Supporting Online Material for

A *Burkholderia pseudomallei* toxin inhibits helicase activity of translation factor eIF4A

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This PDF file includes:

Materials and Methods
Text
Figs. S1 to S13
Tables S1 to S3

Materials and Methods

Bacterial strains, media and reagents

Escherichia coli TOP10F' cells (Invitrogen) were used for cloning and *E. coli* BL21 (DE3) (Invitrogen) used for protein expression and purification. *E. coli* strains were typically grown in Luria Bertani (LB) broth supplemented with ampicillin (25 µg/ml) with agitation at 37˚C. *B. pseudomallei* strain K96243 was obtained from S. Songsivilai, Siriraj Hospital, Thailand and typically grown in LB broth with agitation at 37˚C. Chromosomal DNA was extracted from *B. pseudomallei* by a phenol-chloroform extraction method (*17*). All serum samples were obtained from Defence Science Organisation Laboratories, Singapore from patients diagnosed with melioidosis. All chemicals and reagents were obtained from commercial sources and used as instructed by the manufacturer.

Preparation of protein extract

B. pseudomallei strain K96243 was grown on LB agar plates overnight at 37˚C. The cells were harvested the following day, resuspended in 20 ml PBS, centrifuged at 10,000 x *g* for 15 min and resuspended in 0.5 ml R1 (ReadyPrep Sequential Extraction kit, BioRad). Lysozyme to a final concentration of 10 µg/ml and DNase and RNase at a concentration of 1 µg/ml were added and incubated at room temperature for 30 min. The bacterial suspension was then freeze thawed three times on dry ice. 0.5 ml of R3 (ReadyPrep Sequential Extraction kit, BioRad) was added, incubated for 5 min and centrifuged at 10,000 x *g* for 30 min. The supernatant was collected and stored for analysis.

Identification of the protein encoded by BPSL1549

Two-dimensional gel electrophoresis (2DE), Western blotting and in-gel trypsin digestion were performed as described in (*18*). Matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry and MASCOT was used to identify proteins (again described in (*18*)). Proteins were analysed by online PSORTb v.2.0 (http://www.psort.org/psortb/) to predict subcellular location (*19*) and SignalP 3.0 to infer the presence and location of signal peptides (http://www.cbs.dtu.dk/services/SignalP/) (*20*).

Cloning, expression and purification of proteins

Plasmids used in this study are described in Table S3. Site directed mutagenesis was carried out using the QuickChange II ® Site-Directed Mutagenesis Kit (Stratagene), according to the manufacturer's instructions. The open reading frame encoding BPSL1549 was amplified from *B. pseudomallei* K96243 chromosomal DNA. Primers used for screening *B. thailandensis* strains with no available genome sequence to find BPSL1549 homologues were 5'gatctcgattcgggcaatc and 5'tcctccatccattcgtcgctcg. The protein encoded by BPSL1549 was expressed in *E. coli* BL21 (DE3) cells grown in LB broth containing 1 % (w/v) glucose to the mid-log phase at 37˚C, induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and further cultured for 4 h. Protein expression was detected in culture supernatants by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using a horseradish peroxidase-labelled mouse anti-his IgG antibody. Purification was carried out using immobilised metal affinity chromatography, HisTrap FF columns (GE Healthcare) and an AKTA Fast Protein Liquid Chromatography (FPLC) system (GE Healthcare). *E. coli* cells were sonicated four times for 20 s followed by centrifugation at 27, 000 x *g* for 30 min. The supernatant was filtered through a 0.2 µm filter and applied to the purification column. Purified protein was eluted using a buffer containing 500 mM imidazole and buffer exchanged using a 16/20 desalting column so the resulting protein was buffered in PBS containing 10 % (v/v) glycerol and 2.5 % (w/v) glucose. SDS-

PAGE and brilliant blue R250 stain (Pierce) was used to assess the purity of the protein and the total protein concentration determined using the bicinchoninic acid assay (Pierce).

Crystallization and structure solution

BPSL1549 was overexpressed in *E. coli*, purified and crystallized as previously described (Cruz-Migoni *et al*, in press). Data on native crystals were collected to 1.04 Å resolution and SeMet data sets were collected to 1.9 Å resolution (Table S1). Data for peak, inflection and remote wavelengths were combined with the high resolution native data set and five selenium atoms were identified and used to calculate phases using the ShelX suite (*21*). The map was improved by phase extension to 1.04 Å (Fig S1) and used to construct a molecular model. The structure was refined with ShelX (*22*). Following the final round of refinement hydrogen atoms were introduced with fixed geometry. The programs PROTEP (*23*) and Dali (*24*) were used to compare the BPSL1549 structure with those of all other proteins in the Protein Data Bank (*25*). The structure of the C94S mutant was solved by molecular replacement. The structures were validated using MolProbity (26). Melting curves for the wild type and C94S mutant of the BPSL1549 protein were obtained on a J-810 Spectrophotometer (JAS.CO), using temperature block PFD-425S (JAS.CO) and titrator ATS-429S(JAS.CO). Protein samples were prepared in the buffer contained 0.01M sodium phosphate pH 7.0 at concentration 0.32 mg/ml in the 2mm quartz cuvettes. Melting curves were recorded using Circular Dichroism at 222 nm, in the range of temperatures from 20°C to 100°C at rate of 100°C/hour.

Toxicity towards cultured cells

The BALB/c J774.2 macrophage cell line (European Collection of Animal Cell Cultures) was cultured in Dulbecco's modified Eagle medium (DMEM) containing 4.5 g/L glucose, 2 mM L-glutamine and 1 mM sodium pyruvate, supplemented with 10% fetal bovine serum (FBS; Biowest). Cells were grown at 37°C in a humidified atmosphere of 95% air / 5% CO_2. For toxicity trials, cells (initially 1.6 x 10^4 in 100 μl) were cultured in 96 well plates in the presence/absence of various concentrations of BPSL1549 toxin or the C94S mutant. After 72 h cells were fixed with TCA and cell number assessed using the sulforhodamine B (SRB) assay, where OD at 570nm gives a measure of cellular protein (*27*). Cell numbers in test samples were determined from a standard curve of OD at 570nm vs known cell number. Dose/response curves and statistical analyses were performed using GraphPad Prism 5 software version 5.03 (GraphPad Prism Software). 3T3 Swiss-Albino fibroblasts (Cell Lines Service) were cultured in DMEM containing 4.5 g/L glucose, 4 mM L-glutamine, and 1 mM sodium pyruvate, supplemented with 10% FBS (Hyclone), 100 mg/L streptomycin sulfate and penicillin G (100 000 u/L). Cells were grown at 37°C in a humidified atmosphere of 95% air / 5% CO_2. Cells ($0.7x10^5$) were transfected with purified recombinant protein using the BioPORTER protein delivery reagent QuikEase Kit (Genlantis) in 24-well culture plates grown to 70% density. In brief, protein stocks were diluted to 250 μg/ml with PBS, and one BioPORTER tube was hydrated with 80 μL of the diluted protein solution for 5 min at room temperature before adding 420 μL of serum-free DMEM, giving a final protein concentration in solution of 800nM. Then 125 μL of the 500 μL BioPORTER/protein mix was added to cells grown in 24-well plates and cells were incubated for four hours in the presence of an additional 125 μl serum-free DMEM. Four hours after transfection 250 μL of serum-containing medium was added to the wells and cells were incubated for a further 20 h. For incubations longer than 24 h the BioPORTER/protein mixes were replaced by fresh serum-containing medium and the cells were incubated for up to 72 h. The cytotoxic effect of BPSL1549 towards 3T3 Swiss-Albino fibroblasts was determined by measuring lactate dehydrogenase (LDH) release from the cytosol of cells into the cell culture medium, using the CytoTox 96 nonradioactive cytotoxicity assay kit (Promega). The assay was adapted to quantitate total cell number by measuring the release of LDH from the cytosol of both necrotic and intact (attached)

cells. In brief, cell culture medium (250 μL) was harvested from sample monolayers and intact cells were lysed by freeze-thaw treatment in 250 μL fresh culture medium to measure total LDH release. Sample supernatants and lysates were diluted 1:10 in PBS and samples (50 μL) were transferred to a fresh 96-well enzymatic assay plate, and 50 μL of reconstituted substrate mix was added to each well. The plate was incubated for 30 min at room temperature, protected from light. Absorbance was read at 490 nm using an ELISA plate reader. The absorbance values for each sample were normalized by subtracting the absorbance value obtained for the culture medium background control. Spontaneous LDH release was accounted for by release of LDH from untreated cells. To calculate % cytotoxicity the following calculation was made:

% cytotoxicity= (test supernatant LDH release-background) / (test supernatant LDH release-background) + (test lysate LDH release-background) x 100

Animal challenge

All animal studies were carried out in accordance with the UK Scientific Procedures Act (Animals) 1986. Groups of 6 female Balb/C mice (Charles River) of approximately 6 weeks of age were caged together with free access to food and water and subjected to a 12 h light/dark cycle. Groups of 6 Balb/C mice were immunised intramuscularly with recombinant BPSL1549 or BPSL1549C94S at a concentration of 10 μg delivered in complex with 12.5 μg immune-stimulating complex (ISCOM) AbISCO 100 (Iscomnova, AB) and 12.5 μg of CpG oligodeoxynucleotide (ODN) 10103 (Coley Pharmaceuticals Group), to adjuvant. Alternatively, recombinant BPSL1549 or BPSL1549C94S alone at a concentration of 100 μg was administered intraperitoneally to 6 female Balb/C mice (Charles River). Animals were monitored for 14 days. A group of animals challenged with PBS by the intraperitoneal route was used as a control.

Histology analysis

Animals were culled on days 5, 6 or 9 days post dosing, the spleen, liver, lungs, kidneys, thymus and heart were harvested and examined for gross organ pathology. Three non-immunised animals were also culled, 2 on day 6 and 1 on day 9, blood and organs were harvested. The organs were placed in neutral buffered formalin (NBF). Following primary fixation all organs were resected and further fixed in 10 % NBF for 24 hours prior to histological processing. Tissue processing followed a sequence of dehydration in gradated alcohols, clearing in chloroform and xylene followed by vacuum infiltration with paraffin wax (BDH Ltd) overnight on a Sakura Tissue-Tek E300 vacuum infiltration processor. Wax infiltrated tissue was embedded in wax blocks using a Tissue-Tek IV Sakura wax embedding station. Sections nominally 5μm thick were produced from the wax tissue blocks using a Leica RM2035 rotary microtome and mounted on glass microscope slides (BDH Ltd). Sections were dewaxed with xylene and rehydrated in gradated alcohols before staining with haematoxylin and eosin using a Sakura Linear Stainer II. Stained tissue sections were again dehydrated in graded alcohols and xylene then coverslipped with DPX mounting resin.

