SUPPORTING INFORMATION

The regulatory protein 14-3-3β binds to the IQ motifs of myosin-IC independently of phosphorylation

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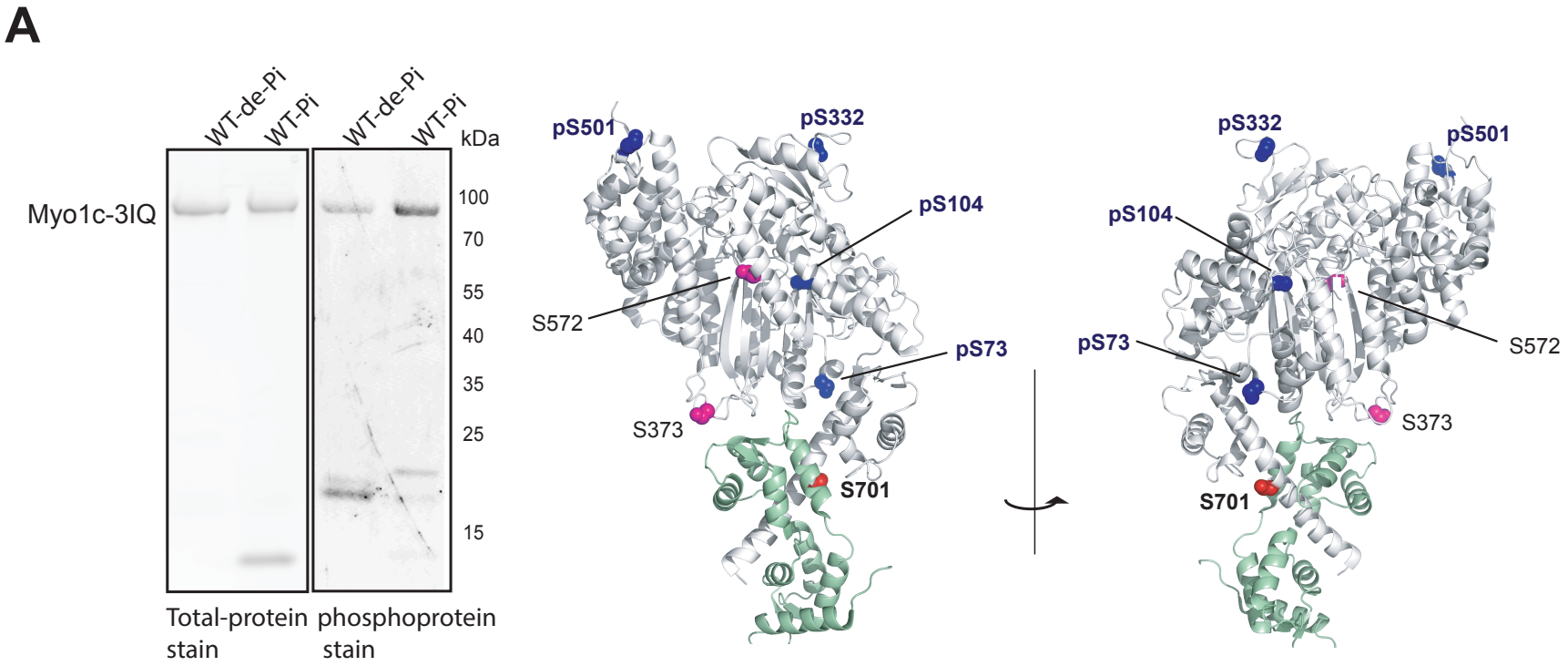
Two Supporting Figures:

