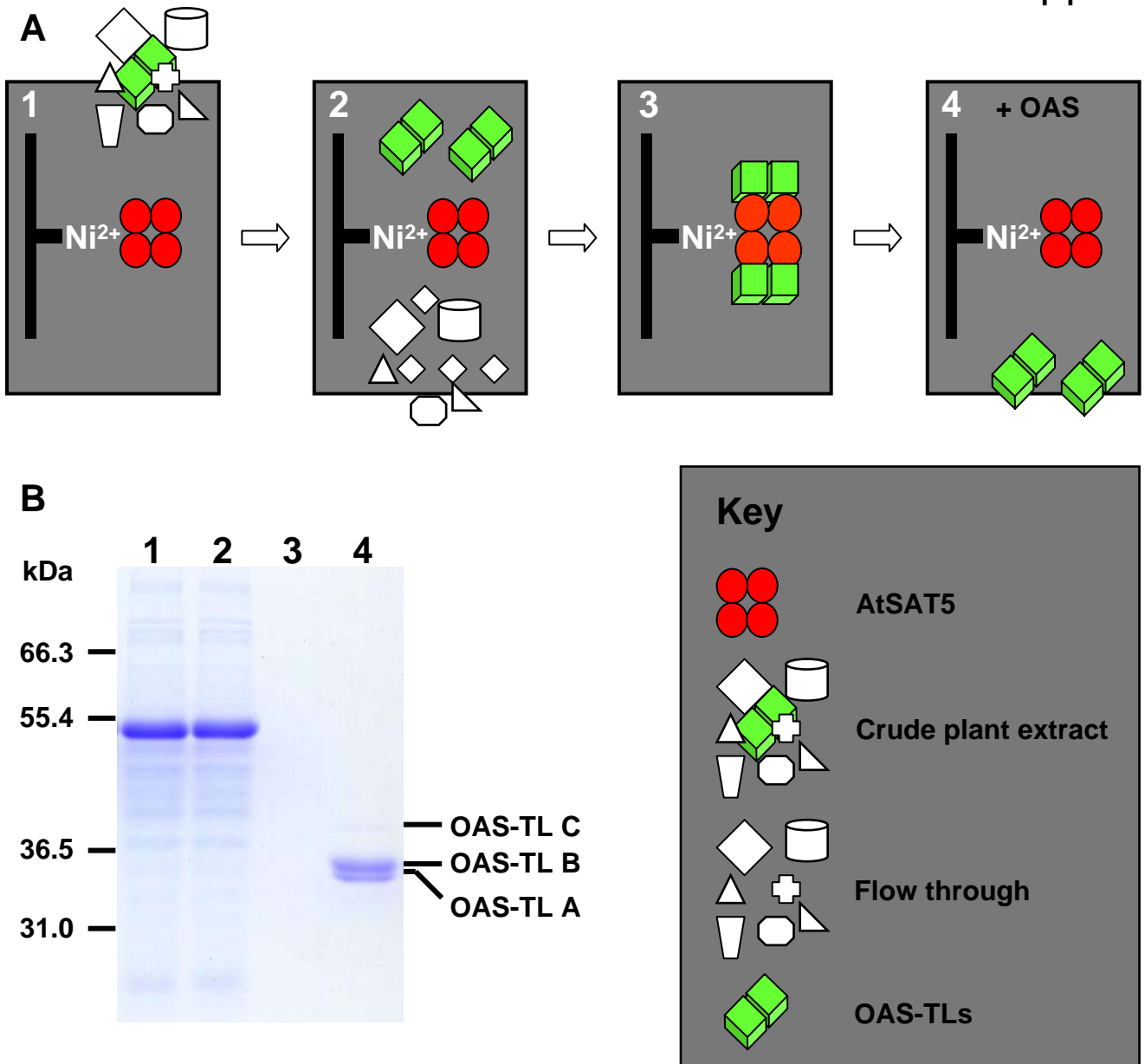


Supplemental Fig. 1: Genomic analysis of *oastI* T-DNA insertion lines

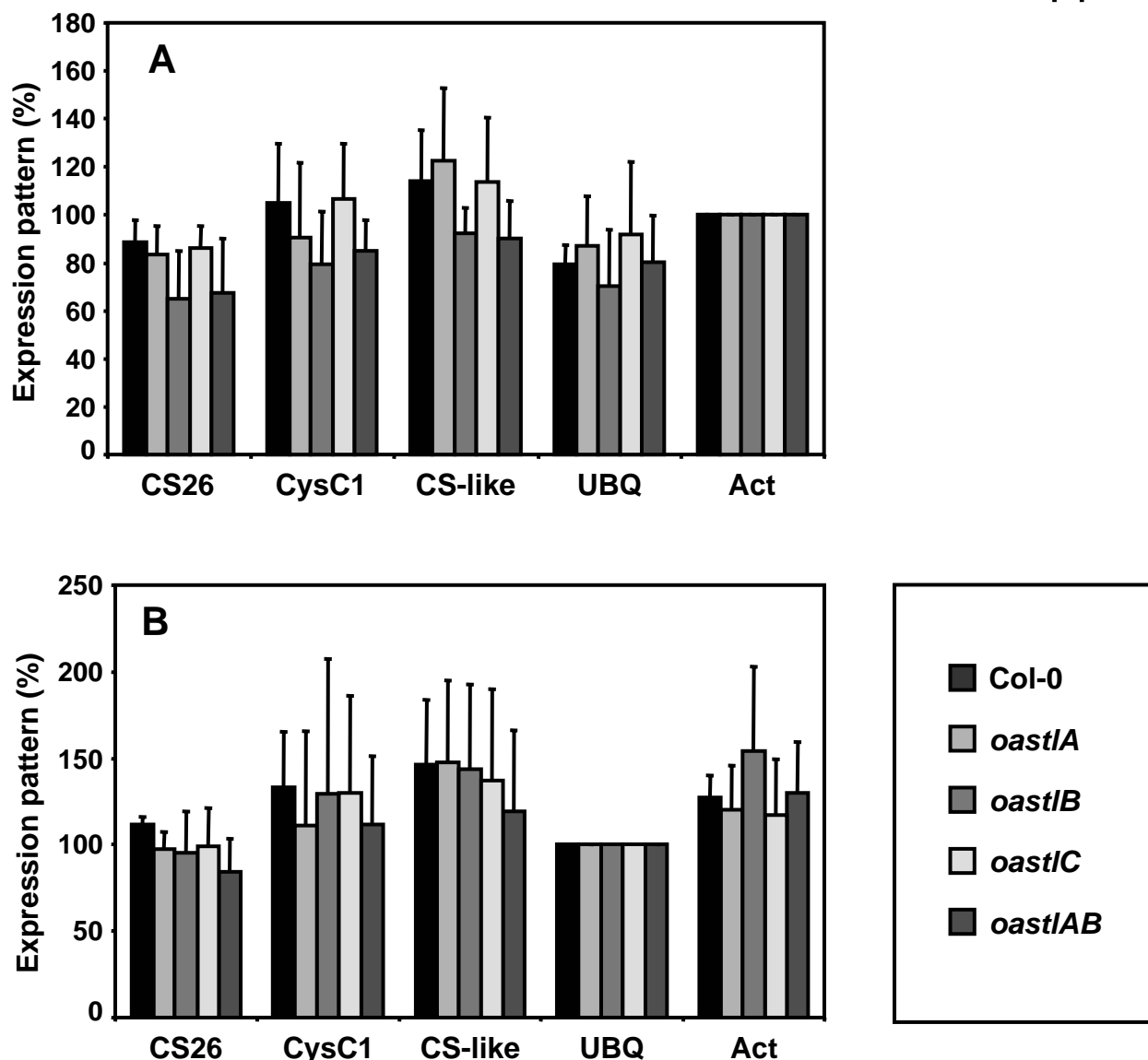
Genomic DNA of wild type and *oastI* T-DNA insertion lines was isolated by using the DNeasy[®] Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufactures instructions. Aliquots of gDNA were digested with *EcoRI* (A), *EcoRV* (B) or *Spe I* (C), separated on a 0.8% (w/v) agarose gel and blotted onto Hybond[™]-N nylon membrane (GE-Healthcare, Germany) by capillary transfer with 10xSSC buffer (0.15 M sodium citrate pH 7, 1.5 M sodium chloride). T-DNA specific fragments of the *nptII* gene as probes were generated by PCR with the primers *nptII*_{for} (5'-AACTCGTCAAGAAGGC GATAGAAGG-3') and *nptII*_{rev} (5'-TGAACAAGATGGATTGCACGCA-3'), and subsequently labeled with α -³²P-dATP and α -³²P-dGTP by using the Megaprime DNA labeling system (GE-Healthcare, Germany). After blocking with 2% fish sperm DNA (SERVA, Germany) in hybridization buffer (6xSSC, 5xDenhardt's and 0.5% (w/v) SDS), the membranes were hybridized with the probe at 65°C in hybridization buffer, washed twice at 65°C with 2xSSC or 1xSSC and 0.5% (w/v) SDS for 15 min, and once with 0.5xSSC and 0.5% (w/v) SDS for 15 min. For signal detection the blots were exposed to X-ray films for 24h.

