

Supplement for

Expression and display of glycoengineered antibodies and antibody fragments with an engineered yeast strain

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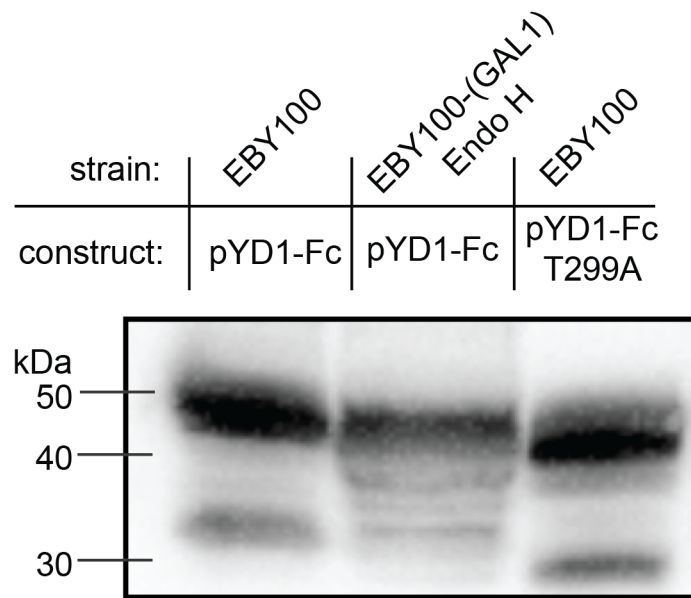
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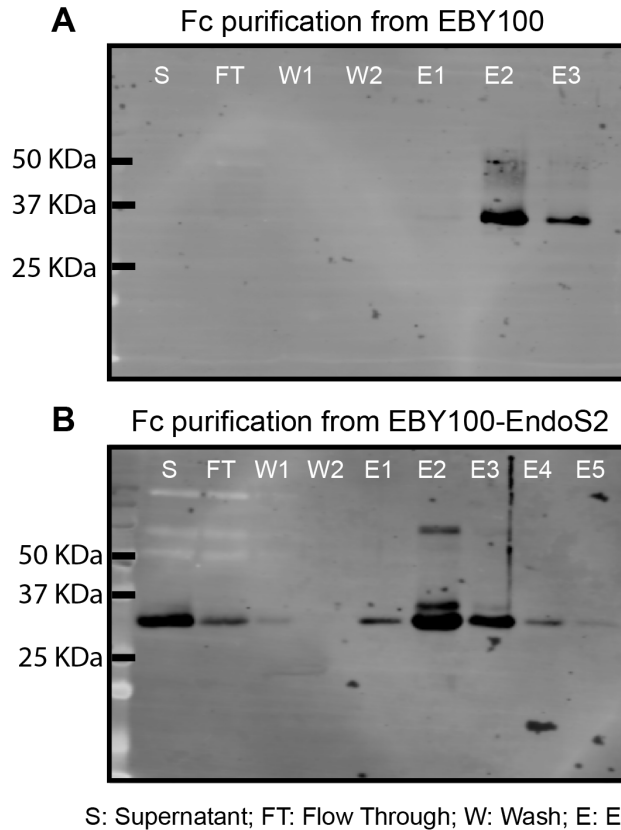
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Table S1. List of primers used for strain creation.

