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SUPPORTING INFORMATION

Isoprenoid Biosynthesis as a Drug Target: Bisphosphonate Inhibition of *Escherichia coli* K12 Growth and Synergistic Effects of Fosmidomycin

By

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Table S1. Bisphosphonate antimicrobial susceptibility test results against *E. coli*. Zone of inhibition results (in mm) in Mueller-Hinton agar media (MH) for all compounds versus *E. coli* W3110 and *E. coli*-pTMV19 in the absence and presence of mevalonate (±MVA).

ID	Chemical Name	*E. coli* W3110	*E.coli*-pTMV19	
		MH	MH	MH+MVA
7	6-Aminohexane-1-hydroxy-1,1-bisphosphonate	0		
8	2-(3-Pyridyl)-1-hydroxy-ethane-1,1-bisphosphonate	0		
9	2-(4-Pyridyl)-1-hydroxy-ethane-1,1-bisphosphonate	0		
10	(1-Hydroxy-1-phosphono-butyl)-phosphonic acid	0		
11	3-aAmino-1-hydroxy-propane-1,1-bisphosphonate	0		
12	3-(3-Pyridyl)-1-hydroxy-propane-1,1-bisphosphonate	0		
13	N-[Methyl(4-phenylbutyl)]-3-aminopropyl-1-hydroxy-1,1-bisphosphonate	19	18	17
14	5-Aminopentane-1-hydroxy-1,1-bisphosphonate	0		
15	2-(4-Aminophenyl)-1-hydroxy-ethane-1,1-bisphosphonate	0		
16	2-(2-Pyridyl)-1-hydroxy-ethane-1,1-bisphosphonate	0		
17	6-Aminohexane-1-hydroxy-1,1-bisphosphonate	0		
18	2-Phenyl-1-hydroxy-ethane-1,1-bisphosphonate	0		
19	2-(4-Pyridylthio)-1-hydroxy-ethane-1,1-bisphosphonate	0		
20	N-(2-(3-Picolyl)) aminomethylene bisphosphonate	7	8	12
21	3-Aminobutane-1-hydroxy-1,1-bisphosphonate	0		
22	1-Hydroxy-pentane-1,1-bisphosphonate	0		
23	2-(4-Imidazole)-1-hydroxy-ethane-1,1-bisphosphonate	0	0	0
24	N-(2-(4-Picolyl)) aminomethylene bisphosphonate	7	9	10
25	Undecane-1-hydroxy-1,1-bisphosphonate	0	0	0
26	Dodecane-1-hydroxy-1,1-bisphosphonate	0		
27	3-(N,N-methylamyl)-amino-1-hydroxy-propane-1,1-bisphosphonate	0		
28	*N*-Octyl-aminomethylene bisphosphonate	0		
29	N-(2,3,4,5,6-pentafluorophenyl) aminomethylene bisphosphonate	0		
30	1-Hydroxy-heptane-1,1-bisphosphonate	0		
31	1-Hydroxy-decane-1,1-bisphosphonate	9	0	0

32	(Cyloheptylamino)methylene-1,1-bisphosphonate	0		
33	(Butylamino)methylene-1,1-bisphosphonate	0		
34	(3-Isoquinolylamine)methylene-1,1-bisphosphonate	0		
35	[[(3-Methylbutyl)amino]methylene]-1,1-bisphosphonate	0		
36	[[(3,5-Dichlorophenyl)amino]methylene]-1,1-bisphosphonate	0	10	8
37	[(Dipropylamino)methylene]-1,1-bisphosphonate	0		
38	[[(6-Methyl-2-pyridinyl)amino]methylene]-1,1-bisphosphonate	7	10	8
39	[[(5-Bromo-2-pyridinyl)amino]methylene]-1,1-bisphosphonate	12	11	8
40	[(Cyclohexylamino)methylene]-1,1-bisphosphonate	7	8	9
41	[[(5-Chloro-2-pyridinyl)amino]methylene]-1,1-bisphosphonate	13	9	12
42	[(Hexahydro-1H-azepin-1yl)methylene]-1,1-bisphosphonate	0	0	0
43	1-(2-Pyridyl)ethane-1,2-bisphosphonate	0	0	0
44	N-nonyl-aminomethylene-1,1-bisphosphonate	0		
45	[(3-Pyridinylamino)methylene]-1,1-bisphosphonate	0		
46	[(2-Pyridinylamino)methylene]-1,1-bisphosphonate	7	8	12
47	1-Piperidinylmethylene-1,1-bisphosphonate	0		
48	4-Thiomorpholinylmethylene-1,1-bisphosphonate	0		
49	Propylaminomethylene-1,1-bisphosphonate	0		
50	N-2-chloro-(3-pyridyl)-aminomethylene-1,1-bisphosphonate	0		
51	N-6-chloro-(3-pyridyl)-aminomethylene-1,1-bisphosphonate	0		
52	(4-Butylphenyl)aminomethylene-1,1-bisphosphonate	0		
53	(4-Butoxyphenyl)aminomethyelene-1,1-bisphosphonate	0		
54	(2-Methylbutyl)aminomethylene-1,1-bisphosphonate	0		
55	Cyclopentylaminomethylene-1,1-bisphosphonate	0		
56	Cyclooctylaminomethylen-1,1-bisphosphonate	7	8	0
57	(4,6-Dimethyl-2-pyridinyl)-aminomethylene-1,1-bisphosphonate	11	7	0
58	[[(3-Methylbutyl)amino]methylene]-1,1-bisphosphonate	0		
59	1-Hydroxy-2-imidazolyl-ethylidene-1,1-bisphosphonate	0		
60	1-(3-Pyridyl)ethane-1,2-bisphosphonate	0		
61	1-(4-Pyridyl)ethane-1,2-bisphosphonate	0		
62	[[(Cyclobutyl)amino]methylene]-1,1-bisphosphonate	0		
63	Cyclopropyl-1-hydroxy- methylene-1,1-bisphosphonate	0		
64	Cyclobutyl-1-hydroxy- methylene-1,1-bisphosphonate	0		
65	[2-(2-Pyridinylamino)ethylidene]-1,1-bisphosphonate	0		
66	[2-(3-Pyridinylamino)ethylidene]-1,1-bisphosphonate	0		

67	2-Butyl-1-hydroxyl-octane-1,1-bisphosphonate	0	0	0
68	Tetradecyl-1-hydroxy-1,1-bisphosphonate	0		
69	Hexadecyl-1-hydroxy-1,1-bisphosphonate	0		
70	Octadecyl-1-hydroxy-1,1-bisphosphonate	0		
71	N-[Methyl(4-phenylethyl)]-3-aminopropyl-1-hydroxy-1,1-bisphosphonate	16	14	14
72	N-[Methyl(4-phenylpropyl)]-3-aminopropyl-1-hydroxy-1,1-bisphosphonate	18	13	15
73	[(3-Ethylphenyl)aminomethylene]-1,1-bisphosphonate	0		
74	[(4-Hexylphenyl)aminomethylene]-1,1-bisphosphonate	0	0	0
75	[(4-Heptylphenyl)aminomethylene]-1,1-bisphosphonate	0	0	0
76	[(5-Indanyl)aminomethylene]-1,1-bisphosphonate	0		
77	[(3,5-Di-tert-butyl-phenyl)aminomethylene]-1,1-bisphosphonate	0		
78	(Biphenyl-4-yl)- aminomethylene-1,1-bisphosphonate	9	8	7
79	[(Biphenyl-3-ylamino)-phosphono-methyl]-phosphonic acid	8	8	0
80	[1-Hydroxy-3-[methyl(2-phenoxyethyl)amino]propylidene-1,1-bisphosphonate	11	15	11
81	(Butylethyl)aminomethylene-1,1-bisphosphonate	0		
82	(3,5-Dibromo-2-pyridinyl)-aminomethylene-1,1-bisphosphonate	0		
83	(5-Iodo-2-pyridinyl)-aminomethylene-1,1-bisphosphonate	7	7	7
84	(3-Pyridinyl)-methyl-imidodiphosphate	0		
85	(3-Benzyloxy-2-pyridinyl)-aminomethylene-1,1-bisphosphonate	0		
86	(9-Ethyl-9H-3-carbazolyl)-aminomethylene-1,1-bisphosphonate	0	0	0
87	(1-Hydroxy-2-imidazo[1,2-a]pyridin-2-yl-1-ethyl)-1,1-bisphosphonate	0		
88	Azocan-1-yl-methylene-1,1-bisphosphonate	0		
89	[(3.5-Dibromo-6-methyl-pyridin-2-yl)-aminomethylene]-1,1-bisphosphonate	0		
90	[(2-Methyl-1-piperidinyl)-methylene]-1,1-bisphosphonate	0		
91	[(3-Methyl-1-piperidinyl)-methylene]-1,1-bisphosphonate	0		
92	[(4-Methyl-1-piperidinyl)-methylene]-1,1-bisphosphonate	0		
93	[(2-Ethyl-1-piperidinyl)-methylene]-1,1-bisphosphonate	0		
94	[(2,6-Dimethyl-1-piperidinyl)-methylene]-1,1-bisphosphonate	0		
95	[(3,5-Dimethyl-1-piperidinyl)-methylene]-1,1-bisphosphonate	0		
96	[(4-Ethyl-phenyl)aminomethylene]-1,1-bisphosphonate	0		
97	(3-Phosphonomethyl-benzyl)-phosphonic acid	0		
98	[(Benzyl-methyl-amino)-methylene]-1,1-bisphosphonate	0		

