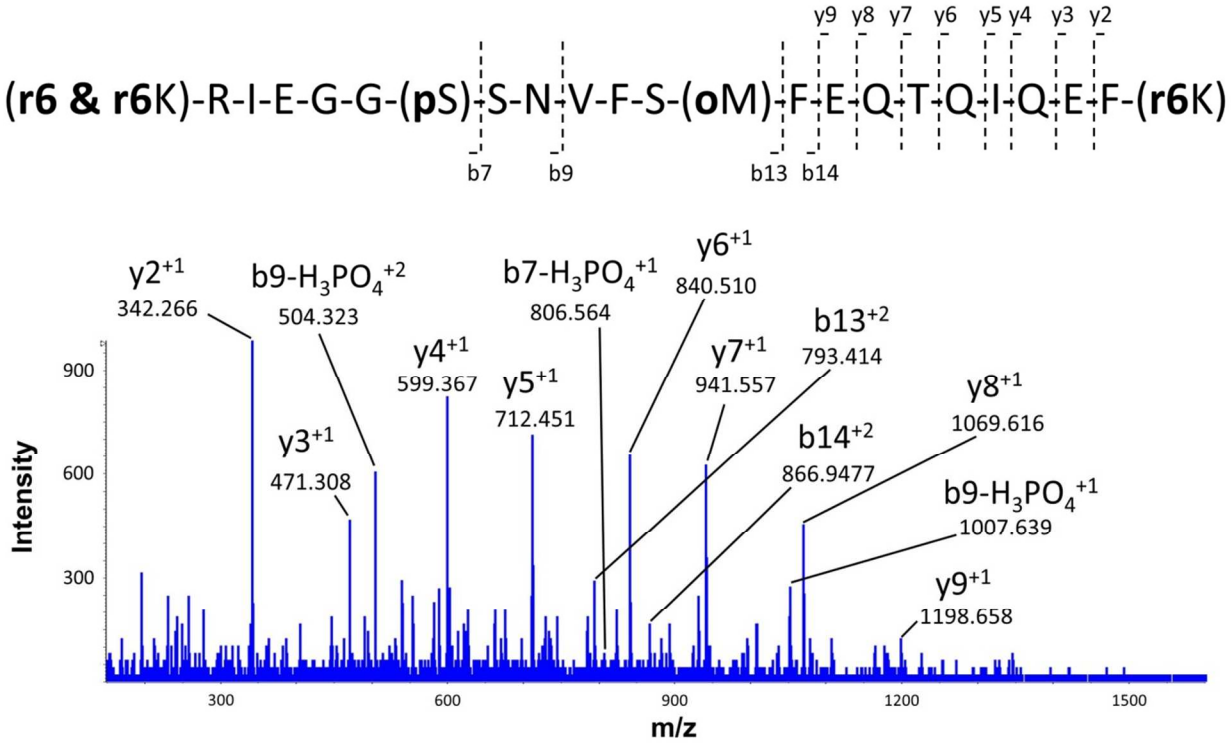
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 8: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 1060.119 (+5), that represents 1437-AVNVYGTSEPSQESELTAVGEKPEEPKDEVEV(**pS**)DDDEKEPEVDYR-1481. The N-terminus of the peptide and the primary amine group of lysine residue @22 @27 and @38 of the peptide are d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

8. Myosin regulatory light chain 2, ventricular/cardiac muscle isoform [Mus musculus]/Ser-14

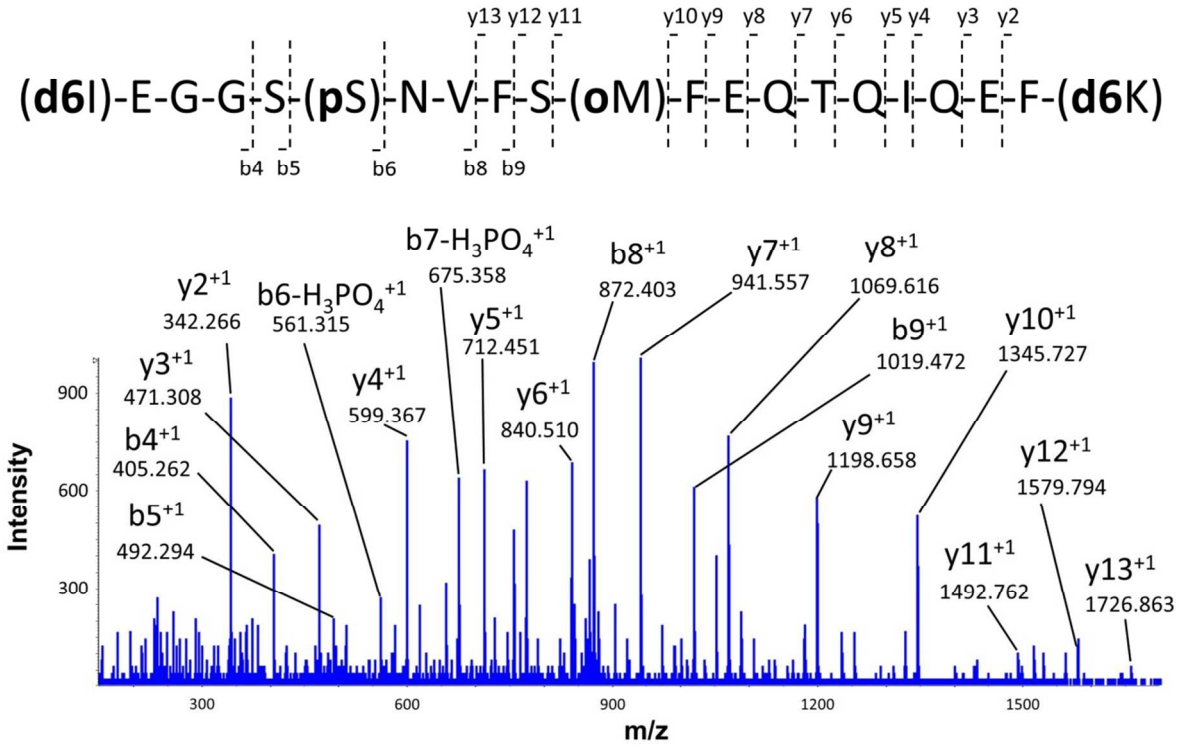
[r6: RABA:d(6)-tag; **o:** oxidation; **p:** phosphorylation**]**



Supplementary Figure 9: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 733.3889 (+4), that represents 8-KRIEGG(**pS**)SNVFSMFEQTQIQEFK-30. The N-terminus of the peptide and the primary amine group of lysine residue @1 and @23 of the peptide are d6-RABA-tagged. The methionine residue @13 is oxidized. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

9. Myosin regulatory light chain 2, ventricular/cardiac muscle isoform [Mus musculus]/Ser-15

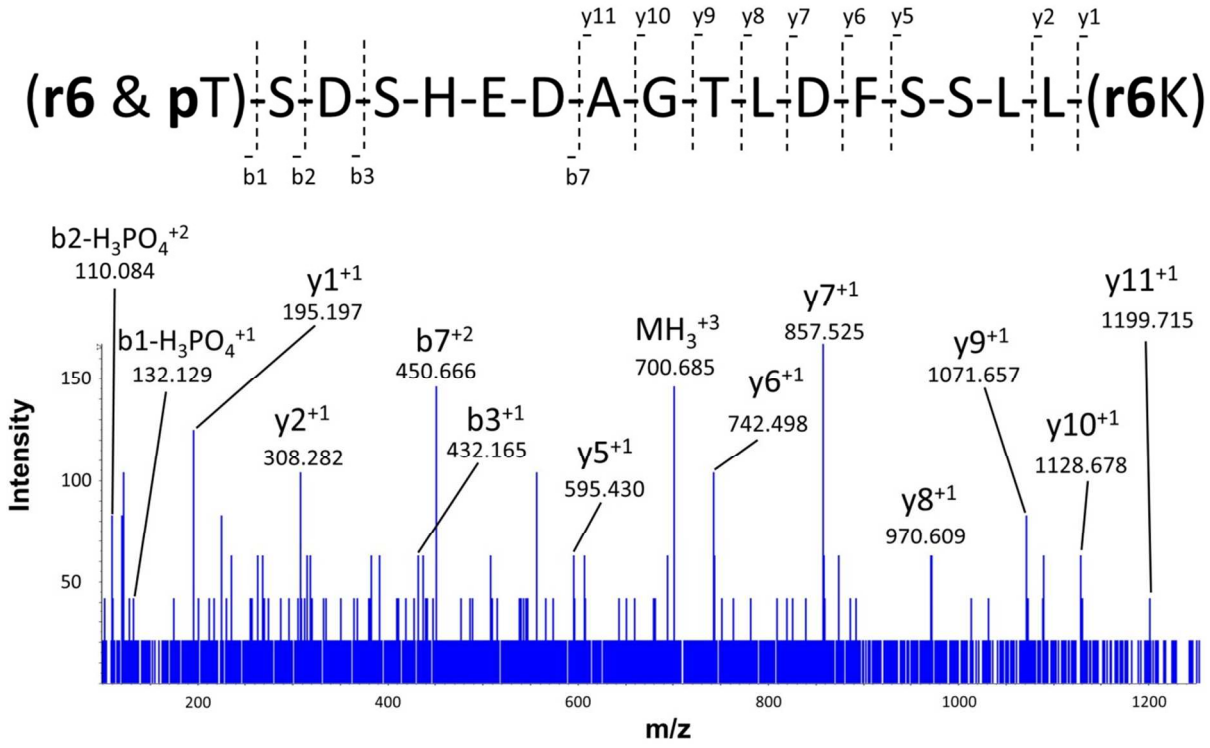
[r6: RABA:d(6)-tag; **o:** oxidation; **p:** phosphorylation**]**



Supplementary Figure 10: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 866.7604 (+3), that represents 10-IEGGS(**pS**)NVFSMFEQTQIQEFK-30. The N-terminus of the peptide and the primary amine group of lysine residue @21 of the peptide are d6-RABA-tagged. The methionine residue @11 is oxidized. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

