Ron et al

SUPPLEMENTAL DATA

Hairy root transformation using *Agrobacterium rhizogenes* as a tool for exploring cell type-specific gene expression and function using tomato as a model

Mily Ron, Kaisa Kajala, Germain Pauluzzi, Dongxue Wang, Mauricio A. Reynoso, Kristina Zumstein, Jasmine Garcha, Sonja Winte, Helen Masson, Soichi Inagaki, Fernán Federici, Neelima Sinha, Roger Deal, Julia Bailey-Serres, Siobhan M. Brady.

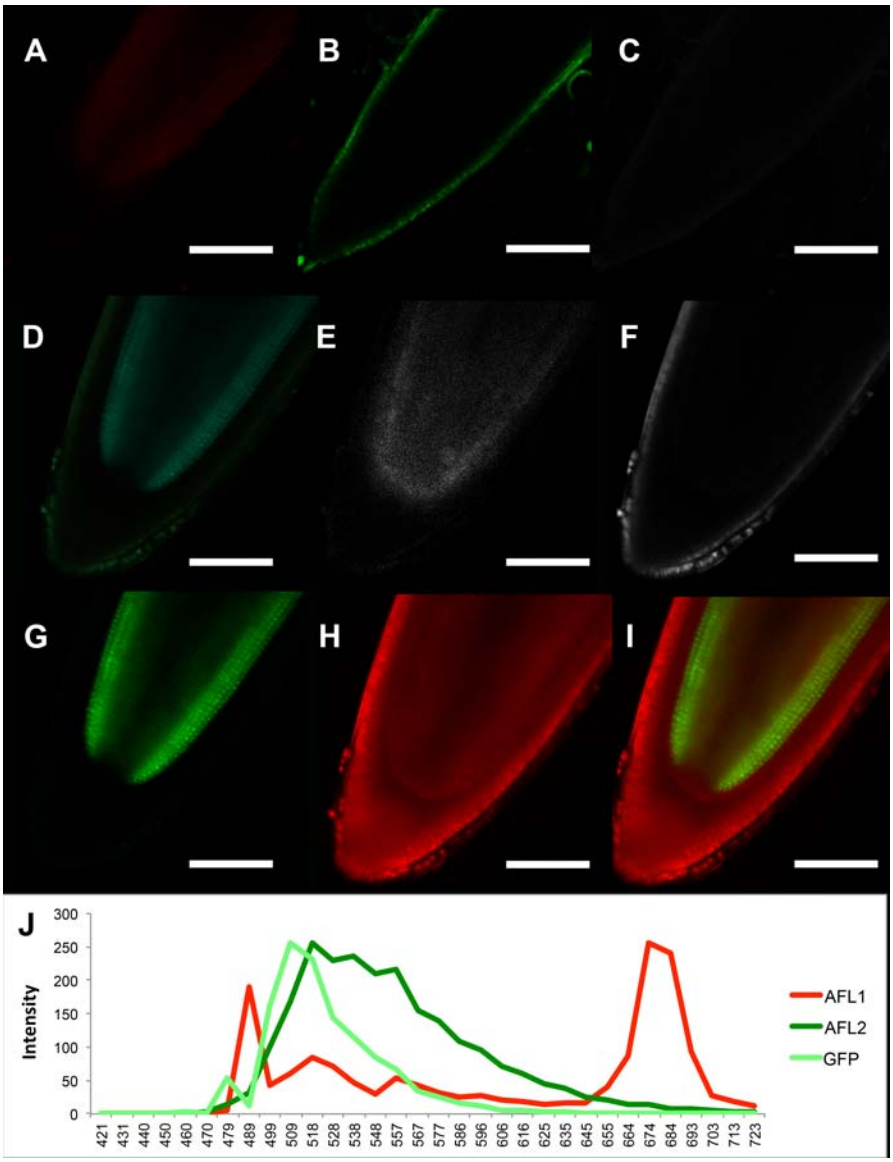


Figure S1: Use of spectral scanning and linear unmixing to resolve light emitted from GFP and autofluorescence.

