Supporting Information

Nicotiana benthamiana α-galactosidase A1.1 can functionally complement human α-galactosidase A deficiency associated with Fabry disease

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Running title: *A plant α-Galactosidase showing great similarity to human enzyme.*

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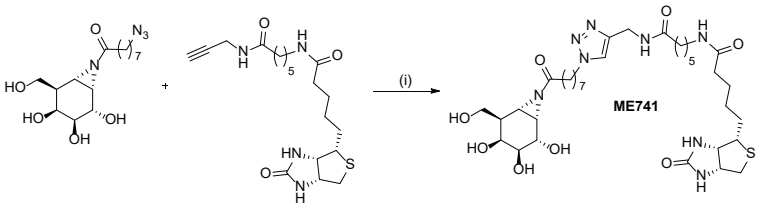
Scheme S1. Synthesis of ME741 (Biotinylated α-Galactosidase ABP)
α-Galactose configured biotinylated cyclophellitol aziridine ME741 was synthesized by copper–catalyzed click chemistry of azido cyclophellitol aziridine intermediate with biotin alkyne (Figure S1).



Scheme S1. Synthesis of biotinylated α-Galactosidase ABP ME741. Reagents and conditions: (i) CuSO4·H2O, sodium ascorbate, DMF, rt, 18 h, 26%.

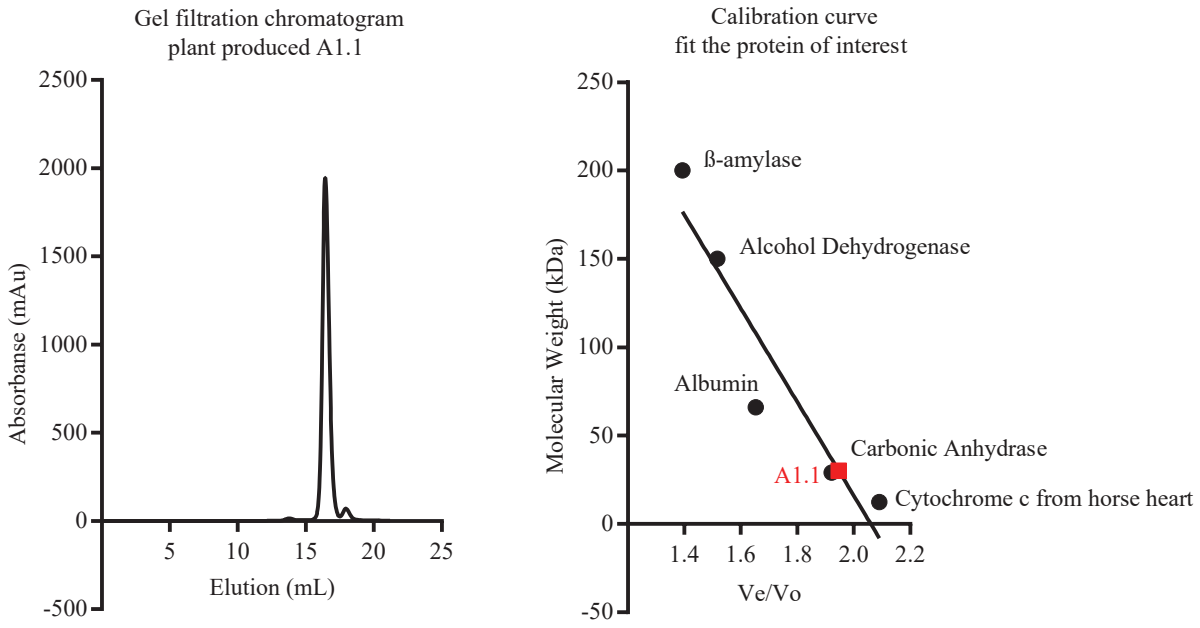


Figure S1. Analytical gel filtration chromatography analysis of A1.1. Left panel. Gel filtration chromatogram of the *N.benthamiana* produced A1.1, after its application in Superdex™ 200 Increase 10/300 GL. Right panel. Gel filtration chromatography calibration curve of proteins with known molecular weight, fitting the results of the plant produced A1.1. In black circle, β-amylase, alcohol dehydrogenase, albumin, carbonic anhydrase, cytochrome c from horse heart and Dextran. In red box A1.1.

