Supplementary information

Aichi virus 3C protease modulates LC3- and SQSTM1/p62-involved antiviral response

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Table S1. Primer sequences for AiV viral gene cloning.

| Target | Primer sequences (5’ to 3’) | Cloning site |
|--------|-----------------------------|--------------|
| AiV-L  | F CCGCCGAATTCA ATG GCTGCAACACGGGTTCGTA  | EcoR I |
|        | R CCCGGGATCC TCA TTGCCCCGTGAAGGTGGTGA  | BamH I |
| AiV-VP0| F CCGCCGAATTCA ATG GGAACCTCGGTCAACAAA  | EcoR I |
|        | R CCCGGGATCC TCA CTGTTGGGCGAGGTAG       | BamH I |
| AiV-VP3| F CGATAGATCTG ATG CACTGGAAGACTCGCACC    | Bgl II |
|        | R CCCGGGATCC TCA CTGGGAAGTGAGGGCAG      | BamH I |
| AiV-VP1| F CCGCCGAATTCA ATG ACCCTCCACCAAGACCCCTC| EcoR I |
|        | R CCCGGGATCC TCA CTAGGTTGGGCGAGCTG      | BamH I |
| AiV-2A | F CCGCCGAATTCA ATG GTCCACTGGGCCATCC     | EcoR I |
|        | R ATCCCTCTAGA TCA CTGTCCGCTGATGCTGG     | Xba I |
| AiV-2B | F CGATAGATCTG ATG GGCCTCCCTCAACCTCT     | Bgl II |
|        | R CCCGGGATCC TCA TTGGAGTTCAAGGGTGCCC    | BamH I |
| AiV-2C | F CCGCCGAATTCA ATG GGGCTCAAAAGACCTACAC  | EcoR I |
|        | R CCCGGGATCC TCA CTGGCAGTGTAGGAGGA      | BamH I |
| AiV-3A | F CCGCCGAATTCA ATG GGTAAACCGGGTCTCG     | EcoR I |
|        | R CCCGGGATCC TCA TTGGGGTTCGCCGTCG       | BamH I |
| AiV-3B | F CCGCCGAATTCA ATG GCTGCTACTCTGCTATC    | EcoR I |
|        | R CCCGGGATCC TCA TTGGCGCTGAGTGCGC       | BamH I |
| AiV-3C | F CCGCCGAATTCA ATG GGAATTCCTCCCAGCTG    | EcoR I |
|        | R CCCGGGATCC TCA TTGGCTGGGTGGTGGGAAAT   | BamH I |
| AiV-3D | F CCGCCGAATTCA ATG TCTTCTATGTCCCACTG    | EcoR I |
|        | R CCCGGGATCC TCA GCCAGCCACGGATGTGAG     | BamH I |
| AiV-3C H42D | TACCTTCTGGTCCCACGGACCTCCGTGAAACCCCA  | Site-directed mutagenesis |
| AiV-3C C143S  | CGACCTTCGAGGTCTGTCGGGATCCCCCGCTTGT  | Site-directed mutagenesis |

F: forward primer; R: reverse primer

Bold letter: Insert other nucleotides for transcription start site

Below line: Insert restriction enzyme sequences for gene cloning
Table S2. Primer sequences for qPCR.

