Supplemental Tables and Supplemental Figure Legends

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Figure S3. HPLC and spectrophotometric analysis of GSH-TNT conjugates. HPLC chromatogram showing the three TNT-GSH conjugation products formed at pH 9.5 by (A) GST-U24 and (B) GST-U25. Samples were analyzed at 250 nm. (C) Absorption spectra and absorption maxima of the GSH-TNT conjugates.

Figure S4. Change in TNT conjugating activity of glutathione transferases (GSTs) with increasing temperature. (A) GST-U24, (B) GST-U25. Assays, containing 100 mM phosphate buffer pH 9.0, 150 μg GST, 5 mM glutathione, 200 μM TNT in a total volume of 250 μl, were performed for 1 hour. Results are mean of three replicate measurements ± SD.

Figure S5. Lineweaver-Burk double reciprocal plots for (A) GST-U24 and (B) GST-U25. The reaction mix contained 50 mM Tris-HCl, pH 6.5, 0.5 mM EDTA, 5 mM GSH, 0.25 mM NADPH, 0.6 unit/ml glutathione reductase, 2.5-10 μM TNT and 5 and 30 μg of enzyme for GST-U25 and GST-U24 respectively in a final volume of 190 μl. The reaction was initiated

32 by the addition of cumene hydroperoxide and glutathione peroxidase activity monitored
33 spectrophotometrically using an NADPH-linked assay. Results are mean of three technical
34 replica ± SD.
35
36 **Figure S6.** Conjugation activity in protein extracts from Arabidopsis rosette leaves using 1-
37 chloro-2,4,-dinitrobenzene substrate. Wild type (WT), GST-U24 and GST-U25
38 overexpressing lines, results are mean of three biological replica ± SD.
39
40 **Figure S7.** Rate of TNT uptake and TNT-conjugate formation by Arabidopsis in liquid
41 culture. Three-week-old wild type (WT), (A) GST-U24 and (B) GST-U25 overexpressing
42 Arabidopsis lines were grown in flasks containing ½ MS, 20 mM sucrose and 200 mM TNT.
43 NPC, no plant control. Results are mean of five biological replica ± SE. (C) Levels of TNT-
44 conjugates in the tissues of plants after one day. Results are mean of three biological replica ±
45 SD.

46 **Supplemental Table I**

47 Root surface area of GST-U24 overexpressing lines relative to wild type. Plants were grown
48 vertically on agar plates containing ½ MS medium plus a range of TNT concentrations. Each
49 ratio is derived from the mean of three replicate plates containing 12-15 seedlings per plate.
50

Age of seedlings (days after germination)	TNT conc^n in the agar (µM)	Root surface area relative to wild type at the same concentration and time point						
		GST-U24 overexpression lines						
		1	2	3	4	5	6	7
9 days	2	1.04	1.62	1.04	1.16	1.24	1.39	1.17
	7	1.08	1.13	1.18	1.45	1.31	1.27	1.18
	15	1.10	1.07	1.24	1.15	1.16	0.94	1.16
14 days	2	1.20	1.40	1.00	1.18	1.49	1.70	1.44
	7	1.10	1.40	1.77	1.74	2.03	2.37	2.04
	15	2.49	2.01	2.95	1.33	2.49	1.31	2.17
20 days	2	1.34	1.23	0.91	1.26	1.08	1.22	1.27
	7	1.44	1.22	1.45	1.41	1.45	1.61	1.63
	15	2.40	2.04	2.37	1.93	1.95	1.26	1.93

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54 **Supplemental Table II**