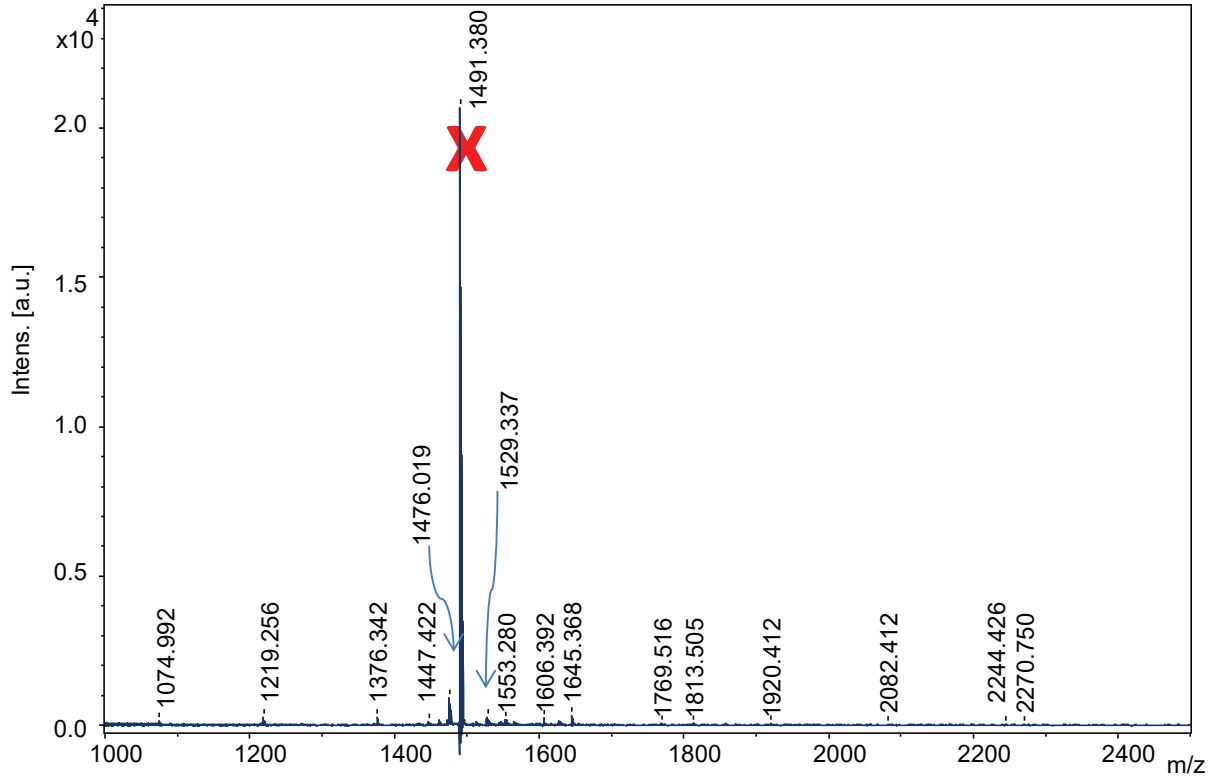
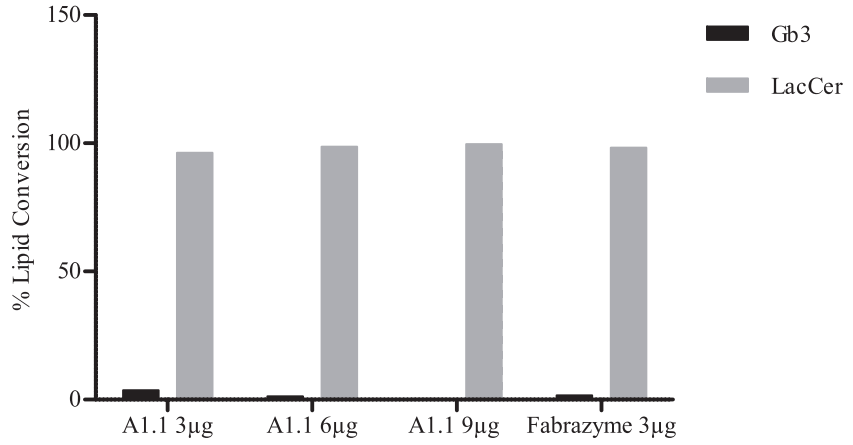


