SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection

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Autophagy is an essential cellular process affecting virus infections and other diseases and Beclin1 (BECN1) is one of its key regulators. Here, we identified S-phase kinase-associated protein 2 (SKP2) as E3 ligase that executes lysine-48-linked poly-ubiquitination of BECN1, thus promoting its proteasomal degradation. SKP2 activity is regulated by phosphorylation in a hetero-complex involving FKBP51, PHLPP, AKT1, and BECN1. Genetic or pharmacological inhibition of SKP2 decreases BECN1 ubiquitination, decreases BECN1 degradation and enhances autophagic flux. Middle East respiratory syndrome coronavirus (MERS-CoV) multiplication results in reduced BECN1 levels and blocks the fusion of autophagosomes and lysosomes. Inhibitors of SKP2 not only enhance autophagy but also reduce the replication of MERS-CoV up to 28,000-fold. The SKP2-BECN1 link constitutes a promising target for host-directed antiviral drugs and possibly other autophagy-sensitive conditions.
Macautophagy (subsequently referred to as autophagy) is a conserved cytosolic degradation process in eukaryotes that maintains macromolecule, energy and organelle homeostasis. It requires a fine-tuned sequence of molecular actions largely executed by autophagy-related genes (ATGs). In the initial steps, a phagophore consisting of a double-membrane sheet is nucleated, amplified and finally closed to form a vesicular structure called an autophagosome that engulfs cellular material for degradation. Degradation is accomplished after the fusion of autophagosomes (AP) with lysosomes to form autolysosomes (AL).

A more recently emerged regulator of autophagy is the HSP90 cochaperone FK506 binding protein (FKBP)51. In general, FKBP51 is a stress-regulated protein involved in numerous pathways, likely through scaffolding regulatory protein interactions. FKBP51-induced autophagy is accompanied by elevated levels of Beclin1 (BECN1) through a yet unknown mechanism. This multifunctional protein is crucial in autophagy and membrane trafficking processes. In mammals, BECN1 is an essential component of two phosphatidylinositol 3-kinase (PI3K) complexes through its interaction with either Barkor (BECN1-associated autophagy-related key regulator)/ATG14 or UVRAG (UV resistance-associated gene). The ATG14 complex is important for the initial nucleation steps of autophagy, whereas the UVRAG containing complex functions in endocytic trafficking and autophagosomal maturation. ATG14 is also found on mature autophagosomes where it enhances tethering and fusion of autophagosomes with lysosomes.

The regulation of BECN1 through post-translational modification is well documented, and in particular involves phosphorylation and ubiquitination. The oncopgenic kinase AKT1 phosphorylates BECN1 at positions S234, S295, which leads to sequestration of this peripheral membrane binding protein to the cytoskeleton with the result of inhibition of autophagy. Several other autophagy-regulating kinases (both stimulatory and inhibitory) also target BECN1. Furthermore, distinct E3 ligases of different types modify BECN1 with K11-, K48- or K63-linked poly-ubiquitins, complemented by ubiquitin-specific peptidases (USPs). Depending on the site and type of linkage of BECN1 ubiquitination, autophagy is inhibited or enhanced; the underlying mechanisms involve degradation of BECN1 and modulation of its protein interactions.

Studies investigating the interplay between autophagy and viruses revealed that some viruses use parts of the autophagy machinery for their own replication but also have evolved strategies to escape autophagic degradation. Congruently, autophagy-inducing agents can have antiviral effects. In case of coronaviruses (CoV), the formation of replication complexes at double membrane vesicles (DMV) and, most likely, of infectious particles depend on ER-derived membranes, as does autophagy. Thus, current knowledge suggests that CoV interact differentially with components of the autophagic pathway with the potential for both the utilization of autophagy components for virus replication and for the attenuation of autophagy. Substances inhibiting the generation of DMVs have been shown to be broadly reactive against CoV replication in vitro. Importantly, this included CoV of different genera including the emerging Middle East respiratory syndrome (MERS)-CoV, a paradigmatic emerging virus that entails considerable pandemic risks due to its prevalence in dromedary populations in Africa and the Arabian Peninsula and its transmission via the respiratory tract causing a severe form of pneumonia in humans. Since its discovery in 2012, the WHO has reported 2468 human MERS-CoV infections in 27 countries, of which at least 851 were fatal.

Currently, there is no clinically-approved treatment for MERS-CoV infection. While holding promise as a broad-range host-directed antiviral target, in general, the role of autophagy in the MERS-CoV life cycle, if any, is unknown. Nevertheless, a kinome analysis of MERS-CoV-infected cells detected phosphorylation changes of several regulatory kinases including AKT1 and mTOR that have been linked to autophagy in other studies. Although pharmacological interventions of broadly active AKT1- and mTOR-pathway inhibitors showed promise for antiviral therapy against several viruses including MERS-CoV, the identification of downstream molecules may facilitate a more targeted therapeutic approach with putative reduced side effects in patients.

A role of autophagy is suggested for another CoV, the mouse hepatitis virus (MHV), by the observation that multiplication of, and immune evasion by the virus is highly dependent on the formation of convoluted membranes and DMVs that are reminiscent of autophagosomes. However, the link of MHV replication to these DMVs did not appear to involve autophagy as the deletion of the pivotal autophagy genes ATG7 or ATG5 did not affect MHV replication. Of note, also, the induction of autophagy by starvation did not significantly change MHV replication. On the other hand, results of an earlier study employing ATG5 knockout cells suggested that autophagy is required for the formation of DMV-bound MHV replication complexes thereby significantly enhancing the efficiency of viral replication. Furthermore, pharmacological or genetic manipulation of autophagy showed that replication of another CoV, the Transmissible Gastroenteritis virus (TGEV), is negatively regulated by autophagy. In contrast, another study reported enhancement of TGEV replication by autophagy. Thus, no general role of autophagy in CoV replication could be established yet.

Here, we aim to elucidate the mechanisms controlling BECN1 protein levels. We find that S-phase kinase-associated protein 2 (SKP2) executes lysine-48-linked poly-ubiquitination of BECN1; its activity is regulated through phosphorylation under the control of FKBP51 involving AKT1 and PHLPP. Small molecule inhibitors of SKP2 enhance autophagy and reduce replication of MERS-CoV, pointing to the prospect of their therapeutic use.

Results

FKBP51 increases BECN1 stability. In search for a mechanism of the previously reported increase of the pivotal autophagy regulator BECN1 driven by FKBP51, we considered effects on mRNA and protein level. In direct comparison to the highly homologous FKBP52, a known counter-player of FKBP51, FKBP51 increased BECN1 levels upon ectopic expression (Fig. 1a). Regulation of BECN1 protein stability through the ubiquitin-proteasome system was indicated by using the proteasome inhibitor MG132, which increased the levels of BECN1 and the extent of its ubiquitination (Fig. 1b, Supplementary Fig. 1a). The use of ammonium chloride to inhibit lysosome-mediated proteolysis confirmed proteasomal degradation of BECN1 (Supplementary Fig. 1b). Ectopic expression of FKBP51 was similarly efficient in stabilising BECN1 as proteasome inhibition by MG132 (Fig. 1c, d). A protein degradation assay based on a pulse-chase using Halo-tagged BECN1 confirmed that FKBP51 stabilises BECN1 (Fig. 1e, f). These results also revealed a high turn-over rate of BECN1 (1/2 < 1 h).

