

Supplementary Information

Table S1. Percent identity matrix of the catalytic D1 domain of Mef2 and the characterized members of the GH107 family

	MfFcna	FFA1	Fhf1	FFA2	FWf1	FWf3	P5AFcna	P19DFcna	Mef2	Fda1	Fda2	SVI_0379
MfFcna/34-418*	100	83	72	63	53	38	16	17	14	23	23	26
FFA1/31-415*	83	100	72	62	54	37	16	17	14	23	23	25
Fhf1/29-447*	72	72	100	68	54	38	17	17	16	25	24	25
FFA2/41-433*	63	62	68	100	52	39	17	17	16	23	22	25
FWf1/15-439*	53	54	54	52	100	40	17	16	13	24	25	23
FWf3/213-589*	38	37	38	39	40	100	15	18	15	23	24	22
P5AFcna/36-393*	16	16	17	17	17	17	100	72	39	22	23	22
P19DFcna/31-393*	17	17	17	17	16	18	72	100	40	23	24	22
Mef2/22-383*	14	14	16	16	13	15	39	40	100	21	23	20
Fda1/23-372*	23	23	25	23	24	23	22	23	21	100	72	44
Fda2/84-439*	23	23	24	22	25	24	23	24	23	72	100	40
SVI_0379/26-367*	26	25	25	25	23	22	22	22	20	44	40	100

* Indicate the amino acid sequence numbers used for the analysis

Table S2. Thermostability by DLS of Mef2 with Ca²⁺ and without Ca²⁺

Mef2 Stability	T_m (°C)
Mef2 - Ca ²⁺	37.9 ± 0.05
Mef2 + Ca ²⁺	44.4 ± 0.03

Table S3. Monosaccharide composition of fucoidan fraction 4 (FeF4) purified from enzymatic extracts of *F. evanescence*

FeF4		
Neutral monosaccharides (mol%)	Fucose	88.1 ± 0.0
	Rhamnose	0.8 ± 0.0
	Galactose	7.3 ± 0.3
	Glucose	0.7 ± 0.1
	Xylose	1.1 ± 0.1
	Mannose	0.2 ± 0.0
Uronic acids (%)	Guluronic acid	0.1 ± 0.1
	Glucuronic acid	0.5 ± 0.2
	Mannuronic acid	1.1 ± 0.5
Sulfate (%)		38.1 ± 1.2

The data are given in %mol (relative level) of total carbohydrates analyzed in the fucoidan fractions

Table S4. Monosaccharide composition of fucoidan fractions purified from enzymatic extracts of *S. latissima* from Ocean Rainforest

		SIF1	SIF2	SIF3	SIF4
Neutral monosaccharides (mol%)	Fucose	31.7 ^c ± 2.2	69.0 ^a ± 0.8	58.6 ^b ± 0.3	63.1 ^b ± 0.5
	Rhamnose	0.4 ^c ± 0.1	1.0 ^b ± 0.0	0.8 ^{b,c} ± 0.0	2.0 ^a ± 0.1
	Galactose	0.5 ^d ± 0.1	17.4 ^c ± 0.2	34.8 ^a ± 0.4	22.1 ^b ± 0.0
	Glucose	0.3 ^d ± 0.0	0.4 ^c ± 0.0	0.5 ^b ± 0.0	0.9 ^a ± 0.0
	Xylose	2.4 ^c ± 0.3	5.1 ^b ± 0.2	2.7 ^c ± 0.1	7.3 ^a ± 0.3
	Mannose	0.6 ^d ± 0.1	2.1 ^a ± 0.1	1.0 ^c ± 0.0	1.8 ^b ± 0.0
Uronic acids (%)	Guluronic acid	2.5 ^a ± 0.1	0.2 ^b ± 0.1	0.1 ^b ± 0.0	0.2 ^b ± 0.0
	Glucuronic acid	1.3 ^{b,c} ± 0.3	3.7 ^a ± 0.4	1.1 ^c ± 0.0	2.3 ^b ± 0.0
	Mannuronic acid	60.2 ^a ± 3.2	1.2 ^b ± 0.1	0.4 ^b ± 0.3	0.5 ^b ± 0.1
Sulfate (%)		11.3 ^c ± 1.0	28.4 ^b ± 2.1	35.6 ^a ± 0.4	32.1 ^a ± 3.1

The data are given in %mol (relative level) of total carbohydrates analyzed in the fucoidan fractions

Different superscript letters a,b,c indicate statistically different values ($p < 0.05$) between the values in the fucoidan fractions

Table S5. Monosaccharide composition of *S. latissima* MMP after Mef2 hydrolysis

MMP		
Neutral monosaccharides (mol%)	Fucose	48.1 ± 0.0
	Rhamnose	1.0 ± 0.6
	Galactose	44.0 ± 3.7
	Glucose	0.3 ± 0.2
	Xylose	2.3 ± 0.3
	Mannose	1.7 ± 1.9
Uronic acids (%)	Guluronic acid	1.6 ± 1.2
	Glucuronic acid	0.7 ± 0.1
	Mannuronic acid	0.3 ± 0.5
Sulfate (%)		34.2 ± 1.8

The data are given in %mol (relative level) of total carbohydrates analyzed in the fucoidan fractions

Table S6. Monosaccharide composition and sulfate content of fractionated *S. latissima* MMP

Fractions	Monosaccharide composition	Sulfate, %
	Fuc:Gal:Rha:Glu:Xyl:Man:GuluA:GluA:ManA (mol:mol)	
MF1	1 : 0.29 : 0.07: 0.02 : 0.14 : 0.02: 0.00: 0.02: 0.02	52.1 ± 0.8
MF2	1 : 0.42 : 0.06: 0.02 : 0.12 : 0.02: 0.00: 0.01: 0.02	48.2 ± 1.9
MF3	1 : 0.56 : 0.04: 0.02 : 0.07 : 0.01: 0.00: 0.01: 0.01	52.1 ± 0.8
MF4	1 : 0.52 : 0.03: 0.02 : 0.06 : 0.01: 0.00: 0.00: 0.01	54.8 ± 2.9
MF5	1 : 0.73 : 0.03: 0.02 : 0.07 : 0.01: 0.00: 0.00: 0.01	27.8 ± 1.6
MF6	1 : 0.65 : 0.03: 0.02 : 0.06 : 0.01: 0.00: 0.00: 0.02	28.7 ± 3.3

Table S7. Monosaccharide composition of fucoidan fraction 3 from different brown seaweed species

		<i>S. polycystum</i>	<i>S. oligocystum</i>	<i>S. serratum</i>	<i>T. ornata</i>	<i>H. cuneiformis</i>	<i>S. mcclurei</i>
Neutral monosaccharides (%mol)	Fucose	46.7 ^d ± 4.5	66.4 ^c ± 3.4	81.5 ^{a,b} ± 0.8	85.6 ^a ± 0.3	72.7 ^{b,c} ± 0.0	78.5 ^{a,b} ± 1.7
	Rhamnose	0.5 ^a ± 0.4	0.8 ^a ± 0.6	0.2 ^a ± 0.0	0.0 ± 0.0	0.3 ^a ± 0.4	0.3 ^a ± 0.0
	Galactose	26.1 ^a ± 4.9	24.3 ^{a,b} ± 2.3	9.7 ^c ± 0.4	9.9 ^c ± 0.5	20.2 ^{a,b} ± 0.4	15.4 ^{b,c} ± 0.5
	Glucose	1.3 ^a ± 0.0	0.7 ^b ± 0.3	0.2 ^{b,c} ± 0.0	0.2 ^{b,c} ± 0.0	0.2 ^c ± 0.1	0.1 ^c ± 0.1
	Xylose	18.3 ^a ± 2.6	3.8 ^b ± 0.1	3.7 ^b ± 0.2	0.8 ^b ± 0.0	2.6 ^b ± 0.1	2.8 ^b ± 0.3
	Mannose	0.0 ± 0.0	1.1 ^a ± 0.0	0.6 ^b ± 0.0	0.20 ^c ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Uronic acids (%mol)	Guluronic acid	0.7 ^a ± 0.2	0.1 ^a ± 0.0	0.1 ^a ± 0.0	0.2 ^a ± 0.0	0.4 ^a ± 0.2	0.1 ^a ± 0.1
	Glucuronic acid	0.6 ^a ± 0.1	1.0 ^a ± 0.1	0.5 ^a ± 0.0	0.5 ^a ± 0.2	1.0 ^a ± 0.4	0.5 ^a ± 0.2
	Mannuronic acid	5.2 ^a ± 0.5	1.8 ^a ± 0.0	3.4 ^a ± 0.1	2.5 ^a ± 0.0	2.4 ^a ± 0.3	2.0 ^a ± 2.1
Sulfate (%)		24.6 ^c ± 0.8	27.7 ^c ± 1.8	38.1 ^a ± 4.0		29.4 ^{b,c} ± 2.3	31.4 ^b ± 2.6

