Title

Induction of autophagy by PI3K/MTOR and PI3K/MTOR/BRD4 inhibitors suppresses HIV-1 replication

Journal

Journal of Biological Chemistry, 293(16)

ISSN

0021-9258

Authors

Campbell, Grant R

Bruckman, Rachel S

Herns, Shayna D

et al.

Publication Date

2018-04-01

DOI

10.1074/jbc.ra118.002353

Peer reviewed

JBC Papers in Press. Published on February 23, 2018 as Manuscript RA118.002353
The latest version is at http://www.jbc.org/cgi/doi/10.1074/jbc.RA118.002353

Induction of autophagy by PI3K/MTOR and PI3K/MTOR/BRD4 inhibitors suppresses HIV-1 replication

Grant R. Campbell[1], Rachel S. Bruckman[1†], Shayna D. Herns[1], Shweta Joshi[2,3], Donald L. Durden[2,3,4], and Stephen A. Spector[1,3*]

[1] Division of Infectious Diseases, Department of Pediatrics, University of California San Diego, La Jolla, CA 92093-0672
[2] Division of Pediatric Hematology-Oncology, Department of Pediatrics, University of California San Diego, La Jolla, CA 92093-0819
[3] Rady Children's Hospital, San Diego, CA 92123
[4] SignalRx Pharmaceuticals, Inc., San Diego, CA 92130

Running title: *PI3K/MTOR inhibitors suppress HIV-1 replication*

[†]Present address: Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, Illinois, United States of America

*To whom correspondence should be addressed: Stephen A. Spector, MD, Division of Infectious Diseases, Department of Pediatrics, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0672, USA; Tel.: (858) 534-7055; Fax: (858) 534-7411; E-mail: saspector@ucsd.edu

Keywords: autophagy; dactolisib; NVP-BEZ235; human immunodeficiency virus; HIV; JQ1; macrophage; SF2523

Abstract

In this study, we investigated the effect of the dual phosphatidylinositol 3-kinase/mechanistic target of rapamycin (PI3K/MTOR) inhibitor dactolisib (NVP-BEZ235), the PI3K/ MTOR/bromodomain-containing protein 4 (BRD4) inhibitor SF2523 and the BET inhibitor JQ1 on the productive infection of primary macrophages with human immunodeficiency type-1 (HIV). These inhibitors did not alter the initial susceptibility of macrophages to HIV infection. However, dactolisib, JQ1 and SF2523 all decreased HIV replication in macrophages in a dose dependent manner via degradation of intracellular HIV through autophagy. Macrophages treated with dactolisib, JQ1 or SF2523 displayed an increase in LC3B lipidation combined with SQSTM1 degradation without inducing increased cell death. LC3B-II levels were further increased in the presence of pepstatin A suggesting that these inhibitors induce autophagic flux. RNA interference for *ATG5* and *ATG7*, and pharmacological inhibitors of autophagosome-lysosome fusion, and of lysosomal hydrolases, all blocked the inhibition of HIV. Thus, we demonstrate that the mechanism of PI3K/MTOR and PI3K/MTOR/BRD4 inhibitor suppression of HIV requires the formation of autophagosomes, as well as their subsequent maturation into autolysosomes. These data provide further evidence in support of a role for autophagy in the control of HIV infection and open new avenues for the use of this class of drugs in HIV therapy.

Although CD4+ T cells are the predominant human immunodeficiency virus type-1 (HIV[††]) reservoir, other cell types including macrophages and microglial cells also serve as occult sites of HIV persistence (1). These infected cells are broadly disseminated across numerous tissues, including sites that may be relatively inaccessible to host defenses or treatment strategies, such as the lymphatic tissue and the central nervous system, where concentrations of antiviral drugs are lower than in peripheral blood (2,3). HIV can spread through cell-to-cell transmission in the presence of antiretroviral therapy (ART) concentrations that effectively inhibit extra-cellular viral infection of susceptible cells (4). Moreover, as

infected cells can persist undetected by the immune system, and are unaffected by ART, lifelong treatment is required for continued viral suppression. Extensive efforts are underway to develop effective strategies for eliminating these reservoirs. One strategy, often called "shock and kill", has emerged as a potential strategy to eradicate HIV but effective drugs are still lacking. Thus, there is an urgent need for the development of new therapeutic strategies.

HIV survival is dependent on its ability to exploit host cell machinery for replication and dissemination and to circumvent cellular processes that prevent its growth. One such mechanism of avoiding the innate immune system is the ability of HIV to inhibit or subvert macroautophagy (hereafter referred to as autophagy). Autophagy is a degradation pathway that occurs at basal levels in all cells whereby cytosolic double membrane-bound compartments termed autophagosomes engulf and sequester cytoplasmic constituents such as sub-cellular organelles and microbial pathogens. These autophagosomes then fuse with lysosomes forming autophagolysosomes (autolysosomes), resulting in the degradation of the engulfed components, generating nutrients and macromolecular precursors. Autophagy is upregulated in response to stress such as starvation, drug treatment and infection. Of the more than 35 human autophagy-associated genes currently known to be involved in autophagy, 10 are known to be essential for HIV replication (reviewed in 5). However, although HIV may require the early stages of autophagy, it must control the antiviral proteolytic and degradative late stages of autophagy to avoid its degradation. In HIV-infected macrophages, Nef binds to and sequesters beclin 1 (BECN1) to the Golgi complex leading to the phosphorylation and cytosolic sequestration of transcription factor EB, culminating in the blocking of the degradative stages of autophagy and the accumulation of autophagic factors (6-8). Despite the down-modulation of autophagy by HIV, inducers of autophagy including 1α,25-dihydroxycholecalciferol (9,10), amino acid starvation (11), hydroxamate histone deacetylase inhibitors (12), sirolimus (8,9), toll-like receptor (TLR) 8 ligands (13), romidepsin (12), and a cell-permeable autophagy-inducing peptide termed Tat–beclin (derived from the region of BECN1 that interacts with HIV Nef and conjugated to the basic region of HIV Tat) (8), overcome the imposed phagosome maturation block leading to inhibition of HIV. Therefore, autophagy shows promise as a target for drug therapy.

The phosphatidylinositol 3-kinase (PI3K) pathway is a major focus for drug development due to its function as a key regulator of cell growth and survival, and cancer genetic studies suggest that the PI3K pathway is frequently altered in human tumors (14); *PIK3CA* (encoding phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) is the second most frequently mutated oncogene, and *PTEN* (phosphatase and tensin homolog) is one of the most frequently mutated tumor suppressor genes (15,16). Key targets of PI3K include the AKT serine/threonine kinase 1 (AKT1) and the mechanistic target of rapamycin (MTOR) pathway. Drugs targeting the PI3K/AKT1/MTOR axis include pan-PI3K inhibitors, isoform-selective PI3K inhibitors, sirolimus and analogues, active-site MTOR inhibitors, and dual-PI3K/MTOR and AKT1 inhibitors. Dactolisib (BEZ235, NVP-BEZ235) is an imidazoquinoline derivative that has dual ATP-competitive inhibition of PI3K and MTOR (17). By inhibiting MTOR through binding at the ATP-cleft, dactolisib prevents the phosphorylation of the ribosomal protein S6 kinase B2 (RPS6KB2) and other MTOR complex 1 (MTORC1) downstream effectors, leading to a release of autophagy inhibition by MTORC1. It is also effective in blocking AKT1 activation as it prevents the feedback activation of PI3K signaling normally observed with MTOR inhibitors, such as sirolimus (18). Dactolisib affects cell growth through PI3K/AKT1 and MTORC1 inhibition (19,20), and induces autophagy in tumor cells (21,22). Moreover, dactolisib also interferes with the growth of lymphocytic choriomeningitis virus through the inhibition of viral budding (23) and rescued mice from a lethal influenza infection (24).

The bromodomain (BRD) and extra terminal domain (BET) family of proteins (BRDT, BRD2, BRD3 and BRD4) are potential new candidates in the "shock and kill" strategy

for HIV eradication (25). BET BRD4 inhibitors act as acetylated lysine mimetics and thus disrupt the binding interaction of BET proteins with acetylated lysine residues on histones such as H3K27 (26). BET bromodomain inhibitors such as JQ1 (27) reactivate the transcription of HIV in latent T cell and monocyte infection models (28-30). Thus, design of PI3K/MTOR/BRD4 inhibitors where BRD4 inhibitors can induce activation of latent virus and PI3K/MTOR inhibitor can induce autophagy could be an effective strategy to eradicate HIV infected T cells or macrophages. SF2523 is a novel and only existing PI3K/MTOR/BRD4 inhibitor (31). SF2523 is i) a pan PI3K inhibitor at K_i, of 19 nM and 35 nM for PIK3CA and PIK3CD respectively; ii) inhibits BRD4 binding to *MYCN* promoter equivalent to JQ1 (1 μM) and blocks *MYCN* transcription; iii) pharmacodynamically inhibits AKT activation by blocking phosphorylation of AKT at Ser^{473} in macrophages; iv) blocks BRD4 binding to acetyl-lysine binding site on histone H3K27; v) blocks M1-M2 macrophage transition and augments immunity (32), and vi) has shown potent *in vivo* tumor activity against a large number of tumor types with no observable toxicity (33,34). SF2523 has been formulated for oral absorption, has excellent pharmacokinetic and absorption, distribution, metabolism, and excretion properties and is slated to enter clinical trials in the near future (CA192656). These characteristics in a single pill opened new opportunities to explore this drug for HIV therapy as it has a potential to induce both the "shock" and the "kill" arms of the "shock and kill" treatment strategy.

In this study we investigated the effect of dactolisib, JQ1 and SF2523 on the susceptibility of macrophages to HIV infection. We report that these inhibitors have no impact on the initial infection events, but through the induction of autophagy induce the degradation of intracellular viral particles that leads to a reduction in viral release. Moreover, our data demonstrate that the dactolisib-mediated autophagic degradation of HIV requires the nucleation and formation of autophagosomes and their subsequent maturation, which leads to a decrease in virion release.

Results

Dactolisib induces autophagy in human primary macrophages

Previous studies demonstrated that addition of dactolisib to continuous transformed cell lines or primary carcinomas leads to the induction of autophagy (18,19,22,35-39). However, because the effects of drugs on primary cells can be different than those observed in continuous cell lines, we investigated the ability of dactolisib to induce autophagy in primary human macrophages. During autophagy, cytosolic microtubule-associated protein 1 light chain 3 beta (MAP1LC3B or LC3B) I is converted to LC3B-II by an ubiquitin-like system that involves autophagy-related (ATG) 7, ATG3 and the ATG12–ATG5 complex. The ATG12–ATG5 complex ligates LC3B-II to the nascent autophagosome membrane through phosphatidylethanolamine with the LC3B-II associated with the inner membrane degraded after fusion of the autophagosome with lysosomes. Therefore, the conversion of LC3B-I to LC3B-II and its turnover is an indicator of autophagy induction and flux (40). Exposure of macrophages to dactolisib for 24 h led to a dose dependent increase in LC3B-II similar to that observed with sirolimus, an inducer of autophagy through inhibition of MTORC1 (Fig. 1*A*).

To verify that the increase in LC3 lipidation in dactolisib treated cells versus control cells represents increased autophagic flux rather than an accumulation of LC3B‐II, the degradation of the polyubiquitin‐binding protein sequestosome 1 (SQSTM1 or p62) was quantified. Inhibition of autophagy leads to an increase in SQSTM1 protein levels while autolysosomes degrade SQSTM1‐ and LC3‐positive bodies during autophagic flux (41). In these experiments, dactolisib treatment led to a dose-dependent decrease in SQSTM1 protein levels corresponding to the induction of autophagic flux (Fig. 1*A*). Of interest, dactolisib treatment led to the dose-dependent increase in the expression of beclin 1 (BECN1) (Fig. 1*A*). Unlike other BH3-only proteins, the overexpression of BECN1 does not function as a pro-apoptotic molecule, but instead leads to

increased autophagy (42). As both a decrease in SQSTM1 and an increase in BECN1 levels correlate with enhanced autophagy, the observed decrease in the SQSTM1:BECN1 protein ratio in dactolisib treated cells suggests augmented autophagy induction (Fig. 1*A*) (40).

To further establish the induction of autophagic flux in dactolisib treated macrophages, and not simply the activation of LC3B transcription, additional experiments were conducted with macrophages in the presence of the established acid protease inhibitor, pepstatin A. Pepstatin A prevents the degradation of LC3B-II during autophagosome-lysosome fusion, resulting in an accumulation of LC3B-II over time as autophagy is initiated and sustained. Macrophages were treated with 10 μg/mL pepstatin A prior to dactolisib treatment, and lysed after subsequent 24 h dactolisib treatment. Blots of cell lysates confirmed autophagic flux, with significant LC3B-II accumulation relative to basal conditions in the vehicle control (Fig. 1*B*). These data indicate that in the presence of dactolisib treatment autophagy goes to completion.

Although we observed an increase in autophagic markers in the absence of visible pyknosis, karyorrhexis, or plasma membrane blebbing, it was important to confirm that the cells were not undergoing cell death at the concentrations being used, as the induction of excessive autophagy can cause cell death (43) and dactolisib exerts cytotoxic effects in cancer cells (18). Importantly, we did not observe significant dactolisib-mediated cell death as measured by lactate dehydrogenase (LDH) release at the concentrations used in this study (Fig. 1*C*). Moreover, based on the selective thermal denaturation of apoptotic ssDNA using low heat and formamide and subsequent detection using a monoclonal antibody, a more specific indicator of apoptosis than TUNEL (44), we also observed no significant difference in ssDNA accumulation using Welch's unequal variances *t* test ($P > 0.05$; Fig. 1*D*).

Dactolisib decreases HIV release from human macrophages

Our laboratory's data and that of others indicate that autophagy is necessary for HIV to establish a productive infection (5). However, once a permissive infection is established HIV down-regulates autophagy to facilitate cell survival and to promote viral replication (6,7). Previous studies have shown that micromolar concentrations of LY 294002, which inhibits class I PI3K as well as MTORC1, casein kinase 2, and polo like kinase 1 (45), suppresses viral infection of macrophages post-viral entry and post-reverse transcription but prior to HIV gene expression (46). Conversely, wortmannin, an inhibitor that is used at nanomolar concentrations in cell culture to inhibit autophagy (40), and inhibits PI3K and PLK1, but not MTORC1 at these concentrations (45), fails to have any effect on HIV replication in macrophages (9). Therefore, as the pharmacological induction of autophagy is known to inhibit HIV (5,8-10,12,13,47), we next determined whether dactolisib-induced autophagy influenced HIV infection and replication in primary macrophages by comparing the extent to which dactolisib treatment influences HIV p24 antigen accumulation in the supernatants of productively infected macrophages. Dactolisib induced a dose-dependent decrease in HIV p24 antigen release into the culture supernatants that became significant by day 3 post-infection at all tested concentrations ($P < 0.01$; Fig. 2*A*). Interestingly, even the low dose of 25 nM dactolisib was sufficient to decrease extracellular HIV p24 antigen accumulation by 83% ($P = 0.003$). Moreover, the magnitude of the inhibition increased until cultures were discontinued on day 10 post-infection (Fig. 2*A*). Importantly, we observed that at 10 d post-HIV-infection dactolisib induced no significant cytotoxic effects at the concentrations used (Fig. 2*B*). Moreover, we also observed no significant difference in ssDNA accumulation at the same time point (Fig. 2*C*).

To understand how dactolisib affects HIV replication, we examined sequential steps of viral replication. Treatment of cells with dactolisib did not significantly affect binding of HIV to dactolisib-treated cells, as measured by ELISA of cell-associated p24 Gag protein ($P > 0.3$; Fig. 3*A*). Moreover, the quantity of trypsin-resistant intracellular p24 Gag in cells exposed to virus for 5 h in both untreated cells and dactolisib-treated cells were similar ($P > 0.29$;

Fig. 3*B*). As controls, sirolimus and maraviroc were used. Sirolimus is known to down regulate C-C motif chemokine receptor 5 (CCR5) expression as well as to induce autophagy through the inhibition of MTOR (48) and maraviroc is a CCR5 antagonist (49); thus, they provide a control for CCR5 binding. Collectively, these results suggest that dactolisib has no effect on either HIV binding or entry.

We next measured viral infection using qPCR for the presence and quantity of strong-stop HIV DNA (with LTR R/U5 primers) 8 h post-infection. Macrophages were treated with dactolisib for 4 h prior to infection with HIV. Following 8 h of infection, dactolisib had no effect on HIV reverse transcription ($P > 0.1$; Fig. 3*C*). As Nef is expressed in abundance during the early phase of HIV infection (50), we also analyzed the translation of Nef by immunoblotting. We observed a slight, but non-significant increase in Nef production in dactolisib treated cells over vehicle controls ($P > 0.18$; Fig. 3*D*). We then assessed for productive HIV infection by assaying for Tat activity using TZM-bl cells. TZM-bl cells were pretreated with dactolisib for 4 h then exposed to HIV. At the concentrations tested, dactolisib had no significant inhibitory effect on the productive infection of TZM-bl cells with HIV ($P > 0.5$; Fig. 3*E*).

In our next series of experiments, we assessed whether the decrease in cell culture supernatant of HIV p24 antigen release was due to dactolisib inducing the production of replication-incompetent viral particles. 1 ng p24 antigen from cell-free supernatants derived from cells treated with dactolisib and infected for 10 d was used to infect fresh macrophages in the absence of dactolisib. We observed no difference in HIV-replicative fitness post-dactolisib treatment indicating that the virus being released is replication competent ($P > 0.19$; Fig. 3*F*). Next, we examined the effect of dactolisib on HIV replication at early time points post-infection by treating macrophage cultures exposed to HIV with dactolisib at 4 h pre-exposure, at the time of infection and at 3, 5, and 7 days post-infection. At every time point, each concentration of dactolisib induced a significant decrease in cell culture supernatant HIV p24 antigen accumulation (Fig. 3*G*) in the absence of toxic cellular effects.

