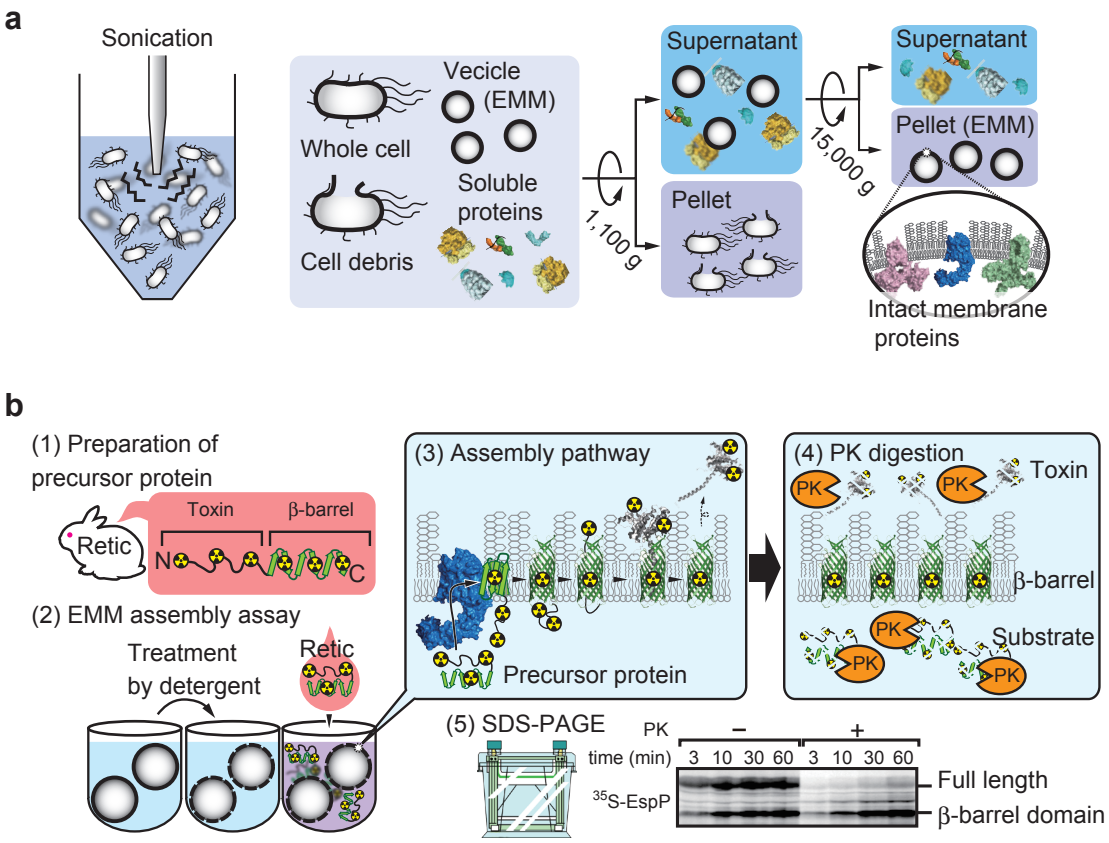
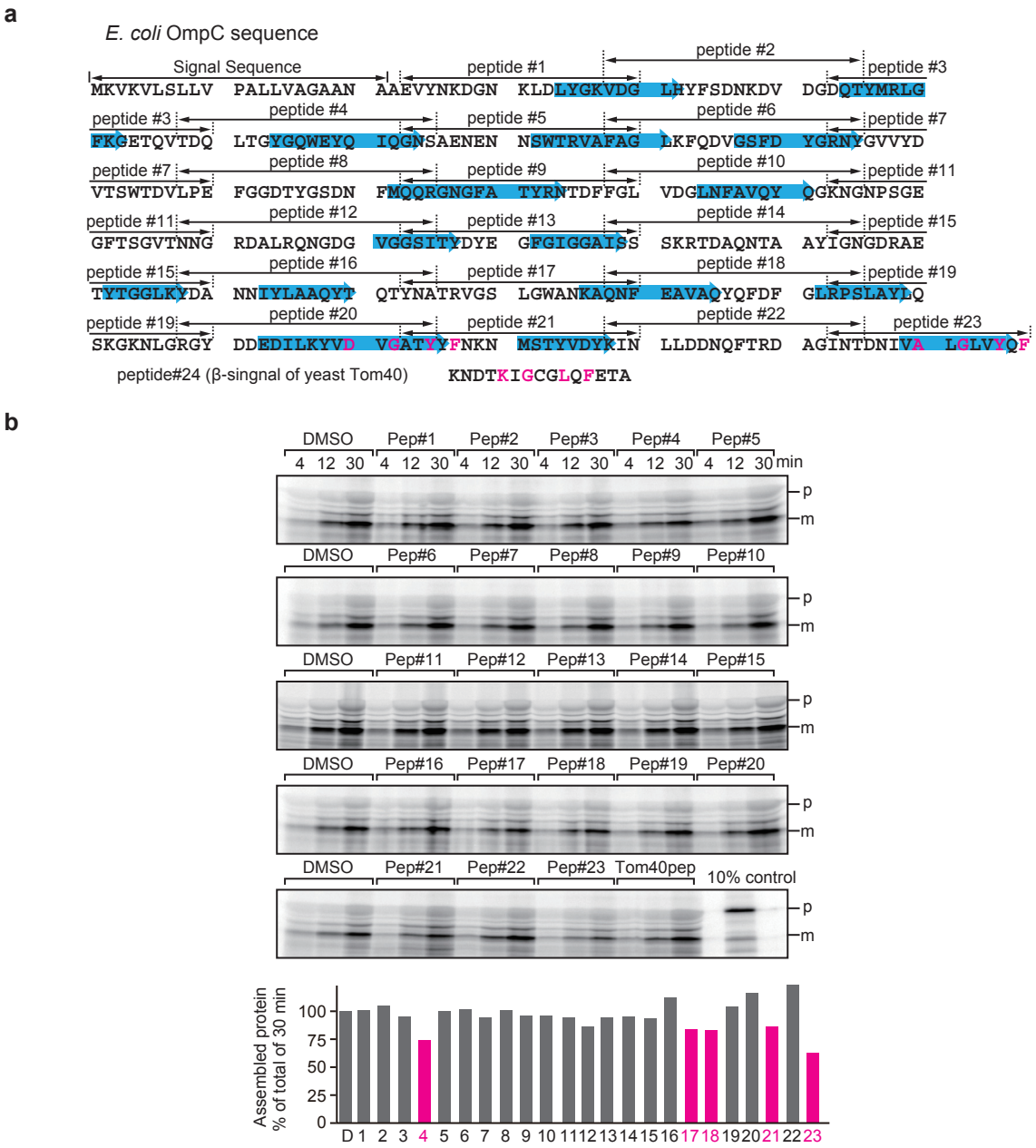


