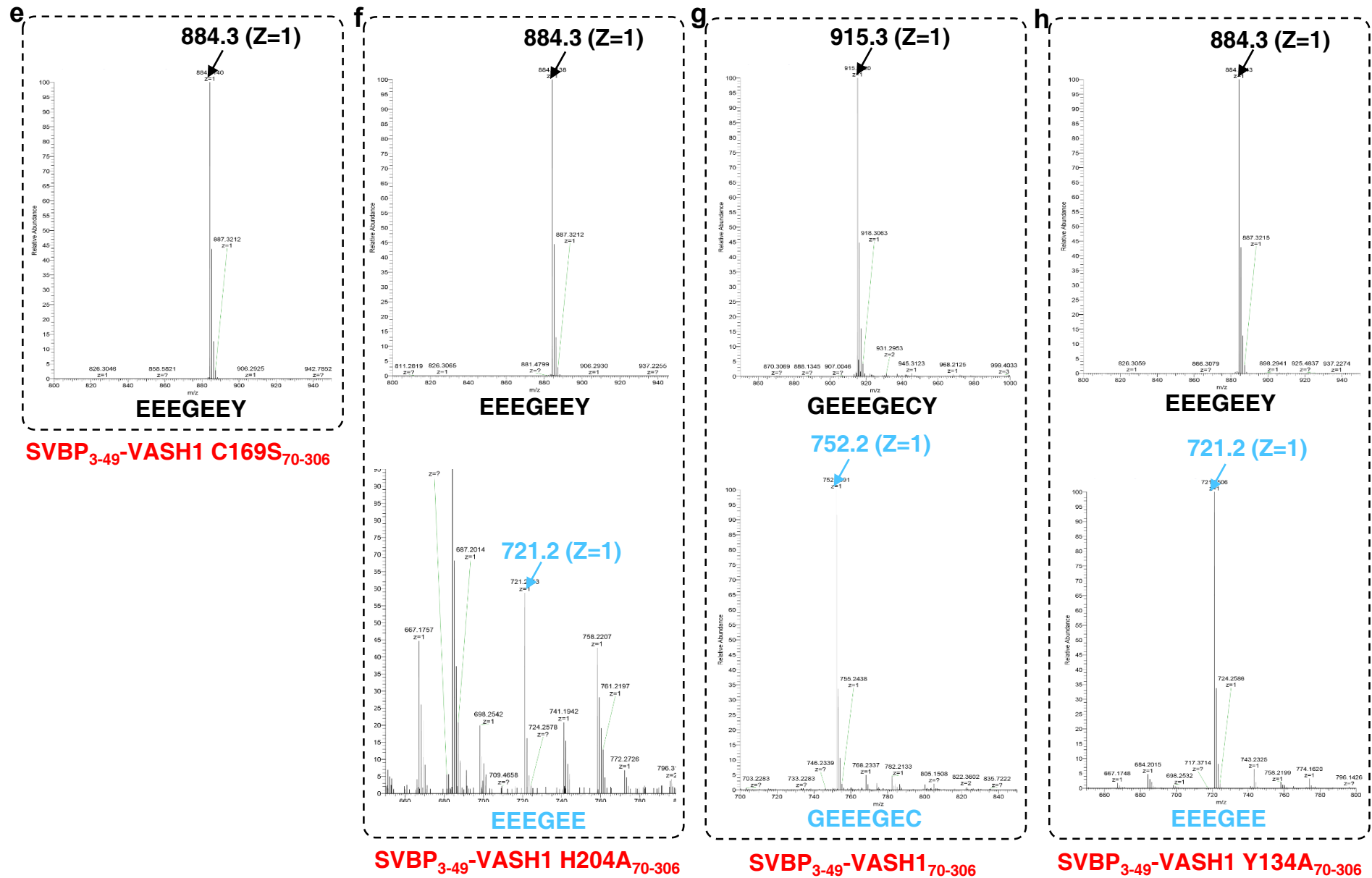
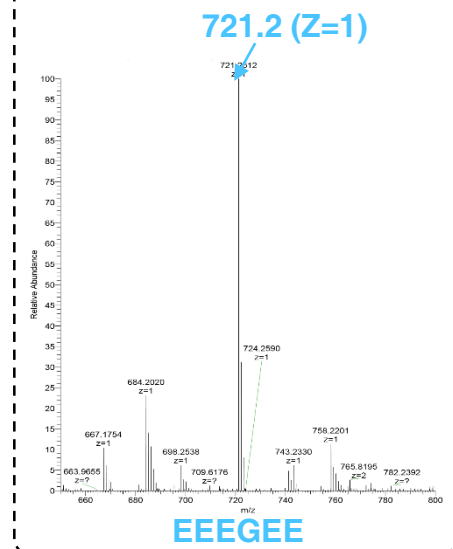
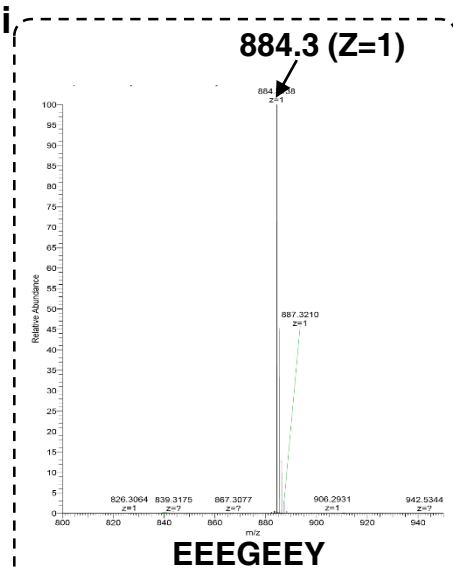
