Supplementary Material

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Characterization of the glycans involved in sea urchin *Paracentrotus lividus* reversible adhesion

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Table S1 - Summary of the dilutions of the 22 lectins and fluorophores used in lectin histochemistry and lectin blotting, as well as, inhibitory (hapten) monosaccharide concentrations used in control experiments in enzyme linked lectin assays**.**

Lectin	Tube Foot sections /protein extracts				Adhesive secretion		Inhibition	
	Lectin histochemistry		Lectin blotting		Lectin histochemistry		Enzyme-linked lectin assay	
	Lectin (µg/ml)	Alexa 488 strep.	Lectin (µg/ml)	HRP strep.	Lectin (µg/ml)	Alexa 488 strep.	Hapten monosaccharide	Success
GSL II	3.5	1:200	1	1:5000	35	1:200	Chitin Hydrolysate 1:4	No
Con A	2.5	1:200	1	1:5000	3.5	1:200	α-Methylmannoside 0.4 M	Yes
LCA	2	1:200	1	1:5000	5	1:200	α-Methyglucoside 0.2 M	No
PSA	1.5	1:200	1	1:5000	5	1:200	α-Methyglucoside 0.2 M	No
PHA-E	2	1:800	1	1:5000	25	1:200	Acetic acid 100 mM	Partial
PHA-L	35	1:200	1	1:5000	40	1:200	Acetic acid 100 mM	Partial
DBA	1	1:200	1	1:5000	3	1:200	N-Acetylgalactosamine 0.4 M	Partial
VVA	2	1:200	1	1:5000	3,5	1:200	Not tested	
ECL	2	1:800	1	1:5000	3.5	1:200	Lactose 0.4 M	No
SNA	2	1:800	1	1:5000	3,5	1:200	Sialic acid 0.4 M	Yes
MAL II	3.5	1:200	1	1:5000	3,5	1:200	Sialic acid 0.4 M	Yes
WGA	2	1:800	1	1:5000	20	1:100	N-Acetylglucosamine 0.2 M	Yes
sWGA	3	1:800	1	1:5000	1	1:100	Chitin Hydrolysate 1:4	No
STL	3.5	1:200	1	1:5000	3.5	1:200	Chitin Hydrolysate 1:4	Partial
DSL	1	1:100	1	1:5000	10	1:800	Chitin Hydrolysate 1:4	No
LEL	3.5	1:100	1	1:5000	3.5	1:100	Chitin Hydrolysate 1:4	Partial
Jacalin	3.5	1:200	1	1:5000	1	1:100	Galactose 0.4 M	No
PNA	3,5	1:200	1	1:5000	10	1:200	Galactose 0.2 M	Yes
SBA	35	1:200	1	1:5000	30	1:200	Galactose 0.2 M	No
GSL I	3.5	1:200	1	1:5000	3.5	1:200	Galactose 0.2 M	No
RCA I	3	1:800	1	1:5000	15	1:200	Galactose 0.2 M	No
UEA I	3.5	1:800	1	1:5000	3	1:200	Fucose 0.4 M	Yes

Figure S1. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 3 that detect mannose, D-glucose and N-acetylglucosamine. Lectin histochemistry images obtained by fluorescence microscopy using ConA (**A**), LCA (**B**) and PSA (**C**). The disc surface is oriented downwards in all the pictures. Abbreviations: Con A, Concavaline A; LCA, *Lens culinaris* agglutinin; PSA, *Pisum sativum* agglutinin.

