Supplementary Information for

De novo engineering of programmable and multi-functional biomolecular condensates for controlled biosynthesis

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Supplementary Table S1 Primers used in this study

Primer	Sequence
HPHT-F1	AGGTTTATTTTATGAGCAAAGGAGAAGAACTT
HPHT-R1	TTCATTCTAGAGCGCAACGCAATTAATGTGAGTTA
PGRAC-R	AGTGAAAAGTTCTTCTCCTTTGCTCATAAAATAAACCTCCTTTCTTTTACTTA
PGRAC-F	ACATTAATTGCGTTGCGCTCTAGAATGAAAGGGGATTTTATGCT
HPHT-F2	GGCAGCCCCGACGGCGGATATTAACTGCAGGTCGACGTCCC
HPHT-R2	CTTGGCCCTTTCGACATAAAATAAACCTCCTTTCTTTTACTTAC
SIDP1-F1	AGAAAGGAGGTTTATTTTATGTCGAAAGGGCCAAGGGG
SIDP1-R1	GACCTGCAGTTAATATCCGCCGTCGGGGCTGCCGTA
PHT-TY-YZ-F	ACGAAACAGCCTCTACAAAT
SIDP1-YZ-R	CGGGTGAACCATATCCTCTCC
HPHT-F3	CGGTGGATCAGGTGGAGGCGGTTCTATGTCGAAAGGGCCAAGGGG
HPHT-R3	GCATACTGTTCCAGCATAAAATAAACCTCCTTTCTTTTACTT
SIDP1-RIA-F	ATGCTGGAACAGTATGCAAATCAGCTGGCAGATCAGATTATCAAAGAAGCA
SIDP1-RIA-R	ACCGCCTCCACCTGATCCACCGCCACCTTCGGTTGCTTCTTTGATAATCT
RIAD-YZ-F	CTGGCAGATCAGATTATCAAAGA
HPHT-F4	ATCAGGCGGAGGCGGTTCCATGAGCAAAGGAGAAGAACTTTT
HPHT-R4	CCCTTTCGACATAAAATAAACCTCCTTTCTTTTACTTAC
SIDP1-GFP-F	TAAAAGAAAGGAGGTTTATTTTATGTCGAAAGGGCCAAGGGG
SIDP1-GFP-R	CGCCTGATCCACCGCCACCATAGCCGCCGTCGGGGCTGCCGTA
HPHT-F7	TGCTGATGCCATAGAACCGCCTCCACCTGAT
HPHT-R7	ATCGCGGCGGAGGCGGCTAACTGCAGGTCGACGTCCCCG
FUSN-F	ATCAGGTGGAGGCGGTTCTATGGCATCAGCATCAAATGA
FUSN-R	CGACCTGCAGTTAGCCGCCTCCGCCGCGATCT
FUSN-YZ-R	TGTGAGTATGAGCCGCTTT
HPHT-F8	ACGGAGGGGATGGTTAACTGCAGGTCGACGTCCCC
HPHT-R8	TATTTGATTGATTTGATTCCATGCTTCCACCGCCACCTTCG
RGG-F1	TGGCGGTGGAAGCATGGAATCAAATCAATCAAATAATGG
RGG-R1	GATTGGTTAGATTCCATGCCATCGCCGCCATTATC
RGG-F2	ATGGCGGCGATGGCATGGAATCTAACCAATCAAACAATGG
RGG-R2	TCGATTGATTGCTCTCCATCAGTTTACCGTCGCCGCCATTGTC
RGG-F3	GCGACGGTAAACTGATGGAGAGCAATCAATCGAATAAT
RGG-YZ-R	CCGCGTGAAAAGTTTGATGAT
HPHT-F9	GTACGGCGGCTATTAACTGCAGGTCGACGTCC

HPHT-R9	CCGCCTCGTGGCCCTTTCGACATAGAACCGCCTCCACCTG
SIDP2-F	GGTGGAGGCGGTTCTATGTCGAAAGGGCCACGAGGCGGA
SIDP2-R	CCTGCAGTTAATAGCCGCCGTACGGGGAG
SIDP2-YZ-R	TCGCCGCCCCGCCCATAA
SIDP4-F	ATGTCGAAAGGGCCAGGTTATCCTTCAGAT
SIDP4-R	GCCACGGCCATCAGATGGGTAA
SIDP4-YZ-R	GGATATCCCCCTCTACCATCG
HPHT-F11	GGAGGTGAAATGTACACATGCTGGAACAGTATGCAA
HPHT-R11	ACATACCACCTATCAACTAGTATAAAAAACGCCCG
Pveg-F	TTATTAACGTTGATATAATTTAAATTTTATTTGACAAAA
Pveg-R	ACCCCCTACCCCAAAAATAAACCTCCTTTCTTTTACTTACCCT
Pveg-YZ-R	TGACAAAAATGGGCTCGTG
HPHT-F12	CAATAAATGTAGTGATAGCGGTACCAAAGGAGGTGAAATGTACACAT
HPHT-R12	AGAAACATTTGGCTAAACTAGTATAAAAAACGCCCGG
VIOE-F	ATGGAAAACCGGGAACCGC
VIOE-R	CTAACGCTTGGCGGCGAAGA
VIOE-YZ-R	ACCATCCTCAGCGGCGTA
VIOC-F	ATGAAAAGAGCAATCATAGTCGGA
VIOC-R	TCAGTTGACCCTCCCTATCT
VIOC-YZ-R	ACCTTGCGCAGCTCGGTG
VIOA-F	ATGAAGCATTCTTCCGATATCT
VIOA-R	TCACGCGGCGATGCGCTG
VIOA-YZ-R	TGTCGACGATCAGCACCT
VIOB-F	ATGAGCATTCTGGATTTTCCACG
VIOB-R	TCAGGCCTCTCTCGAAAGC
VIOB-YZ-R	ATCAGCTCGTACACGCCC
PspovG-YZ-F	CTTTTTTATTTACCTTATGCCCGAAA
Gmd-F	ATGTCAAAAGTCGCTCTCA
Gmd-R	TGAACCAGAACCTGAACCTGACTCCAGCGCGATCGCCA
Gmd-YZ-R	AAATCTTCCGGCTGTTCC
WcaG-F	ATGAGTAAACAACGAGTTTTTATTGC
WcaG-R	CCCCCGAAAGCGGTCTTGAT
WcaG-YZ-R	GCTGCCCCATACCACCAC
ManB-F	ATGCTAACTTGCTTTAAAGCTTAT
ManB-R	CTTGTTCAGTAACTCAAGGATAAGT

ManB-YZ-R	TTGCGCATACGTTCTTTAATAAAGG
ManC-F	ATGAGCTCACCTCTTATTCCG
ManC-R	ATCTTCAAATCGAAGGATATCATC
ManC-YZ-R	CCTACATCATTCCAACCGATA
FutC-F	ATGGCATTTAAGGTTGTTCAGATTT
FutC-R	TGCATTATATTTTTGTGATTTAACTTCAAA
FutC-YZ-R	TTTGATAATCAATGCCCAGTTGG
Hp43NMK-F1	GGAAAAAGAAGAAGCCAAATAATAATGATGAAAGCTTGGCGTAAT
Hp43NMK-R1	GACTGGAAAGCGGGCAGTGAGCCATTACAGCTTTGGCAAAAAA
Pgrac-F3	CTGTAATGGCTCACTGCCCGCTTTCCAGT
Pgrac-R3	GATCAGTTCAGAAACCATAAAATAAACCTCCTTTCTTTTACTTACC
mKate-F	GAAAGGAGGTTTATTTTATGGTTTCTGAACTGATCAAAGA
mKate-R	TACCGCTACCTGAACCACGGTGACCCAGTTTAGA
RIDD-R	AGCTTTCATCATTATTATTTGGCTTCTTCTTTTTCCAG
RIDD-F	ACAGTTCACATTCACGCAGGCTACCGCTACCTGAACCAC
RIDD-YZ-R	GAAATGCCATCGGACGTT
Hp43NMK-F2	GATAGCGGTACCCTCGAATGGTTTCTGAACTGATCAAAGA
Hp43NMK-R2	TGTTACCCCTATAAGTTAGGCCATTACAGCTTTGGCAAAAAAA
xylA-F	CCAAAGCTGTAATGGCCTAACTTATAGGGGTAACACTTAAAAAA
xylA-R	GATCAGTTCAGAAACCATTCGAGGGTACCGCTATCAC
XylA-YZ-R	AACCACTCCTTTGTTTATCCAC
HPB-Trs-F1	GATCCGTAAACGTCTGTAAATTATGGAAAGGCGTGCC
HPB-Trs-R1	GTGTACATTCCTCTCTTACCTATAATGGTACCGCTATCACTTT
Trs-F1	AGAGAGGAATGTACACATGGATGAATTTGAAATGATTAAACGC
Trs-R1	GCCTTTCCATAATTTACAGACGTTTACGGATCGG
Trs-CX-R	TGTTATAGTCGCCGATTTTGC
HP43NMK-R1	TGGAAAGCGGGCAGTGAGCCATTACAGCTTTGGCAA
HP43NMK-F1	CACACCACCACCACCACCACTAATGATGAAAGCTTGGCGT
PGRAC-F1	AGCTGTAATGGCTCACTGCCCGCTTTCCAG
PGRAC-R1	TAAATGCCATAAAATAAACCTCCTTTCTTTTACTTACCC
CX-F2-TY	CCCAGTTGGCAGCCAATG
HPB-Trs-F2	CGAGAGATAAACTGTAAATTATGGAAAGGCGTGCCTGACAAGG
HPB-Trs-R2	ATGGACCCTGAACCAGAGCCTGATCCCAGACGTTTACGG
F2-F1	CAGGCTCTGGTTCAGGGTCCATGGCACACGTTCGGGGGT
F2-R1	CTTTCCATAATTTACAGTTTATCTCTCGGTGTCCGT

F2-YZ-R	TTCCAGAAAAGTATTTGCCCTT
HPB-Trs-F3	CGAGAGATAAACTGTAAATTATGGAAAGGCGTGCCTGACAAGG
HPB-Trs-R3	GCGTTAATGGCATGTGTACATTCCTCTCTTACCTATA
DIV-TY-F1	TAGGTAAGAGAGGAATGTACACATGCCATTAACGCCAAATGA
DIV-TY-R1	AACCAGAGCCTGATCCAGACCCAAAGTGTCCGATTCTTTCATCAA
Trs-F2	GGGTCTGGATCAGGCTCTGGTTCAATGGATGAATTTGAAATGATTAAACG
DIV-YZ-F	AGACGTTTACAAAAAGTTTTCGC
DIVMUT-F1	AAAAAGTTTTTGCGGATATGATGAAGATGAAGTAAATGAA
DIVMUT-R1	ATCATATCCGCAAAAACTTTTTGTAAACGTCTTG
HPB-Trs-F4	TACGGAGATGGCGGCTATATGGCACACGTTCGGGG
HPB-Trs-R4	TTGGCCCTTTCGACATGGACCCTGAACCAGAGCC
SIDP3-F	TCAGGGTCCATGTCGAAAGGGCCAAGGGGAAGTC
SIDP3-R	CGTGTGCCATATAGCCGCCATCTCCGTATGGA
SIDP3-YZ-R	CCTGCCATCCCCGTAAGG
HPB-Trs-F5	CCAGATGGTAGGGGCTACGGGATGGCACACGTTCGGGGGT
HPB-Trs-R5	CCATAGCCCCTTGGCCCTTTCGACATGGACCCTGAACCAGAG
SIDP1-F11	CAGGGTCCATGTCGAAAGGGCCAAGGGGCTATG
SIDP1-R11	GAACGTGTGCCATCCCGTAGCCCCTACCATC
SIDP1-YZ-R	CCGTCCGGAGAGCCGTAA
HPB-Trs-F6	TAGGGGCTACGGGTAAATTATGGAAAGGCGTGCC
SIDP1-R21	GGCACGCCTTTCCATAATTTACCCGTAGCCCCTACCATC
HPADK-R1	TGAGCTCTACAAATAACGCGCGAAAAACGCGAGC
HPADK-F1	TGGGTCACCGTTAACTGCAGGTCGACGTCCCCGGG
mKate-F1	AAGGAGGTTTATTTTATGGTTTAGGAACTGATCAAAGA
mKate-R1	TCGACCTGCAGTTAACGGTGACCCAGTTTAGACG
PVEG-F1	ACCACCTATCAATTTTATGCTTCAGAACGCTCGGTTGC
PVEG-R1	TCAGTTCCTAAACCATAAAATAAACCTCCTTTCTT
P43-R1	TCTGAAGCATAAAATTGATAGGTGGTATGTTTTCGC
P43-F1	CTTCTCCCTAGCTCATGTGTACATTTCACCTCCTTTGG
GFP-F1	GCGTTTTTCGCGCGTTATTTGTAGAGCTCATCCATGCCA
GFP-R1	TGAAATGTACACATGAGCTAGGGAGAAGAAC
GFP-YZ-F	AAGATATAGTGCGTTCCTGTAC
mKate-YZ-F	TTTTCAAACAGTCTTTCCCGGA
HPADK-R2	CGGGTTCATTAGATCCGCGCGAAAAACGCGAGCG
HPADK-F2	AGGTACCTTAGGATCTCGACGAGCTCCGTCTTTAT

