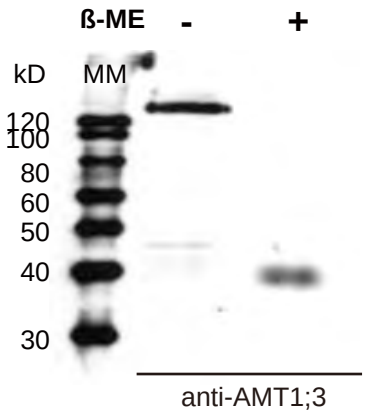
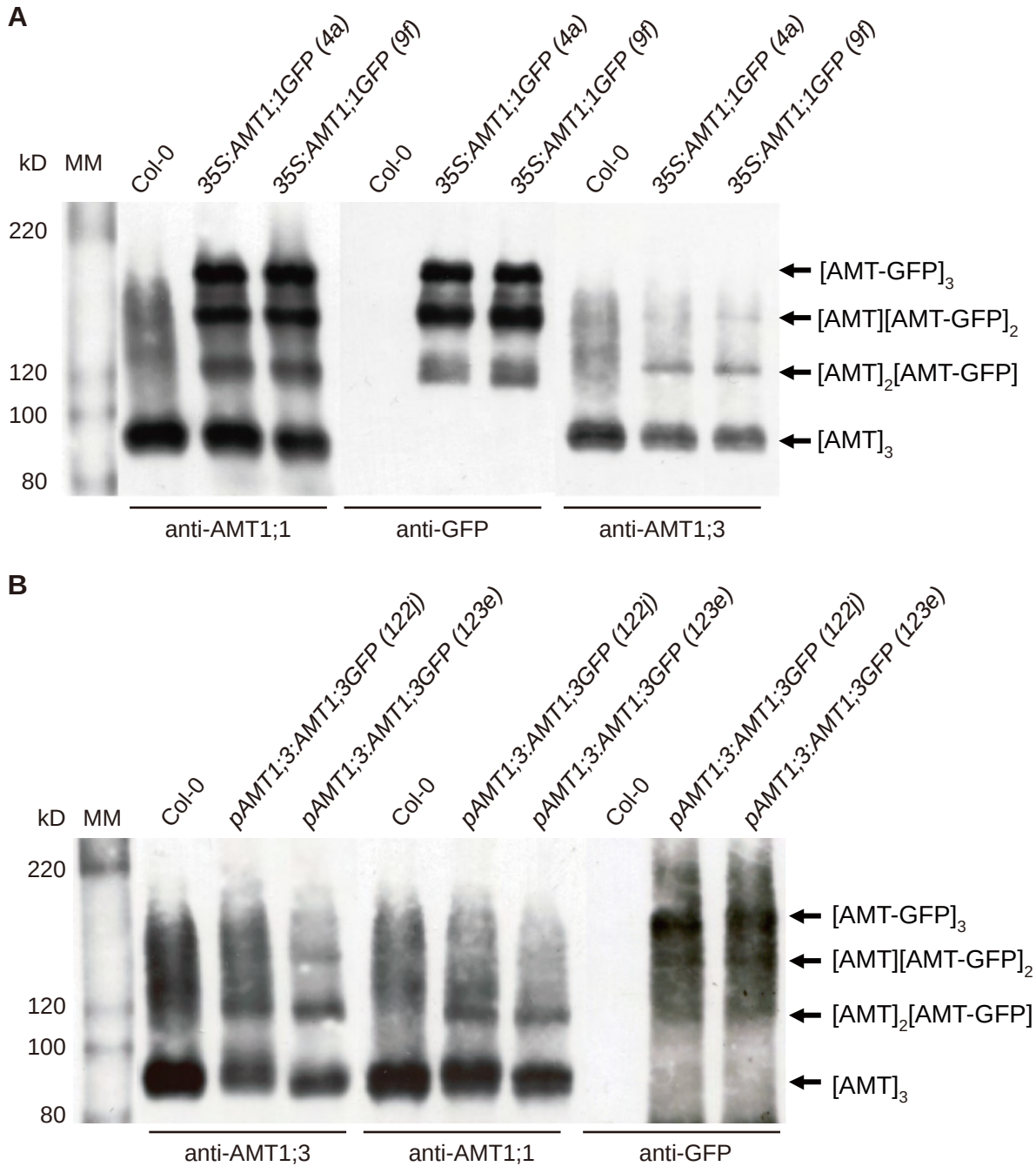