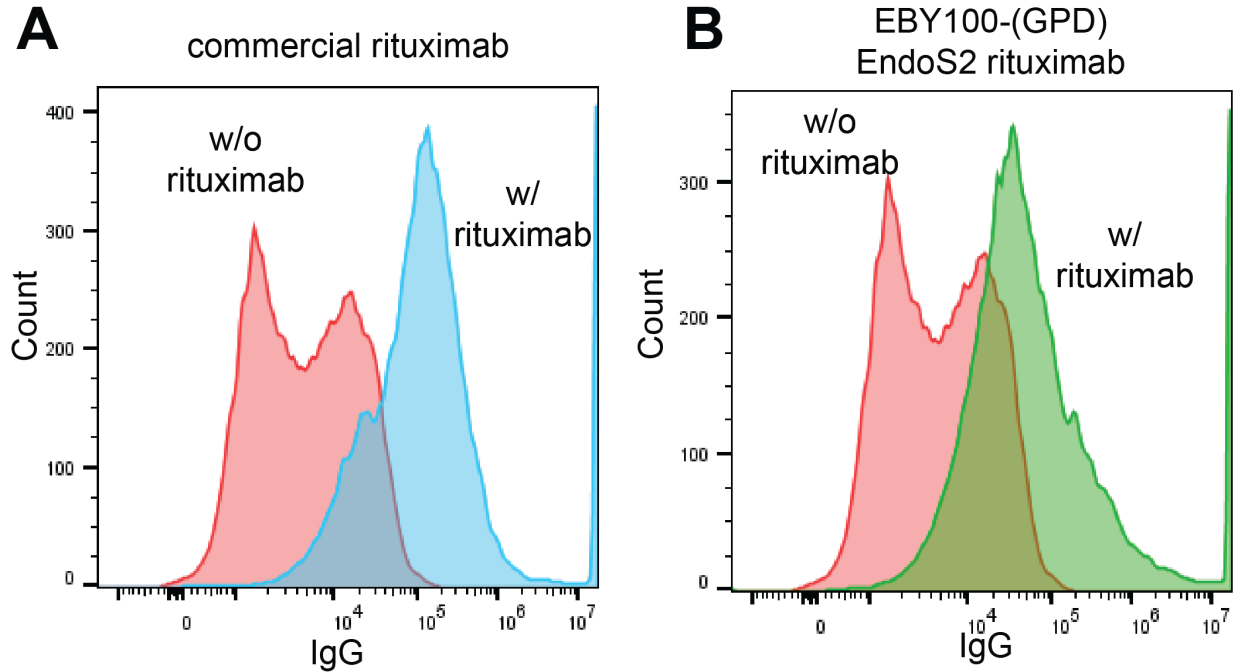
Primer Name	Sequence	Purpose
ESFor	5'GCC GCT CTA GAA TGAAGTTTCTG '3	Amplification Forward primer
ESRev	5'GCCGGACC TAA GCT TCT ATT AAAG '3	Amplification Reverse primer
ESSeq_F	5' GTT TTC TGC TGG TGC CGT C '3	Sequencing forward primer
ESSeq_R	5' GCC AAG CTC GGA ATT AAC CCT C '3	Sequencing reverse primer
ESYC_F	5' GCA TCT TAC GAT ACC TGA GTA TTC CC	Yeast Colony PCR – EndoS2 insert- Forward
ESYC_R	5' CAA GAC AGG ACG GCA CCA GC '3	Yeast colony PCR-EndoS2- Reverse primer
YCol-F	5' GTCAGTAATTGCGGTTCTCAC '3	Yeast Colony PCR – pYD1 insert – Forward
YCol-R	5' GCCTTTCGCTTTGGAAATTGTC '3	Yeast Colony PCR – pYD1 insert -Reverse
HCFFor	CTAGCGTCGACATGAGGTTCCCGTC	Amplify app8 for fusion PCR with Heavy
AFHCR	CTGTAATTGAACTTGGGCTTCTGCTTCTC	Amplify app8 for fusion PCR with Heavy
AFHCFFor	GAG AAG CAG AAG CCC AAG TTC AAT TAC AG	Amplify Heavy chain for fusion PCR with app8
HCR	CGT AGC GCT AGC TTA TTA CTT TCC CGG	Amplify Heavy chain for fusion PCR with app8
LCFor	GTT GAA TTC ATG AGG TTC CCG TCT ATT TTT ACG	Amplify app8 for fusion PCR with Light Chain
AFLCR	GAACAATCTGGGCTTCTGCTTCTCTC	Amplify app8 for fusion PCR with Light Chain
AFLCFFor	GAG AGA AGC AGA AGC CCA GAT TGT TC	Amplify Light Chain for fusion PCR with app8
LCR	GATCTGCGGCCGCTTATCAACATTCTC	Amplify Light Chain for fusion PCR with app8
GALI	5' ATTTTCGGTTTGTATTACTTC 3'	DNA Sequencing Forward Primer (From
GALI reverse	5' GTTCTTAATACTAACATAACT 3'	DNA Sequencing Reverse Primer (From
GALI0	5' GGTGGTAATGCCATGTAATATG 3'	DNA Sequencing Forward Primer (From
GALI0	5' GGCAAGGTAGACAAGCCGACAAC 3'	DNA Sequencing Reverse Primer (From



