Unfoldase-mediated protein translocation through an α-hemolysin nanopore

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Supplementary Figures 1-7

S1:

MGSSHHHHHHGSGLVPRGSASMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKT
TPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGGGSSGGS
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AANDENYALAA

S2-35:

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EYRSGGSGSGGSGSGGSGMGSSHHHHHHGSGLVPRGSASMSDSEVNQEAKPEVKPEVKPE
THINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMED
NDIIEAHREQIGGGGSSGGSGGSGSSGDGGSSGGSGGSGSSGDGGSSGGSGGDGSSGDGG
SDGDSDGSDGDGDSDGDDAANDENYALAA

S2-148:

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GSGSSGSSGGSGMGSSHHHHHHGSGLVPRGSASMSDSEVNQEAKPEVKPEVKPETHINLKV
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S1-RQA:

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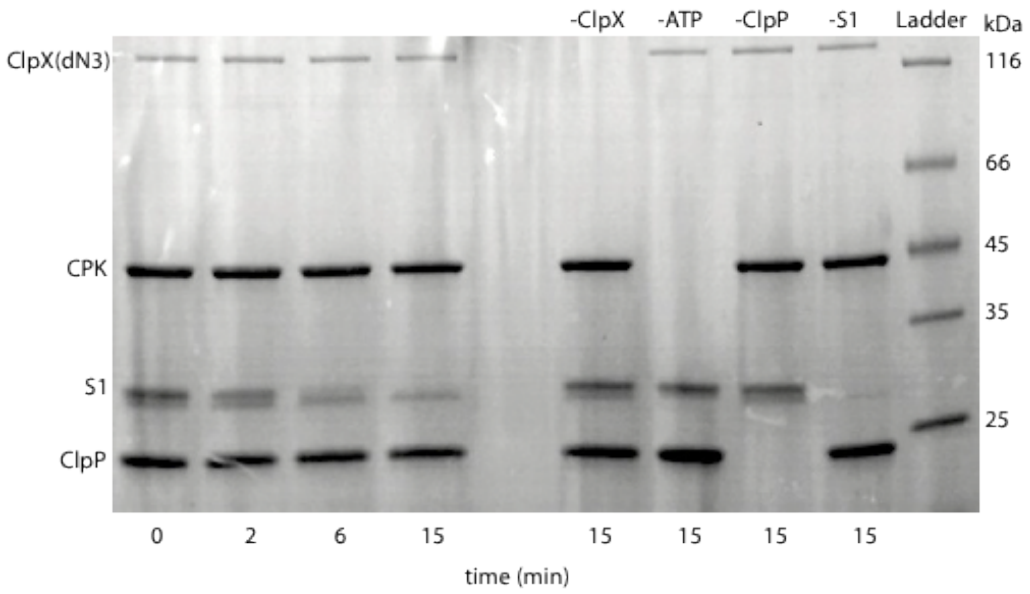
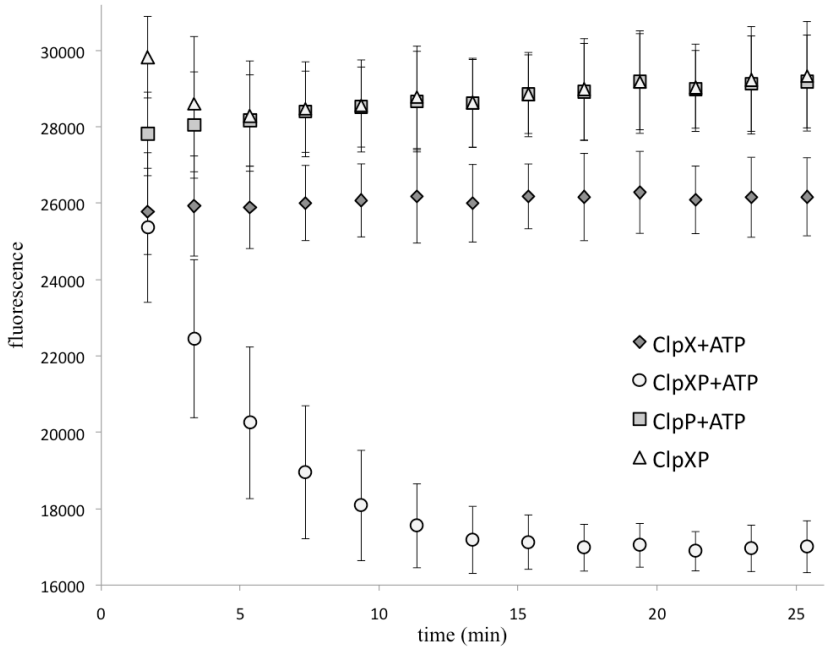
AANDENYALAARQA

