Electronic Supplementary Information

Overexpression of a serine hydroxymethyltransferase increases biomass production and reduces recalcitrance in the bioenergy crop *Populus*†

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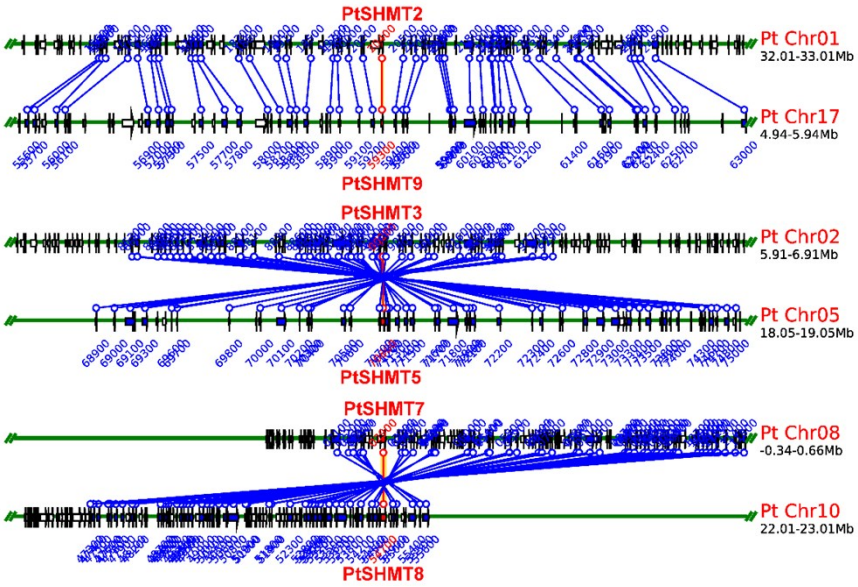


Fig. S1 Duplication of *PtSHMTs* in 500 kb up- and down-stream intervals of the target gene.

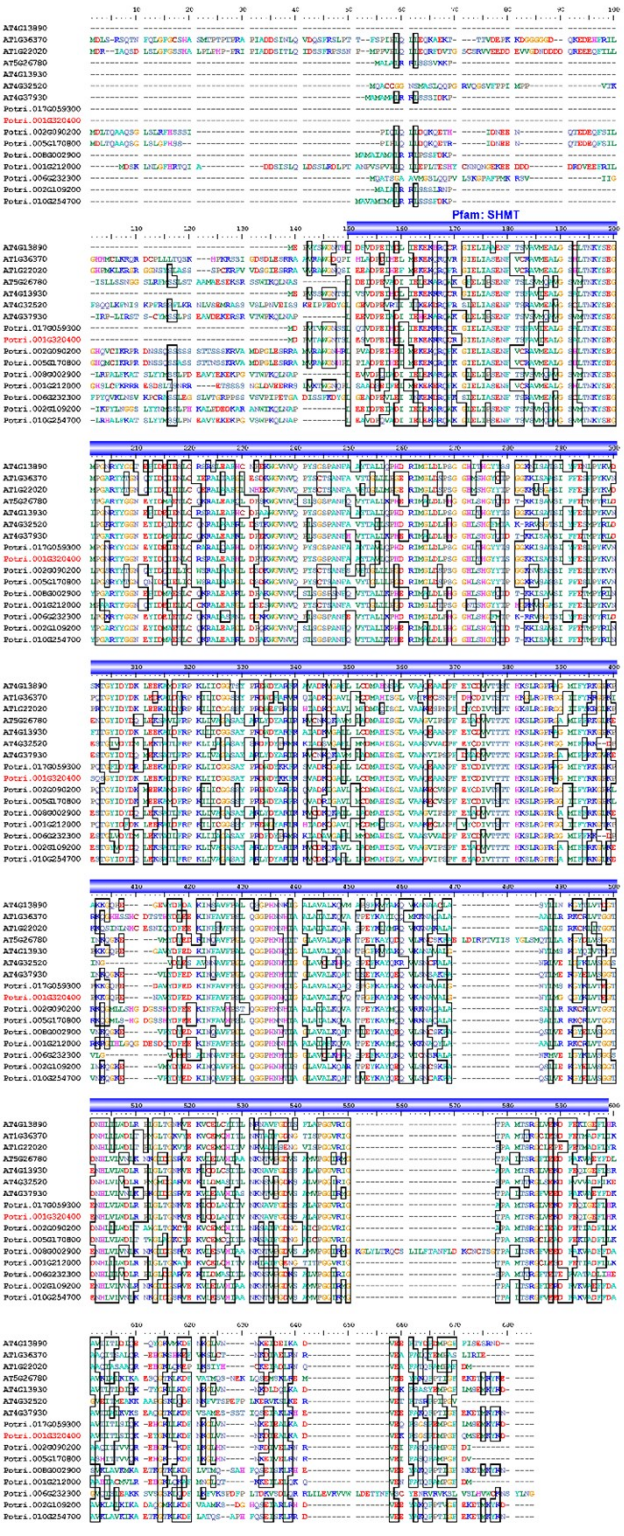
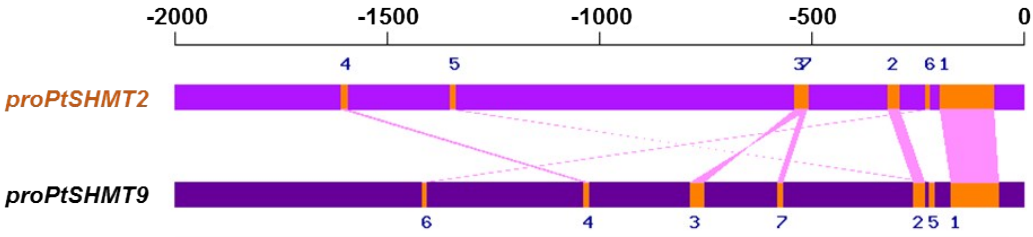


