Supporting information

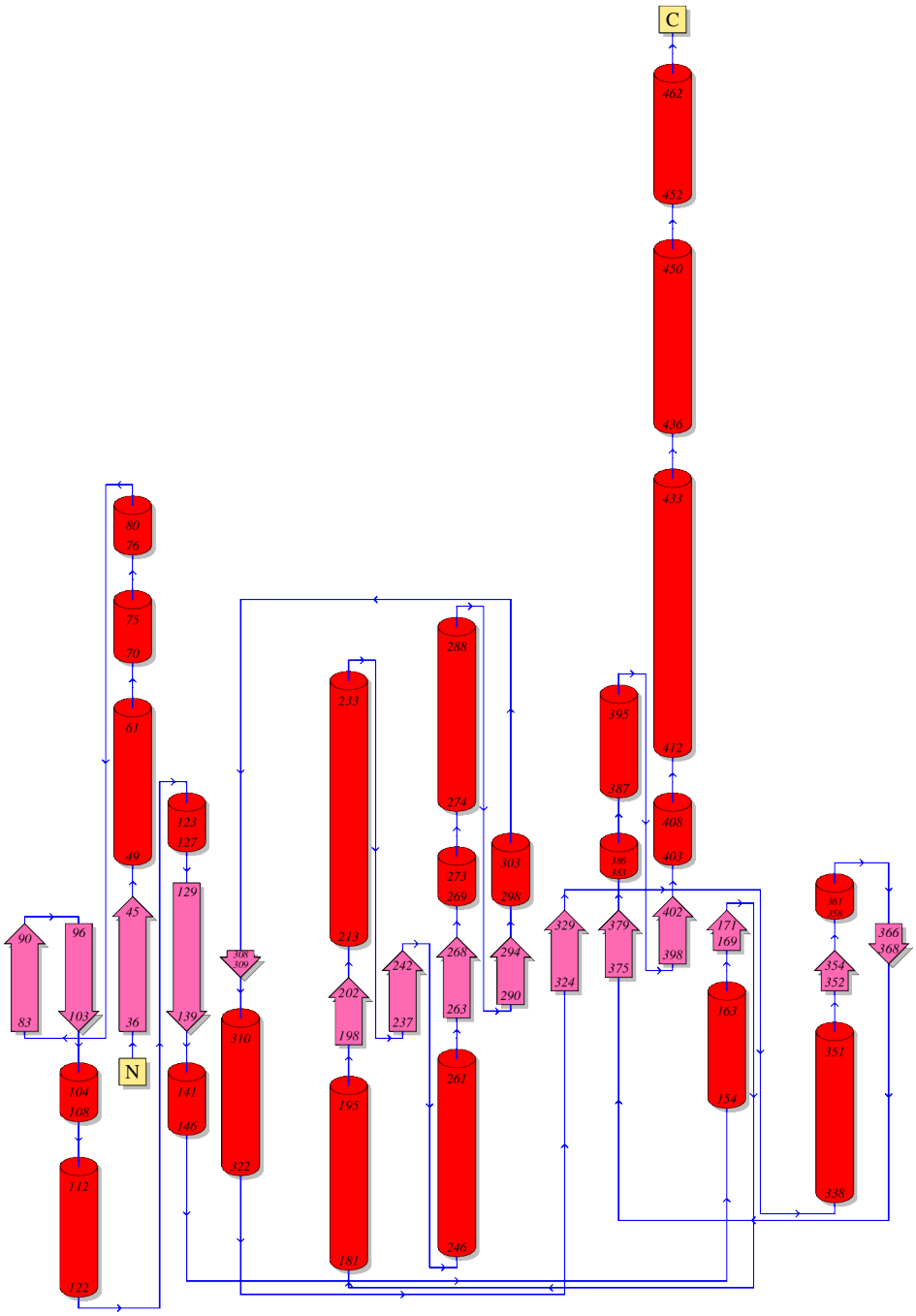

Figure S1. Secondary structure of Bs2Est

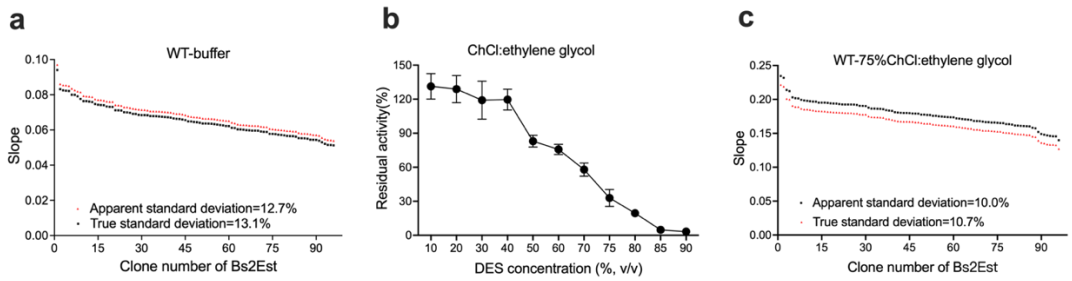

Figure S2. Screening system of Bs2Est in ChCl:ethylene glycol. (a) The slope of Bs2Est WT in the buffer. (b) Residual activity of Bs2Est WT at different ChCl:ethylene glycol concentrations. (c) Slope of Bs2Est WT in 75% ChCl:ethylene glycol (Concentration which can obtain residual activity of 30–40% of the BSLA WT was used in the incubation step for the Bs2Est activity assay). The standard deviation to evaluate the applicability of the 96-well MTP-based screening system for directed Bs2Est WT evolution. The