Supplemental material

Structure-function analysis of MurJ reveals a solvent-exposed cavity containing residues essential for peptidoglycan biogenesis in *Escherichia coli*

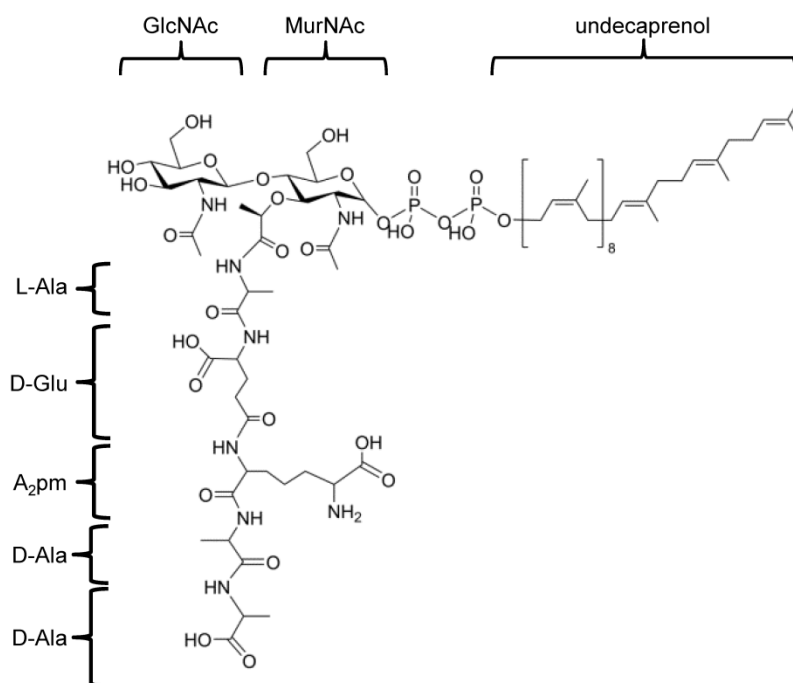Emily K. Butler, Rebecca M. Davis, Vase Bari, Paul A. Nicholson, Natividad Ruiz

FIGURE S1. Lipid II structure. In the *E. coli* lipid II, the C55 isoprenoid carrier molecule undecaprenol is linked by a pyrophosphate to the disaccharide-pentapeptide moiety composed of GlcNAc, MurNAc, and the L-Ala-γ-D-Glu-A$_2$pm-D-Ala-D-Ala stem peptide. GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylmuramic acid; A$_2$pm, *meso*-diaminopimelic acid.

A

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MurJ/1-439      1 - - - - - - - - MNLLKSL - - - - - - - - - - - - - AAVSSMTMF - - - - - - - - - - - - - - - - - - - - - -  16
PfMATE/1-461    1 MSEKTTKGVQLLRGDPKKAIVRLSIPMMIGMSVQTLYNLADGIWVSGLGPESLAAVGLFF  60

MurJ/1-439     17 - - - - - - - - - SRVLGFARDAIVARIFGAGMATDA - - - - - - - - - - - - - - - - - - - - - - - - - -  40
PfMATE/1-461   61 PVFMGIIALAAGLGVGTSSAIARRIGARDKEGADNVAVHSLILSLILGVTITITMLPAID 120

MurJ/1-439     41 - FFV - - - - - - - AFKLPNLLRRIFAEGAFSQAFVPILAEYKSKQGEDATRVFVSYVSGL - L  91
PfMATE/1-461  121 SLFRSMGAKGEAVELAIEYARVLLAGAFIIVFNNVGNGILRGEG - DANRAMLAMVLGSGL 179

MurJ/1-439     92 TL - - - - - - - - - - - - - - - - - - - ALAVVTVAGMLAAPWVIMVTAPGFADTADKFALTSQLL 131
PfMATE/1-461  180 NIVLDPIFIYTLGFGVVGAAYATLLSMVVTSLFIAYWLFVKRDTYVDITLRDFSPSREIL 239

MurJ/1-439    132 KITFPYILLISLASLVGAILNTWNRFSIPAFAPTL - LNISMI - - - GFALFAAPYFNPPVL 187
PfMATE/1-461  240 K - - - - - - - - - - - - - - - - - - DILRVGLPSSLSQLSMSIAMFFLNSVAITAGGENGVAVF 279

MurJ/1-439    188 ALAWAVTVGGVLQLVYQLPHLKKIGMLVLPRINFHDAGAMRVVKQMGPAILGVSVSQISL 247
PfMATE/1-461  280 TSAWRITMLGIVPIL - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - 294

MurJ/1-439    248 IINTIFASFLASGSVSWMYYADRLMEFPSGVLGVALGTILLPSLSKSFASGNHDEYNRLM 307
PfMATE/1-461  295 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - GMAAATT - - SVTGAAYGERNVEKLETAY 320

MurJ/1-439    308 DWGLRLCFLLALPSAVALGILSGPLTVSLFQYGKFTAFD - - - ALMTQRALIAYS - VGLIG 363
PfMATE/1-461  321 LYAIKIAFMIELAVV - AFIMLFAPQVAYLFTYSESAQVIKGDLISALRTLPVFLVLTPFG 379

MurJ/1-439    364 LIVVKVLAPGFYSRQDIKTPVKIAIVTLILTQLMNLAFIGP - - - - - - LKHAGLSL - - SIG 415
PfMATE/1-461  380 M - - - - - MTSAMF - - QGIGEGEKSLILTIFRTLVMQVGFAYIFVHYTTLGLRGVWIGIVIG 432

MurJ/1-439    416 LAACLNASLLYWQLRKQKIFTPQP - - - - - -                              439
PfMATE/1-461  433 NMVAAIVG - FLWGRMRISALKKTSATGGKR                               461
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B

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MurJ/1-439      1 - - - - - - - - - - - MNLLKSLAAVSSMTMFSRVLGFARDAIVARIFGAGMATDAFFVAFKLP  48
NorM-VC/1-461   1 MENSVHRYKKEASNLIKLATPVLIASVAQTGMGFVDTIMAGGVSAIDMAAVSIAASIWLP  60

MurJ/1-439     49 NLLRRIFAEGAFSQAFVPILAEYKSKQGEDATRVFVSYVSGLLT - LALAVVTVAGMLAAP 107
NorM-VC/1-461  61 SI - - - - LFGVGLLMALVPVVAQLNGAGRQHK - IPF - EVHQGLILALLVSVPIIAVLFQTQ 114

MurJ/1-439    108 WVIMV - - - - - - - - - - - - - - - - - - TAPGF - - - - - - ADTADKFALTSQ - - - - - - - - - LLK 132
NorM-VC/1-461 115 FIIRFMDVEEAMATKTVGYMHAVIFAVPAYLLFQALRSFTDGMSLTKPAMVIGFIGLLLN 174

MurJ/1-439    133 ITFPYILLIS - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - LASLVGAILNTWNRFSIPA 161
NorM-VC/1-461 175 IPLNWIFVYGKFGAPELGGVGCGVATAIVYWIMLLLLLFYIVTSKRLAHVKVFETFHKPQ 234

MurJ/1-439    162 FAPTLLNISMIGFALFAAPYFNPPVLALAWAVTVGGVLQLVYQLPHLKKIGMLVLPRINF 221
NorM-VC/1-461 235 - PKELIRLFRLGFPVAAALFFEVTLFAVV - - - - - - - - - - - - - - - - - - - - ALLV - - - - - - 266

MurJ/1-439    222 HDAGAMRVVKQMGPAILGVSVSQISLIINTIFASFLASGSVSWMYYADRLMEFPSGVLGV 281
NorM-VC/1-461 267 - - - - - - - - - APLGSTVV - - - - - - - - - - - - - - - - - - AAHQVA - LNFSSLVFMFPMSI - GA 296

MurJ/1-439    282 ALGTILLPSLSKSFASGNHDEYNRLMDWGLRLCFLLALPSAVALGILSGPLTVSLFQY - - 339
NorM-VC/1-461 297 AVS - - - - - - IRVGHKLGEQDTKGAAI - - - - - - - - - - - - - - AANVGLMTGLATACITALLT 336

MurJ/1-439    340 GKFTAF - DALMTQR - ALIAYSVGL - - - - - - - - IGLIVVKVLAPGFYS - RQDIKTPVKIAIV 389
NorM-VC/1-461 337 VLFREQIALLYTENQVVVALAMQLLLFAAIYQCMDAVQVVAAGSLRGYKDMTAIFHRTFI 396

MurJ/1-439    390 - - - - - - - - TLILTQLMNLAFIGPLKHAGL - - - - SLSIGLAACLNASLLYWQLRKQKIFTP 437
NorM-VC/1-461 397 SYWVLGLPTGYILGMTNWLTEQPLGAKGFWLGFIIGLSAAALMLGQRLYWLQKQSDDVQL 456

