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Supplementary Information for

Fusidic acid resistance through changes in the dynamics of the drug target

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Supplementary Materials and Methods

Site directed mutagenesis. All single amino acid mutations were introduced using the Quikchange site directed mutagenesis kit (Agilent), following the manufacturer's instructions. Multiple mutations were introduced using the Quikchange Multi site directed mutagenesis kit, following the manufacturer's instructions. The primers used in mutagenesis are shown in Table S3. Successful introduction of the mutations was confirmed by Sanger sequencing (Genewiz) using the T7 and T7term universal sequencing primers as well as the primers listed in Supplementary Table 1 for sequencing *fusA*.

Protein overexpression and purification. All proteins were expressed and purified as described previously (1). Briefly, *E. coli* BL21 Gold pET-28a-*fusB*, pET-29b-*fusAC3* or pET-3a-*fusA* cells were cultured in LB broth (unlabelled proteins) or 100 % ^{2}H M9 minimal media containing 3 g/l ^{13}C-^{1}H glucose and 1 g/l ^{15}N ammonium chloride at 37 °C, 200 rpm until an OD_{600nm} of 0.6. IPTG was added to a final concentration of 1 mM and cells were cultured at 25 °C (FusB or EF-G_{C3}) or 18 °C (EF-G), 200 rpm overnight. For ILVA methyl detected experiments, *E. coli* BL21 Gold pET-29b-*fusAC3* or pET-3a-*fusA* cells were cultured in 100 % ^{2}H M9 minimal media containing 1 g/l ^{15}N ammonium chloride and 3 g/l ^{12}C-^{2}H glucose at 37 °C with shaking at 200 rpm until an OD_{600nm} of 0.5. 120 mg/l 3-methyl-^{13}C-^{1}H, ^{2}H α-ketoisovaleric acid sodium salt, 60 mg/l methyl-^{13}C-^{1}H, ^{2}H α-ketobutyric acid sodium salt, 2.5 g ^{2}H succinic acid and 0.8 g/l methyl-^{13}C-^{1}H, ^{2}H L-alanine was then added and the culture was incubated at 37 °C with shaking at 200 rpm for 30 min to exhaust non-methyl labelled precursors. Protein expression was then induced with 1 mM IPTG and cultures were incubated at 18 °C with shaking at 200 rpm overnight. Cultures were centrifuged at 4500 ×*g* 4 °C for 30 min to pellet the cells.

Cells were lysed by sonication on ice in lysis buffer (50 mM NaH_2PO_4, 10 mM imidazole, 300mM NaCl, pH 8.0) containing benonaze (NEB) and Complete EDTA-free protease inhibitors

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(Roche) and the lysate cleared by centrifugation at 11000 x*g*, 4 °C for 15 min. Proteins were purified at 4 °C using Ni-NTA affinity columns, washing the column in 8 × column volume lysis buffer, then 8 × column volume lysis buffer containing 20 mM imidazole before eluting protein in lysis buffer containing 250 mM imidazole. Proteins were then dialysed overnight in 2 l 20 mM TrisHCl, 300mM sodium chloride, 1 mM DTT, pH 8.0 followed by further purification using size exclusion columns (S75 or S200) in 20 mM TrisHCl, 300mM sodium chloride, 1 mM DTT, pH 8.0 at 4 °C as previously described (1, 2).

NMR – Chemical shift differences. ^{1}H-^{15}N chemical shift differences between apo and mutant EF-G_{C3} bound to FusB were calculated using the metric $\Delta = [(\delta^{15}N)^2 + (5 \times \delta\ ^{1}H)^2]^{0.5}$ (3).

SI References

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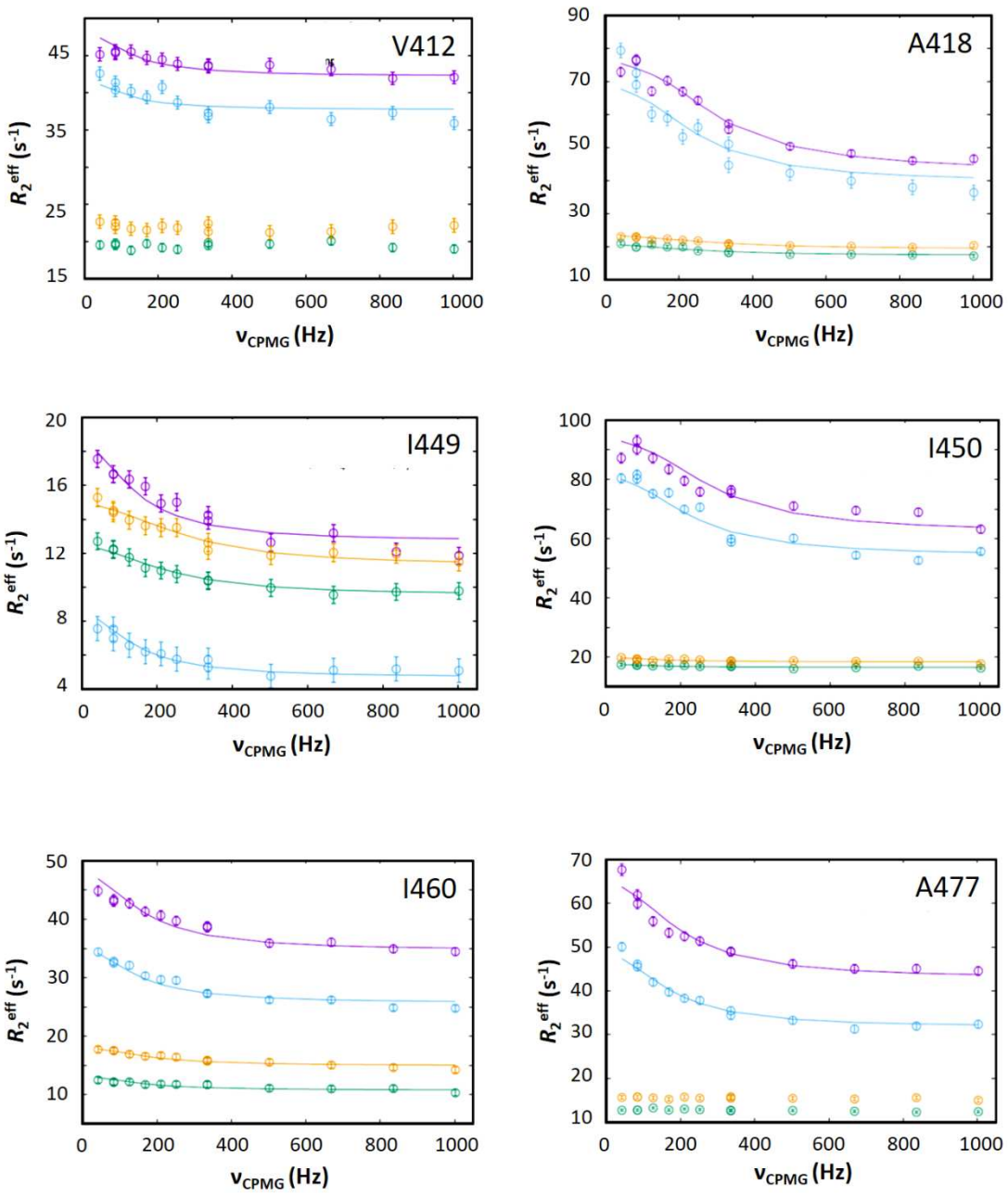
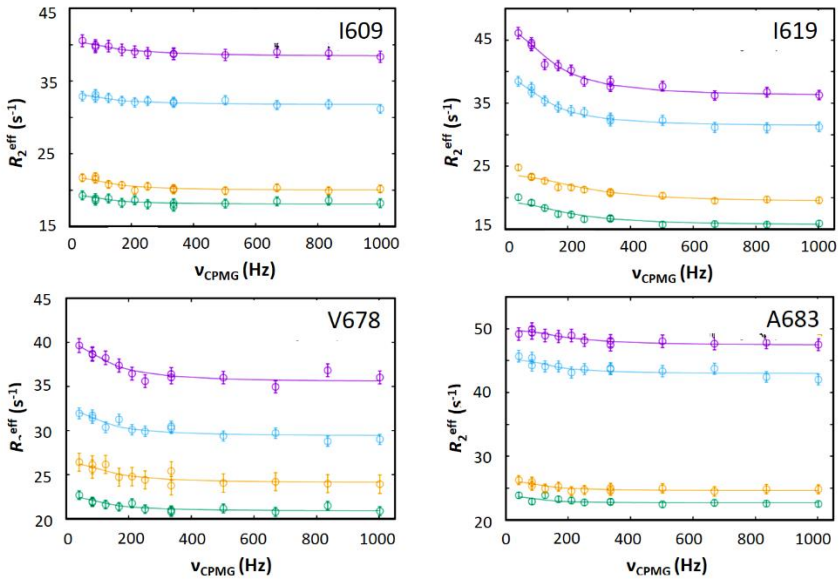


Fig. S1. Comparison of relaxation dispersion effects in domain III of EF-G_{C3} in the presence and absence of FusB. Methyl relaxation dispersion curves measured for residues in domain III in EF-G_{C3}:FusB at 950 MHz (magenta) and 750 MHz (blue) and in apo EF-G_{C3} at 950 MHz (orange) and 750 MHz (green), showing significant dispersion effects in the complex that are absent or greatly reduced in the unbound protein. All relaxation dispersion data were acquired at 30 °C with a relaxation delay of 24 ms. Errors in R_2^{eff} were determined through repeated measurement of 2 data points per experiment. Where error bars are not visible, they are within the limits of the data point. For each relaxation dispersion experiment N=13 at each field.

