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1 ***Escherichia coli* SPFH membrane microdomain proteins HflKC**  
2 **contribute to aminoglycoside and oxidative stress tolerance**

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34  
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39 *Escherichia coli*

40

**41 ABSTRACT**

42

43 Many eukaryotic membrane-dependent functions are often spatially and temporally  
44 regulated by membrane microdomains (FMMs) also known as lipid rafts. These domains  
45 are enriched in polyisoprenoid lipids and scaffolding proteins belonging to the Stomatin,  
46 Prohibitin, Flotillin, and HflK/C (SPFH) protein superfamily that was also identified in  
47 Gram-positive bacteria. By contrast, little is still known about FMMs in Gram-negative  
48 bacteria. In *Escherichia coli* K12, 4 SPFH proteins, YqiK, QmcA, HflK, and HflC, were  
49 shown to localize in discrete polar or lateral inner-membrane locations, raising the  
50 possibility that *E. coli* SPFH proteins could contribute to the assembly of inner-membrane  
51 FMMs and the regulation of cellular processes.

52 Here we studied the determinant of the localization of QmcA and HflC and showed that  
53 FMM-associated cardiolipin lipid biosynthesis is required for their native localization  
54 pattern. Using Biolog phenotypic arrays, we showed that a mutant lacking all SPFH genes  
55 displayed increased sensitivity to aminoglycosides and oxidative stress that is due to the  
56 absence of HflKC. Our study therefore provides further insights into the contribution of  
57 SPFH proteins to stress tolerance in *E. coli*.

58

**59 IMPORTANCE**

60

61 Eukaryotic cells often segregate physiological processes in cholesterol-rich functional  
62 membrane micro-domains. These domains are also called lipid rafts and contain proteins  
63 of the Stomatin, Prohibitin, Flotillin, and HflK/C (SPFH) superfamily, which are also  
64 present in prokaryotes but were mostly studied in Gram-positive bacteria. Here, we  
65 showed that the cell localization of the SPFH proteins QmcA and HflKC in the Gram-  
66 negative bacteria *E. coli* is altered in absence of cardiolipin lipid synthesis. This suggests  
67 that cardiolipins contribute to *E. coli* membrane microdomain assembly. Using a broad  
68 phenotypic analysis, we also showed that HflKC contribute to *E. coli* tolerance to  
69 aminoglycosides and oxidative stress. Our study, therefore, provides new insights into the  
70 cellular processes associated with SPFH proteins in *E. coli*.

## 71 INTRODUCTION

72

73 In addition to separating the intracellular content of cells from the environment, lipid  
74 bilayer membranes also contribute to specialized functions, including cross-membrane  
75 transport, enzymatic activity, signaling as well as anchoring of cytoskeletal and  
76 extracellular structures (1, 2). In eukaryotes, these membrane-dependent functions are  
77 often spatially and temporally regulated by functional membrane microdomains (FMMs)  
78 called lipid rafts (3-5). FMMs compartmentalize membrane cellular processes in  
79 cholesterol- and sphingolipid-enriched membrane regions formed upon lipid-lipid, lipid-  
80 protein and protein-protein interactions (5-7). A family of membrane proteins called  
81 SPFH proteins (for Stomatin/Prohibitin/Flotillin/HflK/C) has been shown to localize in  
82 eukaryotic lipid rafts and to recruit and provide a stabilizing scaffold to other raft-  
83 associated proteins (8-13).

84

85 Whereas most prokaryotes lack sphingolipids and cholesterol (14), the Gram-positive  
86 bacteria *Bacillus subtilis* and *Staphylococcus aureus* can also compartmentalize cellular  
87 processes in functional membrane microdomains (FMMs) (14-16). Although whether  
88 bacterial FMMs display a distinct lipidic composition still needs to be established, they  
89 have been reported to be potentially enriched in polyisoprenoid lipids as well as in  
90 cardiolipins at the cell poles (14-16)

91

92 FMMs also contain SPFH proteins, including flotillins, and a pool of proteins involved  
93 in diverse cellular processes (17) (14, 16). In *B. subtilis*, flotillins FloT and FloA  
94 colocalize in membrane foci and contribute to the assembly of membrane protein  
95 complexes (18) (15). Lack of flotillins impairs biofilm formation, sporulation, protease  
96 secretion, motility, and natural competence, indicating that the formation of FMMs also  
97 plays critical cellular roles in *B. subtilis* (15, 18-22).

98 SPFH proteins are also present in Gram-negative bacteria, and *Escherichia coli* K12 even  
99 possesses four genes, *yqiK*, *qmcA*, *hflK*, and *hflC*, which encode proteins with an SPFH  
100 domain and a N-terminal transmembrane segment (23). QmcA and YqiK are predicted to  
101 face the cytoplasmic compartment, while HflK and HflC are predicted to be exposed in  
102 the periplasm, forming the HflKC complex negatively regulating the protease activity of

103 FtsH against membrane proteins (24-27). HflC and QmcA are detected Fluorescent  
104 microscopy also showed that *E. coli* SPFH proteins HflC and QmcA are localized in  
105 discrete polar or lateral membrane foci (28), raising the possibility that *E. coli* SPFH  
106 proteins could localize in inner-membrane FMMs and regulate specific cellular processes  
107 (29). However, apart from the functional and structural description of HflKC as a  
108 regulator of the FtsH membrane protease (24, 27, 30) and a recent study suggesting that  
109 YqiK is involved in cell motility and resistance to ampicillin (31), the functions of FMM  
110 in *E. coli* and other Gram-negative bacteria are still poorly understood.

111 In this study, we used fluorescent and super-resolution microscopy to perform a detailed  
112 analysis of QmcA and HflC membrane localization signals. We then showed that the  
113 integrity of QmcA and HflC protein domains is required for their inner membrane  
114 localization, and that the lack of cardiolipin and isoprenoid lipids known to associate with  
115 FMMs alters their localization. Moreover, using single and multiple SPFH gene mutants,  
116 we showed that HflKC SPFH proteins contribute to aminoglycosides and oxidative stress  
117 resistance. Our study therefore provides new insights into the determinants of cellular  
118 localization and the function associated with *E. coli* SPFH proteins.

## 119 RESULTS

120

### 121 Chromosomal *E. coli* SPFH fluorescent fusion proteins show distinct localization 122 patterns.

123

124 To investigate the determinant of cell localization of *E. coli* SPFH proteins, we first  
125 tagged YqiK and QmcA, which C-termini are predicted to be in the cytoplasm (26), with  
126 a C-terminal monomeric super folder green fluorescent protein (msfGFP). We then  
127 tagged HflC and HflK, which C-termini are predicted to be in the periplasm (26), with  
128 the C-terminal monomeric red fluorescent protein mCherry. All these fusions were  
129 expressed under their own promoter from their native chromosomal location (Sup. Fig.  
130 S1). Epifluorescence and super-resolution microscopy confirmed the previously reported  
131 polar localization of HflK and HflC (28), with 94% and 91% polar localization pattern  
132 for HflC-mCherry and HflK-mCherry, respectively (n=150) (Fig. 1B and Sup. Fig. S2).  
133 By contrast, C-terminally tagged QmcA-GFP showed punctate foci distributed  
134 throughout the cell body, with 96% of the cells harboring 5 foci or more (n=150) (Fig.  
135 1A). However, we could not detect YqiK-GFP, possibly due to its low native  
136 chromosomal expression level. We then used anti-GFP or mCherry antibodies to perform  
137 immunodetection on cytoplasmic as well as inner and outer membrane fractions of *E. coli*  
138 strains expressing either HflC-mCherry or QmcA-GFP. In agreement with previous  
139 results (26, 29), both fusion proteins were detected in the inner membrane fraction and  
140 showed minimal degradation profile (Fig. 2 and Sup. Fig. S3 and S4). Moreover, the  
141 functionality of the HflKC fusion could be demonstrated (see result sections below).

142

### 143 Domain swap analysis shows that protein integrity is essential for QmcA-GFP and 144 HflC-mCherry localization.

145

146 To identify HflC and QmcA membrane localization signals, we constructed multiple  
147 fluorescently tagged truncated versions of both proteins. We tagged with msfGFP a  
148 QmcA protein reduced to its transmembrane region and SPFH domain (QmcA-GFP is  
149 therefore now named TM<sup>QmcA</sup>-SPFH<sup>QmcA</sup>-GFP in Figure 3) and, separately, one reduced  
150 to the QmcA transmembrane region only (TM<sup>QmcA</sup>-GFP) (Fig. 3A). To test the role of the  
151 QmcA transmembrane region, we also swapped TM<sup>QmcA</sup> in the three constructs by the  
152 single-spanning TM domain of the phage coat protein Pf3 (TM<sup>Pf3</sup>), which orients  
153 subsequent amino acids to the cytosol (38) (Fig. 3A). Similarly, in addition to the full

154 length HflC-mCherry, we tagged with mCherry the HflC transmembrane region and  
155 SPFH domain (HflC-mCherry is therefore now named TM<sup>HflC</sup>-SPFH<sup>HflC</sup>-mCherry in  
156 Figure 3) and, separately, only its TM region (TM<sup>HflC</sup>-mCherry) (Fig. 3B). We also  
157 swapped the HflC TM region with the single-spanning TM region of colistin M immunity  
158 protein (TM<sup>Cmi</sup>), which orients subsequent amino acids to the periplasm (39) (Fig. 3B).  
159 Epifluorescence microscopy of HflC and QmcA derivative fusions showed that in  
160 addition to native full-length constructs only full-length constructs with swapped TM  
161 (TM<sup>PB</sup>-QmcA-GFP and TM<sup>Cmi</sup>-HflC-mCherry) displayed significant punctate foci or  
162 polar localization, respectively (Fig. 3AB), although at reduced frequency compared to  
163 native QmcA-GFP and HflC-mCherry (Sup. Fig. S5). Finally, we prepared inner and  
164 outer membrane fractions of *E. coli* strains expressing each QmcA and HflC derivative,  
165 and we observed that all these constructs were still mainly located in the inner membrane  
166 fraction. This indicates that, while we observed that QmcA-GFP and HflC-mCherry  
167 derivatives exhibit altered cell localization, they do not exhibit significant mis-  
168 localization and remain located in the inner membrane (Sup. Fig. S3 and Fig S4). These  
169 results therefore indicated that specific QmcA and HflC localization requires the  
170 combination of a transmembrane and full cytoplasmic (QmcA) or periplasmic (HflC)  
171 domain.

172

### 173 **Lack of cardiolipin and isoprenoid lipid synthesis alters the cell localization of** 174 **QmcA and HflC.**

175

176 FMMs were proposed to be enriched with negatively charged cardiolipins and  
177 isoprenoids, which promote the localization of polar proteins and modulation of  
178 membrane lipid fluidity (15, 18, 32-37). We first tested whether alteration of cardiolipin  
179 synthesis could cause mis-localization of *E. coli* SPFH proteins QmcA or HflKC in a  
180 mutant lacking the major cardiolipin synthases *clsABC* (38). Whereas a super-resolution  
181 microscopy analysis only showed an alteration of the number of QmcA-GFP punctates  
182 (1-5 cluster per bacterium) compared to WT (10-15 cluster per bacterium) (Fig. 4A and  
183 Sup. Fig. S6), the localization of HflC-mCherry showed a drastic loss of polar localization  
184 pattern (Fig. 4B and Supp. Fig. S4). We then used an *idi* mutant with reduced isoprenoid  
185 lipid synthesis due to the lack of isomerization of isopentenyl diphosphate (IPP) into  
186 dimethylallyl diphosphate (DMAPP) (39, 40). Whereas QmcA-GFP punctate localization  
187 was not affected, HflC-mCherry polar localization was abolished in the  $\Delta idi$  mutant (Fig.

188 4A and Sup. Fig. S6). These results demonstrated that the alteration of cardiolipin and, in  
189 a lesser extent, isoprenoid lipid synthesis pathway affects HflC fusion protein localization  
190 in *E. coli*.

191

192 **Phenotypic analysis of *E. coli* SPFH mutant shows that only the absence of HflKC**  
193 **increases *E. coli* sensitivity to aminoglycosides and oxidative stress.**

194

195 To identify potential phenotypes and functions associated with the *E. coli* SPFH proteins  
196 YqiK, QmcA, HflK and HflC, we introduced single and multiple deletions of the  
197 corresponding SPFH genes. We observed that neither single mutants nor the quadruple  
198  $\Delta hflK$ ,  $\Delta hflC$ ,  $\Delta qmcA$ , and  $\Delta yqiK$  (hereafter referred to  $\Delta SPFH$  mutant) displayed any  
199 significant growth defects in rich or minimal media (Fig. 5A and Sup. Fig. S7A).  
200 Considering the role of SPFH proteins in the activation of inner-membrane kinases  
201 involved in *B. subtilis* biofilm formation (15), we tested adhesion and biofilm capacity of  
202 WT and  $\Delta SPFH$  strains but could not detect any significant differences between these two  
203 strains. We then used Biolog<sup>TM</sup> phenotypic microarrays to perform a large-scale  
204 phenotypic assay comparing an *E. coli* WT and  $\Delta SPFH$  mutant (Sup. Table S1). This  
205 analysis revealed that the  $\Delta SPFH$  mutant is metabolically less active when grown in the  
206 presence of various aminoglycosides (tobramycin, capreomycin, sisomicin and  
207 paromomycin) or when exposed to paraquat (Fig. 5B and Sup. Fig. S7BC). Consistently,  
208 the minimal inhibitory concentration (MIC) for tobramycin of the  $\Delta SPFH$  mutant was 3-  
209 fold lower than that of the WT MIC (Fig.5C), and the sensitivity of the  $\Delta SPFH$  mutant to  
210 paraquat was increased compared to the WT (Fig. 5D and Sup. Fig. S7D). Test of  
211 individual SPFH-gene mutants for their sensitivity to tobramycin and paraquat showed  
212 that the HflKC complex is the sole responsible for both phenotypes, as both single *hflK*  
213 and *hflC* or a double *hflKC* mutants displayed increased sensitivity to tobramycin and  
214 oxidative stress (Fig. 5CD and Sup. Fig. S5E). This phenotype could be complemented  
215 upon the introduction of a plasmid expressing *hflKC* genes in the double *hflKC* mutant  
216 and C-terminally tagged HflC-mCherry and HflK-mCherry displayed wildtype MIC for  
217 tobramycin and paraquat, indicating that both fusions were functional and relevant  
218 proxies for the bacterial localization of the HflKC complex (Sup. Fig. S8).

219

220



## 221 **Contribution of HflC localization to tobramycin and paraquat tolerance**

222

223 Whereas the MIC determination for tobramycin and paraquat of an  $\Delta idi$  mutant showed  
224 no significant difference compared to the WT, the MIC for tobramycin and paraquat of a  
225  $\Delta clsABC$  mutant was reduced by 2 to 3-fold (Fig.5CD). Considering the scaffolding role  
226 of HflKC and the importance of cell localization, we tested the localization and  
227 contribution to aminoglycoside and stress tolerance of AcrA, a protein previously  
228 identified in *E. coli* inner-membrane potentially associated with FMM (28). AcrA is an  
229 efflux pump involved in the transport of a wide range of substrates including  
230 aminoglycosides (41). However, while an *acrA* deletion did not alter *E. coli* MIC  
231 profile to tobramycin and paraquat as much as a  $\Delta hflC$  mutant (Fig.5CD), the expression  
232 of AcrA-GFP from their native chromosomal context did not lead to any distinct polar  
233 co-localization with HflC in exponential or stationary phase conditions (Sup. Fig. S9).

234

235 Taken together, these results indicate that the HflKC SPFH protein complex contributes  
236 to oxidative and antibiotic stress resistance.

237

238

239 **DISCUSSION**

240

241 SPFH-domain proteins have been identified in most organisms (16, 42) and extensively  
242 studied in eukaryotes (3, 5, 43). By contrast, prokaryotic SPFH proteins and proteins  
243 associated with functional membrane microdomains (FMMs) are much less understood.  
244 This is particularly the case for Gram-negative bacteria, in which potential FMMs  
245 functions are mostly inferred from studies performed in *B. subtilis* and *S. aureus*. In this  
246 study, we investigated the functions and the localization determinants of *E. coli* SPFH  
247 proteins. Focusing on QmcA and HflKC SPFH proteins, we used a domain deletion and  
248 replacement approach and showed that most of the tested domain replacement variants  
249 correctly localized to the inner membrane but failed to display WT protein localization  
250 patterns. This indicates that inner-membrane localization alone is not sufficient for the  
251 correct subcellular distribution of HflC and QmcA, whose localization signals likely rely  
252 on oligomerization for focal flotillin cluster formation (44), which could be abolished in  
253 the domain deletion constructs, potentially explaining the observed localization defects.

254

255 QmcA and HflKC SPFH proteins display different localization patterns and could be part  
256 of different FMMs, potentially using different localization signals. The punctate  
257 localization pattern displayed by QmcA-GFP fusion was also observed in the case of *E.*  
258 *coli* YqiK expressed from plasmid and other Gram-positive bacteria flotillin  
259 homologues(15, 16, 36, 45, 46). Interestingly, *B. subtilis*, *B. anthracis* and *S. aureus*  
260 flotillin genes are physically associated with a gene encoding a NfeD protein, which could  
261 contribute to protein-protein interactions within flotillin assemblies (47, 48). Consistently,  
262 *E. coli qmcA* gene is located upstream of the NfeD-like *ybbJ* gene, like NfeD-like *yqiJ*  
263 gene is located upstream of *yqiK* (see Supplementary Figure S1). This further supports  
264 the notion that QmcA and YqiK could be considered as *E. coli* FloA/FloT homologs.

265

266 By contrast, the *hflKC* transcription unit is not closely associated with a *nfeD*-like gene,  
267 suggesting that HflKC may not be a flotillin. However, while QmcA and YqiK have an  
268 opposite orientation to HflK and HflC, they are structurally similar proteins and the four  
269 *E. coli* SPFH proteins could therefore share some degrees of functionalities. The  
270 topological similarity between HflK and HflC might contribute to HflKC complex

271 formation, and its interaction with FtsH protease, resulting in a large periplasmic FtsH-  
272 HflKC complex localized at the cell pole (24, 25, 49-52)

273 Along with phosphatidylethanolamine and phosphatidylglycerol, cardiolipins are the  
274 primary constituent components of *E. coli* membranes that concentrate into cell poles and  
275 dividing septum (53-56). It was indeed observed that the composition of *E. coli* membrane  
276 lipids at cell poles is altered in a *clsABC* cardiolipin deficient mutant, compensated by an  
277 increased amount of phosphatidylglycerol (32, 57). Several studies also reported that  
278 cardiolipin-enriched composition in membranes at cell poles influences both the  
279 localization and activity of inner membrane proteins, such as respiratory chain protein  
280 complexes and the osmosensory transporter ProP (33, 34, 58-60). We showed here that,  
281 similarly to ProP, HflC and QmcA localization patterns are affected in a  $\Delta clsABC$  mutant,  
282 suggesting that HflKC and QmcA complexes could act as scaffolds for cardiolipin-  
283 enriched FMM cargo proteins. Isoprenoid lipids such as farnesol, carotenoids, and  
284 hopanoids have been proposed to be constituents of bacterial FMMs or to interact with  
285 SPFH proteins and FMM-associated proteins (14). Consistently, blocking the *S. aureus*  
286 carotenoid synthetic pathway by zaragozic acid leads to flotillin mis-localization (15),  
287 and the inactivation of farnesol synthesis in a *B. subtilis yisP* mutant impairs focal  
288 localization of the FMM-associated sensor kinase KinC (15). We showed that interfering  
289 with the *E. coli idi* isoprenoid biosynthesis pathway also strongly alters the localization  
290 of HflC. This further indicates that isoprenoid lipids contribute to the formation or  
291 integrity of FMMs, possibly by altering isoprenoid-dependent membrane fluidity, as  
292 shown in *S. aureus* and *B. subtilis* FMMs (14, 61).

293

294 Our investigation of the phenotypes displayed by an *E. coli* mutant lacking all SPFH  
295 protein genes showed that the absence of HflKC leads to an increased susceptibility to  
296 oxidative stress and aminoglycosides. The HflKC complex was previously shown to  
297 modulate the quality control proteolytic activity of FtsH by regulating the access of  
298 misfolded membrane protein products to FtsH (24, 25, 62). *E. coli*  $\Delta hflK$  and  $\Delta hflC$   
299 mutant strains were also shown to accumulate increased amounts of hydroxyl radical,  
300 suggesting that HflK and HflC could influence tolerance to aminoglycosides and  
301 oxidative stress by suppressing excessive hydroxyl radical production. Alternatively,  
302 HflK and HflC could contribute to tobramycin resistance via FtsH-dependent proteolytic

303 activity (63) or favoring FMM formation and the assembly of membrane proteins and  
304 lipids, such as cardiolipin, involved in the transport and movement of aminoglycosides  
305 within cells and cell membranes. Consistently, several proteins associated with  
306 aminoglycosides transport potentially associated with FMMs composition, including  
307 transporters and several components of the AcrAB-TolC efflux pump (28), suggesting  
308 that deletion of *hflK* or *hflC* could reduce the activity of these proteins in FMMs and  
309 enhance entry of aminoglycosides. Whereas the susceptibility to aminoglycosides indeed  
310 partly relies on the AcrAB-TolC efflux pump (41, 64-66), we found that lack of the AcrA  
311 only moderately decreases the MIC to tobramycin, compared to a *hflKC* mutation. We  
312 also showed that the alteration of the cardiolipin pathway in a  $\Delta$ *clsABC* mutant altered  
313 both the localization and sensitivity to tobramycin and paraquat of a HflC-GFP fusion.  
314 This suggests that cardiolipin could be required for the correct localization of HflKC to  
315 FMMs at cell poles. However, we observed that, although an *idi* isoprenoid pathway  
316 mutant affects HflC localization, it does not show altered sensitivity. We cannot rule out  
317 that that the effect of  $\Delta$ *clsABC* mutant on resistance to aminoglycosides and oxidative  
318 stress could be unrelated to its impact on HflKC polar localization. Alternatively, the lack  
319 of effect of  $\Delta$ *idi* mutant may be due to an uncomplete inactivation of the pathway since  
320 lycopene production in a  $\Delta$ *idi* mutant is reduced by 1/3 but not totally abolished, due to  
321 the fact that the *idi* protein is a reversible isomerase.(39).

322 In conclusion, the present study provides new insights into the functions of *E. coli* SPFH  
323 proteins and some of their interacting partners and further experiments will be needed to  
324 fully uncover the roles played by this intriguing family of membrane proteins in Gram-  
325 negative bacteria.

326

## 327 MATERIALS & METHODS

328

### 329 Bacterial strains and growth conditions

330 Bacterial strains and plasmids used in this study are described in Sup. Table S2, and  
331 further explained in Sup. Fig. S1 and Figure 3. Unless stated otherwise, all experiments  
332 were performed in lysogeny broth (LB) or M63B1 minimal medium supplemented with  
333 0.4% glucose (M63B1.G) at 37 °C. Antibiotics were used as follows: kanamycin (50  
334 µg/mL); chloramphenicol (25 µg/mL); ampicillin (100 µg/mL); and zeocin (50 µg/mL).  
335 All compounds were purchased from Sigma-Aldrich (St Louis, MO, USA) except for  
336 Zeocin (InvivoGen, Santa Cruz, CA, USA).

337

### 338 Mutant construction

339 *Generation of mutants in E. coli:* Briefly, *E. coli* deletion or insertion mutants used in this  
340 study originated either from the *E. coli* Keio collection of mutants (67) or were generated  
341 by  $\lambda$ -red linear recombination using pKOBEG (Cm<sup>R</sup>) or pKOBEGA (Amp<sup>R</sup>) plasmids  
342 (68) using primers listed in Sup. Table S3. P1vir transduction was used to transfer  
343 mutations between different strains. When required, antibiotic resistance markers flanked  
344 by two FRT sites were removed using the Flp recombinase (69). Plasmids used in this  
345 study were constructed using an isothermal assembly method, Gibson assembly (New  
346 England Biolabs, Ipswich, MA, USA) using primers listed in Sup. Table S3. The integrity  
347 of all cloned fragments, mutations, and plasmids was verified by PCR with specific  
348 primers and DNA sequencing

349

#### 350 *Construction of deletion mutants*

351  $\Delta yqiK$ ,  $\Delta qmcA$ ,  $\Delta hflK$ ,  $\Delta hflC$ ,  $\Delta clsA$ ,  $\Delta clsB$ ,  $\Delta clsC$ ,  $\Delta idi$ ,  $\Delta acrA$ , deletions were  
352 transferred into *E. coli* MG1655strep by P1vir phage transduction from the corresponding  
353 mutants in the *E. coli* BW25113 background of the Keio collection (67). The associated  
354 kanamycin marker was then removed using the Flp recombinase expressed from the  
355 plasmid pCP20 (69). (Details regarding the construction of all other strains used in this  
356 study are presented in Sup. Table S2).

357

#### 358 *Construction of GFP and mCherry fusions*

359 See Supplementary Methods section in Supplementary Materials

360

#### 361 *Construction of complemented strains*

362 The *hflKC* genes were amplified from MG1655*strep* using primers listed in  
363 Supplementary Table S3 and cloned at the downstream of the IPTG-inducible promoter  
364 of a pZS\*12 vector using the Gibson assembly to generate plasmids pZS\*12-HflKC Then,  
365 these plasmids were introduced into  $\Delta$ *hflKC* mutants, respectively, to construct  
366 complemented mutants (Sup. Table S2). A pZS\*12 empty vector was also introduced into  
367 wildtype and  $\Delta$ *hflKC* mutants. Mutants harbouring these pZS\*12 plasmids were  
368 incubated and used for the below experiments in the presence of IPTG (1 mM) and  
369 ampicillin.

370

#### 371 **Epifluorescence microscopy**

372 Bacteria were incubated into 5 mL of fresh LB medium and harvested at OD<sub>600</sub> 0.4 for  
373 samples in exponential phase or OD<sub>600</sub> 2.0 for stationary phase. After washing twice with  
374 M63B1 medium, cells corresponding to 1 mL of the bacterial culture were pelleted by  
375 centrifugation and resuspended into 0.1 mL of M63B1 medium for exponential samples,  
376 or 1 mL of the medium for stationary samples. Ten  $\mu$ L aliquots of the cell suspension  
377 were immobilized on glass slides previously covered with freshly made M63B1 medium  
378 0.8% agarose pads. Cells were observed using a ZEISS Definite focus fluorescent  
379 microscope (Carl Zeiss, Oberkochen, Germany), equipped with an oil-immersion  
380 objective lens microscope (Pln-Apo 63X/1.4 oil Ph3). GFP or mCherry fluorescence was  
381 excited with a ZEISS Colibri LED illumination system and the fluorescence signal was  
382 detected with Zeiss FS38 HE (Carl Zeiss) or Semrock HcRed filters (Semrock, Rochester,  
383 NY, USA). GFP, and mCherry fluorescence images were taken at 1000, and 2000 msec.  
384 exposure, respectively. Image processing was performed using ImageJ and Adobe  
385 Photoshop. For each tested strain, the subcellular localization patterns of 50 randomly  
386 selected bacteria were evaluated four times (a total of 200 cells), and each frequency was  
387 expressed as percentiles.

388

#### 389 **Super-resolution microscopy**

390 Bacteria were imaged using single-molecule localization microscopy and stochastic

391 optical reconstruction microscopy (SMLM-STORM), using a previously described  
392 method (70). Overnight cultures were fixed with PFA 4%, permeabilized with Triton  
393 0,05%, and labeled with either GFP monoclonal FluoTag®-Q — Sulfo-Cyanine 5 (Cy5),  
394 or RFP monoclonal FluoTag®-Q – Cy5, which are single-domain antibodies (sdAb)  
395 conjugated to Cy5. Labeling was performed at 1:250 (concentration), and washing steps  
396 were carried out three times using Abbelight's SMART kit buffer. For imaging,  
397 Abbelight's imaging system was used with NEO software. Abbelight's module was added  
398 to an Olympus IX83 with 100x TIRF objective, N.A. 1.49. We used Hamamatsu's  
399 sCMOS Flash 4 camera and a 647nm 500mW Oxxius laser, with an astigmatic lens, to  
400 allow for 3D imaging of the sample (71).

401

#### 402 **Inner membrane separation**

403 *E. coli* overnight cultures were diluted into 1 L of fresh LB medium to OD<sub>600</sub> of 0.02 and  
404 incubated at 37°C and 180 rpm until reaching OD<sub>600</sub> 0.4. Cells were harvested and washed  
405 once with 10 mM HEPES (pH 7.4) and stored at -20°C for at least 1 h. Bacteria were then  
406 resuspended in 10 mL of 10 mM HEPES (pH 7.4) containing 100 µL of Benzonase (3.10<sup>4</sup>  
407 U/mL) and were passed through a FRENCH press (Thermo) at 20,000 psi. The lysate was  
408 centrifugated at 15,000 g for 15 min at 4 °C to remove cell debris, and aliquots of the  
409 suspension were stored at 4 °C as the whole extract. Then, the suspension was centrifuged  
410 at 100,000 g for 45 min at 4 °C to separate supernatant and pellets, and aliquots of the  
411 supernatant were stored at 4 °C as the cytosolic and periplasmic fractions. The pellets  
412 were suspended into 600 µL of cold 10 mM HEPES (pH 7.4) and homogenized by using  
413 2 mL tissue grinder (Kontes Glass, Vineland, NJ, USA). Discontinuous sucrose gradients  
414 with the following composition were placed into ultracentrifugation tube: bottom to top  
415 0.5 mL of 2 M sucrose, 2.0 mL of 1.5 M sucrose, and 1.0 mL of 0.8 M sucrose, and 500  
416 µL of the homogenized samples were placed on the top of sucrose gradients. The  
417 gradients were centrifuged at 100,000 g for 17.5 h at 4 °C. Subsequently, 400 µL of  
418 aliquots were collected into 11 microtubes from top to bottom, and the samples were  
419 proceeded to the immunodetection method, as described below.

420

#### 421 **Immunodetection of inner membrane proteins**

422 Aliquots of samples were suspended in 4× Laemmli buffer (BioRad) with 2-  
423 Mercaptoethanol (Sigma) and incubated for 5 min at 98 °C. The protein samples (10 µL  
424 each) were run on 4-20 % Mini-PROTEAN TGX Stain-Free™ precast Gels (BioRad)  
425 in 1× TGX buffer and then transferred to nitrocellulose membrane using a Trans-Blot®  
426 Turbo™ Transfer System (BioRad). Subsequently, the membranes were blocked using  
427 blocking buffer consisting of 5% skim milk in PBS with 0.05% Tween 20 (PBST) for 2  
428 h at 4 °C with agitation. The membranes were then incubated in PBST containing 1%  
429 skim milk with first antibodies, polyclonal rabbit antiserum raised against ExbB and TolC  
430 (kindly given by Dr. Philippe Delepelaire), GFP (Invitrogen, A6455, Thermo Fisher  
431 Scientific, Indianapolis, IN, USA) and mCherry (Invitrogen, PA5-34974) at 1:20,000  
432 overnight at 4 °C with agitation. The membranes were washed in PBST and incubated in  
433 PBST containing 1% skim milk with a secondary antibody, anti-rabbit IgG conjugated  
434 with horseradish peroxidase (Cell signaling, 7074S), at 1:10,000 for 2 h at 25 °C with  
435 agitation. After washing the excess secondary antibody, specific bands were visualized  
436 using the ECL prime detection method (GE Healthcare) and imaged with an imaging  
437 system, iBright™ CL1500 (Invitrogen).

438

#### 439 **Microbial growth phenotypic analysis**

440 A high-throughput analysis for microbial growth phenotypes using a colorimetric reaction,  
441 Phenotype MicroArrays (Biolog Inc., Hayward, CA, USA), was performed in accordance  
442 with the manufacturer's protocol. Briefly, several colonies of *E. coli* grown on LB agar  
443 were transferred in 10 mL of a mixture of Biolog IF-0a media (BioLog) and sterilized  
444 water into a sterile capped test tube. The suspension was mixed gently, and the turbidity  
445 was adjusted to achieve the appropriate transmittance using the Biolog turbidimeter  
446 (BioLog). The cell suspension was diluted with the IF-0a plus dye mix, as mentioned in  
447 the manufacturer's protocol. 100 µL of the mixture suspension was inoculated into PM  
448 plates 1-3 and 9-20 and incubated for 72 h at 37 °C. The absorbance of each well was  
449 taken every 15 min. The OmniLog software (BioLog) was used to view and edit data, to  
450 compare data lists, and to generate reports.

451

#### 452 **Monitoring of bacterial growth**



453 An overnight culture of *E. coli* was diluted into fresh LB and M63B1 supplemented with  
454 0.4% glucose medium to OD<sub>600</sub> of 0.05, and 200 µL aliquots were cultured in the presence  
455 or absence of paraquat (Methyl viologen dichloride hydrate, Sigma-Aldrich) in 96-well  
456 microplates at 37 °C for 24 h with shaking. The absorbance of each culture at 600 nm was  
457 measured every 15 min for 24 h using a microplate reader (Tecan Infinite, Mannedorf,  
458 Switzerland).

459

#### 460 **Susceptibility of *E. coli* against Tobramycin and Paraquat**

461 The broth microdilution method was used to determine the MIC (minimum inhibitory  
462 concentration) values of Tobramycin (Sigma-Aldrich) and Paraquat (Sigma-Aldrich) in  
463 96-well microtiter plates. Briefly, 100 µL of LB medium was distributed into each well  
464 of the microtiter plates. Tobramycin was 2-fold serially diluted in each well. Five µL of  
465 approximately  $1 \times 10^7$  CFU/mL of *E. coli* was inoculated into each well, and the plates  
466 were incubated at 37°C for 24 h. The lowest concentration that visibly inhibited bacterial  
467 growth was defined as the MIC. All strains were evaluated in biological and technical  
468 triplicates.

469 The spot assay was performed to evaluate the susceptibility of *E. coli* against  
470 paraquat. Briefly, an overnight culture of *E. coli* was diluted into fresh LB medium to  
471 OD<sub>600</sub> of 0.05. Ten µL of the diluted culture was spotted on LB plates containing either  
472 no or 100 µM paraquat. The plates were incubated at 37°C for 24 h, and the photographs  
473 were taken. All strains were evaluated in triplicate.

474

#### 475 **Statistical analysis**

476 Data analysis was performed using GraphPad Prism 9.5 software (GraphPad, La Jolla,  
477 CA, USA). All data are expressed as mean ( $\pm$  standard deviation, SD) in figures.  
478 Statistical analysis was performed using unpaired non-parametric Mann-Whitney test.  
479 Differences were considered statistically significant for *P* values of <0.05.

