

Supplemental data

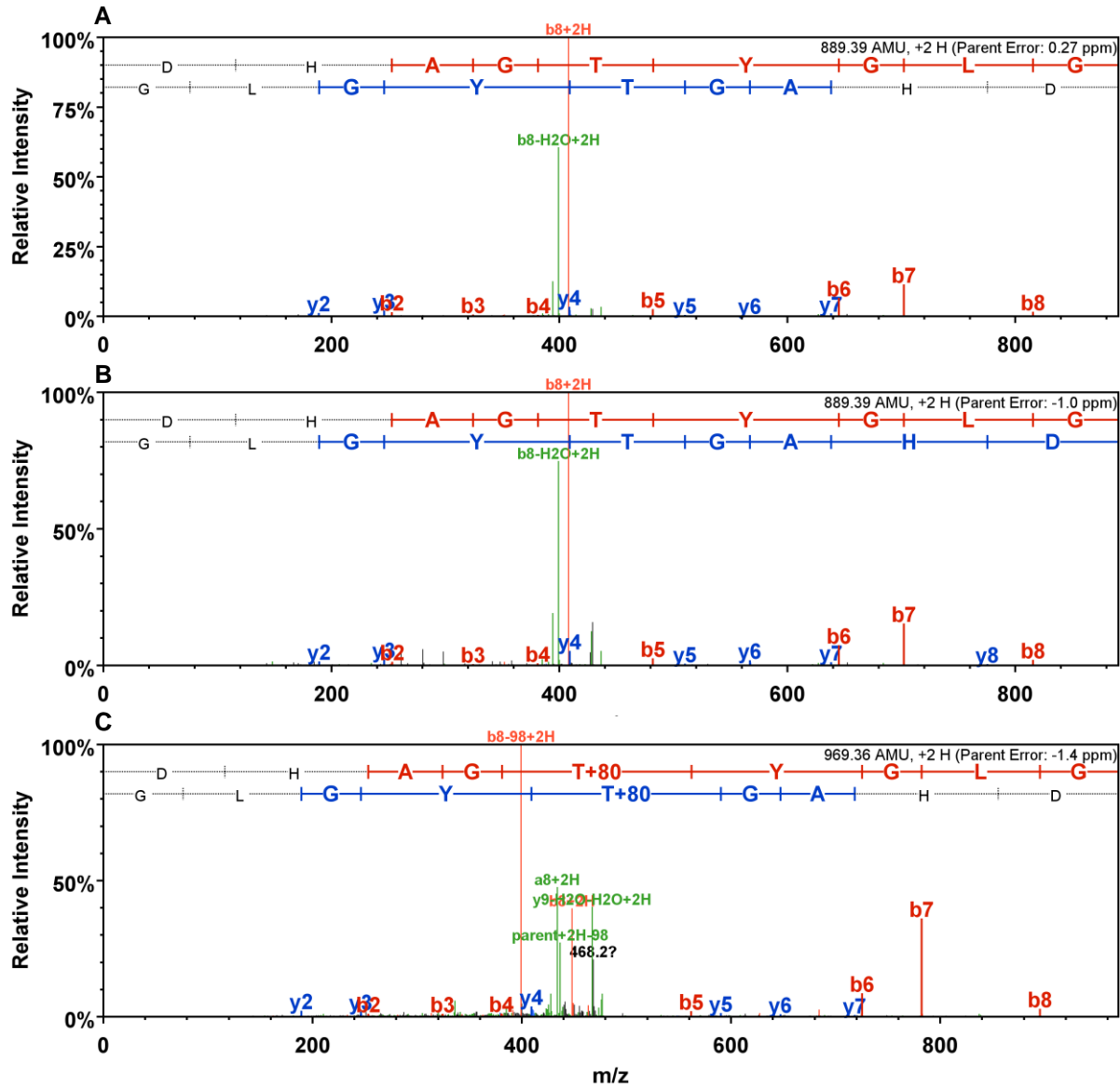


Figure S1. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr17 was phosphorylated by Chk2 only. The corresponding nonphosphopeptide detected in the control and Chk1-treated sample are also shown. The unannotated peak at m/z 468.2 in (C) corresponds to parent-H₂O-NH₃+2H.

