Cell-free and *in vivo* characterization of Lux, Las, and Rpa quorum activation systems in *E. coli*

Supporting Information

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Materials and methods

TX-TL:

TX-TL was prepared using BL-21 Rosetta2 cells (Novagen) as previously described.[10] All TX-TL reactions were conducted at 5uL final volume with a maximum of 25% final volume consisting of DNA. TX-TL extract was 33% final concentration, TX-TL buffer 42% final concentration, and DNA 25%. Any extra volume was filled with Nuclease-Free Water (Ambion). All liquid transfers for TX-TL preparation were performed by an acoustic liquid handling robot, the Echo 525 (LabCyte). After liquid transfer was complete plates were spun at 2000g for 2 minutes to spread the TX-TL across the well. Plates were then sealed with a polylofein seal (Thermo Fisher Scientific) to prevent evaporation. Plates were read in a BioTek Synergy H1M incubated at 29°C with excitation wavelength of 485nm and emission wavelength of 515nm for deGFP. All plasmids used for TX-TL were purified using NucleoBond Xtra Midiprep kit (Macherey-Nagel). AHLs (Sigma Aldrich) were suspended in DMSO and were added such that the final concentration of DMSO in each reaction was fixed at 1%. All TX-TL experiments had negative controls for both auto fluorescence of the TX-TL (wells containing only extract, buffer, and water, with no DNA or AHL added), and for background or "leak" of the DNA (TX-TL containing extract, buffer, water, DNA, but no AHL). For all experiments shown we subtracted the auto fluorescence of TX-TL from the time traces and displayed the "leak" of the DNA clearly in Figure 1a (0nM AHL condition). All TX-TL experiments were conducted at a final concentration of 1% DMSO, the agent used to solubilize the AHL, to control for any confounding variable introduced by the DMSO itself. All TX-TL negative controls were run in quadruplicate for each experiment.

In vivo:

JM109 Mix & Go cells (Zymo Research) were co-transformed with a plasmid constitutively expressing the transcription factor on p15a origin containing chloramphenicol resistance and a plasmid with the AHL promoter regulating GFP expression on a ColE1 origin containing kanamycin resistance. Three colonies from each plate were inoculated into LB containing chloramphenicol (34ug/mL) and kanamycin (50ug/mL) overnight and grown until saturation. Cultures were then diluted 1:100 fold into M9CA (TekNova) containing chloramphenicol (34ug/mL) and kanamycin (50ug/mL) and grown until mid-log phase (0.3-0.6 OD600). Cultures were then diluted into M9Ca containing chloramphenicol (34ug/mL) and kanamycin (50ug/mL) and 1uM of the appropriate AHL, or 0.1% DMSO as a negative control. 150uL of culture was grown in 96-well flat-bottom round-well plates (PerkinElmer). Cells were grown in a BioTek Synergy H1m with constant linear shaking and 37°C incubation. An excitation wavelength of 485nM and emission wavelength of 515nM for deGFP was used. 600nM absorbance was used for calculating cell density. As with the TX-TL experiments, all *in vivo* experiments also were conducted at the same DMSO concentration to avoid a confounding variable. Background values were calculated for each of the nine strains by running DMSO only (no AHL) conditions. These background values were then subtracted from each trace. All *in vivo* negative controls were run in triplicate for each experiment.

Data Analysis:

Data analysis was performed using custom Python scripts. All raw data and code used in this manuscript is available at github.com/adhalleran/QS . All sequence files for constructs used in this manuscript are also available at github.com/adhalleran/QS .

Supporting Table 1:
Sequences used in this study. All .gb files are available here: https://github.com/adhalleran/QS/tree/master/Sequences and via email upon request.

J23106 is part of the Anderson promoter library. BCD2 was selected from the library generated by Mutalik *et al.*, and ECK120033736 was picked from Chen *et al.*.[1, 2]
Highlighted regions correspond to the promoter, 5' UTR, CDS, and terminator respectively.
Unhighlighted regions are scars generated by Type IIS restriction enzyme-mediated assembly.

Constitutive LuxR J23106-BCD2-LuxR- ECK120033736 TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC TACT GGGCCCAAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCT TAATCATGCTAAGGAGGTTTTCT AATG ATGAAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCTTGTAGAAG CAATAATGATATTAATCAATGCTTATCTGATATGACTAAAATGGTACATTGTGAATATTATTT ACTCGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATCCTAGATAATTACCC TAAAAAATGGAGGCAATATTATGATGACGCTAATTTAATAAAATATGATCCTATAGTAGATTA TTCTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAAAACAATGCTGTAAATAAAAA ATCTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTTATCACTGGGTTTAGTTTCCCTA TTCATACGGCTAACAATGGCTTCGGAATGCTTAGTTTTGCACATTCAGAAAAAGACAACTAT ATAGATAGTTTATTTTTACATGCGTGTATGAACATACCATTAATTGTTCCTTCTCTAGTTGAT AATTATCGAAAAATAAATATAGCAAATAATAAATCAAACAACGATTTAACCAAAAGAGAAAAA GAATGTTTAGCGTGGGCATGCGAAGGAAAAAGCTCTTGGGATATTTCAAAAATATTAGGTT GCAGTGAGCGTACTGTCACTTTCCATTTAACCAATGCGCAAATGAAACTCAATACAACAAA CCGCTGCCAAAGTATTTCTAAAGCAATTTTAACAGGAGCAATTGATTGCCCATACTTTAAAA ATTAATAA AGGT CTAACGCATGAGAAAGCCCCCGGAAGATCACCTTCCGGGGGCTTTTTTATTGCGCCCTTG AGA
Constitutive LasR J23106-BCD2-LasR- ECK120033736 TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC TACT GGGCCCAAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCT TAATCATGCTAAGGAGGTTTTCT AATG GCCTTGGTTGACGGTTTTCTTGAGCTGGAACGCTCAAGTGGAAAATTGGAGTGGAGCGCC ATCCTCCAGAAGATGGCGAGCGACCTTGGATTCTCGAAGATCCTGTTCGGCCTGTTGCCT AAGGACAGCCAGGACTACGAGAACGCCTTCATCGTCGGCAACTACCCGGCCGCCTGGCG CGAGCATTACGACCGGGCTGGCTACGCGCGGGTCGACCCGACGGTCAGTCACTGTACCC AGAGCGTACTGCCGATTTTCTGGGAACCGTCCATCTACCAGACGCGAAAGCAGCACGAGT TCTTCGAGGAAGCCTCGGCCGCCGGCCTGGTGTATGGGCTGACCATGCCGCTGCATGGT GCTCGCGGCGAACTCGGCGCGCTGAGCCTCAGCGTGGAAGCGGAAAACCGGGCCGAGG CCAACCGTTTCATAGAGTCGGTCCTGCCGACCCTGTGGATGCTCAAGGACTACGCACTGC AAAGCGGTGCCGGACTGGCCTTCGAACATCCGGTCAGCAAACCGGTGGTTCTGACCAGC CGGGAGAAGGAAGTGTTGCAGTGGTGCGCCATCGGCAAGACCAGTTGGGAGATATCGGT TATCTGCAACTGCTCGGAAGCCAATGTGAACTTCCATATGGGAAATATTCGGCGGAAGTTC GGTGTGACCTCCCGCCGCGTAGCGGCCATTATGGCCGTTAATTTGGGTCTTATTACTCTCT AATAA AGGT CTAACGCATGAGAAAGCCCCCGGAAGATCACCTTCCGGGGGCTTTTTTATTGCGCCCTTG AGA
Constitutive RpaR J23106-BCD2-RpaR- ECK120033736 TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC TACT

