Supporting Information

SI Materials and Methods

RT-qPCR

The 25 µl qRT-PCR reaction mixture included 1 µl of cDNA or DNA, 12.5 µl of 2X SYBER Green Master Mix (Applied Biosystems®), 5 µM of primers and nuclease free water to make up the total volume. The PCR was performed in RT-7500 system (Applied Biosystems®) with the initial denaturation for 2 min at 50°C and 10 min at 95°C followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. Each of the reactions was performed three times independently, in triplicate, and the results were normalized with constitutively expressing 18S ribosomal RNA gene of *B. mori.* All the primer sequences are provided in the Table S1.

Luciferase constructs

3'UTR sequence of Ran mRNA (Ran-3'UTR) containing the 23bp long bmnpv-miR-1 binding site along with some flanking sequence was amplified (primer sequences are provided in Table S1) and cloned into pmirGLO vector according to the manufacturer's instructions using double restriction digestion with SacI and XhoI. Insert orientation was determined by amplifying plasmid with insert and vector primers in combination and the concentration of selected plasmids (pmirGLO-Ran-3'UTR) was quantified using Nanodrop 2000c spectrophotometer (Thermo Scientific®) as well as by comparing it with Lambda DNA/HindIII Marker*, 2* (Fermentas®) on an agarose gel. An unrelated target sequence of Pro-phenoloxidase 3"UTR (pmirGLO-PPO-3'UTR) was also cloned in the same vector as described above (Sequence details are given in Table S1).

dsRNA Preparation for RNAi

Ran and Dicer-2 cDNA (primers sequence details are given in Table S1) was cloned into pCRII-TOPO vector (Invitrogen®) and amplified using M13 primers. The generated templates with flanking T7 and SP6 promoters were utilized for *in vitro* transcription. The sense and antisense RNA strands were transcribed using T7 and SP6 RNA polymerases (Ambion®), respectively. Residual DNA template was removed by treatment with DNase I and RNA was purified using lithium chloride precipitation. Equimolar amount of both the RNA strands were annealed by heating at 95°C for 5 min followed by slow cooling at room temperature for 12 hrs. To monitor annealing, RNAs were electrophoresed on a 2% agarose gel compared with its dsDNA template and then finally quantified by Nanodrop 2000c spectrophotometer. Similarly, dsRNA for GFP (Green Fluorescent Protein) was also synthesized and used as an experimental negative control.

DNA and Protein isolation

DNA was isolated from different *B. mori* fat body samples using DNeasy Blood & Tissue Kit (Qiagen) according to manufacturers' instructions and Protein extraction was done by grinding tissues in 1X PBS in presence of protease inhibitor cocktail followed by centrifugation.

Densitometry analysis

For Northern and Western blots, bands intensity was evaluated by Densitometry using ImageJ software (available at http://rsb.info.nih.gov/ij; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). In each case band intensity

was normalized against denominator gene (endogenous control, for Northern blot 5S ribosomal RNA and for Western blot α-tubulin).

Table S1. A list of all the primer and probe sequences used

Real time PCR primers	
Ran	Forward: 5’ GCCTGCCCTTCTGCCACCAG 3’
	Reverse: 5’ TGGGCACTGGGTACATCCGT 3’
18S rRNA	Forward: 5’ CGATCCGCCGACGTTACTACA 3’
	Reverse: 5’ GTCCGGGCCTGGTGAGATTT 3’
BmNPV *ie-1*	Forward: 5’ GTCCGTTGTCCGTGTGCGCT 3’
	Reverse: 5’ CGGCGCCGTTGGGATTTGTG 3’
Primers used for cloning	
pmirGLO vector	Forward: 5’ TGACCGGCAAGTTGGACGCC 3’
	Reverse: 5’ GGCCGCCCCAAGGGGTTATG 3’
Ran	Forward: 5’ TGATGAGCTCGCCTGCCCTTCTGCCACCAG 3’
	Reverse: 5’ ACTGCTCGAGACGCTACACTGAACACATTTGCATGA 3’
Pro-phenoloxidase (PPO)	Forward: 5’ CGACTATTGAGCTCTTCTCACGGATCTCGGTCTT 3’
	Reverse: 5’ ACATTTTGCTCGAGCGCAAGTTCATGACCAACAG 3’
Primers for RNAi	
Ran	Forward: 5’ CCATACGAACCGCGGGCCAA 3’
	Reverse: 5’ TGGGCACTGGGTACATCCGT 3’
Dicer-2	Forward: 5’ ACCGAAGAGGAAGTAATGACCGGT 3’
	Reverse: 5’ ACGACGAGTGAGACAGAGCGT 3’
DNA probes for Northern blot analysis	
bmo-miR-1	5’ CTCCATACTTCTTTACATTCCA 3’
bmo-miR-276a	5’ AGAGCACGGTATGAAGTTCCTA 3’
bmo-miR-8	5’ GACATCTTTACCTGACAGTATTA 3’
bmo-let-7	5’ ACTATACAACCTACTACCTCA 3’
***B. mori* lysine tRNA**	5’ CGCCCAACGTGGGGCTCGAACCCACGACCCTGAGATTAAGAGTCTCATGCTCTAC 3’
***B. mori* 5S rRNA**	5’ GTTGCTTGACTTCGGTGATCGGACGAGAACCGGTGTATTCAACATGGTATGGACG 3’
bmnpv-miR-1	5’ AGCTGTACGCCGCCCATTTGG 3’

