Supplementary Methods

Materials. Ammonium hydroxide (NH_4OH, 28-30 wt %), tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), fluorescein isothiocyanate (FITC), and 3-aminopropyltrimethoxysilane (APTMS) were from Acrôs [NJ, US]. (3-Trihydroxysilyl)propylmethylphosphonate (THPMP) and N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TMAPS) were from Gelest [PA, US]. Rhodamine B isothiocyanate (RITC), propidium iodide (PI) and fluorescein diacetate (FDA) were from Sigma-Aldrich Chemical [MO, US]. mCherry monoclonal antibody (Cat. # 632543) was from Clontech Laboratories [CA, US]. Colloidal gold (12 nm)-AffiniPure Donkey anti-mouse IgG was from Jackson Immuno Research [Suffolk, UK]. Sodium azide (Cat. #30175) was from Serva [Germany]. Ultrapure deionized (D.I.) water was generated by a Millipore Milli-Q plus system.

Synthesis of F-MSNs and R-MSNs. Dye-functionalized MSNs, RITC-MSNs and FITC-MSNs were prepared by co-condensation. First, *N*-1-(3-trimethoxysilylpropyl)-*N*'-fluoresceyl thioruea (FITC-APTMS) was formed by stirring FITC ethanolic solution containing APTMS (5 ml of 99.5% ethanol, 1 mg FITC, and 0.56 mmole APTMS) in the dark for 24 h. Separately, 0.58 g CTAB was dissolved in 300 g of 0.17 M NH_4OH at 40°C, and 5 ml of 0.2 M dilute TEOS (in ethanol) was added with stirring. Stirring continued for 5 h, then 5 ml FITC-APTMS (in ethanol) and 5 ml of 1.1 M TEOS (in ethanol) were added with vigorous stirring for 1 h. The mixture was then incubated at 40°C for 24 h and centrifuged at 15000 rpm for 30 min. The product was washed with ethanol several times. Finally, surfactant was removed by heating in acidic ethanol (1 g HCl/50 ml ethanol) at 60°C for 24 h. R-MSNs were synthesized by the same procedure, except that RITC was

used.

Synthesis of APTMS/F-MSNs, APTMS/R-MSNs, TMAPS/F-MSNs, and TMAPS/R-MSNs. TMAPS and APTMS were grafted onto the external surface of surfactant-containing Dye-MSNs by refluxing 2.8 mmol of the corresponding trimethoxysilyl derivatives with 0.2 g Dye-MSNs in ethanol for 12 h. After removing surfactant templates, the desired MSN derivatives were obtained.

Synthesis of THPMP/F-MSNs and THPMP/R-MSNs. For THPMP modification, the pH of the surfactant-containing F-MSN suspension (aged for 22 h in aqueous ammonium) was adjusted to 10.0 with NH_4OH (28-30%), then 10 ml of 56 mM aqueous THPMP solution was added with vigorous stirring at 40°C for 2 h. The mixture was centrifuged and washed with ethanol several times. After the surfactant was removed by extraction in acidic ethanol, THPMP/F-MSNs were collected. THPMP/R-MSNs were prepared by the same procedure, except a surfactant-containing R-MSN suspension was used.

Characterization of organically functionalized MSNs with TEM. The morphologic features and size of each MSN product were characterized by TEM (Philips CM 100) at 80 KV, and images were recorded by use of a Gatan Orius CCD camera. Ethanolic suspensions of samples were dropped onto a carbon-coated copper grid, air-dried and examined.

Characterization of organically functionalized MSNs in aqueous solution. The zeta potentials of organically functionalized MSNs were characterized in aqueous solution at various pH levels by use of a Zetasizer Nano (Malvern; Worcestershire,

United Kingdom). Samples were prepared by diluting 3.5 mg of each MSN in 10 ml D.I. water. After ultrasonication for 3 min, solutions were transferred to 1-ml capillary cells, and zeta values were read immediately. The pH value was adjusted with 0.1 N HCl or NaOH by automatic titration. Each zeta value was measured in triplicate.

For DLS assay, 0.35 mg of each organically functionalized MSN was suspended in 1 ml D.I. water. After ultrasonication for 3 min, hydrodynamic diameters were measured in triplicate.

