

Supplementary Fig 1. Sequence analysis of *TNO1*.

A. TNO1 DNA sequence

TNO1

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1  ATGCACGAGAAGGATGATCTTCCGCAGGATTCTATAGCTGATGGAATTGAGAATGATGAC 60
61  GAGTCTAATGGGCAAGAAGAAGAAGAGCTTGATCCTGACCAGGGAACAGCTTTCGTTGAT 120
121 AGTAAGGAAGATATGTTTGTGATGCTCCTGAAGAGTTGAATTTTGATACACCTAGCAAG 180
181 GAAGCTCTTACCACAGATGACGATGATAATGATGACCTTGGAACCTATTTCAATATTGAG 240
241 AAGGGGGATTGGGAGAAGGAACCTGCAGGGCTTCAGGAGCAGTTTAAGCTGTTGACTGGT 300
301 GAGAATGATTTGACAGGTGAAGATGGGAACACTACTGTGGATATTGTCAGTCGCTTCTCG 360
361 AAGTTCCTTAAAACTGCCAAGGAAGAGCGGATTTCAGCATGAGGTTGCACTCAAGGAGCTT 420
421 CACGGGGTTATTAGTGGGAGGGACGATGAGATTGCTGATCTCACCACAAAAATCTCTGAG 480
481 CTTTCTTCGTCGCAGCCGGTTTCCGAAATGGGTGATCAGGCACAGAACTTGGAGCACCTT 540
541 GAGGCTGCAACGGATAGGATTATGGTTTCTCTTAGTAATGTATTTGGGGAAGGGGAGTTG 600
601 CAGTATGGTTCTTCTATCTCTGAAAAGCTTGCTCATCTGGAGAACCGAGTTTCGTTTTTA 660
661 GGTGCAAAGTATACTGAGTTTTACTATGGTGCTGATCAGTTAAGGAAGTGTGGCTAGT 720
721 GATGTGTTGGATCTTAGTTTCCAAGAGGATTTTGGTTCAGCTCTGGTGCTGCTTGTCT 780
781 GAGCTATTTGAGCTCAAACAGAAGGAAGCAGCCTTTTTTGAAGGACTTAGTCATCTAGAA 840
841 GATGAGAATAGGAACCTTGTGTAACAAGTGAACAGAGAGAAAGAAATGTGTGAGTCAATG 900
901 AGAACAGAATTTGAAAAATTGAAGGCAGAGCTTGAGCTAGAAAAGACTAAGTGACTAAC 960
961 ACAAAGAAAAGCTCAGCATGGCCGTAACAAAGGGGAAGGCGTTAGTTCAGAACCGGGAT 1020
1021 GCTCTGAAGCATCAATTGTCTGAAAAACAACAGAGCTTGCGAATAGGTTGACTGAATTA 1080
1081 CAAGAGAAGGAGATTGCCCTTGAAAGTTCTGAAGTAATGAAGGGGAGCTGGAACAATCG 1140
1141 TTAACCGAAAAGACGGATGAACTTGAGAAATGCTATGCTGAATTGAATGATAGGTCCGTA 1200
1201 TCCCTGGAAGCATATGAGCTAACAAAGAAGGAGTTGGAACAGTCTCTGGCTGAAAAACA 1260
1261 AAAGAACTTGAAGAGTGTTTGACGAACTACAAGAGATGTCAACAGCATTGGATCAATCT 1320
1321 GAACTCGACAAAGGCGAGTTAGCAAAATCCGATGCTATGGTTGCATCATATCAGGAAATG 1380
1381 TTATCGGTGAGGAACCTCTATCATTGAAAATATTGAAACTATCCTGTCAAACATATATACA 1440
1441 CCTGAAGAAGGTCACTCTTTTGATATCGTTGAAAAAGTAAGGTCACTTGCAGAAGAGAGG 1500
1501 AAAGAGCTCACAAATGTTTCCCAGGAATACAACAGACTAAAAGATTTGATCGTTTCCATT 1560
1561 GACTTACCAGAGGAGATGTCCCAATCCAGCTTAGAAAAGTCGCCTAGCTTGGCTTAGAGAA 1620
1621 TCTTTTCTCCAGGGAAAAGATGAAGTTAATGCCTTGCAAAACCGGATTGAAAGTGAAGC 1680
1681 ATGTCTCTTTCAGCAGAAATGGAGGAGAAAAGTAACATTAGAAAGGAAGTGGATGATTTA 1740
1741 AGTTTCAGTTTGAAAAAATGGAGGAACTGCAGAGCGAGGTTTCGTTGGAGAGGGAGGAA 1800
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1801 ATTGTGAGGAGACTTGTGGAACCTCTGGCTTGATGACAGAAGGAGTCGAAGATCATACT 1860