Construction of an unmarked in-frame *bpsl1549* deletion mutant in *B.pseudomallei*

Plasmid pDM4-1549, was created to remove 618 bp of *bpsl1549* leaving both the predicted promoter region and stop codon intact. The upstream flanking region (1031 bp) was amplified from *B. pseudomallei* K96243 chromosomal DNA using the primers 5'tctagatacatgctcgcgtccgcg and 5'ggatccaagcaataggcggcagcg. The downstream flanking region (794 bp) was amplified using primers 5'ggatccgttgggcatggtcgtacatc and 5'tctagaccttcagcgtgatctggttg. The PCR products were cloned into pSC-A using the Strataclone^{TM} PCR cloning kit (Stratagene) and the sequence of each of the flanking

regions was confirmed (Cogenics).The flanking regions were cloned together in pSC-A to create the deletion construct flanked by *Xba*I sites and with a central *Bam*H1 site. The deletion construct was excised from pSC-A using *Xba*I, ligated with suitably digested plasmid pDM4 (*28*) and electroporated into *E. coli* DH5α λ*pir*. Subsequently pDM4-1549 was mobilised into *B. pseudomallei* K96243 using conjugation (*29*). Transconjugants were selected on 100 µg/ml ampicillin and 50 µg/ml chloramphenicol and gene deletion mutants then selected by plating approximately 10^5 bacteria onto LB agar (without NaCl) containing 10% (w/v) sucrose. After growth at 24 ºC for 2 -5 days (*30*) colonies were then screened for chloramphenicol sensitivity. The resulting deletion of the *bpsl1549* gene was confirmed by PCR and Southern hybridisation.

Infection of animals and determination of the median lethal dose of *B. pseudomalleiΔ1549*

B.pseudomallei wild type and *Δ1549* strains were grown in LB broth overnight with agitation at 37˚C. The following day a series of dilutions were made at a concentration of 5.8 x 10^6, 5.8 x 10^5, 5.8 x 10^4, 5.8 x 10^3, 5.8 x 10^2 and 5.8 x 10^1 cfu/ml. For each dilution, 100 µl was administered to groups of six Balb/C mice intraperitoneally. After challenge all animals were handled under containment level III conditions within an isolator compliant with British Standard BS5726. The animals were monitored for signs of disease for 5 weeks and culled at pre-determined humane end points. The median lethal dose was calculated at day 35 post challenge, using the method described by Reed and Muench (*31*) .

***B. pseudomallei* Transcriptome Profiling**

A detailed analysis of the *B. pseudomallei* microarray compendium will be presented in a separate report (Ong et al., manuscript in prep). Briefly, bacterial mRNAs were profiled on a high-density *B. pseudomallei* tiling array representing both strands of the *B. pseudomallei* K96243 genome covering all 5855 annotated protein-coding genes (7.2 Mb) (Nimblegen) (50-mers, 15-base overlap) (*32*). Total bacterial RNAs were isolated using Trizol, treated with TURBO DNase I (Ambion), and bacterial mRNAs enriched using the MicrobExpress (Ambion) kit. Strand-specific cDNAs were synthesized using SuperScript II reverse transcriptase (Invitrogen). Purified cDNAs were labeled with either Cy5 or Cy3 fluorescent dyes (Cy5-ULS, Cy3-ULS, Kreatech Diagnostics), and hybridized to arrays as previously described (*33*). Individual Cy5 and Cy3 microarray profiles were acquired using an Axon scanner and normalized using the LOWESS algorithm. Microarray profiles were median-normalized prior to data analysis. Differentially expressed probes were identified using Genespring GX11 software, using a 2.0-fold change cut-off. Log2-transformed fold change of *bpsl1549* was computed by measuring the median value of fold-changes of all probes corresponding to the *bpsl1549* genic region. Error bars were computed using standard deviations of transformed fold-changes of probes within genic regions. P-values were computed using the Wilcoxon Sign Rank test comparing expression levels of *bpsl1549* probes between test and reference conditions.

Co-immunoprecipitation Experiment

Twelve wells from 24 well plates of 293T cells each transfected with 700 ng FLAG-tagged BAP (Bacterial Alkaline Phosophatase), BPSL1549 or BPSL1549C94S were lysed in IP lysis buffer (50mM HEPES pH7.5, 100mM NaCl, 1mM EDTA, 1mM DTT, 0.5% Triton X-100, 10% glycerol) 24h post-transfections. Extracts were subjected to immunoprecipitation for 1 hour at 4 ºC using 30 µl slurry M2-FLAG agarose beads (*Sigma*). Bound proteins were eluted by competition with 0.1 mg/ml 3xFLAG peptide (*Sigma*), before analysis by Western immunoblotting using mouse monoclonal α-FLAG (*Sigma*) or rabbit α-eIF4A1 (*Cell Signalling*).

Mass Spectrometry Identification of BPSL1549 target

293T cells grown in 15 cm plates were washed with PBS and lysed in buffer (50mM HEPES pH7.5, 100mM NaCl, 0.5% Triton X-100) supplemented with 1 mM PMSF and Complete/EDTA free protease inhibitors (*Roche*). Total 293T whole cell extract (WCE - 2 mg) was added to recombinant hexa-histidine tagged YloQ or BT immobilised on TALON/Cobalt beads (*Clontech*). Proteins were eluted from washed beads with buffer containing 1 M NaCl and analysed by SDS-PAGE stained with Instant Coomassie blue (*Expedeon*) and mass spectrometry or by western immunoblotting with rabbit α-eIF4A1 (*Cell Signalling*). For ESI TOF MS/MS analysis following SDS PAGE, in gel tryptic digestion was performed. Briefly proteins were subjected to in gel digestion using 200 ng trypsin/band at 37 °C overnight. Peptides were extracted from the gel using acetonitrile, dried under vacuum and resuspended in 0.1% final concentration of TFA. 5 μL was used for LC-MS/MS analysis. Peptides were separated using an Ultimate 3000 liquid chromatography system (Dionex, UK), using a 150 mm × 75 μm i.d. PepMap reversed phase column (Dionex, UK). Linear gradient elution was performed from 95% buffer A (0.1% formic acid) to 50 % buffer B (0.1% formic acid, 95 % acetonitrile) at a flow rate of 300 nl/min in 60 mins. MS/MS analysis was performed using a maXis UHR TOF mass spectrometer (Bruker Daltonics) using an automated acquisition approach. MS and MS/MS scans (m/z 50-3000) were acquired in positive ion mode. Lock mass calibration was performed using HP 1221.990364. Line spectra data was then processed into peak list by Data analysis using the following settings. The sum peak finder algorithm was used for peak detection using a signal to noise (S/N) ratio of 10, a relative to base peak intensity of 0.1% and an absolute intensity threshold of 100. Spectra were deconvoluted and the peak lists exported as Mascot Generic Files (MGF) and searched using Mascot 2.2 server (Matrix Science) The Swissprot database was searched using the following parameters (analysis peptide tolerance = ± 0.1 Da, MS/MS tolerance = ±0.1 Da, peptide charge 2+ and 3+. Tryptic enzyme specificity with up to two missed cleavages was applied to all searches. Oxidized methionine, deamidation (NQR) were used as a variable modifications for the tryptic digests. All tandem MS spectra of the modifications identified were manually verified.

Deamidation of eIF4A by BPSL1549

3 x 6 cm plates of 293T cells were each transfected with 4 μg p3X-FLAG-eIF4A and either 2 μg pcDNA MycHisA or pcDNA MycHisA-BPSL1549. PBS-washed cells were lysed 30 hours post-transfections in 1.2 ml lysis buffer (50mM HEPES pH 7.5, 100mM NaCl, 1mM EDTA, 1mM DTT, 0.5% Triton X-100) containing 1 mM PMSF and *Complete* (*Roche*) protease inhibitors. Extracts were supplemented with 750 mM NaCl and subjected to immunopurification onto 100 μl slurry M2-FLAG agarose beads (*Sigma*). Bound proteins were eluted by competition with 140 μl lysis buffer containing 0.1 mg/ml 3xFLAG peptide (*Sigma*) before analysis by SDS-PAGE stained with Instant blue (*Expedeon*). Mass spectrometry analysis was performed on cut eIF4A bands. *In vitro* deamidation of eIF4A by BPSl1549 was performed in buffer contained 0.1 M NaCl and 0.05M HEPES pH 7.3 at 37°C. eIF4A was used at 36 μM and BPSL1549 at a concentration of 0.065 μM. Samples were preheated at 37°C. To start the reaction 10μl of BPSL1549 solution was added to 90 μl of eIF4A solution, so the ratio BPSL1549/eIF4A in the reaction mixture was 1/5000. Samples (5μl) were taken from the reaction mixture at the indicated times and denatured in 20 μl of the boiling Nu PAGE SDS sample buffer (Invitrogen). Samples were applied on a Nu PAGE 4-12% BT gel (Invitrogen) to separate eIF4A from BPSL1549.

Reporter Assays

For enzymatic assays carried out with BPSL1549 expression plasmids, 3 wells from 24 well plates of 293T cells were each transfected with 200 ng p3X-FLAG vector or 190 ng p3X-FLAG + 10 ng p3X-FLAG-BPSL1549 or 190 ng p3X-FLAG + 10 ng p3X-FLAG-BPSL1549C94S and either 50 ng pGL3 (for Luciferase assays) or pcDNA-LacZ (for β-galactosidase assays). Transfections were carried out in triplicate and luciferase and β-galactosidase assays measured in triplicate 36 hours post-transfection. For qRT-PCR analysis, total RNA from 3 wells from a 24 well plate of similarly transfected 293T were extracted with 750 µl TRIZOL-LS as indicated by the manufacturer (*Invitrogen*). RNA was treated with RNAse free DNaseI (*Roche*) before heat inactivation. 2 µg RNA (quantified by spectrophotometry at OD_{260}) were used for cDNA synthesis using $poly(dN)_6$ random priming as described by the manufacturer (Bioscript kit from *Bioline*) in presence or in absence of reverse transcriptase (*Bioscript* from *Bioline*). 35 µl H_2O were added to 20 µl cDNA reactions and 1 µl diluted cDNA with 5 ng/µl primers were used in 10 µl quantitative PCRs (*Quantace*) run on a Rotorgene 6000 (*Qiagen*). For enzymatic assays carried out with eIF4A expression plasmids, 3 wells from 24 well plates of 293T cells were each transfected with 200 ng p3X-FLAG-eIF4A wild type or mutant plasmids and either 50 ng pGL3 (for Luciferase assays) or pcDNA-LacZ (for β-galactosidase assays). Transfections were carried out in triplicate and luciferase and β-galactosidase assays measured in triplicate 24 hours post-transfection.

Metabolic cell labeling

Eight wells from 24 well plates of 293T cells were each transfected with 700 ng FLAG-tagged BAP, BPSL1549 or BPSL1549C94S plasmids and 2 wells of each were labelled every 12 hours for 48 hours. For labeling, PBS-washed cells were starved with 500 µl minimal medium lacking L-methionine and L-cysteine (*Gibco*) for 1 hour, before incubation for 1 hour with 480 µl labelling medium containing 60 µCi/ml ^{35}S-L-methionine and ^{35}S-L-cysteine (Express Protein Labelling Mix, *Perkin Elmer*). Cells were washed 3 times with 500 µl PBS before lysis in 80 µl reporter lysis buffer (*Roche*). Radioactivity emitted by total extracts was counted with scintillant liquid using a Beckman counter.

