

TIR-NBS-LRR genes play a role in plant defence against biotic stress in *Solanum lycopersicum*

NAMO DUBEY^{1,2#}, ANJALI CHAUDHARY^{1,2#}, KUNAL SINGH^{1,2*}

¹CSIR-Institute of Himalayan Bioresource Technology, Palampur, India

²Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

[#]Authors contributed equally

*Corresponding author: kunal@ihbt.res.in

ELECTRONIC SUPPLEMENTARY MATERIAL (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

Supplementary Table 1: Primer sequences for the 7 SITNLs and reference gene (Elongation factor 1 α) used in qRT-PCR

Transcript identity	Primer nomenclature	Primer sequence
SITNLC1G1	SL_TNL8800_1F	5'-TCATCACGCCAGACTACGAA-3'
	SL_TNL8800_1R	5'-CACCCAGCCTCAGTAAATGC-3'
SITNLC11G2	SL_TNL11060_1F	5'-CTTCATTTCTTGCGGTTTCG-3'
	SL_TNL11060_1R	5'-CAAGCGTAATCCATCAGACACT-3'
SITNLC11G3	SL_TNL11350_1F	5'-CCCTAATCTCACGACCTCCAT-3'
	SL_TNL11350_1R	5'-CAGGGAACACAAAGGACACTC-3'
SITNLC1G3	SL_TNL66020_1F	5'-AATGGGCGGAGTTGGTAAA-3'
	SL_TNL66020_1R	5'-GCAAGAAAACAAGCAGCATC-3'
SITNLC8G2	SL_TNL81260_1F	5'-TTGGAGAAGGAGTGGAGAGA-3'
	SL_TNL81260_1R	5'-CAGCGTTACAGCCCTCAATA-3'
SITNLC5G3	SL_TNL7850_1F	5'-CCTCTTGCTGGCTTATGGGAT-3'
	SL_TNL7850_1R	5'-CAAAACGAACTCCACACTCCTT-3'
SITNLC8G1	SL_TNL5510_1F	5'-TCGGGAGAACAGAGCAGACT-3'
	SL_TNL5510_1R	5'-AGATTGCTTCCACTGCTTTTCG-3'
Elongation factor 1 α	ef1 α _RT_1F	5'-GATGGTCAGACCCGTGAACA-3'
	ef1 α _RT_1R	5'-CCTTGGAGTACTTCGGGGTG-3'

