Supplemental Material

Table S1. E4bp4 interacting partners from yeast-two-hybrid screen

Gene Name	Positive clones	Protein function
C18orf25	1	Unknown
CHD1	1	DNA helicase binding protein
DSP	2	Cytoskeletal protein
FLJ13057	5	Unknown
FLJ544447	1	Unknown
FLNA	1	Cytoskeletal protein
HIPK1	1	Serine/threonine protein kinase
HIPK3	5	Serine/threonine protein kinase
PIAS1	20	SUMO E3 ligase
PIAS3	2	SUMO E3 ligase
RANBP2	1	SUMO E3 ligase
RNF111	1	SUMO-targeted ubiquitin ligase
SETX	2	DNA helicase
SNRP70	2	Splicesomal ribonucleoprotein
SORL1	1	Neuronal apolipoprotein E receptor
TLK2	2	Tousled-like serine/threonine kinase
U5-200KD	1	RNA helicase
ZBTB16	1	Zinc finger transcription factor
ZMYM5	5	Zinc Finger MYM-Type Protein
ZNF198	5	Zinc Finger Transcriptional cofactor
ZNF237	5	Zinc Finger Transcriptional cofactor

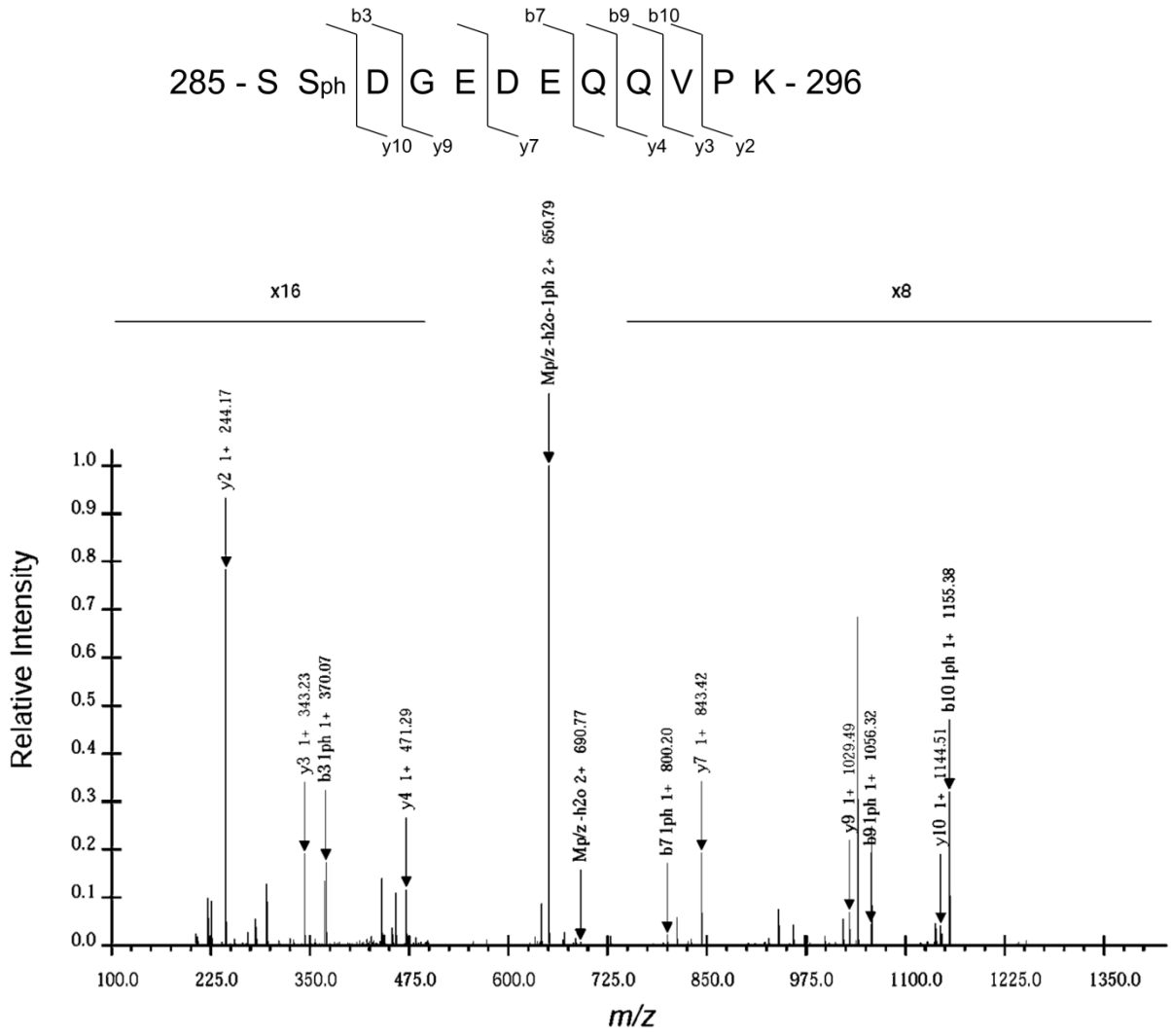

Figure S1. Annotated CID tandem mass spectrum for S286 E4bp4 peptide

Annotated CID tandem mass spectrum of the +2 ion at m/z 699.77, confirming phosphorylation of E4bp4 at S286. Tandem mass spectra acquired with an electrospray ionization LTQ/Orbitrap mass spectrometer from purified FLAG-E4bp4. FLAG-E4bp4 was expressed in HEK-293T cells and purified by immunoprecipitation with anti-FLAG