

## Supporting Information

Profiling of  $\beta$ -Lactam Selectivity for Penicillin-Binding Proteins in *Streptococcus pneumoniae* D39

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TABLE S1. Inhibition of Boc-FL binding to PBPs in *S. pneumoniae* with a number of  $\beta$ -lactam antibiotics.

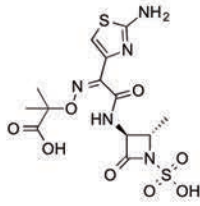
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<sup>e</sup> Equal contribution

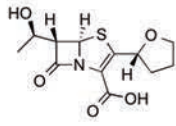
TABLE S2. Inhibition of Boc-FL binding to PBP1b in *S. pneumoniae* IU1945 and IU6647 with amoxicillin, doripenem and meropenem.

**Monobactam**



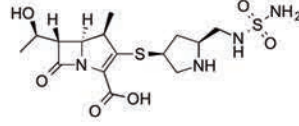
aztreonam

**Penem**

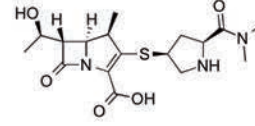


faropenem

**Carbapenem**

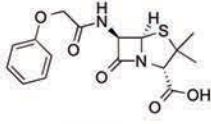


doripenem

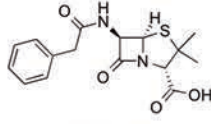


meropenem

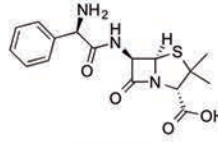
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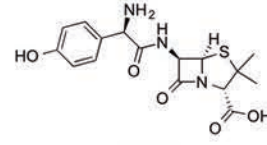
penicillin V



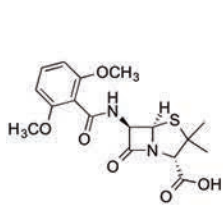
penicillin G



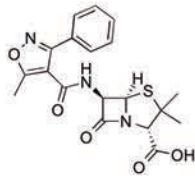
ampicillin



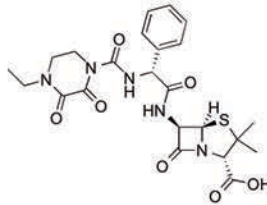
amoxicillin



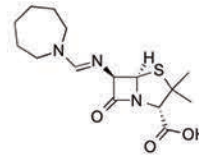
methicillin



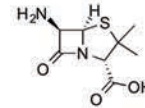
oxacillin



piperacillin

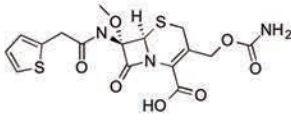


mecillinam

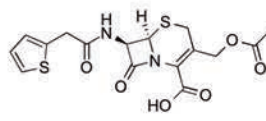


6-APA

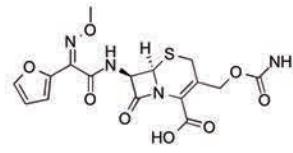
**Cephalosporin**



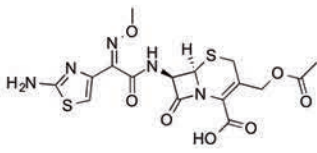
cefoxitin



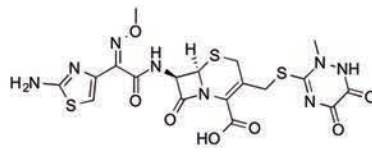
cephalothin



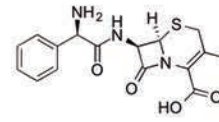
cefuroxime



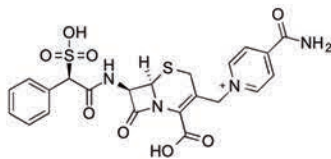
cefotaxime



ceftriaxone



cephalexin



cefsulodin

FIG. S1. Structures of the  $\beta$ -lactam antibiotics used in this study.

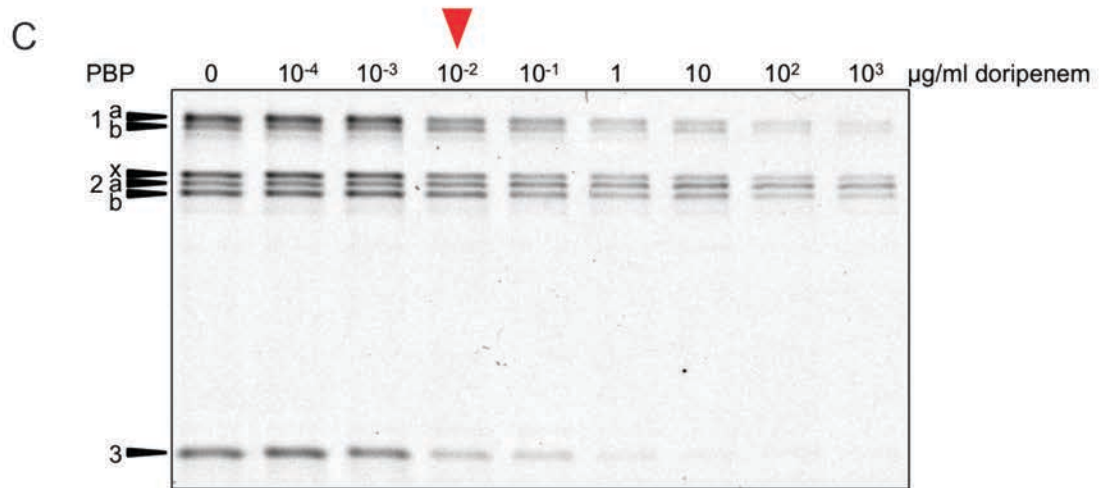
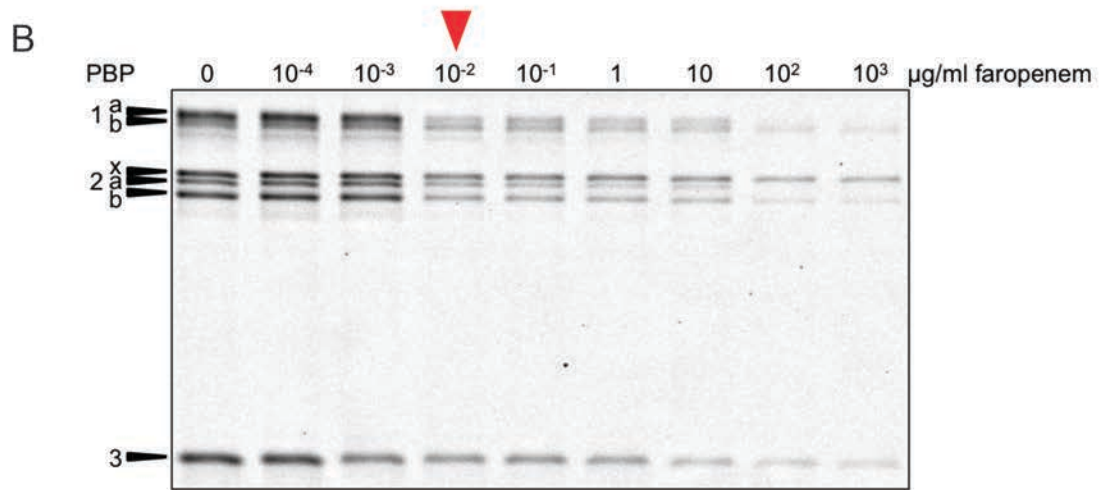
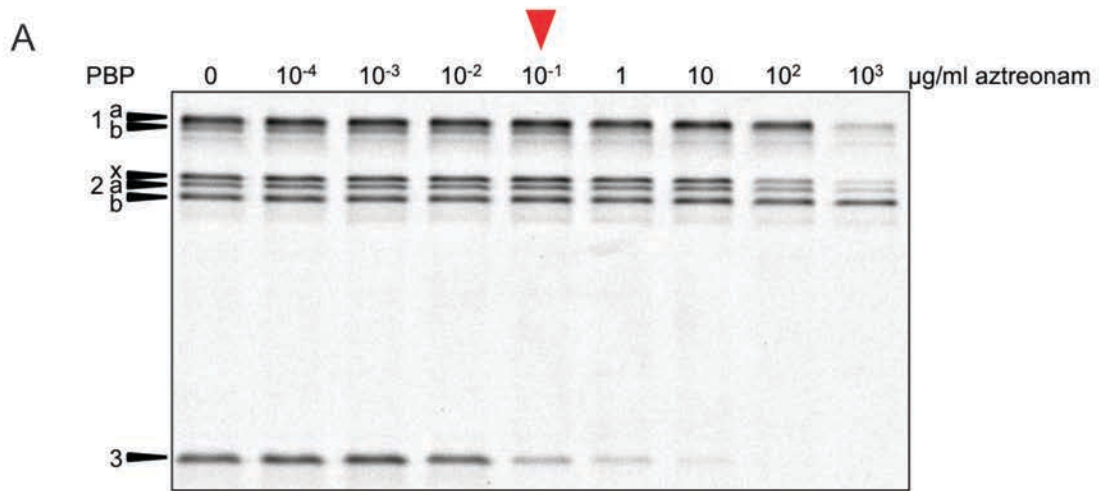


