Figure S1

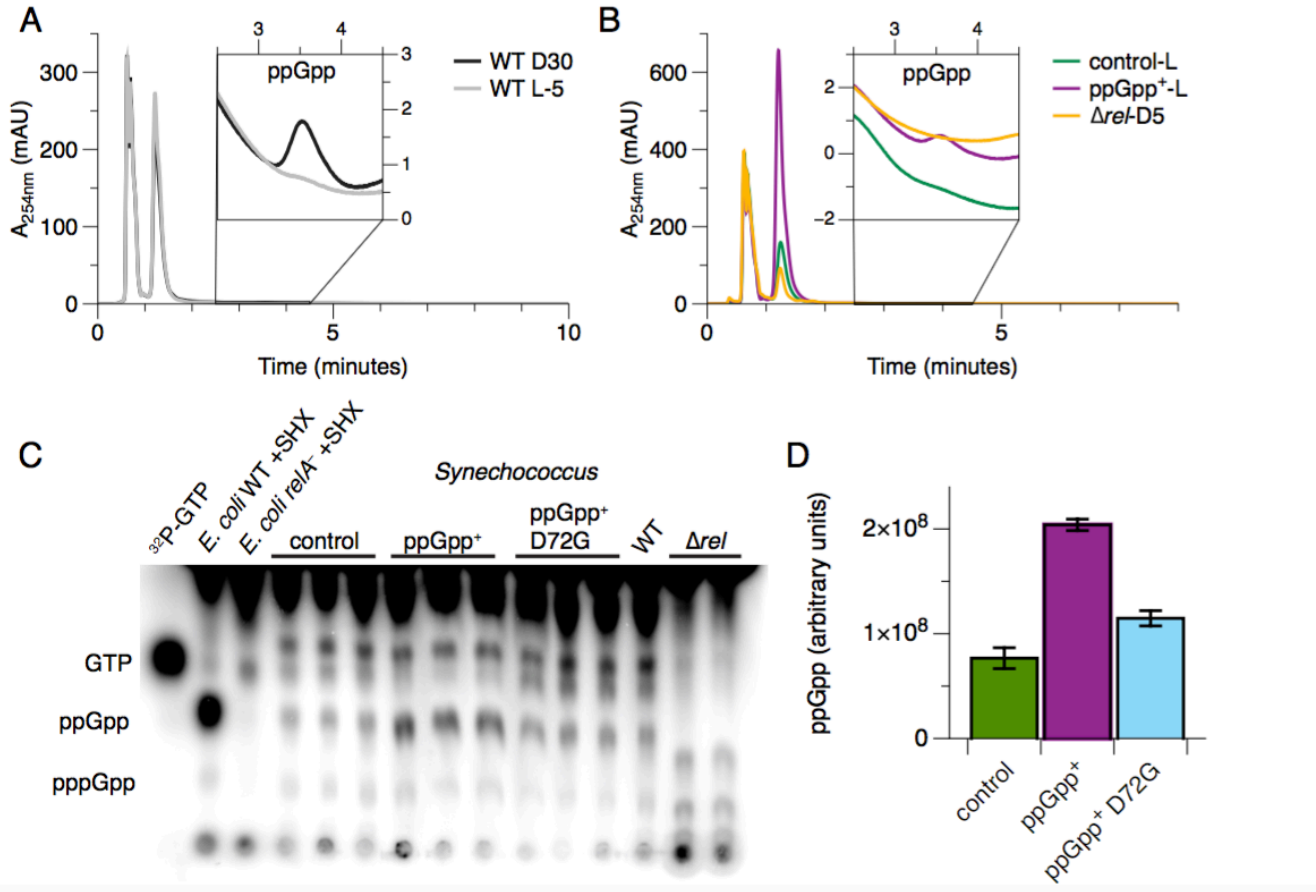

Fig. S1. Measurement of ppGpp in *Synechococcus* lysates by HPLC and ^{32}P-TLC. (*A, B*) Representative traces from HPLC analysis. *Synechococcus* cell extracts were run on an anion exchange column, monitoring absorbance at 254 nm. Under these conditions, ppGpp elutes at ~3.5 minutes, as shown in the enlarged plots. (*A*) Traces from experiment shown in Figure 1A. Gray line, wild-type (WT) cells in the light at t = -5 minutes (5 minutes before shift to darkness); black line, wild-type cells after 30 minutes in the dark. (*B*) Traces from experiment shown in Figure 1B. Green line, control cells (WT-Cm^R) in the light; purple line, ppGpp$^+$ cells in the light after 17 hours of IPTG induction; yellow line, Δ*rel* cells after 5 minutes in the dark. (*C*) *E. coli* and *Synechococcus* (control = WT-Kan^R) were grown in low-phosphate media to mid-log phase. $H_3{}^{32}PO_4$-containing media was added to cultures along with IPTG where appropriate, and they were incubated at 30°C for at least 1 generation time. *E. coli* cultures were treated with serine hydroxamate (SHX) to mimic amino acid starvation for 10 minutes before harvesting. Cells were washed to remove excess $H_3{}^{32}PO_4$ and lysed in 6.5 M formic acid. Cell extracts and [γ-^{32}P]-GTP (a standard) were spotted onto PEI-cellulose TLC plates, run in 1.5 M KH_2PO_4 (pH 3.4) for 30 minutes, and exposed to a phosphorscreen for 24 hours. Imaging and quantitation were performed using a Typhoon phosphorimager. (*D*) Quantitation of ppGpp spot intensities from TLC plate shown in panel C. Data are presented as mean ± SD (n = 3).

