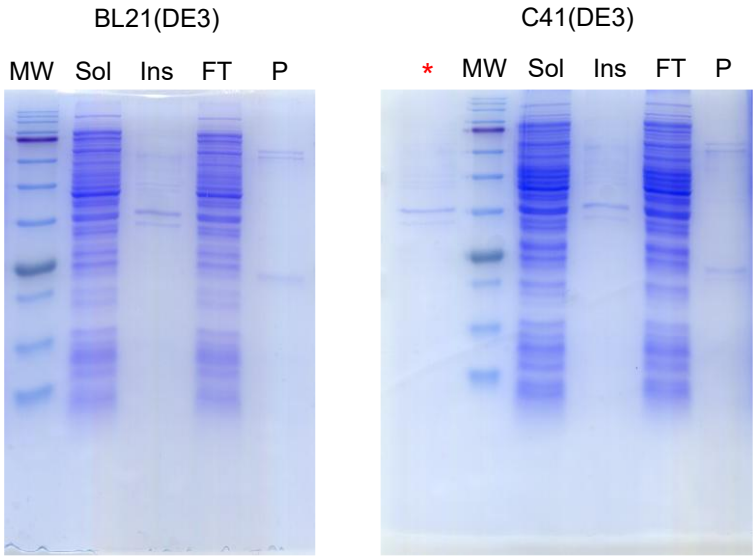
Table S1. Nucleotide and amino acid sequences of the recombinant proteins used in this study.

