Yoshimoto, K., Takamura, H., Kadota, I., Motose, H. & Takahashi, T.

Chemical control of xylem differentiation by thermospermine, xylemin, and auxin

Supplementary Figures S1-S8



Supplementary Figure S1 | Tetraamine content in wild-type seedlings grown with xylemin. Wild-type seedlings were grown for 10 days in the MS liquid medium without xylemin (Mock), grown for 9 days and treated for 24 h with 30  $\mu$ M xylemin (24 h), or grown for 10 days with 30  $\mu$ M xylemin (10 d). Benzoylated polyamines were measured by HPLC. Data are displayed as averages ± SD (n = 3). Asterisk indicates a significant difference from the value in the mock treatment (Student *t*-test, P < 0.01).



Supplementary Figure S2 | Changes in the responsiveness to xylemin during vascular development. The wild type seedlings were germinated and grown for 10 days in the liquid medium. Xylemin was added at various times of culture [1, 4, 6, 12, or 24 hours (h), or 10 days (at the start of culture) before fixation and observation] and xylem differentiation in hypocotyls was observed.



## WT

# WT+xylemin

acl5

Supplementary Figure S3 | Effect of xylemin on xylem differentiation in the root. Seedlings of the wild type were germinated and grown for 7 days in the medium without (WT) or with 50  $\mu$ M xylemin (+ xylemin). The *acl5-1* mutant seedlings were germinated and grown for 7 days in the medium without xylemin. Arrows indicate xylem vessel elements.



1 mm

Supplementary Figure S4 | Effect of xylemin on xylem differentiation in *acl5-1* and *sac51-d*. Seedlings of the *acl5-1* or *sac51-d* mutants were germinated and grown for 7 days in the medium without (Mock) or with 50  $\mu$ M xylemin (+Xylemin).



Supplementary Figure S5 | Effect of xylemin on gene expression in the wild type (WT) and *sac51-d*. Total RNA was isolated from seedlings of the wild type or *sac51-d* germinated and grown for 7 days in the liquid medium without (-) or with 50  $\mu$ M xylemin (+). All transcript levels are relative to those in mock-treated seedlings. Data are displayed as averages ± SD (*n* = 3). Asterisk indicates significant difference from the value in the mock treatment (Student *t*-test, *P* < 0.05)



**Supplementary Figure S6 | A model of thermospermine-mediated negative feedback regulation of auxin-inducible xylem differentiation.** A putative model of thermospermine-mediated negative feedback regulation.



#### Supplementary Figure S7 | Synthetic scheme of xylemin.



#### Supplementary Figure S8 | <sup>1</sup>H NMR spectrum of synthesized xylemin.