

Table S1. Primers used in this study.

Primer name	Sequence (5'-3')
pMA0911-BLA702-F	GCAGCCGCACCGTTTAACGG
pMA0911-BLA702-R	ATGTAAATCGCTCCTTTTTAGGTGGC
SP-F	GCCACCTAAAAAGGAGCGATTACATATGTTGATCAACAAAAGCA AAAAGTTTTTC
SP-R	CCGTAAACGGTGCGGCTGCGGCTTTTGCAACTTCCCCATTC
LipA-F	GCCACCTAAAAAGGAGCGATTACATATGAAATTTGTGAAACGCA GAATTATTGCG
LipA-R	CCGTAAACGGTGCGGCTGCGGCTTTTCGCGCTCGGTTG
YncM-F	GCCACCTAAAAAGGAGCGATTACATATGGCTAAACCGCTGTCAA AAGG
YncM-R	CCGTAAACGGTGCGGCTGCCGCATCAGCTGCTGGCAG
CBM68-F	CCTAAAAAGGAGCGATTACATATGCCCCAAAACAACAGTCG
CBM68-R	CCGTAAACGGTGCGGCTGCACCAAGATCATTCCGTCATATGCA TAC
pMA0911-F	GAATTCGTCGACAAGCTTGGTACC
pMA0911-R	ATGTAAATCGCTCCTTTTTAGGTGGC
0046ytnA-F	GCCACCTAAAAAGGAGCGATTACATATGCAAAAACAAAACAA GAGCTGCAC
0046ytnA-R	GGTACCAAGCTTGTCGACGAATTCTCAGCTGATATTCGTTTCGCT GGC
0572comEA-F	GCCACCTAAAAAGGAGCGATTACATATGAATTGGTTGAATCAGC ATAAGAAAGC
0572comEA-R	GGTACCAAGCTTGTCGACGAATTCTCACTTTACTGTAATGGAAGA CTTTATTTTCTC
1521motA-F	CCACCTAAAAAGGAGCGATTACATATGGATAAAACTTCGTTAAT CGGTATTATTCTTG
1521motA-R	GGTACCAAGCTTGTCGACGAATTCTCATGCTTCTTCTCTTTTTTC TCGCC
1951crh-F	GCCACCTAAAAAGGAGCGATTACATATGGTTCAACAGAAAGTG GAAGTTCG
1951crh-R	GGTACCAAGCTTGTCGACGAATTCTTAACTTCTTCTTGAACGTA AGCAGCC
2143SpoIIQ-F	GCCACCTAAAAAGGAGCGATTACATATGAGAGAGGAAGAAAAG AAAACCTCTCAAG
2143SpoIIQ-R	GGTACCAAGCTTGTCGACGAATTCTTAAGACTGTTCAAGTGTCTTC TGTTGTGC
pDG1730-F	GGGCAAGGCTAGACGGGAC
pDG1730-R	TCTTGACACTCCTTATTTGATTTTTTG
1730-amyE-up1	CAAAAAATCAAATAAGGAGTGCAAGAATGTTTGCAAAACGATT CAAAACCTC

1730-amyE-up2	CTTTTCATATCCTGATATCGGCCGAAGCTTCTAGGATCCGATCAG ACCAG
BLA702-F	CGGCCGATATCAGGATATGAAAAAG
BLA702-R	GGCGCGCCAAACTGGACAC
Spc-F	GTGTCCAGTTTGGCGGCCATCGAATTCCTGCAGCCCTG
Spc-R	GATCCCCCTATGCAAGGGTTTATTG CAATAAACCCCTTGCATAGGGGGATCTCGACATGGATGAGCGATGA TG
1730-amyE-down-F	GTCCCGTCTAGCCTTGCCCTCAATGGGGAAGAGAACCGCTTAAG CAAAAAATCAAATAAGGAGTGTCAAGAGTTTTCCCTTAAACAA ATATTTGAACACGATTTTGGC
1730-amyE-down-R	CTTTTCATATCCTGATATCGGCCGGCAAACAAAAACAGTCAGGA CAC
1730-nprB-up-F	CAATAAACCCCTTGCATAGGGGGATCATGAAAACGAATCAAGTTAA TGACCGC
1730-nprB-up-R	GTCCCGTCTAGCCTTGCCCAACACCACATCCTTCCTATTTTGAAT CTACC
1730-NprB-down-F	CAACCGAGCGGAAAGCGATGAAGAAAATCGCAATAGCAGCTAT CAC
1730-NprB-down-R	GGTACCAAGCTTGTGCGACGAATTCTTATTTAGAATTGCTTGAAGA TGAAGAAGTGCTG
PrsA-F	GAATTCGTCGACAAGCTTGGTACC
PrsA-R	CGCTTTCGCGCTCGGTTG
pMA0911-LipA-F	CCGTTACACTAGAAAACCGAAAGAC
pMA0911-LipA-R	TCATCACCGAAACGCGCG
Test-pMA0911-F	GGGTGAGCAAAAACAGGAAGG
Test-pMA0911-R	GCCGGAAGCGAGAAGAATC
Test-pDG1730-F	
Test-pDG1730-R	

Table S2. Macromolecular protein transporters in this study.

