

## Supplementary information

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# The temperature sensor TWA1 is required for thermotolerance in *Arabidopsis*

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In the format provided by the  
authors and unedited

**Supplementary Table 1. Primers used for genotyping and cloning.**

<b>PRIMER</b>	<b>5'→3' sequence</b>	<b>REACTION</b>
cpl3-10_f	TGGCGCCAATCACACGTCGCGCTGAC	G: <i>cpl3-10</i>
cpl3-10_r	CAACGAAGTACTCTCCAAAACACCAC	
jam1-2_f	ATGAATATGAGTGATTTAGGTTGGG	G: <i>jam1-2</i>
jam1-2_r	TTATATATCACCAGAGACCTGTG	
jam2-2_f	CTTTAGGCTCTCTGCAATATCCATCG	G: <i>jam2-2</i>
jam2-2_r	GAGAAAATCTCGGTGGAAGAGACTTG	
jam3-2_f	ATGGGTCAAAAGTTTTGGGAGAATC	G: <i>jam3-2</i>
jam3-2_r	TTACTGTGATAGAGAGGCAAGGAG	
tpl-8_f	TTGGTTCCTCGCAAAGATTAG	G: <i>tpl-8</i>
tpl-8_r	GCTATGCTGTTTATTGCAGGC	
tpr2-2_f	TCCGTCACTCGTGTAGCATGGAG	G: <i>tpr2-2</i>
tpr2-2_r	GTATCAAACCTGAACAACCTCCAGCTG	
tpr4-2_f	TTGATTGTCGGGATTAGAGTAAAG	G: <i>tpr4-2</i>
tpr4-2_r	CCAACAGAAGCTGTAATCTTAGAGC	
GK-o8409_extended	ATATTGACCATCATACTCATTGCTGATCC	G: GABI-Kat lines
JMLBa1	TGGTTCACGTAGTGGGCCATCG	G: SALK lines
EcoRI-TWA1_f	CCGGAATTCATGTCTGGAAGCCAAGAGCCTAG	C: pGAD424
TWA1-BglII_r	GATCAGATCTTTAAAAACCATCACCACCGAGCTCATCCG	C: pGAD424
SmaI-C-JAM2_f	GATCCCCGGGCATGAATATTGGTCGCCTAGTGTGG	C: pGAD424
JAM2-SalI_r	GATCGTCGACCTATCTACCTGATGATGTTCTTGACTG	C: pGAD424
EcoRI-twa1-1_f	CCGGAATTCATGTCTGGAAGCCAAGAGCCTAG	C: pGAD424
twa1-1-BglII_r	GATCAGATCTTCAAACATCCATGGTAGTATTCAAATCCC	C: pGAD424
SmaI-AI-TWA1_f	TTGTCCCAGGTATGTCTGGAAGCCAAGAGCC	C: pGAD424
AI-TWA1-BglII_r	TTGTAGATCTTTAAAAACCATCACCACCA	C: pGAD424
SmaI-Sa-TWA1_f	TTGTCCCAGGTATGTCTGGAACCAACAGCC	C: pGAD424
SalI-Sa-TWA1_r	TTGTGTCGACCTAAAACCCGTCACCACCACCA	C: pGAD424
SmaI-NotI-TPL-f	GATCCCCGGGGCGGCCGCATGTCTTCTCTTAGTAGAGA	C: pBRIDGE
TPL-NotI-SalI-r	GATCGTCGACGCGGCCGCTCATCTCTGAGGCTGATCAG	C: pBRIDGE
TPR1-BamHI_f	ATTAGGATCCCGATGTCTTCTCTGAGCAGAGAGC	C: pBRIDGE
TPR1-SalI_r	ATTAGTCGACTCATCTCTGAGGCTGGTCAG	C: pBRIDGE
EcoRI-TPR2-fwrd	GATCGAATTCATGTCGTCTTTGAGCAGAGAG	C: pBRIDGE
TPR2-SalI_rev	GATCGTCGACTTACCTTTGAATCTGATCCGAACCTTG	C: pBRIDGE
TPR3-BamHI_f	ATTAGGATCCCGATGTCGTCGTTGAGTCGAGAG	C: pBRIDGE
TPR3-SalI_r	ATTAGTCGACTCATCTTTGTAACGTCTGAGTTTG	C: pBRIDGE
TPR4-BamHI_f	ATTAGGATCCCGATGTCGTCGACTCAGCAGAGAAC	C: pBRIDGE
TPR4-SalI_r	ATTAGTCGACCTACGAATCACTCGGTTGTTGATCTG	C: pBRIDGE
EcoRI-JAM1_f	gatcGAATTCATGAATATGAGTGATTTAGGTTGGGATG	C: pBRIDGE
JAM1-SalI_r	GATCGTCGACTTATATATCACCAGAGACCTGTGA	C: pBRIDGE
BamHI-JAM2_f	GATCGGATCCATGAATATTGGTCGCCTAGTGTGG	C: pBRIDGE
JAM2-SalI_r	GATCGTCGACCTATCTACCTGATGATGTTCTTGACTG	C: pBRIDGE
SmaI-C-JAM3_fwrd	GATCCCCGGGCATGGGTCAAAAGTTTTGGG	C: pBRIDGE
JAM3-BamHI_r	GATCGGATCCTTACTGTGATAGAGAGGCAAGGAGC	C: pBRIDGE