Supplementary Table 2: Properties of identified SLTNs with their ids

Gene	Alternate Nomenclature	Protein	Protein length	MW (Da)	pI	Gravy index	Localization
Solyc01g008800.2	SLTNLC1G1	Solyc01g008800.2.1	1542	176473.5	5.76	−0.295	Nucleus
Solyc01g014840.3	SLTNLC1G2	Solyc01g014840.3.1	1326	151126.8	7.07	−0.209	Nucleus
Solyc01g066020.2	SLTNLC1G3	Solyc01g066020.2.1	856	98402.33	6.07	−0.202	Nucleus
Solyc01g113620.2	SLTNLC1G4	Solyc01g113620.2.1	1105	126209.7	8.77	−0.182	Cytoplasm
Solyc01g102840.3	SLTNLC1G5	Solyc01g102840.3.1	607	69327.26	6.13	−0.412	Chloroplast
Solyc01g102850.2	SLTNLC1G6	Solyc01g102850.2.1	1462	166296.5	8.63	−0.308	Chloroplast
Solyc01g102880.2	SLTNLC1G7	Solyc01g102880.2.1	2357	267960.8	7.23	−0.236	Nucleus
Solyc02g032200.3	SLTNLC2G1	Solyc02g032200.3.1	1214	139012.9	6.38	−0.211	Vacuole
Solyc02g032650.3	SLTNLC2G2	Solyc02g032650.3.1	1218	139209.2	7.94	−0.236	Nucleus
Solyc02g082050.3	SLTNLC2G3	Solyc02g082050.3.1	1228	139016.2	8.17	−0.239	Nucleus
Solyc04g007320.2	SLTNLC4G1	Solyc04g007320.2.1	1251	142280.7	7.97	−0.154	Chloroplast
Solyc04g056570.3	SLTNLC4G2	Solyc04g056570.3.1	1154	130928.6	7.52	−0.089	Nucleus
Solyc05g006620.3	SLTNLC5G1	Solyc05g006620.3.1	1424	159725.3	6.33	−0.115	Nucleus
Solyc05g006630.3	SLTNLC5G2	Solyc05g006630.3.1	1354	152619.4	6.46	−0.169	Nucleus
Solyc05g007850.2	SLTNLC5G3	Solyc05g007850.2.1	1146	131911.8	5.46	−0.291	Chloroplast
Solyc07g052770.2	SLTNLC7G1	Solyc07g052770.2.1	973	111402.8	5.78	−0.148	Nucleus
Solyc07g052800.2	SLTNLC7G2	Solyc07g052800.2.1	384	44216.08	6.36	−0.143	Cytoplasm
Solyc07g055610.2	SLTNLC7G3	Solyc07g055610.2.1	580	67310.02	9.07	−0.354	Cytoplasm
Solyc08g005510.2	SLTNLC8G1	Solyc08g005510.2.1	1170	134100.7	6.83	−0.221	Chloroplast
Solyc08g081260.1	SLTNLC8G2	Solyc08g081260.1.1	997	113848.1	9.37	−0.345	Plasma membrane
Solyc09g007710.3	SLTNLC9G1	Solyc09g007710.3.1	1072	122278.4	6.32	−0.201	Cytoplasm
Solyc09g092410.3	SLTNLC9G2	Solyc09g092410.3.1	1051	119569.2	6.18	−0.18	Nucleus
Solyc11g011080.2	SLTNLC11G1	Solyc11g011080.2.1	1101	126428.2	8.24	−0.385	Nucleus
Solyc11g011060.2	SLTNLC11G2	Solyc11g011060.2.1	2871	325867.6	5.43	−0.168	Chloroplast
Solyc11g011350.2	SLTNLC11G3	Solyc11g011350.2.1	1171	133392.6	6.59	−0.23	Nucleus
Solyc11g013750.2	SLTNLC11G4	Solyc11g013750.2.1	1118	126697.5	6.53	−0.131	Cytoplasm
Solyc11g062150.2	SLTNLC11G5	Solyc11g062150.2.1	430	50097.2	6.61	−0.075	Cytoplasm

Supplementary Table 3: Duplication pairs of tomato TNL with their counterparts

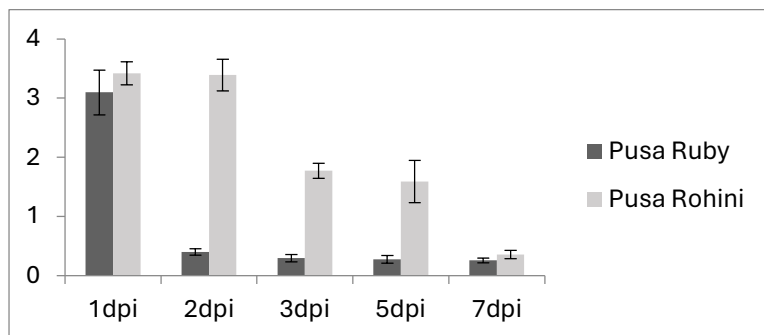
Gene Id (TNL)	Gene Id	Annotatoin	Pvalue
Solyc01g014840	Solyc07g055610	TNL	2,00E-135
Solyc01g113620	Solyc05g006620	TNL	2,00E-87
Solyc02g032650	Solyc02g082050	TNL	3,00E-117
Solyc08g005510	Solyc09g092410	TNL	5,00E-148
Solyc01g008800	Solyc02g072480	Receptor like kinase, RLK	2,00E-11
Solyc01g008800	Solyc03g006100	Receptor like kinase, RLK	9,00E-15
Solyc01g008800	Solyc04g015630	NBS-LRR, resistance protein	4,00E-14
Solyc01g008800	Solyc07g055380	NBS-LRR, resistance protein	9,00E-139
Solyc01g008800	Solyc10g085460	CC-NBS-LRR, resistance protein	1,00E-13
Solyc01g008800	Solyc11g064830	Zinc finger family protein	3,00E-81
Solyc01g008800	Solyc11g069925	NLR-like Disease Resistance Protein	1,00E-17
Solyc01g014840	Solyc07g055380	NBS-LRR, resistance protein	5,00E-136
Solyc05g006620	Solyc02g068820	Receptor like kinase, RLK	2,00E-18
Solyc05g007850	Solyc02g084890	CC-NBS-LRR, resistance protein	4,00E-16
Solyc05g006620	Solyc03g005960	Receptor like kinase, RLK	7,00E-13
Solyc09g092410	Solyc03g006750	CC-NBS-LRR, resistance protein	8,00E-11
Solyc05g006620	Solyc03g115410	LRR domain containing protein	2,00E-12
Solyc05g006620	Solyc07g063360	TIR, resistance protein fragment	1,00E-14
Solyc05g006620	Solyc09g015170	Receptor like kinase, RLK	6,00E-11
Solyc09g007710	Solyc05g053990	CC-NBS-LRR, resistance protein	2,00E-11
Solyc11g011060	Solyc05g053990	CC-NBS-LRR, resistance protein	3,00E-11
Solyc07g052770	Solyc12g096880	TIR, resistance protein fragment	6,00E-60
Solyc09g092410	Solyc07g055380	NBS-LRR, resistance protein	2,00E-119
Solyc07g055610	Solyc12g096880	TIR, resistance protein fragment	2,00E-31
Solyc09g092410	Solyc08g075640	CC-NBS-LRR, resistance protein	2,00E-13
Solyc09g007710	Solyc11g071995	CC-NBS-LRR, resistance protein	1,00E-19