Tomato hairy roots produced with *A. rhizogenes* without a binary vector were excited at 488nm and scanned spectrally across the visible light range in 9.7nm sections. Zen 2011 software was allowed to autodetect the spectral components of this autofluorescence, and these spectra were saved as autofluorescence (AFL) references (**J**). When this tomato hairy root spectral scan was resolved using these two autofluorescence references (**A**, **B**), the residual channel had no signal (**C**).

The sample roots were scanned spectrally as described above, and the saved

autofluorescence and nls-GFP spectra were used in resolving the GFP signal. As an example, a tomato hairy root expressing *SlCO2pro-nls-GFP 35Spro-TagRFP-LTI6b* was scanned spectrally. **D** shows the spectral scan result with each 9.7nm channel shown with a representative color presenting each channel. The spectral scan was successfully resolved by linear unmixing to the two components of autofluorescence (**E, F**) and GFP (**G**), and residual channel had no signal (not shown). The sample was also excited at 561 nm to visualize the plasma membrane bound TagRFP signal (**G**). The GFP signal from the spectral scan was overlaid with the TagRFP signal to allow clear identification of the cell-types expressing GFP (**H**). The spectra used to resolve AFL and GFP are shown in **J**.Scale bars represent 100 μm.

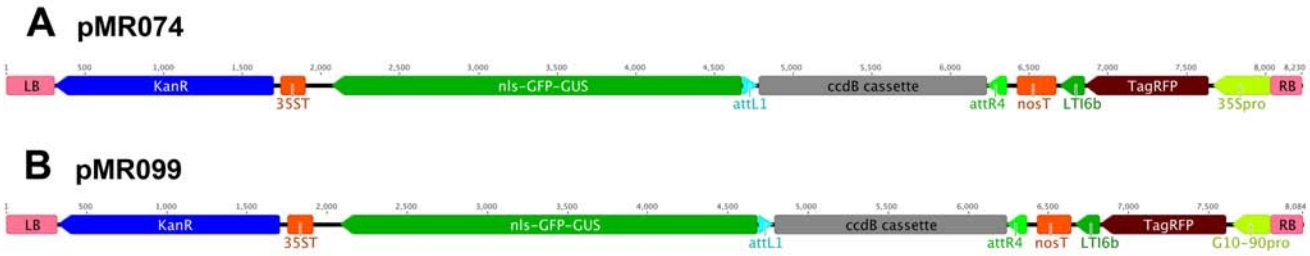
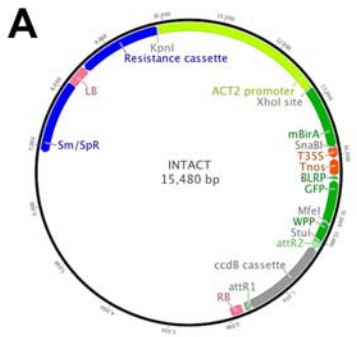
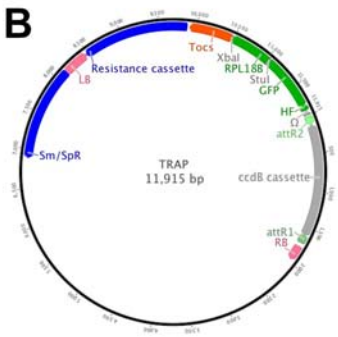


Figure S2: Maps of vectors for transcriptional fusions.
Vector maps were created using Geneious version 7.0.5 created by Biomatters. Available from http://www.geneious.com





C

Optimizable element	In tomato
INTACT & TRAP: Resistance marker	Kanamycin
INTACT & TRAP: Promoter driving resistance marker	*Nopaline synthase*
INTACT: *ACT2pro* driving BirA	2.9kb upstream of ATG of Solyc11g005330
INTACT: *BirA* codon use optimization	Arabidopsis
INTACT: nuclear envelope localization signal	WPP domain of AtRan1GAP
TRAP: RPL18B CDS	At3g05590

Figure S3: Vector maps of TRAP and INTACT backbones : (**A, B**) and list of modules changed for tomato backbone (**C**).