RBTA-F1	TTTTCGCGCGGATCTAATGAACCCGGGAATACTG
RBTA-R1	AGCTCGTCGAGATCCTAAGGTACCTAATTGCCTA
HPADK-R3	AAGTACTTACCCCAAAAAAAACTGCAGGTCGACAAAAACG
HPADK-F3	CAGCTTTGTTCCCCGACGAGCTCCGTCTTTATTT
RBTA-F2	CGACCTGCAGTTTTTTTTGGGGTAAGTACTTCAGCTTTGTT
RBTA-R2	CTCGTCGGGGAACAAAGCTGAAGTACTT
RBTA-YZ-F	TTGGGGTAAGTACTTCAGCT
HPADK-R4	GCATGGACGAACTCTATAAATAATAAAGACGGAGCTCGTCGGGGAAC
HPADK-F4	TCACCTTTCTAAACCATGTGTACATTTCACCTCCTTTG
CFP-F1	CTCCGTCTTTATTATTTATAGAGTTCGTCCATGCC
CFP-R1	GTACACATGGTTTAGAAAGGTGAAGAATT
CFP-YZ-F	TTCAACTCAATCCGGTTTAC
HPHT-D-F1	CGGACACTTTATGTCGAAAGGGCCAAGGGGCTATGGCTCTCCGGA
HPHT-D-R1	CGTTAATGGCATAAAATAAACCTCCTTTCTTTTACTT
DIV-TY-F2	AAGGAGGTTTATTTTATGCCATTAACGCCAAATGAT
DIV-TY-R2	GGCCCTTTCGACATAAAGTGTCCGATTCTTTCATC
GNA1-F	ATGAGCCATATCTTCGACGCA
GNA1-F	TTAAAAGCGCTGGGTCATA
GNA1-YZ-R	TAAAGATTTGCCAAGTGACACCA
AGEM-TY-F	TTATTTCTCCGAGATGTCAGAAAGG
AGEM-TY-R	ATGGACTTTAAGAAGTTAGCGGA
AGEM-YZ-R	CGAAGGTACGCTTAGCGAT
AGE-TY-F	ATGGATTTCAAGAAGCTGGC
AGE-TY-R	TTATTTTTCGCTGATATCACTAAGAACC
AGE-YZ-R	CTTTTCAATTAAGTCCGGACGG

Supplementary Table S2 Plasmids used in this study

Plasmid	Characteristics	Ref.
pHTa0	ColE1 Amp^r, Cm^r, *E. coli-B. subtilis* shuttle vector, *Δ*P$_{grac}$*::gfp*	Lab stock
pP43-NmCherry	ColE1 Amp^r, RepB Kan^r, *E. coli-B. subtilis* shuttle vector, P$_{43}$-mCherry, an N-terminal coding sequence of abrB was added into mCherry to improve its expression	Lab stock
pADK15-sfGFP	p15A Kan^r, Rep60 Kan^r, *E. coli-B. subtilis* shuttle vector, P$_{veg}$-*gfp*	Lab stock
pHT28a	Kan^r, *E. coli* vector for expression of N-terminally 6xHis-tagged	Lab stock

	proteins with a thrombin site	
pHT-XCR6	pHT01 derivate, xylR-P_{xylA}-FnCas12a-NgAgo	Lab stock
pcrF11	ColE1 Kan^r, Kan^r, *E. coli-B. subtilis* shuttle vector, expressing crRNA by P_{veg}	Lab stock
pBUA-Trs	pHT01 derivate, OMeY-aaRS gene cloned under the control of P_{43} promoter and mutant tRNA under the control of P_{224} promoter	Lab stock
pHT28a-1	pHT28a derivate, P_{T7}-*SIDP1-gfp*	This work
pHT28a-2	pHT28a derivate, P_{T7}-*SIDP2-gfp*	This work
pHT28a-3	pHT28a derivate, P_{T7}-*SIDP3-gfp*	This work
pHT28a-4	pHT28a derivate, P_{T7}-*SIDP4-gfp*	This work
pHT28a-5	pHT28a derivate, P_{T7}-*SIDP5-gfp*	This work
pHT28a-6	pHT28a derivate, P_{T7}-*SIDP6-gfp*	This work
pHT28a-7	pHT28a derivate, P_{T7}-*SIDP7-gfp*	This work
pHT28a-8	pHT28a derivate, P_{T7}-*SIDP8-gfp*	This work
pHT28a-9	pHT28a derivate, P_{T7}-*SIDP9-gfp*	This work
pHT28a-10	pHT28a derivate, P_{T7}-*SIDP10-gfp*	This work
pHT28a-11	pHT28a derivate, P_{T7}-*SIDP11-gfp*	This work
pHT28a-12	pHT28a derivate, P_{T7}-*SIDP12-gfp*	This work
pHT28a-13	pHT28a derivate, P_{T7}-*SIDP13-gfp*	This work
pHT28a-14	pHT28a derivate, P_{T7}-*SIDP1*	This work
pHT-P_{grac}-GFP	pHTa0 derivate, $P_{grac100}$-*gfp*	This work
pHT-P_{grac}-SIDP1-GFP	pHT-P_{grac}-GFP derivate, $P_{grac100}$-*SIDP1-gfp*	This work
pHT-P_{grac}-RIAD-SIDP1-GFP	pHT-P_{grac}-GFP derivate, $P_{grac100}$-RIAD-*SIDP1-gfp*	This work
pHT-SIDP1	pHT-P_{grac}-GFP derivate, $P_{grac100}$-RIAD-*SIDP1*	This work
pHT-SIDP2	pHT-P_{grac}-GFP derivate, $P_{grac100}$-RIAD-*SIDP2*	This work
pHT-FUSN	pHT-P_{grac}-GFP derivate, $P_{grac100}$-RIAD-*fusn*	This work
pHT-3RGG	pHT-P_{grac}-GFP derivate, $P_{grac100}$-RIAD-*3rgg*	This work
pHT-SIDP4	pHT-P_{grac}-GFP derivate, $P_{grac100}$-RIAD-*SIDP4*	This work
p43NMK-P_{grac}-mKate-RIDD	pP43-NmCherry derivate, $P_{grac100}$-mKate-RIDD	This work
p43NMK-P_{xylA}-mKate-RIDD	pP43-NmCherry derivate, P_{xylA}-mKate-RIDD	This work
pBUA-Trs-F2	pBUA-Trs derivate, P_{43}-*TyrRS-F2*-Ter-P_{224}-tRNA	This work

pBUA-div-Trs-F2	pBUA-Trs derivate, P_{43}-*div-TyrRS-F2*-Ter-P_{224}-tRNA	This work
pBUA-divmut-Trs-F2	pBUA-Trs derivate, P_{43}-*divmut-TyrRS-F2*-Ter-P_{224}-tRNA	This work
pBUA-Trs-SIDP3-F2	pBUA-Trs derivate, P_{43}-*TyrRS-SIDP3-F2*-Ter-P_{224}-tRNA	This work
pBUA-Trs-SIDP1-F2	pBUA-Trs derivate, P_{43}-*TyrRS-SIDP1-F2*-Ter-P_{224}-tRNA	This work
pBUA-div-Trs-SIDP1-F2	pBUA-Trs derivate, P_{43}-*div-TyrRS-SIDP1-F2*-Ter-P_{224}-tRNA	This work
pBUA-Trs-SIDP1	pBUA-Trs derivate, P_{43}-*TyrRS-SIDP1*-Ter-P_{224}-tRNA	This work
pBUA-RIAD-Trs-SIDP1-F2	pBUA-Trs derivate, P_{43}-RIAD-*TyrRS-SIDP1-F2*-Ter-P_{224}-tRNA	This work
pBUA-RIAD-Trs-F2	pBUA-Trs derivate, P_{43}-RIAD-*TyrRS-F2*-Ter-P_{224}-tRNA	This work
pADK-1	pADK15-sfGFP derivate, P_{43}-GFP^{3TAG}-6RTBA-Ter-P_{veg}-$mKate^{3TAG}$	This work
pADK-2	pADK15-sfGFP derivate, P_{43}-CFP^{3TAG}-6RTBA-Ter-P_{veg}-$mKate^{3TAG}$	This work
pADK-3	pADK15-sfGFP derivate, P_{43}-GFP^{3TAG}-RTBA-Ter-P_{veg}-$mKate^{3TAG}$	This work
pADK-4	pADK15-sfGFP derivate, P_{43}-rpsC-GFP-Ter-P_{veg}-manA-$mKate^{3TAG}$-6RTBA	This work
pHT-P_{grac}-div-SIDP1-GFP	pHT-P_{grac}-SIDP1-GFP derivate, $P_{grac100}$-*div-SIDP1-gfp*	This work
pHT-P_{grac}-divmut-SIDP1-GFP	pHT-P_{grac}-SIDP1-GFP derivate, $P_{grac100}$-*divmut-SIDP1-gfp*	This work
p43NMK-P_{grac}-AGE	p43NMK-P_{grac}-mKate-RIDD derivate, $P_{grac100}$-*age*-6xHis	This work
p43NMK-P_{grac}-AGE^{OMeY}	p43NMK-P_{grac}-mKate-RIDD derivate, $P_{grac100}$-*age*^{OMeY}-6xHis	This work

Supplementary Table S3 Sequences of genetic parts used in this study

Name	Sequence
SIDP1	MSKGP-$(RGYGSPDG)_{20}$-GY
SIDP2	MSKGP-$(RGGDSPYG)_{20}$-GY
SIDP3	MSKGP-$(RGSPYGDG)_{20}$-GY
SIDP4	MSKGP-$(GYPSDGRG)_{20}$-GY
SIDP5	MSKGP-$(DGRGSPYG)_{20}$-GY
SIDP6	MSKGP-$(RGAGSPDG)_{20}$-GY
SIDP7	MSKGP-$(RGTGSPDG)_{20}$-GY
SIDP8	MSKGP-$(RGYGSPDGY)_{20}$-GY
SIDP9	MSKGP-$(GYGSPDG)_{20}$-GY
SIDP10	MSKGP-$(KGYGSPDG)_{20}$-GY