Numbers	Gene name	Functional description
0127	<i>ytxE</i>	ABC transporter ATP-binding protein
0059	<i>ytrF</i>	ABC transporter permease
4127	<i>trpP</i>	tryptophan transporter
3624	<i>gabP</i>	GABA permease
1239	<i>fliP</i>	flagellar biosynthesis protein
3363	<i>thiT</i>	energy-coupled thiamine transporter
2619	<i>yyaD</i>	putative transporter
1522	<i>motB-2</i>	hypothetical protein
1238	<i>fliQ</i>	flagellar biosynthesis protein
1235	<i>flhA</i>	flagellar biosynthesis protein
1945	<i>yvcS</i>	lantibiotic ABC transporter permease
1878	<i>sipV</i>	type I signal peptidase
1819	<i>yitP</i>	hypothetical protein
4131	<i>ecsB</i>	ABC transporter permease

1237	<i>fliR</i>	flagellar biosynthesis protein FliR
1254	<i>fliF</i>	flagellar basal-body MS-ring/collar protein
0062	<i>bceA</i>	bacitracin ABC transporter ATP-binding protein
3115	<i>yvrO</i>	putative ABC transporter (ATP-binding protein)
0750	<i>oxaAB</i>	Sec-independent factor for membrane protein insertion
1236	<i>flhB</i>	flagellar biosynthesis protein
1087	<i>yndE</i>	putative spore germination integral inner membrane protein
4229	<i>ydgF</i>	putative amino acid permease
3467	<i>yfkT</i>	putative spore germination integral inner membrane transporter
2907	<i>tatD</i>	3' -> 5' ssDNA/RNA exonuclease
2790	<i>yelH</i>	putative ABC transporter (ATPase component)
1571	<i>thiV</i>	thiamine transporter, permease component
1820	<i>yitO</i>	putative integral inner membrane protein with HTTM domain
2791	<i>gerKB</i>	spore germination receptor subunit
2858	<i>ycgH</i>	putative amino acid transporter
0058	<i>ytrE</i>	ABC transporter ATP-binding protein
2968	<i>ybxG</i>	putative amino acid permease
0063	<i>bceB</i>	ABC transporter (permease)
2936	<i>ybgB</i>	conserved protein of unknown function
2200	<i>ywkB</i>	putative metabolite transporter
2477	<i>yxdL</i>	ABC transporter ATP-binding protein
2066	<i>glrBB</i>	glycine receptor, beta b
1447	<i>yknY-2</i>	ABC transporter ATP-binding protein
1682	<i>yjKA</i>	putative ABC transporter (permease)
3139	<i>gerAB</i>	component of the germination receptor GerA; putative transporter
2476	<i>yxdM</i>	ABC transporter permease
0168	<i>secDF</i>	protein translocase subunit
2381	<i>cydD</i>	thiol reductant ABC exporter subunit
3063	<i>sdpB</i>	export step of killing factor
0099	<i>pomA</i>	proline-specific permease
2978	<i>skfF</i>	sporulation killing factor biosynthesis and export; ABC transporter (permease)
3038	<i>yvbW</i>	putative leucine permease
1521	<i>motA</i>	flagellar motor protein
1951	<i>crh</i>	corticotropin releasing hormone
2143	<i>spoIIQ</i>	stage II sporulation protein
0572	<i>comEA</i>	membrane bound high-affinity DNA-binding receptor
0046	<i>ytnA</i>	putative amino acid permease

Table S3. The N20 sequences of the macromolecular protein transporters.

