

Supplementary Tables and Figures

Table S1. Classification of PKA-C α binding partners in *PKAC α -YFP* and *Fyn* WT (light) or *PKAC α -YFP* and *Empty Vector* (heavy) SILAC experiment

Table S2. Classification of PKA-C α binding partners in *PKAC α -YFP* and *Fyn* WT (light) or *PKAC α -YFP* and *Fyn* KD (heavy) SILAC experiment

Table S3. Phosphopeptides identified in complex with PKA-C α

Figure S1. Fyn expression in *PKAC α -YFP* and *Fyn* WT (light) or *PKAC α -YFP* and *Fyn* KD (heavy) SILAC experiment.

Figure S2. Fragmentation spectra of phosphorylation sites on proteins in complex with PKA-C α .

Table S1. Classification of PKA- α binding partners in *PKA α -YFP + Fyn WT* (light) or *PKA α -YFP + Empty Vector* (heavy) SILAC experiment.

UNIPROT ACCESSION	GENE SYMBOL	PROTEIN NAME	SPECTRAL COUNT	FDR	LOG ₂ (H:L)
NOVEL PKA BINDING PARTNERS					
Q53H12	AGK	Acylglycerol kinase, mitochondrial	5	5.1E-03	-1.005
O95831	AIFM1	Apoptosis-inducing factor 1, mitochondrial	7	9.2E-03	-0.714
Q9NVI7	ATAD3A	ATPase family AAA domain-containing protein 3A	6	9.4E-05	-1.467
Q5T9A4	ATAD3B	ATPase family AAA domain-containing protein 3B	9	9.4E-05	-1.063
P06576	ATP5B	ATP synthase subunit beta, mitochondrial	7	5.4E-03	-0.790
P36542	ATP5C1	ATP synthase subunit gamma, mitochondrial	5	5.8E-01	-0.150
P78371	CCT2	T-complex protein 1 subunit beta	3	8.6E-02	-0.706
P50991	CCT4	T-complex protein 1 subunit delta	3	1.5E-02	-1.164
P48643	CCT5	T-complex protein 1 subunit epsilon	3	3.3E-03	-1.624
P50990	CCT8	T-complex protein 1 subunit theta	8	1.1E-04	-1.141
Q5SW79	CEP170	Centrosomal protein of 170 kDa	12	6.3E-09	-1.613
P31689	DNAJA1	DnaJ homolog subfamily A member 1	3	1.5E-01	-0.566
O75190	DNAJB6	DnaJ homolog subfamily B member 6	4	2.2E-03	-1.355
O60762	DPM1	Dolichol-phosphate mannosyltransferase subunit 1	5	1.2E-02	-0.848
P55010	EIF5	Eukaryotic transcription initiation factor 5	3	8.0E-09	1.243
Q15717	ELAVL1	ELAV-like protein 1	6	2.5E-02	-0.646
P50402	EMD	Emerin	6	2.4E-04	-1.318
P35637	FUS	RNA-binding protein FUS	4	9.0E-03	-1.052
P06241	FYN	Tyrosine-protein kinase Fyn	7	1.4E-09	-3.690
P09488	GSTM1	Glutathione S-transferase Mu 1	3	2.1E-03	-1.781
P28161	GSTM2	Glutathione S-transferase Mu 2	5	6.5E-04	-1.363
Q16891	IMMT	MICOS complex subunit MIC60	12	3.9E-10	-1.889
Q6PKG0	LARP1	La-related protein 1	3	8.0E-03	0.691
Q71RC2	LARP4	La-related protein 4	9	4.4E-09	0.853
P20700	LMNB1	Lamin-B1	3	2.5E-02	-1.032
Q9BQG0	MYBBP1A	Myb-binding protein 1A	3	3.2E-01	-0.370
P35580	MYH10	Myosin-10	7	8.3E-02	-0.430
P35579	MYH9	Myosin-9	9	6.3E-04	-0.894
O95613	PCNT	Pericentrin	4	6.8E-03	-1.117
O43175	PHGDH	D-3-phosphoglycerate dehydrogenase	12	2.1E-03	-0.632
P61247	RPS3A	40S ribosomal protein S3a	7	5.6E-01	-0.135
P62753	RPS6	40S ribosomal protein S6	9	8.5E-01	-0.041
Q3ZCQ8	TIMM50	Mitochondrial import inner membrane translocase subunit TIM50	5	3.0E-02	-0.686
Q13748	TUBA3C	Tubulin alpha-3C/D chain	4	2.8E-01	-0.344
A6NHL2	TUBAL3	Tubulin alpha chain-like 3	7	1.1E-02	-0.686
Q13885	TUBB2A	Tubulin beta-2A chain	8	1.0E-01	-0.370

