Supplementary figures

Fig. S1. (A) The structure of L-azidohomoalanine (Aha). (B) The structure of TAMRA-alkyne. (C) A combined SILAC-BONCAT approach for quantifying differences in protein translation. Reference cultures were not treated with AI-1 at time 0 min. Otherwise, they were treated identically to experimental cultures. Experiments were performed in triplicate with one isotope label swap experiment. (D) Gel showing enrichment of Aha-labeled proteins using the DADPS tag. F – flow-through, W1-5 – washes, E – elution. The band marked by * is monomeric avidin. (E) The structures of the alkyne DADPS tag and the alkyne fragment released upon cleavage.

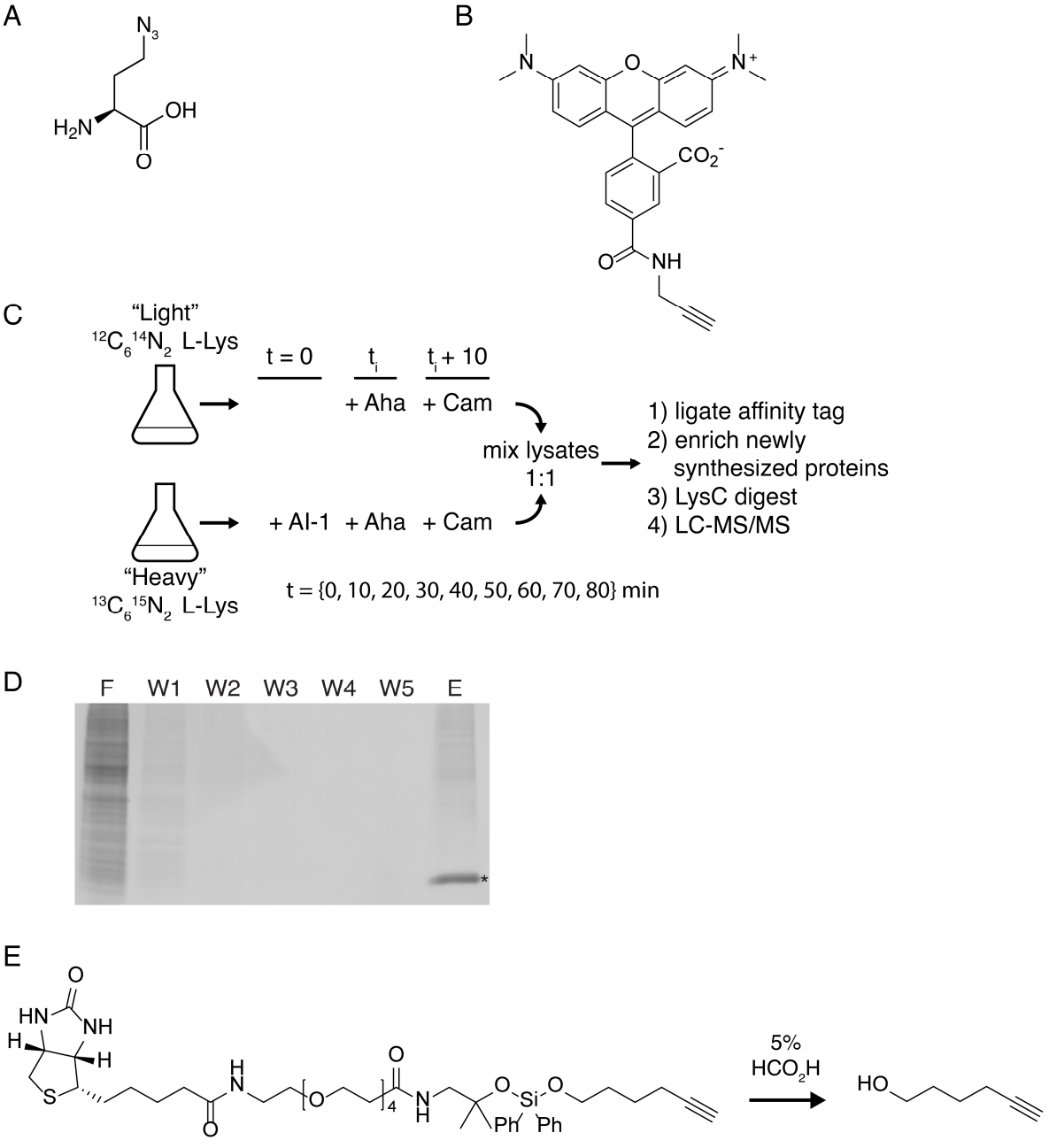
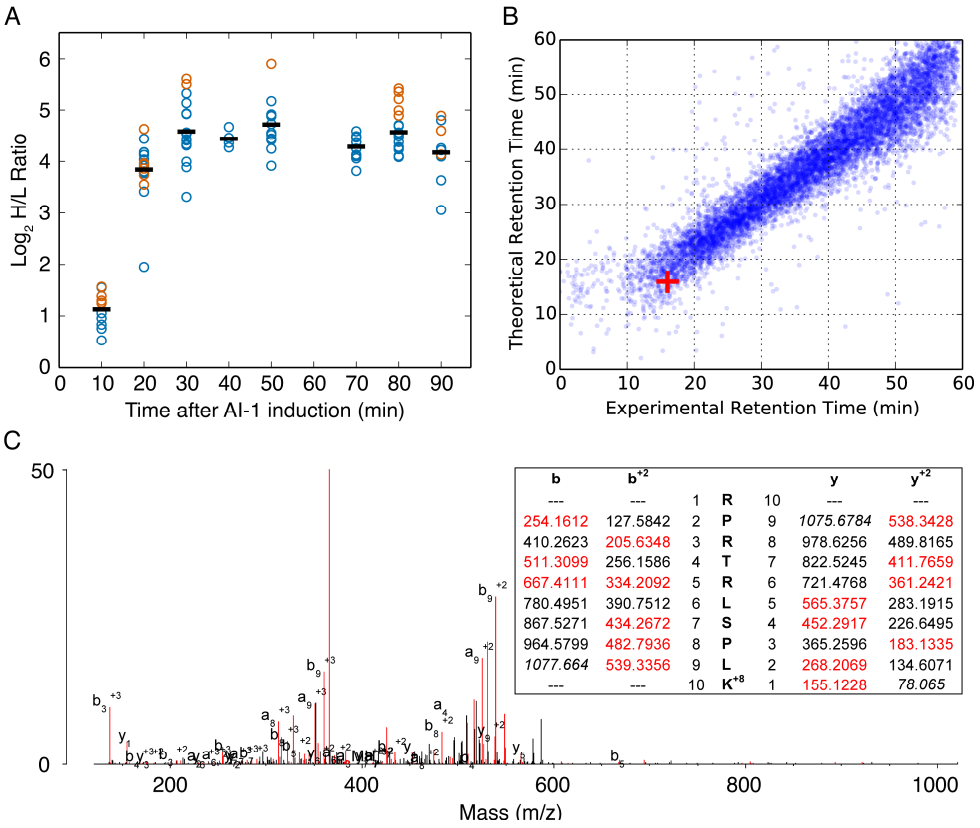