SmaI-C-MYC2_fwd	CCCGGGcATGACTGATTACCGGCTACAACC	C: pBRIDGE
MYC2-PstI_r	GATCCTGCAGTTAACCGATTTTTGAAATCAAACCTTGCTC	C: pBRIDGE
Sal-AIHVR-TWA1_f	TTGTGTCGACATGTCTGGAAGCCAAGAGCCTAGG	C: pGREG574
TWA1-SalI_r	TTGTGTCGACTTAAAAACCATCACCACCGA	C: pGREG574
EcoRI-TWA1_f	CCGGAATTCATGTCTGGAAGCCAAGAGCCTAG	C: pSK_AscI_p35SS1-3xHA-tag-10xAla-nosTER
TWA1-BglII_r	GATCAGATCTTTAAAAACCATCACCACCGAGCTCATCCG	C: pSK_AscI_p35SS1-3xHA-tag-10xAla-nosTER
PstI-ATG-ΔN-TWA1_f	GATCCTGCAGATGGATGAACTTTCAGAAATGATGAAGAG	C: pSK_AscI_p35SS1-nosTER
ΔN-TWA1-BglII_r	GATCAGATCTTTAAAAACCATCACCACCGAGCTCATCCG	C: pSK_AscI_p35SS1-nosTER
EcoRI-twa1-1_f	CCGGAATTCATGTCTGGAAGCCAAGAGCCTAG	C: pSK_AscI_p35SS1-nosTER
twa1-1-SalI_r	GATCGTCGACTCAAACATCCATGGTAGTATTCAAATCCC	C: pSK_AscI_p35SS1-nosTER
EcoRI-JAM1-f	GATCGAATTCATGAATATGAGTGATTTAGGTTGGG	C: pSK_AscI_p35SS1-nosTER
SalI-JAM1-r	gatcGTCGACTTATATATCACCAGAGACCTGTG	C: pSK_AscI_p35SS1-nosTER
SmaI-JAM2-f	GATCCCCGGGATGAATATTGGTCGCCTAGTGTGG	C: pSK_AscI_p35SS1-nosTER
JAM2-SalI_r	GATCGTCGACCTATCTACCTGATGATGTTCTTGACTG	C: pSK_AscI_p35SS1-nosTER
SmaI-C-JAM3_fwd	GATCCCCGGGCATGGGTCAAAGTTTTGGG	C: pSK_AscI_p35SS1-nosTER
JAM3-BamHI_r	GATCGGATCCTTACTGTGATAGAGAGGCAAGGAGC	C: pSK_AscI_p35SS1-nosTER
SmaI-NotI-TPL-f	GATCCCCGGGGCGGCCGCATGTCTTCTTAGTAGAGA	C: pSK_AscI_p35SS1-nosTER
TPL-NotI-SalI-r	GATCGTCGACGCGGCCGCTCATCTCTGAGGCTGATCAG	C: pSK_AscI_p35SS1-nosTER
SmaI-NotI-TPR2-fwd	GATCCCCGGGGCGGCCGCATGTCGCTTTGAGCAGAGAG	C: pSK_AscI_p35SS1-nosTER
TPR2-PstI-SalI-rev	GATCGTCGACCTGCAGTTACCTTTGAATCTGATCCGAACTTG	C: pSK_AscI_p35SS1-nosTER
SalI-TPR4_f	GATCGTCGACATGTCGTCACTCAGCAGAGAAC	C: pSK_AscI_p35SS1-nosTER
TPR4-BglII_r	GATCAGATCTCTACGAATCACTCGGTTGTTGATCTG	C: pSK_AscI_p35SS1-nosTER
SmaI-AI-TWA1_f	TTGTCCCAGGATGTCTGGAAGCCAAGAGCC	C: pSK_AscI_p35SS1-nosTER
AI-TWA1-SacI_r	TTGTGAGCTCTTAAAAACCATCACCACCA	C: pSK_AscI_p35SS1-nosTER
SmaI-Sa-TWA1_f	TTGTCCCAGGATGTCTGGAACCAACAGCC	C: pSK_AscI_p35SS1-nosTER
SalI-Sa-TWA1_r	TTGTGTCGACCTAAAACCCGTCACCACCACCA	C: pSK_AscI_p35SS1-nosTER
pHSFA2-SacII_fwd	GATCCCCGGGCTTTGCCAATTCCTCTGTCCTCG	C: pSK_AscI_pHSFA2-LUC-nosTER
pHSFA2-BamHI_rev	GATCGGATCCTTCCACTTTCAGTTCCTCCATTTTCGTTG	C: pSK_AscI_pHSFA2-LUC-nosTER
BglII-3UTR-TWA1_f	GATCAGATCTAATAAAGGTTAGTATTCAATTTGCTTTAACCTTC	C: TWA1-3'UTR
3'UTR-TWA1-BglII_r	GATCAGATCTGAAAAGAAAAGACTATAAATGTGTGTAG	
NotI-pTWA1_f	GATCGCGGCCGCCTTTGTTCTTTCACTTCAACTCTTTGG	C: TWA1 promoter + TWA1-5'UTR
5'UTR-TWA1-SmaI_r	GATCCCCGGGAGTTGAACTTCGAGGGTCTAAT	

