Supplemental materials to:

Functional Characterization of the Poplar Atypical Aspartic Protease Gene *PtAP66* in Wood Secondary Cell Wall Deposition

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Table S1. All primers used in this study

Purpose	Primer Name	Sequence (5′-3′)
Primers for subcellular localization vector construction		
PtAP66/*Potri.019G002100*	PtAP66-F	CACCATGGCTAATATGTCTTCTTTGTCT
	PtAP66-R	TAGTTCATCACACTGTGTTGGAAG
Primers for CRISPR/Cas9 vectors construction		
Cas9/gRNA-PtAP66-A	DT1-BsF/PtAP66-A	ATATATGGTCTCGATTGTTGTCTTTAGTCGTAGCACGTT
	DT1-F0/PtAP66-A	TGTTGTCTTTAGTCGTAGCACGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtAP66-A	AACGTGCCATTGCTTTGAATCTCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtAP66-A	ATTATTGGTCTCTAAACGTGCCATTGCTTTGAATCTC
Cas9/gRNA-PtAP66-B	DT1-BsF/PtAP66-B	ATATATGGTCTCGATTGAACATGTTTGAGCTTCGCTGTT
	DT1-F0/PtAP66-B	TGAACATGTTTGAGCTTCGCTGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtAP66-B	AACGTGTCCATTATTGCAGAATCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtAP66-B	ATTATTGGTCTCTAAACGTGTCCATTATTGCAGAATC
Primers used to identify Cas9-gRNAs targeted sites		
gPtAP66-1/2/3/4-identify	Identify PtAP66-F	GTCCTTGTCTAACCTACACAACC
	Identify PtAP66-R	ATATTCGCAGCCATCGCTGCAAG
Primers used for RT-qPCR		
PtAP66/*Potri.019G002100*	PtAP66-RT-F	CAAGTGTGAAAGCATCAGACAGTG
	PtAP66-RT-R	CATGCTTGCATCGGCAATCATATAG
GFP	GFP-F	ATGGTGAGCAAGGGCGAGGAGCT
	GFP-R	CTTGTACAGCTCGTCCATGCCGT
PtrCesA4/*Potri.002G257900*	PtrCesA4-F	GAGTTAAGGAAGATGGAGAGGTGT
	PtrCesA4-R	TGCACTGAGGACAGGACTGGTTGC

PtrCesA7A/Potri.006G181900	PtrCesA7A-F	TCGCCTTTCTCTCAGATACGAACG
	PtrCesA7A-R	TTACCCGTAACAAGAGGGGGTTCC
PtrCesA7B/Potri.018G103900	PtrCesA7B-F	CCCCTCTAGTCACGGGCAACACAC
	PtrCesA7B-R	AAGGTGCACATTGAAGCACCATCG
PtrCesA8A/Potri.011G069600	PtrCesA8A-F	GTTGGCCTCTGTCTTCTCTCTTGTC
	PtrCesA8A-R	CAATCTATAGAAATGCAGGTTTCAC
PtrCesA8B/Potri.004G059600	PtrCesA8B-F	GTTGGCCTCTGTCTTCTCTCTTGTT
	PtrCesA8B-R	CAATCAATGGAAATGCAGGTCTCCG
PtrGT47A-1/Potri.001G068100	PtrGT47A-1-F	TCTCACACCCATGGGCT
	PtrGT47A-1-R	AACGCTGGAGTAACGGG
PtrGT47C/Potri.009G006500	PtrGT47C-F	GTGGGACCCAGACGTTAGGC
	PtrGT47C-R	CTATTGGTTTGGAAGCCTCACG
PtrCSLA1/Potri.008G026400	PtrCSLA1-F	ACATATTCCTCTTCCTCCAATCC
	PtrCSLA1-R	CAAGAAAGATTCTTGCTAAGAGTG
PtrMYB021/Potri.009G053900	PtrMYB021-F	CCTTAACAGTGGTGATCATGGTTT
	PtrMYB021-R	TTACTAGTGATGTTGCAGTTTTTGT
PtrMYB074/Potri.015G082700	PtrMYB074-F	CATAAGTTAGAGTCGGTGTTTCA
	PtrMYB074-R	CAGCGACGTTAACGGATAGCTAT
PtActin2	PtActin2-RT-F	AACATGGGATTGTTAGCAACTGG
	PtActin2-RT-R	TCCATCACCAGAATCCAGCACA
AtActin2	ATActin2-F	TCTTCTTCCGCTCTTTCTTTCC
	ATActin2-R	TCTTACAATTTCCCGCTCTGC

Note: The sites for Topo cloning are underlined.