Supplementary Figure S1. Western blot showing Aga2p-Fc fusion mobility in the EBY100-(GAL1)-Endo H strain compared to an aglycosylated control (T299A).



Supplementary Figure S2. Fc purification using Protein A starting with protein expressed from (A) the unmodified yeast strain EBY100 and (B) the glycoengineered yeast strain EBY100-(GPD)-EndoS2. Smearing in E2 in panel A likely represents extensive N-glycan processing.



Supplementary Figure S3. Rituximab binding to Raji B cells (A) 400 nM commercial Rituximab (B) 400 nM Rituximab from the EBY100-(GPD) EndoS2 strain.

Protein Sequences:

EndoS2 Protein Sequence [Signal Peptide – EndoS2 – Flag tag- HDEL]

MKFSAGAVLSWSSLLASSVFAQQEAEKTVQTGKTDQQVGAKLVQEIREGKRGPLYA
GYFRTWHDRASTGIDGKQHPENTMAEVPKEVDILFVFHDHTASDSPFWSELKDSYVH
KLHQQGTALVQTIGVNELNGRTGLSKDYPDTPEGNKALAAAIKAFVTDGRGVDGLDIDI
EHEFTNKRTPEEDARALNVFKEIAQLIGKNGSDKSKLLIMDTTSLVENNPIFKGIAEDLDY
LLRQYYGSQGGAEVDTINSDWNQYQNYIDASQFMIGFSFFEESASKGNLWFDVNEYD
PNNPEKGKDIEGTRAKKYAEWQPSTGGLKAGIFSVAIDRDGVAHVPSTYKNRTSTNLQR
HEVDNISHTDYTVSRNLKTLMTEDKRYDVIDQKDIPDPALREQIIQQVGQYKGDRLRYN
KTLVLTGDKIQNLKGLEKLSKLQKLELRQLSNVKEITPELLPESMKKDAELVMVGMTGL
EKLNL SGLNRQTLTGIDVNSITHLTSFDISHNSLDLSEKSEDRKLLMTLMEQVSNHQKIT
VKNTAFENQKPKGYYPQTYDTKEGHYDVDNAEHDILTDFVFGTVTKRNTFIGDEEAFAI
YKEGAVDGRQYVSKDYTEAFRKDYKGYKVHLTASNLGETVTSKVTATTDETYLVDV
SDGEKVVHHMKNIGSGAIMMENLAKGAKVIGTSGDFEQAKKIFDGEKSDRFFTWGQT

NWIAFDLGEINLAKEWRLFNAETNTEIKTDSSLNVAKGRLQILKDTTIDLEKMDIKNRKE
YLSNDENWTDVAQMDDAKAIFNSKLSNVLSRYWRFCVDGGASSYYPQYTELQILGQRL
SNDVANTLKDDYKDDDDKHDEL

Heavy Chain of Rituximab (Protein Sequence without app8)

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGD
TSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAG
TTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light Chain of Rituximab (Protein Sequence without app8)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRF
SGSGSGTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLS
KADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

Human IgG1 Fc construct (Protein Sequence without app8)

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK