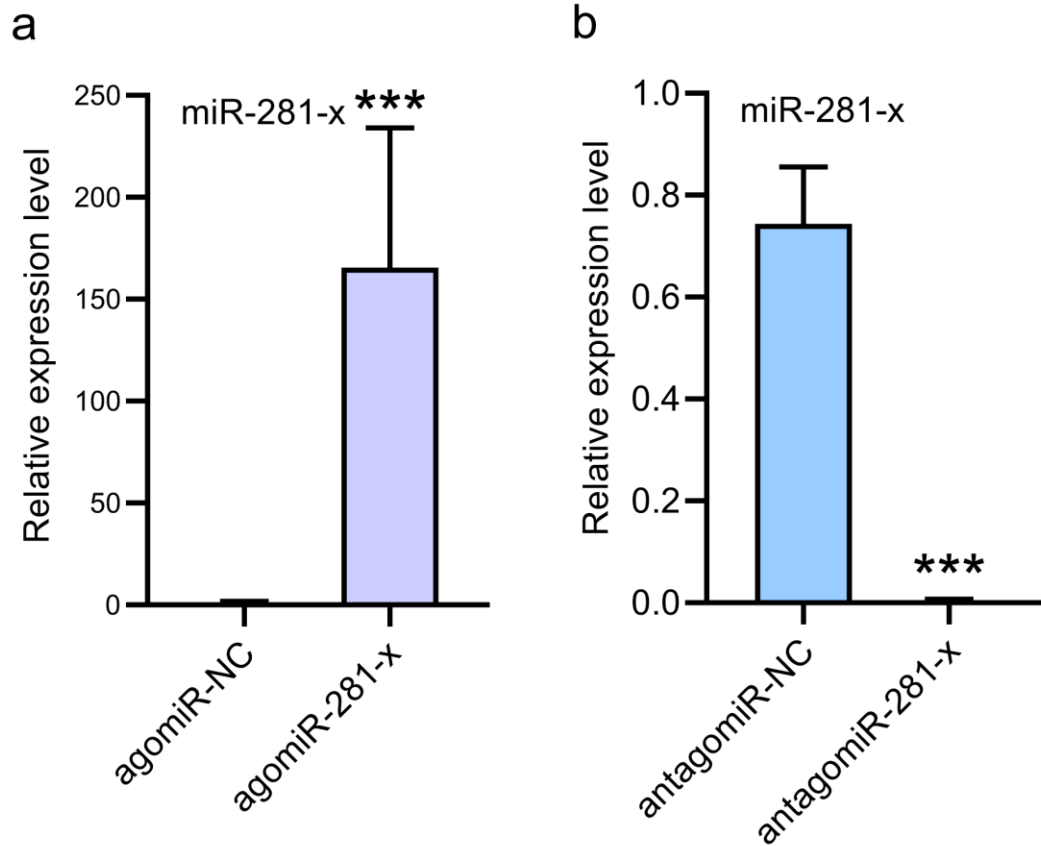


1 **Supplementary Information**

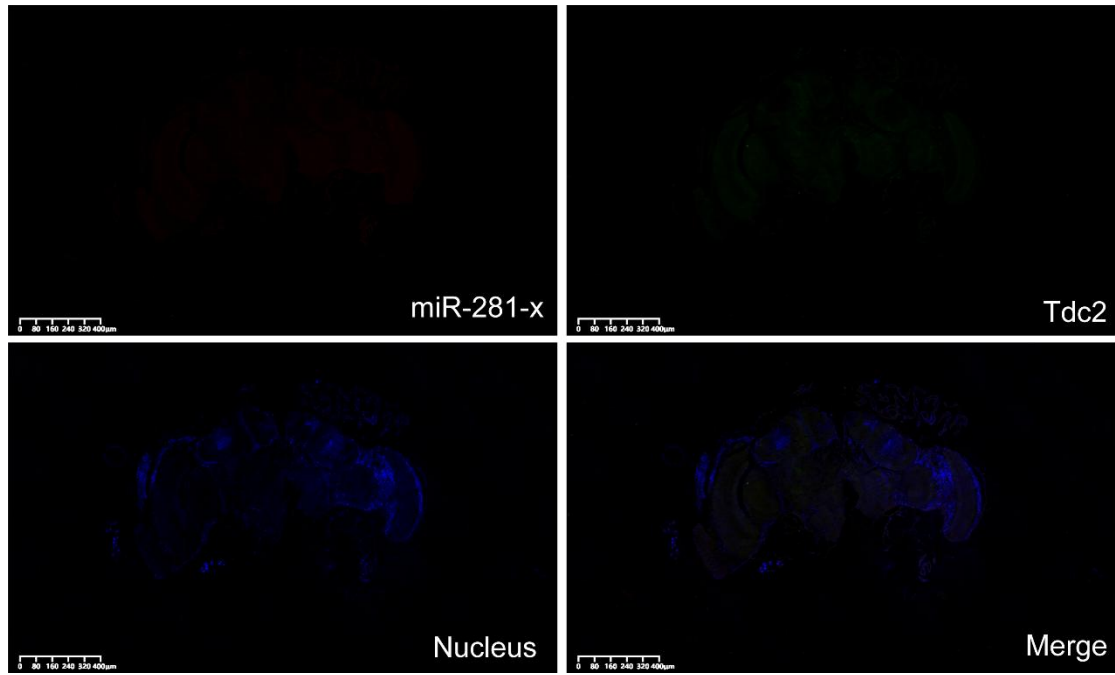