FIG. S2 (continued on next page)

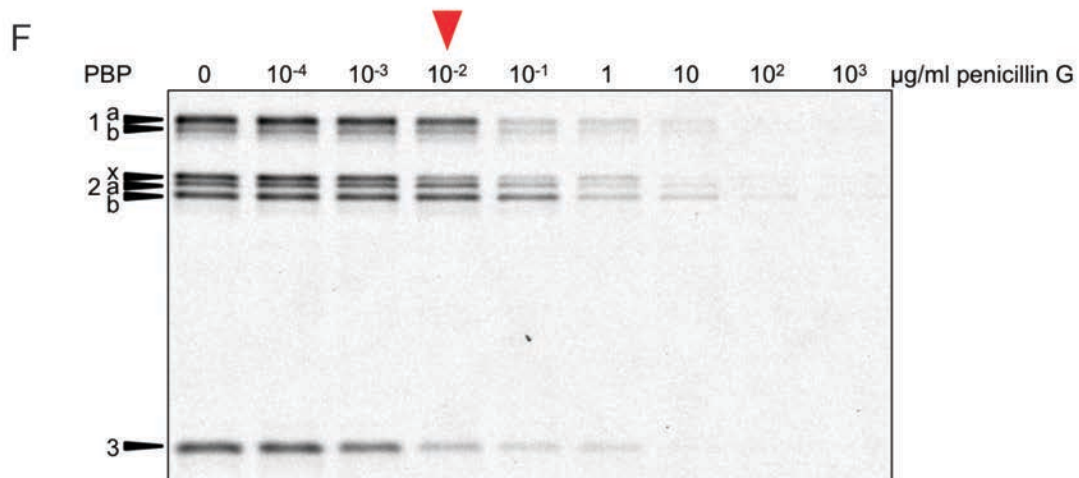
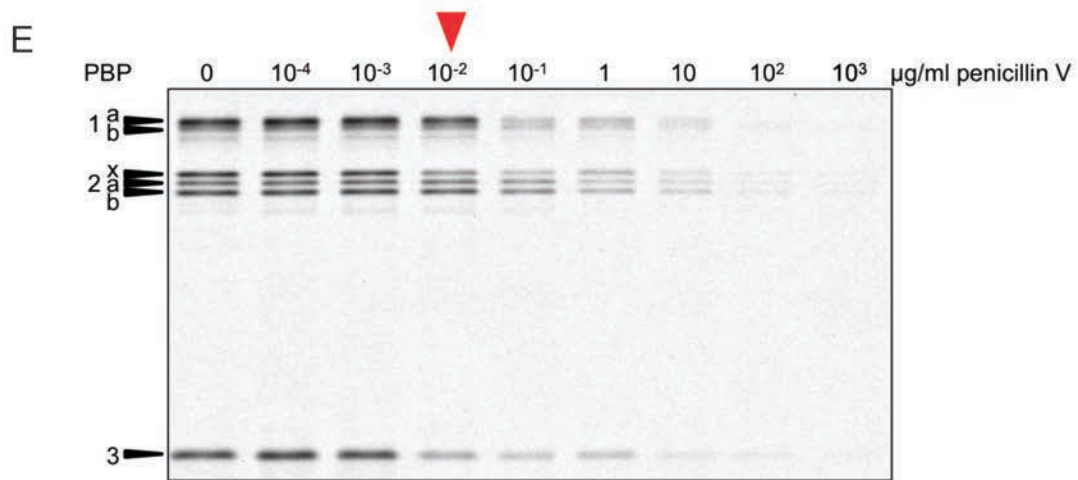
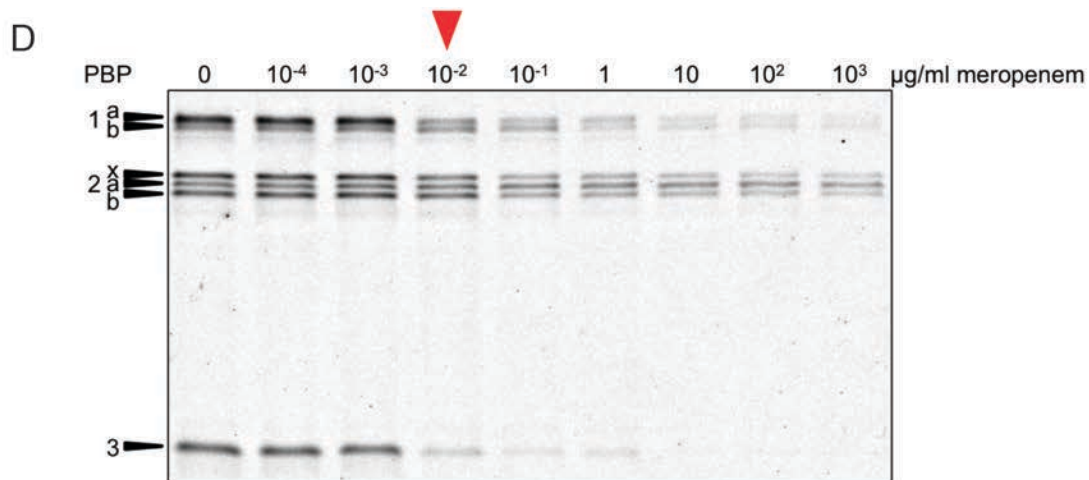


FIG. S2 (continued)