SIDP11	MSKGP-$(EGYGSPDG)_{20}$-GY
SIDP12	MSKGP-$(AGYGSPDG)_{20}$-GY
SIDP13	MSKGP-$(TGYGSPDG)_{20}$-GY
$SIDP1^{TAG}$	MSKGP-$(RGYGSPDG)_{10}$-TAG-$(RGYGSPDG)_{10}$-GY
RBS1	TACCTCGTTAAAAGGATACAATTAA
RBS2	CATAAAGGGGGAAAGGGGTATTAGA
RBS3	GCGACGAAACGGAGAGAGGTATTTTA
RIAD	ATGCTGGAACAGTATGCAAATCAGCTGGCAGATCAGATTATCAAAGAAGCAACCGAA
RIDD	CTGCGTGAATGTGAACTGTATGTTCAGAAACATAATATTCAGGCCCTGCTGAAAGATAGCATTGTTCAGCTGTGTACCGCACGTCCGGAACGTCCGATGGCATTTCTGCGCGAATATTTTGAACGTCTGGAAAAAGAAGAAGCCAAA
FUSN	ATGGCATCAGCATCAAATGATTATACACAACAAGCAACACAAAGCTATGGCGCATATCCGACACAACCGGGCCAAGGATACTCACAACAATCATCACAACCTTATGGACAACAATCATATAGCGGCTATTCACAATCAACAGATACAAGCGGCTATGGCCAATCAAGCTACTCAAGCTACGGCCAATCACAAAATACGGGCTATGGCACACAATCAACACCGCAAGGATACGGCTCAACGGGCGGCTATGGCTCAAGCCAAAGCAGCCAATCAAGCTATGGCCAACAATCATCATATCCGGGCTATGGACAACAACCGGCACCGTCATCAACAAGCGGCAGCTATGGCTCATCATCACAAAGCAGCTCATATGGCCAACCGCAAAGCGGCTCATACTCACAGCAGCCTTCATATGGCGGCCAACAGCAAAGCTACGGACAACAGCAGAGCTATAACCCGCCGCAAGGCTACGGACAACAAAATCAGTATAATTCAAGCAGCGGCGGAGGCGGAGGCGGCGGAGGAGGCGGAAATTATGGCCAAGATCAATCATCAATGTCAAGCGGCGGAGGCAGCGGCGGCGGCTATGGCAATCAAGATCAAAGCGGAGGCGGAGGAAGCGGAGGCTACGGACAACAAGATCGCGGCGGAGGCGGC*
RGG	ATGGAATCAAATCAATCAAATAATGGCGGAAGCGGCAACGCGGCACTGAATCGCGGCGGCAGATACGTTCCGCCGCATCTGAGAGGCGGCGACGGCGGAGCGGCAGCGGCAGCATCAGCGGGCGGCGATGATCGCAGAGGCGGCGCGGGCGGAGGCGGCTATAGAAGAGGCGGAGGCAATAGCGGCGGAGGAGGAGGAGGCGGATATGATAGAGGCTACAATGATAATAGAGATGATAGAGATAACAGAGGCGGCAGCGGCGGCTATGGCAGAGATAGAAATTATGAAGATAGAGGCTATAATGGCGGAGGCGGAGGCGGAGGAAACAGAGGCTACAATAACAACAGAGGAGGAGGCGGAGGCGGCTATAATAGACAAGATAGAGGCGATGGCGGCTCATCAAATTTTTCAAGAGGAGGCTACAACAATCGCGATGAAGGCTCAGATAATAGAGGAAGCGGCAGATCATATAATAATGATAGAAGAGATAATGGCGGCGATGGC*
GNA1	ATGAGCCATATCTTCGACGCATCTGTACTGGCTCCACATATTCCTAGTAACCTTCCT

GATAATTTCAAGGTGAGACCACTGGCAAAGGATGATTTTTCGAAGGGATATGTCGA

CCTGCTGTCACAATTGACGTCAGTTGGAAACCTTGACCAAGAAGCATTTGAGAAAC

GATTTGAGGCGATGAGAACAAGCGTACCGAATTATCACATCGTAGTAATTGAGGAT

TCCAACAGCCAGAAAGTGGTGGCGTCTGCTAGTTTGGTTGTTGAAATGAAATTCAT

TCATGGGGCCGGATCAAGGGGTCGTGTTGAAGATGTTGTCGTCGATACAGAAATG

CGCCGGCAAAAATTAGGTGCCGTGCTTTTAAAAACTTTGGTGTCACTTGGCAAATC

TTTAGGCGTCTACAAAATAAGCCTCGAATGCGTCCCGGAATTACTCCCGTTCTATT

CCCAATTTGGCTTTCAGGATGACTGTAATTTTATGACCCAGCGCTTT*

ATGGATTTCAAGAAGCTGGCTGAACAATATAAGGCTGAATTAATGGAGAATGTCTT

GCCCTTCTGGCTTCAGCACTCGCAGGACAAGGAATTTGGTGGTTATTTCACGTGCT

TAAAGCGCAACGGCGAGGTGTTTGATACAGACAAATTTATCTGGTTGCAAGGTCGT

GAAGTTTGGATGTTCGCCATGTTGTACAACAAGGTGGAGAAACGCCAGGAGTGGT

TGGACTGCGCAATTCAAGGTGGAGAATTTTTAAAAAAGTACGGGCACGATGGGAG

TTACAACTGGTACTTCTCGCTGGACCGTGAGGGCCATCCTCTGGTGGAACCCTAC

AATATTTTTTCTTATACCTTTGCGACAATGGCGTTTGGTCAGTTGTCTTTAGCCACT

GGCAACCAAGAGTATGCTGATATTGCGAAACGTACTTTTGACATCGTCTTAAGTAA

GGTAAATAACCCAAAAGGAAAGTGGAACAAGTTACACCCCGGAACTCGTGACCTG

AAAGGGTTCGCGCTTCCGATGATCTTGTGTAACTTGGCCCTGGAAATCGAGCACTT

AGE GTTGGATGAAGACTTCCTTGTGAAGACTATGGATACTTGCATTCACGAAGTAATGG

ACGTGTTTTACCGCCCCGAGCTTGGAGGACTGATTGTGGAGAACGTGCTTGCTGA

TGGCTCATTAAGTGATAGTTTCGAGGGCCGCCAGGTAACCCCTGGGCACGCAATC

GAGGCAATGTGGTTCATTATGGACCTGGGGAAACGTCTGAACCGTCCGGACTTAA

TTGAAAAGGCGAAGAACATCACGCTGACTATGCTTAACTACGGATGGGATAAACAA

TATGGAGGTATCTACTATTTCATGGACCGCTTAGATAACCCCCCACAACAGCTTGA

GTGGGATCAGAAACTTTGGTGGGTGCACATTGAGACTCTGATTTCATTACTTAAGG

GATATCACTTGACAGGCGATCGTCAGTGCCTGGAGTGGTTTGAGAAAGTGCATGA

TTATGTTTGGTCGCATTTTAAAGACCAAGAATACCCAGAATGGTACGGATACCTGA

ACCGCCGTGGCGAGGTCTTACTTCCGCTTAAGGGCGGGAAATGGAAAGGATGTTT

CCACGTCCCACGTGGATTGTACCAATGCTGGAAGGTTCTTAGTGATATCAGCGAAA

AA*

ATGGACTTTAAGAAGTTAGCGGAACAGTATAAAGCAGAGCTG**CTG**GAAAACGTGCT

TCCATTTTGGTTGCAACACTCGCAGGATAAGGAGTTTGGGGGCTACTTTACGTGCT

AGE^{OMeY} TGAAACGT**GAT**GGAGAAGTCTTTGATACTGACAAGTTTATTTGGTTGCAGGGTCGT

CAAGTCTGGATGTTTGCAATGTTATATAATAAAGTGGAGAAGCGCCAAGAGTGGTT

AGACTGTGCGATCCAAGGAGGAGAGTTTTTGAAGAAATACGGTCATGACGGTTCC

TATAACTGGTATTTCTCATTGGACCGTGAGGGCCATCCCTTAGTGGAGCCATACAA

TATTTTTTCTTACACCTTTGCTACGATGGCATTCGGTCAGTTGAGTCTTGCTACAGG

AAATCAAGAATACGCGGACATCGCTAAGCGTACCTTCGACATCGTATTATCTAAAG

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ATCTGACCGGCGAC**GAG**CAGTGTTTGGAATGGTTTGAAAAAGTACATGACTATGTC

TGGAGTCACTTTAAGGAC**CCG**GAGTATCCGGAATGGTACGGTTATCTGAACCGTC

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GGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTA

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GGCCAACACTTGTCACTACTCTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCG

GATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACA

GGAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTC

GFP AAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAA

GAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGT

ATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCC

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CTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAA*

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CGACGGCGATGTCAATGGACACAAGTTCAGCGTGTCAGGTGAGGGTGAAGGCGA

TGCCACCTATGGCAAATTAACTCTCAAGTTCATATGCACCACCGGCAAACTGCCTG

CFP

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	CGTTAACAACCACCACTTCAAATGCACCTCTGAAGGTGAAGGTAAACCGTACGAAG
	GTACCCAGACCATGCGTATCAAAGCTGTTGAAGGTGGTCCGCTGCCGTTCGCTTTt
	GACATCCTGGCTACCTCTTTCATGTACGGTTCTAAAACCTTCATCAACCACACCCA
	GGGTATCCCGGACTTtTTCAAACAGTCTTTCCCGGAAGGTTTCACCTGGGAACGTG
	TTACCACCTACGAAGACGGTGGTGTTCTGACCGCTACCCAGGACACCTCTCTGcaa
mKate	GACGGTTGCCTGATCTACAACGTTAAAATCCGTGGTGTTAACTTCCCGTCTAACGG
	TCCGGTTATGCAGAAAAAAACCCTGGGTTGGGAAGCTTCTACCGAAACCCTGTAC
	CCGGCTGACGGTGGTCTGGAAGGTCGTGCTGACATGGCTCTGAAACTGGTTGGT
	GGTGGTCACCTGATCTGCAACCTGAAAACCACCTACCGTTCTAAAAAACCGGCTAA
	AAACCTGAAAATGCCGGGTGTTTACTACGTTGACCGTCGTCTGGAACGTATCAAAG
	AAGCTGACAAAGAAACCTACGTTGAACAGCACGAAGTTGCTGTTGCTCGTTACTGC
	GACCTGCCGTCTAAACTGGGTCACCGT*

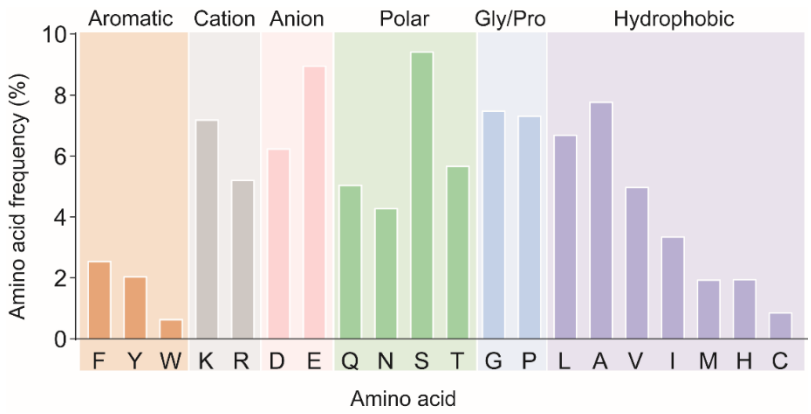
Supplementary Table S4 Strains used in this study

Strain	Characteristics	Ref.
E. coli JM109	*recA1, endA1, thi, gyrA96, supE44, hsdR17Δ (lac-proAB)/F'[traD36,proAB$^+$, lacI^q, lacZΔ M15]*	Lab stock
E. coli BL21 (DE3)	F$^-$, *ompT*, *hsd*SB (rB$^-$mB$^-$), *gal*, *dcm*	Lab stock
B. subtilis 168	*trpC2*	Lab stock
B. subtilis S5	*B. subtilis 168* Δ*gamP*Δ*gamA*Δ*nagA*Δ*nagB*Δ*ldh*Δ*pta::lox72*, P$_{43}$-*glmS*	Lab stock
MT1	Δ*nprE*::P$_{43}$-*manC*-*gmd*-Ter-P$_{43}$-*wcaG*, Δ*bpr*::P$_{43}$-*manB*-Ter-P$_{43}$-*futC*	Lab stock
E. coli BL21-X	*E. coli* BL21 (DE3) derivate, with pHT28a derivate plasmid for protein purification	This work
B. subtilis Trs	*B. subtilis* 168 derivate, pBUA-Trs	This work
B. subtilis A1	*B. subtilis* Trs derivate, p43NMK-P$_{grac}$-AGE	This work

