Supporting Information

Hsp33 confers bleach resistance by protecting elongation factor Tu against oxidative degradation in *Vibrio cholerae*

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Supplemental Experimental Procedure

E. coli EF-Tu^{Q97P} mutagenesis - The *E. coli* EF-Tu^{Q97P} variant was generated by site-specific mutagenesis using the forward primer 5' CCGGTGCTGCTCCGATGGACGGCGC and the reverse primer 5' GCGCCGTCCATCGGAGCAGCACCGG. Plasmid pBR322:*tufA*$^{E.c.}$ (see Table 1) was used as DNA template. A typical 50 µl reaction contained 0.3 ng/µl DNA template, 0.2 mM dNTPs, 3% DMSO, 1 U Phusion polymerase and 25 pmole/µl of each primer in HF buffer supplied by the Phusion polymerase kit (Finnzymes). The PCR program consisted of 1 cycle of 30 sec at 98°C, 30 cycles of 1) 30 sec at 98°C, 2) 30 sec at 70°C, and 3) 3 min 45 sec at 72°C, followed by 1 cycle of 10 min at 72°C, and hold at 4°C. The parental plasmid was digested by adding 40 U DpnI restriction enzyme (NEB) to the PCR products. The reaction was incubated at 37°C for 3 h. The DNA was concentrated to 5 µl using Pellet Paint (Novagen) and transformed into XL-10 Gold Ultracompentent cells (Stratagene). The transformants were streaked twice on selective growth plates and the plasmid was purified using Wizard Plus SV Minipreps kit (Promega). DNA sequencing was performed and correct clones were transformed into O395 and O395Δ*hslO* strains.

Protein identification by mass spectrometry – The identification of protein spots from 2D gels was conducted as described (Shevchenko *et al.*, 2006) with only very minor modifications. Spot analysis was performed by MS/MS analysis using

MALDI TOF. Peptide identification was conducted using the Mascot software with default parameters. The search was done against the Swiss-Prot database.

Thiol trapping with NEM - Bacterial strains were cultivated in LB media at 37°C until OD_{600} of 0.4–0.5 was reached. Then, 3 mM HOCl was added to the medium and incubation was continued for 20 min. Before and after the stress treatment, aliquots of 1 ml were taken and acidified with trichloroacetic acid (TCA) to a final concentration of 10%. After 30 min of incubation on ice, precipitated proteins were pelleted by centrifugation (13, 000 rpm, 20 min, 4°C). The protein pellet was resuspendend in DAB buffer (6 M Urea, 200 mM Tris-HCl pH8.5, 10 mM EDTA, and 0.5% w/v SDS) supplemented with 100 mM N-ethylmaleimide (NEM) to irreversibly alkylate all reduced cysteines. Samples were incubated for 30 min at 25°C, split into two aliquots and supplemented with either reducing (5 mM DTT) or non-reducing Laemmli-buffer. To enhance the efficiency of the protein transfer onto the nitrocellulose membrane after separation on SDS-PAGE, gels were incubated with 3% mercaptoethanol for 1 hr prior to the westernblot. EF-Tu was visualized using polyclonal antibodies against *E. coli* EF-Tu (provided by Dr. J. Beckwith).

Supplemental References

Shevchenko, A., H. Tomas, J. Havlis, J.V. Olsen and M. Mann (2006) In-gel digestion for mass spectrometric characterization of proteins and proteomes. *Nat Protoc* **1**: 2856-2860.

