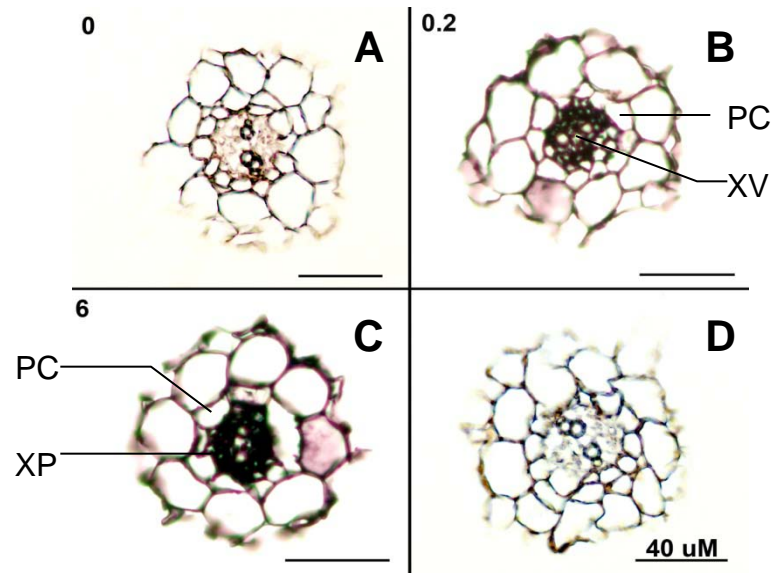
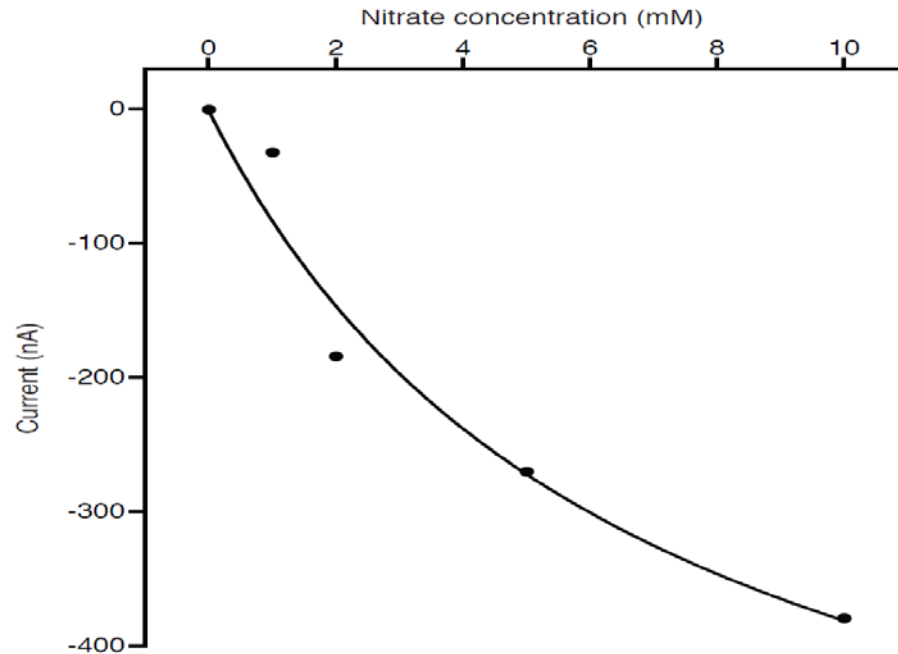