Polysome gradients

4 x 6cm plates of 293T cells grown to low density were each transfected with 6 µg p3X-FLAG or 6 µg p3X-FLAG-BPSL1549 plasmids and the medium was changed 8 hours post-transfection. Cells were then washed with 1ml PBS containing 0.1 mg/ml cycloheximide 24 hours post-transfection, before lysis in 1.2 ml SGB buffer (20mM TRIS pH8, 140 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT) containing 0.1 mg/ml cycloheximide and RNAse/protease inhibitors. Cell lysis was achieved 5 minutes on ice and extracts were subjected to two successive centrifugations performed at 4ºC (5 min at 5000rpm and 5min at 10000rpm). Subsequently, 500 µl extract was loaded onto 13 ml pre-equilibrated 50% to 10% sucrose gradients run in SW41 rotor (*Beckman*) for 3 hours at 35000rpm, 4ºC (as described from Stanford University at http://genome-www.stanford.edu/yeast_translation). UV-absorbance of 500 µl fractions collected at 0.5 ml/min from top (10% sucrose) to bottom (50% sucrose) was recorded before fractionated proteins were analysed by western immunoblotting with rabbit polyclonal α-PABP (a kind gift from S. Morley).

Stress granule analysis

Immunofluorescence experiments were performed on transfected HeLa cells grown on coverslips 24 hours post-transfection using mouse α-TIAR antibody at 1/500 (*BD Biosciences*). Cells were treated with 2.5 mM Na Arsenate for 1 hour when indicated. For western blot analysis, 12 wells from 24 well plates of 293T cells grown to low density were each transfected with 700 ng FLAG-tagged GFP or BT

plasmids. Medium was replaced 8 hours post-transfections. Cells were treated with 2.5 mM Na Arsenate for 1 hour when indicated. PBS-washed cells were lysed in a total of 200 µl IP lysis buffer containing protease inhibitors and total cell extract were analysed by western immunoblotting with mouse monoclonal α-FLAG (*Sigma*), rabbit α-eIF2α (*Invitrogen*), rabbit α-eIF2α[pS^{52}] (*Invitrogen*), and mouse monoclonal β-tubulin (*Sigma*) antibodies.

Helicase assays

Unwinding of duplex RNA was monitored by following the displacement of a short ^{32}P-end radiolabeled strand (GCUUUACGGU) from the duplex formed with a long non-labeled strand (AAAAACAAAACAAAACAAAACAAAACUAGCACCGUAAAGC) essentially as described in (*34*). Briefly, 2 µg recombinant UAP56-6His or 6His-eIF4A (aa20-406) and mutants synthesised in *E. coli* were incubated in the presence or absence of recombinant Magoh-6His or eIF4B-6His at a 4:1 molar ratio for 10 minutes at room temperature prior addition of duplex RNA and ATP. Products of reactions were run on 15% native polyacrylamide gels in TBE buffer before autoradiography.

m7-GTP capture assays

For pull down analysis, PBS-washed 293T cells grown for 48 hours in 1 x 15 cm plate were lysed in 2 ml IP lysis buffer (50 mM HEPES pH7.5, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5 % Triton X-100, 10 % glycerol) supplemented with 1 mM PMSF and *Complete* (*Roche*) protease inhibitors and RNase inhibitors (*Bioline*). Extract was incubated with 30 µl slurry Protein G-sepharose or 7-methyl GTP-sepharose 4B (*GE Healthcare*) in presence or absence of either 100 µg purified recombinant SC35 (9-101)-6His or BPSL1549-6His or BPSL1549C94S-6His for 2 hours at 4ºC. Bound proteins were eluted with SDS-PAGE loading buffer and analysed by western immunoblotting using rabbit polyclonal α-eIF4E, α-eIF4G1, α-PABP antibodies (a kind gift from S. Morley) and rabbit α-eIF4A1 (*Cell Signalling*). For eIF4A recycling assays, PBS-washed 293T cells grown for 48 hours in 2 x 15 cm plates were lysed in 4 ml IP lysis buffer supplemented with 1 mM PMSF and *Complete* (*Roche*) protease inhibitors and RNase inhibitors (*Bioline*). Extract was incubated with 30 µl slurry ProtG-sepharose or 7-methyl GTP-sepharose 4B in presence or absence of either 100 µg purified recombinant SC35 (9-101)-6His or BPSL1549-6His toxin for o/n at 4ºC. IP lysis buffer-washed beads were further incubated with/without 1 µg or 5 µg purified recombinant 6His-eIF4A (20-406) in 1 ml IP lysis buffer for 1 hour at 4ºC. Bound proteins were eluted with SDS-PAGE loading buffer and analysed by western immunoblotting using rabbit polyclonal α-eIF4E (a kind gift from S. Morley), rabbit α-eIF4A1 (*Cell Signalling*) and α-polyHis HRP conjugate (*Sigma*).

***In vitro* pull down assay**

For pulldown assays, 20 µg purified recombinant SC35 (9-101)-6His, BPSL1549-6His or BPSL1549C94S-6His were first immobilised onto 40 µl slurry TALON/Cobalt beads (*Clontech*) before various ^{35}S-labelled eIF4A proteins synthesised in T7-rabbit reticulocytes (*Promega*) were incubated with the beads in PBS + 0.1% Tween for 1 hour at 4ºC. Eluted proteins were analysed by SDS-PAGE stained with Coomassie blue and by Phosphoimage.

ATPase assays

ATPase assays were essentially performed as described in (*35*). Reactions of 50 µl were stopped by the addition of 10 µl 0.5M EDTA and supplemented with 140 µl H_20 before addition of 800 µl of Malachite green-Phosphomolybdenum reagent (*36*).

ATP binding

For ATP binding analysis, 1 µg FLAG-tagged eIF4A immunopurified from 293T cells transfected with either pcDNA MycHisA or pcDNA MycHisA-BPSL1549 (see paragrah de-amidation) was incubated in IP lysis buffer with γ^{32}P-ATP (*Perkin Elmer*) in various ATP:protein molar ratios (1:10, 1:100 and 1:1000). Reactions were UV-irradiated on ice before analysis on SDS-PAGE stained with Coomassie blue and by PhosphoImaging.

***In vivo* RNA binding to eIF4A**

For *in vivo* RNA binding analsyis, 4 x 6cm plates of 293T cells were each transfected with 4 µg p3X-FLAG or 4µg p3X-FLAG-eIF4A and either 2 µg pcDNA MycHisA or pcDNA MycHisA-BPSL1549. Cells from 2 plates were UV-irradiated or not on ice with 0.3 J/cm^2 in 500 µl PBS (24 hours post-transfections) and lysed in 1.2 ml IP lysis buffer (50mM HEPES pH7.5, 100mM NaCl, 1 mM EDTA, 1mM DTT, 0.5% Triton X-100, 10% glycerol) containing 1 mM PMSF and *Complete* (*Roche*) protease inhibitors. Extracts were supplemented with 0.35 M NaCl before FLAG immunopurification using 150 µl slurry FLAG M2 agarose beads (*Sigma*) for 2 hours at 4ºC. Beads were washed with IP Lysis buffer and incubated with 10 µg RNase A in 400 µl IP lysis buffer for 15 minutes at 37ºC. Bound proteins were eluted by competition with 60 µl IP lysis buffer containing 0.1 mg/ml 3xFLAG peptide (*Sigma*) for 30 minutes at 4ºC. Digested mRNA bound to eIF4A were end-labelled with Polynucleotide Kinase (*New England Biolabs*) and reactions were resolved on SDS-PAGE stained with Coomassie blue or analysed by phosphoimage.

Supporting Online Material (SOM) Text

BPSL1549 toxicity in mice

The gene encoding BPSL1549 was cloned and expressed in *E. coli* as a his-tagged protein and purified. A dose of 100 µg of recombinant BPSL1549 given by the intraperitoneal route was lethal to 100% of Balb/C mice by day 14. When injected in the presence of ISCOMs and CpG, the toxicity of BPSL1549 was markedly increased and 10 µg of the toxin was sufficient to kill mice. We have not investigated the basis for this increase in toxicity which may, in any event, not be relevant to the biology of the toxin, given the intracellular lifestyle of *B. pseudomallei*. Toxicity was abolished when BPSL1549 was heated before injection or if mice were treated with the C94S mutation. To confirm that the C94S mutation did not alter the stability or structure of BPSL1549 we examined their CD spectra and further used CD to determine a melting curve. These studies showed that the wild type and mutant proteins had no significant differences in their CD spectra or thermal stability (Fig. S3B,C). We further purified and crystallized the C94S mutant and determined its high resolution structure which was essentially identical to the wild type BPSL1549 (RMSD 0.3Å) (Table S1). To investigate the pathology associated with BPSL1549, 100 µg of protein was administered to 9 mice intraperitoneally, and on days 5, 6 and 9, mice were culled, blood collected by cardiac puncture and organs harvested. Three control mice were culled to investigate the possibility of tissue pathology in pre-symptomatic animals. At 6 days, challenged animals were starting to show signs of illness and by day 9 they were all ruffled, pinched and hunched. Microscopy revealed that hepatocytes in animals which had been challenged with purified BPSL1549 9 days previously, showed megaocytosis and pyknotic nuclei and mitotic figures were visible in some cells. Mononuclear and neutrophil infiltration and haemosiderin accumulation within Kupffer cells was also observed. None of the control animals showed any of these pathological changes. No signs of gross pathology or weight changes were evident in any of the other organs examined (spleen, lungs, kidneys, thymus and heart) compared to the controls (data not shown).

bpsl1549* is consistently present in *B. pseudomallei

BLASTP or TBLASTN searches, using the *bpsl1549* open reading frame, revealed matches with a protein encoded on chromosome 1 of the following *B. pseudomallei* strains: K96243, 1710b, 1106a, 668, 1106b, 1655, 1710a, 406e, 576, MSHR346, Pasteur 52237 and S13. A PCR-based screen of 13 clinical and animal isolates from Malaysia and the reference K96243 strain confirmed the presence of the BPSL1549 gene. This was further validated by comparative genomic hybridization of 4 isolates on a customized *B. pseudomallei* oligonucleotide array (data not shown).

Expression of *bpsl1549* is associated with multiple virulence cues

A protein extract from *B. pseudomallei* strain K96243 was separated by 2D PAGE and silver stained to visualise individual spots. Western blotting using pooled convalescent sera taken from melioidosis patients identified several immunoreactive proteins expressed during infection, one of which was subsequently identified by mass spectrometry as BPSL1549. To identify specific environmental and biological factors regulating expression of *bpsl1549*, we surveyed a global microarray compendium of *B. pseudomallei* exposed to more than 80 physical, chemical, and genetic perturbations. We observed a striking association between *bpsl1549* expression and conditions relevant to microbial virulence and survival in mammalian hosts (Table S2). Specifically, *bpsl1549* was highly upregulated upon *B. pseudomallei* exposure to 30% human serum (13-fold), taurine, an animal-specific amino acid (3-fold; (*32*)); and physiological insulin concentrations (2.5-fold) (Fig. 2E). However, certain conditions known to be virulence cues, for example iron supplementation, did not result in upregulation of *bpsl1549*. Conversely, *bpsl1549* was downregulated under conditions of extreme stress not commonly associated with the mammalian milieu, including nutrient deprivation (17-fold), and antibiotic, osmotic, and temperature stress (4-12 fold). Intriguingly, *B. pseudomallei* mutants genetically disrupted in Type III and VI signaling, two major *B. pseudomallei* virulence systems (*37*);(*38*) also exhibited *bpsl1549* downregulation (2.9-6.4 fold, Fig. 2E). These observations are consistent with *bpsl1549* expression being required to mount a successful mammalian infection, supporting an intimate linkage between *bpsl1549* regulation and the canonical *B. pseudomallei* virulence machinery.

