Supplemental Materials

Sheet-like clay nanoparticles deliver RNA into developing tomato pollen to efficiently silence a target gene

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Supplemental Table S1

Supplemental Figures S1-S9

Supplemental Datasets S1-S3

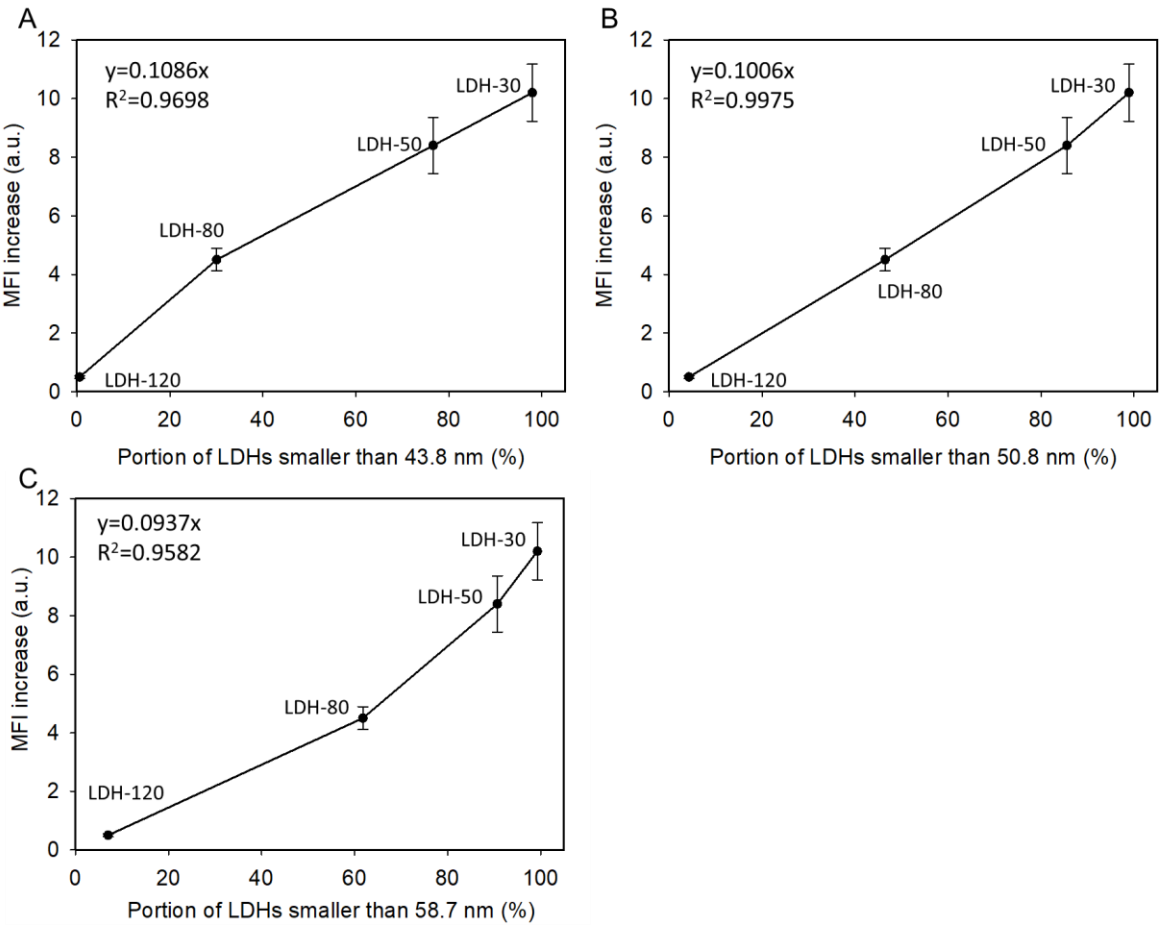
Supplemental Table S1. Characteristic parameters of LDH-30, LDH-50, LDH-80, LDH-120 suspension in deionized water and their stability in 2-(N-morpholino)ethanesulfonic acid (MES) (20 mM, pH 6.0) buffer Characteristic parameters of LDH-30, LDH-50, LDH-80, LDH-120 suspension in deionized water and their stability in 2-(N-morpholino)ethanesulfonic acid (MES) (20 mM, pH 6.0) buffer