Fig. S2. Confirmation of LuxR peptide (RPRTRLSPLK) quantitation. (A) Masses in the range of 1222.7622 ± 2 ppm; the predicted mass of the RPRTRLSPLK peptide. Orange and blue markers represent normalized ratios of peptides from label swap experiments. (B) An additive model of polypeptide chromatography accurately predicts the retention time of the RPRTRLSPLK peptide. The measured and calculated retention times were 16.02 min and 15.09 min, respectively. (C) Fragmentation spectra of candidate masses in the 1222.7622 ± 2 ppm range were matched to the RPRTRLSPLK peptide by ProteinProspector (v 5.12.4). Red text and lines denote matched fragmentation spectra of the RPRTRLSPLK peptide.



b	b^{+2}				y	y^{+2}
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254.1612	127.5842	2	P	9	*1075.6784*	538.3428
410.2623	205.6348	3	R	8	978.6256	489.8165
511.3099	256.1586	4	T	7	822.5245	411.7659
667.4111	334.2092	5	R	6	721.4768	361.2421
780.4951	390.7512	6	L	5	565.3757	283.1915
867.5271	434.2672	7	S	4	452.2917	226.6495
964.5799	482.7936	8	P	3	365.2596	183.1335
1077.664	539.3356	9	L	2	268.2069	134.6071
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Fig. S3. Summary of measurements from proteomics experiments. (A) Sorted list of MS intensities for all quantified proteins. (B) MS/MS spectra per protein. Number of peptides (C) and quantifications (D) for each protein, calculated separately for each time point.

