

Supporting materials

FIG. S1. SDS-polyacrylamide gel electrophoresis of GFPP. Proteins were denatured at 100°C for 10 min in 0.1% SDS and 1% 2-mercaptoethanol before being loaded in a 4% (wt/vol) stacking gel and separated in a 15% (wt/vol) separation gel. The gel was stained with Coomassie bright blue R250. The molecular weight markers from Thermo Scientific are shown at the left side of the panel.

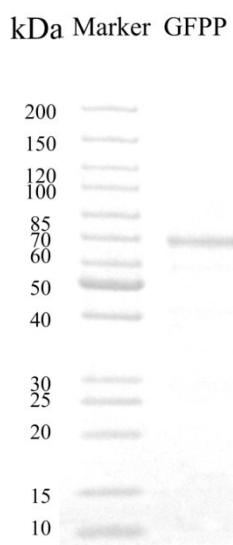


FIG. S2. Multiple amino acid sequence alignment of GFPP from different organisms. Multiple alignments were performed with the ClustalW program using the BioEdit sequence alignment software. The corresponding nucleotide sequences encoding *M. alpina* GFPP has been deposited in the GenBank/EMBL database with Accession No. 1825641. Accession numbers for the other protein sequences above are: Human GFPP (AAH32308.1), Mouse GFPP (AAI10553.1), *Dictyostelium discoideum* GFPP (XP_638295.1), *Medicago truncatula* (XP_003626575.1), *Bacteroides fragilis* GFPP (KFX74805.1), *Arabidopsis thaliana* GFPP (NP_563620.1), *Pisum sativum* UDP-sugar pyrophosphorylase (BAD66876.1), *Leishmania major* UDP-sugar pyrophosphorylase (ABY79093.1), and human UDP-N-acetylglucosamine pyrophosphorylase (NP003106). The motif was boxed and other residues are marked with asterisks.

GFPP M. alpina MSSNNQAAPHTGIDDSQVLAQVHAKLKKLQRLKHPATAIPRTQA QPFDWVITAGAAQRKCVNFKSRLVGLVQRAKHVLDLDFPGSS 98
GFPP Mouse MASREATLRRLRRSEBRGK VVAAGRFWDVAITAADPKELATKLSLRLRREPLGVQHDFVDPACAK 74
GFPP Human MAAARDPPPSREATQLRLRRSEBRGK LVAAGRFWDVAITAADPKELATKLSLRLRREPLGVQHDFVDPACAK 78
GFPP D. discoideum --MMELGFENDILLNQYTHSRKRTDYKGLRLEKQENKNGH-LDISSVWVWVITAILSLKVEYVQVVRKANKQNGPSPFIPVITISDPFVGR 94
GFPP Medicago ELVSVVGEKKMERRRRRKEDLSSLKMSWHLRSVR HPSVPTADALITLASPQSAHLITSSNRKAGRTGRISPTLTTAHTDFPLRR 114
GFPP/FUK B. fragilis KLLSLPPLNQSFEHLERLNKRTDFCTSDPWGKKKSGGGTAWLLKECYNEDGHSFSGWLGKRRLLHAGGSRGLPGYAPSGVITPVPVFRWFRG 102
GFPP/FUK Arabidopsis --RKKADLATVLRMSWHLRSVR HPTVPTADALITLASPQSAELTVDWVRKRAKNGRTIASVITVLAETDFPLRR 79
USP Pisum sativum MASSLGDNFLLSPQGRBIVKMLLDNGDDHFRDWPNGVDDD-EK-SAFDQVLDVSSYPGGLVAIINNAKRLA-DSKAGNPDGTFPSVIT 96
USP Leishmania major MTFNFSNLSLQALR--EELCTPGLDQG--HLEEGWPTVDECN--ERGIALLTDVFQSNMYPGGAIVHNGHDAEESSEVDFAAEPLPLIFET 93
UDP-GlcNAc Human --MNINDKTLTKAGTEHLLRFWNEL--EAAQQVELYAEQADDFDLNFDFDRAIEFQNSSHQKVDARLDFVPRVGLGS 79