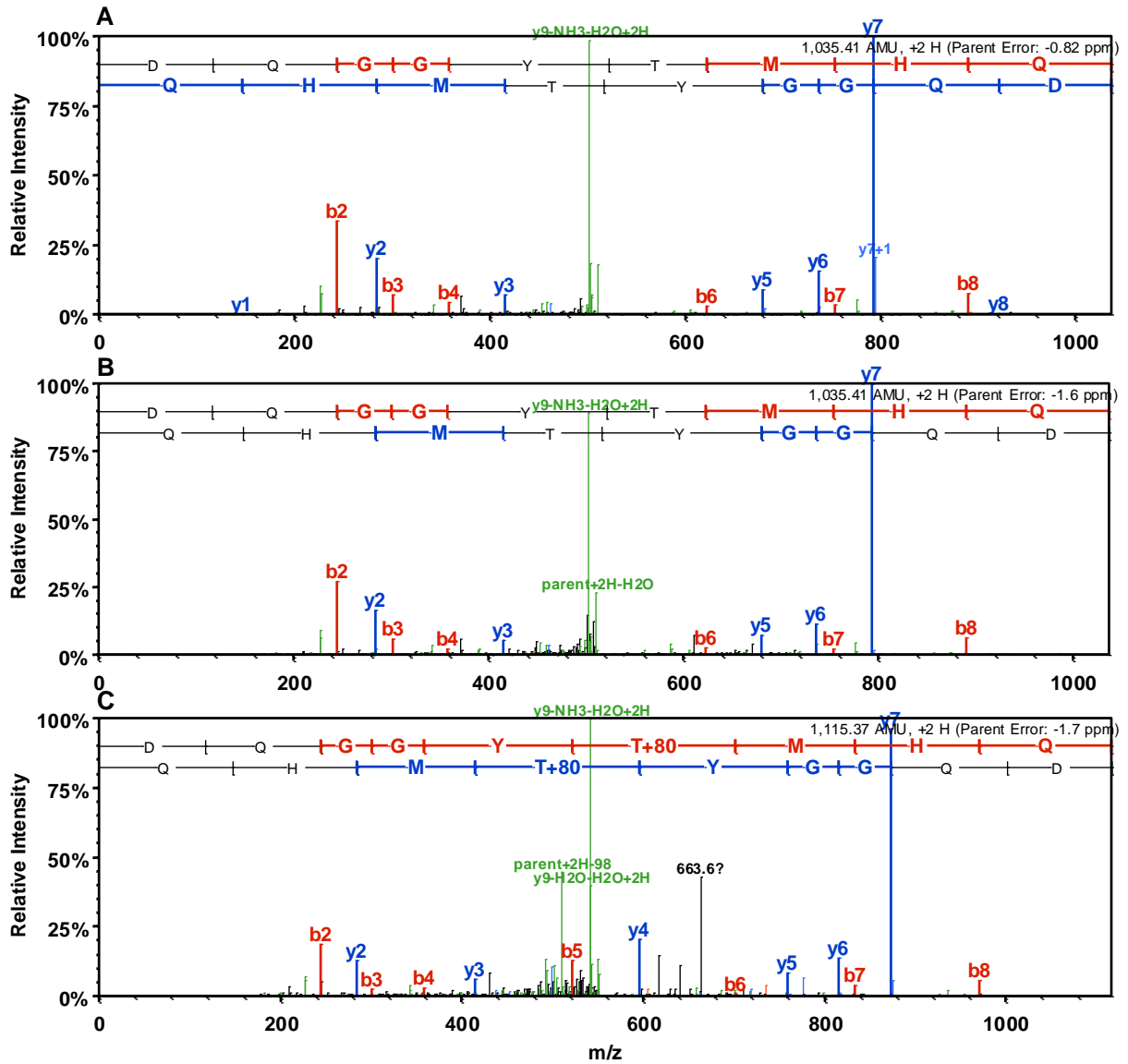


Figure S2. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr30 was phosphorylated by Chk2 only. The corresponding nonphosphopeptide detected in the control and Chk1-treated sample are also shown. The unannotated peak at m/z 663.6 in (C) was identified not to be a part of the peptide of interest.

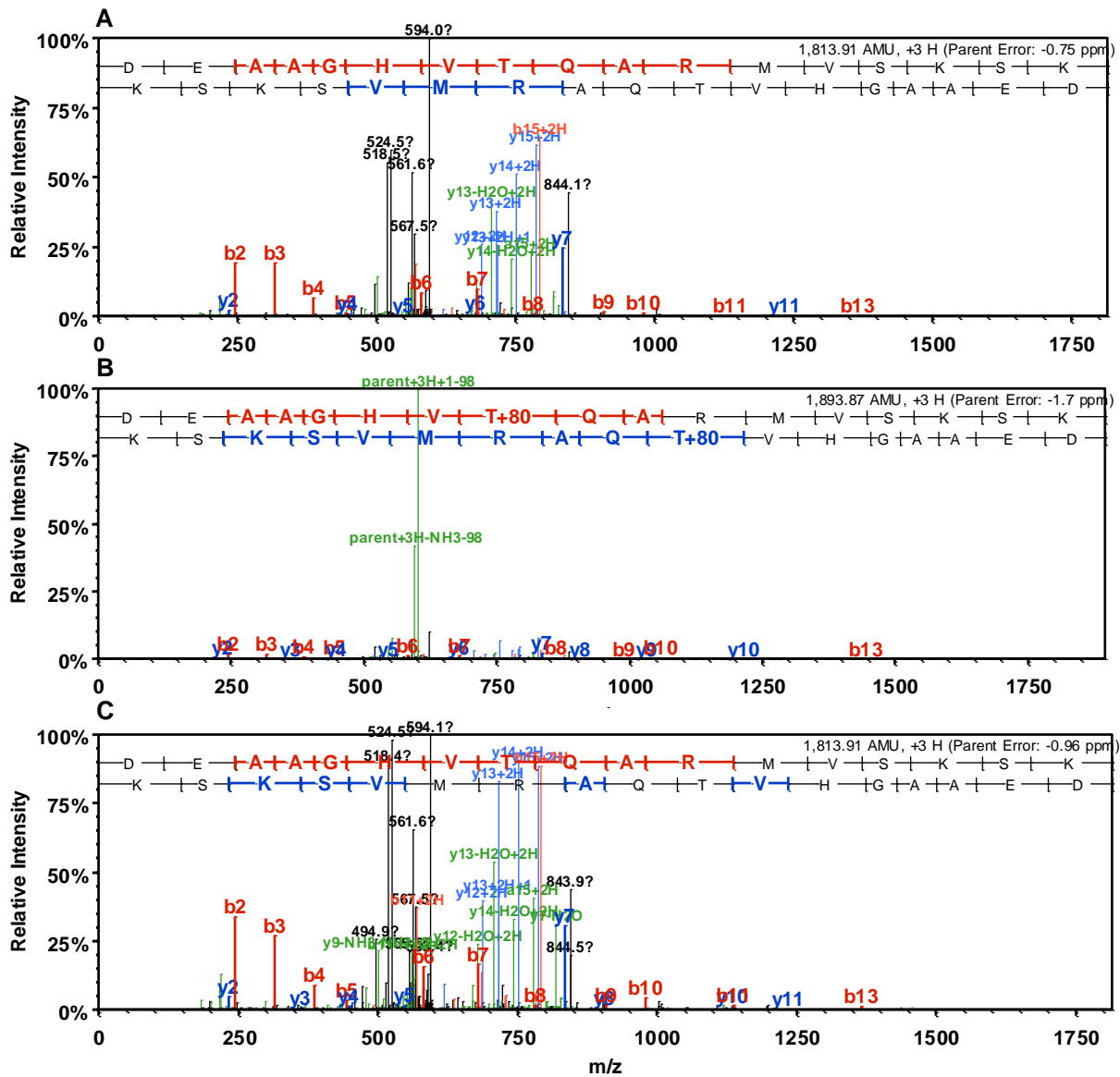


Figure S3. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr123 was phosphorylated by Chk1 only. The corresponding nonphosphopeptide detected in the control and Chk2-treated sample are also shown. The unannotated peaks in the spectra are identified as follows: (A) m/z 518.5: $a_{15}+3H$; m/z 524.5: $y_{15}+3H$; m/z 561.6: $y_{16}-NH_3+3H$; m/z 567.5: $y_{16}+3H$; m/z 594.0: $M-NH_3-H_2O+3H$; m/z 844.1: $b_{16}+H_2O+2H$ (C) m/z 494.9: $y_{14}-NH_3+3H$; m/z 518.4: $a_{15}+3H$; m/z 524.5: $y_{15}+3H$; m/z 561.6: $y_{16}-NH_3+3H$; m/z 567.5: $y_{16}+3H$; m/z 594.1: $M-NH_3-H_2O+3H$; m/z 843.9: $b_{16}+H_2O+2H$; m/z 844.5: $b_{16}+H_2O+2H$

