SUPPLEMENT

Table S1. Chemical structures, common names and abbreviations of camalexin and GS.

Table S2. Primers used for quantitative RT-PCR analysis.

Supplementary Figure legends

Figure S1. Transcriptional changes in tryptophan pathway-related genes in response to *P. brassicae*.

(a) Quantitative RT-PCR analysis of basal expression levels of target genes in 4-week-old non-infected Col-0 plants. Expression levels were normalized relative to the expression level (dashed line) of the reference gene *PTB* (At3g01150). The values represent the mean (±SE) of 3 independent experiments.

(b) Quantitative RT-PCR analysis of pathogen-induced transcriptional changes at 6 hpi and 24 hpi in leaves of 4-week-old plants. The dashed line indicates no change of expression, while values below and above stand for down- and up-regulation of the given gene, respectively. The values represent the mean (±SE) of 3 independent experiments (**P < 0.001, *P < 0.05, ns = not significant; randomization test (Pfaffl *et al.,* 2002)).

Figure S2. Analysis of foliar thiol levels in uninfected Col-0, *cyp79B2 cyp79B3* and *pad2-1* plants.

Glutathione and cysteine levels were analysed in 5-week-old plants. The values represent the mean (±SE) of 4 independent replicates. Bars with different letters differ at P < 0.05 (Tukey's HSD test).

Figure S3. Effect of mutations in the camalexin- or GS-pathway on the accumulation of selected tryptophan-derived secondary metabolites.

Four-week-old plants were inoculated with zoospores of *P. brassicae* and secondary metabolites were analysed 48 hpi.

(a) and (b) Accumulation of the major iGS I3M and 4MO-I3M in camalexin-deficient mutants (*pad3-1* and *cyp71A13*) compared to *myb51* with partially reduced iGS levels. The values represent the mean (±SE) of 3 independent experiments, each with triplicate samples of 5-8 leaves of 5-8 plants. I3M = indol-3-yl-methyl GS; 4MO-I3M = 4-methoxy-indol-3-yl-methyl GS. The mutants *pad3-1* and *cyp71A13* (all with $P > 0.05$) didn't show altered levels of I3M and 4MO-I3M upon *P. brassicae* infection compared to Col-0 (genotype-treatment effect, factorial ANOVA). The levels of I3M and 4MO-I3M in the mutant *myb1* were significantly lower than wild type levels ($P < 0.05$, genotype effect; factorial ANOVA).

(c) Accumulation of camalexin in *myb51* and camalexin-deficient mutants. Values (±SE) report the results of 3 independent experiments, each with triplicate samples of 5 mock- or zoospore-inoculated leaves of 5 different plants. nd = not detected (detection limit of 1 pmol camalexin). The mutants *pad3-1*, *cyp71A13* and *myb51* accumulate camalexin after *P. brassicae* challenge significantly different to the parental background Col-0 (all mutants with $P < 0.05$, genotype-treatment effect; factorial ANOVA).

