SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURES

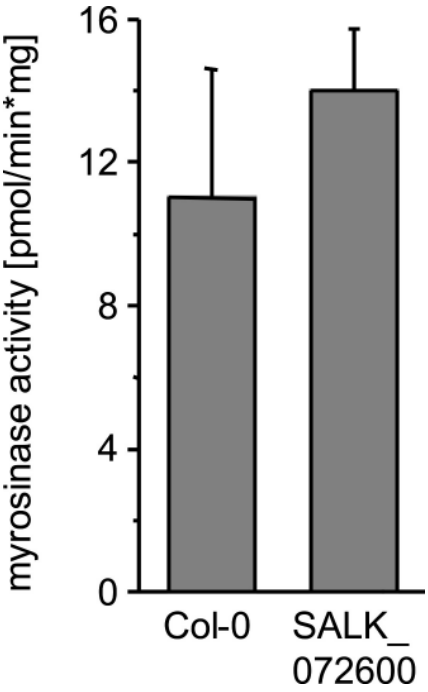

Fig. S1: Myrosinase activity in Col-0 and SALK_072600 rosette leaves. Myrosinase activity determined in rosette leaf extracts using the glucose release assay and allylglucosinolate as substrate was expressed as the amount (pmol) of glucose formed per minute and mg total protein. Means ± SD (n=3).