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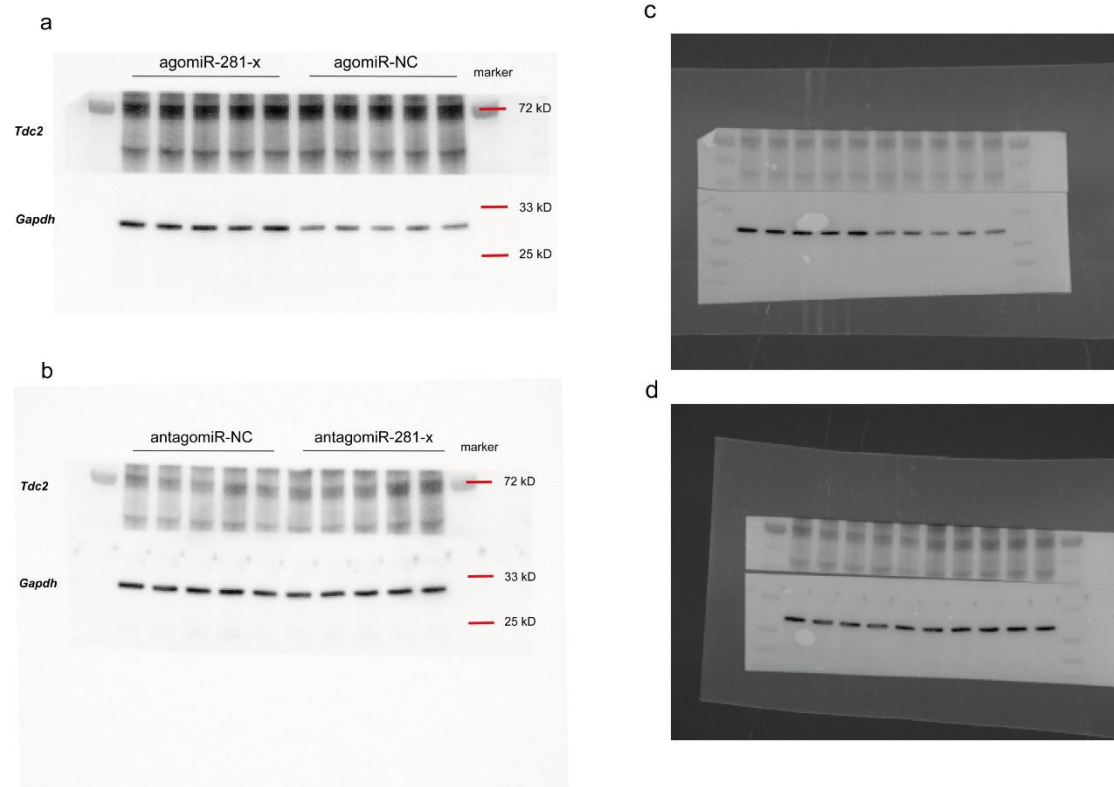
3