<https://doi.org/10.17221/127/2023-PPS>
Supplementary table 4: Collinear pairs of tomato TNL with their counterparts in *Arabidopsis thaliana*

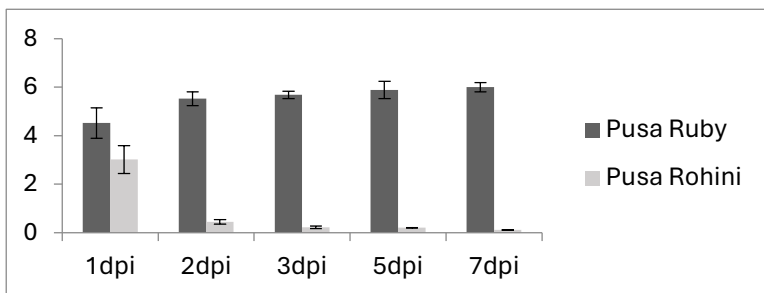
Solanum chromo- some	Protein Id of solanum (domain)	<i>Arabidopsis chro- mosome</i>	Protein Id of <i>Arabidopsis</i> (domain)
Sl2	Solyc02g082050.3.1 (TIR-NBS-LRR-C_JID)	At2	AT2G17050.1 (NBS-LRR-TIR)
Sl5	Solyc05g007850.2.1 (TIR-NBS-LRR)	At1	AT1G69550.1 (TIR-NBS-LRR-C_JID)
Sl5	Solyc05g006620.3.1 (TIR-NBS-LRR)	At1	AT1G69550.1 (TIR-NBS-LRR-C_JID)
Sl5	Solyc05g006620.3.1 (TIR-NBS-LRR)	At1	AT1G27170.2 (TIR-NBS-LRR)
Sl7	Solyc07g055380.1.1 (NBS-LRR-C_JID)	At1	AT1G69550.1 (TIR-NBS-LRR-C_JID)
Sl7	Solyc07g055610.2.1 (TIR-NBS)	At1	AT1G57830.1 (TIR)
Sl12	Solyc12g097000.2.1 (TIR)	At1	AT1G57830.1 (TIR)

Supplementary Table 5. Expression values of SITNL genes at different time points in *Pusa Ruby* and *Pusa Rohini* post *Alternaria solani* inoculation

SITNLC1G1/Solyc01g008800	Pusa Ruby	Pusa Rubey (SE)	Pusa Rohini	Pusa Rohini (SE)
1dpi	3.098359707	0.378151223	3.422170535	0.195502748
2dpi	0.398046014	0.053260502	3.392965986	0.270093505
3dpi	0.293656392	0.06301362	1.774792967	0.127343993
5dpi	0.273310319	0.063385255	1.58919979	0.356637086
7dpi	0.256745745	0.041474635	0.353603353	0.070945264