Characterization of organically functionalized MSNs in 1/2 MS and BY-2 culture medium. The pH of 1/2 MS and BY-2 culture medium was adjusted with 1 N HCl and 1 N NaOH to 5.2 and 5.7, respectively. Samples were prepared by diluting 0.35 mg of each MSN product in 1 ml 1/2 MS (pH 5.2) or BY-2 medium (pH 5.7). After ultrasonication for 3 min, zeta values and hydrodynamic sizes were measured in triplicate.

Table S1. Two-way ANOVA analysis of uptake efficiency of various MSNs by *Arabidopsis* root cells. Four root segments from each type MSNs treated plants were examined by CLSM and the number of cell harboring MSNs were recorded to evaluate the interaction between MSNs and plants. Twenty four plants from 4 independent batches were examined for each type of MSN. SS, sum of squares; df, degree of freedom; MS, mean square; F, F variable.

	SS	df	MS	F	P-value
Duplicate	399.396	3	133.132	8.469	1.870E-05
MSNs particle	385.250	3	128.417	8.169	2.807E-05
Interaction	140.396	9	15.600	0.992	0.446
Within	5784.917	368	15.720		
Total	6709.958	383			

Table S2. Efficiency of MSN internalization and transgene expression by plants. Three root segments from TMAPS/F-MSN with or without DNA treated plants were examined by CLSM and the number of plants harboring mCherry or MSNs were recorded to estimate the efficiency of this system. Three independent experiments were conducted from different batches of plants.

	Plant Stage	Experiment	Number of Plants	Number of Plants with Particle/Reporter	Efficiency
MSN Internalization	Vegetative	1	20	1	5.0%
		2	21	1	4.8%
		3	21	0	0.0%
		mean	20.7	0.7	3.3±0.0%
	Flowering	1	24	12	50.0%
		2	21	10	47.6%
		3	20	12	60.0%
		mean	21.7	11.3	52.5±0.1%
MSN-mediated Transformation	Flowering	1	30	13	43.3%
		2	42	23	54.8%
		3	29	12	41.4%
		mean	33.7	16.0	46.5±0.1%

Table S3. Two-way ANOVA analysis of uptake efficiency of TMAPS/F-MSNs in *Arabidopsis* roots incubated at 4°C and room temperature. Sampling 4 root segments from TMAPS/F-MSN treated plants at low temperature or room temperature and recording cell numbers harbored MSNs by CLSM to investigate the mechanism of MSNs internalization. Fifteen plants from 3 independent batches were examined for each treatment. SS, sum of squares; df, degree of freedom; MS, mean square; F, F variable.

	SS	df	MS	F	P-value
Duplicate	42.050	2	21.025	1.533	0.220
Temperature treatment	0.675	1	0.675	0.049	0.825
Interaction	51.450	2	25.725	1.876	0.158
Within	1563.150	114	13.712		
Total	1657.325	119			

Table S4. Two-way ANOVA analysis of uptake efficiency of TMAPS/F-MSNs in *Arabidopsis* roots incubated with or without sodium azide. Sampling 4 root segments of the same length from TMAPS/F-MSN with or without sodium azide treated plants and recording cell numbers harbored MSNs by CLSM to analyze the energy effect. Fifteen plants from 3 independent batches were examined for each treatment. SS, sum of squares; df, degree of freedom; MS, mean square; F, F variable

	SS	df	MS	F	P-value
Duplicate	2.033	2	1.017	0.248	0.780
NaN_3 treatment	6.533	2	3.267	0.798	0.452
Interaction	29.833	4	7.458	1.822	0.127
Within	699.800	171	4.092		
Total	738.200	179			

