Supplemental figure legends:

Supplemental Figure 1 – A) Depiction of represented neutral, underrepresented and domain genes. On the X-axis, the 3 genes are represented 5' to 3' to relative scale, with number of transposon (Tn) reads at that TA site on the Y axis. Many TA sites had more than 10 reads. B) OD_{600} of *S. marcescens* ATCC 13880 TIS library during the antibiotic screen. C) Beta-lactamase (AmpC) activity under conditions of the antibiotic screen. As expected, cefoxitin, which is a strong inducer of AmpC, results in large increases in beta-lactamase activity, as determined through hydrolysis of nitrocefin, a chromogenic cephalosporin substrate. D,E) "Volcano" plot illustrating candidate genes important for ciprofloxacin (in D) and cefepime (in E) susceptibility. On the X-axis is the log_2fold-change (Log_2FC) in insertion-mutant abundance in antibiotic (CIP and FEP) compared to no drug. The Y-axis is the inverse Mann-Whitney U *p*-value (1/MWU p-val), which roughly measures the concordance between mutants with insertions at individual TA sites across a gene. Genes were depleted (red) if $Log_2FC \leq 2$ and 1/MWU p-val ≥ 100. Genes were enriched (blue) if $Log_2FC \geq 2$ and 1/MWU p-val ≥ 100.

Supplemental Figure 2 – *YdgH* locus. Sigma-70 promoters with scores of 90 or greater in BacPP and rho-independent terminators identified by ARNold are indicated.

Supplemental Figure 3 – Growth curves of *S. marcescens* ATCC 13880 Wt or Δ*ydgH* in LB alone or in LB supplemented with the indicated concentrations (in ug/mL) of A) the 3rd generation cephalosporin moxalactam; B) the 3rd generation cephalosporin ceftriaxone; C) the 1st generation cephalosporin cephalexin; D) the anti-Pseudomonal cephalosporins ceftazidime and E) cefepime. Informative concentrations used in calculating the OD600 ratios depicted in the

main text are depicted. Results for the full range of concentrations tested are in Supplemental Table 4.

Supplemental Figure 4 – Growth curves of *S. marcescens* ATCC 13880 Wt or Δ*ydgH* in LB alone or in LB supplemented with the indicated concentrations (in ug/mL) of the penicillins A) carbenicillin or B) piperacillin; and the carbapenems C) imipenem and D) meropenem. E) AmpC activity is not different in Δ*ydgH* compared to Wt, as measured by bulk nitrocefin hydrolysis of clarified supernatant.

Supplemental Figure 5 - Growth curves of *S. marcescens* ATCC 13880 Wt or Δ*ydgH* in LB alone or in LB supplemented with the indicated concentrations (in ug/mL) of the non-beta lactam antibiotics A) ciprofloxacin; B) trimethoprim; C) gentamicin and; D) chloramphenicol.

Supplemental Figure 6 - Growth curves of *S. marcescens* ATCC 13880 Wt or Δ*ydgH* in LB alone or in LB supplemented with the indicated concentrations (in ug/mL) of the antibiotics to which *S.* marcescens ATCC 13880 is intrinsically resistant, A) rifampin; B) bacitracin; and F) polymyxin B). Benzalkonium chloride and benzethonium chloride are depicted in D) and E). SDS concentrations in C) are (v/v).

Supplemental table 1 – Potential transposon insertion sites (genomic TA dinucleotides) along with the amount of observed insertions on the + and - strands are tabulated along with the annotated locus.

Supplemental table 2 – Essential/underrepresented gene analysis of *S. marcescens* ATCC 13880 as determined through TIS and the EL-ARTIST pipeline. Summary statistics of essential/underrepresented genes underlying the analyses in Figure 1 are tabulated.

