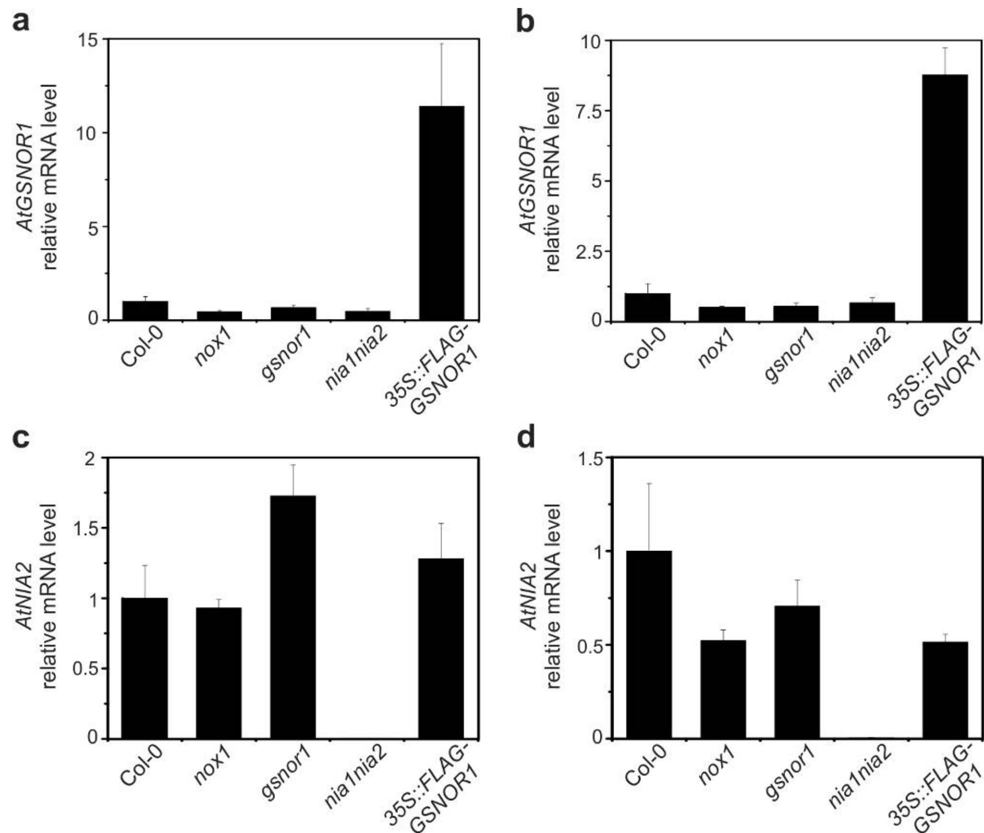
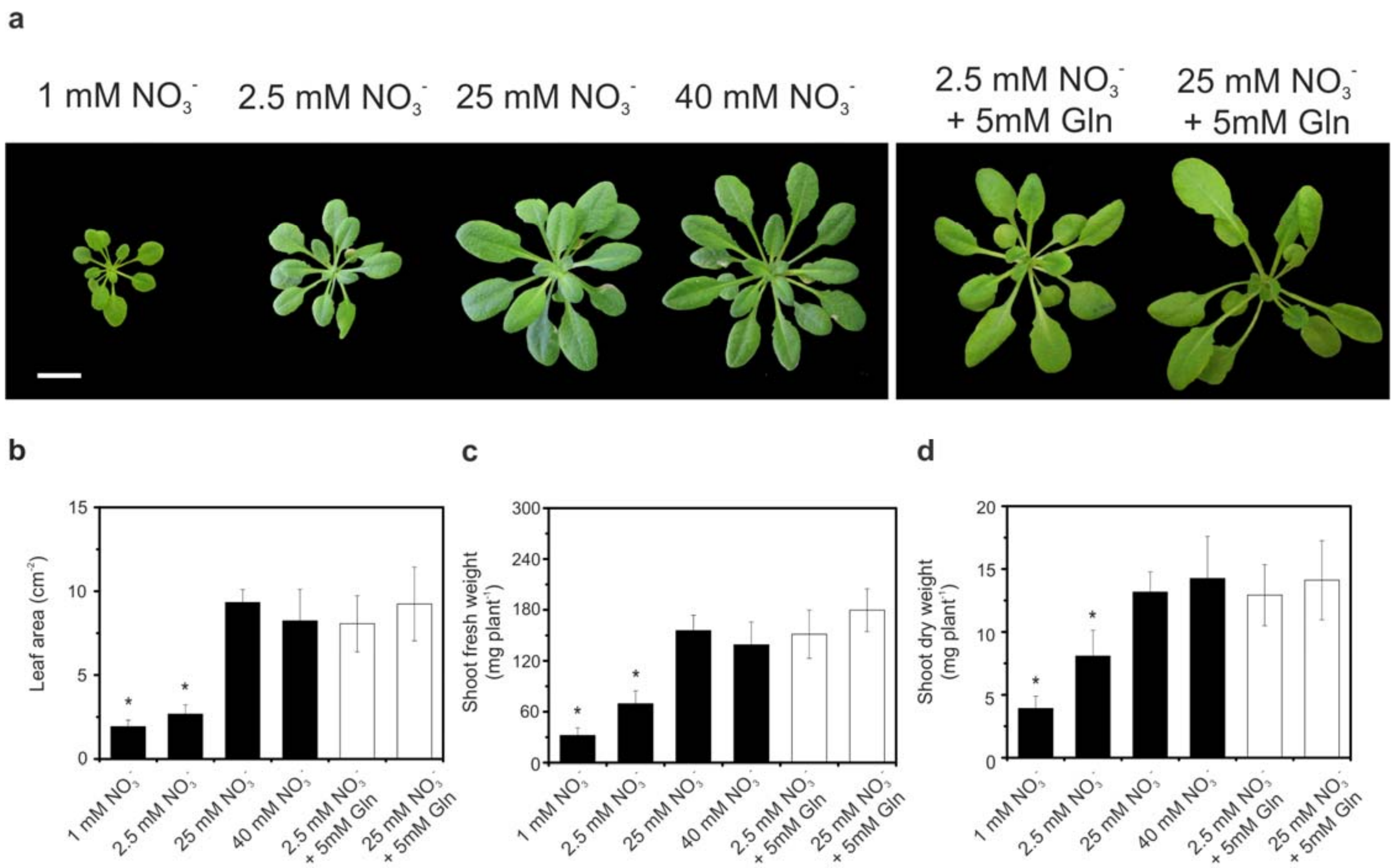
