

Figure. S1. SARS-CoV-2 ORF3a promoted the expression of autophagy-related genes.

Vero-E6 cells ($1-2 \times 10^5$ cells) were transfected with HA-Tag-ORF3a (2 μ g) or empty vector (2 μ g) for 24 h. qPCR was performed to detect the expression level of *ULK1*, *WIP1*, *BECLIN-1*, *Atg5*, *Atg7*, and *LC3*.

Data were analyzed by T-tests or T-tests with Welch's correction. qPCR Data (mean \pm SEM) were representative of three independent experiments. (* $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.001$)

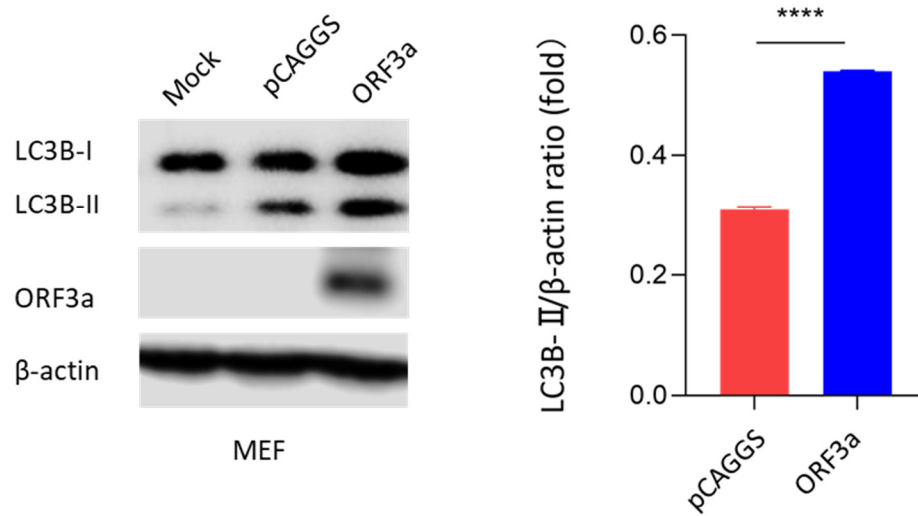


Figure. S2. ORF3a could induce autophagy.

MEF cells ($1-2 \times 10^5$ cells) were transfected with HA-Tag-ORF3a (2 μ g) or empty vector (2 μ g) for 24 h. Western blot was performed to detect LC3B.

Data were analyzed by T-tests. Data were representative of three independent experiments. (, **** $p \leq 0.001$)

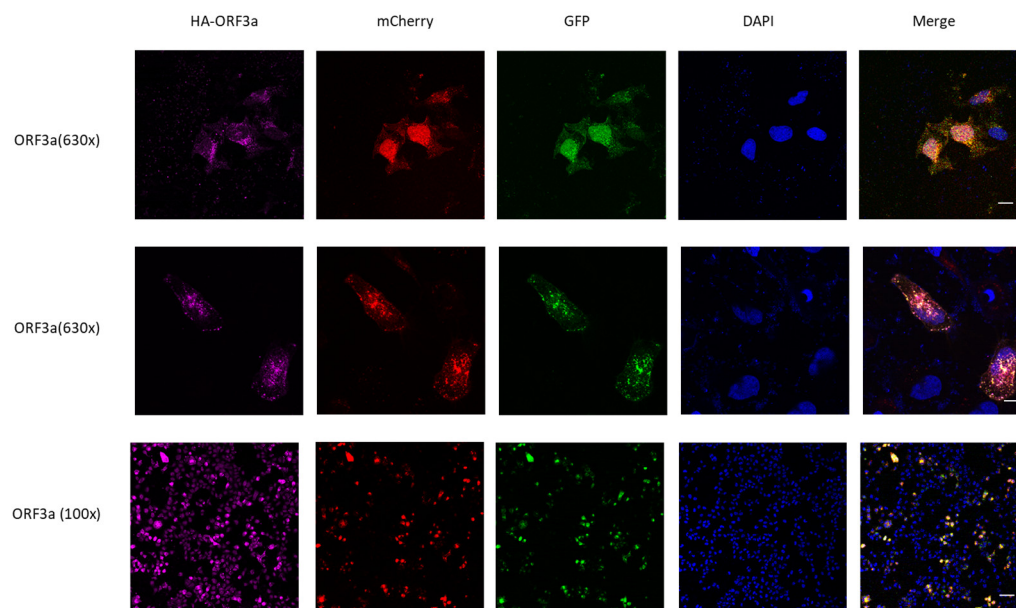


Figure. S3. ORF3a-induced autophagy was an incomplete process.