Figure S1: Phosphorylation of Myo1c with CaMKII

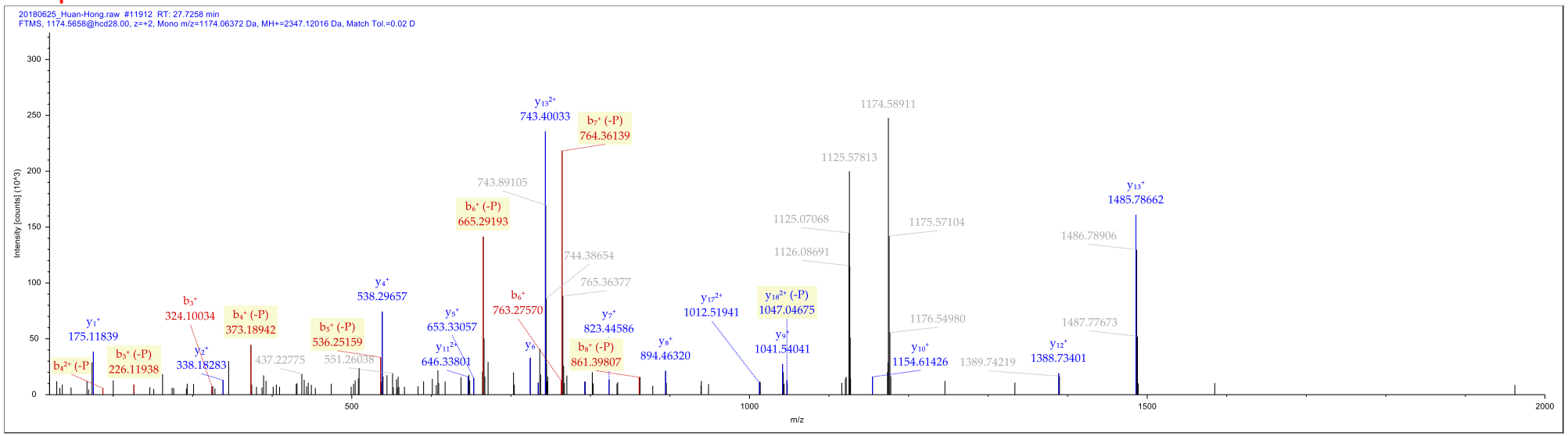
Figure S2: Comparison of sequence and structure of Myo1c IQ motifs and α2 helix of ChREBP

Excel File Containing Mass Spectra Data


A
WT-de-Pi
WT-Pi
WT-de-Pi
WT-Pi
kDa
Myo1c-3IQ
100
70
55
40
35
25
15
Total-protein stain
phosphoprotein stain
pS501
pS332
pS104
S572
pS73
S373
S701
pS332
pS501
pS104
pS73
S572
S373
S701

B

71-GVpSFYEVPPHLFAVADTVYR-90



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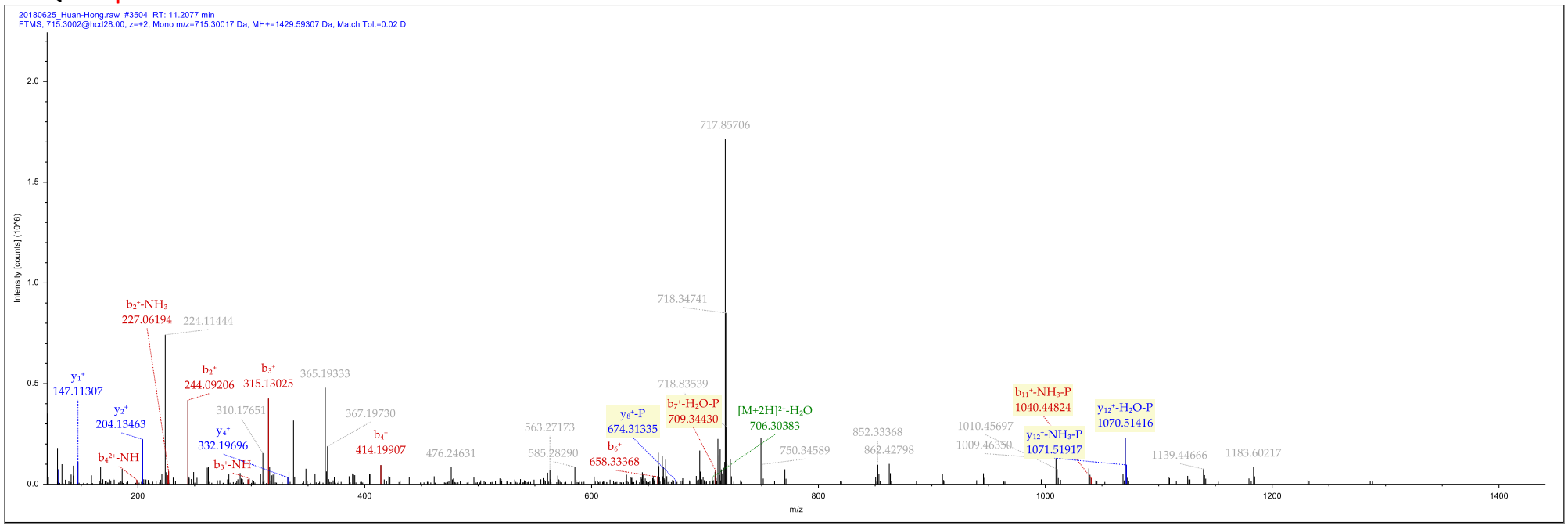

