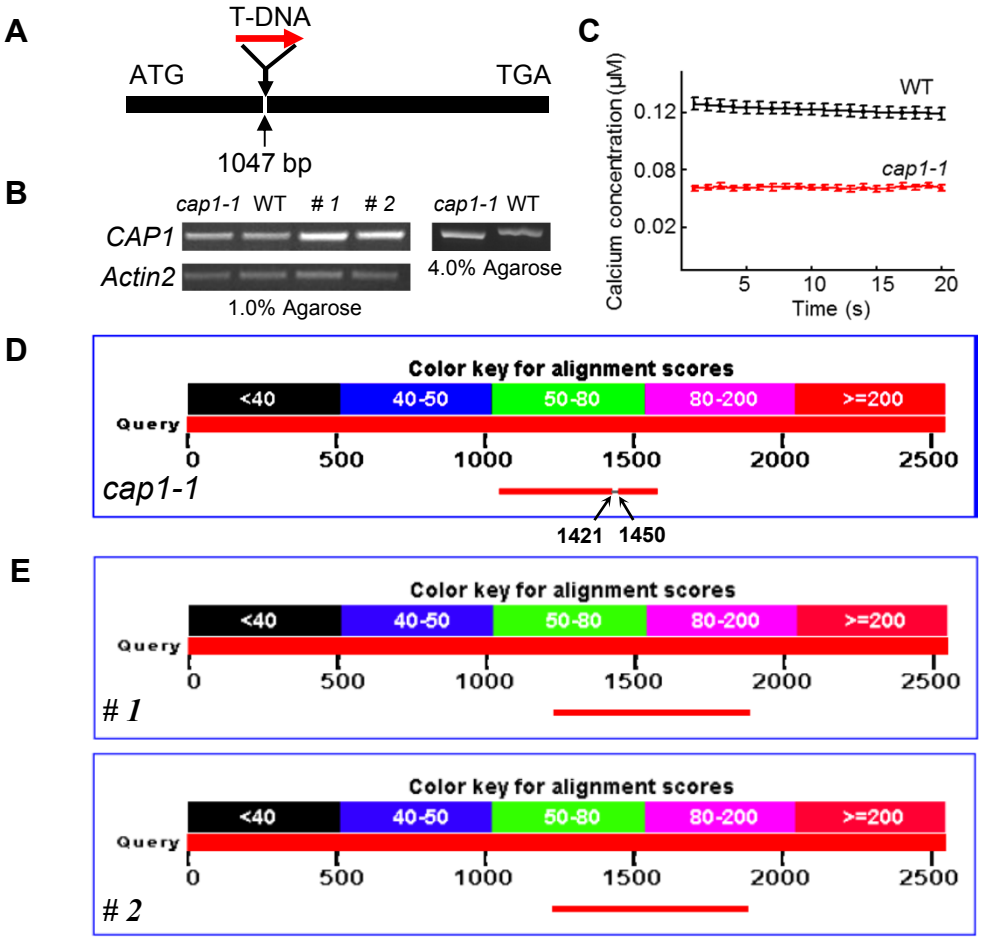
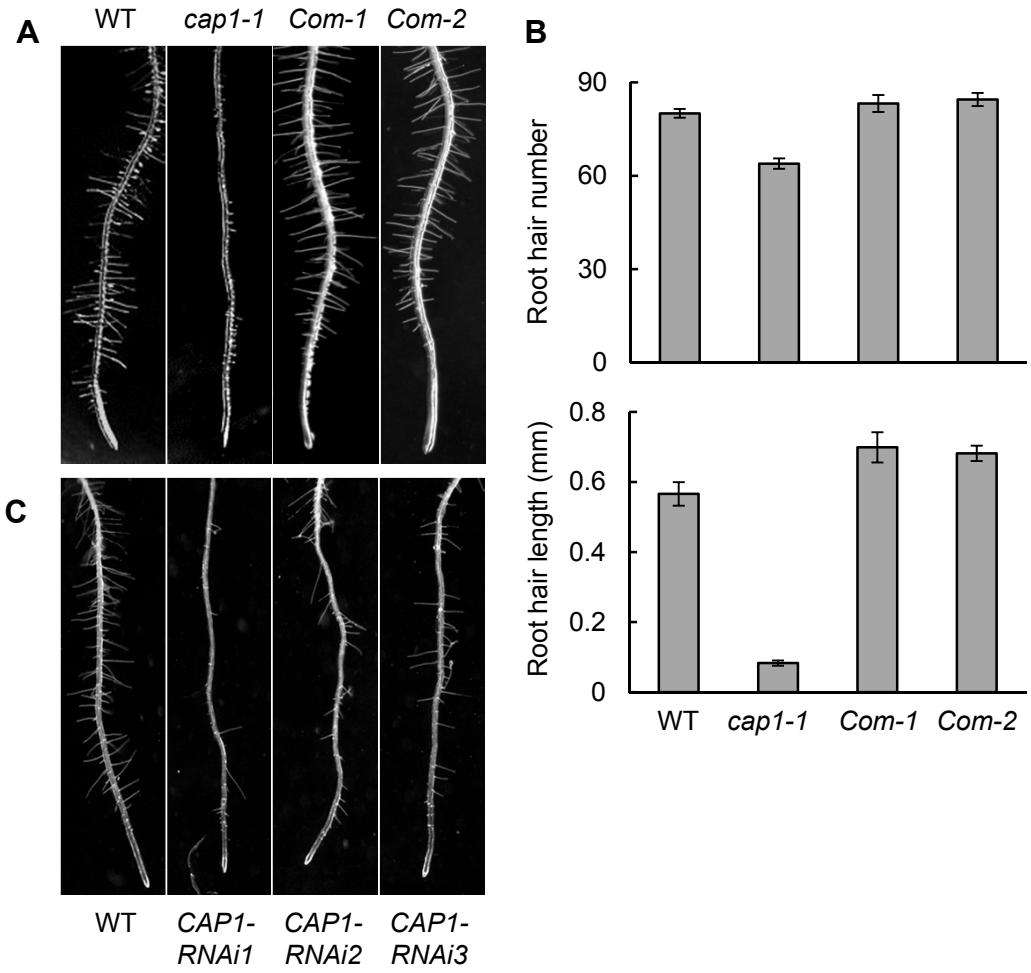
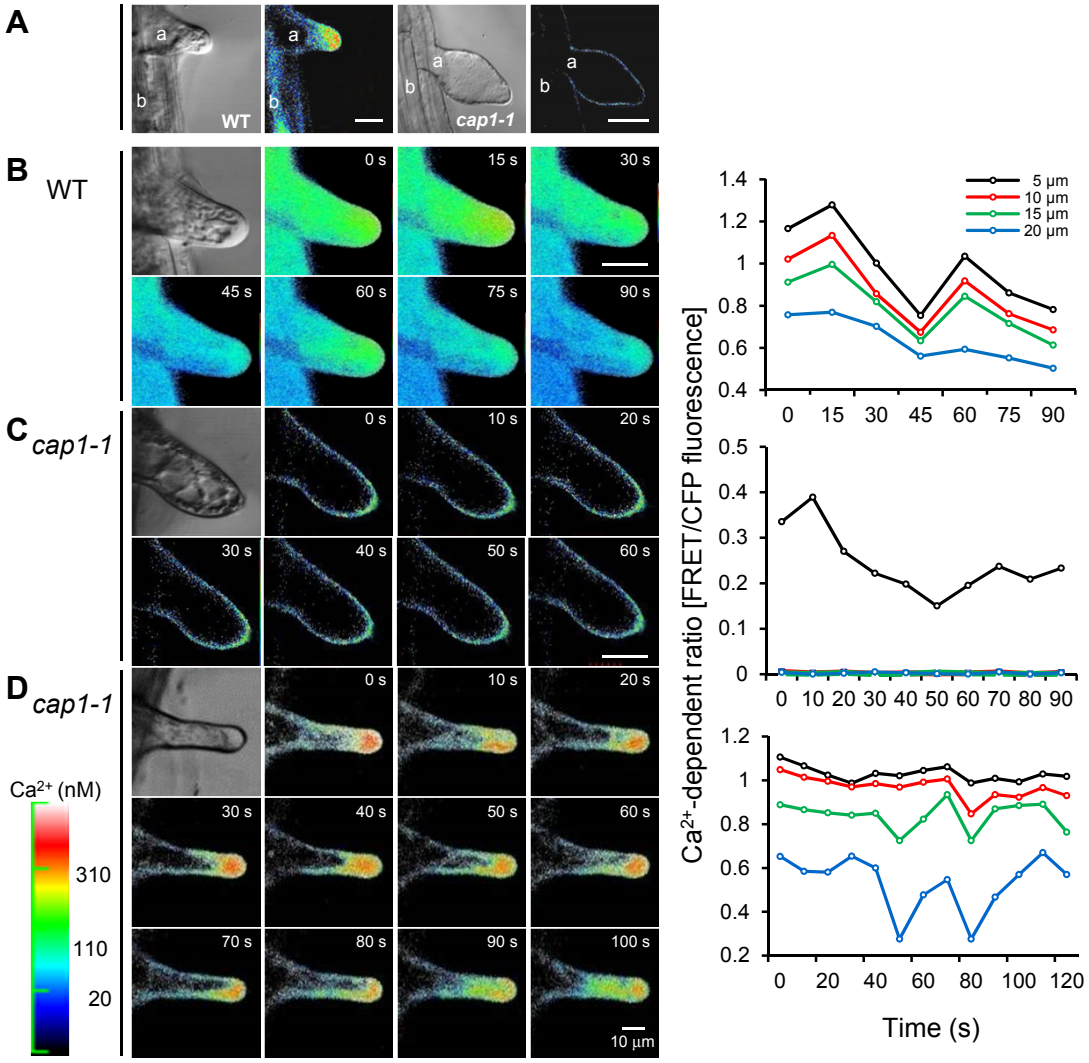


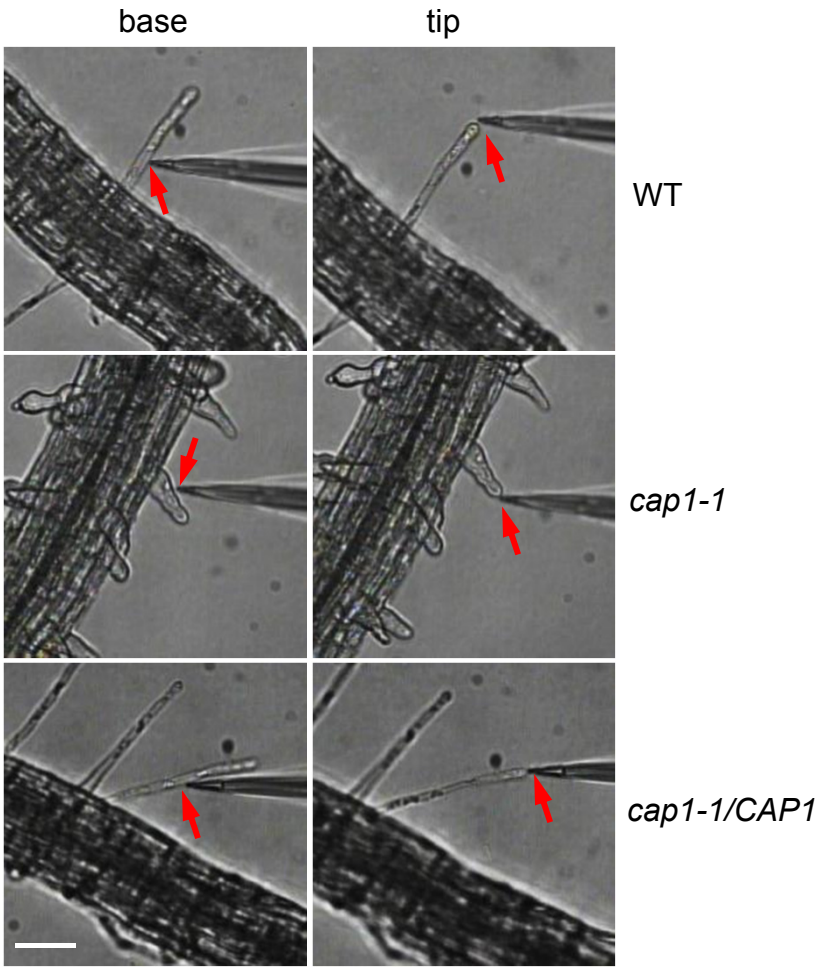
Supplemental Figure 1 online. The *CAP1* T-DNA insertion mutant has a lower cytoplasmic calcium concentration and BLAST results of *cap1-1* and over-expression transcripts. (**A**) Location of T-DNA insertion. The structure of *CAP1* is shown schematically; this gene has no intron. The vertical arrow indicates the T-DNA insertion site in the mutant lines. (**B**) RT-PCR analysis of *CAP1* expression in T-DNA insertion mutant and over-expression lines (*#1* and #2). *CAP1* cDNA was