M2 affinity resin and the purified protein competitively eluted using 3X FLAG peptide. Purified E4bp4 was digested with trypsin and subjected to phosphopeptide enrichment using TiO_2 beads to reduce background from unphosphorylated E4bp4 peptides. Magnification of certain regions of the spectra is highlighted. Above spectra is schematic representation of fragmented peptide with identified ions labelled.

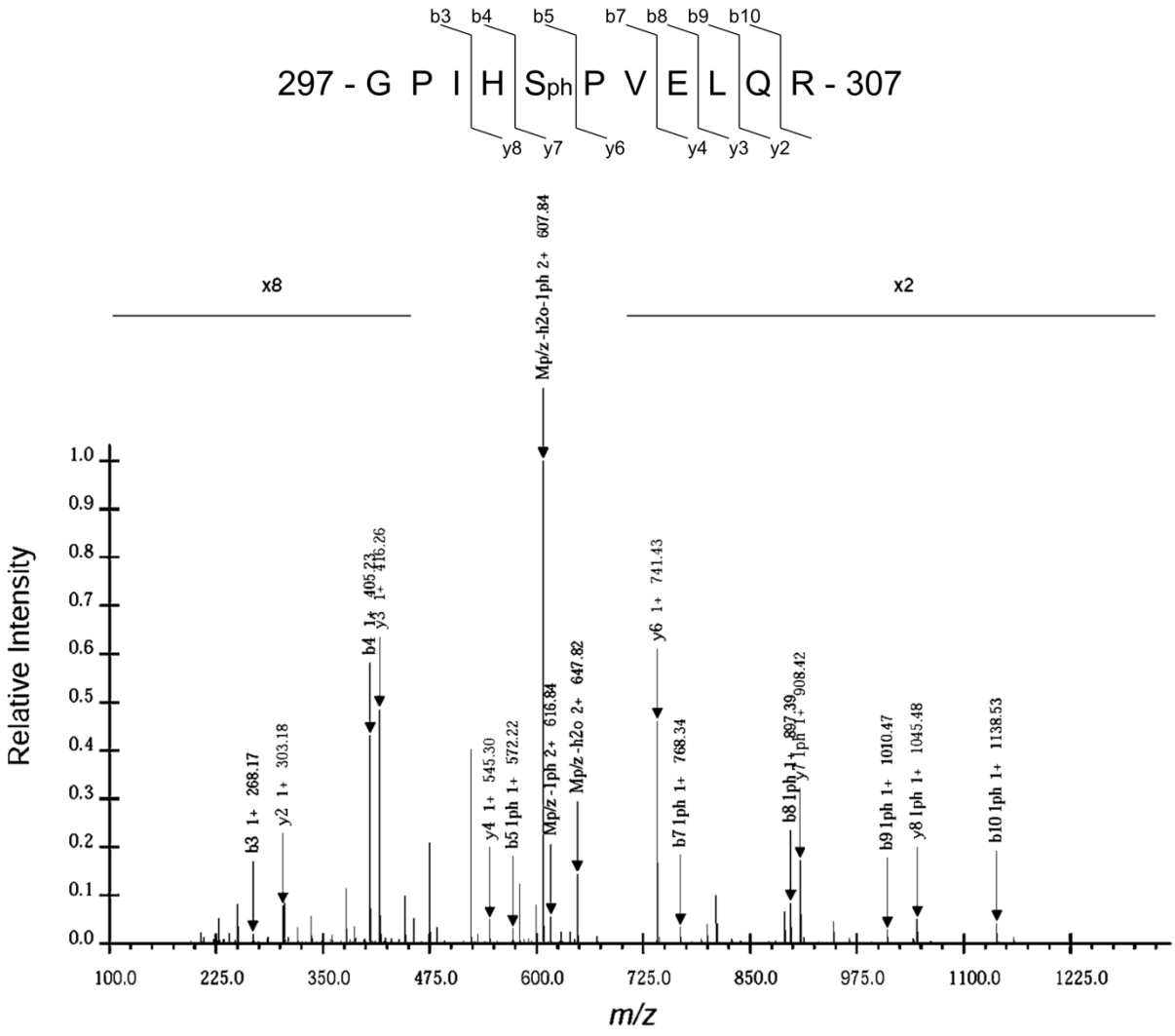

Figure S2. Annotated CID tandem mass spectrum for S301 E4bp4 peptide

Annotated CID tandem mass spectrum of the +2 ion at m/z 656.83, confirming phosphorylation of E4bp4 at S301. Tandem mass spectra acquired with an electrospray ionization LTQ/Orbitrap mass spectrometer from purified FLAG-E4bp4. FLAG-E4bp4 was expressed in HEK-293T cells and purified by immunoprecipitation with anti-FLAG M2 affinity resin and the purified protein competitively eluted using 3X FLAG peptide. Purified E4bp4 was digested with trypsin and subjected to phosphopeptide enrichment using TiO_2 beads to reduce background from unphosphorylated E4bp4 peptides. Magnification of certain regions of the spectra is highlighted. Above spectra is schematic representation of fragmented peptide with identified ions labelled.

