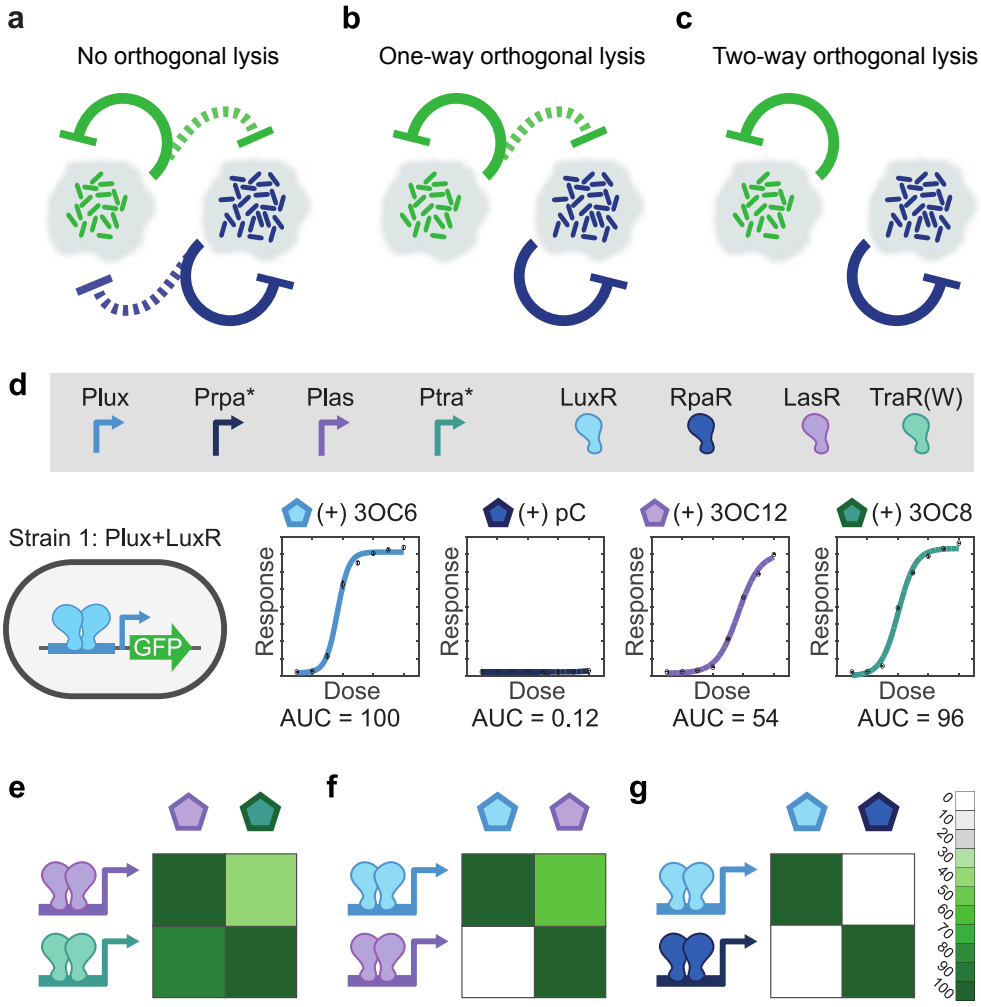
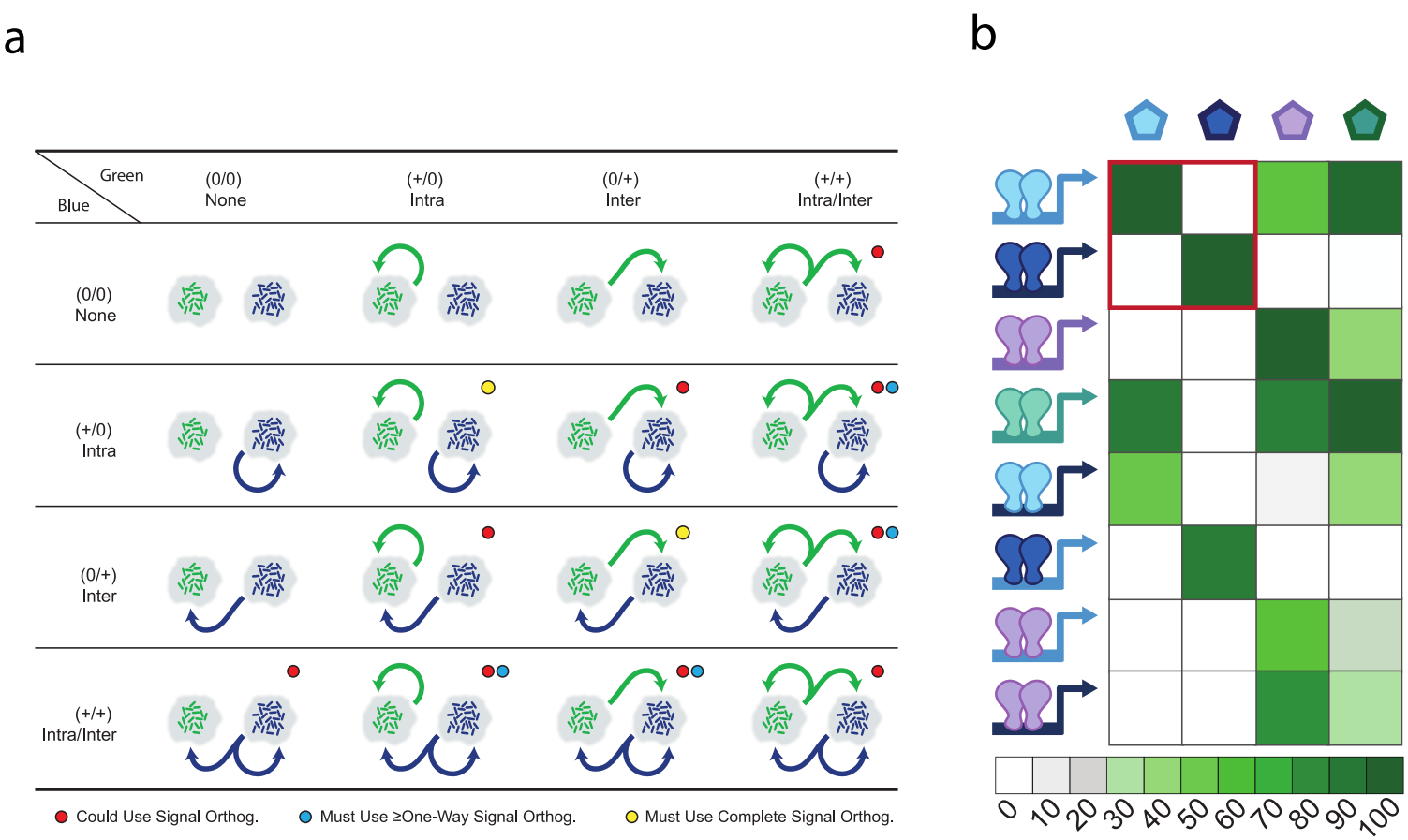
A stabilized microbial ecosystem of self-limiting bacteria using synthetic quorum-regulated lysis

