Supporting Information: Macrolide biosensor optimization through cellular substrate sequestration

Corwin A. Miller[1,*], Joanne M. Ho[1,*], Sydney E. Parks[1], and Matthew R. Bennett[1, 2,†]

[1] Department of Biosciences, Rice University, 6100 Main St., Houston, TX 77005, USA

[2] Department of Bioengineering, Rice University, 6100 Main St., Houston, TX 77005, USA

* These authors contributed equally.

† Correspondence: matthew.bennett@rice.edu

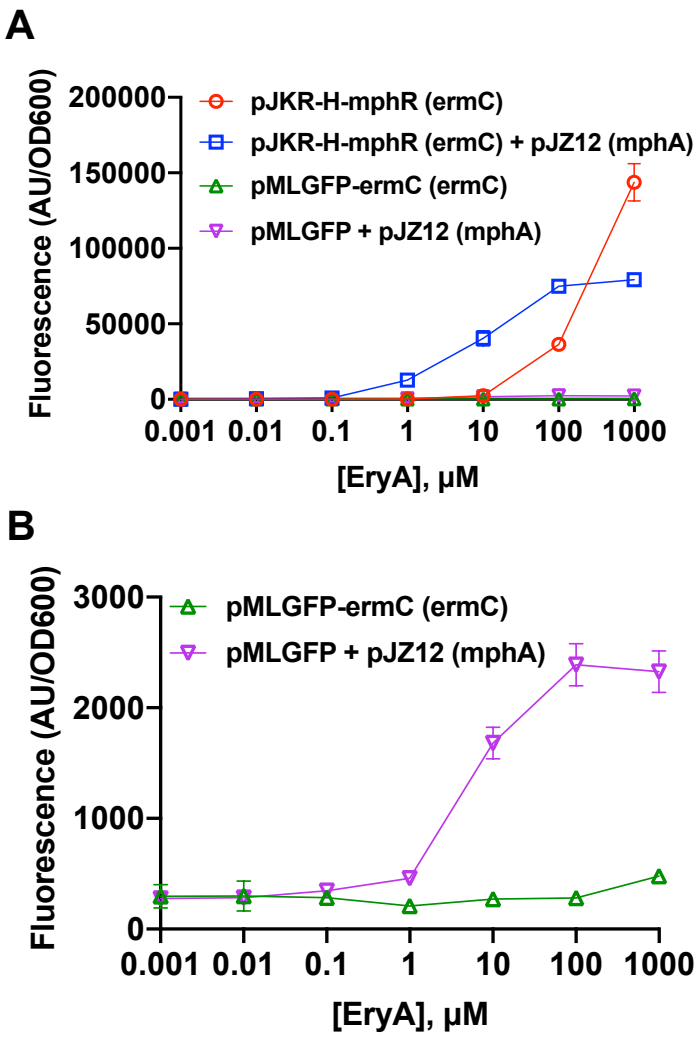

Figure S1. Comparison of previously engineered EryA detection and resistance constructs. (A) Biosensor construct pJKR-H-mphR mediated significantly greater EryA response compared to pMLGFP. Co-transformation with pJZ12 (expressing *mphA*) further increased detection sensitivity. (B) Plasmid pMLGFP combined with pJZ12 exhibited significantly greater response compared to pMLGFP-ermC. Tests were performed using three biological replicates; standard deviations are shown as error bars for each point across these three replicates. Small error bars are not visible for some samples exhibiting small standard deviation.