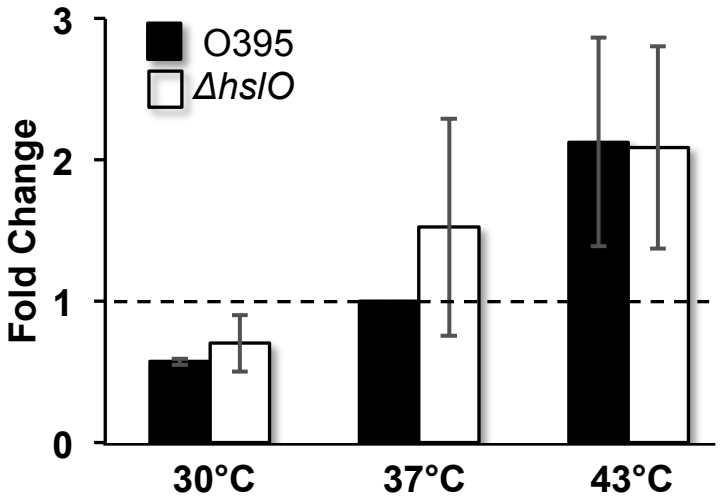

Fig. S1. *V. cholerae* O395 wild type and O395 *ΔhslO* mutant were cultivated in LB medium at the indicated temperatures until OD_{600}=0.45 was reached. Total RNA was isolated and RT-PCR experiments were performed to determine the mRNA levels of EF-Tu (encoded by *tufA* and *tufB*). rRNA levels of the ribosomal gene *rrsD* were used as an internal standard. The mRNA levels of EF-Tu are expressed relative to the mRNA levels present in wild-type O395 at 37°C. The error bars represent the standard deviation of four independent experiments.