Spencer R. Scott, M. Omar Din, Philip Bittihn, Liyang Xiong, Lev Tsimring, and Jeff Hasty

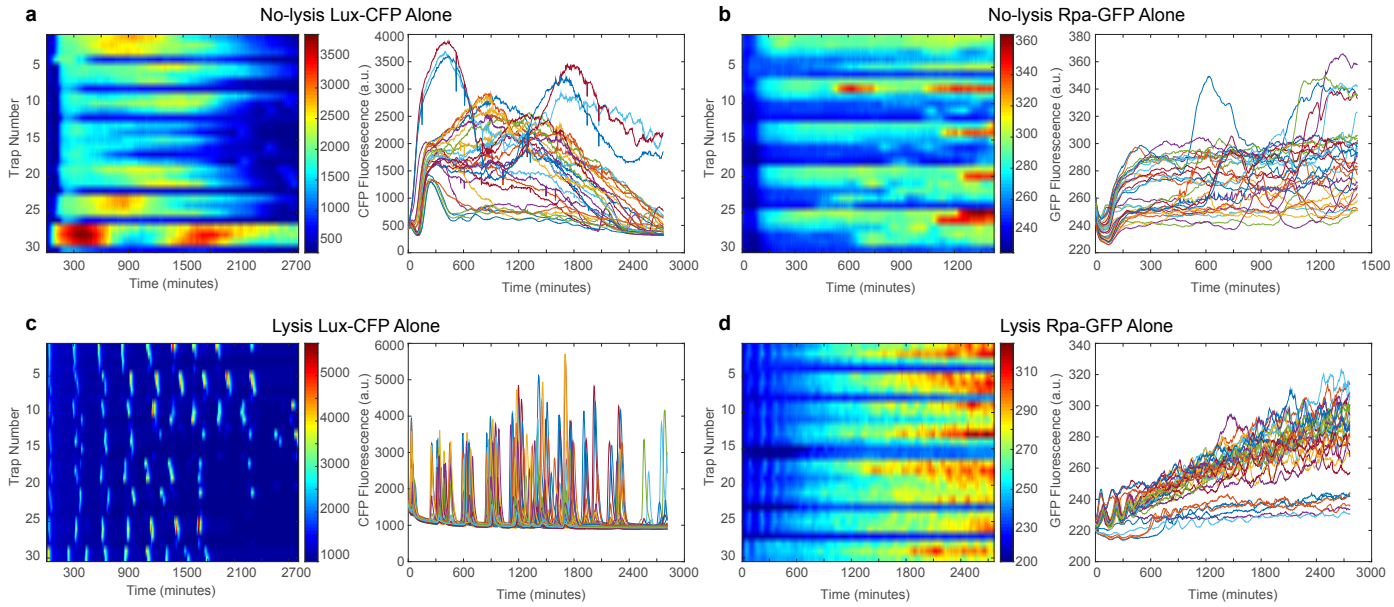
1. **Supplementary Figures 1-5**
2. **Supplementary Table 1**



Supplementary Figure 1. Communication motifs and quorum sensing signaling for synthetic microbial consortia. a-c. Schematic showing some of the QS communication possibilities between two members of a microbial consortia. **a**, With each strain capable of either sending, receiving, both or neither, there are generally 16 possible communication motifs (Supplementary Figure 2a). With the dual-strain consortia interfaced with the synchronized lysis circuit, we can achieve either one-way orthogonal signaling (**b**) or two-way orthogonal signaling (**c**). **d**, Schematic showing how QS systems can be tested and characterized for easy categorization. A strain containing one QS promoter and one QS receptor is subjected to a range of signals and its dose response curve is quantified by the area under its curve (AUC), which becomes the heat map parameter in **e-g**. Signaling homoserine lactones (HSL), 3-oxo-C6 HSL (3OC6), p-Coumaroyl HSL (pC), 3-oxo-C12 HSL (3OC12), and 3-oxo-C8 HSL (3OC8), are represented as pentagons color-coded to their native QS system. Error bars indicate the standard error of the mean (n=3). **e-g**, Heat maps of aggregated QS systems and their AUC responses to different signals; data is representative of 3 technical replicates. This methodology allows for quick identification of signal orthogonal strains classified by their diagonal matrices (**g**). Square matrices with significant induction in all squares indicates two-way signal cross-talk (**e**), while one-way signal cross-talk will only have one square not on the diagonal with a significant value (**f**). Additional combinations of promoters, receptors and signals are shown in Supplementary Figure 2b.

