## Supporting information

Table S1. The DNA sequences of all enzymes used in this study

| Enzymes | Sequences |
| :---: | :---: |
| LmSP | ATGGAAATCCAGAACAAAGCTATGCTGATCACCTACGCTGACTCTCTGG |
|  | GTAAAAACCTGAAAGACGTTCACCAGGTTCTGAAAGAAGACATCGGT |
|  | GACGCTATCGGTGGTGTTCACCTGCTGCCGTTCTTCCCGTCTACCGGTG |
|  | ACCGTGGTTTCGCTCCGGCTGACTACACCCGTGTTGACGCTGCTTTCGG |
|  | TGACTGGGCTGACGTTGAAGCTCTGGGTGAAGAATACTACCTGATGTT |
|  | CGACTTCATGATCAACCACATCTCTCGTGAATCTGTTATGTACCAGGAC |
|  | TTCAAAAAAAACCACGACGACTCTAAATACAAAGACTTCTTCATCCGT |
|  | TGGGAAAAATTCTGGGCTAAAGCTGGTGAAAACCGTCCGACCCAGGCT |
|  | GACGTTGACCTGATCTACAAACGTAAAGACAAAGCTCCGACCCAGGAA |
|  | ATCACCTTCGACGACGGTACCACCGAAAACCTGTGGAACACCTTCGGT |
|  | GAAGAACAGATCGACATCGACGTTAACTCTGCTATCGCTAAAGAATTCA |
|  | TCAAAACCACCCTGGAAGACATGGTTAAACACGGTGCTAACCTGATCC |
|  | GTCTGGACGCTTTCGCTTACGCTGTTAAAAAAGTTGACACCAACGACT |
|  | TCTTCGTTGAACCGGAAATCTGGGACACCCTGAACGAAGTTCGTGAAA |
|  | TCCTGACCCCGCTGAAAGCTGAAATCCTGCCGGAAATCCACGAACACT |
|  | ACTCTATCCCGAAAAAAATCAACGACCACGGTTACTTCACCTACGACTT |
|  | CGCTCTGCCGATGACCACCCTGTACACCCTGTACTCTGGTAAAACCAAC |
|  | CAGCTGGCTAAATGGCTGAAAATGTCTCCGATGAAACAGTTCACCACC |
|  | CTGGACACCCACGACGGTATCGGTGTTGTTGACGCTCGTGACATCCTG |
|  | ACCGACGACGAAATCGACTACGCTTCTGAACAGCTGTACAAAGTTGGT |
|  | GCTAACGTTAAAAAAACCTACTCTTCTGCTTCTTACAACAACCTGGACA |
|  | TCTACCAGATCAACTCTACCTACTACTCTGCTCTGGGTAACGACGACGC |
|  | TGCTTACCTGCTGTCTCGTGTTTTCCAGGTTTTTCGCTCCGGGTATCCCGC |
|  | AGATCTACTACGTTGGTCTGCTGGCTGGTGAAAACGACATCGCTCTGCT |
|  | GGAATCTACCAAAGAAGGTCGTAACATCAACCGTCACTACTACACCCG |
|  | TGAAGAAGTTAAATCTGAAGTTAAACGTCCGGTTGTTGCTAACCTGCT |
|  | GAAACTGCTGTCTTGGCGTAACGAATCTCCGGCTTTCGACCTGGCTGGT |


