SUPPLEMENTAL INFORMATION

Membrane Localization of Small Proteins in *Escherichia coli**

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TABLE S1. **Plasmids used in study.**

TABLE S2. **Strains used in study.**

TABLE S3. **Oligonucleotides used in study.**

TABLE S4. **Data for subcellular fractionation using a sucrose cushion.**

TABLE S5. **Data for subcellular fractionation using sarcosyl.**

TABLE S6. **Data for effect of depletions.**

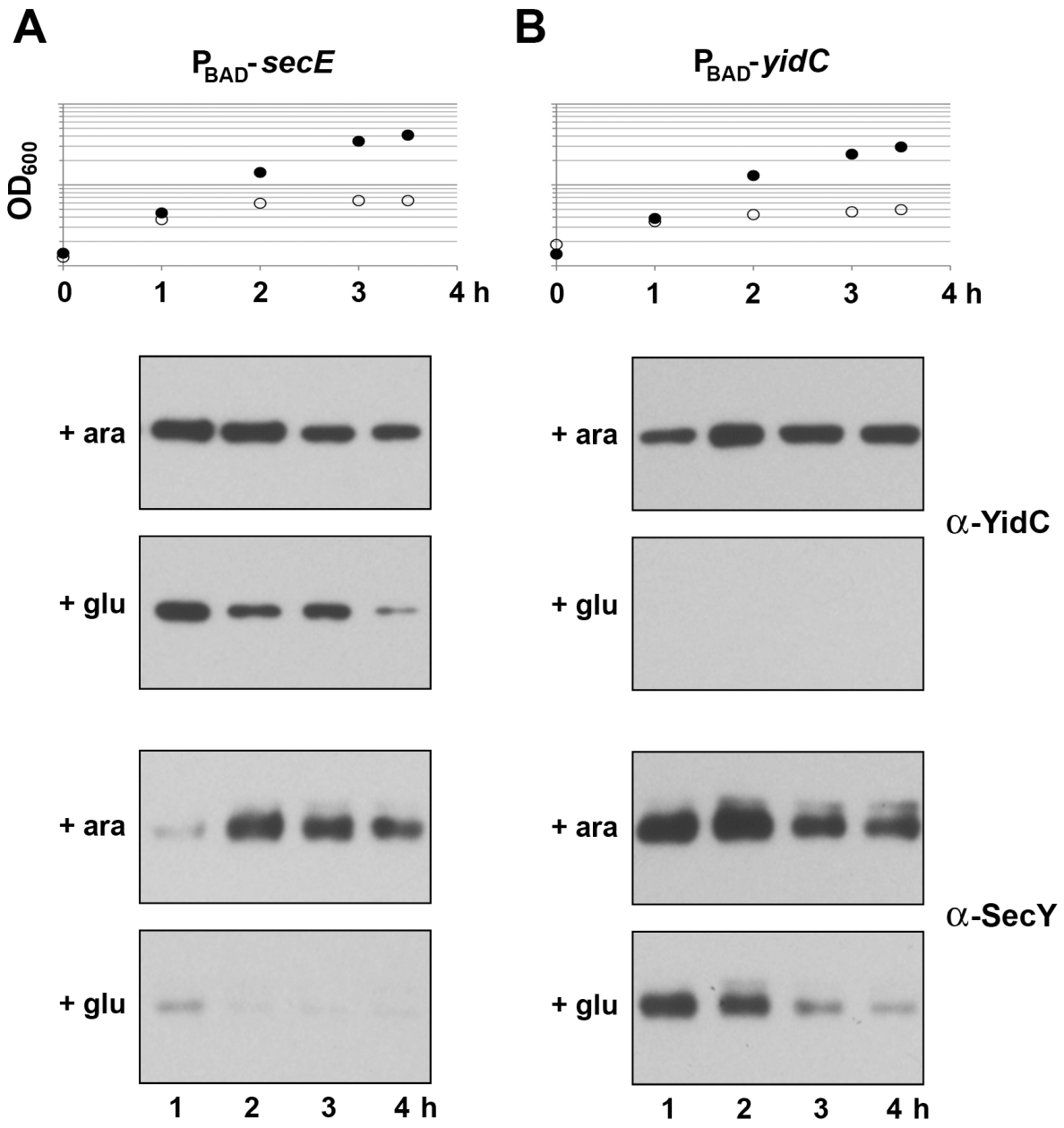

FIGURE S1. **Growth delay upon SecE and YidC depletion.** Growth curve of cells containing P_{BAD}-*secE* (*A*) or P_{BAD}-*yidC* (*B*) chromosomal fusions. Log phase cells growing in LB medium containing 0.2% arabinose