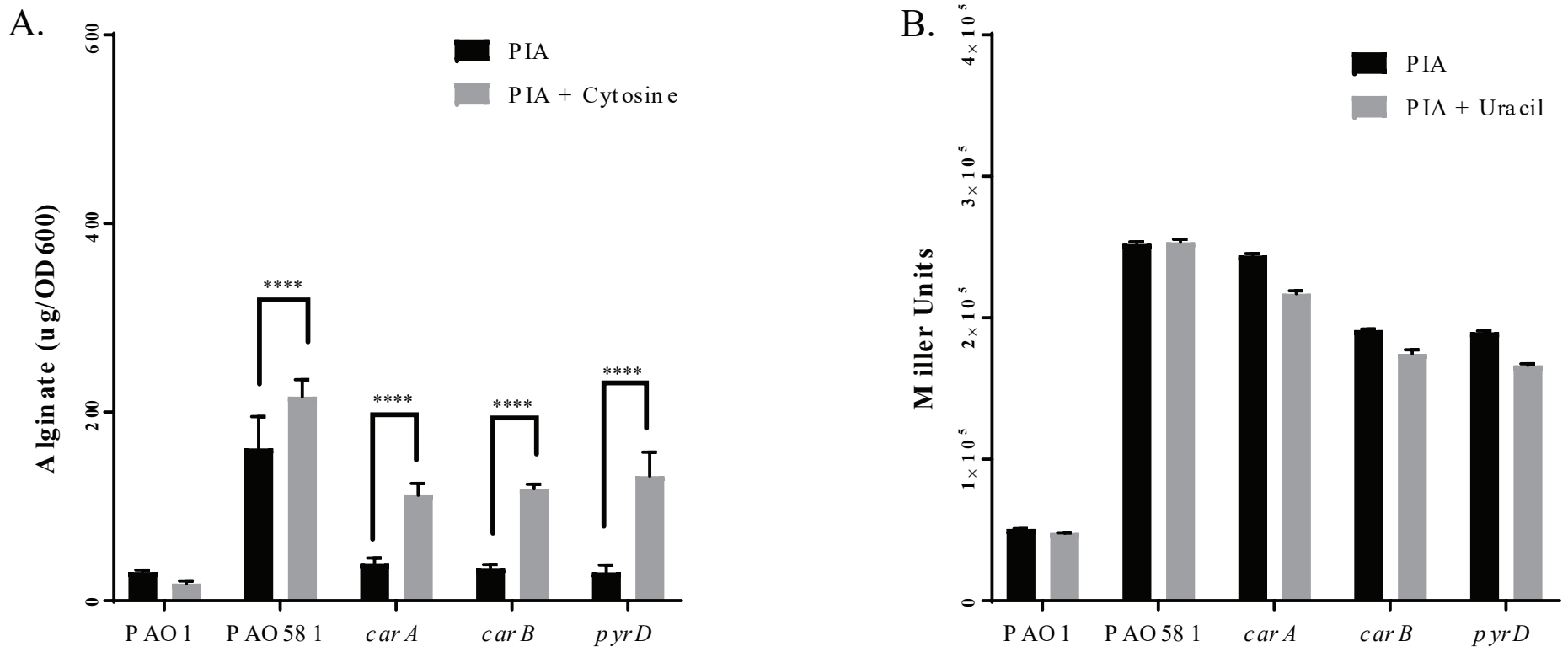

FIG S1. Disruption of *de novo* pyrimidine biosynthesis results in loss of mucoidy. (A) Alginate production for PAO1, PAO581, PAO581*carA*, PAO581*carB*, and PAO581*pyrD* when grown on PIA plates with cytosine at 37ºC for 24 h. Alginate was collected and measured using the carbazole assay. Values shown are mean alginate ± standard deviation of triplicate reads. (B) The β-galactosidase activity of P_{algU} measured using pLP170-P_{algU}-*lacZ* reporter constructs in PAO1, PAO581, PAO581*carA*, PAO581*carB*, and PAO581*pyrD* grown on PIA plates containing carbenicillin and uracil at 37ºC for 24 h. Relative expression mean values shown ± standard deviation of triplicate reads. Levels from three western blots normalized by RNA polymerase levels. Values shown are of mean ratio ± standard deviation performed in triplicates. Two-way ANOVA used with statistical significance at $p<0.01$ (****=$p<0.0001$).