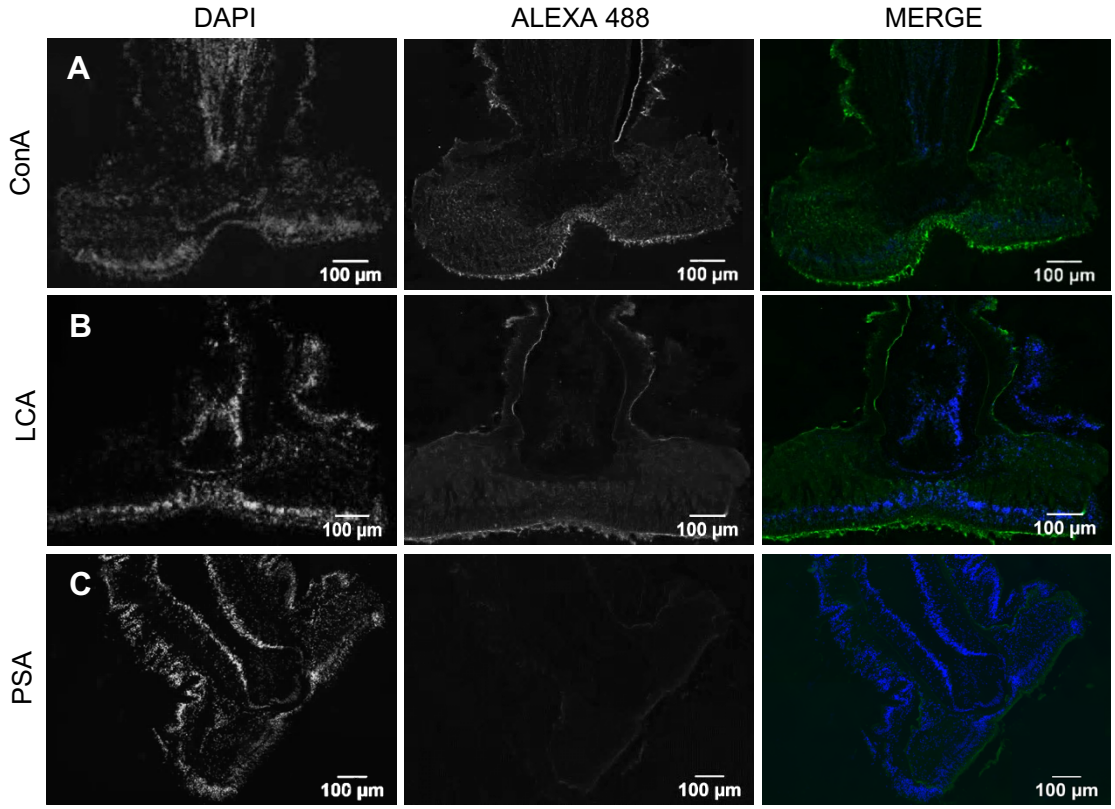

Figure S2. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 2 lectins that detect galactose, N-acetylglucosamine and mannose. Lectin histochemistry images obtained by fluorescence microscopy using PHA-E (**A**) and PHA-L (**B**). The disc surface is oriented downwards in all the pictures.Abbreviations: PHA-E, *Phaseolus vulgaris* erythro agglutinin; PHA-L, *Phaseolus vulgaris* leuco agglutinin.

