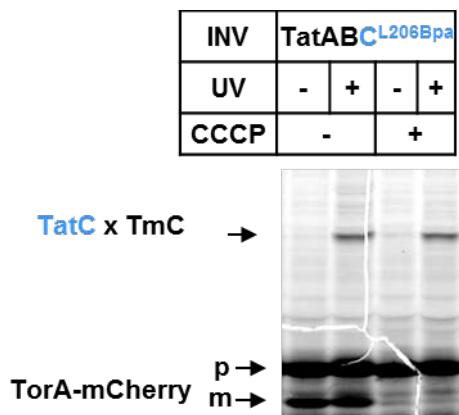
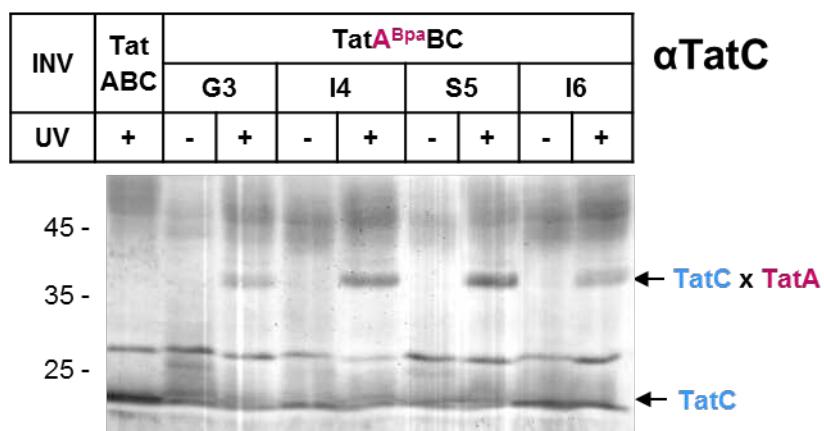
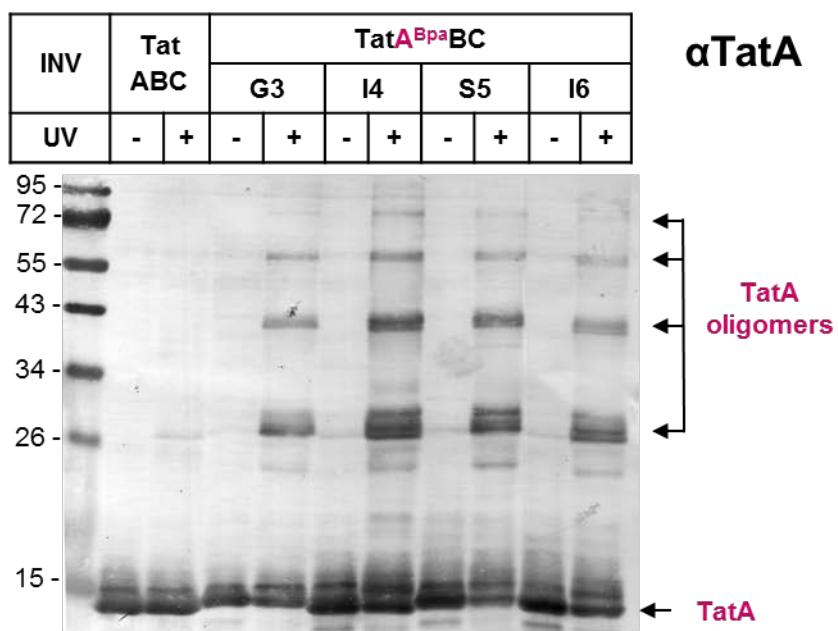


## SUPPLEMENTARY INFORMATION

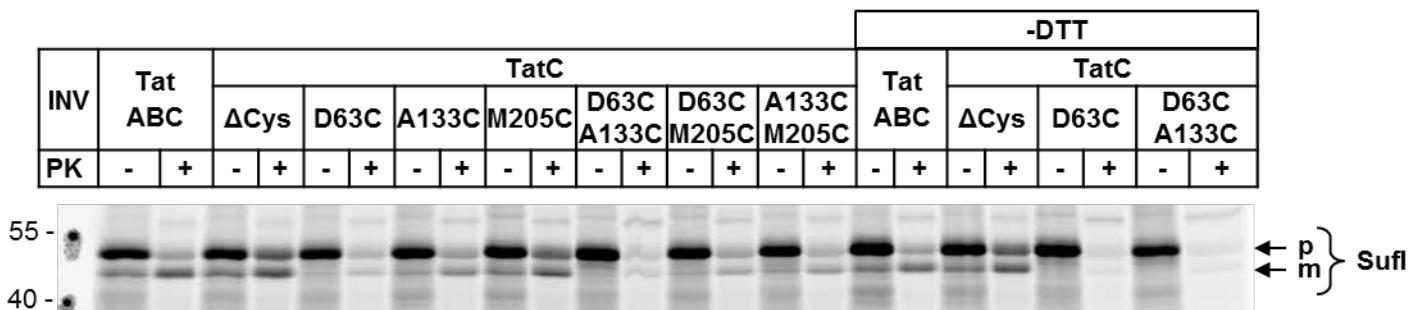
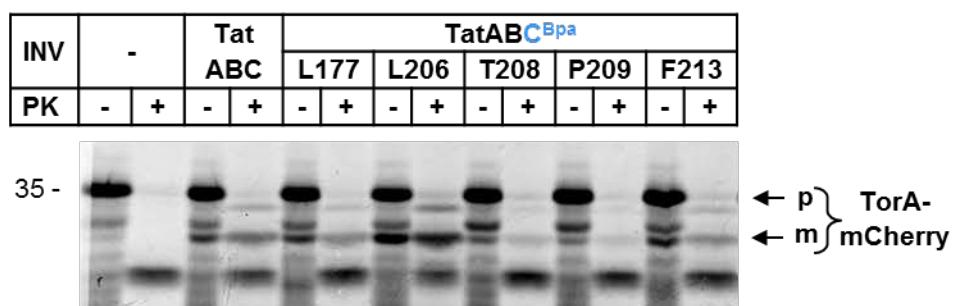
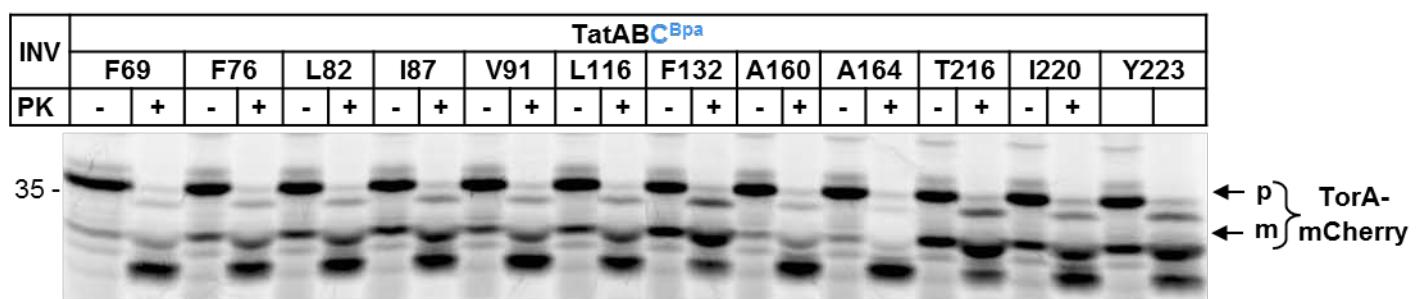
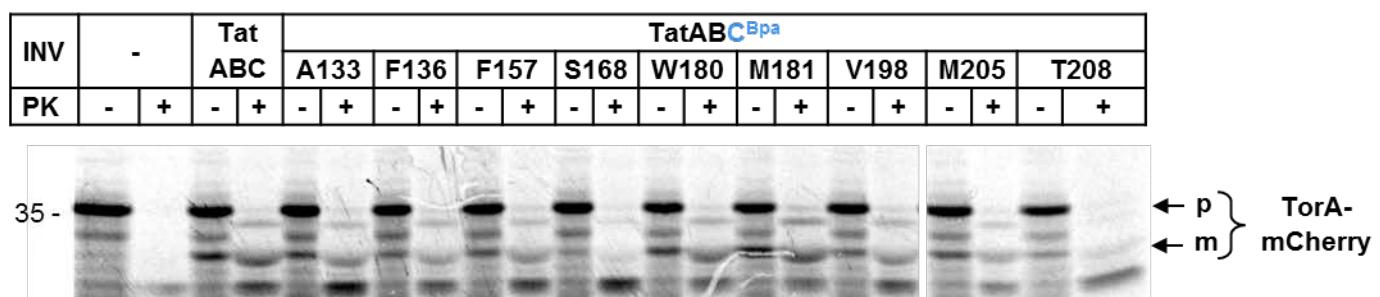
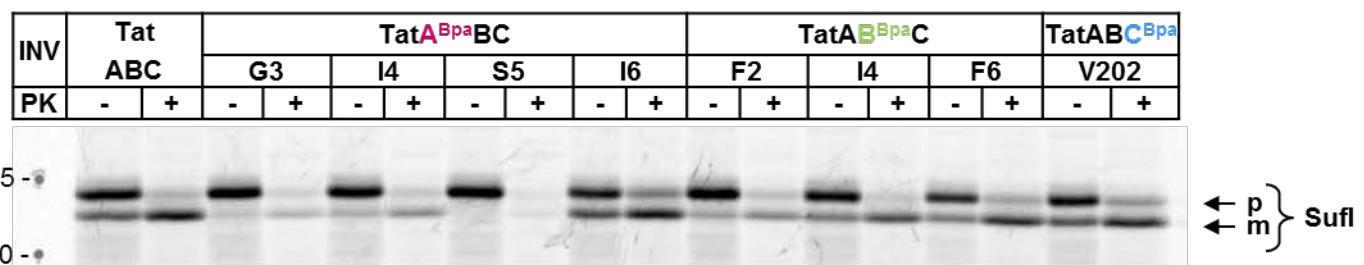
### Supplementary Figures



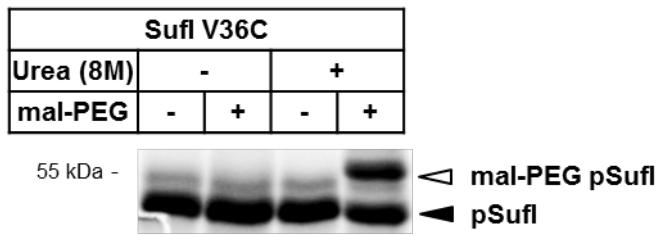
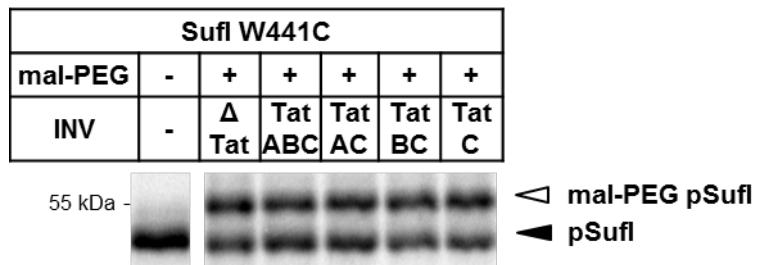
**Supplementary Figure 1. Contacts between TatC and a membrane-inserted RR-precursor do not require the H<sup>+</sup>-motive force.** The model RR-precursor TorA-mCherry was synthesized and radioactively labeled by *in vitro* transcription/translation in the presence of *E. coli* inner membrane vesicles containing TatA, TatB, and the L206Bpa variant of TatC. In samples labeled (+), cross-linking was initiated by irradiation with UV light. Radiolabeled translation products were separated by SDS-PAGE and visualized by phosphorimaging. Cross-linking between TatC and TorA-mCherry (TatC x TmC) was not influenced by the uncoupler CCCP, which, however, blocks transport into the vesicles, as indicated by a lack of conversion of the precursor (p) to the mature form (m) of TorA-mCherry.

