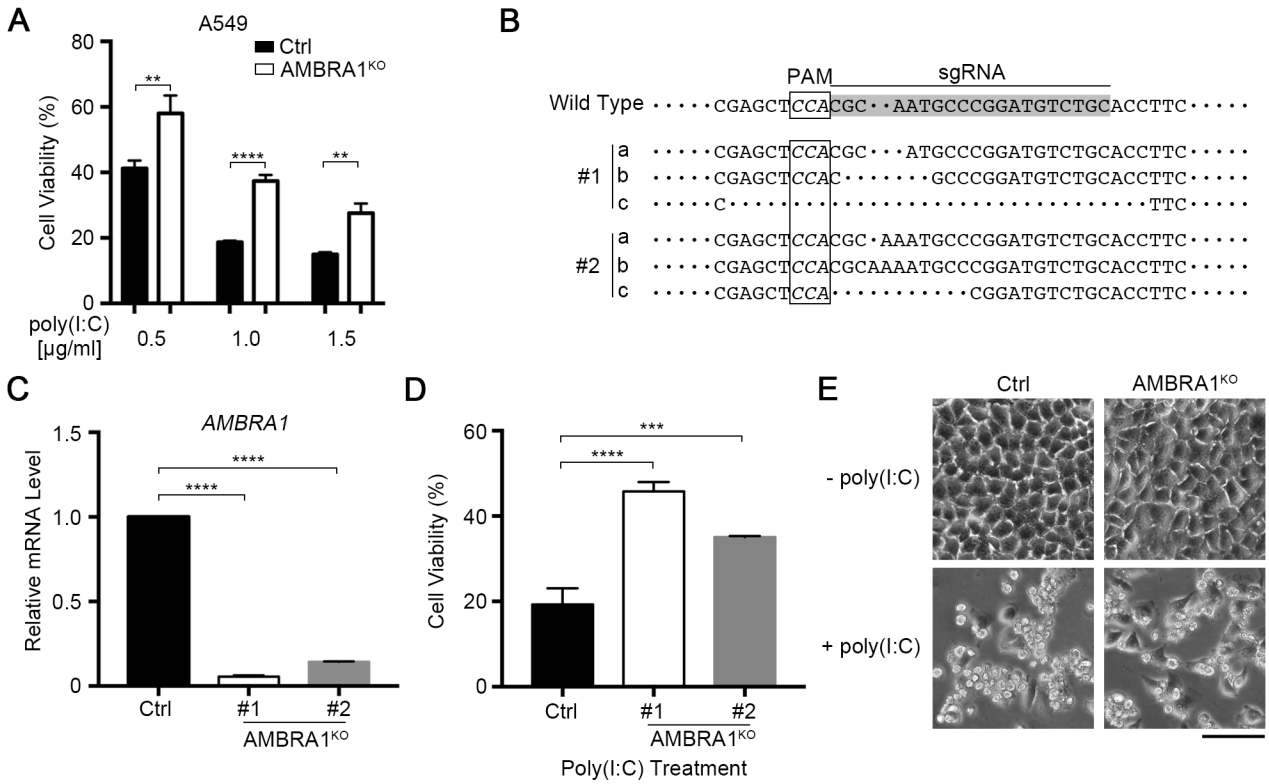

Fig. S1. AMBRA1 promotes dsRNA-induced cell death. (A) Cell viabilities. The control and AMBRA1^{KO} cells were transfected with 0.5, 1, and 1.5 μg/ml poly(I:C). At 24 h post-transfection, cell viabilities were measured by CCK8 assay. P values were calculated by unpaired, two-tailed student's test; ****p < 0.0001, **p < 0.01. (B) Sequences of two HeLa AMBRA1^{KO} cell clones. Genomic DNA was extracted and the region surrounding sgRNA targeting sequences was amplified and sequenced. (C) *AMBRA1* mRNA levels. Total RNAs of the control and two AMBRA1^{KO} HeLa cell clones were extracted and applied for qRT-PCR. Data were presented as mean ± SD from three experiments; P values were calculated by ANOVA with Dunnett's multiple comparison test; ****p < 0.0001. (D) Cell viabilities. The control and two AMBRA1^{KO} HeLa cell clones were transfected with poly(I:C). MTT assay was performed at 24 h post-transfection. Data were presented as mean ± SD from three experiments; P values were calculated by ANOVA with Dunnett's multiple comparison test; ***p < 0.001, ****p < 0.0001. (E) Cell images. The control and AMBRA1^{KO} HeLa cells were transfected with poly(I:C) and then cell images were taken at 24 h post-transfection. Representative images of three independent experiments were shown. Scale bar, 50 μm.

