

Supplementary figure legend

Figure 1S. (a) 2D spectra of the retention time and accurate mass of the beta casein phosphopeptide FQpSEEQQQTEDELQDK from the sensitivity assessment experiment. The monoisotopic mass of the doubly-charged phosphopeptide is 1031.4178. The spectra showed the retention time and peak intensity of the base peaks (1031.4178 ± 3 ppm) from various amount of beta casein that were spiked into serum. The signal-to-noise level is $\sim 1E5$. (b) CID spectrum of the doubly-charged phosphopeptide FQpSEEQQQTEDELQDK . The spectrum labeled to show singly-charged b and y ions, as well as ions corresponding to neutral losses of the phosphate group and water.

Figure 2S. Verification of identified phosphopeptide AIPVAQDLNAPSDWDpSR from secreted phosphoprotein 1 by MS2 spectra comparison. (a) CID spectrum of the phosphopeptide ($2+$ ion m/z 967.9353) obtained from TiO_2 enriched serum phosphopeptides; (b) CID spectrum of synthetic phosphopeptide AIPVAQDLNAPSDWDpSR.

Figure 3S. Example CID and ETD spectra of doubly-charged phosphopeptide HIQETEWQpSQEGK from SPARC-like 1 protein. (a) CID spectrum labeled to show singly-charged b and y ions, as well as ions corresponding to neutral loss of phosphate group and water. (b) ETD spectrum labeled to show singly-charged c and z ions, as well as ions corresponding to unfragmented and charge-reduced parent ion.

Figure 1S.

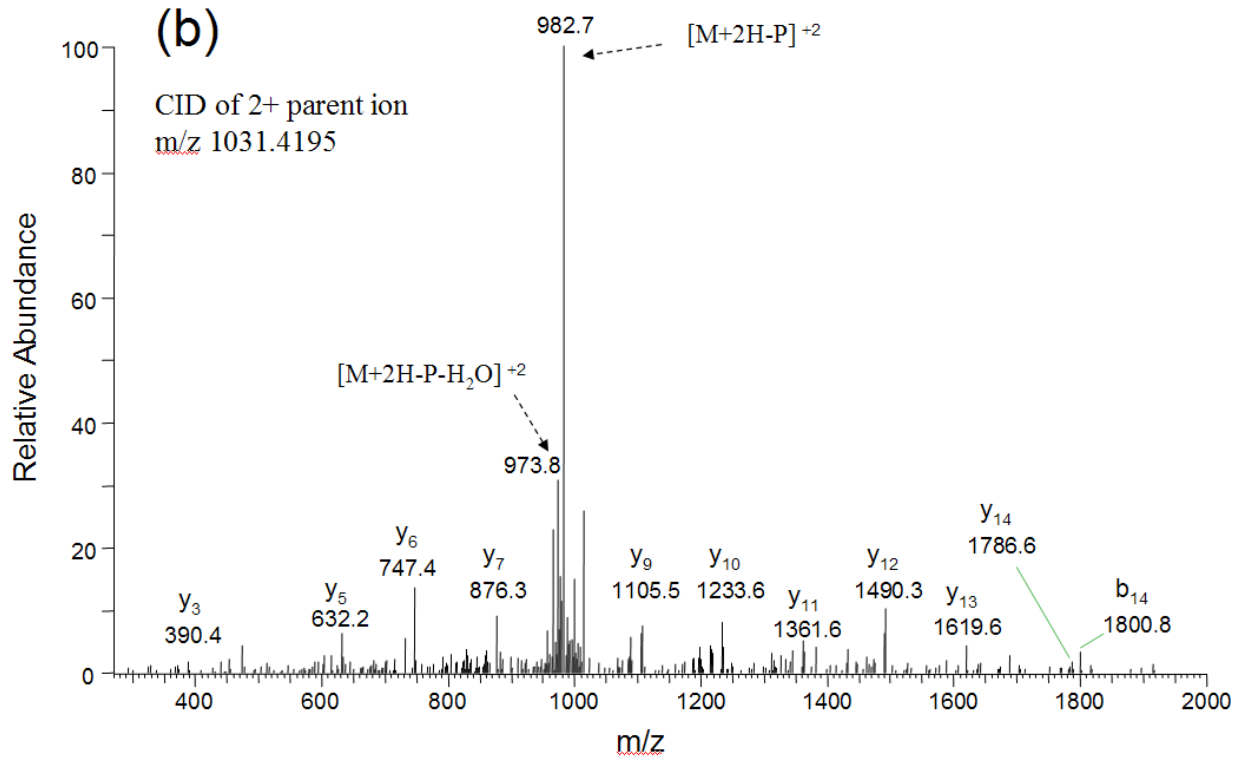
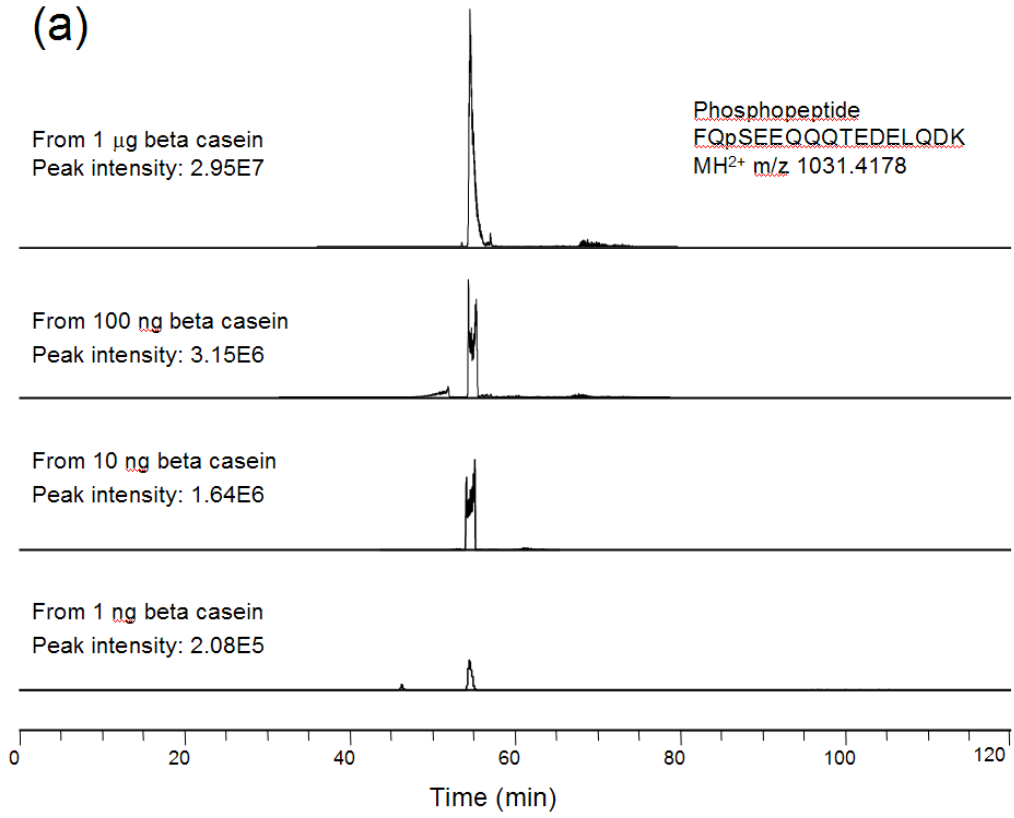