**a****b**

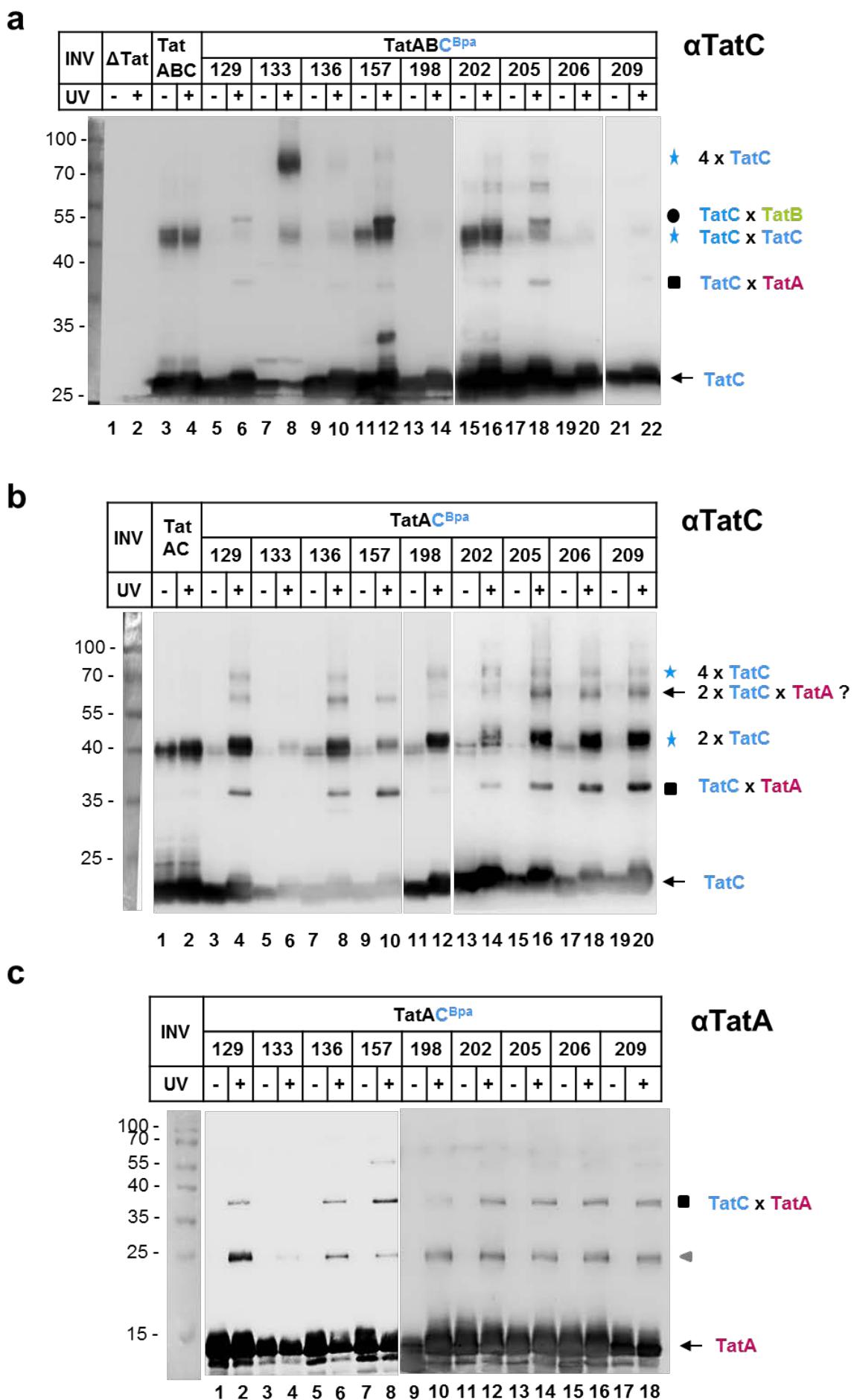
**Supplementary Figure 2. The N-terminus of TatA cross-links to TatC and mediates oligomerization of TatA.** (a, b) Inner membrane vesicles (INV) carrying the indicated Bpa variants in the N-terminus of TatA were probed for TatA adducts on Western blots using antibodies against TatC ( $\alpha$ TatC) and TatA ( $\alpha$ TatA>). Cross-linking was induced by irradiation with UV light (+). Indicated are complexes between TatA and TatC as well as N-terminally colligated oligomers of TatA. TatABC, Bpa-free vesicles.



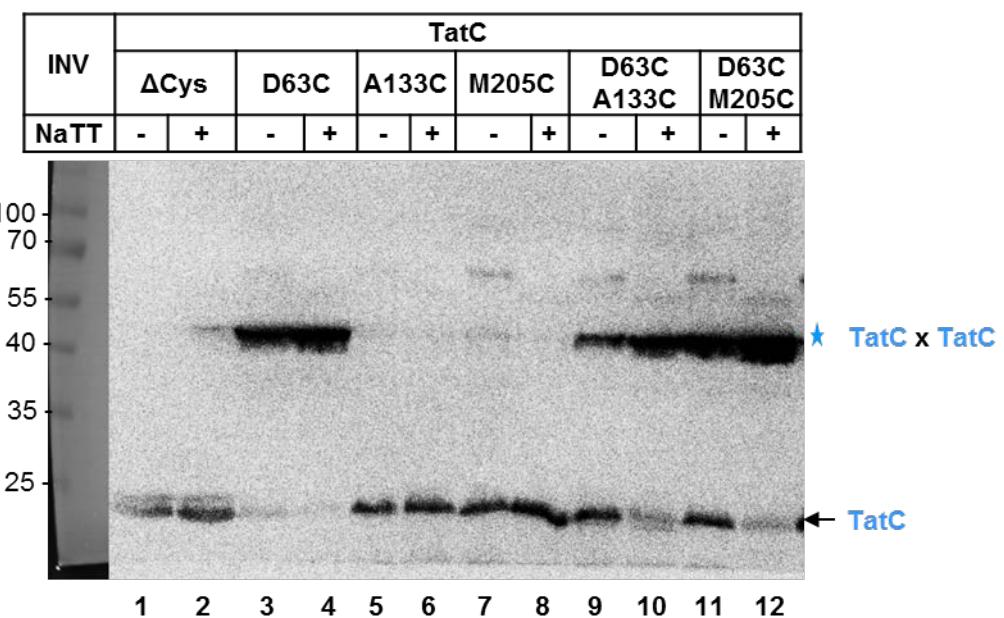
**Supplementary Figure 3. Translocation activity of membrane vesicles carrying Bpa-containing Tat variants used in this study.** The RR-precursors TorA-mCherry and pSufl were synthesized *in vitro* in the presence of membrane vesicles (INV) containing the wild-type TatABC proteins or any of the indicated Bpa variants of TatA ( $A^{Bpa}$ ), TatB ( $B^{Bpa}$ ), or TatC ( $C^{Bpa}$ ). Shown are the radioactively labeled translation products obtained without (-) or after (+) digestion with proteinase K (PK). The positions of the precursors (p) and mature forms (m) of TorA-mCherry and Sufl are indicated. (Bottom panel), vesicles were prepared from *E. coli* strain BL21(DE3) $\Delta$ Tat expressing extra-chromosomally a Cys-less mutant of TatC (TatC $\Delta$ Cys) carrying single or double cysteine mutations where indicated. Assays performed in the total absence of DTT (- DTT) revealed that TatC dimers, which formed through disulfide bridges at position D63, are inactive.



**Supplementary Figure 4. Control experiments to Figure 5.** The surface exposed residue W441 of Sufl is accessible to mal-PEG, whereas residue V36 in the early mature region can be labeled only after denaturation of Sufl with 8M urea. Experimental details are as described in the legend to Fig. 5 and in the Materials section.



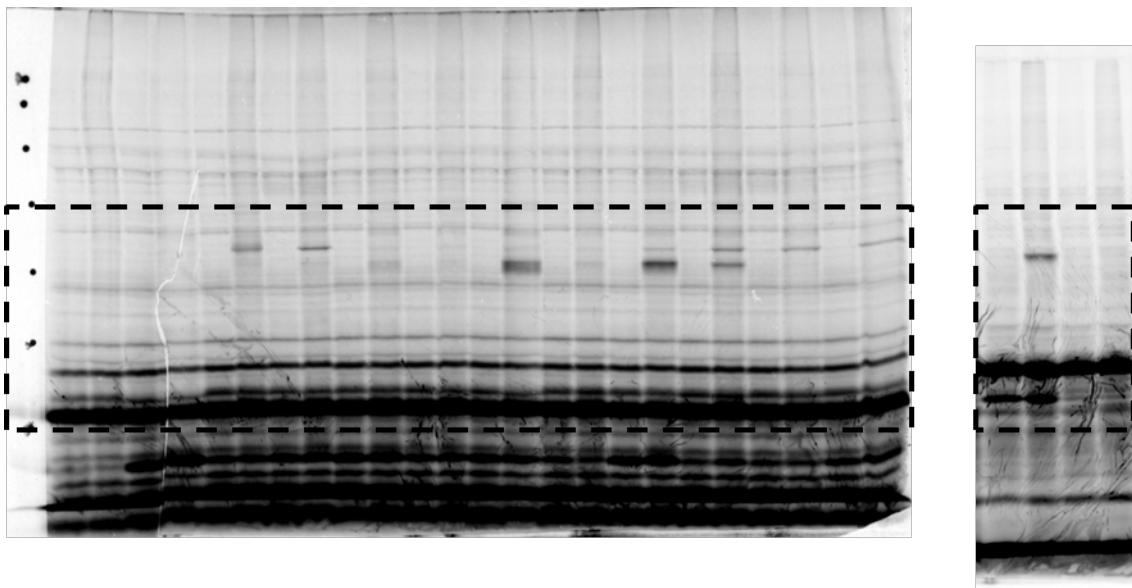
**Supplementary Figure 5. TatB is required for tetramerization of TatC through residue 133, whereas it impairs interaction between TatC and TatA.** **(a)** Membrane vesicles (INV) containing the indicated Bpa variants of TatC were probed for inter-TatC cross-links (blue stars). TatB-TatC adducts (black dots) had been identified as such in Fig. 2a and TatA-TatC adducts (squares) are addressed in **b, c**.  $\Delta$ Tat, vesicles lacking the Tat proteins; TatABC, Bpa-free vesicles. **(b, c)** As in **a**, except that membrane vesicles used were devoid of TatB. UV-dependent adducts to the indicated Bpa variants of TatC were probed by antibodies against TatC ( $\alpha$ TatC) and TatA ( $\alpha$ TatA) revealing the 37 kDa TatA-TatC complexes (squares). TatA dimers formed independently of Bpa, are indicated by a grey arrow head.



