Supporting Information (Appendix)

Identifying N60D mutation in ω subunit of *Escherichia coli* RNA polymerase by bottom-up proteomic approach

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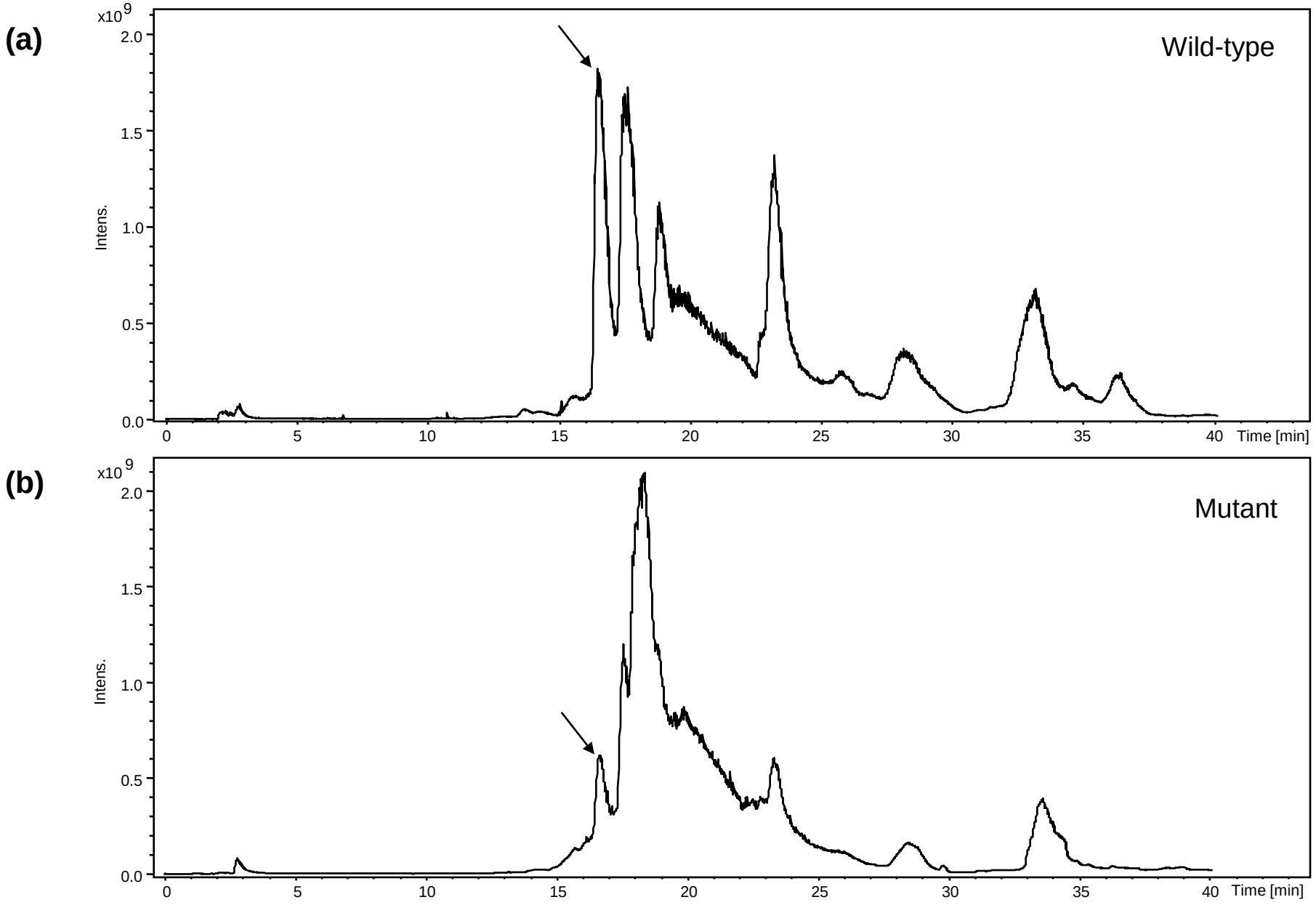

Figure S1. Total ion chromatograms (TICs) from LC-ESI-MS of solubilized and renatured inclusion bodies (IBs) obtained upon expressing plasmids encoding for **(a)** Wild-type and **(b)** Mutant ω subunit of *E. coli* RNAP. The IBs were passed through a reverse phase column, Zorbax 300SB C8 (2.1 mm × 100 mm; 3.5 μm) and a gradient elution was followed using acetonitrile and water, each containing 0.1% formic acid. The peaks that are indicated correspond to the wild-type and mutant ω subunit of *E. coli* RNAP. (Mass spectra of these two proteins are shown in Figure S2)

