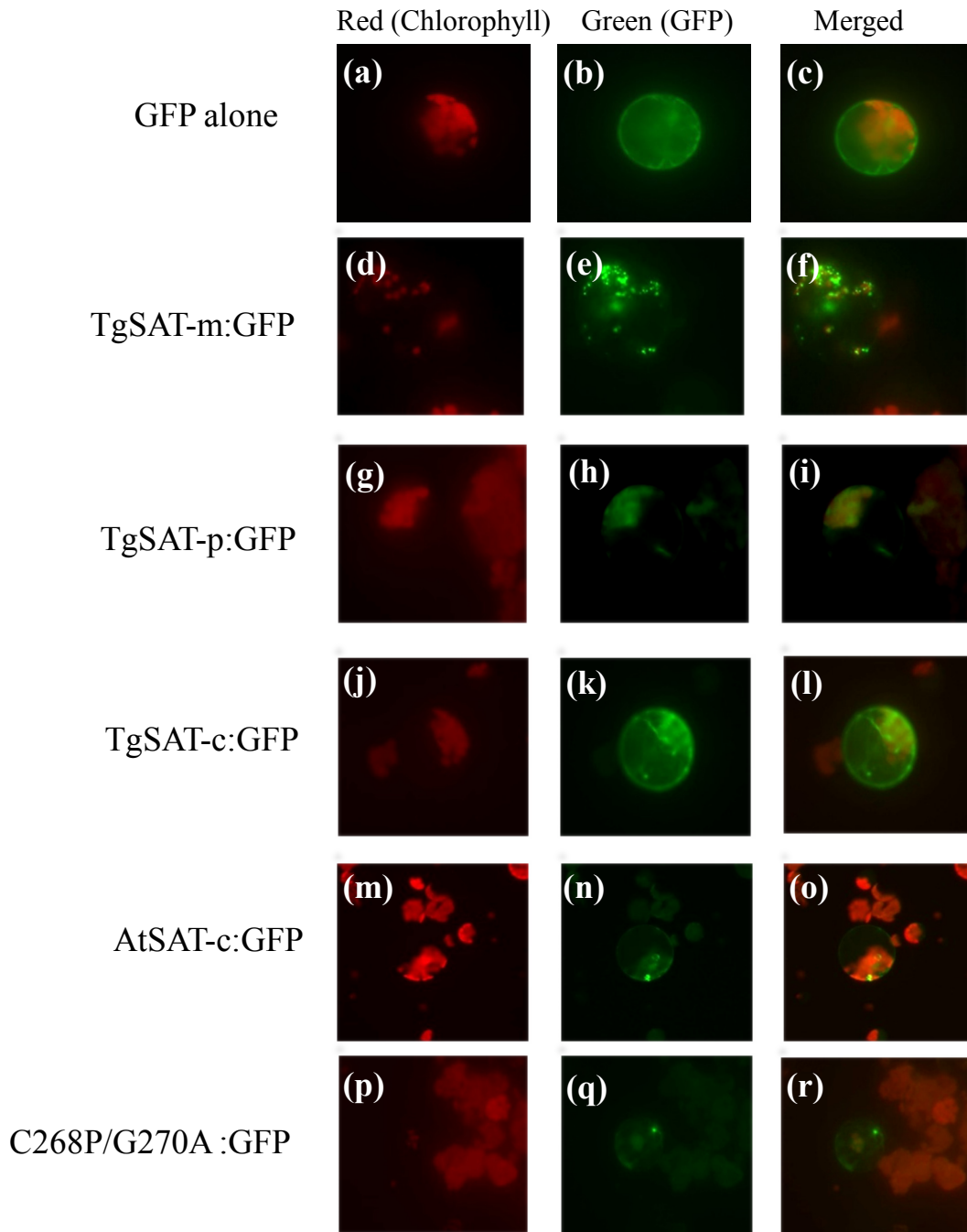


Supplemental Figure 1. Localization of TgSAT:GFP stably expressed in *A. thaliana*. Images taken at wavelengths detecting GFP (a, d, g, j, m, p, and s) and chlorophyll (b, e, h, k, n, q, and t), MitoTracker Red CMXRos (v), and artificially merged (c, f, i, l, o, r, and u). Images of untransformed Col-0 plant are a-c. Stable expression of GFP alone (d-f), TgSAT-p:GFP (g-i), TgSAT-c:GFP (j-l), AtSAT-c:GFP (m-o), C268P/G270A:GFP (p-r), and TgSAT-m:GFP (s-v).