Dactolisib induces autophagy in HIV-infected macrophages by inhibiting MTOR and activating unc-51 like autophagy activating kinase 1

To determine whether the dactolisib-mediated decrease of HIV p24 antigen release could be attributable to autophagy, we assessed the ability of dactolisib to induce autophagy in HIV-infected macrophages. 24 h exposure of HIV-infected macrophages to dactolisib led to a dose-dependent increase in LC3B-II and BECN1 and a dose-dependent decrease in SQSTM1 and the SQSTM1:BECN1 protein ratio suggesting that dactolisib overcomes the HIV-imposed autophagy inhibition and induces autophagy (Fig. 3*H*).

The (unc-51 like autophagy activating kinase 1 (ULK1) kinase complex functions at the initial stages of the canonical autophagy pathway and induces autophagy by phosphorylating beclin 1 and activating PIK3C3 (51-53). Under nutrient-rich conditions MTORC1, consisting of MTOR, the regulatory associated protein of MTOR complex 1 (RPTOR) and MTOR associated protein LST8 homolog (MLST8), phosphorylates ULK1 at Ser^{757} and binds it through RPTOR. Under conditions of stress such as nutrient deprivation or TLR signaling MTORC1 is inhibited leading to global dephosphorylation of ULK1, dissociation of ULK1 from MTORC1 and the induction of autophagosome formation. Therefore, we examined whether ULK1 is involved in dactolisib-mediated autophagy by determining whether dactolisib treatment causes the inhibition of MTOR and thereby the dephosphorylation and activation of ULK1. Dactolisib induced significant and dose-dependent dephosphorylation and activation of ULK1 as monitored by phospho-ULK1 (Ser^{757}) specific antibodies (Fig. 3*I*). We next examined whether dactolisib causes global inhibition of MTORC1 by measuring the phosphorylation status of two well-known MTOR substrates, ribosomal protein S6 kinase, 70kDa, polypeptide 2 (RPS6KB2) and eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1). Dactolisib treatment induced the

dephosphorylation of RPS6KB2 and EIF4EBP1 in a similar manner to ULK1 (Fig. 3*I*) suggesting that dactolisib inhibits MTOR during HIV infection.

Dactolisib-mediated autophagy inhibits HIV dissemination from human macrophages

To determine whether dactolisib-induced autophagy contributes to the dactolisib-mediated decrease of HIV p24 antigen release, we assessed the effect of *ATG5* and *ATG7* silencing on HIV infection post-dactolisib treatment. Both *ATG5* and *ATG7* RNAi were effective in silencing their respective genes over the 10 d infection protocol (Fig. 4*A*) and are efficient at inhibiting LC3B lipidation and the degradation of SQSTM1, and thus autophagy (Fig. 4*B*)(12). *ATG5* silencing reduced the inhibitory effect of 250 nM dactolisib from 94% at day 10 post-infection in the shNS cells ($P < 0.001$) to 32% in sh*ATG5* cells, which was not significantly different from the vehicle treated sh*ATG5* cells ($p = 0.085$; Fig. 4*C*). Similarly, *ATG7* silencing reduced the inhibitory effect of 250 nM dactolisib to just 18% in sh*ATG7* cells (Fig. 4*C*). Importantly, in the presence of shRNA-insensitive *ATG5* or *ATG7* ORF transcripts, the dactolisib mediated decrease in extracellular supernatant HIV p24 antigen accumulation was similar to the non-targeting negative control shRNA (Fig. 4*C*).

We next investigated whether autophagosome acidification, a late stage event during autophagy, is required for the dactolisib-mediated autophagic decrease in HIV release. During autophagy, lysosomes fuse with autophagosomes to form autolysosomes. Macrophages were treated with bafilomycin A_1, an inhibitor of the vacuolar H^+ ATPase and autophagosome-lysosome fusion, and subsequently infected with HIV. Bafilomycin A_1 reversed the dactolisib-mediated decrease of extracellular HIV accumulation (Fig. 5) suggesting that the acidic pH of autolysosomes is required for the autophagy-mediated control of HIV.

After lysosomes fuse with autophagosomes to form autolysosomes, lysosomal hydrolases degrade the sequestered components, which are then released into the cytosol through the actions of lysosomal efflux permeases. We investigated whether lysosomal hydrolases are important for the dactolisib-mediated decrease in HIV release through autophagy using SID 26681509, a novel thiocarbazate specific inhibitor of the lysosome hydrolase cathepsin L. Importantly, in the absence of dactolisib, SID 26681509 induced no net inhibition of HIV (Fig. 5). Moreover, in the presence of dactolisib, SID 26681509 reversed the observed dactolisib-mediated decline in extracellular accumulation of HIV (Fig. 5).

We further examined the effect of dactolisib and the role of autophagy on intracellular HIV p24 antigen levels at a late time point post-infection by treating macrophage cultures exposed to HIV with dactolisib at 7 days post-infection in the presence or absence of bafilomycin A_1. As expected, HIV p24 antigen levels were increased in the presence of bafilomycin A_1 alone (Fig. 6). However, at the concentrations tested, dactolisib induced a significant decrease in intracellular HIV p24 antigen by day 10 post-infection ($P < 0.05$; Fig. 6). Importantly, in each case, bafilomycin A_1 abrogated dactolisib-mediated degradation of HIV. Collectively, these data suggest that dactolisib induces the degradation of HIV through the induction of autophagy, which leads to a reduction in the number of released virions.

PI3K/AKT1/MTOR/BRD4 inhibitor-mediated autophagy inhibits HIV dissemination from human macrophages

To assess whether BRD4 inhibitors or PI3K/BRD4 inhibitors would also induce autophagy and inhibit HIV infection, we examined the effects of JQ1 and SF2523 on HIV infection. Macrophages were pretreated with pepstatin A and then treated with JQ1 or SF2523 for 24 h. Both drugs induced a dose-dependent significant accumulation of LC3B-II relative to basal conditions indicative of autophagic flux (Fig. 7*A*). Both JQ1 and SF2523 also induced the dose-dependent decrease in HIV p24 released into the culture supernatants that became significant by day 3 post-infection at all tested concentrations ($P < 0.01$; Fig. 7*B*) with the magnitude of the decrease increasing until cultures were discontinued at day 10 post-infection (Fig. 7*B*). Importantly, no significant

increase in LDH release was observed at 10 d post-HIV-infection (Fig. 7*C*).

To determine whether the JQ1 and SF2523-mediated decrease of HIV p24 antigen release could be attributable to autophagy, we assessed the ability of JQ1 and SF2523 to induce autophagy in HIV-infected macrophages. 24 h exposure of HIV-infected macrophages to JQ1 and SF2523 led to an increase in LC3B-II and BECN1 and a decrease in SQSTM1 and the SQSTM1:BECN1 protein ratio suggesting that both JQ1 and SF2523 can overcome the HIV-imposed autophagy inhibition and induce autophagy (Fig. 7*D*). We also examined whether ULK1 is involved in JQ1 and SF2523-mediated autophagy by determining whether JQ1 and SF2523 treatment causes the inhibition of MTORC1 and thereby the dephosphorylation and activation of ULK1, RPS6KB2, and EIF4EBP1. Both JQ1 and SF2523 induced significant dephosphorylation and activation of ULK1, RPS6KB2, and EIF4EBP1 (Fig. 7*E*) suggesting that JQ1 and SF2523 both inhibit MTOR during HIV infection.

We next examined the effect of SF2523, and JQ1 on intracellular HIV p24 antigen levels at a late time point post-infection by treating macrophage cultures exposed to HIV with SF2523, and JQ1 at 7 days post-infection in the presence or absence of bafilomycin A_1. At the concentrations tested, both SF2523 and JQ1 induced a significant decrease in intracellular HIV p24 antigen by day 10 post-infection ($P < 0.05$; Fig. 8, *A* and *B*). As expected, bafilomycin A_1 abrogated the PI3K inhibitor mediated degradation of HIV (Fig. 8, *A* and *B*). Collectively, these data suggest that dactolisib, along with SF2523 and JQ1, all induce the degradation of HIV through the induction of autophagy, which leads to a reduction in the number of released virions.

Discussion

HIV infection of macrophages and CD4+ T cells induces autophagy (7,54) and peripheral blood mononuclear cells (PBMC) from elite controllers contain significantly more autophagic vesicles and express more autophagic markers than normal progressors (55). PBMC from elite controllers are also more responsive to sirolimus, with treatment leading to an enhanced autophagic response and a greater reduction in virion production (55), illustrating the importance of autophagy in the cellular antiviral response. Therefore, modulation of autophagy processes is an important mechanism for controlling microbial pathogens (56,57), and the inhibition of HIV through pharmacologic induction of autophagy highlights this role (5,8,9,12,13). However, despite autophagy being an innate antiviral defense mechanism, viruses may also hijack autophagy for their efficient replication in a cell-type and virus specific manner. HIV utilizes autophagosomal membranes as a scaffold for Gag processing and the production of nascent virions (6), while controlling the antiviral proteolytic and degradative late stages of autophagy to avoid its own degradation (6,7,58,59). To our knowledge, these data are the first to describe the effect of a dual PI3K/MTOR inhibitor or PI3K/MTOR/BRD4 inhibitor on HIV infection of macrophages, an important site of HIV infection and persistence. Although dactolisib, JQ1 and SF2523 had no effect on viral binding, entry, reverse transcription, or protein synthesis events, they dose-dependently inhibited HIV through the degradative autophagy pathway that requires the formation of autophagosomes and their subsequent maturation into autolysosomes.

The PI3K/AKT1 pathway regulates a number of different cellular processes including cell proliferation, RNA processing, protein translation, autophagy and apoptosis. In order to replicate in host cells, many viruses evolved strategies to stimulate PI3K activity. In the case of HIV, productive infection of macrophages requires PI3K activity, which triggers key cellular events typically observed during cell survival and activation, namely PTEN reduction, and the membrane localization and increased activity of AKT1 (46,60). These cellular alterations, together with the previously reported reduction in tumor protein 53 (TP53) activity caused by its direct binding to intracellular HIV Tat (60), helps to mechanistically explain the extended survival phenotype of HIV-infected macrophages. Additionally, these cellular effects contribute to viral production and the establishment of macrophages as long-lived viral reservoirs, which are inaccessible to retroviral

therapies. Reactivation of these latent long-lived reservoirs represents a possible strategy for their elimination.

Activated PI3K triggers type I interferon and autophagy responses to counteract viral invasion. While the activation of the class I PI3K suppresses autophagy via the well-established PI3K-AKT1-MTORC1 pathway, the class III PIK3C3 forms a protein complex with BECN1 and PIK3R4 and generates phosphatidylinositol 3-phosphate (PtdIns3P), which is required for the initiation and progression of autophagy (61). Dual inhibitors of PI3K/MTOR are generally considered autophagy activators as they simultaneously inhibit class I PI3K and MTOR, both of which suppress autophagy (37). However, there is some evidence that both dactolisib and PI-103 can suppress autophagic flux in transformed cell lines (62). In the present study, we consistently observed an increase in LC3B lipidation combined with SQSTM1 degradation in human primary macrophages treated with dactolisib, JQ1 and SF2523. We also observed increased LC3B-II levels in the presence of pepstatin A suggesting that these inhibitors induce autophagic flux in primary human macrophages. As a cancer drug, dactolisib showed in early *in vivo* mouse trials to be effective against neoplastic tumors and well-tolerated (20,21,63). While dactolisib is cytostatic in the context of tumors, it does so with limited toxicity (21). We examined the cytotoxic effects of dactolisib, JQ1 and SF2523, and at the doses tested all three drugs had limited cytotoxicity. Importantly, we did not observe any significant increase in cell death, which is consistent with reports that dactolisib does not induce cell death in healthy primary cells (64-68). Thus, these studies demonstrate the important differences that can exist when examining drugs at different concentrations and in different cell types, and highlights the importance of studying primary cells when examining the effects of drugs on HIV replication and pathogenesis. Of note, the PI3K/AKT1/MTOR pathway is a key signaling pathway involved in T cell activation and function. In particular, PI3K activation promotes survival, cell cycle progression, modulates differentiation, and controls the acquisition of effector and memory phenotypes in T cells. Although dactolisib interferes with T cell activation and function it does so at concentrations >100 nM (65), which is less than the dose required to induce autophagy and inhibit HIV. Additional studies are required to examine the effect of dactolisib on healthy human cells in the long-term, particularly important considering the lifelong dependence of HIV-positive persons on antiretroviral therapy.

Targeting host factors used by HIV is a highly attractive antiviral strategy. Unlike current antiviral treatments targeting viral proteins, such as reverse transcriptase, protease and integrase, this strategy can minimize viral escape. During infection, HIV Tat recruits human super elongation complex (SEC) to overcome promoter-proximal pausing of RNA polymerase II. BRD4 competitively blocks this Tat–SEC interaction on the HIV promoter. By dissociating BRD4 from the HIV promoter, BRD4 inhibitors allow Tat recruitment of SEC to stimulate HIV elongation in the absence of global T cell activation (28-30). As BRD4 is also a conserved negative regulator of autophagy, the BET inhibitor JQ1 induces autophagy through BRD4 inhibition (69). Hence blocking of BRD4 and the PI3K/MTOR pathway could reactivate HIV and induce autophagy in infected macrophages, providing an effective HIV therapy regimen.

Currently available antiretroviral therapy has greatly improved life expectancy and quality of life for those infected with HIV. However, multi-drug resistance continues to increase, emphasizing the importance of identifying novel strategies to improve HIV treatment. Since autophagy works at the host cellular level to improve intracellular killing of both replicating and non-replicating HIV the development of resistance is unlikely. The appeal of dactolisib as an anti-HIV therapeutic agent is considerable. Conventional cancer treatment utilizes chemotherapy at the maximally tolerated dose (MTD), or the highest dose shown to be both cytotoxic and tolerable for the patient. Clinical studies including trials of dactolisib alone in pancreatic neuroendocrine tumors (70) and bladder cancers (71) showed that dactolisib was poorly tolerated as the MTD was 300 mg in the phase I study (72). These

studies did demonstrate limited clinical efficacy with several patients with stable disease but given the poor tolerability, these trials did not advance. Dactolisib also has low oral bioavailability with highly variable systemic exposure due to poor aqueous solubility. In cancer therapy, this low oral bioavailability, combined with the reported toxicities, prevent dose escalation to where efficacy could potentially be achieved (73). Thus, to avoid these toxicities and achieve a dose likely to be impact the PI3K/MTOR pathway, a formulation that markedly enhances the oral bioavailability or delivery of the drug via a non-oral route could be considered. In the present study, we show that the concentrations required to inhibit HIV are far lower than those required to induce cell death of tumor cells. In total, the findings presented here suggest that drugs targeting the PI3K/AKT1/MTOR pathway be considered as a potential treatment approach for persons infected with HIV, and that dactolisib may be repurposed as an anti-HIV drug.

Experimental procedures

Ethics Statement – Venous blood was drawn from HIV seronegative subjects using a protocol that was reviewed and approved by the Human Research Protections Program of the University of California, San Diego (Project 09-0660) in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56) and was fully compliant with the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from subjects prior to their participation.

Cells and reagents – Monocyte derived macrophages were obtained from peripheral blood mononuclear cells as previously described (13). All experiments were performed in RPMI 1640 supplemented with 10% (v/v) heat-inactivated FBS (Sigma), and 10 ng/mL colony stimulating factor 1 (Peprotech). TZM-bl cells were obtained through the AIDS Research and Reference Reagent Program, contributed by Drs John C. Kappes and Xiaoyun Wu and Tranzyme Inc. (74). Cell viability was estimated using the LDH Cytotoxicity Detection Kit^{PLUS} and the single-stranded DNA (ssDNA) ELISA (Chemicon) as described previously (75).

Dactolisib (BEZ235, NVP-BEZ235) was purchased from Selleck Chemicals. Bafilomycin A_1, SID 26681509 and sirolimus were purchased from Sigma. JQ1 was a gift from James Bradner, Dana-Farber Cancer Institute, Boston, MA. SF2523 was synthesized as described earlier (34). Bafilomycin A_1 was used at 10 nM and SID 26681509 at 50 nM with pretreatment for 1 h before addition of dactolisib or sirolimus. Maraviroc was purchased from Toronto Research Chemicals and was used at 10 nM.

Virus – $HIV_{Ba\text{-}L}$ was obtained through the AIDS Research and Reference Reagent Program, contributed by Dr. Suzanne Gartner and Dr. Robert Gallo (76,77). Virus stocks and titers were prepared as previously described using the Alliance HIV p24 antigen ELISA (Perkin Elmer) (78). Macrophages were infected with 10^5 $TCID_{50}$/mL $HIV_{Ba\text{-}L}$ per 5×10^5 cells as described previously (13). Viral binding and entry was assessed as described previously (9). Productive infection of TZM-bl cells 48 h post-HIV exposure was detected using the β-Gal Staining Set (Roche).