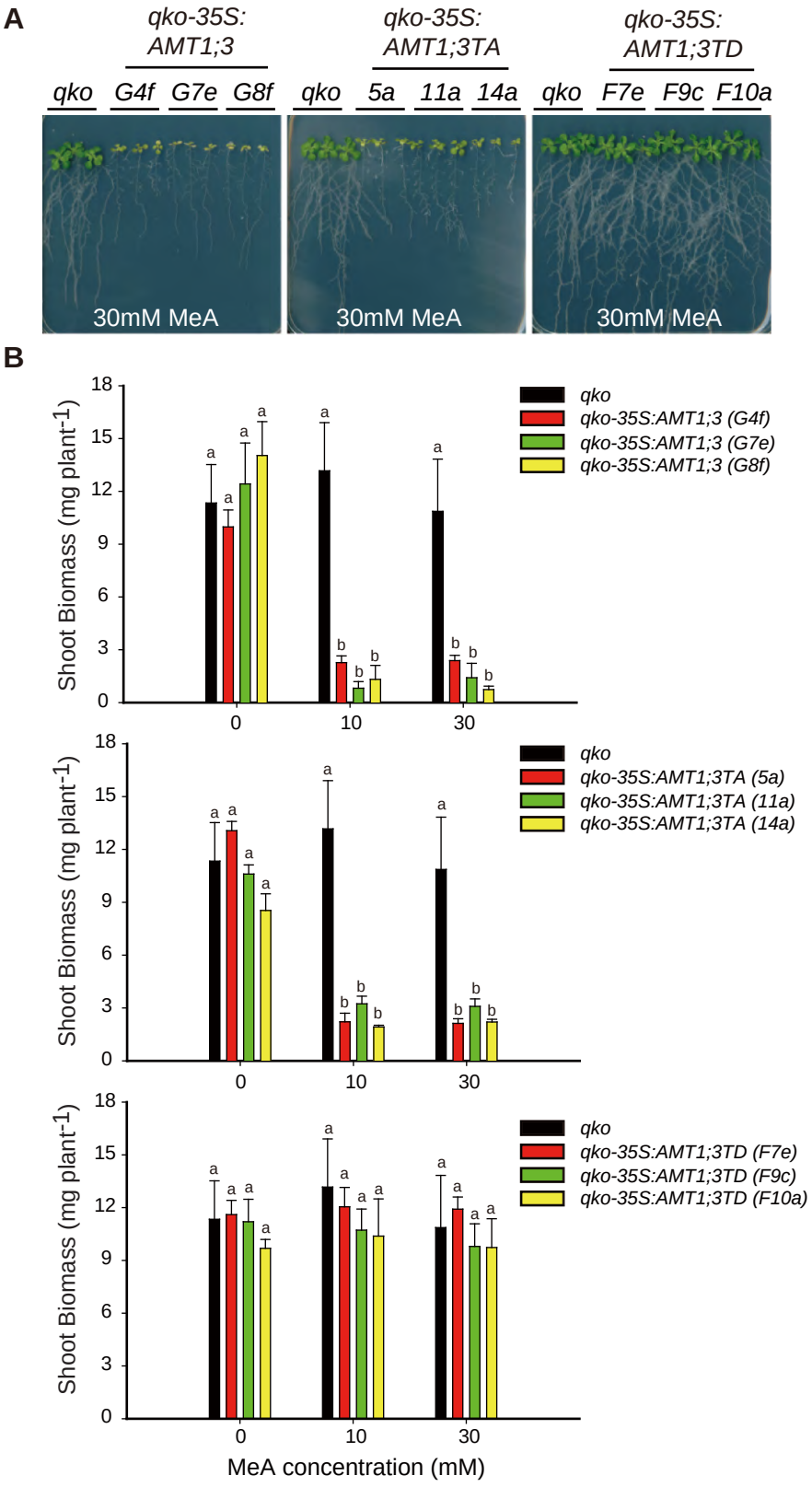