Oligonucleotides used for Ran 3'UTR mutational analysis	
PmirGLO-Ran-*WT*	5' TTCAGAGCTCATATGATCAACGGATGTACCCAGTGCCCATTTTGTGATTGGAGGATCA TGCAAATGTGTCTCGAGTCGA 3'
PmirGLO-Ran-*Mut*	5' TTCAGAGCTCATATGATCAACGGATGTACCCAGTGCATGCTTTGTGATTGGAGGATCA TGCAAATGTGTCTCGAGTCGA 3'

Table S2. The putative targets of four of the selected host miRNAs

Cellular targets (*cis*)		
Host miRNAs	**Accession id**	**Known Function**
bmo-let-7	DQ116721	*B. mori* antimicrobial protein 5Tox.
	AB248080	*B. mori* immune inducible protein.
	HQ179970	*B. mori* 70 kDa ribosomal protein S6 kinase.
	GU354318	*B. mori* aliphatic nitrilase.
	AB208585	*B. mori* MAPK mRNA for p38 map kinase.
	DQ073458	*B. mori* eukaryotic translation initiation factor.
	AB436165	*B. mori* serine protease.
bmo-miR-1	DQ443224	*B. mori* DEAD box polypeptide 5
	EU093074	*B. mori* laccase2 phenoloxidase.
	L27451	*B. mori* chorion factor.
	EF415299	*B. mori* cytochrome P450.
bmo-miR-276a	DQ311319	*B. mori* tyrosine-protein phosphatase.
	DQ311271	*B. mori* fumarylacetoacetate hydrolase.
	AB013386	*B. mori* soluble alkaline phosphatase.
bmo-miR-8	JN021673	*B. mori* gamma-glutamyl transpeptidase.
	AB201474	*B. mori* lipophorin receptors.
	BR000523	*B. mori c*uticle protein.
Viral targets (*trans*)		
Host miRNAs	**Accession id**	**Known Function**
bmo-let-7	---	---
bmo-miR-1	D16231	BmNPV gene for DNA polymerase.
	BMU51009	BmNPV major capsid Bp39 gene.
	D14468	BmNPV gene for p40, virion-specific polypeptide.
	AB009987	BmNPV DNA-directed RNA polymerase component lef8.
	M63416	BmNPV DNA-binding protein.
	AY182246	BmNPV cysteine proteinase gene.
	AY817140	BmNPV cathepsin gene.
bmo-miR-276a	AY779044	BmNPV polyhedrin protein.
	AJ309237	BmNPV bro-III gene.
bmo-miR-8	AY048770	BmNPV immediate early protein (ie-1) gene.
	AY519217	BmNPV lef-8 gene.
	AY616663	BmNPV chitinase A.
	AF063104	BmNPV capsid protein VP39.
	AB009987	BmNPV DNA-directed RNA polymerase component lef8.
	U51009	BmNPV capsid Bp39 gene.
	D16231	BmNPV DNA polymerase.
	L24899	BmNPV hr1 gene.
	AF316871	BmNPV tyrosine phosphatase NPV-PTP gene.