Mass Spectrometry identification of deamidation of eIF4A by BPSL1549

In gel tryptic digestion of eIF4A in conjunction with ESI LC MS/MS analysis was performed as described (see supporting methods) enabling high resolution and low ppm mass accuracy in both the precursor and product mass. The MS analysis identified a single tryptic peptide GIDVQQVSLVINYDLPTNR from eIF4A cotransfected with BPSL1549 or treated with BPSL1549 with an observed increase in mass of 1 Da. The intact MS spectra of the unmodified peptide (1072. 5497 m/z) $[M+2H]^{2+}$ and modified peptide (1073.0619 m/z) $[M+2H]^{2+}$ from eIF4A is shown in Fig S8A and Fig S8B, a difference in mass of 1Da is observed taking into account the peptide is doubly charged. Fig S8C shows the intact mass of the modified peptide from eIF4A following incubation with BPSL1549 in the presence of 50% $H_2{}^{18}O$. The results show incorporation of the stable isotope from the heavy water following the reaction with BPSL1549, therefore confirming enzymatic deamidation of this peptide. The tandem MS analysis of the modified peptide from eIF4A (see Fig. 4A, Fig S8D) revealed unambiguous deamidation of Gln^{339} by virtue of the presence of number of fragment ions including the b_4 (514.2634 m/z), y_{14} (1631.8778) and y_{15} (1760.92480) ions where a +1 Da mass increase is observed following deamidation of Gln^{339}. The tandem MS spectrum of the unmodified peptide is shown in Fig S8E.Mass spectrometry analysis of the enzymatic deamidation of recombinant eIF4A with BPSL1549

was also performed. Following incubation of eIF4A with BPSL1549 at a ratio of 5000:1, the reaction was stopped and analysed using SDS PAGE. In gel tryptic digestion of eIF4A and ESI LC MS/MS analysis was performed. The deamidation reaction was monitored by analysing the extracted ion chromatograms of the unmodified peptide GIDVQQVSLVINYDLPTNR (1072. 5497 m/z) $[M+2H]^{2+}$ and the deamidated peptide GIDVEQVSLVINYDLPTNR (1073.0619 m/z) $[M+2H]^{2+}$ from eIF4A (see Fig. S9). The results show the relative amounts of the unmodified and modified peptide present, a clear difference in retention time of the peptides is observed during the liquid chromatography with the modified peptide eluting later than the unmodified peptide. The results show that after 20 minutes near complete deamidation of Gln^{339} was observed.

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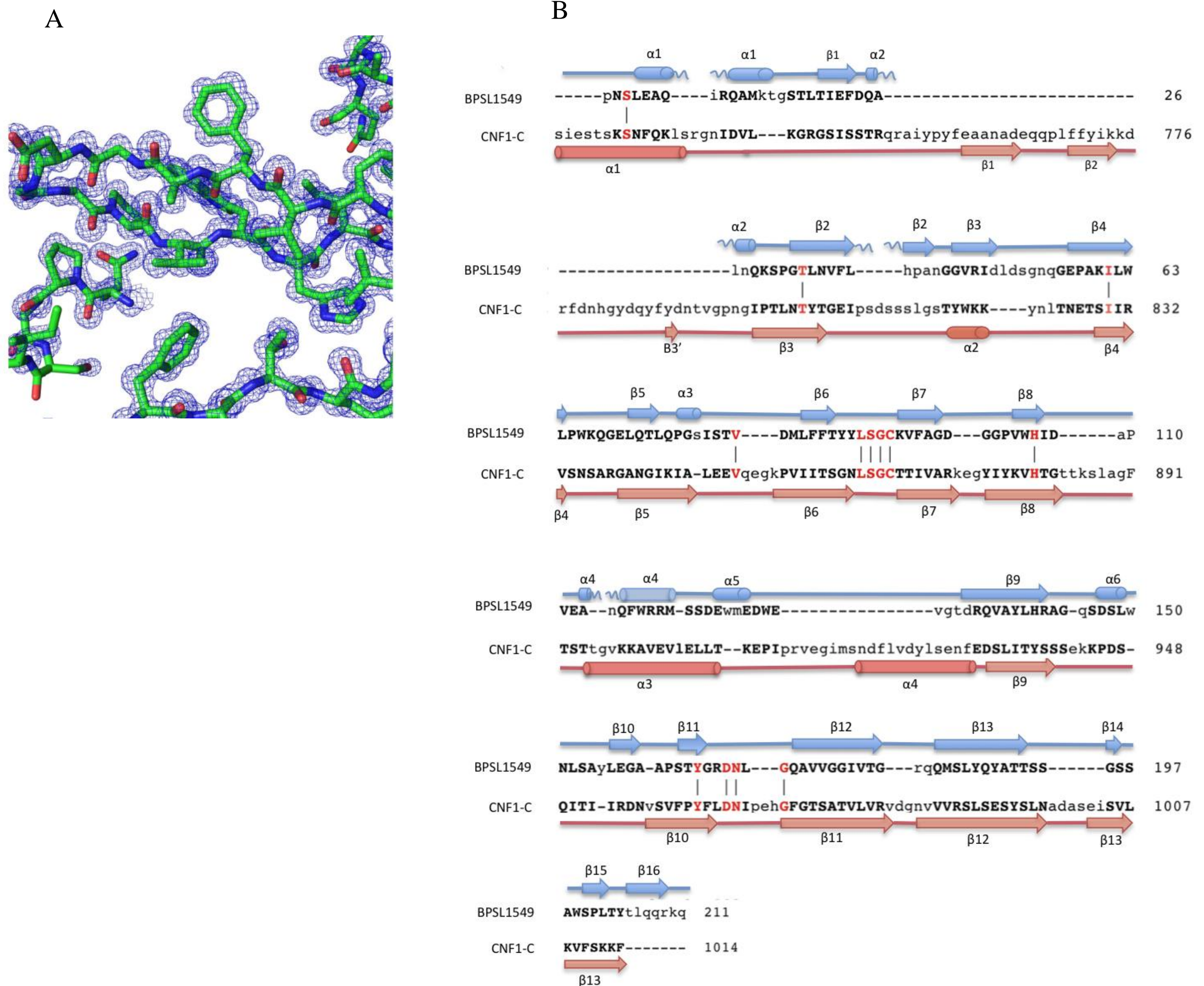


Fig. S1.

Structural analysis of BPSL1549 (A) The initial electron density map of BPSL1549 calculated on phases to 1.9 Å using MAD phasing and phase extended in SHELXE to 1.04 Å resolution contoured at 1 sigma. (B) A structure-based sequence alignment of BPSL1549 and CNF1-C with the elements of secondary structure in the two proteins identified. Conserved residues are identified by a vertical bar between the two sequences.

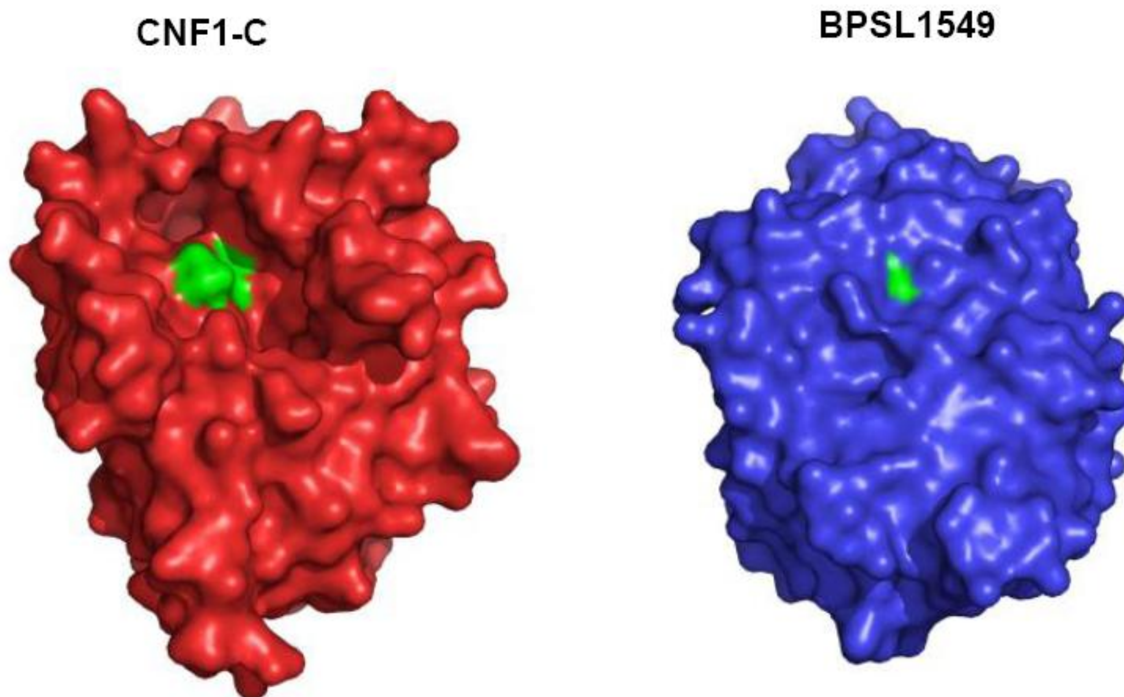
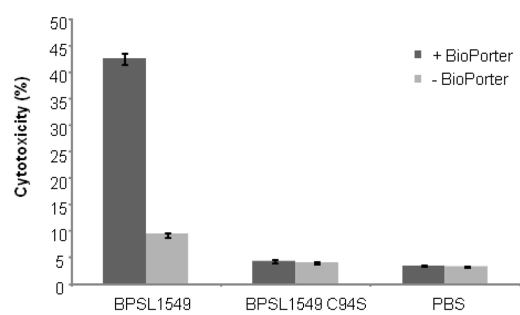


Fig. S2.

A view of the molecular surface of BPSL1549 and CNF1-C. The images show the difference in the shape of the region bordering the active site which can be identified from the position of the sulphur atom of the catalytic cysteine (green).

