Supplementary information

1. Material and methods

1.1. Growth conditions

Escherichia coli/pSB401 (the biosensor strain) and its variants were grown with shaking (180 rpm) in Luria-Bertani (LB) broth supplemented with 20 µg/ml of tetracycline at 37°C. Following overnight incubation, the cells were washed in 0.9% NaCl saline solution and diluted 100 fold in LB to obtain a concentration of approximately 10^6 cells/ml. Isolates of *Pseudomonas aeruginosa* were grown in the same conditions but without antibiotics.

1.2. Modification of LuxR receptor

Error-prone polymerase chain reaction (PCR) was used to modify the LuxR receptor and improve its sensitivity and specificity towards 2-aminoacetophenone (2-AA). Briefly, the *luxR* gene was amplified using GeneMorph II Random Mutagenesis Kit (Aligent technologies, Santa Barbara, CA, USA) with the following conditions: One cycle of 30 seconds at 95°C, then 30 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 58°C and elongation for 1 min at 72°C. Fifty microliters of the reaction mix contained 5 µl of 10× Mutazyme II reaction buffer, 200 µM of each dNTPs, 2.5 ng/µl of each primer, 50 mU/µl of Mutazyme II DNA polymerase and 10 ng of pSB401 plasmid as a template. The primers used for the reaction were LuxR1 and LuxR2 (Table S1).

The PCR products (modified *luxR* gene) were inserted into pSB401 plasmid replacing the native *luxR* gene by the restriction-free (RF) procedure.[1] In this technique, the error-prone PCR product served as a primer and the pSB401 plasmid served as a template. PCR conditions were as follows: One cycle of 30 seconds at 90°C, then 30 cycles of denaturation for 30 seconds at 95°C, annealing for 60 seconds at 60°C and elongation for 5 minutes at 72°C and then one additional cycle of 7 minutes at 72°C. Each 50 µl of the PCR mix contained 100 ng of PCR product, 20 ng of pSB401 plasmid, 200 µM of each of the dNTPs, 10 µl of Phusion high-fidelity buffer X5 (Thermo Fischer Scientific, MO, USA), 0.5 µl of Phusion high-fidelity polymerase enzyme (Thermo Fischer Scientific, MO, USA). The PCR products of this long run were pSB401 plasmid variants expressing modified LuxR receptors. Following the reaction, two microliter of DpnI enzyme (Thermo Fischer Scientific, MO, USA) was

added to 20 microliter of the PCR product for degradation of the old methylated plasmid. Following the DpnI reaction the non-methylated modified plasmids that were generated in the PCR were transferred to *E. coli* cells using standard heat-shock protocol[2]. Resulting colonies were screened for a luminescence response towards 2-AA (described in section 1.3).

1.3. Screening of the variant biosensors harboring the modified LuxR receptors.

Biosensor strains harboring the modified LuxR receptor were exposed to 2-AA and luminescence was compared to that of the biosensor encoding the native LuxR receptor. Cultures of the biosensor strains were prepared as described in section 1.1. One hundred microliters of bacterial culture were added per well to a 96-wel plate (Corning Inc., NY, USA. Cat. number 356701) in four replicates. 2-AA was added at a concentration of 50 μM. The plates were incubated at 37 ºC and the luminescence produced by the biosensor strains was measured during 24 h at 30 min intervals using infinite-F200 plate reader (Tecan Trading AG, Switzerland).

1.4. Sensitivity of the modified LuxR receptor (LuxR-M22) towards 2-AA and acyl homoserine lactone.

The biosensor strains harboring either the native LuxR receptor (*E. coli* pSB401) or the modified LuxR-M22 (*E. coli* pSB401-M22) were exposed to various concentrations of 2-AA (0, 0.1, 1, 2, 10, 50 and 100 μM) in order to determine their detection limit. Luminescence levels were measured during 12 h using the infinite-F200 plate reader as described above.