1861 TCTTCAGATATCAATTTACTTGTGATAGATCATTCGACAAGATAGAAAAGCAAATCAGG 1920
1921 GATTCTAGTGATAGTTCTTATGGCAACGAAGAAATATTTGAAGCCTTTCAAAGTCTCCTT 1980
1981 TATGTGAGAGATCTGGAGTTTTCACTTTGTAAGGAAATGCTAGGAGAGGGAGAGCTGATT 2040
2041 AGCTTTCAGGTAAGCAATCTCTCAGATGAGCTAAAGATCGCATCTCAAGAACTTGCTTTC 2100
2101 GTGAAAGAAGAAAAAATTGCTTTGGAGAAAGATCTAGAGCGATCAGAGGAGAAATCTGCT 2160
2161 TTGCTCAGAGACAACTTTCTATGGCTATCAAGAAAGGCAAGGGACTAGTCCAAGATAGG 2220
2221 GAAAAGTTTAAACTCAGTTGGATGAGAAAAAATCTGAAATCGAAAAGCTGATGCTCGAG 2280
2281 TTGCAGCAGCTAGGTGGTACGGTTGATGGCTACAAGAATCAGATAGATATGTTATCGAGA 2340
2341 GACTTAGAGCGCACGAAAGAGCTAGAGACTGAGCTTGTTGCTACTAAAGAAGAAAGAGAT 2400
2401 CAACTTCAGCAATCCTTATCTCTAATTGACACGTTGTTGCAGAAAGTGATGAAATCAGTT 2460
2461 GAAATTATAGCTCTCCCTGTTGATCTAGCATCTGAAGATCCTTCAGAAAAGATTGACCGA 2520
2521 CTTGCTGGGTACATCCAAGAAGTGCAGCTGGCTAGAGTAGAGGAACAAGAAGAAATAGAA 2580
2581 AAAGTAAAGTCAGAAGTTGATGCGTTAACCAGTAAATTAGCAGAAACCCAAACAGCCCTG 2640
2641 AAGTTGGTTGAGGATGCCTTGCTCTACTGCAGAGGATAACATCAGTCGGCTTACTGAGGAG 2700
2701 AATAGAAATGTCCAAGCTGCCAAGGAAAATGCTGAGCTTGAGCTGCAAAAAGCAGTTGCA 2760
2761 GATGCCTCCTCTGTAGCTAGCGAACTGGATGAAGTTCTTGCAACCAAAGCACACTTGAA 2820
2821 GCTGCACTCATGCAGGCTGAAAGAAATATATCTGATATTATTAGTGAAAAGGAAGAGGCT 2880
2881 CAAGGCAGAACTGCTACTGCAGAGATGGAGCATGAGATGCTGCAAAAAGAAGCTTCAATT 2940
2941 CAGAAGAACAAATTAACAGAAGCACATAGCACCATAAAATTCAGTTGAAGAAACACTTGCT 3000
3001 CAGACAGAAAGCAACATGGATTGCTGTCCAAACAAATTGAAGATGACAAAGTTCTTACT 3060
3061 ACAAGTTTAAAGAATGAGTTAGAGAAGCTTAAAATTGAGGCAGAAATTTGAGCGTAACAAG 3120
3121 ATGGCCGAAGCTTCCTTGACAATAGTATCCCATGAGGAGGCACTTATGAAKGCAGAGAAT 3180
3181 AGTCTTTCTGCTTTACAAGGAGAAATGGTGAAAGCTGAAGGCGAGATATCAACTCTCAGT 3240
3241 AGTAAACTTAATGTATGCATGGAAGAGTTAGCTGGATCAAGCGGAAACTCACAGAGTAAA 3300
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3361 ATTTCCAAGGTGAATGAATTCCTTCAAAGGAAGTTCAAGAGCCTTAGAGACGTGGATGTC 3420
3421 ATTGCTAGAGATATCACACGAAATATTGGTGAGAATGGATTATTGGCAGGGGAAATGGGC 3480
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3541 AACACAGAGCCGGAGAATAGTCAAGGGAGTGAGCTGATGAAGACGAAATTTCTTCATCC 3600
3601 CTTAGGAAGATGGCAGAGGGGGTCAGGCTGAGAAACAAAACCCTCGAGAATAACTTTGAG 3660
3661 GGTTCCTCAACTTCCATTGATACTCTCATAGCGACTTTGATGCAAAACATGACAGCAGCT 3720
3721 AGGGCTGATGTGTTAAATATCGTGGGTCATAATTCATCCTTGGAAGAACAGGTGAGGAGT 3780

