

Supplementary information

Molecular basis for the regulation of human glycogen synthase by phosphorylation and glucose-6-phosphate

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Molecular basis for the regulation of human glycogen synthase by phosphorylation and glucose-6-phosphate

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Supplementary Figures

Supplementary Fig. 1: Sequence alignment of GYS proteins.

Supplementary Fig. 2: Sequence alignment of the N-terminus and CBM21 domain of each PP1 regulatory subunit and the starch binding domain of *R. oryzae* glucoamylase.

Supplementary Fig. 3: PTG pulldowns and BN-PAGE replicates.

Supplementary Fig. 4: Representative thermal shift unfolding curves of each GYS1:GYG1 complex and PTG(CBM21) mutant.

Supplementary Table 1: Sequences of oligonucleotides used for mutagenesis.

Uncropped image of Supplementary Fig. 3a

Uncropped image of Supplementary Fig. 3b

Uncropped image of Supplementary Fig. 3c

sp|P13807|GYS1_HUMAN →β8 α10 →β9 α11 →α12

260 270 280 290 300 310

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sp|P13807|GYS1_HUMAN HVFTTVSITAIEAHLLKRRKPDIVNPNGLNVKKEFSANHEFQNLHAQSKARIQEFVRGHF
sp|P54840|GYS2_HUMAN HVFTTVSITAIEAHMLKRRKPDVVTPNGLNVKKEFSAVHEFQNLHAMYKARIQDFVRGHF
sp|P23337|GYS1_YEAST DVFTTVSITALEAEHLKRRKPDGIIPNGLNVKKEFAVHEFQNLHAKKDKINDFVRGHF
sp|P27472|GYS2_YEAST DVFTTVSITAFEAHLLKRRKPDGIIPNGLNVKKEFAVHEFQNLHAKKDKINDFVRGHF
sp|Q902D9|GYS_CAEL HFTFTVSITGLEAEHLKRRKPDVLTNGLNVKKEFAVHEFQNLHAKKDKINDFVRGHF
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sp|Q921E4|GYS1_MOUSE HVFTTVSITAIEAHLLKRRKPDIVTPNGLNVKKEFSANHEFQNLHAQSKARIQEFVRGHF
sp|Q8VCB3|GYS2_MOUSE HVFTTVSITAIEAHMLKRRKPDVVTPNGLNVKKEFSVHEFQNLHAMYKARIQDFVRGHF
sp|A2RRU1|GYS1_RAT HVFTTVSITAIEAHLLKRRKPDIVTPNGLNVKKEFSVHEFQNLHAMYKARIQDFVRGHF
sp|P17625|GYS2_RAT HVFTTVSITAIEAHMLKRRKPDVVTPNGLNVKKEFSVHEFQNLHAMYKARIQDFVRGHF
sp|P13834|GYS1_RABIT HVFTTVSITAIEAHLLKRRKPDIVTPNGLNVKKEFSANHEFQNLHAQSKARIQEFVRGHF
sp|A7MB78|GYS1_BOVIN HVFTTVSITAIEAHLLKRRKPDIVTPNGLNVKKEFSANHEFQNLHAQSKARIQEFVRGHF

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sp|P13807|GYS1_HUMAN →β10 α13 →β11

320 330 340 350 360

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sp|P54840|GYS2_HUMAN YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
sp|P23337|GYS1_YEAST YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
sp|P27472|GYS2_YEAST YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
sp|Q902D9|GYS_CAEL YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
sp|Q9VFC8|GYS_DROME YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
sp|Q921E4|GYS1_MOUSE YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
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sp|A2RRU1|GYS1_RAT YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
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sp|P13834|GYS1_RABIT YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM
sp|A7MB78|GYS1_BOVIN YCHLDFDDEKLYFFIAGRYEFSNKGADVFLSALRLNYLDRVNG...SDITVMVVFITM

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sp|P13807|GYS1_HUMAN →β12 α14 →TT