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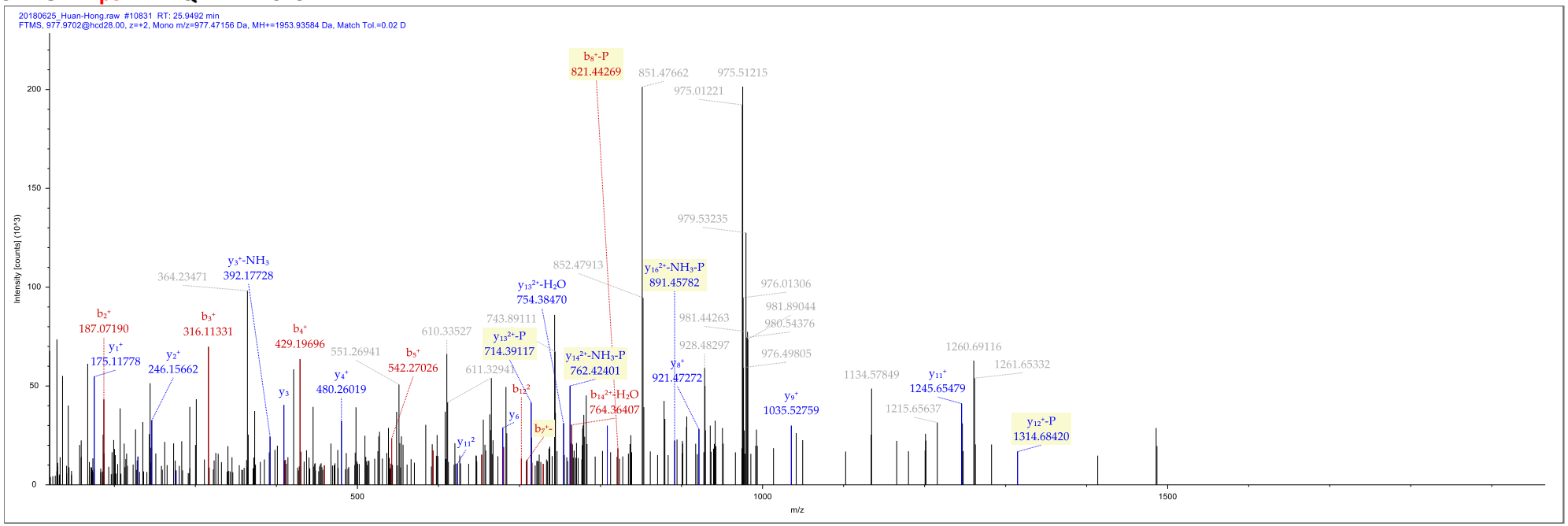