apparent standard deviation was calculated without excluding the background, and the true stand deviation was calculated after deducting the background from the empty vector.

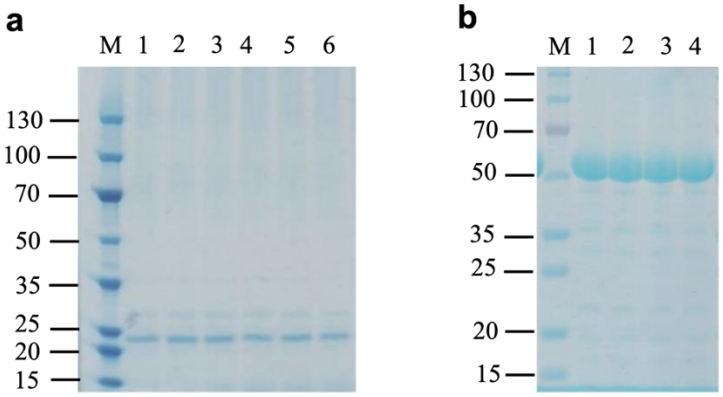

Figure S3. Identification and SDS-PAGE analysis of purified protein. A broad molecular weight standard protein (15-130 kDa) was used as a marker, and the remaining lanes were WT and variants. (a) From left to right was BSLA WT, T66H/G67D, K88E/N89K, M137D/N138D, M137D/N138H, Y161D/S162E/S163E. (b) From left to right was Bs2Est WT, K205H, E286H/E286D, E286K.

Table S1. Primers used for corner engineering of the BSLA gene in pet-22b.

