Supplementary information

Structural and functional implications of *in vivo* phase separation of membrane protein in *Escherichia coli*

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Supplementary Video 1. Time-resolved PALM reconstruction of *E. coli* BW25113 LacY$^{mEos\text{-}Pop}$. 100,000 frames of acquisition were separated in 10,000 consecutive frames segments, and these were used for independent reconstructions. The last 10,000-frame reconstruction was removed from the processing due to the lower number of detected localizations, and nine segments were used to make a time-lapse movie. This procedure was done for non-fixed (**left panel**) and fixed (**right panel**) *E. coli* cells. Fixation was performed by incubating 500 µL of cells at OD_{600} = 0.15 with 100 µL of ice-cold formaldehyde-glutaraldehyde solution (4% w/v formaldehyde, 0.4% w/v glutaraldehyde in 100 mM sucrose dissolved in MQ water). After incubation of cells on ice for 10 min, 1 mL of phosphate-buffered saline (PBS, 0.40 g NaCl, 0.01 g KCl, 0.07 g Na_2HPO_4, 0.01 g KH_2PO_4 in 50 mL MQ water, pH 7.4) was added to stop the reaction by diluting the fixation solution. Cells were spun down and washed 2 times in 500 µL of PBS and resuspended in 100 µl of PBS. In fixed cells the LacY-mEos3.2-PopTag condensates are smaller and "sharper" (with some dispersed signals on the membrane) than in non-fixed cells, which is due to the greater dynamics of the particles.

Supplementary Video 2. Redistribution of phase-separated LacY upon spheroplast formation of *E. coli* cells. *E. coli* BW25113 LacY$^{mEos\text{-}Pop}$ cells were immobilized on a glass slide with agarose pad with a specific composition for spheroplasts formation (0.75% w/v melted agarose, 2M glucose, 5 µL of 200 µg/mL lysozyme plus 5 µL of 0.5 M EDTA (pH 8.0)). The glass slide with immobilized cells was placed on the microscope stage**,** and time series acquisition started with time steps of 2.5 min for 45 min. Spheroplasts formation was observed in real time, and the fluorescent signal from the phase-separated protein disappears from the foci and LacY-mEos3.2-PopTag becomes homogeneously distributed in the membrane.

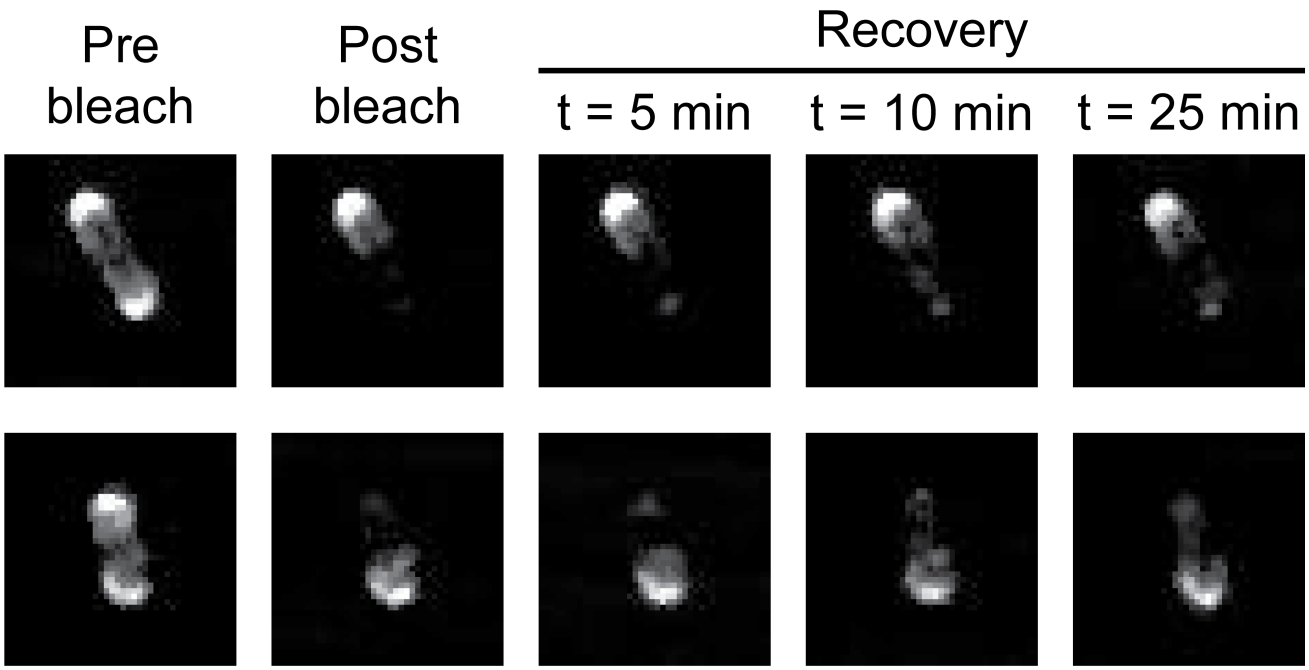


Figure S1. Fluorescence recovery of LacY$^{mEos\text{-}Pop}$. Fluorescence recovery after bleaching of LacY$^{mEos\text{-}Pop}$ at one of the poles. The fluorescent signal from the non-bleached pole is not changing over time, indicating that recovery is predominantly due to diffusion of LacY$^{mEos\text{-}Pop}$ from the lateral membrane.