|  | TCTATCACCGTTGACACCCCGACCGACACCACCATCGTTGTTACCCGTC AGGACGAAAACGGTCAGAACAAAGCTGTTCTGACCGCTGACGCTGCT <br> AACAAAACCTTCGAAATCGTTGAAAACGGTCAGACCGTTATGTCTTCT <br> GACAACCTGACCCAGAACTAA |
| :---: | :---: |
| MaGGP | ATGCTGCTGAAAAACGCTGTTCAGCTGATCTGCTACCCGGACCGTATCG |
|  | GTAACAACCTGAAAGACCTGTACACCGTTGTTGACACCCACCTGTCTG |
|  | AAGCTATCGGTGGTCTGCACATCCTGCCGTTCTTCCCGTCTAACGCTGA |
|  | CGGTGGTTTCTCTCCGCTGACCCACAAAGAAGTTGACCCGAAAGTTGG |
|  | TACCTGGGACGACATCGAAGCTTTCACCGCTAAATACGACCTGTGCGTT |
|  | GACCTGACCGTTAACCACATCTCTGACGAATCTCCGGAATTCACCGACT |
|  | TCATCGCTAACGGTTTCGACTCTGAATACGCTGACCTGTTCGTTCACGT |
|  | TGACAAATTCGGTGAAATCTCTCCGGACGACATGGCTAAAATCCACATC |
|  | CGTAAAGAAAAAGAACCGTTCCGTGAAGTTACCCTGTCTGACGGTACC |
|  | AAAACCCGTGTTTGGTGCACCTTCACCGAACAGCAGATCGACCTGAAC |
|  | TACGAATCTGACCTGGCTTACCAGCTGATGGAATCTTACATCGGTTTCC |
|  | TGACCTCTAAAGGTGTTAACCTGCTGCGTCTGGACGCTTTCGGTTACAC |
|  | CACCAAACGTATCGGTACCTCTTGCTTCCTGGTTGAACCGGAAGTTTAC |
|  | CAGATCCTGGACTGGGTTAACCAGGTTGCTCTGAAACACGGTGCTGAA |
|  | TGCCTGCCGGAAGTTCACGACCACACCTCTTACCAGTACGCTATCTCTC |
|  | GTCGTAACATGCACCCGTACGGTTTCGCTCTGCCGCCGCTGCTGCTGTA |
|  | CTCTCTGCTGGACGCTAACTCTACCTACCTGAAAAACTGGCTGCGTATG |
|  | TGCCCGCGTAACATGGTTACCGTTCTGGACACCCACGACGGTATCTGCA |
|  | TCCCGGACGTTGAAGGTGTTCTGCCGGACGAAAAAATCAAAGTTCTGA |
|  | TCGACAACATCGACGCTCGTTCTGCTGACCCGATCATGCGTCGTTCTGC |
|  | TGCTAACATCCACTCTGTTGGTGCTATCTACCAGCTGACCTGCACCTTCT |
|  | ACGACGCTCTGATGCAGAACGACGACGCTTACATCGCTGCTCGTGCTAT |
|  | CCAGTTCTTCACCCCGGGTATCCCGCAGGTTTACTACGTTGGTCTGCTG |
|  | GCTGGTTGCAACGACCACGAACTGATGGAACAGTCTGGTGAACTGCGT |
|  | GACATCAACCGTCACTACTACACCCTGGAAGAAGTTGAACAGGACATC |