10. Myosin-binding protein C, cardiac-type [Mus musculus]/Thr-281

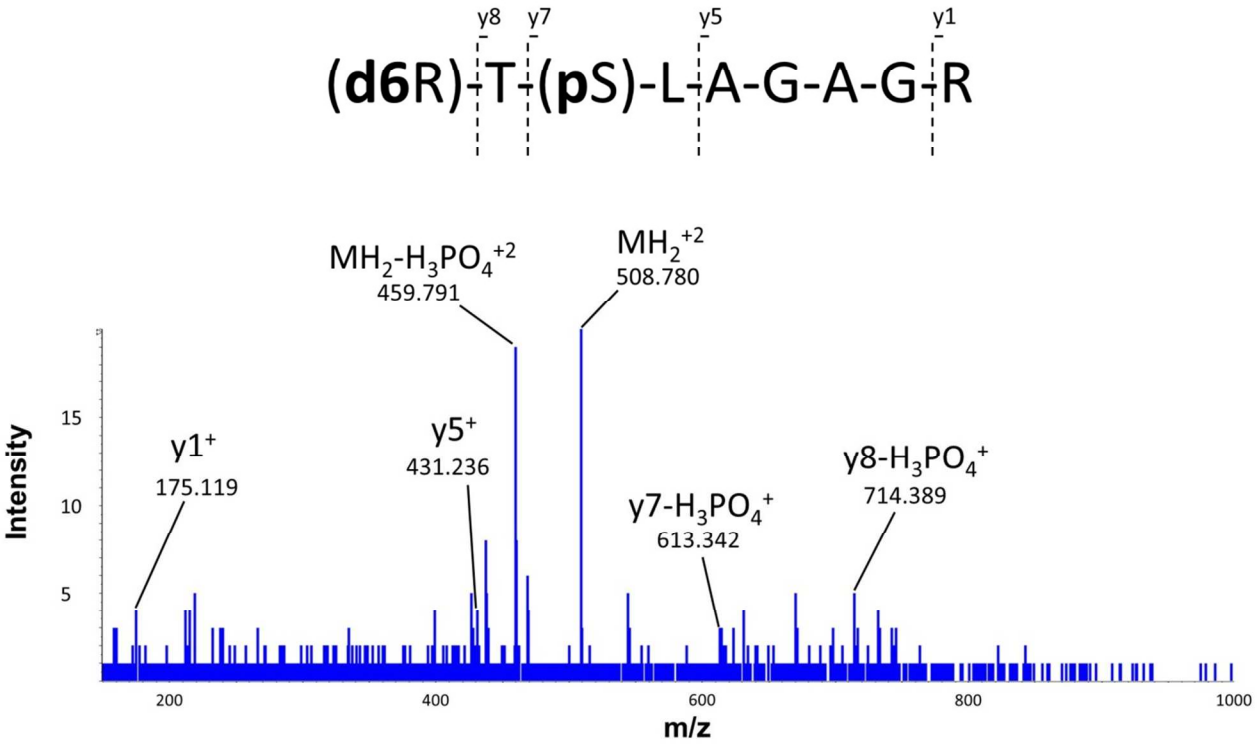
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 11: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 700.4 Da (3+), that represents 281-(**pT**)SDSHEDAGTLDFSSLLK-298. The N-terminus of the peptide and the primary amine group of lysine residue @18 of the peptide are d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

11. Myosin-binding protein C, cardiac-type [Mus musculus]/Ser-273

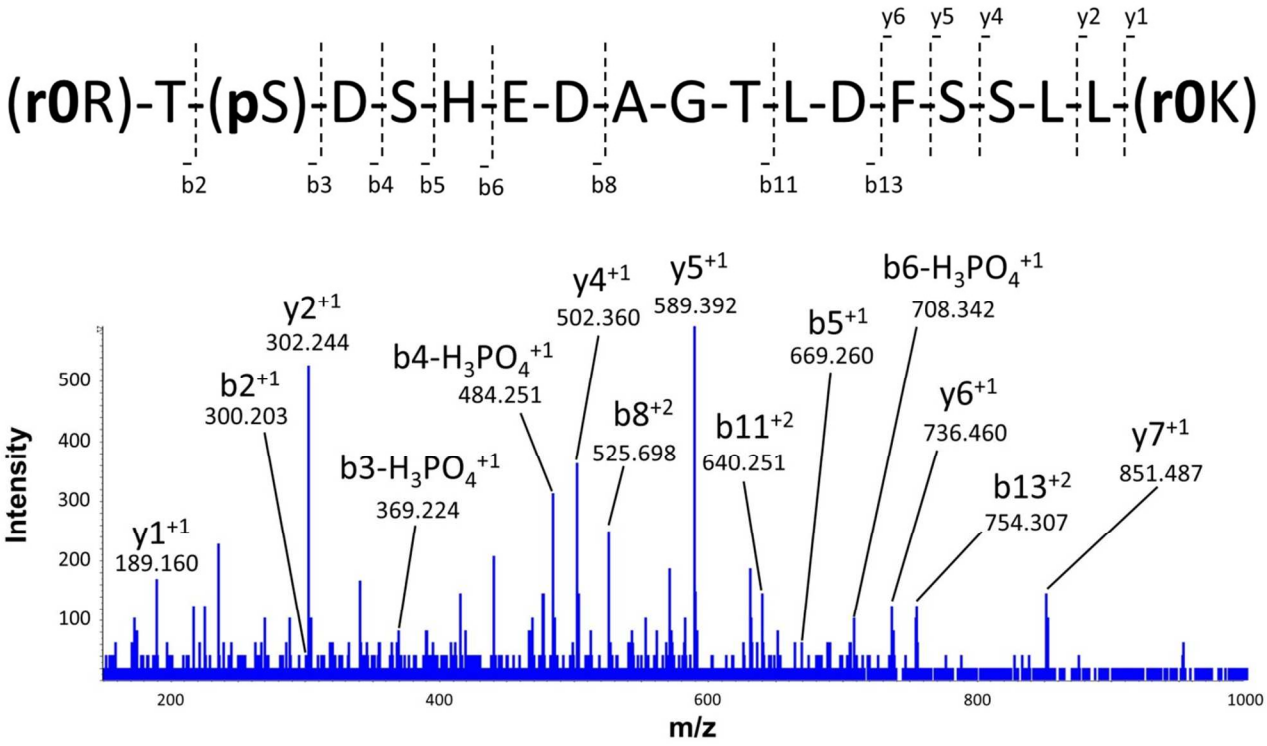
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 12: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 508.7791 Da (+2), that represents 271-RT(**pS**)LAGAGR-279. The N-terminus of the peptide is d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

12. Myosin-binding protein C, cardiac-type [Mus musculus]/Ser-282

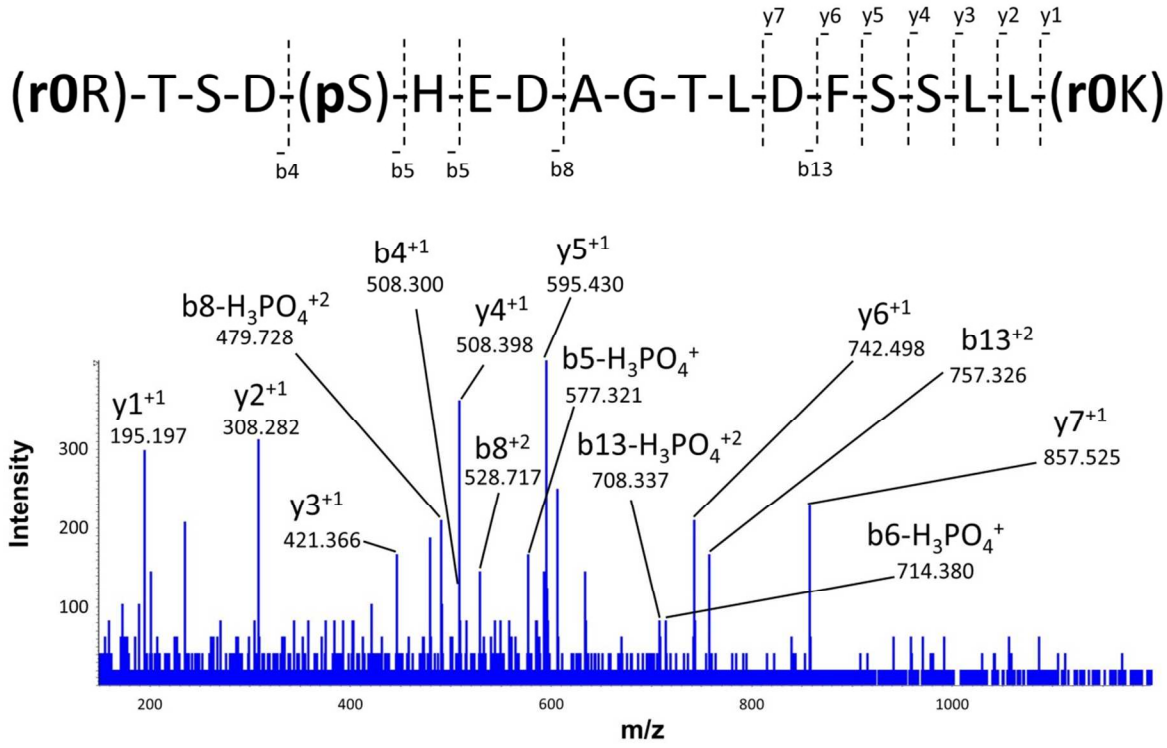
[r0: RABA:d(0)-tag; **p:** phosphorylation**]**



Supplementary Figure 13: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 561.5196 Da (+4), that represents 280-RT(pS)DSHEDAGTLDFSSLLK-298. The N-terminus of the peptide and the primary amine group of lysine residue @19 of the peptide are d0-RABA-tagged. Note: each d0-RABA tagging adds 42 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