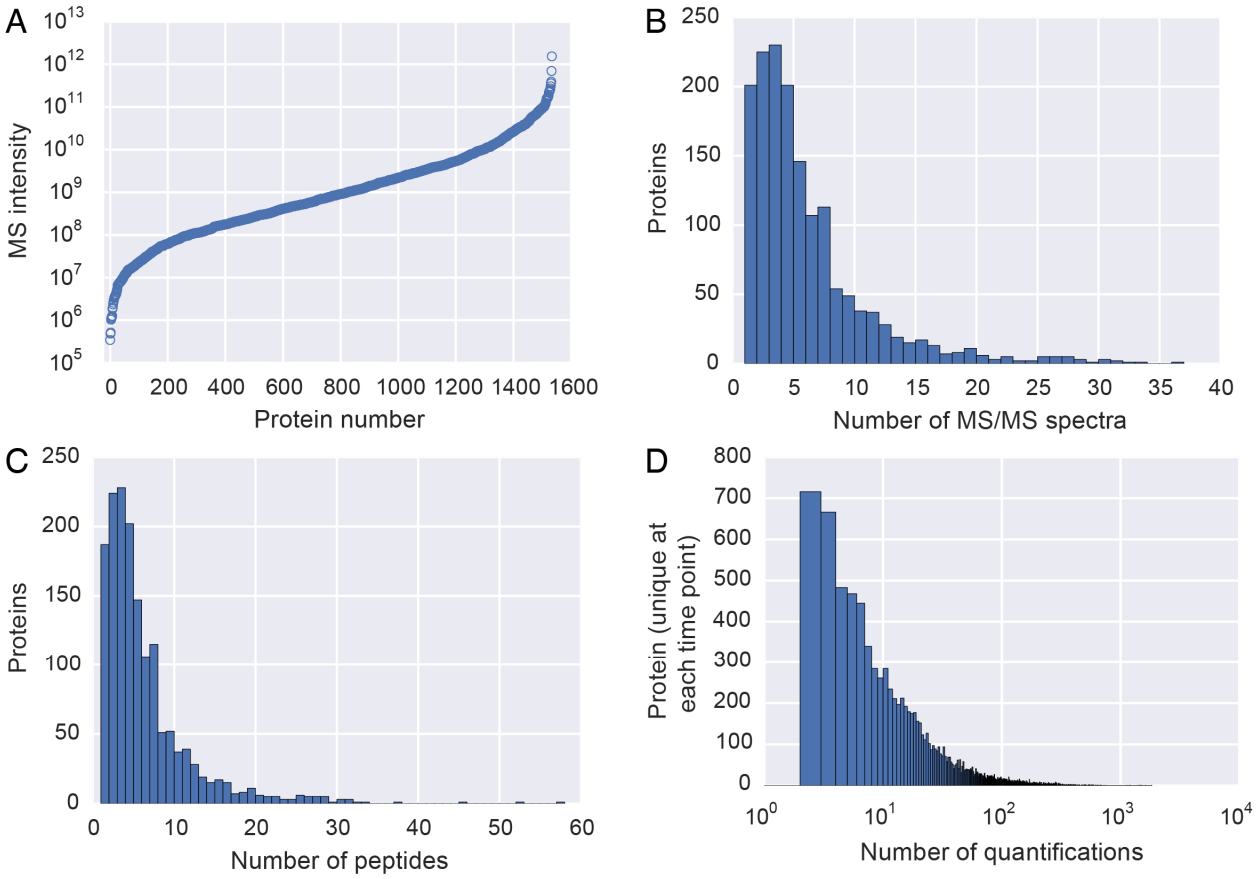


Fig. S4. Comparison of BONCAT proteomics data with the previously measured LuxR, AphA, and quorum-sensing regulons. For each regulon, the subset of genes for which proteins were identified with and without significant regulation is designated.