|  | TGACCCTGCTGCACGTTGACGGTGAACCGTTCATCATGTCTGAAGA |
| :---: | :---: |
|  | CTGCTTCTTTCGAACGTACCCTGGACCTGTCTCAGGGTGTTACCTCTCG |
|  | TAAAGTTTCTCAGCGTATGAAAAACGGTGCTACCATCACCATCCACGAA |
|  | GAAAAATTCGCTTCTTACCGTAAAAAACACGCTGTTCTGATGAAATACA |
|  | CCGTTGAATCTGACCAGGACACCGACGCTGTTCTGGACACCGGTATCG |
|  | ACTACGACGTTTGGTCTATCAACGGTGACCACCTGCAGGGTCACCACT |
|  | ACTTCTCTCACCCGACCGGTGACGGTGTTACCGCTAAAACCGTTTCTTA |
|  | CGAAGACACCGTTACCGTTGTTGAAACCTGCTCTCTGGACGCTGACGC |
|  | TTCTGAAGAAGACTACCAGAACCCGGACGGTTCTGGTCGTACCTTCTC |
|  | TCTGTCTCTGGAAGCTGGTAAACCGGTTACCCTGGAAAAAGCTATGATC |
|  | ATCTACTCTTCTAACGACGTTGACAACCCGCAGGACGAAGCTCTGCTG |
|  | GAAGCTAAACACATGCAGTCTTACGAAGAAGAAAAAGCTGCTAACCGT |
|  | CTGGAATGGGACAACCTGTGGTCTCACTACGACGTTACCATCCAGAAC |
|  | AACATCATCGACCAGGTTGCTCTGCGTTTCAACATCTACCACGCTATCA |
|  | TCGCTACCCCGGTTCACAAATCTCTGCCGATCGGTGCTCGTGGTCTGTC |
|  | TTGCCAGGCTTACCAGGGTGCTGCTTTCTGGGACCAGGAAATCTACAA |
|  | CATGCCGATGTACCTGTACTCTAACCCGGAAATCGCTCGTAACATCCTG |
|  | AAATACCGTCACCGTACCCTGGACGGTGCTCGTCGTAAAGCTAAACGT |
|  | CTGGGTTACGAAGGTGCTTACTACGCTTGGATCTCTGGTAAAACCGGTG |
|  | ACGAACTGTGCCCGGACTTCTTCTTCAAAGACGTTCTGTCTGGTCGTG |
|  | ACATCCGTAACCACTTCAACGACTGGCAGATCCACATCTCTCCGGACAT |
|  | CGCTTACGCTGTTAAAAAATACCACCAGGTTACCGGTGACGACGCTTTC |
|  | ATCCGTGACTACGGTGCTGAAATGATCTTCGAAATCGCTCGTTTCCTGG |
|  | CTTCTCACGCTGTTTACAAACCGATGCGTGGTCGTTACGAATTCATGCG |
|  | TGTTCAGGGTCCGGACGAATACCACGAAAACGTTGACAACAACGCTTT |
|  | CACCAACCACCAGGCTATGTTCACCCTGCAGGCTGCTGACGAACTGCT |
|  | GCAGACCCTGGACGAAAAAACCCTGTCTGCTGTTAAAGAAAAAATCG |
|  | GTCTGTCTGACGACGAAATCTCTCTGTGGCGTGACATGCTGGCTAACAC |
|  | CTACGTTCCGAAACCGGACAAACACGGTATCATCGAACAGTTCGACGG |


|  | TTACTACGACCTGGAAACCATCATCCCGGCTAAAAAAGTTACCGAACG TCTGATCAAAGAAGACGAATACTACGGTTACCCGAACGGTGTTACCGTT CGTACCCAGTGCATCAAACAGGCTGACGTTATCCAGCTGTTCGTTCTGC ACCCGCACCTGTACGACCGTAAAACCGTTGAACTGAACTACGAATTCT ACGAACCGCGTACCCTGCACTTCTCTTCTCTGTCTCCGTCTTCTTACGCT ATCGTTGCTGCTCAGATCGACAAAGTTGAAGAAGCTTACCGTAACTTCC GTAAATCTGTTATGATCGACCTGCTGAACACCAACGAAGCTGTTTCTGG TGGTACCTTCATCGGTGGTATCCACACCGCTGCTAACGGTGCTTCTTGG CAGATGGTTGTTAACGGTTTCGGTGGTCTGTCTGTTCACGGTGACGACA TCCACCTGTCTCCGCGTCTGCCGGACGCTTGGGACGGTTACACCTTCA AAGCTATCGTTAAAGGTCAGACCCTGGAAGTTGACGTTACCAAAGAAC AGATCACCATCACCAACAAATCTGAAGACCGTAAACCGCTGACCCTGC ACATCTTCGGTGAAAAATCTGTTCTGGACTCTGAACGTATCACCAAATC TCGTTAA |
| :---: | :---: |
| MpGGP | ATGCTCCTCAAAAATGCCGTTCAGCTGATCTGCTACCCGAATCGCATCG <br> GCAACAATCTGAAGGATCTCTACACCGTGGTTGACAAGCATCTGAGCG <br> AAGCGATCGGTGGTCTGCATATCCTCCCATTCTTCCCGAGCAACGCCGA <br> TGGTGGTTTCAGTCCGCTGACGCACAAGGAAGTTGACCCAGACTTCGG <br> TACGTGGGATGACATCGAGGCCTTCACGAAAAAATACGATCTGTGTGT <br> GGATCTGACGGTGAATCACATTAGCGACGAGAGCCCAGAGTTCAAAGA <br> CTTTATCGCGCACGGCTTTGACAGCAAATACGCCGATCTGTTTGTGCAC <br> GTTGACAAGTTCGGCGAGATCAGTCCAGACGACATGGCGAAGATCCAT <br> ATCCGCAAGGAGAAGGAACCATTCCGCGAAGTGACGCTGGCGGACGG <br> CACGAAAACGCGTGTTTGGTGTACCTTCACGGAGCAGCAAATCGACCT <br> CAACTATGAGAGCGACCAAGCCTATCGTCTCATGGAGAGTTACATCGGT <br> TTTCTGACCAGCAAAGGCGTTAATCTGCTGCGTCTGGATGCGTTCGGCT <br> ATACCACCAAACGCATCGGCACGAGCTGCTTCCTCGTTGAACCGGAGG <br> TGTACCGCATTCTGGACTGGATCAACGAGGTGGCGCTGAAACACGGCG <br> CGGAATGTCTGCCGGAAGTTCACGACCACACCAGCTACCAGTACGCGA |