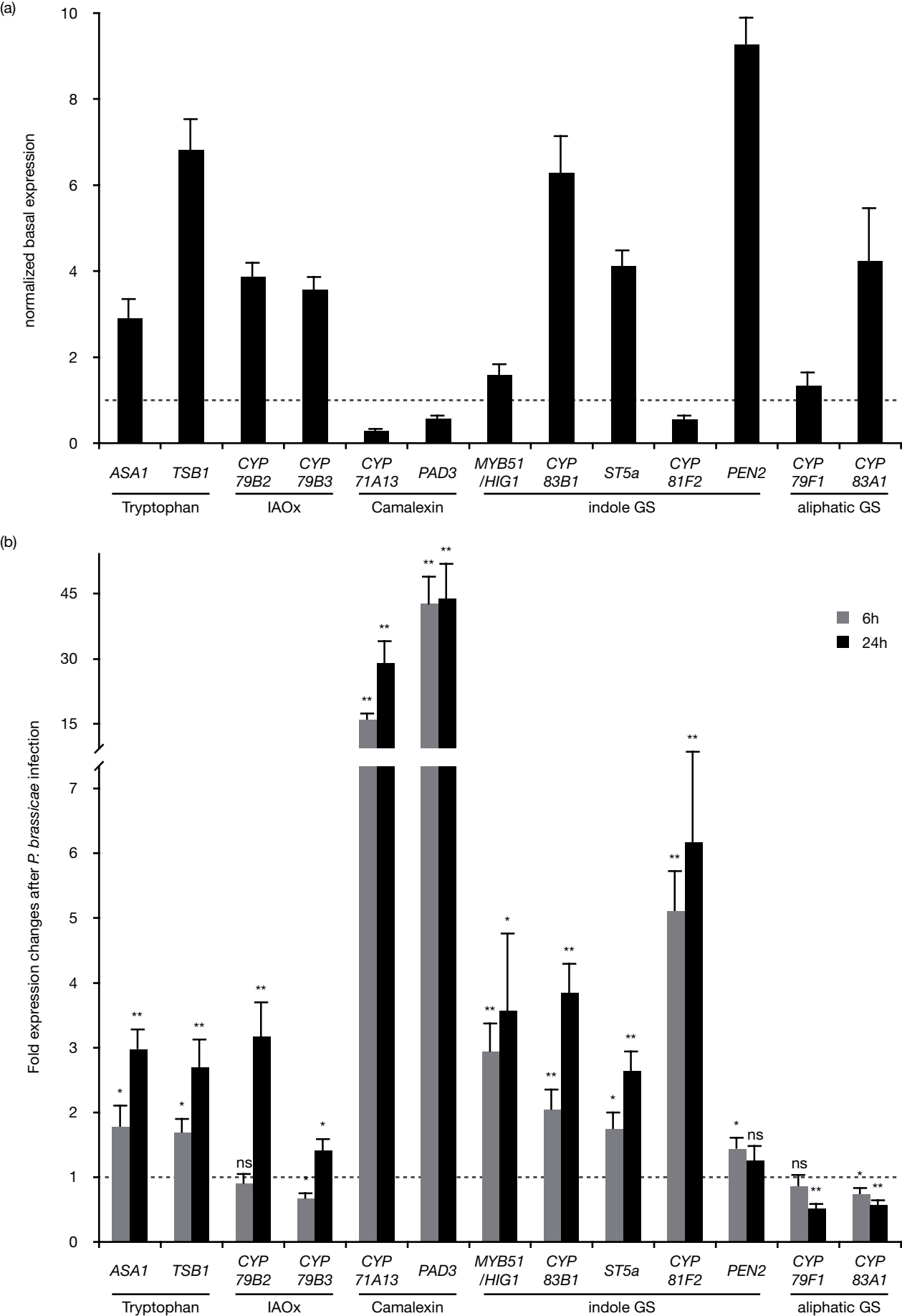
Figure S4. Comparison of transcript accumulation of genes involved in indole metabolism between *pad2-1* and Col-0.