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AGA

pLux GFP
pLux-BCD2-deGFP- ECK120033736
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AGGT
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AGA

pLas GFP
pLas-BCD2-deGFP- ECK120033736
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AGA

pRpa GFP

pRpa-BCD2-deGFP- ECK120033736

GCACCTGTCCGATCGGACAGTATTACGCAAGAAAATGGTTTGTTATAGTCGAATAT TACT
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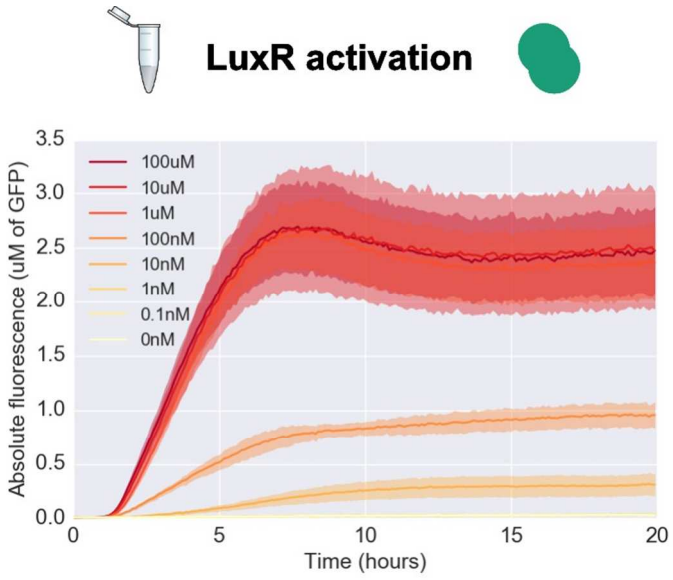
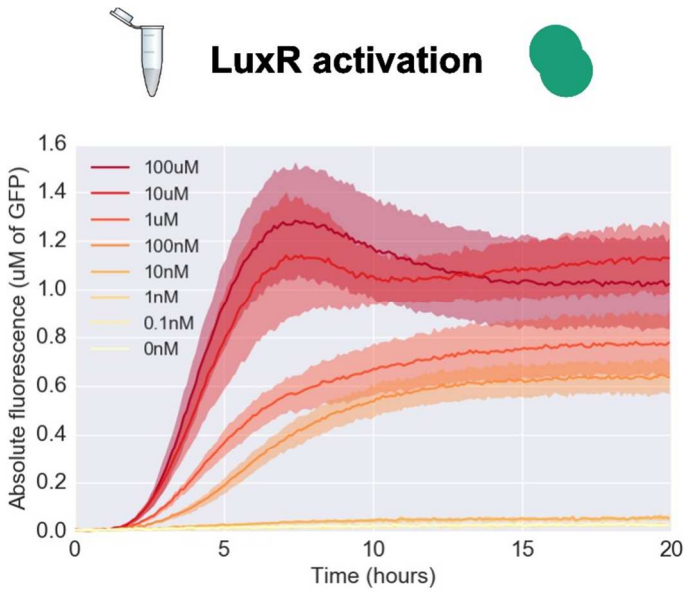
AGGT
CTAACGCATGAGAAAGCCCCCGGAAGATCACCTTCCGGGGGCTTTTTTATTGCGCCCTTG
AGA

References

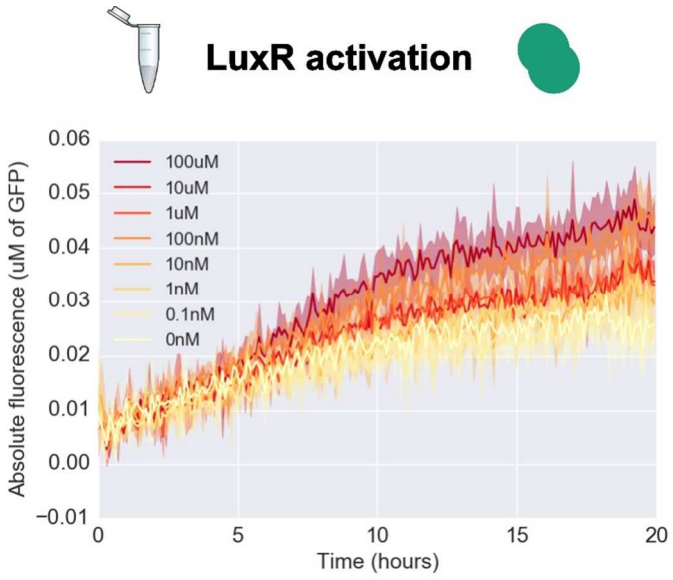
1. Mutalik, V. K., Guimaraes, J. C., Cambray, G., Lam, C., Christoffersen, M. J., Mai, Q.-A., Tran, A. B., Paull, M., Keasling, J. D., and Arkin, A. P. (2013) Precise and reliable gene expression via standard transcription and translation initiation elements, *Nature methods 10*, 354-360.
2. Chen, Ying-Ja, Peng Liu, Alec AK Nielsen, Jennifer AN Brophy, Kevin Clancy, Todd Peterson, and Christopher A. Voigt. (2013) Characterization of 582 natural and synthetic terminators and quantification of their design constraints, *Nature methods 10*, 659-664.