A



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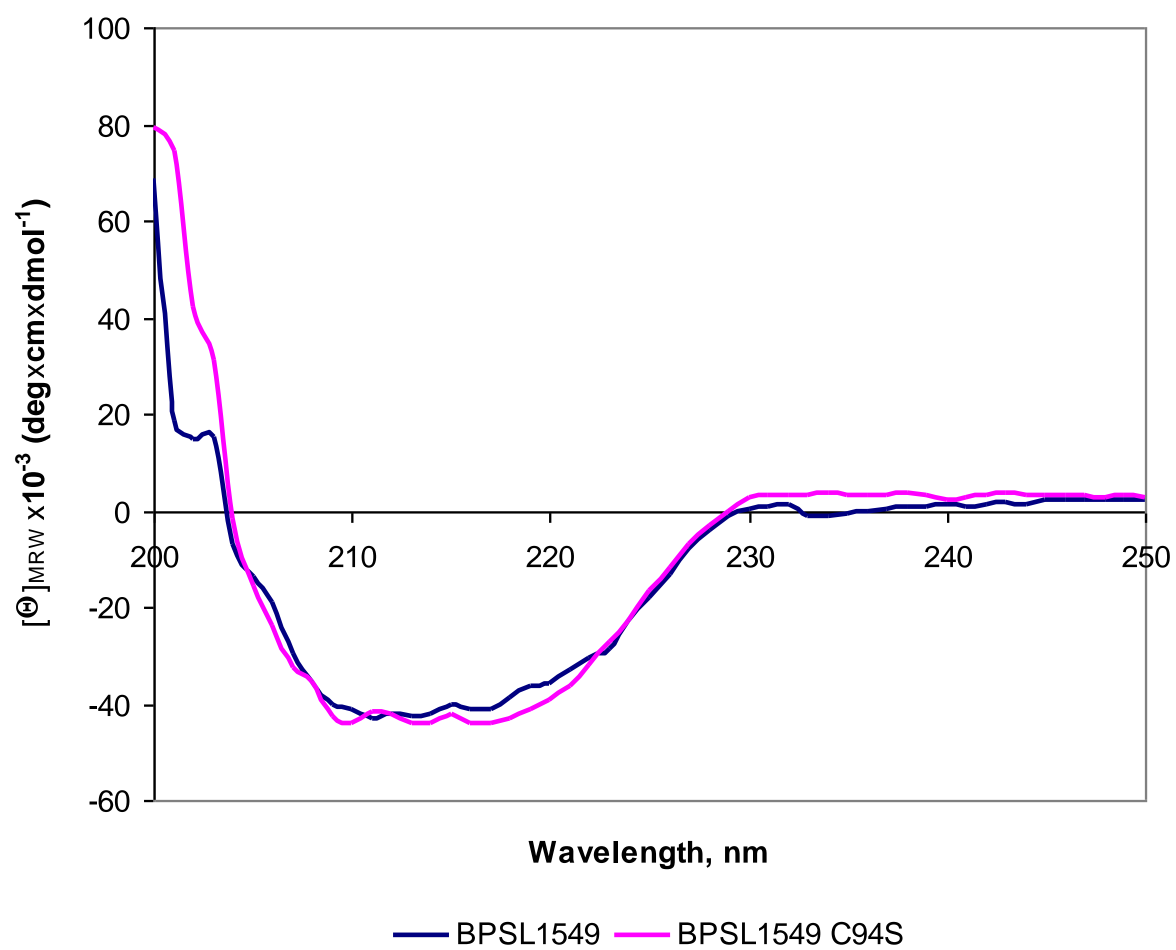


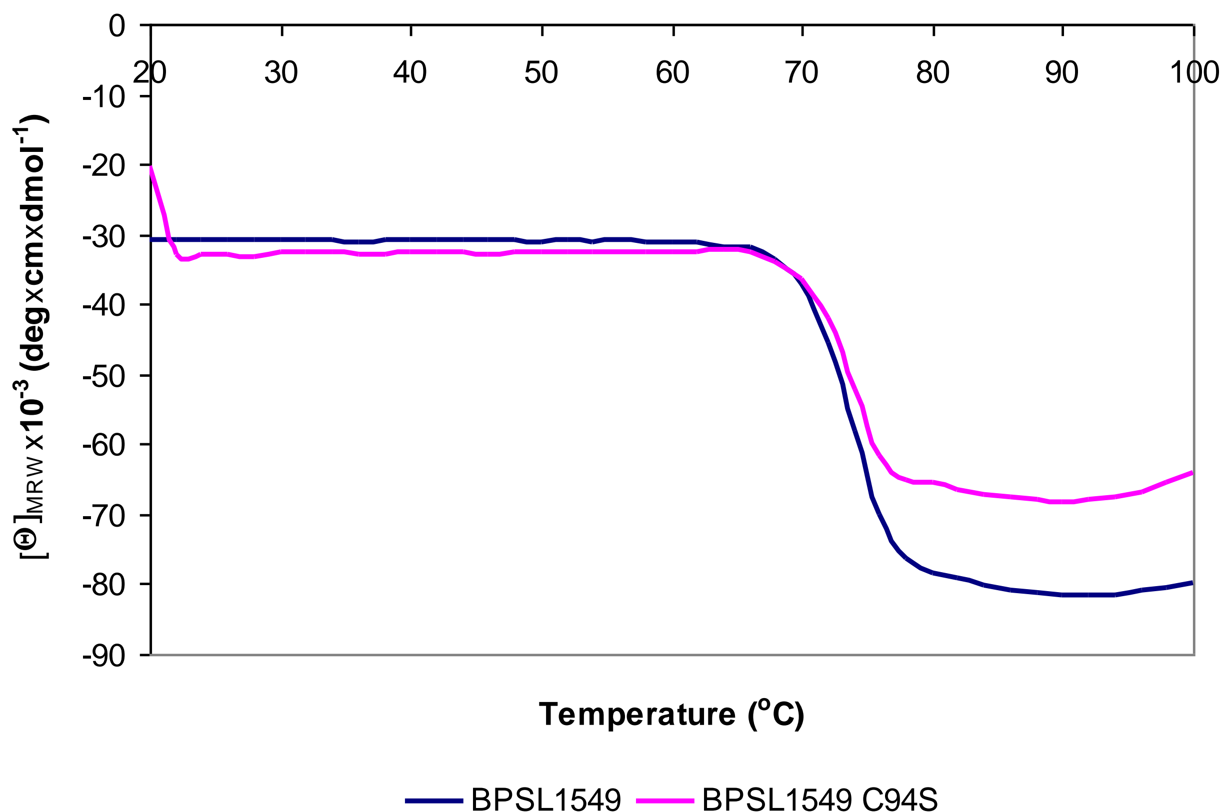


Fig. S3 Effect of BPSL1549 on 3T3 Swiss-Albino fibroblasts. (A) 3T3 Swiss-Albino fibroblasts were treated with 800 nM recombinant BPSL1549 in the presence or absence of the protein delivery reagent BioPORTER, and incubated for 72 h. Cytotoxicity was determined by a modified LDH assay as described in the methods section. Error bars represent standard deviation from the mean. (B) Circular dichroism (CD) spectrum expressed in units of mean residual ellipticity for BPSL1549 and BPSL1549C94S at 20 ºC showing that they have equivalent secondary structure. (C) Mean residual ellipticity at 222nm as a function of temperature for BPSL1549 and BPSL1549C94S indicating their equivalent melting temperature. Both proteins undergo irreversible polymerisation above 70 ºC.

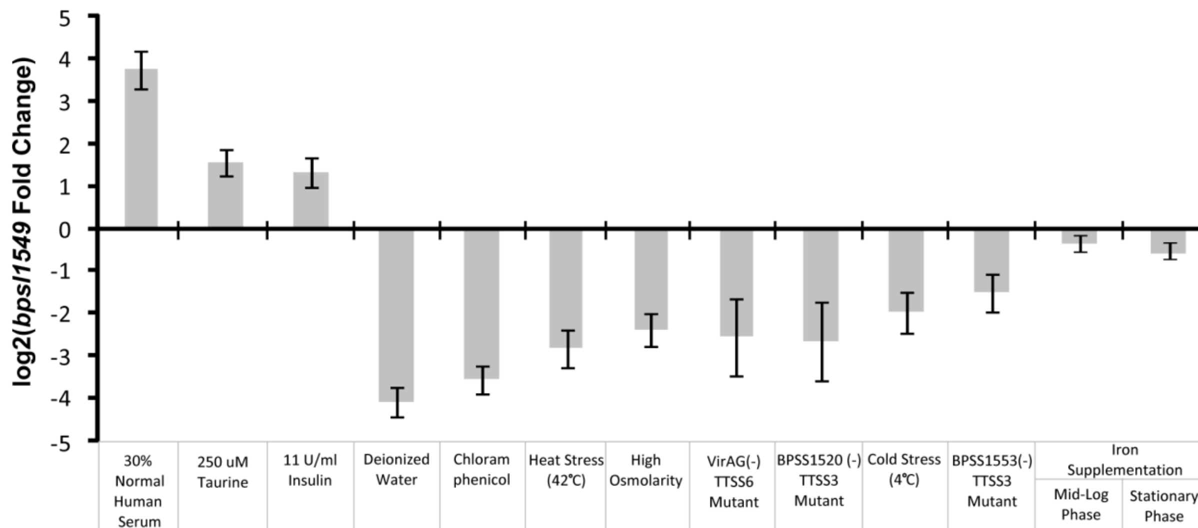
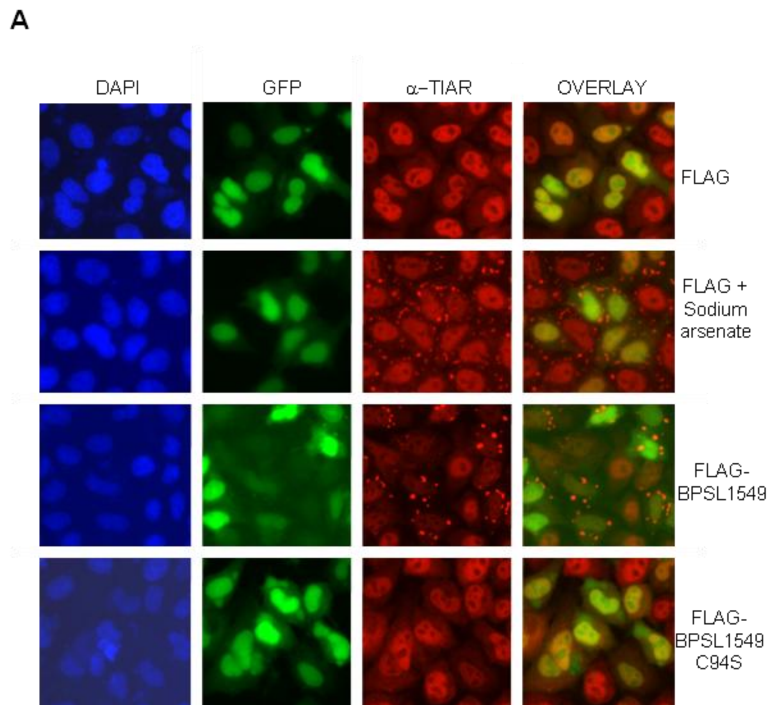


Fig. S4 *bpsl1549* transcriptional regulation. The graph depicts *bpsl1549* expression levels across various perturbations (listed at bottom). See Table S2 for details of experimental and reference conditions. All *bpsl1549* expression alterations, with the exception of iron supplementation, are differentially regulated by a greater than 2 fold change (absolute $\log_2$*bpsl1549* Fold Change>1) and passing the threshold level of significance ($p<0.001$).



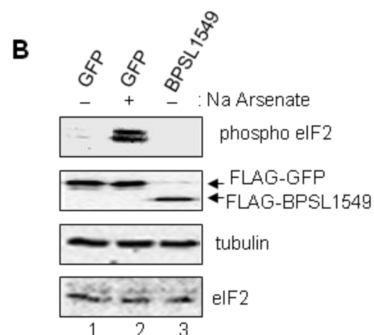


Fig. S5 Stress granule formation in the presence of BPSL1549. (A) Analysis of stress granule formation in HeLa cells transfected with the indicated FLAG expression plasmids. GFP was cotransfected to mark transfected cells. Cells were treated with sodium arsenate for 1 hour prior to fixation were indicated. α –TIAR = immunostaining with an antibody specific for TIAR. TIAR is a marker for stress granules. (B) Western analysis of 293T cell extracts transfected with the indicated plasmids (along the top) probed with the indicated antibodies (right hand side).