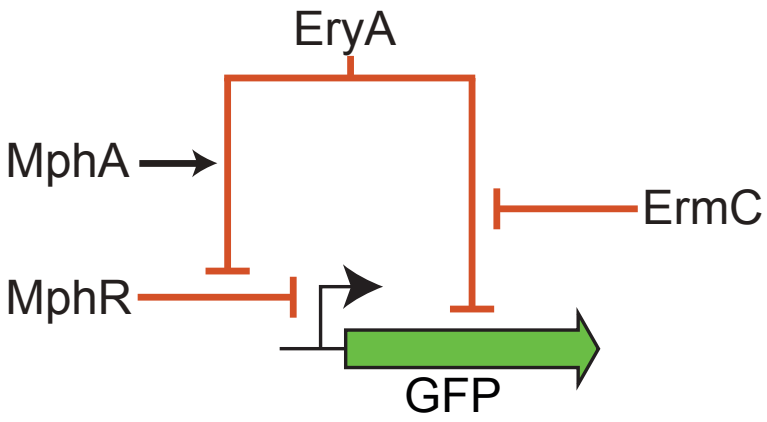

Figure S2. Genetic circuit diagram showing components tested in this work. For all gene circuits tested, EryA (or other macrolide drugs) inhibit MphR repression of GFP, resulting in induction of GFP expression. When MphA is present, this gene potentiates the effects of low concentrations of EryA by increasing its intracellular concentration. In the absence of ErmC, high concentrations of EryA inhibit translation of proteins, including GFP. The presence of ErmC prevents this effect, resulting in increased GFP expression at high concentrations of EryA.

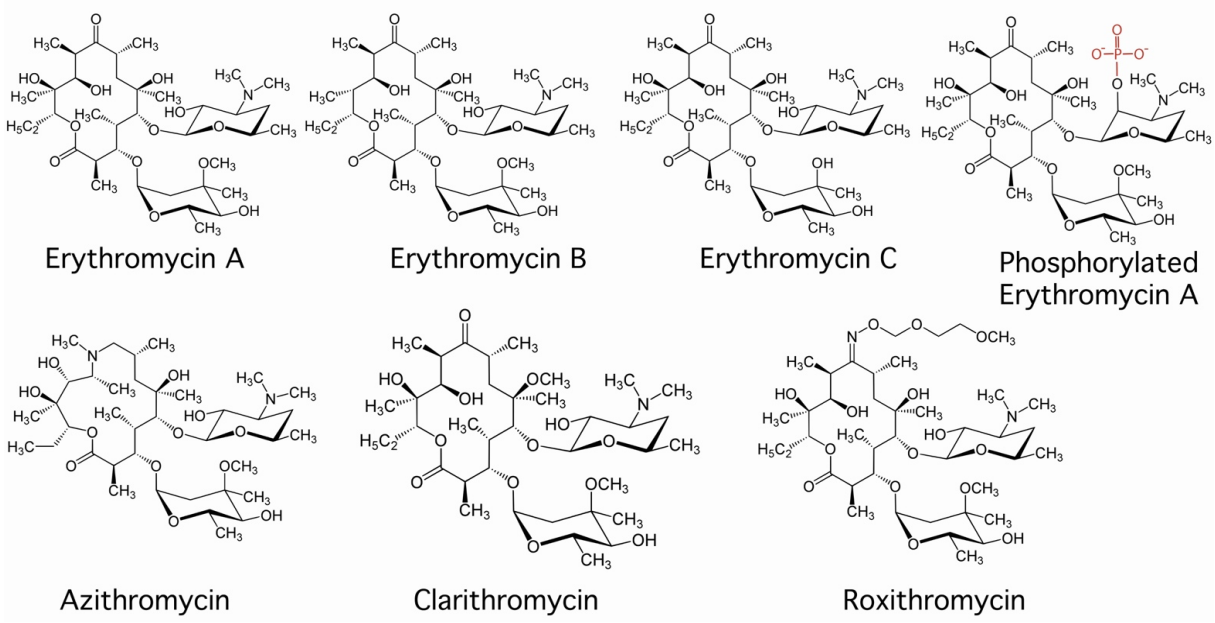

Figure S3. Macrolides used in this study. Phosphorylated EryA was produced within *E. coli* cells following expression of *mphA* and exposure to EryA. All other compounds were commercially sourced.

