**Virologica Sinica**

**Supplementary Data**

**Ebola virus VP35 perturbs type I interferon signaling to facilitate viral replication**

**Zengguo Caoa,1, Chenchen Liua,b,1, Cheng Pengc,1, Yong Ranc, Yulin Yaoa, Gengfu Xiaoa, Entao Lid, Zixi Chene, Xia Chuaia, Sandra Chiud,\***

*a Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, 430071, China*

*b University of Chinese Academy of Sciences, Beijing, 100190, China*

*c National Biosafety Laboratory, Chinese Academy of Sciences, Wuhan, 430020, China*

*d Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, 230026, China*

*e Shenzhen Key Laboratory of Marine Bioresource and Eco-environmental Science, Shenzhen Engineering Laboratory for Marine Algal Biotechnology, Guangdong Provincial Key Laboratory for Plant Epigenetics, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China*

\* Corresponding author.

E-mail address: qiux@ustc.edu.cn (S. Chiu)

1 Zengguo Cao, Chenchen Liu and Cheng Peng contributed equally to this work.

**Supplementary Table S1** Antibodies used in this study.

|  |  |  |
| --- | --- | --- |
| **Antibodies** | **Source** | **Information** |
| Rabbit anti-STAT1 | Cell Signaling Technology (USA) | Cat#14994S |
| Rabbit pSTAT1 | Cell Signaling Technology (USA) | Cat#7649S |
| Rabbit anti-STAT2 | Cell Signaling Technology (USA) | Cat#72604S |
| Rabbit pSTAT2 | Cell Signaling Technology (USA) | Cat#88410S |
| Rabbit anti-Flag | Sigma-Aldrich (USA) | Cat#F7425 |
| Mouse anti-Flag | Cell Signaling Technology (USA) | Cat#8146S |
| Rabbit anti-GAPDH | Sigma-Aldrich (USA) | Cat#G9545 |
| Rabbit anti-Myc | Cell Signaling Technology (USA) | Cat#2278S |
| Mouse anti-Myc | Cell Signaling Technology (USA) | Cat#2276S |
| HRP Goat Anti-Rabbit IgG (H+L) | ABclonal Biotechnology (China) | Cat#AS014 |
| HRP Goat Anti-Mouse IgG (H+L) | ABclonal Biotechnology (China) | Cat#AS003 |
| Goat anti-mouse IgGs conjugated with Alexa 488 | ThermoFisher Scientific (USA) | Cat#A-11001 |
| Goat anti-mouse IgGs conjugated with Alexa 568 | ThermoFisher Scientific (USA) | Cat#A-11004 |
| Goat anti-rabbit IgGs conjugated with Alexa 488 | ThermoFisher Scientific (USA) | Cat#A-11008 |
| Goat anti-rabbit IgGs conjugated with Alexa 568 | ThermoFisher Scientific (USA) | Cat#A-11011 |

**Supplementary Table S2** Primers used in this study.

|  |  |  |
| --- | --- | --- |
| **Target** | **Primers** | **Sequences (5**′ **- 3**′**)** |
| EBOV NP | ZENP-F | CTGACATGGATTACCACAAG |
| EBOV NP | ZENP-R | GGAAACTGTCCGCACTCTC |
| EBOV GP | ZEGP-F | GGGAATGGAGTGGCAACTGA |
| EBOV GP | ZEGP-R | GCTGCTGGTAGACACTCACT |
| Human-GAPDH | Hu-GAPDH-F | TGTTGCCATCAATGACCCCTT |
| Human-GAPDH | Hu-GAPDH-R | CTCCACGACGTACTCAGCG |
| Human-ISG15 | 15-F | CGCAGATCACCCAGAAGATCG |
| Human-ISG15 | 15-R | TTCGTCGCATTTGTCCACCA |
| Human-ISG56 | 56-F | GCTTTCAAATCCCTTCCGCTAT |
| Human-ISG56 | 56-R | ACTTCAAGCACCTTTTCAAAGC |
| Human-OAS1 | OAS1-F | CCAAGCTCAAGAGCCTCATC |
| Human-OAS1 | OAS1-R | GAGCTCCAGGGCATACTGAG |
| Human-IFITM1 | IFITM1-F | TCATCCTGTCACTGGTATTCGGCTC |
| Human-IFITM1 | IFITM1-R | GTGGGTATAAACTGCTGTATCTAGGG |

**Supplementary Figures**

****

**Supplementary Fig. S1** Huh-7 cell were infected with EBOV at an MOI of 1 for 1 h, and then the inoculum was removed and replaced with fresh DMEM containing 2% FBS. At 4 h.p.i. and 24 h.p.i., samples were harvested for cellular RNA extraction.The heatmap was generated to visualize the fold change in expression levels of the target genes at 4 and 24 h.p.i. relative to mock infection group at the same time point, are shown in heatmap. Up-regulated and down-regulated genes are indicated in red and navy, respectively.

****

**Supplementary Fig. S2** Volcano plots showing all differentially expressed genes of EBOV-infected huh-7 cells (24 h.p.i. vs 4 h.p.i.). 2706 up regulated genes (red) and 2519 down regulated genes (green) were selected with –lg (*P* value) > 1.3. Other non-differential genes were represented in blue.

****

**Supplementary Fig. S3** Huh-7 were infected with EBOV at MOI of 0.1 and 1 respectively. Uninfected cells were set as control. Cells were treated with human IFN-α (1,000 U/mL) at 24 h.p.i.. After 24 h of IFN-α treatment, CPE was examined by inverted microscope observations. Scale bar, 400 μm.

****

**Supplementary Fig. S4** HeLa cells were also subjected to the type-I signaling luciferase reporter assay. The data were analyzed by normalizing *Firefly* luciferase values to *Renilla* luciferase values, and then normalized by non-stimulated samples to obtain fold induction. Error bars represent the mean ± S.D. All experiments were performed at least twice. Statistical significance was evaluated by Student’s two-sided *t*-test, \**P* ＜ 0.05, \*\*\**P* ＜ 0.001, \*\*\*\**P* ＜ 0.0001.

****

**Supplementary Fig. S5** The transcription of EBOV in huh-7 cells was quantified at 4 h.p.i. and 24 h.p.i. by mapping RNA-seq reads against the EBOV genome sequence (shown at the bottom). The y-axis represents the number of reads per million mapped reads (RPM).

****

**Supplementary Fig. S6** HEK293T cells were co-transfected with plasmids expressing Flag-tagged VP35 and Myc-tagged JAK1, TYK2, STAT1, or STAT2. At 36 h.p.t., whole cell lysate was incubated with indicated antibody conjugated beads for immunoprecipitation, and detected by Western blotting.