| Viral gene | Primer sequences (5’ to 3’) |
|------------|-----------------------------|
| Human IFNα1 | F CTCGCGCTTTGGCTTTACTG<br>R GCCCAGAGGAGCAGCTTGACT |
| Human IFNβ  | F TGA GCA GTG TGC ACC TGA AA<br>R GCT TGA AGC AAT TGT CCC GT |
| Human RIG-I | F GCA GAG GCC GGC ATG AC<br>R TGT AGG TAG GGT CCA GGG TCT TC |
| Human MDA5  | F TGC TTC TCT AAG TGG GCA GC<br>R TTT TCA CCC TGG CCC TGA AG |
| Human TBK1  | F GGA GAC CCG GCT GGT ATA A<br>R TGA ACA TCC ACT GGA AGG |
| Human IRF3  | F GAC CTT CCA TCG TAG GCC G<br>R AAT CCT CCT GCT GTG CAT CC |
| Human IRF7  | F AGC TGT GCT GGC GAG AAG<br>R TGG AGT CCA GCA TGT GTG TG |
| Human IKKε  | F AAG AGC CGG GAT CAG GTA CA<br>R CAT CTT GTC CAA ACA GCA CTG AA |
| Human ISG15 | F GGT GGA CAA ATG CGA CGA A<br>R ATG CTG GTG GAG GCC CTT A |
| Human IFIT3 | F GCT GAA GGA GAG CAG TTT GTT GA<br>R AGG ACA TCT GTT TGGCAA GGA |
| Human Viperin | F CAA GGA AGA ATG TGA GCA AGA GTA GA<br>R TGA TAT GGT GAC ATG GCT TCA CT |
| Human MyD88 | F GAG CTG GCG GGC ATC AC<br>R TCG AAA CGC TCA GGC AIA TG |
| Human Trim5α | F GCC TGG AAC TCC TGA CAC AAC<br>R CAT GGA CTT CTT GTG GTT TGC A |
| Human Trim25 | F CGA GGT GGA ACT GAA CCA CA<br>R GTG GAT TTT GTG GTG GAC GC |
| Human LC3   | F GGC GCT TAC AGC TCA ATG C<br>R ACC ATG CTG TGT CCG TTC AC |
| Human p62   | F CCA TGT CCT ACG TGA AGG ATG A<br>R CCG CGG GCA CTC TTT TT |
| Human TNFα  | F TGC TCC TCA CCC ACA CCA T<br>R GGA GGT TGA CCT TGG TCT GGT A |
| Human IL-6  | F GCT GCA GGC ACA GAA CCA<br>R GCT GGG CAG AAT GAG ATG AG |
| Human IL-8  | F CTG GCC GTG GCT CTC TTT<br>R CTT GGC AAA ACT GCA CCT TCA |
| Human CXCL10 | F CCT GCA AGC CAA TTT GTG CCA<br>R TGC ATC GAT TTT GCT CCC CT |
| Human GAPDH | F CAA CTG GTC GTG GAC AAC CAT<br>R GCA CGG ACA CTC ACA ATG TTC |

F: forward primer; R: reverse primer
Figure S1. Evaluation of regulation effect of Atg conjugation system in RLR pathway. (A) Luciferase reporter assay of IFNβ, NFκB, AP-1 and ISRE in A549 cells (1×10^5) with overexpression of Atg5, Atg7, Atg12 and mAtg16L1 expression vectors or polyI:C stimulation for 24 h. (B) Immunoblotting assay of RLR signaling, LC3 and p62 protein levels in A549 cells with Atg5, or mAtg16L1. GFP-p62 and V5-MAVS were the positive control.
Figure S2

Figure S2. Analysis of RLR signaling in LC3- and p62-knockdown cells. (A and B)
Immunoblotting assay of RLR signal proteins in shCtrl, shLC3 or shp62 A549 cells with polyI:C stimulation.
Figure S3

**Figure S3. AiV attenuates polyI:C-promoted RLR response.** (A) A549 cells were cotransfected with IFNβ-Luc reporter and pRL-TK for 24 h, and then infected by AiV (MOI = 1). Cells were then stimulated with polyI:C for 24 h and dual luciferase assay was performed. (B) Mock- or AiV-infected A549 cells were stimulated with polyI:C, cell lysates were subjected to immunoblotting with the indicated antibody. (C) IFNβ-Luc reporter, pRL-TK and RIG-I expression vector (300, 600, 1200 ng) were transfected into A549 cells for 24 h, which were then infected by AiV (MOI 1). Dual luciferase assay was performed at 24 h after infection. Data are mean±SD from three independent tests. RIG-I expression and AiV VP1 expression are shown in panel (D). (E) A549 cells were cotransfected with RIG-I expression vector (1200 ng) and AiV 3C vector (300, 600, 1200 ng). Post-transfection 24 h, cell extracts underwent immunoblotting analysis.