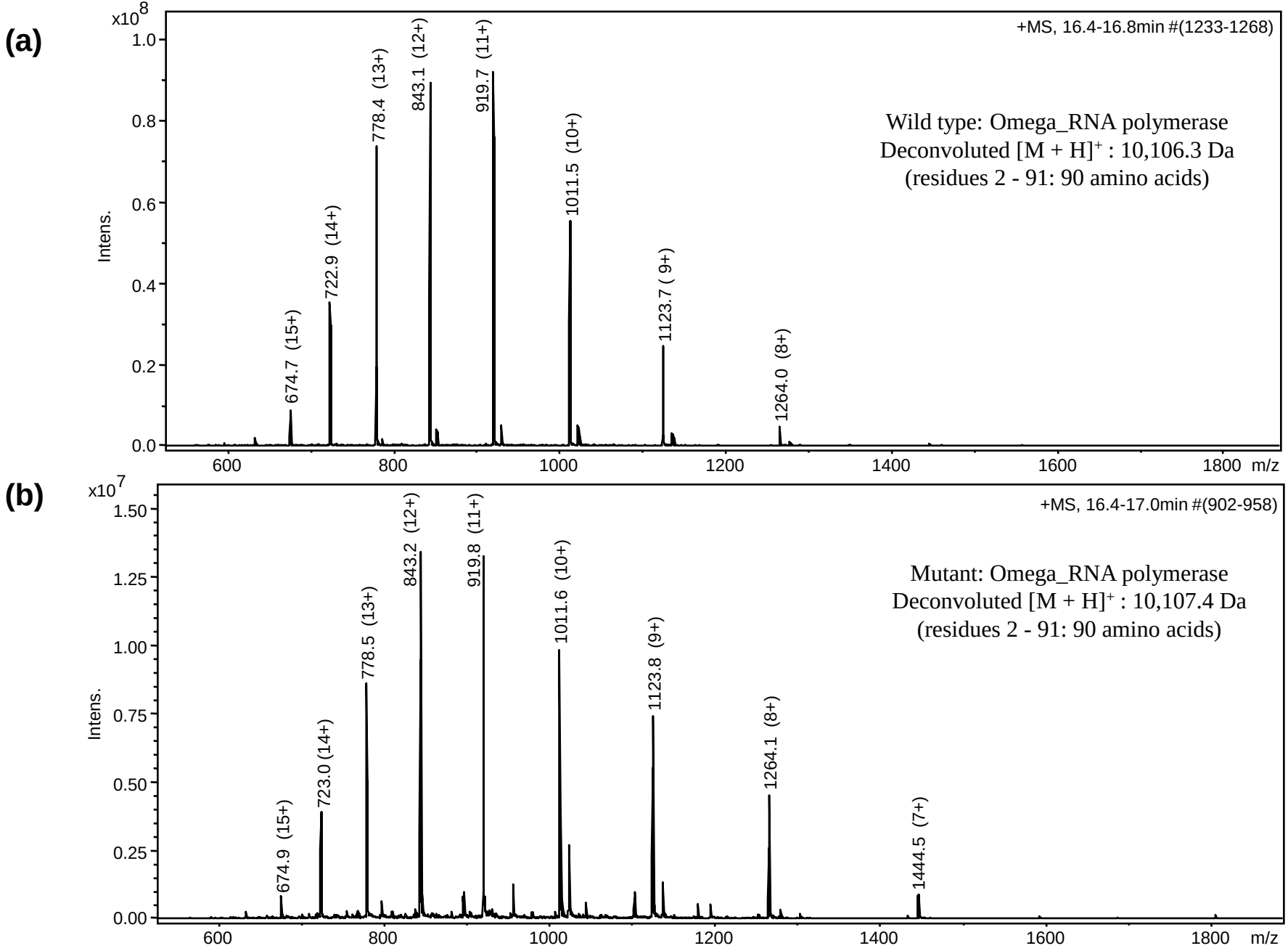

Figure S2. LC-ESI mass spectrum of **(a)** wild-type and **(b)** mutant ω subunit of *E. coli* RNAP. The above mass spectra are obtained upon processing the indicated peak in the respective TIC shown in Figure S1.The molecular mass ($[M + H]^+$) is obtained upon deconvoluting the mass spectrum. The molecular mass of wild-type and mutant suggests loss of N- terminus methionine, which may have occurred during processing the sample. Thus, the molecular mass corresponds to 90 amino acids, i.e., residues 2 - 91.