55 Root surface area of GST-U25 overexpressing lines relative to wild type. Plants were grown
56 vertically on agar plates containing ½ MS medium plus a range of TNT concentrations. Each
57 ratio is derived from the mean of three replicate plates containing 12-15 seedlings per plate.
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Age of seedlings (days after germination)	TNT conc^{n} in the agar (µM)	Root surface area relative to wild type at the same concentration and time point						
		GST-U25 overexpression lines						
		1	2	3	4	5	6	7
9 days	2	0.84	0.63	1.10	0.79	1.26	0.89	1.13
	7	0.84	0.84	1.29	1.11	1.49	1.26	1.02
	15	1.24	1.12	1.35	1.49	1.52	1.20	1.20
14 days	2	1.05	0.61	1.12	1.05	1.54	1.11	1.24
	7	0.85	0.46	1.30	1.16	1.74	1.64	0.88
	15	1.18	0.69	1.24	1.03	1.38	1.17	0.60
20 days	2	2.18	1.55	1.54	1.61	1.55	1.46	1.80
	7	1.39	2.17	1.84	1.48	1.73	1.54	2.21
	15	3.34	1.84	2.66	2.28	3.04	2.18	2.01

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61 **Supplemental Table III**

62 Primer sequences used during cloning of GSTs for expression in *E. coli* and Arabidopsis.
63 Regions in italics are the Ligation Independent Cloning-specific overhangs for cloning into
64 pET YSBLIC (Bonsor et al., 2006). Regions in bold represent enzyme restriction sites used to
65 clone into pART7 (Gleave, 1992).

Primer name	**Sequence (5' to 3')**
GSTU1LICF	*CCAGGGACCAGCA*ATGGCGGAGAAAGAAGAGAGTGTGAAG
GSTU1LICR	*GAGGAGAAGGCGCG*TTAGGCAGACTTAATTGTCTCTGCAATTTTGGT
GSTU3LICF	*CCAGGGACCAGCA*ATGGCCGAGAAAGAAGAGGGTGTGAA
GSTU3LICR	*GAGGAGAAGGCGCG*TTAGACCGCTTTGATTCGTCCTACAATTTTCAT
GSTU4LICF	*CCAGGGACCAGCA*ATGGCGGAGAAAGAAGAGGATGTGAAG
GSTU4LICR	*GAGGAGAAGGCGCG*TTAGGCTGATTTGATTCTTTCTACAACTTTCTTC
GSTU7LICF	*CCAGGGACCAGCA*ATGGCGGAGAGATCAAATTCAGAGGAAG
GSTU7LICR	*GAGGAGAAGGCGCG*TTATCAAGCAGATTTGATATTGAGTTTCTCCATACG
GSTU12LICF	*CCAGGGACCAGCA*ATGGCTCAAAATGGTTCGAATACTACTGTG
GSTU12LICR	*GAGGAGAAGGCGCG*TTACTAAACACTGAATTTCTTTTTGGCAAACTCGAT
GSTU22LICF	*CCAGGGACCAGCA*ATGGCGGATGAAGTGATACTTTTGGATTTTTG
GSTU22LICR	*GAGGAGAAGGCGCG*TTAGACACAGTATATCTTCCTAATCTTATAGGC
GSTU24LICF	*CCAGGGACCAGCA*ATGGCAGATGAGGTGATTCTTCTGGATTTC
GSTU24LICR	*GAGGAGAAGGCGCG*TTACTCCAACCCAAGTTTCTTCCTACGTTC
GSTU25LICF	*CCAGGGACCAGCA*ATGGCAGACGAGGTGATTCTTCTTGATTTC
GSTU25LICR	*GAGGAGAAGGCGCG*TTACTATTCGATTTCGATCCCAAGTTTTTTCCTTAG
GSTU1F	**GAATTC**ATGGCGGAGAAAGAAGAGAG
GSTU1R	**GGATCC**TTAGGCAGACTTAATTGTC