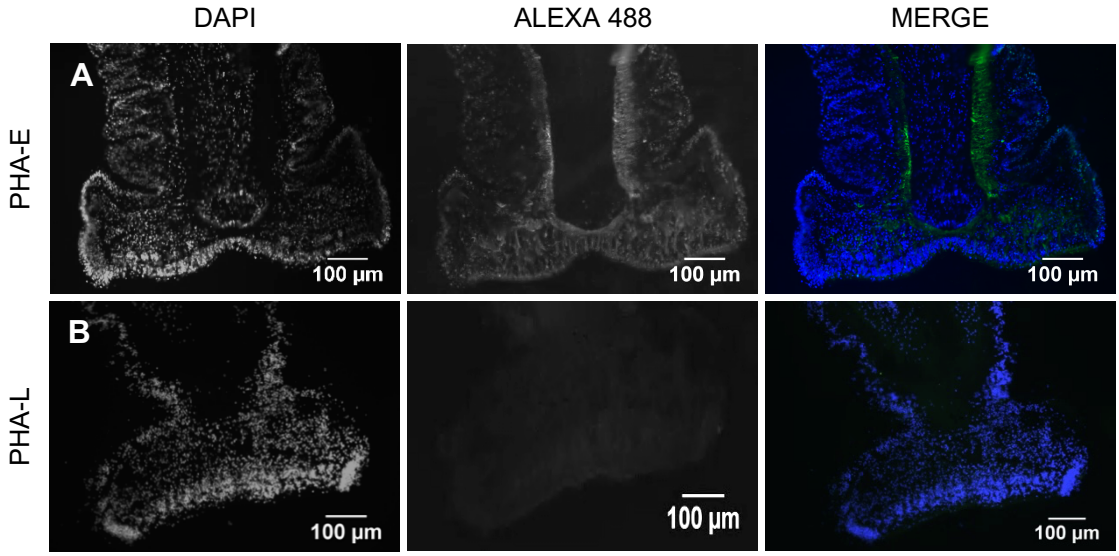

Figure S3. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 2 lectins that detect N-acetylgalactosamine. Lectin histochemistry images obtained by fluorescence microscopy using DBA (**A**) and VVA (**B**). The disc surface is oriented downwards towards the right in all the pictures. Abbreviations: DBA, *Dolichos biflorus* agglutinin; VVA, *Vicia villosa* agglutinin.

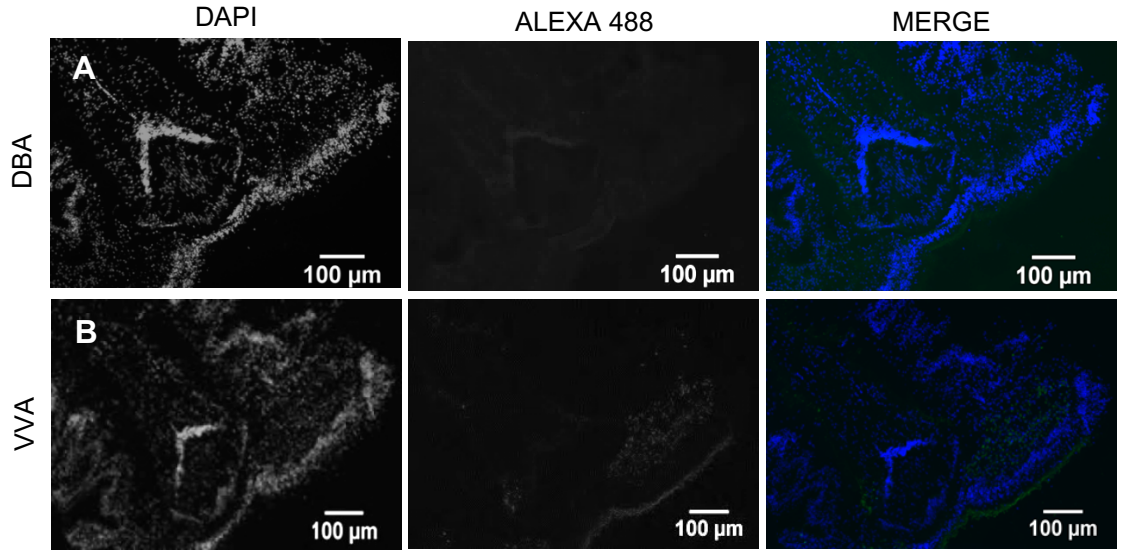

Figure S4. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 1 lectin that detects N-acetillactosamine and N-acetylgalactosamine. Lectin histochemistry images obtained by fluorescence microscopy (**A**). The disc surface is oriented downwards towards the right in all the pictures. Abbreviations: ECL, *Erythrina cristagalli* lectin.