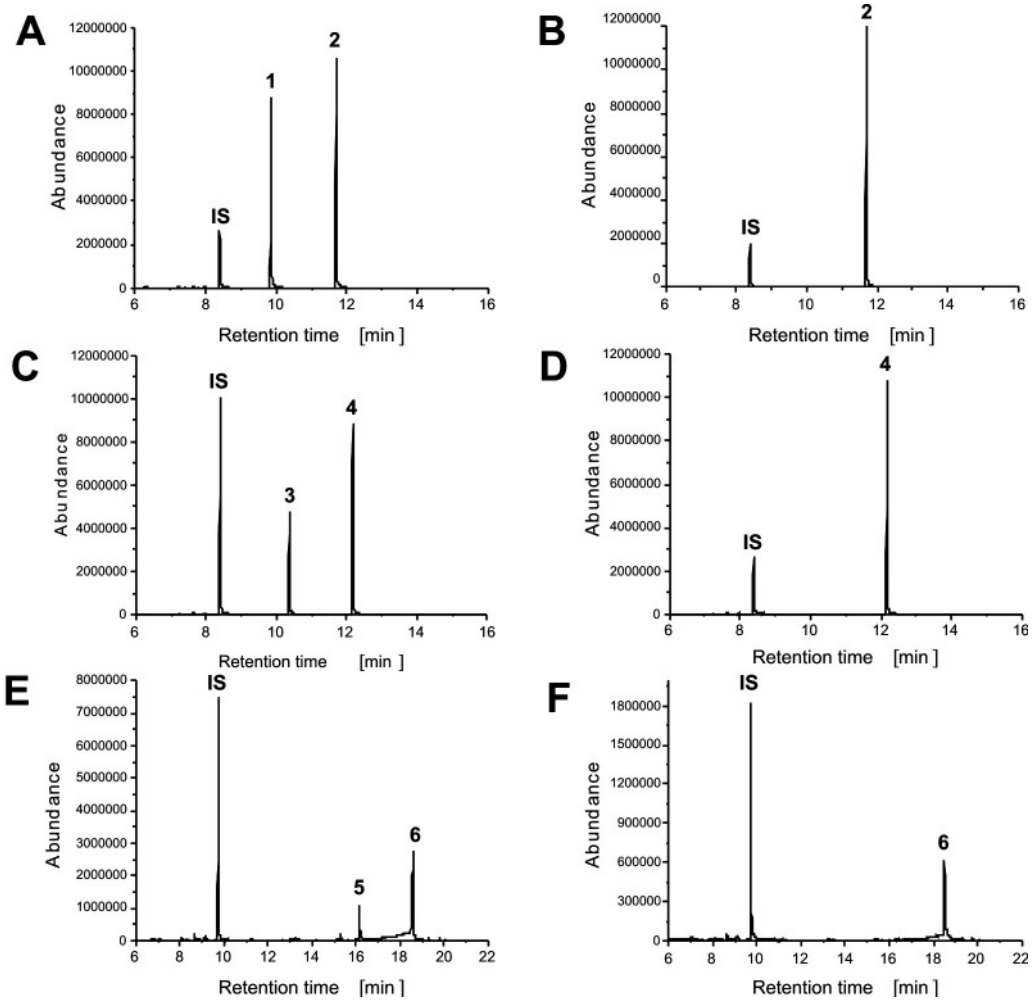

Fig. S2: Effects of AtNSP1 on glucosinolate hydrolysis *in vitro*. Hydrolysis products in enzyme assays with benzylglucosinolate (A-B), 4-methylthiobutylglucosinolate (C-D), and 4-methylsulfinylbutylglucosinolate (E-F). Assays were carried out with (A, C, E) and without (B, D, F) purified AtNSP1 in 50 mM Mes buffer, pH 6.0, containing 1 mM (benzyl-, 4-methylthiobutylglucosinolate) or 3 mM (4-methylsulfinylbutylglucosinolate) substrate for the myrosinase reaction. Depicted are GC-MS chromatograms (total ion current traces) of dichloromethane extracts. 1, benzyl-CN (phenylacetonitrile); 2, benzyl-ITC (1-(isothiocyanatomethyl)benzene); 3, 4-methylthiobutyl-CN (5-methylsulfanyl-pentanenitrile); 4, 4-methylthiobutyl-ITC (1-isothiocyanato-4-methylsulfanyl-butane); 5, 4-methylsulfinylbutyl-CN (5-methanesulfinyl-pentanenitrile); 6, 4-methylsulfinylbutyl-ITC (1-isothiocyanato-4-methanesulfinyl-butane); CN, simple nitrile; ITC, isothiocyanate; IS, internal standard.

