Supplemental Information

Supplemental Figures

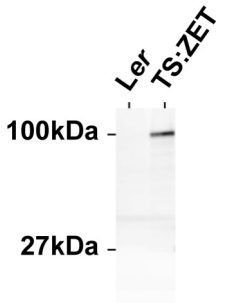

Fig. S1. Western blot using an extract of 6-day *pZET::TS:ZET zet-1* seedlings probed with an anti-GFP antibody. A single band is detected indicating an intact TS:ZET fusion protein. The observed molecular weight of the fusion protein (100 kDa) is larger than the expected one (80 kDa) suggesting posttranslational modification.

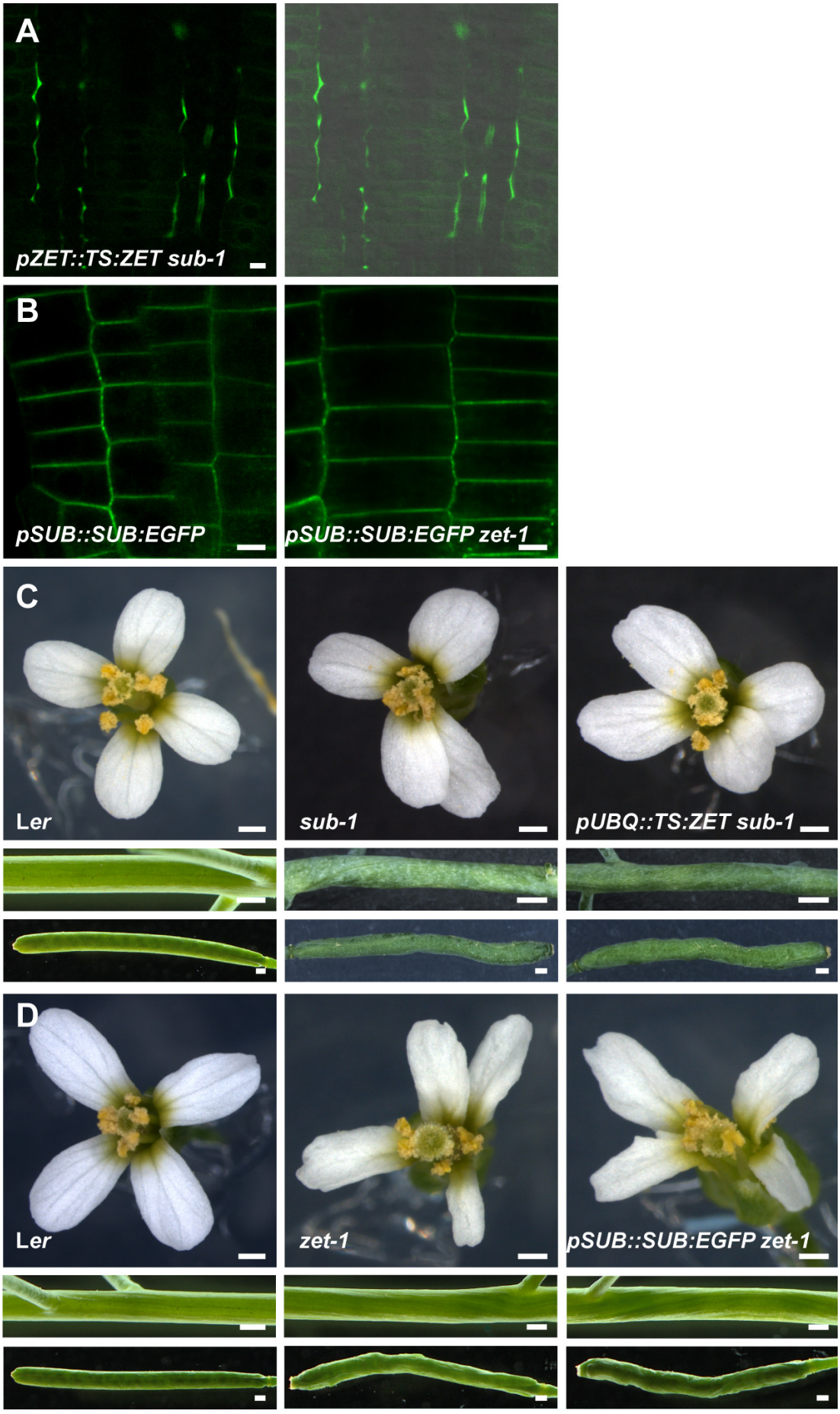

Fig. S2. Analysis of TS:ZET and SUB:EGFP signal localization in different mutant backgrounds. (A) and **(B)** Optical sections through the meristematic region of a 5-day root. **(C)** and **(D)** Upper panels: Stage 13 flower. Middle panel: Siliques. Bottom panel: Stem. Genotypes are indicated. **(A)** Note regular TS:ZET signal distribution in *sub-1* (compare with Fig. 3C). Right panel includes DIC channel. **(B)** The spotty SUB:EGFP signal is identical in wild type (left panel) and *zet-1* (right panel). **(C)** A functional *pUBQ::TS:ZET* transgene does not influence the *sub-1* phenotype. **(D)** A functional *pSUB::SUB:EGFP* transgene does not influence the *zet-1* phenotype. Abbreviations: DIC, differential interference contrast. Scale bars: 10 μm.