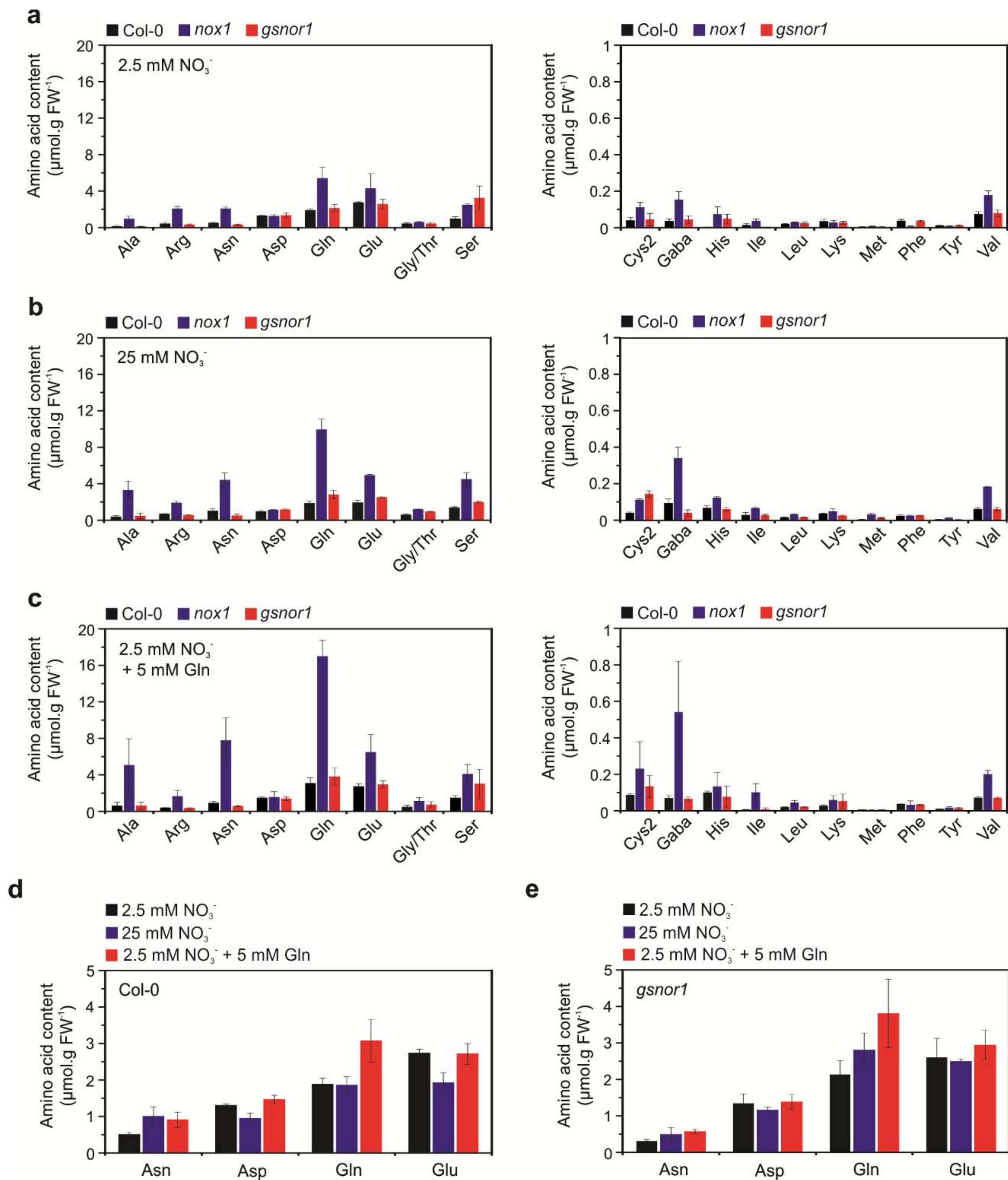