Name	5' --> 3' sequence
F1(5.6)- Forward Primer	TGAACACAATSRCSRCGTTATGGTTC
F1(5.6)- Reverse Primer	GAACCATAACGYSGYSATTGTGTTCA
F1'(5.6)- Forward Primer	TGAACACAATRAARAAGTTATGGTTC
F1'(5.6)- Reverse Primer	GAACCATAACTTYTTYATTGTGTTCA
F2(9.10)- Forward Primer	AGTCGTTATGSRCSRCGGTATTGGAG
F2(9.10)- Reverse Primer	CTCCAATACCGYSGYSCATAACGACT
F2'(9.10)- Forward Primer	AGTCGTTATGRAARAAGGTATTGGAG
F2'(9.10)- Reverse Primer	CTCCAATACCTTYTTYCATAACGACT
F3(15.16)- Forward Primer	GTATTGGAGGGSRCSRCTTCAATTTTGC
F3(15.16)- Reverse Primer	GCAAAATTGAAGYSGYSCCCTCCAATAC
F3'(15.16)-Forward Primer	GTATTGGAGGGRAARAATTCAATTTTGC
F3'(15.16)- Reverse Primer	GCAAAATTGAATTYTTYCCCTCCAATAC
F4(28.29)- Forward Primer	GCTATCTCGTASRCSRCGGCTGGTCGCG
F4(28.29)- Reverse Primer	CGCGACCAGCCGYSGYSTACGAGATAGC
F4'(28.29)-Forward Primer	GCTATCTCGTARAARAAGGCTGGTCGCG
F4'(28.29)- Reverse Primer	CGCGACCAGCCTTYTTYTACGAGATAGC
F5(32.33)- Forward Primer	TCTCAGGGCTGGSRCSRCGACAAGCTGTAT
F5(32.33)- Reverse Primer	ATACAGCTTGTCGYSGYSCCAGCCCTGAGA
F5'(32.33)-Forward Primer	TCTCAGGGCTGGRAARAAGACAAGCTGTAT
F5'(32.33)- Reverse Primer	ATACAGCTTGTCTTYTTYCCAGCCCTGAGA
F6(35.36)- Forward Primer	TGGTCGCGGGACSRCSRCTATGCAGTTGAT
F6(35.36)- Reverse Primer	ATCAACTGCATAGYSGYSGTCCCGCGACCA
F6'(35.36)-Forward Primer	TGGTCGCGGGACRAARAATATGCAGTTGAT
F6'(35.36)- Reverse Primer	ATCAACTGCATATTYTTYGTCCCGCGACCA
F7(38.39)- Forward Primer	GACAAGCTGTATSRCSRCGATTTTTGGGAC
F7(38.39)- Reverse Primer	GTCCCAAAAATCGYSGYSATACAGCTTGTC
F7'(38.39)-Forward Primer	GACAAGCTGTATRAARAAGATTTTTGGGAC
F7'(38.39)- Reverse Primer	GTCCCAAAAATCTTYTTYSATACAGCTTGTC
F8(47.48)- Forward Primer	GACAAGACAGGCSRCSRCTATAACAATGGA
F8(47.48)- Reverse Primer	TCCATTGTTATAGYSGYSGCCTGTCTTGTC
F8'(47.48)-Forward Primer	GACAAGACAGGCRAARAATATAACAATGGA
F8'(47.48)- Reverse Primer	TCCATTGTTATATTYTTYGCCTGTCTTGTC
F9(66.67)-Forward Primer	GTTTTAGATGAASRCSRCGCGAAAAAAGTG
F9(66.67)-Reverse Primer	CACTTTTTTCGCGYSGYSTTCATCTAAAAC
F9'(66.67)-Forward Primer	GTTTTAGATGAARAARAAGCGAAAAAAGTG
F9'(66.67)-Reverse Primer	CACTTTTTTCGCTTYTTYTTCATCTAAAAC
F10 (70.71)- Forward Primer	ACGGGTGCGAAASRCSRCGATATTGTCGCT
F10(70.71)- Reverse Primer	AGCGACAATATCGYSGYSTTTCGCACCCGT
F10' (70.71)- Forward Primer	ACGGGTGCGAAARAARAAGATATTGTCGCT
F10' (70.71)- Reverse Primer	AGCGACAATATCTTYTTYTTTCGCACCCGT