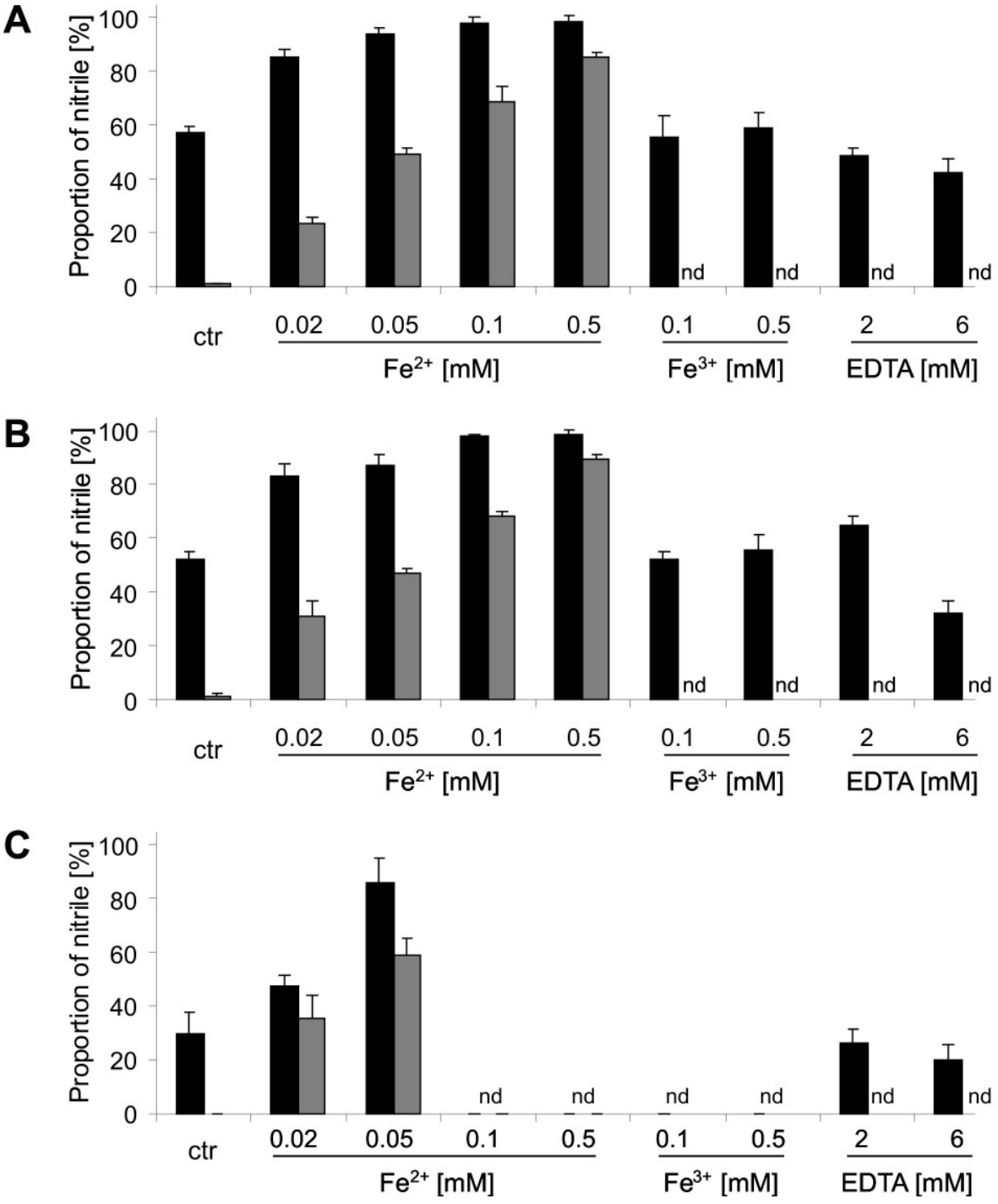

Fig. S3: **Effects of iron salts and EDTA on AtNSP1 activity with various substrates *in vitro*.** Simple nitrile formation in assays with purified AtNSP1 and myrosinase (black bars) and in control assays with myrosinase alone (grey bars) was measured in 50 mM Mes, pH 6.0, containing 1 mM benzylglucosinolate (A), 1 mM 4-methylthiobutylglucosinolate (B), or 3 mM 4-methylsulfinylbutylglucosinolate (C). Fe^{2+} and Fe^{3+} were added as $(NH_4)_2[Fe(SO_4)_2]$ and $FeCl_3$, respectively. Means ± SD of results obtained in three independent experiments. ctr, control (no iron added); nd, not determined

