SUPPLEMENTARY INFORMATION

Screening for transmembrane association in divisome proteins using TOXGREEN, a high-throughput variant of the TOXCAT assay

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Table S1 Test constructs used in this study

Name	Sequence
GpA	LIIFGVMAGVIGT
GpA*	LIIFGVMAIVIGT
PXDC2	LLLLLLLGLLLGILLLVL
F173B	LLLLLLLFLLLGLLLGTL
ANPRC	LLLLLLLSALLGILLGAL
EPHB6	LLLLLLLSLLLGSLLGAL
MUC13	LLLFQLLLTLLGTLLGIL
TNR1B	LLLTGLLALLLGLLLGVL
HFE	LLLTLLLGVLLGILLFVL
SPTC3	LLLFTLLGYLLGTLLGYL
STAB1	LLLVLLLGALLGLLLGAL
MUC15	LLLGILLGALLGALLGVL
ADCK4	LLLLLLLANLLGLLLGLL
ACV1B	LLLLLLLVELLGILLGAL
TNR12	LLLALLLTFLLGLLLGFL
STX17	LLLAALLGGLLGFLLGKL
ROMO1	LLLGFLLGCLLGMLLGAL
VAS1	LLLFFLLGILLGLLLSLL

The inserts sequence were digested with NheI and DpnII and ligated into the compatible NheI-BamHI restriction sites of the pccKAN and pccGFPKAN, plasmids to produce the sequence "...NRAS-TM-ILIN..." The sequences are part of a library of previously studied TOXCAT constructs, which contain predicted helix-helix interfaces from human single-span transmembrane proteins [1], covering a range of CAT expression levels from approximately 25% to 175% relative to the CAT expression of the GpA standard.

Table S2 Divisome sequences used in this study

Bacterial Species	Uniprot code	TM Sequence
FtsB		
Escherichia coli	P0A6S5	LTLLLLAILVWLQYSLWF
Yersinia pestis	Q1C479	LTLLLLVLLGWLQYSLWL
Vibrio cholerae	Q9KUJ3	FALTLSLLLVWLLYTLMW
Haemophilus influenzae	P44035	LILILLSVLVLFQYNFWF
Legionella pneumophila	Q5WV01	IFIILIVALVALQHKLWL
Neisseria meningitidis	P64161	VTVVLSFALVCCQYSLWF
Caulobacter crescentus	Q9A7J8	FLPTTAIFFLIFYFAFHAL
Rickettsia prowazekii	Q9ZDA9	IILNIFLALLLVYFIFHCIY
Bacillus subtilis	P37471	LTVFGALVFLTAIVLASSV
Streptococcus pneumoniae	Q8CZD2	FMGGVLILIMLLFILPTFNLA
Streptococcus pyogenes	Q9A203	FMGWILVSMMFLFILPTYNLV
FtsQ		
Escherichia coli	P06136	ILFLLTVLTTVLVSGWVVLG
Yersinia pestis	Q7CGB1	VIFLLMVLGTILWGGWVVIGW
Vibrio cholerae	Q9KPG9	FFLVVLLLIGGLLYSTISWMW
Haemophilus influenzae	P45067	FAVLLGVFFLLGVYFNWQSIL
Legionella pneumophila	Q5ZSA8	GLRYLTIMGLLILSALLLAGRLG
Neisseria meningitidis	X5EU48	WLLVMMAMLLAASGLVWFY
Caulobacter crescentus	P0CAU8	VALSVAGAALGLGLVVMLATG
Rickettsia prowazekii	Q9ZDS5	ILVLKIVLMIFVCLFVFTKYF
Bacillus subtilis	P16655	ISFIMLFFIMVLIIVYLQTPI
Streptococcus pneumoniae	Q97RU7	FTILFPSLLLLFVSAYLLSP
Streptococcus pyogenes	Q1JG12	LPVLLGALLLMAVSIFMITPY
FtsI		
Escherichia coli	P0AD68	FALLCGCILLALAFLLG
Yersinia pestis	A0A0E1NWU7	FALLCGCILLALVGLIM
Vibrio cholerae	A0A085PFW1	FYLLLFFVLTAFCALVA
Haemophilus influenzae	P45059	YMLSTVLILLGLCALVA
Legionella pneumophila	A0A130BGB2	LVTVAVFFSLILAVLIW
Neisseria meningitidis	E0N6Q4	ISFVLMAIAVLFAGLIA
Caulobacter crescentus	B8H0A0	LVMGFFGFCFVGVSLGAG
Rickettsia prowazekii		
Bacillus subtilis	Q07868	GAAILSICFALFFFVILG
Streptococcus pneumoniae	Q75Y83	LSLLSVFVFAIFLVNFAVIIG
Streptococcus pyogenes	Q1J5G0	MMLLTIFIFFIFIINFMIIIG