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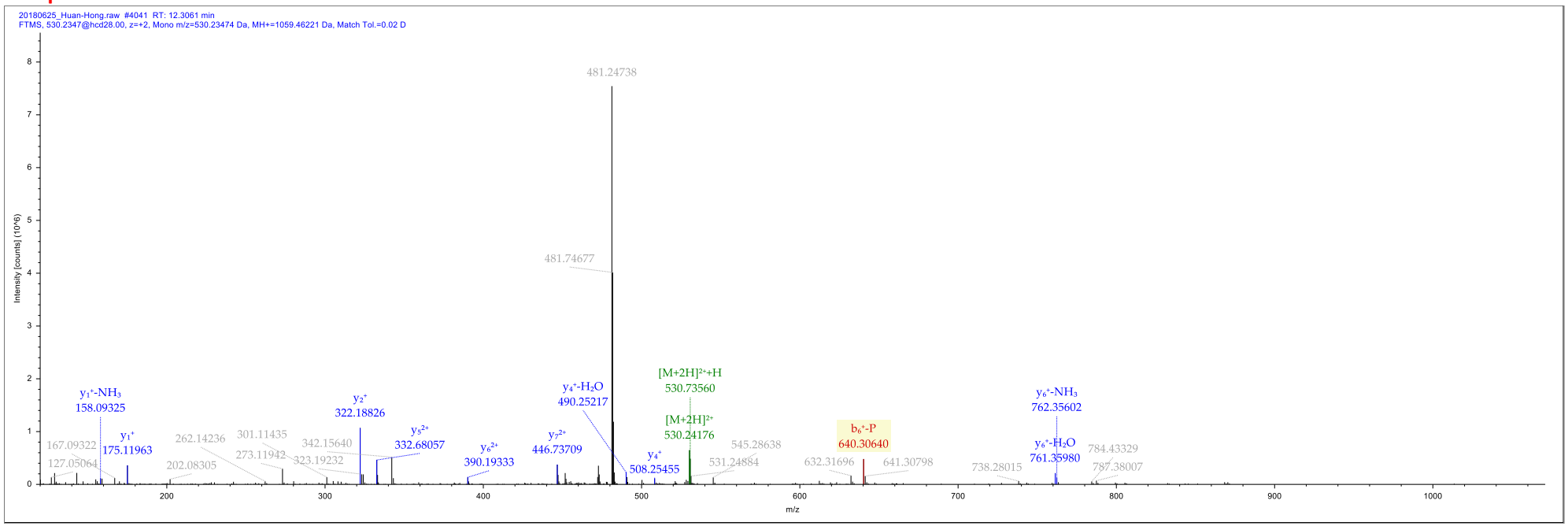

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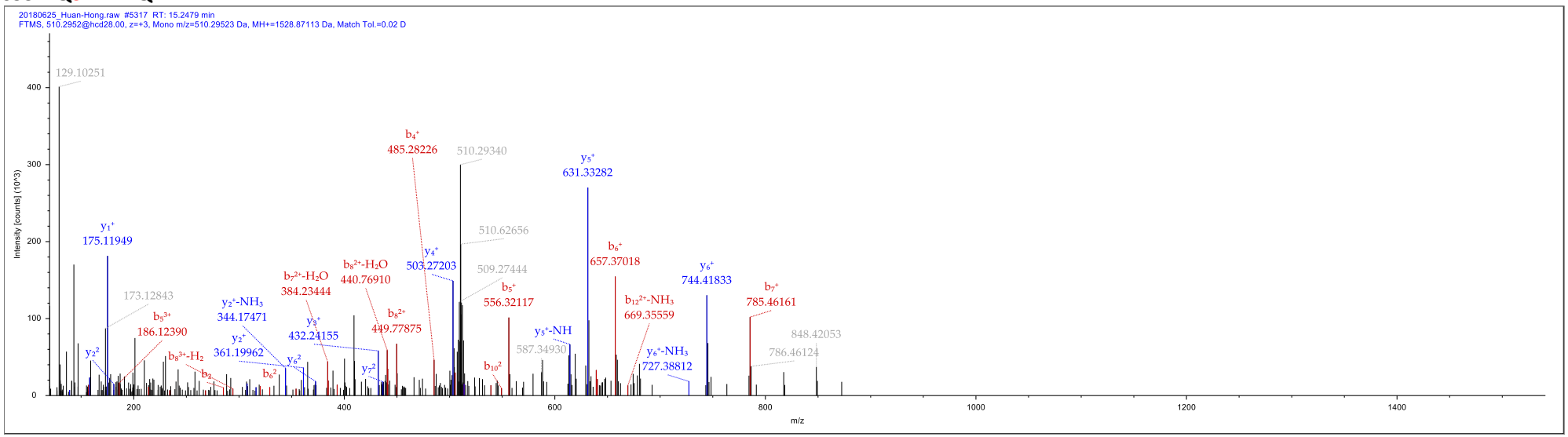