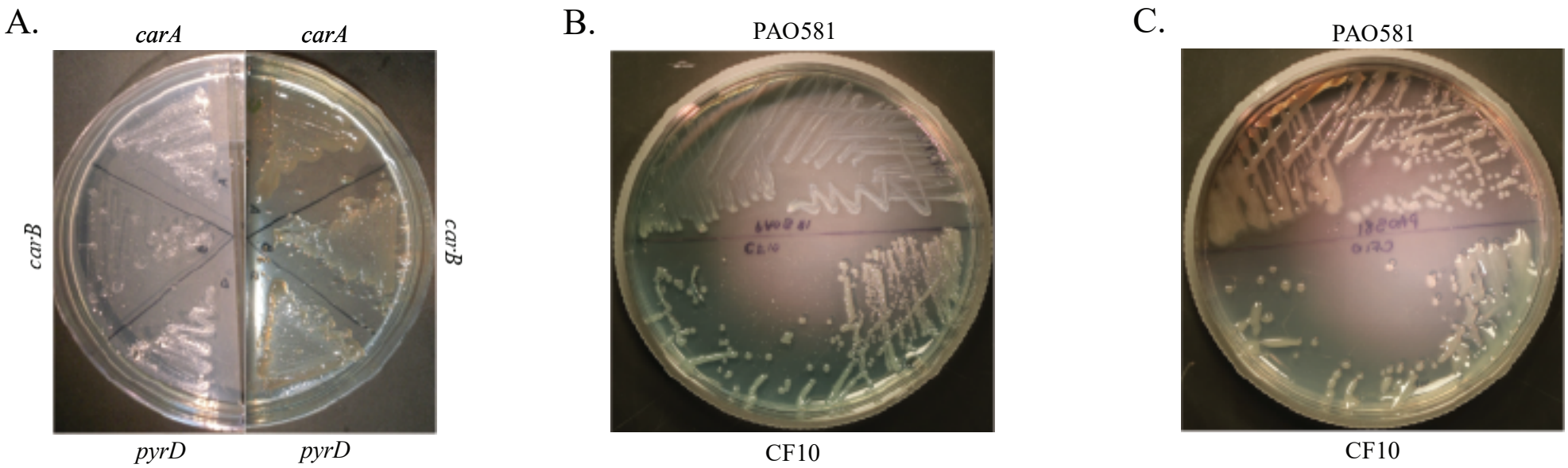

FIG S2. Mucoid phenotype on SCFM2 media indicates that PIA medium has the same effect on maintenance of mucoid phenotype in laboratory and clinical isolates of P. *aeruginosa*. (A) PAO581*carA*, PAO581*carB*, and PAO581*pyrD* grown on SCFM2 media (*left*) and SCFM2 media with uracil (*right*) at 37ºC for 24 h. (B) PAO581(*top*) and CF10 -mucoid CF isolate (*bottom*) grown on SCFM2 media and (C) PIA at 37ºC for 24 h.

