Supplementary Material

SupplementaryTable 1

Database search result of 2D-SW spot "a" in HEK293 nuclear extract

Name	ID	MW	pI	Sequence coverage	Entries matched	Score
C/EBP beta	**NP_005185**	**36106**	**8.55**	**9%**	**2**	**40**
hnRNP A1	**P09651**	**38822**	**9.26**	**12%**	**2**	**122**
PA2G2	**Q9UQ80**	**43759**	**6.13**	**17%**	**4**	**134**

Except keratin, there are other two candidate proteins identified along with C/EBP beta, which are heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) and proliferation associated protein 2G4 (PA2G4). hnRNP A1 is a highly abundant protein involved in post-transcriptional gene expression processes including mRNA and rRNA processing, RNA export, and RNA stability. It can interact with ssDNA and RNA. As its MW and pI is so close to C/EBP, so we speculate that it is contaminated in our sample. Another candidate protein is PA2G4 with MW of 43787Da and pI 6.13. It is a RNA binding protein involved in growth regulation and also a transcriptional co-repressor of androgen receptor-regulated genes and other cell cycle regulatory genes through its interactions with histone deacetylases. Until now, it is not shown that PA2G4 is associated with EP24 oligonucleotide binding.

Supplementary Figure Legends

Figure 1

A, The Enhanced-GFP and C/EBP alpha sequences are shown in Bold and Bold underlined, respectively, in the chimeric fusion protein GFP-C/EBP. B, EMSA competition assay. Bacterial crude extract (1 µg) containing GFP-C/EBP was mixed with 1.5 nM radiolabeled C/EBP oligonucleotide (EP24) in the absence (no competitor, NC) or presence of 100 molar excess of unlabeled C/EBP (CEBP) or irrelevant AP1 oligonucleotide (AP1). The positions of the specific C/EBP complex (C) and the unbound DNA (U) are shown to the right. C, The purified GFP-C/EBP (pure) and the bacterial crude extract (crude), were separated by 12% SDS-PAGE gel and stained with coomassie brilliant blue. D, Comparison of Southwestern blot and Western blot analysis of purified GFP-C/EBP. A serial dilution of purified GFP-C/EBP was simultaneously analyzed by western blot (WB) using C/EBP antibody and Southwestern blotting (SW) analysis using radiolabeled C/EBP oligonucleotide (EP24).

Figure 2

MS/MS spectra of precursor peptides shown in Figure 2A. The precursor ions including 444.2 (2+), 606.6 (2+), 999.6 (1+) and 1566.6 (1+) were analyzed by HPLC-ESI-MS/MS. A series of b- and y-product ions are indicated in the spectra. The amino acid sequence of the precursor peptide is shown on the top of each spectrum.

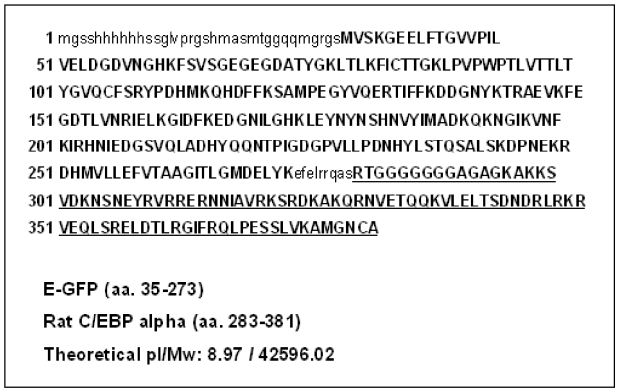
Figure 3

(A) MS/MS spectra of a typical peptide VLELTSDNDR (m/z 581.10, 2+) from 2D-SW blot of purified GFP-C/EBP. (B, C) two peptides, TIFFK (m/z 655.22, 1+) and FSVSGEGEGDATYGK (752.16, 2+) from 2D-SW blot of bacterial crude extract GFP-

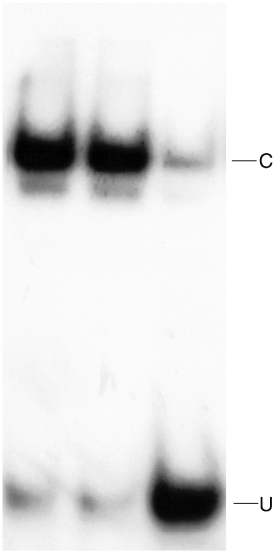
C/EBP. (D) MS/MS spectra of triply charged peptide APPTACYAGAAPAPSQVKSK at m/z 639.18 (2+) from 2D-SW blot of HEK293 nuclear extract. A series of b- and y-fragment ions are indicated in the spectra. The amino acid sequence of the precursor peptide is shown on the top of each spectrum.

