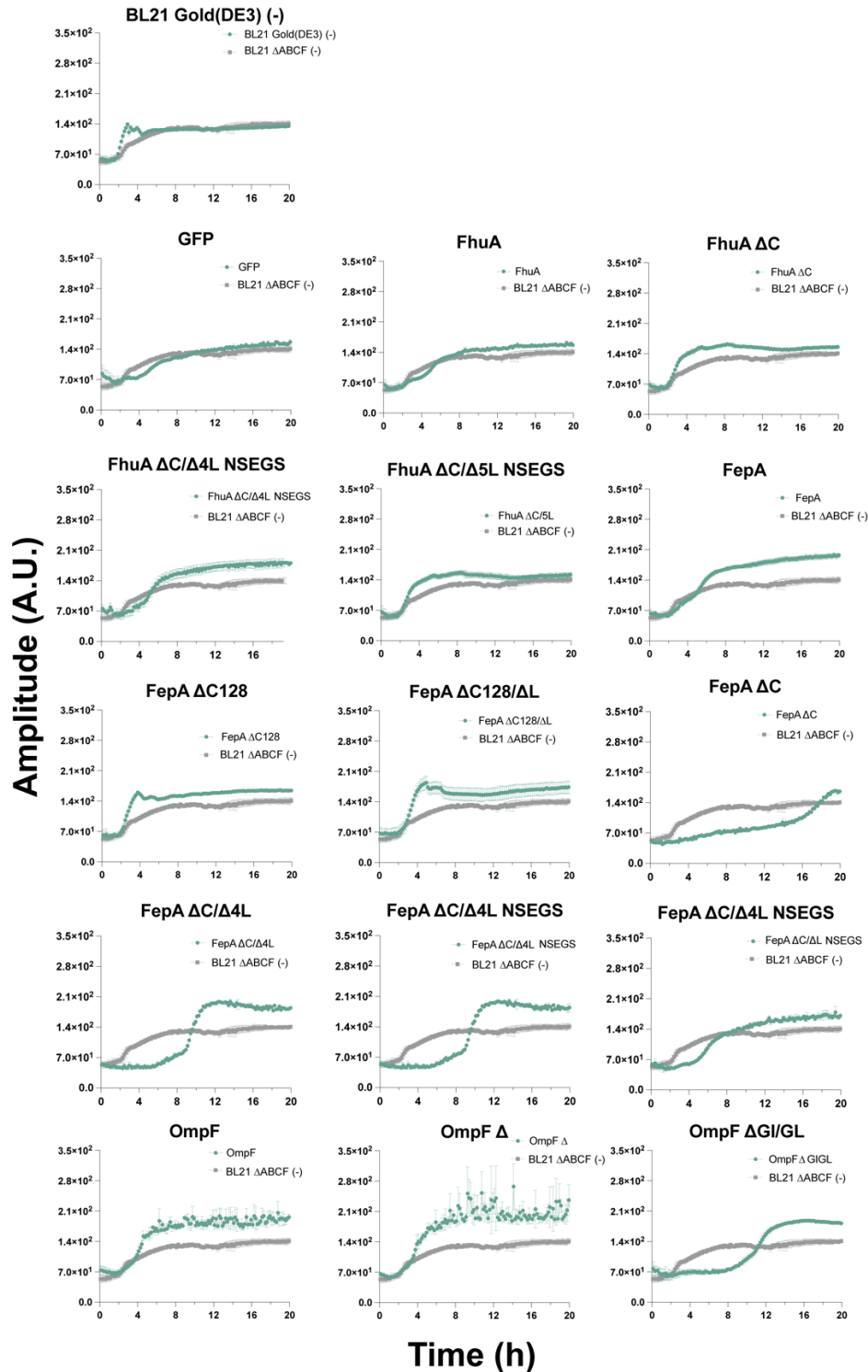
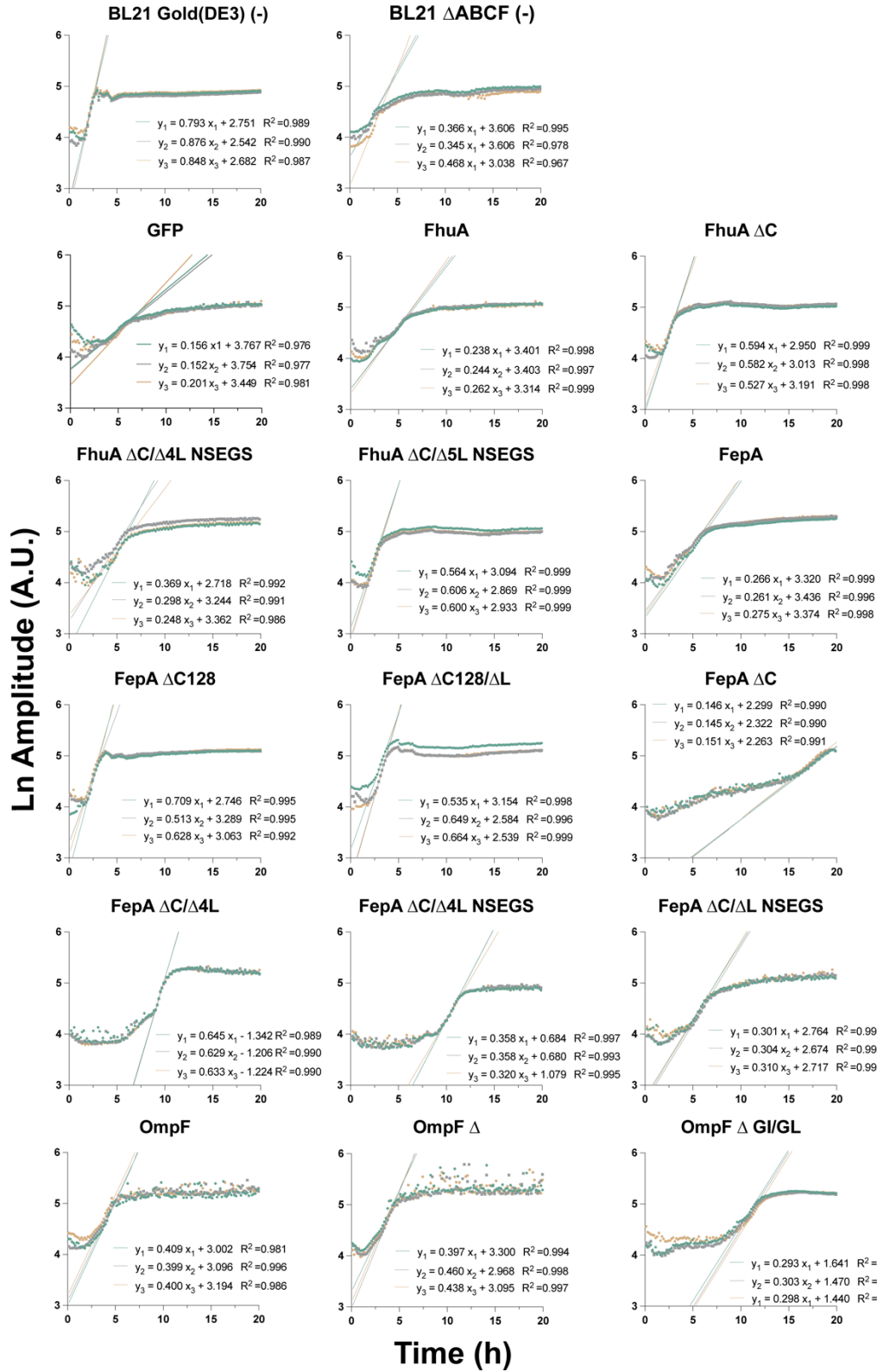


