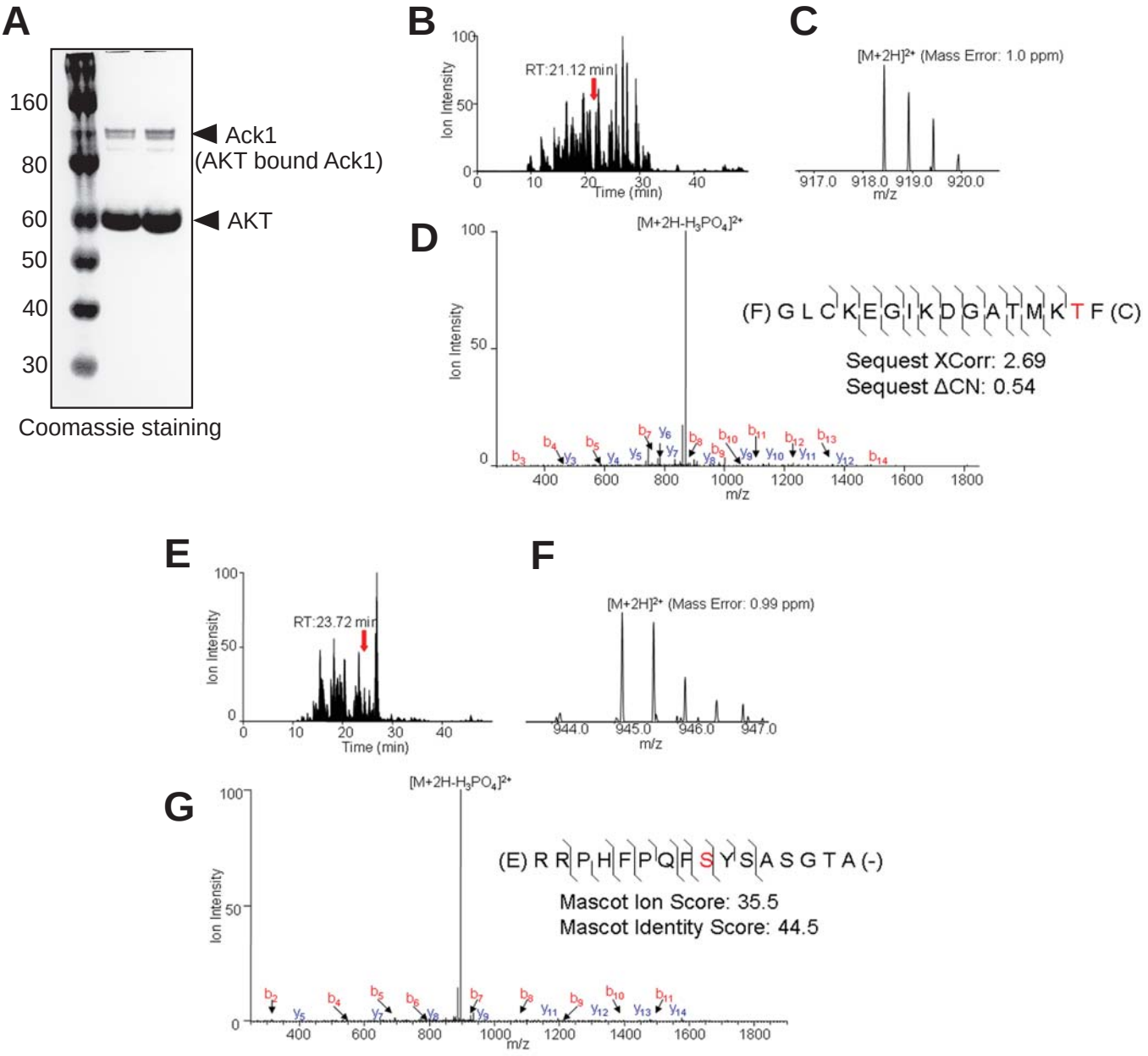

Figure S2. Tyr176-phosphorylated AKT sample also contains Thr308 and Ser473 phosphorylated AKT. (A) Activated Ack1 (caAck) and HA-tagged AKT were coexpressed in HEK293T cells followed by IP with HA-beads. IP AKT was subjected to SDS-PAGE electrophoresis and the gel was stained Coomassie. A prominent band of ~59 kDa corresponding to AKT is seen which was excised and subjected to mass spectrometry as described in methods section. The upper ~113 kDa band corresponds to caAck1 that bound to AKT. **(B)** Purified AKT peptide preparation that lead to the identification of pTyr176-AKT was assessed for other phosphorylation events. A peptide was detected at 21.12 mins in the total ion chromatogram with mass-to-charge ratio 918.43, which represents an error of 1.0 ppm (**C**). **(D)** The tandem mass spectrum matched the sequence, FGLCKEGIKDGATMK**<u>pT</u>**FC indicating that Thr308 in AKT was phosphorylated; the detection of the phosphothreonine y_3 is consistent with this localization. **(E)** Another peptide was detected at 23.72 mins in the total ion chromatogram with mass-to-charge ratio 944.93, which represents an error of 0.99 ppm (**F**). **(G)** The tandem mass spectrum matched the sequence, ERRPHFPQF**<u>pS</u>**YSASGTA indicating that Ser473 in AKT was phosphorylated; the detection of b_8, b_9, y_7 and y_8 is consistent with this localization.