were collected and washed. The cells were then resuspended and split into fresh LB containing 0.2% arabinose (filled circles) or 0.2% glucose (open circles). Growth was monitored by measuring the OD_{600nm} over time. At each time point, 1ml of cells were harvested and subject to immunoblot analysis using antibodies against YidC or SecY. After 3 h of SecE depletion, more than 95% of SecY is depleted, while YidC levels are only reduced by 15%. Similarly, after 3 h of YidC depletion, 99% of YidC is depleted, while SecY levels were reduced by 60%.

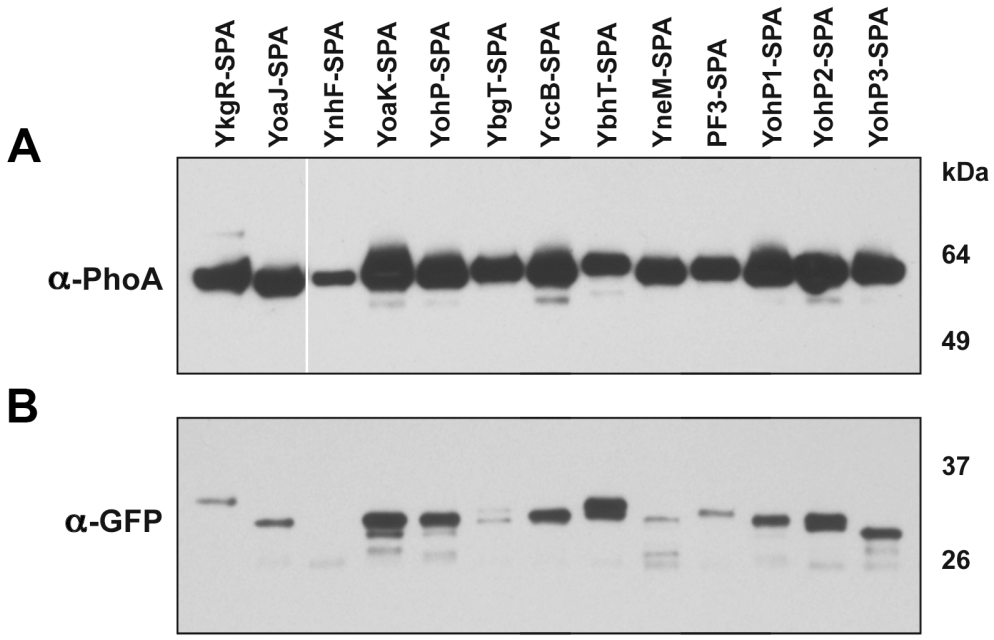

FIGURE S2. **Levels GFP and PhoA fusion proteins.** Immunoblot using antibodies against PhoA (*A*) or GFP (*B*).