A



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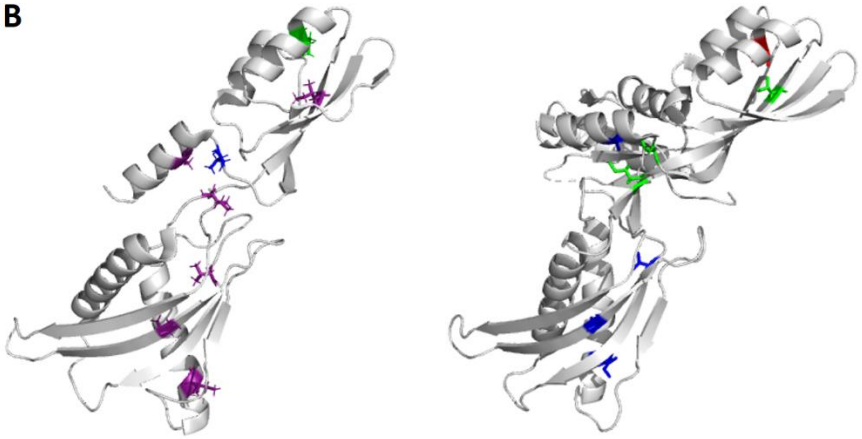

C

Residue	Apo Δω (ppm)	Error (ppm)	Complex Δω (ppm)	Error (ppm)
600	0.32	0.02	0.16	0.05
609	0.301	0.02	0.15	0.05
619	0.67	0.05	0.36	0.13
678	0.36	0.03	0.22	0.07
683	0.27	0.03	0.18	0.06
511	0.25	0.02	0.19	0.06
538	0.27	0.02	0.16	0.05
562	0.24	0.02	0.18	0.06

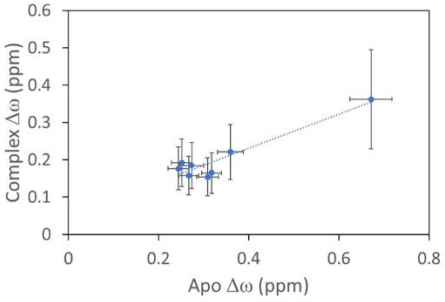

Fig. S2. Comparison of relaxation dispersion effects in EF-G_{C3} outside of domain III in the absence and presence of FusB. (A) Methyl relaxation dispersion curves measured for residues in domains IV and V of EF-G_{C3}:FusB at 950 MHz (magenta) and 750 MHz (blue) and in apo EF-G_{C3} at 950 MHz (orange) and 750 MHz (green), showing similar dispersion effects in the

complex and the unbound protein, suggesting that these effects are not the result of FusB binding and therefore not the cause of FA resistance. All relaxation dispersion data were acquired at 30 °C with a relaxation delay of 24 ms. Errors in R_2^{eff} were determined through repeated measurement of 2 data points per experiment. Where error bars are not visible, they are within the limits of the data point. For each relaxation dispersion experiment *N*=13 at each field. **(B)** Residues within EF-G_{C3} domains IV and V (sticks) showing relaxation dispersion, coloured by $|\Delta\omega|$ ^{13}C (purple 0.1-0.2 ppm, blue 0.2-0.3 ppm, green 0.3-0.4 ppm, red 0.6-0.7 ppm). Left: relaxation dispersion when bound to FusB mapped onto the EF-G_{C3}:FusB complex structure (PDB ID 2MZW (1)). Right: relaxation dispersion in apo EF-G_{C3} mapped onto the EF-G structure (PDB ID 2XEX (4)). Domain III is not shown in the complex as the structure of this domain in the complex could not be determined (1). **(C)** ^{13}C $|\Delta\omega|$ data extracted from relaxation dispersion data in EF-G_{C3} (left) and comparison of apo and FusB bound ^{13}C $|\Delta\omega|$ data (right) (R^2 = 0.88) *N* = 8.

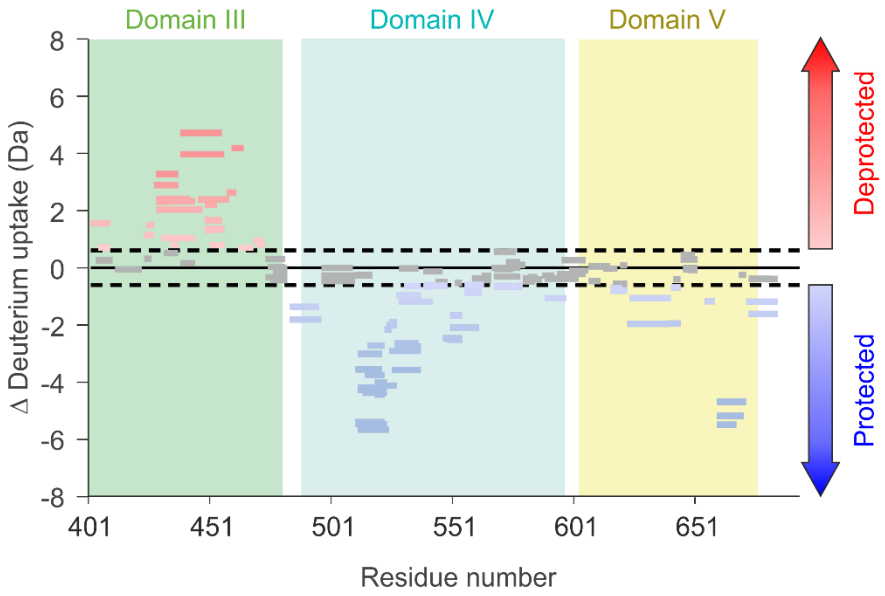

Fig. S3. Wood's plot showing the summed differences in deuterium uptake in EF-G_{C3} over all five HX-MS timepoints, when comparing EF-G_{C3} alone with EF-G_{C3} in the presence of FusB. This figure was generated using Deuteros (5). Peptides coloured in blue or red, respectively, are protected or deprotected from exchange in the presence of FusB. Peptides with no significant difference between conditions, determined using a 99% confidence interval (dotted line), are shown in grey. Domains III, IV and V are shown as green, blue and yellow shaded regions, respectively. Raw HDX-MS data is available on the ProteomeXchange (accession number PXD017491).

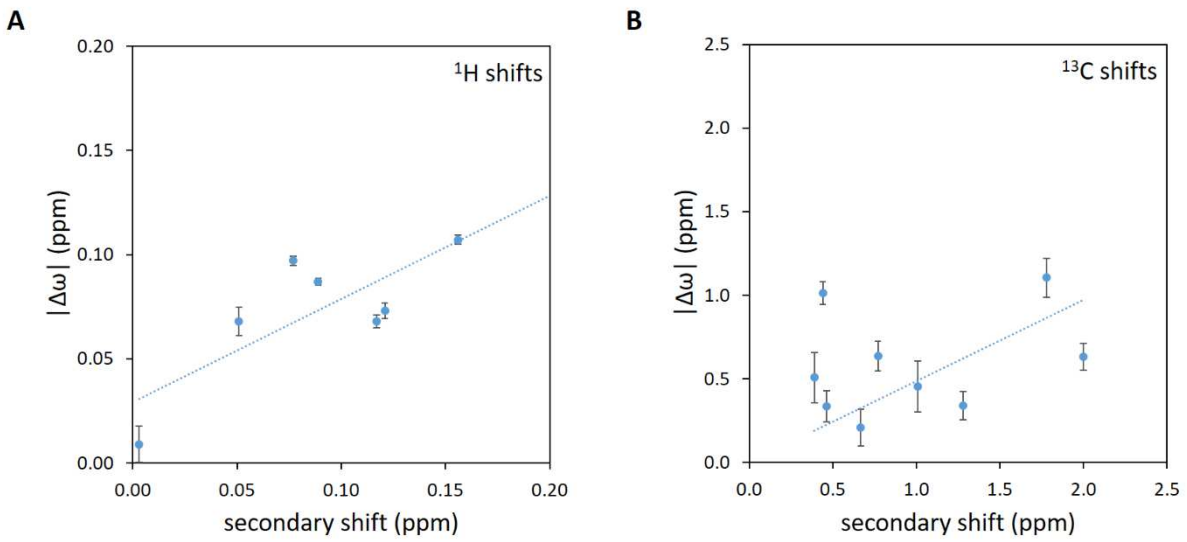

Fig. S4. Comparison of |Δω| between major and minor state with deviation from random coil shifts. Differences in **(A)** ^{1}H and **(B)** ^{13}C chemical shift differences between the major and minor state in EF-G_{C3} bound to FusB determined from relaxation dispersion measurements plotted against the difference between observed chemical shift (major state) and reference chemical shifts for unfolded proteins (6). Where error bars are not visible errors fall within the bounds of the point marker. Dotted lines represent lines of best fit. Correlation between |Δω| and secondary shifts is poor suggesting the minor state does not consist of a fully unfolded domain III.