Sm/SpR - Spectinomycin/streptinomycin resistance cassette for bacterial selection

LB, RB - Left and right border for plant transformation

Resistance cassette - resistance marker for plant selection

ACT2 promoter - *ACTIN2* promoter

mBirA - codon optimized biotin ligase gene with 3x myc-tag

T35S - *35S* terminator

Tnos - *nos* terminator

BLRP - biotin ligase receptor peptide

WPP - WPP domain of *Arabidopsis RanGAP1*

MfeI, SnaBI, StuI, XbaI, XhoI - unique restriction sites

attR2, attR1 - att sites for Gateway cloning

ccdB cassette - *ccdB* gene and chlorampenicol resistance

Tocs - *ocs* terminator

RPL18B - *RPL18B* coding sequence

GFP - green fluorescence protein

HF - His-FLAG-tag

Ω - omega translational enhancer

Nuclear Tagging Fusion (NTF) comprises of WPP, GFP and BLRP.

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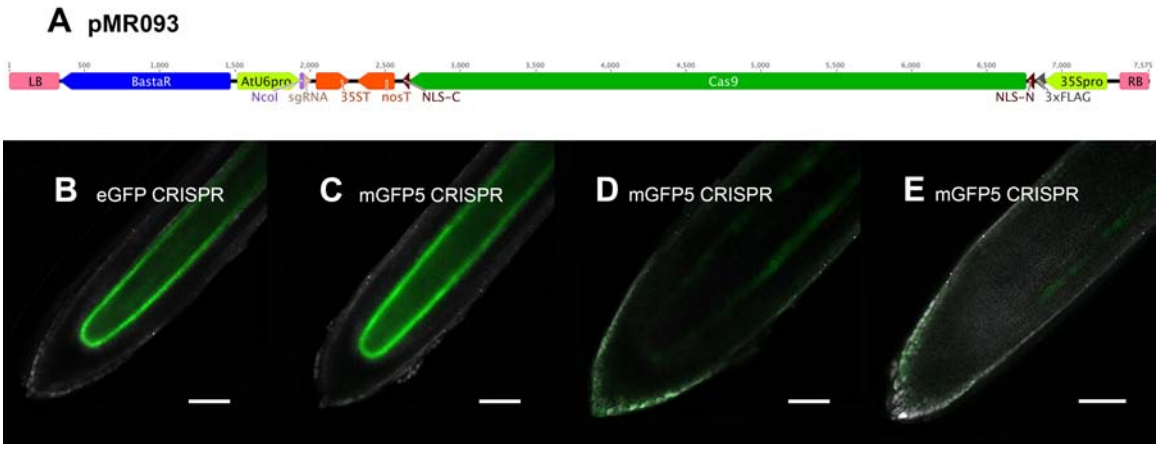

Figure S4: High-efficiency targeted gene editing in tomato plants.
(**A**) Map of vector used for CRISPR induced gene targeting. The CAS9 cassette is driven by the 35S promoter, whereas the sgRNA is driven by the AtU6 promoter. Cloning of the target sequence was done into the NcoI site in the sgRNA sequence. (**B**) A representative root from the transformation with an sgRNA designed to complement *eGFP*. All roots tested showed strong GFP expression like the untransformed control. (**C-E**) Additional roots from the transformation with sgRNA designed to complement mGFP5 showing no affectet GFP expression (C) reduced GFP expression (D) and no GFP expression (E). Scale bars represent 100 μm.
Vector maps were created using Geneious version 7.0.5 created by Biomatters. Available from http://www.geneious.com.

