Supplementary Information

Supplementary Figure Legends

Supplementary Figure 1 Cadmium sensitivity of the *pcs* and *abc* deletion mutants, expression of ABC transporters during Cd exposure and vacuole staining in wild type and the *abc1-4 hmt1* quintuple mutant. **(A)** Cd sensitivity of the *hmt1*, *pcs* and *abc* deletion mutants, including the *pdr1 bfr1* double mutant. Bfr1 and Pdr1 belong to Cluster I of *S. pombe* ABC transporters (1). (**B**) Vacuolar ABC transporter expression during cadmium exposure. Expression data was plotted using publicly available microarrays (http://www.bahlerlab.info/cgi-bin/SPGE/geexview) (2). **(C)** Staining of the vacuolar membrane with FM4-64 to show that vacuole biogenesis and morphology were not affected in the *abc1-4 hmt1* quintuple mutant. Vacuoles were visualized by confocal microscopy after incubation with FM4-64 as descibed in (1).

Supplementary Figure 2 Complementation of the *hmt1* mutant by expression of $hmt1^{+}$. Cd sensitivity of *hmt1* and *abc2 abc3 hmt1* can be rescued by expression of $hmt1^{+}$. The *abc* mutants were transformed with pRep41-Hmt1 using the lithium acetate method and independent transformants were selected on EMMS without Leu. Complemented strains were grown on liquid EMMS without Leu media and 1:10 serial dilutions were spotted on selective plates containing or not cadmium.

Supplementary Figure 3 Phytochelatin transport mediated by *S. pombe* full-size ABC transporters. Microsomal vesicles obtained from the *hmt1* mutant showed a reduced uptake of ^{35}S-PC_2 (black columns) and ^{35}S-PC_2-Cd (light columns) compared to wild type vesicles. ^{35}S-PC transport was decreased in the *hmt1* mutant and abolished in the *abc1-4 hmt1* quintuple mutant (data from 3 replicates ± SE). Asterisk denotes a statistically significant difference compared to wild type ($P < 0.05$).

Supplementary Figure 4 Abc4 expression in the quintuple *abc1-4 hmt1* quintuple mutant and GS-bimane accumulation in the *hmt1* and *abc2 hmt1* mutants. **(A-B)** Abc4 transports GS-bimane into vacuoles but does not restore Cd tolerance in the *abc1-4 hmt1* mutant. **(A)** Expression of Abc4 in the *abc1-4 hmt1* quintuple mutant restored GS-bimane accumulation in vacuoles. However, **(B)** Abc4 did not rescue the Cd sensitivity of the quintuple *abc1-4 hmt1* mutant. **(C)** GS-bimane accumulation in the *hmt1* single and the *abc2 hmt1* double mutants. The *hmt1* mutant and the double mutant, *abc2 hmt1,* show GS-bimane accumulation in vacuoles suggesting that other ABC transporters (i.e. Abc4) have overlapping functions and are able to sequester GS-conjugates into vacuoles.

Supplementary Figure 5 Phytochelatin content in *S. pombe abc* mutants and identification of phytochelatins in *S. pombe* high-molecular-weight complexes (HMWCs). **(A)** Phytochelatins in cell extracts from the different *abc* mutants were quantified by HPLC. The *abc1-4 hmt1* quintuple mutant showed a strong decrease in phytochelatin levels. Expression of either Hmt1 or Abc2 in the quintuple *abc1-4 hmt1* mutant restored the phytochelatin content in cell extracts (n = 3 experiments, ± SD). * denotes a statistically significant difference compared to wild type ($P < 0.05$). ** denotes a statistically significant difference compared to *abc1-4 hmt1* ($P < 0.05$). (**B**) Phytochelatins in high molecular fractions of *S. pombe* cell extracts. Soluble cell extracts from yeast exposed to Cd were loaded onto a Superdex 75 10/300 GL column equilibrated with 20 mM Tris pH 7.7. Fractions (2 ml) were collected, concentrated using 3 kDa cut-off centrifugal

devices. Thiols were labeled, separated and detected by fluorescence HPLC coupled to mass spectrometry as previously described (3).

Supplementary Figure 6 Phylogenetic tree of the 11 *S. pombe* ABC transporters and the *Arabidopsis* MRP/ABCC subfamily. The tree was constructed using the standard parameters suggested in *phylogeny.fr* (4). Amino acid sequences were obtained from TAIR (The Arabidopsis Information Resource, http://www.arabidopsis.org/) and the *Schizosaccharomyces pombe* database (http://genedb.org/genedb/pombe/).