GFPP M. alpina IGGSCSTCLVTRVREHPAD--IISKAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 158
GFPP Mouse IGGSCSTCLVTRVREHPAD--IISKAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 136
GFPP Human IGGSCSTCLVTRVREHPAD--IISKAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 144
GFPP D. discoideum IGGSCSTYVLTSTIIGDDKIKN--MKLILLHAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 175
GFPP Medicago IGGSCSTYVLTSTIIGDDKIKN--MKLILLHAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 188
GFPP/FUK B. fragilis IGGSCSTYVLTSTIIGDDKIKN--MKLILLHAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 167
GFPP/FUK Arabidopsis IGGSCSTYVLTSTIIGDDKIKN--MKLILLHAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 169
USP Pisum sativum IGGSCSTYVLTSTIIGDDKIKN--MKLILLHAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 153
USP Leishmania major IGGSCSTYVLTSTIIGDDKIKN--MKLILLHAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 156
UDP-GlcNAc Human IGGSCSTYVLTSTIIGDDKIKN--MKLILLHAGGCSRPHICARGKIFMTIQAADR-----ARGIQIL 139

GFPP M. alpina ERMVYVYHLLLETFFPFHTSAGHLHSSNPFFSEPKPTIIALHPSSTNGSTHGVYLLHTIDWVTHDRDHPRDQSAALLKCFEHRKFSTI 257
GFPP Mouse ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--QCYDFHRKFLTE 226
GFPP Human ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--SCHDFHRKFLTE 234
GFPP D. discoideum ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--QYKFNQIN 263
GFPP Medicago ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--LVNDLDFHRKFLTE 275
GFPP/FUK B. fragilis ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--AHFQDEDFE 256
GFPP/FUK Arabidopsis ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--LVNDLDFHRKFLTE 255
USP Pisum sativum ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--RVQTKPFGHGDWH 244
USP Leishmania major ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--KLLRKPFGHGDWH 228
UDP-GlcNAc Human ERMAVYVDFPSPNDFGLTTCADHLELVGSVEYIAFDVPGIHALHPSLIAGLHGVVHSDSSQHGDELYR--MAPDGNGGY 227

GFPP M. alpina VIKSVFRVYPDPNDSAN--DVVYDTSCHPPPTANLADMSSLHE--RCVLAWDDISFGDDHPASLSSAQDST--APSSLSPH 339
GFPP Mouse NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--VTREESQ 322
GFPP Human NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--VIREESE 330
GFPP D. discoideum NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--VTFPLPH 349
GFPP Medicago NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--VTFPLPH 339
GFPP/FUK B. fragilis NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--VTFPLPH 344
GFPP/FUK Arabidopsis NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--VTFPLPH 329
USP Pisum sativum NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--DGN-SMV 321
USP Leishmania major NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--SGDPWLV 324
UDP-GlcNAc Human NHRFNVAHQRSFQGNLSGGDITDCLPHTEVYDTSYVYDHRSAKMLDFKSEGINCSITDAYGDFQAAGPGATAEITNTSH--SGDPWLV 297

GFPP M. alpina VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 421
GFPP Mouse VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 409
GFPP Human VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 417
GFPP D. discoideum VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 428
GFPP Medicago VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 431
GFPP/FUK B. fragilis VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 435
GFPP/FUK Arabidopsis VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 411
USP Pisum sativum VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 420
USP Leishmania major VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 424
UDP-GlcNAc Human VQGRHLKQVLAQVSDVVAASVYFVLCVGCISGCTCHKAFMEENINPNDLGVAVVVSQPNLIDPDRNGAQSS 363