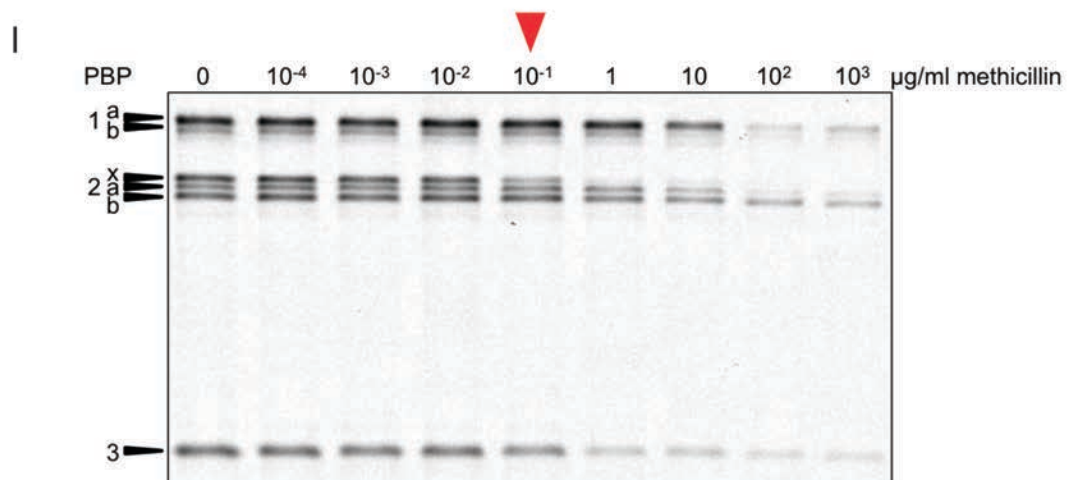
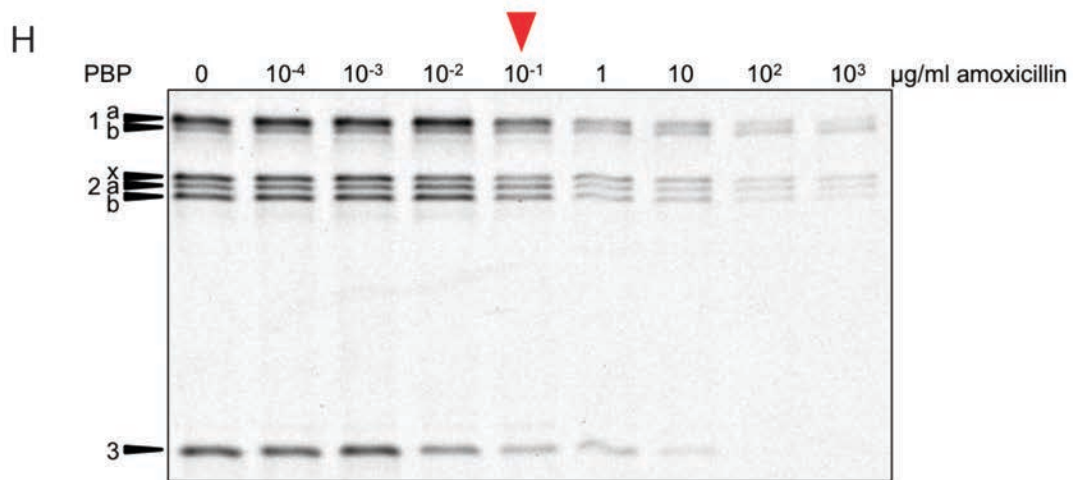
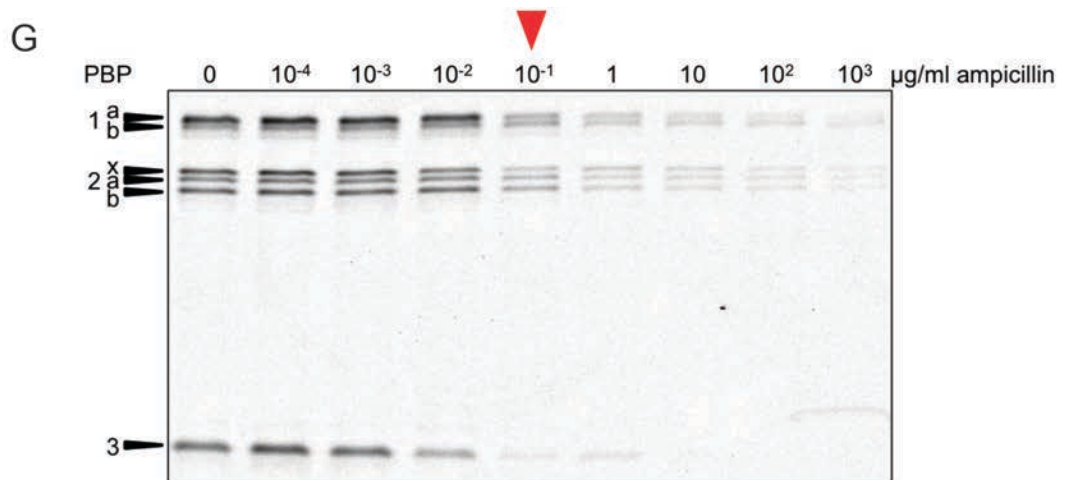


FIG. S2 (continued)



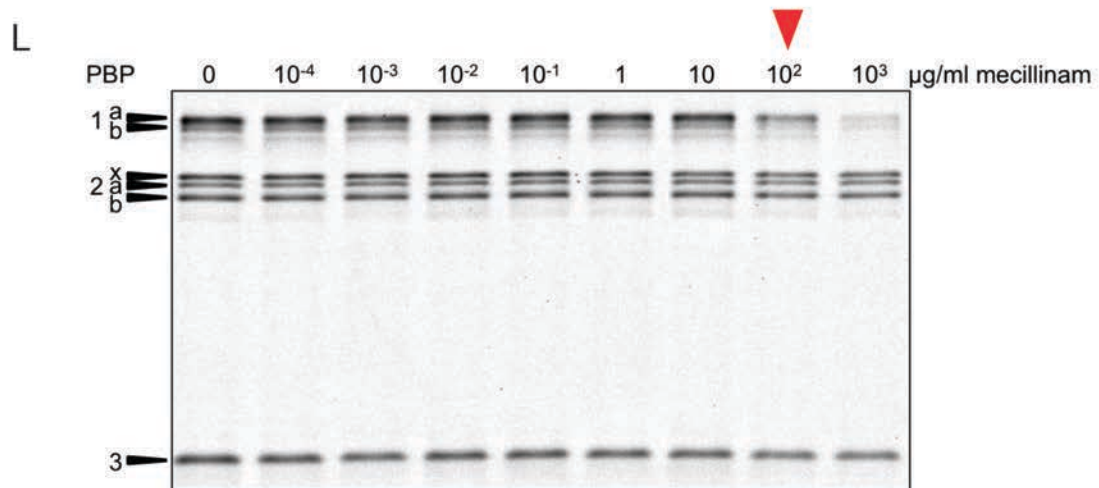
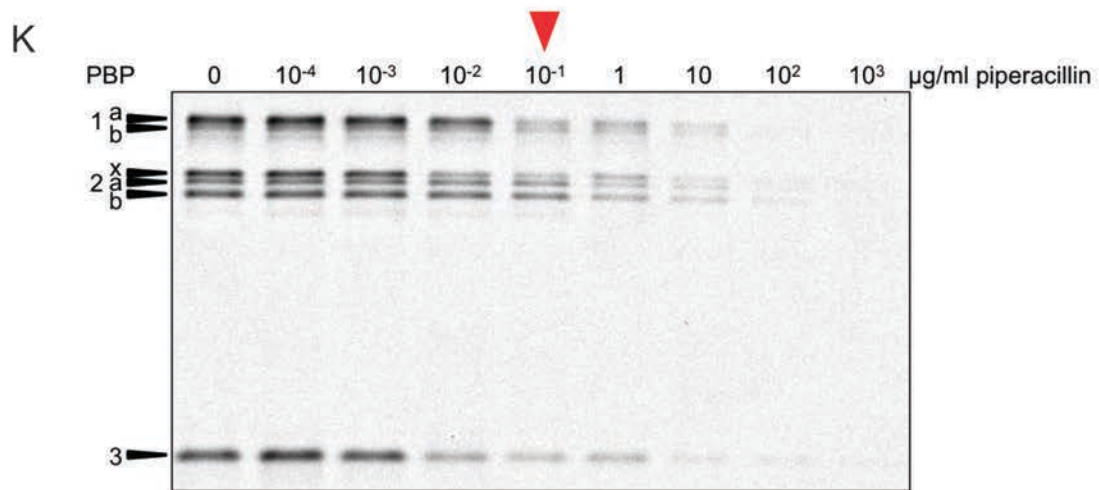
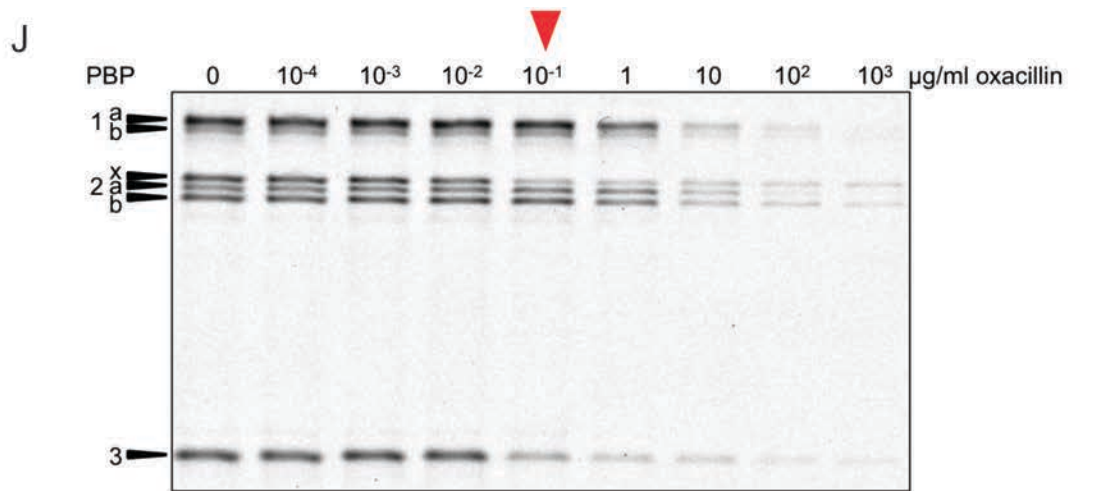
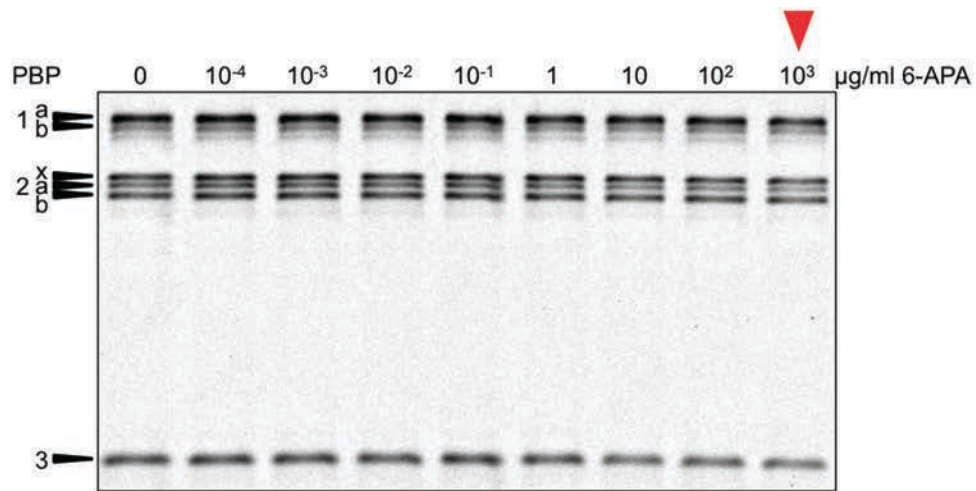
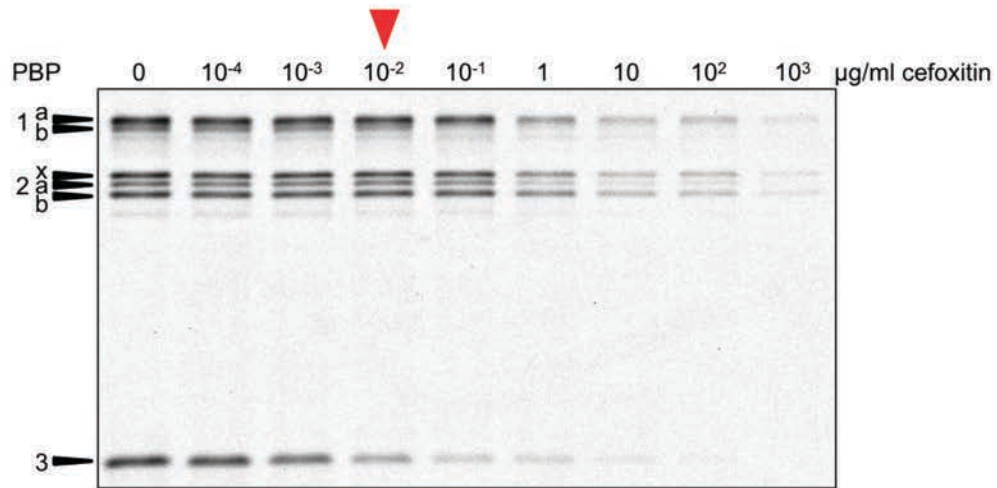