P08670	VIM	Vimentin	35	5.4E-19	-1.459
KNOWN PKA BINDING PARTNERS					
Q92667	AKAP1	A-kinase anchor protein 1, mitochondrial	6	8.9E-01	-0.034
Q9UKA4	AKAP11	A-kinase anchor protein 11	71	5.1E-05	0.282
Q12802	AKAP13	A-kinase anchor protein 13	4	6.5E-04	0.754
Q9Y2D5	AKAP2	A-kinase anchor protein 2	13	3.6E-01	0.139
P24588	AKAP5	A-kinase anchor protein 5	10	7.5E-06	-1.208
Q99996	AKAP9	A-kinase anchor protein 9	607	2.0E-176	-1.427
Q16543	CDC37	Hsp90 co-chaperone Cdc37	39	1.3E-13	-0.952
Q96SN8	CDK5RAP2	CDK5 regulatory subunit-associated protein 2	52	1.5E-28	-1.579
P07900	HSP90AA1	Heat shock protein HSP 90-alpha	110	6.1E-28	-0.924
P08238	HSP90AB1	Heat shock protein HSP 90-beta	57	1.4E-18	-0.951
P11137	MAP2	Microtubule-associated protein 2	7	1.6E-02	0.461
Q15691	MAPRE1	Microtubule-associated protein RP/EB family member 1	13	1.7E-08	-1.416
Q5VU43	PDE4DIP	Myomegalin	164	6.9E-58	-1.287
P22694	PRKACB	cAMP-dependent protein kinase catalytic subunit beta	31	1.5E-01	-0.161
P10644	PRKAR1A	cAMP-dependent protein kinase type I-alpha regulatory subunit	148	3.7E-11	0.522
P31321	PRKAR1B	cAMP-dependent protein kinase type I-beta regulatory subunit	17	1.7E-08	0.643
P13861	PRKAR2A	cAMP-dependent protein kinase type II-alpha regulatory subunit	282	1.5E-19	-0.467
P31323	PRKAR2B	cAMP-dependent protein kinase type II-beta regulatory subunit	61	9.0E-19	-0.921
KNOWN PKA SUBSTRATES					
Q12802	AKAP13	A-kinase anchor protein 13	4	6.5E-04	0.754
Q99996	AKAP9	A-kinase anchor protein 9	607	2.0E-176	-1.427
O60762	DPM1	Dolichol-phosphate mannosyltransferase subunit 1	5	1.2E-02	-0.848
P50402	EMD	Emerin	6	2.4E-04	-1.318
P06241	FYN	Tyrosine-protein kinase Fyn	7	1.4E-09	-3.690
P07900	HSP90AA1	Heat shock protein HSP 90-alpha	110	6.1E-28	-0.924
P08238	HSP90AB1	Heat shock protein HSP 90-beta	57	1.4E-18	-0.951
Q16891	IMMT	MICOS complex subunit MIC60	12	3.9E-10	-1.889
P11137	MAP2	Microtubule-associated protein 2	7	1.6E-02	0.461
P13861	PRKAR2A	cAMP-dependent protein kinase type II-alpha regulatory subunit	282	1.5E-19	-0.467
P31323	PRKAR2B	cAMP-dependent protein kinase type II-beta regulatory subunit	61	9.0E-19	-0.921
P08670	VIM	Vimentin	35	5.4E-19	-1.459

Table S2. Classification of PKA-C α binding partners in *PKAC α -YFP + Fyn WT* (light) or *PKAC α -YFP + Fyn KD* (heavy) SILAC experiment.

