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Supplemental Information

**Genome-wide Integrative Analysis of Zika-Virus-
Infected Neuronal Stem Cells Reveals Roles for
MicroRNAs in Cell Cycle and Stemness**

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Figure S1

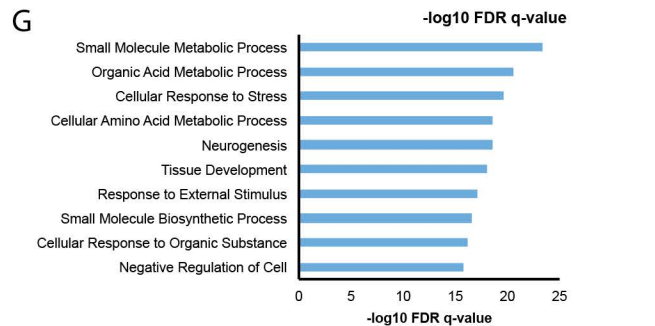
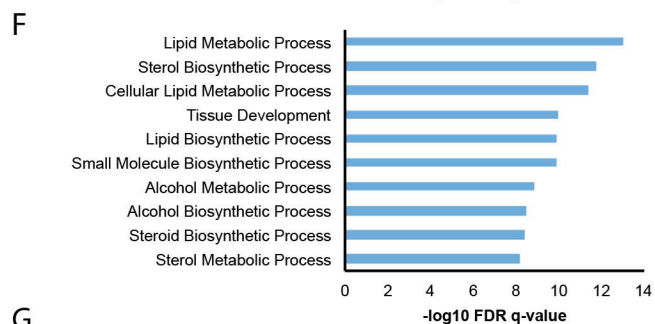
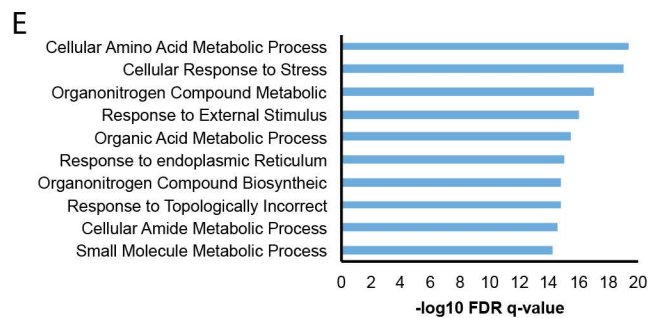
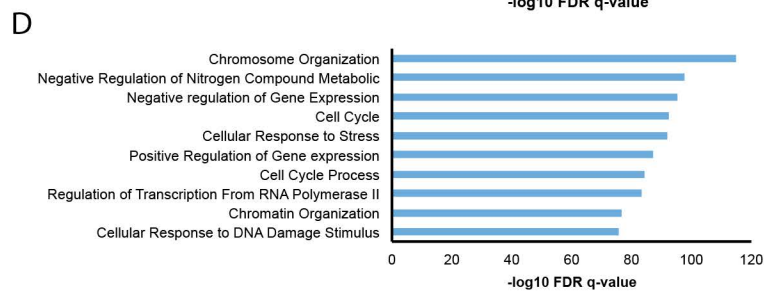
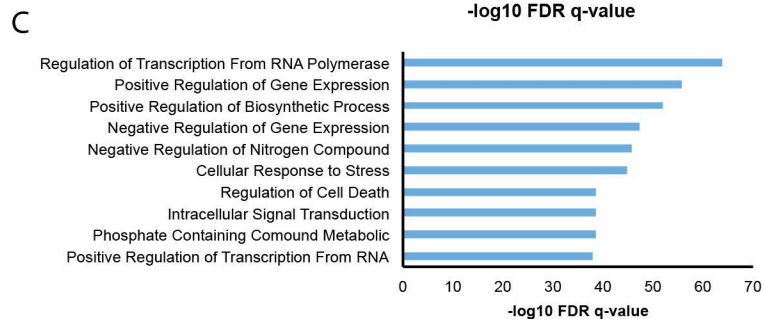
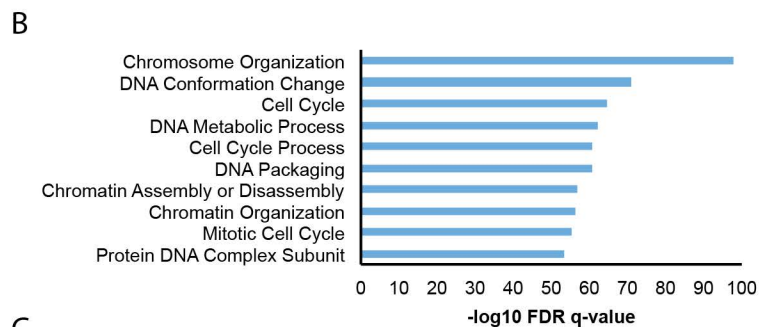
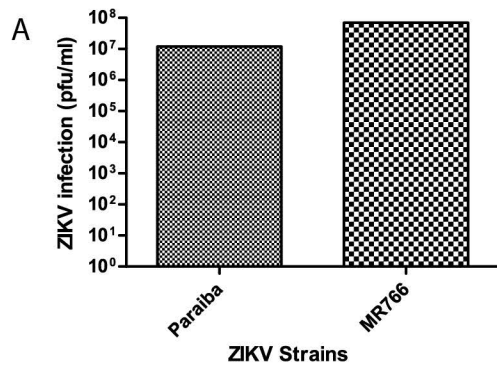


