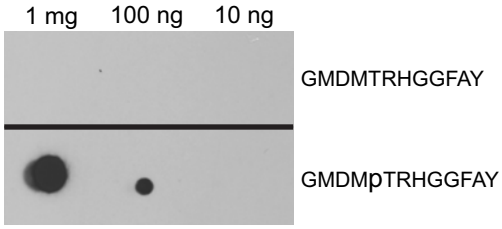
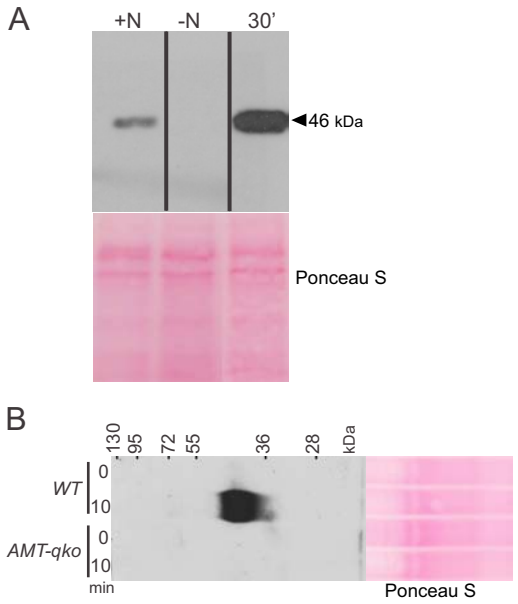
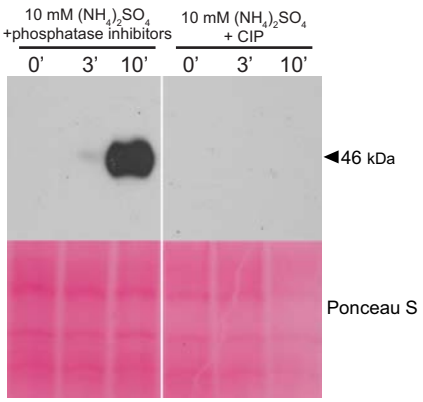
Supplemental Data. Lanquar et al. (2009). Feedback inhibition of ammonium uptake by a phospho-dependent allosteric mechanism in *Arabidopsis*.



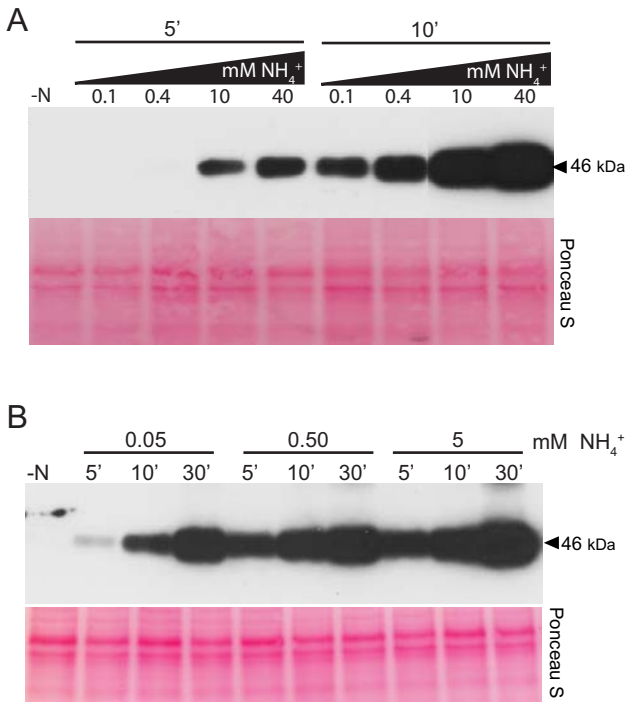
Supplemental Figure 1. The antiserum AMT-P specifically recognizes the phosphorylated (T460) peptide GMDMTRHGGFAY.
AMT1-P specificity analyzed by dot blotting. One µg, 100 ng and 10 ng of phosphorylated and non-phosphorylated peptides were blotted. AMT1-P antiserum only hybridized to the phosphorylated form (n=1).