**Supplemental Figure 1. Amino acid sequence alignments of Arabidopsis AMT1;3 and AMT1;1.** The identical residues are shown in yellow. The conserved C-termini of AMT1;1 and AMT1;3 are enclosed by a black box, while the Thr residue relevant for phosphorylation is enclosed in red (Loqué et al, 2007).



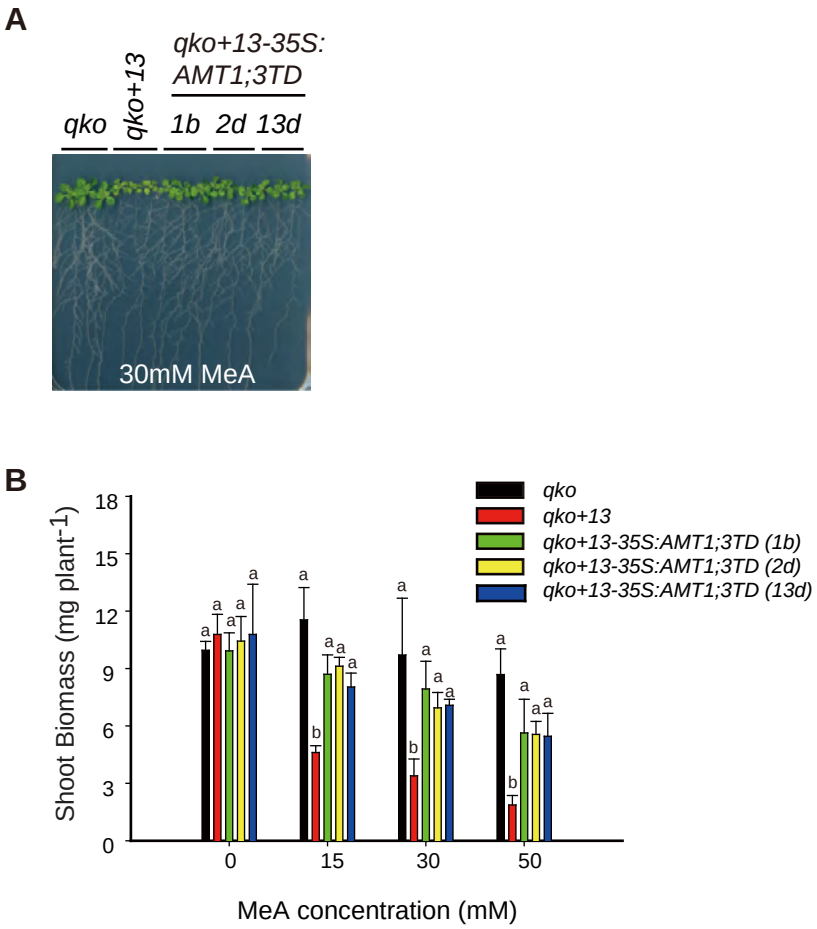

**Supplemental Figure 2. Oligomerization of AMT1;3 in roots.**
Protein gel blot analysis of microsomal membrane fractions from Arabidopsis wild-type (Col-0) roots using the anti-AMT1;3 antibody. Protein samples were pre-treated with ß-mercaptoethanol (ß-ME) at 37°C (+) corresponding to reducing conditions or in the absence of ß-ME at 0°C (-) corresponding to non-reducing conditions. A 10% SDS-PAGE gel was used to separate proteins. Numbers at the left indicate molecular masses in kD according to marker proteins. Protein samples were extracted from six-week-old Arabidopsis plants that were pre-cultured in nutrient solution containing 2 mM ammonium nitrate and harvested after a 4-day period of nitrogen deficiency.