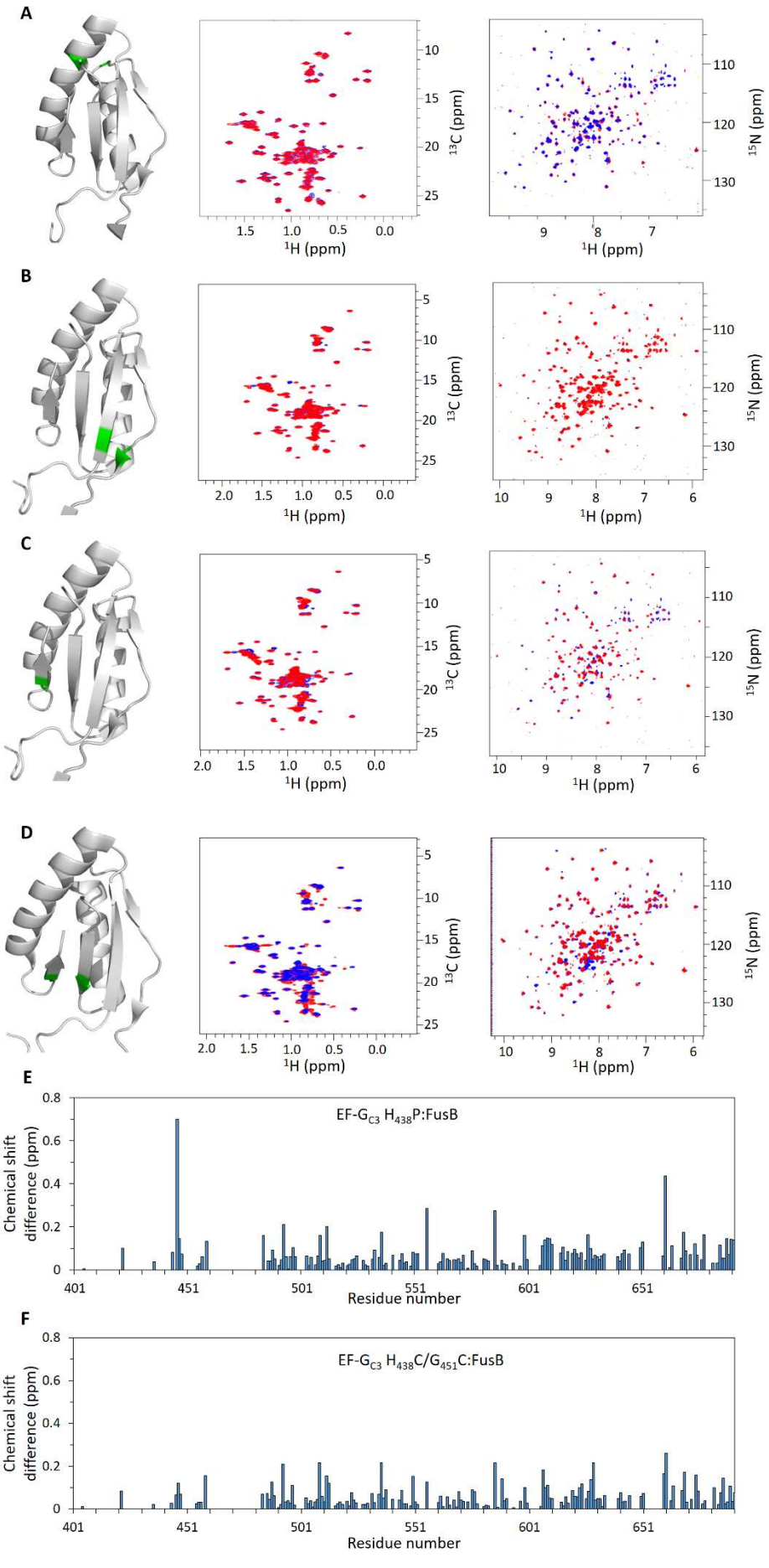
A
B
C
D
E
F
^{13}C (ppm)
^{15}N (ppm)
^{1}H (ppm)
Chemical shift difference (ppm)
Residue number
EF-G$_{C3}$ H$_{438}$P:FusB
EF-G$_{C3}$ H$_{438}$C/G$_{451}$C:FusB

Fig. S5. The positions of mutations and interactions inserted into EF-G_{C3} to disrupt FusB induced dynamics without preventing binding or conformational changes. Mutations and interactions are shown in green on the structure of domain III. ^{1}H-^{13}C-HMQC spectra (left) and ^{1}H-^{15}N-TROSY-HSQC spectra (right) for each mutant bound to FusB (red) are shown overlaid with the spectra of the wild type protein bound to FusB (blue), showing few chemical shift changes. Peaks absent from the mutant ^{15}N-^{1}H-TROSY-HSQC spectra are alanine residues that were not ^{15}N labelled in these samples due to the use of methyl-^{13}C-^{1}H, ^{2}H L-alanine to label the CH_3 group, which did not include ^{15}N labelling. Consequently, all alanines in these samples were missing from these spectra. This indicates that FusB binding and conformational changes are not perturbed by the mutations. **(A)** EF-G_{C3} $N_{470}D$. The substituted aspartate side chain was designed to form a salt bridge with the wild type K_{422} in the α-helix. **(B)** EF-G_{C3} $H_{409}K/M_{479}E$. The substituted residues are expected to form a salt bridge between β-strands. **(C)** EF-G_{C3} $H_{438}P$. The proline substitution is expected to restrain dynamics at the end of the β-strand. **(D)** EF-G_{C3} $H_{438}C/G_{451}C$. The substituted cysteines are expected to form a disulphide bond between the β-strands. **(E)** Combined ^{1}H and ^{15}N chemical shift differences between wild type and mutant EF-G_{C3} bound to FusB for EF-G_{C3} $H_{438}P$ (top) and EF-G_{C3} $H_{438}C/G_{451}C$ (bottom).

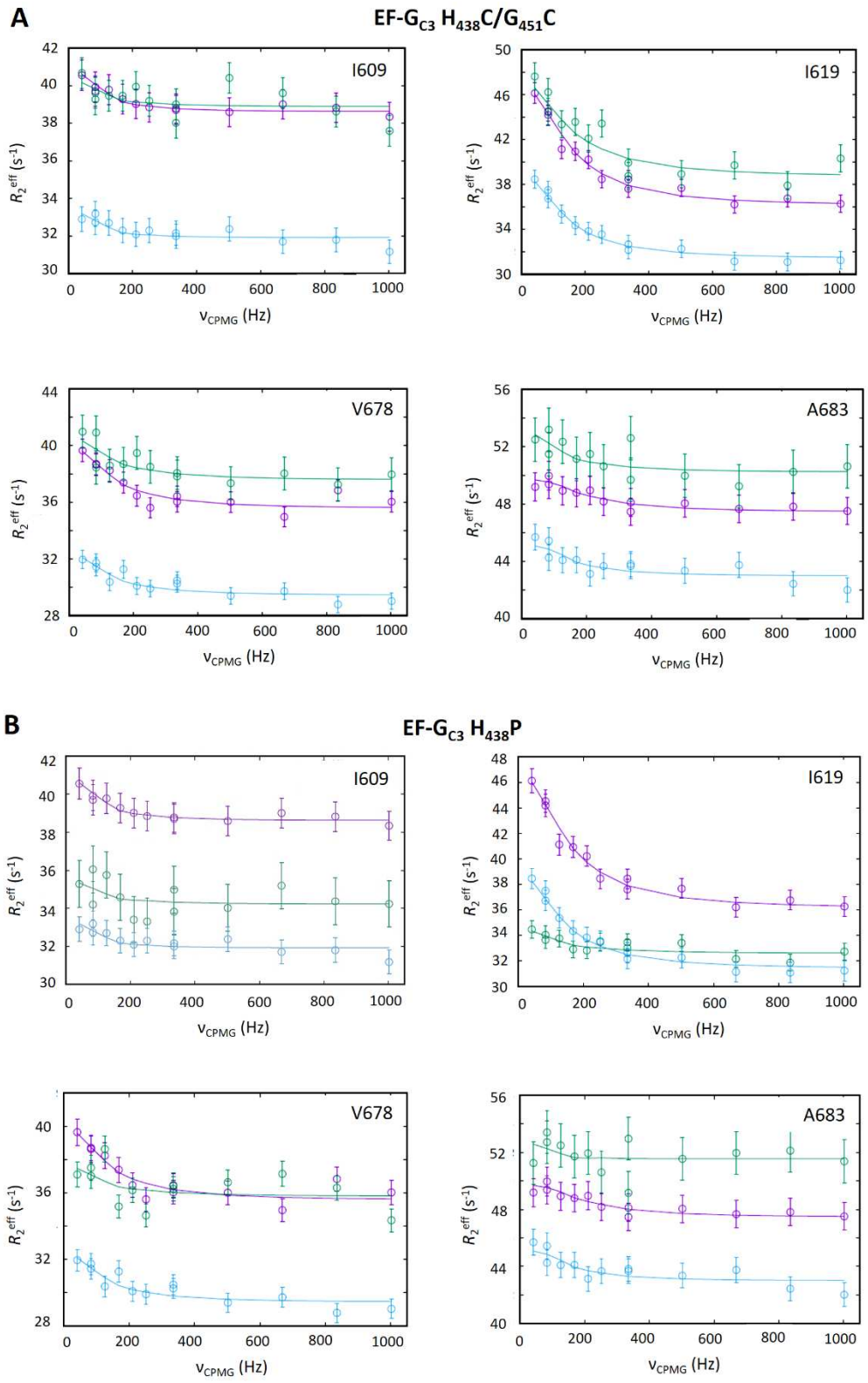

Fig. S6. Comparison of relaxation dispersion profiles outside of domain III between wild type and mutant EF-G$_{C3}$ when bound to FusB. Methyl relaxation dispersion curves measured for residues in domains IV and V of wild type EF-G$_{C3}$:FusB at 950 MHz (magenta) and 750 MHz (blue) and in **(A)** EF-G$_{C3}$ H$_{438}$C/G$_{451}$C at 950 MHz and **(B)** EF-G$_{C3}$ H$_{438}$P at 950 MHz (green),

showing similar dispersion effects in the complex for EF-G_{C3} $H_{453}C/G_{451}C$, suggesting that this mutation does not affect dynamics elsewhere in the protein. For EF-G_{C3} $H_{438}P$, while the magnitude of the dispersion effects are reduced for I_{619} and V_{678}, all residues showing dispersion in the wild type show dispersion in the mutant protein. All relaxation dispersion data were acquired at 30 °C with a relaxation delay of 24 ms. Errors in R_2^{eff} were determined through repeated measurement of 2 data points per experiment. Where error bars are not visible, they are within the limits of the data point. For each relaxation dispersion experiment N=13 at each field.

