

Figure S1. Microbial growth curves of the enrichments on pretreated *A. donax* in different culture media.

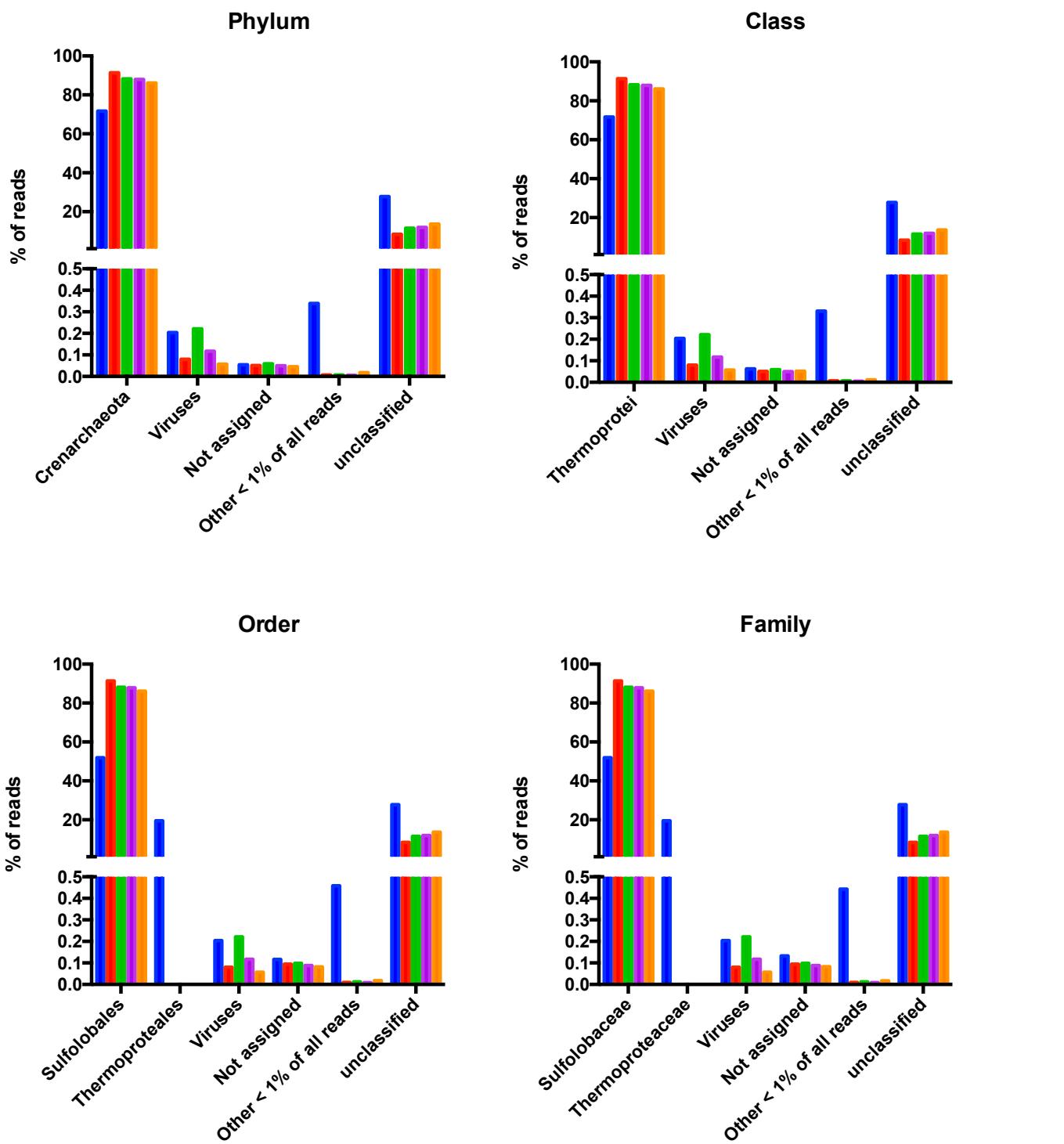


Figure S2. Taxonomic annotation at the level of phylum, class, order, and family.

