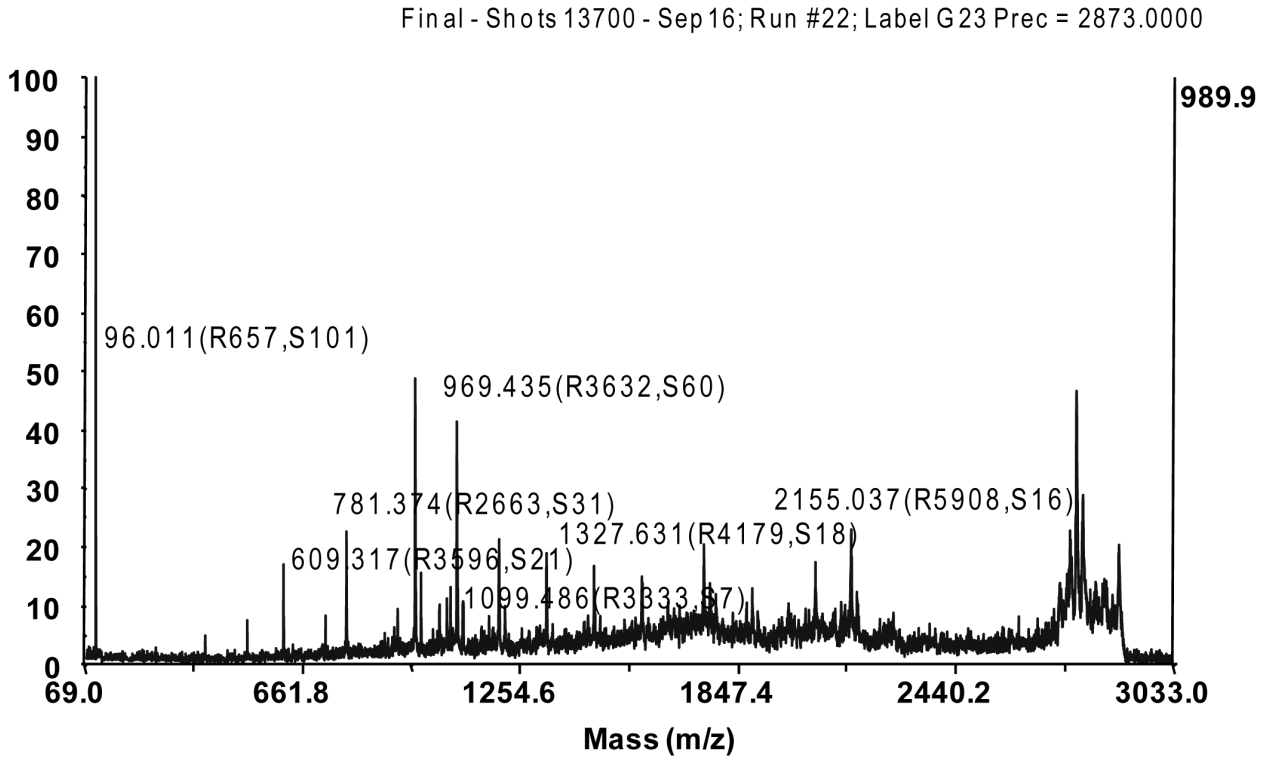
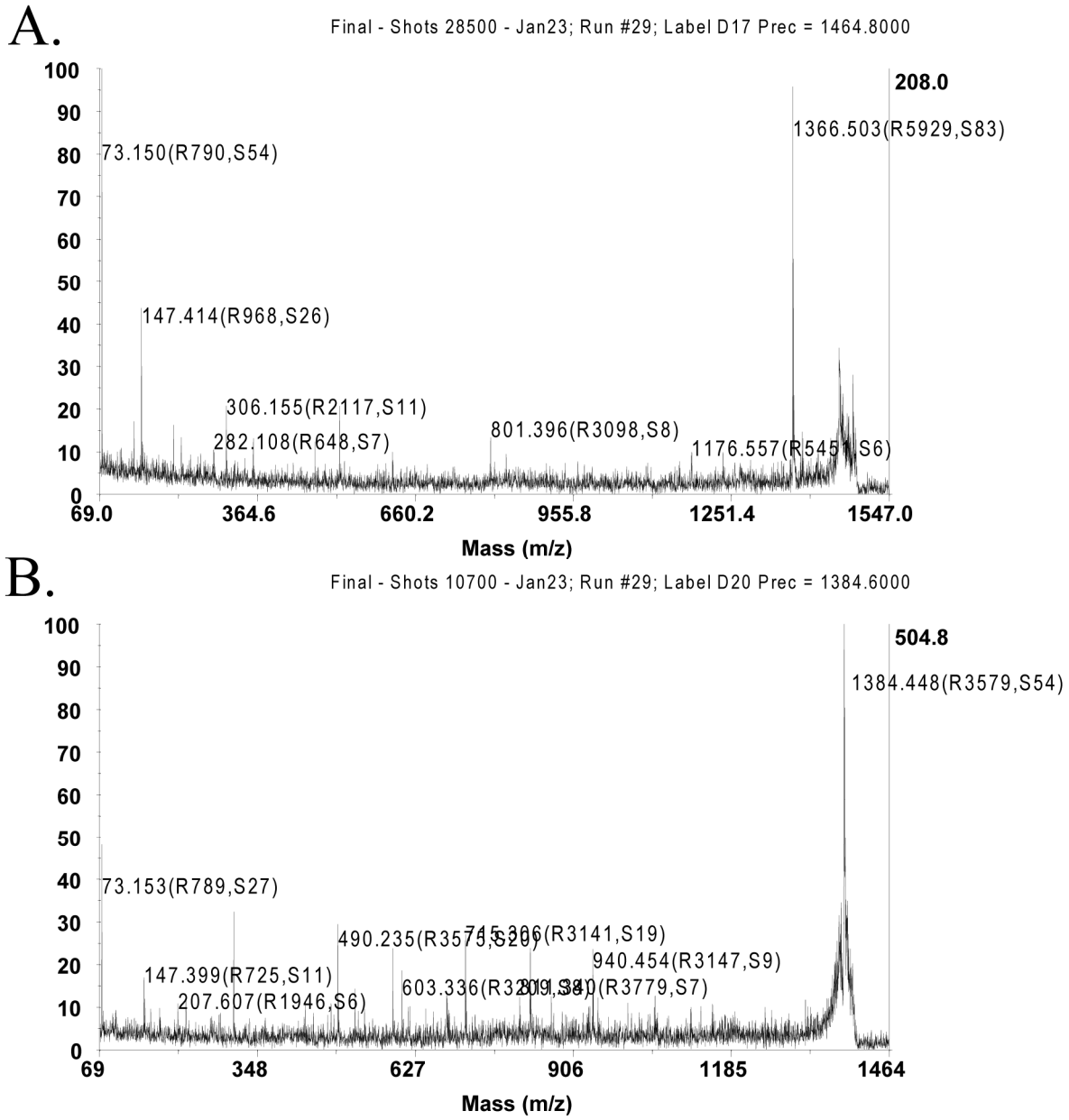
## SUPPLEMENTAL DATA

**Supplemental Table A.**

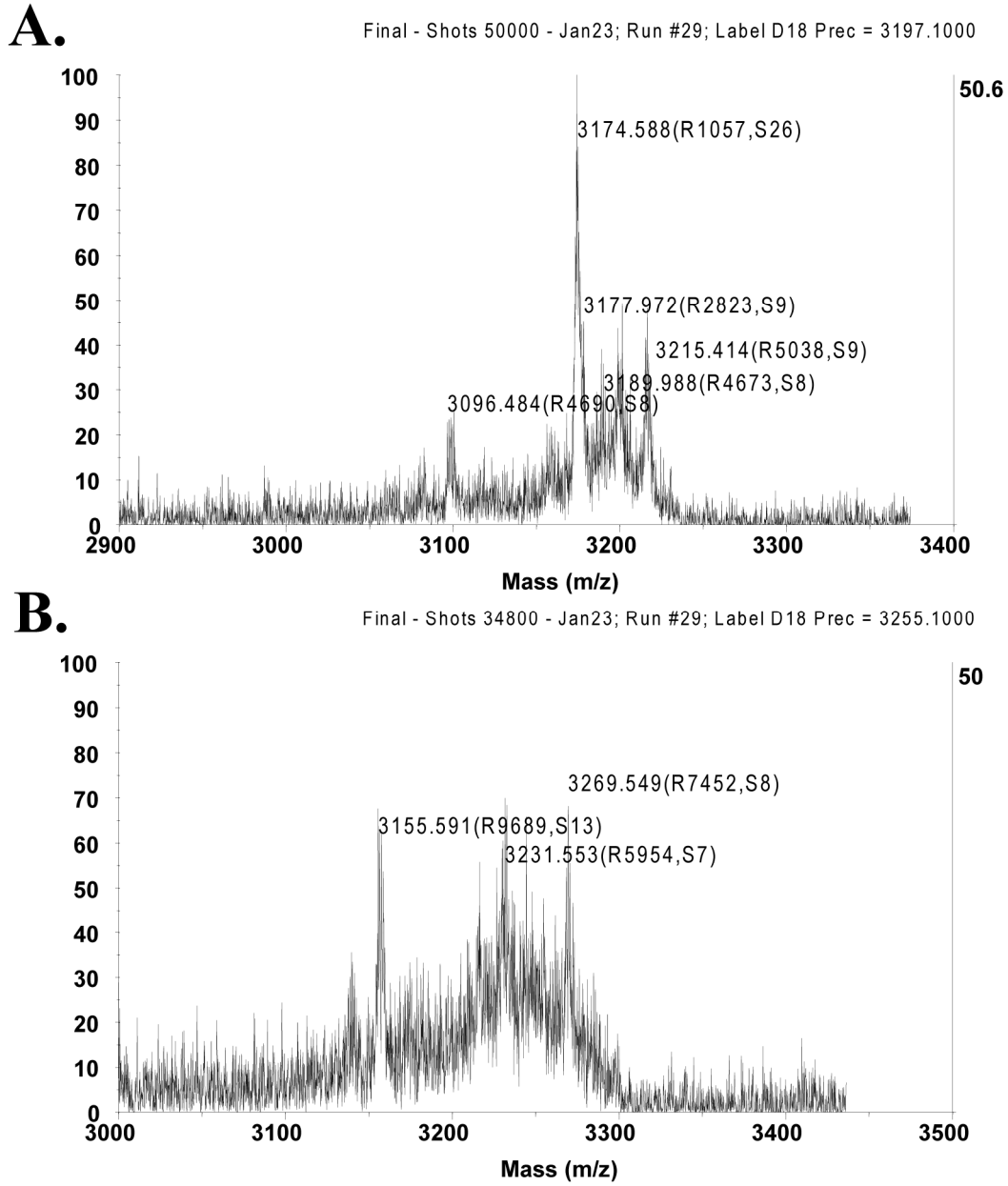
| Primer | Sequence (5'-3') |
|---|---|
| At-CK2A1 5' NdeI | ATA*CATATG*TCGAAAGCTCGTGTGTACACCG |
| At-CK2A1 3' BamHI | AGT*GGATCC*TCATTGACTTCTCATTCTGCTGG |
| | |
| Ta-eIF3c 5' NcoI | AGCT*CCATGG*CGTCTCGTTTTTGGGGAC |
| Ta-eIF3c 3' SacI | AGCT*GAGCTC*CTACCAGGCCTGTTTAGGTTCACC |
| | |
| Ta-eIF2alpha S318A F | TTGCTGGTGATGATGACGCTGAAGATGAGGAAGATAC |
| Ta-eIF2alpha S318A R | GTATCTTCCTCATCTTCAGCGTCATCATCACCAGCAA |
| | |
| Ta-eIF2beta T52A F | GAGGGCTTGTCAGTCGCTGAGTCTGGTGAGG |
| Ta-eIF2beta T52A R | CCTCACCAGACTCAGCGACTGACAAGCCCTC |
| Ta-eIF2beta S54A F | GCTGGAGATGGTGAAGACGCTCTTGATGATCAAGTTG |
| Ta-eIF2beta S54A R | CAACTTGATCATCAAGAGCGTCTTCACCATCTCCAGC |
