Supplementary Figure 1. Colistin sensitivity of LESB65 and LESB65ΔpmrB before and after two-day infection of mouse airways. Post-infection samples are *P. aeruginosa* recovered from 2 day post-infection mouse lung homogenates. Bacteria were recovered by plating homogenates onto Pseudomonas selective agar and then growth for ~30 hours at 37°C. Susceptibility testing was performed by microdilution in Mueller Hinton broth with two-fold decreasing concentrations of colistin (range 64-0.125µg/ml). Post-infection samples are from a single *in vivo* experiment. Frozen stocks of these bacteria were tested for susceptibility on three separate assay days. Data show mean OD_{600} ± standard deviation.

Supplementary Figure 2. Spermidine levels in bacterial lysates. Lysates of overnight cultures were used in a competitive spermidine ELISA. Levels detected were close to the manufacturer’s stated limits of assay sensitivity (<0.28 ng/ml). Data are from five separate overnight cultures per strain, run, in duplicate, on a single ELISA assay plate. Ns = not significant.
Supplementary Figure 3. Spermine levels in cystic fibrosis sputum and mouse respiratory tissue. (A) Concentration of spermine in sputum from people with CF, chronically infected with *P. aeruginosa*, determined by competition ELISA. (A) Spermine levels in 16 study participants. Between 1 and 5 samples were available per participant. No two samples from the same participant were collected at the same visit. (B) Collected samples were defined as baseline, treatment or pulmonary exacerbation, defined by participant clinical data. Whiskers show 10-90 percentile. (C) Concentration of spermine in the sinuses and lungs of mice at 48 hours post intranasal administration of PBS (-) or LESB65 (+). Each circle represents a tissue sample from an individual mouse. Data are from a single experiment.
Supplementary Figure 4. Growth of *P. aeruginosa* in the presence of exogenous spermidine. LESB65, PAO1 and their isogenic *pmrB*-deficient mutants were grown in Mueller Hinton broth in the presence of spermidine (range 0.125-32 mM). Growth was determined after 24 hours by reading absorbance at 600 nM. A concentration of 4 mM was chosen for subsequent assays.
Supplementary Figure 5. Spermidine-NBD coats the *P. aeruginosa* surface. Fluorescence microscopy image of LESB65 in the absence (left) or presence (right) of spermidine-NBD.