FIG. S2 (continued)

M



N



O

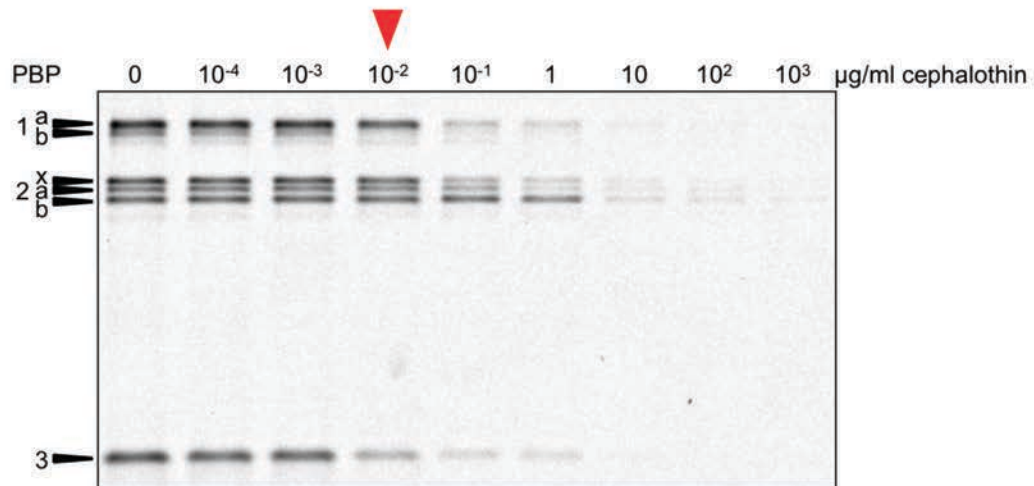


FIG. S2 (continued)