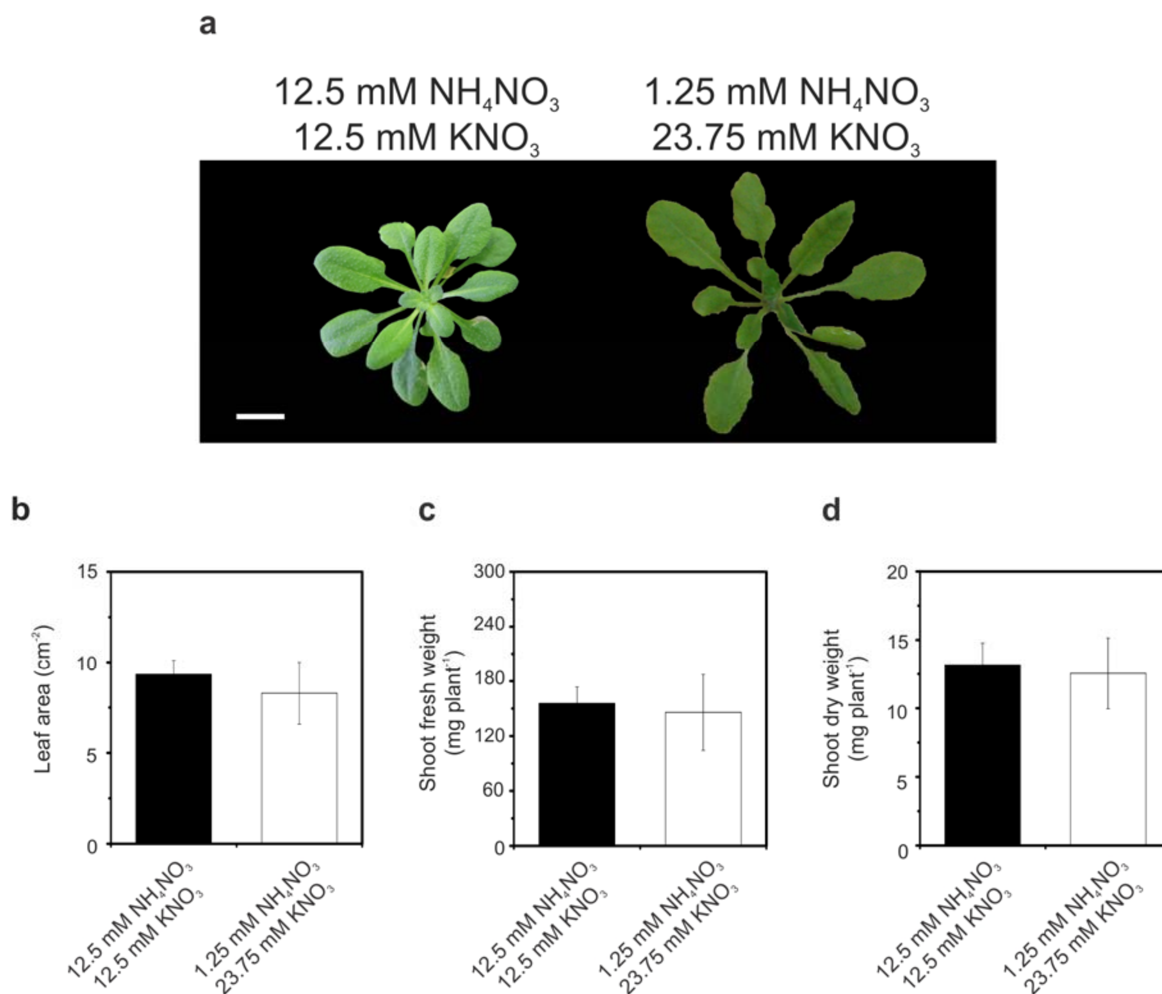
Supplementary Figure 1. Gene expression analysis of *GSNOR1* and *NIA2* in genotypes with altered NO signalling. Relative expression of *GSNOR1* in leaves (**a**) and roots (**b**) and *NIA2* in leaves (**c**) and roots (**d**) of WT plants and genotypes with enhanced (*nox1* and *par2-1*) or impaired (*nia1nia2* and *35S::FLAG-GSNOR1*) (S)NO homeostasis determined by qRT-PCR and normalized to expression of *ACT2.* Analysis of gene expression in roots was carried out on 15 days-old plants grown in petri dishes containing half-strength MS medium (9.4 mM KNO_3 and 10.3 mM NH_4NO_3). For analysis of gene expression in leaves plants were grown in soil and the form and content of N in the soil was not determined. Error bars represent SD (n = 3).