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481

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494

495 **AUTHOR CONTRIBUTIONS:**

496 A.K.W., Y.Y., and J.-M.G. designed the experiments. A.K.W., Y.Y., A.R., R.B.; M.S.,  
497 J.B-B. performed the experiments. A.K.W., Y.Y., A.R., U.V. M.S., R.B., J.-M.B., C.B.,  
498 D.L. and J.-M.G. analyzed the data. Y.Y., A.K.W. and J.-M.G. wrote the paper with  
499 significant contribution from all authors.

500 **DATA AVAILABILITY**

501 The data that support the findings of this study are presented in the paper and/or the  
502 Supplementary Materials. Strains and plasmids are available from the corresponding  
503 author, JMG, upon reasonable request.

504 **COMPETING INTERESTS**

505 The authors declare no competing financial or non-financial interests.

506

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716 **FIGURE LEGENDS**

717

718 **Figure 1. Cell localization patterns of HflC and QmcA.** Epifluorescence microscopy  
 719 of cells expressing QmcA-GFP (A) and HflC-mCherry (B). Representative images are  
 720 shown. Percentages indicate the frequencies of cells showing localization foci.  
 721 Arrowheads indicate polar or punctate localization foci. Scales are indicated as white bars.

722

723 **Figure 2. QmcA and HflC localize to the inner membrane.** SDS-PAGE and  
 724 immunodetection analyses of whole-cell extracts, cytosolic fractions, and inner (IM) or  
 725 outer membrane (OM) fractions prepared from cells expressing QmcA-GFP (A) and  
 726 HflC-mCherry (B). Anti-GFP and anti-mCherry antibodies were used to detect the  
 727 presence of QmcA-GFP and HflC-mCherry, respectively. An anti-ExbB antibody were  
 728 used to detect the inner membrane- (IM) marker ExbB and anti-TolC antibodies to detect  
 729 the outer membrane- (OM) marker TolC.

730

731 **Figure 3. The localization pattern and membrane topology of full-length, domain**  
 732 **swapped or truncated versions of QmcA and HflC.** (A) GFP fusion derivatives of  
 733 QmcA and (B) mCherry fusion derivatives of HflC. The representative images are shown  
 734 in each strain with the frequencies of cells showing punctate (A) or polar localization (B).  
 735 In figure 3A, first panel, the control WT QmcA-GFP is re-named TM<sup>QmcA</sup>-SPFH<sup>QmcA</sup>-  
 736 GFP and the fluorescence microscopy image corresponding to its localization is therefore  
 737 a duplicate of the one presented in Figure 1A. Similarly, In figure 3B, first panel, the  
 738 control WT HflC-mCherry is re-named TM<sup>HflC</sup>-SPFH<sup>HflC</sup>-mCherry and the fluorescence  
 739 microscopy image corresponding to its localization is therefore a duplicate of the one  
 740 presented in Figure 1B. In membrane topology, helical structures represent  
 741 transmembrane (TM) domains; silver, native TM domain of QmcA; pink, Pf3 domain;  
 742 green, native TM domain of HflC; black, Cmi domain. Scale bars are 2  $\mu$ m.

743

744 **Figure 4. Alteration of QmcA and HflC cell localization in *E. coli* cardiolipin and**  
 745 **isoprenoid pathway mutants.** Two-dimensional super-resolution microscopy images of  
 746 WT,  $\Delta$ *clsABC*, and  $\Delta$ *idi* strains expressing QmcA-GFP (A) and HflC-mCherry (B) in  
 747 stationary phase. Number or nature of detected cluster is indicated. Scales are indicated  
 748 as white bars.

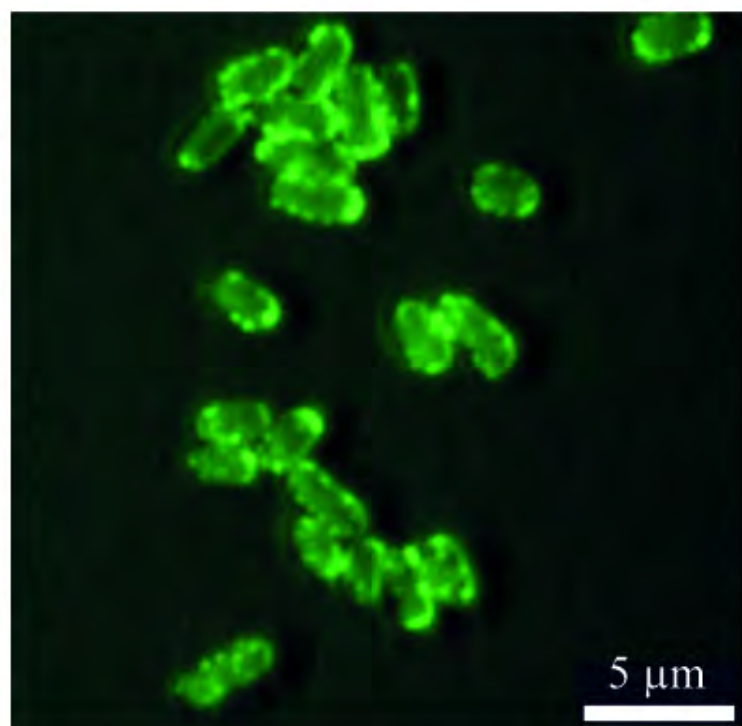
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750 **Figure 5. Phenotypic analysis of *E. coli* SPFH mutants.** A: Bacterial growth curve of  
 751 WT and SPFH gene deletion mutants in LB medium. B: Biolog bacterial growth curve of  
 752 WT and  $\Delta$ SPFH in the presence of tobramycin and paraquat. C: Minimum inhibitory  
 753 concentration (MIC) for tobramycin of *E. coli* WT and indicated mutants. D: Minimum  
 754 inhibitory concentration (MIC) for paraquat of *E. coli* WT and indicated mutants. \* $p$  <  
 755 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001, \*\*\*\* $p$  < 0.0001 compared with WT.

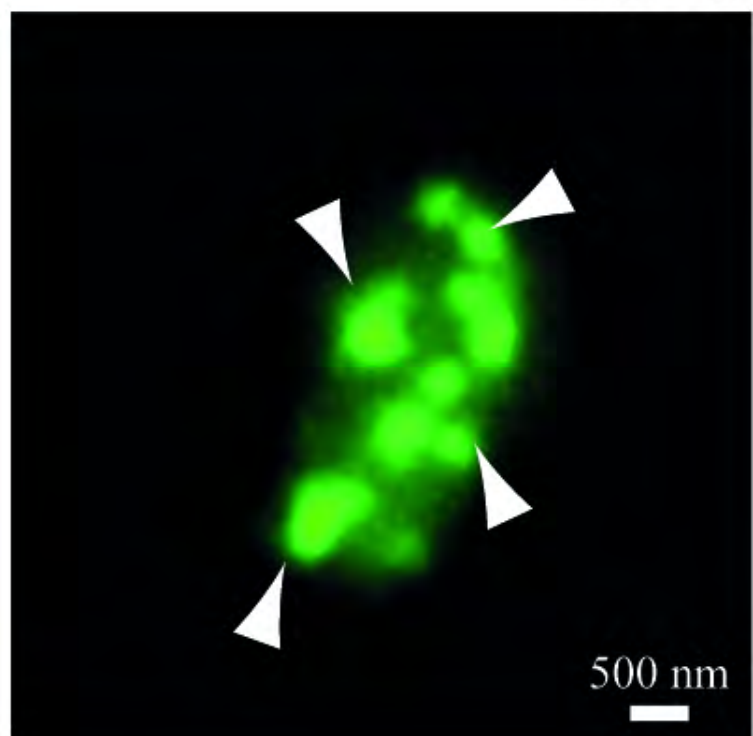
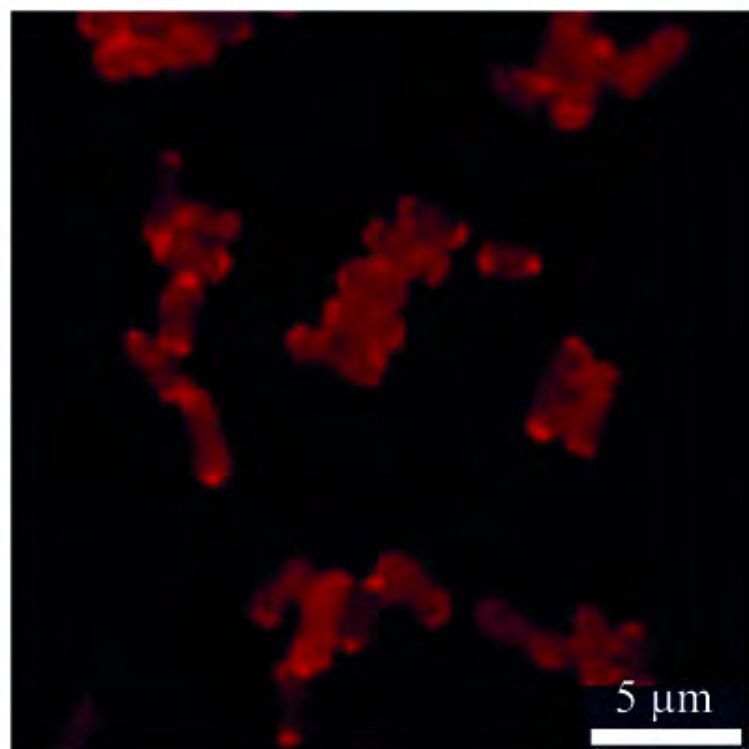
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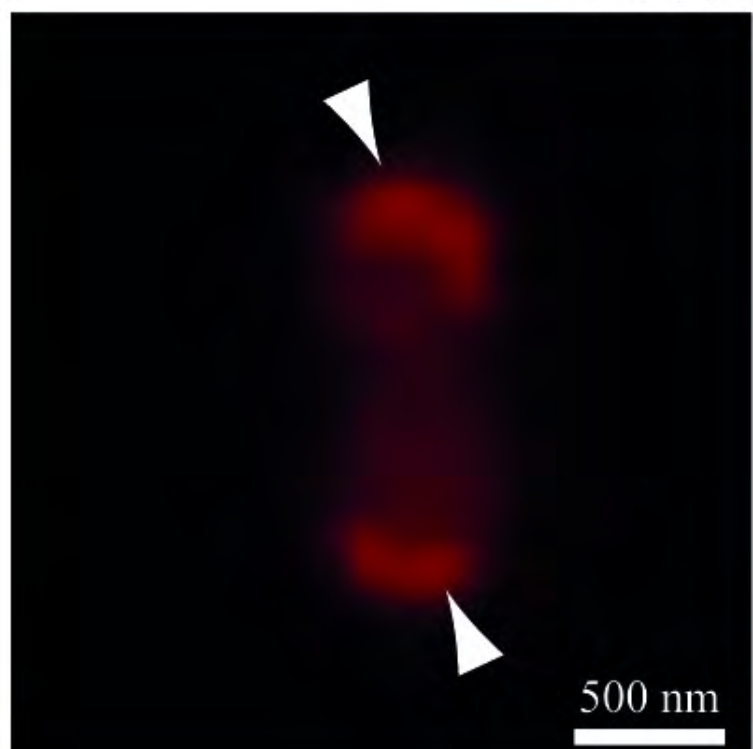


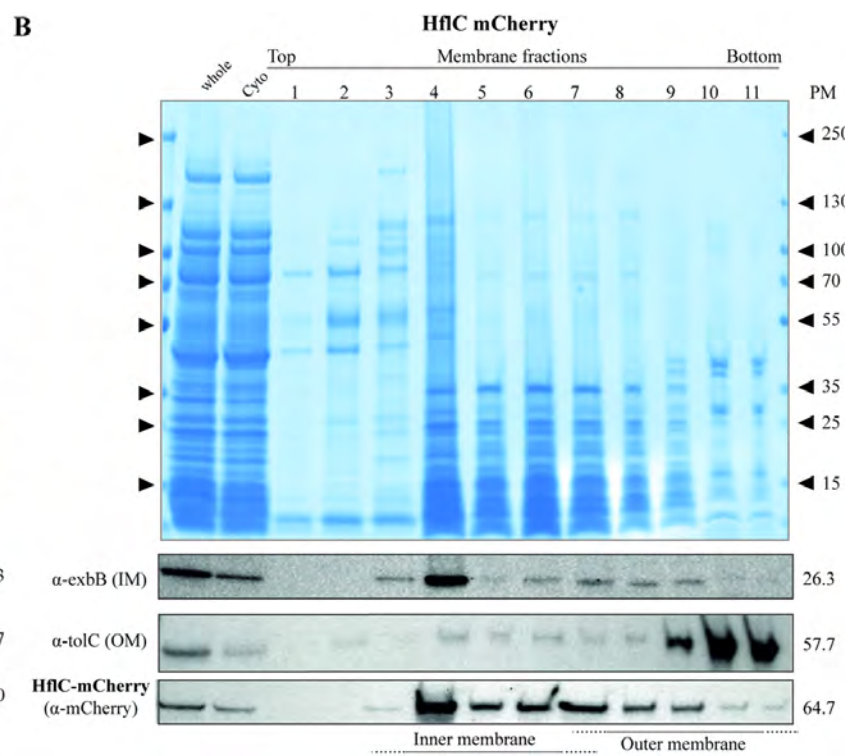
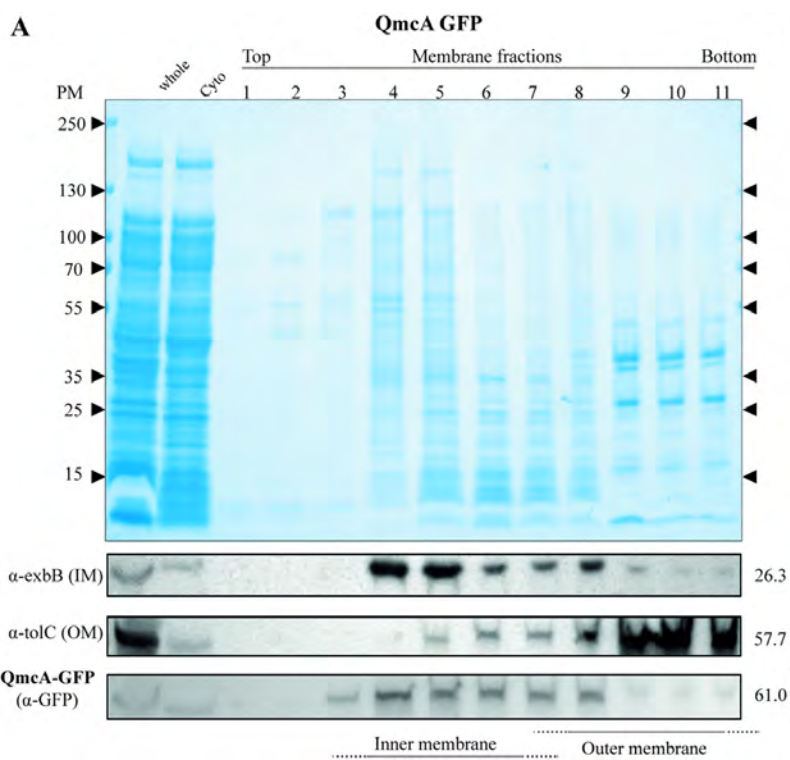
**A***E. coli qmCA-gfp*

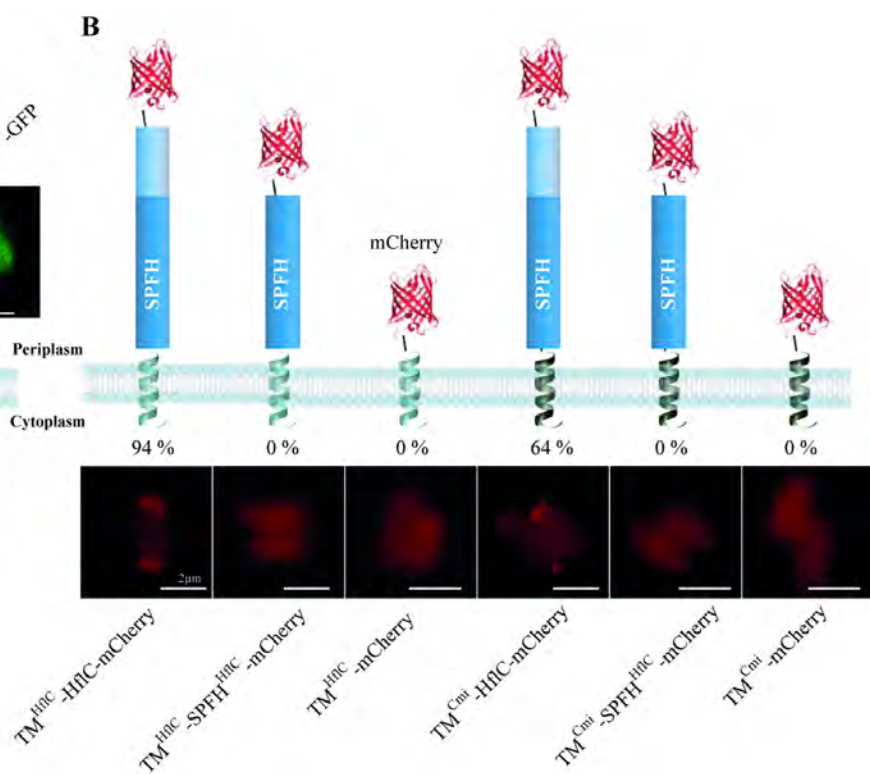
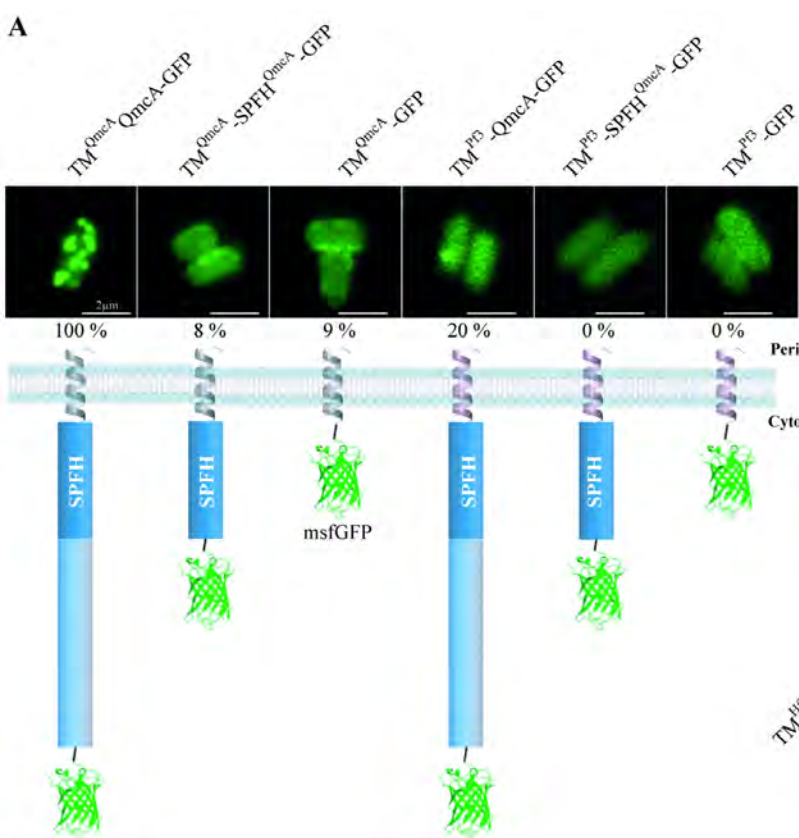
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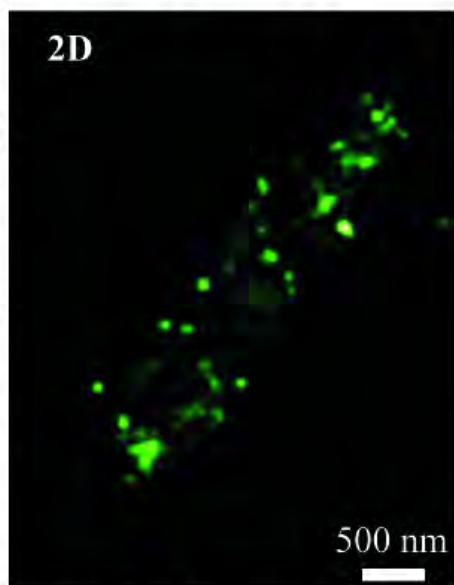
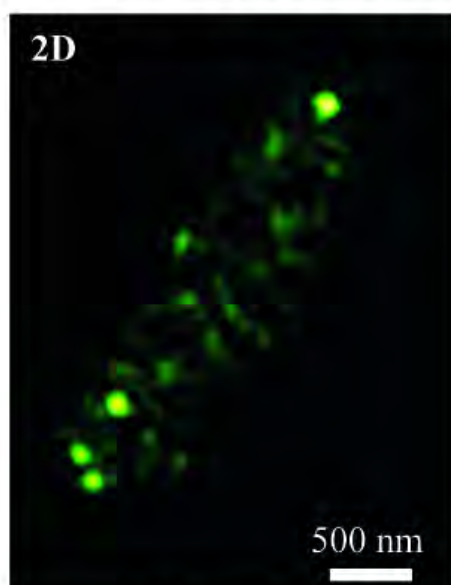
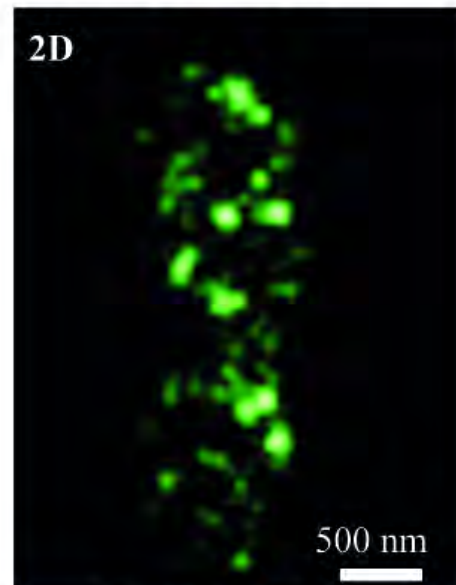
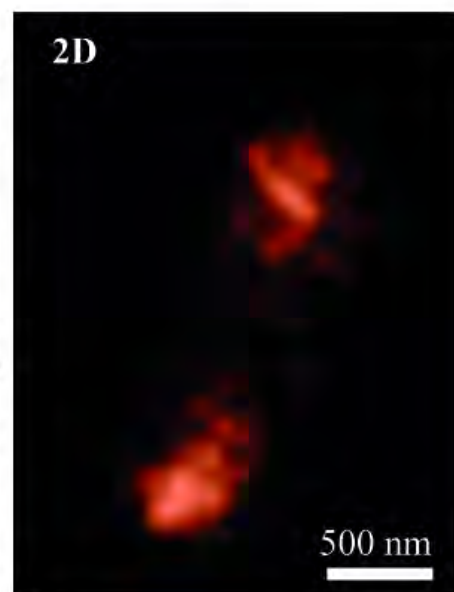
**B***E. coli hflC-mCherry*

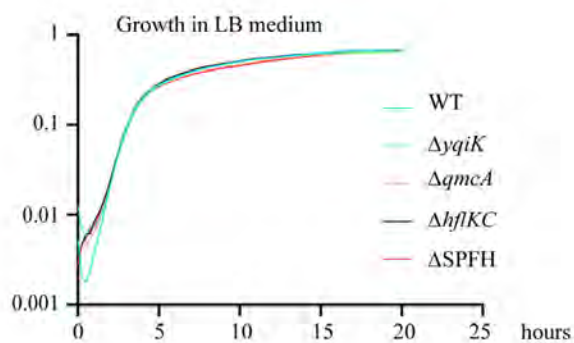
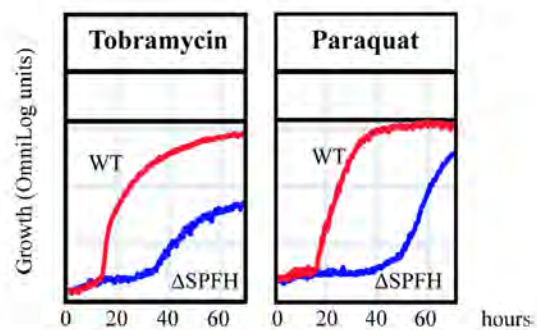
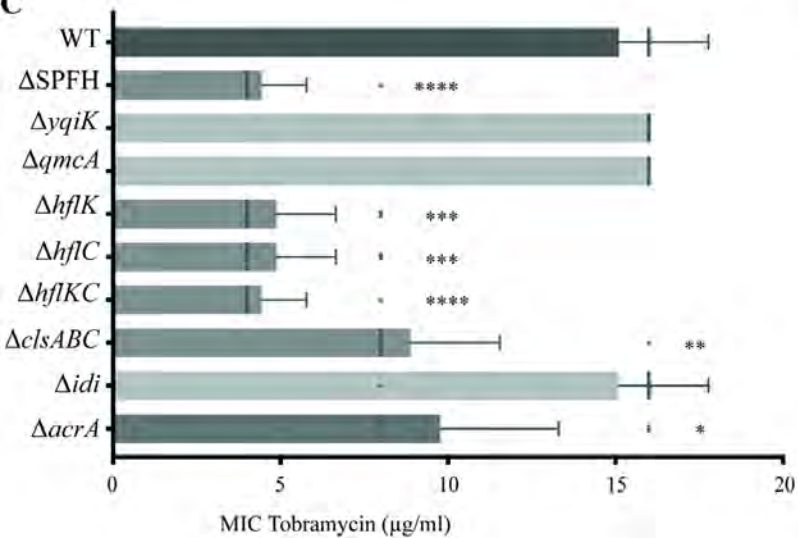
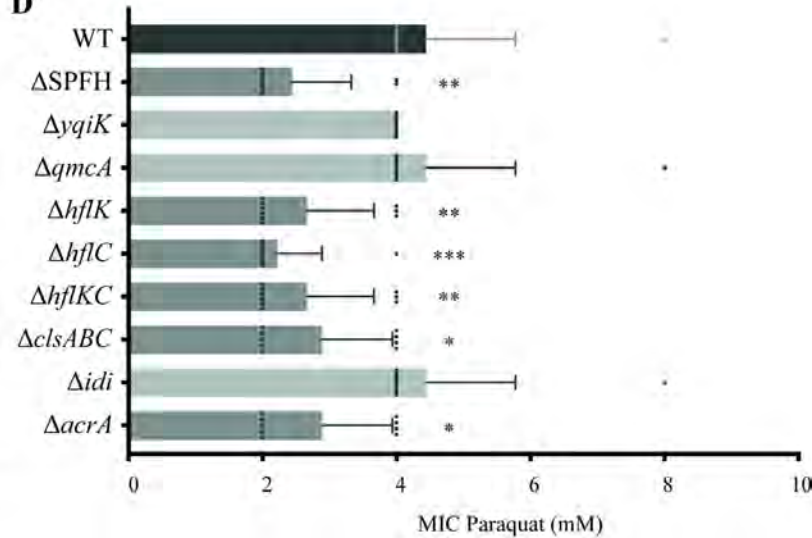
94 %







**A***E. coli* WT*E. coli*  $\Delta$ *clsABC**E. coli*  $\Delta$ *idi**E. coli* *qmCA-gfp*10-15 clusters  
per bacterium1-5 clusters  
per bacterium4-10 clusters  
per bacterium**B***E. coli* *hflC-mCherry*2 polar clusters  
per bacteriumPolar and intracellular  
clustersIntracellular  
clusters

**A****B****C****D**

## SUPPLEMENTARY MATERIALS

*Escherichia coli* SPFH membrane microdomain proteins HflKC contributes to aminoglycoside and oxidative stress tolerance

Wessel AK, Yoshii Y *et al.*

This pdf file includes supplementary methods, supplementary figures S1 to S9 and supplementary Tables S2 and S3. Supplementary Table S1 is provided separately as a dataset.

## SUPPLEMENTARY METHODS

*Construction of YqiK tagged with GFP and HflK tagged with mCherry*

### ***YqiK-GFP chromosomal construct***

The *yqiK* (except for the stop codon), *msfgfp*, and *aphAIII* genes encoding YqiK, msfGFP, and KmFRT cassette were amplified from MG1655 *strep*, MG1655mreB-GFP (1), and MG1655 $\Delta$ *cpxP*::KmFRT (2) chromosomes, respectively. Then, the intermediate plasmid pUC18 *yqiK-gfp-aphAIII* was constructed by combining these three DNA fragments with the linearized backbone vector pUC18, using Gibson assembly (New England Biolabs, Ipswich, MA, USA) and primers described in Table S3. Next, the DNA fragment encoding YqiK-GFP-KmFRT was amplified from the plasmid pUC18*yqiK-gfp-aphAIII* using the long recombining primers described in Table S3. The resulting PCR product was introduced on its native location under the *yqiK* promoter in strain MG1655 *strep* using pKOBEG plasmid by  $\lambda$ -red recombination to produce YqiK-GFP.

### ***HflK-mCherry chromosomal construct***

The DNA gene block corresponding to the entire *hflK* gene except for the stop codon, mCherry, and KmFRT with 40 bp-long homology regions of upstream of the *hflK* start codon and downstream of the *aphAIII* last codon was designed from the DNA sequence of MG1655 *strep*, MG1655mreB-mCherry (3), and MG1655 $\Delta$ *cpxP*::KmFRT (2) chromosomes, respectively, and purchased from Eurofins Genomics (France). Then, the DNA oligonucleotide was introduced on its native location under the *hflK* promoter in MG1655 *strep* using pKOBEG plasmid by  $\lambda$ -red recombination to produce HflK-mCherry.

### *Construction of QmcA-GFP and QmcA-derivatives tagged with GFP*

The *qmcA* and *ybbJ*, *msfgfp*, and *aphAIII* genes encoding QmcA and YbbJ, msfGFP and KmFRT cassette, respectively, were amplified from MG1655 *strep*, MG1655*mrB*-GFP, and MG1655 $\Delta$ *cpxP*::KmFRT (2) chromosomes. The sequence of information on the *pf3* gene was obtained from (4).

#### **• *TM<sup>QmcA</sup>-QmcA-GFP chromosomal construct***

The intermediate plasmid pUC18*qmcA-gfp-aphAIII* was constructed by combining DNA fragments of the entire *qmcA* gene except for the stop codon, msfGFP, the entire *ybbJ* gene followed by 20 bp upstream regions of the start codon, and KmFRT, with the linearized backbone vector pUC18 using Gibson assembly (New England Biolabs). The DNA fragment encoding QmcA-GFP-KmFRT was amplified from the plasmid pUC18*qmcA-gfp-aphAIII* using the long recombining primers described in Table S3, and introduced on its native location under the *qmcA* promoter in strain MG1655 *strep* using pKOBEG plasmid by  $\lambda$ -red recombination to produce TM<sup>qmcA</sup>-QmcA-GFP.

#### ***TM<sup>QmcA</sup>-SPFH<sup>QmcA</sup>-GFP chromosomal construct***

The DNA fragment encoding msfGFP-KmFRT with 40 bp-long upstream and downstream homology regions was amplified from the TM<sup>pf3</sup>-SPFH<sup>QmcA</sup>-GFP chromosomal construct using the long recombining primers described in Table S3. Then, the resulting PCR product was introduced on the chromosome at the downstream position of the *qmcA* SPFH domain region in strain MG1655 *strep* by  $\lambda$ -red recombination to produce TM<sup>QmcA</sup>-SPFH<sup>QmcA</sup>-GFP.

#### **• *TM<sup>QmcA</sup>-GFP chromosomal construct***

The DNA fragment encoding to msfGFP-KmFRT with 40 bp-long upstream and downstream homology regions was amplified from the TM<sup>QmcA</sup>-QmcA-GFP chromosomal construct using long recombining primers described in Table S3. Then, the resulting PCR product was introduced on the chromosome at the downstream position of the *qmcA* TM domain region in strain MG1655 *strep* by  $\lambda$ -red recombination to produce TM<sup>QmcA</sup>-GFP.

#### **• *$\Delta$ qmcA::zeo chromosomal construct***

The *zeo* gene with 40 bp-long upstream and downstream homology regions was amplified from

the MG1655  $\lambda$ ATT:*amp*\_GFPmut3\_Δ*fimAICDFGH*::*zeo*

Δ*yadyadNecpDhtrEyadMLKC*::*cat* (5) using long recombining primers described in Table S3. Then, the resulting PCR product was introduced on the native location under the *qmcA* promoter in strain MG1655 strep by  $\lambda$ -red recombination to generate Δ*qmcA*::*zeo*.

- ***TM<sup>pf3</sup>-QmcA-GFP chromosomal construct***

The plasmid pZS\*12*TM<sup>pf3</sup>-gfp-aphAIII* was constructed via pZS\*12*TM<sup>pf3</sup>-gfp*, by combining DNA fragments of *pf3*, *msfGFP*, and *KmFRT*, with the linearized backbone vector pZS\*12 using Gibson assembly (New England Biolabs) and primers described in Table S3. Then, the intermediate plasmid pZS\*12*TM<sup>pf3</sup>-qmcA-gfp-aphAIII* was constructed by combining the DNA fragment of *qmcA* gene except for the native TM domain region with the linearized vector pZS\*12*TM<sup>pf3</sup>-gfp* using Gibson assembly (New England Biolabs). Next, the DNA fragment encoding *TM<sup>pf3</sup>-QmcA-GFP-KmFRT* with 40 bp-long upstream and downstream homology regions was amplified from the intermediate plasmid using the long recombining primers described in Table S3. The resulting PCR product was introduced on its native location under *qmcA* promoter by  $\lambda$ -red recombination into strain Δ*qmcA*::*zeo* using pKOBEG plasmid to produce *TM<sup>pf3</sup>-QmcA-GFP*.

- ***TM<sup>pf3</sup>-SPFH<sup>QmcA</sup>-GFP chromosomal construct***

The intermediate plasmid pZS\*12*TM<sup>pf3</sup>-SPFH<sup>QmcA</sup>-gfp-aphAIII* was constructed by combining the *SPFH* domain region of *qmcA* gene with the linearized vector PZ\*12 *TM<sup>pf3</sup>-gfp-aphAIII* using Gibson assembly (New England Biolabs). Then, the DNA fragment encoding *TM<sup>pf3</sup>-SPFH<sup>QmcA</sup>-GFP-KmFRT* with 40 bp-long upstream and downstream homology regions was amplified from the plasmid pZS\*12 *TM<sup>pf3</sup>-SPFH<sup>QmcA</sup>-GFP-aphAIII* using the long recombining primers described in Table S3. The resulting PCR product was introduced on its native location under *qmcA* promoter by  $\lambda$ -red recombination into Δ*qmcA*::*zeo* strain using pKOBEG plasmid to produce *TM<sup>pf3</sup>-SPFH<sup>QmcA</sup>-GFP*.