F11(76.77.78)-Forward Primer	AGTGGATATTGTCGCTSRCSRCSRCGGGGGCGCGAACACAC
F11(76.77.78)- Reverse Primer	GTGTGTTCGCGCCCCCGYSGYSGYSAGCGACAATATCCACT
F11'(76.77.78)- Forward Primer	AGTGGATATTGTCGCTRAARAARAAGGGGGCGCGAACACAC
F11'(76.77.78)- Reverse Primer	GTGTGTTCGCGCCCCCTTYTTYTTYAGCGACAATATCCACT
F12(88.89)- Forward Primer	CGAACACACTTTACTACATASRCSRCCTGGACGGCGGAAATAAAGT
F12(88.89)- Reverse Primer	ACTTTATTTCCGCCGTCCAGGYSGYSTATGTAGTAAAGTGTGTTCG
F12'(88.89)- Forward Primer	CGAACACACTTTACTACATARAARAACTGGACGGCGGAAATAAAGT
F12'(88.89)- Reverse Primer	ACTTTATTTCCGCCGTCCAGTTYTTYTATGTAGTAAAGTGTGTTCG
F13(91.92)- Forward Primer	ATAAAAAATCTGSRCSRCGGAAATAAAGTT
F13(91.92)- Reverse Primer	AACTTTATTTCCGYSGYSCAGATTTTTTAT
F13'(91.92)- Forward Primer	ATAAAAAATCTGRAARAAGGAAATAAAGTT
F13'(91.92)- Reverse Primer	AACTTTATTTCCTTYTTYCAGATTTTTTAT
F14(102.103)- Forward Primer	GTCGTGACGSRCSRCGGCGCGAAC
F14(102.103)- Reverse Primer	GTTCGCGCCGYSGYSCGTCACGAC
F14'(102.103)- Forward Primer	GTCGTGACGRAARAAGGCGCGAAC
F14'(102.103)- Reverse Primer	GTTCGCGCCTTYTTYCGTCACGAC
F15(105.106)- Forward Primer	CGTGACGCTTGGCGGCSRCSRCCGTTTGACGACAGGCA
F15(105.106)- Reverse Primer	TGCCTGTCGTCAAACGGYSGYSGCCGCCAAGCGTCACG
F15'(105.106)- Forward Primer	CGTGACGCTTGGCGGCRAARAACGTTTGACGACAGGCA
F15'(105.106)- Reverse Primer	TGCCTGTCGTCAAACGTTYTTYGCCGCCAAGCGTCACG
F16(108.109)- Forward Primer	CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC
F16(108.109)- Reverse Primer	GCGCCTTGCCTGTGYSGYSACGGTTCGCGCCG
F16'(108.109)- Forward Primer	CGGCGCGAACCGTRAARAAACAGGCAAGGCGC
F16'(108.109)- Reverse Primer	GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG
F16(123.124)- Forward Primer	CCGGGAACAGATCCAAATCAAAAGSRCSRCTACACATCCATTTACAGCAGTGCCG
F17(123.124)- Reverse Primer	CGGCACTGCTGTAAATGGATGTGTAGYSGYSCTTTTGATTTGGATCTGTTCCCGG
F17'(123.124)- Forward Primer	CCGGGAACAGATCCAAATCAAAAGRAARAATACACATCCATTTACAGCAGTGCCG
F17'(123.124)- Reverse Primer	CGGCACTGCTGTAAATGGATGTGTATTYTTYCTTTTGATTTGGATCTGTTCCCGG