The data are given in %mol (relative level) of total carbohydrates analyzed in the fucoidan fractions

Different superscript letters a,b,c indicate statistically different values ($p < 0.05$) between the values in the fucoidan samples

FWf3/213-589	ALGERAVNLRGTWN-----SEI-----LVDLLDDRKNLKTNLRLGVSFTN	41
Fp273/234-617	AAGERAVGLRGTV-----GGV-----GD---LESIKHFKTLTTMGASFSS	38
FFA2/41-433	DLGLRADWMRGSLGLLWLPENNYNGNIEGV-----SIEDFLTQIQHLKTVDFVQVGLAS	54
Fhf2/31-439	DLGLRADWMRGSLGLLWLPENNYNGNIEGV-----SIEAFLTQIQHLKTVDFIQVGLAS	54
Fhf1/29-447	NQGLRANWMRGSWAALWLPENNYNGHIEGV-----SIEPFIEQIKELETIDYIQIGLTN	54
FWf4/37-421	EQGLRANWMRGALGLLWLPERNYNGNIEGV-----EIDEFISQVKDLRTIDYVQVPLTS	54
MfFcnA/34-418	NQGLRAEWMRGALGMLWLPERTFNNGNIEGI-----RIDDFLTQIKDIRTVDYVQVPLTS	54
FFA1/31-415	DQGLRAEWMRGSGYGLWLPERNFNNGNIEGI-----RIDEFITQIKDIRTVDYVQVPLTS	54
FWf2/243-679	ALGERADWLRGTVGVTWTPSNYQNGNVEDV-----SIDDFLNQISEVKTISYIQVKLGS	54
Fp279/40-449	KITTRADWLRGSWGLNWKPTDLWNGAGEAL-----TIDLFLEQISHLKTIDYVQVHLNE	54
FWf1/15-439	NQGLRAHWLRGTWGINWKPVNLYNGGHEGL-----SIEPFLNQISHIKTIDYIQVHLGE	54
Fp277/26-435	-VESRGDWLRNSWGINWKPVDVYNGKSESL-----EIDIFLDQISHLKTIDYIQSHLGE	53
SVI_0379/26-367	-TESKAVWMEGKVGVGFRINADR---KD-NIENY-DVDTLVNQIKTIDIGISYVIFNLSD	53
Fda1/23-372	--ETKADWMQGNWGISYRIPGGDINYSGS-HVAEY-NVRAAVEQISAIPGLKWLQVNLNLTN	56
Fda2/84-439	PEDIRASWMQGNWGISFRISGGDISQNES-HVNEY-QVAPAVEQIAAIPGLKWLQVNLNLTN	58
Mef2/22-383	---DDSWLEGSWGITFPVFGGARLDAEV--AGGLDPVSGAQEIVDELSEVGHVITNLSY	54
P5A_FcnA/36-393	GIEYNASWMAGTWGITQRVDGGYKLDNSA---DSSNWQAGAEIIVTNIPAAEYVITSFTH	57
P19D_FcnA/31-393	DSEYTANWMAGAWGITQRVDGGYKL-DASVETGKYDWWAGAEIIVENIPSAGYVITSFTH	59
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FWf3/213-589	GAVLSAEHKVPKHLFDRLSG-----GDVVAPSWGDL--NSDTPDNNDNMLADI	86
Fp273/234-617	GASASQHNAPHKFLLETIALKGV---NGNGPAIDYVVPASWGDL--SLDEPESDFFLDNI	93
FFA2/41-433	PYIYSPVHTAPHPLLERLWEGDL---GPDGKPI-NLVVPRASA-----PDPFLGWL	101
Fhf2/31-439	PYIYSPVHTAPHPLLEKLWEGDL---GPDGKPI-NLVVPRASA-----PDPFLSWL	101
Fhf1/29-447	PNIYSPVHTAPHPIIESLWQGDV---DPDGNPL-NLVVPRASA-----PDPFLSWL	101
FWf4/37-421	PNIFSPVHVAPHSIIESLWEGDT---DSNGDPI-NLVVPRESV-----DDPLLRLWL	101
MfFcnA/34-418	PNIFSPTHVAPHPIIESLWQGDV---DANGDPI-NLVAPRESV-----DDPLLRLWL	101
FFA1/31-415	PNIFSPIHVPHSLIESFWEGDT---DANGDPI-NLVAPRESV-----DDPLLRLWL	101
FWf2/243-679	SYIYSPVFAAPHQVLESFWYNDGVDRDNGEPVQNLVVPWRWNG--TI--AENDPFLDWI	110
Fp279/40-449	SYINSPSHLGTHELLESFWQGDV---SPLGTPM-NLVVPRAST-----GVDPFLEMI	102
FWf1/15-439	SSIKSSVHMGPMSLLESFWEGDT---DANGDPI-NLVVPRASY-----GEDPFLEIV	102
Fp277/26-435	SSIKSSVHIAPHELLESFWQGDV---DATGNPV-NLVVPRASA-----NVDPLFKIL	101
SVI_0379/26-367	AAH-GDAYLAPHSIL-----TTLNPDSTPNAD-----RDLFGELA	87
Fda1/23-372	GAS-GDRFIVPVTEV-----EAINPLSAPNSINDLYDPTLPGRDLFEQLA	100
Fda2/84-439	GAF-GDRFIVPVPEV-----EAINPNSAPNSADLFDPALPGDDLEFQIA	102
Mef2/22-383	FAH-SHYFTLSQNNYINIR-----NEIHPDVVP-----SAANDDIIFDVL	93
P5A_FcnA/36-393	PAH-GHLFTLRTNNNVDV-----SAIHPDMVP-----TLENEKIIFDVI	95
P19D_FcnA/31-393	PAH-GFLYTLRDNENVDV-----AAIHPDMVP-----SLENEKIIFDVI	97
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FWf3/213-589	LKIKAAGFNVKAYTNSENFVGT-----	108
Fp273/234-617	LAIKAAAGFKIKAYLNSENFLGS-----	115
FFA2/41-433	KAIKAAAGLKTEVYVNSANLLWEAF-----G-----	127
Fhf2/31-439	KAIKAAAGLKTEVYVNSANLLQWEAF-----N-----	127
Fhf1/29-447	KAIKAAAGLKTEIYVNSYNLLARVP-----D-----	126
FWf4/37-421	KALKAEGLRVEIYVNSYNLLARNP-----E-----	126
MfFcnA/34-418	KALRAAGLRTEIYVNSYNLLARIP-----E-----	126
FFA1/31-415	KALRAAGLKAEIYVNSYNLLARIP-----E-----	126
FWf2/243-679	TKIKAAAGLKQVYVNSSNMLQRYSDSYEDGRI-----	142
Fp279/40-449	KKSQKAGLKQVYVNSSNLLTRG-A----TF-----	128
FWf1/15-439	KAIRAAGLKVMYVNSSNMLSREGP-----GG-----	129
Fp277/26-435	KKVKAAGMKNQVYVNSSNMLQNES-----T-----	126
SVI_0379/26-367	KAFHAAGIKVIAAATQGPAML-----KHGAAYAFDGVQD-----	122
Fda1/23-372	LAFKAKGIRVVAYIATQGPAML-----KHGAENSMDDEDSIT-----DCKSSKPLVTDL	149
Fda2/84-439	LGLQAKGIKVAYIATQGPAML-----KHGAERSMDFDSDIVDES DGSACKSSRPVSDP	157
Mef2/22-383	DIFKNSDKKIILYISTQYFAR-----ADEDN-----	119
P5A_FcnA/36-393	NIYRAAGKKVILYLSAGPSMA-----EERGD-----	122
P19D_FcnA/31-393	NVYKSAGKKVLLYLNATGPTHA-----ADRNS-----	124
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FWf3/213-589	-NADYLQPFVDSWKEYCDTDPVQAFINSQPYHTGIWNRTEQYEDATATYPNRKYMFCY	167
Fp273/234-617	-NQDHLQEFVDRWKEYCDNDVAVQAFINSQPFHTSVWDTETQTYVDATETYPNRKYMFCY	174
FFA2/41-433	TPISEFPDFSERWKAYCDTPTMQAFIESQPYHKDGVN-----DDRRPYMFCY	175
Fhf2/31-439	GAPSEFPDFSNRWKAYCDTPTVQAFINSEAYHKDGVN-----DDRRPYMFCY	175
Fhf1/29-447	GIPAGYDLSERWEEYCNTNATAQAFIKNNPYISED-D-----PERRKYMFCY	173
FWf4/37-421	DTQTEYPDVSDRWMEWCNTNAAEQAFINTQTYHN---G-----SGRRYMYFCY	171

