fermentation

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Supporting Information for

Improving the synthesis efficiency of amino acids such as L-lysine by assembling artificial cellulosome elements dockerin protein in vivo.

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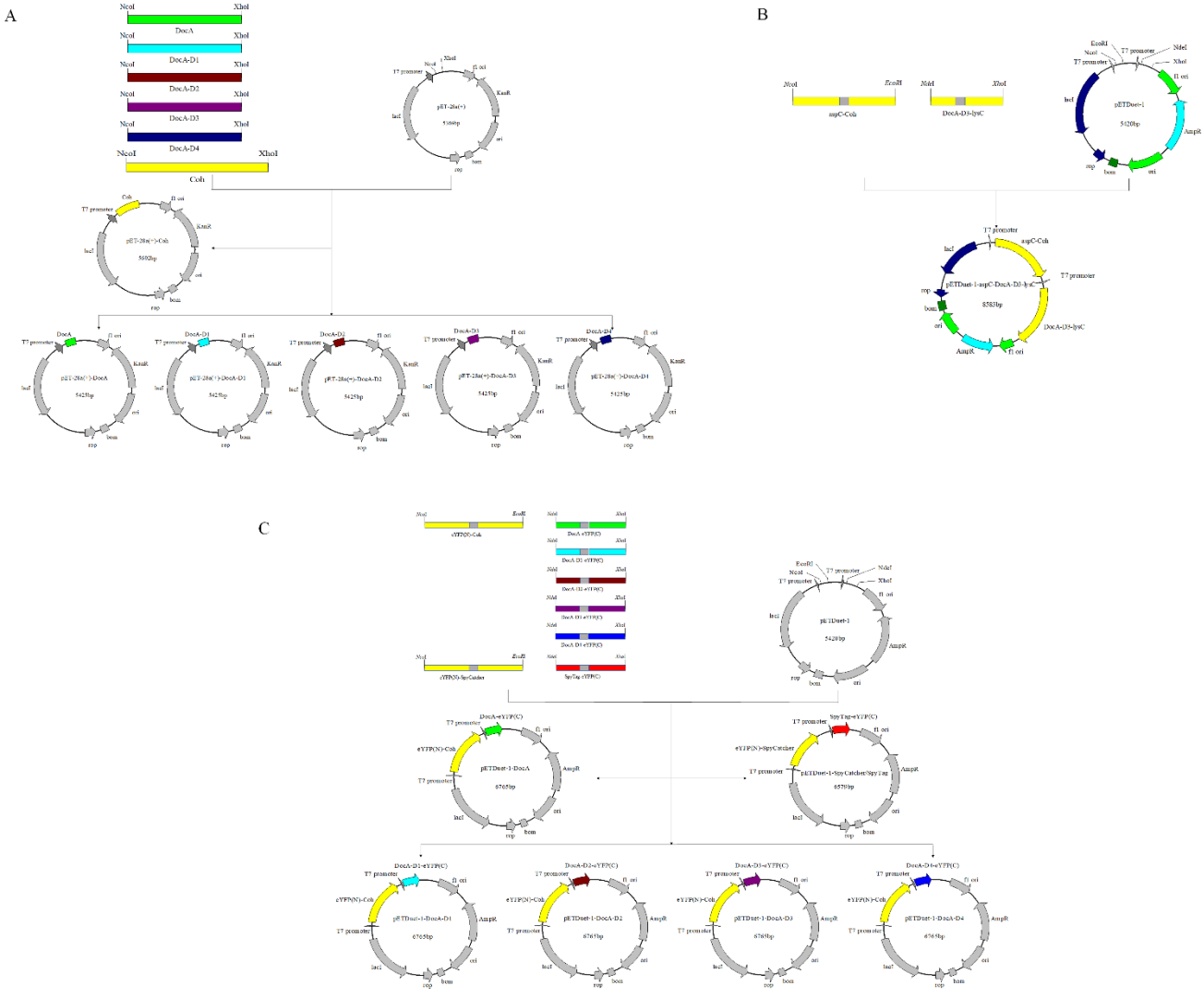
A
NcoI
XhoI
DocA
DocA-D1
DocA-D2
DocA-D3
DocA-D4
Coh
pET-28a(+)
5369bp
pET-28a(+)-Coh
5502bp
pET-28a(+)-DocA
5427bp
pET-28a(+)-DocA-D1
5425bp
pET-28a(+)-DocA-D2
5425bp
pET-28a(+)-DocA-D3
5425bp
pET-28a(+)-DocA-D4
5425bp
B
aspC-Coh
DocA-D3-lysC
pETDuet-1
5420bp
pETDuet-1-aspC-DocA-D3-lysC
8583bp
C
eYFP(C)-Coh
eYFP(N)-SpyCatcher
SpyTag-eYFP(C)
pETDuet-1-DocA
6765bp
pETDuet-1-SpyCatcher/SpyTag
6570bp
pETDuet-1-DocA-D1
6765bp
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6765bp
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6765bp
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6765bp