GSTU1F	**GAATTC**ATGGCGGAGAAAGAAGAGAG
GSTU1R	**GGATCC**TTAGGCAGACTTAATTGTC
GSTU3F	**GAATTC**ATGGCCGAGAAAGAAGAGG
GSTU3R	**GGATCC**TTAGACCGCTTTGATTC
GSTU3R	**GGATCC**TTAGACCGCTTTGATTC
GSTU4F	**GAATTC**ATGGCGGAGAAAGAAGAGG
GSTU4R	**GGATCC**TTAGGCTGATTTGATTC
GSTU7F	**GAATTC**ATGGCGGAGAGATCAA
GSTU7R	**GGATCC**TCAAGCAGATTTGATATTG
GSTU22F	**GAATTC**ATGGCGGATGAAGTG
GSTU22R	**GGATCC**TTAGACACAGTATATCTTCC
GSTU24F	**GGTACC**ATGGCAGATGAGGTGATTCTT
GSTU24R	**TCTAGA**TTACTCCAACCCAAGTTTGTT
GSTU25R	**GGATCC**CTATTCGATTTCGATCC
GSTU25F	**GAATTC**ATGGCAGACGAGGTGA

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68 **Supplemental Table IV**

69 Primer sequences used for qPCR of GSTs in Arabidopsis.

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Primer name	Sequence (5' to 3')
RTU1F	CGTGCCATACGAATACTTGGAA
RTU1R	TTCTTGTGAAGCGGGTTTAGC
RTU3F	ACCAAACATGGACAAACAATCCT
RTU3R	CGACAAATTTGGCCCAGAA
RTU4F	AAGCCCTTTTACTCGTAGAGTTGAGA
RTU4R	TTTGTAGACAAGAACCGGAACCTT
RTU7F	TCCGGTTCTTGTTCATAATGGTA
RTU7R	TCATCGACGAATTTAGACCAGAAT
RTU22F	TCGAAGCATCAGAGAAACTAGCTAAC
RTU22R	CCTCTTAGCCGAAGCCATCA
RTU24F	TCCCTCCGATCCTTACAAGAGA
RTU24R	TCGCCGTAACATTCACCTTTT
RTU25F	TGTCAAATTCGATTACAGAGAACAAG
RTU25R	GGTATTTTCTTATGAACCGGATTCA

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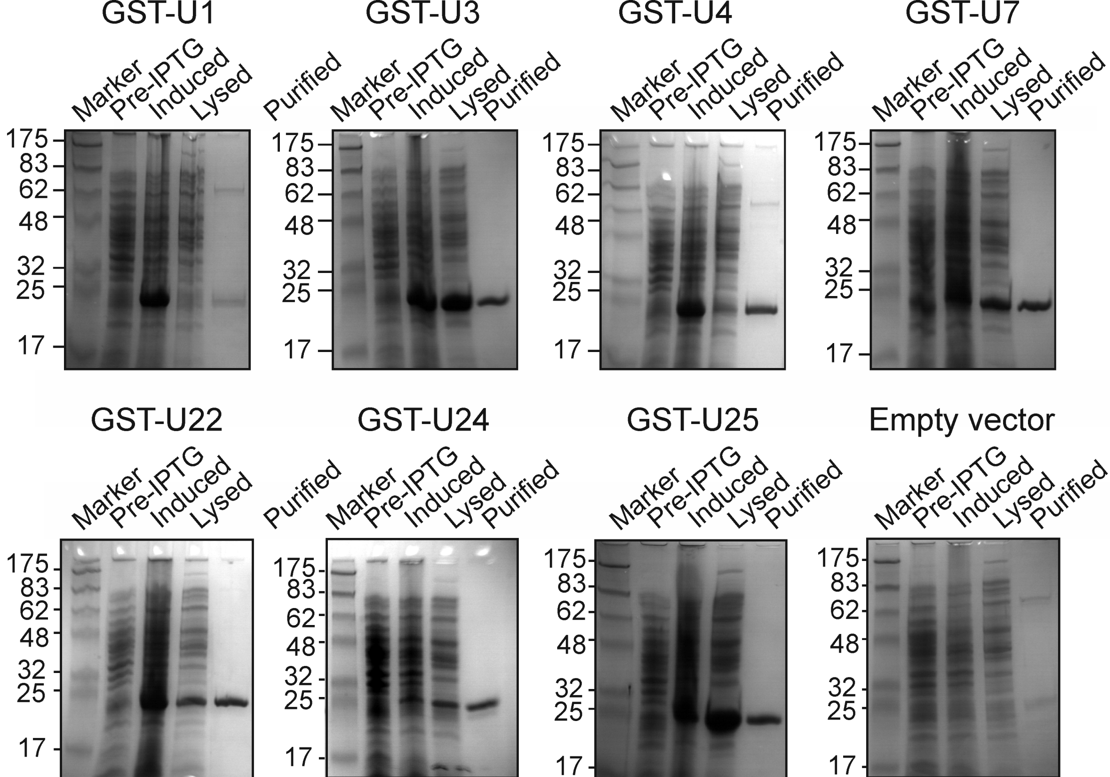