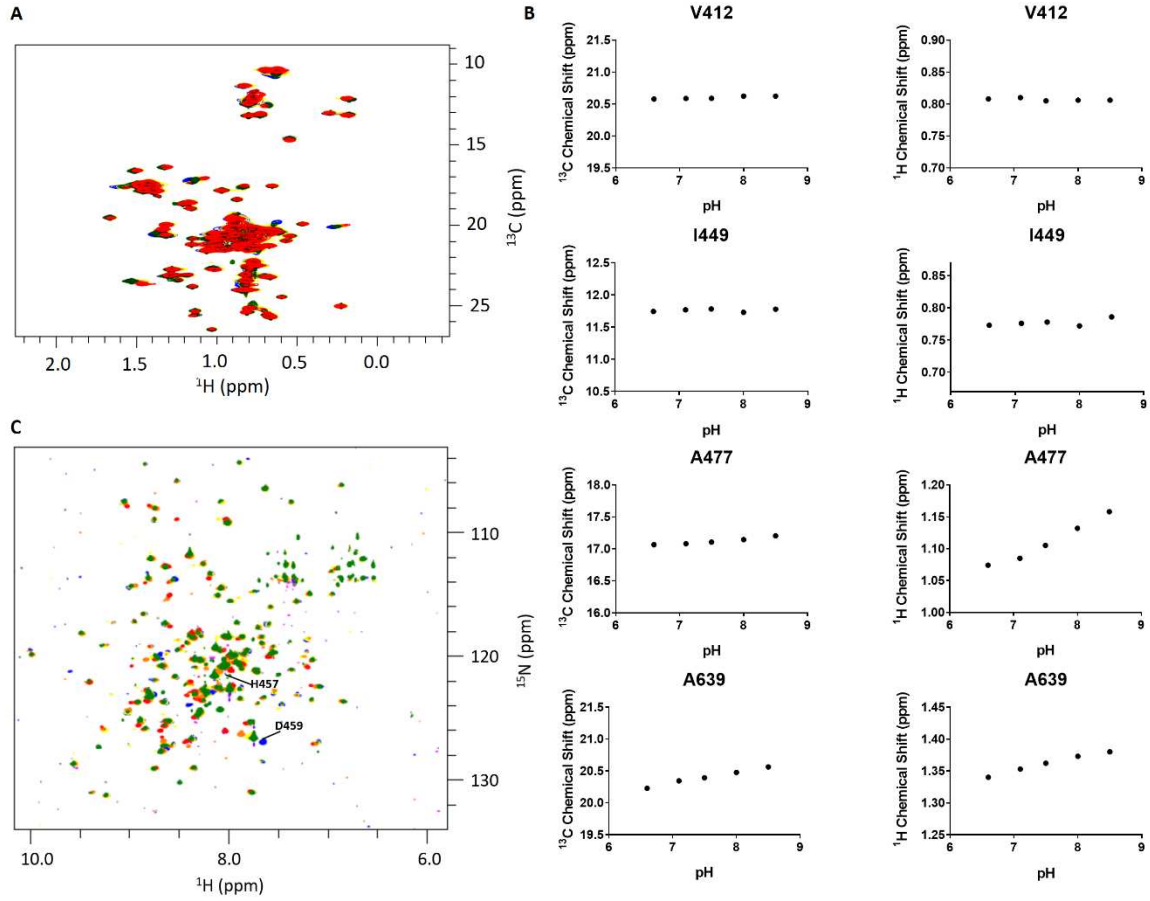

Fig. S7. pH dependent changes in chemical shift in the FusB bound state. (A) ^{1}H-^{13}C-HMQC spectra of EF-G_{C3} bound to FusB recorded at pH 6.6 (red), pH 7.1 (orange), pH 7.5 (yellow), pH 8.0 (green) and pH 8.5 (blue) are overlaid, showing very little change in chemical shift with pH. **(B)** Plots of changes in chemical shift with pH for ^{13}C (left) and ^{1}H (right) in residues within domain III that show relaxation dispersion profiles in the FusB bound state (residues 412-477). The majority of these residues show no significant change in chemical shift with pH. The greatest changes in chemical shift are seen for A_{477} (domain III) and A_{639} (domain V). However, as A_{639} is not one of the residues showing changes in relaxation dispersion in response to FusB binding and A_{477} is not close to the mutated histidine residues and this effect is not widespread throughout the domain, it is unlikely that the dispersion profiles observed are due to the titration of a histidine residue. For each graph *N*=5. **(C)** ^{1}H-^{15}N-TROSY-HSQC spectra of EF-G_{C3} bound to FusB. Spectra recorded at the same pHs as ^{1}H-^{13}C-HMQC spectra **(A)**. Only 2 additional peaks are visible in the pH 6.6 spectrum compared with the pH 8.0 spectrum, suggesting the broadening of domain III peaks is not due to amide exchange at high pH.

Table S1. Summary of |Δω| data within domain III from NMR relaxation dispersion experiments.

Residue	1H |Δω| apo (ppm)	13C |Δω| apo (ppm)	1H |Δω| complex (ppm)	13C |Δω| complex (ppm)
408	< 0.02	1.4 ± 0.1	-[a]	-[a]
412	-[b]	-[b]	0.107 ± 0.002	0.50 ± 0.15
418	< 0.02	1.00 ± 0.07	0.01 ± 0.009	1.1 ± 0.1
427	-[b]	-[b]	0.073 ± 0.004	0.45 ± 0.15
448	-[b]	-[b]	0.068 ±0.007	0.21 ±0.1
449	< 0.02	0.89 ± 0.06	0.068 ± 0.003	0.34 ± 0.09
450	< 0.02	0.44 ± 0.05	< 0.02	1.01 ± 0.07
460	< 0.02	0.74 ± 0.05	0.087 ± 0.002	0.64 ± 0.09
462	< 0.02	1.2 ± 0.1	-[a]	-[a]
471	< 0.02	0.96 ± 0.09	-[a]	-[a]
477	-[b]	-[b]	< 0.02	0.63 ± 0.08

[a]Residue not assigned

[b]No dispersion

Table S2. HX-MS data. Summary of deuterium uptake measurements for EF-G_{C3}. Deuterium uptake is tabulated for all detected peptides at each deuterium incubation timepoint. Data are shown as mean ± standard deviation of three replicate measurements.

Peptide			Incubation Time (min)									
			apo EFG-C3					+ FusB				
Start Res.	End Res.	Sequence	0.5	2	10	30	60	0.5	2	10	30	60
1	9	MEFPEPVIH	1.78 ± 0.01	2.16 ± 0.05	2.56 ± 0.06	2.72 ± 0.01	2.81 ± 0.10	2.65 ± 0.01	2.70 ± 0.05	2.68 ± 0.06	2.69 ± 0.04	2.86 ± 0.06
3	10	FPEPVIHL	1.29 ± 0.03	1.71 ± 0.04	2.16 ± 0.06	2.08 ± 0.05	2.12 ± 0.04	1.87 ± 0.03	1.92 ± 0.01	1.87 ± 0.07	1.90 ± 0.05	2.06 ± 0.04
4	9	PEPVIH	0.90 ± 0.02	1.25 ± 0.03	1.67 ± 0.05	1.47 ± 0.08	1.55 ± 0.03	1.48 ± 0.01	1.59 ± 0.04	1.54 ± 0.02	1.44 ± 0.04	1.50 ± 0.02
11	22	SVEPKSKADQDK	2.62 ± 0.04	3.01 ± 0.05	3.30 ± 0.07	3.15 ± 0.04	3.28 ± 0.12	2.98 ± 0.03	3.04 ± 0.08	3.10 ± 0.06	3.02 ± 0.06	3.16 ± 0.15
23	26	MTQA	0.73 ± 0.03	0.91 ± 0.01	1.06 ± 0.01	1.11 ± 0.02	1.16 ± 0.05	1.02 ± 0.02	1.03 ± 0.03	1.03 ± 0.06	1.05 ± 0.02	1.15 ± 0.07
23	27	MTQAL	0.70 ± 0.03	0.95 ± 0.03	1.29 ± 0.03	1.48 ± 0.03	1.63 ± 0.03	1.39 ± 0.02	1.44 ± 0.02	1.43 ± 0.03	1.45 ± 0.02	1.49 ± 0.09
24	27	TQAL	0.35 ± 0.02	0.55 ± 0.02	0.96 ± 0.02	1.13 ± 0.02	1.25 ± 0.04	1.09 ± 0.04	1.16 ± 0.05	1.13 ± 0.03	1.11 ± 0.06	1.23 ± 0.09
27	37	LVKLQEEDPTF	1.51 ± 0.06	2.07 ± 0.05	3.01 ± 0.13	3.33 ± 0.05	3.62 ± 0.05	3.19 ± 0.13	3.26 ± 0.04	3.32 ± 0.07	3.25 ± 0.04	3.40 ± 0.14
28	34	VKLQEED	0.99 ± 0.05	1.33 ± 0.04	1.75 ± 0.04	2.07 ± 0.06	2.25 ± 0.08	2.04 ± 0.05	2.14 ± 0.06	2.18 ± 0.11	2.15 ± 0.07	2.18 ± 0.13
28	37	VKLQEEDPTF	1.48 ± 0.06	2.09 ± 0.04	2.81 ± 0.06	3.25 ± 0.05	3.42 ± 0.07	3.09 ± 0.06	3.24 ± 0.05	3.32 ± 0.09	3.24 ± 0.04	3.44 ± 0.15
28	42	VKLQEEDPTFHAHTD	1.70 ± 0.08	2.26 ± 0.08	3.01 ± 0.12	3.20 ± 0.11	3.42 ± 0.11	3.09 ± 0.14	3.19 ± 0.17	3.22 ± 0.18	3.19 ± 0.16	3.31 ± 0.21
28	44	VKLQEEDPTFHAHTDEE	1.99 ± 0.05	2.54 ± 0.06	3.32 ± 0.10	3.45 ± 0.11	3.68 ± 0.13	3.33 ± 0.07	3.41 ± 0.17	3.46 ± 0.17	3.47 ± 0.12	3.63 ± 0.15
28	47	VKLQEEDPTFHAHTDEETGQ	3.47 ± 0.04	4.14 ± 0.04	5.09 ± 0.05	5.24 ± 0.14	5.60 ± 0.22	5.04 ± 0.07	4.98 ± 0.12	4.99 ± 0.13	5.13 ± 0.06	5.46 ± 0.30
30	44	LQEEDPTFHAHTDEE	1.46 ± 0.04	1.87 ± 0.05	2.27 ± 0.10	2.17 ± 0.16	2.17 ± 0.06	2.22 ± 0.01	2.07 ± 0.08	2.11 ± 0.05	2.25 ± 0.03	2.32 ± 0.14
31	37	QEEDPTF	0.92 ± 0.02	1.19 ± 0.01	1.42 ± 0.02	1.46 ± 0.02	1.55 ± 0.05	1.39 ± 0.02	1.40 ± 0.01	1.38 ± 0.02	1.41 ± 0.01	1.48 ± 0.07
34	37	DPTF	0.49 ± 0.05	0.77 ± 0.03	0.98 ± 0.02	1.10 ± 0.02	1.13 ± 0.06	1.02 ± 0.03	1.05 ± 0.04	1.07 ± 0.03	1.08 ± 0.03	1.06 ± 0.06
38	44	HAHTDEE	0.64 ± 0.04	0.66 ± 0.06	0.63 ± 0.06	0.60 ± 0.05	0.64 ± 0.07	0.65 ± 0.03	0.69 ± 0.07	0.72 ± 0.08	0.65 ± 0.08	0.60 ± 0.05
38	55	HAHTDEETGQVIIGGMGE	3.33 ± 0.08	3.77 ± 0.18	4.48 ± 0.14	4.72 ± 0.22	5.15 ± 0.21	4.85 ± 0.11	5.32 ± 0.08	5.39 ± 0.14	5.29 ± 0.18	5.31 ± 0.23
38	56	HAHTDEETGQVIIGGMGEL	3.75 ± 0.13	4.45 ± 0.14	5.16 ± 0.21	5.20 ± 0.18	5.62 ± 0.19	5.11 ± 0.20	5.76 ± 0.10	5.81 ± 0.18	5.68 ± 0.21	5.78 ± 0.27
45	58	TGQVIIGGMGELHL	2.86 ± 0.18	3.46 ± 0.07	4.10 ± 0.06	4.38 ± 0.12	4.82 ± 0.10	3.90 ± 0.10	4.42 ± 0.08	4.50 ± 0.08	4.55 ± 0.26	4.64 ± 0.26
48	53	VIIGGM	0.83 ± 0.10	0.99 ± 0.12	1.45 ± 0.02	1.60 ± 0.02	1.80 ± 0.06	1.64 ± 0.04	1.77 ± 0.03	1.78 ± 0.04	1.79 ± 0.03	1.89 ± 0.08
48	54	VIIGGMG	1.51 ± 0.03	1.83 ± 0.05	2.08 ± 0.02	2.30 ± 0.07	2.45 ± 0.03	2.15 ± 0.03	2.33 ± 0.05	2.22 ± 0.05	2.39 ± 0.05	2.39 ± 0.12
48	55	VIIGGMGE	1.31 ± 0.02	1.57 ± 0.01	1.81 ± 0.01	1.95 ± 0.03	2.17 ± 0.03	1.96 ± 0.02	2.10 ± 0.03	2.10 ± 0.05	2.12 ± 0.04	2.19 ± 0.10
48	56	VIIGGMGEL	2.04 ± 0.00	2.29 ± 0.01	2.48 ± 0.01	2.63 ± 0.04	2.83 ± 0.06	2.61 ± 0.03	2.73 ± 0.05	2.72 ± 0.05	2.77 ± 0.05	2.81 ± 0.10
50	56	IGGMGEL	1.36 ± 0.03	1.50 ± 0.05	1.61 ± 0.04	1.69 ± 0.04	1.84 ± 0.03	1.68 ± 0.04	1.77 ± 0.05	1.75 ± 0.05	1.77 ± 0.04	1.84 ± 0.05
57	61	HLDIL	0.19 ± 0.01	0.35 ± 0.02	0.69 ± 0.02	0.97 ± 0.02	1.37 ± 0.02	0.86 ± 0.01	1.16 ± 0.01	1.41 ± 0.01	1.40 ± 0.01	1.38 ± 0.05
59	64	DILVDR	0.31 ± 0.04	0.49 ± 0.03	0.93 ± 0.05	1.24 ± 0.03	1.63 ± 0.04	1.45 ± 0.05	1.73 ± 0.06	1.79 ± 0.09	1.86 ± 0.08	1.95 ± 0.13