Supplemental Figure 2. Localization of transiently expressed TgSAT:GFP in *A. thaliana* protoplasts. Images taken at wavelengths detecting chlorophyll (a, d, g, j, m, and p) and GFP (b, e, h, k, n, and q), and artificially merged (c, f, i, l, o, and r). Transient expression of GFP alone (a-c), TgSAT-m:GFP (d-f), TgSAT-p:GFP (g-i), TgSAT-c:GFP (j-l), AtSAT-c:GFP (m-o), and C268P/G270A:GFP (p-r).

TgSAT-c MPPAGELRHQPPS-EDTQSDTTPSAEAAAAAAILAAAADSEAAGLWTQIK
 AtSAT-c MPPAGELRHQSPSKEKLSVTSQSDAEAAASAAISAAAADAEAAAGLWTQIK

 TgSAT-c AEARRDAEAEPALASYLYSTILSHSSLERSISFHLGNKLCSSSTLLSTLLY
 AtSAT-c AEARRDAEAEPALASYLYSTILSHSSLERSISFHLGNKLCSSSTLLSTLLY

 TgSAT-c DFLNLTFTSDPSLRNATVADLRAARVRDPACISFSHCLLNKYGFLAIQAH
 AtSAT-c DFLNLTFS SDPSLRNATVADLRAARVRDPACISFSHCLLNKYGFLAIQAH

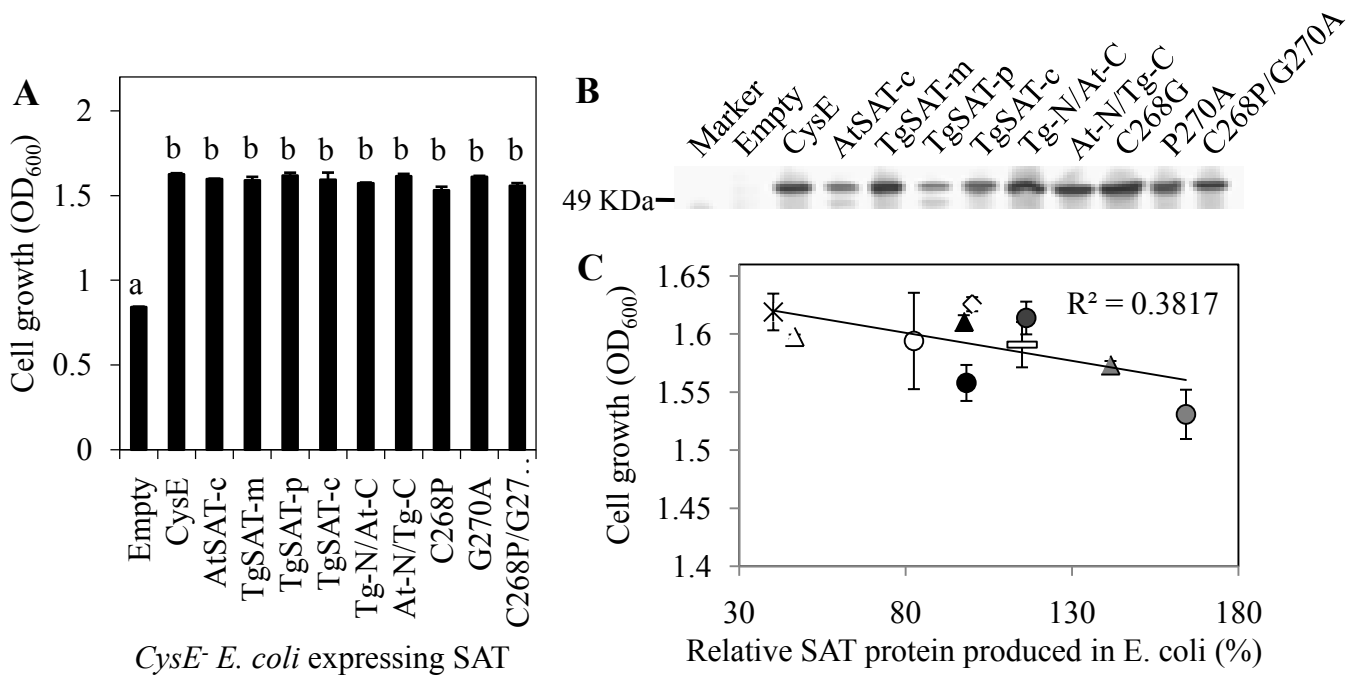
 TgSAT-c RVSHKLWTQSRKPLALALHSRISDVFAVDIHPAARIGKGILLDHATGVVVI
 AtSAT-c RVSHKLWTQSRKPLALALHSRISDVFAVDIHPAAKIGKGILLDHATGVVV