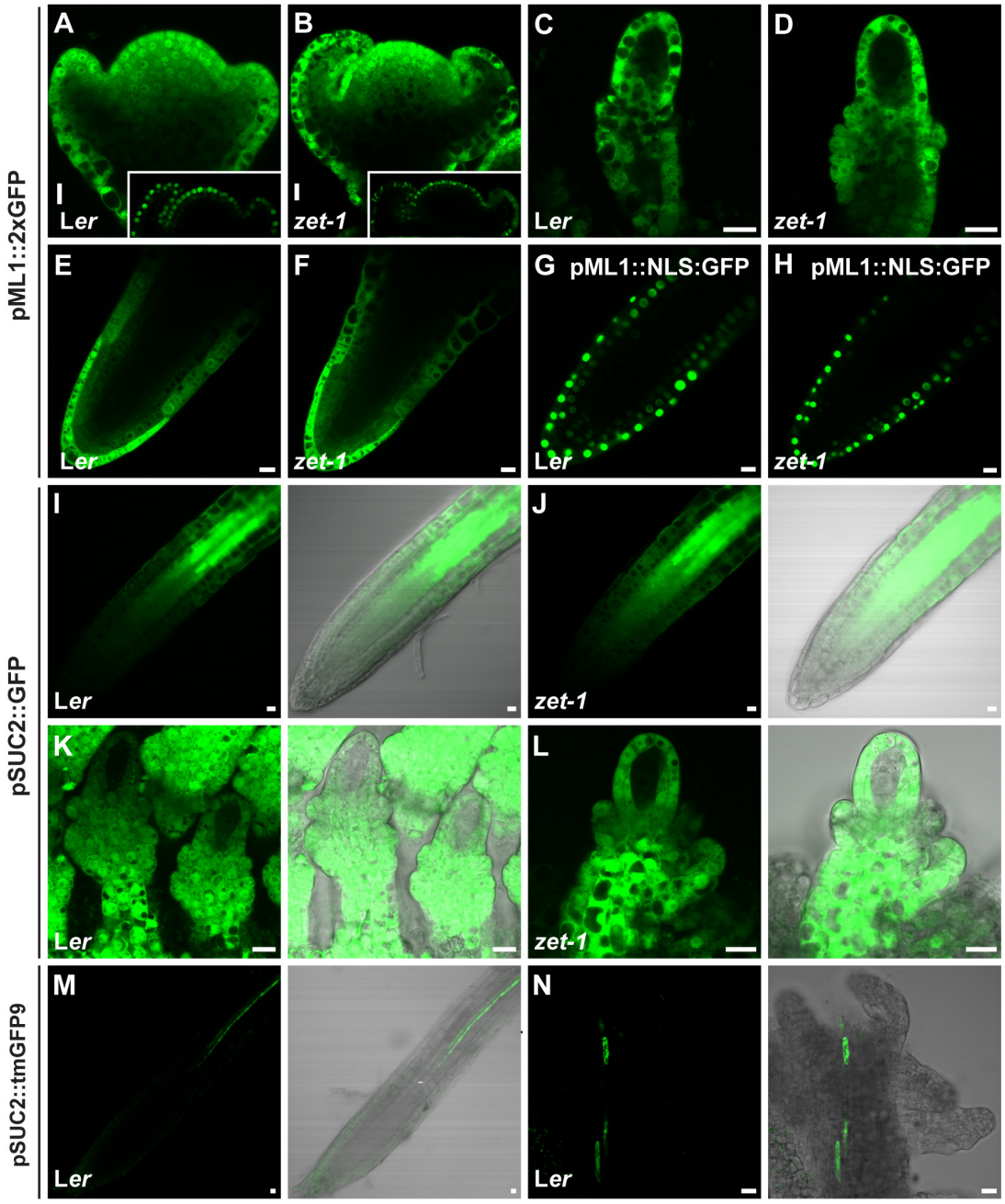

Fig. S3. Inter-cell layer movement of GFP in L*er* and *zet-1*. Longitudinal confocal micrographs of tissue expressing the pML1::2xGFP or pSUC2::GFP reporters, respectively. Genotypes are indicated. (**A**) and (**B**) Stage 3 floral meristems, (**C**) and (**D**), (**K**) and (**L**) Stage 2-III ovules. (**E**) to (**H**) Lateral root tip of 10-day seedling. (**I**) and (**J**), (**M**) Main root tip of 5-day seedling. (**N**) Stage 11 carpel tissue showing placenta and young stage 2-III ovules. (**A**) to (**F**) Free 2xGFP expression driven by the epidermis-specific *ML1* promoter. The diffuse gradient of GFP signal intensity

indicates movement of GFP from the epidermis to sub-epidermal tissue. Signal distribution does not noticeably differ between genotypes. **(A)** and **(B)** Note the epidermis-restricted localization of a nuclear pML1::NLS:GFP reporter (insets). (**G**) and (**H**), Control using a nuclear localized GFP reporter (pML1::NLS:GFP). Note epidermis-specific signal. **(H)** Different optical section from root depicted in Fig. 6B. (**I**) to (**L**) Left panels: confocal micrographs, right panels: overlay with DIC channel. Free GFP expression driven by the *SUC2* promoter. Note unaltered diffusion from the metaphloem companion cells to the lateral cell layers. (**M**) and (**N**), The *SUC2* promoter drives expression of a membrane-anchored GFP (tmGFP9). Signal marks the expression domain of the *SUC2* promoter in companion cells of the metaphloem. Abbreviations: DIC, differential interference contrast. Scale bars: 10 µm.

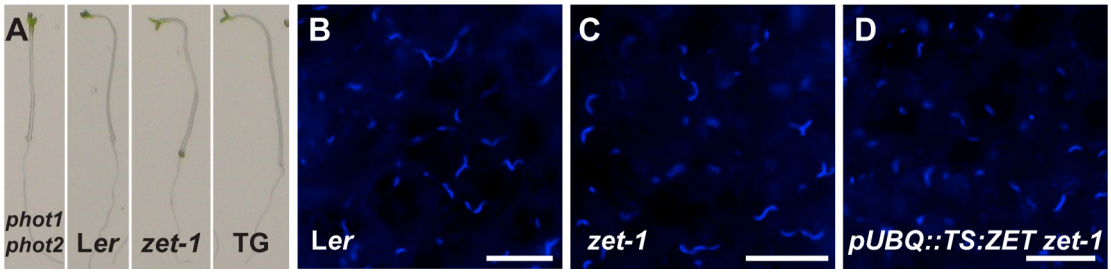

Fig. S4. Phototropism and callose deposition assays. (**A**) Phototropism assay. 3-days-old dark-grown seedlings were illuminated with blue light coming from the left. The *phot1 phot2* control plants lack the blue light receptor responsible for phototropism and are defective in the photropism response (Christie et al., 1998). TG: *pUBQ::TS:ZET zet-1*. Phototropism appears normal in plants with altered *ZET* activity. (**B**) to (**D**) Cotyledon epidermis. Callose deposition upon addition of 1 µM flg22 appears unaltered in plants with altered *ZET* activity. Scale bars: 50 µm.