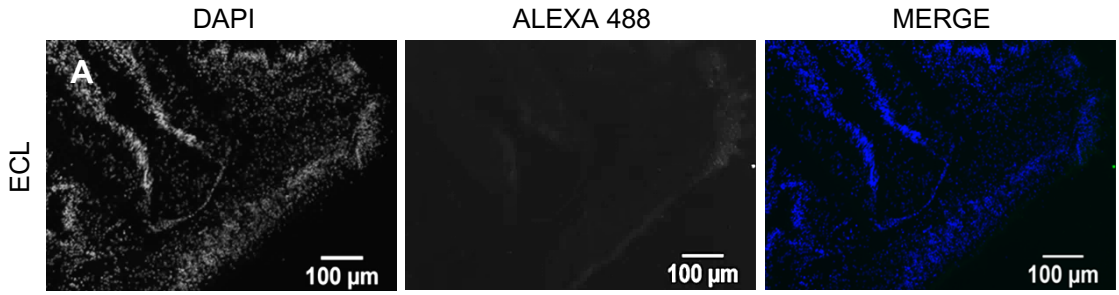

Figure S5. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 2 lectins that detect sialic acid, galactose and N-acetylgalactosamine. Lectin histochemistry images obtained by fluorescence microscopy using SNA (**A**) and MAL II (**B**). The disc surface is oriented downwards in all the pictures. Abbreviations: MAL II, *Maackia amurensis* lectin II; SNA, *Sambucus nigra* agglutinin.

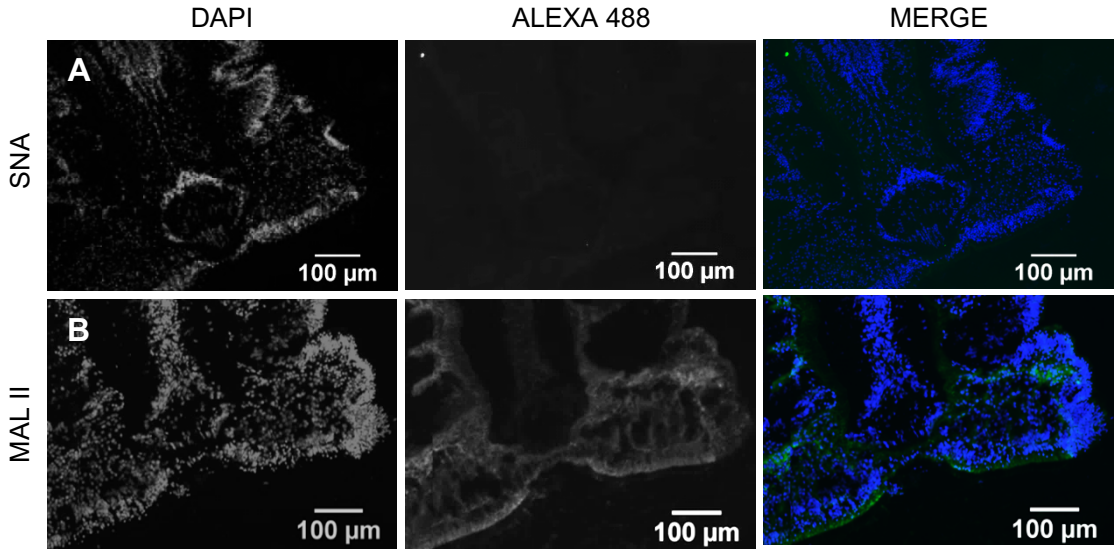

Figure S6. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 2 lectins that detect chitobiose, N-acetylglucosamine and N-acetylgalactosamine. Lectin histochemistry images obtained by fluorescence microscopy using sWGA (**A**) and DSL (**B**). The disc surface is oriented downwards in all the pictures. Abbreviations: DSL, *Datura stramonium* lectin; sWGA, Succinylated wheat germ agglutinin