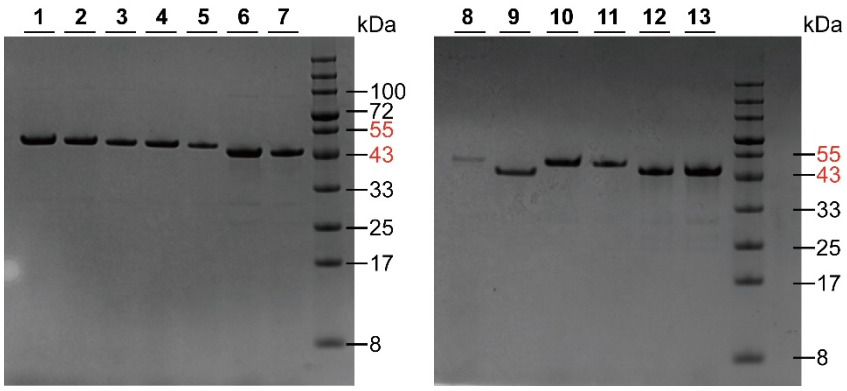
B. subtilis A2	*B. subtilis* Trs derivate, p43NMK-P_{grac}-AGE^{OMeY}	This work
YSC0	*B. subtilis* 168 derivate, pHT-P_{grac}-GFP	This work
YSC1	*B. subtilis* 168 derivate, pHT-P_{grac}-SIDP1-GFP	This work
YSC2	*B. subtilis* 168 derivate, pHT-P_{grac}-RIAD-SIDP1-GFP	This work
YSC3	*B. subtilis* 168 derivate, p43NMK-P_{grac}-mKate-RIDD	This work
YSC4	YSC1 derivate, p43NMK-P_{grac}-mKate-RIDD	This work
YSC5	YSC2 derivate, p43NMK-P_{grac}-mKate-RIDD	This work
YSC6	YSC2 derivate, p43NMK-P_{xylA}-mKate-RIDD	This work
YSC1-2	*B. subtilis* 168 derivate, pHT-SIDP2	This work
YSC1-3	*B. subtilis* 168 derivate, pHT-FUSN	This work
YSC1-4	*B. subtilis* 168 derivate, pHT-3RGG	This work
YSC1-5	*B. subtilis* 168 derivate, pHT-SIDP4	This work
YSC1-21	YSC1-2 derivate, p43NMK-P_{grac}-mKate-RIDD	This work
YSC1-31	YSC1-3 derivate, p43NMK-P_{grac}-mKate-RIDD	This work
YSC1-41	YSC1-4 derivate, p43NMK-P_{grac}-mKate-RIDD	This work
YSC1-51	YSC1-5 derivate, p43NMK-P_{grac}-mKate-RIDD	This work
YSC7	*B. subtilis* 168 derivate, Δ*yesZ*::P_{spovG}-^{RIDD}*VioA*-Ter-P_{43}-^{RIDD}*VioB*-Ter-P_{veg}-^{RIDD}*VioE*	This work
YSC8	*B. subtilis* 168 derivate, Δ*yesZ*::P_{spovG}-^{RIDD}*VioA*-Ter-P_{43}-^{RIDD}*VioB*-Ter-P_{veg}-^{RIDD}*VioE*-Ter-P_{spovG}-^{RIDD}*VioC*	This work
YSC9	YSC7 derivate, pHT-SIDP1	This work
YSC10	YSC8 derivate, pHT-SIDP1	This work
YSC11	*B. subtilis* 168 derivate, pHT-P_{grac}-div-SIDP1-GFP	This work
YSC12	*B. subtilis* 168 derivate, pHT-P_{grac}-divmut-SIDP1-GFP	This work
YSC13	*B. subtilis* 168 derivate, pBUA-Trs-F2	This work
YSC14	*B. subtilis* 168 derivate, pBUA-div-Trs-F2	This work
YSC15	*B. subtilis* 168 derivate, pBUA-divmut-Trs-F2	This work
YSC16	*B. subtilis* 168 derivate, pBUA-Trs-SIDP3-F2	This work
YSC17	*B. subtilis* 168 derivate, pBUA-Trs-SIDP1-F2	This work
YSC18	*B. subtilis* 168 derivate, pBUA-div-Trs-SIDP1-F2	This work
YSC19	*B. subtilis* 168 derivate, pBUA-Trs-SIDP1	This work
YSC20	*B. subtilis* Trs derivate, pADK-1	This work
YSC21	YSC13 derivate, pADK-1	This work
YSC22	YSC14 derivate, pADK-1	This work
YSC23	YSC15 derivate, pADK-1	This work

YSC24	YSC16 derivate, pADK-1	This work
YSC25	YSC17 derivate, pADK-1	This work
YSC26	YSC18 derivate, pADK-1	This work
YSC27	YSC19 derivate, pADK-1	This work
YSC28	YSC13 derivate, pADK-3	This work
YSC29	YSC14 derivate, pADK-3	This work
YSC30	YSC18 derivate, pADK-3	This work
YSC31	YSC13 derivate, pADK-2	This work
YSC32	YSC14 derivate, pADK-2	This work
YSC33	YSC17 derivate, pADK-2	This work
YSC34	YSC18 derivate, pADK-2	This work
YSC35	*B. subtilis* 168 derivate, pBUA-RIAD-Trs-F2, pADK-P_{43}-GFP-RIDD	This work
YSC36	*B. subtilis* 168 derivate, pBUA-RIAD-Trs-SIDP1-F2, pADK-P_{43}-GFP-RIDD	This work
YSC37	YSC13 derivate, pADK-4	This work
YSC38	YSC17 derivate, pADK-4	This work
MT1-1	MT1 derivate, Δ*yesZ*, Δ*ganA*	This work
YW0	MT1-1 derivate, Δ*bpr*::P_{43}-*manB*^{RIDD}-Ter-P_{43}-*futC*	This work
YW1	MT1-1 derivate, Δ*nprE*::P_{43}-*manC*^{RIDD}-*gmd*-Ter-P_{43}-*wcaG*	This work
YW2	MT1-1 derivate, Δ*nprE*::P_{43}-*manC*-^{RIDD}*gmd*-Ter-P_{43}-*wcaG*	This work
YW3	MT1-1 derivate, Δ*nprE*::P_{43}-*manC*-*gmd*-Ter-P_{43}-^{RIDD}*wcaG*	This work
YW4	MT1-1 derivate, Δ*bpr*::P_{43}-*manB*-Ter-P_{43}-*futC*^{RIDD}	This work
YW5	YW1 derivate, Δ*nprE*::P_{43}-*manC*^{RIDD}-^{RIDD}*gmd*-Ter-P_{43}-^{RIDD}*wcaG*, Δ*bpr*::P_{43}-*manB*-Ter-P_{43}-*futC*^{RIDD}	This work
YW6	YW2 derivate, Δ*nprE*::P_{43}-*manC*-^{RIDD}*gmd*-Ter-P_{43}-^{RIDD}*wcaG*, Δ*bpr*::P_{43}-*manB*-Ter-P_{43}-*futC*^{RIDD}	This work
YW7	YW0 derivate, Δ*nprE*::P_{43}-*manC*^{RIDD}-*gmd*-Ter-P_{43}-*wcaG*	This work
YW8	YW7 derivate, Δ*nprE*::P_{43}-*manC*^{RIDD}-^{RIDD}*gmd*-Ter-P_{43}-*wcaG*	This work
YW9	YW8 derivate, Δ*nprE*::P_{43}-*manC*^{RIDD}-^{RIDD}*gmd*-Ter-P_{43}-^{RIDD}*wcaG*	This work
YW10	YW9 derivate, Δ*bpr*::P_{43}-*manB*^{RIDD}-Ter-P_{43}-*futC*^{RIDD}	This work
YW11	YW5 derivate, pHT-SIDP1	This work
YW12	YW6 derivate, pHT-SIDP1	This work
YW13	YW8 derivate, pHT-SIDP1	This work
YW14	YW9 derivate, pHT-SIDP1	This work
YW15	YW10 derivate, pHT-SIDP1	This work

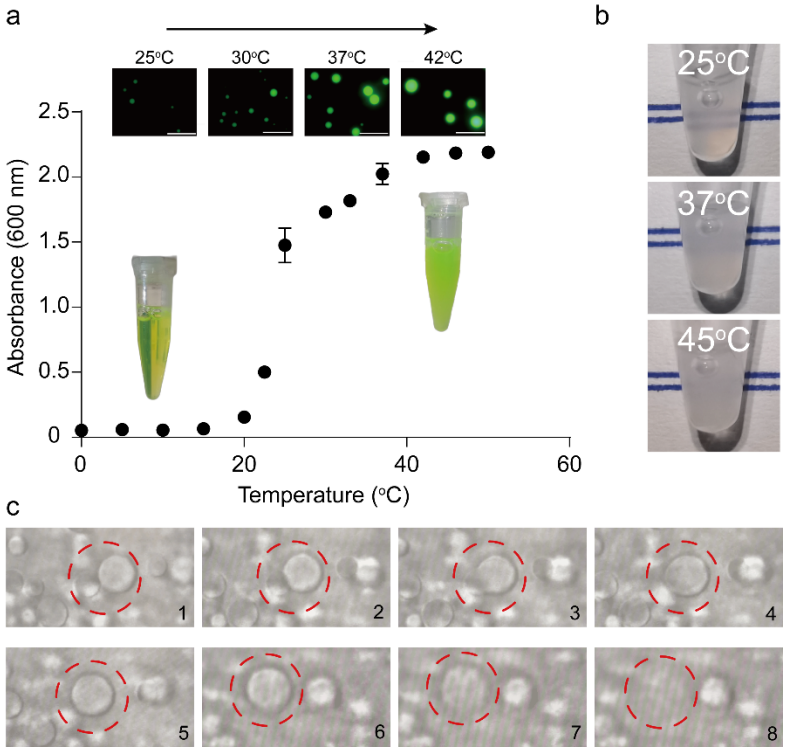
YW16	YW10 derivate, pHT-SIDP2	This work
YW17	YW10 derivate, pHT-FUSN	This work
YW18	YW10 derivate, pHT-3RGG	This work
YW19	YW10 derivate, pHT-SIDP4	This work
MA1	*B. subtilis* S5 derivate, Δ*yesZ*::P_{43}-*GNA1-age*	This work
MA2	MA1 derivate, pBUA-Trs	This work
MAO1	*B. subtilis* S5 derivate, Δ*yesZ*::P_{43}-*GNA1-age*^{OMeY}, pBUA-Trs	This work
MAO2	*B. subtilis* S5 derivate, Δ*yesZ*::P_{43}-*GNA1-age*^{OMeY}, pBUA-div-Trs-SIDP1-F2	This work



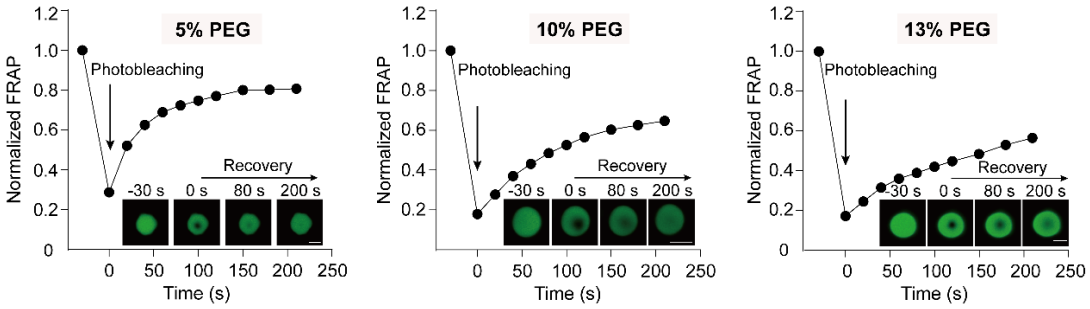
Supplementary Fig. 1 Graph of frequency in amino acid composition of natural IDPs that form biomolecular condensates.



Supplementary Fig. 2 The SDS-PAGE gels of SIDPs. The numbers 1-13 in the image correspond to SIDP1-GFP through SIDP13-GFP, respectively. Source data provided as a Source Data file.