FKBP51 associates with SKP2 regulating BECN1 stability. FKBP51 recently emerged as a scaffold protein organising various regulatory protein complexes. We thus considered an FKBP51-controlled association of BECN1 with E3 ubiquitin ligases or USPs. Based on the results of an FKBP51 mammalian-2-hybrid interaction screen, we first verified the association of
FKBP51 with the USPs 18 and 36, and with the E3 ligase SKP2 by immunoprecipitation in HEK293 cells (Fig. 2a). TRAF6 that is known as an E3 ligase of BECN1 35 and interactor of FKBP51 36 served as a control. Of these FKBP51 interactors, SKP2, USP18, and USP36 have the potential to induce autophagy 37,38. However, inhibition of SKP2 has been previously linked to the induction of autophagy 37,38.

Since we aimed at understanding the mechanism of FKBP51-directed increase in BECN1, we focussed in the following on proteins we found associated with both FKBP51 and BECN1. To assess the effect of SKP2 on BECN1 stability, we monitored the protein levels of BECN1 after the addition of the translation inhibitor cycloheximide (CHX) in the presence of over-expressed SKP2 or TRAF6 (again used as control), respectively. SKP2 affected BECN1 stability, leading to faster proteasomal degradation, while the K63-E3 ligase TRAF6 had no effect (Fig. 2c, d). Similar results were obtained in a pulse-chase experiment showing that SKP2 reduced BECN1 by about 20% compared to the vector control already at 30 min (Fig. 2e, f).

Further corroborating the function of SKP2 on BECN1, siRNA directed against Skp2 produced increased levels of BECN1 and the SKP2 target P27 along with enhanced lipidation of LC3B (typically detected as the ratio LC3B-II/I) (Fig. 2g, Supplementary Fig. 1c). Ectopic expression of SKP2 exerted the opposite effect (Fig. 2h, Supplementary Fig. 1d). Decreasing SKP2 by siRNA prevented BECN1 ubiquitination (Fig. 2i). In addition, stability assays using either CHX or the Halo-tagged BECN1 further established that reducing SKP2 levels with siRNA increases BECN1 stability leading to almost 3-fold higher levels at 90 min (Fig. 2j–m).

Phosphorylation-controlled ubiquitination of BECN1 by SKP2. Next, we examined whether SKP2 ubiquitinates BECN1. Ubiquitination frequently forms chains linking several ubiquitin units to distinct lysine (K) residues. The type of lysine linkage determines protein fate and function 39. Using a set of ubiquitin mutants, we found that SKP2 leads to K48-linked poly-ubiquitination of BECN1. Co-immunoprecipitations of BECN1 in HEK293 cells revealed association with SKP2 only (Fig. 2b), which has not been described as an E3 ligase of BECN1 before. However, inhibition of SKP2 has been previously linked to induction of autophagy 37,38.

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Fig. 2 FKBP51 associates with SKP2 that regulates BECN1 stability. a HEK293 cells were transfected with plasmids expressing the indicated E3 ligases and USPs; after 72 h, cells were either harvested for immunoprecipitation of FKBP51. b BECN1 associates with SKP2, but not with USP18 or USP36. HEK293 cells were transfected with vectors expressing tagged SKP2, USP18 and USP36; after 72 h, cells were harvested for immunoprecipitation with either control IgG or anti BECN1 antibody. c, d SKP2 affects BECN1 stability. HEK293 cells were transfected with plasmids expressing SKP2 or TRAF6 and exposed to MG132 (10 µM, 2 h) where indicated and to cycloheximide (CHX, 30 µg mL⁻¹) after 72 h for the durations indicated to monitor the decay of BECN1. e, f The conditions of c,d were also used in the pulse-chase assay, performed as in Fig. 1e, f, to determine BECN1 stability. g, h Cellular SKP2 levels determine BECN1 levels. SKP2 was either down-regulated by using siRNA (g) or up-regulated by transient transfection (h) and the levels of the indicated proteins were measured by western blotting (P27 served as control). Quantifications for g and h are provided in Supplementary Fig. 1c, d. i Knock-down of SKP2 by siRNA decreases BECN1 ubiquitination (assay as in Fig. 1b). j–m Stability assays were performed like in c–f to determine the effect of SKP2-targeting siRNA on BECN1 stability. WCE = whole cell extract. In all panels, error bars denote the standard error of the mean, derived from n = 3 biologically independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 (two-way ANOVAs, details in Supplementary Table 1). Source data and blot collections are provided as a Source Data file.

or cannot be phosphorylated (S72A)⁴¹. The phosphomimetic mutant still decreased BECN1 stability, but the S72A mutant showed no effect (Fig. 4b, c), consistent with the proposed cascade (Fig. 4a). We further made use of a well-established PHLPP inhibitor⁴² that led to enhanced phosphorylation of AKT1 and SKP2 (Fig. 4d, Supplementary Fig. 1f). A pulse-chase assay showed that the stability of BECN1 decreased by using the PHLPP inhibitor (Fig. 4e, f). This inhibitor had no effect on the stability of the ubiquitination-protected K402R mutant of BECN1, further corroborating the proposed cascade (Fig. 4g, h). In addition, pharmacological inhibition of AKT1 enhanced BECN1 stability in both assays, leaving about twice the amount of BECN1 at 90 min compared to control (Fig. 4i–l). In order to verify that reduced SKP2 activity drives autophagy in our cellular set-up, we used siRNA and determined the degradation of long-lived proteins⁴³. Targeting SKP2 increased proteolysis, but not in the presence of the autophagy inhibitor 3-methyladenine (3-MA, Fig. 4m). Furthermore, we observed decreased levels of P62 (Supplementary Fig. 1g), which typically goes along with enhanced autophagy⁴³. All these data are consistent with an inhibitory effect of SKP2 on autophagy regulated by a phosphorylation cascade involving FKBP51, PHLPP and AKT1.

Small molecule inhibitors of SKP2 increase BECN1 stability. Next, we compared known small molecule inhibitors of SKP2⁴⁴–⁴⁶ and found increased stability of BECN1 after a 90 min exposure to SMER3 (leaving about twice as much BECN1 at 60 and at 90 min compared to vehicle control, p < 0.001), and SMIP004 in particular (virtually no degradation vs. 78% decrease at 90 min, p < 0.001),
while compound C1 had no effect (Fig. 5a, Supplementary Fig. 2a). The pulse-chase assay confirmed SMIP004 as the most efficient compound in stabilising BECN1 leaving threefold more BECN1 protein after 90 min (Fig. 5b). Furthermore, SMIP004 reduced ubiquitination of BECN1 most efficiently (Fig. 5c, last lane). The long-lived protein assay revealed that SMER3 and SMIP004 enhanced protein half-life, while C1 showed no effect (Fig. 5d). Starvation by cultivating the cells in HBSS, which we confirmed to induce autophagy (Supplementary Fig. 2b-d), did not elicit a greater effect than SMIP004 in the long-lived protein assay (Fig. 5d). Furthermore, SMIP004 most profoundly affected not only BECN1, but also LC3B-II/I and P62 (example blot in Fig. 5e, quantifications in Supplementary Fig. 2e-g) that are established markers of autophagy. To analyse autophagy more thoroughly, it is mandatory to include assays that address autophagic protein turn-over, commonly referred to as autophagic flux. Thus, we subjected SMIP004 to a flux assay with the specific inhibitor of vacuolar H+ -ATPases bafilomycin A1 (BafA1), which interferes with lysosome acidification and thus degradation of autophagosome cargo. We revealed a stimulatory effect of SMIP004 on the autophagic flux (Fig. 5f, Supplementary Fig. 2b, c). In addition, it has been shown that ATG14 oligomerizes to dimers and tetramers, which is essential for autophagy by promoting STX17 binding and autophagosome-lysosome fusion. Using capillary-based electrophoresis allowing for better resolution at high molecular weight we observed ATG14 oligomerization to 7–8mers, which was enhanced by SMIP004 (Supplementary Fig. 2h, i). Thus, SMIP004 was used as SKP2-inhibitor (SKP2i) in all subsequent experiments.