Supplementary Figure 1 Amino acid sequences of substrate proteins used in this study. Green, Smt3 domains; yellow, charged tail; red, ssrA tag; orange, linker region; black, affinity purification tag regions; blue, additional residues added to prevent ClpX binding to the ssrA tag.

a

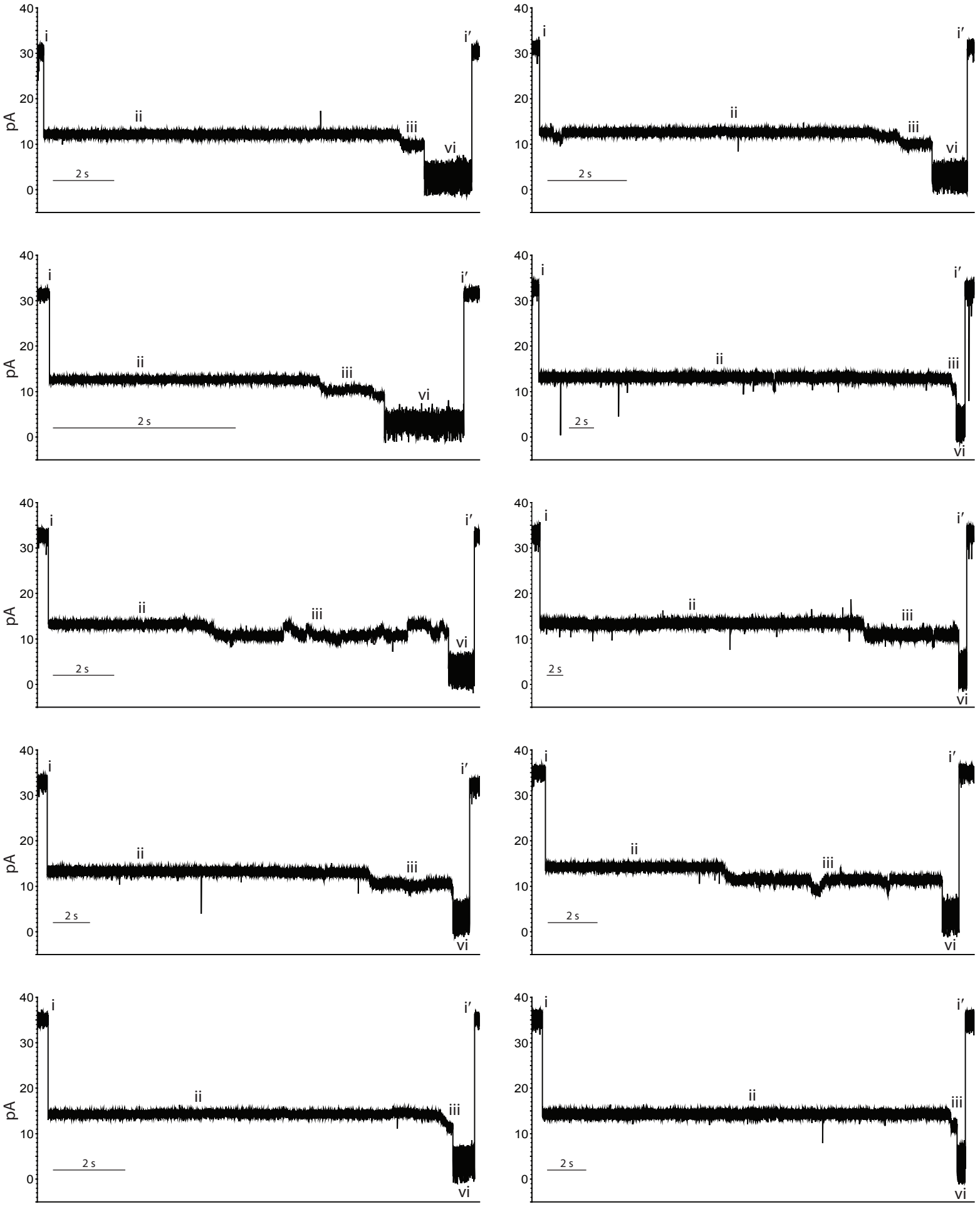


b

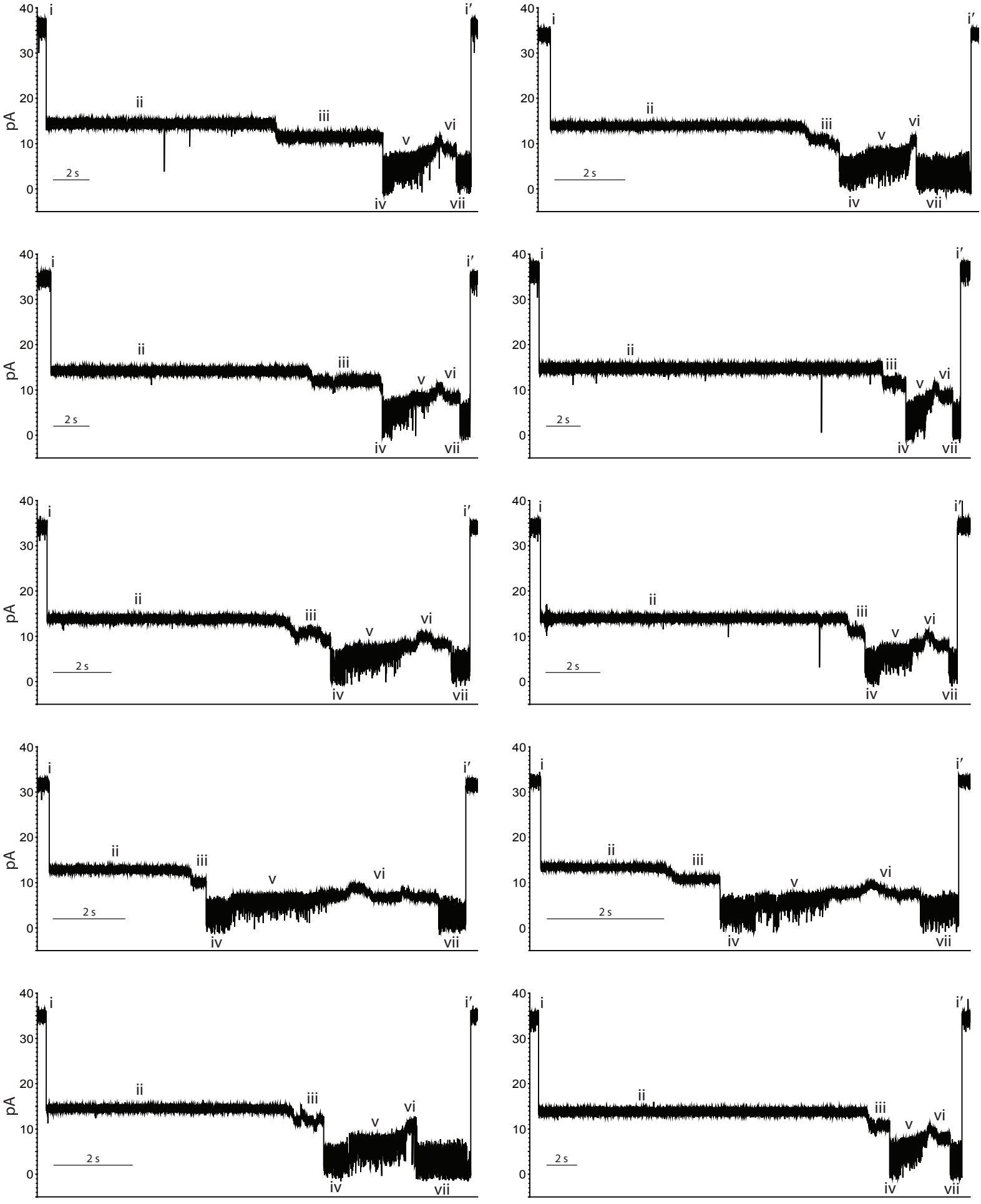