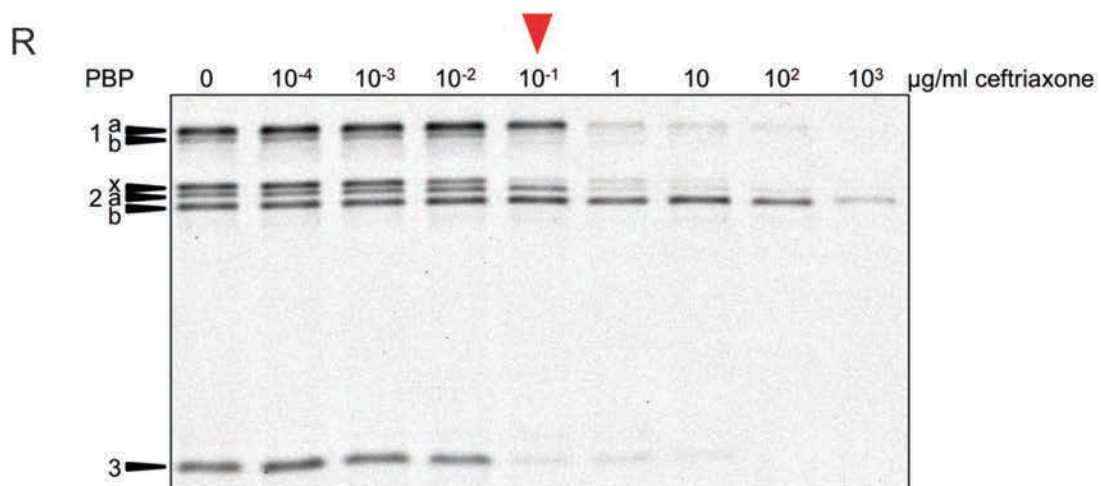
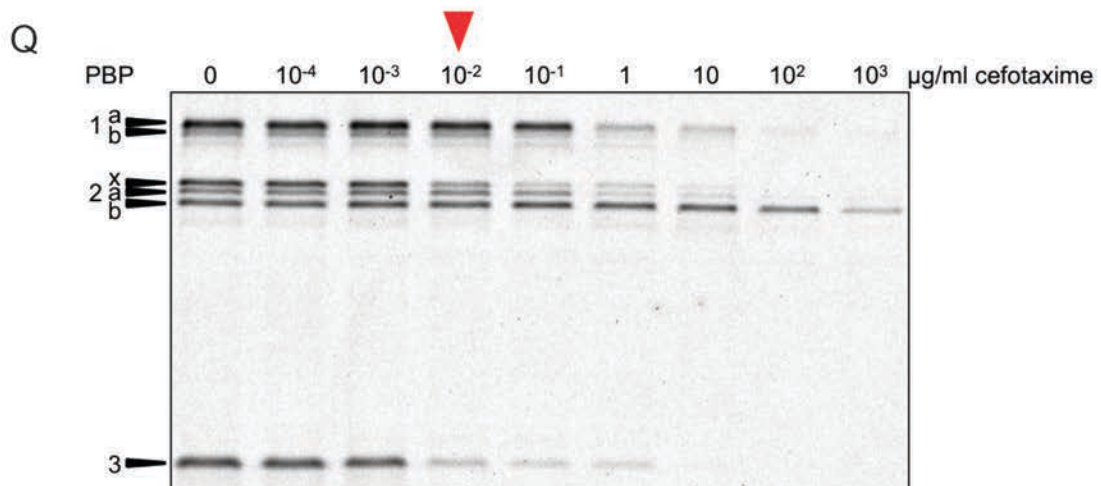
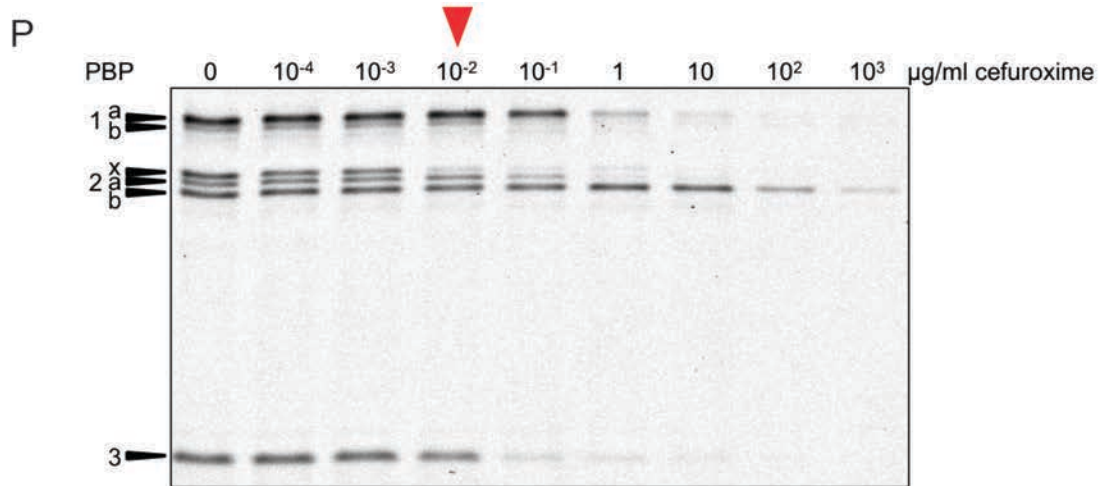


FIG. S2 (continued)

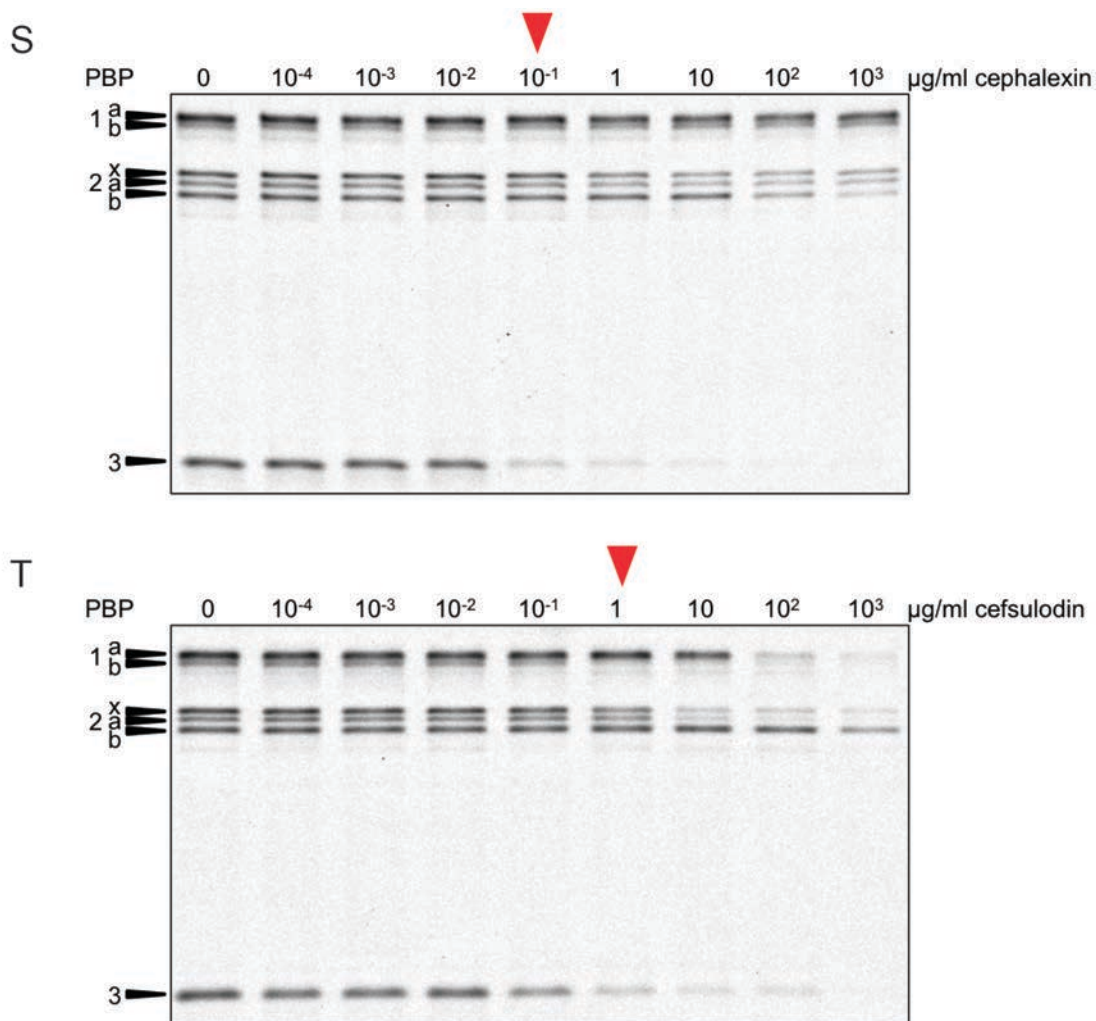


FIG. S2. Representative SDS-PAGE gels for  $\beta$ -lactam titration of PBPs in *S. pneumoniae* IU1945. Whole cells were treated with various concentrations of antibiotics and subsequently labeled with BOCILLIN FL (Boc-FL) (A) aztreonam, (B) faropenem, (C) doripenem, (D) meropenem, (E) penicillin V, (F) penicillin G, (G) ampicillin, (H) amoxicillin, (I) methicillin, (J) oxacillin, (K) piperacillin, (L) mecillinam, (M) (+)-6-Aminopenicillanic acid (6-APA), (N) ceftiofloxacin, (O) cephalothin, (P) cefuroxime, (Q) cefotaxime, (R) ceftriaxone, (S) cephalexin, (T) cefsulodin. Red arrow shows the lowest concentration of antibiotic that resulted in  $\leq 50\%$  Boc-FL labeling for at least one PBP.