 TgSAT-c GETAVIGNNVSILHHVTLGGTGKACGDRHPKIGDGCLIGAGATILGNVKI
 AtSAT-c GETAVIGNNVSILHHVTLGGTGKACGDRHPKIGDGCLIGAGATILGNVKI

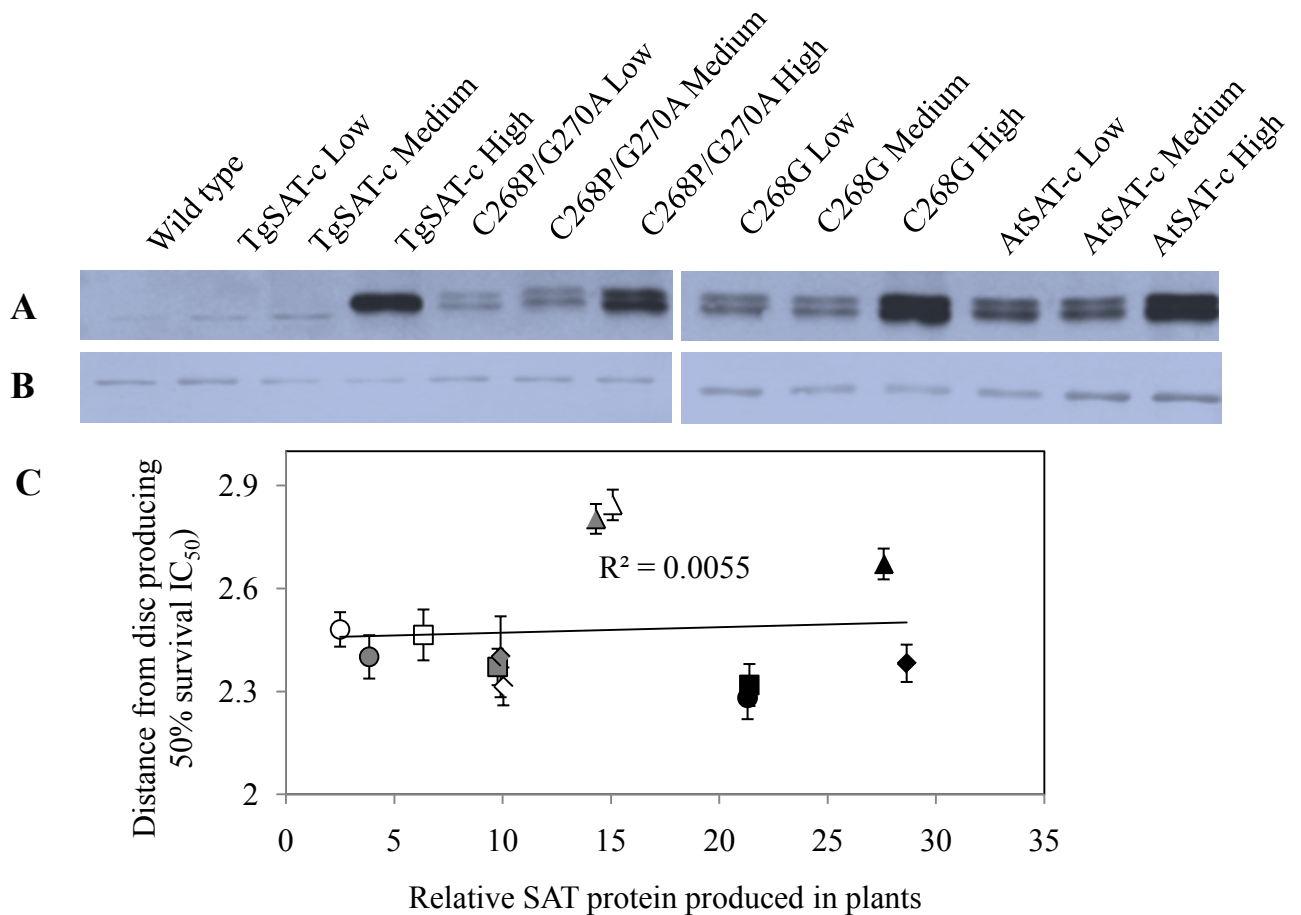
 TgSAT-c GAGAKVGAGSVVLIDVPPRATAVGNPARLVGGKEKPTIHDEECPGESMDH
 AtSAT-c GAGAKVGAGSVVLIDVPCRGTA VGNPARLVGGKEKPTIHDEECPGESMDH