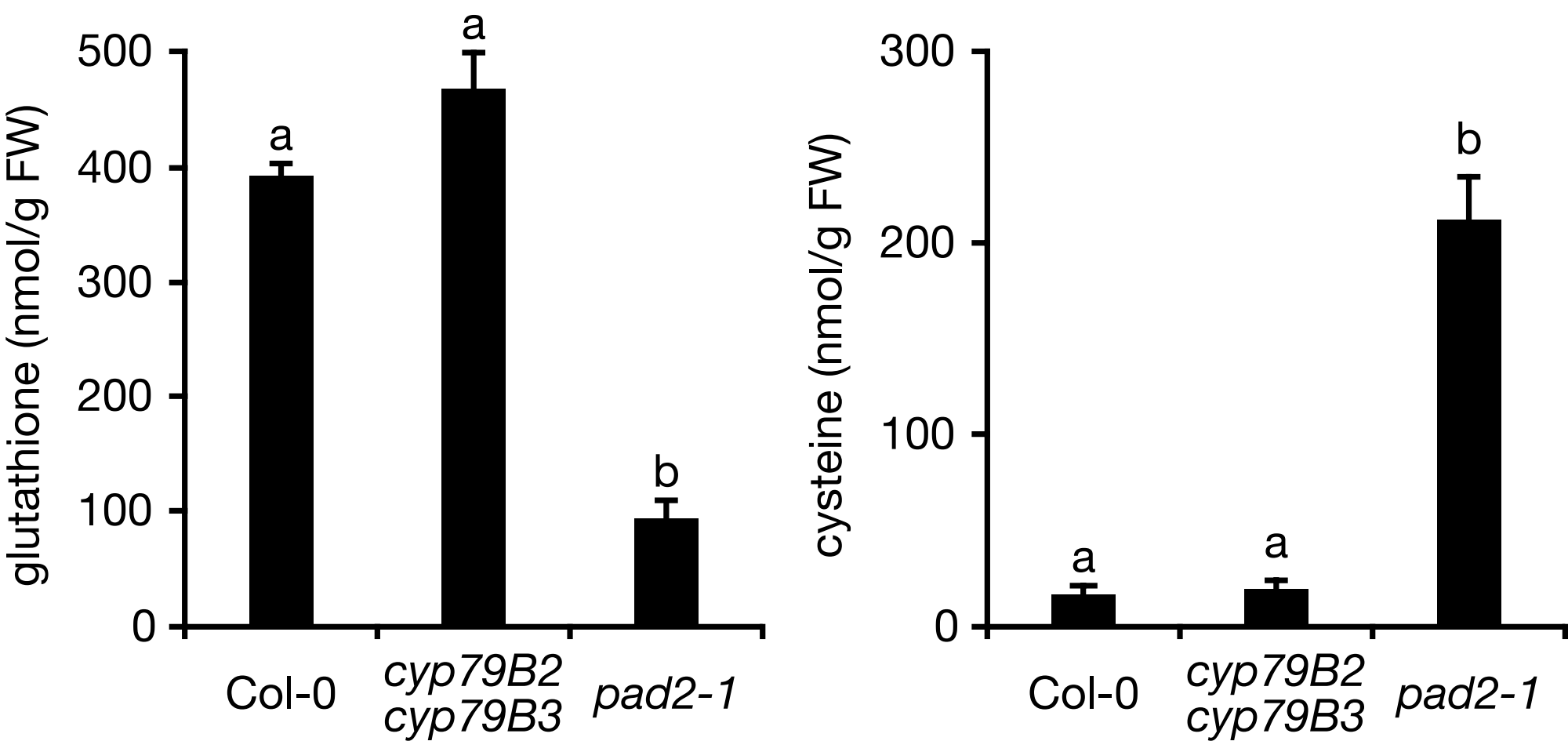
Quantitative RT-PCR analysis of pathogen-induced transcriptional changes at 24 hpi in leaves of 4-week-old plants. Expression levels were normalized to the expression level of the reference gene *PTB* (At3g01150). The values represent the mean (±SE) of 3 independent experiments, each with triplicate samples (*$P < 0.05$, ns = not significant, treatment effect; factorial ANOVA). The tested genes *MYB51*, *CYP83B1*, *ST5a* and *PEN2* are not differentially expressed in *pad2-1* compared to wildtype ($P > 0.05$, genotype-treatment effect; factorial ANOVA).

