

#### Supplemental Figure 1. Phylogeny and conservation of TX and TN protein domains.

Phylogenetic tree of TN and TX protein domains encoded in the *Arabidopsis* Col-0 genome. The tree was generated using ClustalW. Domain structure is based on annotated structures published by Meyers et.al. (2002). The grey boxes represent the TIR domain; red boxes represent the NBS sub-domain; white boxes indicate a variable "X" domain; a thin single line indicates an intron.



### Supplemental Figure 2. Expression levels of *TN* and *TX* genes vary under biotic stresses.

*TN* and *TX* transcript levels were assessed using microarray data available from Genevestigator; we searched a range of biotic stress conditions in this database. Red colors indicate genes that are upregulated relative to the control for a given treatment; green colors indicate genes that were down-regulated relative to the control.



Supplemental Figure 3. Western blots of the TN proteins AtTN10 and AtTN3 using anti-GFP antibodies confirm protein expression and localization in the nucleus and cytoplasm. A) AtTN10 GFP fusion protein transiently overexpressed in *N. benthamiana* leaves displayed nuclear and cytoplasmic localization. The western blot shows a 48 kDa protein corresponding to the AtTN10 GFP fusion protein. B) AtTN3 GFP fusion protein transiently overexpressed in N. benthamiana leaves displayed nuclear and cytoplasmic localization. The western blot shows a 76 kDa protein corresponding to the AtTN10 GFP fusion protein. The confocal images were obtained at 20 µm magnification ...

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### Supplemental Figure 4. Yeast two hybrid interactions of TX and TN proteins with candidate elicitor protein library.

The intricate network of the TX and TN protein interactions with candidate elicitor proteins and other plant proteins is represented in the figure. The figure is generated through cytoscape. **Node legend:** Candidate elicitor proteins: Olive colored circles; TX proteins: green colored circles: TN proteins: orange colored circles; Arabidopsis transcription factors: Pink circles; Lettuce proteins: fluorescent pink; NBS-LRR proteins: cyan. The interconnecting lines in blue represent the interaction between the two corresponding proteins and the edge thickness corresponds to the strength of the interaction between them.



## Supplemental Figure 5. Co-localization of YFP with 3PGDH-CFP or AtTN10-YFP with CFP resulted in cytoplasmic localization.

A) Non-fusion YFP protein co expressed with 3PGDH-CFP protein. B) AtTN10-YFP fusion protein co expressed with CFP non fusion protein in *N. benthamiana* leaves. The confocal images were obtained at 20 µm magnification.

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### Supplemental Figure 7. Model for the function of Arabidopsis TN and TX proteins.

Plant TX and TN proteins may function as accessory proteins in the detection of pathogen effector proteins or their activities, or may function in signal transduction during defense responses. Based on genomic pairs of *TN* and *TNL* genes in the Arabidopsis genome and encoded "fusion proteins", we believe that the TN proteins likely function together with TNL proteins.

Supplemental Table 1. Primers designed for the amplification of transcript abundance through the use of real time PCR.

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No.	Primer Name	Sequence (5' 3')
1	AtTN2 for	GAGACAAGCTCTGACCAATTTG
2	AtTN2 rev	TCACTTGGTGGTGTTACCGAG
3	AtTX10 for	CACCTCGCTGCCGCTTTTGA
4	AtTX10 rev	CACCATGGGGACTCCGCATA
5	AtTN10 for	GCGTGGAACCGAATCATGTGAG
6	AtTN10 rev	TTGTCCACCAGCTTCGAGTCATC
7	AtTN11 for	GAGAAAGTTGCTTCGTGGAGACG
8	AtTN11 rev	CTTGTCAGCAATTTCGTCCACC
9	AtTN21 for	CCTGCTCCTCCAAGGATCTCTT
10	AtTN21 rev	GCGATTCCCTGAAGCTCAAG
11	AtTN3 for	GACCTGTGTGGGCAACATAACCG
12	AtTN3 rev	CCGGAGATCCCAACGATCTTAAC
13	AtTX21 for	GGAGATCAGCGAAATCAATGT
14	AtTX21 rev	TCTTGATCTCAACTAGCTCGTCTAA
15	AtTN13 for	GGAGACATGCTTTGGAGGAAGTCG
16	AtTN13 rev	GAAATCACTTGACGGCGTTGAGA
17	PDF1.2 for	TTTGCTGCTTTCGACGCAC
18	PDF1.2 rev	CGCAAACCCCTGACCATG
19	EDS1 for	CGAAGGGGACATAGATTGGA
20	EDS1 rev	ATGTACGGCCCTGTGTCTTC