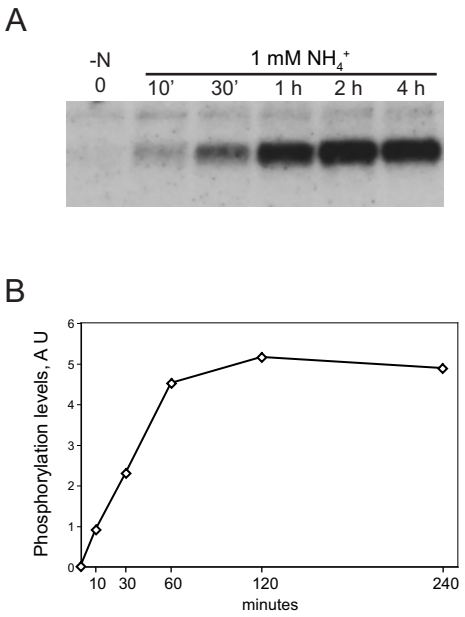
Supplemental Figure 2. Ponceau S staining as loading control for protein gel blots
(A) Ponceau S staining corresponding to protein gel blot shown in Figure 1D.
(B) Ponceau S staining corresponding to protein gel blot shown in Figure 1E.



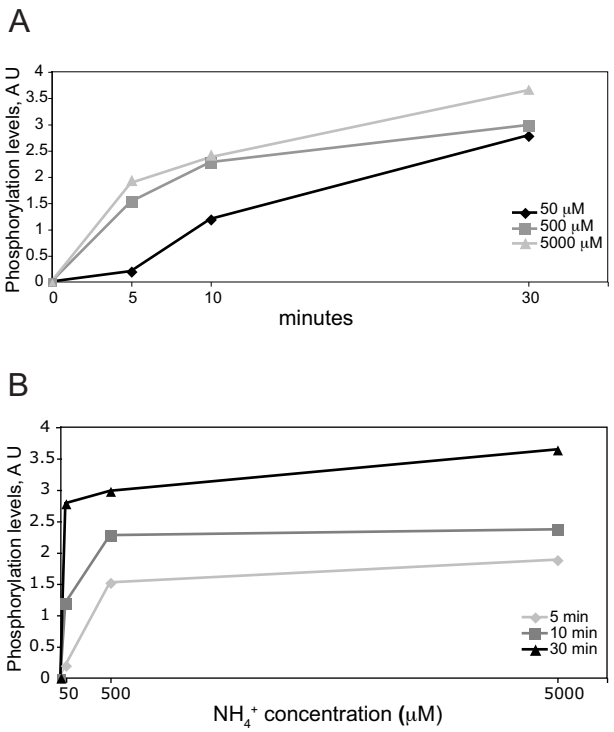
Supplemental Figure 3. CIP treatment leads to a loss of a reaction with the antiserum,
Protein gel blot analysis of root microsomal fractions. Ten mM $(NH_4)_2SO_4$ was re-supplied to nitrogen-starved plants for 3 or 10 min. Microsomal proteins were treated either with calf intestinal alkaline phosphatase (+ CIP) or with phosphatase inhibitors. Ponceau S staining is presented as control for protein loading (n=1).



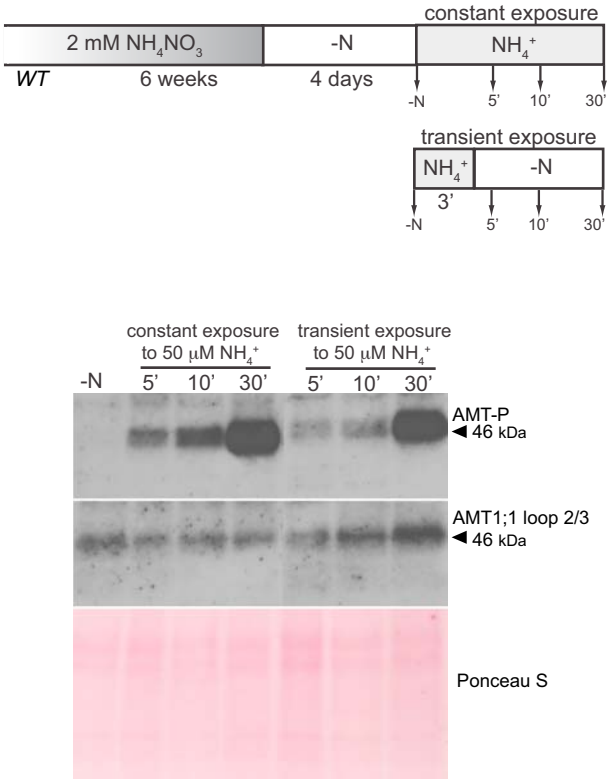
Supplemental Figure 4. Ponceau S staining as loading control for protein gel blots.
(A) Ponceau S staining corresponding to protein gel blot shown in Figure 2A.
(B) Ponceau S staining corresponding to protein gel blot shown in Figure 2B.



