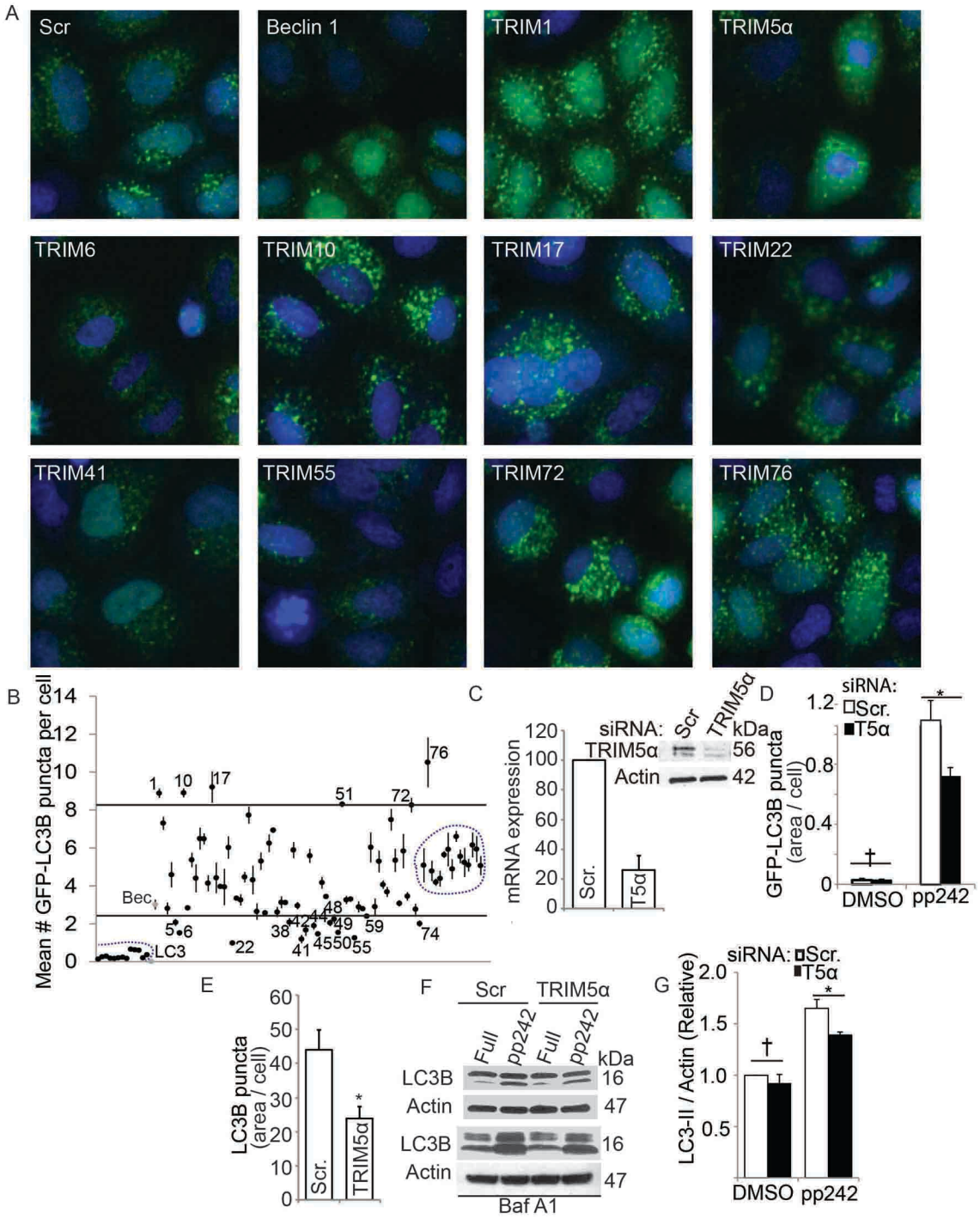
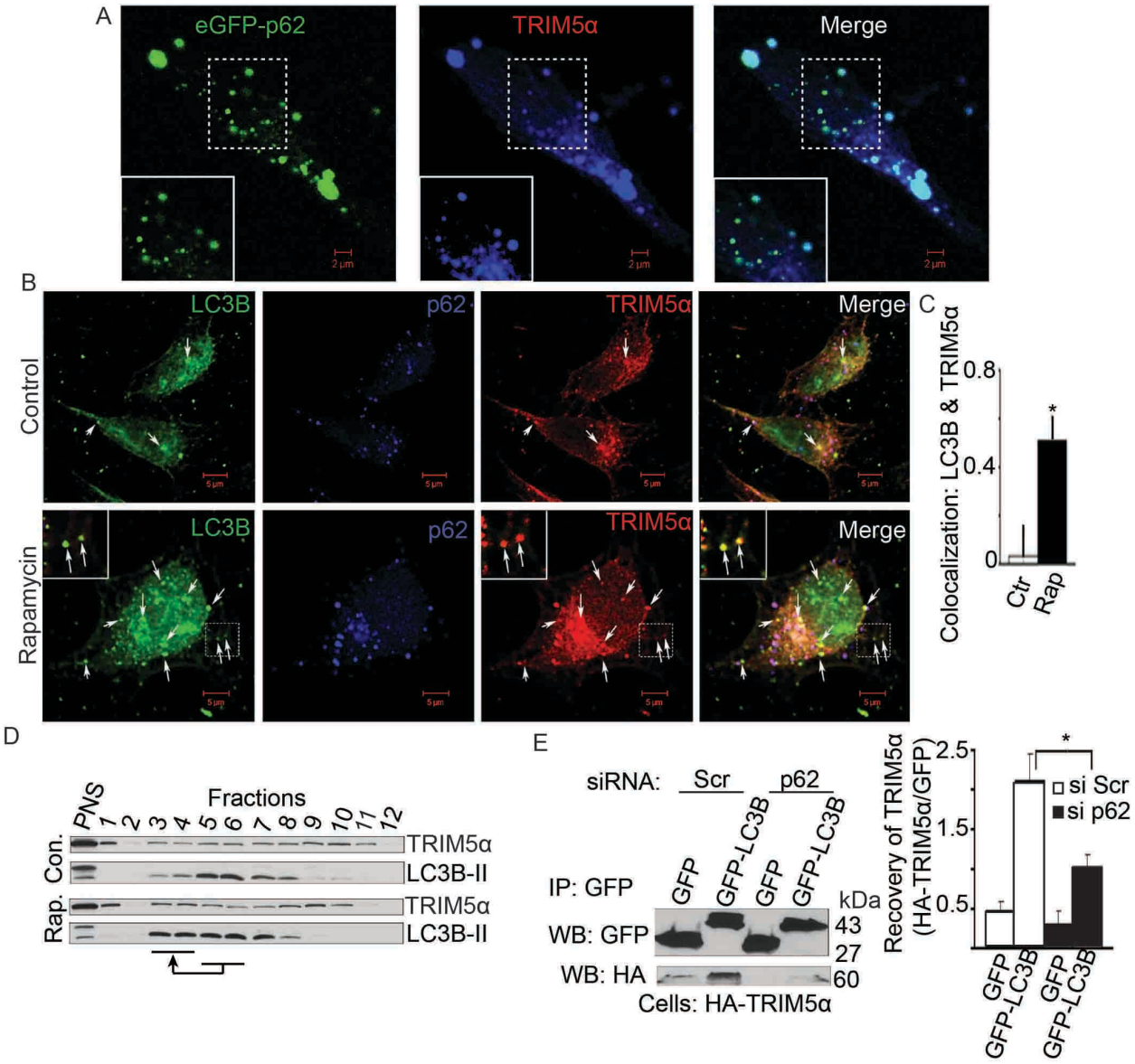


Supplementary Figure S1 related to Figure 1. TRIM proteins regulate autophagy.

(**A**) Representative images (epifluorescence, high content Cellomics HCS scanner) of cells expressing green-fluorescent LC3B transfected with non-targeting siRNA (Scr), siRNA against Beclin 1, or against selected TRIMs (see Fig. 1B) after treatment with pp242. Green, GFP-LC3B. Blue, nuclei. (**B**) High content image analysis of TRIM siRNA screen as in Figure 1, plotted here as number of LC3B puncta per cell. Labeling as in

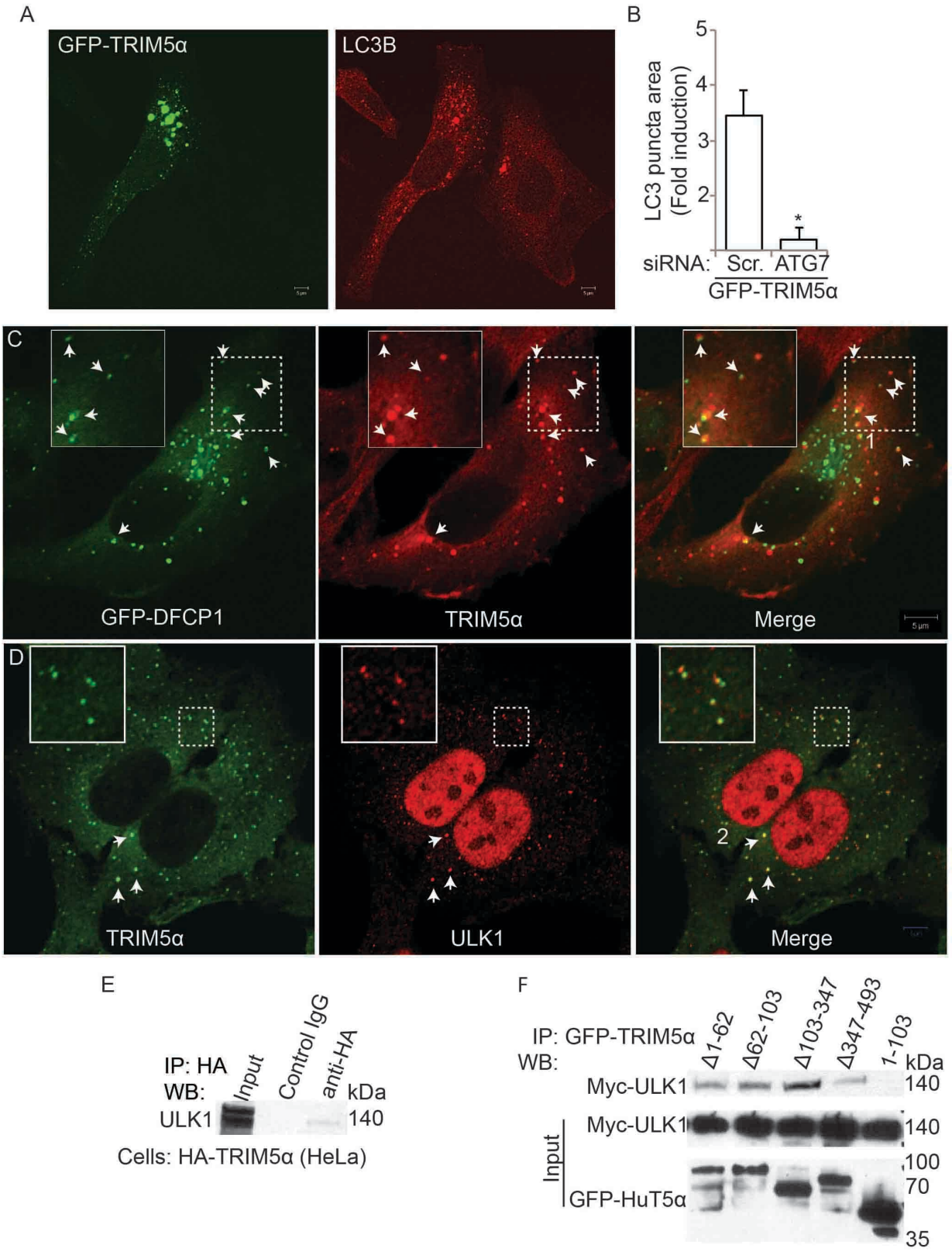