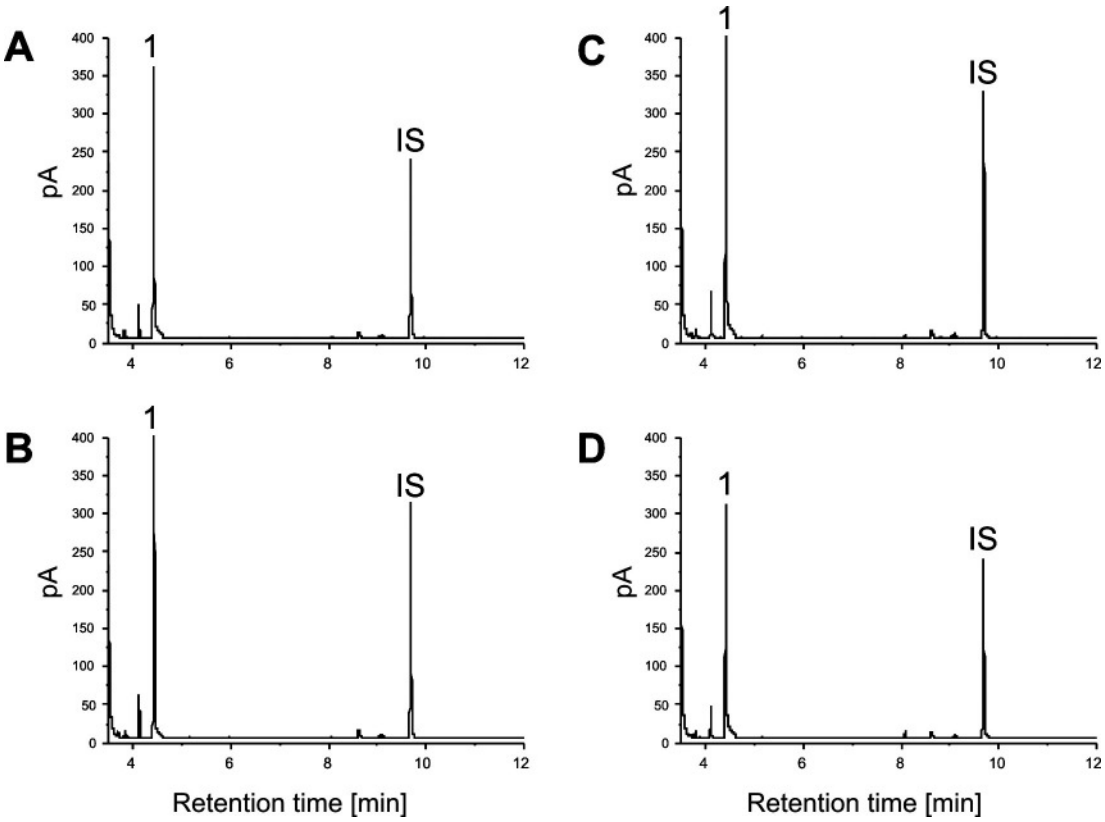

Fig. S4: **Effects of AtNSP2 through 5 on allylglucosinolate hydrolysis *in vitro*.** Hydrolysis products in enzyme assays with AtNSP2 (A), AtNSP3 (B), AtNSP4 (C), and AtNSP5 (D). Assays were carried out with crude extracts of bacteria harbouring the respective expression contructs in 50 mM Mes buffer, pH 6.0, containing 2 mM allylglucosinolate as substrate for the myrosinase reaction. Depicted are GC-FID chromatograms of dichloromethane extracts. 1, simple nitrile formed from allylglucosinolate; IS, internal standard

SUPPLEMENTAL TABLES

Table S1: Publicly available mutant lines of candidate genes screened in this study.

gene locus	ABRC/NASC stock numbers
At3g16400	SALK_072600
At2g33070	SALK_004170 SALK_004179 SALK_057194
At3g16390	SALK_006880 SALK_016880 SALK_108690
At3g16410	CS91216 CS91230 CS92268 CS93227 CS93855
At5g48180	SALK_121606
At3g07720	SALK_020735 SALK_109542 SALK_123411 SALK_137517

Table S2: Glucosinolate content in Col-0 and SALK_072600 rosette leaves. Glucosinolate content (nmol/mg fresh weight) was determined by HPLC after conversion of the glucosinolates to their desulfo-derivatives. Numbers 1 to 5 indicate biological replicates. P shows the significance using a one-tailed t-test. n.s., no significant difference between Col-0 and SALK_072600. 3MSOP, 3-methylsulfinylpropyl-; 4MTB, 4-methylthiobutyl-; 4MSOB, 4-methylsulfinylbutyl-; 7MSOH, 7-methylsulfinylheptyl-; I3M, indol-3-ylmethyl-; 4MOI3M, 4-methoxy-indol-3-ylmethyl-; 1MOI3M, 1-methoxy-indol-3-ylmethyl-; n.d. = not detected.