Supplemental Figure 5. Kinetics of AMT1 phosphorylation after NH_4^+ supply.
(A) Protein gel blot were performed on roots' proteins extract. Phosphorylation levels were monitored using the AMT1-P antiserum (n=3). Growth conditions were as in Figure 3.
(B) Graphic representation of AMT1 phosphorylation. Values were quantified from protein gel blots shown in Supplemental Figure 2A. Values are presented as arbitrary units (A.U.).

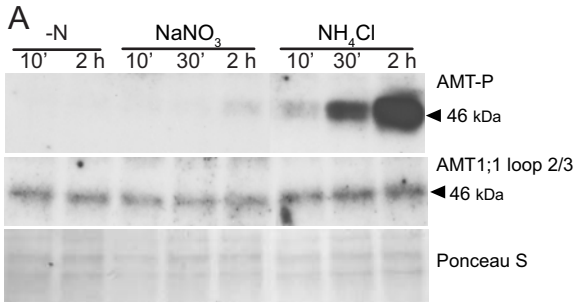


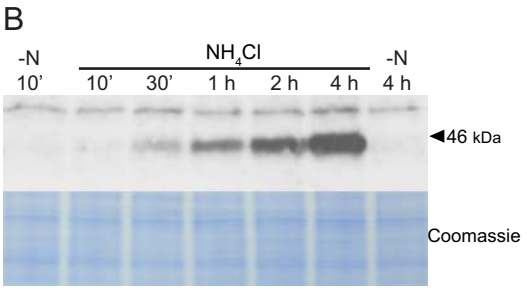
Supplemental Figure 6. Concentration dependence of AMT1 phosphorylation.
Quantitation of AMT1 phosphorylation. Values were quantified from protein gel blots shown in Figure 2B. Values are presented as arbitrary units (A.U.).
(A) Phosphorylation levels plotted against the time course exposure to NH_4^+ at different supply levels.
(B) Phosphorylation levels are plotted against each NH_4^+ concentration for each time point.

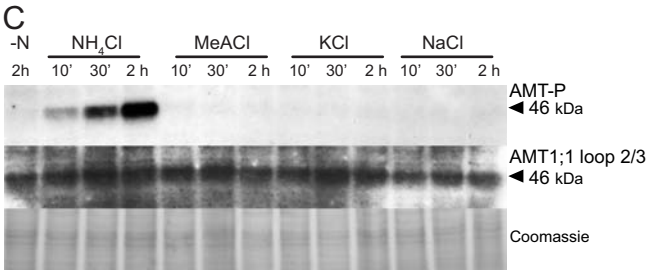


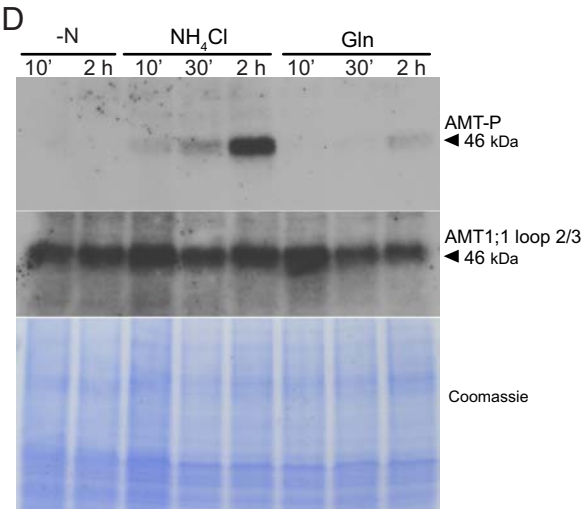
Supplemental Figure 7. AMT1 phosphorylation level resulting from constant exposure to NH_4^+ versus transient exposure to NH_4^+.