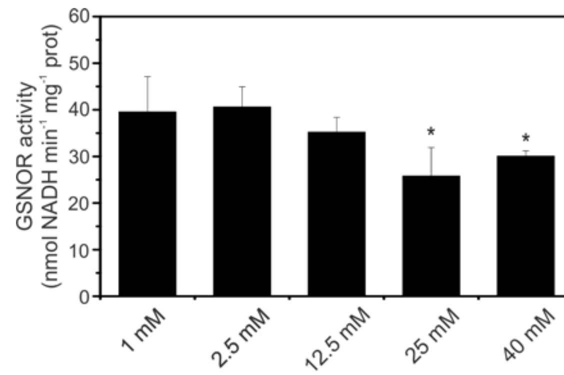
Supplementary Figure 2. Effect of nitrate availability and glutamine on growth vigour of wild-type plants. (**a**) Phenotype, (**b**) leaf area, (**c**) shoot fresh weight and (**d**) shoot dry weight of four-week-old plants grown on perlite:vermiculite (1:1) under 12h/12h light/dark and irrigated three times a week with a MS nutrient solution containing nitrate (half KNO_3 and half NH_4NO_3) and glutamine as indicated. In (**a**) scale bar, 1 cm. In (**b-d**) data points represent means ± SD (n=15). Asterisks indicate statistically significant difference from 25 mM (P < 0.05, Student's t test).