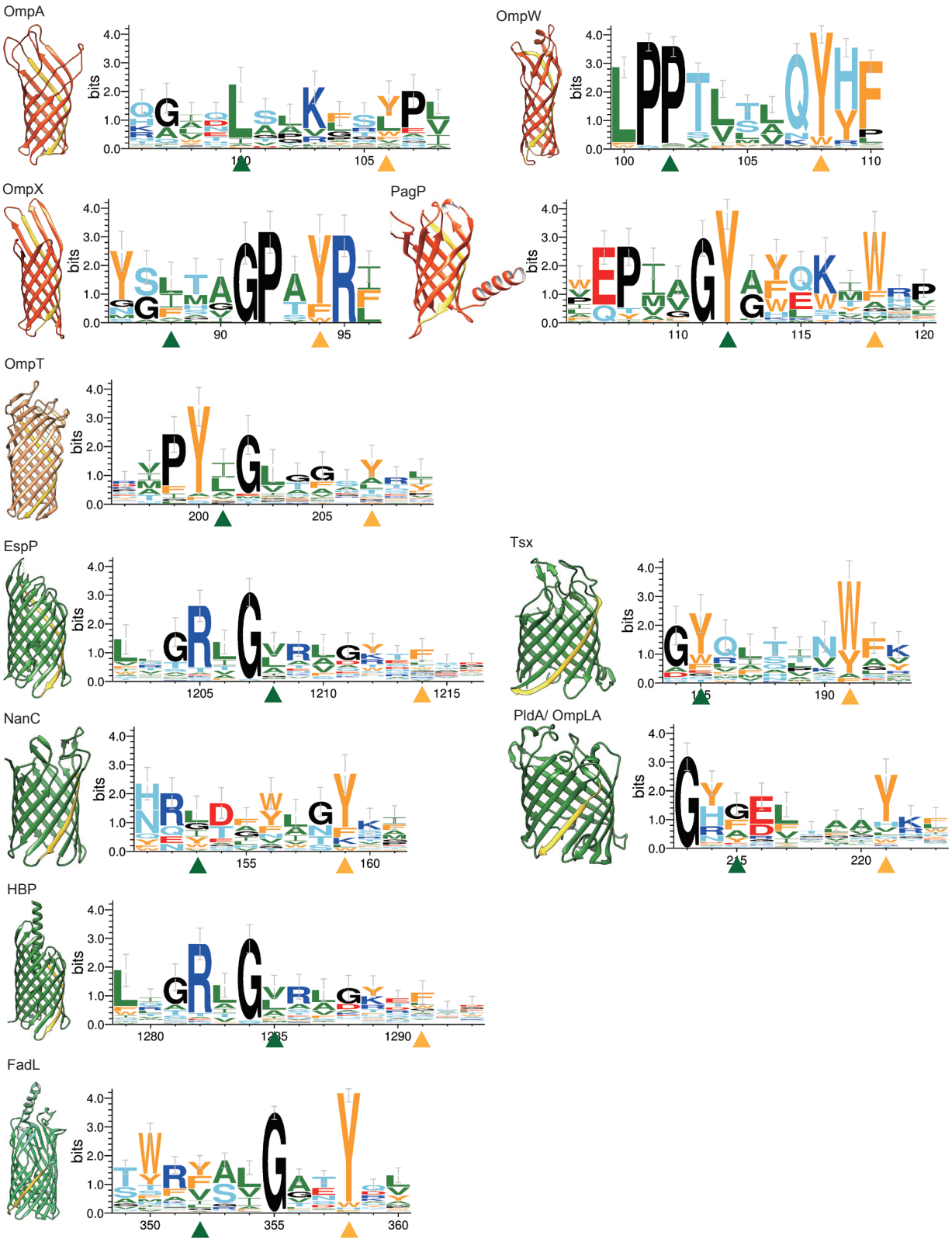
Extended Data Figure 1: Schematic of *E coli* microsomal membrane (EMM) isolation and EMM assembly assay. Relating to Figure 1.