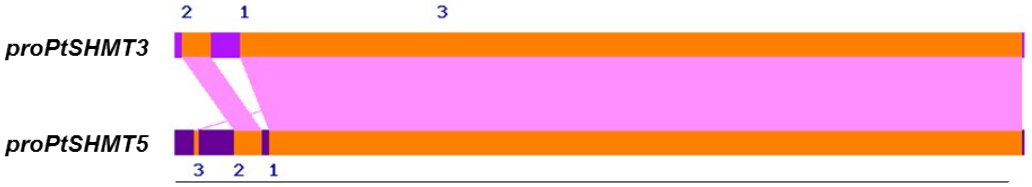
Fig. S2 Alignment of SHMTs in *Arabidopsis* and poplar.

	AT1G22020	AT1G36370	AT4G13890	AT4G13930	AT4G32520	AT4G37930	AT5G26780	Potri.001G212000	**Potri.001G320400**	Potri.002G090200	Potri.002G109200	Potri.005G170800	Potri.006G232300	Potri.008G002900	Potri.010G254700	Potri.017G059300
AT1G22020	ID	0.720	0.456	0.468	0.401	0.400	0.369	0.663	0.485	0.662	0.388	0.658	0.389	0.365	0.386	0.483
AT1G36370	0.720	ID	0.464	0.485	0.394	0.399	0.376	0.670	0.495	0.694	0.394	0.691	0.367	0.367	0.388	0.486
AT4G13890	0.456	0.464	ID	0.811	0.468	0.488	0.465	0.491	0.796	0.525	0.487	0.533	0.439	0.449	0.475	0.779
AT4G13930	0.468	0.485	0.811	ID	0.494	0.503	0.479	0.500	0.902	0.531	0.516	0.536	0.467	0.477	0.503	0.893
AT4G32520	0.401	0.394	0.468	0.494	ID	0.533	0.514	0.414	0.507	0.434	0.532	0.442	0.737	0.496	0.528	0.509
AT4G37930	0.400	0.399	0.488	0.503	0.533	ID	0.822	0.420	0.515	0.445	0.849	0.453	0.508	0.822	0.859	0.511
AT5G26780	0.369	0.376	0.465	0.479	0.514	0.822	ID	0.403	0.485	0.423	0.834	0.428	0.490	0.769	0.815	0.485
Potri.001G212000	0.663	0.670	0.491	0.500	0.414	0.420	0.403	ID	0.514	0.683	0.413	0.685	0.391	0.388	0.410	0.512
Potri.001G320400	0.485	0.495	0.796	0.902	0.507	0.515	0.485	0.514	ID	0.546	0.521	0.552	0.465	0.475	0.509	0.949
Potri.002G090200	0.662	0.694	0.525	0.531	0.434	0.445	0.423	0.683	0.546	ID	0.441	0.945	0.410	0.410	0.433	0.539
Potri.002G109200	0.388	0.394	0.487	0.516	0.532	0.849	0.834	0.413	0.521	0.441	ID	0.446	0.506	0.810	0.854	0.521
Potri.005G170800	0.658	0.691	0.533	0.536	0.442	0.453	0.428	0.685	0.552	0.945	0.446	ID	0.414	0.418	0.441	0.545
Potri.006G232300	0.389	0.367	0.439	0.467	0.737	0.508	0.490	0.391	0.465	0.410	0.506	0.414	ID	0.474	0.501	0.474
Potri.008G002900	0.365	0.367	0.449	0.477	0.496	0.822	0.769	0.388	0.475	0.410	0.810	0.418	0.474	ID	0.903	0.474
Potri.010G254700	0.386	0.388	0.475	0.503	0.528	0.859	0.815	0.410	0.509	0.433	0.854	0.441	0.501	0.903	ID	0.507
Potri.017G059300	0.483	0.486	0.779	0.893	0.509	0.511	0.485	0.512	0.949	0.539	0.521	0.545	0.474	0.474	0.507	ID

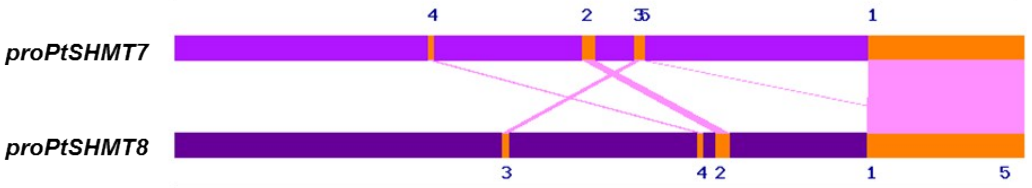
Fig. S3 Pairwise sequence similarity of *Arabidopsis* and poplar SHMTs.



Conserved Region ID	*proPtSHMT2*	*proPtSHMT9*	Identity	Alignment Length	*E*-value	Bit score
1	-196 ~ -72	-172 ~ -60	70.99	131	2.00E-13	64.4
2	-320 ~ -293	-261 ~ -233	89.66	29	8.00E-05	35.6
3	-541 ~ -509	-755 ~ -784	78.79	33	0.04	26.5
4	-1607 ~ -1595	-1037 ~ -1025	100	13	0.14	24.7
5	-1352 ~ -1341	-211 ~ -222	100	12	0.49	22.9
6	-232 ~ -222	-1417 ~ -1407	100	11	1.7	21.1
7	-522 ~ -512	-579 ~ -569	100	11	1.7	21.1