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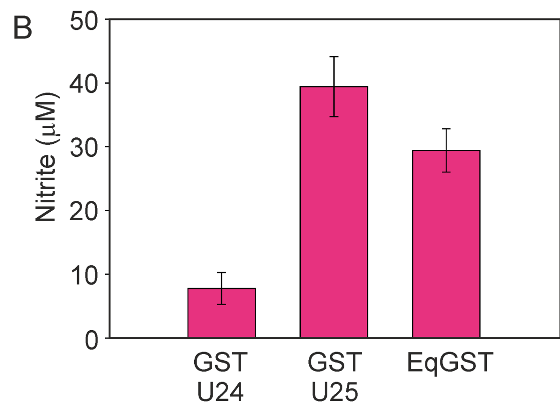

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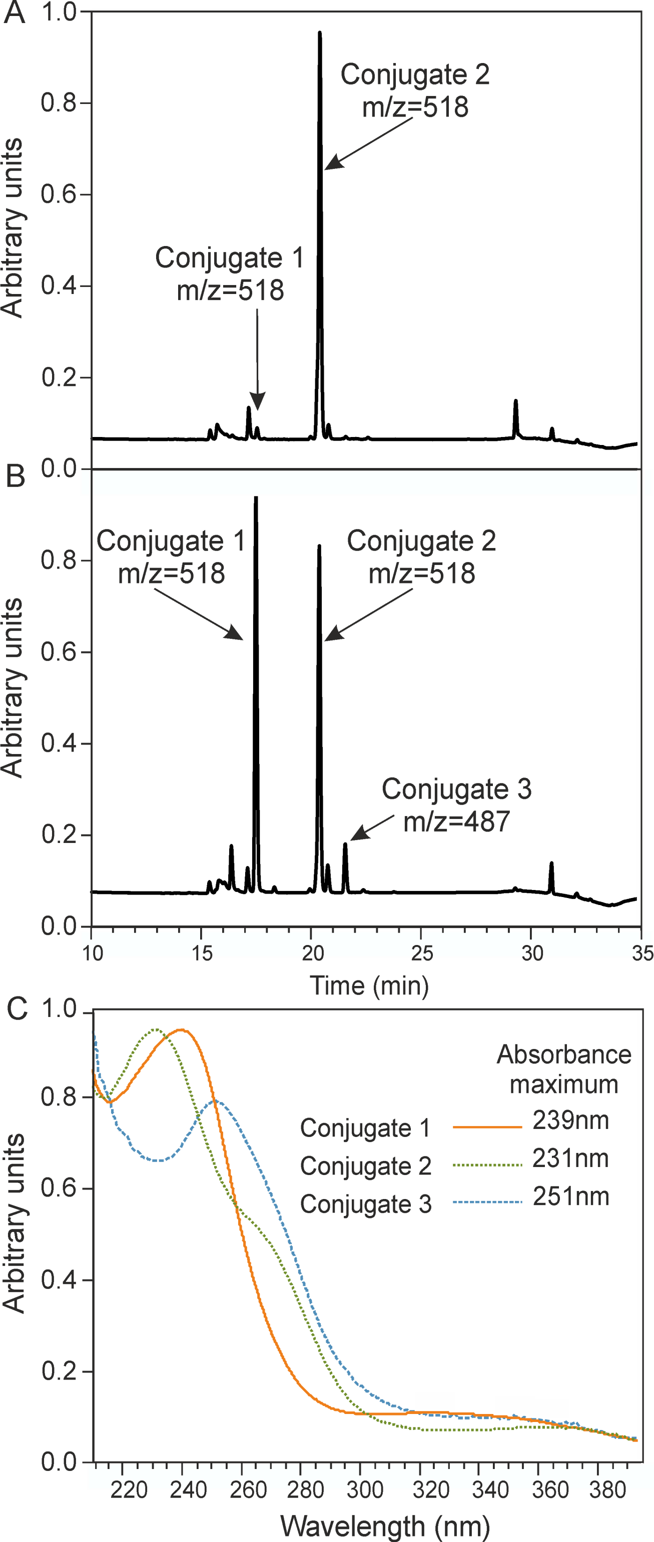