**MERS-CoV decreases autophagy.** Results up to this point suggested that activity of BECN1 and thereby autophagy is limited by SKP2, which in turn is activated by AKT1 and Figs. 3, 4). Since MERS-CoV has been shown to enhance phosphorylation of AKT1, it might reduce autophagy. Infection of VeroB4 cells with MERS-CoV facilitated increased phosphorylation of SKP2 at S72 (Fig. 6a, Supplementary Fig. 3a), with a concomitant decrease of cellular levels of BECN1 (Fig. 6a, Supplementary Fig. 3b) and enhanced K48-polyubiquitinylation of BECN1 (Fig. 6b). Infection with MERS-CoV further led to an increase of the total number of phagocytic vesicles (sum of AL + AP) per cell (Fig. 6c, d). However, the number of successfully formed AL was reduced significantly indicating that AP can form but not fuse with lysosomes when cells are infected. A fusion block was also evidenced by the significantly reduced ATG14 oligomerization, essential for AP-lysosome fusion, in infected cells (Fig. 6e, f) and by the increase of the autophagy target P62 (Supplementary Fig. 3c). The block of vesicle fusion was further tested by the use of BafA1: as expected, LC3B-II/I levels were not further enhanced upon treatment with BafA1 (Fig. 6g). The fusion between AP and lysosome membranes is facilitated by the interaction between tSNARE proteins STX17 (AP-bound) and VAMP8 (lysosome-bound). This interaction is mediated by SNAP29 (Supplementary Fig. 3d). Co-immunoprecipitation revealed that MERS-CoV infection leads to a reduced interaction of STX17 with SNAP29 and VAMP8 (Fig. 6h). This further supports the conclusion that MERS-CoV infection causes a block of AP-lysosome fusion.

Since the results so far raised the possibility that MERS-CoV may benefit from reducing autophagy, we generated VeroB4 cells...
with a knockout of the pivotal autophagy gene ATG5. Compared to WT cells, infection of VeroB4 Atg5-KO cells led to the formation of 52-fold more infectious viral particles (Fig. 7a) while genomic viral RNA copies only increased by 6-fold (Fig. 7b). The efficient formation of DMVs is required for CoV replication and might exploit autophagy or its components. CoV-induced DMV formation is known to depend on viral nonstructural proteins (NSP) 4 and 6. Ectopic expression of MERS-CoV NSP4 and 6 led indeed to an accumulation of LC3-II and of P62 in the case of NSP6, while NSP4 only had a very minor effect on LC3-II (Fig. 7c). This suggested a block of the autophagic flux by NSP6, which was confirmed by using BafA1 (Fig. 7d), altogether suggesting the MERS-CoV-induced inhibition of autophagic flux to be mediated mainly by NSP6.

CoV have group-specific accessory proteins that can modulate viral replication and immune evasion. To assess potential autophagy-related functions, we overexpressed all MERS-CoV accessory genes 3, 4a, 4b, and 5. Protein 4b and 5, but not 3 and 4a, caused accumulation of P62 and enhanced the level of LC3-II similar to BafA1, which produced an additional effect in the presence of p3 or p4a, but not p4b or p5 (Fig. 7e, f). Because p4b and p5 are accessory proteins that can be deleted without lethal effects on virus replication, we constructed recombinant MERS-CoVs lacking the respective ORFs (Supplementary Fig. 4a, b) to evaluate their effect on autophagy in the context of full virus replication. In comparison to the recombinant MERS-CoV the p4b- and p5-deletion variants showed a decreased accumulation of P62 and LC3-II, suggesting that both proteins contribute to the inhibition of the autophagic flux (Supplementary Fig. 4c, d). The p4b- and p5-deleted viruses as well as the WT control virus grew to higher levels in Atg5 knockout Vero cells compared to WT cells (Supplementary Fig. 4e, f). However, the p4b and p5-deleted
viruses showed overall an up to 10-fold decreased replication in both WT and Atg5 knockout cells compared to WT virus suggesting a p4b- and p5-dependent attenuation of virus replication that is independent of ATG5-directed autophagy.

**SKP2 inhibition reduces MERS-CoV replication.** The influence of MERS-CoV infection on SKP2 phosphorylation, BECN1 degradation and its inhibition of the autophagic flux encouraged us to test if SKP2 inhibitors (such as SKP2i) may limit MERS-CoV amplification in infected cells. Indeed, SKP2i caused significant reduction of viral replication (by about 250-fold, Fig. 8a, Supplementary Fig. 5a). To explore the relevance of SKP2 inhibition on viral infection in more general terms, we also tested SKP2i in Sindbis virus (SINV) replication. It is known that SINV induces autophagy but that its replication levels are unaffected by it. We observed that treatment with SKP2i caused a moderate decrease of SINV replication (Supplementary Fig. 5b, c). SKP2i may thus be exploitable as a broader therapeutic antiviral principle.

As high concentrations of SKP2i inhibit the cell cycle, it was mandatory to control for potential cell cycle effects in VeroB4 cells that may partly account for the observed inhibition of MERS-CoV replication. FACS-based analysis of VeroB4 cells revealed effects on cell proliferation occur only at concentrations of SKP2i beyond 10 μM (Supplementary Fig. 6). Accordingly, while SKP2i enhanced the levels of BECN1 already at 1 μM, no significant increase in the cell cycle promoter P27 was observable up to 100 μM (Supplementary Fig. 7a).