Figure 2S.

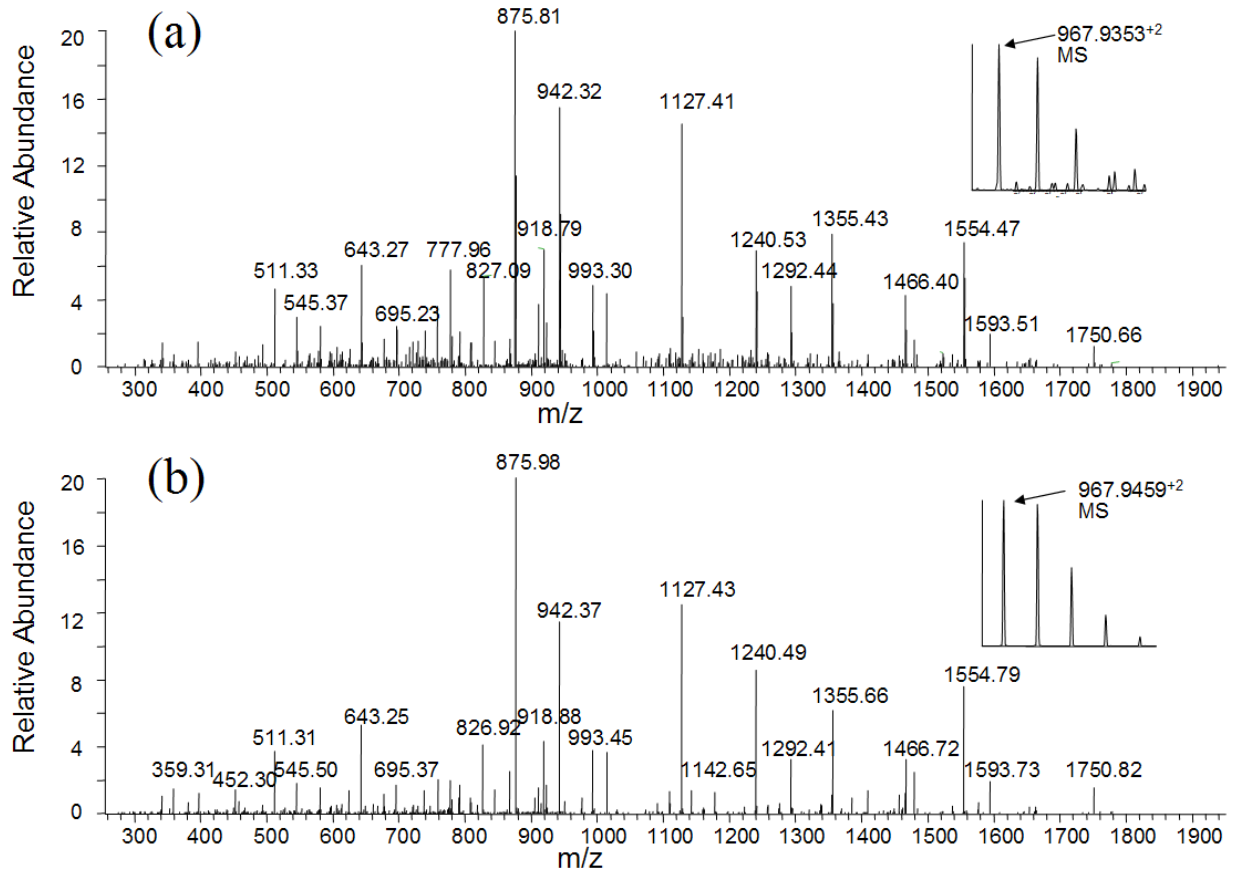


Figure 3S.

