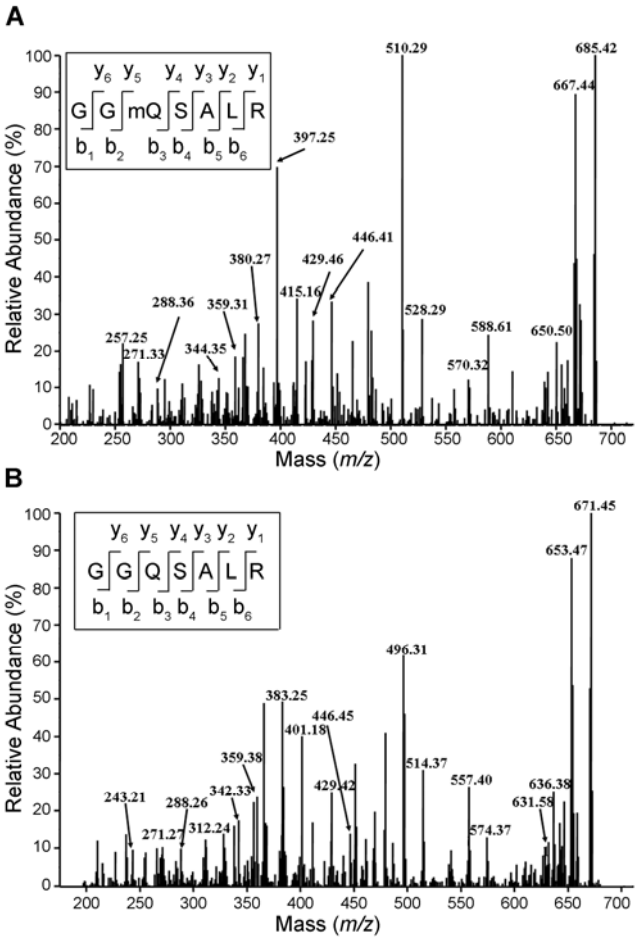
Supplemental Material

Deficiency in a glutamine-specific methyltransferase for the release factor causes mouse embryonic lethality

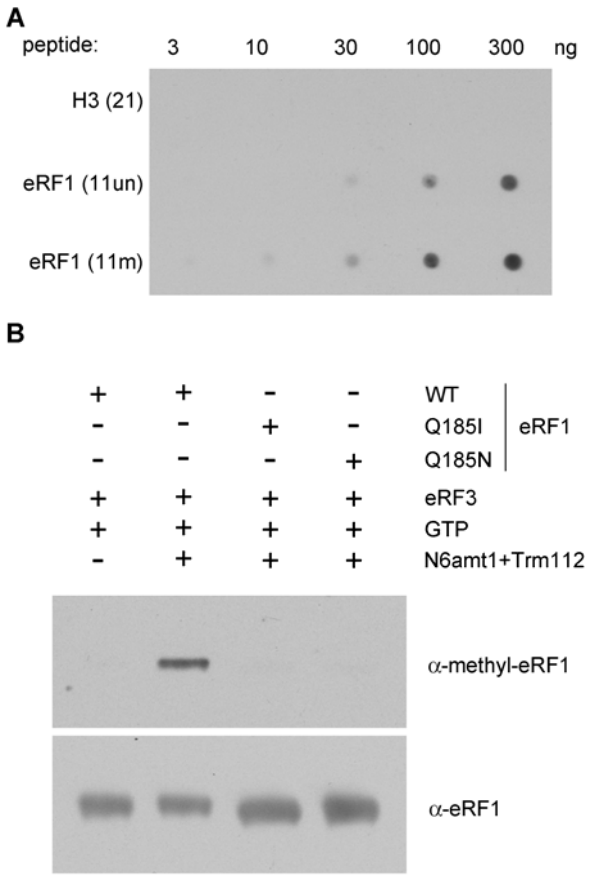
Peng Liu, Song Nie, Bing Li, Zhong-Qiang Yang, Zhi-Mei Xu, Jian Fei, Chyuansheng Lin, Rong Zeng and Guo-Liang Xu

Supplemental FIG. S1.



Supplemental FIG. S1. ESI-MS/MS spectra of Gln185-methylated (A) and Gln185-unmethylated (B) synthetic peptides with an identical sequence to the heptapeptide of eRF1 from trypsin digestion. Note that the spectrum of the peptide with Gln methylation is almost identical to that of the methylated peptide identified in HEK-293T cells (Fig. 1A). The fragmentation pattern of the unmethylated synthetic peptide is similar to that of the peptide derived from recombinant eRF1 (Fig. 1B).

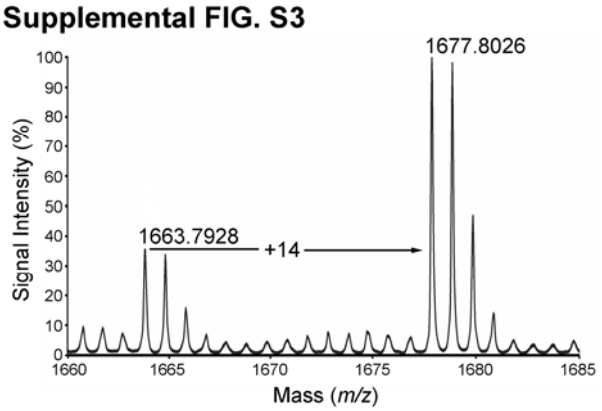
Supplemental FIG. S2.



Supplemental FIG. S2. Characterization of rabbit polyclonal antibodies specific for Gln185-methylated eRF1.

A. Dot blotting assay. Peptides of different amounts were spotted on the membrane and probed with α-methyl-eRF1 antibodies. Peptide H3 (21) with the sequence ARTKQTARKSTGGKAPRKQLA was used as a negative control; eRF1 (11un), HGRGGQSGLRC and eRF1 (11m), HGRGG(mQ)SGLRC.

B. Western blotting assay. The eRF1 wild-type and mutant proteins were incubated *in vitro* with indicated enzyme and co-factors in the presence of cold AdoMet methyl donor and the mixture was then separated by SDS-PAGE for immunoblotting. Anti-methyl-eRF1 polyclonal antibodies were tested for the specific detection of methylated eRF1.



Supplemental FIG. S3. MALDI-TOF MS analysis for Gln185 methylation of endogenous eRF1 from HEK-293T control sample.