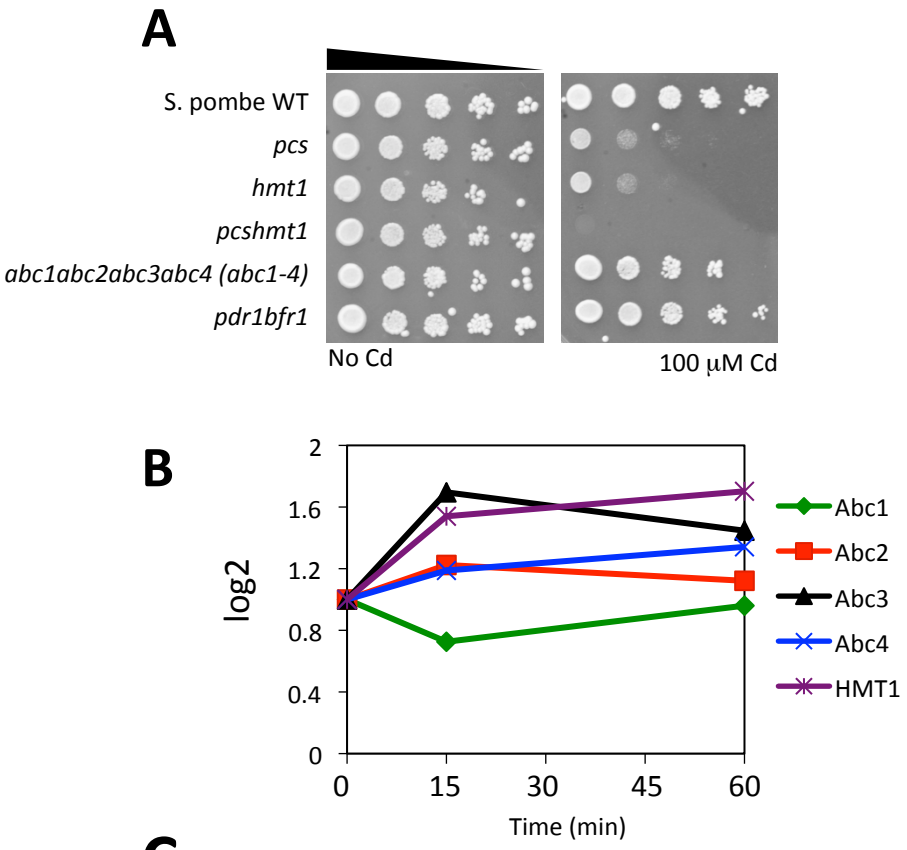
Supplementary Table 1 *Schizosaccharomyces pombe* strains used in this study.

Strain	Genotype	Source
Wild type	h- ura4-D18 leu1-32	
pcs	h- ura4-D18 leu1-32 pcs::kanMX4	This study
hmt1	h- ura4-D18 leu1-32 hmt1::natMX6	This study
pcs hmt1	h- ura4-D18 leu1-32 hmt1::natMX6 pcs::kanMX4	This study
abc2	h- ura4-D18 leu1-32 ade6 abc2::kanMX4	This study
abc2 hmt1	h- ura4-D18 leu1-32 ade6 abc2::kanMX4 hmt1::natMX6	This study
abc3 hmt1	h- ura4-D18 leu1-32 ade6 abc3::kanMX4 hmt1::natMX6	This study
abc4 hmt1	h- ura4-D18 leu1-32 abc4::kanMX4 hmt1::natMX6	This study
abc2 abc3 hmt1	h- ura4-D18 leu1-32 ade6 abc2::kanMX4 abc3::kanMX4 hmt1::natMX6	This study
abc2 abc4 hmt1	h- ura4-D18 leu1-32 abc2::kanMX4 abc4::kanMX4 hmt1::natMX6	This study
abc1 abc2 abc3 abc4	h- ura4-C190T leu1-32 ade7::ura4 abc1::ura4 abc2::ura4 abc3::ura4 abc4::ura4	(1)
abc1 abc2 abc3 abc4 hmt1	h- ura4-C190T leu1-32 ade7::ura4 abc1::ura4 abc2::ura4 abc3::ura4 abc4::ura4 hmt1::natMX6	This study