Figure S2. Determination of A1.1 N-glycosylation profile. MALDI-TOF MS analysis of 2-aminobenzoic acid derivatised PNGase-A possibly released N-glycans of plant produced A1.1. No N-linked glycans were detected.

(A)



(B)

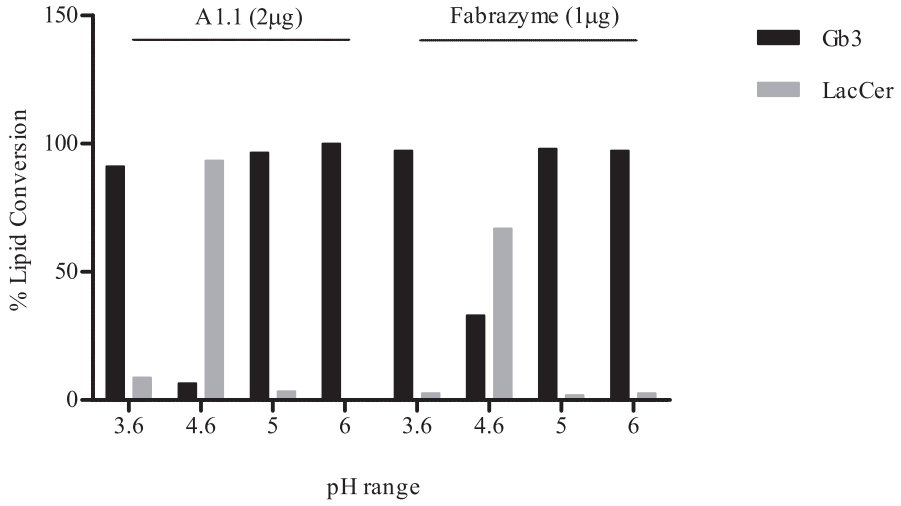


Figure S3. Activity of A1.1 and Fabrazyme towards lipid substrates, as measured by HPLC analysis. (A) HPLC analysis of degradation of C18-Gb3 to LacCer by different µg amounts of A1.1 or Fabrazyme. (B) Degradation of C18-Gb3 to LacCer by A1.1 or Fabrazyme in different pHs (3.6-6).

