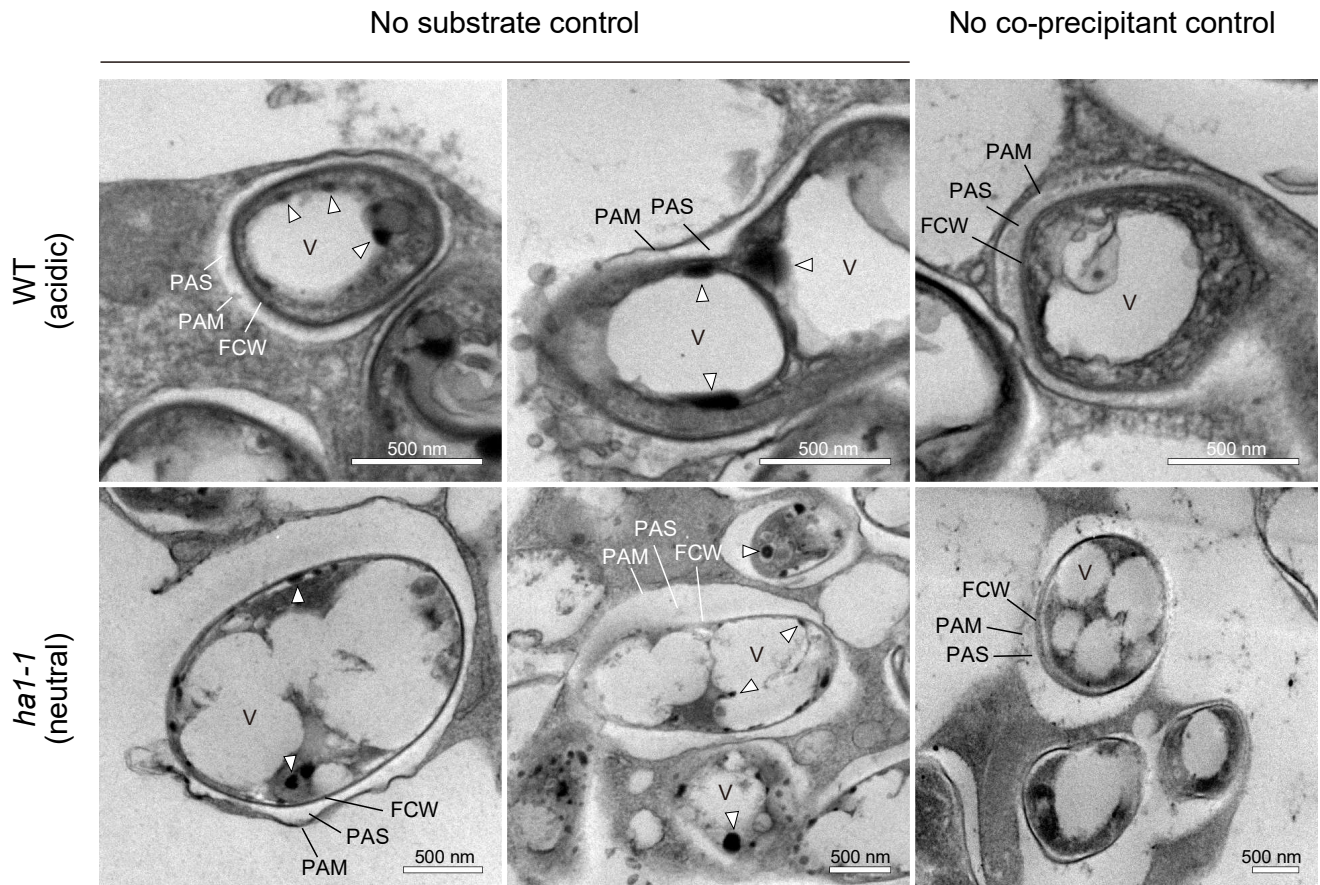


**Supplementary Figure 1** | Negative controls for polyphosphate (polyP) labeling using the polyP binding domain (PPBD) affinity method. Sections of wild-type (WT) *Lotus japonicus* roots colonized by *Rhizophagus irregularis* were incubated without PPBD (no PPBD control) or with an excess of tripolyphosphate (absorption control with polyP<sub>3</sub>) during the PPBD-anti-Xpress antibody incubation. **(A)** Fluorescence microscopy images of Alexa Fluor 488, a secondary antibody conjugate, and differential interference contrast (DIC) images of arbuscules. **(B)** Micrographs of transmission electron microscopy. The right panels show magnified images of the dotted areas in the left panels. FCW: fungal cell wall, PAM: periarbuscular membrane, T: trunk hypha.