MurJ/1-439    438 QP - - -                                                        439
NorM-VC/1-461 457 HLAAK                                                         461
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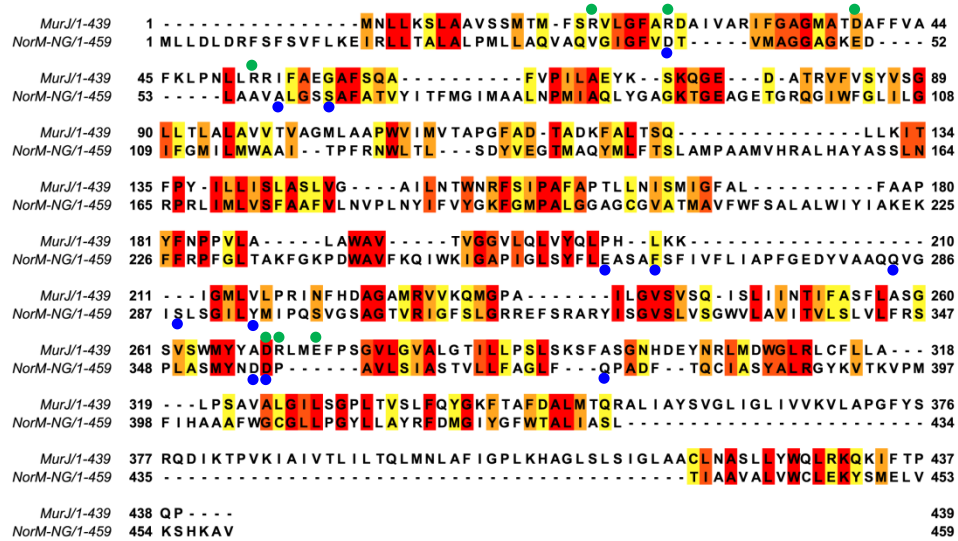
C

FIGURE S2. Amino-acid sequence alignments of MurJ to MATE transporter proteins. ClustalOmega (1) alignment of MurJ (NP_415587) to **A)** PfMATE (Q8U2X0_PYRFU), **B)** NorM-Vc (AAF94694), **C)** NorM-NG (YP_005889265). MurJ sequence identity to these MATE proteins is 22.42%, 21.13%, and 21.87%, respectively. Conserved residues are highlighted in accordance with JalView (2) conservation scores (1 to 10), where yellow = 8, orange = 9, and red = 10. Green dots mark functionally relevant residues in MurJ; blue dots mark functionally relevant residues in the aligned MATE protein sequence (3-5).
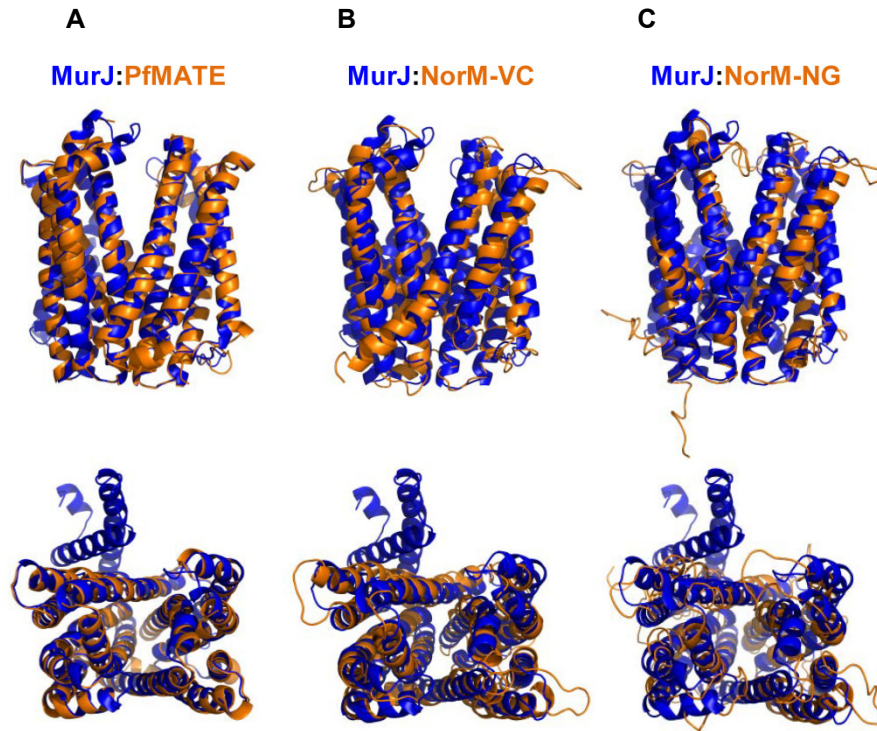
A
MurJ:PfMATE

B
MurJ:NorM-VC

C
MurJ:NorM-NG

FIGURE S3. I-TASSER structural model of MurJ is highly similar to the crystal structure of MOP exporter superfamily proteins. Alignment of the full-length MurJ model structure generated by I-TASSER (blue) and the x-ray crystal structures (orange) of **A)** PfMATE (3VVN), **B)** NorM-VC (3MKU), and **C)** NorM-NG (4HUK) shows high degree of structural similarity throughout TMDs 1-12 (RMSD values of 0.72, 2.61, and 3.10 Å, respectively). Front view from the membrane plane is given at the top; view from the periplasm is given at bottom.
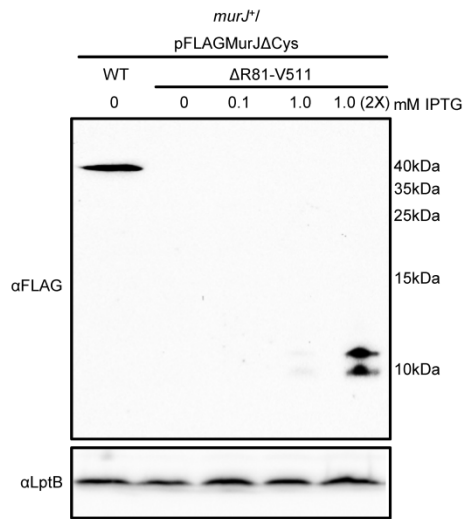
FIGURE S4. A FLAGMurJΔCys fragment of residues 1-80 is unstable. αFLAG immunoblot showing that while full-length FLAGMurJΔCys (WT) is easily detected when expressed in the wild-type strain ($murJ^+$) without IPTG induction, a variant lacking residues R81-V511 is best detected after induction with 1.0 mM IPTG in a concentrated sample (2X). The truncated variant migrates aberrantly in an 18% polyacrylamide SDS gel (expected MW = 9.7kDa), and a degradation product is also detected. To test sample loading, blots were re-probed with αLptB antiserum.
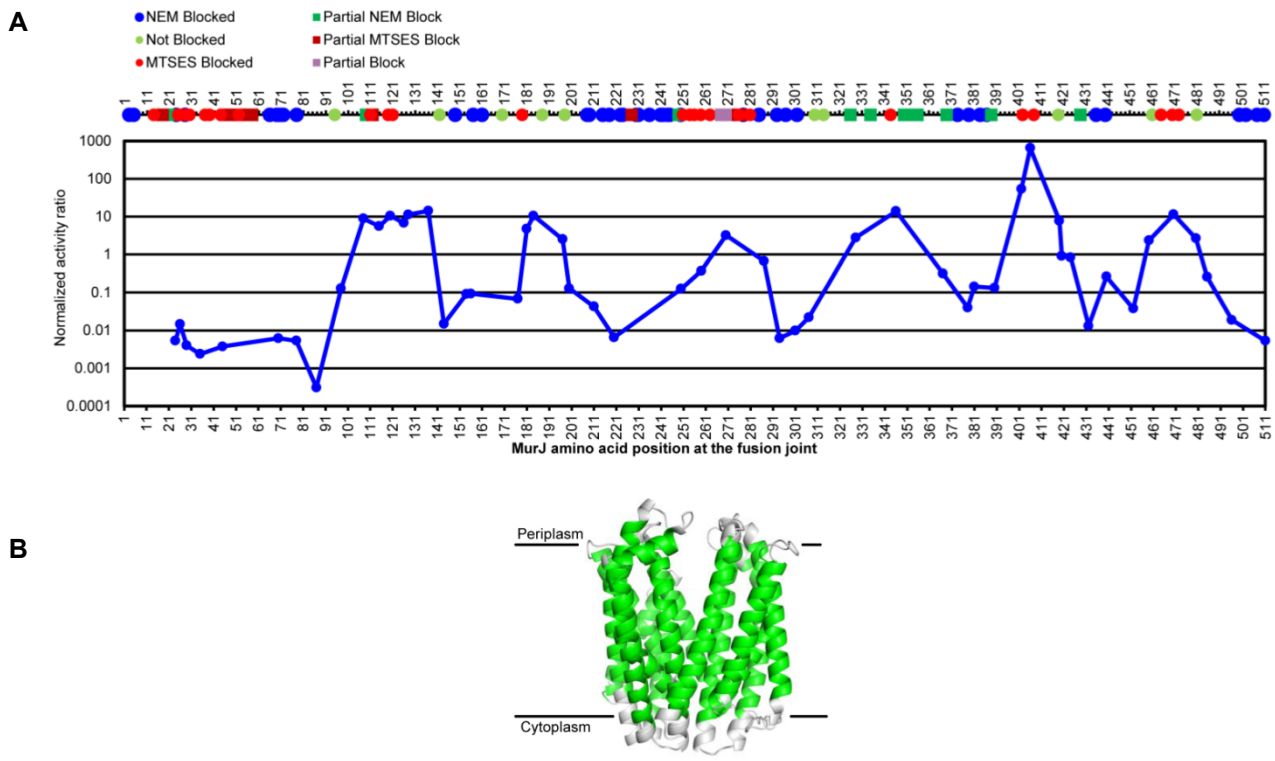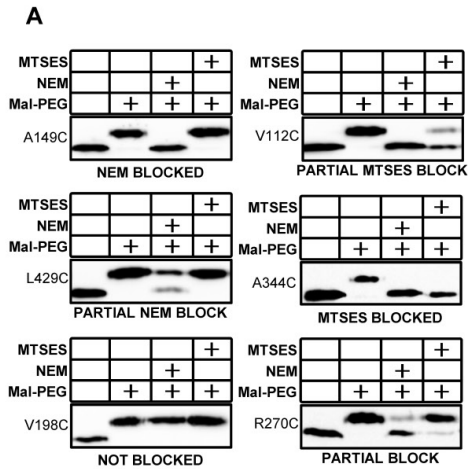
FIGURE S5. **A)** Comparison of SCAM and MurJ truncation-reporter fusion data. **(Top)** Linear representation of SCAM data. **(Bottom)** The normalized activity ratio is given per MurJ truncation-reporter fusion. This is a reproduction of Fig. 3B. **B)** TMDs presented in Fig. 5 are highlighted in green in the MurJ structure model generated by I-TASSER (6, 7).