Our data strongly suggest that of the potential mechanisms of SKP2i, it is the increase of BECN1-directed autophagy that reduces MERS-CoV multiplication. If this is correct, other ways of enhancing BECN1’s effect on autophagy should elicit similar effects. Therefore, we used two recently introduced tools to enhance BECN1 function in autophagy: the BH3 mimetic ABT-737 and a BECN1-derived peptide. These compounds, in contrast to SKP2i, do not change the levels of BECN1, but increase autophagy by redirecting BECN1 function towards the autophagy pathway, which we confirmed in VeroB4 cells (Supplementary Fig. 7b–d, g). Enhanced autophagy was indicated by the increase of LC3B-II (Supplementary Fig. 7e, g) and the reduction of P62 (Supplementary Fig. 7f, g). This was paralleled by inhibition of MERS-CoV replication by up to 60-fold in the case of the BECN1-peptide (TAT-B) (Figure Supplementary Fig. 7h) and up to 10-fold in the case of ABT-737 at 48h p.i. (Supplementary Fig. 7i). These results are in accordance with the notion that increasing BECN1

**Fig. 5 SKP2 inhibitors enhance BECN1 protein stability and autophagy.** a, b Evaluation of small compounds inhibiting SKP2. HEK293 cells were exposed to known SKP2 inhibitors for 24 h, followed by additional exposure to cycloheximide (CHX, 30 μg mL⁻¹) for the times as indicated or vehicle (a). b HEK293 cells were transfected with Halo-tagged BECN1 expressing plasmid and exposed to known SKP2 inhibitors and halogenated dye (100 nM, R110Direct) overnight. Cells were transferred to medium without dye and harvested after the indicated times. Labelled BECN1 was determined and quantified. c HEK293 cells were transfected with control vector (–) or plasmids expressing BECN1-Flag, HA-ubiquitin and myc-SKP2. Cells were exposed to known SKP2 inhibitors (C1, 3 μM; SMER3, 5 μM; SMIP004, 10 μM) for 24 h, BECN1 was precipitated from cell extracts and its ubiquitination and levels were determined by western blotting. d SKP2 inhibitors impact long-lived proteins. HEK293 cells were metabolically labelled with [¹⁴C]-valine overnight, exposed to the indicated drugs or vehicle and the levels of P62 and LC3B-II/I were determined by western blotting (e). The most efficient drug, SMIP004, was analyzed in the flux assay with bafilomycin A1 (BafA1; f). In all panels, error bars denote the standard error of the mean, derived from n = 3 biologically independent experiments. WCE = whole cell extract. **p ≤ 0.01, ***p ≤ 0.001 (two-way ANOVAs, details in Supplementary Table 1). In d, *labels refer to the effects of 3-MA, $labels to the drug effects in comparison to vehicle. Source data and blot collections are provided as a Source Data file.
MERS-CoV blocks autophagic flux. a MERS-CoV decreases BECN1 and increases pSKP2. VeroB4 cells were infected with MERS-CoV (MOI = 0.001) or mock-infected and harvested 48 h later. Representative western blots are shown, quantification in Supplementary Fig. 3a, b. MERS-CoV increases K48-linked poly-ubiquitination of BECN1. BECN1 was immune-precipitated from cell extracts 48 h p.i. and its ubiquitination determined by a ubiquitin K48 linkage specific antibody. c, d MERS-CoV blocks fusion of AP with lysosomes. VeroB4 cells were transfected with tandem fluorescent-tagged LC3B (mRFP and EGFP) and infected with MERS-CoV (MOI = 0.001). Twenty-four hours later, cells were fixed and analyzed for fluorescence. Vesicles with both green and red fluorescence (autophagosomes, AP) and with red fluorescence only (autolysosomes, AL) were counted. d, representative images, scale bar 25 µm. e, f MERS-CoV decreases ATG14 oligomerization. VeroB4 cells were infected with MERS-CoV (MOI = 0.001), cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 min and harvested. ATG14 homo-oligomerization was examined after western blotting (ProteinSimple). g MERS-CoV decreases autophagic flux. VeroB4 cells were infected with MERS-CoV (MOI = 0.001) and incubated with bafilomycin A1 (BafA1, 0.1 µM) for 2 h before samples were taken at 48 h p.i.. The ratios of LC3B-II/I were determined by western blotting. h MERS-CoV affects SNARE protein interactions. VeroB4 cells were infected with MERS-CoV (MOI = 0.001), cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 min and harvested. The SNARE complex protein STX17 was immunoprecipitated and the eluate was probed for interacting VAMP8 and SNAP29 by western blotting (the quantification represents the bands detected at the combined molecular weights of the cross-linked proteins, i.e. STX17 + VAMP8 and STX17 + SNAP29). In all panels, error bars denote the standard error of the mean. derived from *n = 3* biologically independent experiments for b, f, g, h, and *n = 13* (control) or *n = 12* (CoV) different cells for c. WCE = whole cell extract of vehicle exposed cells, i.e. no cross-linking. *p < 0.05, **p < 0.01, ***p < 0.001 (t-test in b, c, f, h, two-way ANOVA in g, details in Supplementary Tables 1, 2). Source data and blot collections are provided as a Source Data file.

Fig. 6 MERS-CoV blocks autophagic flux. a MERS-CoV decreases BECN1 and increases pSKP2. VeroB4 cells were infected with MERS-CoV (MOI = 0.001) or mock-infected and harvested 48 h later. Representative western blots are shown, quantification in Supplementary Fig. 3a, b. MERS-CoV increases K48-linked poly-ubiquitination of BECN1. BECN1 was immune-precipitated from cell extracts 48 h p.i. and its ubiquitination determined by a ubiquitin K48 linkage specific antibody. c, d MERS-CoV blocks fusion of AP with lysosomes. VeroB4 cells were transfected with tandem fluorescent-tagged LC3B (mRFP and EGFP) and infected with MERS-CoV (MOI = 0.001). Twenty-four hours later, cells were fixed and analyzed for fluorescence. Vesicles with both green and red fluorescence (autophagosomes, AP) and with red fluorescence only (autolysosomes, AL) were counted. d, representative images, scale bar 25 µm. e, f MERS-CoV decreases ATG14 oligomerization. VeroB4 cells were infected with MERS-CoV (MOI = 0.001), cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 min and harvested. ATG14 homo-oligomerization was examined after western blotting (ProteinSimple). g MERS-CoV decreases autophagic flux. VeroB4 cells were infected with MERS-CoV (MOI = 0.001) and incubated with bafilomycin A1 (BafA1, 0.1 µM) for 2 h before samples were taken at 48 h p.i.. The ratios of LC3B-II/I were determined by western blotting. h MERS-CoV affects SNARE protein interactions. VeroB4 cells were infected with MERS-CoV (MOI = 0.001), cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 min and harvested. The SNARE complex protein STX17 was immunoprecipitated and the eluate was probed for interacting VAMP8 and SNAP29 by western blotting (the quantification represents the bands detected at the combined molecular weights of the cross-linked proteins, i.e. STX17 + VAMP8 and STX17 + SNAP29). In all panels, error bars denote the standard error of the mean. derived from *n = 3* biologically independent experiments for b, f, g, h, and *n = 13* (control) or *n = 12* (CoV) different cells for c. WCE = whole cell extract of vehicle exposed cells, i.e. no cross-linking. *p < 0.05, **p < 0.01, ***p < 0.001 (t-test in b, c, f, h, two-way ANOVA in g, details in Supplementary Tables 1, 2). Source data and blot collections are provided as a Source Data file.

