

# **Living hydrogels with catalytic function grown from engineered yeast co-cultures**

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**This Supplementary Information contains:**

**Figure S1:** FACS gating strategy

**Figure S2:** Yeast colony PCR

**Figure S3:** Photographs of stationary cultivations

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**Figure S5:** SDS-PAGE of the control cultivations

**Figure S6:** Photographs of casein-containing control cultivation

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**Figure S8:** Photographs of the CNFs

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**Figure S27:** Nucleotide sequence of pATK001

**Figure S28.** Nucleotide sequence of the SmTG secretion construct

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**Table S1:** Dry weight and water content of the living hydrogels

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**Table S5:** Nucleotide sequences of the synthetic genes

**Table S6:** Parts used to assemble the yeast display constructs

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**Table S8:** Parts used to assemble pATK001

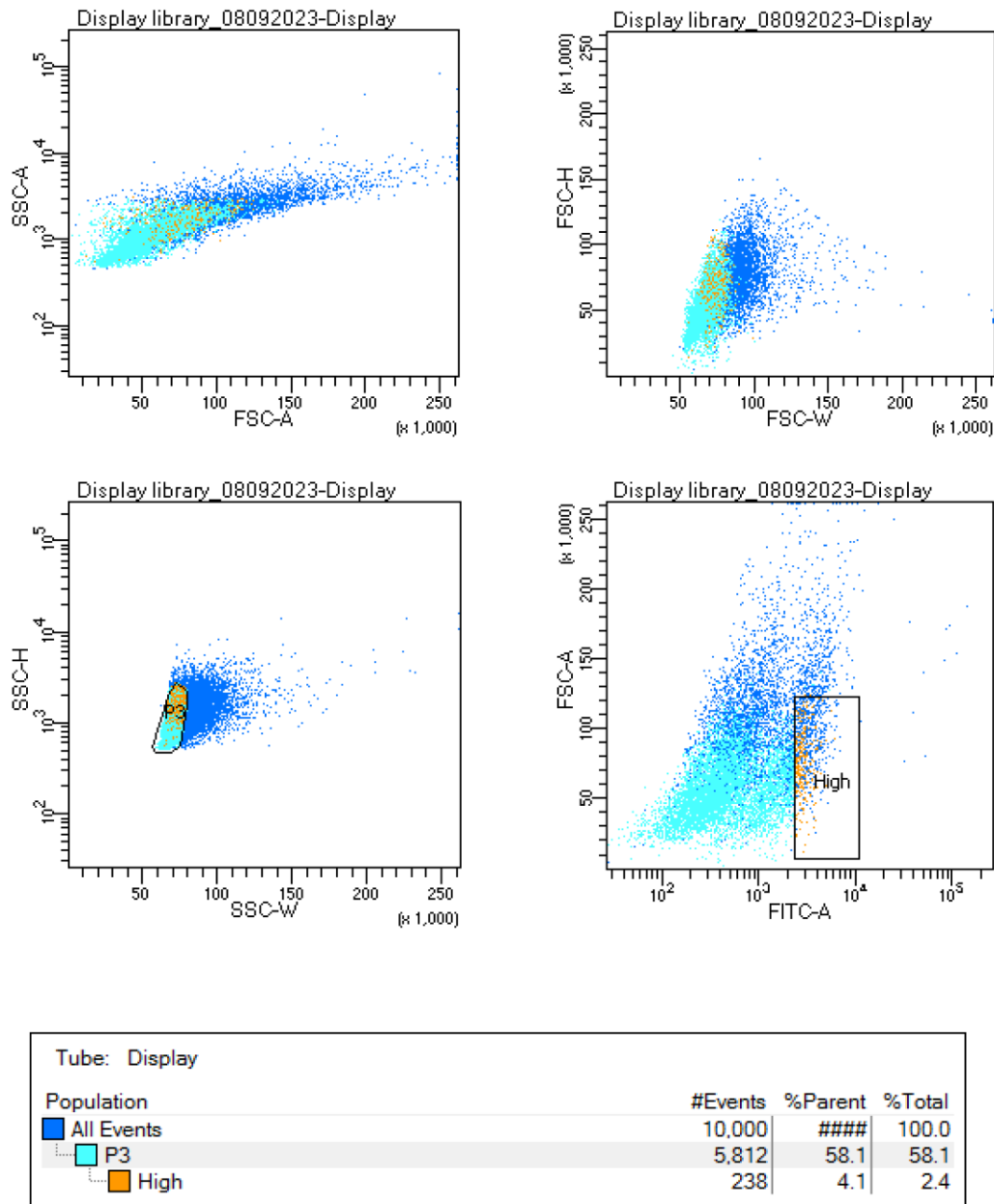
**Table S9.** Parts used to assemble the SmTG secretion construct

**Table S10:** Amino acid sequences of the secreted enzymes

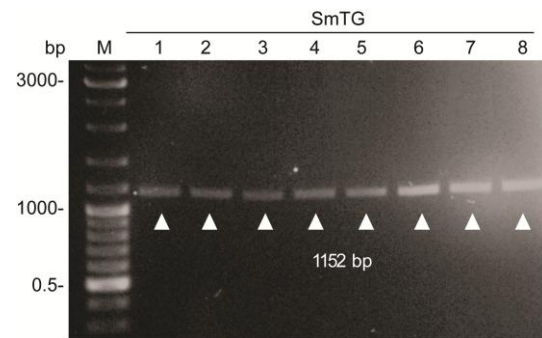
**Table S11:** The data used to generate the Ashby plot shown in Figure S10

**References**

# BD FACSDiva 8.0.3



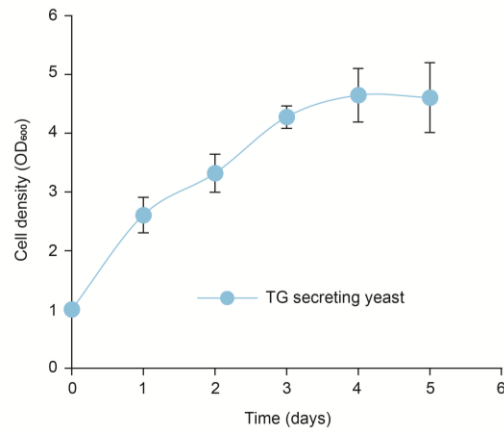
**Supplementary Figure 1. FACS gating strategy for sorting the TG display library.** Yeast cells were gated based on morphology (FSC-A vs SSC-A), followed by selection of single cells to prevent sorting of doublets (FSC-W vs FSC-H and SSC-W vs SSC-H). The gate P3 was generated in plot SSC-W vs SSC-H, but the outline of the gate was based on all three plots: FSC-A vs SSC-A, FSC-W vs FSC-H, and SSC-W vs SSC-H. Finally, P3 gated events with the highest fluorescence intensity were sorted from the “High” gate (FITC-A vs FSC-A). Total events and the percentages of the gated populations are shown in the table at the bottom of the figure.



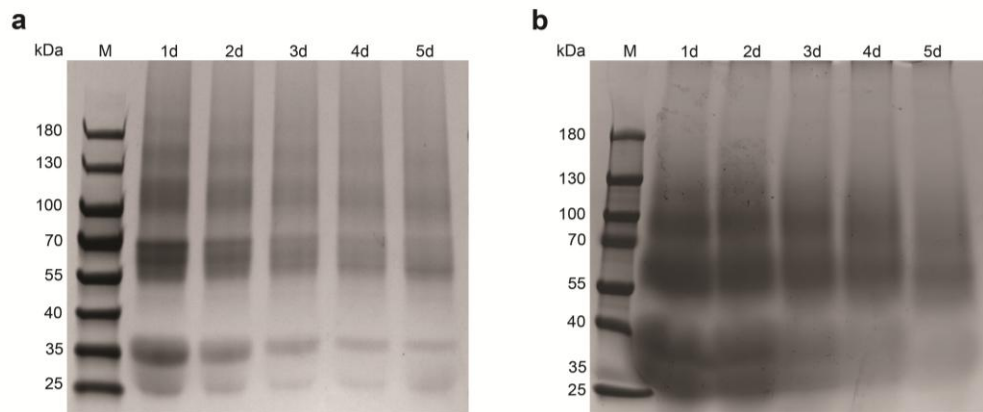
**Supplementary Figure 2. Colony PCR of *S. cerevisiae* yAK003 transformed with *S. mobaraensis* TG.** Lane M = GeneRuler DNA Ladder Mix, Thermo Scientific. Lanes 1–8 = PCR products of expected size (1152 bp), indicated with white arrow.



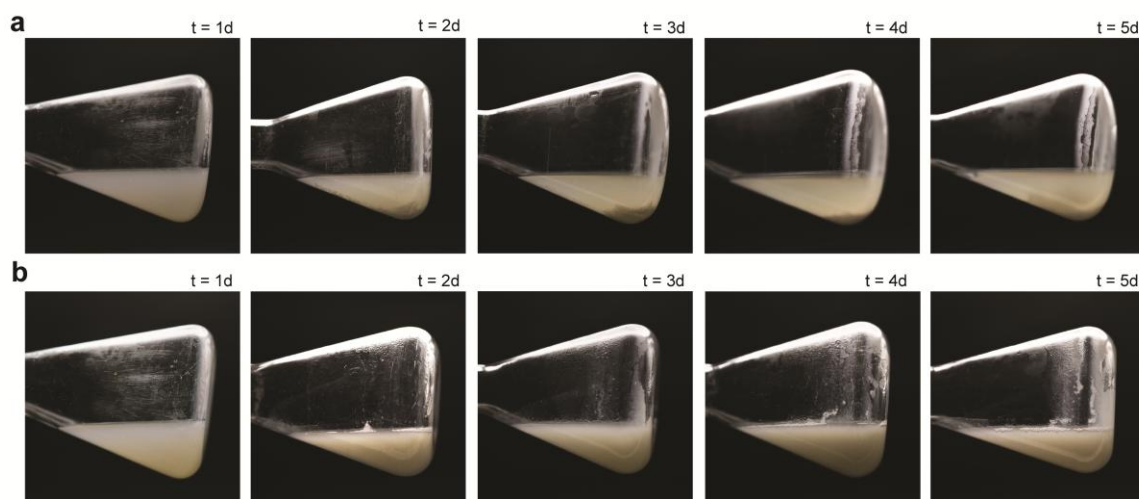
**Supplementary Figure 3. Photographs of stationary yeast cultivations.** The engineered yeast was suspended in MM for a final cell density ( $OD_{600}$ ) of 1.0 and cultivated under stationary cultivation conditions for five days. Galactose was supplied daily for a final concentration of 2 wt.%. The photographs visualize the cell sedimentation. The white arrows indicate the sedimented cell mass.



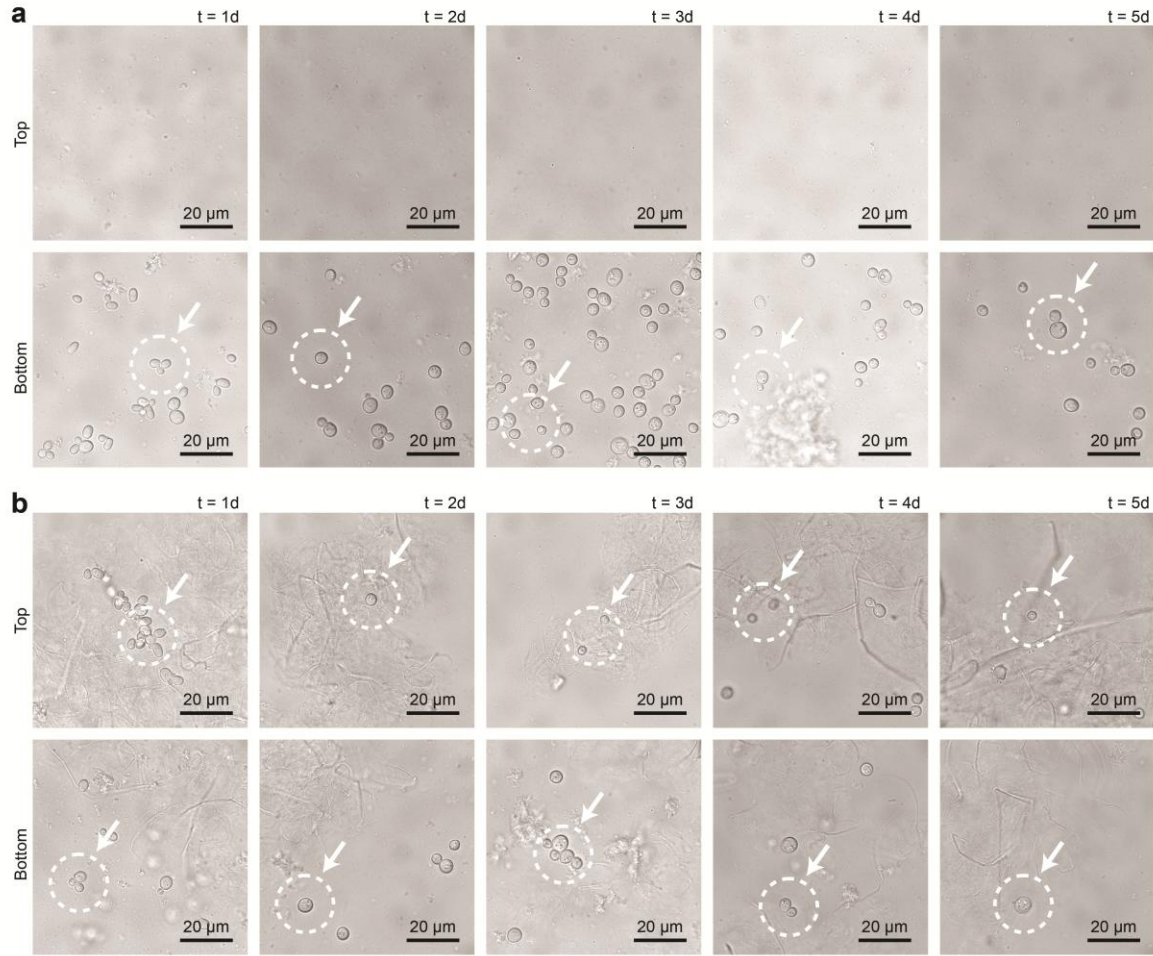
**Supplementary Figure 4. Cell density in the stationary yeast cultivations.** The engineered yeast was suspended in MM for a final cell density (OD<sub>600</sub>) of 1.0 and cultivated under stationary cultivation conditions for five days. Galactose (2 wt.%) was supplied daily to the cells. The cell density (OD<sub>600</sub>) values represent the average of three biological replicates.



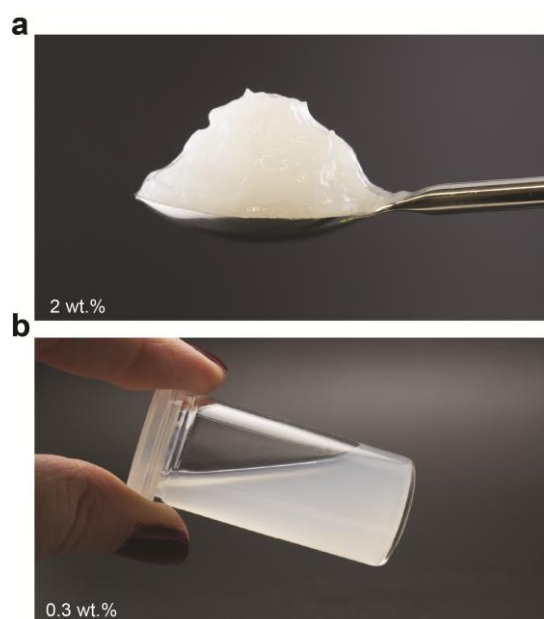
**Supplementary Figure 5. SDS-PAGE of the casein-containing control yeast cultivations.** SDS-PAGE analysis of the yeast culture broth containing a) 2 wt.% casein, and b) 2 wt.% casein and 0.3 wt.% CNFs. In both images, lane M = Thermo Scientific PageRuler Pre-stained protein ladder (10  $\mu$ L), and the other lanes contain the yeast culture supernatant, 2.5  $\mu$ L (a), and 5  $\mu$ L (b) from 1 to 5 days of cultivation, as indicated.



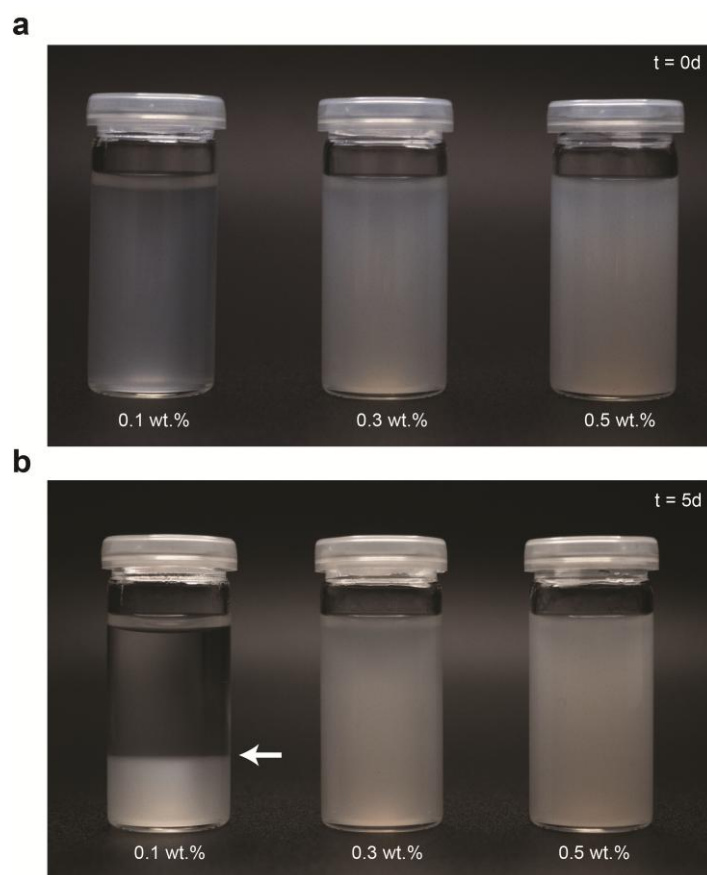
**Supplementary Figure 6. Photographs of the casein-containing control yeast cultivations.** The control yeast yAK003 was suspended in MM for a final cell density ( $OD_{600}$ ) of 1.0 and cultivated under stationary cultivation conditions for five days. The cultivations contained either a) 2 wt.% casein, or b) 2 wt.% casein and 0.3 wt.% CNFs. The cells in both cultivations were supplied with galactose for a final concentration of 2 wt.%.



**Supplementary Figure 7. Light microscopy images of the control yeast cultivations.** The control yeast yAK003 was suspended in MM for a final cell density ( $\text{OD}_{600}$ ) of 1.0 and cultivated under stationary cultivation conditions for five days. The cultivations contained either a) 2 wt.% casein, or b) 2 wt.% casein and 0.3 wt.% CNFs. Samples were collected daily from the top and bottom layers of the cultivation broth for imaging. The white arrows mark the presence of the yeast cells.



**Supplementary Figure 8. Photographs of the prepared cellulose nanofibrils (CNFs).** The CNFs were prepared from wood pulp fibres by mechanical disintegration and dispersed in water. At solid content of a) 2 wt.% the CNFs showed gel-like, self-standing behaviour, while b) a more diluted dispersion with solid content of 0.3 wt.% behaved as a water-like liquid.



**Supplementary Figure 9. Sedimentation test of the CNF dispersions.** The CNFs were dispersed in water for final solid contents of 0.1, 0.3 and 0.5 wt.% after which the vials containing the dispersions were left to stand undisturbed for five days. The photographs show the dispersions a) immediately after preparation, and b) after five days. The white arrow indicates CNF sedimentation in the unstable dispersion.

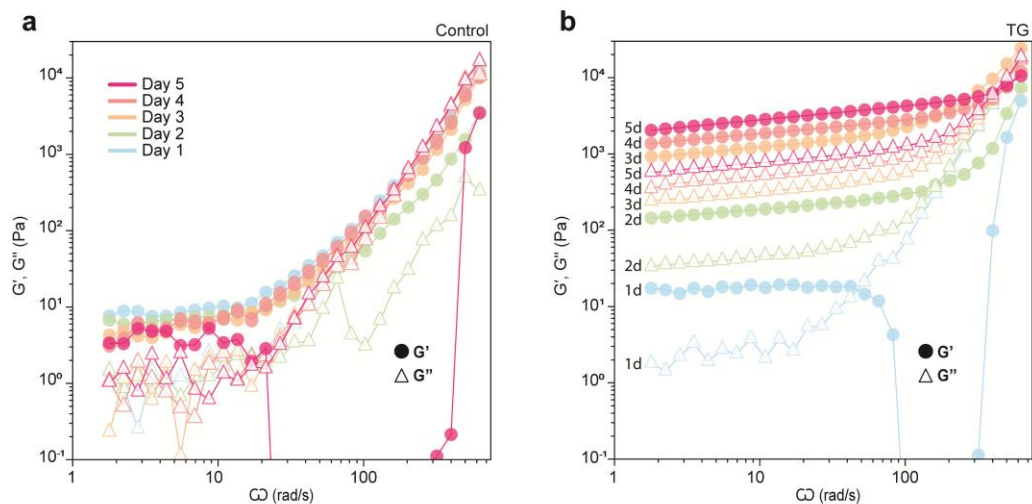


**Supplementary Figure 10. Photographs of stationary CNF-containing yeast cultivations.** The engineered yeast was suspended in MM for a final cell density ( $OD_{600}$ ) of 1.0 and cultivated under stationary cultivation conditions for five days. The cultivations contained 0.3 wt.% CNFs as dispersing agents for the cells. Galactose was supplied daily for a final concentration of 2 wt.%. The photographs visualize the lack of cell sedimentation. The white arrows indicate the bottom of the flask where no sedimented cell mass can be seen.

**Supplementary Table 1.** Dry weight and water content of the living hydrogels.

| Cultivation time (days) | Dry weight (wt.%) <sup>†</sup> | Water content (wt.%) |
|-------------------------|--------------------------------|----------------------|
| 1                       | 4.19 ± 0.01                    | 95.81                |
| 2                       | 4.25 ± 0.01                    | 95.75                |
| 3                       | 4.51 ± 0.02                    | 95.49                |
| 4                       | 5.16 ± 0.02                    | 94.84                |
| 5                       | 6.05 ± 0.01                    | 93.95                |

<sup>†</sup>The dry weights were obtained after oven drying at 105°C. The average values and their standard deviations represent three biological replicates.



**Supplementary Figure 11. Frequency sweeps of the yeast cultures containing casein and CNFs showing all the collected data points up to  $628 \text{ rad s}^{-1}$ .** The frequency sweeps were performed daily for both the a) control yeast, and b) engineered yeast. The storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) were plotted as a function of frequency ( $\text{rad s}^{-1}$ ). The measurements were conducted with a constant strain rate of 0.1% at  $23^\circ\text{C}$ .

