Enhanced heterologous production of glycosyltransferase UGT76G1 by co-expression of endogenous *prpD* and *malK* in *Escherichia coli* and its transglycosylation application in production of rebaudioside

Wenju Shu^{1,2,†} Hongchen Zheng^{1,2,3,†,*} Xiaoping Fu^{2,3,†} Jie Zhen^{2,3} Ming Tan^{2,3} Jianyong Xu^{2,3} Xingya Zhao^{1,2} Shibin Yang^{1,2} Hui Song^{1,2,3,*} YanHe Ma^{2,*}

¹University of Chinese Academy of Sciences, Beijing 100049, China

²Industrial Enzymes National Engineering Laboratory, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

³Tianjin Key Laboratory for Industrial Biological Systems and Bioprocessing Engineering, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

*Corresponding authors. Address: No. 32 West 7th Avenue, Tianjin Airport Economic Area, Tianjin 300308, China. Tel: 086+022+84861934; Fax: 086+022+84861934. E-mail address: zheng_hc@tib.cas.cn (HongChen Zheng); song_h@tib.cas.cn (Hui Song); ma_yh@tib.cas.cn (YanHe Ma).

[†]Wenju Shu, Hongchen Zheng and Xiaoping Fu contributed equally to this work and are listed as co-first authors.

Gene	Fusion expression partner	Protein sizes	Organism
		(kDa)	
Fh8	Fasciola hepatica 8-kDa	7.7	F. hepatica
	antigen		
MBP	Maltose-binding protein	42	Escherichia coli
Smt3	Small ubiquitin modified	11.3	Synthetic
DsbA	Disulphide isomerase I	23.1	Escherichia coli
DsbC	Disulphide isomerase	25.6	Escherichia coli

Table S1 Detail information of fusion partners in this work

Table S2 Primers	used to amplify	the target get	nes in this work

Genes	Primer sequences
UGT76G1	5' -ATGGAAAATAAAACGGAGACCACC -3'
	5' -CAACGATGAAATGTAAGAAACCAAAGA -3'
Fh8	5' -ATGCCGAGCGTTCAGGA -3'
	5' -GCTGCTCAGAATGCTCAC-3'
MBP	5' -ATGAAAATAAAAACAGGTGCACGCA-3'

	5' -CTTGGTGATACGAGTCTGCG-3'
Smt3	5' - ATGAGCGATAGCGAAGTGAA-3'
	5' -ACCACCAATCTGTTCACG-3'
DsbA	5' - ATGAAAAAGATTTGGCTGGCG-3'
	5' -TTTTTTCTCGCTTAAGTATTTCACTGT-3'
DsbC	5' -ATGAAGAAAGGTTTTATGTTGTTTACT-3'
	5' -TTTACCGCTGGTCATTTTTTG-3'
prpD	5' -ATGTCAGCTCAAATCAACAACATC-3'
	5' -TTAAATGACGTACAGGTCGAG-3'
malK	5' -ATGGCGAGCGTACAGCTG-3'
	5' -TTAAACGCCCGGCTCCTTATG-3'



Figure S1 Expression of recombinant UGT76G1 under different plasmids in *E. coli*. A: SDS-PAGE of the whole cell lysates of different recombinant strains which harbouring different recombinant plasmids (1, pET32a-*UGT76G1*; 2, pET22b-*UGT76G1*; 3, pET26b-*UGT76G1*), M means marker of protein molecular weight; B: The different conversion rates from St to RA by the crude enzymes of the different recombinant strains in 12 h. Data are presented as mean±SD (n=3).



Figure S2 Growth profiles of the overexpression strains E. coli BL21 (DE3) M/P-(1-6) -S32U. The

detail information of these strains see Table 1. Data are presented as mean \pm SD (n=3).



Figure S3 SDS-PAGE of the fusion enzyme Smt3-UGT76G1 and its tag deleted enzyme UGT76G1 by enterokinase hydrolysis