Figure 1B. (**C**) Evaluation of TRIM5α knockdown efficiency by q-RT-PCR (graph) and immunoblot (gel). (**D**) Effects of TRIM5α knockdown in HeLa cells on GFP-LC3B puncta (area/cell; Cellomics HC analysis) under basal (DMSO control) and autophagy-inducing (pp242) conditions. T5a, TRIM5α siRNA; Scr, Scrambled control siRNA. Knockdown of TRIM5α with an siRNA Smartpool (Dharmacon: GCAGAAAGUUGAUCAUUGU, GAGAGUAGCUGCCCUGUGU, GGAAUCCUGGUUAAUGUAA, and UUACCAGCCUGAGAACAUA). (**E**) Effect of TRIM5α knockdown on LC3B puncta. HeLa cells were transfected with scrambled siRNA or an siRNA against TRIM5α that was different from any of the siRNAs in the Smartpool (sequence: CUCUGAAACUGAGAUGGUG), and treated with pp242 for 2 h. Cells were stained to detect endogenous LC3B and high content imaging and analysis employed to assess abundance of LC3B puncta. (**F**) Lysates from HeLa cells subjected to TRIM5α knockdown and treated or not with pp242 in the presence of bafilomycin A1 were analyzed by immunoblotting as indicated. (**G**) Quantification of LC3B blots (two examples in F). Data, means ± SE; n ≥ 3 experiments *; $P < 0.05$ (t test).



Supplementary Figure S2 related to Figure 2. TRIM5α is in complexes with p62 and LC3B.