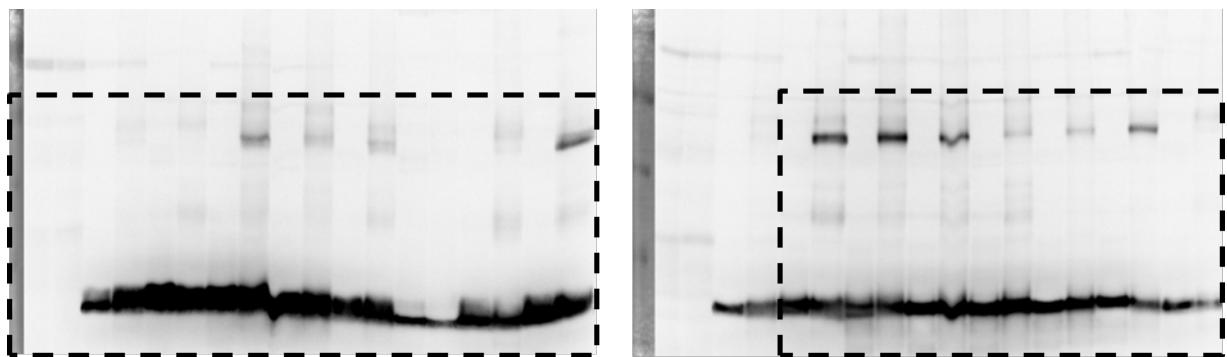
**Supplementary Figure 6. No direct cross-linking between the D63 and A133 residues of two adjacent TatC monomers.** In contrast to the about 13 Å long, bifunctional cysteine cross-linker Bismaleimidohexane (Fig. 7b), sodium tetrathionate (NaTT) did not yield adducts larger than 70 kDa that would have been indicative of tetramerization and hexamerization of TatC through residues D63 and A133.