Figure S1. Gene Set Enrichment Analyses of Differentially Expressed Genes in ZIKV-Infected hNSCs.

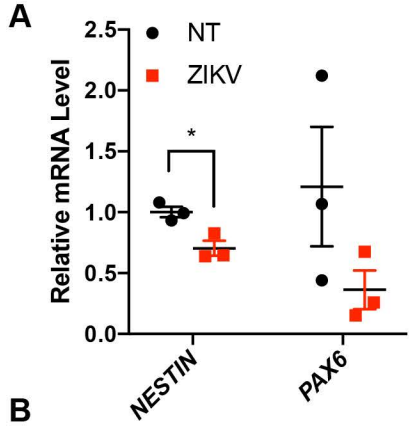
Related to Figure 1.

(A) Plaque-forming assay measuring infectious particles of ZIKV Paraiba and MR766 strains. n=3 biological replicates.

(B–D) GSEA of upregulated (B), downregulated (C) or upregulated or downregulated (D) genes in ZIKV MR766-infected hNSCs at 3 days post-infection at a MOI of 1.

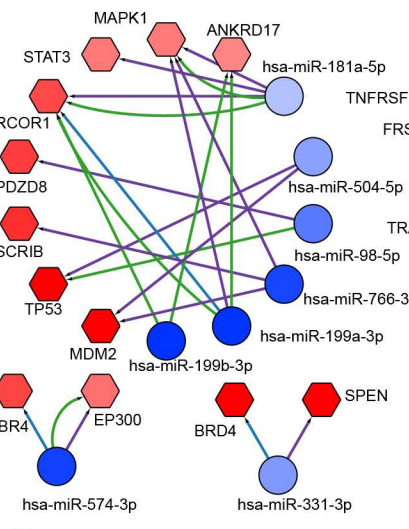
(E–G) As in (B–D) for ZIKV Paraiba-infected hNSCs.

