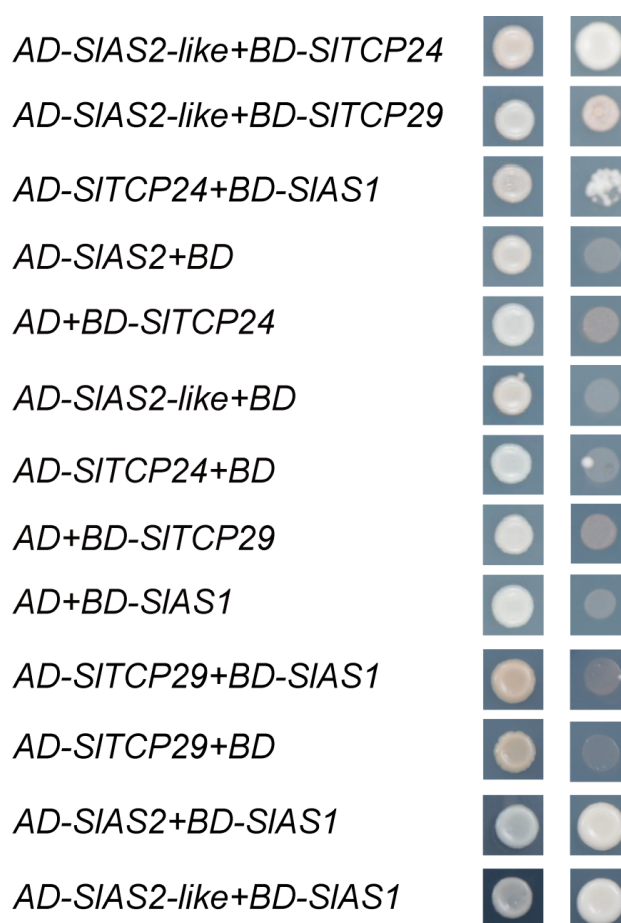
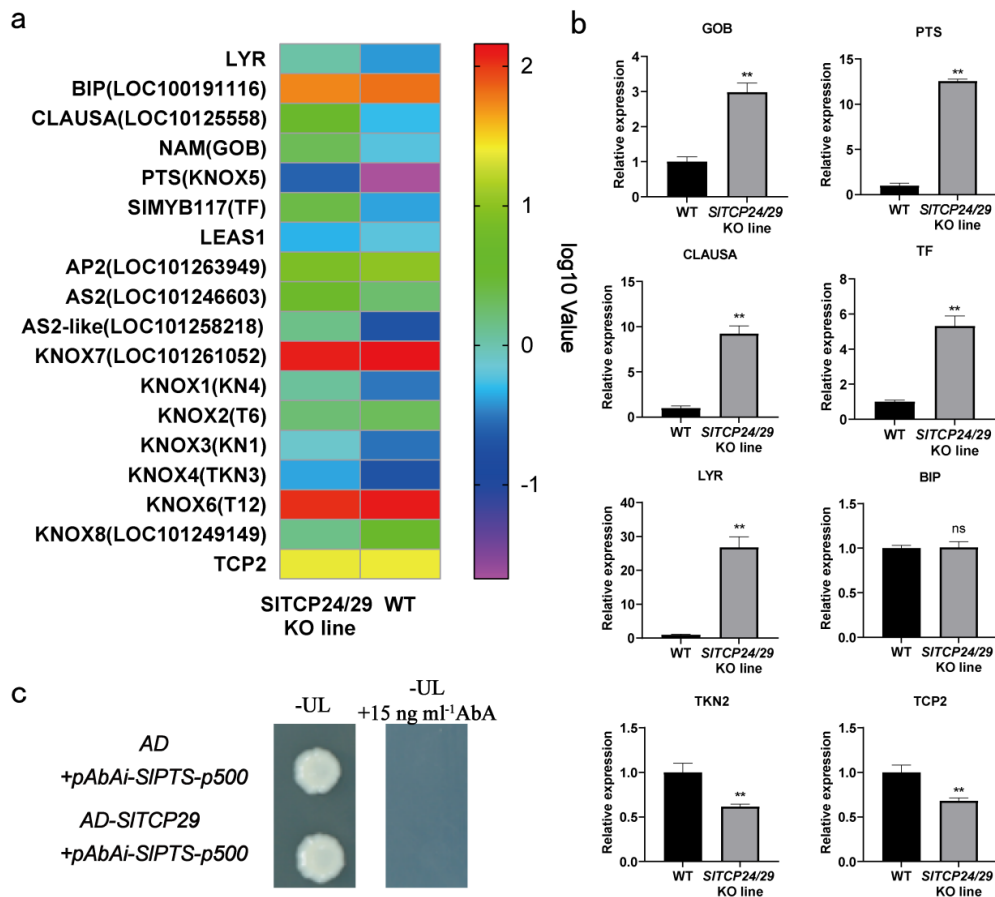