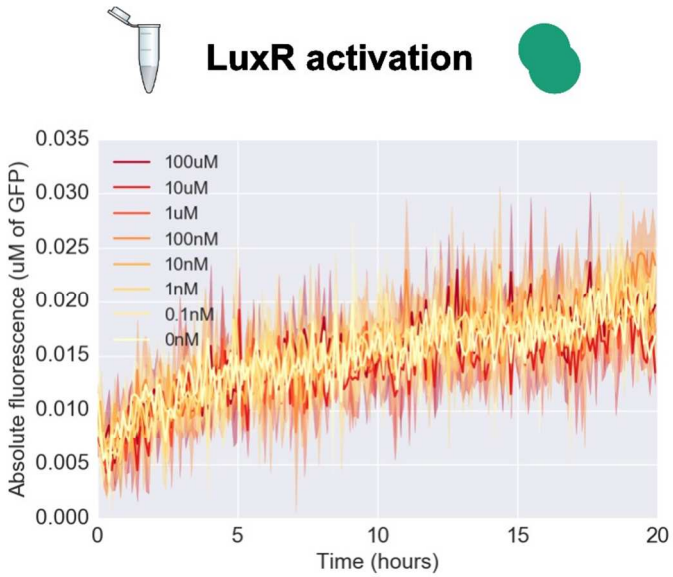
Supporting Figure 1:
TX-TL time traces from LuxR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



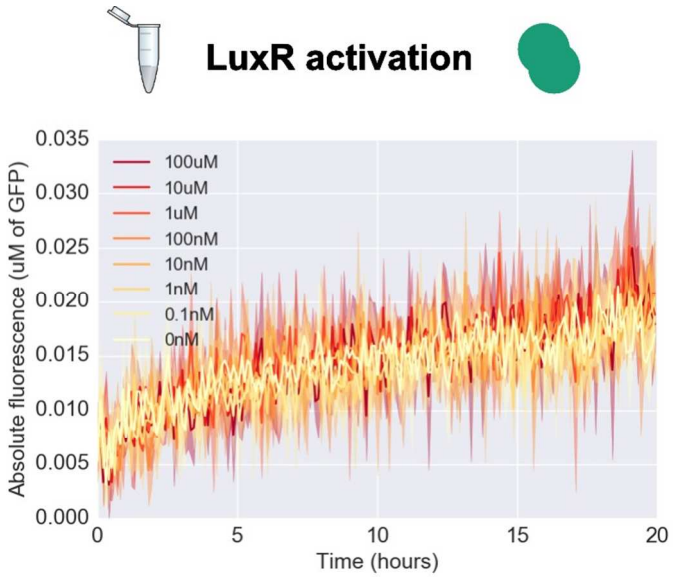
Supporting Figure 2:
TX-TL time traces from LuxR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



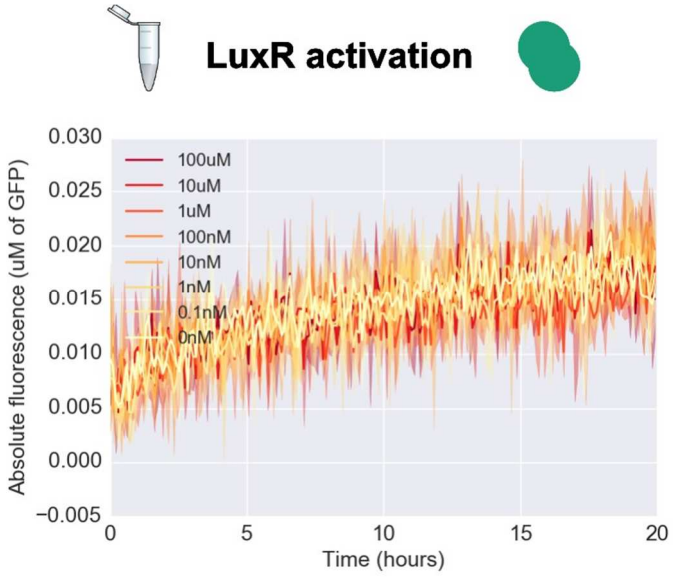
Supporting Figure 3:
TX-TL time traces from LuxR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



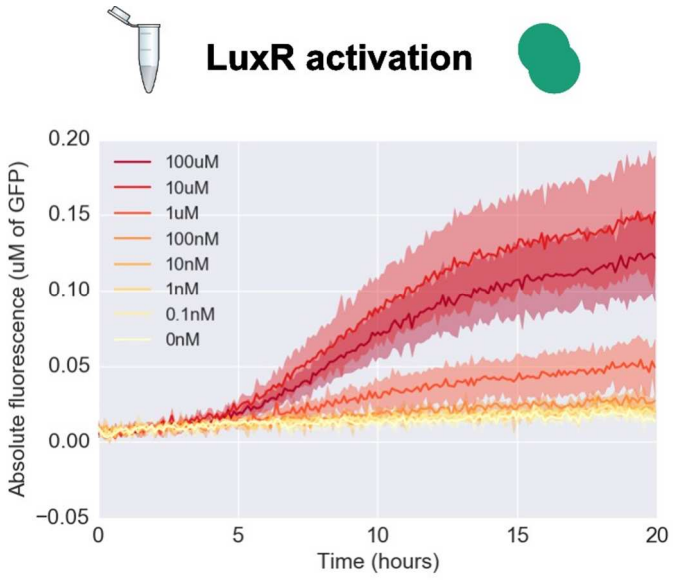
Supporting Figure 4:
TX-TL time traces from LuxR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



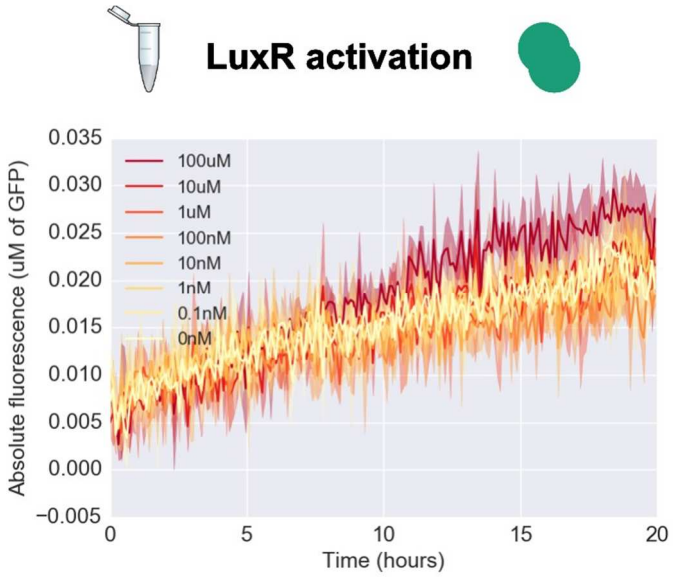
Supporting Figure 5:
TX-TL time traces from LuxR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



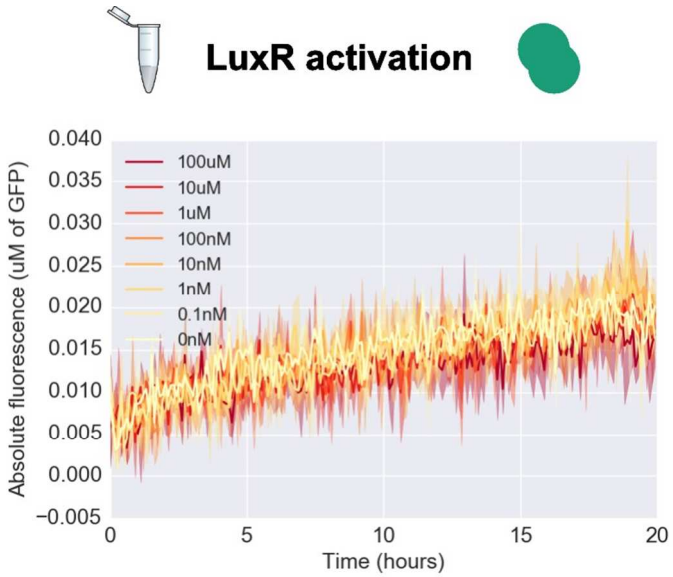
Supporting Figure 6:
TX-TL time traces from LuxR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