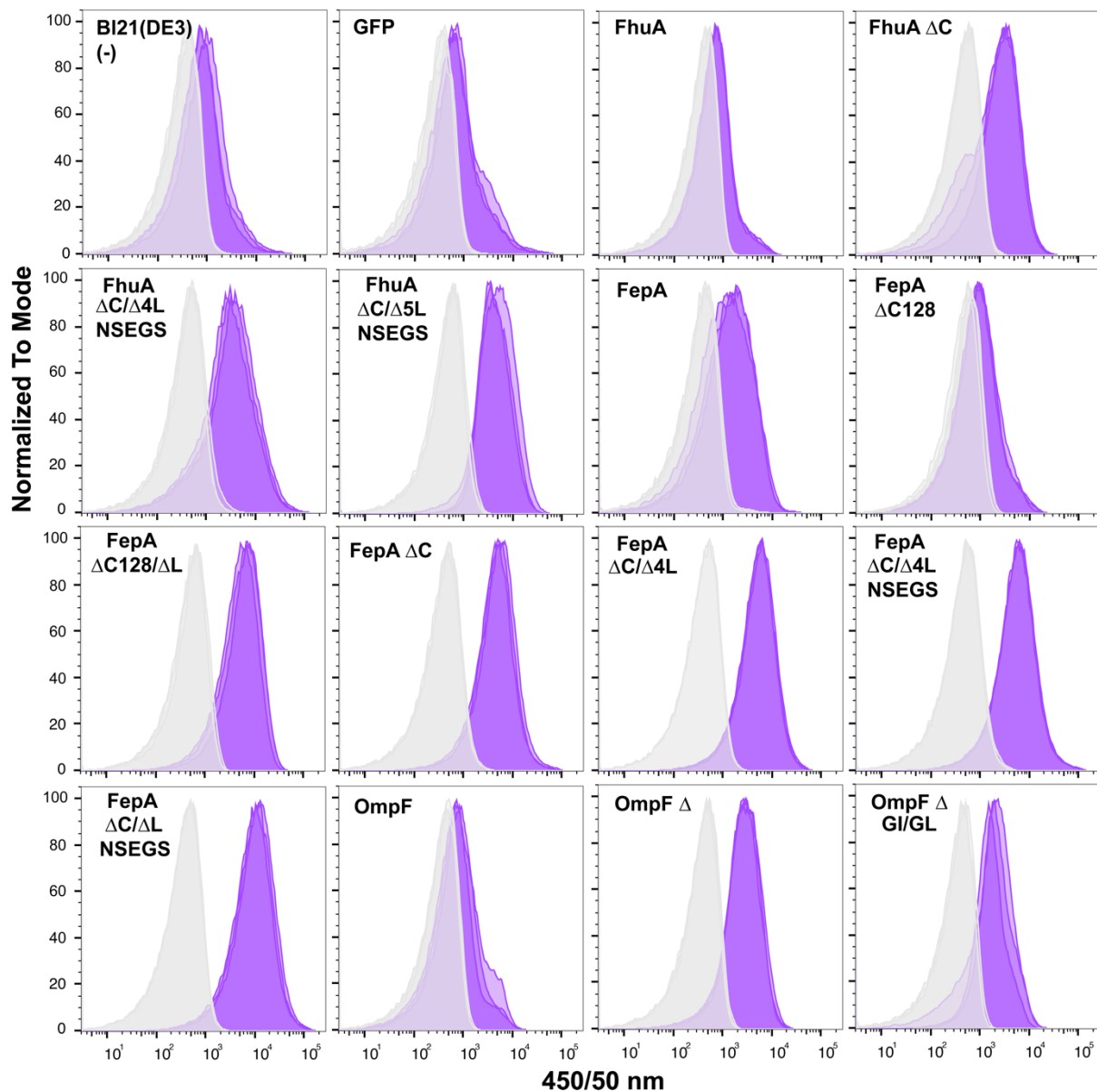
Supplementary Material



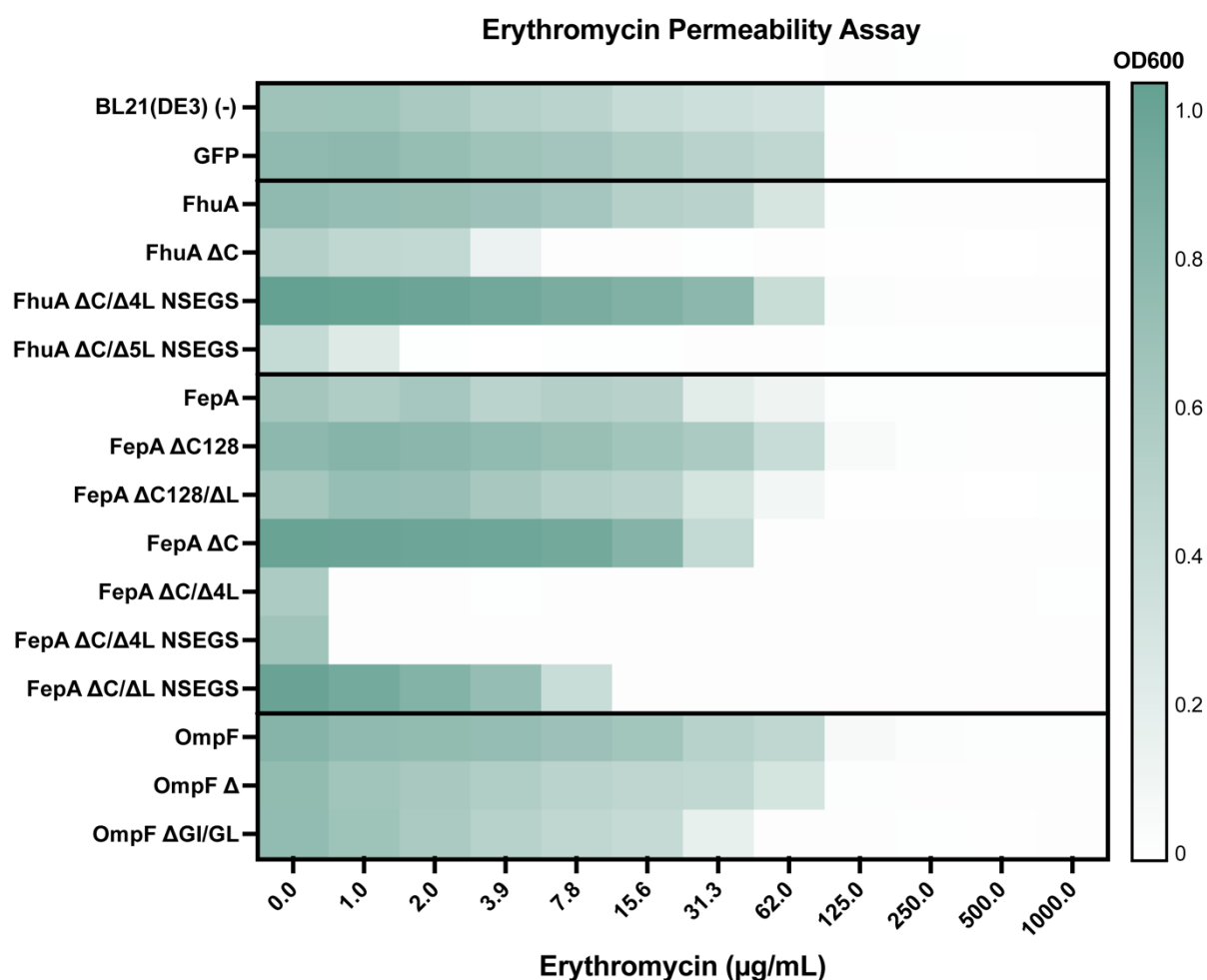
Supplementary Figure 1. Growth behavior of BL21ΔABCF overproducing the indicated proteins. Independent triplicates of BL21ΔABCF strains producing none (grey) or the indicated protein (green) were seeded in a deep-well plate and incubated at 37°C in LB. Growth was monitored as a function of backscatter and the amplitude is plotted. Growth of BL21ΔABCF in grey and the parental strain, BL21 Gold (DE3) carrying a plasmid with no OMP (-), are shown for comparison for the negative controls. The GFP expressing strain serves as an indicator of the effect of metabolic burden on the growth of BL21ΔABCF.



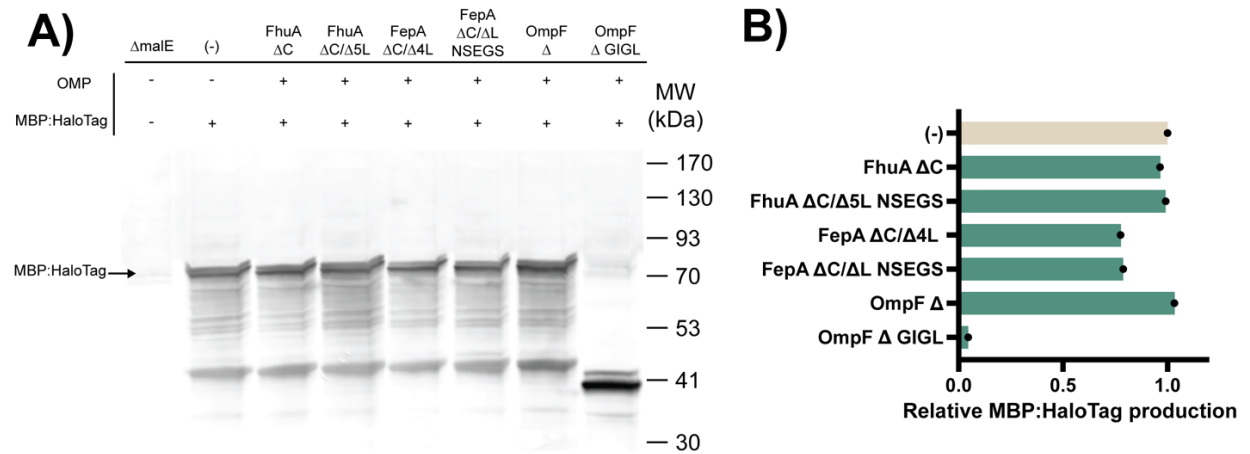
Supplementary Figure 2. Calculation of maximum specific growth rates of the BL21 Δ ABCF strains overproducing the indicated proteins. Independent triplicates of BL21 Δ ABCF strains producing none (-) or the indicated protein were seeded in a deep-well plate and incubated at 37°C in LB. Growth was monitored as a function of backscatter and the amplitude is plotted. The natural logarithmic values of the growth are plotted and were used to determine the maximum specific growth rate for each replicate. BL21 Gold (DE3) strain used for comparison for the negative controls. The GFP expressing strain serves as an indicator of the effect of metabolic burden on the growth of BL21 Δ ABCF.



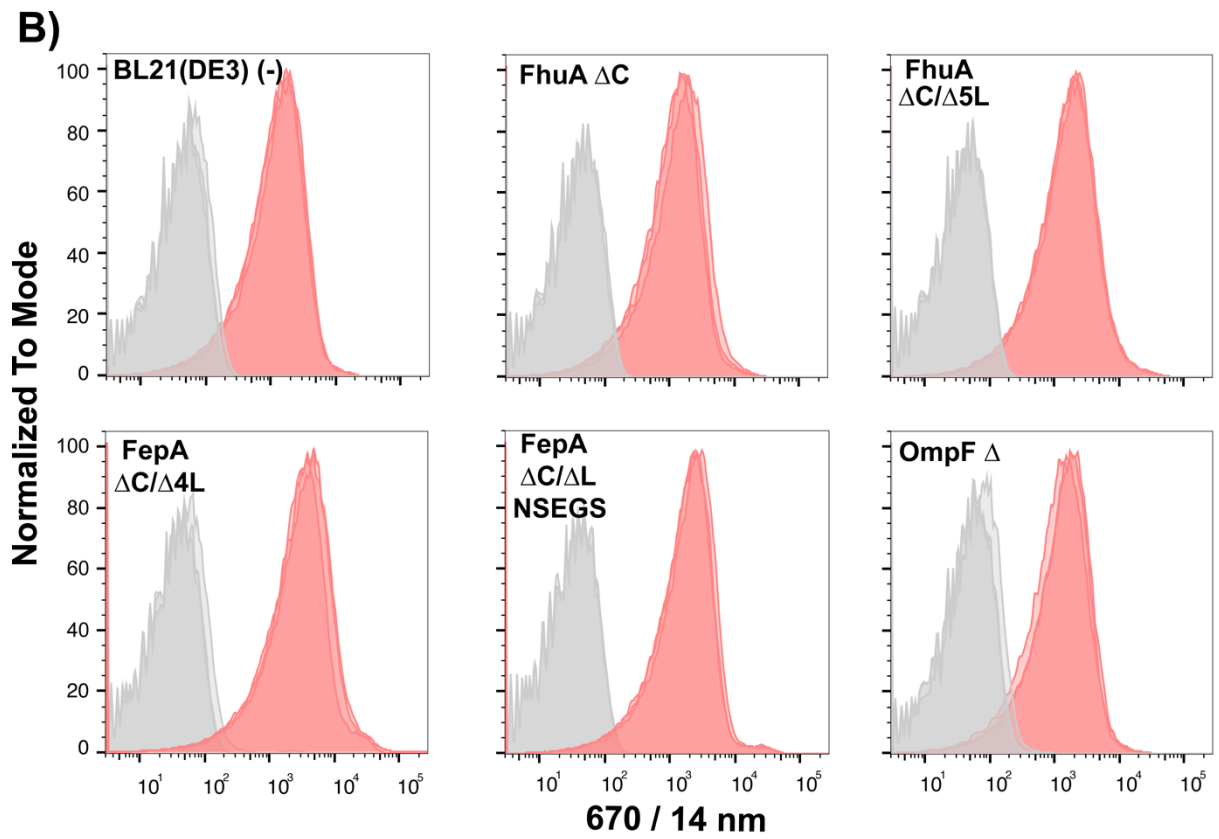
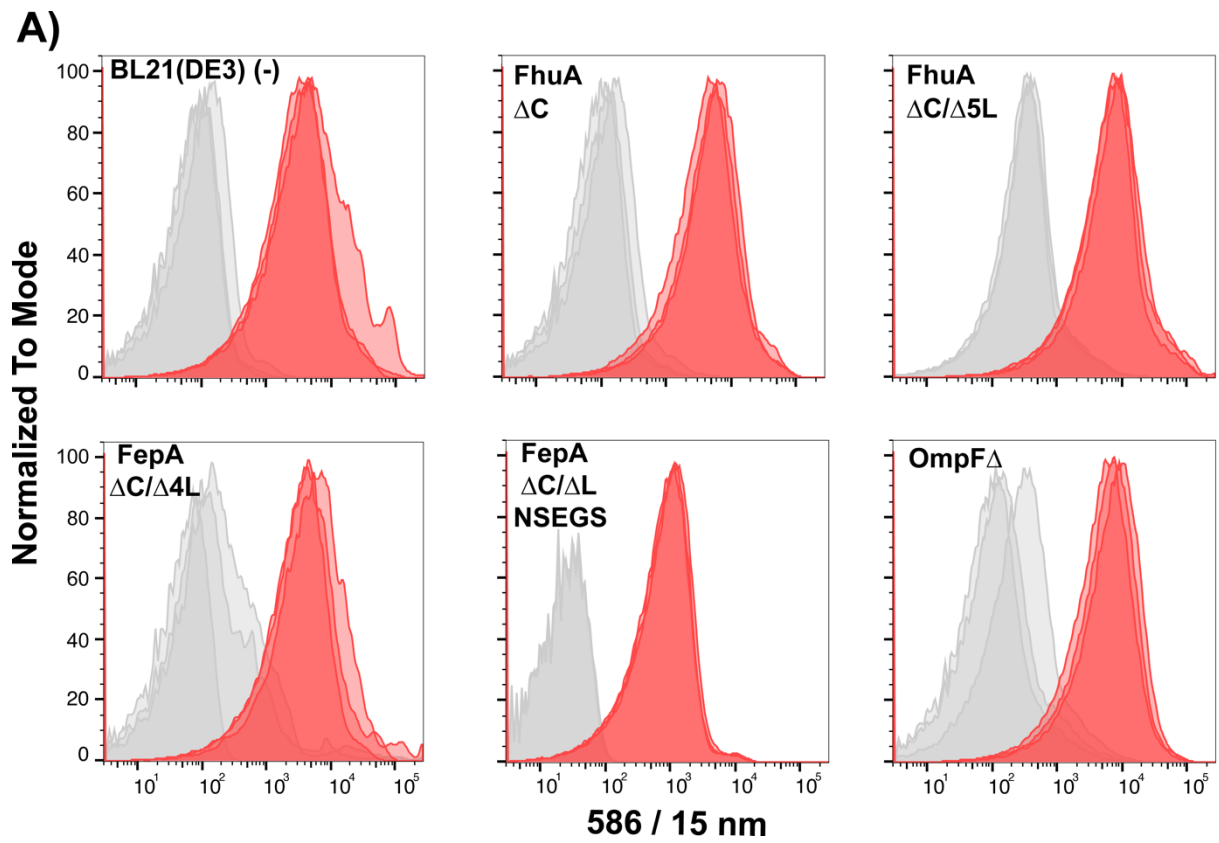
Supplementary Figure 3. Flow cytometry analysis of the effect of OMP overproduction on NPN import. BL21 (DE3) strains overproducing no protein (-) or the indicated protein were grown and incubated with (purple) or without (grey) 10 μ M NPN, washed, resuspended in PBS and fluorescence (ex 355 nm, em 450/50 nm) was analyzed via flow cytometry. This experiment was performed with independent biological triplicates (n=3) with at least 80000 single events. The cell count of the populations displayed in the histograms was normalized to their corresponding mode for visualization purposes.