**Article title: *SITCP24* and *SITCP29* synergistically regulate compound leaf development through interacting with *SIAS2* and directly activating transcription of *SICKX2* in tomato**

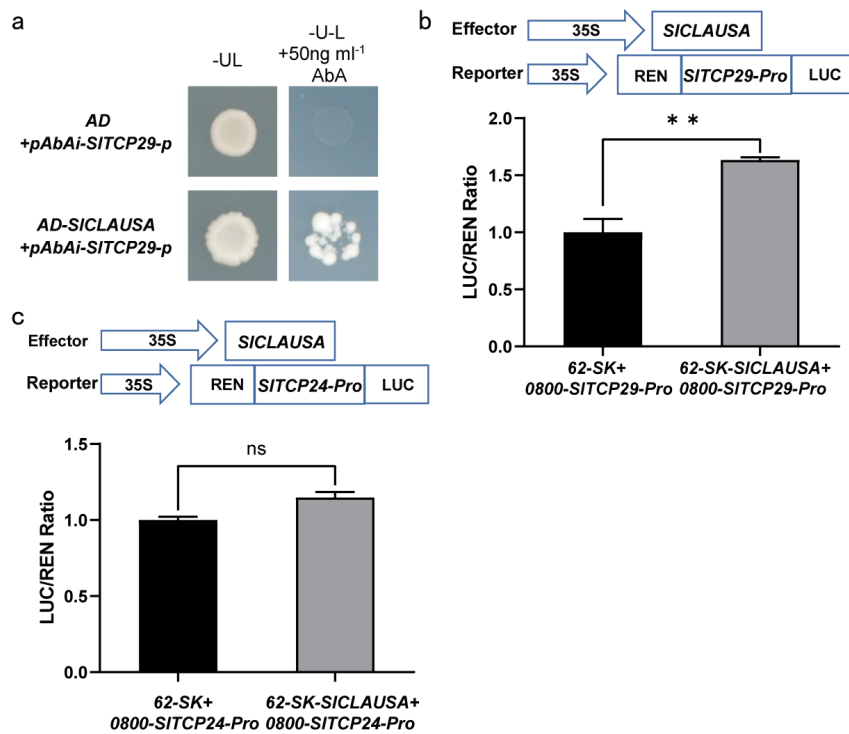
Authors: Guoyu Hu, Danqiu Zhang, Dan Luo, Wenhui Sun, Rijin Zhou, Zonglie Hong, Zhibiao Ye, Changxian Yang, Junhong Zhang\*, Taotao Wang\*



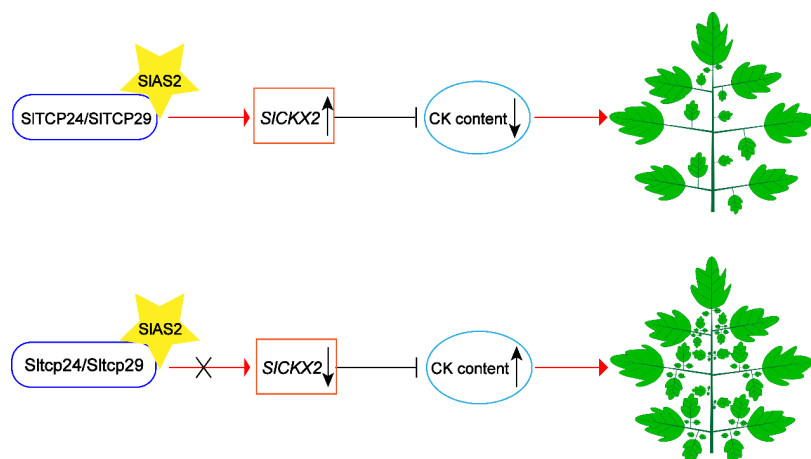
**Supplemental Figure 1.** Interactions of *SIAS1*, *SIAS2*, *SIAS2-like*, *SITCP29*, and *SITCP24* in yeast two-hybrid system.



