Supplementary data

Two marine GH29 *α*-L-fucosidases from an uncultured *Paraglaciecola* sp. specifically hydrolyze fucosyl-*N*-acetylglucosamine regioisomers

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Supplementary Table I:

Nucleotide primers used in the present study. Uracil-containing overhangs for USER-fusion are underscored and codon triplets changed for mutants are in bold.

Primer name	5'-->3'
Primers for pET15b cloning	
Fp231_pET15b_F	ACG GAT CUG AGC TTG CCG CCC AC
Fp231_pET15b_R	AGC CGG AUT TAC TGG CTT ATC TCT AAT GC
Fp239_pET15b_F	ACG GAT CUC AAG CAC ACT CTG AAA AAA C
Fp239_pET15b_R	AGC CGG AUC TAA GGG AGA TAT TCT ATA GTT AAC
Fp240_pET15b_F	ACG GAT CUG CCG ATA AAC CCT ACG AC
Fp240_pET15b_R	AGC CGG AUT TAA AAA TGA TTT CCG TCT AAA TC
Fp251_pET15b_F	ACG GAT CUG AGA CTG AGC ATA GAC TTA AAC
Fp251_pET15b_R	AGC CGG AUT TAC TTT AAT AAC ACT TCA ATG G
Fp284_pET15b_F	ACG GAT CUG CAA GTG ATT ATA CAA GCC TTA C
Fp284_pET15b_R	AGC CGG AUC TAC TGC GCT TCT TTA ACC
Primers for pET9a cloning	
Fp231_Nat.SP_Forw.	AGG CTT AAU ATG CAT CAG CAA CGA G
Fp231_Nat.SP_Rev.	ACT TCC ACU CTG GCT TAT CTC TAA TGC
Fp231_K67_Forw.	ATA TGG CUA AAA GCT TGA CCA AAG AG
Fp231_K417_Rev.	ACT TCC ACU TTT AGA GTC ATA GAT TGC ATC
Fp231_E48_Forw.	AGG CTT AAU GAG AAA AAA CAA GTA TAT GGC
Primers for mutagenesis	
Fp231_H174F_Forw.	GCT CCA AG**T TC**C ATG AAG GTT TTG CCA TGT TTA AGT CTG AAG
Fp231_H174F_Rev.	TTC ATG **GAA** CTT GGA GCC CAT GAC GAT GTA TTT CAT GCC T
Fp231_W225H_Forw.	AAC TCC CTT GAT **CAT** CGC GAT GGT GGT GAT GGT GG
Fp231_W225H_Rev.	CG**A TG**A TCA AGG GAG TTA GAA TAA TAA ACA CCA AAG TCG AGG C
Primers for vector amplification	
pET15b_vec_Forw.	ATC CGG CUG CTA ACA AAG
pET15b_vec_Rev.	AGA TCC CUG ATG ATG ATG ATG ATG GCT
pET9a_vec_Forw.	ATT AAG CCU CAG CAT ATG TAT ATC
pET9a_vec_Rev.	AGT GGA AGU CCG CAC CAC CAC CAC

Supplementary Table II: 96-well format protein crystallization screens used in this study.

Screen	**Supplier**
MCSG-1	Anatrace
MCSG-2	Anatrace
SaltRx 1	Hampton Research
JBScreen Basic HTS	Jena Biosciences
JBScreen Pact ++ HTS	Jena Biosciences
JBScreen Clasic HTS I	Jena Biosciences
JBScreen PEG/Salt HTS	Jena Biosciences
JBS JCSG++ HTS	Jena Biosciences
The PGA Screen™ (MD1-51)	Molecular dimensions
SG1™ Screen (MD1-89)	Molecular dimensions
Clear Strategy™ (MD1-31)	Molecular dimensions
Morpheus® (MD1-47)	Molecular dimensions
MIDAS*plus*™ (MD1-107)	Molecular dimensions

Figure S1: Protein domain structure of GH29 fucosidases in the present study. Protein domain features were predicted using the hmmscan function at EMBL-EBI https://www.ebi.ac.uk/ and SignalP 5.0 (Almagro Armenteros et al. 2019). The total length of each enzyme is indicated in amino acids (AA). SPI: Secretory signal peptide cleaved by Signal Peptidase I, SPII: Lipoprotein signal peptide cleaved by Signal Peptidase II