Samples	[a] Z-average size (nm)	[a,b] TEM lateral diameter (nm)	[c] Crystallite thickness (nm)	Polydispersity index (PDI)	Zeta potential (mV) [a]
LDH-30 in deionized water	32.7 ± 2.2	26.6 ± 9.3	6.0	0.152	38.6 ± 2.2
LDH-30 in MES	37.3 ± 2.6			0.213	
LDH-50 in deionized water	52.1 ± 3.7	47.3 ± 17.7	7.1	0.149	43.7 ± 1.4
LDH-50 in MES	62.3 ± 1.3			0.212	
LDH-80 in deionized water	80.7 ± 3.6	70.6 ± 20.1	9.9	0.216	41.6 ± 1.7
LDH-80 in MES	81.7 ± 4.9			0.228	
LDH-120 in deionized water	122.9 ± 3.2	117.5 ± 29.4	15.3	0.246	47.9 ± 3.3
LDH-120 in MES	142.5 ± 9.9			0.257	

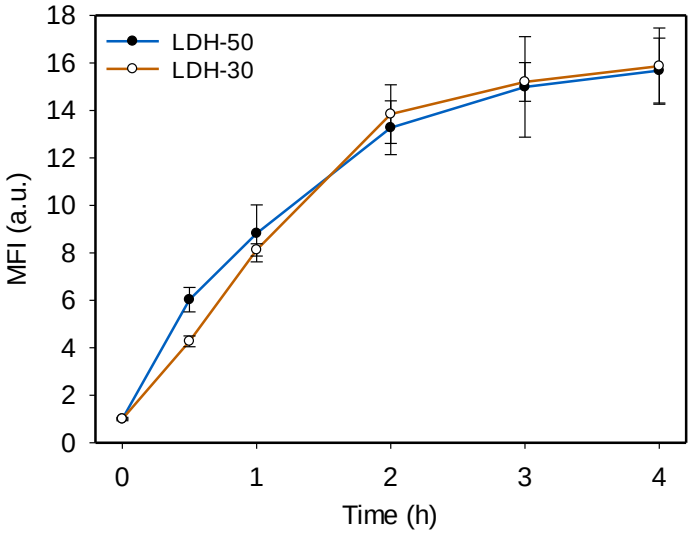
[a] Data shown as mean ± SD

[b] The average lateral diameter measured in TEM images for 100 nanoparticles;

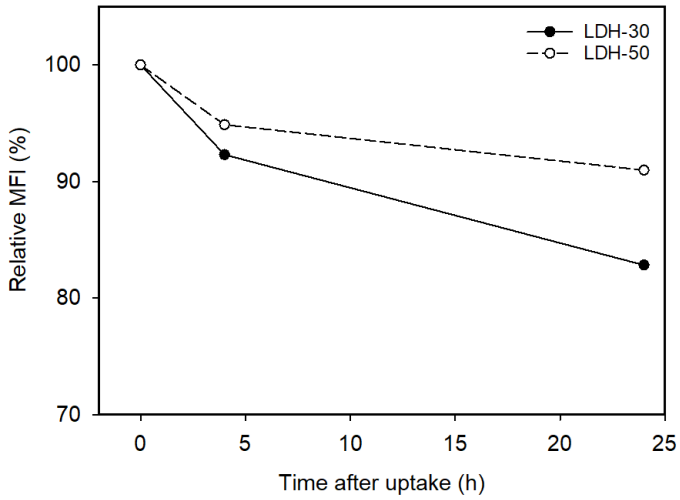
[c] The average thickness of plate LDH nanoparticles estimated from the full width at the half maximum of peak (003) using the Scherrer Equation.