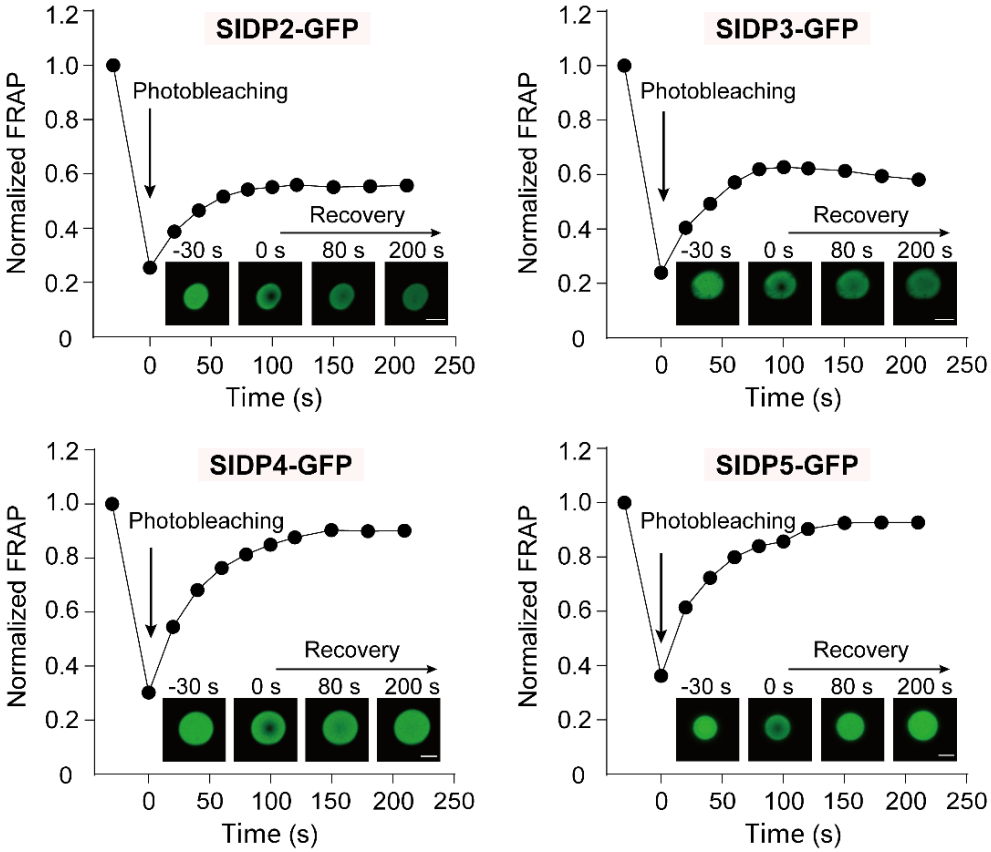


Supplementary Fig. 3 Turbidity analysis of different temperature in physiological buffer. Representative turbidity measurements showed temperature-responsive phase behavior of SIDP1-GFP (a) and SIDP1 (b) at protein concentration of ~50 μM in physiological buffer (150 mM NaCl, pH 7.4, without PEG2000). Both SIDP1-GFP and SIDP1 exhibited lower critical solution temperature phase behavior. Specifically, they were transparent at low temperatures, but upon heating, they underwent phase separation and became visibly turbid. (c) The state changes of SIDP1-GFP condensates during the transition from 37°C to 20°C. Data are presented as mean ± s.d. of three biologically independent replicates. Scale bars = 10 μm. Source data provided as a Source Data file.

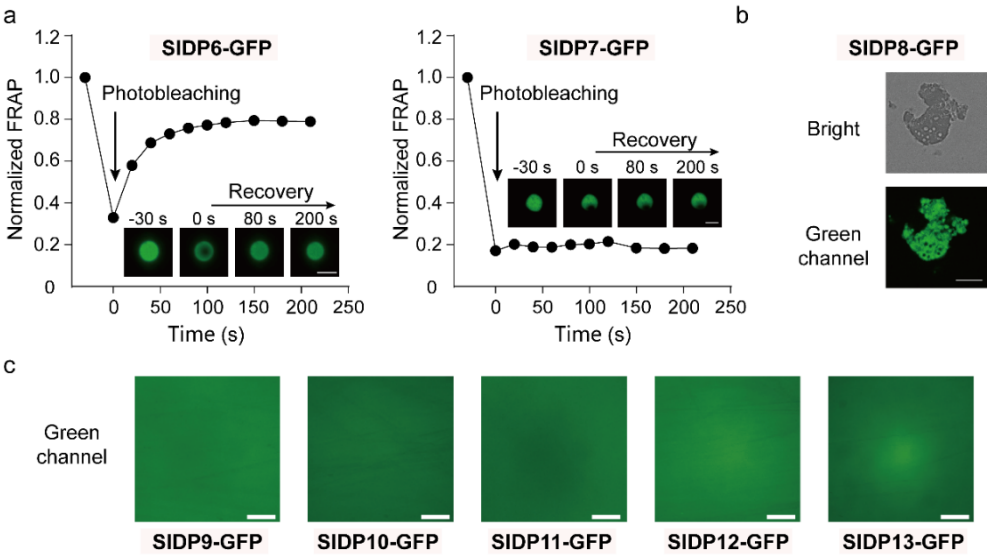


Supplementary Fig. 4 Representative fluorescence recovery of synthetic condensates under varying molecular crowding agent concentrations. Fluorescence recovery after photobleaching (FRAP) measurement of dynamic protein diffusion inside SIDP1-GFP driven droplets with different PEG2000 concentration (~50 μM protein concentration, 150 mM NaCl,

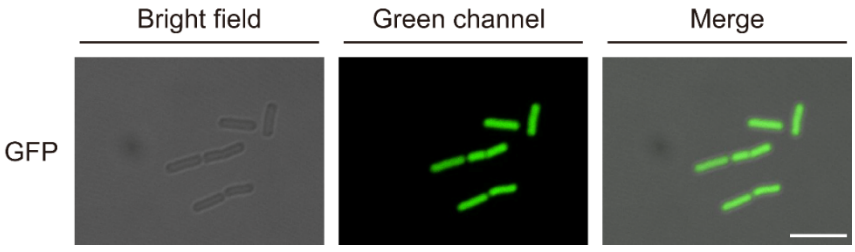
pH=7.4, ~25°C). FRAP recovery curves (top) and images (bottom) were shown. Data are presented as mean ± s.d. of three biologically independent replicates. Scale bars = 5 μm. Source data provided as a Source Data file.



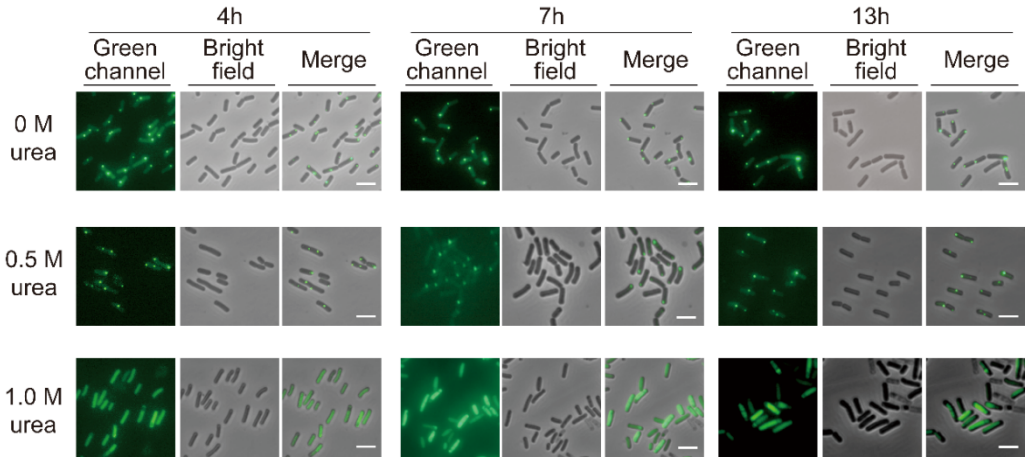
Supplementary Fig. 5 Fluorescence recovery after photobleaching (FRAP) measurement of dynamic protein diffusion inside SIDP2-GFP, SIDP3-GFP, SIDP4-GFP, and SIDP5-GFP droplets under condensate formation buffer. FRAP recovery curves (top) and images (bottom) were shown (~50 μM protein concentration, 150 mM NaCl, pH=7.4, ~25°C, without PEG2000). Data are presented as mean ± s.d. of three biologically independent replicates. Scale bars = 5 μm. Source data provided as a Source Data file.



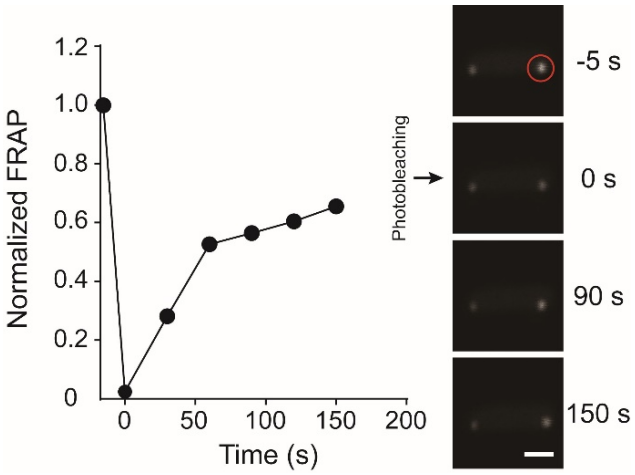
Supplementary Fig. 6 Phase separation of the SIDP6-GFP, SIDP7-GFP, and SIDP8-GFP under condensate formation buffer. (a) Representative FRAP images of ~50 μM SIDP6-GFP and SIDP7-GFP condensates (150 mM NaCl, pH=7.4, ~25°C, without PEG2000). The inset showed images of photobleaching on the droplet. (b) Amorphous and irreversible structures were observed when approximately 50 μM SIDP8-GFP was applied for condensate formation. Scale bars = 5 μm. (c) no condensates were observed when approximately 150 μM SIDP9-GFP, SIDP10-GFP, SIDP11-GFP, SIDP12-GFP and SIDP10-GFP were applied for condensate formation, respectively. Data are presented as mean ± s.d. of three biologically independent replicates. Scale bars = 10 μm. Source data provided as a Source Data file.



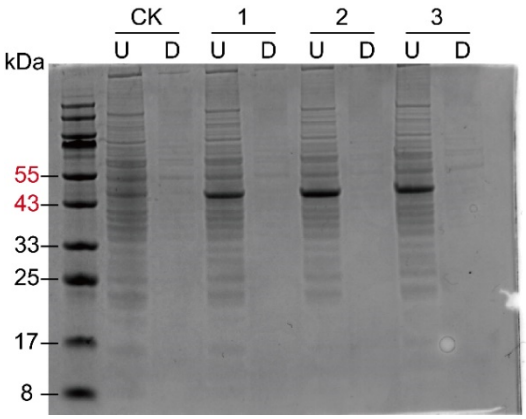
Supplementary Fig. 7 The fluorescence microscopy images of *B. subtilis* expressing GFP at 37°C. Microscopy experiments were repeated in three independent experiments with similar results. Scale bars = 5 μm.



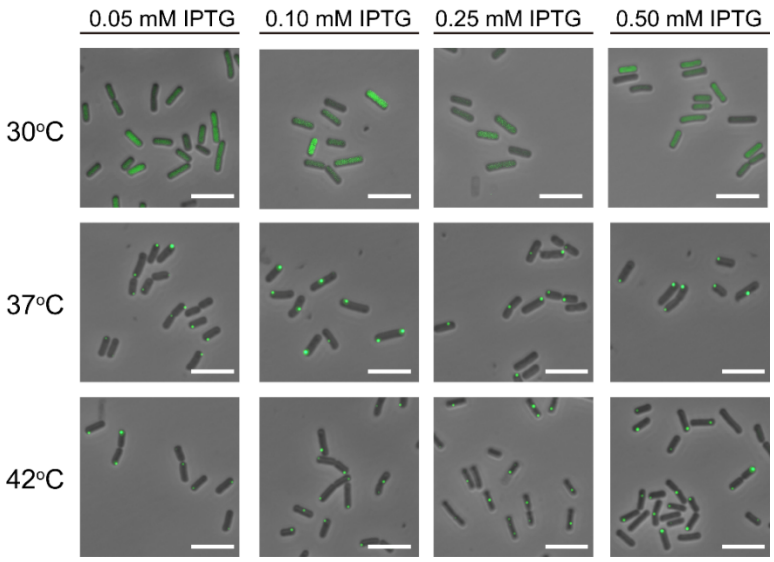
Supplementary Fig. 8 Characterization of dynamic compartments within *B. subtilis* through urea perturbation. Images of SIDP1-GFP expressing cells treated with varying levels of urea. Sampling was performed at 4, 7, and 13 h after induction, and the recombinant *B. subtilis* were harvested and resuspended in PBS buffer with and without the addition of urea. Following incubation at ~25°C for 20 min, the cells were imaged. Microscopy experiments were repeated in three independent experiments with similar results. Scale bars = 5 μm.



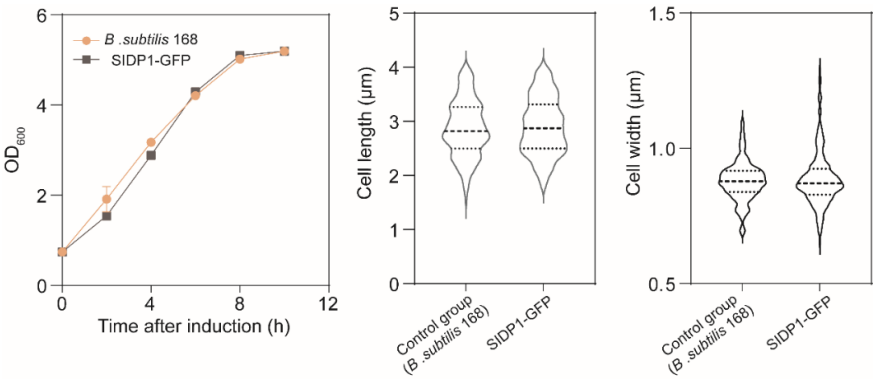
Supplementary Fig. 9 Fluorescence recovery after photobleaching (FRAP) of SIDP1-GFP in cells. The red circle indicates the bleached area. Data are represented as mean ± standard error from $n = 3$ (SIDP1-GFP) biologically independent cells. Representative images shown alongside. Scale bars = 1 μm. Source data provided as a Source Data file.