		Col-0			**SALK_072600**			
Glucosinolate	P	1	2	**mean**	3	4	5	**mean**
3MSOP	n.s.	0.43	0.32	0.37	0.38	0.38	0.33	0.36
4MTB	n.s.	0.28	0.44	0.36	0.50	0.27	0.39	0.39
4MSOB	n.s.	2.99	2.22	2.60	2.79	2.80	2.45	2.68
7MSOH	n.s.	0.04	0.03	0.04	0.03	0.03	0.02	0.03
I3M	n.s.	0.45	0.41	0.43	0.43	0.43	0.39	0.42
4MOI3M	n.s.	0.09	0.11	0.10	0.08	0.08	0.09	0.08
1MOI3M		n.d.	n.d.	n.d.	0.01	0.01	0.01	0.01
total	n.s.	4.28	3.53	3.90	4.22	3.99	3.67	3.96

Table S3: Temperature programs used for GC-MS and GC-FID analyses.

Program no.	Temperature gradient	Application
I	35°C for 3 min, 18°C min^{-1} to 280°C, 30°C min^{-1} to 310°C, 3 min final hold	Analysis of *in vitro* assays with allyl- and benzylglucosinolate
II	35°C for 3 min, 12°C min^{-1} to 280°C, 30°C min^{-1} to 310°C, 3 min final hold	Analysis of *in vitro* assays with 4-methylthiobutyl- and 4-methylsulfinylbutylglucosinolate
III	35°C for 3 min, 12°C min^{-1} to 280°C, 30°C min^{-1} to 300°C, 3 min final hold	Analysis of hydrolysis products in leaf homogenates with or without addition of exogenous glucosinolates

Table S4: Temperature programs used for PCR.

Program no.	Temperature gradient	Application
I	94°C for 2 min; 34 cycles of 94°C for 45 s, 57°C for 45 s, 72°C for 60 s; final incubation at 72°C for 10 min	Verification of T-DNA insertion
II	94°C for 2 min; 33 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 60 s; final incubation at 72°C for 10 min	Expression analysis by RT-PCR
III	95°C for 3 min; 35 cycles of 95°C for 45 s, 60°C for 60 s, 72°C for 120 s; final incubation at 72°C for 10 min	Generation of expression constructs

Table S5: Primers for expression analysis by RT-PCR.

gene locus	**forward primer** (5'- … -3')	**reverse primer** (5'- … -3')
At3g16400 (*AtNSP1*)	GCTACGGCACGACTCAATACAC	CCCACACACACACACACACATTC
At1g54040 (*ESP*)	GCAGCCATGGCTCCGAC	TCATCTAGATTAAGCTGAATTGACCGCATAG
At1g49240 (*actin 8*)	AGCTGTTCTATCACTTTACGCCAG	GATCCCTGCAGCTTCCATCC

Table S6: Primers for the generation of expression constructs. Lower case letters indicate primer overhangs required for USER cloning.

gene locus	**forward primer** (5'- … -3')	**reverse primer** (5'- … -3')
At3g16400 (*AtNSP1*)	ggcttaauATGGCCCAAAAGCTAGAAGC	ggtttaauTTAGGCAGAGTCAATCCCGTAA
At2g33070 (*AtNSP2*)	ggcttaauATGGTGCAAAAGGTGGAAGC	ggtttaauTTATGCAGAGTCAACTCCATAAAAG
At3g16390 (*AtNSP3*)	ggcttaauATGGCCCAAAAGCTAGTTGCACA	ggtttaauTCAGACAGAGTCAATTCCGTAAAAG
At3g16410 (*AtNSP4*)	ggcttaauATGGCCCAAAAGGTTGAAGCAC	ggtttaauTTAGGCAGAGTCAATTCCGTAAAAG
At5g48180 (*AtNSP5*)	ggcttaauATGTGTCCGGTGGAGAAC	ggtttaauTTAATTGACATTAAGATGAGAGAAAC
At3g07720	ggcttaauATGGCAGCGACTCCTATGGA	ggtttaauTCACTTGAGTAGAGTGTTGGGAGTG
At1g54040 (*ESP*)	ggcttaauATGGCTCCGACTTTGAAGGC	ggtttaauTTAAGCTGAATTGACCGCATAGAAG