Bacterial Species	Uniprot code	TM Sequence
FtsL		
Escherichia coli	P0AEN6	FGKLPLCLFICIILTAVTVVTTA
Yersinia pestis	Q7CGA7	LILLVAVLISAVLVVTTA
Vibrio cholerae	Q9KPG0	LLLLVLIFSCAMGVVFMT
Haemophilus influenzae	P45058	LVVLLLIGILVSAMGTIWIT
Legionella pneumophila	Q5WXZ3	LYMLIVLLAAVLVSAFAVIY
Neisseria meningitidis	Q7DDQ4	FLLLLAVCVSAFSVVM
Caulobacter crescentus	Q9RQJ5	VVEVVGLCILLSLVTGVYLA
Rickettsia prowazekii	D5AXG5	FHYLILFITIIAICSLF
Bacillus subtilis	Q07867	VLLVLFAAAVLSVSLLIVS
Streptococcus pneumoniae	Q8DR70	FYFSIAVTTLIVAISIIFM
Streptococcus pyogenes	Q99YK0	AFYTAIIVTAITMAVSIIYL
ZipA/ErzA		
Escherichia coli	P77173	LILIIVGAIAIIALLVHGFWT
Yersinia pestis	P58492	LILIVVGAIAIIALLLHGLWT
Vibrio cholerae	Q9KTD2	FVLIIVGALAIAALLFHGLWT
Haemophilus influenzae	P44113	TILIIVGIVALVALIVHGLWS
Legionella pneumophila	Q5ZSA0	SLILNVLLLIGVLVAIGRLM
Neisseria meningitidis	A0A0H5QES0	YIVLFLAAVLAVVAYNMY
Caulobacter crescentus		
Rickettsia prowazekii		
Bacillus subtilis	O34894	FVIGLLIVLLALFAAGYFF
Streptococcus pneumoniae	Q8DQE5	LMVAIAVILVLAYVVAIFL
Streptococcus pyogenes	P0DA99	LIVAIVLLVIIAYLVGVII
FtsN		
Escherichia coli	P29131	AMVAIAAAVLVTFIGGL
Yersinia pestis	Q7CL21	TVMALAVALLVVFVGGL
Vibrio cholerae	A0A085TJQ3	GLVAIVLLAVFGYGLYLL
Haemophilus influenzae	A0A0N1EX29	VLIFLALFIVLVFVVGLYLL
Legionella pneumophila	A0A0C9P799	LVVIVTFLFGYITASFL
Neisseria meningitidis	X5F9A7	GLSGFFFGLILATVIIAGIL
Caulobacter crescentus	B8GX61	LIISAVVLVTLVVAVVMIL
Rickettsia prowazekii	Q9ZE80	ICLVSLICISGIYFGYQYYQ
Bacillus subtilis		
Streptococcus pneumoniae		
Streptococcus pyogenes		

The inserts sequence were digested with NheI and DpnII and ligated into the compatible NheI-BamHI restriction sites of the pccKAN and pccGFPKAN, plasmids to produce the sequence "...NRAS-TM-ILIN..." The bacterial sequences were selected as identified in the UniProt database [2] and their TM segments were selected as annotated in the TRANSMEM feature of their UniProt record.

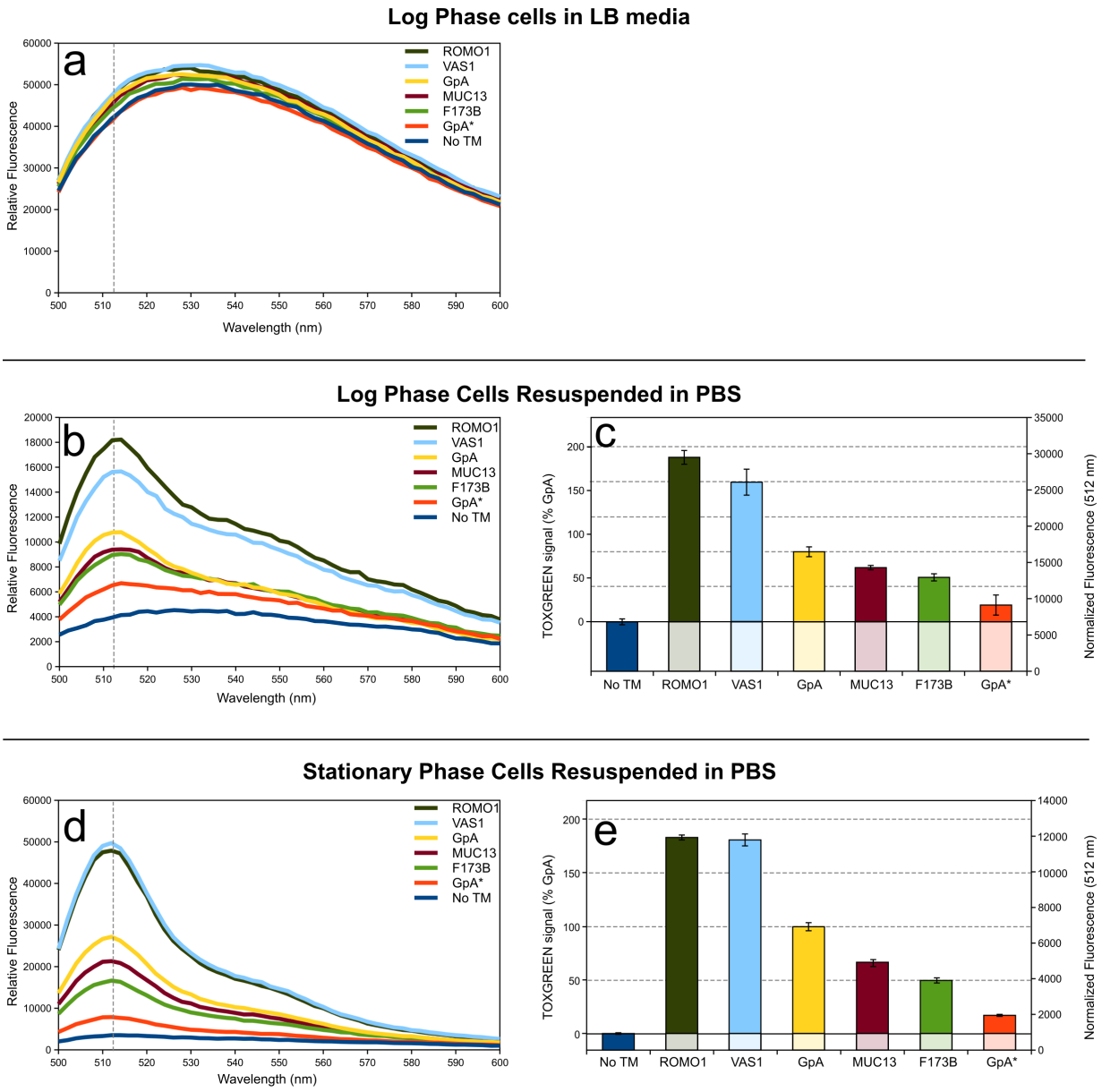

Figure S1. Fluorescence emission scans and TOXGREEN signal calculation in different cell growth conditions. Fluorescence measurements of seven TOXGREEN constructs, including the "no-TM" control, as in Fig. 2. The dashed vertical line indicates the readout wavelength used (512 nm). a) Fluorescence spectra of cells in log phase measured directly in LB culture media. No fluorescent signal is apparent. b) Fluorescence spectra of the same log phase cells harvested by centrifugation and resuspended in the same volume of PBS buffer. c) Conversion of the log phase cell's fluorescence in PBS at 512 nm to TOXGREEN signal. d) Spectra of stationary phase cells, harvested by centrifugation and resuspended in the same volume of PBS buffer. Compared to the same cells in LB culture media (Fig. 2c) the background fluorescence (no-TM construct) is noticeably lower. e) Conversion of stationary phase cell's fluorescence to TOXGREEN signal.

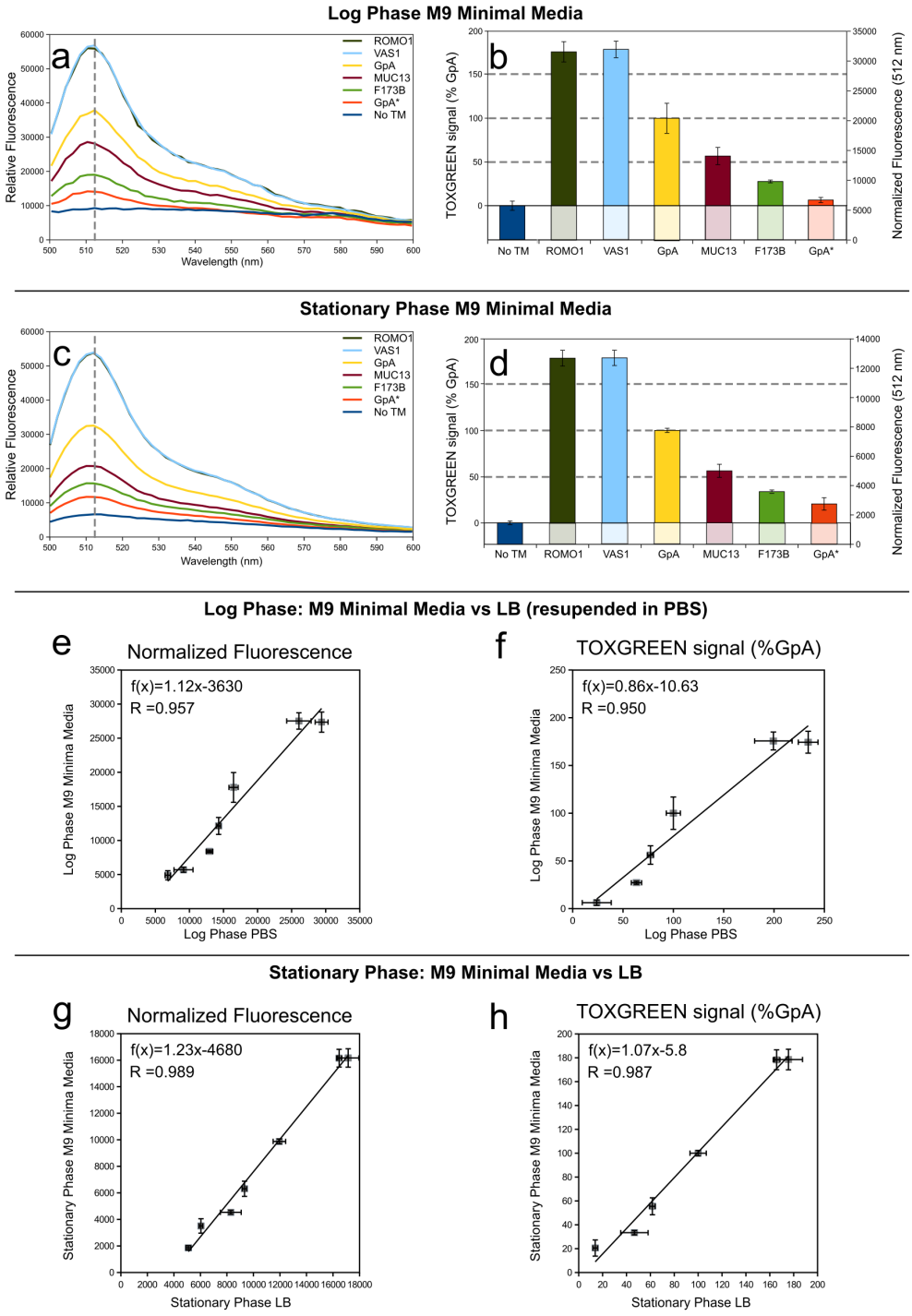

Figure S2. Direct measurements in M9 minimal media in log and stationary phases. a) Fluorescence spectra of cells in log phase grown in M9 minimal media. b) Conversion of log phase cell's fluorescence at 512 nm to TOXGREEN signal. c) Spectra of stationary phase cells grown in M9 minimal media. d) Conversion of stationary phase cell's fluorescence to TOXGREEN signal. e) Comparison of fluorescence of log phase cells grown in M9 minimal media and log phase cells in resuspended in 1X PBS buffer after normalization to cell density. f) Comparison of relative TOXGREEN signal for log phase grown in M9 minimal and log phase cells in 1X PBS buffer. g) Comparison of fluorescence of stationary phase cells grown in M9 minimal media and stationary phase cells in LB after normalization to cell density. h) Comparison of relative TOXGREEN signal for stationary phase grown in M9 minimal and stationary phase cells in LB.

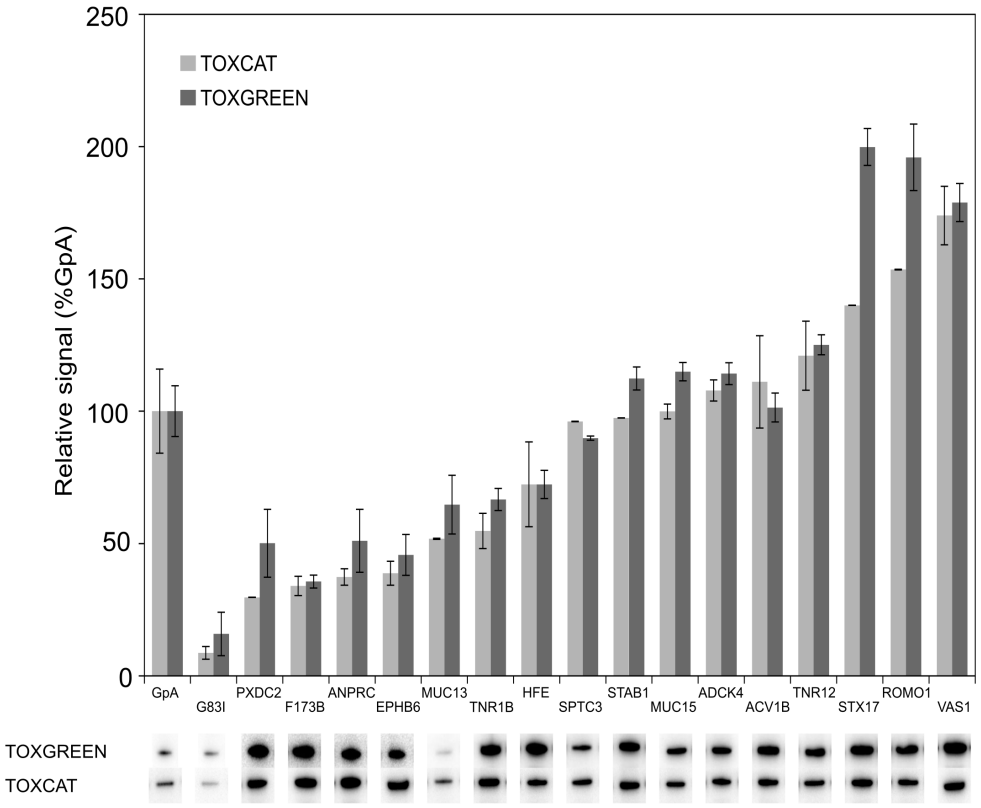

Figure S3. TOXGREEN and TOXCAT are in excellent agreement. Histogram representation of the data reported as XY plot in Fig. 3. Comparison of reporter gene expression between TOXCAT (measured as CAT enzymatic activity in lysates) and TOXGREEN (measured as fluorescence intensity whole cells in stationary phase). The values have been normalized to their respective value of the GpA sample (100%). The expression level of the TOXGREEN and TOXCAT chimeric constructs was assessed by immunoblotting using anti-MBP antibodies (bands displayed under the histogram). Constructs containing the same transmembrane sequence have a similar level of expression in the two assays.