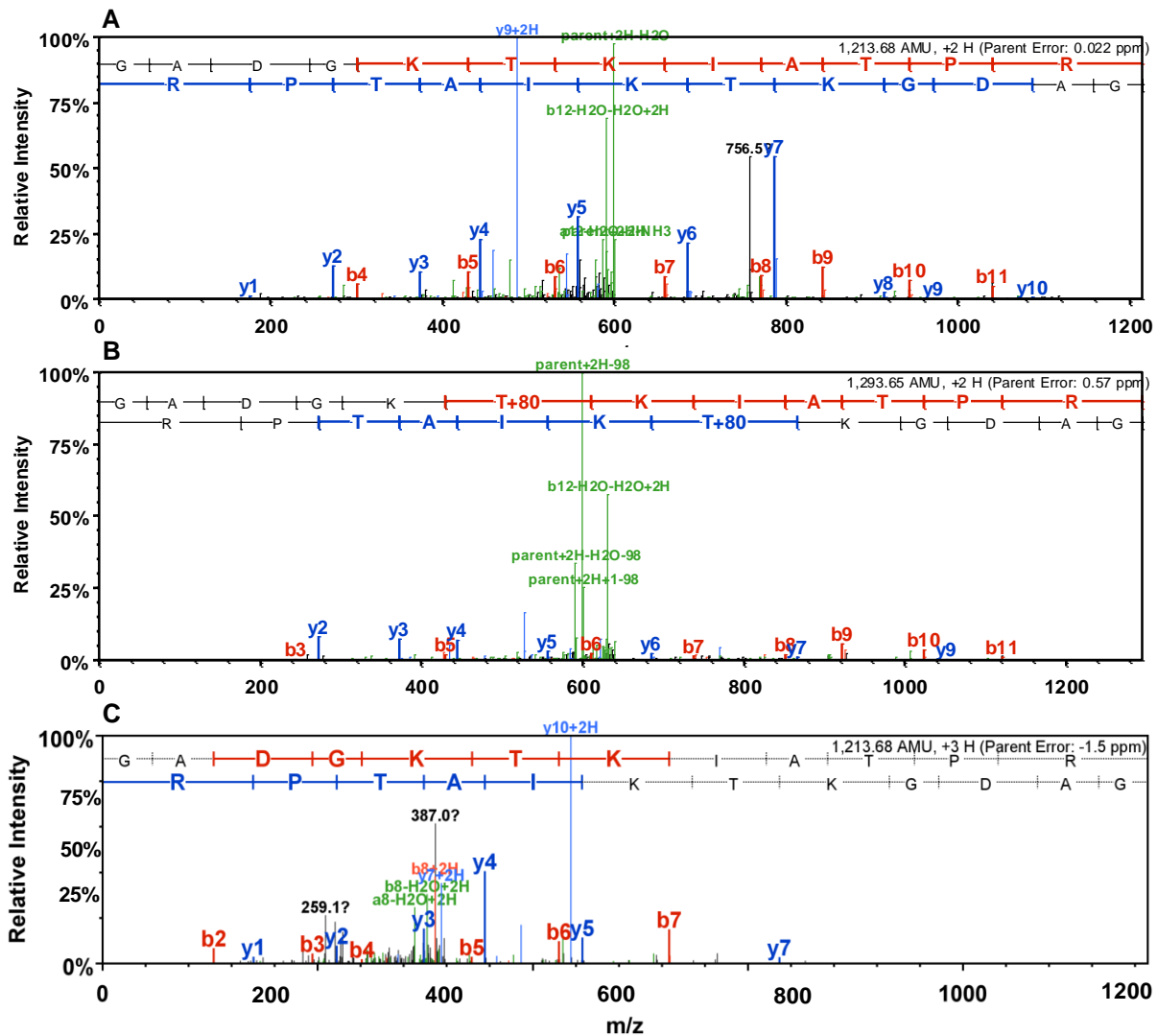


Figure S4. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr149 was phosphorylated by Chk1 only. The corresponding nonphosphopeptide detected in the control and Chk2-treated sample are also shown. The peptide detected in the Chk2-treated sample has a different charge state. The unannotated peaks at m/z 259.1 and 387.0 in (C) correspond to $a_6\text{-NH}_3+2\text{O}+2\text{H}$ and $y_3+\text{O}-2\text{H}$, respectively. The unannotated peak at m/z 756.5 in (A) was identified not to be a part of the peptide of interest.