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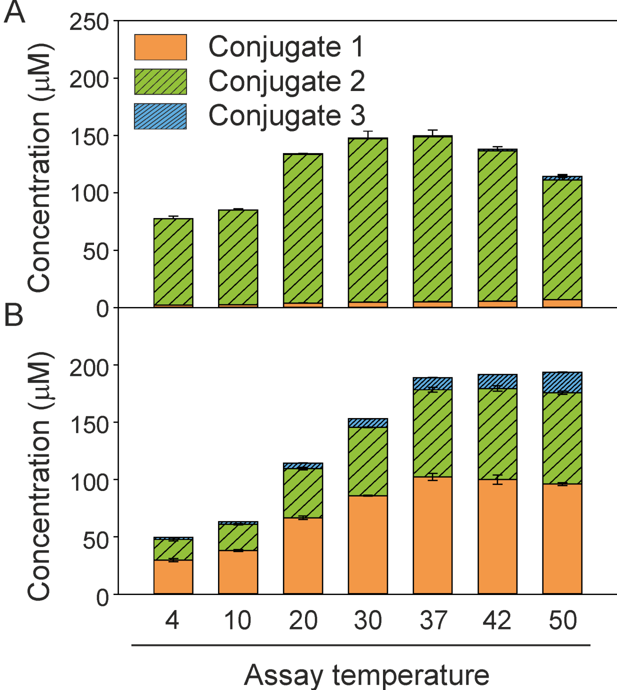

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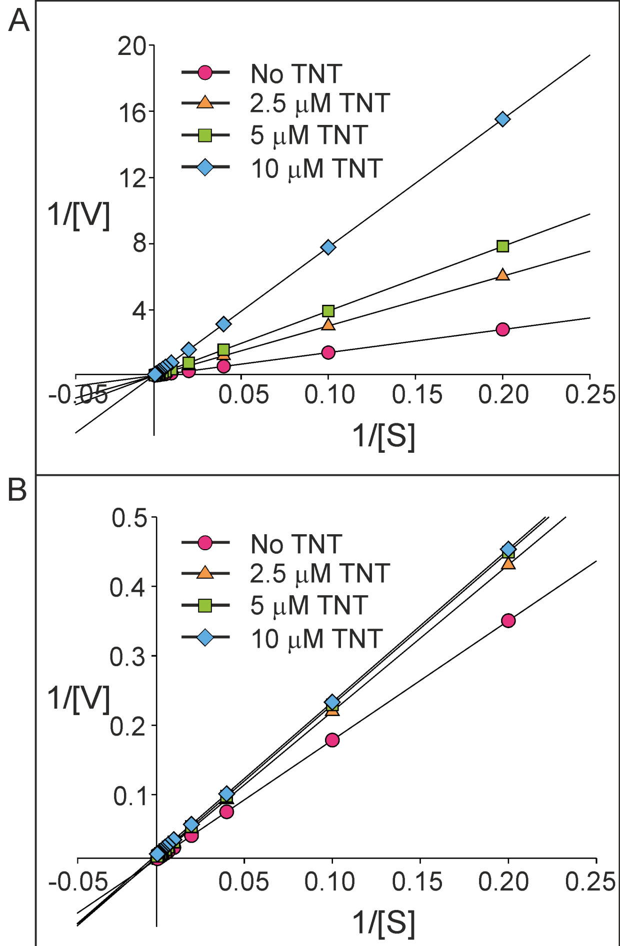