F18(130.131)- Forward Primer	TATACACATCCATTTACSRCSRCGCCGATATGATTGTCAT
F18(130.131)- Reverse Primer	ATGACAATCATATCGGCGYSGYSGTAAATGGATGTGTATA
F18'(130.131)- Forward Primer	TATACACATCCATTTACRAARAAGCCGATATGATTGTCAT
F18'(130.131)- Reverse Primer	ATGACAATCATATCGGCTTYTTYGTAAATGGATGTGTATA
F19(137.138)- Forward Primer	GCAGTGCCGATATGATTGTCSRCSRCTACTTATCAAGATTAGATGG
F19(137.138)- Reverse Primer	CCATCTAATCTTGATAAGTAGYSGYSGACAATCATATCGGCACTGC
F19'(137.138)- Forward Primer	GCAGTGCCGATATGATTGTCRAARAATACTTATCAAGATTAGATGG
F19'(137.138)- Reverse Primer	CCATCTAATCTTGATAAGTATTYTTYGACAATCATATCGGCACTGC
F20(141.142)- Forward Primer	ATGAATTACTTASRCSRCTTAGATGGTGCT
F20(141.142)- Reverse Primer	AGCACCATCTAAGYSGYSTAAGTAATTCAT
F20'(141.142)- Forward Primer	ATGAATTACTTARAARAATTAGATGGTGCT
F20'(141.142)- Reverse Primer	AGCACCATCTAATTYTYTYTAAGTAATTCAT
F21(146.147)- Forward Primer	ACTTATCAAGATTAGATGGTSRCSRCAACGTTCAAATCCATGGCGT
F21(146.147)- Reverse Primer	ACGCCATGGATTTGAACGTTGYSGYSACCATCTAATCTTGATAAGT
F21'(146.147)- Forward Primer	ACTTATCAAGATTAGATGGTRAARAAAACGTTCAAATCCATGGCGT
F21'(146.147)- Reverse Primer	ACGCCATGGATTTGAACGTTTTYTYTYACCATCTAATCTTGATAAGT
F22(151.152)- Forward Primer	GGTGCTAGAAACGTTCAASRCSRCGGCGTTGGACACATCGGC
F22(151.152)- Reverse Primer	GCCGATGTGTCCAACGCCGYSGYSTTGAACGTTTCTAGCACC
F22'(151.152)- Forward Primer	GGTGCTAGAAACGTTCAARAARAAGGCGTTGGACACATCGGC
F22'(151.152)- Reverse Primer	GCCGATGTGTCCAACGCCTTYTTYTTGAACGTTTCTAGCACC
F23(157.158)- Forward Primer	CCATGGCGTTGGACACSRCSRCCTTCTGTACAGCAGCC
F23(157.158)- Reverse Primer	GGCTGCTGTACAGAAGGYSGYSGTGTCCAACGCCATGG
F23'(157.158)- Forward Primer	CCATGGCGTTGGACACRAARAACTTCTGTACAGCAGCC
F23'(157.158)- Reverse Primer	GGCTGCTGTACAGAAGTTYTTYGTGTCCAACGCCATGG

F24(161.162.163)-Forward Primer	GGACACATCGGCCTTCTGSRCSRCSRCCAAGTCAACAGCCTGATT
F24(161.162.163)-Reverse Primer	AATCAGGCTGTTGACTTGGYSGYSGYSCAGAAGGCCGATGTGTCC
F24'(161.162.163)-Forward Primer	GGACACATCGGCCTTCTGRAARAARAACAAGTCAACAGCCTGATT
F24'(161.162.163)-Reverse Primer	AATCAGGCTGTTGACTTGTTYTTYTTYCAGAAGGCCGATGTGTCC
F25(173.174)- Forward Primer	CCTGATTAAAGAAGGGSRCSRCGGCGGGGGCCAGAATACG
F25(173.174)- Reverse Primer	CGTATTCTGGCCCCCGCCGYSGYSCCCTTCTTTAATCAGG
F25'(173.174)- Forward Primer	CCTGATTAAAGAAGGGRAARAAGGCGGGGGCCAGAATACG
F25'(173.174)- Reverse Primer	CGTATTCTGGCCCCCGCCTTYTTYCCCTTCTTTAATCAGG

Table S2. Primers used for corner engineering of the Bs2Est gene in pet-22b.