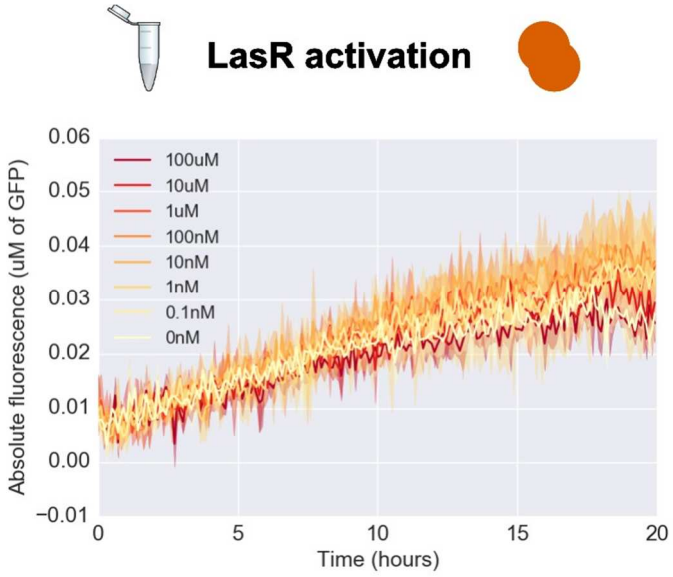
Supporting Figure 7:
TX-TL time traces from LuxR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



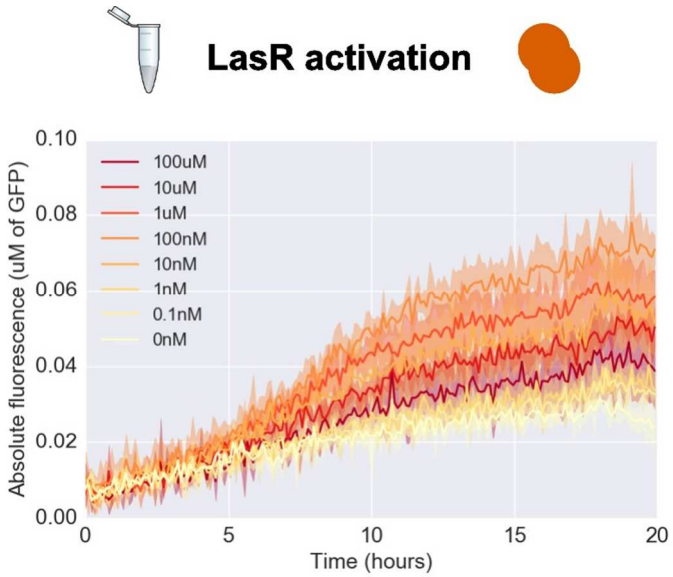
Supporting Figure 8:
TX-TL time traces from LuxR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



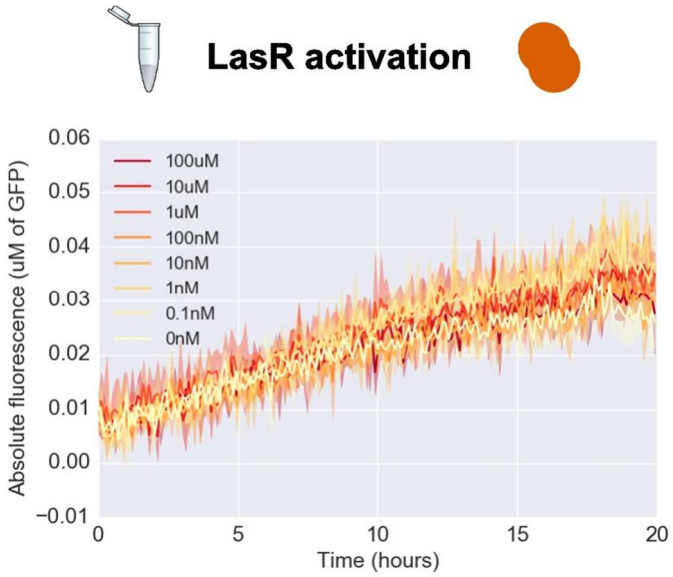
Supporting Figure 9:
TX-TL time traces from LuxR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



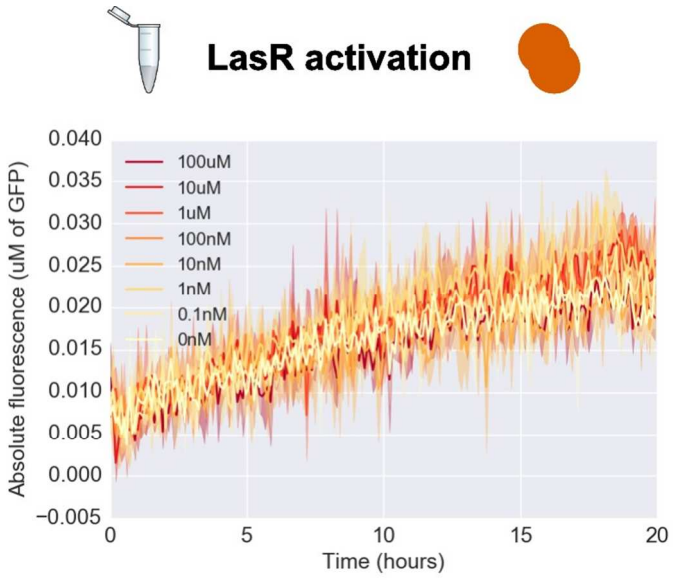
Supporting Figure 10:
TX-TL time traces from LasR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



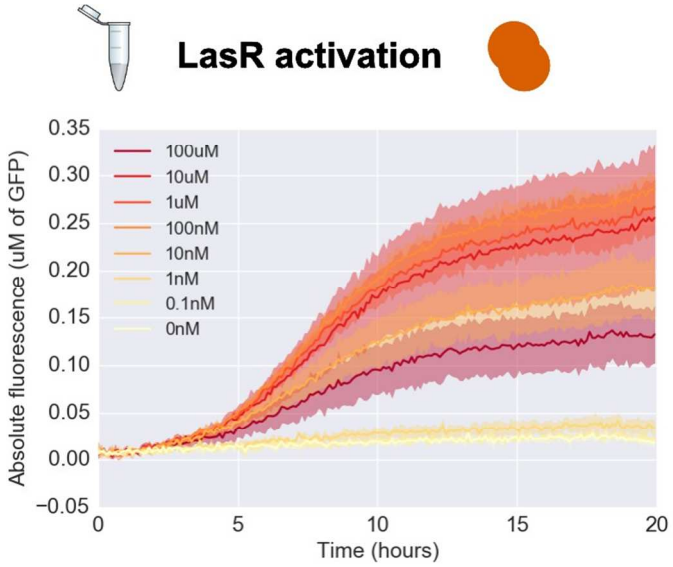
Supporting Figure 11:
TX-TL time traces from LasR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