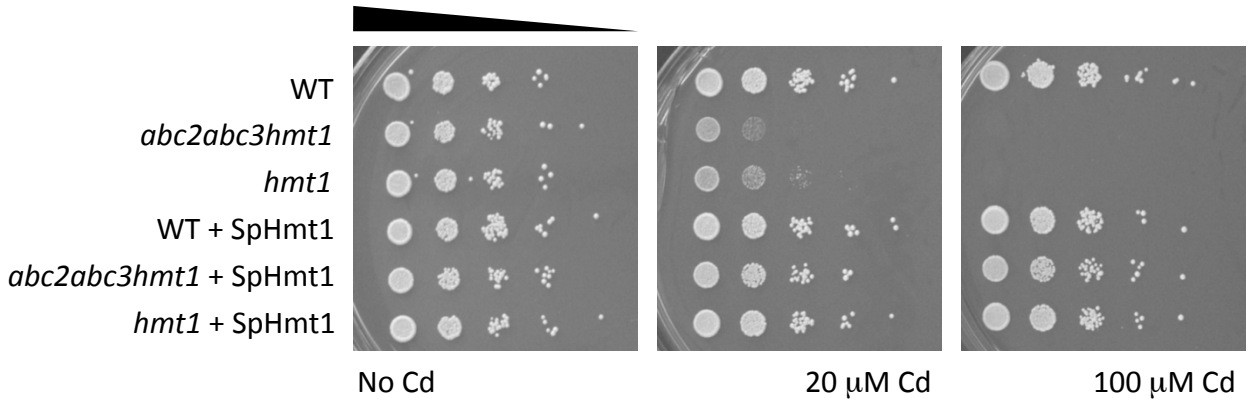
Author Contributions. DGMC and JIS designed the experiments in discussions with PR and OKV. DGMC cloned *abc2*$^+$, *hmt1*$^+$ and performed the experiments in Figs. 1-2, 3A and B, 4A-F, 5 and Supp. Figs 1,2,4, 5B. TOJ performed experiments in Fig. 3A-C. GZA contributed to Abc4 experiments. ZZ and OKV isolated *S. pombe* vacuoles and performed the experiments in Fig. 3F and Supp. Fig. 5A. ZZ, VIK and OKV performed the experiments in Fig. 4G and H. OL and MRR generated the *S. pombe* deletion strains. WYS and EM generated the *S. cerevisiae* Sm14 and Sm15 strains and performed experiments in Supp. Fig. 3. DGMC and JIS wrote the paper.

References

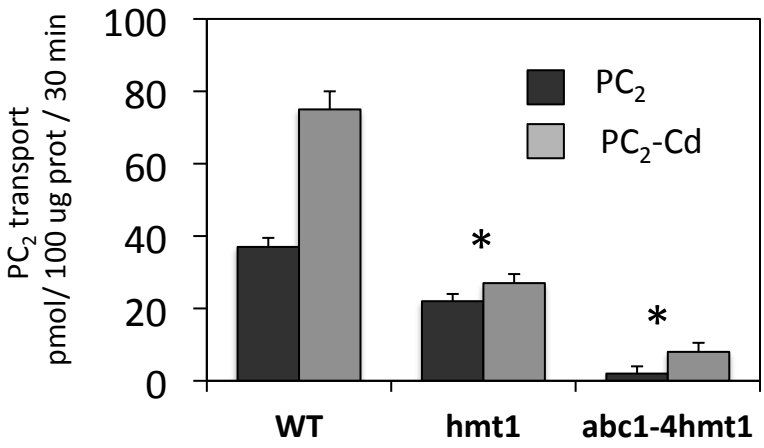
1. Iwaki, T., Giga-Hama, Y., and Takegawa, K. (2006) *Microbiology* **152**, 2309-2321
2. Chen, D., Toone, W. M., Mata, J., Lyne, R., Burns, G., Kivinen, K., Brazma, A., Jones, N., and Bahler, J. (2003) *Mol. Biol. Cell.* **14**, 214-229
3. Mendoza-Cozatl, D. G., Butko, E., Springer, F., Torpey, J. W., Komives, E. A., Kehr, J., and Schroeder, J. I. (2008) *Plant J.* **54**, 249-259
4. Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., and Gascuel, O. (2008) *Nucleic Acids Res.* **36**, W465-469.



