Supplementary information

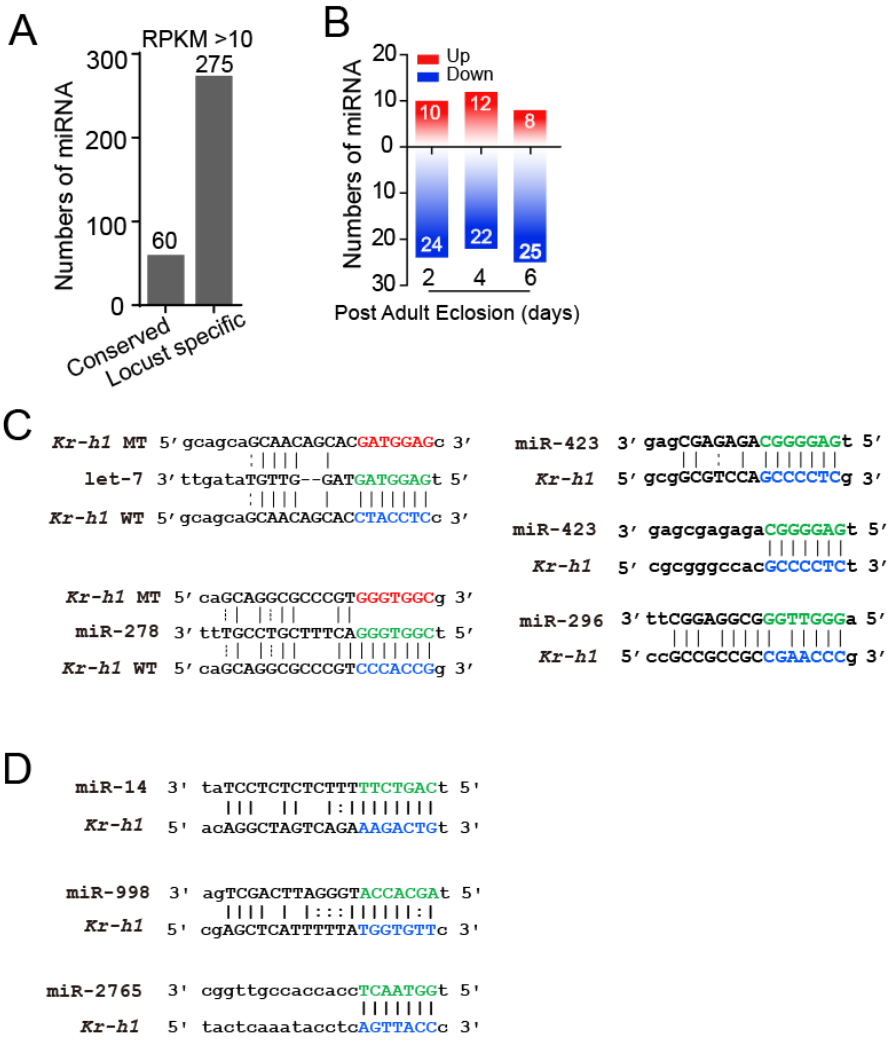

Fig. S1. **miRNA identification and binding site prediction for *Kr-h1*.** (A) Numbers of conserved miRNA and locust specific miRNA with RPKM values > 10. (B) The number of upregulated and downregulated miRNAs in the fat body of adult females at 2, 4 and 6 days post adult eclosion compared to that on the day of adult eclosion. (C, D) Sequence alignment of miRNAs with their predicted binding sites in the CDS (C) and 3′UTR (D) of *Kr-h1*. The binding sites (blue) in *Kr-h1* complementary to the seed region (green) of let-7 and miR-278 were site-mutated to their complementary bases (red) in the luciferase reporter assays.

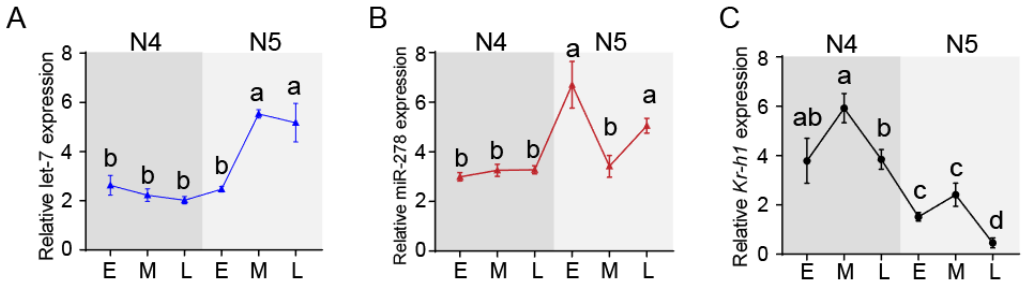

Fig. S2. The temporal expression patterns of let-7, miR-278 and *Kr-h1* in late-instar locust nymphs. qRT-PCR showing the relative levels of let-7 (A), miR-278 (B) and *Kr-h1* (C) expression in the whole body of penultimate 4th (N4) and final 5th (N5) instar nymphs. E, M, and L indicate the early (day 1), middle (day 2 for N4, and day 3 for N5), and late (day 4 for N4, and day 5 for N5) stages, respectively. The duration of 4th and 5th instar nymphs was about 4 days and 5 days, respectively under our reading conditions. In each panel, the means labeled with different letters indicate significant difference at $P < 0.05$. n = 8.