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sp|P13807|GYS1_HUMAN PARTNPNVETLKGQAVRKQLWDTANTVKEKFGKRLYESSLVG...SIPDMNKMIL
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sp|P23337|GYS1_YEAST PAKTNSFVETLKGQAVRKQLWDTANTVKEKFGKRLYESSLVG...SIPDMNKMIL
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sp|A7MB78|GYS1_BOVIN PAKTNSFVETLKGQAVRKQLWDTANTVKEKFGKRLYESSLVG...SIPDMNKMIL

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sp|P13807|GYS1_HUMAN α15 →β13 α16 →β14

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sp|P54840|GYS2_HUMAN DRDDLTIKRAIFSTQR...CSPPVCTHNMIDDSDPILSTIRRIGLFNSSSDRVKVIFH
sp|P23337|GYS1_YEAST KSSDKVLLKRRVILALRRPEGQLPPIVTHNMVDDANDLILNIRVOLFNSSSDRVKVIFH
sp|P27472|GYS2_YEAST KSSDKVLLKRRVILALRRPEGQLPPIVTHNMVDDANDLILNIRVOLFNSSSDRVKVIFH
sp|Q902D9|GYS_CAEL SPADNILLKRRIMGLHN...SSDPPVCTHNMIR...ADPVLSSLRRIGLFNSSSDRVKVIFH
sp|Q9VFC8|GYS_DROME CKDDLVLKRRCMFAMQR...DSMPPVCTHNVADDNDPVLSSIRRCGLFNSSSDRVKVIFH
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sp|P13834|GYS1_RABIT DKEDFTMKRAIFATQR...CSPPVCTHNMIDDSDPILSTIRRIGLFNSSSDRVKVIFH
sp|A7MB78|GYS1_BOVIN DKEDFTMKRAIFATQR...CSPPVCTHNMIDDSDPILSTIRRIGLFNSSSDRVKVIFH

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sp|P13807|GYS1_HUMAN α17 →β15 α18 →β16 α19