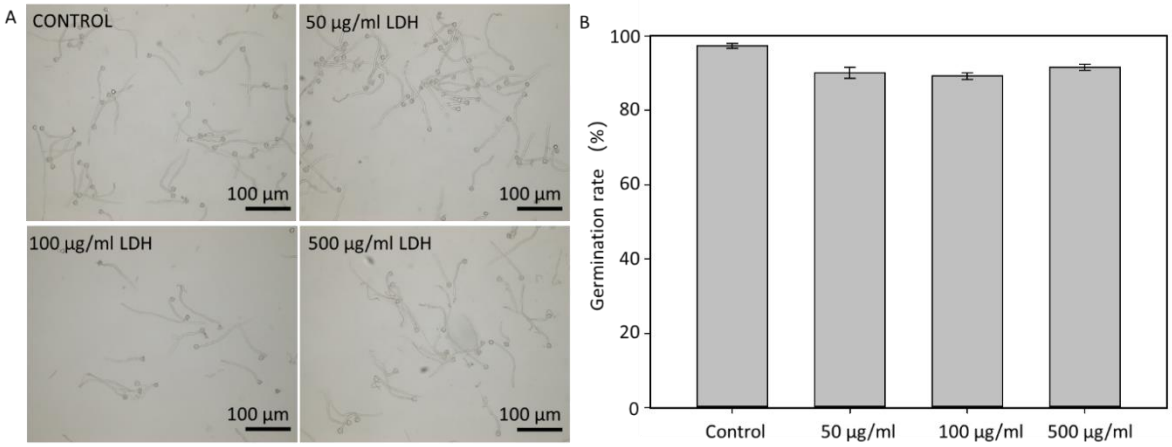
Supplemental Figure S1. Correlation between the fluorescence intensity (MFI) increase (fold increase relative to pollen autofluorescence intensity) and the mass percentage of LDHs. A, the size smaller than 43.8 nm. B, the size smaller than 50.8 nm. C, the size smaller than 58.7 nm. Data are shown as mean ± SEM (n=3).



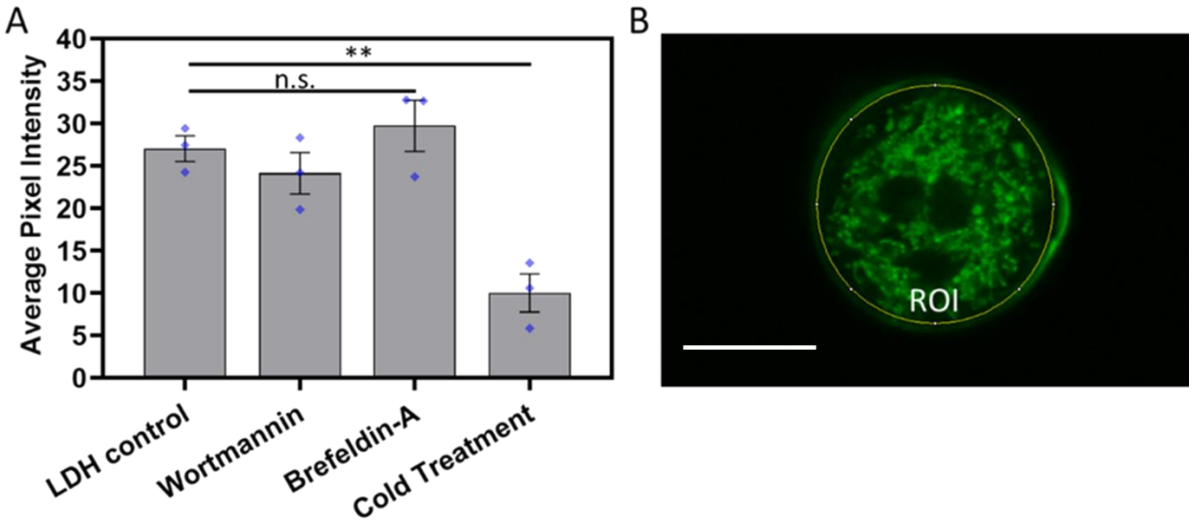
Supplemental Figure S2. Time-dependent uptake profiles of LDH-30 and LDH-50 nanoparticles at 50 mg/L (in terms of fold increase in mean fluorescence intensity (MFI) relative to pollen autofluorescence intensity).