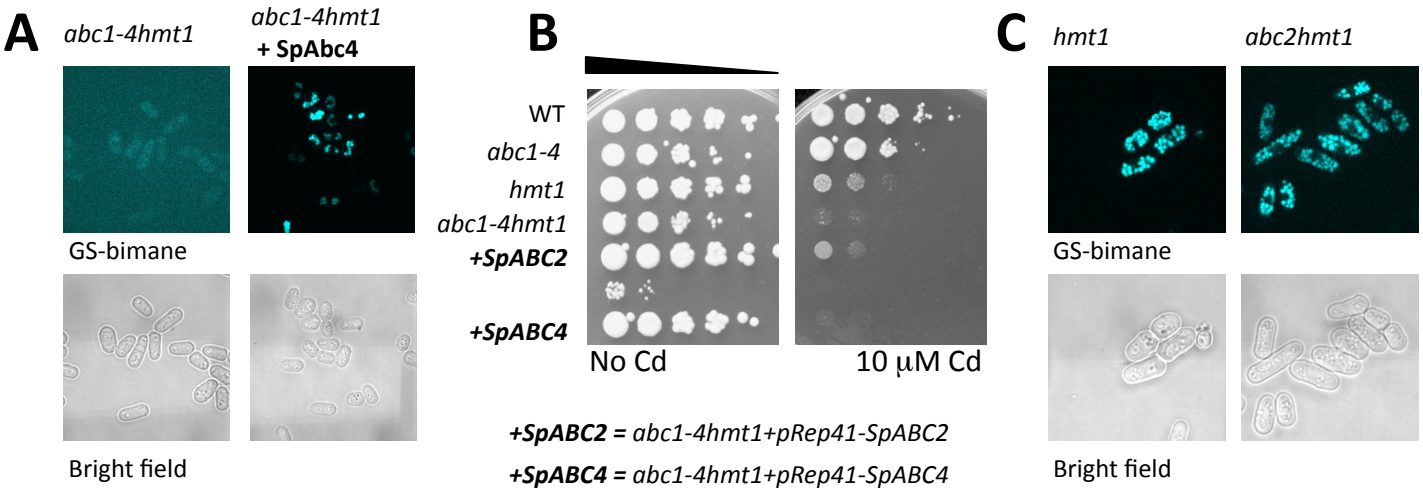
Supp Fig 1



Supp Fig 2

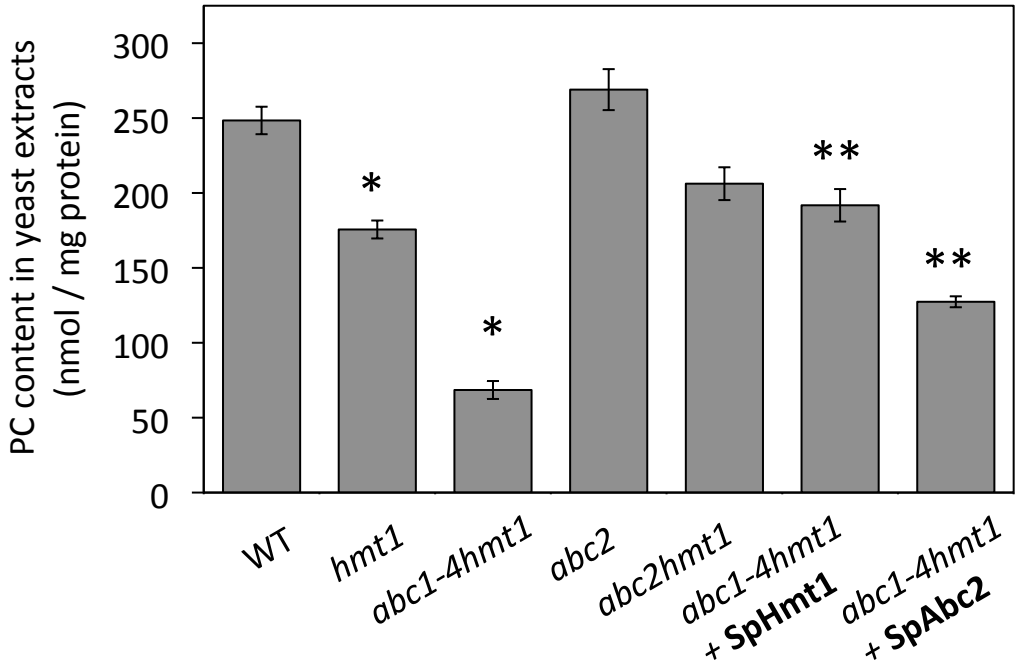


Supp Fig 3

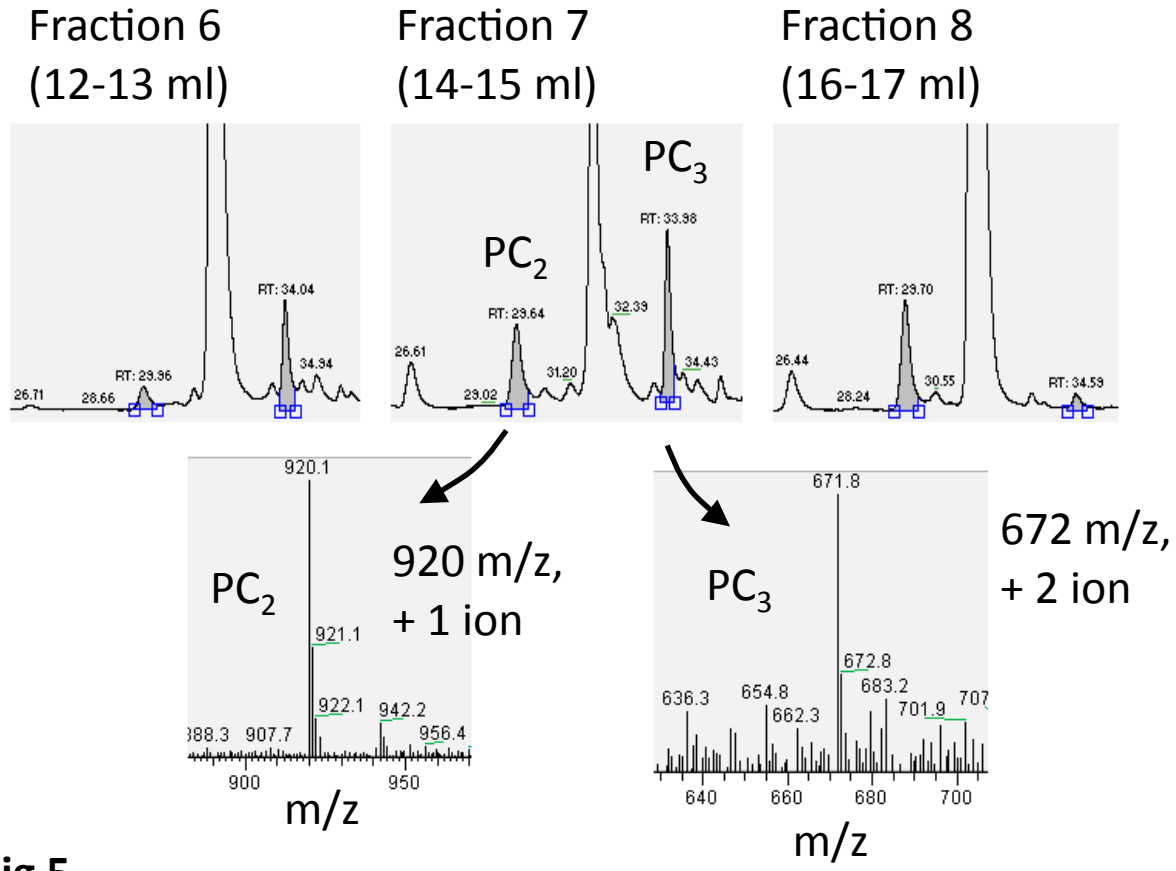


Supp Fig 4

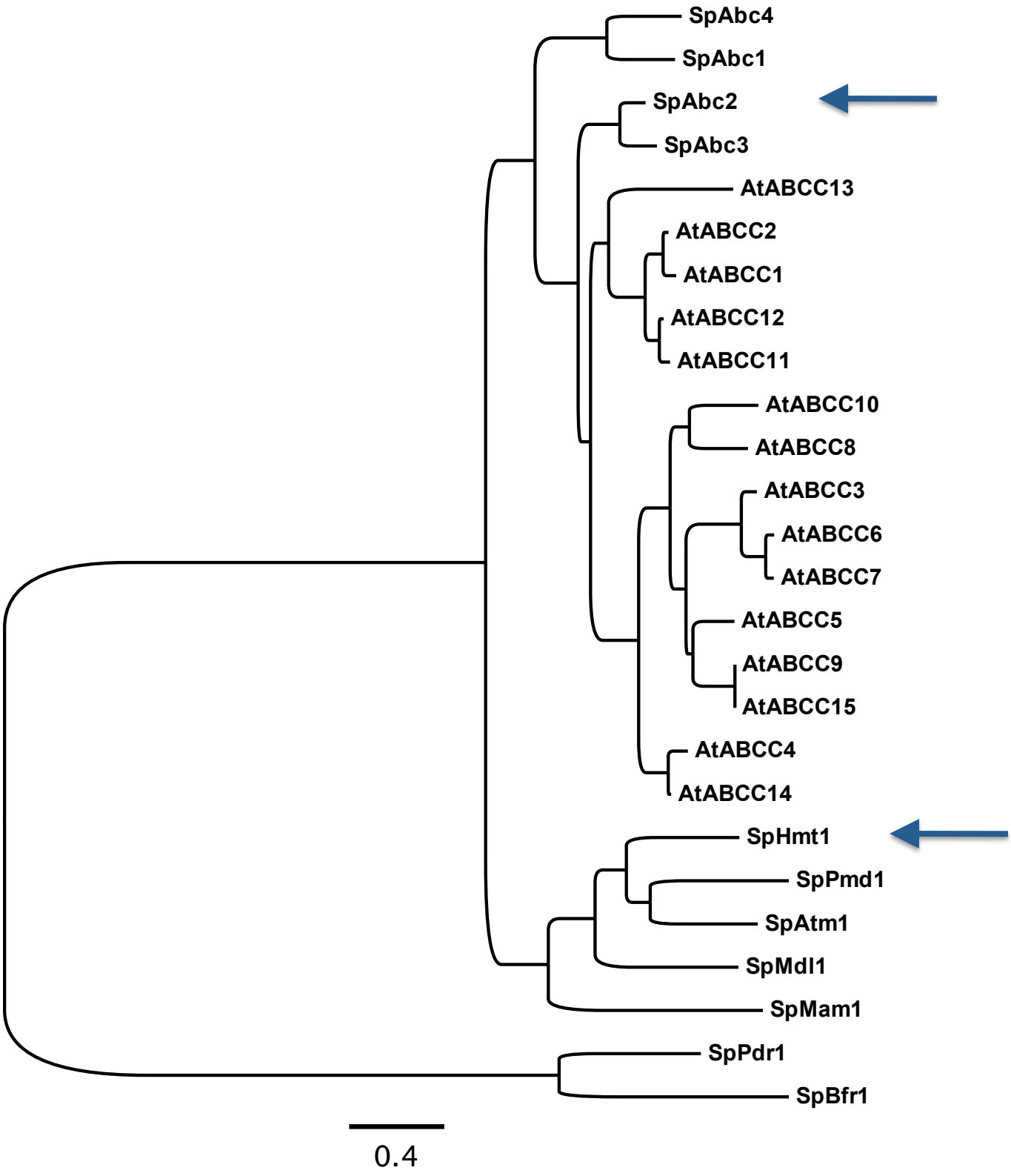
A


PC content in yeast extracts
(nmol / mg protein)
300
250
200
150
100
50
0
*
*
**
**
WT
hmt1
abc1-4hmt1
abc2
abc2hmt1
abc1-4hmt1
+ SpHmt1
abc1-4hmt1
+ SpAbc2

B


Fraction 6
(12-13 ml)
Fraction 7
(14-15 ml)
Fraction 8
(16-17 ml)
PC3
PC2
RT: 34.04
RT: 28.36
RT: 33.38
RT: 29.64
RT: 29.70
RT: 34.59
920.1
PC2
921.1
922.1
942.2
956.4
907.7
900
950
m/z
920 m/z,
+ 1 ion
671.8
PC3
672.8
683.2
636.3
654.8
662.3
701.9
640
660
680
700
m/z
672 m/z,
+ 2 ion

Supp Fig 5


SpAbc4
SpAbc1
SpAbc2
SpAbc3
AtABCC13
AtABCC2
AtABCC1
AtABCC12
AtABCC11
AtABCC10
AtABCC8
AtABCC3
AtABCC6
AtABCC7
AtABCC5
AtABCC9
AtABCC15
AtABCC4
AtABCC14
SpHmt1
SpPmd1
SpAtm1
SpMdl1
SpMam1
SpPdr1
SpBfr1
0.4

Supp Fig 6