|  | TTGGCCGCCGTAATATGCACCCGTATGGCTTTGCCCTCCCGCCACTGCT GCTGTACAGCCTCCTCGACGCGAACAGCGTTTATCTGAAGAACTGGCT GCGTATGTGCCCACGCAATATGGTGACGGTTCTGGACACGCATGACGG CATCTGCATTCCAGATGTGGAAGGTGTGCTGCCGGATGAGAAGATCCG TGCGCTGATCGATAACATCGATGCCCGTAGCGCGGATCCAATTATGCGC CGTAGCGCGGCCAACATTCATAGCGTGGGCGCGATCTACCAGCTCACG TGTACCTTCTACGATGCGCTGATGCAGAACGACGACGCGTACATCGCCG CCCGCGCGATTCAGTTTTTCACGCCGGGCATCCCACAAGTGTACTATGT TGGTCTGCTGGCCGGCTGCAACAACCACGAACTGATGAAACAGAGTG GCGAGCTCCGTGATATCAACCGCCACTACTATACGCTGGACGATGTTGA GCAGCACATCCAAAAACCGGTGGTTCAGCGTCTGCTGGCGCTGATGAC GTTCCGCAGTAACTATCCGGCGTTCGACGGCCACTTTGAGCTGAACTAC AGCAACAACAGCAGCGTTGCCATGGCGTGGCGCCATGGCGACTACTAC TGCCATCTGTTCGTGGACCTCAATTTCAACACGGTGAAGATCGGCTACT ACGACCTCGATACGGCCCAGATGGAAAAGCTGGCGTGTTAA |
| :---: | :---: |
| MsGGP | ATGCTGCTGAAAAACGCGGTGCAACTCATCTGCTACCCAAACCGCATC GGCAAAGATCTGAAAGATCTGCATACCGTGGTGGAAAAGCATCTGAGC GAAGCGATCGGTGGTCTGCATATCCTCCCATTCTTCCCAAGCAACGCCG ATGGCGGTTTCAGCCCACTGACGCACAAAGAGGTGGACCCAGATTTTG GCACGTGGAACGATATCGAGGCGTTCACGCAGAAGTACGATCTGTGTG TGGATCTGACGGTGAACCACATCAGCGACGAGAGCCCGGAGTTCAAA GACTTCATCGTGAACGGCTTTGACAGCAAATACGCCGATCTGTTTGTGC ACGTTGACAAGTTCGGCGAGATCAGTCCAGACGACATGGCGAAGATCC ATATCCGCAAGGAGAAGGAGCCATTCCGCGAAGTTACGCTGGCCGATG GCACCAAAACCCGTGTGTGGTGTACCTTCACGGAGCAGCAAATCGACC TCAACTACGACGCGGATCAAGCCTACACGCTGATGGAGAGCTACATCG GTTTTCTGACCAGCAAAGGCGTTAATCTGCTGCGTCTGGATGCGTTCGG CTATACCACGAAACGTATCGGCACCAGCTGTTTTCTGGTGGAGCCAGA GGTTTACCGCATCCTCGATTGGATCAACGAAGTGGCGCTCAAGCACGG |