Response of these biosensors towards acyl-homoserine-lactone (AHL), the cognate signal of LuxR, was examined using 10 nM of 3-oxo-C6-HSL

1.5. Amplification and sequencing of the modified *luxR-M22* gene.

The amplification of the gene encoding the modified LuxR-M22 receptor was performed using the primers prPSB401-1 and prPSB401-2 (Table S1). The conditions of the reaction were as following: 30 cycles of denaturation for 30 sec at 95ºC, annealing for 30 sec at 58 ºC and elongation for 1 min at 72ºC. Fifty microliter of the reaction mix contained 0.4 μM of each of the primers, 20 ng of pSB401-M22 plasmid,

200 μM of each of the dNTPs, 10 μL of Phusion high fidelity buffer X5 and 0.5 μL of Phusion high fidelity polymerase. The sequence of the PCR product was conducted using standard Sanger method (Hylabs®, Rehovot, Israel).

1.6. In silico docking of 2-AA and 3-oxo-C6-HSL within the binding pocket of native and modified LuxR receptors.

Structural models of both the native (LuxR) and the modified (LuxR-M22) receptors were generated using residue scanning module in Schrodinger software (Schrodinger software LLC., Portland, OR, USA). The modified model was generated from the native receptor model with best docked pose of 3-oxo-C6-HSL. The structure of the modified M-22 receptor was identical to that of the native receptor but with modifications in three amino acid residues: Gly72 instead of Asp72, Ile102 instead of Val102 and Thr135 instead of Met135 (mutations M22A, M22B and M22C, respectively). The modified-receptor structure underwent protein preparation and minimization. Both the native and the modified receptor models were docked with 2-AA and AHL using Glide module in Schrodinger software.

1.7. Generation of single point-mutations in the LuxR receptor.

In order to examine the contribution of each of the three mutations (M22A, M22B and M22C) that composed the modified *luxR* gene (M22 construct), we generated, using the RF technique, three different plasmids each harboring a single mutation. The following primers were used to generate the M22A, M22B and M22C mutations, respectively: prLuxR2 and prM22A; prLuxR2 and prM22B; prLuxR2 and prM22C (Table S1). In order to generate an additional mutated receptor with two single mutations M22A and M22C (variant M22AC), the pSB401-M22A plasmid was used as a template for RF reaction with prLuxR2 and prM22C primers.

The conditions of the PCR reactions were as follows: 30 cycles of denaturation for 30 sec at 95ºC, annealing for 30 sec at 58 ºC and elongation for 1 min at 72ºC. Fifty microliter of the reaction mix contained 0.4 μM of each of the primers, 20ng of pSB401 plasmid, 200μM of each of the dNTPs, 10μL of Phusion high fidelity buffer X5 and 0.5μL of Phusion high fidelity polymerase. The PCR products were then used as primers for a long PCR to generate modified plasmids as described above in

section 1.2. The receptors with the different mutations were encoded within the *E. coli* biosensor replacing the native LuxR encoding gene and their response towards 2-AA was examined as described in section 1.3.

1.8. Identification of 2-AA production in clinical *P. aeruginosa* isolates using the native and modified LuxR biosensors.

In order to detect production of 2-AA by various clinical isolates of *P. aeruginosa*, their volatiles were analyzed using both the native (pSB401) and the modified (pSB401-M22) biosensors. The isolates were inoculated with different biosensors in two separate compartments of Petri dishes. Such a compartmental inoculation enabled only the exchange of volatiles between *P. aeruginosa* culture and the examined reporter strain. Following overnight incubation, the colonies of the reporter strain were scraped from the agar, re-suspended in phosphate buffer saline (PBS; 0.1 M pH=7.4; 10.9 g/l of Na2HPO4, 3.2 g/l of NaH2PO4 and 9 g/l of NaCl) and measured for luminescence in a 96 well plate using infinite-F200 plate reader.