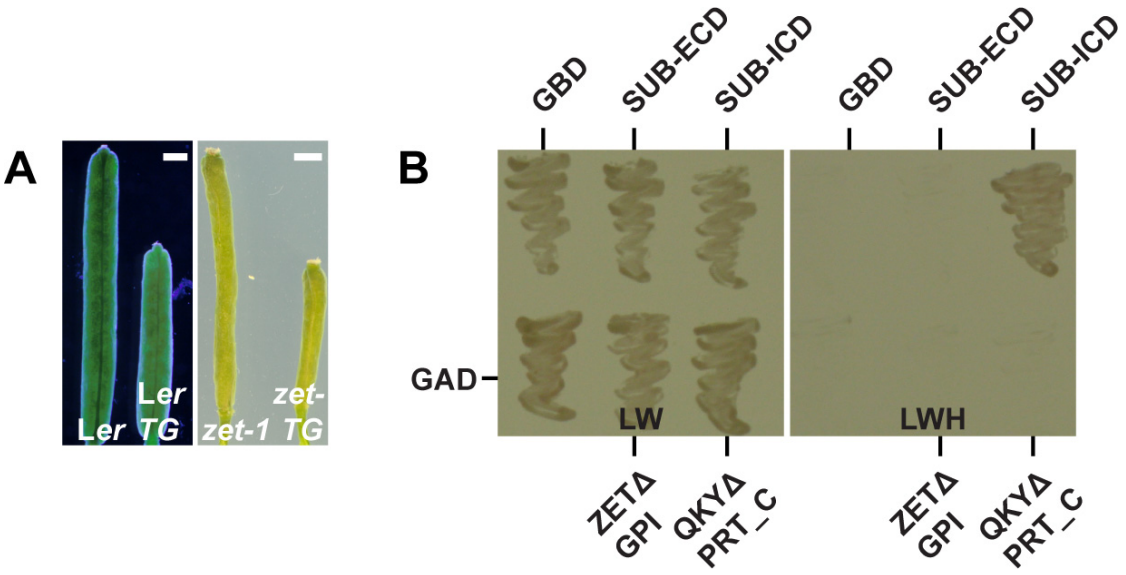

Fig. S5. Analysis of *SUB* and *ZET* interactions. (A) Plants ectopically expressing *SUB:EGFP* in L*er* or *zet-1* exhibit comparably shorter siliques. Genotypes are indicated. Abbreviations: TG: *pUBQ::SUB:EGFP*. Scale bars: 1 mm. **(B)** Yeast two-hybrid assay involving a ZET variant lacking the GPI anchor addition domain (ZETΔGPI), a QKY variant, including all four C2 domains but lacking the PRT_C domain (QKYΔPRT_C) fused to the GAL4 activating domain (GAD) and the extracellular domain (ECD) or intracellular domain (ICD) of SUB fused to the GAL4 DNA-binding domain (GBD), respectively (Vaddepalli et al., 2014). Growth on –LW panel indicates successful transformation of both plasmids and on –LWH panel indicates presence or absence of interaction.

Supplemental Tables

Table S1. Summary of *zet* alleles.

Allele	Mutagen	Mutation#	Amino acid change/transcript	Background	Reference
zet-1	EMS	T>Δ, 265	S88--*	L*er*	this study Fulton et. al. 2009
zet-2	T-DNA Ds Transposon-ET13436	193/LB	M64--*	L*er*	this study

#the coordinates refer to the genomic sequence and relate to the ATG of *ZET* (At1g64760).
--*indicates various aberrant sequences of residues followed by a stop.

Table S2. Primers used in this study.

