

**Supplemental Figure 1. MS/MS spectra from eIF3 showing *in vivo* phosphorylation of Prt1 at S61 and T746 and Tif5 at T191.** (A) An MS/MS spectrum generated from trypsin-digested eIF3 showing *in vivo* phosphorylation of a Prt1 peptide at S61. The spectrum significantly matched the trypsin-generated peptide of Prt1 (Sequest Xcorr = 5.5). The spectrum shows the addition of 80 Da in the  $m/z$  values for the  $y_{8-15}$  and  $b_{15-16}$  ions. The  $m/z$  value of the most prominent ion corresponds to the neutral loss of phosphoric acid (+2) from the precursor ion of 1184.18 (+2). (B) An MS/MS spectrum generated from chymotrypsin-digested eIF3 showing *in vivo* phosphorylation of a Prt1 peptide at S61. The spectrum significantly matched the chymotrypsin-generated peptide of Prt1 (Sequest Xcorr = 4.0). The spectrum shows the addition of 80 Da in the  $m/z$  values for the  $y_{7-16}$  ions. The  $m/z$  value of the most prominent ion corresponds to the neutral loss of phosphoric acid (+2) from the precursor ion of 1362.8 (+2). (C) An MS/MS spectrum generated from trypsin-digested eIF3 showing *in vivo* phosphorylation of a Prt1 peptide at T746. The spectrum significantly matched the trypsin-generated peptide of Prt1 (Sequest Xcorr = 3.5). The spectrum shows the addition of 80 Da in the  $m/z$  values for the  $y_{14-16}$  and  $b_{16}$  ions. The  $m/z$  value of the most prominent ion corresponds to the neutral loss of phosphoric acid (+2) from the precursor ion of 1661.1 (+2). (D) An MS/MS spectrum generated from trypsin-digested eIF3 showing *in vivo* phosphorylation of a Tif5 peptide at T191. The spectrum significantly matched the trypsin-generated peptide of Tif5 (Sequest Xcorr = 4.2). The spectrum shows the addition of 80 Da

in the  $m/z$  values for the  $y_{19-24}$  and  $b_{15-16}$  ions. The  $m/z$  value of the peak at 927.2 corresponds to the neutral loss of phosphoric acid (+3) from the precursor ion of 959.8 (+3).