amplified (~650 bp) for 35 PCR cycles; the positive control *Actin2* was amplified with 24 PCR cycles, and amplified fragments were separated on a 1.0% agarose gel (left panel). Smaller amplified fragments (~250 bp) of *CAP1* across the deletion region were separated on the 4% agarose gel (right panel). WT transcripts display relatively slower mobility. Three biological replicates showed the same results. (**C**) Resting $[Ca^{2+}]_{cyt}$ in seedlings. Values were averaged and plotted (WT = 31; *cap1* = 21). (**D**) Sequence BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) of cDNA fragments derived from *cap1-1*. A sequence deletion from 1422 bp to 1449 bp occurred in the mutant. Query seq = WT *CAP1* cDNA sequence. (**E**) Sequence BLAST of cDNA fragment derived from the two over-expression lines (#1 and #2). The deletion has been rescued in these lines.



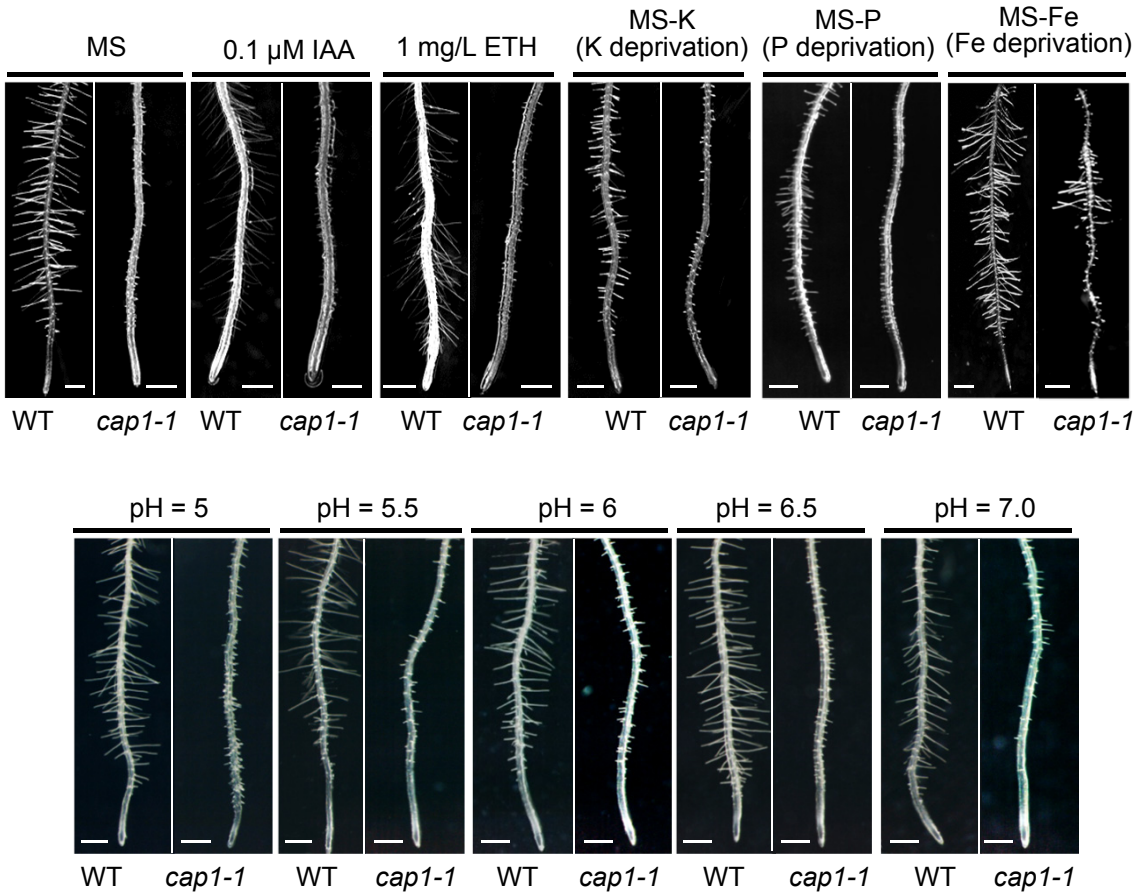
Supplemental Figure 2 online. Root hair phenotype was complemented by *CAP1* driven by the wild-type promoter in mutants and suppression of *CAP1* expression in *Arabidopsis* RNAi knock-down lines. (**A**) WT, *cap1-1*, and complementation lines (*Com-1* and *Com-2,* mutant *cap1-1* transformed with *CAP1* driven by the WT promoter) grow vertically on 1.2% MS medium for 7 d. Root hairs