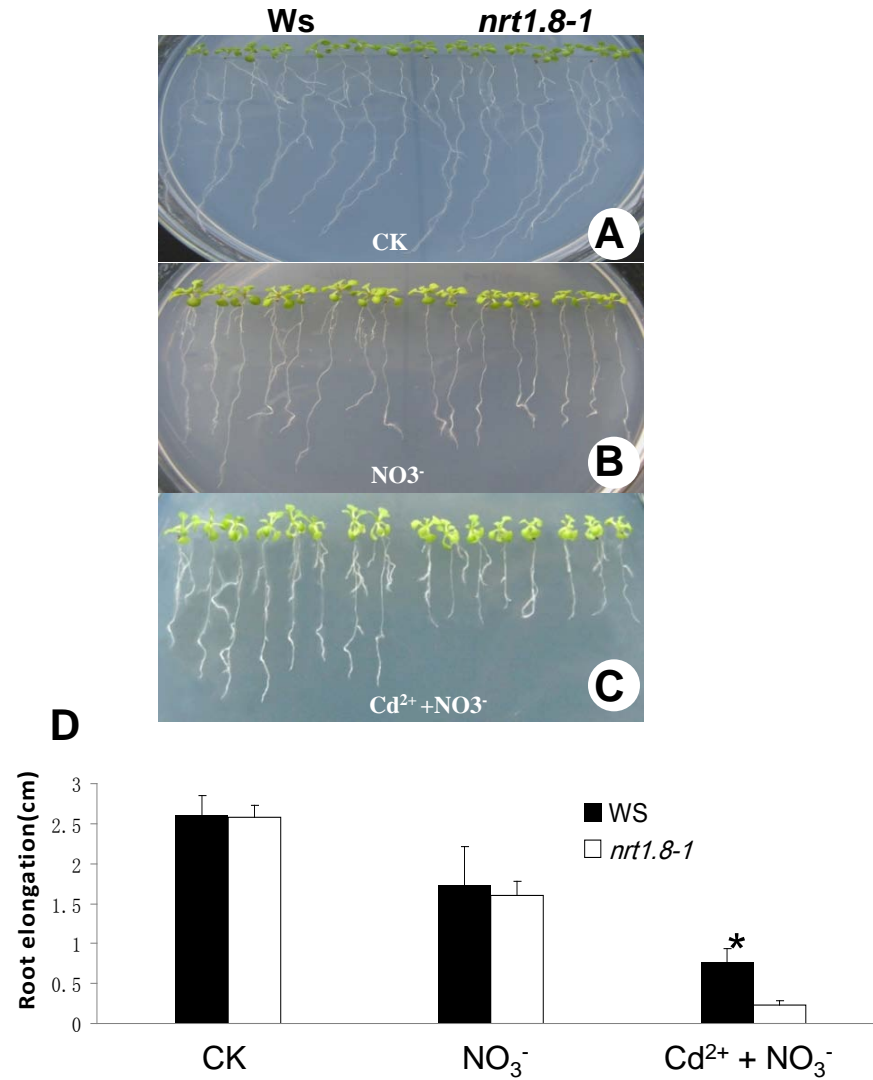
Supplemental Figure 1. Isolation and characterization of T-DNA insertion line *nrt1.8-1* and its complementation lines. (A): Schematic map of the *nrt1.8-1* mutant carrying a T-DNA insertion in the third exon in an antisense orientation. The black boxes and lines represent exons and introns respectively. The arrows indicate the primers used for RT-PCR. L, *NRT1.8* forward primer; R, *NRT1.8* reverse primer. (B): PCR screening using genomic DNA identified a homozygous *nrt1.8-1* line (left), RT-PCR analysis showed knockout of *NRT1.8* transcript in *nrt1.8* homozygote compared to Ws (right). Primer sequences are shown in Materials and Methods. (C) RT-PCR using primers from (A) was performed to identify *NRT1.8/nrt1.8-1* complementation lines; *Actin 2* was used as a loading control.