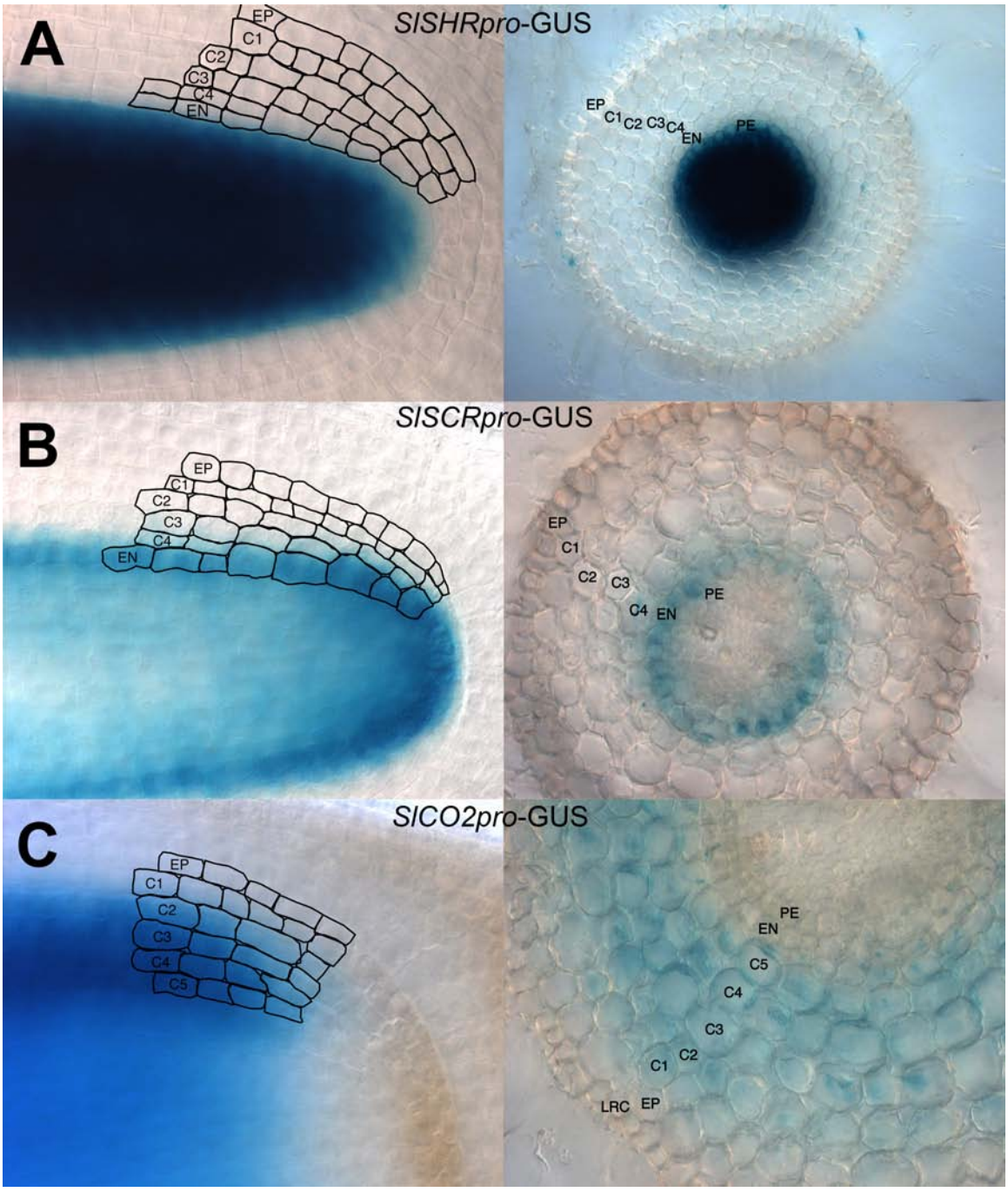

Figure S5: Tissue and cell type-specific expression conferred by the *Solanum lycopersicum* promoters, *SHR, SCR* and *CO2*. Left panel = whole mount image under differential interference contrast, right panel = cross-section. **(A)** The *SHR* promoter confers expression in the stele**. (B)** The *SCR* promoter confers expression in the endodermis and quiescent center. **(C)** The *CO2* promoter confers expression in all layers of the cortex.