To determine whether SKP2i relieves the antagonistic effects of MERS-CoV infection on autophagy, we performed flux assays using either BafA1 or the tandem-labelled LC3B. While BafA1 did not change LC3B lipitation in infected cells as evidenced by constant levels of LC3B-II/I in the absence of SKP2i (as before, Fig. 6g gray bars), it increased LC3B-II/I in the presence of SKP2i (Supplementary Fig. 7j). The fluorescence-based assay revealed that SKP2i enhanced the number of AP while decreasing the number of AL (Fig. 8b, c). Furthermore, SKP2i enhanced ATG14 oligomerization (Fig. 8d, e) and the interaction of autophagic SNARE complex proteins (STX17 with VAMP8 or SNAP29), in MERS-CoV infected cells (Fig. 8f), thereby counteracting the effect of MERS-CoV (see above, Fig. 6h). Thus, SKP2i inhibits MERS-CoV production, most likely by enhancing BECN1 levels, which might also account for its effect on late steps of autophagy. Since very little interaction between BECN1 and ATG14 was observed using either BafA1 or the tandem-labelled LC3B. While BafA1 increased LC3B-II/I in MERS-CoV infected cells, thereby counteracting the

acts through enhancing autophagy as the relevant mechanism for the antiviral effects of SKP2 in vitro.
flux (Fig. 9a, Supplementary Fig. 8a, b, Supplementary Table 3) and comparable to SKP2i (see Fig. 5e, f, Supplementary Fig. 2e, f). NIC and VAL reduced MERS-CoV multiplication by up to 1000-fold at 48 h p.i. (Fig. 9b, Supplementary Fig. 9a–c). Thus, NIC and VAL were characterized further. Both drugs enhanced ATG14 oligomerization about twofold (Supplementary Fig. 9d, e) and increased the number of autolysosomes more than twofold (Supplementary Fig. 9h, i). NIC also affected the autophagic flux in the infected cells similar to SKP2i (Supplementary Fig. 9h, i). Therefore, these three compounds were tested for their dose-effect relationship in a MERS-CoV infection experiment. All compounds showed high efficacy, \( I_{\text{maxSKP2i}} = 28,000 \) fold,
The present work reveals BECN1 as a target of the E3 ligase SKP2\(^3\),\(^7\),\(^37\),\(^38\),\(^46\). SKP2 executes K48-linked ubiquitination at K402 of BECN1, resulting in proteasomal degradation. This action of SKP2 is counteracted by FKBP51 that regulates SKP2 phosphorylation through forming regulatory complexes with AKT1 and PHLP\(_P\). Inhibition of SKP2 increases BECN1 levels, enhances the assembly of lysosomal SNARE proteins and autophagy, and efficiently reduces the replication of MERS-CoV (Fig. 9d).

FKBP51 is a pivotal element of molecular feedback loops of the cellular and physiological stress reaction. It increasingly emerges as a scaffold that chaperones various regulatory protein interactions\(^3\). Here, FKBP51 appears to recruit SKP2 in its inactive form to BECN1, probably by virtue of its interaction with the kinase AKT1 and the phosphatase PHLP\(_P\). Similarly, FKBP51-directed protein interactions increase BECN1 phosphorylation\(^2\), decrease phosphorylation and activity of AKT1\(^3\). Thus, FKBP51 impacts BECN1 at least via two mechanisms, phosphorylation of BECN1 itself\(^2\) and of its regulatory E3 ligase SKP2. Both mechanisms involve AKT1, which exhibits yet another way of influencing BECN1 by phosphorylating USP14, which removes K63-linked ubiquitins from BECN1\(^57\).

In addition to K48-linked poly-ubiquitination by SKP2, BECN1 undergoes ubiquitination also by other E3 ligases, at different sites, and with different types of poly-ubiquitination linkage\(^12\),\(^38\),\(^39\). NEDD4 produces both K63- and K11-linkages at yet to be determined sites of BECN1\(^9\). Decoration of BECN1 with

\(I_{\text{maxNIC}} = 23,000 \fold\) and \(I_{\text{maxVAL}} = 93,000 \fold\); Fig. 9c, Supplementary Fig. 10) and moderate to high potency (IC\(_{50SKP2i} = 9 \mu M\), IC\(_{50NIC} = 0.32 \mu M\) and IC\(_{50VAL} = 84 \mathrm{nM}\).

**Discussion**

The present work reveals BECN1 as a target of the E3 ligase SKP2\(^3\),\(^7\),\(^38\),\(^46\). SKP2 executes K48-linked ubiquitination at K402 of BECN1, resulting in proteasomal degradation. This action of SKP2 is counteracted by FKBP51 that regulates SKP2 phosphorylation through forming regulatory complexes with AKT1 and PHLP\(_P\). Inhibition of SKP2 increases BECN1 levels, enhances the assembly of lysosomal SNARE proteins and autophagy, and efficiently reduces the replication of MERS-CoV (Fig. 9d).

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In addition to K48-linked poly-ubiquitination by SKP2, BECN1 undergoes ubiquitination also by other E3 ligases, at different sites, and with different types of poly-ubiquitination linkage\(^12\),\(^38\),\(^39\). NEDD4 produces both K63- and K11-linkages at yet to be determined sites of BECN1\(^9\). Decoration of BECN1 with
the non-canonical K11-linked ubiquitins by NEDD4 leads to its inactivation through inhibiting its phosphorylation. Both scenarios enhance autophagy, which involves ATG14 oligomerization (probably 7-8mers) as recently described essential step in functional autophagy.

MERS-CoV reduces autophagy through distinct viral proteins leading to blockade of autophagosome-lysosome fusion and ATG14 oligomerization. Compounds inhibiting SKP2 reinstate autophagy and efficiently reduce viral production. Source data are provided as a Source Data file.

**Fig. 9 Some potential SKP2 inhibitors affect autophagy and viral replication.** a VeroB4 cells were treated with various FDA-approved drugs (for abbreviations and concentrations see Supplementary Table 3) and cotreatment with Bafilomycin A1 (0.1 μM) was performed to evaluate the autophagic flux. The graph provides the means ± SEM of three independent experiments, representative western blots are shown in Supplementary Fig. 8b. b VeroB4 cells were infected with MERS-CoV (MOI = 0.001), treated with the indicated drugs, and MERS-CoV genome copies were determined by RT-PCR 24 h and 48 h p.i.; data are presented as (log10) fold difference in relation to the DMSO control. Unprocessed data and PFU results are presented in Supplementary Fig. 9a–c. c Concentration-dependent inhibition of MERS-CoV replication by the SKP2-inhibitor (SKP2i), valinomycin (VAL), and niclosamide (NIC). VeroB4 cells were infected with MERS-CoV (MOI = 0.001) and treated with drug. MERS-CoV genome copies were determined by real-time RT-PCR at 48 h p.i., data present the percentage of remaining MERS-CoV genomes in comparison to vehicle treatment. In all panels, error bars denote the standard error of the mean, derived from n = 3 or n = 6 (c, no drug condition) biologically independent experiments. **p < 0.01, ***p < 0.001 (two-way ANOVA in a, one-way ANOVA in b, details in Supplementary Table 1). d Summary scheme: SKP2 leads to K48-linked poly-ubiquitination and thus degradation of BECN1. The effect of SKP2 can be diminished in two ways, either by chemical inhibition or by FKBP51, which scaffolds protein interactions ultimately leading to SKP2 inactivation through inhibiting its phosphorylation. Both scenarios enhance autophagy, which involves ATG14 oligomerization (probably 7-8mers) as this points to the intriguing possibility that inhibiting SKP2 using small molecule inhibitors might counteract deleterious effects of soluble proteins with polyQ expansions that are held responsible for causing neurodegenerative diseases such as Huntington’s disease and ataxin 3 in spinocerebellar ataxia.