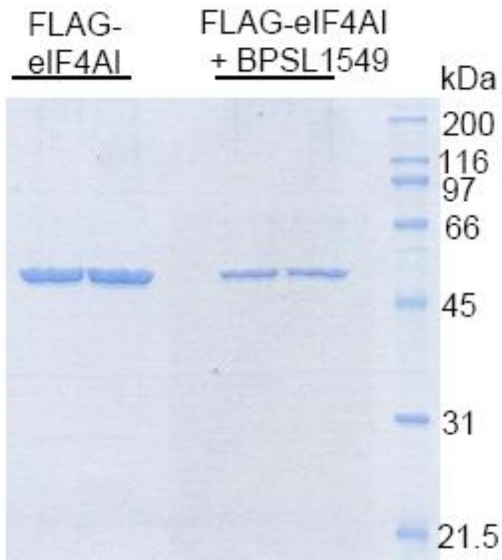


Fig. S6

Purification of eIF4AI modified by BPSL1549 *in vivo.*

Coomassie stained gel of FLAG-eIF4AI immunopurified from human 293T cells transfected with a BPSL1549 expression plasmid as indicated. The purified protein was used in mass spectrometry analysis to identify the BPSL1549 modified residue in eIF4AI **.**

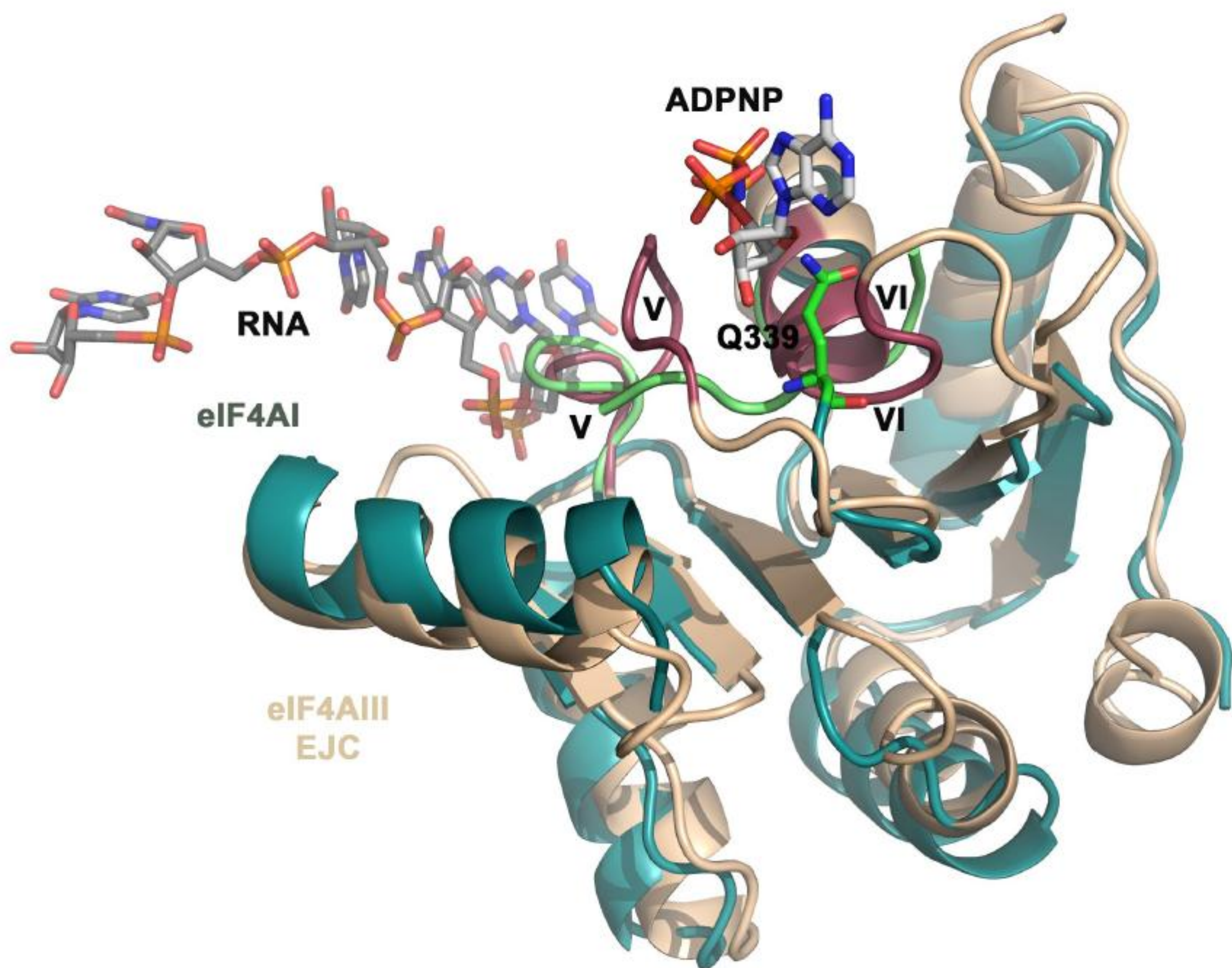


Fig. S7 Cartoon representation showing the position of Gln^{339} in the open structure of eIF4AI on the loop between the ATP and RNA binding sites (turquoise) superimposed on the closed structure of eIF4AIII (light brown). The conserved motifs V and VI in this region are shown in green (eIF4AI) and maroon (eIF4AIII) to illustrate the major conformation change that occurs in this region upon domain closure.

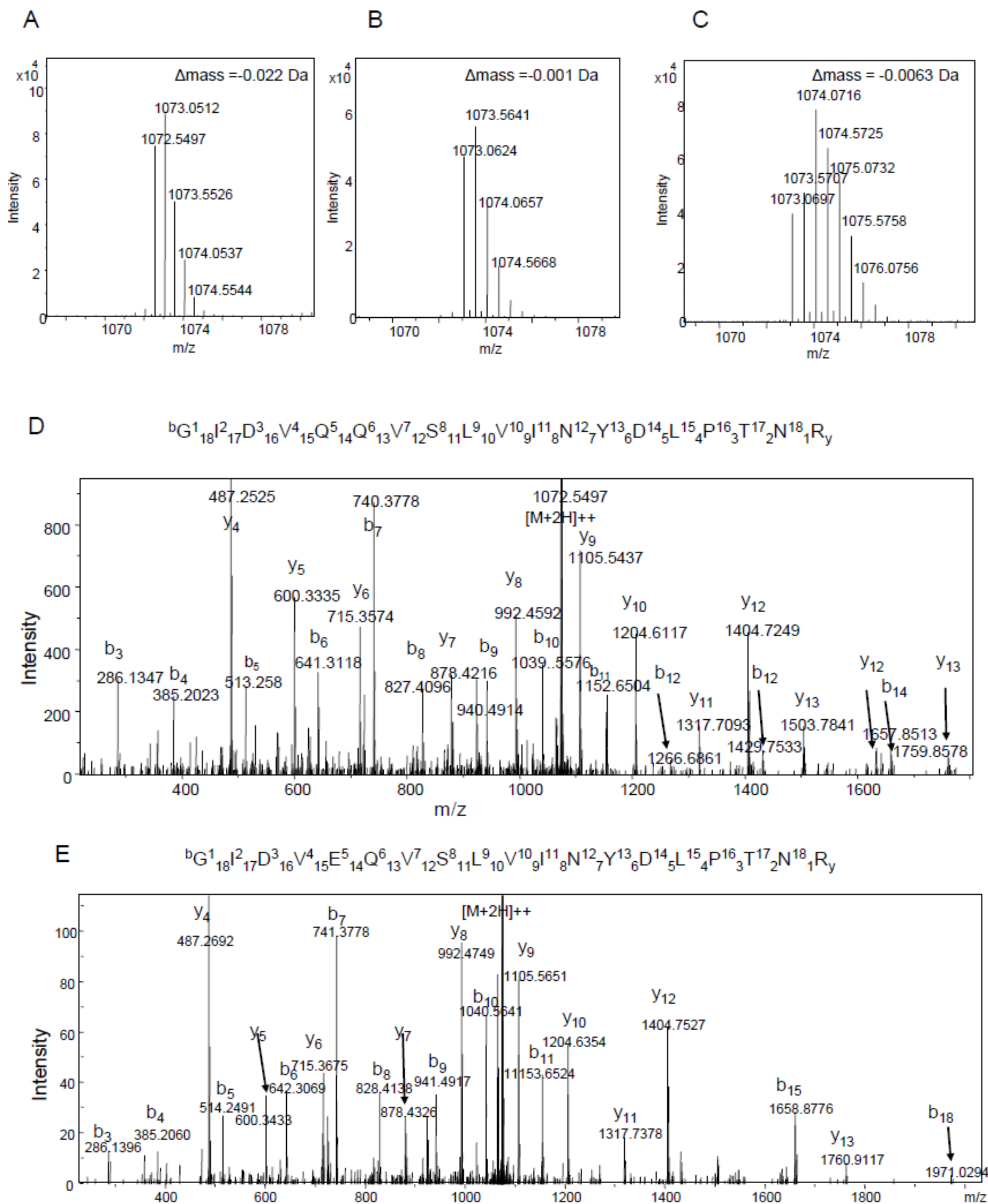


Fig. S8. UHR TOF MS analysis of the Gln[339] containing tryptic peptide from eIF4A. Intact MS analysis of the tryptic peptide GIDV**Q**QVSLVINYDLPTNR [M+2H] $^{2+}$ from recombinant eIF4A (A) and the equivalent deamidated peptide following incubation with BPSL1549 (B). C) Intact MS analysis of the deamidated peptide in the presence of 50% $H_2^{18}O$. Tandem MS analysis of the peptide GIDV**Q**QVSLVINYDLPTNR [M+2H] $^{2+}$ from eIF4A (D) and the deamidated peptide (E). The prominent b and y ions are highlighted. The site of deamindation is indicated in bold in the legend.