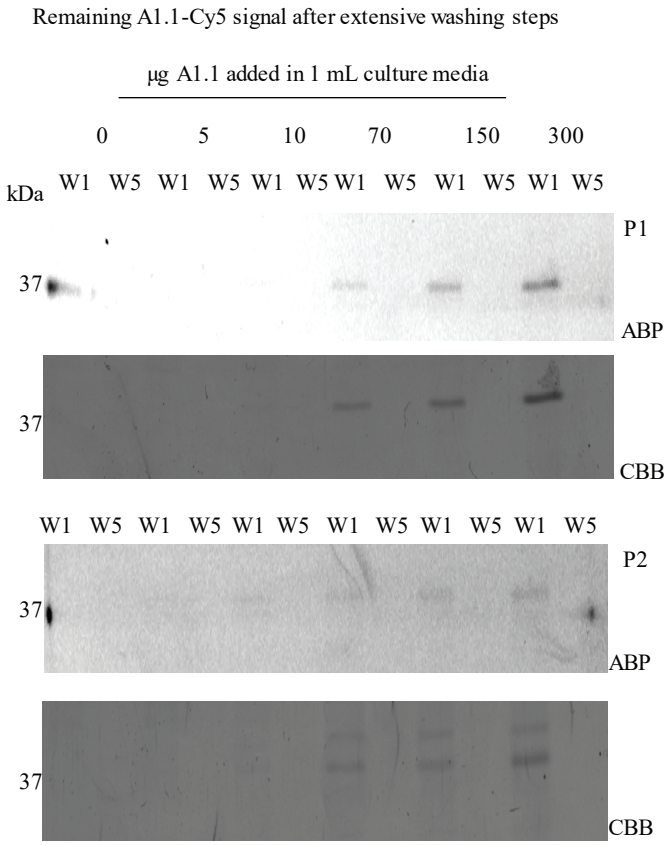


Figure S4. Detection of remaining Cy5 signal after exposure of FD fibroblasts to prelabelled A1.1, from Figure 6. After incubation of the 2 different FD fibroblasts, with prelabelled A1.1, extensive washing steps (x5) of the cells performed. To ensure that all of the not internalized to the cells prelabelled protein is washed out prior to lysate making, samples from both wash 1 and wash 5 (W1 and W5) were analysed in SDS-PAGE, following the fluorescent signal. 10 % of the total wash volume was loaded on the gels. ABP= activity based probe, CBB= Coomassie brilliant blue, P1= patient 1, P2= patient 2.

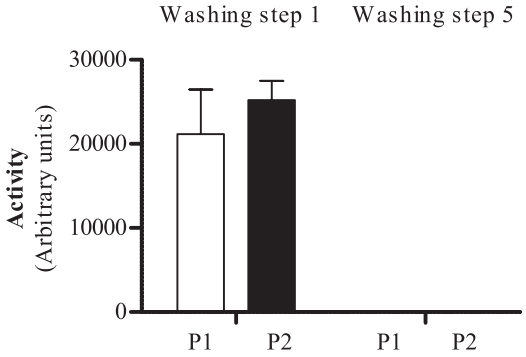


Figure S5. 4MU-α-GAL activities found in washing steps of Figure 7. After treatment with 300 µg/ mL of A1.1, the FD cells were extensively washed with PBS (x5 times). Activities in washing steps 1 and 5 were performed to ensure that no remaining activity is found to the cells prior to lysate making. P1= patient 1, P2= patient 2.

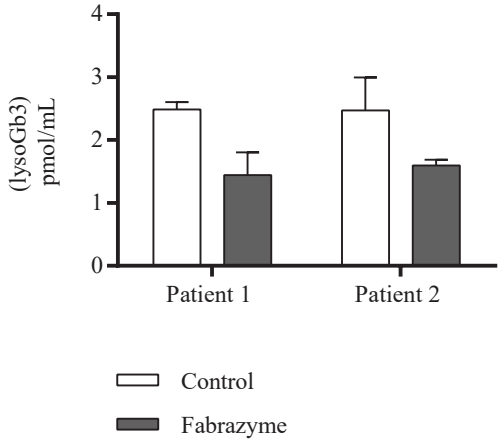


Figure S6. LC-MS/MS analysis of lysoGb3 found in FD fibroblasts after incubation with Fabrazyme. Fibroblasts from FD patients were grown in 12 well plates. Overnight incubation with 5 µg/mL of Fabrazyme took place, followed by lipid extraction and LC-MS/MS analysis. ^{13C}lysoGb3 was used as internal standard. (n=2, error bars indicate mean ± standard deviation).