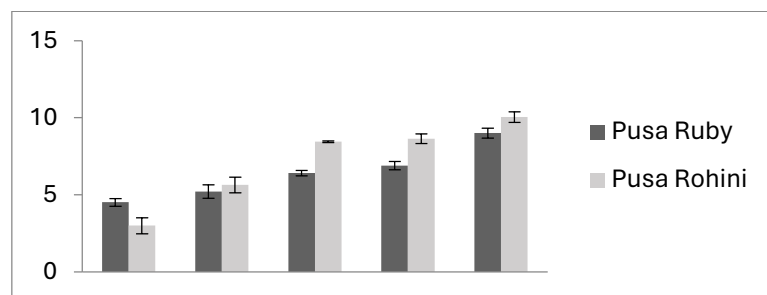


SITNLC1G3/Solyc01g066020	Pusa Ruby	Pusa Rubey (SE)	Pusa Rohini	Pusa Rohini (SE)
1dpi	4.529256523	0.627184609	3.02049385	0.575434058
2dpi	5.530203646	0.285686423	0.44851445	0.090500222
3dpi	5.686846311	0.147946241	0.22445253	0.047678589
5dpi	5.891287743	0.359824263	0.209625247	0.008821113
7dpi	6.007057811	0.194747572	0.112043292	0.005174501

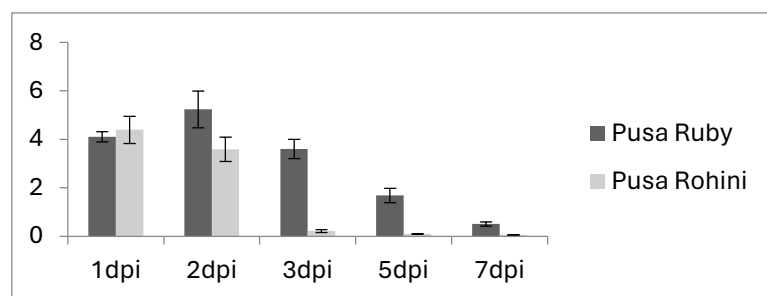


Supplementary Table 5 to be continued...

SITNLC5G3/BS4/Solyc05g007850	Pusa Ruby	Pusa Rubey (SE)	Pusa Rohini	Pusa Rohini (SE)
1dpi	4.514512028	0.249957277	2.990432596	0.519336562
2dpi	5.205985623	0.436354538	5.640063551	0.513089049
3dpi	6.413546367	0.177311538	8.436943957	0.051488994
5dpi	6.895452692	0.275288087	8.644652137	0.303069041
7dpi	9.002413883	0.323656115	10.04507597	0.347083491

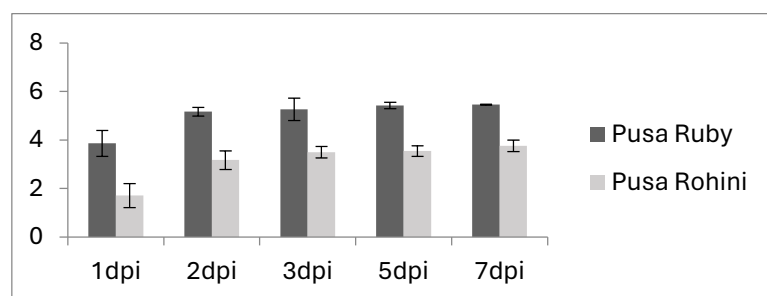


SITNLC8G1/Solyc08g005510	Pusa Ruby	Pusa Rubey (SE)	Pusa Rohini	Pusa Rohini (SE)
1dpi	4.104578608	0.21279481	4.393812728	0.565724635
2dpi	5.236207566	0.757547117	3.59377645	0.501494972
3dpi	3.605558157	0.397706146	0.219613891	0.059684182
5dpi	1.683885479	0.293162586	0.103806315	0.006154414
7dpi	0.508831036	0.085096246	0.056625114	0.012089828

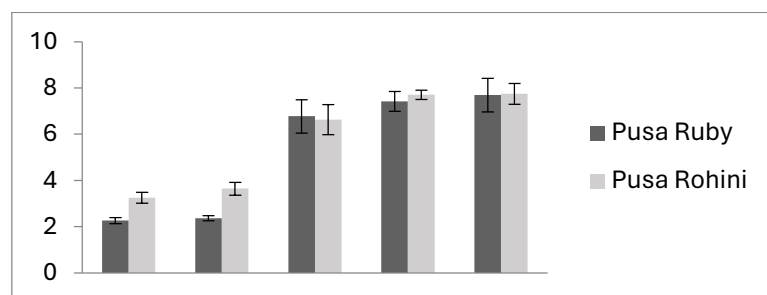


Supplementary Table 5 to be continued...