Figure S2

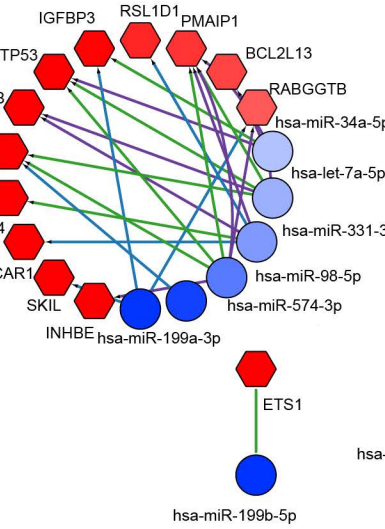


B

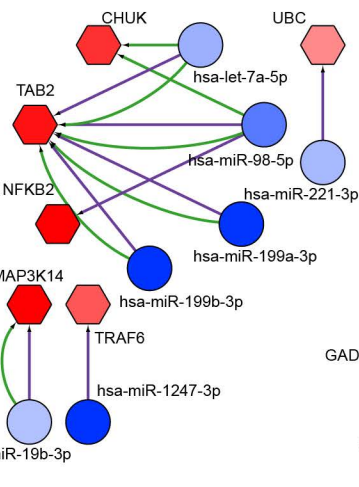
Viral Process



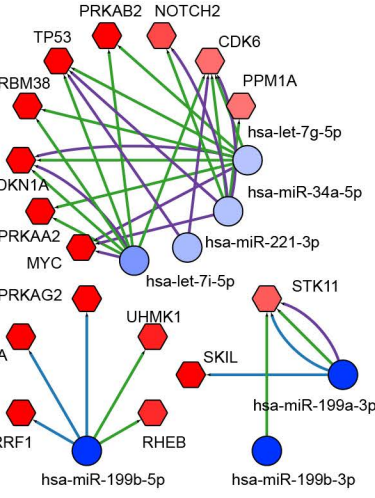
Apoptosis



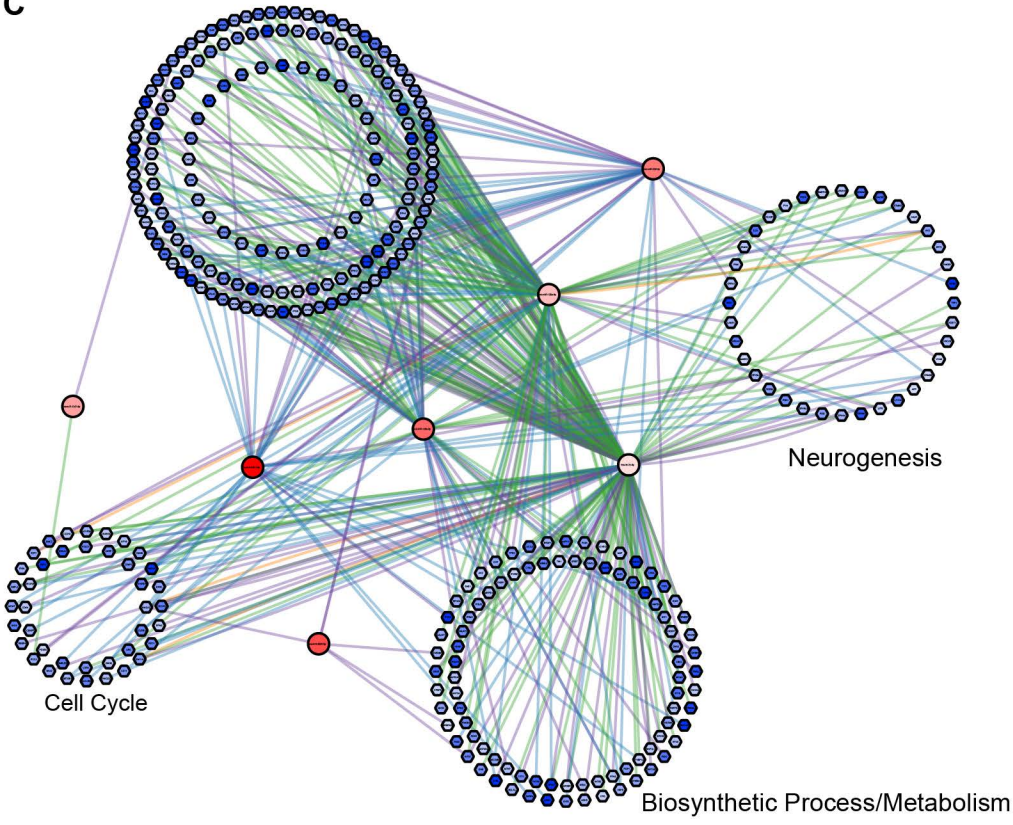
NFKB Signaling



Cell Cycle Arrest



C



D

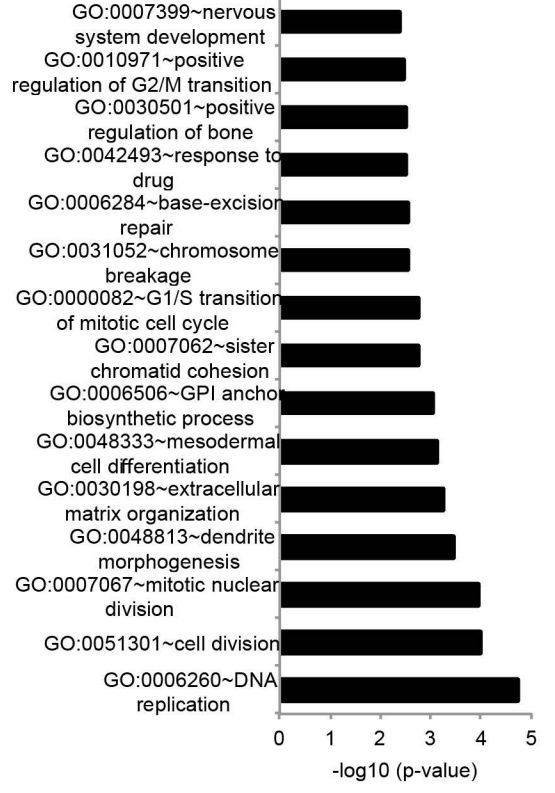


Figure S2. miRNA-Mediated Regulatory Networks in ZIKV-Infected hNSCs. Related to Figure 2.

(A) RT-qPCR analysis of *NESTIN* and *PAX6* mRNA levels in hNSCs 3 days post-infection with ZIKV MR766 or Paraiba at a MOI of 1. Mean \pm SEM of n = 3 biological replicates. * p < 0.05.

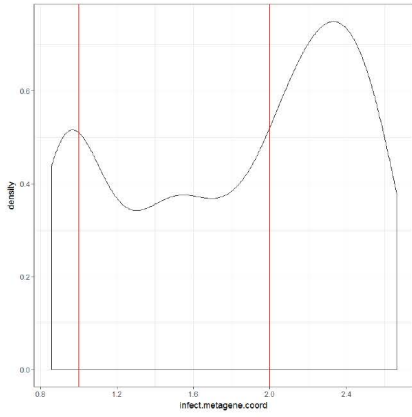