**Supplementary Figure 7. Uncropped images of Figs. 1-7 and Supplementary Figs. 1-6.**

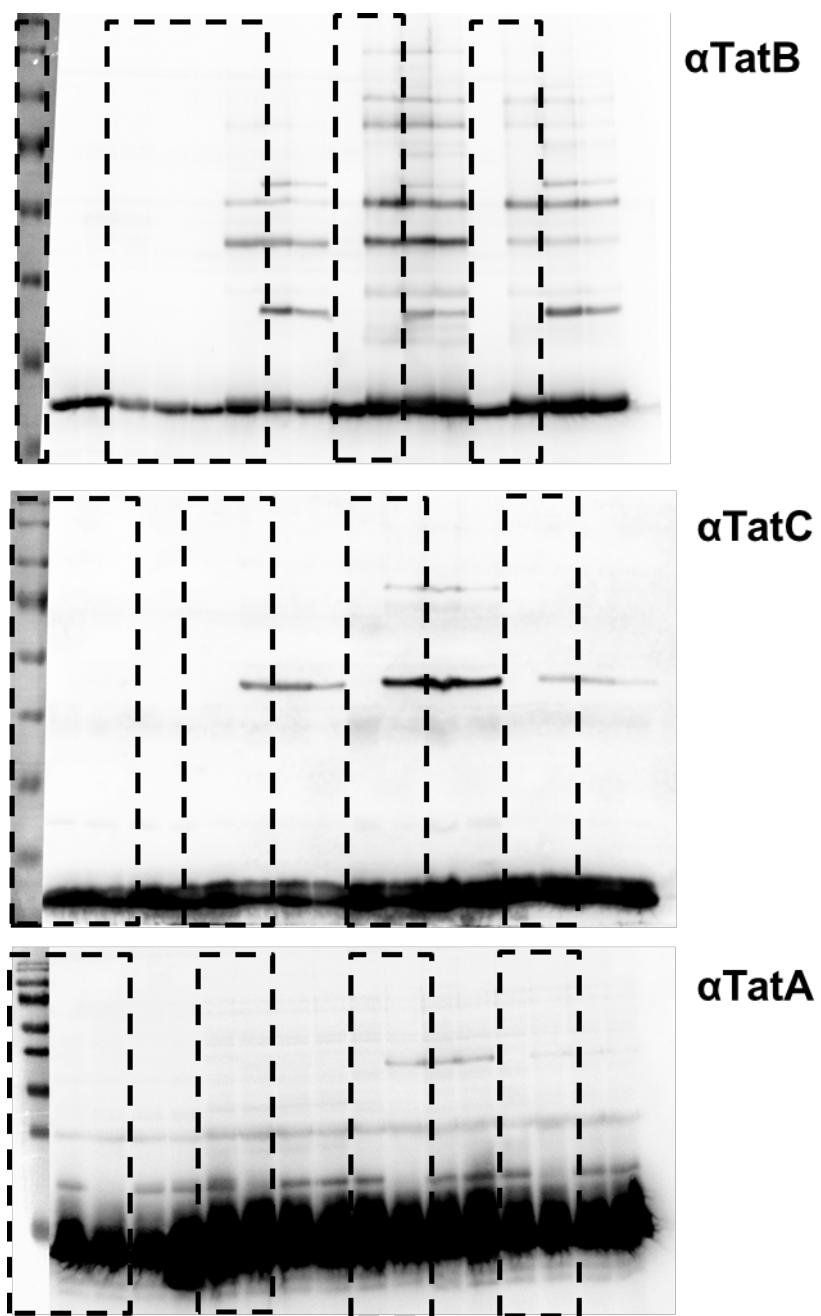
**Uncropped Figure 1b**



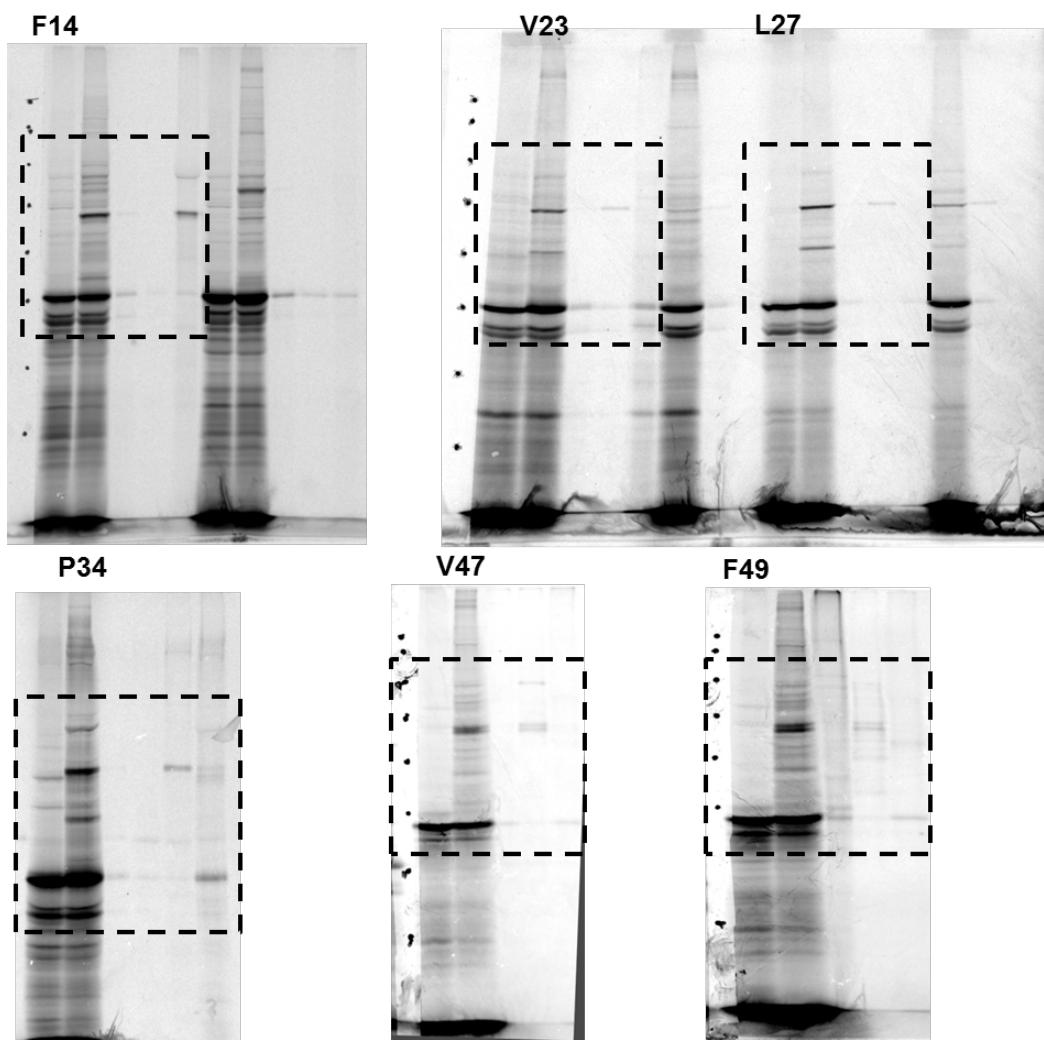
**Uncropped Figure 2a**



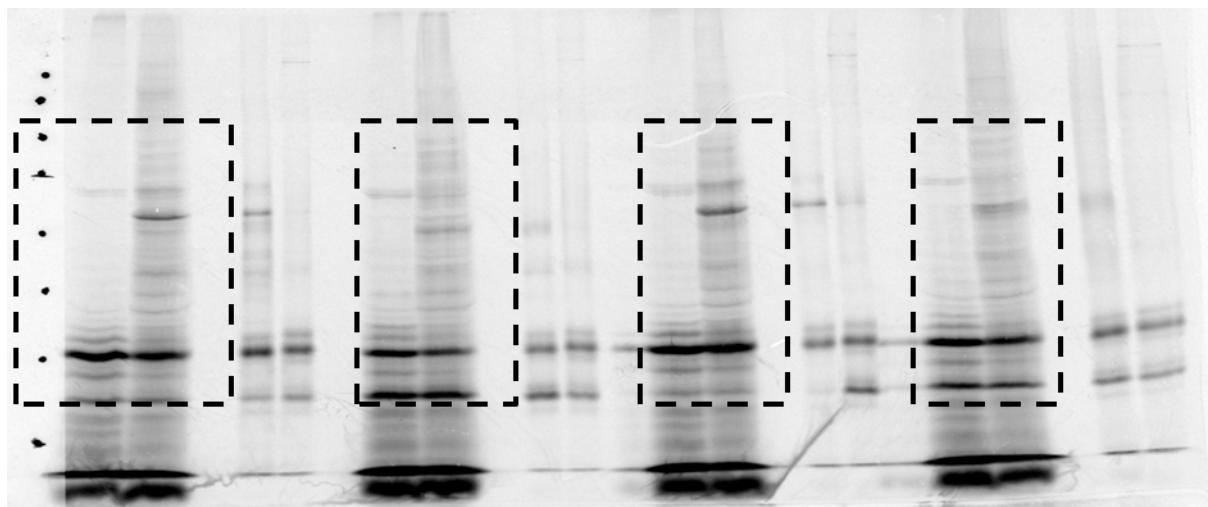
**Uncropped Figure 2d**



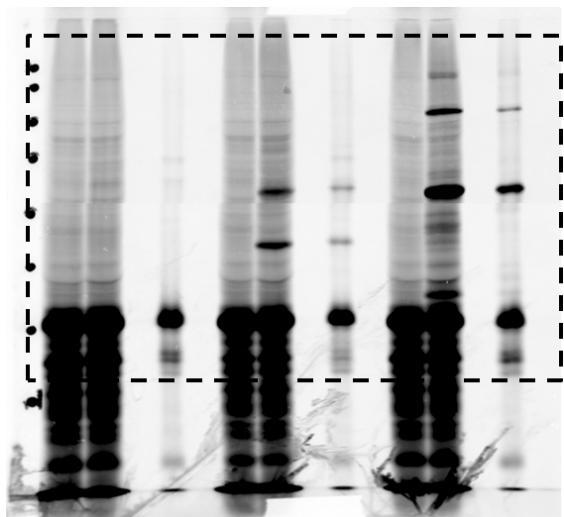
**Uncropped Figure 3b**



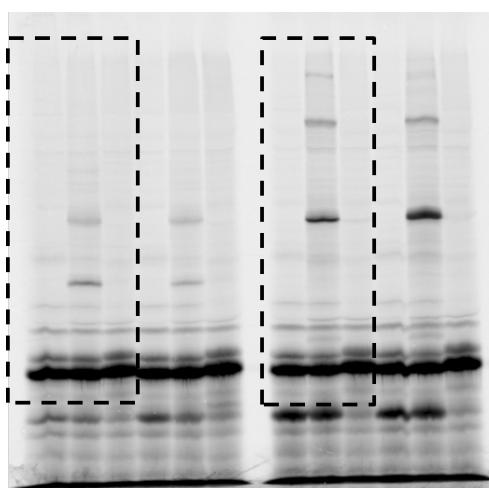
**Uncropped Figure 3c**



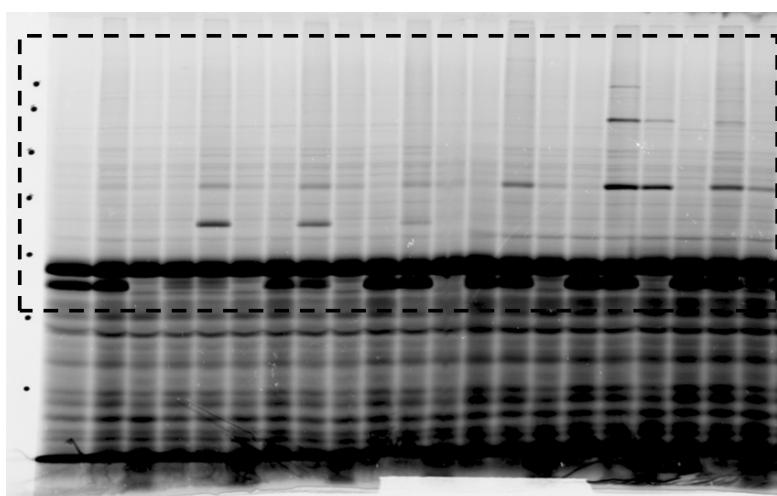
**Uncropped Figure 4a**



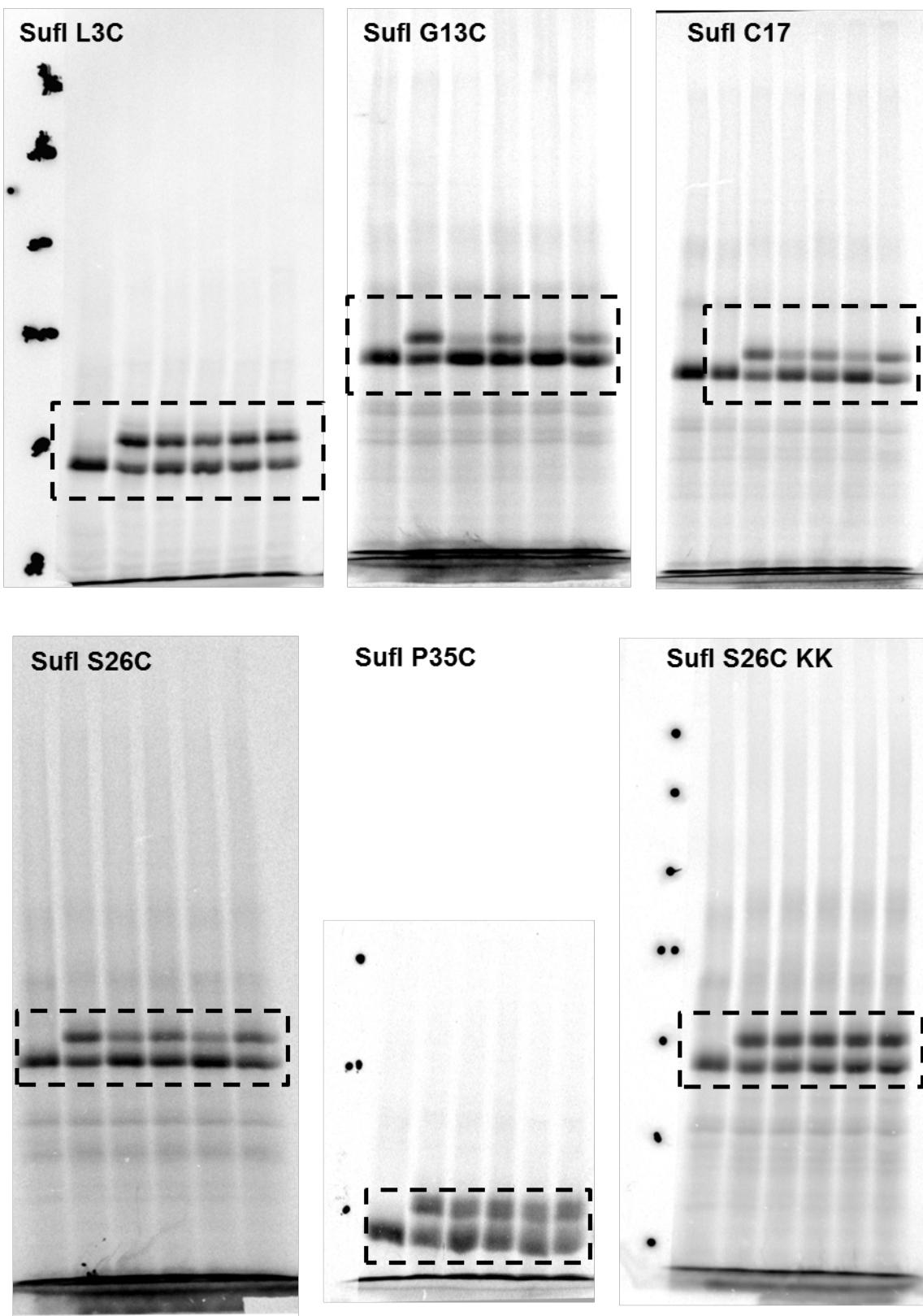
**Uncropped Figure 4b**



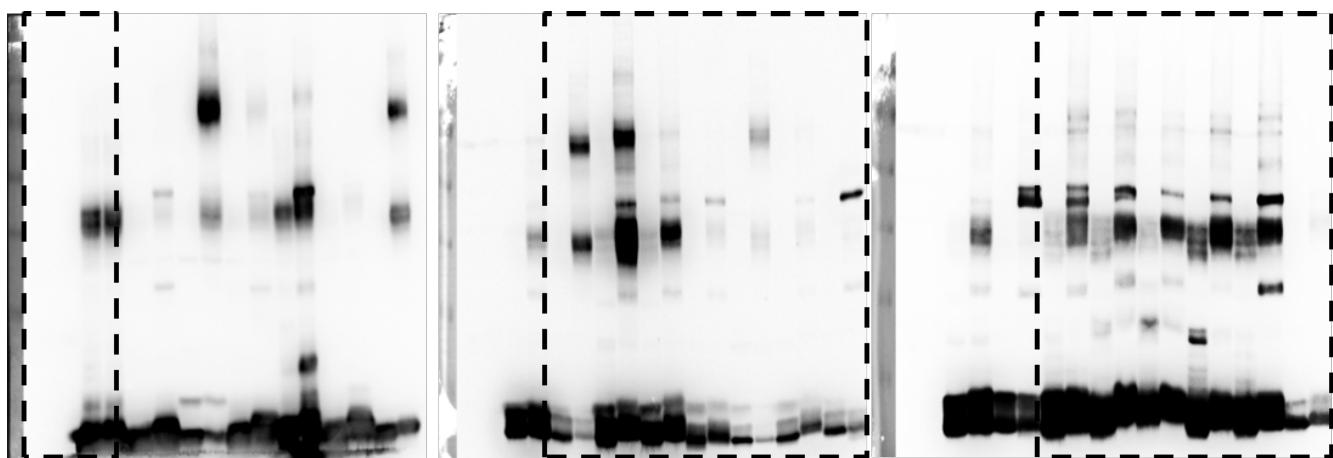