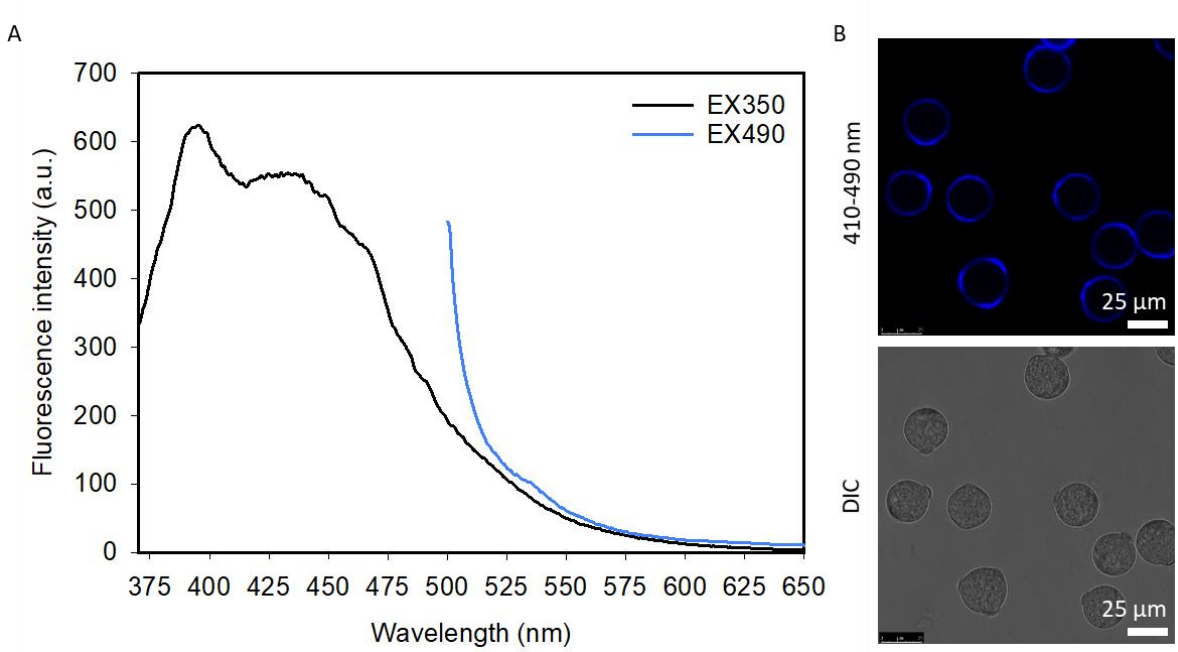
Supplemental Figure S3. Time-dependent fluorescence decay after 2 h uptake of LDH-30-FITC and LDH-50-FITC at 50 mg/L of LDH. Mean fluorescence intensity (MFI) was normalized as the percentage based on the MFI of mature pollen grains incubated for 4 and 24 h post 2 h uptake of LDH-FITC nanoparticles in relation to that measured at 0 h. The dissolved FITC in pollen cytosol is weakly fluorescent.