MfFcnA/34-418	DTQADYDPDVSARWMEWCDTNTNEAQAFINSQTYHE---G-----NGRRKYMFCY	171
FFA1/31-415	DTQEDYDPDVSERWMEWCDTNPNEAQAFIASQDFHE---G-----DGRRKYMFCY	171
Fwf2/243-679	PSPNELPDVTNRWKAYCDANY--TTFINSQPYHTGIYNSATGNYVNSEAEFFPERKYVFCF	200
Fp279/40-449	SNPASFPDITDRWKEWVDTNPEAQAFIHSQPYHTGIWDAESETYVDASETYPERKYMFCY	188
Fwf1/15-439	SNPDYIPNITERWKEWCDTNAEAQAFIASQPYHTGVWDETSKTYINSETEFPERKYMFCY	189
Fp277/26-435	PNPDIIPNITERWKAQVWDSAEAAQAFIASQPYHTGTWDADTQSYVDATATYPERKYMFCY	186
SVI_0379/26-367	ANGHWSSKSMTNWAKWVTNKYGSASDDN-----YKKAY	155
Fda1/23-372	DTQVYCSANMNRWRDYVLEQYPSTSLYR-----SFELA	182
Fda2/84-439	DTQVYCSANMNRWRDYVLEQYPSTSLHH-----SFQLG	190
Mef2/22-383	-----IDIKTNWDQFVTQYP-----SQNDA	139
P5A_FcnA/36-393	-----TDIQAADWEYINWEDG-----DEAAA	144
P19D_FcnA/31-393	-----PEIQDAWDDYVNTNWNG-----DHGAA	146
	* :	
Fwf3/213-589	AEYFLKDYALRYGEHFDSWIFDDGATM--EQNGDNATSGVVEEQRIYQAYANAVHAGNPD	225
Fp273/234-617	AEFVLKDYALRYAKYIDSWIFDDGGTM--VENGDNATSGLVVEEQRIYQAYANAVHAGNPE	232
FFA2/41-433	AEFVLKEYAMRYGDLIDAWCFDAAHVNM--GAAGDDYSTGIDTQRVFQAFADACHAGNPD	234
Fhf2/31-439	AEFILKDYAMRYGDLIDAWCFDAAHVNM--ASAGDDYSTGNIDTQRVYQAFADACHAGNPN	234
Fhf1/29-447	AEFILKEYSERYGDLIDAWCFDSADNIM--GACGDEPQSDDVNHQRIYQAFADACHAGNPN	232
Fwf4/37-421	AEFILKEYAIRYGDLIDAWCFDSADNIMEDECGDNPDSENVEDQRIYQAFANAVHAGNPN	231
MfFcnA/34-418	AEFILKEYAQRYGDLIDAWCFDSADNIMEDECGDDPASEDVNDQRIYQAFADACHAGNPN	231
FFA1/31-415	AEFILKEYANRYGDLIDAWCFDSADNIMEGECGDDPASDDIDDQRIYQAFAEACHSGNPK	231
Fwf2/243-679	AEYILKEYATRYGDLIDAWVFDSGEFM--VSNGDDSESGILEEQRLYEAFANAVHAGNEN	258
Fp279/40-449	AEFVLKEYAVRYGNLIDGWLFDSGDFM--KKNGDNATNGVKAQQIYKAFADACHAGNPM	246
Fwf1/15-439	AEFVLKEYAIRYGDLIDAWCFDSGSWM--GMNGDSQTNGIYEDQMIFNAFKAACHAGNPD	247
Fp277/26-435	AEFVLKVYSERYGDLIDGWLFDSGSFM--YSNGDSATNGVKEDQSLYGAFAKAAQAGNPN	244
SVI_0379/26-367	AEVIAEYARQRYGSLIDGWVFDHSSCA-----NIPLLHKITKANPA	197
Fda1/23-372	MVNIVETLSLRYGSTIDGWVFDHSGFG-----DSELLHAAALAGNND	224
Fda2/84-439	LVNIVETLSLRYGTLIDGWVFDHSGFG-----DYNLLPDAARAGNSN	232
Mef2/22-383	YVELLRGFVEYLKDYADGYWLDTVTA-----NVEDIVDMIREVDPT	180
P5A_FcnA/36-393	WRNLARGYVERFDGLVDGYWLDNSRNLN-----G--EVSDVFAMLRSDVDE	188
P19D_FcnA/31-393	WRNLVEGYAKRFKGLVDGYWLDNSKNMA-----GGQKEIPEFVAMLRDIDPS	193
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Fwf3/213-589	IAIAFNNGRSTVN---Y-----KD--YPFAHAVRFEDFTFGHA	258
Fp273/234-617	IPIAFNNGRSTTA---Y-----AS--TPFAHAVRFEDFTFGHA	265
FFA2/41-433	AAITFNNIGDRDS---D-----PF--VPYVTPSLFEDYKFGHP	268
Fhf2/31-439	AAITFNNIGDRDS---D-----PF--LPYRTPSLFEDYKFGHP	268
Fhf1/29-447	AAISFNNVSGDREL---N-----PF----STATYFDDYTFGHP	263
Fwf4/37-421	AAVAFNNVSGDRVE---N-----PF----STATLFDDYTFGHP	262
MfFcnA/34-418	AAIAFNNVSGDREG---N-----PF----TSATLFDDYTFGHP	262
FFA1/31-415	AAISFNNVSGDREG---N-----PF----TTATYFDDYTFGHP	262
Fwf2/243-679	AAVAFNNVGNPNRHEANAANG-----TV--TPYSHATHFDDFMFGHP	296
Fp279/40-449	AAISFNNVSPNRVT---E-----SL--NPFSEATHYDDYFMFGHP	279
Fwf1/15-439	AALSFFQNSPERDT---E-----EL--NPFSEAVHADDYFMFGHP	280
Fp277/26-435	APVSFNNVSPERDT---E-----EL--NPFSEATHYDDYFMFGHP	277
SVI_0379/26-367	VILAFNNKGQKVPLI-----NNNPGYEDYTSFGHP	225
Fda1/23-372	AAVAFNNEGDKVPLV-----NNPETLDDYTFGHP	252
Fda2/84-439	AAVSLNLEGDIFLS-----NNPEVMEDFTGGHP	260
Mef2/22-383	AVVTTNKGKDYIRLDGTKAVVASNVSITGETSPKVGYNIRHTLDVASTTQDFTNHGV	240
P5A_FcnA/36-393	LTIAVNYDQHYFTDDNGEYLYVDSGLDDEDE---SDYKIVKHVV--TNEYMDFTNGHV	242
P19D_FcnA/31-393	FAIGVNYETHYFEDEDGNYGLKVASDSIDNDND---REYKIIKHVV--TNEYMDFTNGHV	247
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Fwf3/213-589	FGGNNNHAEKI-----N--GNQFNLNRYHITRMTETNGLVHAG-----	294
Fp273/234-617	FGGNNNHAEKE-----N--GNQFNNYRHVTRMTETNGYVHAG-----	301
FFA2/41-433	FGGAGNMVEPR-----DPLYTVNFGICEYMRDNTNGHPYTN-----	303
Fhf2/31-439	FGGAGNMVEPR-----DPLYTVNFGICEYMRDNNGLPYTN-----	303
Fhf1/29-447	FGGAGNMVEPR-----DPLYTYNYHVIEWMSDYNGSAFLVESVNKEISTDGNKILI	314
Fwf4/37-421	FGGAGNMVEN-----ETLYRYNYHVIEWMDYAGDAFLD-----	296
MfFcnA/34-418	FGGAGNMVVP-----EALYTYNHDLVVFMQTNNGYAFRD-----	296
FFA1/31-415	FGGAGNMVEN-----ETLYGYNFGVIEWMDYNGSAFLD-----	296
Fwf2/243-679	YNGGKSIGKDKADYYDTGAYPTNYGRNYAHMLWMQGTGGNVHLYR-D-----SSNPV	347
Fp279/40-449	YNGGTFIGNHD-----N---TNYDRNYRHQKVSETNGNIHSGSLD-----AGTDS	322
Fwf1/15-439	YNGGRDGGSH-----IGTPLYSRNYAHIEKMKETNGYAH-----GSDP	321
Fp277/26-435	YNGGRVIGNHE-----N---GLYERNYAHQKITATNGNVHSGSVA-----AGTDA	320
SVI_0379/26-367	TPLRRSTVS-ND-----NLP---MVESIEASSNGYV-----	252
Fda1/23-372	TPIGSEVSSDDK-----NLP---MLTSIEATLDGIL-----	280
Fda2/84-439	TPIARVSSDDT-----NLP---MLTAIEDAPNGIF-----	288
Mef2/22-383	TSII-KAPTDSW-----GYEFTVGDMIPEPYTE-----	268
P5A_FcnA/36-393	TPLGRGAPPNSW-----AYEETIPDMIEVPWET-----	271