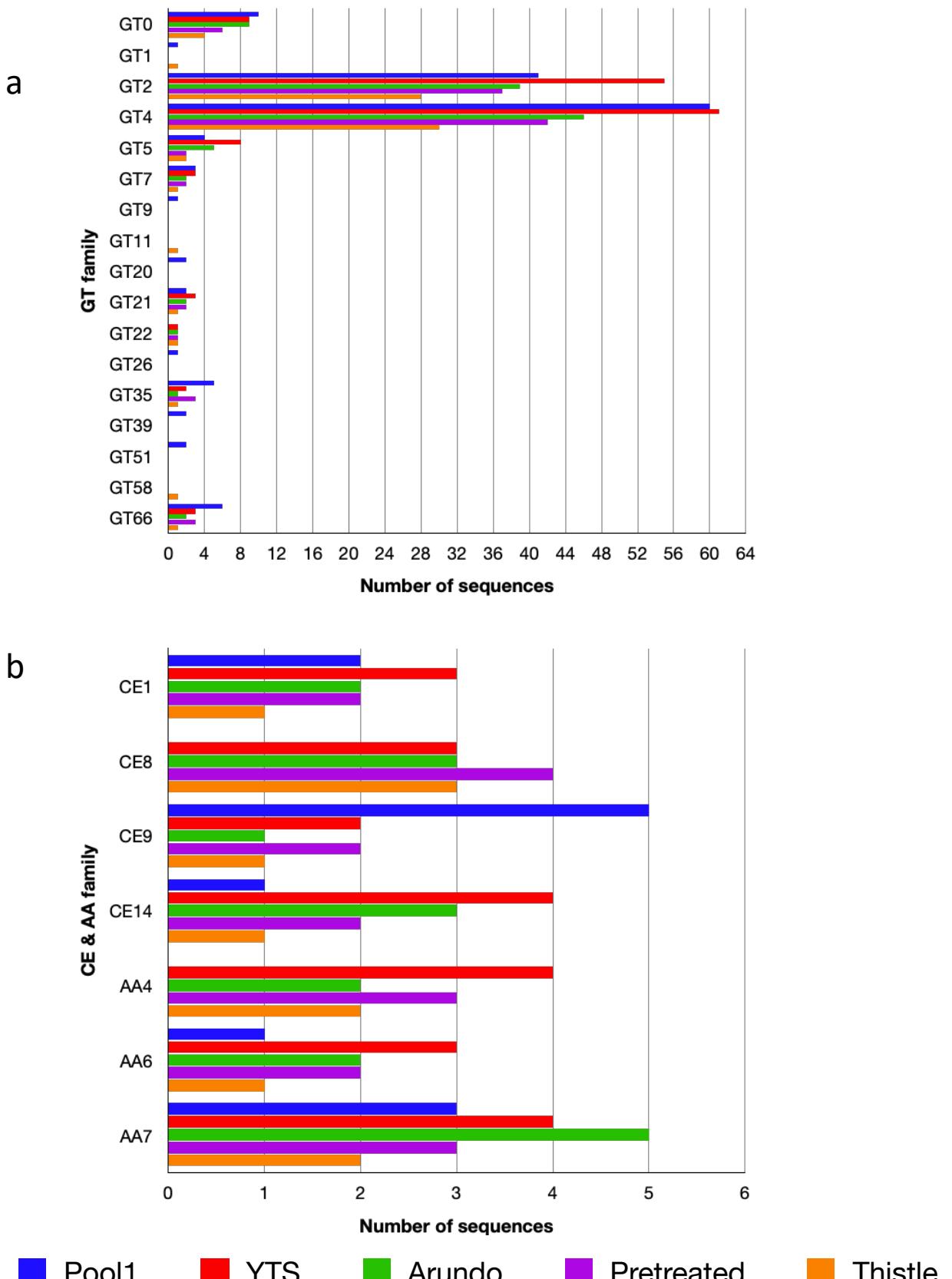


Figure S3. Distribution among the samples of the (a) glycosyltransferase, (b) carbohydrate esterases and auxiliary activities. The number of sequences assigned to each family from Pool1 and the four enrichments is displayed.