 TgSAT-c TSFISEWSDYII
 AtSAT-c TSFISEWSDYII

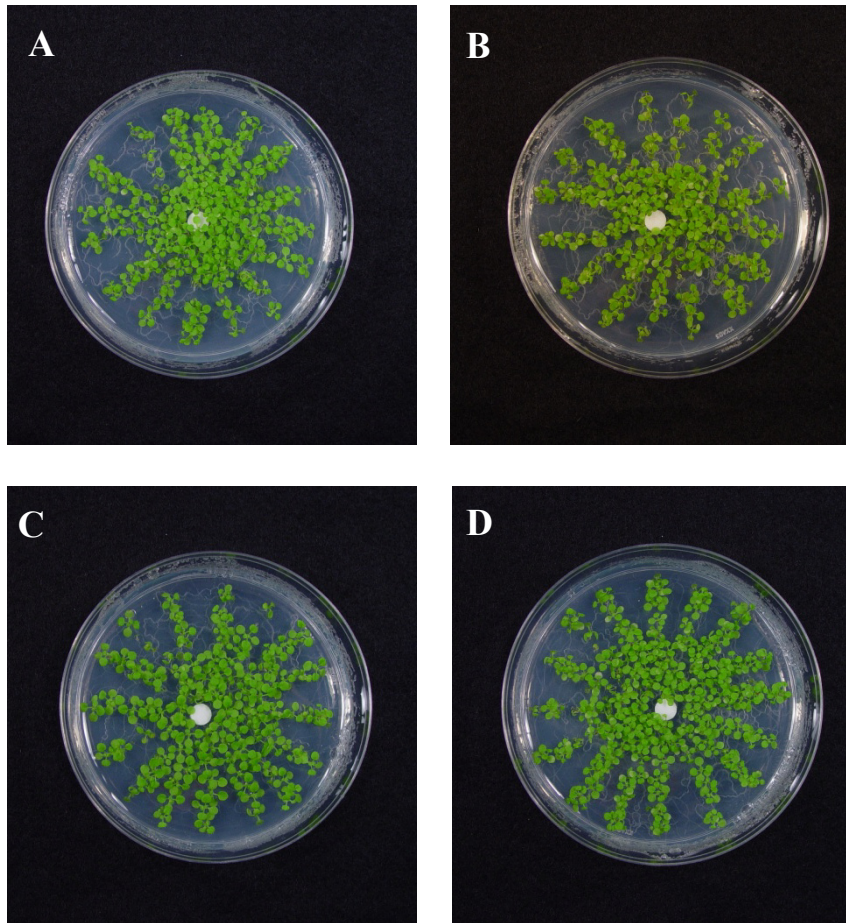
Supplemental Figure 3. Alignment of the predicted amino acid sequences of TgSAT-c and AtSAT-c. Colors represent identical amino acids (blue), conservative amino acid substitution (grey), and non-similar amino acid residues (white). Dashed line indicates the amino acid sequences used in the domain swapped constructs. Black box indicates the amino acids used for the point mutated SATs.