(**a**) *E. coli* microsomal membranes (EMM) were isolated via cell disruption by sonication, cell debris is removed via low-speed centrifugation, finally membranes are harvested after high-speed centrifugation. (**b**) ^{35}S-labelled EspP EMM assembly assay is prefomed as follows: (1) Radio-labelled substrate proteins are translated using rabbit reticulocyte lysate, (2) EMMs are treated with detergent then radio-labelled substrate is added to the EMM. (3) Treatment with detergent allows for substrate to access all available BAM complex, the BAM complex then assists in assembly of substrate. (4) EMM were then incubated with protease. EspP passenger domain and unassembled EspP are digested, while membrane-integrated barrel domain is protected from protease. (5) Samples are boiled and subjected to SDS-PAGE for analysis.

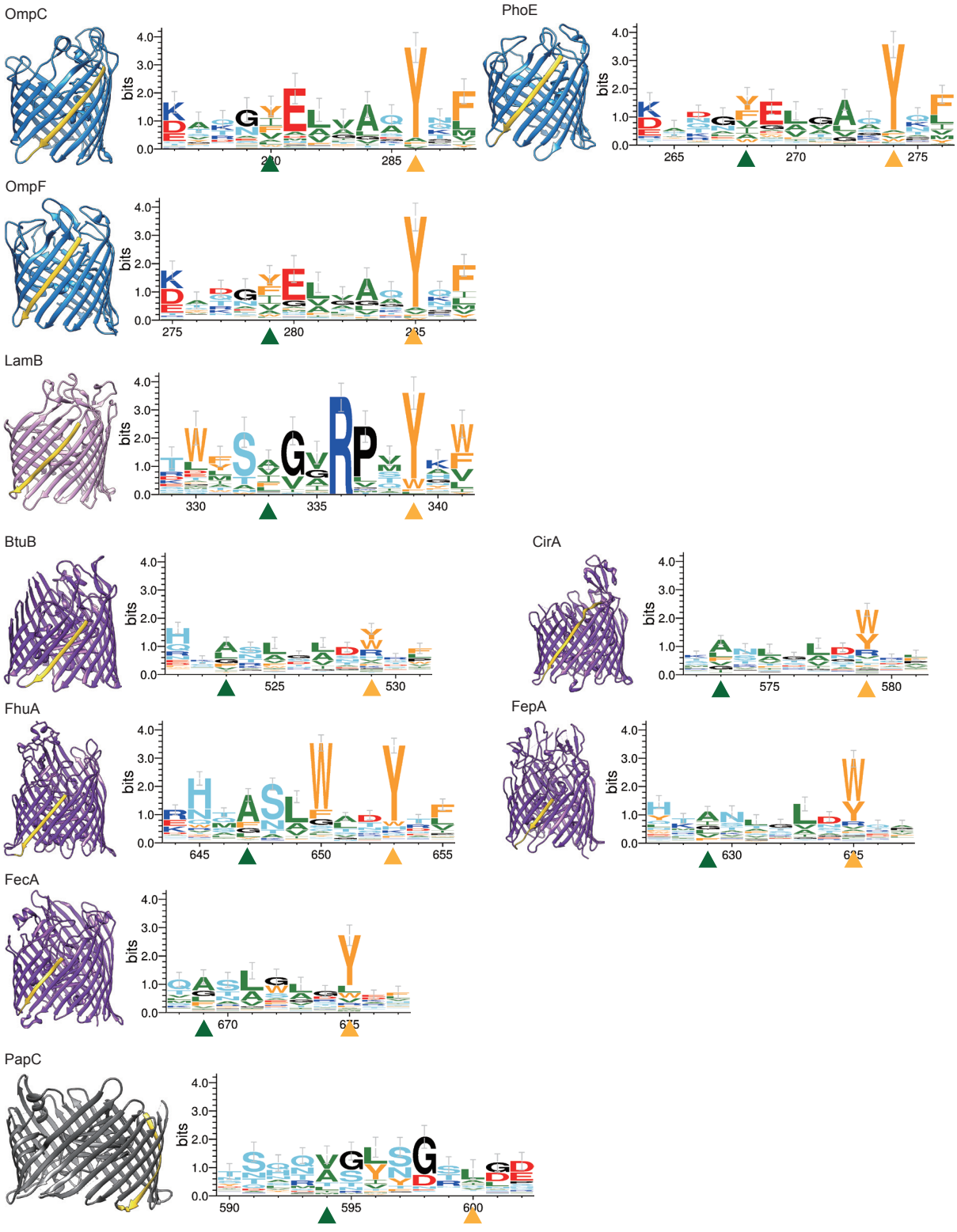


Extended Data Figure 2: Design of peptide inhibitor library from *E. coli* OmpC, relating to Figure 1.

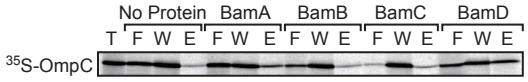