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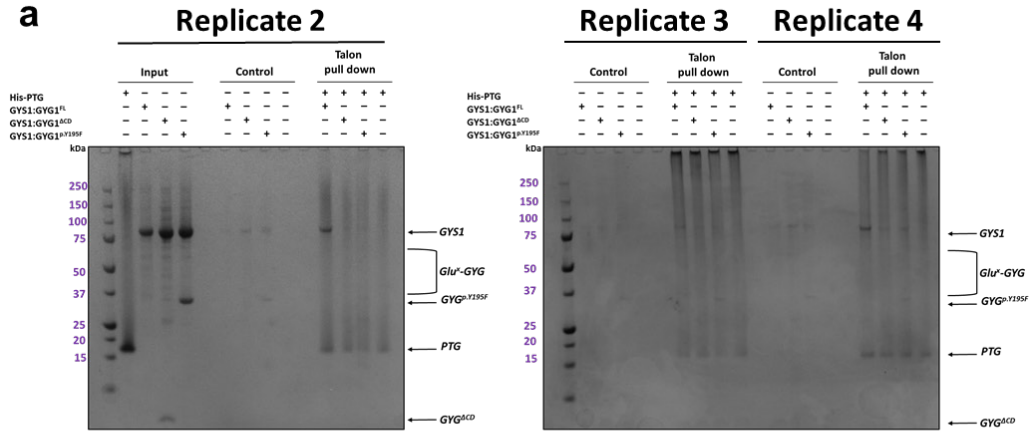
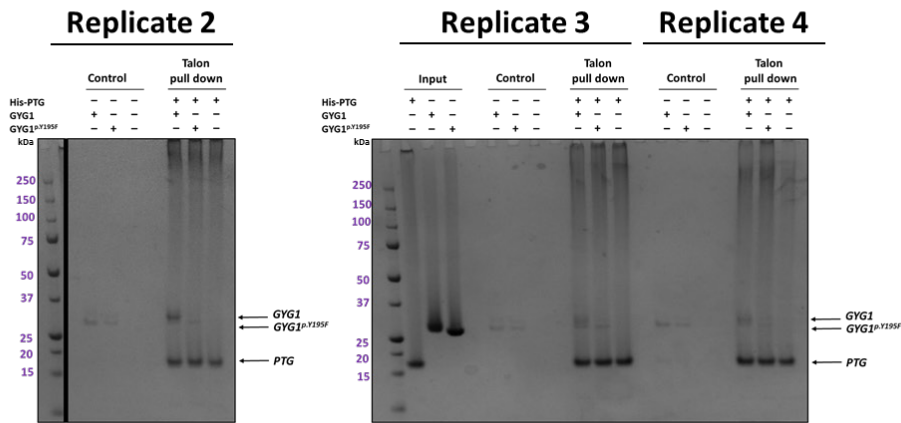
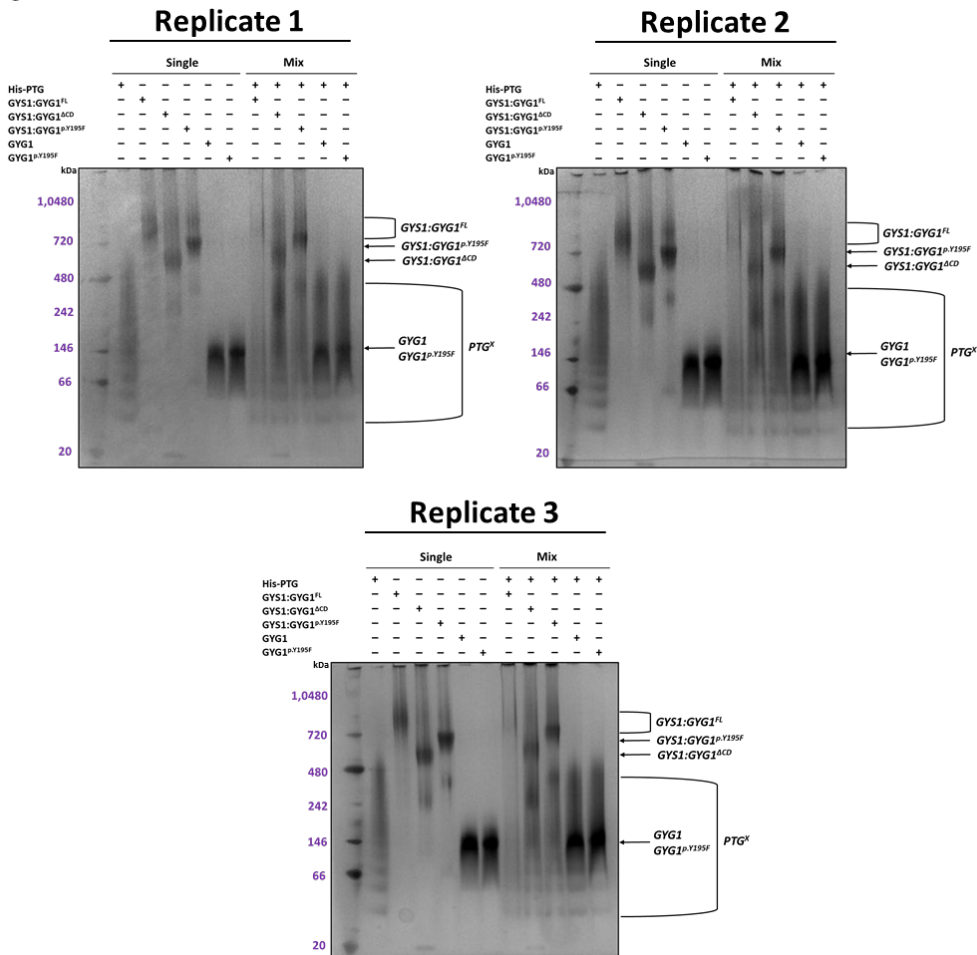
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sp|P23337|GYS1_YEAST PEFLLSSTSPLLPVDYEEFVRGCHLGVPFSYIEPWGYTPAECTVMGIPSVITNLSGFGCFM
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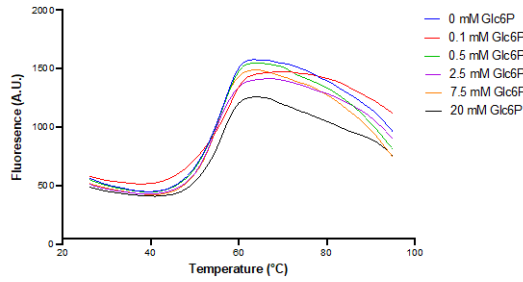