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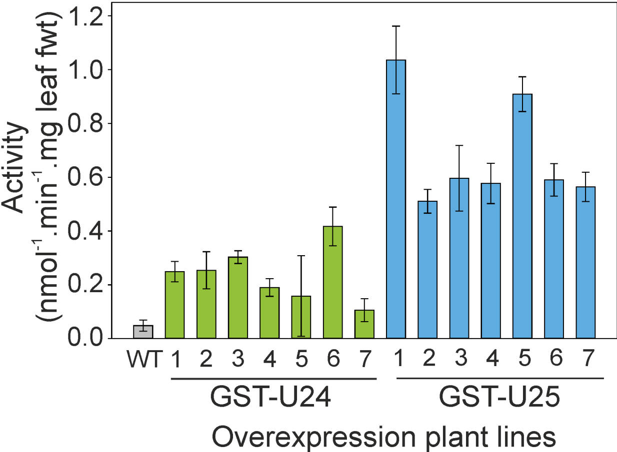

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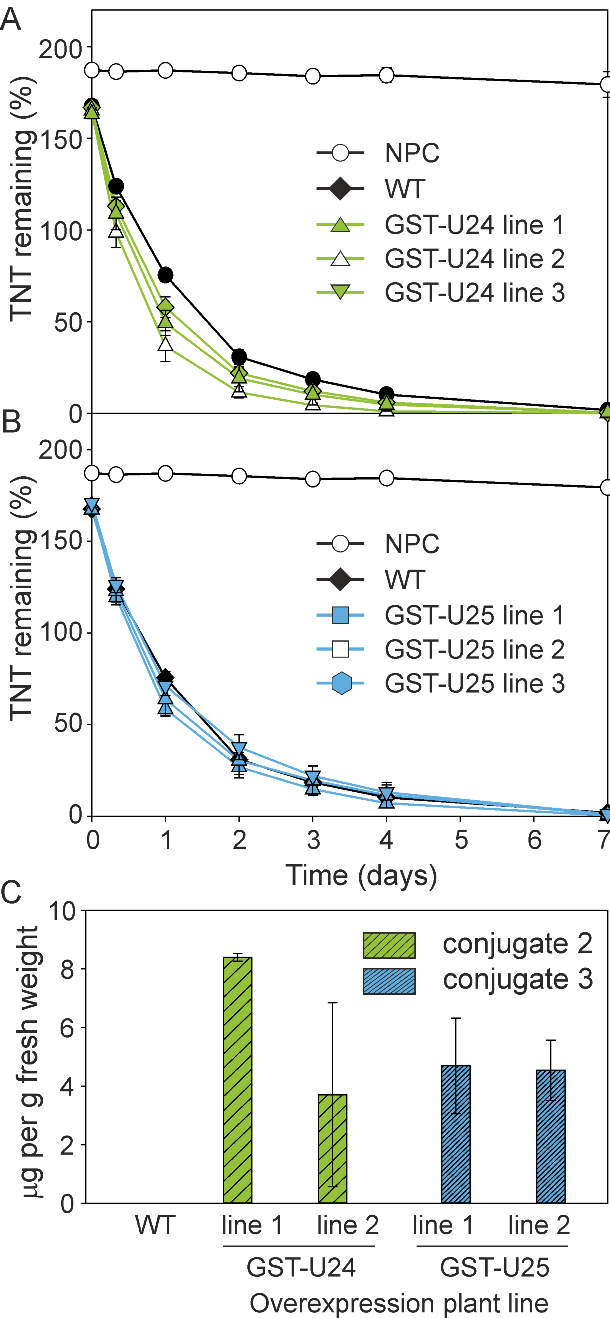

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