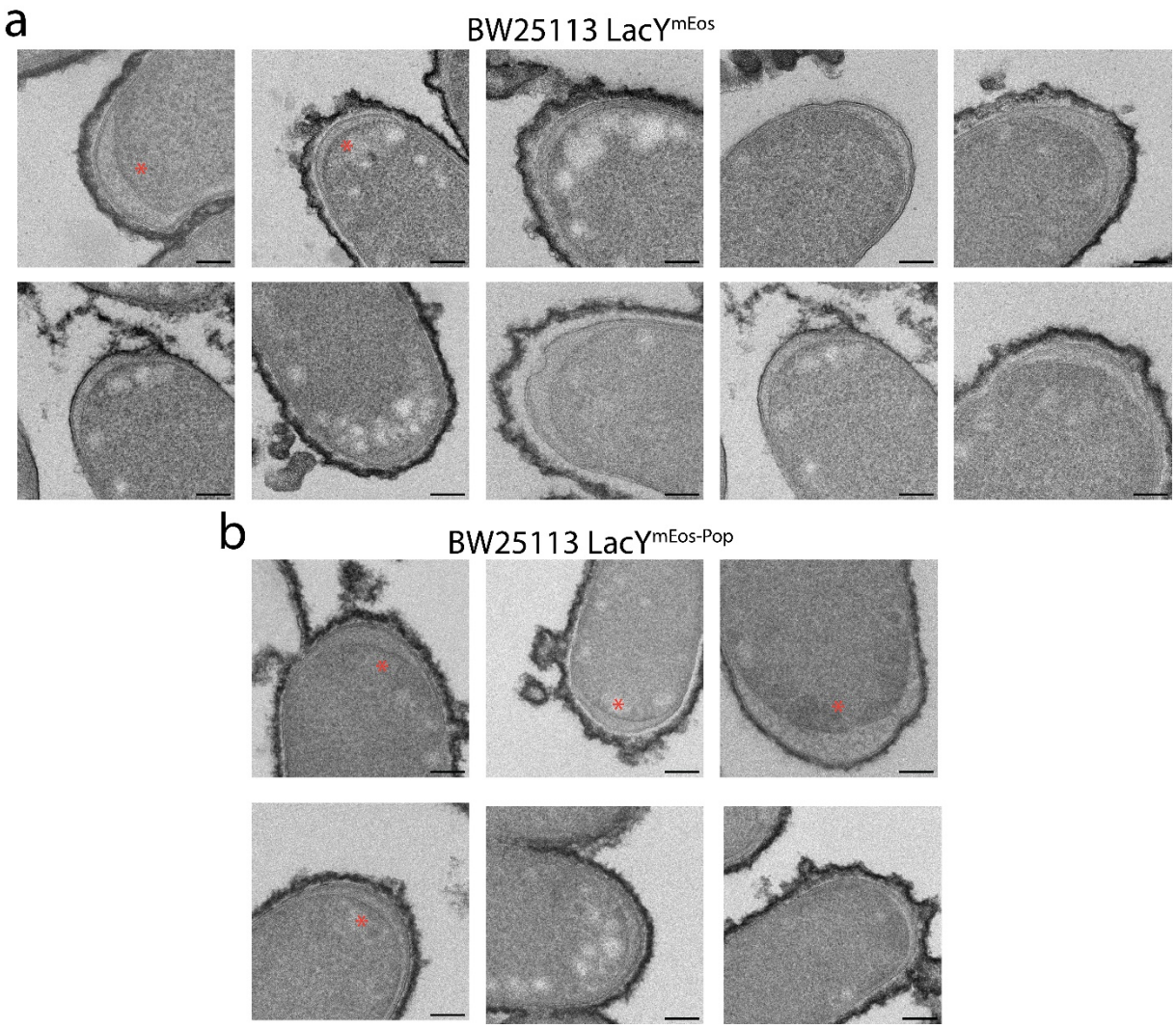


Figure S2. Transmission electron microscopy of BW25113 LacY^{mEos} and BW25113 LacY$^{mEos-Pop}$ cells. Asterix signs are showing electron-dense regions at the cytoplasmic face of the inner membrane. White spots are freezing artifacts. Scale bars are 200 nm.

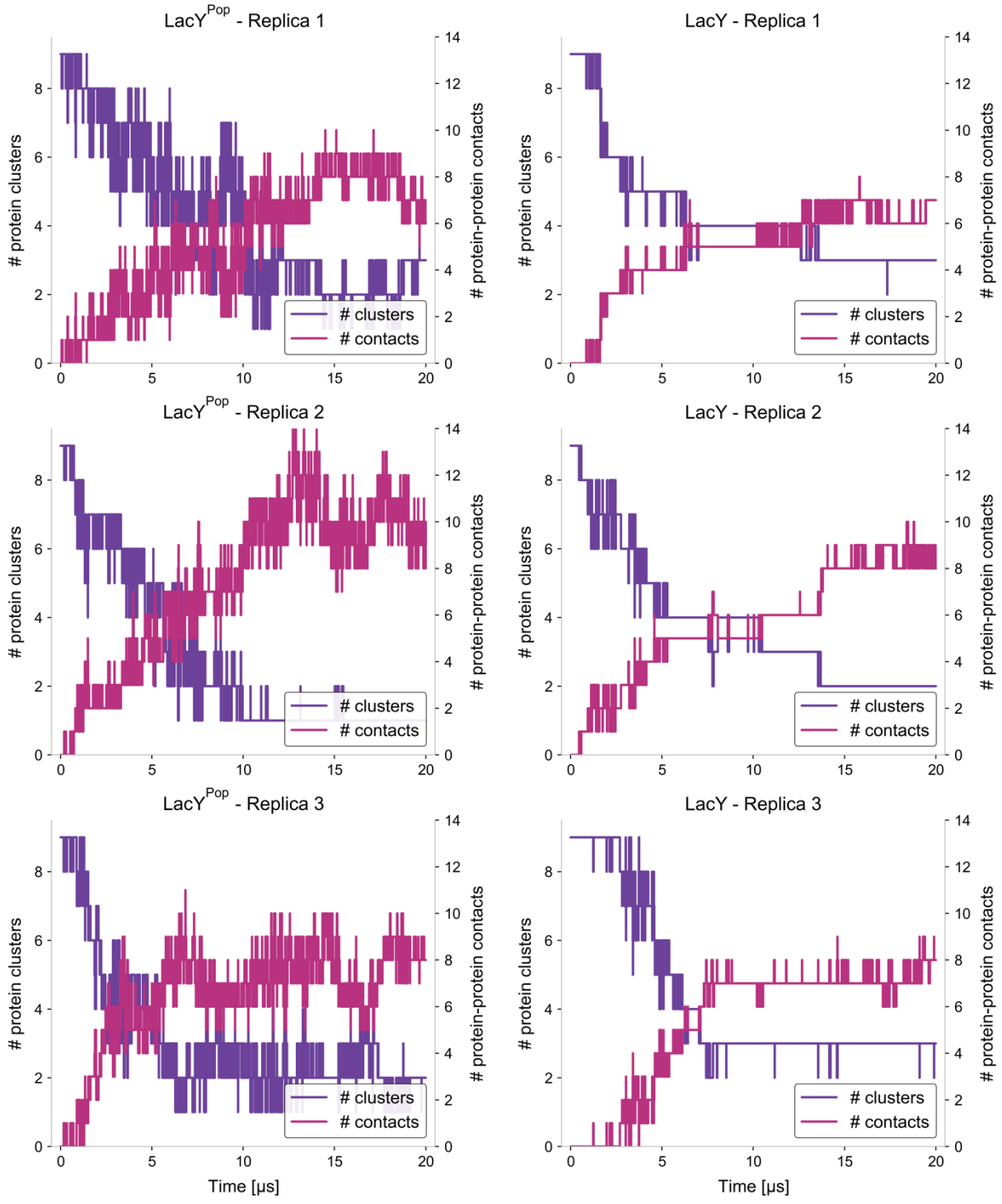


Figure S3. **Individual replicas showing clustering dynamics of LacY and LacY^{Pop}.** Time evolution of protein clustering metrics for all three simulation replicas of LacY^{Pop} (left column) and LacY (right column), showing their time evolution. Each panel shows the number of protein clusters (purple) and protein-protein contacts (magenta) over the 20 μs simulation time.