99	2-(3-Methyl-pyridin-2-ylamino)-1-phosphono-ethanesulfonic acid	0		
100	[(3-Phenoxy-phenyl)aminomethylene]-1,1-bisphosphonate	0		
101	[(2,4-Dichloro-phenyl)aminomethylene]-1,1-bisphosphonate	0		
102	[(2,6-Diisopropyl-phenyl)aminomethylene]-1,1-bisphosphonate	0		
103	(4-Imidazol-1-yl)-propyl-imidodiphosphate	0	0	0
104	[Benzyl-(4-methyl-pyridin-2-yl)-aminomethylene]-1,1-bisphosphonate	0	0	0
105	[(3-Isopropyl-phenyl)aminomethylene]-1,1-bisphosphonate	8	9	0
106	[(6-Ethyl-pyridin-2-yl)aminomethylene]-1,1-bisphosphonate	0		
107	[1-Hydroxy-2-(3-trifluoromethyl-phenyl)-ethyl]-1,1-bisphosphonate	7	7	0
108	[2-(3-Fluoro-phenyl)-1-hydroxy-1-ethyl]-1,1-bisphosphonate	0		
109	1-Hydroxy-nonane-1,1-bisphosphonate	9	7	0
110	[2-(3-Chloro-phenyl)-1-hydroxy-ethylene]-1,1-bisphosphonate	8	11	0
111	(sec-Butylaminomethylene)-1,1-bisphosphonate	0		
112	[2-(3-Bromo-phenyl)-1-hydroxy-ethylene]-1,1-bisphosphonate	12	14	0
113	{[3-(2,5-Dimethyl-benzyloxy)-6-methyl-pyridin-2-yl]-aminomethylene}-1,1-bisphosphonate	0	0	0
114	(1-Hydroxy-2-m-tolyl-ethylene)-1,1-bisphosphonate	0	0	0
115	[1-Hydroxy-(4-octyl-phenyl)-methylene]-1,1-bisphosphonate	0	0	0
116	(1-Hydroxy-2-imidazo[1,2-a]pyridin-3-yl-1-ethylidene)-1,1-bisphosphonate	10	0	0
117	[(3-Benzyl-phenyl)-aminomethylene]-1,1-bisphosphonate	7	8	0
118	[(3-Tert-Butyl-phenyl)-aminomethylene]-1,1-bisphosphonate	7	7	0
119	[(1-Naphthalen-1-yl-ethyl)-aminomethylene]-1,1-bisphosphonate	0	0	0
120	[(5-Bromo-3-methyl-pyridin-2-yl)-aminomethylene]-1,1-bisphosphonate	0	0	0
121	(2-Benzoimidazol-1-yl-1-hydroxy-ethylene)-1,1-bisphosphonate	14	0	13
122	[(1-Naphthalen-1-yl-ethyl)-aminomethylene]-1,1-bisphosphonate	0	0	0
123	[(2-Pyridinyl)-thiomethylene]-1,1-bisphosphonate	0	0	0
	Fosmidomycin	20	19	0
	Chloramphenicol	24	20	19
	Kanamycin	21	22	17
	Novobiocin	9	9	15
	Tetracycline	21	21	22
	Streptomycin	15	16	7
	Neomycin	15	18	12

Table S2. *E. coli* Common Feature Generation Log Excerpt.

```
Summary of parameters:
  HyposReported    10
  Spacing         300
  MinPoints         4
  MinSubsetPoints           4
  SuperpositionError        1
  Misses            1
  FeatureMisses     1
  CompleteMisses          0.0e+00
  ToleranceFactor           1
  CheckSuperposition        1
  WeightVariation           0.3
  MappingCoeff    0.0e+00
  Mem      60
  IdealHBondGeomOnly      0.0e+00
  VariableWeight          0.0e+00
  VariableTolerance       0.0e+00

Constraints on numbers of features:
Name:   HYDROPHOBIC     NegIonizable      positive    Total
Min:         0              0               0          3
Max:         5              5               5          10

Summary of lead molecules selected:
            Name     Principal MaxOmitFeat Chirality Confs Feats
                210P        2      1                  131  5.02
                209P        1      1                  239  5.00
                219P        1      1                  249  5.00
                 56P        1      1                   66  5.12

Summary of feature definitions:
  H: HYDROPHOBIC
     Hydrophobic:  Tolerance= 160
  N: NegIonizable
     NegIon-pnt:  Tolerance= 160
  P: positive
     #Any Atom#2:  Tolerance= 160


Summary of feature definition hit statistics:
           HYDROPHOBIC  hits/lead:  mean= 1.03  stddev=  0.06
          NegIonizable  hits/lead:  mean= 3.00  stddev=  0.00
              positive  hits/lead:  mean= 1.00  stddev=  0.00

Summary of feature definition hits by molecule:
                        H      N      P
                210P   1.02   3.00   1.00
                209P   1.00   3.00   1.00
                219P   1.00   3.00   1.00
                 56P   1.12   3.00   1.00
                 48P             Not used
                 255             Not used
                 84P             Not used
                 217             Not used

Summary of ranking coefficients:
  Constant: -6.6
  Dispersion: 0.0054
  H: 1.9
  N: 3.8
  P: 2.7

Processing:
 Generating hypos with NH: [c]. 399
 Generating hypos with PH: [c]. 133
 Generating hypos with NN: [c]. 262
 Generating hypos with PN: [c]. 393
 Generating hypos with NNH: [c]. 266
 Generating hypos with PNH: [c]. 399
 Hypos with features NH not desired. Freeing memory.
 Hypos with features PH not desired. Freeing memory.
 Generating hypos with NNN: [c]. 0
 Generating hypos with PNN: [c]. 262
 Hypos with features NN not desired. Freeing memory.
 Hypos with features PN not desired. Freeing memory.
 Generating hypos with PNNH: [c]. 532
 Reprocessing hypos with PNNH: 5 remaining.
```

```
 Hypos with features NNH not desired. Freeing memory.
 Hypos with features PNH not desired. Freeing memory.
 Hypos with features PNN not desired. Freeing memory.
 Inspecting hypos with PNNH


Exporting hypotheses:
1 PNNH  Rank: 46.9362  DH: 1111  PH: 0000 Max Fit: 4

2 PNNH  Rank: 45.4283  DH: 1111  PH: 0000 Max Fit: 4

3 PNNH  Rank: 42.2135  DH: 1111  PH: 0000 Max Fit: 4

4 PNNH  Rank: 40.9805  DH: 1111  PH: 0000 Max Fit: 4

5 PNNH  Rank: 39.5424  DH: 1111  PH: 0000 Max Fit: 4

Halted normally.
CPU time = 7 s  System time = 0 s
```