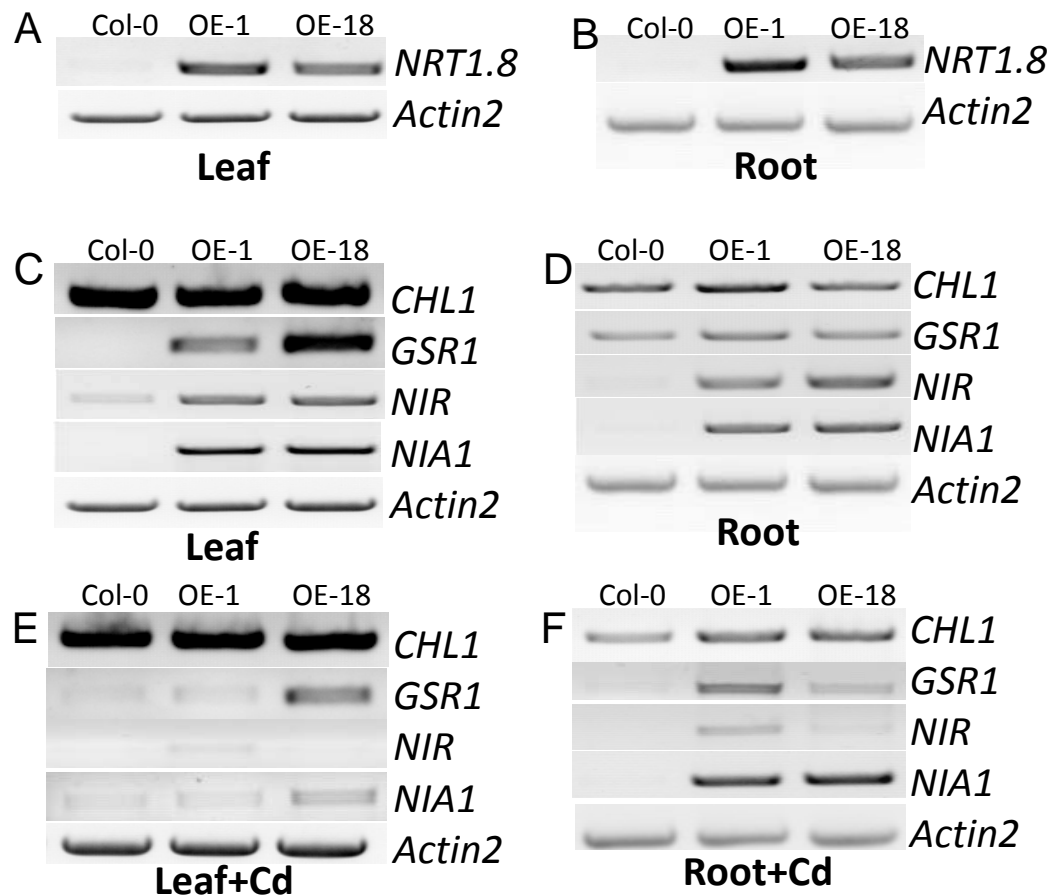
Supplemental Figure 2. *NRT1.8* expression is up-regulated in response to external NO_3^- . Plants were grown and pretreated as described in Methods, then transferred to control (A), 0.2 mM nitrate (B), or 6mM nitrate (C) for 2h. In-situ hybridizations using anti-sense primer (A-C) and sense primer as a control (D) were performed. PC, pericycle cells; XV, xylem vessel; XP, xylem parenchyma. Bar=40 μm



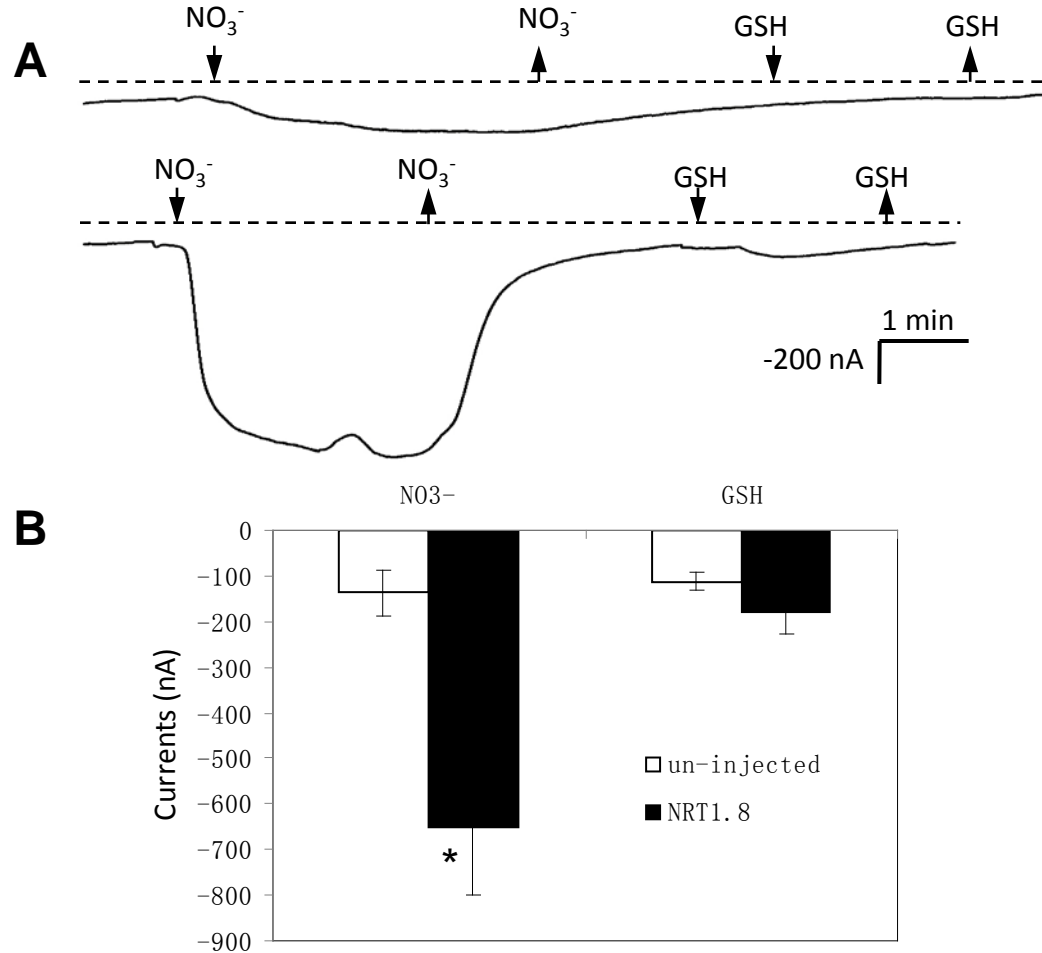
Supplemental Figure 3. NRT1.8 is a low-affinity nitrate transporter. Representative example of current responses of a *NRT1.8*-injected oocyte to increasing external nitrate concentrations. The oocyte membrane potential was clamped to -20 mV. Data were fitted to the Michaelis-Menten equation, resulting for this dataset in values for the parameters K_m and V_{max} of 7 mM and -638 nA, respectively.