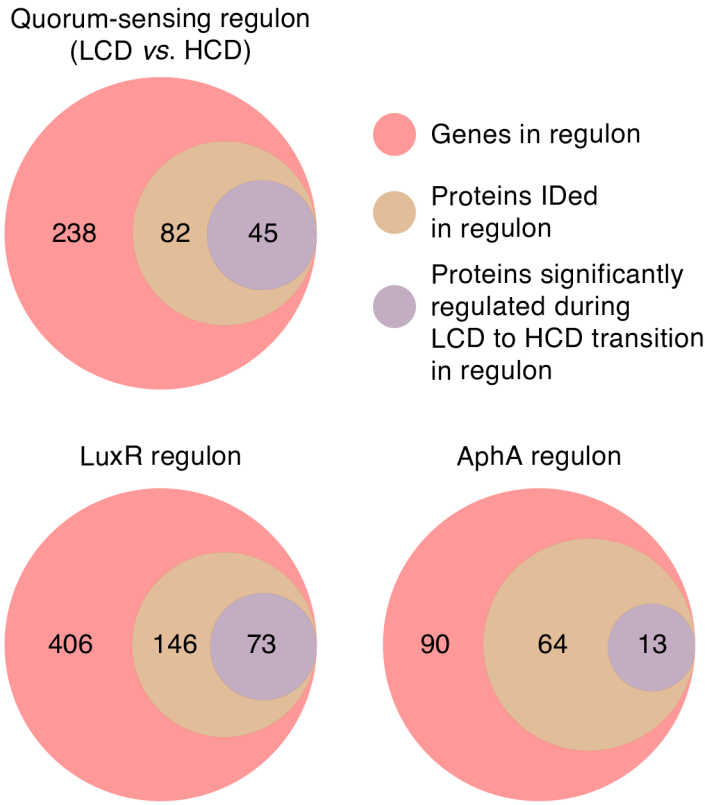
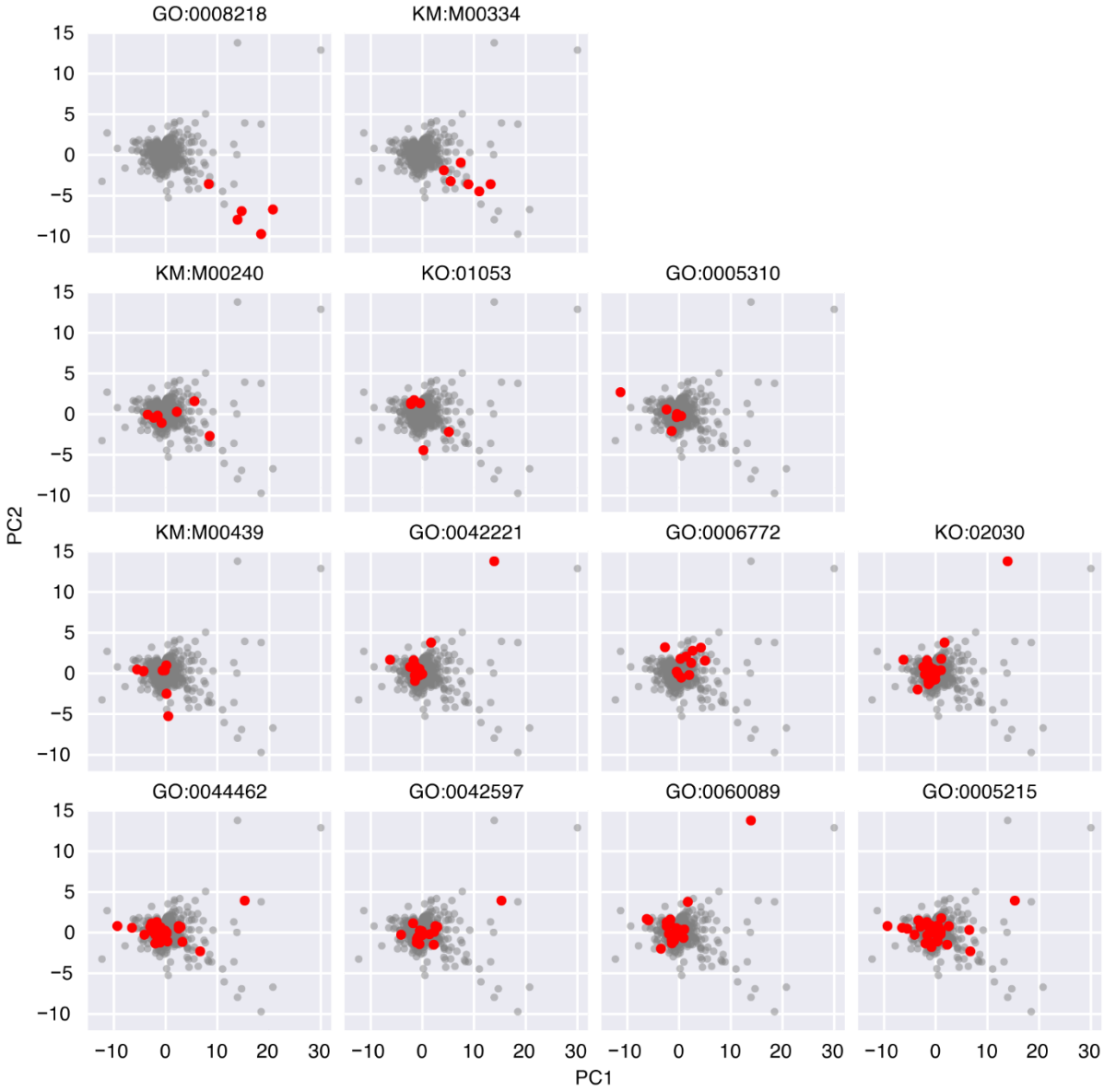