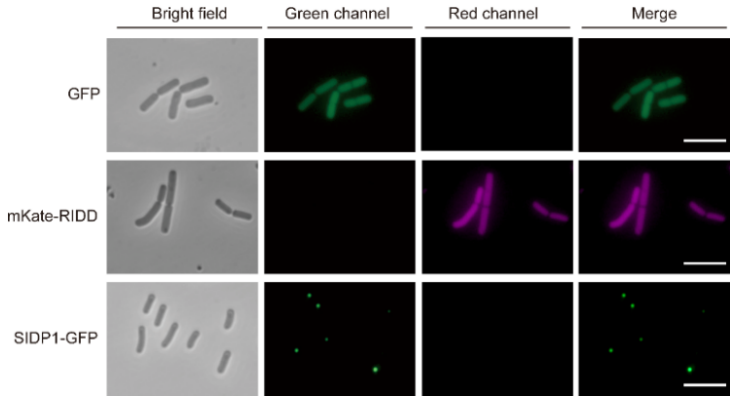
Supplementary Fig. 10 The solubility of the intracellular condensates. "CK" represents the *B. subtilis* 168. Besides, 1, 2, and 3 represent experimental groups where SIDP1-GFP, SIDP2-GFP, and SIDP4-GFP were expressed under isopropyl-β-d-thiogalactoside (IPTG) induction concentrations of 0.5 mM, respectively. In addition, "U" represents the supernatant fluid after sonication, and "D" represents the sediment. Source data provided as a Source Data file.



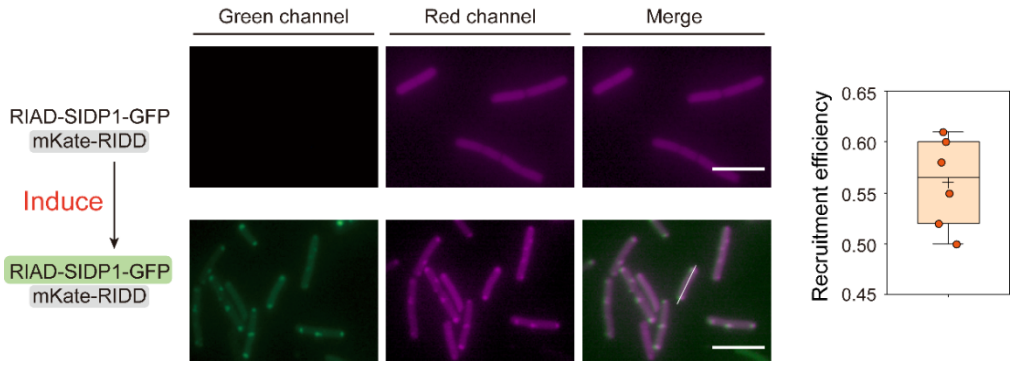
Supplementary Fig. 11 Representative images of cellular condensate formation in *B. subtilis* expressing SIDP1-GFP under varying levels of isopropyl-β-d-thiogalactoside (IPTG) and temperatures. Microscopy experiments were repeated in three independent experiments with similar results. Scale bars = 5 μm.



Supplementary Fig. 12 Effects of formation of synthetic condensates on the cell growth and morphology. The cell growth (left, data are presented as mean ± s.d. of three biologically independent replicates), length (middle, *n* = 204 biologically independent cells), and width (right, *n* = 204 biologically independent cells) of *B. subtilis* expressing SIDP1-GFP, and the control group (*B. subtilis* 168) harboring empty vector. Source data provided as a Source Data file.

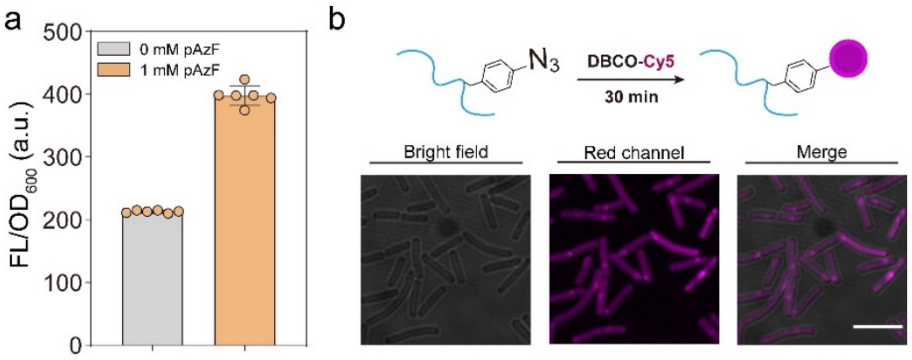


Supplementary Fig. 13 Co-expression of GFP, mKate-RIDD, and untagged SIDP1-GFP serves as a control related to Fig. 3a. Microscopy experiments were repeated in three independent experiments with similar results. Scale bars = 5 μm.

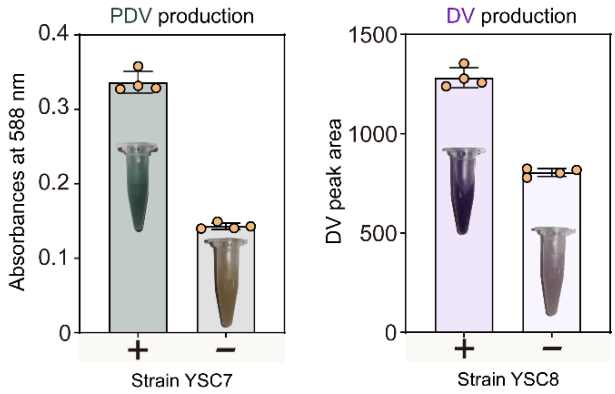


Supplementary Fig. 14 Representative images of *B. subtilis* expressing RIAD-SIDP1-GFP

and mKate-RIDD driven by isopropyl-β-d-thiogalactoside (IPTG) and xylose operon, respectively. The first three columns indicate the dual-channel imaging and merging images of RIAD-SIDP1-GFP and mKate-RIDD. The fourth column represents the ratio of mKate fluorescence intensity of condensates to that of the whole cell (*n* = 6 biologically independent cells). The box plot shows the mean value as a black cross, the center as a black line, box extending between the 25th and 75th percentiles, and whiskers indicating minimum and maximum data points. Scale bars = 5 μm. Source data provided as a Source Data file.

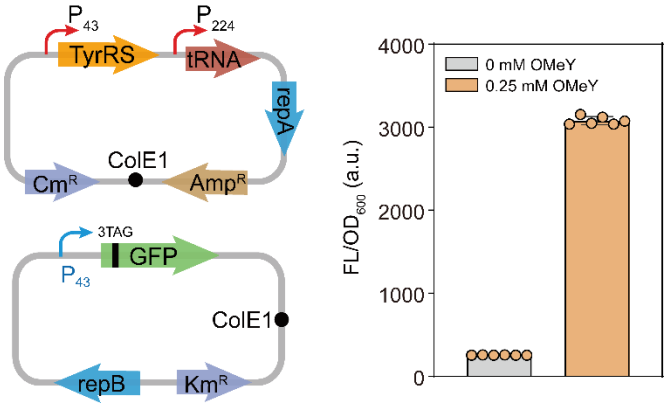


Supplementary Fig. 15 Validation of whether small molecule compounds can diffuse into cellular condensates. (a) Testing the feasibility of p-Azido-L-phenylalanine (pAzF) incorporation system using the reporter protein GFP^{3TAG}. (b) Labelling of cellular condensates with a small molecule fluorescent dye. The synthetic condensates are formed by SIDP1^{TAG} with pAzF residues, which present a bio-orthogonal azide that can be labelled in situ with DBCO-Cy5. DBCO-Cy5 mixture can diffuse into the cellular SIDP1 condensates and label the azide group. Data are presented as mean ± s.d. of six biologically independent replicates. Scale bars = 5 μm. Source data provided as a Source Data file.

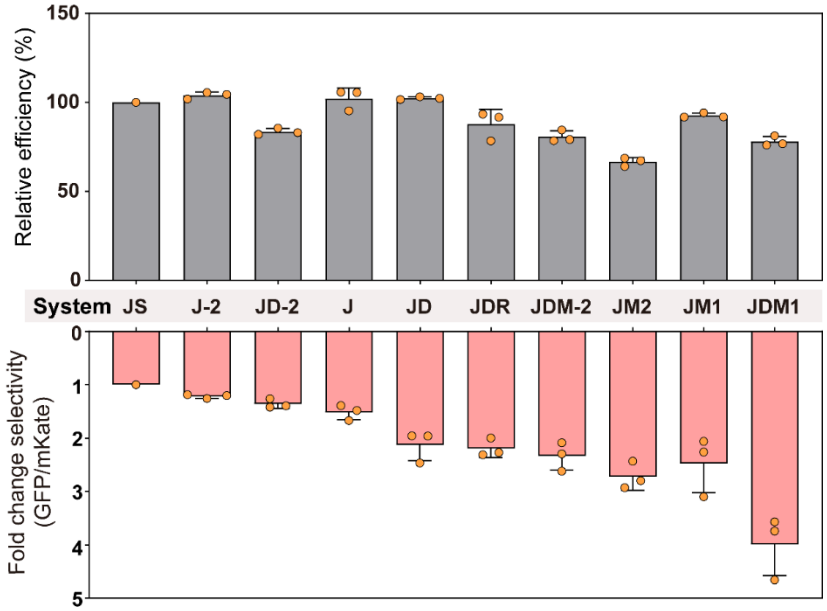


Supplementary Fig. 16 Microplate reader and high-performance liquid chromatography quantification of prodeoxyviolacein (PDV) and desoxyviolacein (DV), respectively. The

"+" represents the presence of cellular condensates, while the "-" indicates the absence. Data are presented as mean ± s.d. of four biologically independent replicates. Source data provided as a Source Data file.

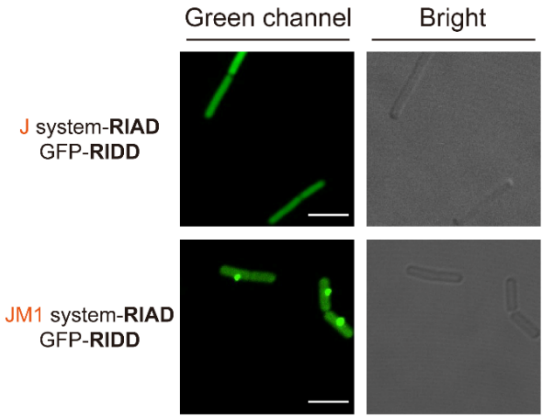


Supplementary Fig. 17 Construction of *O*-methyl-tyrosine (OMeY) incorporation tool in *B. subtilis*. The OMeY incorporation tool was constructed based on the pHT01 plasmid by integrating TyrRS and tRNA under the control of P_{43} and P_{224}, respectively. Testing the specific incorporation using the reporter protein GFP^{3TAG}. The histogram represents the degree of GFP expression after replacing one original codons by amber stop codons in the sequence from the third codon (3TAG). Data are presented as mean ± s.d. of six biologically independent replicates. Source data provided as a Source Data file.