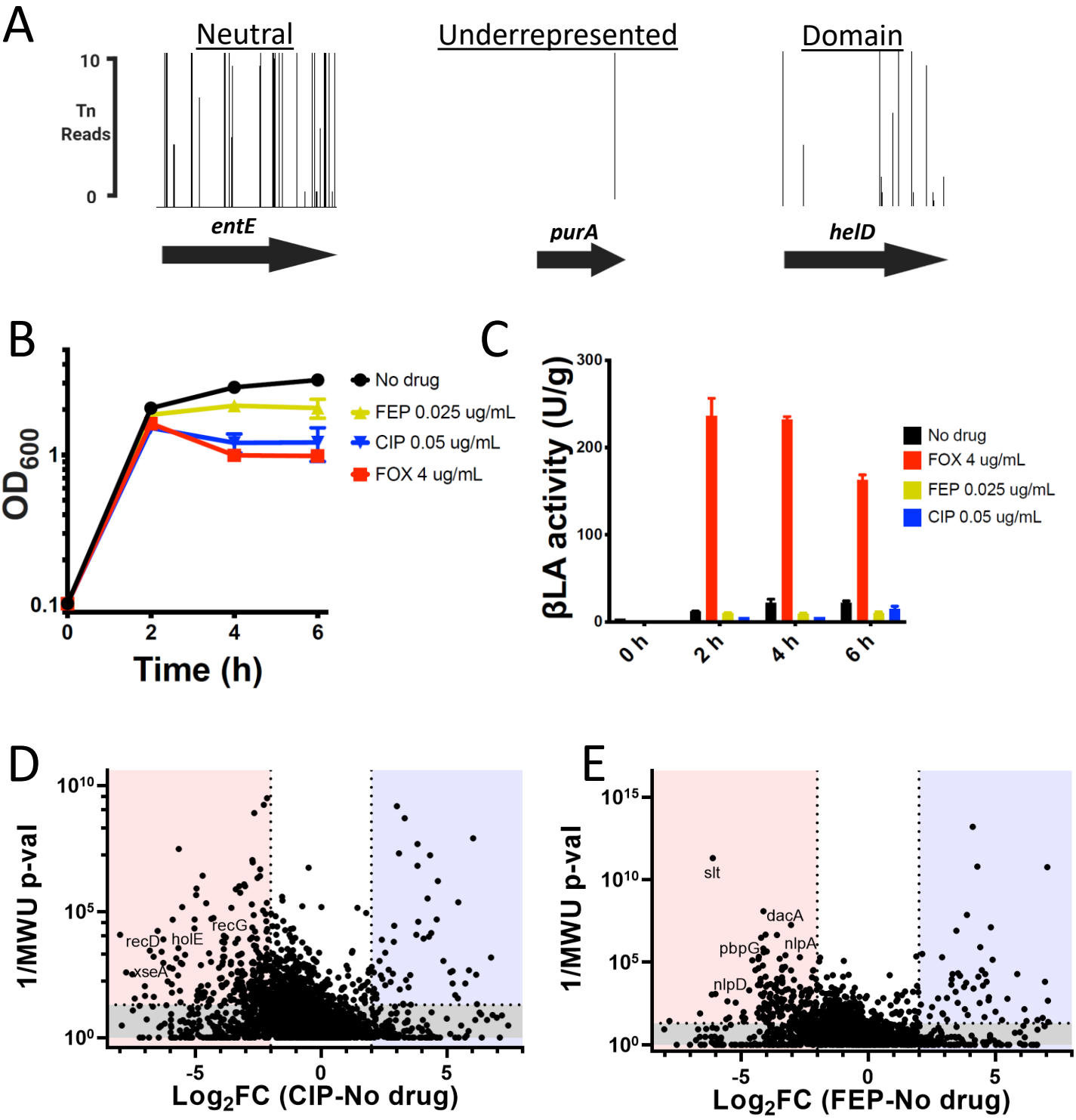
Supplemental table 3 – Essential/underrepresented genes unique to *S. marcescens* ATCC 13880 compared to both from *E. coli* K12 using either TIS and the EL-ARTIST pipeline or based on single gene deletion attempts resulting in the KEIO collection.