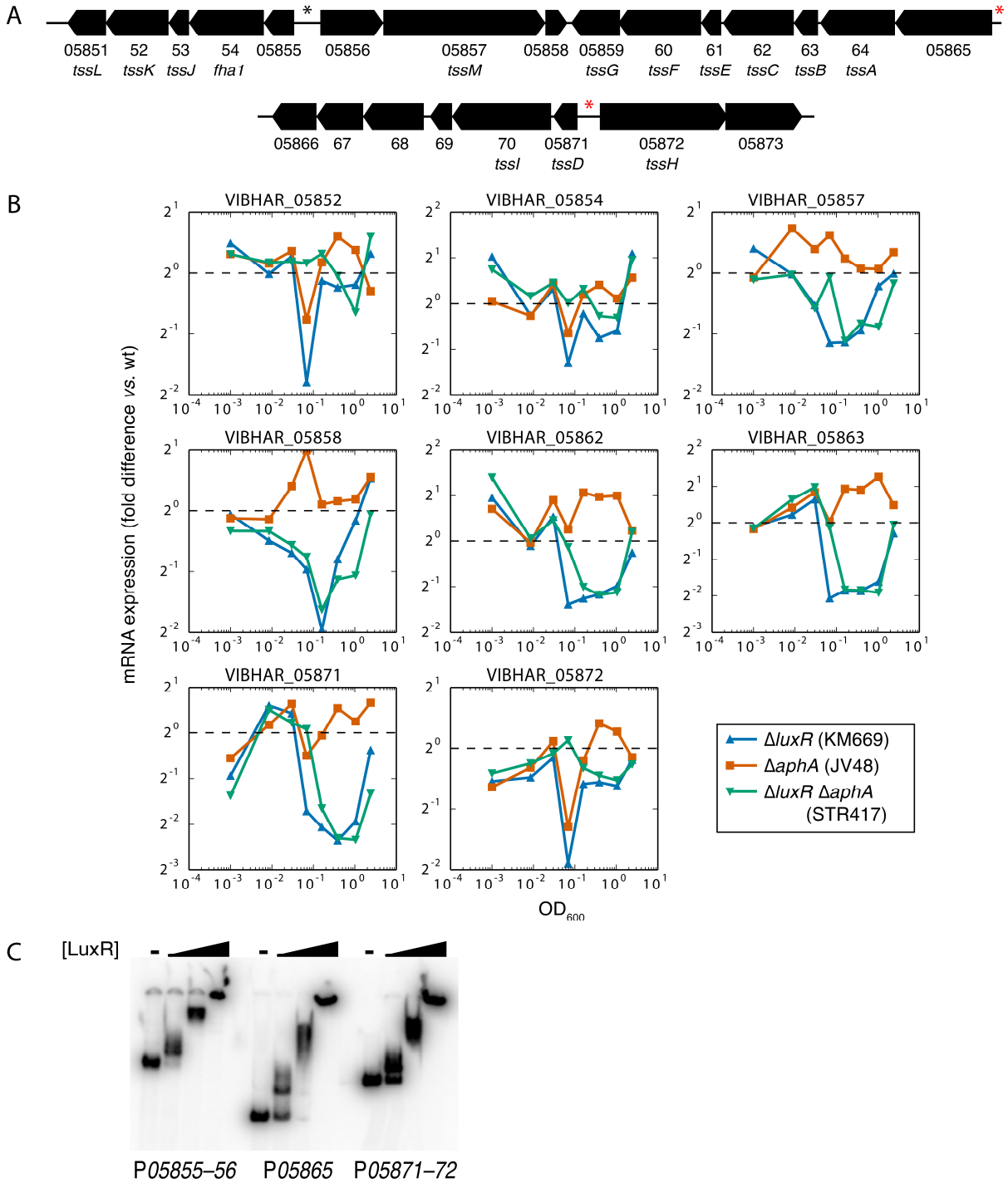