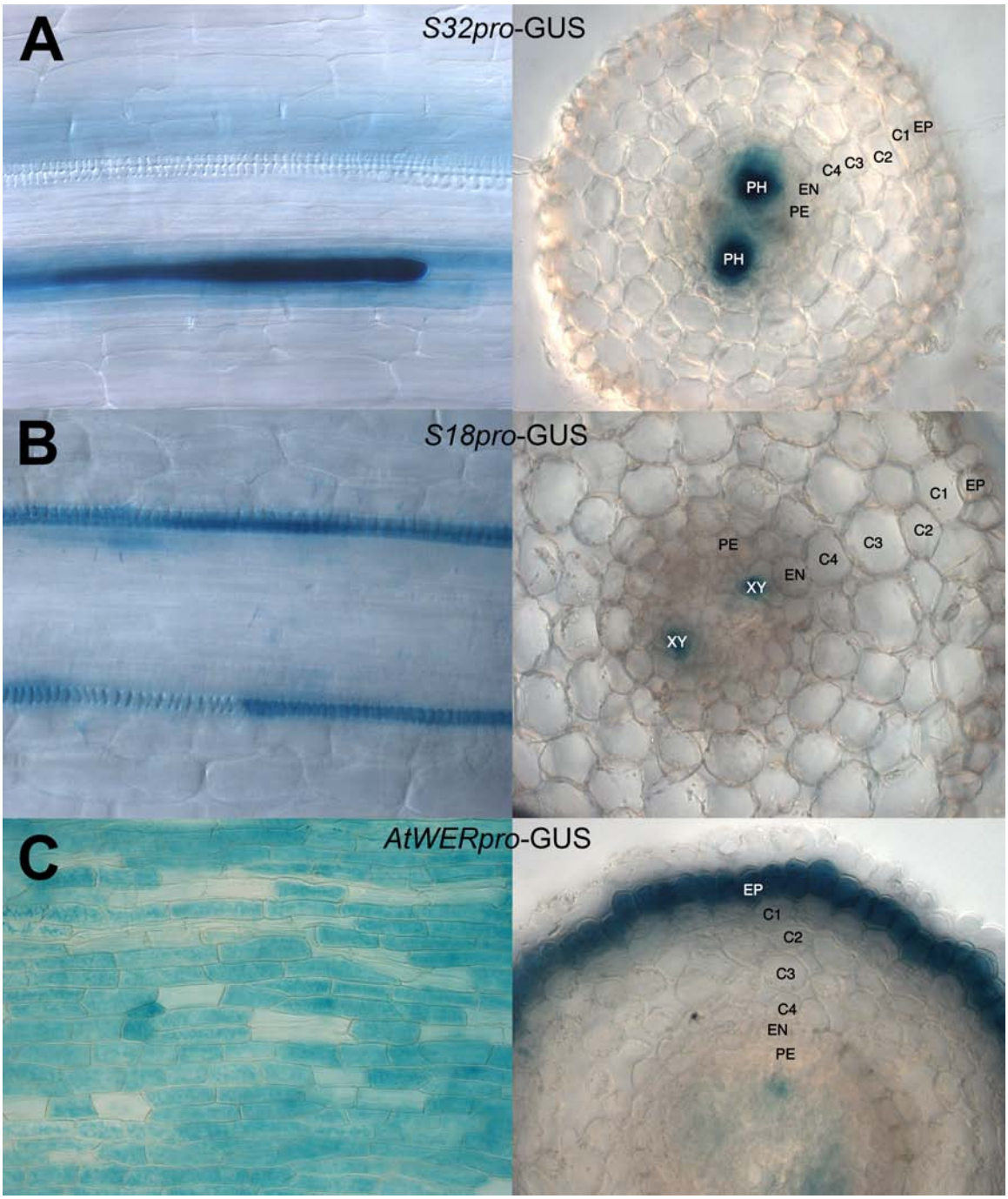

Figure S6: Cell type and tissue-specific expression conferred by the Arabidopsis thaliana promoters in tomato hairy roots. Left panel = whole mount image under differential interference contrast, right panel = cross-section. **(A)** The *S32* promoter confers expression in the protophloem and metaphloem**. (B)** The *S18* promoter confers expression in the protoxylem and metaxylem. **(C)** The *WER* promoter confers expression in the epidermis.

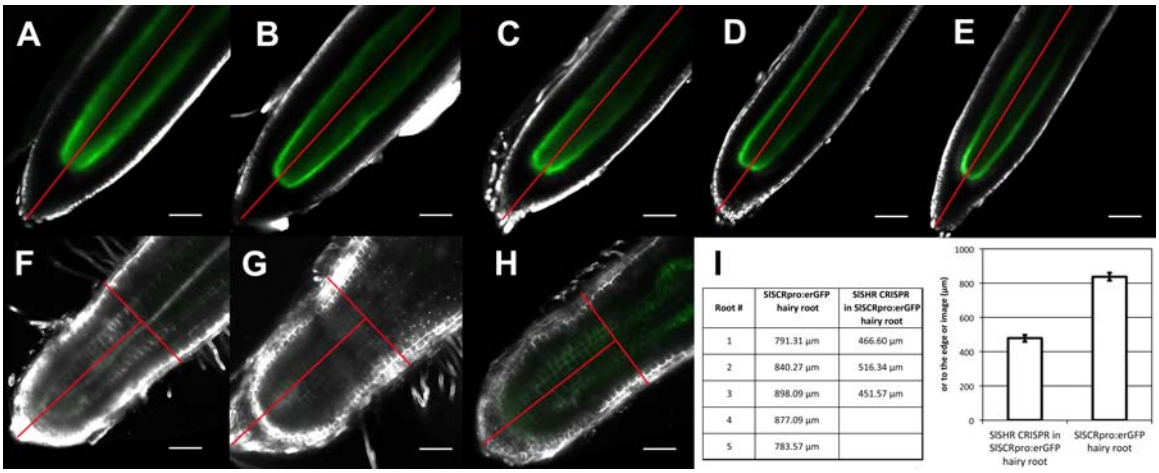

Figure S7: CRISPR/Cas9 editing of *SlSHR* leads to a shorter root meristem.

(**A-E**) Root tips of control hairy root (containing no CRISPR binary vector) induced from the *SlSCRpro-mGFP5* transgenic line show no start of the maturation zone (as indicated by root hairs) in the field of view. The distance from root tip was measured to the edge of the field of view as shown with the red line.

(**F-H**) Root tips of hairy roots transformed with a CRISPR/Cas9 construct with sgRNA designed against the endogenous *SHR* coding sequence. These roots show strongly reduced *SlSHRpro-mGFP5* expression and shorter meristematic and elongation zones. The distance from the root tip to the start of the maturation zone was measured as indicated by the first visible root hair and as shown with the red lines.

(**I**) Quantification of the length from root tip to the first visible root hair or to the edge of the field of view (whichever was shorter) for roots shown in A-H. The difference in average lengths was significant (student's t-test: p=0.000019). For the control roots (n=5) the average was 838 µm and for the CRISPR/Cas9 SlSHR edited roots (n=3) it was 478 µm.

A-H were imaged with 20x objective and constant settings of 488nm excitation, 70% laser power, 1.87 Airy unit pinhole, 583 gain. Scale bars represent 100 µm. GFP expression is shown in green with both autofluorescence spectra and residuals shown in white. The levels of the AFL2 channel (as defined in Fig S1J) have been adjusted for increased intensity and to show the outline of the root and the root hairs more clearly while the levels of other channels are untouched.