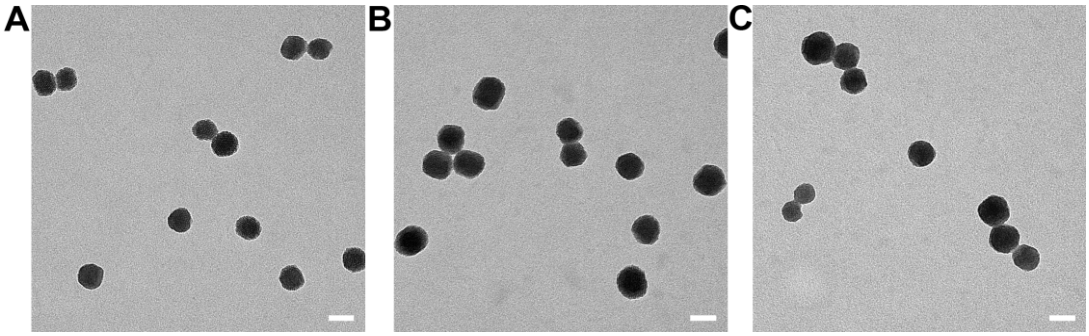

Fig. S1 Transmission electron microscopy (TEM) of organically functionalized mesoporous silica nanoparticles (MSNs). (A) F-MSNs, (B) APTMS/F-MSNs, and (C) THPMP/F-MSNs. Scale bars: 50 nm.

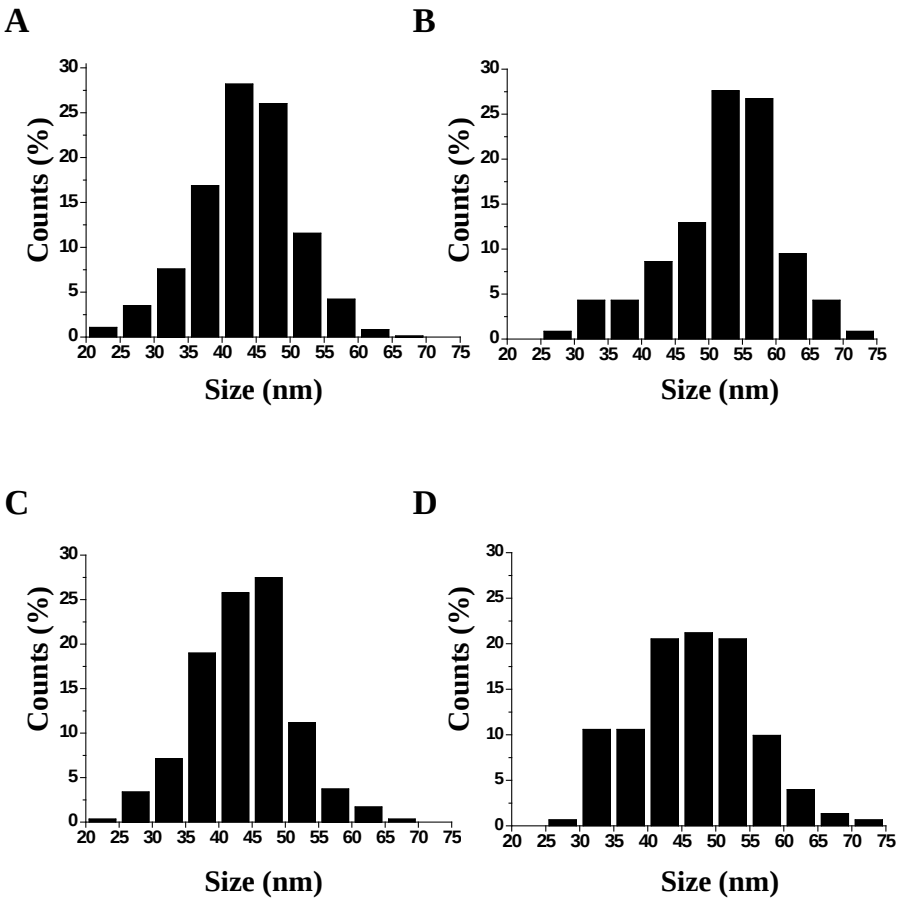

Fig. S2 TEM histograms of size of organically functionalized MSNs. (A) F-MSNs, (B) TMAPS/F-MSNs, (C) APTMS/F-MSNs, and (D) THPMP/F-MSNs.