700-QSLATK-705

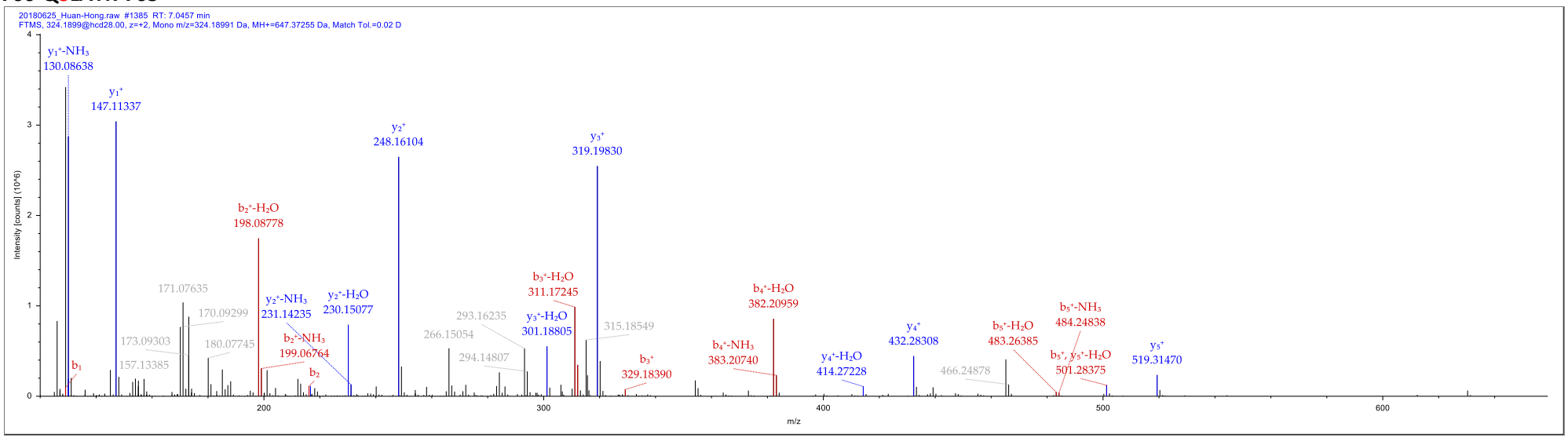

Figure S1: Phosphorylation of Myo1c with CaMKII. (A) Myo1c-3IQ WT treated with phosphatase and CaMKII was detected with SYPRO Ruby Protein Gel Stain (total-protein stain) and Pro-Q Diamond Phosphoprotein Gel Stain (phophoprotein stain), respectively (left panel). Cartoon representation of Mass spectra analysis of Myo1c-3IQ (PDB ID: 4BYF) phosphorylated with CaMKII (right panel). The amino acids (S73, S104, S332 and S501) phosphorylated by CamKII were colored blue. The amino acids (S572 and S373), which have been phosphorylated when expressed in SF9 cells, were colored magenta. S701 was colored red. (B) Detailed information of MS/MS analysis of phosphorylated residues (S73, S104, S332 and S501) and unphosphorylated residue S701 of Myo1c-3IQ treated by CaMKII. All fragment ions were generated with high resolution (35,000) and matched within 10 ppm accuracy.