62	68	VDRMKKE	0.81 ± 0.05	1.26 ± 0.05	1.94 ± 0.12	1.89 ± 0.05	1.99 ± 0.02	1.70 ± 0.09	1.62 ± 0.04	1.63 ± 0.04	1.78 ± 0.04	1.86 ± 0.11
68	72	EFNVE	0.90 ± 0.02	1.14 ± 0.01	1.35 ± 0.02	1.47 ± 0.03	1.56 ± 0.05	1.46 ± 0.03	1.50 ± 0.02	1.44 ± 0.03	1.47 ± 0.03	1.49 ± 0.09
69	73	FNVEC	1.02 ± 0.02	1.28 ± 0.04	1.44 ± 0.02	1.62 ± 0.03	1.73 ± 0.03	1.56 ± 0.02	1.59 ± 0.04	1.53 ± 0.07	1.59 ± 0.03	1.62 ± 0.09
73	81	CNVGAPMVS	2.96 ± 0.04	3.30 ± 0.05	3.35 ± 0.09	3.33 ± 0.03	3.42 ± 0.10	3.33 ± 0.05	3.32 ± 0.06	3.27 ± 0.09	3.35 ± 0.08	3.40 ± 0.15
73	82	CNVGAPMVSY	3.03 ± 0.03	3.37 ± 0.04	3.60 ± 0.07	3.61 ± 0.07	3.89 ± 0.07	3.39 ± 0.03	3.39 ± 0.04	3.37 ± 0.05	3.44 ± 0.04	3.57 ± 0.12
74	81	NVGAPMVS	2.31 ± 0.03	2.72 ± 0.05	2.76 ± 0.05	2.69 ± 0.08	2.78 ± 0.11	2.67 ± 0.04	2.66 ± 0.08	2.56 ± 0.09	2.66 ± 0.08	2.74 ± 0.05
75	82	VGAPMVSY	2.16 ± 0.04	2.34 ± 0.03	2.48 ± 0.07	2.47 ± 0.03	2.64 ± 0.05	2.25 ± 0.04	2.29 ± 0.08	2.27 ± 0.03	2.36 ± 0.03	2.48 ± 0.06
76	82	GAPMVSY	1.50 ± 0.02	1.63 ± 0.05	1.73 ± 0.02	1.74 ± 0.05	1.86 ± 0.02	1.67 ± 0.07	1.64 ± 0.09	1.59 ± 0.03	1.68 ± 0.05	1.73 ± 0.12
78	82	PMVSY	1.16 ± 0.01	1.21 ± 0.02	1.21 ± 0.01	1.32 ± 0.01	1.39 ± 0.03	1.20 ± 0.03	1.28 ± 0.02	1.26 ± 0.03	1.27 ± 0.05	1.26 ± 0.05
83	95	RETFKSSAQVQGK	2.21 ± 0.16	2.50 ± 0.12	2.44 ± 0.13	2.43 ± 0.12	2.41 ± 0.12	1.97 ± 0.11	2.13 ± 0.12	2.12 ± 0.12	2.19 ± 0.12	2.21 ± 0.11
83	96	RETFKSSAQVQGKF	2.39 ± 0.24	2.79 ± 0.26	2.76 ± 0.23	2.73 ± 0.20	2.75 ± 0.16	2.04 ± 0.21	2.30 ± 0.23	2.28 ± 0.22	2.44 ± 0.23	2.55 ± 0.21
96	109	FSRQSGGRGQYGDV	3.12 ± 0.11	3.36 ± 0.11	3.59 ± 0.12	3.69 ± 0.11	4.02 ± 0.16	3.11 ± 0.15	3.26 ± 0.18	3.50 ± 0.20	3.78 ± 0.15	4.12 ± 0.18
96	110	FSRQSGGRGQYGDVH	3.14 ± 0.10	3.38 ± 0.08	3.66 ± 0.09	3.72 ± 0.09	4.01 ± 0.14	3.02 ± 0.11	3.21 ± 0.13	3.49 ± 0.17	3.68 ± 0.13	4.03 ± 0.16
96	112	FSRQSGGRGQYGDVHIE	2.97 ± 0.05	3.27 ± 0.05	3.59 ± 0.06	3.60 ± 0.07	3.88 ± 0.11	2.94 ± 0.09	3.14 ± 0.13	3.43 ± 0.18	3.59 ± 0.14	3.96 ± 0.13
97	110	SRQSGGRGQYGDVH	2.92 ± 0.07	3.20 ± 0.09	3.39 ± 0.11	3.44 ± 0.08	3.73 ± 0.15	2.81 ± 0.08	2.96 ± 0.07	3.17 ± 0.09	3.45 ± 0.09	3.82 ± 0.15
110	117	HIEFTPNE	0.62 ± 0.09	0.67 ± 0.11	0.55 ± 0.10	0.58 ± 0.08	0.58 ± 0.08	0.47 ± 0.09	0.59 ± 0.10	0.54 ± 0.15	0.58 ± 0.09	0.55 ± 0.08
110	121	HIEFTPNETGAG	2.09 ± 0.09	2.38 ± 0.06	2.36 ± 0.07	2.43 ± 0.06	2.45 ± 0.11	1.34 ± 0.06	1.57 ± 0.06	1.64 ± 0.07	1.74 ± 0.08	1.86 ± 0.05
110	122	HIEFTPNETGAGF	2.32 ± 0.01	2.63 ± 0.01	2.65 ± 0.03	2.61 ± 0.02	2.63 ± 0.06	1.23 ± 0.03	1.43 ± 0.04	1.50 ± 0.02	1.58 ± 0.04	1.70 ± 0.02
110	123	HIEFTPNETGAGFE	2.32 ± 0.03	2.65 ± 0.01	2.69 ± 0.05	2.66 ± 0.01	2.73 ± 0.04	1.30 ± 0.03	1.49 ± 0.04	1.50 ± 0.04	1.58 ± 0.04	1.70 ± 0.03
111	117	IEFTPNE	0.60 ± 0.02	0.63 ± 0.01	0.57 ± 0.01	0.56 ± 0.01	0.57 ± 0.01	0.47 ± 0.03	0.54 ± 0.04	0.56 ± 0.02	0.56 ± 0.02	0.53 ± 0.02
111	121	IEFTPNETGAG	1.98 ± 0.12	2.24 ± 0.09	2.27 ± 0.13	2.37 ± 0.16	2.36 ± 0.16	1.34 ± 0.16	1.53 ± 0.08	1.71 ± 0.20	1.77 ± 0.09	1.87 ± 0.12
111	122	IEFTPNETGAGF	2.07 ± 0.11	2.30 ± 0.20	2.29 ± 0.15	2.37 ± 0.15	2.41 ± 0.14	1.16 ± 0.08	1.39 ± 0.09	1.46 ± 0.08	1.53 ± 0.10	1.63 ± 0.10
111	123	IEFTPNETGAGFE	2.19 ± 0.02	2.38 ± 0.06	2.41 ± 0.03	2.47 ± 0.03	2.55 ± 0.06	1.32 ± 0.05	1.51 ± 0.02	1.58 ± 0.02	1.64 ± 0.03	1.74 ± 0.02
111	124	IEFTPNETGAGFEF	2.18 ± 0.05	2.30 ± 0.03	2.32 ± 0.05	2.33 ± 0.03	2.36 ± 0.06	0.98 ± 0.03	1.19 ± 0.02	1.20 ± 0.02	1.20 ± 0.03	1.24 ± 0.04
113	123	FTPNETGAGFE	2.09 ± 0.08	2.27 ± 0.12	2.32 ± 0.10	2.42 ± 0.11	2.59 ± 0.20	1.21 ± 0.13	1.39 ± 0.10	1.47 ± 0.10	1.58 ± 0.14	1.67 ± 0.15
114	122	TPNETGAGF	1.71 ± 0.05	1.92 ± 0.04	1.98 ± 0.03	2.00 ± 0.03	2.00 ± 0.03	0.98 ± 0.02	1.09 ± 0.01	1.17 ± 0.13	1.21 ± 0.02	1.40 ± 0.09
115	122	PNETGAGF	1.18 ± 0.03	1.38 ± 0.06	1.59 ± 0.08	1.37 ± 0.04	1.32 ± 0.04	0.68 ± 0.02	0.78 ± 0.01	0.84 ± 0.03	0.87 ± 0.06	0.94 ± 0.02
118	122	TGAGF	0.90 ± 0.03	1.11 ± 0.04	1.18 ± 0.04	1.18 ± 0.03	1.24 ± 0.06	0.32 ± 0.03	0.27 ± 0.04	0.13 ± 0.06	0.25 ± 0.12	0.21 ± 0.09
118	123	TGAGFE	0.90 ± 0.03	1.10 ± 0.04	1.10 ± 0.03	1.22 ± 0.03	1.23 ± 0.02	0.29 ± 0.04	0.32 ± 0.03	0.32 ± 0.05	0.31 ± 0.03	0.30 ± 0.02
122	125	FEFE	0.61 ± 0.02	0.63 ± 0.01	0.58 ± 0.01	0.58 ± 0.02	0.57 ± 0.01	0.18 ± 0.02	0.17 ± 0.02	0.14 ± 0.02	0.17 ± 0.01	0.16 ± 0.01
122	127	FEFENA	1.06 ± 0.01	1.11 ± 0.01	1.14 ± 0.02	1.22 ± 0.02	1.32 ± 0.03	0.34 ± 0.05	0.34 ± 0.03	0.32 ± 0.04	0.35 ± 0.04	0.37 ± 0.04
123	127	EFENA	0.51 ± 0.04	0.56 ± 0.01	0.59 ± 0.01	0.68 ± 0.01	0.77 ± 0.02	0.26 ± 0.01	0.24 ± 0.01	0.18 ± 0.01	0.21 ± 0.01	0.21 ± 0.02
124	127	FENA	0.44 ± 0.03	0.48 ± 0.03	0.56 ± 0.02	0.61 ± 0.03	0.71 ± 0.02	0.17 ± 0.02	0.18 ± 0.02	0.16 ± 0.01	0.19 ± 0.01	0.19 ± 0.02
124	137	FENAIVGGVVPREY	3.36 ± 0.12	3.79 ± 0.14	3.98 ± 0.12	4.01 ± 0.13	4.49 ± 0.14	2.79 ± 0.11	3.10 ± 0.09	3.35 ± 0.09	3.68 ± 0.09	3.80 ± 0.16