A

MTSES				+
NEM			+	
Mal-PEG		+	+	+

A149C

NEM BLOCKED

MTSES				+
NEM			+	
Mal-PEG		+	+	+

V112C

PARTIAL MTSES BLOCK

MTSES				+
NEM			+	
Mal-PEG		+	+	+

L429C

PARTIAL NEM BLOCK

MTSES				+
NEM			+	
Mal-PEG		+	+	+

A344C

MTSES BLOCKED

MTSES				+
NEM			+	
Mal-PEG		+	+	+

V198C

NOT BLOCKED

MTSES				+
NEM			+	
Mal-PEG		+	+	+

R270C

PARTIAL BLOCK

B

Category	NEM Block	MTSES Block	# Mutants
NEM Blocked	100	0	42
Partial NEM Block	<100	0	15
Not Blocked	0	0	10
Partial MTSES Block	100	<100	16
MTSES Blocked	100	100	25
Partial Block	>50	>50	2
		Total:	110

C

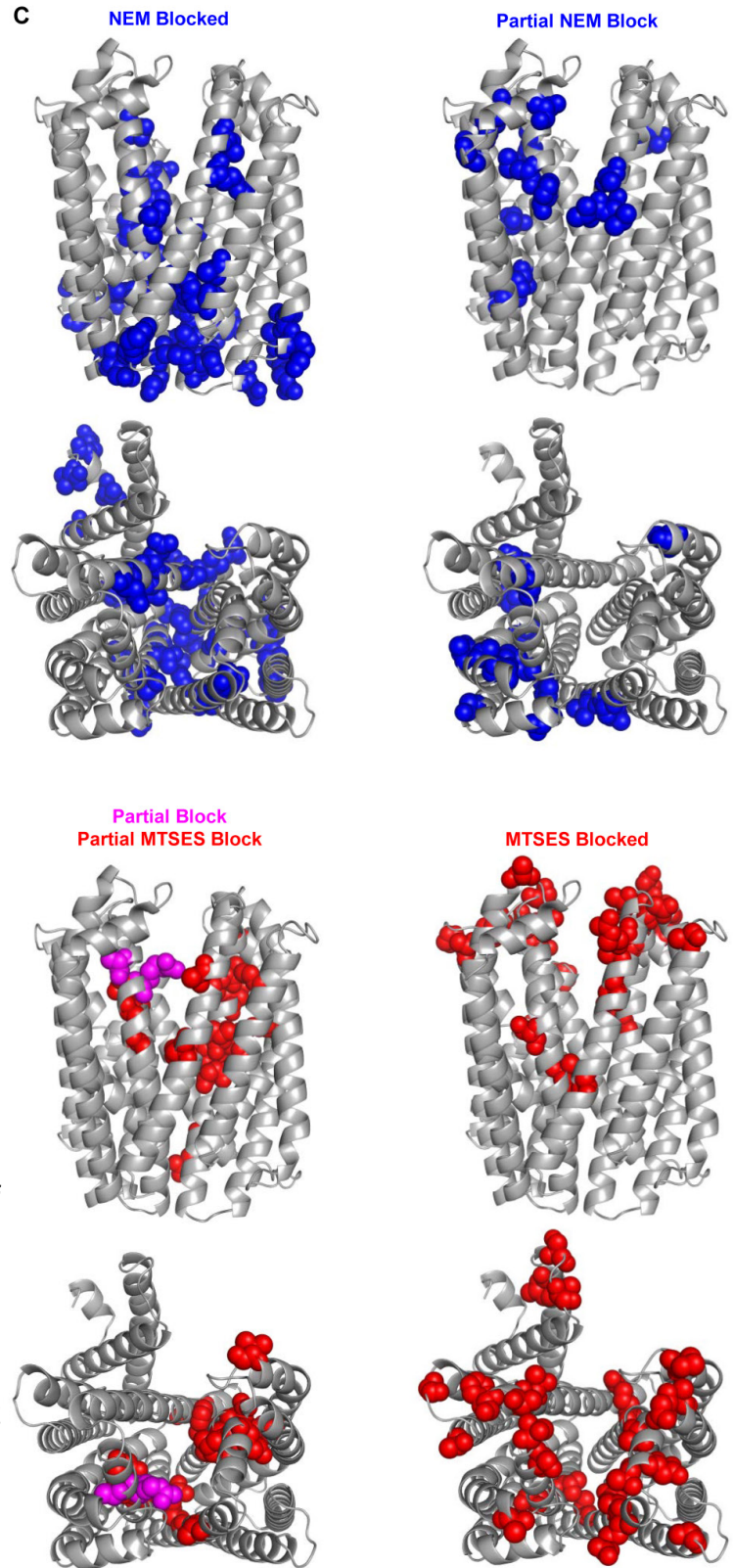**FIGURE S6. A)** Representative αFLAG immunoblot results from each SCAM result category. **B)** Table defining SCAM categories and summarizing results. **C)** Overlay of SCAM results on the structural model of MurJ. Category and colors are indicated in the text above each column. **C** For each category, the front view **(top)** from the membrane plane and view from the periplasm **(bottom)** is given.
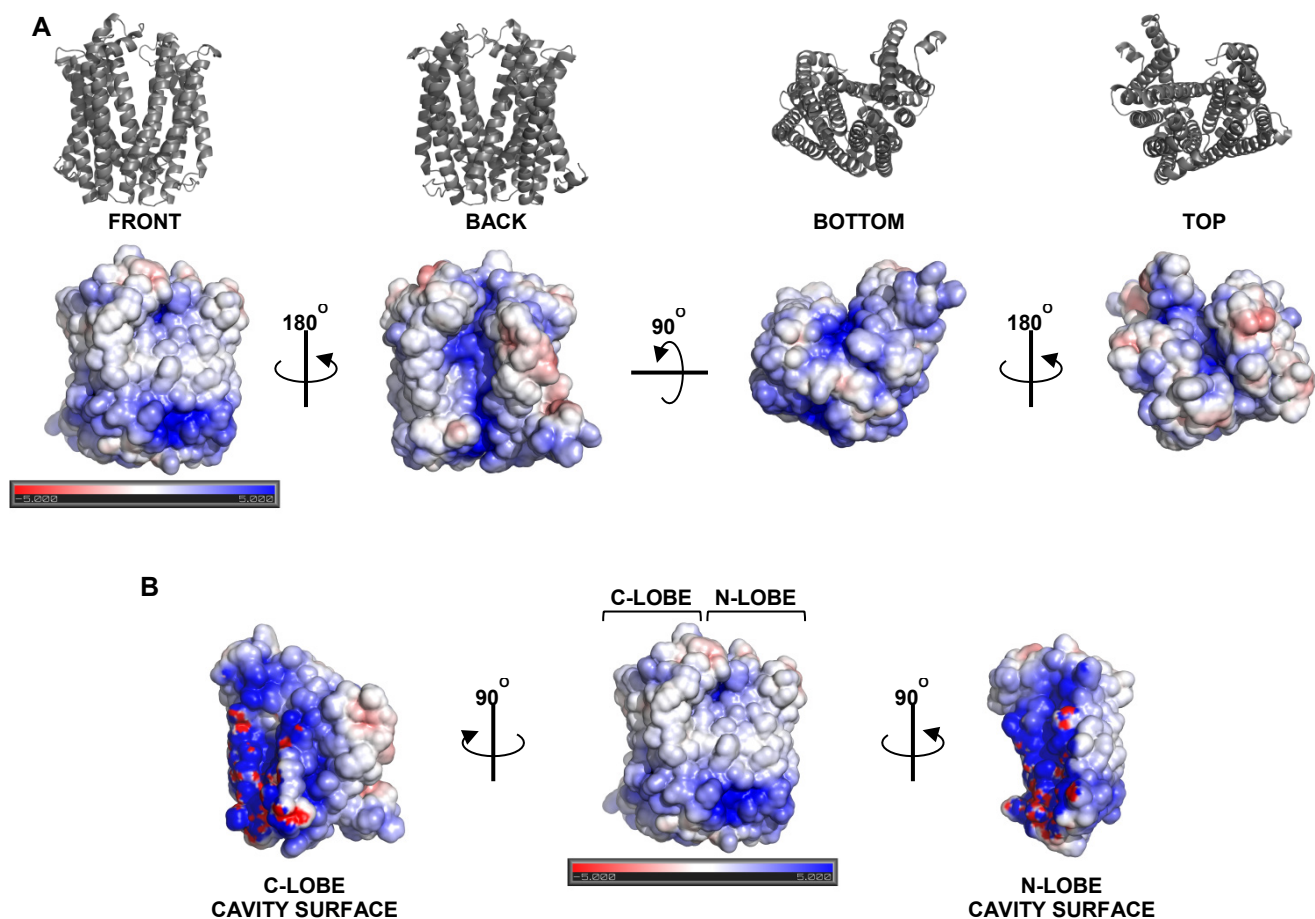
8

FIGURE S7. Electrostatic potential of the MurJ model structure. A) The MurJ structure is given as the cartoon representation at **top** for each solvent-accessible surface electrostatic representation at **bottom**. **B)** Electrostatic potential of the surface corresponding to each half of the MurJ cavity (N- and C- lobes). The N-terminal lobe shows the protein fragment that includes TMDs 1-6; the C-terminal lobe shows the protein fragment that includes TMDs 7-14. Lobes are shown with the surface of the cavity in front. Surfaces are colored according to charge from red (-5 kT/e) to blue (+5 kT/e). Solvent-accessible electrostatic potential was calculated using APBS (8) in PyMol Molecular Graphics System, Version 1.5.0.4 (Schrödinger, LLC, Portland, Oregon).