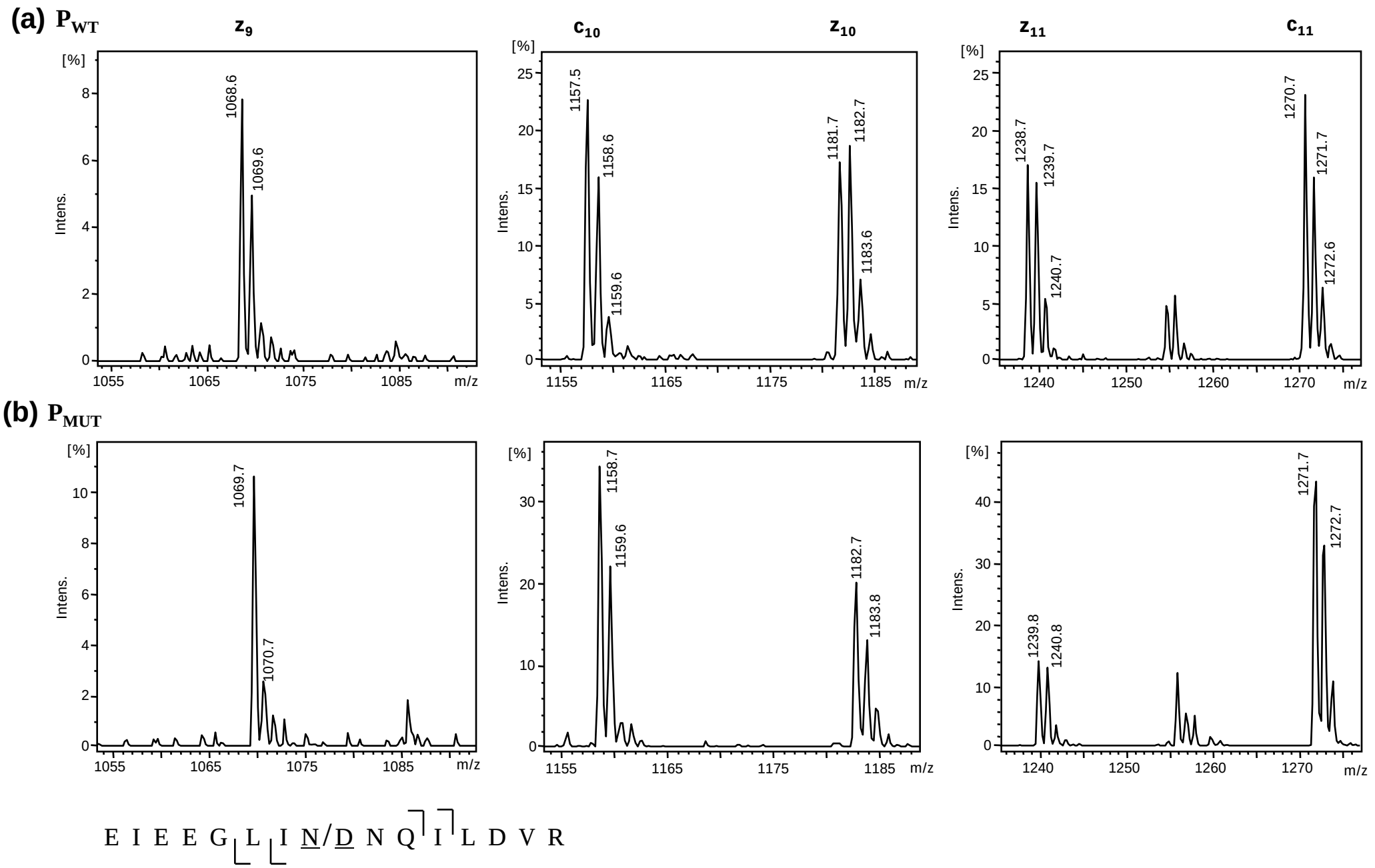

Figure S3. Expansion of the LC-ESI-ETD MS/MS spectra of $[M + 3H]^{3+}$ precursor ion of **(a)** P_{WT} and **(b)** P_{MUT} shown in Figure 1, edited between m/z 1055 - m/z 1275. The signals of z_9, z_{10}, c_{10}, c_{11} along with their respective isotopic peaks are shown. See Table 1 for the calculated m/z values of these *c*- and *z*- ions.