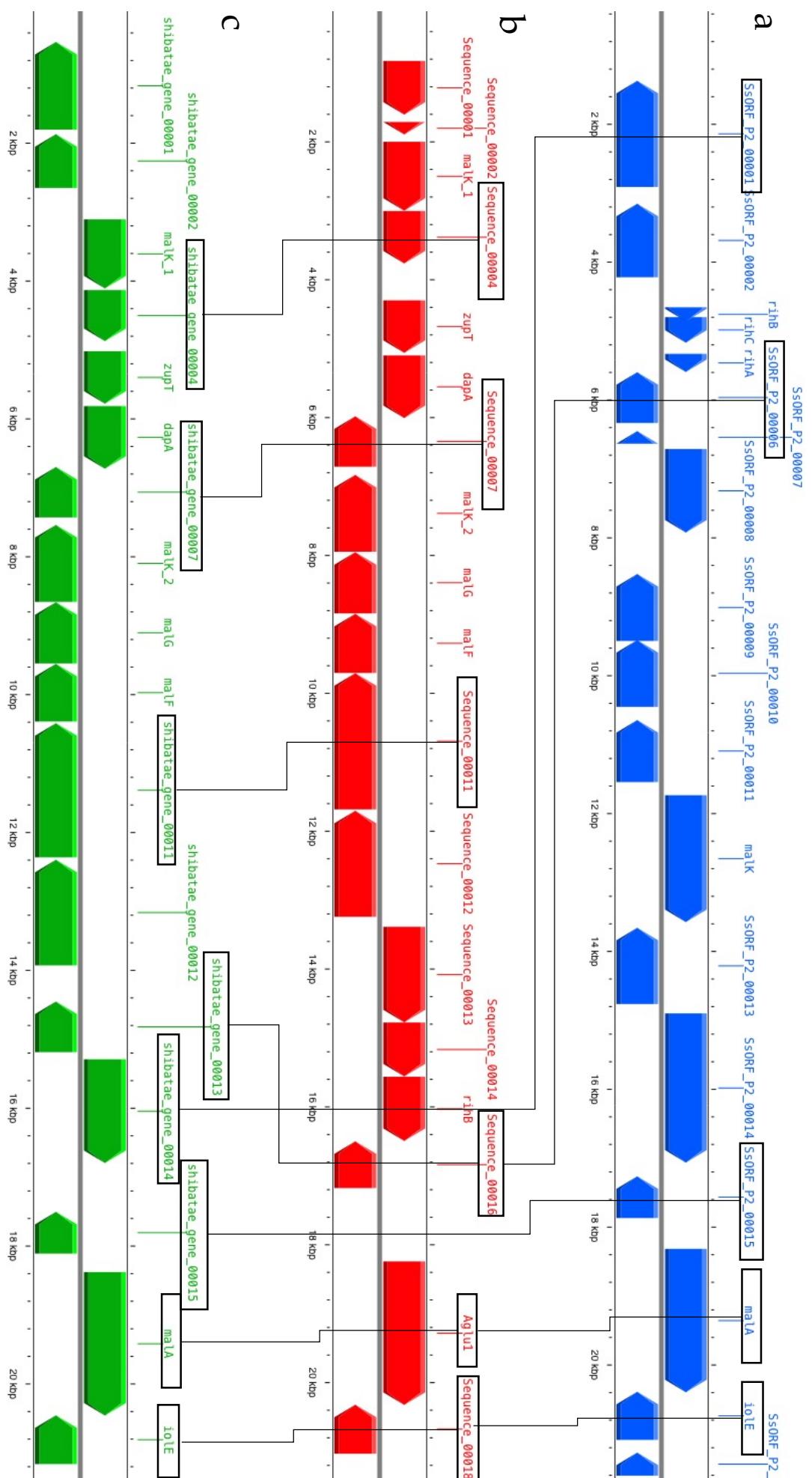


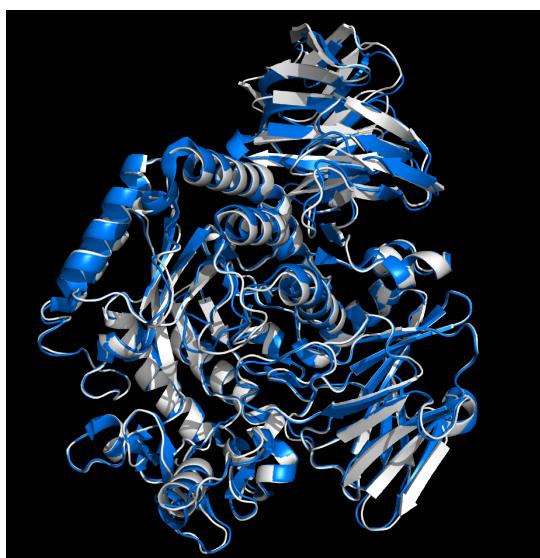
Figure S4. Genomic environments of *S. solfataricus* P2 *malA* (a), the contig containing *aglu1* gene (b) and *S. shibatae* *malA* (c). Callouts and connectors indicate homologous unannotated ORFs with amino acid identity  $\geq 80\%$