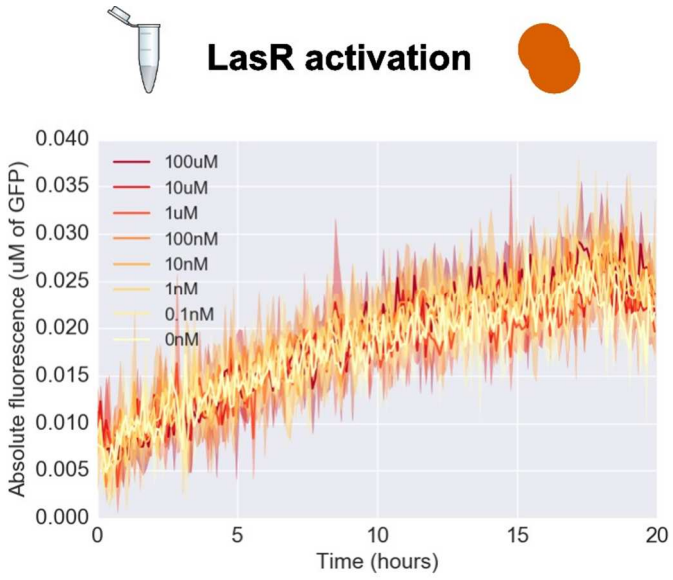
Supporting Figure 12:
TX-TL time traces from LasR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



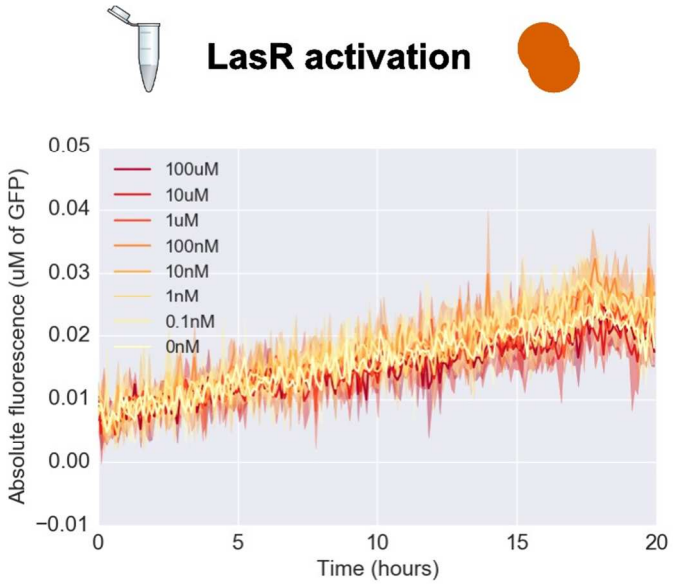
Supporting Figure 13:
TX-TL time traces from LasR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



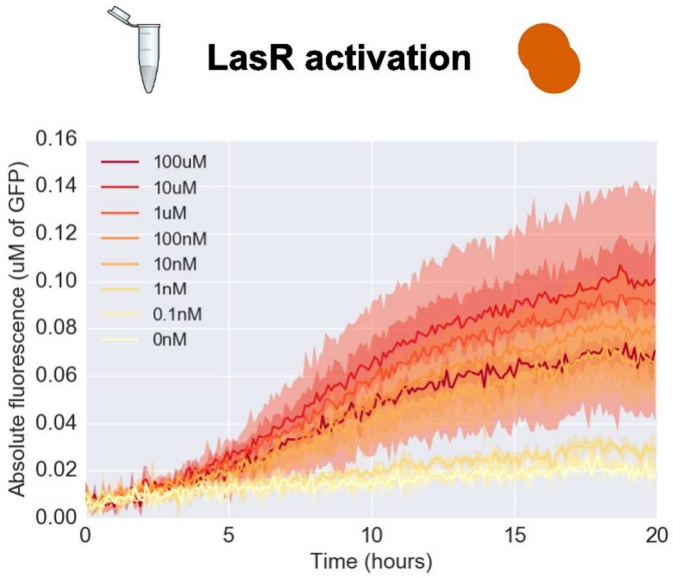
Supporting Figure 14:
TX-TL time traces from LasR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



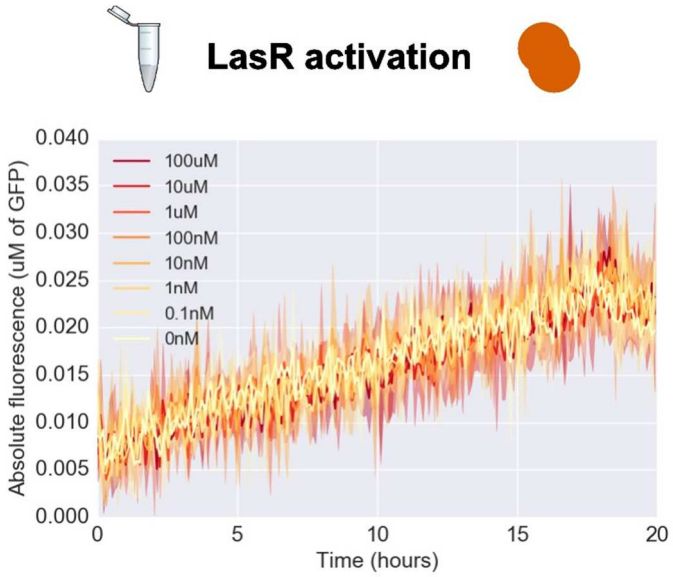
Supporting Figure 15:
TX-TL time traces from LasR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



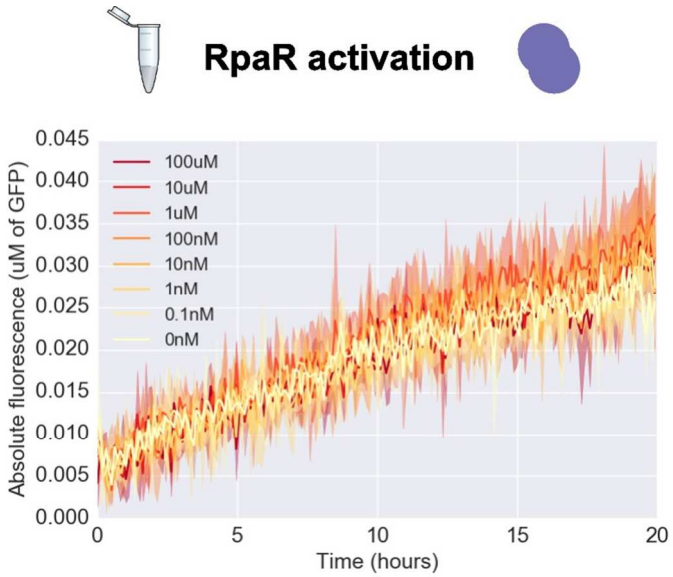
Supporting Figure 16:
TX-TL time traces from LasR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



