

# Supplementary Materials: Silencing of Two Insulin Receptor Genes Disrupts Nymph-Adult Transition of Alate Brown Citrus Aphid

Bi-Yue Ding, Feng Shang, Qiang Zhang, Ying Xiong, Qun Yang, Jin-Zhi Niu, Guy Smagghe and Jin-Jun Wang

**Figure S1.** Nucleotide and deduced amino acid sequences of *AcInR1* from *Aphis (Toxoptera) citricida*. The start codon (ATG), stop codon (TGA) are highlighted in black. The furin-like cysteine-rich (Fu) region is underlined with red wavy lines. Three fibronectin type 3 (FN3) domains are labeled with yellow solid underlines. A single transmembrane (TM) region is highlighted with a box. An “NPXY” motif is shown in green. The highly conserved tyrosine kinase domain (TyrKc) is indicated in blue. A triple tyrosine cluster (YXXXYY) is indicated in red.

**Figure S2.** Nucleotide and deduced amino acid sequences of *AcInR2* from *Aphis (Toxoptera) citricida*. The start codon (ATG), stop codon (TGA) are highlighted in black. The furin-like cysteine-rich (Fu) region is underlined with red wavy lines. Three fibronectin type 3 (FN3) domains are labeled with yellow solid underlines. A single transmembrane (TM) region is highlighted with a box. An “NPXY” motif is shown in green. The highly conserved tyrosine kinase domain (TyrKc) is indicated in blue. A triple tyrosine cluster (YXXXYY) is indicated in red.

**Table S1.** Primer sequences used for cloning, quantitative real-time PCR and double-stranded RNA (dsRNA) synthesis.

Experiments	Primer Names and Sequence (5' to 3')	Amplification Efficiency	Product Length
Full-length confirmation	<i>InR1-F</i> : ATGTGTGGAGAGTGTGACAA	-	4473
	<i>InR1-R</i> : TCAGCATATACTGGTATTAT		
	<i>InR2-F</i> : ATGCCGTCCATGAACGGGTG	-	3963
	<i>InR2-R</i> : TTACATTGGCGATAAATGCC		
qPCR analysis	Q- <i>InR1-F</i> : ATGGTCGAGATGCTTGGA	98.2%	217
	Q- <i>InR1-R</i> : TGTGGAGGACTTGGAGTTCC		
	Q- <i>InR2-F</i> : TTCCCGAACCTGATGCTGAT	96.3%	204
	Q- <i>InR2-R</i> : GCTCACTCCATTGGCGATT		
dsRNA synthesis	Q- <i>EF1α-F</i> : GATGCACCTGGTCACAGAGA	96.5%	194
	Q- <i>EF1α-R</i> : CCATCTGTTCACACCAAACG		
	ds <i>AcInR1-F</i> : taatcgactcaatagggTTGTGGGATTGGAGTACTC	-	511
	ds <i>AcInR1-R</i> : taatcgactcaatagggCACTTGTCACCATTAGA		
dsRNA synthesis	ds <i>AcInR2-F</i> : taatcgactcaatagggAACCTAAATTGTGTCACG	-	609
	ds <i>AcInR2-R</i> : taatcgactcaatagggGACTTCGATCTCCAGAGAC		
	ds <i>GFP-F</i> : taatcgactcaatagggCAGTTCTTGTGAATTAGATG	-	436
	ds <i>GFP-R</i> : taatcgactcaatagggTTGGTTGTCTCCCATGATG		

*InR*, insulin receptor; dsRNA, double-stranded RNA; *EF1α*, elongation factor 1α; F, forward; R, reverse; Ac, *Aphis (Toxoptera) citricida*. The lower case letters represent the T7 promoter sequences for efficient in vitro transcription in dsRNA synthesis.