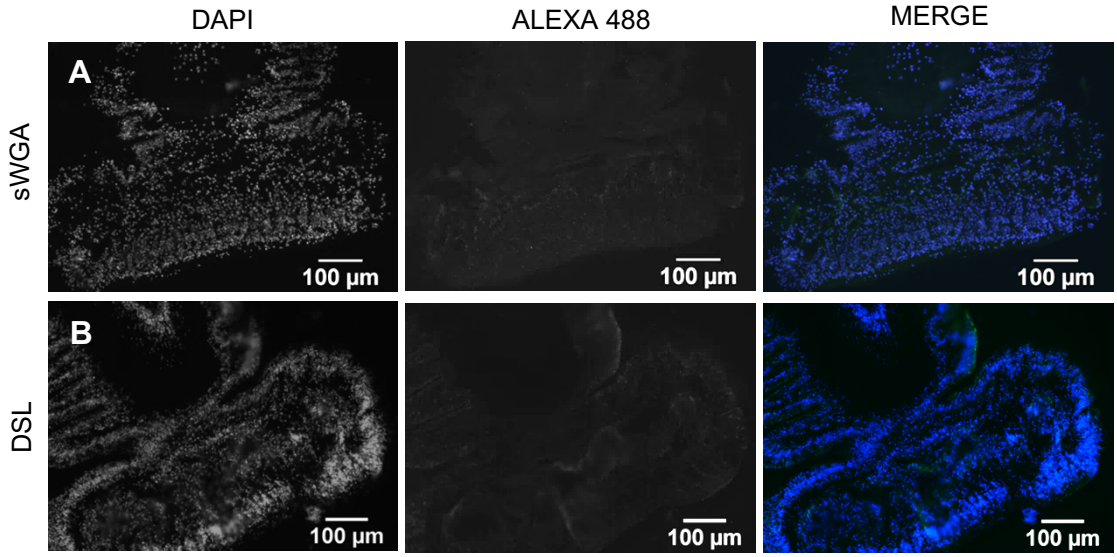

Figure S7. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 3 lectins that detect galactose and N-acetylgalactosamine. Lectin histochemistry images obtained by fluorescence microscopy using PNA (**A**), GSL I (**B**) and RCA I (**C**). The disc surface is oriented downwards in all the pictures. Abbreviations: GSL I, *Griffonia simplicifolia* lectin I; PNA, Peanut agglutinin; RCA I, *Ricinus communis* agglutinin I.