Supplementary Figure 2 Bulk phase assays of ClpX/ATP-dependent unfolding and translocation of substrate proteins bearing the long, charged ssrA-tagged C-terminal tail (see Online Methods for details). (**a**) SDS/PAGE gel showing substrate protein S1 degradation by ClpXP in the presence of ATP. Lanes 1-4 are a time course of S1 digestion in the presence of ClpX, ClpP, and ATP. Reactions minus ClpX (lane 6), minus ClpP (lane 7), or minus ATP (lane 8) showed no comparable degradation. Lane 9 is absent S1. (**b**) ClpX(P)/ATP-dependent quenching of a titin-GFP-tagged variant of the substrate protein S2-35 (C-terminus>charged-flexible tail>Smt3>titin>GFP>Smt3>N-terminus). ClpX+ATP n=4, ClpXP+ATP n=4, ClpP+ATP n=4, ClpXP(no ATP) n=3.

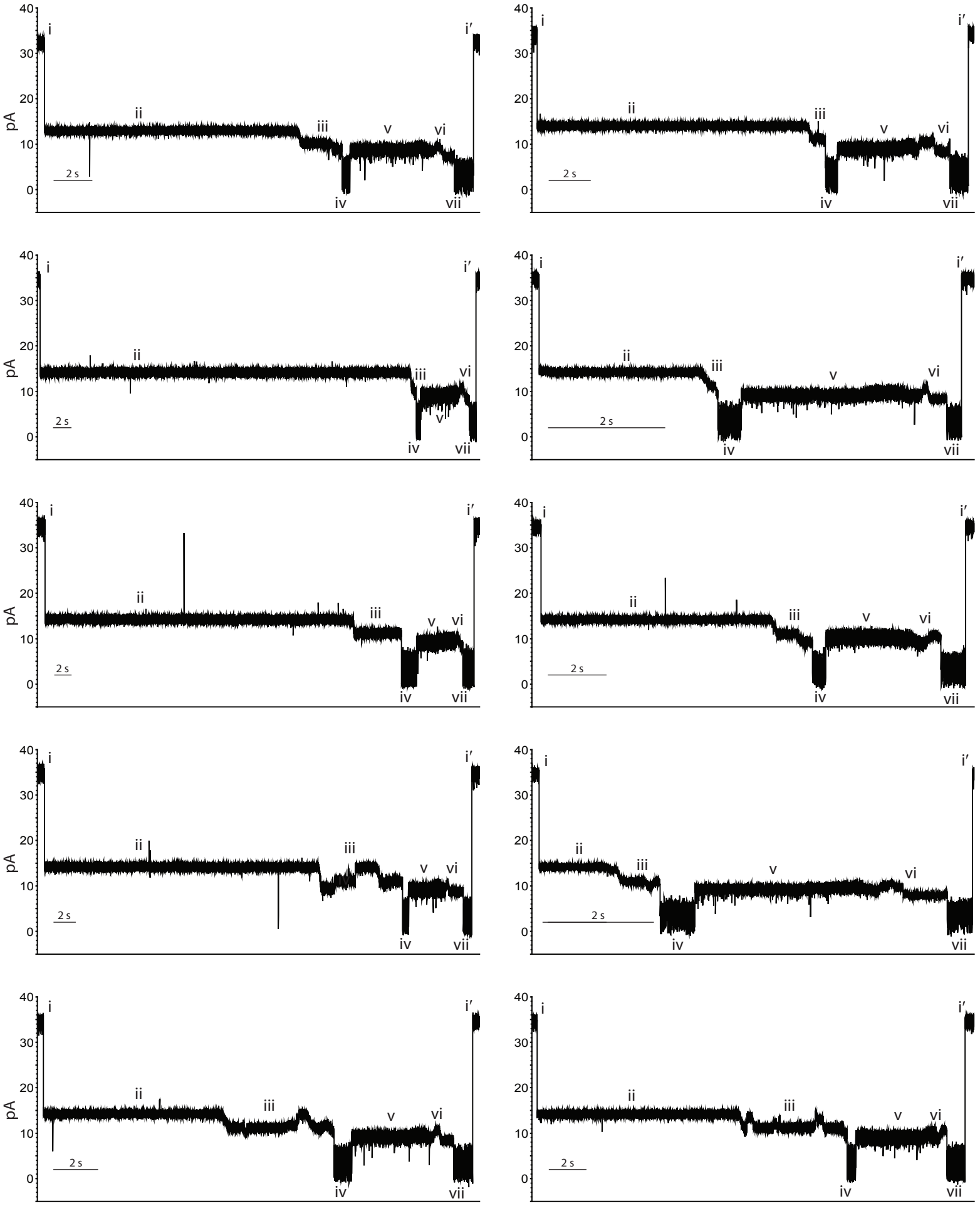
Supplementary Figure 3 Example traces arising from ClpX/ATP-dependent protein substrate translocation. (**a**) ClpX/ATP-dependent S1 translocation events.