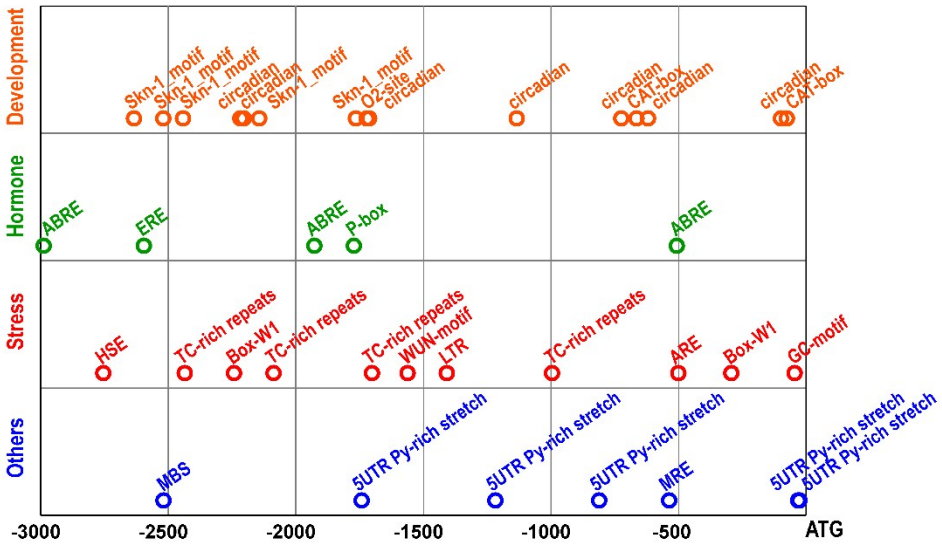
Conserved Region ID	*proPtSHMT3*	*proPtSHMT5*	Identity	Alignment Length	*E*-value	Bit score
1	-1846 ~ -5	-1778 ~ -6	72.91	1938	0.00E+00	1148
2	-1982 ~ -1917	-1861 ~ -1796	86.36	66	7.00E-18	78.8
3	-1380 ~ -1370	-1954 ~ -1944	100	11	1.7	21.1



Conserved Region ID	*proPtSHMT7*	*proPtSHMT8*	Identity	Alignment Length	*E*-value	Bit score
1	-364 ~ -1	-369 ~ -1	72.01	393	1.00E-60	221
2	-1041 ~ -1012	-724 ~ -695	80	30	0.01	28.3
3	-916 ~ -903	-1228 ~ -1215	92.86	14	1.7	21.1
4	-1402 ~ -1392	-768 ~ -758	100	11	1.7	21.1
5	-903 ~ -893	-55 ~ -45	100	11	1.7	21.1

Fig. S4 *cis*-acting elements evolution between *PtSHMT* paralogous pairs.

We compared the similarity of *cis*-acting elements located in the promoter of paralogous *PtSHMT* genes. The promoters of *PtSHMT3* and *PtSHMT5* were highly conserved.



Classification	*cis*-acting elements	Description	#
Development	circadian	involved in circadian control	7
	Skn-1_motif	required for endosperm expression	5
	CAT-box	related to meristem expression	2
	O2-site	involved in zein metabolism regulation	1
Hormone	ABRE	involved in the abscisic acid responsiveness	3
	ERE	ethylene-responsive element	1
	P-box	gibberellin-responsive element	1
Stress	TC-rich repeats	involved in defense and stress responsiveness	4
	Box-W1	fungal elicitor responsive element	2
	HSE	involved in heat stress responsiveness	1
	WUN-motif	wound-responsive element	1
	LTR	involved in low-temperature responsiveness	1
	ARE	essential for the anaerobic induction	1
	GC-motif	involved in anoxic specific inducibility	1
Others	5UTR Py-rich stretch	conferring high transcription levels	5
	MBS	MYB Binding Site	1
	MRE	MYB binding site involved in light responsiveness	1

Fig. S5 *cis*-acting elements located in the promoter of *PtSHMT2*.