Numbers	Gene name	N20 sequence (5' - 3')
0127	<i>ytxE</i>	ATGTGCAAAAATTCAAAGCGGCAG
0059	<i>ytrF</i>	AAACTTCCATCAAGAACATTTCGGC

4127	<i>trpP</i>	ATCTTGCAAAATGTACTCGTCATC
3624	<i>gabP</i>	ATGTGCGCCATACAACGGATCTGG
1239	<i>fliP</i>	ATGTGCAATCTATGCCCCAAACC
3363	<i>thiT</i>	AAACAGTTTCCGATTGCTATTTGT
2619	<i>yyaD</i>	AAACAGCTTCCTTTCTGGTGGGGA
1522	<i>motB-2</i>	ATGTAGGCCGAAGCAAAAACCGGC
1238	<i>fliQ</i>	ATGTTCAAATCCAGGAACAGACTT
1235	<i>flhA</i>	ATGTGTCAGTTGGCTTTGGAATC
1945	<i>yvcS</i>	ATGTTTTAGTCAGACTTGCCGTA
1878	<i>sipV</i>	ATGTCCAGGAAGGAAACGAATTGT
1819	<i>yitP</i>	ATGTGCCAAGTCGGCAAGGAACAG
4131	<i>ecsB</i>	ATGTGACGAAAGCAGCGTTTCTAT
1237	<i>fliR</i>	ATGTGATCAGGCATTTCC6AATTT
1254	<i>fliF</i>	ATGTTGCCACAGCGGGATACCTG
0062	<i>bceA</i>	ATGTCAGAAGCAGAGAACATCCGC
3115	<i>yvrO</i>	ATGTAGCAGCGGGTGGCGATAGCG
0750	<i>oxaAB</i>	ATGTTTCAGTCTCCGATTATGATT
1236	<i>flhB</i>	ATGTGACAGCGGGAAATGGCGATG
1087	<i>yndE</i>	ATGTCCCGACATATTGTAGGAAAA
4229	<i>ydgF</i>	ATGTCTACAAGTATTTGTCGCTGT
3467	<i>yfkT</i>	ATGTTCCAAAAAGCCGCGATGATC
2907	<i>tatD</i>	AAACACAGGACATTTATGACTGGC
2790	<i>yelH</i>	ATGTAGCAGCGGGTATCAATTACA
1571	<i>thiV</i>	ATGTTGTTCAAGGGCTCGGGGCTG
1820	<i>yitO</i>	ATGTTCTCAGCAGGAATGGGTGGA
2791	<i>gerKB</i>	ATGTTCTCAATTGGCTTATCCGAT
2858	<i>ycgH</i>	ATGTCCCGAAAAGCATTCCGCAAA
0058	<i>ytrE</i>	ATGTTCAAGAGCTTTAGCTCATCC
2968	<i>ybxG</i>	ATGTATCAAGGAGTTGAGCTGATC
0063	<i>bceB</i>	ATGTACGCAGCCATTAATAATAA
2936	<i>ybgB</i>	AAACTAATGAATCGGCATCTGCGT
2200	<i>ywkB</i>	ATGTACAGCC6ACATTCGCATTTA
2477	<i>yxdL</i>	ATGTGCAGCTCGGATTTGTGTTTC
2066	<i>glrBB</i>	ATGTCAGCAATTTGCTGTATGCT
1447	<i>yknY-2</i>	ATGTAGCAATTTAGCTTCTTCCG
1682	<i>yjKA</i>	ATGTATTCAATTAATATTGTCACT
3139	<i>gerAB</i>	ATGTTTTGAGTCCTTTGAGCTTAA
2476	<i>yxdM</i>	ATGTGCAAAGCGTGACAATACAAT
0168	<i>secDF</i>	ATGTTCTCAGGCAATTGAGAAAGC
2381	<i>cydD</i>	ATGTAACAACGCTTTACAAGATGG
3063	<i>sdpB</i>	ATGTAGCGATTGAAAAATAAAGAG
0099	<i>pomA</i>	ATGTATCCATCTCGAAATGGAGCG
2978	<i>skfF</i>	ATGTTCCAACATTCATCTGGGATA
3038	<i>yvbW</i>	ATGTGAACCAAGTCAATTCCGTTC

1521	<i>motA</i>	ATGTGCTCAGCTTGCACGCCGCGA
1951	<i>crh</i>	ATGTCCAGGGAGAAGATGAACAAG
2143	<i>spoIIQ</i>	ATGTAATCAAGTGATCGGAAAATC
0572	<i>comEA</i>	ATGTTCTCAGGCAATTGAGAAAGC
0046	<i>ytnA</i>	ATGTTGGCAGGAAATTGGCTCTCA