HeLa cells ($1-2 \times 10^5$ cells) were co-transfected with mCherry-GFP-LC3B (1 μ g) and

HA-Tag-ORF3a (1 μ g) for 24 h. Immunofluorescence was performed to assess autophagy status. Scale bars: 4 μ m.

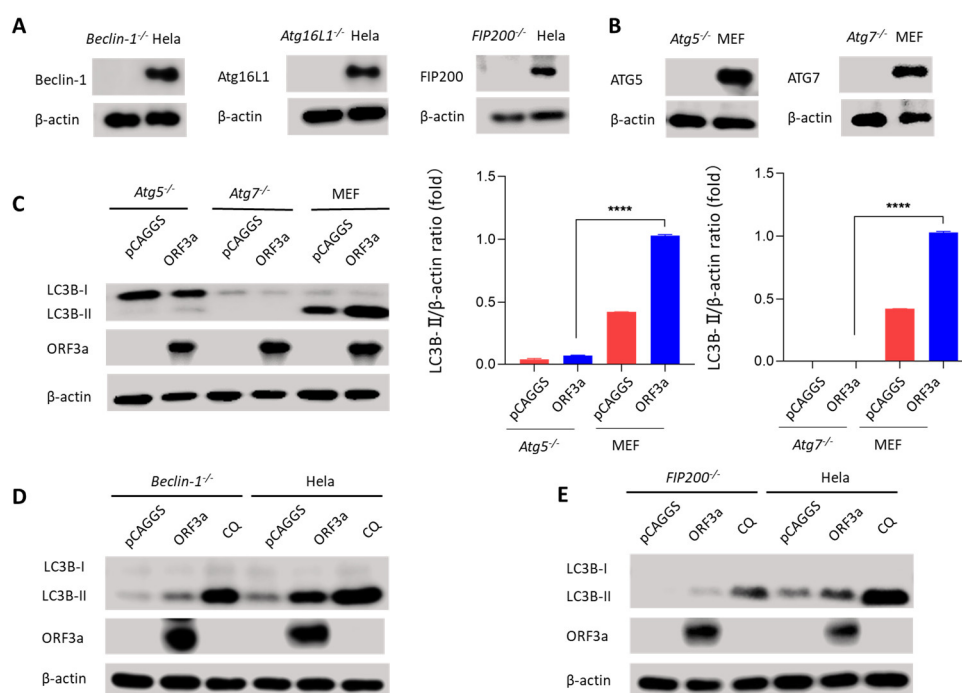


Figure. S4. ORF3a-induced autophagy was classic.

(A) *Beclin-1*, *Atg16L1*, and *FIP200* KO HeLa cells ($1-2 \times 10^5$ cells) were lysed to verify successful knockout.

(B) *Atg5* and *Atg7* KO MEF cells ($1-2 \times 10^5$ cells) were lysed to verify successful knockout.

(C) *Atg5* KO, *Atg7* KO, and WT MEF cells ($1-2 \times 10^5$ cells) were transfected with HA-Tag-ORF3a (2 μ g) or empty vector (2 μ g) for 24 h. Western blot was performed to detect LC3B.

(D and E) HeLa cells ($1-2 \times 10^5$ cells) and *Beclin-1* / *FIP200* KO HeLa cells ($1-2 \times 10^5$ cells) were transfected with HA-Tag-ORF3a (2 μ g) or empty vector (2 μ g) for 24 h or treated with CQ (50 nM) for 6 h. Western blot was performed to detect LC3B.

One-way ANOVAs followed by Dunnett's multiple comparisons test were used in comparing multiple groups. Data were representative of 3 independent experiments.

(**** $p \leq 0.001$)