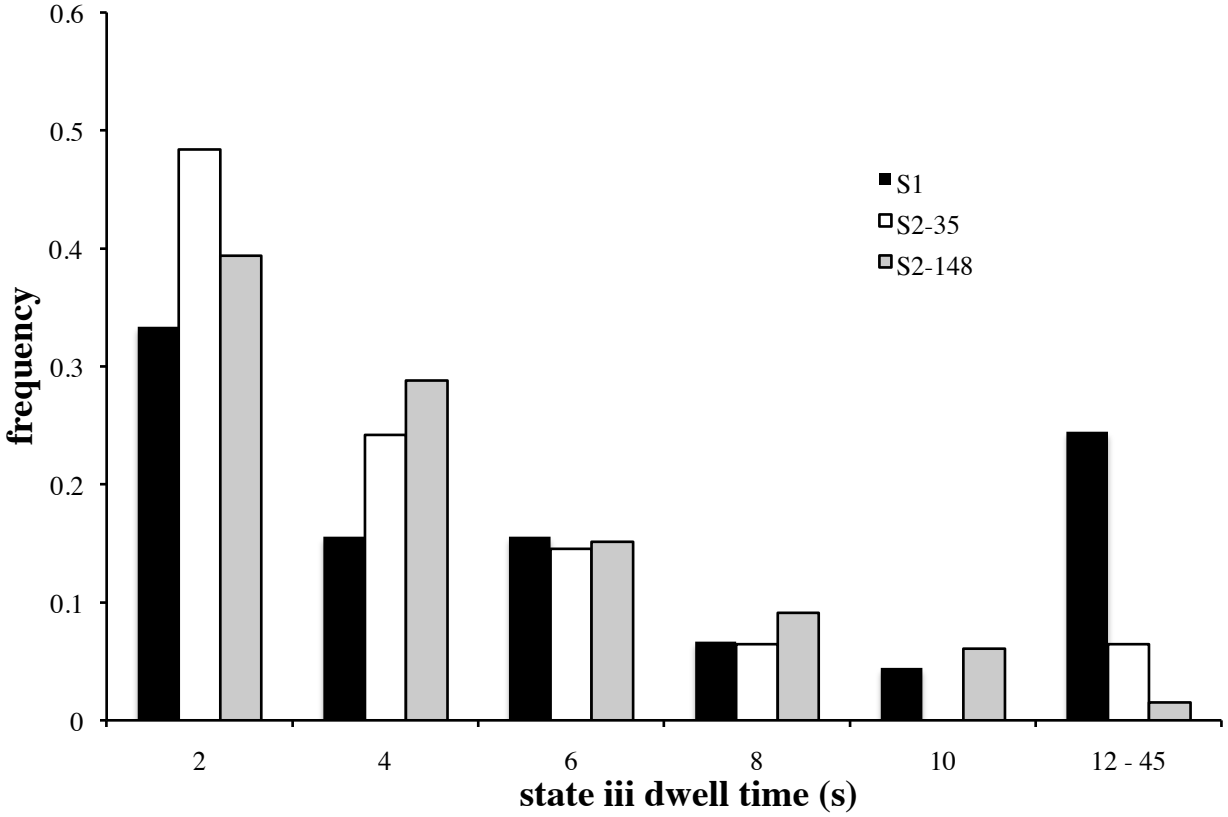


Supplementary Figure 3 Example traces arising from ClpX/ATP-dependent protein substrate translocation. (**b**) ClpX/ATP-dependent S2-35 translocation events.

