

Supporting Information for:

Light Activation of *Staphylococcus aureus* Toxin YoeB_{Sa1} Reveals Guanosine-Specific
Endoribonuclease Activity

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Table S1. Sequences of primers used in this study.

Primer	Sequence
PP1QC1F	5'-CTTGCCTTATCTACTTTTCTAGAGGGCCCTATTC-3'
PP1QC1R	5'-GAATAGGGCCCTCTAGAAAAGTAAGATAAGGCAAG-3'
PP1QC2F	5'-CTTGGTACCGGAATTCTACGAATTTTCTTTTGTATTTC-3'
PP1QC2R	5'-GAAATAAACAAAAGAAAAATTCGTAGAATCCGGTACCAAG-3'
PP2QCF	5'-GTTGCGCTCTATTCTTCTAGAGGGCCCTATTC-3'
PP2QCR	5'-GAATAGGGCCCTCTAGAAGAATAGAGCGCAAC-3'
PP4QCF	5'-GCCCTAATCCCACTCTAGAGGGCCCTATTC-3'
PP4QCR	5'-GAATAGGGCCCTCTAGAGTGGGATTAGGGC-3'
PP6QCF	5'-GGTACGGGATCCCTCTAGAGGGCCC-3'
PP6QCR	5'-GGGCCCTCTAGAGGGATCCCGTACC-3'
PP7QCF	5'-GAAAAATTCGTAGAATTCCTCTAGAGGGCCCTATTC-3'
PP7QCR	5'-GAATAGGGCCCTCTAGAGGAATTCTACGAATTTTTC-3'
PP8QCF	5'-GAAACTAAAGTAAGATTCTAGAGGGCCCTATTCTATAGTG-3'
PP8QCR	5'-CACTATAGAATAGGGCCCTCTAGAATCTTACTTTAGTTTC-3'
PP9QCF	5'-GACAGTAGAATAGAGCTCTAGAGGGCCCTATTC-3'
PP9QCR	5'-GAATAGGGCCCTCTAGAGCTCTATTCTACTGTC-3'
PP10QCF	5'-CCAATGGGTAGGATTCTAGAGGGCCCTATTC-3'
PP10QCR	5'-GAATAGGGCCCTCTAGAATCCTACCCATTGG-3'
PP12QCF	5'-GACTCCAACCTCATCTAGAGGGCCCTATTC-3'
PP12QCR	5'-GAATAGGGCCCTCTAGATGAGGTTGGAGTC-3'
YefM _{Sa1} -NdeI-F	5'-GCGATACATATGATTATTA-3'
YefM _{Sa1} -QC-F	5'-GACAAAAGTAAATGATGATAGTGATATGGTAACTGTAACATCTACTG-3'
YefM _{Sa1} -QC-R	5'-CAGTAGATGTTACAGTTACCATATCACTATCATCATTTACTTTTGTC-3'
YefM _{Sa1} -XhoI-R	5'-AAAATTCTCGAGTTATTATTATTATACATCTATATCTTTCGTTATAGTTTTC-3'
YefM _{Sa2} -NdeI-F	5'-AAAAAACATATGATTATCACTAGCCCTAC-3'
YefM _{Sa2} -HindIII-R	5'-AAAAAAAAGCTTAAAGATTATCCCAATCAATATCATC-3'
YefM-YoeB _{Sa1} -F	5'-CGCTCGACAGAATTTAAAGG-3'
YefM-YoeB _{Sa1} -R	5'-CCTTCCAATGCACCATTTC-3'
YefM-YoeB _{Sa2} -F	5'-GGTATAGACGACCCTGTGCTG-3'
YefM-YoeB _{Sa2} -R	5'-GCCCTACAGAAGCGAGAAAAG-3'
YoeB _{Sa1} -HindIII-R	5'-AAAAAAAAGCTTAAATAATGGTATTTACATGATGCTAT-3'
YoeB _{Sa1} -NdeI-F	5'-AAAAAACATATGGCTAGGTTAAATATTACGT-3'
YoeB _{Sa1} -Y88F-HindIII-R	5'-AAAAAAAAGCTTAAAAATGGTATTTACATGATGCTAT-3'
YoeB _{Sa1} -Y88X-HindIII-R	5'-AAAAAAAAGCTTACTAATGGTATTTACATGATGC-3'
YoeB _{Sa2} -NdeI-F	5'-AAAAGTCATATGAGCAATTACACGGTTAAG-3'
YoeB _{Sa2} -HindIII-R	5'-AAAAAAAAGCTTAATCATAATGTGACCATGC-3'