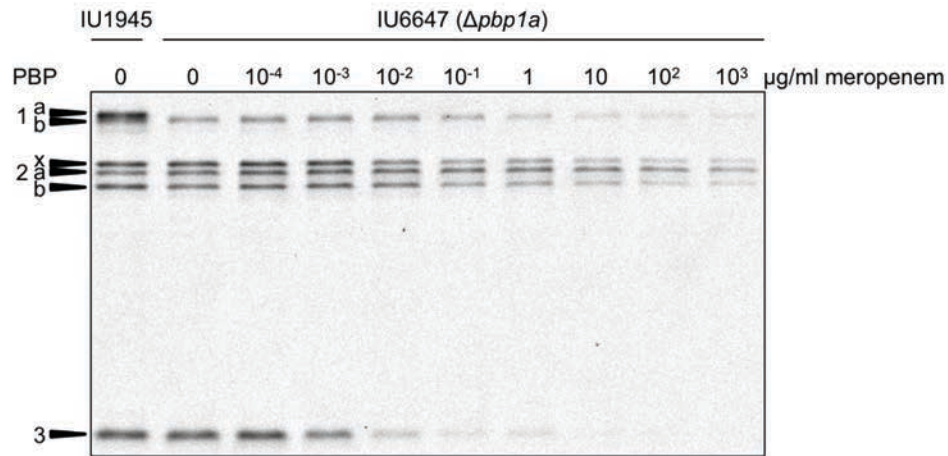


FIG. S3. Representative SDS-PAGE gel for  $\beta$ -lactam (meropenem) titration of PBPs in *S. pneumoniae* IU6647 (IU1945  $\Delta pbp1a::P-erm$ ).

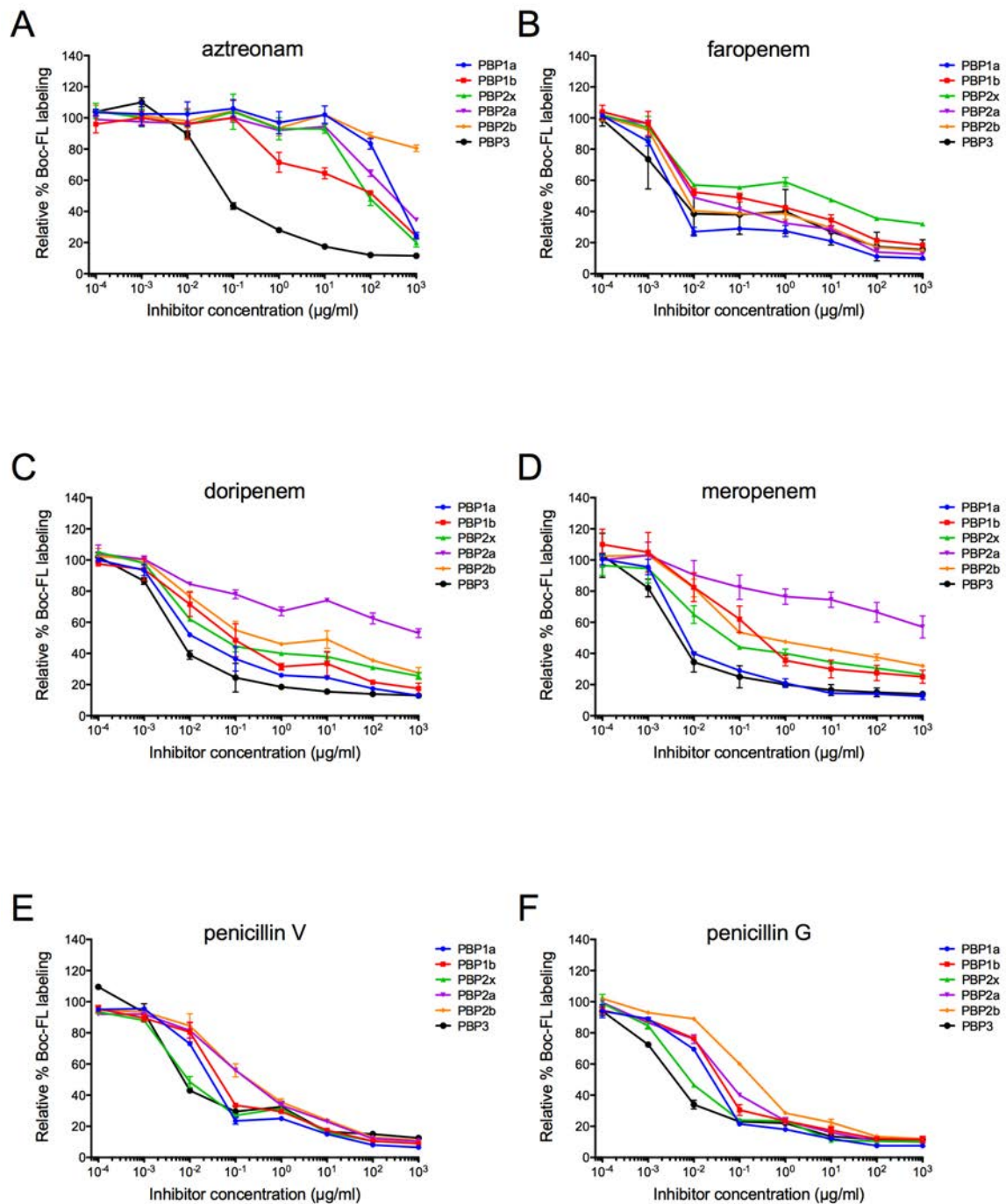


FIG. S4 (continued on next page)