**Table S2.** Sequences and relevant information used for phylogenetic analysis of the insulin receptor genes.

Genes	GenBank No. or Gene ID	Species
<i>BgInR</i>	CDI30232.1	<i>Blattella germanica</i>
<i>ZnInR1</i>	KDR13786.1	<i>Zootermopsis nevadensis</i>
<i>ClInR1</i>	XP_014256336.1	<i>Cimex lectularius</i>
<i>HhInR1</i>	XP_014273515.1	<i>Halyomorpha halys</i>
<i>NlInR1</i>	AIY24638.1	<i>Nilaparvata lugens</i>
<i>AcInR1</i>	KX507134	<i>Aphis (Toxoptera) citricida</i>
<i>DnInR1</i>	XP_015375915.1	<i>Diuraphis noxia</i>
<i>ApInR1</i>	XP_008185917.1	<i>Acyrtosiphon pisum</i>
<i>MpInR1</i>	MYZPE13164_G006_v1.0_000181710	<i>Myzus persicae</i>
<i>PhInR</i>	XP_002430961.1	<i>Pediculus humanus corporis</i>
<i>NvInR</i>	XP_003425750.1	<i>Nasonia vitripennis</i>
<i>AmInR1</i>	NP_001233596.1	<i>Apis mellifera</i>
<i>BiInR1</i>	XP_003490625.1	<i>Bombus impatiens</i>
<i>SiInR1</i>	XP_011158641.1	<i>Solenopsis invicta</i>
<i>CfInR1</i>	XP_011251746.1	<i>Camponotus floridanus</i>
<i>HsaInR1</i>	XP_011144146.1	<i>Harpegnathos saltator</i>
<i>OnInR</i>	AFQ20827.1	<i>Onthophagus nigriventris</i>
<i>TcInR1</i>	XP_008199415.1	<i>Tribolium castaneum</i>
<i>BmInR</i>	XP_012549124.1	<i>Bombyx mori</i>
<i>PxInR</i>	XP_011567916.1	<i>Plutella xylostella</i>
<i>AaInR</i>	AAB17094.1	<i>Aedes aegypti</i>
<i>DmInR</i>	ACY01695.1	<i>Drosophila melanogaster</i>
<i>GmInR</i>	AFQ01096.1	<i>Glossina morsitans morsitans</i>
<i>BdInR</i>	XP_011210333.1	<i>Bactrocera dorsalis</i>
<i>CcInR</i>	XP_004518075.1	<i>Ceratitis capitata</i>
<i>CfInR2</i>	XP_011263722.1	<i>Camponotus floridanus</i>

**Table S2.** *Cont.*

<b>Genes</b>	<b>GenBank No. or Gene ID</b>	<b>Species</b>
<i>SiInR2</i>	XP_011175961.1	<i>Solenopsis invicta</i>
<i>HsaInR2</i>	XP_011140707.1	<i>Harpegnathos saltator</i>
<i>AmInR2</i>	XP_394771.4	<i>Apis mellifera</i>
<i>BiInR2</i>	XP_003484422.1	<i>Bombus impatiens</i>
<i>ZnInR2</i>	KDR21367.1	<i>Zootermopsis nevadensis</i>
<i>CInR2</i>	XP_014242610.1	<i>Cimex lectularius</i>
<i>HhInR2</i>	XP_014273071.1	<i>Halyomorpha halys</i>
<i>NlInR2</i>	AIY24639.1	<i>Nilaparvata lugens</i>
<i>TcInR2</i>	AHF20215.1	<i>Tribolium castaneum</i>
<i>AcInR2</i>	KX507135	<i>Aphis (Toxoptera) citricida</i>
<i>DnInR2</i>	XP_015363980.1	<i>Diuraphis noxia</i>
<i>ApInR2</i>	XP_001942660.2	<i>Acyrtosiphon pisum</i>
<i>MpInR2</i>	MYZPE13164_G006_v1.0_000187240	<i>Myzus persicae</i>
<i>HsInR</i>	NP_001073285.1	<i>Homo sapiens</i>