3781 GTGGAAAATATTGTTTCGTGAACAGGAGAACTATATCTGCATTACAAAAAGATTGTCA 3840
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 4021 GCAGAGAAGGCATGTGCTACTCTTAAACTCTTTGAGACAACAAATAATGCAGCTGCCACT 4080
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 4141 TTAGAAAGAGATCTAAACCAAATAAGGTTTCAAGTTCTGAGGCCAAGGTGGAATCTCTC 4200
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 4261 TGGCATGAAAAAAGGTGGAAGTGTCTACGTTATATGATAAACTATTGGTGCAAGAGCAA 4320
 4321 GAGGCAAAGGAACATCTAATTCAGCTTCTGATATGCGAACCCTTTTTGACAAAATAAAT 4380
 4381 GGTATTGAAGTGCCATCAGTAGATCTAGTCAACGGATTAGATCCACAGAGTCCATATGAT 4440
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 4561 GGTCTAAAGAAGGCGACTGAAGCAGAGAGTACGACCGAGCTAGAGTTAGTGAAGGCAAAG 4620
 4621 AATGAACTGTCCAAGCTAATATCTGGCTTGGAAAACTGCTGGGTATATTGGCAAGCAAT 4680
 4681 AATCCTGTTGTAGACCCAACTTCTCCGAGTCATGGACACTCGTACAAGCACTAGAAAAA 4740
 4741 AAGATAACTTCCCTTCTCCTAGAAATCAGAGAGTTCAAAATCAAGGGCCCAAGAACTTGGT 4800
 4801 TTAAAGTTGGCCGGTAGCGAGAACTTGTGATAAACTATCATTAAGAGTCAAAGAGTTT 4860
 4861 GAAGAGAACTTCAAACCAAAGCAATTCAGCCTGATATTGTTCAAGAAAGAAGCATCTTC 4920
 4921 GAAACACCGAGAGCACCTTCTACCTCAGAGATATCCGAGATTGAGGACAAGGGAGCCTTG 4980
 4981 GGAATAAAATCAATATCACCGGTGCCTACAGCAGCACAAAGTGAGAACAGTGAGGAAGGGA 5040
 5041 TCGACGGATCATCTTTCAATCAACATAGATTAGAGTCCGAGCATCTGATGAACAACAAC 5100
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 5161 ACGCAAGGAAAGATAATAGCAGATCGGGTTGATGGAATATGGGTCTCAGGTGGAAGAGTA 5220
 5221 TTGATGAGCCGTCCTCAAGCAAGGCTTGGCGTTATGGTATACAGTCTCTTATTGCATCTG 5280
 5281 TGGCTCCTAGCCTCCATCTTGTA 5304