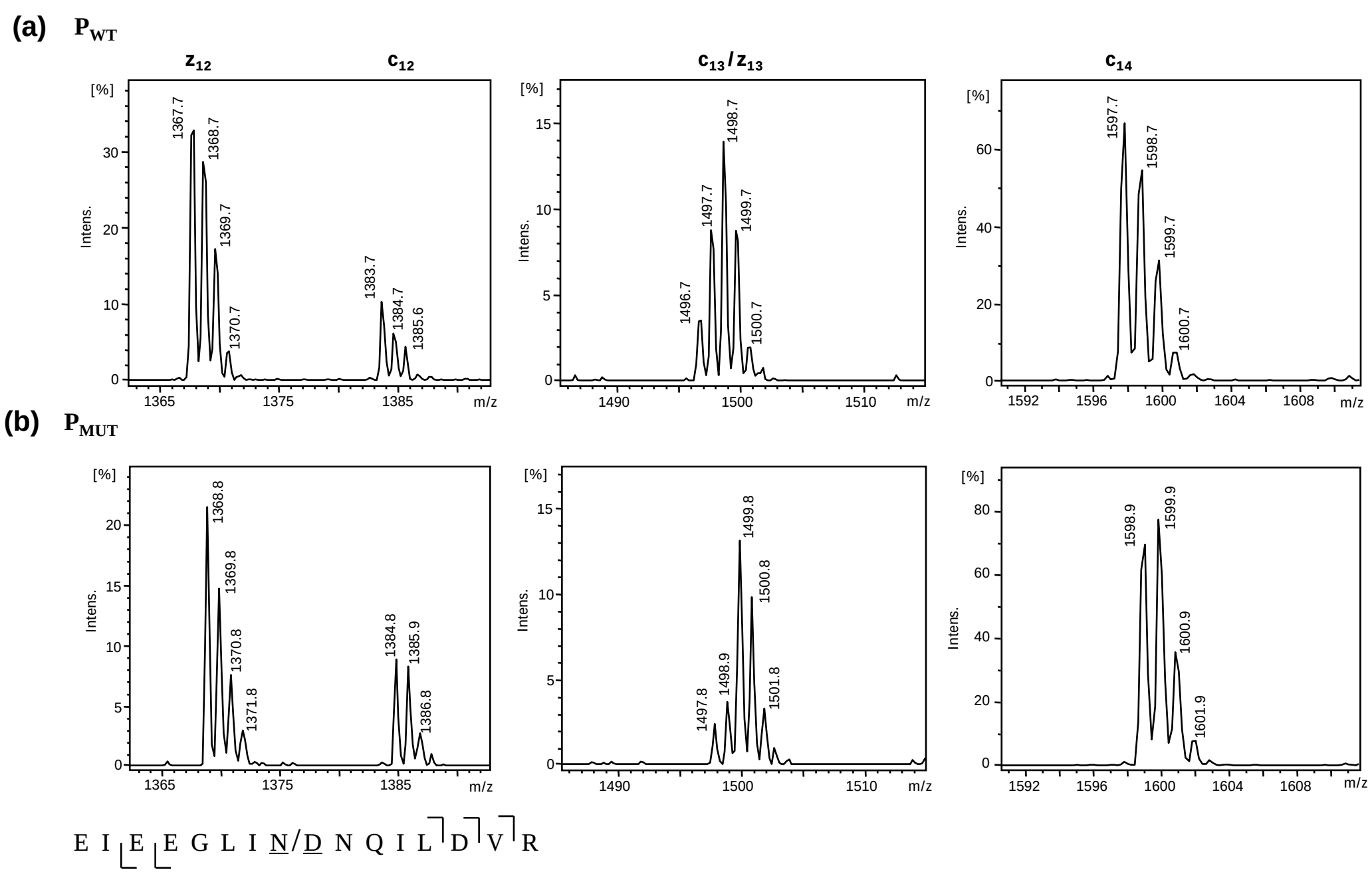

Figure S4. Expansion of the LC-ESI-ETD MS/MS spectra of $[M + 3H]^{3+}$ precursor ion of **(a)** P_{WT} and **(b)** P_{MUT} shown in Figure 1, edited between m/z 1365 - m/z 1610. The signals of z_{12}, z_{13}, c_{12}, c_{13}, c_{14} along with their respective isotopic peaks are shown. See Table 1 for the calculated m/z values of these *c*- and *z*- ions.