P19D_FcnA/31-393	TPMQQGAPPNSW-----GYEETIPHMIEKPWDS-----	276
FWf3/213-589	GNWDWDDKIVGNFHSKLSSTAWKYGPN-QAWEQADFNQWNLEAIQAGGSMTWGGSFNRA-	352
Fp273/234-617	GNWEWDDRIVGNFHSKLSSTAWTYGPN-QAWEEDFLQWNLEAMHAGGMSWDGSRPRT-	359
FFA2/41-433	DDIDWNDNVVAHFFPKQSTTSWNDGGT-PCLTDEEFVEWNNVGLINGGGITWGTPLVITN	362
Fhf2/31-439	DGIDWNDNVVAHFFPKQSTTSWNDGGT-PCLTDGEFVEWNNVGLINGGGITWGTPLVITN	362
Fhf1/29-447	ENRAWNDNVVSHFFPKQSATSWNAGNT-PCLTDEEFVEWTSTGIIDGGAITWGTPLVRTN	373
FWf4/37-421	DTRDWNKVVSHFFPKQSSTSWNAGST-PCLTDEQFVEWTTNGIIDGGAITWGTPLIRTN	355
MfFcnA/34-418	DTRTWNDNVVAHFFPKQSTTSWNAGNT-PCLTDEQFVEWTSTGIVNGGGITWGTPLVRTN	355
FFA1/31-415	DDRTWNDNVVSHYFPKQSATSWNAGNT-PCLTDDQFVEWTSKGIIDGGAITWGTPLIRTN	355
FWf2/243-679	DEWEFDNKVVGGFYPPMSTTSWQGGAT-QALEQVDFELWNQVAIDHGGTIIWGTALEIYN	406
Fp279/40-449	QNWDWDDKVVGHFDPMPMSRTRWNSGAD-PGLSDEDFLLWNYESAVGGGAISWGAPLYSP-	380
FWf1/15-439	QTWTWDDNVVAHYDPPMSTTSWNGGNT-PALTDEEFNLWNLEAVQNGGAISWGLPLVKK-	379
Fp277/26-435	HDWDWDDKVIHGFDPMPMSTTSWNGGNT-PALTDDEFNLWNLESAGGGGAISWGLALVGK-	378
SVI_0379/26-367	D-DGAGHKSLGHMFPLG-NTWNSGL--SVWPLSKAVDWITRVNNACGAWTWNVATQDTN	308
Fda1/23-372	TGSGDDVGSVGHMFPLQ-ESWNGGT--VVFSEAKGSDWLNRLKAGGAFTWALSQDSND	337
Fda2/84-439	TGTGDDVDALGHMFPLQ-ETWNGGT--VVFSEAKGTEWLNRVTRAGGALTWALSHEG--	343
Mef2/22-383	--YLGNP-VIKHAWFPVR-YRWHSTRELLYDTERS YRTVKSIIIDAGASVTLAN TVDYNT	324
P5A_FcnA/36-393	--YDGSKYALKHGWFPPIR-NSWSGSKAELMFDEQAYRFVRTVTDGGAAMTWSTTQ--DN	326
P19D_FcnA/31-393	--VDGNHYALMHGWFPPIR-FSWSGSGAELMFETE QAYRFVRTITD GGAAMTWSTTQ--KK	331
	* .	
FWf3/213-589	-----ETAI-YDWVYVLLLEGLDDYLVQYGNP-----	377
Fp273/234-617	-----SDDL-YTWAYDLLKGLDDHLAKYQKP-----	384
FFA2/41-433	LNNASANLTL-QPYALRQLELVDADLSANQFP-----	393
Fhf2/31-439	LNNASPNLTL-QPYALRQLELVDADLSVNQYPGAPNWARQYTVLPEII	409
Fhf1/29-447	LNN-APDLTL-QPYALRQLALTDEYLKQNFPGAPNWARQTRLSEVI	419
FWf4/37-421	LEN-SPELVL-RDYALTQLTLTDDFLKEFQYP-----	385
MfFcnA/34-418	LEN-APVLTTL-QPYALNQFELTDTYLKEFQSP-----	385
FFA1/31-415	LEN-SPVLTTL-RDYAVTQFELTDAYLKEFQSP-----	385
FWf2/243-679	LNNANPSLIA-RDWAIQIQGADDYFASLYNS-----	437
Fp279/40-449	PG-TGEQLLI-REWGMAQLELIDADLAERQEP-----	410
FWf1/15-439	SG-TNEQLVG-TDWALAQLNGMDAHLMELEAPGAPNWSRQETVLSEAK	425
Fp277/26-435	SSNDNAKLVA-TDWALAQLTGMDAHLAQLQEP-----	409
SVI_0379/26-367	SKLADHAITFMNDVTAKIIISNGGTRCTVTIDPS-----	341
Fda1/23-372	ELGGGGARLI-SE-----P-----	350
Fda2/84-439	SVSGGEAMLI-SA-----P-----	356
Mef2/22-383	GRMPPEMVMKEVNRRLLL--NEDCEAYTRPAGARLEGE-----	362
P5A_FcnA/36-393	GYMTADEMSIMIEISNRMTQTPKPDYSVYERP-----	358
P19D_FcnA/31-393	GYMSADEMDIMIEINNRMTPQAPKLDYEAYERP-----	363