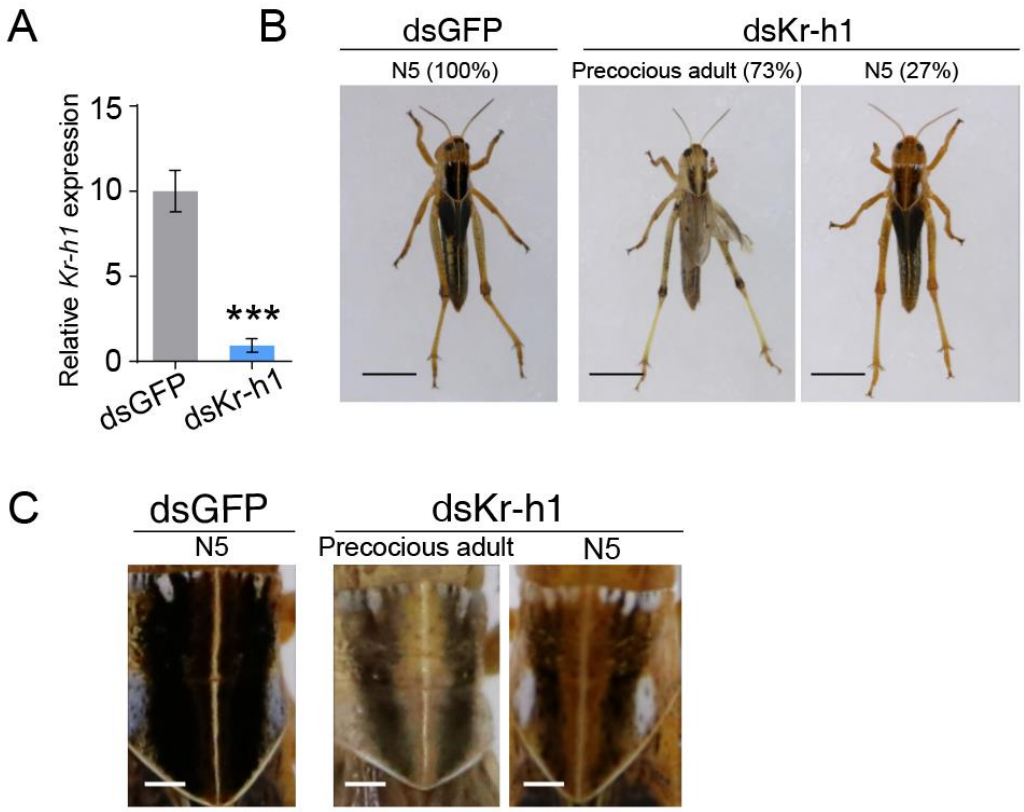

Fig. S3. Effect of *Kr-h1* knockdown on locust metamorphosis. (A) RNAi-mediated knockdown efficiency of *Kr-h1* in the whole body of final 5th instar nymphs previously subjected to dsKr-h1 treatment at the penultimate 4th nymphal instar. ***, $P < 0.001$ compared to the dsGFP control. n = 7-8. (B) Representative phenotypes and percentage of precocious metamorphosis at the final instar nymphs (N5) previously subjected to dsKr-h1 treatment at the penultimate 4th nymph instar (3 replicates with 7-8 locusts in each treatment). Scale bar, 1 cm. (C) Enlarged images of the pronotum shown in (B). Scale bar, 0.1 cm.

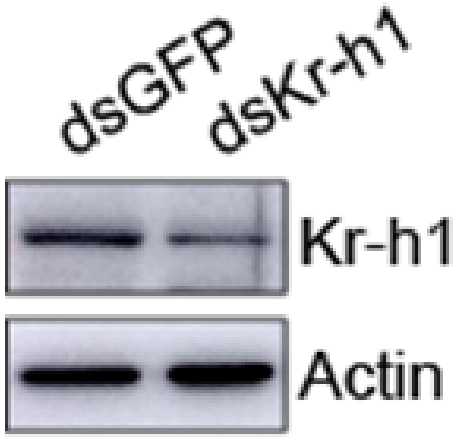

Fig. S4. Specificity of the antiserum. The antiserum specificity was verified by western blot using protein extracted from fat body of locusts treated with dsKr-h1 versus dsGFP.

Table S1. Primers used in qRT-PCR, mutation and RNAi.

Primer Name	Sequence (5' to 3')
qRT-PCR	
qKr-h1-F	ACTTCGTCTTCTGGAATGA
qKr-h1-R	GGCAATCGGTATTACACTTAG
qlet-7-F	GCTGAGGTAGTAGGTTGTATAGTT
qmiR-278-F	GGTGGGACTTTCGTCCGTTT
qU6	ACACTCCAGCTGGGTCAAAATCGTGAAGCG
qVgA-F	CCCACAAGAAGCACAGAACG
qVgA-R	TTGGTCGCCATCAACAGAAG
qActin-F	AATTACCATTGGTAACGAGCGATT
qActin-R	TGCTTCCATACCCAGGAATGA
site mutation	
mut-let-7-F	CCTCTGCTTGATGATGGAGTACTCGCAGCAGGCGCCCGTCCCAC
mut-let-7-R	CGAGTACTCCATCATCAAGCAGAGGTCGCGACCGCCCCCGGGCGT
mut-miR-278-F	GCGCCCGTTGATGACGCGCACGCGGGCCACGCCCCTCTCTCC
mut-miR-278-R	CGTGCGCGTCATCAACGGGCGCCTGCTGCGAGTACAGGTAGT
dsRNA synthesis	
dsKr-h1-F	GTCAAGGAGAACCTGAGCGTGC
dsKr-h1-R	TGCTGCTGCTCCGAGTGGCT
dsGFP-F	CACAAGTTCAGCGTGTCCG
dsGFP-R	GTTCACCTTGATGCCGTTC