Primer Name	Sequence (5'-3')
CER453151_F	GCTCTGTTAGGTACGCCTTTTGTTACAAAC
CER453151_R	GTGAGTAACGTGCATGTTGTTGGAATC
F13011_F	AGTGATTGGATGGTCGGTATG
F13011_R	TGGTTTTGGTGAGTTCTGCT
530(TaqI)_F	TCTGAATCTGAAACCACGACCAAGG
530(TaqI)_R	GGAGTCCACTCAGGTAACTTTTTCC
840(ClaI)_F	GCTGATGTATTGGATTTGAGTCGGT
840(ClaI)_R	AAGCCGAAGAGCCACAACAGGAAAT
ZETsense_Insitu_F	TAATACGACTCACTATAGGGTTCCCCAAAACCAAAA AGTTTAC
ZETsense_InsituM_R	ATCTGTAAGCACTGCCTGCATTA
ZETas_Insitu_F	TTCCCCAAAACCAAAAAGTTTAC
ZETas_InsituM_R	TAATACGACTCACTATAGGGATCTGTAAGCACTGCC TGCATTA
EGFP_sense_F	TAATACGACTCACTATAGGGGTCGAGCTGGACGGC GACGT
EGFP_sense_R	GCGCTTCTCGTTGGGGTCTTTGCTCAGGG
EGFP_as_F	GTCGAGCTGGACGGCGACGT
EGFP_as_R	TAATACGACTCACTATAGGGGCGCTTCTCGTTGGGG TCTT
PDCB1_EcoRI_F	CCGAATTCTGGTGTGTGTGTAAGACAGGGC
PDCB1_Xho1_R	TGCTCGAGGCTGTCTGTCGTGTAATCCGGG
ZETGH_EcoRI_F	CCGAATTCTTGGGTGTGAATTGGGGAACAA
ZETX8_XhoI_R	TGCTCGAGATTGCATTGTCCTTGAGATATA
ZET_Entry clone_F_KpnI	TAGGTACCATGTCGAATCTGTTGGCTC TC
ZET_Entryclone_R_ XhoI	TAGACTCGAGTCAAAACATCATCCCTGATAAC
ZET_NdeI_F (Y2H)	TGCATATGTTGGGTGTGAATTGGGGAAC
ZET_EcoRI_R (Y2H)	GAGAATTCATTGCATTGTCCTTGAGATA
ZETpro_F	ATGAGCTCTGATGGAGAGTAAGGAGAGG
ZETpro_R	TAACCGGTCGATTTTCACCTGAGAAAGAT
ZET_EcoRI_F	AGTGAATTCTTGGGTGTGAATTGGGGAA
ZET_BamHI_R	TCAAGGACAATGCAATTTCCGGATCCAT
ZET F448*_F	CAAGGACAATGCAATTGACCTATTCAGATTGTGG
ZET F448*_R	CCACAATCTGAATAGGTCAATTGCATTGTCCTTG

Supplemental Materials and Methods

Map-Based Cloning of *ZET*

To map the *ZET* locus at high resolution, an F2-mapping population was generated by outcrossing *zet-1* (L*er*) to wild-type (Col). The F2 progeny were screened for *zet* individuals based on twisted inflorescence morphology. Genomic DNA was isolated and used for PCR-based amplification of molecular markers. The *zet-1* mutation was initially mapped to a single region on the lower arm of chromosome 1 between markers CER453151 and F13011. Further fine-mapping placed *zet-1* in a 138 kb interval between the two CAPS markers 530(TaqI) and 840(ClaI). Candidate genes were analyzed by T-DNA insertion mutant analysis and/or sequence determination revealing that *zet-1* carries a mutation in At1g64760. A second *zet* mutant carrying a mutation in At1g64760 (*zet-2*) was identified in the Cold Spring Harbor GeneTrap Ds-transposon insertion line collection (Sundaresan et al., 1995). Finally, the mutant *zet-1* phenotype could be fully complemented by a construct encoding a T-Sapphire:ZET fusion protein driven by the native *ZET* promoter (pZET::TS:ZET) (Fig. 1).

Supplemental References

Christie, J. M., Reymond, P., Powell, G. K., Bernasconi, P., Raibekas, A. A., Liscum, E. and Briggs, W. R. (1998). Arabidopsis NPH1: a flavoprotein with the properties of a photoreceptor for phototropism. *Science* **282**, 1698-1701.

Sundaresan, V., Springer, P., Volpe, T., Haward, S., Jones, J. D., Dean, C., Ma, H. and Martienssen, R. (1995). Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes Dev* **9**,

1797-1810.

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