Due to the tremendous variability of viral proteins, there is huge interest in host cell-encoded pathways that could be exploited for fighting viral infection. The present study as well as earlier work suggest autophagy as a pivotal component of virus-host interaction and potential broad-range antiviral target. For MERS-CoV replication, it remains to be elucidated whether blocking basal autophagy is mandatory or only auxiliary; nevertheless, the virus encodes at least three proteins that limit autophagy when expressed ectopically, NSP6, p4b and p5. Although the detailed molecular mechanisms are yet to be analysed, MERS-CoV NSP6 may also play a role in inhibiting the expansion of autophagosomes as
shown for other betacoronaviruses. MERS-CoV p4b has phospho-
phosphate function inhibiting the activation of RNase L, which
itself can activate autophagy. MERS-CoV p5 is located in the
endoplasmic reticulum-Golgi intermediate compartment and
was shown to inhibit IFN-beta induction, which may constitute
another link to autophagy.

Deletion of p4b or p5 resulted in reduced MERS-CoV growth.
These results differ from previous observations, which may be
differed to different cell lines and cDNA clone construction strategies.
In addition, we used a very low MOI and a type I
IFN-deficient cell line (VeroB4), which allows to determine minor
growth differences. The absence of p4b and p5 during MERS-CoV
replication may also result in lower LC3B levels compared to
MERS-CoV wild-type-infected but also to the mock-infected control
cells. This points to a more complex interaction between virus
replication and autophagy; p4b and p5 may counteract a response of
the host to viral infection, but the functional relevance of their
link to autophagy requires further investigation. Apparently, sev-
eral CoV proteins collaborate to reprogram the membrane traffic
in the cell for DMV formation serving as compartments of viral
genome replication and transcription. Therefore, the finding that
SKP2 is tightly linked to intracellular membrane traffic and turnover,
shows that MERS-CoV blocks autophagy not only to evade degradation but also to improve membrane availability.

It appears that most of the potential treatments arising from
other observations of reduction of viral replication upon autophagy
stimulation are not easily applicable in vivo because of side
effects and inefficient peptide delivery. Examples are rapamycin
and vitamin D which restricted the replication of HIV in primary
human macrophages, and the autophagy-inducing BECN1-
derived peptide which limited the replication of a number of
viruses in vitro. From the present results, therapeutic induction
of autophagy by inhibition of SKP2 emerges as a promising
approach. We should note that all the substances tested here have
or may have additional targets. While we cannot exclude the
possibility that for each of the compounds it is this additional
target that is responsible for the antiviral effect, we consider this
scenario as less likely; this assessment is also based on the
observation of the antiviral effects of compounds targeting
BECN1, which we establish here as client of SKP2. Nevertheless,
futuristic experiments should assess the effect of these drugs in
autophagy-defective cells such as the Atg5 knockout cells.

It further remains to be investigated whether inhibiting SKP2
will affect all autophagy-sensitive viruses. SINV, which was not
overly influenced by Atg5 knockout despite indications of
degradation of viral components through autophagy, reacted
modestly to SKP2. The reason for this is unknown; it may relate
to the involvement of other immune pathways influencing SINV,
for example the JAK/STAT pathway or stress granule forma-
tion, or to autophagy functioning in the absence of Atg5.
FDA-approved drugs known to act as SKP2 inhibitors are available. We show that some of these drugs are effective in
reducing MERS-CoV replication in vitro, for example nicosam-
ide and pyrvinium pamoate. Even though there are reports of
low absorption of these drugs, others found considerable
absorption and serum levels of nicosamide reached 1–20 µM,
concentrations we show here to efficiently limit MERS-CoV
replication. In addition, inhalable formulations of nicosamide
have been developed. SKP2-targeting compounds also may
have potential against other infections and other clinical condi-
tions that are influenced by autophagy induction.

Methods
Chemicals. The following chemicals were used for treatment of cells: BaF1 (Alfa
Aesar, J61835), 17-AAG (Sigma-Aldrich, A8476), 6-AN (Sigma-Aldrich, A68203),
AMI (Sigma-Aldrich, 1019701), BRO (Sigma-Aldrich, 1076501), CEL (Sigma-
Aldrich, PZ0008), CLO (Sigma-Aldrich, C7291), ETO (Sigma-Aldrich, E1383),
(MET (Sigma-Aldrich, M60055000), PAR (Sigma-Aldrich, P8623), RAP (Tocris,
Columbia, US 14373; Tocris, US4327, Tocris, Germany 3073; Tocris, UK 3735; Tocris,
Biotin (AnaSpec, AS65467), Tat-scr (AnaSpec, AS65468), GOS (Sigma-Aldrich, G8761),
HAL (Tocris, 0931), MIN (Sigma-Aldrich, M95511), NIC (Sigma-Aldrich, N3510), PIM (Sigma-
Aldrich, P17937), PRT (Tocris, 0610), PER (Sigma-Aldrich, SML1200), PEP (Sigma-
Aldrich, P6402), PVR (Sigma-Aldrich, 1992001), RA5 (Sigma-Aldrich, SML0124),
C11 (Sigma-Aldrich, SML0115), CAM (Sigma-Aldrich, SML0122), TAT-B (AnaSpec,
SML0120), TS648), TOP (Tocris, 4562), VAL (Sigma-Aldrich, V0627), PHLPP1 NSC117079
(GlaxoLabs, GLXC-03994), C4+Valine (Perkin Elmer, NEC291EU050UC), Akiti X
(Cayman Chemical, 14865) MK-2206 HCl (Cayman Chemical, 11593).

Co-Immunoprecipitation (CoIP) with crosslinker. CoIP of endogenous STX17 or
BECN1 were performed in VeroB4 cells. The PBS-washed cell pellet was incubated
with 75 µM DS (disuccinimidyl suberate, Thermo Fisher Scientific, 21655) and
0.1% benzolase (Novagen). Crosslinking was performed for 30 min at room
temperature followed by 2 h at 4 °C while rotating. After washing with PBS,
crosslinking was quenched in tris-buffered saline (pH 7.0) for 20 min at 4 °C. Cells
were lysed in CoIP-buffer containing 20 mM Tris-HCl pH 8.0, 100 mM NaCl,
1% EDTA, 0.5% Igepal CA-630 complemented with protease inhibitor
cocktail (Sigma, P7271). This was followed by incubation on an overhand shaker
for 20 min at 4 °C. 1.2 mg of lysate was incubated with 2.5 µg STX17 or
BECN1 antibody overnight at 4 °C. 20 µl of BSA-blocked Protein G Dynabeads
(Invitrogen, 100–300) were added to the lysate–antibody mix followed by 3 h incubation
at 4 °C. The beads were washed three times with PBS and protein-antibody complexes
were eluted by boiling for 5 min at 95 °C in Laemmli loading buffer. Fifteen
microgram of the cell lysates or 5 µl of the immunoprecipitates were separated by
SDS-PAGE.

