

**Title: Intron-retained splice variants of the VND6 and SND1 transcription factors are dominant negatives that cross-regulate VND6 and SND1 members in *Populus trichocarpa*.**

Ying-Chung Lin<sup>1,\*</sup> (ylin14@ncsu.edu), Hao Chen,<sup>1</sup> Quanzi Li,<sup>1,2</sup> Rui Shi,<sup>1</sup> Sermsawat Tunlaya-Anukit,<sup>1</sup> Peng Shuai,<sup>1,3</sup> Wei Li,<sup>1,4</sup> Huiyu Li,<sup>1,4</sup> Ying-Hsuan Sun,<sup>5</sup> **Ronald R. Sederoff,<sup>1</sup>** and **Vincent L. Chiang<sup>1</sup>**

<sup>1</sup> Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina 27695

<sup>2</sup> State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry, Beijing 100091, China

<sup>3</sup> College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, China

<sup>4</sup> State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin, China

<sup>5</sup> Department of Forestry, National Chung Hsing University, Taichung 40227, Taiwan

Vascular-Related NAC-Domain 6 (VND6) is a key transcription factor (TF) involved in xylem and secondary cell wall differentiation. We discovered a splice variant of PtrVND6, called PtrVND6-C1<sup>IR</sup>, which is a dominant negative regulator of full-size PtrVND6 members. PtrVND6-C1<sup>IR</sup> lacks a transactivation domain and DNA binding ability, and can be translocated from the cytosol into the nucleus as a heterodimeric partner with any full-size PtrVND6 member. The formation of heterodimers between PtrVND6-C1<sup>IR</sup> and the full-size PtrVND6 disrupts the function of the full-size PtrVND6, thereby repressing transcription of PtrVND6 direct targets in its network. Secondary Wall-Associated NAC Domain 1 (SND1) also affects secondary cell wall biosynthesis. We previously demonstrated that the splice variant of PtrSND1-A2, PtrSND1-A2<sup>IR</sup>, can inhibit the PtrSND1 transcription network through the same mechanism. Using laser capture microdissection, we found that PtrVND6-C1<sup>IR</sup>, PtrSND1-A2<sup>IR</sup>, and all full-size PtrVND6 and PtrSND1 are expressed in both fiber and vessel cells. We further discovered that either PtrVND6-C1<sup>IR</sup> or PtrSND1-A2<sup>IR</sup> can inhibit both PtrVND6 and PtrSND1 transcription by the same mechanism. The cross-regulation between the PtrSND1 and PtrVND6 families through their splice variants suggests a general mechanism for the function of xylem specific NAC TFs controlling wood formation.