Immunoblotting – ATG5 (D5G3), ATG7 (D12B11), BECN1 (3738), EIF4EBP1 (53H11), phospho-EIF4EBP1 ($Thr^{37/46}$; 236B4), RPS6KB2 (49D7), phospho-RPS6KB2 (Thr^{389}; 9205), ULK1 (D9D7), and phospho-ULK1 (Ser^{757}; 6888) antibodies were obtained from Cell Signaling. SQSTM1 (ab56416), HIV p24 (39/5.4A), and HIV Nef (3D12) antibodies were obtained from Abcam. β-actin (ACTB; AC-74) and microtubule-associated protein 1 light chain 3 beta (LC3B; NB100-2220) antibodies were obtained from Sigma and Novus Biologicals respectively. Cell lysates were prepared using 20 mM HEPES, 150 mM NaCl, 1 mM EDTA supplemented with 1% (v/v) 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol (all Sigma) and 1% (v/v) Halt protease and phosphatase inhibitor cocktail (Thermo Scientific). Cell lysates were resolved using 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol buffered 12% polyacrylamide gel (Novex) and transferred to 0.2 μm pore-size PVDF membranes (Thermo Scientific), followed by detection with alkaline phosphatase tagged secondary antibodies (Invitrogen) and 0.25 mM disodium 2-chloro-5-(4-methoxyspiro[1,2-dioxetane-3,2′-(5-

chlorotricyclo[3.3.1.1$^{3.7}$]decan])-4-yl]-1-phenyl phosphate supplemented with 5% (v/v) Nitro-Block II (both Applied Biosystems). Relative densities of the target bands compared to the reference ACTB bands were calculated using ImageJ (NIH). Each data point was normalized to the vehicle then $\log_2$ transformed.

shRNA transduction – Lentiviral transduction of macrophages with MISSION lentiviral particles containing shRNAs targeting *ATG5* (SHCLNV-NM_004849/TRCN0000150940), *ATG7* (SHCLNV-NM_006395/TRCN0000007584), or scrambled non-target negative control (SHC002V) was performed according to the manufacturer's protocol (Sigma). SHC002V was used as non-targeting negative control as it activates the RNA-induced silencing complex and the RNAi pathway, but does not target any human gene allowing the examination of the effects of shRNA transduction and RNAi activation on gene expression. Macrophages were transduced with non-specific scrambled shRNA (shNS) or target shRNA and selected using 3 mg L^{-1} puromycin (Gibco). Five days later, cells were analyzed for target gene silencing and used in experiments. Validation was performed by phenotypic rescue using custom shRNA-insensitive open reading frame (ORF) cDNA lentiviral vectors (LentiORF; GeneCopoeia). For rescue experiments, macrophages were transduced with target shRNA with a neomycin resistance marker and LentiORF constructs. Selection was performed using 0.1 g L^{-1} G418 (Gibco) combined with 3 mg L^{-1} puromycin (for *ATG7*) or 0.2 g L^{-1} hygromycin B (Gibco) (for *ATG5*) as selection agents (12).

Quantitative real-time PCR (qPCR) – Strong-stop HIV DNA quantification was measured using qPCR with the LightCycler 1.5 instrument and FastStart DNA Master SYBR Green I (both Roche Applied Science). Primers and run conditions were as previously described (78). Data were analyzed using the Pfaffl method (79). The ratio between HIV LTR DNA and *polymerase (RNA) II (DNA directed) polypeptide A* (*POLR2A*) was calculated and normalized so that HIV LTR DNA in untreated cells equals 1.00. Data was then $\log_2$ transformed.

Statistics – Data were assessed for symmetry, or skewness, using Pearson's skewness coefficient. Fold change data were $\log_2$ transformed to convert the ratio to a difference that better approximates the normal distribution on a log scale. Comparisons between groups were performed using the paired, two-tailed, Student's *t* test. However, if the ratio of the standard deviations between two groups is greater than 2, then the Welch's unequal variances *t* test was used. Differences were considered to be statistically significant when $P < 0.05$ and is indicated on plots and charts with *. All data are presented as individual data points representing individual donors on scatter plots overlain with the mean ± 95 % confidence interval (CI), unless stated otherwise.

Acknowledgements: This work was supported, in whole or in part, by the National Institute of Neurological Disorders and Stroke of the NIH (www.ninds.nih.gov) (R01 NS084912 and R01 NS104015 to SAS), the National Cancer Institute (www.cancer.gov) (R01 CA172513 and R42 CA192656 to DLD), the California HIV/AIDS Research Program (www.californiaaidsresearch.org) (ID12-SD-255 to GRC), and the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (impaactnetwork.org). Overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network was provided by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) under Award Numbers UM1AI068632 (IMPAACT LOC), UM1AI068616 (IMPAACT SDMC) and UM1AI106716 (IMPAACT LC), with co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (NIMH). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest: DLD declares a financial conflict of interest with SignalRx Pharmaceuticals. The relationship between DLD and SignalRx has been reviewed by the conflict of interest office within UC San Diego. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author contributions: Conceived and designed the experiments: GRC RSB SJ DLD SAS. Performed the experiments: GRC RSB SDH. Analyzed the data: GRC RSB SDH SAS. Contributed reagents/materials/analysis tools: GRC DLD SAS. Wrote the paper: GRC RSB SDH SJ DLD SAS.

References

1. Kandathil, A. J., Sugawara, S., and Balagopal, A. (2016) Are T cells the only HIV-1 reservoir? *Retrovirology* **13**, 86
2. Fletcher, C. V., Staskus, K., Wietgrefe, S. W., Rothenberger, M., Reilly, C., Chipman, J. G., Beilman, G. J., Khoruts, A., Thorkelson, A., Schmidt, T. E., Anderson, J., Perkey, K., Stevenson, M., Perelson, A. S., Douek, D. C., Haase, A. T., and Schacker, T. W. (2014) Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 2307-2312
3. Calcagno, A., Di Perri, G., and Bonora, S. (2014) Pharmacokinetics and pharmacodynamics of antiretrovirals in the central nervous system. *Clin. Pharmacokinet.* **53**, 891-906
4. Sigal, A., Kim, J. T., Balazs, A. B., Dekel, E., Mayo, A., Milo, R., and Baltimore, D. (2011) Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. *Nature* **477**, 95-98
5. Campbell, G. R., and Spector, S. A. (2013) Inhibition of human immunodeficiency virus type-1 through autophagy. *Curr. Opin. Microbiol.* **16**, 349-354
6. Kyei, G. B., Dinkins, C., Davis, A. S., Roberts, E., Singh, S. B., Dong, C., Wu, L., Kominami, E., Ueno, T., Yamamoto, A., Federico, M., Panganiban, A., Vergne, I., and Deretic, V. (2009) Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J. Cell Biol.* **186**, 255-268
7. Campbell, G. R., Rawat, P., Bruckman, R. S., and Spector, S. A. (2015) Human immunodeficiency virus type 1 Nef inhibits autophagy through transcription factor EB sequestration. *PLoS Pathog.* **11**, e1005018
8. Shoji-Kawata, S., Sumpter, R., Leveno, M., Campbell, G. R., Zou, Z., Kinch, L., Wilkins, A. D., Sun, Q., Pallauf, K., MacDuff, D., Huerta, C., Virgin, H. W., Helms, J. B., Eerland, R., Tooze, S. A., Xavier, R., Lenschow, D. J., Yamamoto, A., King, D., Lichtarge, O., Grishin, N. V., Spector, S. A., Kaloyanova, D. V., and Levine, B. (2013) Identification of a candidate therapeutic autophagy-inducing peptide. *Nature* **494**, 201-206
9. Campbell, G. R., and Spector, S. A. (2011) Hormonally active vitamin D3 (1α,25-dihydroxycholecalciferol) triggers autophagy in human macrophages that inhibits HIV-1 infection. *J. Biol. Chem.* **286**, 18890-18902
10. Campbell, G. R., and Spector, S. A. (2012) Vitamin D inhibits human immunodeficiency virus type 1 and Mycobacterium tuberculosis infection in macrophages through the induction of autophagy. *PLoS Pathog.* **8**, e1002689
11. Zhou, D., and Spector, S. A. (2008) Human immunodeficiency virus type-1 infection inhibits autophagy. *AIDS* **22**, 695-699
12. Campbell, G. R., Bruckman, R. S., Chu, Y. L., and Spector, S. A. (2015) Autophagy induction by histone deacetylase inhibitors inhibits HIV type 1. *J. Biol. Chem.* **290**, 5028-5040
13. Campbell, G. R., and Spector, S. A. (2012) Toll-like receptor 8 ligands activate a vitamin D mediated autophagic response that inhibits human immunodeficiency virus type 1. *PLoS Pathog.* **8**, e1003017
14. Lui, V. W., Hedberg, M. L., Li, H., Vangara, B. S., Pendleton, K., Zeng, Y., Lu, Y., Zhang, Q., Du, Y., Gilbert, B. R., Freilino, M., Sauerwein, S., Peyser, N. D., Xiao, D., Diergaarde, B., Wang, L., Chiosea, S., Seethala, R., Johnson, J. T., Kim, S., Duvvuri, U., Ferris, R. L., Romkes, M., Nukui, T., Kwok-Shing Ng, P., Garraway, L. A., Hammerman, P. S., Mills, G. B., and Grandis, J. R. (2013) Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. *Cancer Discov.* **3**, 761-769
15. Samuels, Y., and Ericson, K. (2006) Oncogenic PI3K and its role in cancer. *Curr. Opin. Oncol.* **18**, 77-82
16. Song, M. S., Salmena, L., and Pandolfi, P. P. (2012) The functions and regulation of the PTEN tumour suppressor. *Nat. Rev. Mol. Cell Biol.* **13**, 283-296

17. *Kinase Profiling Inhibitor Database* (2018) MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, Dundee, UK, http://www.kinase-screen.mrc.ac.uk/screening-compounds/345906
18. Chiarini, F., Grimaldi, C., Ricci, F., Tazzari, P. L., Evangelisti, C., Ognibene, A., Battistelli, M., Falcieri, E., Melchionda, F., Pession, A., Pagliaro, P., McCubrey, J. A., and Martelli, A. M. (2010) Activity of the novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 against T-cell acute lymphoblastic leukemia. *Cancer Res.* **70**, 8097-8107
19. Fan, Q. W., Cheng, C., Hackett, C., Feldman, M., Houseman, B. T., Nicolaides, T., Haas-Kogan, D., James, C. D., Oakes, S. A., Debnath, J., Shokat, K. M., and Weiss, W. A. (2010) Akt and autophagy cooperate to promote survival of drug-resistant glioma. *Science signaling* **3**, ra81
20. Serra, V., Markman, B., Scaltriti, M., Eichhorn, P. J., Valero, V., Guzman, M., Botero, M. L., Llonch, E., Atzori, F., Di Cosimo, S., Maira, M., Garcia-Echeverria, C., Parra, J. L., Arribas, J., and Baselga, J. (2008) NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res.* **68**, 8022-8030
21. Maira, S. M., Stauffer, F., Brueggen, J., Furet, P., Schnell, C., Fritsch, C., Brachmann, S., Chene, P., De Pover, A., Schoemaker, K., Fabbro, D., Gabriel, D., Simonen, M., Murphy, L., Finan, P., Sellers, W., and Garcia-Echeverria, C. (2008) Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol. Cancer Ther.* **7**, 1851-1863
22. Chang, Z., Shi, G., Jin, J., Guo, H., Guo, X., Luo, F., Song, Y., and Jia, X. (2013) Dual PI3K/mTOR inhibitor NVP-BEZ235-induced apoptosis of hepatocellular carcinoma cell lines is enhanced by inhibitors of autophagy. *Int. J. Mol. Med.* **31**, 1449-1456
23. Urata, S., Ngo, N., and de la Torre, J. C. (2012) The PI3K/Akt pathway contributes to arenavirus budding. *J. Virol.* **86**, 4578-4585
24. Smallwood, H. S., Duan, S., Morfouace, M., Rezinciuc, S., Shulkin, B. L., Shelat, A., Zink, E. E., Milasta, S., Bajracharya, R., Oluwaseum, A. J., Roussel, M. F., Green, D. R., Pasa-Tolic, L., and Thomas, P. G. (2017) Targeting Metabolic Reprogramming by Influenza Infection for Therapeutic Intervention. *Cell reports* **19**, 1640-1653
25. Karn, J. (2013) A new BET on the control of HIV latency. *Cell Cycle* **12**, 545-546
26. Hewings, D. S., Rooney, T. P., Jennings, L. E., Hay, D. A., Schofield, C. J., Brennan, P. E., Knapp, S., and Conway, S. J. (2012) Progress in the development and application of small molecule inhibitors of bromodomain-acetyl-lysine interactions. *J Med Chem* **55**, 9393-9413
27. Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W. B., Fedorov, O., Morse, E. M., Keates, T., Hickman, T. T., Felletar, I., Philpott, M., Munro, S., McKeown, M. R., Wang, Y., Christie, A. L., West, N., Cameron, M. J., Schwartz, B., Heightman, T. D., La Thangue, N., French, C. A., Wiest, O., Kung, A. L., Knapp, S., and Bradner, J. E. (2010) Selective inhibition of BET bromodomains. *Nature* **468**, 1067-1073
28. Lu, P., Qu, X., Shen, Y., Jiang, Z., Wang, P., Zeng, H., Ji, H., Deng, J., Yang, X., Li, X., Lu, H., and Zhu, H. (2016) The BET inhibitor OTX015 reactivates latent HIV-1 through P-TEFb. *Sci Rep* **6**, 24100
29. Li, Z., Guo, J., Wu, Y., and Zhou, Q. (2013) The BET bromodomain inhibitor JQ1 activates HIV latency through antagonizing Brd4 inhibition of Tat-transactivation. *Nucleic Acids Res* **41**, 277-287
30. Banerjee, C., Archin, N., Michaels, D., Belkina, A. C., Denis, G. V., Bradner, J., Sebastiani, P., Margolis, D. M., and Montano, M. (2012) BET bromodomain inhibition as a novel strategy for reactivation of HIV-1. *J Leukoc Biol* **92**, 1147-1154
31. Singh, A., Joshi, S., Garlich, J. R., Morales, G., Kutateladze, T., Andrews, F., and Durden, D. (2016) A novel dual epigenetic/kinase inhibitory platform: SF2523 and analogues as first-in-class PI3K/BRD4 inhibitors. *Cancer Res.* **76**, LB-211
32. Joshi, S., Singh, A. R., Zulcic, M., and Durden, D. L. (2014) A macrophage-dominant PI3K isoform controls hypoxia-induced HIF1alpha and HIF2alpha stability and tumor growth, angiogenesis, and metastasis. *Mol Cancer Res* **12**, 1520-1531

33. Andrews, F. H., Singh, A. R., Joshi, S., Smith, C. A., Morales, G. A., Garlich, J. R., Durden, D. L., and Kutateladze, T. G. (2017) Dual-activity PI3K-BRD4 inhibitor for the orthogonal inhibition of MYC to block tumor growth and metastasis. *Proc Natl Acad Sci U S A* **114**, E1072-E1080
34. Morales, G. A., Garlich, J. R., Su, J., Peng, X., Newblom, J., Weber, K., and Durden, D. L. (2013) Synthesis and cancer stem cell-based activity of substituted 5-morpholino-7H-thieno[3,2-b]pyran-7-ones designed as next generation PI3K inhibitors. *J. Med. Chem.* **56**, 1922-1939
35. Cerniglia, G. J., Karar, J., Tyagi, S., Christofidou-Solomidou, M., Rengan, R., Koumenis, C., and Maity, A. (2012) Inhibition of autophagy as a strategy to augment radiosensitization by the dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. *Mol. Pharmacol.* **82**, 1230-1240
36. Ji, Y., Di, W., Yang, Q., Lu, Z., Cai, W., and Wu, J. (2015) Inhibition of autophagy increases proliferation inhibition and apoptosis induced by the PI3K/mTOR inhibitor NVP-BEZ235 in breast cancer cells. *Clin. Lab.* **61**, 1043-1051
37. Kuger, S., Corek, E., Polat, B., Kammerer, U., Flentje, M., and Djuzenova, C. S. (2014) Novel PI3K and mTOR inhibitor NVP-BEZ235 radiosensitizes breast cancer cell lines under normoxic and hypoxic conditions. *Breast Cancer (Auckl.)* **8**, 39-49
38. Yang, X., Niu, B., Wang, L., Chen, M., Kang, X., Wang, L., Ji, Y., and Zhong, J. (2016) Autophagy inhibition enhances colorectal cancer apoptosis induced by dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. *Oncol. Lett.* **12**, 102-106
39. Yu, Y., Yu, X., Ma, J., Tong, Y., and Yao, J. (2016) Effects of NVP-BEZ235 on the proliferation, migration, apoptosis and autophagy in HT-29 human colorectal adenocarcinoma cells. *Int. J. Oncol.* **49**, 285-293
40. Klionsky, D. J., Abdelmohsen, K., Abe, A., Abedin, M. J., Abeliovich, H., Acevedo Arozena, A., Adachi, H., Adams, C. M., Adams, P. D., Adeli, K., Adhihetty, P. J., Adler, S. G., Agam, G., Agarwal, R., Aghi, M. K., Agnello, M., Agostinis, P., Aguilar, P. V., Aguirre-Ghiso, J., Airoldi, E. M., Ait-Si-Ali, S., Akematsu, T., Akporiaye, E. T., Al-Rubeai, M., Albaiceta, G. M., Albanese, C., Albani, D., Albert, M. L., Aldudo, J., Algul, H., Alirezaei, M., Alloza, I., Almasan, A., Almonte-Beceril, M., Alnemri, E. S., Alonso, C., Altan-Bonnet, N., Altieri, D. C., Alvarez, S., Alvarez-Erviti, L., Alves, S., Amadoro, G., Amano, A., Amantini, C., Ambrosio, S., Amelio, I., Amer, A. O., Amessou, M., Amon, A., An, Z., Anania, F. A., Andersen, S. U., Andley, U. P., Andreadi, C. K., Andrieu-Abadie, N., Anel, A., Ann, D. K., Anoopkumar-Dukie, S., Antonioli, M., Aoki, H., Apostolova, N., Aquila, S., Aquilano, K., Araki, K., Arama, E., Aranda, A., Araya, J., Arcaro, A., Arias, E., Arimoto, H., Ariosa, A. R., Armstrong, J. L., Arnould, T., Arsov, I., Asanuma, K., Askanas, V., Asselin, E., Atarashi, R., Atherton, S. S., Atkin, J. D., Attardi, L. D., Auberger, P., Auburger, G., Aurelian, L., Autelli, R., Avagliano, L., Avantaggiati, M. L., Avrahami, L., Awale, S., Azad, N., Bachetti, T., Backer, J. M., Bae, D. H., Bae, J. S., Bae, O. N., Bae, S. H., Baehrecke, E. H., Baek, S. H., Baghdiguian, S., Bagniewska-Zadworna, A., Bai, H., Bai, J., Bai, X. Y., Bailly, Y., Balaji, K. N., Balduini, W., Ballabio, A., Balzan, R., Banerjee, R., Banhegyi, G., Bao, H., Barbeau, B., Barrachina, M. D., Barreiro, E., Bartel, B., Bartolome, A., Bassham, D. C., Bassi, M. T., Bast, R. C., Jr., Basu, A., Batista, M. T., Batoko, H., Battino, M., Bauckman, K., Baumgarner, B. L., Bayer, K. U., Beale, R., Beaulieu, J. F., Beck, G. R., Jr., Becker, C., Beckham, J. D., Bedard, P. A., Bednarski, P. J., Begley, T. J., Behl, C., Behrends, C., Behrens, G. M., Behrns, K. E., Bejarano, E., Belaid, A., Belleudi, F., Benard, G., Berchem, G., Bergamaschi, D., Bergami, M., Berkhout, B., Berliocchi, L., Bernard, A., Bernard, M., Bernassola, F., Bertolotti, A., Bess, A. S., Besteiro, S., Bettuzzi, S., Bhalla, S., Bhattacharyya, S., Bhutia, S. K., Biagosch, C., Bianchi, M. W., Biard-Piechaczyk, M., Billes, V., Bincoletto, C., Bingol, B., Bird, S. W., Bitoun, M., Bjedov, I., Blackstone, C., Blanc, L., Blanco, G. A., Blomhoff, H. K., Boada-Romero, E., Bockler, S., Boes, M., Boesze-Battaglia, K., Boise, L. H., Bolino, A., Boman, A., Bonaldo, P., Bordi, M., Bosch, J., Botana, L. M., Botti, J., Bou, G., Bouche, M., Bouchecareilh, M., Boucher, M. J., Boulton, M. E., Bouret, S. G., Boya, P., Boyer-Guittaut, M., Bozhkov, P. V., Brady, N., Braga, V. M., Brancolini, C., Braus, G. H., Bravo-San Pedro, J. M., Brennan, L. A., Bresnick, E. H., Brest, P., Bridges, D.,