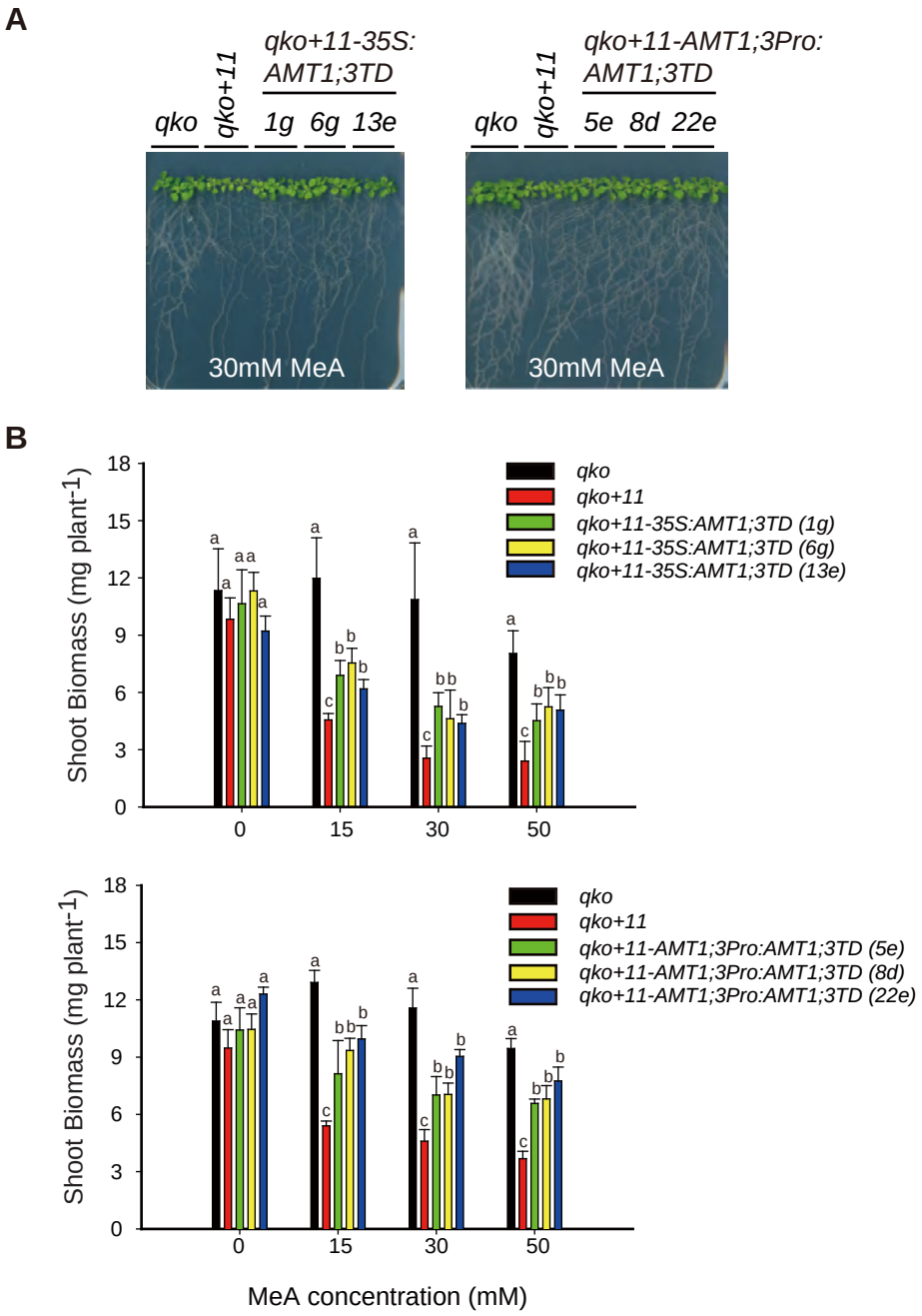

**Supplemental Figure 3. Hetero-trimerization of AMT1;3 and AMT1;1.**
**(A)** Protein gel blot analysis of microsomal membrane fractions from Arabidopsis roots of wild-type (Col-0), or two independent transgenic plants expressing an *AMT1;1-GFP* fusion construct (*35S:AMT1;1GFP*) using anti-AMT1;1, anti-GFP or anti-AMT1;3 antibody. Protein gel blots correspond to those shown in Figure 2A-C and are shown here for better comparison of molecular weights and data in Figure S3B.
**(B)** Protein gel blot analysis of microsomal membrane fractions from Arabidopsis roots of wild-type (Col-0), or two independent transgenic plants expressing an *AMT1;3-GFP* fusion construct (*pAMT1;3:AMT1;3GFP*) using anti-AMT1;3, anti-AMT1;1 or anti-GFP antibody. Protein samples were extracted from six-week-old Arabidopsis plants that were pre-cultured in nutrient solution containing 2 mM ammonium nitrate and harvested after a 4-day period of N deficiency. The protein samples were pretreated at 0°C (non-reducing conditions) in the absence of ß-ME and a 6% SDS-PAGE gel was used to separate proteins. Numbers at the left indicate molecular masses in kD according to marker proteins. According to the shift in their molecular weight, possible combinations of oligomers are indicated at the right.




**Supplemental Figure 4. Functional expression of C-terminally modified AMT1;3 variants in the quadruple insertion line *qko* as revealed by methylammonium (MeA) sensitivity.**
**(A)** Growth phenotype of *qko* and three independent transgenic lines of *qko-35S:AMT1;3*, *qko-35S:AMT1;3TA*, or *qko-35S:AMT1;3TD* on agar supplied with 30 mM MeA.
**(B)** Shoot fresh weight of plants as the same lines in **(A)** on agar supplied with 0-30 mM MeA. The plants were grown on agar containing different concentration of MeA in the presence 1 mM nitrate and 1% sucrose for 12 days after preculture on ½ MS medium containing 5 mM nitrate as sole nitrogen source for 7 days. Bars indicate means ± SD; n=4. Significant differences at $p<0.01$ according to Fisher's LSD test are indicated by different letters.