1.9. Analysis of *rhl* and *las* QS systems in clinical isolates of *P. aeruginosa*.

In order to assess whether isolate R11 did not activate LuxR biosensor due to a non-active QS system that led to a lack in 2-AA production, we examined the supernatant with two different biosensor strains. The following biosensor strains were used: *P. aeruginosa* JP2/pKD-rhlA which responds to the *rhl*-regulated signal C4-HSL and *P. aeruginosa* JP2/pKD201 that responds to the *las*-regulated signal 3oxo-C12-HSL[3]. Briefly, the clinical isolate R11, together with the clinical isolate R12 and *P. aeruginosa* PA14 strain as positive controls, were grown overnight. Following overnight incubation, the supernatant of the culture was collected and added to fresh cultures of each of the biosensor strain at 20% v/v. One hundred microliters from each of the treatments, in six replicates, were transferred to 96-well plate and the luminescence produced by the *P. aeruginosa* biosensor strains was measured every half hour in a plate reader for 24 h.

1.10. Amplification and sequencing of *lasR* gene.

The amplification of the gene encoding for LasR receptor of *P. aeruginosa* was performed using the primers prLasR1-F, prLasR1-R, prLasR2-F and prLasR2-R (Table S1). The PCR was conducted with DNA extracted from *P. aeruginosa* PA14, and two *P. aeruginosa* isolates from lung infection samples (isolates R11 and R12).

The conditions of the reaction were the same as described in section 1.5 but with an annealing temperature of 61 °C and elongation time of 80 sec. Fifty microliter of the reaction mix contained the same materials as described in section 1.5 but with genomic DNA of *P. aeruginosa* instead of plasmid as a template. The sequence of the PCR product was conducted using standard Sanger method (Hylabs®, Rehovot, Israel).

1.11. Analysis of sputum samples using the modified biosensor strain.

In order to examine the diagnostic potential of the modified biosensor, 24 sputum samples were collected from lungs of subjects suffering from lung infection. All subjects provided informed written consent for their samples to be tested according to procedures that were approved by the Edith Wolfson Hospital Helsinki Committee. The samples were analyzed for presence of *P. aeruginosa* using both standard culturing methods [4] and the pSB401-M22 biosensor. For analysis with the biosensor ca. 300 µL of each sample were placed in a 12-wells culture plate (black walls, transparent bottom; Cellvis, CA, USA). Three hundred microliters of PBS buffer were used as control. Ten microliter of the biosensor strain, prepared as described above, were immobilized on 200 µL of LB-agar that were solidified at 12 different places on the lid of the 12 well plate. In this manner, each well had a biosensor culture immobilized directly above it on the position of its lid. There was no direct contact between the biosensor and the sputum samples, allowing only the transfer of volatile substances between each sample and its biosensor. The luminescence produced by the biosensor cultures with and without the exposure to sputum samples was measured using a plate reader during 18 h of incubation at 37°C. Following incubation, a relative activation was calculated for each of the samples as follows: the maximal luminescence produced during 18 h of incubation divided by maximal luminescence produced by control treatment. A sample exhibiting relative activation of above 1.5 was considered as a positive sample in the biosensor assay.

Results of the biosensor were compared to the culture in order to determine the sensitivity and specificity of the biosensor assay. The following calculations were carried out: Sensitivity = A/(A+C) x 100; Specificity=D/(D+B) x 100; Positive predictive value (PPV) = A/(A+B) x 100; Negative predictive value (NPV)= D/(D+C) x 100. A= number of positive samples according to biosensor; B= false positive; C= false negative; D= negative samples according to biosensor.

2. Supplementary tables and figures.

Table 1. Primers used in the presented study.

Primer	Sequance	Target
prLuxR1	atgccgacgacacatacaga	*luxR*
prLuxR2	tgggcaatcaattgctcctgt	*luxR*
prM22A	ggaggcaatattatgatggcgc	*luxR* with M22A mutation
prM22B	caatggcttcggaacgcttag	*luxR* with M22B mutation
prM22C	ggaatatatttgaaaacaatgct	*luxR* with M22C mutation
PrPSB401-1	tcgtcgtggtattcactccag	pSB401 plasmid downstream of *luxR*
PrPSB401-2	cacattcttgcccgcctgat	pSB401 plasmid upstream of *luxR*
prLasR1-F	accctctaggacgggtatcg	Chromosome of *P. aeruginosa*, upstream of *lasR* gene
prLasR1-R	agcaatggcttcacacgaga	Chromosome of *P. aeruginosa*, downstream of *lasR* gene
prLasR2-F	ttctcggactgccgtacaac	Chromosome of *P. aeruginosa*, upstream of *lasR* gene
prLasR2-R	ttatcgaactcttcgcgccg	Chromosome of *P. aeruginosa*, downstream of *lasR* gene

A.

