



Figure S3 – Src phosphorylates recombinant hYVH1 *in vitro*. *A*, HeLa cells expressing myc-Src Y530F were subjected to anti-Src immunoprecipitation and an *in vitro* kinase assay was performed using recombinant hYVH1 C115S purified from *E.coli* cells. Following the *in vitro* kinase assay, samples were analyzed by anti-phosphotyrosine immunoblotting (α -pTyr). *B*, In vitro kinase assay using recombinant wild type Src and recombinant hYVH1 C115S. Following the kinase assay phosphorylation of hYVH1 was analyzed by anti-phosphotyrosine immunoblotting (α -pTyr). *C*, Representative MS/MS fragment ion spectrum of a monophosphorylated tryptic peptide at m/z 1017.9⁺² corresponding to amino acids 164-180 of hYVH1. Analysis of the fragmentation pattern unambiguously mapped the phosphorylation site to Tyr179.