Figure S2

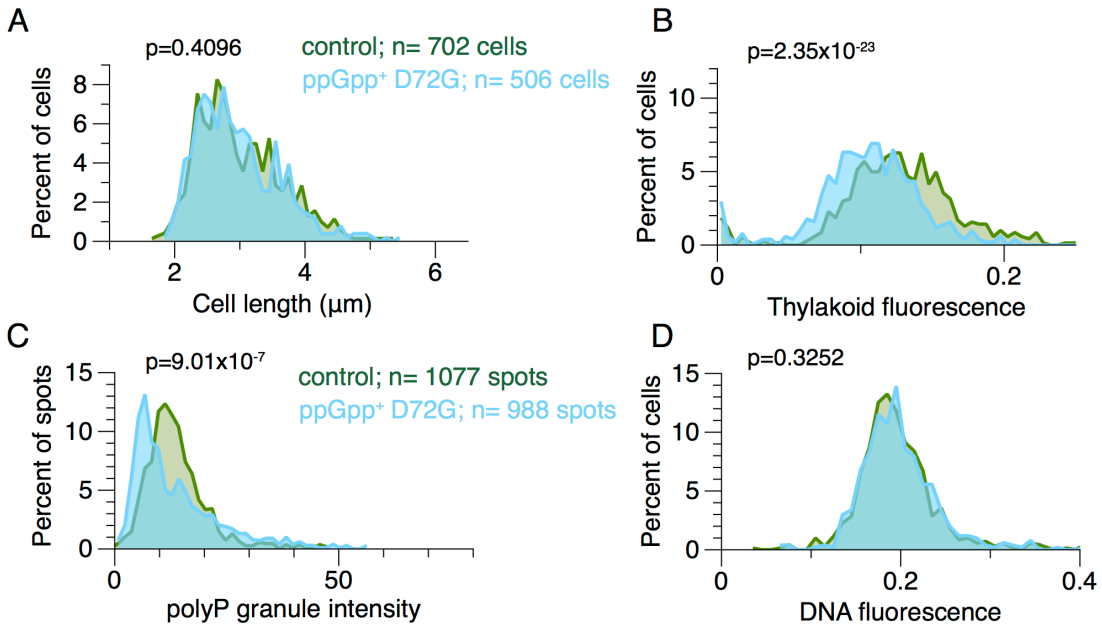

Fig. S2. The phenotypes of ppGpp$^+$ D72G cells are similar to those of a wild-type control strain. Microscopy images were analyzed using MicrobeTracker and SpotFinder, bacterial image analysis programs written in Matlab. Images were acquired from two independent cultures at 26 hours after induction with 50 µM IPTG, as in Figure 2. Histogram colors are the same in all panels: green, control; light blue, ppGpp$^+$ D72G. The same number of cells (control: n = 702 cells; ppGpp$^+$ D72G: n = 506 cells) was used for each analysis shown. Data were analyzed with the Mann-Whitney U-test, and p-values are indicated on the histograms.

Figure S3

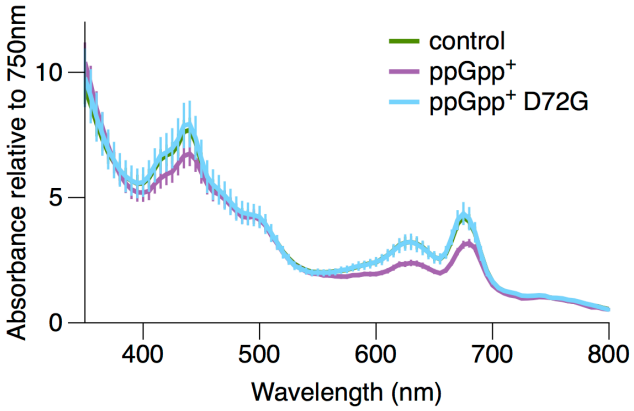

Fig. S3. (p)ppGpp induces chlorosis (bleaching) in *Synechococcus*. *Synechococcus* cultures of the indicated strains were grown to mid-log phase and induced with 50 µM IPTG for 44 hours. Absorbance scans of cultures were performed using a Tecan M1000 plate reader, reading absorbance between 350-800 nm at 5 nm intervals. An identical absorbance scan was performed using sterile media, and these values were subtracted from culture readings. Spectra from the control and ppGpp$^+$ D72G strains are nearly identical and are therefore hard to resolve. Data are presented as mean ± SD (n = 3).