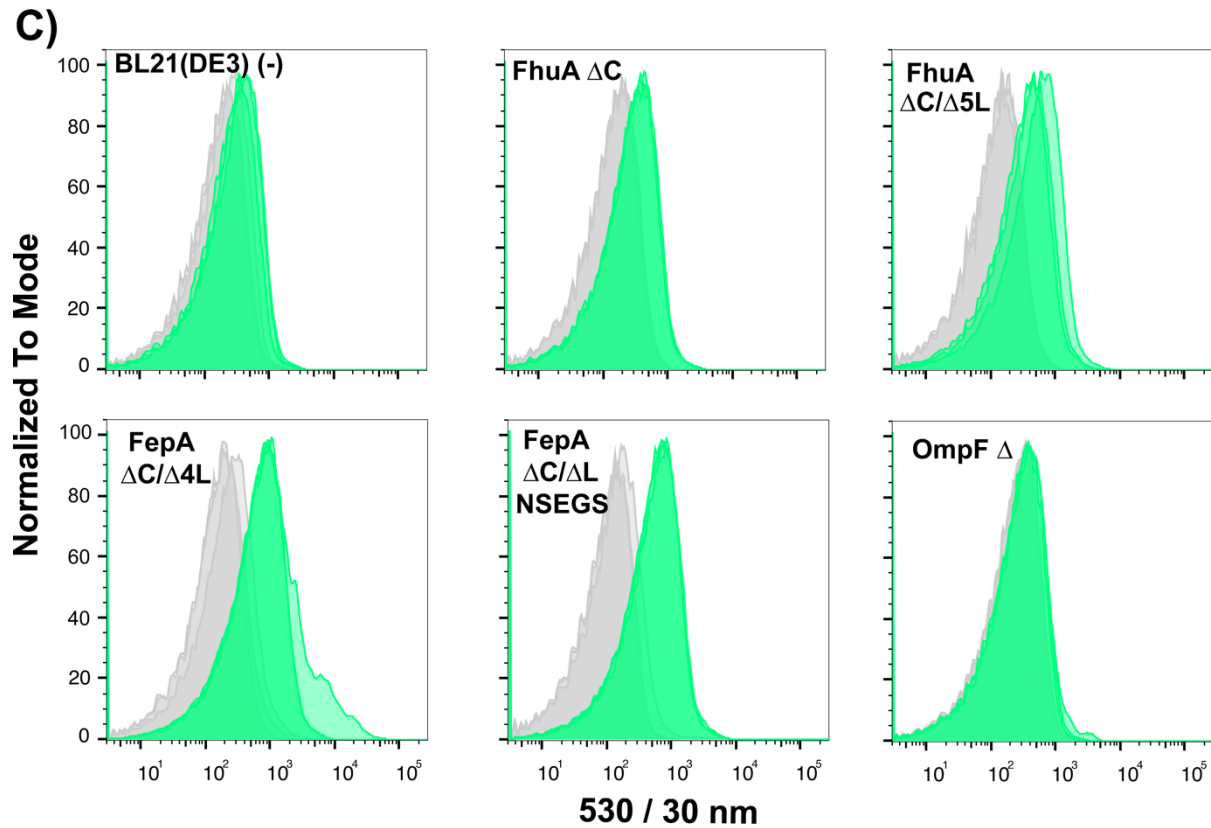
Supplementary Figure 4. Erythromycin permeability assay. BL21 (DE3) strains overproducing no protein (-) or the indicated protein were grown in LB supplemented with 50 mg·L⁻¹ of kanamycin, 50 μM IPTG and the indicated concentration of erythromycin. This experiment was performed with independent biological triplicates (n=3). Heat map graph displays the OD₆₀₀ values observed for the OMP overproducing strains and the indicated erythromycin concentrations after 18 h of incubation at 37°C. The data shown for FhuA ΔC/Δ4L NSEGS correspond to cell cultures that possessed a disrupted version of the OMP gene when analyzing after the experiment.



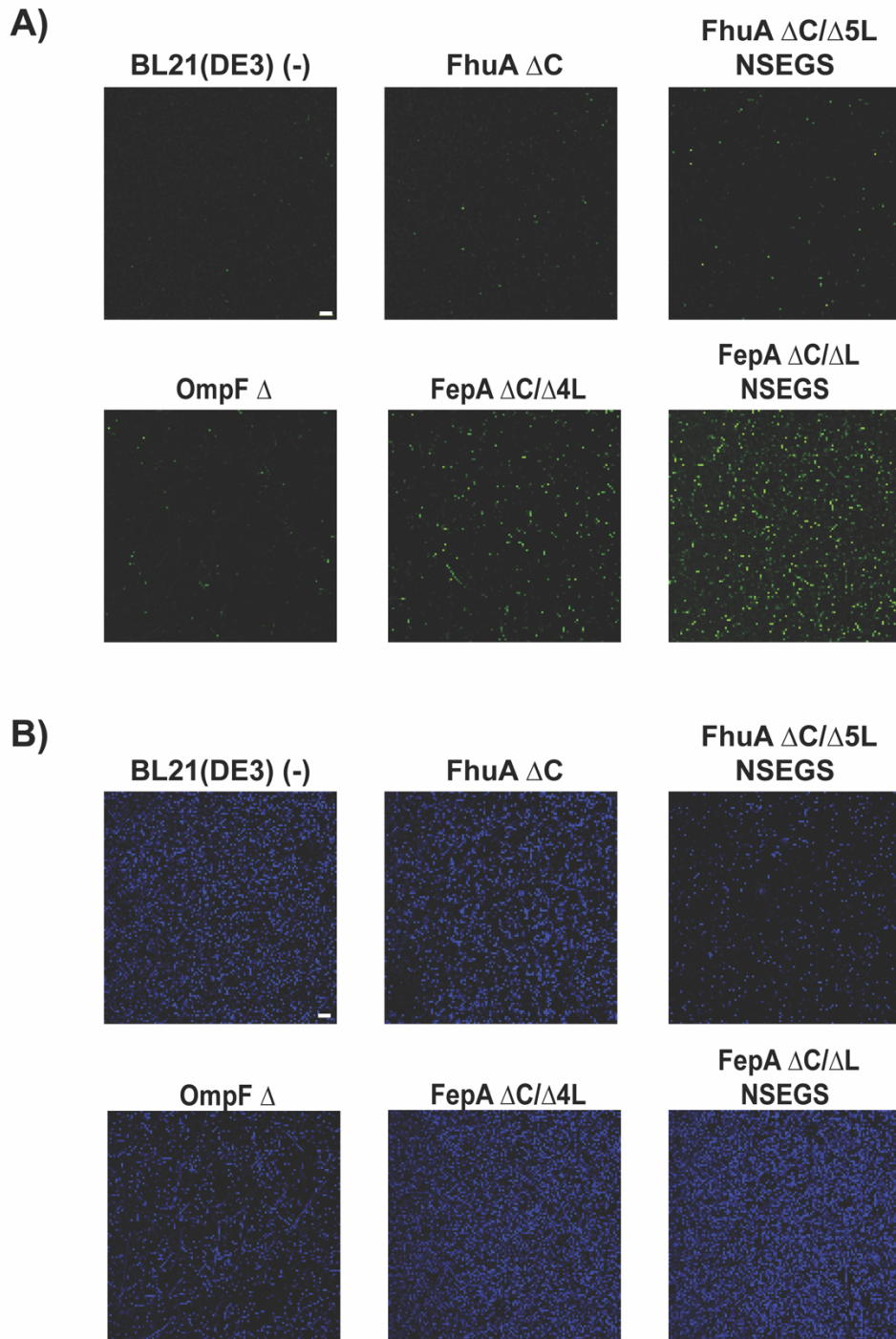
Supplementary Figure 5. MBP:HaloTag production in BL21(DE3) $\Delta malE$ overproducing different OMPs. A) Western Blot for MBP:HaloTag quantification. Cell lysates were electrophoresed by A 4-20% SDS-PAGE electrophoresis was carried out with the cell lysates of the indicated strains and transferred on to nitrocellulose membrane before treating them with a primary anti-MBP antibody and secondary fluorescent antibody for detection. The expected sizes for MBP, HaloTag and the MBP:HaloTag fusion protein are 43.4, 33.5 and 78.7 kDa respectively. B) Quantification of cellular MBP:HaloTag normalized to the MBP:HaloTag present in the BL21(DE3) $\Delta malE$ strain overproducing MBP:HaloTag but no OMP.