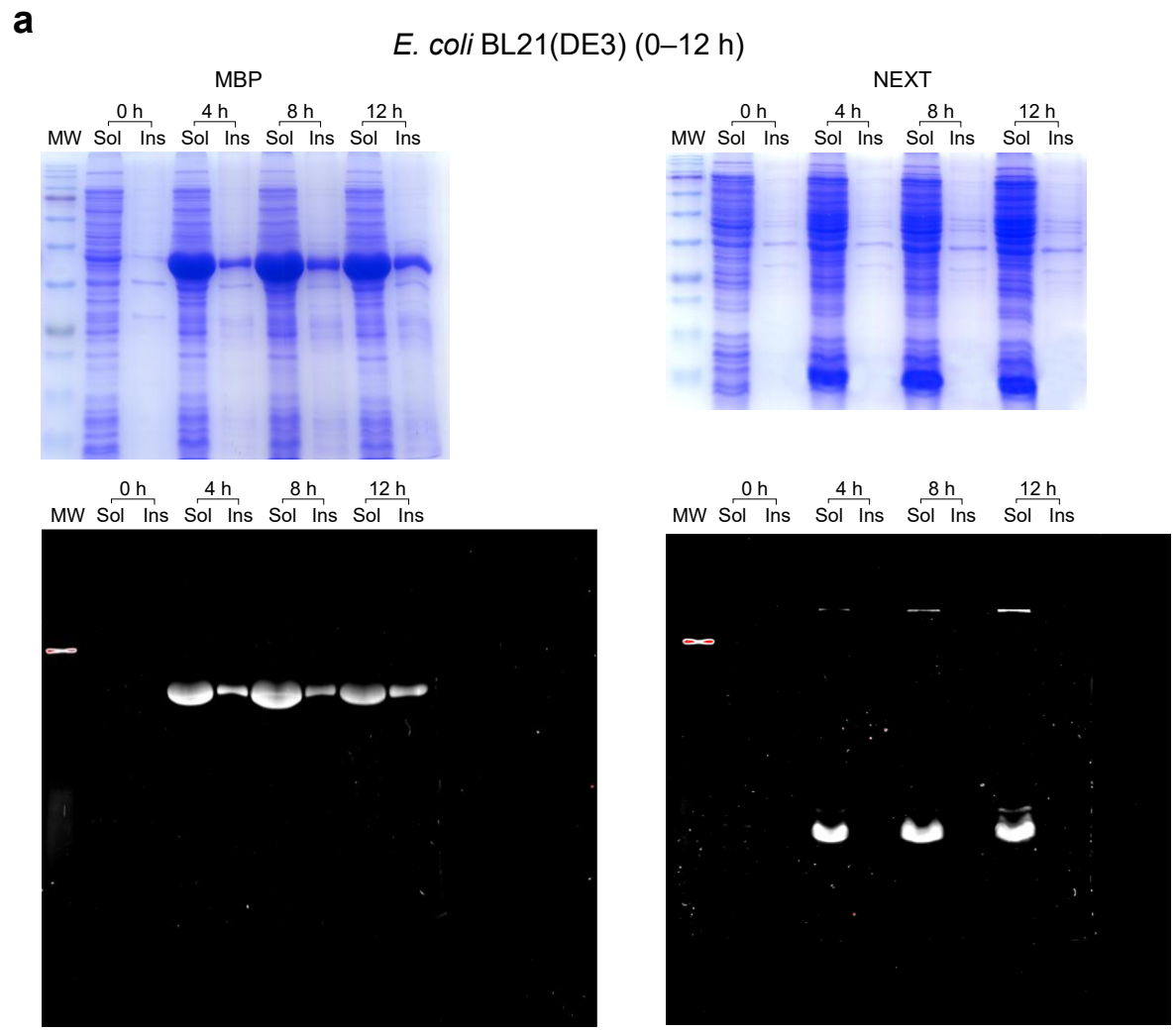
Protein	Type	Sequence[a]
R5-His_6	DNA	CATATGTCCAGCAAAAAATCTGGCTCCTATTCAGGCTCGAAAGGTTCTAAA CGTCGCATTCTGCTCGAG*CACCACCACCACCACCAC*TGA
	Protein	MSSKKSGSYSGSKGSKRRILLE*HHHHHH*
MBP-R5-His_6	DNA	CATATGAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAA GGCTATAACGGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGG AATTAAAGTCACCGTTGAGCATCCGGATAAACTGGAAGAGAAATTCCCAC AGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGGCACACGACC GCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACA AAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACA ACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGATTTA TAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGG CGCTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAAC CTGCAAGAACCGTACTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTAT GCGTTCAAGTATGAAAACGGCAAGTACGACATTAAAGACGTGGGCGTGGA TAACGCTGGCGCGAAAGCGGGTCTGACCTTCCTGGTTGACCTGATTAAAA ACAAACACATGAATGCAGACACCGATTACTCCATCGCAGAAGCTGCCTTTA ATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCCAAC ATCGACACCAGCAAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAG GGTCAACCATCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCC GCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCCTCGAAAACTATCTGCT GACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCG TAGCGCTGAAGTCTTACGAGGAAGAGTTGGTGAAAGATCCGCGTATTGCC GCCACTATGGAAAACGCCCAGAAAGGTGAAATCATGCCGAACATCCCGCA GATGTCCGCTTTCTGGTATGCCGTGCGTACTGCGGTGATCAACGCCGCCAG CGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTGGCGGA GGTGGCAGCGGCGGTGGAGGCTCC**GAAAACCTGTACTTCCAATCC**AGCA AAAAATCTGGCTCCTATTCAGGCTCGAAAGGTTCTAAACGTCGCATTCTGC TCGAG*CACCACCACCACCACCAC*TGA
	Protein	MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVA ATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLI AYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFT WPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADT DYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVG VLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELVK DPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQ TGGGGSGGGGS**ENLYFQS**SKKSGSYSGSKGSKRRILLE*HHHHHH*
NEXT-R5-His_6	DNA	CATATGGCTGTTCAACATAGCAATGCCCCATTGATTGACTTGGGCGCGGAA ATGAAAAAACAGCACAAGGAGGCAGCTCCCGAAGGCGCTGCGCCGGCTC AAGGTAAGGCACCTGCCGCGGAAGCCAAAAAAGAAGAAGCACCTAAACC AAAACCCGTTGTGGGCGGAGGTGGCAGCGGCGGTGGAGGCTCC**GAAAAC** **CTGTACTTCCAATCC**AGCAAAAAATCTGGCTCCTATTCAGGCTCGAAAGG TTCTAAACGTCGCATTCTGCTCGAG*CACCACCACCACCACCAC*TGA
	Protein	MAVQHSNAPLIDLGAEMKKQHKEAAPEGAAPAQGKAPAAEAKKEEAPKPKP VVGGGGSGGGGS**ENLYFQS**SKKSGSYSGSKGSKRRILLE*HHHHHH*
MBP-TEVp	DNA	CATATGAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAA GGCTATAACGGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGG AATTAAAGTCACCGTTGAGCATCCGGATAAACTGGAAGAGAAATTCCCAC AGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGGCACACGACC GCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACA

	AAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACA ACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGATTTA TAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGG CGCTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAAC CTGCAAGAACCGTACTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTAT GCGTTCAAGTATGAAAACGGCAAGTACGACATTAAAGACGTGGGCGTGGA TAACGCTGGCGCGAAAGCGGGTCTGACCTTCCTGGTTGACCTGATTAAAA ACAAACACATGAATGCAGACACCGATTACTCCATCGCAGAAGCTGCCTTTA ATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCCAAC ATCGACACCAGCAAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAG GGTCAACCATCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCC GCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCCTCGAAAACTATCTGCT GACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCG TAGCGCTGAAGTCTTACGAGGAAGAGTTGGTGAAAGATCCGCGTATTGCC GCCACTATGGAAAACGCCCAGAAAGGTGAAATCATGCCGAACATCCCGCA GATGTCCGCTTTCTGGTATGCCGTGCGTACTGCGGTGATCAACGCCGCCAG CGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTGGCGGA GGTGGCAGCGGCGGTGGAGGCTCC**GAAAACCTGTACTTCCAATCC**ATGG GAGAAAGCTTGTTTAAGGGACCACGTGATTACAACCCGATATCGAGCACCA TTTGTCATTTGACGAATGAATCTGATGGGCACACAACATCGTTGTATGGTAT TGGATTTGGTCCCTTCATCATTACAAACAAGCACTTGTTTAGAAGAAATAAT GGAACACTGTTGGTCCAATCACTACATGGTGTATTCAAGGTCAAGAACACC ACGACTTTGCAACAACACCTCATTGATGGGAGGGACATGATAATTATTCGC ATGCCTAAGGATTTCCCACCATTTCCTCAAAAGCTGAAATTTAGAGAGCCA CAAAGGGAAGAGCGCATCTGTCTTGTGACAACCAACTTCCAAACTAAGAG CATGTCTAGCATGGTGTCAGACACTAGTTGCACATTCCCTTCATCTGATGGC ATATTCTGGAAGCATTGGATTCAAACCAAGGATGGGCAGTGTGGCAGTCCA TTAGTATCAACTAGAGATGGGTTCATTGTTGGTATACACTCAGCATCGAATT TCACCAACACAAACAATTATTTCACAAGCGTGCCGAAAAACTTCATGGAAT TGTTGACAAATCAGGAGGCGCAGCAGTGGGTTAGTGGTTGGCGATTAAAT GCTGACTCAGTATTGTGGGGGGGCCATAAAGTTTTCATGGTGAAACCTGAA GAGCCTTTTCAGCCAGTTAAGGAAGCGACTCAACTCATGAATCGTCGTCGC CGTCGCTAACTCGAG
Protein	MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVA ATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLI AYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFT WPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADT DYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVG VLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELVK DPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQ T**GGGGSGGGGSENLYFQS**MGESLFKGPRDYNPISSTICHLTNESDGHTTSLYG IGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGRDMIIIRMP KDFPPFPQKLKFREPQREERICLVTTNFQTKSMSSMVSDTSCTFPSSDGIFWKH WIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEA QQWVSGWRLNADSVLWGGHKVFMVKPEEPFQPVKEATQLMNRRRRR

[a]The restriction site sequences are underlined, and the His_6-tag sequences are italicized. The flexible $(G_4S)_2$ linker sequences are highlighted in gray shading, and the TEV protease cleavage sites are shown in bold.