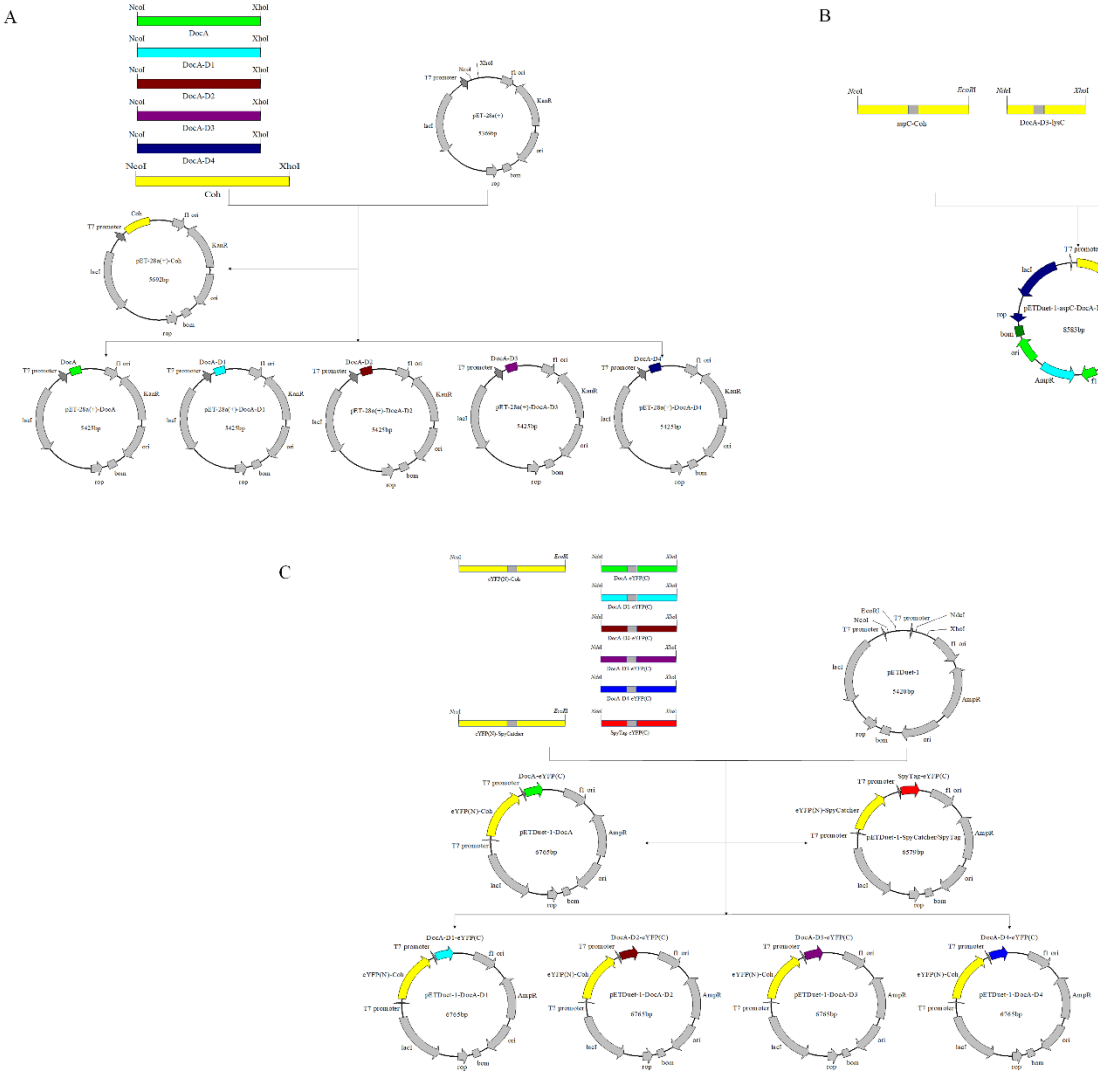

Figure S1. The construction and the schematic representation of the recombinant plasmids. A The construction and schematic representation of recombinant plasmid used in protein expression and purification; B The construction of assembly engineering strains QDE-DocA-D3; C The construction and schematic representation of the recombinant plasmid used in BIFC-FC.