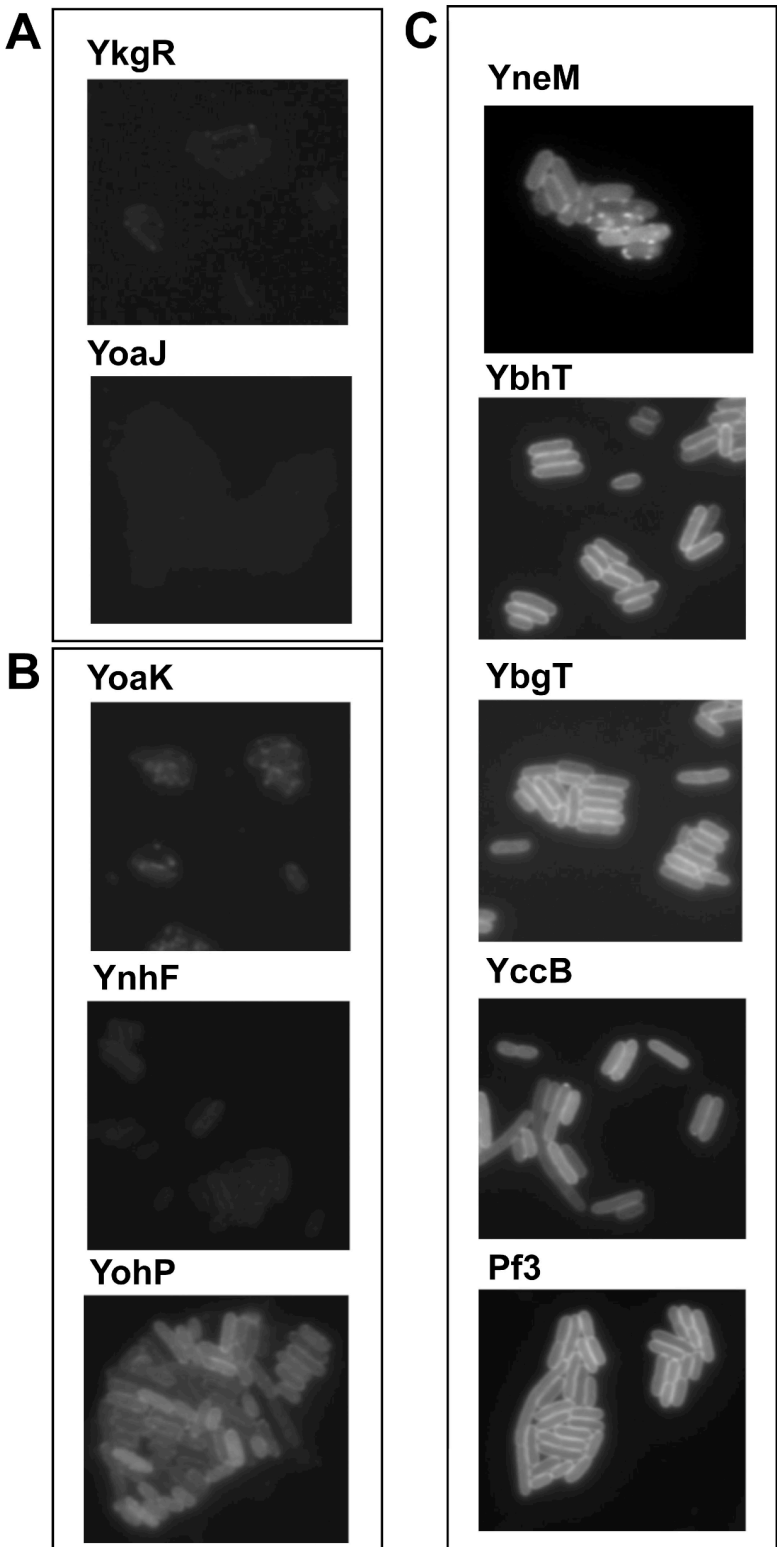

FIGURE S3. **Microscopy of small protein-GFP fusions.** Images were taken for small proteins with an orientation C_{out}-N_{in} (*A*), $C_{in/out}$-$N_{in/out}$ (*B*) and C_{in}-N_{out} (*C*). Overnight cultures were diluted 1:50 in LB containing 25 µg/ml chloramphenicol and 50 µg/ml kanamycin and incubated at 37˚C for 90 min. To induce T7 RNA polymerase, cells were grown for another 4 h in the presence of 0.4 mM IPTG. Cells were spread on a thin layer of agarose between a slide and cover slip and examined under the microscope. The images were taken at the 100x magnification.

A

YkgR

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ECOLI          MKENKVQQISHKLINIVVFVAIVEYAYLFLHFY
ESPE4          MKENKVQQISHKLINIVVFVVIVEYAYLFLHFY
SHIBO          MKENKVQQISHKLINIVVFVAIVEYAYLFLHFY
EFERG          MKENKIQQISHKLINIVVFVAIVEYAYLFLHFY
EALBE          MKENKIQKISNKLINIVVFVAVVEYAYLFLHFY
               *****:*:**:*********.:***********
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YoaJ

```
ECOLI           MKKTTIIMMGVAIIVVLGTELGWW
KLEPN           MKKSTIIMLVVAIVAVAGTQFGWW
ENT38           VRKTTIIMLIIAVVAVAGSQLGWW
SALTY           MRKTTMVLMGIALIAVAGTELGWW
                ::*:*:::: :*::.* *:::***
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B

YnhF