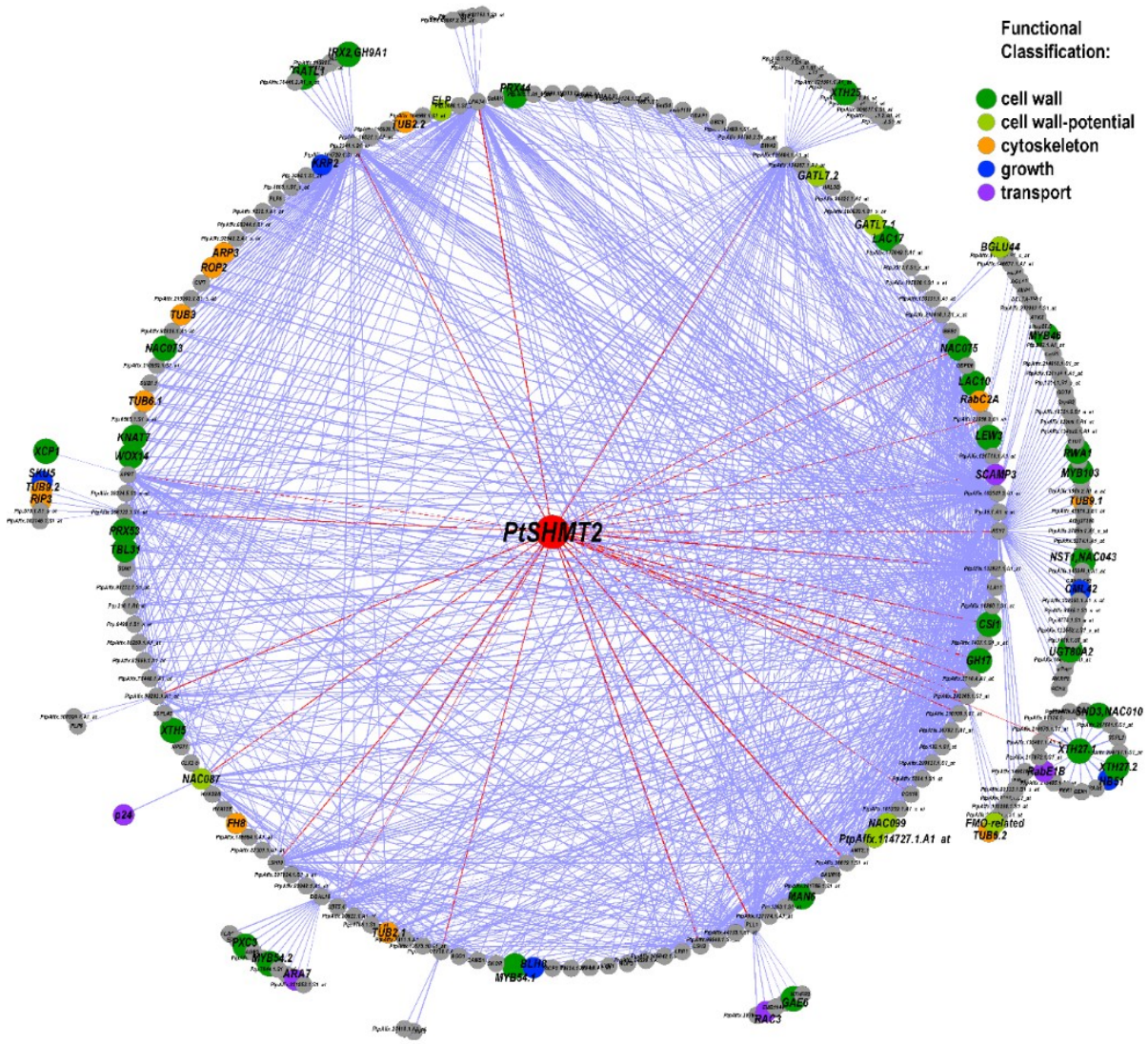


Fig. S6 Co-expression network of *PtSHMT2*.

Based on the co-expression network of *PtSHMT2*, many cell wall- or growth-related genes were co-expressed with *PtSHMT2*, implying *PtSHMT2* might be involved in cell wall related biological process.