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pSB401-M22  ATGGGTATGAAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCT
pSB401      ATGGGTATGAAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCT
            ************.******************:****************************

pSB401-M22  TGTAGAAGCAATAATGATATTAATCAGTGCTTATCTGATATGACTAAAATGGTACATTGT
pSB401      TGTAGAAGCAATAATGATATTAATCAGTGCTTATCTGATATGACTAAAATGGTACATTGT
            **************************.**********************************

pSB401-M22  GAATATTATTTACTTGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATT
pSB401      GAATATTATTTACTCGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATT
            ************** ********************************************

pSB401-M22  CTAGATAATTACCCTAAAAAATGGAGGCAATATTATGATGGCGCTAATTTAATAAAATAT
pSB401      CTAGATAATTACCCTAAAAAATGGAGGCAATATTATGATGACGCTAATTTAATAAAATAT
            ****************************************.*******************

pSB401-M22  GATCCTATAGTAGATTATTCTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAA
pSB401      GATCCTATAGTAGATTATTCTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAA
            ************************************************************

pSB401-M22  AACAATGCTATAAATAAAAAATCTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTT
pSB401      AACAATGCTGTAAATAAAAAATCTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTT
            *********.**************************************************

pSB401-M22  ATCACTGGGTTTAGTTTCCCTATTCATACGGCTAACAATGGCTTCGGAACGCTTAGTTTT
pSB401      ATCACTGGGTTTAGTTTCCCTATTCATACGGCTAACAATGGCTTCGGAATGCTTAGTTTT
            ********************************************** **********

pSB401-M22  GCACATTCAGAAAAAGACAACTATATAGATAGTTTATTTTTACATGCGTGTATGAACATA
pSB401      GCACATTCAGAAAAAGACAACTATATAGATAGTTTATTTTTACATGCGTGTATGAACATA
            ************************************************************

pSB401-M22  CCATTAATTGTTCCTTCTCTAGTTGATAATTATCGAAAAATAAATATAGCAAATAATAAA
pSB401      CCATTAATTGTTCCTTCTCTAGTTGATAATTATCGAAAAATAAATATAGCAAATAATAAA
            ************************************************************

pSB401-M22  TCAAACAACGATTTAACCAAAAGAGAAAAAGAATGTTTAGCGTGGGCATGCGAAGGAAAA
pSB401      TCAAACAACGATTTAACCAAAAGAGAAAAAGAATGTTTAGCGTGGGCATGCGAAGGAAAA
            ************************************************************

pSB401-M22  AGCTCTTGGGATATTTCAAAAATATTAGGCTGCAGTGAGCGTACTGTCACTTTCCATTTA
pSB401      AGCTCTTGGGATATTTCAAAAATATTAGGCTGCAGTGAGCGTACTGTCACTTTCCATTTA
            ************************************************************

pSB401-M22  ACCAATGCGCAAATGAAACTCAATACAACAAACCGCTGCCAAAGTATTTCTAAAGCAATT
pSB401      ACCAATGCGCAAATGAAACTCAATACAACAAACCGCTGCCAAAGTATTTCTAAAGCAATT
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pSB401-M22  TTAACAGGAGCAATTGATTGCCCATACTTTAAAAATTAA
pSB401      TTAACAGGAGCAATTGATTGCCCATACTTTAAAAATTAA
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B.