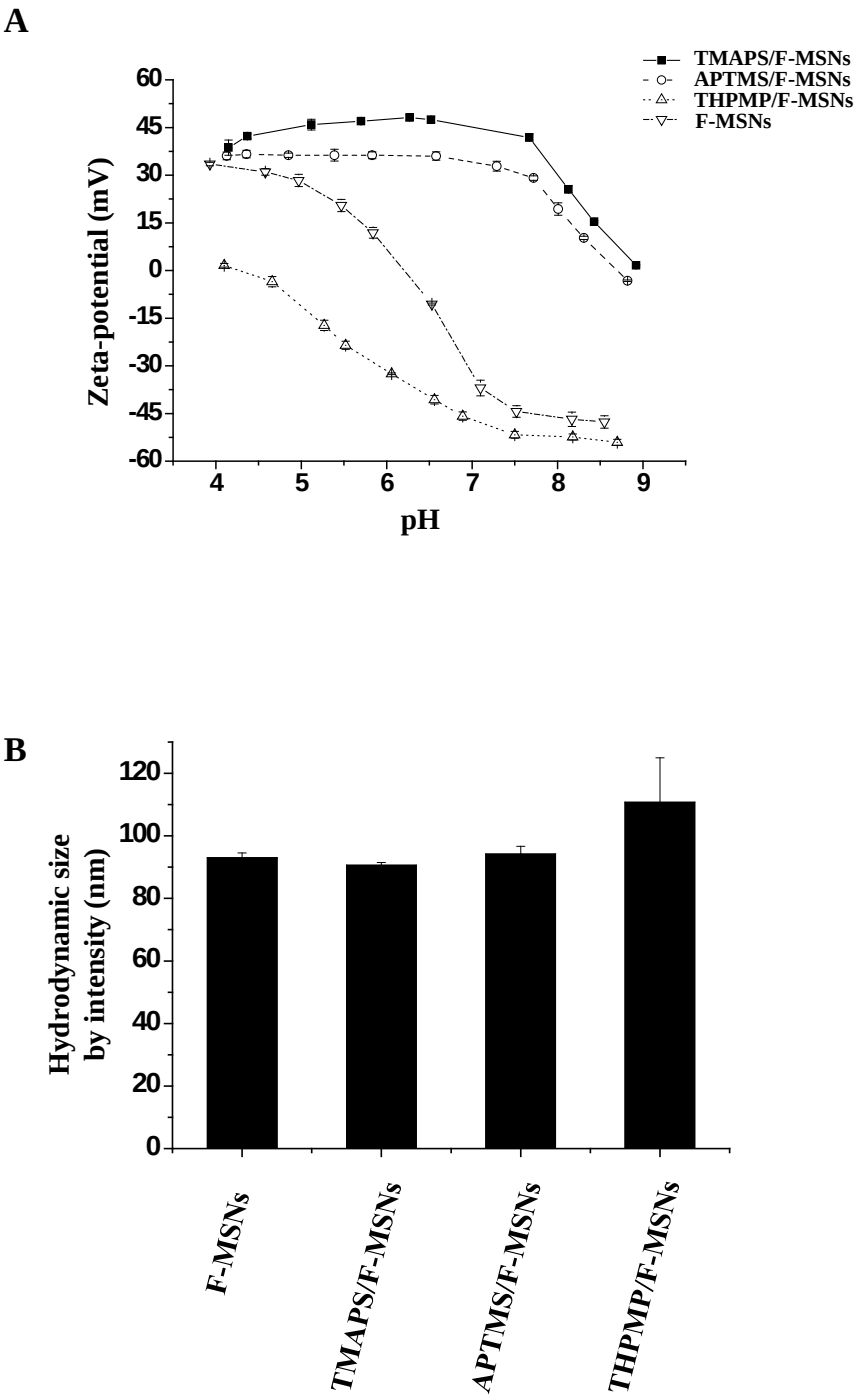

Fig. S3 (A) Zeta potential and (B) hydrodynamic size of organically functionalized MSNs in aqueous solution. Data are mean±SD, n = 3.