**Supplemental Figure 2.** Expression levels of genes related to compound leaf development in *SITCP24/29-KO* line. **a** Heatmap of expression levels of compound leaf development-related genes in *SITCP24/29-KO* line identified from transcriptome analysis. **b** Validation of the transcriptome data using qRT-PCR. The bars show the mean  $\pm$  SE ( $n = 3$ ). A One-way ANOVA and Dunnett's test were conducted. '\*' and '\*\*' indicate statistically significant differences with  $P < 0.05$  and  $P < 0.01$ , and 'ns' means no significant difference. **c** Yeast one-hybrid assay showing lack of binding of *SITCP29* to the *SIPTS* promoter. Yeast strain Y1H Gold was transformed with the bait vector *pAbAi-SIPTs-p500* and the prey vector *AD-SITCP29* and plated on SD/-Leu-Ura medium with or without aureobasidin A ( $15 \text{ ng mL}^{-1}$ ).



**Supplemental Figure 3.** Activation of *SITCP29* expression by SICLAUSA. **a** Yeast one-hybrid assay showing the binding of SICLAUSA to the *SITCP29* promoter. Yeast strain Y1H Gold was transformed with the bait vector *pAbAi-SITCP29* and the prey vector *AD-SICLAUSA*. Yeast cells were plated on SD/-Ura-Leu medium (-UL) in the absence or presence of aureobasidin A (AbA, 50 ng mL<sup>-1</sup>). **b** Dual-luciferase reporter assays showing the activation of the *SITCP29* promoter by SICLAUSA. Values are means  $\pm$  SD (n=6). \*\* indicates statistically significant differences at  $P < 0.01$ . A One-way ANOVA and Dunnett's test were conducted. **c** Dual-luciferase reporter assays showing the lack of activation of the *SITCP24* promoter by SICLAUSA. Values are means  $\pm$  SD (n=6), 'ns' means no significant difference.



**Supplemental Figure 4.** Model of proposed *SITCP24* and *SITCP29* regulation of compound leaf development in tomato. In the wildtype tomato plants, *SITCP24* and *SITCP29* form homo- and hetero-dimers that further interact SIAS2. Formation of the protein complex promotes the expression *SICKX2*, encoding cytokinin oxidase that degrades cytokinin. As a result, the reduced

level of cytokinin leads to development of normal compound leaves with simple leaflets. In the *SITCP24/29-KO* double-gene knockout plants, the lack of functional *SITCP24* and *SITCP29* fails to activate *SICKX2*, leading to reduced cytokinin oxidase activity and accumulation of cytokinin in developing leaves. As a result, the elevated level of cytokinin leads to development of more complex compound leaves in the knockout mutant plants.