Table S2. The conversion increased through optimization of conditions

| Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | pH | LmSP:MaGGP <br> $(\mathrm{U} / \mathrm{mL})$ | Sucrose:glycerol <br> $(\mathrm{mM})$ | PBS concentration <br> $(\mathrm{mM})$ | Conversion <br> $(\%)$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 30 | 7.0 | $5: 5$ | $200: 200$ | 50 | 61 |
| 37 | 7.0 | $5: 5$ | $200: 200$ | 50 | 65 |
| 37 | 6.5 | $5: 5$ | $200: 200$ | 50 | 72 |
| 37 | 6.5 | $10: 5$ | $200: 200$ | 50 | 75 |
| 37 | 6.5 | $10: 5$ | $280: 200 / 200: 240$ | 50 | $91 / 92$ |
| 37 | 6.5 | $10: 5$ | $200: 240$ | 10 | 98 |



Figure S1. (a) SDS-PAGE analysis of purified recombinant enzymes for $\alpha$ GG production. (b) Sequence alignment of glucosylglycerol phosphorylase from different sources. MpGGP: glucosylglycerol phosphorylase from Marinobacter psychrophilus, MsGGP: glucosylglycerol phosphorylase from Marinobacter salinus, AcGGP: glucosylglycerol phosphorylase from Azoarcus communis.


Figure S2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of purified recombinant enzymes. Mp: glucosylglycerol phosphorylase from Marinobacter psychrophilus, Ms: glucosylglycerol phosphorylase from Marinobacter salinus, Ac: glucosylglycerol phosphorylase from Azoarcus communis.


Figure S3. HPLC analysis of the in vitro cascade reactions to produce $\alpha \mathrm{GG}$ using sucrose/ maltose and glycerol as substrate. The proof-of-concept cascade reaction to produce $\alpha \mathrm{GG}$ with sucrose and glycerol as substrates was conducted in 50 mM PBS ( pH 7.0 ), 200 mM glycerol, 200 mM sucrose, as well as $5 \mathrm{U} / \mathrm{mLL} \mathrm{LmSP}$ and $5 \mathrm{U} / \mathrm{mL}$ MaGGP. The reaction medium was conducted at $30{ }^{\circ} \mathrm{C}$ for 12 h . The proof-of-concept cascade reaction to produce $\alpha \mathrm{GG}$ with maltose and glycerol as substrates was conducted in 50 mM PBS ( pH 6.5 ), 200 mM glycerol, 200 mM maltose, as well as $5 \mathrm{U} / \mathrm{mL}$ LaMP and $5 \mathrm{U} / \mathrm{mL}$ BsGGP. The reaction medium was conducted at $30{ }^{\circ} \mathrm{C}$ for 12 h.


Figure S4. Time courses of $\alpha$ GG production under 5 mM and 10 mM PBS respectively. The reaction containing $10 \mathrm{U} / \mathrm{mL} \mathrm{LmSP}, 5 \mathrm{U} / \mathrm{mL}$ MaGGP, 200 mM sucrose and 240 mM glycerol was conducted at $37^{\circ} \mathrm{C}$ in PBS buffer ( pH 6.5 ).


Figure S5. The HPLC chromatograms for product mixture, purified and standard $\alpha \mathrm{GG}$. They were determined by Agilent HPLC equipped with a Sugar-Pak column and a refractive index detector. Different samples were applied to this HPLC with a mobile phase of deionized water at a flow rate of $0.4 \mathrm{~mL} / \mathrm{min}$ and the column was operated at $80{ }^{\circ} \mathrm{C}$.


Figure S6. Thermal stability of LmSP (a) and MaGGP (b) at $37{ }^{\circ} \mathrm{C}$.
The enzymes were treated at $37{ }^{\circ} \mathrm{C}$ for a certain period and then determined the residual activity. (a) The activity of LmSP was measured at $30{ }^{\circ} \mathrm{C}$ in 50 mM PBS buffer containing 50 mM sucrose for 10 min and stopped at boiling temperature for 10 min . One unit of enzyme activity was defined as the enzyme amount catalyzing the consumption of $1 \mu \mathrm{~mol}$ sucrose per min. (b) The activity of MaGGP was measured at $30{ }^{\circ} \mathrm{C}$ in 50 mM PBS buffer containing $50 \mathrm{mM} \alpha-\mathrm{G} 1 \mathrm{P}$ and 50 mM glycerol for 10 min and stopped at boiling temperature for 10 min . One unit of enzyme activity was defined as the enzyme amount catalyzing the consumption of $1 \mu \mathrm{~mol}$ glycerol per min.