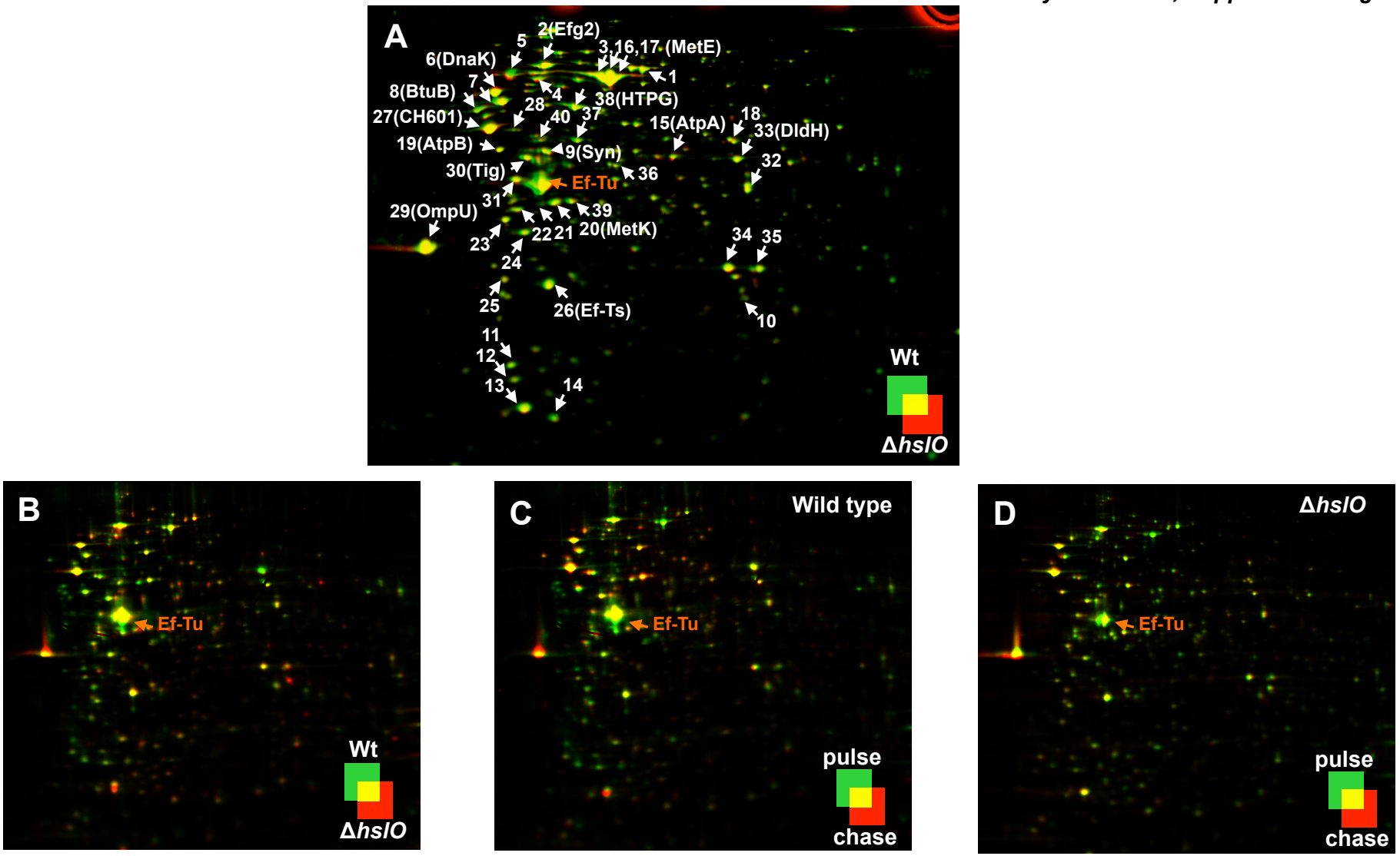

Fig. S2. Comparison of steady state protein levels (**A**) and newly translated proteins (**B**) in *V. cholerae* O395 wt and *ΔhslO* mutant. Representative false-colored overlays of either Coommassie-stained 2D-gels (A) or autoradiographs after 2 min pulse with ^{35}S-Met (B) of wt (green) and *ΔhslO* mutant (red) are shown. Arrows indicate the positions of the 40 most abundant protein spots, which were used for data analysis**. C.** and **D.** Extent of protein degradation within a 4 h chase in wt (**C**) or *ΔhslO* mutant (**D**). Representative false-colored overlays of autoradiographs after 2 min pulse (green) and 4h chase (red) are shown.