```
ECOLI          MSTDLKFSLVTTIIVLGLIVAVGLTAALH-
SALTY          MSTDLKFSLITTLIVLGVIVAGGLTAALH-
ENT38          MNTDLKFSLTTTIIVLGLIVAASFTAILH-
KLEPN          MDTNLKFSLITTIIALGVIVAFSLTAILH-
SERPR          MDTDLKMSLFTTVCALAVIIAFSFTAALN-
PHOPR          MEADLKFALITTGVVFAILIGFGLTAIGA-
VIBHB          MEHDLKSALLIVVTIFAVLLSFGIIAITTA
               *. :** :*  .   :.:::. .: *
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YoaK

```
ECOLI           MRIGIIFPVVIFITAVVFLAWFFIGGYAAPGA-
SERPR           MKIGYLFPVSIIITAVVLLAWFIIGGYAMPA--
ENT38           MRIGIVFPVVIFITAVVFLTWFFVGGYAAPGA-
SALTY           MRVGILFPVVIFITAILFLAWFFIGGYAAPGA-
CITKO           MKLGIIFPVVIFITAVTFLAWFFIGGYATPGA-
KLEPN           MKLGILFPVAIFIIAVVFLGWFFVGGYAAPGGA
LACPL           MRLGIPLTAVVFVVALVEMFWFHLVGLA-----
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YohP

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YEREN           -MKIILWIIAIIFIVGLLTITGVFKLIF
SODGL           -MKIFLWIIAIIFIVGLLTLTGVFKLIF
SALTY           -MKILLWAILIIFLIGLLVVTGVFKMIF
ENT38           -MKILLWVVLIIFLIGLLVVTGVFKMIF
ECOLI           -MKIILWAVLIIFLIGLLVVTGVFKMIF
PSE14           MLKFLGSTVGIIFLIGLVVVIALFKLVF
RHOS1           MLKFIGGTVGIIFLVGLLVIIGILALIF
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C

YbgT

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ECOLI          MWYFAWILGTLLACSFGVITALALEHVESGKAGQEDI
CITSP          MWYFAWILGTLLACAFGIITALALEHVESGKAGQED-
KLESP          MWYFAWILGTLLACAFGVITALALEHVEATKAGKEE-
KLEPN          MWYFAWILGTLLACAFGVITALALEHVEATKAGKKE-
ENTSP          MWYFAWILGTLLACAFGVITALALEHVEASKAGEEN-
SENTE          MWYFAWILGTLLACAFGIITALALEHVEAGKTGQEE-
SERPR          MWYFAWILGTLLACAFGIITALALEHHEDSKAQQDD-
ERWCA          MWYFAWILGTLLACSLGIITALAIEQSEASKAAEED-
PHOLU          MWYFAWILGTLLACSFAIIAALALEHNEEEKVAKNS-
               **************::.:*:***:*: *  *. :..
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YccB

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ECOLI          MWYLLWFVGILLMCSLSTLVLVWLDPRLKS
SHDYS          MWYLLWFIGILLMCSLSTLVLVWLDPRLKS
EDWTA          MWYLLWFVGILLMCSLSTLILVWLEPRLK-
EALBE          MWYLLWFVGILLMCSLSTVVLVWLEPRLKG
CITSP          MWYLFWFVGILLMCALSTLALVWLESRQK-
SENTE          MWYLLWFVGILLMCSLSTLVLVWLESRQQ-
EFERG          MWYQLWFVGILLMCAITSLVLVWLEPRFES
               *** :**:******:::::: ****:.* :
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YbhT

```
ECOLI          MLELLKSLVFAVIMVPVVMAIILGLIYGLGEVFNIFSGVGKKDQPGQNH-
EFERG          MLELLKSLVFAVIMVPIVMAIILGLIYGLGEVFNIFSGIGQKDQSRQNH-
SENTE          MLELLKSLVFAVIMVPVVMAIILGLIYGLGEVFNIFSGIGQKDQSRQNR-
ENTSP          MLELLKSLVFAVIMVPVVMAIILGAIYGLGEVFNVFSNIGHKDHPKKQH-
CITFR          MLELLKSLVFAVIMVPVVMAIILGLIYGLGEVFNIFSGIGQKDQSRQNH-
KLEPN          MLELLKSLVFAVIMVPVVMAVILGLIYGLGEVFNLFSGVGHKDRSQQNH-
ERWPY          MLELLKGLAVAVLMVPVVMAIILGLIWGLGEVFNVISKLGHRDGSSAN--
ERWTA          MLELLKGLAIAILMVPVVMAIILGLIWGLGEVFNVISKFGHRDDSAEN--
ERWCA          MLELLKSLLFAVAMVPVMMVIIMGAIYCLGEVFNVLSRIGHSDGQRAKNQ
CROSA          MFELLKSLAFAVIMVPVVMAVILGLIWGLGEVFNIFSGIGHKDQSKQH--
SEROD          MLELLKSLLFAVVMVPVVMAIILGLIYGLAEVFNIFSKVGRSKENRTQH-
YERPE          MFELLKSLVFAVVMVPVVMAVILGLIYGLGEVFNVISKTGHPKE------
DICZA          MFELLKSLVFAVCMVPVMMALILGAIYGLGEVFNLFSAFGHRESSAAR--
               *:****.* .*: ***::*.:*:* *: *.******:*  *: .
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YneM