Table S3. *E. coli* FPPS Common Feature Hypothesis Generation Log.

```
Summary of parameters:
  HyposReported           10
  Spacing        300
  MinPoints        4
  MinSubsetPoints          3
  SuperpositionError       1
  Misses           3
  FeatureMisses            1
  CompleteMisses         0.0e+00
  ToleranceFactor          1
  CheckSuperposition       1
  WeightVariation          0.3
  MappingCoeff   0.0e+00
  Mem      60
  IdealHBondGeomOnly     0.0e+00
  VariableWeight         0.0e+00
  VariableTolerance      0.0e+00

Constraints on numbers of features:
Name:   HYDROPHOBIC     NegIonizable      positive    Total
Min:         0              0               0          3
Max:         5              5               5          5

Summary of lead molecules selected:
            Name     Principal MaxOmitFeat Chirality Confs Feats
                266P        2      1                   117  5.00
                261P        1      1                   131  5.00
                 91P        1      1                   103  4.00
                210P        2      1                   241  5.02
                209P        1      1                   189  5.00
                 21P        1      1                    70  5.00

Summary of feature definitions:
  H: HYDROPHOBIC
     Hydrophobic:  Tolerance= 160
  N: NegIonizable
     NegIon-pnt:  Tolerance= 160
  P: positive
     #Any Atom#2:  Tolerance= 160


Summary of feature definition hit statistics:
           HYDROPHOBIC  hits/lead:  mean= 0.84  stddev=  0.41
          NegIonizable  hits/lead:  mean= 3.00  stddev=  0.00
              positive  hits/lead:  mean= 1.00  stddev=  0.00


Summary of feature definition hits by molecule:
                         H      N      P
                266P   1.00   3.00   1.00
                261P   1.00   3.00   1.00
                 91P   0.00   3.00   1.00
                210P   1.02   3.00   1.00
                209P   1.00   3.00   1.00
                 21P   1.00   3.00   1.00

Summary of ranking coefficients:
  Constant: -6.6
  Dispersion: 0.0054
  H: 1.9
  N: 3.8
  P: 2.7

Processing:
 Generating hypos with NH: [c].. 938
 Generating hypos with PH: [c].. 301
 Generating hypos with NN: [c].. 716
 Generating hypos with PN: [c].. 1073
 Generating hypos with NNH: [c].. 558
 Generating hypos with PNH: [c].. 829
 Hypos with features NH not desired. Freeing memory.
 Hypos with features PH not desired. Freeing memory.
 Generating hypos with NNN: [c].. 0
 Generating hypos with PNN: [c].. 715
 Hypos with features NN not desired. Freeing memory.
 Hypos with features PN not desired. Freeing memory.
 Generating hypos with PNNH: [c].. 1004
```

```
 Reprocessing hypos with PNNH: 5 remaining.
 Hypos with features NNH not desired. Freeing memory.
 Hypos with features PNH not desired. Freeing memory.
 Hypos with features PNN not desired. Freeing memory.
 Inspecting hypos with PNNH

Exporting hypotheses:
1 PNNH  Rank: 62.1099  DH: 111011  PH: 000100
2 PNNH  Rank: 61.53  DH: 111011  PH: 000100
3 PNNH  Rank: 57.6212  DH: 111011  PH: 000100
4 PNNH  Rank: 57.175  DH: 111011  PH: 000100
5 PNNH  Rank: 56.6978  DH: 111011  PH: 000100
Halted normally.
CPU time = 9 s  System time = 0 s
```

Table S4. *E. coli* isoprenoid, menaquinone and ubiquinone biosynthesis pathway genes and their relative changes in gene expression. UP and DOWN represent increases or decreases in gene transcription, compared to the control values.

Gene	Blattner Number	Treatment					Product
		13	Fosm	13-Fosm	Carb	Cipro	
dxs	b0420	DOWN	DOWN	UP			1-deoxyxylulose-5-phosphate synthase
dxr	b0173	UP	UP				1-deoxyxylulose-5-phosphate reductoisomerase
ispD	b2747						4-diphosphocytidyl-2C-methyl-D-erythritol synthetase
ispE	b1208						4-diphosphocytidyl-2C-methyl-D-erythritol kinase
ispF	b2746		UP				2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
ispG	b2515			UP			1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase
ispH	b0029			UP	UP		1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase
idi	b2889			UP			isopentenyl diphosphate isomerase
ispA	b0421	DOWN	DOWN	UP	UP	UP	geranyl/farnesyl diphosphate synthase
ispB	b3187	DOWN	DOWN				octaprenyl diphosphate synthase
uppS	b0174	UP	UP				undecaprenyl diphosphate synthase
menA	b3930				DOWN		1,4-dihydroxy-2-naphthoate octaprenyltransferase
menB	b2262			UP	UP	UP	naphthoate synthase
menC	b2261						O-succinylbenzoate-CoA synthase
menD	b2264	DOWN					putative 2-hydroxyglutarate synthase/SHCHC synthase/2-oxoglutarate decarboxylase
menE	b2260					DOWN	O-succinylbenzoic acid-CoA ligase
menF	b2265		UP				isochorismate synthase, menaquinone-specific
pqqL	b1494	UP	UP	DOWN	DOWN	DOWN	putative zinc peptidase
purR	b1658	DOWN	DOWN			DOWN	PurR transcriptional repressor
ubiA	b4040			DOWN	DOWN		4-hydroxybenzoate octaprenyltransferase
ubiB	b3835					UP	2-octaprenylphenol hydroxylase
ubiC	b4039		UP	DOWN			chorismate pyruvate lyase
ubiD	b3843					UP	3-octaprenyl-4-hydroxybenzoate decarboxylase
ubiE	b3833					UP	S-adenosylmethionine:2-DMK

					methyltransferase/2-octaprenyl-6-methoxy-1,4-benzoquinone methylase
ubiF	b0662			UP	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone hydroxylase
ubiG	b2232	DOWN	DOWN		3-demethylubiquinone 3-methyltransferase/2-octaprenyl-6-hydroxyphenol methylase
ubiH	b2907			UP	2-octaprenyl-6-methoxyphenol hydroxylase
ubiX	b2311	UP	UP	UP	3-octaprenyl-4-hydroxybenzoate decarboxylase 2

Table S5. *E. coli* peptidoglycan biosynthesis genes and their relative changes in gene expression. UP and DOWN represent increases or decreases in gene transcription, compared to the control values.

Gene	Blattner Number	Treatment					Product
		13	Fosm	13-Fosm	Carb	Cipro	
amiA	b2435						N-acetylmuramoyl-L-alanine amidase 1
amiB	b4169			UP	UP	UP	N-acetylmuramoyl-L-alanine amidase 2
amiC	b2817			UP			N-acetylmuramyl-L-alanine amidase
ampC	b4150						beta-lactamase; penicillin resistance
ampG	b0433						AmpG muropeptide MFS transporter
ampD	b0110						regulates ampC
cycA	b4208						CycA serine/alanine/glycine APC transporter
dacB	b3182				UP	UP	D-alanyl-D-alanine carboxypeptidase, fraction B; penicillin-binding protein 4
ddlA	b0381			DOWN			D-alanine-D-alanine ligase A
ddlB	b0092			UP		UP	D-alanine-D-alanine ligase B
ddpX	b1488					DOWN	D-Ala-D-Ala dipeptidase
emtA	b1193				DOWN	DOWN	murein transglycosylase E
ftsI	b0084			UP			essential cell division protein FtsI; penicillin-binding protein 3
glmM	b3176			UP		UP	phosphoglucosamine mutase
glmS	b3729					UP	subunit of L-glutamine:D-fructose-6-phosphate aminotransferase
glmU	b3730					UP	subunit of N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase
hipA	b1507			DOWN	DOWN	DOWN	HipA transcriptional activator
hipB	b1508						HipB transcriptional activator
lpp	b1677			UP	UP	UP	murein lipoprotein
mepA	b2328						subunit of murein DD-endopeptidase, penicillin-insensitive
mipA	b1782						Scaffolding protein that interacts with murein polymerase and murein hydrolase
mltA	b2813			UP			membrane-bound lytic murein transglycosylase A
mltB	b2701			UP	UP		membrane-bound lytic murein transglycosylase B
mltC	b2963				UP		membrane-bound lytic murein transglycosylase C