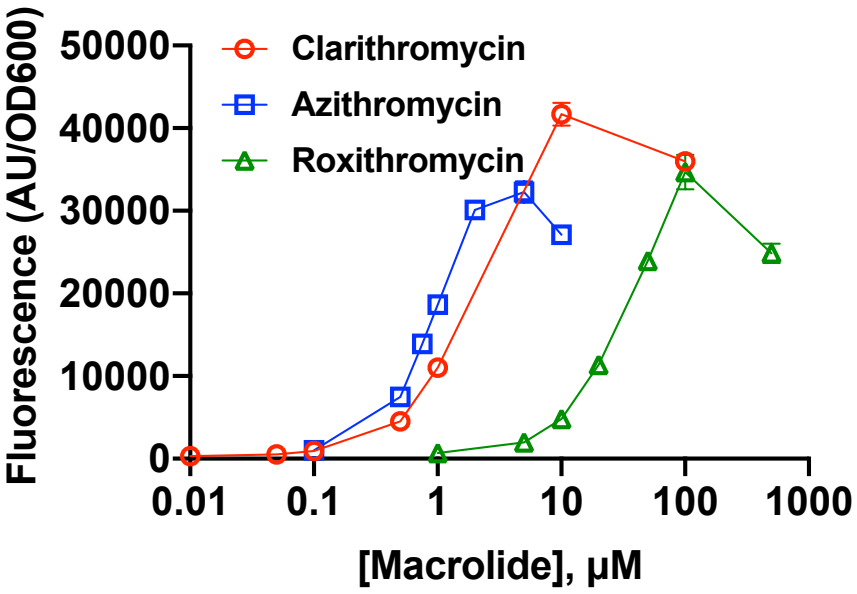

Figure S4. Detection of macrolide derivatives using biosensor construct pCM54. Construct pCM54 contains *mphA* as the sole macrolide resistance gene. For each compound, detection was impaired at high concentrations, likely due to effects of the antibiotics on cell fitness. Tests were performed using three biological replicates; standard deviations are shown as error bars for each point across these three replicates. Small error bars are not visible for some samples exhibiting small standard deviation.