**Supplementary Table 2.** Compressive properties of the living hydrogel.

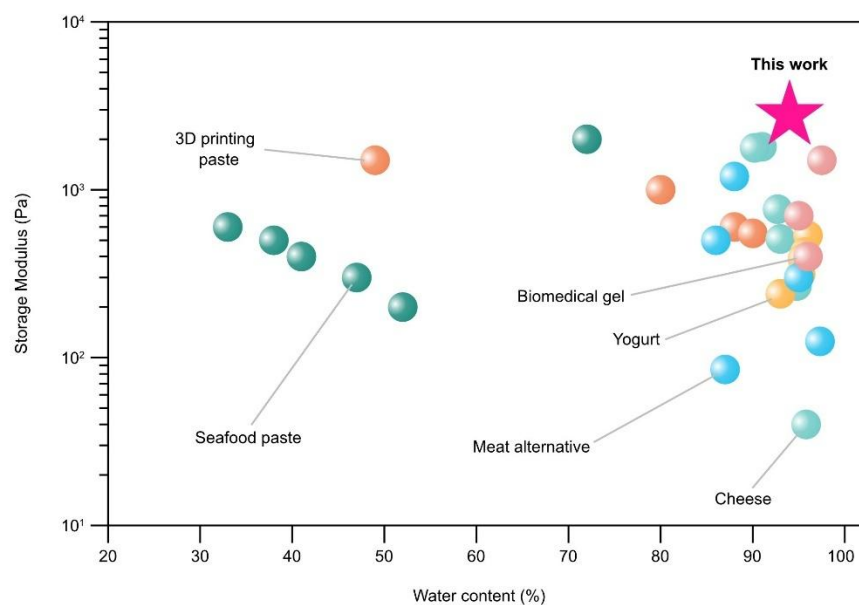
| Cultivation time (days) | Compressive strength (Pa) | Elastic modulus (Pa) |
|-------------------------|---------------------------|----------------------|
| 1                       | nd. <sup>‡</sup>          | nd.                  |
| 2                       | nd.                       | nd.                  |
| 3                       | 456                       | 353                  |
| 4                       | 818                       | 553                  |
| 5                       | 914                       | 577                  |

<sup>†</sup>Reported at strain rate of 50%.

<sup>‡</sup>Not determined.

**Supplementary Table 3.** Data used to generate the Ashby plot in Figure 4e.

| <b>Crosslinker</b> | <b>Storage modulus (Pa)</b> | <b>Water content (%)</b> | <b>Reference</b> |
|--------------------|-----------------------------|--------------------------|------------------|
| Engineered yeast   | 2840                        | 94.0                     | This work*       |
| TG                 | 1099                        | 94.0                     | 1                |
| TG                 | 40                          | 85.0                     | 2                |
| TG                 | 1000                        | 84.2                     | 3                |
| TG                 | 60                          | 86.0                     | 4                |
| TG                 | 500                         | 87.0                     | 5                |
| TG                 | 80                          | 96.0                     | 6                |
| Glutaraldehyde     | 1000                        | 99.8                     | 7                |
| Glutaraldehyde     | 35                          | 99.5                     | 8                |
| Glutaraldehyde     | 30                          | 90.0                     | 9                |
| Glutaraldehyde     | 40                          | 99.5                     | 10               |
| Glutaraldehyde     | 400                         | 91.5                     | 11               |
| Genipin            | 80                          | 95.0                     | 12               |
| Genipin            | 1635                        | 90.0                     | 13               |
| Genipin            | 200                         | 92.0                     | 14               |
| Genipin            | 250                         | 85.0                     | 15               |
| Genipin            | 140                         | 85.0                     | 15               |
| EDC NHS            | 2000                        | 89.0                     | 16               |
| EDC NHS            | 1750                        | 99.7                     | 17               |
| EDC NHS            | 2200                        | 99.0                     | 18               |
| Click chemistry    | 200                         | 95.0                     | 19               |
| Click chemistry    | 600                         | 88.0                     | 20               |
| Click chemistry    | 1000                        | 88.0                     | 21               |
| Click chemistry    | 1000                        | 95.0                     | 21               |



**Supplementary Figure 12. Ashby plot.** Storage modulus plotted against water content of the living hydrogel, as compared with other hydrogel materials. The values used to generate the Ashby plot are presented in Supporting Table 11.

**Supplementary Table 4.** Amino acid sequences of the surface displayed TGs.

| Name and annotation                                                                      | Amino acid sequence <sup>†</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>BaTG display</b><br>SP w/ Kex2–BaTG–CBM3a–<br>Linker–AGA2–Linker–HA-tag               | MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAVL PFSNSTNNGLLFI<br>NTTIASIAAKEEGVSLDKREAEASMIHSGQVLRPQDIANWQTEESLVPYLNELINSPVQFDYGS<br>TELMFEARLRHIVEAARELHGSGAKFATFAKTYGNTAYWRVTPEGALELKYRIPASKAIRDIE<br>NGAFYAFECATAIVVIYYLAVLKTIGEERFDRFRDITLYDWHYEHLPIYTETGRHFLRGDCLYF<br>KNPEFNPAKAQWRGENVIVMGNDQYFAHGLGILTAEQIIQRLNSLRKNAVQSAYLLSQATRL<br>DAPALYQIMHGSANTPVSGNLKVEFYNSNPSTTTNSINPQFKVTNTGSSAIDL SKLTLRYYTVD<br>DGQKDQTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNNADTYLEISFTGGTLEPGAHVQ<br>IQGRFAKNDWSNYTQSNDSYFKSASQFVEWDQVTAYLNGVLVWGKEPGGSGSGSGSGSGS<br>GSQELTTICEQIPSPSTLESTPYSLSTTTILANGKAMQGVFEYYKSVTFVSNCGSHPSTTSKGSPI<br>N TQYVFGSGSGSYPYDVPDYA*                                                                                                                                                        |
| <b>BsTG display</b><br>SP w/ Kex2–BsTG–CBM3a–<br>Linker–AGA2–Linker–HA-tag               | MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAVL PFSNSTNNGLLFI<br>NTTIASIAAKEEGVSLDKREAEASMIHSGQLLRPQDIENWQIDQDLNPLLKEMIETPVQFDYHS<br>IAEMLFELKLRMNIVAAAKTLHKSGAKFATFLKTYGNTTYWRVSPEGALELKYRMPPSKAIRDI<br>AENGPFYAFECATAIVVIYYLALIDTIGEDKFNASFDRIILYDWHYEKLPIYTETGHHFFLGDCLYF<br>KNPEFDQPKAQWRGENVILLGEDKYFAHGLGILNGKQIIDKLNSFRKKGALQSAAYLLSQATRL<br>DVPSLFRIVRGSANTPVSGNLKVEFYNSNPSTTTNSINPQFKVTNTGSSAIDL SKLTLRYYTVD<br>GQKDQTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNNADTYLEISFTGGTLEPGAHVQ<br>IQGRFAKNDWSNYTQSNDSYFKSASQFVEWDQVTAYLNGVLVWGKEPGGSGSGSGSGSGSGS<br>GSQELTTICEQIPSPSTLESTPYSLSTTTILANGKAMQGVFEYYKSVTFVSNCGSHPSTTSKGSPI<br>N TQYVFGSGSGSYPYDVPDYA*                                                                                                                                                 |
| <b>PaTG display</b><br>SP w/ Kex2–PaTG–CBM3a–<br>Linker–AGA2–Linker–HA-tag               | MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAVL PFSNSTNNGLLFI<br>NTTIASIAAKEEGVSLDKREAEASRLGPLWSLPMPGNKGVTLSESMA PGDIAELGRSAELAFR<br>VRFEGALPPREQLYWRALTMERFDGRRWAQAPQWSGEDALHWQKRGPELRYDVIMQPSSQP<br>WLFALDVAQTDQTDTRLMSDFHLQRRQPVEQRLFYRVSSWPQALRESSIDPRTRWRNLQLPM<br>HGNPRARALADELRQAHAQPQALVAALLQRFNHEPFAYTLKPPATGADGVDDFLDTRSGFCA<br>HYAGAMAFVLRAAGIPARVVAGYQGELNPAGNYLLVHQFDAHAWVEYWQPEQGWSVDP<br>TYQVAPERIEQGGLQALAGDSEYLADAPLSPLRYRGLPWLNDMRLAWDSLNYGWQRWVLAY<br>QGEQQGAFLQRWFGGLDPGSANTPVSGNLKVEFYNSNPSTTTNSINPQFKVTNTGSSAIDL SK<br>LTLRYYTVDGQKDQTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNNADTYLEISFTGG<br>TLEPGAHVQIQGRFAKNDWSNYTQSNDSYFKSASQFVEWDQVTAYLNGVLVWGKEPGGSGS<br>GSGSGSGSGSGSQELTTICEQIPSPSTLESTPYSLSTTTILANGKAMQGVFEYYKSVTFVSNCGSH<br>PSTTSKGSPI NTQYVFGSGSGSYPYDVPDYA*                    |
| <b>SmTG display</b><br>SP w/o Kex2–Pro-Kex2–SmTG–<br>CBM3a–Linker–AGA2–Linker–<br>HA-tag | MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAVL PFSNSTNNGLLFI<br>NTTIASIAAKEEGVSGDNGAGEETKSYAETYRLTADDVANINALQESAPAASSAGPSFRAPSLD<br>KREAEADSDDRVTPPAEPLDRMPDPYRPSYGRAETVVNNYIRK WQQVYSHRDGRKQQMTEE<br>QREWLSYGCVGVTWVNSGQYPTNRLAFASFDEDRFKNELKNGRPRSGETRAEFEGRVAKESF<br>DEEKGFQRRAREVASVMNRALENAHDESAYLDNLKKELANGNDALRNEDARSPFYALSALRNTPS<br>FKERNNGNHDPSRMKAVIYSKHFVWSGQDRSSADKRKYGDPDAFRPAPGTGLVDMSRDRNIP<br>RSPTSPGEGFVNFDYGWFGAQTEADADKTVWTHGNHYHAPNGSLGAMHVVYESKFRQWSEG<br>YSDFDRGAYVITFIPKSWNTAPDKVKQGWPGSANTPVSGNLKVEFYNSNPSTTTNSINPQFKV<br>TNGSSAIDL SKLTLRYYTVDGQKDQTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNN<br>ADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSNDSYFKSASQFVEWDQVTAYLNGVL<br>VWGKEPGGSGSGSGSGSGSGSGSQELTTICEQIPSPSTLESTPYSLSTTTILANGKAMQGVFEYYK<br>SVTFVSNCGSHPSTTSKGSPI NTQYVFGSGSGSYPYDVPDYA* |

<sup>†</sup>Minimal, flexible AS and GS linkers are indicated with light grey. The asterisk symbol (\*) signifies the stop codon.

**Supplementary Table 5. Nucleotide sequences of the synthetic genes and their flanking regions.<sup>†</sup>**

| Gene                           | Codon-optimised nucleotide sequence <sup>‡</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Signal peptide w/ Kex2</b>  | GCATCGTCTCATCGGTCTCATATGAGATTCCCATCTATTTTACAGCTGTTTTGTTTGCTGCATCTTCAGCATTAGCTG<br>CACCAGTTAATACTACAACCTGAAGATGAACTGCTCAAATTCAGCTGAAGCAGTTATTGGTTATTGGATTGGAG<br>GGTGACTTTGATGTTGCAGTTTGGCATTCTTAACCTCAACAAACAACGGTTTGGTTTATTAATACAACATTGGCT<br>TCTATTGCTGCAAAGGAAGAAGGTGTTTCATTGGATAAGAGAGAAGCAGAAAGCTTCTGAGACCTGAGACGGCAT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Signal peptide w/o Kex2</b> | GCATCGTCTCATCGGTCTCATATGAGATTCCCATCTATTTTACAGCTGTTTTGTTTGCTGCATCTTCAGCATTAGCTG<br>CACCAGTTAATACTACAACCTGAAGATGAACTGCTCAAATTCAGCTGAAGCAGTTATTGGTTATTGGATTGGAG<br>GGTGACTTTGATGTTGCAGTTTGGCATTCTTAACCTCAACAAACAACGGTTTGGTTTATTAATACAACATTGGCT<br>TCAATTGCTGCAAAGGAAGAAGGTGTTGGTCTTCTGAGACCTGAGACGGCAT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>BaTG</b>                    | GCATCGTCTCATCGGTCTCATTTCTATGATCATCATCTCTGGTCAAGTTTTAAGACCACAAGATATCGAAACTGGCAA<br>ACAGAAGAATCATTGGTTCCATATTTGAACGAATTGATTAATTCTCCAGTTCAATTCGATTACGGTTCAATCACTGAA<br>TTGATGTTTGAAGCTAGATTAAGAAGACATATCGTTGAAGCTGCAAGAGAATTGCATGGTTCTGGTGCTAAGTTCCG<br>AATCTTCGCTAAGACATACGGTAACACTGCATACCTGGAGAGTTACACCAGAAGGTGCTTTGGAAATTGAAGTACAGA<br>ATCCCAGCATCAAAGGCTATCAGAGATATCATCGAAAACGGTGCATTCTACGCTTTTGAATGTGCTACAGCAATCGT<br>TGTTATATATTCTGGCTGTTTGAAGACTATCGGTGAAGAAAGATTTCGATAGAAGATTTCAGAGATATCACTTTGTA<br>TGATTGGCATTACGAACATTGGCAATCTATACTGAAACAGGTAGACATTCTTGAGAGGTGACTGTTTGTAATCTCA<br>AGAACCCAGAATTCAATCCAGCTAAGGCACAATGGAGAGGTGAAAACGTTATCGTTATGGGTAACGATCAATACTT<br>CGCACATGGTTTGGGTATCTTGACAGCTGAACAAATCATCAAAGATTGAACCTTTTGAGAAGAAAGAATGCAGTT<br>CAATCTGCTTATTGTTATCACAAGCTACTAGATTGGATGCTCCAGCATTATACCAAATTATGCATGGATCTCTGAGACC<br>TGAGACGGCAT                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>BsTG</b>                    | GCATCGTCTCATCGGTCTCATTTCTATGATCATTGTTTCTGGTCAATTGTTAAGACCACAAGATATCGAAACTGGCAA<br>ATCGATCAAGATTGGAACCATTTGTTGAAGGAAATGATCGAAACTCCAGTTCAATTCGATTACCACTTCTATTGCAGA<br>ATTGATGTTTGAAGTTGAAGTTGAGAATGAATATTGTTGCTGCAGCTAAGACATTGCATAAGTCAGGTGCAAAGTTCCG<br>CTACATTTTGAAGACATACGGTAACACTACATACTGGAGAGTTTCTCCAGAAGGTGCATTGGAATTGAAGTACAGA<br>ATGCCACCATCAAAGGCAATCAGAGATATCGCTGAAAATGGTCCATTTTACGCTTTTGAATGTGCAACTGCTATCGT<br>TATCATCTATTACTTGGCATTGATCGATACAATCGGTGAGGATAAGTTTAATGCTTCATTTCGATAGAATCATTTTGTATG<br>ATTGGCATTACGAAAAGTTGCCAATCTATACTGAAACAGGTCATCATTTCTTTTGGGTGACTGTTTGTACTTCAAGA<br>ACCCGAATTTCGATCCCAAAAAGCAATGAGAGGTGAAAACGTTATCTTGTGGTGAGGATAAGTACTTCCG<br>TCATGTTTGGGTATCTTGAACGGTAAACAAATCATCGATAAATTGAACCTTTTGAAGAAGAAAGGTGCATTGCAAT<br>CTGCTTACTTGTATCACAAGCTACTAGATTGGATGTTCCATCTTTGTTTAGAATTGTTAGAAGATCTCTGAGACCTGA<br>GACGGCAT                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>PaTG</b>                    | GCATCGTCTCATCGGTCTCATTTCTAGATTGGGTCCATTATGGTCTTTGCCAATGCCAGGTAATAAGGGTGTTACTGGT<br>TTATCTGAATCAATGGCTCCAGGTGACATTGCAGAAATTAGGTAGATCAGCTGAATTGGCTTTTAGAGTTAGATTGA<br>AGGTGCTTTGCCACCAAGAGACAATTATATTGGAGAGCATTGACAAATGGAAGATTGATGGTAGAAGATTGGGCT<br>CAAGCACCACAATGGTCTGGTGAAGATGCTTTACATTGGCAAAAAGAGAGGTCCAGAATTGAGATACGATGTTATTAT<br>GCAACCATCTTCACAACCATGGTTATTTGCTTTGGATGTTGCACAACTGATCAAACGATACAAAGATTAATGTCAG<br>ATTTCATTTGCAAGAAGACAACCAAGTTGAACAAAGATTATTTTATAGAGTTTCTTCATGGCCACAAGCTTTGAGA<br>GAATCTTCAATTGATCCAAGAACAAGATGGAGAAATTTGCAATTACCAATGCATGGTAATCCAAGAGCTAGAGCATT<br>AGCTGATGAATTGAGACAAGCAGATGCTCAACCACAAGCTTTAGTTGCTGCATTGTCAGAAAGATTCAATCATGAA<br>CCATTCCGATACATTGAAAGCACCAGCTACAGGTGCAGATGGTTGATGATTCTTCTGATAGATGATGTTGCTGCTC<br>TTCTGTGCTCATTACGAGGTGCTATGGCATTGTTTGAAGAGCTGCAGGTATTCCAGCTAGAGTTGTTGCAGGTAT<br>CAAGGTGGTGAATTGAATCCAGCTGGTAACTACTTGTGGTTTCATCAATTCGATGCTCATGATGGGTGGAATATTGG<br>CAACCAAGAACAAGGTGGTTATCTGTTGATCCACATCAAGATTGCTCCAGAAAGAGTTGCTCCAGAAAGTTGAAC<br>AAGCTTTGGCTGGTGACTCTGAATATTGGCTGATGCACCAATTATCACCATTGAGATACAGAGGTTTACCATGGTTG<br>AATGATATGAGATTAGCTTGGGATTCATTGAATTATGGTTGGCAAAAGATGGGTTTGGCTTACCAAGGTGAACAACA<br>AGGTGCATTTTACAAAGATGGTTTGGTGGTTTGGATCCAAGATCTCTGAGACCTGAGACGGCAT                                                       |
| <b>SmTG</b>                    | GCATCGTCTCATCGGTCTCATTTCTGATAATGGTGCTGGTGAAGAACTAAGTCATACGCAGAACTTACAGATTGAC<br>AGCTGATGATGTTGCAAAACATCAACGCTTTGCAAGAATCTGCACCAGCTGCATCTTCAGCTGGTCCATCTTTTAGAG<br>CACCATCTTTGGATAAAAGAGAAGCTGAAGCAGATTGATGATAGAGTTACTCCAGCTGAACCAATTAGATAG<br>AATGCCAGATCCATATAGACCATACACGGTAGAGCAGAAACAGTTGTTAACAACATACATCAGAAAGTGGAACAA<br>GTTTACTCTCATAGAGATGGTAGAAAACAACAAATGACTGAAGAACAAGAGAATGGTTGTCATATGGTTGTGTTG<br>GTGTTACTTGGGTAAATTCTGGTCAATACCCAACAATAGATTGGCTTTTGCATCTTTTCGATGAAGATAGATTCAAAA<br>ACGAATTGAAAAATGGTAGACCAAGATCAGGTGAACAAGAGCTGAATTGAAGGTAGAGTTGCAAGGAATCTT<br>TCGATGAAGAAAAGGGTTTTCAAGAGCAAGAGAAGTTGCTTCAGTTATGAATAGAGCATTGGAAAATGCTCATGA<br>TGAATCTGCTTATTGGATAATTGAAGAAAGAAATGGCAACGGTAACGATGCTTTGAGAAAACGAAGATGCTAGAT<br>CACCATTTTACTCTGCATTGAGAAACACTCCATCTTTAAAGAAAGAAACGGTGGTAACCATGATCCATCTAGAATG<br>AAGGCTGTTATATATCAAAGCATTTTTGGTCTGGTCAAGATAGATCTTCATCTGCTGATAAGAGAAAGTACGGTGA<br>CCCAGATGCTTTTAGACCAGCTCCAGGTACTGGTTTGGTTGATATGCTAGAGATAGAAACATCCAAAGATCACC<br>CATCTCCAGGTGAAGGTTTCGTTAACTTCGATTACGGTTGGTTTGGTGTCTAAACAGAAAGCTGATGCAGATAAAC<br>TGTTTGGACACATGGTAATCATTATCATGCTCCAAATGGTTTCATTAGGTGCAATGCATGTTTACGAATCTAAGTTT<br>ACAATGGTCAGAAAGTTATCTGATTGTTGATAGAGGTGCTTACGTTATCATCTTTTATTCCAAAGTCTTGGAAACAGC<br>ACCAGATAAAGTTAAACAAGGTGGCCAAGATCTCTGAGACCTGAGACGGCAT |
| <b>(His)<sub>6</sub>tag</b>    | CGTAGATCGTCTCATCGGTCTCATATCCATCATCATCATCACTAAAGGCTGAGACCTGAGACGGCATCAGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

<sup>†</sup>The flanking regions are marked with the following colours: Red = BsaI binding site, Turquoise = BsmBI binding site, Yellow = part-type specific overhang, Green = nucleotides added for integration into the YTK entry vector, Grey = variable nucleotides, Orange = nucleotides added to retain the native reading frame. Once the construct is assembled, they will encode part of a flexible GS or AS linker.