Bringer, M. A., Brini, M., Brito, G. C., Brodin, B., Brookes, P. S., Brown, E. J., Brown, K., Broxmeyer, H. E., Bruhat, A., Brum, P. C., Brumell, J. H., Brunetti-Pierri, N., Bryson-Richardson, R. J., Buch, S., Buchan, A. M., Budak, H., Bulavin, D. V., Bultman, S. J., Bultynck, G., Bumbasirevic, V., Burelle, Y., Burke, R. E., Burmeister, M., Butikofer, P., Caberlotto, L., Cadwell, K., Cahova, M., Cai, D., Cai, J., Cai, Q., Calatayud, S., Camougrand, N., Campanella, M., Campbell, G. R., Campbell, M., Campello, S., Candau, R., Caniggia, I., Cantoni, L., Cao, L., Caplan, A. B., Caraglia, M., Cardinali, C., Cardoso, S. M., Carew, J. S., Carleton, L. A., Carlin, C. R., Carloni, S., Carlsson, S. R., Carmona-Gutierrez, D., Carneiro, L. A., Carnevali, O., Carra, S., Carrier, A., Carroll, B., Casas, C., Casas, J., Cassinelli, G., Castets, P., Castro-Obregon, S., Cavallini, G., Ceccherini, I., Cecconi, F., Cederbaum, A. I., Cena, V., Cenci, S., Cerella, C., Cervia, D., Cetrullo, S., Chaachouay, H., Chae, H. J., Chagin, A. S., Chai, C. Y., Chakrabarti, G., Chamilos, G., Chan, E. Y., Chan, M. T., Chandra, D., Chandra, P., Chang, C. P., Chang, R. C., Chang, T. Y., Chatham, J. C., Chatterjee, S., Chauhan, S., Che, Y., Cheetham, M. E., Cheluvappa, R., Chen, C. J., Chen, G., Chen, G. C., Chen, G., Chen, H., Chen, J. W., Chen, J. K., Chen, M., Chen, M., Chen, P., Chen, Q., Chen, Q., Chen, S. D., Chen, S., Chen, S. S., Chen, W., Chen, W. J., Chen, W. Q., Chen, W., Chen, X., Chen, Y. H., Chen, Y. G., Chen, Y., Chen, Y., Chen, Y., Chen, Y. J., Chen, Y. Q., Chen, Y., Chen, Z., Chen, Z., Cheng, A., Cheng, C. H., Cheng, H., Cheong, H., Cherry, S., Chesney, J., Cheung, C. H., Chevet, E., Chi, H. C., Chi, S. G., Chiacchiera, F., Chiang, H. L., Chiarelli, R., Chiariello, M., Chieppa, M., Chin, L. S., Chiong, M., Chiu, G. N., Cho, D. H., Cho, S. G., Cho, W. C., Cho, Y. Y., Cho, Y. S., Choi, A. M., Choi, E. J., Choi, E. K., Choi, J., Choi, M. E., Choi, S. I., Chou, T. F., Chouaib, S., Choubey, D., Choubey, V., Chow, K. C., Chowdhury, K., Chu, C. T., Chuang, T. H., Chun, T., Chung, H., Chung, T., Chung, Y. L., Chwae, Y. J., Cianfanelli, V., Ciarcia, R., Ciechomska, I. A., Ciriolo, M. R., Cirone, M., Claerhout, S., Clague, M. J., Claria, J., Clarke, P. G., Clarke, R., Clementi, E., Cleyrat, C., Cnop, M., Coccia, E. M., Cocco, T., Codogno, P., Coers, J., Cohen, E. E., Colecchia, D., Coletto, L., Coll, N. S., Colucci-Guyon, E., Comincini, S., Condello, M., Cook, K. L., Coombs, G. H., Cooper, C. D., Cooper, J. M., Coppens, I., Corasaniti, M. T., Corazzari, M., Corbalan, R., Corcelle-Termeau, E., Cordero, M. D., Corral-Ramos, C., Corti, O., Cossarizza, A., Costelli, P., Costes, S., Cotman, S. L., Coto-Montes, A., Cottet, S., Couve, E., Covey, L. R., Cowart, L. A., Cox, J. S., Coxon, F. P., Coyne, C. B., Cragg, M. S., Craven, R. J., Crepaldi, T., Crespo, J. L., Criollo, A., Crippa, V., Cruz, M. T., Cuervo, A. M., Cuezva, J. M., Cui, T., Cutillas, P. R., Czaja, M. J., Czyzyk-Krzeska, M. F., Dagda, R. K., Dahmen, U., Dai, C., Dai, W., Dai, Y., Dalby, K. N., Dalla Valle, L., Dalmasso, G., D'Amelio, M., Damme, M., Darfeuille-Michaud, A., Dargemont, C., Darley-Usmar, V. M., Dasarathy, S., Dasgupta, B., Dash, S., Dass, C. R., Davey, H. M., Davids, L. M., Davila, D., Davis, R. J., Dawson, T. M., Dawson, V. L., Daza, P., de Belleroche, J., de Figueiredo, P., de Figueiredo, R. C., de la Fuente, J., De Martino, L., De Matteis, A., De Meyer, G. R., De Milito, A., De Santi, M., de Souza, W., De Tata, V., De Zio, D., Debnath, J., Dechant, R., Decuypere, J. P., Deegan, S., Dehay, B., Del Bello, B., Del Re, D. P., Delage-Mourroux, R., Delbridge, L. M., Deldicque, L., Delorme-Axford, E., Deng, Y., Dengjel, J., Denizot, M., Dent, P., Der, C. J., Deretic, V., Derrien, B., Deutsch, E., Devarenne, T. P., Devenish, R. J., Di Bartolomeo, S., Di Daniele, N., Di Domenico, F., Di Nardo, A., Di Paola, S., Di Pietro, A., Di Renzo, L., DiAntonio, A., Diaz-Araya, G., Diaz-Laviada, I., Diaz-Meco, M. T., Diaz-Nido, J., Dickey, C. A., Dickson, R. C., Diederich, M., Digard, P., Dikic, I., Dinesh-Kumar, S. P., Ding, C., Ding, W. X., Ding, Z., Dini, L., Distler, J. H., Diwan, A., Djavaheri-Mergny, M., Dmytruk, K., Dobson, R. C., Doetsch, V., Dokladny, K., Dokudovskaya, S., Donadelli, M., Dong, X. C., Dong, X., Dong, Z., Donohue, T. M., Jr., Doran, K. S., D'Orazi, G., Dorn, G. W., 2nd, Dosenko, V., Dridi, S., Drucker, L., Du, J., Du, L. L., Du, L., du Toit, A., Dua, P., Duan, L., Duann, P., Dubey, V. K., Duchen, M. R., Duchosal, M. A., Duez, H., Dugail, I., Dumit, V. I., Duncan, M. C., Dunlop, E. A., Dunn, W. A., Jr., Dupont, N., Dupuis, L., Duran, R. V., Durcan, T. M., Duvezin-Caubet, S., Duvvuri, U., Eapen, V., Ebrahimi-Fakhari, D., Echard, A., Eckhart, L., Edelstein, C. L., Edinger, A. L., Eichinger, L., Eisenberg, T., Eisenberg-Lerner, A., Eissa, N. T., El-Deiry, W. S., El-Khoury, V., Elazar, Z., Eldar-Finkelman, H., Elliott, C.

J., Emanuele, E., Emmenegger, U., Engedal, N., Engelbrecht, A. M., Engelender, S., Enserink, J. M., Erdmann, R., Erenpreisa, J., Eri, R., Eriksen, J. L., Erman, A., Escalante, R., Eskelinen, E. L., Espert, L., Esteban-Martinez, L., Evans, T. J., Fabri, M., Fabrias, G., Fabrizi, C., Facchiano, A., Faergeman, N. J., Faggioni, A., Fairlie, W. D., Fan, C., Fan, D., Fan, J., Fang, S., Fanto, M., Fanzani, A., Farkas, T., Faure, M., Favier, F. B., Fearnhead, H., Federici, M., Fei, E., Felizardo, T. C., Feng, H., Feng, Y., Feng, Y., Ferguson, T. A., Fernandez, A. F., Fernandez-Barrena, M. G., Fernandez-Checa, J. C., Fernandez-Lopez, A., Fernandez-Zapico, M. E., Feron, O., Ferraro, E., Ferreira-Halder, C. V., Fesus, L., Feuer, R., Fiesel, F. C., Filippi-Chiela, E. C., Filomeni, G., Fimia, G. M., Fingert, J. H., Finkbeiner, S., Finkel, T., Fiorito, F., Fisher, P. B., Flajolet, M., Flamigni, F., Florey, O., Florio, S., Floto, R. A., Folini, M., Follo, C., Fon, E. A., Fornai, F., Fortunato, F., Fraldi, A., Franco, R., Francois, A., Francois, A., Frankel, L. B., Fraser, I. D., Frey, N., Freyssenet, D. G., Frezza, C., Friedman, S. L., Frigo, D. E., Fu, D., Fuentes, J. M., Fueyo, J., Fujitani, Y., Fujiwara, Y., Fujiya, M., Fukuda, M., Fulda, S., Fusco, C., Gabryel, B., Gaestel, M., Gailly, P., Gajewska, M., Galadari, S., Galili, G., Galindo, I., Galindo, M. F., Galliciotti, G., Galluzzi, L., Galluzzi, L., Galy, V., Gammoh, N., Gandy, S., Ganesan, A. K., Ganesan, S., Ganley, I. G., Gannage, M., Gao, F. B., Gao, F., Gao, J. X., Garcia Nannig, L., Garcia Vescovi, E., Garcia-Macia, M., Garcia-Ruiz, C., Garg, A. D., Garg, P. K., Gargini, R., Gassen, N. C., Gatica, D., Gatti, E., Gavard, J., Gavathiotis, E., Ge, L., Ge, P., Ge, S., Gean, P. W., Gelmetti, V., Genazzani, A. A., Geng, J., Genschik, P., Gerner, L., Gestwicki, J. E., Gewirtz, D. A., Ghavami, S., Ghigo, E., Ghosh, D., Giammarioli, A. M., Giampieri, F., Giampietri, C., Giatromanolaki, A., Gibbings, D. J., Gibellini, L., Gibson, S. B., Ginet, V., Giordano, A., Giorgini, F., Giovannetti, E., Girardin, S. E., Gispert, S., Giuliano, S., Gladson, C. L., Glavic, A., Gleave, M., Godefroy, N., Gogal, R. M., Jr., Gokulan, K., Goldman, G. H., Goletti, D., Goligorsky, M. S., Gomes, A. V., Gomes, L. C., Gomez, H., Gomez-Manzano, C., Gomez-Sanchez, R., Goncalves, D. A., Goncu, E., Gong, Q., Gongora, C., Gonzalez, C. B., Gonzalez-Alegre, P., Gonzalez-Cabo, P., Gonzalez-Polo, R. A., Goping, I. S., Gorbea, C., Gorbunov, N. V., Goring, D. R., Gorman, A. M., Gorski, S. M., Goruppi, S., Goto-Yamada, S., Gotor, C., Gottlieb, R. A., Gozes, I., Gozuacik, D., Graba, Y., Graef, M., Granato, G. E., Grant, G. D., Grant, S., Gravina, G. L., Green, D. R., Greenhough, A., Greenwood, M. T., Grimaldi, B., Gros, F., Grose, C., Groulx, J. F., Gruber, F., Grumati, P., Grune, T., Guan, J. L., Guan, K. L., Guerra, B., Guillen, C., Gulshan, K., Gunst, J., Guo, C., Guo, L., Guo, M., Guo, W., Guo, X. G., Gust, A. A., Gustafsson, A. B., Gutierrez, E., Gutierrez, M. G., Gwak, H. S., Haas, A., Haber, J. E., Hadano, S., Hagedorn, M., Hahn, D. R., Halayko, A. J., Hamacher-Brady, A., Hamada, K., Hamai, A., Hamann, A., Hamasaki, M., Hamer, I., Hamid, Q., Hammond, E. M., Han, F., Han, W., Handa, J. T., Hanover, J. A., Hansen, M., Harada, M., Harhaji-Trajkovic, L., Harper, J. W., Harrath, A. H., Harris, A. L., Harris, J., Hasler, U., Hasselblatt, P., Hasui, K., Hawley, R. G., Hawley, T. S., He, C., He, C. Y., He, F., He, G., He, R. R., He, X. H., He, Y. W., He, Y. Y., Heath, J. K., Hebert, M. J., Heinzen, R. A., Helgason, G. V., Hensel, M., Henske, E. P., Her, C., Herman, P. K., Hernandez, A., Hernandez, C., Hernandez-Tiedra, S., Hetz, C., Hiesinger, P. R., Higaki, K., Hilfiker, S., Hill, B. G., Hill, J. A., Hill, W. D., Hino, K., Hofius, D., Hofman, P., Hoglinger, G. U., Hohfeld, J., Holz, M. K., Hong, Y., Hood, D. A., Hoozemans, J. J., Hoppe, T., Hsu, C., Hsu, C. Y., Hsu, L. C., Hu, D., Hu, G., Hu, H. M., Hu, H., Hu, M. C., Hu, Y. C., Hu, Z. W., Hua, F., Hua, Y., Huang, C., Huang, H. L., Huang, K. H., Huang, K. Y., Huang, S., Huang, S., Huang, W. P., Huang, Y. R., Huang, Y., Huang, Y., Huber, T. B., Huebbe, P., Huh, W. K., Hulmi, J. J., Hur, G. M., Hurley, J. H., Husak, Z., Hussain, S. N., Hussain, S., Hwang, J. J., Hwang, S., Hwang, T. I., Ichihara, A., Imai, Y., Imbriano, C., Inomata, M., Into, T., Iovane, V., Iovanna, J. L., Iozzo, R. V., Ip, N. Y., Irazoqui, J. E., Iribarren, P., Isaka, Y., Isakovic, A. J., Ischiropoulos, H., Isenberg, J. S., Ishaq, M., Ishida, H., Ishii, I., Ishmael, J. E., Isidoro, C., Isobe, K. I., Isono, E., Issazadeh-Navikas, S., Itahana, K., Itakura, E., Ivanov, A. I., Iyer, A. K., Izquierdo, J. M., Izumi, Y., Izzo, V., Jaattela, M., Jaber, N., Jackson, D. J., Jackson, W. T., Jacob, T. G., Jacques, T. S., Jagannath, C., Jain, A., Jana, N. R., Jang, B. K., Jani, A., Janji, B., Jannig, P. R., Jansson, P. J., Jean, S., Jendrach, M., Jeon, J. H., Jessen, N., Jeung, E. B., Jia, K., Jia, L., Jiang, H., Jiang, H., Jiang, L., Jiang, T., Jiang,