B. TNO1 amino acid sequence

TNO1 1 MHEKDDLDPQDSIADGIENDDESNGQEEEELDPDQGTAFVDSKEDMFVDAPEELNFDTPSK 60
61 EALTTDDDDNDDLGHFNIEKGDWEKELAGLQEQQFKLLTGENDLTGEDGNTTVDIVSRFS 120
121 KFLKTAKEERIQHEVALKELHGVISGRDDEIADLTTKISELSSSQPVSEMGDQAQNLEHL 180
181 EAATDRIMVSLSNVFGEGELQYGSSISEKLAHLENRV**SFLGAI**YTEFYYGADQLRKCLAS 240
241 DVLDSLQFQEDFGSALGAACSELFELKQKEAAFFEGLSHLEDENRNRFVEQVNREKEMCESM 300
301 RTEFEKLKAELELEKTKCTNTKEKLSMAVTKGKALVQNRDALKHQLSEKTTELANRLTEL 360
361 QEKEIALESSEVMKGQLEQSLTEKTDELEKCYAELNDRSVSLEAYELTKKELEQSLAEKT 420
421 KELEECLTKLQEMSTALDQSELDKGELAKSDAMVASIQEMLSVRNSIIENIETILSNIYT 480
481 PEEGHSFDIVEKVR**SLAEER**KELTNVSQEYNRLKDLIVSIDLPEEMSQSSLESRLAWLRE 540
541 SFLQGKDEVNALQNRIESVSMSSAEMEKEKNIRKELDDLFSFLKMEETAERGSLEREE 600
601 IVRRLVETSGLMTEGVEDHTSSDINLLVDRSFDKIEKQIRDSSDSSYGNEEIFEAFQSL 660
661 YVRDLEFSLCKEMLGEGELISFQVSNLSDELKIASQELAFVKEEKIALEKDLERSEEKSA 720
721 LLRDKLSMAIKKGKGLVQDREKFKTQLDEKKSEIEKLMLELQQLGGTVDGYKNQIDMLSR 780
781 DLERTKELETELVATKEERDQLQQSLSLIDTLLQKVMKSVEIIALPVDLASEDPSEKIDR 840
841 **LAGYIQEVQLAR** **VEEQEEIEKVK** **SEVDALTS**LAETQTALKLVEDALSTAEDNISRLTEE 900
901 NRVQAAKENAELELQKAVADASSVASELDEVLATKSTLEAALMQAERN**NISDIISEKEEA** 960
961 **QGH**TATAEMEHEMLQKEASIQKNKLTEAHSTINSLEETLAQTESNMDSLSKQIEDDKVLT 1020
1021 TSLKNELEKLEKIEAEFERNKMAEASLTIVSHEEALMKAENSLSALQGEMVKAEGEISTLS 1080
1081 SKLNCMEELAGSSGNSQSKSLEIITHLDNLQMLLKDGGLISKVNEFLQRKFKSLRDVDV 1140
1141 IARDITRNIGENGLLAGEMGNAEDDSTEAKSLLSDLDNSVNTPEPENSQGSAADEDEISS 1200
1201 LRKMAEGVRLRNKTLENNFEGFSTSIDTLIATLMQNMTAAR**ADVLNIVGHNSSLEEQVR** **S** 1260
1261 **VENIVRE**QENTISALQKDLSSLISACGAAARELQLEVKNLLELVQFQENENGEMESTE 1320
1321 DPQELHVSECAQRIKELSSAAEKACATLKLFFETTNNAAATVIRDMENRLTEASVALEKAV 1380
1381 LERDLNQTKVSSSEAK**KVESLEELRQDLKLQLENLRV**KEEKWHEKKVELSTLYDKLLVQEQ 1440
1441 EAKEHLIPASDMRTLFDKINGIEVPSVDLVNGLDPQSPYDVKKLFAIVDSVTEMQHQIDI 1500
1501 LSYGQKELNSTLAEKDLEIQGLKKATEAESTTELELVKAKNELSKLISGLEKLLGILASN 1560
1561 NPVVDPNFSESWTLVQALEKKITSILLSESSKSRAQELGLKLAGSEKLVDKLSLRVKEF 1620
1621 EEKLQTKAIQPDIVQERSIFETPR**APSTSEISEIEDK**GALGIKISISPVPTAAQVRTVRKG 1680
1681 STDHLSINIDSESEHLMNNNETDEDKGVFKSLNMSGLIPTQGKIIADRVDDGIWVSGGRV 1740
1741 LMSRPQAR**RLGVMVYSLLLHLWLLASII** 1767