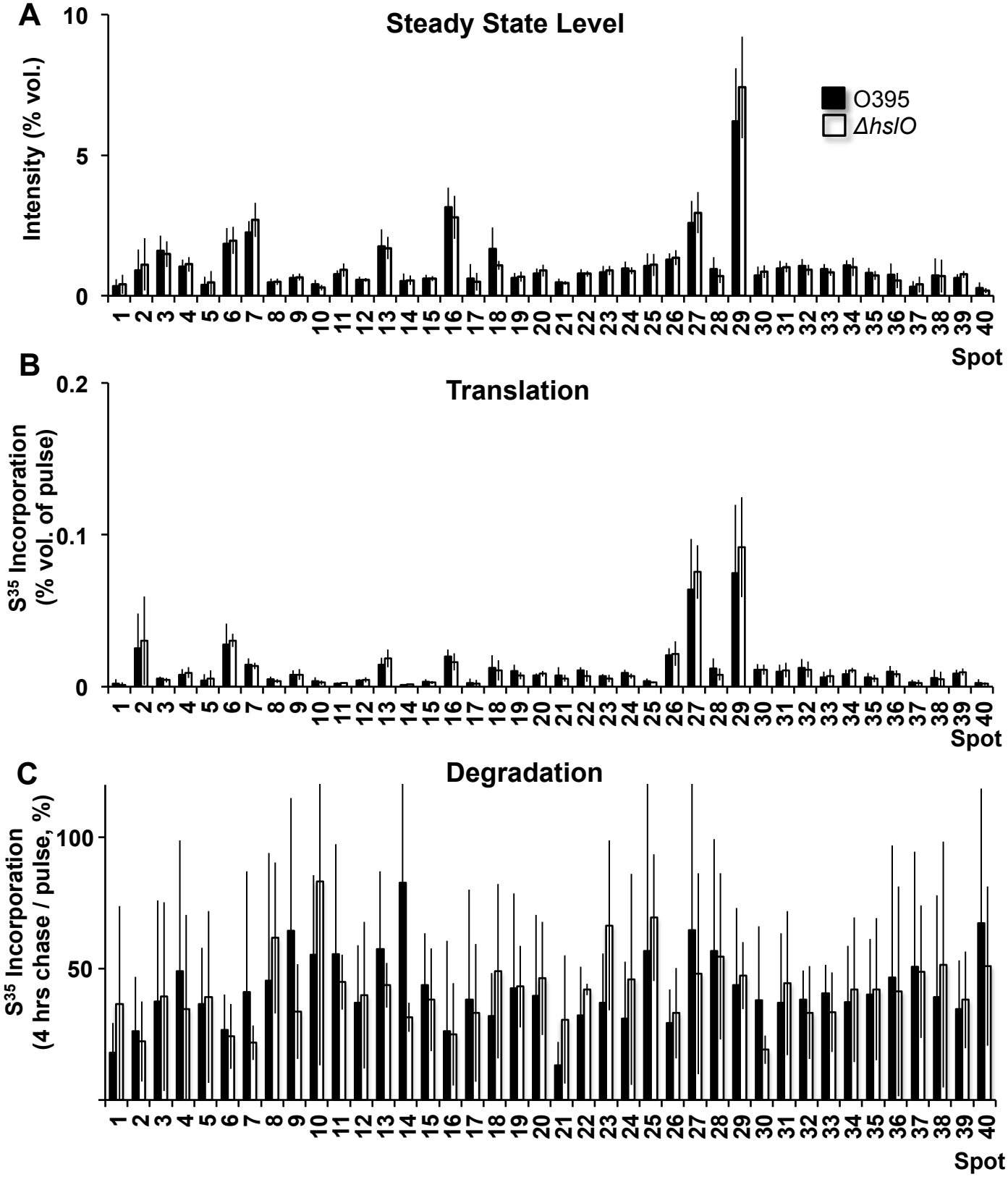

Fig. S3. Analysis of steady state levels (A), translation levels (B), and percentage degradation (C) of 40 most abundant proteins in *V. cholerae* O395 wild type (black bars) and O395 *ΔhslO* mutant (white bars). The error bars represent the standard deviation/error of four independent experiments. The corresponding data for EF-Tu can be found in Fig. 3B-D.

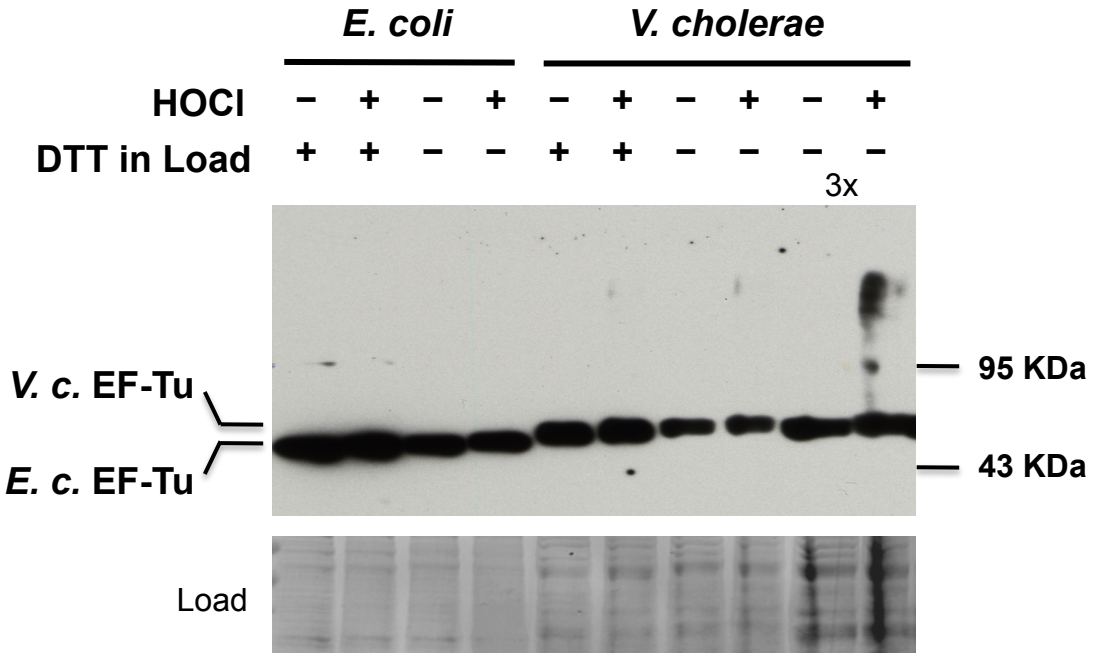

Fig. S4. Monitoring disulfide bond formation in EF-Tu. *E. coli* MC4100 and *V. cholerae* O395 were grown in LB medium at 37°C under aerobic conditions until mid-log phase was reached. Cells were treated with 3 mM HOCl for 20 min. Samples were taken and cysteines were labeled with NEM while all oxidized cysteines were left untreated. Protein samples were then split into two aliquots and resuspended in either reducing or non-reducing loading buffer prior to SDS-PAGE analysis. To enhance transfer efficiency of higher molecular weight EF-Tu complexes, proteins were reduced in gel immediately before the westernblot using β-mercaptoethanol. Without in-gel reduction, no higher molecular weight complexes were detected and only very faint bands were visible in the *V. cholerae* samples prepared under non-reducing conditions. These results suggest that *V. cholerae* EF-Tu forms reversible inter- and intramolecular disufide bonds.