**Supplemental Table 1. Primers used in experiments of this work**

Primers	Sequences (5'-3')	Experiments	
35S-AS2-FW	CATTGGAGAGGACAGCTCGAGATGGCTTCATCTTCTCATTATCA	Over-expression of AS2	
35S-AS2-RV	TCTCATAAAGCAGGACTCTAGACTATCCTGTATCAAAAATTGTTACTTCC		
AD-AS2-like-FW	ACGTACCAGATTACGCTCATATGATGTCATCTTCATCATCATTATCG	Yeast two hybrid	
AD-AS2-like-RV	TACGATTCATCTGCAGCTCGAGCCTAAGATGGATCAACCGGTGT		
AD-AS2-FW	ACGTACCAGATTACGCTCATATGATGGCTTCATCTTCTCATTATCA		
AD-AS2-RV	TACGATTCATCTGCAGCTCGAGCCTATCCTGTATCAAAAATTGTTACTTCC		
AD-TCP24-FW	ACGTACCAGATTACGCTCATATGATGGAGGAGATTGGAAGTGA		
AD-TCP24-RV	TACGATTCATCTGCAGCTCGAGCTCATAAAAAAACAATAACACA		
BD-AS1-FW	TCTCAGAGGAGGACCTGCATATGATGAGGGAGAGGCAACCGGTG		
BD-AS1-RV	TTATGCGGCCGCTGCAGGTGACGCTTAGCGGCCCATAGG		
BD-TCP24-FW	TCTCAGAGGAGGACCTGCATATGATGGAGGAGATTGGAAGTGA		
BD-TCP24-RV	TTATGCGGCCGCTGCAGGTGACGCTCATAAAAAAACAATAACACA		
BD-TCP29-FW	TCTCAGAGGAGGACCTGCATATGATGAGTAAACAAGGAGGATGA		
BD-TCP29-RV	TTATGCGGCCGCTGCAGGTGACGCTAGTATAATATGATAAGCCTGT		
AD-TCP29-FW	ACGTACCAGATTACGCTCATATGATGAGTAAACAAGGAGGATGA	Yeast two hybrid and Yeast one hybrid	
AD-TCP29-RV	TACGATTCATCTGCAGCTCGAGCTAGTATAATATGATAAGCCTGT		
AD-CLAU-FW	ACGTACCAGATTACGCTCATATGATGGAAATTGTGGAAG	Yeast one hybrid	
AD-CLAU-RV	TACGATTCATCTGCAGCTCGAGCTCATAATGCAATAGATAGA		
pAbai-CKX2-FW	AGCTTGAATTCGAGCTCGGTACCGTTGTTTGGAGACACATTAGATG		
pAbai-CKX2-RV	ACATACAGAGCACATGCCTCGAGAAAGAAACCGTATGAAGACTGAC		
pAbai-PTS-500-FW	AGCTTGAATTCGAGCTCGGTACCAAAAAAAGTAAGTAGGAGTA		
pAbai-PTS-500-RV	ACATACAGAGCACATGCCTCGAGGTCTTATTATGTAGAAGAAG		
pAbai-TCP29-Pro2000-FW	AGCTTGAATTCGAGCTCGGTACCATGAGACCAAGGAAATACCG		
pAbai-TCP29-Pro2000-RV	ACATACAGAGCACATGCCTCGAGTCTTGGAAATTGCACTCCTTCTC		
pAbai-TCP29-Pro600-FW	AGCTTGAATTCGAGCTCGGTACCTGATTATGGCTTTGGA		
pAbai-TCP29-Pro600-RV	ACATACAGAGCACATGCCTCGAGATATGGAGGTGAGTGGG		
pGreen-0800-CKX2-FW	CACTATAGGGCGAATTGGGTACCGTTGTTTGGAGACACATTAGATG		Dual-luciferase reporter assays
pGreen-0800-CKX2-RV	TATGTTTTGGCGTCTCCATGGAAAGAAACCGTATGAAGACTGAC		
pGreen-0800-TCP24-FW	CACTATAGGGCGAATTGGGTACCTGTACTTACACGATAAGCCTGGTG		
pGreen-0800-TCP24-RV	TATGTTTTGGCGTCTCCATGGGAGGATAGATAGAGTGGAGAGCC		
pGreen-0800-TCP29-600-FW	CACTATAGGGCGAATTGGGTACCTGATTATGGCTTTGGA		
pGreen-0800-TCP29-600-RV	TATGTTTTGGCGTCTCCATGGATATGGAGGTGAGTGGG		
pGreen62-SK-AS2-FW	GCCGCTCTAGAAGTGGATCCATGGCTTCATCTTCTCATTATCA		
pGreen62-SK-AS2-RV	TTGGTACCGGGCCCCCTCGAGCTATCCTGTATCAAAAATTGTTACTTCC		
pGreen62-SK-CLAUSA-FW	GCCGCTCTAGAAGTGGATCCATGGGAAATTGTGGAAG		