**Supplemental Figure 5. Inhibition of the ammonium transport capacity in roots of the triple insertion line *qko+AMT1;3 (qko+13)* by ectopic expression of *AMT1;3TD* as revealed by methylammonium (MeA) sensitivity.**
**(A)** Growth phenotype of *qko*, *qko+13*, and three independent transgenic lines of *qko+13-35S:AMT1;3TD* on agar supplied with 30 mM MeA.
**(B)** Shoot fresh weight of plants as the same lines in **(A)** on agar supplied with 0-50 mM MeA.
The plants were grown on agar containing different concentration of MeA in the presence 1 mM nitrate and 1% sucrose for 12 days after preculture on ½ MS medium containing 5 mM nitrate as sole nitrogen source for 7days. Bars indicate means ± SD; n=4. Significant differences at $p<0.01$ according to Fisher's LSD test are indicated by different letters.

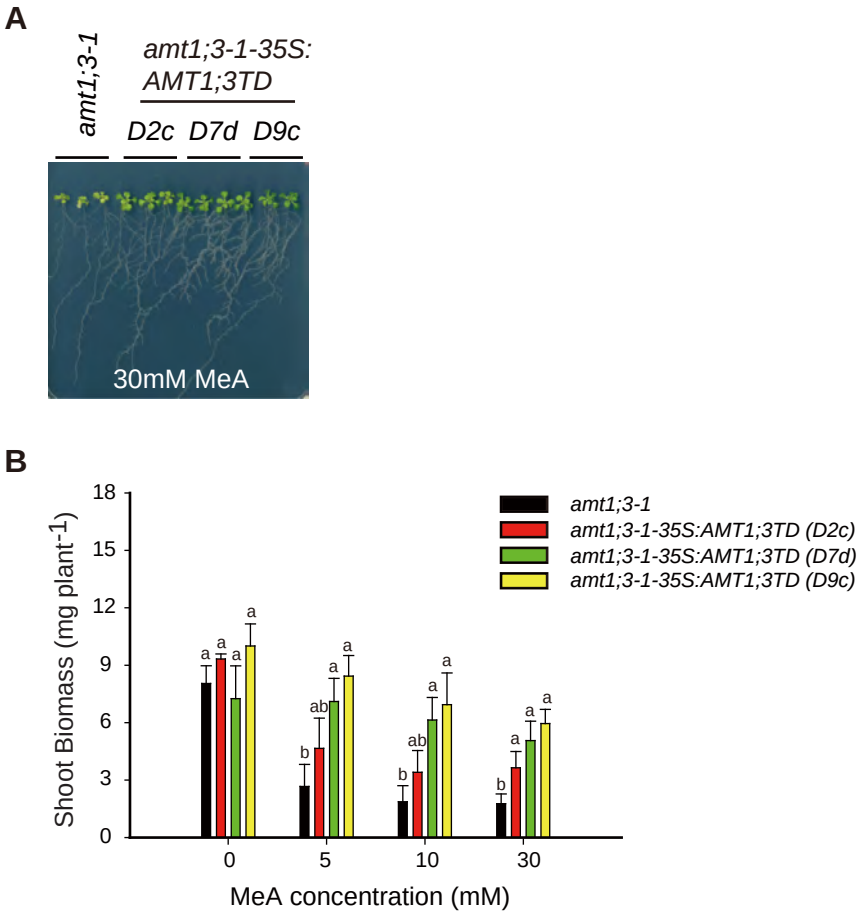



**Supplemental Figure 6. Inhibition of the ammonium transport capacity in roots of the triple insertion line *qko+AMT1;1 (qko+11)* by ectopic expression of *AMT1;3TD* as revealed by methylammonium (MeA) sensitivity.**