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pSB401-M22      MGMKNINADDTYRIINKIKACRSNNDINQCLSDMTKMVHCEYYLLAIIYPHSMVKSDISI
pSB401          MGMKNINADDTYRIINKIKACRSNNDINQCLSDMTKMVHCEYYLLAIIYPHSMVKSDISI
                ************************************************************

pSB401-M22      LDNYPKKWRQYYDGANLIKYDPIVDYSNSNHSPINWNIFENNAINKKSPNVIKEAKTSGL
pSB401          LDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWNIFENNAVNKKSPNVIKEAKTSGL
                *************.****************************:****************

pSB401-M22      ITGFSFPIHTANNGFGTLSFAHSEKDNYIDSLFLHACMNIPLIVPSLVDNYRKINIANNK
pSB401          ITGFSFPIHTANNGFGMLSFAHSEKDNYIDSLFLHACMNIPLIVPSLVDNYRKINIANNK
                *************** ****************************************

pSB401-M22      SNNDLTKREKECLAWACEGKSSWDISKILGCSERTVTFHLTNAQMKLNTTNRCQSISKAI
pSB401          SNNDLTKREKECLAWACEGKSSWDISKILGCSERTVTFHLTNAQMKLNTTNRCQSISKAI
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pSB401-M22      LTGAIDCPYFKN
pSB401          LTGAIDCPYFKN
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Figure S1. Sequence and alignment of *luxR* gene from the native pSB401 biosensor plasmid (pSB401), and the modified pSB401-M22 biosensor plasmid (pSB401-M22). A) Nucleic acid sequence of the gene. B) Amino acids sequence of the gene. M22A mutation is marked with blue, M22B with yellow and M22C with green.

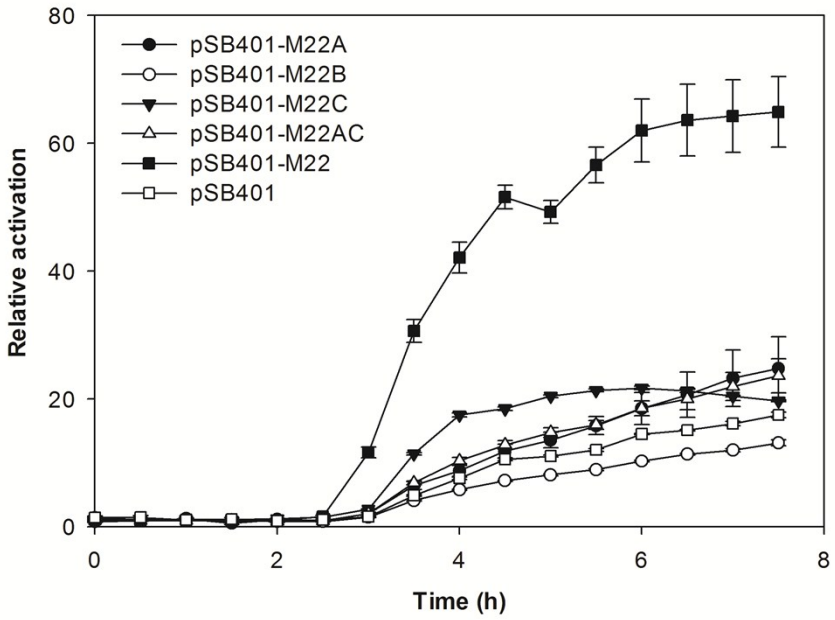

Figure S2. Effect of single point-mutations in the *luxR* gene on the response of the receptor to 2-AA. Relative activation of biosensors strain harboring native and modified LuxR receptors by 50μM of 2-AA. Relative activation is defined as luminescence produced by the biosensor in addition of 2-AA divided by the luminance without addition of 2-AA. The examined biosensors were: (i) pSB401, expressing the native *luxR* gene, (ii) pSB401-M22, expressing *luxR* with three point mutations, (iii) pSB401-M22A, expressing *luxR* with single point mutation M22A, (iv) pSB401-M22B, expressing *luxR* with single point mutation M22B, (v) pSB401-M22C, expressing *luxR* with single point mutation M22C and (vi) pSB401-M22AC, expressing *luxR* with two point mutations M22A and M22C. Error bars represent a standard deviation of four replicates.