UNIPROT ACCESSION	GENE SYMBOL	PROTEIN NAME	SPECTRAL COUNT	FDR	LOG ₂ (H:L)
NOVEL PKA BINDING PARTNERS					
P23526	AHCY	Adenosylhomocysteinase	4	4.8E-44	0.772
P04075	ALDOA	Fructose-bisphosphate aldolase A	3	4.6E-22	0.579
P04083	ANXA1	Annexin A1	12	2.3E-111	-3.043
P27708	CAD	CAD protein	3	1.6E-10	0.395
Q86VP6	CAND1	Cullin-associated NEDD8-dissociated protein 1	4	2.4E-75	1.576
P40227	CCT6A	T-complex protein 1 subunit zeta	3	6.1E-38	0.772
P55060	CSE1L	Exportin-2	4	3.9E-63	0.945
Q92841	DDX17	Probable ATP-dependent RNA helicase DDX17	4	1.4E-46	0.773
Q14204	DYNC1H1	Cytoplasmic dynein 1 heavy chain 1	10	2.3E-61	0.659
P60842	EIF4A1	Eukaryotic initiation factor 4A-I	6	8.0E-59	0.771
Q92616	GCN1L1	eIF-2- α kinase activator GCN1	9	1.7E-50	0.880
O60812	HNRNPCL1	Heterogeneous nuclear ribonucleoprotein C-like 1	3	5.0E-05	0.254
O00425	IGF2BP3	Insulin-like growth factor 2 mRNA-binding protein 3	3	8.9E-06	0.277
O00410	IPO5	Importin-5	4	1.4E-35	0.665
Q7Z794	KRT77	Keratin, type II cytoskeletal 1b	5	1.2E-55	-1.862
Q659C4	LARP1B	La-related protein 1B	5	8.1E-03	-0.146
Q71RC2	LARP4	La-related protein 4	5	6.7E-24	0.517
Q9BQG0	MYBBP1A	Myb-binding protein 1A	4	2.1E-37	0.693
P11940	PABPC1	Polyadenylate-binding protein 1	33	1.1E-39	0.296
Q13310	PABPC4	Polyadenylate-binding protein 4	9	1.0E-09	0.227
P78527	PRKDC	DNA-dependent protein kinase catalytic subunit	60	3.9E-117	0.583
P62269	RPS18	40S ribosomal protein S18	4	3.9E-21	0.499
O75746	SLC25A12	Calcium-binding mitochondrial carrier protein Aralar1	9	3.6E-116	1.098
Q9UJS0	SLC25A13	Calcium-binding mitochondrial carrier protein Aralar2	7	9.9E-72	0.912
Q12931	TRAP1	Heat shock protein 75 kDa, mitochondrial	3	3.3E-08	0.340
Q13509	TUBB3	Tubulin beta-3 chain	4	1.5E-23	0.529
Q9HAV4	XPO5	Exportin-5	3	8.5E-37	0.759
KNOWN PKA BINDING PARTNERS					
Q92667	AKAP1	A-kinase anchor protein 1, mitochondrial	3	7.1E-05	0.250
Q9UKA4	AKAP11	A-kinase anchor protein 11	38	3.2E-12	0.152
Q12802	AKAP13	A-kinase anchor protein 13	7	2.6E-16	0.342
P24588	AKAP5	A-kinase anchor protein 5	4	6.6E-96	1.209
Q99996	AKAP9	A-kinase anchor protein 9	354	2.1E-14	0.099
Q16543	CDC37	Hsp90 co-chaperone Cdc37	18	1.6E-08	-0.177
Q96SN8	CDK5RAP2	CDK5 regulatory subunit-associated protein 2	41	6.6E-02	0.045
Q5VU43	PDE4DIP	Myomegalin	71	6.6E-06	0.075