Name	5' --> 3' sequence
F1(33.34)- Forward Primer	TATGCCAAGCCGSRCSRCGGACAATGGC
F1(33.34)- Reverse Primer	TATGCCAAGCCGRAARAAGGACAATGGC
F1'(33.34)- Forward Primer	GCCATTGTCCGYSGYSCGGCTTGGCATA
F1'(33.34)- Reverse Primer	GCCATTGTCCTTYTTYCGGCTTGGCATA
F2(38.39)- Forward Primer	TCGGACAATGGSRCSRCAAAGCACCTGAGC
F2(38.39)- Reverse Primer	TCGGACAATGGRAARAAAAGCACCTGAGC
F2'(38.39)- Forward Primer	GCTCAGGTGCTTTGYSGYSCCATTGTCCGA
F2'(38.39)- Reverse Primer	GCTCAGGTGCTTTTTYTTYCCATTGTCCGA
F3(114.115)- Forward Primer	CTAGGAGCGGGCSRCSRCCCATTGTATGAC
F3(114.115)- Reverse Primer	CTAGGAGCGGGCRAARAACCATTGTATGAC
F3'(114.115)- Forward Primer	GTCATACAATGGGYSGYSGCCCGCTCCTAG
F3'(114.115)- Reverse Primer	GTCATACAATGGTTYTTYGCCCGCTCCTAG
F4(119.120)- Forward Primer	GAGCCATTGTATSRCSRCTCAAAACTTGCG
F4(119.120)- Reverse Primer	GAGCCATTGTATRAARAATCAAAACTTGCG
F4' (119.120)- Forward Primer	CGCAAGTTTTGAGYSGYSATACAATGGCTC
F4' (119.120)- Reverse Primer	CGCAAGTTTTGATTYTTYATACAATGGCTC
F5(125.126)- Forward Primer	TCAAAACTTGCGSRCSRCGGAGAAGTCATT
F5(125.126)- Reverse Primer	TCAAAACTTGCGRAARAAGGAGAAGTCATT
F5' (125.126)- Forward Primer	AATGACTTCTCCGYSGYSCGCAAGTTTTGA
F5' (125.126)- Reverse Primer	AATGACTTCTCCTTYTTYCGCAAGTTTTGA
F6(128.129)- Forward Primer	GCGGCACAGGGASRCSRCATTGTCGTTACA
F6(128.129)- Reverse Primer	GCGGCACAGGGARAARAAATTGTCGTTACA
F6'(128.129)- Forward Primer	TGTAACGACAATGYSGYSTCCCTGTGCCGC
F6'(128.129)- Reverse Primer	TGTAACGACAATTTYTTYTCCCTGTGCCGC