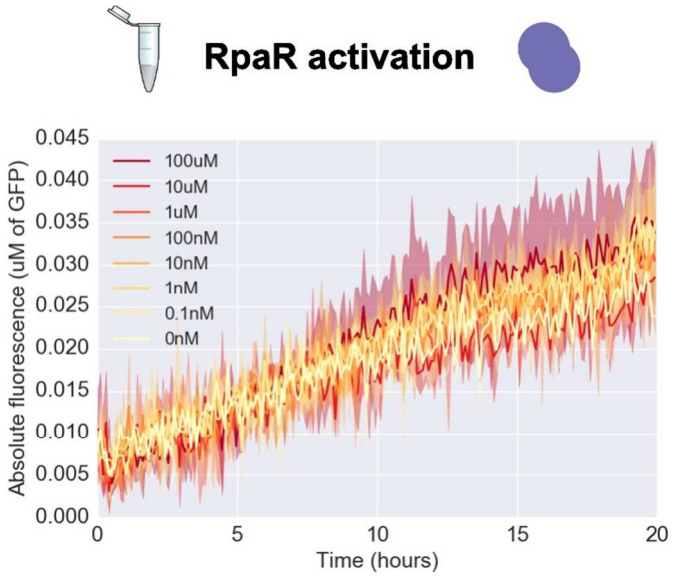
Supporting Figure 17:
TX-TL time traces from LasR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



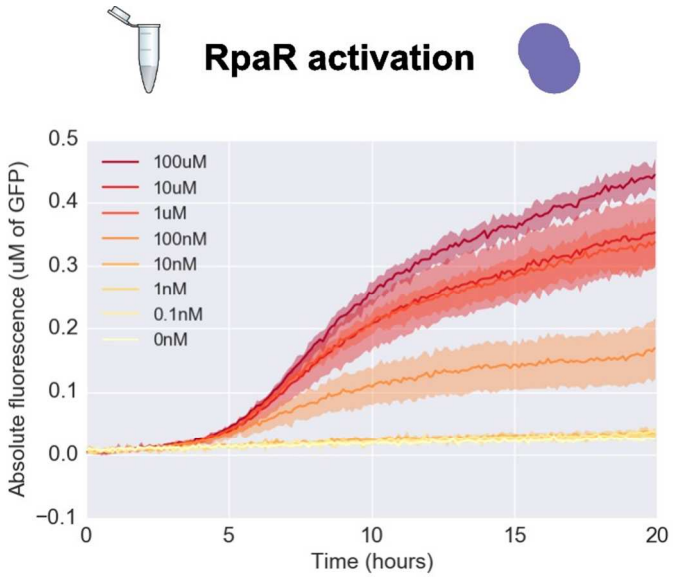
Supporting Figure 18:
TX-TL time traces from LasR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



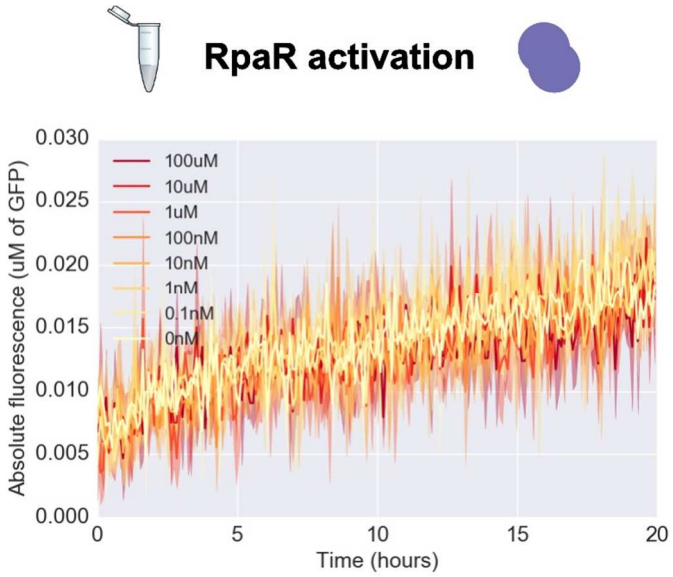
Supporting Figure 19:
TX-TL time traces from RpaR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



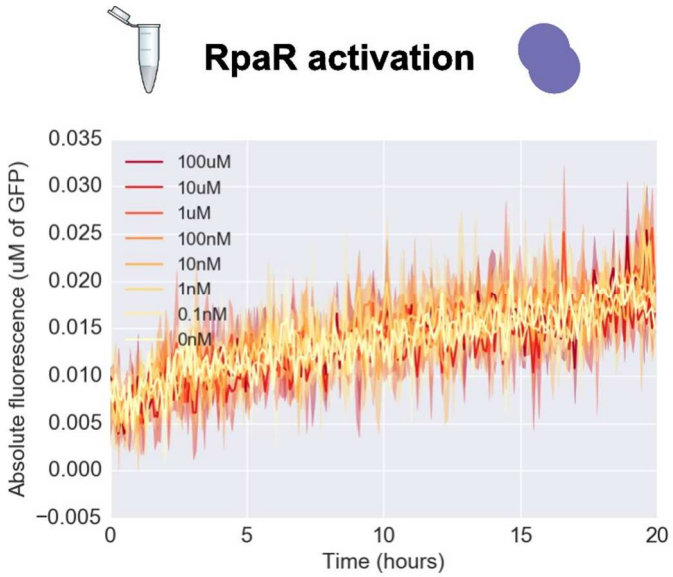
Supporting Figure 20:
TX-TL time traces from RpaR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



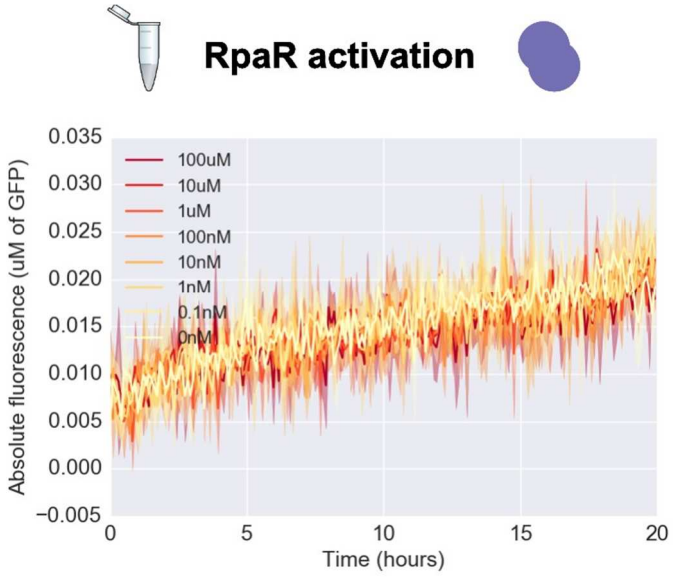
Supporting Figure 21:
TX-TL time traces from RpaR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