13. Myosin-binding protein C, cardiac-type [Mus musculus]/Ser-284

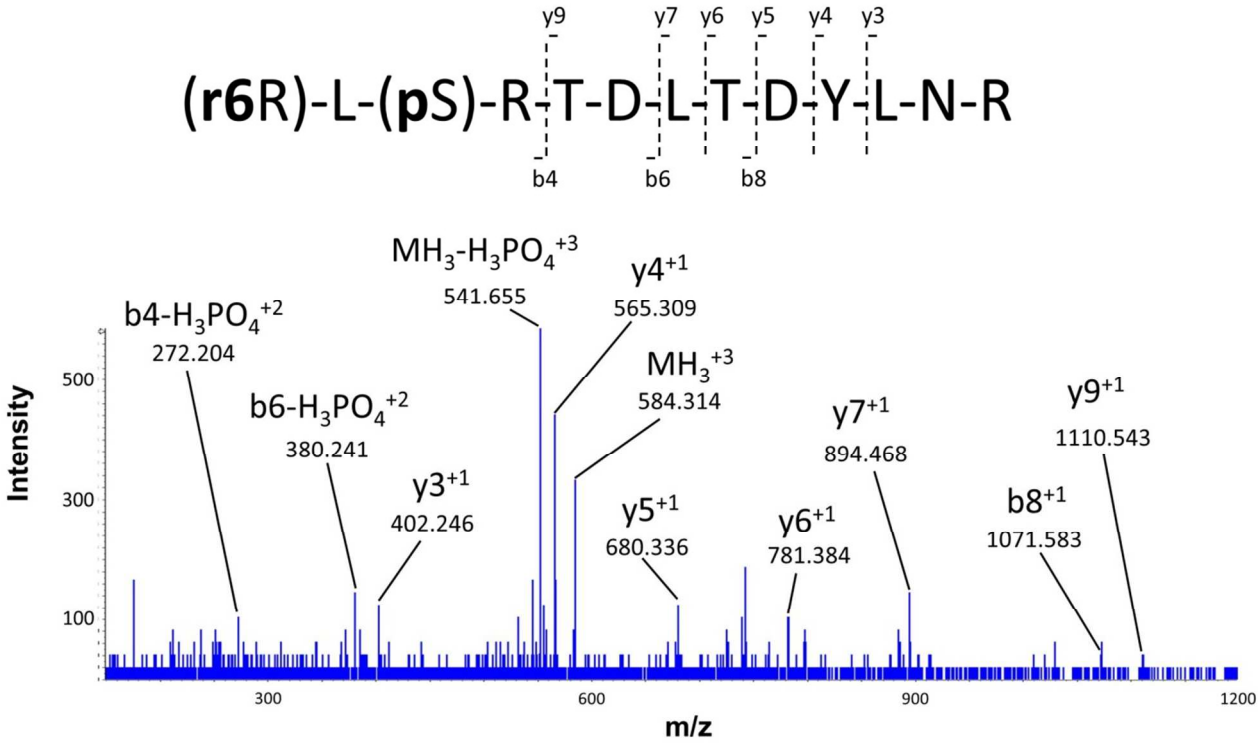
[r0: RABA:d(0)-tag; **p:** phosphorylation**]**



Supplementary Figure 14: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 564.5389 Da (+4), that represents 280-RTSD(**pS**)HEDAGTLDFSSLLK-298. The N-terminus of the peptide and the primary amine group of lysine residue @19 of the peptide are d0-RABA-tagged. Note: each d0-RABA tagging adds 42 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

14. Cytochrome b-c1 complex subunit 1, mitochondrial precursor [Mus musculus]/Ser-212

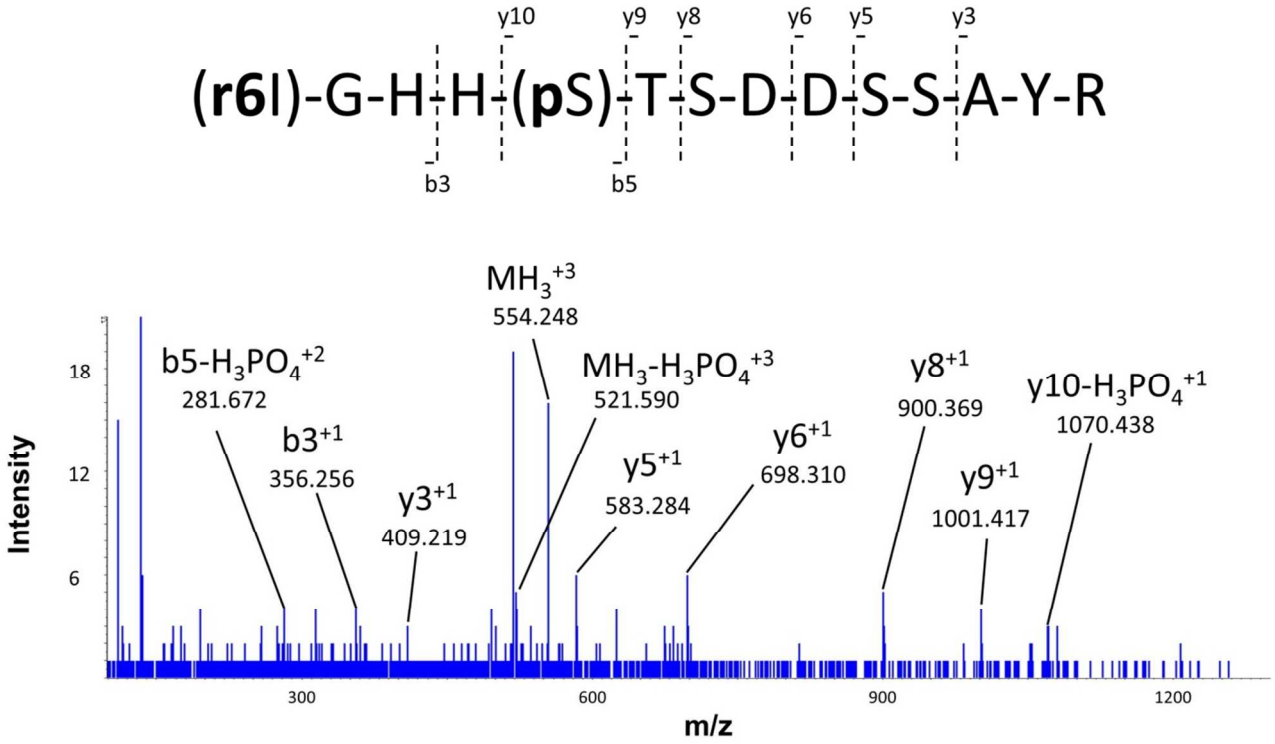
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 15: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 584.3072 (+3), that represents 210-RL(**pS**)RTDLTDYLNR-222. The N-terminus of the peptide is d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

15. 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial [Mus musculus]/Ser-338

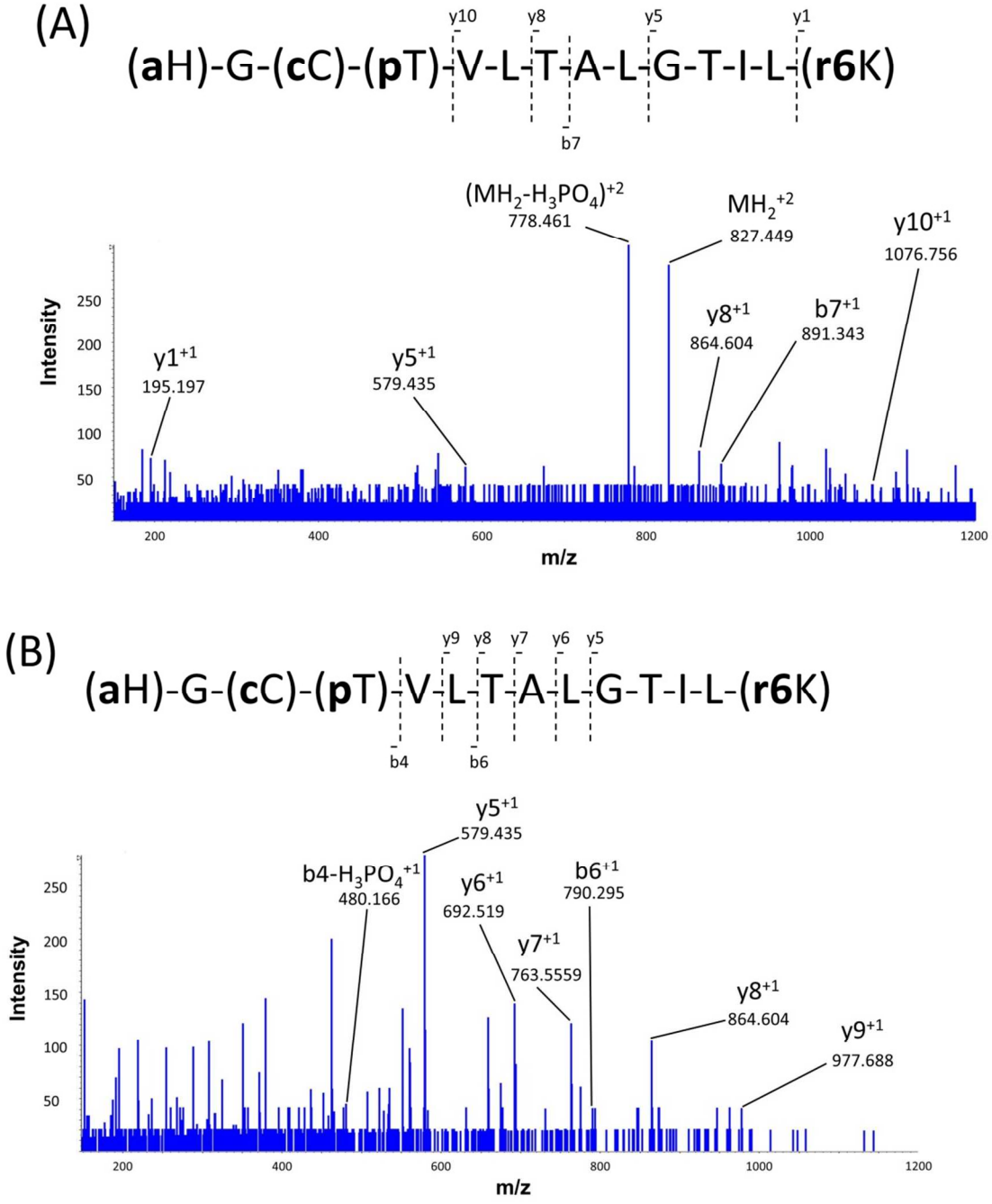
[r6: RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 16: MS/MS fragmentation spectrum obtained for the precursor ion at m/z: 554.2445 (+3), that represents 334-IGHH(**pS**)TSDDSSAYR-347. The N-terminus of the peptide is d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

16. Myoglobin [Mus musculus]/Thr-68:
(Note: MS/MS spectra obtained for both doubly protonated 2+ peptide ion and triply protonated 3+ peptide ion are evaluated simultaneously for high confidence validation of sequence information)