Supplementary Fig. 2 | Sequence alignment of the CBM21 domain of PP1 regulatory subunit PPP1R3 family and *R. oryzae* glucoamylase. Only the N-terminus and CBM21 domain of each PP1 regulatory subunit and *R. oryzae* glucoamylase are shown. Key motifs are highlighted.

a**b****c**

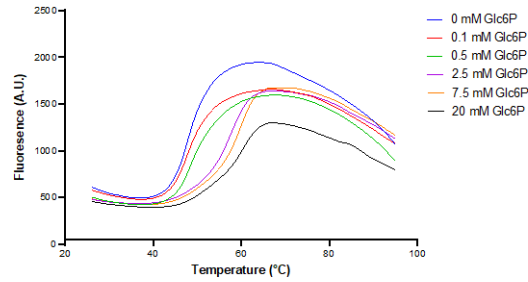
Supplementary Fig. 3 | PTG pulldowns and BN-PAGE replicates. a, Coomassie stained SDS-PAGE replicates of PTG(CBM21) pulldowns against GYS1:GYG1^{FL}, GYS1:GYG1^{p.Y195F}, or GYS1:GYG1^{ΔCD}. **b**, Coomassie stained SDS-PAGE replicates of PTG(CBM21) pulldowns against GYG1^{FL} or GYG1^{p.Y195F}. **c**, Blue native PAGE shift replicates of PTG(CBM21) incubated with GYS1:GYG1^{FL}, GYS1:GYG1^{p.Y195F}, GYS1:GYG1^{ΔCD}, GYG1 alone, or GYG1^{p.Y195F} alone. Complex formation between PTG(CBM21) and GYS1:GYG1^{FL} was inferred from the disappearance of bands compared to other reaction lanes. CBM21 appears as oligomers but behaves as a monomer with some dimer in solution (data not shown).

a

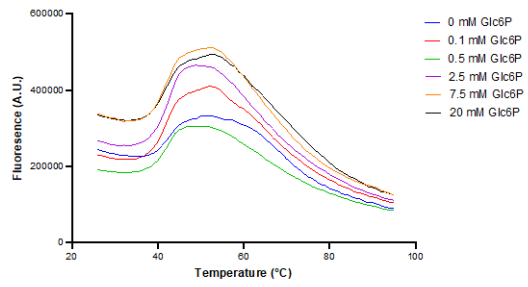
GYS1:GYG1 Δ CD WT
As-purified Glc6P Titration

**b**

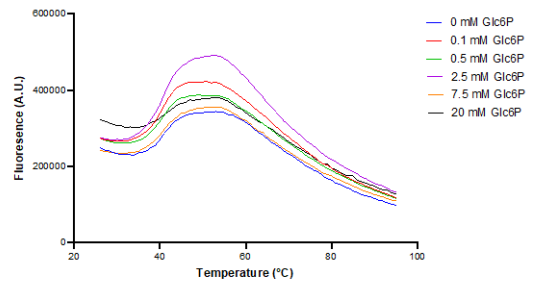
GYS1:GYG1 Δ CD WT
PP1c Glc6P Titration

**c**

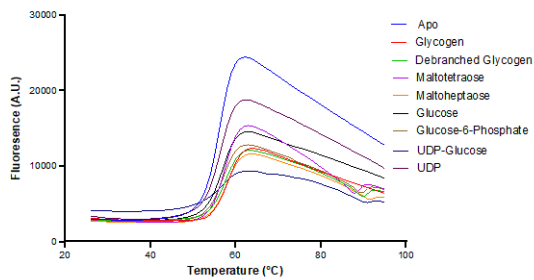
GYS1^{p.R582A+p.R586A}:GYG1 Δ CD
As-purified Glc6P Titration

