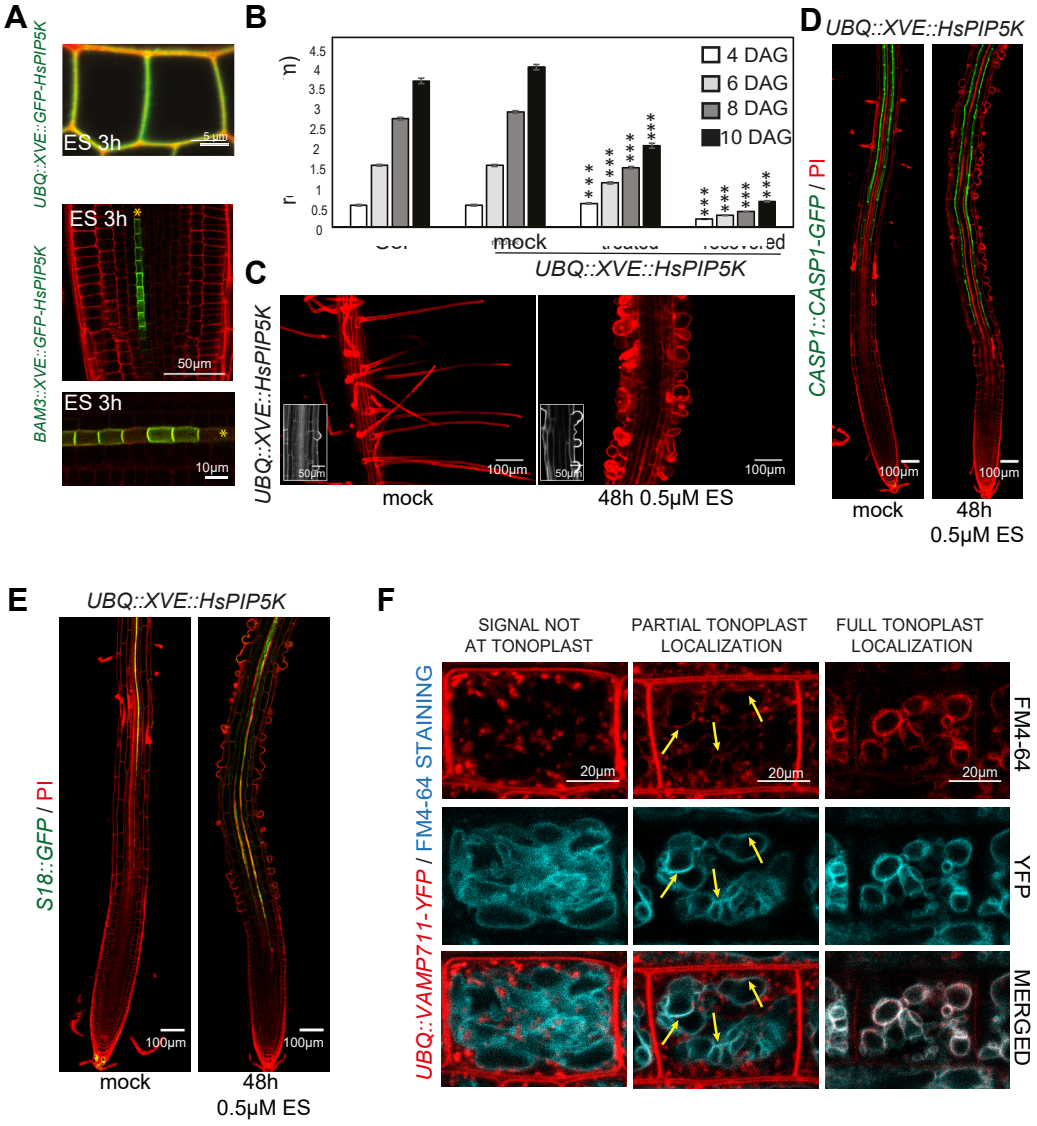

Fig. S1. Effect of a disturbed PtdIns(4,5)P_2 homeostasis in root development. (**A**) Plasma membrane localization of *GFP-HsPIP5K* in the root meristem of a PI-stained root of imaged using confocal microscopy. In the lower panel, magnification of a protophloem strand from *BAM3::XVE::GFP-HsPIP5K* treated with estradiol for 3h. Yellow asterisks mark protophloem strand. (**B**) Root growth quantification of the indicated genotypes after 4, 6, 8 and 10 days after germination. Plants were grown in standard media and transferred for 4 days to ES-included plates (treated) or transferred to a standard media after 4 days growing on ES-supplemented plates (recovered). (**C**) Root hair phenotypes of 6 day old seedlings after 48h 0.5µM ES treatment. Roots were stained with PI and immediately visualized using confocal microscopy. Black and white images in corners show root hair initiation. (**D**) Analysis of *CASP1::CASP1-GFP* expression in PI-stained roots subjected to ES treatments. (**E**) Premature xylem differentiation in a 6-day-old seedlings as revealed by the early expression of the xylem reporter *S18::GFP.* (**F**) Analysis of FM4-64 uptake dynamics in an epidermal cell harbouring the vacuolar marker VAMP711-YFP. Three different scenarios (absence, partial and full co-localization) are represented. Yellow arrows mark portion of the vacuole stained with FM4-64. Error bars represent standard errors (n=20) and asterisks indicate a statistically significant difference between mock and ES-treated roots by Students t-Test ($P \geq 0.0001$).