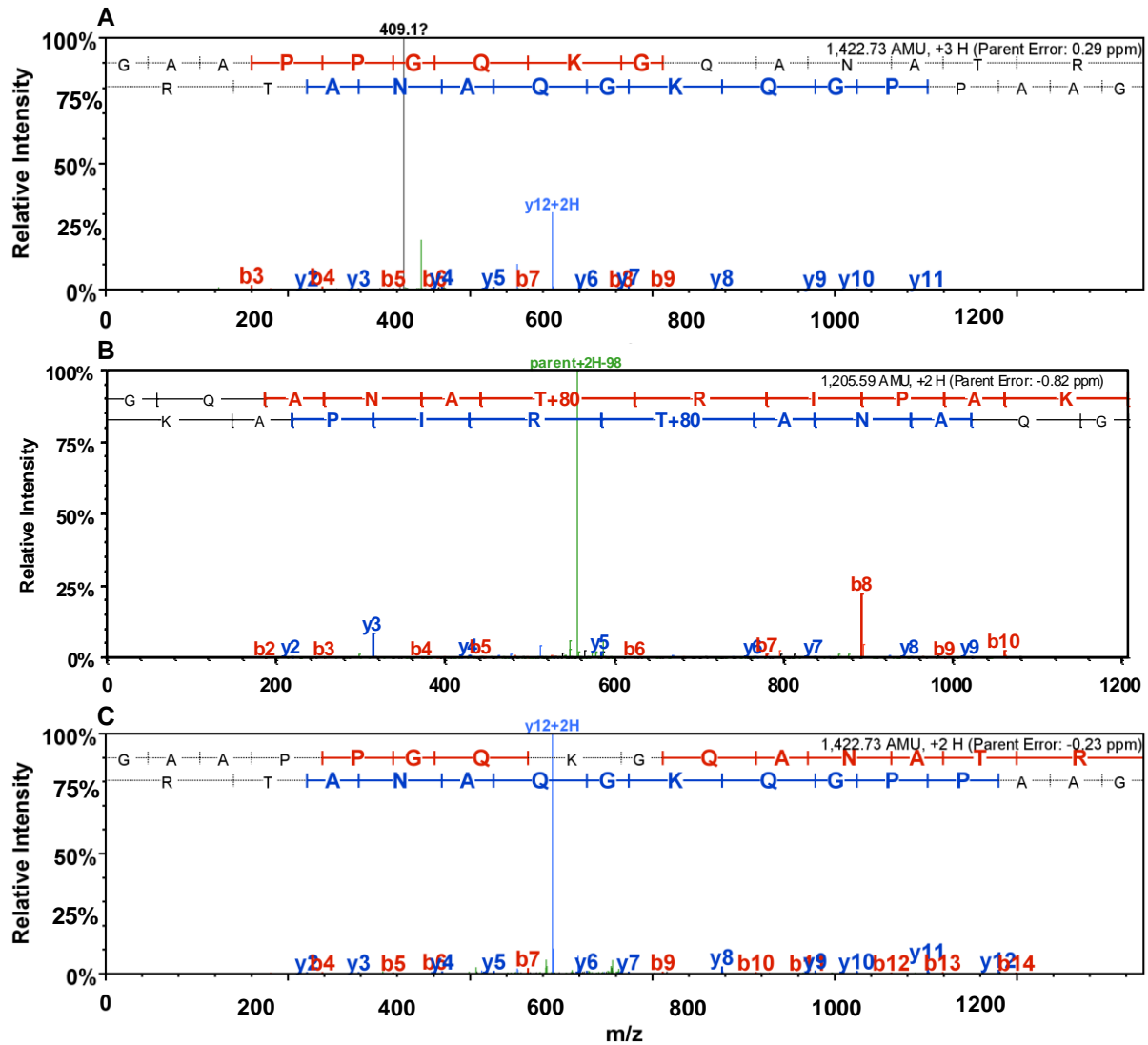


Figure S5. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr169 was phosphorylated by Chk1 only. The exact corresponding nonphosphopeptide was not detected in either the control or Chk2-treated sample. A different nonphosphopeptide containing the residue of interest was detected instead, for both the control and Chk2-treated sample. The unannotated peak at m/z 409.1 in (A) corresponds to $y_{12}+3H$.

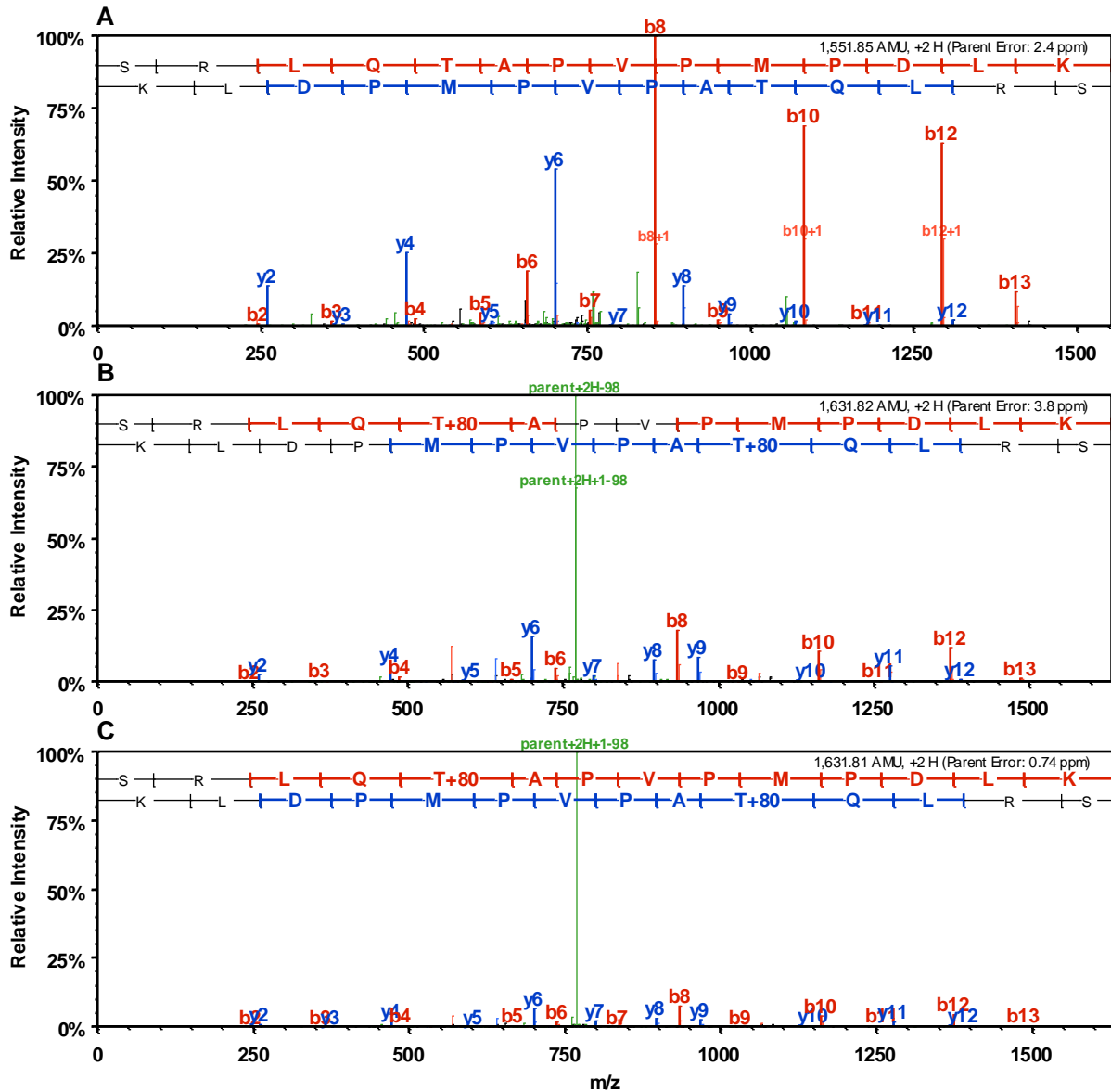


Figure S6. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr245 was phosphorylated by Chk1 and Chk2. The corresponding nonphosphopeptide detected in the control is also shown.

