Supplemental data

The periplasmic membrane proximal domain of MacA acts as a switch in stimulation of ATP hydrolysis by MacB transporter

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Table S1. Peptide masses of whole-length MacA^{wt} and MacA^{G353A} and their major tryptic fragments

Peptide	Mass of MacA and its peptides (Da) based on:	
	MALDI-TOF	**AA sequence**
MacA^{WT} whole length	41,155	41,443
M1-K139	15,338	15,511
A140-Q371 + 6His	26,094	25,950
MacA^{G353A} whole length	41,500	41,457
M1-R334	36,666	36,804
M1-K326	36,022	35,862
M1-K324	35,883	35,634
A140-Q371 + 6His	26,096	25,950
A140-R334	20,455	20,141

Figure S1 Modali and Zgurskaya

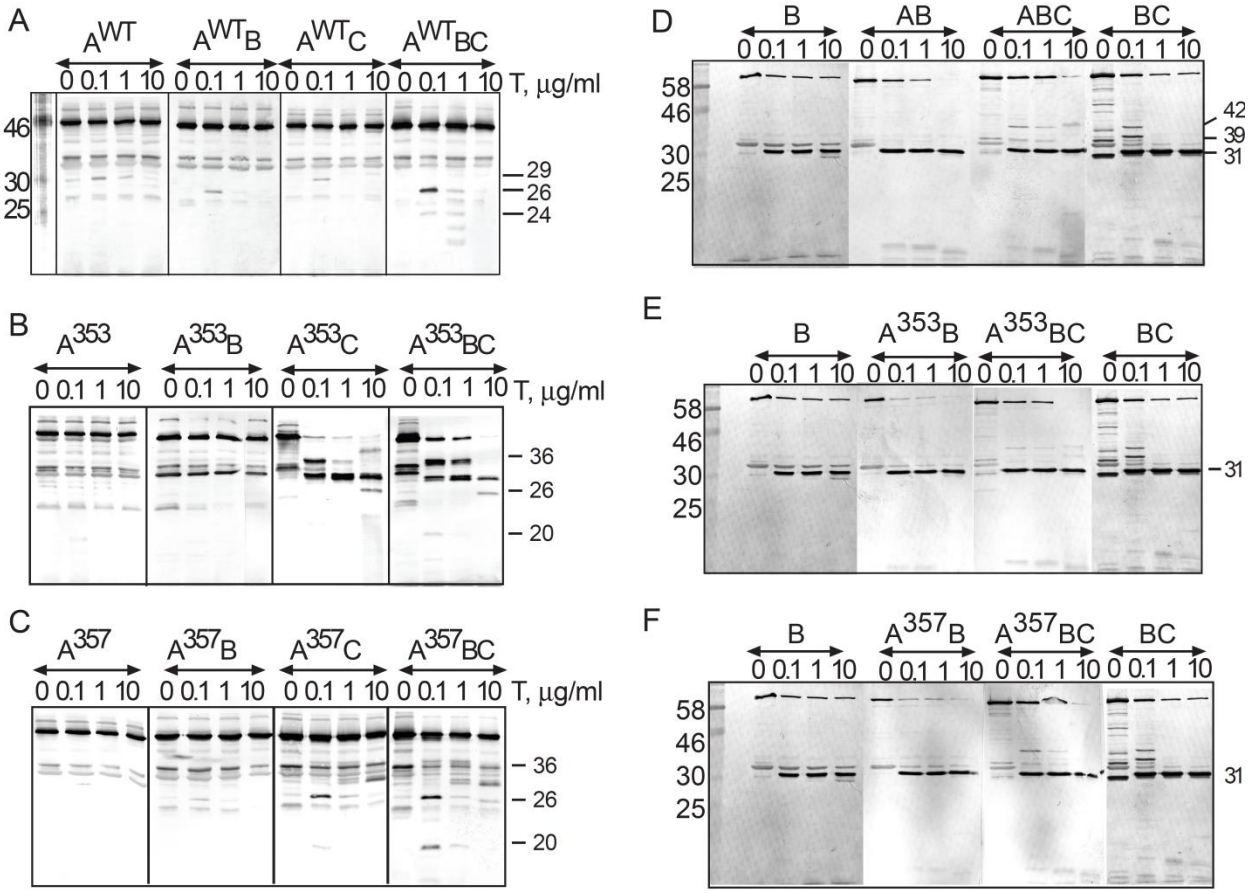

Figure S1. ***In vivo*** **tryptic digest of MacA and MacB produced in cells with different genetic backgrounds. A, B, C.** *E. coli* W4680AD (Δ*acrAB*, Δ*acrD*) and ECM2115 (MC4100 Δ*acrAB* Δ*tolC*) cells carrying plasmids producing MacA variants alone (A^{WT}, A^{353}, A^{357}) or in the presence of MacB ($A^{WT}B$, $A^{353}B$, $A^{357}B$) were treated with trypsin at indicated concentrations and analyzed by anti-MacA immunoblotting. **D.