TABLE S1. Strains used in this study

STRAIN	RELEVANT GENOTYPE[a]	REFERENCE
DH5α	*fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	
DY378	λcI857 Δ(*cro-bioA*)	(9)
MC4100	*araD*139, Δ(*lac*)U169, *strA*, *thi*	(10)
NR754	*araD*[+] revertant of MC4100	(11)
NR1648	NR754 *murJ*::*kan* (pRC7MurJ)	This study
NR2066	NR754 *murJ*::*FRT* (pRC7MurJ)	This study
NR2117	NR754 *murJ*::*FRT hsdR*::*kan* (pRC7MurJ)	This study
NR2040	NR754 *murJ*::*kan* (pEcMurJwtCterm)	This study
NR2075	NR754 *murJ*::*kan* (pMurJ)	This study
NR2132	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJ)	This study
NR1628-1	DH5α (pMurJTOP1533)	This study
NR1693	DH5α (pMurJTOP72)	This study
NR1725-1	DH5α (pMurJTOP78)	This study
NR1995	DH5α (pMurJTOP87)	This study
NR1762	DH5α (pMurJTOP105)	This study
NR1971	DH5α (pMurJTOP135)	This study
NR1747	DH5α (pMurJTOP210)	This study
NR1797	DH5α (pMurJTOP234)	This study
NR1748	DH5α (pMurJTOP261)	This study
NR1798	DH5α (pMurJTOP294)	This study
NR1799	DH5α (pMurJTOP324)	This study
NR2008	DH5α (pMurJTOP345)	This study
NR1749	DH5α (pMurJTOP360)	This study
NR1727	DH5α (pMurJTOP378)	This study
NR1750	DH5α (pMurJTOP384)	This study
NR1988	DH5α (pMurJTOP411)	This study
NR1990	DH5α (pMurJTOP432)	This study
NR1801	DH5α (pMurJTOP462)	This study
NR1763	DH5α (pMurJTOP468)	This study
NR1802	DH5α (pMurJTOP531)	This study
NR2009	DH5α (pMurJTOP543)	This study
NR1803	DH5α (pMurJTOP552)	This study
NR1764	DH5α (pMurJTOP591)	This study
NR1996	DH5α (pMurJTOP600)	This study
NR2010	DH5α (pMurJTOP633)	This study
NR1765	DH5α (pMurJTOP660)	This study
NR1804	DH5α (pMurJTOP750)	This study
NR1989	DH5α (pMurJTOP777)	This study
NR1805	DH5α (pMurJTOP810)	This study
NR1806	DH5α (pMurJTOP861)	This study
NR1997	DH5α (pMurJTOP882)	This study
NR2016	DH5α (pMurJTOP903)	This study
NR1728	DH5α (pMurJTOP921)	This study
NR1807	DH5α (pMurJTOP984)	This study
NR1808	DH5α (pMurJTOP1038)	This study
NR1916	DH5α (pMurJTOP1101)	This study
NR1917	DH5α (pMurJTOP1134)	This study
NR1726-1	DH5α (pMurJTOP1143)	This study
NR1991	DH5α (pMurJTOP1170)	This study
NR2017	DH5α (pMurJTOP1206)	This study
NR1970	DH5α (pMurJTOP1218)	This study
NR1998	DH5α (pMurJTOP1257)	This study
NR1969	DH5α (pMurJTOP1260)	This study

STRAIN	RELEVANT GENOTYPE[a]	REFERENCE
NR2023	DH5α (pMurJTOP1272)	This study
NR2012	DH5α (pMurJTOP1296)	This study
NR2024	DH5α (pMurJTOP1320)	This study
NR2018	DH5α (pMurJTOP1356)	This study
NR2011	DH5α (pMurJTOP1377)	This study
NR2019	DH5α (pMurJTOP1410)	This study
NR2013	DH5α (pMurJTOP1440)	This study
NR2021	DH5α (pMurJTOP1455)	This study
NR2014	DH5α (pMurJTOP1488)	This study
NR2131	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCys)	This study
NR2569	NR754 (pFLAGMurJΔCysΔR81-V511)	This study
NR2202	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysL3C)	This study
NR2296	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysK5C)	This study
NR2184	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysT14C)	This study
NR2323	NR754 (pFLAGMurJΔCysR18C)	This study
NR2679	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysR18A, pRC7MurJ)	This study
NR2203	NR754 murJ::FRT hsdR::kan (pFLAGMurJΔCysV19C)	This study
NR2501	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysL20C)	This study
NR2204	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA23C)	This study
NR2356	NR754 (pFLAGMurJΔCysR24C)	This study
NR2680	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysR24A, pRC7MurJ)	This study
NR2297	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysD25C)	This study
NR2185	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA26C)	This study
NR2502	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV28C)	This study
NR2186	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA29C)	This study
NR2298	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysR30C)	This study
NR2214	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA37C)	This study
NR2254	NR754 (pFLAGMurJΔCysD39C)	This study
NR2681	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysD39A, pRC7MurJ)	This study
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NR2324	NR754 (pFLAGMurJΔCysR52C)	This study
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STRAIN	RELEVANT GENOTYPE[a]	REFERENCE
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NR2464	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysK231C)	This study
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NR2506	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysI245C)	This study
NR2477	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysI249C)	This study
NR2478	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysN250C)	This study
NR2283	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysT251C)	This study
NR2479	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA254C)	This study
NR2480	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysF256C)	This study
NR2144	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysS259C)	This study
NR2419	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysS263C)	This study
NR2481	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA268C)	This study
NR2465	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysD269C)	This study
NR2684	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysD269A)	This study
NR2483	NR754 (pFLAGMurJΔCysR270C)	This study
NR2685	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysR270A, pRC7MurJ)	This study
NR2284	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysM272C)	This study
NR2359	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysE273C)	This study
NR2686	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysE273A)	This study
NR2484	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysP275C)	This study
NR2358	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysS276C)	This study
NR2485	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysG277C)	This study
NR2249	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV278C)	This study
NR2285	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV281C)	This study
NR2286	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysT285C)	This study
NR2319	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysK293C)	This study
NR2134	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysS297C)	This study
NR2466	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysE302C)	This study
NR2318	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysG310C)	This study
NR2145	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJC419S)	This study
NR2253	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysG326C)	This study
NR2252	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysS335C)	This study
NR2226	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA344C)	This study
NR2507	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysT350C)	This study
NR2360	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA353C)	This study
NR2508	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA356C)	This study
NR2433	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV369C)	This study

STRAIN	RELEVANT GENOTYPE[a]	REFERENCE
NR2227	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysF374C)	This study
NR2250	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysD379C)	This study
NR2251	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV384C)	This study
NR2509	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA387C)	This study
NR2459	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV389C)	This study
NR2228	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysG403C)	This study
NR2535	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysA408C)	This study
NR2133	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJC314S)	This study
NR2301	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysL429C)	This study
NR2302	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysT436C)	This study
NR2454	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysG440C)	This study
NR2455	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysG461C)	This study
NR2361	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysI465C)	This study
NR2211	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysS470C)	This study
NR2456	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysT473C)	This study
NR2320	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysL481C)	This study
NR2457	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysG500C)	This study
NR2362	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV503C)	This study
NR2363	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysR508C)	This study
NR2229	NR754 *murJ*::*FRT hsdR*::*kan* (pFLAGMurJΔCysV511C)	This study

[a] For pMurJTOP plasmids, the number indicates the bp within the *murJ* coding sequence at which *murJ* was truncated.