Figure S1. Multiple alignment of the catalytic domain (D1) of the fucoidanases Mef2 with other GH107 family members. The conserved catalytic amino acid aspartate (red) and histidine (blue) are indicated. Conserved amino acids around the -1 subsite are indicated purple. Changes in conserved positions (orange and underlined) are indicated. Mef2 (GenBank: ON099398), Fhf1 (RefSeq: WP_066217780), Fhf2 (RefSeq: WP_066217784.1), Fhf3 (RefSeq: WP_066217796.1), FFA1 (RefSeq: WP_057784217.1), FFA2 (RefSeq: WP_057784219.1), Fda1 (GenBank: AAO00508.1), Fda2 (GenBank: AAO00509.1), MfFcnA (GenBank: CAI47003.1), P5AFcnA (GenBank: AYF59291.1), P19DFcnA (GenBank: AYF59292.1), SVI_0379 (GenBank: BAJ00350.1), FWF1 (GenBank: ANW96097.1), FWF2 (GenBank: ANW96098.1), FWF3 (GenBank: ANW96115.1), FWF4 (GenBank: ANW96116.1), Fp273 (GenBank: AYC81238.1), Fp277 (GenBank: AYC81239.1), Fp279 (GenBank: AYC81240.1).

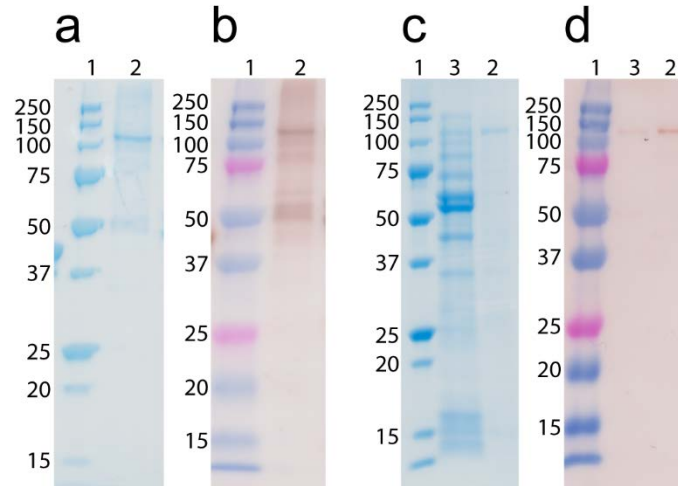


Figure S2. Recombinant expression and purification of the Mef2 fucoidanase. SDS-PAGE of a) purified Mef2 (2)(expression at 20 °C, O/N) and c) crude (3) and purified Mef2 (2)(expression at 37 °C, 4h). Western blot of b) purified Mef2 (2)(expression at 20 °C, O/N) and d) crude (3) and purified Mef2 (2)(expression at 37 °C, 4h). 1) Protein standards.

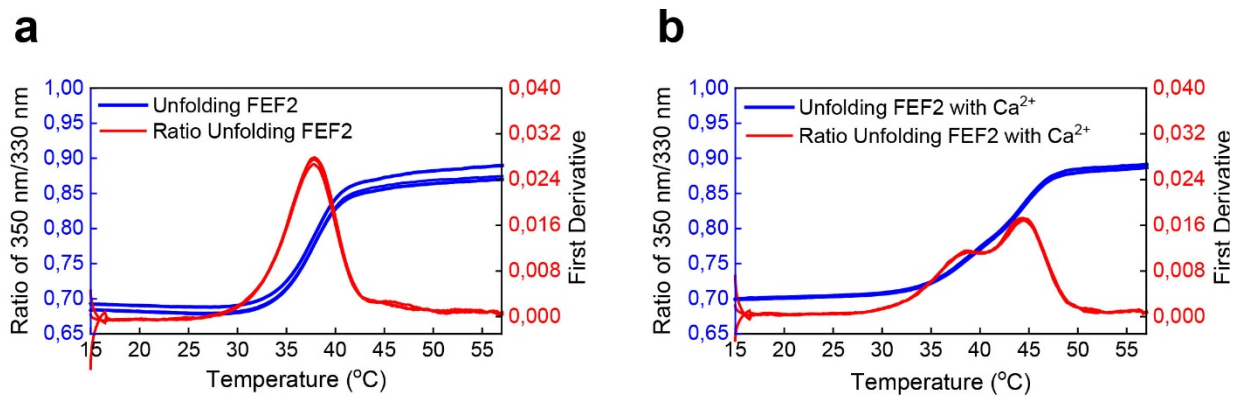


Figure S3. Thermostability assessments of Mef2 by DLS. a) Thermostability of Mef2 without Ca^{2+} and b) with Ca^{2+} .

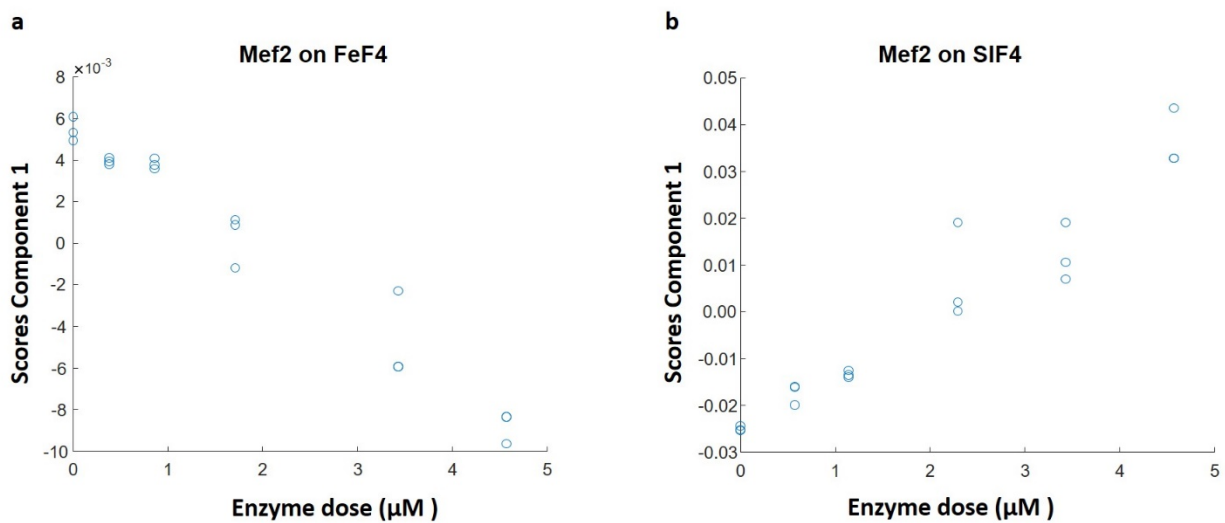


Figure S4. PARAFAC first component scores versus enzyme dosage were plotted to build calibrations. a) for Mef2 on *F. evanescens* FeF4 fucoidans with $R^2=0.97$, b) for Mef2 on *S. latissima* SIF4 fucoidans with $R^2=0.98$.

