SUPPLEMENTAL FIGURES AND LEGENDS TO ACCOMPANY:

Pseudomonas aeruginosa Quorum-Sensing Molecule Homoserine Lactone Modulates Inflammatory Signaling through PERK and eI-F2α[1]

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Short Title: HSL-C12 Modulates Inflammation through PERK

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SUPPLEMENTAL FIGURE 1

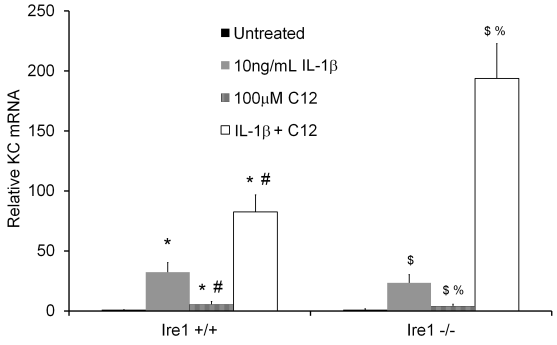

Fig. S1. HSL-C12 effects on KC gene expression are not IRE-1α-dependent. IRE-1α $^{-/-}$ and IRE-1α-corrected MEF were rinsed with fresh media then treated for 4 hours with IL-1β (10 nM), HSL-C12 (100 μM) or IL-1β + HSL-C12. Cells were then treated with Trizol, and cDNA was prepared for qPCR analysis, where results are given as RQ score normalized to RPS17 cDNA. Averages +/- Std error; n = 3 biological replicates for all conditions. For experiments on IRE-1α-corrected MEF: * comparisons to control; # comparison to IL-1β. For experiments on IRE-1α $^{-/-}$ MEF, $^{\$}$ comparison to control; $^{\%}$ comparison to IL-1β.

SUPPLEMENTAL FIGURE 2

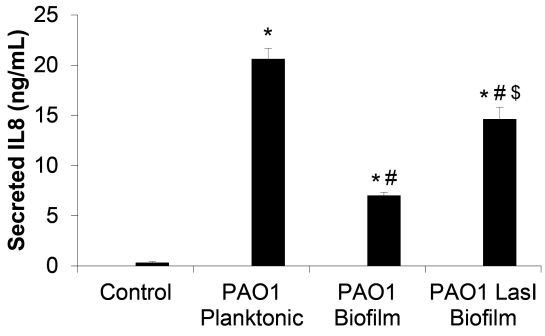

Fig. S2. Compared to planktonic *P. aeruginosa*, biofilm *P. aeruginosa* inhibit IL8 secretion by Calu-3 airway epithelial cells; this inhibition is mediated in part by lasI, the enzyme responsible for production of HSL-C12 in *P. aeruginosa*. Calu-3 cells, an airway epithelial cell line, was grown to confluence on filters. *P. aeruginosa* strains PAO1 and PAO1lasI (missing lasI gene, which encodes the enzyme responsible for the last step of synthesis in HSL-C12) were grown in LB media ("planktonic") or as biofilms on small, permeable filters on the surface of agar plates to permit growth as biofilms ("biofilm") (Schwarzer et al, 2012. *Cell Micro* 14: 698-709). Planktonic PAO1 and biofilm PAO1 and PAO1lasI were resuspended in tissue culture MEM at 10^8 cfu/ml. 100 µl of the suspensions were added to the apical surface of the Calu-3 monolayers, which were placed in the incubator for 4 hrs. Samples (100 µl) were taken from the basolateral surface of the epithelial monolayers at t = 0 and t = 4 hrs, and IL8 was assayed by ELISA. IL8 secreted during this time was largest during exposure to planktonic PAO1, less during exposure to biofilm PAO1 and intermediate during exposure to biofilm PAO1lasI. Data are averages +/- Std errors, n = 3 biological replicates, each measured in triplicate. * comparsion to control; # comparison to PAO1 planktonic; $ comparison to PAO1 biofilm.