P10644	PRKAR1A	cAMP-dependent protein kinase type I-alpha regulatory subunit	64	6.0E-01	-0.009
P31321	PRKAR1B	cAMP-dependent protein kinase type I-beta regulatory subunit	10	3.2E-11	0.377
P13861	PRKAR2A	cAMP-dependent protein kinase type II-alpha regulatory subunit	173	7.9E-68	0.252
P31323	PRKAR2B	cAMP-dependent protein kinase type II-beta regulatory subunit	23	4.7E-16	0.201
O14980	XPO1	Exportin-1	13	4.1E-63	0.615
KNOWN PKA SUBSTRATES					
Q12802	AKAP13	A-kinase anchor protein 13	7	2.6E-16	0.342
Q99996	AKAP9	A-kinase anchor protein 9	354	2.1E-14	0.099
P04075	ALDOA	Fructose-bisphosphate aldolase A	3	4.6E-22	0.579
P04083	ANXA1	Annexin A1	12	2.3E-111	-3.043
P27708	CAD	CAD protein	3	1.6E-10	0.395
P13861	PRKAR2A	cAMP-dependent protein kinase type II-alpha regulatory subunit	173	7.9E-68	0.252
P31323	PRKAR2B	cAMP-dependent protein kinase type II-beta regulatory subunit	23	4.7E-16	0.201

Table S3. Phosphopeptides identified in complex with PKA-C α . Experimental conditions each phosphopeptide was identified in is indicated, along with the PKA-C α -normalized H:L ratio for that peptide. Amino acid modifications (phosphorylation = *, heavy isotopes of carbon and nitrogen = #) are indicated after the modified amino acid in the given peptide. Annotated spectra are included in Figure S2. H:L ratios of all phosphopeptides identified were statistically lower ($p < 0.05$) from that of the average H:L for each individual protein. Spectral counts (*) refer to the sum of spectra assigned to peptides housing the indicated phosphorylation site, including heavy and light peptides as well as alternative cleavages.

Light SILAC Condition	Heavy SILAC Condition	Gene Name	Log ₂ (H:L)	Peptide	Site	Spectrum (Figure S2)	Spectral Count (pY)*
YFP-PKACA + Fyn WT	YFP-PKACA + EV	AKAP11	-0.431	SVS*PTFLNPSDENLK	S1242	A	2
YFP-PKACA + Fyn WT	YFP-PKACA + EV	PRKAR1A	-0.731	TDSREDEIS*PPPPNPVVK	S83	B	2
YFP-PKACA + Fyn WT	YFP-PKACA + Fyn KD	PRKAR1A	-2.470	EDEIS*PPPPNPVVK#	S83	C	4
YFP-PKACA + Fyn WT	YFP-PKACA + Fyn KD	PRKAR1B	-2.211	SNSQSDSHDEEV*PTPPNPVVK	S83	D	1
YFP-PKACA + Fyn WT	YFP-PKACA + Fyn KD	PRKAR2A	-2.861	VADAKGDS*ESEDEDEDLEVPVPSR	S78	E	2
YFP-PKACA + Fyn WT	YFP-PKACA + Fyn KD	PRKAR2A	-3.041	VADAKGDS*ES*EEDEDEDLEVPVPSR	S78, S80	F	1

Figure S1. Fyn expression in *PKACα-YFP + Fyn WT* (light) or *PKACα-YFP + Fyn KD* (heavy) SILAC experiment. Western blots of whole cell lysates from this SILAC experiment demonstrate even levels of PKA-Cα (α-GFP) and Fyn WT/KD (α-Fyn). The α-pY blot confirms Fyn kinase activity in the conditions where Fyn WT is expressed. A strong signal is also observed at 75 kDa in the condition where Fyn WT and PKA-Cα were co-expressed. A strong signal is also observed at 75 kDa in the condition where Fyn WT and PKA-Cα were co-expressed.