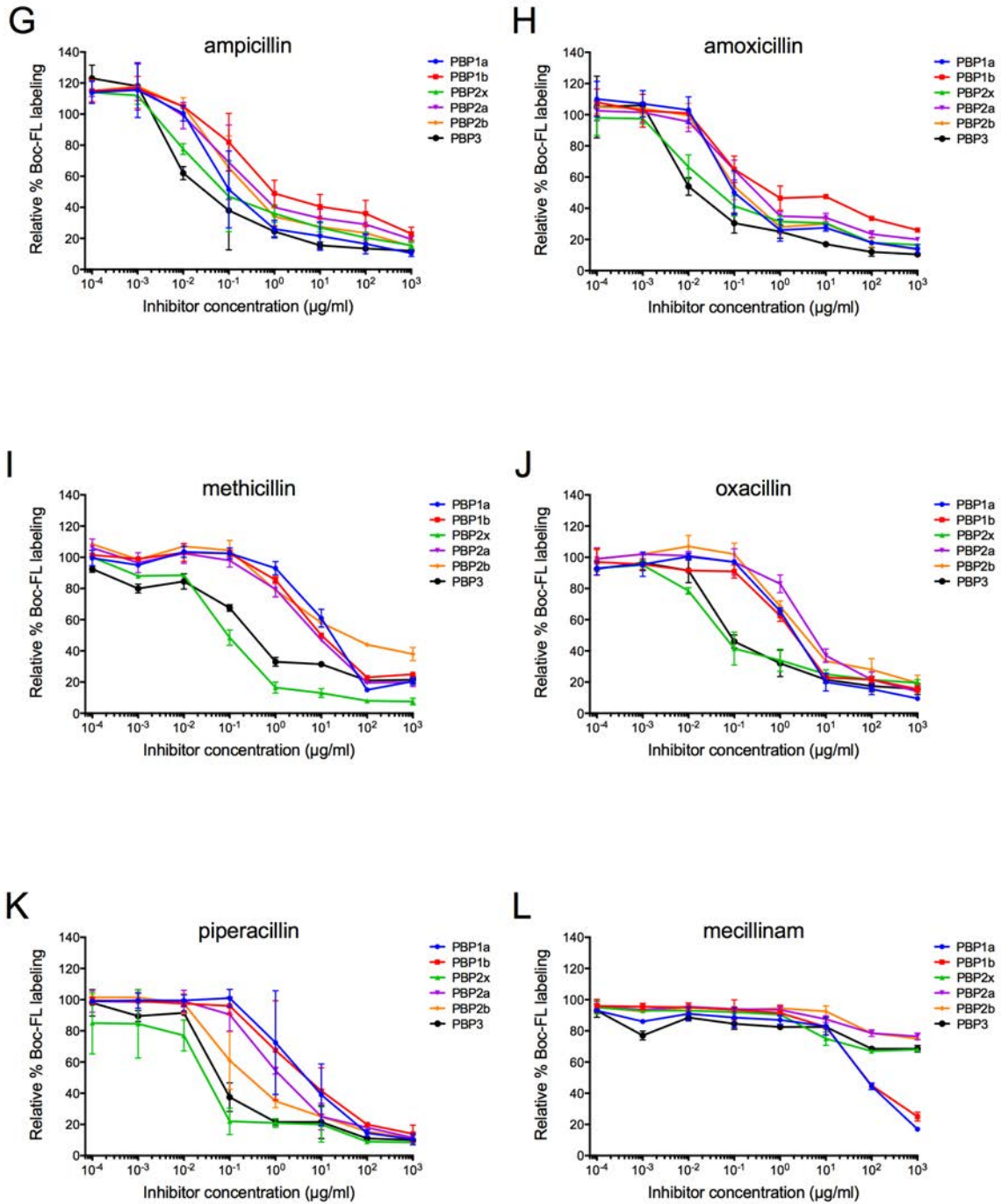


FIG. S4 (continued)



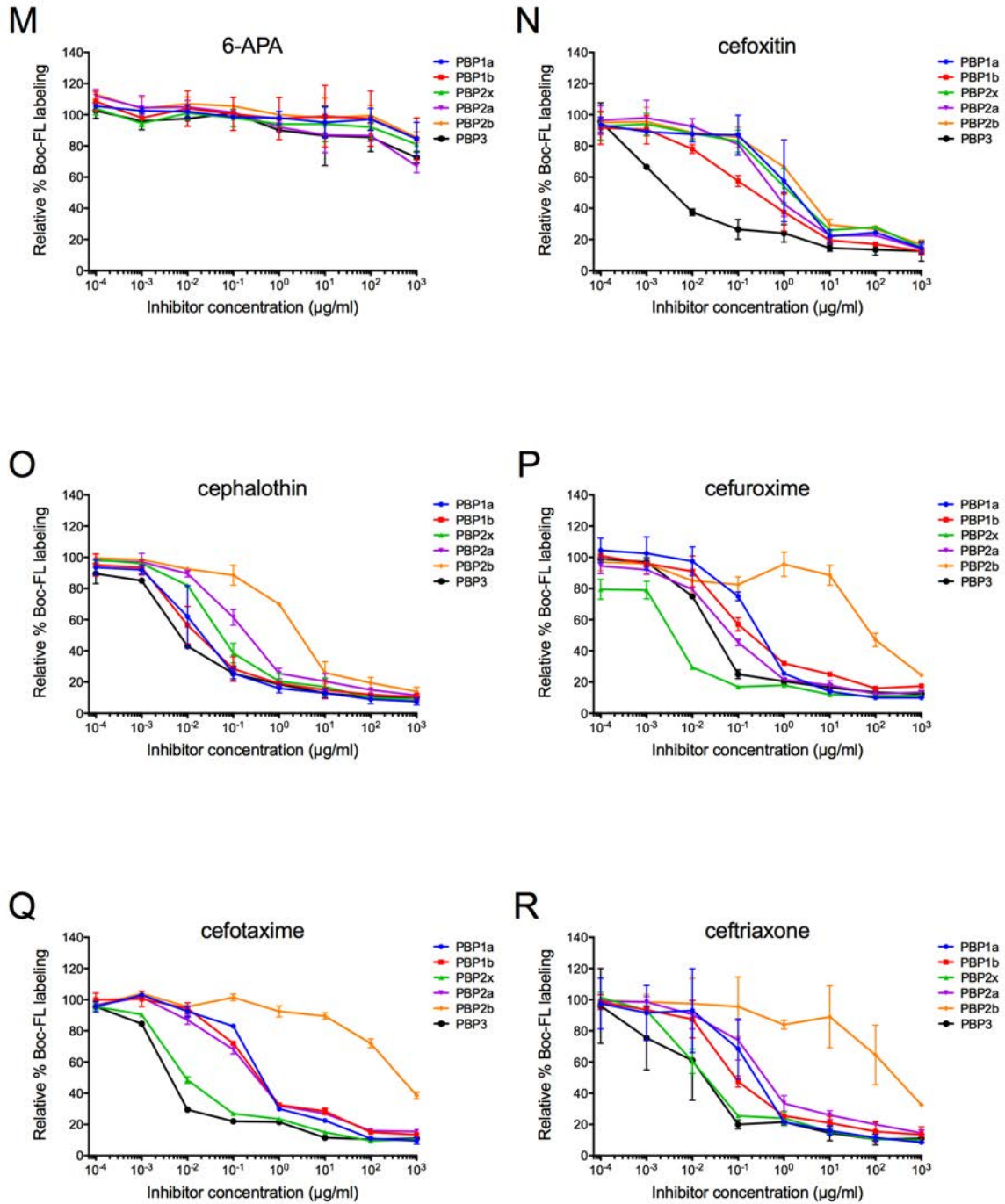


FIG. S4 (continued)



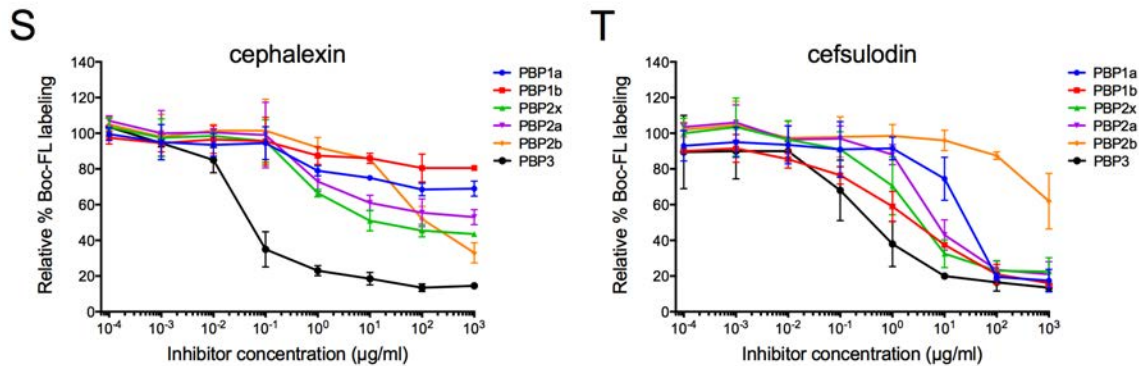


FIG. S4. Graphs of PBP fluorescent gel-band quantitation. (A) aztreonam (B) faropenem, (C) doripenem, (D) meropenem, (E) penicillin V, (F) penicillin G, (G) ampicillin, (H) amoxicillin, (I) methicillin, (J) oxacillin, (K) piperacillin, (L) mecillinam, (M) 6-APA, (N) ceftiofur, (O) cephalothin, (P) cefuroxime, (Q) cefotaxime, (R) ceftriaxone, (S) cephalalexin, (T) cefsulodin. Standard deviation from two independent experiments plotted.