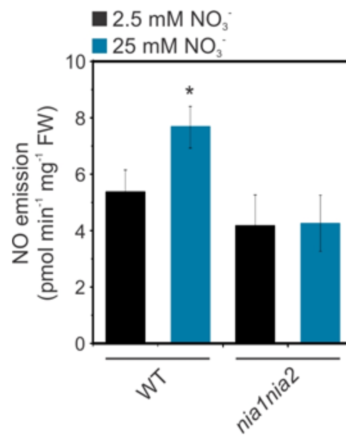
Supplementary Figure 3. Free amino acid contents in leaves: effect of nitrate and glutamine availability. (**a-c**) Free amino acid profile in WT, *nox1* and *gsnor1* and (**d,e**) contents of primary transported amino acids in WT and *gsnor1* leaves of four-week-old plants irrigated with MS nutrient solution containing 2.5 mM nitrate, 25 mM nitrate or 2.5 mM nitrate and 5 mM glutamine, as indicated. Data represent means ± SD of three independent analyses. Cys2, cystine; Gaba, gamma-aminobutiric acid.



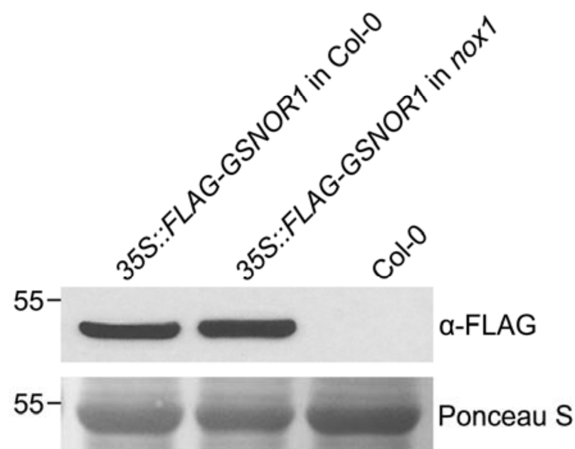
Supplementary Figure 4. Growth vigour of WT plants irrigated with different concentrations of NH_4^+ in the nutrient solution. Phenotype (**a**), leaf area (**b**), shoot fresh weight (**c**) and shoot dry weight (**d**) of four-week-old plants grown on perlite:vermiculite (1:1) under 12h/12h light/dark and irrigated three times a week with a MS nutrient solution containing 12.5 mM NH_4NO_3 and 12.5 mM KNO_3 or 1.25 mM NH_4NO_3 and 23.75 mM KNO_3, as indicated. In (**a**) scale bar, 1 cm. In (**b-d**) data points represent means ± SD (n = 15 plants).



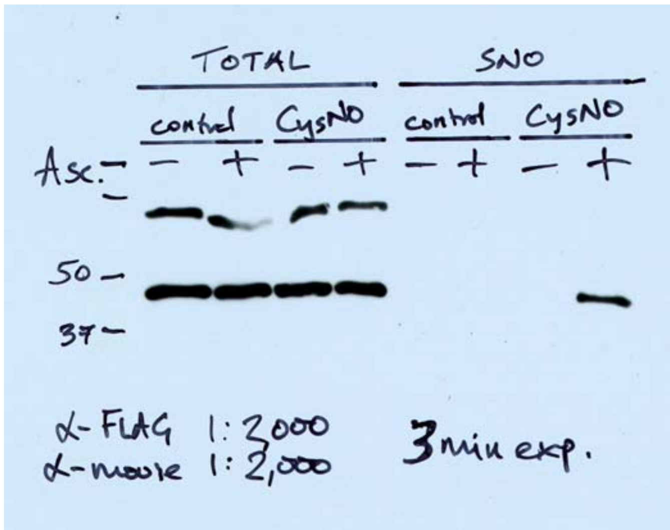
Supplementary Figure 5. GSNOR activity in wild-type plants cultivated under different nitrate availability. Plants were grown on perlite:vermiculite (1:1) under 12h/12h light/dark and irrigated three times a week with a MS nutrient solution containing nitrate as indicated (half KNO_3 and half NH_4NO_3). Asterisks indicate statistically significant difference from 2.5 mM ($P < 0.05$, Student's *t* test).

