## **Supporting Information**

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SI Text



**Fig. S1.** Schematic of *A. thaliana* SEX4 depicting the domain topography and secondary structure of chain A in the asymmetric unit. The chloroplast targeting peptide (cTP) is gray, the dual specificity phosphatase (DSP) domain is pink, the carbohydrate binding module family 48 (CBM48) is green, the C-terminal domain is blue, and linker regions are black. The different subdomains within the DSP domain are labeled per standard nomenclature (33).  $\alpha$ -helices and  $\beta$ -sheets within the DSP domain are numbered 1–8 and 1–5, respectively, the  $\beta$ -sheets within the CBM are numbered 6–13, and the  $\alpha$ -helices in the C-terminal domain 9–10. The active site cysteine (C198) and aspartic acid (D166) are highlighted by a red box. F167 is highlighted by an asterisk. Residues known to disrupt binding to glucans are highlighted with a green box.



**Fig. S2.** DSP domain structure. (*A*) The phosphatase active site P-loop of SEX4 including bound phosphate ion (pink and red) shown with 2Fo-Fc electron density map contoured at  $1.6\sigma$ . (*B*) The DSP domain of SEX4 displayed in the classical orientation and with each subdomain colored. Green, recognition domain; purple, variable insert; beige, D-loop; red, active site; salmon, R-motif.



**Fig. S3.** Multiple sequence alignment of the DSP domain of *A. thaliana* SEX4, *H. sapiens* VHR, and *H. sapiens* laforin. Numbering refers to the amino acids of SEX4. The different subdomains within the DSP domain are labeled per standard nomenclature (33). SEX4 secondary structure is displayed in gray.  $\alpha$ -helices 3 and 4 are in tan because they are shared between SEX4 and the predicated secondary structure of laforin. The black  $\alpha$ -helices and  $\beta$ -sheet are predicted secondary elements of laforin and VHR.

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**Fig. S4.** Multiple sequence alignment of SEX4 orthologs. Secondary structure and elements are labeled as in Fig. S1. The catalytic residues D166 and C198 are boxed in red. F167 is marked with a black asterisk. F214 within the AYLM motif is boxed in blue. The  $\alpha$ 5 region of the DSP and the  $\beta$ 7/8 loop of the CBM that pack together are both boxed in yellow. Residues within  $\alpha$ 8 of the DSP domain that pack against  $\beta$ 10 and the end of the CBM are all marked with red asterisks.



**Fig. S5.** F167 is critical for SEX4 function as a glucan phosphatase. (*A*) Ribbon diagram of SEX4 (residues 90–379) with the active site S198 labeled with a red stick, F167 in yellow, and W278 in green. This is the same view as in Fig. 1C. (*B*) Ribbon diagram as in *B* but rotated 90°. This is the same orientation as the zoom in Fig. 4A. (*C*) Multiple sequence alignment of the D-loop of SEX4 orthologs and multiple DSPs. (*D*) Release of phosphate by  $\Delta$ 89-SEX4 (WT) and SEX4 mutants utilizing the specific glucan substrate amylopectin. Error bars indicate means  $\pm$  the standard deviation. (*E*) Specific activity of  $\Delta$ 89-SEX4 (WT) and SEX4 mutants against the generic substrate *p*-NPP.

Table S1. Percent similarity and identity of SEX4 ortholgs to A. thaliana SEX4 full-length protein
(At-SEX4), the DSP domain (At-SEX4 DSP), and the CBM (At-SEX4 CBM48) (similarity/identity)

	At-SEX4	At-SEX4 DSP	At-SEX4 CBM48
A. thaliana	100/100	100/100	100/100
B. distachyan	68/57	86/75	71/57
C. reinhardtii	49/34	71/56	52/34
C. sinensis	64/54	93/86	76/64
G. hirsutum	73/62	93/86	76/62
G. max	72/60	90/83	74/65
M. truncatula	71/58	89/81	71/57
N. tabacum	73/64	90/85	68/62
O. sativa	67/57	89/77	66/55
Phaseolis spp.	71/59	86/77	74/62
P. bicolor	68/58	87/77	70/62
P. patens	56/42	72/60	63/43
P. sitchensis	61/48	88/75	67/57
P. trichocarpa	73/61	92/83	70/62
R. communis	73/63	93/82	72/68
S. lycopersicum	73/62	89/83	71/60
S. tuberosum	73/62	89/82	71/60
V. vinifera	71/60	91/83	72/65
Z. mays	68/57	87/76	68/60

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