

SUPPLEMENTAL TABLE S1. Promoter and riboswitch sequences used in this study. Predicted transcriptional start sites are bolded and underlined. Riboswitch sequences were obtained from Topp et al., Appl Environ Microbiol, 76(23):7881-4, 2010, and were cloned immediately after the promoter sequences listed. Aptamer sequences are underlined. ATG start codons immediately precede *myc-yfp* or *sacB*, with the exception of the riboswitch F EcoRI vector control.

Name	Sequence
<i>conII</i> promoter	ACCGGTTTTCGAATTGACAATTAATCATCGGCTCGTATAATGGTACC <u> </u>
<i>trc</i> promoter with <i>lacO</i>	TGAAATGAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGG <u> </u> AATTGTGAGCGGATAACAATTTACAGGTACC
Riboswitch A	<u>GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACC</u> <u>CTGAGAAGGGGCAACAAGATG</u>
Riboswitch B	<u>GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACC</u> <u>CTGAGAAGGGGCAACAAGATG</u>
Riboswitch C	CGGTACCTGATAAGATAGGGGTGATACCAGCATCGTCTT <u>GATGCCCTTGGCAGCACCAAGGGACAACAAGATG</u>
Riboswitch D	<u>GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACC</u> <u>CTGCTAAGGTAACAACAAGATG</u>
Riboswitch E	<u>GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACC</u> <u>CTGCTAAGGAGGTAACAACAAGATG</u>
Riboswitch F	<u>GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACC</u> <u>CTGCTAAGGAGGCAACAAGATG</u>
Riboswitch F EcoRI	<u>GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACC</u> <u>CTGCTAAGGAGGCAACAAGATGAATTC</u>
Riboswitch F, no aptamer	TGCTAAGGAGGCAACAAGATG