X., Jiang, X., Jiang, X., Jiang, Y., Jiang, Y., Jimenez, A., Jin, C., Jin, H., Jin, L., Jin, M., Jin, S., Jinwal, U. K., Jo, E. K., Johansen, T., Johnson, D. E., Johnson, G. V., Johnson, J. D., Jonasch, E., Jones, C., Joosten, L. A., Jordan, J., Joseph, A. M., Joseph, B., Joubert, A. M., Ju, D., Ju, J., Juan, H. F., Juenemann, K., Juhasz, G., Jung, H. S., Jung, J. U., Jung, Y. K., Jungbluth, H., Justice, M. J., Jutten, B., Kaakoush, N. O., Kaarniranta, K., Kaasik, A., Kabuta, T., Kaeffer, B., Kagedal, K., Kahana, A., Kajimura, S., Kakhlon, O., Kalia, M., Kalvakolanu, D. V., Kamada, Y., Kambas, K., Kaminskyy, V. O., Kampinga, H. H., Kandouz, M., Kang, C., Kang, R., Kang, T. C., Kanki, T., Kanneganti, T. D., Kanno, H., Kanthasamy, A. G., Kantorow, M., Kaparakis-Liaskos, M., Kapuy, O., Karantza, V., Karim, M. R., Karmakar, P., Kaser, A., Kaushik, S., Kawula, T., Kaynar, A. M., Ke, P. Y., Ke, Z. J., Kehrl, J. H., Keller, K. E., Kemper, J. K., Kenworthy, A. K., Kepp, O., Kern, A., Kesari, S., Kessel, D., Ketteler, R., Kettelhut, I. D., Khambu, B., Khan, M. M., Khandelwal, V. K., Khare, S., Kiang, J. G., Kiger, A. A., Kihara, A., Kim, A. L., Kim, C. H., Kim, D. R., Kim, D. H., Kim, E. K., Kim, H. Y., Kim, H. R., Kim, J. S., Kim, J. H., Kim, J. C., Kim, J. H., Kim, K. W., Kim, M. D., Kim, M. M., Kim, P. K., Kim, S. W., Kim, S. Y., Kim, Y. S., Kim, Y., Kimchi, A., Kimmelman, A. C., Kimura, T., King, J. S., Kirkegaard, K., Kirkin, V., Kirshenbaum, L. A., Kishi, S., Kitajima, Y., Kitamoto, K., Kitaoka, Y., Kitazato, K., Kley, R. A., Klimecki, W. T., Klinkenberg, M., Klucken, J., Knaevelsrud, H., Knecht, E., Knuppertz, L., Ko, J. L., Kobayashi, S., Koch, J. C., Koechlin-Ramonatxo, C., Koenig, U., Koh, Y. H., Kohler, K., Kohlwein, S. D., Koike, M., Komatsu, M., Kominami, E., Kong, D., Kong, H. J., Konstantakou, E. G., Kopp, B. T., Korcsmaros, T., Korhonen, L., Korolchuk, V. I., Koshkina, N. V., Kou, Y., Koukourakis, M. I., Koumenis, C., Kovacs, A. L., Kovacs, T., Kovacs, W. J., Koya, D., Kraft, C., Krainc, D., Kramer, H., Kravic-Stevovic, T., Krek, W., Kretz-Remy, C., Krick, R., Krishnamurthy, M., Kriston-Vizi, J., Kroemer, G., Kruer, M. C., Kruger, R., Ktistakis, N. T., Kuchitsu, K., Kuhn, C., Kumar, A. P., Kumar, A., Kumar, A., Kumar, D., Kumar, D., Kumar, R., Kumar, S., Kundu, M., Kung, H. J., Kuno, A., Kuo, S. H., Kuret, J., Kurz, T., Kwok, T., Kwon, T. K., Kwon, Y. T., Kyrmizi, I., La Spada, A. R., Lafont, F., Lahm, T., Lakkaraju, A., Lam, T., Lamark, T., Lancel, S., Landowski, T. H., Lane, D. J., Lane, J. D., Lanzi, C., Lapaquette, P., Lapierre, L. R., Laporte, J., Laukkarinen, J., Laurie, G. W., Lavandero, S., Lavie, L., LaVoie, M. J., Law, B. Y., Law, H. K., Law, K. B., Layfield, R., Lazo, P. A., Le Cam, L., Le Roch, K. G., Le Stunff, H., Leardkamolkarn, V., Lecuit, M., Lee, B. H., Lee, C. H., Lee, E. F., Lee, G. M., Lee, H. J., Lee, H., Lee, J. K., Lee, J., Lee, J. H., Lee, J. H., Lee, M., Lee, M. S., Lee, P. J., Lee, S. W., Lee, S. J., Lee, S. J., Lee, S. Y., Lee, S. H., Lee, S. S., Lee, S. J., Lee, S., Lee, Y. R., Lee, Y. J., Lee, Y. H., Leeuwenburgh, C., Lefort, S., Legouis, R., Lei, J., Lei, Q. Y., Leib, D. A., Leibowitz, G., Lekli, I., Lemaire, S. D., Lemasters, J. J., Lemberg, M. K., Lemoine, A., Leng, S., Lenz, G., Lenzi, P., Lerman, L. O., Lettieri Barbato, D., Leu, J. I., Leung, H. Y., Levine, B., Lewis, P. A., Lezoualc'h, F., Li, C., Li, F., Li, F. J., Li, J., Li, K., Li, L., Li, M., Li, M., Li, Q., Li, R., Li, S., Li, W., Li, W., Li, X., Li, Y., Lian, J., Liang, C., Liang, Q., Liao, Y., Liberal, J., Liberski, P. P., Lie, P., Lieberman, A. P., Lim, H. J., Lim, K. L., Lim, K., Lima, R. T., Lin, C. S., Lin, C. F., Lin, F., Lin, F., Lin, F. C., Lin, K., Lin, K. H., Lin, P. H., Lin, T., Lin, W. W., Lin, Y. S., Lin, Y., Linden, R., Lindholm, D., Lindqvist, L. M., Lingor, P., Linkermann, A., Liotta, L. A., Lipinski, M. M., Lira, V. A., Lisanti, M. P., Liton, P. B., Liu, B., Liu, C., Liu, C. F., Liu, F., Liu, H. J., Liu, J., Liu, J. J., Liu, J. L., Liu, K., Liu, L., Liu, L., Liu, Q., Liu, R. Y., Liu, S., Liu, S., Liu, W., Liu, X. D., Liu, X., Liu, X. H., Liu, X., Liu, X., Liu, X., Liu, Y., Liu, Y., Liu, Z., Liu, Z., Liuzzi, J. P., Lizard, G., Ljujic, M., Lodhi, I. J., Logue, S. E., Lokeshwar, B. L., Long, Y. C., Lonial, S., Loos, B., Lopez-Otin, C., Lopez-Vicario, C., Lorente, M., Lorenzi, P. L., Lorincz, P., Los, M., Lotze, M. T., Lovat, P. E., Lu, B., Lu, B., Lu, J., Lu, Q., Lu, S. M., Lu, S., Lu, Y., Luciano, F., Luckhart, S., Lucocq, J. M., Ludovico, P., Lugea, A., Lukacs, N. W., Lum, J. J., Lund, A. H., Luo, H., Luo, J., Luo, S., Luparello, C., Lyons, T., Ma, J., Ma, Y., Ma, Y., Ma, Z., Machado, J., Machado-Santelli, G. M., Macian, F., MacIntosh, G. C., MacKeigan, J. P., Macleod, K. F., MacMicking, J. D., MacMillan-Crow, L. A., Madeo, F., Madesh, M., Madrigal-Matute, J., Maeda, A., Maeda, T., Maegawa, G., Maellaro, E., Maes, H., Magarinos, M., Maiese, K., Maiti, T. K., Maiuri, L., Maiuri, M. C., Maki, C. G., Malli, R., Malorni, W.,

Maloyan, A., Mami-Chouaib, F., Man, N., Mancias, J. D., Mandelkow, E. M., Mandell, M. A., Manfredi, A. A., Manie, S. N., Manzoni, C., Mao, K., Mao, Z., Mao, Z. W., Marambaud, P., Marconi, A. M., Marelja, Z., Marfe, G., Margeta, M., Margittai, E., Mari, M., Mariani, F. V., Marin, C., Marinelli, S., Marino, G., Markovic, I., Marquez, R., Martelli, A. M., Martens, S., Martin, K. R., Martin, S. J., Martin, S., Martin-Acebes, M. A., Martin-Sanz, P., Martinand-Mari, C., Martinet, W., Martinez, J., Martinez-Lopez, N., Martinez-Outschoorn, U., Martinez-Velazquez, M., Martinez-Vicente, M., Martins, W. K., Mashima, H., Mastrianni, J. A., Matarese, G., Matarrese, P., Mateo, R., Matoba, S., Matsumoto, N., Matsushita, T., Matsuura, A., Matsuzawa, T., Mattson, M. P., Matus, S., Maugeri, N., Mauvezin, C., Mayer, A., Maysinger, D., Mazzolini, G. D., McBrayer, M. K., McCall, K., McCormick, C., McInerney, G. M., McIver, S. C., McKenna, S., McMahon, J. J., McNeish, I. A., Mechta-Grigoriou, F., Medema, J. P., Medina, D. L., Megyeri, K., Mehrpour, M., Mehta, J. L., Mei, Y., Meier, U. C., Meijer, A. J., Melendez, A., Melino, G., Melino, S., de Melo, E. J., Mena, M. A., Meneghini, M. D., Menendez, J. A., Menezes, R., Meng, L., Meng, L. H., Meng, S., Menghini, R., Menko, A. S., Menna-Barreto, R. F., Menon, M. B., Meraz-Rios, M. A., Merla, G., Merlini, L., Merlot, A. M., Meryk, A., Meschini, S., Meyer, J. N., Mi, M. T., Miao, C. Y., Micale, L., Michaeli, S., Michiels, C., Migliaccio, A. R., Mihailidou, A. S., Mijaljica, D., Mikoshiba, K., Milan, E., Miller-Fleming, L., Mills, G. B., Mills, I. G., Minakaki, G., Minassian, B. A., Ming, X. F., Minibayeva, F., Minina, E. A., Mintern, J. D., Minucci, S., Miranda-Vizuete, A., Mitchell, C. H., Miyamoto, S., Miyazawa, K., Mizushima, N., Mnich, K., Mograbi, B., Mohseni, S., Moita, L. F., Molinari, M., Molinari, M., Moller, A. B., Mollereau, B., Mollinedo, F., Mongillo, M., Monick, M. M., Montagnaro, S., Montell, C., Moore, D. J., Moore, M. N., Mora-Rodriguez, R., Moreira, P. I., Morel, E., Morelli, M. B., Moreno, S., Morgan, M. J., Moris, A., Moriyasu, Y., Morrison, J. L., Morrison, L. A., Morselli, E., Moscat, J., Moseley, P. L., Mostowy, S., Motori, E., Mottet, D., Mottram, J. C., Moussa, C. E., Mpakou, V. E., Mukhtar, H., Mulcahy Levy, J. M., Muller, S., Munoz-Moreno, R., Munoz-Pinedo, C., Munz, C., Murphy, M. E., Murray, J. T., Murthy, A., Mysorekar, I. U., Nabi, I. R., Nabissi, M., Nader, G. A., Nagahara, Y., Nagai, Y., Nagata, K., Nagelkerke, A., Nagy, P., Naidu, S. R., Nair, S., Nakano, H., Nakatogawa, H., Nanjundan, M., Napolitano, G., Naqvi, N. I., Nardacci, R., Narendra, D. P., Narita, M., Nascimbeni, A. C., Natarajan, R., Navegantes, L. C., Nawrocki, S. T., Nazarko, T. Y., Nazarko, V. Y., Neill, T., Neri, L. M., Netea, M. G., Netea-Maier, R. T., Neves, B. M., Ney, P. A., Nezis, I. P., Nguyen, H. T., Nguyen, H. P., Nicot, A. S., Nilsen, H., Nilsson, P., Nishimura, M., Nishino, I., Niso-Santano, M., Niu, H., Nixon, R. A., Njar, V. C., Noda, T., Noegel, A. A., Nolte, E. M., Norberg, E., Norga, K. K., Noureini, S. K., Notomi, S., Notterpek, L., Nowikovsky, K., Nukina, N., Nurnberger, T., O'Donnell, V. B., O'Donovan, T., O'Dwyer, P. J., Oehme, I., Oeste, C. L., Ogawa, M., Ogretmen, B., Ogura, Y., Oh, Y. J., Ohmuraya, M., Ohshima, T., Ojha, R., Okamoto, K., Okazaki, T., Oliver, F. J., Ollinger, K., Olsson, S., Orban, D. P., Ordonez, P., Orhon, I., Orosz, L., O'Rourke, E. J., Orozco, H., Ortega, A. L., Ortona, E., Osellame, L. D., Oshima, J., Oshima, S., Osiewacz, H. D., Otomo, T., Otsu, K., Ou, J. J., Outeiro, T. F., Ouyang, D. Y., Ouyang, H., Overholtzer, M., Ozbun, M. A., Ozdinler, P. H., Ozpolat, B., Pacelli, C., Paganetti, P., Page, G., Pages, G., Pagnini, U., Pajak, B., Pak, S. C., Pakos-Zebrucka, K., Pakpour, N., Palkova, Z., Palladino, F., Pallauf, K., Pallet, N., Palmieri, M., Paludan, S. R., Palumbo, C., Palumbo, S., Pampliega, O., Pan, H., Pan, W., Panaretakis, T., Pandey, A., Pantazopoulou, A., Papackova, Z., Papademetrio, D. L., Papassideri, I., Papini, A., Parajuli, N., Pardo, J., Parekh, V. V., Parenti, G., Park, J. I., Park, J., Park, O. K., Parker, R., Parlato, R., Parys, J. B., Parzych, K. R., Pasquet, J. M., Pasquier, B., Pasumarthi, K. B., Patschan, D., Patterson, C., Pattingre, S., Pattison, S., Pause, A., Pavenstadt, H., Pavone, F., Pedrozo, Z., Pena, F. J., Penalva, M. A., Pende, M., Peng, J., Penna, F., Penninger, J. M., Pensalfini, A., Pepe, S., Pereira, G. J., Pereira, P. C., Perez-de la Cruz, V., Perez-Perez, M. E., Perez-Rodriguez, D., Perez-Sala, D., Perier, C., Perl, A., Perlmutter, D. H., Perrotta, I., Pervaiz, S., Pesonen, M., Pessin, J. E., Peters, G. J., Petersen, M., Petrache, I., Petrof, B. J., Petrovski, G., Phang, J. M., Piacentini, M., Pierdominici, M., Pierre, P., Pierrefite-Carle, V., Pietrocola, F., Pimentel-Muinos, F. X., Pinar, M., Pineda, B., Pinkas-Kramarski, R., Pinti, M.,