Figure S7. The HPLC profiles and standard curve of sucrose.
Sucrose was determined by the HPLC system (Agilent 1200 series) equipped with a refractive index detector and fitted with chromatographic column (Sugar-Pak ${ }^{\mathrm{TM}}, 6.5 \times 300 \mathrm{~mm}$ ). A mobile phase of deionized water was used at a flow rate of $0.4 \mathrm{~mL} / \mathrm{min}$ and the column was operated at $80^{\circ} \mathrm{C}$. The retention time of sucrose is 10.7 min .


Figure S8. The HPLC profiles and standard curve of maltose.
Maltose was determined by the HPLC system (Agilent 1200 series) equipped with a refractive index detector and fitted with chromatographic column (Sugar-Pak ${ }^{\mathrm{TM}}, 6.5 \times 300 \mathrm{~mm}$ ). A mobile phase of deionized water was used at a flow rate of $0.4 \mathrm{~mL} / \mathrm{min}$ and the column was operated at $80{ }^{\circ} \mathrm{C}$. The retention time of maltose is 10.3 min .


Figure S9. The HPLC profiles and standard curve of fructose.
Fructose was determined by the HPLC system (Agilent 1200 series) equipped with a refractive index detector and fitted with chromatographic column (Sugar-Pak ${ }^{\mathrm{TM}}, 6.5 \times 300 \mathrm{~mm}$ ). A mobile phase of deionized water was used at a flow rate of $0.4 \mathrm{~mL} / \mathrm{min}$ and the column was operated at $80^{\circ} \mathrm{C}$. The retention time of fructose is 16.5 min .


Figure S10. The HPLC profiles and standard curve of glucose.
Glucose was determined by the HPLC system (Agilent 1200 series) equipped with a refractive index detector and fitted with chromatographic column (Sugar-Pak ${ }^{\mathrm{TM}}, 6.5 \times 300 \mathrm{~mm}$ ). A mobile phase of deionized water was used at a flow rate of $0.4 \mathrm{~mL} / \mathrm{min}$ and the column was operated at $80^{\circ} \mathrm{C}$. The retention time of glucose is 12.7 min .


Figure S11. The HPLC profiles and standard curve of glycerol.
Glycerol was determined by the HPLC system (Agilent 1200 series) equipped with a refractive index detector and fitted with chromatographic column (Sugar-Pak ${ }^{\mathrm{TM}}, 6.5 \times 300 \mathrm{~mm}$ ). A mobile phase of deionized water was used at a flow rate of $0.4 \mathrm{~mL} / \mathrm{min}$ and the column was operated at $80^{\circ} \mathrm{C}$. The retention time of glycerol is 18.0 min .


Figure S12. The HPLC profiles and standard curve of $\alpha G G$.
$\alpha$ GG was determined by the HPLC system (Agilent 1200 series) equipped with a refractive index detector and fitted with chromatographic column (Sugar-Pak ${ }^{\mathrm{TM}}, 6.5 \times 300 \mathrm{~mm}$ ). A mobile phase of deionized water was used at a flow rate of $0.4 \mathrm{~mL} / \mathrm{min}$ and the column was operated at $80^{\circ} \mathrm{C}$. The retention time of $\alpha \mathrm{GG}$ is 12.2 min .


Figure S13. The time course for enzyme activity determination of MaGGP
To determinate the enzyme activity of MaGGP, the reaction containing $50 \mathrm{mM} \alpha-\mathrm{G} 1 \mathrm{P}$ and glycerol was performed at $30^{\circ} \mathrm{C}$ in 50 mM PBS buffer ( pH 7.0 ) for 50 min .