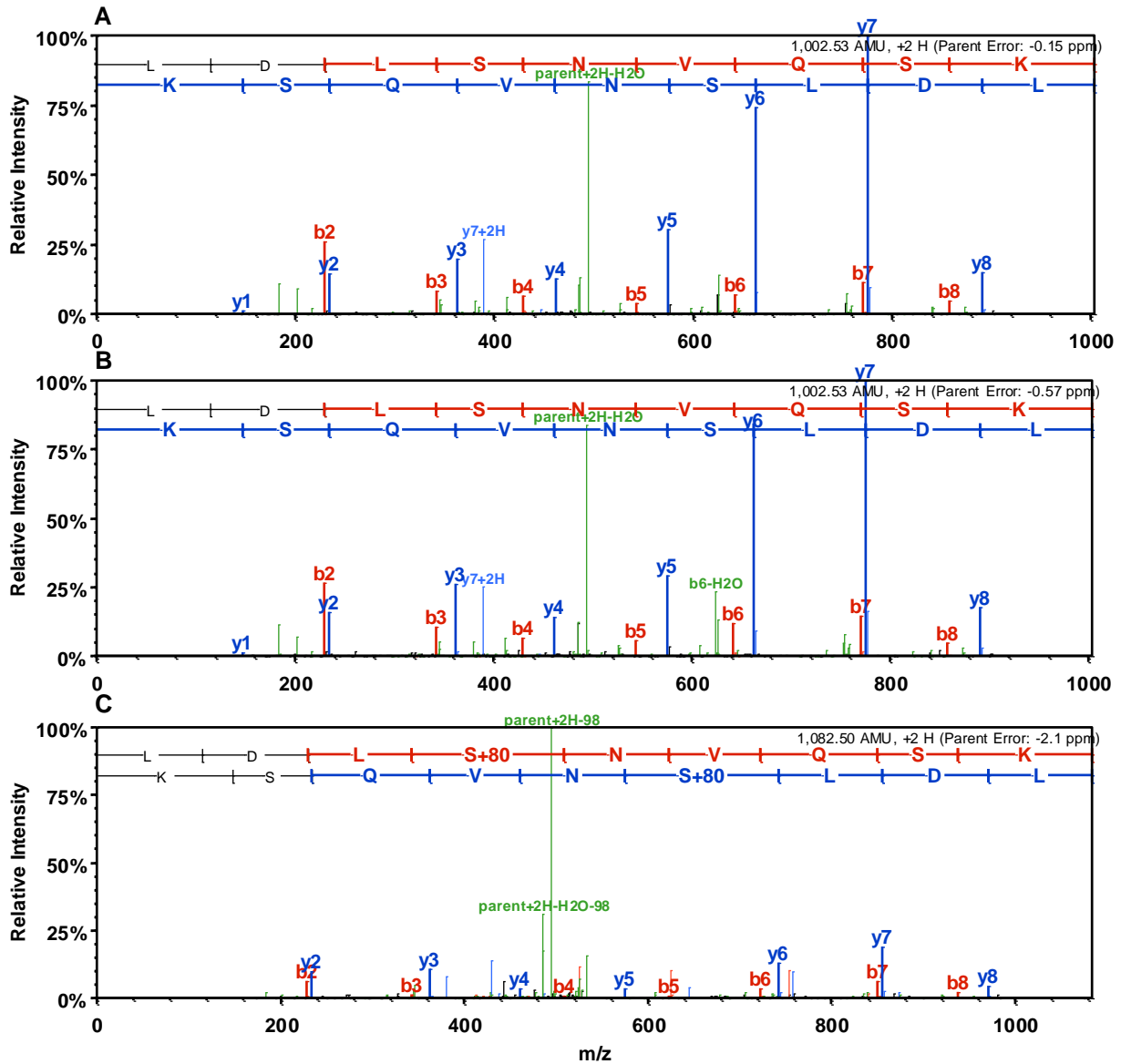


Figure S7. MS/MS spectra (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Ser285 was phosphorylated by Chk2 only. The corresponding nonphosphopeptide detected in the control and Chk1-treated sample are also shown.