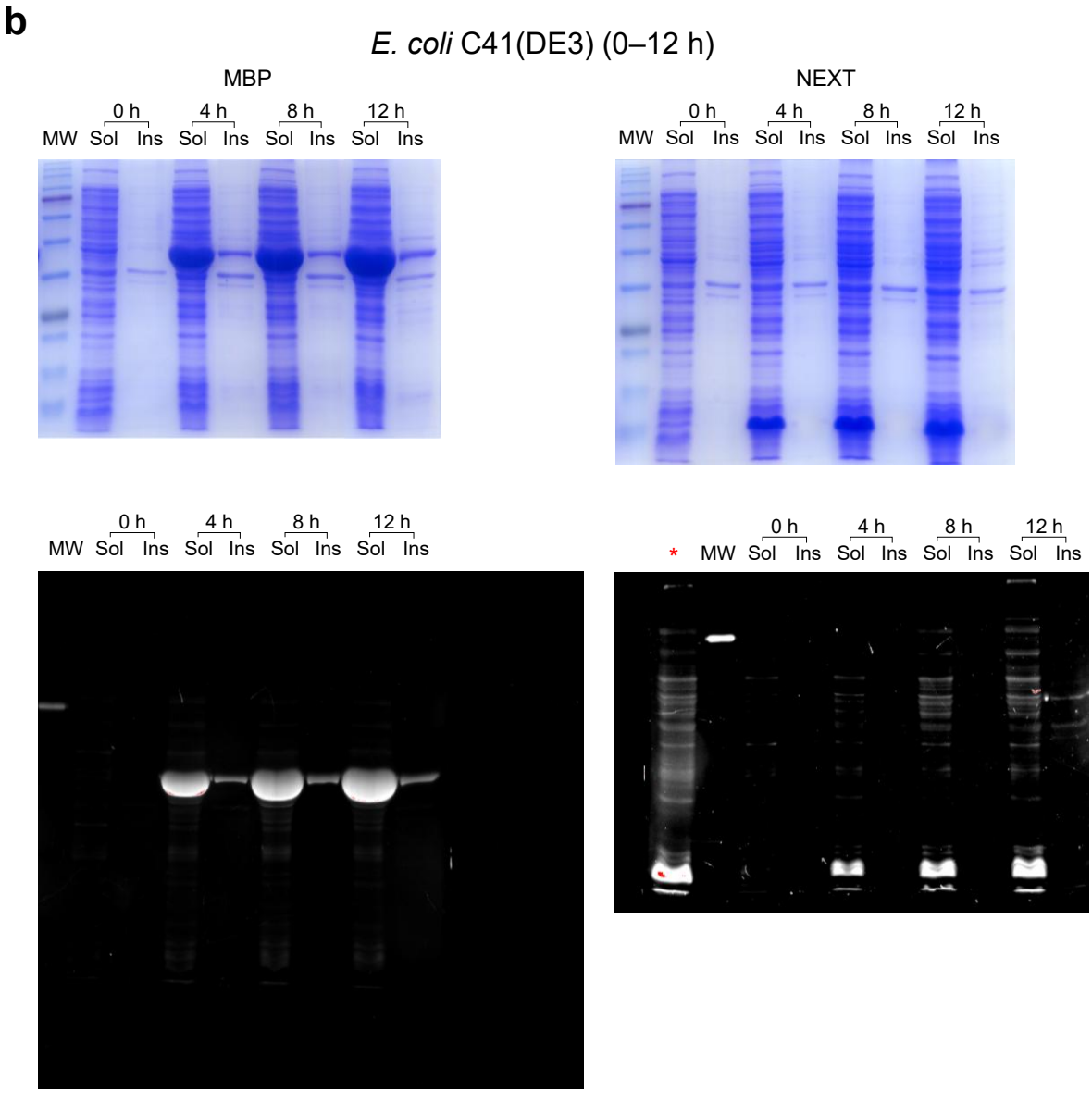


Supplementary Figure S1. Original SDS-PAGE gel images corresponding to Fig. 2. Original Coomassie-stained gel images for direct expression of R5-His_6 in *E. coli* BL21(DE3) (left) and C41(DE3) (right) are shown, with lane identities indicated above each gel. The red asterisk (*) indicates a sample lane not used in the present study. Some gel edges were not captured during the original scanning, and the images are presented as originally acquired. MW, molecular mass marker; Sol, soluble fraction; Ins, insoluble fraction; FT, flow-through; P, purified fraction (Ni-NTA eluate).