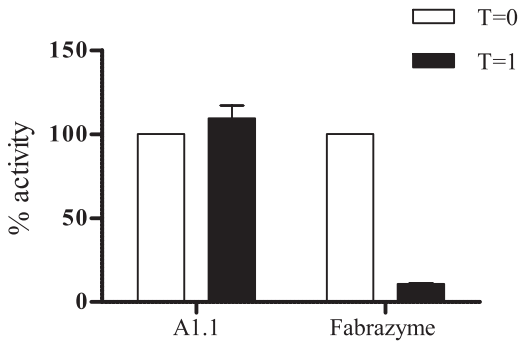


Figure S7. 4MU-α-Gal activity of recombinant galactosidases after incubation in FD serum, before and after ERT. A1.1 and Fabrazyme were incubated in Fabry serum at a concentration of 1 mg/mL each, before and after ERT (T=0, T=1, respectively). (n=2, error bars indicate mean ± standard deviation).

Table S1. Proteomics analysis using Swissprot (version June 2017)

	Sample	Protein Accession code	Protein Name	Organism	Covarage % AA	ppm	Score	Sequence
Apoplast #1	DMSO							
	Competition	Q8RX86	α-galactosidase 2	*Arabidopsis thaliana*	2	-4	28	EVIAVNQDK
	Pull down	Q8RX86	α-galactosidase 2	*Arabidopsis thaliana*	2	-7	56	EVIAVNQDK
		Q42656	α-galactosidase	*Coffea arabica*	4	-5 -4	29 13	GSTFPSGIK ALADYVHSK
		Q9FT97	α-galactosidase 1	*Arabidopsis thaliana*	3	-4 -12	23 35	STFPSGIK YPVMTR
Apoplast #2	DMSO							
	Competition	Q8RX86	α-galactosidase 2	*Arabidopsis thaliana*	2	-8	54	EVIAVNQDK
	Pull down	Q42656	α-galactosidase	*Coffea arabica*	8	-6 -8 -8 -7	38 22 30 52	GSTFPSGIK ALADYVHSK YPIMSK APLLIGCDIR
		Q8RX86	α-galactosidase 2	*Arabidopsis thaliana*	2	8	56	EVIAVNQDK
		Q9FT97	α-galactosidase 1	*Arabidopsis thaliana*	7	-7 5 -8	10 31 29	STFPSGIK LGIYSDAGYFTCSK YPVMTR

Table S2.Proteomics analysis using Swissprot (version June 2017, including N.benthamiana α-galactosidase sequence).

	Sample	Coverage (% of AA)	Start–End	ppm	Score	Sequence	AA identified
Plant leaf lysate #1	DMSO	0					
	Competition	0					
	Pull down	7	139 - 152	4	39	LGIYSDAGSQTCSK	29
			213 - 245	6	17	TTGDISDNWDSMTSR	
Apoplast #1	DMSO	0					
	Competition	0					
	Pull down	17	118 - 126	-5	29	GSTFPSGIK	70
			127 - 135	-4	13	ALADYVHSK	
			139 - 152	1	82	LGIYSDAGSQTCSK	
			153 - 166	-7	42	QMTGSLGHEEQDAK	
			231 - 245	8	22	TTGDISDNWDSMTSR	
			314 - 322	-7	56	EVIAVNQDK	
Apoplast #2	DMSO	0					
	Competition	11	118 - 126	-8	20	GSTFPSGIK	46
			139 - 152	2	45	LGIYSDAGSQTCSK	
			153 - 166	-6	57	QMTGSLGHEEQDAK	
			314 - 322	-8	54	EVIAVNQDK	
	Pull down	33	118 - 126	-6	38	GSTFPSGIK	135
			127 - 135	-8	22	ALADYVHSK	
			139 - 152	1	48	LGIYSDAGSQTCSK	
			153 - 166	2	34	QMTGSLGHEEQDAK	
			192 - 197	-8	30	YPIMSK	
			231 - 245	4	80	TTGDISDNWDSMTSR	
			291 - 300	-7	52	APLIIGCDLR	
			301 - 313	-12	40	SMDQTAHEILSNK	
			314 - 322	8	56	EVIAVNQDK	
			348 - 355	- 9	10	VALVLWNR	
			381 - 388	- 5	34	DLWAHSTK	
			393 - 405	- 9	12	GQISASIDSHDCR	
			406 - 412	-13	33	MYVLTPK	