(**a**) Primary sequence of the *E. coli* OMP OmpC divided into peptide regions. The sequence was divided into 23 peptides made up of 15 amino acids. Highlighted in blue arrows are the 16 β-sheets which make up OmpC. Below OmpC sequece, the canonical β-signal of yeast-Tom40, a mitochondrial β-barrel protein, used as the peptide #24 control. (**b**) The inital inhibitor peptide screen of EspP assembly assay . Peptides were added at final concentration 100 μM into EMM assembly assay. Peptides 4, 17, 18, 20, and 23 displayed the highest degree of inhibition compared to DMSO (magenta bars). p, precursor form; m, mature form. Densitometry of EspP assembly at 30 minutes was quantified to determine the peptides with the highest potential of inhibition.



Extended Data Figure 3 (legend next page)



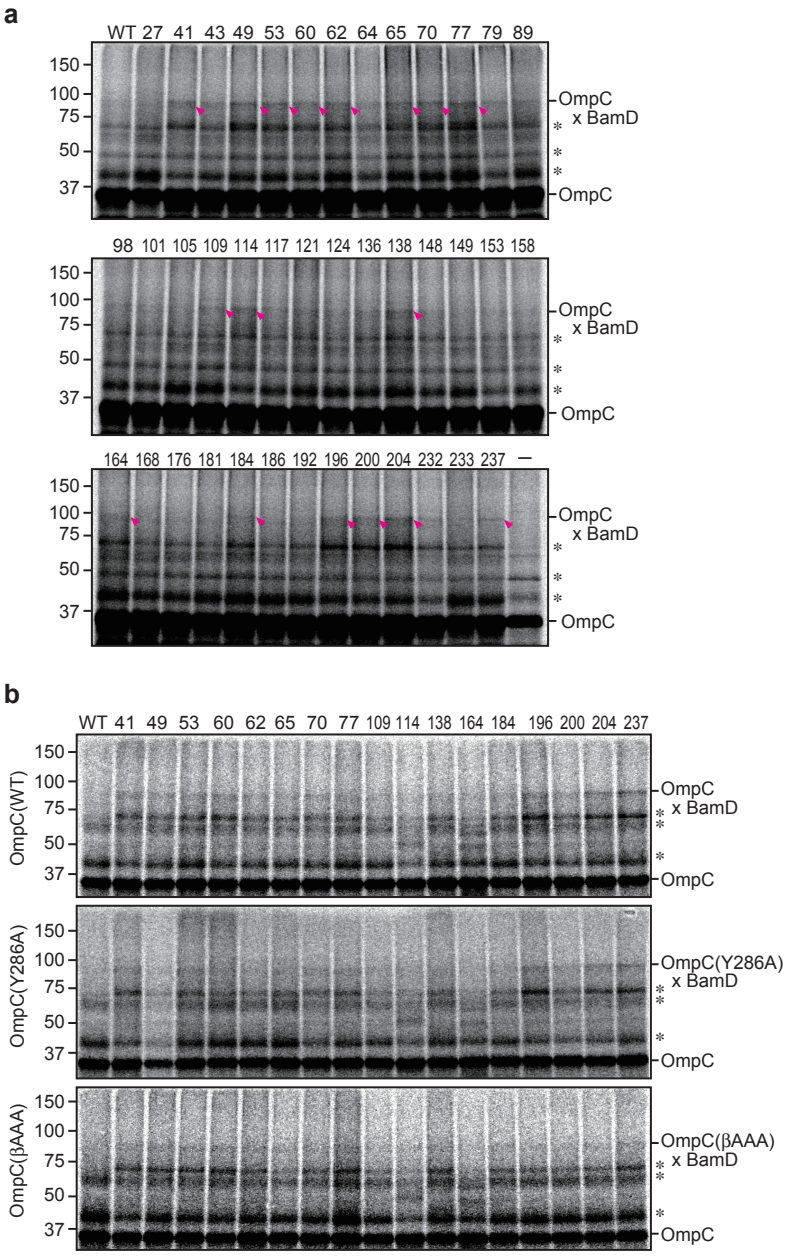
Extended Data Figure 4 (legend next page)



Extended Data Figure 5- Ni-NTA pull-down of ^{35}S-OmpC with BAM complex subunits, relating to Figure 5.