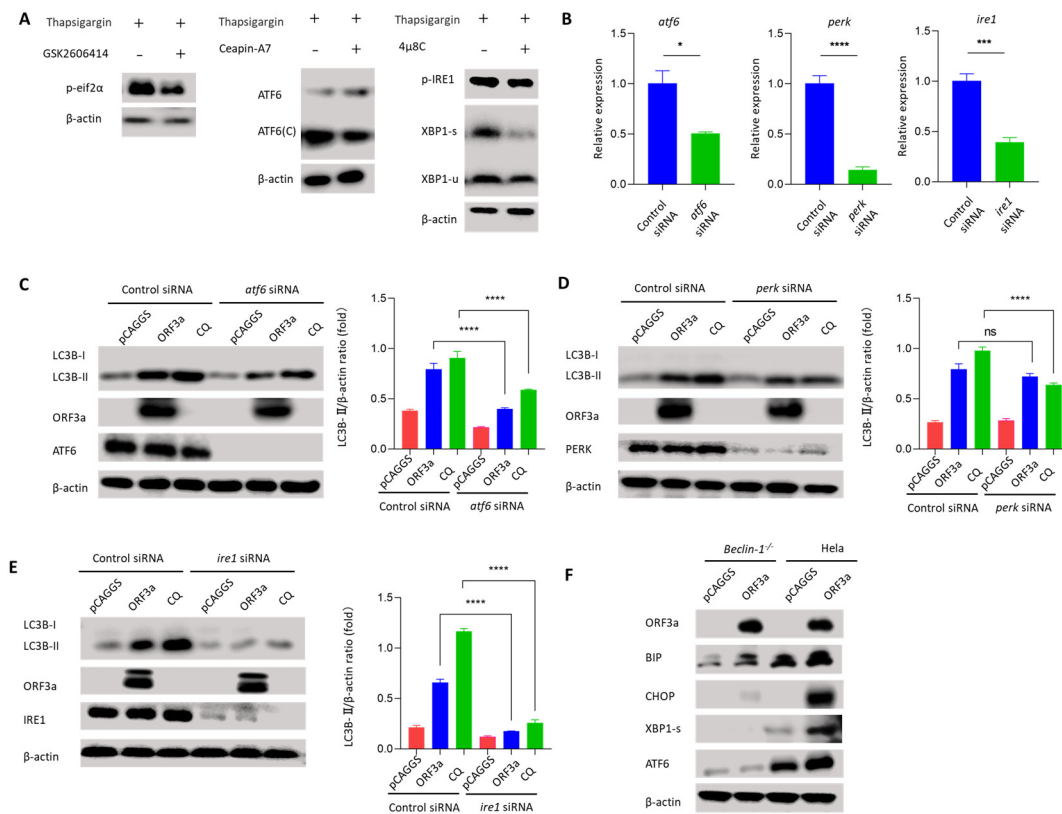


Figure. S5. ORF3a induced autophagy through unfolded protein response.

(A) 293T cells ($1-2 \times 10^5$ cells) were pretreated with Ceapin-A7 (10 nM), GSK2606414 (5 nM), 4μ8C (10 nM) for 24 h and treated with Thapsigargin (2 μM) for 2 h before harvest. Western blot was performed to detect relative UPR proteins.

(B) HeLa cells ($1-2 \times 10^5$ cells) were transfected with *atf6* / *perk* / *ire1* siRNA (50 nM) or control siRNA (50 nM) for 24 h. qPCR was performed to detect the expression level of *atf6*, *perk*, *ire1*.

(C, D and E) HeLa cells ($1-2 \times 10^5$ cells) were transfected with HA-Tag-ORF3a (2 μg) or empty vector (2 μg) and *atf6* / *perk* / *ire1* siRNA (50 nM) or control siRNA (50 nM) for 36 h. HeLa cells ($1-2 \times 10^5$ cells) were transfected with *atf6* / *perk* / *ire1* siRNA (50 nM) or control siRNA (50 nM) for 36 h and treated with CQ (50 nM) for 6 h before harvest. Western blot was performed to detect LC3B.

(F) HeLa cells ($1-2 \times 10^5$ cells) and *Beclin-1* KO HeLa cells ($1-2 \times 10^5$ cells) were transfected with HA-Tag-ORF3a (2 μg) or empty vector (2 μg) for 24 h Western blot was performed to detect relative UPR proteins.

Data were analyzed by T-tests or T-tests with Welch's correction. One-way ANOVAs followed by Dunnett's multiple comparisons test were used in comparing multiple groups. Data were representative of three independent experiments. ($*p \leq 0.05$, $***p \leq$

0.001, *** $p \leq 0.001$)

Supplementary Table S1.