Toxicity assays. LDH assay. To assess membrane disruption and cell death the release of lactate dehydrogenase (LDH) into the growth medium of HEK293 or
VeroB4 cells was measured. This assay (LDH cytotoxicity detection system) was
carried out according to the manufacturer’s protocol (Clontec, Mountain View, CA,
USA). As positive control 0.02% Triton X-100 was added to the medium 1–2 h prior
performing the assay; empty wells were used with medium only, as negative control.
MTT assay. HEK293 or VeroB4 cells were incubated in the presence of 0.5 mg
ml−1 tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium (MTT) for 4 h at
37 °C and 5% CO2. Read-out of MTT assay was carried out as described previously.

Western blot analysis. Protein extracts were obtained by lysing cells in RIPA buffer
(150 mM NaCl, 1% IGEPA CA-630, 0.5% Sodium deoxycholate, 0.1% SDS 50 mM
Tris (pH 8.0)) freshly supplemented with protease inhibitor (Merck Millipore,
Darmstadt, Germany), 5 mM DTT (Sigma Aldrich, D11480), 1 mM EDTA, 0.5% IGEPAL CA-630 complemented
with protease inhibitor cocktail (Sigma, P7271). This was followed by incubation on an overhand shaker
for 20 min at 4 °C. 1.2 mg of lysate was incubated with 2.5 µg STX17 or
BECN1 antibody overnight at 4 °C. 20 µl of BSA-blocked Protein G Dynabeads
(Invitrogen, 100–300) were added to the lysate–antibody mix followed by 3 h incubation
at 4 °C. The beads were washed three times with PBS and protein-antibody complexes
were eluted by boiling for 5 min at 95 °C in Laemmli loading buffer. Fifteen
microgram of the cell lysates or 5 µl of the immunoprecipitates were separated by
SDS-PAGE.

In the case of ATG14 oligomerization, lysates were analyzed by capillary electrophoresis on Wes™ (ProteinSimple) using the 60–440 kDa cartridges.
In general, protein quantification was performed by normalization to the intensity of Actin, which was determined on the same membrane.
For quantification of lipidated LC3B, the intensity of LC3B-II was always related to
the signal intensity of the corresponding LC3B-1, following the guidelines for the
determination of autophagy.

RIPA lysis of MERS-CoV-infected VeroB4 cells. Whole cell lysates were prepared
with RIPA lysis buffer containing 150 mM NaCl, 1% IGEPA CA-630, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris (pH 8.0). 1% protease inhibitor
cocktail III (Calbiochem), 0.1% benzolase (Cell Signaling Technology, #9100), 0.1% dithiothreitol [0.1 mM] and
0.1% phenylmethylsulfonyl fluoride (Sigma-Aldrich, 57917) were freshly added. The cell lysates were sonicated and
centrifuged at 15,000 g for 5 min at 4 °C. The resulting supernatant was collected and 50 µl of the resulting lysate was loaded onto a 4%–12% Novex Tris-
acetate polyacrylamide gel (Invitrogen, 41550 for ion exclusion and 41540 for non-
ion exclusion) and run at 200 V for 90 min. The resulting gel was stained with
Coomassie Blue R250 and destained with 10% acetic acid.

In summary, we present evidence that SKP2 is an
efficient client of autophagy. It blocks autophagy not only to evade
degradation but also to improve membrane availability. It
potentially affects the replication of other viruses besides MERS-CoV.
Furthermore, SKP2 is an efficient client of autophagy. It blocks autophagy not only to evade
degradation but also to improve membrane availability.
transferred to a microcentrifuge tube. Cells were pelleted by centrifugation at 4 °C and 300 x g for 5 min. The supernatant was removed carefully and cells were resuspended in lysis buffer and incubated for 20 min at 4 °C for efficient lysis. Then, SDS loading buffer (4x NuPAGE LDS, ThermoFisher Scientific) was added and samples were boiled at 95 °C for 10 min. Samples were stored at −20 °C and processed as indicated in the respective sections.

**Cells.** VeroB4 (DSMZ [German Collection of Microorganisms and Cell Cultures] number ACC33) were cultivated in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 10% bovine serum (FSA, AGC GGTGATGAAAGAGGCAATATGGAATGTTG TCTTGCTATCATGCTATGATATAGGGATGCTCTGCTAGTTGTAACAACTTAAAC3′). They were used as described in83.

**Virus infection**

VeroB4 cells were plated at 6-well plates at a cell density of 5 x 10^5/well in VeroB4 medium and transfected with lipofectamine 3000 according to the manufacturer’s protocol. For the Atg5 knock-out, the pSpCas9-BB-2A-Puro (PX459) V2.0 plasmid containing Atg5-targeting gRNA (sequence: 5′-TATCCCCCTTGAATATATC-3′) purchased from GenScript was used. Forty-eight after transfection, 10 µg/mL puromycin (InVivoGen, anti-pr-1) was added to the medium. After 48 h, the medium was changed to puromycin-free VeroB4 medium and, another 48 h later, cells were sorted into a 96-well plate at a density of one cell per well using the BD FACSMelody cell sorter (BD Biosciences). Cells were maintained in conditioned medium for 1 week until a colony was formed from a single cell. All the colonies were then trypsinized and expanded individually. Atg5 knock-out was examined for each clone by western blot analysis using anti-Atg5 purchased from Santa Cruz (sc-131358).

**Plaque assay**

VeroB4 cells were electroplated as described above with siRNA (sense 5′-GGGCGGUUCAUGAUAAUTT-3′; anti-sense 5′-AUUAAUAUACUUGCAGCGACGTT-3′; anti-sense 5′-ACGGGACCGUGGAAATTT-3′) and and FLAG-HA-USP36 each led to the appearance of another band at higher molecular weight in western blots. Irrespective of the exact nature of these bands, specific co-precipitation with FKBP51 was only observable for the bands at the predicted molecular weight. Experiments were performed as described elsewhere85.

**Generation of Atg5 KO VeroB4.** Vero B4 were plated in 6-well plates at a cell density of 5 x 10^5/well in VeroB4 medium and transfected with lipofectamine 3000 according to the manufacturer’s protocol. For the Atg5 knock-out, the pSpCas9-BB-2A-Puro (PX459) V2.0 plasmid containing Atg5-targeting gRNA (sequence: 5′-TATCCCCCTTGAATATATC-3′) purchased from GenScript was used. Forty-eight after transfection, 10 µg/mL puromycin (InVivoGen, anti-pr-1) was added to the medium. After 48 h, the medium was changed to puromycin-free VeroB4 medium and, another 48 h later, cells were sorted into a 96-well plate at a density of one cell per well using the BD FACSMelody cell sorter (BD Biosciences). Cells were maintained in conditioned medium for 1 week until a colony was formed from a single cell. All the colonies were then trypsinized and expanded individually. Atg5 knock-out was examined for each clone by western blot analysis using anti-Atg5 purchased from Santa Cruz (sc-131358).