were recovered in both transformation lines. (**B**) Lengths and numbers of root hairs; means ± SE are indicated (n = 100 root hairs for WT and *cap1-1*; n = 60 for the *Com-1* and *Com-2* lines). (**C**) *CAP1*-silenced *Arabidopsis* roots at 7 d post germination.



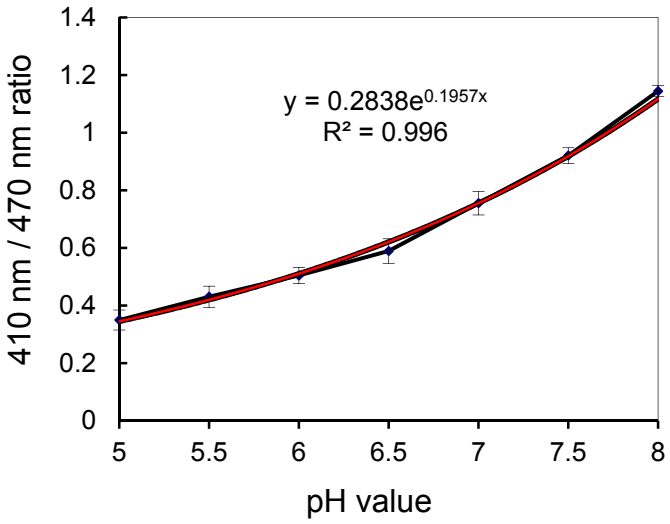
Supplemental Figure 3 online. FRET-based YC3.6 shows levels of $[Ca^{2+}]_{cyt}$ and oscillation of tip-focused Ca^{2+} gradients only in wild-type root hairs. (**A**) Cytosolic Ca^{2+} levels of root hair cells (a) and their adjacent epidermal cells (b) in WT and *cap1-1*. Tip-focused Ca^{2+} gradients oscillated in growing WT root hairs (**B**), but no oscillation was detected in *cap1-1* root hairs (**C**). Cytosolic Ca^{2+} levels were pseudocolor-coded according to the scale at the left. Images were taken every 15 s for WT and every 10 s for *cap1-1*. (**D**) The Ca^{2+} gradient and oscillation in root tips were recovered in *cap1* when seedlings were grown in ammonium-deficient medium. Quantitative analysis of cytosolic Ca^{2+} oscillations in the root hair is shown on the right of each panel. Ca^{2+} levels were measured in 10 μm^2 regions of interest along the root hair length. An increase in the FRET/CFP ratio reflects an increase in cytoplasmic Ca^{2+} level. Bars = 10 μm.