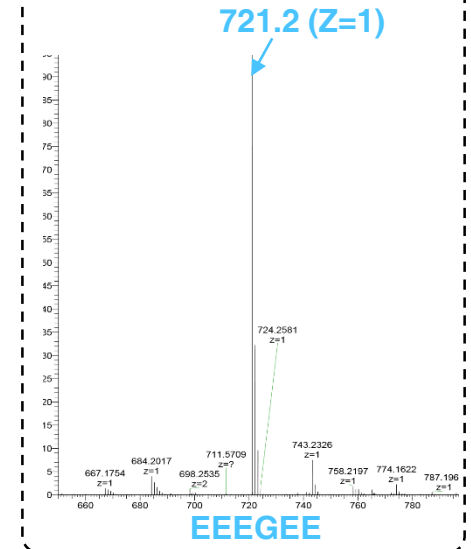
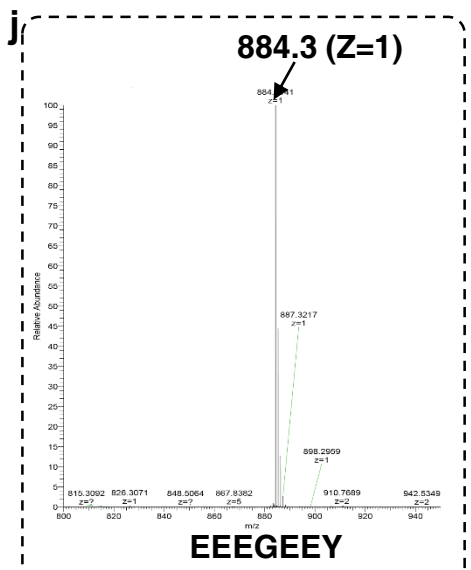