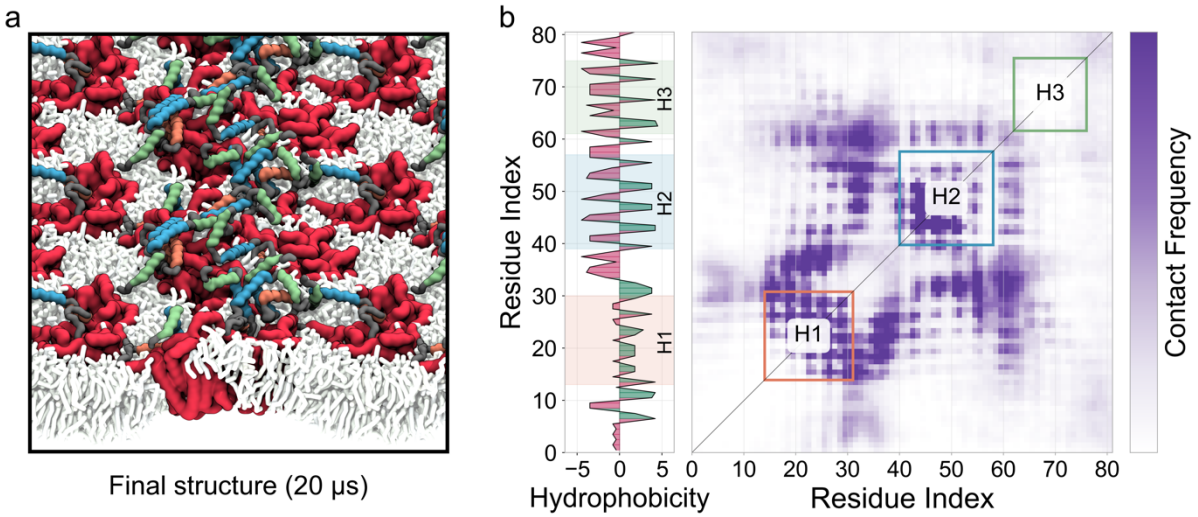


Figure S4. High-density simulation analysis of LacY^{Pop}. (a) Representative snapshot of the high-density LacY^{Pop} simulation (20 μs), initialized with PopTags in an extended conformation away from the membrane. LacY proteins (red) are embedded in the lipid bilayer (white) with PopTag domains colored by helical segments (helix 1 in orange, helix 2 in blue, helix 3 in green). **(b)** Contact map analysis of inter-PopTag interactions with corresponding hydrophobicity profile (left). The map shows significant interactions between helices, particularly involving helix 1 (orange box) and helix 2 (blue box), with helix 3 (green box) participating less. This pattern demonstrates how PopTag's amphipathic helices preferentially interact with each other rather than embedding in the membrane in this high-density configuration.

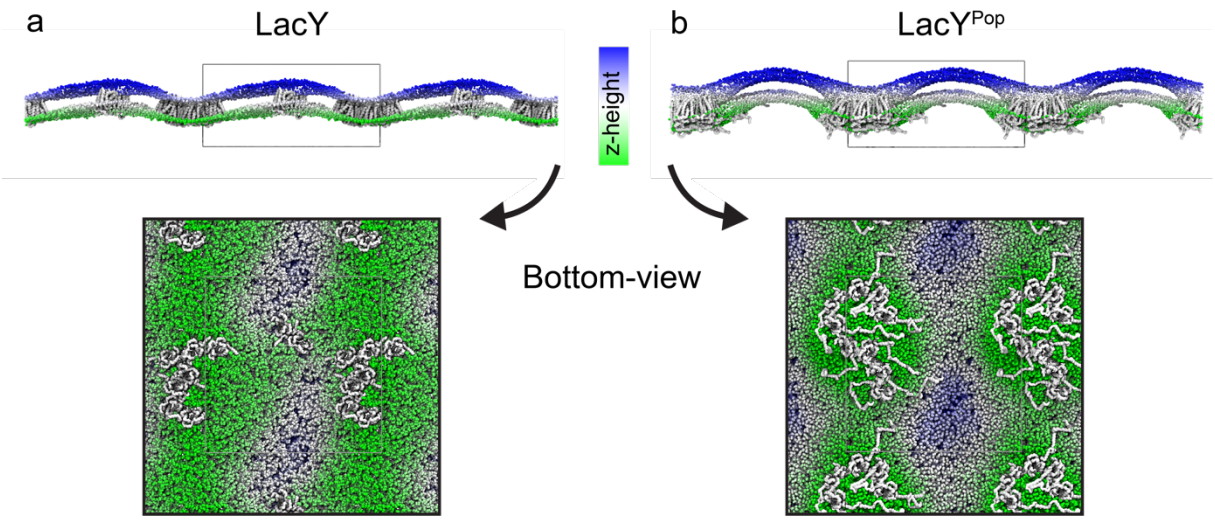


Figure S5. Membrane curvature comparison between LacY and LacY^{Pop}. Side view (top) and bottom view (bottom) of membrane deformation by **(a)** LacY and **(b)** LacY^{Pop} at the end of a 20 µs simulation. The membrane is colored according to z-height (green to blue). LacY^{Pop} induces more pronounced local membrane curvature compared to LacY.

