

Figure 1a. Extracted ion chromatograms of tryptic phosphopeptides ASTIFLSKsQTDVR (1) , WsPAIIS (2), YSscSTIFLDDSTVSQPNLK (3) and SASADNLTLPWsPAIIS (4) derived from Cyclin Y expressed in the presence of Wt PCTAIRE(A) , KD PCTAIRE (B) or in the absence of PCTAIRE (C). Chromatograms are scaled to the same absolute intensity (100% = 1.6×10^6 cps).

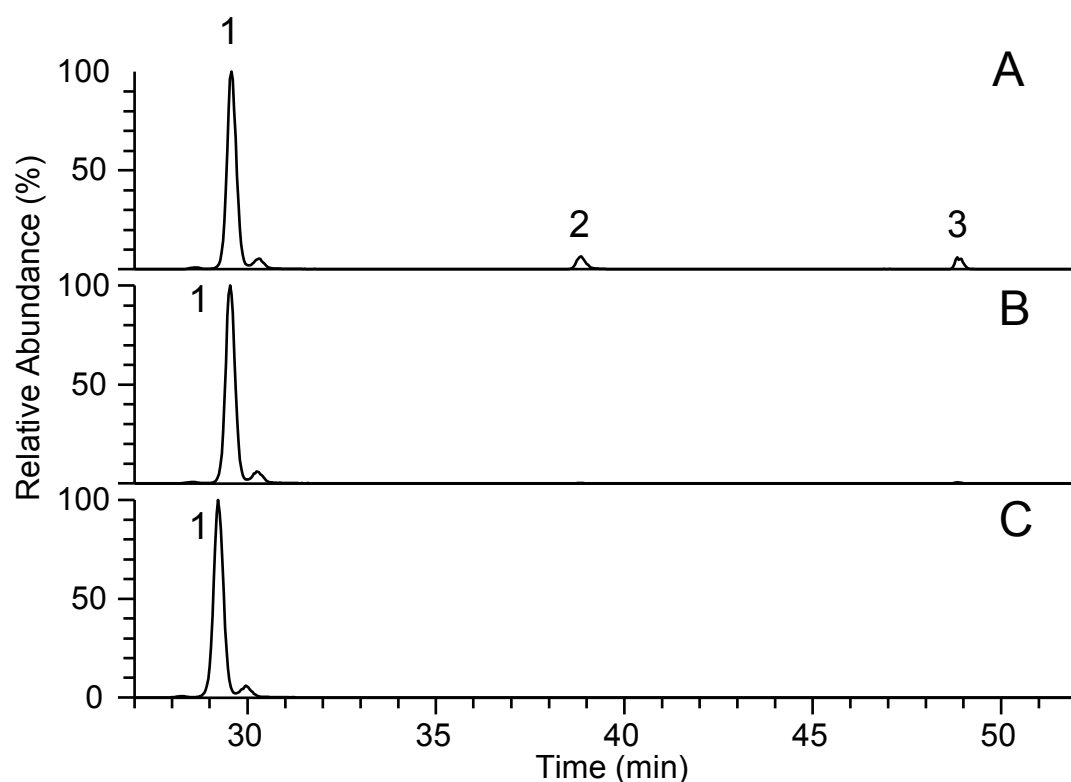
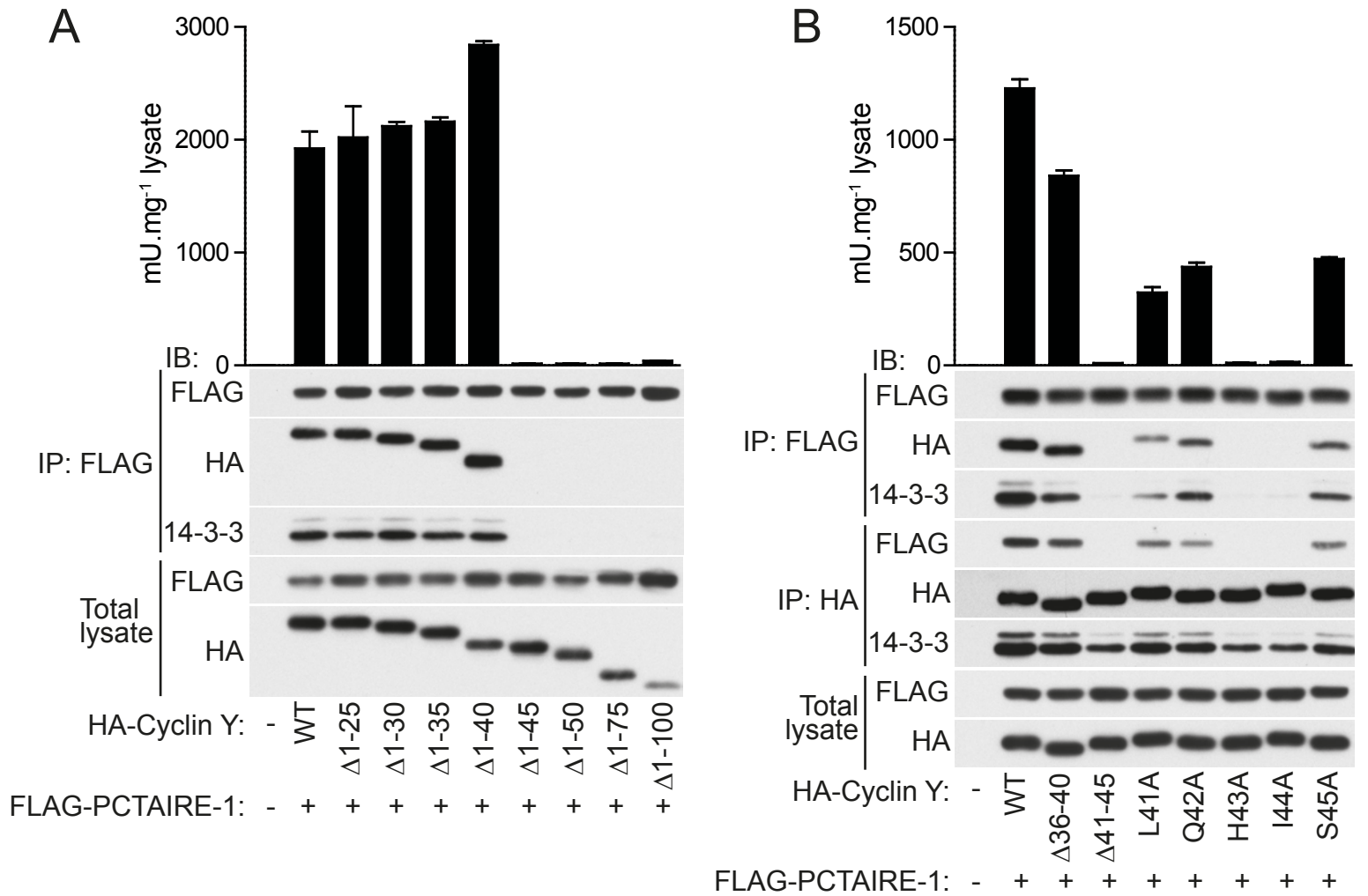