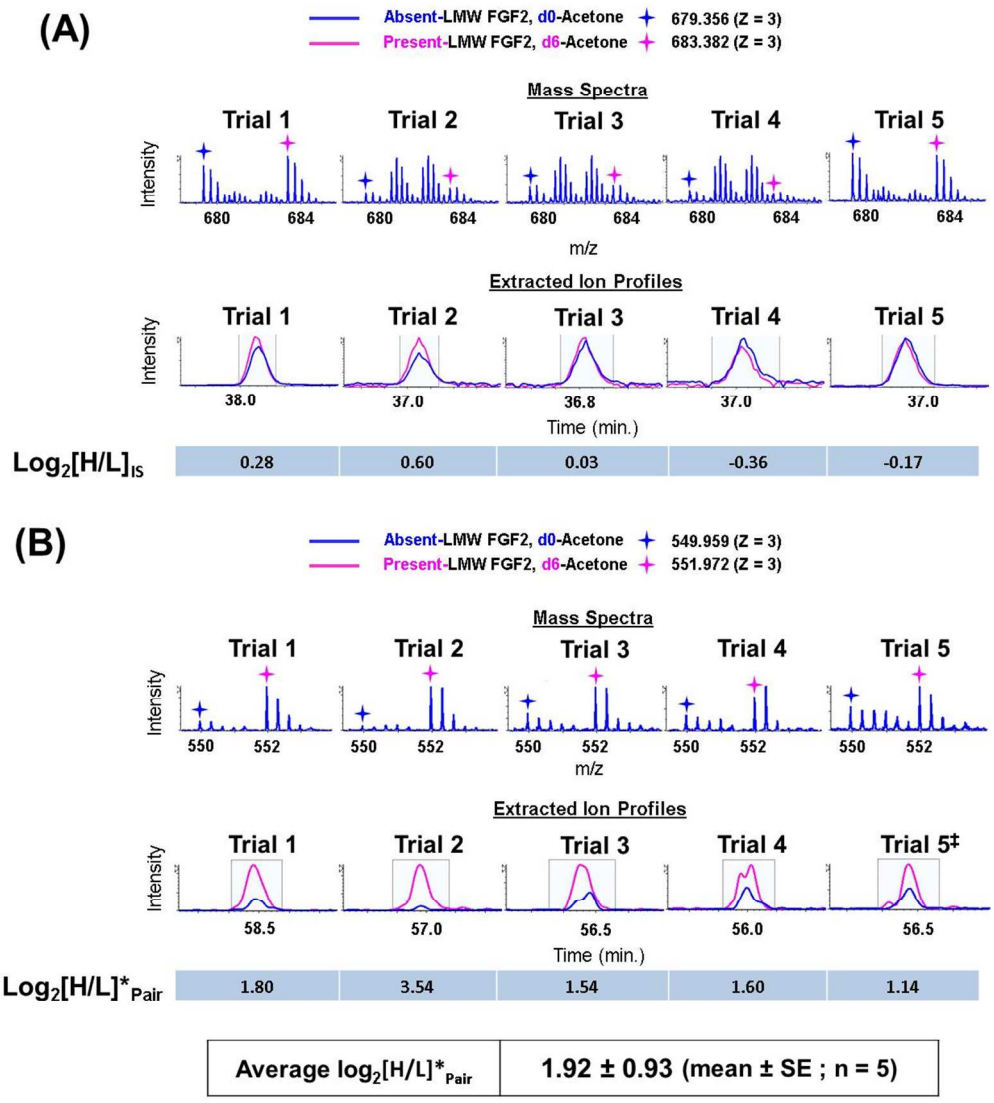
[a: acetyl; **c:** carbamidomethyl; **r6:** RABA:d(6)-tag; **p:** phosphorylation**]**



Supplementary Figure 2: MS/MS fragmentation spectra obtained for the precursor ions at m/z A) 827.4479 Da (+2) and B) 551.9666 Da (3+), which represent 65-HGC(**pT**)VLTALGTILK-78. The N-terminus is acetylated and the cystine residue @3 is carbamidomethylated. The primary amine group of lysine residue @14 of the peptide is d6-RABA-tagged. Note: each d6-RABA tagging adds 48 Da to the peptide mass. Fragmentation was performed by application of collision energy, 43.0 in vendor-defined arbitrary units.

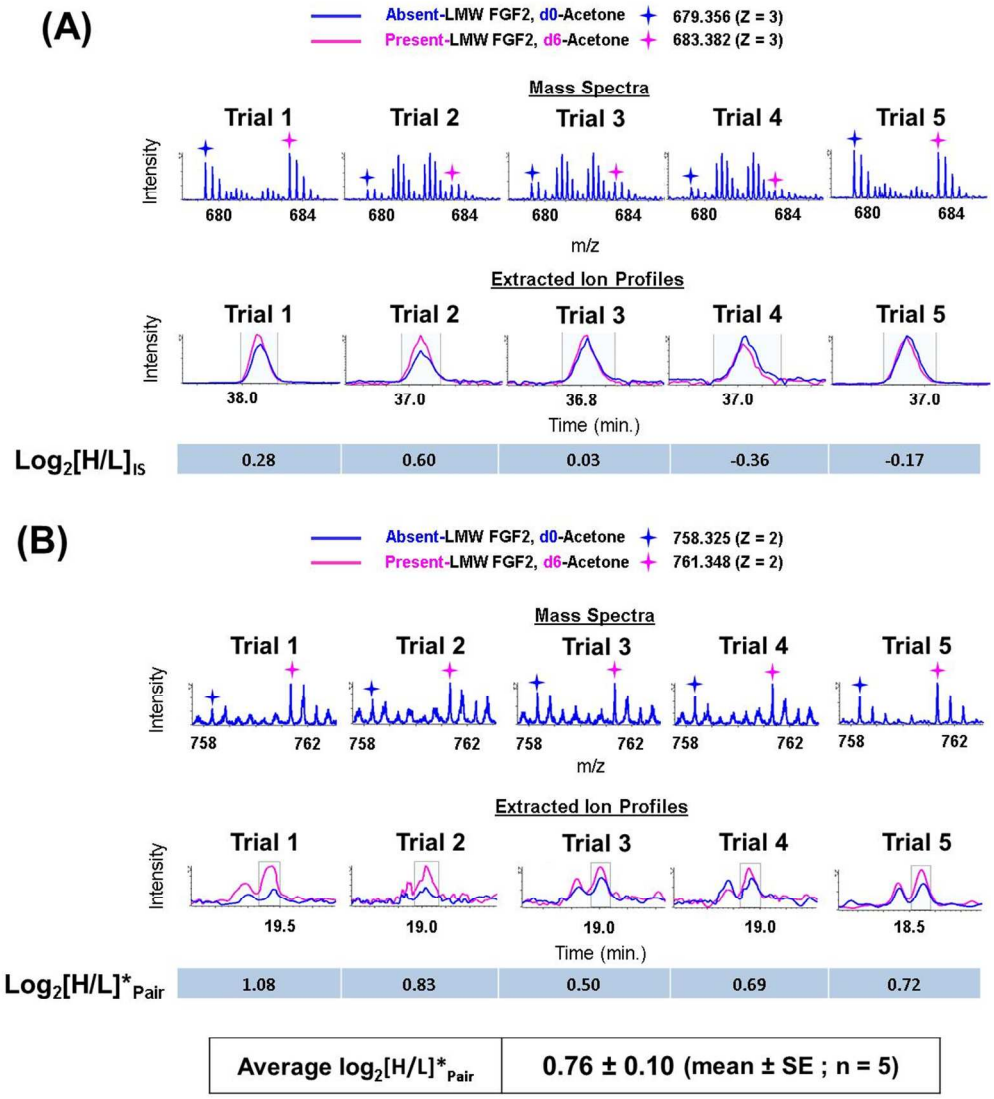
A. Relative Quantification Figures of Phosphopeptides that are Determined as Targets with Statistical High Confidence

17. Myoglobin/ Thr-68



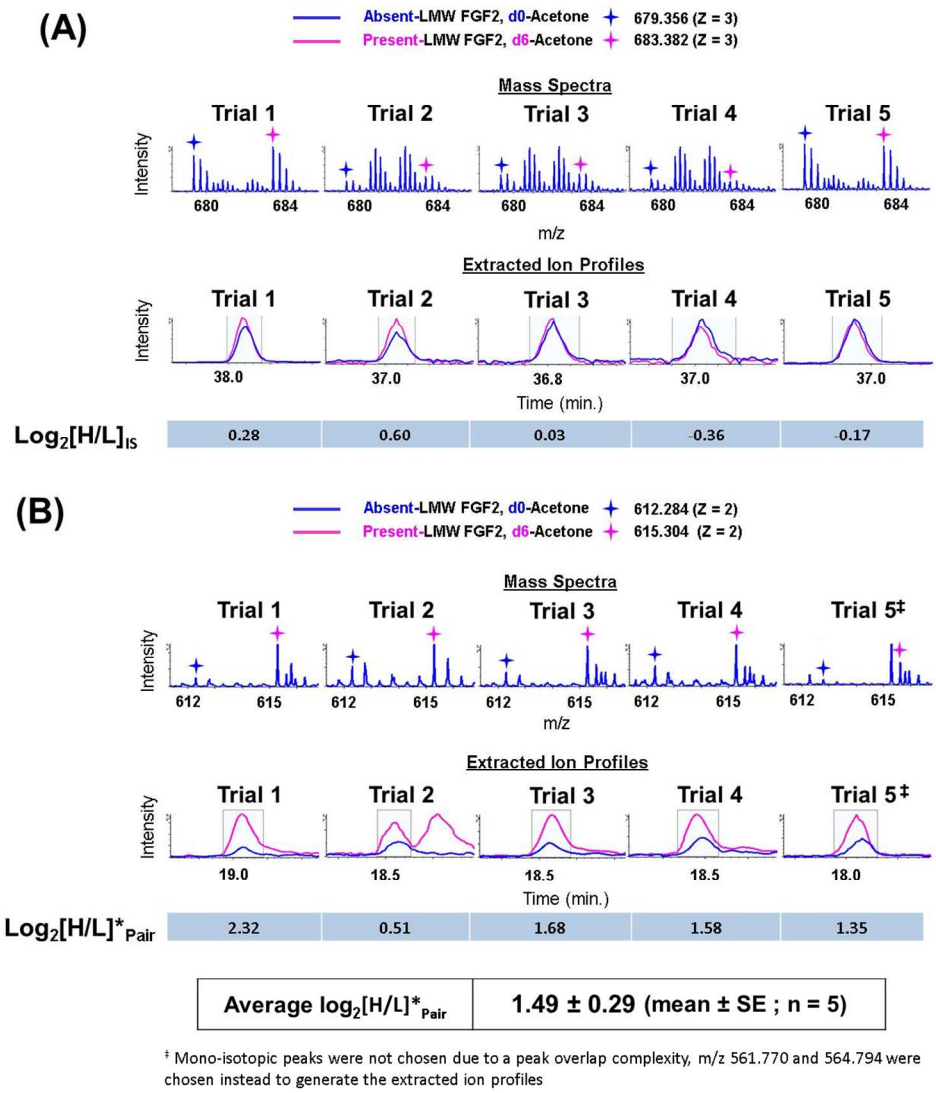
Supplemental Figure 17: Relative quantitative comparison of phosphopeptide—65-HGC(pT)VLTALGTILK-78, in LMW FGF2 "non-expressed" and FGF2 HMWKO (LMW FGF2 "expressed") mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of myoglobin [Mus musculus]/Thr-68 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—65-HGC(pT)VLTALGTILK-78 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 549.959 and 551.972 represents 65-HGC(pT)VLTALGTILK-78 with modifications Acetyl@N-term; Carbamidomethyl(C)@3; RABA(K)@14 and charge state 3+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