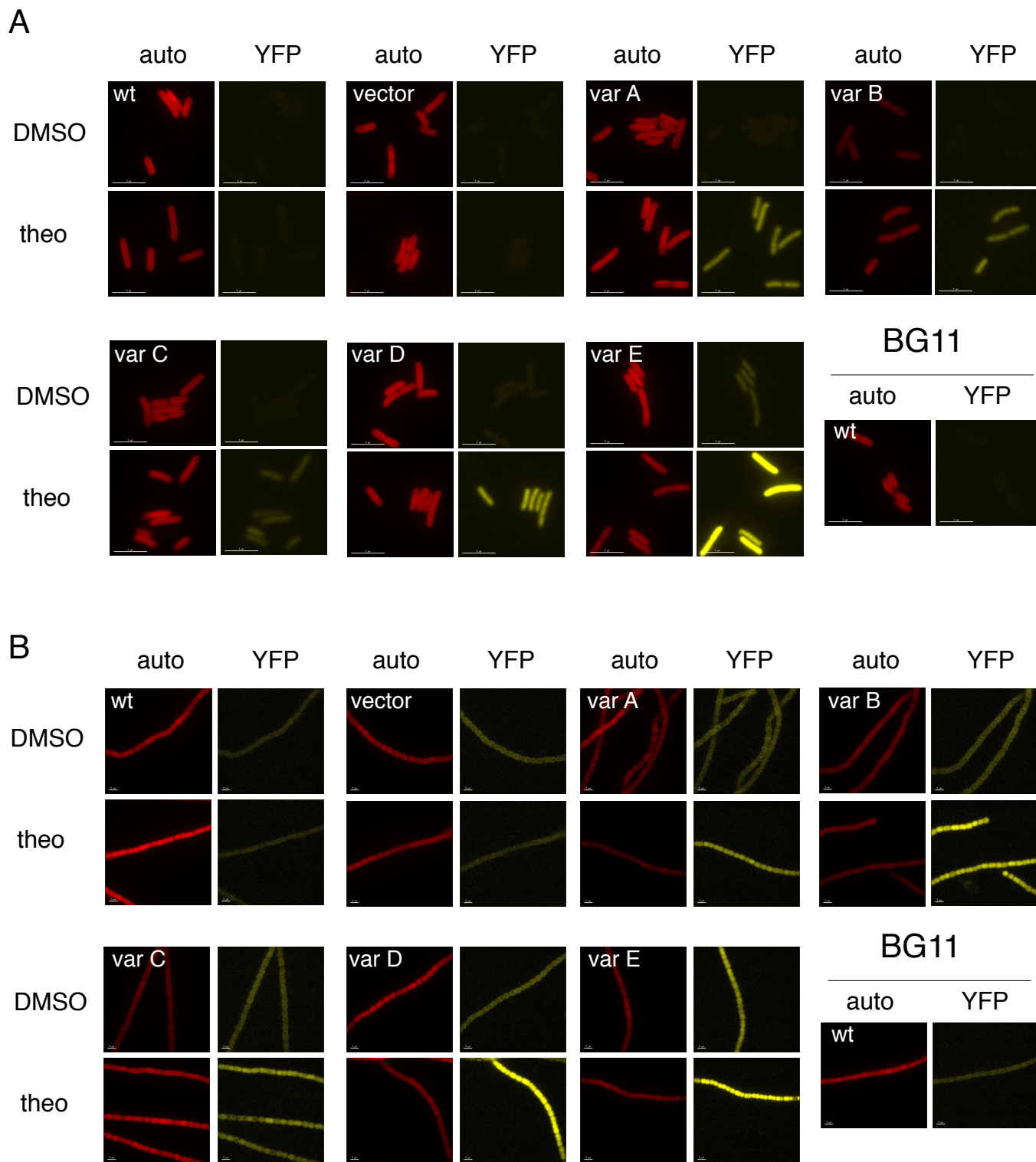


FIG S1 Fluorescence micrographs of model laboratory strains expressing different riboswitch variants regulating expression of YFP. (A) *Synechococcus elongatus* PCC 7942 and B. *Anabaena* sp. PCC 7120. Images on the left show autofluorescence from photosynthetic pigments and images on the right show YFP expression. Cultures were treated with no additions (BG11), 1% DMSO, or 2 mM theophylline for one day. Scale bars depict 5 microns.