A

α2 helix of ChREBP	121	R	L	N	N	A	I	W	R	A	W	Y	131
IQ1 (698-720)	RRQSLA	T	K	I	Q	A	A	W	R	G	F	H	WRQKFL
IQ2 (721-743)	RVKRSA	I	C	I	Q	S	W	W	R	G	T	L	GRRKAA
IQ3 (744-766)	KRKWAA	Q	T	I	R	R	L	I	R	G	F	I	LRHSPR

B

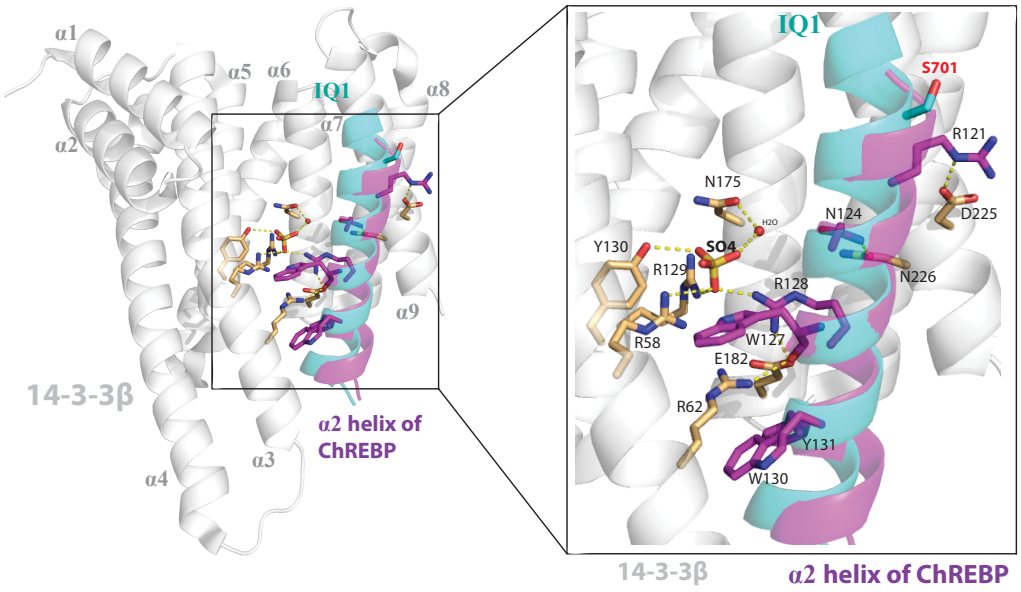

Figure S2: Comparison of sequence and structure of Myo1c IQ motifs and α2 helix of ChREBP. (A) Alignment of amino acid sequence of α2 helix of ChREBP and IQ motifs of Myo1c. The residues of α2 helix of ChREBP in gray are those in contact with 14-3-3β. (B) Overview of 14-3-3β binding α2 helix of ChREBP (PDB ID: 4GNT) and aligned IQ1 motif of Myo1c (aa. 698-720, PDB ID: 4BYF). 14-3-3β, α2 helix of ChREBP and IQ1 motif were labeled in gray, magnet and cyan, respectively. Nine helices were labeled as α1-9 (left). The enlarged box showed detailed view of the interaction of 14-3-3β with α2 helix of ChREBP and aligned IQ1 motif of Myo1c. A free sulfate (SO4) mediates the interaction between R58, R129 and Y130 triad of 14-3-3β and R128 of α2 helix of ChREBP. Hydrogen bond contacts exist between N124 of α2 helix of ChREBP and N226 of 14-3-3β, and between W127 of α2 helix of ChREBP and R58 of 14-3-3β, and between R128 of α2 helix of ChREBP and E182 of 14-3-3β. Van der Waal contacts mainly exist between W127, W130 of α2 helix of ChREBP and R62 of 14-3-3β, and between Y131 of α2 helix of ChREBP and E182 of 14-3-3β. Residue S701 on IQ1 motif of Myo1c was labeled Red.