SSo3051	--KFCEIESGELFVGKMWPGTTVYPDFFREDTREWWAGLISEWLSQG-VDGIWL <b>D</b> MNEPT	325
<b>Aglu1</b>	--KFCEIETGELFVGKMWPGTTVYPDFFREDTREWWAGLISEWLSQG-VDGIWL <b>D</b> MNEPT	325
ST2525	--KYCETDKGELFVGKLWPGNSVYPDFFREDTREWWAGLISEWLSQG-IDGIWL <b>D</b> MNEPT	318
Saci_1160	--NYVENEDGTIYADILWPGLSVFPDFLNSKTRREWWRNLVKEWKVNKENNIDGIWL <b>D</b> MNEPS	323
PTO0092	--KYVINSNNEIYISRLWPGNCFLNFLDADSINYWKSCVKEFAENV--DGIWL <b>D</b> MNEPA	319
Ta0298	GGYFVKYPDGNVMYAPVWPEMAAFPDFTDEKAREWYASKYDFMRSMG-VSGFWH <b>D</b> MNEPA	413
XyLS	-----CIILGTTAFNPFKDECREFWS-YVKGFYDLG-IDAYWL <b>D</b> ASEPE	358
	: . * : . . . . * * .**	
SSo3051	KGFRTSHRNEIFILSRAGYAGI <b>Q</b> RYAFI-WTG <b>D</b> NTPSWDDLKQLQLVLGLSISGVPFVG	443
<b>Aglu1</b>	EGFRKSGRSEVFILSRAGYAGI <b>Q</b> RYAFI-WTG <b>D</b> NTPSWDDLRLQLQLVLGLSISGIPFVG	443
ST2525	EGFK--NKEEVFILTRGGYAGI <b>Q</b> KFAGI-WTG <b>D</b> NTPSWDDLKQLQLILGLSISGIAYIG	422
Saci_1160	PSVD-----FVLSRAGYSGI <b>Q</b> RYAAI-WTG <b>D</b> NTTSWSDLTLQLALTLLGLSISGVPYVG	414
PTO0092	EALK-EIKDEFFILSRSGYPGI <b>Q</b> RYAAI-WTG <b>D</b> NKASDDDLKLQISMIVSMNLSGIMICG	416
Ta0298	DHLS--KVERPFFILSRSGWAGISRYGWI-WTG <b>D</b> TETSWKELKQNIITIMHMSMSGITLTG	507
XyLS	EGQRRISENKRVVILTRSAFAGQQRHSAISWSGDVLGDWATLRAQIPAGLNFSISGIPYWT	455
	.::*:*.:. * . . . * *;** . * : . : .::**:	

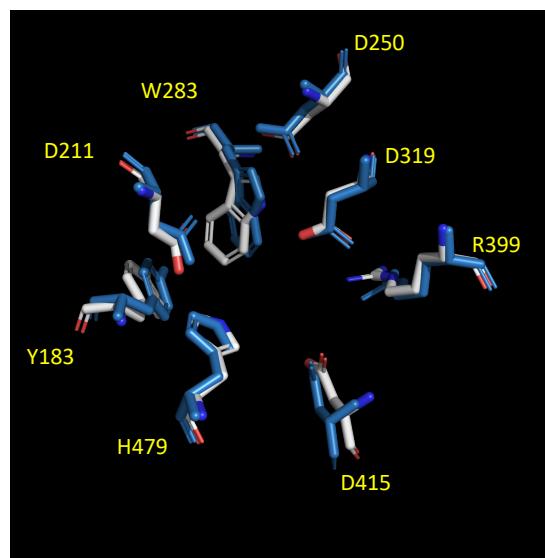
Figure S5. Multialignment of the regions close to catalytic residues (in red) of the aminoacidic sequences of the GH31 enzymes: SSo3051 MalA from *S. solfataricus*, Aglu1 (in red), ST2525 from *S. tokodaii* str.7, Saci\_1160 MalA from *S. acidocaldarius* DSM639, PTO0092 AglA from *P. torridus* DSM 9790, Ta0298 AglA from *Thermoplasma acidophilum* DSM 1728 and XylS (Sso3022) from *S. solfataricus* P2.