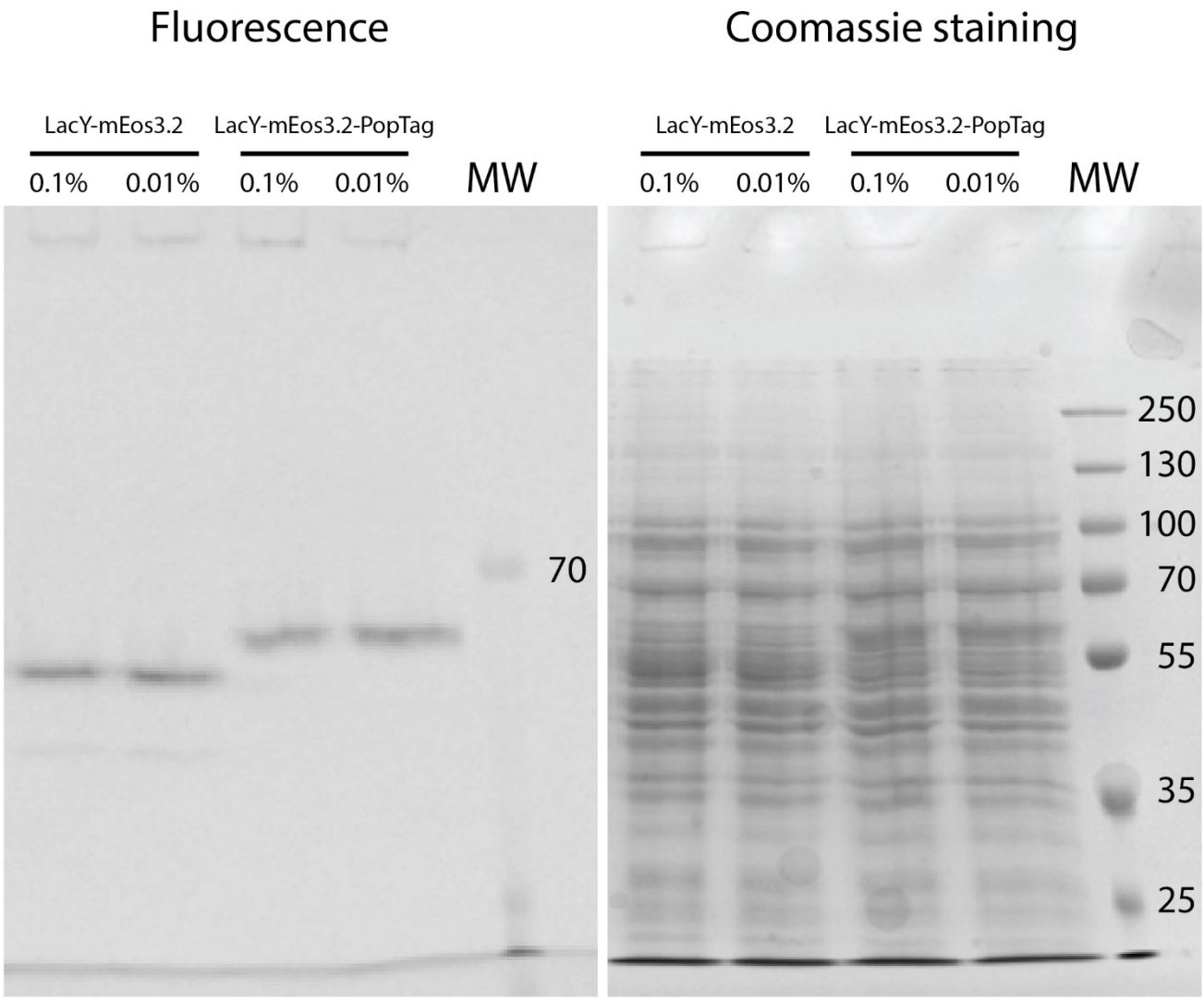


Figure S6. SDS-PAGE gel of LacY-expressing cells. Coomassie Brilliant Blue-stained gel (**right panel**) of *E. coli* BW25113 *ΔlacY* LacY^{mEos} and BW25113 *ΔlacY* LacY$^{mEos\text{-}Pop}$ lysates after 4h of arabinose induction. The in-gel fluorescence at 480 nm excitation (**left panel**) was used to evaluate the expression of tagged and non-tagged protein in cells. Arabinose concentrations of 0.01 and 0.1% w/v were tested. In all experiments, we observed similar expression levels of LacY-mEos3.2 and LacY-mEos3.2-PopTag, which we used to normalize the ^{14}C-lactose uptake data; the same cell samples were used for determining the uptake and expression levels. PageRuler™ Plus Prestained Protein Ladder (Thermo Scientific) was used as a marker of molecular weight.

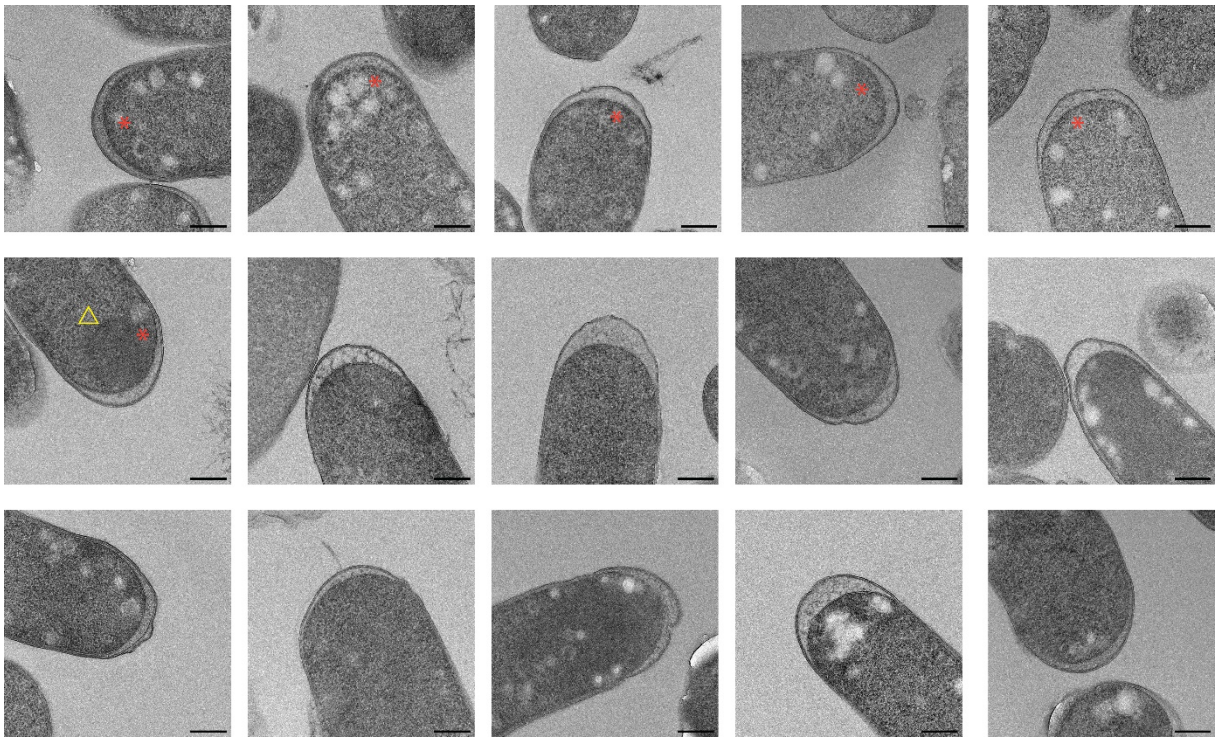
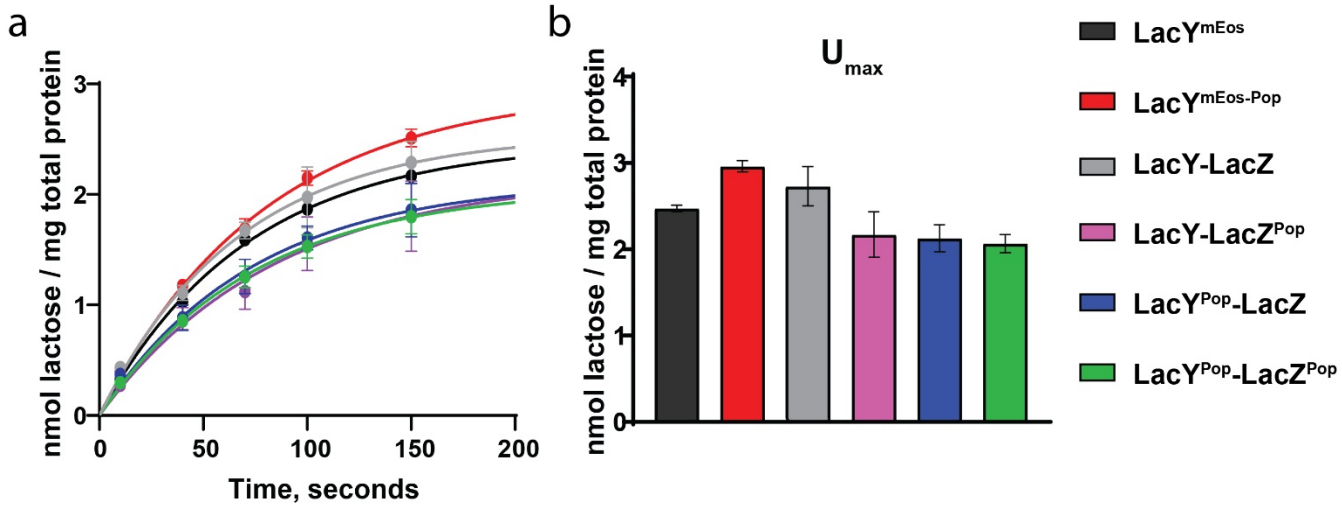


Figure S7. Transmission electron microscopy of BW25113 LacY$^{mEos\text{-}Pop}$ LacZ$^{mRuby\text{-}Pop}$ cells. Asterix signs are showing electron-dense regions at the cytoplasmic face of the inner membrane; yellow triangle shows large cytoplasmic electron-dense region. White spots are freezing artifacts. Scale bars are 200 nm.