| Ta-eIF2beta T85A F | CTTGTCAGTCACTGAGGCTGGTGAGGCAAGCTT |
| Ta-eIF2beta T85A R | AAGCTTGCCTCACCAGCCTCAGTGACTGACAAG |
| | |
| Ta-eIF5 S451E F | TGGCTCCAGAGCGCCGAGGAGGACGAGGAGTGAGGATC |
| Ta-eIF5 S451E R | GATCCTCACTCCTCGTCCTCCTCGGCGCTCTGGAGCCA |
| Ta-eIF5 S451A F | CCAGAGCGCCGAGGCCGACGAGGAGTGAGG |
| Ta-eIF5 S451-A R | CCTCACTCCTCGTCGGCCTCGGCGCTCTGG |
| Ta-eIF5 S209A F | AGAAGGGTGCTGGGGGCGCTGATGAGGAACATGTCT |
| Ta-eIF5 S209A R | AGACATGTTCCTCATCAGCGCCCCCAGCACCCTTCT |
| Ta-eIF5 T240A F | ATGATGTACAGTGGGCGGCTGACACGTCAGCAGAGGC |
| Ta-eIF5 T240A R | GCCTCTGCTGACGTGTCAGCCGCCCACTGTACATCAT |



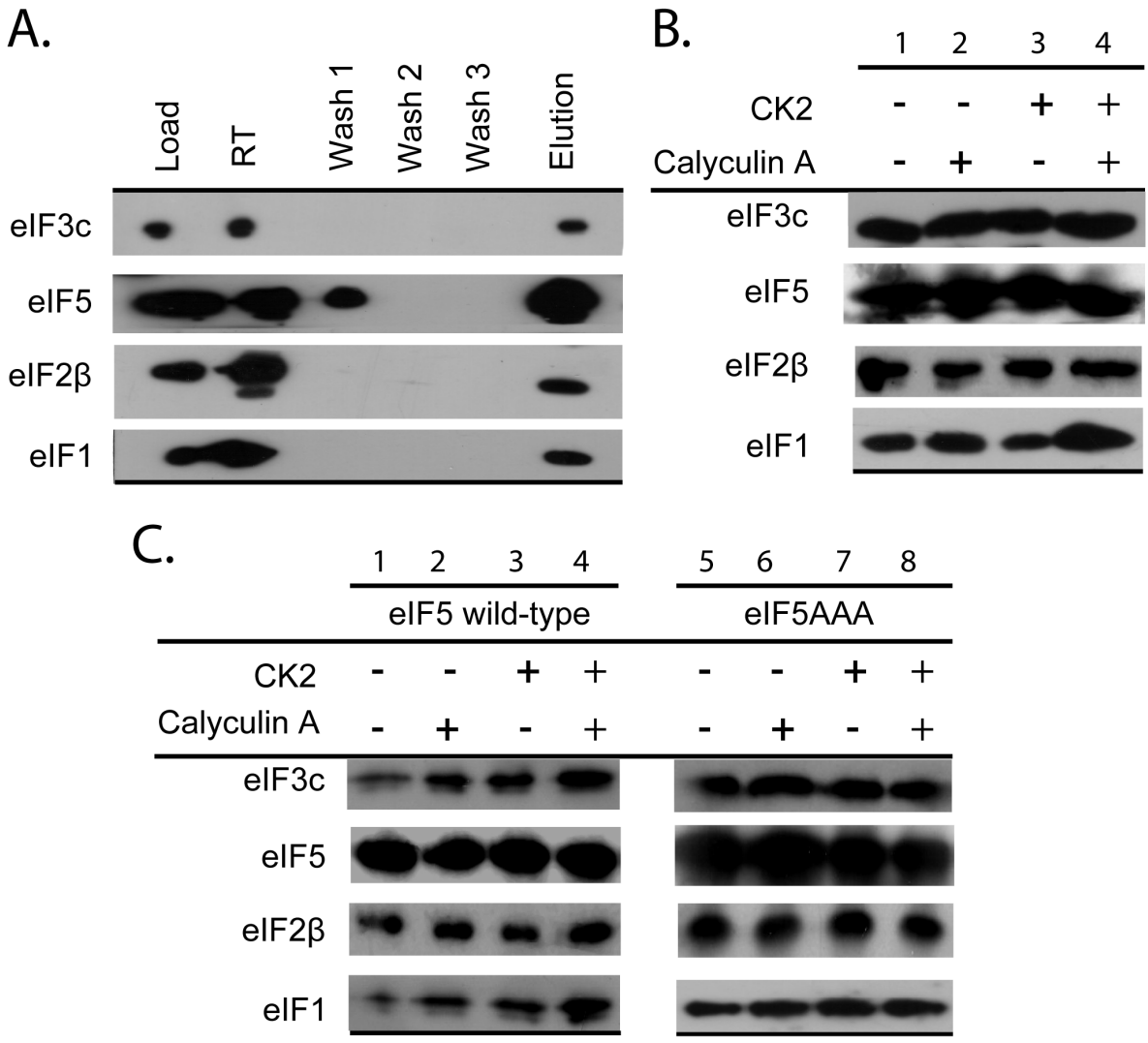

**Supplemental Fig. A:** Native wheat eIF2α is phosphorylated on S318. MS/MS of phosphopeptide 2868.09 (307LAGEEVDGDDDSEEEDNGMGDVDFTK332) matched 20 fragment ions. The following ions were observed: $y_3$ -$y_{13}$ with no Pi, $y_{14}$, $y_{15}$ & $y_{19}$ plus 80 Da, $y_{19}$ minus 98, and loss of 98 Da (phosphoric acid) from the parent ion.




**Supplemental Fig. B**. Native wheat eIF5 peptides provide evidence for S209 phosphorylation. **Panel A**. eIF5 phosphopeptide 1464.64 (*205*GAGG**S**DEEHVSSPR*218* plus 80 Da) is observed, but the only matching fragment ion is the 98 Da neutral loss of phosphoric acid at 1366. The other fragment ions result from neighboring, intense parent ions that are not gated out by the timed ion selector (TIS). **Panel B**. eIF5 peptide 1384.69 (205-218) was observed with three matching ions ($y_6$ -$y_8$). These ions are the dominant fragment ions in the MS/MS of the same peptide observed *in vitro* at higher s/n.




**Supplemental Fig C.** Native wheat eIF5 contains phosphopeptides that provide evidence for T240 phosphorylation. **Panel A**. Phosphopeptide 3197.1 shows neutral loss of 98 at 3096, while peptide 3175 (from isoform TC234445) comes through TIS at resolution 200. **Panel B.** eIF5 phosphopeptide 3255.1 (219DADFAAAADGDDDDDDDVQWATDTSAEAAR248) is observed at plus 80 Da, however only the loss of phosphoric acid at 3155.6 is the only fragment ion. Other fragment ions observed in the MS/MS are from non-phosphorylated fragments from 3115 & 3173. Due to their strong intensity, these fragments are not gated out by the TIS set to 100 resolution.




**Supplemental Figure D**. Immunoprecipitation from wheat germ extracts. Native eIF5 was immunoprecipitated from wheat germ S30 extracts with Protein A-Sepharose crosslinked to affinity purified polyclonal rabbit antibodies raised against recombinant wheat eIF5. The beads were washed (3 x 1 ml) with binding buffer and proteins were eluted with 15 µl of 1X SDS loading buffer. Proteins were separated by SDS-PAGE and eIF3c, eIF5, eIF2β, and eIF1 were visualized using the One-Step Complete IP-Western kit (Genescript). **Panel A**. Immunoprecipitation was performed in the presence of calyculin A (250 nM) and the following are shown 0.5% load, 1% run-through, 1% wash 1, 1% wash 2, 1% wash 3, 100% elution. **Panel B**. A series of immunoprecipitations were performed in the presence and absence of calyculin A (250 nM) and/or CK2 holoenzyme (10 pmol). Shown is the elution from each reaction. **Panel C**. eIF5-depleted extracts were supplemented with 5 µg of recombinant eIF5 or eIF5AAA and immunoprecipitation of eIF5 was performed in the presence and absence of calyculin A and/or CK2 holoenzyme.