**SUPPLEMENTARY TABLE 1.** Minimal-inhibitory concentrations of  $\beta$ -lactam, fluoroquinolone,

and aminoglycoside antibiotics for <i>P</i> .	aeruginosa	peptidoglycan-a	active enzyme mutants.
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	Minimum-Inhibitory Concentrations (µg/mL) <sup>b</sup>						
Strain <sup>a</sup>	PP	СТ	TZ	IP	CI	ТМ	
PAO1	4	12	0.75	1	0.125	1.5	
ponA (PBP1a)	4	8	0.75	1	0.125	1	
mrcB (PBP1b)	3	8	0.5	1	0.125	1	
pbpC (PBP3a)	4	12	1	1.5	0.125	1.5	
dacB (PBP4)	64	>256	16	1	0.125	1	
dacC (PBP6)	4	8	0.5	1	0.125	1	
pbpG (PBP7)	4	12	0.75	1	0.125	1	
mgtA	4	8	1	2	0.125	1	
mltA	4	8	0.5	1.5	0.125	1	
mltB	8	12	1.5	1.5	0.19	1	
mltD	4	8	0.75	1.5	0.125	1	
mltF	2	6	0.38	1	0.125	1	
mltF2	4	8	0.75	1	0.125	1	
slt	1.5	6	0.19	1	0.125	1	
sltB1	12	16	1	0.75	0.125	1.5	
sltG	4	12	1	1.5	0.125	1	
sltH	4	8	0.75	1	0.125	1	

<sup>a</sup>Protein names are listed in parentheses when different from gene name.

<sup>b</sup>Abbreviations: PP, piperacillin; CT, cefotaxime; TZ, ceftazidime; IP, imipenem; CI,

ciprofloxacin; TM, tobramycin.



SUPPLEMENTARY FIGURE 1. Loss of PG-active enzymes does not affect bacterial

**growth.** Bacterial growth ( $OD_{600nm}$ ) was monitored over a 24-hour period to detect abnormalities due to the loss of specific PG-active enzymes. The assay was performed using a plate-based assay (described below). N=3. Error bars represent the mean ± standard error.

## **Bacterial growth assays**

Overnight bacterial cultures were sub-cultured 1:200 in Mueller-Hinton broth (MHB; Becton, Dickinson and Company, Mississauga, ON, Canada) and 300  $\mu$ L of each sample was aliquotted into triplicate wells of a sterile 100-well Honeycomb 2 plate (Oy Growth Curves Ab Ltd). Plates were incubated for 24 hours at 37°C with medium shaking in a Bioscreen C plate reader (Oy Growth Curves Ab Ltd) with OD<sub>600nm</sub> reads every hour. Growth data were analyzed using GraphPad Prism software V5.0C.