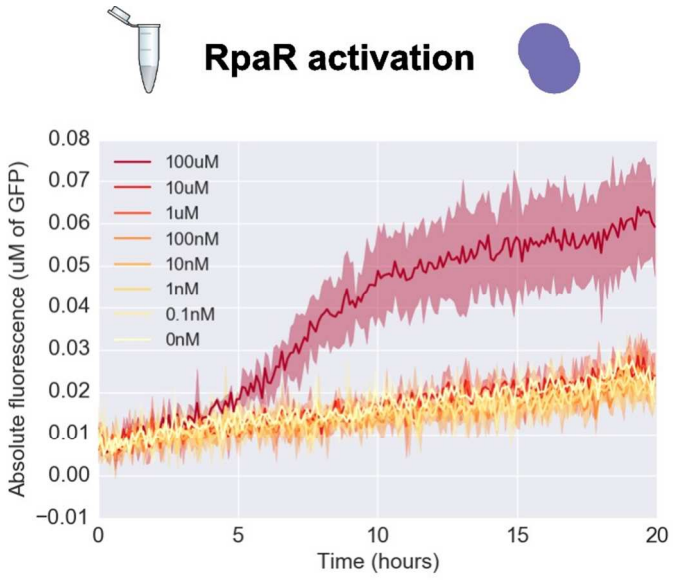
Supporting Figure 22:
TX-TL time traces from RpaR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



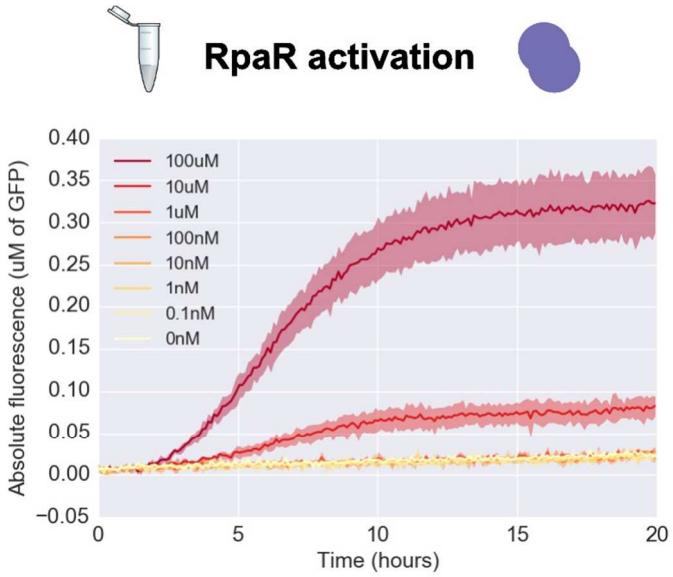
Supporting Figure 23:
TX-TL time traces from RpaR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



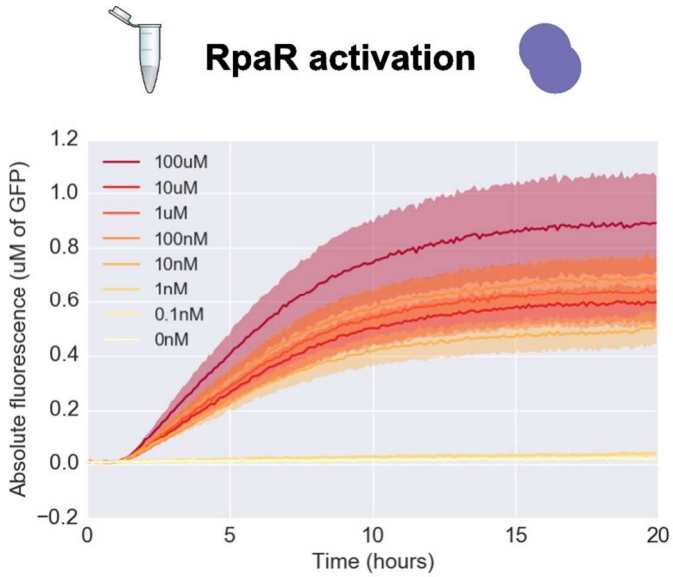
Supporting Figure 24:
TX-TL time traces from RpaR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



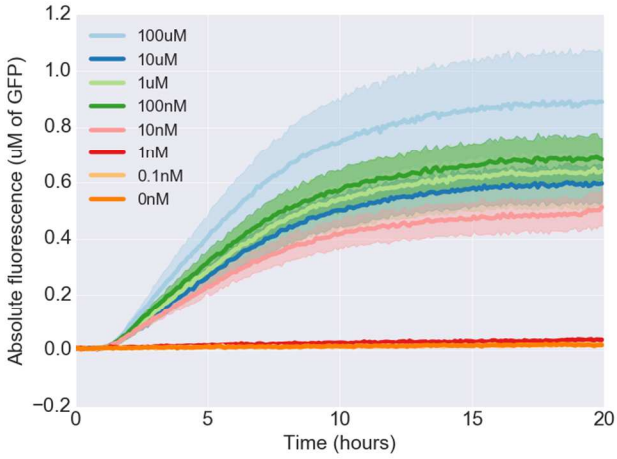
Supporting Figure 25:
TX-TL time traces from RpaR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



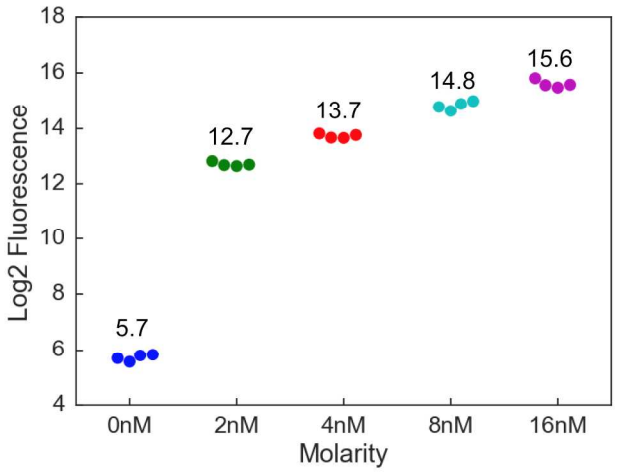
Supporting Figure 26:
TX-TL time traces from RpaR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



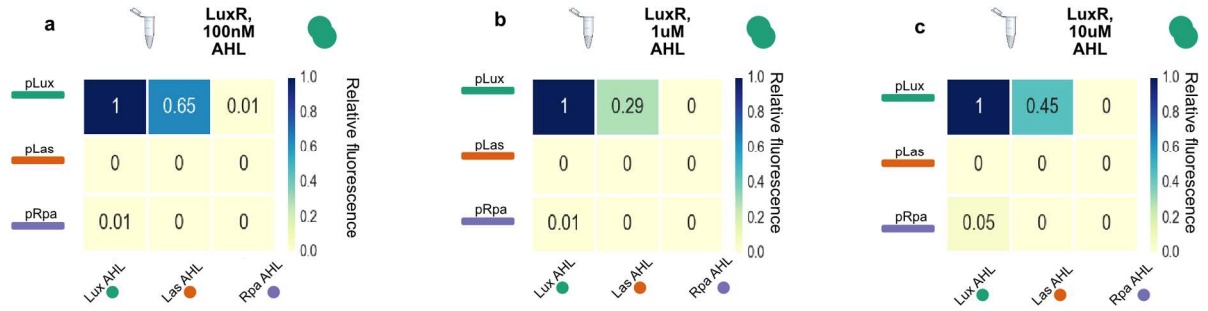
Supporting Figure 27:
TX-TL time traces from RpaR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.