**Uncropped Figure 4c**



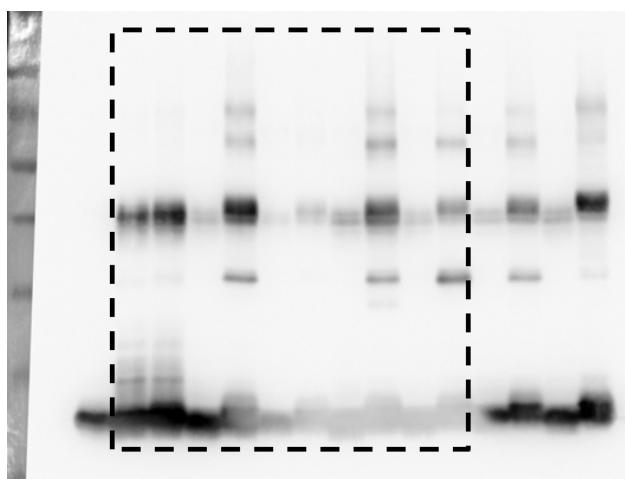
**Uncropped Figure 5c**



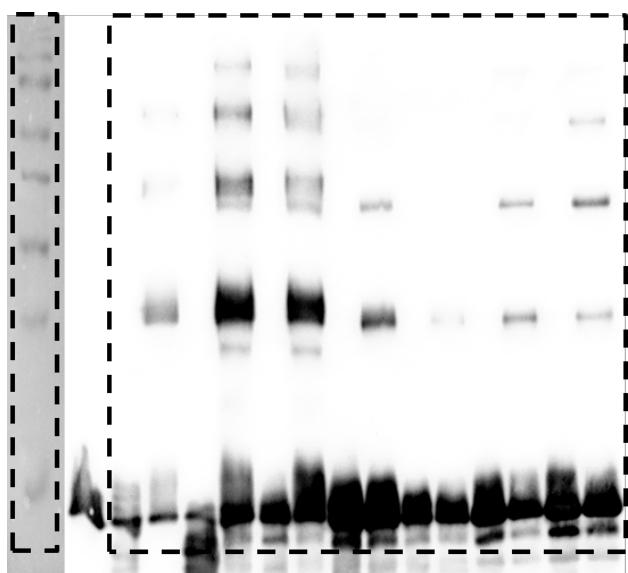
**Uncropped Figure 6a**



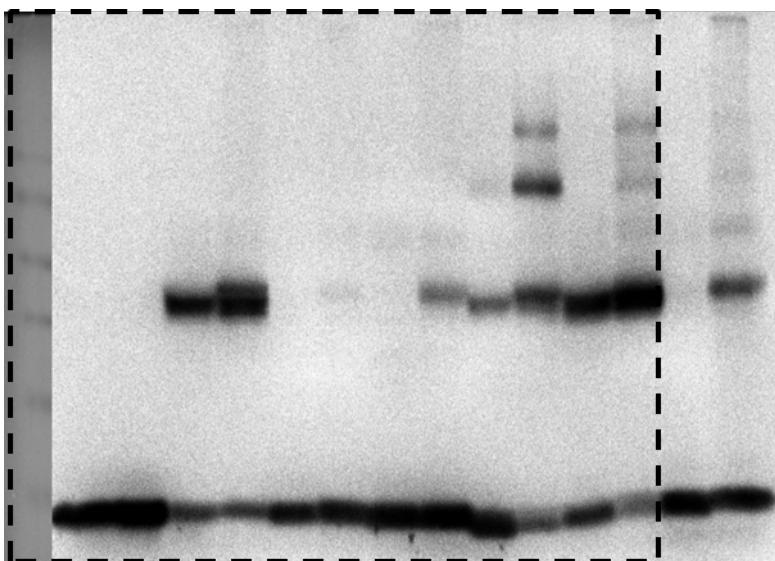
**Uncropped Figure 6b**



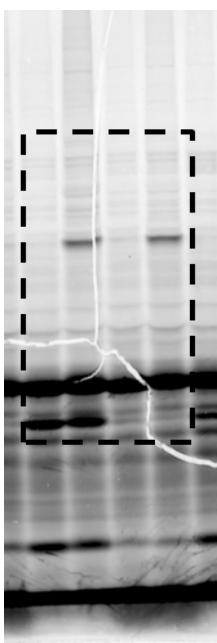
**Uncropped Figure 6c**



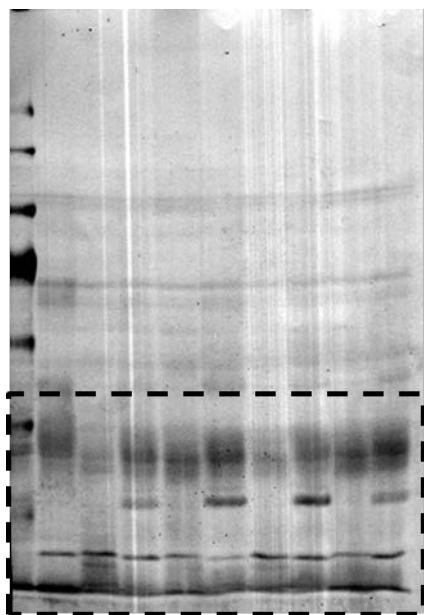
**Uncropped Figure 7b**



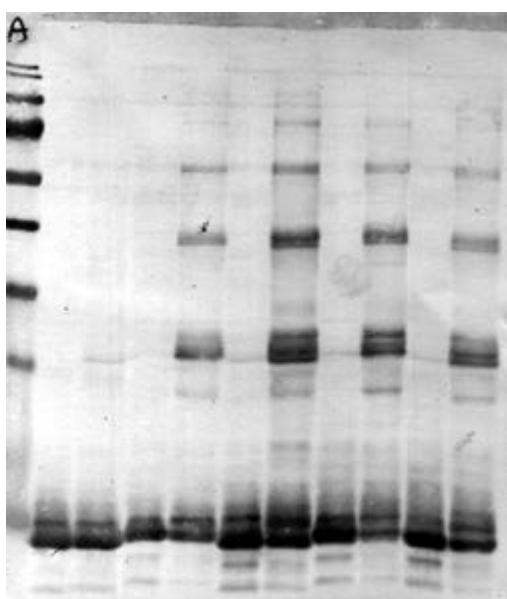
**Uncropped Supplementary Figure 1**



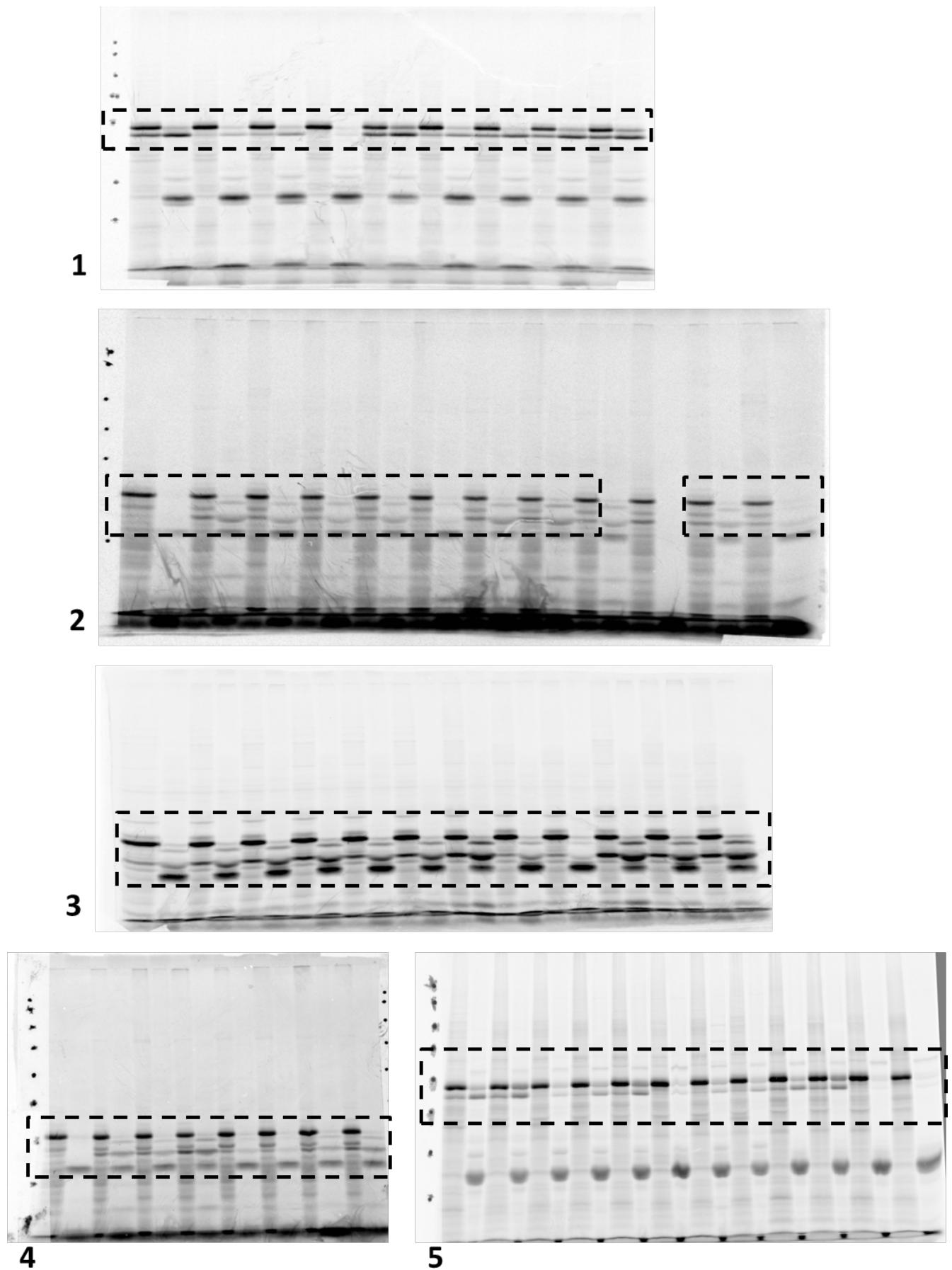
**Uncropped Supplementary Figure 2a**



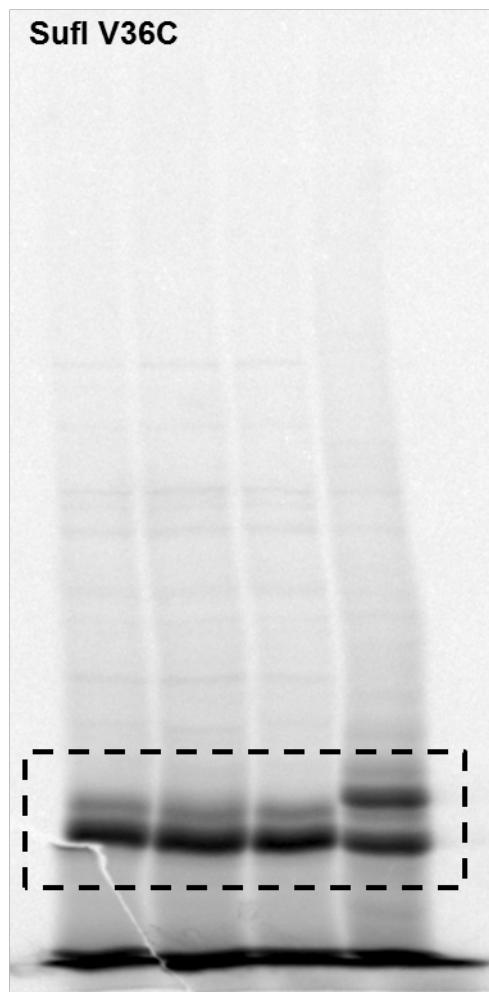
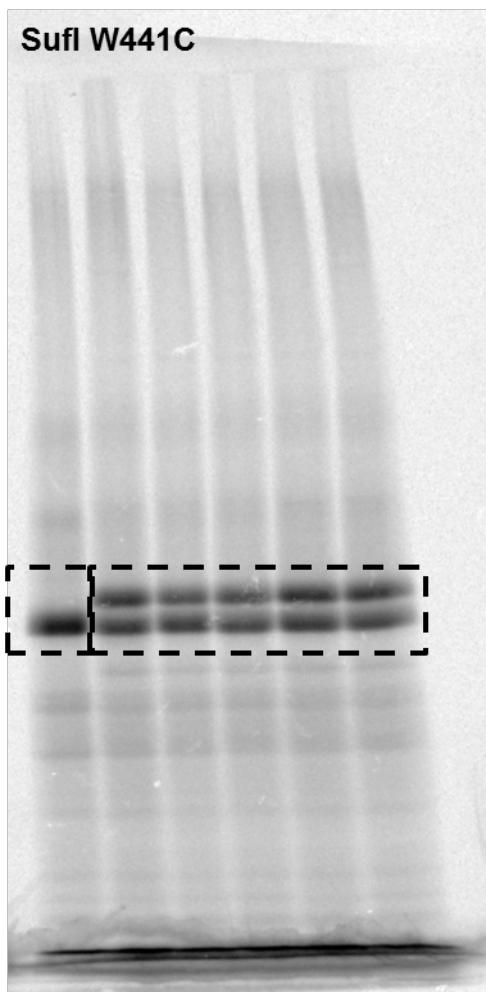
**Uncropped Supplementary Figure 2b**