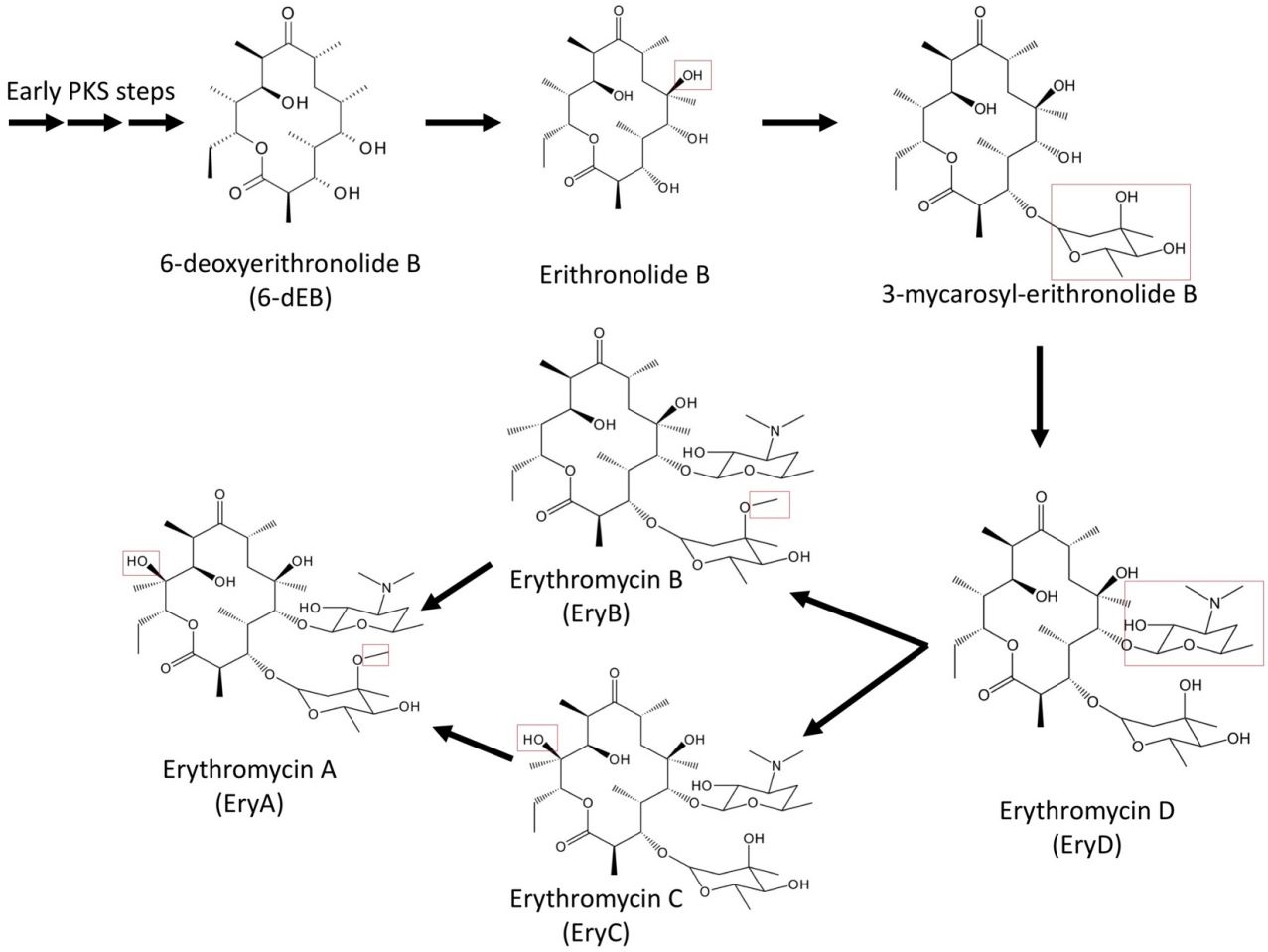

Figure S5. Macrolide precursors formed during EryA biosynthesis. Compound 6-dEB is biosynthesized first, with successive arrows indicating subsequent macrolide compounds formed during biosynthesis. Red boxes indicate modified regions relative to the prior precursor. As subsequent modifications to EryD can be performed in any order, EryB and EryC both serve as immediate precursors to EryA.