mraY	b0087		DOWN		phospho-N-acetylmuramoyl-pentapeptide transferase
mrcA	b3396	UP		UP	peptidoglycan synthetase; penicillin-binding protein 1A
mrcB	b0149	UP	UP	UP	peptidoglycan synthetase; penicillin-binding protein 1B
mrdA	b0635				cell shape; peptidoglycan synthetase; penicillin-binding protein 2
mreC	b3250	UP	UP	UP	rod shape-determining protein
mreD	b3249			UP	rod shape-determining protein
mtgA	b3208		DOWN		putative peptidoglycan enzyme
murA	b3189				UDP-N-acetylglucosamine enolpyruvoyl transferase
murB	b3972		UP	UP	UDP-N-acetylmuramate dehydrogenase
murC	b0091	UP			UDP-N-acetylmuramate-alanine ligase
murD	b0088	DOWN	DOWN	DOWN	UDP-N-acetylmuramoylalanine-D-glutamate ligase
murE	b0085				UDP-N-acetylmuramoylalanyl-D-glutamate 2,6-diaminopimelate ligase
murF	b0086				D-alanyl-D-alanine-adding enzyme
murG	b0090				N-acetylglucosaminyl transferase
murI	b3967				subunit of glutamate racemase
pbpC	b2519			DOWN	putative peptidoglycan enzyme
pbpG	b2134			DOWN	penicillin-binding protein 7
prc	b1830	DOWN	DOWN	DOWN	tail-specific protease
slt	b4392	UP		UP	soluble lytic murein transglycosylase
dacC	b0839			DOWN	penicillin-binding protein 6
dacA	b0632				D-alanyl-D-alanine carboxypeptidase, fraction A; penicillin-binding protein 5
dacD	b2010			DOWN	DD-carboxypeptidase, penicillin-binding protein 6b

Table S6. *E. coli* DNA replication genes and their relative changes in gene expression. UP and DOWN represent increases or decreases in gene transcription, compared to the control values.

Gene	Blattner Number	Treatment					Product
		13	Fosm	13-Fosm	Carb	Cipro	
argP	b2916						ArgP transcriptional activator
cspD	b0880			UP			DNA replication inhibitor
dfp	b3639			UP	UP	UP	P-pantothenate cysteine ligase/P-pantothenoylcysteine decarboxylase
diaA	b3149					UP	DiaA protein
dinB	b0231					UP	DNA polymerase IV (Y-family DNA polymerase; translesion DNA synthesis)
dnaA	b3702					UP	DnaA-ATP transcriptional dual regulator
dnaB	b4052						chromosome replication; chain elongation; part of primosome
dnaC	b4361			UP	UP	UP	chromosome replication; initiation and chain elongation
dnaE	b0184					UP	DNA polymerase III, alpha subunit
dnaG	b3066					UP	DNA biosynthesis; DNA primase
dnaN	b3701					UP	DNA polymerase III, beta-subunit
dnaQ	b0215			DOWN			DNA polymerase III, epsilon subunit
dnaT	b4362			UP	UP	UP	DNA biosynthesis; primosomal protein i
dnaX	b0470						DNA polymerase III, tau and gamma subunits; DNA elongation factor III
gyrA	b2231						DNA gyrase, subunit A
gyrB	b3699					UP	DNA gyrase, subunit B
hda	b2496					DOWN	regulator of DnaA that prevents premature reinitiation of DNA replication
helD	b0962			UP			DNA helicase IV
holA	b0640						DNA polymerase III, delta subunit
holB	b1099			UP			DNA polymerase III, delta prime subunit
holC	b4259			DOWN		UP	DNA polymerase III, chi subunit
holD	b4372			UP	UP	UP	DNA polymerase III, psi subunit
holE	b1842						DNA polymerase III, theta subunit
hrpA	b1413			UP			helicase, ATP-dependent
hrpB	b0148						helicase, ATP-dependent
lhr	b1653					DOWN	member of ATP-dependent helicase superfamily II
mioC	b3742					UP	flavoprotein involved in biotin synthesis

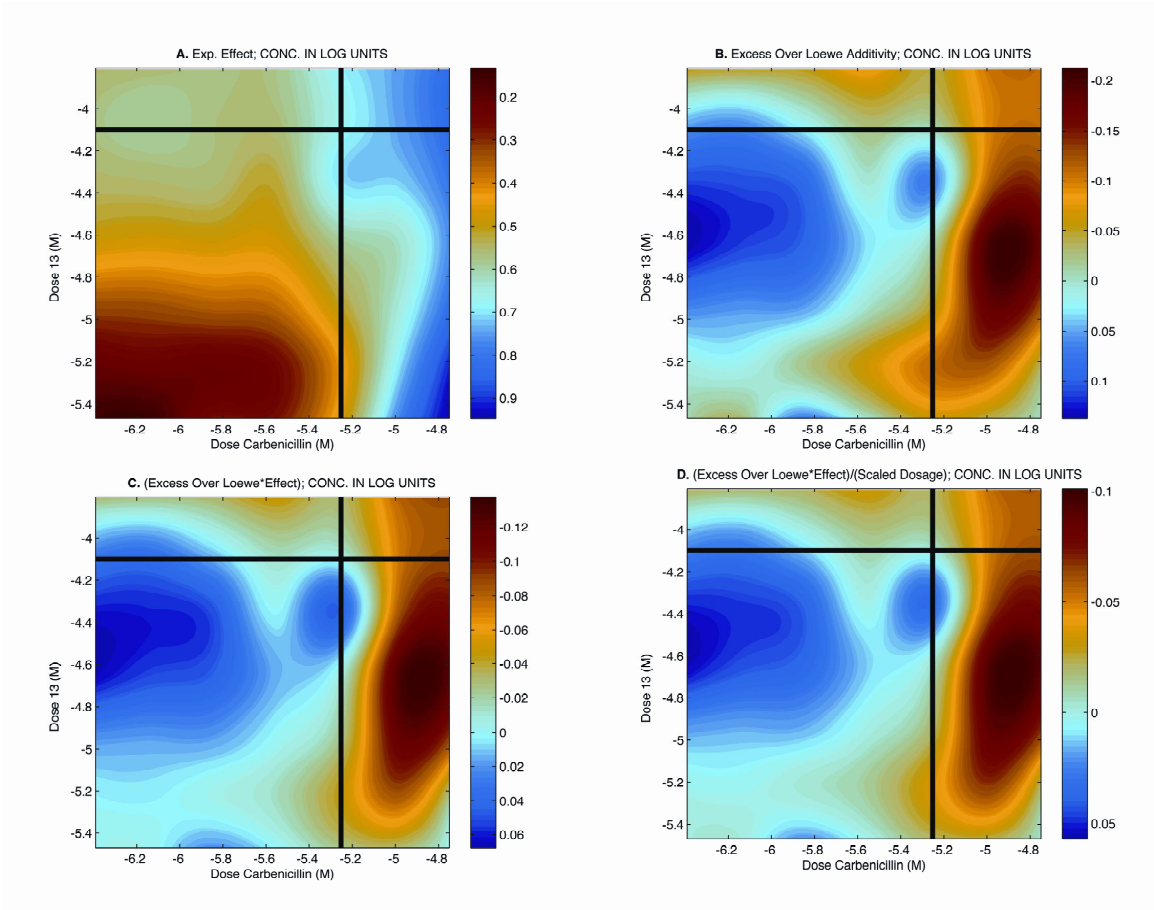
parC	b3019				Topoisomerase IV subunit A
parE	b3030			UP	Topoisomerase IV subunit B
pioO	b3322	DOWN			calcium-binding protein required for initiation of chromosome replication
polA	b3863			UP	DNA polymerase I, 3' --> 5' polymerase, 5' --> 3' and 3' --> 5' exonuclease
polB	b0060				DNA polymerase II
priA	b3935			UP	primosomal protein N'(= factor Y)(putative helicase)
priB	b4201		UP	UP	primosomal replication protein N
priC	b0467	UP	UP		primosomal replication protein N''
rarA	b0892	UP			recombination factor
rdgC	b0393			DOWN	nonspecific DNA binding protein; nucleoid component
recF	b3700			UP	ssDNA and dsDNA binding, ATP binding
recG	b3652	DOWN			RecG, DNA helicase, resolution of Holliday junctions, branch migration
recQ	b3822			UP	ATP-dependent DNA helicase
rep	b3778			UP	rep helicase, a single-stranded DNA dependent ATPase
rnhA	b0214				RNase HI, degrades RNA of DNA-RNA hybrids, participates in DNA replication
rob	b4396	UP	UP	UP	Rob transcriptional activator
sbmC	b2009		DOWN	UP	DNA gyrase inhibitor
seqA	b0687				SeqA, negative modulator of initiation of replication
spf	b3864	UP	DOWN	UP	Spf RNA
topA	b1274			DOWN	DNA topoisomerase I
topB	b1763			DOWN	DNA topoisomerase III
tus	b1610				DNA-binding protein; inhibition of replication at Ter sites
umuC	b1184				SOS mutagenesis and repair
umuD	b1183	DOWN		UP	SOS mutagenesis; error-prone repair; processed to UmuD'; forms complex with UmuC
uvrD	b3813	UP	UP	UP	DNA-dependent ATPase I and helicase II
ycdX	b1034			DOWN	putative DNA polymerase subunit