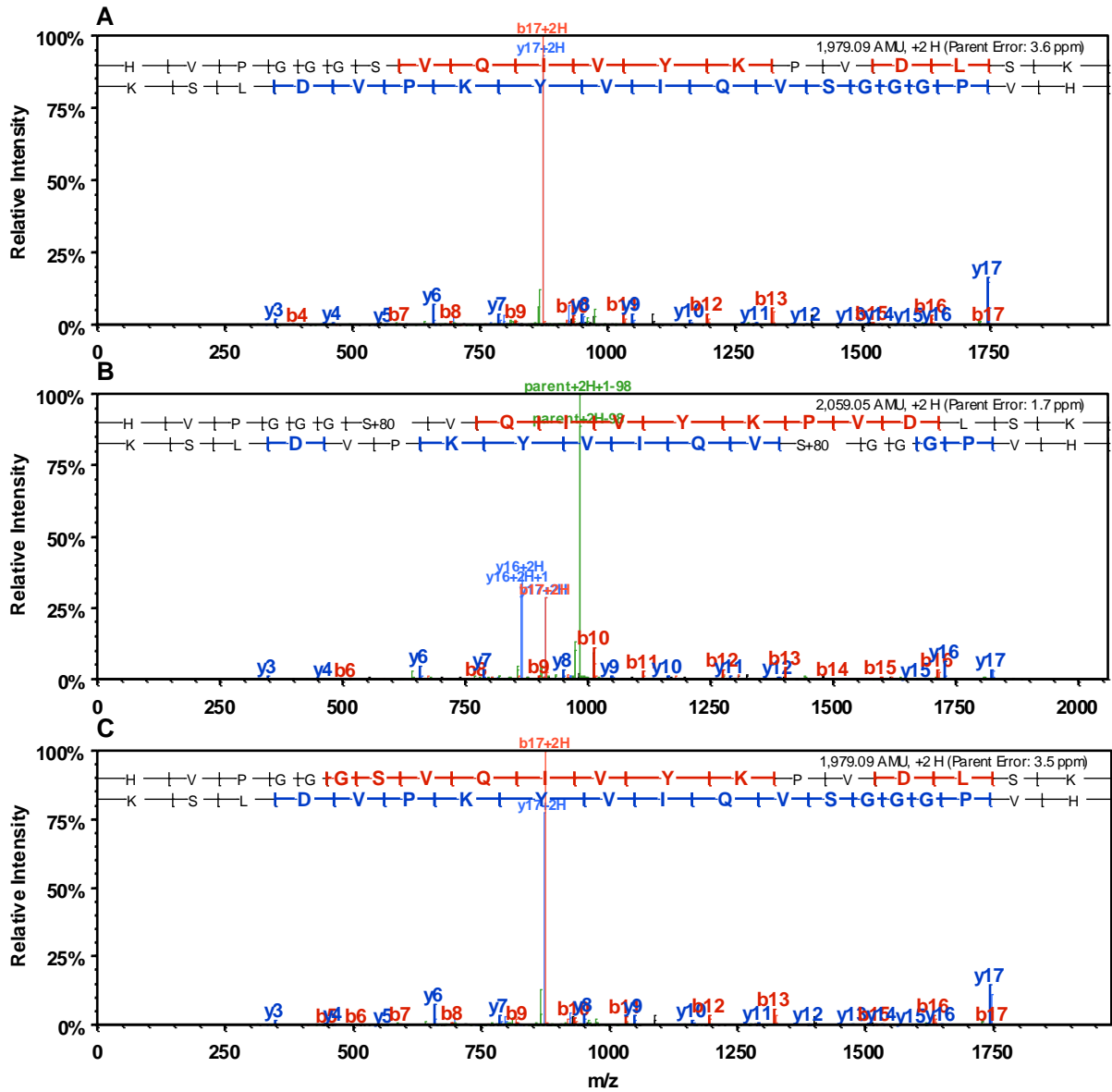


Figure S8. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Ser305 was phosphorylated by Chk1 only. The corresponding nonphosphopeptide detected in the control and Chk2-treated sample are also shown.

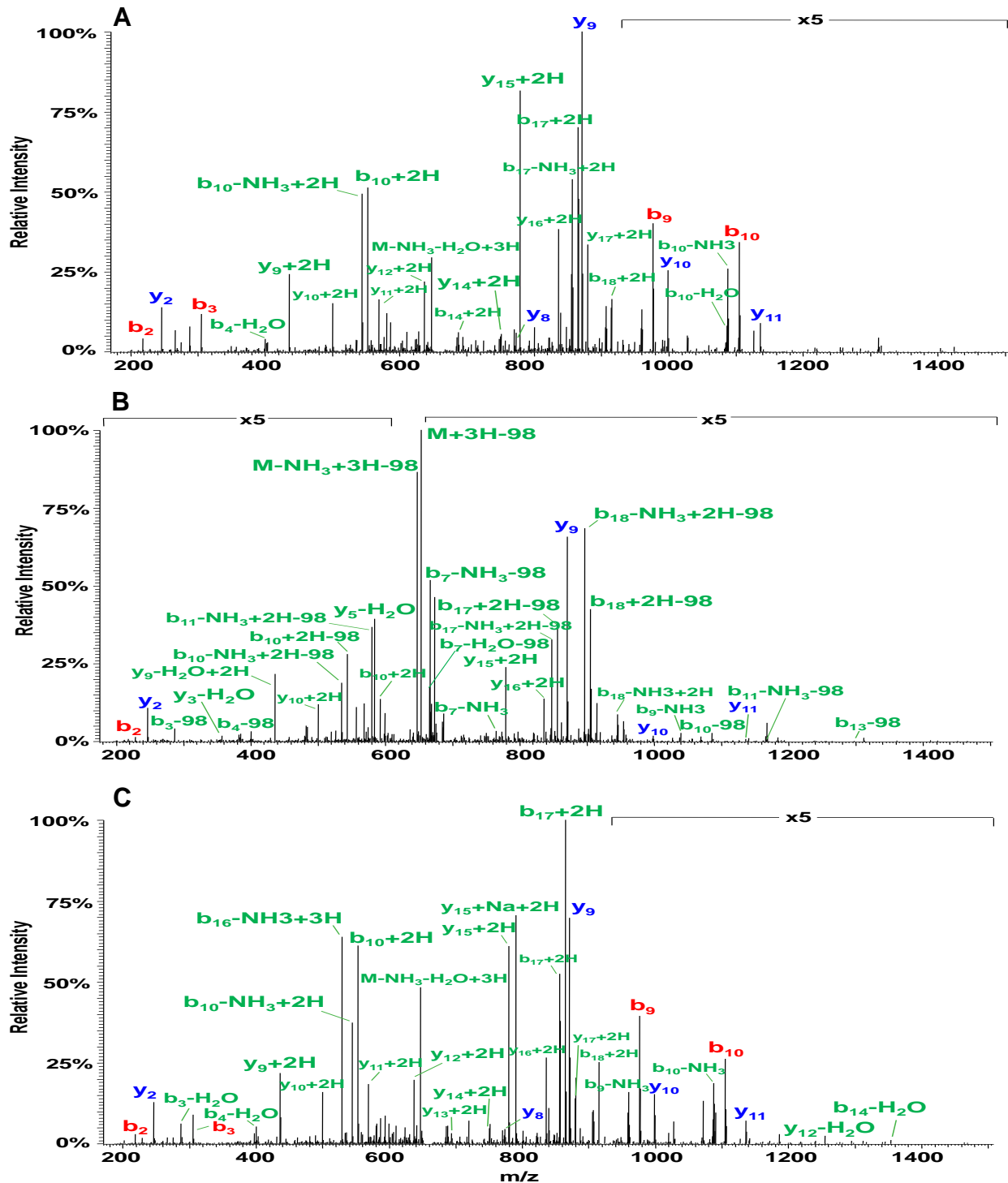


Figure S9. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Ser324 (cGS₃₂₄LGNIHHKPGGGQVEVK) was phosphorylated by Chk1 only. The corresponding nonphosphopeptide detected in the control and Chk2-treated sample are also shown. Phosphorylation, in this case, was established after manual inspection of MS/MS and MS³ scans.