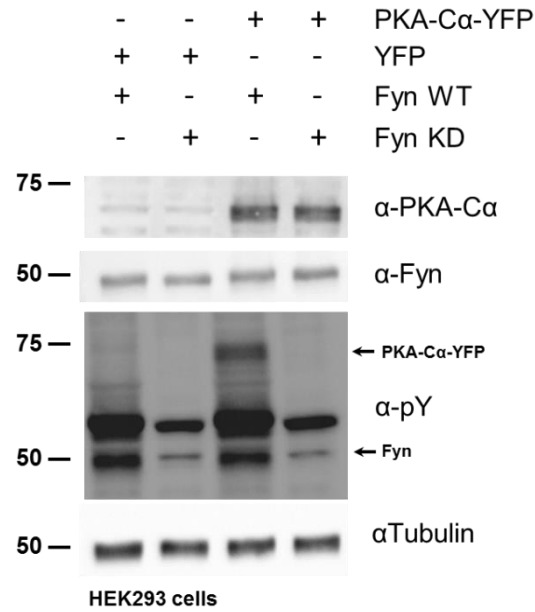
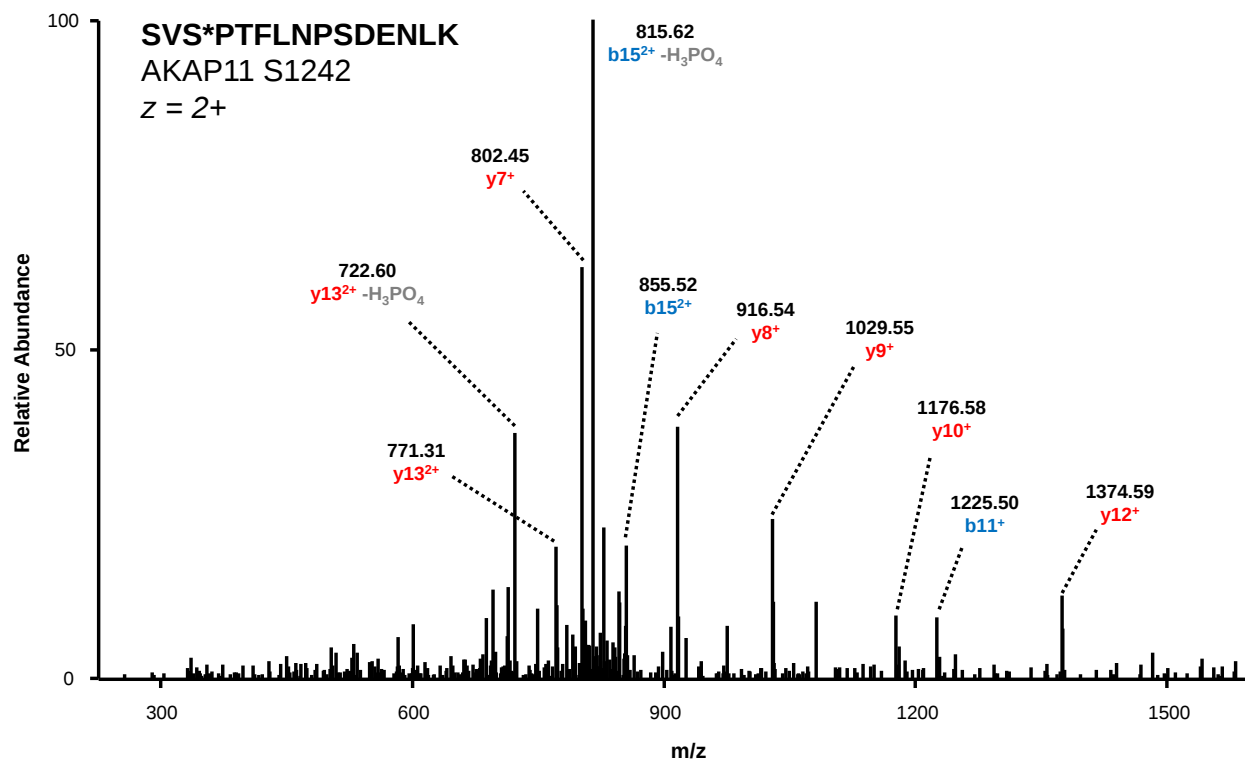