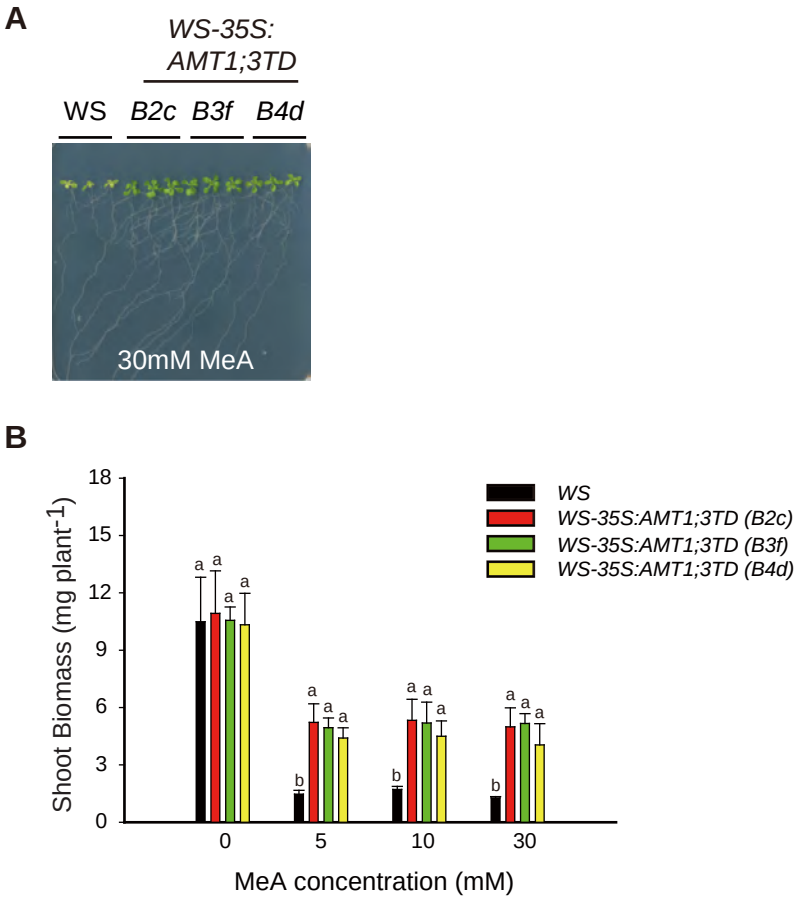
**(A)** Growth phenotype of *qko*, *qko+11* and three independent transgenic lines of *qko+11-35S:AMT1;3TD* and *qko+11-AMT1;3Pro:AMT1;3TD* on agar supplied with 30 mM MeA.

**(B)** Shoot fresh weight of plants as the same lines in **(A)** on agar supplied with 0-50 mM MeA.

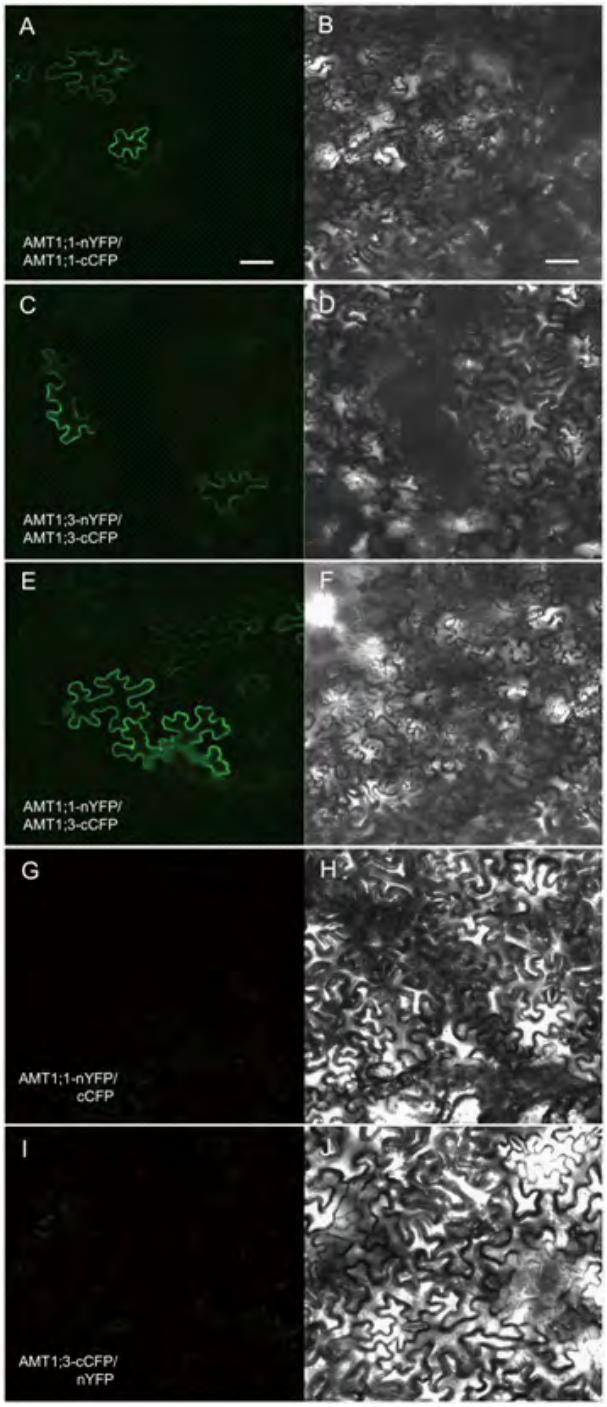
The plants were grown on agar containing different concentration of MeA in the presence 1 mM nitrate and 1% sucrose for 12 days after preculture on ½ MS medium containing 5 mM nitrate as sole nitrogen source for 7days. Bars indicate means ± SD; n=4. Significant differences at $p<0.01$ according to Fisher's LSD test are indicated by different letters.