- ***TM<sup>pf3</sup>-GFP chromosomal construct***

The DNA fragment encoding *TM<sup>pf3</sup>-GFP-KmFRT* with 40 bp-long upstream and downstream homology regions was amplified from pZS\*12*TM<sup>pf3</sup>-gfp-aphAIII* using the long recombining primers described in Table S3. The resulting PCR product was introduced on its native location under *qmcA* promoter by  $\lambda$ -red recombination into Δ*qmcA*::*zeo* strain using pKOBEG plasmid to produce *TM<sup>pf3</sup>-GFP*.



### *Construction of HflC derivatives tagged with mCherry*

The *hflC*, *mCherry*, and *cat* genes encoding HflC, mCherry, and CmFRT cassette, respectively, were amplified from MG1655 *strep*, MG1655mr $\epsilon$ B-mCherry, and MG1655 $\Delta$ yfcV-P::CmFRT (6) chromosomes. The sequence of information on *cmi* gene was obtained from (4).

#### **• *TM<sup>HflC</sup>-HflC-mCherry chromosomal construct***

The intermediate plasmid pUC18 $TM^{HflC}$ -*HflC-mCherry-aphAIII* was constructed by combining DNA fragments of the *hflC* gene except for the stop codon, mCherry, and KmFRT, with the linearized backbone vector pUC18 using Gibson assembly (New England Biolabs) and primers described in Table S3. The DNA fragment encoding HflC-mCherry-KmFRT with 40 bp-long upstream and downstream homology regions was amplified from the plasmid pUC18 $TM^{HflC}$ -*HflC-mCherry-aphAIII* using the long recombining primers described in Table S3 and introduced on its native location under the *hflC* promoter in strain *MG1655 strep* using pKOBEG plasmid by  $\lambda$ -red recombination to  $TM^{HflC}$ -HflC-mCherry.

#### **• *TM<sup>HflC</sup>-SPFH<sup>HflC</sup>-mCherry chromosomal construct***

The DNA fragment encoding mCherry-CmFRT with 40 bp-long upstream and downstream homology regions was amplified from the  $TM^{cmi}$ -SPFH $^{HflC}$ -mCherry chromosomal construct, using the long recombining primers described in Table S3. The resulting PCR product was introduced on the chromosome at the downstream position of the *hflC* SPFH domain region by  $\lambda$ -red recombination into *MG1655 strep* strain using pKOBEGA plasmid to produce  $TM^{HflC}$ -SPFH $^{HflC}$ -mCherry.

#### **• *TM<sup>HflC</sup>-mCherry chromosomal construct***

The DNA fragment encoding mCherry-KmFRT with 40 bp-long upstream and downstream homology regions was amplified from the plasmid pUC18 $TM^{HflC}$ -*HflC-mCherry-aphAIII* using the long recombining primers described in Table S3. The resulting PCR product was introduced on the chromosome at the downstream position of the *hflC* TM domain region by  $\lambda$ -red recombination into *MG1655 strep* strain using pKOBEGA plasmid to produce  $TM^{HflC}$ -mCherry.

#### **• *TM<sup>cmi</sup>-HflC-mCherry chromosomal construct***

The plasmid pZS\*12 $TM^{cmi}$ -mCherry-cat was constructed, via pZS\*12 $TM^{cmi}$ -mCherry, by combining DNA fragments encoding  $TM^{cmi}$ , mCherry, and CmFRT, with the linearized backbone vector pZS\*12 using Gibson assembly (New England Biolabs) and the primers described in Table S3. Then, the intermediate plasmid pZS\*12 $TM^{cmi}$ -hflC-mCherry-cat was constructed by combining the DNA fragment of hflC gene except for the native TM domain region with the linearized vector pZS\*12 $TM^{cmi}$ -mCherry-cat using Gibson assembly (New England Biolabs). Then, the DNA fragment encoding  $TM^{cmi}$ -HflC-mCherry-CmFRT with 40 bp-long upstream and downstream homology regions was amplified from the intermediated plasmid using the long recombining primers described in Table S3. The resulting PCR product was introduced on its native location under hflC promoter by  $\lambda$ -red recombination into  $\Delta hflC::KmFRT$  strain using pKOBEGA plasmid to produce  $TM^{cmi}$ -HflC-mCherry.

•  ***$TM^{cmi}$ -SPFH<sup>HflC</sup>-mCherry chromosomal construct***

The intermediated plasmid pZS\*12 $TM^{cmi}$ -SPFH<sup>HflC</sup>-mCherry-cat was constructed by combining the hflC SPFH domain region with the linearized initial plasmid pZS\*12 $TM^{cmi}$ -mCherry-cat, which was used for generating  $TM^{cmi}$ -HflC-mCherry chromosomal construct, using Gibson assembly (New England Biolabs). Then, the DNA fragment encoding  $TM^{cmi}$ -SPFH<sup>HflC</sup>-mCherry-CmFRT with 40 bp-long upstream and downstream homology regions was amplified from the intermediated plasmid using the long recombining primers described in Table S3. The resulting PCR product was introduced on its native location under hflC promoter by  $\lambda$ -red recombination into  $\Delta hflC::KmFRT$  strain using pKOBEGA plasmid to produce  $TM^{cmi}$ -SPFH<sup>HflC</sup>-mCherry.

•  ***$TM^{cmi}$ -mCherry chromosomal construct***

The DNA fragment encoding  $TM^{cmi}$ -GFP-CmFRT with 40 bp-long upstream and downstream homology regions was amplified from the plasmid pZS\*12 $TM^{cmi}$ -mCherry-cat using the long recombining primers described in Table S3. The resulting PCR product was introduced on its native location under hflC promoter by  $\lambda$ -red recombination into  $\Delta hflC::KmFRT$  strain using pKOBEGA plasmid to produce  $TM^{cmi}$ -mCherry.

*Construction of QmcA-GFP and HflC-mCherry in the  $\Delta clsABC$  and  $\Delta idi$  mutants*

• ***QmcA-GFP chromosomal construct in the  $\Delta clsABC$  strain***

$TM^{QmcA}$ -QmcA-GFP-KmFRT mutation from  $TM^{QmcA}$ -QmcA-GFP chromosomal construct

was introduced into strain  $\Delta clsABC$  by P1 vir phage transduction ( $\Delta clsABC$  QmcA-GFP).

- ***TM<sup>QmcA</sup>-QmcA-GFP chromosomal construct in the  $\Delta idi$  strain***

TM<sup>QmcA</sup>-QmcA-GFP-KmFRT mutation from TM<sup>QmcA</sup>-QmcA-GFP chromosomal construct was introduced into strain  $\Delta idi$  by P1 vir phage transduction ( $\Delta idi$  QmcA-GFP).

- ***TM<sup>HflC</sup>-HflC-mCherry chromosomal construct in the  $\Delta clsABC$  strain***

TM<sup>HflC</sup>-HflC-mCherry-KmFRT mutation from TM<sup>HflC</sup>-HflC-mCherry chromosomal construct was introduced into strain  $\Delta clsABC$  by P1 vir phage transduction ( $\Delta clsABC$  HflC-mCherry).

- ***TM<sup>HflC</sup>-HflC-mCherry chromosomal construct in the  $\Delta idi$  strain***

TM<sup>HflC</sup>-HflC-mCherry-KmFRT mutation from TM<sup>HflC</sup>-HflC-mCherry chromosomal construct was introduced into strain  $\Delta idi$  by P1 vir phage transduction ( $\Delta idi$  HflC-mCherry).

- ***AcrA-GFP***

The DNA fragment encoding GFP-KmFRT region with 40 bp-long upstream and downstream homology regions was amplified from the TM<sup>pf3</sup>-SPFH<sup>QmcA</sup>-GFP chromosomal construct using the long recombining primers described in Table S3. The resulting PCR product was introduced on chromosome at the downstream position of the *acrA* gene (without stop codons) in *MG1655 strep* by  $\lambda$ -red recombination, to produce a GFP fusion. The mutation harboring KmFRT was introduced into a *MG1655 strep* strain having clean background by P1 vir phage transduction (*AcrA-GFP*).

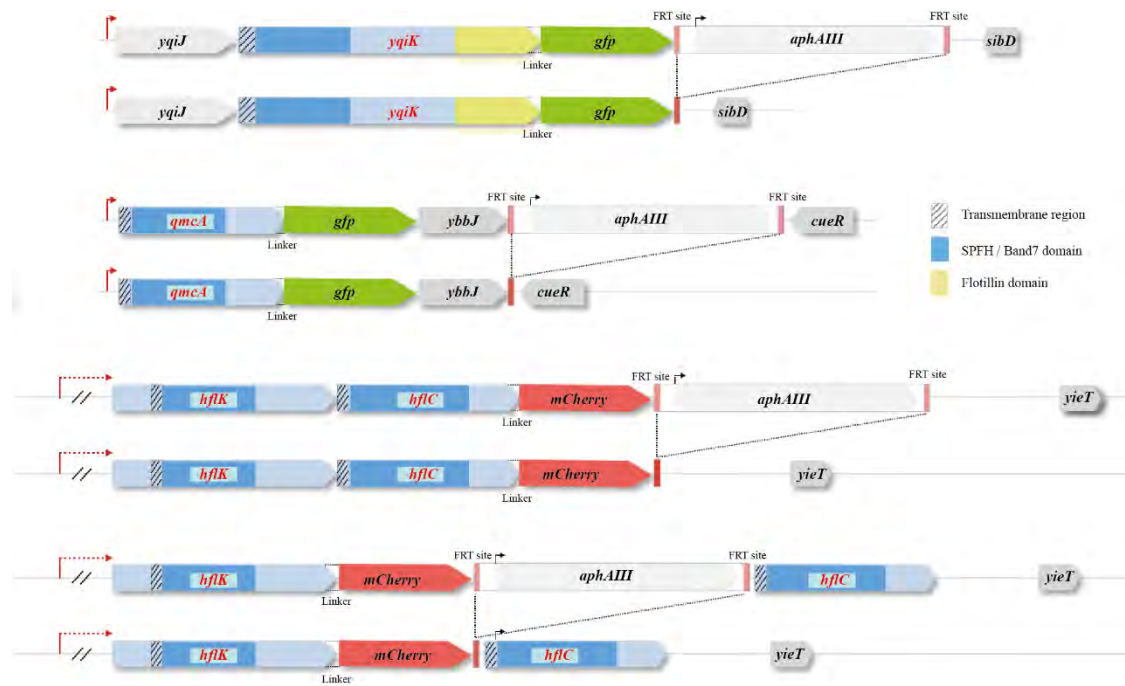
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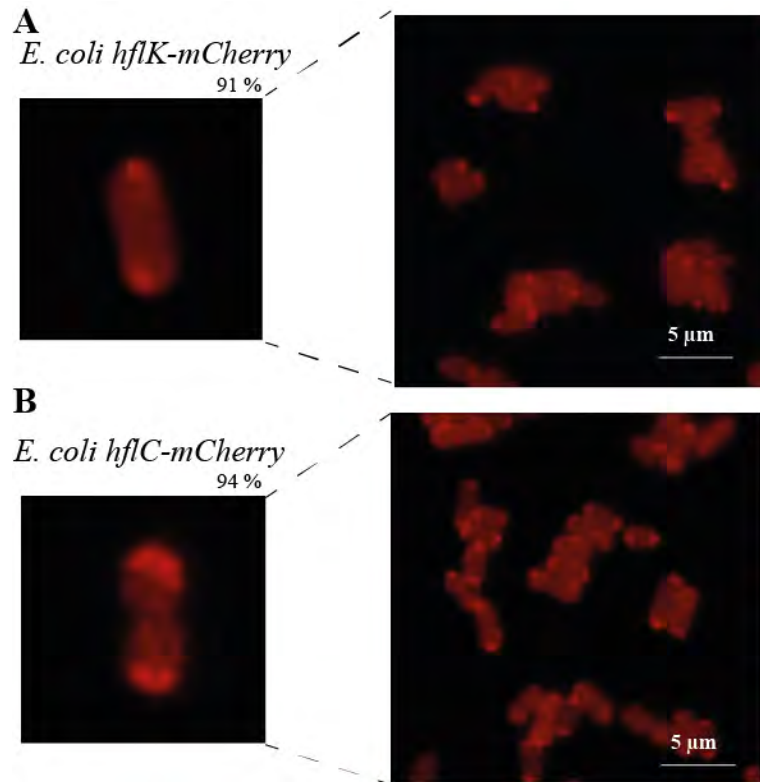
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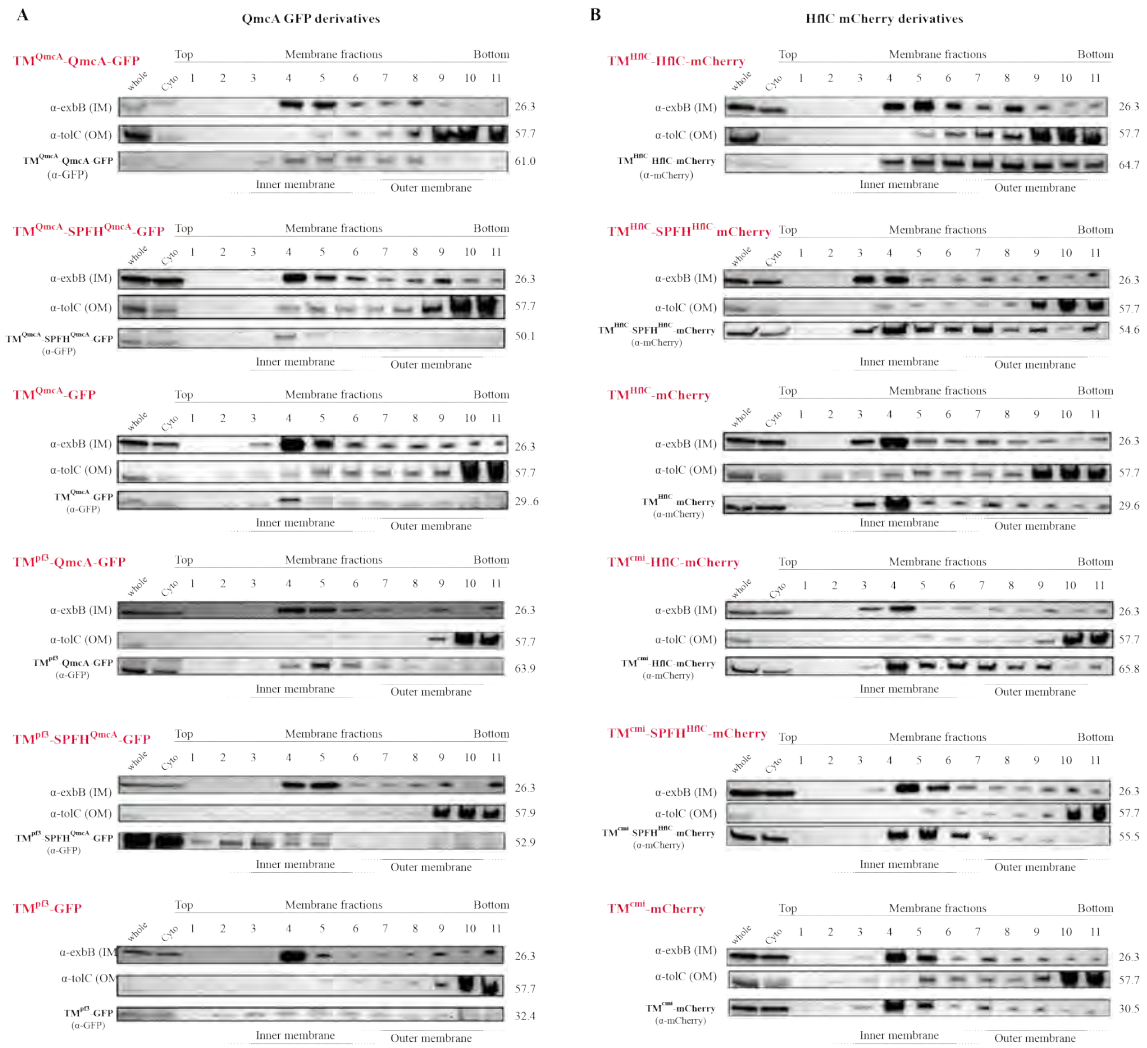
## SUPPLEMENTARY FIGURES



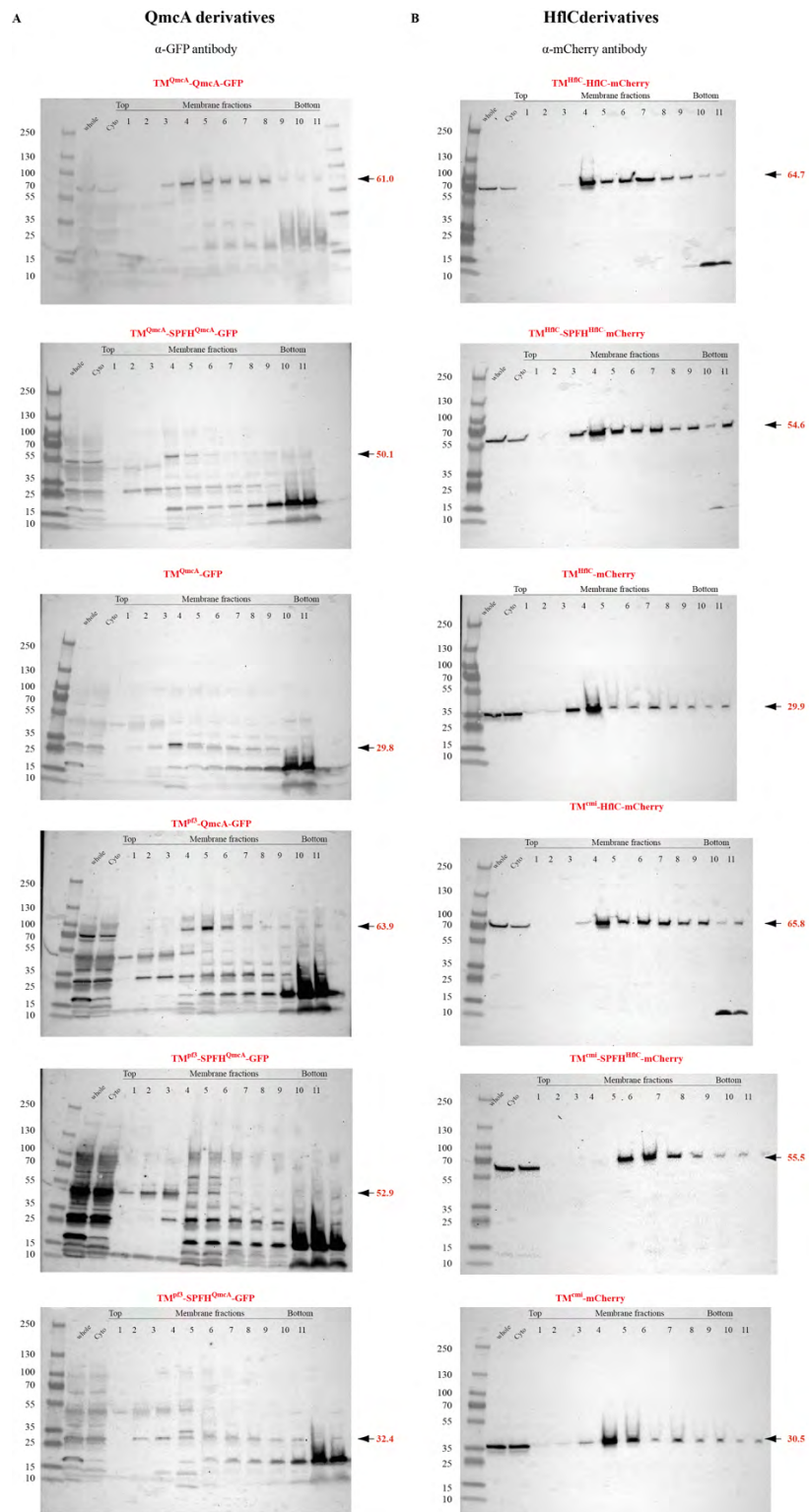
**Supplementary Figure S1. Schematic representation of chromosomal GFP or mCherry fusions to genes encoding *E. coli* SPFH proteins.** SPFH protein-encoding genes, *yqiK*, *qmcA*, are fused with monomeric super folder green fluorescent protein GFP (msfGFP) and *hflK*, and *hflC* to monomeric red fluorescent protein mCherry, respectively. Fusions were constructed as described in the Materials and Method section and the associated kanamycin antibiotic resistance marker (*aphAIII*) was inserted downstream of the fusion genes and removed using pCP20 plasmid, encoding the *flp* flippase.



**Supplementary Figure S2. Localization pattern of HflK and HflC.** Epifluorescence microscopic images of cells expressing HflK-mCherry (A) and HflC-mCherry (B) in exponential phase with high and low magnifications.

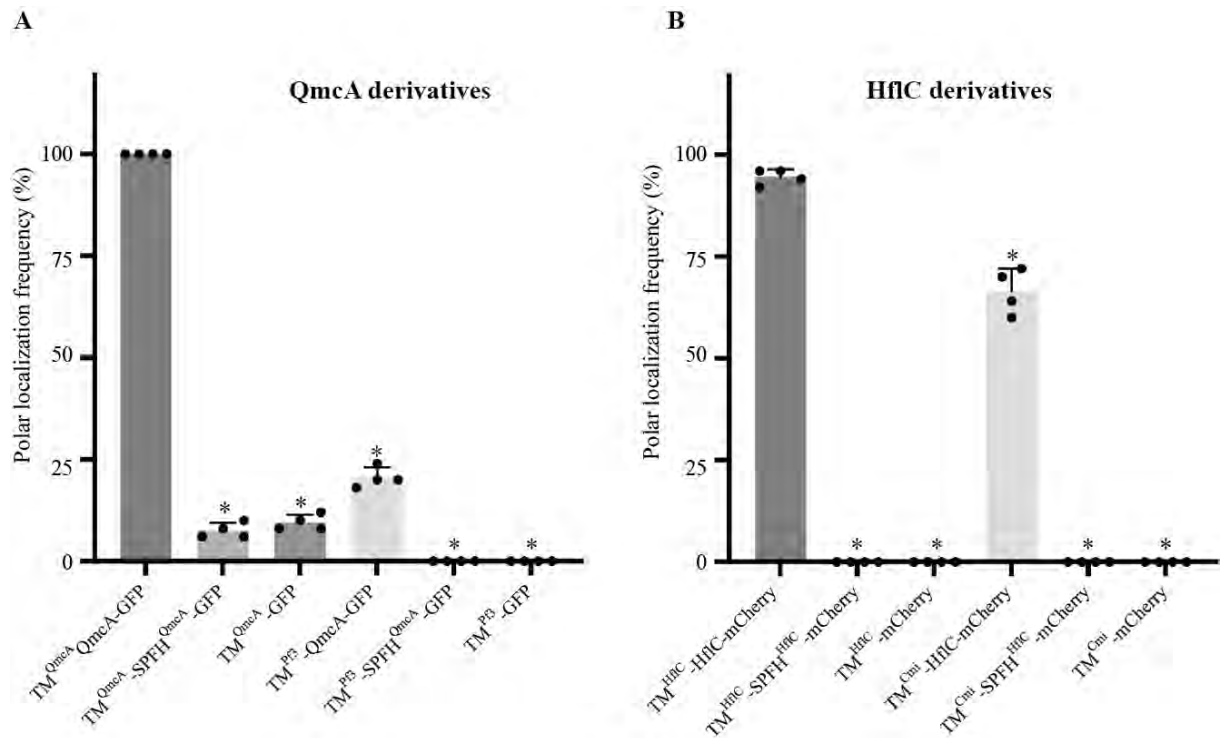


**Supplementary Figure S3. Inner membrane localization of QmcA and HflC in domain swap constructs.** SDS-PAGE and immunodetection analyses using proteins of whole-cell extract, cytosolic fraction, and membrane fractions prepared from cells expressing GFP fusion derivatives of QmcA (A) or mCherry fusion derivatives of HflC. (B). In immunodetection analysis, anti-ExbB antibodies were used to detect the inner membrane- (IM) marker ExbB and anti-TolC antibodies to detect the outer membrane- (OM) marker TolC. Anti-GFP and anti-mCherry antibodies were used to detect the expressions of GFP and mCherry fused proteins, respectively.

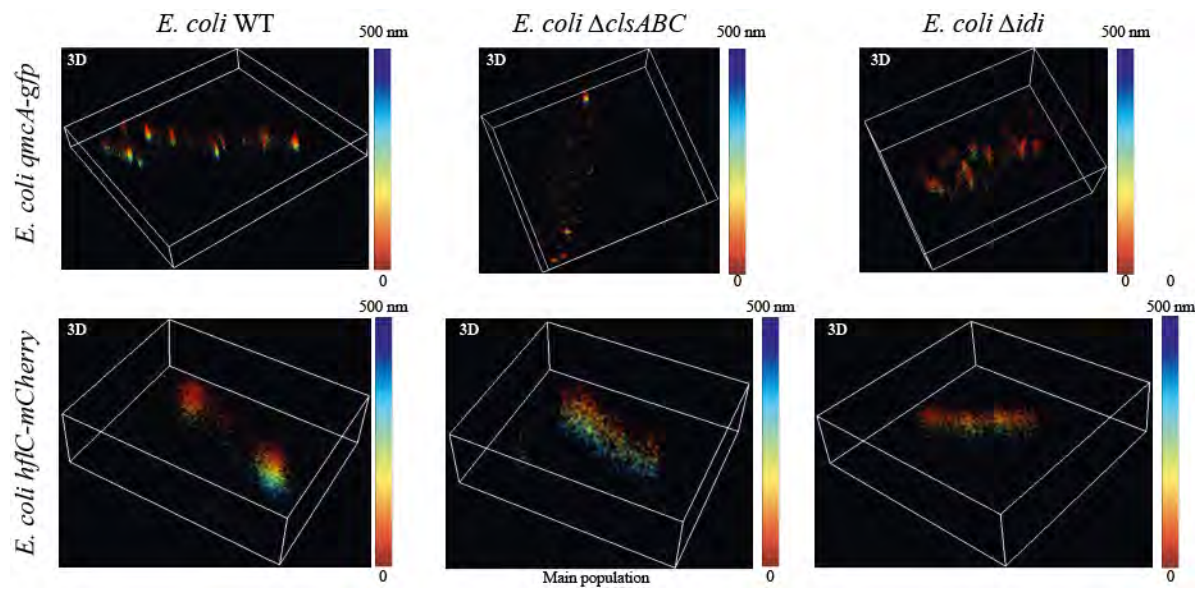


**Supplementary Figure S4. Detection of QmcA and HflC fusion derivatives in domain swap constructs. A:** immunodetection of GFP fusion derivatives of QmcA using anti-GFP antibody. **B:** immunodetection of mCherry fusion derivatives of HflC using anti-mCherry antibody.

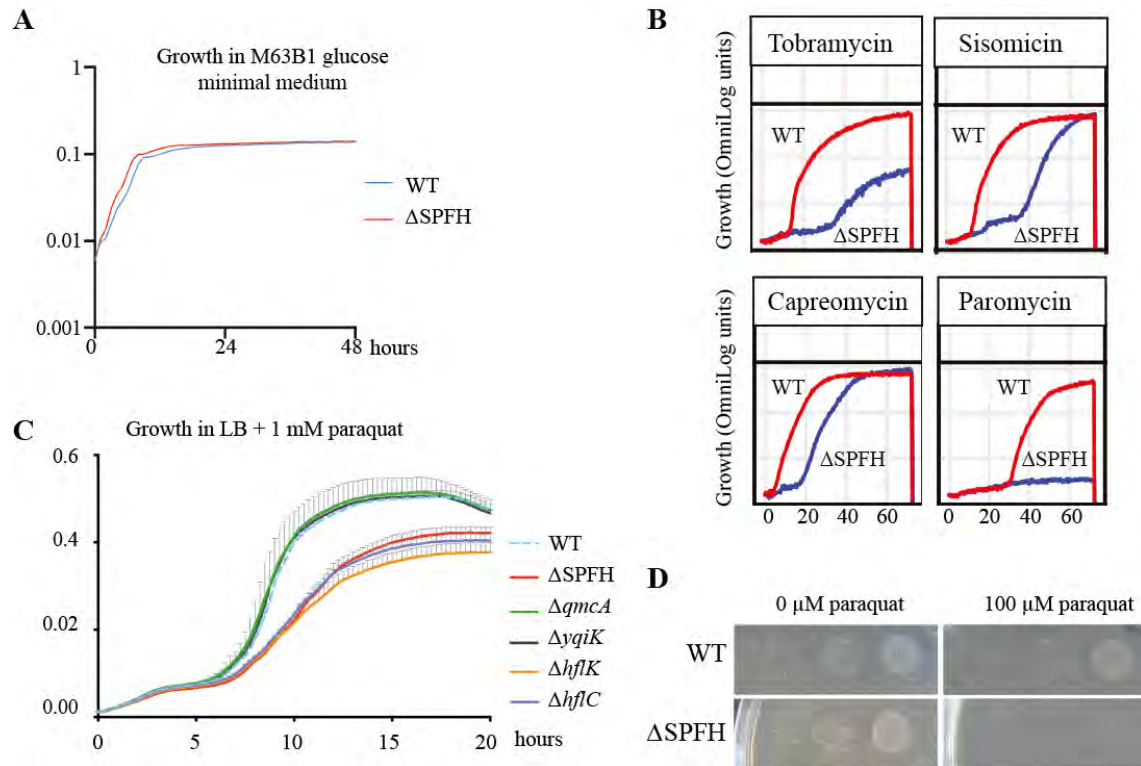




**Supplementary Figure S5.** Quantification data for punctate or polar localizations in QmcA and HflC derivatives. **A:** QmcA derivatives. **B:** HflC derivatives. \* $p < 0.05$  compared with TM<sup>QmcA</sup>-QmcA-GFP or TM<sup>HflC</sup>-HflC-mCherry.

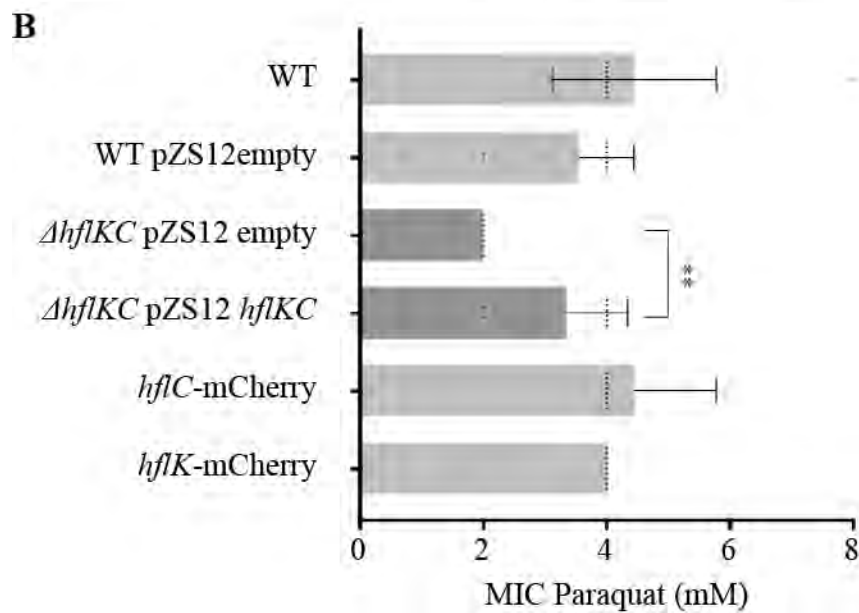
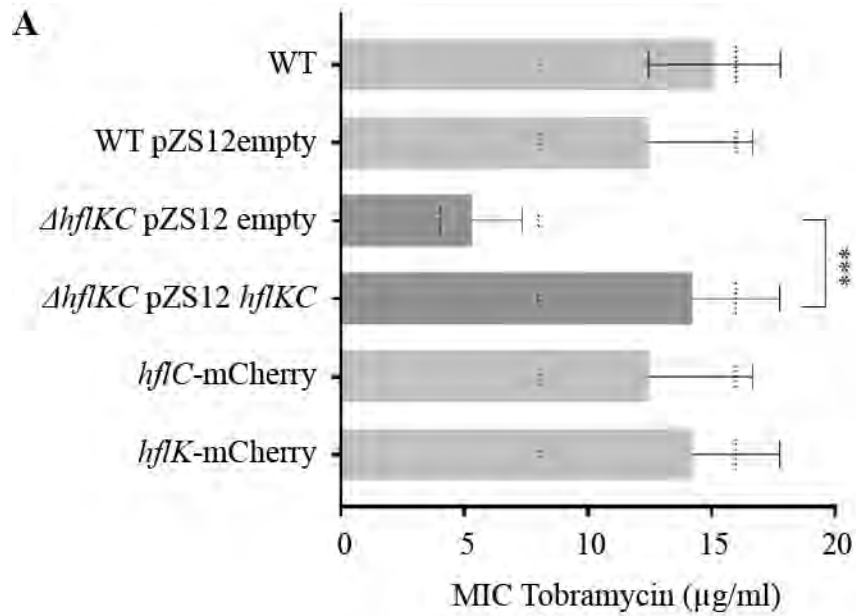


**Supplementary Figure S6. The subcellular localization of QmcA and HflC in mutants with the alteration of cardiolipin or isoprenoid synthetic pathway.** Three-dimensional super-resolution microscopy images of WT,  $\Delta clsABC$ , and  $\Delta idi$  strains expressing QmcA-GFP and HflC-mCherry in stationary phase, with colors corresponding to depth location along the Z axis, 0-500 nm, with 0 nm expressed in red, and 500 nm expressed in deep blue.

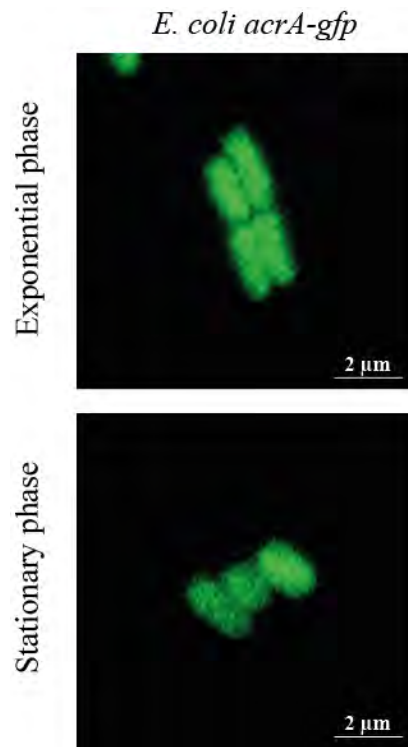


**Supplementary Figure S7. Phenotypic analysis of *E. coli* SPFH mutant**

(A) Growth curve of *E. coli* WT and  $\Delta$ SPFH mutant in M63B1 glucose minimal medium. (B) Biolog metabolic activity curve of WT and  $\Delta$ SPFH in presence of tobramycin, capreomycin, sisomicin, and paromycin. (C) Bacterial growth curve of WT and  $\Delta$ SPFH in the presence of 0.8 mM paraquat. (D) Paraquat susceptibility assay of WT and  $\Delta$ SPFH spotted on M63B1 glucose agar containing 100  $\mu$ M paraquat. (E) Growth curve of WT and indicated SPFH gene mutants in the presence of 0.8 mM paraquat.