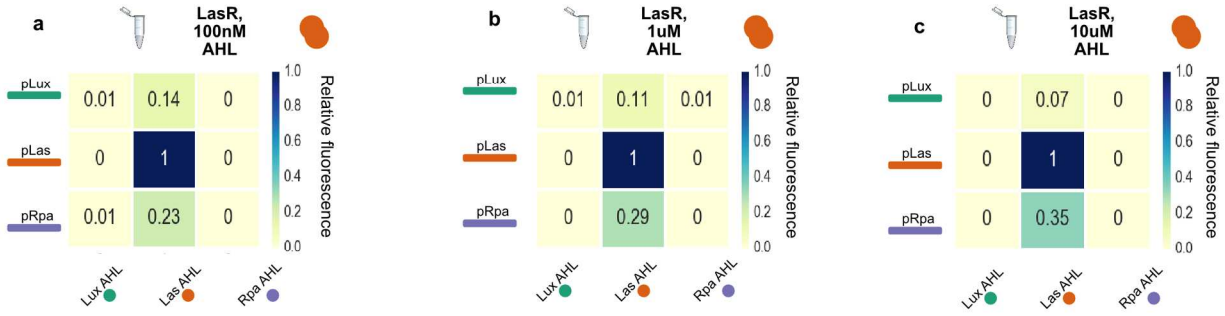
Supporting Figure 28:
Figure 1a rendered with a divergent color map rather than the sequential shown in 1a.



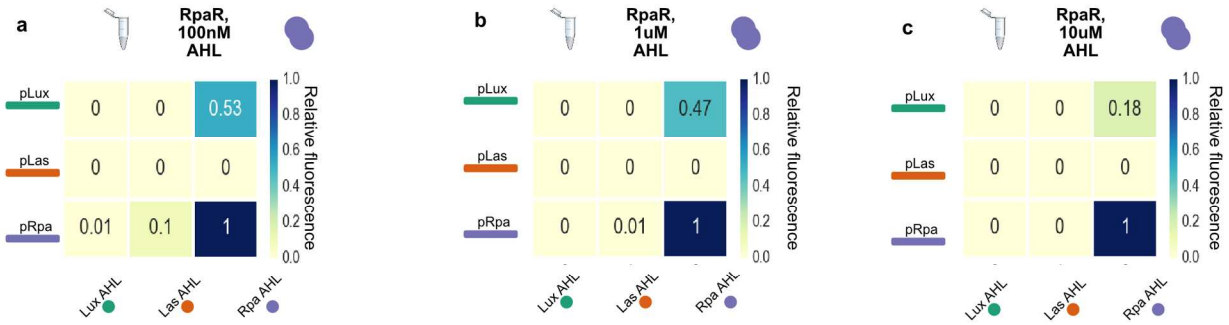
Supporting Figure 29:
Titration of RFP DNA causes a linear increase in reporter expression. Fluorescence for each replicate was calculated as the 95th percentile value obtained across the time course. Each dot is a single replicate. Numbers above each set of four replicates is the mean of the Log2 fluorescence.



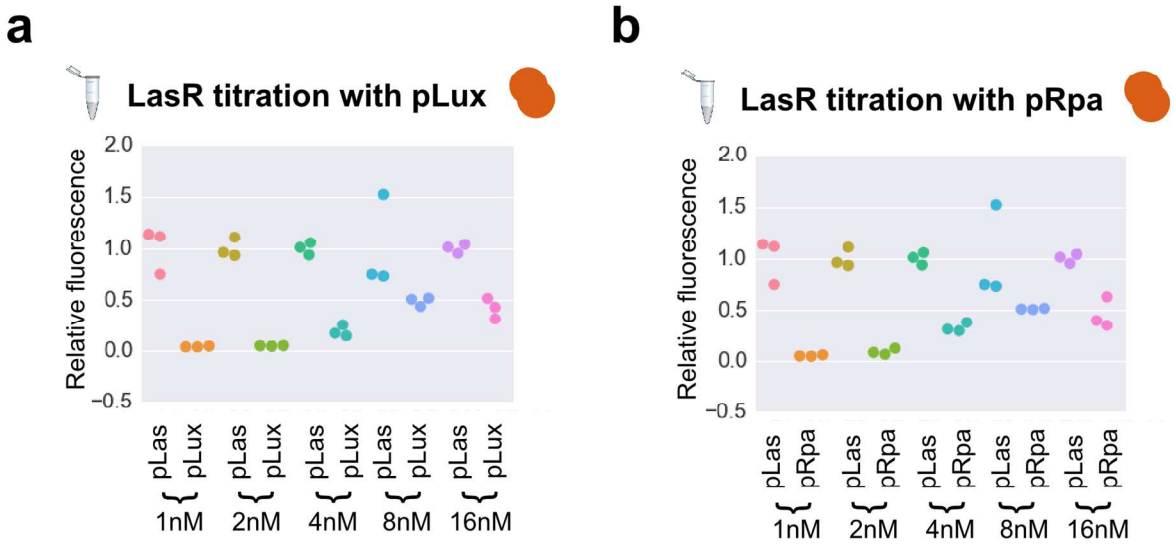
Supporting Figure 30: Crosstalk heatmap from figure 1b, shown at three different AHL concentrations ((a)100nM, (b)1uM, and (c)10uM).



Supporting Figure 31: Crosstalk heatmap from figure 1c, shown at three different AHL concentrations ((a)100nM, (b)1uM, and (c)10uM).



Supporting Figure 32: Crosstalk heatmap from figure 1d, shown at three different AHL concentrations ((a)100nM, (b)1uM, and (c)10uM).



Supporting Figure 33:
(a) LasR titration with pLux at 1uM AHL. Relative fluorescence calculated at each concentration of LasR DNA (1-16nM) as 95th percentile fluorescence / mean of three pLas-GFP 95th percentile fluorescence at the same concentration of LasR. (E) LasR titration with pRpa.