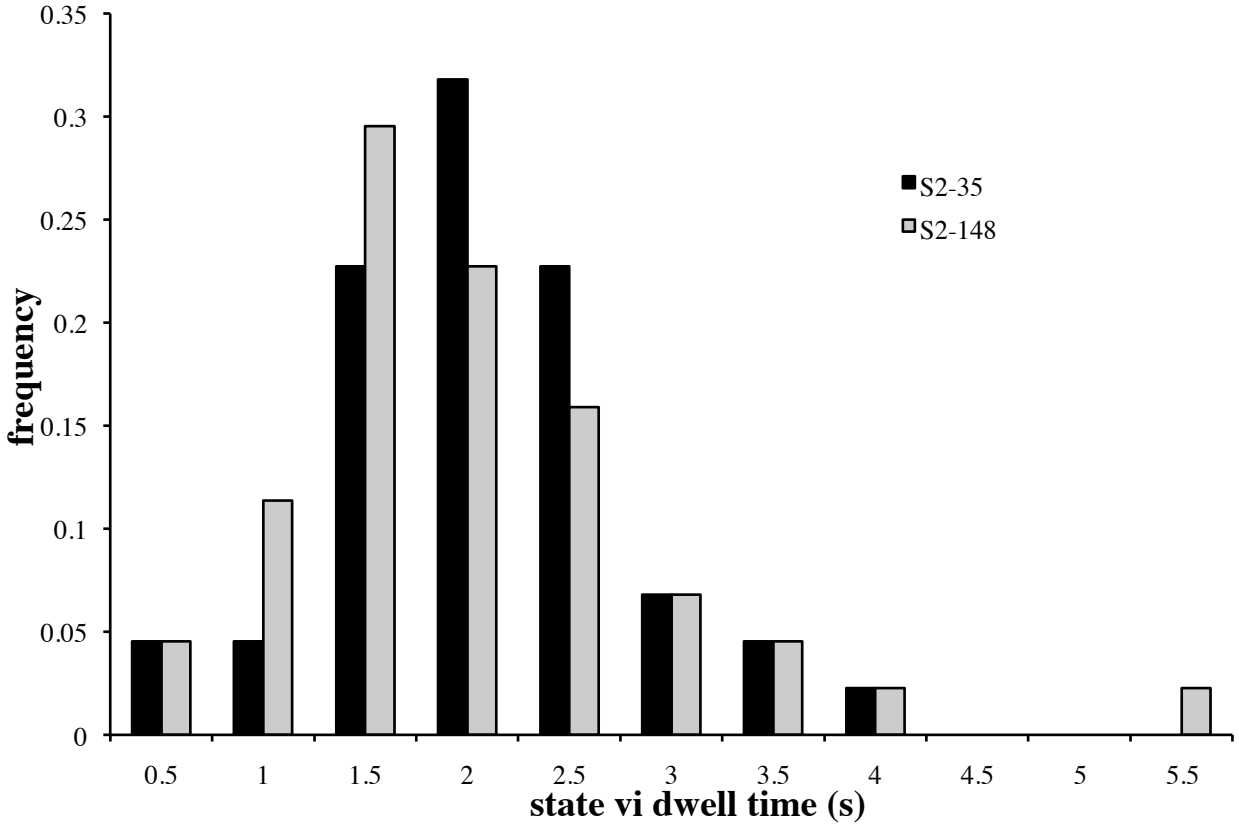


Supplementary Figure 3 Example traces arising from ClpX/ATP-dependent protein substrate translocation. (**c**) ClpX/ATP-dependent S2-148 translocation events.