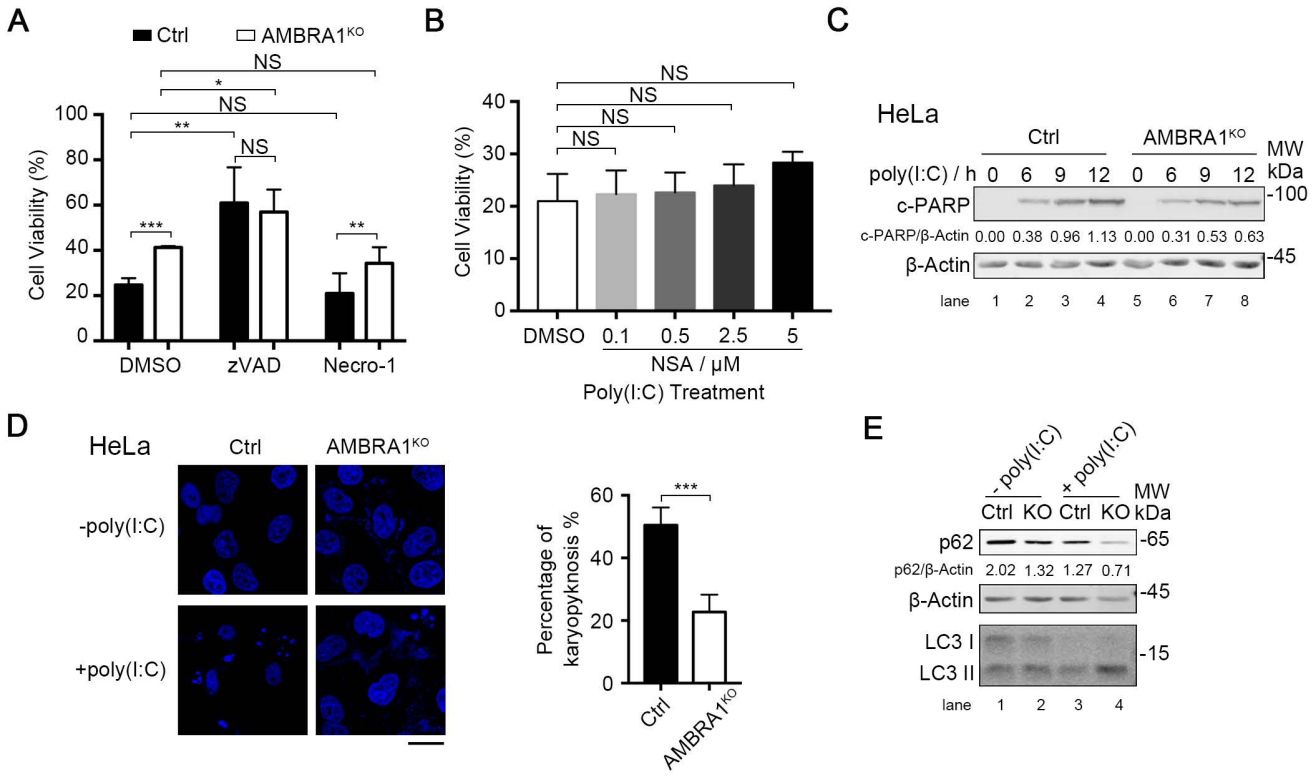

Fig. S2. AMBRA1 promotes the dsRNA-induced apoptosis. (A) Cell viability. The control and AMBRA1^{KO} A549 cells were pretreated with DMSO, z-VAD(OMe)-FMK (zVAD) (50 μM) or Necrostatin-1 (Necro-1) (100 μM), followed by transfection with poly(I:C). The MTT assay was performed at 24 h post-transfection. Data were presented as mean ± SD from three experiments; P values were calculated by unpaired, two-tailed student's test; ***p < 0.001, **p < 0.01, and NS, not significant, or by ANOVA with Dunnett's multiple comparison test; **p < 0.01, *p < 0.05, and NS, not significant. (B) Cell viability. A549 cells were pretreated with DMSO or of Necrosulfonamide (NSA) at indicated concentrations for 1 h, followed by transfection with poly(I:C). The MTT assay was performed at 24 h post-transfection. Data were presented as mean ± SD from three experiments; P values were calculated by ANOVA with Dunnett's multiple comparison test; NS, not significant. (C) Western blot. The control and AMBRA1^{KO} HeLa cells were treated with poly(I:C) for indicated time and then c-PARP was measured by western blot. α-Tubulin was probed as an internal control. Representative blots of three independent experiments were presented. (D) Immunofluorescence microscopy. The control and AMBRA1^{KO} HeLa cells were transfected with poly(I:C) for 12 h and then stained with hoechst. Cell images were taken to reveal karyopyknosis. Representative images of three independent experiments were shown. Scale bar, 20 μm. P values were calculated by unpaired, two-tailed student's test; ***p < 0.001. (E) Western blot. The control and AMBRA1^{KO} A549 cells

were stimulated with poly(I:C). At 9 h post-transfection, the whole cell lysates were collected for detection of p62 and LC3 I/II levels. β-actin was probed as an internal control. Representative blots of three independent experiments were presented.