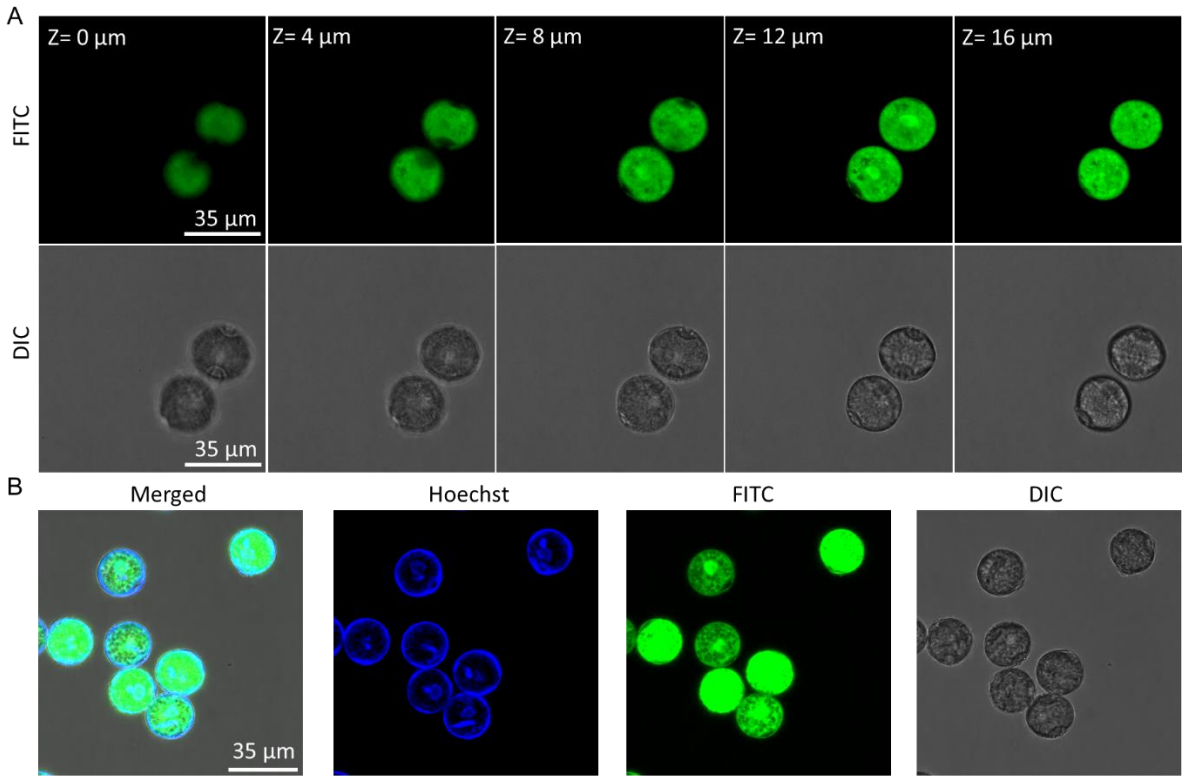
Supplemental Figure S4. Pollen *in-vitro* germination in the presence of LDH-50 nanoparticles. A, Microscope images. B, statistical analysis of the in-vitro germination rate of mature pollen grains. Data shown as mean ± SEM for n=2 biological replicates.



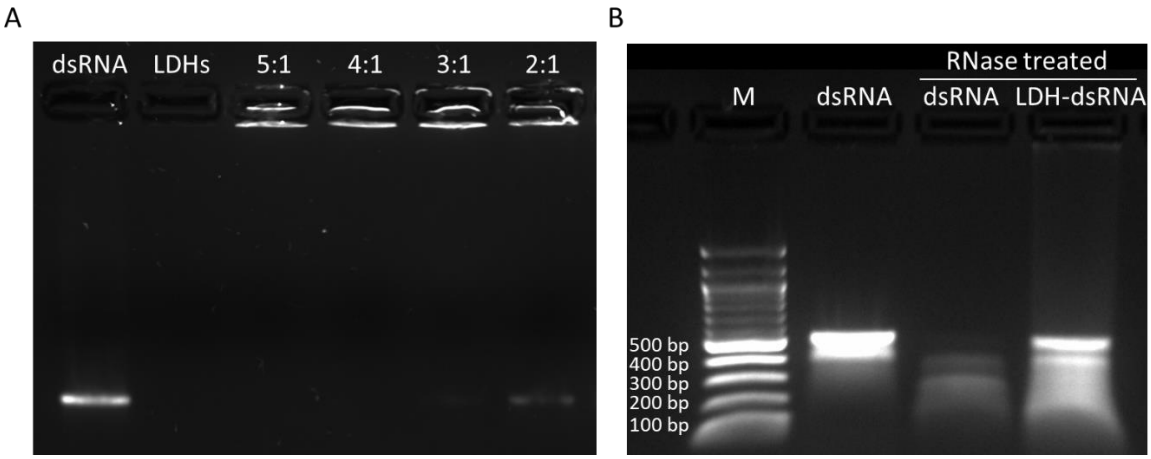
Supplemental Figure S5. Confocal microscope image analysis of average pixel intensity inside the pollen. A, Average pixel intensity of fluorescein isothiocyanate labelled LDH nanoparticles (LDH-FITC) inside the pollen. **, P<0.005 and n.s., no significant difference based on one-way ANOVA analysis. B, Example of selected region of interest (ROI), same image as in Figure 7 indicating LDHs stick on pollen wall (scale bar 15 µm). Data shown as mean ± SEM for n=3 biological replicates (blue diamonds).