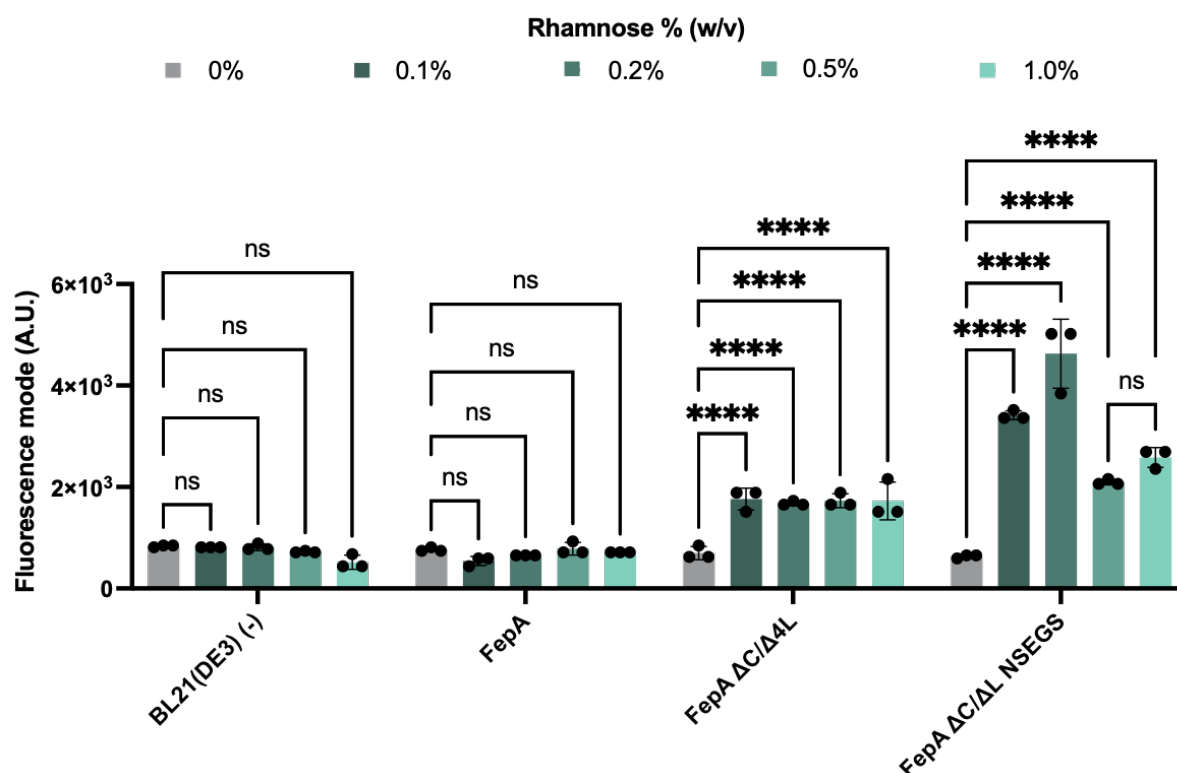
Supplementary Figure 6. Caption in next page.



Supplementary Figure 6. Flow cytometry analysis of the effect of OMP overproduction on HaloTag dyes import. BL21 (DE3) strains overproducing the MBP:HaloTag fusion protein, and no protein (-) or the indicated protein were grown and incubated with (color) or without (grey) 1 μ M of HaloTag TMR (A), HaloTag JF646(B) or HaloTag AF488 (C), washed, resuspended in PBS and fluorescence was analyzed via flow cytometry (ex 561nm, em 686/15nm; ex 640nm, em 670/14nm; ex 488nm, em 530/30nm). This experiment was performed with independent biological triplicates (n=3) with at least 80000 single events. The cell count of the populations displayed in the histograms was normalized to their corresponding mode for visualization purposes.



Supplementary Figure 7. Channel-split microscopy images of the effect of OMP production on HaloTag protein labeling with HaloTag-AF488. BL21 (DE3) strains overproducing the MBP:HaloTag fusion protein, and no protein (-) or the indicated protein were grown incubated with (color) or without (grey) 1 μ M of HaloTag TMR (A), HaloTag JF646(B) or HaloTag AF488 (C), washed, resuspended in PBS and fluorescence was analyzed via microscopy. A) Images obtained with confocal microscopy of BL21(DE3) cells overproducing periplasmic MBP:Halo and the indicated OMP stained with DAPI and HaloTag-AF488 for the AF-488 channel. Scale bar, 10 μ m. B) Images obtained with confocal microscopy of BL21(DE3) cells overproducing periplasmic MBP:Halo and the indicated OMP stained with DAPI and HaloTag-AF488 for the DAPI channel. Scale bar, 10 μ m.



Supplementary Figure 8. Flow cytometry analysis of the effect of different expression induction levels on NPN import. Fluorescence mode of OMP overproducing strains and controls after induction with different concentrations of L-rhamnose. BL21 (DE3) strains overproducing no protein (-) or the indicated OMP, which gene expression was regulated by the rhamnose-inducible promoter P_{RhaBAD} , were grown and incubated with 0, 0.1, 0.2, 0.5 or 1.0% (w/v) L-Rhamnose. After wash and resuspension in PBS, cells were incubated with (purple) or without (grey) 10 μ M NPN, washed and resuspended in PBS again. Cell fluorescence (ex 355 nm, em 450/50 nm) was analyzed via flow cytometry. This experiment was performed with independent biological triplicates (n=3) with at least 80000 single events. The values displayed here correspond to the statistical mode of the indicated population.

Supplementary Table 1. Complete set of OMPs and their different engineered versions assessed in this study.

OMP	Version	Structure mutated	Reference
OmpF	Wild type		Rosenbusch et al. 1992
	Δ	ΔG109_L114	Saint et al. 1996
	ΔGIGL	ΔG109_L114 + Residues for trimer formation	This study
FhuA	Wild type		Coulton et al. 1986
	ΔC	ΔA1_P160	Mohammad et al. 2011
	ΔC/Δ4L NSEGS	ΔA1_P160 ΔY243-N273::NSEGS ΔY315-A342::NSEGS ΔD394_N419::NSEGS ΔN682_R694::NSEGS	Mohammad et al. 2011
	ΔC/Δ5L NSEGS	ΔA1_P160 ΔY243-N273::NSEGS ΔY315-A342::NSEGS ΔD394_N419::NSEGS ΔG640_S652::NSEGS ΔN682_R694::NSEGS	Wolfe et al. 2016
	Wild type		Lundrigan and Kadner 1986
	ΔC	ΔQ1_S150	This study
	ΔC128	ΔQ1_P128	This study
	ΔC128/ΔL	ΔQ1_P128 ΔK167_E169 ΔD201_G224 ΔG262_D279 ΔE319_Q335 ΔS378_P403 ΔS470_L502 ΔV541_G549 ΔT595_I602 ΔQ631_I651 ΔL683_P715	This study
FepA	ΔC/Δ4L	ΔQ1_S150 ΔD201_G224 ΔS378_P403 ΔS470_L502 ΔL683_P715	This study
	ΔC/Δ4L NSEGS	ΔQ1_S150 ΔQ199_E226::NSEGS ΔD376_S405::NSEGS ΔA468_A504::NSEGS ΔK681_T715::NSEGS	This study
	ΔC/ΔL NSEGS	ΔQ1_P128 ΔE165_T172::NSEGS ΔQ199_E226::NSEGS ΔY260_T281::NSEGS ΔI317_F337::NSEGS ΔD376_S405::NSEGS ΔA468_A504::NSEGS ΔG539_D551::NSEGS ΔK593_E604::NSEGS ΔG629_P653::NSEGS ΔK681_T715::NSEGS	This study

Supplementary Table 2. Analysis of the permeability of *E. coli* strains overproducing OMPs to the aromatic hydrophobic molecule NPN. BL21 (DE3) strains overproducing no protein (-) or the indicated protein were grown and incubated with (purple) or without (grey) 10 μ M NPN, washed, resuspended in PBS and fluorescence (ex 355 nm, em 450/50 nm) was analyzed via flow cytometry. This experiment was performed with independent biological triplicates (n=3) with at least 80000 single events. The values shown correspond to the average and standard deviation of the triplicates for the fluorescence mean, median and mode of the studied populations.

Fluorescence signal (A.U.)

Overproduced protein	NPN (μ M)	Mean	Median	Mode
(-)	0	373.0 $\pm$ 11.4	316.3 $\pm$ 10.0	437.3 $\pm$ 49.9
GFP	0	327.0 $\pm$ 7.5	275.3 $\pm$ 7.1	371.7 $\pm$ 39.3
FhuA	0	391.3 $\pm$ 6.1	348.7 $\pm$ 6.4	472.0 $\pm$ 65.2
FhuA Δ C	0	512.7 $\pm$ 18.8	435.0 $\pm$ 13.0	641.7 $\pm$ 60.7
FhuA Δ C/ Δ 4L NSEGS	0	494.7 $\pm$ 49.9	405.0 $\pm$ 30.3	499.0 $\pm$ 44.5
FhuA Δ C/ Δ 5L NSEGS	0	549.7 $\pm$ 39.8	478.7 $\pm$ 33.1	631.3 $\pm$ 43.4
FepA	0	436.3 $\pm$ 23.7	338.7 $\pm$ 16.9	441.3 $\pm$ 86.3
FepA Δ C128	0	532.7 $\pm$ 55.6	469.3 $\pm$ 50.9	587.7 $\pm$ 55.4
FepA Δ C128/ Δ L	0	575.7 $\pm$ 46.1	504.7 $\pm$ 42.5	650.0 $\pm$ 29.0
FepA Δ C	0	462.0 $\pm$ 16.6	383.7 $\pm$ 14.4	507.3 $\pm$ 57.6
FepA Δ C/ Δ 4L	0	887.3 $\pm$ 753.2	745.7 $\pm$ 623.8	968.7 $\pm$ 796.2
FepA Δ C/ Δ 4L NSEGS	0	551.0 $\pm$ 21.1	447.0 $\pm$ 9.8	512.7 $\pm$ 12.7
FepA Δ C/ Δ L NSEGS	0	418.7 $\pm$ 8.4	355.3 $\pm$ 5.5	498.0 $\pm$ 22.0
OmpF	0	406 $\pm$ 19.0	359.7 $\pm$ 16.2	476.7 $\pm$ 37.5
OmpF Δ	0	429 $\pm$ 22.0	379.0 $\pm$ 17.5	529.7 $\pm$ 48.1
OmpF Δ GIGL	0	395 $\pm$ 26.9	352.7 $\pm$ 24.9	442.3 $\pm$ 21.9

Fluorescence signal (A.U.)

Overproduced protein	NPN (μ M)	Mean	Median	Mode
(-)	10	1354.0 $\pm$ 276.4	737.7 $\pm$ 127.6	796.3 $\pm$ 149.5
GFP	10	1394.3 $\pm$ 136.0	612.3 $\pm$ 30.9	673.7 $\pm$ 92.0
FhuA	10	918.3 $\pm$ 36.3	623.0 $\pm$ 21.9	699.7 $\pm$ 17.9
FhuA Δ C	10	3144.7 $\pm$ 439.7	2323.0 $\pm$ 422.2	3475.3 $\pm$ 327.5
FhuA Δ C/ Δ 4L NSEGS	10	5306.3 $\pm$ 688.8	3140.0 $\pm$ 481.1	6363.0 $\pm$ 164.5
FhuA Δ C/ Δ 5L NSEGS	10	5717.0 $\pm$ 581.9	4324.0 $\pm$ 391.9	4173.3 $\pm$ 1136.8
FepA	10	2068.0 $\pm$ 103.5	1267.7 $\pm$ 175.7	1607.3 $\pm$ 344.1
FepA Δ C128	10	1334.0 $\pm$ 20.9	840.3 $\pm$ 8.0	973.3 $\pm$ 111.0
FepA Δ C128/ Δ L	10	7027.0 $\pm$ 825.5	5790.0 $\pm$ 757.7	6760.7 $\pm$ 1470.6
FepA Δ C	10	6357.0 $\pm$ 624.3	4753.7 $\pm$ 458.2	5421.0 $\pm$ 1277.4
FepA Δ C/ Δ 4L	10	6604.3 $\pm$ 332.8	5217.0 $\pm$ 235.7	6090.3 $\pm$ 307.7
FepA Δ C/ Δ 4L NSEGS	10	7964.3 $\pm$ 176.7	5568.7 $\pm$ 128.3	5916.7 $\pm$ 397.0
FepA Δ C/ Δ L NSEGS	10	12867.0 $\pm$ 1239.3	9628.3 $\pm$ 954.4	12109.3 $\pm$ 1661.7
OmpF	10	1151.7 $\pm$ 138.0	700.3 $\pm$ 54.2	788.7 $\pm$ 54.1
OmpF Δ	10	3300.0 $\pm$ 141.6	2643.0 $\pm$ 74.0	2777.3 $\pm$ 144.3
OmpF Δ GIGL	10	2373.7 $\pm$ 373.1	1818.3 $\pm$ 316.3	1978.7 $\pm$ 429.4

Supplementary Table 3. Values of the replicates of the erythromycin MIC assay. BL21 (DE3) strains overproducing no protein (-) or the indicated protein were grown in media with varying concentration of erythromycin ranging from 1 mg/mL to 1 µg/mL. The values for each of the independent biological triplicates (n=3), is indicated. The data shown for FhuA Δ C/ Δ 4L NSEGS correspond to cell cultures with disrupted version of the OMP gene.