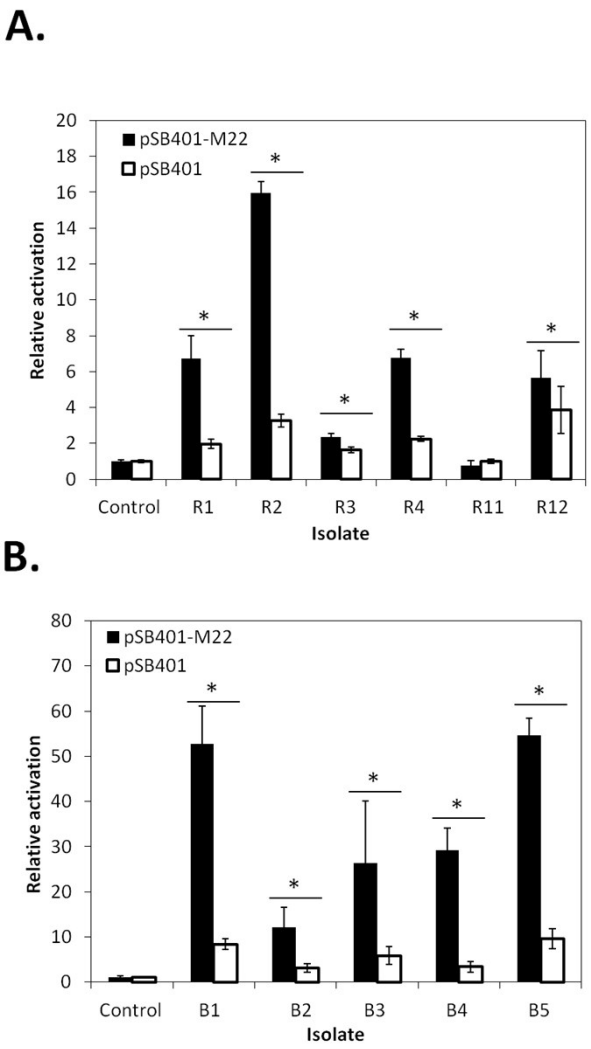

Figure S3. Activation of LuxR and LuxR-M22 biosensor strains by volatiles produced by clinical isolates of *P. aeruginosa*. Relative activation of selected biosensor strains harboring native (pSB401) and modified (pSB401-M22) LuxR receptors by volatiles produced by different clinical isolates of *P. aeruginosa*. Relative activation is defined as luminescence produced by the biosensor in exposure to volatiles of a *P. aeruginosa* isolate divided by the luminescence without the exposure. The clinical isolates were isolated from **A)** Lung infections (R) and **B)** bloodstream infections (B). Error bars represent standard deviation of four replicates. Asterics indicate on statistical difference between the activation of pSB401 and pSB401-M22 biosensors according to one way t-test ($p<0.05$). Results showing 11 representative strains. All examined strains except R11 exhibited similar activation patterns.