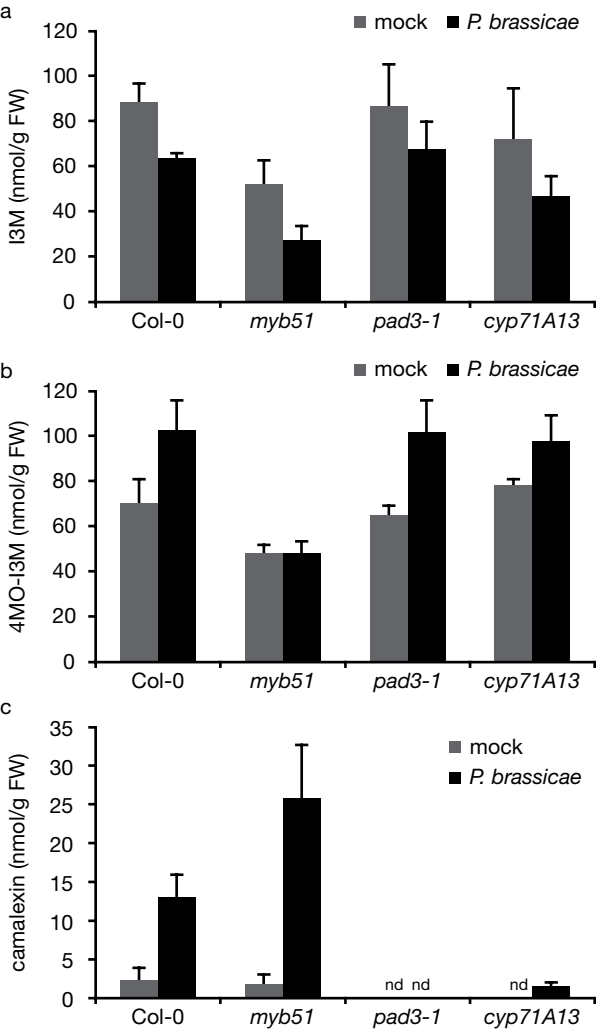
Figure S5. Analysis of antimicrobial activity of GS and camalexin towards *P. brassicae*.

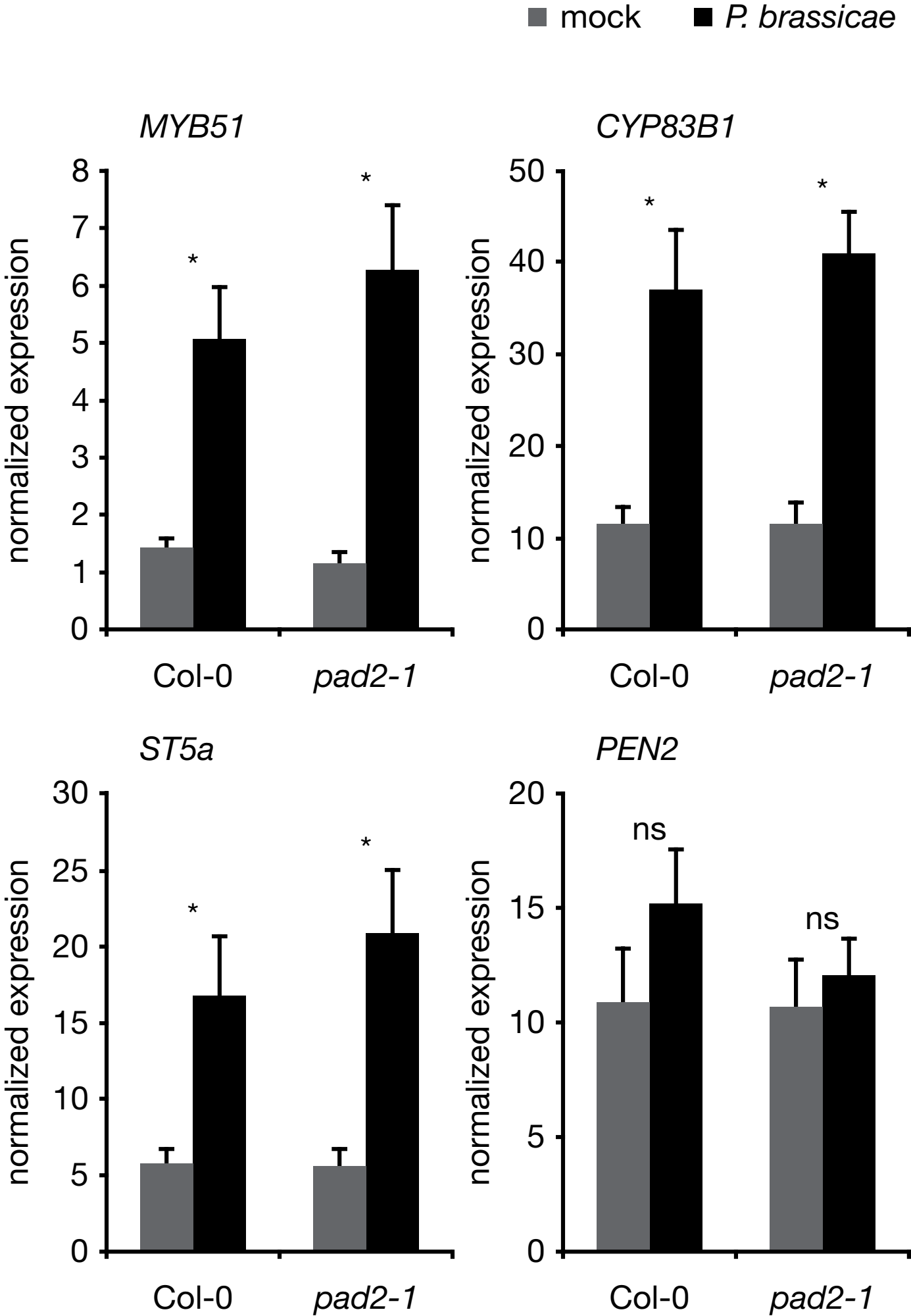
(a) Growth inhibition of *P. brassicae* by leaf extract containing GS extracted from wildtype Arabidopsis. Zoospores of *P. brassicae* were allowed to germinate on V8-agar plates for 1h before filter discs containing test solutions were applied. Inhibition zones around the filter discs were recorded 5 days later. The leaf extract containing GS was applied separately and in combination with either commercial myrosinase or protein extract from Arabidopsis. The physiological concentration present in a leaf corresponds to 1x while 4x corresponds to a 4-fold concentrated solution and 0.5x corresponds to a 2-fold dilution.