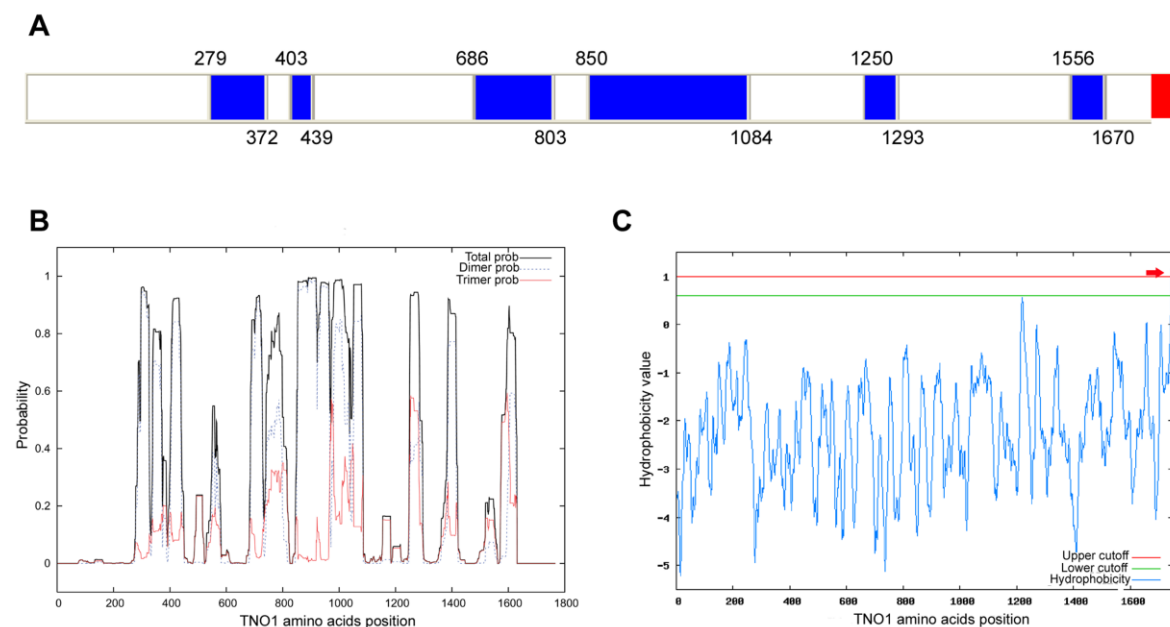
A. *TNO1* cDNA sequence.

Sequence of the open reading frame of *TNO1*. The extra exon encoded by the *TNO1* sequence compared with that in the TAIR database is marked in red.

B. *TNO1* amino acid sequence.

The extra amino acids predicted by our sequence are marked in red, and the predicted transmembrane domain is marked in green. Nine peptides identified by tandem mass spectrometry are marked in blue.

Supplementary Fig 2. TNO1 domain prediction.



A. Position of predicted coiled-coil domains and *trans*-membrane domain.

Positions are relative to the initiation amino acid. Blue boxes represent coiled-coil domains, and the red box represents a transmembrane domain.