pGreen62-SK-CLAUSA-RV	TTGGTACCGGGCCCCCTCGAGTCATAATGCAATAGATAGA	
pGreen62-SK-TCP24-FW	GCCGCTCTAGAAGTACTAGTGGATCCATGGAGGAGATTGCAACTGA	
pGreen62-SK-TCP24-RV	TTGGTACCGGGCCCCCTCGAGTCATAAAAAAACAATAACACA	
pGreen62-SK-TCP29-FW	GCCGCTCTAGAAGTACTAGTGGATCCATGAGTAACAAGGAGGATGA	
pGreen62-SK-TCP29-RV	GCCGCTCTAGAAGTACTAGTGGATCCATGAGTAACAAGGAGGATGA	
pGreen62-SK-TCP29-RV	TTGGTACCGGGCCCCCTCGAGTTACTTCTTTCTTTCTTTCTGATC	
pGreen62-SK-TCP29-RV	TTGGTACCGGGCCCCCTCGAGCTTCTTTCTTTCTTTCTGATCTG	
pHBT-AS2-ha-FW	CTCCCCTTGCTCCGTGGATCCATGGCTTACTTCTTATTATCA	Co-IP
pHBT-AS2-ha-RV	AACGTCGTATGGTAAGGCCTTCTGTATCAAAATTGTTACTTCC	
pHBT-TCP24-gfp-FW	CTCCCCTTGCTCCGTGGATCCATGGAGGAGATTGCAACTGA	
pHBT-TCP24-gfp-RV	CATCGTATGGGTACATAGGCCTTAAAAAACAATAACACA	
pHBT-TCP29-flag-FW	CTCCCCTTGCTCCGTGGATCCATGAGTAACAAGGAGGATGA	
pHBT-TCP29-flag-RV	TCCTTGTAGTCAGAAGGCCTGTATAATATGATAAGCCTGT	
pHBT-TCP29-ha-FW	CTCCCCTTGCTCCGTGGATCCATGAGTAACAAGGAGGATGA	
pHBT-TCP29-ha-RV	AACGTCGTATGGTAAGGCCTGTATAATATGATAAGCCTGT	
CR-AS2-FW	GAATCTAACAGTGTAGTTTGCGCAATTTCTGAGGCTGATCGTTTTAGAGCTAGAAATAG	CRISPR/Cas9-mediated gene knockout
CR-AS2-RV	GCTATTCTAGCTCTAAAAGTACTGATCTCGAAGGCGCAAACACTACTGTTAGATT	
CR-TCP24-NEW-FW	GAATCTAACAGTGTAGTTTGATTGTAAAGTTTATGGTGTTTAGAGCTAGAAATAGC	
CR-TCP24-NEW-RV	GCTATTCTAGCTCTAAAAGTACTGATCTCGAAGGCGCAAACACTACTGTTAGATT	
CR-TCP29/TCP24-FW	GAATCTAACAGTGTAGTTTGATTGTAAAGTTTATGGTGTTTAGAGCTAGAAATAGC	
CR-TCP29/TCP24-RV	GCTATTCTAGCTCTAAAAGTACTGATCTCGAAGGCGCAAACACTACTGTTAGATT	
CR-TCP29-NEW-FW	GAATCTAACAGTGTAGTTTGAAAAGAGTGGTGACCTAGGGTTTTAGAGCTAGAAATAGC	
CR-TCP29-NEW-RV	GCTATTCTAGCTCTAAAAGTACTGATCTCGAAGGCGCAAACACTACTGTTAGATT	
TCP24-CR-NEW-YJ-FW	CAAGCCCAAGAAGGTACCCC	Detection of gene editing
TCP24-CR-NEW-YJ-RV	CCACCCGCAATAGCTCAGTGA	
TCP24-CR-YJ-FW	CAACAAGGAAGATGAGCAG	
TCP24-CR-YJ-RV	GGAAAGTGACAATCCAGAA	
TCP29-CR-NEW-YJ-FW	TGTAACAGGGGTATGGTGCT	
TCP29-CR-NEW-YJ-RV	TCTCAGAATTGCTACAACCTGG	
TCP29-CR-YJ-FW	TCCAAGAATGAGTAACAAGG	
TCP29-CR-YJ-RV	AAAGTGACAATCCAGAACC	
AS2-CR-YJ-FW	TGGCTTCATCTTCTTATTATCATC	
AS2-CR-YJ-RV	GAGTGGTGCCTCCTCATT	
TCP29-LUC-C-FW	TACGCGTCCCGGGCGGTACCATGAGTAACAAGGAGGATGA	Luciferase complementation Imaging Analysis
TCP29-LUC-C-RV	ACGAAAGCTCTGCAGGTCGACCTAGTATAATATGATAAGCCTGT	
TCP29-LUC-N-FW	CGAGCTCGGTACCCGGGATCCATGAGTAACAAGGAGGATGAGCAG	
TCP29-LUC-N-RV	CGCGTACGAGATCTGGTCGACCTTCTTTCTTTCTTTCTGATCTG	
AS2-LUC-N-FW	CGAGCTCGGTACCCGGGATCCATGGCTTACTTCTTATTATCA	
AS2-LUC-N-RV	CGCGTACGAGATCTGGTCGACTCCTGTATCAAAATTGTTACTTCC	
LUC-C-TCP24-FW	TACGCGTCCCGGGCGGTACCATGGAGGAGATTGCAACTGA	
LUC-C-TCP24-RV	ACGAAAGCTCTGCAGGTCGACTCATAAAAAAACAATAACACA	
TCP29-mybsite-mt-FAM-FW	AAACTCTCAGATCTGGACAAAGTGG	EMSA
TCP29-mybsite-mt-RV	CCACTTTGTCCAGATCTGAGAGTTT	
TCP29-mybsite-wt-FAM-FW	AAACTCTCAGATCCAACAGAAGTGG	