Figure S4

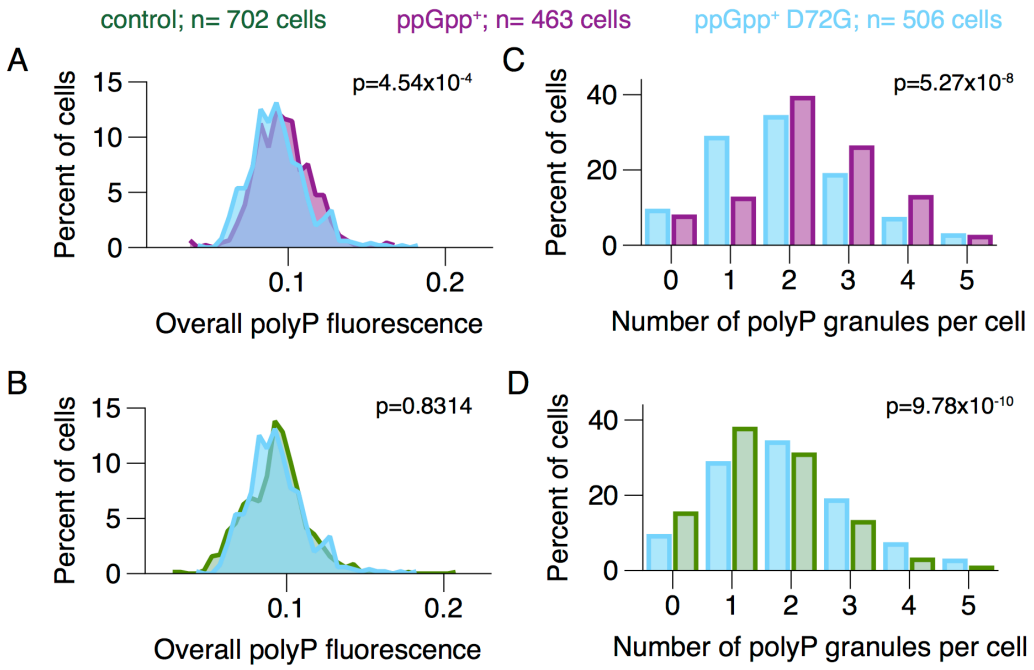

Fig. S4. (p)ppGpp does not dramatically change overall polyP levels when normalized to cell area, but has a slight effect on polyP granule number. Microscopy images were analyzed using MicrobeTracker and SpotFinder. Images were acquired from two independent cultures at 26 hours after induction with 50 µM IPTG, as in Figure 2. Histogram colors are the same in all panels: green, control; purple, ppGpp$^+$; light blue, ppGpp$^+$ D72G. The same number of cells (control: n = 702 cells; ppGpp$^+$: n = 463 cells; ppGpp$^+$ D72G: n = 506 cells) was used for each analysis shown. Data were analyzed with the Mann-Whitney U-test, and p-values are indicated on the histograms. (*A, B*) Histograms of overall polyP fluorescence (AU; normalized to cell area) in control, ppGpp$^+$, and ppGpp$^+$ D72G cultures. A CFP filter set (excitation 436 nm; emission 480 nm) was used to image DAPI-polyP fluorescence; at these wavelengths, DAPI-DNA fluorescence signal is negligible. (*C, D*) Higher (p)ppGpp levels slightly increase the number of polyP granules in *Synechococcus*. Histograms show the number of polyP granules present per cell in each strain.

Figure S5

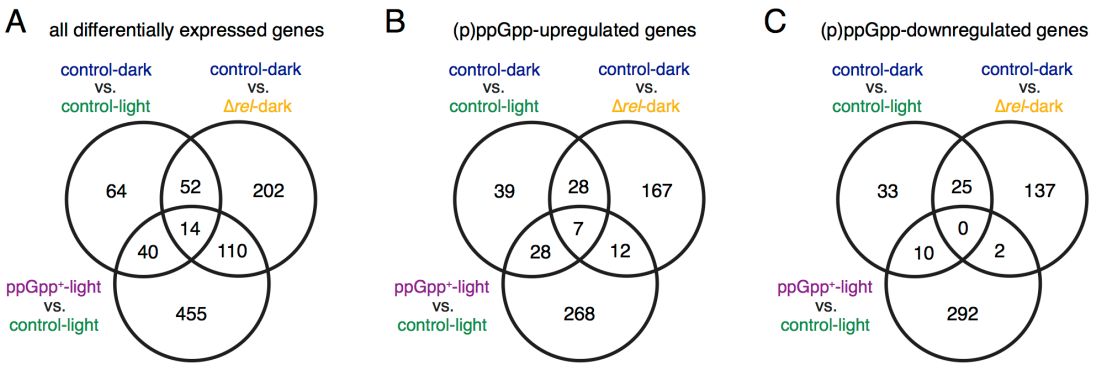

Fig. S5. Venn diagrams comparing (p)ppGpp-regulated genes across RNA-seq comparisons. (*A*) Overlap among all differentially expressed genes across comparisons. (*B*) Overlap among genes upregulated by (p)ppGpp across comparisons. Genes included here showed expression values at least one standard deviation higher than calculated based on the regression line. See Table S1 for a list of the 7 genes consistently upregulated by (p)ppGpp across all three comparisons. (*C*) Overlap among genes downregulated by (p)ppGpp across comparisons. Genes included here showed expression values at least one standard deviation lower than calculated based on the regression line. See Tables S2 and S3 for lists of the 75 genes upregulated or 37 genes downregulated, respectively, by (p)ppGpp in at least two comparisons.