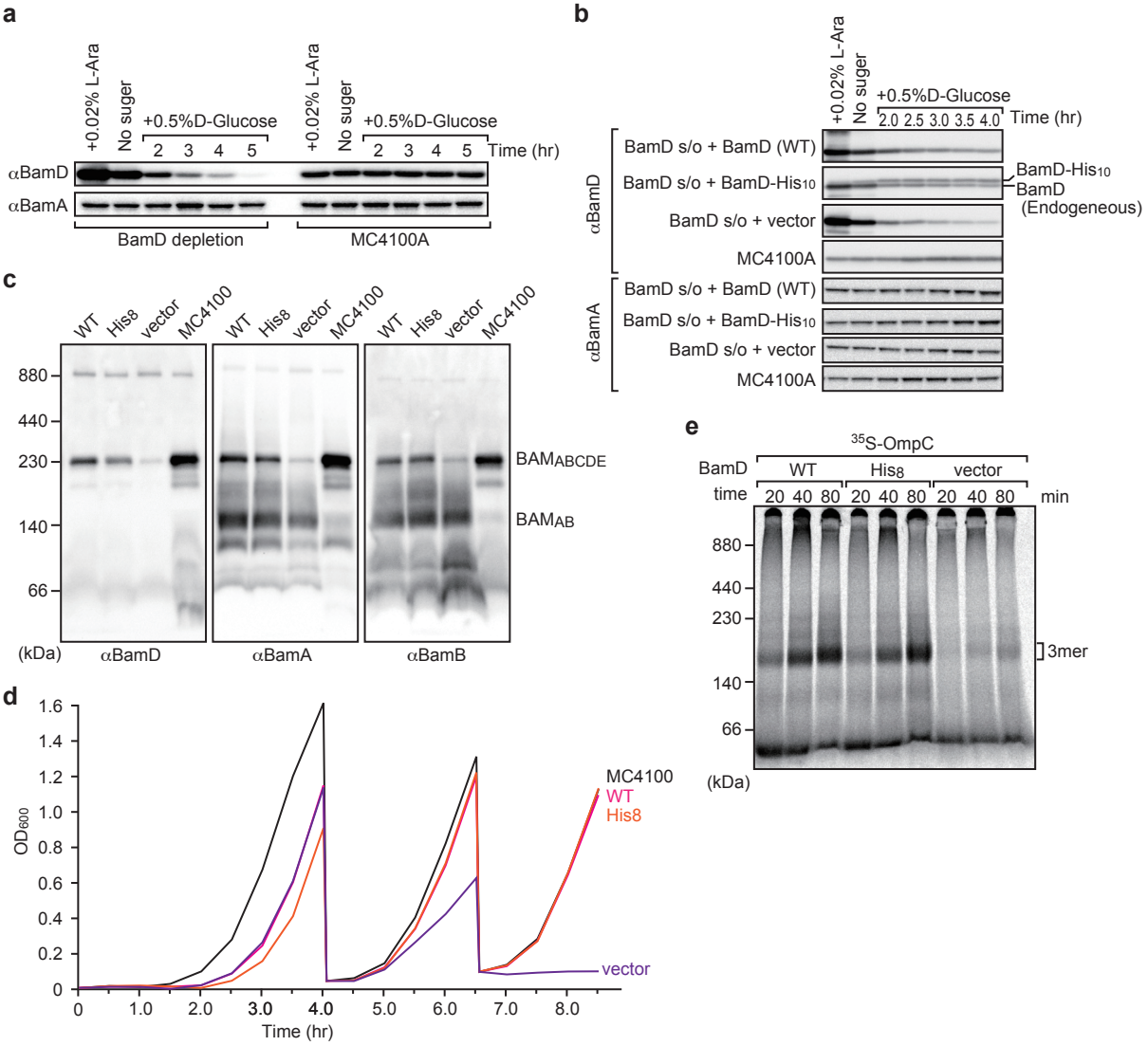
In vitro Ni-NTA pull-down of BAM Complex components with ^{35}S-labelled OmpC. ^{35}S-labelled OmpC was incubated with purified 6xHis-tagged BAM complex proteins, either BamA, BamB, BamC, and BamD, and co-purified via Ni-NTA. T- 5% total input of 35S-OmpC, F- flow-through, W- wash, E- Elution.

In this study, we excluded BamE for the following reasons: (i) We could not yield enough amount of purified BamE, (ii) There is no evidence of BamE interact with substrate protein in previous studies.