4 **Supplementary Figure 1. The expression level of miR-281-x of honeybees** (a)injected with
 5 miR-281-x-agomir (b)and miR-281-x-antagomir. The data are presented as the mean $\pm$ SEM; The
 6 asterisks outside the strip indicate the significant difference between controls and the treatments
 7 by Student's t-test. *** $P < 0.001$.

8

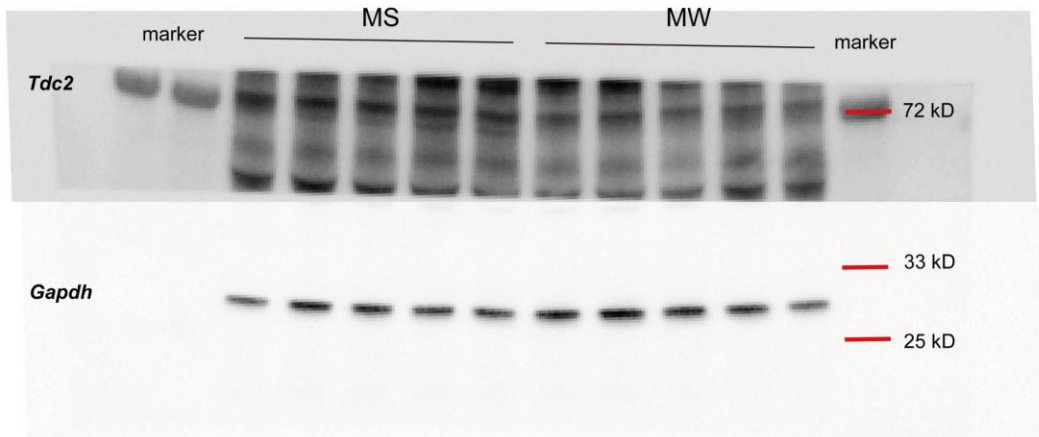


Supplementary Figure 2. The negative control for co-localization of miR-281-x and *Tdc2* in the honeybee brain.