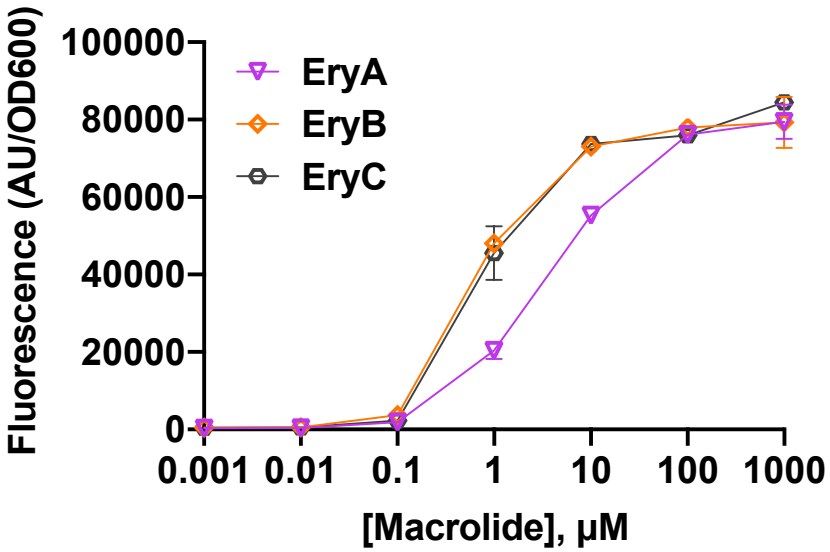

Figure S6. Detection of EryA and two precursors using cells containing biosensor construct pCM120. While precursor EryB produced a similar fluorescent response compared to EryA, somewhat reduced sensitivity was observed for EryC. This difference can likely be attributed to poor solubility of EryC (see text). Tests were performed using three biological replicates; standard deviations are shown as error bars for each point across these three replicates. Small error bars are not visible for some samples exhibiting small standard deviation.