Table S2. The Cas9/gRNA-targeted mutations in *PtAP66* genes

Gene: Potri.019G002100 (*PtAP66*)					
Vector: Cas9/gRNA-*PtAP66*-A **gRNA**: g*PtAP66*-1 (TTTGTCTTTAGTCGTAGCACTGG); g*PtAP66*-2 (AAGATTCAAAGCAATGGCACTGG) **Target sequences**: *PtAP66*-1, TTTGTCTTTAGTCGTAGCACTGG; *PtAP66*-2, AAGATTCAAAGCAATGGCACTGG					
Line (#)	**g*PtAP66*-1** TTTGTCTTTAGTCGTAGCACTGG	**Mutation types** (Number of plasmids containing the cloned PCR products)		**g*PtAP66*-2** AAGATTCAAAGCAATGGCACTGG	**Mutation types** (Number of plasmids containing the cloned PCR products)	
ptap66-1#	TTTGTCTTTAGTCGTAG(T)CACTGG TTTGTCTTTAGTCGTAGCACTGG	+1 (×12) 0 (×8)	heterozygous	AAGATTCAAAGCA.....CTGG AAGATTCAAAGCAATGGCACTGG AAGATTCAAAGC.....CACTGG	-6 (×11) 0 (×7) -5 (×2)	chimera
ptap66-2#	TTTGTCTTTAGTCGTAG..... TTTGTCTTTAGTCGTAGCACTGG	-196 (×11) 0 (×9)	heterozygous	.....ACTGG AAGATTCAAAGCAATGGCACTGG	-196 (×11) 0 (×9)	heterozygous
ptap66-3#	TTTGTCTTTAGTCGTAG(A)CACTGG TTTGTCTTTAGTCGTAG(T)CACTGG	+1 (×10) +1 (×10)	biallelic	AAGATTCAAAGCAA..... AAGATTCAAA.....CTGG	-11 (×10) -9 (×10)	biallelic
ptap66-4#	TTTGTCTTTAGTCGTAGC..... TTTGTCTTTAGTCGTAG(T)CACTGG	-197 (×12) +1 (×8)	biallelic	.....TGG AAGATTCAAAGC.....CACTGG	-197 (×12) -5 (×8)	biallelic
Vector: Cas9/gRNA-*PtAP66*-B **gRNA**: g*PtAP66*-3 (CCGAGCGAAGCTCAAACATGTTG); g*PtAP66*-4 (TATTCTGCAATAATGGACACAGG) **Target sequences**: *PtAP66*-3, CCGAGCGAAGCTCAAACATGTTG; *PtAP66*-4, TATTCTGCAATAATGGACACAGG						
Line (#)	**g*PtAP66*-3** CCGAGCGAAGCTCAAACATGTTG	**Mutation types** (Number of plasmids containing the cloned PCR products)		**g*PtAP66*-4** TATTCTGCAATAATGGACACAGG	**Mutation types** (Number of plasmids containing the cloned PCR products)	
ptap66-5#	CCGAG.GAAGCTCAAACATGTTG CCGAGC.....	-1 (×11) -220 (×9)	biallelic	TATTCTGCAATAATGG.CACAGG .....ACAGG	-1 (×11) -220 (×9)	biallelic
ptap66-6#	CCGAGC..... CCGAGCGAAGCTCAAACATGTTG	-219 (×13) 0 (×7)	heterozygous	.....CACAGG TATTCTGCAATAATGGACACAGG	-219 (×13) 0 (×7)	heterozygous

ptap66-7#	CCGAGC**.** CCGAGCGAAGCTCAAACATGTTG	-219 (×14) 0 (×6)	heterozygous	**.**CACAGG TATT**.**TGCAATAA**.**CACAGG	-219 (×14) -5 (×6)	biallelic
ptap66-8#	CCGAGCGAAGCTCAAACATGTTG CCGAG**.**GAAGCTCAAACATGTTG	0 (×9) -1 (×11)	heterozygous	TATTCTGCAATAATGGACACAGG TATTCTGCAATAA(T)TGGACACAGG	0 (×9) +1 (×11)	heterozygous
ptap66-9#	CCGAGC**.**AAGCTCAAACATGTTG CCGAGC**.**	-1 (×11) -220 (×9)	biallelic	TATTCTGCAATAATGGA(T)CACAGG **.**ACAGG	+1 (×11) -220 (×9)	biallelic
ptap66-10#	CCGAG**.**GAAGCTCAAACATGTTG	-1 (×10)	heterozygous	TATTCTGCAATAATGG**.**CACAGG	-1 (×10)	heterozygous
ptap66-11#	CCGAGC**.** CCGAGCGAAGCTCAAACATGTTG	-220 (×12) 0 (×8)	heterozygous	**.**ACAGG TATTCTGCAATAATGGACACAGG	-220 (×12) 0 (×8)	heterozygous
ptap66-12#	CCGAGC**.** CCGAGCGAAGCTCAAACATGTTG	-220 (×11) 0 (×9)	heterozygous	**.**ACAGG TATTCTGCAATAATGGACACAGG	-220 (×11) 0 (×9)	heterozygous

Biallelic, two alleles of the gene were edited and the different mutations were induced. Chimeric, more than three different allelic mutations in a target editing site of the gene.