# Supplemental Table 2. Primers designed for the amplification of *TX* and *TN* transcripts for cloning and generation of pENTRY gateway vectors.

No.	Primer Name	Sequence (5' 3')
1	AtTN10 for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTTCTCATACTGCAACTAAGTATGATG
2	AtTN11 for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTTCTCCTACTGCGACTAAGTATG
3	AtTN13 for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTTATAGAAAATTCAGGTTACACC
4	AtTN21 for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAAGGGAATAGAAGAAGAAGCAAG
5	AtTX21 for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACGAACTCTTCTCAGTCGCGG
6	AtTN3 for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTGCATCTACTTCTTCTTCTTC
7	AtTN2 for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTACTTCTTATTCTTTTTGTTGGC
8	AtTN2 rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGAAGATTCAGTCCCGGATATAGGTG
9	AtTN10 rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCTATTGTTGCATAAATCGTCTTCTTGTTTG
10	AtTN11 rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCACCAGATCTACCACTTAGACAACCTAACC
11	AtTN21 rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCGCTATAACAATTCAGTGAAGAGACTTTG
12	AtTN3 rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCTCCAATCCTTTCTTCAACTTCTAAC

Locus	MC 24	MR 06	MR 12	MR 24	MS 06	MS 12	MS 24	MS 96	NCA 01	NCL 01	NCL 02	NCR 01	NDL 01	NDL 02	NDR 01	NGS 01
LOC_Os01g 55530	27	18	18	13	29	3	16	9	84	23	17	53	30	23	69	37
LOC_Os08g 38970	0	0	0	0	0	3	0	0	311	0	0	49	1	0	56	42
LOC_Os09g 30380	1	0	1	0	0	3	2	0	3	0	0	0	1	2	6	7
Locus	NIP 01	NLA 01	NPO 01	NSL 01	NSL 02	NSR 01	NST 01	NYL 01	NYL 02	NYR 01	PLA 01	PLA 02	PLC 01	PLC 02	PLW 01	PLW 02
LOC_Os01g 55530	67	21	7	19	11	79	115	69	34	81	0	0	0	0	12	13
LOC_Os08g 38970	35	1	0	0	1	53	12	1	4	48	0	0	0	0	0	0
LOC_Os09g 30380	11	1	0	0	2	4	9	0	0	6	0	0	0	0	0	0
Locus	PSC 01	PSI 01	PSI 02	PSL 01	PSL 02	PSN 01	PSN 02	PSY 01	PSY 02	NSO 01	NMR 01					
LOC_Os01g 55530	0	0	0	0	0	38	57	0	12	54	104					
LOC_Os08g 38970	34	0	0	59	48	0	0	0	0	51	56					
LOC_Os09g 30380	0	0	0	0	0	0	0	0	0	21	7					