(B) Integrative networks of mRNAs downregulated and miRNAs upregulated in ZIKV-infected hNSCs involved in “viral process,” “apoptosis,” “NF κ B signaling,” and “cell cycle arrest” based on gene ontology annotation.

(C) miRNA–mRNA network of commonly upregulated miRNAs and downregulated mRNAs in ZIKV MR766- and Paraiba-infected hNSCs. Genes are clustered based on gene ontology annotation.

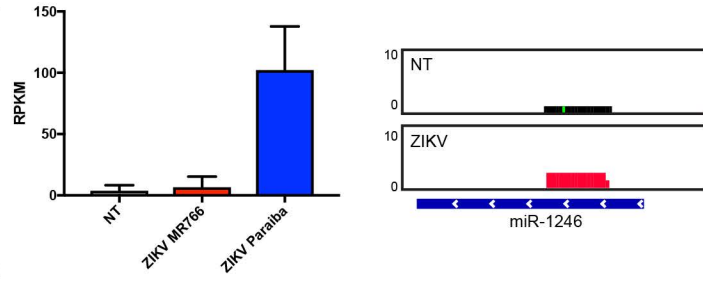
(D) Gene ontology analyses of putative mRNA targets of commonly upregulated miRNAs in ZIKV MR766- and Paraiba-infected hNSCs (shown in Figure 2A-B).