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136 **Figure S8. ^{14}C-lactose uptake by *E. coli* cells co-expressing tagged (PopTag) and non-tagged**
137 **LacY and LacZ.** (a) Lactose uptake profiles of *E. coli* BW25113 ΔlacY expressing LacY^{mEos} and
138 LacY$^{mEos-Pop}$ alone or in a heterocondensates. (b) Maximum levels (U_{max}) of ^{14}C-lactose taken
139 up. Significance levels are presented as asterisk signs: (ns) for p>0.05 and (****) for p<0.0001.

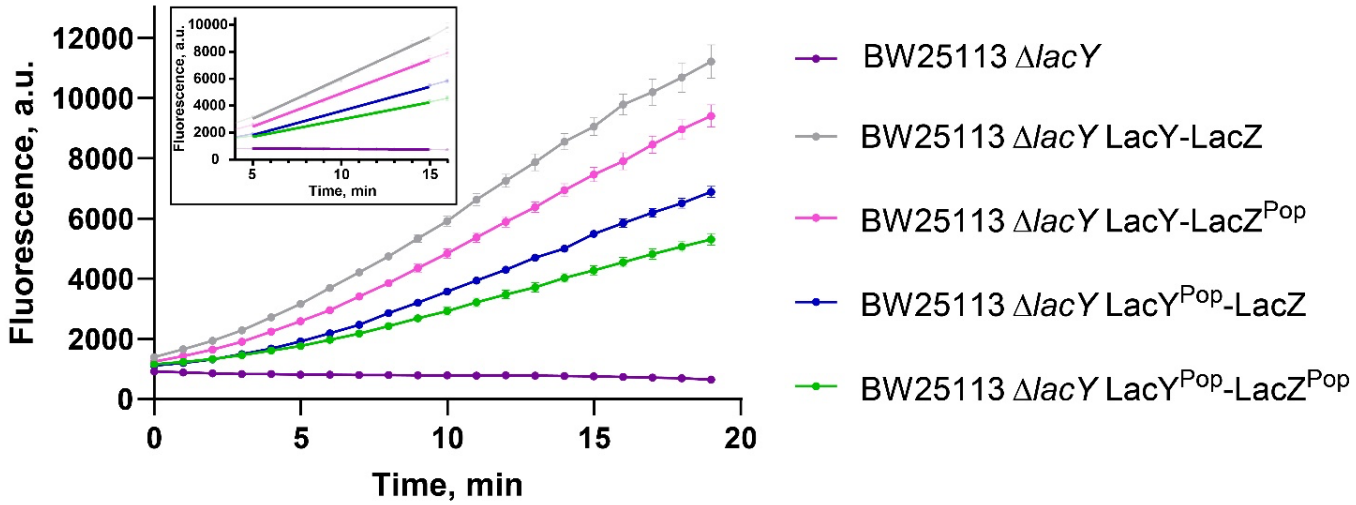

Figure S9. Increase of β-MUG emission at 445 nm upon cleavage by LacZ. The linear parts of the curves (inset: from 5 to 15 min) were used to calculate the *in vivo* β–galactosidase activity of *E. coli* BW25113 *ΔlacY* (r^2=0.19), negative control; BW25113 *ΔlacY* LacY-LacZ (r^2=0.92); BW25113 *ΔlacY* LacY-LacZ^{Pop} (r^2=0.92); BW25113 *ΔlacY* LacY^{Pop}-LacZ (r^2=0.95); and BW25113 *ΔlacY* LacY^{Pop}-LacZ^{Pop} (r^2=0.84). The initial non-liner increase in fluorescence (up to 5 min) is due to the temperature adjustment from 4 to 30 °C, and the slight leveling-off after 15 min is due to depletion of β-MUG (initial concentration was 50 μM). Spectra of β-MUG emission at t=0 and t=20 min are presented in Figure S8. Data presented as mean ± SEM, n = 4.