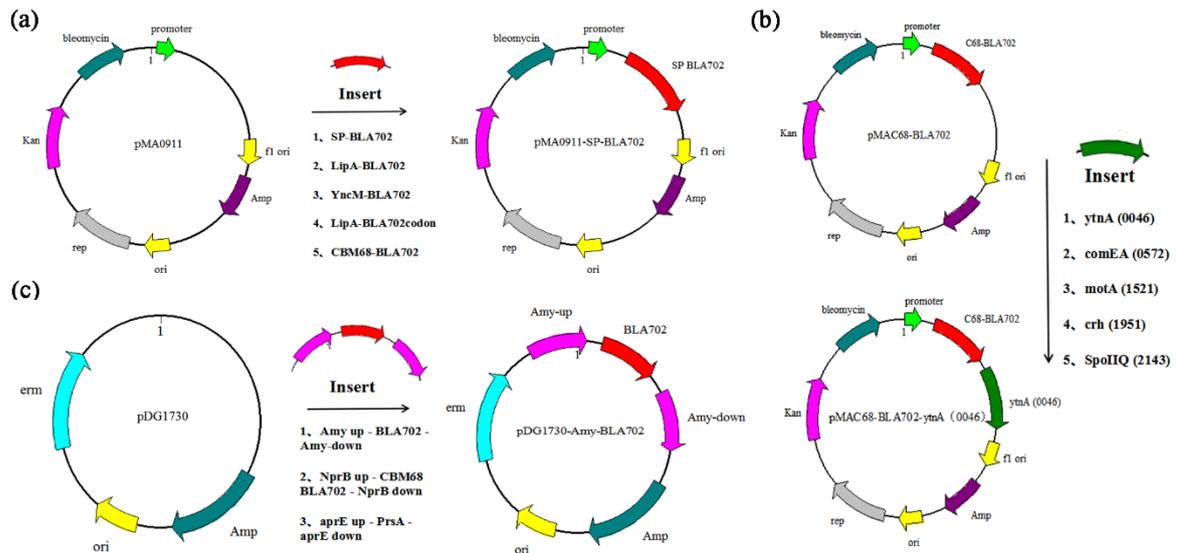


Figure S1. Schematic diagrams of plasmids construction. (a) Plasmids construction for secretory expression of BLA702 guided by different signal peptides or CBM68; (b) Plasmids construction for enhancing the secretory expression of CBM68-BLA702 by overexpressing the key macromolecular protein transporters; (c) Plasmids construction for integrating different target genes into different sites in the genome of *B. subtilis* SCK6 or *B. subtilis* 0127.

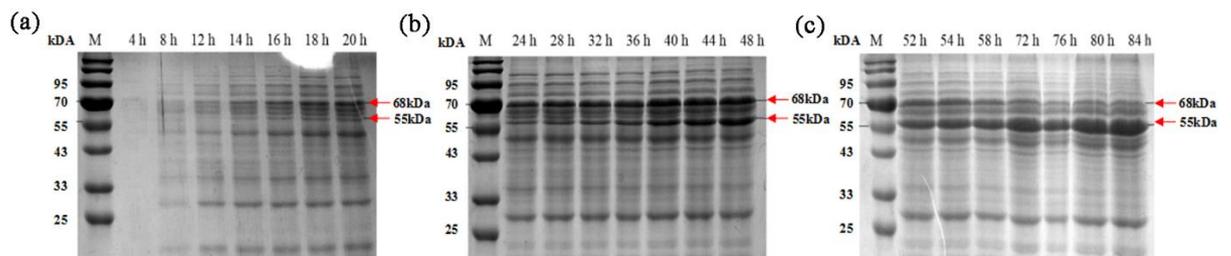


Figure S2. SDS-PAGE of the extracellular proteins of *B. subtilis* SCK6 (pMAC68-BLA702).

(a) 4-20 h fermentation; (b) 24-48 h fermentation; (c) 52-84 h fermentation.