SITNLC8G2/Solyc08g081260	Pusa Ruby	Pusa Rubey (SE)	Pusa Rohini	Pusa Rohini (SE)
1dpi	3.867610148	0.536016622	1.70677182	0.494672525
2dpi	5.173882554	0.175589022	3.175947956	0.382003083
3dpi	5.271144923	0.460326877	3.498394046	0.236366317
5dpi	5.429658561	0.133691813	3.543653155	0.216930343
7dpi	5.464248536	0.021867174	3.764136843	0.235912321

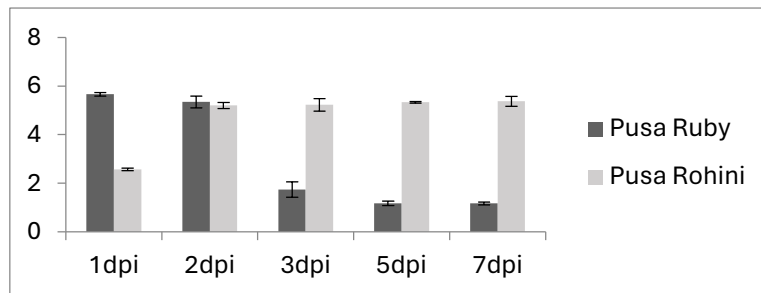


SITNLC11G3/Solyc11	Pusa Ruby	Pusa Rubey (SE)	Pusa Rohini	Pusa Rohini (SE)
1dpi	2.25770071	0.130398194	3.251458629	0.240647674
2dpi	2.361646533	0.111529564	3.642497516	0.281100735
3dpi	6.776374905	0.720897205	6.635425319	0.646617102
5dpi	7.420501619	0.429391261	7.7150844	0.202835996
7dpi	7.695731477	0.731401811	7.753715475	0.452474521



Supplementary Table 5 to be continued...

SITNLC11G2/Solyc11g011060	Pusa Ruby	Pusa Rubey (SE)	Pusa Rohini	Pusa Rohini (SE)
1dpi	5.670881413	0.073364584	2.573050597	0.051913565
2dpi	5.34977033	0.238614589	5.208428092	0.125254069
3dpi	1.740337808	0.31411788	5.230275276	0.255283147
5dpi	1.174949493	0.093148717	5.339558723	0.032586267
7dpi	1.170196891	0.056417761	5.371595931	0.203116693