**d**

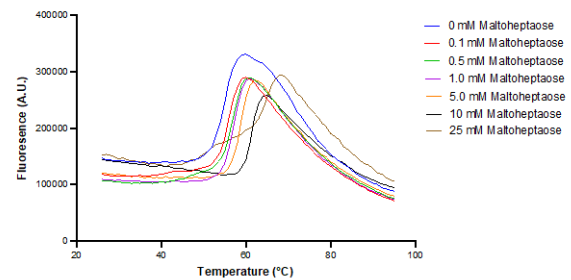
GYS1^{p.R582A+p.R586A}:GYG1 Δ CD
PP1c Glc6P Titration

**e**

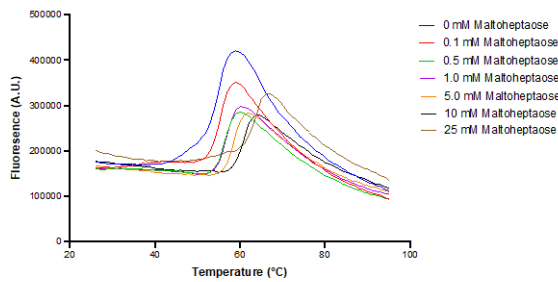
PTG Sugar Screen

**f**

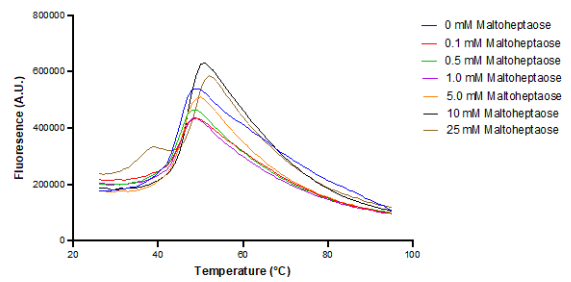
PTG WT Maltoheptaose Titration

**g**

PTG^{p.Y203R} Maltoheptaose Titration

**h**

PTG^{p.W246R} Maltoheptaose Titration

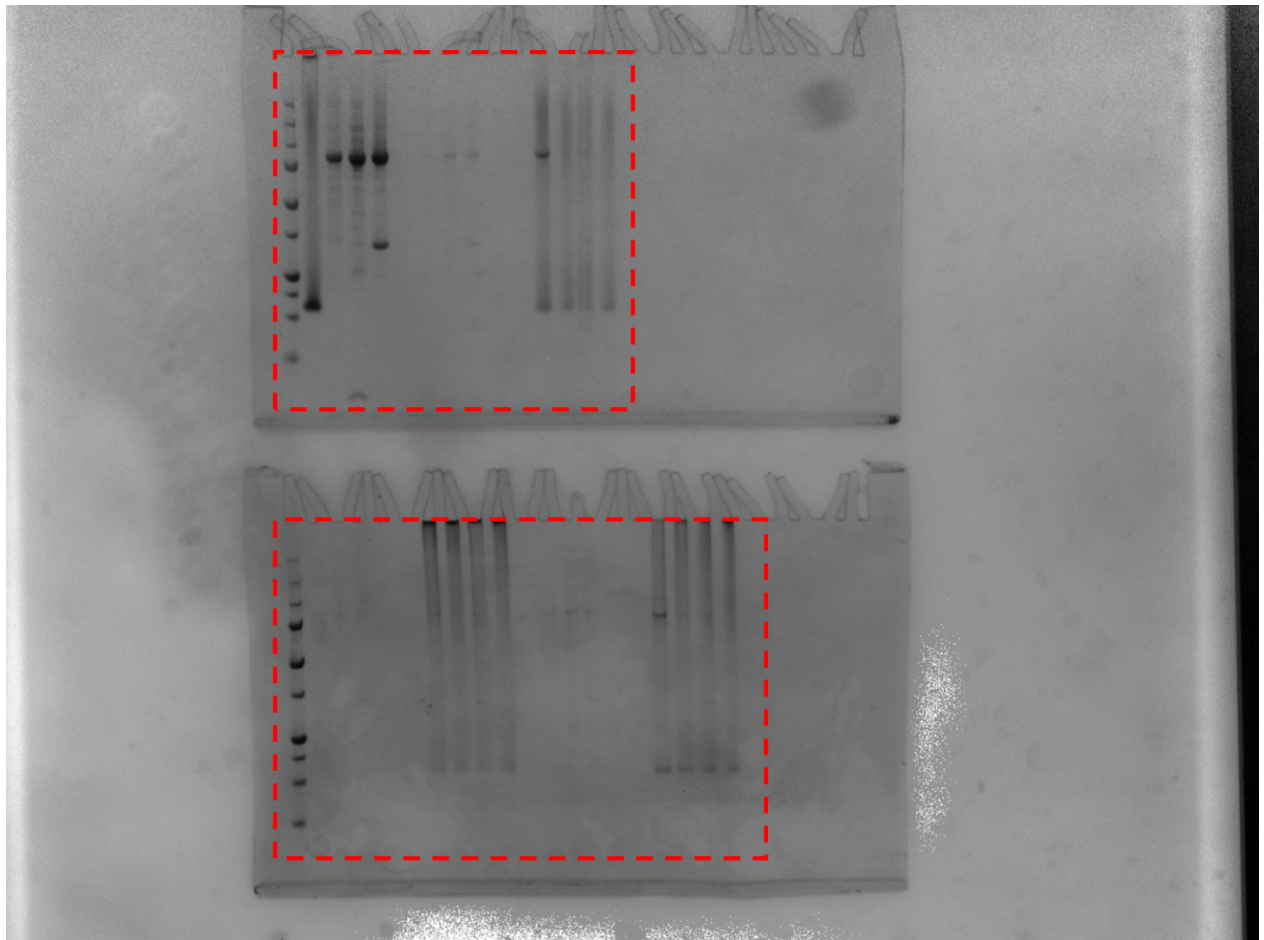


Supplementary Fig. 4 | Representative thermal shift unfolding curves of each GYS1:GYG1 complex and PTG(CBM21) mutant. a, GYS1:GYG1^{ΔCD} WT as-purified, titrated with increasing Glc6P. **b,** GYS1:GYG1^{ΔCD} WT + PP1c, titrated with increasing Glc6P. **c,** GYS1^{p.R582A+p.R586A}:GYG1^{ΔCD} as-purified, titrated with increasing Glc6P. **d,** GYS1^{p.R582A+p.R586A}:GYG1^{ΔCD} + PP1c, titrated with increasing Glc6P. **e,** PTG(CBM21) screened against various sugars and ligands at 1 mM each. **f,** PTG(CBM21) WT, titrated with increasing maltoheptaose. **g,** PTG(CBM21)^{p.Y203R}, titrated with increasing maltoheptaose. **h,** PTG(CBM21)^{p.W246R}, titrated with increasing maltoheptaose.