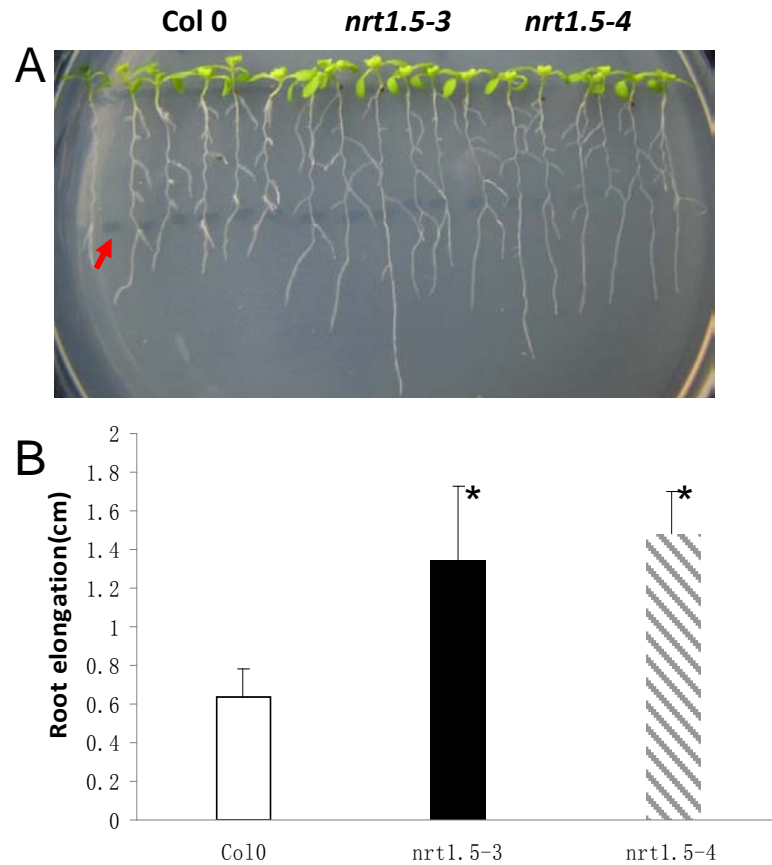
Supplemental Figure 4. Cadmium sensitivity in *nrt1.8-1* is increased at higher nitrate levels. Cd^{2+} sensitivity was evaluated in control (A), 50mM NO_3^- (B), and 50uM Cd^{2+} plus 50mM NO_3^- (C). Root elongation after treatments was determined (D), values are mean \pm SD, n = 8 plants. * $P < 0.001$. Images are not at same scale.