GFPP M. alpina PLYIENVLAPEAFHPCSTAVD--SCLPRDAIPKSCVFTIQLDREVTFTFLSKDDIRHMPASVITATLSDEEAIAEADRLYIPRVVPSRMIP 519
GFPP Mouse VLEISKQ--PEISGNCNIS--SSLAKTVPVPSFLCSASRLIN--GHRYSTVFGQDNLKNKSATLEDIHALQFGG--CFLSCDDNPK 498
GFPP Human VLEISKQ--PEISGNCNIS--SYLTKAALPAPSVCSSRLIN--RCRYATWVFGQDNLKNKSATLEDIHALQFGG--CFLSCDDNPK 507
GFPP D. discoideum VLEISKQ--PEISGNCNIS--LFEFNSNEQQQQQQQQQQQQQQQQQQSSSNTYIEIPSNFVITQLSI--CNVYVITPQDIDDKSTNPKFQ 526
GFPP Medicago VLEISKQ--PEISGNCNIS--LFEFNSNEQQQQQQQQQQQQQQQQQQSSSNTYIEIPSNFVITQLSI--CNVYVITPQDIDDKSTNPKFQ 525
GFPP/FUK B. fragilis VLEISKQ--PEISGNCNIS--LFEFNSNEQQQQQQQQQQQQQQQQQQSSSNTYIEIPSNFVITQLSI--CNVYVITPQDIDDKSTNPKFQ 533
GFPP/FUK Arabidopsis VLEISKQ--PEISGNCNIS--LFEFNSNEQQQQQQQQQQQQQQQQQQSSSNTYIEIPSNFVITQLSI--CNVYVITPQDIDDKSTNPKFQ 503
USP Pisum sativum VLEISKQ--PEISGNCNIS--LFEFNSNEQQQQQQQQQQQQQQQQQQSSSNTYIEIPSNFVITQLSI--CNVYVITPQDIDDKSTNPKFQ 510
USP Leishmania major VLEISKQ--PEISGNCNIS--LFEFNSNEQQQQQQQQQQQQQQQQQQSSSNTYIEIPSNFVITQLSI--CNVYVITPQDIDDKSTNPKFQ 521
UDP-GlcNAc Human VLEISKQ--PEISGNCNIS--LFEFNSNEQQQQQQQQQQQQQQQQQQSSSNTYIEIPSNFVITQLSI--CNVYVITPQDIDDKSTNPKFQ 523

GFPP M. alpina PHQAHPLTGATVEAPSAGS--VNAPVFEVARTAQ--SSRLADHRRDRI--NCLDGAPAEVVCN--PDVSRPGADVGVNLSNAARKAREYE 608
GFPP Mouse ATIKLPLS--GNKMNISLWARTAPPCSSLS--SATASGILSAYVNHSPNLSDPN--LLSDFEVLVYAVDMLAYREHIFLSSNNKIQ 584
GFPP Human VTEPLPLS--GNKTCISLWARTAPPCSSLS--SATITSELDNAYVNHSPNLSTYK--LLSDFEVLVYAVDMLAYREHIFLSSNNKSS 593
GFPP D. discoideum LKLYNL--SINTSDNNNNNS--NANAPPSISNIPKSLKLTNGLKTKT--QTFPSNNEQPPQQQQQSYYSL--EONRELLSKQFIRRSNTNLLNKNK 626
GFPP Medicago ETLWGS--SGTDVQV--NSHFPPLPYAQM--KSWM--VYVQTE--NLSLWRA--QRTISEEHRSHRT--CIGSSHQADLAAGIA- 612
GFPP/FUK B. fragilis NRMLRAR--ILKLDGKDYRPEEQAA--LDLDGGLB--SNRSEPKLDTYS--GIVWGRVPRVDMAGGWIDTPPYSLSYG--VNLATEINGPPLGVTV 630
GFPP/FUK Arabidopsis ESILWSS--YVAQDR--WNAH--PILTYSEM--KASW--GDDSNKR--IKLWRS--QRYSE--EFGHSIN--PONGSS--HQAD--AGGIAK 591
USP Pisum sativum SISQRSI--LAIKGRK--FID--LDGALVDAV--D--AEVNSG--VQ--GWALEPVD--YKDSSEPE--LRIG--RPNK--VQ--VEKKYSEPFKFD 598
USP Leishmania major HLDQHSI--LIVEGR--VHESLE--GALTIRGPTD--SMALPH--VRNAYV--RAGWSVH--ILSLCAGRSR--SADRIG--M--KKTAM--V--D--CKTG--ESEA 618
UDP-GlcNAc Human WWLNAGG--HFID--ENGSE--PARE--L--K--DAND--PI--Q--SPLI--STAGE--I--E--SY--WAD--E--H--AP--I--D--GVH--V--KNGI-- 505

Fig. S3. Phylogenetic tree of amino acid hydroxylases from different organisms. The tree was constructed using the neighbor-joining method with ClustalW NJplot. The horizontal branch length is proportional to the amino acid substitution rate per site.