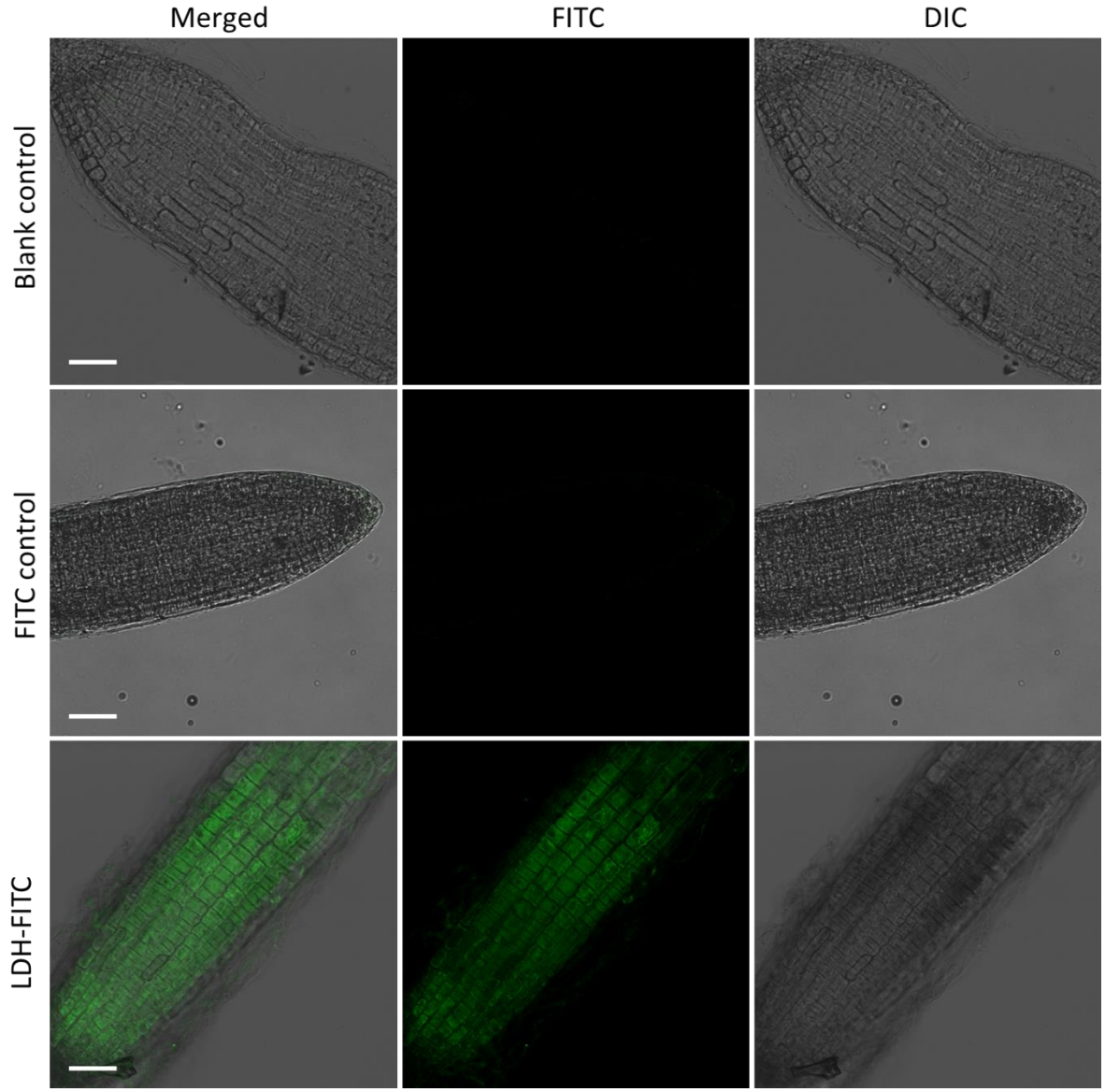
Supplemental Figure S6. Auto-fluorescence of mature pollen grains. A, Fluorescence spectrum of pollen auto-fluorescence (λ_{ex} = 350 nm and 490 nm). B, confocal microscope image of pollen auto-fluorescence (λ_{ex} = 405 nm).