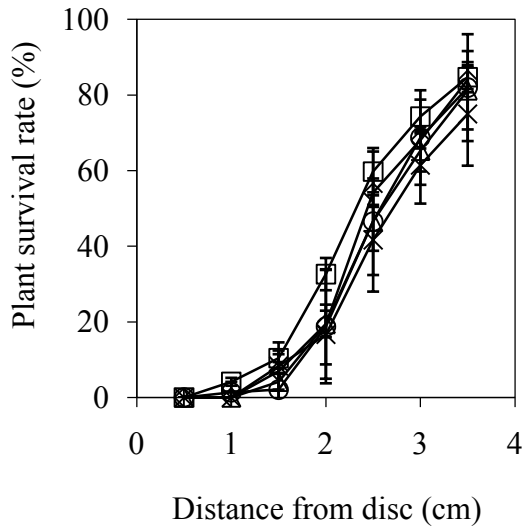
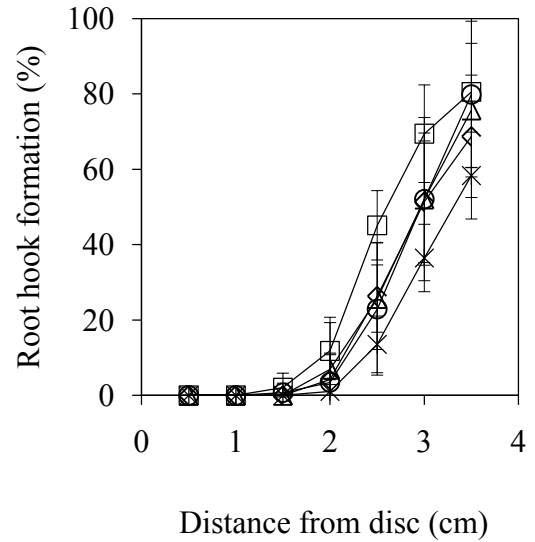
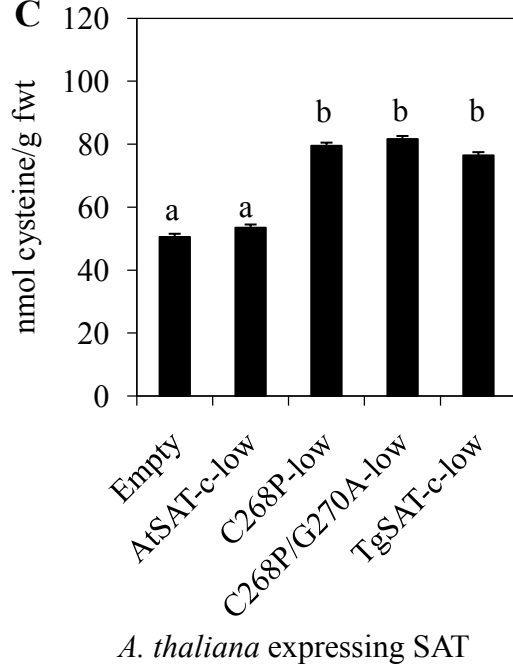
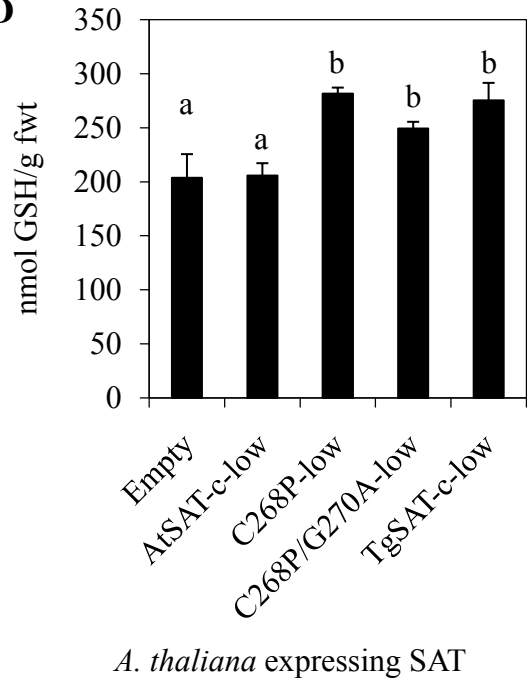
Supplemental Figure 4. (A) Cell growth of *CysE*- *E. coli* expressing various SATs in the absence of Ni²⁺. Letters above the bars represent results of a one-way ANOVA, the same letters were not significantly different ($P > 0.05$). Data represent the average (\pm SE) of three independent samples with three replicates each ($n=9$). (B) Protein levels of each SAT expressed in *E. coli* determined by Western blot with anti-SAT antibody as a primary antibody. (C) The relationship between the cell growth of *E. coli* expressing various SATs and SAT protein produced in the absence of Ni²⁺. Symbols represent *CysE* from *E. coli* (open diamond), *AtSAT-c* (open triangle), *Tg-N/At-C* (grey triangle), *G270A* (closed triangle), *C268P* (grey circle), *TgSAT-c* (open circle), *C268P/G270A* (closed circle), *At-N/Tg-C* (dark grey circle), *TgSAT-p* (star), and *TgSAT-m* (open rectangle). Produced SAT proteins were measured using ImageJ software. Relative SAT protein produced in *E. coli* were calculated based on the SAT protein expression level in *CysE* transformed *E. coli* (%).