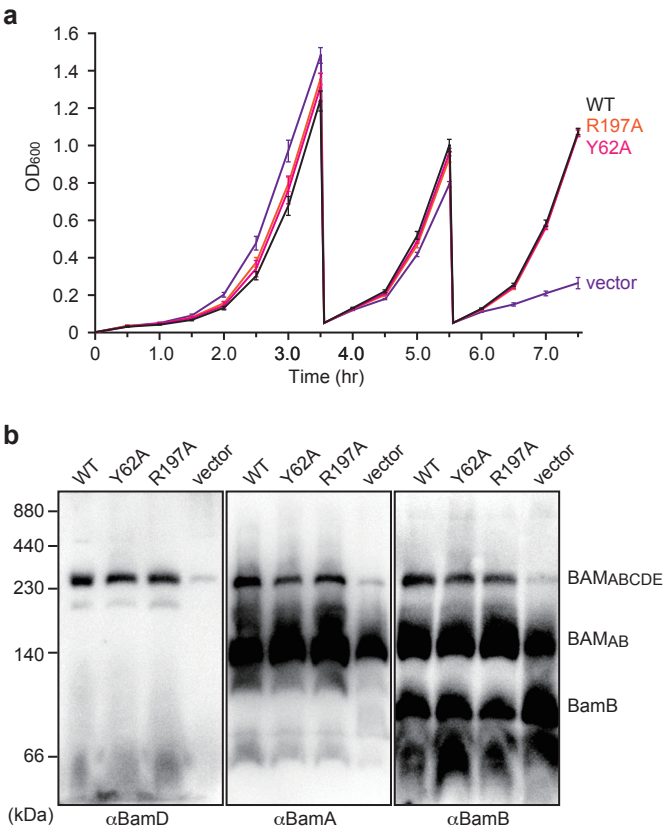
Extended Data Figure 6- Identification of amino acid crosslink products between BamD and OmpC, relating to Figure 4.

(**a**) BamD expressing unnatural amino acids were purified, incubated with purified OmpC substrate, UV-irradiated, and analyzed by SDS-PAGE. Numbers above each lane indicate the amino acid position that was mutated to BPA. Pink arrows designate crosslink product of BamD-OmpC, while asterisks signify non-specific products. (**b**) Residues that produced crosslinking products from Figure S9A were incubated with WT, -5 β-strand mutant (Y286A), or β-signal mutant (Y365A, Q366A, F367A).



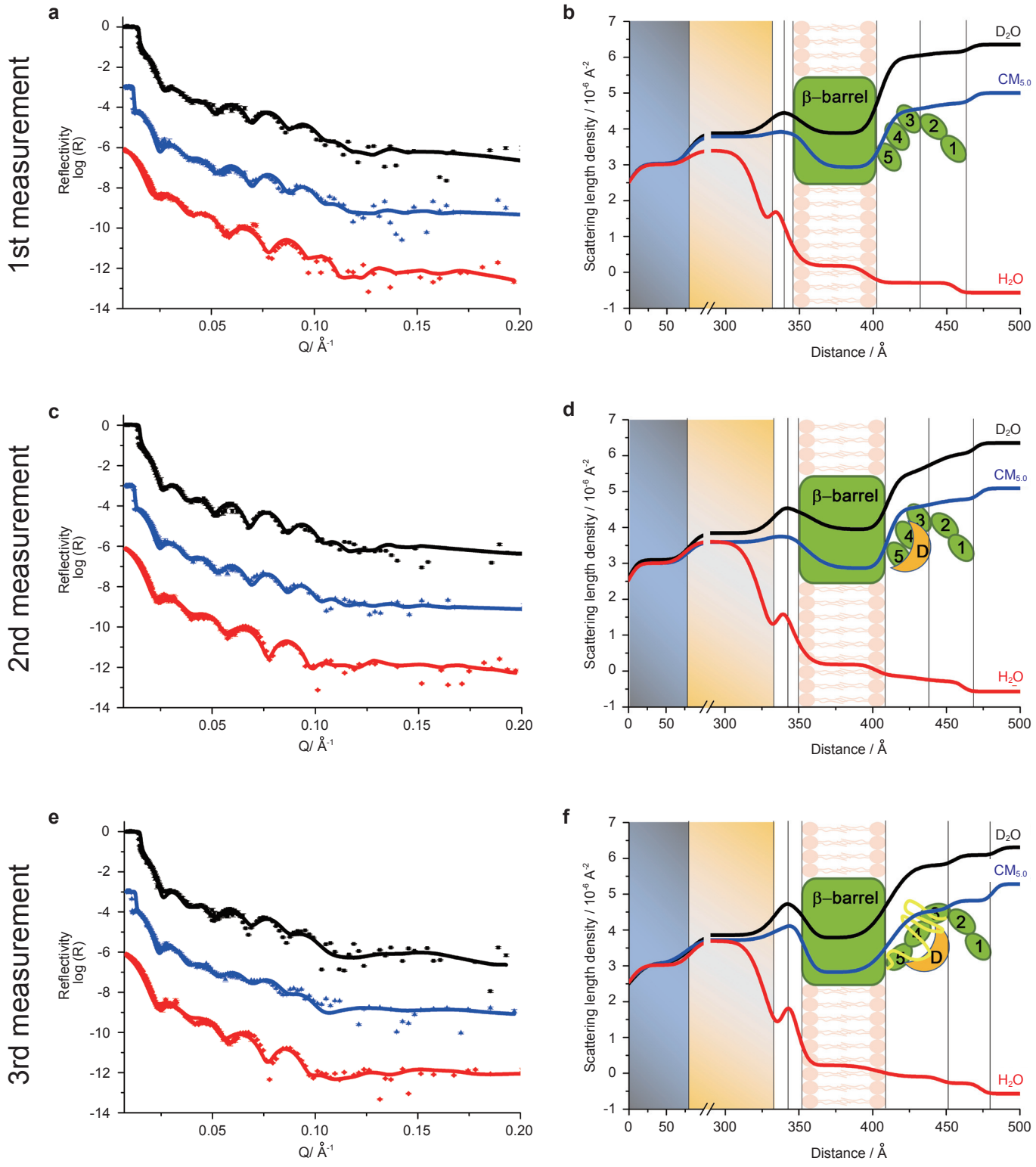
Extended Data Figure 7- Characterization of BamD depletion strain, relating to Figure 6.