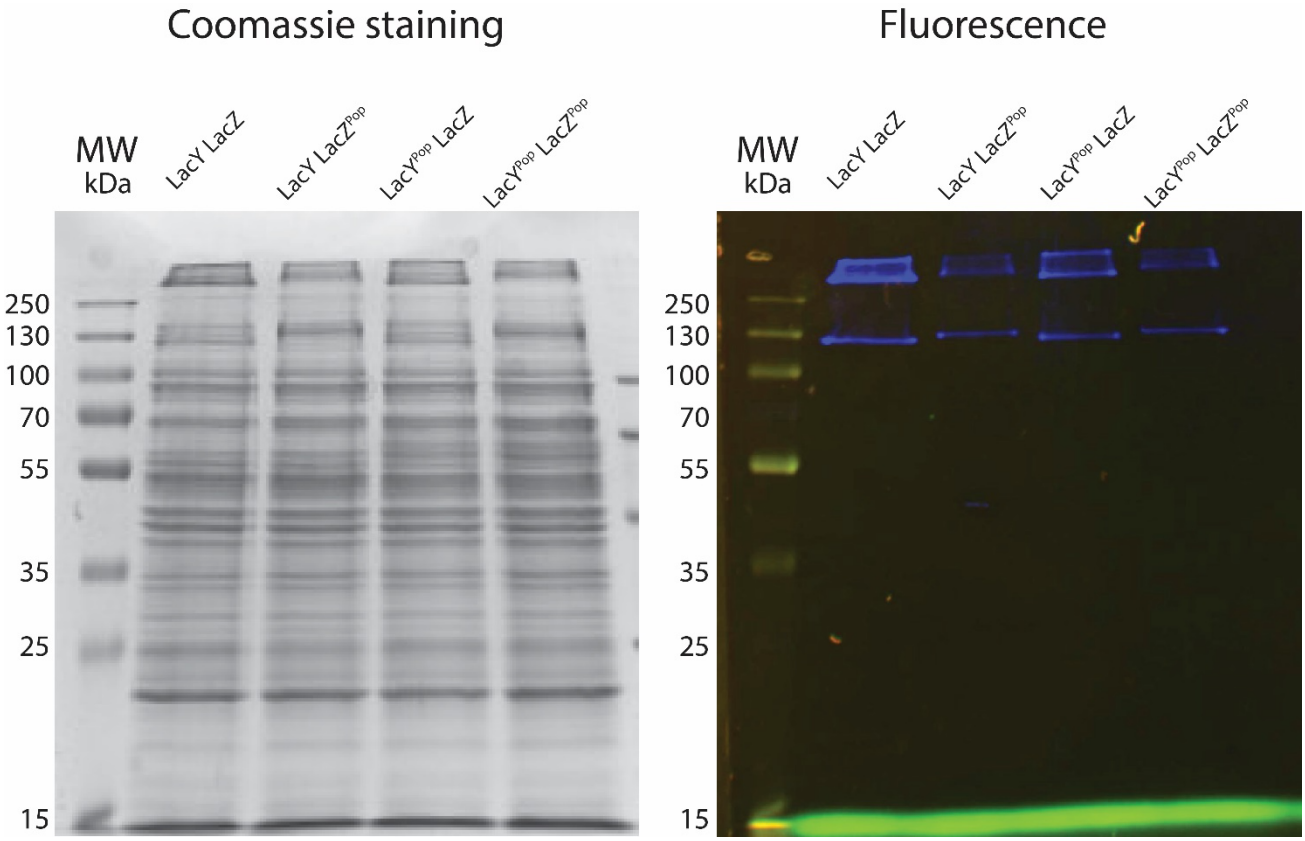

Figure S10. SDS-PAGE of LacY and LacZ co-expressing cells. Coomassie Brilliant Blue-stained gel (left panel) of *E. coli* BW25113 LacY^{mEos}-LacZ^{mRuby}, BW25113 LacY^{mEos}-LacZ$^{mRuby\text{-}Pop}$, BW25113 LacY$^{mEos\text{-}Pop}$-LacZ^{mRuby} and BW25113 LacY$^{mEos\text{-}Pop}$-LacZ$^{mRuby\text{-}Pop}$ lysates after 4h arabinose and rhamnose induction. The in-gel fluorescence at 560 nm excitation (right panel) was used to evaluate the amount of condensated and non-condensated LacZ-mRuby in cells. Mean fluorescence of all LacZ bands (~145 kDa monomer, dimer and tetramer) was used to determine the relative amounts of expressed protein. Arabinose and rhamnose concentrations of 0.1 and 0.000001% w/v, respectively, were tested. Relative expression levels of LacY-mEos3.2 and LacY-mEos3.2-PopTag are comparable to the values presented in Supplementary Figure 1 (Coomassie Brilliant Blue staining).

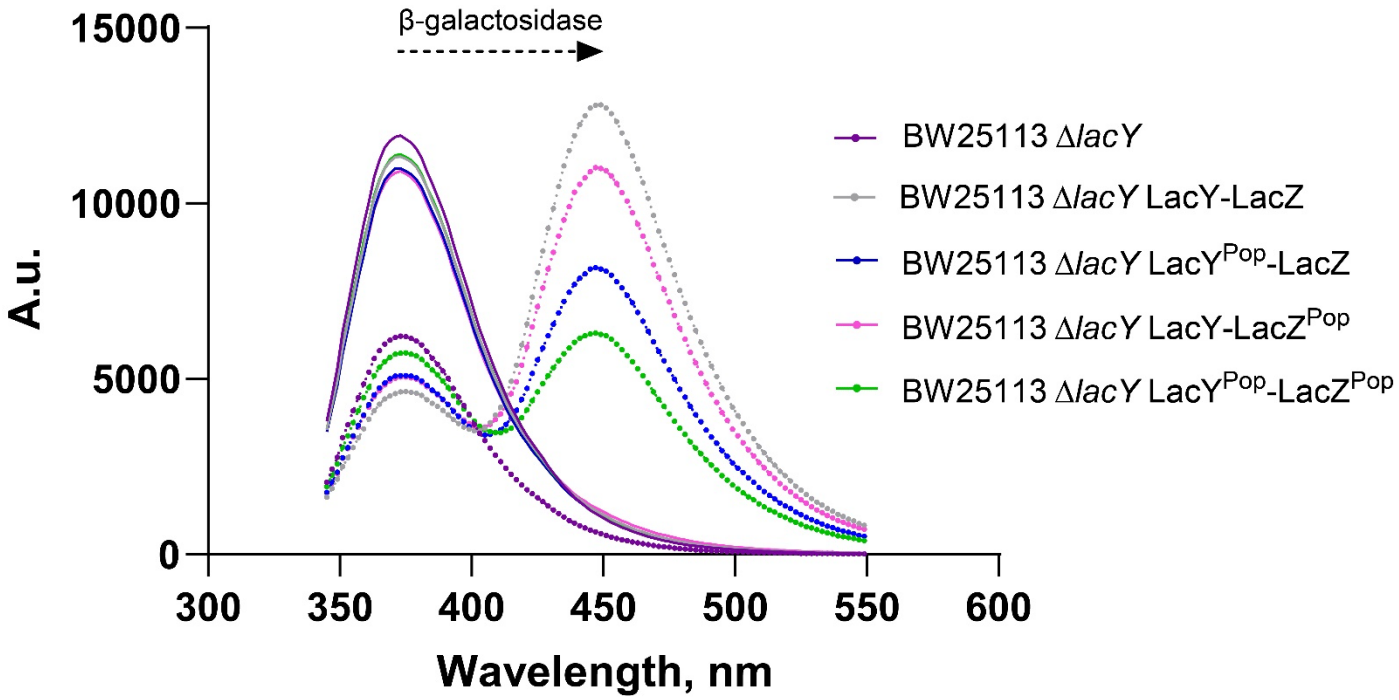

Figure S8. Emission spectra of β-MUG. Solid lines are spectra of β-MUG recorded in cells in 96-well plates at t=0. Dotted lines are the spectra after 20 min of incubation. The spectral shift to longer wavelength, from 375 to 445 nm, reflects β-galactosidase activity, which is not present in the negative control – *E. coli* BW25113 *ΔlacY*, lacking both *lacY* and *lacZ* genes. Data presented as mean values from n = 4 independent measurements.

Supplementary Table 1. Sequences of proteins used in this work. Linker sequences are marked in blue, mEos3.2 in green, mRuby is red and PopTag is colored magenta.