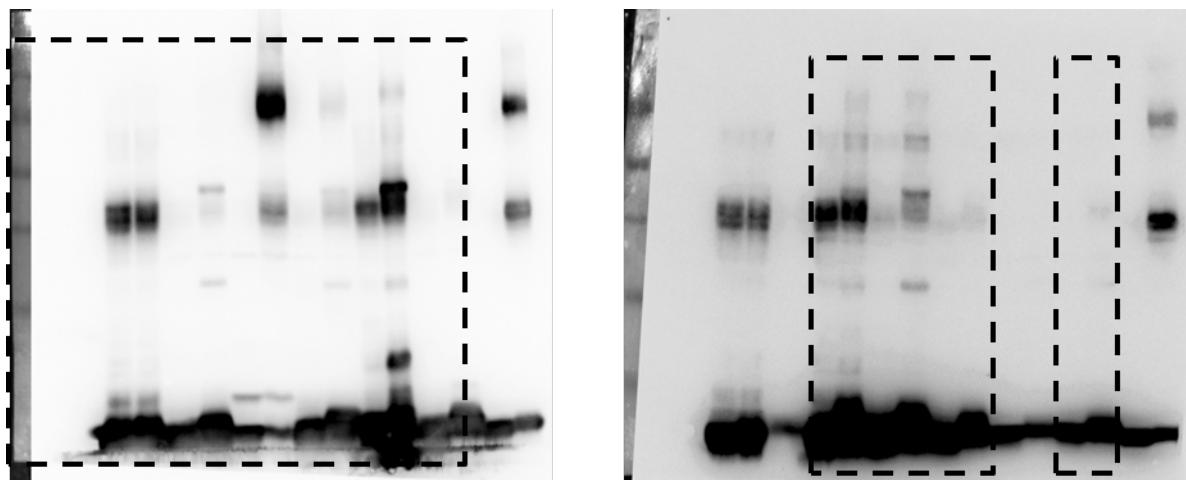
### Uncropped Supplementary Figure 3



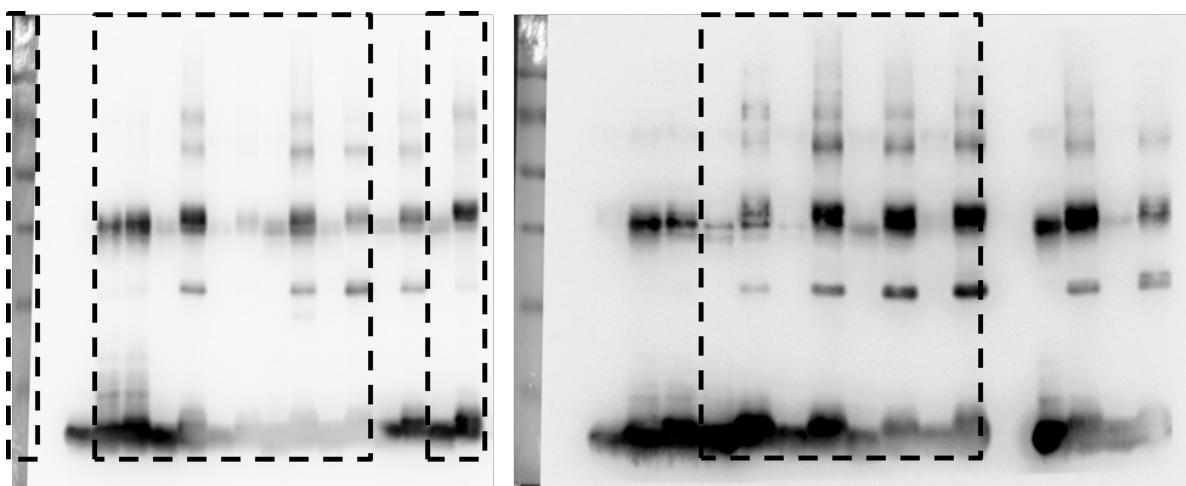
### Uncropped Supplementary Figure 4



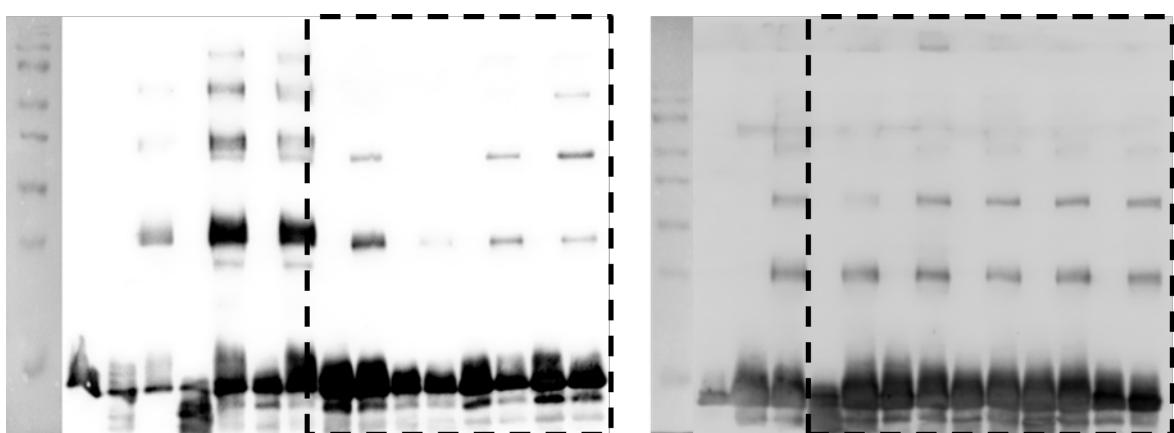
**Uncropped Supplementary Figure 5a**



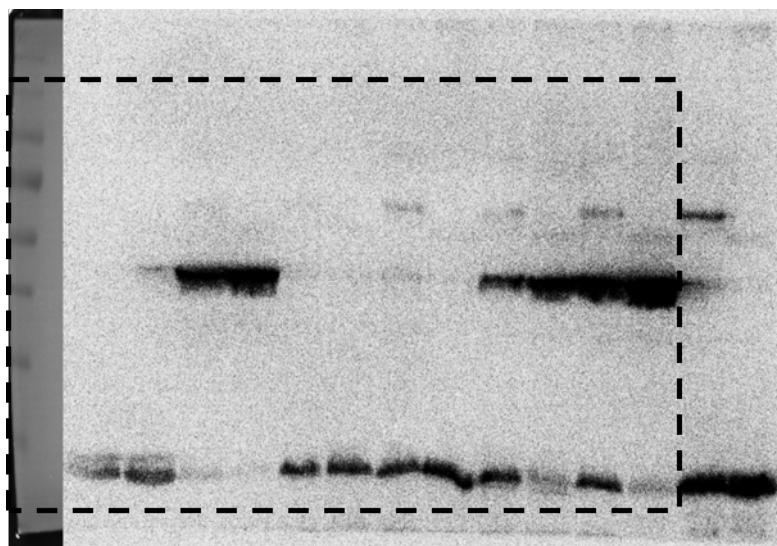
**Uncropped Supplementary Figure 5b**



**Uncropped Supplementary Figure 5c**



**Uncropped Supplementary Figure 6**



## Supplementary Tables

**Supplementary Table 1.** Quantitative data depicted in Fig. 5b

|                 | Exp.I | Exp.II | Exp.III | mean  | S.D.  |
|-----------------|-------|--------|---------|-------|-------|
| <b>ΔTat-INV</b> |       |        |         |       |       |
| <b>L3C</b>      |       | 0.610  | 0.673   | 0.642 |       |
| <b>A11C</b>     | 0.536 | 0.607  |         | 0.572 |       |
| <b>G13C</b>     | 0.468 | 0.530  |         | 0.499 |       |
| <b>II4C</b>     | 0.551 | 0.629  |         | 0.590 |       |
| <b>A15C</b>     | 0.501 | 0.656  |         | 0.579 |       |
| <b>L16C</b>     | 0.417 | 0.698  | 0.766   | 0.627 | 0.185 |
| <b>C17</b>      | 0.497 | 0.655  | 0.648   | 0.600 | 0.089 |
| <b>A18C</b>     | 0.617 | 0.716  |         | 0.666 |       |
| <b>G19C</b>     | 0.503 | 0.575  |         | 0.539 |       |
| <b>A20C</b>     | 0.370 | 0.532  |         | 0.451 |       |
| <b>V21C</b>     | 0.556 | 0.589  | 0.657   | 0.600 | 0.051 |
| <b>P22C</b>     | 0.234 | 0.258  |         | 0.246 |       |
| <b>L23C</b>     | 0.538 | 0.590  |         | 0.564 |       |
| <b>A25C</b>     | 0.483 | 0.541  |         | 0.512 |       |
| <b>S26C</b>     | 0.442 | 0.481  |         | 0.461 |       |
| <b>A28C</b>     | 0.502 | 0.350  |         | 0.426 |       |
| <b>G29C</b>     | 0.491 | 0.347  |         | 0.419 |       |
| <b>Q30C</b>     | 0.293 | 0.640  |         | 0.466 |       |
| <b>Q31C</b>     | 0.314 | 0.155  |         | 0.234 |       |
| <b>Q32C</b>     | 0.147 | 0.258  |         | 0.202 |       |
| <b>P33C</b>     | 0.116 | 0.094  |         | 0.105 |       |
| <b>L34C</b>     | 0.148 | 0.081  |         | 0.114 |       |
| <b>P35C</b>     | 0.500 | 0.454  | 0.720   | 0.558 | 0.142 |
| <b>V36C</b>     | 0.062 | 0.046  |         | 0.054 |       |
| <b>W441C</b>    | 0.492 | 0.532  |         | 0.512 |       |
| <b>S26CKK</b>   | 0.530 | 0.349  |         | 0.439 |       |