## Supplemental Table 3. Expression data from rice orthologs of Arabidopsis TIR-NBS genes.

MC : leaves from 3week old Pi9 transgenic plants; MR : leaves from 3week old Pi9 transgenic plants after rice blast inoculation (KJ201); MS : leaves from 3week old Nipponbare plants after rice blast inoculation (KJ201); NCA: Nipponbare 35 day callus; NCL : Two week old Nipponbare plant leaves stressed in cold for 24hrs; NCR : Two week old Nipponbare plant roots stressed in cold for 24hrs ; NDL : Two week old Nipponbare plant leaves stressed in drought for 24hrs; NDR : Two week old Nipponbare plant leaves stressed in drought for 24hrs; NGS : Nipponbare, 3day germinating seed; NIP : Nipponbare, 90 day immature panicle; NLA : Nipponbare, 60 day mature leaves; NPO : Nipponbare, mature pollen; NSL : 2 week Nipponbare plant leaves stressed in salt; NSR : 2 week Nipponbare plant roots stressed in salt; NST : 60 day Nipponbare stem; NYL : 2 week old Nipponbare plant leaves, untreated; NYR : 2 week old Nipponbare plant roots untreated; PLA : Beet armyworm damaged 2 week old Nipponbare plant leaves 24 hrs after infestation; PLC : Nipponbare control plant leaves after mechanical damage; PLW : Rice Water Weevil damaged 2 month old Nipponbare plant leaves 24 hrs after infestation; PSC : 6 day old seedlings developed from high milling quality cultivar Cypress; PSI : 6 day old seedlings developed from high taste quality cultivar ILpumbyeo; PSL : 6 day old seedlings developed from low milling quality cultivar LaGrue; PSN: 6 day old seedlings from Nipponbare control; PSY: 6 day old seedlings developed from low taste quality cultivar YRK; NSO :Nipponbare mature stigma and ovary; NMR : Nipponbare 60 day mature roots; The libraries are available at http://mpss.udel.edu/rice

### Supplemental Table 4. Phylogeny and conservation of TN protein domains among monocots and other basal angiosperms.

		gymn	osperm	basal angio	sperms		monoco	ots	mag	Inoliids	
Gene	type	Zamia	Amborella (454)	Aristolochia -454	Nuphar (454)	Asparagus	Acorus	Banana (M.acuminata)	Persea	Saruma	Liriodendron
AtTN21	TN	-	62/75	47/67	63/84	77/84	-	60/72	70/83	84/88	46/73
AtTN17	TN	-	61/85	67/87	52/73	43/70	-	57/71	39/60	42/70	59/79
AtTN10	TN	-	45/64	-	48/65	-	-	-	-	-	-
AtTN11	TN	-	42/66	-	45/62	-	-	-	-	-	43/65
AtTN3	TN	41/58	51/68	-	33/52	-	-	-	-	-	-
RPM1	CNL	35/55	41/67	32/52	41/65	-	-	31/50	45/64	39/68	38/67
RPP1	TNL	41/56	46/67	37/58	40/59	31/57	36/60	31/49	36/51	36/53	36/53

Each plant was grouped according to their families. The data represents a tblastx result of each of the TN protein from *Arabidopsis* blasted against the monocot genomes (*Asparagus* and *Acorus*) and other lower monocot genomes which includes magnoliids (*Persea, Samura* and *Liriodendron*) and basal angiosperms (*Amborella, Aristolochia* and *Nuphar*). Each of the cell values is presented as a ratio which represents a percent identity by percent positives. The tblastx is performed against the genomes that were sequenced and are available at

(<u>http://jlmack.plantbio.uga.edu/blast/blast.html</u>). Banana genome data was obtained from (<u>http://banana-genome.cirad.fr/index.html</u>).