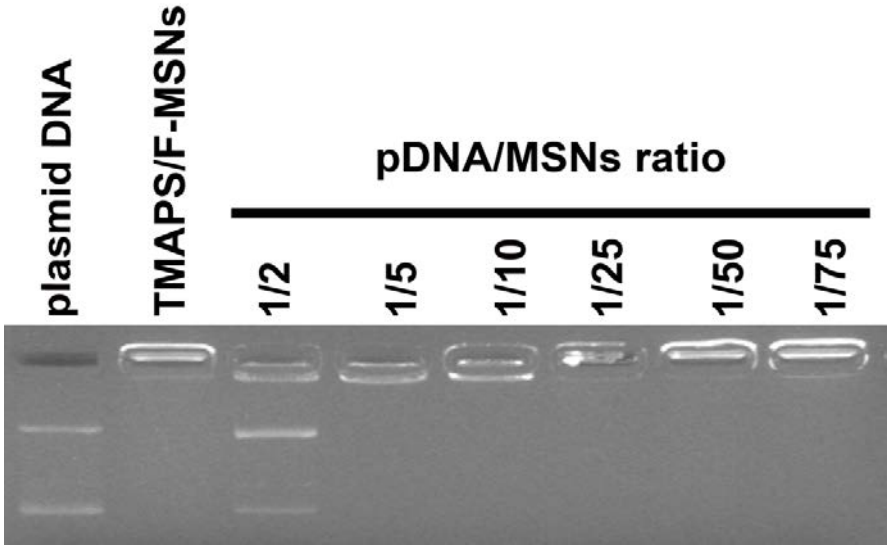

Fig. S4 Agarose gel electrophoresis assay of DNA-loaded TMAPS/F-MSNs at various ratios of DNA to MSNs. One microgram DNA (4.5 kbp) was incubated with TMAPS/F-MSNs (2, 5, 10, 25, 50, and 75 μg) in 1/2 MS medium (pH 5.2) for 30 min. The complexes underwent 1.5% agarose gel electrophoresis. DNA was visualized by ethidium bromide staining. No free DNA bands were observed with > 5 μg TMAPS/F-MSNs.