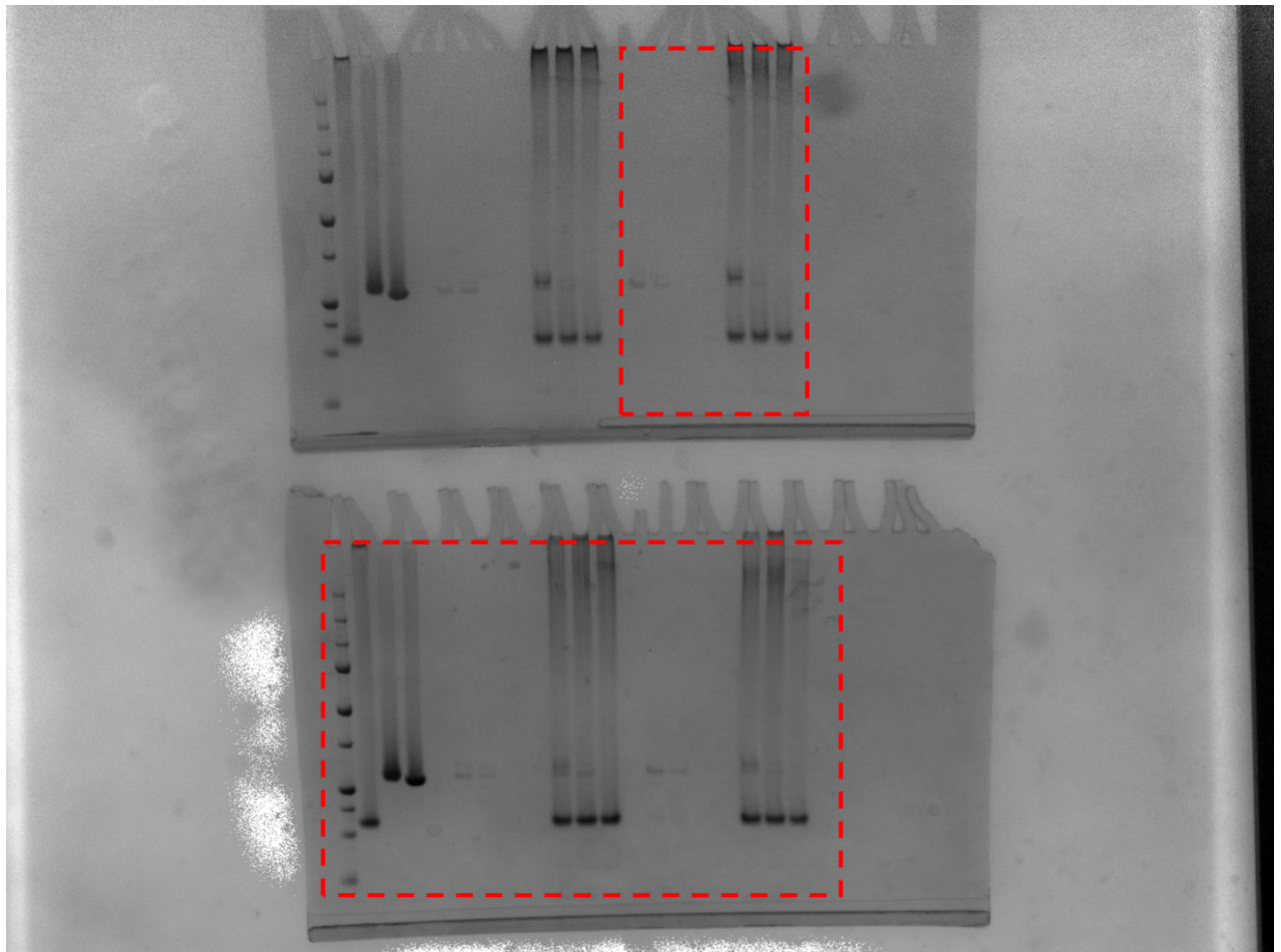
Supplementary Table 1 | Oligonucleotide sequences used for mutagenesis

Primer	Sequence
GYS1_R582A+R586A_Fr	GGCCATCATCCAGGCCAACCGCACGGAGCGCCTC
GYS1_R582A+R586A_Rv	GCCTGGATGATGGCCTGCCGCCGGCTCTGCTG
PTG_W246R_Fr	AGTCTTTCGGGACAACAATGATGGTCAGAATT
PTG_W246R_Rv	TTGTCCCGAAAGACTTGCCCATTAGCA
PTG_Y203R_Fr	CTGTGTCCGTATGAAAAATGTGTATGGTGGCACAG
PTG_Y203R_Rv	TTCATACGGACACAGTCTACGTCAGTGTAG

Uncropped image of Supplementary Fig. 3a



Uncropped image of Supplementary Fig. 3b



Uncropped image of Supplementary Fig. 3c