125	137	ENAIVGGVVPREY	3.30 ± 0.03	3.54 ± 0.04	3.75 ± 0.05	3.87 ± 0.03	4.17 ± 0.09	2.44 ± 0.03	2.80 ± 0.08	3.17 ± 0.13	3.28 ± 0.07	3.38 ± 0.13
126	136	NAIVGGVVPRE	3.37 ± 0.08	3.62 ± 0.08	3.68 ± 0.07	3.80 ± 0.07	4.08 ± 0.09	2.57 ± 0.07	2.96 ± 0.07	3.38 ± 0.07	3.50 ± 0.06	3.52 ± 0.10
126	137	NAIVGGVVPREY	3.10 ± 0.08	3.32 ± 0.09	3.42 ± 0.10	3.48 ± 0.11	3.75 ± 0.10	2.30 ± 0.09	2.67 ± 0.10	3.02 ± 0.12	3.12 ± 0.11	3.18 ± 0.12
127	137	AIVGGVVPREY	3.06 ± 0.03	3.21 ± 0.03	3.14 ± 0.06	3.10 ± 0.03	3.30 ± 0.05	2.37 ± 0.06	2.82 ± 0.05	3.16 ± 0.06	3.23 ± 0.08	3.26 ± 0.08
128	136	IVGGVVPRE	2.33 ± 0.07	2.43 ± 0.08	2.38 ± 0.08	2.42 ± 0.07	2.52 ± 0.10	2.16 ± 0.09	2.33 ± 0.09	2.45 ± 0.09	2.53 ± 0.09	2.57 ± 0.12
128	142	IVGGVVPREYIPSVE	3.30 ± 0.02	3.50 ± 0.03	3.39 ± 0.04	3.39 ± 0.02	3.51 ± 0.05	2.91 ± 0.01	3.12 ± 0.02	3.19 ± 0.02	3.27 ± 0.06	3.39 ± 0.07
130	137	GGVVPREY	1.86 ± 0.05	2.05 ± 0.05	2.08 ± 0.05	1.93 ± 0.05	2.00 ± 0.05	1.72 ± 0.02	1.79 ± 0.03	1.83 ± 0.04	1.88 ± 0.02	2.05 ± 0.07
138	142	IPSVE	0.55 ± 0.04	0.54 ± 0.04	0.48 ± 0.04	0.50 ± 0.04	0.48 ± 0.04	0.39 ± 0.00	0.41 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.35 ± 0.01
138	146	IPSVEAGLK	0.68 ± 0.06	0.73 ± 0.06	0.62 ± 0.07	0.64 ± 0.05	0.63 ± 0.05	0.70 ± 0.06	0.72 ± 0.07	0.61 ± 0.05	0.59 ± 0.05	0.55 ± 0.05
138	148	IPSVEAGLKDA	0.72 ± 0.05	0.77 ± 0.03	0.71 ± 0.05	0.75 ± 0.03	0.80 ± 0.03	0.68 ± 0.03	0.68 ± 0.01	0.56 ± 0.01	0.61 ± 0.01	0.58 ± 0.02
143	148	AGLKDA	0.38 ± 0.07	0.41 ± 0.03	0.38 ± 0.03	0.42 ± 0.03	0.43 ± 0.03	0.34 ± 0.03	0.32 ± 0.02	0.25 ± 0.00	0.29 ± 0.02	0.30 ± 0.03
146	154	KDAMENGVL	1.03 ± 0.05	1.38 ± 0.02	1.47 ± 0.02	1.65 ± 0.04	1.72 ± 0.03	0.67 ± 0.07	0.84 ± 0.11	1.02 ± 0.04	1.11 ± 0.08	1.14 ± 0.07
147	154	DAMENGVL	0.84 ± 0.03	1.15 ± 0.02	1.33 ± 0.01	1.41 ± 0.02	1.50 ± 0.03	0.46 ± 0.03	0.66 ± 0.02	0.82 ± 0.06	0.87 ± 0.03	0.93 ± 0.05
148	154	AMENGVL	0.87 ± 0.03	1.23 ± 0.03	1.37 ± 0.02	1.52 ± 0.04	1.64 ± 0.02	0.54 ± 0.03	0.70 ± 0.02	0.87 ± 0.01	0.97 ± 0.03	1.03 ± 0.01
149	154	MENGVL	0.54 ± 0.04	0.77 ± 0.03	0.86 ± 0.04	0.89 ± 0.04	0.93 ± 0.03	0.30 ± 0.05	0.37 ± 0.04	0.47 ± 0.04	0.58 ± 0.04	0.61 ± 0.04
149	161	MENGVLAGYPLID	1.22 ± 0.04	1.61 ± 0.03	1.66 ± 0.04	1.78 ± 0.02	1.81 ± 0.04	0.82 ± 0.02	1.07 ± 0.03	1.23 ± 0.04	1.37 ± 0.05	1.48 ± 0.02
151	154	NGVL	0.43 ± 0.02	0.62 ± 0.02	0.66 ± 0.01	0.66 ± 0.02	0.66 ± 0.01	0.28 ± 0.03	0.42 ± 0.02	0.55 ± 0.03	0.62 ± 0.02	0.63 ± 0.02
155	161	AGYPLID	0.31 ± 0.06	0.32 ± 0.09	0.26 ± 0.08	0.28 ± 0.05	0.26 ± 0.05	0.24 ± 0.04	0.19 ± 0.04	0.07 ± 0.02	0.12 ± 0.06	0.04 ± 0.08
155	162	AGYPLIDV	0.37 ± 0.02	0.41 ± 0.01	0.41 ± 0.01	0.44 ± 0.03	0.51 ± 0.03	0.27 ± 0.05	0.27 ± 0.05	0.24 ± 0.05	0.24 ± 0.05	0.23 ± 0.05
155	163	AGYPLIDVK	0.35 ± 0.05	0.41 ± 0.04	0.41 ± 0.05	0.44 ± 0.07	0.52 ± 0.06	0.36 ± 0.07	0.35 ± 0.06	0.28 ± 0.04	0.25 ± 0.05	0.28 ± 0.04
158	163	PLIDVK	0.23 ± 0.04	0.25 ± 0.05	0.26 ± 0.03	0.25 ± 0.03	0.27 ± 0.03	0.24 ± 0.02	0.20 ± 0.04	0.16 ± 0.03	0.14 ± 0.03	0.15 ± 0.03
162	166	VKAKL	0.17 ± 0.02	0.16 ± 0.00	0.13 ± 0.02	0.15 ± 0.01	0.11 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.06 ± 0.02	0.05 ± 0.02
163	166	KAKL	0.11 ± 0.03	0.12 ± 0.03	0.10 ± 0.03	0.10 ± 0.03	0.08 ± 0.04	0.05 ± 0.01	0.04 ± 0.02	-0.02 ± 0.01	0.03 ± 0.03	-0.03 ± 0.01
166	178	LYDGSYHDVDSSE	1.53 ± 0.03	1.66 ± 0.04	1.74 ± 0.03	1.69 ± 0.03	1.72 ± 0.06	1.63 ± 0.02	1.61 ± 0.05	1.55 ± 0.03	1.77 ± 0.07	1.78 ± 0.07
167	175	YDGSYHDVD	1.07 ± 0.06	1.15 ± 0.08	1.15 ± 0.08	1.13 ± 0.05	1.21 ± 0.06	1.04 ± 0.04	1.09 ± 0.05	1.13 ± 0.05	1.16 ± 0.06	1.25 ± 0.10
167	176	YDGSYHDVDS	1.44 ± 0.12	1.56 ± 0.02	1.59 ± 0.08	1.51 ± 0.05	1.61 ± 0.06	1.74 ± 0.16	1.55 ± 0.05	1.70 ± 0.16	1.55 ± 0.04	1.72 ± 0.07
167	178	YDGSYHDVDSSE	1.98 ± 0.10	2.17 ± 0.10	2.21 ± 0.12	2.13 ± 0.10	2.28 ± 0.13	2.02 ± 0.12	2.07 ± 0.17	2.05 ± 0.11	2.07 ± 0.14	2.24 ± 0.15
167	179	YDGSYHDVDSSEM	2.79 ± 0.08	2.84 ± 0.09	2.97 ± 0.10	2.91 ± 0.12	2.98 ± 0.14	2.69 ± 0.02	2.67 ± 0.11	2.71 ± 0.02	2.74 ± 0.06	3.02 ± 0.06
172	178	HDVDSSE	1.52 ± 0.03	1.60 ± 0.03	1.55 ± 0.04	1.45 ± 0.03	1.55 ± 0.09	1.55 ± 0.03	1.57 ± 0.02	1.58 ± 0.05	1.53 ± 0.06	1.63 ± 0.09
172	180	HDVDSSEMA	2.14 ± 0.09	2.30 ± 0.13	2.24 ± 0.15	2.38 ± 0.12	2.51 ± 0.19	2.12 ± 0.06	2.27 ± 0.11	2.33 ± 0.12	2.44 ± 0.14	2.50 ± 0.31
179	182	MAFK	0.17 ± 0.03	0.18 ± 0.03	0.23 ± 0.03	0.29 ± 0.03	0.36 ± 0.04	0.15 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	0.19 ± 0.03	0.21 ± 0.02
179	184	MAFKIA	0.16 ± 0.08	0.20 ± 0.08	0.25 ± 0.08	0.34 ± 0.08	0.40 ± 0.09	0.15 ± 0.09	0.16 ± 0.08	0.15 ± 0.07	0.20 ± 0.09	0.22 ± 0.08
180	184	AFKIA	0.16 ± 0.02	0.20 ± 0.02	0.24 ± 0.02	0.29 ± 0.03	0.36 ± 0.04	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	0.21 ± 0.02
181	184	FKIA	0.11 ± 0.06	0.13 ± 0.05	0.14 ± 0.06	0.20 ± 0.03	0.25 ± 0.04	0.09 ± 0.05	0.04 ± 0.03	0.07 ± 0.03	0.08 ± 0.03	0.07 ± 0.05