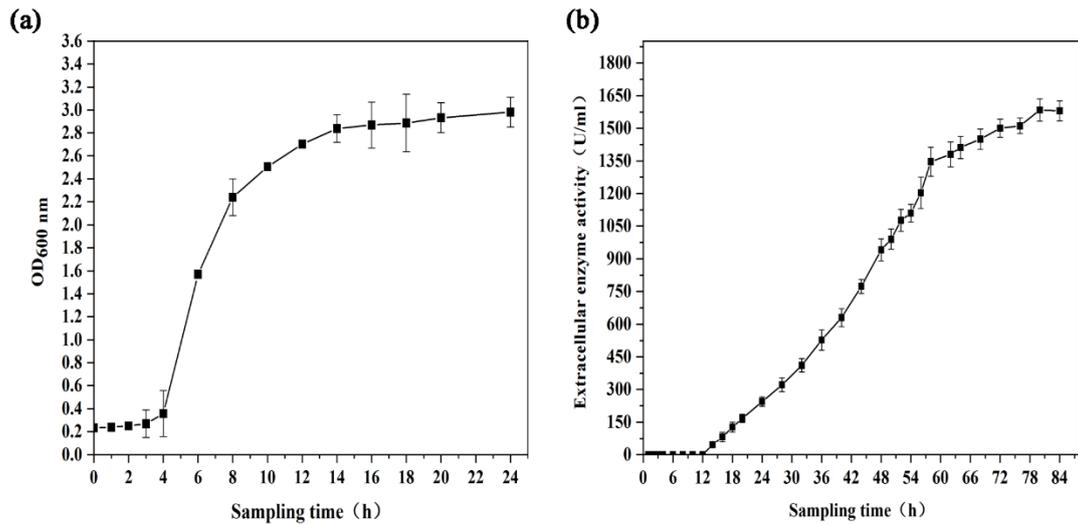


Figure S3. Growth profile (a) and extracellular amylase activity profile (b) of *B. subtilis* SCK6 (pMAC68-BLA702).

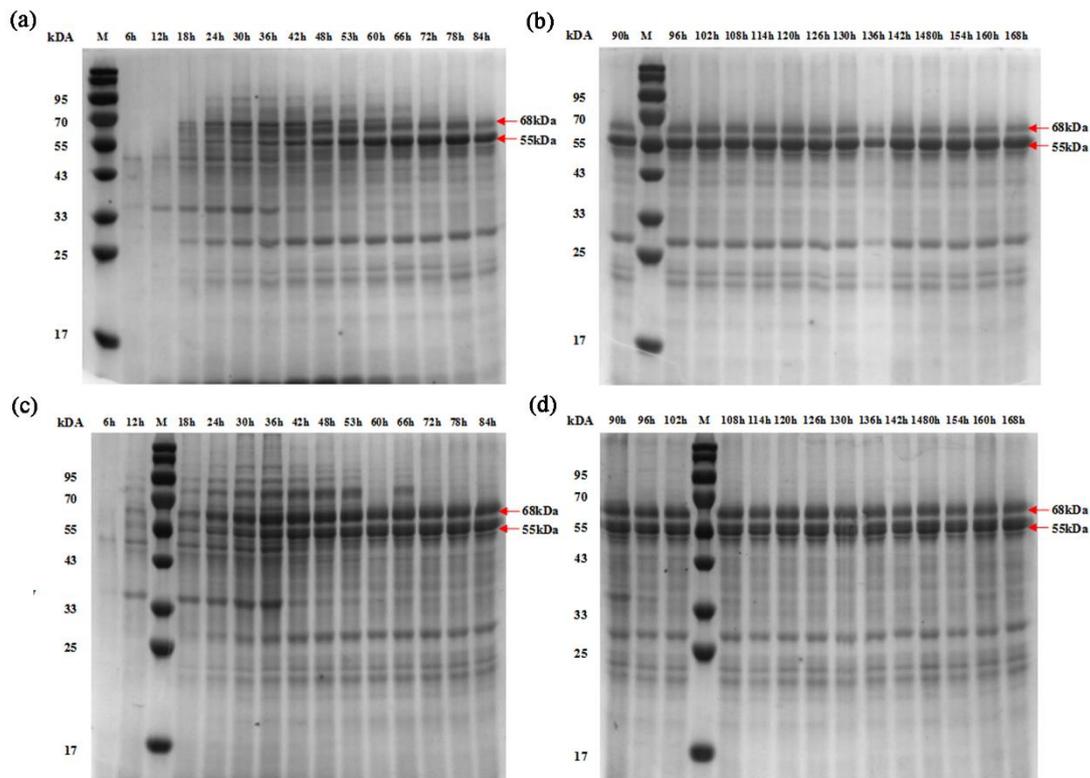


Figure S4. SDS-PAGE of the extracellular proteins of *B. subtilis* 0127 (pMAC68-BLA702) and *B. subtilis* 0127 (AmyE::BLA702-NprB::CBM68-BLA702-PrsA). (a) 6-84 h fermentation of *B. subtilis* 0127 (pMAC68-BLA702); (b) 90-168 h fermentation of *B. subtilis* 0127 (pMAC68-BLA702); (c) 6-84 h fermentation of *B. subtilis* 0127 (AmyE::BLA702-NprB::CBM68-BLA702-PrsA); (d) 90-168 h fermentation of *B. subtilis* 0127 (AmyE::BLA702-NprB::CBM68-BLA702-PrsA).