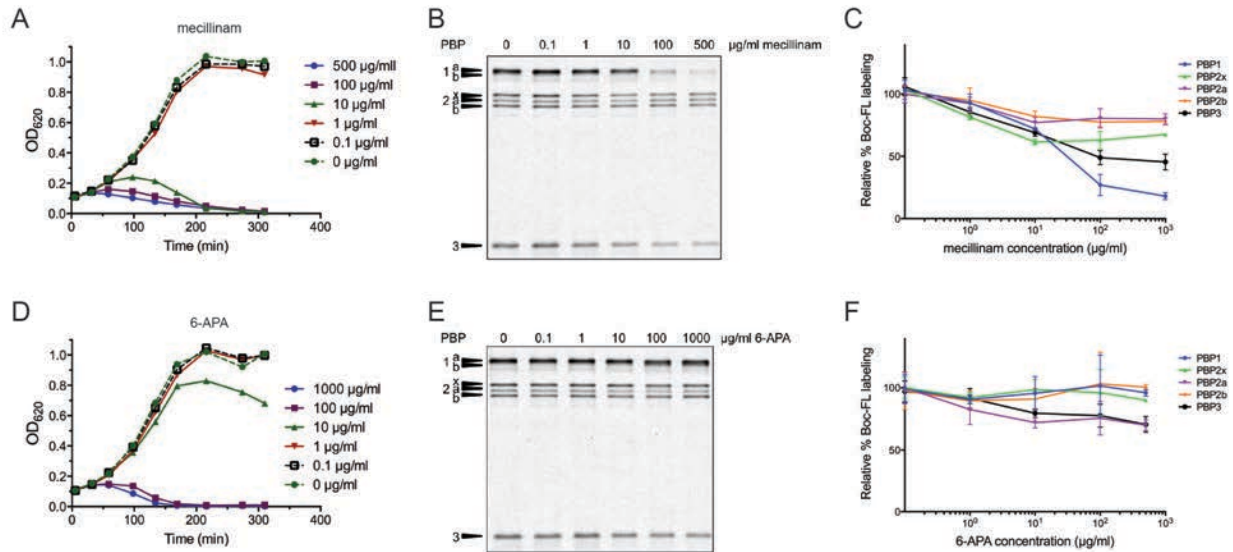


FIG. S5. Analysis of mecillinam and 6-APA titrations in BHI broth. Antibiotic titration in BHI and growth monitoring were performed as described previously (A. mecillinam, D. 6-APA) (1). Boc-FL labeling and SDS-PAGE gel analysis were performed as it was described in Materials and Methods (B, C. mecillinam, E, F. 6-APA). Antibiotic-treated cells, which were used for growth curves and Boc-FL labeling for gel-based analysis, were from the same culture tube.

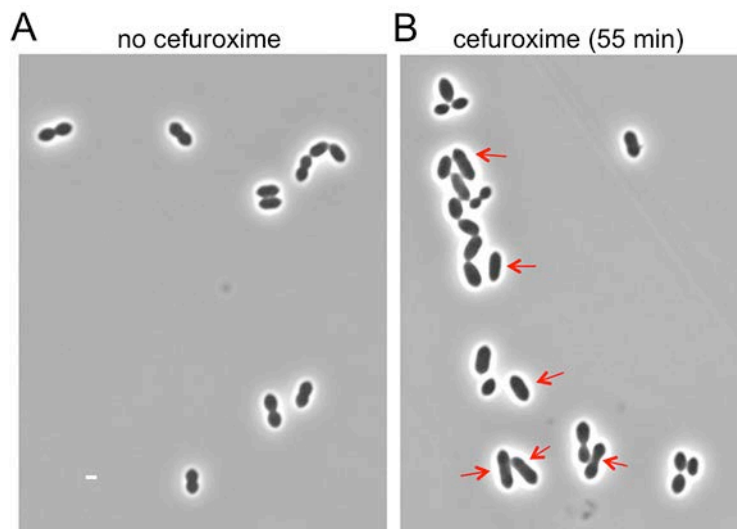


FIG. S6. Inhibition of PBP2x activity with cefuroxime leads to elongation of *S. pneumoniae* cells. Representative phase-contrast images of IU1945 with (A) no treatment or (B) 55 min after addition of cefuroxime at 0.006  $\mu\text{g/ml}$ . Arrows point to elongated cells in cefuroxime-treated cultures. Scale bar, 1  $\mu\text{m}$ . The experiment was performed twice with similar results.

TABLE S1 Inhibition of Boc-FL binding to PBPs in *S. pneumoniae* with a number of  $\beta$ -lactams

$\beta$ -lactam	Concentration <sup>a</sup> ( $\mu$ g/ml)	Relative % Boc-FL Labeling <sup>b</sup>					
		PBP1a	PBP1b	PBP2x	PBP2a	PBP2b	PBP3
Monobactam							
Aztreonam	0.1	106	100	104	100	105	<b>43</b>
Penem							
Faropenem	0.01	<b>27</b>	52	57	<b>49</b>	<b>40</b>	<b>48</b>
Carbapenem							
Doripenem	0.01	52	72	62	85	77	<b>39</b>
Meropenem	0.01	<b>40</b>	83	65	91	82	<b>35</b>
Penicillin							
Penicillin V	0.01	73	81	<b>48</b>	81	84	<b>43</b>
Penicillin G	0.01	70	76	<b>47</b>	76	89	<b>34</b>
Ampicillin	0.1	52	82	<b>47</b>	69	65	<b>38</b>
Amoxicillin	0.1	<b>50</b>	65	<b>41</b>	64	54	<b>31</b>
Methicillin	0.1	102	103	<b>48</b>	98	105	67
Oxacillin	0.1	97	91	<b>42</b>	97	102	<b>46</b>
Piperacillin	0.1	101	96	<b>22</b>	90	61	<b>38</b>
Mecillinam	100	<b>45</b>	<b>45</b>	67	78	78	68
6-APA	1000	85	84	81	67	85	72
Cephalosporin							
Cefoxitin	0.01	88	78	88	92	89	<b>38</b>
Cephalothin	0.01	74	56	82	90	93	<b>43</b>
Cefuroxime	0.01	97	91	<b>30</b>	79	85	75
Cefotaxime	0.01	92	94	<b>49</b>	87	96	<b>30</b>
Ceftriaxone	0.1	68	<b>48</b>	<b>26</b>	74	96	<b>20</b>
Cephalexin	0.1	95	96	96	99	101	<b>35</b>
Cefsulodin	1	91	59	71	88	99	<b>38</b>

<sup>a</sup> Concentration of antibiotic required to reduce the subsequent labeling of Boc-FL to  $\leq 50\%$  for at least one PBP.

<sup>b</sup> Inhibition of  $\geq 50\%$  of Boc-FL labeling is indicated by bolded text. Average of two independent experiments.

TABLE S2 Inhibition of Boc-FL binding to PBP1b in *S. pneumoniae* IU6647 (IU1945  $\Delta$ *pbp1a*::P-*erm*) and IU1945 with amoxicillin (AMX), doripenem (DOR) and meropenem (MEM).

Concentration ( $\mu$ g/ml)	Relative % Boc-FL Labeling								
	AMX (WT, trial 1)	AMX (WT, trial 2)	AMX ( $\Delta$ <i>pbp1a</i> )	DOR (WT, trial 1)	DOR (WT, trial 2)	DOR ( $\Delta$ <i>pbp1a</i> )	MEM (WT, trial 1)	MEM (WT, trial 2)	MEM ( $\Delta$ <i>pbp1a</i> )
0	100	100	100	100	100	100	100	100	100
$10^{-4}$	114	102	95	98	97	87	103	117	105
$10^{-3}$	110	95	102	99	89	91	96	114	102
$10^{-2}$	100	102	89	77	66	91	76	89	100
$10^{-1}$	59	71	72	56	41	62	56	68	75
$10^0$	41	52	58	33	30	48	33	38	58
$10^1$	48	47	52	39	28	40	26	34	47
$10^2$	33	34	50	21	22	33	24	31	46
$10^3$	25	27	38	20	15	30	22	28	39
<b>IC<sub>50</sub> (<math>\mu</math>g/ml)</b>	<b>0.054</b>	<b>0.28</b>	<b>0.17</b>	<b>0.053</b>	<b>0.017</b>	<b>0.14</b>	<b>0.046</b>	<b>0.071</b>	<b>0.13</b>
	Average = 0.17 $\pm$ 0.12			Average = 0.035 $\pm$ 0.018			Average = 0.059 $\pm$ 0.012		

## REFERENCES

1. Land AD, Tsui HC, Kocaoglu O, Vella SA, Shaw SL, Keen SK, Sham LT, Carlson EE, Winkler ME. 2013. Requirement of essential Pbp2x and GpsB for septal ring closure in *Streptococcus pneumoniae* D39. *Molec Microbiol* **90**:939-955.