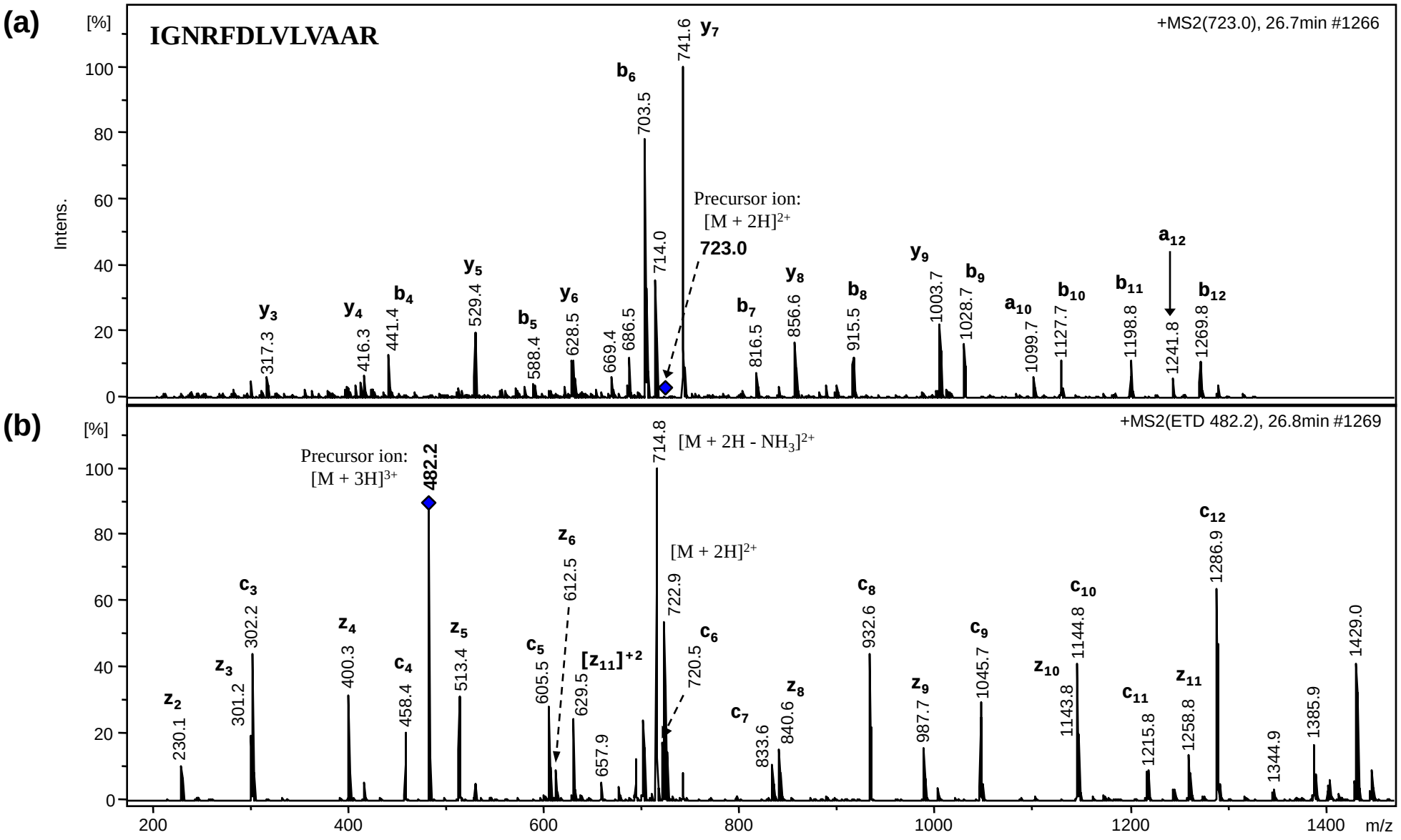

Figure S5. (a) LC-ESI-CID MS/MS spectrum of $[M + 2H]^{2+}$ precursor ion *m/z* 723.0 of tryptic peptide, **IGNRFDLVLVAAR**, residues (13 - 25) of ω subunit of *E. coli* RNAP. **(b)** LC-ESI-ETD MS/MS spectrum of $[M + 3H]^{3+}$ precursor ion *m/z* 482.2 of same tryptic peptide.