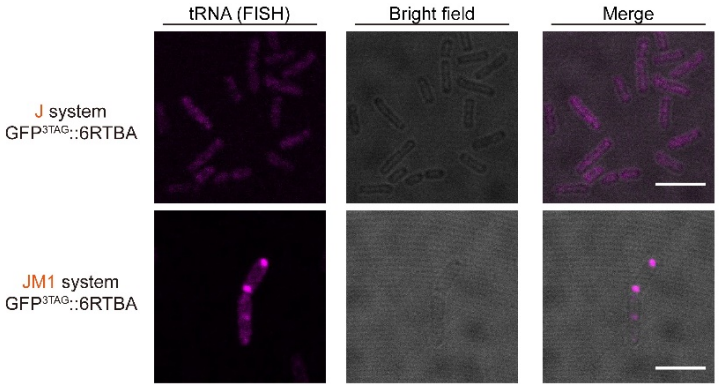


Supplementary Fig. 18 Testing the spatially separated orthogonal translation in *B. subtilis*. The bottom bars (normalized to JS system) represent the fold change in the ratios of

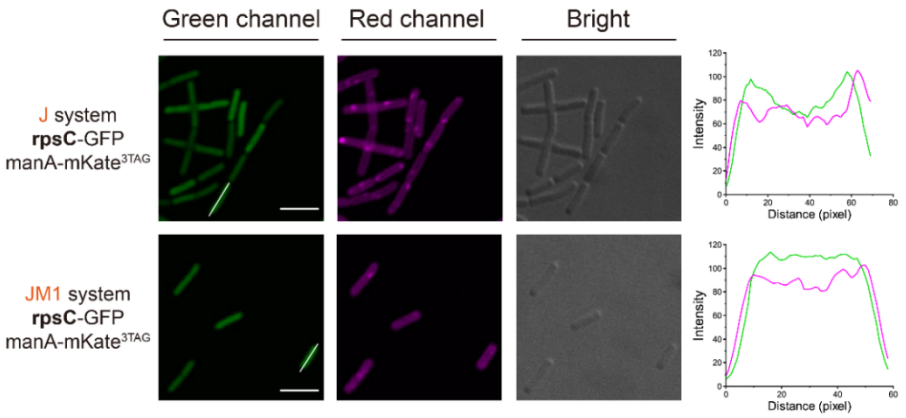
the mean fluorescence intensities of GFP versus mKate for all the systems tested. The top bars represent the relative efficiency as defined by the mean fluorescence intensity of GFP for each condition divided by JS system (SIDP1 and TyrRS) control. Data are presented as mean ± s.d. of three biologically independent replicates. Source data provided as a Source Data file.



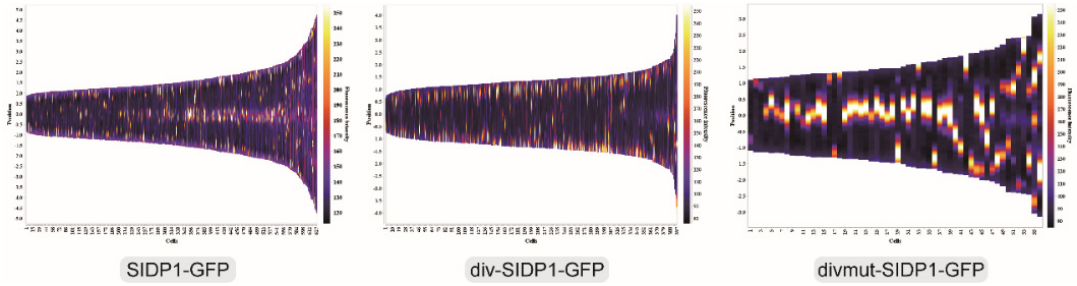
Supplementary Fig. 19 The formation of functional condensates in *B. subtilis*. The functional condensates were formed by TyrRS-SIDP1-F2 fusion protein. Microscopy experiments were repeated in three independent experiments with similar results. Scale bars = 5 μm.



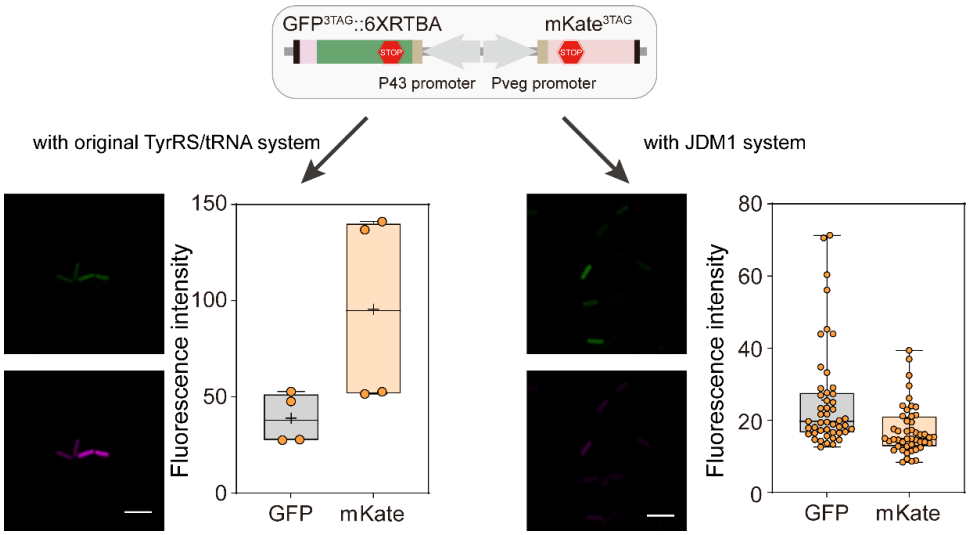
Supplementary Fig. 20 Fluorescence in situ hybridization (FISH) imaging of recombinant *B. subtilis* with J and JM1 system, respectively. For simplicity, a single-color GFP^{3TAG}::6RTBA reporter was used instead of the dual-color reporter. Microscopy experiments were repeated in three independent experiments with similar results. Scale bar = 5 μm.



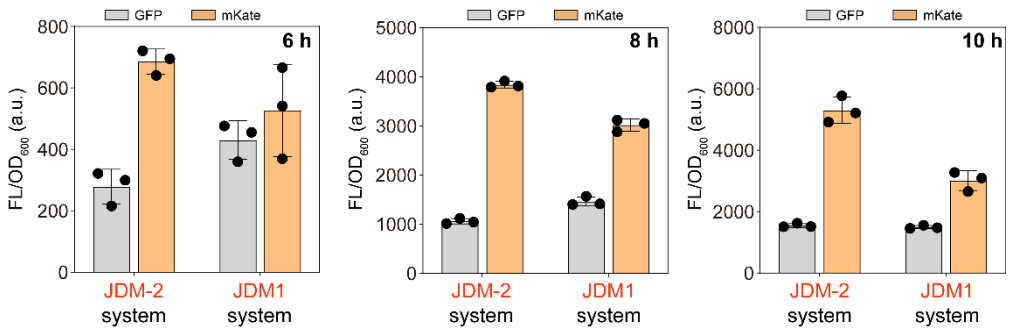
Supplementary Fig. 21 Synthetic condensates enrich ribosomes for orthogonal translation. The representative confocal images of recombinant *B. subtilis* with J and JM1 system are shown, respectively. Shown from left to right are ribosomal protein rspC (green), mKate^{3TAG} (red), merge, and line profiles for the rspC and mKate^{3TAG} channels (green and red curves, respectively). Microscopy experiments were repeated in three independent experiments with similar results. Scale bar = 5 μm.



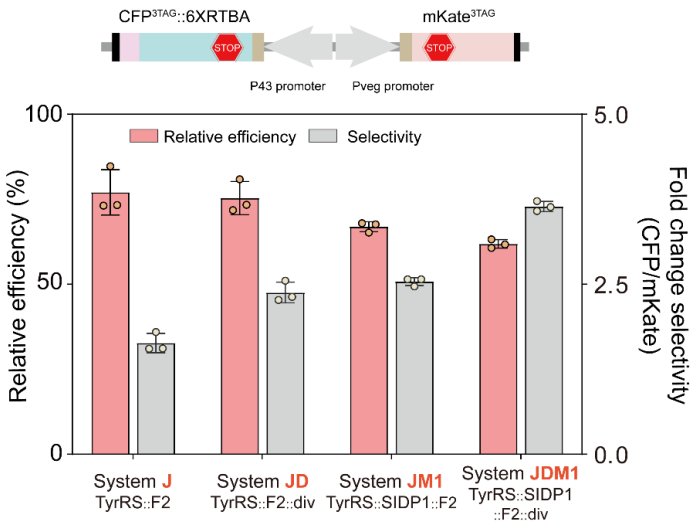
Supplementary Fig. 22 The intracellular spatial distribution of different fusion protein-driven condensates. Localization patterns of SIDP1-GFP, div-SIDP1-GFP (fusing the N-terminal 60 amino acids of the gene *divIVA* to SIDP1-GFP), and divmut-SIDP1-GFP (fusing the N-terminal 60 amino acids of the gene *divIVA*^{R18C} to SIDP1-GFP) were quantified, respectively. Vertical heatmaps representing intensities of fluorescent protein across the long cell axis were generated using ImageJ.



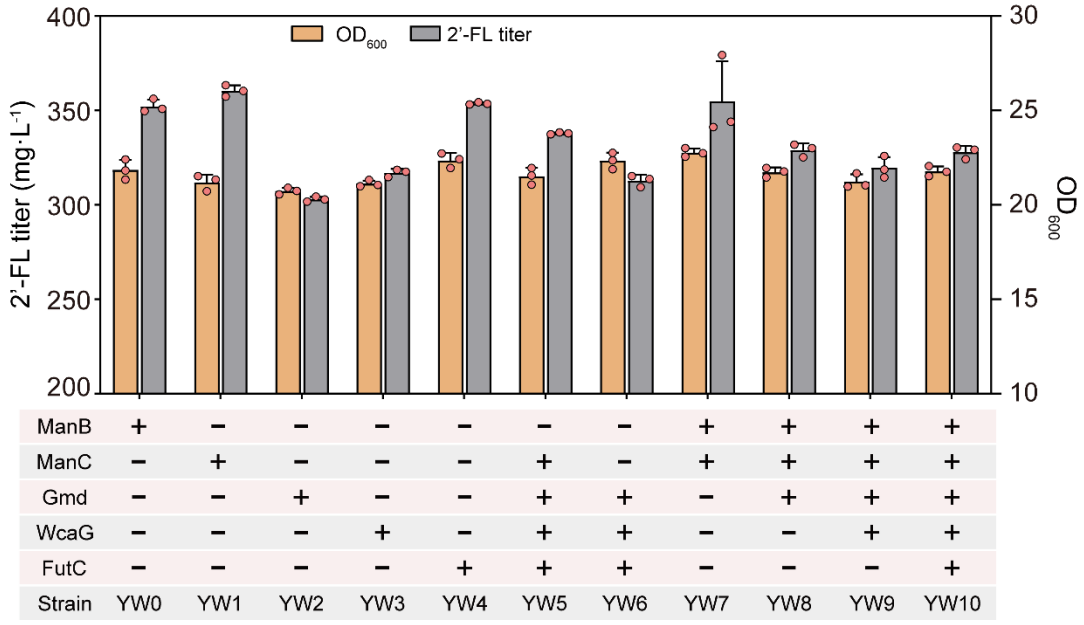
Supplementary Fig. 23 The representative confocal images of the spatially separated orthogonal translation system. The left column shows the representative confocal images of the strain YSC20 (with original TyrRS/tRNA system), while the representative confocal images of strain expressing the JDM1 system are on the right. Besides, the corresponding fluorescence intensities have also been calculated and provided (left, *n*= 4 biologically independent cells; right, *n*= 47 biologically independent cells). The box plots show the mean value as a black cross, the center as a black line, box extending between the 25th and 75th percentiles, and whiskers indicating minimum and maximum data points. Source data provided as a Source Data file.



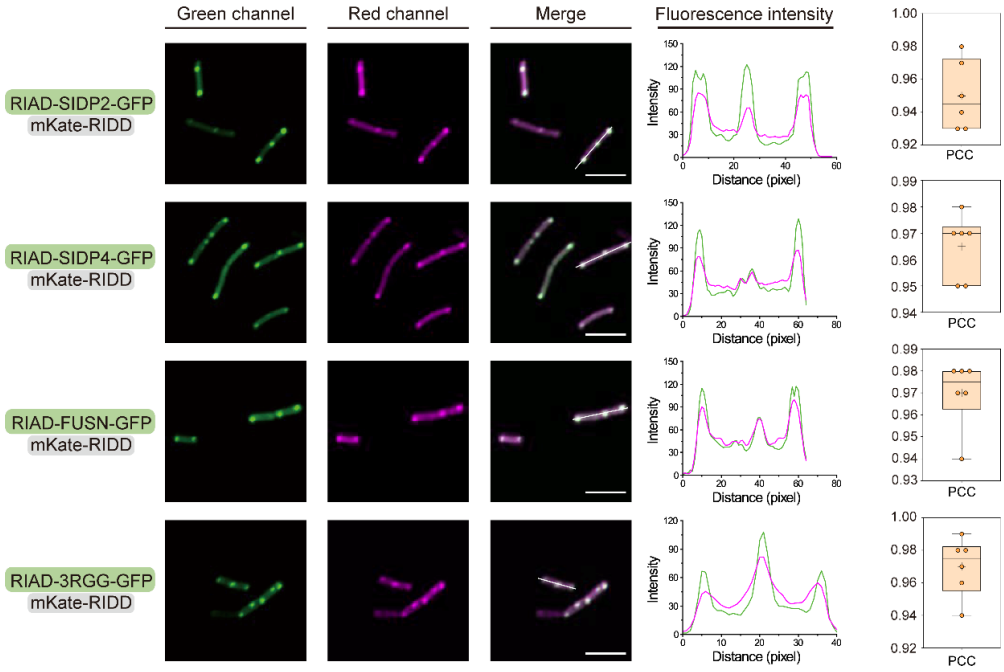
Supplementary Fig. 24 Trends of the dual-reporter fluorescence intensity within recombinant *B. subtilis* with JDM-2 and JDM1 system, respectively. Data are presented as mean ± s.d. of three biologically independent replicates. Source data provided as a Source Data file.