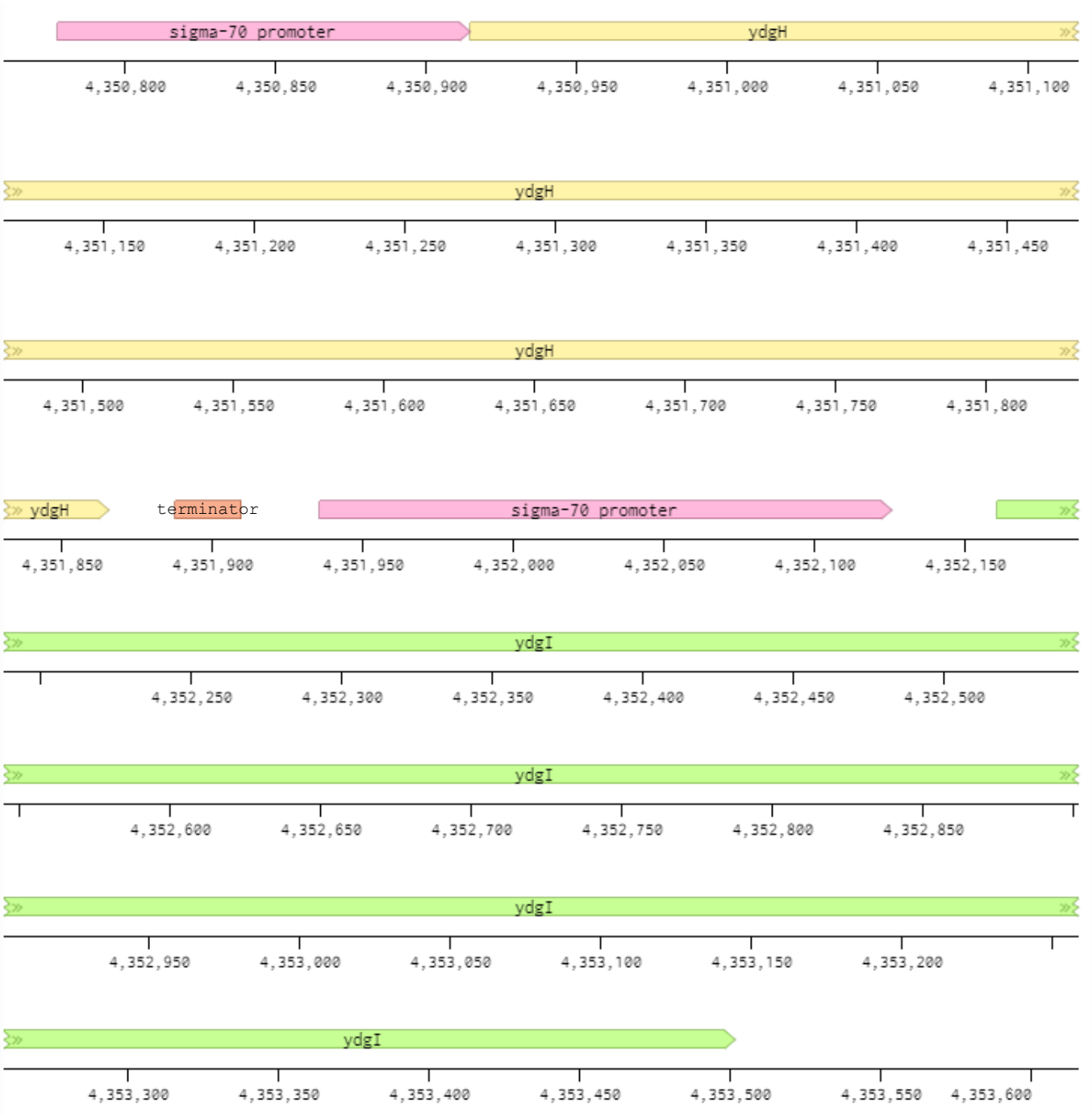
Supplemental table 4 – Full TIS analysis identifying candidate genes important for outgrowth in no drug, cefoxitin, cefepime, or ciprofloxacin.