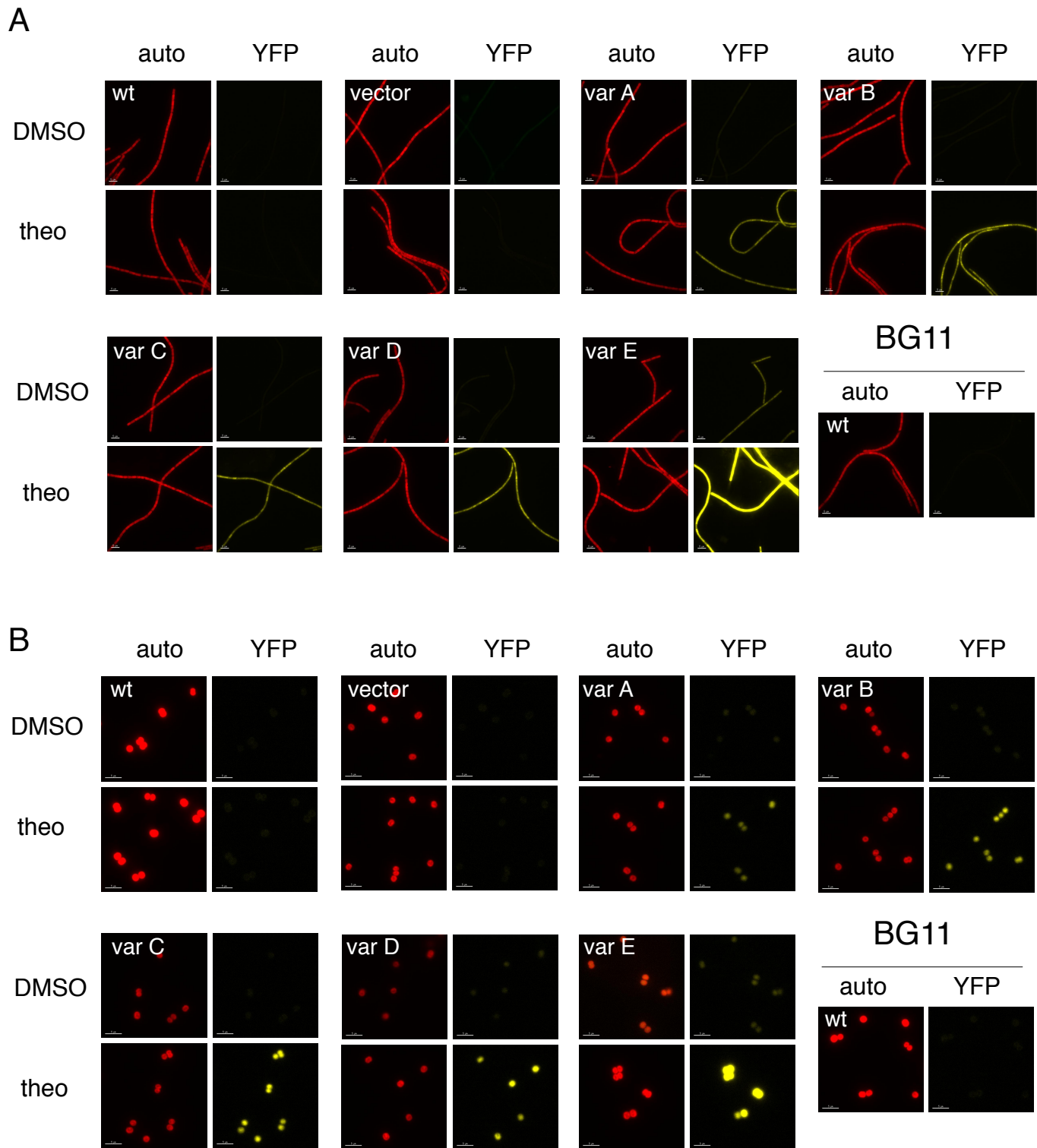
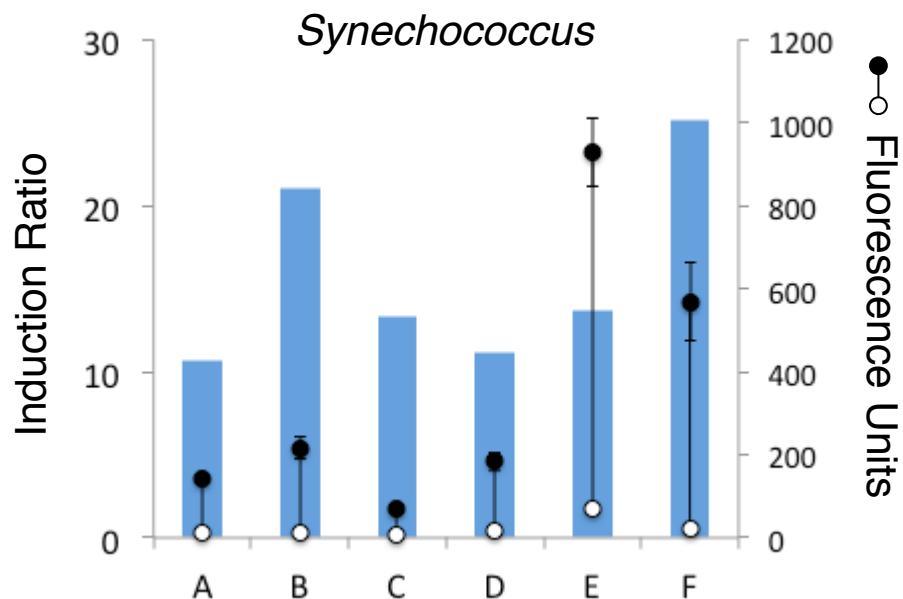