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Ec_tufA     MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFDQIDNAPEEKARG 60
Ec_tufB     MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFDQIDNAPEEKARG 60
Vc_A2774    MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAICTVLAKVYGGKARDFASIDNAPEERERG 60
Vc_A2723    MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAICTVLAKVYGGKARDFASIDNAPEERERG 60
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Ec_tufA     ITINTSHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTREHI 120
Ec_tufB     ITINTSHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTREHI 120
Vc_A2774    ITINTSHVEYDTPNRHYAHVDCPGHADYVKNMITGAAQMDGGILVVAATDGPMPQTREHI 120
Vc_A2723    ITINTSHVEYDTPNRHYAHVDCPGHADYVKNMITGAAQMDGGILVVAATDGPMPQTREHI 120
            ************.*************************.******************

Ec_tufA     LLGRQVGVPYIIVFLNKCDMVDDEELLELVEMEVRELLSQYDFPGDDTPIVRGSALKALE 180
Ec_tufB     LLGRQVGVPYIIVFLNKCDMVDDEELLELVEMEVRELLSQYDFPGDDTPIVRGSALKALE 180
Vc_A2774    LLGRQVGIPYIIVFMNKCDMVDDEELLELVEMEVRELLSEYDFPGDDLPVIQGSALGALN 180
Vc_A2723    LLGRQVGIPYIIVFMNKCDMVDDEELLELVEMEVRELLSEYDFPGDDLPVIQGSALGALN 180
            *******:******:************************:******* *:::**** **:

Ec_tufA     GDAEWEAKILELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGIIKVG 240
Ec_tufB     GDAEWEAKILELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGIIKVG 240
Vc_A2774    GEAQWEAKIVELAEALDTYIPEPERAVDMAFLMPIEDVFSIQGRGTVVTGRIERGILKVG 240
Vc_A2723    GEAQWEAKIVELAEALDTYIPEPERAVDMAFLMPIEDVFSIQGRGTVVTGRIERGILKVG 240
            *:*:*****:*** **:********:* .**:********.*********:****:***

Ec_tufA     EEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRGIKREEIERGQVLAKPGTIK 300
Ec_tufB     EEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRGIKREEIERGQVLAKPGTIK 300
Vc_A2774    DEVAIVGIKETVKTTCTGVEMFRKLLDEGRAGENVGALLRGTKREEVERGQVLAKPGSIT 300
Vc_A2723    DEVAIVGIKETVKTTCTGVEMFRKLLDEGRAGENVGALLRGTKREEVERGQVLAKPGSIT 300
            :** ******* *:**********************.**** ****:**********:*.

Ec_tufA     PHTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTIELPEGVEMVMPGDNIKMV 360
Ec_tufB     PHTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTIELPEGVEMVMPGDNIKMV 360
Vc_A2774    PHTKFESEVYVLSKDEGGRHTPFFKGYRPQFYFRTTDVTGSIELPEGVEMVMPGDNVKMV 360
Vc_A2723    PHTKFESEVYVLSKDEGGRHTPFFKGYRPQFYFRTTDVTGSIELPEGVEMVMPGDNVKMV 360
            **********:*****************************:***************:***

Ec_tufA     VTLIHPIAMDDGLRFAIREGGRTVGAGVVAKVLS 394
Ec_tufB     VTLIHPIAMDDGLRFAIREGGRTVGAGVVAKVLG 394
Vc_A2774    VDLIAPIAMDEGLRFAIREGGRTVGAGVVAKIIA 394
Vc_A2723    VDLIAPIAMDEGLRFAIREGGRTVGAGVVAKIIA 394
            * ** *****:*********************::.
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Fig. S5. Sequence comparison between *E. coli* EF-Tu (encoded by *tufA* and *tufB* genes) and *V. cholerae* EF-Tu (encoded by A2774 and A2723 genes). Cysteine residues are highlighted.