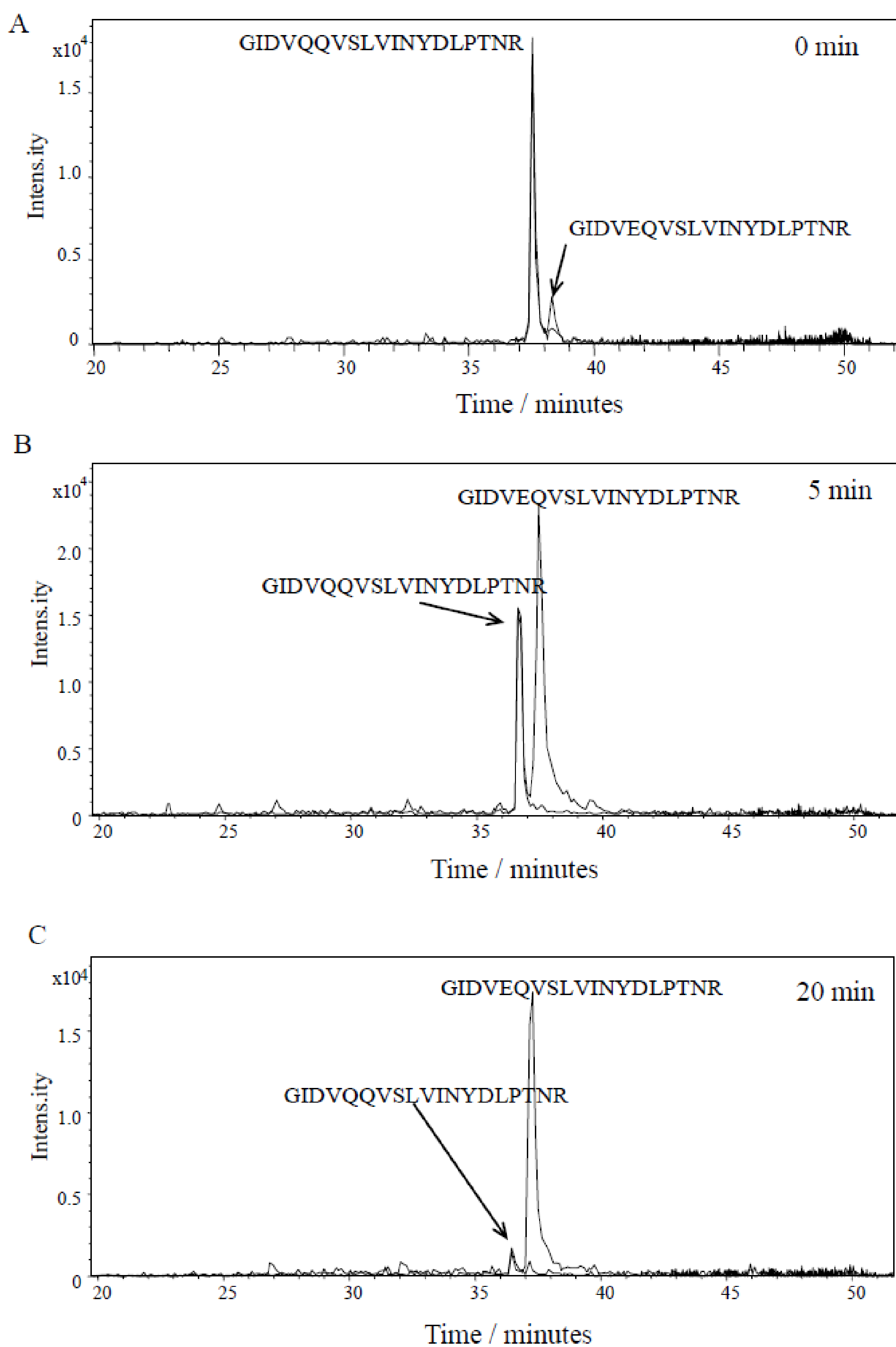


Figure S9. Electrospray ionisation mass spectrometry analysis of the enzymatic deamidation of recombinant eIF4A with BPSL1549. Extracted ion chromatograms of the unmodified peptide GIDVQQVSLVINYDLPTNR (1072. 5497 m/z) $[M+2H]^{2+}$ and the deamidated peptide GIDVEQVSLVINYDLPTNR (1073.0619 m/z) $[M+2H]^{2+}$ were generated following incubation of recombinant eIF4A with BPSL1549 at a ratio of 5000:1. The reaction was stopped after A) 0 mins B) 5 mins C) 20 mins. The results show that near complete deamidiation of Gln^{339} was achieved after 20 mins.

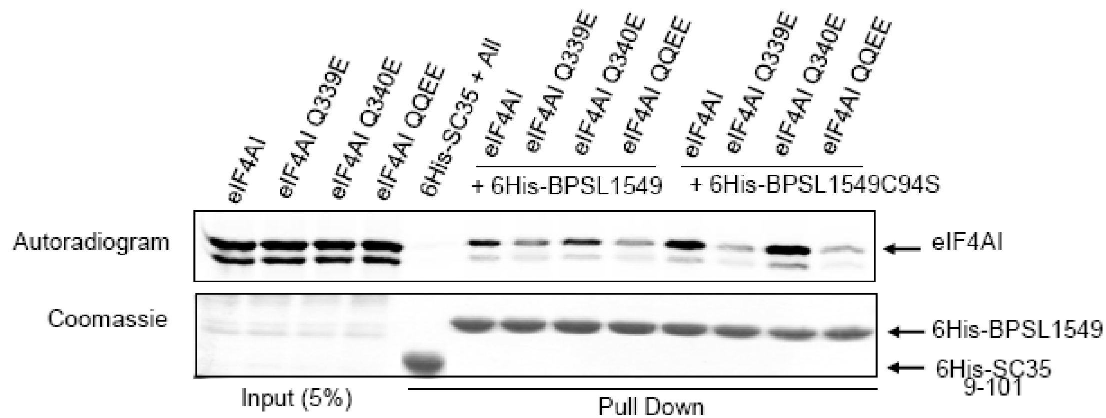


Fig. S10.

Pulldown interaction assay between BPSL1549 or BPSL1549C94S and eIF4AI and point mutants. SC35 is a splicing factor that is used as a negative control. eIF4AI QQEE has a double mutation of Q339 and Q340 to glutamate. Wild type BPSL1549 binds its substrate, eIF4AI, efficiently and this interaction is reduced in the eIF4AIQ339E mutant, which represents the product after BT modification of eIF4AI. The catalytically inactive mutant BPSL1549C94S binds more efficiently to eIF4AI than wild type BPSL1549, but still interacts with eIF4AIQ339E poorly.

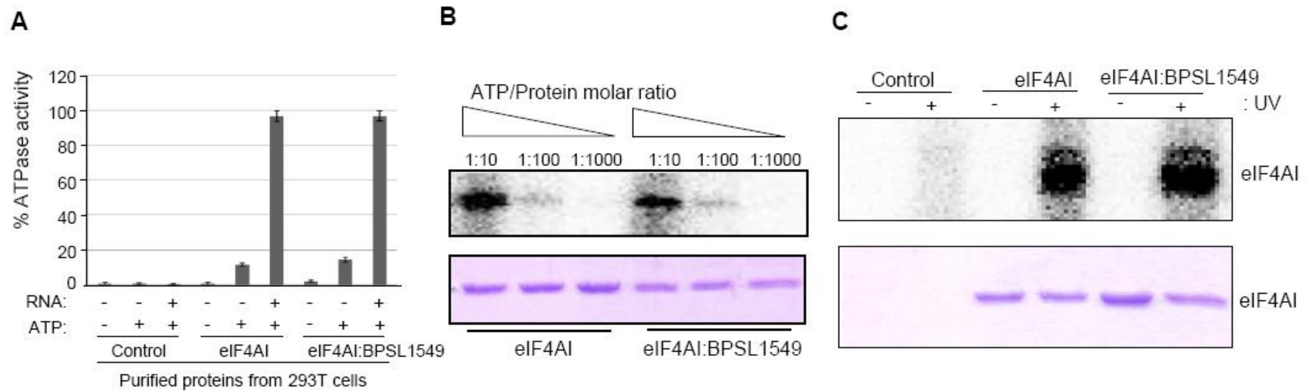


Fig. S11.

Biochemical properties of eIF4AI following modification by BPSL1549

A) ATPase activities for FLAG-eIF4AI purified from human 293T cells as in Fig. S1A. Values are the averages from 3 independent assays and error bars represent the SD. B) ATP crosslinking to eIF4AI immunopurified from 293T cells as in Fig. S1A. C) UV cross linking of RNA to eIF4AI *in vivo*. 293T cells were transfected with FLAG-eIF4AI +/- a BPSL1549 expression vector and empty FLAG vector for the control. 24 hours post transfection, RNA was crosslinked to proteins *in vivo* by irradiating cells with UV. eIF4AI was immunopurified using FLAG agarose, treated with RNAse and the remaining crosslinked RNA fragment was end labelled with ^{32}P.

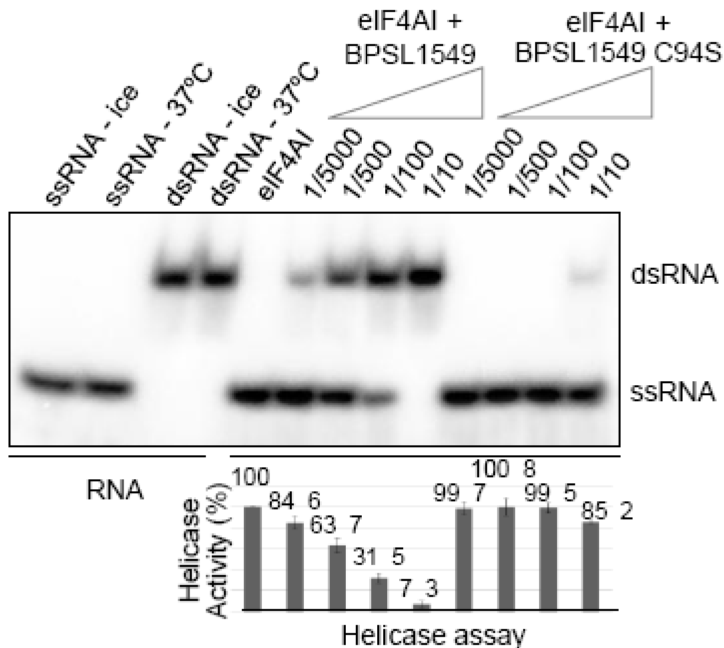


Fig. S12.
Helicase activity for eIF4AI following incubation with BPSL1549.
6His eIF4AI 20-406 was incubated with various molar ratios of BPSL1549 or BPSL1549C94S for 30 min at 37°C prior to the helicase assay. (molar ratios are expressed as BPSL1549/BPSL1549C94S:eIF4A). 4 independent experiments were done to obtain the graph (lower panel). The y axis is helicase activity in % calculated as a ratio: (ssRNA)/(dsRNA + ssRNA)*100. A representative gel is shown in the upper panel.

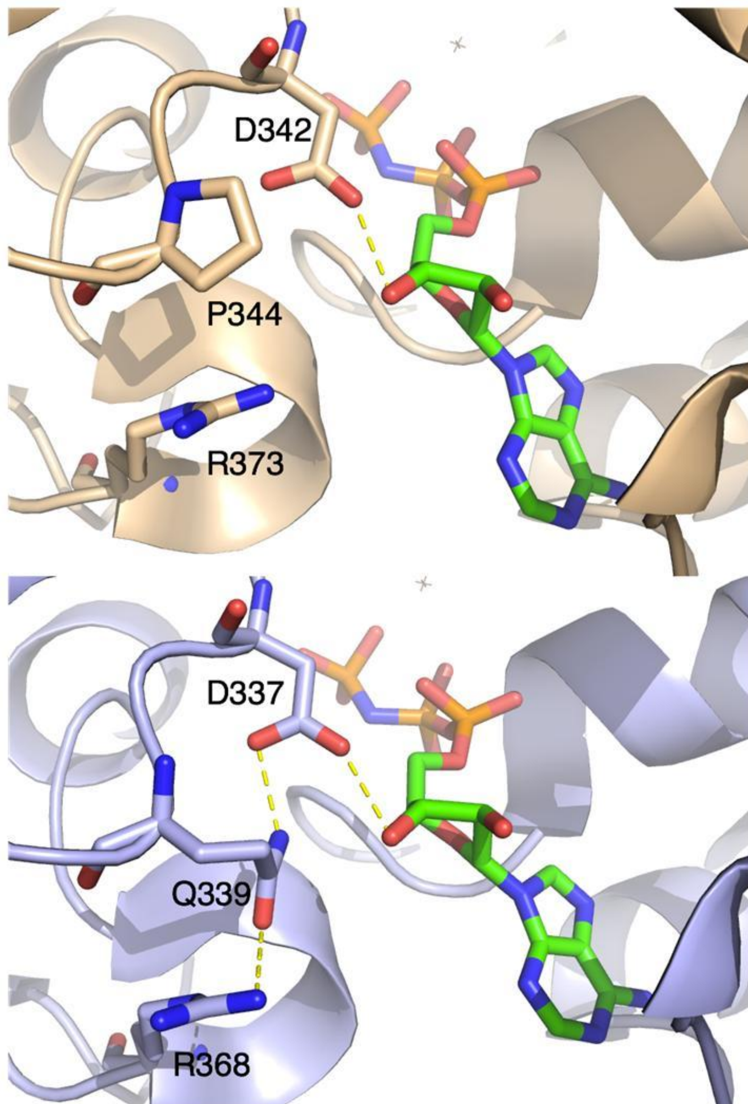


Fig. S13. Structural model of eIF4AI in the closed conformation. The ATP binding site in eIF4AIII (top) centered around D^{342}. The protein is shown as a light brown cartoon, with the ADPNP shown in green. The hydrogen bond between D342 and the 3'OH of the adenine ribose is highlighted as a yellow dashed line (Bottom). A model of the same region of eIF4AI (light blue), based on the eIF4AIII structure. In eIF4AI proline 344 is replaced by a glutamine. Plausible interactions are made between D^{337}, Q^{339} and R^{368} (eIF4AI numbering), which would be disrupted when Q^{339} is deamidated by BPSL1549.