Gene ID	Gene Symbol	Species	Sequence 5' to 3'
60	<i>β-actin</i> -F	human	CATGTACGTTGCTATCCAGGC
60	<i>β-actin</i> -R	human	CTCCTTAATGTCACGCACGAT
8408	<i>ULK1</i> -F	human	GGCAAGTTCGAGTTCTCCCG
8408	<i>ULK1</i> -R	human	CGACCTCCAAATCGTGCTTCT
9474	<i>ATG5</i> -F	human	AAAGATGTGCTTCGAGATGTGT
9474	<i>ATG5</i> -R	human	CACTTTGTCAGTTACCAACGTCA
10533	<i>ATG7</i> -F	human	CAGTTTGCCCCTTTTAGTAGTGC
10533	<i>ATG7</i> -R	human	CCAGCCGATACTCGTTCAGC
8678	<i>Beclin-1</i> -F	human	CCATGCAGGTGAGCTTCGT
8678	<i>Beclin-1</i> -R	human	GAATCTGCGAGAGACACCATC
81631	<i>LC3</i> -F	human	GATGTCCGACTTATTCGAGAGC
81631	<i>LC3</i> -R	human	TTGAGCTGTAAGCGCCTTCTA
9776	<i>ATG13</i> -F	human	TTGCTATAACTAGGGTGACACCA
9776	<i>ATG13</i> -R	human	CCCAACACGAACTGTCTGGA
55062	<i>WIP1</i> -F	human	ACTAAAGCCGGGTATAAGCTGT
55062	<i>WIP1</i> -R	human	CGGGATTTCATTGCTTCCGTG
22241	<i>ULK1</i> -F	mouse	AAGTTCGAGTTCTCTCGCAAG
22241	<i>ULK1</i> -R	mouse	ACCTCCAGGTCGTGCTTCT
56208	<i>Beclin-1</i> -F	mouse	ATGGAGGGGTCTAAGGCGTC
56208	<i>Beclin-1</i> -R	mouse	TGGGCTGTGGTAAGTAATGGA
55062	<i>WIP1</i> -F	mouse	CTGCTTCTCTTTCAACCAAGACT
55062	<i>WIP1</i> -R	mouse	ACGTCAGGGATTTCATTGCTT
67443	<i>LC3</i> -F	mouse	TTATAGAGCGATACAAGGGGGAG
67443	<i>LC3</i> -R	mouse	CGCCGTCTGATTATCTTGATGAG
74244	<i>ATG7</i> -F	mouse	TCTGGGAAGCCATAAAGTCAGG
74244	<i>ATG7</i> -R	mouse	GCGAAGGTCAGGAGCAGAA
11793	<i>ATG5</i> -F	mouse	TGTGCTTCGAGATGTGTGGTT
11793	<i>ATG5</i> -R	mouse	ACCAACGTCAAATAGCTGACTC
11461	<i>β-actin</i> -F	mouse	GGCTGTATTCCCCTCCATCG
11461	<i>β-actin</i> -R	mouse	CCAGTTGGTAACAATGCCATGT
22926	<i>ATF6</i> -F	human	TCCTCGGTCAGTGGACTCTTA
22926	<i>ATF6</i> -R	human	CTTGGGCTGAATTGAAGGTTTGT
9452	<i>PERK</i> -F	human	ACGATGAGACAGAGTTGCGAC
9452	<i>PERK</i> -R	human	ATCCAAGGCAGCAATTCTCCC
2081	<i>IRE1</i> -F	human	CACAGTGACGCTTCCTGAAAC
2081	<i>IRE1</i> -R	human	GCCATCATTAGGATCTGGGAGA

Supplementary Table S2.

Gene ID	Gene Symbol	Species	Sequence 5' to 3'
9452	<i>Siperk-1</i> -F	human	GCCAUAAUGGACAUAGUGATT
9452	<i>Siperk-1</i> -R	human	UCACUAUGUCCAUAUUGGCTT
9452	<i>Siperk-2</i> -F	human	CAAUCUUGCAGUAUCCAUAATT
9452	<i>Siperk-2</i> -R	human	UAUGGAUACUGCAAGAUUGTT

9452	<i>siperk-3-F</i>	human	CCUCAAGCCAUCCAACAUAUU
9452	<i>siperk-3-R</i>	human	AAUAUGUUGGAUGGCUUGAGG
2081	<i>siirel-1-F</i>	human	CCCAUCAACCUCUCUUCUGUA
2081	<i>siirel-1-R</i>	human	UACAGAAGAGAGGUUGAUGGG
2081	<i>siirel-2-F</i>	human	CAUCGUUCACAGAGACCUATT
2081	<i>siirel-2-R</i>	human	UAGGUCUCUGUGAACGAUGTT
2081	<i>siirel-3-F</i>	human	CGAUCGUGAAGCAGUUAGATT
2081	<i>siirel-3-R</i>	human	UCUAAACUGCUUCACGAUCGTT
22926	<i>siatf6-1-F</i>	human	GCAGCAACCAAUUAUCAGUUU
22926	<i>siatf6-1-R</i>	human	AAACUGAUAAUUGGUUGCUGC
22926	<i>siatf6-2-F</i>	human	GCUGGAUGAAGUUGUGUCATT
22926	<i>siatf6-2-R</i>	human	UGACACAACUUCAUCCAGCTT
22926	<i>siatf6-3-F</i>	human	GGUUCAUAGACAUGAAGUATT
22926	<i>siatf6-3-R</i>	human	UACUUCAUGUCUAUGAACCTT

Supplementary clone map.