Table S2. PCR results with MRSA total genomic DNA. Hospital 1 is Carle Foundation Hospital (Urbana, IL), Hospital 2 is Delnor Community Hospital (Geneva, IL), Hospital 3 is Memorial Medical Center (Springfield, IL), and NARSA is Network on Antimicrobial Resistance in *Staphylococcus aureus*.

Isolate	Toxin-Antitoxin System		Isolate	Toxin-Antitoxin System	
	<i>yefM-yoeB_{Sa1}</i>	<i>yefM-yoeB_{Sa2}</i>		<i>yefM-yoeB_{Sa1}</i>	<i>yefM-yoeB_{Sa2}</i>
Hospital 1			S5	(+)	(+)
C1	(+)	(+)	S6	(+)	(+)
C2	(+)	(+)	S7	(+)	(+)
C3	(+)	(+)	S8	(+)	(+)
C4	(+)	(+)	S9	(+)	(+)
C5	(+)	(+)	S10	(+)	(+)
C6	(+)	(+)	S11	(+)	(+)
C7	(+)	(+)	S12	(+)	(+)
C8	(+)	(+)	S13	(+)	(+)
C9	(+)	(+)	S14	(+)	(+)
C10	(+)	(+)	S15	(+)	(+)
C11	(+)	(+)	S16	(+)	(+)
C12	(+)	(+)	S17	(+)	(+)
C13	(+)	(+)	S18	(+)	(+)
C14	(+)	(+)	S19	(+)	(+)
C15	(+)	(+)	S20	(+)	(+)
C16	(+)	(+)	S21	(+)	(+)
C17	(+)	(+)	S22	(+)	(+)
C18	(+)	(+)	S23	(+)	(+)
C19	(+)	(+)	S24	(+)	(+)
C21	(+)	(+)	S25	(+)	(+)
C26	(+)	(+)	S26	(+)	(+)
Hospital 2			NARSA		
G1	(+)	(+)	NRS3	(+)	(+)
G2	(+)	(+)	NRS4	(+)	(+)
G3	(+)	(+)	NRS17	(+)	(+)
G4	(+)	(+)	NRS18	(+)	(+)
G5	(+)	(+)	NRS21	(+)	(+)
G6	(+)	(+)	NRS22	(+)	(+)
G8	(+)	(+)	NRS23	(+)	(+)
G9	(+)	(+)	NRS24	(+)	(+)
G10	(+)	(+)	NRS26	(+)	(+)
G11	(+)	(+)	NRS27	(-)	(-)
G12	(+)	(+)	NRS28	(+)	(+)
G13	(+)	(+)	NRS29	(+)	(+)
G14	(+)	(+)	NRS51	(+)	(+)
Hospital 3			NRS68	(+)	(+)
S1	(+)	(+)	NRS73	(+)	(+)
S2	(+)	(+)	NRS74	(+)	(+)
S3	(+)	(+)	NRS76	(+)	(+)
S4	(+)	(+)	NRS382	(+)	(+)

Figure S1. Clustal W multiple sequence alignment of *yefM-yoeB_{SaI}* PCR products from 9 of the 77 clinical MRSA strains found to contain *yefM-yoeB_{SaI}* by PCR. Bases highlighted in bold on the reference sequence from the *S. aureus* COL genome indicate the location of the primers used to detect the *yefM-yoeB_{SaI}* sequence.