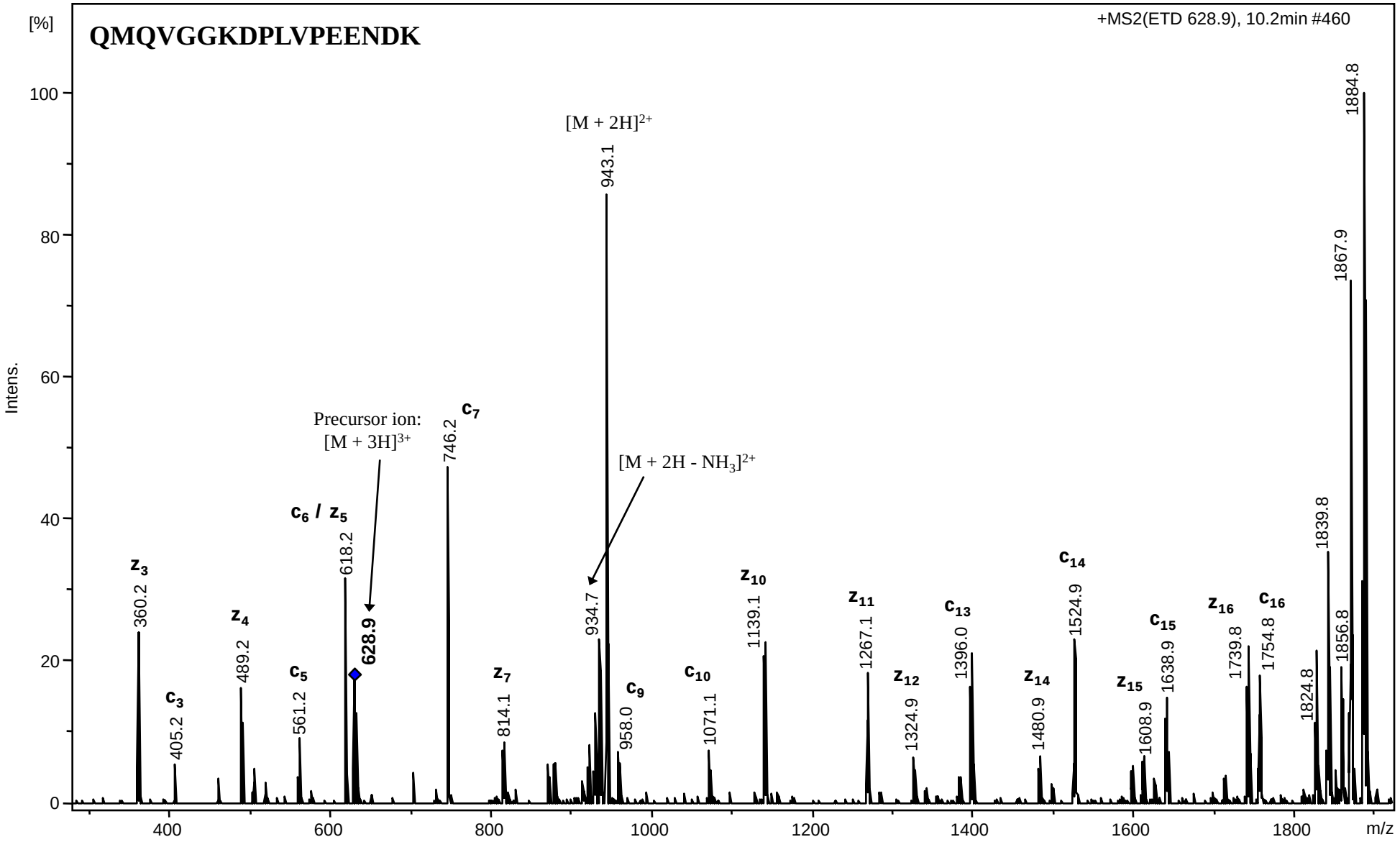

Figure S6. LC-ESI-ETD MS/MS spectrum of $[M + 3H]^{3+}$ precursor ion *m/z* 628.9 of **QMQVGGKDPLVPEENDK**, residues (29 - 45) of ω subunit of *E. coli* RNAP.

