**Material and Methods**

**Study organisms and culture conditions**

Using three strains**,** NPAO1 lasI/rhlI lasB:lux; NPAO1 lasI/rhlI lasI:lux and NPAO1lasI/rhlI rhlI:lux. These strains were characterized as lasB:luxCDABE genomic reporter fusion in NPAO1∆lasI/rhlI, lasI:luxCDABE genomic reporter fusion in NPAO1∆lasI/rhlI and rhlI:luxCDABE genomic reporter fusion in NPAO1∆lasI/rhlI, respectively. All strains were streaked out in Luria-Bertani (LB) agar at 37°C for 24 h, then a single colony was subculture in 10 ml LB and incubated at 37°C under shaking conditions (180 rpm) for 24 h.

**Preparing signal molecules**

3-oxo-C12-HSL and C4-HSL signals were prepared in methanol at 7 different concentrations ranged from 0.1, 0.5, 1, 2, 3, 4 and 5 µM. All concentrations of each signal were prepared from 100 mM stock.

**Biological experiment**

All overnight strains were centrifuged and washed three times using PBS then re-suspended the cells in LB and diluted to an OD600 of 0.05. 200 µl of each culture was transferred to a black 96-well plate with a clear bottom then transfer the combinatorial signals at the indicated different concentrations. C4-HSL was kept at a constant concentration in each experiment and different 3O-C12-HSL concentrations were added, ranged from 0-5 µM. Each combinatorial signal experiment was carried out in five replicates. Methanol was used instead of signal molecules as a control. The plates were incubated in BioSpa at 37°c for 18 h. Measurements of OD600 and RLU (Relative Luminescence Units) were collected every h.