<sup>‡</sup>The nucleotide sequences were codon-optimised for *S. cerevisiae*.

```

1  GGTCTCATAT GAGATTCCCA TCTATTTTTC CAGCTGTTTT GTTGTCTGCA TCTTCAGCAT TAGCTGCACC AGTTAATACT ACAACTGAAG ATGAAACTGC
101 TCAAATTCCT GCTGAAGCAG TTATTGGTTA TTTGGATTTG GAGGGTGACT TTGATGTTGC AGTTTGGCCA TTTTCTAACT CAACAAACAA CGGTTTGTGT
201 TTTATTAAATA CAACTATTGC TTCATTGCTC GCAAAGGAAG AAGGTGTTTC ATTGATAAAG AGAGAAGCAG AAGCTTCTTG AGACCAGACC AATAAAAAAC
301 GCCCGGCGCG AACCGAGCGT TCTGAACAAA TCCAGATGGA GTTCTGAGGT CATTACTGGA TCTATCAACA GGAGTCCAAG CGAGCTCGAT ATCAAATTAC
401 GCGCCGCGCT GCCACTCATC GCAGTACTGT TGTAATTCAT TAAGCATTCT GCCGACATGG AAGCCATCAC AAACGGCATG ATGAACCTGA ATCGCCAGCG
501 GCATCAGCAC CTTGTGCGCT TGCGTATAAT ATTTGCCCAT GGTGAAACG GGGGCGAAGA AGTTGTCCAT ATTTGGCCAGG TTTAAATCAA AACTGGTGAA
601 ACTCACCCAG GGATTGGCTG AAACGAAAAA CATATTCTCA ATAAACCTTT TAGGGAAATA GGCCAGGTTT TCACCGTAAC ACGCCACATC TTGCGAATAT
701 ATGTGTAGAA ACTGCCGGAA ATCGTCGTGG TATTCACCTC AGAGCGATGA AAACGTTTCA GTTGTCTCAT GGAAAAACGGT GTAACAAGGG TGAACACTAT
801 CCCATATCAC CAGCTCACCG TCTTTCATTG CCATACGAAA TTCCGGATGA GCATTTCATCA GCGGGGCAAG AATGTGAATA AAGGCCGGAT AAAACTTGTG
901 CTTATTTTTC TTTACGGTCT TAAAAAGGC CGTAATATCC AGCTGAACGG TCTGGTTATA GGTACATTGA GCAACTGACT GAAATGCCTC AAAATGTTCT
1001 TTACGATGCC ATTTGGATAT ATCAACGGTG GTATATCCAG TGATTTTTTT CTCCATTTTA GCTTCCTTAG CTCCTGAAAA TCTCGATAAC TCAAAAAATA
1101 CGCCCGGTAG TGATCTTATT TCATTATGTT GAAAGTTGGA ACCTCTTACG TGCCCGATCA ATCATGACCA AAATCCCTTA ACGTGAGTTT TCGTCCACT
1201 GAGCGTCAGA CCCCGTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAACA AAAAAACCA CGCTACCAAG
1301 GGTGGTTTGT TTGCCGGATC AAGAGCTACC AACTCTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAATA CTGTCTTCT AGTGTAGCCG
1401 TAGTTAGGCC ACCACTTCAA GAACCTCTGA GCACCGCCTA CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC
1501 TTACCGGGTT GGACTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCTGT CACACAGCCC AGCTTGGAGC GAACGACCTA
1601 CACCGAACTG AGATACCTAC AGCGTGAGCT ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG GGTGCGAACA
1701 GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG TCCTGTGCGG TTTGCGCAC TCTGACTTGA GCGTCGATTT TTGTGATGCT
1801 CGTCAGGGGG GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTCTCTGG CCTTTTGCTG GCCTTTTGCT CACATGTTCT TTCCTGCGTT
1901 ATCCCTGAT TCTGTGGATA ACCGTAGTC

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**Supplementary Figure 13. The nucleotide sequence for the part plasmid pATK002.** The part plasmid was generated by cloning the synthesised yeast  $\alpha$ -mating factor secretion signal with the Kex2 site into the YTK entry vector pYTK001. The insert is highlighted with orange colour.

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1 GGTCTCATAT GAGATTCCCA TCTATTTTTC CAGCTGTTTT GTTGTGTCGA TCTTCAGCAT TAGCTGCACC AGTTAATACT ACAACTGAAG ATGAAACTGC
101 TCAAAATCCA GCTGAAGCAG TTATTGGTTA TTTGGATTG GAGGGTGACT TTGATGTTGC AGTTTGGCCA TTTTCTAACT CAACAAACAA CGGTTTGTG
201 TTTATTAATA CAACTATTGC TTCAATTGCT GCAAAAGAAG AAGGTGTGG TTCTTGAGAC CAGACCAATA AAAAACGCCC GCGGCAACC GAGCGTCTG
301 AACAAATCCA GATGGAGTTC TGAGGTCATT ACTGGATCTA TCAACAGGAG TCCAAGCGAG CTCGATATCA AATTACGCCC CGCCCTGCCA CTCATCGCAG
401 TACTGTTGTA ATTCAATTAAG CATTCTGCCG ACATGGAAGC CATCACAAAC GGCATGATGA ACCTGAATCG CCAGCGGCAT CAGCACCTTG TCGCCTTGCG
501 TATAATATTT GCCCATGGTG AAAACGGGGG CGAAGAAGTT GTCCATATTG GCCACGTTTA AATCAAAACT GGTGAAACTC ACCCAGGGAT TGGCTGAAAC
601 GAAAAACATA TTCTCAATAA ACCCTTTAGG GAAATAGGCC AGGTTTTTTC CGTAACACGC CACATCTTGC GAATATATGT GTAGAAACTG CCGGAAATCG
701 TCGTGGTATT CACTCCAGAG CGATGAAAAC GTTTCAGTTT GCTCATGGAA AACGGGTGAA CAAGGGTGAA CACTATCCCA TATCACCAGC TCACCGTCTT
801 TCATTGCCAT ACGAAATTCC GGATGAGCAT TCATCAGGCG GCAAGAATG TGAATAAAG CCGGATAAAA CTTGTGCTTA TTTTCTTTA CGGTCTTTAA
901 AAAGGCCGTA ATATCCAGCT GAACGGTCTG GTTATAGGTA CATTGAGCAA CTGACTGAAA TGCCTCAAAA TGTTCCTTAC GATGCCATTG GGATATATCA
1001 ACGGTGGTAT ATCCAGTGAT TTTTTCCTCC ATTTTAGCTT CCTTAGCTCC TGAAAATCTC GATAACTCAA AAAATACGCC CGGTAGTGAT CTTATTTTAT
1101 TATGGTGAAA GTTGGAACTT CTTACGTGCC CGATCAATCA TGACCAAAAT CCCTTAACGT GAGTTTTTCG TCCACTGAGC GTCAGACCCC GTAGAAAAGA
1201 TCAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA
1301 GCTACCAACT CTTTTTCCGA AGGTAAGTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC
1401 TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT GCGGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT
1501 AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG
1601 TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CCGCAGGGTC GGAACAGGAG AGCGCAGCAG GGAGCTTCCA
1701 GGGGGAAACG CCTGGTATCT TTATAGTCTT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA
1801 AAAACGCCAG CAACGCGGCC TTTTACCGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTTC TGCCTTATCC CCTGATTCTG TGGATAACCG
1901 TAGTC

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**Supplementary Figure 14. The nucleotide sequence for the part plasmid pATK003.** The part plasmid was generated by cloning the synthesised yeast  $\alpha$ -mating factor secretion signal without the Kex2 site encoding gene into the YTK entry vector pYTK001. The insert is highlighted with purple colour.