18. Microtubule-associated protein tau isoform a / Ser-191



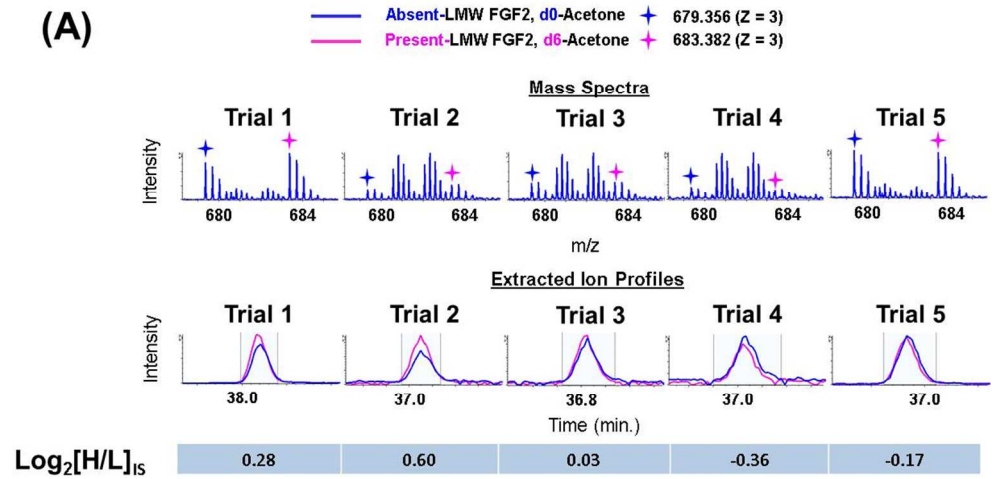
Supplemental Figure 18: Relative quantitative comparison of phosphopeptide—184-SGYSSPG(pS)PGTPGSR-198, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Microtubule-associated protein tau isoform a [Mus musculus] /Ser-191 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—184-SGYSSPG(pS)PGTPGSR-198 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 758.325 and 761.348 represents 184-SGYSSPG(pS)PGTPGSR-198 with modifications RABA@N-term and charge state 2+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

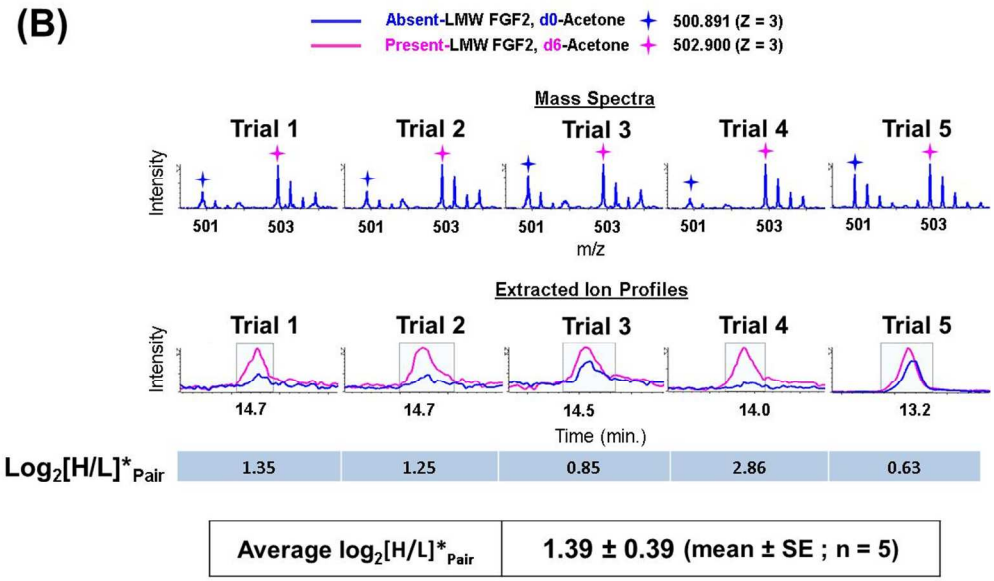
19. Microtubule-associated protein tau isoform a / Ser-393



Supplemental Figure 19: Relative quantitative comparison of phosphopeptide—385-SPVVSGDT(pS)PR-395, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Microtubule-associated protein tau isoform a [Mus musculus]/Ser-393 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—385-SPVVSGDT(pS)PR-395 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 612.284 and 615.304 represents 385-SPVVSGDT(pS)PR-395 with modifications RABA@N-term and charge state 2+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

20. Gap junction alpha-1 protein / Ser-297

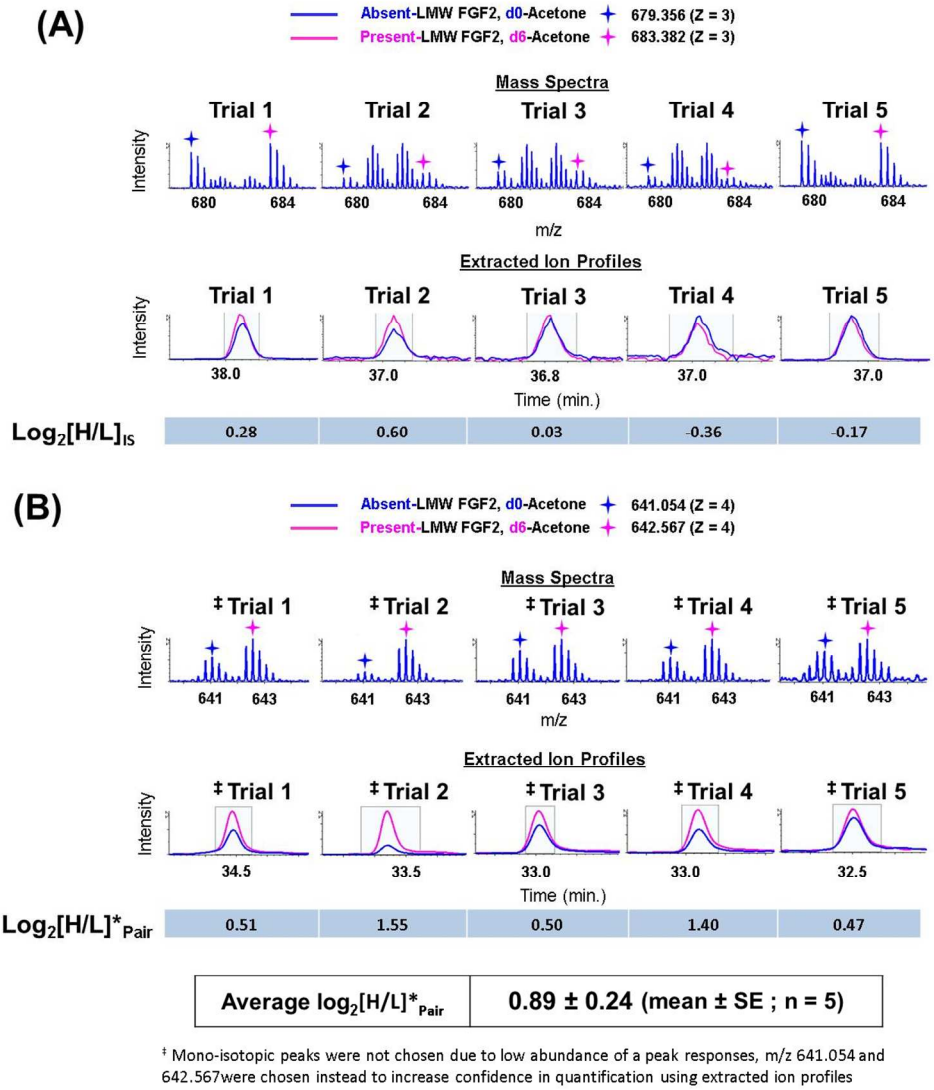




Average $\log_2[H/L]^*_{Pair}$	1.39 ± 0.39 (mean ± SE ; n = 5)

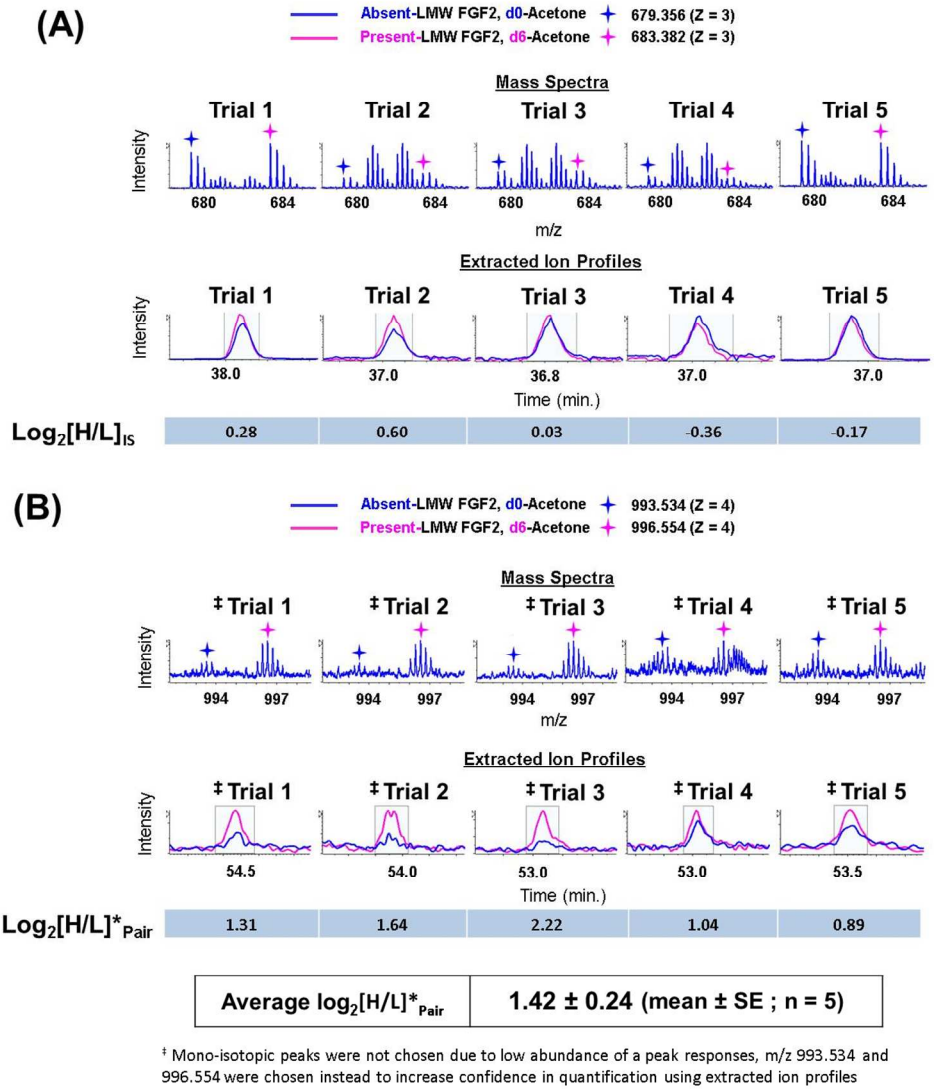
Supplemental Figure 20: Relative quantitative comparison of phosphopeptide—288-LVTGDRNNS(pS)CR-299, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of gap junction alpha-1 protein [Mus musculus]/Ser-297 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—288-LVTGDRNNS(pS)CR-299 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 500.891 and 502.900 represents 288-LVTGDRNNS(pS)CR-299 with modifications RABA@N-term, Carbamidomethyl(C)@11 and charge state 3+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $\log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