The stringent washing conditions ensured the absence of cross-hybridization with the wild type gDNA. Restriction of *oastI*A gDNA with *EcoRV* resulted in three detectable signals, which is in agreement with a triplicate insertion of the T-DNA at the *OastI*A locus. Since the *nptII* gene is cloned via *EcoRI* in the pD991 vector (Thomas-Jack-lines), the restriction of gDNA from *oastI*A with *EcoRI* results in only one signal. The *oastI*B and *oastI*C insertion lines were obtained from the Salk collection and for that reason carried the T-DNA of the pROK2 vector. In these T-DNA insertion lines, not only the T-DNA sequence of the pROK2 vector, but rather the complete vector containing the T-DNA sequence in addition to the first copy of the T-DNA sequence had integrated into the corresponding gene (D). Therefore similar signal patterns for *oastI*B and *oastI*C were obtained after restriction of gDNA with *EcoRI* (A) or *EcoRV* (B). In both cases a signal (V1 or V2) was detectable that corresponds to the backbone of the pROK2 vector. Restriction of *oastI*B and *oastI*C gDNA with *Spe I*, which does not cut in the pROK2 vector at all, results in only one signal, demonstrating that the multiple copies of the *nptII* gene are inserted at only one locus in both, *oastI*B and *oastI*C. The presence of the backbone of the pROK2 vector in the gDNA was confirmed by PCR (V3, E, lane 2 and 4) with the primers P1004 (5'-GCATTCTTGGCATAGTGGTCGC-3') and P1005 (5'-GCATGACAAGTCATCGGG CAT-3'). The identity of the amplified DNA was evidenced by restriction with *EcoRV* (E, lane 5 and 6).



Supplemental Fig. 2: SAT-affinity purification of plant OAS-TL proteins.

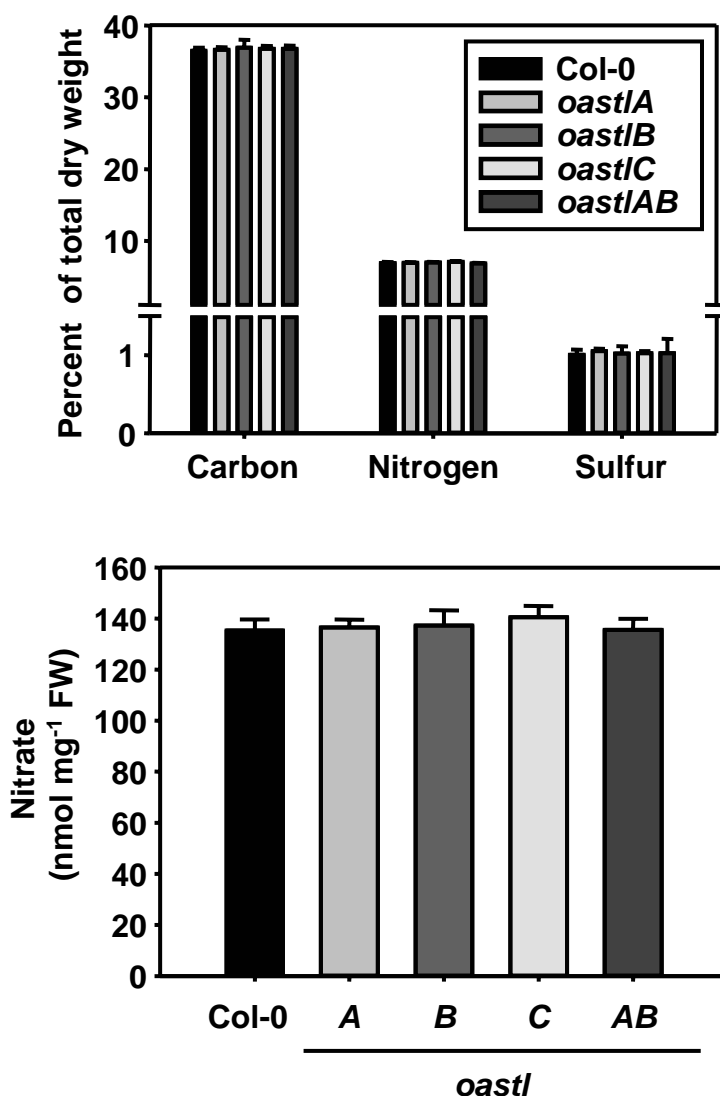
A) Schematic overview of the SAT-affinity purification method for plant OAS-TL proteins. Recombinant AtSAT5 was over-expressed and immobilized on a HiTrapTM-column (GE-Healthcare). Expression and immobilization of AtSAT5 is not shown in the purification scheme. 300 mg of crude plant extract (see material and method section) was loaded on the column (1) and the flow through was collected (2). The column was washed with 50 mM Tris, pH 8, 250 mM NaCl and 80 mM imidazole (3) and the plant OAS-TL proteins were eluted from the column by dissociation of the cysteine synthase complex with 1 mM OAS. **B)** The resulting protein fractions were separated by SDS-PAGE using a 12.5 % Polyacrylamide gel. The identity of OAS-TL A, B and C were confirmed by identification of MALDI-TOF sequencing of trypsin digested fragments.