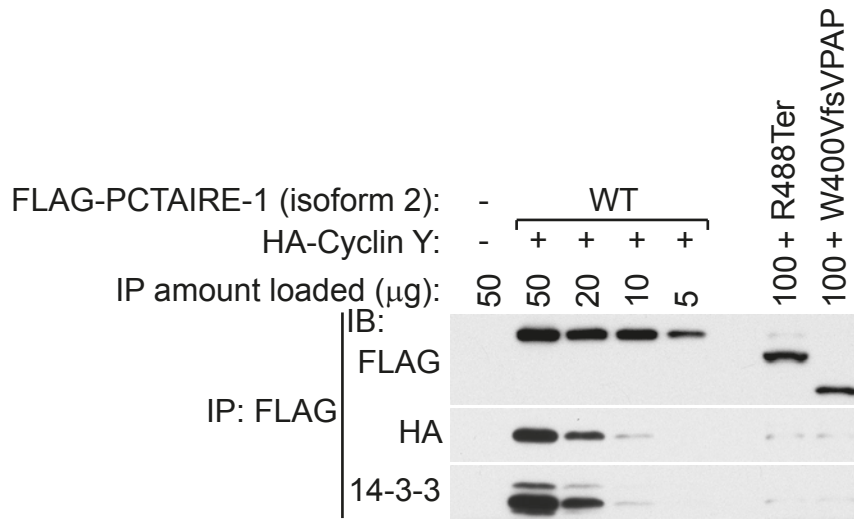
Figure 1b. Extracted ion chromatograms of tryptic phosphopeptides SAsADNLTLP (1) , WsPAIIS (2), (3) and SASADNLTLPWsPAIIS (3) derived from Cyclin Y expressed in the presence of Wt PCTAIRE (A) , KD PCTAIRE (B) or in the absence of PCTAIRE (C) . Chromatograms are scaled to the same absolute intensity (100% = 3×10^7 cps).