Supplemental table 5 – Tabulation of those genes with $Log_2FC \leq 2$ and 1/MWU p-val ≥ 100 used to generate the Venn diagram in Figure 2D.

Supplemental table 6 – Raw data from all final concentrations of chemical/antibiotic stressors used to generate main text figure 5 and the corresponding supplemental figures.

Supplemental table 7 – Primers used for creation of pTOX3 allelic exchange vectors and pBAD33-*ydgH*.


A
Neutral
Underrepresented
Domain
10
Tn
Reads
0
entE
purA
helD
B
OD600
1
0.1
0
2
4
6
Time (h)
No drug
FEP 0.025 ug/mL
CIP 0.05 ug/mL
FOX 4 ug/mL
C
βLA activity (U/g)
300
200
100
0
0 h
2 h
4 h
6 h
No drug
FOX 4 ug/mL
FEP 0.025 ug/mL
CIP 0.05 ug/mL
D
1/MWU p-val
10^10
10^5
10^0
recD
holE
recG
xseA
-5
0
5
Log2FC (CIP-No drug)
E
1/MWU p-val
10^15
10^10
10^5
10^0
slt
dacA
pbpG
nlpA
nlpD
-5
0
5
Log2FC (FEP-No drug)


sigma-70 promoter
ydgH
4,350,800
4,350,850
4,350,900
4,350,950
4,351,000
4,351,050
4,351,100
ydgH
4,351,150
4,351,200
4,351,250
4,351,300
4,351,350
4,351,400
4,351,450
ydgH
4,351,500
4,351,550
4,351,600
4,351,650
4,351,700
4,351,750
4,351,800
ydgH
terminator
sigma-70 promoter
4,351,850
4,351,900
4,351,950
4,352,000
4,352,050
4,352,100
4,352,150
ydgI
4,352,250
4,352,300