(a)



(b)



(c)

Figure S6 . a) Different domains and subdomains of *S. solfataricus* MalA 3D structure (PDB ID 2G3M). The different colours indicate the domains N (blue), A (red), C (yellow), D (orange), and subdomains B (green) and B' (purple). b) Overall superimposition of *S. solfataricus* MalA (blue) and Aglu1 model (white). c) Close-up of the superimposed aminoacid residues in the active sites of the Aglu1 model (white) and MalA (blue).

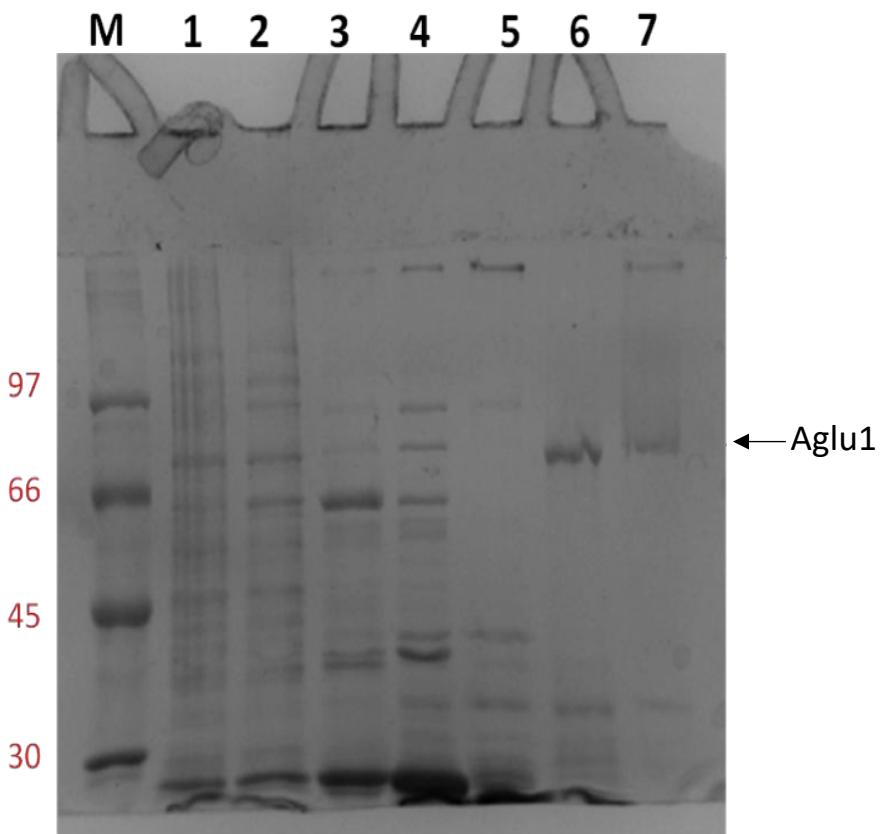


Figure S7 Purification steps of Aglu1. Lane M, molecular weight markers, lane 1) crude extract (7 µg); lanes 2–5) heat treatment at 50 °C (7 µg), 60 °C (10 µg), 70 °C (10 µg) and 80 °C (10 µg) respectively; lane 6, Q-Sepharose 16/10 (5 µg); lane 7, Phenyl Sepharose 26/10 (3 µg).

