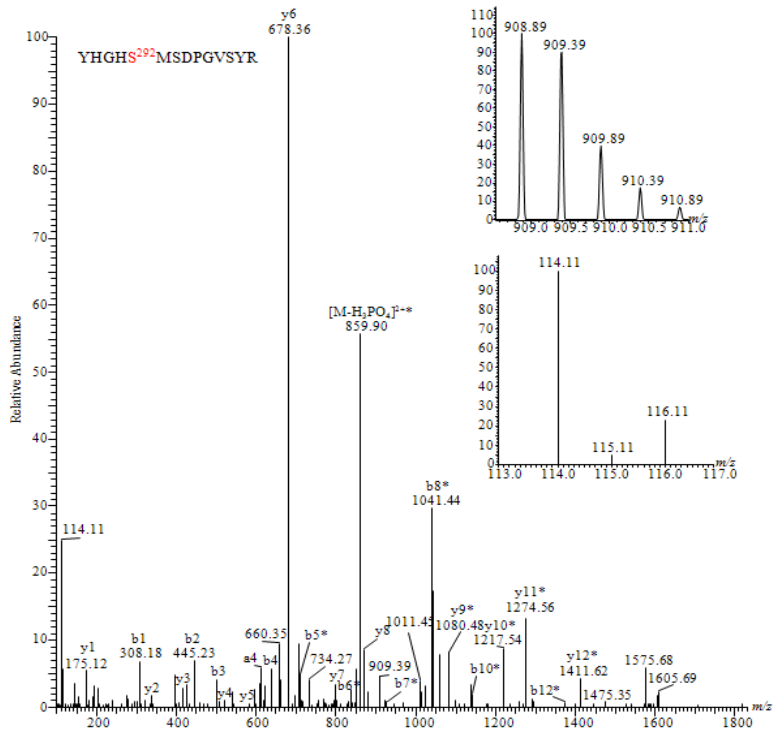


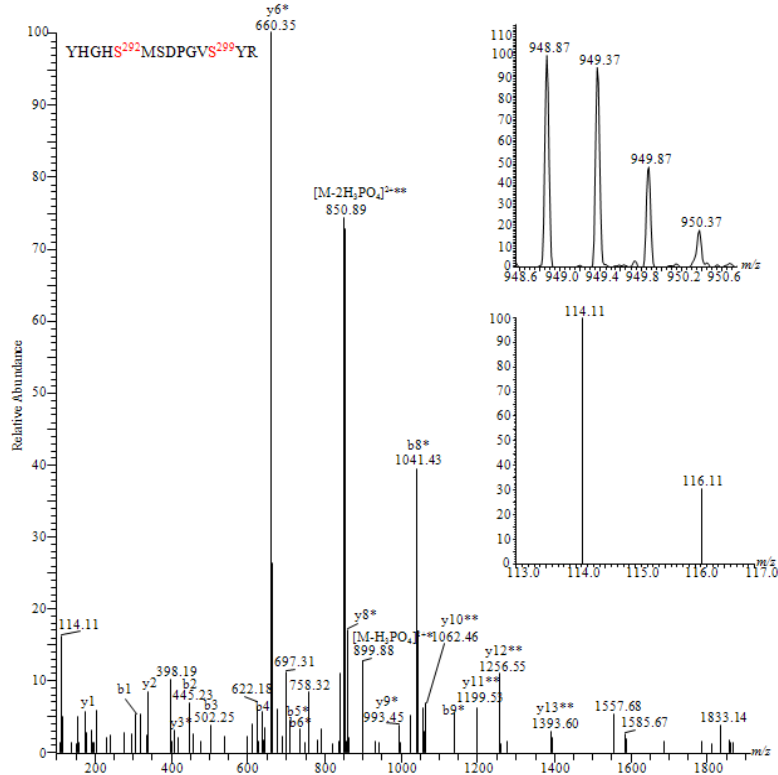
Supplemental Figure 1: Representative HCD-MS/MS spectra of iTRAQ-labeled phosphopeptides identified from pig heart mitochondrial proteins. Insets showed the precursor ion masses of phosphopeptides and iTRAQ ratios representing the change between control mitochondria and mitochondria in response to 0.65 μ M free calcium. (A). YHGHS*MSDPGVSYR (PDH E1 α subunit) showed 3.3-fold dephosphorylation at Ser292; (B). YHGHS*MSDPGVS*YR (PDH E1 α subunit) demonstrated a total of 4.4-fold dephosphorylation at both Ser292 and Ser299; (C). YGMGTS*VER (PDH E1 α subunit) showed 2.7-fold dephosphorylation at Ser231; (D). TGTAEVSS*ILEER from α subunit of ATP synthase F1 complex (Complex V of ETC) showed no change in phosphorylation.