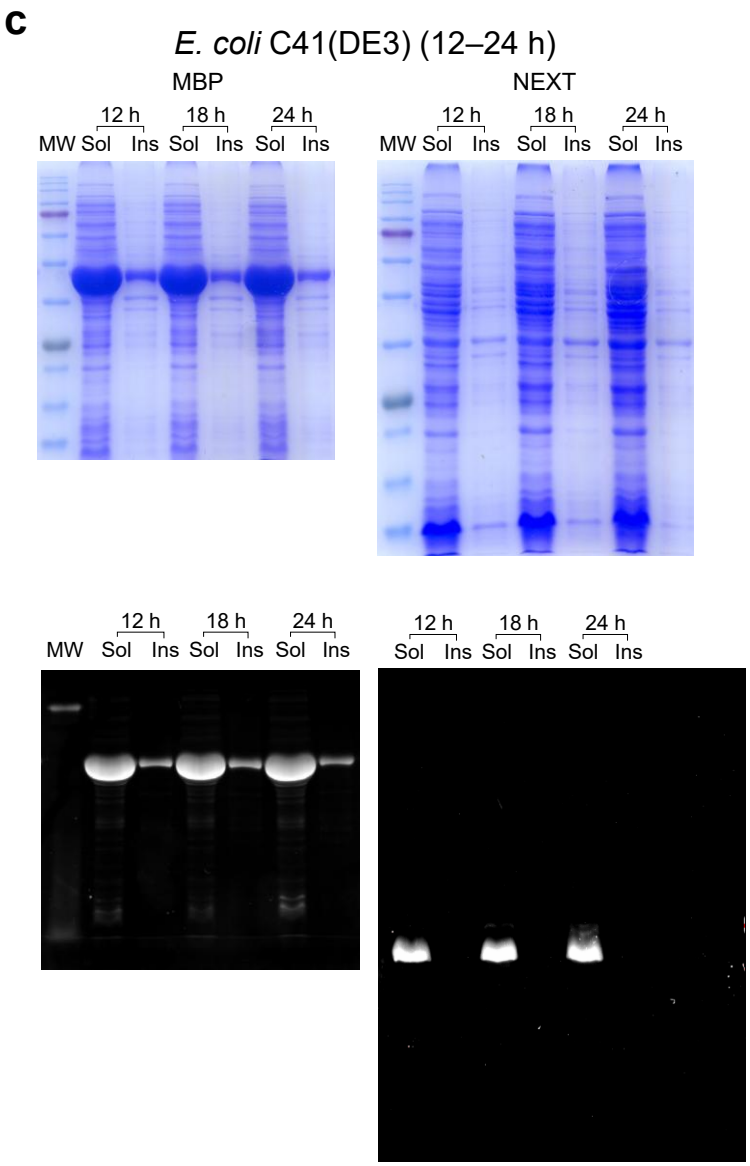


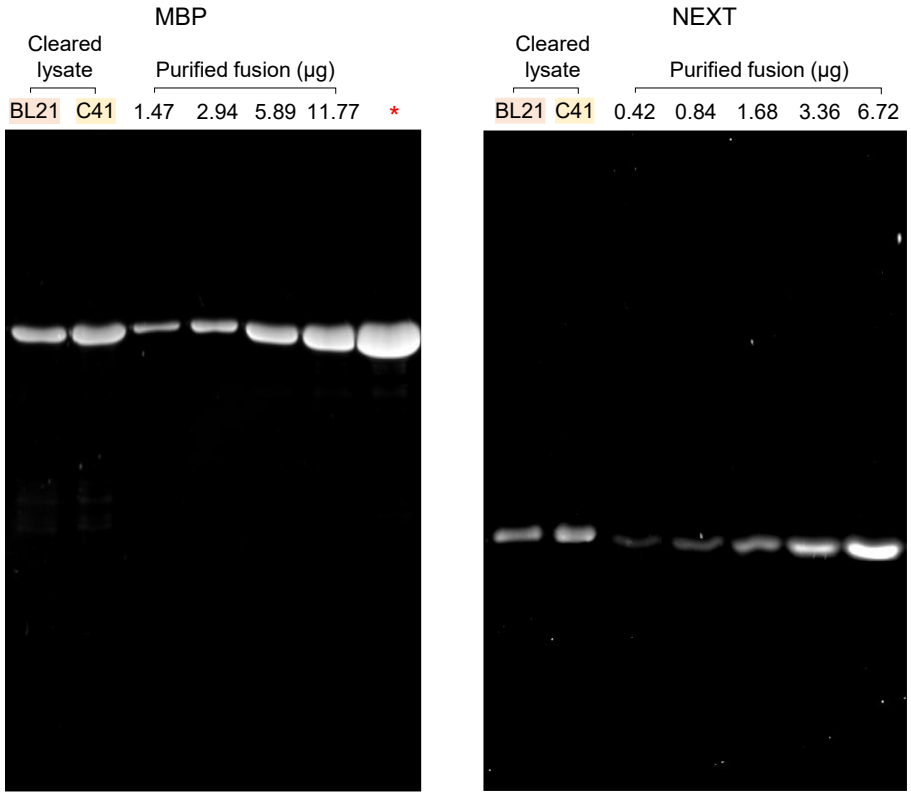
Supplementary Figure S2. Original SDS-PAGE gel images corresponding to Fig. 3. Original Coomassie-stained (upper) and in-gel His-tag-stained (lower) images are shown for MBP-R5-His_6 (left) and NEXT-R5-His_6 (right) expressed in *E. coli* BL21(DE3) at 0–12 h **(a)** and C41(DE3) at 0–12 h **(b)** or 12–24 h **(c)** after IPTG induction. The two C41(DE3) time courses were obtained from separate cultures and analyzed on separate gels. Lane identities and sampling times are indicated above each gel. The red asterisk (*) indicates a sample lane not used in the present study. MW, molecular mass marker; Sol, soluble fraction; Ins, insoluble fraction.