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COL_MBSa1      TTAATAATGGTATTTACATGATGCTATTTTTATATGATTGTCATCTACTGTATAAACCAA
C2             -----
C6             -----
G1             -----
G8             -----
S10           -----
S22           -----
NRS28         -----
NRS51         -----
NRS68         -----

COL_MBSa1      TCTATGTTTCGTGATTGATACGTCTACTATAATACCCAGTCAGATTTCGATTTTAACTTTTC
C2             -----
C6             -----
G1             -----
G8             -----
S10           -----
S22           -----
NRS28         -----
NRS51         -----
NRS68         -----

COL_MBSa1      AGGCTTACCTATACCCTTCCAATGCACCATTTCTGTCAATACTTTTAAGTAACTCATTAAT
C2             -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
C6             -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
G1             -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
G8             -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
S10           -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
S22           -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
NRS28         -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
NRS51         -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
NRS68         -----CCTTCCAATGCACCATTTCGTCAATACTTTTAAGTAACTCATTAAT
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COL_MBSa1      CTTCTTCACCATTTTTTTT-ATCGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
C2             CTTCTTCACCATTTTTTTT-ATCGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
C6             CTTCTTCACCATTTTTTTT-ATTGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
G1             CTTCTTCACCATTTTTTTTATTGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
G8             CTTCTTCACCATTTTTTTT-ATCGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
S10           CTTCTTCACCATTTTTTTT-ATTGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
S22           CTTCTTCACCATTTTTTTT-ATCGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
NRS28         CTTCTTCACCATTTTTTTT-ATCGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
NRS51         CTTCTTCACCATTTTTTTT-ATCGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
NRS68         CTTCTTCACCATTTTTTTT-ATTGTTCTGCTGAAAATACTTATAATCTTCAAAGGCTTGAG
                ***** ** *****

COL_MBSa1      GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
C2             GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
C6             GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
G1             GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
G8             GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
S10           GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
S22           GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
NRS28         GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
NRS51         GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
NRS68         GCGAAAACGTAATATTTAACCTAGCCATTATACATCTATATCTTTCGTTATAGTTTTCC
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Figure S1, cont.

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COL_MBSa1      ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
C2             ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGATTCTGTTGGAG
C6             ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
G1            ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
G8            ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
S10           ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
S22           ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
NRS28         ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
NRS51         ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
NRS68         ACGTTCTAGATCTGCAATTGATTGAGCTAAGTGTTTCAGCATTATTTGGGTTCTGTTGGAG
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COL_MBSa1      GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
C2             GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
C6             GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
G1            GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
G10           GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
S22           GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
NRS28         GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
NRS51         GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
NRS68         GTAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
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COL_MBSa1      ATCAGTAGATGTTACAGTTACCATATCACTATCATCATTTACTTTTGTGCATAAAGTGCCTT
C2             ATCAGTAGAGGTTACAGTTACCATATCACTATCATCATTTACTTTTGTGCATAAAGTGCCTT
C6             ATCAGTAGATGTTACAGTTACCATATCACTATCATCATTTACTTTTGTGCATAAAGTGCCTT
G1            ATCAGTAGATGTTACAGTTACCATATCACTATCATCATTTACTTTTGTGCATAAAGTGCCTT
S10           ATAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
S22           ATAAAGTGTTCATCATGGAGTTATAATCTGATTCTGACATGATTACTACGTTTTTATC
NRS28         ATCAGTAGATGTTACAGTTACCATATCACTATCATCATTTACTTTTGTGCATAAAGTGCCTT
NRS51         ATCAGTAGATGTTACAGTTACCATATCACTATCATCATTTACTTTTGTGCATAAAGTGCCTT
NRS68         ATCAGTAGATGTTACAGTTACCATATCACTATCATCATTTACTTTTGTGCATAAAGTGCCTT
*****
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COL_MBSa1	TAAATTCGTGCGAGCG TATGAATAATTTTAAATAATCAT	Sequence Identity
C2	TAAATTCGTGCGAGCG-----	99%
C6	TAAATTCGTGCGAGCG-----	99%
G1	TAAATTCGTGCGAGCG-----	99%
G8	TAAATTCGTGCGAGCG-----	100%
S10	TAAATTCGTGCGAGCG-----	99%
S22	TAAATTCGTGCGAGCG-----	100%
NRS28	TAAATTCGTGCGAGCG-----	100%
NRS51	TAAATTCGTGCGAGCG-----	100%
NRS68	TAAATTCGTGCGAGCG-----	99%

Figure S2. Clustal W multiple sequence alignment of *yefM-yoeB_{Sa2}* PCR products from 9 of the 77 clinical MRSA strains found to contain *yefM-yoeB_{Sa2}* by PCR. Bases highlighted in bold on the reference sequence from the *S. aureus* COL genome indicate the location of the primers used to detect the *yefM-yoeB_{Sa2}* sequence.