(**a**) Optimization of BamD depletion as compared to parent-strain MC41000A. BamD levels were monitored by immunoblotting after growth in media containing glucose to repress chromosomal BamD expression as shown in Figure 6B. BamA protein levels were found to be unchanged even after BamD was unable to be detected by immunoblotting. (**b**) Expression of plasmid-born BamD and BamD harboring a poly-histidine tag versus empty vector and MC4100A strain. Both tagged and non-tagged BamD were detected at similar levels. (**c**) BamA expression remained unchanged between all samples. BN-PAGE Western blot analysis of BAM complex formation in BamD depletion strain. Expression of non-tagged and His-tagged BamD did not affect BAM complex formation. BamD depleted cells were unable to form the full BAM (BamABCDE) complex but did form a smaller complex (BamAB). (**d**) Growth curve of BamD depletion strain expressing His-tagged and non-tagged BamD as followed by optical density at 600 nm. Cells were grown in non-permissive conditions (+ glucose) and were subcultured into fresh LB media containing glucose two times, after which BamD depletion strain was incapable of growing. (**e**) Assembly of ^{35}S-labelled OmpC in BamD depletion strain expressing plasmid-borne tagged and non-tagged BamD as followed by EMM assembly assay. OmpC was able to be assembled by both tagged and non-tagged BamD, while BamD depleted cells were unable to assemble OmpC into EMMs.