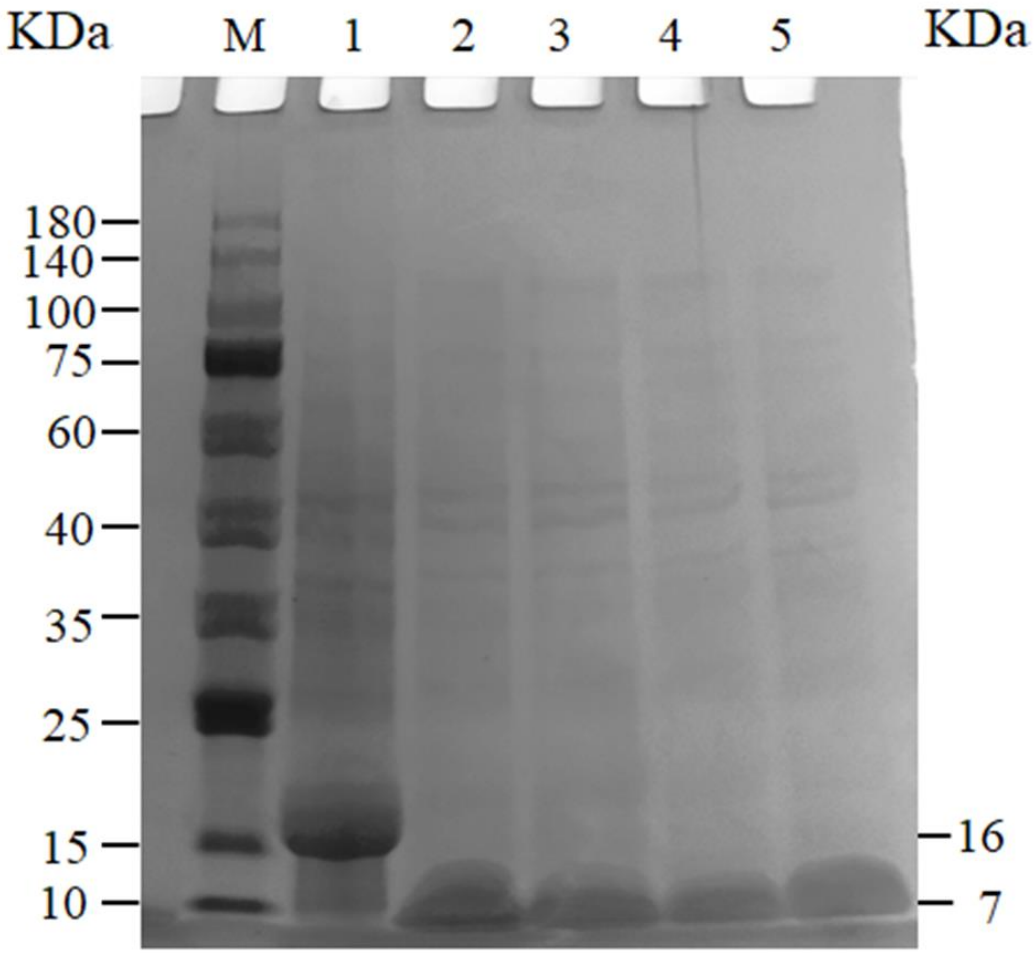

Figure S2. Electrophoretogram of purified proteins. M: marker 180 KDa; 1: 7 kDa of the initial docking protein DocA; 2: 16 kDa of the adhesion protein Coh; 3: 7 kDa of the docking protein mutant DocA-D1; 4: 7 kDa of the docking protein mutant DocA-D2; 5: 7 kDa of the docking protein mutant DocA-D3; 6: 7 kDa of the docking protein mutant DocA-D4.