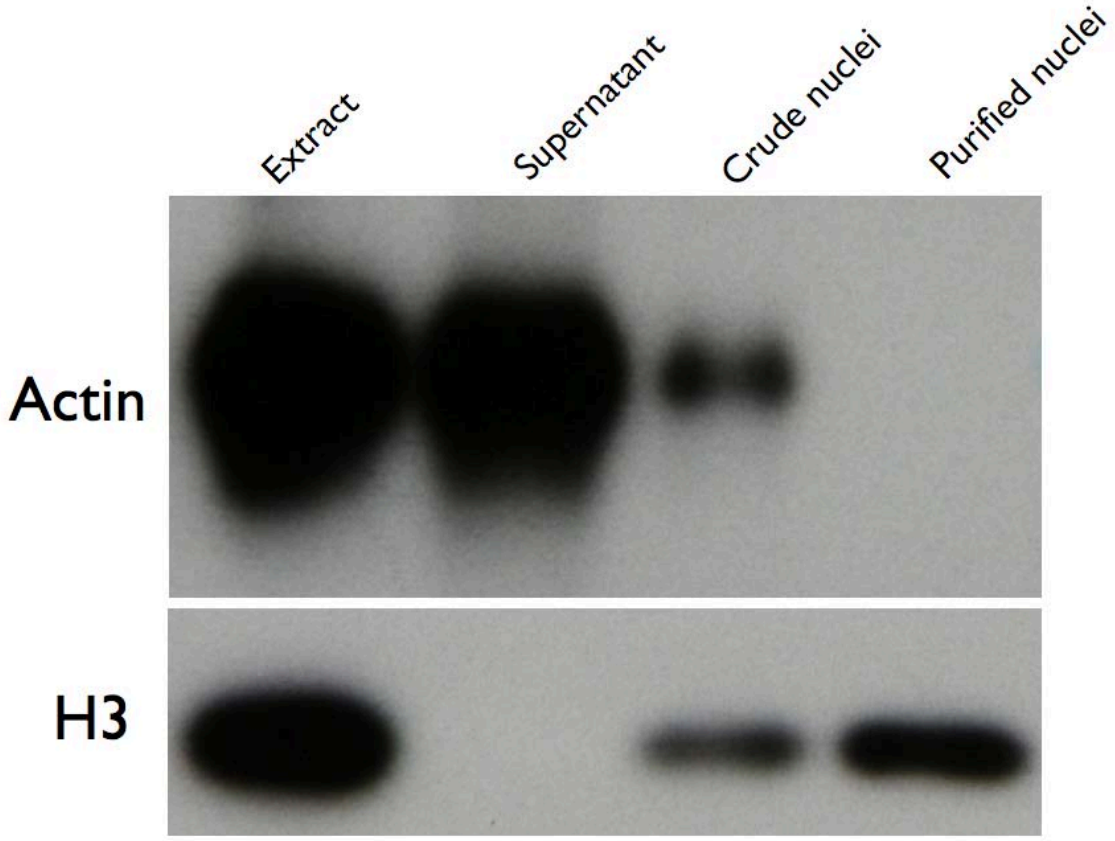

Figure S8. INTACT-purified nuclei are free of cytoplasmic contamination. Western blots show the distribution of Actin 8 (cytoplasmic marker; upper panel) and histone H3 (nuclear marker; lower panel) in various fractions during INTACT purification of nuclei from tomato roots. Roots transformed with *35Spro:NTF* and *SlACT2pro:mBirA* transgenes were homogenized in nuclei purification buffer to generate the “Extract” fraction. The extract was then centrifuged to separate the “Supernatant” and “Crude nuclei” fractions. The crude nuclei pellet was resuspended in nuclei purification buffer and labeled nuclei were then captured with streptavidin-coated magnetic beads to generate the “Purified nuclei” fraction.