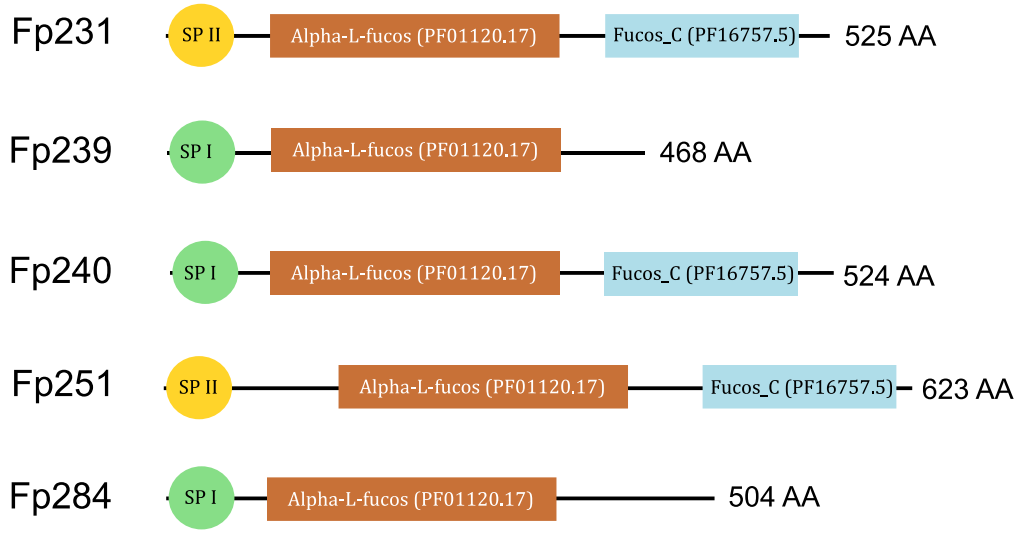


Figure S2: SDS-PAGE of recombinant GH29 fucosidases after IMAC purification. Theoretical molar masses: Fp239 (52.4 kDa), Fp240 (59.4 kDa), Fp251 (70.3 kDa), and Fp284 (56.8 kDa).

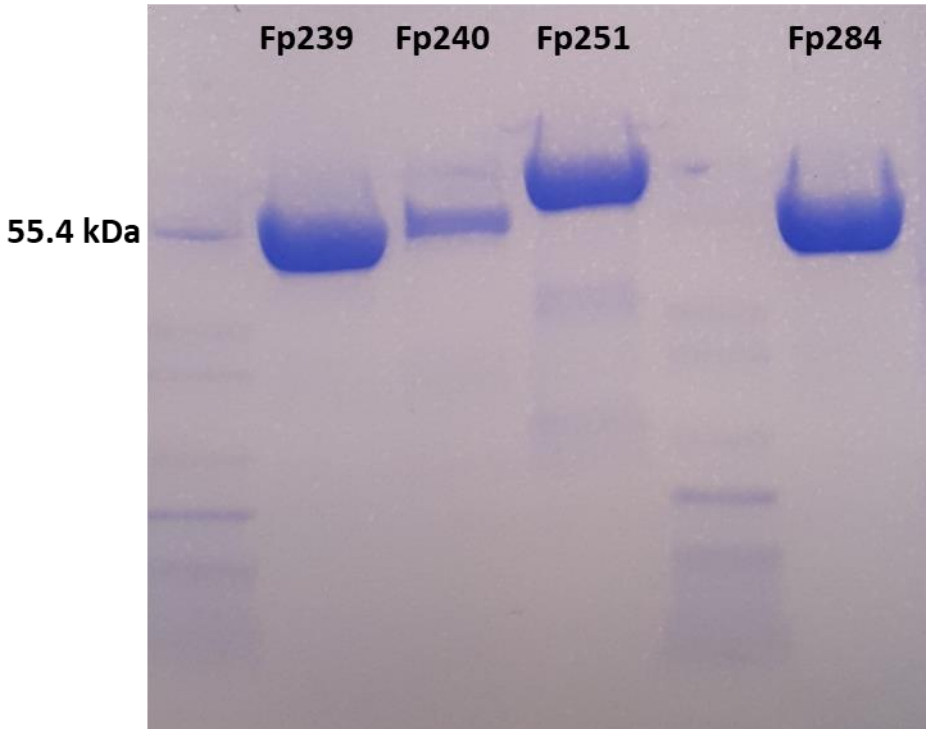


Figure S3: Activity of Fp231 in the presence of NaCl measured towards 1 mM CNP-Fuc.