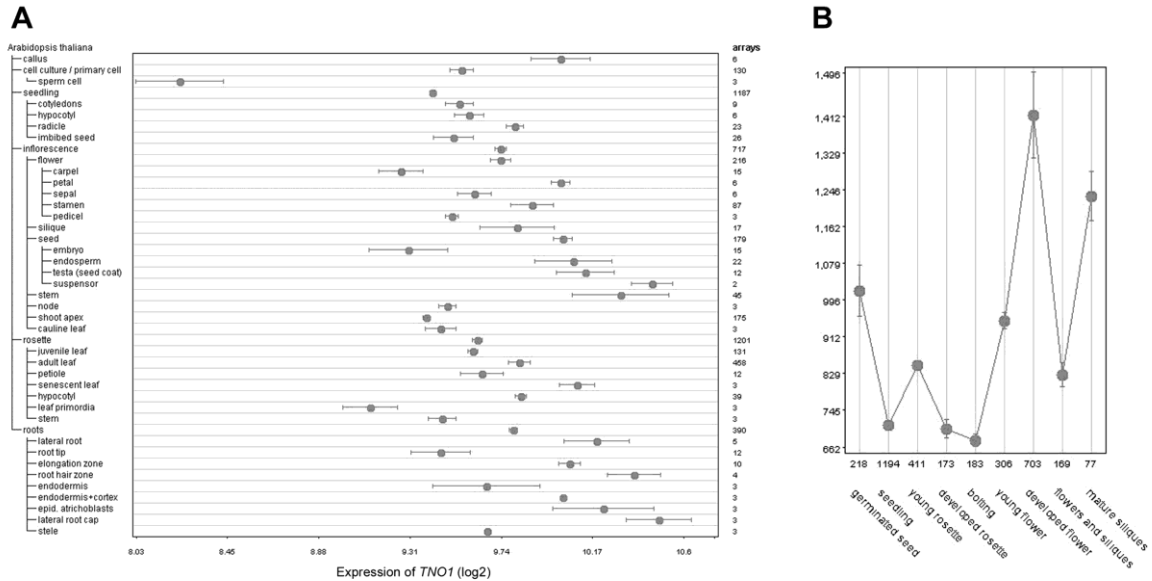
B. Probability of coiled-coil domain.

Probability of coiled-coil domains as determined by Multicoil, a coiled-coil domain prediction algorithm. Total probability, black solid line; dimer probability, black dotted line; trimer probability, red solid line.

C. Hydrophobicity value of TNO1.

Hydrophobicity value of TNO1 is determined by TopPred, a transmembrane topology prediction algorithm; amino acids 1749-1767 are predicted to form a transmembrane domain.

Supplementary Fig 3. Expression pattern of *TNO1* by microarray analysis.



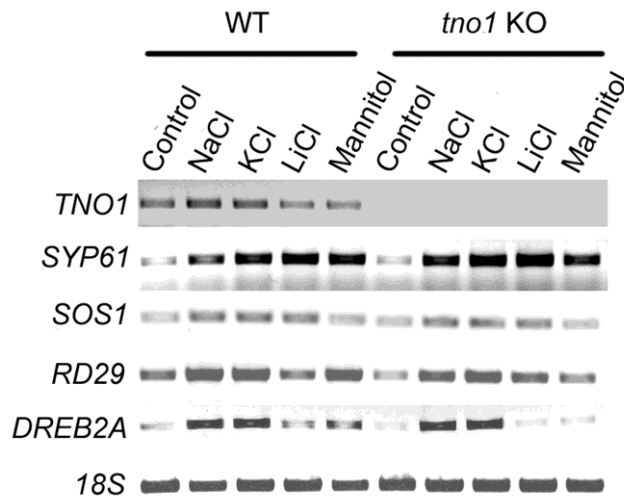
A. Expression of *TNO1* in diverse tissues in Arabidopsis.

Expression of *TNO1* in diverse tissues in Arabidopsis was determined using GENEVESTIGATOR. The number of arrays used to determine the transcript level of *TNO1* is shown at right. The scale is logarithmic. Error bars indicate standard error.

B. Expression of *TNO1* during development

Expression of *TNO1* during development was determined using GENEVESTIGATOR. X-axis represents developmental stage and the number of arrays used to calculate the mean intensities are indicated. Y-axis represents the intensity of *TNO1* transcript. Error bars indicate standard error.

Supplementary Fig 4. Salt/ osmotic stress responsive gene expression in wild-type and *tno1* mutant.



RT-PCR was performed with *TNO1*, *SYP61*, *SOS1*, *DREB2A* and *RD29*-specific primers using RNA extracted from wild-type and *tno1* mutant seedlings after the indicated treatments. Treatments were as follows: control (no treatment), NaCl (300 mM NaCl), KCl (300 mM), LiCl (40 mM LiCl), Mannitol (600 mM).