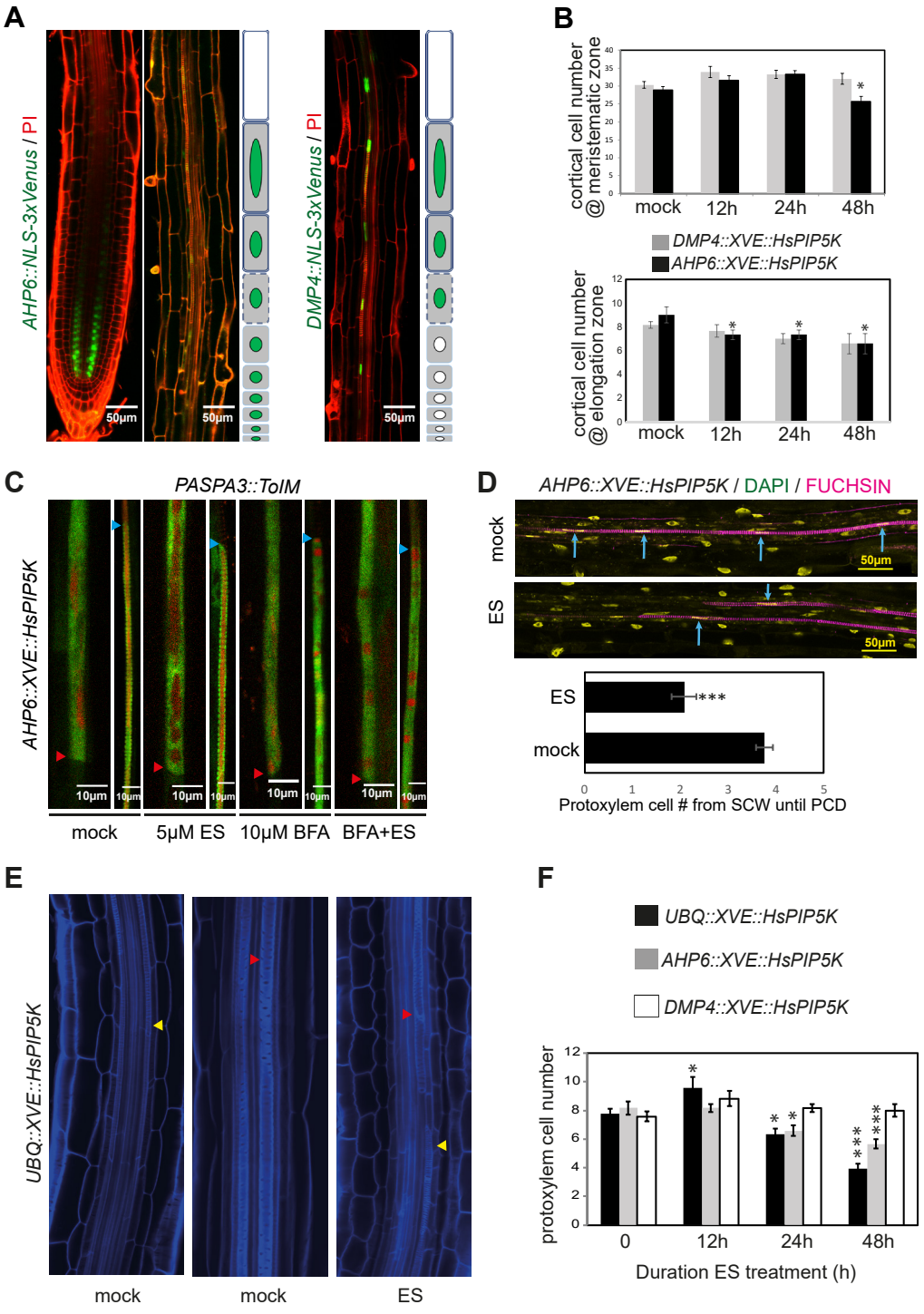

Fig. S2. A protoxylem-specific genetic tool to increase PtdIns(4,5)P_2 levels. (**A**) Analysis of *AHP6* and *DMP4* expression by confocal microscopy analysis in 5-day-old roots stained with PI. Schematic representation of *AHP6* and *DMP4* expression in protoxylem strands before and after cell wall thickening. (**B**) Quantification of meristematic activity and elongation rate expressed by number of cortical cells in *AHP6::XVE::HsPIP5K* and *DMP4::XVE::HsPIP5K* roots subjected to 10µM ES treatments. (**C**) Vacuolar morphology in the first xylem cell expressing *PASPA3::ToIM* (red triangle) and the last cell before PCD completion (blue triangle) of indicated treatments. *AHP6::XVE::HsPIP5K* was induced for 24h with ES. (**D**) Quantification of nucleated xylem cells after SCW formation in *AHP6::XVE::HsPIP5K* roots induced for 24h with 5µM ES. 7-day-old roots were fixed and stained with DAPI and fuchsin and visualized by confocal microscopy. (**E**) Confocal microscopy analysis of protoxylem and metaxylem differentiated elements in calcofluor-stained 5-day-old roots upon 48h of 0.5µM ES treatment. Yellow triangle mark first protoxylem differentiating cell whereas red triangle points to the first metaxylem differentiated cell based on cell wall morphology. (**F**) Quantification of metaxylem differentiation rate in the indicated transgenic lines based on the number of protoxylem cells between first protoxylem differentiated element and the appearance of the first metaxylem differentiated cell assessed by cell wall morphology. *UBQ::XVE::HsPIP5K* was exposed to 0.5µM ES whereas *AHP6::XVE::HsPIP5K* and *DMP4::XVE::HsPIP5K* lines were exposed to 10µM ES for the indicated times. Data represents mean ± S.E. and asterisks marks a statistically significant difference by Student's t-Test ($P \geq 0.0001$, n=12).