Figure S6

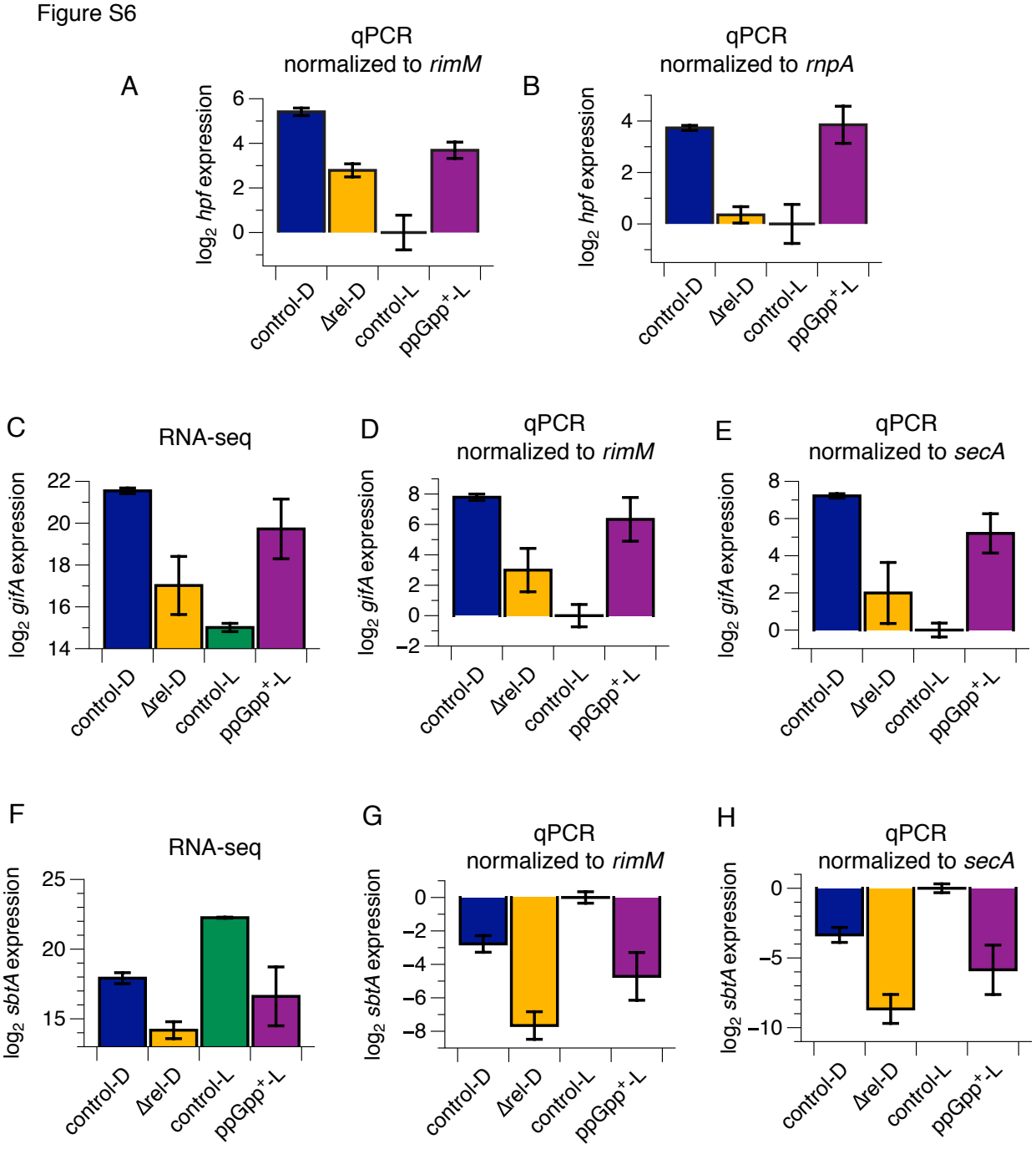

Fig. S6. Verification of *hpf, gifA,* and *sbtA* gene regulation by qPCR. Three candidate reference genes were selected for qPCR after confirming similar expression levels across conditions from our RNA-seq data. All data are presented as mean ± SEM (n = 4 biological replicates). (*A*) *hpf* expression normalized to *rimM* expression and plotted on a $\log_2$ scale relative to control-L. (*B*) *hpf* expression normalized to *rnpA* expression and plotted on a $\log_2$ scale relative to control-L. (*C*) Rockhopper-normalized *gifA* expression values from RNA-seq, plotted on a $\log_2$ scale. (*D*) *gifA* expression normalized to *rimM* expression and plotted on a $\log_2$ scale relative to control-L. (*E*) *gifA* expression normalized to *secA* expression and plotted on a $\log_2$ scale relative to control-L. (*F*) Rockhopper-normalized *sbtA* expression values from RNA-seq, plotted on a $\log_2$ scale. (*G*) *sbtA* expression normalized to *rimM* expression and plotted on a $\log_2$ scale relative to control-L. (*H*) *sbtA* expression normalized to *secA* expression and plotted on a $\log_2$ scale relative to control-L.