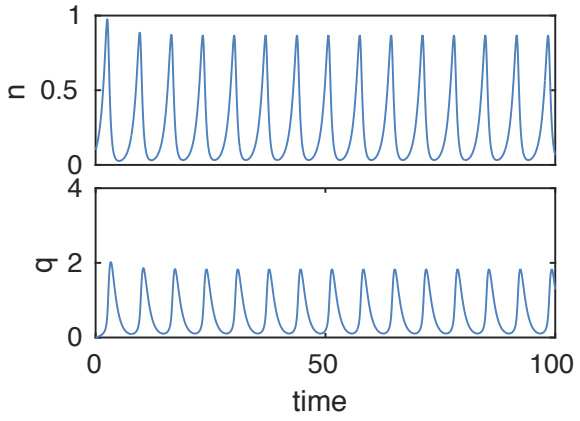


Supplementary Figure 2. Possible Two-Strain Quorum Sensing Communication Motifs. **a**, Each strain is capable of no, intra, inter or both intra and inter-communication. This gives 16 general QS dynamics between two strains. Certain communication motifs require different level of signal orthogonality. Yellow dot means that motif requires complete signal orthogonality. Blue dot means that motif requires at least one-way signal orthogonality. Red dot means the motif doesn't necessarily need signal orthogonality, but it could utilize that dynamic. **b,** Example heat map of aggregated QS systems and their AUC responses to different signals. The meaning of the column and row label pictograms is the same as in Supplementary Figure 1.



Supplementary Figure 3. Phenotypes of Lysis and Non-Lysis Monocultures. **a,** Fluorescence intensity heat map of individual traps plotted against time and raw CFP fluorescence time-series of non-lysis Lux-CFP cells grown alone. **b**, Fluorescence intensity heat map of individual traps plotted against time and raw GFP fluorescence time-series of non-lysis Rpa-GFP cells grown alone. **c**, Fluorescence intensity heat map of individual traps plotted against time and raw CFP fluorescence time-series of oscillatory lysing Lux-CFP cells grown alone. **d**, Fluorescence intensity heat map of individual traps plotted against time and raw GFP fluorescence time-series of constantly lysing Rpa-GFP cells grown alone.

Supplementary Figure 4. Plasmid maps of the main DNA constructs used in this study. Arrows in Red represent LuxR. Dark red arrows represent LuxI. Orange elements represent the pLuxI promoter. Yellow arrows represent RpaR. Green arrows represent sfGFP. Light blue arrows represent CFP. Dark blue arrows represent RpaI. Pink arrows represent the lysis gene E. Purple elements represent antibiotic resistance markers. Black elements represent origins of replication. Gray elements represent transcription terminators. See Methods section for more details.



Supplementary Figure 5. Dynamics of the model equation 1 in the Methods section. n represents the population of cells, and q represents the amount of fluorescent protein in the system.

Strain #	Strain Name	Host Bacterium	Plasmid(s)
1	MOD41	JS006, BW25113	pTD103luxI(-LAA)sfGFP + pZA35 X174E (+LuxR)
2	MOD29	JS006, BW25113	pTD103luxI sfGFP + pZA35 X174E (+LuxR)
3	MOD42	JS006, BW25113	pTD103luxI(TS) sfGFP + pZA35 X174E (+LuxR)
4	SRS732	SL1344	pTD103-LuxI-CFP
5	SRS800	SL1344	pTD103-LuxI-CFP + pZA35-X174E (+LuxR)
6	SRS840	SL1344	pTD103-RpaR-RPaI-LAA-sfGFP
7	SRS841	SL1344	pTD103-RpaR-RPaI-LAA-sfGFP + pZA35-X174E (+RpaR)

Supplementary Table 1. A list of strains used in this study and their respective chassis and plasmid(s). JS006, BW25113 is a lab strain E. coli chassis. SL1344 is an attenuated Salmonella enterica subsp. *enterica* serovar Typhimurium host. The components of the plasmids listed in the table are shown in Supplementary Figure 4.