Fig. S5. Gene ontology analysis. Proteins from the significantly regulated gene ontology groups (Fig. 6*B*) are shown on the PCA plot. Groups were scored based on the average distance of proteins from the origin, and groups with fewer than 4 members were excluded. Ontology analysis used a combination of groups from the Gene Ontology (GO) database, and the KEGG Orthology (KO) and KEGG Module (KM) databases.



GO:0008218 bioluminescence
KM:M00334 type VI secretion system
KM:M00240 iron complex transport system
KO:01053 biosynthesis of siderophore group nonribosomal peptides
GO:0005310 bicarboxylic acid transmembrane transporter activity
KM:M00439 oligopeptide transport system
GO:0042221 response to chemical
GO:0006772 thiamine metabolic process
KO:02030 bacterial chemotaxis
GO:0044462 external encapsulating structure part
GO:0042597 periplasmic space
GO:0060089 molecular transducer activity
GO:0005215 transporter activity

Fig. S6. (A) The type VI secretion genes in *V. harveyi* are organized into five putative operons. The black asterisk symbol marks the location of the LuxR binding site previously identified by ChIP. Red askterisk symbols mark newly identified LuxR binding sites. (B) Up-regulation of type VI secretion operons at HCD is LuxR-dependent and AphA-independent. Results are from van Kessel et al. (2013). These data show relative gene expression values from Δ*aphA* (JV48), Δ*luxR* (KM669), and Δ*aphA* Δ*luxR* (STR417) *V. harveyi* strains relative to wild-type (BB120; wt) at varying cell densities. (C) EMSAs for reaction mixtures containing 0, 10, 100, or 1000 nM LuxR incubated with 1 nM radiolabeled DNA substrate corresponding to the three TSSS promoter regions for *VIBHAR_05855–56*, *VIBHAR_05865*, or *VIBHAR_05871–72*.



Supplementary tables

Table S1. Calculation of Aha incorporation based on total evidence counts, MS-MS counts, and MS intensity provides estimates in a range of 13−17%.

Measure of abundance	Aha peptides	Met peptides	All peptides	Calculated Aha incorporation
Evidence Counts	26,496	131,808	158,304	16.7%
MS-MS Counts	23,285	147,918	171,203	13.6%
MS Intensity	5.09×10^{11}	2.72×10^{12}	3.23×10^{12}	15.8%

Table S2. The weights used to transform protein ratios into principal component space. The mean (μ) and standard deviation (σ) of each sample were used to standardize the original variables prior to multivariate analysis.

	10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min	90 min	Variance accounted for
PC1	0.128	0.254	0.300	0.343	0.399	0.325	0.378	0.387	0.393	50%
PC2	0.583	0.407	0.428	0.196	-0.018	-0.360	-0.259	-0.220	-0.168	13%
PC3	0.782	-0.491	-0.258	-0.127	0.023	0.224	0.109	0.038	0.018	9%
PC4	-0.162	-0.652	0.385	0.526	0.092	0.026	-0.257	-0.212	0.064	7%
PC5	0.042	0.296	-0.473	0.523	-0.273	0.392	-0.119	-0.386	0.147	6%
PC6	-0.020	0.088	0.446	-0.335	-0.181	0.736	-0.202	-0.091	-0.239	5%
PC7	0.029	-0.080	0.294	-0.100	-0.684	-0.135	0.406	-0.152	0.472	4%
PC8	-0.031	-0.047	0.042	0.321	-0.175	0.018	0.593	0.032	-0.713	3%
PC9	0.045	-0.022	-0.039	0.228	-0.475	-0.015	-0.375	0.757	-0.061	3%
μ	0.001	-0.004	0.028	0.003	0.050	0.025	0.052	0.047	0.021	-
σ	0.203	0.250	0.311	0.259	0.451	0.381	0.444	0.575	0.375	-

Table S3. Timings of significantly regulated proteins that are directly regulated by LuxR and the Qrr sRNAs.