Figure S7

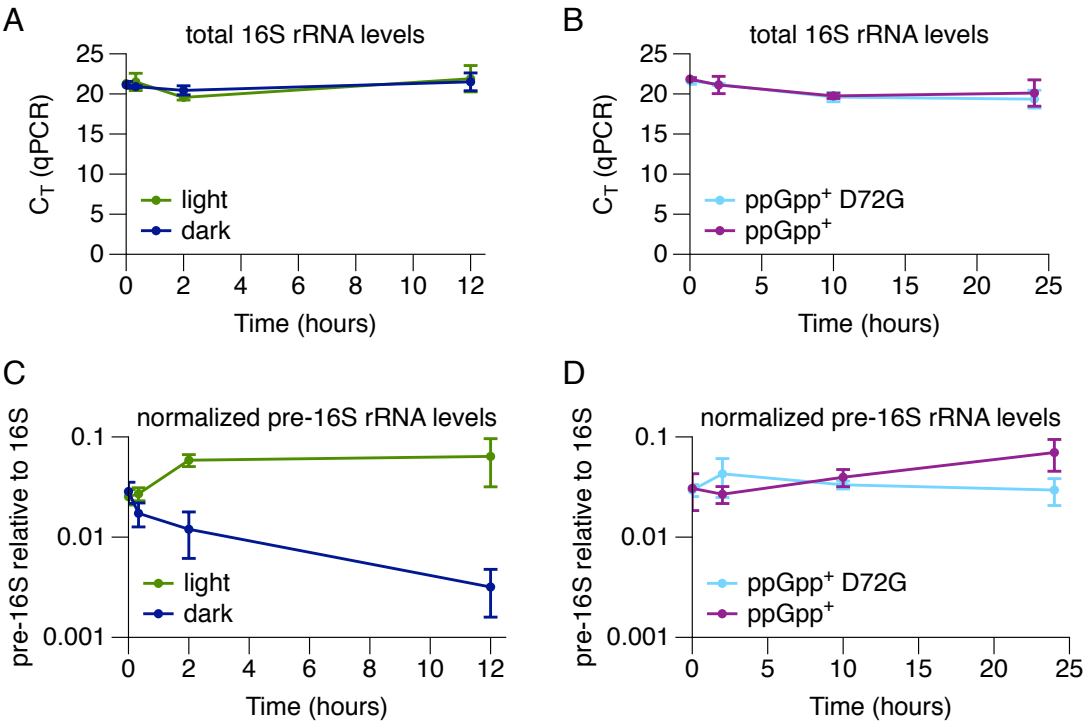

Fig. S7. Levels of ribosomal RNA precursors decrease in the dark, but do not decrease in the ppGpp$^+$ strain. For the indicated conditions, RNA was isolated, reverse transcribed, and qPCR was performed to amplify sequences from mature 16S rRNAs and pre-16S rRNAs (primers within the 5' leader sequence of the rRNA transcript, which is cleaved during rRNA maturation). All data are presented as mean ± SD (n = 3 biological replicates). (*A, B*) Total 16S rRNA levels are plotted as the threshold cycle (C_T) measured by qPCR, and do not change measurably under any of the indicated conditions. (*C, D*) Pre-16S rRNA levels are plotted relative to total 16S rRNA levels from the same sample (using the calculation 2^(16S C_T – pre-16S C_T)). Levels of pre-16S rRNAs decrease over time when cells are incubated in the dark (*C*; dark begins at time = 0 hours), but are not affected by induction of high (p)ppGpp levels in the ppGpp$^+$ strain (*D*; cultures were induced with 50 μM IPTG at time = 0 hours).

Figure S8

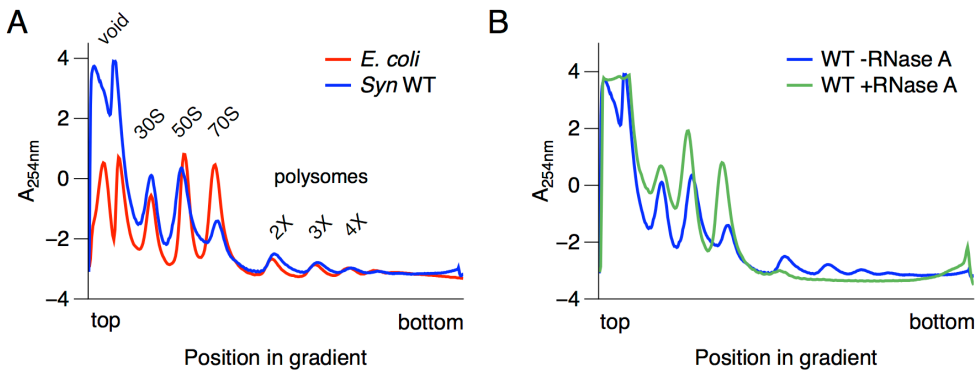

Fig. S8. Verification of ribosomal peak identities in polysome traces. (*A, B*) Polysome profiles from *E. coli* and *Synechococcus* lysates analyzed by sucrose density gradient centrifugation. Cultures were grown to mid-log phase (in constant light for *Synechococcus*). Two minutes before harvesting, all cultures were treated with chloramphenicol at 0.5 mg/ml to arrest translation elongation and were rapidly cooled on ice before centrifugation. Cells were lysed shortly before preparation of 10-40% sucrose gradients, ultracentrifugation, and analysis. Abundance of RNA species was monitored by absorbance at 254 nm (A_{254nm}). (*A*) Comparison of *E. coli* and wild-type *Synechococcus* (*Syn*) polysome traces. Top labels indicate the identity of each peak. (*B*) Treatment of wild-type *Synechococcus* lysates with RNase A confirms the identity of polysome peaks. *Synechococcus* lysates were treated with RNase A for 10 minutes to cleave mRNAs linking polysomes before continuing with the sucrose density gradient centrifugation protocol.

Table S1. Genes upregulated by (p)ppGpp across all three RNA-seq comparisons.

			RNA-seq expression values			
locus tag	**gene name**	**gene description**	**control-D**	**Δ*rel*-D**	**control-L**	**ppGpp$^+$-L**
Synpcc7942_0182		hypothetical protein	135	43	10	41
Synpcc7942_0900	*gifA*	hypothetical protein	15228	3075	166	7825
Synpcc7942_1212		hypothetical protein	77	21	8	30
Synpcc7942_1724		hypothetical protein	97	18	27	866
Synpcc7942_2352	*lrtA*	sigma 54 modulation protein / SSU ribosomal protein S30P	27694	4836	1073	8799
Synpcc7942_2411		hypothetical protein	108	31	19	83
Synpcc7942_2412	*phb, hflC*	SPFH domain, Band 7 family protein	337	83	35	126

Table S2. Genes upregulated by (p)ppGpp across at least two RNA-seq comparisons.