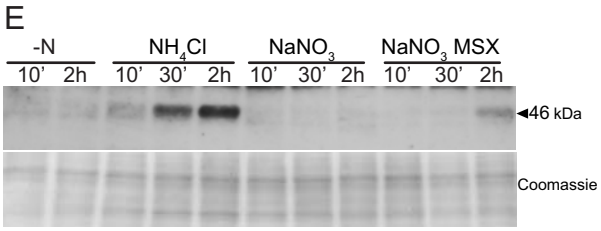
For the constant exposure, plants were exposed to 50 µM NH_4^+ (as $(NH_4)_2SO_4$) for 5, 10 and 30 min and collected. For the transient exposure, plants were exposed to 50 µM NH_4^+ (as $(NH_4)_2SO_4$) for 3 min and transferred back to nitrogen-free medium (-N) and samples were collected 5, 10 and 30 min after transfer. Protein gel blots were performed using the AMT1-P antiserum and the 'AMT1;1 loop 2/3' antiserum (n=1).



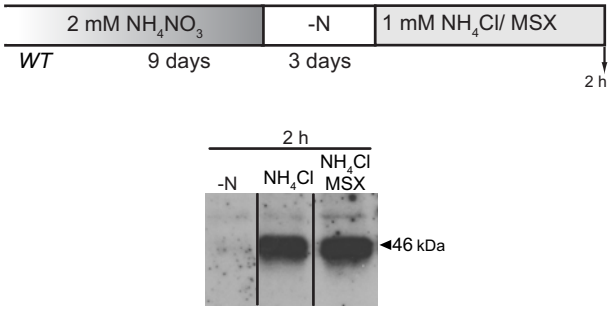




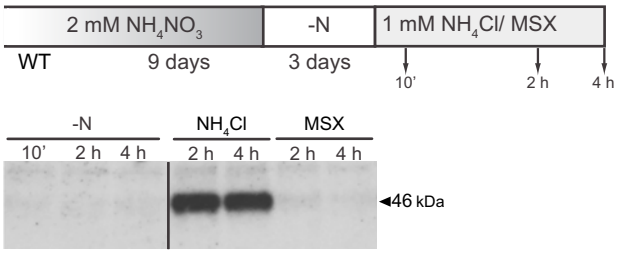




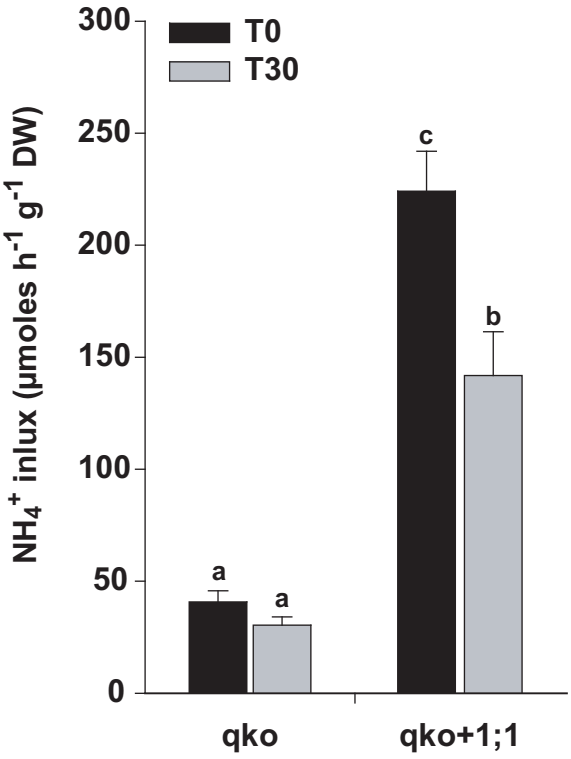
Supplemental Figure 8. Control for protein loading.
(A) Ponceau S staining of blot Figure 3A.
(B) Coomassie in gel staining for Blot Figure 3B.
(C) Coomassie in gel staining for Blot Figure 4.
(D) Coomassie in gel staining for Blot Figure 5A.
(E) Coomassie in gel staining for Blot Figure 5B.