Erythromycin minimal growth inhibitory concentration (µg/ml)

Overproduced protein	Rep.1	Rep.2	Rep.3	Mean
(-)	125.0	125.0	125.0	125.0
GFP	125.0	125.0	125.0	125.0
FhuA	125.0	125.0	125.0	125.0
FhuA Δ C	3.9	3.9	7.8	5.2
FhuA Δ C/ Δ 4L NSEGS	125.0	125.0	125.0	125.0
FhuA Δ C/ Δ 5L NSEGS	1.0	2.0	1.0	1.3
FepA	31.3	62.5	31.3	41.7
FepA Δ C128	125.0	125.0	125.0	125.0
FepA Δ C128/ Δ L	62.5	125.0	62.5	83.3
FepA Δ C	62.5	62.5	62.5	62.5
FepA Δ C/ Δ 4L	1.0	1.0	1.0	1.0
FepA Δ C/ Δ 4L NSEGS	1.0	1.0	1.0	1.0
FepA Δ C/ Δ L NSEGS	15.6	15.6	7.8	13.0
OmpF	125.0	125.0	125.0	125.0
OmpF Δ	125.0	125.0	125.0	125.0
OmpF Δ GIGL	62.5	62.5	31.3	52.1

Supplementary Table 4. Summary of the data obtained for the analysis of the effect of OMP production on growth, on outer membrane permeability to NPN and on *E. coli* sensitivity to erythromycin. Data was normalized using the GFP overproducing strain as reference.

Overproduced protein	Growth of BL21 Δ ABCF		NPN import to periplasm		Reduction in erythromycin MIC	
	$\mu_{\max}$ (h ⁻¹)	Norm.	Fluorescence mode (A.U.)	Norm.	MIC (μ g·mL ⁻¹)	Norm.
GFP	0.15	1.00	674	1.00	125.0	1.00
FhuA	0.25	1.67	700	1.04	125.0	1.00
FhuA Δ C	0.57	3.80	3475	5.16	5.2	0.04
FhuA Δ C/ Δ 4L NSEGS	0.31	2.07	3380	5.01	125.0	1.00
FhuA Δ C/ Δ 5L NSEGS	0.59	3.93	6363	9.44	1.3	0.01
FepA	0.27	1.80	1607	2.38	31.3	0.33
FepA Δ C128	0.62	4.13	973	1.44	125.0	1.00
FepA Δ C128/ Δ L	0.62	4.13	6761	10.03	83.3	0.67
FepA Δ C	0.15	1.00	5421	8.04	62.5	0.50
FepA Δ C/ Δ 4L	0.64	4.27	6090	9.04	1.0	0.01
FepA Δ C/ Δ 4L NSEGS	0.35	2.33	5917	8.78	1.0	0.01
FepA Δ C/ Δ L NSEGS	0.31	2.07	12109	17.97	13.0	0.10
OmpF	0.40	2.67	789	1.17	125.0	1.00
OmpF Δ	0.43	2.87	2777	4.12	125.0	1.00
OmpF Δ GIGL	0.30	2.00	1978	2.93	52.1	0.42

Supplementary Table 5. Values of the replicates of the coumarin de-allylation in *E. coli* periplasm. BL21 Gold (DE3) strains overproducing Sav S112M K121R, and no protein (-) or the indicated protein were grown and incubated with 1 μ M of the biotinylated ruthenium cyclopentadienyl (Biot-HQ)CpRu cofactor for 1h, washed, and then incubated with 100 μ M allyl carbamate. The product concentration was quantified by measuring the fluorescence intensity with a Tecan Infinite® M1000 at an excitation of 394 nm and an emission of 460 nm and the values were normalized by dividing by the OD₆₀₀ of the respective culture. This experiment was performed with independent biological triplicates (n=3) and the values shown correspond to their average and standard deviation.

Coumarin Fluorescence		
OMP	Fluorescence/OD600 (A.U.)	Normalized Fluorescence/OD600 (A.U.)
(-)	7.73E+04 $\pm$ 1.12E+04	1.00E+00 $\pm$ 1.45E-01
FhuA Δ C	1.14E+05 $\pm$ 1.77E+04	1.48E+00 $\pm$ 2.29E-01
FhuA Δ C/ Δ 5L NSEGS	2.76E+05 $\pm$ 3.24E+04	3.57E+00 $\pm$ 4.20E-01
FepA Δ C/ Δ 4L	2.35E+05 $\pm$ 1.24E+04	2.83E+00 $\pm$ 1.50E-01
FepA Δ C/ Δ L NSEGS	2.34E+05 $\pm$ 3.63E+04	2.83E+00 $\pm$ 4.39E-01
OmpF Δ	2.06E+05 $\pm$ 1.34E+04	2.67E+00 $\pm$ 1.74E-01
OmpF Δ GiGL	2.24E+05 $\pm$ 2.86E+03	2.90E+00 $\pm$ 3.70E-02

Supplementary Table 6. Analysis of the permeability of *E. coli* strains overproducing OMPs to HaloTag TMR dye. BL21 (DE3) strains overproducing the MBP:HaloTag fusion protein, and no protein (-) or the indicated protein were grown incubated with (color) or without (grey) 1 μ M of HaloTag TMR, washed, resuspended in PBS and fluorescence was analyzed via flow cytometry. This experiment was performed with independent biological triplicates (n=3) with at least 5000 counted cells. This experiment was performed with independent biological triplicates (n=3) with at least 80000 single events. The average and standard deviation of the fluorescence means, medians and modes are indicated.

Fluorescence signal TMR (561 nm, 586/15 nm)

Sample	OMP	Mean	Median	Mode
DMSO	(-)	1.17E+02 $\pm$ 3.48E+01	8.60E+01 $\pm$ 2.09E+01	1.20E+02 $\pm$ 2.73E+01
	FhuA Δ C	1.59E+02 $\pm$ 5.42E+01	1.03E+02 $\pm$ 2.60E+01	1.43E+02 $\pm$ 2.52E+01
	FhuA Δ C/ Δ 5L	6.25E+02 $\pm$ 4.51E+01	3.33E+02 $\pm$ 2.54E+01	3.82E+02 $\pm$ 2.83E+01
	FepA Δ C/ Δ 4L	1.03E+03 $\pm$ 8.47E+02	1.14E+02 $\pm$ 5.69E+01	1.04E+02 $\pm$ 3.54E+01
	FepA Δ C/ Δ L NSEGS	2.95E+01 $\pm$ 1.53E-01	2.46E+01 $\pm$ 4.62E-01	1.94E+01 $\pm$ 0.00E+00
	OmpF Δ	3.88E+02 $\pm$ 1.92E+02	1.69E+02 $\pm$ 9.99E+01	2.15E+02 $\pm$ 1.45E+02
TMR (561 nm, 586/15 nm)	(-)	7.46E+03 $\pm$ 3.94E+03	3.64E+03 $\pm$ 1.03E+03	4.00E+03 $\pm$ 7.08E+02
	FhuA Δ C	7.13E+03 $\pm$ 4.09E+02	4.64E+03 $\pm$ 6.58E+02	5.46E+03 $\pm$ 9.25E+02
	FhuA Δ C/ Δ 5L	1.17E+04 $\pm$ 1.32E+03	6.94E+03 $\pm$ 6.08E+02	9.23E+03 $\pm$ 6.19E+02
	FepA Δ C/ Δ 4L	7.33E+03 $\pm$ 3.26E+03	4.00E+03 $\pm$ 1.04E+03	5.32E+03 $\pm$ 1.61E+03
	FepA Δ C/ Δ L NSEGS	1.14E+03 $\pm$ 7.61E+01	8.37E+02 $\pm$ 5.84E+01	1.25E+03 $\pm$ 1.18E+02
	OmpF Δ	8.57E+03 $\pm$ 1.37E+03	6.22E+03 $\pm$ 1.16E+03	9.20E+03 $\pm$ 1.85E+03

Supplementary Table 7. Analysis of the permeability of *E. coli* strains overproducing OMPs to HaloTag JF646 dye. BL21 (DE3) strains overproducing the MBP:HaloTag fusion protein, and no protein (-) or the indicated protein were grown incubated with (color) or without (grey) 1 μ M of HaloTag JF646, washed, resuspended in PBS and fluorescence was analyzed via flow cytometry. This experiment was performed with independent biological triplicates (n=3) with at least 5000 counted cells. This experiment was performed with independent biological triplicates (n=3) with at least 80000 single events. The average and standard deviation of the fluorescence means, medians and modes are indicated.

Fluorescence signal JF646 (640nm, 670/14nm)

Sample	OMP	Mean	Median	Mode
DMSO	(-)	5.48E+01 $\pm$ 4.50E+00	4.37E+01 $\pm$ 3.98E+00	5.49E+01 $\pm$ 1.34E+01
	FhuA Δ C	4.64E+01 $\pm$ 1.56E+00	3.79E+01 $\pm$ 1.93E+00	4.72E+01 $\pm$ 8.70E-15
	FhuA Δ C/ Δ 5L	4.67E+01 $\pm$ 9.64E-01	3.79E+01 $\pm$ 1.10E+00	4.72E+01 $\pm$ 8.70E-15
	FepA Δ C/ Δ 4L	7.61E+01 $\pm$ 5.71E+01	3.82E+01 $\pm$ 4.79E+00	4.01E+01 $\pm$ 2.36E+01
	FepA Δ C/ Δ L NSEGS	4.26E+01 $\pm$ 2.00E-01	7.50E+01 $\pm$ 5.77E-01	2.65E+01 $\pm$ 0.00E+00
	OmpF Δ	5.37E+01 $\pm$ 2.49E+00	4.28E+01 $\pm$ 9.00E-01	4.72E+01 $\pm$ 8.70E-15
JF646 (640nm, 670/14nm)	(-)	1.70E+03 $\pm$ 7.88E+01	1.28E+03 $\pm$ 7.70E+01	1.86E+03 $\pm$ 1.25E+02
	FhuA Δ C	1.75E+03 $\pm$ 2.57E+02	1.29E+03 $\pm$ 1.84E+02	1.69E+03 $\pm$ 2.24E+02
	FhuA Δ C/ Δ 5L	2.54E+03 $\pm$ 2.42E+01	1.66E+03 $\pm$ 4.14E+01	2.14E+03 $\pm$ 2.96E+02
	FepA Δ C/ Δ 4L	4.49E+03 $\pm$ 4.81E+02	3.11E+03 $\pm$ 3.26E+02	4.48E+03 $\pm$ 5.53E+02
	FepA Δ C/ Δ L NSEGS	2.59E+03 $\pm$ 1.72E+02	1.87E+03 $\pm$ 1.55E+02	2.64E+03 $\pm$ 4.15E+02
	OmpF Δ	1.79E+03 $\pm$ 1.59E+02	1.33E+03 $\pm$ 1.46E+02	1.66E+03 $\pm$ 1.24E+02

Supplementary Table 8. Analysis of the permeability of *E. coli* strains overproducing OMPs to HaloTag AF488 dye. BL21 (DE3) strains overproducing the MBP:HaloTag fusion protein, and no protein (-) or the indicated protein were grown incubated with (color) or without (grey) 1 μ M of HaloTag AF488, washed, resuspended in PBS and fluorescence was analyzed via flow cytometry. This experiment was performed with independent biological triplicates (n=3) with at least 5000 counted cells. This experiment was performed with independent biological triplicates (n=3) with at least 80000 single events. The average and standard deviation of the fluorescence means, medians and modes are indicated.