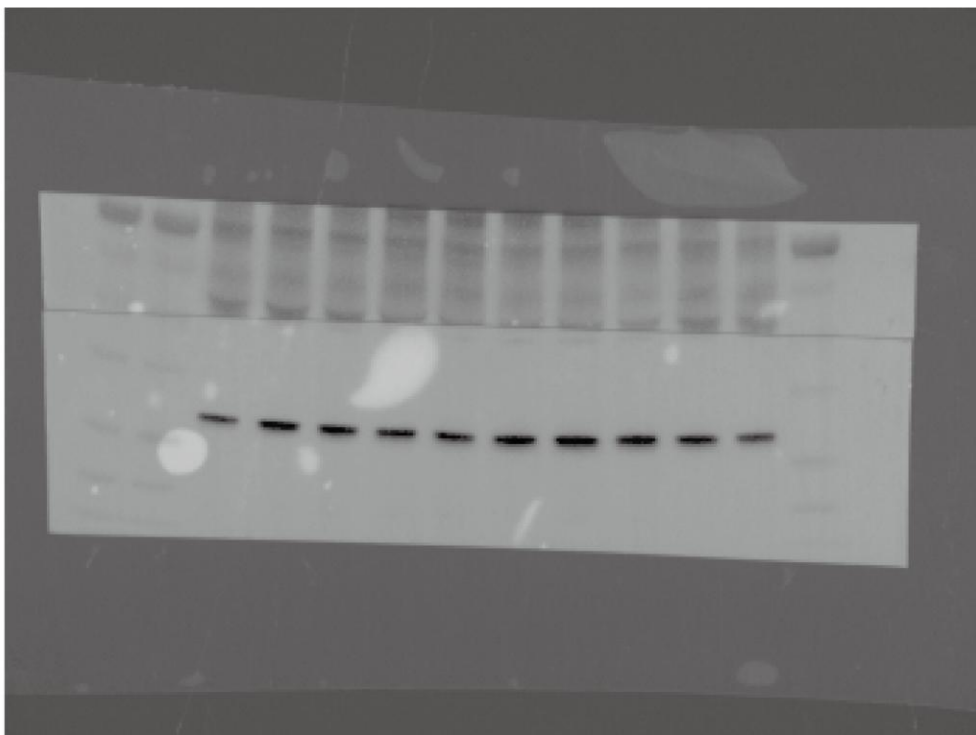


Supplementary Figure 3. The western blot scans of *Tdc2*. The western blot scans of *Tdc2* in the honeybee brains injected with (a) agomiR-281-x and (b) antagomiR-281-x. (c-d) There