TABLE S2. Primers used in this study

PRIMER NAME	SEQUENCE 5'-3'	PURPOSE
5EcoR1PgfA	cggaattctggattagaacaccgatgaat	pRC7*murJ* construction
3Hind3PgfA	cccaagcttggaatgcattgttacaccgt	pRC7*murJ* construction
pEcMurJwtCterm	gtaagggtcgacctgcagccaagcttaa	pFLAGMurJ construction
3pEcMurJwtCterm	accgtccggcgggcaaattctttaac	pFLAGMurJ construction
5MurJNtHisDEL	tattaaaatcgctggccgccgtcag	pFLAGMurJ construction
3pEcMurJntHISdel	aattcatagttaatttctcctctttaatg	pFLAGMurJ construction
5NtermFlgMmurJ	gattataaagatgacgatgacaaaaatttattaaaatcgctggccgccgt	pFLAGMurJ construction
3NtermFlgMmurJ	catagttaatttctcctctttaatg	pFLAGMurJ construction
3pFlgMurJdel81-511	gtggcgtcttcaccctgcttactt	pFLAGMurJ truncation
5murJP1	ggtagactattcgcctctttcagcgcctgccttgcaggcg ttttgcccgtgggtctggattagaacaccgatggtgtaggctggagctgcttc	Chromosomal *murJ* deletion
3murJP2	cgg cgg taaaaagat ttc gat ctg aag attatctccggcctgcac tgcaggccggaatgcattgttacatatgaatatcctccta	Chromosomal *murJ* deletion
5MurJ10UPXba	gctctagaacaccgatgaattt	MurJ truncation-reporter fusions
3MurJ11DOWNBamHI	atgcattgttaggatcccaccgtccg	MurJ truncation-reporter fusions
5phoAEcoRV	tcgatatcgttctggaaaaccg	MurJ truncation-reporter fusions
3MurJEcoRV72	tcgatatctcgtgcgaagccaagcac	MurJ truncation-reporter fusions
3MurJEcoRV78	tcgatatctgcgtctcgtgcgaagcca	MurJ truncation-reporter fusions
3MurJEcorV87	tcgatatcggcgacaattgcgtctcgtgc	MurJ truncation-reporter fusions
3MurJEcorV105	tcgatatcccctgcgccaaagattctg	MurJ truncation-reporter fusions
3MurJEcorV135	tcgatatcaaaagcgacgaaaaaggcg	MurJ truncation-reporter fusions
3MurJEcorV210	tcgatatcttccgccagaatcggtacaaatg	MurJ truncation-reporter fusions
3MurJEcorV234	tcgatatcgtcttcaccctgcttactttta	MurJ truncation-reporter fusions
MurJEcorV261	tcgatatcaacataagagacaaagacccgc	MurJ truncation-reporter fusions
3MurJEcorV294	tcgatatccacaaccgccagcgcaagtg	MurJ truncation-reporter fusions
3MurJEcorV324	tcgatatcccacggtgcggcgagcatg	MurJ truncation-reporter fusions
3MurJEcoRV345	tcgatatctggcgcggtcaccatgatcac	MurJ truncation-reporter fusions
3MurJEcorV360	tcgatatctgtgtcagcgaagcctggc	MurJ truncation-reporter fusions
3MurJEcoRV378	tcgatatccagggcaaatttgtcagc	MurJ truncation-reporter fusions
3MurJEcorV384	tcgatatcgctggtcagggcaaatttgtc	MurJ truncation-reporter fusions
3MurJEcorV411	tcgatatcataggggaaaggtaatctttag	MurJ truncation-reporter fusions
3MurJEcorV432	tcg ata tcc gcc agg gag atc agc aag	MurJ truncation-reporter fusions
3MurJEcorV462	ttgatatcccacgtattcagaatcgctccc	MurJ truncation-reporter fusions
3MurJEcorV468	tcgatatcgcggttccacgtattcagaatc	MurJ truncation-reporter fusions
3MurJEcorV531	tcgatatcaaacagcgcgaaaccaatcatgc	MurJ truncation-reporter fusions
3MurJEcoRV543	tcgatatcgtacggtgcggcaaacagcg	MurJ truncation-reporter fusions
3MurJEcorV552	tcgatatctgggttaaagtacggtgcg	MurJ truncation-reporter fusions
3MurJEcorV591	tcgatatcgccgccgaccgtaacagc	MurJ truncation-reporter fusions
3MurJEcorV600	tcgatatcctgcaggacgccgccg	MurJ truncation-reporter fusions
3MurJEcoRV633	tcgatatcgatcttcttcaggtgcggtag	MurJ truncation-reporter fusions
3MurJEcorV660	tcgatatcgttaatgcgcggcaggac	MurJ truncation-reporter fusions
3MurJEcorV750	tcgatatcgttgatgattaaggagatctggc	MurJ truncation-reporter fusions
3MurJEcorV777	tcgatatcggaagcaagaaacgaggc	MurJ truncation-reporter fusions
3MurJEcorV810	tcgatatcgcggtcggcgtaatacat	MurJ truncation-reporter fusions
3MurJEcorV861	tcgatatccaaaatggtgccaagcgccac	MurJ truncation-reporter fusions
3MurJEcorV882	tcgatatcacttttcgacagcgacggcagc	MurJ truncation-reporter fusions
3MurJEcoRV903	tcgatatccatcatgattgccactggcaa	MurJ truncation-reporter fusions
3MurJEcorV921	tcgatatccatcaaacggttgtattcatcatg	MurJ truncation-reporter fusions
3MurJEcorV984	tcgatatccaagaatgcccaacgcaaccg	MurJ truncation-reporter fusions
3MurJEcorV1038	tcgatatccatcaaacgcggtaaatttaccg	MurJ truncation-reporter fusions
3MurJEcorV1101	tcgatatccactacaatcaggccaatcaaac	MurJ truncation-reporter fusions

PRIMER NAME	SEQUENCE 5'-3'	PURPOSE
3MurJEcorV1134	tcgatatcctggcgggaataaaagcc	MurJ truncation-reporter fusions
3MurJEcorV1143	tcgatatctttaatgtcctggcggg	MurJ truncation-reporter fusions
3MurJEcorV1170	tcgatatccgtaacgatggcaattttcac	MurJ truncation-reporter fusions
3MurJ1206EcoRV	tc gatatc aataaacgccaggttcatc	MurJ truncation-reporter fusions
3MurJEcorV1218	tcgatatctttcaacggaccaataaacgc	MurJ truncation-reporter fusions
3MurJ1257EcoRV	tcgatatcacacgccgccagaccaatag	MurJ truncation-reporter fusions
3MurJEcorV1260	tcgatatccagacacgccgccagacc	MurJ truncation-reporter fusions
3MurJ1272EcoRV	tcgatatccagcgaagcattcagacacg	MurJ truncation-reporter fusions
3MurJ1296EcoRV	tcgatatcctgcttacgcaactgccag	MurJ truncation-reporter fusions
3MurJEcoRV1320	tcgatatcgccgggttgcggggtaaagat	MurJ truncation-reporter fusions
3MurJ1356EcoRV	tcgatatctaccgccaccaccagacgc	MurJ truncation-reporter fusions
3MurJEcoRV1377	tcgatatcaagcacgccagacatcaccag	MurJ truncation-reporter fusions
3MurJEcoRV1410	tcgatatctgaccactccggcatgatatg	MurJ truncation-reporter fusions
3MurJ1440EcoRV	tcgatatcacgcagtaaacgccagggca	MurJ truncation-reporter fusions
3MurJEcoRV1455	tcgatatccacgaccgccattaaacgcag	MurJ truncation-reporter fusions
3MurJ1488EcoRV	tcgatatccagtgcagcgaagtacgcgg	MurJ truncation-reporter fusions
5pEcMurJC314S	ggggttgcgtcttagcttcctgttggcg	SCAM
3pEcMurJC314S	cgccaacaggaagctaagacgcaacccc	SCAM
5pEcMurJC419S2	tggtctggcggcgagtctgaatgcttcg	SCAM
3pEcMurJC419S2	cgaagcattcagactcgccgccagacca	SCAM
5pFlgMurJL3C	ggattataaagatgacgatgacaaaaattgcttaaaatcgctggccgcc	SCAM
3pFlgMurJL3C	ggcggccagcgattttaagcaattttgtcatcgtcatctttataatcc	SCAM
5pFlgMurJK5C	gattataaagatgacgatgacaaaaatttattatgctcgctggccgccgtcag	SCAM
3pFlgMurJK5C	ctgacggcggccagcgagcataataaattttgtcatcgtcatctttataatc	SCAM
5MurJT14C	cgccgtcagctcgatgtgcatgttttcgcgtgtg	SCAM
3MurJT14C	cacacgcgaaaacatgcacatcgagctgacggcg	SCAM
5MurJR18C	gatgaccatgttttcgtgtgtgcttggcttcgc	SCAM
3MurJR18C	gcgaagccaagcacacacgaaaacatggtcatc	SCAM
5MurJR18A	cgatgaccatgttttcggctgtgcttggcttcgcac	Mutagenesis
3MurJR18A	gtgcgaagccaagcacagccgaaaacatggtcatcg	Mutagenesis
5MurJV19C	gatgaccatgttttcgcgttgccttggcttcgcacgagacg	SCAM
3MurJV19C	cgtctcgtgcgaagccaaggcaacgcgaaaacatggtcatc	SCAM
5MurJL20C	ccatgttttcgcgtgtgtgtggcttcgcacgagacg	SCAM
3MurJL20C	cgtctcgtgcgaagccacacacacgcgaaaacatgg	SCAM
5MurJA23C	cgcgtgtgcttggcttctgccgagacgcaattgtcgc	SCAM
3MurJA23C	gcgacaattgcgtctcggcagaagccaagcacacgcg	SCAM
5MurJR24C	gtgcttggcttcgcatgcgacgcaattgtcgcc	SCAM
3MurJR24C	ggcgacaattgcgtcgcatgcgaagccaagcac	SCAM
5MurJR24A	cgcgtgtgcttggcttcgcagcagacgcaattg	Mutagenesis
3MurJR24A	caattgcgtctgctgcgaagccaagcacacgcg	Mutagenesis
5MurJD25C	tgtgcttggcttcgcacgatgcgcaattgtcgcc	SCAM
3MurJD25C	ggcgacaattgcgcatcgtgcgaagccaagcaca	SCAM
5MurJA26C	gcttggcttcgcacgagactgcattgtcgccagaatctttg	SCAM
3MurJA26C	caaagattctggcgacaatgcagtctcgtgcgaagccaagc	SCAM
5MurJV28C	tggcttcgcacgagacgcaatttgcgccagaatctttg	SCAM
3MurJV28C	caaagattctggcgcaaattgcgtctcgtgcgaagcca	SCAM
5MurJA29C	cacgagacgcaattgtctgcagaatctttggcgcag	SCAM
3MurJA29C	ctgcgccaaagattctgcagacaattgcgtctcgtg	SCAM
5MurJR30C	gacgcaattgtcgcctgcatctttggcgcaggg	SCAM
3MurJR30C	ccctgcgccaaagatgcaggcgacaattgcgtc	SCAM
5MurJA37C	atctttggcgcagggatgtgcaccgacgccttttttcgtc	SCAM
3MurJA37C	gacgaaaaaggcgtcggtgcacatccctgcgccaaagat	SCAM