Locus	Gene	CAF	INF	LEF	ROF	SIF	AP1	AP3	AGM	INS	ROS	SAP	SA4	SA52	LES	GSE	CAS	SIS
At1g17610	AtTN1												4	103	4			
At1g47370	AtTX1			9		19		3		4								3
At1g65390	AtTX10			5				6	3						2			
At1g66090	AtTN3	21		8		7								1			14	
At1g72850	AtTN4	7	2	21	13	7	1	11			25			7	4			
At1g72870	AtTN5				5	3				1	6					3	8	
At1g72890	AtTN6	141	6	13	21	3	2	4	2		17		14	13	20	2	191	
At1g72900	AtTN7	200		10		6	3		40	7		16		805	199		1382	
At1g72910	AtTN8													9	15			
At1g72920	AtTN9	26	4	10	2	4											5	
At1g72930	AtTN10	81	25	192	3	49	21	80	30	35	17	60	248	1499	369	1	318	70
At1g72940	AtTN11	177	5	6	9		6	32	13	13	3	60	6	117	93	10	215	0
At1g72950	AtTN12	30	6		18			5								8	2	28
At2g03300	AtTX12																	
At2g20145	AtTX13	79		2	2						5			12	4		73	
At2g32140	AtTX14														4			
At3g04210	AtTN13	70	58	285			13	48	10	24		26	316	2008	654	12	6	6
At4g04110	AtTN14																	
At4g09420	AtTN15	22			76						24				2	10	28	
At4g11340	AtTX15																	
At4g16990	AtTN16		66	476		50			1	6				20	90	14		
At4g19920	AtTX17	112			10											6	4	
At4g23440	AtTN17	24	58	12	8	12	34	28	35	15	26	20		1	38	7	20	
At4g23513	AtTX21	15									15						132	
At5g44920	AtTX25	600	6	15	695	5	1	7	9	6	128			30		2	343	3
At5g45000	AtTX26																	
At5g45070	AtTX27	8			150						20							
At5g45080	AtTX28				32						22							
At5g45090	AtTX29			2	4													
At5g45220	AtTX30	12	13														12	
At5g46480	AtTN19																	
At5g48780	AtTN20		4			1					5	1					2	
At5g56220	AtTN21	31	11	9	42	2	34	44	38	26	46	33			9	18	15	49

Supplemental Table 5. Expression of *TN* and *TX* genes from public MPSS database libraries.

CAF/CAS=callus, INF/INS=inflorescence, LEF/LES=leaf, ROF/ROS=root, SIF/SIS=silique, SA4/52=salicylic acid, GSE=germinated seedlings, AP1/AP3/AGM=mutants.

\* values in different libraries are indicated in TPM (Transcripts Per Million).

#	TX/TN protein	HR response (+/-)
1	AtTN3	+
2	AtTN10	+
3	AtTN11	+
4	AtTN21	+
5	AtTX21	+
6	AtTN1	-
7	AtTN2	-
8	AtTN6	-
9	AtTN13	-
10	AtTN15	-
11	AtTN19	-
12	AtTX6	-
13	AtTX8	-
14	AtTX10	-
15	AtTX16	-

Supplemental Table 6. *Arabidopsis* TX and TN proteins used for HR assays on *N. benthamiana*.

HR response is represented as plus (+) or minus (-) sign representing the presence or absence of HR.

Supplemental Table 7. Stable transgenic overexpression lines generated in *Arabidopsis* ecotype Col-0.

Gene	Annotation	Lines analyzed					
At1g17610	AtTN1	10 lines					
At1g17615	AtTN2	20 lines					
At1g61105	AtTX9	20 lines					
At1g57830	AtTX6	10 lines					
At1g57850	AtTX7	14 lines					
At1g60320	AtTX8	11 lines					
At1g65390	AtTX10	15 lines					
At1g72850	AtTN4	12 lines					
At1g72930	AtTN10	8 lines					
At1g72940	AtTN11	10 lines					
At2g32140	AtTX14	13 lines					
At4g11345	AtTX16	13 lines					
At4g09420	AtTN15	10 lines					
At3g04210	AtTN13	10 lines					
At4g23440	AtTN17	8 lines					
At4g23513	AtTX21	15 lines					
At5g56220	AtTN21	15 lines					
At5g46480	AtTN19	12 lines					
At5g44910	AtTX24	17 lines					
At5g45000	AtTX26	14 lines					
At5g40090	AtTN18	10 lines					
*At1g66090	AtTN3	10 lines					
pK2GW7	control	8 lines					

Number of lines analyzed were tabulated in the third column.