Name	Sequence
LacY-mEos3.2 ($LacY^{mEos}$)	MYYLKNTNFWMFGLFFFFYFFIMGAYFPFFPIWLHDINHISKSDTGIIFAAISLFSLLFQPLFGLLSDKLGL RKYLLWIITGMLVMFAPFFIFIFGPLLQYNILVGSIVGGIYLGFCFNAGAPAVEAFIEKVSRRSNFEFGRA RMFGCVGWALCASIVGIMFTINNQFVFWLGSGCALILAVLLFFAKTDAPSSATVANAVGANHSAFSL KLALELFRQPKLWFLSLYVIGVSCTYDVFDQQFANFFTSFFATGEQGTRVFGYVTTMGELLNASIMFFA PLIINRIGGKNALLLAGTIMSVRIIGSSFATSALEVVILKTLHMFEVPFLLVGCFKYITSQFEVRFSATIYLVC FCFFKQLAMIFMSVLAGNMYESIGFQGAYLVLGLVALGFTLISVFTLSGPGPLSLLRRQVNEVAGGTGG SGSAIKPDMKIKLRMEGNVNGHHFVIDGDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNR VFAKYPDNIQDYFKQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPVM QKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGVKLPGAHFVDHCIEILS HDKDYNKVKLYEHAVAHSGLPDNARR-
LacY-mEos3.2-PopTag ($LacY^{mEos\text{-}Pop}$)	MYYLKNTNFWMFGLFFFFYFFIMGAYFPFFPIWLHDINHISKSDTGIIFAAISLFSLLFQPLFGLLSDKLGL RKYLLWIITGMLVMFAPFFIFIFGPLLQYNILVGSIVGGIYLGFCFNAGAPAVEAFIEKVSRRSNFEFGRA RMFGCVGWALCASIVGIMFTINNQFVFWLGSGCALILAVLLFFAKTDAPSSATVANAVGANHSAFSL KLALELFRQPKLWFLSLYVIGVSCTYDVFDQQFANFFTSFFATGEQGTRVFGYVTTMGELLNASIMFFA PLIINRIGGKNALLLAGTIMSVRIIGSSFATSALEVVILKTLHMFEVPFLLVGCFKYITSQFEVRFSATIYLVC FCFFKQLAMIFMSVLAGNMYESIGFQGAYLVLGLVALGFTLISVFTLSGPGPLSLLRRQVNEVAGGTGG SGSAIKPDMKIKLRMEGNVNGHHFVIDGDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNR VFAKYPDNIQDYFKQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPVM QKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGVKLPGAHFVDHCIEILS HDKDYNKVKLYEHAVAHSGLPDNARRGSGSGSVAEQLVGVSAASAAASAFGSLSSALLMPKDGRTLE DVVRELLRPLLKEWLDQNLPRIVETKVEEEVQRISRGRGA-
LacY-PopTag ($LacY^{Pop}$)	MYYLKNTNFWMFGLFFFFYFFIMGAYFPFFPIWLHDINHISKSDTGIIFAAISLFSLLFQPLFGLLSDKLGL RKYLLWIITGMLVMFAPFFIFIFGPLLQYNILVGSIVGGIYLGFCFNAGAPAVEAFIEKVSRRSNFEFGRA RMFGCVGWALCASIVGIMFTINNQFVFWLGSGCALILAVLLFFAKTDAPSSATVANAVGANHSAFSL KLALELFRQPKLWFLSLYVIGVSCTYDVFDQQFANFFTSFFATGEQGTRVFGYVTTMGELLNASIMFFA PLIINRIGGKNALLLAGTIMSVRIIGSSFATSALEVVILKTLHMFEVPFLLVGCFKYITSQFEVRFSATIYLVC FCFFKQLAMIFMSVLAGNMYESIGFQGAYLVLGLVALGFTLISVFTLSGPGPLSLLRRQVNEVAGSGSG SVAEQLVGVSAASAAASAFGSLSSALLMPKDGRTLEDVVRELLRPLLKEWLDQNLPRIVETKVEEEVQR ISRGRGA-
LacZ-mRuby ($LacZ^{mRuby}$)	MTMITDSLAVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEARTDRPSQQLRSLNGEWRFAWF PAPEAVPESWLECDLPEADTVVVPSNWQMHGYDAPIYTNVTYPITVNPPFVPTENPTGCYSLTFNVD ESWLQEGQTRIIFDGVNSAFHLWCNGRWVGYGQDSRLPSEFDLSAFLRAGENRLAVMVLRWSDGS YLEDQDMWRMSGIFRDVSLLHKPTTQISDFHVATRFNDDFSRAVLEAEVQMCGELRDYLRVTVSLW QGETQVASGTAPFGGEIIDERGGYADRVTLRLNVENPKLWSAEIPNLYRAVVELHTADGTLIEAEACD VGFREVRIENGLLLLNGKPLLIRGVNRHEHHPLHGQVMDEQTMVQDILLMKQNNFNAVRCSHYPNH PLWYTLCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRMVQRDRNHPSVIIWSLGN ESGHGANHDALYRWIKSVDPSRPVQYEGGGADTTATDIICPMYARVDEDQPFPAVPKWSIKKWLSL PGETRPLILCEYAHAMGNSLGGFAKYWQAFRQYPRLQGGFVWDWVDQSLIKYDENGNPWSAYGG DFGDTPNDRQFCMNGLVFADRTPHPALTEAKHQQQFFQFRLSGQTIEVTSEYLFRHSDNELLHWMV ALDGKPLASGEVPLDVAPQGKQLIELPELPQPESAGQLWLTVRVVQPNATAWSEAGHISAWQQWR LAENLSVTLPAASHAIPHLTTSEMDFCIELGNKRWQFNRQSGFLSQMWIGDKKQLLTPLRDQFTRAPL DNDIGVSEATRIDPNAWVERWKAAGHYQAEAALLQCTADTLADAVLITTAHAWQHQGKTLFISRKT YRIDGSGQMAITVDVEVASDTPHPARIGLNCQLAQVAERVNWLGLGPQENYPDRLTAACFDRWDLP LSDMYTPYVFPSENGLRCGTRELNYGPHQWRGDFQFNISRYSQQQLMETSHRHLLHAEEGTWLNID GFHMGIGGDDSWSPSVSAEFQLSAGRYHYQLVWCQKGGTGGSNSLIKENMRMKVVLEGSVNGHQ FKCTGEGEGNPYMGTQTMRIKVIEGGPLPFAFDILATSFMYGSRTFIKYPKGIPDFFKQSFPEGFTWER VTRYEDGGVITVMQDTSLEDGCLVYHAQVRGVNFPSNGAVMQKKTKGWEPNTEMMYPADGGLR GYTHMALKVDGGGHLSCSFVTTYRSKKTVGNIKMPGIHAVDHRLERLEESDNEMFVVQREHAVAKF AGLGGG-
LacZ-mRuby-PopTag ($LacZ^{mRuby\text{-}Pop}$)	MTMITDSLAVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEARTDRPSQQLRSLNGEWRFAWF PAPEAVPESWLECDLPEADTVVVPSNWQMHGYDAPIYTNVTYPITVNPPFVPTENPTGCYSLTFNVD ESWLQEGQTRIIFDGVNSAFHLWCNGRWVGYGQDSRLPSEFDLSAFLRAGENRLAVMVLRWSDGS YLEDQDMWRMSGIFRDVSLLHKPTTQISDFHVATRFNDDFSRAVLEAEVQMCGELRDYLRVTVSLW