**Supplemental Figure 7. Inhibition of the ammonium transport capacity in roots of the single insertion line *amt1;3-1* by ectopic expression of *AMT1;3TD* as revealed by methylammonium (MeA) sensitivity.**
**(A)** Growth phenotype of *amt1;3-1* and three independent transgenic lines of *amt1;3-1-35S:AMT1;3TD* on agar supplied with 30 mM MeA.
**(B)** Shoot fresh weight of plants as the same lines in **(A)** on agar supplied with 0-30 mM MeA.
The plants were grown on agar containing different concentration of MeA in the presence 1 mM nitrate and 1% sucrose for 12 days after preculture on ½ MS medium containing 5 mM nitrate as sole nitrogen source for 7days. Bars indicate means ± SD; n=4. Significant differences at $p<0.01$ according to Fisher's LSD test are indicated by different letters.

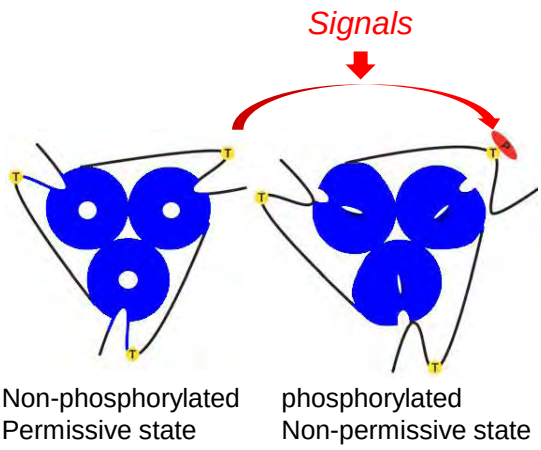



**Supplemental Figure 8. Inhibition of the ammonium transport capacity in roots of Arabidopsis wild-type plants by ectopic expression of *AMT1;3TD* as revealed by methylammonium (MeA) sensitivity.**
**(A)** Growth phenotype of the wild-type (WS) and three independent transgenic lines of *WS-35S:AMT1;3TD* on agar supplied with 30 mM MeA.
**(B)** Shoot fresh weight of plants as the same lines in **(A)** on agar supplied with 0-30 mM MeA.
The plants were grown on agar containing different concentration of MeA in the presence 1 mM nitrate and 1% sucrose for 12 days after preculture on ½ MS medium containing 5 mM nitrate as sole nitrogen source for 7days. Bars indicate means ± SD; n=4. Significant differences at $p<0.01$ according to Fisher's LSD test are indicated by different letters.