			RNA-seq expression values			
locus tag	**gene name**	**gene description**	**control-D**	**Δ*rel*-D**	**control-L**	**ppGpp^{+}-L**
Synpcc7942_0049	*pilA*	pilin polypeptide PilA-like	36111	2658	14369	62878
Synpcc7942_0108		sulfiredoxin	62	5	10	6
Synpcc7942_0147		hypothetical protein	453	209	78	237
Synpcc7942_0182		hypothetical protein	135	43	10	41
Synpcc7942_0193		hypothetical protein	723	221	58	244
Synpcc7942_0195		hypothetical protein	3918	585	67	121
Synpcc7942_0196		Beta-carotene 15,15'-dioxygenase	622	109	39	44
Synpcc7942_0243	*hliC*	possible high light inducible polypeptide HliC	1012	215	392	9045
Synpcc7942_0251		Exonuclease	941	400	40	244
Synpcc7942_0252	*cp12*	hypothetical protein	14391	5430	363	2738
Synpcc7942_0253		hypothetical protein	6310	383	106	119
Synpcc7942_0267	*gidB*	glucose-inhibited division protein B	3005	933	105	534
Synpcc7942_0291		hypothetical protein	853	121	118	141
Synpcc7942_0316	*digD, orf7.5*	hypothetical protein	4333	370	705	426
Synpcc7942_0398		hypothetical protein	369	77	222	607
Synpcc7942_0465		hypothetical protein	640	83	127	140
Synpcc7942_0497		hypothetical protein	186	110	41	145
Synpcc7942_0623	*trxB*	thioredoxin reductase	696	107	100	82
Synpcc7942_0700		hypothetical protein	5329	1310	546	1007
Synpcc7942_0741		Phage tail protein I	4	8	1	8
Synpcc7942_0781	*ppsA, pps*	phosphoenolpyruvate synthase	1840	659	46	144
Synpcc7942_0801	*sodB, sod1*	Superoxide dismutase	1227	268	916	1761
Synpcc7942_0834		hypothetical protein	106	83	10	76
Synpcc7942_0900	*gifA*	hypothetical protein	15228	3075	166	7825
Synpcc7942_0905		hypothetical protein	128755	83391	4074	42319
Synpcc7942_0906		hypothetical protein	2095	2217	158	1882
Synpcc7942_1002	*psaD*	photosystem I reaction center subunit II	17725	3646	7833	8142
Synpcc7942_1089	*clpB, clpB1, clpBI*	ATPase	444	48	71	66
Synpcc7942_1120		hypothetical protein	20	3	12	127
Synpcc7942_1147		hypothetical protein	44	2	14	8
Synpcc7942_1150		hypothetical protein	290	37	22	36
Synpcc7942_1153	*digE*	hypothetical protein	108	11	11	10
Synpcc7942_1209		hypothetical protein	544	224	99	282
Synpcc7942_1211		probable molybdopterin-guanine dinucleotide biosynthesis protein A	101	63	18	75
Synpcc7942_1212		hypothetical protein	77	21	8	30
Synpcc7942_1302		hypothetical protein	3005	273	133	143
Synpcc7942_1303		hypothetical protein	1424	203	126	136
Synpcc7942_1314	*ftsH*	FtsH-2 peptidase. Metallo peptidase. MEROPS family M41	1586	200	295	138
Synpcc7942_1320		hypothetical protein	201	23	12	23
Synpcc7942_1373		hydrogenase accessory protein	2898	538	565	1245
Synpcc7942_1389	*psbAII, psbA, psbA2*	photosystem q(b) protein	27451	2081	14196	16499
Synpcc7942_1426	*rbcL*	ribulose bisophosphate carboxylase	3935	604	1668	3245
Synpcc7942_1427	*rbcS*	ribulose 1,5-bisphosphate carboxylase small subunit	9456	1280	3089	7402
Synpcc7942_1460		hypothetical protein	476	180	89	406

Synpcc7942_1542	*isiA*	iron-stress chlorophyll-binding protein	203	33	51	79
Synpcc7942_1646		hypothetical protein	1750	744	256	527
Synpcc7942_1648		putative ferric uptake regulator, FUR family	511	43	50	35
Synpcc7942_1649		rubrerythrin	1375	15	132	49
Synpcc7942_1656	*katG, cpx*	catalase/peroxidase HPI	531	278	42	345
Synpcc7942_1689	*rhdA, rhd*	Rhodanese-like	94	8	26	4
Synpcc7942_1722	*sbpA*	Thiosulphate-binding protein	110	11	26	18
Synpcc7942_1724		hypothetical protein	97	18	27	866
Synpcc7942_1845		hypothetical protein	3582	1204	288	3809
Synpcc7942_2043	*speH*	S-adenosylmethionine decarboxylase proenzyme	237	43	238	550
Synpcc7942_2082	*fus*	elongation factor G	591	136	71	56
Synpcc7942_2119		RNA methyltransferase TrmH, group 3	634	124	559	1041
Synpcc7942_2125		hypothetical protein	456	111	109	140
Synpcc7942_2126		hypothetical protein	657	93	142	191
Synpcc7942_2301		hypothetical protein	383	278	57	189
Synpcc7942_2352	*lrtA*	sigma 54 modulation protein / SSU ribosomal protein S30P	27694	4836	1073	8799
Synpcc7942_2401	*hspA, hsp16.6*	heat shock protein Hsp20	7874	255	87	90
Synpcc7942_2411		hypothetical protein	108	31	19	83
Synpcc7942_2412	*phb, hflC*	SPFH domain, Band 7 family protein	337	83	35	126
Synpcc7942_2478	*psb28-2*	photosystem II reaction center W protein	1490	202	87	132
Synpcc7942_2485		hypothetical protein	1233	419	102	383
Synpcc7942_2486		hypothetical protein	1245	511	167	507
Synpcc7942_2529	*gifB*	hypothetical protein	3874	1199	152	3766
Synpcc7942_2600	*ctaB, cyoE*	protoheme IX farnesyltransferase	354	125	46	218
Synpcc7942_2601	*ctaA*	putative cytochrome aa3 controlling protein	392	201	55	277
Synpcc7942_2602	*ctaC*	cytochrome c oxidase subunit II	670	494	112	1543
Synpcc7942_2603	*ctaD*	Cytochrome-c oxidase	251	258	60	898
Synpcc7942_B2634		hypothetical protein	118	91	16	122
Synpcc7942_B2645		hypothetical protein	19142	2537	1526	2072
Synpcc7942_B2646		two-component sensor histidine kinase	2269	388	269	241
Synpcc7942_B2657		hypothetical protein	27	37	8	55