**Supplementary Figure 2** | Negative controls for enzyme cytochemistry of phosphatase activity. Transmission electron micrographs show acid and neutral phosphatase activity in arbuscule-containing cortical cells of wild-type (WT) and *ha1-1*, respectively. Mycorrhizal roots were incubated in the absence of  $\beta$ -glycerophosphate (no substrate control) or cerium salt (no co-precipitant control) in the reaction buffer. In the no substrate control, a few precipitates (white triangles) were observed in fungal vacuoles. FCW: fungal cell wall, PAM: periarbuscular membrane, PAS: periarbuscular space, V: vacuole.

**Supplementary Table 1 | Primers used in this study.**

Gene name	Primer sequence (5'→3')	Reference
Screening of <i>LORE1</i> insertion mutants		
<i>HA1</i> (plant ID: 30006854)	TTTCATTGTGGAAGCTGGACATTTTGA	This study
	TGGTTCACATAGGCTGTCTCAGCA	
P2 primer	ACCTTGTTGCTTCAGCCATGG	Urbanski <i>et al.</i> 2012.
RT-PCR		
<i>HA1</i>	AAGCGCGGAGATTATCAAGA	This study
	TTTACCACCAGATCCCAAGC	
<i>EF2</i>	AGTTGCTTTGGTTGGTTTGG	This study
	AAGTGGGGTACCTTGCCTCT	
qRT-PCR		
<i>Lotus japonicus</i>		
<i>EF2</i>	GGTGGCTGCTGCTGGAATTA	This study
	ACGGGTATCAGTCATACGGACAT	
<i>PT1</i>	TGCCGGCCGAGATTTTC	This study
	GCAGCTGAGATTCCATGACAAG	
<i>PT2</i>	GGGCCTAATGCCACCACAT	This study
	CGGAATCTAGCCGGGAAAA	
<i>PT3</i>	CCTCACGCGGAGGAAATG	This study
	GTTGATGCTCTTGCAACCTTGTA	
<i>PT4</i>	TCCGGGCTCTCCTTTGG	Kojima <i>et al.</i> 2014.
	AGAAGCATAGCGTTCCCATCA	
<i>AMT2;2</i>	ACACATGCTTGCACTGCTACC	Guether <i>et al.</i> 2009.
	CTGCCCATCCTTGAACAACCC	
<i>RAM2</i>	GCCCGTAGCCATGGTGAA	This study
	CCCTCTTGCACTGGTTCCA	
<i>Rhizophagus irregularis</i>		
<i>EF1β</i>	CCCATGCAGCTCGATGGTA	Kobae <i>et al.</i> 2015.
	TGCCAGGAAGTGAAGAAAATGA	
<i>VTC1</i>	GAGAATCAGGTCCTTATGATGATAGGT	This study
	CACCCACGAGGAAAAAGCA	
<i>VTC2</i>	GCACGACAGACGTACAAGTTACAA	This study
	AATGGGCACGCGTTCAAA	
<i>VTC4</i>	CATGGTGTGCAACACTTATGG	This study
	GTGGAAGCCAGAAAGGAAAAAG	
<i>PPN1</i>	GAAAGATTTAACAATACCGAGTTGGAT	This study
	TGCGCTATTAAGTCCATCATTAAACA	
<i>PPN2</i>	TCGACCACCTAATGCTTTTGG	This study
	ATCGCAATTATATTCTCCCCATTTT	
<i>PPN3</i>	CCGAATAGTGACCCATCCAAA	This study
	CATTCCGATTGTGGAGTTCCA	

Guether et al. 2009. Plant Physiology 150: 73–83; Kobae et al. 2015. Mycorrhiza 25: 411–417;  
Kojima et al. 2014. Plant & Cell Physiology 55: 928–941; Urbanski et al. 2012. Plant Journal 69: 731–741.