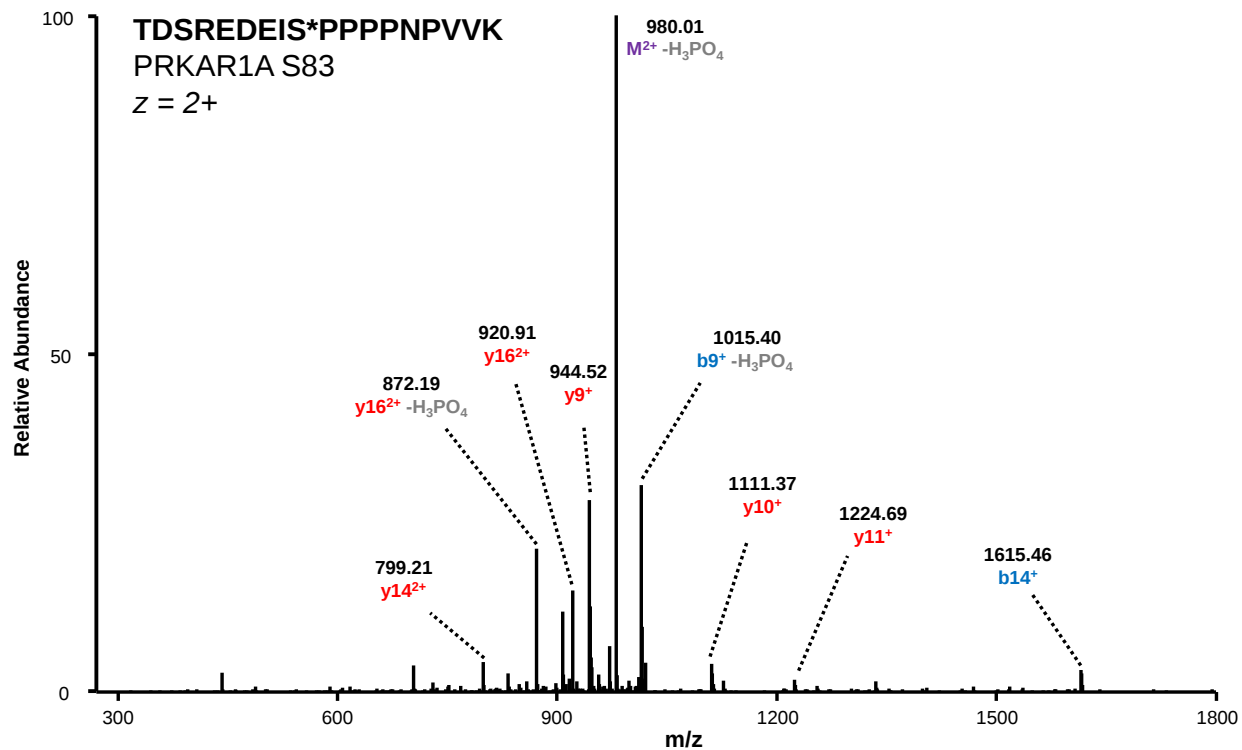