Supplemental Figure 2: (A). HCD-MS/MS spectra of iTRAQ-labeled mitofilin phosphopeptide (KS*IQSGPLK) identified from pig heart mitochondria with significant dephosphorylation by 6.7-fold upon de-energization; (B). HCD-MS/MS spectra of iTRAQ-labeled AIF phosphopeptide (VLPNAIVQS*VGVSGGK) showing an increase in phosphorylation by 1.9-fold with calcium.

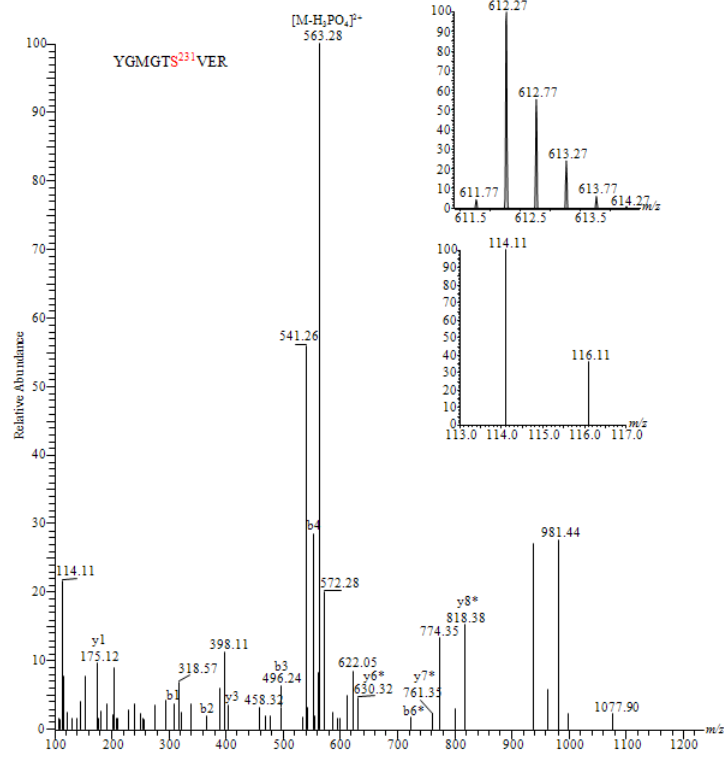