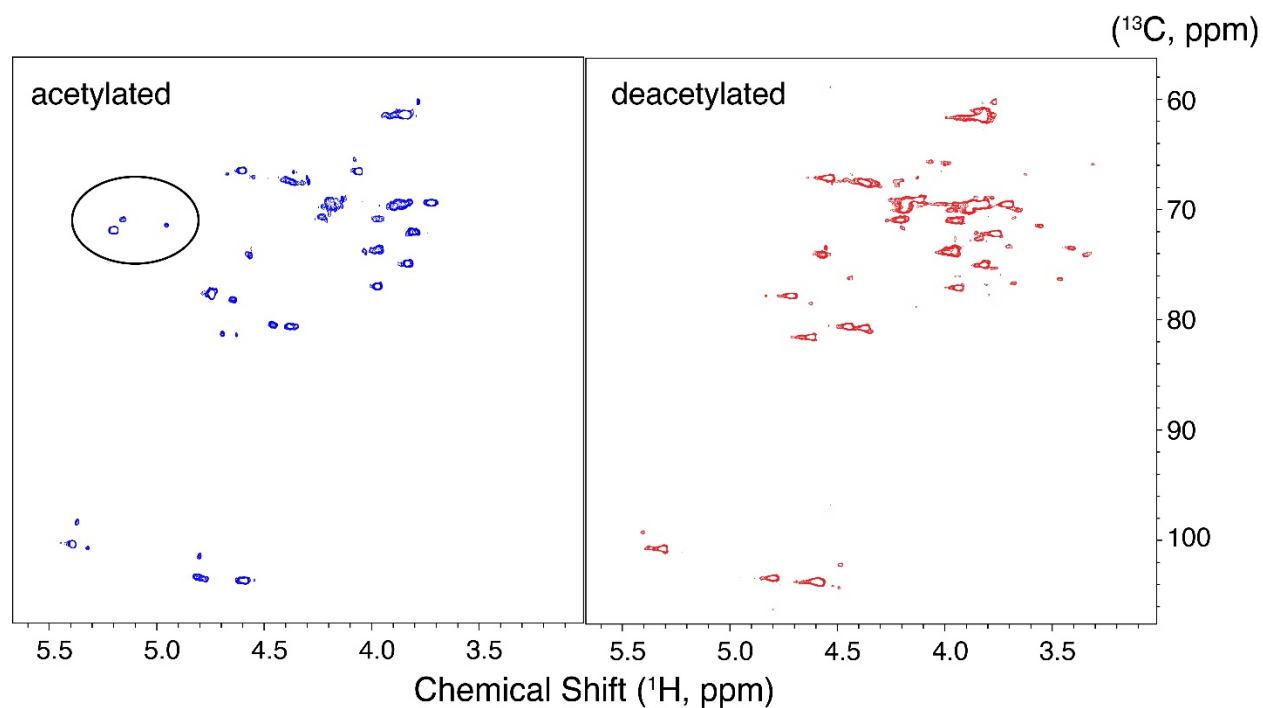


Figure S5. NMR analysis of deacetylation of *S. latissima* fucoidans. Circle indicates acetylations, which were gone after the deacetylation process.

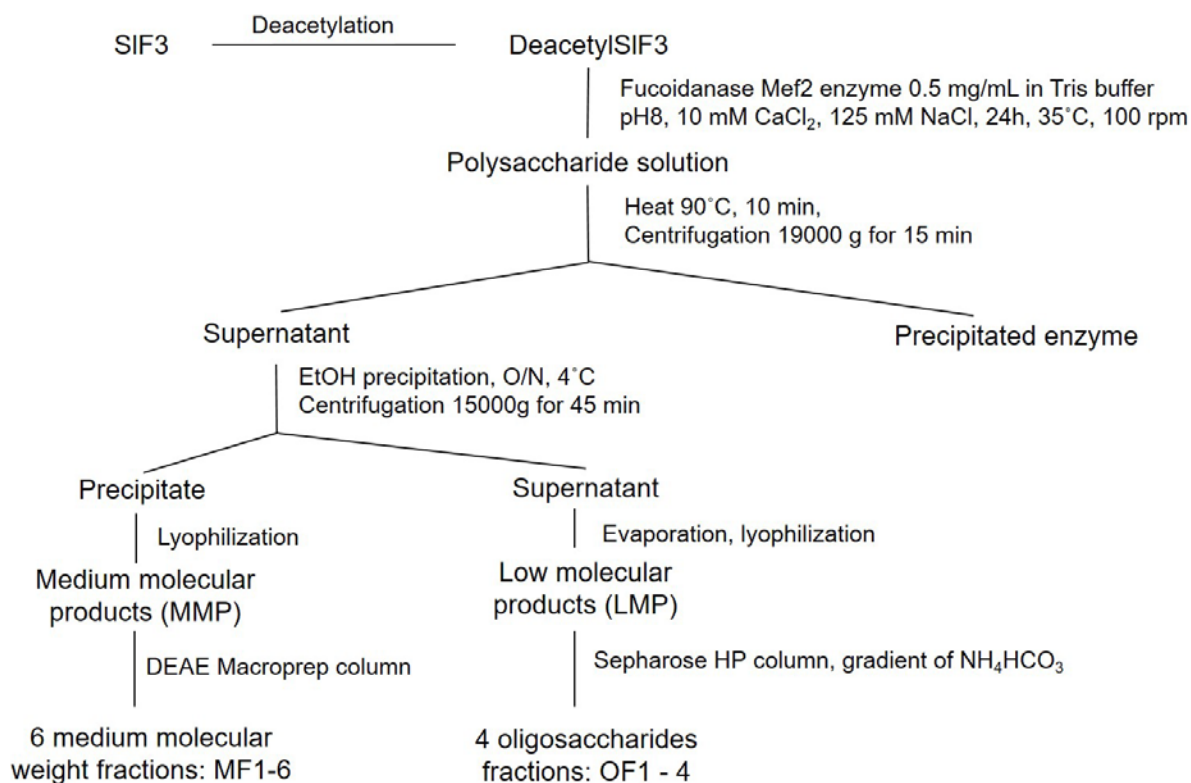


Figure S6. Flow-chart of enzymatic hydrolysis of fucoidans from *S. latissima* and further product separation