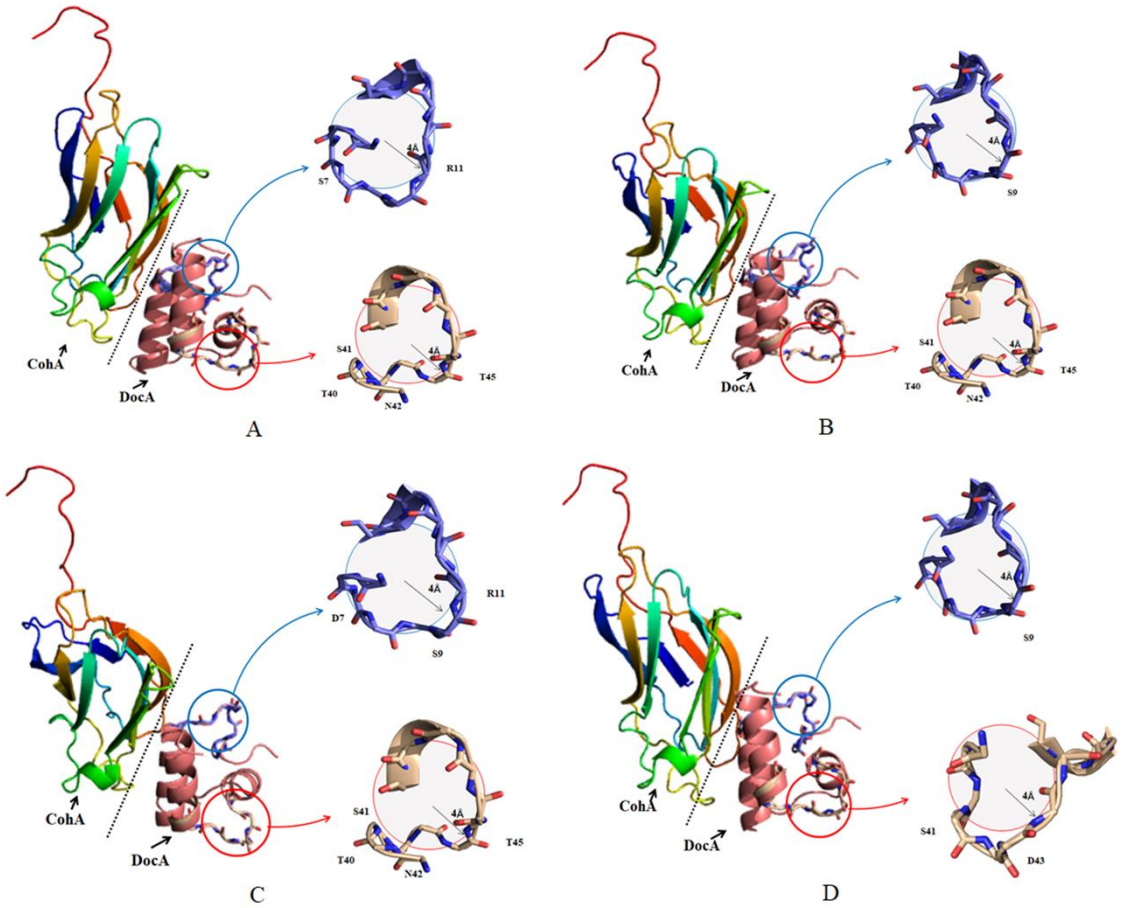

Figure S3. Changes in the key amino acid positions of DocA mutants. A The structure of DocA-D1/ Coh. B The structure of DocA-D2/ Coh. C The structure of DocA-D3/ Coh. D The structure of DocA-D4/ Coh.

Table S1. The list of verification primers in this study. Introduced restriction sites (RS) are underlined.