** *E. coli* W4680AD (Δ*acrAB*, Δ*acrD*) and ECM2115 (MC4100 Δ*acrAB* Δ*tolC*) cells carrying plasmids producing MacB alone or in the presence of $MacA^{wt}$ were treated with trypsin at indicated concentrations and analyzed by anti-MacB immunoblotting**. E, F.** The same as **D** but MacB was co-expressed with $MacA^{G353A}$ and $MacA^{G357A}$ variants ($A^{353}B$, $A^{357}B$). The composition of complexes is shown above the patterns with TolC indicated by “C” when present in cells.

Figure S2 Modali and Zgurskaya

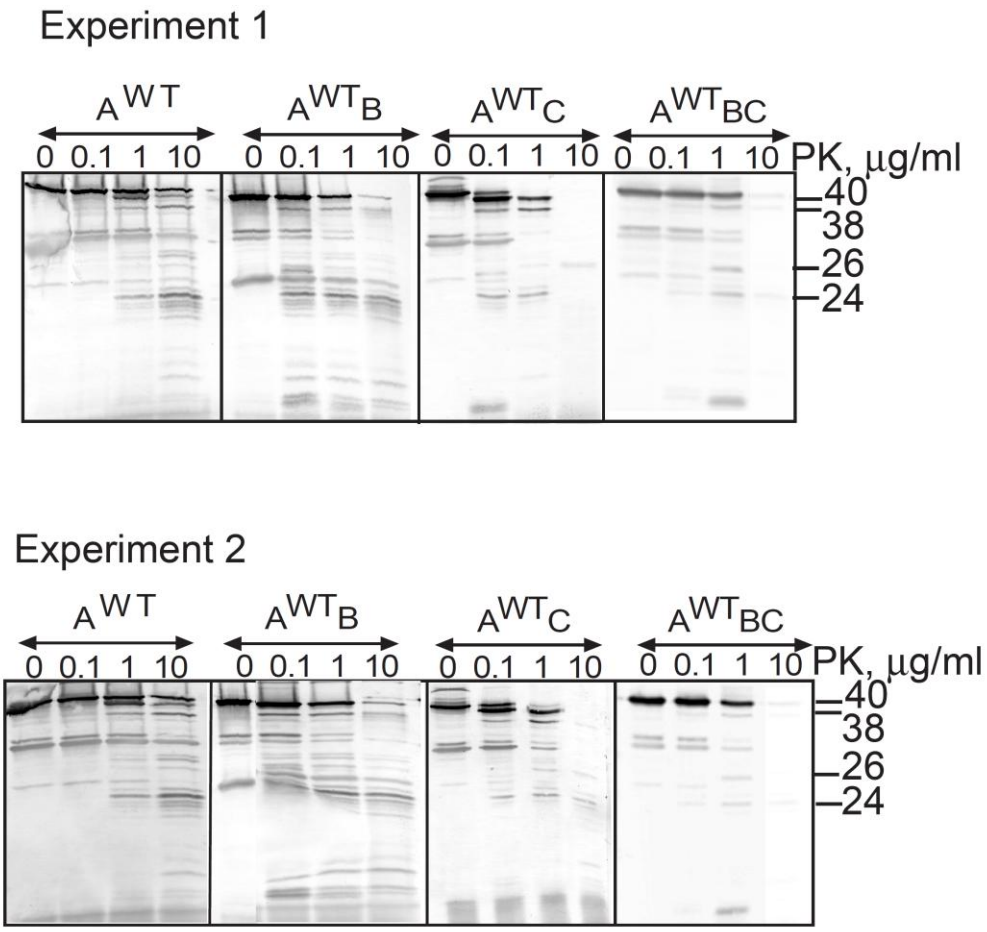

Figure S2. Differences in proteolytic patterns of MacA are highly reproducible. The *in vivo* proteolytic profiles of MacA^{WT} generated by treatment with PK are shown (see Fig. 3 for details). The composition of complexes is shown above the patterns with MacB and TolC indicated by “B” and “C” when present in cells. Two independent experiments are shown.