```

1 GGTCTCATTC TATGATCATC ATCTCTGGTC AAGTTTAAAG ACCACAAGAT ATCGCAAACCT GGCAAAACAGA AGAATCATTG GTTCCATATT TGAACGAATT
101 GATTAATTCT CCAGTTCAAT TCGATTACGG TTCAATCACT GAATTGATGT TCGAAGCTAG ATTAAGAAGA CATATCGTTG AAGCTGCAAG AGAATTGCAT
201 GGTCTCTGGT CTAAGTTCCG AACTTTCGCT AAGACATACG GTAACACTGC ATACTGGAGA GTTACACCGA AAGGTGCTTT GGAATTGAAG TACAGAATCC
301 CAGCATCAAA GGCTATCAGA GATATCATCG AAAACGGTGC ATTTACGCT TTTGAAITGT CTACAGCAAT CGTTGTTATA TATTACTTGG CTGTTTGAAG
401 GACTATCGGT GAAGAAAGAT TCGATAGAAG ATTCAGAGAT ATCATTGTGT ATGATTGGCA TTACGAACAT TTGCCAATCT ATACTGAAAC AGGTAGACAT
501 TTCTTGAGAG GTGACTGTTT GTACTTCAAG AATCCAGAAAT TCAATCCAGC TAAGGCACAA TGGAGAGGTG AAAACGTTAT CGTTATGGGT AACGATCAAT
601 ACTTCGCACA TGGTTTGGGT ATCTTGACAG CTGAACAAAT CATCCAAAGA TTGAACTCTT TGAGAAGAAA GAATGCAGTT CAATCTGCTT ATTTGTTATC
701 ACAAGCTACT AGATTGGATG CTCCAGCATT ATACCAAATT ATGCATGGAT CCGTGAACCA GACCAATAAA AAACGCCCGG CGGCAACCGA GCGTCTGTAA
801 CAAATCCAGA TGGAGTTCTG AGGTCATTAC TGGATCTATC AACAGGAGTC CAAGCGAGCT CGATATCAAA TTACGCCCCG CCCTGCCACT CATCGCAGTA
901 CTGTTGTAAT TCATTAAGCA TTCTGCCGAC ATGGAAGCCA TCACAAACGG CATGATGAAC CTGAATCGCC AGCGGCATCA GCACCTTGTC GCCTTGCGTA
1001 TAATATTTCG CCATGGTGAA AACGGGGGCG AAGAAGTTGT CCATATTGGC CACGTTTAAA TCAAAACTGG TGAAACTCAC CCAGGGATTG GCTGAAACGA
1101 AAAACATATT CTCAATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA CATCTTGCGA ATATATGTGT AGAAACTGCC GGAAATCGTC
1201 GTGGTATTCA CTCAGAGCG ATGAAAACGT TTCAGTTTGC TCATGGAAAA CGGTGTAACA AGGGTGAACA CTATCCCATTA TCACCAGCTC ACCGTCTTTC
1301 ATTGCCATAC GAAATTCCGG ATGAGCATTC ATCAGGCGGG CAAGAATGTG AATAAAGGCC GGATAAAACT TGTGCTTATT TTTCTTTACG GTCTTTAAAA
1401 AGGCCGTAAT ATCCAGCTGA ACGGTCTGGT TATAGGTACA TTGAGCAACT GACTGAAATG CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC
1501 GGTGGTATAT CCAGTGATTT TTTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACTCAAAA AATACGCCCG GTAGTGATCT TATTTTCATTA
1601 TGGTGAAAGT TGGAACCTCT TACGTGCCCG ATCAATCATG ACCAAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC
1701 AAAGGATCTT CTTGAGATCC TTTTCTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTCCGG GATCAAGAGC
1801 TACCAACTCT TTTTCCGAAG GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTTT TTCTAGTGTA GCCCTAGTTA GGCCACCACT TCAAGAACTC
1901 TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCACTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGGACTC AAGACGATAG
2001 TTACCCGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG
2101 AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG
2201 GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA
2301 AACCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA
2401 CTC

```

**Supplementary Figure 15.** The nucleotide sequence for part the plasmid pATK004. The part plasmid was generated by cloning the synthesised BaTG encoding gene into the YTK entry vector pYTK001. The insert is highlighted with green colour.

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1 GGTCTCATTC TATGATCATT GTTCTTGGTC AATTGTTAAG ACCACAAGAT ATCGAAAAC TGGCAAATCGA TCAAGATTG AACCATTGT TGAAGGAAAT
101 GATCGAAACT CCAGTTCAAT TCGATTACCA TTCTATTGCA GAATTGATGT TCGAATTGAA GTTGAGAATG AATATTGTTG CTGCAGCTAA GACATTGCAT
201 AAGTCAGGTG CAAAGTTCGC TACATTTTGT AAGACATACG GTAACACATC ATACTGGAGA GTTCTCTCCG AAGGTGCATT GGAATTGAAG TACAGAATGC
301 CACCATCAAA GGCATCAGA GATATCGCTG AAAATGGTCC ATTTTACGCT TTCGAATGTG CAACTGCTAT CGTTATCATC TATTACTTGG CATTGATCGA
401 TACAATCGGT GAGGATAAGT TTAATGCTTC ATTGATAGA ATCATTGTGT ATGATTGGCA TTACGAAAAG TTGCCAATCT ATACTGAAAC AGGTCATCAT
501 TTCTTTTGG GTGACTGTTT GTACTTCAAG AACCCAGAAT TCGATCCACA AAAAGCACAA TGGAGAGGTG AAAACGTTAT CTTGTTGGGT GAGGATAAGT
601 ACTTCGCTCA TGGTTTGGGT ATCTTGAACG GTAAACAAAT CATCGATAAA TTGAACTCTT TTAGAAAAGAA AGGTGCATTG CAATCTGCTT ACTTGTTATC
701 ACAAGCTACT AGATTGGATG TTCCATCTTT GTTTAGAATT GTTAGAGGAT CGTGAGACCA GACCAATAAA AACGCCCCG CGGCAACCGA GCGTCTGTAA
801 CAAATCCAGA TGGAGTTCTG AGGTCATTAC TGGATCTATC AACAGGAGTC CAAGCGAGCT CGATATCAAA TTACGCCCCG CCCTGCCACT CATCGCAGTA
901 CTGTTGTAAT TCATTAAGCA TTCTGCCGAC ATGGAAGCCA TCACAAACGG CATGATGAAC CTGAATCGCC AGCGGCATCA GCACCTTGTC GCCTTGCGTA
1001 TAATATTGTC CCATGGTGAA AACGGGGCGG AAGAAGTTGT CCATATTGGC CACGTTTAAA TCAAAACTGG TGAAACTCAC CCAGGGATTG GCTGAAACGA
1101 AAAACATATT CTCAATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA CATCTTGCGA ATATATGTGT AGAAACTGCC GGAAATCGTC
1201 GTGGTATTCA CTCAGAGCG ATGAAAACGT TTCAGTTTGC TCATGGAAAA CGGTGTAACA AGGGTGAACA CTATCCCATC TCACCAGCTC ACCGTCTTTC
1301 ATTGCCATAC GAAATCCCG ATGAGCATTC ATCAGGCGGG CAAGAATGTG AATAAGGCC GGATAAAACT TGTGCTTATT TTTCTTTACG GTCTTTAAAA
1401 AGGCCGTAAAT ATCCAGCTGA ACGGTCGTGT TATAGGTACA TTGAGCAACT GACTGAAATG CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC
1501 GGTGGTATAT CCAAGTATTT TTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACTCAAAA AATACGCCCG GTAGTGATCT TATTTTATTA
1601 TGGTGAAAGT TGGAACCTCT TACGTGCCCG ATCAATCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC
1701 AAAGGATCTT CTTGAGATCC TTTTCTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTCCGG GATCAAGAGC
1801 TACCAACTCT TTTTCCGAG GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTTT TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC
1901 TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCACTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGGACTC AAGACGATAG
2001 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG
2101 AGCTATGAGA AAGCGCCACG CTTCCCAAG GGAGAAAGGC GGACAGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG
2201 GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG CCTATGAAAA
2301 AACCCAGCA ACGCGCCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA
2401 GTC

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**Supplementary Figure 16. The nucleotide sequence for the part plasmid pATK005.** The part plasmid was generated by cloning the synthesised BsTG encoding gene into the YTK entry vector pYTK001. The insert is highlighted with blue colour.

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1 GGTCTCATTC TAGATTGGGT CCATTATGGT CTTTGCCAAT GCCAGGTAAT AAGGGTGTTA CTGGTTTATC TGAATCAATG GCTCCAGGTG ACATTGCAGA
101 ATTAGGTAGA TCAGCTGAAT TGGCTTTTAG AGTTAGATTT GAAGGTGCTT TGCCACCAAG AGAACAATTA TATTGGAGAG CATTGACAAT GGAAAGATTT
201 GATGGTAGAA GATGGGCTCA AGCACCACAA TGGTCTGGTG AAGATGCTTT ACATTGGCAA AAGAGAGGTC CAGAATTGAG ATACGATGTT ATTAATGCAAC
301 CATCTTCACA ACCATGGTTA TTTGCTTTGG ATGTTGCACA AACTGATCAA ACTGATACAA GATTAATGTC AGATTTCAT TTGCAAAGAA GACAACCACT
401 TGAACAAAGA TTATTTTATA GAGTTTCTTC ATGGCCACAA GCTTTGAGAG AATCTTCAAT TGATCCAAGA ACAAGATGGA GAAATTTGCA ATTACCAATG
501 CATGGTAATC CAAGAGCTAG AGCATTAGCT GATGAATTGA GACAAGCACA TGCTCAACCA CAAGCTTTAG TTGCTGCATT GTTGCAAAGA TTCATCATG
601 AACCATTCGC ATACACTTTG AAGCCACCAG CTACAGGTGC AGATGGTGTT GATGATTCTT TGTTTCGATC TAGATCTGGT TTCTGTGCTC ATTACGCAGG
701 TGCTATGGCA TTTGTTTTAA GAGCTGCAGG TATTCCAGCT AGAGTTGTTG CAGGTTATCA AGGTGGTGAA TTGAATCCAG CTGGTAACTA CTTGTTGGTT
801 CATCAATTGC ATGCTCATGC ATGGGTTGAA TATTGGCAAC CAGAACAAGG TTGGTTATCT GTTGATCCAA CATACCAAGT TGCTCCAGAA AGAATTGAAC
901 AAGGTTTAGA ACAAGCTTTG GCTGGTGACT CTGAATATTT GGCTGATGCA CCATTATCAC CATTGAGATA CAGAGGTTTA CCATGGTTGA ATGATATGAG
1001 ATTAGCTTGG GATTCAATTGA ATTTATGGTTG GCAAAGATGG GTTTTGGCTT ACCAAGGTGA ACAACAAGGT GCATTTTAC AAAGATGGTT TGGTGGTTTG
1101 GATCCAGGAT CCTGAGACCA GACCAATAAA AAACGCCCGG CGGCAACCGA GCGTCTGAA CAAATCCAGA TGGAGTTCTG AGGTCATTAC TGGATCTATC
1201 AACAGGAGTC CAAGCGAGCT CGATATCAAA TTACGCCCGG CCCTGCCACT CATCGCAGTA CTGTTGTAAT TCATTAAGCA TTCTGCCGAC ATGGAAGCCA
1301 TCACAAACGG CATGATGAAC CTGAATCGCC AGCGGCATCA GCACCTTGTG GCCTTGCGTA TAATATTGTC CCATGGTGAA AACGGGGGCG AAGAGTTGT
1401 CCATATTGGC CACGTTTTAA TCAAACCTGG TGAACCTCAC CCAGGGATTG GCTGAAACGA AAAACATATT CTCATAAAC CCTTTAGGGA AATAGGCCAG
1501 GTTTTCACCG TAACACGCCA CATCTTGCGA ATATATGTGT AGAAACTGCC GGAAATCGTC GTGGTATTCA CTCAGAGCG ATGAAAACGT TTCAGTTTGC
1601 TCATGGAAAA CGGTGTAACA AGGGTGAACA CTATCCCATC TCACCAGCTC ACCGTCTTTC ATTGCCATAC GAAATCCCG ATGAGCATTC ATCAGGCGGG
1701 CAAGAATGTG AATAAAGGCC GGATAAAACT TGTGCTTATT TTTCTTTACG GTCTTTAAAA AGGCCGTAAT ATCCAGCTGA ACGGTCTGGT TATAGGTACA
1801 TTGAGCAACT GACTGAAATG CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC GGTGGTATAT CCAGTGATTT TTTTCTCCAT TTTAGCTTCC
1901 TTAGCTCCTG AAAATCTCGA TAACCTCAAAA AATACGCCCG GTAGTGATCT TATTTCTATTA TGGTGAAAGT TGGAACTCT TACGTGCCCG ATCAATCATG
2001 ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTCCTG CGCGTAATCT
2101 GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGTGGT TTGTTTCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC
2201 GCAGATACCA AATACTGTTT TTCTAGTGTA GCCGTAGTTA GGCCACCAC TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA
2301 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGAGCTC AAGACGATAG TTACCGGATA AGGCGCGAGC GTCCGGCTGA ACGGGGGGTT
2401 CGTGACACAA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC
2501 GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGG AGCTTCCAGG GGGAACGCC TGGTATCTTT ATAGTCTGTG CGGGTTTCGC
2601 CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT
2701 GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA GTC

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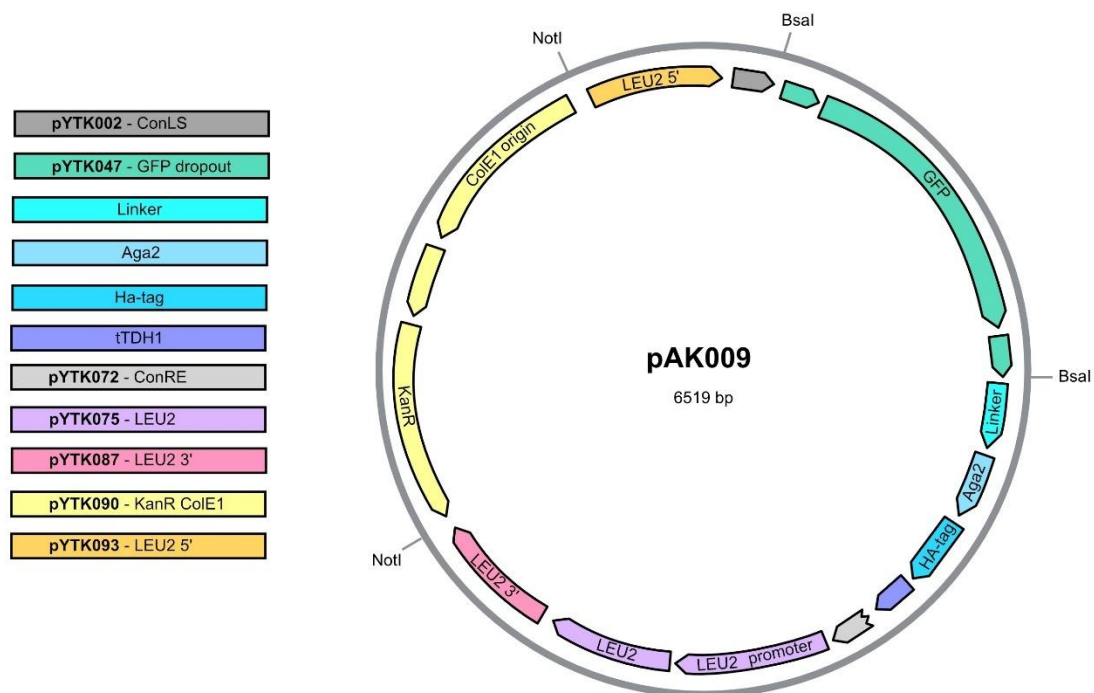
**Supplementary Figure 17. The nucleotide sequence for the part plasmid pATK006.** The part plasmid was generated by cloning the synthesised PaTG encoding gene into the YTK entry vector pYTK001. The insert is highlighted with light blue colour.

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1 GGTCTCATTC TGATAATGGT GCTGGTGAAG AAACATAAGTC ATACGCAGAA ACTTACAGAT TGACAGCTGA TGATGTTGCA AACATCAACG CTTTGCAAGA
101 ATCTGCAACCA GCTGCATCTT CAGCTGGTCC ATCTTTTAGA GCACCATCTT TGGATAAAAG AGAAGCTGAA GCAGATTGAG ATGATAGAGT TACTCCACCA
201 GCTGAACCAT TAGATAGAAT GCCAGATCCA TATAGACCAT CATACGGTAG AGCAGAAACA GTTGTTAACA ACTACATCAG AAAGTGGCAA CAAGTTTACT
301 CTCATAGAGA TGGTAGAAAA CAACAAATGA CTGAAGAACA AAGAGAATGG TTGTCAATG GTTGTGTTGG TGTACTTGG GTTAATCTG GTCAATACCC
401 AACAAATAGA TTGGCTTTTG CATCTTTTGA TGAAGATAGA TTCAAAAACG AATTGAAAAA TGGTAGACCA AGATCAGGTG AAACAAGAGC TGAATTTGAA
501 GGTAGAGTTG CAAAGGAATC TTTCGATGAA GAAAAGGGTT TTCAAAGAGC AAGAGAAGTT GCTTCAGTTA TGAATAGAGC ATTTGAAAAAT GCTCATGATG
601 AATCTGCTTA TTTGGATAAT TTGAAGAAAG AATTGGCAAA CGGTAACGAT GCTTTGAGAA ACGAAGATGC TAGATCACC ATTTACTCTG CATTGAGAAA
701 CACTCCATCT TTTAAAGAAA GAAACGGTGG TAACCATGAT CCATCTAGAA TGAAGGCTGT TATATATCA AAGCATTTT GGTCTGGTCA AGATAGATCT
801 TCATCTGCTG ATAAGAGAAA GTACGGTGAC CCAGATGCTT TTAGACCAGC TCCAGGTACT GGTGTTGGTTG ATATGCTAG AGATAGAAAC ATCCCAAGAT
901 CACCAACATC TCCAGGTGAA GGTTCCTGTA ACTTCGATTA CGGTGCTTTT GGTGCTCAA CAGAAGCTGA TGCAGATAAA ACTGTTTGG A CACATGTTAA
1001 TCATTATCAT GCTCCAAATG GTTCATTAGG TGCAATGCAT GTTTACGAAT CTAAGTTTAG ACAATGGTCA GAAGGTTATT CTGATTTTGA TAGAGGTGCT
1101 TACGTTATCA CTTTTATTCC AAAGTCTTGG AACACAGCAC CAGATAAAGT TAAACAAGGT TGGCCAGGAT CCTGAGACCA GACCAATAAA AAACGCCCGG
1201 CGGCAACCGA GCGTCTGTA CAAATCCAGA TGGAGTTCTG AGGTCAATAC TGGATCTATC AACAGGAGTC CAAGCGAGCT CGATATCAA TTACGCCCCG
1301 CCCTGCCACT CATCGCAGTA CTGTTGTAAT TCATTAAGCA TTCTGCCGAC ATGGAAGCCA TCACAAACGG CATGATGAAC CTGAATCGCC AGCGGCATCA
1401 GCACCTTGTC GCCTTGCGTA TAATATTGTC CCATGGTGAA AACGGGGCGC AAGAAGTTGT CCATATTGGC CACGTTTAAA TCAAAACTGG TGAAACTCAC
1501 CCAGGGATTG GCTGAAACGA AAAACATATT CTCATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA CATCTTGCGA ATATATGTGT
1601 AGAAACTGCC GGAATCGTC GTGGTATTCA CTCCAGAGCG ATGAAAACGT TTCAGTTTGC TCATGGAATA CGGTGTAACA AGGGTGAACA CTATCCATA
1701 TCACCAAGCTC ACCGCTCTTC ATTGCCATAC GAAATTCGG ATGAGCATTC ATCAGCGCGG CAAGAATGTG AATAAAGGCC GGATAAAACT TGTGCTTATT
1801 TTTCTTTACG GTCTTTAAAA AGGCCGTAAT ATCCAGCTGA ACGGTCTGTT TATAGGTACA TTGAGCAACT GACTGAAATG CCTCAAAATG TTCTTTACGA
1901 TGCCATTGGG ATATATCAAC GGTGGTATAT CCAGTGATTT TTTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACCTCAAAA AATACGCCCG
2001 GTAGTGATCT TATTTTATTA TGGTGAAAGT TGAACCTCT TACGTGCCCG ATCAATCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT
2101 CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTCTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGTGGT
2201 TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGT TCAGCAGAGC GCAGATACCA AATACTGTTT TTCTAGTGTA GCCGTAGTTA
2301 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG
2401 GGTGAGACTC AAGACGATG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA
2501 ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAAGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG
2601 CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG
2701 GGGGGCGGAG CCTATGAAAA AACGCCAGCA ACGCGGCCCT TTTACGGTTC CTGGCCTTTT GCTGCGCTTT TGCTCACATG TTCTTCTCTG CGTTATCCCG
2801 TGATTCTGTG GATAACGGTA GTC

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**Supplementary Figure 18. The nucleotide sequence for the part plasmid pATK007.** The part plasmid was generated by cloning the synthesised SmTG encoding gene into the YTK entry vector pYTK001. The insert is highlighted with turquoise colour.



**Supplementary Figure 19. Vector map for the surface-display vector pAK009.** The identifiers of the YTK parts used to assemble the vector are also indicated. The parts without the YTK identifiers were synthesised and inserted into the vector backbone by Gibson assembly. In pAK009, the inverted BsaI sites flanking the GFP dropout cassette enable cloning of the insert as a fusion to the Aga2 and a HA-tag. NotI digestion yields homology arms for integration into the yeast genome to the *LEU2* locus.

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1 GGTCTCAAGC AGTGGTAGTG GGTCCGGTTC TGGTTCAGGC TCAGGTTTCAG GTTCTCAGGA ACTGACAACAT ATATGCGAGC AAATCCCCCTC ACCAACTTTA
101 GAATCGACGC CGTACTCTTT GTCAACGACT ACTATTTTGG CCAACGGGAA GGCAATGCAG GGAGTTTGTG AATATTACAA ATCAGTAACG TTTGTACGTA
201 ATTGCGGGTTC TCACCCCTCA ACAACTAGCA AAGGCAGCCC CATAAACACA CAGTATGTTT TTGGGTCTCG CAGCGGCTCA TACCCTACAG ACGTTCCAGA
301 CTACCGTTAA CTCGAGATAA AGCAATCTTG ATGAGGATAA TGATTTTTTT TTGAATATAC ATAAATACTA CCGTTTTTCT GCTAGATTTT GTGATGACGT
401 AAAATAAGTAC ATATTACTTT TTAAGCCAAG ACAAGATTAA GCATTAACCT TACCCCTTTC TTTCTAAGTT TCAATATTAG TTATCACTGT TTAAGAGTTA
501 TGGCGAGAAC GTCCGGCGGT AAAATATATT ACCCTGAACG CGTCTCAAGC AGTTTACAGAG ATGTTACGAA CCACATAGTC ACTCGAGTAC ATCGAGGAGA
601 ACTTCTAGTA TATCTACATA CTAATATATTA TTGCCTTATT AAAAATGGAA TCCCAACAAT TACATCAAAA TCCACATTCT CTTCAAAATC AATTGTCCTG
701 TACTTCCTTG TTCATGTGTG TTCAAAAACG TTATATTTAT AGGATAATTA TACTCTATTT CTCACAAAGT AATTGGTGTG TTGGCCGAGC GGTCTAAGGC
801 GCCTGATTCA AGAAATATCT TGACCGCAGT TAACTGTGGG AATACTCAGG TATCGTAAGA TGCAAGAGTT CGAATCTCTT AGCAACCACT AATTTTTTCC
901 TCAACATAAC GAGAACACAC AGGGGCGCTA TCGCACAGAA TCAAAATCGA TGAATGAAA TTTTTTGTTA ATTTTCAGAG TCGCCTGACG CATATACCTT
1001 TTTCAACTGA AAAATTGGGA GAAAAAGGAA AGGTGAGAGC GCCGGAACCG GCTTTTCATA TAGAATAGAG AAGCGTTCAT GACTAAATGC TTGCATCACA
1101 ATACTTTAAG TTGACAATAT TATTTAAGGA CCTATTGTTT TTTCCAATAG GTGGTTAGCA ATCGTCTTAC TTTCTAAGTT TTTCTACCTT TTACATTTCA
1201 GCAATATATA TATATATATT TCAAGGATAT ACCATTCTAA TGCTCTGCCC TAAGAAGATC GTCTGTTTGC CAGGTGACCA CGTTGGTCAA GAAATCACAG
1301 CCGAAGCCAT TAAGGTCTCT AAAGCTATTT CTGATGTTCG TTCCAATGTC AAGTTCGATT TCGAAAATCA TTTAATTGGT GGTGCTGCTA TCGATGCTAC
1401 AGGTGTTTCCA CTTCAGAGAT AGGCGCTGGA AGCCTCCAAG AAGGCTGATG CCGTTTTGTT AGGTGCTGTG GGTGGTCTTA AATGGGGAAC CGGTAGTGTG
1501 AGACCTGAAC AAGGTTTACT AAAAATCCGT AAAGAACCTC AATTGTACGC CAACTTAAGA CCATGTAAC TTGCATCCGA CTCCTTTTA GACTTAICTC
1601 CAATCAAGCC ACAATTGTCT AAAGGTACTG ACTTCGTTGT TGTCAAGAGA TTAGTGGGAG GTATTTACTT TGGTAAGAGA AAGGAGGAGC AATGGATGAG
1701 TGTCGCTTGG GATAGTGAAC AATACACCGT TCCAGAAGTG CAAAGAAATC CAAGAATGGC CGCTTTCATG GCCCTACAAC ATGAGGCCAC ATTGCCTATT
1801 TGTCGCTTGG AATAAGCTAA TGTTTTGGCC TCTTCAAGAT TATGGAGAAA AACTGTGGAG GAAACCATCA AGAACCAATT TCTTACATTG AAGATTCAAC
1901 ATCAATTGAT TGATTCGTGC GCCATGATCT TAGTTAAGAA CCCAACCCAC TCAATAGGTA TTATAATCAC CAGCAACATG TTTGGTGAAT CATCTCCGA
2001 TGAAGCCTCC GTTATCCGAG GTTCTTGGG TTTGTTGCCA TCTGCGTCTT TGGCTCTCTT GCCAGACAAG AACACCCGAT TTGTTTGTGA CGAACCATGC
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2201 AAGTGAAGGC CATTTGAAGAT GCAGTTAAAA AGGTTTTGGG TGCAGGTATC AGAAGTGGT ATTTAGGTGG TTCCACAGAT ACCACCGAAG TCGGTGATGC
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2501 ATAGGCATGA GTACTCGTAT CGCATGTCCG TCGCACACGA AATTCAAAAA TGGAAATATG TCATAGGGTA GACGAAATC ATACGCAAT CTACATCAT
2601 TTATCAAGAA GGAGAAAAAG GAGGATGTAA AGGAATACAG GTAAGCAAA TGTACTAAT GCCTCAACGT GATAGGAAA AAGAAATGCA CTTTAACATT
2701 AATATTGACA AGGAGGAGG CACCACACAA AAGTTAGGT GTAACAGAAA ATCATGAAAC TATGATTCCT AATTTATATA TTGGAGGATT TTCTTAAAA
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2901 CCTCAAGAA TTTACTCTGT CAGAAACGCG CTTAACGAGC TAGTCGACCT CCTCTCAGT ACTAAATCTA CCAATACCAA ATCTGATGGA AGAATGGGCT
3001 AATGCATCAT CTTTACCAG CGCGGAGCGG CCGCGTGTAA CAACCAATTA ACCAATTCTG ATTAGAAAAA CTCATCGAGC ATCAAAATGA ACTGCAATTT
3101 ATTCAATACA GGATTTCFAA TACCATATTT TTGAAAAAGC CGTTTCTGTA ATGAAAGAGA AAATCAACCG AGGCAGTTCC ATAGGATGCG AAGATCTCTG