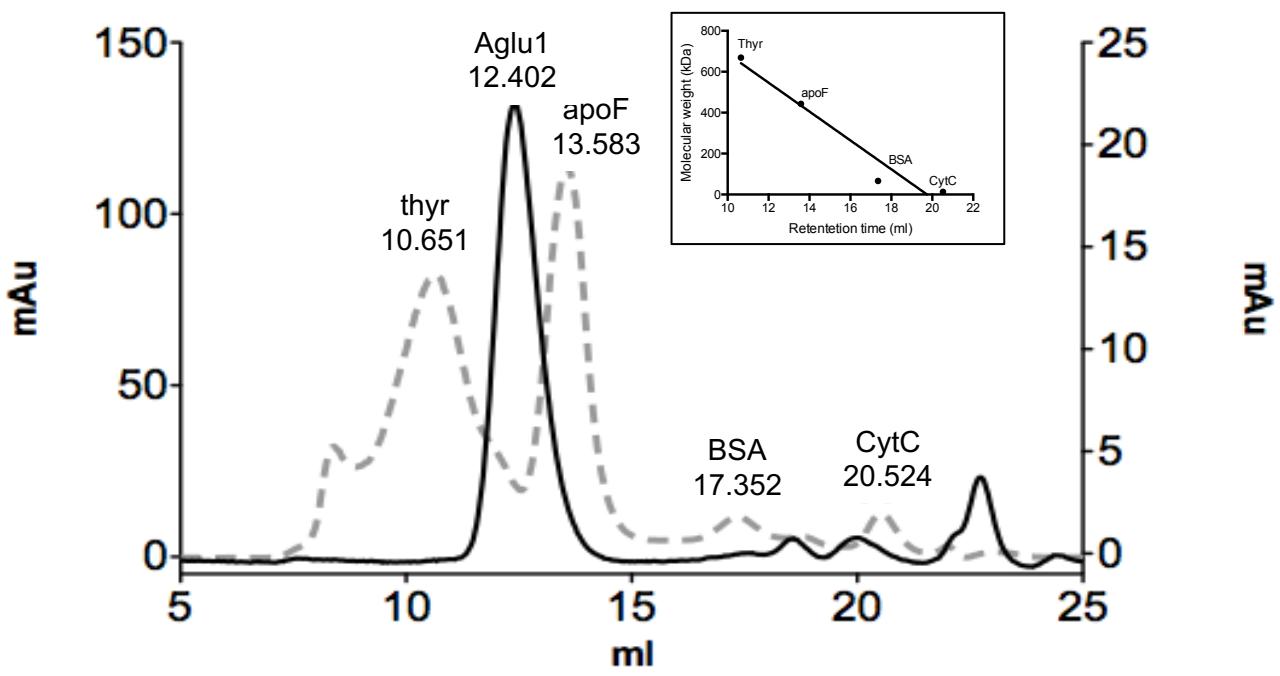


Figure S8. Elution profile of Aglu1 and molecular weight markers. Inset: graph of the calibration line obtained by plotting the retention time and the molecular weight.