Fluorescence signal AF488 (488 nm, 530/30 nm)

Sample	OMP	Mean	Median	Mode
DMSO	(-)	2.21E+02 $\pm$ 2.40E+01	1.80E+02 $\pm$ 2.22E+01	2.24E+02 $\pm$ 0.00E+00
	FhuA Δ C	1.73E+02 $\pm$ 4.04E+00	1.41E+02 $\pm$ 4.58E+00	2.24E+02 $\pm$ 0.00E+00
	FhuA Δ C/ Δ 5L	1.54E+02 $\pm$ 2.52E+00	1.23E+02 $\pm$ 1.73E+00	1.68E+02 $\pm$ 2.48E+01
	FepA Δ C/ Δ 4L	2.57E+02 $\pm$ 7.00E+01	1.83E+02 $\pm$ 4.04E+01	2.45E+02 $\pm$ 7.82E+01
	FepA Δ C/ Δ L NSEGS	1.57E+02 $\pm$ 4.65E+01	1.17E+02 $\pm$ 1.76E+01	1.48E+02 $\pm$ 1.96E+01
	OmpF Δ	2.82E+02 $\pm$ 4.04E+00	2.33E+02 $\pm$ 2.65E+00	3.39E+02 $\pm$ 1.73E+01
AF488 (488 nm, 530/30 nm)	(-)	3.60E+02 $\pm$ 3.40E+01	2.92E+02 $\pm$ 2.97E+01	3.97E+02 $\pm$ 7.02E+01
	FhuA Δ C	3.57E+02 $\pm$ 8.08E+00	2.83E+02 $\pm$ 8.62E+00	4.18E+02 $\pm$ 3.70E+01
	FhuA Δ C/ Δ 5L	5.15E+02 $\pm$ 1.25E+02	3.98E+02 $\pm$ 1.03E+02	5.08E+02 $\pm$ 7.49E+01
	FepA Δ C/ Δ 4L	1.28E+03 $\pm$ 9.27E+01	7.27E+02 $\pm$ 1.05E+02	9.16E+02 $\pm$ 9.12E+01
	FepA Δ C/ Δ L NSEGS	6.86E+02 $\pm$ 3.00E+01	5.26E+02 $\pm$ 1.81E+01	7.43E+02 $\pm$ 3.30E+01
	OmpF Δ	3.61E+02 $\pm$ 3.69E+01	2.84E+02 $\pm$ 1.46E+01	3.82E+02 $\pm$ 3.40E+01

Supplementary Table 9. Microscopy analysis of the permeability of *E. coli* strains overproducing OMPs to HaloTag AF488 dye. BL21 (DE3) strains overproducing the MBP:HaloTag fusion protein, and no protein (-) or the indicated protein were grown incubated with (color) or without (grey) 1 μ M of HaloTag AF488, washed, resuspended in PBS and fluorescence was analyzed via microscopy. This experiment was performed with independent biological triplicates (n=3) with at least 5000 counted cells. The mean, integrated density and raw integrated density of the fluorescence signal of the triplicates are indicated.

OMP	HaloTag AF488-stained cells (%)	Mean	IntDen	Raw IntDen
(-)	3.20E+00 $\pm$ 1.47E+00	2.48E+01 $\pm$ 2.71E+00	3.80E+01 $\pm$ 1.12E+01	1.17E+03 $\pm$ 3.46E+02
FhuA Δ C	2.99E+00 $\pm$ 1.46E+00	2.25E+01 $\pm$ 1.59E+00	3.38E+01 $\pm$ 8.83E+00	1.04E+03 $\pm$ 2.72E+02
FhuA Δ C/ Δ 5L	9.55E+00 $\pm$ 2.13E+00	2.86E+01 $\pm$ 3.21E+00	4.85E+01 $\pm$ 1.08E+01	1.49E+03 $\pm$ 3.31E+02
FepA Δ C/ Δ 4L	1.64E+01 $\pm$ 4.90E+00	3.63E+01 $\pm$ 3.45E+00	1.40E+02 $\pm$ 3.68E+01	4.31E+03 $\pm$ 1.13E+03
FepA Δ C/ Δ L NSEGS	2.05E+01 $\pm$ 4.94E+00	3.52E+01 $\pm$ 3.68E+00	1.82E+02 $\pm$ 5.46E+01	5.59E+03 $\pm$ 1.68E+03
OmpF Δ	6.14E+00 $\pm$ 8.20E-01	2.66E+01 $\pm$ 1.22E+00	6.30E+01 $\pm$ 1.08E+01	1.94E+03 $\pm$ 3.33E+02

Supplementary Table 10. List of *E. coli* strains used in this study.

Strain	Genotype	Source
DH5α	F ϕ 80lacZ Δ M15 Δ (lacZYA-argF) U169 recA1 endA1 hsdR17 (rK ϕ mK ϕ) phoA supE44 λ -thi-1 gyrA96 relA1	Thermo Fisher Scientific
NEB[®] Turbo	F' proA+B+ lacIq Δ lacZM15 / fhuA2 Δ (lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)TetS endA1 thi-1 Δ (hsdS-mcrB)S	New England Biolabs
BL21(DE3)	F ϕ ompT hsdSB (rB ϕ , mB ϕ) gal dcm (DE3)	Merck
BL21(DE3) ΔmalE	F ϕ ompT hsdSB (rB ϕ , mB ϕ) gal dcm (DE3) Δ malE	This study
BL21 Gold(DE3)	B F ϕ ompT hsdS(rB ϕ mB ϕ) dcm+ Tetr gal λ (DE3) endA Hte	Agilent Technologies
BL21 ΔABCF	B F ϕ ompT hsdS(rB ϕ mB ϕ) dcm+ Tetr gal λ (DE3) endA Hte Δ ompA Δ ompC Δ ompF Δ lamB	Meuskens et al. 2017

Supplementary Table 11. List of primers used in this study.

Name	DNA sequence (5'-3')	Description
IC18NOV01	GCCTAGGCCGCGGCCGCGCGTAATACGACTCACTATAGGGGAAT TGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTGTTAACTT TAAGAAGGAGATATACATATGGCGCGTTCCAAAACGTC	Amplification of FhuA Δ C For and T7 addition
IC18NOV02	CCAGTCACGACGCGGCCGCACAAAAACCCCTCAAGACCCG	Amplification of FhuA Δ C Rev
IC18NOV14	GCCTAGGCCGCGGCCGCGCGTAATACGACTCACTATAGGGGAAT TGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTGTTAACTT TAAGAAGGAGATATACATATGAACAAGAAGATTTCATCCCTG	Amplification of FepA For and T7 addition
IC18NOV15	CCAGTCACGACGCGGCCGCACAAAAACCCCTCAAGACCCGTT TAGAGGCCCAAGGGGTATGCTAGTTATTGCTCAGCGGTCAGA AGTGGGTGTTACGC	Amplification of FepA For
IC19JAN07	GCCGTTTCAGCAGTAATAAGG	Sequencing of FhuA
IC19JAN08	ATCTGGCACGTAAATACGTC	Sequencing of FhuA
IC19JAN09	GCACGTGGCGTAGAAATCG	Sequencing of FhuA
IC19JAN10	GCCTAGGCCGCGGCCGCGCGTAATACGACTCACTATAGGGGAAT TGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTGTTAACTT TAAGAAGGAGATATACATATGGCGCGTTCCAAAACGTC	Amplification of FhuA For and T7 addition
IC19JAN11	GAAGTCGAAATCGGTATTCACCGGATTGTACAGATTGAGCAGTG	Amplification of FhuA Rev
IC19MAR05	GCGGAAAAGGACCCTTGTC	Amplification of CamR cassette For
IC19MAR06	CCAATAATTACGATTTAAATGATCGG	Amplification of CamR cassette Rev
IC19APR07	GCCTAGGCCGCGGCCGCGCGTAATACGACTCACTATAGGGGAAT TGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTGTTAACTT TAAGAAGGAGATATACATATGGCTCGCTCCAAAACGG	Amplification of FhuA Δ C/ Δ 5L NSEGS and T7 addition For
IC19APR08	CCAGTCACGACGCGGCCGCACAAAAACCCCTCAAGACCCGTT TAGAGGCCCAAGGGGTATGCTAGTTATTGCTCAGCGGTTAAA AACGAAAGGTTGCGGTG	Amplification of FhuA Δ C/ Δ 5L NSEGS Rev
IC19APR09	TAATACGACTCACTATAGGG	Sequencing of FhuA Δ C/ Δ 5L NSEGS
IC19APR10	TAGTTATTGCTCAGCGGTGG	Sequencing of FhuA Δ C/ Δ 5L NSEGS
IC19MAY01	TGCGGCCGCGTCGTGACTG	Amplification of pSEVA empty vectors For
IC19MAY02	CGCGCGGCCGCGGCCTAG	amplification of pSEVA empty vectors Rev
IC19SEPT03	TGATGCCTTTAATTAAAGGAATCCGTCTCAGGAGTAATACGACT CACTATAGGGGAATTGTGAGCGGATAACAATCCCCTCTAGAAAT AATTTGTTAACTTTAAGAAGGAGATATACATATGCGTAAAGGC GAAGAG	Amplification of GFP and addition of T7 For

Name	DNA sequence (5'-3')	Description
IC19SEPT04	GTTTTCCCAGTCACGACAAGCTTCGTCTCATGACAGCGCAAAAA ACCCCTCAAGACCCGTTTAGAGGCCCAAGGGGTTATGCTAGTT ATTGCTCAGCGGTCATTTGTACAGTTCATCCATAC	Amplification of GFP Rev
IC19OCT03	AGGCTGGCCGTAGGCCGCGCGGCGCAGGATAGGTGAAGTAG	Amplification of pSEVA 36X plasmid For
IC19OCT04	CCTAGACAGCTGGGCGCGCCGGCGCGCCGGATATATTC	Amplification of pSEVA 36X plasmid Rev
IC20JAN02	TTAATTGGACCGCGGTCCGC	Amplification of KanR cassette For
IC20JAN03	CCTGTATTACTGTTTATGTAAGCAGACAG	Amplification of KanR cassette Rev
IC20JAN04	ACATAAACAGTAATACAAGGTTCAAATATGTATCCGCTCATG	Amplification of AmpR cassette For
IC20JAN05	GCGGACCGCGGTCCAATTAATTACCAATGTTTAATCAGGCTC	Amplification of AmpR cassette Rev
IC20JUL01	TTTAAGAAGGAGATATACATATGAACAAGAAGATTCATTCCCTGG CCTTGTTGGTCAATCTGGGGATTATGGGGTAGCGCAGGCAGCA GCTGCGCGTTATGGC	Amplification of FepA Δ C128 For
IC20JUL02	ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTTC	Amplification of FepA Δ C128 Rev
IC20JUL03	TTTAAGAAGGAGATATACATATGAACAAGAAGATTCATTCCCTGG CCTTGTTGGTCAATCTGGGGATTATGGGGTAGCGCAGGCAGCC GCCGCTCGTTATGG	Amplification of FepA Δ C128/ Δ L For
IC20OCT01	CTGACCACCTTTACCCCTAATC	Amplification of pRha promoter system For
IC20OCT02	CATCCGGCAATCAATGCCTG	Amplification of pRha promoter system Rev
IC21JUL03	TTTAAGAAGGAGATATACATATGATGAAGCGCAATATTCTGG	Amplification of OmpF For
IC21JUL04	TGCTAGTTATTGCTCAGCGGTTAGAACTGGTAAACGATACC	Amplification of OmpF Rev
IC21SEP01	ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGG	Amplification pSEVA 29X backbone Rev
IC21SEP02	GTTTAACTTTAAGAAGGAGATATACATATG	Amplification of OmpF For
IC21SEP03	GCCGCCTAGGCCGCGGCCGCTCAAAAGTGAGTGTTGACGC	Amplification of FepA Δ C128/ Δ L Rev
IC21SEP04	GCCGCCTAGGCCGCGGCCGCTTAAAAACGAAAGGTTGCGGTG	Amplification of FhuA Δ C/ Δ 5L Rev
IC21SEP05	GCCGCCTAGGCCGCGGCCGCTTAGAAACGGAAGGTTGCG	Amplification of FhuA Δ C rev
IC21SEP06	AATTCCTTTAATTAAAGGCATC	Amplification pSEVA 29X with pBAD Rev
IC21SEP07	CCTCTAGAAATAATTTTGTTTAAC	Amplification pSEVA 29X with pBAD For
IC20OCT01	CTGACCACCTTTACCCCTAATC	Amplification of pRha promoter system For
IC20OCT02	CATCCGGCAATCAATGCCTG	Amplification of pRha promoter system Rev