3201 TATCGGTCTG CATTCTCGAT TCGTCCAACA TCAATACAAT CTATTAATTT CCCCTCGTCA AAAATAAGGT TATCAAGTGA GAAATACGCA TAGTGACGA
3301 CTGAATCCGG TGAGAAATGG AAAAGCTTAT GCATTTCTTT CCAGACTTGT TCAACAGGCC AGCCATTACG CTCGTATCA AAATCACTCG CATCAACCAA
3401 ACCGTTATTC ATTCGTGATT GCGCCTGAGC GAGGCGAAAT ACGCGATTCG TGTAAAAAGG ACAATTACAA ACAGGAATCG AATGCAACCG GCGCAGGAAC
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3601 CATCAGGAGT ACGGATAAAA TGCTTGATGG TCGGAAGAGG CATAAATTCG GTCAGCCAGT TTAGTCTGAC CATCTCATCT GTAACATCAT TGGCAACGCT
3701 ACCTTTGACA TGTTTCAGAA ACAACTCTGG CGCATCGGCC TTCCCATGTA ATCGATAGAT TGTGCGACCT GATTGCGCGA CATTATCGCG AGCCCATTTA
3801 TACCATATATA AATCAGCATC CATGTTGAAA TTTAATCGCG GCCTGGAGCA AGACGTTTCC CGTTGAATAT GGCTCATAAC ACCCCTTGTA TTACTGTTTA
3901 TGTAAGCACA CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC AGAGATTTTG AGACACAACG TGGCTTTGTT GAATAAATCG
4001 AACTTTTTGT GAGTTGAAGG ATCAGTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAATC AAAGGATCTT
4101 CTTGAGATCC TTTTTTCTGT CGCGTAATCT GCTGCTTGCA AAAAAAAGAA CCACCGCTAC CAGCGGTGGT TTGTTTGCAG CATCAAGAGT TACCAACTCT
4201 TTTCCGAGG TCACTCGGCT TCAGCAGAGC GCAGATACCA AATACTGTTT TCTTAGTATA GCCGTAGTTA GGCACCACTT TCAAGAACTC TGACGACCG
4301 CCTACATATC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCGAGTGG CGATAAGTCG TGTCTTACCG GGTGGAATC AAGACGATAG TTACCGGATA
4401 AGGCGCAGCG GTCCGGGCTGA ACGGGGGGTT CGTGACACCA CCGCAGCTTG GAGCGAACGA CTTACACCGA CTGAGATAC CTACAGGATG AGCTATGAGA
4501 AAGCGCCACG CTTCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTGAAGC GCAGGGTCTG AACAGGAGAG CGCACGAGG AGCTTCCAGG GGGAAACGCC
4601 TGGTATCTTT ATAGTCTCTG CGGGTTTCGC CACCTCTGAC TTGAGGCTGC ATTTTGTGA TGCTCTGTCG GGGGGCGGAG CCTATGGAAA AACGCCAGCA
4701 ACGCGGCCCT TTTACGGTTC CTGGCCCTTT GCTGGCCTTT TGCTCACATG TCTTTCTCTG CGTTATCCCG TGATTCTGTG GATAACCGTG CGGCCGCCAA
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4901 GACATGTCTT TTTTGATTT GGAATCCAAC CACTCAAGTG ACTCTGTCTA TTACTTTTGC GAAAAATATA CCCACAATT GCCCATCGAA AGTGAATCGC
5001 AAACACACCT CAGACTGGCA CCGACAAAGC AAAGATTATA CAGACAGAGT ACTTTATACG TACCGTTAAG TCTCAAGCAA AGGGTTTTCT TATTTACTGA
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5201 ATATTTTTATA TTGACTTTTC TGATACATTA TCACATCGAC TGTTCATTGT GCAAAATGAAC CACGGGCATT GACTATTTTT CAGGTTACTA CTATATATA
5301 TCATCAGCGG CAAGGATTGT ACCCTGAATT CGCATCTAGA CTGATGAGC GTGGTAGAGC CACAAACAGC CGGTACAAGC AACGATCTCC AGGACCATCT
5401 GAATCATGCG CGGATGACAC GAACTCACGA CGGCGATCAC AGACATTAAC CCACAGTACA GACACTGCGA CAACGTGGCA ATTCGTGCGA ATACAACTGT
5501 AGACCGAAAG TGAACGTGA TTTTCATGCT CATTTTGAAC ATTTTGTAAA TCTTATTATA TAATGTGTGC GGCAATTAC ATTTAATTTA TGAATGTTTT
5601 CTTAAACATCG CGGCAACTCA AGAAACGGCA GGTTCGGATC TTAGCTACTA GAGAAAGAGG AGAAATACTA GATGCGTAAA GGCGAAGAGC TGTTCACCTG
5701 TGTCGTCCTT ATTCTGGTGG AACTGGATGG TGATGTCAAC GGTCTAAGT TTTCCGTGCG TGGCGAGGGT GAAGGTGACG CAACTAATGG TAACTGACG
5801 CTGAAGTTCA TCTGTACTAC TGGTAAACTG CCGGTTCTTT GGCGAGCTCT GGTAAACGAG CTGACTTATG GTGTTCAAGT CTTGTCTCTG TATCCGGACC
5901 ATATGAAGCA GCATGACTTC TTCAAGTCCG CCATGCCGGA AGGCTATGTG CAGGAACGCA CGATTTCCTT TAAGGATGAC GGCACGTACA AAACGCGTGC
6001 GGAAGTGAAA TTTGAAGGCG ATACCTCTGT AAACCGCATT GAGCTGAAAG GCATTGACTT TAAAGAGGAC GGCAATATCC TGGGCCATAA GCTGGAATAC
6101 AATTTTAAAC GCCACAATGT TTACATCACC GCCGATAAAC AAAAAAATGG CATTAAAGCG AATTTTAAAA TTCGCCACA CGTGGAGGAT GGCAGCGTGC
6201 AGCTGGCTGA TCACTACCA GAAACACTC CAATCGGTGA TGGTCTGTT CTGCTGCCAG ACAATCACTA TCTGAGCAGC CAAAGCGTTC TGTCTAAGA
6301 TCCGAACGAG AAACGCGATC ATATGTTTCT GCTGGAGTTC GTAACCGCAG CGGGCATCAC GCATGGTATG GATGAAGTGT ACAAATGACC AGGCATCAAA
6401 TAAACAGAAA GGCTCAGTGC AAAGACTGGG CTTTCTGTTT TATCTGTGTT TGTCGGTGA ACGTCTCTTA CTAGAGTCAC ACTGGCTCAC CTTCCGGTGG
6501 GCCTTCTGCG GTTTATAAG

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**Supplementary Figure 20. The nucleotide sequence for pAK009.** The vector parts are marked with the following colours: Turquoise = Linker, Blue = Aga2, Light blue = Ha-tag, Purple = TDH1 terminator, Light grey = ConRE (partial), Lilac = LEU2, Pink = LEU2 3' homology, Yellow = KanR-ColE1, Orange = LEU2 5' homology, Dark grey = ConLS, Green = GFP dropout cassette.

**Supplementary Table 6.** Parts used to assemble the yeast display constructs and their access information.

| Plasmid | YTK part no. | Encodes                           | <i>E. coli</i> marker | Accessible                                        |
|---------|--------------|-----------------------------------|-----------------------|---------------------------------------------------|
| pYTK009 | 2            | TDH3 promoter                     | CamR                  | YTK toolkit <sup>†</sup>                          |
| pAK009  | –            | Backbone with AGA2, HA-tag, tTDH1 | KanR                  | Ellis group collection, AK Toolkit <sup>‡</sup>   |
| pATK002 | 3a           | SP w/ Kex2                        | CamR                  | Linder group collection, ATK Toolkit <sup>§</sup> |
| pATK003 | 3a           | SP w/o Kex2                       | CamR                  | Linder group collection, ATK Toolkit              |
| pATK004 | 3b           | BaTG                              | CamR                  | Linder group collection, ATK Toolkit              |
| pATK005 | 3b           | BsTG                              | CamR                  | Linder group collection, ATK Toolkit              |
| pATK006 | 3b           | PaTG                              | CamR                  | Linder group collection, ATK Toolkit              |
| pATK007 | 3b           | Pro-Kex2-SmTG                     | CamR                  | Linder group collection, ATK Toolkit              |
| pAK003  | 3c           | CBM3a                             | CamR                  | Ellis group collection<br>AK Toolkit              |

<sup>†</sup>The original YTK toolkit is available at Addgene. <sup>‡</sup>AK Toolkit that extends the YTK toolkit with yeast display and secretion parts is also available at Addgene. <sup>§</sup>ATK Toolkit that extends the YTK toolkit with enzyme and protein parts is currently only available upon request from Linder group, Aalto University.

1 AACGCGAGTTC GAGTTTATCA TTATCAATAC TGCCATTTC AAGAATACGT AAATAATTAA TAGTAGTGAT TTTCTTAAC TTAATTAGT AAAAAATTAG  
101 CCTTTTAAAT CTGGTGTAA CCGTACATGC CCAAAATAGG GGGGGGGTTA CACAGAATAT ATAACATCGT AGGTGTCTGG GTGACAGATT TTGTTCTGGC  
201 ATCCACTAAA TATAATGGAG CCCGCTTTTT AAGCTGGCAT CCAGAAAAAA AAAGAATCCC AGCACCACAA TATTGTTTTC TTCACCAACC ATCAGTTCAT  
301 AGGTCCATT TCTTAGCGCA ACTACAGAGA ACAGGGGAC AAACAGGCAA AAAACGGGCA CAACCTCAAT GGAGTGATGC AACCTGCCTG GAGTAAATGA  
401 TGACACAAAG CAAATTGACC ACGCATGTAT CTATCTCAAT TTCTTATACC TTCTGTCTCT TCTGATTGGG AAAAAGCTGA AAAAAAGGT  
501 TGAACACAGT TCCCTGAAT TATTCCCCCTA CTGACTAAT AAGTATATAA AGACGGTAGG TATTGATTGT AATTCTGTAA ATCTATTCTT TAAAGTCTCT  
601 AAATCTTACT TTTATAGTTA GTCTTTTTTT TAGTTTTAAA ACACCAAGAA CTTAGTTTCG AATAAACACA CATAAACAAA CAAAGATCT ATGAGATTCC  
701 CATCTATTTT TACAGCTGTT TTGTTTGCTG CATCTTCAGC ATTAGCTGCA CCAGTTAATA CTACAACCTA AGATGAAACT GCTCAAATTC CAGCTGAAGC  
801 AGTTATTGGT TATTTGGATT TGGAGGGTGA CTTTGATGTT SCAGTTTTTC CATTTTCTAA CTCACACAAAC AACGGTTTGT TGTTTATTA TACAACATT  
901 GCTTCTATTG CTGCAAAAGGA AGAAGGTGTT TCATTGGATA AGAGAGAAAG AGAAGCTTCT ATGATCATCA TCTCTGGTCA AGTTTTAAGA CCACAAGATA  
1001 TCGCAAACTG GCAACAGAA GAATCATTGG TTCCATATTT GAACGAATTG ATTAATTCTC CAGTTCAATT CGATTACGGT TCAATCACTG AATTGATGTT  
1101 CGAAGCTAGA TTAAGAAGAC ATATCGTTGA AGCTGCAAGA GAATTGTCAT GTTCTGGTGC TAAGTTTCGCA ACTTTCGCTA AGACATACGG TAACACTGCA  
1201 TACTGGAGAG TTACACCAGA AGGTGCTTTG GAATTGAAGT ACAGAAATCCC AGCATCAAG GCTATCAGAG ATATCATCGA AAACGGTGCA TTCTACGCTT  
1301 TTGAATGTGC TACAGCAATC GTTGTATAT ATTACTTGGC TGTTTTGAAG ACTATCGGTG AAGAAAGATT CGATAGAAGA TTCAGAGATA TCACTTTGTA  
1401 TGATTGGCAT TACGAACATT TGCCAATCTA TACTGAAACA GGTAGACATT TCTTGAGAGG TGACTGTTTG TACTTCAAGA ACCCAGAATT CAATCCAGCT  
1501 AAGGCACAAT GGAGAGGTGA AAACGTTATC GTTATGGGTA ACGATCAATA CTTCGCACAT GGTTTGGGTA TCTTGACAGC TGACAAAATC ATCCAAAGAT  
1601 TGAACCTCTT GAGAAGAAAG AATGCAGTTC AATCTGCTTA TTTGTTATCA CAAGCTACTA GATTGGATGC TCCAGCATTA TACCAAATTA TGCATGGATC  
1701 CGCAAAATACA CCGGTATCAG GCAATTTGAA GGTGGAATTC TACACAGCA ATCCTTCAGA TACTACTAAC TCAATCAATC CTCAGTTCAA GGTACTAAT  
1801 ACCGGAAGCA GTGCAATTGA TTGTGCCAAA CTCACATTGA GATATTATTA TACAGTAGAC GGACAGAAAG ATCAGACCTT CTGGTGTGAC CATGCTGCAA  
1901 TAATCGGCAG TAACGGCAGC TACAACGGAA TTACTTCAA TGTAAGAGGA ACATTTGTAA AAATGAGTTC CTCACAAAT AACCGACAGA CCTACCTTGA  
2001 AATAAGCTTT ACAGGCGGAA CTCTTGAACC GGTGCACAT GTTCAGATAA AAGTGAATT TGCAAAAGAT GACTGGAGTA ACTATACACA CTCAATGAC  
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2201 GTGGGTCGCG TTCTGGTTCA GGCTCAGGTT CAGGTTCTCA GGAATCAGCA ACTATATGCG AGCAAAATCCC CTCACCAACT TTAGAATCCA CGCGTACTC  
2301 TTTGTCAACG ACTACTATT TTGCCCAACG GAAGGCAATG CAAGGAGTTT TTGAATATTA CAAATCAGTA ACGTTTGTCA GTAATTGCGG TTTCTACCCC  
2401 TCAACAACTA CCAAGGCAG CCCCATAAAC ACACAGTATG TTTTGGGCT TGGCAGCGGC TCATACCCTT ACGACGTTC AGACTACGCT TAACTCGAGA  
2501 TAAAGCAATC TTGATGAGGA TAATGATTTC TTTTGAATA TACATAATA TACCCGTTTT TCTGCTAGAT TTTGTGATGA CTGAAATAG TACATATTAC  
2601 TTTTAAAGCC AAGACAAGAT TAAGCATTAA CTTTACCCTT TCTTCTCAA GTTTCAATAT TAGTTATCAC TGTTTAAAG TTATGGCGAG AAGCTCGCGG  
2701 GTTAAATAT ATTACCCTGA ACGCGTCTCA AGCAGTTACA GAGATGTTAC GAAACACTAG TGCAGTGCAG TACATCGAGG AGAATCTCTA GTATATCTAC  
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2901 GTGTTCAAAA ACGTTATATT TATAGGATAA TTATACTCTA TTTCTCAACA AGTAATTGGT TGTTTGGCGC AGCGGCTCAA GGCGCTGAT TCAAGAAATA  
3001 TCTTGACCGC AGTTAACTGT GGGAACTACT AGGTATCGTA GTTCGAATCT CTTAGCAACC ATTTATTTTT TCCCTCAACT TCCAGGAACA  
3101 CACAGGGGCG CTATCGCACA GAATCAAAAT CGATGACTGG AAATTTTTTG TTAATTTTCA AGGTGCGCTG ACGCATATAC CTTTTCAC TGAAAAATTG  
3201 GGAGAAAAAG GAAAGGTGAG ACGCGCGGAA CCGGCTTTTC ATATAGATAA GAGAAGCGTT CATGACTAAA TGCTTGATC ACAATCTTG AAGTTGACAA  
3301 TATTATTGTA GAGCCTATTG TTTTTCCTAA TAGGTGGTTA GCAATGCTCT TCTTTCTTAA CTTTCTTACT TACGACATAT ATATATATAT  
3401 ATTTCAAGGA TATACCATTC TAATGTCTGC CCCTAAGAAG ATCGTCTGTT TGCCAGGTGA CCACGTTGGT CAAGAAATCA CAGCGGAGCG CATTAAAGTT  
3501 CTTAAAGCTA TTTCTGATGT TCGTTCCAAT GTCAAGTTCTG ATTTCAAGAA CATTTTAATT GGTGGTGCTG CTATCGATGC TACAGGTGTT CCAGTTCCAG  
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3701 ACTAAGAACT CGTAAGAAAC TTCAATTGTA CGCCAACCTA AGACCTATGA ACTTTGCATC GACTCTCTTT TTAGACTTAT TTAGCAATTA GCGCAATTT  
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4001 TAATGTTTTG CCGCTTTCAA GATTATGGAG AAAAAGCTGT GAGGAAACGA TCAAGAACGA ATTTCTTACA TTGAAGGTCA AACATCAATT GATTGATTCT  
4101 GCGGCAATCA TCCTTAGTTA GAACCCCAAC CACCTAAATG GTATTATCAT CACCAGCAAC ATGTTTGGTG ATATCATCTC CGATGAAGCC TCCGTTATCC  
4201 CAGGTTCCTT CCGTTGTTTG CCATCTGCTG CTTTGGCCTC TTTTGCAGAC AAGAACACCG CATTGTTGTT GTAGCAACCA CTTGCTCCAGA  
4301 TTTGCCAAAG AATAAGGTGA ACCCTATCGC CACTATCTTG TCTGCTGCAA TGATGTTGAA ATTTGCTATT AACTTGCCTG AAGAAGGTAA GGCATTTGAA  
4401 GATGCGATTA AAAAGGTTTT GGATGCAGGT ATCAGAACTG GTGATTTAGG TGGTTCCAAC AGTACCACCG AAGTCCGTTA TGCTTCCGC GAAGAAGTTA  
4501 AGAAATTCCT TGCTTACCTT TTTTTCCTAG TCTCTTTAGA CTATTCTACT CTTATGTCAGC TAAAAAAATC TAGGAAATAT TAGGATAAAT GAGGATAAAT  
4601 AACCGGCTAG TGGCATTCAT ATACCGCTCT GTTTACATCT ACATCTACCA TTTGAGTGT ATATCTCGCA ACGTTGGCGT TAAATAGGCA GGAGTACTCG  
4701 TATCGCATGT CCGTGGCGACA CGAAATTACA AAATGGAATA GTTTCATAGG GTAGACGAAA CTATATACGC AATCTACATA CATTATTCAA ATTAATATTA ACAAGGAGAA  
4801 AAGGAGGATG TAAAGGAATA CAGGTAAGCA AATTGATAC ATAGGCTCAA CGTGATAAGG AAAAAGAAAT GCACCTTTAAT ATTAATATTA ACAAGGAGAA  
4901 GGGCAGACGA CAAAAGGTTA GGTGTAACAG AAAATCATGA AACTATTAT CTAAATTTAT ATATTGGAGG ATTTTCTCTA TCGAACTTTT GCTGAGTTTA  
5001 ATAAAAACA CTCATGACC TGACCAATTTG ATGGAGTTTA AGTCAATACC TTTTGAACC ATTTCCATA ATGGTGAAAG TTCCCTCAAG AATTTTACTC  
5101 TGTGAGAAAC GGCCTTAACG ACGTAGTCGA CCTCCTCTC AGTACTAAAT CTACCAATAC CAAATCTGAT GGAAGAATGG GCTAATGCAT CATCCTTACC  
5201 CAGCGCCGAG CGGCGCGGTG TTACAACCAA TTAACCAATT CTGATTAGAA AAACCTCATCG AGCATCAAA TTTATTTCATA TACAGATTAT CATGAGTTAT  
5301 CAATACCATTA TTTTGAATA AGCGTTTCT GTAAATGAAG AGAAAACTCA CCGAGGCAGT TCCATAGGAT GGCAAGATCC TGGTATCGGT CTGCGATTCC  
5401 GACTCGTCA ACATCAATAC AACCTATTA TTTCCCTCG TCAAAAAATA GTTTATCAAG TGAGAAATCA CCATGAGTGA GCACTGAATC CGGTGAGAA  
5501 GGCAAAAGCT TATGATTTT TTTCCAGACT TGTTCACAG GCCAGCCATT ACGTCTGCTA TCAAAATCAC TCGCATCAAC CAAACCGTTA TTCATTCTG  
5601 ATTGCGCCTG ACGGAGGCGA AATACGCGAT CGCTGTTAAA AGGACATTA CAAACAGGAA TCGAATGCAA CCGGCGCAGG AACATCAGCA CGCATCAAC  
5701 AATATTTCAG GATATTCTCT GATATTCTCT ATGTTCTTTC TCCCGGGAT CCGAGTGGTG AGTAACCATG CATCATGCGG AGATACCGTA AAGACAGTA  
5801 AAATGCTTGA TGGTCCGAAG AGGCATAAAT TCCGTCAGCC AGTTTAGTCT GACCATCTCA TCTGTAACAT CATTGGCAAC GCTACCTTTG CCATGTTTCA  
5901 GAAACCACT TGGCGCATCG GGCTTCCCAT ACAATCGATA GATTGTGCGA CCGATTGCCC CGACATTATC GCGAGGCCAT TTATACCCAT ATAAATCAGC  
6001 ATCCATGTTG GAATTTAAT GCGGCTTGA GCAAGACGTT TCCCGTTGAA TATGGTCTAT AACACCCCTT GTATTACTGT TTATGTAAGC AGACAGTTT  
6101 ATTTGTTATG ATGATATATT TTTATCTTGT GCAATGTAAC ATCAGAGATT TTGAGACACA ACGTGGCTTT GTTGAATAAA TCGAACTTTT GCTGAGTTTA  
6201 AGGATCAGTC ATGACCAAAA TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAA AGTCAAAAGGAT CTTCTTGAGA TCCTTTTTTT  
6301 CTGCGCGTAA TCTGCTGCTT GCAACAAAAA AAACCCAGCG TACCAGCGGT GGTTTGTTTG CCGGATCAAG AGCTACCAC TCTTTTTCG AAGTAACTG  
6401 GCTTCAGCAG AGCGCAGATA CCAAAATCTG TTCTTCTAGT TTAGGCCAACC ACTTCAAGAA CTCTGTAGCA CCGCTACAT ACCTCGCTCT  
6501 GCTAATCCTG TACCCAGTGG CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAGACGA TAGTTACCGG ATAGGCGCA GCGGTGCGG  
6601 TGAACGCGGG GTTCGTGCAC ACAGCCAGC TTGGAGCGAA GCACTACAG CAACTACAG GTAGCTATG AGAAAGCGCC AGCTTCCCG CCGGTGCGG  
6701 AAGGGAGAAA GCGCGACAGG TATCCGGTAA GCGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC AGGGGGAAC GCCTGTGATC TTTATAGTCC  
6801 TGTCGGGTTT CGCCACCTCT GACTTGAGCG TCGATTTTTG TGATGCTCTG CAGGCGGGCG GAGCCTATGG AAAAAACGCA GCAACGCGCG CTTTTACGG  
6901 TTCTGTCGCT TTTGCTGGCC TTTTGTCTAC ATGTTCTTTC CTGCGTATTCT CCGGTATTCT GTGGATAACC GTGCGGCGCG CAATACCCAG AAGAACAGTA  
7001 AAATAAAGCA AGGTACGTGA AATTAATATT TTTAAATGGT TCTAACCGAT GCCGAAGAAC TGCGCAGTCC GGTTATAACG TCTGACATGT CTTTTTTGA  
7101 TTTGGAATCC AACCATCAA GTGACTCTGT TCATTTACTT TGCAGAAAAAT ATACCCACAA ATTGCCATC GAAAGTGAAT ATGACCAAC CTTAGACTG  
7201 GCACCGACAA AGCAAGATT ATACAGACAG AGTACTTTAT ACGTACCGTT AAGTCTCAAG CAAAGGGTTT TCTTATTATC TGAACGGGTA AAGAGTATCT  
7301 GGGCGCGCTT GCCAAGATG AACCCGAATA AGTATTTCAA AGTTGCACTT GCGTTAGCCG TCCTGACACC TCCTGATATT TGGATATTTT ATATTGACT  
7401 TCGTGTACAT TGATCACATC GACTGTCTTA TTGGCAAATG AACCCAGGGC ATTGACTATT TTTGAGTTA CTACTATATA TTATCATCAC GGGCAAGGAT  
7501 TGTACCTCGA ATTCGATCT AGACTGATGA GACGTGGTAG AGCCACAAAC AGCCGGTACA AGCAACGATC TCCAGGACCA TCTGAATCAT GCGCGGATGA  
7601 CACGAACCTA CGAGCGCGAT CACAGACATT AACCCACAGT ACACAGCACT CGACAACGCT GCAATTGCTG CCAATAC

**Supplementary Figure 21.** The nucleotide sequence of BaTG surface display construct. The surface display parts are marked with the following colours: Blue = TDH3 promoter, Orange = SP w/ Kex2, Turquoise = BaTG, Green = CBM3a, Grey = Linkers, Yellow = AGA2, Pink = HA-tag, Red = tTDH1.

1 AACGCGATTC GAGTTTATCA TTATCAATAC TGCCATTTC AAGAATACGT AAATAATTAA TAGTAGTGAT TTTCTAACT TTATTAGTC AAAAAATTAG  
101 CCTTTTAAAT CTGCTGTAA CCGTACATGC CCAAAATAGG GGGGGGGTTA CACAGAATAT ATAACATCGT AGGTGTCGTG GTGAACAGTT TATTCTCGGC  
201 ATCCCACTAAA TATAATGGAG CCCGCTTTTT AAGCTGGCAT CCAGAAAAAA AAAGAATCCC AGCACCACAAA TATTGTTTTC TTCACCAACC ATCAGTTCAT  
301 AGGTCCATTCT TCTTAGCGCA ACTACAGAGA ACAGGGGCAC AAACAGGCAA AAAACGGGCA CAACCTCAAT GGAGTGATGC AACCTGCCTG GAGTAAATGA  
401 TGACACAAAG CAATGTACCC ACGCATGTAT CTATCTCATT TTCTTACACC TTCTATTACC TTCTGCTCTC TCTGATTTCG AAAAAGCTGA AAAAAAGGT  
501 TGAACACAGT TCCCTGAAAT TATTCCCCCTA CTGACTAAT AAGTATATAA AGACGGTAGG TATTGATTGT AATTCTGTAA ATCTATTCTT TAAACTTCTT  
601 AAAATCTACT TTTATAGTTA GTCTTTTTTT TAGTTTTTAA ACACCAAGAA CTTAGTTTTC AATAAACACA CATAAACAAA CAAAGATCT ATGAGATTCC  
701 CATCTATTGT TACAGCTGTT TTGTTTGCTG CATCTTCAGC ATTAGCTGCA CAGTTTAATA CTACAACCTA AGATGAAACT GCTCAAAATC CAGCTGAAGC  
801 AGTTATTGGT TATTGGATT TGGAGGGTGA CTTTGATGTT GCAGTTTTCG CATTTTCTAA CTCACACAAAC AACGGTGTGT TGTTTTATTA TACAACATT  
901 GCTTCTATTG CTGCAAAGGA AGAAGGTGTT TCATTGGATA AGAGAGAAGC AGAAGCTTCT ATGATCATTG TTTCTGGTCA ATTGTTAAGA CCACAAGATA  
1001 TCGAAAACTG GCAAACTCGAT CAAGATTTGA ACCCATTTGT GAAGGAAATG ATCGAAATC CAGTTCAATT CGATTACCAT TCTATTGCAG AATTGATGTT  
1101 CGAATTGAAG TTGAGAATGA ATATTGTTGC TGCAGCTAAG ACATTGCATA AGTCAGGTGC AAAGTTCGCT ACATTTTTGA AGACATACGG TAACACTACA  
1201 TACTGGAGAG TTTCTCCAGA AGGTGCATTG GAATTGAAGT ACAGAAATGCC ACCATCAAAG GCAATCAGAG ATATCGCTGA AAATGGTCCA TTTTACGCTT  
1301 TCGAATGTGC AACTGCTATC GTTATCATCT ATTACTTGGC ATTGATCGAT ACAATCGGTG AGGATAAGTT TAATGCTTCA TTCGATAGAA TCATTTTGTA  
1401 TGATTGGCAT TACGAAAAGT TGCCAAATCTA TACTGAAACA GGTCTCATTT TCTTTTTGGG TGACTGTTTG TACTTCAAGA ACCCAGAATT CGATCCACAA  
1501 AAAGCACAAT GGAGAGGTGA AAACGTTATC TTGTTGGGTG AGGATAAGTA CTTGCTCAT GGTTTGGGTA TCTTGAACGG TAAACAACTC ATCGATAAAT  