Pinton, P., Piperdi, B., Piret, J. M., Platanias, L. C., Platta, H. W., Plowey, E. D., Poggeler, S., Poirot, M., Polcic, P., Poletti, A., Poon, A. H., Popelka, H., Popova, B., Poprawa, I., Poulose, S. M., Poulton, J., Powers, S. K., Powers, T., Pozuelo-Rubio, M., Prak, K., Prange, R., Prescott, M., Priault, M., Prince, S., Proia, R. L., Proikas-Cezanne, T., Prokisch, H., Promponas, V. J., Przyklenk, K., Puertollano, R., Pugazhenthi, S., Puglielli, L., Pujol, A., Puyal, J., Pyeon, D., Qi, X., Qian, W. B., Qin, Z. H., Qiu, Y., Qu, Z., Quadrilatero, J., Quinn, F., Raben, N., Rabinowich, H., Radogna, F., Ragusa, M. J., Rahmani, M., Raina, K., Ramanadham, S., Ramesh, R., Rami, A., Randall-Demllo, S., Randow, F., Rao, H., Rao, V. A., Rasmussen, B. B., Rasse, T. M., Ratovitski, E. A., Rautou, P. E., Ray, S. K., Razani, B., Reed, B. H., Reggiori, F., Rehm, M., Reichert, A. S., Rein, T., Reiner, D. J., Reits, E., Ren, J., Ren, X., Renna, M., Reusch, J. E., Revuelta, J. L., Reyes, L., Rezaie, A. R., Richards, R. I., Richardson, D. R., Richetta, C., Riehle, M. A., Rihn, B. H., Rikihisa, Y., Riley, B. E., Rimbach, G., Rippo, M. R., Ritis, K., Rizzi, F., Rizzo, E., Roach, P. J., Robbins, J., Roberge, M., Roca, G., Roccheri, M. C., Rocha, S., Rodrigues, C. M., Rodriguez, C. I., de Cordoba, S. R., Rodriguez-Muela, N., Roelofs, J., Rogov, V. V., Rohn, T. T., Rohrer, B., Romanelli, D., Romani, L., Romano, P. S., Roncero, M. I., Rosa, J. L., Rosello, A., Rosen, K. V., Rosenstiel, P., Rost-Roszkowska, M., Roth, K. A., Roue, G., Rouis, M., Rouschop, K. M., Ruan, D. T., Ruano, D., Rubinsztein, D. C., Rucker, E. B., 3rd, Rudich, A., Rudolf, E., Rudolf, R., Ruegg, M. A., Ruiz-Roldan, C., Ruparelia, A. A., Rusmini, P., Russ, D. W., Russo, G. L., Russo, G., Russo, R., Rusten, T. E., Ryabovol, V., Ryan, K. M., Ryter, S. W., Sabatini, D. M., Sacher, M., Sachse, C., Sack, M. N., Sadoshima, J., Saftig, P., Sagi-Eisenberg, R., Sahni, S., Saikumar, P., Saito, T., Saitoh, T., Sakakura, K., Sakoh-Nakatogawa, M., Sakuraba, Y., Salazar-Roa, M., Salomoni, P., Saluja, A. K., Salvaterra, P. M., Salvioli, R., Samali, A., Sanchez, A. M., Sanchez-Alcazar, J. A., Sanchez-Prieto, R., Sandri, M., Sanjuan, M. A., Santaguida, S., Santambrogio, L., Santoni, G., Dos Santos, C. N., Saran, S., Sardiello, M., Sargent, G., Sarkar, P., Sarkar, S., Sarrias, M. R., Sarwal, M. M., Sasakawa, C., Sasaki, M., Sass, M., Sato, K., Sato, M., Satriano, J., Savaraj, N., Saveljeva, S., Schaefer, L., Schaible, U. E., Scharl, M., Schatzl, H. M., Schekman, R., Scheper, W., Schiavi, A., Schipper, H. M., Schmeisser, H., Schmidt, J., Schmitz, I., Schneider, B. E., Schneider, E. M., Schneider, J. L., Schon, E. A., Schonenberger, M. J., Schonthal, A. H., Schorderet, D. F., Schroder, B., Schuck, S., Schulze, R. J., Schwarten, M., Schwarz, T. L., Sciarretta, S., Scotto, K., Scovassi, A. I., Screaton, R. A., Screen, M., Seca, H., Sedej, S., Segatori, L., Segev, N., Seglen, P. O., Segui-Simarro, J. M., Segura-Aguilar, J., Seki, E., Seiliez, I., Sell, C., Semenkovich, C. F., Semenza, G. L., Sen, U., Serra, A. L., Serrano-Puebla, A., Sesaki, H., Setoguchi, T., Settembre, C., Shacka, J. J., Shajahan-Haq, A. N., Shapiro, I. M., Sharma, S., She, H., Shen, C. J., Shen, C. C., Shen, H. M., Shen, S., Shen, W., Sheng, R., Sheng, X., Sheng, Z. H., Shepherd, T. G., Shi, J., Shi, Q., Shi, Q., Shi, Y., Shibutani, S., Shibuya, K., Shidoji, Y., Shieh, J. J., Shih, C. M., Shimada, Y., Shimizu, S., Shin, D. W., Shinohara, M. L., Shintani, M., Shintani, T., Shioi, T., Shirabe, K., Shiri-Sverdlov, R., Shirihai, O., Shore, G. C., Shu, C. W., Shukla, D., Sibirny, A. A., Sica, V., Sigurdson, C. J., Sigurdsson, E. M., Sijwali, P. S., Sikorska, B., Silveira, W. A., Silvente-Poirot, S., Silverman, G. A., Simak, J., Simmet, T., Simon, A. K., Simon, H. U., Simone, C., Simons, M., Simonsen, A., Singh, R., Singh, S. V., Singh, S. K., Sinha, D., Sinha, S., Sinicrope, F. A., Sirko, A., Sirohi, K., Sishi, B. J., Sittler, A., Siu, P. M., Sivridis, E., Skwarska, A., Slack, R., Slaninova, I., Slavov, N., Smaili, S. S., Smalley, K. S., Smith, D. R., Soenen, S. J., Soleimanpour, S. A., Solhaug, A., Somasundaram, K., Son, J. H., Sonawane, A., Song, C., Song, F., Song, H. K., Song, J. X., Song, W., Soo, K. Y., Sood, A. K., Soong, T. W., Soontornniyomkij, V., Sorice, M., Sotgia, F., Soto-Pantoja, D. R., Sotthibundhu, A., Sousa, M. J., Spaink, H. P., Span, P. N., Spang, A., Sparks, J. D., Speck, P. G., Spector, S. A., Spies, C. D., Springer, W., Clair, D. S., Stacchiotti, A., Staels, B., Stang, M. T., Starczynowski, D. T., Starokadomskyy, P., Steegborn, C., Steele, J. W., Stefanis, L., Steffan, J., Stellrecht, C. M., Stenmark, H., Stepkowski, T. M., Stern, S. T., Stevens, C., Stockwell, B. R., Stoka, V., Storchova, Z., Stork, B., Stratoulias, V., Stravopodis, D. J., Strnad, P., Strohecker, A. M., Strom, A. L., Stromhaug, P., Stulik, J., Su, Y. X., Su, Z., Subauste, C. S., Subramaniam, S., Sue, C. M., Suh, S. W., Sui, X., Sukseree, S., Sulzer, D., Sun, F.

L., Sun, J., Sun, J., Sun, S. Y., Sun, Y., Sun, Y., Sun, Y., Sundaramoorthy, V., Sung, J., Suzuki, H., Suzuki, K., Suzuki, N., Suzuki, T., Suzuki, Y. J., Swanson, M. S., Swanton, C., Sward, K., Swarup, G., Sweeney, S. T., Sylvester, P. W., Szatmari, Z., Szegezdi, E., Szlosarek, P. W., Taegtmeyer, H., Tafani, M., Taillebourg, E., Tait, S. W., Takacs-Vellai, K., Takahashi, Y., Takats, S., Takemura, G., Takigawa, N., Talbot, N. J., Tamagno, E., Tamburini, J., Tan, C. P., Tan, L., Tan, M. L., Tan, M., Tan, Y. J., Tanaka, K., Tanaka, M., Tang, D., Tang, D., Tang, G., Tanida, I., Tanji, K., Tannous, B. A., Tapia, J. A., Tasset-Cuevas, I., Tatar, M., Tavassoly, I., Tavernarakis, N., Taylor, A., Taylor, G. S., Taylor, G. A., Taylor, J. P., Taylor, M. J., Tchetina, E. V., Tee, A. R., Teixeira-Clerc, F., Telang, S., Tencomnao, T., Teng, B. B., Teng, R. J., Terro, F., Tettamanti, G., Theiss, A. L., Theron, A. E., Thomas, K. J., Thome, M. P., Thomes, P. G., Thorburn, A., Thorner, J., Thum, T., Thumm, M., Thurston, T. L., Tian, L., Till, A., Ting, J. P., Titorenko, V. I., Toker, L., Toldo, S., Tooze, S. A., Topisirovic, I., Torgersen, M. L., Torosantucci, L., Torriglia, A., Torrisi, M. R., Tournier, C., Towns, R., Trajkovic, V., Travassos, L. H., Triola, G., Tripathi, D. N., Trisciuoglio, D., Troncoso, R., Trougakos, I. P., Truttmann, A. C., Tsai, K. J., Tschan, M. P., Tseng, Y. H., Tsukuba, T., Tsung, A., Tsvetkov, A. S., Tu, S., Tuan, H. Y., Tucci, M., Tumbarello, D. A., Turk, B., Turk, V., Turner, R. F., Tveita, A. A., Tyagi, S. C., Ubukata, M., Uchiyama, Y., Udelnow, A., Ueno, T., Umekawa, M., Umemiya-Shirafuji, R., Underwood, B. R., Ungermann, C., Ureshino, R. P., Ushioda, R., Uversky, V. N., Uzcategui, N. L., Vaccari, T., Vaccaro, M. I., Vachova, L., Vakifahmetoglu-Norberg, H., Valdor, R., Valente, E. M., Vallette, F., Valverde, A. M., Van den Berghe, G., Van Den Bosch, L., van den Brink, G. R., van der Goot, F. G., van der Klei, I. J., van der Laan, L. J., van Doorn, W. G., van Egmond, M., van Golen, K. L., Van Kaer, L., van Lookeren Campagne, M., Vandenabeele, P., Vandenberghe, W., Vanhorebeek, I., Varela-Nieto, I., Vasconcelos, M. H., Vasko, R., Vavvas, D. G., Vega-Naredo, I., Velasco, G., Velentzas, A. D., Velentzas, P. D., Vellai, T., Vellenga, E., Vendelbo, M. H., Venkatachalam, K., Ventura, N., Ventura, S., Veras, P. S., Verdier, M., Vertessy, B. G., Viale, A., Vidal, M., Vieira, H., Vierstra, R. D., Vigneswaran, N., Vij, N., Vila, M., Villar, M., Villar, V. H., Villarroya, J., Vindis, C., Viola, G., Viscomi, M. T., Vitale, G., Vogl, D. T., Voitsekhovskaja, O. V., von Haefen, C., von Schwarzenberg, K., Voth, D. E., Vouret-Craviari, V., Vuori, K., Vyas, J. M., Waeber, C., Walker, C. L., Walker, M. J., Walter, J., Wan, L., Wan, X., Wang, B., Wang, C., Wang, C. Y., Wang, C., Wang, C., Wang, C., Wang, D., Wang, F., Wang, F., Wang, G., Wang, H. J., Wang, H., Wang, H. G., Wang, H., Wang, H. D., Wang, J., Wang, J., Wang, M., Wang, M. Q., Wang, P. Y., Wang, P., Wang, R. C., Wang, S., Wang, T. F., Wang, X., Wang, X. J., Wang, X. W., Wang, X., Wang, X., Wang, Y., Wang, Y., Wang, Y., Wang, Y. J., Wang, Y., Wang, Y., Wang, Y. T., Wang, Y., Wang, Z. N., Wappner, P., Ward, C., Ward, D. M., Warnes, G., Watada, H., Watanabe, Y., Watase, K., Weaver, T. E., Weekes, C. D., Wei, J., Weide, T., Weihl, C. C., Weindl, G., Weis, S. N., Wen, L., Wen, X., Wen, Y., Westermann, B., Weyand, C. M., White, A. R., White, E., Whitton, J. L., Whitworth, A. J., Wiels, J., Wild, F., Wildenberg, M. E., Wileman, T., Wilkinson, D. S., Wilkinson, S., Willbold, D., Williams, C., Williams, K., Williamson, P. R., Winklhofer, K. F., Witkin, S. S., Wohlgemuth, S. E., Wollert, T., Wolvetang, E. J., Wong, E., Wong, G. W., Wong, R. W., Wong, V. K., Woodcock, E. A., Wright, K. L., Wu, C., Wu, D., Wu, G. S., Wu, J., Wu, J., Wu, M., Wu, M., Wu, S., Wu, W. K., Wu, Y., Wu, Z., Xavier, C. P., Xavier, R. J., Xia, G. X., Xia, T., Xia, W., Xia, Y., Xiao, H., Xiao, J., Xiao, S., Xiao, W., Xie, C. M., Xie, Z., Xie, Z., Xilouri, M., Xiong, Y., Xu, C., Xu, C., Xu, F., Xu, H., Xu, H., Xu, J., Xu, J., Xu, J., Xu, L., Xu, X., Xu, Y., Xu, Y., Xu, Z. X., Xu, Z., Xue, Y., Yamada, T., Yamamoto, A., Yamanaka, K., Yamashina, S., Yamashiro, S., Yan, B., Yan, B., Yan, X., Yan, Z., Yanagi, Y., Yang, D. S., Yang, J. M., Yang, L., Yang, M., Yang, P. M., Yang, P., Yang, Q., Yang, W., Yang, W. Y., Yang, X., Yang, Y., Yang, Y., Yang, Z., Yang, Z., Yao, M. C., Yao, P. J., Yao, X., Yao, Z., Yao, Z., Yasui, L. S., Ye, M., Yedvobnick, B., Yeganeh, B., Yeh, E. S., Yeyati, P. L., Yi, F., Yi, L., Yin, X. M., Yip, C. K., Yoo, Y. M., Yoo, Y. H., Yoon, S. Y., Yoshida, K. I., Yoshimori, T., Young, K. H., Yu, H., Yu, J. J., Yu, J. T., Yu, J., Yu, L., Yu, W. H., Yu, X. F., Yu, Z., Yuan, J., Yuan, Z. M., Yue, B. Y., Yue, J., Yue, Z., Zacks, D. N., Zacksenhaus, E., Zaffaroni, N., Zaglia, T., Zakeri, Z., Zecchini, V., Zeng, J.,

Zeng, M., Zeng, Q., Zervos, A. S., Zhang, D. D., Zhang, F., Zhang, G., Zhang, G. C., Zhang, H., Zhang, H., Zhang, H., Zhang, H., Zhang, J., Zhang, J., Zhang, J., Zhang, J., Zhang, J. P., Zhang, L., Zhang, L., Zhang, L., Zhang, L., Zhang, M. Y., Zhang, X., Zhang, X. D., Zhang, Y., Zhang, Y., Zhang, Y., Zhang, Y., Zhang, Y., Zhao, M., Zhao, W. L., Zhao, X., Zhao, Y. G., Zhao, Y., Zhao, Y., Zhao, Y. X., Zhao, Z., Zhao, Z. J., Zheng, D., Zheng, X. L., Zheng, X., Zhivotovsky, B., Zhong, Q., Zhou, G. Z., Zhou, G., Zhou, H., Zhou, S. F., Zhou, X. J., Zhu, H., Zhu, H., Zhu, W. G., Zhu, W., Zhu, X. F., Zhu, Y., Zhuang, S. M., Zhuang, X., Ziparo, E., Zois, C. E., Zoladek, T., Zong, W. X., Zorzano, A., and Zughaier, S. M. (2016) Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* **12**, 1-222

41. Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., Stenmark, H., and Johansen, T. (2005) p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J. Cell Biol.* **171**, 603-614
42. Lin, F., Ghislat, G., Luo, S., Renna, M., Siddiqi, F., and Rubinsztein, D. C. (2015) XIAP and cIAP1 amplifications induce Beclin 1-dependent autophagy through NFkappaB activation. *Hum. Mol. Genet.* **24**, 2899-2913
43. Kroemer, G., and Levine, B. (2008) Autophagic cell death: the story of a misnomer. *Nat. Rev. Mol. Cell Biol.* **9**, 1004-1010
44. Frankfurt, O. S., and Krishan, A. (2001) Enzyme-linked immunosorbent assay (ELISA) for the specific detection of apoptotic cells and its application to rapid drug screening. *J. Immunol. Methods* **253**, 133-144
45. Bain, J., Plater, L., Elliott, M., Shpiro, N., Hastie, C. J., McLauchlan, H., Klevernic, I., Arthur, J. S., Alessi, D. R., and Cohen, P. (2007) The selectivity of protein kinase inhibitors: a further update. *Biochem. J.* **408**, 297-315
46. François, F., and Klotman, M. E. (2003) Phosphatidylinositol 3-kinase regulates human immunodeficiency virus type 1 replication following viral entry in primary CD4+ T lymphocytes and macrophages. *J. Virol.* **77**, 2539-2549
47. Campbell, G. R., and Spector, S. A. (2012) Autophagy induction by vitamin D inhibits both Mycobacterium tuberculosis and human immunodeficiency virus type 1. *Autophagy* **8**, 1523-1525
48. Donia, M., McCubrey, J. A., Bendtzen, K., and Nicoletti, F. (2010) Potential use of rapamycin in HIV infection. *Br. J. Clin. Pharmacol.* **70**, 784-793
49. Fatkenheuer, G., Pozniak, A. L., Johnson, M. A., Plettenberg, A., Staszewski, S., Hoepelman, A. I., Saag, M. S., Goebel, F. D., Rockstroh, J. K., Dezube, B. J., Jenkins, T. M., Medhurst, C., Sullivan, J. F., Ridgway, C., Abel, S., James, I. T., Youle, M., and van der Ryst, E. (2005) Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat. Med.* **11**, 1170-1172
50. Klotman, M. E., Kim, S., Buchbinder, A., DeRossi, A., Baltimore, D., and Wong-Staal, F. (1991) Kinetics of expression of multiply spliced RNA in early human immunodeficiency virus type 1 infection of lymphocytes and monocytes. *Proc. Natl. Acad. Sci. U. S. A.* **88**, 5011-5015
51. Itakura, E., and Mizushima, N. (2010) Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy* **6**, 764-776
52. Russell, R. C., Tian, Y., Yuan, H., Park, H. W., Chang, Y. Y., Kim, J., Kim, H., Neufeld, T. P., Dillin, A., and Guan, K. L. (2013) ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat. Cell Biol.* **15**, 741-750
53. Ganley, I. G., Lam du, H., Wang, J., Ding, X., Chen, S., and Jiang, X. (2009) ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J. Biol. Chem.* **284**, 12297-12305
54. Espert, L., Varbanov, M., Robert-Hebmann, V., Sagnier, S., Robbins, I., Sanchez, F., Lafont, V., and Biard-Piechaczyk, M. (2009) Differential role of autophagy in CD4 T cells and macrophages during X4 and R5 HIV-1 infection. *PLoS One* **4**, e5787
55. Nardacci, R., Amendola, A., Ciccosanti, F., Corazzari, M., Esposito, V., Vlassi, C., Taibi, C., Fimia, G. M., Del Nonno, F., Ippolito, G., D'Offizi, G., and Piacentini, M. (2014) Autophagy plays