\* We could not develop transgenics for At1g66090 after repeating for three times in Col-0 background. We were however able to generate transgenics in *eds-1* background in *Arabidopsis*.

Supplemental Table 8. Salk T-DNA knockout lines for TX and TN gene
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#	Gene	Annotation	Salk accession number
1	At1g60320	AtTX8	salk_013760
2	At1g65390	AtTX10	salk_132166
3	At1g72860	TNL	salk_058326
4	At1g72890	AtTN6	salk_099669
5	At1g72900	AtTN7	salk_051714
6	At1g72920	AtTN9	salk_023426
7	At1g72940	AtTN11	salk_070394
8	At3g04210	AtTN13	salk_056759
9	At4g11340	AtTX15	salk_053459
10	At4g23440	AtTN17	cs839709
11	At5g44900	AtTX23	salk_014983
12	At5g45070	AtTX27	salk_020832
13	At5g45220	AtTX30	salk_055052
14	At5g56220	AtTN21	salk_114218, salk_114410

Salk accession number corresponds to the lines that were tested for the respective TX or TN gene.

# Supplemental Table 9. Summary of TX and TN phenotypes. A. Transcriptional data and overexpression phenotypes

<i>TX/TN</i> gene	Annota tion	Signature abundance (in TPM) ª	Gene Expres sion librari es #	Leaf control	SA	SA Foliar spray	MeJA exogenous Application	Overexpression Phenotypes
At1g17615	AtTN2	4-103	3	4	103	NA	NA	Stunted, curly leaves
At1g66090	AtTN3	1-21	5	0	1	Yes	yes	HR response in N. benthamiana
At1g72930	AtTN10	1-1499	17	369	1499	No	yes	Resistance to <i>P.syringae</i> DC3000 & <i>F.oxysorum strain</i> O-685
At1g72940	AtTN11	3-215	15	93	117	Yes	yes	NA
At4g23513	AtTX21	15-132	3	0	0	NA	NA	Resistance to <i>P.syringae</i> DC3000 & <i>F.oxysorum strain</i> O-685
At5g56220	AtTN21	9-49	14	9	0	No	No	Resistance to P.syringae DC3000

### **B.** Protein analysis results and related phenotypic data.

TX/TN gene	Annotation	Free SA levels in transgenics compared to control	Ability to induce stromules	Conservation with other plant genomes	Localization	<b>Protein interactions</b>
At1g17615	AtTN2	Higher by 4 fold	NA	With other TNLs	Cytoplasm	Pto66 (AvrPto) of P. syringae and Ral011t (R. solanacearum), Mor003
At1g66090	AtTN3	NA	NA	With other TNLs	Nuc+Cyto	Pto42 (HopY), Brl028 (B. Lactucae), At3g14470 (NB-LRR)
At1g72930	AtTN10	Higher by 7 fold	Yes in NRIP1	With other TNLs	Nuc+Cyto	Rbp001 (root knot nematode effector),Urf004
At1g72940	AtTN11	Higher by 2 fold	NA	With other TNLs	membrane	Hpp001 (Hyaloperonospora parasitica)
At4g23513	AtTX21	Higher by 2 fold	NA	With Musa	Cytoplasmic aggregates	Pto66 (AvrPto) of P. syringae
At5g56220	AtTN21	Higher by 7 fold	NA	Highly conserved	membrane	P. syringae - Psy(2), Pto(5), Pph (3) R. solanacearum – Ral (6) P. flourescens - Pfl003, B. lactucae - Brl(6), H. parasitica – Hpp001, Hpp021 nematode – Rbp001, Rbp002, M. oryza - Mor003; Pvx001 plant proteins: At5g63020 (NB-LRR), At1g12210, At4g16950, At4g36150, At5g63020, AtACT, AtEDS1, AtPBS1, AtTGA1, AtTGA2, AtTGA3, AtTGA7, AtMPK4, LeCMPG1, AtTN21