**Supplementary Figure S8. Susceptibility to tobramycin and paraquat of complemented mutants.** Minimum inhibitory concentrations (MICs) for tobramycin (A) and paraquat (B) of *E. coli* Wt,  $\Delta hflKC$  strains harboring empty vector and *hflKC*- complemented strains or strain expressing *hflC* or *hflK*-mCherry constructs from their native chromosomal context. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Supplementary Figure S9. Localization of AcrA-GFP fusion protein does not show polar localization pattern when expressed from native chromosomal context.** Epifluorescence microscopic images of cells expressing AcrA-GFP in exponential and stationary phases.

**Supplementary Table S2. Bacterial strains and plasmids used in this study**

<i>E. coli</i> strains and plasmids	Genotype or description	Antibiotic resistance(s)	Reference
<b>Strains</b>			
BW25113 $\Delta$ <i>yqiK</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>qmcA</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>hflK</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>hflC</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>clsA</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>clsB</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>clsC</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>idi</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
BW25113 $\Delta$ <i>acrA</i> ::Km <sup>f</sup> rt	Keio collection	Km <sup>R</sup>	(1)
MG1655 <i>strep</i>	Spontaneous streptomycin-resistant mutant of K-12 wild-type strain	Strep <sup>R</sup>	(2)
MG1655mreB-GFP	Source of <i>msfgfp</i> gene	No resistance	Gift from Dr. Sven van Teeffelen
MG1655mreB-mCherry	Source of <i>mCherry</i> gene	No resistance	Gift from Dr. Sven van Teeffelen
MG1655 $\Delta$ <i>cpxP</i> ::Km <sup>F</sup> RT	Source of kanamycine resistance cassette	Km <sup>R</sup>	(3)
MG1655 $\Delta$ <i>yfcV-P</i> ::Cm <sup>F</sup> RT	Source of chloramphenicol resistance cassette	Cm <sup>R</sup>	(4)
DH5alpir pSW25 <i>tetAccdB</i>	Source of spectinomycin resistance cassette	Spec <sup>R</sup>	(5)

MG1655_λATT:amp_GFPmut3_ΔfimAICDFGH::zeo ΔyadyadNecpDhtrEyadMLKC::cat	Source of zeocin resistance cassette	Zeo <sup>R</sup>	(6)
ΔyqiK	P1 vir transduction from BW25113ΔyqiK::Kmftr into MG1655 strep	Strep <sup>R</sup>	This study
ΔqmcA	P1 vir transduction from BW25113ΔqmcA::Kmftr into MG1655 strep	Strep <sup>R</sup>	This study
ΔhflK	P1 vir transduction from BW25113ΔhflK::Kmftr into MG1655 strep	Strep <sup>R</sup>	This study
ΔhflC	P1 vir transduction from BW25113ΔhflC::Kmftr into MG1655 strep	Strep <sup>R</sup>	This study
ΔhflKC	Double mutant of ΔhflK and ΔhflC. MG1655strepR, ΔhflKC::Cmftr	Strep <sup>R</sup>	This study
ΔyqiJK	Double mutant of ΔyqiJ and ΔyqiK. MG1655strepR, ΔyqiJK::Spec	Strep <sup>R</sup> , Spec <sup>R</sup>	This study
ΔqmcA, ΔyqiJK	Triple mutant of ΔqmcA and ΔyqiJK. MG1655strepR, ΔqmcA, ΔyqiJK::Spec	Strep <sup>R</sup> , Spec <sup>R</sup>	This study
ΔSPFH	Quintuple mutant of ΔyqiK, ΔqmcA, ΔhflK, and ΔhflC. MG1655strepR, ΔqmcA, ΔyqiJK::Spec, ΔhflKC::Cmftr	Strep <sup>R</sup> , Spec <sup>R</sup>	This study
ΔclsA	P1 vir transduction from BW25113ΔclsA::Kmftr into MG1655 strep	Strep <sup>R</sup>	This study
ΔclsB	P1 vir transduction from BW25113ΔclsB::Kmftr into MG1655 strep	Strep <sup>R</sup>	This study
ΔclsC	P1 vir transduction from BW25113ΔclsC::Kmftr into MG1655 strep	Strep <sup>R</sup>	This study

$\Delta clsAB$	Double mutant of $\Delta clsA$ and $\Delta clsB$ . P1 vir transduction from BW25113 $\Delta clsA::Kmfrt$ into $\Delta clsB$	Strep <sup>R</sup>	This study
$\Delta clsABC$	Triple mutant of $\Delta clsA$ , $\Delta clsB$ , and $\Delta clsC$ . P1 vir transduction from BW25113 $\Delta clsC::Kmfrt$ into $\Delta clsAB$	Strep <sup>R</sup>	This study
$\Delta idi$	P1 vir transduction from BW25113 $\Delta idi::Kmfrt$ into MG1655 <i>strep</i>	Strep <sup>R</sup>	This study
$\Delta acrA$	P1 vir transduction from BW25113 $\Delta acrA::Kmfrt$ into MG1655 <i>strep</i>	Strep <sup>R</sup>	This study
$\Delta qmcA::zeo$	MG1655 <i>strep</i> , $\Delta qmcA::zeo$	Strep <sup>R</sup> , Zeo <sup>R</sup>	This study
<i>yqiK-gfp</i>	MG1655 <i>strep</i> , <i>yqiK::gfp</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<i>qmcA-gfp</i>	MG1655 <i>strep</i> , <i>qmcA::gfp</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<i>hflK-mCherry</i>	MG1655 <i>strep</i> , <i>hflK::mCherry</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<i>hflC-mCherry</i>	MG1655 <i>strep</i> , <i>hflC::mCherry</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
$TM^{qmcA}$ -SPFH $^{qmcA}$ - <i>gfp</i>	MG1655 <i>strep</i> , $\Delta qmcA::TM^{qmcA}$ -SPFH $^{qmcA}$ - <i>gfp</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
$TM^{qmcA}$ - <i>gfp</i>	MG1655 <i>strep</i> , $\Delta qmcA::TM^{qmcA}$ - <i>gfp</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
$TM^{Pj3}$ - <i>qmcA-gfp</i>	MG1655 <i>strep</i> , $\Delta qmcA::TM^{Pj3}$ - <i>qmcA-gfp</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
$TM^{Pj3}$ -SPFH $^{qmcA}$ - <i>gfp</i>	MG1655 <i>strep</i> , $\Delta qmcA::TM^{Pj3}$ -SPFH $^{qmcA}$ - <i>gfp</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
$TM^{Pj3}$ - <i>gfp</i>	MG1655 <i>strep</i> , $\Delta qmcA::TM^{Pj3}$ - <i>gfp</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
$TM^{hflC}$ -SPFH $^{hflC}$ - <i>mCherry</i>	MG1655 <i>strep</i> , $\Delta hflC::TM^{hflC}$ -SPFH $^{hflC}$ - <i>mCherry</i> Cmfrt	Strep <sup>R</sup> , Cm <sup>R</sup>	This study
$TM^{hflC}$ - <i>mCherry</i>	MG1655 <i>strep</i> , $\Delta hflC::TM^{hflC}$ - <i>mCherry</i> Kmfrt	Strep <sup>R</sup> , Km <sup>R</sup>	This study
$TM^{cmi}$ - <i>hflC-mCherry</i>	MG1655 <i>strep</i> , $\Delta hflC::TM^{cmi}$ - <i>hflC-mCherry</i> Cmfrt	Strep <sup>R</sup> , Cm <sup>R</sup>	This study
$TM^{cmi}$ -SPFH $^{hflC}$ - <i>mCherry</i>	MG1655 <i>strep</i> , $\Delta hflC::TM^{cmi}$ -SPFH $^{hflC}$ - <i>mCherry</i> Cmfrt	Strep <sup>R</sup> , Cm <sup>R</sup>	This study
$TM^{cmi}$ - <i>mCherry</i>	MG1655 <i>strep</i> , $\Delta hflC::TM^{cmi}$ - <i>mCherry</i> Cmfrt	Strep <sup>R</sup> , Cm <sup>R</sup>	This study



<i>ΔclsABC qmcA-gfp</i>	P1 vir transduction from <i>qmcA-gfp</i> into <i>ΔclsABC</i>	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<i>Δidi qmcA-gfp</i>	P1 vir transduction from <i>qmcA-gfp</i> into <i>Δidi</i>	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<i>ΔclsABC hflC-mCherry</i>	P1 vir transduction from <i>hflC-mCherry</i> into <i>ΔclsABC</i>	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<i>Δidi hflC-mCherry</i>	P1 vir transduction from <i>hflC-mCherry</i> into <i>Δidi</i>	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<i>acrA-gfp</i>	MG1655 <i>strep</i> , <i>acrA::gfp</i> Km <sup>R</sup>	Strep <sup>R</sup> , Km <sup>R</sup>	This study
<b>Plasmids</b>			
pKOBEG	<i>oriR101ts araC</i> arabinose-inducible λred γβα operon	Cm <sup>R</sup>	(7)
pKOBEGA	pKOBEG derivative	Amp <sup>R</sup>	(7)
pCP20	Rep(Ts) Flp <sup>+</sup>	Cm <sup>R</sup> , Amp <sup>R</sup>	(8)
pUC18	High-copy-number, lac promoter cloning vector, ampicillin resistant (Amp <sup>R</sup> )	Amp <sup>R</sup>	(9)
pZS*12	Tightly regulated P <sub>LtetO-1</sub> Low copy-number vector ampicillin resistant (Amp <sup>R</sup> )	Amp <sup>R</sup>	(10)
pUC18yqiK-gfp-aphAIII	Assembly of <i>YqiK-mCherry-aphAIII</i> into pUC18	Amp <sup>R</sup> , Km <sup>R</sup>	This study
pUC18TM <sup>QmcA</sup> -QmcA-gfp-aphAIII	Assembly of <i>TM<sup>QmcA</sup>-QmcA-gfp-aphAIII</i> into pUC18	Amp <sup>R</sup> , Km <sup>R</sup>	This study
pZS*12TM <sup>p/3</sup> -gfp	Assembly of <i>TM<sup>p/3</sup>-gfp</i> into pZS*12	Amp <sup>R</sup>	This study
pZS*12TM <sup>p/3</sup> -gfp-aphAIII	Assembly of <i>aphAIII</i> into PZ*12TM <sup>p/3</sup> -gfp	Amp <sup>R</sup> , Km <sup>R</sup>	This study
pZS*12TM <sup>p/3</sup> -QmcA-gfp-aphAIII	Assembly of <i>qmcA</i> into PZ*12TM <sup>p/3</sup> -gfp-aphAIII	Amp <sup>R</sup> , Km <sup>R</sup>	This study
pZS*12 TM <sup>p/3</sup> -SPFH <sup>QmcA</sup> -gfp-aphAIII	Assembly of <i>SPFH<sup>qmcA</sup></i> into PZ*12TM <sup>p/3</sup> -gfp-aphAIII	Amp <sup>R</sup> , Km <sup>R</sup>	This study
pUC18TM <sup>HflC</sup> -HflC-mCherry-aphAIII	Assembly of <i>TM<sup>HflC</sup>-HflC-mCherry-aphAIII</i> into pUC18	Amp <sup>R</sup> , Km <sup>R</sup>	This study
pZS*12TM <sup>cmi</sup> -mCherry	Assembly of <i>cmi-mCherry</i> into pZS*12	Amp <sup>R</sup>	This study
pZS*12TM <sup>cmi</sup> -mCherry-cat	Assembly of <i>Cm<sup>R</sup></i> into PZ*12 <i>cmi-mCherry</i>	Amp <sup>R</sup> , Cm <sup>R</sup>	This study

pZS*12TM <sup>cmi</sup> - <i>hflC</i> - <i>mCherry-cat</i>	Assembly of <i>hflC</i> into PZ*12 <i>cmi-mCherry-Cmfrt</i>	Amp <sup>R</sup> , Cm <sup>R</sup>	This study
pZS*12TM <sup>cmi</sup> -SPFH <sup>HflC</sup> - <i>mCherry-cat</i>	Assembly of SPFH <sup>HflC</sup> into PZ*12 <i>cmi-mCherry-Cmfrt</i>	Amp <sup>R</sup> , Cm <sup>R</sup>	This study
pZS*12- <i>HflKC</i>	Assembly of <i>hflKC</i> into pZS*12	Amp <sup>R</sup>	This study

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**Supplementary Table S3. Primers used for generating chromosomal mutants or assembling plasmids in this study**

Purpose	Name	Sequence (5'-3')
For amplifying the DNA fragment to generate $\Delta hflKC$ strain	Up. $\Delta hflKC$ -CmFRT-5	gacaacagggatcaccgcatacaaatatggagcacaacgtgtaggctggagctgcttcgaag
	Down. $\Delta hflKC$ -CmFRT-3	ggatgcggggctttattgacctgtaccgcagtcgtatacatatgaatatcctccttagttcc
For amplifying the DNA fragment to generate $\Delta yqiJK$ strain	Up. $\Delta yqiJK$ -Spec-5	caaagtaaattgctcactaaatggacattggagtgatgctcaattgccgaataatac
	Down. $\Delta yqiJK$ -Spec-3	ccccggcttttggccagggtattctctggaagagggtcattggctggcaccagaagcagttt
For amplifying the DNA fragment to generate $\Delta qmcA::zeo$ strain	Up. $\Delta qmcA$ -Zeo-5	caattttgctgattatggacggtagacaacaggaggttttccgttctggatccgagcacgtgttgac
	Down. $\Delta qmcA$ -Zeo-3	gactgagccagaaaatgtggatgaacgaccattaactccatctcagtcctctcctcgccacg
For assembling the pUC18yqiK-gfp-aphAIII plasmid	Up.GFP_pUC18yqiK-gfp-5	cacccttaacctcaacaactcccgtcgaagaaaaagcagagggcggcgccggcagcatgagtaaaggtgaagaac
	Down.GFP_pUC18yqiK-gfp-3	caaaatcttctgtcgttattgtagagttcatccatg
	Up.KmFRT_pUC18yqiK-gfp-5	cgacagaaagattttgggagcaaatgatgtgttaggc
	Down.KmFRT_pUC18yqiK-gfp-3	ccccggcttttggccagggtattctctggaagagggtcagggttactcatatgaatac
	Up.linearized pUC18_pUC18yqiK-gfp-5	gccccaaaagccgggggaattcgtaatcatggtcatag
	Down.linearized pUC18_pUC18yqiK-gfp-5	gttgaggtaagggtgggatcctctagagtcgacctg
For amplifying the DNA fragment to generate YqiK-GFP strain	Up.yqiK_YqiK-GFP-KmFRT-5	Same as Up.GFP_pUC18yqiK-gfp-5
	Down.KmFRT_YqiK-GFP-KmFRT-3	Same as Down.KmFRT_pUC18yqiK-gfp-3
For assembling the pUC18 <sup>TM</sup> <sub>QmcA</sub> -QmcA-gfp-aphAIII plasmid	Up.qmcA-gfp_pUC18qmcA-gfp-5	ccgagctggtgaaagacagcgcacaacacggactcagccaggcggcgccggcagcatgagtaaaggtgaagaactgttc
	Down.qmcA-gfp_pUC18qmcA-gfp-3	ggctgagtcctgtttgttgcttattgtagagttcatccatgccgtgctgatacctgctg
	Up.ybbJ_pUC18qmcA-gfp-5	cagcaggtatcacgcacggcatggatgaactctacaataagccaacaacggactcagcc
	Down.ybbJ_pUC18qmcA-gfp-3	caaaatcttctgtcgttaagacgagacggctcggatag
	Up.KmFRT_pUC18qmcA-gfp-5	cgacagaaagattttgggagcaaatgatgtgttaggc

	Down.KmFRT_pUC18qmcA-gfp-3	gaaaatctctccggctgctgcatcatcgggcagggtgatcagggttactcatatgaatc
	Up.linearized pUC18_pUC18qmcA-gfp-5	cagccggagagatttcgaatcgtaatcatggtcatag
	Down.linearized pUC18_pUC18qmcA-gfp-5	ctttaccagctcggggatcctctagatcgacctg
For amplifying the DNA fragment to generate TM <sup>qmcA</sup> -QmcA-GFP strain	Up.qmcA_QmcA-GFP-KmFRT-5	Same as Up.qmcA-gfp_pUC18qmcA-gfp-5
	Down.KmFRT_QmcA-GFP-KmFRT-3	Down.KmFRT_pUC18qmcA-gfp-3
For amplifying the DNA fragment to generate TM <sup>qmcA</sup> -SPFH <sup>qmcA</sup> -GFP strain	UP.SPFHqmcA_SPFHqmcA-GFP-KmFRT-5	gcggaaggatccgtcaggcggaaatcctcaagccgaagtgatgaattaaacaacaatgagtaaagg
	Down.KmFRT_SPFHqmcA-GFP-KmFRT-3	gaaaatctctccggctgctgcatcatcgggcagggtgatcagggttactcatatgaatc
For amplifying the DNA fragment to generate TM <sup>qmcA</sup> -GFP strain	Up.TMqmcA_TMqmcA-GFP-KmFRT-5	ctcattttgtcgcgctggtcattgtcggcggggtgcaaatcgtagaattaaacaacaatgagtaaagg
	Down.KmFRT_TMqmcA-GFP-KmFRT-3	Same sequence as Down.KmFRT_SPFHqmcA-GFP-KmFRT-3
For assembling the pZS*12TM <sup>pf3</sup> -gfp plasmid	Up.pf3-GFP_pZS*12pf3-gfp-5	gtgagcggataacaagatacatgcaatccgtgattactg
	Down.pf3-GFP_pZS*12pf3-gfp-3	ctttactcatgttgtttaaattcaagaattg
	Up.GFP_pZS*12pf3-gfp-5	taacaacaacatgagtaaagggtgaagaac
	Down.GFP_pZS*12pf3-gfp-3	tctagactcagctaattaagtcattttagagtcatcc
	Up.linearized pZS*12 pZS*12pf3-gfp-5	ctacaaatgactaattagctgagctagag
	Down.linearized pZS*12 pZS*12pf3-gfp-3	cggattgcatgtatcttattatccgctc
For assembling the pZS*12TM <sup>pf3</sup> -gfp-aphAIII plasmid	Up.KmFRT_pZS*12pf3-gfp-KmFRT-5	cgaaaggctcagtcgaacgacagaaagatttgggag
	Down.KmFRT_pZS*12pf3-gfp-KmFRT-3	cgaaaggcccagtcctcagggttactcatatgaatacc
	Up. linearized pZS*12_pZS*12pf3-gfp-KmFRT-5	ggatattcatatgagtaaaccctgaagactgggcctttcg
	Down. linearized pZS*12_pZS*12pf3-gfp-KmFRT-3	ctttctgctgctgactgagcctttcgtttatttg
For assembling the pZS*12TM <sup>pf3</sup> -qmcA-gfp-aphAIII plasmid	Up. qmcA_pZS*12pf3-qmcA-gfp-KmFRT-5	tgatcaaagcgcaattctttatctaccgcagggtatc
	Down. qmcA_pZS*12pf3-qmcA-gfp-KmFRT-3	actcatgttgtttaaattctggctgagtcctgtttgtggc

	Up. linearized pZS*12_pZS*12pf3-gfp-Kmfrt-5	ggatattcatatgagtaaaccctgaagactgggccttctcg
	Down. linearized pZS*12_pZS*12pf3-gfp-Kmfrt-3	ggtacgataagaattgcgctttgatcc
For assembling the pZS*12TM <sup>Pf3</sup> -SPFH <sup>QmcA</sup> -gfp-aphAIII plasmid	Up. SPFHqmcA_pZS*12pf3-SPFHqmcA-gfp-KmFRT-5	Same as Up. qmcA_pZS*12pf3-qmcA-gfp-KmFRT-5
	Down. SPFHqmcA_pZS*12pf3-SPFHqmcA-gfp-KmFRT-3	actcatgttgtttaaattcacctcggctttgaggatttcc
	Up. Linearised pZS*12_pZS*12pf3-SPFHqmcA-gfp-KmFRT-5	caaagccgaaggtgaatttaacaacaacatgagtaaagg
	Down. Linearised pZS*12_pZS*12pf3-SPFHqmcA-gfp-KmFRT-3	Same as Down. linearized pZS*12_pZS*12pf3-gfp-Kmfrt-3
For amplifying the DNA fragments to generate TM <sup>Pf3</sup> -QmcA-GFP, TM <sup>Pf3</sup> -SPFH <sup>QmcA</sup> -GFP, and TM <sup>Pf3</sup> -GFP strains	Up.Pf3_pf3-(QmcA)-GFP-KmFRT-5	caatttctgctattatggacggtagaacaggaggttttccgatgcaatccgtgattactgatg
	Down.KmFRT_pf3-(QmcA)-GFP-KmFRT-3	Same sequence as Down.KmFRT_SPFHqmcA-GFP-KmFRT-3
For assembling the pUC18TM <sup>HflC</sup> -HflC-mCherry-aphAIII plasmid	Up.hflC-mCherry_pUC18hflC-mCherry-KmFRT-5	gatttctccgctacatgaagacgccgacttccgaacgcgtggcggcggcggcagcatggttccaagggcgaggag
	Down. hflC-mCherry_pUC18hflC-mCherry-KmFRT-3	caaaatctttctgctgttattgtacagctcatccatg
	Up.KmFRT_pUC18hflC-mCherry-KmFRT-5	cgacagaaagatttgggagcaaatgatgtgttaggc
	Down.KmFRT_pUC18hflC-mCherry-KmFRT-3	gaggatgcgggtggcttattgacctgtaccgagctgtatatcagggttactcatatgaatc
	Up.linearized pUC18_pUC18hflC-mCherry-KmFRT-5	caataaagccaccgcatcctcgaattcgaatcatggtcatag
	Down.linearized pUC18_pUC18hflC-mCherry-KmFRT-3	catgtagcggaaagaatcggatcctctagagtcgacctg
For amplifying the DNA fragment to generate TM <sup>HflC</sup> -HflC-mCherry strain	Up.hflC_HflC-mCherry-KmFRT-5	Same as Up.hflC-mCherry_pUC18hflC-mCherry-5
	Down.KmFRT_HflC-mCherry-KmFRT-3	gaggatgcgggtggcttattgacctgtaccgagctgtatatcagggttactcatatgaatc
For amplifying the DNA fragment to generate TM <sup>hflC</sup> -SPFH <sup>hflC</sup> -mCherry strain	Up.SPFHhflC_SPFHhflC-mCherry-CmFRT-5	gagcgtgaagcggtagcgcgtcaccgttcacaaggtcaggaatttaacaacaacatggttccaagg
	Down.CmFRT_SPFHhflC-mCherry-CmFRT-3	ggatgcgggtggcttattgacctgtaccgagctgtatatcaggaatcctccttagttcctattcc
For amplifying the DNA fragment to generate TM <sup>hflC</sup> -mCherry strain	Up.TMhflC_TMhflC-mCherry-KmFRT-5	catcgtgctgtagtgctttacatgtctgtcttctgctcggcggcggcggcagcatg
	Down.KmFRT_TMhflC-mCherry-KmFRT-3	gaggatgcgggtggcttattgacctgtaccgagctgtatatcagggttactcatatgaatc
	Up.cmi-pZS*12cmi-mcherry-5	gaaaggtaccatgaaagtattgattgatgaaattttttttt

For assembling the pZS*12TM <sup>cmi</sup> -mCherry plasmid	Down.cmi-pZS*12cmi-mCherry-3	tggaacctgtgtgtaaatcgcc
	Up.mCherry_pZS*12cmi-mCherry-5	taacaacaacatggttccaaggcgag
	Down.mCherry_pZS*12cmi-mCherry-3	ctaattaagcttattgtacagctcatccatgc
	Up.linearized pZS*12_pZS12cmi-mCherry-5	gtacaataagcttaattagctgagctagagg
	Down.linearized pZS*12_pZS12cmi-mCherry-3	tcacttcatggtaccttctcctttaatg
For assembling the pZS*12TM <sup>cmi</sup> -mCherry-cat plasmid	Up.CmFRT_pZS*12cmi-mCherry-CmFRT-5	gctcagtcgagtgtaggctggagctgcttc
	Down. CmFRT_pZS*12cmi-mCherry-CmFRT-5	gcccagtctcatatgaatatcctccttagtctctattcc
	Up.linearized pZS*12_pZS*12cmi-mCherry-CmFRT-5	tattcatatgaagactgggccttctgctttatc
	Down.linearized pZS*12_pZS*12cmi-mCherry-CmFRT-3	cagcctacactcgactgagccttctgctttattg
For assembling the pZS*12TM <sup>cmi</sup> -hflC-mCherry-cat plasmid	Up.hflC_pZS*12cmi-mCherry-CmFRT-5	agataaaggcgtcgtcaagaaggtagcgcc
	Down.hflC_pZS*12cmi-mCherry-CmFRT-3	tggtaaattcacgcttgaggaaagtcgg
	Up.linearized pZS*12_pZS*12cmi-hflC-mCherry-CmFRT-5	cgcaacgcgtgaatttaacaacaacatggtttcc
	Down.linearized pZS*12_pZS*12cmi-hflC-mCherry-CmFRT-3	ctttgacgacgctttatctcgtcctcaaaaaaac
For assembling the pZS*12TM <sup>cmi</sup> -SPFH <sup>hflC</sup> -mCherry-cat plasmid	Up. SPFHhflC_pZS*12cmi-SPFHhflC-mCherry-CmFRT-5	Same as Up.hflC_pZS*12cmi-mCherry-CmFRT-5
	Down. SPFHhflC_pZS*12cmi-SPFHhflC-mCherry-CmFRT-3	tggtaaattcctgacctgtgaacggtg
	Up.linearized pZS*12_pZS*12cmi-SPFHhflC-mCherry-CmFRT-5	acaaggtcaggaatttaacaacaacatggtttcc
	Down.linearized pZS*12_pZS*12cmi-SPFHhflC-mCherry-CmFRT-5	Same as Down.linearized pZS*12_pZS*12cmi-hflC-mCherry-CmFRT-3
For amplifying the DNA fragments to generate TM <sup>cmi</sup> -HflC-mCherry, TM <sup>cmi</sup> -SPFH <sup>hflC</sup> -mCherry, TM <sup>cmi</sup> -mCherry	Up.cmi_cmi-(HflC)-mCherry-CmFRT-5	ccaacgcgcagcgtaacgactaccagcgtcaggggaataacgatgaaagtgattagcatgaa
	Down.CmFRT_cmi-(HflC)-mCherry-CmFRT-3	ggatgcggtggctttattgacctgtaccgagctgtatacatatgaatatcctccttag
For amplifying the DNA fragment to generate AcrA-GFP strain	Up.acrA_AcrA-GFP-KmFRT-5	ccagcaagccgcaagcgggtgctcagcctgaacagctcaagtctgaatttaacaacaacatgagtaaagggtgaagaac
	Down.KmFRT_AcrA-GFP-KmFRT-3	cgggcgatcgataaagaattaggcatgtcttaacggctcctgtttaagtcagggttactcatatgaatcctcc

For assembling the pZS12* <i>hflKC</i> plasmid	Up.hflKC_pZS*12-HflKC-5	cattaaagaggagaaaggtaccatggcgtggaatcagcccggtaataacgg
	Down.hflKC_pZS*12-HflKC -3	gatgcctctagactcagctaattaagctttaacgcgttgcggaagtgcg
	Up.linearized pZS*12_pZS*12 -5	aagctaattagctgagtctag
	Down.linearized pZS*12_pZS*12-3	ggtaccttctcctcttaataaattcgg



## PM1 MicroPlate™ Carbon Sources

A1 Negative Control	A2 L-Arabinose	A3 N-Acetyl-D-Glucosamine	A4 D-Saccharic Acid	A5 Succinic Acid	A6 D-Galactose	A7 L-Aspartic Acid	A8 L-Proline	A9 D-Alanine	A10 D-Trehalose	A11 D-Mannose	A12 Dulcitol
B1 D-Serine	B2 D-Sorbitol	B3 Glycerol	B4 L-Fucose	B5 D-Glucuronic Acid	B6 D-Gluconic Acid	B7 D,L- $\alpha$ -Glycerol-Phosphate	B8 D-Xylose	B9 L-Lactic Acid	B10 Formic Acid	B11 D-Mannitol	B12 L-Glutamic Acid
C1 D-Glucose-6-Phosphate	C2 D-Galactonic Acid- $\gamma$ -Lactone	C3 D,L-Malic Acid	C4 D-Ribose	C5 Tween 20	C6 L-Rhamnose	C7 D-Fructose	C8 Acetic Acid	C9 $\alpha$ -D-Glucose	C10 Maltose	C11 D-Melibiose	C12 Thymidine
D-1 L-Asparagine	D2 D-Aspartic Acid	D3 D-Glucosaminic Acid	D4 1,2-Propanediol	D5 Tween 40	D6 $\alpha$ -Keto-Glutaric Acid	D7 $\alpha$ -Keto-Butyric Acid	D8 $\alpha$ -Methyl-D-Galactoside	D9 $\alpha$ -D-Lactose	D10 Lactulose	D11 Sucrose	D12 Uridine
E1 L-Glutamine	E2 M-Tartaric Acid	E3 D-Glucose-1-Phosphate	E4 D-Fructose-6-Phosphate	E5 Tween 80	E6 $\alpha$ -Hydroxy Glutaric Acid- $\gamma$ -Lactone	E7 $\alpha$ -Hydroxy Butyric Acid	E8 $\beta$ -Methyl-D-Glucoside	E9 Adonitol	E10 Maltotriose	E11 2-Deoxy Adenosine	E12 Adenosine
F1 Glycyl-L-Aspartic Acid	F2 Citric Acid	F3 M-Inositol	F4 D-Threonine	F5 Fumaric Acid	F6 Bromo Succinic Acid	F7 Propionic Acid	F8 Mucic Acid	F9 Glycolic Acid	F10 Glyoxylic Acid	F11 D-Cellobiose	F12 Inosine
G1 Glycyl-L-Glutamic Acid	G2 Tricarballic Acid	G3 L-Serine	G4 L-Threonine	G5 L-Alanine	G6 L-Alanyl-Glycine	G7 Acetoacetic Acid	G8 N-Acetyl- $\beta$ -D-Mannosamine	G9 Mono Methyl Succinate	G10 Methyl Pyruvate	G11 D-Malic Acid	G12 L-Malic Acid
H1 Glycyl-L-Proline	H2 p-Hydroxy Phenyl Acetic Acid	H3 m-Hydroxy Phenyl Acetic Acid	H4 Tyramine	H5 D- Psicose	H6 L-Lyxose	H7 Glucuronamide	H8 Pyruvic Acid	H9 L-Galactonic Acid- $\gamma$ -Lactone	H10 D-Galacturonic Acid	H11 Phenylethylamine	H12 2-Aminoethanol