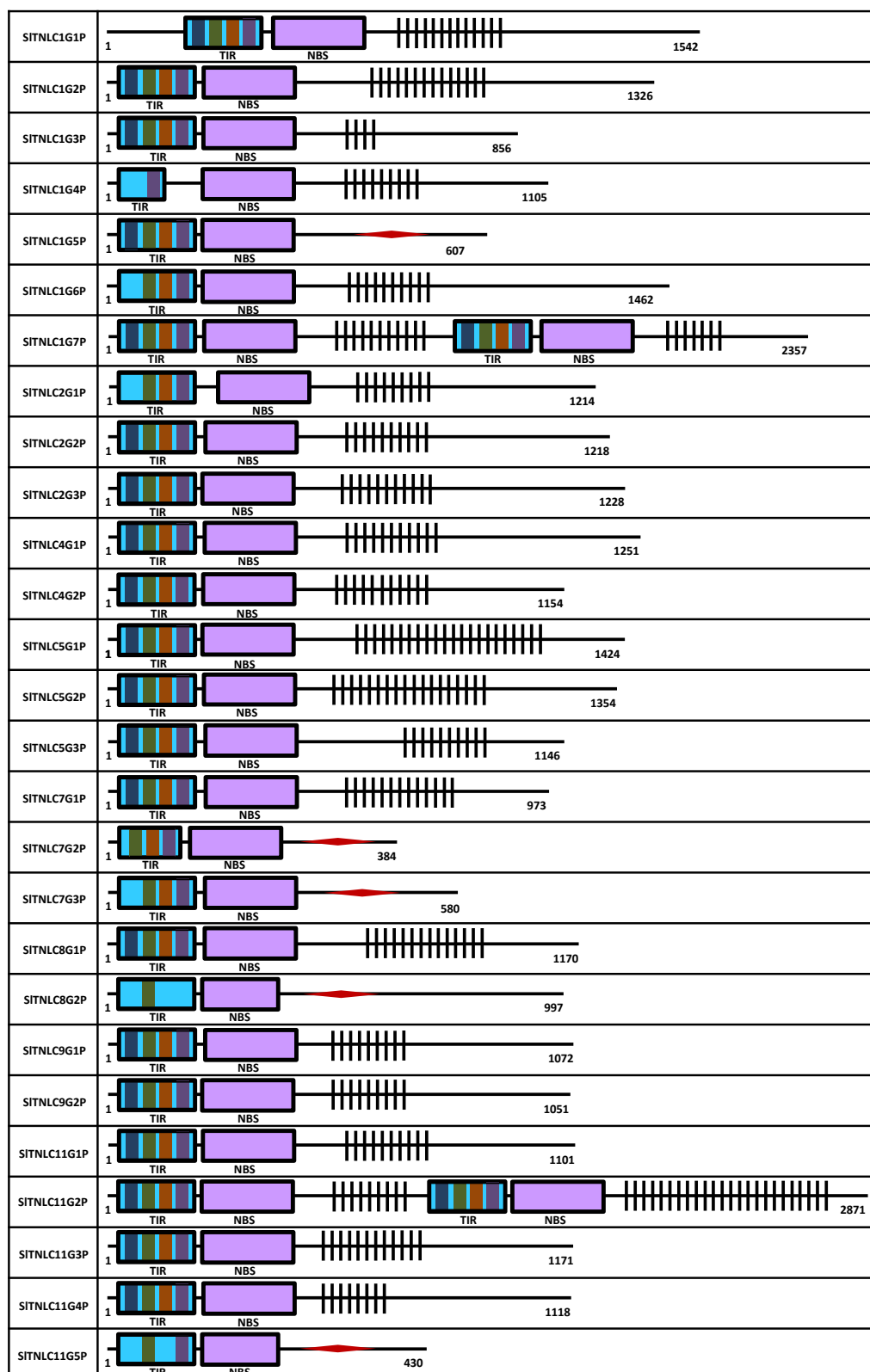


Figure S1. Diagrammatic representation of All three domains (TIR-NBS-LRR) in identified SITNLCs. LRR identified by CDD search are shown by vertical lines while those identified manually are represented by red diamonds.