Table S1 Data Collection and Refinement Statistics

Table S1A – data collection statistics

Protein sample	Native	SeMet			C94S
Space group			$P2_12_12_1$		
Unit cell parameters					
a (Å)	37.0		37.0		37.0
b(Å)	45.4		45.4		45.2
c (Å)	111.4		111.1		115.7
Temperature (K)	100		100		100
X-ray source	DLS I04		ESRF ID29		DLS I03
Detector	ADSC Q315		ADSC Q315		Pilatus 6M
		Peak	**Inflection**	**Remote**	
Resolution range (Å)	25 –1.02	20-1.9	20-1.9	20-1.9	37-1.09
	(1.1-1.04)	(2.0-1.9)	(2.0-1.9)	(2.0-1.9)	(1.12-1.09)
Unique reflections	102825(4222)	15292(2115)	15288(2111)	15295(2127)	77711(4489)
R_{merge}†	0.09 (0.459)	0.065(0.15)	0.067(0.15)	0.062(0.15)	0.034 (0.354)
Completeness (%)	99.0 (87.7)	99.1(96.4)	99.1(96.4)	99.1 (96.8)	95.5 (75.6)
Anomalous completeness	__	96.1(87.2)	95.9(86.5)	96.5(89.0)	__
Multiplicity	5.2(2.2)	3.3(2.9)	3.3(2.9)	3.3(3.0)	3.2 (1.8)
Anomalous multiplicity	__	1.7(1.5)	1.7(1.5)	1.7(1.6)	__
Mean $(I)/\sigma(I)$	18.8 (1.7)	14.7(6.7)	14.3(6.3)	15.8(7.2)	15.0 (2.4)

Values in parentheses are for data in the highest resolution shell.

† $R_{merge} = \Sigma_{hkl} \Sigma_i | I_i - I_m| / \Sigma_{hkl} \Sigma_i I_i$, where I_i and I_m are the observed intensity and mean intensity of related reflections, respectively. R-factor=$\Sigma|F_{obs}-F_{calc}|/ \Sigma F_{obs}$.

Table S1B – Refinement statistics

Refinement statistics	Native	C94S
Resolution (Å)	25 -1.04	37-1.09
R-factor/ R-free	0.129/ 0.160	0.141/ 0.168
Number of protein residues	210	210
No. of protein atoms	1754	1766
No. of Bromide ions	1	0
No. of water molecules	369	294
Total number of atoms	2124	2061
Rmsd bond lengths (Å)	0.011	0.009
Rmsd bond angle 1-3 distances (Å)	0.027	0.025
Average B factor (main chain) ($Å^2$)	10	10
Average B factor (side chain) ($Å^2$)	15	15
Average B factor (waters) ($Å^2$)	29	29
Poor rotamers (%) [A]	1.16 %	0 %
Ramachandran plot Favoured/ allowed/ outliers (%) [A]	97/ 3/ 0	97/ 3/ 0

[A] calculated using Molprobity.

Table S2 Conditions Inducing *bpsl1549* Regulation

Conditions Inducing *BPSL1549* Upregulation						
	TEST CONDITION			REFERENCE CONDITION		
Fold Change	Specific Condition	Duration of incubation (hrs)	Temp of incubation	Reference Condition	Duration of incubation (hrs)	Temp of incubation
16.5468	30% Normal Human Serum (NHS) in 1x PBS	16hrs	37°C	1x PBS	16hrs	37°C
5.7439	Chemically Defined Medium (CDM), Mid Log	8hrs	37°C	LB Media	8hrs	37°C
4.1326	250uM Na2SO4, Mid-Log Phase, Modified M63 Media	24hrs	37°C	LB Media	8hrs	37°C
3.4746	RpoE mutant, Cold stress (4°C), LB Media	16hrs	4°C	Wild-type, Cold stress (4°C), LB Media	16hrs	4°C
3.0229	250uM Taurine, Mid-Log, Modified M63 Media	24hrs	37°C	LB Media	30hrs	37°C
2.9875	0.01% Bleach, LB Media	16hrs	37°C	LB Media	16hrs	37°C
2.7224	11U/ml Insulin, 1x PBS Media	16hrs	37°C	1x PBS	16hrs	37°C
2.5032	Bp008::pmlI:Tc (QS mutant), LB Media	24hrs	37°C	Wild-type, LB Media	24hrs	37°C

Conditions Inducing *bpsl1549* Downregulation						
	TEST CONDITION			REFERENCE CONDITION		
Fold Change	Specific Condition	Duration of incubation (hrs)	Temp of incubation	Reference Condition	Duration of incubation (hrs)	Temp of incubation
21.0156	Deionized water/Nutrient Deprivation	24hrs	37°C	LB Media	24hrs	37°C
10.6041	8ug/ml chloramphenicol (1X MIC), MHB Media	16hrs	37°C	MHB Media	16hrs	37°C
7.6543	Heat stress (42°C), LB Media	16hrs	42°C	LB Media	16hrs	28°C
5.4408	2M Sorbitol, LB Media	16hrs	37°C	LB Media	16hrs	37°C
4.9194	ΔVirAG (T6SS5 mutant – BPSS1494/1495), Exposed to RAW Macrophages	5hrs	37°C	LB Media	4hrs	37°C
4.8595	ΔBPSS1520 (TTSS3 mutant), Exposed to RAW macrophages	5hrs	37°C	LB Media	4hrs	37°C
4.4815	Cold stress (4°C), LB Media	16hrs	4°C	LB Media	16hrs	28°C
4.1599	200uM CdSO4, 1x TSBDC Media	24hrs	37°C	1x TSBDC Media	24hrs	37°C

3.5271	2ug/ml ceftazidime (1X MIC), MHB Media	16hrs	37°C	MHB Media	16hrs	37°C
2.5799	ΔBPSS1553 (TTSS3 mutant), LB Media	3hrs	37°C	LB Media	3hrs	37°C

Table S3 Plasmids used in this study

Plasmid name	Description	Tag(s)	Source
pcDNA-LacZ	Full length LacZ subcloned into pcDNA	None	L. Roaden
pcDNAMycHisA-BPSL1549	Full length BPSL1549 cloned as HindIII/XhoI PCR fragment into pcDNAMycHisA	Myc + 6 His (3')	This study
pET9a-eIF4A	Full length BPSL1549 cloned as NdeI/BamHI PCR fragment into pET9a	None	This study
pET9a-eIF4A Q339E	Full length BPSL1549 Q339E cloned as NdeI/BamHI PCR fragment into pET9A	None	This study
pET9a-eIF4A Q340E	Full length BPSL1549 Q340E cloned as NdeI/BamHI PCR fragment into pET9A	None	This study
pET9a-eIF4A QQEE	Full length BPSL1549 QQ339-340EE cloned as NdeI/BamHI PCR into pET9A	None	This study
pET9a-6His eIF4A (20-406)	*BPSL1549 37-1218* cloned as NdeI/BamHI PCR fragment into pET9a	6His (5')	This study
pET9a-6His eIF4A (20-406) Q339E	*BPSL1549 37-1218* Q339E cloned as NdeI/BamHI PCR fragment into pET9a	6His (5')	This study
pET9a-6His eIF4A (20-406) Q340E	*BPSL1549 37-1218* Q340E cloned as NdeI/BamHI PCR fragment into pET9a	6His (5')	This study
pET15b-eIF4B	Full length eIF4B	6His	C. Hellen
pET24b-SC35 (9-101)	*SC35 28-303* cloned as NdeI/XhoI PCR fragment into pET24b	6His (3')	Clayton et al., 2010
pET24b-Magoh	Full length Magoh cloned as NdeI/XhoI PCR fragment into pET24b	6His (3')	This study
pET24b-UAP56	Full length UAP56 clones as NdeI/XhoI PCR fragment into pET24b	6His (3')	This study
p3X-FLAG	CMV-driven expression vector for mammalian cells	3xFLAG(5')/Myc(5')	Sigma
p3X-FLAG-BAP	Bacterial Alkaline Phosphatase cloned into p3X-FLAG	3xFLAG(5')	Sigma
p3X-FLAG-GFP	eGFP cloned as EcoRI/XbaI PCR fragment into p3X-FLAG	3xFLAG(5')/Myc(5')	This study
p3X-FLAG-BPSL1549	Full length BPSL1549 cloned as HindIII/XbaI fragment into p3X-FLAG	3xFLAG (5')	This study
p3X-FLAG-BPSL1549 C94S	Full length BPSL1549 C94S cloned as HindIII/XbaI fragment into p3X-FLAG	3xFLAG (5')	This study
p3X-FLAG-eIF4A	Full length eIF4A cloned as HindIII/XbaI into p3X-FLAG	3xFLAG(5')/Myc(5')	This study
p3X-FLAG-eIF4A Q339E	eIF4A Q339E directed mutagenesis of p3X-FLAG-eIF4A	3xFLAG(5')/Myc(5')	This study
p3X-FLAG-eIF4A Q340E	eIF4A Q340E directed mutagenesis of p3X-FLAG-eIF4A	3xFLAG(5')/Myc(5')	This study
p3X-FLAG-eIF4A QQEE	eIF4A QQ339-340EE directed mutagenesis of p3X-FLAG-eIF4A	3xFLAG(5')/Myc(5')	This study
pGL3	Luciferase reporter construct		Promega
pET15-BPSL1549	Full length BPSL1549 cloned into pET15B	6His (5')	This study
pET15-BPSL1549C94S	Full length BPSL1549 C94S cloned into pET15B	6His (5')	This study
pSCA	StrataClone PCR cloning vector	None	Agilent
pDM4	Suicide vector, *sacBR oriT oriR6K* Chl^r	None	ref. 28
pDM4-1549	pDM4 containaing the 1549 deletion construct	None	This study