Figure S2. Fragmentation spectra of phosphorylation sites on proteins in complex with PKA-C α . Spectra we acquired in a linear ion trap mass analyzer. Peptide charge state is indicated. Amino acid modifications (phosphorylation = *, heavy isotopes of carbon and nitrogen = #) are indicated after the modified amino acid in the given peptide.

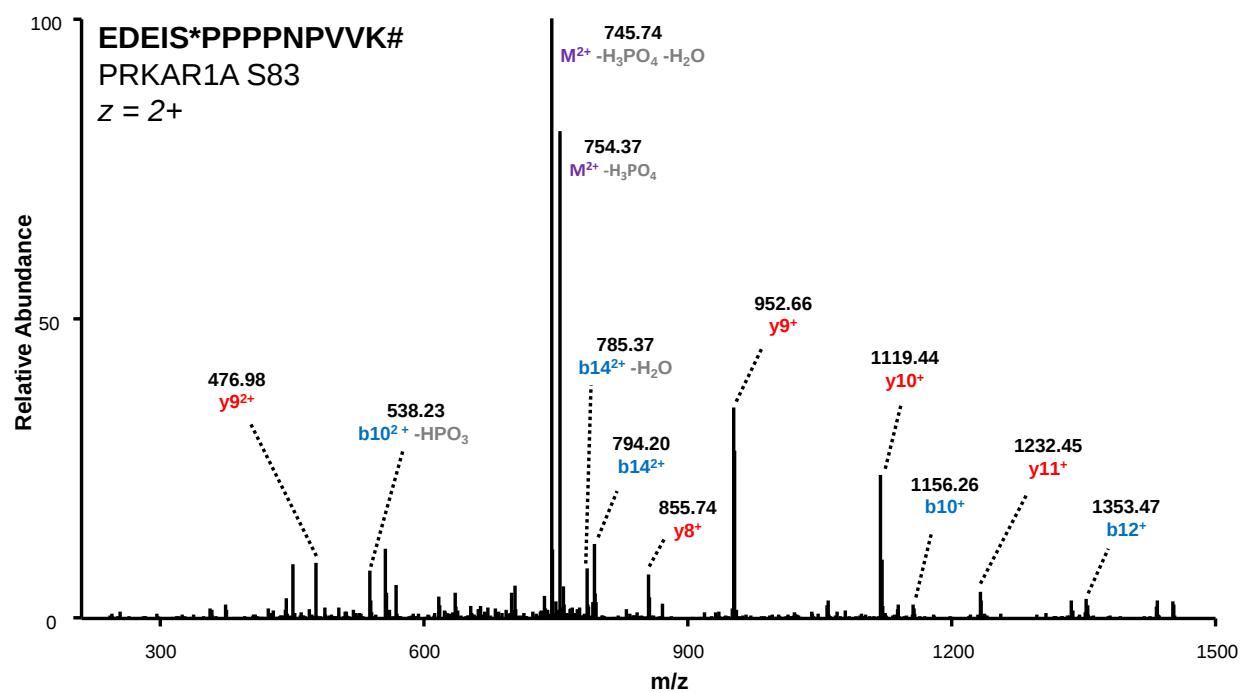
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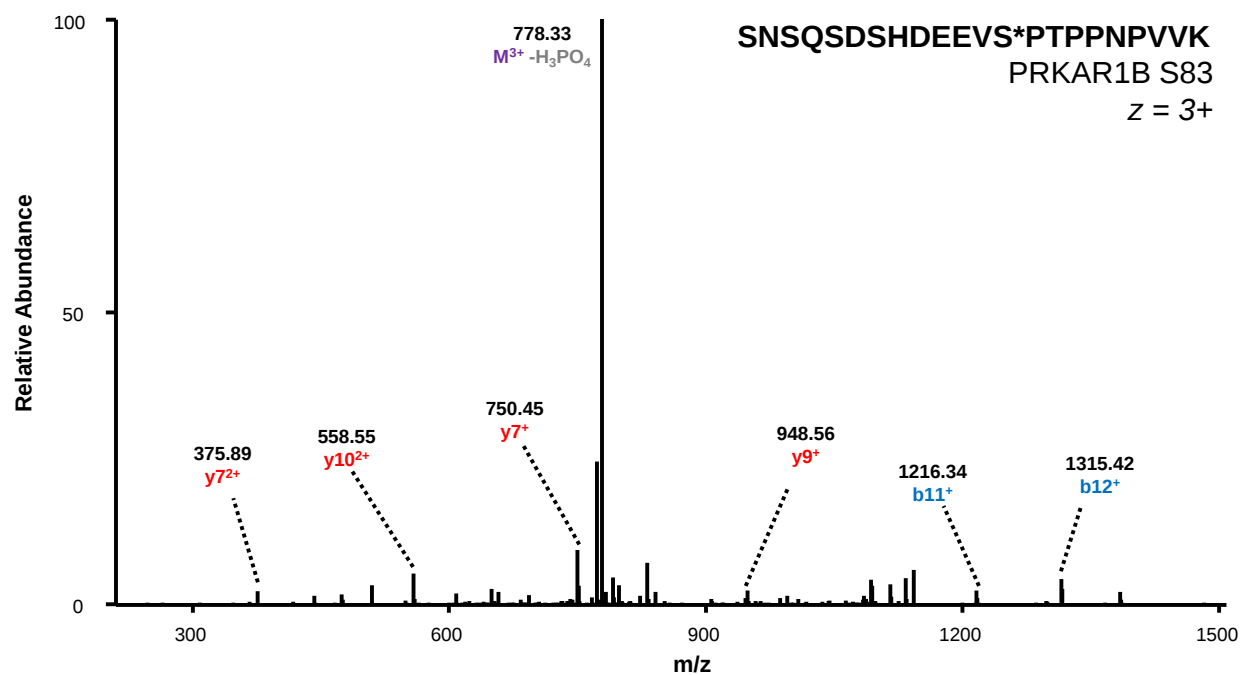
B