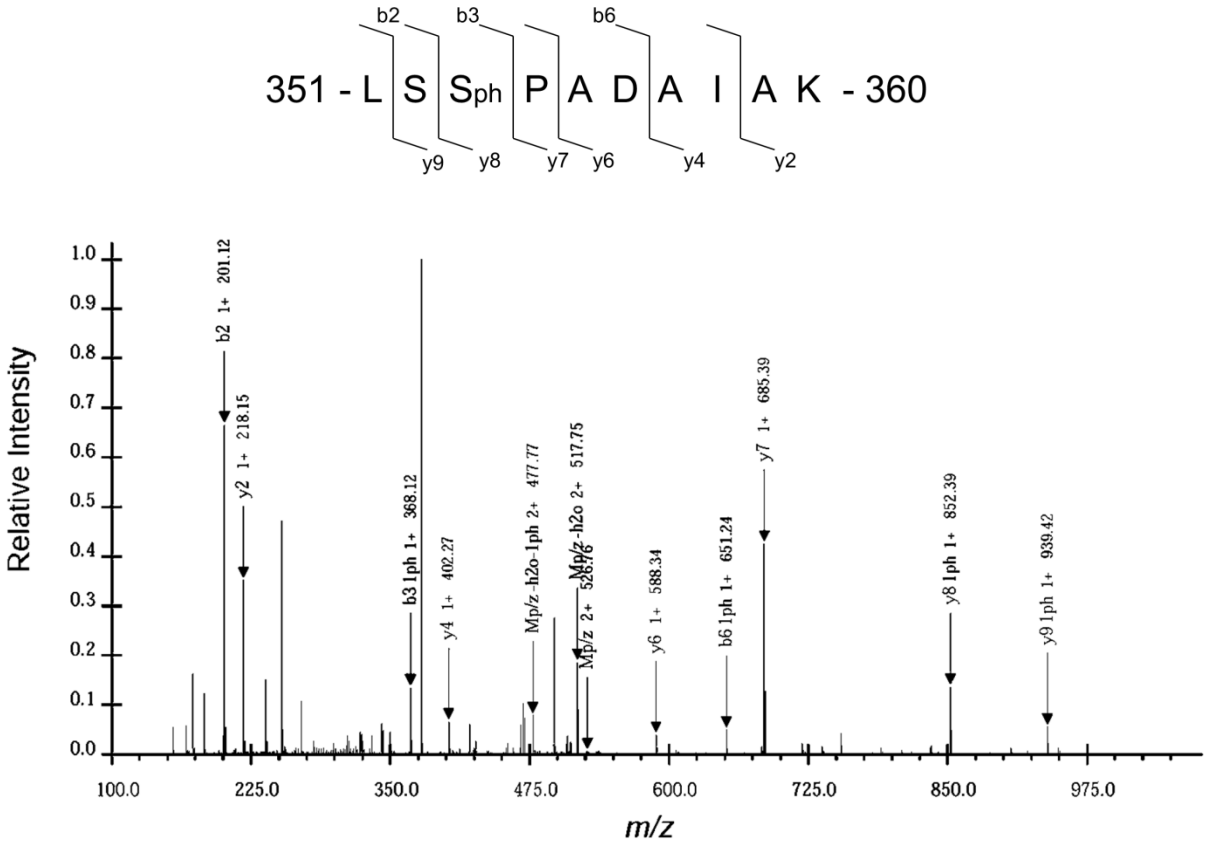

Figure S3. Annotated CID tandem mass spectrum for S353 E4bp4 peptide

Annotated CID tandem mass spectrum of the +2 ion at m/z 526.76, confirming phosphorylation of E4bp4 at S301. Tandem mass spectra acquired with an electrospray ionization LTQ/Orbitrap mass spectrometer from purified FLAG-E4bp4. FLAG-E4bp4 was expressed in HEK-293T cells and purified by immunoprecipitation with anti-FLAG M2 affinity resin and the purified protein competitively eluted using 3X FLAG peptide. Purified E4bp4 was digested with trypsin and subjected to phosphopeptide enrichment using TiO_2 beads to reduce background from unphosphorylated E4bp4 peptides. Magnification of certain regions of the spectra is highlighted. Above spectra is schematic representation of fragmented peptide with identified ions labelled.