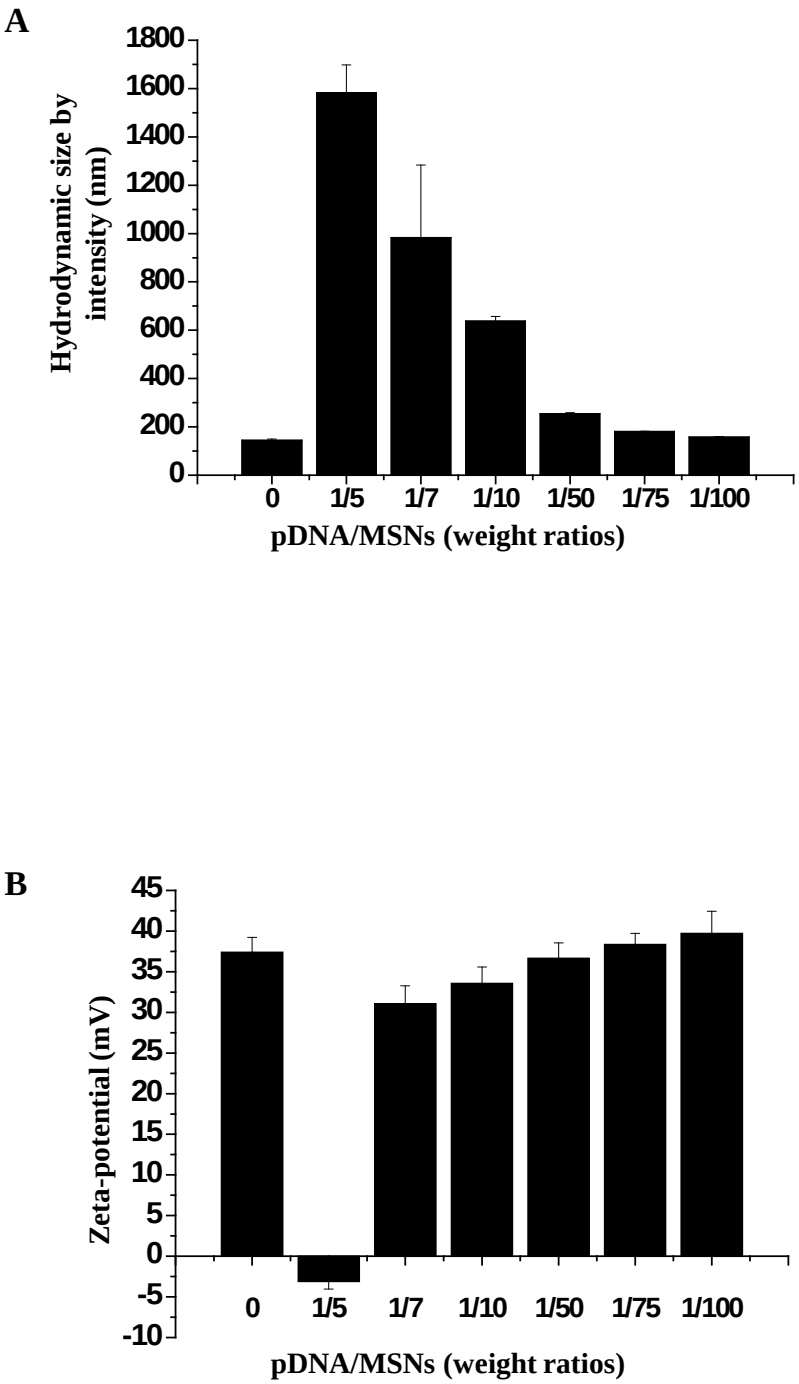

Fig. S5 Characterization of plasmid DNA-loaded TMAPS/F-MSNs by dynamic light-scattering (DLS) and zeta potentials. (A) Mean particle hydrodynamic size and (B) surface charge (zeta potentials) of the plasmid DNA-loaded TMAPS/F-MSNs at various ratios of DNA to MSNs (w/w) in 1/2 MS medium (pH 5.2). Data are mean±SD, n = 3.

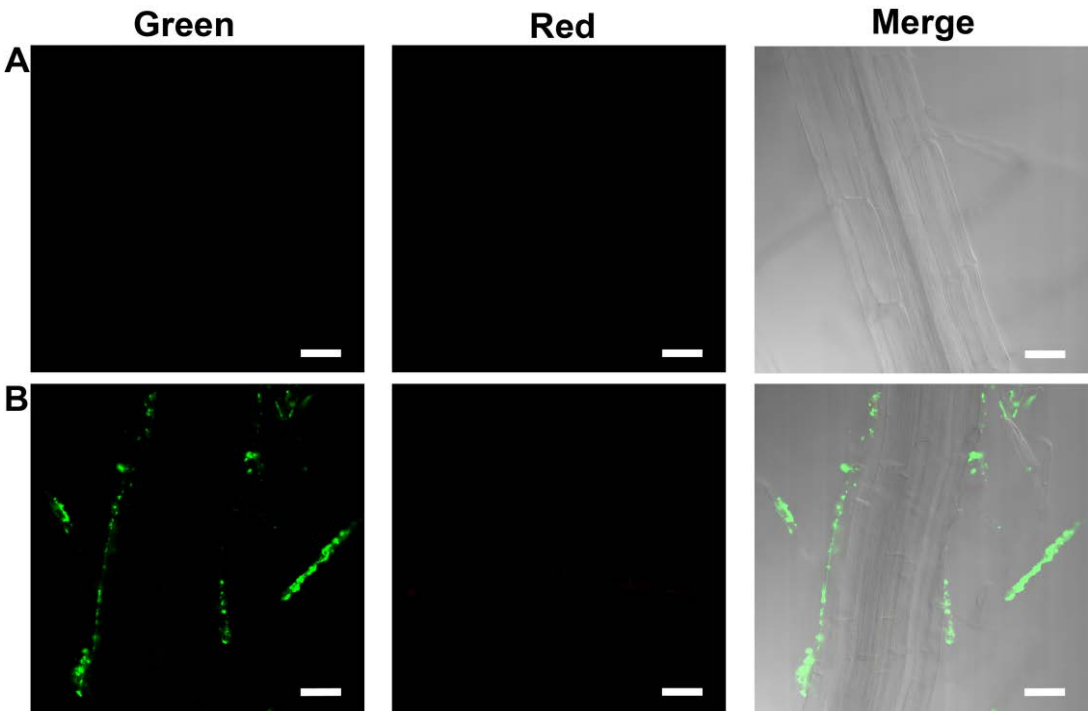

Fig. S6 Confocal microscopy of *Arabidopsis* roots treated with plasmid DNA or TMAPS/F-MSNs as the negative control. No gene expression (mCherry protein; red) was observed in the red channels of roots after incubation with either 0.2ug/mL plasmid DNA (A) or 20ug/mL TMAPS/F-MSNs (B) for 48 h at 24 °C in 1/2 MS. Green signals on the root surfaces (B) are the TMAPS/F-MSNs. Scale bars: 50 μm.