Supplemental Figure 5. Over-expression of *NRT1.8* alters the expression of key genes in the nitrate assimilation pathway. *c-myc* tag was fused to the C-terminus of *NRT1.8* ORF. Primers crossing *NRT1.8* and *c-myc* ORFs were used to determine overexpression of *NRT1.8* in leaves (A) and roots (B) of WT (Col-0) and representative transgenic lines (OE-1, OE-18). The expression pattern of selected marker genes in the nitrate assimilation pathway was analyzed in Col-0, OE-1 and OE-18 in leaves (C) and roots (D) under control conditions, or leaves (E) and roots (F) treated with 20 μ M Cd²⁺ for 3 days. The amplification cycles were 28 and 20 for the marker genes and *Actin 2* respectively. *CHL1*: Dual-affinity nitrate uptake transporter *AtNRT1.1* (At1g12110); *NIA1*: nitrate reductase 1 (At1g77760); *NIR*: nitrite reductase (At2g15620); *GSR1*: cytosolic glutamine synthase 1 (At5g37600). *Actin2* was used as a loading control. The expression of these genes were not analyzed in *nrt1.8* mutant plants due to their low expression levels under control conditions.



Supplemental Figure 6. Analysis of substrate selectivity for NRT1.8 (A) Representative inward currents elicited by 10 mM NO₃⁻ or 10 mM GSH at pH 5.5 and a holding potential of -100 mV were recorded for un-injected control (top) and *NRT1.8* cRNA injected oocytes (bottom). (B) Quantification of the currents recorded; **P* < 0.05, *n* = 3 for both injected and uninjected oocytes from three separate batches.



Supplemental Figure 7. *nrt1.5* mutants show increased tolerance to cadmium stress. Normally and equally grown 5 day old seedlings were transferred to $\frac{1}{2}$ x MS medium supplemented with $50 \mu\text{M}$ Cd^{2+} , and allowed another 8 days to grow. **(A)** Cd^{2+} sensitivity was evaluated, the red arrow indicates the start points (black-dotted line on the plate) for root elongation after treatment; **(B)** root elongation after treatments was determined. Values were mean \pm SD, $n = 6$ plants, * $P < 0.001$.

Supplemental Data. Li et al. (2010). Plant Cell 10.1105/tpc.110.075242

Supplemental Table 1. Transcriptomic analysis of nitrate assimilation pathway under Cd²⁺ stress.