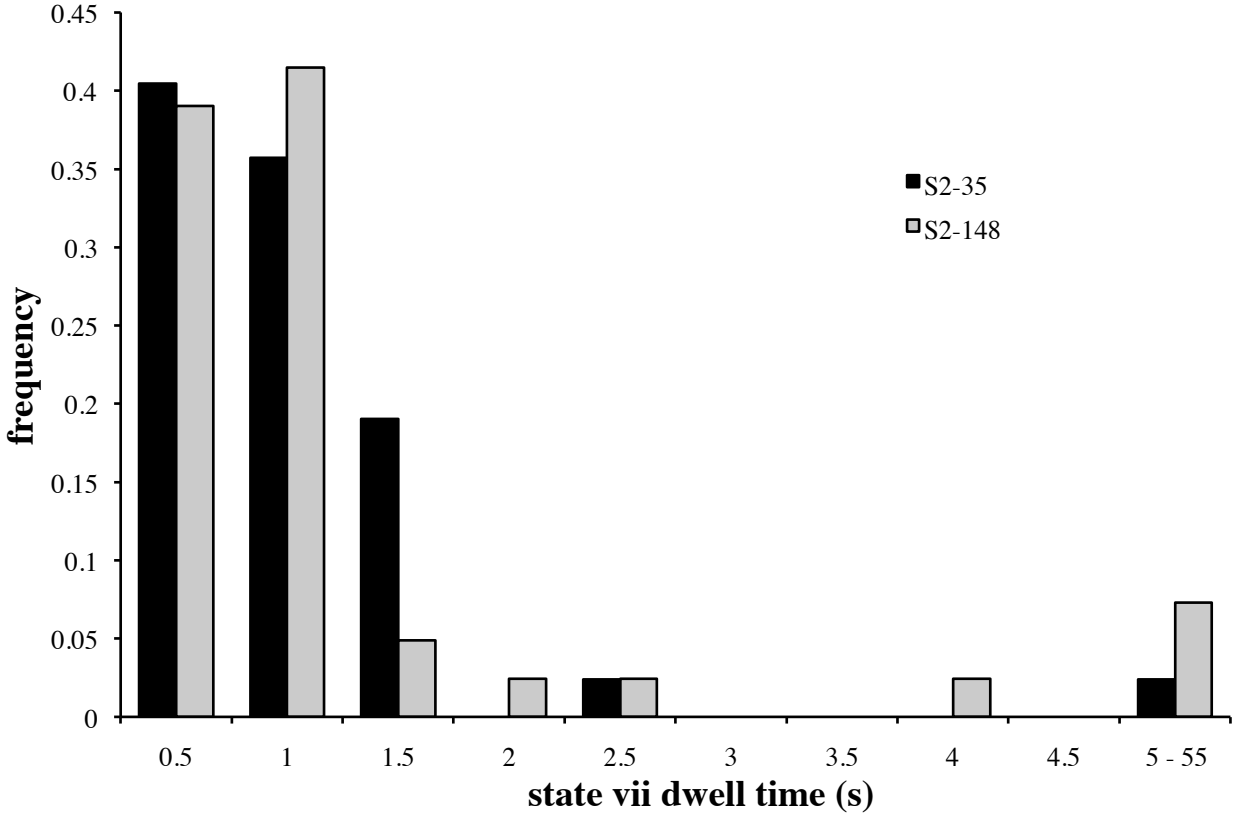




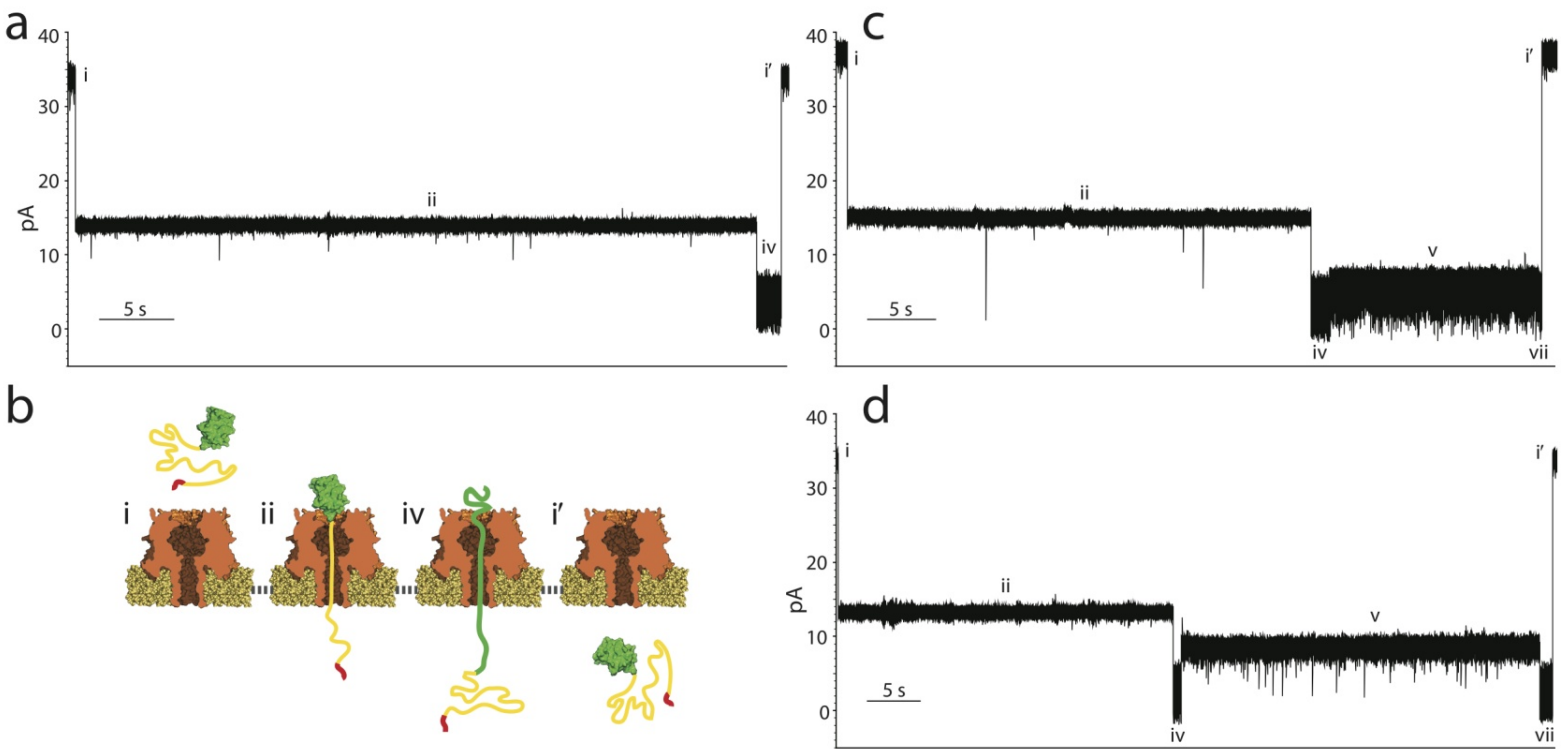
Supplementary Figure 4 Comparison of ionic current state iii dwell times for ClpX/ATP-dependent translocation events. Black bars: median = 4.15 s, IQR = 7.52, n = 45. White bars: median = 2.12 s, IQR = 3.23, n = 62. Gray bars: median = 2.76 s, IQR = 3.98, n = 66.



Supplementary Figure 5 Comparison of ionic current state vi dwell times for ClpX/ATP-dependent translocation events. Black bars: median = 1.80 s, IQR = 0.97, n = 44. Gray bars: median = 1.61 s, IQR = 0.97, n = 44.



Supplementary Figure 6 Comparison of ionic current state vii dwell times for ClpX/ATP-dependent translocation events. Analyzed events included ClpX/ATP-dependent ramping states iii and vi. Black bars: median = 0.63 s, IQR = 0.93, n = 42. Gray bars: median = 0.66 s, IQR = 0.90, n = 41.



Supplementary Figure 7 Ionic current traces showing translocation of the three model proteins absent ClpX/ATP-dependent mechanical work (no ramping states iii/vi). Following state ii, all protein substrates we examined eventually unfolded and translocated due to the 180 mV applied potential. All events exhibited more widely distributed state dwell times compared to ClpX-mediated events (**Fig. 3**). (**a**) S1 translocation. Note the absence of state iii compared to **Figure 2a**. (**b**) Model of ClpX/ATP-independent protein S1 translocation. Cartoons i-i′ correspond to ionic current states i-i′ in **a**. (**c**) S2-35 translocation. Note absence of ramping states iii and vi compared to **Figure 2c**. (**d**) S2-148 translocation. Note absence of ramping states iii and vi compared to **Figure 2d**.