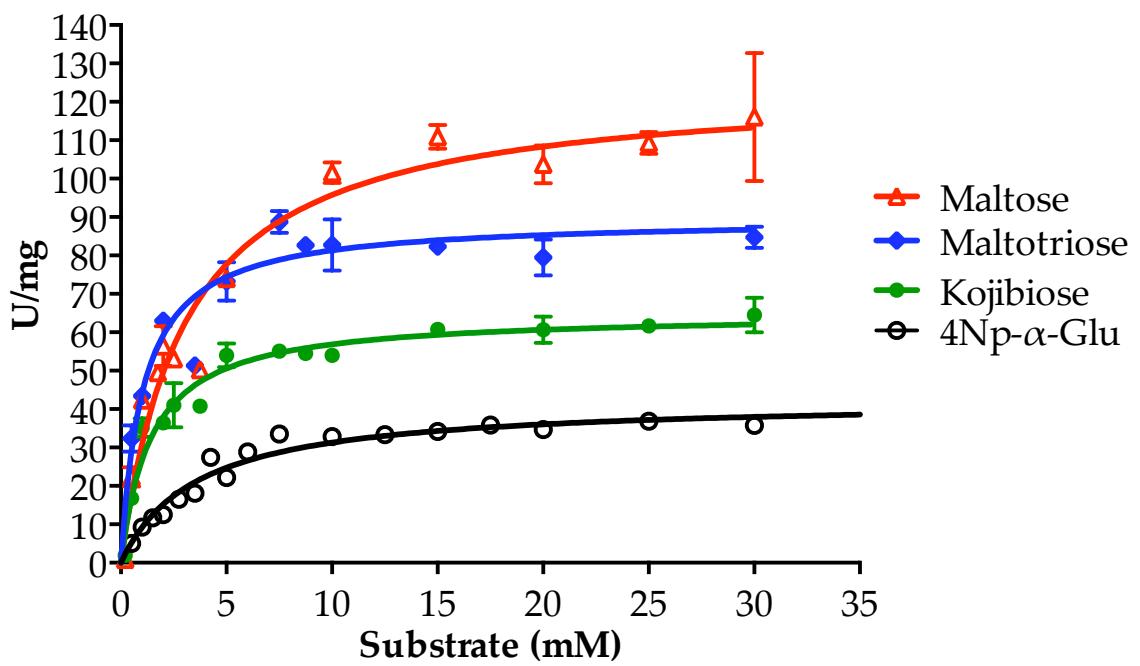


Figure S9. Kinetic curves of Aglu1 on Maltose, Maltotriose, Kojibiose and 4Np- $\alpha$ -Glu.

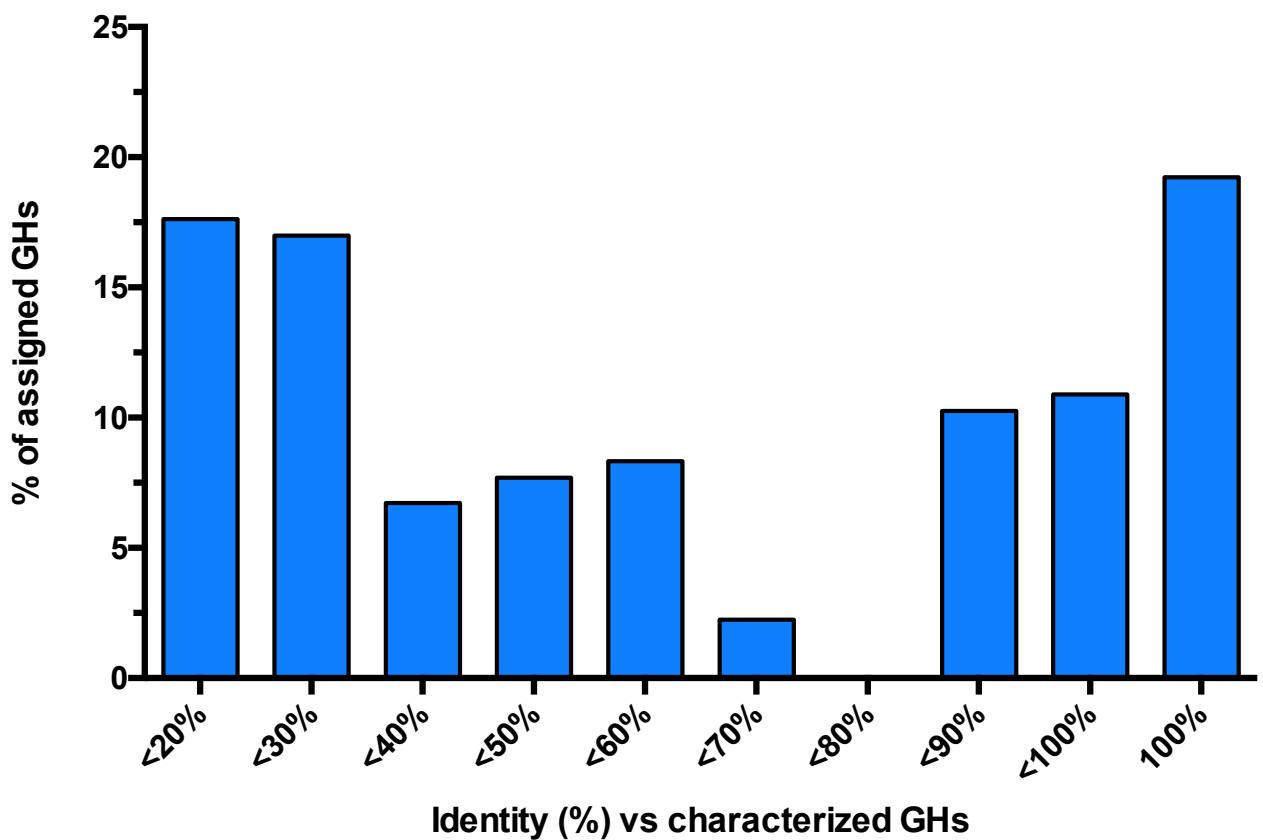


Figure S10. Percentage of the assigned GHs in the samples against the GHs characterized from [www.cazy.org](http://www.cazy.org)