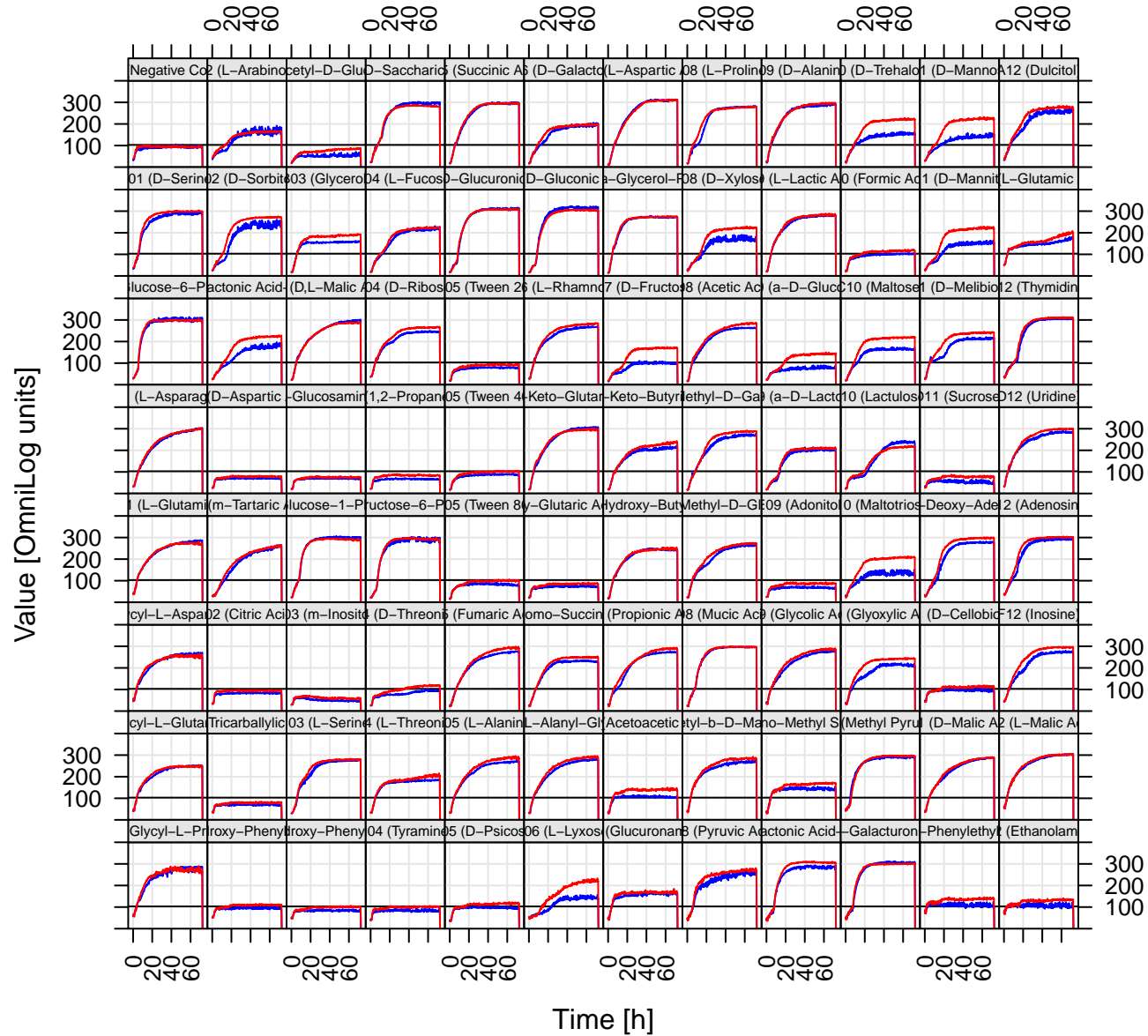
## PM2A MicroPlate™ Carbon Sources

A1 Negative Control	A2 Chondroitin Sulfate C	A3 $\alpha$ -Cyclodextrin	A4 $\beta$ -Cyclodextrin	A5 $\gamma$ -Cyclodextrin	A6 Dextrin	A7 Gelatin	A8 Glycogen	A9 Inulin	A10 Laminarin	A11 Mannan	A12 Pectin
B1 N-Acetyl-D-Galactosamine	B2 N-Acetyl-Neuraminic Acid	B3 $\beta$ -D-Allose	B4 Amygdalin	B5 D-Arabinose	B6 D-Arabitol	B7 L-Arabitol	B8 Arbutin	B9 2-Deoxy-D-Ribose	B10 l-Erythritol	B11 D-Fucose	B12 3-O- $\beta$ -D-Galactopyranosyl-D-Arabinose
C1 Gentobiose	C2 L-Glucose	C3 Lactitol	C4 D-Melezitose	C5 Maltitol	C6 $\alpha$ -Methyl-D-Glucoside	C7 $\beta$ -Methyl-D-Galactoside	C8 3-Methyl Glucose	C9 $\beta$ -Methyl-D-Glucuronic Acid	C10 $\alpha$ -Methyl-D-Mannoside	C11 $\beta$ -Methyl-D-Xyloside	C12 Palatinose
D1 D-Raffinose	D2 Salicin	D3 Sedoheptulose	D4 L-Sorbose	D5 Stachyose	D6 D-Tagatose	D7 Turanose	D8 Xylitol	D9 N-Acetyl-D-Glucosaminitol	D10 $\gamma$ -Amino Butyric Acid	D11 $\beta$ -Amino Valeric Acid	D12 Butyric Acid
E1 Capric Acid	E2 Caproic Acid	E3 Citraconic Acid	E4 Citramalic Acid	E5 D-Glucosamine	E6 2-Hydroxy Benzoic Acid	E7 $\gamma$ -Hydroxy Benzoic Acid	E8 $\beta$ -Hydroxy Butyric Acid	E9 $\gamma$ -Hydroxy Butyric Acid	E10 $\alpha$ -Keto Valeric Acid	E11 Itaconic Acid	E12 5-Keto-D-Gluconic Acid
F1 D-Lactic Acid Methyl Ester	F2 Malonic Acid	F3 Mellblionic Acid	F4 Oxalic Acid	F5 Oxalomalic Acid	F6 Quinic Acid	F7 D-Ribono-1,4-Lactone	F8 Sebacic Acid	F9 Sorbic Acid	F10 Succinamic Acid	F11 D-Tartaric Acid	F12 L-Tartaric Acid
G1 Acetamide	G2 L-Alaninamide	G3 N-Acetyl-L-Glutamic Acid	G4 L-Arginine	G5 Glycine	G6 L-Histidine	G7 L-Homoserine	G8 Hydroxy-L-Proline	G9 L-Isoleucine	G10 L-Leucine	G11 L-Lysine	G12 L-Methionine
H1 L-Ornithine	H2 L-Phenylalanine	H3 L-Pyroglutamic Acid	H4 L-Valine	H5 D,L-Carnitine	H6 Sec-Butylamine	H7 D,L-Octopamine	H8 Putrescine	H9 Dihydroxy Acetone	H10 2,3-Butanediol	H11 2,3-Butanone	H12 3-Hydroxy 2-Butanone

# PM01 (Carbon Sources)

ΔSPFH-1

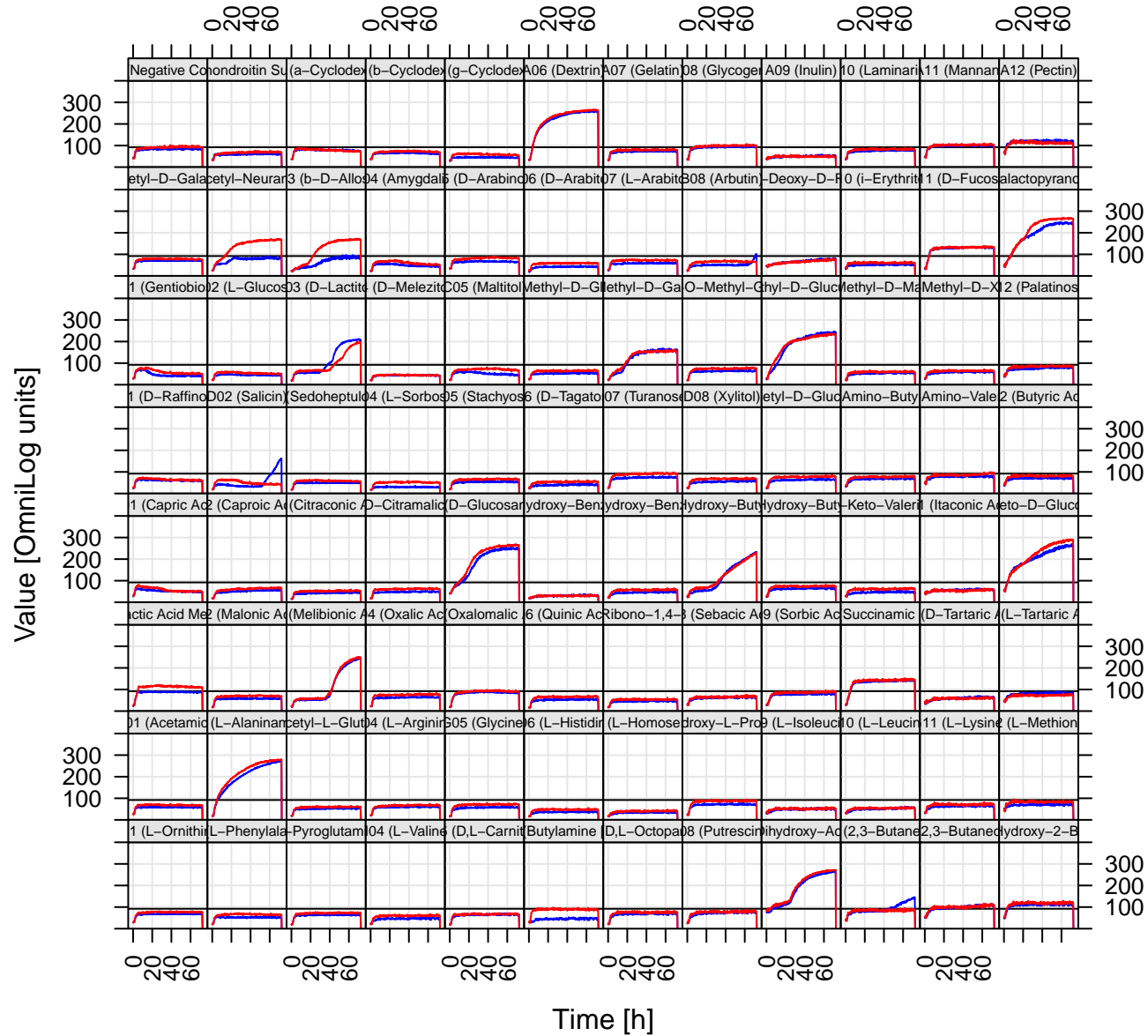
WT-1





# PM02 (Carbon Sources)

ΔSPFH-1  
WT-1





## PM3B MicroPlate™ Nitrogen Sources

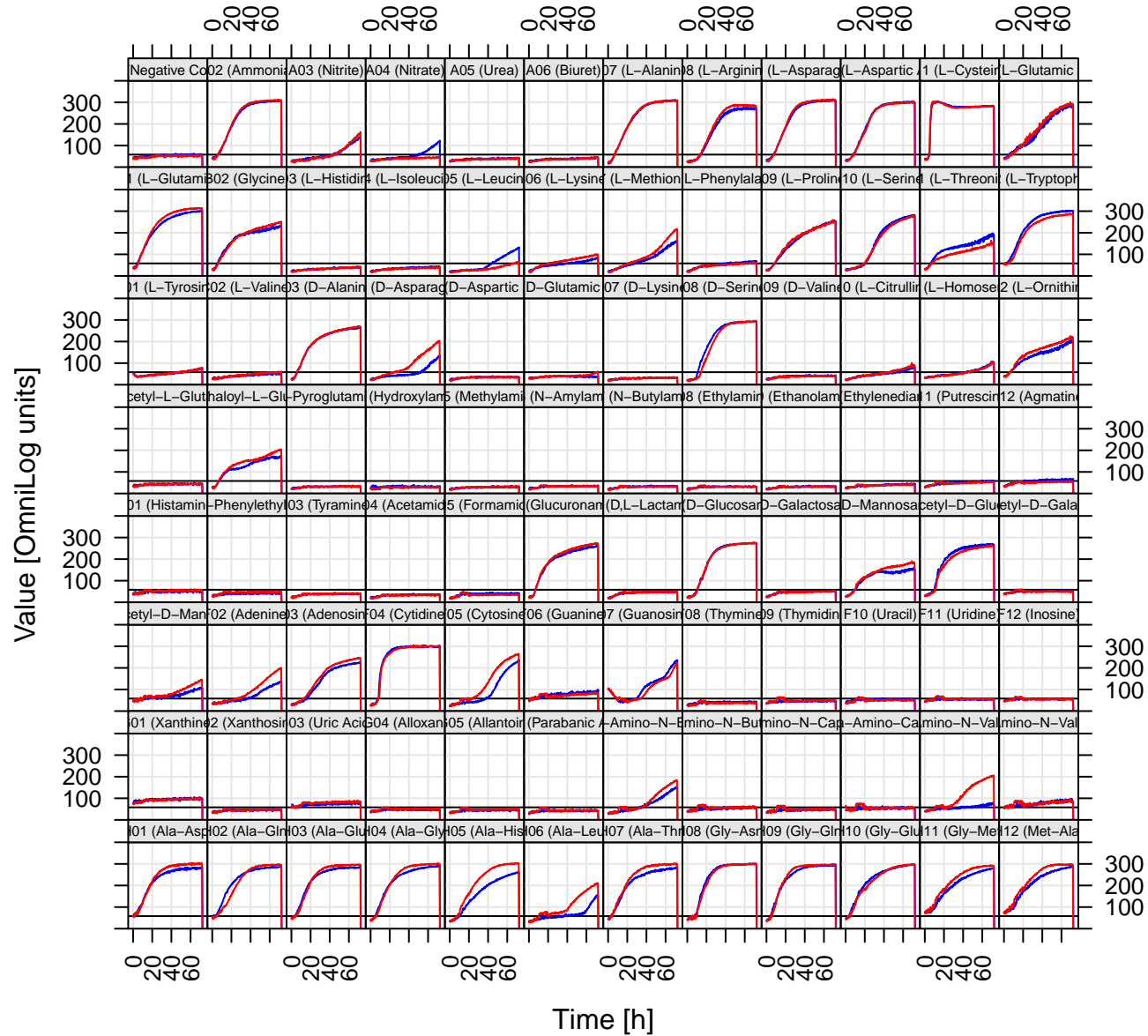
A1 Negative Control	A2 Ammonia	A3 Nitrite	A4 Nitrate	A5 Urea	A6 Biuret	A7 L-Alanine	A8 L-Arginine	A9 L-Asparagine	A10 L-Aspartic Acid	A11 L-Cysteine	A12 L-Glutamic Acid
B1 L-Glutamine	B2 Glycine	B3 L-Histidine	B4 L-Isoleucine	B5 L-Leucine	B6 L-Lysine	B7 L-Methionine	B8 L-Phenylalanine	B9 L-Proline	B10 L-Serine	B11 L-Threonine	B12 L-Tryptophan
C1 L-Tyrosine	C2 L-Valine	C3 D-Alanine	C4 D-Asparagine	C5 D-Aspartic Acid	C6 D-Glutamic Acid	C7 D-Lysine	C8 D-Serine	C9 D-Valine	C10 L-Citrulline	C11 L-Homoserine	C12 L-Ornithine
D-1 N-Acetyl-D,L- Glutamic Acid	D2 N-Phthaloyl-L- Glutamic Acid	D3 L-Pyroglutamic Acid	D4 Hydroxylamine	D5 Methylamine	D6 N-Amylamine	D7 N-Butylamine	D8 Ethylamine	D9 Ethanolamine	D10 Ethylene diamine	D11 Putrescine	D12 Agmatine
E1 Histamine	E2 β-Phenylethyl- amine	E3 Tyramine	E4 Acetamide	E5 Formamide	E6 Glucuronamide	E7 D,L-Lactamide	E8 D-Glucosamine	E9 D- Galactosamine	E10 D- Mannosamine	E11 N-Acetyl-D- Glucosamine	E12 N-Acetyl-D- Galactosamine
F1 N-Acetyl-D- Mannosamine	F2 Adenine	F3 Adenosine	F4 Cytidine	F5 Cytosine	F6 Guanine	F7 Guanosine	F8 Thymine	F9 Thymidine	F10 Uracil	F11 Uridine	F12 Inosine
G1 Xanthine	G2 Xanthosine	G3 Uric Acid	G4 Alloxan	G5 Allantoin	G6 Parabanic Acid	G7 D,L-α-Amino-N- Butyric Acid	G8 γ-Amino-N- Butyric Acid	G9 ε-Amino-N- Caproic Acid	G10 D,L-α-Amino- Caprylic Acid	G11 δ-Amino-N- Valeric Acid	G12 α-Amino-N- Valeric Acid
H1 Ala-Asp	H2 Ala-Gln	H3 Ala-Glu	H4 Ala-Gly	H5 Ala-His	H6 Ala-Leu	H7 Ala-Thr	H8 Gly-Asn	H9 Gly-Gln	H10 Gly-Glu	H11 Gly-Met	H12 Met-Ala

## PM4A MicroPlate™ Phosphorus and Sulfur Sources

A1 Negative Control	A2 Phosphate	A3 Pyrophosphate	A4 Trimeta- phosphate	A5 Tripoly- phosphate	A6 Triethyl Phosphate	A7 Hypophosphite	A8 Adenosine- 2'- monophosphate	A9 Adenosine- 3'- monophosphate	A10 Adenosine- 5'- monophosphate	A11 Adenosine- 2',3'- cyclic monophosphate	A12 Adenosine- 3',5'- cyclic monophosphate
B1 Thiophosphate	B2 Dithiophosphat e	B3 D,L-α-Glycerol Phosphate	B4 β-Glycerol Phosphate	B5 Carbamyl Phosphate	B6 D-2-Phospho- Glyceric Acid	B7 D-3-Phospho- Glyceric Acid	B8 Guanosine- 2'- monophosphate	B9 Guanosine- 3'- monophosphate	B10 Guanosine- 5'- monophosphate	B11 Guanosine- 2',3'- cyclic monophosphate	B12 Guanosine- 3',5'- cyclic monophosphate
C1 Phosphoenol Pyruvate	C2 Phospho- Glycolic Acid	C3 D-Glucose-1- Phosphate	C4 D-Glucose-6- Phosphate	C5 2-Deoxy-D- Glucose 6- Phosphate	C6 D- Glucosamine-6- Phosphate	C7 6-Phospho- Gluconic Acid	C8 Cytidine- 2'- monophosphate	C9 Cytidine- 3'- monophosphate	C10 Cytidine- 5'- monophosphate	C11 Cytidine- 2',3'- cyclic monophosphate	C12 Cytidine- 3',5'- cyclic monophosphate
D1 D-Mannose-1- Phosphate	D2 D-Mannose-6- Phosphate	D3 Cysteamine-S- Phosphate	D4 Phospho-L- Arginine	D5 O-Phospho-D- Serine	D6 O-Phospho-L- Serine	D7 O-Phospho-L- Threonine	D8 Uridine- 2'- monophosphate	D9 Uridine- 3'- monophosphate	D10 Uridine- 5'- monophosphate	D11 Uridine- 2',3'- cyclic monophosphate	D12 Uridine- 3',5'- cyclic monophosphate
E1 O-Phospho-D- Tyrosine	E2 O-Phospho-L- Tyrosine	E3 Phosphocreatin e	E4 Phosphoryl Choline	E5 O-Phosphoryl- Ethanolamine	E6 Phosphono Acetic Acid	E7 2-Aminoethyl Phosphonic Acid	E8 Methylene Diphosphonic Acid	E9 Thymidine- 3'- monophosphate	E10 Thymidine- 5'- monophosphate	E11 Inositol Hexaphosphate	E12 Thymidine 3',5'- cyclic monophosphate
F1 Negative Control	F2 Sulfate	F3 Thiosulfate	F4 Tetrathionate	F5 Thiophosphate	F6 Dithiophosphat e	F7 L-Cysteine	F8 D-Cysteine	F9 L-Cysteinyl- Glycine	F10 L-Cysteic Acid	F11 Cysteamine	F12 L-Cysteine Sulfonic Acid
G1 N-Acetyl-L- Cysteine	G2 S-Methyl-L- Cysteine	G3 Cystathionine	G4 Lanthionine	G5 Glutathione	G6 D,L-Ethionine	G7 L-Methionine	G8 D-Methionine	G9 Glycyl-L- Methionine	G10 N-Acetyl-D,L- Methionine	G11 L- Methionine Sulfoxide	G12 L-Methionine Sulfone
H1 L-Djenkoic Acid	H2 Thiourea	H3 1-Thio-β-D- Glucose	H4 D,L-Lipoamide	H5 Taurochoic Acid	H6 Taurine	H7 Hypotaurine	H8 p-Amino Benzene Sulfonic Acid	H9 Butane Sulfonic Acid	H10 2- Hydroxyethane Sulfonic Acid	H11 Methane Sulfonic Acid	H12 Tetramethylene Sulfone

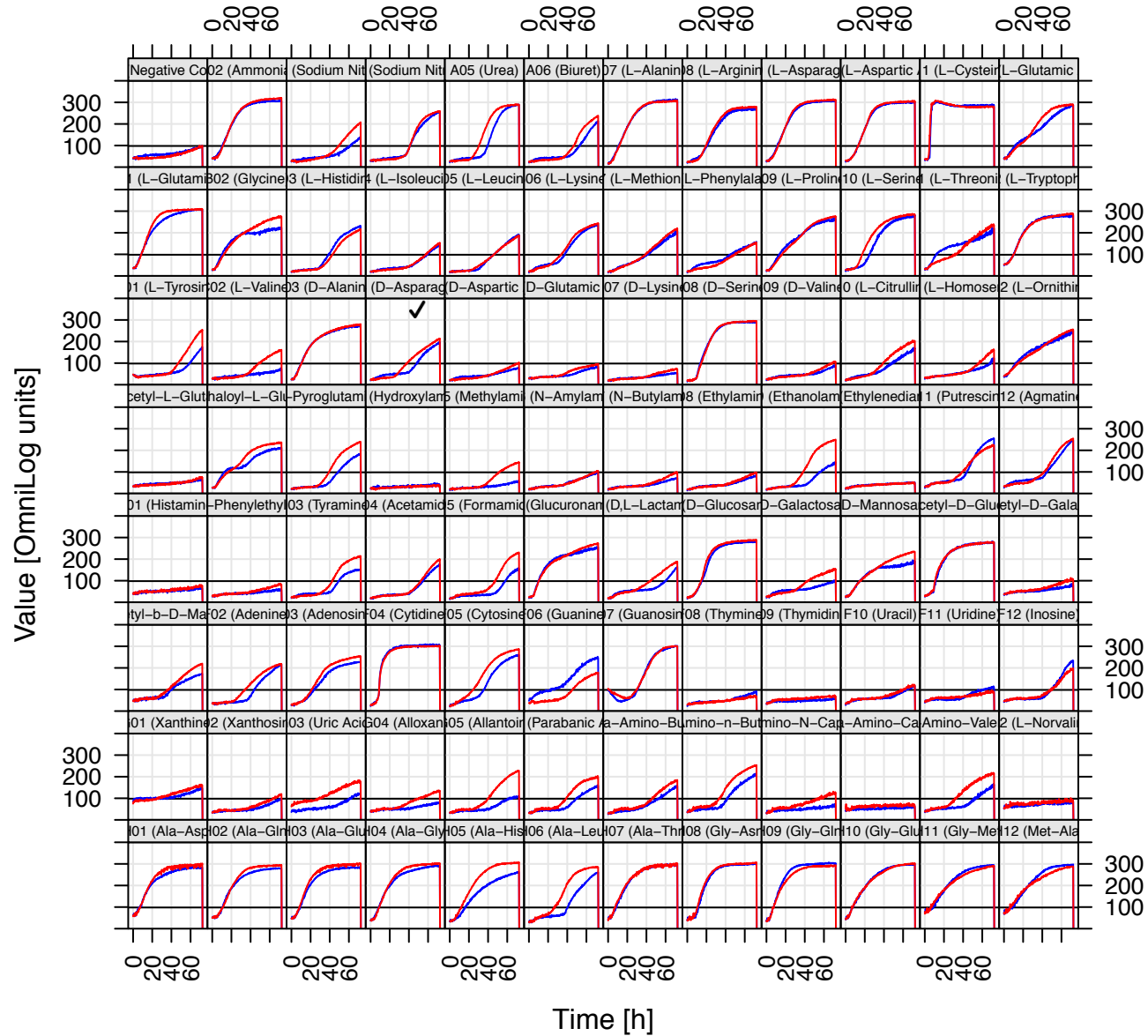
# PM3 (Nitrogen Sources)

ΔSPFH-1  
WT-1



# PM3 (Nitrogen Sources)

ΔSPFH-2  
WT-2





## PM9 MicroPlate™ Osmolytes

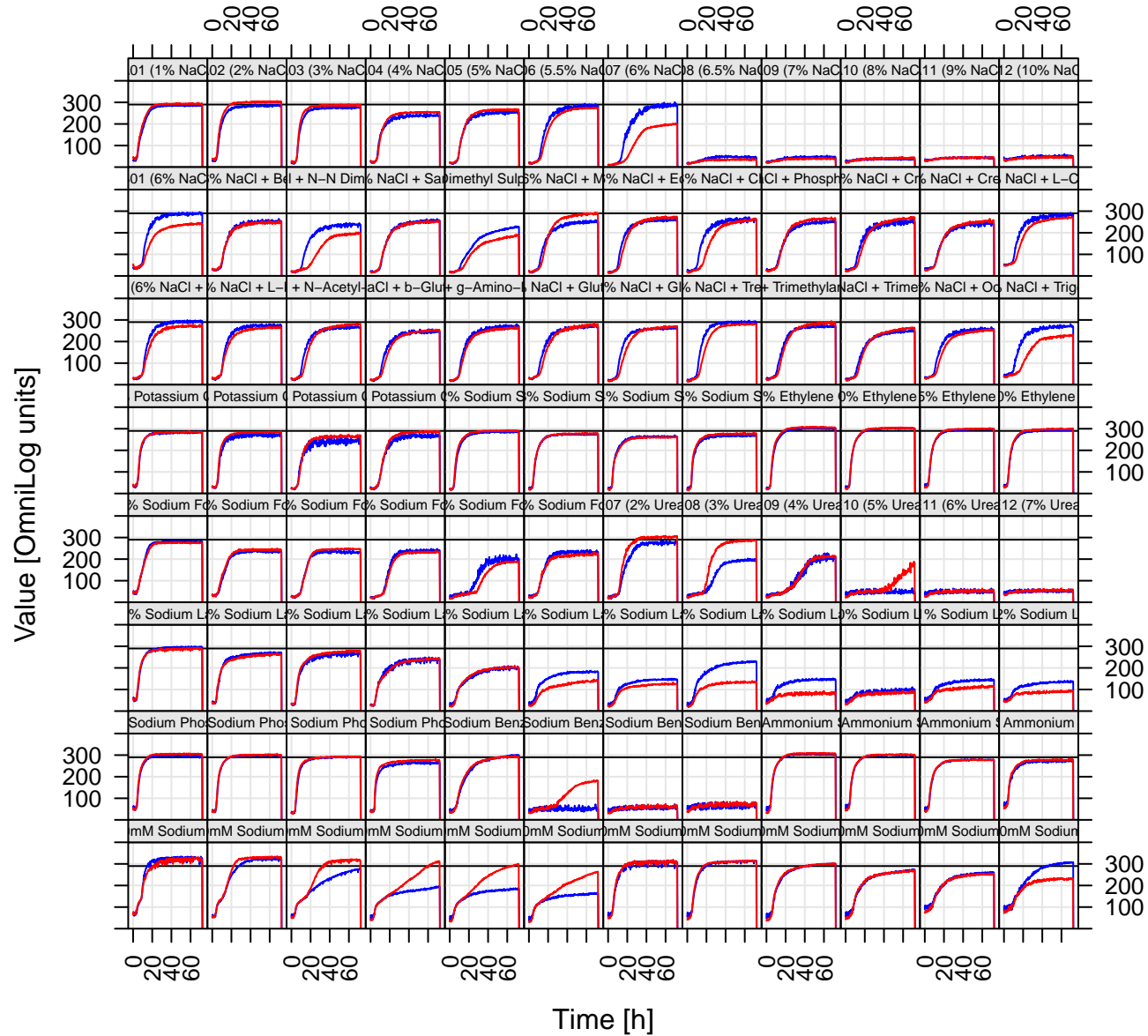
A1 NaCl 1%	A2 NaCl 2%	A3 NaCl 3%	A4 NaCl 4%	A5 NaCl 5%	A6 NaCl 5.5%	A7 NaCl 6%	A8 NaCl 6.5%	A9 NaCl 7%	A10 NaCl 8%	A11 NaCl 9%	A12 NaCl 10%
B1 NaCl 6%	B2 NaCl 6% + Betaine	B3 NaCl 6% + N-N Dimethyl glycine	B4 NaCl 6% + Sarcosine	B5 NaCl 6% + Dimethyl sulphonyl propionate	B6 NaCl 6% + MOPS	B7 NaCl 6% + Ectoine	B8 NaCl 6% + Choline	B9 NaCl 6% + Phosphoryl choline	B10 NaCl 6% + Creatine	B11 NaCl 6% + Creatinine	B12 NaCl 6% + L- Carnitine
C1 NaCl 6% + KCl	C2 NaCl 6% + L-proline	C3 NaCl 6% + N-Acethyl L-glutamine	C4 NaCl 6% + β-Glutamic acid	C5 NaCl 6% + γ-Amino -n- butyric acid	C6 NaCl 6% + Glutathione	C7 NaCl 6% + Glycerol	C8 NaCl 6% + Trehalose	C9 NaCl 6% + Trimethylamine -N-oxide	C10 NaCl 6% + Trimethylamine	C11 NaCl 6% + Octopine	C12 NaCl 6% + Trigonelline
D-1 Potassium chloride 3%	D2 Potassium chloride 4%	D3 Potassium chloride 5%	D4 Potassium chloride 6%	D5 Sodium sulfate 2%	D6 Sodium sulfate 3%	D7 Sodium sulfate 4%	D8 Sodium sulfate 5%	D9 Ethylene glycol 5%	D10 Ethylene glycol 10%	D11 Ethylene glycol 15%	D12 Ethylene glycol 20%
E1 Sodium formate 1%	E2 Sodium formate 2%	E3 Sodium formate 3%	E4 Sodium formate 4%	E5 Sodium formate 5%	E6 Sodium formate 6%	E7 Urea 2%	E8 Urea 3%	E9 Urea 4%	E10 Urea 5%	E11 Urea 6%	E12 Urea 7%
F1 Sodium Lactate 1%	F2 Sodium Lactate 2%	F3 Sodium Lactate 3%	F4 Sodium Lactate 4%	F5 Sodium Lactate 5%	F6 Sodium Lactate 6%	F7 Sodium Lactate 7%	F8 Sodium Lactate 8%	F9 Sodium Lactate 9%	F10 Sodium Lactate 10%	F11 Sodium Lactate 11%	F12 Sodium Lactate 12%
G1 Sodium Phosphate pH 7 20mM	G2 Sodium Phosphate pH 7 50mM	G3 Sodium Phosphate pH 7 100mM	G4 Sodium Phosphate pH 7 200mM	G5 Sodium Benzoate pH 5.2 20mM	G6 Sodium Benzoate pH 5.2 50mM	G7 Sodium Benzoate pH5.2 100mM	G8 Sodium Benzoate pH 5.2 200mM	G9 Ammonium sulfate pH8 10mM	G10 Ammonium sulfate pH 8 20mM	G11 Ammonium sulfate pH 8 50mM	G12 Ammonium sulfate pH8 100mM
H1 Sodium Nitrate 10mM	H2 Sodium Nitrate 20mM	H3 Sodium Nitrate 40mM	H4 Sodium Nitrate 60mM	H5 Sodium Nitrate 80mM	H6 Sodium Nitrate 100mM	H7 Sodium Nitrite 10mM	H8 Sodium Nitrite 20mM	H9 Sodium Nitrite 40mM	H10 Sodium Nitrite 60mM	H11 Sodium Nitrite 80mM	H12 Sodium Nitrite 100mM

## PM10 MicroPlate™ pH

A1 pH 3.5	A2 pH 4	A3 pH 4.5	A4 pH 5	A5 pH 5.5	A6 pH 6	A7 pH 7	A8 pH 8	A9 pH 8.5	A10 pH 9	A11 pH 9.5	A12 pH 10
B1 pH 4.5	B2 pH 4.5 + L-Alanine	B3 pH 4.5 + L-Arginine	B4 pH 4.5 + L-Asparagine	B5 pH 4.5 + L-Aspartic Acid	B6 pH 4.5 + L-Glutamic Acid	B7 pH 4.5 + L-Glutamine	B8 pH 4.5 + Glycine	B9 pH 4.5 + L-Histidine	B10 pH 4.5 + L-Isoleucine	B11 pH 4.5 + L-Leucine	B12 pH 4.5 + L-Lysine
C1 pH 4.5 + L-Methionine	C2 pH 4.5 + L- Phenylalanine	C3 pH 4.5 + L-Proline	C4 pH 4.5 + L-Serine	C5 pH 4.5 + L-Threonine	C6 pH 4.5 + L-Tryptophan	C7 pH 4.5 + L-Tyrosine	C8 pH 4.5 + L-Valine	C9 pH 4.5 + Hydroxy- L-Proline	C10 pH 4.5 + L-Ornithine	C11 pH 4.5 + L-Homoarginine	C12 pH 4.5 + L-Homoserine
D-1 pH 4.5 + Anthranilic acid	D2 pH 4.5 + L-Norleucine	D3 pH 4.5 + L-Norvaline	D4 pH 4.5 + α- Amino-N- butyric acid	D5 pH 4.5 + p- Aminobenzoate	D6 pH 4.5 + L-Cystelic acid	D7 pH 4.5 + D-Lysine	D8 pH 4.5 + 5-Hydroxy Lysine	D9 pH 4.5 + 5-Hydroxy Tryptophan	D10 pH 4.5 + D,L-Diamino pimelic acid	D11 pH 4.5 + Trimethyl amine-N-oxide	D12 pH 4.5 + Urea
E1 pH 9.5	E2 pH 9.5 + L-Alanine	E3 pH 9.5 + L-Arginine	E4 pH 9.5 + L-Asparagine	E5 pH 9.5 + L-Aspartic Acid	E6 pH 9.5 + L-Glutamic Acid	E7 pH 9.5 + L-Glutamine	E8 pH 9.5 + Glycine	E9 pH 9.5 + L-Histidine	E10 pH 9.5 + L-Isoleucine	E11 pH 9.5 + L-Leucine	E12 pH 9.5 + L-Lysine
F1 pH 9.5 + L-Methionine	F2 pH 9.5 + L- Phenylalanine	F3 pH 9.5 + L-Proline	F4 pH 9.5 + L-Serine	F5 pH 9.5 + L-Threonine	F6 pH 9.5 + L-Tryptophan	F7 pH 9.5 + L-Tyrosine	F8 pH 9.5 + L-Valine	F9 pH 9.5 + Hydroxy- L-Proline	F10 pH 9.5 + L-Ornithine	F11 pH 9.5 + L-Homoarginine	F12 pH 9.5 + L-Homoserine
G1 pH 9.5 + Anthranilic acid	G2 pH 9.5 + L-Norleucine	G3 pH 9.5 + L-Norvaline	G4 pH 9.5 + Agmatine	G5 pH 9.5 + Cadaverine	G6 pH 9.5 + Putrescine	G7 pH 9.5 + Histamine	G8 pH 9.5 + Phenylethylamin e	G9 pH 9.5 + Tyramine	G10 pH 9.5 + Creatine	G11 pH 9.5 + Trimethyl amine-N-oxide	G12 pH 9.5 + Urea
H1 X-Caprylate	H2 X-α-D- Glucoside	H3 X-β-D- Glucoside	H4 X-α-D- Galactoside	H5 X-β-D- Galactoside	H6 X-α- D- Glucuronide	H7 X-β- D- Glucuronide	H8 X-β-D- Glucosaminid e	H9 X-β-D- Galactosaminid e	H10 X-α-D- Mannoside	H11 X-PO4	H12 X-SO4

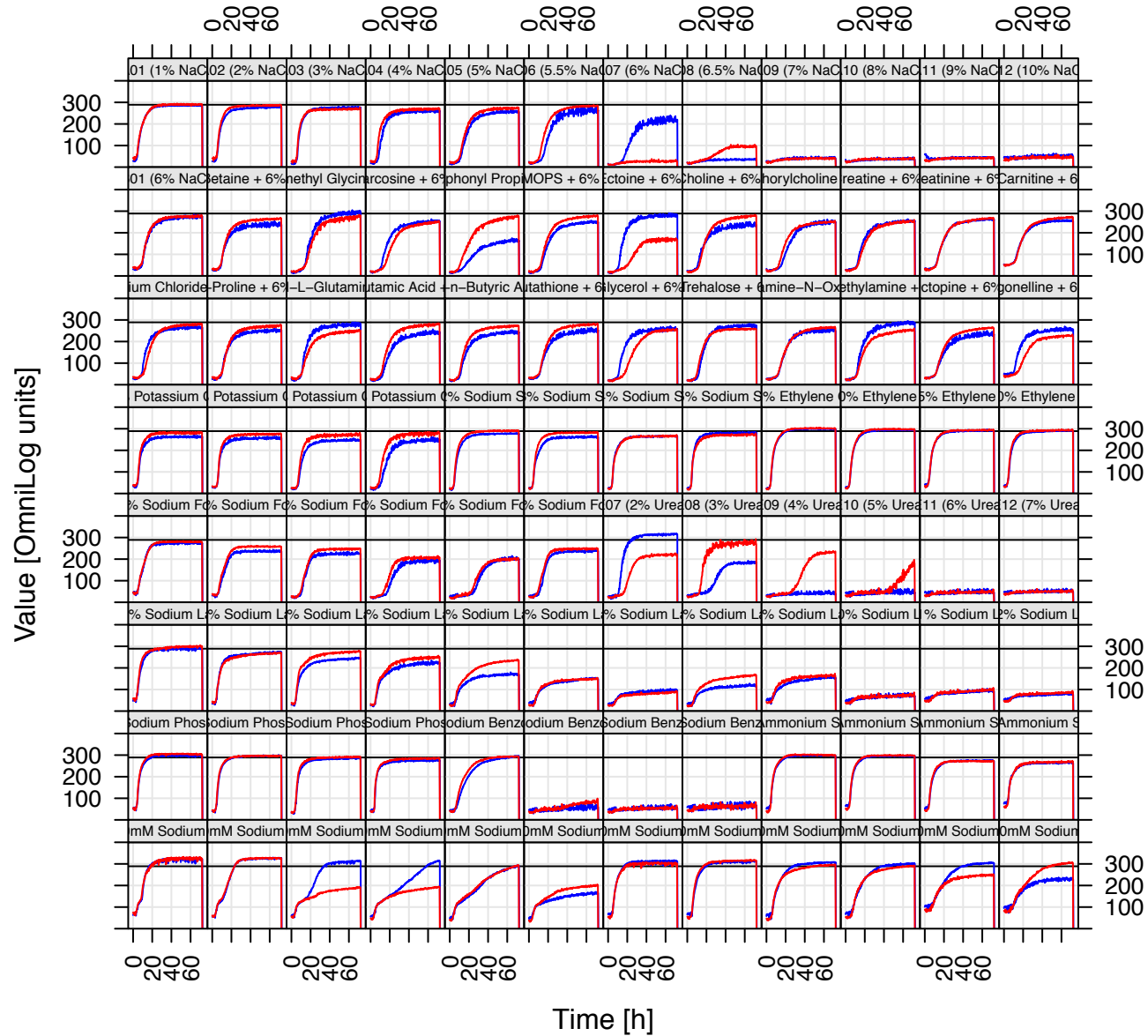
# PM09 (Osmolytes)

$\Delta$ SPFH-1  
WT-1



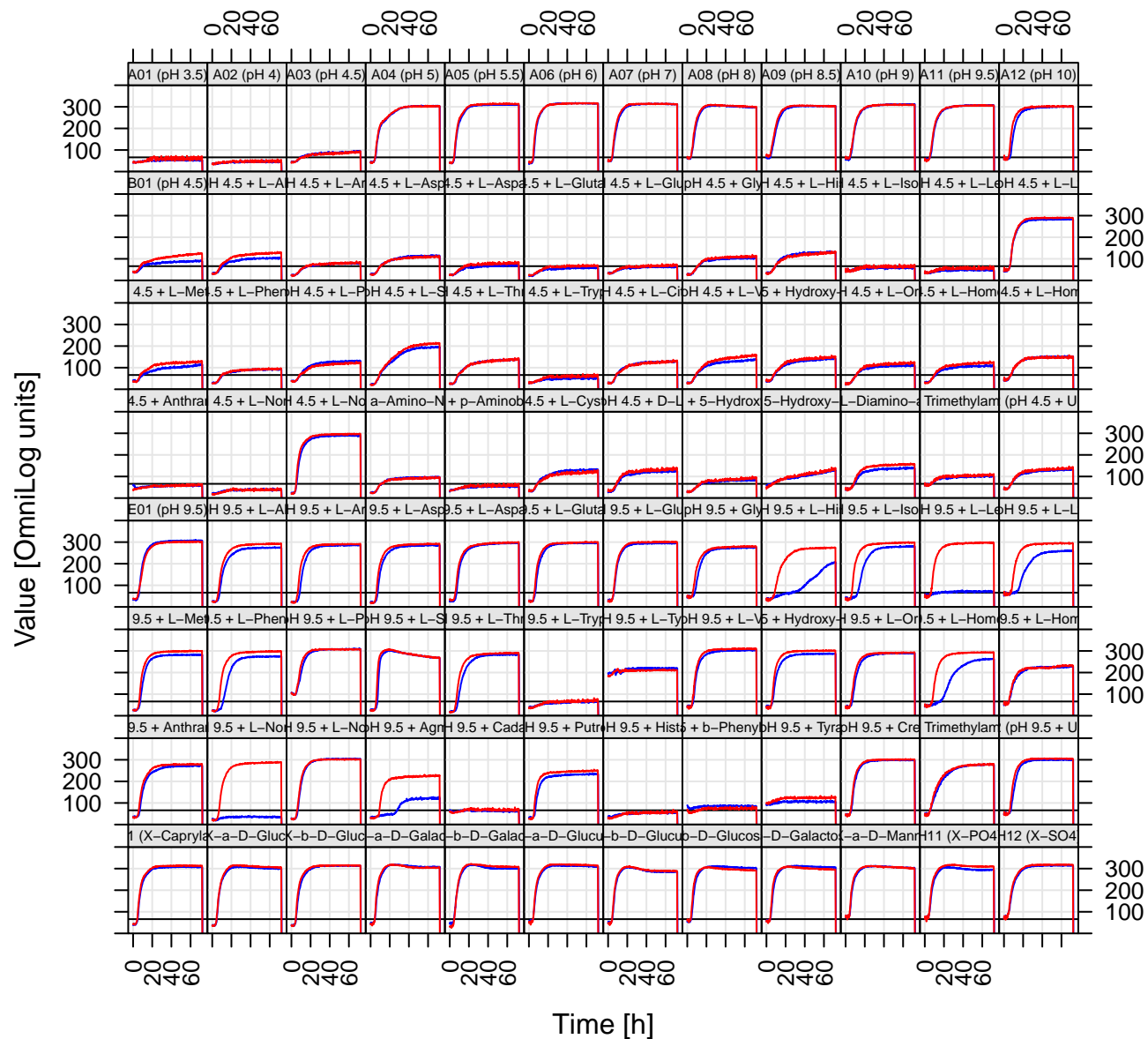
# PM09 (Osmolytes)

ΔSPFH-2  
WT-2



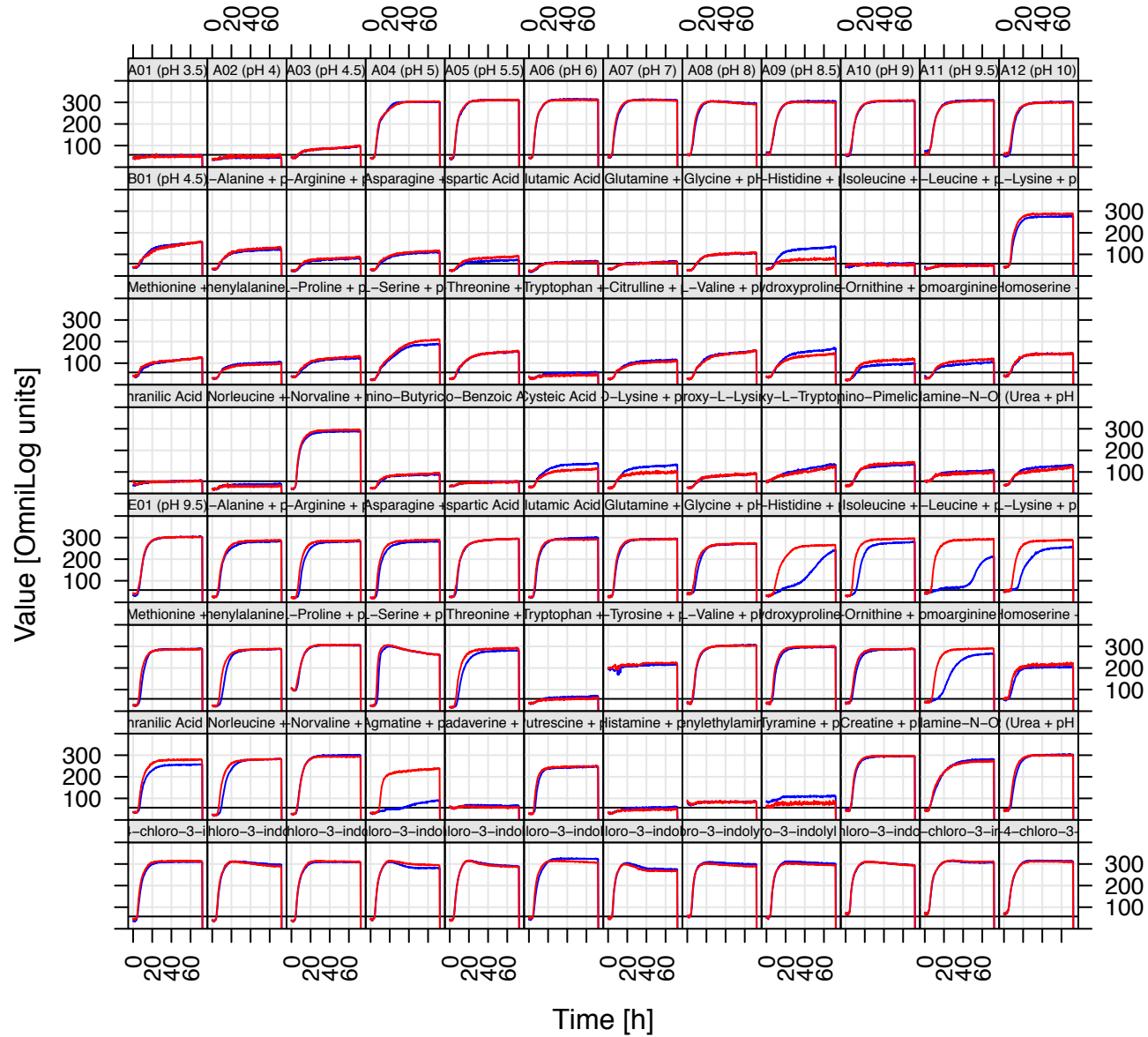
# PM10 (pH)

ΔSPFH-1  
WT-1



# PM10 (pH)

$\Delta$ SPFH-2  
WT-2



**PM11C MicroPlate™**

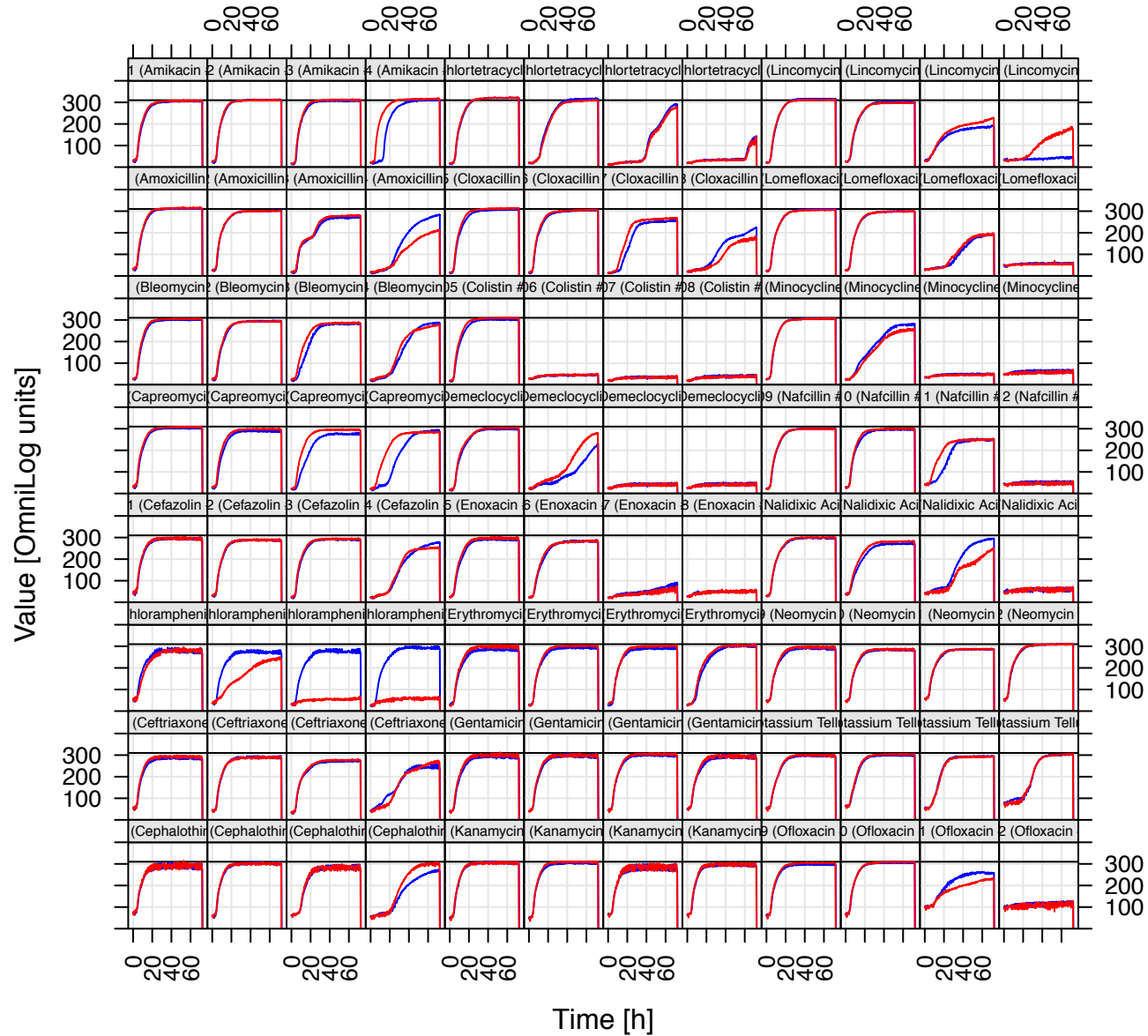
A1 Amikacin  1	A2 Amikacin  2	A3 Amikacin  3	A4 Amikacin  4	A5 Chlortetracycline  1	A6 Chlortetracycline  2	A7 Chlortetracycline  3	A8 Chlortetracycline  4	A9 Lincomycin  1	A10 Lincomycin  2	A11 Lincomycin  3	A12 Lincomycin  4
B1 Amoxicillin  1	B2 Amoxicillin  2	B3 Amoxicillin  3	B4 Amoxicillin  4	B5 Cloxacillin  1	B6 Cloxacillin  2	B7 Cloxacillin  3	B8 Cloxacillin  4	B9 Lomefloxacin  1	B10 Lomefloxacin  2	B11 Lomefloxacin  3	B12 Lomefloxacin  4
C1 Bleomycin  1	C2 Bleomycin  2	C3 Bleomycin  3	C4 Bleomycin  4	C5 Colistin  1	C6 Colistin  2	C7 Colistin  3	C8 Colistin  4	C9 Minocycline  1	C10 Minocycline  2	C11 Minocycline  3	C12 Minocycline  4
D1 Capreomycin  1	D2 Capreomycin  2	D3 Capreomycin  3	D4 Capreomycin  4	D5 Demeclocycline  1	D6 Demeclocycline  2	D7 Demeclocycline  3	D8 Demeclocycline  4	D9 Nafcillin  1	D10 Nafcillin  2	D11 Nafcillin  3	D12 Nafcillin  4
E1 Cefazolin  1	E2 Cefazolin  2	E3 Cefazolin  3	E4 Cefazolin  4	E5 Enoxacin  1	E6 Enoxacin  2	E7 Enoxacin  3	E8 Enoxacin  4	E9 Nalidixic acid  1	E10 Nalidixic acid  2	E11 Nalidixic acid  3	E12 Nalidixic acid  4
F1 Chloramphenicol  1	F2 Chloramphenicol  2	F3 Chloramphenicol  3	F4 Chloramphenicol  4	F5 Erythromycin  1	F6 Erythromycin  2	F7 Erythromycin  3	F8 Erythromycin  4	F9 Neomycin  1	F10 Neomycin  2	F11 Neomycin  3	F12 Neomycin  4
G1 Ceftriaxone  1	G2 Ceftriaxone  2	G3 Ceftriaxone  3	G4 Ceftriaxone  4	G5 Gentamicin  1	G6 Gentamicin  2	G7 Gentamicin  3	G8 Gentamicin  4	G9 Potassium tellurite  1	G10 Potassium tellurite  2	G11 Potassium tellurite  3	G12 Potassium tellurite  4
H1 Cephalothin  1	H2 Cephalothin  2	H3 Cephalothin  3	H4 Cephalothin  4	H5 Kanamycin  1	H6 Kanamycin  2	H7 Kanamycin  3	H8 Kanamycin  4	H9 Ofloxacin  1	H10 Ofloxacin  2	H11 Ofloxacin  3	H12 Ofloxacin  4

**PM12B MicroPlate™**

A1 Penicillin G  1	A2 Penicillin G  2	A3 Penicillin G  3	A4 Penicillin G  4	A5 Tetracycline  1	A6 Tetracycline  2	A7 Tetracycline  3	A8 Tetracycline  4	A9 Carbenicillin  1	A10 Carbenicillin  2	A11 Carbenicillin  3	A12 Carbenicillin  4
B1 Oxacillin  1	B2 Oxacillin  2	B3 Oxacillin  3	B4 Oxacillin  4	B5 Penimepicycline  1	B6 Penimepicycline  2	B7 Penimepicycline  3	B8 Penimepicycline  4	B9 Polymyxin B  1	B10 Polymyxin B  2	B11 Polymyxin B  3	B12 Polymyxin B  4
C1 Paromomycin  1	C2 Paromomycin  2	C3 Paromomycin  3	C4 Paromomycin  4	C5 Vancomycin  1	C6 Vancomycin  2	C7 Vancomycin  3	C8 Vancomycin  4	C9 D,L-Serine hydroxamate  1	C10 D,L-Serine hydroxamate  2	C11 D,L-Serine hydroxamate  3	C12 D,L-Serine hydroxamate  4
D1 Sisomicin  1	D2 Sisomicin  2	D3 Sisomicin  3	D4 Sisomicin  4	D5 Sulfamethazine  1	D6 Sulfamethazine  2	D7 Sulfamethazine  3	D8 Sulfamethazine  4	D9 Novobiocin  1	D10 Novobiocin  2	D11 Novobiocin  3	D12 Novobiocin  4
E1 2,4-Diamino-6,7-diisopropyl-pteridine  1	E2 2,4-Diamino-6,7-diisopropyl-pteridine  2	E3 2,4-Diamino-6,7-diisopropyl-pteridine  3	E4 2,4-Diamino-6,7-diisopropyl-pteridine  4	E5 Sulfadiazine  1	E6 Sulfadiazine  2	E7 Sulfadiazine  3	E8 Sulfadiazine  4	E9 Benzethonium chloride  1	E10 Benzethonium chloride  2	E11 Benzethonium chloride  3	E12 Benzethonium chloride  4
F1 Tobramycin  1	F2 Tobramycin  2	F3 Tobramycin  3	F4 Tobramycin  4	F5 Sulfathiazole  1	F6 Sulfathiazole  2	F7 Sulfathiazole  3	F8 Sulfathiazole  4	F9 5-Fluoroorotic acid  1	F10 5-Fluoroorotic acid  2	F11 5-Fluoroorotic acid  3	F12 5-Fluoroorotic acid  4
G1 Spectinomycin  1	G2 Spectinomycin  2	G3 Spectinomycin  3	G4 Spectinomycin  4	G5 Sulfa-methoxazole  1	G6 Sulfa-methoxazole  2	G7 Sulfa-methoxazole  3	G8 Sulfa-methoxazole  4	G9 L-Aspartic-β-hydroxamate  1	G10 L-Aspartic-β-hydroxamate  2	G11 L-Aspartic-β-hydroxamate  3	G12 L-Aspartic-β-hydroxamate  4
H1 Spiramycin  1	H2 Spiramycin  2	H3 Spiramycin  3	H4 Spiramycin  4	H5 Rifampicin  1	H6 Rifampicin  2	H7 Rifampicin  3	H8 Rifampicin  4	H9 Dodecyltrimethyl ammonium bromide  1	H10 Dodecyltrimethyl ammonium bromide  2	H11 Dodecyltrimethyl ammonium bromide  3	H12 Dodecyltrimethyl ammonium bromide  4

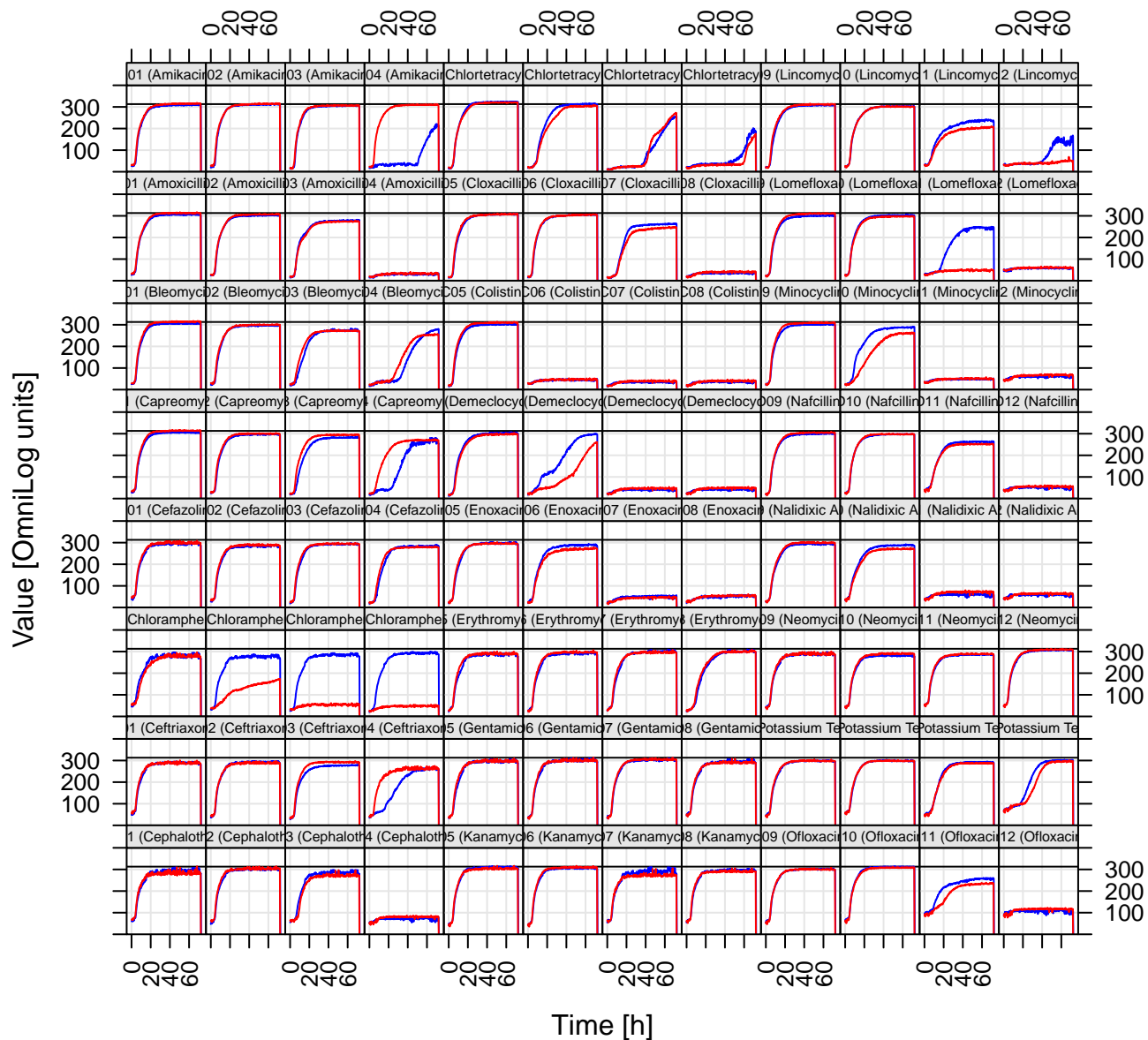
# PM11 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



# PM11 (Chemicals)

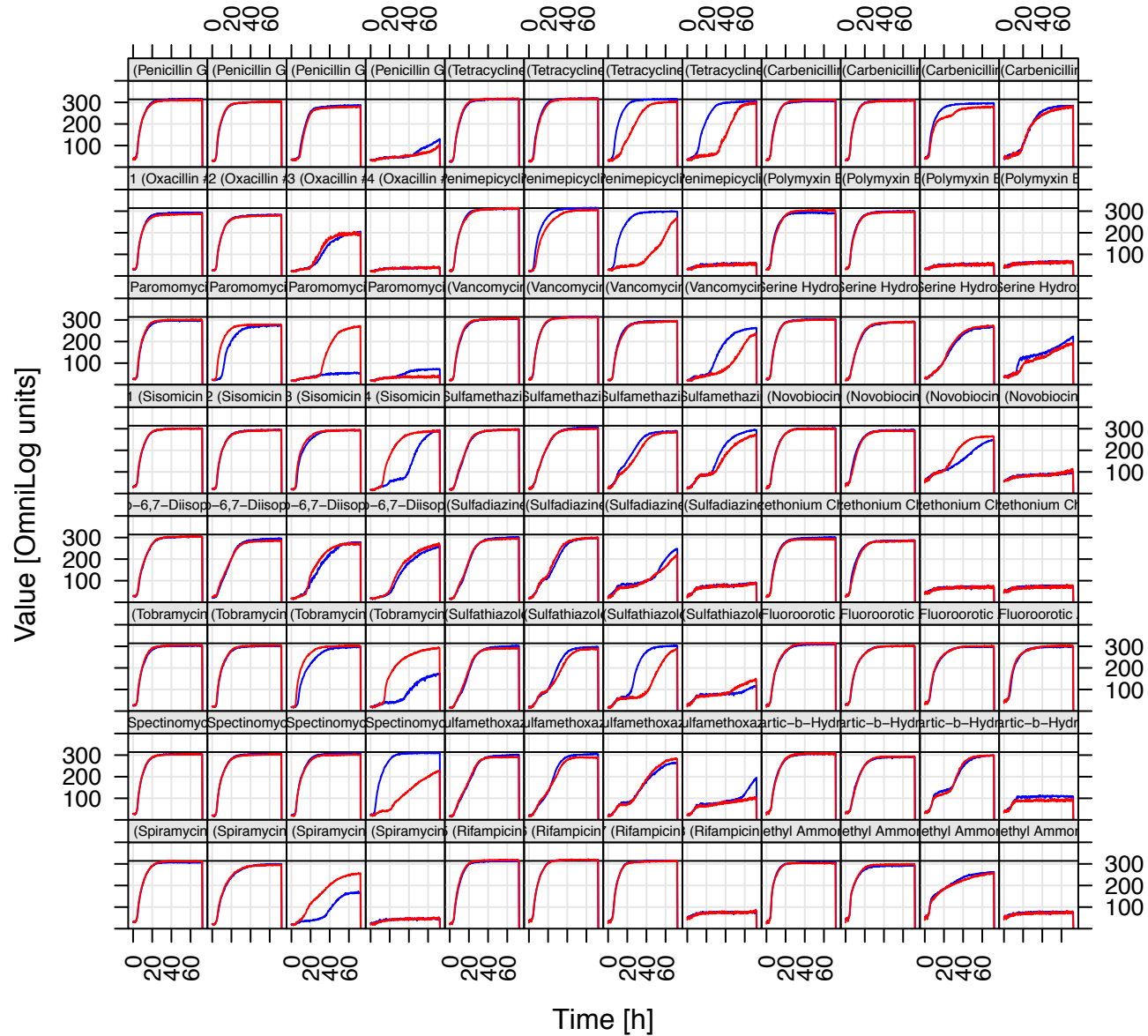
$\Delta$ SPFH-1  
WT-1





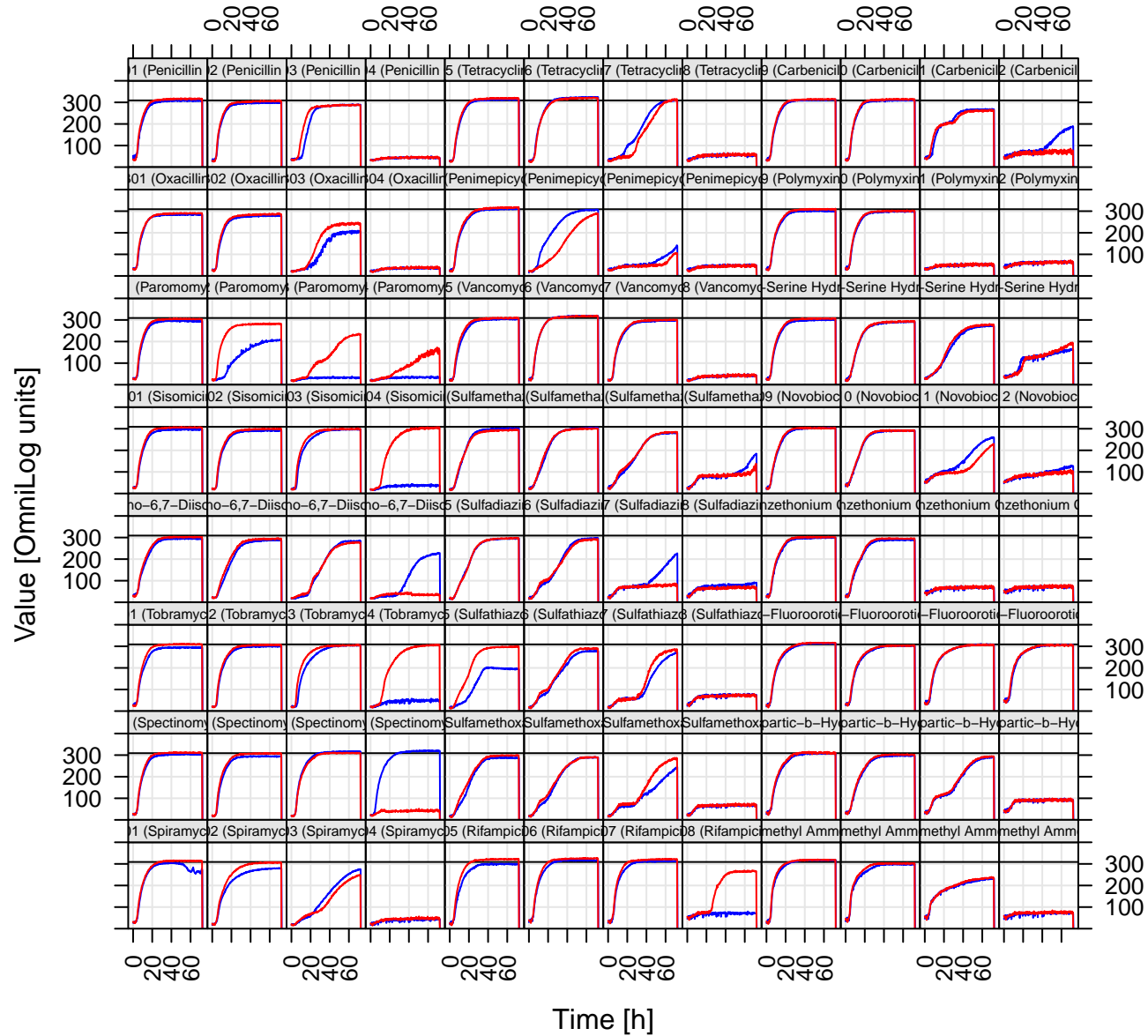
# PM12 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



# PM12 (Chemicals)

$\Delta$ SPFH-1  
WT-1



**PM13B MicroPlate™**

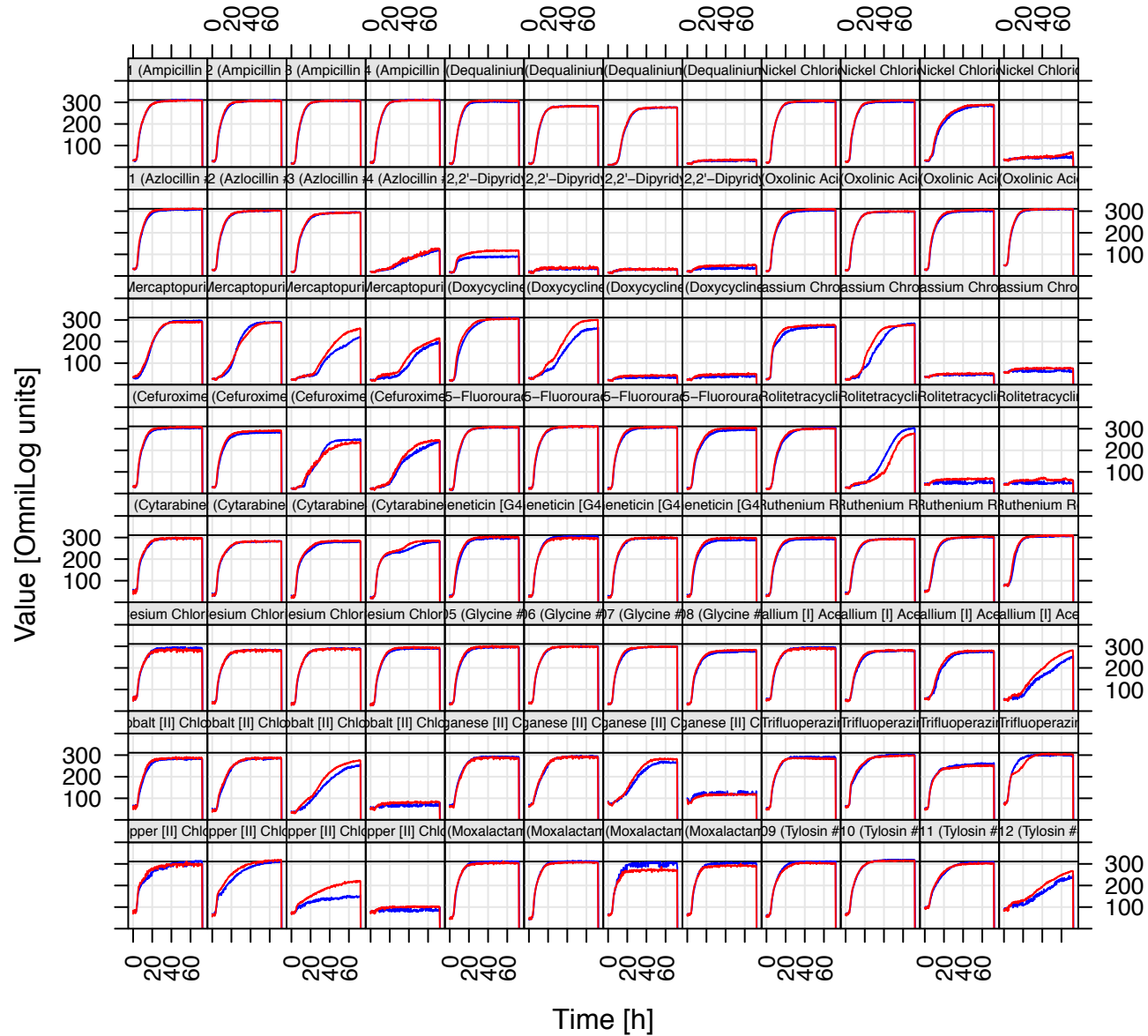
A1 Ampicillin  1	A2 Ampicillin  2	A3 Ampicillin  3	A4 Ampicillin  4	A5 Dequalinium chloride  1	A6 Dequalinium chloride  2	A7 Dequalinium chloride  3	A8 Dequalinium chloride  4	A9 Nickel chloride  1	A10 Nickel chloride  2	A11 Nickel chloride  3	A12 Nickel chloride  4
B1 Azlocillin  1	B2 Azlocillin  2	B3 Azlocillin  3	B4 Azlocillin  4	B5 2, 2'-Dipyridyl  1	B6 2, 2'-Dipyridyl  2	B7 2, 2'-Dipyridyl  3	B8 2, 2'-Dipyridyl  4	B9 Oxolinic acid  1	B10 Oxolinic acid  2	B11 Oxolinic acid  3	B12 Oxolinic acid  4
C1 6-Mercapto- purine  1	C2 6-Mercapto- purine  2	C3 6-Mercapto- purine  3	C4 6-Mercapto- purine  4	C5 Doxycycline  1	C6 Doxycycline  2	C7 Doxycycline  3	C8 Doxycycline  4	C9 Potassium chromate  1	C10 Potassium chromate  2	C11 Potassium chromate  3	C12 Potassium chromate  4
D1 Cefuroxime  1	D2 Cefuroxime  2	D3 Cefuroxime  3	D4 Cefuroxime  4	D5 5-Fluorouracil  1	D6 5-Fluorouracil  2	D7 5-Fluorouracil  3	D8 5-Fluorouracil  4	D9 Rolitetracycline  1	D10 Rolitetracycline  2	D11 Rolitetracycline  3	D12 Rolitetracycline  4
E1 Cytosine-1-beta- D-arabino- furanoside  1	E2 Cytosine-1-beta- D-arabino- furanoside  2	E3 Cytosine-1-beta- D-arabino- furanoside  3	E4 Cytosine-1-beta- D-arabino- furanoside  4	E5 Geneticin (G418)  1	E6 Geneticin (G418)  2	E7 Geneticin (G418)  3	E8 Geneticin (G418)  4	E9 Ruthenium red  1	E10 Ruthenium red  2	E11 Ruthenium red  3	E12 Ruthenium red  4
F1 Cesium chloride  1	F2 Cesium chloride  2	F3 Cesium chloride  3	F4 Cesium chloride  4	F5 Glycine  1	F6 Glycine  2	F7 Glycine  3	F8 Glycine  4	F9 Thallium (I) acetate  1	F10 Thallium (I) acetate  2	F11 Thallium (I) acetate  3	F12 Thallium (I) acetate  4
G1 Cobalt chloride  1	G2 Cobalt chloride  2	G3 Cobalt chloride  3	G4 Cobalt chloride  4	G5 Manganese chloride  1	G6 Manganese chloride  2	G7 Manganese chloride  3	G8 Manganese chloride  4	G9 Trifluoperazine  1	G10 Trifluoperazine  2	G11 Trifluoperazine  3	G12 Trifluoperazine  4
H1 Cupric chloride  1	H2 Cupric chloride  2	H3 Cupric chloride  3	H4 Cupric chloride  4	H5 Moxalactam  1	H6 Moxalactam  2	H7 Moxalactam  3	H8 Moxalactam  4	H9 Tylosin  1	H10 Tylosin  2	H11 Tylosin  3	H12 Tylosin  4

**PM14A MicroPlate™**

A1 Acriflavine  1	A2 Acriflavine  2	A3 Acriflavine  3	A4 Acriflavine  4	A5 Furaltadone  1	A6 Furaltadone  2	A7 Furaltadone  3	A8 Furaltadone  4	A9 Sanguinarine  1	A10 Sanguinarine  2	A11 Sanguinarine  3	A12 Sanguinarine  4
B1 9-Aminoacridine  1	B2 9-Aminoacridine  2	B3 9-Aminoacridine  3	B4 9-Aminoacridine  4	B5 Fusaric acid  1	B6 Fusaric acid  2	B7 Fusaric acid  3	B8 Fusaric acid  4	B9 Sodium arsenate  1	B10 Sodium arsenate  2	B11 Sodium arsenate  3	B12 Sodium arsenate  4
C1 Boric Acid  1	C2 Boric Acid  2	C3 Boric Acid  3	C4 Boric Acid  4	C5 1-Hydroxy- pyridine -2- thione  1	C6 1-Hydroxy- pyridine -2- thione  2	C7 1-Hydroxy- pyridine -2- thione  3	C8 1-Hydroxy- pyridine -2- thione  4	C9 Sodium cyanate  1	C10 Sodium cyanate  2	C11 Sodium cyanate  3	C12 Sodium cyanate  4
D1 Cadmium chloride  1	D2 Cadmium chloride  2	D3 Cadmium chloride  3	D4 Cadmium chloride  4	D5 Iodoacetate  1	D6 Iodoacetate  2	D7 Iodoacetate  3	D8 Iodoacetate  4	D9 Sodium dichromate  1	D10 Sodium dichromate  2	D11 Sodium dichromate  3	D12 Sodium dichromate  4
E1 Cefoxitin  1	E2 Cefoxitin  2	E3 Cefoxitin  3	E4 Cefoxitin  4	E5 Nitrofurantoin  1	E6 Nitrofurantoin  2	E7 Nitrofurantoin  3	E8 Nitrofurantoin  4	E9 Sodium metaborate  1	E10 Sodium metaborate  2	E11 Sodium metaborate  3	E12 Sodium metaborate  4
F1 Chloramphenicol  1	F2 Chloramphenicol  2	F3 Chloramphenicol  3	F4 Chloramphenicol  4	F5 Piperacillin  1	F6 Piperacillin  2	F7 Piperacillin  3	F8 Piperacillin  4	F9 Sodium metavanadate  1	F10 Sodium metavanadate  2	F11 Sodium metavanadate  3	F12 Sodium metavanadate  4
G1 Chelerythrine  1	G2 Chelerythrine  2	G3 Chelerythrine  3	G4 Chelerythrine  4	G5 Carbenicillin  1	G6 Carbenicillin  2	G7 Carbenicillin  3	G8 Carbenicillin  4	G9 Sodium nitrite  1	G10 Sodium nitrite  2	G11 Sodium nitrite  3	G12 Sodium nitrite  4
H1 EGTA  1	H2 EGTA  2	H3 EGTA  3	H4 EGTA  4	H5 Promethazine  1	H6 Promethazine  2	H7 Promethazine  3	H8 Promethazine  4	H9 Sodium orthovanadate  1	H10 Sodium orthovanadate  2	H11 Sodium orthovanadate  3	H12 Sodium orthovanadate  4

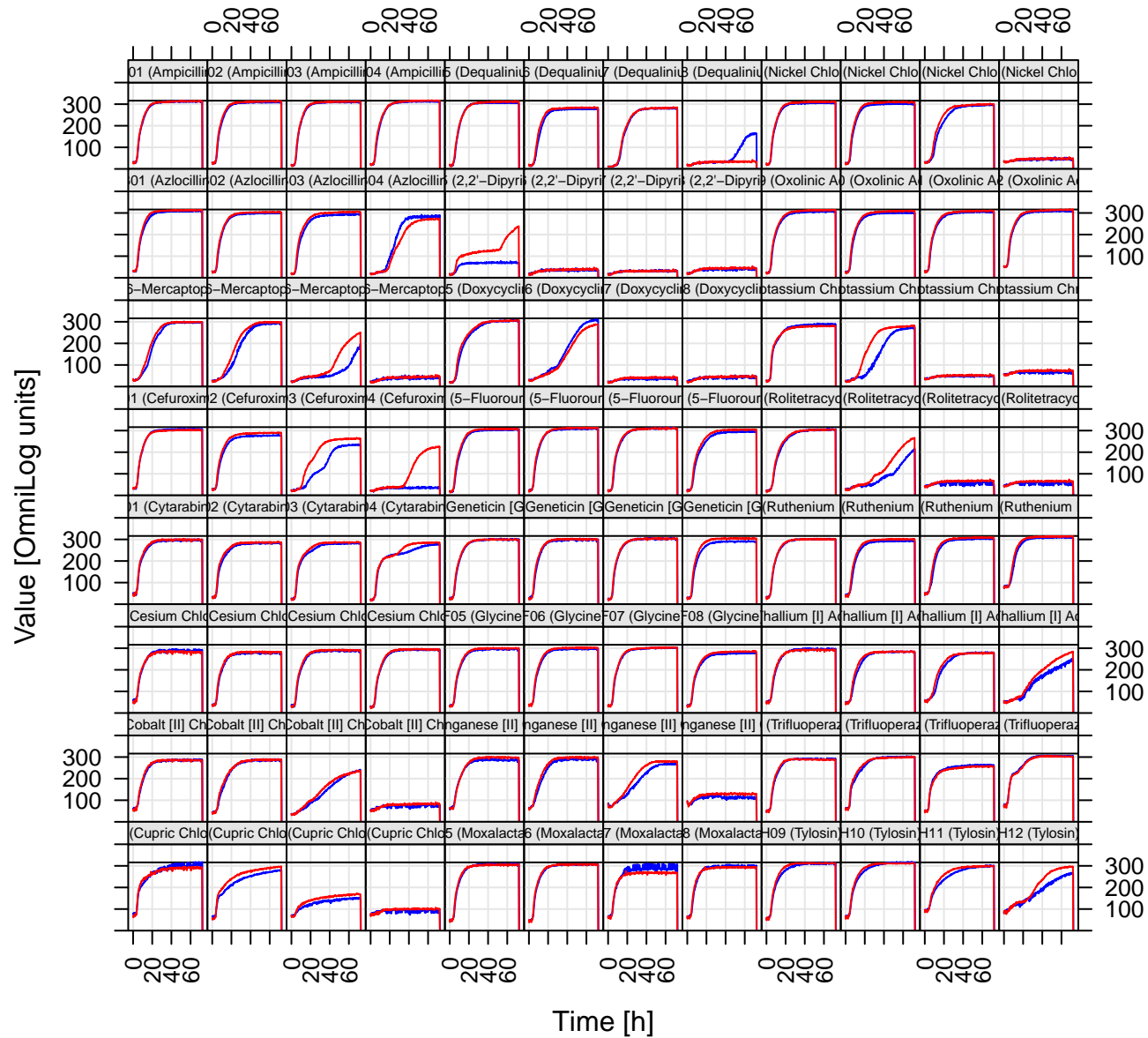
# PM13 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



# PM13 (Chemicals)

ΔSPFH-1  
WT-1







**PM15B MicroPlate™**

A1 Procaine  1	A2 Procaine  2	A3 Procaine  3	A4 Procaine  4	A5 Guanidine hydrochloride  1	A6 Guanidine hydrochloride  2	A7 Guanidine hydrochloride  3	A8 Guanidine hydrochloride  4	A9 Cefmetazole  1	A10 Cefmetazole  2	A11 Cefmetazole  3	A12 Cefmetazole  4
B1 D-Cycloserine  1	B2 D-Cycloserine  2	B3 D-Cycloserine  3	B4 D-Cycloserine  4	B5 EDTA  1	B6 EDTA  2	B7 EDTA  3	B8 EDTA  4	B9 5,7-Dichloro-8-hydroxyquinoline  1	B10 5,7-Dichloro-8-hydroxyquinoline  2	B11 5,7-Dichloro-8-hydroxyquinoline  3	B12 5,7-Dichloro-8-hydroxyquinoline  4
C1 5,7-Dichloro-8-hydroxyquinoline  1	C2 5,7-Dichloro-8-hydroxyquinoline  2	C3 5,7-Dichloro-8-hydroxyquinoline  3	C4 5,7-Dichloro-8-hydroxyquinoline  4	C5 Fusidic acid  1	C6 Fusidic acid  2	C7 Fusidic acid  3	C8 Fusidic acid  4	C9 1,10-Phenanthroline  1	C10 1,10-Phenanthroline  2	C11 1,10-Phenanthroline  3	C12 1,10-Phenanthroline  4
D1 Phleomycin  1	D2 Phleomycin  2	D3 Phleomycin  3	D4 Phleomycin  4	D5 Domiphen bromide  1	D6 Domiphen bromide  2	D7 Domiphen bromide  3	D8 Domiphen bromide  4	D9 Nordihydroguaiaretic acid  1	D10 Nordihydroguaiaretic acid  2	D11 Nordihydroguaiaretic acid  3	D12 Nordihydroguaiaretic acid  4
E1 Alexidine  1	E2 Alexidine  2	E3 Alexidine  3	E4 Alexidine  4	E5 5-Nitro-2-furaldehyde semicarbazone  1	E6 5-Nitro-2-furaldehyde semicarbazone  2	E7 5-Nitro-2-furaldehyde semicarbazone  3	E8 5-Nitro-2-furaldehyde semicarbazone  4	E9 Methyl viologen  1	E10 Methyl viologen  2	E11 Methyl viologen  3	E12 Methyl viologen  4
F1 3,4-Dimethoxybenzyl alcohol  1	F2 3,4-Dimethoxybenzyl alcohol  2	F3 3,4-Dimethoxybenzyl alcohol  3	F4 3,4-Dimethoxybenzyl alcohol  4	F5 Oleandomycin  1	F6 Oleandomycin  2	F7 Oleandomycin  3	F8 Oleandomycin  4	F9 Puromycin  1	F10 Puromycin  2	F11 Puromycin  3	F12 Puromycin  4
G1 CCCP  1	G2 CCCP  2	G3 CCCP  3	G4 CCCP  4	G5 Sodium azide  1	G6 Sodium azide  2	G7 Sodium azide  3	G8 Sodium azide  4	G9 Menadione  1	G10 Menadione  2	G11 Menadione  3	G12 Menadione  4
H1 2-Nitroimidazole  1	H2 2-Nitroimidazole  2	H3 2-Nitroimidazole  3	H4 2-Nitroimidazole  4	H5 Hydroxyurea  1	H6 Hydroxyurea  2	H7 Hydroxyurea  3	H8 Hydroxyurea  4	H9 Zinc chloride  1	H10 Zinc chloride  2	H11 Zinc chloride  3	H12 Zinc chloride  4

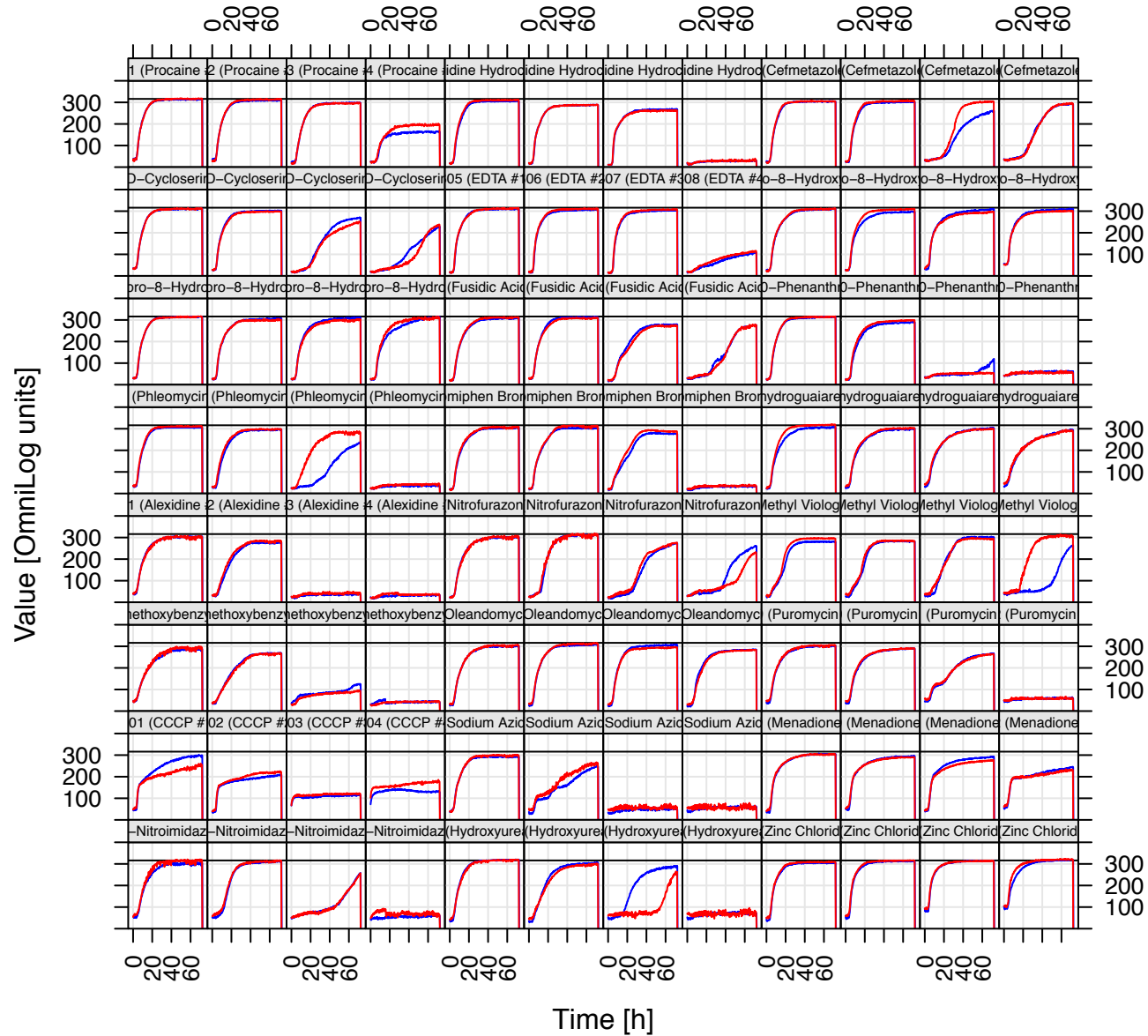
**PM16A MicroPlate™**

A1 Cefotaxime  1	A2 Cefotaxime  2	A3 Cefotaxime  3	A4 Cefotaxime  4	A5 Phosphomycin  1	A6 Phosphomycin  2	A7 Phosphomycin  3	A8 Phosphomycin  4	A9 5-Chloro-7-iodo-8-hydroxyquinoline  1	A10 5-Chloro-7-iodo-8-hydroxyquinoline  2	A11 5-Chloro-7-iodo-8-hydroxyquinoline  3	A12 5-Chloro-7-iodo-8-hydroxyquinoline  4
B1 Norfloxacin  1	B2 Norfloxacin  2	B3 Norfloxacin  3	B4 Norfloxacin  4	B5 Sulfanilamide  1	B6 Sulfanilamide  2	B7 Sulfanilamide  3	B8 Sulfanilamide  4	B9 Trimethoprim  1	B10 Trimethoprim  2	B11 Trimethoprim  3	B12 Trimethoprim  4
C1 Dichlofluamid  1	C2 Dichlofluamid  2	C3 Dichlofluamid  3	C4 Dichlofluamid  4	C5 Protamine sulfate  1	C6 Protamine sulfate  2	C7 Protamine sulfate  3	C8 Protamine sulfate  4	C9 Cetylpyridinium chloride  1	C10 Cetylpyridinium chloride  2	C11 Cetylpyridinium chloride  3	C12 Cetylpyridinium chloride  4
D1 1-Chloro -2,4-dinitrobenzene  1	D2 1-Chloro -2,4-dinitrobenzene  2	D3 1-Chloro -2,4-dinitrobenzene  3	D4 1-Chloro -2,4-dinitrobenzene  4	D5 Diamide  1	D6 Diamide  2	D7 Diamide  3	D8 Diamide  4	D9 Cinoxacin  1	D10 Cinoxacin  2	D11 Cinoxacin  3	D12 Cinoxacin  4
E1 Streptomycin  1	E2 Streptomycin  2	E3 Streptomycin  3	E4 Streptomycin  4	E5 5-Azacytidine  1	E6 5-Azacytidine  2	E7 5-Azacytidine  3	E8 5-Azacytidine  4	E9 Rifamycin SV  1	E10 Rifamycin SV  2	E11 Rifamycin SV  3	E12 Rifamycin SV  4
F1 Potassium tellurite  1	F2 Potassium tellurite  2	F3 Potassium tellurite  3	F4 Potassium tellurite  4	F5 Sodium selenite  1	F6 Sodium selenite  2	F7 Sodium selenite  3	F8 Sodium selenite  4	F9 Aluminum sulfate  1	F10 Aluminum sulfate  2	F11 Aluminum sulfate  3	F12 Aluminum sulfate  4
G1 Chromium chloride  1	G2 Chromium chloride  2	G3 Chromium chloride  3	G4 Chromium chloride  4	G5 Ferric chloride  1	G6 Ferric chloride  2	G7 Ferric chloride  3	G8 Ferric chloride  4	G9 L-Glutamic-g-hydroxamate  1	G10 L-Glutamic-g-hydroxamate  2	G11 L-Glutamic-g-hydroxamate  3	G12 L-Glutamic-g-hydroxamate  4
H1 Glycine hydroxamate  1	H2 Glycine hydroxamate  2	H3 Glycine hydroxamate  3	H4 Glycine hydroxamate  4	H5 Chloroxylenol  1	H6 Chloroxylenol  2	H7 Chloroxylenol  3	H8 Chloroxylenol  4	H9 Sorbic acid  1	H10 Sorbic acid  2	H11 Sorbic acid  3	H12 Sorbic acid  4



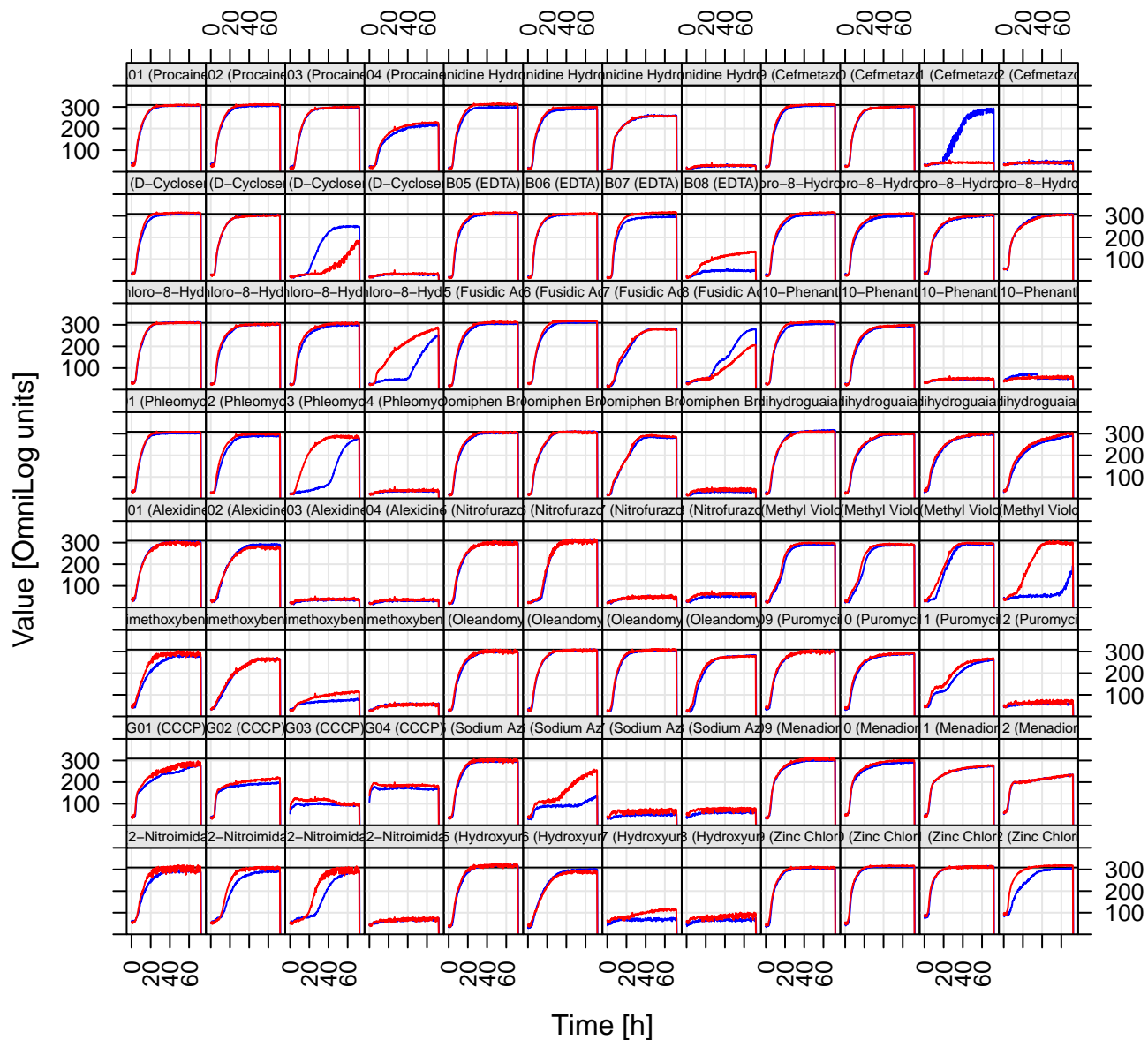
# PM15 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



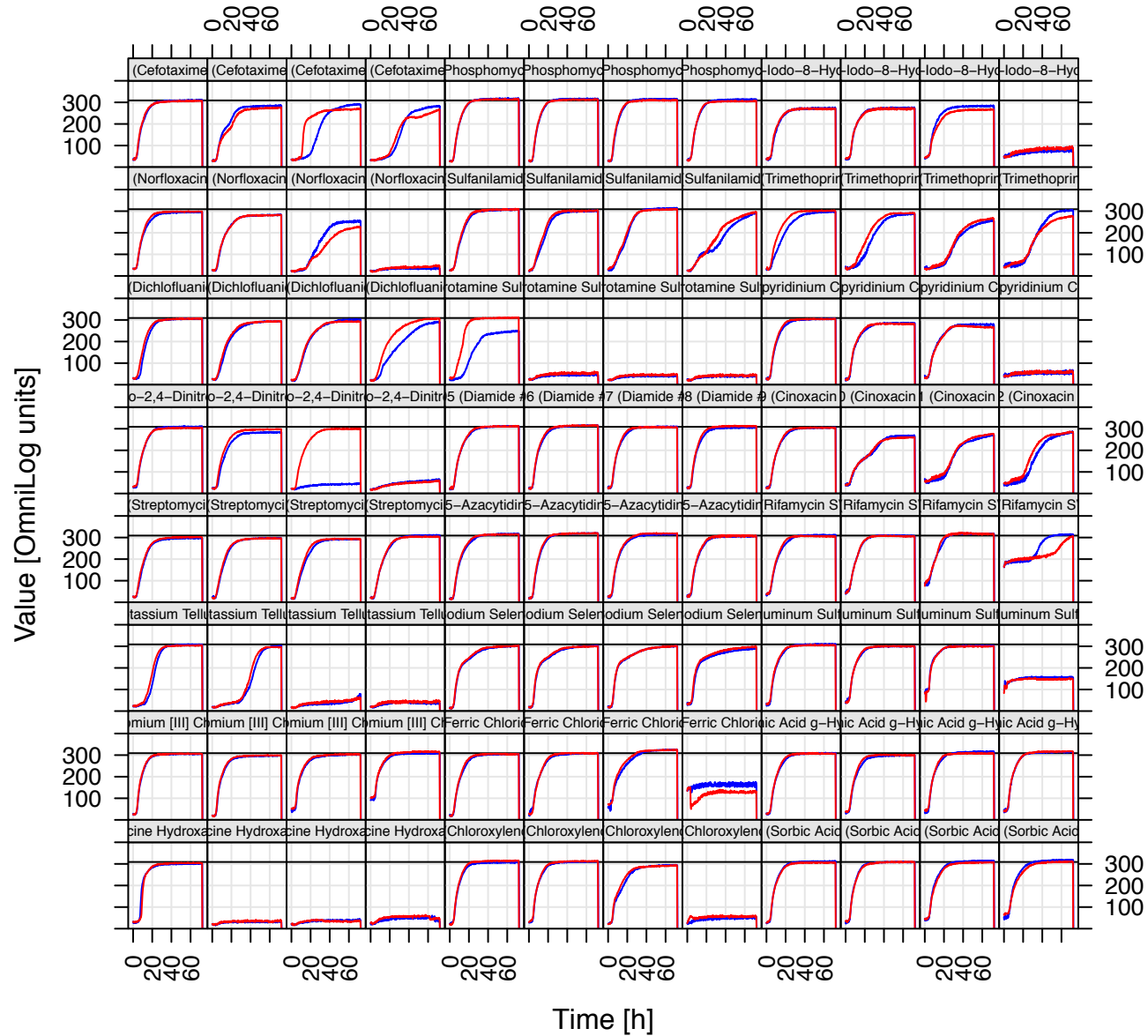
# PM15 (Chemicals)

$\Delta$ SPFH-1  
WT-1



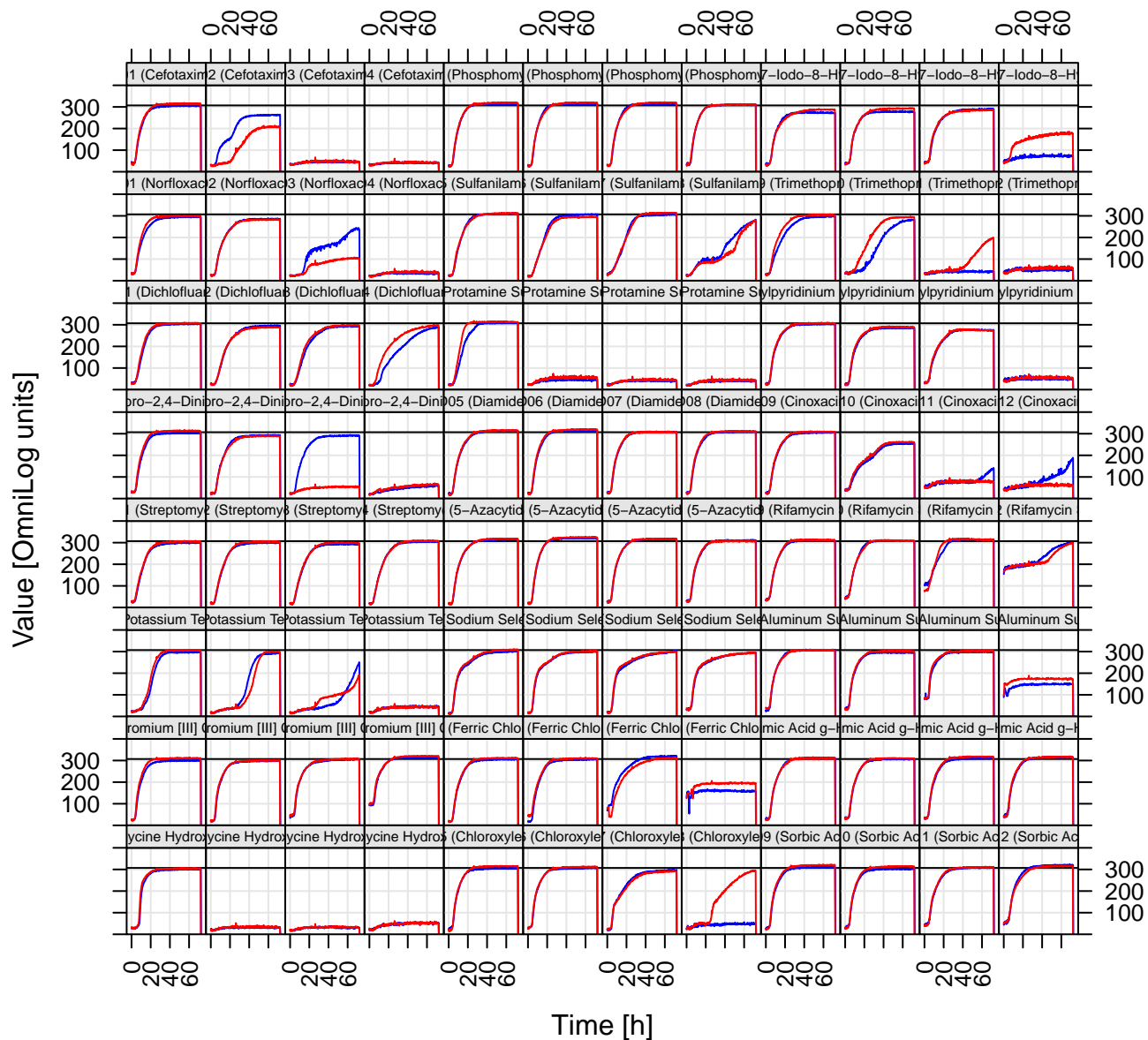
# PM16 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



# PM16 (Chemicals)

ΔSPFH-1  
WT-1



**PM17A MicroPlate™**

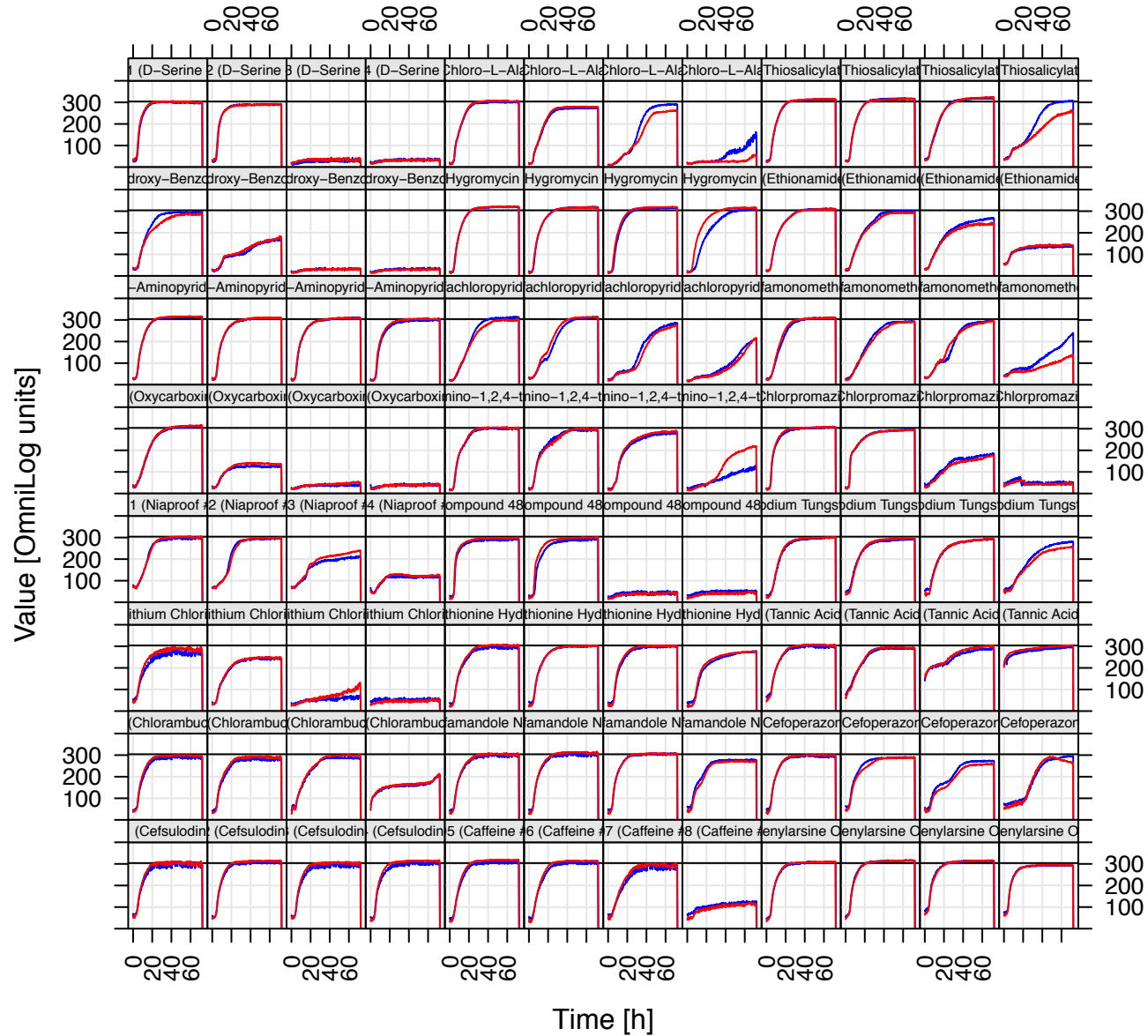
A1 D-Serine  1	A2 D-Serine  2	A3 D-Serine  3	A4 D-Serine  4	A5 β-Chloro-L-alanine hydrochloride  1	A6 β-Chloro-L-alanine hydrochloride  2	A7 β-Chloro-L-alanine hydrochloride  3	A8 β-Chloro-L-alanine hydrochloride  4	A9 Thiosalicylic acid  1	A10 Thiosalicylic acid  2	A11 Thiosalicylic acid  3	A12 Thiosalicylic acid  4
B1 Sodium salicylate  1	B2 Sodium salicylate  2	B3 Sodium salicylate  3	B4 Sodium salicylate  4	B5 Hygromycin B  1	B6 Hygromycin B  2	B7 Hygromycin B  3	B8 Hygromycin B  4	B9 Ethionamide  1	B10 Ethionamide  2	B11 Ethionamide  3	B12 Ethionamide  4
C1 4-Aminopyridine  1	C2 4-Aminopyridine  2	C3 4-Aminopyridine  3	C4 4-Aminopyridine  4	C5 Sulfachloro-pyridazine  1	C6 Sulfachloro-pyridazine  2	C7 Sulfachloro-pyridazine  3	C8 Sulfachloro-pyridazine  4	C9 Sulfamonomethoxine  1	C10 Sulfamonomethoxine  2	C11 Sulfamonomethoxine  3	C12 Sulfamonomethoxine  4
D1 Oxycarboxin  1	D2 Oxycarboxin  2	D3 Oxycarboxin  3	D4 Oxycarboxin  4	D5 3-Amino-1,2,4-triazole  1	D6 3-Amino-1,2,4-triazole  2	D7 3-Amino-1,2,4-triazole  3	D8 3-Amino-1,2,4-triazole  4	D9 Chlorpromazine  1	D10 Chlorpromazine  2	D11 Chlorpromazine  3	D12 Chlorpromazine  4
E1 Niaproof  1	E2 Niaproof  2	E3 Niaproof  3	E4 Niaproof  4	E5 Compound 48/80  1	E6 Compound 48/80  2	E7 Compound 48/80  3	E8 Compound 48/80  4	E9 Sodium tungstate  1	E10 Sodium tungstate  2	E11 Sodium tungstate  3	E12 Sodium tungstate  4
F1 Lithium chloride  1	F2 Lithium chloride  2	F3 Lithium chloride  3	F4 Lithium chloride  4	F5 DL-Methionine hydroxamate  1	F6 DL-Methionine hydroxamate  2	F7 DL-Methionine hydroxamate  3	F8 DL-Methionine hydroxamate  4	F9 Tannic acid  1	F10 Tannic acid  2	F11 Tannic acid  3	F12 Tannic acid  4
G1 Chlorambucil  1	G2 Chlorambucil  2	G3 Chlorambucil  3	G4 Chlorambucil  4	G5 Cefamandole nafate  1	G6 Cefamandole nafate  2	G7 Cefamandole nafate  3	G8 Cefamandole nafate  4	G9 Cefoperazone  1	G10 Cefoperazone  2	G11 Cefoperazone  3	G12 Cefoperazone  4
H1 Cefsulodin  1	H2 Cefsulodin  2	H3 Cefsulodin  3	H4 Cefsulodin  4	H5 Caffeine  1	H6 Caffeine  2	H7 Caffeine  3	H8 Caffeine  4	H9 Phenylarsine oxide  1	H10 Phenylarsine oxide  2	H11 Phenylarsine oxide  3	H12 Phenylarsine oxide  4

**PM18C MicroPlate™**

A1 Ketoprofen  1	A2 Ketoprofen  2	A3 Ketoprofen  3	A4 Ketoprofen  4	A5 Sodium pyrophosphate decahydrate  1	A6 Sodium pyrophosphate decahydrate  2	A7 Sodium pyrophosphate decahydrate  3	A8 Sodium pyrophosphate decahydrate  4	A9 Thiamphenicol  1	A10 Thiamphenicol  2	A11 Thiamphenicol  3	A12 Thiamphenicol  4
B1 Trifluorothymidine  1	B2 Trifluorothymidine  2	B3 Trifluorothymidine  3	B4 Trifluorothymidine  4	B5 Pipemidic Acid  1	B6 Pipemidic Acid  2	B7 Pipemidic Acid  3	B8 Pipemidic Acid  4	B9 Azathioprine  1	B10 Azathioprine  2	B11 Azathioprine  3	B12 Azathioprine  4
C1 Poly-L-lysine  1	C2 Poly-L-lysine  2	C3 Poly-L-lysine  3	C4 Poly-L-lysine  4	C5 Sulfisoxazole  1	C6 Sulfisoxazole  2	C7 Sulfisoxazole  3	C8 Sulfisoxazole  4	C9 Pentachlorophenol  1	C10 Pentachlorophenol  2	C11 Pentachlorophenol  3	C12 Pentachlorophenol  4
D1 Sodium m-arsenite  1	D2 Sodium m-arsenite  2	D3 Sodium m-arsenite  3	D4 Sodium m-arsenite  4	D5 Sodium bromate  1	D6 Sodium bromate  2	D7 Sodium bromate  3	D8 Sodium bromate  4	D9 Lidocane  1	D10 Lidocane  2	D11 Lidocane  3	D12 Lidocane  4
E1 Sodium metasilicate  1	E2 Sodium metasilicate  2	E3 Sodium metasilicate  3	E4 Sodium metasilicate  4	E5 Sodium m-periodate  1	E6 Sodium m-periodate  2	E7 Sodium m-periodate  3	E8 Sodium m-periodate  4	E9 Antimony (III) chloride  1	E10 Antimony (III) chloride  2	E11 Antimony (III) chloride  3	E12 Antimony (III) chloride  4
F1 Semicarbazide  1	F2 Semicarbazide  2	F3 Semicarbazide  3	F4 Semicarbazide  4	F5 Tinidazole  1	F6 Tinidazole  2	F7 Tinidazole  3	F8 Tinidazole  4	F9 Aztreonam  1	F10 Aztreonam  2	F11 Aztreonam  3	F12 Aztreonam  4
G1 Triclosan  1	G2 Triclosan  2	G3 Triclosan  3	G4 Triclosan  4	G5 3,5-Diamino-1,2,4-triazole (Guanazole)  1	G6 3,5-Diamino-1,2,4-triazole (Guanazole)  2	G7 3,5-Diamino-1,2,4-triazole (Guanazole)  3	G8 3,5-Diamino-1,2,4-triazole (Guanazole)  4	G9 Myricetin  1	G10 Myricetin  2	G11 Myricetin  3	G12 Myricetin  4
H1 5-fluoro-5'-deoxyuridine  1	H2 5-fluoro-5'-deoxyuridine  2	H3 5-fluoro-5'-deoxyuridine  3	H4 5-fluoro-5'-deoxyuridine  4	H5 2-Phenylphenol  1	H6 2-Phenylphenol  2	H7 2-Phenylphenol  3	H8 2-Phenylphenol  4	H9 Plumbagin  1	H10 Plumbagin  2	H11 Plumbagin  3	H12 Plumbagin  4

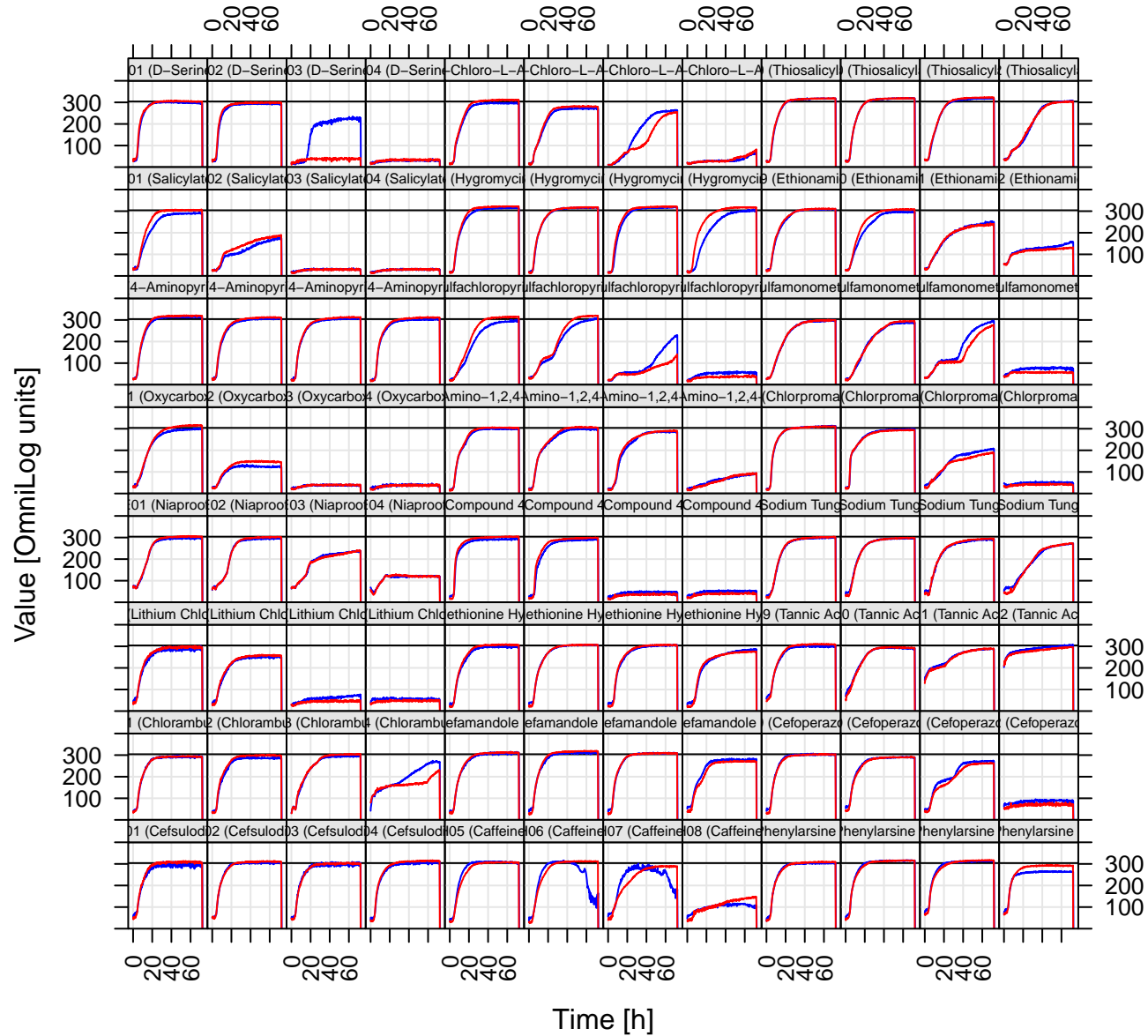
# PM17 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



# PM17 (Chemicals)

$\Delta$ SPFH-1  
WT-1

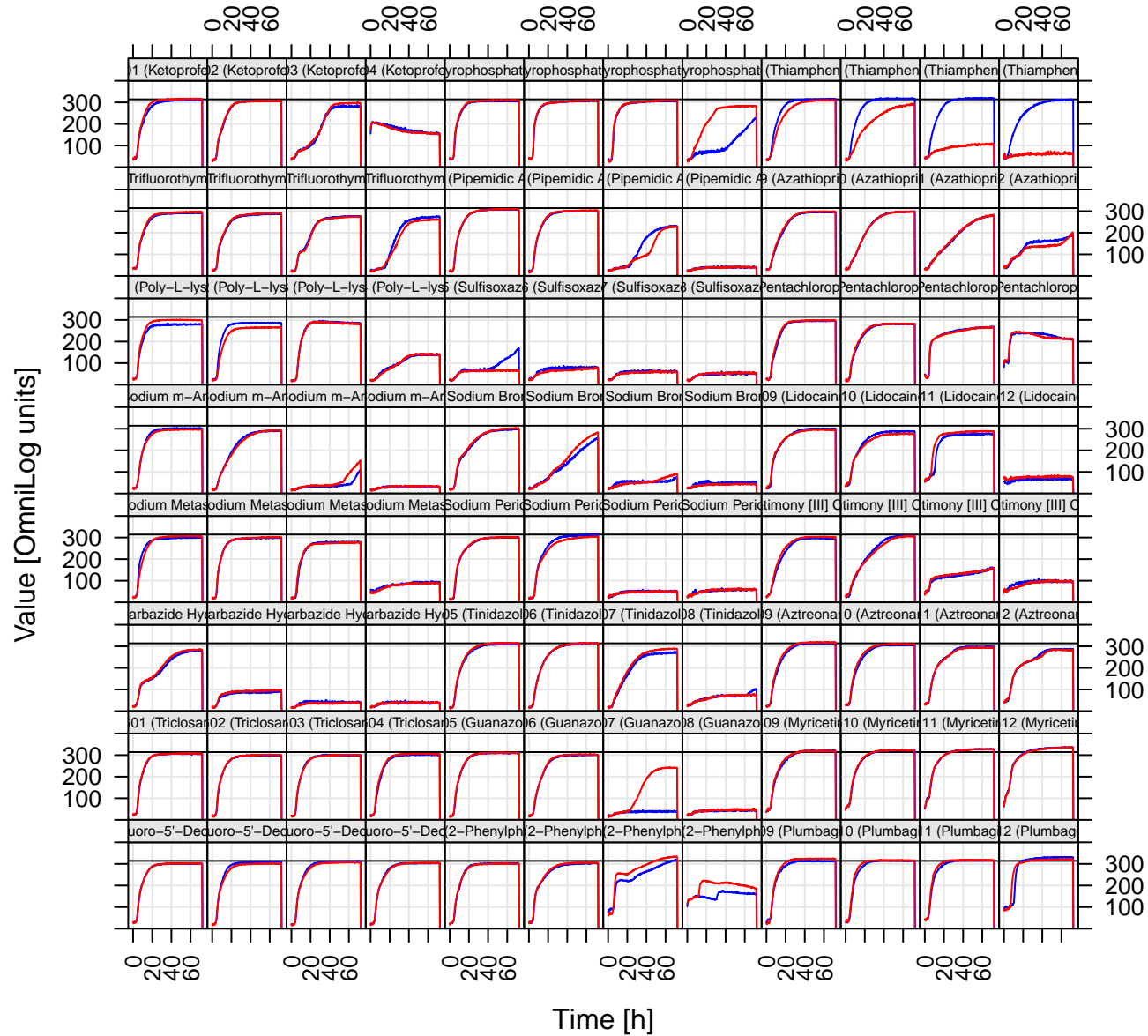






# PM18 (Chemicals)

ΔSPFH-1  
WT-1



**PM19 MicroPlate™**

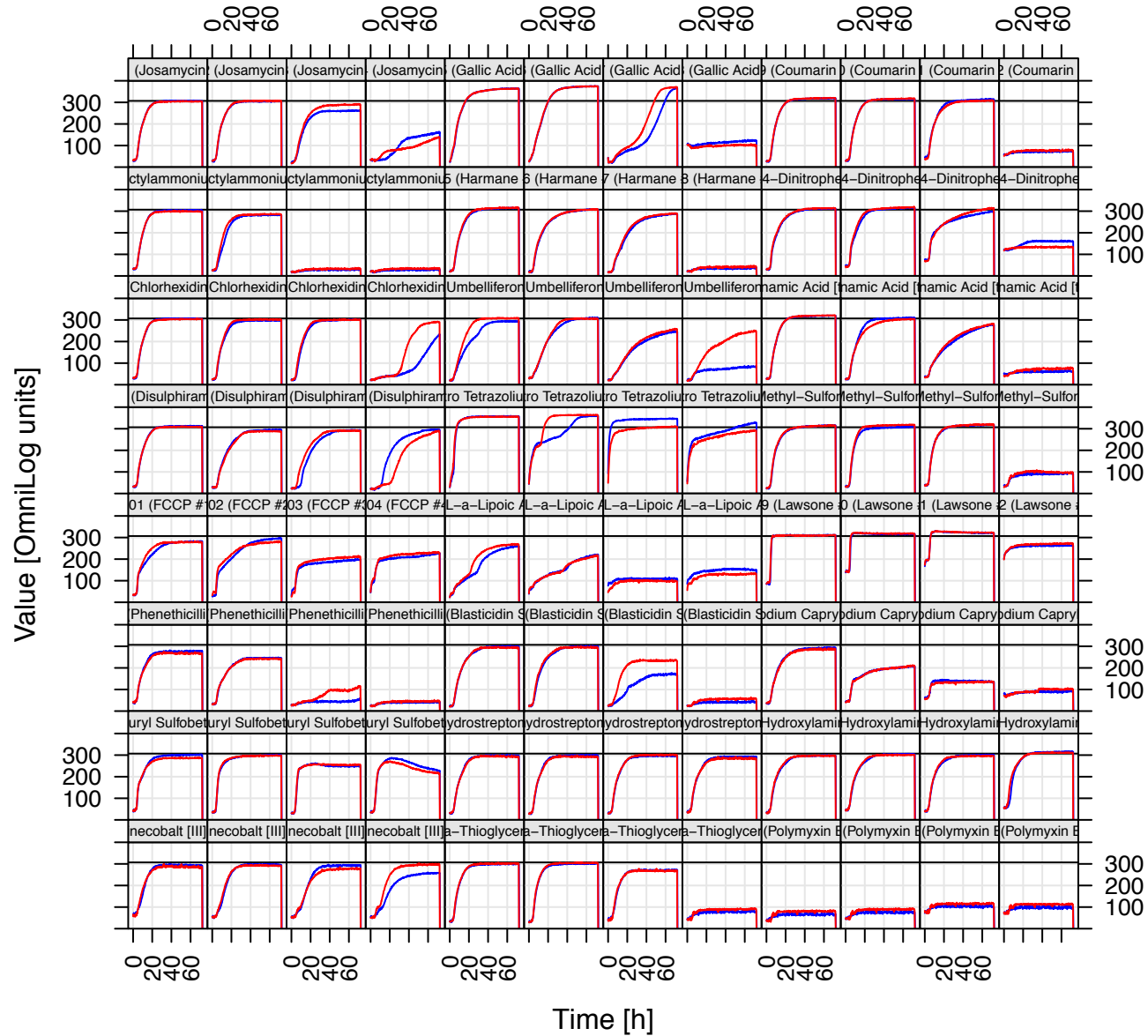
A1 Josamycin  1	A2 Josamycin  2	A3 Josamycin  3	A4 Josamycin  4	A5 Gallic acid  1	A6 Gallic acid  2	A7 Gallic acid  3	A8 Gallic acid  4	A9 Coumarin  1	A10 Coumarin  2	A11 Coumarin  3	A12 Coumarin  4
B1 Methyltriocetyl- ammonium chloride  1	B2 Methyltriocetyl- ammonium chloride  2	B3 Methyltriocetyl- ammonium chloride  3	B4 Methyltriocetyl- ammonium chloride  4	B5 Harmene  1	B6 Harmene  2	B7 Harmene  3	B8 Harmene  4	B9 2,4-Dinitrophenol  1	B10 2,4-Dinitrophenol  2	B11 2,4-Dinitrophenol  3	B12 2,4-Dinitrophenol  4
C1 Chlorhexidine  1	C2 Chlorhexidine  2	C3 Chlorhexidine  3	C4 Chlorhexidine  4	C5 Umbelliferone  1	C6 Umbelliferone  2	C7 Umbelliferone  3	C8 Umbelliferone  4	C9 Cinnamic acid  1	C10 Cinnamic acid  2	C11 Cinnamic acid  3	C12 Cinnamic acid  4
D1 Disulphiram  1	D2 Disulphiram  2	D3 Disulphiram  3	D4 Disulphiram  4	D5 Iodonitro Tetrazolium Violet  1	D6 Iodonitro Tetrazolium Violet  2	D7 Iodonitro Tetrazolium Violet  3	D8 Iodonitro Tetrazolium Violet  4	D9 Phenyl- methyl- sulfonyl- fluoride (PMSF)  1	D10 Phenyl- methyl- sulfonyl- fluoride (PMSF)  2	D11 Phenyl- methyl- sulfonyl- fluoride (PMSF)  3	D12 Phenyl- methyl- sulfonyl- fluoride (PMSF)  4
E1 FCCP  1	E2 FCCP  2	E3 FCCP  3	E4 FCCP  4	E5 D,L-Thioctic Acid  1	E6 D,L-Thioctic Acid  2	E7 D,L-Thioctic Acid  3	E8 D,L-Thioctic Acid  4	E9 Lawsone  1	E10 Lawsone  2	E11 Lawsone  3	E12 Lawsone  4
F1 Phenethicillin  1	F2 Phenethicillin  2	F3 Phenethicillin  3	F4 Phenethicillin  4	F5 Blasticidin S  1	F6 Blasticidin S  2	F7 Blasticidin S  3	F8 Blasticidin S  4	F9 Sodium caprylate  1	F10 Sodium caprylate  2	F11 Sodium caprylate  3	F12 Sodium caprylate  4
G1 Lauryl sulfobetaine  1	G2 Lauryl sulfobetaine  2	G3 Lauryl sulfobetaine  3	G4 Lauryl sulfobetaine  4	G5 Dihydro- streptomycin  1	G6 Dihydro- streptomycin  2	G7 Dihydro- streptomycin  3	G8 Dihydro- streptomycin  4	G9 Hydroxylamine  1	G10 Hydroxylamine  2	G11 Hydroxylamine  3	G12 Hydroxylamine  4
H1 Hexammine cobalt (III) chloride  1	H2 Hexammine cobalt (III) chloride  2	H3 Hexammine cobalt (III) chloride  3	H4 Hexammine cobalt (III) chloride  4	H5 Thioglycerol  1	H6 Thioglycerol  2	H7 Thioglycerol  3	H8 Thioglycerol  4	H9 Polymyxin B  1	H10 Polymyxin B  2	H11 Polymyxin B  3	H12 Polymyxin B  4

**PM20B MicroPlate™**

A1 Amitriptyline  1	A2 Amitriptyline  2	A3 Amitriptyline  3	A4 Amitriptyline  4	A5 Apramycin  1	A6 Apramycin  2	A7 Apramycin  3	A8 Apramycin  4	A9 Benserazide  1	A10 Benserazide  2	A11 Benserazide  3	A12 Benserazide  4
B1 Orphenadrine  1	B2 Orphenadrine  2	B3 Orphenadrine  3	B4 Orphenadrine  4	B5 D,L-Propranolol  1	B6 D,L-Propranolol  2	B7 D,L-Propranolol  3	B8 D,L-Propranolol  4	B9 Tetrazolium violet  1	B10 Tetrazolium violet  2	B11 Tetrazolium violet  3	B12 Tetrazolium violet  4
C1 Thioridazine  1	C2 Thioridazine  2	C3 Thioridazine  3	C4 Thioridazine  4	C5 Atropine  1	C6 Atropine  2	C7 Atropine  3	C8 Atropine  4	C9 Ornidazole  1	C10 Ornidazole  2	C11 Ornidazole  3	C12 Ornidazole  4
D1 Proflavine  1	D2 Proflavine  2	D3 Proflavine  3	D4 Proflavine  4	D5 Ciprofloxacin  1	D6 Ciprofloxacin  2	D7 Ciprofloxacin  3	D8 Ciprofloxacin  4	D9 18-Crown-6 ether  1	D10 18-Crown-6 ether  2	D11 18-Crown-6 ether  3	D12 18-Crown-6 ether  4
E1 Crystal violet  1	E2 Crystal violet  2	E3 Crystal violet  3	E4 Crystal violet  4	E5 Dodine  1	E6 Dodine  2	E7 Dodine  3	E8 Dodine  4	E9 Hexa- chlorophene  1	E10 Hexa- chlorophene  2	E11 Hexa- chlorophene  3	E12 Hexa- chlorophene  4
F1 4-Hydroxy- coumarin  1	F2 4-Hydroxy- coumarin  2	F3 4-Hydroxy- coumarin  3	F4 4-Hydroxy- coumarin  4	F5 Oxytetracycline  1	F6 Oxytetracycline  2	F7 Oxytetracycline  3	F8 Oxytetracycline  4	F9 Pridinol  1	F10 Pridinol  2	F11 Pridinol  3	F12 Pridinol  4
G1 Captan  1	G2 Captan  2	G3 Captan  3	G4 Captan  4	G5 3,5-Dinitro- benzene  1	G6 3,5-Dinitro- benzene  2	G7 3,5-Dinitro- benzene  3	G8 3,5-Dinitro- benzene  4	G9 8-Hydroxy- quinoline  1	G10 8-Hydroxy- quinoline  2	G11 8-Hydroxy- quinoline  3	G12 8-Hydroxy- quinoline  4
H1 Patulin  1	H2 Patulin  2	H3 Patulin  3	H4 Patulin  4	H5 Tolyfluanid  1	H6 Tolyfluanid  2	H7 Tolyfluanid  3	H8 Tolyfluanid  4	H9 Troleandomycin  1	H10 Troleandomycin  2	H11 Troleandomycin  3	H12 Troleandomycin  4

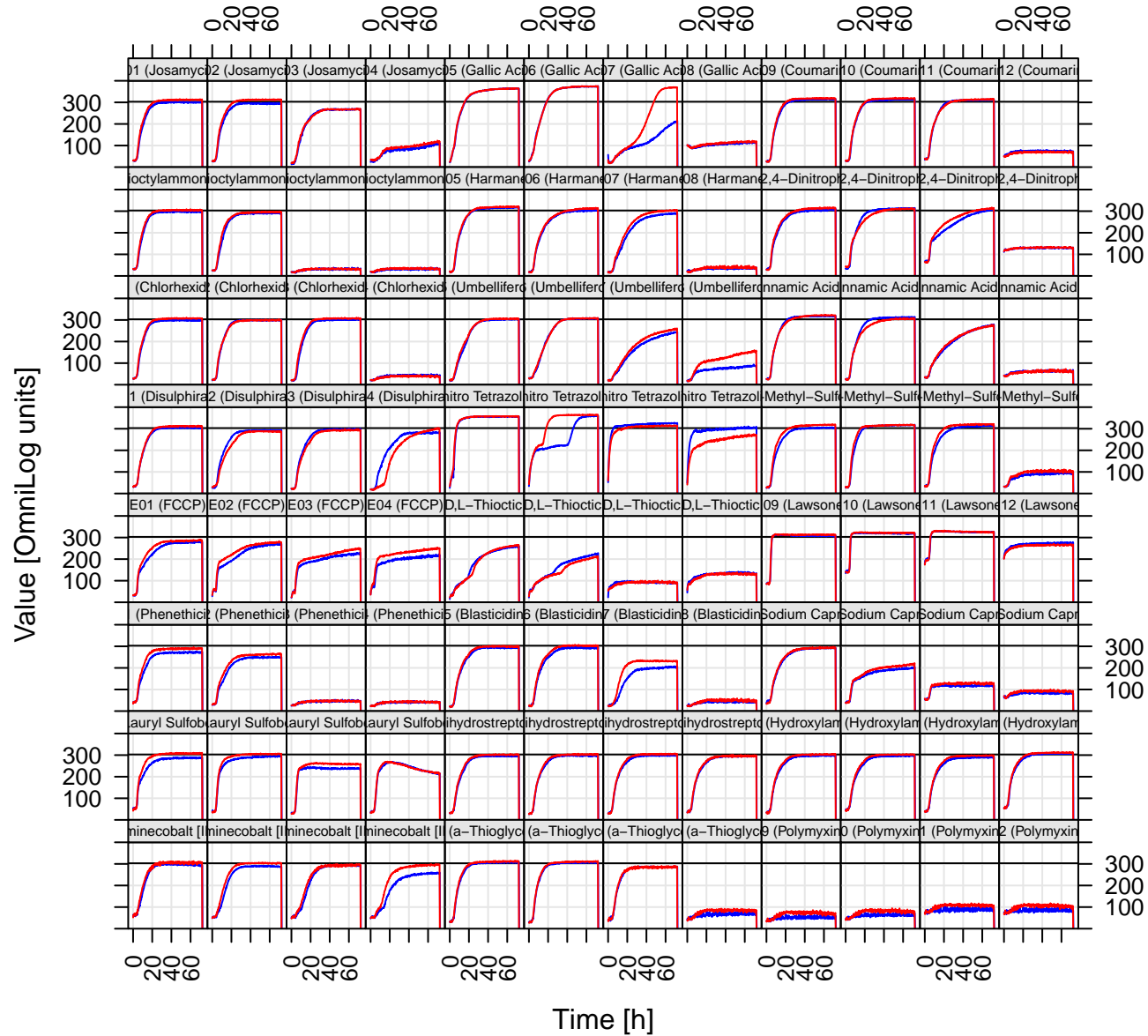
# PM19 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



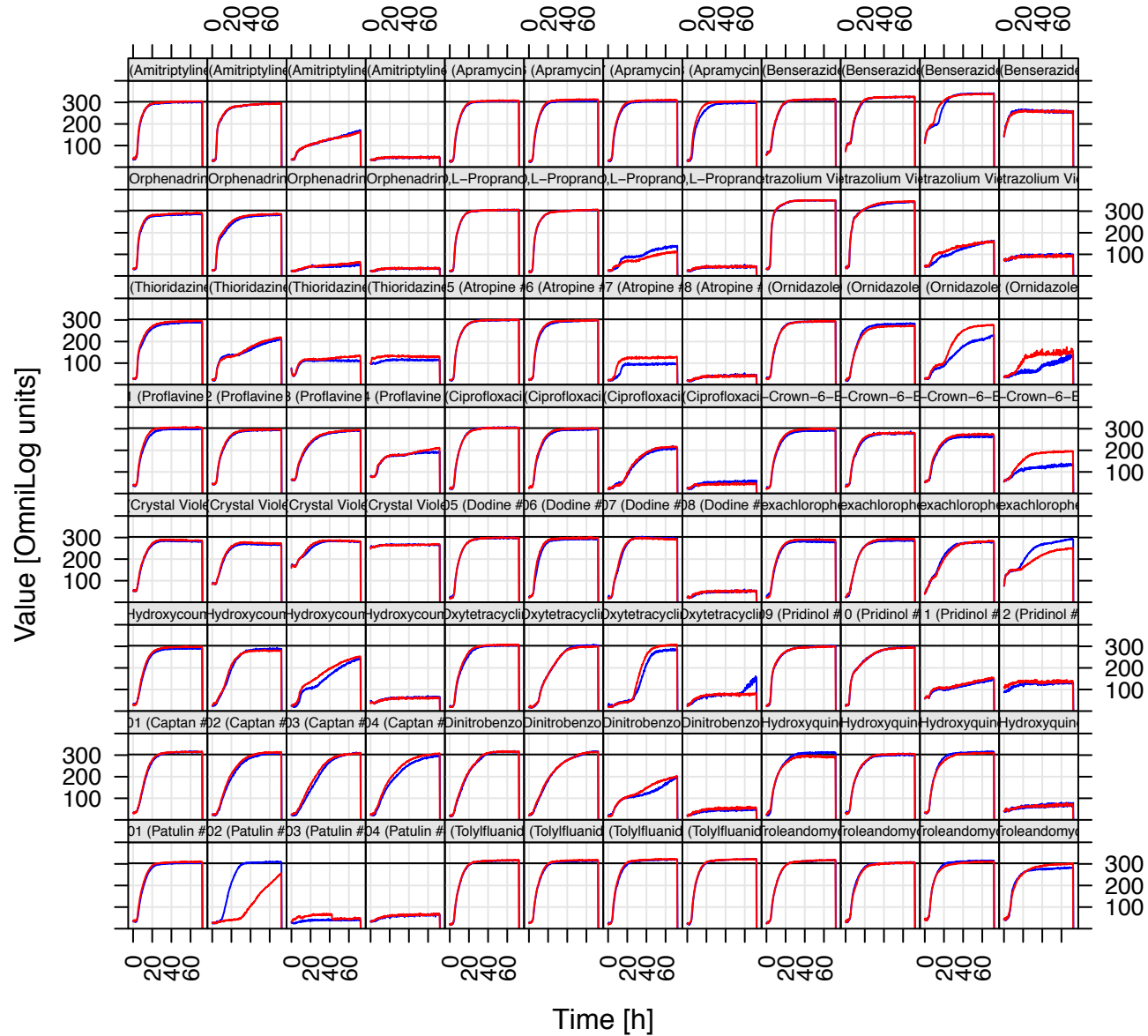
# PM19 (Chemicals)

ΔSPFH-1  
WT-1



# PM20 (Chemical Sensitivity Bacteria)

ΔSPFH-2  
WT-2



# PM20 (Chemicals)

ΔSPFH-1  
WT-1