Table S3. Genes downregulated by (p)ppGpp across at least two RNA-seq comparisons.

			RNA-seq expression values			
locus tag	**gene name**	**gene description**	**control-D**	**Δ*rel*-D**	**control-L**	**ppGpp$^+$-L**
Synpcc7942_0293		hypothetical protein	2	37	37	99
Synpcc7942_0358	*dc13, dcl3, trmB*	tRNA (guanine-N(7))-methyltransferase	1	9	11	15
Synpcc7942_0703	*smf*	DNA processing protein DprA, putative	97	362	53	22
Synpcc7942_0718		hypothetical protein	0	1	2	3
Synpcc7942_0719		hypothetical protein	0	3	2	10
Synpcc7942_0724		hypothetical protein	0	1	1	3
Synpcc7942_0915	*aroQ*	3-dehydroquinate dehydratase	21	83	111	323
Synpcc7942_0919		hypothetical protein	25	151	134	191
Synpcc7942_0995	*ycf20*	conserved hypothetical protein YCF20	3	27	24	16
Synpcc7942_1008	*purU*	formyltetrahydrofolate deformylase	2	26	15	14
Synpcc7942_1165		hypothetical protein	0	1	1	3
Synpcc7942_1185		hypothetical protein	8	53	47	38
Synpcc7942_1186	*ribD, ribG*	putative riboflavin-specific deaminase	1	10	9	6
Synpcc7942_1205		phage_integrase-like	0	2	2	4
Synpcc7942_1353		hypothetical protein	5	13	30	3
Synpcc7942_1471		hypothetical protein	16	22	241	67
Synpcc7942_1472		hypothetical protein	29	29	565	133
Synpcc7942_1473	*ndhD5*	putative monovalent cation/H+ antiporter subunit D	10	6	311	42
Synpcc7942_1474		putative monovalent cation/H+ antiporter subunit C	8	8	350	52
Synpcc7942_1475	*sbtA*	sodium-dependent bicarbonate transporter	247	22	4472	569
Synpcc7942_1489	*cmpB, ntrB*	nitrate transport permease	9	3	92	36
Synpcc7942_1538		hypothetical protein	0	2	4	6
Synpcc7942_1547		hypothetical protein	0	7	4	11
Synpcc7942_1560		hypothetical protein	2	12	16	22
Synpcc7942_1564		hypothetical protein	0	5	4	5
Synpcc7942_1744		hypothetical protein	22	22	149	35
Synpcc7942_1970		N-acyl-L-amino acid amidohydrolase	1	7	9	19
Synpcc7942_2085		probable anion transporting ATPase	16	69	59	21
Synpcc7942_2194		hypothetical protein	1	12	8	27
Synpcc7942_2234		NADH dehydrogenase I subunit N	148	146	794	110
Synpcc7942_2235	*trmD*	tRNA (guanine-N(1)-)-methyltransferase	46	70	380	47
Synpcc7942_B2618		transcriptional regulator, BadM/Rrf2 family	0	2	2	11
Synpcc7942_B2620	*srpA*	putative catalase	0	3	2	7
Synpcc7942_B2623	*srpD, cysK*	cysteine synthase A	0	1	2	3
Synpcc7942_B2629	*srpKLM*	sulfonate ABC transporter, periplasmic sulfonate-binding protein, putative	0	1	1	1
Synpcc7942_B2641		hypothetical protein	0	3	2	8
Synpcc7942_B2663	*srpH*	putative serine acetyltransferase	0	2	1	2

Table S4. Plasmids and primers used in this study.