Name	DNA sequence (5'-3')	Description
IC21SEP08	GATGCCTTTAATTAAAGGAATTTTATGACAACTTGACGGCTACAT C	Amplification of pBAD promoter system For
IC21SEP09	GTAAACAAAATTATTTCTAGAGGCAGTAGAGAGTTGCGATAAA AAGC	Amplification of pBAD promoter system Rev
IC22MAR01	AGGGTACCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATA TACATCTCGAGCCAACCACTGAG	Amplification of HALO domain For
IC22MAR02	AAGACTAGTCGAGCGGATAACAATTTACACAGGAGGCCGTTA GTGGTGATGGTGATGATG	Amplification of HALO domain Rev
IC22MAR03	CGGCCTCTGTGTGAAATTG	Amplification of pSEVA 36X backbone for MBP:Halo expression For
IC22MAR04	ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGG	Amplification of pSEVA 36X for MBP:Halo expression Rev
IC22MAR05	ATGTCCAGACCTGCAGGC	Amplification of pSEVA 36Xwith pBAD prom For
IC22MAR06	ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAG	Amplification of pSEVA 36Xwith pBAD prom Rev
IC22MAR07	CTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACATATGGC GCGTTCCAAAACCTG	Amplification of FhuA to clone it with pBAD promoter For
IC22MAR08	TTGATGCCTCTAGAGCTTGCATGCCTGCAGGTCTGGACATTAGA AACGGAAGGTTGCGG	Amplification of FhuA to clone it with pBAD promoter Rev
IC22MAR09	CTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACATATGGC TCGCTCCAAAACGG	Amplification of FhuA $\Delta C/\Delta 5L$ NSEGS to clone it with pBAD promoter For
IC22MAR10	TTGATGCCTCTAGAGCTTGCATGCCTGCAGGTCTGGACATTAAA AACGAAAGGTTGCGGTG	Amplification of FhuA $\Delta C/\Delta 5L$ NSEGS to clone it with pBAD promoter Rev
IC22MAR11	CTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACATATGAA CAAGAAGATTCATCCCTG	Amplification of FepA to clone it with pBAD promoter For
IC22MAR12	TTGATGCCTCTAGAGCTTGCATGCCTGCAGGTCTGGACATTCAG AAGTGGGTGTTACGC	Amplification of FepA to clone it with pBAD promoter For
IC22MAR13	TTGATGCCTCTAGAGCTTGCATGCCTGCAGGTCTGGACATTCAA AGTGAGTGTTGACGC	Amplification of FepA $\Delta C128/\Delta L$ to clone it with pBAD promoter Rev
IC22MAR18	CTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACATATGCG TAAAGGCGAAGAGCT	Amplification of GFP to clone it with pBAD promoter For
IC22MAR19	TTGATGCCTCTAGAGCTTGCATGCCTGCAGGTCTGGACATTCATT TGTACAGTTCATCCATACCATG	Amplification of GFP to clone it with pBAD promoter Rev
IC22MAR20	CTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACATATGAT GAAGCGCAATATTCT	Amplification of Omp to clone it with pBAD promoter For

Name	DNA sequence (5'-3')	Description
IC22MAR21	TTGATGCCTCTAGAGCTTGCATGCCTGCAGGTCTGGACATTAGA ACTGGTAAACGATAC	Amplification of Omp to clone it with pBAD promoter Rev
IC22MAR22	CTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACATAGAA AAAGAGCACTCTGGC	Amplification of Omp Δ to clone it with pBAD promoter For
IC22MAR23	TTGATGCCTCTAGAGCTTGCATGCCTGCAGGTCTGGACATGAAC TGGTAAACGATACCC	Amplification of Omp Δ to clone it with pBAD promoter Rev
IC22JUN01	TCCTCAGTGGTTGGCTCGAGCTTGGTGATACGAGTCTGC	Amplification of pSEVA 36X with pRha For
IC22JUN02	CAATTCACACAGGAGGCCGCTTGGTGATACGAGTCTGCG	Amplification of MBP TU for 3' fusion with Halo
IC22JUN03	CGCGTCTTATCAGGCCT	Amplification of 5' malE and its flanking regions for colony PCR For
IC22JUN04	GAGGTTGCCGTATAAAGAAAC	Amplification of 5' malE and its flanking regions for colony PCR For
IC22JUN05	AGTCCGTTTAGGTGTTTTACGAGCACTTCACCAACAAGGACCA TAGATTCATATGAATATCCTCCTTAGTTCCTATTC	Amplification of KanR cassette from pKD4 with malE flanking regions Rev
IC22JUN06	GTTTTGTAGGCCGACAAGGCGTTCACGCCGATCCGGCATTTC ACAGCAGTG TAGGCTGGAGCTGCTTC	Amplification of KanR cassette from pKD4 with malE flanking regions For
IC23JUN10	TATACCGTTGTGGATGCACTGGTTC	Addition of L10 to FhuA Δ C/ Δ 5L to clone FhuA Δ C/ Δ 4L For
IC23JUN11	AGTGCATCCACAACGGTATAACTCCCACTTTAAAGGAGTTAGCC GGATCACCATAACTGGAGCCGGTATAGCGACCACCGGTG	Addition of L10 to FhuA Δ C/ Δ 5L to clone FhuA Δ C/ Δ 4L Rev
IC23SEPT01	ATGAAAATAAAAACAGGTGCACG	Amplification of malE for colony PCR For
IC23SEPT02	TTACTTGGTGATACGAGTCTG	Amplification of malE for colony PCR Rev

Supplementary Table 11. List of plasmids used in this study.

Plasmid	Description	Reference
pT7-FhuA	pT7-FhuA cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FhuA ΔC	pT7-FhuA ΔC cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FhuA ΔC/Δ4L NSEGS	pT7-FhuA ΔC/Δ4L cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FhuA ΔC/Δ5L NSEGS	pT7-FhuA ΔC/Δ5L cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FepA	pT7-FepA cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FepA ΔC128	pT7-FepA ΔC128 cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FepA ΔC128/ΔL	pT7-FepA ΔC128/ΔL cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FepA ΔC	pT7-FepA ΔC cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FepA ΔC/Δ4L	pT7-FepA ΔC/Δ4L cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FepA ΔC/Δ4L NSEGS	pT7-FepA ΔC/Δ4L NSEGS cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-FepA ΔC/ΔL NSEGS	pT7-FepA ΔC/ΔL NSEGS cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-OmpF	pT7-OmpF cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-OmpF Δ	pT7-OmpF Δ cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
pT7-OmpF Δ GIGL	pT7-OmpF Δ GIGL cloned in pSEVA291 (pBR322-ROP, Kan ^R)	This study
prhaBAD-FhuA ΔC	prhaBAD-FhuA ΔC cloned in pSEVA371 (pSC101, Cam ^R)	This study
prhaBAD-FhuA ΔC/Δ5L NSEGS	prhaBAD-FhuA ΔC/Δ5L NSEGS cloned in pSEVA371 (pSC101, Cam ^R)	This study
prhaBAD-FepA ΔC/Δ4L	prhaBAD-FepA ΔC/Δ4L cloned in pSEVA371 (pSC101, Cam ^R)	This study
prhaBAD-FepA ΔC/ΔL NSEGS	prhaBAD-FepA ΔC/ΔL NSEGS cloned in pSEVA371 (pSC101, Cam ^R)	This study
prhaBAD-OmpF Δ	prhaBAD-OmpF Δ cloned in pSEVA371 (pSC101, Cam ^R)	This study
prhaBAD-OmpF Δ GIGL	prhaBAD-OmpF Δ GIGL cloned in pSEVA371 (pSC101, Cam ^R)	This study
pSAV S112M K121R	pT7-OmpA-SAV S112M K121R cloned in pSEVA 291 (pBR322-ROP, Kan ^R)	Vornholt et al. ⁷⁰
MBP-HaloTag	prhaBAD-MBP-HaloTagL cloned in pSEVA361 (p15A, Cam ^R)	This study
pKD4	FRT- <i>kan</i> -FRT (R6K, Kan ^R , Carb ^R)	Datsenko and Wanner ⁸²
pKD46	araC-P _{BAD} -gam-beta-exo (pSC101 (ts), Carb ^R)	Datsenko and Wanner ⁸²
pCP20	<i>flp</i> expression. (pSC101 (ts), Cam ^R , Carb ^R)	Datsenko and Wanner ⁸²

Supplementary Table 12. Effect of different expression induction levels on on outer membrane permeability to bulky hydrophobic molecules. MICs of strains overproducing the indicated OMP and controls when incubated with the indicated concentrations of rhamnose. (-) represents the MIC obtained for strain BL21(DE3) containing a plasmid without gene for overexpression. BL21 (DE3) strains overproducing no protein (-) or the indicated OMP were grown in LB supplemented with 50 mg·L⁻¹ of kanamycin, 50 µM IPTG, the indicated concentrations of rhamnose, and varying concentrations of erythromycin ranging from 1 mg/mL to 0 mg/mL. After 18 h of incubation at 37°C, cell density was analyzed by measuring the OD600 of the samples. This experiment was performed with independent biological triplicates (n=3), and the value displayed here corresponds to the average of the three replicates.