were original images of agomiR-281-x and antagomiR-281-x, respectively.

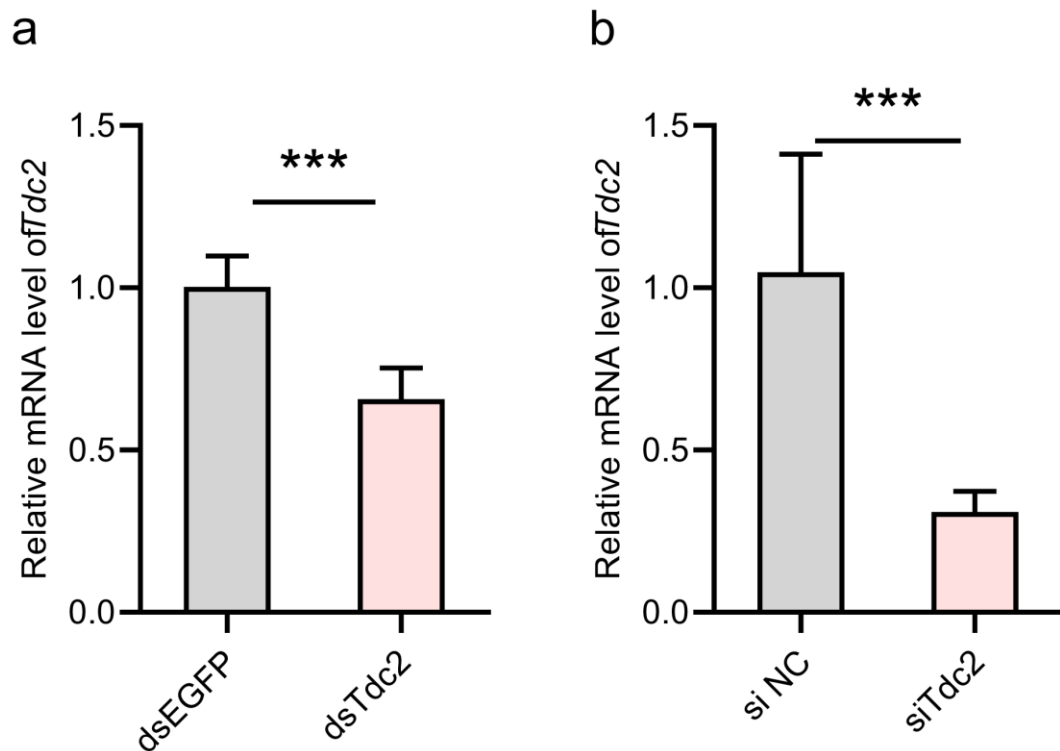
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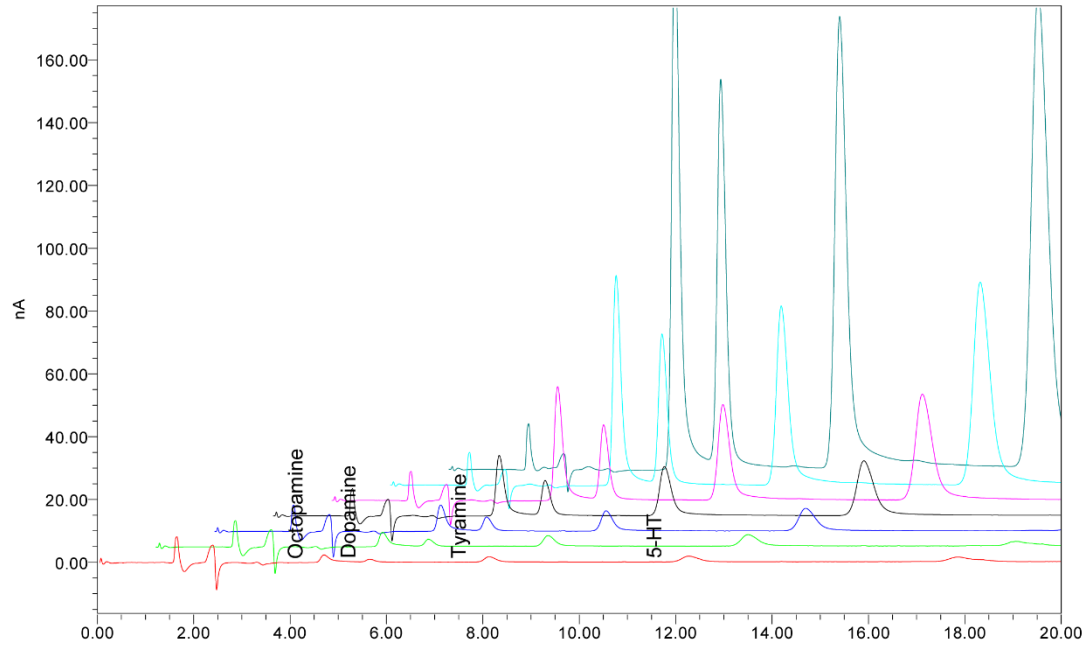
b