1601 TGAACCTCTT TAGAAGGAAA GGTGCATTGC AATCTGCTTA CTGTTATACA CAAGCTACTA GATTGGATGT TCCATCTTTG TTTAGAATTG TTAGAGGATC  
1701 CGCAAAATACA CCGGTATCAG GCAATTTGAA GGTGGAATTC TACAAACAGA ATCCTTCAGA TACTACTAAC TCAATCAACT CTCAGTTCAA GGTACTTAAT  
1801 ACCGGAAGCA GTGCAATTGA TTTGTCCAAA CTCACATTGA TACAGTAGAC GGACAGAAAG ATCAGACCTT CTGCTGTGAC TACTCTGCAG TAACCTGCAA  
1901 TAATCGCGAG TACCGCGCAG TACAACGGAA TACTTCCAAA TGTAAAAGGA ACATTTGTAA AAATGAGTTC CTCACAAATAT ACCCAGACAGA CCTACCTTGA  
2001 AATAAGACTT ACAGCGGGAA CTCITTGAACC GGTGACAT GTTCAGATAGT TGCAAAAGAT GACTGGAGTA ACTATACACA GTCAAAATGAC  
2101 TACTCATTCA AGTCTGCTTC ACAGTTTGTG GAATGGGATC AGGTAACAGC ATACTTGAAC GGTGTTCTTG TATGGGGTAA AGAACCCTGT GGCAGTGGTA  
2201 GTGGGTCCGG TTTCTGGTTC GGCTCAGGTT CAGGTTCTCA GGAATGACA ACTATATCGG AGCAAAATCCC CTCACCAACT TTAGAATCCA CGCGTACTC  
2301 TTTGTCAACG ACTACTATT TGGCCAAACG GAAGGCAATG CAAGAGATTT TTGAATATTA CAAATCAGTA ACGTTTGTCA GTAATTGCGG TTTCTACCCC  
2401 TCAACAACCTA GCAAGGCGAG CCCCATAAAC ACACAGTATG TTTTGGGTC TGGCAGCGGC TCATACCCAT ACGACGTTC AGACTACGCT TAACTCAGGA  
2501 TAAAGCAATC TTGATGAGGA TAATGATTTT TTTTGAATA TACATAAATA TACCGCTTTT TCTGCTAGAT TTTGTGATGA TCTAAATAG TCAATATTAC  
2601 TTTTAAAGCC AAGACAAGAT TAAGCATTAA CTTTACCCTT TCTTCTCAA GTTTCAATAT TAGTTATCAC TGTTTAAAG TTATGGCGAG AACGTCGGCG  
2701 GTTAAATAT ATTACCCTGA ACGCTCTCA AGCAGTTACA GAGATGTTAC GAACCACTAG TGCCTGCGAG TACATCGAGG AGAATCTCTA GTATATCTAC  
2801 ATACCTAATA TTTTGGCTTT ATTAAAAATG GAATCCCAAC AATTGACAT TAATTCACAT AAATCCACAT TCTCTTCAAA ATCAATGTTG TGTGCTATGT TTGTTCAATG  
2901 GTGTTCAAAA ACGTTATATT TATAGGATAA TTATACTCTA TTTCTCAAGA AGTAATTGGT TGTTTGGCGG AGCGGCTCAA GCGCGCTGAT TCAAGAAATA  
3001 TCTTGACCCG AGTTAAGCTG AGGTATCTGA AGATGACATA GTTCGAATCT CTTAGCAACC ATTTATTTTT TCCCTCAACAT TACAGAGACA AACCAGAACAA  
3101 CACAGGGGGC GTATCGCAGA GAATCAAAAT CGATGACTGG AAATTTTTTG TTAATTTTCAG AGGTGCGCTG ACGCATATAC CTTTTCAC TCAGAAAATTG  
3201 GGAGAAAAGT AAGAGGTGAG AGCGCCGGAA CCGCTTTTCT ATATAGAATA GAGAAGCGTT CATGACTAAA TCGTTGCATC ACAATACTGT AAGTTGACAA  
3301 TATTATTATA GGACCTATTG TTTTTCCAA TAGGTGGTTA GCAATCGTCT TACTTTCTAA CTTTCTTAC CTTTACATT TCAGCAATAT ATATATATAT  
3401 ATTTCAAGGA TATACCATTC TAATGTCGTC CCCTAAGAAG ATCGTCGTTT TGCCAGGTGA CCACGTTGGT CAAGAAATCA CAGCCGAGTC CATTAAAGTT  
3501 CTTAAAGCTA TTTCTGATGT TCGTTCCAAT GTCAAGTTCT ATTTTCGAAA TCTTTTAATT GGTGGTGTG CTATCGATGC TACAGGTGTT CCACCTCCAG  
3601 ATGAGGCGCT GGAAGCCTCC AAGAAGGCTG ATGCCGTTTT GTTAGGTGCT GTGGGTGGT CTAAATGGG AACCGGTAGT GTTAGACCTG AACAGGTTT  
3701 ACTAAAAATC CTAAGAAGTA TTCAATTGTA CGCCAACCTA AGTACCATCT GTTTGTCATC CGACTCTCTT TTAGACTTAT CTCCAATCAA GCCCAATTG  
3801 GCTAAAGGTA CTGACTTCGT TGTGTGCTAGA GAATTAGTGG GAGGTATTTA CTTTGGTAA AGAAAGGAGG ACGATGGTGA TGGTGTGCTG TGGGATAGTG  
3901 AACAATACAC CGTTCAGAA GTGCAAGAA TCACAAGAAT GGCGCTTTC ATGCGCCCTAC AACATGAGCC ACCATGGCTT ATTTGGTCTG TGGATAAAGC  
4001 TAATGTTTTG CCGCTTCAA GATTATGGAG AAAAATCTGT TATGACAGTA ATTTCTTACA TTGAAGGTTT AACATCAATT GATTGATTCT GATTGATTCT  
4101 GCCGCCATGA TCCTAGTTAA GAACCCAAAC CACCTAAATG GTATTATAAT CACCAGCAAC ATGTTTGGT ATATCATCTC CGGTAAAGCT TCCGTATTCC  
4201 CAGGTTCCCT GGGTTTGGTG CCATCTGCGT CTTGGCCCTG AAGAACACCG CATTGGTGT GTAGCAACCA CACTGAAGCC TCGTCCAGA  
4301 TTTGCCAAAG AATAAGGTA ACCCTATCGC CACTATCTTG TCTGCTGAG TGAATTTGAA ATGTGCTATTG AACTTGGCTG AAGAAGTAA GGCCATTTGA  
4401 GATGCAATGA AAAAGGTTTT GGATGCAAGT ATCAGAATG CTGTTTGAAG TGGTTCCAAG AGTACCACCG AAGTCGGTGA TCGTGTGCTG GAAGAAGTTA  
4501 AGAAAACTCT TGCTTAACCT GGAGGACCTT TCTCTTTAGA CTATTCTACT CTTATGCAGC TAAAAAATC TAGGAAATAT GTATTAACTA GGAGTAAAT  
4601 AACCGGCTAG TGGCATTCAT ATGACCGTCT GTTTACATCT ACATTCACAT TTTGAGTGT ATATCTCGCA ACGTTGGCGT TAAATAGGCA GGAGTACTCG  
4701 TATCGCATGT CGGTGGCAGA CGAAATTACA AAATGGAATA TGTTTCATAGG GTAGACGAAA CTATATACGC AATCTACATA CATTATACAA GAAGGAGAAA  
4801 AAGGAGGATG TAAAGGAATA CAGTAAGCA AATTGATACT AATGGCTCAA CGTGATAAGG AAAAAGAATT GCACCTTTAC ATTAATATTG ACAAGGAGGA  
4901 GGGCAGCACA CAAAAGTTA GGTGTAACAG AAAATCATGA AACTATGATT CTTAATTTAT ATATTGGAGG ATTTTCTCTA ATTAATAAATA AATAACAACA  
5001 ATAAAAACA CTCATGACC TGACCAATTTG ATGGAGTTTA AGTCAATACC TTTCTGAACC ATTTCCCAT AATGGTGAAG TTCCTCAAG AATTTTACTC  
5101 TGTCAGAAAC GGCCTTAACG ACGTAGTCGA CCTCCTCTC AGTACTAAAT CTACCAATAC CAAATCTGAT GGAAGAATGG GCTAATGCAT CATCCTTACC  
5201 CAGCGCGAG CGGCGCGGT TTAACAACAA TTAACCAATT CTGATTAGAA AAACCTCATCG AGCATCAAAAT AGCATGAAA TTTATTCATA TATTGATTAT  
5301 CAATACCAT TTTTGAAGAA AGCGTTTCT GTAATGAAG AGAAAACTCA CCGAGGCAGT TCCATAGGAT GGCAAGATCC TGGTATCGGT CTGCGATTCC  
5401 GACTCGTCCA ACATCAATAC AACCTATTAA TTTCCCTCG TCAAAAATAA GGTATCAAG TGAGAAATCA CCATGAGTGA GCACTGAATC CGGTGAGAA  
5501 GGCAAAAGCT TATGCAATTC TTTCCAGACT TGTTCACAG GCCAGCAAT ACGTCTGCTA TCAAAATCAC TCGCATCAAC CAAACCGTTA TTTATCTGT  
5601 ATGCGCTGT AGCGAGGCGA AATACGCGAT CGCTGTTAAA AGGACAATTA CAAACAGGAA TCGAATGCAA CCGCGCGAGG AACACTGCCA CGGCATCAAC  
5701 AATATTTTCA CCTGAATCAG GATATCTTCT TAATACCTGG AATGCTGTTT TCCCGGGGAT CGCAGTGGT AGTAACCATG CATCATCAGG AGTACGGATA  
5801 AAATGCTTGA TGGTCCGAAG AGGCATAAAT TCCGTACGCC AGTTTAGTCT GACCATCTCA TCTGTAACAT CATTGGCAAC GCTACCTTTG CCATGTTTCA  
5901 GAAACAATC TGGCGCATCG GGCTTCCCAT ACAATCGATA GATTGTGCGA GATTGTTGCC CGACATTATC GCGAGGCCAT TTATACCAT ATAAATCAGC  
6001 ATCCATGTTG GAATTTAATC GCGGCTCGGA GCAAGACGTT TCCCGTTGAA TATGGCTCAT AACACCCCTT GTATTACTGT TTATGTAAGC AGACAGTTTT  
6101 ATTTGTTTAT ATGATATATT TTTATCTTGT GCAATGTAAC ATCAGAGATT TTGAGACACA ACGTGGCTTT GTTGAATGAA TCGAATCTTT GCTGAGTTGA  
6201 AGGATCAGCT ATGACCAAAA TCCCTTAAAG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAAGGAT CTTCTTGAGA TCCTTTTTCT  
6301 CTGCGCGTAA TCTGCTGCTT GCAAAACAAA AAACCAACCG TACCAGCGGT GGTTTGTTTG CCGGATCAAG AGCTACCAAC TCTTTTCCG AAGGTAACTG  
6401 GCTTCAGCAG AGCGCAGATA CCAAACTAGT TCTTCTAGT GTAGCGCTAG TTAGGCCACC ACTTCAAGAA CTCTGTAGCA CGCCTACAT ACCTCGCTCT  
6501 GCTAATCCGT TTACAGTGG CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAGACGA TAGTTACCGG ATAGGCGGCA GCGGTGCGGC  
6601 TGAACGGGGG GTTCGTGCAC ACAGCCACAG CGACCTACAG CAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACCTGCTCCG TTTATGCTCC  
6701 AAGGCGGAAA GCGCGCAGG TATCCGGTAA GCGGCAAGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC AGGGGGAAC GCCTGGTATCT TTTATGTCC  
6801 TGTGCGGTTT GCGCACCTCT GACTTGAGCG TCGATTTTTG TAGTGTCTGT TAGGCGGGCG GAGCCTATGG AAAAACGCCA GCAACCGCGC CTTTTACGG  
6901 TTTCTGGCCT TTTGCTGGCC TTTTGTCTAC ATGTTCTTTC CTGCGTTATC CCCTGATTCT GTGGATAACC GTGCGGCGCC CAATACCCAG AAGAACAGTA  
7001 AAATAAAGCA AGGTACGTGA AATTAATATT TTTAAATGGT TCTAACCGAT GCCGAAGAAC TGCGCAGTCC GGTTATAACG TCTGACATGT CTTTATTGTA  
7101 TTTGGAATCC AACCACTCAA GTGACTCTGT TCATTTACTT TGCAGAAAAAT ATACCCACAA ATTGCCCATC GAAAGTGAAT CGCAACACCT CTTCAGACTG  
7201 GCACCGACAA AGCAAGATT ATACAGACAG AGTACTTTAT ACGTACCGTT AAGTCTCAAG CAAAGGGTTT TCTTATTATC TGAACGGGTA AAGAGTATCT  
7301 GGGCCGGCTT GCCAAGATGC AAACCGAATA AGTATTTCAA AGTTGCAATT GCCTTAGCCG CCTTGACACC ATTGCTATT TGGATATTT ATATTGACTT  
7401 TCGTGTATCT TGATTCATCT GACTTGTCTA TTGCAAAATG AACCACGGGC ATTGACTATT TTTGAGTTA CTACTATATA TTATCATCAC GGGCAAGGAT  
7501 TGTACCTCTA ATTGCATCT AGACTGTATG GACGTGGTAG AGCCACAACT AGCCGGTACA AGCAACGAT TCCAGGACCA TCTGAATCAT GCGCGGATGA  
7601 CACGAACCTCA CCGCGCGGAT CACAGACATT AACCCACAGT AACCAACACT CGACAACCTG GCAATTCTGC TCAATCTGC

**Supplementary Figure 22. The nucleotide sequence of BsTG surface display construct.** The surface display parts are marked with the following colours: Blue = TDH3 promoter, Orange = SP w/ Kex2, Purple = BsTG, Green = CBM3a, Grey = Linkers, Yellow = AGA2, Pink = HA-tag, Red = tTDH1.

1 AACGCGATTC GAGTTTATCA TTATCAATAC TGCCATTTC AAGAATACGT AAATAATTAA TAGTAGTGAT TTTCTTAAC TTTATTTAGT AAAAAATTAG  
101 CCTTTTAAAT CTGCTGTAAC CCGTACATGC CCAAAATAGG GGGCGGGTTA CACAGAATAT ATAACATCGT AGGTGTCTGG GTGAACAGTT TTATCCTGGC  
201 ATCCCACTAAA TATAATGGAG CCCGCTTTTT AAGCTGGCAT CCAGAAAAAA AAAGAATCCC AGCACCACAAA TATTGTTTTC TTCACCAACC ATCAGTTCAT  
301 AGGTCCATTCT TCTTAGCGCA ACTACAGAGA ACAGGGGCAC AAACAGGCCA AAAACGGGCA CAACCTCAAT GGAGTGATGC AACCTGCCTG GAGTAAATGA  
401 TGACACAAAGG CAAATTGACCG ACGCATGTAT CTATCTCAAT TTCTTACACC TTCTTATACC TTCTGTCTCT TCTGATTGG AAAAAGCTGA AAAAAAGGT  
501 TGAAACCACTG TCCCTGAAAT TATTCCCCCTA CTGACTAAT AAGTATATAA AGACGGTAGG TATTGATTGT AATTCTGTAA ATCTATTCTT TAAACTTCTT  
601 AAATTTCTACT TTTATAGTTA GTCTTTTTTT TAGTTTTTAA ACACCAAGAA CTTAGTTTTC AATAAACACA CATAAACAAA CAAAAGATTG ATGAGATTCC  
701 CATCTATTTT TACAGCTGTT TTGTTTGCTG CATCTTCAGC ATTAGCTGTA CCAGTTTAATA CTACAACCTGA AGATGAAACT GCTCAATTC CAGCTGAAGC  
801 AGTTATTGGT TATTTTGGATT TGGAGGGTGA CTTTGATGTT GCAATTTTGC CATTTTCTAA CTCACACAAAC AACGGTGTGT TGTTTATTAA TACAACATT  
901 GCTTCTATTG CTGCAAGGA AGAAGGTGTT TCATTGGATA AGAGAGAAGC AGAAGCTTCT AGATTGGGTC CATTATGGTC TTTGCCAATG CACGGTAATA  
1001 AGGGTGTTC TGGTTTATCT GAATCAATGG CTCCAGGTGA CATTGCGAAT TTAGGTAGAT CAGCTGAATT GGCTTTTGA GTTAGATTG AAGGTGCTTT  
1101 GCCACCAAGA GAACAATTAT ATTGGAGAGC ATTGACAATG GAAAGATTGT ATGGTAGAAG ATGGGCTCAA GCACCACAAT GGCTGTGGTA AGATGCTTTA  
1201 CATTGGCACA AGAGAGGTCC AGAATTGAGA TACGATGTTA TTATGCAACC ATCTTCACAA CCATGGTTAT TTGCTTTGGA TGTGTCACAA ACTGATCAAA  
1301 CTGATACAAG ATTAATGTCA GATTTCATT TGCAAGAAG ACAACCAAGT GAACAAAGAT TATTTTATAG AGTTTCTTCA TGGCCACAAG CTTTGAGAGA  
1401 ATCTTCAATT GATCCAAGAA CAAGATGGAG AAATTGCAA TTACCAATGC ATGGTAATCC AAGAGCTAGA GCATTAGCTG ATGAATTGAG ACAAGCACAT  
1501 GCTCAACCCAC AAGCTTTAGT TGCTGCATTG TTGCAAAGAT TCAATCATGA ACCATTGCGA TACACTTTGA AGCCACCAGC TACAGGTGCA GATGGTGTG  
1601 ATGATTTCTT GTTCGATACT AGATCTGGT TCTGTGCTCA TTACGCAAGT GCTATGGCAT TTGTTTTAAG AGCTGCAGGT ATTCGAGGT GAGTGTGTTG  
1701 AGGTTATCAA GGTGGTGAAT TGAATCCAGC TGGTAACATC TTGTTGGTTC ATCAATTGCA TGCTCATGCA TGGGTGTAAT ATTGGAACAT AGACAAGGT  
1801 TGGTTATCTG TTGATCCAAC ATACCAAGTT GCTCCAGAAA GAATTGACCA AGGTTTAGAA CAAGCTTTGG CTGGTAGACT TGAATATTTC GCTGATGAC  
1901 CATTATCACC ATTAGATATC AGAGGTTTAC CATGGTTGAA TTAGTTGAGA TTAGCTTGGG ATTCATTGAA TTATGGTTGG CAAAGATGGG TTTTGGGTTA  
2001 CCAAGGTGAA CAACTAGGTC CATTTTTCAG AAGATGGTTT GGTGGTTTGG ATCCAGGATC CGCAATACGA CCGGTATGCA GCAATTGAA GGTGTAATTC  
2101 TACAACAGCA ATCTTTCAGA TACTACTAAC TCAATCAATC CTCAGTTCAA GGTACTAAT ACCGGAAGCA GTGCAATTGA TTTGTCCAAA CTCACATTGA  
2201 GATATTATTA TACAGTAGAC GGACAGAAAG ATCAGACCTT CTGGTGTGAC CATGCTGCAA TAATCGGCAG TAACGGCAGC TACACGGAAA TTACTTCAAA  
2301 TTGAAAAAGA CATTGTGTA AATAGAGTTC CTCACAAAT CTCAACCTGA CCTACCTTGA AATAAGCTTT ACAGGGCGGA TCTTTGAACC GGCTGACCAT  
2401 TGTCCAGATA AAGTAGATT TGCAAGAAGT GACTGGAGTA ACTATAACCA CTCAATGAC TACTCATTA AGTCTGCTTC ACAGTTTGT GAATGGGATC  
2501 AGGTAACAGC ATCTTTGAAC GGTGTTCTTG TATGGGGTAA AGAACCCGTT GGCAGTGGTA GTGGGTCCGG TTCTGGTTCA CAGTTTCTCA CAGTTTCTCA  
2601 GGAAGTACGA ACTATATGCG AGCAAAATCCC CTCACCAACT TTAGAATCGA CGCCGTACTC TTTGTCAACG ACTACTATTT TGGCCAAACG GAAGGCAATG  
2701 CAAGGAGTTT TTGAATATTA CAAATCAGTA ACGTTTGTCA GTAATTGCGG TTCTCACCCC TCAACAACCTA GCAAGGCGAG CCCCATAAAC ACACAGTATG  
2801 TTTTGGGTC TGGCAGCGCG TCATACCCAT ACGAGCTTCC AGACTAGCTG TAAGTGCAGA TAAAGCAATC TTGATGAGGA TAATGATTTT TTTTGAATA  
2901 TACATAAAAT CTACCGTTTT TCTGCTAGAT TTTGTGATGA CGTAATAAAG TACATATTAC TTTTAAAGCC AAGACAAGAT TAAGCATTTA CTTTACCTTT  
3001 TTTTCTCAA GTTCTCAATAT TAGTTATCAC TGTTTAAAGG TTATGGCGAG ACGTCTCGCG GTTAAATAT ATTACCCTGA CCGCGTCTCA ACGCATTTCA  
3101 GAGATTATTC GAACCACTAG TGCAGTGCAG TACATCGAGG AGAATCTCTA GTATATCATC ATACCTAATA TTATTGCTTT ATTAAGGATG GAATCCCAAC  
3201 AATTACAGTA AAATCCACAT TCTCTTCAA ATCAATTGTC CTGACTTCTA GTTTCATGT GTGTTCAAAA ACGTTATATT TATAGATAA TTATACTCTA  
3301 TTTCTCAACA AGTAATTGGT TGTTTGGCGC AGCGGTCTAA GCGCGCTGAT TCAAGAAATA TCTTGACCCG AGTTAACTGT GGGAATACTC AGGTATCGTA  
3401 AGATGCAAGA GTTCGAATCT CTTAGCAACC ATTAATTTTT TCCTCAACAT AACGAGAACA CACAGGGGCG CTATCGCAGA GAATCAAAAT CGATGACTGT  
3501 AAAATTTTTTG TTAATTTGAA AGGTGCGCTG ACGCATATAC CTTTTTCAAC TGAAAAATTG GGAGAAAAAG GAAAGGTGAG AGCGCGGGAA CCGGCTTTTC  
3601 ATATAGATAA GAGAGCGCT CATGACTAAA TGCTTGCATT ACAATCATCT AAGTTGACAA TATTATTAA GGACCTATTG TTTTTCCTAA TAGGTGGTTA  
3701 GCAATCGTCT TACTTCTCAA CTTTTCTTAC TTTTACATT TCAGCAATAT ATATATATAT ATTTCAAGGA TATACCATTC TAAATGCTCG CTTCAAGAA  
3801 ATCGTCGTTT TGCCAGGTGA CCACGTTGGT CAAGAAATCA CAGCCGAAGC CATTAAGGTT CTTAAAGCTA TTTCTGATGT TCGTTCCAA GTCAAGTTTC  
3901 ATTTTCGAAA TCATTTAAT GGTGGTGTCT CTATCGATGC TACAGGTGTT CCACCTCCAG ATGAGGCGCT GGAAGCCTCC AAGAAGGCTG ATGCGCTTTT  
4001 GTTAGGTGCT CTAAATGGG CTAAAGGTGTT AACAGGTTTT ACTAAAAATC CTGAATTTGA TTTCAATTGT TCCCAATTTA CGCCAACTTA  
4101 AGACCATGTA ACTTTGCTAT CGACTCTCTT TTAGACTTAT TCGGAATCAA GCCCAATTT GCTAAAGGTA CTGACTTCGT TGTGTGCTGA GAAATAGTGG  
4201 GAGGTATTTA CTTTGGTAAG AGAAAGGAGG ACGATGGTGA TGCTGTCTGT TGGGATAGTG AACAAATACAC CGTTCACAGA TGGCAAGAAA TCACAAAGG  
4301 GGCGCGTTCT ATGCGCCTAC AACATGAGCC ACCATTGCCT ATTTGTCCT TGGATAAAGC TAATGTTTTG GCCTCTTCAA GATTATGGAG AAAACATGTC  
4401 GAGGAACACA TCAAGAACGA ATTTCTTCAA TTGAAGTTC AACATCTCTA GTTGTATTCT GCCGCCATCT TCCTAGTTAA GAACCCAAAC CACCTAATGT  
4501 GTATTATAAT CACCAGCAAC ATGTTTGGTG ATATCATCTC CATGGAAGCC TCCGTTATCC CAGGTTCCCT GGGTTTGTG CATCTGCGT CTTGGGCTTC  
4601 TTTGCCAGAC AAGAACACCG CATTTTGGTT GTACGAACCA TGCCACGGTT CTGCTCCAGA TTTGCCAAG AATAAGGTA ACCCTATCGC CACTATCTGT  
4701 TCTGCTGCAA TGATGTTGAA ATTGTCAATT AACTTGCCTG AAGAAGGTAA GGCCATTGAA GATGCACTGA AAAAGGTTTT GATGCAAGT ATCAGAAGT  
4801 GTGATTTAGG TGGTTCCAAC AGTACCACCG AAGTCGGTGA TGCTGTCCGC GAAGAAGTTA AGAAAACTCT TGCTTAACCT GGAGGACCCCT TCTCTTTAGA  
4901 CTATTCTACT CTTATGACAG TAAAAAATTC TAGGAAATAT GTTAACTATA GAAGTAATAA AACCGGCTAG TGGCATTCAT ATGACCGCTG TTTTACATCT  
5001 ACATACACA TTTGAGTGT ATATCTCGCA ACGTTGGCGT TAAATAGGCA GGAGTACTCG TATCGCATGT CGGTGCGACA GCAAAATACA AAATGGAATA  
5101 TGTTTCATAGG GTAGACGAAA CTATATACCG AATCTACATA CATTATTCAA GAAGGAGAAA AAGGAGGATG TAAAGGAATA CAGGTAAAGTA AATTGATACT  
5201 AATGGCTCAA CGTGATAAGG AAAAAGAAAT GCACTTTAAAT ATTAATATTG ACAAGGAGGA GGGCACCACA CAAAAGGTTA GAGTGAACAG AAAATCATGA  
5301 AACTATGATT CCTAATTTAT ATATTGGAGG ATTTTCTCTA AAAAAAATAA AATAACAACA ATAAAAACA CTCATGACC TGACCATTTG ATCGAGTTTA  
5401 AGTCAATACC TCTTTGAACC ATTTCCCATTA ATGGTGAAAG TTCCCTCAAG AATTTTACTC TGTGAGAAC GGCCTTAAAC AGCTATGACA CCTCCTCTC  
5501 AGTACTAAT CTACCAATAC CAAATCTGAT GGAAGAATGG GCTAATGCAT CATCCTTACC CAGCGCCGAG CGGCGCGGTG TTACAACCAA TTAACCAATT  
5601 CTGATTAGAA AAATCATCG AGCATCAAT GAAATGCAA TTTATTCTA TACAGGATTAT CAATACCTA TTTTGAATA AGCCGTTTCT GTAATGAAG  
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5801 TCAAAAAATA GGTATATCAG TGAGAAATCA CCATGAGTGA CGACTGAAAT CGGTGAGAAT GGCAAAAGCT TATGCAATTT TTTCCAGACT TGTTCACAG  
5901 GCGAGCCATT ACGCTCGTCA TCAAAATCAC TCGCATCAAC CAAACGGTTA TCCATTCTGT ATTGCGCCTG AGCGAGGCGA AATACGGAT CTGTTTAAA  
6001 AGGCAATTA CAAACAGGAA TCGAATGCAA CCGCGCGAGG AACACTGCCA CGCATCAAC AATATTTTCA CCTGAATCAG GATATCTCT TAATACCTGT  
6101 AATGCTGTTT TCCCGGGGAT CGCAGTGGTG AGTAACCATG CATCATCAGG AGTACGGATA AAATGCTTGA TGGTCCGAAG AGGCATAAAT TCCGTCAGCC  
6201 AGTTTAGTCT GACCATCTCA TCTGTAACAT CATTGGCAAC GCTACCTTTG CCATGTTTCA GAAACAACCT TGGCGCATCG GGCTTCCCAT ACAATCGATA  
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6501 ATCAGAGATT TTGAGACACA ACGTGGCTTT GTTGAATAAA TCGAATTTT GCTGAGTTGA AGGATCAGT ATGACCAAAA TCCCTTAAAC TGAGTTTTCG  
6601 TTCCACTGAG CGTCAGACCC CGTAGAAAA ATCAAAAGGAT CTTCTTGTGA TCCTTTTTTT CTGCGGTAA TCTGCTGCTT GCAAAAAA AAACACCCCG  
6701 TACCAGCGGT GGTTTGTTTG CCGGATCAAG AGCTACCAAC TCTTTTTTCC AAGGTAACGT GCTTCAGCAG AGCGCAGATA CCAATACCTG TTCCTTCTAG  
6801 GTAGCCGTAG TTAGGCCAC ACTTCAAGAA CTCGTAGCA CCGCTACAT ACCTCGCTCT GCTAATCCTG TTACCACTGG CTGCTGCCAG TGGCGATAAG  
6901 TCGTGTCTTA CCGGGTTGCA CTCAGACGA TAGTTACCGG ATAAGGCGCA CGCGTCGGG TGAAACGGGG GTTCTGTCAC ACAGCCGAGC TTGGAGCGAA  
7001 CGACCTACAC CGAAGTGA TACCTACAGC GTGAGCTATG AGAAGAGCCG ACGCTTCCCG AAGGGAGAAA GCGCGACAGG TATCCGGTAA GCGGACGGT  
7101 CGGAACAGGA GAGCGCACGA GGGAGCTTCC AGGGGGAAC GCCTGGTATC TTTATAGTCT TGTGCGGTTT CGCCACCTCT GCACTGAGC TCGATTTTTG  
7201 TGATGCTCTG CAGGGGGGCG GAGCCTATGG AAAAAAGCCA GCAACGCGCG CTTTTTACGG TTCTTGGCCT TTTGCTGGCT TTTGCTTCTC ATGTCTTTCT  
7301 CTGGGTTATC CCCTGATTCT GTGGATAACC GTGCGGCGCG CAATACCCAG AATAAACGA AAATAAAGCA AGGTACGTTGA AATTAATATT TTTAAATGGT  
7401 TCTAACCAGT GCCGAAGAAC TGCGCAGTCC GGTATAACG TCTGACATGT CCTTTTTTGA TTTGGAATCC AACCACTCAA GTGACTCTGT TCATTTACTT  
7501 TGCGAAAAAT ATACCCACAA ATTGCCCATC GAAAGTGAAT CGCAACCCAC CTTGAGACT GCACCGACAA AGCAAGAGT ATACAGACAG AGTACTTTAT  
7601 ACGTACCGTT AAGTCTCAAG CAAAGGGTTT TCTTATTATC TGAACGGGTA AAGAGTATCT GGGCCGGCTT GCCAAGATGC AAACCGGAATA AGTATTTCAA  
7701 AGTTGCAATTT GCCTTAGCCG TCCTGACACC ATTTGGCTATT TGGATATTTT ATATTGACTT TCGTGTACAT TGATCACAT GACTGTTCTA TTGGCAAGT  
7801 AACCAAGCGG ATTGACTATT TTTCAAGTTA CTACTATATA TTATCATCAC GCGCAAGGAT TGTACCTGTA ATTCGATCT GACCTGATGA GACGTGGTAG  
7901 AGCCACAAC AGCCGGTACA AGCAACGATC TCCAGGACCA TCTGAATCAT GCGCGGATGA CACGAACCTA CGACGGCGAT CACAGACATT AACCCACAGT  
8001 ACAGACACTG CGACAACGTG GCAATTCTGC GCAATAC

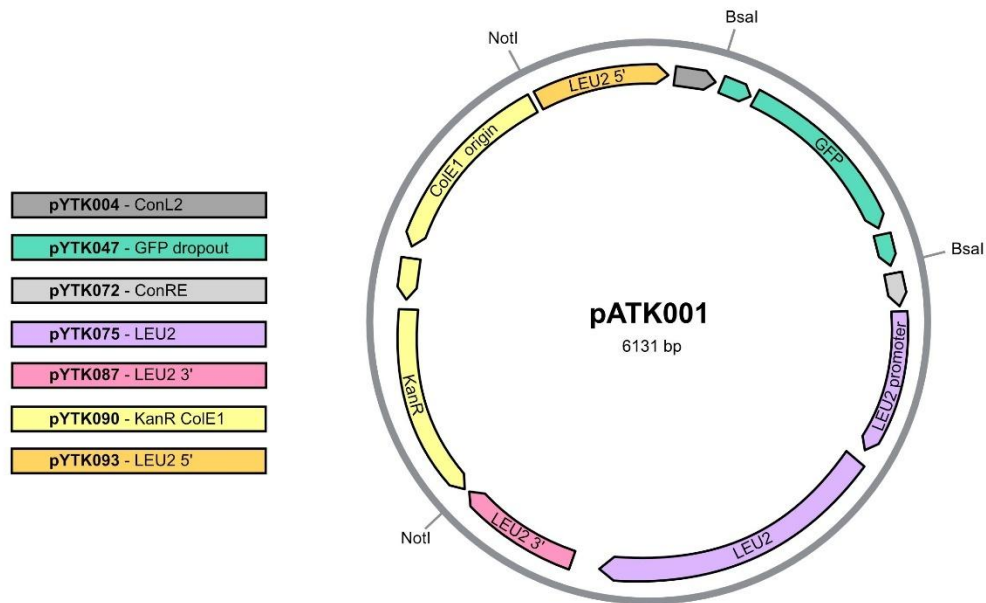
**Supplementary Figure 23. The nucleotide sequence of PaTG surface display construct.** The surface display parts are marked with the following colours: Blue = TDH3 promoter, Orange = SP w/ Kex2, Pale blue = PaTG, Green = CBM3a, Grey = Linkers, Yellow = AGA2, Pink = HA-tag, Red = tTDH1.

1 AACGCGAGTTC GAGTTTATCA TTATCAATAC TGCCATTTC AAGAATACGT AAATAATTAA TAGTAGTGAT TTTCTTAAC TTTATTAGTC AAAAAATTAG  