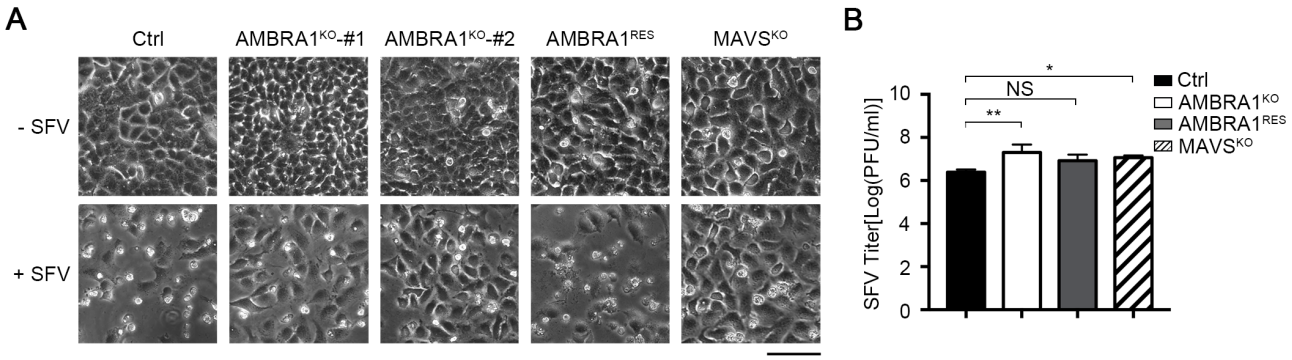

Fig. S3. Effects of AMBRA1 or MAVS depletion on the cell death induced by SFV and viral replication. (A) The control, AMBRA1^{KO}, AMBRA1^{RES}, and MAVS^{KO} A549 cells were infected with SFV. Cell images were taken at 48 h p.i. Representative images of three independent experiments were shown. Scale bar, 50 μm. (B) Viral titers. The supernatants were collected at 24 h p.i. for plaque assay. Data were presented as mean ± SD from three experiments; P values were calculated by ANOVA with Dunnett's multiple comparison test; **p < 0.01, *p < 0.05, and NS, not significant.

Table S1. Sequences of oligos used in CRISPR/Cas9 gene editing

Genes	Primers	Sequences (5'-3')	Targeting region
AMBRA1	sg-AMBRA1-5F	GCAGACATCCGGGCATTGCG	Exon 11
	sg-AMBRA1-3R	CGCAATGCCCGGATGTCTGC	
MAVS	sg-MAVS-5F	CTGTGAGCTAGTTGATCTCG	Exon 3
	sg-MAVS-3R	CGAGATCAACTAGCTCACAG	

Table S2. Sequences of primers used in PCR amplification of gene fragments

Genes	Sequences (5'-3')
AMBRA1-5F	ATTTGCGGCCGCATGAAGGTTGTCCCAGAAAAGAATGC
AMBRA1-FLAG-3R	TTGCGGCCGCCTACTTATCGTCGTCATCCTTGTAATCACCA CCACCCCTGTTCCGTGGTTCTCCCCTAG
AMBRA1-NGG-5F	TGATAGATCAAGGCACCGAGCTGCACGTAAT
AMBRA1-NGG-3R	CAGACATTCGTGCATTACGTGCAGCTCG
AMBRA1-5F	CGGAATTCATGAAGGTTGTCCCAGAAAAGAATGC
AMBRA1-HA-3R	CCAAGCTTCTAAGCGTAATCTGGAACATCGTATGGGTAACC ACCACCCCTGTTCCGTGGTTCTCCCCTAG
AMBRA1-5F	CTCTAGATCGCGAACGCGTATGAAGGTAGTGCCTGAAAAG A
AMBRA1-HA-3R	GCCGCCCTCGAGGAATTCCTAAGCGTAATCTGGAACATCGT ATGGGTAACCACCACCCCTGTTCCGTGGTTCTCCCCTAG
MDA5-5F	CGGGATCCATGTCGAATGGGTATTCCACA
MDA5-FLAG-3R	CCGCTCGAGCTACTTATCGTCGTCATCCTTGTAATCACCAC CACCATCCTCATCACTAAATAAACAGC
RIG-I-5F	CCCAAGCTTATGACCACCGAGCAACGACG
RIG-I-FLAG-3R	CCGCTCGAGTCACTTATCGTCGTCATCCTTGTAATCACCAC CACCTTTGGACATTTCTGCTGG
1-173-5F (MAVS)	CCAAGCTTATGCCGTTTGCTGAAGACAA
1-173-FLAG-3R (MAVS)	CCCTCGAGCTACTTATCGTCGTCATCCTTGTAATCACCACC ACCTGGATTCCTTGGGATGGCTCT
AMBRA1-TA clone-5F	AAGACAACCCCCCAAGACACA
AMBRA1-TA clone-3R	TGGCTAACCATCATCCGTCAAGAG

Table S3. Sequences of primers used in qRT-PCR.

Genes	Sequences (5'-3')
AMBRA1-5F	AGCTCCACGCAATGCCCGGAT
AMBRA1-3R	CAGACTGTCCATCACCGATCACT
β-actin-5F	GCTCCTCCTGAGCGCAAG
β-actin-3R	CATCTGCTGGAAGGTGGACA
SFV-5F	CCGGAGGACGCACAGAAGTTG
SFV-3R	TGCGACGGCCACAATCGGAAG