21. Septin-2 a / Ser-218



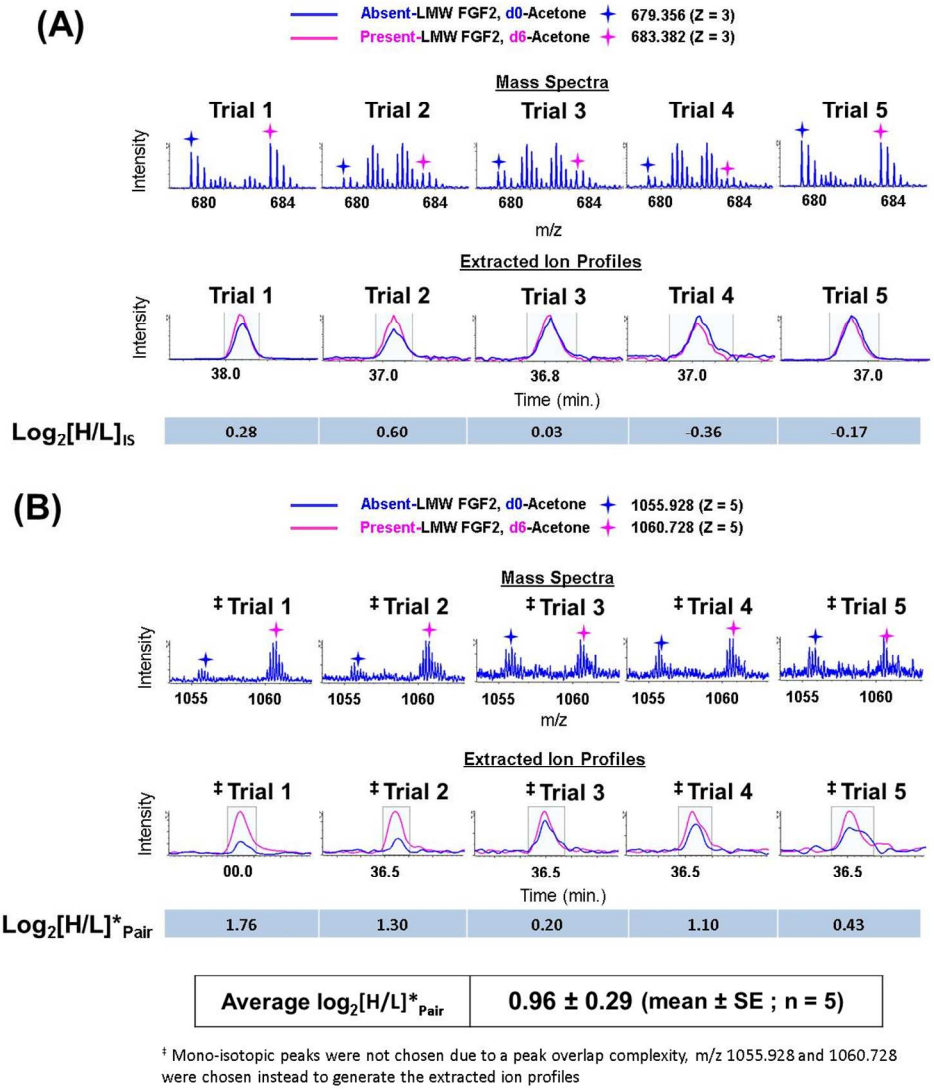
Supplemental Figure 21: Relative quantitative comparison of phosphopeptide—210-IYHLPDAE(pS)DEDEDFKEQTR-229, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Septin-2 a [Mus musculus]/Ser-218 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—210-IYHLPDAE(pS)DEDEDFKEQTR-229 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 640.529 and 642.038 represents 210-IYHLPDAE(pS)DEDEDFKEQTR-229 with modifications RABA@N-term and charge state 4+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

22. Heat shock protein beta-6 / Ser-157



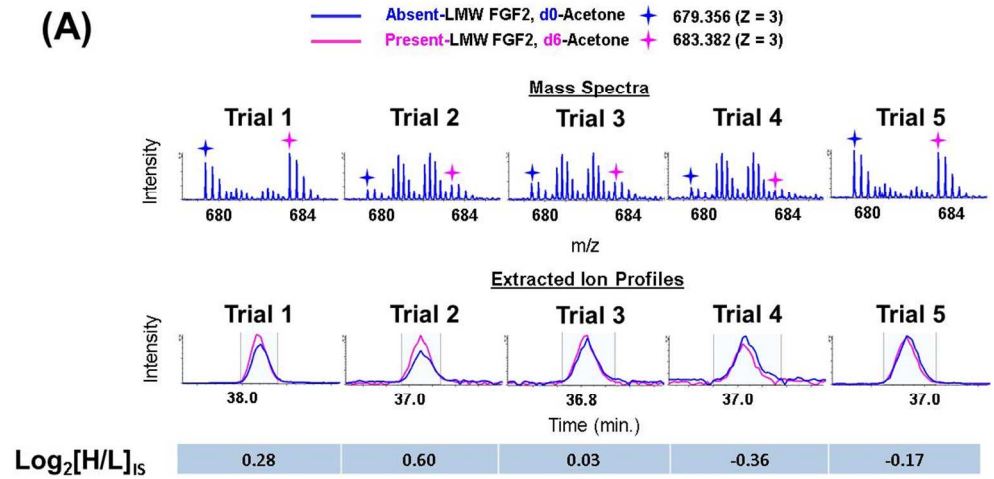
Supplemental Figure 22: Relative quantitative comparison of phosphopeptide—123-LPPGVDPAAVTSALSPEGVLSIQATPASAQAQLP(pS)PPAAK-162, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Heat shock protein beta-6 [Mus musculus]/Ser-157 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—123-LPPGVDPAAVTSALSPEGVLSIQATPASAQAQLP(pS)PPAAK-162 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 993.034 and 993.056 represents 123-LPPGVDPAAVTSALSPEGVLSIQATPASAQAQLP(pS)PPAAK-162 with modifications RABA@N-term, RABA(K)@40 and charge state 4+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

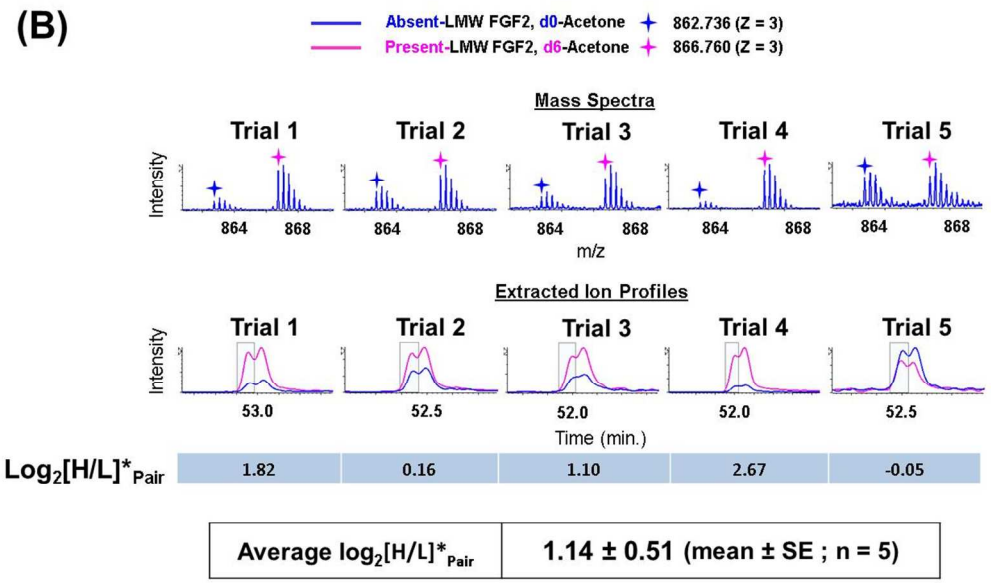
23. Myosin light chain kinase, smooth muscle / Ser-1469



Supplemental Figure 23: Relative quantitative comparison of phosphopeptide—1437-AVNVYGTSEPSQESELTAVGEKPEEPKDEVEV(pS)DDDEKEPEVDYR-1481, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Myosin light chain kinase, smooth muscle / Ser-1469 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—1437-AVNVYGTSEPSQESELTAVGEKPEEPKDEVEV(pS)DDDEKEPEVDYR-1481 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 1055.319 and 1060.119 represents 1437-AVNVYGTSEPSQESELTAVGEKPEEPKDEVEV(pS)DDDEKEPEVDYR-1481 with modifications RABA@N-term, RABA(K)@22, RABA(K)@27, RABA(K)@38 and charge state 5+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target