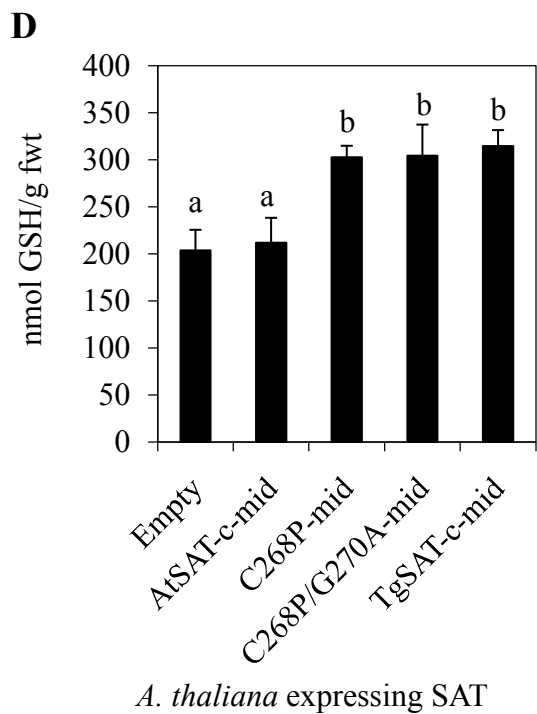
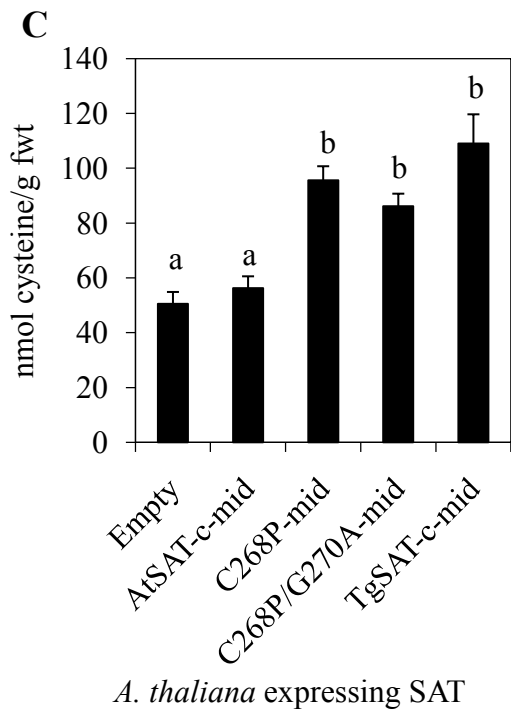
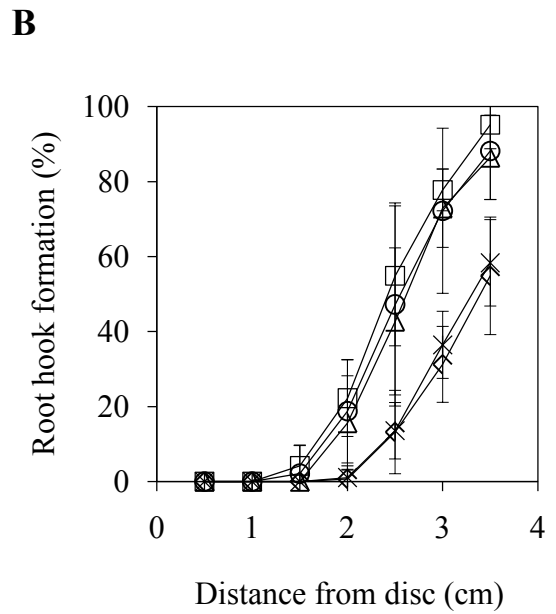
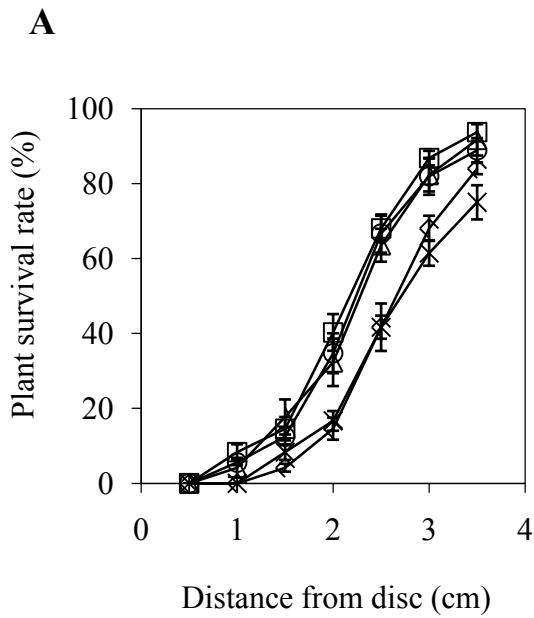
Supplemental Figure 5. Protein levels of each SAT expressed in *A. thaliana* determined by Western blot with anti-SAT (A) and anti-Rubisco (B) as a primary antibody, respectively. Three independent plant lines were selected for each construct based upon the level of SAT accumulation. Low, Medium, and High represent the lowest, medium, and highest SAT protein level from at least 15 to 20 independent transformants for each construct. (C) The relationship between SAT protein produced and the distance from the central Ni^{2+} acetate soaked disc (50 μ l of 100 mM Ni acetate) that allowed 50% survival (IC_{50}) in lowest, medium, and highest SAT protein produced in plants. Symbols represent lowest (open diamond), medium (grey diamond), and highest (closed diamond) *AtSAT-c* protein level, lowest (open square), medium (grey square), and highest (closed square) *TgSAT-c* protein level, lowest (open triangle), medium (grey triangle), and highest (closed triangle) *C268P* protein level, and lowest (open circle), medium (grey circle), and highest (closed circle) *C268P/G270A* protein level. Produced SAT proteins were measured using ImageJ software. Relative SAT protein produced in plants were calculated based on the SAT protein expression level in empty vector transformed *A. thaliana* (folds).