AGI Index/Alias	Exp. 1	Exp.2	Exp.3	AGI Index /Alias	Exp. 1	Exp.2	Exp.3
NRT1 family				At3g25280	2.31	1.23	1.1
At1g12110 (NRT1.1)	-1.98	-1.98	1.5	At1g27040	1.26	-1.28	1.1
At1g69850 (NRT1.2)	-1.16	-1.10	1.2	At5g62730	1.43	-1.42	1.1
At3g21670 (NRT1.3)	-2.88	-1.01	1.1	At5g19640	1.06	-9.20	1.7
At2g26690 (NRT1.4)	-1.77	-1.30	2.0	At3g54140	1.09	-1.50	-1.8
At1g32450 (NRT1.5)	-2.04	-1.37	-4.0	At5g01180	1.31	1.13	1.4
At1g27080 (NRT1.6)	1.29	-1.20	1.1	At1g62200	1.41	-1.09	1.2
At1g69870 (NRT1.7)	1.96	-1.32	1.2	At2g02020	-1.35	-1.48	1.1
At4g21680 (NRT1.8)	5.37	7.94	26.0	At2g02040	1.24	-1.02	1.3
At5g46050	-2.97	-2.30	-2.9	NRT2 family			
At5g46040	-2.01	-1.30	-3.5	At1g08090 (NRT2.1)	1.37	-1.04	1.1
At2g40460	-1.10	-2.92	-1.7	At1g08110 (NRT2.2)	1.88	1.32	1.4
At2g37900	-1.03	-2.04	-1.1	At5g60780 (NRT2.3)	-1.16	3.47	5.12
At3g53960	1.30	-1.98	-1.7	At5g60770 (NRT2.4)	1.00	1.14	1.4
At3g01350	-1.58	-3.30	-5.2	At1g12940 (NRT2.5)	-1.13	2.02	3.5
At5g14940	-1.83	-2.29	-2.8	At3g45060 (NRT2.6)	1.14	1.58	2.4
At1g72120	1.21	-1.36	-1.58	At5g14570 (NRT2.7)	1.04	1.46	2.2
At1g22550	-2.27	-2.04	-2.7	Nitrate/nitrite assimilatory genes			
At1g22570	-1.33	-1.68	-1.2	At1g77760(NIA1)	-3.41	-4.8	-2.27
At1g72130	1.07	-1.03	1.5	At1g37130(NIA2)	1.07	1.65	1.39
At1g72140	1.11	-2.10	-1.4	At2g15620(NiR)	-2.94	-3.49	-1.97
At1g22540	1.63	-1.13	-1.0	At5g40850	-2.37	-2.06	-1.48
At5g28470	8.41	-1.39	2.1	At4g05390	-1.40	1.1	-1.25
At1g69860	1.33	-1.57	1.5	At1g30510	-1.99	-1.14	-1.97
At1g18880	-1.14	-1.03	-1.4	At4g32360	-1.27	-1.45	-1.42
At5g62680	-1.55	-1.13	-1.7	At5g66190	-1.11	1.25	1.39
At3g47960	2.55	2.61	1.3	At1g20020	-2.31	1.06	1.67
At5g11570	-1.13	-2.55	-1.0	At5g66810	1.20	1.27	1.24
At3g16180	-1.85	-1.22	-1.5	At2g27510	-1.10	1.02	1.32
At1g52190	-4.30	-3.79	-1.4	At4g14890	-2.22	-4.38	-1.49
At1g68570	-1.97	-1.76	-1.3	At1g32550	1.26	-1.09	1.16
At3g45650	2.05	1.94	1.2	At5g07950	-1.02	-9.21	-1.40
At3g45710	-1.78	-1.98	-1.2	At1g10960	2.68	1.04	1.20
At3g45700	-13.40	-5.50	-3.41	At1g60950	-1.21	-1.99	-1.39
At3g45680	-2.18	-4.69	-1.06	At1g24280	-3.69	-2.34	-1.77
At3g45690	1.28	-1.26	1.2	At5g13110	-1.51	-1.06	1.09
At3g45720	-1.12	-1.42	1.3	At5g41670	-1.75	-1.06	-1.72
At2g38100	1.14	-1.05	1.4	At1g64190	-1.67	-1.47	-1.27
At5g13400	1.39	-1.11	1.2	At3g60750	-1.90	-1.51	-1.22
At1g33440	-1.01	-1.23	-1.1	At2g45290	-1.18	1.08	1.07
At1g59740	3.64	1.92	1.7	At5g13240	-1.09	1.27	1.21
At3g25260	1.02	-1.04	1.4	At1g12230	-1.28	-1.17	-1.45

Note: 4-week old *Arabidopsis* plants were exposed to 200uM Cd²⁺ for 6h. Root tissues were washed before harvesting as described (Gong et al., 2003 *PNAS*), RNA was extracted and subject to hybridization to *Arabidopsis* whole genome ATH1 arrays by UCSD Genechip Core facility and Capitalbio Co. (Beijing, China) respectively. Data were extracted and normalized according to manufacturer's standard protocol. Three independent experiments were performed (Exp.1, 2, and 3). Columns 1 and 5 show index number or assigned name of each gene, and columns 2, 3, 4 and columns 6, 7, 8 show fold change in Cd²⁺ treated roots compared to control. “-” represents signal deduction by Cd²⁺ treatment. Gene list in nitrate uptake and assimilation pathway is according to Wang R et al., (2004, *Plant Physiol.*)

Supplemental Table 2:

Accession numbers of the sequences corresponding to Figure 1A:

CHL1 (At1g12110), NRT1.2 (At1g69850), NRT1.3 (At3g21670), NRT1.4 (At2g26690), NRT1.5 (At1g32450), NRT1.6 (At1g27080), NRT1.7 (At1g69870), NRT1.8 (At4g21680), AT1G18880, AT1G22540, AT1G22550, AT1G22570, AT1G27040, AT1G33440, AT1G52190, AT1G59740, AT1G62200, AT1G68570, AT1G69860, AT1G72130, AT1G72140, AT2G02020, AT2G02040 (PTR2B), AT2G37900, AT2G38100, AT2G40460, AT3G01350, AT3G16180, AT3G25260, AT3G25280, AT3G43790, AT3G45650, AT3G45660, AT3G45680, AT3G45690, AT3G45700, AT3G45710, AT3G45720, AT3G47960, AT3G54140 (PTR1), AT5G01180, AT5G11570, AT5G13400, AT5G19640, AT5G28470, AT5G46040, AT5G46050 (PTR3), AT5G62680, AT5G62730, AT3G54450, AT1G72120, AT1G72125, AT3G53960.