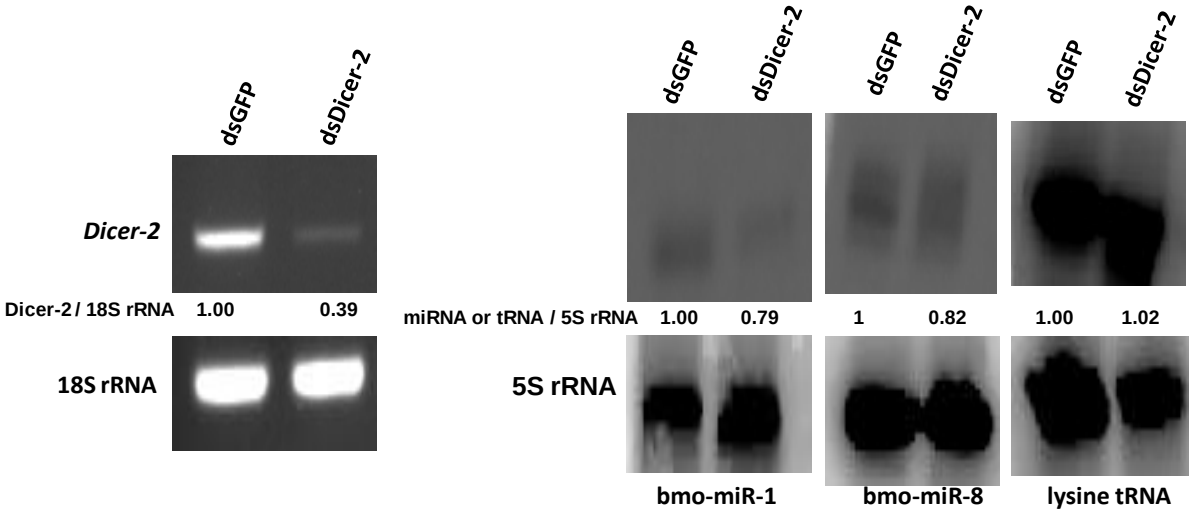

Figure S1. *B. mori* miRNAs and lysine tRNA expression analysis upon Dicer-2 knockdown. (A) RT-PCR analysis of dsRNA-mediated Dicer-2 knockdown in fat body tissues. DsRNA against GFP and dsRNA against Dicer-2 are represented as dsGFP and dsDicer-2 respectively.18S rRNA was used as loading control. (B) Northern blot analysis of bmo-miR-1, bmo-miR-8 and lysine tRNA expression upon Dicer-2 knockdown in fat body tissues. Band ratio was determined by densitometry and normalized against endogenous control 5S rRNA, is given.

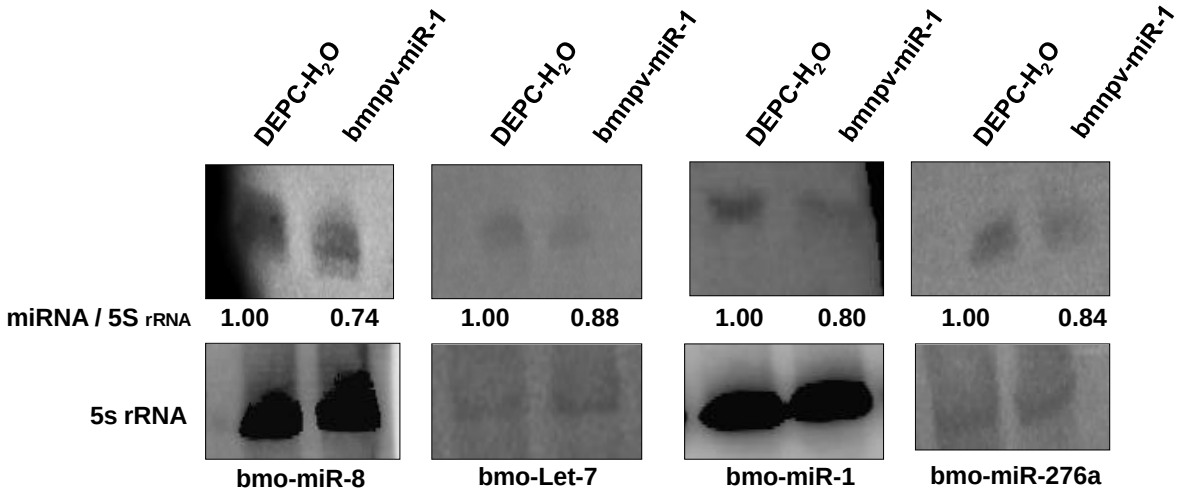

Figure S2. Host miRNAs expression after 4 days of bmnpv-miR-1 duplex administration in the *B. mori* larvae determined by Northern blot. Band ratio was determined by densitometry and normalized against endogenous control 5S rRNA, is given.