4,352,350
4,352,400
4,352,450
4,352,500
ydgI
4,352,600
4,352,650
4,352,700
4,352,750
4,352,800
4,352,850
ydgI
4,352,950
4,353,000
4,353,050
4,353,100
4,353,150
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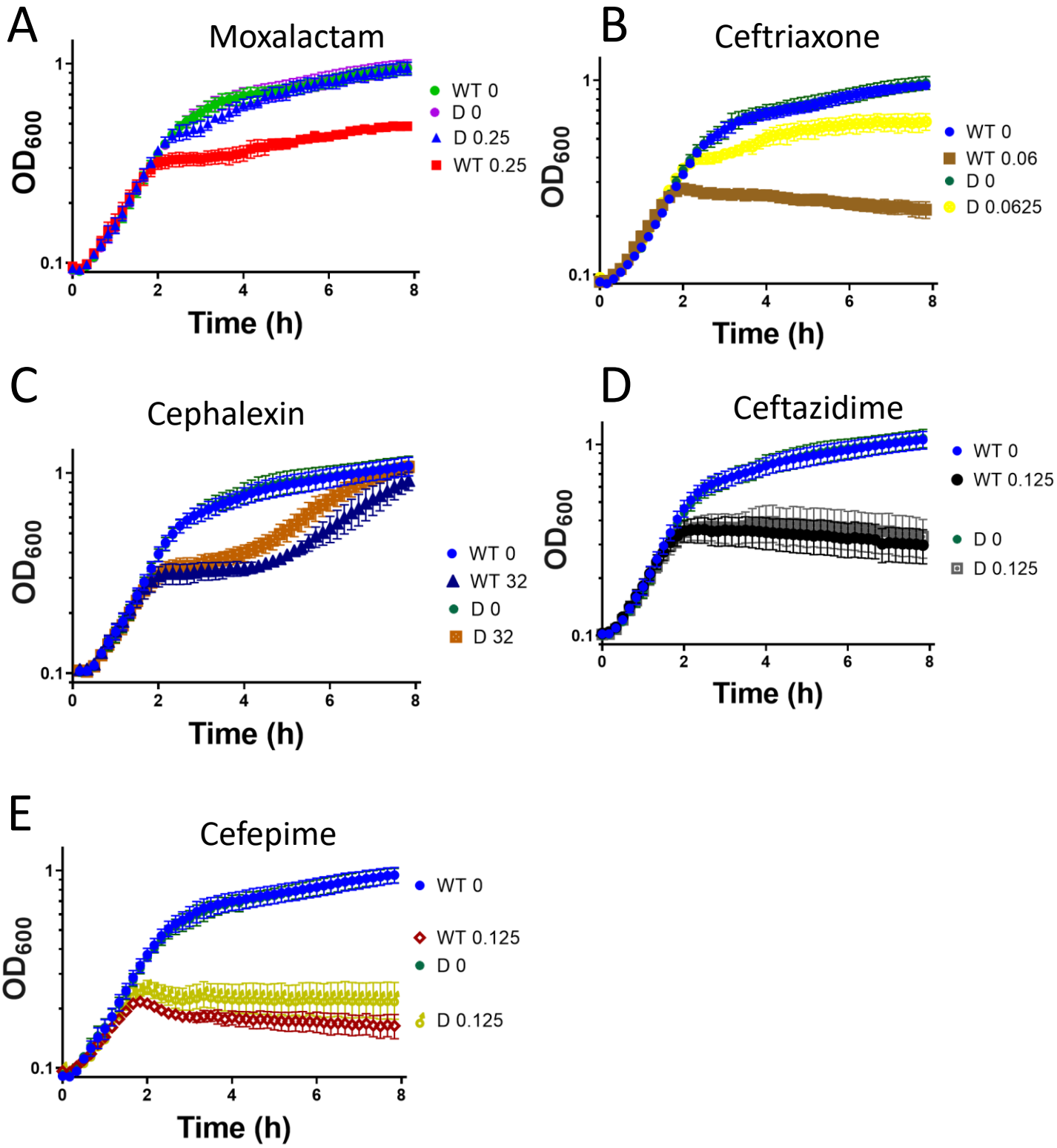
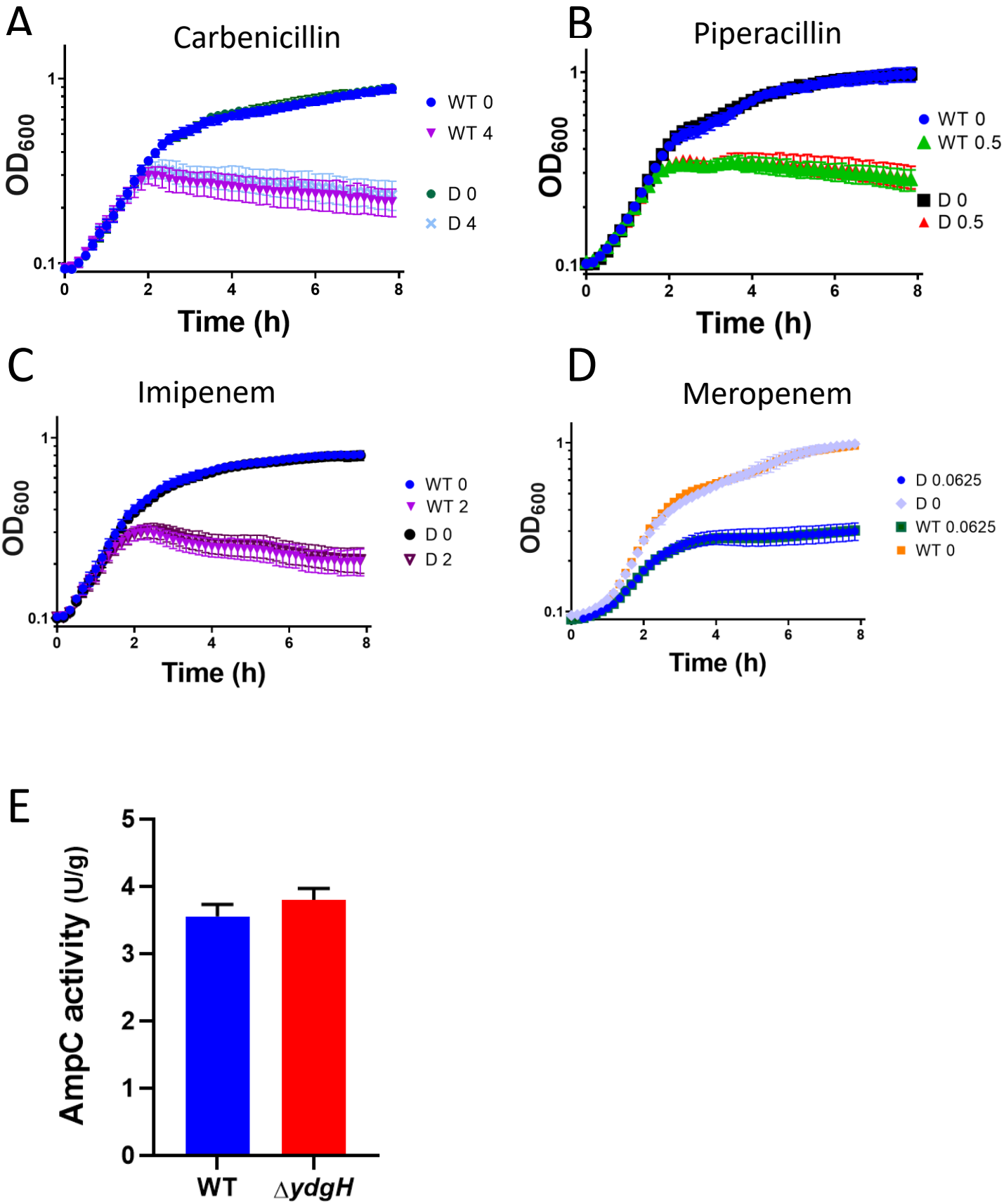
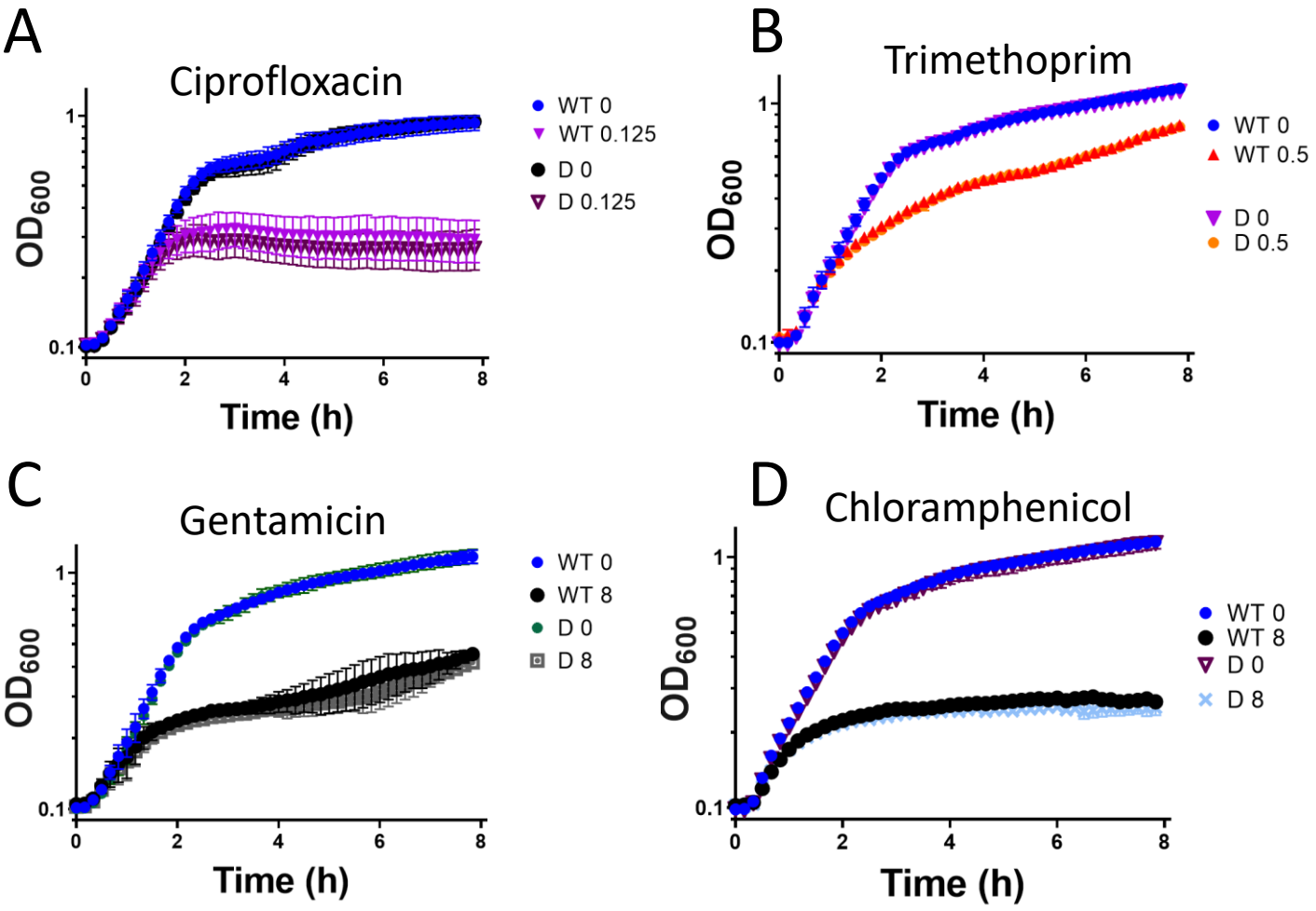
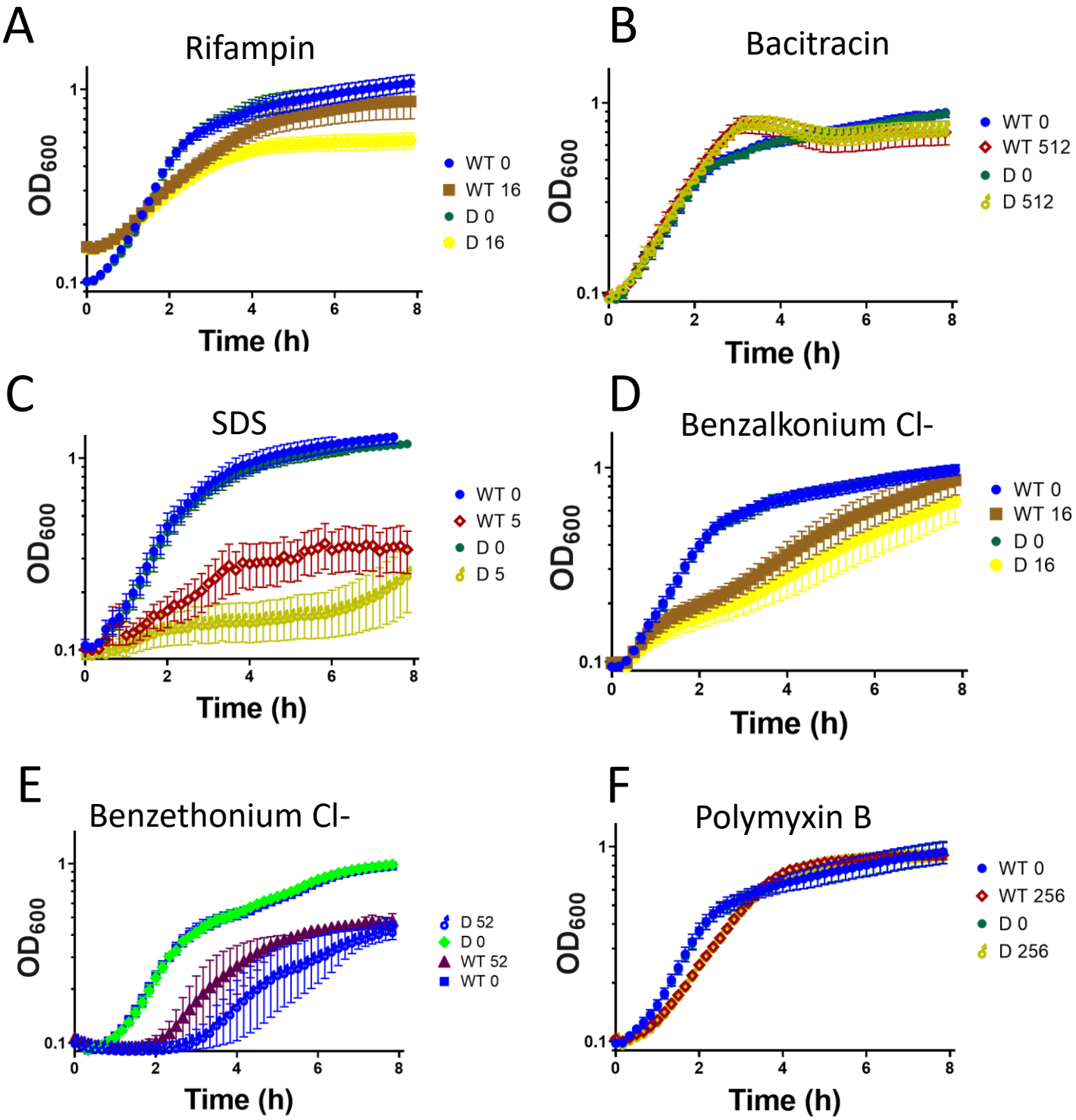
4,353,500
4,353,550
4,353,600