**Virus infection.** Infection of HEK293 cells was performed at a multiplicity of infection (MOI) of 1. Cells were detached after 24 h, trypsinized and expanded individually. Atg5-knockout was examined for each clone by western blot analysis using anti-Atg5 purchased from Santa Cruz (sc-131358). Cells were infected with SARS-CoV-2 at a fixed (1% PFA for 1 h) incubated at 37 °C and 5% CO2. Vero B4 were plated in 6-well plates at a cell density of 5 x 10^5/well in VeroB4 medium and transfected with lipofectamine 3000 according to the manufacturer’s protocol. For the Atg5 knock-out, the pSpCas9-BB-2A-Puro (PX459) V2.0 plasmid containing Atg5-targeting gRNA (sequence: 5′-TATCCCCCTTGAATATATC-3′) purchased from GenScript was used. Forty-eight after transfection, 10 µg/mL puromycin (InVivoGen, anti-pr-1) was added to the medium. After 48 h, the medium was changed to puromycin-free VeroB4 medium and, another 48 h later, cells were sorted into a 96-well plate at a density of one cell per well using the BD FACSMelody cell sorter (BD Biosciences). Cells were maintained in conditioned medium for 1 week until a colony was formed from a single cell. All the colonies were then trypsinized and expanded individually. Atg5 knock-out was examined for each clone by western blot analysis using anti-Atg5 purchased from Santa Cruz (sc-131358).

**Plaque assay.** SINV infectious particles were quantified by plaque assay as previously described84 with minor modifications. Briefly, VeroB4 cells were seeded in a concentration of 3.5 x 10^5/well in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin and 1% sodium pyruvate at 37 °C and 5% CO2. HEK293 cells (ATCC, Manassas, VA, USA CRL-1573) were cultivated in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin and 1% sodium pyruvate at 37 °C and 5% CO2. For transfection HEK293 and VeroB4 were detached and resuspended in 100 µL (50 mM HEPES pH 7.3, 90 mM NaCl, 5 mM KCl, 0.15 mM CaCl2) transfected using the Nucleofector II system (program # T020).

**Virus infection**

Vero B4 were seeded at a density of 1.5 x 10^5 cells/well in 24-well plates, 1 day after plating, infection was started by using the virus master mix.Two days later cells were fixed, washed twice with 1x PBS and refilled with DMEM (supplemented as described above). Samples were taken at the indicated time points. All virus infection experiments were performed under biosafety level 3 conditions with enhanced respiratory personal protection equipment.

**Real-time RT-PCR assay**

Real-time RT-PCR assays were performed as previously described86. More detailed information is available for the detection of MERS-dehL and MERS-del5 eukaryotic genomes. Primers were upFwd (5′-GCAACGGGGGATCTAGGT-3′), upRev (5′-GGCTTACCTGGAGAC-3′), and downFwd (5′-TGCTATCTGGGTCTTCTTC-3′). For the detection of MERS-dehL and MERS-del5 eukaryotic genomes, primers were upFwd (5′-GCAACGGGGGATCTAGGT-3′), upRev (5′-GGCTTACCTGGAGAC-3′), and downFwd (5′-TGCTATCTGGGTCTTCTTC-3′). Sequence details are available82.

**Plasmids and construction of MERS-CoV cDNA clones.** For heterologous expression, nsp4 and nsp6 were PCR amplified from MERS-CoV cDNA and cloned into the eukaryotic expression plasmid pCAGGS along with a C-terminal Flag tag (MERS-nsp4-F: 5′-AGAGAATTGCGGCCACATGGCGTCTCAGTATGATTTAGTATC-3′; MERS-nsp4-R: 5′-TCTTGCGGGGCGTTATTTATGGATGAGTGTTG-3′). For MERS-nsp6-F: 5′-AGAGAATTGCGGCCACATGGCGTCTCAGTATGATTTAGTATC-3′; MERS-nsp6-R: 5′-TCTTGCGGGGCGTTATTTATGGATGAGTGTTG-3′). Sequence details are available82.

**Plasmids and construction of MERS-CoV cDNA clones.** For heterologous expression, nsp4 and nsp6 were PCR amplified from MERS-CoV cDNA and cloned into the eukaryotic expression plasmid pCAGGS along with a C-terminal Flag tag (MERS-nsp4-F: 5′-AGAGAATTGCGGCCACATGGCGTCTCAGTATGATTTAGTATC-3′; MERS-nsp4-R: 5′-TCTTGCGGGGCGTTATTTATGGATGAGTGTTG-3′). For MERS-nsp6-F: 5′-AGAGAATTGCGGCCACATGGCGTCTCAGTATGATTTAGTATC-3′; MERS-nsp6-R: 5′-TCTTGCGGGGCGTTATTTATGGATGAGTGTTG-3′). Sequence details are available82.
Determination of IC₅₀. To estimate the maximal viral inhibition (Iₘ₅₀) and the drug concentration achieving viral inhibition halfway between baseline and maximum (IC₅₀), we first normalized the dose-response curves for each drug by dividing by cell viability, and then fitted the normalized viral inhibition curves to a sigmoid curve using the Sigmoid and Nonlinear Least Squares functions in R version 3.1.0 (https://www.R-project.org/) as previously described (http://krycha.info/2012/07/08/tutorials-fitting-a-sigmoid-function-in-r/). Based on the sigmoid-fitted curves, we then determined numerically the Iₘ₅₀ and IC₅₀.

Flow cytometry. Cells were washed in PBS and fixed with cold ethanol (70%) for 30 min at 4 °C. After washing with PBS cells were treated with ribonuclease A (100 μg mL⁻¹ Sigma Aldrich) and stained with Vybrant DyeCycle Orange (Invitrogen, V35005) as described by the manufacturer’s manual. Cells were analyzed with the FACS Calibur (BD Biosciences). Acquired data were analyzed with FlowJo software (Tree Star).

Statistical analysis. When two groups were compared, the student’s t-test was applied. For three or more group comparisons, one or two-way analysis of variance (ANOVA) was performed, as appropriate, followed by Tukey post hoc test, as appropriate. All t-test t and p-values and ANOVA F and p-values are reported in the legends to the Supplementary Figures; significance results of the contrast tests are further indicated by asterisks in the graphs. All statistical tests were two-tailed and p < 0.05 was considered statistically significant. For the complete set of raw data, see the data source file.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability Candidate ubiquitination sites on BECN1 were taken from the mUbiSiDa data base (originally at http://202.195.183.4:8000/mUbiSiDa.php, now part of omicX at https://omictools.com/mubisida-tool). The source data underlying Figs. 1, 2, 4–9, and Supplementary Figs. 1–10 are provided as a Source Data file. All other data is available from the authors upon request.

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Author contributions

NCC, V.M.C., C.D., M.A.M., T.R. designed and conceived the work. N.C.G., D.N., V.M.C., C.D., M.A.M., T.R. wrote the main paper text. N.C.G., prepared all experimental details. N.C.G., C.D., M.A.M., T.R. performed the main experiments and data analysis. M.A.M. was supported by the European Research Council starting grant (# 281338, Gex Molemb, awarded to Elisabeth Binder) within the FP7 framework. CD was supported by the German Research Council (DFG) grant DFG SPP1596 (DR 772/10–2) and SFB-T664 (TRR 84/3, A07), the Federal Ministry of Education and Research (BMBF) grant RAPID (#01KI1723A) and the European Horizon 2020 grants COMPARE (# 643476) and EVAg (#653316). MAM was supported by the VW Foundation (#93345).” The SKP2 mutant plasmids were a kind gift from Wenyi Wei.

Competing interests

The authors declare no competing interests.
Additonal information

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