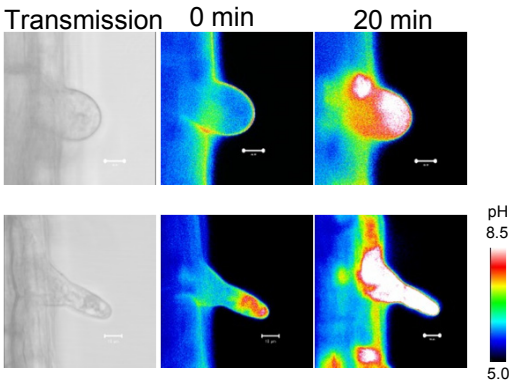
Supplemental Figure 4 online. Ion-selective vibration microelectrode recording of Ca^{2+} fluxes at root hairs surfaces in 7-d-old seedlings. Red arrows show the positions corresponding to the root hair apex (tip) and below the hair's midpoint (base). Bar = 50 µm.



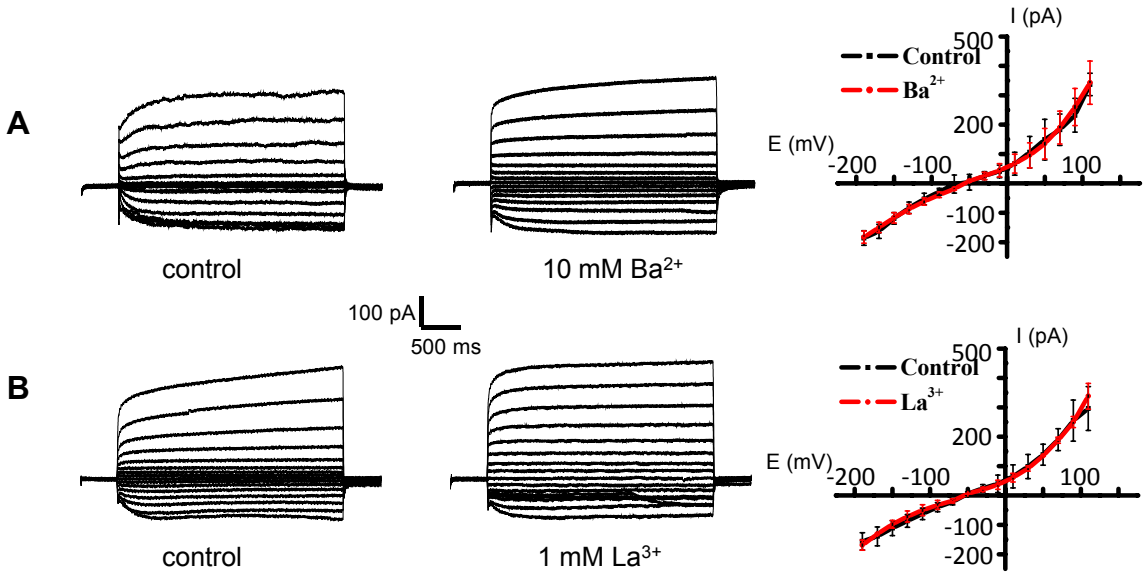
Supplemental Figure 5 online. Effects of auxin (IAA), ethylene (ETH), various nutrients, and pH on the growth of root hairs in wild-type and *cap1-1* plants. Seedlings were grown on the indicated medium for 7 d. Representatives of 30 seedlings for each genotype on different media in three separate experiments are presented. Bars = 0.5 mm.