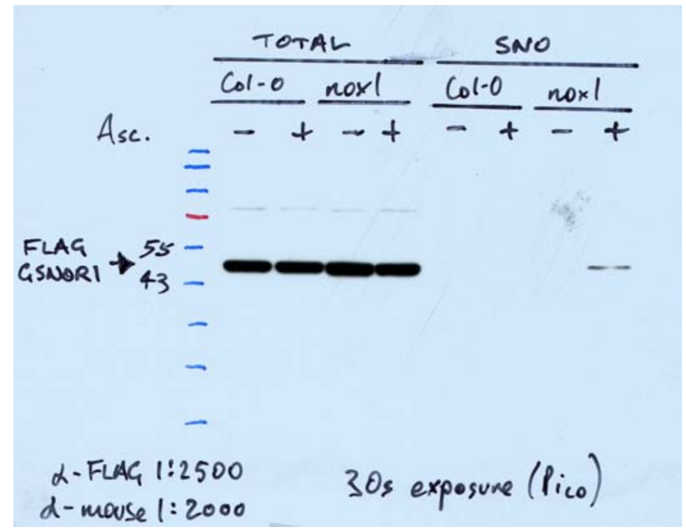


Supplementary Figure 6. NO emission by leaves of wild-type (WT) and *nia1 nia2* plants cultivated under low (2.5 mM) and high (25 mM) nitrate availability. NO concentration was measured using the electrochemical sensor ISO-NOP connected to a free radical analyser Apollo 4000 (World Precision Instruments, Sarasota, FL). Leaves (200 mg) were harvest and washed tree times with deionized water before incubation in 0.5 mM phosphate buffer (pH 7.8) and 5% DMSO. Analyses were carried out after electrode stabilization. Signal from 3 to 5 min of capture were used to construct a linear fit. The NO electrode was calibrated with *S*-nitroso-N-acetyl-penicillamine (SNAP) in 0.1 M $CuCl_2$ according to Zhang (2004, Front. Biosci. 9: 3434). Data points represent means ± SD of three independent experiments. Asterisks indicate significant differences from the WT grown at low nitrate (Student's *t* test, $P < 0.05$).



Supplementary Figure 7. Accumulation of FLAG-GSNOR1 protein in both wild-type and *nox1* backgrounds. Protein was extracted from wild-type Col-0 and *nox1* plants that were untransformed or transformed with *35S::FLAG-GSNOR1*. Proteins were analysed by SDS-PAGE and western blotting using an anti-FLAG antibody. Ponceau S staining confirmed equal loading. The position of a 55 kDa marker is indicated.





Supplementary Figure 8. *In vitro* and *in vivo S*-nitrosylation of GSNOR1. Full scans of the blots presented in Figs. 4d (left) and 4e (right) of the main text.

Supplementary Table 1. Primers sequences.

ACT2F	CGTACAACCGGTATTGTGCTGG
ACT2R	CTCTCTCTGTAAGGATCTTCATG
GSNOR1F	GGTCTCTTTCCTTGTATTCTAG
GSNOR1R	GCATTCACGACACTCAGCTTG
NIA2F	CTCAGTACCTAGACTCTTTGC
NIA2R	ACCGTGAACCGTGAAACTAC
NRT1.1F	ACACGCTCATGGTCCAACAG
NRT1.1R	AGATTAACGCTTCGCCGATACC
NRT2.1F	GCTTGCACGTTACCTGTGACC
NRT2.1R	GCGTCCACCCTCTGACTTGG