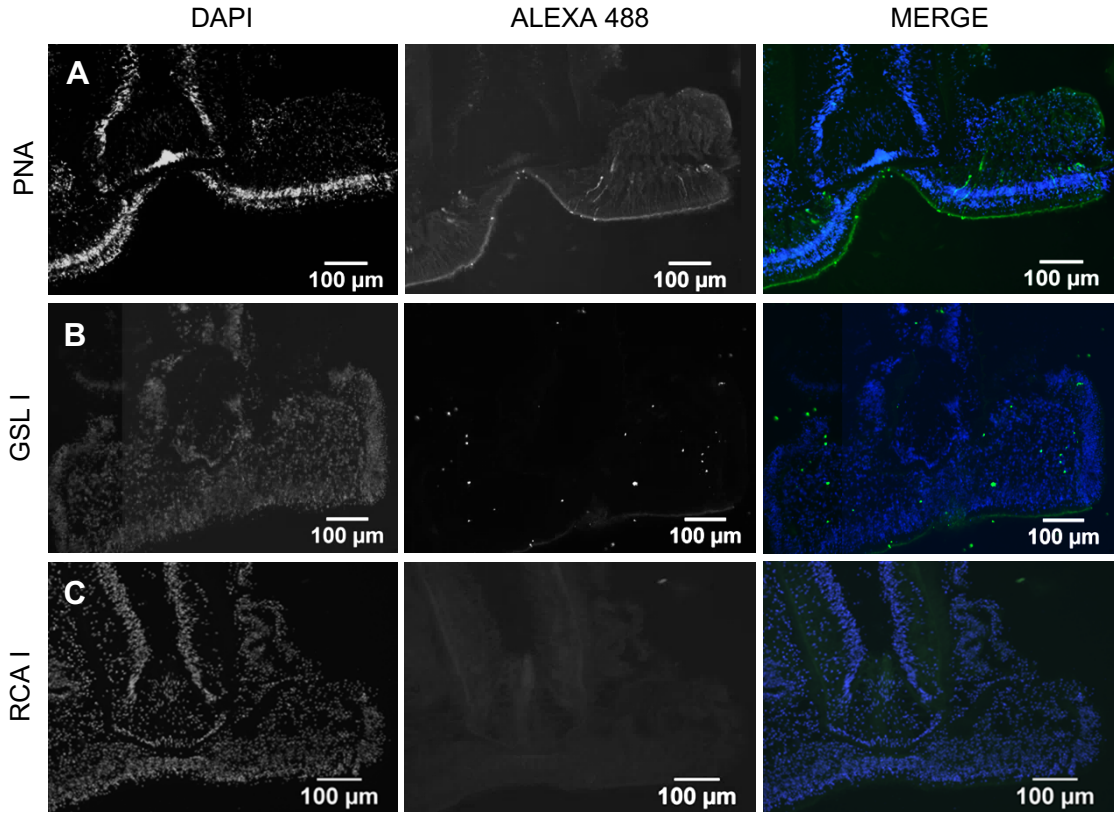

Figure S8. Labelling of *Paracentrotus lividus* tube foot longitudinal sections using 1 lectin to detect fucose. Lectin histochemistry images obtained by fluorescence microscopy using UEA I (**A**). The disc surface is oriented downwards in all the pictures. Abbreviations: UEA I, *Ulex europaeus* agglutinin I.

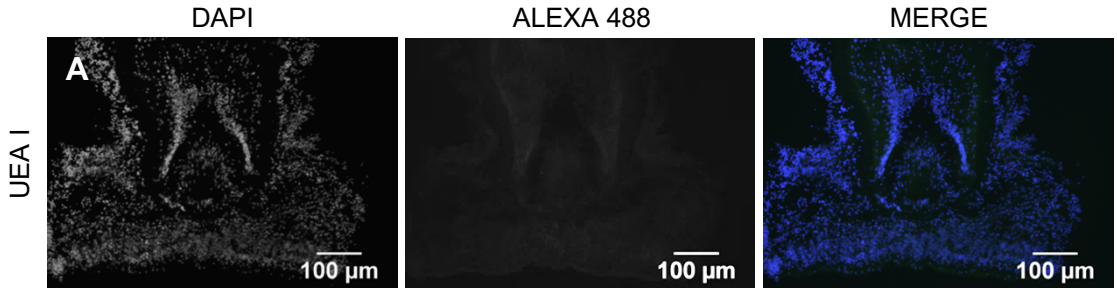

Figure S9. Labelling *Paracentrotus lividus* adhesive footprints obtained with the 9 lectins. Lectin histochemistry images obtained by fluorescence microscopy. ConA, LCA and PSA were used to detect the presence of mannose, D-glucose and N-acetylglucosamine (**A-C**, respectively) ; PHA-E (**D**) and PHA-L (**E**) to detect galactose, N-acetylglucosamine and mannose; ECL (**F**) was used to detect the presence of N-acetillactosamine and N-acetylgalactosamine; DSL (**G**) was used to detect chitobiose, N-acetylglucosamine and N-acetylgalactosamine; GSL I and RCA I to detect galactose and N-acetylgalactosamine (**H-I**, respectively). Abbreviations: Con A, Concavaline A; DSL, *Datura stramonium* lectin; ECL, *Erythrina cristagalli* lectin; GSL I, *Griffonia simplicifolia* lectin I; LCA, *Lens culinaris* agglutinin; PHA-E, *Phaseolus vulgaris* erythro agglutinin; PHA-L, *Phaseolus vulgaris* leuco agglutinin; PSA, *Pisum sativum* agglutinin; RCA I, *Ricinus communis* agglutinin I.