Supplemental Figure 6. Growth of transgenic *A. thaliana* expressing various SATs for 13 days in the absence of Ni treatment. Figures represent plants transformed with empty vector (A), *AtSAT-c* (B), *C268P/G270A* (C), and *TgSAT-c* (D).

A**B****C****D**

Supplemental Figure 7. Nickel resistance of transgenic *A. thaliana* expressing various SATs grown on plates for 13 days in the presence of a central Ni acetate soaked filter paper disc (50 μ l of 100 mM Ni acetate). Data for transgenic lines accumulating low levels of AtSAT-c, C268P, C268P/G270A, and TgSAT-c are presented. Both plant growth determined as formation of secondary leaves (A), a root hook (B), and seedling Cys (C) and GSH (D) content are presented from transgenic plants transformed with various SATs. In (A) and (B), symbols mean empty vector (stars), *AtSAT-c* (open diamonds), *C268P* (open triangles), *C268P/G270A* (open circles), and *TgSAT-c* (open squares) transformed plants. Each data point represented an average \pm SE of plants from three low SAT accumulating lines grown on three independent plates per line. Same letter within graphs represents lines that are not significantly different ($P > 0.05$, $n=9$).



Supplemental Figure 8

Supplemental Figure 8. Nickel resistance of transgenic *A. thaliana* expressing various SATs grown on plates for 13 days in the presence of a central Ni acetate soaked filter paper disc (50 μ l of 100 mM Ni acetate). Data for transgenic lines accumulating medium levels of AtSAT-c, C268P, C268P/G270A, and TgSAT-c are presented. Both plant growth determined as formation of secondary leaves (A), a root hook (B), and seedling Cys (C) and GSH (D) content are presented from transgenic plants transformed with various SATs. In (A) and (B), symbols mean empty vector (stars), *AtSAT-c* (open diamonds), *C268P* (open triangles), *C268P/G270A* (open circles), and *TgSAT-c* (open squares) transformed plants. Each data point represented an average \pm SE of plants from three medium SAT accumulating lines (data for C268P represents two independent medium SAT accumulating lines). Same letter within graphs were not significantly different ($P>0.05$, $n=9$ or $n=6$).