Supplementary Figure S2 (continued)

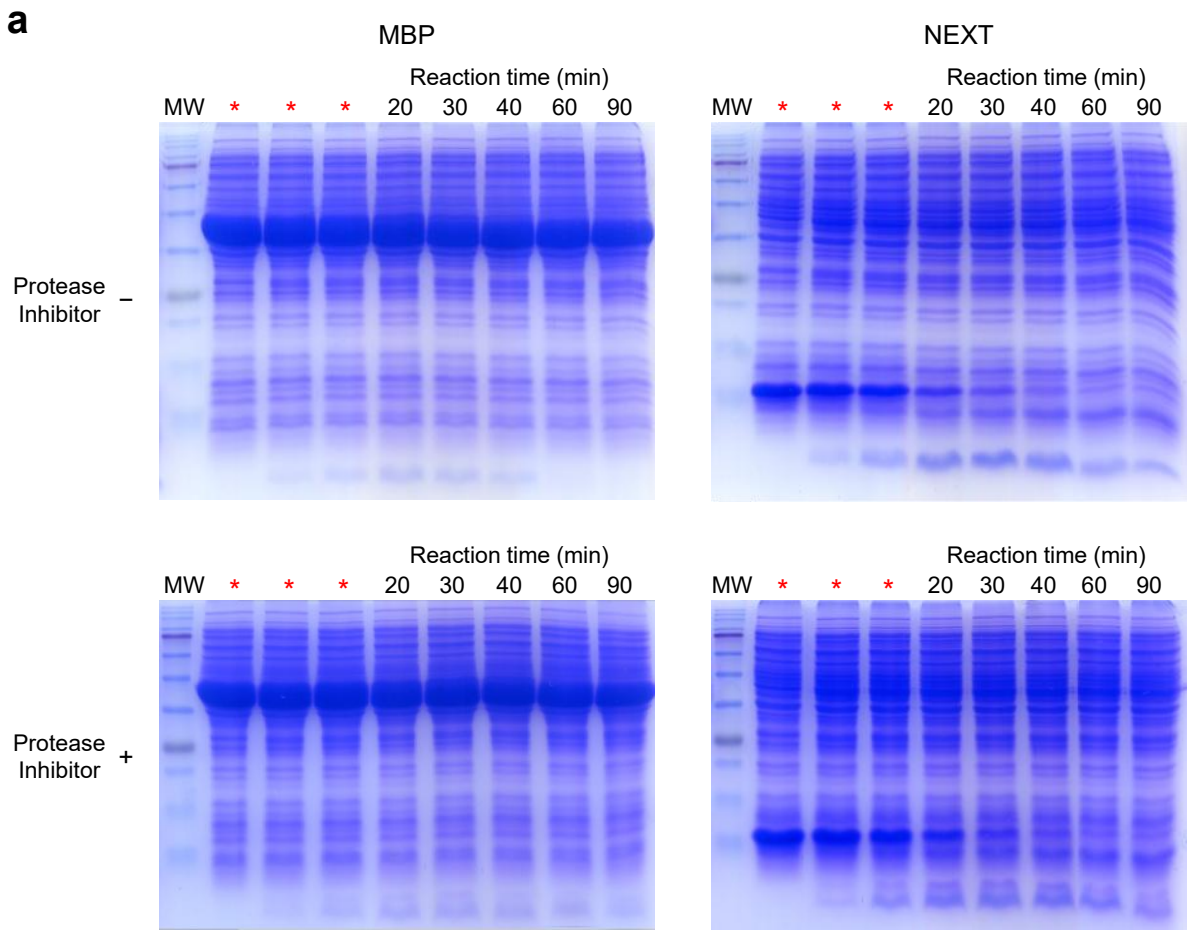


Supplementary Figure S2 (continued)



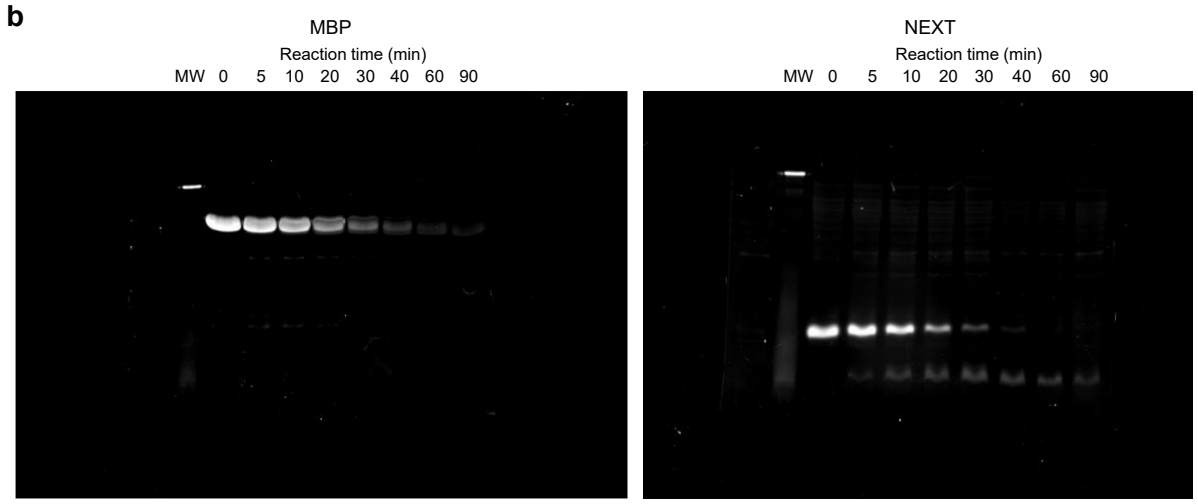


Supplementary Figure S3. Original SDS-PAGE gel images corresponding to Fig. 4. Original in-gel His-tag-stained images corresponding to Fig. 4a are shown for MBP-R5-His_6 (left) and NEXT-R5-His_6 (right). Cleared lysates from *E. coli* BL21(DE3) (BL21) and C41(DE3) (C41) were analyzed alongside known amounts of the corresponding purified fusion proteins. Lane identities and protein loading amounts are indicated above each gel. The red asterisk (*) indicates a sample lane not used in the present study.



Supplementary Figure S4. Original SDS-PAGE gel images corresponding to Fig. 5. (a) Coomassie-stained images of cleavage reactions for MBP-R5-His_6 (left) and NEXT-R5-His_6 (right) without (−, upper) or with (+, lower) a protease inhibitor cocktail. **(b)** In-gel His-tag-stained images showing the time-dependent disappearance of uncleaved MBP-R5-His_6 (left) and NEXT-R5-His_6 (right). **(c)** Coomassie-stained images of Ni-NTA purification following 60 or 90 min of cleavage of the MBP fusion (left and center, respectively) and 60 min of cleavage of the NEXT fusion (right). A protease inhibitor cocktail was included in **(b)** and **(c)**. Lane identities and reaction times (min) are indicated above each gel. Red asterisks (*) indicate lanes not included in the corresponding panels of Fig. 5. MW, molecular mass marker; Sol, soluble fraction; Ins, insoluble fraction; Rxn, reaction mixture after cleavage; FT, flow-through; P, purified fraction (Ni-NTA eluate).

Supplementary Figure S4 (continued)



Supplementary Figure S4 (continued)