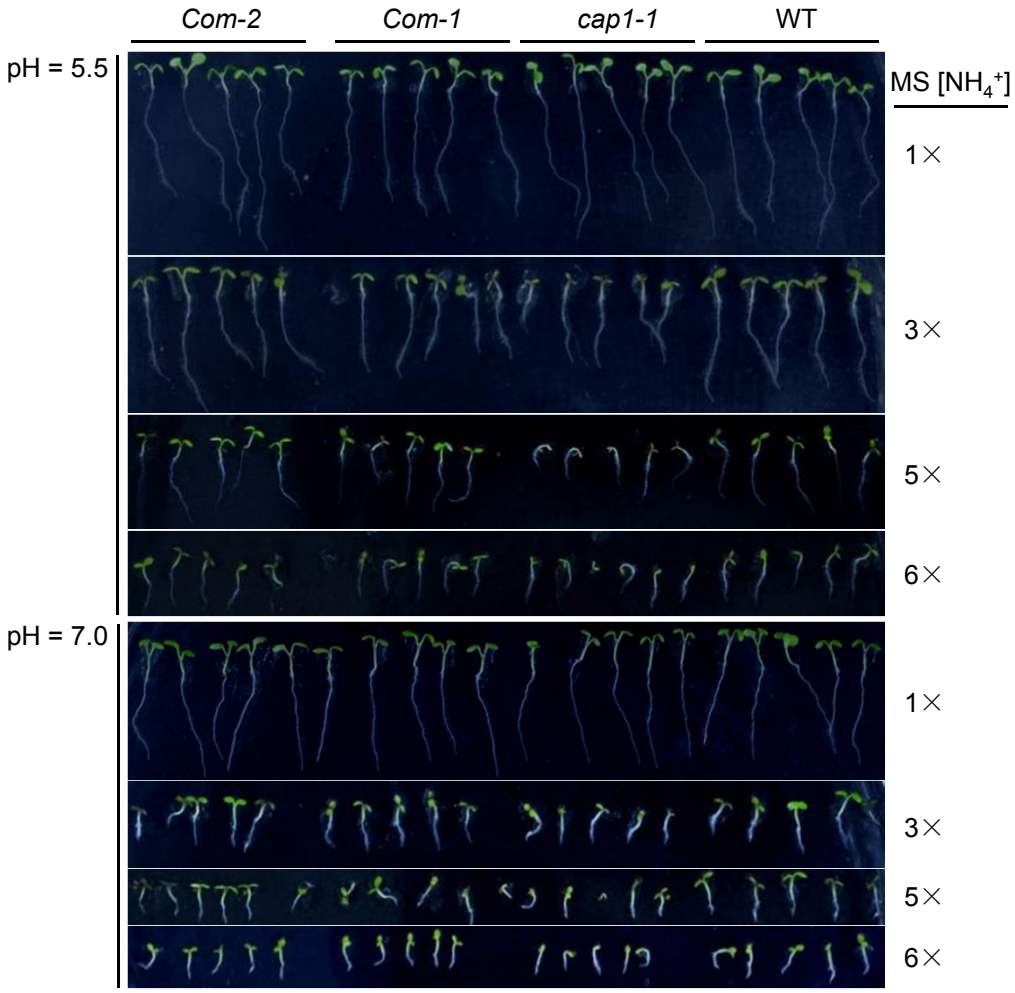
Supplemental Figure 6 online. Standard curve of the calibrated 410 nm/470 nm ratio in different pH solutions. Black line indicates the standard calibration curve, and red line is the trendline generated by Microsoft Excel. Root hair cells of *Arabidopsis* were used, bars represent means ± SE (n = 6).



Supplemental Figure 7 online. High levels of NH_4^+ enhances root hair pH_c. Fluorescence was monitored before and 20 min after 100 mM NH_4^+ was added to WT root hairs. Representative figures are shown (n = 6). Bars = 10 μm.



Supplemental Figure 8 online. Characterization of ammonium current recording on the whole-cell model using Ba^{2+} and La^{3+}. (**A**) Typical time-dependent currents recorded in WT root hair cell protoplasts in normal bathing solution (left, control) and bathing solution with 10 mM Ba^{2+} (middle) and the current-voltage relationships for the control (n = 4) and 10 mM Ba^{2+} (n = 5) in the bathing solutions (*I-V* curve, right) are shown. *I-V* curves plot means ± SE. (**B**) The calcium-channel inhibitor La^{3+} (1 mM) showed no influence on the current. WT root hair protoplasts were used. Control, n = 6; La^{3+}-treated, n = 5.



Supplemental Figure 9 online. *cap1-1* was more sensitive to high levels of ammonium than WT and the complementary lines. WT, *cap1-1*, and two complementary lines (*com-1, 2*) were sown on media with increasing ammonium (NH_4Cl) levels (MS [NH_4^+] = 20.6 mM, concentration of ammonium in MS medium). At pH 5.5, *cap1-1* growth was inhibited more severely than that of other lines on media with increasing NH_4^+ levels; while seedlings grew more slowly on higher pH (7.0) medium, inhibition of *cap1-1* growth was more severe than in other lines at high NH_4^+ levels. Results from one of the three repeated experiments are shown here. Photographs were taken after vertical growth in a growth chamber for 9 d.