LuxR-regulated genes			Qrr sRNA-regulated genes		
Gene	Time first significant (min)	Log_2 H/L ratio	Gene	Time first significant (min)	Log_2 H/L ratio
VIBHAR_06238	40–50	3.11	VIBHAR_00417	0–10	0.66
VIBHAR_06244	40–50	3.24	VIBHAR_06667	10–20	0.78
VIBHAR_02988	70–80	-0.73	VIBHAR_06666	10–20	0.92
VIBHAR_06253	70–80	-1.07	VIBHAR_02446	10–20	0.75
VIBHAR_01749	70–80	0.97	VIBHAR_03459	0–10	1.13
VIBHAR_01762	20–30	-0.69	VIBHAR_02959	0–10	0.74
VIBHAR_00081	50–60	-0.85	VIBHAR_00046	10–20	-1.22
VIBHAR_03197	40–50	-0.82			
VIBHAR_06838	50–60	-3.13			
VIBHAR_05086	40–50	0.96			
VIBHAR_02986	30–40	-0.64			
VIBHAR_04809	0–10	0.60			
VIBHAR_02041	70–80	1.08			
VIBHAR_06007	40–50	-0.94			
VIBHAR_01133	20–30	-0.77			
VIBHAR_02617	20–30	-1.00			
VIBHAR_06860	70–80	1.54			
VIBHAR_01256	50–60	-1.03			
VIBHAR_03248	70–80	-1.63			
VIBHAR_05968	80–90	-0.73			
VIBHAR_01398	70–80	-0.64			

Table S4. Oligonucleotides used in this study.

Name	Sequence
P05855-56	GGGCGAAAGATATCAAGTCTCTCTT
P05855-56	ATTTTCCAATTCCAACTGATTATATGAAGG
P05865	GTTGCTCTTCACTAGCGCTCTTG
P05865	CCTTGTTTCAAGGCTGGTATTTAAA
P05871-22	ATATGCTGGAGTTGGCATCGTTATT
P05871-22	TTTATTCTTTAGAGGAAAAGAGGTGGTC
aphA qRT-PCR	ATCCATCAACTCTAGGTGATAAAC
aphA qRT-PCR	CGTCGCGAGTGCTAAGTACA
luxO qRT-PCR	GCATTCCTGATCTTATTCTGCTCG
luxO qRT-PCR	TCCATCCCCGTCATATCAGGTA
luxR qRT-PCR	GCAAAGAGACCTCGTACTAGG
luxR qRT-PCR	GCGACGAGCAAACACTTC
02788 qRT-PCR	TGTTTAACAGTATACGTACTCGAATCG
02788 qRT-PCR	TCAGTAAATGCATCGGTAGTCAT
05853 qRT-PCR	CAACAGGCATTACGCCAG
05853 qRT-PCR	CGCAAAATAACTGGAGAGGATTG
05857 qRT-PCR	CGATTTTGGTTTCTACCATAGTGG
05857 qRT-PCR	CAATCCATAGATATAGCTTATCTGCATCT
05861 qRT-PCR	GTTATCGTCTTTTAGAGCGGATTG
05861 qRT-PCR	AGGTGAGAATGAATGGATTCGAT
05864 qRT-PCR	GATTGATATCGAACGTTTGCTTACG
05864 qRT-PCR	CTTCACTTCGGATTCCATCATTTC
05871 qRT-PCR	GTGAAACTCAAGGTCACATCAC
05871 qRT-PCR	AGTTCTTGAACTAGGAACTCATCAA
05872 qRT-PCR	GTCTCTGAAAAAGCAAACGAAGT
05872 qRT-PCR	GATTGTCTTAATAAGTTCTCAGAACAATCA