an important role in the containment of HIV-1 in nonprogressor-infected patients. *Autophagy* **10**, 1167-1178
56. Mizushima, N., Levine, B., Cuervo, A. M., and Klionsky, D. J. (2008) Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069-1075
57. Levine, B., and Deretic, V. (2007) Unveiling the roles of autophagy in innate and adaptive immunity. *Nat. Rev. Immunol.* **7**, 767-777
58. Gomez-Mora, E., Robert-Hebmann, V., Garcia, E., Massanella, M., Clotet, B., Cabrera, C., Blanco, J., and Biard-Piechaczyk, M. (2017) Brief report: Impaired CD4 T-Cell response to autophagy in treated HIV-1-infected individuals. *J. Acquir. Immune Defic. Syndr.* **74**, 201-205
59. Borel, S., Robert-Hebmann, V., Alfaisal, J., Jain, A., Faure, M., Espert, L., Chaloin, L., Paillart, J. C., Johansen, T., and Biard-Piechaczyk, M. (2015) HIV-1 viral infectivity factor interacts with microtubule-associated protein light chain 3 and inhibits autophagy. *AIDS* **29**, 275-286
60. Chugh, P., Bradel-Tretheway, B., Monteiro-Filho, C. M., Planelles, V., Maggirwar, S. B., Dewhurst, S., and Kim, B. (2008) Akt inhibitors as an HIV-1 infected macrophage-specific anti-viral therapy. *Retrovirology* **5**, 11
61. Yu, X., Long, Y. C., and Shen, H. M. (2015) Differential regulatory functions of three classes of phosphatidylinositol and phosphoinositide 3-kinases in autophagy. *Autophagy* **11**, 1711-1728
62. Button, R. W., Vincent, J. H., Strang, C. J., and Luo, S. (2016) Dual PI-3 kinase/mTOR inhibition impairs autophagy flux and induces cell death independent of apoptosis and necroptosis. *Oncotarget* **7**, 5157-5175
63. Schnell, C. R., Stauffer, F., Allegrini, P. R., O'Reilly, T., McSheehy, P. M., Dartois, C., Stumm, M., Cozens, R., Littlewood-Evans, A., Garcia-Echeverria, C., and Maira, S. M. (2008) Effects of the dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 on the tumor vasculature: implications for clinical imaging. *Cancer Res.* **68**, 6598-6607
64. Rahmani, M., Aust, M. M., Attkisson, E., Williams, D. C., Jr., Ferreira-Gonzalez, A., and Grant, S. (2013) Dual inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism. *Cancer Res.* **73**, 1340-1351
65. Herrero-Sánchez, M. C., Rodríguez-Serrano, C., Almeida, J., San Segundo, L., Inogés, S., Santos-Briz, A., García-Briñón, J., Corchete, L. A., San Miguel, J. F., del Cañizo, C., and Blanco, B. (2016) Targeting of PI3K/AKT/mTOR pathway to inhibit T cell activation and prevent graft-versus-host disease development. *J. Hematol. Oncol.* **9**, 113
66. Baumann, P., Mandl-Weber, S., Oduncu, F., and Schmidmaier, R. (2009) The novel orally bioavailable inhibitor of phosphoinositol-3-kinase and mammalian target of rapamycin, NVP-BEZ235, inhibits growth and proliferation in multiple myeloma. *Exp. Cell Res.* **315**, 485-497
67. Zheng, Y., Yang, J., Qian, J., Zhang, L., Lu, Y., Li, H., Lin, H., Lan, Y., Liu, Z., He, J., Hong, S., Thomas, S., Shah, J., Baladandayuthapani, V., Kwak, L. W., and Yi, Q. (2012) Novel phosphatidylinositol 3-kinase inhibitor NVP-BKM120 induces apoptosis in myeloma cells and shows synergistic anti-myeloma activity with dexamethasone. *J. Mol. Med. (Berl.)* **90**, 695-706
68. McMillin, D. W., Ooi, M., Delmore, J., Negri, J., Hayden, P., Mitsiades, N., Jakubikova, J., Maira, S. M., Garcia-Echeverria, C., Schlossman, R., Munshi, N. C., Richardson, P. G., Anderson, K. C., and Mitsiades, C. S. (2009) Antimyeloma activity of the orally bioavailable dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. *Cancer Res.* **69**, 5835-5842
69. Sakamaki, J. I., Wilkinson, S., Hahn, M., Tasdemir, N., O'Prey, J., Clark, W., Hedley, A., Nixon, C., Long, J. S., New, M., Van Acker, T., Tooze, S. A., Lowe, S. W., Dikic, I., and Ryan, K. M. (2017) Bromodomain Protein BRD4 Is a Transcriptional Repressor of Autophagy and Lysosomal Function. *Mol Cell* **66**, 517-532 e519
70. Fazio, N., Buzzoni, R., Baudin, E., Antonuzzo, L., Hubner, R. A., Lahner, H., WW, D. E. H., Raderer, M., Teule, A., Capdevila, J., Libutti, S. K., Kulke, M. H., Shah, M., Dey, D., Turri, S.,

Aimone, P., Massacesi, C., and Verslype, C. (2016) A phase II study of BEZ235 in patients with everolimus-resistant, advanced pancreatic neuroendocrine tumours. *Anticancer Res.* **36**, 713-719

71. Seront, E., Rottey, S., Filleul, B., Glorieux, P., Goeminne, J. C., Verschaeve, V., Vandenbulcke, J. M., Sautois, B., Boegner, P., Gillain, A., van Maanen, A., and Machiels, J. P. (2016) Phase II study of dual phosphoinositol-3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibitor BEZ235 in patients with locally advanced or metastatic transitional cell carcinoma. *BJU Int.* **118**, 408-415
72. Bendell, J. C., Kurkjian, C., Infante, J. R., Bauer, T. M., Burris, H. A., 3rd, Greco, F. A., Shih, K. C., Thompson, D. S., Lane, C. M., Finney, L. H., and Jones, S. F. (2015) A phase 1 study of the sachet formulation of the oral dual PI3K/mTOR inhibitor BEZ235 given twice daily (BID) in patients with advanced solid tumors. *Invest. New Drugs* **33**, 463-471
73. Wise-Draper, T. M., Moorthy, G., Salkeni, M. A., Karim, N. A., Thomas, H. E., Mercer, C. A., Beg, M. S., O'Gara, S., Olowokure, O., Fathallah, H., Kozma, S. C., Thomas, G., Rixe, O., Desai, P., and Morris, J. C. (2017) A phase Ib study of the dual PI3K/mTOR inhibitor dactolisib (BEZ235) combined with everolimus in patients with advanced solid malignancies. *Target. Oncol.* **12**, 323-332
74. Wei, X., Decker, J. M., Liu, H., Zhang, Z., Arani, R. B., Kilby, J. M., Saag, M. S., Wu, X., Shaw, G. M., and Kappes, J. C. (2002) Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. *Antimicrob. Agents Chemother.* **46**, 1896-1905
75. Campbell, G. R., Watkins, J. D., Loret, E. P., and Spector, S. A. (2011) Differential induction of rat neuronal excitotoxic cell death by human immunodeficiency virus type 1 clade B and C Tat proteins. *AIDS Res. Hum. Retroviruses* **27**, 647-654
76. Gartner, S., Markovits, P., Markovitz, D. M., Kaplan, M. H., Gallo, R. C., and Popovic, M. (1986) The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**, 215-219
77. Popovic, M., Gartner, S., Read-Connole, E., Beaver, B., and Reitz, M. (1988) Cell tropism and expression of HIV-1 isolates in natural targets. in *Retroviruses of Human AIDS and Related Animal Diseases, Colloque Des Cent Gardes* (Girard, M., and Valette, L. eds.), Pasteur Vaccins, Marnes-La-Coquette, France. pp 21-27
78. Campbell, G. R., and Spector, S. A. (2008) CCL2 increases X4-tropic HIV-1 entry into resting CD4+ T cells. *J. Biol. Chem.* **283**, 30745-30753
79. Pfaffl, M. W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29**, e45

FOOTNOTES

[††]Abbreviations used are: AKT serine/threonine kinase 1, AKT1; antiretroviral therapy, ART; autophagy-related, ATG; beclin 1, BECN1; bromodomain, BRD; bromodomain and extra terminal domain, BET; *β*-galactosidase, *β*-gal; C-C motif chemokine receptor 5, CCR5; eukaryotic translation initiation factor 4E binding protein 1, EIF4EBP1; human immunodeficiency virus type-1, HIV; lactate dehydrogenase, LDH; maximally tolerated dose, MTD; mechanistic target of rapamycin, MTOR; mechanistic target of rapamycin complex 1, MTORC1; microtubule-associated protein 1 light chain 3 beta, MAP1LC3B; peripheral blood mononuclear cells, PBMC; phosphatidylinositol 3-kinase, PI3K; phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, PIK3C; phosphatase and tensin homolog, PTEN; phosphatidylinositol 3-phosphate, PtdIns3P; polo like kinase 1, PLK1; polymerase (RNA) II (DNA directed) polypeptide A, POLR2A; ribosomal protein S6 kinase, 70kDa, polypeptide 2, RPS6KB2; regulatory associated protein of MTOR complex 1, RPTOR; sequestosome 1, SQSTM1; super elongation complex, SEC; 50% tissue culture infective dose, $TCID_{50}$; toll-like receptor, TLR; unc-51 like autophagy activating kinase 1, ULK1.

FIGURES

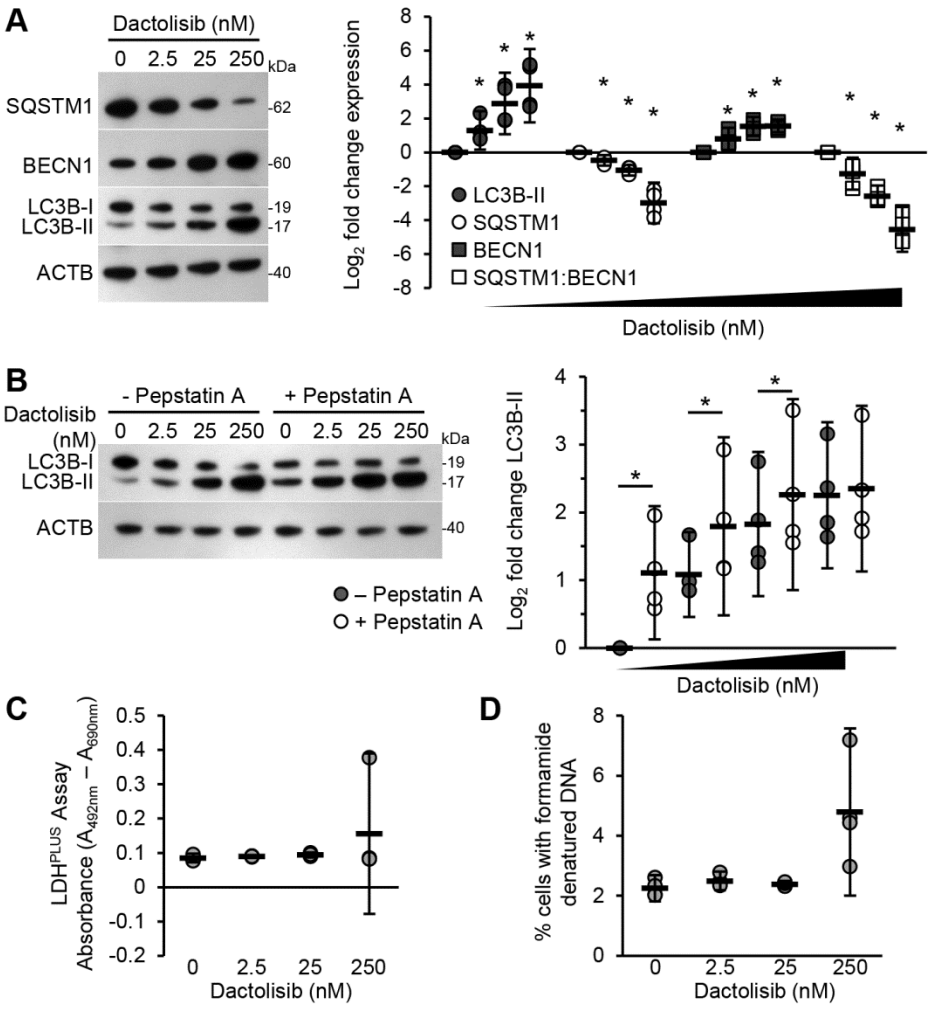

FIGURE 1. **Dactolisib induces autophagy in human macrophages.**
(**A**) Macrophages were treated for 24 h with dactolisib. *Left*, representative immunoblots of LC3B isoforms, BECN1 and SQSTM1 using antibody to LC3B, BECN1, SQSTM1 and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.
(**B**) Macrophages were pretreated with pepstatin A before incubation with dactolisib for 24 h. *Left*, representative immunoblots of LC3B isoforms using antibody to LC3B, and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.
(**C**) Aliquots of supernatant from (**A**) were spectrophotometrically tested for LDH using the LDH^{PLUS} assay. $n = 4$.
(**D**) Quantification of the number of cells with apoptotic ssDNA using the ssDNA ELISA 96 h after dactolisib treatment. $n = 4$.

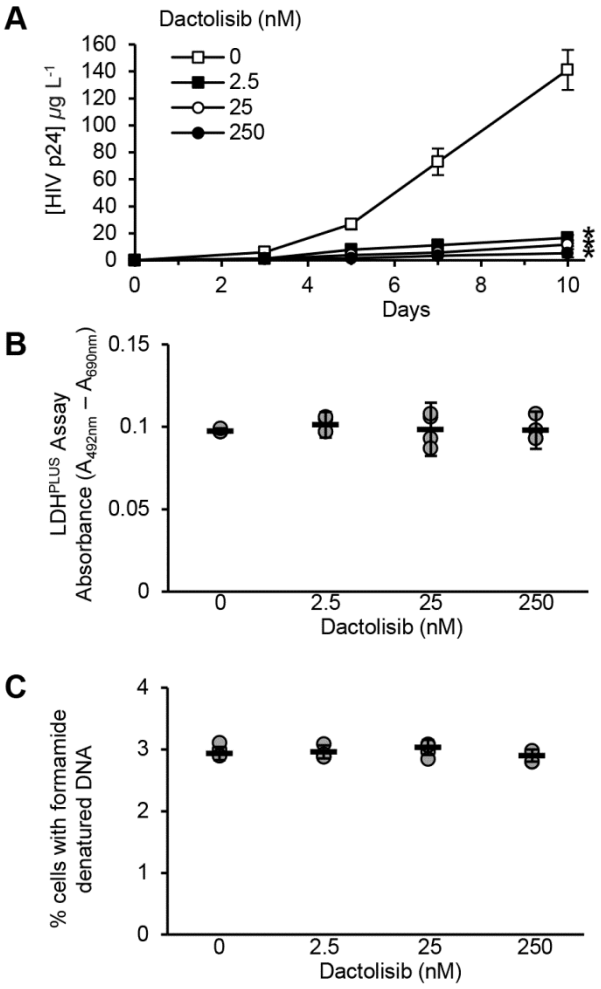

FIGURE 2. **Dactolisib inhibits HIV replication.**
(**A**) Macrophages were incubated with increasing concentrations of dactolisib before exposure to HIV. ELISA performed for supernatant HIV p24 antigen over 10 d. Data are reported as mean ± s.e.m., $n = 4$.
(**B**) After 10 d HIV infection, aliquots of supernatant from part (A) were spectrophotometrically tested for LDH using the LDH^{PLUS} assay. $n = 4$.
(**C**) After 10 d HIV infection, cells from (A) were fixed and permeabilized and the percentage of cells with apoptotic ssDNA quantified by ELISA. $n = 4$.

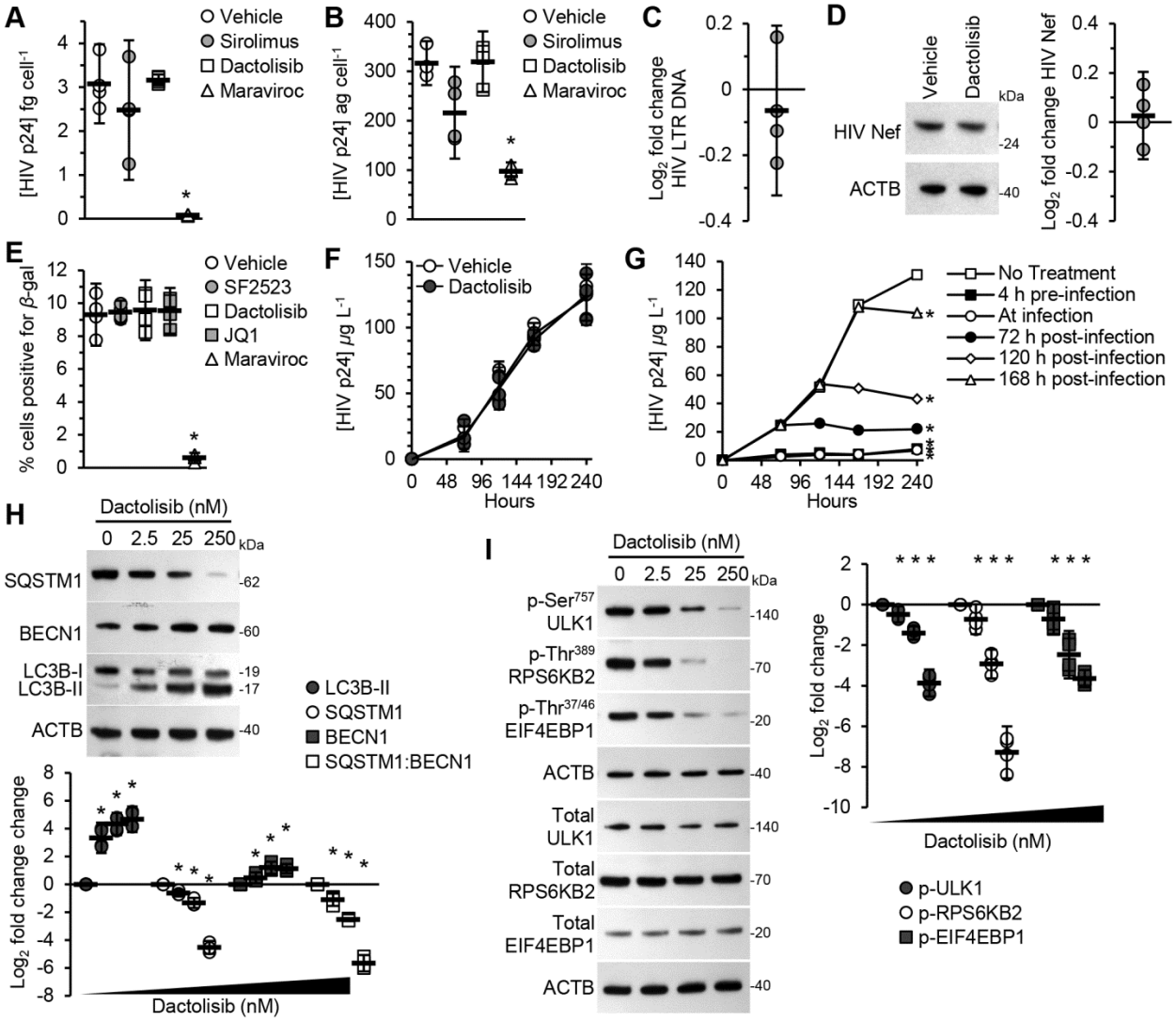

FIGURE 3. **Dactolisib does not affect HIV entry or release.**

(**A**) Macrophages were treated with 250 nM dactolisib, 100 nM sirolimus, or 10 nM maraviroc for 4 h before exposure to HIV. Binding was measured at 3 h post exposure by washing cells extensively, then lysing and analyzing p24 by ELISA. $n = 4$.