Supplemental Figure S7. Confocal images testify the internalization of fluorescein isothiocyanate labelled LDH nanoparticles (LDH-FITC). A, Z-stack confocal microscope images of pollen uptake of LDH-50 nanoparticles (LDH-50-FITC). The usual size of early bicellular pollen is 20-30 µm in diameter. B, Confocal image of pollen uptake of LDH-50-FITC and co-stained pollen wall and nuclei with Hoechst 33342.



Supplemental Figure S8. LDH-50 nanoparticles loading and protection of dsRNA (505 bp). A, dsRNA loading onto LDH-50 nanoparticles at different LDH:dsRNA mass ratios. LDH-dsRNA complexes did not migrate and the fluorescence was observed in the loading well. B, treatment of naked dsRNA and LDH-dsRNA with RNase A. dsRNA from treated LDH-dsRNA was released prior to gel electrophoreses. M, 100 bp ladder.



Supplemental Figure S9. LDH-50 nanoparticle uptake by 1 week-old *Arabidopsis* root. Fluorescein isothiocyanate labelled LDH nanoparticles (LDH-FITC) at 100 mg/L or fluorescein isothiocyanate (FITC control) (5 mg/L) were co-incubated with 1 week-old *Arabidopsis thaliana* root for 4 h in pH 6.0 2-(N-morpholino)ethanesulfonic acid (MES) buffer in the dark at room temperature, (Scale bar, 40 µm).

Supplemental Dataset S1

Cy3 labelled CMV2b dsRNA sequence:

TATGTAATTGAACGTAGGTGCAATGACAAACGTCGAACTCCAACTGGCTCGTATG
GTGGAGGCGAAGAAGCAGAGACGAAGGTCTCACAAACAGAATCGACGGGAACG
AGGTCACAAAAGTCCCAGCGAGAGAGCTCGTTCAAATCTCAGACTATTCCGCTTC
CTACCATTCTATCAGATAGATGGTTCGGAACTGACAGGGTCATGCCGCCATATGA
ACATGGCGGAGTTACCCGAGTCTGAGGCCTCTCGTTTAGAGTTATCGGCGGAAG
ACCATGATTTTGACGATACAGATTGGTT

Supplemental Dataset S2

GUS dsRNA sequence (505 bp, 460-964 nt of GUS, GenBank Accession AJ298139.1):

GAAAAGTGTACGTATCACCGTTTGTGTGAACAACGAACTGAACTGGCAGACTAT
CCCGCCGGGAATGGTGATTACCGACGAAAACGGCAAGAAAAAGCAGTCTTACTT
CCATGATTTCTTTAACTATGCCGGAATCCATCGCAGCGTAATGCTCTACACCACG
CCGAACACCTGGGTGGACGATATCACCGTGGTGACGCATGTCGCGCAAGACTGT
AACCACGCGTCTGTTGACTGGCAGGTGGTGGCCAATGGTGATGTCAGCGTTGAA
CTGCGTGATGCGGATCAACAGGTGGTTGCAACTGGACAAGGCACTAGCGGGACT
TTGCAAGTGGTGAATCCGCACCTCTGGCAACCGGGTGAAGGTTATCTCTATGAAC
TGTGCGTCACAGCCAAAAGCCAGACAGAGTGTGATATCTACCCGCTTCGCGTCG
GCATCCGGTCAGTGGCAGTGAAGGGCGAACAGTTCCTGATTAACCACAAACCGT
TCTACTTTACTGGCTTT

Supplemental Dataset S3

Primers for RT-qPCR:

LAT52 forward: AGA CCA CGA GAA CGA TAT TTG C

LAT52 reverse: TTC TTG CCT TTT CAT ATC CAG ACA

GUS forward: AAT CAA AAA ACT CGA CGG CCT GTG

GUS reverse: AAC TGC CTG GCA CAG CAA TTG C