PRIMER NAME	SEQUENCE 5'-3'	PURPOSE
5MurJD39C	gcagggatggcaacctgcgcctttttcgtcgc	SCAM
3MurJD39C	gcgacgaaaaaggcgcaggttgccatccctgc	SCAM
5MurJD39A2	agggatggcaaccgcggcctttttcgtcg	Mutagenesis
3MurJD39A2	cgacgaaaaaggccgcggttgccatccct	Mutagenesis
5MurJA44C	gcaaccgacgcctttttcgtctgttttaaacttcctaacttgtt	SCAM
3MurJA44C	aacaagttaggaagtttaaaacagacgaaaaaggcgtcggttgc	SCAM
5MurJK46C	cgacgcctttttcgtcgcttttgccttcctaacttgttacgccg	SCAM
3MurJK46C	cggcgtaacaagttaggaaggcaaaaagcgacgaaaaaggcgtcg	SCAM
5MurJK46A	cgacgcctttttcgtcgctttgcacttcctaacttgttacg	Mutagenesis
3MurJK46A	cgtaacaagttaggaagtgcaaaagcgacgaaaaaggcgtcg	Mutagenesis
5MurJL47C	accgacgcctttttcgtcgctttaaatgtcctaacttgttacg	SCAM
3MurJL47C	cgtaacaagttaggacatttaaaagcgacgaaaaaggcgtcggt	SCAM
5MurJN49C	ttcgtcgcttttaaacttccttgcttgttacgccgtatctttgc	SCAM
3MurJN49C	gcaaagatacggcgtaacaagcaaggaagtttaaaagcgacgaa	SCAM
5MurJR52C	gtcgcttttaaacttcctaacttgttatgccgtatctttgcc	SCAM
3MurJR52C	ggcaaagatacggcataacaagttaggaagtttaaaagcgac	SCAM
5MurJR52A	cggcaaagatacgggctaacaagttaggaagtttaaaagcga	Mutagenesis
3MurJR52A	tcgcttttaaacttcctaacttgttagcccgtatctttgccg	Mutagenesis
5MurJR53C	ttcctaacttgttacgctgtatctttgccgaaggg	SCAM
3MurJR53C	cccttcggcaaagatacagcgtaacaagttaggaa	SCAM
5MurJI54C	cctaacttgttacgccgttgctttgccgaaggggcatt	SCAM
3MurJI54C	aatgcccccttcggcaaagcaacggcgtaacaagttagg	SCAM
5MurJS55C	ctaacttgttacgccgtatctgtgccgaagggg	SCAM
3MurJS55C	ccccttcggcacagatacggcgtaacaagttag	SCAM
5MurJA56C	tcctaacttgttacgccgtatcttttgcgaaggggcattt	SCAM
3MurJA56C	aaatgcccccttcgcaaaagatacggcgtaacaagttagga	SCAM
5MurJE57C	gttacgccgtatctttgcctgcggggcattttcccaggcat	SCAM
3MurJE57C	atgcctgggaaaatgccccgcaggcaaagatacggcgtaac	SCAM
5MurJG58C	acgccgtatctttgccgaatgcgcattttcccagg	SCAM
3MurJG58C	cctgggaaaatgcgcattcggcaaagatacggcgt	SCAM
5MurJP66C	gggcattttcccaggcatttgtatgcattctggcggaatataaaagt	SCAM
3MurJP66C	actttttatattccgccagaatgcatacaaatgcctgggaaaatgccc	SCAM
5MurJA69C	ccaggcatttgtaccgattctgtgcgaatataaaagtaagcagggtg	SCAM
3MurJA69C	caccctgcttactttttatattcgcacagaatcggtacaaatgcctgg	SCAM
5MurJE70C	ggcatttgtaccgattctggcgtgctataaaagtaagcagggtgaag	SCAM
3MurJE70C	cttcaccctgcttactttttatagcacgccagaatcggtacaaatgcc	SCAM
5MurJY71C	accgattctggcggaatgtaaaagtaagcagggtg	SCAM
3MurJY71C	caccctgcttactttttacattccgccagaatcggt	SCAM
5MurJK72C	ttgtaccgattctggcggaatattgcagtaagcagggtgaagacg	SCAM
3MurJK72C	cgtcttcaccctgcttactgcaatattccgccagaatcggtacaa	SCAM
5MurJS85C	acgcgggtctttgtctgttatgtttctggcctg	SCAM
3MurJS85C	caggccagaaacataacagacaaagacccgcgt	SCAM
5MurJL95C	cctgctgacacttgcgtgcgcggttgtgacggtcg	SCAM
3MurJL95C	cgaccgtcacaaccgcgcacgcaagtgtcagcagg	SCAM
5murJV109C	ccgcaccgtggtgcatcatggtgacc	SCAM
3murJV109C	ggtcaccatgatgcaccacggtgcgg	SCAM
5MurJM111C	gcaccgtgggtgatctgcgtgaccgcgccaggc	SCAM
3MurJM111C	gcctggcgcggtcacgcagatcacccacggtgc	SCAM
5MurJV112C	caccgtgggtgatcatgtgcaccgcgccaggcttc	SCAM
3MurJV112C	gaagcctggcgcggtgcacatgatcacccacggtg	SCAM
5MurJD119C	ccgcgccaggcttcgcttgcacagctgacaaatttgc	SCAM
3MurJD119C	gcaaatttgtcagctgtgcaagcgaagcctggcgcgg	SCAM

PRIMER NAME	SEQUENCE 5'-3'	PURPOSE
5MurJA121C	gcgccaggcttcgctgacacatgtgacaaatttgc	SCAM
3MurJA121C	gcaaatttgtcacatgtgtcagcgaagcctggcgc	SCAM
5MurJS142C	ccctatatcttgctgatctgcctggcgtcg	SCAM
3MurJS142C	cgacgccaggcagatcagcaagatataggg	SCAM
5MurJA149C	ctggcgtcgctggtgggatgcattctgaatacgtggaac	SCAM
3MurJA149C	gttccacgtattcagaatgcatcccaccagcgacgccag	SCAM
5MurJN152C	ggtgggagcgattctgtgtacgtggaaccgcttc	SCAM
3MurJN152C	gaagcggttccacgtacacagaatcgctcccacc	SCAM
5MurJA161C	gaaccgcttctcgattccgtgctttgctccaacactgctta	SCAM
3MurJA161C	taagcagtgttggagcaaagcacggaatcgagaagcggttc	SCAM
5MurJS170C	caacactgcttaacatctgcatgattggtttcgcg	SCAM
3MurJS170C	cgcgaaaccaatcatgcagatgttaagcagtgttg	SCAM
5MurJA179C	ggtttcgcgctgtttgcctgcccgtactttaacccaccg	SCAM
3MurJ179C	cggtgggttaaagtacgggcaggcaaacagcgcgaaacc	SCAM
5MurJA188C	cccaccggtgctgtgcctggcgtgggctg	SCAM
3MurJA188C	cagcccacgccaggcacagcaccggtggg	SCAM
5MurJV198C	tacggtcggcggctgcctgcagctggtg	SCAM
3MurJV198C	caccagctgcaggcagccgccgaccgta	SCAM
5MurJL208C	ggtgtatcagctaccgcactgcaagaagatcggcatgctgg	SCAM
3MurJL208C	ccagcatgccgatcttcttgcagtgcggtagctgatacacc	SCAM
5MurJK209C	tatcagctaccgcacctgtgcaagatcggcatgctggtc	SCAM
3MurJK209C	gaccagcatgccgatcttgcacaggtgcggtagctgata	SCAM
5MurJV215C	gaagatcggcatgctgtgcctgccgcgcattaac	SCAM
3MurJV215C	gttaatgcgcggcaggcacagcatgccgatcttc	SCAM
5MurJR218C	atgctggtcctgccgtgcattaacttccacg	SCAM
3MurJR218C	cgtggaagttaatgcacggcaggaccagcat	SCAM
5MurJA224C	ccgcgcattaacttccacgattgcggagcaatgc	SCAM
3MurJA224C	gcattgctccgcaatcgtggaagttaatgcgcgg	SCAM
5MurJR228C	gatgccggagcaatgtgcgtggtgaaacaga	SCAM
3MurJR228C	tctgtttcaccacgcacattgctccggcatc	SCAM
5MurJV230C	cggagcaatgcgcgtgtgcaaacagatgggaccgg	SCAM
3MurJV230C	ccggtcccatctgtttgcacacgcgcattgctccg	SCAM
5MurJK231C	gcaatgcgcgtggtgtgccagatgggaccggcg	SCAM
3MurJK231C	cgccggtcccatctggcacaccacgcgcattgc	SCAM
5MurJA236C	gtgaaacagatgggaccgtgcatccttggcgtctctgtg	SCAM
3MurJA236C	cacagagacgccaaggatgcacggtcccatctgtttcac	SCAM
5MurJS241C	ggcgatccttggcgtctgtgtgagcca	SCAM
3MurJS241C	tggctcacacagacgccaaggatcgcc	SCAM
5MurJV242C	ccggcgatccttggcgtctcttgcagccagatctc	SCAM
3MurJV242C	gagatctggctgcaagagacgccaaggatcgccgg	SCAM
5MurJQ244C	atccttggcgtctctgtgagctgcatctccttaatcatcaacacc	SCAM
3MurJQ244C	ggtgttgatgattaaggagatgcagctcacagagacgccaaggat	SCAM
5MurJI245C	ggcgtctctgtgagccagtgctccttaatcatcaacac	SCAM
3MurJI245C	gtgttgatgattaaggagcactggctcacagagacgcc	SCAM
5MurJS246C	ctctgtgagccagatctgcttaatcatcaacacca	SCAM
3MurJS246C	tggtgttgatgattaagcagatctggctcacagag	SCAM
5MurJN250C	gccagatctccttaatcatctgcaccattttgcctcgtttc	SCAM
3MurJN250C	gaaacgaggcaaaaatggtgcagatgattaaggagatctggc	SCAM
5MurJT251C	cagatctccttaatcatcaactgcattttgcctcgtttcttgc	SCAM
3MurJT251C	gcaagaaacgaggcaaaaatgcagttgatgattaaggagatctg	SCAM
5MurJA254C	cagatctccttaatcatcaacaccattttttgctcgtttcttgcttcc	SCAM
3MurJA254C	ggaagcaagaaacgagcaaaaaatggtgttgatgattaaggagatctg	SCAM