(**B**) Macrophages treated with 250 nM dactolisib, 100 nM sirolimus, or 10 nM maraviroc for 4 h before exposure to HIV. Entry was measured at 5 h post exposure by washing cells extensively, followed by trypsinization, lysing and subsequent analysis of p24 by ELISA. $n = 4$.

(**C**) Macrophages were treated with 250 nM dactolisib for 4 h before exposure to HIV. DNA was extracted at 8 h post-infection for qPCR analysis of pre-integration strong-stop HIV DNA. *POLR2A* was amplified as a control. Results are expressed as the ratio between the HIV LTR DNA and *POLR2A*, normalized to the vehicle control then $\log_2$ transformed. $n = 4$.

(**D**) Macrophages were treated with 250 nM dactolisib for 4 h before exposure to HIV. Macrophages were lysed at 5 h post exposure and subjected to immunoblotting for both HIV Nef and ACTB. $n = 4$.

(**E**) Percentage of TZM-bl cells productively infected with HIV after 4 h treatment with 250 nM dactolisib, nM SF2523, nM JQ1, or 10 nM maraviroc for 4 h before exposure to HIV. $n = 4$.

(**F**) Macrophages were incubated with 1 ng p24 antigen from the 10 d aliquots of cell-free supernatants post-250 nM dactolisib treatment for four hours then cultured for 10 days with ELISA performed for HIV p24 antigen. Data are reported as mean ± s.e.m., $n = 4$.

(**G**) Macrophages were incubated with 250 nM dactolisib at different time points with respect to infection with HIV. ELISA performed for HIV p24 antigen over 10 d. Data are reported as mean ± s.e.m., $n = 4$.
(**H**) HIV-infected macrophages were treated for 24 h with dactolisib. *Left*, representative immunoblots of LC3B isoforms, BECN1 and SQSTM1 using antibody to LC3B, BECN1, SQSTM1 and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.
(**I**) HIV-infected macrophages were treated for 24 h with dactolisib. *Left*, representative immunoblots of phospho-ULK1 (Ser^{757}), phospho-EIF4EBP1 ($Thr^{37/46}$), phospho-RPS6KB2 (Thr^{389}), total ULK1, total EIF4EBP1, total RPS6KB2 and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.

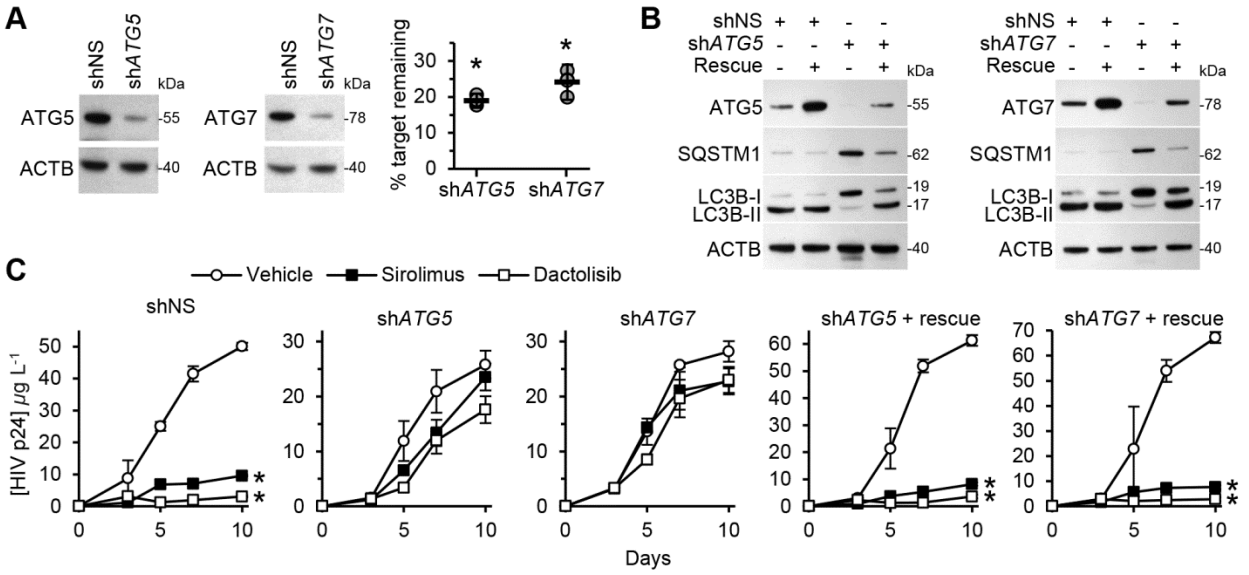

FIGURE 4. **Dactolisib-mediated decrease of HIV p24 antigen release from macrophages requires ATG5 and ATG7.**

(**A**) Macrophages were transduced with *ATG5* shRNA (sh*ATG5*), *ATG7* shRNA (sh*ATG7*), or scrambled shRNA (shNS). Samples were pooled and analyzed by western blotting. *Left*, representative western blots performed using antibodies raised to ATG5, ATG7, and ACTB. *Right*, densitometric analysis of western blots. $n = 4$.

(**B**) Macrophages were transduced as in part A with the corresponding rescue shRNA-insensitive open reading frame (ORF) lentiviral vectors, then were treated with 250 nM dactolisib for 24 h, harvested and analyzed for autophagy proteins. Representative western blots of LC3B isoforms, ATG5, ATG7, and SQSTM1 using antibodies raised to LC3B, SQSTM1, ATG7, and ACTB is presented. $n = 4$.

(**C**) Macrophages transduced as in part A and B were incubated with 250 nM dactolisib or 100 nM sirolimus for 4 h before HIV infection under continuous drug treatment for 10 d. ELISA performed for supernatant HIV p24 antigen content. Data are reported as mean ± s.e.m., $n = 4$.

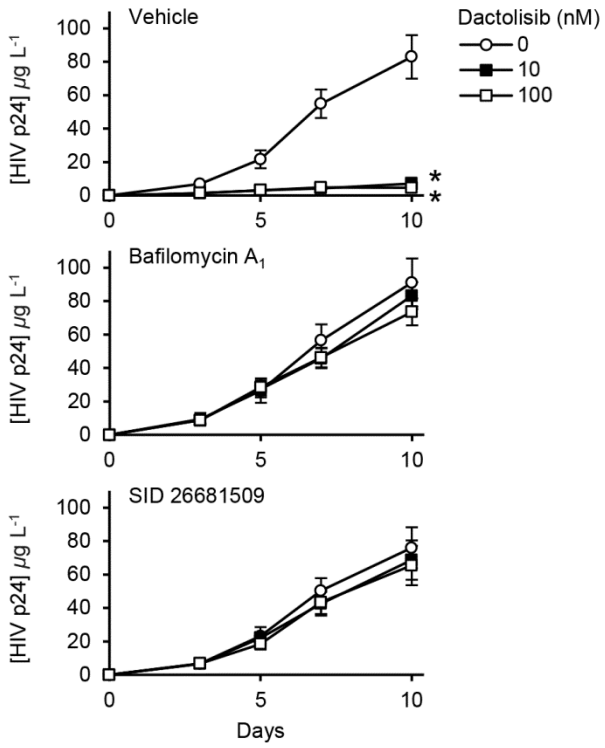

FIGURE 5. **Dactolisib- mediated decrease of HIV p24 antigen release from macrophages requires the completion of autophagic flux.**
Macrophages were incubated with bafilomycin A_1, SID 26681509 or vehicle prior to treatment with 0-100 nM dactolisib for 4 h before HIV infection under continuous drug treatment for 10 d. ELISA performed for supernatant HIV p24 antigen content. Data are reported as mean ± s.e.m., $n = 4$.

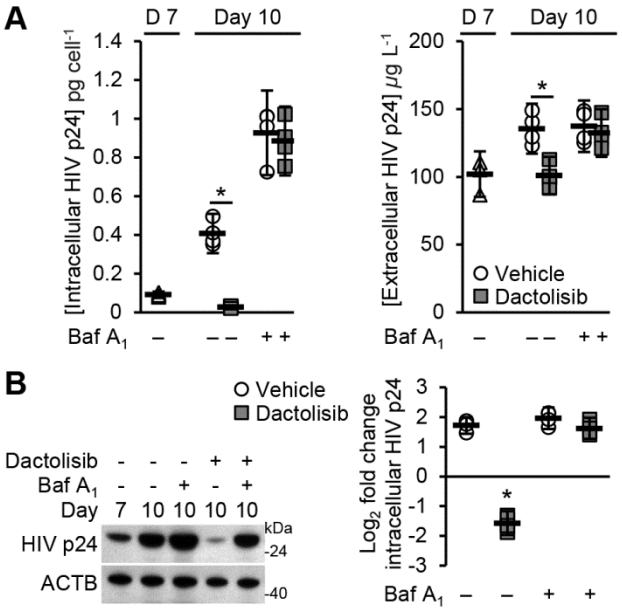

FIGURE 6. **Dactolisib induces the lysosomal degradation of intracellular HIV.**
Macrophages were infected with HIV for 7 d then treated with 250 nM dactolisib in the presence or absence of 10 nM bafilomycin A_1 for 3 d.
(**A**) ELISA was performed for both extracellular and intracellular HIV p24 antigen at 7 and 10 d post-infection. $n = 4$.
(**B**) *Top*, representative immunoblots of HIV p24 antigen using antibody to HIV p24 antigen and ACTB. *Bottom*, densitometric analysis of immunoblots. $n = 4$.

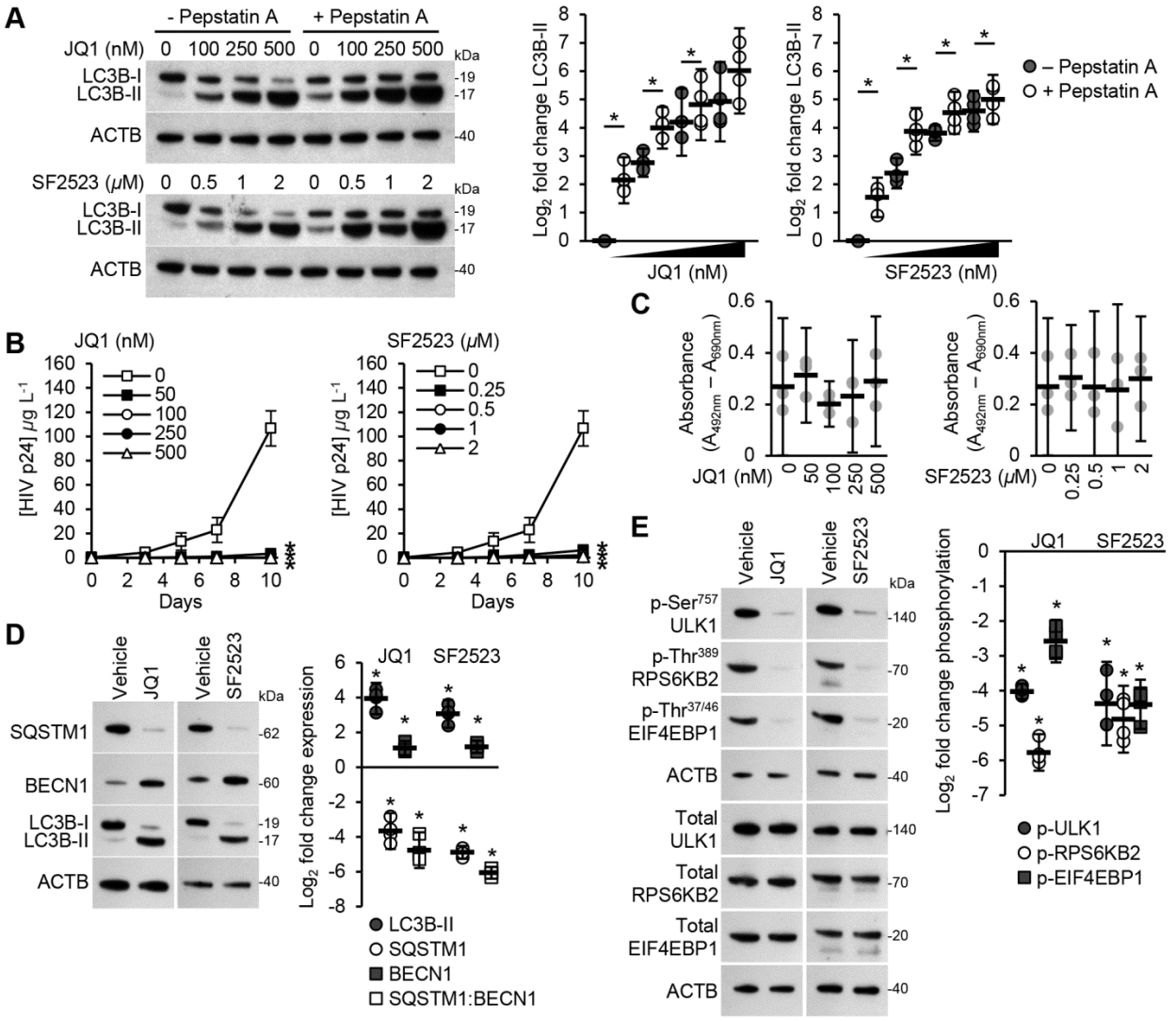

FIGURE 7. **SF2523 and JQ1 induce autophagy and inhibit HIV replication.**
(**A**) Macrophages were pretreated with pepstatin A before incubation with JQ1 and SF2523 for 24 h. *Left*, representative immunoblots of LC3B isoforms using antibody to LC3B, and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.
(**B**) Macrophages were incubated with increasing concentrations of JQ1 and SF2523 before exposure to HIV. ELISA performed for supernatant HIV p24 antigen over 10 d. Data are reported as mean ± s.e.m., $n = 4$.
(**C**) After 10 d HIV infection, aliquots of supernatant from cells in part (B) were spectrophotometrically tested for LDH using the LDH^{PLUS} assay. $n = 4$.
(**D**) Macrophages were infected with HIV for 7 d then treated with 250 nM JQ1 or 500 nM SF2523 for 24 h. *Left*, representative immunoblots of LC3B isoforms, BECN1 and SQSTM1 using antibody to LC3B, BECN1, SQSTM1 and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.
(**E**) Macrophages were infected with HIV for 7 d then treated with 250 nM JQ1 or 500 nM SF2523 for 24 h. *Left*, representative immunoblots of phospho-ULK1 (Ser^{757}), phospho-EIF4EBP1 (Thr$^{37/46}$), phospho-RPS6KB2 (Thr^{389}), total ULK1, total EIF4EBP1, total RPS6KB2 and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.

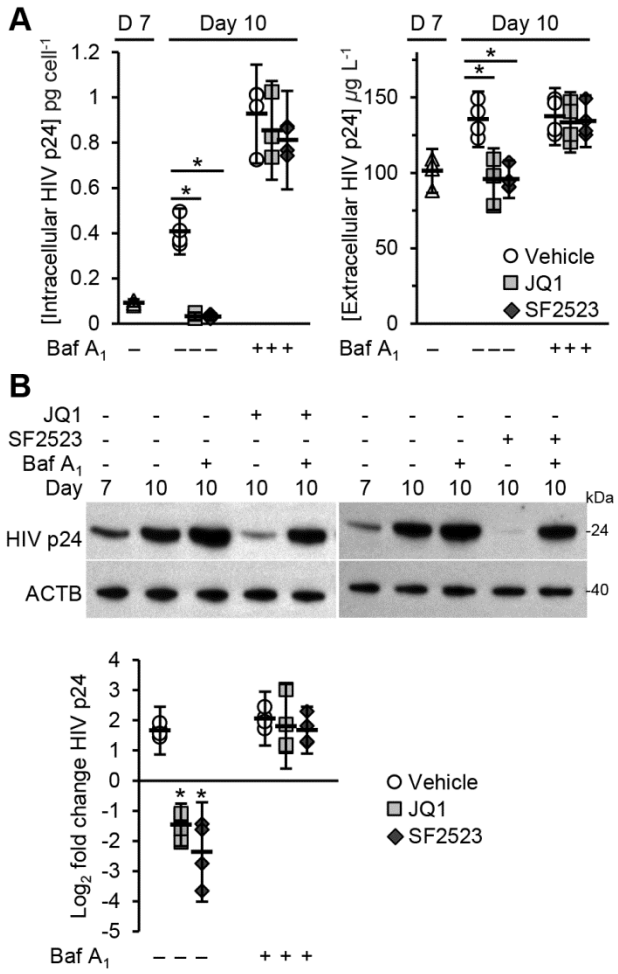

FIGURE 8. **SF2523 and JQ1-mediated induction of autophagy inhibits HIV replication.**
(**A**) Macrophages were infected with HIV for 7 d then treated with 250 nM JQ1 or 500 nM SF2523 in the presence or absence of 10 nM bafilomycin A_1 for 3 d. ELISA was performed for both extracellular and intracellular HIV p24 antigen at 7 and 10 d post-infection. $n = 4$.
(**B**) Macrophages were infected with HIV for 7 d then treated with 250 nM JQ1 or 500 nM SF2523 in the presence or absence of 10 nM bafilomycin A_1 for 3 d. *Left*, representative immunoblots of HIV p24 antigen using antibody to HIV p24 antigen and ACTB. *Right*, densitometric analysis of immunoblots. $n = 4$.

Induction of autophagy by PI3K/MTOR and PI3K/MTOR/BRD4 inhibitors suppresses HIV-1 replication

Grant R. Campbell, Rachel S. Bruckman, Shayna D. Herns, Shweta Joshi, Donald Durden and Stephen A. Spector

J. Biol. Chem. published online February 23, 2018
