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**Supplementary Data**

**A novel IFNbeta-induced long non-coding RNA ZAP-IT1 interrupts Zika virus replication in cells**

**Yanxia Huanga,1, Yu Sua,1, Li Shenb, Zhiting Huoc, Cancan Chend, Tao Suna, Xu Tianc, Ning Lia, Chao Yanga,\***

*a Department of Neurosurgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China*

*b Department of Microbiology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, 510080, China*

*c Department of Immunology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, 510080, China*

*d Department of Pathology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, 510080, China*

\* Corresponding author.

*E-mail addresses*: chyalbtrac@126.com (C. Yang)

1 Yanxia Huang and Yu Su contributed equally to this work.

**Supplementary Table S1** Sequences of primers used in qRT-PCR.

|  |  |  |
| --- | --- | --- |
| **Gene** | **Primer** | **Sequence (5**′**–3**′**)** |
| *LAP3-AS1* | 5F | GACCAGCAACAACCACAGCCAAGG |
| 3R | GAAGTGTGCTGGTCATCCCACCAT |
| *LINC21762* | 5F | GGCTGTGGTCCTCTCTCGACACCA |
| 3R | GGCATTTCCATTAGCACTCCATCT |
| *VAMP1-AS1* | 5F | AAGATGAAAGACTCTGCAACAACA |
| 3R | GCGGGGAAATGCCATTTAGGACAA |
| *ZAP-IT1* | 5F | AAAGCACCCAAGATGTGCCACCAG |
| 3R | TAGCTTTTTCTTCTGTGCACCTCC |
| *ZIKV NS1* | 5F | GTCAGAGCAGCAAAGACAA |
| 3R | CAGCCTCCTTTCCCTTAACA |
| *β-actin* | 5F | GCTCCTCCTGAGCGCAAG |
| 3R | CATCTGCTGGAAGGTGGACA |
| *U6* | 5F | CGCTTCGGCAGCACATATAC |
| 3R | CGAATTTGCGTGTCATCCTTG |
| *IFN-β* | 5F | AAACTCATGAGCAGTCTGCA |
| 3R | AGGAGATCTTCAGTTTCGGAGG |
| *MX1* | 5F | GCACACACCCAACTGTCAGCGA |
| 3R | CCCATGTCCGAAACTCTCTGCGG |
| *ZAP* | 5F | GAATTTATGCAAATATTCTCA |
| 3R | GAAAACGACAGTTCCCTCGGG |

**Supplementary Table S2** Sequences of oligos used in plasmid construction.

|  |  |  |
| --- | --- | --- |
| **Gene name** | **Primer** | **Sequence (5**′**–3**′**)** |
| LAP3-AS1 | 5F | CACGTGACAGGACCGAGCGATGGG |
| 3R | TCATCTTCCGATTTAAAATTTTTT |
| LINC21762 | 5F | CTGTTTGTCCATCTGTCCAATTAC |
| 3R | GGCATTTCCATTAGCACTCCATCT |
| VAMP1-AS1 | 5F | AAGGACTCACATGCATCCTCACCC |
| 3R | TGGGAAGAAAAAGAGGTTTAATTG |
| ZAP-IT1 | 5F | GTGATCTGTGAAAATGGTTCA |
| 3R | GAATTTATTCCCGTGCTG |

**Supplementary Table S3** Sequences of primers used in CRISPR/Cas9 gene editing for ZAP-IT1.

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer** | | **Sequence (5**′**–3**′**)** | **Targeting region** |
| sgRNA1 | 5F | GGCCATCAAGAGTACGCCTA | N terminal |
| 3R | TAGGCGTACTCTTGATGGCC |
| sgRNA2 | 5F | TGTTCCTAAACCAGAAGAGG | C terminal |
| 3R | CCTCTTCTGGTTTAGGAACA |

**Supplementary Table S4** Non-coding RNA profiling by array.

|  |  |  |
| --- | --- | --- |
| **LncRNA** | **Normalized signal** | **Fold Change** |
| LAP3-AS1 | 5.031327392 | 3.100706353 |
| LINC21762 | 7.415824642 | 3.879791443 |
| VAMP1-AS1 | 4.817324742 | 2.011495915 |
| ZAP-IT1 | 5.871964441 | 2.47964136 |

**Supplementary Table S5** The Ct values of lncRNAs detected by qRT-PCR in uninfected cells.

|  |  |  |  |
| --- | --- | --- | --- |
| **LncRNA** | **A549** | **Macrophages** | **LN229** |
| LAP3-AS1 | 28.40 | 28.94 | 27.40 |
| LINC21762 | 29.43 | 31.59 | 28.47 |
| VAMP1-AS1 | 29.58 | 31.88 | 31.21 |
| ZAP-IT1 | 28.99 | 28.13 | 24.42 |

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**Supplementary Fig. S1** Natural abundance of lncRNAs in different cells. Total RNAs of wild-type A549 cells (**A**), monocyte-differentiated macrophages (**B**) and LN229 cells (**C**) were extracted. qRT-PCR assay was performed to detect LAP3-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1 level. Human *U6* level was measured as an internal control. Experiments were independently repeated for three times. Data were shown as mean ± standard deviation.

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**Supplementary Fig. S2** Homologous of ZAP-IT1 and the confirmation of ZAP-IT1 knockout efficiency by DNA sequencing. **A** Homologous of ZAP-IT1 from different species are shown from UCSC genome browser. **B** Genomic DNA of ZAP-IT1Ko cell was extracted and then the region surrounding the sgRNA targeting sequence was amplified and sequenced.

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**Supplementary Fig. S3** Replication of VSV and HSV-1 in ZAP-IT1 knockout cell. **A, B** Control cells and ZAP-IT1Ko cells were infected with DENV2 NGC (MOI = 5), JEV (MOI = 5). The supernatant of DENV2 and JEV was collected at 24 h.p.i. and the viral titers were determined by plaque forming assay. **C, D** Control cells and ZAP-IT1Ko cells were infected with VSV (MOI = 1), HSV-1 (MOI = 1). The supernatant of VSV and HSV-1 was collected at 24 h.p.i. and the viral titers were determined by plaque forming assay. Experiments were independently repeated for three times. Data were shown as mean ± standard deviation. \*\*\* *P* < 0.001. NS, not significant.