



Hair dsRNase -Protein Rep 1 Hair dsRNase -Protein Rep 2 Nonhair ssRNase +Protein Rep 1 Nonhair ssRNase +Protein Rep 2 Hair ssRNase +Protein Rep 1 Hair ssRNase +Protein Rep 1 Nonhair dsRNase -Protein Rep 2 Nonhair dsRNase -Protein Rep 1 Nonhair dsRNase +Protein Rep 2 Nonhair dsRNase +Protein Rep 2 Hair dsRNase +Protein Rep 1 Hair dsRNase +Protein Rep 1 Hair ssRNase -Protein Rep 2 Hair ssRNase -Protein Rep 1 Nonhair ssRNase -Protein Rep 2 Nonhair ssRNase -Protein Rep 1

## Figure S1: High quality PIP-seq was performed on highly pure nuclei, Related to Figures 1-6.

(A) Western blot analysis of whole root tissue, hair cell nuclei, and nonhair cell nuclei. H3 is used as a positive control for purified nuclei, while CNX1, EIF1A, and ALDOLASE are markers of the membrane of the endoplasmic reticulum, the plasma membrane, and cytoplasm. The non-nuclear protein markers are absent from the nuclear samples. (B) Clustering analysis of all 16 PIP-seq libraries. The TAIR10 genome was divided into 100 nt bins and mapped reads were counted for each bin. The libraries were then clustered with the most similar libraries (the biological replicates) clustering together. (C) Comparison of average PhastCons scores between PPSs (green bars) and equal sized flanking regions (orange bars) for all PPSs. Error bars indicate SEM. \*\*\* denotes *p* value <  $1 \times 10^{-10}$ , Fisher's t-test.



## Figure S2: PPSs identified by PIP-seq are highly reproducible, Related to Figures 1-6.

(A-B) The overlap in PPSs present in both dsRNase and ssRNase libraries in hair cells (A) and nonhair cells (B). (C-D) The overlap in PPSs between biological replicates of root hair cell (C) and root nonhair cell (D) nuclei. Overlap is defined by at least one nucleotide overlapping between PPSs.





(A-B) The overlap in PPSs identified in dsRNase (A) and ssRNase (B) treated samples in the nuclei of root hair (green) and nonhair (purple) cells. (C) The overlap in PPSs only found in transcripts that are expressed in both root hair (green) and nonhair cells (purple) with more than 50 reads. Overlap is defined by at least one nucleotide overlapping between PPSs.



Figure S4: Numerous RBPs are identified via RNA-affinity chromatography, Related to Figures 3-7.

A heatmap showing enrichment of proteins in motif sample compared to the scramble and bead-only controls after RNA affinity chromatography. These are candidate RBPs that have more than 2-fold enrichment in at least one sample. Red text indicates an annotated RBP.



Figure S5:  $\alpha$ -SE and  $\alpha$ -ABH1/CBP80 efficiently isolate endogenous proteins, Related to Figures 3-4.

(A-B) Western blot of immunoprecipitations performed with  $\alpha$ -SE (A),  $\alpha$ -

ABH1/CBP80 (B), or rabbit IgG (negative control) performed on whole seedlings.



Figure S6: GRP8 and SE influence root hair cell fate independently of the CAPRICE/WEREWOLF transcription factor network, Related to Figures 3-7. (A) Western blot of nuclear lysate from root hair and nonhair cells examining SE expression using H3 as a loading control. There is no detectable difference in SE levels once normalizing to H3. (B) The lengths of root hair cells in Col-0 and se-1 show similar epidermal cell lengths. (C) RT-qPCR using RNA from roots of WS (red) or cpc-1 (light green) plants measuring GL2 (positive control) and SE transcript levels. The results show no significant difference in SE abundance between genotypes. \* indicates p value < 0.05; Welch's t-test. Error bars indicate SEM. (D) RT-qPCR using RNA from root tissue of Col-0 (red) and se-1 (blue) plants measuring components of the CAPRICE/WEREWOLF transcription factor network. The results show no significant difference in the abundance of the tested transcripts between the two genotypes. Error bars indicate SEM. (E) RTaPCR based measurements of CAX4. MOR1, and PKL levels in the roots of WS (red) and cpc-1 (light green) plants showing no significant difference in the abundance of these transcripts between genotypes. Error bars indicate SEM. (F)

Western blot using protein lysates from root hair and nonhair cell nuclei measuring GRP7 and GPR8 protein levels. There is no noticeable difference between cell types when normalized to the H3 loading control. See Figure S6A for the H3 loading control results. (G) Measurement of the lengths of root hair cells in plants with altered GRP7 or GRP8 levels. The results show similar epidermal cell lengths in all tested genotypes. (H) RT-qPCR based measurements of *GRP7* and *GRP8* levels in the roots of WS (red) and *cpc-1* (light green) plants reveals no significant difference in their abundance. Error bars indicate SEM. (I) RT-qPCR of transcripts involved in the CAPRICE/WEREWOLF transcription factor network using RNAs from the the roots of Col-0 (red) and *GRP8ox* (green) plants. The results reveal no significant difference in the abundance of any of the tested transcripts between the two genotypes. Error bars indicate SEM.



## Figure S7: GRP8 is a novel regulator of root hair cell fate and phosphate stress response in *Arabidopsis*, Related to Figure 5-7.

(A) The first 100 nt of 3' UTRs annotated in TAIR10 were taken and intersected with all hair or nonhair cell PPSs. MEME was used to identify significantly (E-value < 0.05) enriched motifs in this region for hair cell PPSs, while no significant motifs were identified for nonhair cell PPSs. The figure shows the three hair cell specific motifs found in this region, an area that shows a hair cell specific decrease in RNA secondary structure and an increase in RBP binding. (B-C) RT-qPCR screen of numerous phosphate starvation response genes in the roots of 8-day-old Col-0 (red bars), *GRP8*ox (green bars), or *grp7-1;8i* (purple bars) seedlings after 3-days of control (B) or phosphate starvation (C) treatment. \*, \*\*, and \*\*\* denote *p* value < 0.05, 0.01, or 0.001, respectively, Welch's t-test. Error bars indicate SEM.