**Supplementary Table 2. Primers used for qPCR expression analysis.**

<b>PRIMER</b>	<b>5'→3' sequence</b>	<b>REACTION</b>	<b>REFERENCE</b>
TIP41L_f	GTGAAAACCTGTTGGAGAGAAGCAA	qPCR reference gene (At4g34270)	
TIP41L_r	TCAACTGGATACCCTTTTCGCA		
UBC9_f	TCACAATTTCCAAGGTGCTGC	qPCR reference gene (AT4G27960)	
UBC9_r	TCATCTGGGTTTGGATCCGT		
UBI10_f	GGCCTTGTATAATCCCTGATGAAT AAG	qPCR reference gene (At4g05320)	
UBI10_r	AAAGAGATAACAGGAACGGAAAC ATAGT		
HSFA2_f	GTGTTGAGGTTGGGCAATACG	HSFA2 (AT2G26150)	Ayako et al, 2011 Plant and Cell Physiology, <a href="https://doi.org/10.1093/pcp/pcr045">https://doi.org/10.1093/pcp/pcr045</a>
HSFA2_r	TTGCTGTTGCCTCAACCTAACTAC		
HSP17.6_f	GCACGTGTTCAAGGCGGATTAC	HSP17.6 (AT1G53540)	Volkov et al, 2003 Journal of Experimental Botany, <a href="https://doi.org/10.1093/jxb/erg244">https://doi.org/10.1093/jxb/erg244</a>
HSP17.6_r	TCACACATTTTGGTCTTAAACAAT TCC		
HSP18.2_f	CCGTTCTCGCAAGACTTATGG	HSP18.2 (AT5G59720)	
HSP18.2_r	GAAGCGTTTGCCAACGCAGAA		
HSP70	CATGACCGCGTCGAAATCATCG	HSP70 (AT3G12580)	Zhang et al 2019, Plant Signaling & Behavior, <a href="https://doi.org/10.1080/15592324.2019.1670596">https://doi.org/10.1080/15592324.2019.1670596</a>
HSP70	GCCAGTGACTCTTATCCGCTTGAA C		
HSP21_f	CGCTTAACCATGGACGTCTCTC	HSP21 (AT4G27670)	Kim et al 2012, G3 Genes/Genomes/Genetics, <a href="https://doi.org/10.1534/g3.112.003368">https://doi.org/10.1534/g3.112.003368</a>
HSP21_r	CTGACACTCCACTTCCTCCTC		
HSP26.5_f	TGTGAAAGAGGTTTGGTCGG3	HSP26.5 (AT1G52560)	designed with Primer3Plus (bioinformatics.nl)
HSP26.5_r	TGTTACGCCAGAGGCTTTT		
TWA1_qP	CTCCACTCAGATTCCACAACC	TWA1 (Atg513590)	
TWA1_qP	TCCAATGCTCTTCCCTTAC		
cis7_f	GACGTGGCAGGACGAAACGG	qPCR pRD29B promoter fragments, ChIP samples	
cis7_r	CTCTCTGGCTTCTGTCTCTTTAC		
cis6_f	CATCGATAGGCTTCTCTAAAGATC		
cis6_r	CAGCCAGTTGCTCTCTACGTG		
cis5_f	GCCAAATAACTACATGATGGGCC		
cis5_r	GATCTTTAGAGAAGCCTATCGATG		
cis4_f	GCTGAATTGATTTTTTCTTTTGCCG		
cis4_r	GCCCATCATGTAGTTATTTGGC		
cis3_f	GAGTACGTTATGCCGTTTTAAATG		
cis3_r	CGGCAAAAGAAAAAATCAATTCAG C		