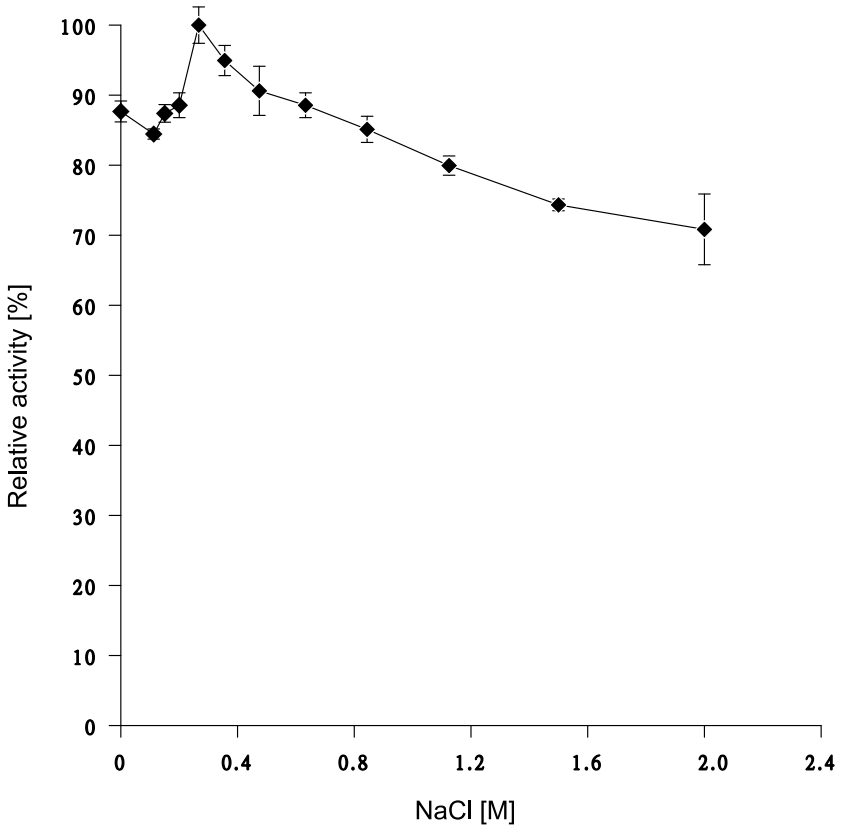


Figure S4: Michaelis-Menten plots of hydrolysis of CNP-Fuc and Fuc(α1,4)GlcNAc by Fp231

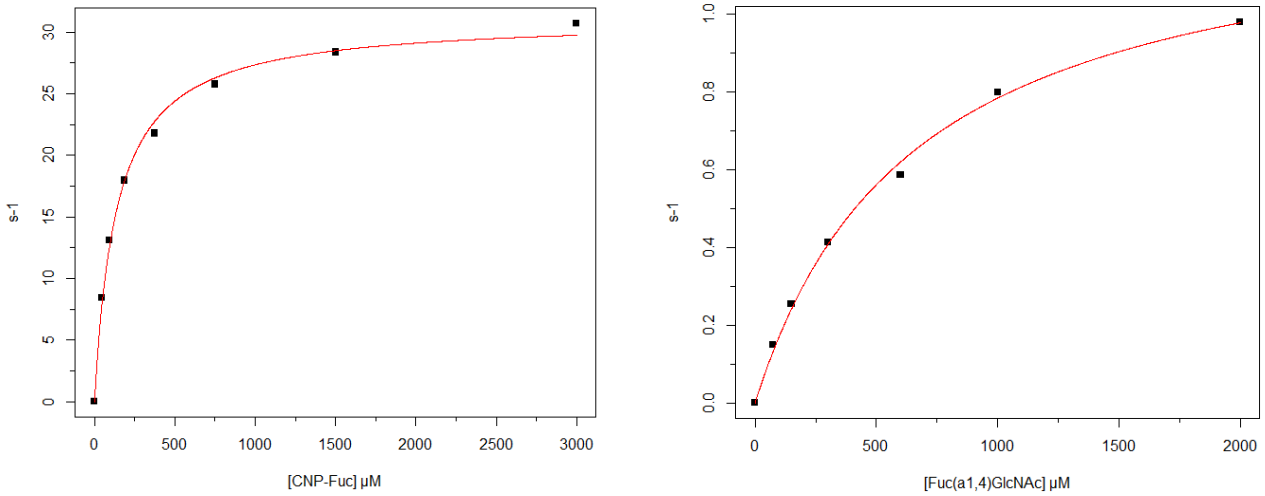
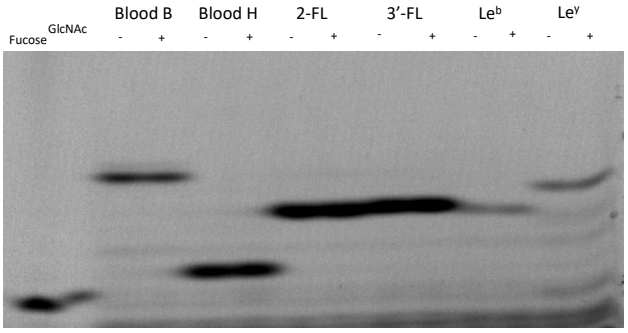