**Supplementary Table 2.** Quantitative data depicted in Fig. 5d

|                   | Exp.I | Exp.II | Exp.III | mean  | S.D.  |
|-------------------|-------|--------|---------|-------|-------|
| <b>ΔTat-INV</b>   |       |        |         |       |       |
| <b>L3C</b>        |       | 0.610  | 0.673   | 0.642 |       |
| <b>G13C</b>       | 0.468 | 0.530  |         | 0.499 |       |
| <b>C17</b>        | 0.497 | 0.655  | 0.648   | 0.600 | 0.089 |
| <b>S26C</b>       | 0.442 | 0.481  |         | 0.461 |       |
| <b>P35C</b>       | 0.500 | 0.454  | 0.720   | 0.558 |       |
| <b>W441C</b>      | 0.492 | 0.532  |         | 0.512 |       |
| <b>S26CKK</b>     | 0.530 | 0.349  |         | 0.439 | 0.128 |
| <b>TatABC-INV</b> |       |        |         |       |       |
| <b>L3C</b>        |       | 0.476  | 0.515   | 0.495 |       |
| <b>G13C</b>       | 0.152 | 0.137  |         | 0.145 |       |
| <b>C17</b>        | 0.244 | 0.378  | 0.298   | 0.307 | 0.068 |
| <b>S26C</b>       | 0.218 | 0.263  |         | 0.240 |       |
| <b>P35C</b>       | 0.394 | 0.331  | 0.500   | 0.408 |       |
| <b>W441C</b>      | 0.418 | 0.419  |         | 0.418 |       |
| <b>S26CKK</b>     | 0.525 | 0.394  |         | 0.459 | 0.093 |
| <b>TatAC-INV</b>  |       |        |         |       |       |
| <b>L3C</b>        |       | 0.413  | 0.476   | 0.444 |       |
| <b>G13C</b>       | 0.274 | 0.266  |         | 0.270 |       |
| <b>C17</b>        | 0.337 | 0.373  | 0.270   | 0.326 | 0.052 |
| <b>S26C</b>       | 0.339 | 0.342  |         | 0.340 |       |
| <b>P35C</b>       | 0.446 | 0.333  | 0.328   | 0.369 |       |
| <b>W441C</b>      | 0.459 | 0.400  |         | 0.429 |       |
| <b>S26CKK</b>     | 0.504 | 0.408  |         | 0.456 | 0.067 |
| <b>TatBC-INV</b>  |       |        |         |       |       |
| <b>L3C</b>        |       | 0.497  | 0.553   | 0.525 |       |
| <b>G13C</b>       | 0.127 | 0.109  |         | 0.118 |       |
| <b>C17</b>        | 0.216 | 0.269  | 0.278   | 0.254 | 0.033 |
| <b>S26C</b>       | 0.132 | 0.203  |         | 0.167 |       |
| <b>P35C</b>       | 0.388 | 0.221  | 0.414   | 0.341 |       |
| <b>W441C</b>      | 0.545 | 0.593  |         | 0.569 |       |
| <b>S26CKK</b>     | 0.492 | 0.445  |         | 0.469 | 0.033 |
| <b>TatC-INV</b>   |       |        |         |       |       |
| <b>L3C</b>        |       | 0.573  | 0.618   | 0.596 |       |
| <b>G13C</b>       | 0.302 | 0.311  |         | 0.307 |       |
| <b>C17</b>        | 0.446 | 0.446  | 0.516   | 0.469 | 0.041 |
| <b>S26C</b>       | 0.423 | 0.394  |         | 0.409 |       |
| <b>P35C</b>       | 0.500 | 0.389  | 0.535   | 0.474 |       |
| <b>W441C</b>      | 0.545 | 0.514  |         | 0.529 |       |
| <b>S26CKK</b>     | 0.494 | 0.383  |         | 0.438 | 0.079 |

**Supplementary Table 3.** Plasmids used in this study

| Name                   | Vector  | Insert                | Reference |
|------------------------|---------|-----------------------|-----------|
| pSup-BpaRS-6TRN(D286R) |         |                       | 1         |
| pEVOL-pBpF             |         |                       | 2         |
| p8737                  | pET22b+ | TatABCD               | 3         |
| p8737-tatAC            | pET22b+ | TatAC                 | 4         |
| pFAT588                | pQE     | TatCHis               | 5         |
| pFAT75CH $\Delta$ A    | pQE     | TatBCHis              |           |
| pPJ3                   | pET22b+ | TorA-mCherry          | 6         |
| pPJ5                   | pET22b+ | TorA(KK)-mCherry      |           |
| pPJ11                  | pET22b+ | TorA-MalE             | 7         |
| pKSM Sufl-RR           | pKSM    | Sufl                  | 8         |
| pEJ                    | pET22b+ | Sufl                  |           |
| pETRick                | pET22b+ | Sufl(KK)              |           |
| pLJ1                   | pET22b+ | Sufl $\Delta$ Cys     |           |
| pLJ2                   | pET22b+ | Sufl(KK) $\Delta$ Cys |           |
| pUNITATCC4             | pQE60   | TatABC $\Delta$ Cys   | 9         |

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**Supplementary Table 4.** List of primers (mutations are given in bold)

|           |     |  |
|-----------|-----|--|
| SufI NdeI | for | GCCATATGTCACTCAGTCGGCG   |
| SufI Xhol | rev | GCCTCGAGTACCGGATTGACC  |
| SufI KK F | for | CATATGTCACTCAGTAAGAAGCAGTTCATTCAGGCATCGG   |
| SufI KK R | rev | CCGATGCCTGAATGAAC TGCTTACTGAGTGACATATG   |
| TatA G3   | for | GGAACATGTATGGG <b>TAG</b> ATCAGTATTGGCAG   |
|           | rev | CTGCCAAATACTGAT <b>CTA</b> ACCCATACATGTTCC   |
| TatA I4   | for | CATGTATGGG <b>TGG</b> <b>TAG</b> AGTATTGGCAGTTATTG                                   |
|           | rev | CAATAACTGCCAAATACT <b>CTA</b> ACCACCCATACATG   |
| TatA S5   | for | GTATGGG <b>TGG</b> TAT <b>CTA</b> AGATTGGCAGTTATTG                                   |
|           | rev | CAATAACTGCCAAAT <b>CTA</b> AGATACCACCCATAC   |
| TatA I6   | for | GGTGGTATCAG <b>TAG</b> GGCAGTTATTGATTATTG  |
|           | rev | CAATAATCAATAACTGCC <b>ACTA</b> CTGATACCACC   |
| TatB F2   | for | GCAGGTGTAATCCGT <b>TAG</b> GTAGGATATCGGTTTAGCG                                       |
|           | rev | CGCTAAAACCGATAT <b>CCT</b> ACACGGATTACACCTGC   |
| TatB I4   | for | GGTGTAA <b>CCG</b> TGTTGATT <b>AGGG</b> TTTAGCGA <b>ACT</b> GC                       |
|           | rev | GCAGTTCGCTAAAC <b>CC</b> <b>CTA</b> ATCAAACACGGATTACACC                              |
| TatB F6   | for | CCGTGTTGATATCGG <b>TAG</b> AGCGA <b>ACT</b> GCTATTGG                                 |
|           | rev | CCAATAGCAGTCG <b>CTA</b> ACCGATATCAAACACGG   |
| TatC F69  | for | GGCCTGCCGTT <b>CTA</b> GC <b>GG</b> CGATCAAGC  |
|           | rev | GCTTGATCGGCG <b>CTA</b> GA <b>AC</b> GGCGAGGCC                                       |
| TatC F76  | for | GCCGATCAAG <b>CTGAC</b> <b>CTA</b> GTGGTGC <b>CTGATTC</b>                            |
|           | rev | GAATCAGCGACACC <b>ATCTA</b> GGTCAGCTTGATCGGC   |
| TatC L82  | for | GGTGTGCTGATT <b>TAG</b> TCAGCGCCGGTG   |
|           | rev | CACCGGCCG <b>CTGACT</b> AAATCAGCGACACC   |
| TatC I87  | for | CTGTCAGCGCCGG <b>TAG</b> CTCTATCAGGTGTGG   |
|           | rev | CCACAC <b>CTGATAGAG</b> <b>CTACAC</b> CCGGCG <b>CTGACAG</b>                          |
| TatC V91  | for | CCGGTGATTCTCTATCAG <b>TAG</b> GGCATTATCGCCCC   |
|           | rev | GGGGCGATAAA <b>ATGCC</b> ACT <b>ACTG</b> ATAGAGAA <b>ATCACCGG</b>                    |
| TatC L116 | for | GCTGCTGGTTCCAGCT <b>TTAG</b> CTGTTTATCGGCATGG  |
|           | rev | CCATGCCGATATAAA <b>ACAG</b> <b>CTAAG</b> AGCTGGAAACCAGCAGC                           |
| TatC V129 | for | GCATTGCC <b>CTACTTGT</b> <b>TAG</b> TTCCG <b>CTGG</b> CATTGG                         |
|           | rev | CCAAATGCCAGCGGAA <b>ACTACAC</b> AAAGTAGGCCGA <b>ATGC</b>                             |
| TatC L132 | for | CTACTTGTGG <b>CTTCCG</b> <b>CTAG</b> GCATTGG <b>CTTCC</b> TTGCC                      |
|           | rev | GGCAAGGAAG <b>CCAAATGCC</b> <b>CTAC</b> GGAAAGACCACAAAGTAG                           |
| TatC A133 | for | CTTGTGG <b>CTTCCG</b> <b>CTAG</b> TTGG <b>CTTCC</b> TTGCC                            |
|           | rev | GGCAAGGAAG <b>CCAAACTAC</b> AGCGGAAAGACCACAAAG                                       |
| TatC F136 | for | CTTCCG <b>CTGG</b> CATTGG <b>CTAG</b> CTGCCA <b>ATACCG</b> CG                        |
|           | rev | CGCGGTATTGG <b>CAAG</b> <b>CTAG</b> CCAA <b>ATGCC</b> AGCGGAAAG                      |
| TatC F157 | for | CGCCAG <b>CTATTA</b> AG <b>CTAG</b> GTATGG <b>CGCTG</b> TTATGG                       |
|           | rev | CCATAAACAGCG <b>CCATAAC</b> CTAG <b>CTTAA</b> ATAG <b>CTGG</b> CG                    |
| TatC A160 | for | CTATTAAG <b>CTTCG</b> TTAT <b>GTAG</b> CTGTTATGG <b>CGTT</b> GG                      |
|           | rev | CCAAACGCC <b>CATAAC</b> AG <b>CTAC</b> ATAAC <b>GAAG</b> CTTAA <b>ATAG</b>           |
| TatC A164 | for | CTTCGTTATGG <b>CGCTG</b> TTAT <b>GTAG</b> TTGG <b>GTCT</b> CC <b>CTT</b> GA<br>AGTGC |
|           | rev | GCACTTCAAAGGAGAC <b>CCAAACTAC</b> ATAACAGCG <b>CCATA</b>                             |