Table S7. *E. coli* SOS response genes and their relative changes in gene expression. UP and DOWN represent increases or decreases in gene transcription, compared to the control values.

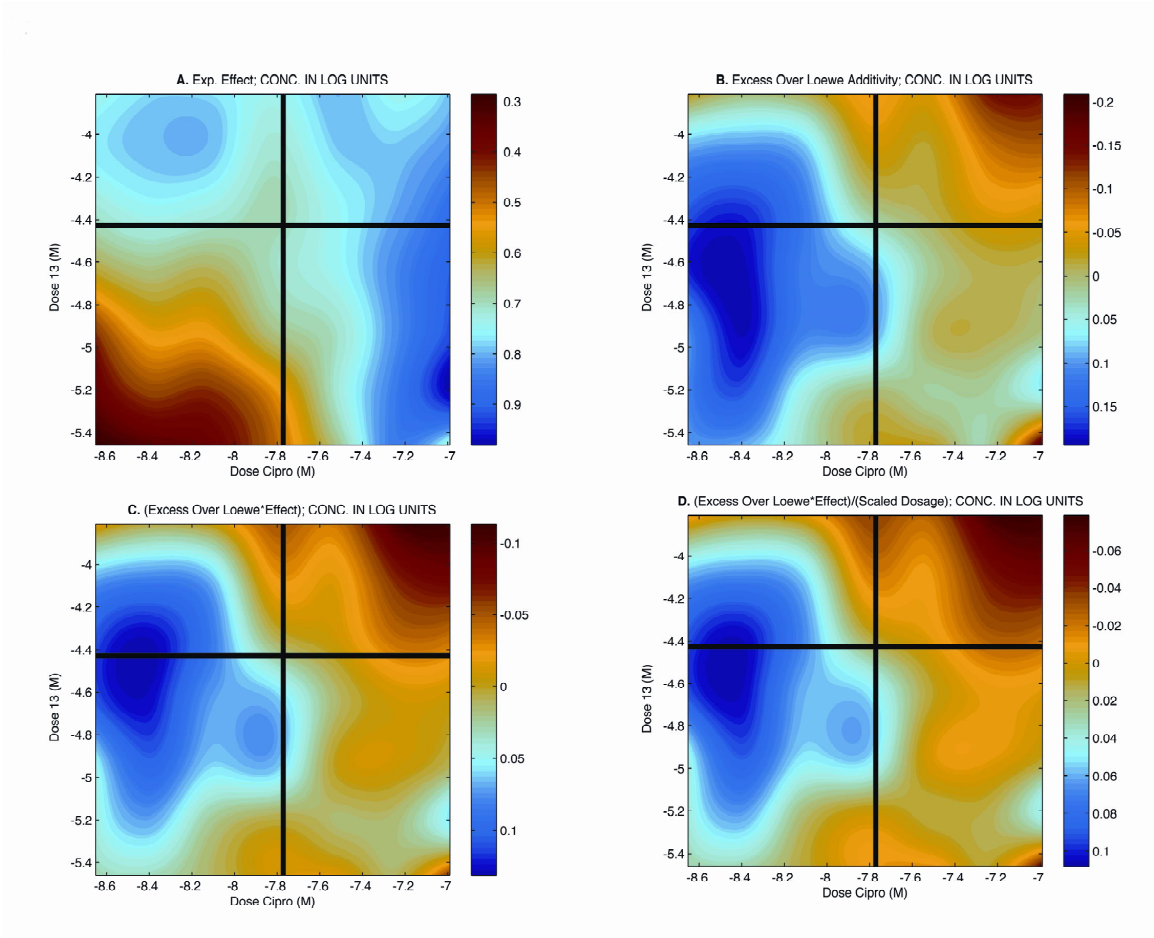
Gene	Blattner Number	Treatment					Product
		13	Fosm	13-Fosm	Carb	Cipro	
cho	b1741	DOWN				DOWN	DNA repair endonuclease that acts together with UvrC in incision repair of some DNA lesions
dinB	b0231						DNA polymerase IV (Y-family DNA polymerase; translesion DNA synthesis)
dinF	b4044	UP		DOWN	DOWN		DinF MATE Transporter
dinG	b0799			UP			ATP-dependent helicase
dinI	b1061	UP				UP	damage-inducible protein I
ftsK	b0890						essential cell division protein FtsK
lexA	b4043			DOWN	DOWN	UP	LexA transcriptional repressor
polB	b0060	DOWN					DNA polymerase II
recA	b2699	DOWN		UP		UP	DNA strand exchange and renaturation, DNA-dependent ATPase, DNA- and ATP-dependent coprotease
recG	b3652			DOWN			DNA helicase, resolution of Holliday junctions, branch migration
recN	b2616					UP	protein used in recombination and DNA repair
recX	b2698						inhibitor of RecA
rfaG	b3631	UP		DOWN	DOWN	UP	lipopolysaccharide core biosynthesis; glucosyltransferase I
ruvA	b1861			UP		UP	branch migration of Holliday structures; repair
ruvB	b1860			UP			branch migration of Holliday structures; repair helicase
ruvC	b1863					DOWN	Holliday junction nuclease; resolution of structures; repair
sbmC	b2009				DOWN	UP	DNA gyrase inhibitor
ssb	b4059			UP		UP	ssDNA-binding protein
sulA	b0958					UP	suppressor of lon; inhibits cell division and ftsZ ring formation
umuC	b1184						SOS mutagenesis and repair
umuD	b1183	UP		DOWN		UP	SOS mutagenesis; error-prone repair; processed to UmuD'; forms complex with UmuC
uvrA	b4058	DOWN				UP	excision nuclease subunit A
uvrB	b0779			UP			DNA repair; excision nuclease subunit B
uvrD	b3813	DOWN		UP	UP	UP	DNA-dependent ATPase I and

					helicase II
ybfE	b0685				hypothetical protein, transcriptionally regulated by LexA
ydjM	b1728		DOWN		hypothetical protein
yebG	b1848	DOWN		UP	hypothetical protein; gene is part of SOS regulon
yjiW	b4347	DOWN	DOWN		hypothetical protein