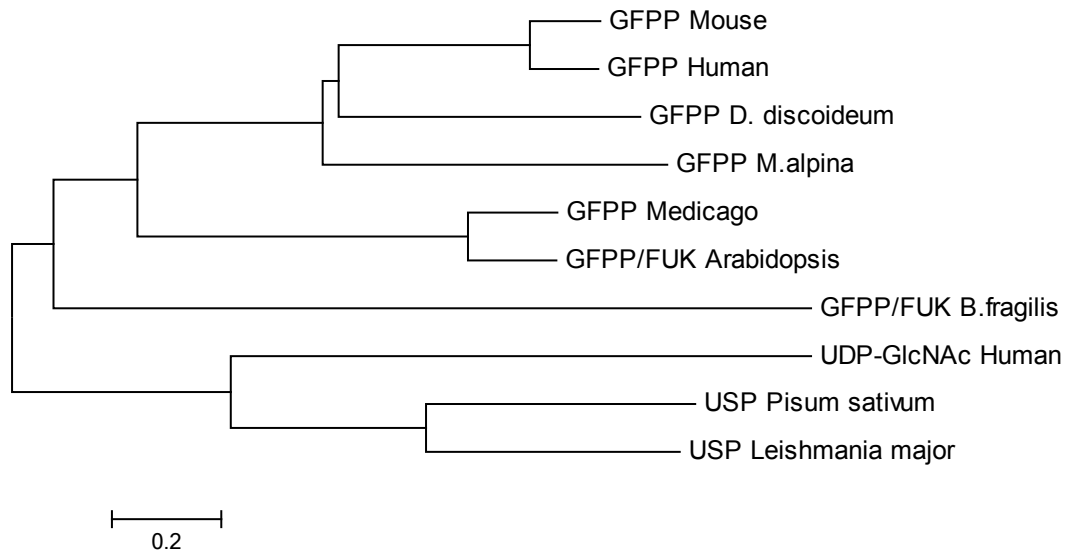


Figure S4. LC-MS chromatographs of GFPP reaction with the addition of MgCl₂ (C, D), MnCl₂ (E, F), KCl (G, H), CuCl₂ (I, J), CoCl₂ (K, L), FeCl₂ (M, N), CaCl₂ (O, P), ZnCl₂ (Q, R), NiSO₄ (S, T), and HgCl₂(U, V); LC-MS chromatographs of GFPP reaction with the addition of MgCl₂ and EDTA (W, X); Extracted ion chromatogram (XIC) of L-fucose-1-phosphate standard (A, m/z 243.03); XIC of GDP-L-fucose standard (B, m/z 588.08); XIC of L-fucose-1-phosphate (C, E, G, I, K, M, O, Q, S, U and W; m/z 243.03); XIC of the GDP-L-fucose (D, F, H, J, L, N, P, R, T, V and X; m/z 588.08).