A
Moxalactam
OD600
Time (h)
WT 0
D 0
D 0.25
WT 0.25
B
Ceftriaxone
OD600
Time (h)
WT 0
WT 0.06
D 0
D 0.0625
C
Cephalexin
OD600
Time (h)
WT 0
WT 32
D 0
D 32
D
Ceftazidime
OD600
Time (h)
WT 0
WT 0.125
D 0
D 0.125
E
Cefepime
OD600
Time (h)
WT 0
WT 0.125
D 0
D 0.125


A
Carbenicillin
OD600
Time (h)
WT 0
WT 4
D 0
D 4
B
Piperacillin
WT 0
WT 0.5
D 0
D 0.5
C
Imipenem
WT 0
WT 2
D 0
D 2
D
Meropenem
D 0.0625
D 0
WT 0.0625
WT 0
E
AmpC activity (U/g)
WT
ΔydgH


A
Ciprofloxacin
OD600
Time (h)
WT 0
WT 0.125
D 0
D 0.125
B
Trimethoprim
OD600
Time (h)
WT 0
WT 0.5
D 0
D 0.5
C
Gentamicin
OD600
Time (h)
WT 0
WT 8
D 0
D 8
D
Chloramphenicol
OD600
Time (h)
WT 0
WT 8
D 0
D 8


A
Rifampin
OD600
Time (h)
WT 0
WT 16
D 0
D 16
B
Bacitracin
WT 0
WT 512
D 0
D 512
C
SDS
WT 0
WT 5
D 0
D 5
D
Benzalkonium Cl-
WT 0
WT 16
D 0
D 16
E
Benzethonium Cl-
D 52
D 0
WT 52
WT 0
F
Polymyxin B
WT 0
WT 256
D 0
D 256