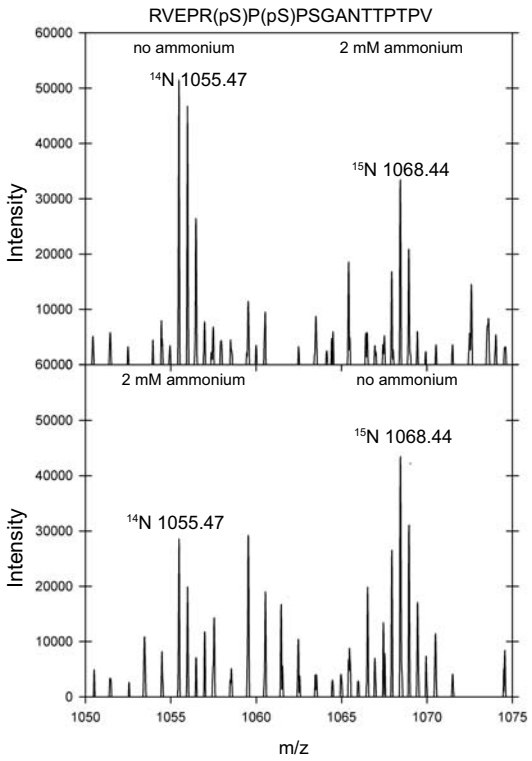
Supplemental Figure 9. Effect of NH_4^+ + MSX on AMT1 phosphorylation.
Growth conditions were as described in Figure 5. Roots were exposed to 1 mM of NH_4Cl +/- 1 mM MSX for 2 h. Protein gel blots were performed using the AMT1-P antiserum (n=1).



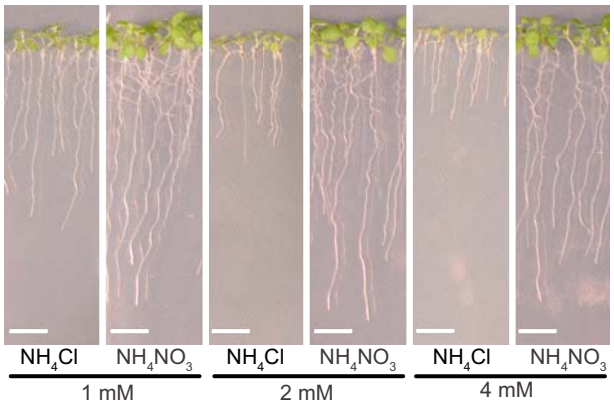
Supplemental Figure 10. Effect of MSX on AMT1 phosphorylation.
Growth conditions were as described in Figure 5. Roots were exposed to 1 mM of NH_4Cl or 1 mM MSX for 2 h and 4 h. Protein gel blots were performed using the AMT1-P antiserum (n=3).



Supplemental Figure 11. Ammonium uptake is repressed by ammonium re-supply.
Uptake of ^{15}N-labeled ammonium into roots of AMT-qko+1;1, and AMT-qko plants. Six-week-old plants were pre-cultured hydroponically under continuous supply of 2 mM ammonium nitrate (+N) and then deprived of nitrogen for 4 days (-N) prior to re-supply of 150 µM NH_4^+ (75 µM $(NH_4)_2SO_4$) for indicated times. Bars indicate means ± SD (n = 10 plants). Significant differences are indicated by different letters for each genotype (Two-way ANOVA, Fisher LSD, $p<0.001$).



Supplemental Figure 12. NH_4^+ induces the decrease of phosphorylation of S488 and S490.
Mass spectrum of labeled and unlabeled form of the doubly phosphorylated C-terminal peptide RVEPR(pS)P(pS)PSGANTTPTPV. (Top) ^{15}N-labeled plants were exposed to a pulse of 2 mM ammonium. (Bottom) Unlabeled (^{14}N) plants were exposed to 2 mM ammonium for 10 min. 1055.47 corresponds to the mass of the ^{14}N peptide and 1068.44 of the ^{15}N peptide. Ion intensities of labeled and unlabeled peptides are a measure of the relative abundance of these peptides in the two combined samples.



Supplemental Figure 13. Arabidopsis is sensitive to high NH_4Cl concentrations.
Arabidopsis seedlings were grown for seven days on medium containing either NH_4Cl or NH_4NO_3. Twenty µM of KNO_3 was supplied in both cases. Scale bar is 2 cm.