Figure S3

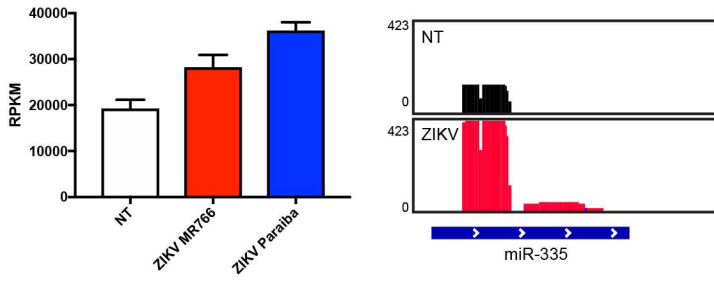
A



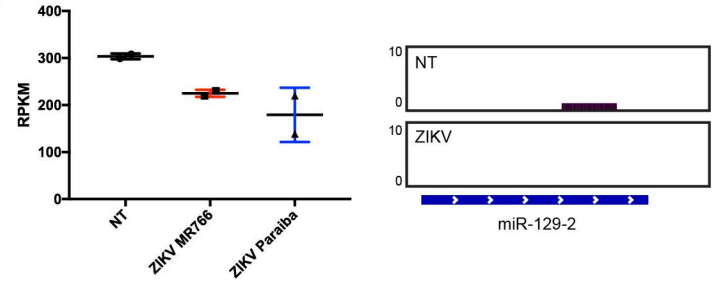
B



C



D



E

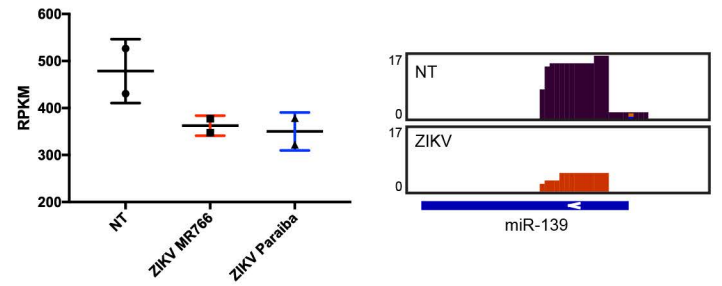


Figure S3. Related to Figure 3.

(A) Metagene analysis of AGO-iCLIP-seq binding sites in ZIKV Paraiba infected hNSCs 4 days post-infect at MOI 1 shows enrichment in 3'-UTRs.

(B and C) miRNA-seq analysis in ZIKV MR766 and Paraiba infected hNSCs 3 days post-infect at MOI 1 (left) and AGO binding maps (right) in ZIKV Paraiba infected hNSCs 4 days post-infect at MOI 1 show upregulation and enhanced binding of *miR-1246* (B) and *miR-335* (C).

(D and E) miRNA-seq analysis in ZIKV MR766 and Paraiba infected hNSCs 3 days post-infect at MOI 1 (left) and AGO binding maps in ZIKV Paraiba infected hNSCs 4 days post-infect at MOI 1 (right) show downregulation and decreased binding of *miR-129-2* (D) and *miR-139* (E).

Figure S4. Related to Figure 4.

(A) Scatterplot showing the ranking and combined score of miRNAs predicted to regulate genes associated with microcephaly based on the Harmonizome databased. *hsa-miR-124-3p* (rank 1) is highlighted in red.

(B) miRNA-seq analysis in ZIKV MR766 and Paraiba infected hNSCs 3 days post-infect at MOI 1 show *miR-124-3p* expression following viral infection. Scatterplot mean and standard deviation of n=2 biological replicates.

(C) AGO binding maps of mock-infected (NT) and Paraiba ZIKV-infected hNSCs show significantly enriched loading of *miR-124-3p* in the RISC 4 days after ZIKV infection at MOI 1.

(D) Venn diagram of putative *miR-124-3p* target mRNAs (TargetScan) and significantly ($p < 0.05$) downregulated genes in ZIKV MR766- and Paraiba-infected hNSCs.

(E) Sequence conservation of putative *miR-124-3p*-binding site within *TFRC*.

(F) TargetScan prediction of the *miR-124-3p* targeting site within the *TFRC* 3'-UTR.

(G) Immunostaining of ZIKV envelope flavivirus group antigen (ZIKVE, green) and neural progenitor marker SOX2 (red) in the hippocampus (top row) and subventricular zone (bottom row) of a ZIKV Paraiba-infected *Ifnar^{-/-}* mouse brain 6 days post-infection. Nuclei were stained with DAPI (gray or blue). Right-most column shows enlargement of the merged panels. SGZ = subgranular zone, DG = dentate gyrus, LV = lateral ventricle, STR = striatum. Scale bars, 200 μ M and 50 μ M. Dashed lines indicate separation between subgranular zone and dentate gyrus and hillus (top panels) and striatum and lateral ventricle (bottom panels).

(H) Immunostaining of ZIKV envelope antigen (ZIKV, green) and the mature neuronal marker NeuN (red) in the hippocampus of uninfected (top) or ZIKV Paraiba-infected (bottom) *Ifnar^{-/-}* mice 6 days post-infection. Labeling and scale bars are as for (D). Dashed lines indicate separation between subgranular zone and dentate gyrus and hillus regions.