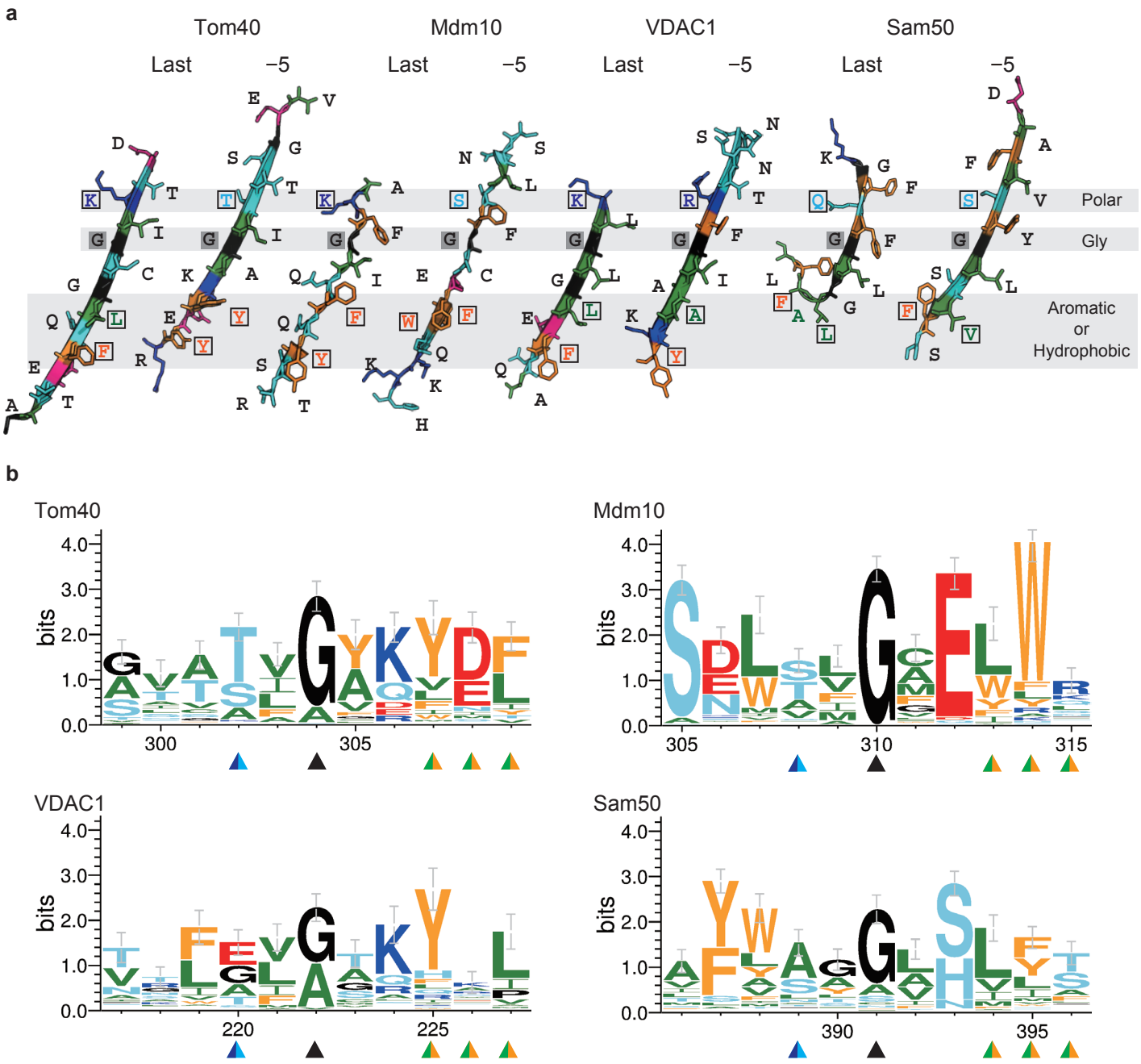


Extended Data Figure 8- Effect of BamD mutations on growth and BAM complex formation, relating to Figure 6.

(**a**) Growth curve of BamD-depletion strain expressing BamD and mutant strains from plasmids. Cells were grown in glucose containing media to repress endogenous BamD expression.WT: black line, Y62A: magenta, R197A: orange, and empty vector: purple. (**b**) BN PAGE Western blot analysis of BAM complex formation in BamD depletion expressing mutant BamD. BamD harboring either mutation was able to form full BAM complex.



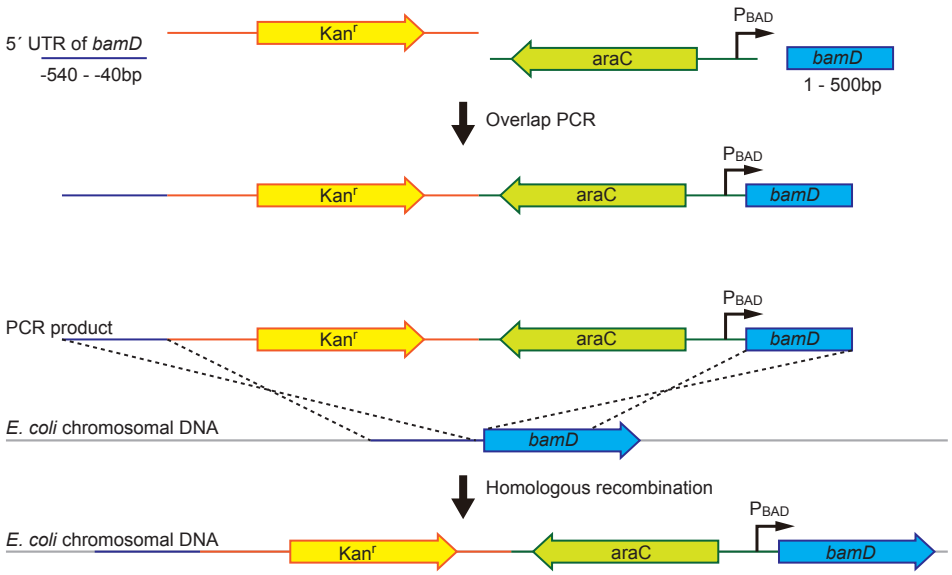
Extended Data Figure 9 (legend next page)



Extended Data Figure 10- Mitochondrial OMPs -5 strand also contains mitochondrial β signal like sequence.

(**a**) Analysis of the -5 strand and the last strand of yeast-Tom40 (6ucu), yeast-Mdm10 (7btx), human-VDAC1 (3EMN), and yeast-Sam50 (7btx). Residues comprising the mitochondrial β signal are highlighted by gray boxes. Aromatic, hydrophobic, positively charged, negatively charged, polar, and glycine are shown by orange, green, blue, red, sky blue, and black, respectively.

(**b**) Sequence logos of -5 strand were generated using WebLogo 3. The positions of residues comprising the mitochondrial β signal are highlighted by an arrowhead. Amino acid characters are shown by colour as in A.



Extended Data Figure 11- Schematic model of construction of bamD-depletion strain.

Indicated four DNA fragments were amplified, respectively, and then combined by the overlap PCR method. PCR fragment was introduced into MC4100A strain haboring pKD46 plasmid. The final DNA map described the bottom.