101 CCTTTTAAAT CTGCTGTAAC CCGTACATGC CCAAAATAGG GGGCGGGTTA CACAGAATAT ATAACATCGT AGGTGCTCGG GTGACAGATT TATTCCTGGC  
201 ATCCCACTAAA TATAATGGAG CCCGCTTTTT AAGCTGGCAT CCAGAAAAAA AAAGAATCCC AGCACCACAAA TATTGTTTTC TTCACCAACC ATCAGTTCAT  
301 AGGTCCATTCT TCTTAGCGCA ACTACAGAGA ACAGGGGCAC AAACAGGCAG AAAACGGGCA CAACCTCAAT GGAGTGATGC AACCTGCCTG GAGTAAATGA  
401 TGACACAAAGG CAATTTAGCCC ACGCATGTAT CTATCTCATT TTCTTACACC TTCTTATACC TTCTGCTCTC TCTGATTTGG AAAAAGCTGA AAAAAAGGT  
501 TGAAACCAAGT TCCCTGAAAT TATTCCCCCTA CTGACTAAT AAGTATATAA AGACGGTAGG TATTGATTGT AATTCTGTAA ATCTATTCTC TAAACTTCTT  
601 AAATTTCTACT TTTATAGTTA GTCTTTTTTT TAGTTTTAAA ACACCAAGAA CTTAGTTTTCG AATAAACACA CATAAACCAA CAAAGATTCT ATGAGATTCC  
701 CATCTATTTT TACAGCTGTT TTGTTTGCTG CATCTTCAGC ATTAGCTGCA CCAGTTTAATA CTACAACCTGA AGATGAAACT GCTCAAACTC CAGCTGAAGC  
801 AGTTATTGGT TATTGGATT TGGAGGGTGA CTTTGATGTT GCAGTTTTCG CATTTTCTAA CTCACACAAAC AACGGTGTGT TGTTTATTA TACAACATT  
901 GCTTCAATTG CTGCAAAAGA AGAAGGTGTT GGTTCGTATA ATGTTGCTGG TGAAGAACT AAGTCATACG CAGAACTTA CAGATTGACA GCTGATGATG  
1001 TTGCAAAACAT CAACGCTTTG CAAGAATCTG CACCAGCTGC ATCTTCAGCT GGTCCATCTT TTAGAGCACC ATCTTTGGAT AAAAGAGAAG CTGAAGCAGA  
1101 TTCAGATGAT AGAGTTACTC CACCAGCTGA ACCATTAGAT AGAATGCCAG ATCCATATAG ACCATCATAC GGTAGAGCAG AACACAGTTG TAACAACTAC  
1201 ATCAGAAAGT GGCACCAAGT TTACTCTCAT AGAGATGGTA GAAACACACA AATGACTGAA GAACAAAGAG AATGGTTGTC ATATGGTTGT GTTGGTGTTA  
1301 CTTGGGTAA TTCTGGTCAA TACCCAACAA ATAGATTGGC TTTTGCATCT TTCGATGAAG ATAGATTCAA AAACGAATTG AAAAATGGTA GACCAAGATC  
1401 AGGTGAAACA AGAGCTGAAT TTGAAGTAG AGTTGCAAAAG GAATCTTTTCG ATGAAGAAAA GGGTTTTCAA AGAGCAAGAG AAGTTGCTTC AGTTATGAAT  
1501 AGAGCATTTG AAAATGCTCA TGATGAATCT GCTTATTTTG ATAATTGAA GAAAGAATTG GCACACGGTA ACGATGCTTT GAGAAACGAA GATGCTAGAT  
1601 CACCATTTTA CTCTGCAATT AGAAACACTC CATCTTTTAA AGAAAGAAAC GGTGGTAACC ATGATCCATC TAGAATTGAAG CTTGTTATAT ATTCAAAGCA  
1701 TTTTTTGTCT GGTCAAGATA GATCTTCATC TGCTGATAAG AGAAGATTAC GTGACCAGA TGCTTTTGA CCAGCTCCAG GTACTGTTT GGTGTGATGT  
1801 TCTAGAGTAC GAACATCCAC ACATCTCCAG GTGAAGTTT CTGTAACCTC GATTACGGTT GGTTTGCTGC TCAACACAGA CCGATGACAG  
1901 ATAAACTCTG TTGACACAT GGTAACTATT ATCATGCTCC AAATGTGTTA TTGAGTGCAA TGCAATGTTA CGAATCTAAG TTTAGACAACT GGTGCAAGG  
2001 TTATTCTGAT TTTGATAG GTGCTTACGT TATCATTTTT ATTCGAACAT AGCACCAGAT AAAGTTTAAAC CTTGGTGGCC AGGATTGGCC  
2101 AATACACCGG TATCAGGCAA TTTGAAGGTT GAATTTCTACA ACAGCAATCC TTCAGATACT ACTAATCTCA TCAATCTCTCA GTTCAAGGTT ACTAATACCG  
2201 GAAGCAGTGC AATTGATTG TCCAACTCA CATTGAGATA TTATTATACA GTAGACGGAC AGAAAGATCA GACCTTCTCG TGTCAGCATC CTGCAATTAAT  
2301 CGGCAGTAAC GGCAGTACA ACGGAATTAC TTCAAATGTA AAAGAAACAT GAGTTCTCTCA ACAAAATACG CAGACACCTA CTTGTAATAA  
2401 AGCTTTACAG GCGGAACCTCT TGAACCGGGT GCACATGTT AGATACAAGG TAGATTGCA AAGAATGACT GGAGTAACTA TACACAGTCA AATGACTACT  
2501 CATTCAAGTC TGCTTACAG TTTGTTGAAT GGGATCAGGT AACAGCATGT TTGAACGGTG TCTTGTGATG GGGTAAAGAA CCCCGTGGCA TCGGTGTTGG  
2601 GTCCGGTTCT GGTTCAGGCT CAGGTTTCAGG TTCTCAGGAA CTGACAACATA TATGCGAGCA AATCCCTCTCA CCACTTTAG AATCGACGCC GTACTCTTTG  
2701 TCAACGACTA CTATTTTGGC CAACGGGAAG GCAATGCAAG GAGTTTGTGA ATATTACAAA TCAGTAACGT TTGTCAGTAA TTGCGGTTCT CACCCCTCAA  
2801 CAACATGATA AGGCAGCCCC ATAAACACAC AGTATGTTTT TGGTCTTGGC AGCGGCTCAT ACCCATACGA CGTTCCTAGAC TACGCTTAAAC TCGAGTTAAA  
2901 GCAATCTTGA TGAGGATAAT GATTTTTTTT TGAATATACA TAAATACTAC CGTTTTTCTG CTAGATTTTG TGATGACGTA AATAAGTACA TATTACTTTT  
3001 TAAGCCCAAGA CAAGATTAA GATTAACTTT ACCCTTTTCT TCTTAAGTTT CAATATTAGT TATCACTGTT TAAAGATTAT TCGCGAGAACG TCGCGGGTTA  
3101 AAATATATTA CCCTGAAGCC GTCTCAAGCA GTTACAGAGA TGTTACGAAG CACATGTGCA CTGCAGTACA TCGAGGAGAA CTTCTAGTAT ATCTACATAT  
3201 CTAATATTAT TCCCTTTTAA AAAATGGAAT CCCAACAAAT CCATCAAAAT CCATCTCTCT TCAAAAATCA ATTGTCTCTGT TCTAGTCTGT TATGTTGATC  
3301 TCAAAAACGT TATATTATTA GGATAATTAT ACTCTATTTT TCAACAAGTA ATGGTTGTT TGGCCGAGCG GTCTAAGGCG CCTGATTCAA GAAATATCTT  
3401 GACCGCAGTT AACTGTGGGA ATACTCAGGT ATCGTAAGAT GCAAGAGTTC GAATCTCTTA GCAACCATTA TTTTTTTCTT CAACATAACG AGAACACACA  
3501 GGGCGCCTAT CCGCAGAAAT CAAATTCGAT GACTGGAAAT TTTTTTTTAA TTTTCAAGGT CGCCTGACCG ATATACCTTT TTTCAACTGAA AAATTTGGGAG  
3601 AAAAAGGAAA GGTGAGAGCG CCGGAACCGG CTTTTCATAT AGAATAGAGA AGCCTTCATG ACTAAATGCT TGCATCACAA TACTTGAAT TGACAAATAT  
3701 ATTTAAGGAC CTATTGTTTT TTCCAATAGG TGGTAGCAA TCGTCTTACT TTCTAACCTT TCTTACCTTT TACATTTTCA ATATATATAT ATATATATTT  
3801 CAAGGATATA CCATTCTAAT GTCTGCCCTC AAGAAGATCG TCGTTTTGCC AGGTGACCAC GTTGGTCAAG AAATCACAGC CGAAGCCATT AAGGTTCTTA  
3901 AAGCTATTTT TGATGTTCTG TCCAATGTCA AGTTCGATT CGAAATCTAT TTAATTGGT GTGCTGCTAT CGATGTCTACA GGTGTCCAC TTCCAGATGA  
4001 GGGCGTGGAA GCGTCCAAGA AGGCTGATGC GCTTTTGTTA GTGCTGTGGT GTGGTCTCTAA ATGGGGAACC GGTAGTGTTA GACCTGAACA AGGTTTACTA  
4101 AAAATCCGTA AAGAATCTCA ATTTAGCCG AACTTAAGAC CATGTAACCT TGCAATCCGAC TCTCTTTTAG ACTTATCTCC ATCAACGCA CAATTTGCTA  
4201 AAGGTACTGA TCTCGTTGTT GTGAGGAGG TATTACTTTT GTTAAGAGAA AGGAGGACGA TGGTGATGTT GTGCTGTGGG ATGCTGAACA  
4301 ATATAGGCTT CCAGAAAGTGC AAAGAATCAC AAGAATGGCC GCTTTTCTAG CCCTTACAACA TGAGCCACCA TTGCTTATTT GTCCTTGGTA TAAAGCTAAT  
4401 GTTTTGCCTT CTTCAAGATT ATGGAGAAAA ACTGTGGAGG AACTCATCAA GAACGAATTT CTACATTGA AGGTTTCAAC TCAATTGATT GATTCTGCCG  
4501 CCATGATCCT AGTTAAGAAC CCAACCCACC TAAATGGTAT TATAATCACC AGCAACATGT TTGGTGATAT CATCTCCGAT GAAGCCCTCCG TTATCCCAGG  
4601 TTCTTGGGTT TTGTTGCCAT CTGCGTCTTT GGCCTCTTTT CCAGACAGTA ACACCCGATT TGGTTGTGAC GAACCATGCC ACGGTTCTCG TCCAGATTG  
4701 CCAAGAATA AGGTCAACCC TATCGCCACT ATCTTGTCTG CTCGAATTG GTTGAAATTG TCATTGAAC TGCCTGAAGA AGGTGAAGCG ATTTGAAGAT  
4801 CAGTTTAAAA GGTTTGGAT GTAGGTATCA GAACCTGGTA TTTAGTGGT TCCACACAGTA CCACCGAAGT CGGTGATGCT GTCCCGGAAG AAGTTAAGAA  
4901 AATCCTTGAT TAACTCGGAG GACCTTCTCT TTAGACTAT TTAGCTGTT TCGACGTAAA AAATCTTAGG AAATATTGAT TAACTAGGAG TAAATTAACC  
5001 GGCTAGTGGC ATTCATATAG CCGTCTGTTT ACATCTACAT CACACATTTT GAGTGTATAT CTCGCAACGT TGGCGTTAAA TAGGCAGGAG TACTCTGATC  
5101 GCATGTCGTT GCGACACGAA ATTACAAAAA GGAATATGTT CATAGGATAG ACGAAGATAT ATACGCAATC TACATACATT TATCAAGAAG GAGAAAAAGG  
5201 AGGATGTAAA GGAATACAGG TAAGCAAAAT GCTCAAGCTG CTCGAAGTGT ATAAGGAAAA AGAATTGCAT TTTAATCATT TATTGACAA GAGGAGGGCG  
5301 ACCACACAAA AAGTTAGGTT TAACAGAAAA TCATGAAACT ATGATTCCTA ATTTATATAT TGGAGGATT TCTCTAAAAA AAAAAATAA CAACAAATAA  
5401 AAAACACTCA ATGACCTGAC CATTGTATGG AGTTTAAAGT AACACCTTTT GAAACCATTT CCCATAATGG TGAAAGTTTC CTCAAGAATT TACTCTGTCT  
5501 AGAAACGGCC TTAACGACGT AGTCGACCTC CTCTTCAGTA CTAATCTAC CAATACCAAA TCTGATGAAA GAATGGGCTA ATGCATCATC CTTACCAGC  
5601 CGCGAGCGGC CCGCTGTTAC AACCAATTAA CCAATTCTGA TTAGAATAA CTATCGAGCA TCAATGAAA CTGCAATTTA TGCATCATG GATTATCAAT  
5701 ACCATATTTT TGA AAAAGGCC GTTCTGTAA TGAAGGAGAA AACTCACCGA GGCAGTTCCA TAGGATGGCA AGATCCTGGT ATCGGTCTGC GATTCCGACT  
5801 CGTCCAACAT CAATACCAACC TATTAATTTT CCCTCGTCAA AAATAAGGTT ATCAAGTGAG AAATCACCAT GAGTGACAG TGAATCCGGT GAGAAATGGCA  
5901 AAAGCTTATG CATTCTTTTC CAGACTTGTG CAACAGGCCA GCCATTACGC TCGTCACTAA AATCACTCGC ATCAACCAAA CCGTTATTCA TCTGTATTG  
6001 CGCTCGAGCG AGGCGAAATA CGCGATCGCT GTTAAAAGGA CAATTACAAA GAGGAATCGA ATGCAACCGG CGCAGGAACA CTGCCAGCG CTACCAATA  
6101 TTTTCACTG ATCAGGATA TTCTTCTAAT ACCTGGAATG CTGTTTTCCC GGGGATCGCA GTGGTGAGTA ACCATGAGTA CTAGGAGTA CGGATAAAAT  
6201 GCTTGATGTT CGGAAGAGGC ATAAATTCGG TCAGCCAGTT TAGTCTGACC ATCTCATCTG TAACATCATT GGCACCGCTA CCTTTGCCAT GTTTCAGAAA  
6301 CAACCTGTCG GCATCGGGCT TCCCATACAA TCGATAGATT GTGCGACCTG ATTGCCCGAC ATTTATCGCA GCCCATTTAT ACCCATATAA ATCAGCATCC  
6401 ATGTTTGAAT TTAATCGCG CCTGGAGCAA GACGTTTTCC GTTGCAATAT GTGCAATAACA CCCCTGTAT TACTGTTTAT TTAAGCAGAC AGTTTATTATG  
6501 TTCATGATGA TATATTTTTA TCTTGTGCAA TGTAACATCA GAGATTTTGA GACACAACGT GGCCTTTGTT AATAAATCGA ACTTTTGTCT AGTTGAAGGA  
6601 TCAGTCATGA CCAAAATCCC TTAACGTGAG TTTTCGTTC CAGTACGCTC AGCCCCCGTA GAAAGATCA AAGGATCTCT TTGAGATCCT TTTTTTCTGC  
6701 GCGTAACTCTG CTGCTTGCAA ACAAAAAAAC CACCGTACC AGCGGTGGT TGTTTGCCGG ATCAAGAGCT ACCAATCTCT TTCCGAGACT TAACTGGCTT  
6801 CAGCAGAGCTC CAGATACCAA ATACTGTTCT TCTAGTGTAG CCGTAGTGTG GACCACACTT CAAGAACTCT GTAGCACCGC CTACACTCT CCGTCTGCTA  
6901 ATCTCTGTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGGCGACGG TCGGGCTGAA  
7001 CGGGGGGTTG GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACGCAA CTAGATACC TACAGCGTGA GCTATGAGAA AGCGCCACG TTCCGGAAGG  
7101 GAGAAAGGCG GACAGGTATC CGGTAAGCGG CAGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCTT GGTATCTTTA TAGTCTGTCT  
7201 GGGTTCTGCG ACCTCTGACT TGAGCGTCGA TTTTGTGAT GCTCGTCAAG GGGGCGGAGC CTATGGA AAAA ACGCCAGCAA CGCGGCTTT TTACGGTTCC  
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7501 GAATCCAACC ACTCAAGTGA CTCTGTTTCT TTACTTTGCG AAAAAATATC CCACAATTTG CCCATCGAAA GTGAATCGCA AACCACCTCT AGACTGGCAC  
7601 CGACAAAAGCA AAGATTATAC AGACAGAGTA CTTTATACGT ACCGTTAAGT CTCAGACAAA GGGTTTTCTT ATTTACTGAA CGGGTAAAGA GATCTGGGCG  
7701 CGGCTTGCCA AGATGCAAAAC CGAATAAGTA TTTCAAAGTT GCAATTTGCT TAGCCGTCCT GACACCATG GCTATTTGGA TATTTATAT TGACTTTCTG  
7801 GTACATTGAT CACATCGACT GTTCTATTGG CAAATGAACC ACGGGCATTG ACTATTTTTT AGGTACTAC TATATATTAT TATCATGGG AAGGATTGTA  
7901 CCCTGAATTC GCATCTAGAC TGATGAGAGC TGGTAGAGCC ACAACAGGCC GGTACAAGCA ACGATCTCCA GGACCATCTG AATCATGCGC GGATGACAGC  
8001 AACTCACGAC GGCATCACA GACATTAACC CACAGTACAG ACACGCGAC AACGTGGCAA TCTGTCGCAA TAC

**Supplementary Figure 24. The nucleotide sequence of SmTG surface display construct.** The surface display parts are marked with the following colours: Blue = TDH3 promoter, Purple = SP w/o Kex2, Turquoise = Pro-Kex2-SmTG, Green = CBM3a, Grey = Linkers, Yellow = AGA2, Pink = HA-tag, Red = tTDH1.

**Supplementary Table 7.** The primers used in yeast colony PCR.

| <b>Primer</b> | <b>Oligonucleotide sequence (5'→3')</b> | <b>T<sub>m</sub> (C°)</b> |
|---------------|-----------------------------------------|---------------------------|
| BaTG Forward  | GATCATCATCTCTGGTCAAG                    | 50                        |
| BaTG Reverse  | CCATGCATAATTTGGTATAATG                  | 47                        |
| BsTG Forward  | GGTCAATTGTTAAGACCAC                     | 47                        |
| BsTG Reverse  | CTCTAACAATTCTAAACAAAGATGG               | 51                        |
| PaTG Forward  | CATTATGGTCTTTGCCAATG                    | 48                        |
| PaTG Reverse  | CCAAACCACCAAACCATC                      | 48                        |
| SmTG Forward  | GATAATGGTGCTGGTGAAGAAAC                 | 53                        |
| SmTG Reverse  | CCAACCTTGTTAACTTTATCTG                  | 50                        |



**Supplementary Figure 25. Vector map for the genome-integration vector pATK001.** The identifiers of the YTK parts used to assemble the vector are also indicated. In pATK001, the inverted BsaI sites flanking the GFP dropout cassette enable cloning of the insert by BsaI reaction. NotI digestion yields homology arms for integration into the yeast genome to the *LEU2* locus.

**Supplementary Table 8.** The YTK part plasmids<sup>†</sup> used to assemble the genome-integration vector pATK001.

| Plasmid | YTK part no. | Encodes          | <i>E. coli</i> marker |
|---------|--------------|------------------|-----------------------|
| pYTK004 | 1            | ConL2            | CamR                  |
| pYTK047 | 234r         | GFP dropout      | CamR                  |
| pYTK072 | 5            | ConRE            | CamR                  |
| pYTK075 | 6            | LEU2             | CamR                  |
| pYTK087 | 7            | LEU2 3' homology | CamR                  |
| pYTK090 | 8a           | KanR-ColE1       | KanR                  |
| pYTK093 | 8b           | LEU2 5' homology | CamR                  |

<sup>†</sup>The part plasmids originated from the MoClo-YTK kit.

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1 AACGTGAGAC CGAAGTGA ACGTGATTTC ATGCGTCATT TTGAACATTT TGTAAATCTT ATTTAATAAT GTGTGCGGCA ATTCACATTT AATTTATGAA
101 TGTGTTCTTA ACATCGCGGC AACTCAAGAA ACGGCAGGTT CGGATCTTAG CTACTAGAGA AAGAGGAGAA ATACTAGATG CGTAAGGCGC AAGAGCTGTT
201 CACTGGGTGC GTCCCTATTTC TGGTGGAACT GGATGGTGAT GTCAACGGTC ATAAGTTTTC CGTGGGTGAG GTGACGCAAC TAATGGTAAA
301 CTGACCGTGA AGTTTCTCTG TACTACTGGT AAACCTCGCG TCCCTTGGCC GACTCTGGTA ACGACGCTGA CTTATGGTGT TCAGTGGCTT GCTCGTTATC
401 CGGACCATAT GAAGCAGCAT GACTTCTTCA AGTCCGCCAT GCCGGAAGGC TATGTGCAGG AACGCACGAT TTCCTTTAAG GATGACGGCA CGTACAAAC
501 GCGTGGCGGA GTGAAATTTG AAGGCGATAC CCTGGTAAAC CGCATTAAGC TGAAGGCGAT TGACTTTAA GAGGACGGCA ATATCTCTGG CCATAAGCTG
601 GAATACAAAT TTAACAGCCA CAATGTTTAC ATCACCGCCG ATAAACGAAA AAATTGGCATT AAAGCGAATT TTAAAAATTC CCACAACCTG GAGGATGGCA
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901 ATCAAAATAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCCT TCGTTTTATC TGTGTTTGT CGGTGAACGC TCTCTACTAG AGTCACACTG GCTCACCTTC
1001 GGGTGGGCCCT TTCTGCGCTT ATAGGTCTCA GCTGAGCATG AGACGGAAT CTGCTCGTCA GTGGTGTCTA CACTGACGAA TCATGTACAG ATCATACCGA
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1301 AAAATCAATT GTCCTGTACT TCCTTGTTC TGTGTGTTCA AAAACGTTAT ATTTATAGGA TAATTATACT CTATTTCTCA ACAAGTAATT GGTGTTTGG
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3001 TCTTAGGAAA TATGATTTAA CTAGGAGTAA AATAACCGCG TAGTGGCTCT CATATAGCCG TCTGTTTACA TCTACATCAC ACATTTCCAG TGTATATCTC
3101 GCACCGTTGG CGTTAAATAG GCAGGAGTAC TCGTATCGCA ACAAAGTGGC ACAGAAATTT ATATGTTTCA ATAGTTTCAAT ACTGTTTCAAT AACTATATTA
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5801 ACCATTGGCT ATTTGGATAT TTTATATTGA CTTTCTGTGA CATTGATGAC ATCGACTGTT CTATTGGCAA ATGAACCAAG GGCATTGACT ATTTTTCAGG
5901 TTACTACTAT ATATTATCAT CACGGGCAAG GATTGTACCC TGAATTCCGA TCTAGAATAA ATCGCAGCCA AGTGAGTGAA TAGATGACGC ACCACGCTCA
6001 GACACGGCCA CATCGTATCT CACAGGAGCA AGCGCGATAG GAGCACTCAC ACATAGTACG GTGATCCGCT GACTCCTTTG CCCAAATAAG ACGTGAGGCC
6101 GTCTCAGATG CACACACTGG CTTAAGATGA C

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**Supplementary Figure 26. Nucleotide sequence of the genome-integration vector pATK001.** The vector parts are marked with the following colours: Green = GFP dropout cassette, Light grey = ConRE, Purple = LEU2, Pink = LEU2 3' homology, Yellow = KanR-ColE1, Orange = LEU2 5' homology, Dark grey = ConL2.

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1  GGTCTCAATC CCATCATCAT CATCATCACT AATGGCTGAG ACCAGACCAA TAAAAACGC CCGGCGGCAA CCGAGCGTTC TGAACAAATC CAGATGGAGT
101 TCTGAGGTCA TTACTGGATC TATCAACAGG AGTCCAAGCG AGCTCGATAT CAAATTACGC CCCGCCCTGC CACTCATCGC AGTACTGTTG TAATTCATTA
201 AGCATTCCTGC CGACATGGAA GCCATCACAA ACGGCATGAT GAACCTGAAT CGCCAGCGGC ATCAGCACCTT TGTGCGCTTG CGTATAATAT TTGCCCATGG
301 TGAAAAACGGG GGCAGAAGAG TTGTCCATAT TGGCCACGTT TAAATCAAAA CTGGTGAAAC TCACCCAGGG ATTGGCTGAA ACGAAAAACA TATTCTCAAT
401 AAACCCCTTA GGGAAATAGG CCAGGTTTTC ACCGTAACAC GCCACATCTT GCGAATATAT GTGTAGAAAC TGCCGGAAAT CGTCGTGGTA TTCACTCCAG
501 AGCGATGAAA ACGTTTCAGT TTGCTCATGG AAAACGGTGT AACAAGGGTG AACACTATCC CATATCACCA GCTCACCGTC TTTCATTGCC ATACGAAATT
601 CCGGATGAGC ATTCATCAGG CGGGCAAGAA TGTGAATAAA GGCCGGATAA AACTTGTGCT TATTTTCTT TACGGTCTTT AAAAAGGCCG TAATATCCAG
701 CTGAACGGTC TGGTATAGG TACATTGAGC AACTGACTGA AATGCCCAA AATGTTCTTT ACGATGCCAT TGGGATATAT CAACGGTGGT ATATCCAGTG
801 ATTTTTTTCT CCATTTTAGC TTCCTTAGCT CCTGAAAATC TCGATAACTC AAAAAATACG CCCGGTAGTG ATCTTATTTT ATTATGGTGA AAGTTGGAAC
901 CTCTTACGTG CCCGATCAAT CATGACCAA ATCCCTTAAC GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG
1001 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAAACAA AAAACCAACG CTACCAGCGG TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTC
1101 GAAGGTAACT GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTTCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA
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1301 AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC
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1501 CTTTATAGTC CTGTCCGGTT TCGCCACCTC TGACTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG
1601 CCTTTTACG GTTCTGGCC TTTTGTGTC CTTTGTGCTA CATGTTCTTT CCTGCGTTAT CCCCTGATTG TGTGGATAAC CGTAGTC

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**Supplementary Figure 27. The nucleotide sequence for the part plasmid pATK008.** The part plasmid was generated by cloning the synthesised (His)<sub>6</sub>-tag encoding gene into the YTK entry vector pYTK001. The insert is highlighted with pink colour.

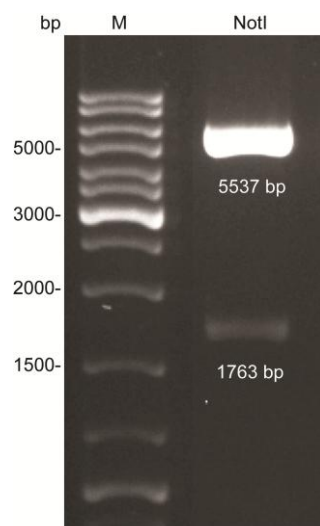
**Supplementary Table 9.** Parts used to assemble the SmTG secretion construct and their access information.

| Plasmid | YTK part no. | Encodes                   | <i>E. coli</i> marker | Source                                            |
|---------|--------------|---------------------------|-----------------------|---------------------------------------------------|
| pATK001 | —            | Genome integration vector | KanR                  | Linder group collection, YTK toolkit <sup>†</sup> |