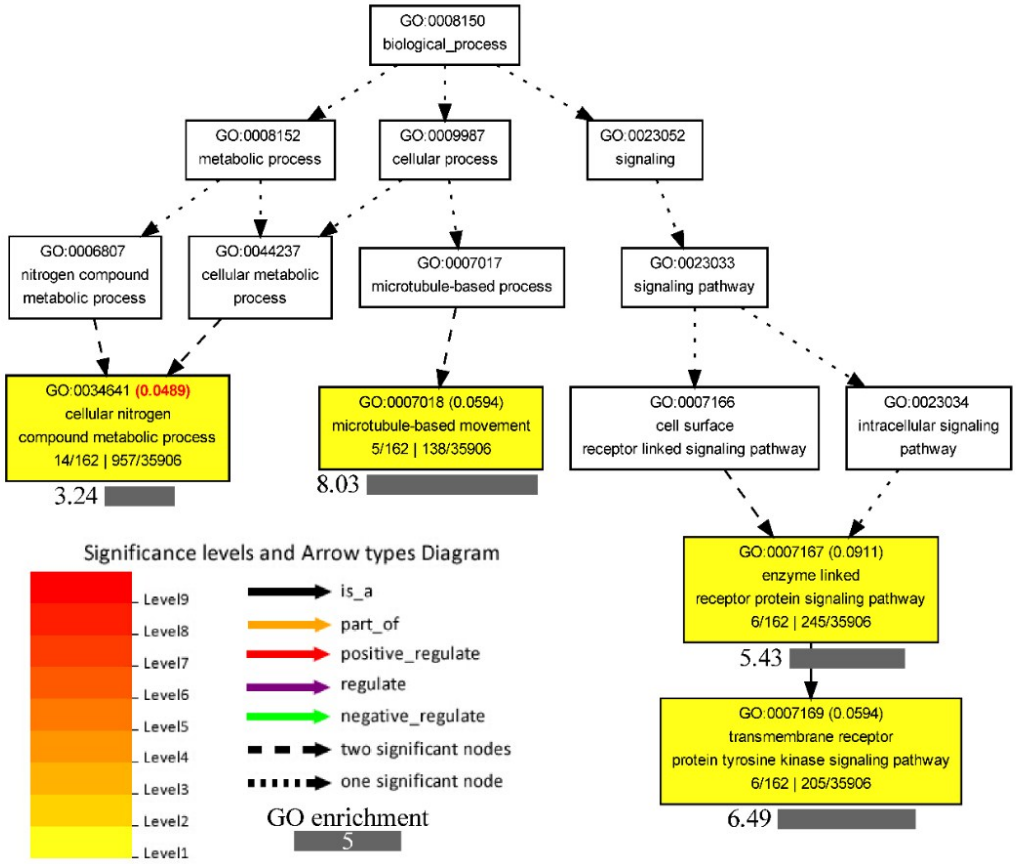


Fig. S7 GO enrichment of genes co-expressed with *PtSHMT2*.

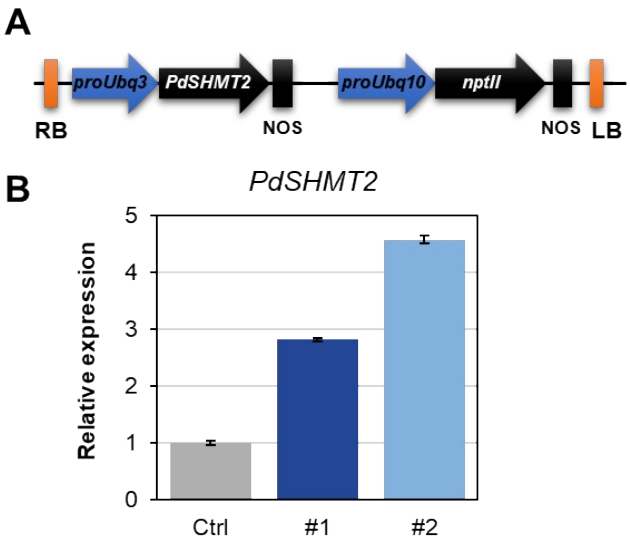


Fig. S8 Confirmation of *PdSHMT2* transgenic poplar.

(A) Gene construct used for generating *Populus* transgenic plants overexpressing *PdSHMT2*. **(B)** qRT-PCR analysis of *PdSHMT2* expression in two independent transgenic lines. Total RNA was extracted from mature leaf.