181	185	FKIAA	0.16 ± 0.04	0.19 ± 0.05	0.19 ± 0.04	0.25 ± 0.05	0.26 ± 0.06	0.15 ± 0.03	0.13 ± 0.02	0.12 ± 0.03	0.15 ± 0.03	0.17 ± 0.03
182	187	KIAASL	0.29 ± 0.04	0.32 ± 0.01	0.24 ± 0.02	0.27 ± 0.01	0.26 ± 0.04	0.22 ± 0.01	0.23 ± 0.02	0.17 ± 0.01	0.18 ± 0.01	0.15 ± 0.01
187	192	LALKEA	0.25 ± 0.09	0.26 ± 0.09	0.21 ± 0.08	0.25 ± 0.06	0.26 ± 0.09	0.19 ± 0.09	0.23 ± 0.08	0.15 ± 0.05	0.19 ± 0.06	0.19 ± 0.06
188	192	ALKEA	0.28 ± 0.02	0.28 ± 0.02	0.23 ± 0.03	0.26 ± 0.03	0.28 ± 0.04	0.24 ± 0.03	0.23 ± 0.03	0.16 ± 0.01	0.23 ± 0.03	0.20 ± 0.01
188	197	ALKEAAKKCD	1.36 ± 0.07	1.65 ± 0.02	1.78 ± 0.02	1.77 ± 0.07	1.92 ± 0.05	1.16 ± 0.04	1.38 ± 0.02	1.57 ± 0.05	1.62 ± 0.04	1.67 ± 0.03
188	202	ALKEAAKKCDPVILE	1.36 ± 0.12	1.63 ± 0.11	1.77 ± 0.11	1.78 ± 0.11	1.91 ± 0.11	1.26 ± 0.09	1.55 ± 0.12	1.69 ± 0.11	1.74 ± 0.09	1.81 ± 0.07
193	205	AKKCDPVILEPMM	0.70 ± 0.07	0.70 ± 0.13	0.71 ± 0.17	0.64 ± 0.13	0.67 ± 0.08	0.67 ± 0.11	0.73 ± 0.14	0.58 ± 0.10	0.64 ± 0.12	0.59 ± 0.21
195	205	KCDPVILEPMM	0.18 ± 0.04	0.23 ± 0.04	0.23 ± 0.04	0.22 ± 0.04	0.26 ± 0.04	0.31 ± 0.03	0.36 ± 0.05	0.24 ± 0.03	0.26 ± 0.03	0.22 ± 0.04
198	201	PVIL	0.18 ± 0.03	0.20 ± 0.04	0.13 ± 0.03	0.16 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.13 ± 0.02	0.07 ± 0.02	0.11 ± 0.02	0.06 ± 0.03
198	202	PVILE	0.20 ± 0.02	0.22 ± 0.02	0.18 ± 0.03	0.18 ± 0.02	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.04	0.08 ± 0.02	0.11 ± 0.03	0.10 ± 0.04
198	205	PVILEPMM	0.21 ± 0.04	0.23 ± 0.04	0.18 ± 0.03	0.19 ± 0.03	0.16 ± 0.04	0.19 ± 0.04	0.19 ± 0.03	0.13 ± 0.03	0.16 ± 0.03	0.15 ± 0.03
198	206	PVILEPMMK	0.24 ± 0.04	0.28 ± 0.04	0.26 ± 0.05	0.23 ± 0.07	0.24 ± 0.04	0.30 ± 0.08	0.31 ± 0.05	0.18 ± 0.08	0.17 ± 0.05	0.17 ± 0.05
202	205	EPMM	0.11 ± 0.01	0.13 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
206	211	KVTIEM	0.28 ± 0.03	0.31 ± 0.04	0.27 ± 0.04	0.32 ± 0.04	0.29 ± 0.05	0.25 ± 0.05	0.23 ± 0.05	0.17 ± 0.04	0.18 ± 0.04	0.16 ± 0.05
206	215	KVTIEMPEEY	0.83 ± 0.05	0.93 ± 0.05	1.04 ± 0.06	1.14 ± 0.05	1.20 ± 0.04	0.87 ± 0.04	0.99 ± 0.05	1.07 ± 0.04	1.12 ± 0.06	1.15 ± 0.04
212	215	PEEY	0.46 ± 0.01	0.53 ± 0.02	0.66 ± 0.02	0.75 ± 0.02	0.79 ± 0.01	0.46 ± 0.02	0.53 ± 0.02	0.64 ± 0.02	0.72 ± 0.02	0.80 ± 0.04
215	218	YMGD	0.52 ± 0.02	0.55 ± 0.02	0.51 ± 0.02	0.46 ± 0.02	0.48 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.48 ± 0.02	0.50 ± 0.02	0.52 ± 0.02
215	220	YMGDIM	1.20 ± 0.01	1.52 ± 0.01	1.67 ± 0.02	1.66 ± 0.02	1.73 ± 0.04	0.93 ± 0.01	1.24 ± 0.01	1.53 ± 0.02	1.62 ± 0.02	1.73 ± 0.01
215	221	YMGDIMG	1.66 ± 0.02	1.97 ± 0.03	2.11 ± 0.04	2.11 ± 0.02	2.19 ± 0.06	1.40 ± 0.01	1.68 ± 0.01	1.94 ± 0.02	2.05 ± 0.01	2.18 ± 0.04
215	222	YMGDIMGD	1.66 ± 0.01	1.99 ± 0.02	2.13 ± 0.02	2.11 ± 0.02	2.19 ± 0.05	1.38 ± 0.01	1.68 ± 0.02	1.96 ± 0.02	2.06 ± 0.02	2.20 ± 0.04
216	221	MGDIMG	1.21 ± 0.06	1.50 ± 0.08	1.63 ± 0.03	1.70 ± 0.04	1.75 ± 0.08	1.02 ± 0.06	1.29 ± 0.03	1.53 ± 0.03	1.60 ± 0.03	1.74 ± 0.03
216	222	MGDIMGD	1.11 ± 0.03	1.39 ± 0.03	1.51 ± 0.03	1.49 ± 0.04	1.53 ± 0.04	0.91 ± 0.02	1.17 ± 0.02	1.40 ± 0.03	1.44 ± 0.04	1.56 ± 0.02
219	222	IMGD	0.54 ± 0.01	0.56 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.53 ± 0.01	0.57 ± 0.03	0.56 ± 0.01	0.54 ± 0.02	0.54 ± 0.01	0.56 ± 0.02
222	240	DVTSRRGRVDGMEPRGNAQ	5.45 ± 0.16	5.88 ± 0.16	5.89 ± 0.20	5.75 ± 0.16	5.93 ± 0.23	4.95 ± 0.18	5.26 ± 0.19	5.32 ± 0.19	5.53 ± 0.18	5.87 ± 0.23
223	240	VTSRRGRVDGMEPRGNAQ	4.66 ± 0.13	4.88 ± 0.12	4.89 ± 0.13	4.72 ± 0.13	4.87 ± 0.15	4.40 ± 0.15	4.53 ± 0.17	4.52 ± 0.18	4.63 ± 0.16	4.87 ± 0.19
239	244	AQVVNA	0.51 ± 0.03	0.60 ± 0.02	0.66 ± 0.02	0.78 ± 0.03	0.97 ± 0.06	0.19 ± 0.01	0.25 ± 0.02	0.32 ± 0.01	0.34 ± 0.00	0.46 ± 0.05
240	244	QVVNA	0.30 ± 0.03	0.34 ± 0.03	0.34 ± 0.04	0.40 ± 0.02	0.48 ± 0.04	0.20 ± 0.02	0.20 ± 0.01	0.18 ± 0.01	0.25 ± 0.02	0.35 ± 0.03
241	244	VVNA	0.28 ± 0.03	0.27 ± 0.03	0.27 ± 0.03	0.34 ± 0.01	0.37 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.19 ± 0.01	0.25 ± 0.02	0.30 ± 0.03
244	250	AYVPLSE	0.20 ± 0.03	0.36 ± 0.05	0.42 ± 0.05	0.44 ± 0.05	0.45 ± 0.04	0.31 ± 0.02	0.44 ± 0.02	0.49 ± 0.04	0.55 ± 0.01	0.52 ± 0.06
244	251	AYVPLSEM	0.31 ± 0.09	0.40 ± 0.08	0.51 ± 0.09	0.59 ± 0.13	0.58 ± 0.11	0.38 ± 0.10	0.47 ± 0.10	0.60 ± 0.10	0.66 ± 0.12	0.57 ± 0.12
245	250	YVPLSE	0.60 ± 0.05	0.65 ± 0.04	0.57 ± 0.04	0.65 ± 0.07	0.72 ± 0.06	0.65 ± 0.03	0.66 ± 0.03	0.56 ± 0.06	0.67 ± 0.03	0.59 ± 0.10
245	251	YVPLSEM	0.28 ± 0.11	0.39 ± 0.11	0.46 ± 0.12	0.52 ± 0.11	0.52 ± 0.11	0.30 ± 0.09	0.40 ± 0.10	0.46 ± 0.09	0.51 ± 0.10	0.50 ± 0.09
247	251	PLSEM	0.20 ± 0.01	0.28 ± 0.01	0.31 ± 0.01	0.36 ± 0.02	0.36 ± 0.02	0.19 ± 0.03	0.25 ± 0.02	0.31 ± 0.02	0.33 ± 0.03	0.35 ± 0.02
254	258	YATSL	1.35 ± 0.02	1.50 ± 0.03	1.68 ± 0.01	1.74 ± 0.01	1.86 ± 0.07	1.27 ± 0.01	1.31 ± 0.01	1.37 ± 0.01	1.44 ± 0.04	1.56 ± 0.05