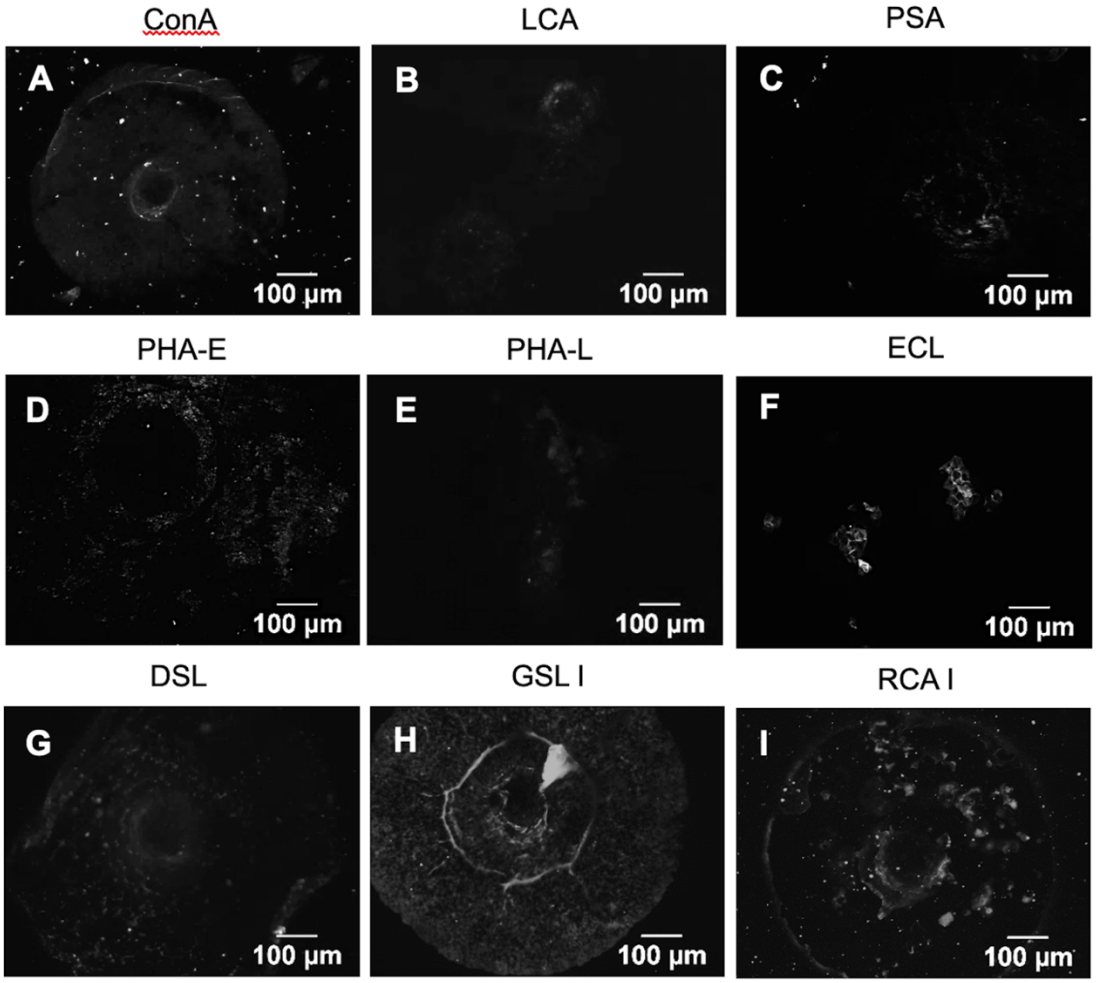

Figure S10. Glycan abundance in *Paracentrotus lividus* tube feet disc and stem extracts obtained with the 17 lectins. Enzyme linked lectin assay comparing the obtained absorbance values. ConA, LCA and PSA were used to detect the presence of mannose, D-glucose and N-acetylglucosamine; PHA-E and PHA-L to detect galactose, N-acetylglucosamine and mannose; DBA and VVA to detect N-acetylgalactosamine; ECL was used to detect the presence of N-acetillactosamine and N-acetylgalactosamine; SNA and MAL II to detect sialic acid, galactose and N-acetylgalactosamine; sWGA and DSL to detect chitobiose, N-acetylglucosamine and N-acetylgalactosamine; Jacalin, PNA, GSL I and RCA I to detect galactose and N-acetylgalactosamine; UEA I was used to detect fucose in the extracts. Each bar represents the mean and standard deviation (N=8). T-test was performed to compare absorbance values for each lectin; * $p < 0.05$ and ** $p < 0.001$. Abbreviations: Con A, Concavaline A; DBA, *Dolichos biflorus* agglutinin; D-Glc, D-glucose; DSL, *Datura stramonium* lectin; ECL, *Erythrina cristagalli* lectin; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; GSL I, *Griffonia simplicifolia* lectin I; LacNAc, N-acetillactosamine; LCA, *Lens culinaris* agglutinin; MAL