OMP	Erythromycin MIC (µg·mL ⁻¹)				
BL21(DE3)(-)	125	125	125	125	125
FepA	125	125	125	125	125
FepA ΔC/Δ4L	125	15.6	15.6	15.6	15.6
FepA ΔC/ΔL NSEGS	125	15.6	15.6	15.6	15.6
	0	0.1	0.2	0.5	1
	Rhamnose % (w/v)				

[illegible]

TTAATTAAGCGGATAACAATTTCACACAGGAGGCCGCTAGGCCGCGGCCGCGCTAATCAGCTACTATAGGGGAATTGTGAGCGGATAACAATCCCTCTAGAAAATAATTTTGTTAA
TTTAAAGAAGAGATATACATAGGCTCGCTCCAAACGGCCAGCCTAAAACATAGCTCGCTGCTAAATTCGACTGTTGTGTTCGAACCCGAGTACGGCTATAGGCTTTATGCACAGCGACTGAA
AGAAGTTCAGTTTAAAGCAGGCACGTAGCTGTTTCAGACCGCTTTGATTAGCAGTAACCGTGGATGAGTGATGTTGTTTATAGCTATGCTGTCAGACGTTCTGGCTGGGAGTGCACAAATGCA
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GGTGTGGATTATATGCGTATGCGCAATGATAATTAATGCTGTTTGGCTATAATTAGAAGGTAGCAGCGGTCCGATATCGTATTTGAATAAACAGAAACAGACCGGGTGTATGTTCAGGAT
AGGCACAGTGGGATAAAGTTCGGTTACCTCGGGTGGCTGTTATGATTGGGCACATCAGGAAGACCTGTAATCGGTCAGGCCACCCGATAAACGTGATGATAAACAGTTTACATGGCGT
GGTGGTGTGAATTACCTGTTGATAAATGGCGTGGCCGCTGTTTACGTAATAGCGAAGCTTTGAACGAGCAGCCAGGTGGGTAAAGATGGCAATTTGTTTGCACAGCAAGAACGAAACAG
TATGAAGTGGGCGTTAAATATGTTCCGGAAGATCGTCGATTGTGTTACCGTGCCGCTTTAATCTGACCAAAACCAATAATCTGATGGCAGATCCGGAAGGTAGCTTTTATGCTGGTGAAG
TGGGTGAATTCGTGCGAGTGGTTGTAATTTGAACCAAGCAGCACTGAGCCGCAAGGCTTAAGTTGTTGGCAGCTACTACTACCGTAGCAGAATAACCCAGTACCACCTATAAAG
GTAATACACCGGCACAGGTTCCGAAACATAGCAAGCGCTGGGCGAGATTATACCTTTTTGATGGTCCGCTGTCTGGCTGACCTGGGCGACCGGTGGTGCCTATACCGGCTCCAGTTATGG
TGATCCGGCTACCTCTTTAAAGTGGGAAGTTATACCGTGTGGATGACGATGTTCTGTTATGATCGGCTGTCTGGTATGGCAGTAGCAATTTGTCACGTCATGTGAATAGTGAAGGTAGC
CAGGTTGTGTCACCGCAACCTTCGTTTTAACCGCTGAGCAACTAGTACGATGCTTGGGCGCTTAAACCGGCTGTAGGGGTTTTGTCGGCCGCGCTGTGCTAGCTGGGAAACCTC
GGCGACTAGTCTTGGACTCCTGTTGATAGATCCAGTAATGACCTCAGAACTCCATCTGGATTTTGTGAGAAGCTCGGTTCCGCGCGGGCGTTTTTATGGTGAGAATCCAGGGGTCCCCAATA
ATTACGATTTAAATTTGTGTCCAAATCTCGATGTACATTCGACAGAAATAAATAATCATCATGAACAATAAGGAGCTGTGCTTACATACAGAGTAATACAGGGGTGTTATGAGCCATAT
TACGGCTGAAACGAGCTGTAGCCGTCGGCTGTGAACGACAACTGGATGGGATCTGTAGGCTATGAATGGAACGAGCGGTGATACAGTGGGTGAGCGCGGCACCAATTATGCTCTGTATG
GCAACCGGATCGCGCGAACTGTTTCTGAAACATGGCAAGGCGACGCTGGCGAACGATGTGACCGATGAAATGGTGGCTGTAAGTGGCTGACCGAATTTATCCGCTGCCACCAATTAA
CATTTTATTCGCAACCCGAGTATGGCTGGTGGCTGACCAACCGCATTCGGGCAAGAACCCGGCTTTACGTTGTGCGAAGTAATTCGGATAGCGCTGGCCGCT
TTTTCGGTGGCTGTGATAGCAATTCGGTGTGCAACTGCGGTTTAAACGAGATCGTGTGTTCTGTGCGCCAGGCGACAGCGCTATGAACCAACGCGCTGGTGATGACGAGATTGTAGT
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GGCAAATGATTGGTGGCTGATTGATGTGGCGGTGGGCGATTGGGATCGGGATCGTTACAGGATCTGGCCATTCGTGGAAGTCTGCGGCGAATTTAGCCGAGCTCAAAAACGCTCTGTTCC
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TCTCTTGAGATCTTTTTTTTTCGGCGTAATCTGCTGTGCAAAACAAAAAACCCGCTACACGGGTGGTTTGTTCGGGATCAAGAGCTACCAACTTTTTTCGAAGGTAACTGGCTC
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TTACCGCAAGGACCGTAATGTTACTGAATCCCGCATCATCTATCGCGACGAAAGTATGCGTACCGCTGCTCTCCGATGGTTTATGCACTGACGCGCAAGGCTTGAACCTGAAATCGCGATCGCATCCCAAC
GGATGTTACGTGCATAAAGCCATGATTCACTGTGCGCGCTGCGGCTTCGGATTGCTGGTATCTATGACAAGGATGAAGCCGAGCGCATTGTCGAAAATACTGCATACACTGCAGAACGTCAGCC
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CTTTCACAGCGTTTCTGGGTGAGCAAAAAAGGCAAAATGCCGCAAAAAAGGGAATAGGGCGACACGGAAATGTTGAATCTCATACTCTTCTTTTCAATATTATTGAAGCATTTA
TCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAAGTGCCACCTG

[illegible]

Supplementary Data 2. Protein sequences of the OMPs used in this study:

FhuA

MARSKTAQPKHSLRKIAV VVATAVSGMSVYAQA AVEPKEDTITVTAAPAPQESAWGPAATIA
ARQSATGKTKDTPIQKVPQSI SVVTAEEMALHQPKSVKEALSYTPGVSVGTRGASNTYDHLI
IRGFAAEGQSQNNYLNGLKLQGNFYNDVIDPYMLERAIEIMRGPVSVLYGKSSPGGLLMVS
KRPTTEPLKEVQFKAGTDSL FQTGFDFSDSLDDDGVS YRLTGLARSANAQQKGSEEQRYAI
APAF TWRPDDKTNFTFLSYFQNEPETGYYGWLPKEGTVEPLPNGKRLPTDFNEGAKNNTYSR
NEKMGVYSFDHEFNDTFTVRQNLRFAENKTSQNSVYGYGVCSDPANAYSKQCAALAPADKGH
YLARKYVVDDEKLQNF SVDTQLQSKFATGDI DHTLLTGVD FMRMRNDINAWFGYDDSVPLLN
LYNPVNTDFDFNAKDPANSGPYRI LNKQKQTGVYVQDQAQWDKVLVTLGGRYDWADQESLNR
VAGTTDKRDDKQFTWRGGVNYLFDNGVTPYFSYSESFEPSSQVGKDGNIFAPSKGKQYEVGV
KYVPEDRPIVVTGAVYNLTKTNNLMADPEG SFFSVEGGEIRARGVEIEAKAALSASVNVVGS
YTYTDAEYTTDTTYKGNTPAQVPKHMASLWADYTF FDGPLSGLTLGTGGRYTGSSYGDPANS
FKVGSYTVVDALVRYDLARVGMAGSNVALHVNNLFDREYVASC FNTYGCFWGAERQVVATAT
FRF

FhuA ΔC

MARSKTAQPKHSLRKIAV VVATAVSGMSVYAQA -----

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NEKMGVYSFDHEFNDTFTVRQNLRFAENKTSQNSVYGYGVCSDPANAYSKQCAALAPADKGH
YLARKYVVDDEKLQNF SVDTQLQSKFATGDI DHTLLTGVD FMRMRNDINAWFGYDDSVPLLN
LYNPVNTDFDFNAKDPANSGPYRI LNKQKQTGVYVQDQAQWDKVLVTLGGRYDWADQESLNR
VAGTTDKRDDKQFTWRGGVNYLFDNGVTPYFSYSESFEPSSQVGKDGNIFAPSKGKQYEVGV
KYVPEDRPIVVTGAVYNLTKTNNLMADPEG SFFSVEGGEIRARGVEIEAKAALSASVNVVGS
YTYTDAEYTTDTTYKGNTPAQVPKHMASLWADYTF FDGPLSGLTLGTGGRYTGSSYGDPANS
FKVGSYTVVDALVRYDLARVGMAGSNVALHVNNLFDREYVASC FNTYGCFWGAERQVVATAT
FRF

FhuA ΔC/Δ4L NSEGS

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-----LKEVQFKAGTDSL FQTGFDFSDSLDDDG VYSYRLTGLARSANAQQKGSEEQRYAI
APAFTWRPDDKTNFTFLSYFQNEPETG-----NSEGS-----TYSR
NEKMGVYSFDHEFNDTFTVRQNLRFAENKTSQNSVYG-----NSEGS-----
---RKYVVDDEKLQNF SVDTQLQSKFATGDI DHTLLTGVD FMRMRNDINAWFGY-----
---NSEGS-----SGPYRI LNKQKQTGVYVQDQAQWDKVLVTLGGRYDWADQESLNR
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KYVPEDRPIVVGTGAVYNLTKTNNLMADPEGSFFSVEGGEIRARGVEIEAKAALSASVNVVGS
YTYTDAEYTTDTTYKGNTPAQVPKHMASLWADYTFFDGPLSGLTLGTGGRYTGSSYGDPANS
FKVGSYTVVDALVRYDLARVGMAGSNVALHV-----NSEGS-----QVVATAT
FRF

FhuA ΔC/Δ5L NSEGS

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-----LKEVQFKAGTDSL FQTGFDFSDSLDDDG VYSYRLTGLARSANAQQKGSEEQRYAI
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NEKMGVYSFDHEFNDTFTVRQNLRFAENKTSQNSVYG-----NSEGS-----
---RKYVVDDEKLQNF SVDTQLQSKFATGDI DHTLLTGVD FMRMRNDINAWFGY-----
---NSEGS-----SGPYRI LNKQKQTGVYVQDQAQWDKVLVTLGGRYDWADQESLNR
VAGTTDKRDDKQFTWRGGVNYLFDNGVTPYFSYSESFEPSSQVGKDGNI FAPSKGKQYEVGV
KYVPEDRPIVVGTGAVYNLTKTNNLMADPEGSFFSVEGGEIRARGVEIEAKAALSASVNVVGS
YTYTDAEYTTDTTYKGNTPAQVPKHMASLWADYTFFDGPLSGLTLGTGGRYT-----NSEGS
-----YTVVDALVRYDLARVGMAGSNVALHV-----NSEGS-----QVVATAT
FRF

FepA

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FepA $\Delta C128$

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HF

FepA $\Delta C128/\Delta L$

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YALTWNGGWDNGVTTSNWVQYEHTRNSRIP-----DFVDIDLDDVMLHSE
VNLPIDFLVNQTLTLGTEWNQQRMKDL-----YSKAEIFSL
FAENNMELTDSTIVTPGLRFDHHSIVGNNWSPALNISQGLGDDFTLKMGIARAYKAP-----
-----KAETSINKEIGLEFKRDGWLAVGTWFRNDYRNKI

EAGY-----TDLYQWDNVPKAVVEGLEGSLNVPVSETVMWTNNITYMLKSENKT-----
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 HF

FepA ΔC

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FepA ΔC/Δ4L

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 HF

FepA ΔC/Δ4L NSEGS

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FAENNMELTDSTIVTPGLRFDHHSIVGNNWSPALNISQGLGDDFTLKMGIARAYKAP-----
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HF

FepA ΔC/ΔL NSEGS

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VNLPIDFLVNQTLTLGTEWNQQRMKDL-----NSEGS-----YSKAEIFSL
FAENNMELTDSTIVTPGLRFDHHSIVGNNWSPALNISQGLGDDFTLKMGIARAYKAP-----
-----NSEGS-----KAETSINKEIGLEFKRDGWLAVGTWFRNDYRNKI
EAGY---NSEGS---TDLYQWDNVPKAVVEGLEGLSNVPVSETVMWTNNITYMLKSENKT-NSE
GS---PEYTLNSTLSWQAREDLSMQTTFTWYKG-----NSEGS-----SPYSIVGLS
ATWDVTKNVSLTGGVDNLFDKR-----NSEGS-----GRTWYMSVNT
HF

OmpF

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OmpF Δ

MKKSTLALVVMGIVASASVQA AEIYNKDG NKVDLYGKAVGLHYFSKGNGENSYGGNGDMTYA
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VVYDAL-----PEFGGDTAYSDDFFVGRVGGVATYRNSNFFGLVDGLNFAVQYLGKNERDT
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OmpF Δ GI/GL

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