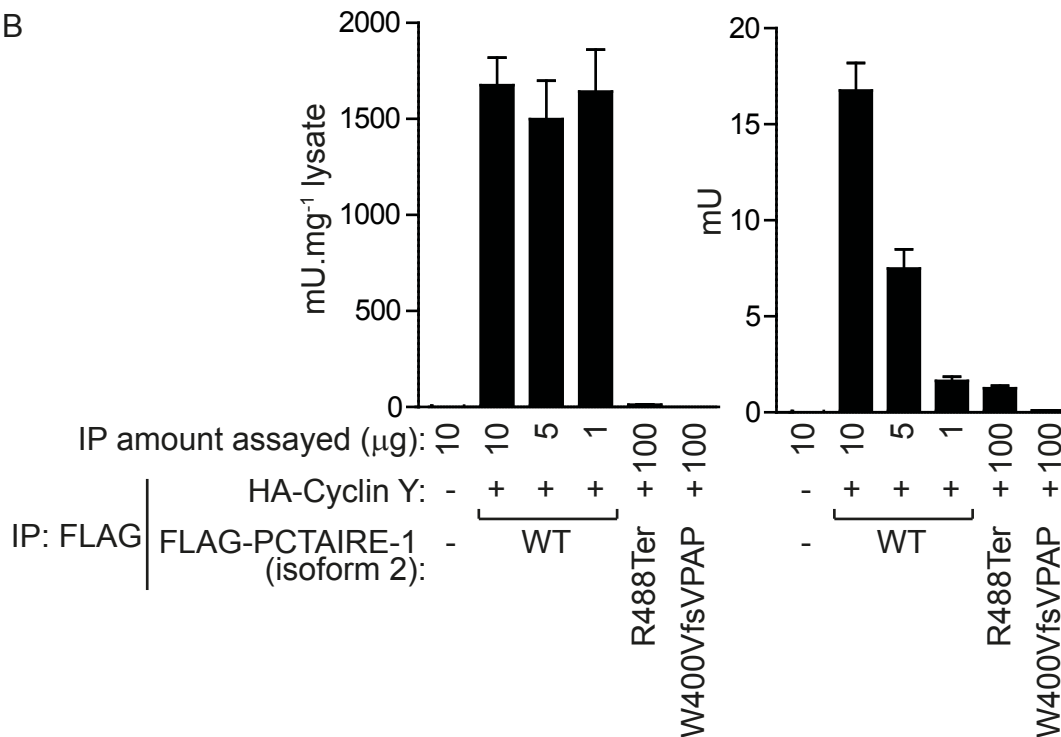
Mapping of cyclin Y residues required for PCTAIRE-1 interaction. (A) HA-WT-cyclin Y or a series of N-terminal truncations were co-expressed with FLAG-WT-PCTAIRE-1 in COS-1 cells and total lysates immunoblotted using the indicated antibodies. Lysates were immunoprecipitated using FLAG-agarose and either immunoblotted or assayed for kinase activity. (B) HA-WT-cyclin Y, N-terminal deletions, or Ala-mutants of residues 41-45 were co-expressed with FLAG-WT-PCTAIRE-1 in COS-1 cells and total lysates immunoblotted using the indicated antibodies. Lysates were immunoprecipitated using either FLAG- or HA-agarose and either immunoblotted or the FLAG-immunoprecipitates assayed for PCTAIRE-1 kinase activity. Results are expressed as mean± standard deviation and are representative of 3 independent experiments.

Supplementary Figure 2

A



B



Disease-associated human PCTAIRE-1 variants abolish cyclin Y binding and kinase activity. (A) Isoform 2 of FLAG-WT-PCTAIRE-1 or disease-associated variants were co-expressed with HA-WT-cyclin Y in COS-1 cells and varying amounts of total lysates immunoprecipitated using FLAG-agarose and immunoblotted using the indicated antibodies. (B) The indicated varying amounts of lysates from (A) were immunoprecipitated using FLAG-agarose and assayed for kinase activity. Left panel shows activity relative to amount of lysate, whereas right panel compares total activity irrespective of lysate amount. Both results shown in (B) were generated from the same assay.

Supplementary Figure 3