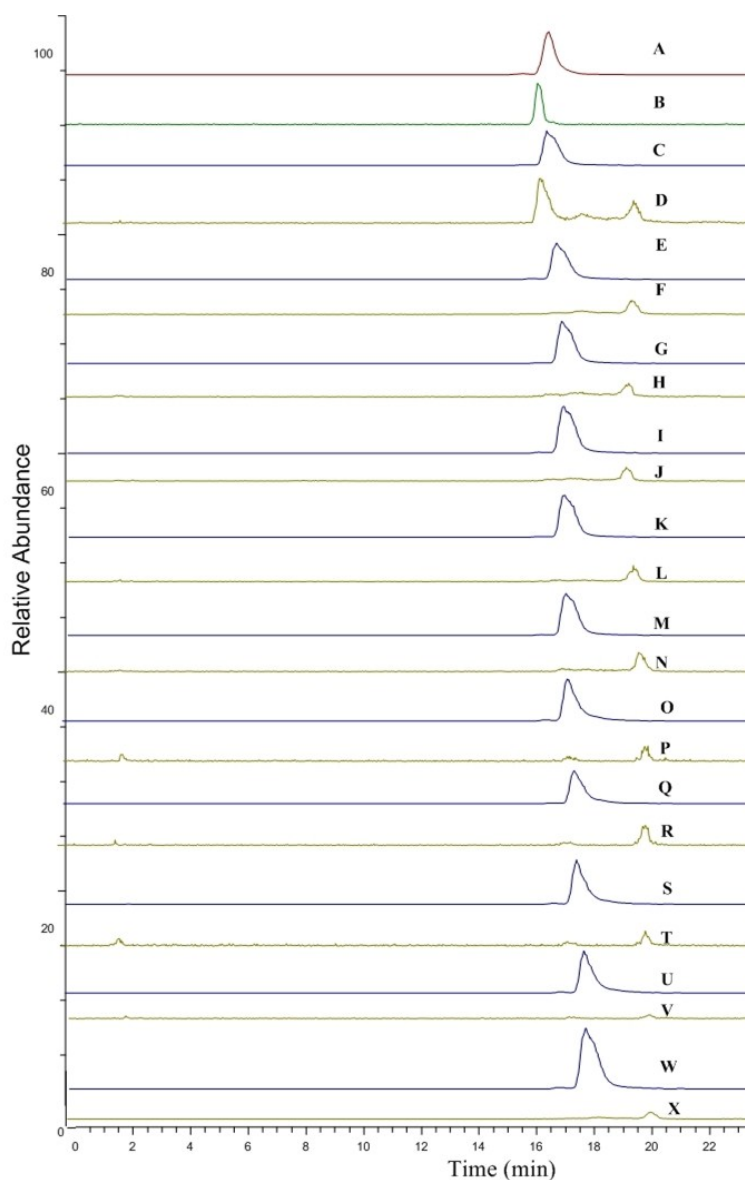


Figure S5. The kinetic parameters of GFPP for L-fucose-1-phosphate.

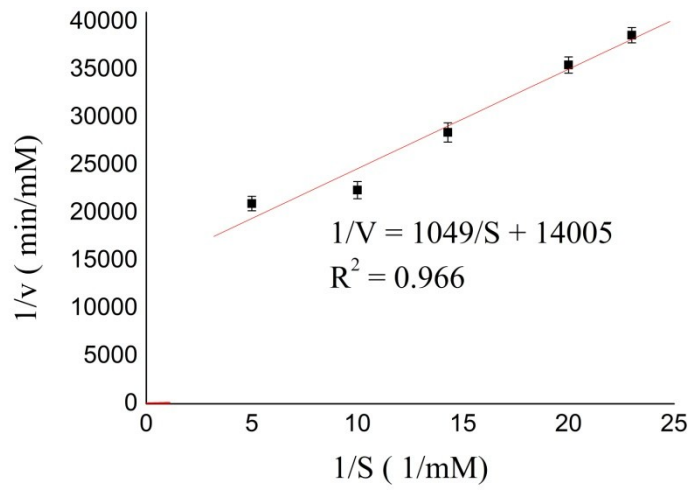


Table S2. Gradient elution of the chromatographic separation

Time (min)	Flow rate (mL/min)	B%
0	0.1	80
5	0.1	80
21	0.1	56
22	0.2	40
26	0.2	40
27	0.2	80
34	0.2	80
35	0.1	80

Table S3. Summary of GDP-L-fucose content in *M. alpina*.

Samples	Dry cell weight (g/L)	GDP-L-fucose concentration (mg/L)	Specific GDP-L-fucose content (mg / g cell)
Control	11.76±0.32	2.50±0.0047	0.21±0.0015
1day fucose (10 nM)	12.36±0.27	2.64±0.0052	0.21±0.0019
3days fucose (10 nM)	11.67±0.37	3.76±0.0053	0.32±0.0014
3days Mg ²⁺ (10 nM)	12.34±0.29	3.12±0.0046	0.25±0.0016
3days fucose (1 nM) and Mg ²⁺ (10 nM)	12.30±0.32	2.68±0.0049	0.22±0.0015
3days fucose (5 nM) and Mg ²⁺ (10 nM)	11.43±0.33	2.92±0.0044	0.26±0.0013
3days fucose (10 nM) and Mg ²⁺ (10 nM)	12.31±0.37	7.02±0.0049	0.57±0.0013
3days fucose (20 nM) and Mg ²⁺ (10 nM)	12.53±0.29	8.15±0.0051	0.65±0.0018
3days fucose (30 nM) and Mg ²⁺ (10 nM)	12.62±0.35	8.58±0.0052	0.68±0.0015