17

PRIMER NAME	SEQUENCE 5'-3'	PURPOSE
5MurJF256C	caacaccatttttgcctcgtgtcttgcttccggtt	SCAM
3MurJF256C	aaccggaagcaagacacgaggcaaaaatggtgttg	SCAM
5murJS259C	cctcgtttcttgcttgtggttcggtgtcttg	SCAM
3murJS259C	caagacaccgaaccacaagcaagaaacgagg	SCAM
5MurJS263C	gcttccggttcggtgtgttggatgtattacgcc	SCAM
3MurJS263C	ggcgtaatacatccaacacaccgaaccggaagc	SCAM
5MurJA268C	ggttcggtgtcttggatgtattactgcgaccgcttaatggag	SCAM
3MurJA268C	ctccattaagcggtcgcagtaatacatccaagacaccgaacc	SCAM
5MurJD269C	gtcttggatgtattacgcctgccgcttaatggagtttccg	SCAM
3MurJD269C	cggaaactccattaagcggcaggcgtaatacatccaagac	SCAM
5MurJD269A	ttggatgtattacgccgcgcgcttaatggagtttc	Mutagenesis
3MurJD269A	gaaactccattaagcgcgcggcgtaatacatccaa	Mutagenesis
5MurJR270C	ggatgtattacgccgactgcttaatggagtttccg	SCAM
3MurJR270C	cggaaactccattaagcagtcggcgtaatacatcc	SCAM
5MurJR270A	tggatgtattacgccgacgccttaatggagtttccgtc	Mutagenesis
3MurJR270A	gacggaaactccattaaggcgtcggcgtaatacatcca	Mutagenesis
5MurJM272C	gtattacgccgaccgcttatgcgagtttccgtccggtgtg	SCAM
3MurJM272C	cacaccggacggaaactcgcataagcggtcggcgtaatac	SCAM
5MurJE273C	tgtattacgccgaccgcttaatgtgctttccgtccggtgtg	SCAM
3MurJE273C	cacaccggacggaaagcacattaagcggtcggcgtaataca	SCAM
5MurJE273A	gaccgcttaatggcttttccgtccg	Mutagenesis
3MurJE273A	ggcgtaatacatccaagacaccg	Mutagenesis
5MurJP275C	acgccgaccgcttaatggagttttgctccggtgtgctg	SCAM
3MurJP275C	cagcacaccggagcaaaactccattaagcggtcggcgt	SCAM
5MurJS276C	cgcttaatggagtttccgtgcggtgtgctg	SCAM
3MurJS276C	cagcacaccgcacggaaactccattaagcg	SCAM
5MurJG277C	ggagtttccgtcctgtgtgctgggcgt	SCAM
3MurJG277C	acgcccagcacacaggacggaaactcc	SCAM
5MurJV278C	gagtttccgtccggttgcctgggcgtggcgctt	SCAM
3MurJV278C	aagcgccacgcccaggcaaccggacggaaactc	SCAM
5MurJV281C	tccggtgtgctgggctgcgcgcttggcaccatt	SCAM
3MurJV281C	aatggtgccaagcgcgcagcccagcacaccgga	SCAM
5MurJT285C	gcgtggcgcttggctgcattttgctgccgt	SCAM
3MurJT285C	acggcagcaaaatgcagccaagcgccacgc	SCAM
5MurJK293C	tttgctgccgtcgctgtcgtgcagttttgccagtggcaatc	SCAM
3MurJK293C	gattgccactggcaaaactgcacgacagcgacggcagcaaa	SCAM
5MurJS297C	gctgtcgaaaagttttgcctgtggcaatcatgatgaata	SCAM
3MurJS297C	tattcatcatgattgccacaggcaaaacttttcgacagc	SCAM
5MurJE302C	aaaagttttgccagtggcaatcatgattgctacaaccgtttgatggactg	SCAM
3MurJE302C	cagtccatcaaacggttgtagcaatcatgattgccactggcaaaacttttt	SCAM
5MurJG310C	aaccgtttgatggactggtgcttgcgtctttgtttcctg	SCAM
3MurJG310C	caggaaacaaagacgcaagcaccagtccatcaaacggtt	SCAM
5MurJG326C	agtgcggttgcgttgtgcattctttccggtc	SCAM
3MurJG326C	gaccggaaagaatgcacaacgcaaccgcact	SCAM
5MurJS335C	ggtccgttgaccgtttgcctgttccagtacggt	SCAM
3MurJS335C	accgtactggaacaggcaaacggtcaacggacc	SCAM
5MurJA344C	ctgttccagtacggtaaatttacctgctttgatgcgctgatgacc	SCAM
3MurJA344C	ggtcatcagcgcatcaaagcaggtaaatttaccgtactggaacag	SCAM
5MurJT350C	cgtttgatgcgctgatgtgccagcgggcgttaattg	SCAM
3MurJT350C	caattaacgcccgctggcacatcagcgcatcaaacg	SCAM
5MurJA353C	cgctgatgacccagcggtgcttaattgcctactcggt	SCAM
3MurJA353C	accgagtaggcaattaagcaccgctgggtcatcagcg	SCAM

PRIMER NAME	SEQUENCE 5'-3'	PURPOSE
5MurJA356C	cccagcgggcgttaatttgctactcggtgggg	SCAM
3MurJA356C	cccaccgagtagcaaattaacgcccgctggg	SCAM
5MurJV369C	ggtttgattggcctgattgtagtgaaatgcttggctcctggcttt	SCAM
3MurJV369C	aaagccaggagccaagcatttcactacaatcaggccaatcaaacc	SCAM
5MurJF374C	gttggctcctggctgttattcccgccagg	SCAM
3MurJF374C	cctggcgggaataacagccaggagccaac	SCAM
5MurJD379C	ctggcttttattcccgccagtgcattaaaacgccagtgaaaa	SCAM
3MurJD379C	ttttcactggcgttttaatgcactggcgggaataaaagccag	SCAM
5MurJV384C	cgccaggacattaaaacgccatgcaaaattgccatcgttacgctg	SCAM
3MurJV384C	cagcgtaacgatggcaattttgcatggcgttttaatgtcctggcg	SCAM
5MurJA387C	ggacattaaaacgccagtgaaaatttgcatcgttacgctgattttaac	SCAM
3MurJA387C	gttaaaatcagcgtaacgatgcaaattttcactggcgttttaatgtcc	SCAM
5MurJV389C	aacgccagtgaaaattgccatcgtacgctgattttaacgcaattg	SCAM
3MurJV389C	caattgcgttaaaatcagcgtacagatggcaattttcactggcgtt	SCAM
5MurJG403C	caattgatgaacctggcgtttatttgtccgttgaaacatg	SCAM
3MurJG403C	catgtttcaacggacaaataaacgccaggttcatcaattg	SCAM
5MurJA408C	ggcgtttattggtccgttgaaacattgcgggctgtcactt	SCAM
3MurJA408C	aagtgacagcccgcaatgtttcaacggaccaataaacgcc	SCAM
5MurJL429C	gaatgcttcgctgctttactggcagtgccgtaagcagaaaat	SCAM
3MurJL429C	attttctgcttacggcactgccagtaaagcagcgaagcattc	SCAM
5MurJT436C	agttgcgtaagcagaaaatcttttgcccgcaacccgg	SCAM
3MurJT436C	ccgggttgcgggcaaaagattttctgcttacgcaact	SCAM
5MurJG440C	ccccgcaaccctgctggatggcg	SCAM
3MurJG440C	cgccatccagcagggttgcgggg	SCAM
5MurJG461C	gatgtctggcgtgctttatgtatgttacatatcatgcc	SCAM
3MurJG461C	ggcatgatatgtaacatacataaaagcacgccagacatc	SCAM
5MurJI465C	gcgtgcttttaggtatgttacattgcatgccggagtggtc	SCAM
3MurJI465C	gaccactccggcatgcaatgtaacatacctaaaagcacgc	SCAM
5MurJS470C	tcatgccggagtggtgcttgggtaccatgccc	SCAM
3MurJS470C	gggcatggtacccaagcaccactccggcatga	SCAM
5MurJT473C	ggagtggtcattgggttgcatgccctggcgttta	SCAM
3MurJT473C	taaacgccagggcatgcaacccaatgaccactcc	SCAM
5MurJL481C	gccctggcgtttactgcgttgcatggcggtcgt	SCAM
3MurJL481C	acgaccgccatgcaacgcagtaaacgccagggc	SCAM
5MurJG500C	ctgcactggcggtactgtgcttcaaagttaaagaat	SCAM
3MurJG500C	attctttaactttgaagcacagtaccgccagtgcag	SCAM
5MurJV503C	gcggtactgggcttcaaatgtaaagaatttgcccgccg	SCAM
3MurJV503C	cggcgggcaaattctttacatttgaagcccagtaccgc	SCAM
5MurJR508C	aaagttaaagaatttgcctgccggacggtgtaaggg	SCAM
3MurJR508C	cccttacaccgtccggcaggcaaattctttaacttt	SCAM
5MurJV511C	tttgcccgccggacgtgctaagggtcgacctgc	SCAM
3MurJV511C	gcaggtcgacccttagcacgtccggcgggcaaa	SCAM