Protein	Primer name	Sequence (5′→3′)	Notes
Coh	Coh-F	TACCATGGGCTCTGATGGCGTTGTTGTTGA	*Nco*I
	Coh-R	GCTCGAGGGTCGCGCCTTTGGTCGGG	*Xho*I
DocA	DocA-F	ACCATGGGCGTTCTGCTGGGCGATGTTAAC	*Nco*I
	DocA-R	GTGCTCGAGTTTATCGATAACACGCAGCAG	*Xho*I
DocA-D1	DocA-D1-F	TACCATGGGCGTCCTGCTGGGTGATGTTAG	*Nco*I
	DocA-D1-R	GTGCTCGAGGATAACACGCAGCAGATAACG	*Xho*I
DocA-D2	DocA-D2-F	ACCATGGGCGTGCTGCTGGGTGATGTTAAC	*Nco*I
	DocA-D2-R	TGCTCGAGGATAACACGCAGCAGGTAAC	*Xho*I
DocA-D3	DocA-D3-F	TACCATGGGCGTCCTGCTGGGTGATGTTG	*Nco*I
	DocA-D3-R	GCTCGAGGATAACACGCAGCAGATAACGA	*Xho*I
DocA-D4	DocA-D4-F	TACCATGGGCGTGCTGCTGGGTGATGT	*Nco*I
	DocA-D4-R	TGCTCGAGGATAACACGCAGCAGATAACGG	*Xho*I
eYFP-DocA	EY-coh-F1	TACCATGGGCATGAGCAAGGGCGAGGAACT	*Nco*I
	EY-coh-F2	TGGCAGCAGCGATGGCGTGGTTGTGGA	
	EY-coh-R	GCTCGAATTCTTAGGTCGCACCTTTGGTCG	*Eco*RI
	DocA-EY-F	TATACATATGGTGCTGCTGGGTGACGTTAA	*Nde*I
	DocA-EY-R1	GCCACCGCTAATAACACGCAGCAGG	
	DocA-EY-R2	CAGACTCGAGTTATTTGTACAGTTCG	*Xho*I

	DocA-D1-EY-F	ATATACATATGGTACTATTAGGTGATGT	*Nde*I
eYFP-DocA-D1	DocA-D1-EY-R1	ACCGCTGATGACACGTAACAGGTA	
	DocA-D1-EY-R2	AGACTCGAGTTATTTGTACAGTTCGT	*Xho*I
	DocA-D2-EY-F	ATATACATATGGTACTATTAGGTGATGTTA	*Nde*I
eYFP-DocA-D2	DocA-D2-EY-R1	ACCGCTGATCACGCGCAACAGGTA	
	DocA-D2-EY-R2	ACTCGAGTTATTTGTACAGTTCGT	*Xho*I
	DocA-D3-EY-F	ATACATATGGTACTATTAGGGGATGTTGACGG	*Nde*I
eYFP-DocA-D3	DocA-D3-EY-R1	ACCGCTGATGACACGGAGCAGGTAACG	
	DocA-D3-EY-R2	CCAGACTCGAGTTATTTGTACAGTTCGTCCAT	*Xho*I
	DocA-D4-EY-F	ATATACATATGGTACTATTAGGAGATG	*Nde*I
eYFP-DocA-D4	DocA-D4-EY-R1	CCGCTAATAACACGCAACAGGTAAC	
	DocA-D4-EY-R2	ACTCGAGTTATTTGTACAGTTCG	*Xho*I
	EY-Scat-F1	GGAGATATACCATGGGCATGTCAAAAGGAGAA	*Nco*I
	EY-Scat-F2	GCGGTGGTTCTATGAGCTATTATCATCACC	
eYFP-Spy-	EY-Scat-R	CCGAGCTCGAATTCGATGTGTGCGTCGC	*Eco*RI
Catcher/SpyTag	Stag-EY-F	GATATACATATGGCTCACATAGTAATGGTTGA	*Nde*I
	Stag-EY-R1	CACCGCTTTTCGTCGGTTTAT	
	Stag-EY-R2	GACTCGAGTTACTTATACAGCTCATCCATACC	*Xho*I