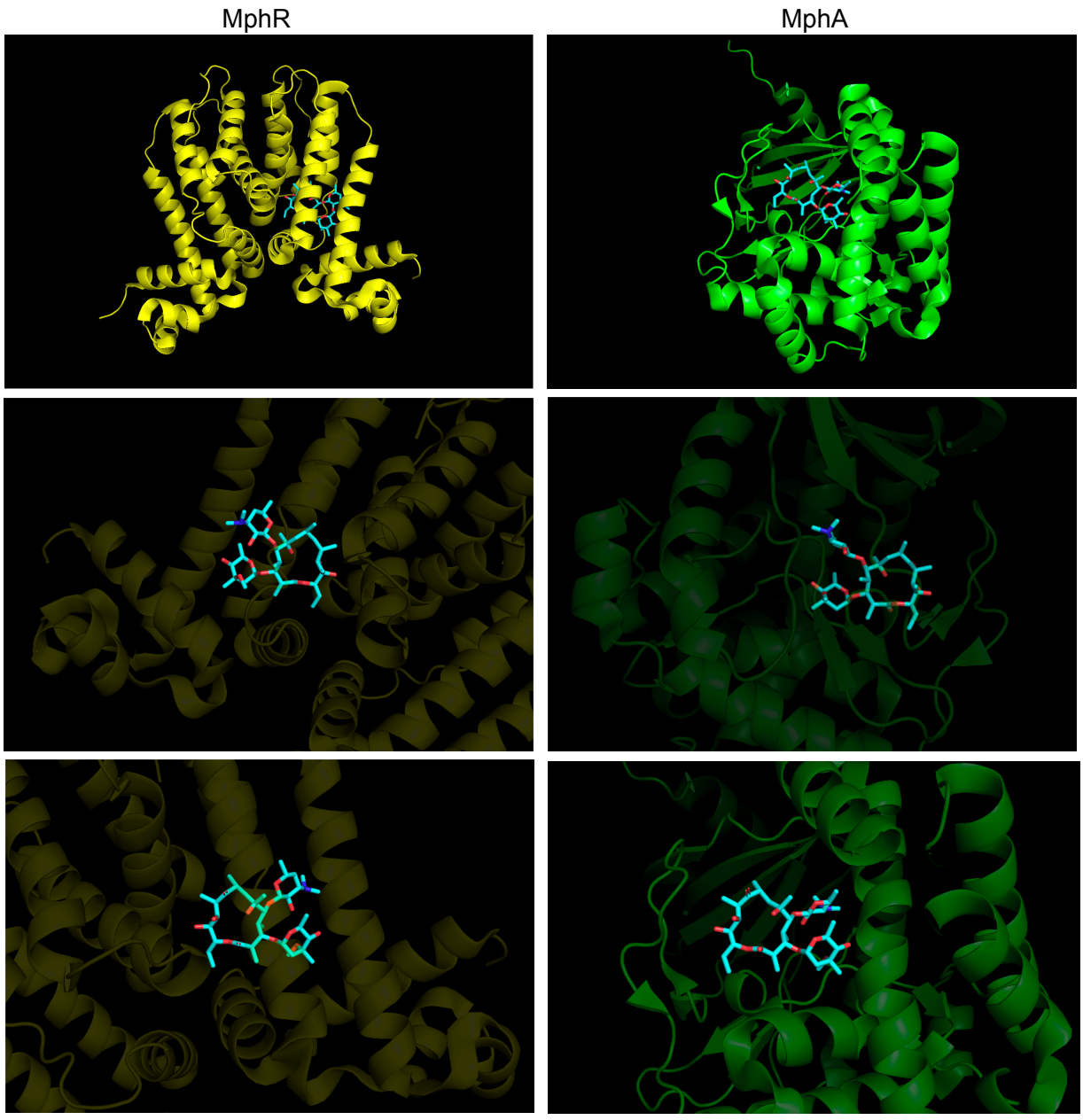

Figure S7. EryA in separate co-complexes with MphR and MphA. In the left column, EryA (shown in teal) is depicted from multiple angles in co-complex with MphR (shown in yellow) from PDB: 3FRQ[1]. In the right column, EryA is depicted in co-complex with MphA (shown in green) from PDB: 5IGP[2].

Plasmid	Description	Insert 1	Insert 2	Insert 3	Insert 4	Resistance	Origin
pCM54	*ermC* in pJKR-H-mphR replaced with *mphA*	PmphR, sfGFP	mphR	mphA	-	Ampicillin	pUC
pCM98	MphR mutation T17R introduced to pCM54	PmphR, sfGFP	mphR-T17R	mphA	-	Ampicillin	pUC
pCM120	*mphA* cassette inserted into pJKR-H-mphR	PmphR, sfGFP	mphR	mphA	ermC	Ampicillin	pUC
pCM121	MphR mutation T17R introduced to pCM120	PmphR, sfGFP	mphR-T17R	mphA	ermC	Ampicillin	pUC
pJKR-H-mphR	see Rogers (2015)	PmphR, sfGFP	mphR	-	ermC	Ampicillin	pUC
pMLGFP	see Kasey (2017)	PmphR, GFP	mphR	-	-	Ampicillin	pBR322
pMLGFP-ermC	*ermC* cassette inserted into pMLGFP	PmphR, GFP	mphR	-	ermC	Ampicillin	pBR322
pJZ12	see Kasey (2017)	-	-	mphA	-	Tetracycline	p15A

Table S1. Strains and plasmids used in this study. For insertion of *mphA*, plasmid pJZ12 was used as template (including promoter and terminator regions). For insertion of *ermC*, plasmid pJKR-H-mphR was used as template (including promoter and terminator regions).

Plasmid	Antibiotic	Two-fold increase parameter (nM)
pJKR-H-mphR	EryA	397
pCM54	EryA	33.4
pCM120	EryA	40.2
pCM121	EryA	13.2
pJKR-H-mphR	Clr	9,787
pCM120	Clr	52.2
pJKR-H-mphR	Azr	3,340
pCM120	Azr	45.6
pJKR-H-mphR	Rxr	23,997
pCM120	Rxr	2,414

Table S2. Macrolide response curves using multiple biosensor plasmids were analyzed to determine the EryA concentration at which a two-fold increase in sfGFP signal was observed, with concentrations denoted here as "Two-fold increase parameter". For each macrolide compound, constructs expressing *mphA* (pCM54 and pCM120) showed significantly lower (and thus more sensitive) two-fold increase parameters compared to tests performed in the absence of *mphA* (using pJKR-H-mphR).

Supplementary References

[1] Zheng, J., Sagar, V., Smolinsky, A., Bourke, C., LaRonde-LeBlanc, N., and Cropp, T. A. (2009) Structure and function of the macrolide biosensor protein, MphR(A), with and without erythromycin, *J Mol Biol 387*, 1250-1260.

[2] Fong, D. H., Burk, D. L., Blanchet, J., Yan, A. Y., and Berghuis, A. M. (2017) Structural Basis for Kinase-Mediated Macrolide Antibiotic Resistance, *Structure 25*, 750-761 e755.