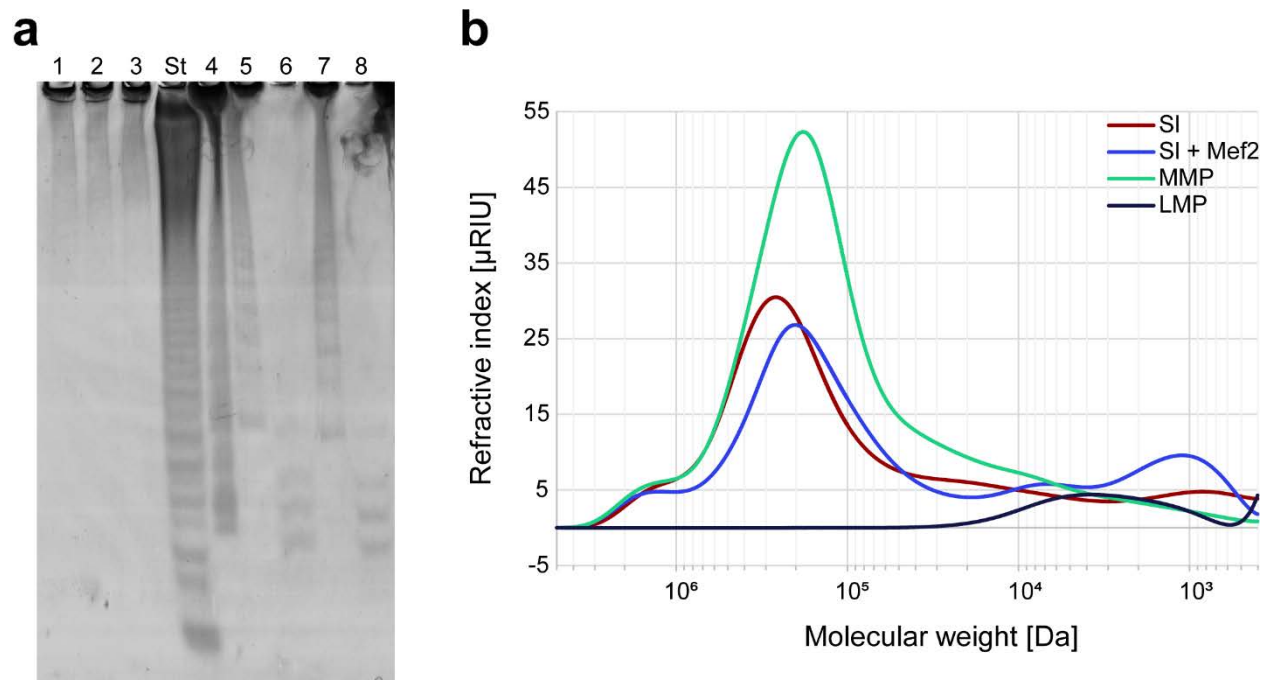


Figure S7. Medium and low molecular weight fucoidans after Mef2 hydrolysis of fucoidans from *S. latissima*. a) 1) DeSIF3; 2) DeSIF3 + heat 90 °C, 10 min; 3) DeSIF3 + heat 90 °C, 1 hour; 4) DeSIF3 + Mef2; 5) MMP from DeSIF3 + Mef2; 6) LMP from DeSIF3 + Mef2; 7) MMP (DeSIF3 + Mef2) + Mef2; 8) LMP (DeSIF3 + Mef2) + Mef2. The standard (St) is the product profile of FFA2 treatment of fucoidan from *F. evanescens*. b) HP-SEC chromatograms of MMP and LMP of Mef2 on fucoidan from *S. latissima*.

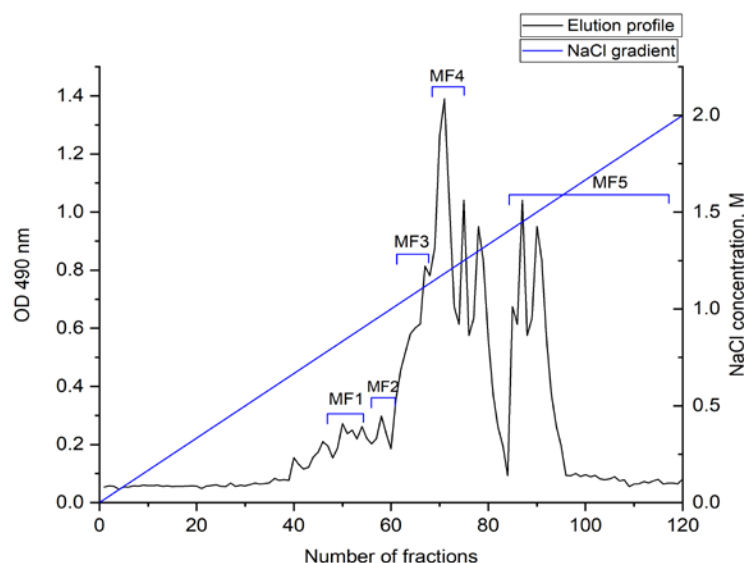


Figure S8. Separation of *S. latissima* MMP products on DEAE macrorep column. The total sugar content was detected by the phenol-sulfuric acid method.

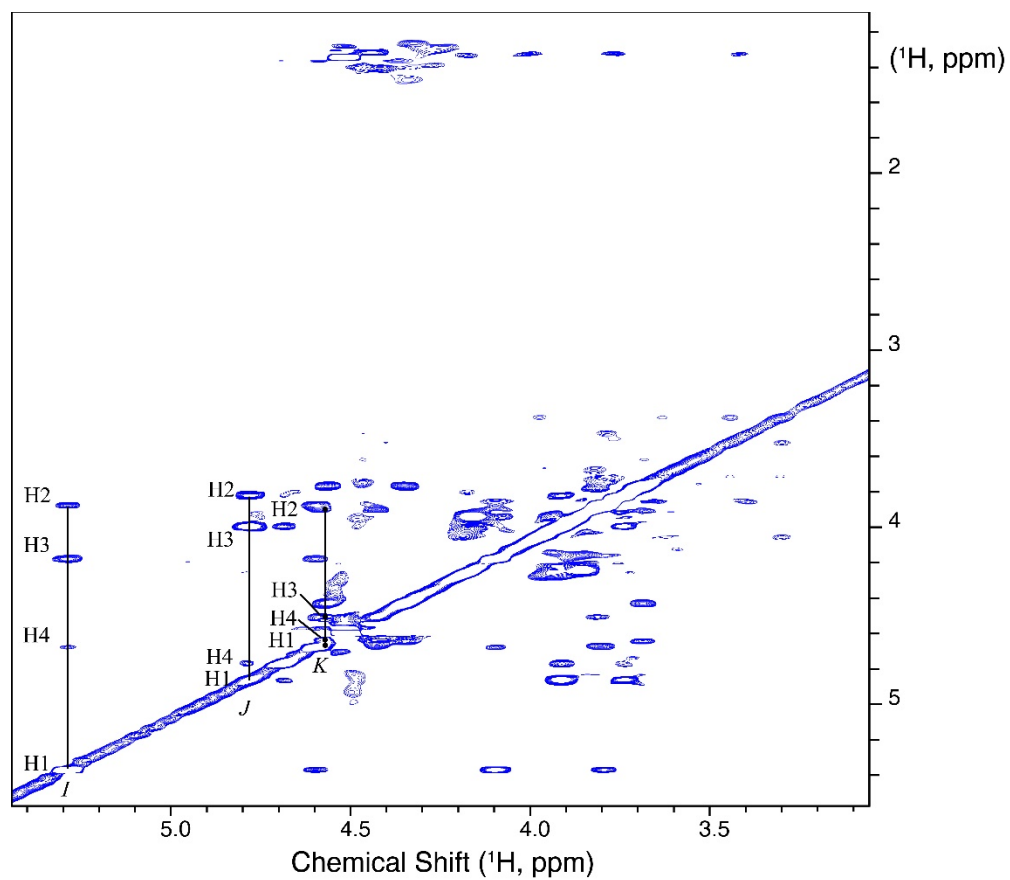


Figure S9. ^1H - ^1H TOCSY NMR spectrum for the deacetylated *S. latissima* MMP. The spectrum shows the correlations between H1, H2, H3 and H4 for the three spin systems I, J and K in the MMP described in Figure 7d and in Table 3.

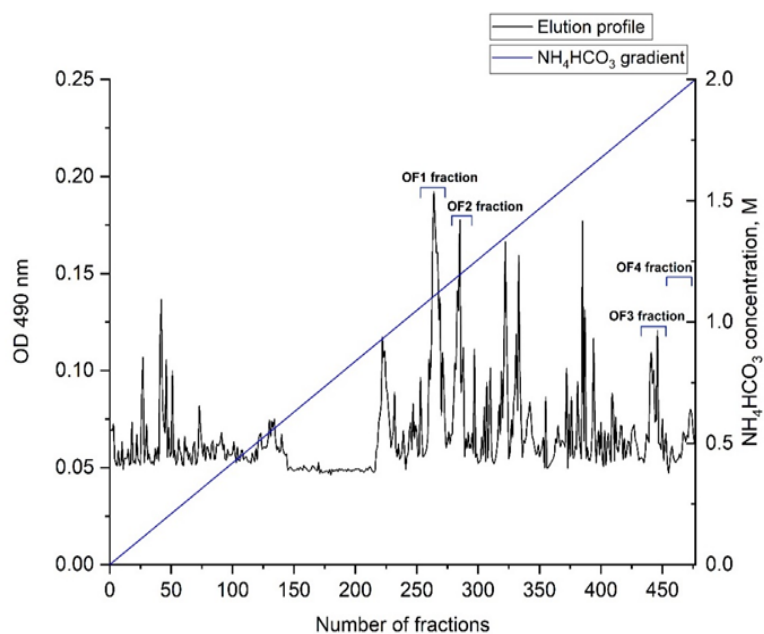


Figure S10. Separation of *S. latissima* LMP products on Q sepharose High Performance column. The total sugar content was detected by the phenol-sulfuric acid method.