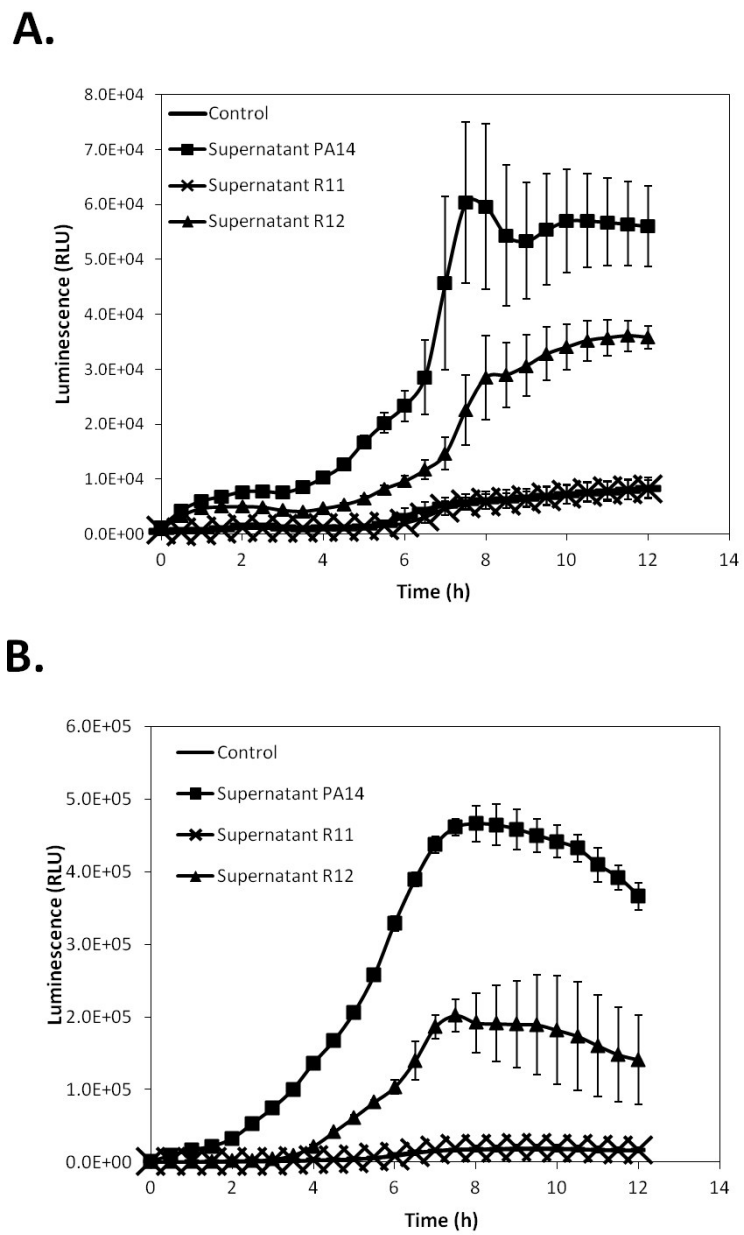

Figure S4. Use of QS biosensors for analysis of QS signal production in clinical isolates of *P. aeruginosa*. Luminescence produced by the *P. aeruginosa* JP2/pKD201 and *P. aeruginosa* JP2/pKD-rhlA biosensors in response to supernatants from cultures of the clinical *P. aeruginosa* isolates R11, R12 and PA14. A) Luminescence produced by the *P. aeruginosa* JP2/pKD201 biosensor sensitive to *las*-regulated 3-oxo-C12-HSL. B) Luminescence produced by the *P. aeruginosa* JP2/pKD-rhlA biosensor sensitive to *rhl*-regulated C4-HSL. Error bars represent standard deviation of 4 replicates. Control treatment indicates luminescence produced by the biosensor without addition of isolates supernatants.

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R11        PTVSHCTQSVLPIFWEPSIYQTRKQHEFFEEASAAGLVYGLTMPLHGARGELGALSLSVEAE
R12        PTVSHCTQSVLPIFWEPSIYQTRKQHEFFEEASAAGLVYGLTMPLHGARGELGALSLSVEAE
PA14       PTVSHCTQSVLPIFWEPSIYQTRKQHEFFEEASAAGLVYGLTMPLHGARGELGALSLSVEAE
           ************************************************************

R11        SRVEANRFMESVLPTLWMLKDYALQSGAGLAFEHPVNKPVVLTSREKEVLQWCAIGKTSWEI
R12        NRAEANRFMESVLPTLWMLKDYALQSGAGLAFEHPVSKPVVLTSREKEVLQWCAIGKTSWEI
PA14       NRAEANRFMESVLPTLWMLKDYALQSGAGLAFEHPVSKPVVLTSREKEVLQWCAIGKTSWEI
           .*.*********************************.************************

R11        SVICNCSEANVNFHMGNIRRK
R12        SVICNCSEANVNFHMGNIRRK
PA14       SVICNCSEANVNFHMGNIRRK
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Figure S5. Partial amino acids sequence and alignment of LasR receptors from *P. aeruginosa* PA14 and two *P. aeruginosa* strains isolated from lung infections (R11 and R12).

3. References

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4. V. Brown and E. Lowbury, *J. Clin. Pathol.*, 1965, **18**, 752–756.