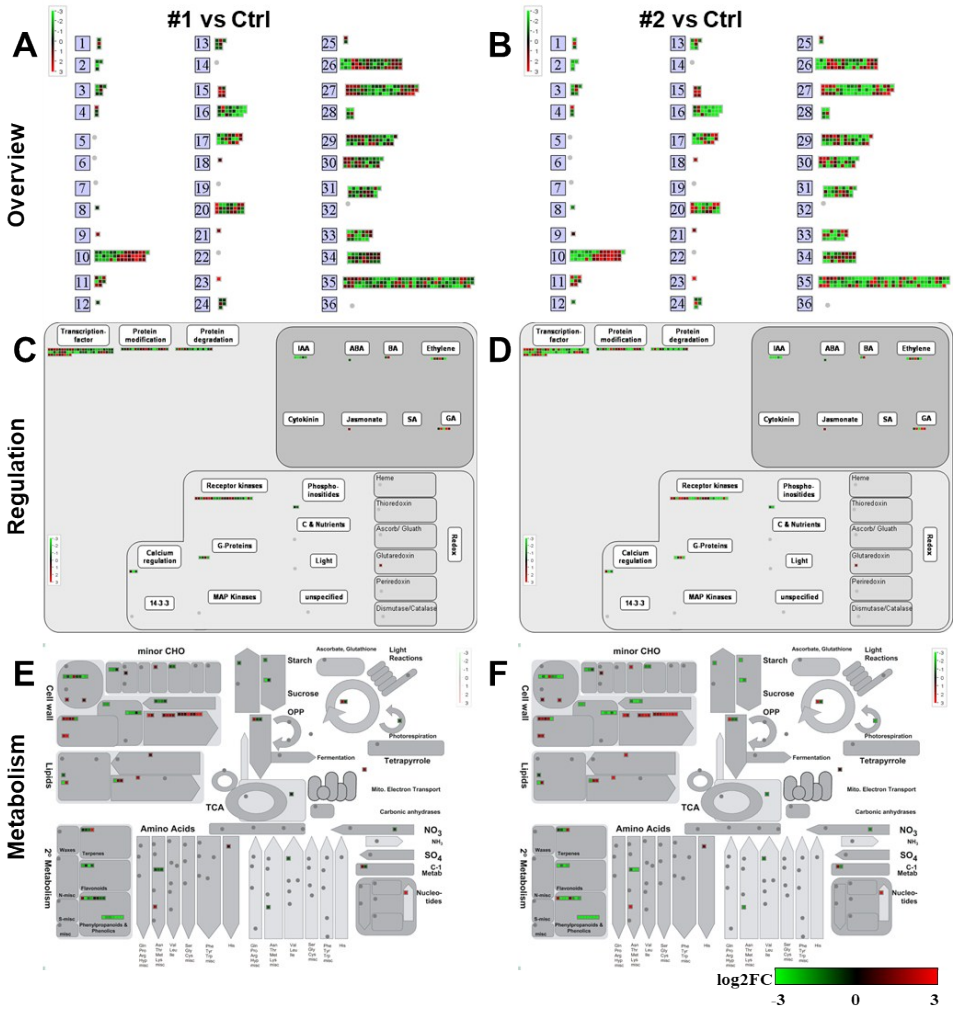


Fig. S9 Functional classification of DEGs in two *PdSHMT2* overexpression lines based on MapMan.

(A and B) Overview of DEGs. (C and D) Overview of regulation related DEGs. (E and F) Overview of metabolism related DEGs. The expression data was transformed using log2 fold change compared to vector control plants. Red represents up-regulated and green represents down-regulated genes.