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|               |     | ACGAAG  |
| TatC F165     | for | GGCGCTTTATGGCG <b>TAGGGTGTCTCCTTGAAGTGC</b>       |
|               | rev | GCACTCAAAGGAGACACC <b>TACGCCATAAACAGCGCC</b>      |
| TatC S168     | for | GTTATGGCGTTGGTGT <b>CAGTTGAAGTGCCGGTAG</b>        |
|               | rev | CTACCGGCACTCAA <b>ACTAGACACCAAACGCCATAAAC</b>     |
| TatC L177     | for | GAAGTGCCGGTAGCAATTGTGT <b>AGCTGTGCTGGATGGG</b>    |
|               | rev | CCCATCCAGCACAG <b>CTACACAATTGCTACC GGCACTTC</b>   |
| TatC W180     | for | GCAATTGTGCTGCTGT <b>CAGATGGGGATTACCTCGCC</b>      |
|               | rev | GGCGAGGTAATCCCCAT <b>CTAGCACAGCAGCACAATTGC</b>    |
| TatC M181     | for | GCAATTGTGCTGCTGT <b>GGTAGGGGATTACCTCGCC</b>       |
|               | rev | GGCGAGGTAATCCCC <b>TACCAAGCACAGCAGCACAATTGC</b>   |
| TatC V198     | for | AAAAAAACGCCGTATGTGCTGT <b>AGGGTGCATTGTTG</b>      |
|               | rev | CAACGAATGCAC <b>CCCTACAGCACATACGGCGTTTTG</b>      |
| TatC V202     | for | GTGCTGGTTGGTGCATTCT <b>AGGTCGGGATGTTGCTGAC</b>    |
|               | rev | GTCAGCAACATCCC <b>GACCTAGAACATGCACCAACCAGCAC</b>  |
| TatC M205     | for | GTTGGTGCATTGTT <b>GGGATGTTAGCTGTGACGCCGCC</b>     |
|               | rev | GGCGGCGTCAGCAA <b>CTACCCGACAACGAATGCACCAAC</b>    |
| TatC L206     | for | GCATTGTTGTCGGGATG <b>TAGCTGACGCCGCCGGATG</b>      |
|               | rev | CATCCGGCGGC <b>GT CAGCTACATCCCGACAACGAATGC</b>    |
| TatC T208     | for | GTTGTCGGGATGTTGCTGT <b>AGCCGCCGGATGTTCTC</b>      |
|               | rev | GAGAAGACATCCGGCG <b>GCTACGTCAGCAACATCCCGACAAC</b> |
| TatC P209     | for | GTTGTCGGGATGTTGCTGACGT <b>AGCCGGATGTTCTC</b>      |
|               | rev | GAGAAGACATCCGG <b>C TACGTCAGCAACATCCCGACAAC</b>   |
| TatC F213     | for | GCTGACGCCGCCGGATG <b>TAGTCGCAAACGCTGTTGG</b>      |
|               | rev | CCAACAGCGTT <b>GCGACTAGACATCCGGCGGCTCAGC</b>      |
| TatC T216     | for | GGATGTTCTCTCGCAATAG <b>CTGTTGGCGATCCCG</b>        |
|               | rev | CGGGATGCCAACAG <b>CTATTGCGAGAACATCC</b>           |
| TatC I220     | for | CGCAAACGCTGTTGGCG <b>TAGCCGATGTACTGTTG</b>        |
|               | rev | CAAACAGACAGTACATCGG <b>CTACGCCAACAGCGTTGCG</b>    |
| TatC Y223     | for | GCTGTTGGCGATCCCGATG <b>TAGTGTCTGTTGAAATCGGTG</b>  |
|               | rev | CACCGATTCAAACAGAC <b>ACTACATCGGGATGCCAACAGC</b>   |
| <hr/>         |     |   |
| TatC A133C    | for | CTACTTGTGGCTTCCGCTGT <b>GTGTTGGCTCCTTGC</b>       |
|               | rev | GCAAGGAAGCCAAA <b>ACACAGCGGAAAGACCACAAAGTAG</b>   |
| TatC D63C     | for | GTTCACCGATGATGCCAC <b>CTGTGTGGCTCGCCGTT</b>       |
|               | rev | GAACGGCGAGGCCAC <b>ACAGGTGGCGATCATCGTTAAC</b>     |
| TatC M205C    | for | GGTGCATTGTTGTCGGG <b>TGTGCTGACGCCGCCG</b>         |
|               | rev | CGGCGGCGTCAGCAA <b>ACACCCGACAACGAATGCACC</b>      |
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| TorA SS F14_f | for | CATCACGTCGGCG <b>TAGCTGGCACAACTCGGCGGC</b>        |
| TorA SS F14_r | rev | GCCGCCGAGTTGTGCCAG <b>CTAACGCCGACGTGATG</b>       |
| TorA SS V23_f | for | CAACTCGGCGGCTAAC <b>CTAGGCCGGATGCTGG</b>          |
| TorA SS V23_r | rev | CCAGCATCCGGC <b>CTAGGTTAGCCGCCGAGTTG</b>          |
| TorA SS L27_f | for | CCGTCGCCGGGATG <b>TAGGGGCCGTATTGTTAACGC</b>       |

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| TorA SS<br>L27_r        | rev | GCGTTAACAAATGACGGCCCC <b>TAC</b> ATCCCAGGCGACGG  |
| TorA SS<br>P34_f        | for | CCGTCATTGTTAACGT <b>AG</b> CGACGTGCGACTGCG   |
| TorA SS<br>P34_r        | rev | CGCAGTCGCACGTCG <b>CTAC</b> GTTAACAAATGACGG  |
| TorA-MalE<br>V47        | for | GCGGCGACTGACG <b>C</b> TTAGGAATTGATATCATCGAAG  |
|                         | rev | CTTCGATGATATCGAATT <b>C</b> TAAGCGTCAGTCGCCGC  |
| TorA-MalE<br>F49        | for | CGACTGACG <b>C</b> TGTCGAAT <b>A</b> GGGATATCATCGAAGAAGG   |
|                         | rev | CCTTCTTCGATGATAT <b>C</b> CTATTGACAGCGTCAGTCG  |
| TorA-<br>mCherry<br>V47 | for | GCGGCGACTGACG <b>C</b> TTAGGAATTGATGGTGAGCAAGGG  |
|                         | rev | CCCTTGCTCACCATGAATT <b>C</b> TAAGCGTCAGTCGCCGC   |
| TorA-<br>mCherry<br>F49 | for | GCGACTGACG <b>C</b> TGTCGAAT <b>A</b> GGGATGGTGAGCAAGGG  |
|                         | rev | GCCCTTGCTCACCAT <b>C</b> TATTGACAGCGTCAGTCG  |
| SufI C17A               | for | GGG ATT GCA CTT <b>GCG</b> GCA GGC GCT G   |
|                         | rev | C AGC GCC TGC <b>CGC</b> AAG TGC AAT CCC   |
| SufI C295A              | for | GTG TCG ATC ACC <b>GCG</b> GGC GAA GCG G   |
|                         | rev | C CGC TTC GCC <b>CGC</b> GGT GAT CGA CAC   |
| SufI L3C                | for | GGAGATATACATATGTCAT <b>G</b> CAGTCGGCGTCAGTTCATTC  |
|                         | rev | GAATGAACTGACGCCGACT <b>G</b> CATGACATATGTATATCTCC  |
| SufI A11C               | for | CGTCAGTTCATTCA <b>G</b> TCAGTCGGGGATTGCACTTGCG   |
|                         | rev | CGCAAGTGCAATCCCCGAG <b>C</b> ACTGAATGAACTGACG  |
| SufI G13C               | for | GTCAGTTCATTCAAGGCATCG <b>G</b> CATTGCACTTGCAGG   |
|                         | rev | CCTGCCGCAAGTGAAT <b>G</b> CACGATGCCTGAATGAACTGAC   |
| SufI I14C               | for | CAGGCATCGGGGG <b>T</b> GTGCACTTGCAGG   |
|                         | rev | GCCTGCCGCAAGTGCACACCCCCGATGCCTG  |
| SufI A15C               | for | GGCATCGGGGATT <b>T</b> GCCTTGCAGG  |
|                         | rev | CGCCTGCCGCAAGG <b>C</b> AAATCCCCGATGCC   |
| SufI L16C               | for | CGGGGATTGCAT <b>T</b> GTGCAGG  |
|                         | rev | GCCTGCCG <b>C</b> ACATGCAATCCCC  |
| SufI A18C               | for | GGGATTGCACTTGC <b>G</b> CTGCAGG  |
|                         | rev | GGGAAACAGCG <b>C</b> ACGCAAGTGAATCCC   |
| SufI G19C               | for | GGATTGCACTTGC <b>G</b> GCAT <b>G</b> C <b>G</b> CTGTTCCCCTGAAGG  |
|                         | rev | CCTTCAGGGGAAACAG <b>G</b> C <b>G</b> ATGCCGAAGTGAATCC  |
| SufI A20C               | for | GGGATTGCACTTGC <b>G</b> GCAGG <b>T</b> GTGTTCCCCTGAAGG   |
|                         | rev | GGCCTTCAGGGGAAAC <b>A</b> C <b>A</b> GC <b>C</b> CTGCCGAAGTGAATCCC                                     |
| SufI V21C               | for | GC <b>A</b> CTTGC <b>G</b> GCAGG <b>C</b> G <b>T</b> GTCCC <b>C</b> TAAGGCCAGC                         |
|                         | rev | GCTGCC <b>T</b> TCAGGG <b>A</b> CAAGGCC <b>C</b> TCGCCGAAGTGC  |
| SufI P22C               | for | GC <b>G</b> GCAGGC <b>C</b> G <b>T</b> TT <b>G</b> C <b>C</b> TAAGGCCAGCGC                             |
|                         | rev | GC <b>G</b> C <b>T</b> GG <b>C</b> CTTCAG <b>G</b> C <b>A</b> ACAGGCC <b>C</b> TCGCCGC                 |
| SufI L23C               | for | GCAGGC <b>G</b> C <b>T</b> TT <b>C</b> CC <b>T</b> GC <b>A</b> AGGCCAGCGCAGC                           |
|                         | rev | GCTGC <b>G</b> C <b>T</b> GG <b>C</b> CT <b>T</b> <b>G</b> C <b>A</b> AGGCC <b>A</b> AGCGC <b>C</b> TC |
| SufI A25C               | for | GCTGTTCCC <b>C</b> TAAGT <b>G</b> C <b>A</b> GC <b>G</b> CAGGCCGG                                      |
|                         | rev | CCC <b>G</b> G <b>C</b> TC <b>G</b> C <b>G</b> C <b>T</b> <b>G</b> C <b>A</b> AGGCC <b>A</b> ACAGC     |
| SufI S26C               | for | CCTGAAGGCC <b>T</b> GTGCAGGCCGG <b>C</b> AAAC  |
|                         | rev | GTTC <b>G</b> CC <b>G</b> CTGC <b>A</b> AGGCC <b>T</b> TCAGG   |
| SufI A28C               | for | CCTGAAGGCCAGCG <b>C</b> <b>A</b> T <b>G</b> C <b>GG</b> CAACAGCAACC                                    |

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|            | rev | GGTTGCTGTTGCCCGCATGCGCTGGCCTTCAGG          |
| SufI G29C  | for | GGCCAGCGCAGCCTGCCAACAGCAACCGC              |
|            | rev | GCGGTTGCTGTTGCCAGGCTGCCTGGCC               |
| SufI Q30C  | for | GCGCAGCCGGGTGCCAGCAACCGCTACC               |
|            | rev | GGTAGCGGTTGCTGGCACCCGGCTGCGC               |
| SufI Q31C  | for | CGCAGCCGGCAATGCCAACCGCTACCCG               |
|            | rev | CGGGTAGCGGTTGGCATTGCCCGGCTGCG              |
| SufI Q32C  | for | GCCGGGCAACAGTGCCCCTACCCGTTCCG              |
|            | rev | CGGAACGGGTAGCGGGCACTGTTGCCCGC              |
| SufI P33C  | for | GGGCAACAGCAATGCCTACCCGTTCCGCG              |
|            | rev | CGGCAGAACGGTAGGCATTGCTGTTGCC               |
| SufI L34C  | for | GGCAACAGCAACCGTGCCTCCGCTACTGAAATCTC        |
|            | rev | GCGCGGAACGGGCA CGGTTGCTGTTGCC              |
| SufI P35C  | for | GCAACAGCAACCGCTATGCCTCCGCCGCTACTGAAATCTC   |
|            | rev | GAGATTCAAGTAGCGGCGGAACGCATAGCGGTTGCTGTTGCC |
| SufI V36C  | for | CAACCGCTACCCGTCCGCCGCTACCTG                |
|            | rev | CAAGTAGCGGCGGAACAGGGTAGCGGTTG              |
| SufI W441C | for | GGTCAGCCTCCTGCCGCGCACTTCCCG                |
|            | rev | CGGGAAGTGC CGCAGGAAGGCTGACC                |