(b) Growth inhibition of *P. brassicae* by camalexin was tested in V8-agar plates containing different concentrations of camalexin. The diameter of the colonies was recorded after 4 days. The values represent the mean (±SE) of 6 replicates. The effects at all concentrations differ from each other at $P < 0.05$ (Tukey's HSD test).

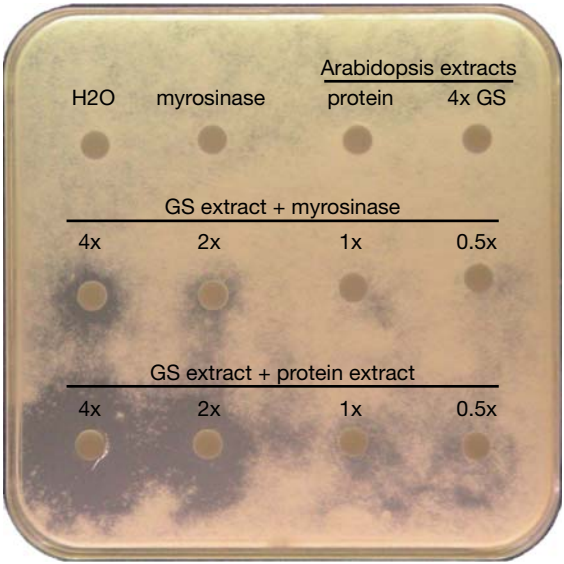

(a)
normalized basal expression
10
8
6
4
2
0
ASA1
TSB1
CYP 79B2
CYP 79B3
CYP 71A13
PAD3
MYB51 /HIG1
CYP 83B1
ST5a
CYP 81F2
PEN2
CYP 79F1
CYP 83A1
Tryptophan
IAOx
Camalexin
indole GS
aliphatic GS
(b)
Fold expression changes after P. brassicae infection
6h
24h
45
30
15
7
6
5
4
3
2
1
0
ns
*
**
ASA1
TSB1
CYP 79B2
CYP 79B3
CYP 71A13
PAD3
MYB51 /HIG1
CYP 83B1
ST5a
CYP 81F2
PEN2
CYP 79F1
CYP 83A1
Tryptophan
IAOx
Camalexin
indole GS
aliphatic GS


glutathione (nmol/g FW)
500
400
300
200
100
0
a
a
b
Col-0
cyp79B2
cyp79B3
pad2-1
cysteine (nmol/g FW)
300
200
100
0
a
a
b
Col-0
cyp79B2
cyp79B3
pad2-1