259	267	RSNTQGRGT	2.39 ± 0.04	2.69 ± 0.11	2.96 ± 0.06	2.95 ± 0.07	3.08 ± 0.09	1.26 ± 0.07	1.51 ± 0.05	1.73 ± 0.06	1.93 ± 0.06	2.15 ± 0.06
259	270	RSNTQGRGTYTM	3.46 ± 0.09	3.87 ± 0.14	4.03 ± 0.17	4.06 ± 0.15	4.25 ± 0.20	2.23 ± 0.09	2.65 ± 0.11	2.99 ± 0.12	3.18 ± 0.14	3.43 ± 0.15
259	271	RSNTQGRGTYTMY	3.15 ± 0.06	3.52 ± 0.08	3.69 ± 0.08	3.75 ± 0.06	3.93 ± 0.11	2.03 ± 0.05	2.46 ± 0.05	2.78 ± 0.04	2.92 ± 0.06	3.15 ± 0.06
268	271	YTMY	0.65 ± 0.03	0.66 ± 0.02	0.59 ± 0.02	0.60 ± 0.02	0.59 ± 0.02	0.59 ± 0.02	0.58 ± 0.01	0.55 ± 0.00	0.55 ± 0.01	0.56 ± 0.02
271	284	YFDHYAEVPKSIAE	2.67 ± 0.12	3.10 ± 0.19	3.39 ± 0.26	3.53 ± 0.20	3.68 ± 0.23	2.23 ± 0.07	2.67 ± 0.04	3.20 ± 0.06	3.46 ± 0.11	3.63 ± 0.20
272	281	FDHYAEVPKS	1.71 ± 0.03	1.81 ± 0.03	1.90 ± 0.11	1.68 ± 0.02	1.76 ± 0.08	1.62 ± 0.09	1.72 ± 0.12	1.70 ± 0.15	1.71 ± 0.10	1.73 ± 0.11
272	284	FDHYAEVPKSIAE	2.39 ± 0.10	2.87 ± 0.06	3.17 ± 0.10	3.23 ± 0.09	3.39 ± 0.12	1.93 ± 0.10	2.31 ± 0.14	2.80 ± 0.16	3.03 ± 0.14	3.36 ± 0.11
276	284	AEVPKSIAE	1.78 ± 0.06	2.23 ± 0.07	2.61 ± 0.10	2.77 ± 0.07	2.90 ± 0.09	1.70 ± 0.13	2.06 ± 0.09	2.52 ± 0.08	2.72 ± 0.14	2.91 ± 0.09

Table S3. Primer sequences used in this study. FusA-S1-S4 primers were used for sequencing while FusA-H_{409}K and FusA-M_{479}E were used in Quikchange Multi reactions to create the H_{409}K/M_{479}E mutant, FusA-H_{438}C and FusA-G_{451}C were used in Quikchange Multi reactions to create the H_{438}C/G_{451}C mutant and the FusA-H_{438}P-F and R and FusA-N_{470}D-F and R primer pairs were used in Quikchange reactions to create the H_{438}P and N_{470}D mutations respectively. For mutagenic primers the base mismatches are shown in bold.

Primer	Sequence
FusA-H_{409}K	5'-GGAATTCCCAGAGCCAGTTATT**A**A**G**TTATCAGTAGAGCCAAAATC-3'
FusA-M_{479}E	5'-GTTGAATGTAACGTAGGTGCTCCA**GA**GGTTTCATATCGTGAAACAT-3'
FusA-N_{470}D-F	5'-CACCTACGTTACATTC**A**ACGTCGAATTCTTTCTTCATACGGTC-3'
FusA-N_{470}D-R	5'-GACCGTATGAAGAAAGAATTCGACGTTGAATGTAACGTAGGTG-3'
FusA-H_{438}P-F	5'-CTTCGTCAGTGTGTGCA**G**GGAATGTTGGGTCTTCT-3'
FusA-H_{438}P-R	5'-AGAAGACCCAACATTCC**C**TGCACACACTGACGAAG-3'
FusA-H_{438}C	5'CAAGAAGAAGACCCAACATTC**TG**TGCACACACTGACGAAGAAAC-3'
FusA-G_{451}C	5'-CTGGACAAGTTATCATC**T**GTGGTATGGGTGAGCTT-3'
FusA-S1	5'-TTACGTGTACTTGACGGAGCA-3'
FusA-S2	5'-TGTGGTACAGCTTTCAAAAACAA-3'
FusA-S3	5'-TGACCAAGATAAAATGACTCAAGC-3'
FusA-S4	5'-TTGCTGCATCATTAGCACTTAAA-3'

Table S4. **HX-MS Data Summary Table.** SD = standard deviation, CI = confidence interval.

Data Set	**EF-G_{C3}**	**EF-G_{C3} + FusB**
HDX reaction details	10 mM potassium phosphate pD 8.0, 300 mM NaCl, 82 % D_2O, 4 ºC	10 mM potassium phosphate pD 8.0, 300 mM NaCl, 82 % D_2O, 4 ºC
HDX time course (min)	0.5, 2, 10, 30, 60 min	
HDX control samples	Maximally-labelled controls were not performed.	
Back-exchange	*ca*. 30 %	
# of Peptides	137	137
Sequence coverage	94%	94%
Average peptide length / Redundancy	8.8 / 4.3	8.8 / 6.99
Replicates (biological or technical)	3 (technical)	3 (technical)
Repeatability	0.04 (average SD)	0.05 (average SD)
Significant differences in HDX (delta HDX > X D)	Reference	0.3 Da (0.5, 2, 10, 30 min, 99 % CI), 0.43 Da (60 min, 99 % CI), 0.61 Da (99 % CI) in summed data