F7(137.138)- Forward Primer	ACATTGAACTATSRCSRCGGGCCGTTTGGC
F7(137.138)- Reverse Primer	ACATTGAACTATRAARAAGGGCCGTTTGGC
F7'(137.138)- Forward Primer	GCCAAACGGCCCGYSGYSATAGTTCAATGT
F7'(137.138)- Reverse Primer	GCCAAACGGCCCTTYTTYATAGTTCAATGT
F8(143.144)- Forward Primer	GGGCCGTTTGGCSRCSRCCACTTGTCTTCA
F8(143.144)- Reverse Primer	GGGCCGTTTGGCRAARAACACTTGTCTTCA
F8'(143.144)- Forward Primer	TGAAGACAAGTGGYSGYSGCCAAACGGCCC
F8'(143.144)- Reverse Primer	TGAAGACAAGTGTTYTTYGCCAAACGGCCC
F9(155.156)- Forward Primer	GAGGCGTATTCTSRCSRCCTTGGGCTTTTA
F9(155.156)- Reverse Primer	GAGGCGTATTCTRAARAACTTGGGCTTTTA
F9' (155.156)- Forward Primer	TAAAAGCCCAAGGYSGYSAGAATACGCCTC
F9' (155.156)- Reverse Primer	TAAAAGCCCAAGTTYTTYAGAATACGCCTC
F10(176.177)- Forward Primer	AATATTTCAGCGSRCSRCGGTGATCCCGAT
F10(176.177)- Reverse Primer	AATATTTCAGCGRAARAAGGTGATCCCGAT
F10'(176.177)- Forward Primer	ATCGGGATCACCGYSGYSCGCTGAAATATT
F10'(176.177)- Reverse Primer	ATCGGGATCACCTTYTTYCGCTGAAATATT
F11(206.207)- Forward Primer	CCTGCGGCAAAASRCSRCTTCCAGAAAGCA
F11(206.207)- Reverse Primer	CCTGCGGCAAAARAARAATTCCAGAAAGCA
F11'(206.207)- Forward Primer	TGCTTTCTGGAAGYSGYSTTTTGCCGCAGG
F11'(206.207)- Reverse Primer	TGCTTTCTGGAATTYTTYTTTTGCCGCAGG
F12(205.206)- Forward Primer	ATGCCTGCGGCASRCSRCCTGTTCCAGA
F12(205.206)- Reverse Primer	ATGCCTGCGGCARAARAACTGTTCCAGA
F12'(205.206)- Forward Primer	TCTGGAACAGGYSGYSTGCCGCAGGCAT
F12'(205.206)- Reverse Primer	ATGCCTGCGGCARAARAACTGTTCCAGA
F13(221.222)- Forward Primer	GCTTCTCGAACGSRCSRCAAAGAACAAGCG
F13(221.222)- Reverse Primer	GCTTCTCGAACGRAARAAAAGAACAAGCG
F13'(221.222)- Forward Primer	CGCTTGTTCTTTGYSGYSCGTTCGAGAAGC
F13'(221.222)- Reverse Primer	CGCTTGTTCTTTTTYTTYCGTTCGAGAAGC
F14(238.239)- Forward Primer	TTACAGGTCCTTSRCSRCAACGAGGGCCAA
F14(238.239)- Reverse Primer	TTACAGGTCCTTRAARAAAACGAGGGCCAA
F14'(238.239)- Forward Primer	TTGGCCCTCGTTGYSGYSAAGGACCTGTAA
F14'(238.239)- Reverse Primer	TTGGCCCTCGTTTTYTTYAAGGACCTGTAA
F15(242.243)- Forward Primer	GGGATTAACGAGSRCSRCCTGGATAAATTG
F15(242.243)- Reverse Primer	GGGATTAACGAGRAARAACTGGATAAATTG
F15'(242.243)- Forward Primer	CAATTTATCCAGGYSGYSCTCGTTAATCCC
F15'(242.243)- Reverse Primer	CAATTTATCCAGTTYTTYCTCGTTAATCCC
F16(264.265)- Forward Primer	GATCAGCTTCGGSRCSRCGAAAAAGAAA
F16(264.265)- Reverse Primer	GATCAGCTTCGGRAARAAGAAAAAGAAA
F16'(264.265)- Forward Primer	TTTCTTTTTCGYSGYSCCGAAGCTGATC
F16'(264.265)- Reverse Primer	ATTTTCTTTTTCTTYTTYCCGAAGCTGATC
F17(286.287)- Forward Primer	AAAACGCTGCCTSRCSRCCCAGAAAAAGCG
F17(286.287)- Reverse Primer	AAAACGCTGCCTRAARAACCAGAAAAAGCG
F17'(286.287)- Forward Primer	CGCTTTTTCTGGGYSGYSAGGCAGCGTTTT
F17'(286.287)- Reverse Primer	CGCTTTTTCTGGTTYTTYAGGCAGCGTTTT