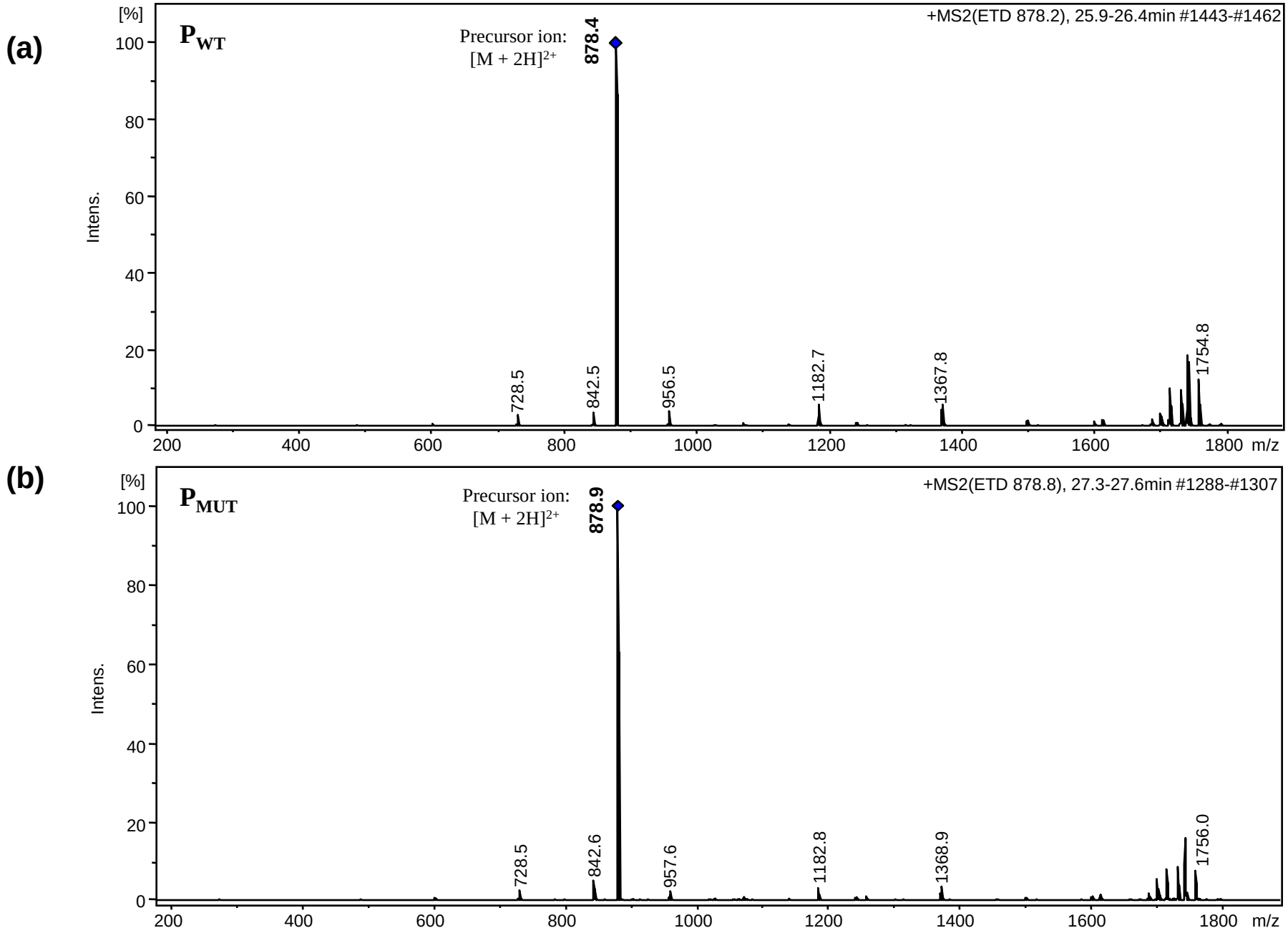

Figure S7. LC-ESI-ETD MS/MS spectrum of **(a)** $[M + 2H]^{2+}$ precursor ion m/z 878.4 of P_{WT} and **(b)** $[M + 2H]^{2+}$ precursor ion m/z 878.9 of P_{MUT}. P_{WT}: EIEEGLI**N**NQILDVR and P_{MUT}: EIEEGLI**D**NQILDVR (residues 53 - 67 of wild-type and mutant of ω subunit of *E. coli* RNAP).