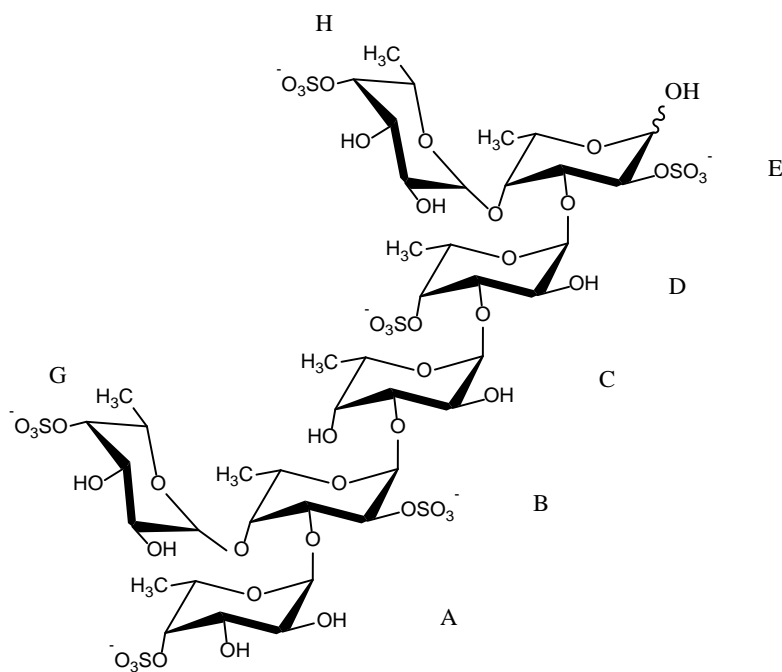


Figure S11. The proposed structure of the *S. latissima* OF2 fraction

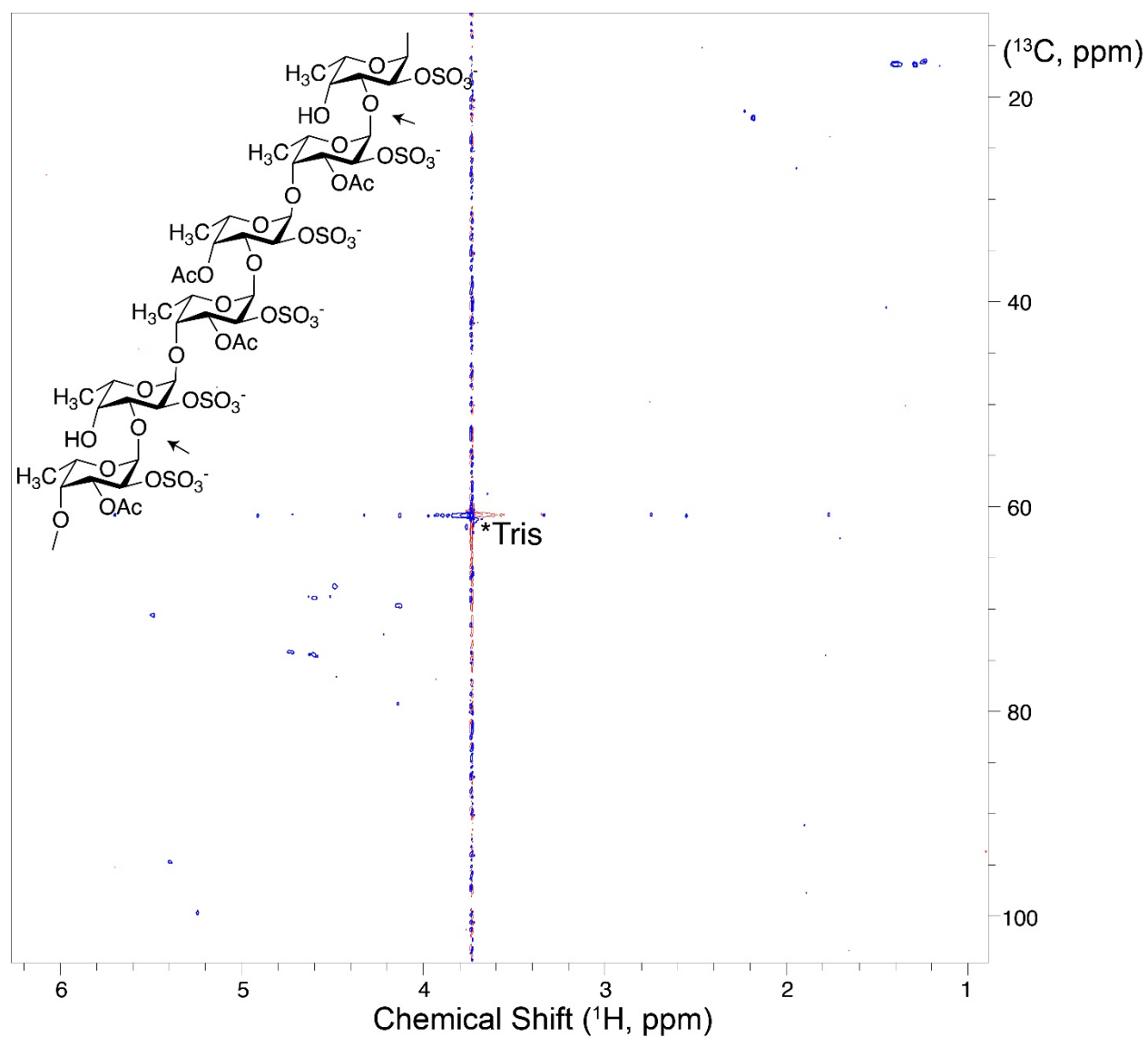


Figure S12. ^1H - ^{13}C NMR spectra of LMP from *F. evanescens* fucoidan after Mef2 hydrolysis. Upon cleavage of *F. evanescens* fucoidans, the reducing end is not visible due to limitations with regards to material and due to length of the product. Arrows indicate possible Mef2 cleavage sites.

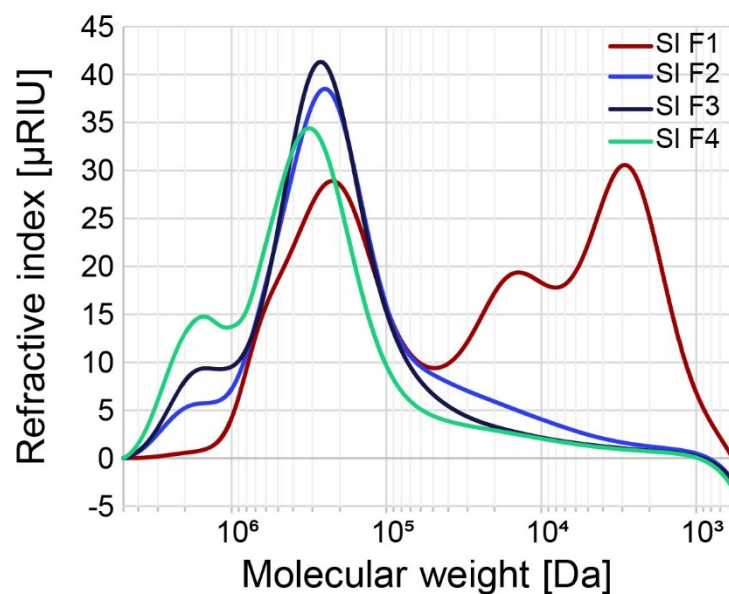


Figure S13. HP-SEC chromatogram of fucoidan fractions from *S. latissima* from Ocean Rainforest after IEX purification. The SIF1 fraction contains low molecular weight compounds (likely partially degraded alginates) of around 2 - 12 kDa and a proportion of HMP compounds between ~200–600 kDa. The SIF2 and SIF3 contain HMP fucoidan compounds ranging from ~250 kDa to over 800 kDa. The SIF4 contain more HMP compounds ranging from ~300 kDa to over 800 kDa. Pullulan was used as standard.