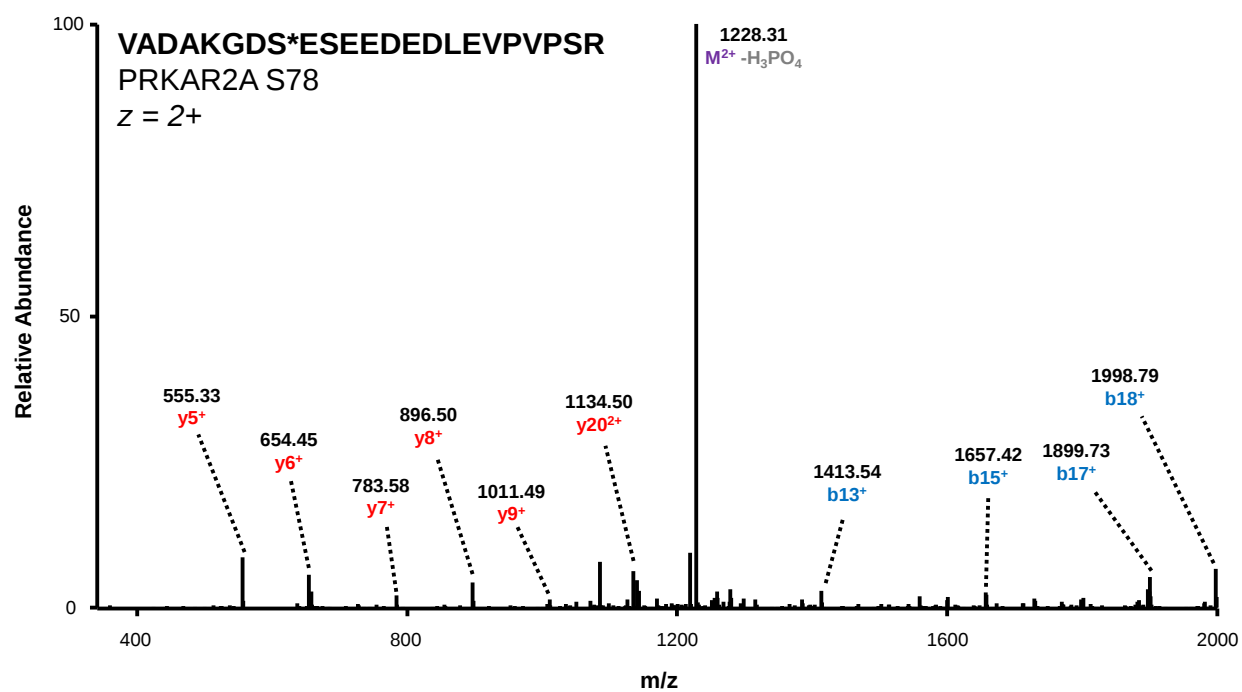
C



D



E



F