FIG S2 Fluorescence micrographs of model production strains expressing different riboswitch variants regulating expression of YFP. (A) *Leptolyngbya* sp. BL0902 and (B) *Synechocystis* sp. WHSyn. Images on the left show autofluorescence from photosynthetic pigments, and images on the right show YFP expression. Cultures were treated with no additions (BG11), 1% DMSO, or 2 mM theophylline for one day. Scale bars depict 5 microns.

A



B

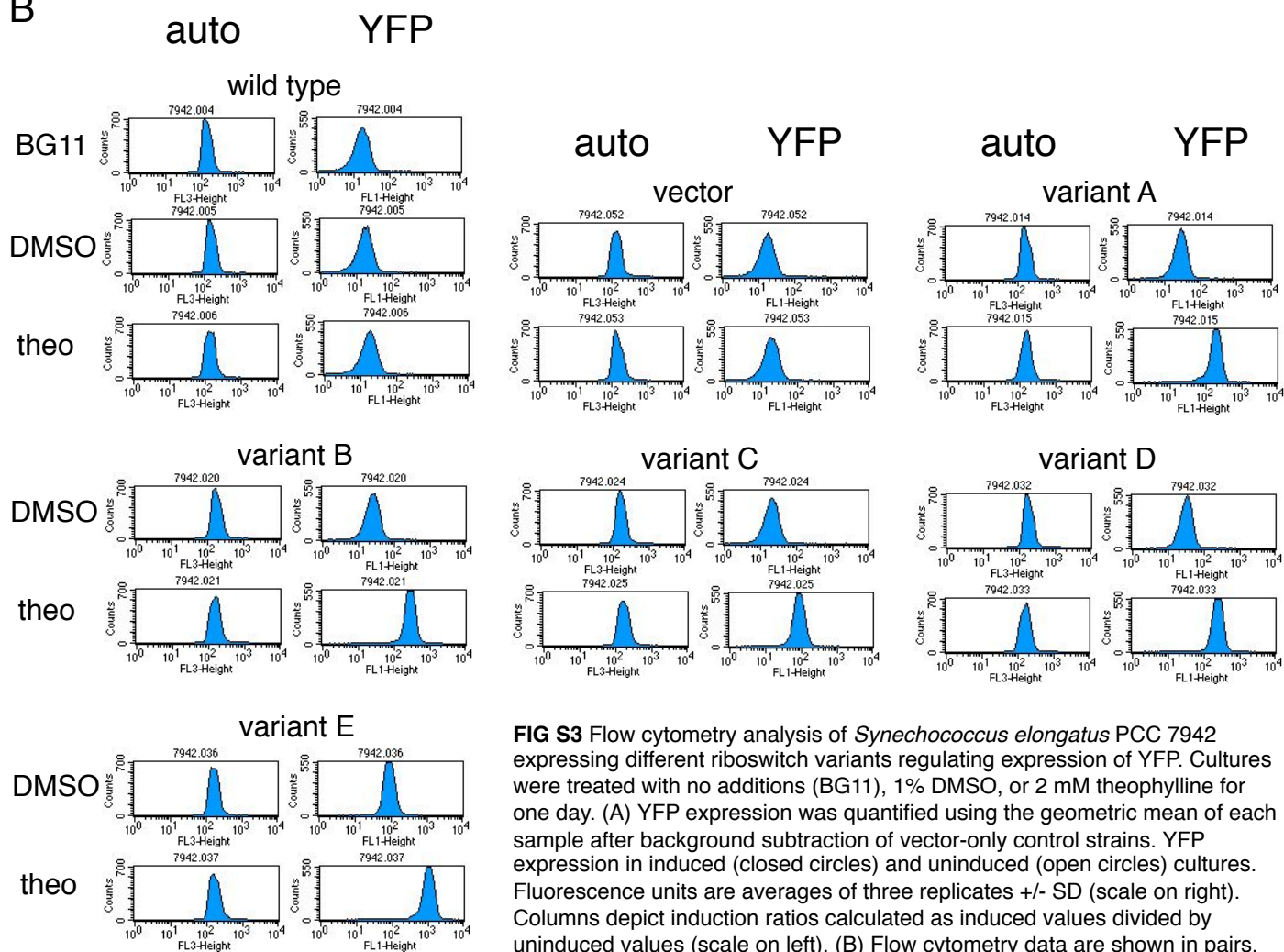
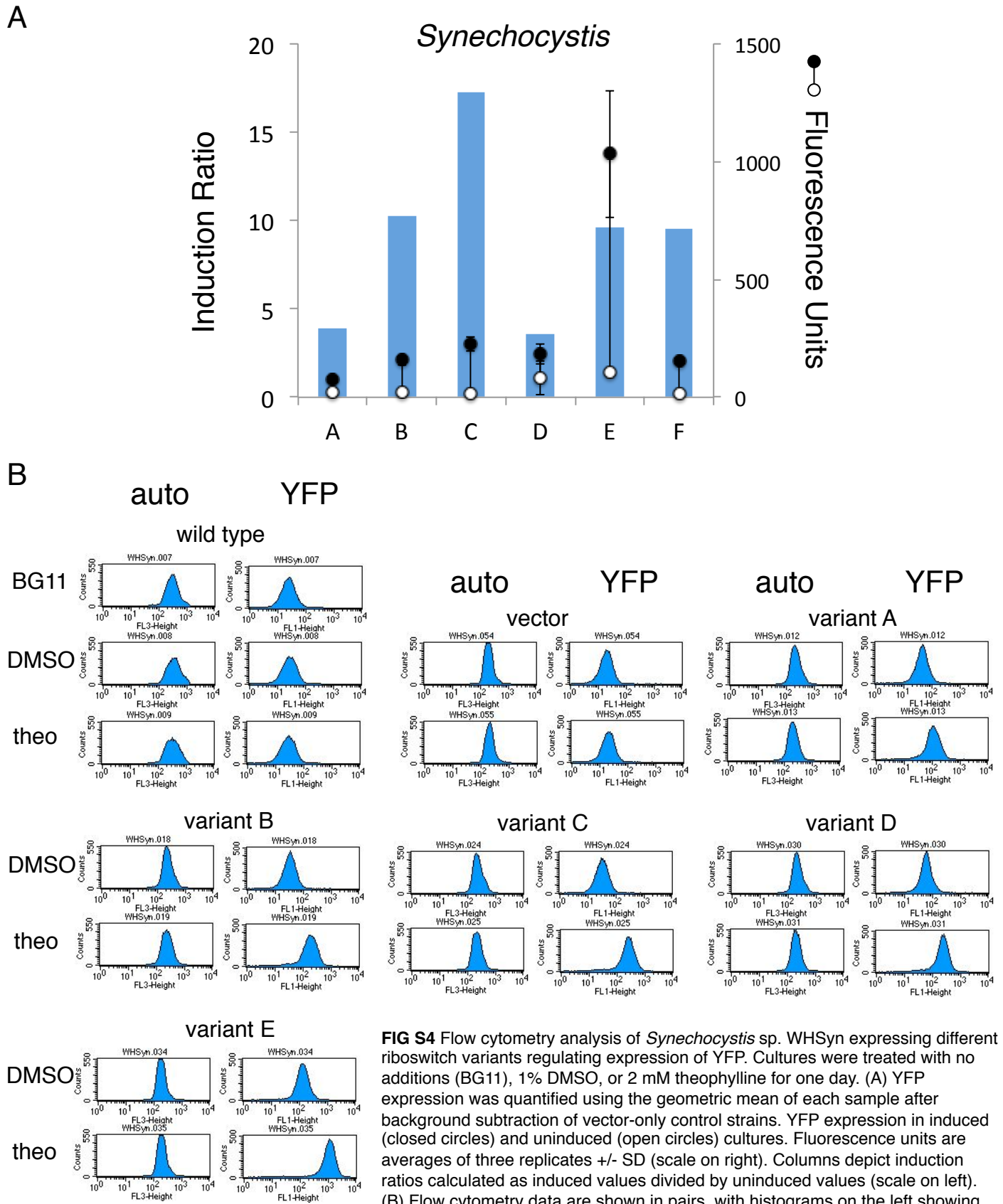