24. Myosin regulatory light chain 2, ventricular/cardiac muscle isoform / Ser-14

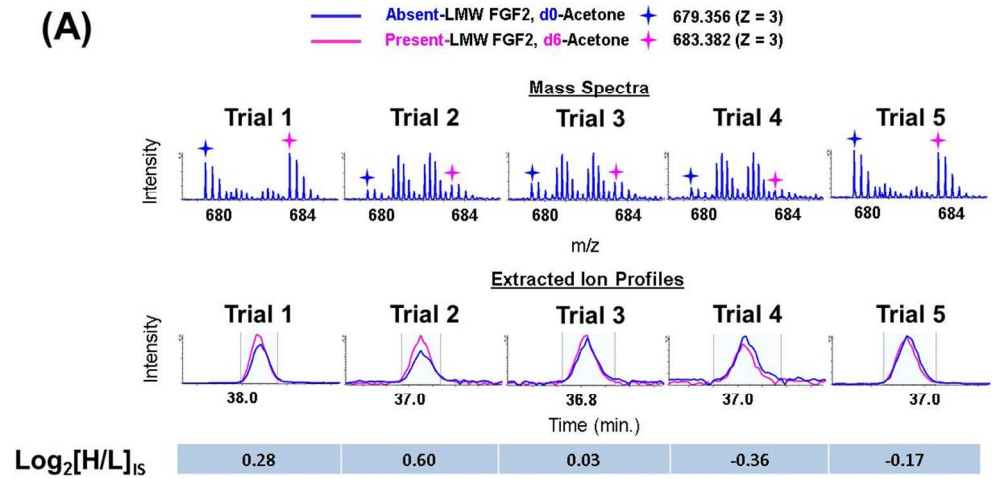


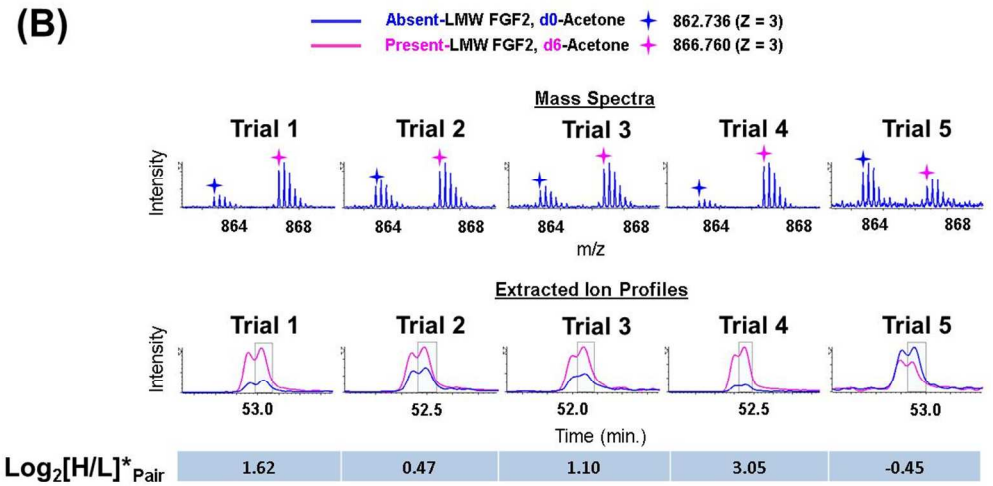


Average $log_2[H/L]^*_{Pair}$	1.14 ± 0.51 (mean ± SE ; n = 5)

Supplemental Figure 24: Relative quantitative comparison of phosphopeptide—8-KRIEGG(pS)SNVFSMFEQTQIQEFK-30 OR 8-KRIEGGS(pS)NVFSMFEQTQIQEFK-30, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Myosin regulatory light chain 2, ventricular/cardiac muscle isoform [Mus musculus]/Ser-14 OR Ser-15 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—8-KRIEGG(pS)SNVFSMFEQTQIQEFK-30 OR 8-KRIEGGS(pS)NVFSMFEQTQIQEFK-30 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 862.736 and 866.760 represents 8-KRIEGG(pS)SNVFSMFEQTQIQEFK-30 OR 8-KRIEGGS(pS)NVFSMFEQTQIQEFK-30 with modifications RABA@N-term, RABA(K)@21, Oxidation(M)@11, and charge state 3+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

25. Myosin regulatory light chain 2, ventricular/cardiac muscle isoform / Ser-15

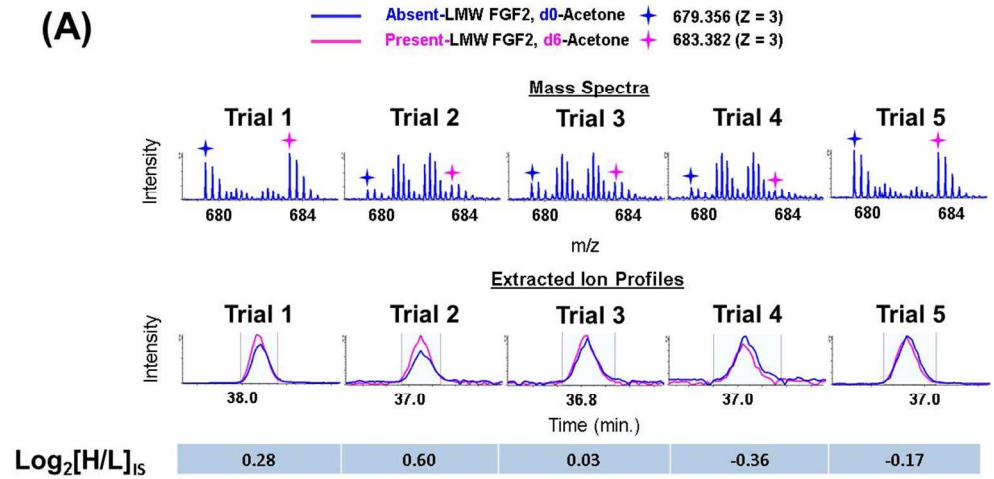


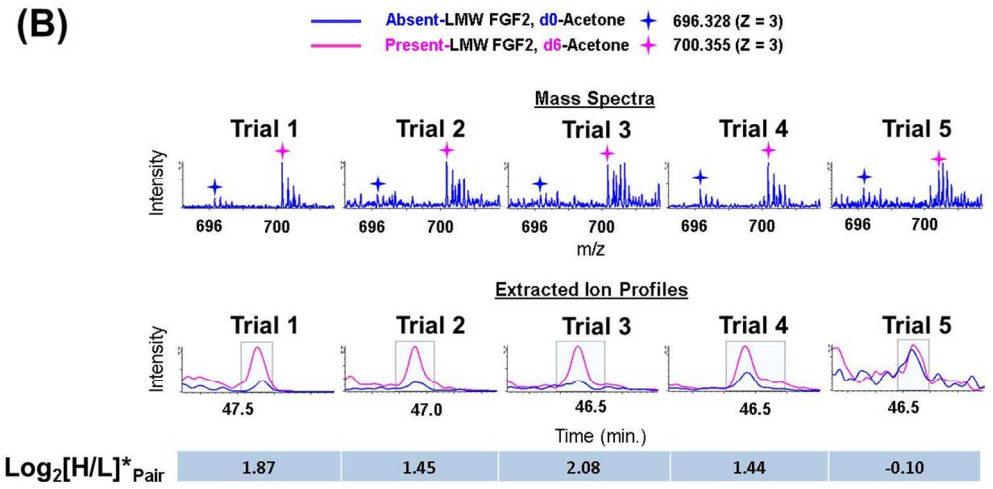


Average $log_2[H/L]^*_{Pair}$	1.16 ± 0.59 (mean ± SE ; n = 5)

Supplemental Figure 25: Relative quantitative comparison of phosphopeptide—8-KRIEGG(pS)SNVFSMFEQTQIQEFK-30 OR 8-KRIEGGS(pS)NVFSMFEQTQIQEFK-30, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Myosin regulatory light chain 2, ventricular/cardiac muscle isoform [Mus musculus]/Ser-14 OR Ser-15 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—8-KRIEGG(pS)SNVFSMFEQTQIQEFK-30 OR 8-KRIEGGS(pS)NVFSMFEQTQIQEFK-30 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 862.736 and 866.760 represents 8-KRIEGG(pS)SNVFSMFEQTQIQEFK-30 OR 8-KRIEGGS(pS)NVFSMFEQTQIQEFK-30 with modifications RABA@N-term, RABA(K)@21, Oxidation(M)@11, and charge state 3+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

26. Myosin-binding protein C, cardiac-type / Thr-281

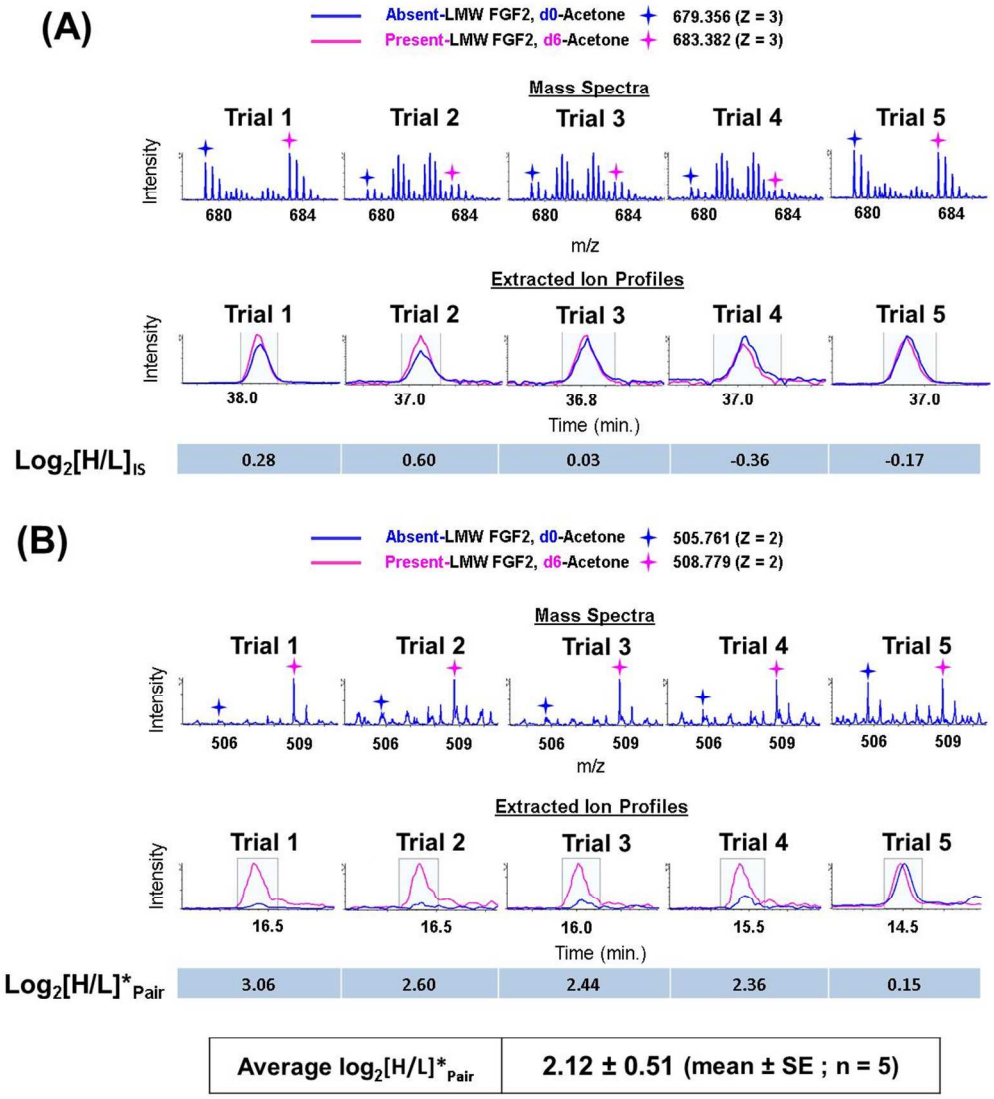




Average $log_2[H/L]^*_{Pair}$	1.35 ± 0.38 (mean ± SE ; n = 5)