	QGETQVASGTAPFGGEIIDERGGYADRVTLRLNVENPKLWSAEIPNLYRAVVELHTADGTLIEAEACD VGFREVRIENGLLLLNGKPLLIRGVNRHEHHPLHGQVMDEQTMVQDILLMKQNNFNAVRCSHYPNH PLWYTLCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRMVQRDRNHPSVIIWSLGN ESGHGANHDALYRWIKSVDPSRPVQYEGGGADTTATDIICPMYARVDEDQPFPAVPKWSIKKWLSL PGETRPLILCEYAHAMGNSLGGFAKYWQAFRQYPRLQGGFVWDWVDQSLIKYDENGNPWSAYGG DFGDTPNDRQFCMNGLVFADRTPHPALTEAKHQQQFFQFRLSGQTIEVTSEYLFRHSDNELLHWMV ALDGKPLASGEVPLDVAPQGKQLIELPELPQPESAGQLWLTVRVVQPNATAWSEAGHISAWQQWR LAENLSVTLPAASHAIPHLTTSEMDFCIELGNKRWQFNRQSGFLSQMWIGDKKQLLTPLRDQFTRAPL DNDIGVSEATRIDPNAWVERWKAAGHYQAEAALLQCTADTLADAVLITTAHAWQHQGKTLFISRKT YRIDGSGQMAITVDVEVASDTPHPARIGLNCQLAQVAERVNWLGLGPQENYPDRLTAACFDRWDLP LSDMYTPYVFPSENGLRCGTRELNYGPHQWRGDFQFNISRYSQQQLMETSHRHLLHAEEGTWLNID GFHMGIGGDDSWSPSVSAEFQLSAGRYHYQLVWCQKGGTGGSNSLIKENMRMKVVLEGSVNGHQ FKCTGEGEGNPYMGTQTMRIKVIEGGPLPFAFDILATSFMYGSRTFIKYPKGIPDFFKQSFPEGFTWER VTRYEDGGVITVMQDTSLEDGCLVYHAQVRGVNFPSNGAVMQKKTKGWEPNTEMMYPADGGLR GYTHMALKVDGGGHLSCSFVTTYRSKKTVGNIKMPGIHAVDHRLERLEESDNEMFVVQREHAVAKF AGLGGGGSGSGSVAEQLVGVSAASAAASAFGSLSSALLMPKDGRTLEDVVRELLRPLLKEWLDQNLP RIVETKVEEEVQRISRGRGA-
LacZ-PopTag (LacZ^{Pop})	MTMITDSLAVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEARTDRPSQQLRSLNGEWRFAWF PAPEAVPESWLECDLPEADTVVVPSNWQMHGYDAPIYTNVTYPITVNPPFVPTENPTGCYSLTFNVD ESWLQEGQTRIIFDGVNSAFHLWCNGRWVGYGQDSRLPSEFDLSAFLRAGENRLAVMVLRWSDGS YLEDQDMWRMSGIFRDVSLLHKPTTQISDFHVATRFNDDFSRAVLEAEVQMCGELRDYLRVTVSLW QGETQVASGTAPFGGEIIDERGGYADRVTLRLNVENPKLWSAEIPNLYRAVVELHTADGTLIEAEACD VGFREVRIENGLLLLNGKPLLIRGVNRHEHHPLHGQVMDEQTMVQDILLMKQNNFNAVRCSHYPNH PLWYTLCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRMVQRDRNHPSVIIWSLGN ESGHGANHDALYRWIKSVDPSRPVQYEGGGADTTATDIICPMYARVDEDQPFPAVPKWSIKKWLSL PGETRPLILCEYAHAMGNSLGGFAKYWQAFRQYPRLQGGFVWDWVDQSLIKYDENGNPWSAYGG DFGDTPNDRQFCMNGLVFADRTPHPALTEAKHQQQFFQFRLSGQTIEVTSEYLFRHSDNELLHWMV ALDGKPLASGEVPLDVAPQGKQLIELPELPQPESAGQLWLTVRVVQPNATAWSEAGHISAWQQWR LAENLSVTLPAASHAIPHLTTSEMDFCIELGNKRWQFNRQSGFLSQMWIGDKKQLLTPLRDQFTRAPL DNDIGVSEATRIDPNAWVERWKAAGHYQAEAALLQCTADTLADAVLITTAHAWQHQGKTLFISRKT YRIDGSGQMAITVDVEVASDTPHPARIGLNCQLAQVAERVNWLGLGPQENYPDRLTAACFDRWDLP LSDMYTPYVFPSENGLRCGTRELNYGPHQWRGDFQFNISRYSQQQLMETSHRHLLHAEEGTWLNID GFHMGIGGDDSWSPSVSAEFQLSAGRYHYQLVWCQKGSGSGSVAEQLVGVSAASAAASAFGSLSSA LLMPKDGRTLEDVVRELLRPLLKEWLDQNLPRIVETKVEEEVQRISRGRGA-

182

183 **Supplementary Table 2. List of primers used in the work.**

№	Used for	Template	Direction	5’ -> 3’ sequence
1	Amplification of *lacY* gene	pACYC_LacY-mEos3.2	Forward	aattaaccauggtggtggatttg
2			Reverse	atccaccagtuccaccagcgacttcattcac
3	Amplification of pBAD vector with *mEos3.2* sequence for *lacY* insertion	pBAD_mEos3.2	Forward	aactggtggauccggtagc
4			Reverse	atggttaatucctcctgtt
5	Amplification of *PopTag* sequence from *popZ* gene	pMA-RQ_popZ-mRuby	Forward	agcggaagcguggcagaacagctg
6			Reverse	agatcttttatuaggcaccacgacc
7	Amplification of pBAD vector with *lacY-mEos3.2* gene for *PopTag* insertion	pBAD_LacY-mEos3.2	Forward	aataaaagatcugcagctgg
8			Reverse	acgcttccgcutccgcttccacgacgtgcattatc
9	Removing *mEos3.2* gene from *lacY-mEos3.2* fusion	pBAD_LacY-mEos3.2	Forward	aataaaagatcugcagctgg
10			Reverse	agatcttttatuaagcgacttcattcacctg
11	Removing *mEos3.2* gene from *lacY-mEos3.2-PopTag* fusion	pBAD_LacY-mEos3.2-PopTag	Forward	agcggaagcguggcagaacagctg
12			Reverse	acgcttccgcutccagaaccagcaacttcattcacctgacgacgc
13	Amplification of *lacZ* gene	Genomic DNA of *E. coli* W3110	Forward	atgaccatgatuacggatt
14			Reverse	agggatcctuatttttgacaccagaccaactg
15	Amplification of pACYC vector for *lacZ* insertion	pACYC_LacY-mEos3.2	Forward	aataaggatcccugcgccc
16			Reverse	aatcatggtcauggtcgaacacctcctg
17	Amplification of *mRuby* gene	pMA-RQ_popZ-mRuby	Forward	atggtgtcaaaaagguggcacaggtggtagc
18			Reverse	agggatccttatuaaccaccacctaaacctg
19	Amplification of pACYC vector with *lacZ* gene for *mRuby* insertion	pACYC_LacZ	Forward	aataaggatcccugcgccc
20			Reverse	accttttgacaccauaccaactggtaatgg
21	Amplification of *PopTag* sequence from *popZ* gene	pBAD_LacY-mEos3.2-PopTag	Forward	aagtggaaguggaagcgtggcagaaca
22			Reverse	agggatccttatuaggcaccacgaccac
23	Amplification of pACYC vector with *lacZ-mRuby* gene for *PopTag* insertion	pACYC_LacZ-mRuby	Forward	aataaggatcccugcgccc
24			Reverse	acttccactuccaccaccacctaaacctgc
25	Removing *mRuby* gene from *lacZ-mRuby-PopTag* fusion	pACYC_LacZ-mRuby-PopTag	Forward	aagtggaaguggaagcgtggcagaaca
26			Reverse	acttccactucctttttgacaccataccaact
27	Amplification of *lacY-mEos3.2-PopTag* gene for insertion to pAYCY vector	pBAD_LacY-mEos3.2-PopTag	Forward	atgaagtcgcuggtggaac
28			Reverse	agatcttttatuaggcaccacgacc
29	Amplification of pBAD vector for insertion of *lacY-mEos3.2-PopTag* gene	pACYC_LacY-mEos3.2	Forward	aataaaagatcuaaggatccccgcgc
30			Reverse	agcgacttcautcacctgac

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