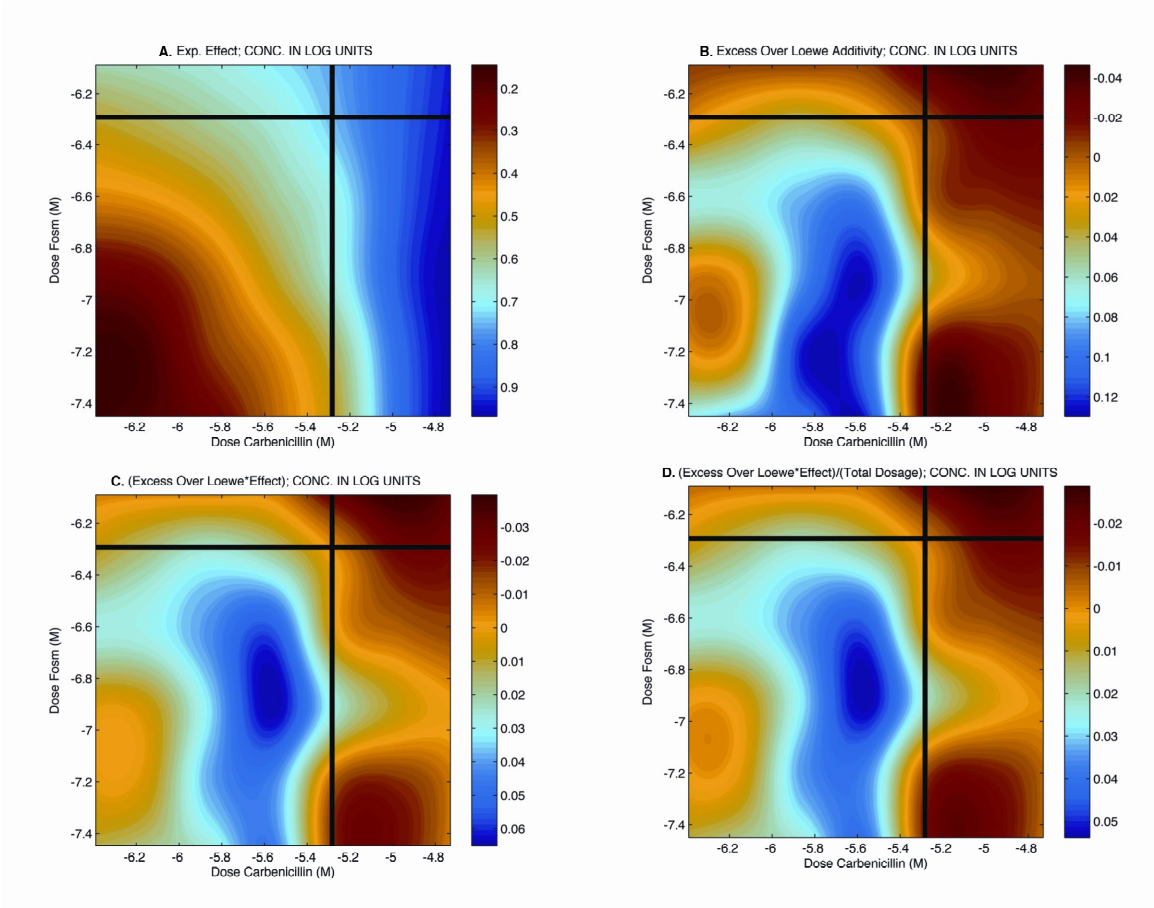
Figures S1. **A.** Contour surface for the fractional effect of the drug combination **13**-carbenicillin. **B.** Contour plot of Loewe additivity difference response surface. Z-axis values greater than zero are indicative of Loewe synergy. **C.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect. **D.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values and divided by a scaled dosage value for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect and lowest combination dosage.


A. Exp. Effect; CONC. IN LOG UNITS
B. Excess Over Loewe Additivity; CONC. IN LOG UNITS
C. (Excess Over Loewe*Effect); CONC. IN LOG UNITS
D. (Excess Over Loewe*Effect)/(Scaled Dosage); CONC. IN LOG UNITS
Dose 13 (M)
Dose Carbenicillin (M)

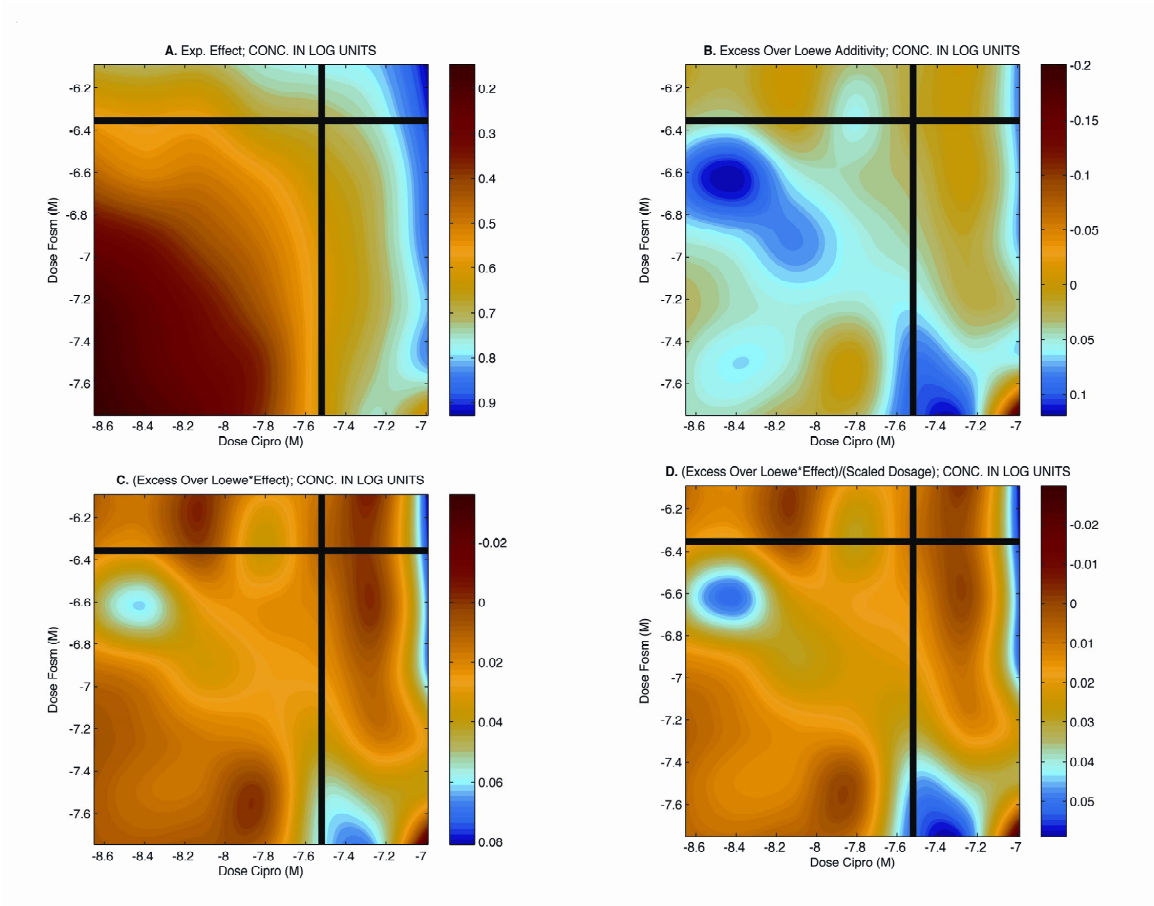
Figures S2. **A.** Contour surface for the fractional effect of the drug combination **13**-ciprofloxacin. **B.** Contour plot of Loewe additivity difference response surface. Z-axis values greater than zero are indicative of Loewe synergy. **C.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect. **D.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values and divided by a scaled dosage value for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect and lowest combination dosage.


A. Exp. Effect; CONC. IN LOG UNITS
B. Excess Over Loewe Additivity; CONC. IN LOG UNITS
C. (Excess Over Loewe*Effect); CONC. IN LOG UNITS
D. (Excess Over Loewe*Effect)/(Scaled Dosage); CONC. IN LOG UNITS
Dose 13 (M)
Dose Cipro (M)

Figures S3. **A.** Contour surface for the fractional effect of the drug combination fomidomycin-carbenicillin. **B.** Contour plot of Loewe additivity difference response surface. Z-axis values greater than zero are indicative of Loewe synergy. **C.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect. **D.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values and divided by a scaled dosage value for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect and lowest combination dosage.