TCP29-mybsite-wt-FW	AAACTCTCAGATCCAACAGAAGTGG	
TCP29-mybsite-wt-RV	CCACTTCTGTGGATCTGAGAGTTT	
pET15d-CLAU-MBP-FW	TCTGTTCCAGGGCCGCATATGATGGGAAATTGTGGAAG	
pET15d-CLAU-MBP-RV	TGTTAGCAGCCGGATCCTCGAGTCATAATGCAATAGATAGA	
pET15d-TCP24-MBP-FW	TCTGTTCCAGGGCCGCATATGATGGAGGAGATTGGAAGTGA	
pET15d-TCP24-MBP-RV	TGTTAGCAGCCGGATCCTCGAGTCATAAAAAAACAATTAACACA	
pET15d-TCP29-MBP-FW	TCTGTTCCAGGGCCGCATATGATGAGTAACAAGGAGGATGA	
pET15d-TCP29-MBP-RV	TGTTAGCAGCCGGATCCTCGAGTACTCTTTTCTTTTCTTTCTG	
Actin-q-FW	GTCCCTTCCAGCCATCCAT	qRT-PCR
Actin-q-RV	ACCACTGAGCACAATGTTACCG	
CKX2-q-NEW-FW	TTAGCACCTAAATCCTGGACTG	
CKX2-q-NEW-RV	CATGAAAGAGGTCAGCATTCTG	
potato leaf-q-FW	TCATATGTAGAGCCATTCCAC	
potato leaf-q-RV	CACATCATTTCTCATTGCCAT	
mouse ear-q-FW	TCGTCTTTGACTGCTTATCTC	
mouse ear-q-RV	CACCACTACTACTACTGCTACG	
LYR-q-FW	ACTCAAACCTCAACTATAGCCC	
LYR-q-RV	TTCTGGACTCCCTACATGACTA	
GOB-q-FW	CTACGTGATCGGAAGTATCCAA	
GOB-q-RV	TTCATACCAACAAGTGCACATG	
PTS-q-FW	TGGTCTTTGGTTCAAACCTCAC	
PTS-q-RV	TGATGATCCATTGTGTGTGAGA	
BIP-q-FW	ATTGCAGCATAGAGTAGAGGAC	
BIP-q-RV	CAGCTATCGTTTTCTCTTCGTG	
TF-q-FW	CTTGTTGCTATTTATGGCCCTC	
TF-q-RV	GTTCTTCACTGCATTGTCTGTT	
CLAU-q-FW	AGAATAGAGAGGTGCAGCATAAC	
CLAU-q-RV	ACGTTTCATCATCATCTTCTCGA	
9930-q-FW	TTCGAAGACCTTTTGAGACCTT	
9930-q-RV	ATCTGCTAAAAGTCGATCCTGT	
53400-q-FW	GAACTGTTGAAAATAGGCCTGG	
53400-q-RV	TCACGACATTATCAATGGTGGA	
8330-q-FW	CGAGTCATCGTTGTTTCATGATC	
8330-q-RV	AGCTTCATCTGTTGTAACCTCT	
14990-q-FW	TGACTGGTTTCTTGGTGACGG	
14990-q-RV	TCCCCCTTCTCTGTGGACTG	
8310-q-FW	TCCTCAATATCAAGACGAGTCG	
8310-q-RV	AAGTTGAACAGACATTCCAACG	
16220-q-FW	AAAGGGGGTTCTTCTCGAATAG	
16220-q-RV	GCCATAAACGGTGACATATGG	
IPT3-q-FW	TGCTACTATGACGCACTAGAAG	
IPT3-q-RV	GAAAGATGTTGGGTGTACGAAC	
79930-q-FW	AACGTTGAGTGTACAGCTTTC	
79930-q-RV	ATAGCCATTGTTTATTACGCG	

16210-q-FW	AACAATGGTTTCTTGGTTACGG	
16210-q-RV	TCACAACGTTCTCAATAGTGGA	
CKX5-q-FW	CATTCTATCTTCTGCCATGCC	
CKX5-q-RV	CTAGCAAATGTTCCCATCGAG	
53120-q-FW	CCTTGGATTAGCCACTATTC	
53120-q-RV	CGAAATCACAGGGCTAAAGATG	
TKN2-q-FW	TCGTCTTGACTGCTTATCTC	
TKN2-q-RV	CACCACTACTACTGCTACG	
TCP2-q-FW	GCTGTGGATTGGCTTATCAAAA	
TCP2-q-RV	GCAGATGCAGTACCAATTGTAG	