Supplemental Figure 1A



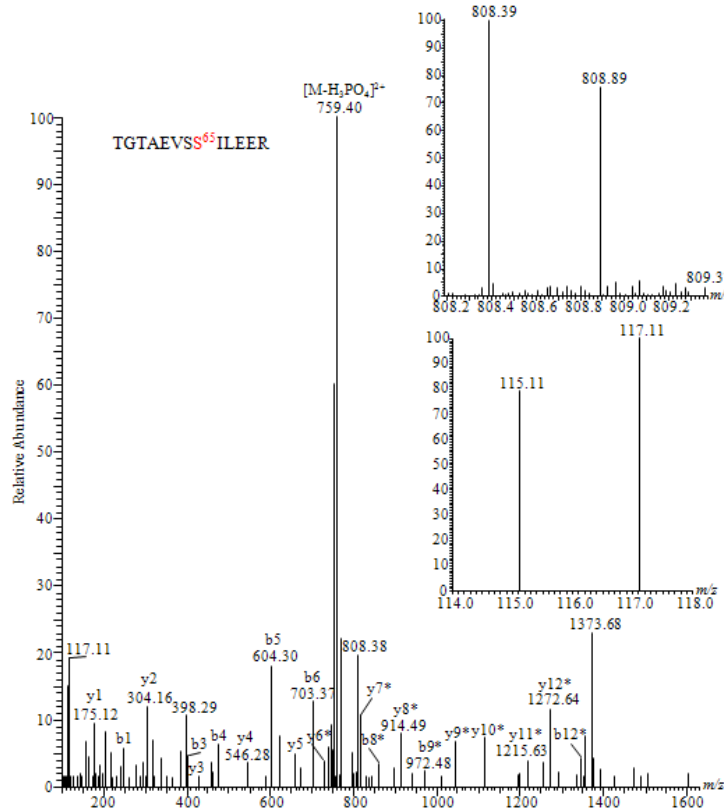
Supplemental Figure 1B



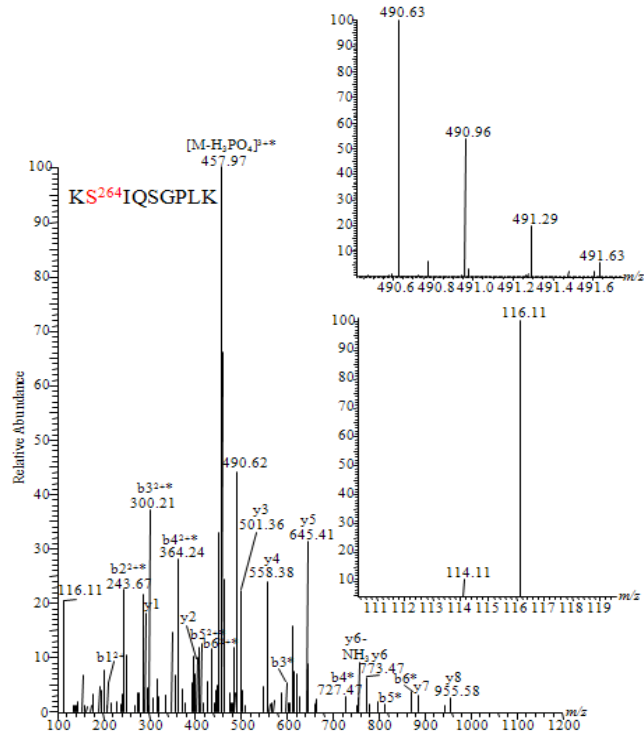
Supplemental Figure 1C



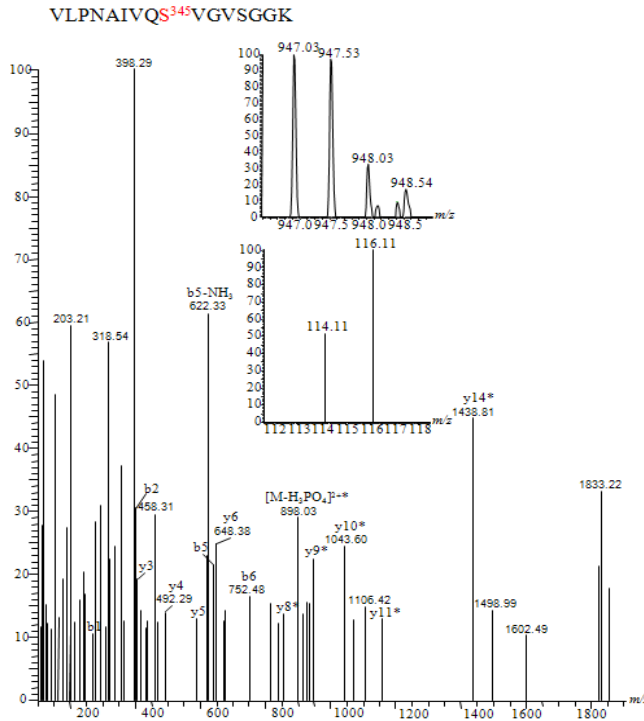
Supplemental Figure 1D



Supplemental Figure 2A



Supplemental Figure 2B



Supplemental Table I. iTRAQ Analysis of Pig Mitochondrial Phosphoproteome Upon DCA Treatment