Figure S2 (To be continued)

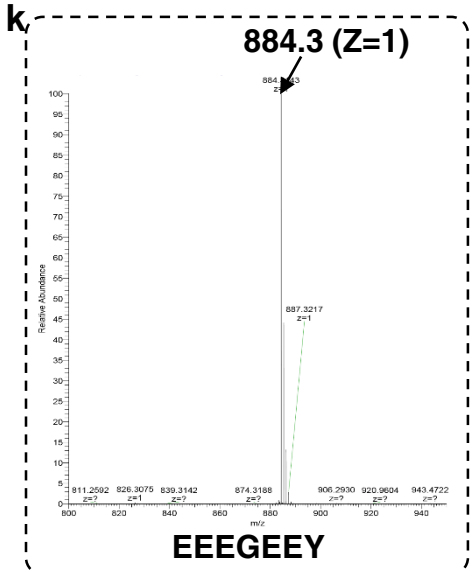




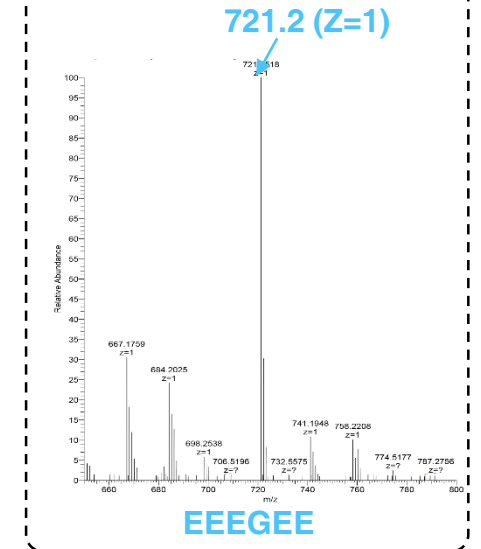
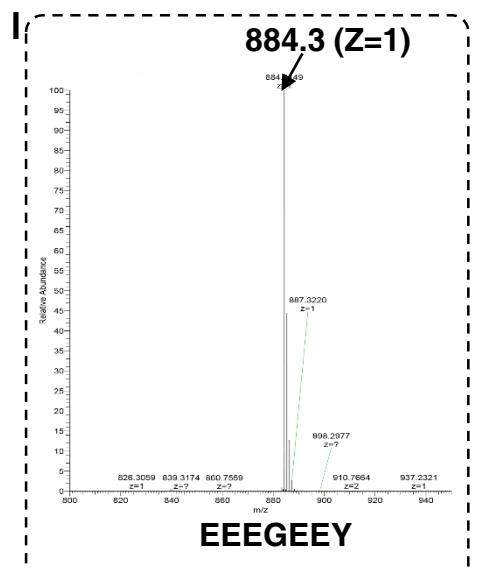
**SVBP<sub>3-49</sub>-VASH1 K146A<sub>70-306</sub>**



**SVBP<sub>3-49</sub>-VASH1 S221A<sub>70-306</sub>**



**SVBP<sub>3-49</sub>-VASH1 R222A<sub>70-306</sub>**



**SVBP<sub>3-49</sub>-VASH1 K146A/R222A<sub>70-306</sub>**

**Figure S2**

**Figure S2. Mass spectra data of  $\alpha$ -tubulin peptides digested by SVBP/VASH1 and its variants.** **a** Spectra of peptides without enzyme as the controls. Spectra of the peptide EEGEEY detyrosinated by **b** SVBP<sub>1-66</sub>-VASH1<sub>70-306</sub>, **c** SVBP<sub>3-49</sub>-VASH1<sub>70-306</sub>, **d** SVBP<sub>3-49</sub>-VASH1 C169A<sub>70-306</sub>, **e** SVBP<sub>3-49</sub>-VASH1 C169S<sub>70-306</sub>, **f** SVBP<sub>3-49</sub>-VASH1 H204A<sub>70-306</sub>, **h** SVBP<sub>3-49</sub>-VASH1 Y134A<sub>70-306</sub>, **i** SVBP<sub>3-49</sub>-VASH1 K146A<sub>70-306</sub>, **j** SVBP<sub>3-49</sub>/VASH1 S221A<sub>70-306</sub>, **k** SVBP<sub>3-49</sub>-VASH1 R222A<sub>70-306</sub>, **l** SVBP<sub>3-49</sub>-VASH1 K146A/R222A<sub>70-306</sub>, and **g** Spectra of the peptide GEEEGECY detyrosinated by SVBP<sub>3-49</sub>-VASH1<sub>70-306</sub>. Molecular weights and sequences of detyrosinated peptides are labelled in blue. The intensities of peptides detected by the mass spectrometry are shown in Table S1.