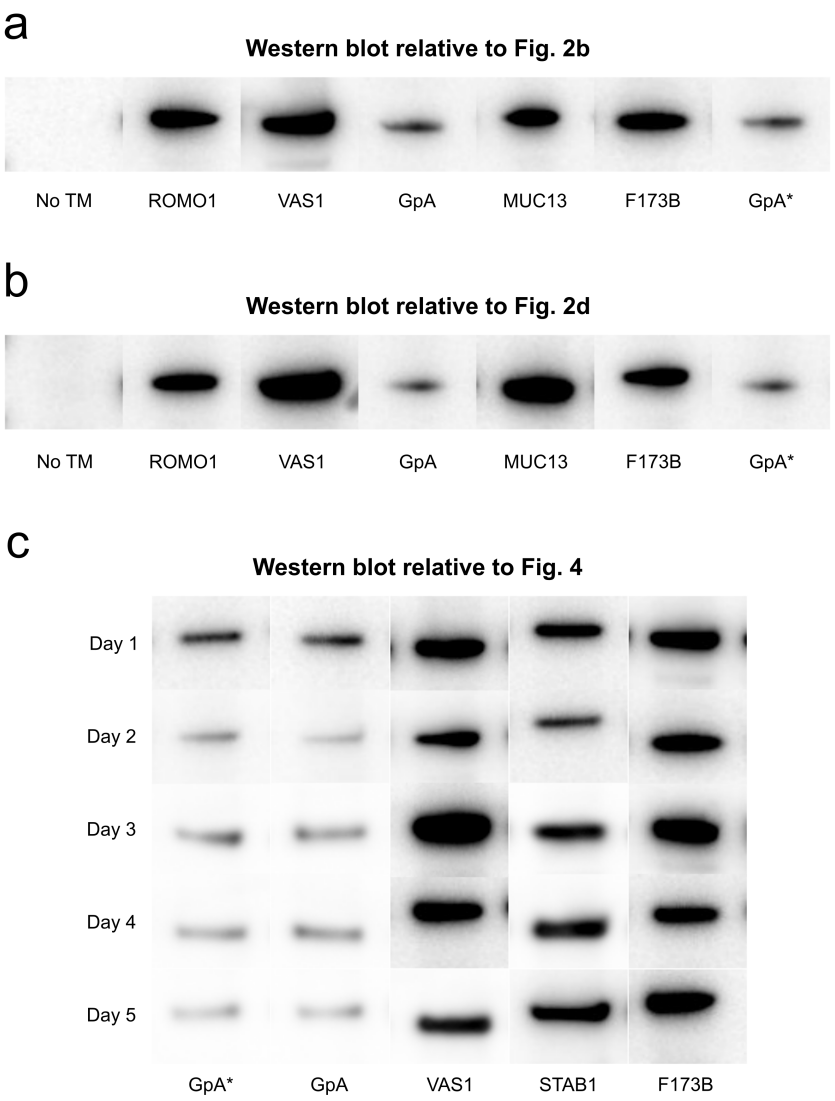

Figure S4. Western blots of log and stationary phase cell cultures (Fig. 2) and of multi-day variability tests (Fig. 4). Immunoblotting using anti-MBP antibodies to control for the expression level of the chimeras for the seven constructs used for TOXGREEN testing. a) Immunoblots of log phase cells concentrated in 3× PBS. b) Immunoblots of stationary phase cells in LB. c) Immunoblots of stationary phase cells in LB, taken over 5 days.

References

[1] B.K. Mueller, S.M. Anderson, S. Subramaniam, E. Lange, A. Senes, in preparation (2016).
[2] UniProt Consortium, UniProt: a hub for protein information, Nucleic Acids Res., 43 (2015) D204-212.