II, *Maackia amurensis* lectin II; Man, mannose; PHA-E, *Phaseolus vulgaris* erythro agglutinin; PHA-L, Phaseolus vulgaris leuco agglutinin; PNA, Peanut agglutinin; PSA, *Pisum sativum* agglutinin; RCA I, *Ricinus communis* agglutinin I; SNA, *Sambucus nigra* agglutinin; sWGA, Succinylated wheat germ agglutinin; UEA I, *Ulex europaeus* agglutinin I; VVA, *Vicia villosa* agglutinin.

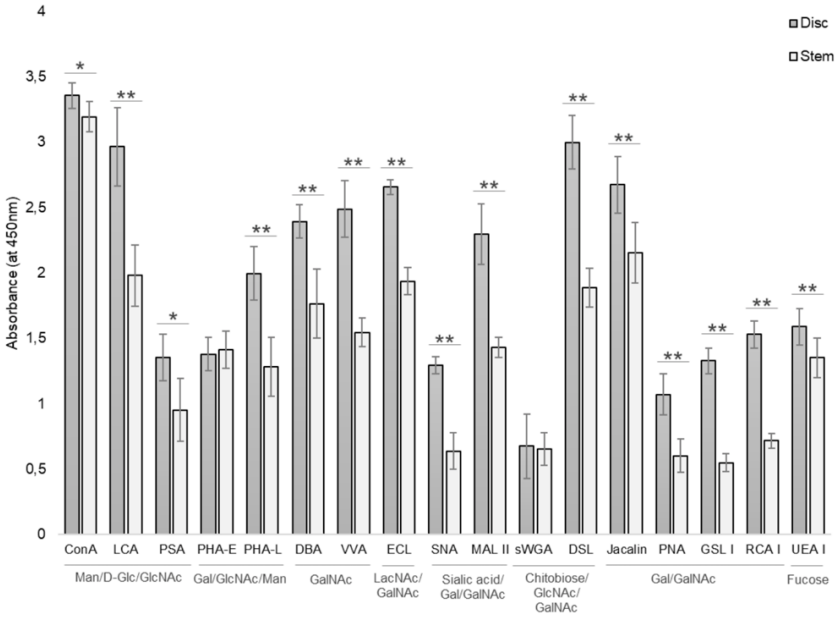

Figure S11. Glycoproteins present in *Paracentrotus lividus* tube feet disc and stem extracts detected using the 17 lectins. One-dimensional gel electrophoresis protein profiles and corresponding lectin blots. ConA, LCA and PSA were used to detect the presence of mannose, D-glucose and N-acetylglucosamine; PHA-L was used to detect galactose, N-acetylglucosamine and mannose; DBA to detect N-acetylgalactosamine; SNA to detect sialic acid, galactose and N-acetylgalactosamine; sWGA and DSL to detect chitobiose, N-acetylglucosamine and N-acetylgalactosamine in the extracts. Abbreviations: Con A, Concavaline A; DBA, *Dolichos biflorus* agglutinin; DSL, *Datura stramonium* lectin; LCA, *Lens culinaris* agglutinin; PHA-L, *Phaseolus vulgaris* leuco agglutinin; PSA, Pisum sativum agglutinin; SNA, *Sambucus nigra* agglutinin; sWGA, Succinylated wheat germ agglutinin.