Supplementary Figure 1

A



B



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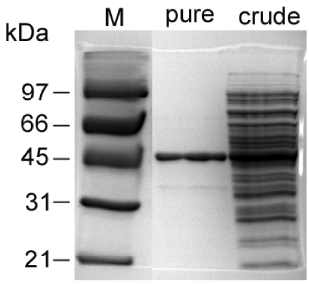

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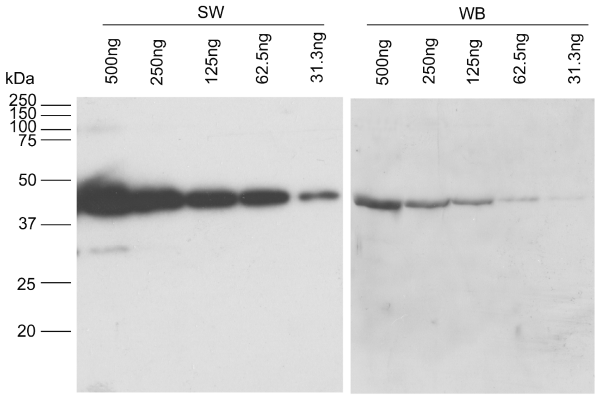
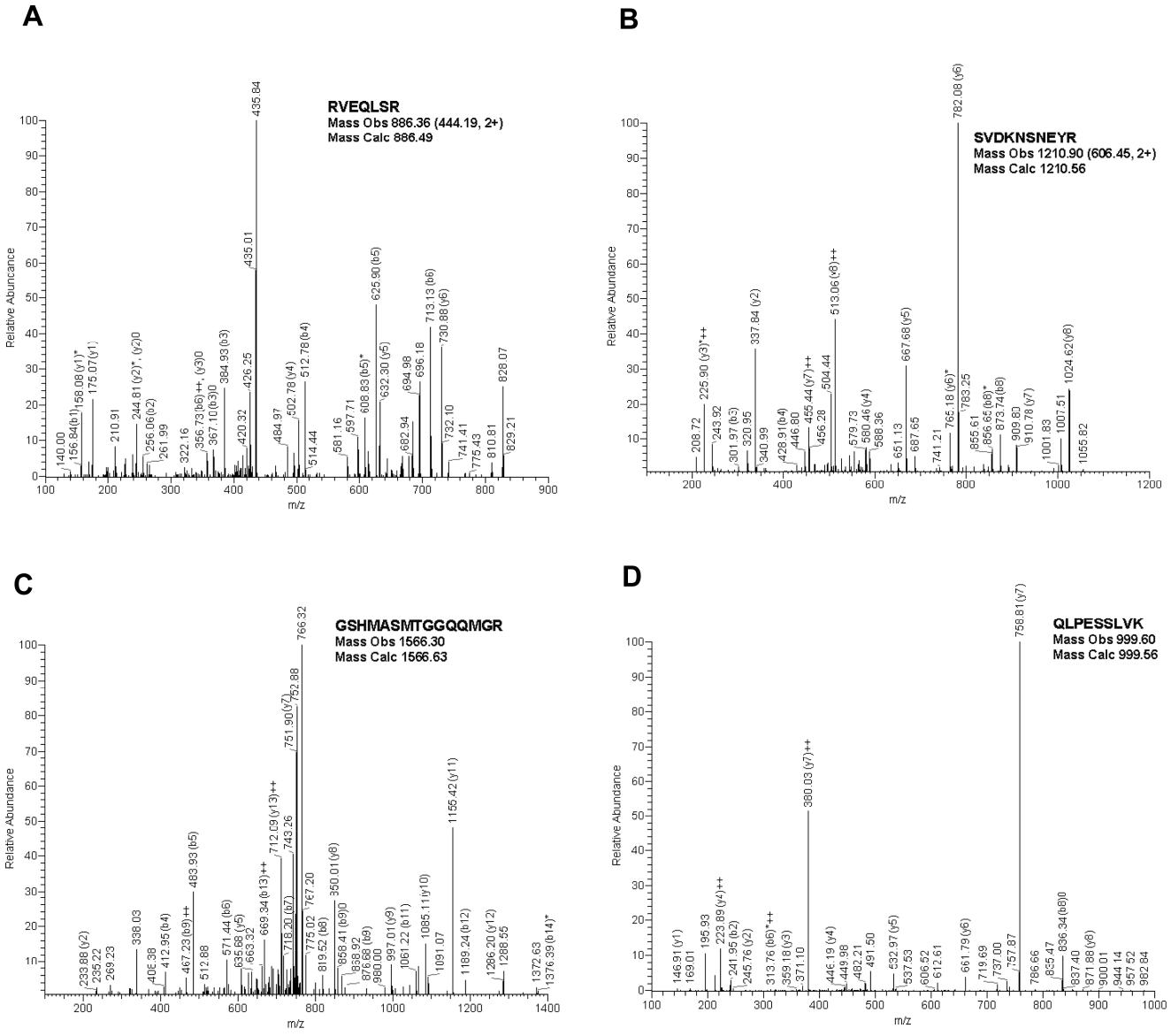


Supplementary Figure 2



Supplementary Figure 3