| pYTK030 | 2            | pGAL1                     | CamR                  | YTK toolkit                                       |
| pATK002 | 3a           | SP w/ Kex2                | CamR                  | Linder group collection, ATK toolkit <sup>‡</sup> |
| pATK003 | 3a           | SP w/o Kex2               | CamR                  | Linder group collection, ATK toolkit              |
| pATK007 | 3b           | Pro-Kex2-SmTG             | CamR                  | Linder group collection, ATK toolkit              |
| pATK008 | 4a           | (His) <sub>6</sub> -tag   | CamR                  | Linder group collection, ATK toolkit              |
| pYTK066 | 4b           | tTDH1                     | CamR                  | YTK toolkit                                       |

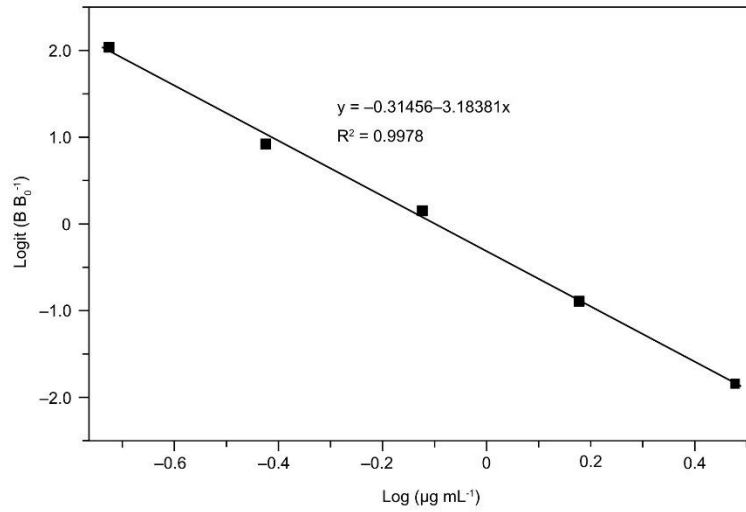
<sup>†</sup>The original YTK toolkit is available at Addgene. <sup>‡</sup> ATK Toolkit that extends the YTK toolkit with enzyme and protein parts is currently only available upon request from Linder group, Aalto University.

1 AACGCCCCAT TATCTTAGCC TAAAAAACC TTCTCTTTGG AACTTTTCAGT AATACGCTTA ACTGCTCATT GCTATATTGA AGTACGGATT AGAAGCCGGC  
101 GAGCGGGCGA CAGCCCTCCG ACGGATGACT CTCTCCGCTG CGTCCTCGTC ATCACC GGTC GCGTTCCGTA AACGCAGATG TGCCTCGCGC CGCACTGCTC  
201 CGAACCAATA AGATTCTACA ATACTAGCTT TTATGGTTAT GAAGAGGAAA AATTGGCAGT AACCTGGCCC CACAAACCTT CAAATTAAAC AATCAAATTA  
301 ACAACCATAG GATGATAATG CGATTAGTTT TTTAGCCTTA TTTCTGGGGT AATTAAATCAG CGAAGCGATG ATTTTGTATC TATTAACAGA TATATAAATG  
401 GAAAAGCTGC ATAAACCATT TAACATAATC TTTCAACATT TTCAGTTTGT ATTACTTCTT ATTCAAAATG CATAAAAAGT TCAACAAAAA ATTTGTTAATA  
501 TACCTCTATA CTTTAAACGTC AAGGAGAAAA AACTATAAGA TCTATGAGAT TCCCATCTAT TTTTACAGCT GTTTTGTTTG CTGCATCTCT AGCATAGCT  
601 GCACCGCTTA ATACTACAAC TGAAGATGAA ACTGCTCAAA TTCAGCTGTA AGCAGTTTAT GTTTATTGTTG ATTTGGAGGG CAGCTTTGAT GTTGCACTTT  
701 TGCCATTTC TAACTCAACA AACACCGGTT TGTGTTTAT TAATACAAC ATTGCTTCAA TTGCTGCAAA AGAAGAAGGT GTTGGTTCTG ATATGGTGTG  
801 TGGTGAAGAA ACTAAGTCAT ACGCAGAAAC TTACAGATTG ACAGCTGATG ATGTTGCAAA CATCAACGCT TTGCAAGAT CTGCACCAGC TGCATCTTCA  
901 GCTGGTCCAT CTTTTAGAGC ACCATCTTTG GATAAAGAG AAGCTGAAGC AGATTAGAT GATAGAGTTA CTCCACCAGC TGAACCATTA GATAGATGCT  
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1101 ACAAACTACT GAAGAACAAA GAGAATGGTT GTCATATGGT TGTGTTGGTG TTACTTGGGT TAATTCTGGT CAATACCCAA CAAATAGATT GGCTTTTGCA  
1201 TCTTTCCGATG AAGATAGATT CAAAAACGAA TTGAAAAATG GTAGACCAAG ATCAGGTGAA ACAAGAGCTG AATTGGAAGG TAGAGTTGCA AAGGAATCTT  
1301 TCGATGAAGA AAAGGGTTTT CAAAGAGCAA GAGAAGTTGC TTCAGTTATG AATAGAGCAT TGGAAAAATG TCATGATGAA TCTGCTTATT TGGATAATTT  
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6301 CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCGAG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT  
6401 CCTGTCCGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCT GTGAGGGGG CGGAGCCTAT GGA AAAACG CAGCAACGCG GCCTTTTTTAC  
6501 GGTTCCTGGC CTTTGTCTGG CTTTGTCTC ACATGTTCTT TCCTGCGTTA TCCCTGATT CTGTGGATAA CCGTGGCGCC GCCAATACCC AGAAGAACAG  
6601 TAAAAATAAG CAAGGTACGT GAAATTAATA TTTTAAATG GTTCTAACCG ATGCCGAAGA ACTGCGCAGT CCGGTTATAA CGTCTGACAT GTCTTTTTTT  
6701 GATTTGGAA CCAACCACT AAGTGACTCT GTTCATTAC TTTGCGAAAA ATATACCCAC AAATTGCCCA TCGAAAGTGA ATCGCAAAACC ACCTTACAGC  
6801 TGGCACCGCA AAAGCAAGA TTATACAGAC AGAGTACTTT ATAGCTACCG TTAAGTCTCA AGCAAGGGT TTTCTTATT ACTGAACGG TAAAGAGTAT  
6901 CTGGCCCGCG TTGCCAAGAT GCAAAACGAA TAAGTATTTT AAAGTTGCAAT TGCCCTTAGC CGTCTGACA CCATGTGCTA TTTGGATAT TATATTGAC  
7001 TTTGCTGTAC ATTGATCACA TCGACTGTTT TATTGGCAAA TGAACCAAGC GCATTTGACTA TTTTTCAGGT TACTACTATA TATTATCATC ACGGGCAAGG  
7101 ATTTGATACC GAATTCGCAT CTAGAATAAA TCGGACCAA GTGAGTGAAT AGATGACGCA CCACGGTCAG ACACGGCCAC ATCTATCTCT ACAGGAGCAA  
7201 GCGGATAGG AGCACTACA CATAGTACGG TATCCGCTG ACTCCTTTGC CCAATAAGA CGTGAGCCG TCTCAGATGC ACACACTGGC TTAAGATGAC

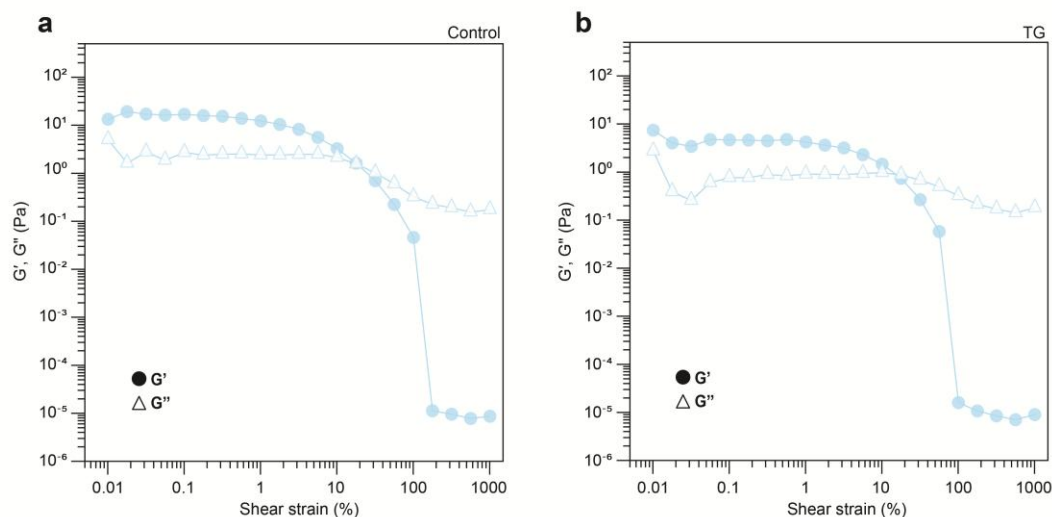
**Supplementary Figure 28. Nucleotide sequence of the SmTG secretion construct.** The parts for SmTG secretion are marked with the following colours: Blue = pGal1, Purple = SP w/o Kex2, Turquoise = Pro-Kex2-SmTG, Pink = (His)<sub>6</sub>-tag, Red = tTDH1.



**Supplementary Figure 29. NotI linearized SmTG secretion construct.** The two bands released upon NotI cleavage corresponded to the genome-integration cassette encoding the SmTG (5537 bp), and the kanamycin resistance and ColE1 parts (1763 bp).



**Supplementary Figure 30. Standard curve of ELISA.** The standard curve presents the logit transformation of absorbance values measured for a His-protein standard at 412 nm against their log concentration. The logit transformation was used to linearise the absorbance values before performing the linear regression fitting.



**Supplementary Figure 31. Amplitude sweeps of the yeast cultures containing casein and CNFs.**

The amplitude sweeps were performed for both the a) control yeast, and b) TG secreting yeast cultivations. The storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) were plotted as a function of shear strain (%). The measurements were conducted with a constant frequency of  $6.28 \text{ rad s}^{-1}$  at  $23^\circ\text{C}$ .

**Supplementary Table 10.** Amino acid sequences of the enzymes secreted from yeast.

| Name and annotation                                       | Amino acid sequence <sup>†</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>SmTG</b></p> <p>SP w/o Kex2–Pro-Kex2-SmTG–6×HIS</p> | <p>MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAVLPFSNST<br/> NNGLLFINTTASIAAKEEGVSGDNGAGEETKSYAETYRLTADDVANINALQESAPA<br/> ASSAGPSFRAPSLDKREAEADSDDRVTTPAEPLDRMPDPYRPSYGRAETVVNNYIR<br/> KWQQVYSHRDGRKQQMTEEQREWLSYGCVGVTWVNSGQYPTNRLAFASFDEDR<br/> FKNELKNGRPRSGETRAEFEGRVAKESFDEEKGFQRRAREVASVMNRALENAHDES<br/> AYLDNLKKELANGNDALRNEDARSPFYALSALRNTPSFKERNNGGNHDP SRMKAVIYS<br/> KHFWSGQDRSSADKRKYGDPDAFRPAPGTGLVDMSRDRNIPRSPTSPGEGFVNFD<br/> YGWFGAQTEADADKTVWTHGNHYHAPNGSLGAMHVYESKFRQWSEGYSDFDR<br/> GAYVITFIPKSWNTAPDKVKQGWPGSHHHHHH*</p> |
| <p><b>β-lactamase</b></p> <p>SP-β lactamase–CBDcex</p>    | <p>MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAVLPFSNST<br/> NNGLLFINTTASIAAKEEGVSLDKRGSHPETLVKVKDAEDQLGARVGYIELDLNSG<br/> KILESFRPEERFPMMSFTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYSPVTEK<br/> HLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTSLDRWEP<br/> ELNEAIPNDERDITMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVGAPLLRS<br/> ALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEI<br/> GASLIKHWGSGGPAGCQVLWGVTNQWNTGFTANVTVKNTSSAPVDGWTLTFSFPS<br/> GQQVTQAWSSTVTQSGSAVTVRNAPWNGSIPAGGTAQFGFNGSHTGTNAAPTAFS<br/> LNGTPCTVGGSTG*</p>               |

<sup>†</sup>Minimal, flexible AS and GS linkers are indicated with light grey. The asterisk symbol (\*) signifies the stop codon.

**Supplementary Table 11.** The data used to generate the Ashby plot shown in Supporting Figure 12.

| Application      | Storage modulus (Pa) | Water content (wt.%) | Reference        |
|------------------|----------------------|----------------------|------------------|
| Living hydrogel  | 2840                 | 94.0                 | <b>This work</b> |
| Cheese           | 40                   | 95.8                 | 22               |
| Cheese           | 267                  | 94.8                 | 23               |
| Cheese           | 509                  | 93.0                 | 23               |
| Cheese           | 766                  | 92.7                 | 23               |
| Cheese           | 1803                 | 91.0                 | 23               |
| Cheese           | 1777                 | 90.2                 | 23               |
| Yoghurt          | 534                  | 96.0                 | 23               |
| Yoghurt          | 425                  | 95.7                 | 23               |
| Yoghurt          | 389                  | 95.4                 | 23               |
| Yoghurt          | 314                  | 95.2                 | 23               |
| Yoghurt          | 240                  | 93.0                 | 23               |
| Seafood paste    | 200                  | 52.0                 | 24               |
| Seafood paste    | 300                  | 47.0                 | 24               |
| Seafood paste    | 400                  | 41.0                 | 24               |
| Seafood paste    | 500                  | 38.0                 | 24               |
| Seafood paste    | 600                  | 33.0                 | 24               |
| Seafood paste    | 2000                 | 72.0                 | 25               |
| Meat alternative | 1200                 | 88.0                 | 26               |
| Meat alternative | 85                   | 87.0                 | 27               |
| Meat alternative | 500                  | 86.0                 | 27               |

**Supplementary Table 11.** Continues.

| <b>Application</b> | <b>Storage modulus<br/>(Pa)</b> | <b>Water content<br/>(wt.%)</b> | <b>Reference</b> |
|--------------------|---------------------------------|---------------------------------|------------------|
| Meat alternative   | 300                             | 95.0                            | 28               |
| Meat alternative   | 125                             | 97.3                            | 29               |
| 3D printing paste  | 600                             | 88.0                            | 20               |
| 3D printing paste  | 1500                            | 49.0                            | 30               |
| 3D printing paste  | 550                             | 90.0                            | 31               |
| 3D printing paste  | 1000                            | 80.0                            | 32               |
| Biomedical gel     | 1500                            | 97.5                            | 33               |
| Biomedical gel     | 400                             | 96.0                            | 34               |
| Biomedical gel     | 700                             | 95.0                            | 35               |

## References

1. Jin, M. & Zhong, Q. Transglutaminase cross-linking to enhance elastic properties of soy protein hydrogels with intercalated montmorillonite nanoclay. *J. Food Eng.* **115**, 33–40 (2013).
2. Moguilansky, S., Friedman, N. & Davidovich-Pinhas, M. The effect of transglutaminase on the structure and texture of plant-protein based bigel. *Food Hydrocoll.* **162**, 110981 (2025).
3. Wang, Y. *et al.* Construction of transglutaminase covalently cross-linked hydrogel and high internal phase emulsion gel from pea protein modified by high-intensity ultrasound. *J. Sci. Food Agric.* **103**, 1874–1884 (2023).
4. Liu, Q., Zhang, M., Bhandari, B. & Yang, C. Modification of pork-skin jelly by enzymatic cross-linking: melting resistance and quality improvement. *Int. J. Food Sci. Technol.* **56**, 2357–2364 (2021).
5. Dickinson, E. & Yamamoto, Y. Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. *J. Agric. Food Chem.* **44**, 1371–1377 (1996).
6. Bönisch, M. P., Huss, M., Weitzl, K. & Kulozik, U. Transglutaminase cross-linking of milk proteins and impact on yoghurt gel properties. *Int. Dairy J.* **17**, 1360–1371 (2007).
7. Nashchekina, Y. A. *et al.* Biological and rheological properties of collagen cross-linked with glutaraldehyde. *Technical Physics 2020 65:9* **65**, 1535–1540 (2020).
8. Tian, Z., Li, C., Duan, L. & Li, G. Physicochemical properties of collagen solutions cross-linked by glutaraldehyde. *Connect. Tissue Res.* **55**, 239–247 (2014).
9. Wang, Y., Mo, X., Sun, X. S. & Wang, D. Soy protein adhesion enhanced by glutaraldehyde crosslink. *J. Appl. Polym. Sci.* **104**, 130–136 (2007).
10. Hense, D. & Strube, O. I. Glutaraldehyde cross-linking of salt-induced fibrinogen hydrogels. *ACS Biomater. Sci. Eng.* **10**, 6927 (2024).
11. Caillard, R., Remondetto, G. E. & Subirade, M. Rheological investigation of soy protein hydrogels induced by Maillard-type reaction. *Food Hydrocoll.* **24**, 81–87 (2010).
12. Chiou, B. Sen *et al.* Rheological and mechanical properties of cross-linked fish gelatins. *Polymer (Guildf)*. **47**, 6379–6386 (2006).
13. Zhou, E. *et al.* Genipin as a natural cross-linker strengthens pea protein gel: a mechanistic study. *Food Hydrocoll.* **174**, 112417 (2026).
14. Song, F., Zhang, L. M., Yang, C. & Yan, L. Genipin-crosslinked casein hydrogels for controlled drug delivery. *Int. J. Pharm.* **373**, 41–47 (2009).

15. Butler, M. F., Ng, Y. F. & Pudney, P. D. A. Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. *J. Polym. Sci. A Polym. Chem.* **41**, 3941–3953 (2003).
16. Ghanbari, M., Salavati-Niasari, M., Mohandes, F., Dolatyar, B. & Zeynali, B. *In vitro* study of alginate–gelatin scaffolds incorporated with silica NPs as injectable, biodegradable hydrogels. *RSC Adv.* **11**, 16688–16697 (2021).
17. Iwashita, M. *et al.* Brain-stiffness-mimicking tilapia collagen gel promotes the induction of dorsal cortical neurons from human pluripotent stem cells. *Scientific Reports* 2019 9:1 **9**, 3068 (2019).
18. Pugliese, R. & Gelain, F. Cross-linked self-assembling peptides and their post-assembly functionalization via one-pot and *in situ* gelation system. *International Journal of Molecular Sciences* 2020, Vol. 21, Page 4261 **21**, 4261 (2020).
19. Xiong, Y. *et al.* Mechanical properties of low-molecular-weight peptide hydrogels improved by thiol-ene click chemistry. *Langmuir* **39**, 16750–16759 (2023).
20. Huang, J. *et al.* A click chemistry-mediated all-peptide cell printing hydrogel platform for diabetic wound healing. *Nature Communications* 2023 14:1 **14**, 7856- (2023).
21. Cengiz, N. Glutathione-responsive multifunctionalizable hydrogels via amine-epoxy “click” chemistry. *Eur. Polym. J.* **123**, 109441 (2020).
22. Swaminathan, A. V. *et al.* Impact of pre-acidification on the functionality and insoluble calcium levels of low-moisture part-skim mozzarella made from high-casein milk. *J. Dairy Sci.* **108**, 173–189 (2025).
23. Mookoonlall, A., Pfannstiel, J., Struch, M., Berger, R. G. & Hinrichs, J. Structure modification of stirred fermented milk gel due to laccase-catalysed protein crosslinking in a post-processing step. *Innovative Food Science & Emerging Technologies* **33**, 563–570 (2016).
24. Zhu, L. *et al.* Effects of moisture content on the physicochemical properties and swallowing function of surimi gel. *Food Chem.* **508**, 148372 (2026).
25. Liu, C. *et al.* Improvement of the rheological properties and freeze-thawing stability of Pickering emulsions filled emulsified surimi gels: Effects of the interfaces of surimi particles and konjac glucomannan. *Food Hydrocoll.* **162**, 111000 (2025).
26. Li, C. *et al.* Effect of partial hydrolysis with papain on the characteristics of transglutaminase-crosslinked tofu gel. *J. Food Sci.* **83**, 3092–3098 (2018).
27. Dickinson, E. Enzymic crosslinking as a tool for food colloid rheology control and interfacial stabilization. *Trends Food Sci. Technol.* **8**, 334–339 (1997).
28. Liu, H., Nardin, C. & Zhang, Y. Novel soft food gels using beta-lactoglobulin via enzymatic crosslinking as agar gel alternatives. *Food Hydrocoll.* **155**, 110213 (2024).

29. Raak, N., Rohm, H. & Jaros, D. Enzymatic cross-linking of casein facilitates gel structure weakening induced by overacidification. *Food Biophysics* 2017 12:2 **12**, 261–268 (2017).
30. Liu, Z. *et al.* Correlating rheology with 3D printing performance based on thermo-responsive  $\kappa$ -carrageenan/*Pleurotus ostreatus* protein with regard to interaction mechanism. *Food Hydrocoll.* **131**, 107813 (2022).
31. Lou, F. *et al.* Synergistic enhancement of 3D printing performance in soybean protein isolate gel inks by ionic/nonionic polysaccharides: A network rearrangement mechanism revealed by rheology and the sequence of physical processes analysis. *Food Hydrocoll.* **166**, 111352 (2025).
32. Liu, Z. *et al.* Insights into the structural evolution of wheat starch-soy protein isolate gel network during 3D printing based on nonlinear rheology. *Food Hydrocoll.* **174**, 112419 (2026).
33. McGill, M., Coburn, J. M., Partlow, B. P., Mu, X. & Kaplan, D. L. Molecular and macro-scale analysis of enzyme-crosslinked silk hydrogels for rational biomaterial design. *Acta Biomater.* **63**, 76–84 (2017).
34. Ding, D. *et al.* From soft self-healing gels to stiff films in suckerin-based materials through modulation of crosslink density and  $\beta$ -sheet content. *Advanced Materials* **27**, 3953–3961 (2015).
35. Veiga, A. *et al.* Biofunctional silk sericin hydrogels: A versatile platform with potential for tissue healing and regeneration. *Colloids Surf. B Biointerfaces* **255**, 114916 (2025).