plasmid name	**reference**
pNS2 (Kan^R)	Clerico, EM, Ditty, JL, and SS Golden (2007) Methods Mol Biol 362: 115-129
pNS2-BSU11600	this study
pNS2-BSU11600_D72G	this study
pNS2-Synpcc7942_1377	this study
pNS3 (Cm^R)	Clerico, EM, Ditty, JL, and SS Golden (2007) Methods Mol Biol 362: 115-129
pUC-ΔSynpcc7942_1377(rel)-Cm^R	this study
pUC-ΔSynpcc7942_2352(hpf)-Kan^R	this study
primer name	**primer sequence**
for plasmid construction	
BSU11600-F-BsaI-GG	CACACCA GGTCTC A GTCC GATGACAAACAATGGGAGCG
BSU11600-R-stop-BsaI-GG	CACACCA GGTCTC A CGCT CTATTGTTGCTCGCTTCCTT
BSU11600-D72G-F-GG	CACACCA GGTCTC A GGTA TTGCTGGCCTTAGAATCATG
BSU11600-D72G-R-GG	CACACCA GGTCTC A TACC CTGCATGGTTTCAATTTCATGC
Synpcc7942_1377-F-BsaI-GG	CACACCA GGTCTC A GTCC ccgtcagccggtttgccg
Synpcc7942_1377-R-stop-BsaI-GG	CACACCA GGTCTC A CGCT tcacagctcatcatcgctgccg
rel-KO-up-F-BsaI-GG	CACACCA GGTCTC A CGCT ctggtcagcaagacttccagca
rel-KO-up-R-BsaI-GG	CACACCA GGTCTC A TACT cgaacgtgcgatcgctcc
rel-KO-down-F-BsaI-GG	CACACCA GGTCTC A TAGC tcactcagctatccaactgatg
rel-KO-down-R-BsaI-GG	CACACCA GGTCTC A GTCC ctggatctgaatccacacgatc
Cm^R-F-BsaI-GG	CACACCA GGTCTC A AGTA cactggagcacctcaa
Cm^R-R-BsaI-GG	CACACCA GGTCTC A GCTA ctgccaccgctgagc
hpf-KO-up-F-BsaI-GG	CACACCA GGTCTC A CGCT gctaacgaactgctgatactgg
hpf-KO-up-R-BsaI-GG	CACACCA GGTCTC A TACT aaatcgcctcccagacaag
hpf-KO-down1-F-BsaI-GG	CACACCA GGTCTC A TAGC tctcaacttgatcaacccttttctcac
hpf-KO-down1-R-BsaI-GG	CACACCA GGTCTC A acga ccaccattggcctgcg
hpf-KO-down2-F-BsaI-GG	CACACCA GGTCTC A tcgt ctctcggaagaggatggtttcg
hpf-KO-down2-R-BsaI-GG	CACACCA GGTCTC A GTCC cgcagaagcagcagttcatgg
Kan^R-F-BsaI-GG	CACACCA GGTCTC A AGTA agcttagatcgacctgcag
Kan^R-R-BsaI-GG	CACACCA GGTCTC A GCTA gcgctgaggtctgcctcg
for qPCR	
Syn7942_2352-qPCR-F (hpf)	CCTTGGCACAGCTACAACTAG
Syn7942_2352-qPCR-R (hpf)	CCTTCTCTGAGTTGCCTTCG
Syn7942_0900-qPCR-F (gifA)	TCGCGTGAACTGGTGATG
Syn7942_0900-qPCR-R (gifA)	TGAATGGTGCCGTTGTAGTG
Syn7942_1475-qPCR-F (sbtA)	TCATCGGGTCAAAATCTGGC
Syn7942_1475-qPCR-R (sbtA)	CCTTCGTAGACACTTTCTGGAC
Syn7942_2259-qPCR-F (rimM)	ATCTCTACATCGTGCAACTGG
Syn7942_2259-qPCR-R (rimM)	AAGACGTGAAATTCTCCCTCG
Syn7942_1615-qPCR-F (rnpA)	CTTCCCAGCACTGTATCGAG
Syn7942_1615-qPCR-R (rnpA)	CGCTTGTGAACTTTGAGACTG
Syn7942_0289-qPCR-F (secA)	CGAACGCTACTTCATCCTTCAG
Syn7942_0289-qPCR-R (secA)	AGCCCTCACTCTTATACTCCAG
Syn7942_16SrRNA-qPCR-F	TGGAAACGACTGCTAATACCC
Syn7942_16SrRNA-qPCR-R	TCATCCTCTCAGACCAGCTAC
Syn7942_pre-16SrRNA-qPCR-F	TGGGTTCGGGAAAACTTACG
Syn7942_pre-16SrRNA-qPCR-R	CGTTCGACTTGCATGTGTTAAG

Table S5. Strains used in this study.

Strain name	Strain genotype	Resistance	Reference
Synechococcus elongatus PCC 7942 wild-type (WT)	WT	–	–
control (WT-Kan^{R})	pNS2	Kan	this study
control (WT-Cm^{R})	pNS3	Cm	this study
control (WT-Cm^{R}/Kan^{R})	pNS2 + pNS3	Cm, Kan	this study
ppGpp^{+}	pNS2-BSU11600	Kan	this study
ppGpp^{+} D72G	pNS2-BSU11600_D72G	Kan	this study
Δ*rel*	ΔSynpcc7942_1377-Cm^{R}	Cm	this study
Δ*rel* (Cm^{R}/Kan^{R})	ΔSynpcc7942_1377-Cm^{R} +pNS2	Cm, Kan	this study
Δ*rel* + *rel*	ΔSynpcc7942_1377-Cm^{R} + pNS2-Synpcc7942_1377	Cm, Kan	this study
Δ*hpf*	ΔSynpcc7942_2352-Kan^{R}	Kan	this study
Escherichia coli W3110 wild-type (CF1943)	WT	–	Michael Cashel (NIH)
E. coli W3110 *relA251::Kan* (CF1944)	*relA::Kan*	Kan	Michael Cashel (NIH)