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COL_MBSa2      TTAATCATAATGTGACCATGCCGATAGTATTAATACTTCTTTATTTTCGATCATCTACGGT
C6              -----GGT
C18             -----GGT
G5              -----GGT
G10            -----GGT
G14            -----GGT
S14            -----GGT
S15            -----GGT
NRS28          -----GGT
NRS76          -----GGT
                ***

COL_MBSa2      ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
C6              ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
C18             ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
G5              ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
G10            ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
G14            ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
S14            ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
S15            ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
NRS28          ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
NRS76          ATAGACGACCCTGTGCTGATGGTTAATTCTTCTTGAATATCGCTCTAAATATTTAGGCTC
                *****

COL_MBSa2      TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
C6              TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
C18             TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
G5              TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
G10            TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
G14            TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
S14            TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
S15            TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
NRS28          TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
NRS76          TAATTTTTCAAAGATTGTGTTATTTTATACGGATCATTTTTTAAAGTCTCAACAATTTTC
                *****

COL_MBSa2      TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
C6              TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
C18             TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
G5              TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
G10            TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
G14            TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
S14            TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
S15            TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
NRS28          TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
NRS76          TAAAAATGACTTCTTTAAATAAGAATGTTTTATTTTCTTTAAATCTGATTTTCGCTGAATT
                *****

COL_MBSa2      TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
C6              TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
C18             TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
G5              TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
G10            TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
G14            TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
S14            TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
S15            TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
NRS28          TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
NRS76          TTTAATCTTAACCGTGAATTGCTCATTAAAGATTATCCCAATCAATATCATCTATATTT
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Figure S2, cont.

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COL_MBSa2      GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
C6             GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
C18           GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
G5            GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
G10           GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
G14           GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
S14           GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
S15           GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
NRS28         GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
NRS76         GTTGTACCACTATTATCTTTTTCTCTTTCTTACTTTGTCCATTGTACCAGTAGATTCA
*****

COL_MBSa2      AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
C6            AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
C18           AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
G5            AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
G10           AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
G14           AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
S14           AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
S15           AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
NRS28         AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
NRS76         AGATATATTGTCTCTTGTATACTTTTCCAATCTTCTAAACCTATAATCACAGCATTATTT
*****

COL_MBSa2      TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
C6            TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
C18           TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
G5            TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
G10           TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
G14           TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
S14           TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAAC-----
S15           TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
NRS28         TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
NRS76         TCGGCATTATTGCCACTAATATAAAATTGGTTCGTGATTATTATTAACATTTTTTAGTAAT
*****

COL_MBSa2      TGATAAAAAATCTTTTCTCGCTTCTGTAGGGCTAGTGATAATCAT      Sequence Identity
C6            TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          100%
C18           TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          100%
G5            TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          100%
G10           TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          100%
G14           TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          99%
S14           -----                                          100%
S15           TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          100%
NRS28         TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          99%
NRS76         TGATAAAAAATCTTTTCTCGCTTCTGTAGGGC-----          99%
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Figure S3. MALDI-MS analysis of YoeB_{Sa1} specificity toward fluorogenic substrate. Peak fractions were collected from blue HPLC trace in Figure 5B, concentrated, and submitted for MALDI-MS at the University of Illinois Mass Spectrometry Laboratory. *A*, MALDI spectrum of the first elution peak (calculated MW: 3115.0 Da; observed MW: 3115.3 Da). *B*, MALDI spectrum of the early portion of the second elution peak (calculated MW: 2602.8 Da; observed MW: 2602.9 Da). *C*, MALDI spectrum of the later portion of the second elution peak (calculated MW: 2602.8 Da; observed MW: 2604.3 Da).