Supplemental Fig. 3: Transcriptional expression pattern of *CS26*, *CysC1* and *CS-like* in the *oastI* mutants *A*, *B*, *C* and *AB* compared to the wild type *Col-0* determined by quantitative PCR.

(A) based on ubiquitin (*UBQ*, *At3g62250*) as reference gene; (B) based on actin (*Act*, *At5g09810*) as reference gene. Total RNA was independently extracted from two leaf batches of each genotype using TRIzol reagent (Invitrogen, Carlsbad, CA). First strand cDNA was synthesized by M-MLV reverse transcriptase (Promega, Madison, WI) and products were amplified by PCR using *Taq* DNA polymerase (NEB, Frankfurt, Germany). After determination of a cycle number at the beginning of the exponential amplification phase for each gene, equivalent amounts of each PCR reaction were loaded on the same gel that was subsequently stained with ethidium bromide (0.1 mg ml⁻¹). The absolute integrated OD-values were determined by Gel-Pro[®] Express (MediaCybernetics, Silver Spring, USA). Values were calculated as the percentage of the OD compared to actin and ubiquitin as reference, respectively, for each genotype. Consequently, the diagramm shows no expression level, but the relative expression pattern for each gene in wild type and mutants. Gels and calculation were carried out twice for each of the two cDNA charges.

Following numbers of amplification were chosen: *CS26*, 28 cycles; *CS-like*, 23 cycles; *CysC1*, 24 cycles; *UBQ*, 24 cycles; *Actin*, 23 cycles. Fragments were amplified by the use of the primers *CysC1_for* (5' TTACTGAGGAGAGACGATCCCC 3'), *CysC1_rev* (5' GCCATGAAAGCCAACTGATTCC 3'), *CS26_for* (5' AAAATGGCTTTC GCGTCTCCTTCG 3'), *CS26_rev* (5' GCTCC GCTGTTTTCTGCCTCGTT 3'), *CS-like_for* (5' TAGAACCACGGGTCCGGAGATTTG 3'), *CS-like_rev* (5' GAGTTTCCCTA CGTTTTT AGGCC 3'), *Act_for* (5' AAAATGGCCGATGGTGAGG 3'), *Act_rev* (5' GAGTGAGTCTGT GAGATCCCCG 3'), *UBQ5_for* (5' GTGGTGCTAAGAAGAGGAAGA 3') and *UBQ5_rev* (5' TCAAGCTTCAACTCCTTCTTT 3').



Supplemental Fig. 4: Carbon, nitrogen, sulfur and nitrate levels in leaves of wild type and *oastl* T-DNA insertion lines.

A) Approximately 20 mg of dry leaf material from wild type and *oastl* T-DNA insertion lines were analysed by using an elemental analyser (VarioMAX CNS, Elementar, Hanau, Germany). The amount of Carbon, Nitrogen and Sulfur is shown as percent of total dry weight. No significant differences in the *oastl* insertion lines were observed (n=7). **B)** Nitrate was extracted from leaves of wild type and *oastl* T-DNA insertion lines as described in material and methods. Five individual plants for each line were extracted and quantified separately by using a ICS 1000 (Dionex, Idstein, Germany) as described for determination of sulfate. No significant differences were observed between wild type and the *oastl* insertion lines.