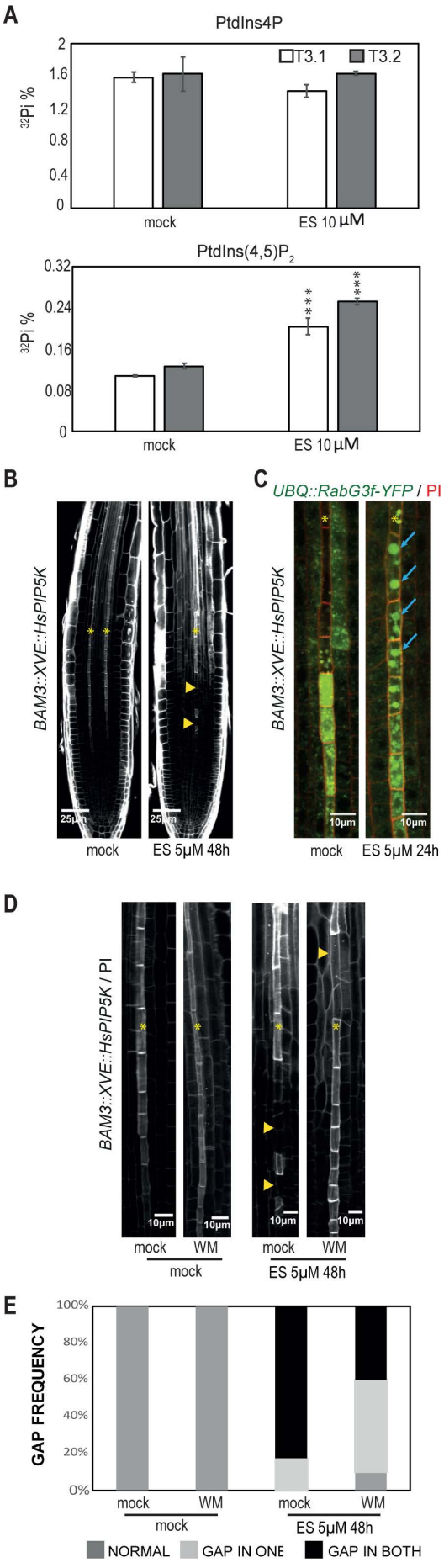

Fig. S3. A protophloem-specific genetic tool to increase PtdIns(4,5)P_2 levels. (**A**) Quantification of normalized PtdIns4P and PtdIns(4,5)P_2 levels in *BAM3::XVE::HsPIP5K* roots of two independent transgenic lines upon ES treatments. Error bars represent standard errors (n=3) and asterisks indicate a statistically significant difference between mock and ES-treated roots by Students t-Test ($P \geq 0.001$). (**B**) Analysis by confocal microscopy of root meristem and protophloem continuity in *HsPIP5K*-induced roots stained with Calcofluor White. (**C**) Analysis of vacuolar morphology in protophloem strands of *BAM3::XVE::HsPIP5K* roots upon ES treatment. Blue arrows indicate the presence of big vacuolar compartments when increasing PtdIns(4,5)P_2 levels. (**D, E**) Wortmannin (WM) treatment partially rescues the gap phenotype of *HsPIP5K*-induced roots. Protophloem strands of calcofluor stained-roots were visualized by confocal microscopy and the appearance of gaps in one or both strands was quantified (n=15 roots). Asterisk marks protophloem strands and yellow arrows mark gap cells.