TABLE S3. SCAM results

aa change [a,b]	% NEM block [c]	% MTSES block	SCAM Result	Location
L3C	100	0	NEM Blocked	N-terminal tail
K5C	100	0	NEM Blocked	N-terminal tail
T14C	**100**	**100**	**MTSES Blocked**	**TMD1**
R18C	**100**	**<50**	**Partial MTSES Block**	**TMD1**
V19C	<50	0	Partial NEM Block	TMD1
L20C	**>50**	**0**	**Partial NEM Block**	**TMD1**
A23C	<50	0	Partial NEM Block	TMD1
R24C	**100**	**0**	**NEM Blocked**	**TMD1**
D25C	**100**	**100**	**MTSES Blocked**	**TMD1**
A26C	**100**	**<50**	**Partial MTSES Block**	**TMD1**
V28C	**100**	**0**	**NEM Blocked**	**TMD1**
A29C	**100**	**100**	**MTSES Blocked**	**TMD1**
R30C	**100**	**100**	**MTSES Blocked**	**TMD1**
A37C	100	100	MTSES Blocked	Periplasmic loop
D39C	100	100	MTSES Blocked	Periplasmic loop
A44C	**100**	**100**	**MTSES Blocked**	**TMD2**
K46C	**100**	**<50**	**Partial MTSES Block**	**TMD2**
L47C	**100**	**<50**	**Partial MTSES Block**	**TMD2**
N49C	**100**	**>50**	**Partial MTSES Block**	**TMD2**
R52C	**100**	**100**	**MTSES Blocked**	**TMD2**
R53C	**100**	**<50**	**Partial MTSES Block**	**TMD2**
I54C	**100**	**<50**	**Partial MTSES Block**	**TMD2**
F55C	**100**	**<50**	**Partial MTSES Block**	**TMD2**
A56C	**100**	**>50**	**Partial MTSES Block**	**TMD2**
E57C	**100**	**>50**	**Partial MTSES Block**	**TMD2**
G58C	**100**	**<50**	**Partial MTSES Block**	**TMD2**
P66C	**100**	**0**	**NEM Blocked**	**TMD2**
A69C	**100**	**0**	**NEM Blocked**	**TMD2**
E70C	100	0	NEM Blocked	Cytoplasmic loop
Y71C	100	0	NEM Blocked	Cytoplasmic loop
K72C	100	0	NEM Blocked	Cytoplasmic loop
D78C	100	0	NEM Blocked	Cytoplasmic loop
L95C	0	0	Not Blocked	TMD3
V109C	<50	0	Partial NEM Block	TMD3
M111C	100	100	MTSES Blocked	Periplasmic loop
V112C	100	50	Partial MTSES Block	Periplasmic loop
D119C	100	100	MTSES Blocked	Periplasmic loop
A121C	100	100	MTSES Blocked	Periplasmic loop
S142C	0	0	Not Blocked	TMD4
A149C	**100**	**0**	**NEM Blocked**	**TMD4**
F157C	**100**	**0**	**NEM Blocked**	**TMD5**
A161C	**100**	**0**	**NEM Blocked**	**TMD5**
S170C	0	0	Not Blocked	TMD5
A179C	100	100	MTSES Blocked	Periplasmic loop
A188C	0	0	Not Blocked	TMD6
V198C	0	0	Not Blocked	TMD6
L208C	100	0	NEM Blocked	Cytoplasmic loop
K209C	100	0	NEM Blocked	Cytoplasmic loop
V215C	100	0	NEM Blocked	Cytoplasmic loop
R218C	100	0	NEM Blocked	Cytoplasmic loop

aa change [a,b]	% NEM block [c]	% MTSES block	SCAM Result	Location
D223C	100	0	NEM Blocked	Cytoplasmic loop
A224C	**100**	**0**	**NEM Blocked**	**TMD7**
R228C	**100**	**<50**	**Partial MTSES Block**	**TMD7**
V230C	**100**	**0**	**NEM Blocked**	**TMD7**
K231C	**100**	**0**	**NEM Blocked**	**TMD7**
A236C	**100**	**0**	**NEM Blocked**	**TMD7**
S241C	**100**	**0**	**NEM Blocked**	**TMD7**
V242C	**100**	**0**	**NEM Blocked**	**TMD7**
Q244C	**100**	**0**	**NEM Blocked**	**TMD7**
I245C	**100**	**0**	**NEM Blocked**	**TMD7**
I249C	<50	0	Partial NEM Block	TMD7
N250C	**100**	**0**	**NEM Blocked**	**TMD7**
T251C	**100**	**100**	**MTSES Blocked**	**TMD7**
A254C	100	100	MTSES Blocked	Periplasmic loop
F256C	100	100	MTSES Blocked	Periplasmic loop
S259C	100	100	MTSES Blocked	Periplasmic loop
S263C	100	100	MTSES Blocked	Periplasmic loop
A268C	**>50**	**<50**	**Partial Block**	**TMD8**
D269C	**100**	**50**	**Partial MTSES Block**	**TMD8**
R270C	**50**	**<50**	**Partial Block**	**TMD8**
M272C	**<50**	**0**	**Partial NEM Block**	**TMD8**
E273C	**100**	**>50**	**Partial MTSES Block**	**TMD8**
P275C	<50	0	Partial NEM Block	TMD8
S276C	**100**	**100**	**MTSES Blocked**	**TMD8**
G277C	**100**	**50**	**Partial MTSES Block**	**TMD8**
V278C	**100**	**0**	**NEM Blocked**	**TMD8**
V281C	**100**	**100**	**MTSES Blocked**	**TMD8**
T285C	**100**	**0**	**NEM Blocked**	**TMD8**
K293C	100	0	NEM Blocked	Cytoplasmic loop
S297C	100	0	NEM Blocked	Cytoplasmic loop
E302C	100	0	NEM Blocked	Cytoplasmic loop
G310C	0	0	Not Blocked	TMD9
C314	0	0	Not Blocked	TMD9
G326C	<50	0	Partial NEM Block	TMD9
S335C	<50	0	Partial NEM Block	TMD9
A344C	100	100	MTSES Blocked	Periplasmic loop
T350C	<50	0	Partial NEM Block	TMD10
A353C	<50	0	Partial NEM Block	TMD10
A356C	<50	0	Partial NEM Block	TMD10
V369C	50	0	Partial NEM Block	TMD10
F374C	**100**	**0**	**NEM Blocked**	**TMD10**
D379C	100	0	NEM Blocked	Cytoplasmic loop
V384C	**100**	**0**	**NEM Blocked**	**TMD11**
A387C	**100**	**0**	**NEM Blocked**	**TMD11**
V389C	<50	0	Partial NEM Block	TMD11
G403C	100	100	MTSES Blocked	Periplasmic loop
A408C	100	100	MTSES Blocked	Periplasmic loop
C419	0	0	Not Blocked	TMD12
L429C	<50	0	Partial NEM Block	TMD12
T436C	100	0	NEM Blocked	Cytoplasmic loop
G440C	100	0	NEM Blocked	Cytoplasmic loop

aa change [a,b]	% NEM block [c]	% MTSES block	SCAM Result	Location
G461C	0	0	Not Blocked	TMD13
I465C	100	100	MTSES Blocked	Periplasmic loop
S470C	100	100	MTSES Blocked	Periplasmic loop
T473C	100	100	MTSES Blocked	Periplasmic loop
L481C	0	0	Not Blocked	TMD14
G500C	100	0	NEM Blocked	C-terminal tail
V503C	100	0	NEM Blocked	C-terminal tail
R508C	100	0	NEM Blocked	C-terminal tail
V511C	100	0	NEM Blocked	C-terminal tail

[a] C314 and C419 are native Cys

[b] Results in boldface indicate blocked residues (>50% NEM) predicted to reside within the membrane plane

[c] Percent reflects amount of detected protein not labeled by Mal-PEG

TABLE S4. Comparison of TMD predictions of MurJ with our structural model

	aa encompassing TMDs[a]					
TMD	TOPPRED	HMMTOP	TMHMM	SOSUI	MINNOU	This study
1	2-23	27-51	26-45	2-24	1-31	6-32
2	27 - 47	82-106	82-104	29-51	38-71	41-69
3	88 - 108	125-149	133-152	87-109	78-111	83-109
4	133 - 153	162-181	159-181	131-153	121-153	124-154
5	161 - 181	186-203	186-203	157-179	161-178	157-178
6	247 - 267	234-258	237-259	183-205	186-210	186-207
7	272 - 292	275-292	274-296	236-258	224-255	224-253
8	313 - 333	313-337	316-338	274-296	263-289	264-292
9	354 - 374	354-373	353-375	311-333	307-337	303-337
10	385 - 405	386-403	382-404	354-376	345-376	347-374
11	447 - 467	408-427	408-427	381-403	382-402	380-402
12	481 - 501	440-463	444-466	408-429	406-430	410-429
13		482-501	481-503	440-462	440-464	444-463
14				481-502	474-500	478-499

[a] TOPPRED, HMMTOP, TMHMM, SOSUI, MINNOU prediction servers (12-16).

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