SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Cells, viruses, and inhibitors

HeLa, 293T, and FRhK4 cells (from ATCC) were cultured in DMEM containing 10% fetal calf serum. Primary rhesus CD4+ T cells were enriched by depletion of CD8+ cells from peripheral blood-derived non-adherent lymphocytes, activated with concanavalin A, and maintained in RPMI supplemented with 1% human serum, 10% fetal calf serum, 50 μM β-mercaptoethanol, and human 10 ng mL^{-1} IL-2. HeLa cells stably expressing mRFP-GFP-LC3B (from D. Rubinsztein, Cambridge University) were used for TRIM5α siRNA screen and maintained in complete DMEM containing 500 μg mL^{-1} G418 while HeLa cells stably expressing HA-TRIM5α (from J. Sodroski, Harvard University) were maintained in media containing 1 μg mL^{-1} of puromycin as a positive selection agent. Single cycle HIV-1 or SIV_{mac239} viruses were generated by co-transfection of plasmids encoding the NL43 or SIV_{mac239} clones lacking the env gene and VSV-G protein into 293T cells. HIV-1 RT was determined according to the manufacturer's protocol (Enz Chek, Invitrogen). HIV-1 proviral DNA was quantified as previously described (Campbell et al., 2004). Working concentrations for inhibitors were as follows: pp242, 10 μg ml^{-1}; e64d, 10 μg ml^{-1}; pepstatin A, 10 μg ml^{-1}; rapamycin, 50 μg ml^{-1}; MG132, 500 ng ml^{-1}; bafilomycin A1, 60 ng ml^{-1}.

Plasmids, siRNA, and transfection

HA- and GFP-tagged TRIM5α expression plasmids (from J. Sodroski) have been described previously(Song et al., 2005; Stremlau et al., 2004), as have those for FLAG-Beclin 1 (Shoji-Kawata et al., 2013) and eGFP-DFCP1 (from N. Ktistakis) (Axe et al., 2008). The TRIM5α mutants were generated from GFP-TRIM5α (rhesus or human) expression clone by site-directed mutagenesis and mutations confirmed by sequencing. All constructs in pENTR or pDONR221 were generated by BP cloning and expression vectors were made using the LR reaction (Gateway; Invitrogen). All siRNA smart pools were from Dharmacon. With the exception of the siRNA transfections for the TRIM screens (with siRNA printed into the 96 well plates), siRNA were delivered to cells by nucleoporation (Amaxa). Plasmid transfections were performed by either $CaPO_4$ or nucleoporation (Amaxa).

GST pull-down assays

GST and GST-tagged proteins were expressed in *Escherichia coli* BL21(DE3) or SoluBL21 (Amsbio). GST and GST-fusion proteins were purified and immobilized on glutathione-coupled sepharose beads (Amersham Bioscience, Glutathione-sepharose 4 Fast Flow) and pull-down assays with in vitro translated [^{35}S]-labeled proteins were done as described previously (Pankiv et al., 2007). The [^{35}S] labeled proteins were

produced using the TNT T7 Quick Coupled Transcription/Translation System (Promega) in the presence of [^{35}S] L-methionine. The proteins were eluted from washed beads by boiling for 5 min in SDS-PAGE gel loading buffer, separated by SDS-PAGE, and radiolabeled proteins detected in a Fujifilm bioimaging analyzer BAS-5000 (Fuji).

Peptide array synthesis

Peptide array synthesis methods have been previously published (Kramer et al., 1996).

Immunoblotting, immunolabeling for microscopy and co-immunoprecipitation

These procedures were as previously described (Kyei et al., 2009).

Subcellular fractionation

Subcellular organellar fractionation by isopycnic centrifugation in sucrose gradents was carried out as previously described (Kyei et al., 2009).

Proximity ligation assay

Proximity ligation assay (PLA) was performed as described (Pilli et al., 2012). PLA reports direct in situ interactions between proteins revealed as fluorescent dots, the products of in situ PCR that generates a fluorescent product physically attached to antibodies against the two proteins being interrogated by PLA. When the antibodies bound to proteins in situ are < 16 nm apart (FRET distance) positive PCR signals emerge that are revealed by imaging as fluorescent puncta. PLA results were reported as average number of red puncta per cell or total intensity of the PLA signal (sum of all puncta intensity) within green-fluorescent (transfected) cells using ImageJ software.

Infection

Cells were exposed to virus at 4°C for 1 h to allow binding but not entry. Unbound virus was removed by washing and bound virus was allowed to infect cells under basal or induced autophagy conditions (starvation or rapamycin) at 37^{o}C for 4 h. Samples were then prepared for analysis of p24, reverse transcriptase, or proviral DNA. For assays with luciferase, siRNA-treated and infected (as above) cells were maintained in full media for 48 h following the 4 h infection period.

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