Protein Description	Accession#	Phosphopeptides	<i>m/z/z</i>	^a iTRAQ Ratio (CTL/DCA)	^d Ion Score/ Expect; <i>p</i> -value
ADP/ADT translocator protein	gi 339920	DFLAGGVAAAISK	529.95/3	^c ND	31/0.072
		YFPTQALNFAFK	907.96/2	0.89±0.13	71/0.00011
		YFPTQALNFAFK	907.96/2	1.35	64/0.00051
		AAAYFGVYDTAK	787.38/2	0.94±0.01	68/0.00035
		^b Ac-M*TDAAVSFPKDFLAGGVAAAISK	898.46/3	^c ND	36/0.61
Mitochondrial creatine kinase	gi 338237	LGYILTC*PSNLGTGLR	980.00/2	1.02±0.13	59/0.0018
		FSKILENLR	744.41/2	0.99	24/0.61
		RGTGGVDTAAVADVVDISNIDR	830.40/3	0.98	68/0.00038
VDAC1	gi 47522750	LTFDSSFSPNTGKK	1021.02/2	0.99±0.08	41/0.013
		KLETAVNLAWTAGNSNTR	772.06/3	0.94	1e-5
		VNNSSLIGLGYTQTLKPGIK	824.45/3	0.95	1e-5
		VTGSLETK	601.82/2	1.02	35/0.021
VDAC3	gi 6755967	^b Ac-C*NTPTYC*DLGK	797.82/2	0.98	65.00014
Malate dehydrogenase	gi 89574151	IQEAGTEVVK	721.38/2	0.99	48/0.032
		AGAGSATLSMAYAGAR	839.89/2	0.91	33/0.036
Apoptosis inducing factor 1	gi 194228272	VLPDAIVQSVGVSGGK	947.53/2	0.95	62/0.00085
ATP synthase, OSCP	gi 57108786	GEVPC*TVTTASPLDEATLTELK	900.78/3	0.77	47/0.031
PR domain containing 16	gi 41349472	SPPPTLSDPILR	506.29/3	1.02	0.04878

Paired basic amino acid cleaving system 4	gi 4505577	SYC*IVGIAAYNAK	863.95/2	0.98	1e-5
Predicted: hypothetical protein	gi 194037207	AISAPTSPTR	612.81/2	°ND	1e-5
Predicted: 2,4-dienoyl-CoA reductase	gi 194037076	STEAFPSK	617.80/2	°ND	0.01408
Succinate-CoA ligase, ADP-forming, β subunit	gi 11321583	INFDSNSAYR	705.81/2	°ND	1e-5
ATPase type 13A4	gi 66932949	IGSLEDWLSAK	765.44/2	0.98	0.032258
SRA stem-loop-interacting RNA-binding protein	gi 148233424	ALQGDQTSDEEKDF ALQGDQTSDEEKDF	975.93/2 1015.91/2	0.95 1.09	1e-5 1e-5
Similar to ubiquinol-cytochrome c reductase binding protein	gi 194037026	^b Ac-ASRPAVAASSK	655.83/2	1.02	45/0.22

Supplemental Table II. iTRAQ Analysis of Pig Mitochondrial Phosphoproteome Upon Calcium Activation

Protein Description	Accession#	Phosphopeptides	<i>m/z/z</i>	^a iTRAQ Ratio (CTL/Ca ²⁺)	^d Ion Score/ Expect; <i>p</i> -value; XCorr
ADP/ADT translocator protein	gi 339920	AA ^b YFGV ^b YDTAK	787.38/2	1.12±0.16	77/4.3e-5
		^b Ac-M*TDAAV ^b SFPKDFLAGGVAAAISK	898.46/3	^c ND	41/0.019
		DFLAGGVAAAISK	794.42/2	^c ND	44/0.08
		YFPTQALNFAFK	907.96/2	0.93±0.16	51/0.0094
Mitochondrial creatine kinase	gi 338237	LGYILTC*PSNLGTGLR	980.00/2	0.92±0.08	66/0.00036
		GTGGVDTA ^b AVADV ^b DISNIDR	1167.55/2	^c ND	1e-5
		FSKILENLR	496.62/3	^c ND	20/0.34
Branched chain alpha ketoacid dehydrogenase kinase	gi 2583173	STSATDTHHVEMAR	883.88/2	1.03	88/1e-6
Malate dehydrogenase	gi 89574151	AGAGSATLSMAYAGAR	839.89/2	0.98	82/4.7e-6
		DVF ^b TK	489.26/2	0.68	20/0/069
VDAC1	gi 47522750	LTFDSSFS ^b PNTGK	884.92/2	1.04±0.29	40/0.19
		DVF ^b TKGYGFGLIK	979.02/2	1.28	2.65
		VNNSSLIGVGYTQTLRPGVK	824.45/3	1.02	25/0.013
Succinyl-CoA synthetase, β subunit	gi 3766197	INFDSNSAYR	705.81/2	1.19	44/0.018
Hydroxysteroid (17-beta) dehydrogenase 10	gi 4758504	LVGQGASAVLLDLPNSGGEAQAK	855.47/3	^c ND	1e-5
Similar to nebulin	gi 194043732	GYDLRPDAISK	573.00/3	1.05	0.047