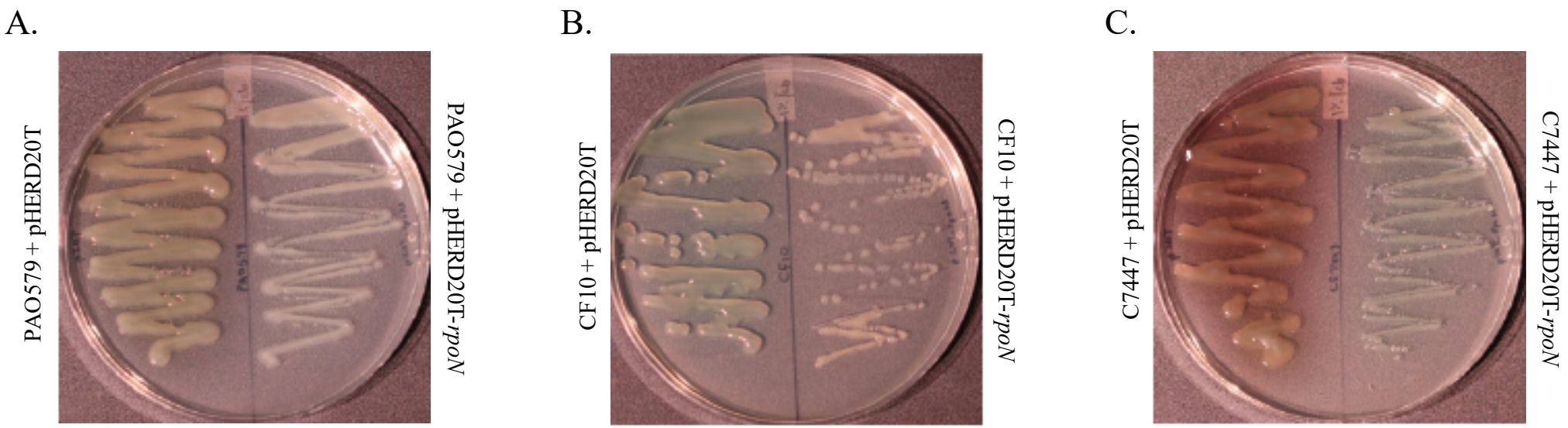

FIG S3. Overexpression of *rpoN* results in reduction of mucoidy in CF Isolates and PAO579. Image showing (A) PAO579, and stable mucoid CF isolates (B) CF10 and (C) CF7447 carrying pHERD20T (*left*) and pHERD20T-*rpoN* (*right*) grown on PIA plates with carbenicillin and 1% arabinose at 37ºC for 36h.

	Aztreonam****	Meropenem****	Imipenem****	Ceftazidime	Cefepime**	Levofloxacin	Ciprofloxacin	Tobramycin
PAO581	20.0 ± 0.8	25.0 ± 0.47	22.0 ± 0.82	19.3 ± 0.94	20.3 ± 1.24	17.0 ± 0.82	20.3 ± 0.47	9.3 ± 0.47
PAO581*algD*	24.0 ± 0	29.7 ± 0.47	30.3 ± 0.47	19.3 ± 0.47	21.7 ± 0.47	14.7 ± 0.47	21.7 ± 0.47	11.3 ± 0.47
PAO581*carA*	23.3 ± 0.47	27.7 ± 0.47	34.0 ± 0.82	25.3 ± 0.47	25.3 ± 1.24	26.0 ± 0.82	29.7 ± 0.47	17.0 ± 0.82
PAO581*carB*	25.3 ± 1.24	32.3 ± 2.05	34.7 ± 0.47	27.0 ± 1.41	29.0 ± 0.82	25.7 ± 2.62	28.3 ± 2.35	14.0 ± 0.82
PAO581*pyrD*	32.3 ± 0.95	30.3 ± 0.47	36.0 ± 1.41	34.0 ± 0.82	27.7 ± 2.5	20.0 ± 0.82	25.0 ± 0	18.7 ± 0.94

Table S1: Zone of inhibition (mm) for strains grown on PIA with indicated antibiotic disk. Antibiotic disk diffusion assay performed using BD Sensi-Disc ™ according to the standard protocol. Values shown are of the average zone (mm) ± standard deviation for each strain when grown on PIA at 37^{o}C for 24h with the indicated disk measured in triplicates. Two-way ANOVA with multiple comparisons was used to calculate the statistical significance with $p<0.01$ (**=$p<0.001$, ****=$p<0.0001$).

	Aztreonam****	Meropenem****	Imipenem****	Ceftazidime	Cefepime**	Levofloxacin	Ciprofloxacin	Tobramycin
PAO581	19.3 ± 0.94	25.7 ± 0.47	23.7 ± 0.94	18.7 ± 0.47	20.7 ± 0.47	18.3 ± 0.47	22.3 ± 0.47	10.3 ± 0.47
PAO581*algD*	19.7 ± 0.47	27.3 ± 0.94	23.7 ± 0.47	19.7 ± 0.47	21.3 ± 0.47	19.3 ± 0.47	20.3 ± 0.47	9.3 ± 0.47
PAO581*carA*	30.7 ± 0.47	31.7 ± 0.47	26.7 ± 0.47	25.7 ± 0.47	27.0 ± 0.82	25.3 ± 1.24	33.3 ± 0.47	16.7 ± 0.47
PAO581*carB*	29.7 ± 0.47	36.3 ± 0.94	34.7 ± 0.47	29.7 ± 0.47	29.7 ± 0.47	26.0 ± 0	27.7 ± 0.94	15.0 ± 0.82
PAO581*pyrD*	34.0 ± 0.82	35.0 ± 0.82	35.0 ± 0.82	34.3 ± 0.47	33.0 ± 0.82	17.7 ± 0.47	25.0 ± 0	18.7 ± 1.7

Table S2: Zone of inhibition (mm) for strains grown on PIA with uracil with indicated antibiotic disk. Antibiotic disk diffusion assay performed using BD Sensi-Disc ™ according to the standard protocol. Values shown are of the average zone (mm) ± standard deviation for each strain when grown on PIA with uracil at 37ºC for 24h with the indicated disk measured in triplicates. Two-way ANOVA with multiple comparisons was used to calculate the statistical significance with $p<0.01$ (****=$p<0.0001$).