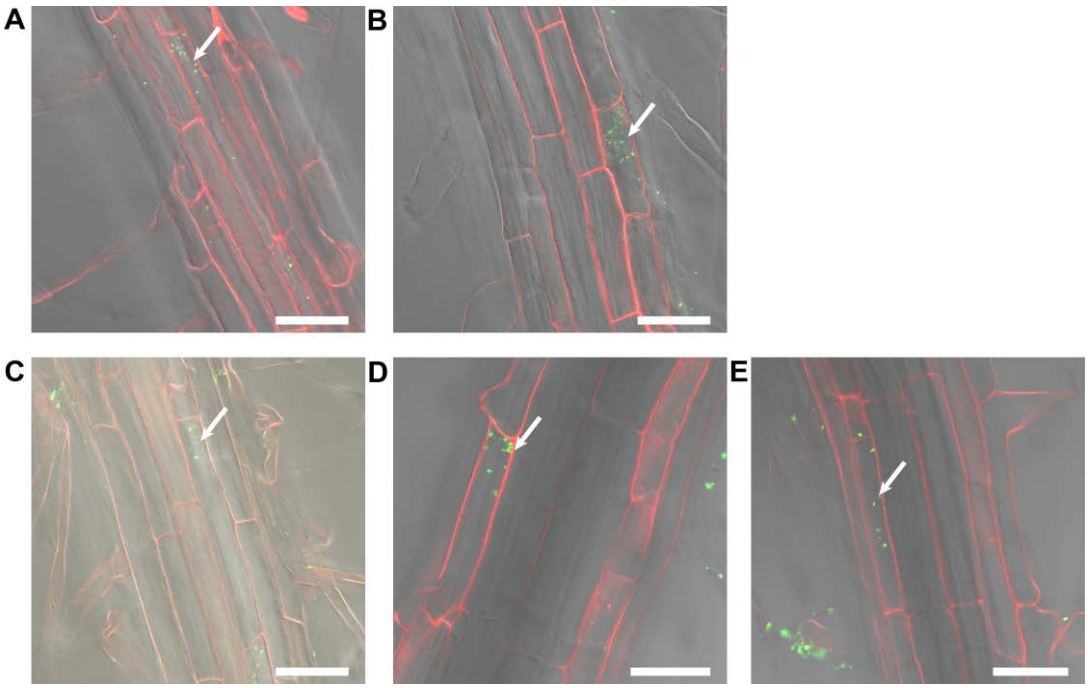

Fig. S7 Assessment of MSN uptake route by treating *Arabidopsis* roots with 4 ˚C or sodium azide. Confocal microscopy of *Arabidopsis* roots exposed to (A) room temperature (RT) as a control or (B) 4 ˚C for 36 h, then 20 μg TMAPS/F-MSNs at RT or 4 ˚C for 12 h. *Arabidopsis* roots were treated with (C) 1/2 MS medium only as control and (D) 0.05 mM and (E) 0.1 mM sodium azide in medium at 24˚C for 30 min, then 20 μg TMAPS/F-MSNs for 4 h. Particles in green (white arrows) were detected inside root cells. Roots were stained with propidium iodide (red) to reveal cell viability and label cell walls. Scale bars: 50 μm.

Supplementary animation legends

3D Animation S1. Accumulation of MSNs in *Arabidopsis* roots. TMAPS/F-MSNs accumulated in some epidermal cells in root maturation zone of *Arabidopsis* after 4-h incubation.

3D Animation S2. Polar distribution of MSNs in *Arabidopsis* roots. Polar distribution of TMAPS/F-MSNs in epidermal cells of *Arabidopsis* roots after 24-h incubation.