Figure S1. Multiple sequence alignment of PtAP66 with some APs in plants.
PtAP66 and PtAP5 are from *Populus*. ASPR1, CDR1, PCS1 and PASPA3 are from Arabidopsis. Nucellin is from barley. Cardosin A is from Cardoons. Cardosin A and PASPA3 have the PSI domain and are the typical APs. The PSI sequences are in blue frame. Nucellin belongs to the Nucellin-like APs. ASPR1, CDR1, PCS1, PtAP66 and PtAP5 are the atypical APs. Both the atypical and Nucellin-like APs do not have the PSI domain. The two conserved aspartic acid catalytic residues of PtAP66 are marked with red asterisk.

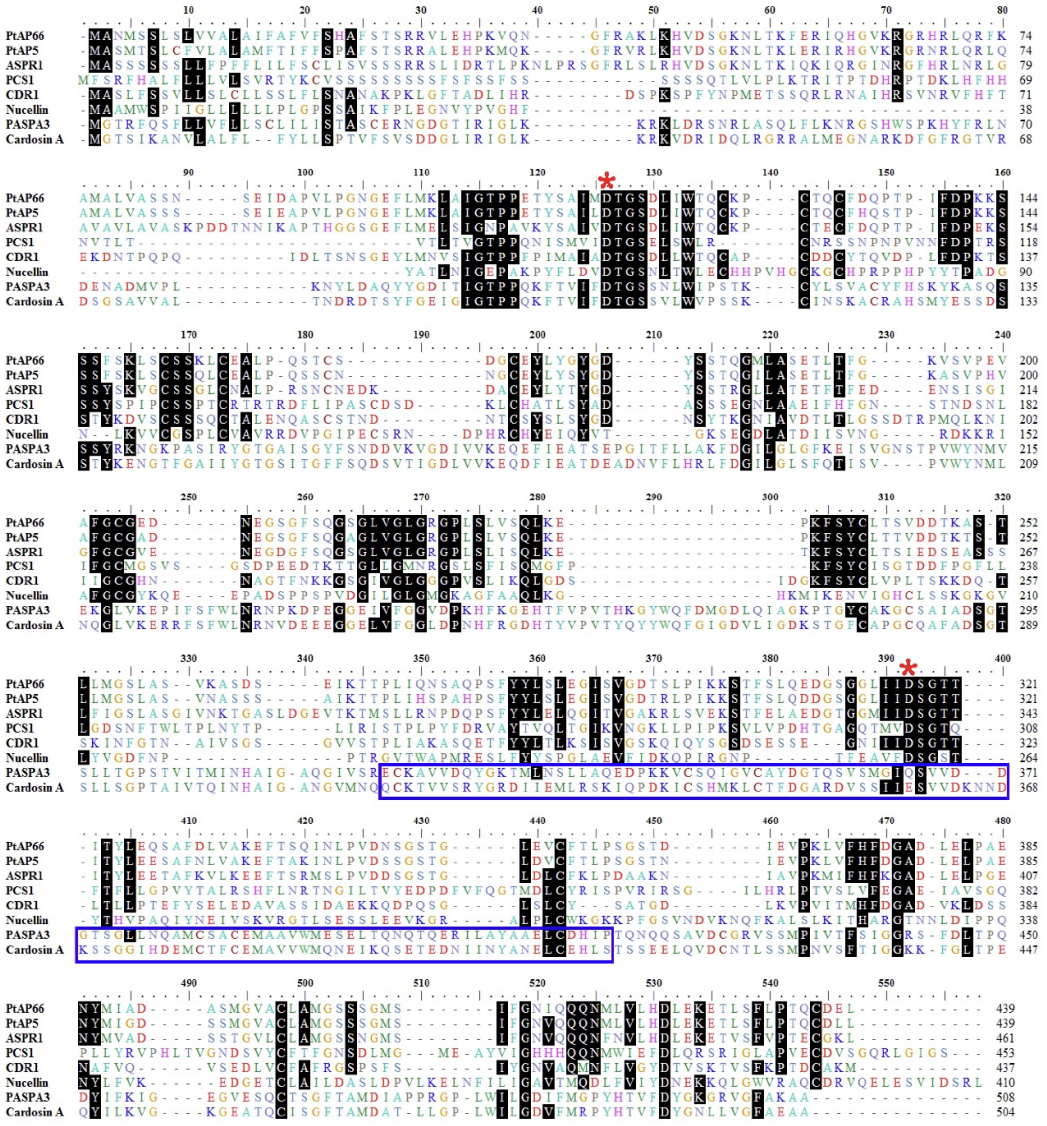

Figure S2. Phenotypes analyses of 4-month-old WT and the *ptap66* mutant plants. The WT and *ptap66* mutant plants grown in the greenhouse, these plants were measured for internode number (a), internode length (b), leaf length (c) and leaf width (d) at 4-month-old. Error bars represent ± SD values of six biological replicates.