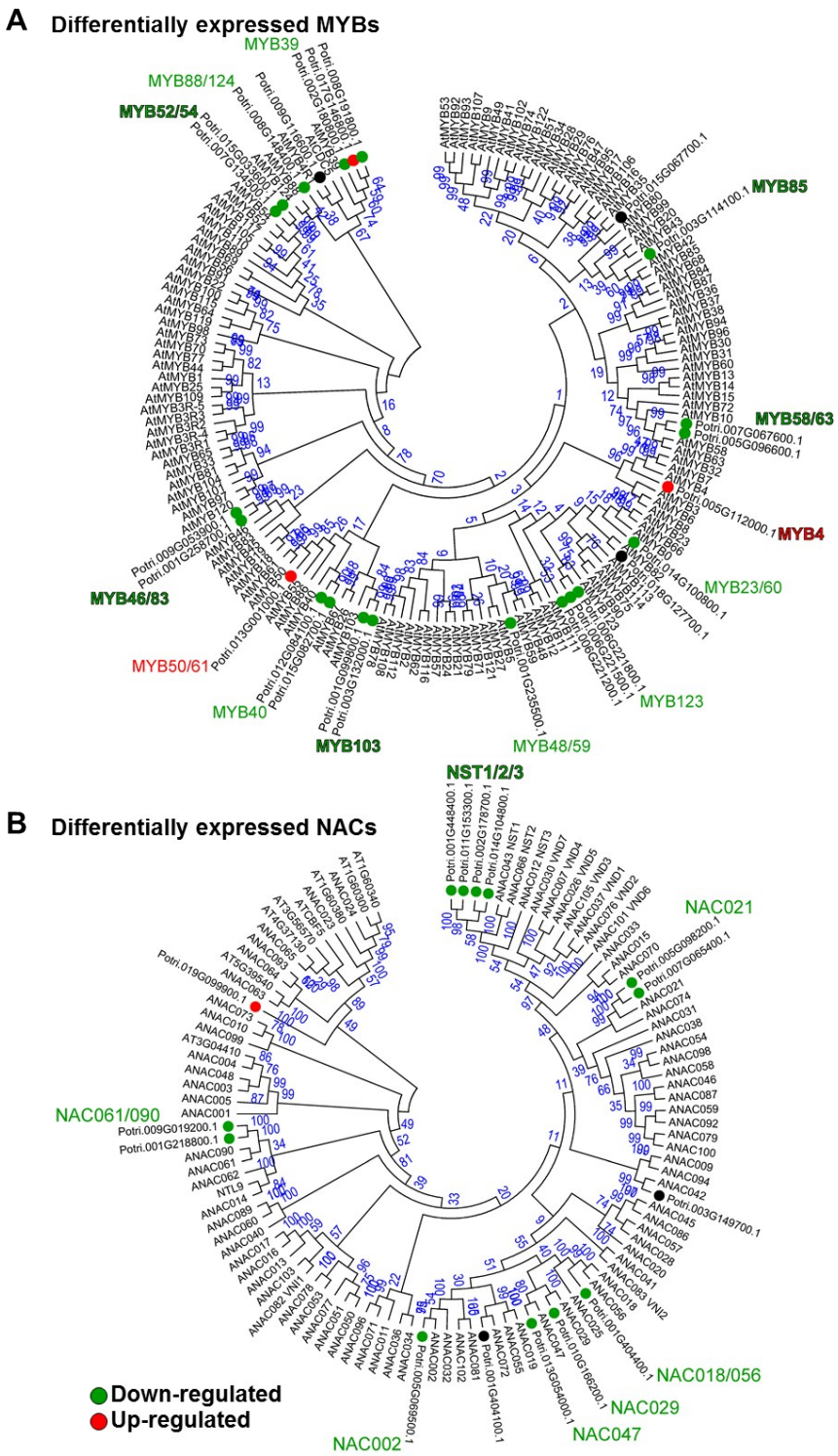


Fig. S10 Phylogenetic relationships of differentially expressed *MYBs* (A) and *NACs* (B) with *Arabidopsis MYB* or *NAC* genes.

Bold labelled are known TFs involved in cell wall biosynthesis regulatory network.

Table S1. Gene ID and sequences of SHMT proteins from 12 species

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Table S2. Differentially expressed genes (DEGs) in *PdSHMT2* overexpression lines.

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Table S3. Expression patterns of core-DEGs in *PdSHMT2* overexpression lines.

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Table S4. qRT-PCR primers used for this study.

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