**Virologica Sinica**

**Supplementary Data**

SARS-CoV-2 Nsp8 induces mitophagy by damaging mitochondria

**Shan Zong a, 1, Yan Wu b, 1, Weiling Li a, Qiang You a, Qian Peng a, Chenghai Wang a, Pin Wan a, Tao Bai d, Yanling Ma e, Binlian Sun a,\* and Jialu Qiao a,c,\***

*a Wuhan Institute of Biomedical Sciences, School of Medicine, Jianghan University, Wuhan, 430056, China*

*b State Key Laboratory of Virology, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, 430071, China*

*c Hubei Key Laboratory of Wudang Local Chinese Medicine Research, Hubei University of Medicine, Shiyan, 442000, China*

*d Division of Gastroenterology, Tongji Medical College, Hua Zhong University of Science and Technology, Wuhan, 430030, China*

*e Department of Respiratory and Critical Care Medicine, Hubei Province Clinical Research Center for Major Respiratory Diseases, NHC Key Laboratory of Pulmonary Diseases, Union Hospital, Tongji Medical College, Hua Zhong University of Science and Technology, Wuhan, 430030, China*

\* Corresponding authors.

*E-mail addresses*: jialuqiao@jhun.edu.cn (J. Qiao), binlian17@jhun.edu.cn (B. Sun)

1 Shan Zong and Yan Wu contributed equally to this work.

**Supplementary Table S1** Primers for plasmid construction used in this study.

|  |  |
| --- | --- |
| Primer | Sequence 5`–3` |
| Nsp1-F | CGGAATTCGGATGGAGAGCCTTGTCCCTG |
| Nsp1-R | CCCTCGAGTTACCCTCCGTTAAGCTCACG |
| Nsp2-F | GCGTCGACCGCATACACTCGCTATGTCGATAAC |
| Nsp2-R | GGGGTACCTTAACCGCCTTTGAGTGTGAAGG |
| Nsp3-F | CGGAATTCGGGCACCAACAAAGGTTACTTTTG |
| Nsp3-R  | CCCTCGAGTTACCACCCTTAAGTGCTATCTTTG |
| Nsp4-F | CGGAATTCGGAAAATTGTTAATAATTGGTTGAAGC |
| Nsp4-R | CCCTCGAGTTACTGCAAAACAGCTGAGGTGAT |
| Nsp5-F | CGGAATTCGGAGTGGTTTTAGAAAAATGGCATT |
| Nsp5-R | CCCTCGAGTTATTGGAAAGTAACACCTGAGCATT |
| Nsp6-F | GCGTCGACCAGTGCAGTGAAAAGAACAATCAAG |
| Nsp6-R | GGGGTACCTTACTGTACAGTGGCTACTTTGATACAAG |
| Nsp7-F | CGGAATTCGGTCTAAAATGTCAGATGTAAAGTGCA |
| Nsp7-R | CCCTCGAGTTATTGTAAGGTTGCCCTGTTGTC |
| Nsp8-F | CGGAATTCGGGCTATAGCCTCAGAGTTTAGTTCC |
| Nsp8-R | CCCTCGAGTTACTGTAATTTGACAGCAGAATTGG |
| Nsp9-F | CGGAATTCGGAATAATGAGCTTAGTCCTGTTGCA |
| Nsp9-R | CCCTCGAGTTATTGTAGACGTACTGTGGCAGCT |
| Nsp10-F | CGGAATTCGGGCTGGTAATGCAACAGAAGTG |
| Nsp10-R | CCCTCGAGTTACTGAAGCATGGGTTCGC |
| Nsp12-F | CGGAATTCGGTGCACAATCGTTTTTAAACG |
| Nsp12-R | CCCTCGAGTTACTGTAAGACTGTATGCGGTGTG |
| Nsp13-F | GCGTCGACCGCTGTTGGGGCTTGTGTTC |
| Nsp13-R | GGGGTACCTTATTGTAAAGTTGCCACATTCCTAC |
| Nsp14-F | CGGAATTCGGGCTGAAAATGTAACAGGACTCTTT |
| Nsp14-R | CCCTCGAGTTACTGAAGTCTTGTAAAAGTGTTCCAG |
| Nsp15-F | GCGTCGACCAGTTTAGAAAATGTGGCTTTTAATG |
| Nsp15-R | GGGGTACCTTATTGTAATTTTGGGTAAAATGTTTCT |
| Nsp16-F | CGGAATTCGGTCTAGTCAAGCGTGGCAACC |
| Nsp16-R | CCCTCGAGTTAGTTGTTAACAAGAACATCACTAGAAAT |
| GFP-Nsp8-F | CCGCTCGAGGCTATAGCCTCAGAGTTTAGTTCC |
| GFP-Nsp8-R | GAAT GCGGCCGC TTACTGTAATTTGACAGCAGAATTG |
| Nsp8-N-F | CCCTCGAGGGATGGCTATAGCCTCAGAGTTTAGTT |
| Nsp8-N-R | TTGCGGCCGCAATTACAACTTTCTAAGCATAGTGAAAAG |
| Nsp8-C-F | CCCTCGAGGGATGGATAATGATGCACTCAACAA |
| Nsp8-C-R | TTGCGGCCGCAATTACTGTAATTTGACAGCAGAATTG |
| GFP-Nsp8-N-F | CCGCTCGAGCGGATGGCTATAGCCTCAGAGTTTAGTT |
| GFP-Nsp8-N-R | TTGCGGCCGCAATTACAACTTTCTAAGCATAGTGAAAAG |
| GFP-Nsp8-N-F | CCGCTCGAGCGGATGGATAATGATGCACTCAACAA |
| GFP-Nsp8-N-R | TTGCGGCCGCAATTACTGTAATTTGACAGCAGAATTG |
| pCDH-Nsp8-F | CGGAATTCATG GCTATAGCCTCAGAGTTTAGTTCC |
| pCDH-Nsp8-R | CGGGATCC CTGTAATTTGACAGCAGAATTGG |

****

**Supplementary Fig S1.** Generation of Nsp8 expression cell lines. **A** A549 or A549-Nsp8 cells (Nsp8 expression cell lines) were measured fluorescence intensity by flow cytometry. **B** A549 cells were transfected with 2 μg GFP-Nsp8 plasmid for 24 h. Then, A549 or A549-Nsp8 cells were detected by fluorescence microscopy. Scale bar = 200 μm. **C** A549 or A549-Nsp8 cells were collected and lysed. LC3, Flag-Nsp8, and β-actin (loading control) were analyzed by Western blotting.

****

**Supplementary Fig S2.** Analysis of SARS-CoV-2 non-structural proteins in autophagy. **A** A549 cells were transfected with 2 μg Flag-Nsp1 to Flag-Nsp10 and Flag-Nsp12 to Flag-Nsp16 plasmids for 24 h. Flag-Nsp1 to Flag-Nsp16 without Flag-Nsp11 and LC3 expression levels were detected by Western blotting. **B** A549 cells were transfected with 2 μg indicated plasmids for 24 h or treated with 50 μmol/L CQ for 6 h or EBSS for 1 h before harvesting. LC3, Flag-Nsp8, and β-actin (loading control) were analyzed by Western blotting.