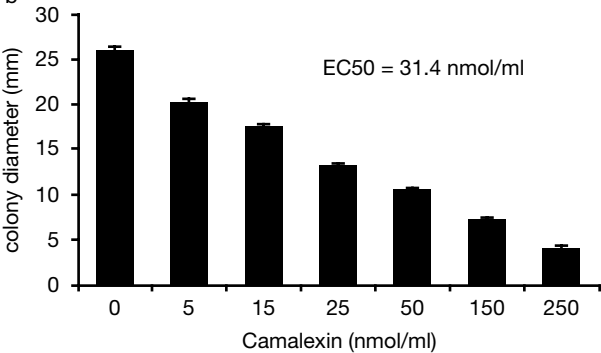

a
mock
P. brassicae
I3M (nmol/g FW)
120
100
80
60
40
20
0
Col-0
myb51
pad3-1
cyp71A13
b
mock
P. brassicae
4MO-I3M (nmol/g FW)
120
100
80
60
40
20
0
Col-0
myb51
pad3-1
cyp71A13
c
mock
P. brassicae
camalexin (nmol/g FW)
35
30
25
20
15
10
5
0
nd nd
nd
Col-0
myb51
pad3-1
cyp71A13


mock
P. brassicae
MYB51
CYP83B1
ST5a
PEN2
normalized expression
Col-0
pad2-1
*
ns
0
1
2
3
4
5
6
7
8
10
15
20
25
30
40
50

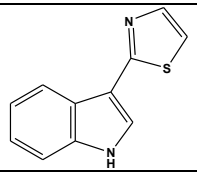
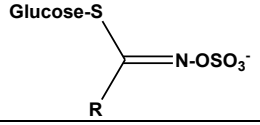
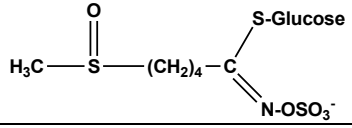
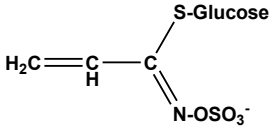
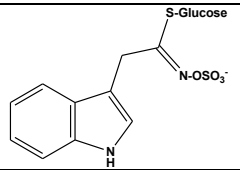
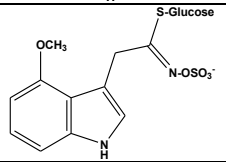
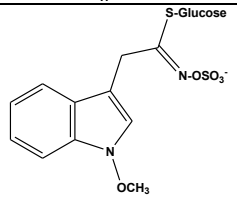
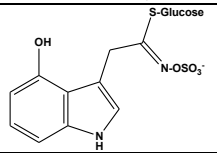
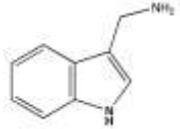
a


Arabidopsis extracts
H2O
myrosinase
protein
4x GS
GS extract + myrosinase
4x
2x
1x
0.5x
GS extract + protein extract
4x
2x
1x
0.5x

b


30
25
20
15
10
5
0
colony diameter (mm)
EC50 = 31.4 nmol/ml
0
5
15
25
50
150
250
Camalexin (nmol/ml)

Supplementary Table S1

Abb.	Systematic name	Common name	Structure
	3-thiazol-2'-yl-indole	Camalexin	
GS	ß-thioglucoside-*N*-hydroxysulfates	Glucosinolates	 (general structure; R = animo acid derivative)
4MSB	4-methyl-sulphinyl-butyl GS	Glucoraphanin	
Sin	Prop-2-enyl GS	Sinigrin	
I3M	indole-3-yl-methyl GS	Glucobrassicin	
4MO-I3M	4-methoxy-indole-3-yl-methyl GS	4-Methoxy Glucobrassicin	
1MO-I3M	1-methoxy-indole-3-yl-methyl GS	Neoglucobrassicin	
4HO-I3M	4-hydroxy-indole-3-yl-methyl GS		
I3A	Indol-3-ylmethylamine		