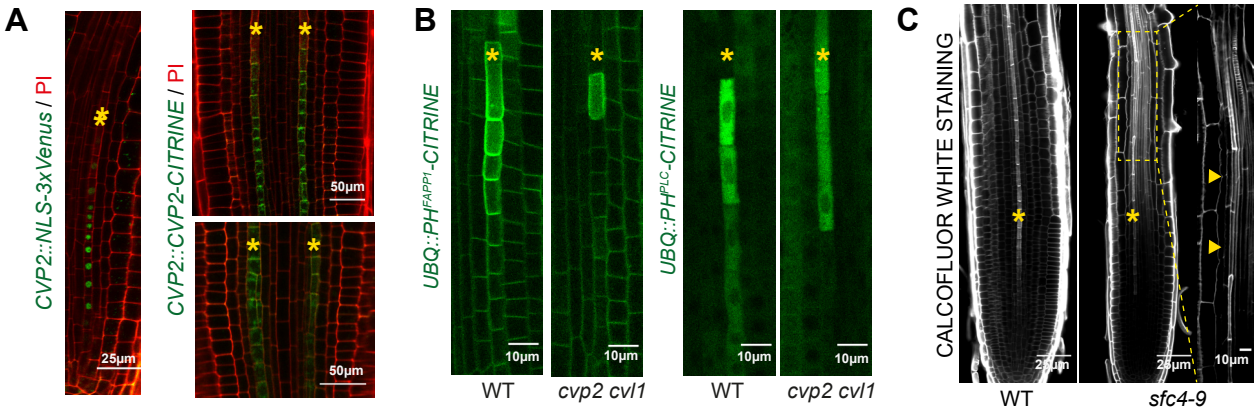

Fig. S4. CVP2 and VAN3/SCARFACE impact on protophloem differentiation. (**A**) Confocal microscopy analysis of *CVP2* expression pattern and subcellular localization in a 5-day-old root tip. CVP2-CITRINE is polarly localized at the PM in protophloem cells near the enucleation zone (upper panel) whereas is mainly localized in the inner subcellular compartments in provascular cells (lower panel). (**B**) Subcellular distribution of PtdIns4P biosensor *UBQ::PH^{FAPP1}-CITRINE* and PtdIns(4,5)P_2 biosensor *UBQ::PH^{PLC}-CITRINE* in wild type and *cvp2 cvl1* protophloem strands. (**C**) Analysis of root meristem and protophloem continuity in *scf4-9* 6-day-old roots stained with calcofluor white and visualized by confocal microscopy. Asterisks mark protophloem strands, while yellow triangle mark gap cells.

SUPPLEMENTARY TABLE S1. PCR Primers Used in This Study

OBJECTIVE	GENE NAME	SIZE (bp)	PRIMER NAME	5'-SEQUENCE-3'
Tissue specific *HsPIP5K* expression (protoxylem)	AHP6 promotor	1594	pAHP6_PmeI_F	ATT GTT TAA ACC ACG GGG CGC AAA GAA GCA TGA C
			pAHP6R_BstBI_R	ATT TTC GAA CAA CGG CAC ACC CGT CTT GTC
Tissue specific *HsPIP5K* expression (protoxylem)	DMP4 promotor	1358	pDMP4_PmeI_F	ATT GTT TAA ACC CAG CGC AGG TAC ATG TTT AA
			pDMP4_BstBI_R	ATT TTC GAA ATC TTT GAA GTT GTT TCC TTT GTC C
Tissue specific *HsPIP5K* expression (protoxylem)	BAM3 promotor	2139	pBAM3_SbfI_F	ATT CCT GCA GGC TGC TTC CCT AGT TTA TCT AAT AAA TCT GAT G
			pBAM3_BstBI_ReV	ATT TTC GAA TGT AAC ATC AGA AAA ATA AAA ACA AAA ATT TGT CC
CVP2 translational reporter	CVP2 cDNA	2229	CVP2cDNA_F	CTG CCC TCA TTG TCT CGC CTC
			CVP2cDNA_R	GAA GAA GCT GAG CTC GGT GTA TCC
AHP6 expression pattern	AHP6 promotor	1594	pAHP6_attB4_F	GGG GAC AAC TTT GTA TAG AAA AGT TGT CCA CGG GGC GCA AAG AAG CAT GAC
			pAHP6R_attB1r_R	GGG GAC TGC TTT TTT GTA CAA ACT TGT CAA CGG CAC ACC CGT CTT GTC
DMP4 expression pattern	DMP4 promotor	1358	pDMP4_attB4_F	GGG GAC AAC TTT GTA TAG AAA AGT TGT CCC AGC GCA GGT ACA TGT TTA A
			pDMP4_attB1r_R	GGG GAC TGC TTT TTT GTA CAA ACT TGT ATC TTT GAA GTT GTT TCC TTT GTC C
Preparation of GFP-HsPIP5K construct to be inserted into XVE inducible vector pMDC7	HsPIP5K	1650	HsPIP5K_attB1_F	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT CAT GGC GTC GGC CTC CTC CGG
			HsPIP5Kstop_attB2_R	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA TTA ATG GGT GAA CTC TGA CTC TGC
	GFP	716	eGFP_attB1_F	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT CAT GGT GAG CAA GGG CGA GGA G