



Figure S2. Mass spectrometric analysis of Src-YEEI and Hck-YEEI tail and activation loop peptides. A) ESI-MS/MS spectra of Src-YEEI peptic peptide YFTSTESQpY⁵²⁷EEIP ($[M+H]^+ = 1683.68$ Da), which is derived from the C-terminal tail, indicates that Tyr527 is phosphorylated (numbering as per crystal structure of c-Src; PDB ID: 2SRC). The mass difference between fragment ions b₈ and b₉ (blue) indicates the presence of a phosphate group attached to the tyrosine residue (243 Da). The y ions are colored in red. B) ESI-MS/MS spectra of Src-YEEI peptic peptide Y⁴¹⁶TARQGAKF ($[M+H]^+ = 1041.54$ Da), which maps to the activation loop in the kinase domain, indicates that Tyr416 is not phosphorylated (163 Da). The mass difference between fragment ions y₈ and y₉ (red) corresponds to the unphosphorylated tyrosine. C) ESI-MS/MS spectra of Hck-YEEI peptic peptide ESQpY⁵²⁷EEIP ($[M+H]^+ = 1074.40$ Da), derived from the C-terminal tail, indicates that Tyr527 is phosphorylated. The mass difference between fragment ions b₃ and b₄ (blue) indicates the presence of a phosphate group attached to the tyrosine residue. D) ESI-MS/MS spectra of the Hck-YEEI peptic peptide Y⁴¹⁶TAREGAKF ($[M+H]^+ = 1042.54$ Da), derived from the activation loop, indicates that Tyr416 is not phosphorylated. The mass difference between fragment ions y₈ and y₉ (red) corresponds to the unphosphorylated tyrosine.