F18(294.295)- Forward Primer	AAAGCGATCGCASRCSRCGCTGCTTCCG
F18(294.295)- Reverse Primer	AAAGCGATCGCARAARAAGCTGCTTCCG
F18'(294.295)- Forward Primer	CGGAAGCAGCGYSGYSTGCGATCGCTTT
F18'(294.295)- Reverse Primer	CGGAAGCAGCTTYTTYTGCGATCGCTTT
F19(347.348)- Forward Primer	GTTGCCGATTTGSRCSRCCGTTCTCTGGAA
F19(347.348)- Reverse Primer	GTTGCCGATTTGRAARAACGTTCTCTGGAA
F19'(347.348)- Forward Primer	TTCCAGAGAACGGYSGYSCAAATCGGCAAC
F19'(347.348)- Reverse Primer	TTCCAGAGAACGTTYTTYCAAATCGGCAAC
F20(349.350)- Forward Primer	GATTTGTATCCGSRCSRCCTGGAAAGCCAA
F20(349.350)- Reverse Primer	GATTTGTATCCGRAARAACTGGAAAGCCAA
F20'(349.350)- Forward Primer	TTGGCTTTCCAGGYSGYSCGGATACAAATC
F20'(349.350)- Reverse Primer	TTGGCTTTCCAGTTYTTYCGGATACAAATC
F21(375.376)- Forward Primer	GCATCCGCACAGSRCSRCTACGCCCCTGTC
F21(375.376)- Reverse Primer	GCATCCGCACAGRAARAATACGCCCCTGTC
F21'(375.376)- Forward Primer	GACAGGGGCGTAGYSGYSCTGTGCGGATGC
F21'(375.376)- Reverse Primer	GACAGGGGCGTATTYTTYCTGTGCGGATGC
F22(401.402)- Forward Primer	GCGTTTCACGCASRCSRCCTTCCTTTTGTC
F22(401.402)- Reverse Primer	GACAAAAGGAAGGYSGYSTGCGTGAAACGC
F22'(401.402)- Forward Primer	GCGTTTCACGCARAARAACTTCCTTTTGTC
F22'(401.402)- Reverse Primer	GACAAAAGGAAGTTYTTYTGCGTGAAACGC
F23(421.422)- Forward Primer	GCAAAAGCGGAGSRCSRCGATGAGGTGAAA
F23(421.422)- Reverse Primer	GCAAAAGCGGAGRAARAAGATGAGGTGAAA
F23'(421.422)- Forward Primer	TTTCACCTCATCGYSGYSCTCCGCTTTTGC
F23'(421.422)- Reverse Primer	TTTCACCTCATCTTYTTYCTCCGCTTTTGC
F24(443.444)- Forward Primer	TTCGCCAAAACASRCSRCCCAAGCACCGAA
F24(443.444)- Reverse Primer	TTCGCCAAAACARAARAACCAAGCACCGAA
F24'(443.444)- Forward Primer	TTCGGTGCTTGGGYSGYSTGTTTTGGCGAA
F24'(443.444)- Reverse Primer	TTCGGTGCTTGGTTYTTYTGTTTTGGCGAA

Table S3. Kinetic characterization of the purified BSLA variants in buffer and DES.

Variant	Buffer			30 %ChCl:acetamide=1:2			30 %TBPB:ethylene glycol =1:2			95% ChCl:ethylene glycol =1:2		
	K_M (mM)	K_{cat} (min^{-1})	K_{cat}/K_M ($min^{-1}mM^{-1}$)	K_M (mM)	K_{cat} (min^{-1})	K_{cat}/K_M ($min^{-1}mM^{-1}$)	K_M (mM)	K_{cat} (min^{-1})	K_{cat}/K_M ($min^{-1}mM^{-1}$)	K_M (mM)	K_{cat} (min^{-1})	K_{cat}/K_M ($min^{-1}mM^{-1}$)
WT	0.004	0.189	44.704	0.145	0.188	1.297	0.365	0.396	1.085	0.0886	0.208	2.348
T66H/G67D	0.025	0.606	24.240	0.073	0.128	1.753	0.383	0.299	0.781	0.157	0.223	1.420
K88E/N89K	0.013	0.189	14.538	0.140	1.434	10.243	0.217	2.110	9.724	0.226	2.174	9.619
Y161D/S162E/S163E	0.017	0.233	13.706	0.026	0.039	1.500	0.530	0.246	0.464	0.0654	0.0899	1.375
M137D/N138H	0.0529	0.507	9.584	0.122	0.526	4.311	0.118	0.532	4.508	0.114	0.498	4.368
M137D/N138D	0.0812	0.563	6.933	0.124	0.560	4.516	0.150	0.590	3.933	0.116	0.518	4.466

Table S4. Kinetic characterization of the purified Bs2Est in buffer and DES.

Variant	Buffer			75% (v/v) ChCl:ethylene glycol		
	K_M (mM)	K_{cat} (min^{-1})	K_{cat}/K_M ($min^{-1}mM^{-1}$)	K_M (mM)	K_{cat} (min^{-1})	K_{cat}/K_M ($min^{-1}mM^{-1}$)
WT	0.0398	0.532	13.367	0.139	0.277	1.993
205H	0.0079	0.090	11.416	0.042	0.105	2.500
E286H/E286D	0.0187	0.203	10.856	0.248	0.239	0.964
E286K	0.0214	0.123	5.747	0.048	0.111	2.313