Supplementary Figure 4. The western blot scans of *Tdc2*. (a) The western blot scans of *Tdc2* in the MS and MW bee brains and (b) was original image of (a).



Supplementary Figure 5. The expression level of *Tdc2* of honeybees (a) injected with *dsEgfp* and *dsTdc2* (b) and siNC and *siTdc2*. The data are presented as the mean $\pm$ SEM; The asterisks outside the strip indicate the significant difference between controls and the treatments by Student's t-test. *** $P < 0.001$.



Supplementary Figure 6. HPLC-ECD chromatogram of biogenic amines. Depicts a representative calibration curve using octopamine, dopamine, tyramine and serotonin standards ranging 5 ng to 0.5 μ g. Red line: 5ng/mL; green line: 10 ng/mL; black line: 20 ng/mL; purple line: 50ng/mL; pink line: 100 ng/mL; blue line: 200 ng/mL; cyan line: 500 ng/mL

Gene/miRNA	Sequence (5' to 3')	Experiments
miR-281-x	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATAC GACCTGTCG	miRNA cDNA Synthesis
ame-miR-1-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATAC GACCTCCTC	
ame-miR-12-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATAC GACCCGTCC	
ame-miR-10-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATAC GACCTTCGG	
ame-miR-375-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATAC GACTAACTC	
ame-miR-9a-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATAC GACTCTCGC	
ame-miR-263a-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATAC GACGTGTTC	
U6	TATCAGGCATTTCTCCACCAG	
miR-281-x-F	GCGCGAAGAGAGACCA	qPCR
ame-miR-1-3p-F	CGCGCGGGAAGAAAGAA	
ame-miR-12-5p-F	GCGCGCGGAGAACACA	
ame-miR-10-5p-F	CGCGCGCGACCCGAGA	
ame-miR-375-3p-F	GCGTTTGTTTCGTTGGCTC	
ame-miR-9a-5p-F	CGCGCGCGCGCGGACA	
ame-miR-263a-5p-F	GCGGAAAGGCACGGAA	
Reverse miR primer	AGTGCAGGGTCCGAGGTATT	
U6-F	CGGAAGGAAGAAGAGTCACCA	
U6-R	TATCAGGCATTTCTCCACCAG	
Tdc2-F	CCGAGCCTTGGGAGAACATT	dsRNA/siRNA synthesis
Tdc2-R	TTCCCGAACCAATCGCAGA	
Actin-F	TTGTATGCCAACACTGTCCTTT	
Actin-R	TGGCGCGATGATCTTAATTT	
dsTdc2-F	CGTTGGTCGTCGATCCTCTT	
dsTdc2-R	CAGCATCTTTCGCCTGCATC	
T7-dsTdc2-F	TAATACGACTCACTATAGGGCGTTGGTCGTCGATCCTCTT	
T7-dsTdc2-R	TAATACGACTCACTATAGGGCAGCATCTTTCGCCTGCATC	dsRNA/siRNA synthesis
dsEgfp-F	ATGAGCAAGGGCGAGGAAGCTG	

dsEgfp-R	CTTGTACAGCTCGTCCATGCC	
T7-dsEgfp-F	GGATCCTAATACGACTCACTATAGGATGAGCAAGGGCGAGG AACTG	
T7-dsEgfp-R	GGATCCTAATACGACTCACTATAGGCTTGTACAGCTCGTCCA TGCC	
siTdc2-F	GGCGAAAGAUGCUGCUGAU(dT)(dT)	
siTdc2-R	AUCAGCAGCAUCUUUCGCC(dT)(dT)	
pmirGLO-F	CGAGGTGCCTAAAGGACTGACCG	Luciferase report assay
pmirGLO-R	ACTGCATTCTAGTTGTGGTTTGTCC	
Mutation-F	AGTCTcgagagaTTGGATCGCGACAGAGATGC	
Mutation-R	TCCAAtctctcgAGACTTATATAAACAGATCGATAAGCAAGTA	
agomir-NC	UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT	miRNA mimic/ inhibitor synthesis
antagomir NC	CAGUACUUUUGUGUAGUACAA	
miR-281-x-agomi r	AAGAGAGCUAUCCAUCGACAGU UGUCGAUGGAUAGCUCUCUUUU	
miR-281-x-antag omir	ACUGUCGAUGGAUAGCUCUCUU	

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39 **Supplementary Table 2.** Probe sequence used in this study.

Gene/miRNA	Sequence (5' to 3')	Experiments
DIG-miR-281-x	ACTGTCGATGGATAGCTCTCTT	FISH
DIG-Tdc2	GAATCGATGGAAAGGAATTTCCGGCTGG	
	CAACAAGCAGTCGTGCCTAAAGTGGT	
	TACCAGCCATCAGGTACTTCAATTCCGG	
	CAAGCGCGAAACGAATAGTGAACCGT	
	GCGACAATTCACTGGTATCAGCAGCATC	

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41

42 **Supplementary Data 1.** Target Genes of seven differentially expressed miRNAs.

43 <..\..\supplementary figure data table\Supplementary data 1.html>

44

45 **Supplementary Data 2.** The data of all figures.
46 <..\..\supplementary figure data table\Supplementary Data 2 .html>
47

48 **Video Recordings of MS and MW bees**

49 [..\..\supplementary figure data table\Video of strong grooming bee \(MS\) .mp4](..\..\supplementary figure data table\Video of strong grooming bee (MS) .mp4)

50 **Video 1. MS (strong grooming) bee**

51 [..\..\supplementary figure data table\Video of weak grooming bee \(MW\) .mp4](..\..\supplementary figure data table\Video of weak grooming bee (MW) .mp4)

52 **Video 2. MW (weak grooming) bee**

53