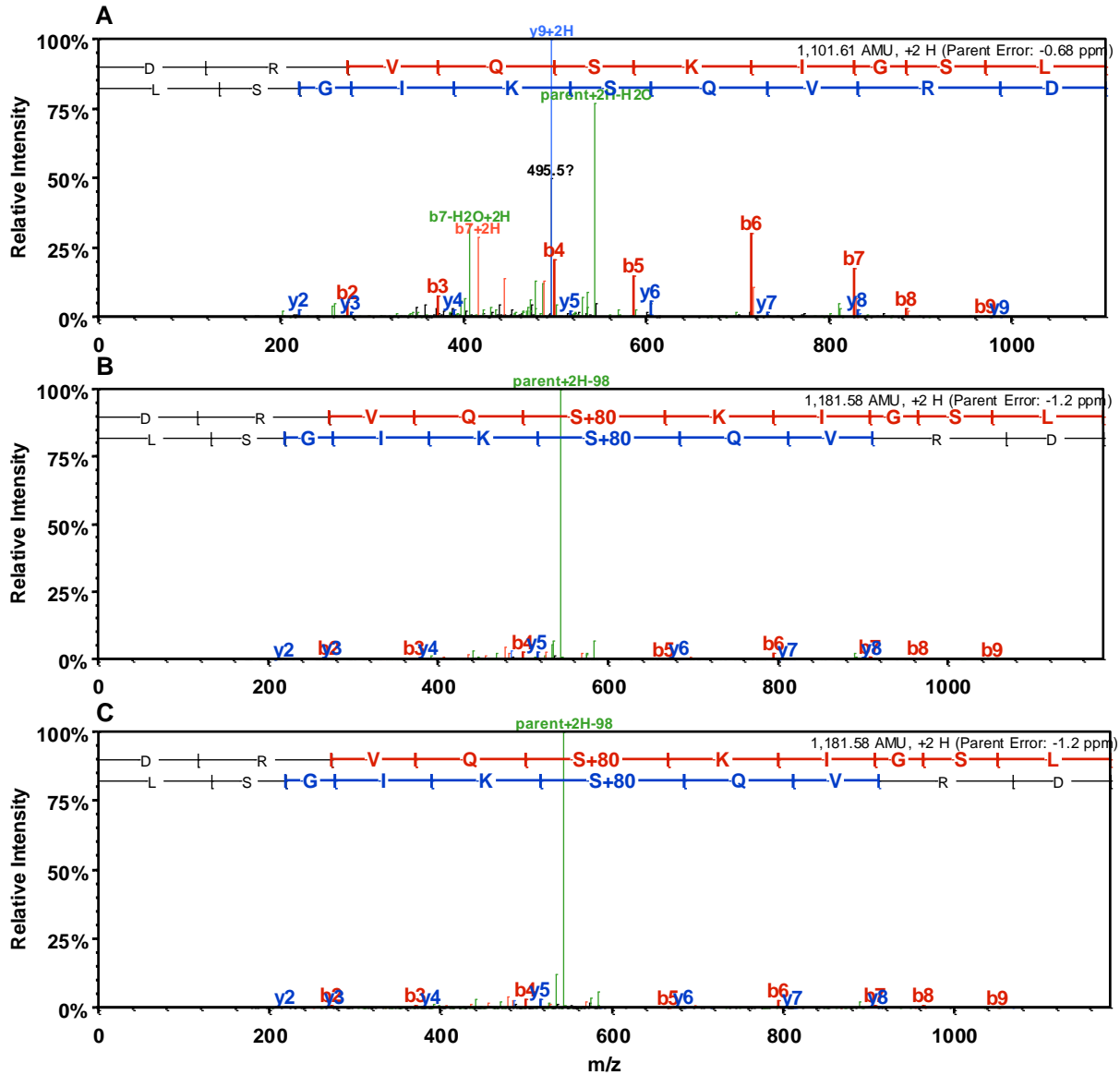


Figure S10. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Ser352 was phosphorylated by Chk1 and Chk2. The corresponding nonphosphopeptide detected in the control is also shown. The unannotated peak at m/z 495.5 in (A) corresponds to b_9+H_2O+2H .