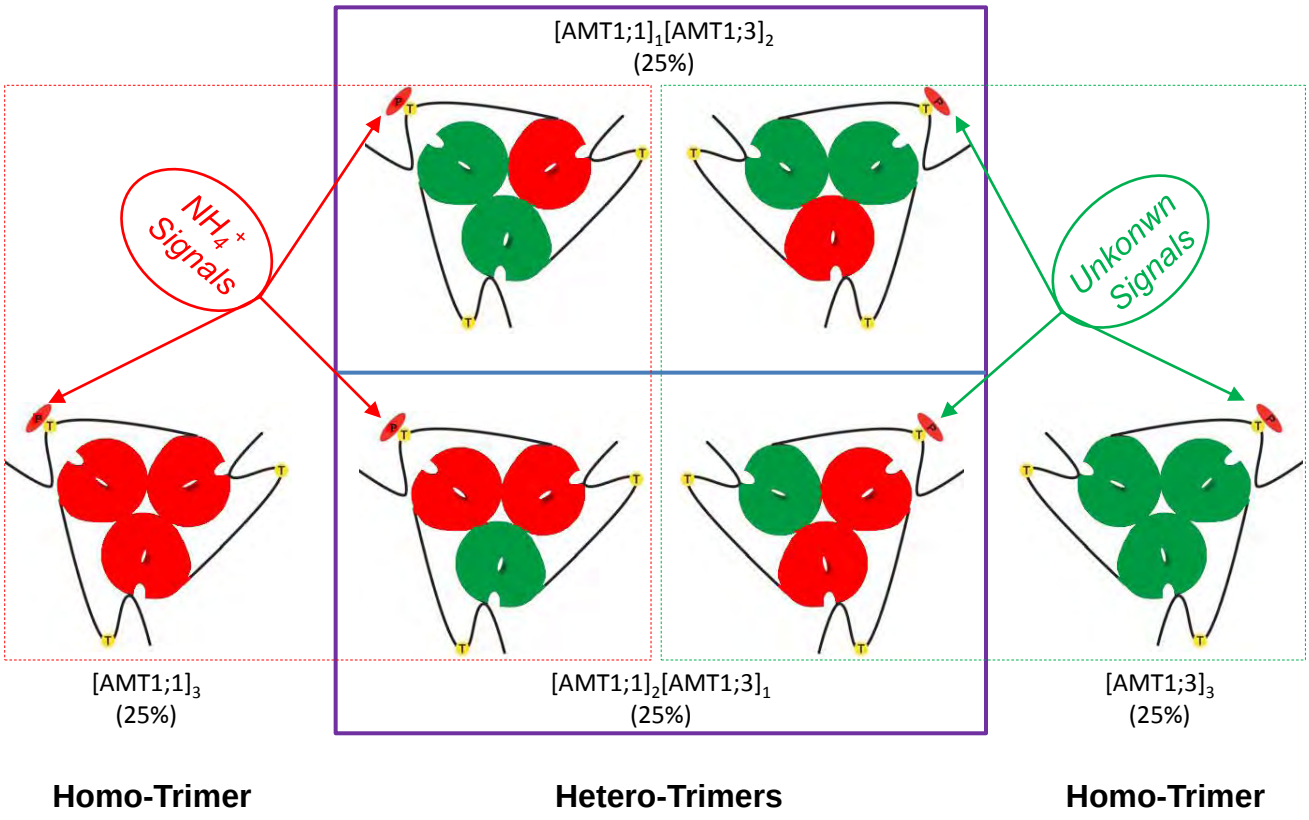



**Supplemental Figure 9. Split-YFP-based interaction assays for AMT1;1 and AMT1;3.**
**(A, B)** Reconstitution of YFP fluorescence from AMT1;1-nYFP + AMT1;1-cCFP;
**(C, D)** AMT1;3-nYFP + AMT1;3-cCFP;
**(E, F)** AMT1;1-nYFP + AMT1:3-cCFP;
**(G, H)** AMT1;1-nYFP + cCFP;
**(I, J)** AMT1;3-cCFP + nYFP.
Transient coexpression in *Nicotiana benthamiana* leaves of the indicated constructs. YFP-derived fluorescence and bright field images are shown on the right and left, respectively. Bars = 20 µm.

**A**




**B**




**Supplemental Figure 10. Model for the *trans*-inactivation of a trimeric AMT complex by phosphorylated AMT1;1 or AMT1;3.**
**(A)** Ammonium transport activity (permissive state) of a trimeric AMT complex requires *trans*-activation of each subunit through interaction of a pore with a non-phosphorylated C terminus of a neighbouring AMT subunit. Phosphorylation of a conserved threonine residue in the C terminus inactivates the whole complex (Loqué et al., 2007).
**(B)** Phosphorylation of AMT1;1 mediated by external ammonium (Lanquar et al., 2009) or of AMT1;3 by yet unidentified signals triggers *trans*-inactivation of the neighbouring subunit which, in turn, *trans*-inactivates the next subunit. Reciprocal interactions between AMT1;1 and AMT1;3 subunits in heteromeric AMT complexes then allow signal exchange across AMT1 isoforms.

**Supplemental References:**

**Lanquar, V., Loqué, D., Hormann, F., Yuan, L., Bohner, A., Engelsberger, W.R., Lalonde, S., Schulze, W.X., von Wirén, N., and Frommer, W.B.** (2009). Feedback Inhibition of Ammonium Uptake by a Phospho-Dependent Allosteric Mechanism in Arabidopsis. Plant Cell **21:** 3610-3622.

**Loqué, D., Lalonde, S., Looger, L.L., von Wirén, N., and Frommer, W.B.** (2007). A cytosolic trans-activation domain essential for ammonium uptake. Nature **446:** 195-198.