Supplementary Table S2

Gene	Description	AGI#	Primer pair	
EXPG[1]	*Expressed gene*	At4g26410	5'-GAGCTGAAGTGGCTTCCATGAC-3'	5'-GGTCCGACATACCCATGATCC-3'
PTB[1]	*Polypyrimidine tract-binding*	At3g01150	5'-GATCTGAATGTTAAGGCTTTTAGCG-3'	5'-GGCTTAGATCAGGAAGTGTATAGTCTCTG-3'
PPR[1]	*similar to pentatricopeptide repeat-containing protein*	At1g62930	5'-GAGTTGCGGGTTTGTTGGAG-3'	5'-CAAGACAGCATTTCCAGATAGCAT-3'
SAND[1]	*Sand family protein*	At2g28390	5'-AACTCTATGCAGCATTTGATCCACT-3'	5'-TGATTGCATATCTTTATCGCCATC-3'
TSB1	*Tryptophan synthase beta-subunit1*	At5g54810	5'-GCTTACCTCGAGAAGCTATGTCC-3'	5'-CCACTGTCTGAACATCTTTATCTCC-3'
ASA1	*Antranylate synthase alpha 1*	At5g05730	5'-AACGATGTTGGAAAGGTTACG-3'	5'-CGTCCCAGCAAGTCAAACC-3'
CYP79B2	*Cytochrome-P450-79B2*	At4g39950	5'-CTCGCGAGACTTCTTCAAGG-3'	5'-CCATAACCAACGGTTTAGCC-3'
CYP79B3	*Cytochrome-P450-79B3*	At2g22330	5'-ACGTACGGCGAGGATAATTC-3'	5'-CAAAAGGACCAAAACCGAAC-3' 5'-CTTCTTCCCTCCACAACTGG-3'
CYP83A1	*Cytochrome-P450-83A1*	At4g13770	5'-AGAGAGTCAAGCCCGAAACC-3'	5'-TTCCCGCCACTACAATATCC-3'
CYP83B1	*Cytochrome-P450-83B1*	At4g31500	5'-TTCATGAACGAGCACAAAGG-3'	5'-CATTGCAATCCCAAGATGC-3'
MYB51/ HIG1	*Myb transcription factor 51/ high indole glucosinolates 1*	At1g18570	5'-TCGAACTTTGACGTTGATGG-3'	5'-CGAAATTATCGCAGTACATTAGAGG-3'
ST5a	*Sulfotransferase-5a*	At1g74100	5'-ATGGCTGCTCGTATTGATGG-3'	5'-CCGCACCAAATAACAGAAGG-3'
CYP81F2	*Cytochrome-P450-81F2*	At5g57220	5'-TGGCTATGCGTAAACTCGTG-3'	5'-CCGGTAAACTTCAAAATGGTG-3'
PEN2	*Penetration2*	At2g44490	5'-CGAGTGGAACAGTGGATATGG-3'	5'-CATTTTCGGGTATCGTCTAAGC-3'
CYP71A13	*Cytochrome-P450-71A13*	At2g30770	5'-ACTCGTCTTATTAGTGTTGCATAGC-3'	5'-CCATTGGTATCGATGTTTGC-3'
Cyp71B15/ PAD3	*Cytochrome-P450-71B15/ Phytoalexin-deficient 3*	At3g26830	5'-GGGTACCATACTTGTTGAGATGG-3'	5'-TTGATGATCTCTTTGGCTTCC-3'

[1]Primer pairs from: **Czechowski, T., Stitt, M., Altmann, T., Udvardi, M. K. and Scheible, W. R.** (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. *Plant Physiol*, **139**, 5-17.