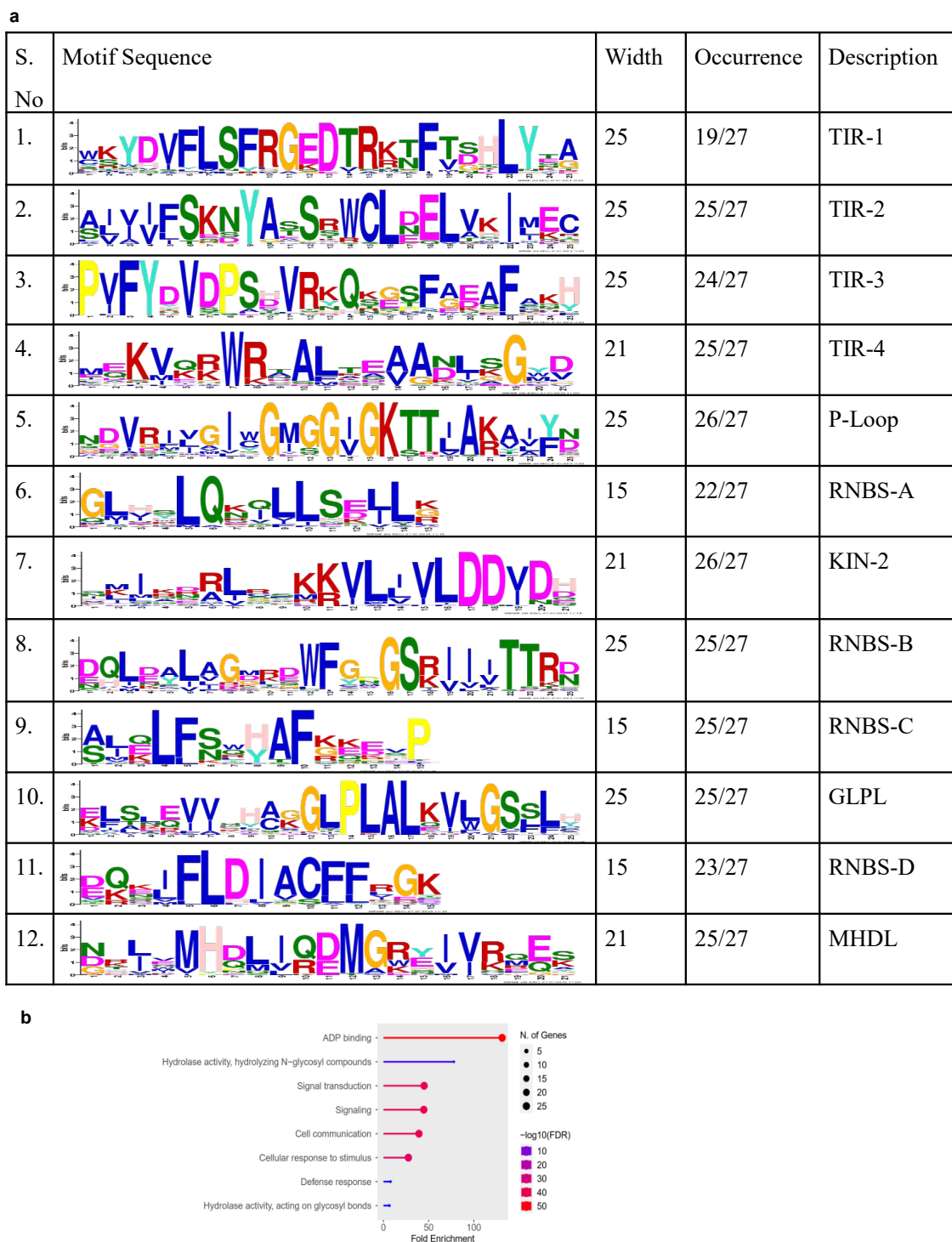


Figure S2. (a) Motif analysis by MEME showed presence of all conserved motifs from NBS and TIR domain. (b) Gene ontology terms related to signal transduction and signalling were enriched

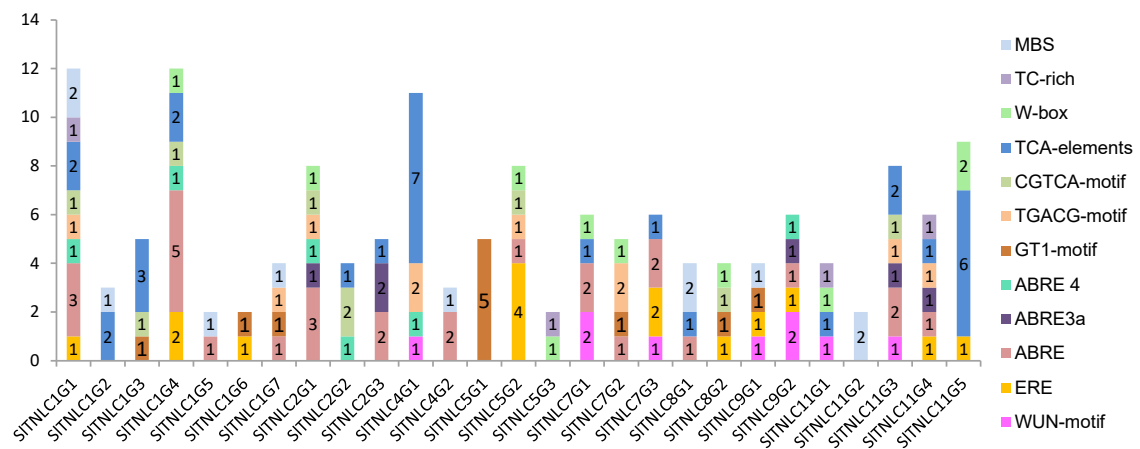


Figure S3. All TNL transcripts are encoded by genes with multiple introns except for STNLC8G2/Solyc08g081260 which is intronless