A. Exp. Effect; CONC. IN LOG UNITS
B. Excess Over Loewe Additivity; CONC. IN LOG UNITS
C. (Excess Over Loewe*Effect); CONC. IN LOG UNITS
D. (Excess Over Loewe*Effect)/(Total Dosage); CONC. IN LOG UNITS
Dose Fosm (M)
Dose Carbenicillin (M)

Figures S4. **A.** Contour surface for the fractional effect of the drug combination fosmidomycin-ciprofloxacin. **B.** Contour plot of Loewe additivity difference response surface. Z-axis values greater than zero are indicative of Loewe synergy. **C.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect. **D.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values and divided by a scaled dosage value for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect and lowest combination dosage.


A. Exp. Effect; CONC. IN LOG UNITS
B. Excess Over Loewe Additivity; CONC. IN LOG UNITS
C. (Excess Over Loewe*Effect); CONC. IN LOG UNITS
D. (Excess Over Loewe*Effect)/(Scaled Dosage); CONC. IN LOG UNITS
Dose Fosm (M)
Dose Cipro (M)

Figures S5. **A.** Contour surface for the fractional effect of the drug combination carbenicillin-ciprofloxacin. **B.** Contour plot of Loewe additivity difference response surface. Z-axis values greater than zero are indicative of Loewe synergy. **C.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect. **D.** Contour plot of Loewe additivity difference response values multiplied by fractional effect values and divided by a scaled dosage value for all experimental concentrations. Dark blue here is indicative of Loewe synergy optimized for highest fractional effect and lowest combination dosage.

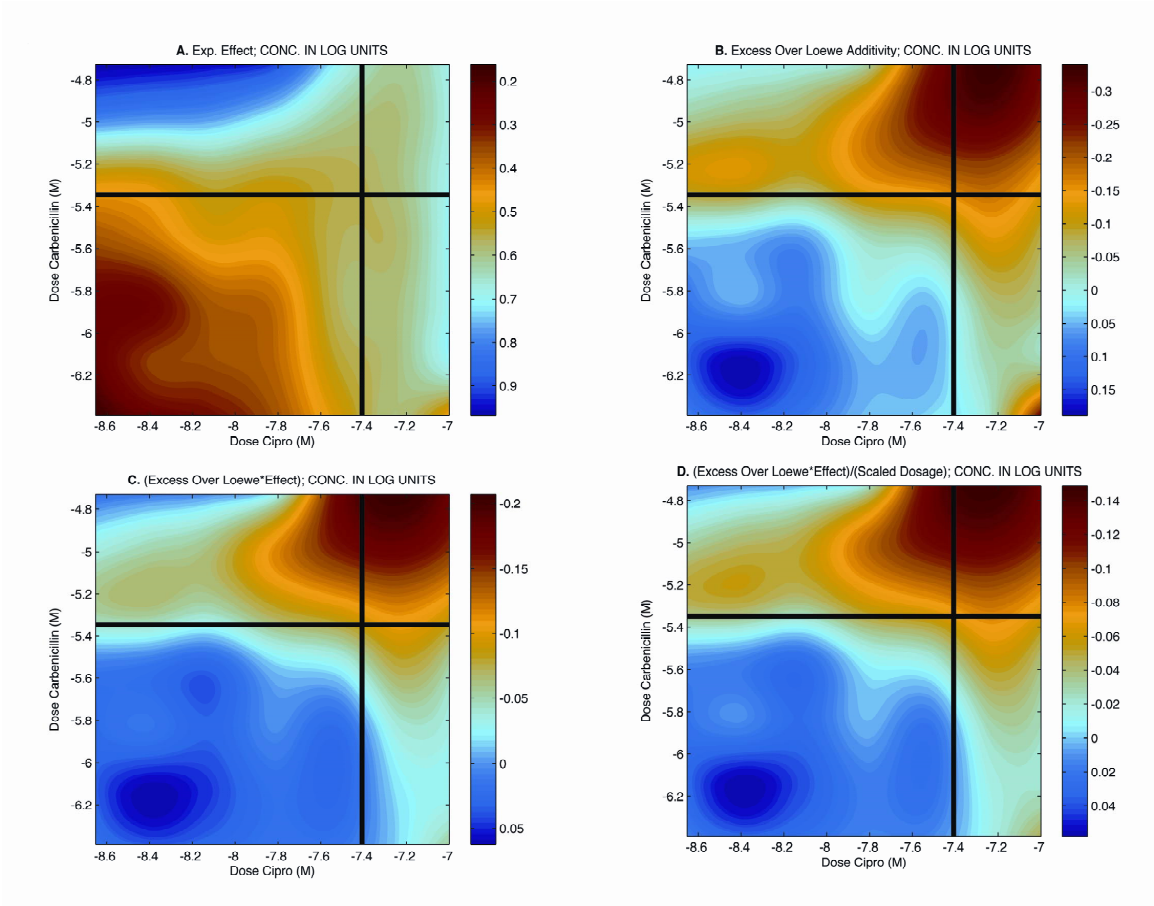

A. Exp. Effect; CONC. IN LOG UNITS
B. Excess Over Loewe Additivity; CONC. IN LOG UNITS
C. (Excess Over Loewe*Effect); CONC. IN LOG UNITS
D. (Excess Over Loewe*Effect)/(Scaled Dosage); CONC. IN LOG UNITS
Dose Carbenicillin (M)
Dose Cipro (M)

Figure S6. IC_{50} isobolograms for each drug combination. For readability, certain values for two or more points with similar CI values are given as an approximate range.