Figure S5: FACE gels showing activity of Fp231 on oligosaccharides (supplementing Figure 3B). Oligosaccharide substrates (**A** and **B**), or algal fucoidans from *Fucus vesiculosus*, *Fucus serratus* and *Ascophyllum nodosum* (**B**), were incubated with (+) and without (–) Fp231 (5 µM) at room temperature for 24 h and the reaction products were fluorescently labeled and separated in acrylamide gels. Enzyme activity was evaluated as a change in mobility of the fluorescent products. Monosaccharide standards (Fuc and GlcNAc) are included for comparison.

A)



B)

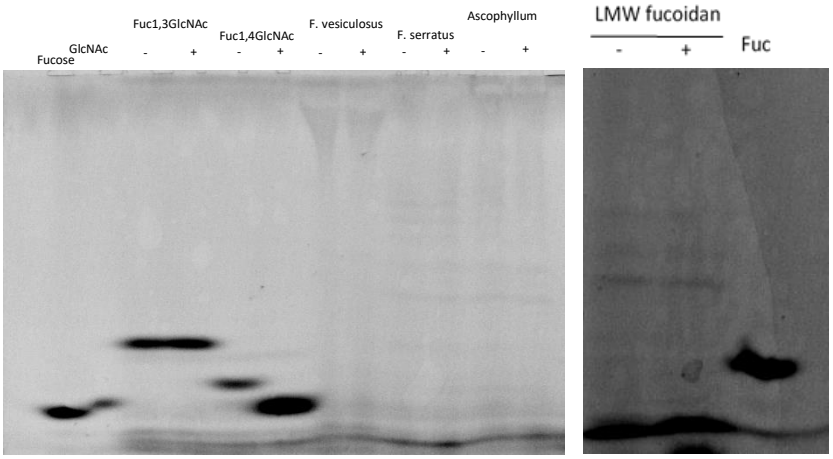
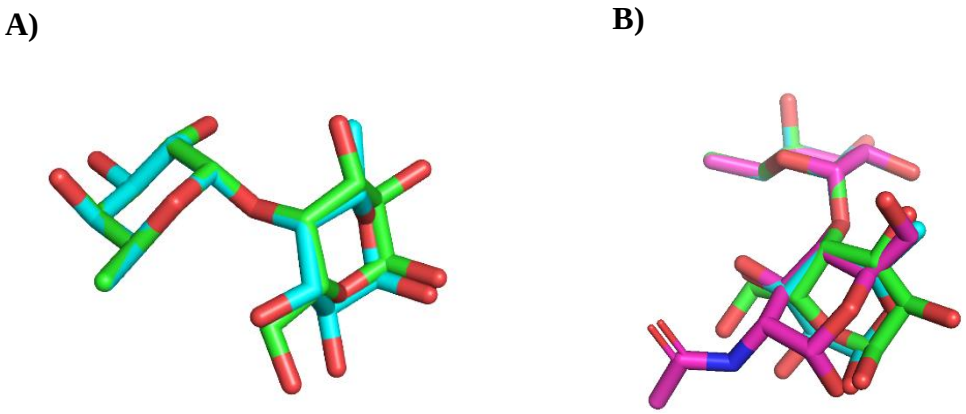


Figure S6: Overlay of oligosaccharide structures. **A)**: Fuc(α1,4)Gal (green sticks) and Fuc(α1,4)Fuc (cyan sticks); **B)**: Fuc(α1,4)GlcNAc (pink sticks), Fuc(α1,4)Gal (green sticks) and Fuc(α1,4)Fuc (cyan sticks)



References

Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H. 2019. SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol*, 37:420-423.