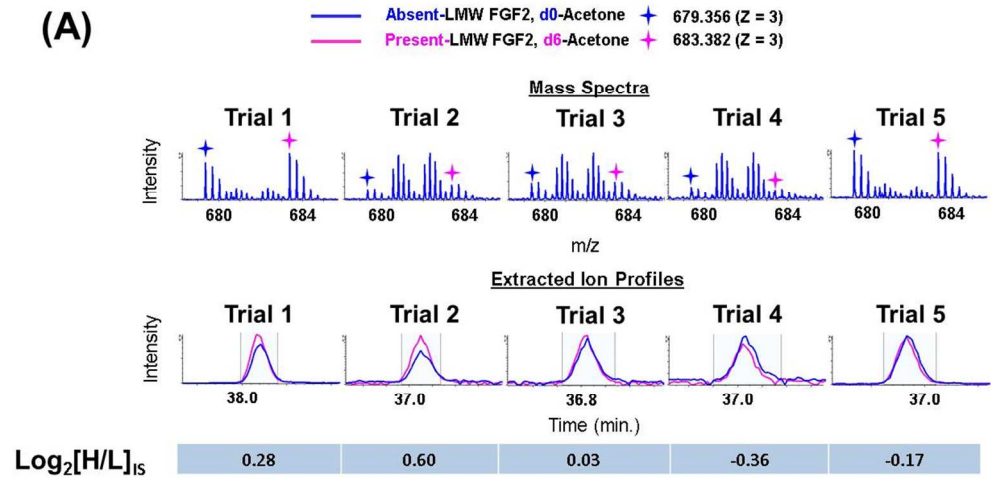
Supplemental Figure 26: Relative quantitative comparison of phosphopeptide—281-(pT)SDSHEDAGTLDFSSLLK-298, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Myosin-binding protein C [Mus musculus]/Thr-281 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—281-(pT)SDSHEDAGTLDFSSLLK-298 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 696.328 and 700.355 represents 281-(pT)SDSHEDAGTLDFSSLLK-298 with modifications RABA@N-term, RABA(K)@18 and charge state 3+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note: **Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio.* Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

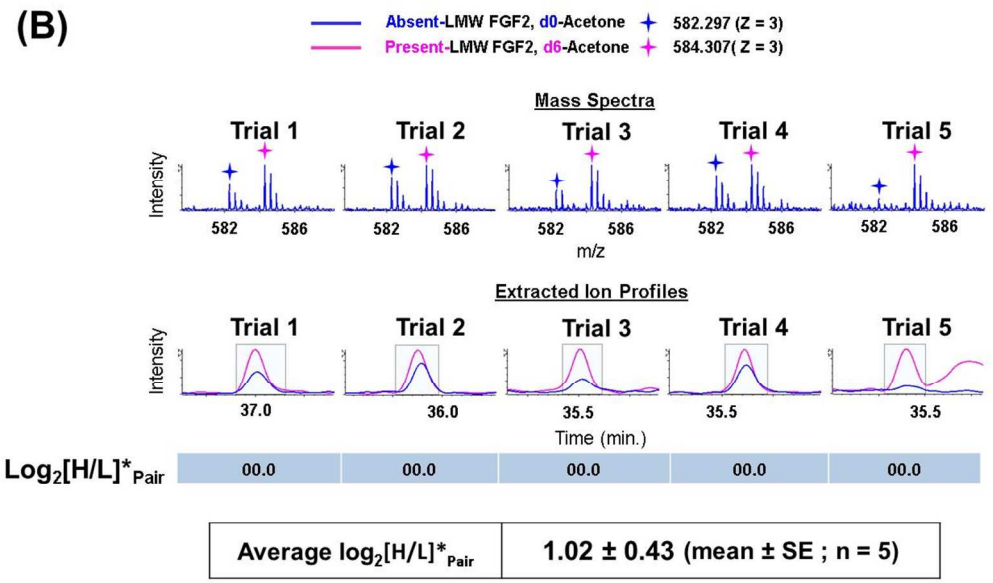
27. Myosin-binding protein C, cardiac-type / Ser-273



Supplemental Figure 27: Relative quantitative comparison of phosphopeptide—271-RT(pS)LAGAGR-279, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Myosin-binding protein C [Mus musculus]/Ser-273 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—271-RT(pS)LAGAGR-279 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 505.761 and 508.779 represents 271-RT(pS)LAGAGR-279 with modifications RABA@N-term and charge state 2+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

28. Cytochrome b-c1 complex subunit 1, mitochondrial precursor / Ser-212

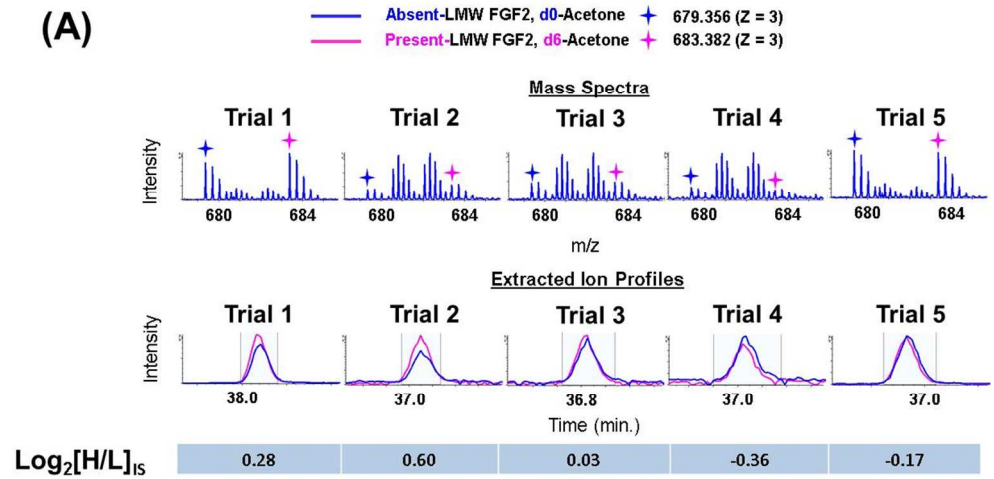


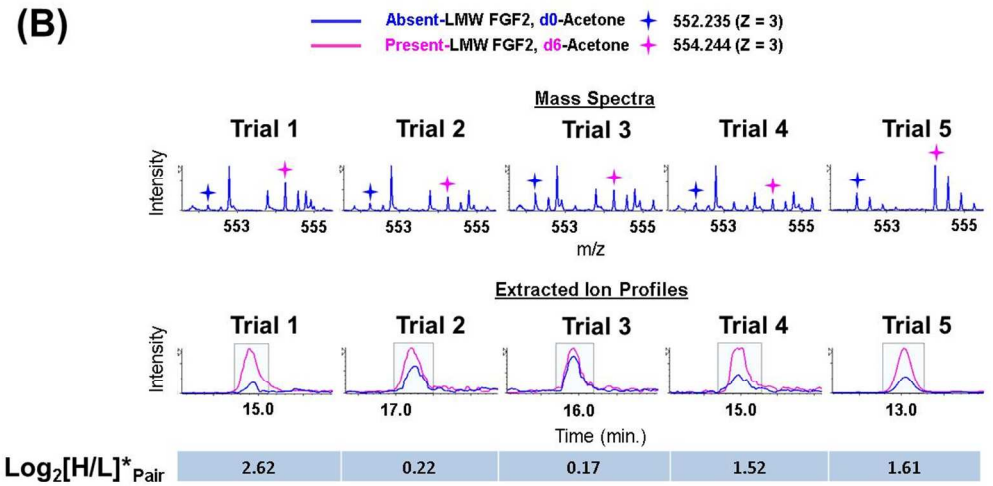


Average $log_2[H/L]^*_{Pair}$	1.02 ± 0.43 (mean ± SE ; n = 5)

Supplemental Figure 28: Relative quantitative comparison of phosphopeptide—210-RL(pS)RTDLTDYLNR-222, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of Cytochrome b-c1 complex subunit 1, mitochondrial precursor [Mus musculus]/Ser-212 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate" numbers. Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—210-RL(pS)RTDLTDYLNR-222 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 582.297 and 584.307 represents 210-RL(pS)RTDLTDYLNR-222 with modifications RABA@N-term and charge state 3+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.

29. 2-Oxoisovalerate dehydrogenase subunit alpha, mitochondrial / Ser-338





Average $log_2[H/L]^*_{Pair}$	1.23 ± 0.46 (mean ± SE ; n = 5)

Supplemental Figure 29: Relative quantitative comparison of phosphopeptide—334-IGHH(pS)TSDDSSAYR-347, in LMW FGF2 "non-expressed" and LMW FGF2 "expressed" mouse hearts subjected to 60 minutes of ischemia and 5 minutes of reperfusion. This phosphopetide represents the protein phosphorylation of 2-Oxoisovalerate dehydrogenase subunit alpha, mitochondrial [Mus musculus]/Ser-338 (A) Relative quantification figure of external standard phosphopeptide pair—m/z 679.356 and 683.385 representing 104-YKVPQLEIVPN(pS)AEER-119 with modifications RABA@N-term, RABA(K)@2 and charge state 3+. Biologically distinct replicates that are denoted with "replicate numbers". Below each replicate number are the respective averaged MS spectra and respective extracted ion chromatograms (XICs) for the phosphopeptide pair. Respective $Log_2[H/L]_{IS}$ values are indicated to denote procedural errors associated to each replicate. (B) Relative quantification figure of phosphopeptide—334-IGHH(pS)TSDDSSAYR-347 with significant phosphorylation changes between the two types of hearts. Mass spectral responses representing the phosphopeptide pair—m/z 552.235 and 554.244 represents 334-IGHH(pS)TSDDSSAYR-347 with modifications RABA@N-term and charge state 3+. Respective $Log_2[H/L]^*_{Pair}$ values indicate differences in phosphorylation associated to each replicate. Note:*Ratio of H/L of a phoosphopeptide pair is normalized to fully labeled casein phosphopeptide H/L ratio. Average $log_2[H/L]^*_{Pair}$ with standard error (SE) is indicated to signify that this protein/phosphosite is a potential target.