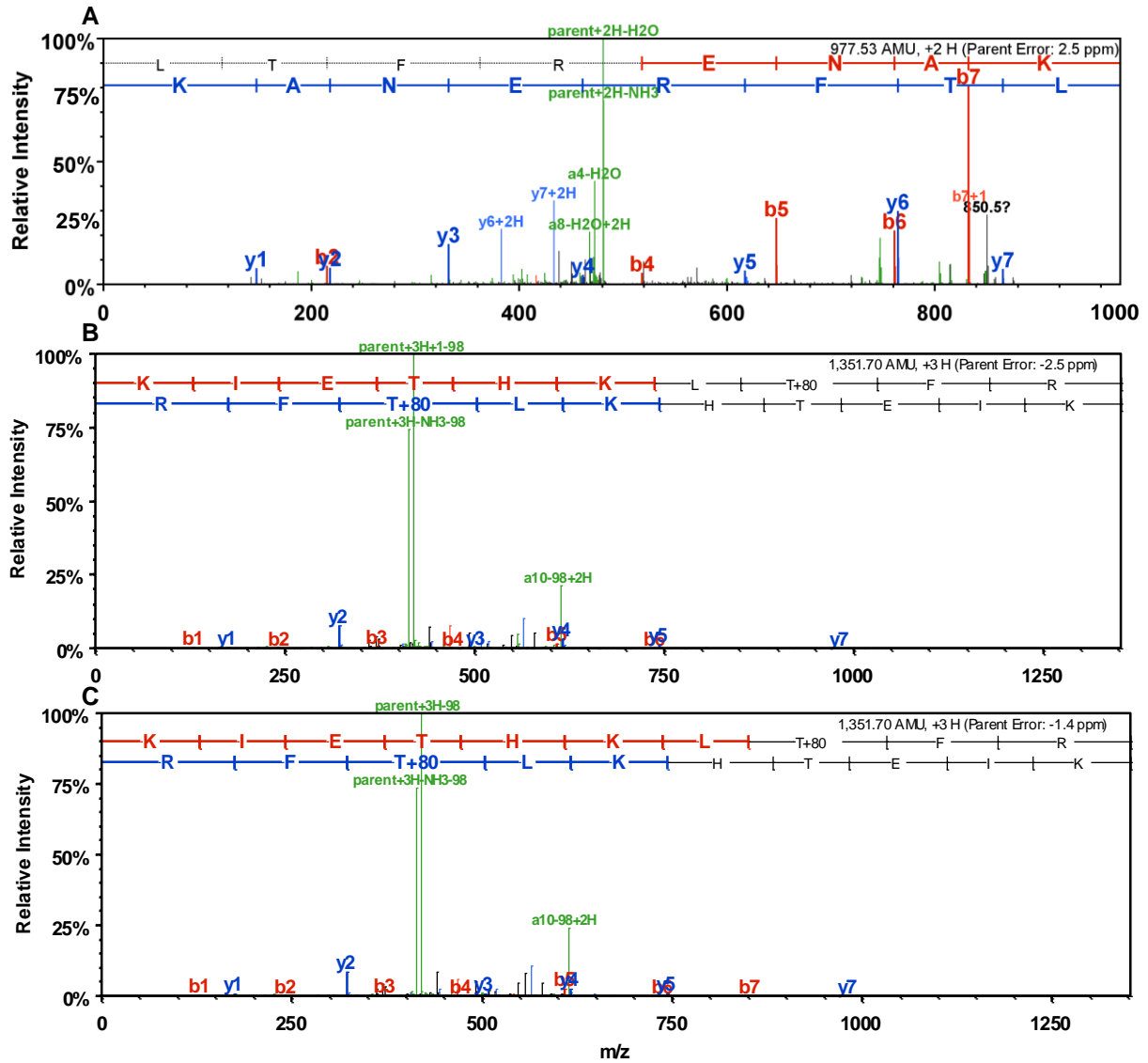


Figure S11. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr377 was phosphorylated by Chk1 and Chk2. The exact corresponding nonphosphopeptide was not detected in the control. A different nonphosphopeptide containing the residue of interest was detected instead. The unannotated peak at m/z 850.5 in (A) corresponds to b_7+H_2O .

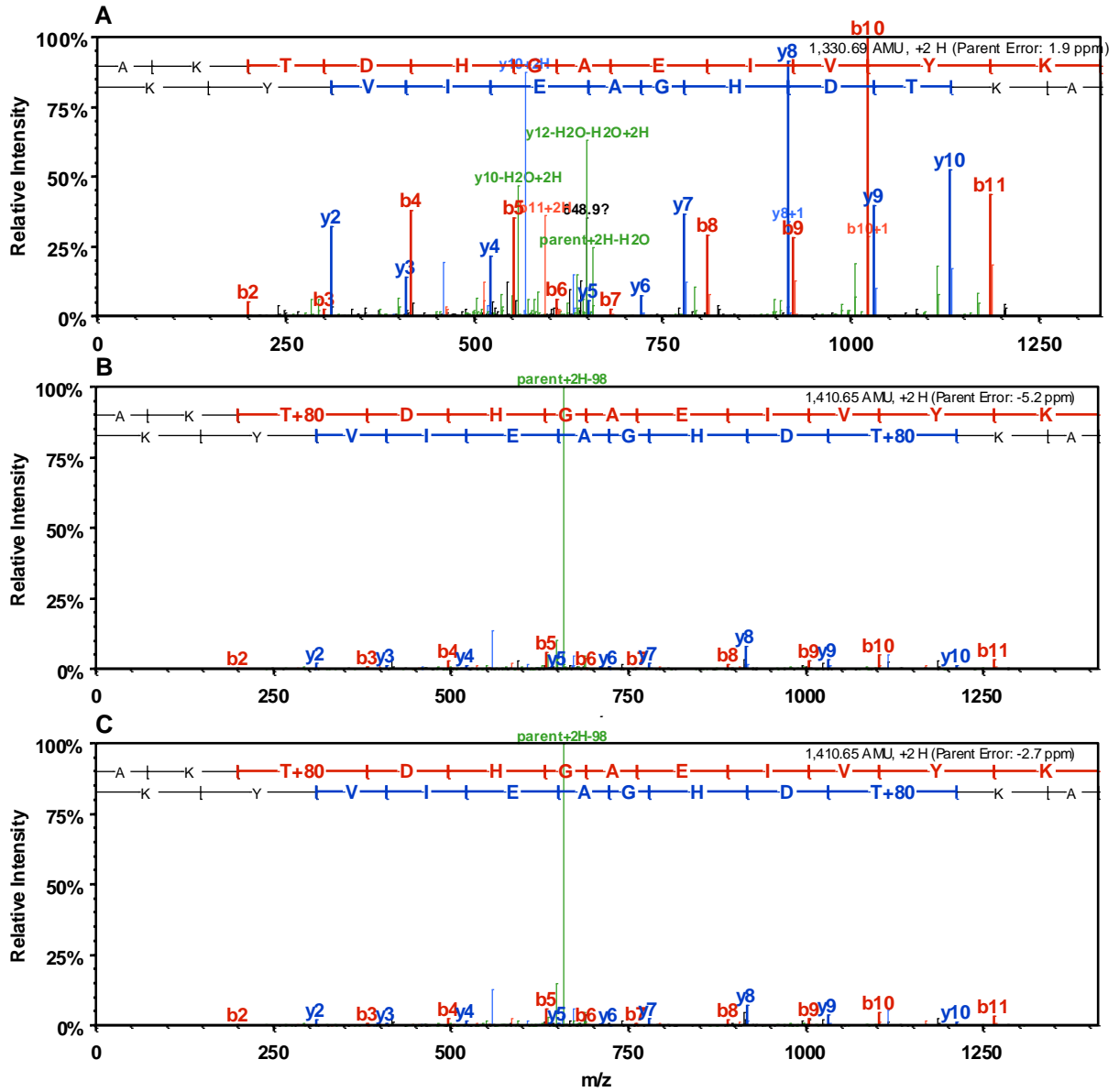


Figure S12. MS/MS spectra of (A) control, (B) Chk1-treated and (C) Chk2-treated samples showing that Thr386 was phosphorylated by Chk1 and Chk2. The corresponding nonphosphopeptide detected in the control is also shown.