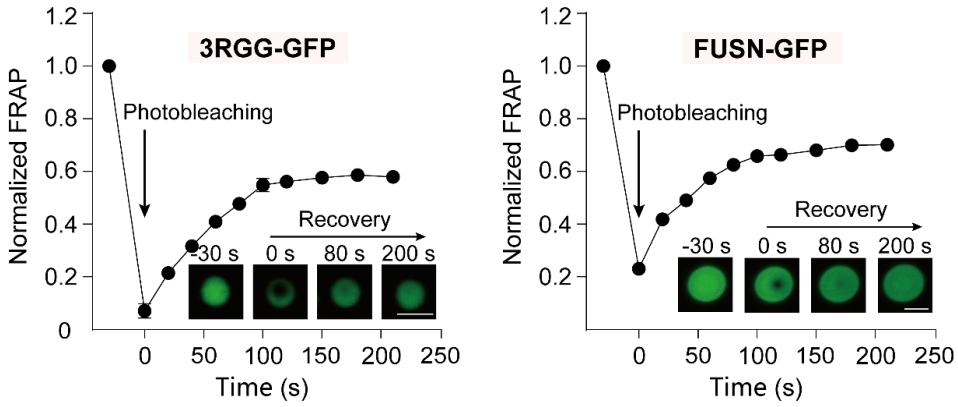
Supplementary Fig. 25 Validation of the universality of the spatially separated orthogonal translation system. The target gene was substituted with CFP, and the 6RTBA loop was additionally fused to the 3' untranslated region of CFP for functional characterization. Data are presented as mean ± s.d. of three biologically independent replicates. Source data provided as a Source Data file.



Supplementary Fig. 26 The effects of fusion RIDD peptide on the 2'-fucosyllactose (2'-FL) production. The "+" signifies that the enzyme is fused with the RIDD peptide, while "-" represents enzymes that are not fused. Data are presented as mean ± s.d. of three biologically independent replicates. Source data provided as a Source Data file.

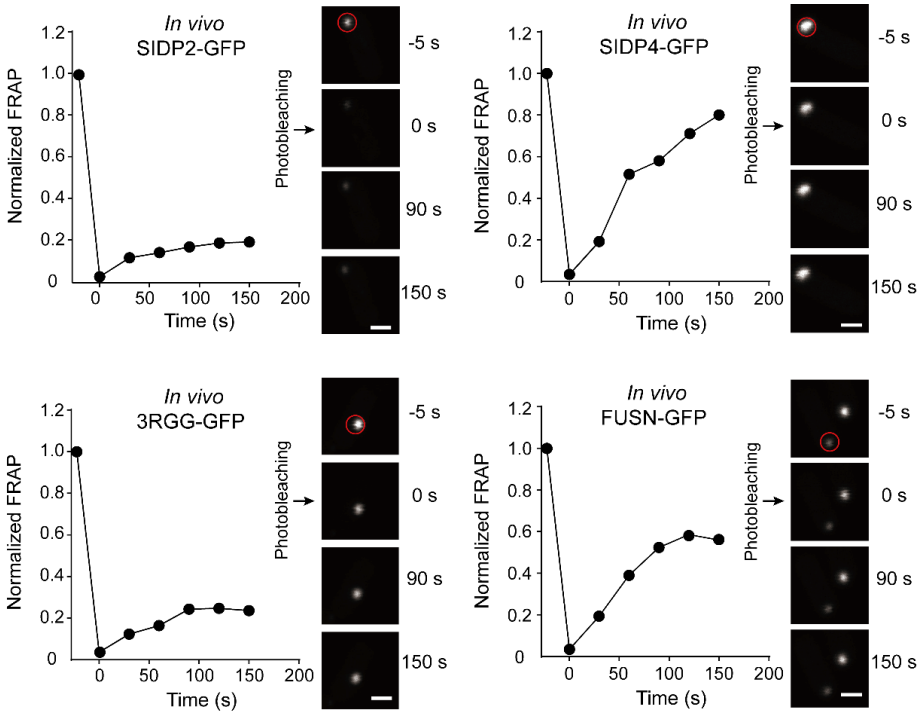


Supplementary Fig. 27 Colocalization of multiple fluorescent proteins in compartments upon coexpression of mKate-RIDD and RIAD fused to SIDP2-GFP, SIDP4-GFP, FUSN-GFP, and 3RGG-GFP, respectively. Representative images showed that all four fusion proteins can form cellular condensates within *B. subtilis* and can recruit target fluorescent protein. The box plots show the mean value as a black cross, the center as a black line, box extending between the 25th and 75th percentiles, and whiskers indicating minimum and maximum data points (*n* = 6 biologically independent cells). Scale bars = 5 μm. Source data provided as a Source Data file.

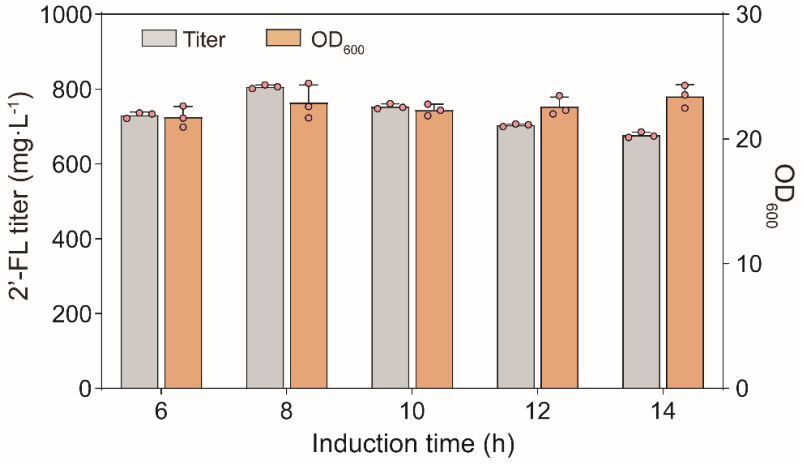


Supplementary Fig. 28 Fluorescence recovery after photobleaching (FRAP) measurement of 3RGG-GFP and FUSN-GFP condensates *in vitro*. FRAP recovery curves (top) and representative images (bottom) are shown (~50 μM protein concentration, 150 mM NaCl, pH=7.4, ~25°C, without PEG2000). Data are presented as mean ± s.d. of three biologically independent replicates. Scale bars = 5 μm. Source data provided as a Source Data

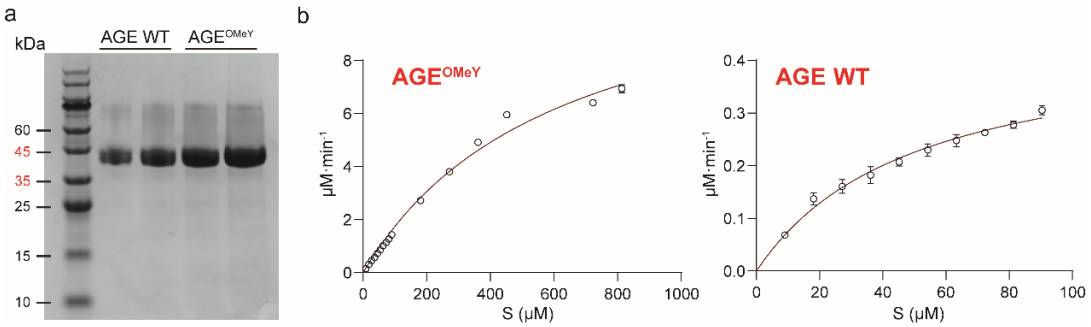
file.



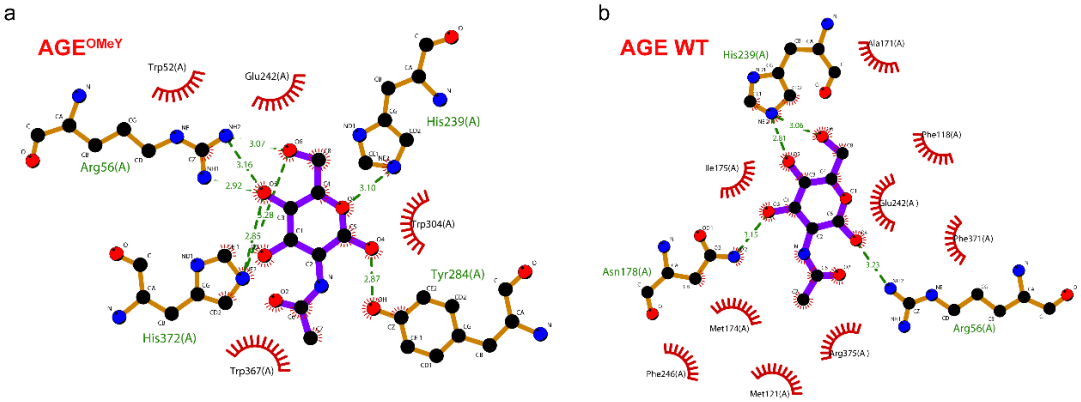
Supplementary Fig. 29 Fluorescence recovery after photobleaching (FRAP) of SIDP2-GFP, SIDP4-GFP, 3RGG-GFP and FUSN-GFP in *B. subtilis*. The red circle indicates the bleached area. Data are represented as mean ± standard error from $n = 3$ biologically independent cells. Representative images shown alongside. Scale bars = 1 μm. Source data provided as a Source Data file.



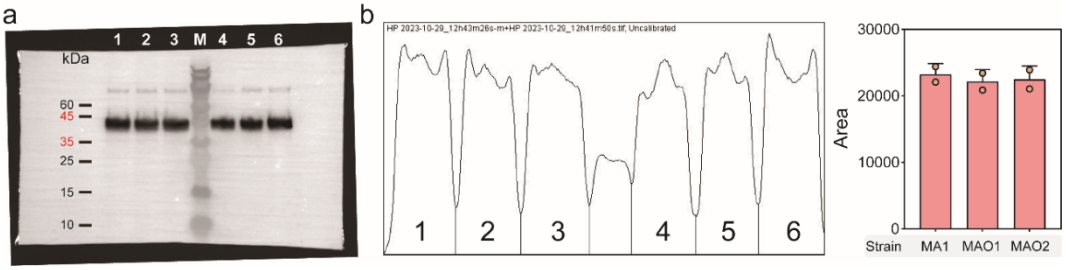
Supplementary Fig. 30 Effects of induction time for cellular condensates formation on 2'-fucosyllactose (2'-FL) production. Data are presented as mean ± s.d. of three biologically independent replicates. Source data provided as a Source Data file.



Supplementary Fig. 31 In vitro assays of catalytic activity on GlcNAc substrate by AGE WT and its mutant AGE^{OMeY}. (a) SDS-PAGE analysis of the AGE wild type (WT) and its mutant AGE^{OMeY}. (b) Nonlinear fitting for AGE WT and AGE^{OMeY} kinetic constant measurements. Data are presented as mean ± s.d. of three biologically independent replicates. Source data provided as a Source Data file.



Supplementary Fig. 32 Geometric constraints of enzyme-substrate interactions for catalytic activity with GlcNAc. Schematic representation of the interactions between AGE^{OMeY} (a) or AGE (b) and GlcNAc, based on Alphafold2 model structure. Hydrogen bonds are shown as dashed lines.



Supplementary Fig. 33 Western blots of AGE and AGE^{OMeY} from *B. subtilis* cell lysates. (a) Lane 1 and 2, strain MA1 (AGE); lane 3 and 4, strain MAO1 (AGE^{OMeY} with aaRS/tRNA$_{OMeY}$ system); lane 5 and 6, strain MAO2 (AGE^{OMeY} with JDM1 system). lane M, protein molecular

weight marker. (b) Gray values of lane 1-6 by using ImageJ. Data are expressed as the mean ± SD. Source data provided as a Source Data file.