FIG S3 Flow cytometry analysis of *Synechococcus elongatus* PCC 7942 expressing different riboswitch variants regulating expression of YFP. Cultures were treated with no additions (BG11), 1% DMSO, or 2 mM theophylline for one day. (A) YFP expression was quantified using the geometric mean of each sample after background subtraction of vector-only control strains. YFP expression in induced (closed circles) and uninduced (open circles) cultures. Fluorescence units are averages of three replicates \pm SD (scale on right). Columns depict induction ratios calculated as induced values divided by uninduced values (scale on left). (B) Flow cytometry data are shown in pairs, with histograms on the left showing the distribution of autofluorescence (FL3), and histograms on the right showing the distribution of YFP expression (FL1) in log scale, with signal intensity (X-axis) versus number of cells (Y-axis).



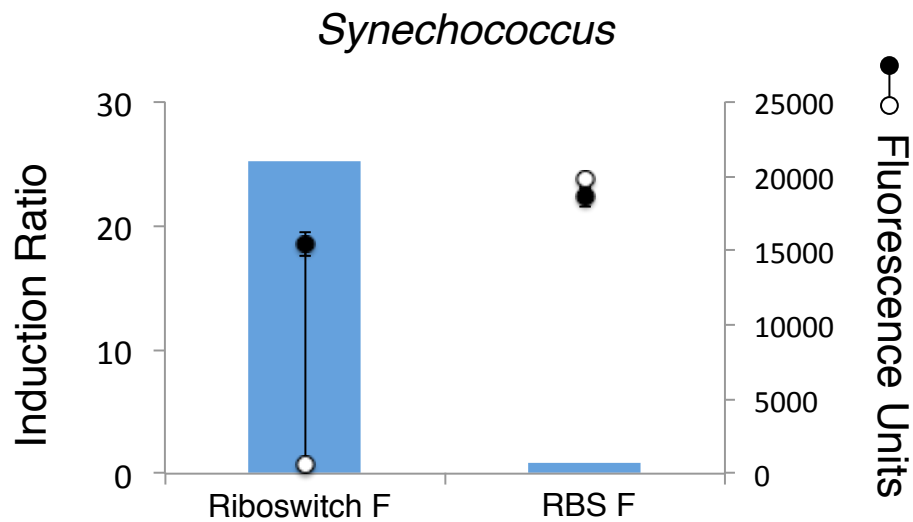


FIG S5 Expression under riboswitch F regulation compared to constitutive translation from only ribosome binding site F (RBS F) in *Synechococcus elongatus* PCC 7942. Transcription in both strains was driven by the P_{conII} promoter. The RBS F construct was constructed by deleting the aptamer region of riboswitch F (see Supplemental Table 1). Strains were treated with 2 mM theophylline or 1% DMSO negative control for one day. Fluorescence units (scale on right) of YFP expression in induced (closed circles) and uninduced (open circles) cultures. Values given are averages of three replicates \pm SD. Columns depict induction ratios, calculated as induced values divided by uninduced values (scale on left). Measurements were made using a fluorescence plate reader after normalization of cultures by OD_{750} .