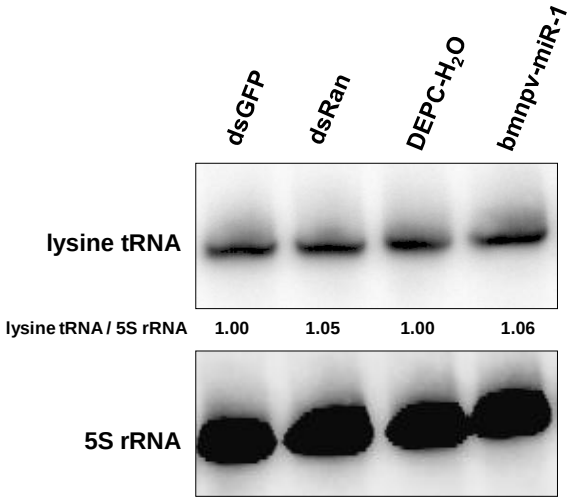

Figure S3. Northern blots showing, *B. mori* lysine tRNA expression after 4 days of bmnpv-miR-1 and Ran dsRNA administration in BmNPV infected larvae. Band ratio was determined by densitometry and normalized against endogenous control 5S rRNA, is given.

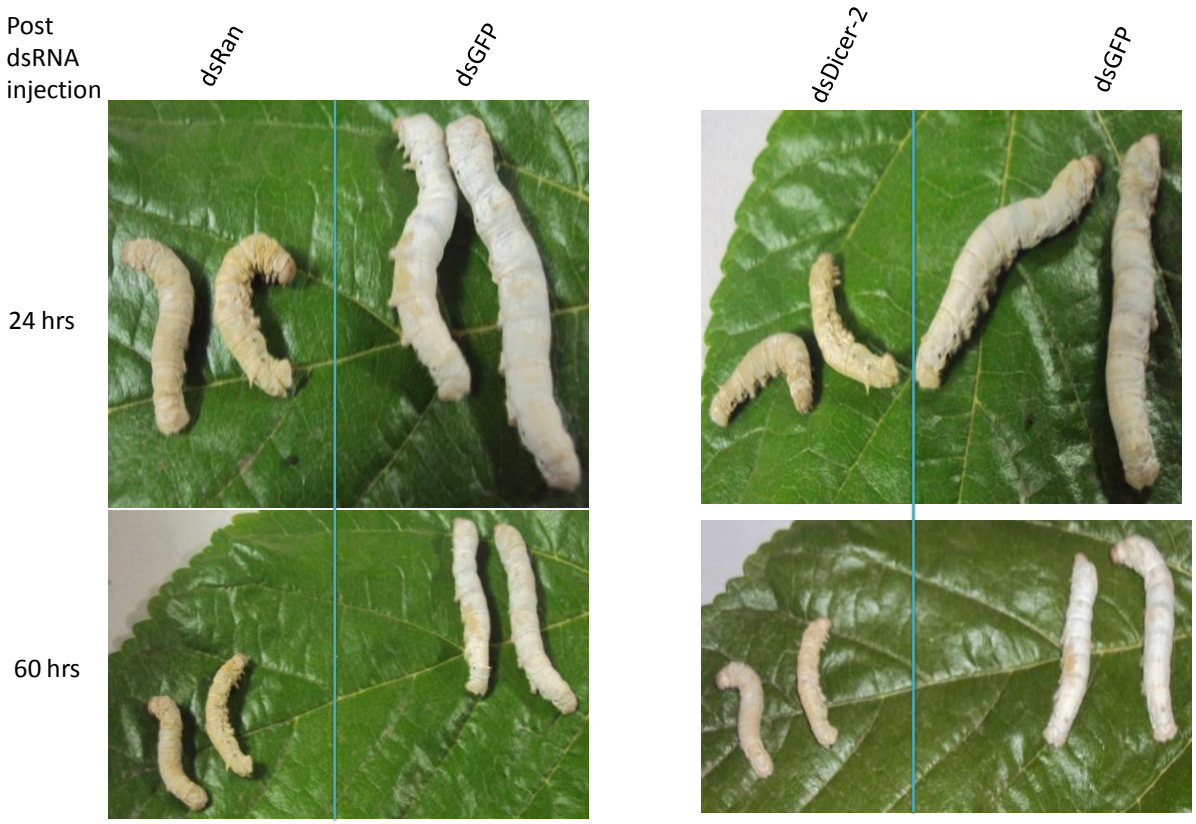

Figure S4. Comparing phenotypes of Ran and Dicer-2 knockdown *B. mori* larvae. Similar phenotypes were observed upon dsRNA-mediated knockdown of Ran and Dicer-2 in the BmNPV infected larvae.