Predicted: 2,4-dienoyl-CoA reductase	gi 194037076	STEAFPSK	617.80/2	°ND	1e-5
Phospholamban	gi 47523502	RASTIEMPQAR	806.40/2	0.83	0.049363
ATP synthase, F1 α subunit	gi 187370717	VLSIGDGIAR	612.83/2	0.67	21/0.43
Cytochrome Bc1 complex, chain B	gi 3891849	GGLGLSGAK	564.31/2	0.67	24/0.41
Aspartate aminotransferase	gi 223046	IASTILTSPDLR	755.91/2	0.67	24/0.29
ATP synthase, γ subunit	gi 543875	THSDQFLVTFK	564.29/3	1.16	29/0.13
Prohibitin	gi 4505773	KLEAAEDIA YQLSR	659.01/3	°ND	20/0.11
Cytochrome c oxidase, subunit Vb	gi 55926217	LVSHQLAH	564.79/2	0.83	30/0.41
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13	gi 119605232	TLQILRENLEEEAIIMK	804.44/3	0.97	27/0.29

Ubiquinol-cytochrome c reductase complex 11 kDa protein	gi 73977070	LFNSLK	545.31/2	0.86	21/0.91
2-oxoglutarate/malate carrier protein (OGCP)	gi 57086345	LGIYTVLFR	717.89/2	0.71	24/2.7

Supplemental Table III. iTRAQ Analysis of Pig Phosphoproteome of De-energized Versus Energized Mitochondria

Protein Description	Accession#	Phosphopeptides	<i>m/z/z</i>	³ iTRAQ Ratio (D/E)	⁴ Ion Score/Expect
ADP/ADT translocator protein	gi 339920	AAYFGVYDTAK	787.38/2	1.20	46/0.021
		AA ^Y FGVYDTAK	787.38/2	1.10	44/0.039
		YFPTQALNFAFK	907.96/2	1.01±0.05	53/0.0054
Mitochondrial creatine kinase	gi 338237	LGYILTC*PSNLGTGLR	979.99/2	1.40±0.13	53/0.0056
		FSKILENLR	744.41/2	1.04±0.01	25/0.27
		GTGGVDTAAVADV ^Y DISNIDR	799.75/3	1.01	23/0.61
Branched chain alpha ketoacid dehydrogenase kinase	gi 2583173	STSATDTHHVEMAR	589.59/3	1.07±0.05	41/0.05
Malate dehydrogenase	gi 89574151	AGAGSATLSMAYAGAR	839.89/2	1.01±0.04	72/4.7e-05
VDAC1	gi 47522750	LTFDSSFSPNTGK	681.02/3	1.24±0.04	35/0.28
		VNNSSLIGVGYTQTLRPGVK	824.46/3	1.13	50/0.0053
Succinyl-CoA synthetase, β subunit	gi 3766197	INFDSNSAYR	705.81/2	0.89±0.26	31/0.31
Apoptosis inducing factor	gi 4406579	SLSAIDR	493.24/2	0.75	25/0.11
2-oxoglutarate carrier	gi 11693170	FLFGGLAGM*GATV ^F VQPLDLVC*NR	565.29/5	0.99	12/0.72

Cytochrome c oxidase subunit Vb	gi 55926217	LVSHQLAH	376.87/3	1.33	17/0.15
Heat shock protein 27kDa	gi 50916342	QLSSGVSEIQQTADR	614.97/3	°ND	43/0.054
Electron transfer flavoprotein	gi 26344475	HHPSIQPTLEGWK	949.49/2	1.01	15/0.31
Cardiac alpha tropomyosin	gi 148222268	AISEELDHALNDM*TSI	999.96/2	1.08	50/0.0098
Catenin, alpha 3	gi 223942084	TPEELEDVSDLEEDHEVR	789.02/3	°ND	75/3.3e-05
B-cell receptor associated protein 37	gi 1666876	ISSPTGSKDLQM*VNISLR	1205.61/2	0.85	14/0.68
Progesterone receptor membrane component 1	gi 5729875	EGEPTVYSDEEHPKDESAR	888.73/3	°ND	61/0.00085