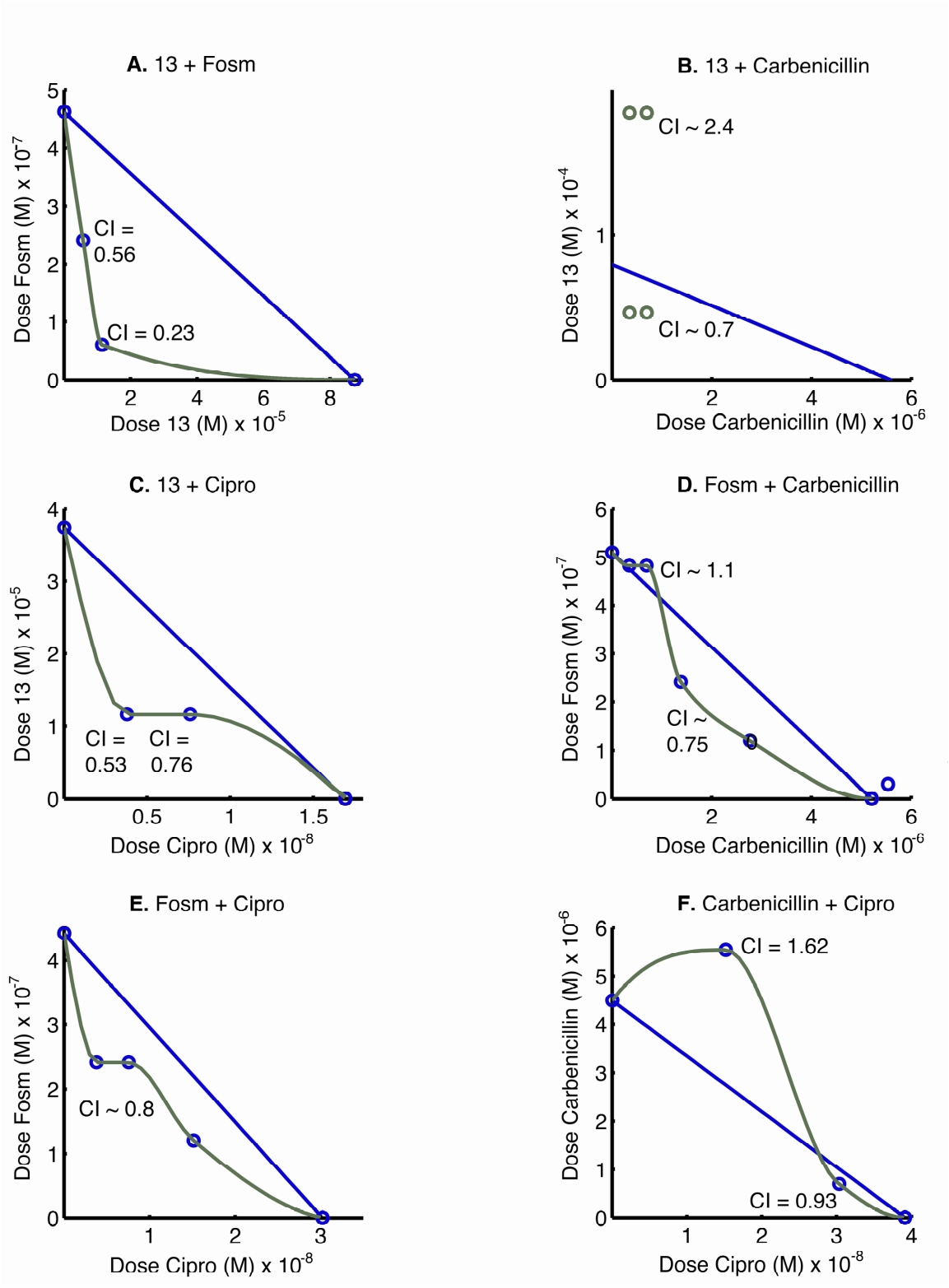
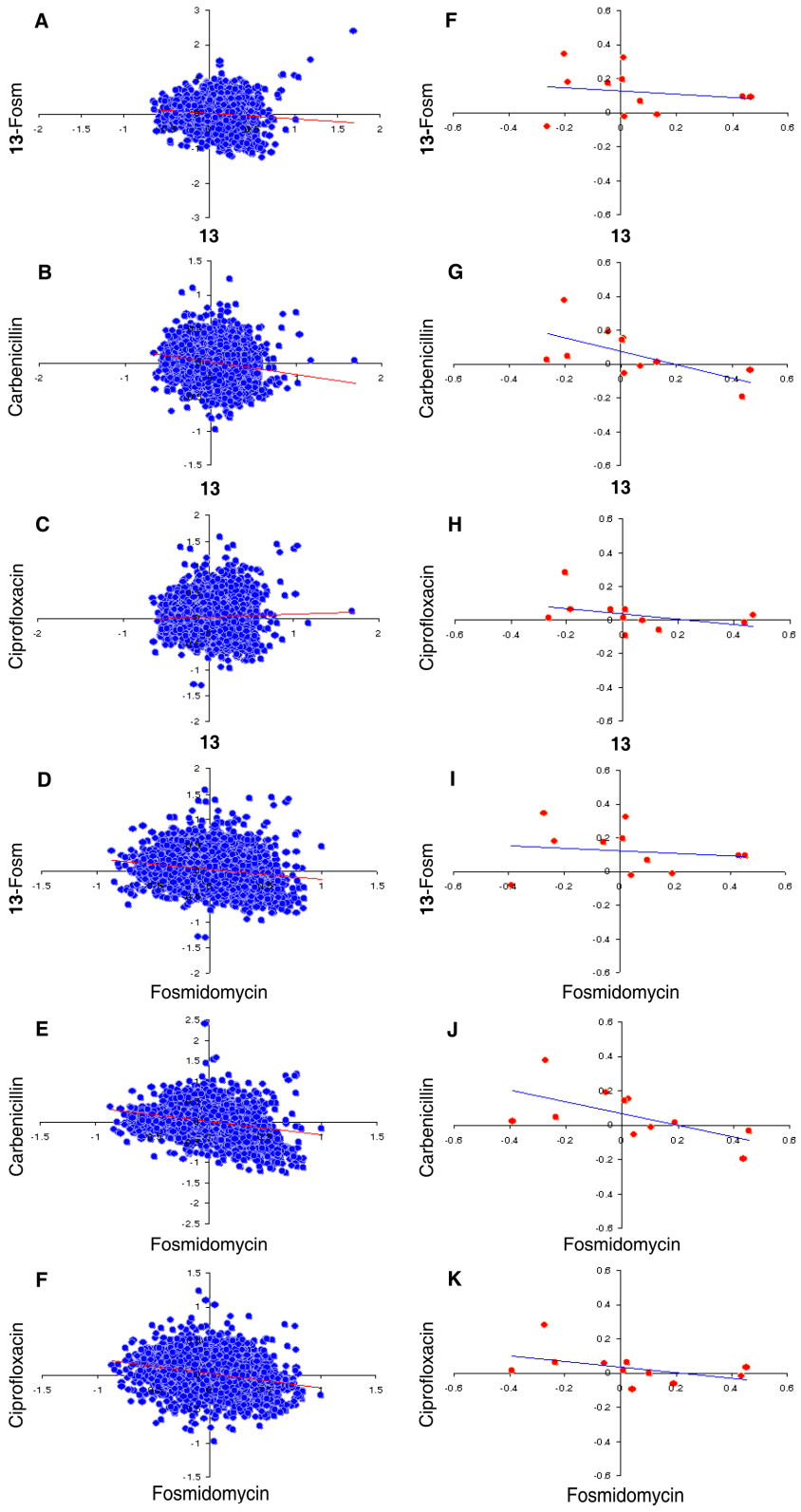

A. 13 + Fosm
Dose Fosm (M) x 10^-7
Dose 13 (M) x 10^-5
CI = 0.56
CI = 0.23
B. 13 + Carbenicillin
Dose 13 (M) x 10^-4
Dose Carbenicillin (M) x 10^-6
CI ~ 2.4
CI ~ 0.7
C. 13 + Cipro
Dose 13 (M) x 10^-5
Dose Cipro (M) x 10^-8
CI = 0.53
CI = 0.76
D. Fosm + Carbenicillin
Dose Fosm (M) x 10^-7
Dose Carbenicillin (M) x 10^-6
CI ~ 1.1
CI ~ 0.75
E. Fosm + Cipro
Dose Fosm (M) x 10^-7
Dose Cipro (M) x 10^-8
CI ~ 0.8
F. Carbenicillin + Cipro
Dose Carbenicillin (M) x 10^-6
Dose Cipro (M) x 10^-8
CI = 1.62
CI = 0.93

Figure S7. Relative increases and decreases in *E. coli* gene expression levels on treatment with **13** or fosmidomycin compared to the combination **13**-fosmidomycin, carbenicillin, or ciprofloxacin. Each point represents the $\log_2$ ratio of the estimated mean signal intensity determined for one treatment to the estimated mean signal intensity of untreated cells. (A,B,C,D,E,F) The 4345 ORFs contained in the Affymetrix GeneChip® antisense *E. coli* genome array. (G,H,I,J,K) The eleven isoprenoid biosynthesis pathway genes. (A,F) **13** versus combination **13**-fosmidomycin (R^2 = 0.0162 and R^2 = 0.0254 respectively). (B,G) **13** versus carbenicillin (R^2 = 0.0428 and R^2 = 0.3903 respectively). (C,H) **13** versus ciprofloxacin (R^2 = 0.0018 and R^2 = 0.1498 respectively). (D,I) Fosmidomycin versus combination **13**-fosmidomycin (R^2 = 0.0227 and R^2 = = 0.0879 respectively). (E,J) Fosmidomycin versus carbenicillin (R^2 = 0.0741 and R^2 = 0.3705 respectively). (F,K) Fosmidomycin versus ciprofloxacin (R^2 = 0.0301 and R^2 = 0.1963 respectively). The data sets are all poorly correlated while the **13** versus fosmidomycin data sets are highly correlated (Figure 7).


A
13-Fosm
13
B
Carbenicillin
13
C
Ciprofloxacin
13
D
13-Fosm
Fosmidomycin
E
Carbenicillin
Fosmidomycin
F
Ciprofloxacin
Fosmidomycin
F
13-Fosm
13
G
Carbenicillin
13
H
Ciprofloxacin
13
I
13-Fosm
Fosmidomycin
J
Carbenicillin
Fosmidomycin
K
Ciprofloxacin
Fosmidomycin