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ECOLI          MLGNMNVFMAVLGIILFSGFLAAYFSHKWDD
CITKO          MLGNMNVFMAVLGIILFSGFLAAYFSHKWDD
EALBE          MLGNMNIFMAVLGIILFSGFLAAYFSHKWDN
CITFR          MLGNMNIFMAVLGIILFSGFLAAYFSHRWDD
SALTY          MLGSINLFIVVLGIILFSGFLAAWFSHKWDD
PELCA          MLGNININFIAVLGGILFLSFLAAYLSPKWDD
CROTU          MPGAADLFICMPGIVLLAGFLAAYCSNKWDD
DICDA          MFEQVTVYLAILAVIVVPGFLAAFLSPKWDD
SEROR          -MGNMSVFIGFLGIISTLGLLAAYLSTKWDD
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FIGURE S4. **Conservation of charged residues in small protein sequences.** The sequences of small proteins with an orientation (*A*) N_{in}-C_{out}, (*B*) dual orientation and (*C*) C_{in}-N_{out}. The sequences were identified by tBLASTn then aligned with CLUSTALW (http://align.genome.jp/). '*' indicates that the residues are identical in all sequences and ':' and '.', respectively, indicate conserved and semi-conserved substitutions as defined by ClustalW. The organism codes are defined in UniProtKB/Swiss-Prot (http://www.uniprot.org/docs/speclist). The positive residues (K and R) are in red and the negative (D and E) in blue. The gray box corresponds to the TM domain predicted by TMpred.

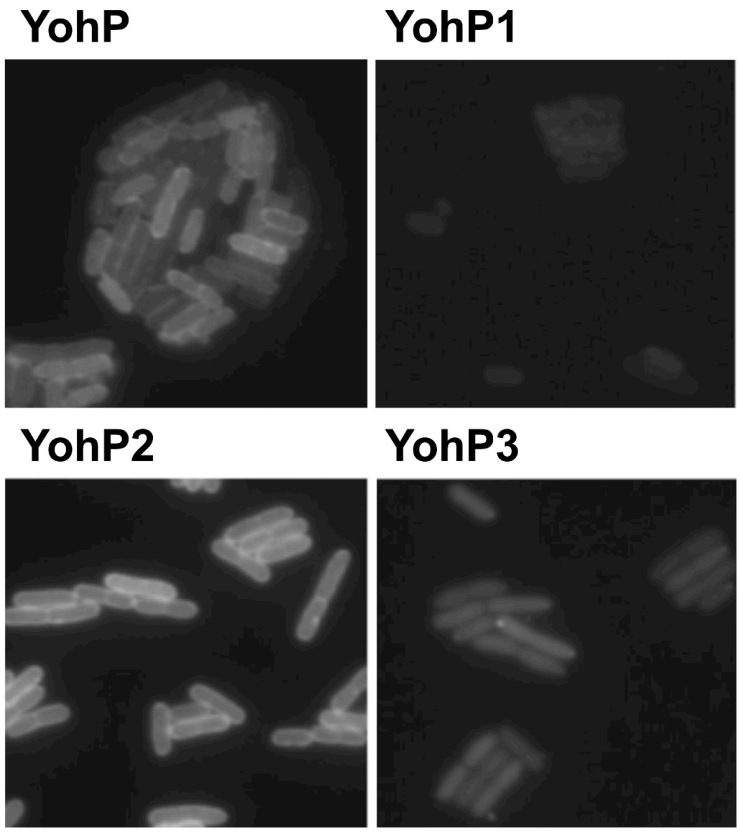

FIGURE S5. **Microscopy of mutant YohP-GFP fusions.** Cells were grown and examined as for supplemental Fig. S3.