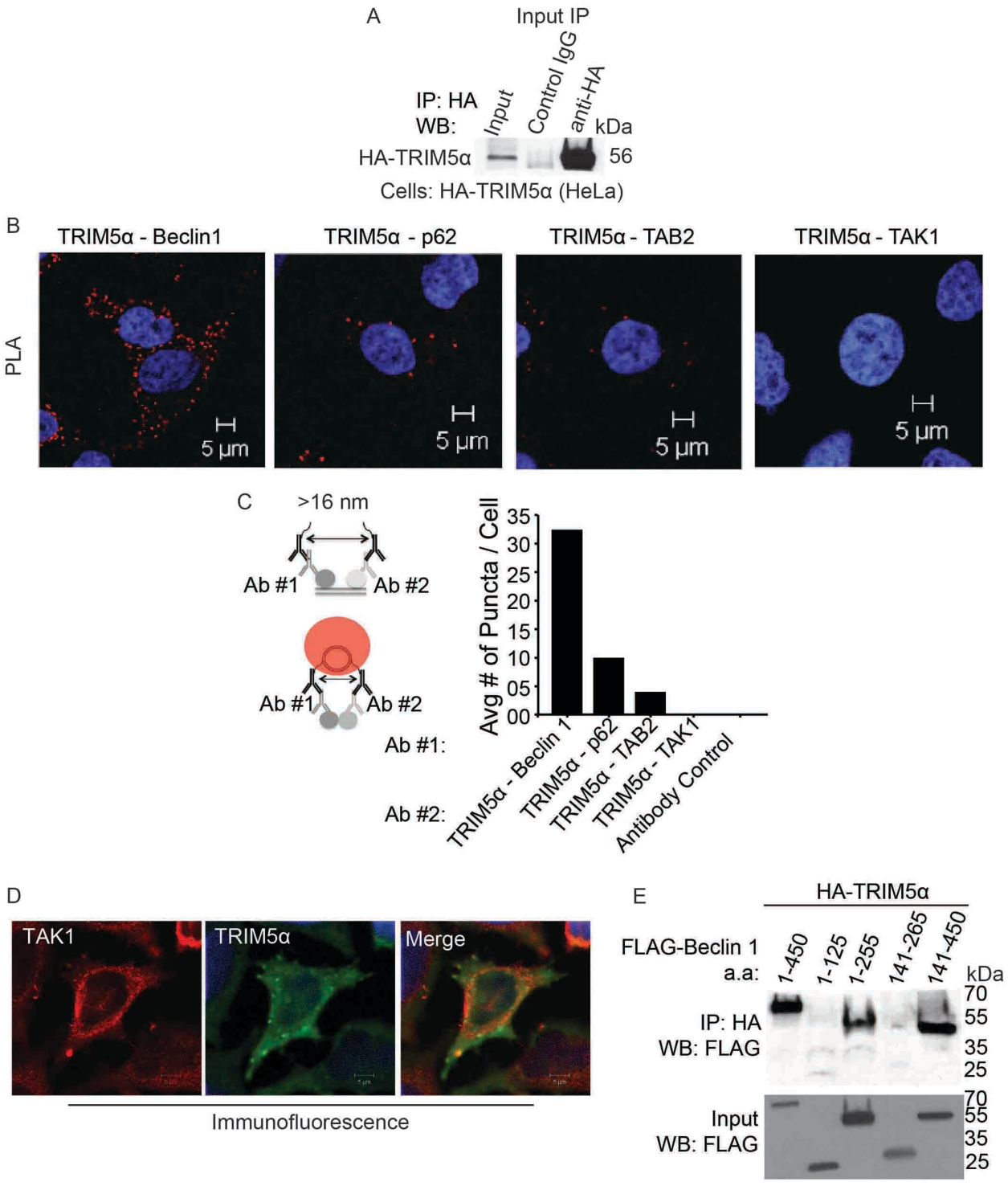
(**A**) Confocal immunofluorescence microscopy of cells expressing GFP-p62 (green) and HA-tagged TRIM5α (blue). (**B**) Confocal immunofluorescent microscopy of LC3B, p62, and HA-RhTRIM5α under basal and rapamycin-induced autophagy conditions. Arrows, overlaps between LC3B and HA-TRIM5α. (**C**) Pearson's coefficient of co-localization (calculated using SLIDEBOOK 5.0, Intelligent Imaging Innovations) between LC3B and HA-TRIM5α. Pearson's colocalization coefficient ($R_r=\Sigma[(S_{1i}-S_{1avg})x(S_{2i}-S_{2avg})]/[\Sigma(S_{1i}-S_{1avg})^2x\Sigma(S_{2i}-S_{2avg})^2]^{1/2}$ (R_r values range: ≥-1 R_r ≤+1) was calculated using SLIDEBOOK 5.0 (Intelligent Imaging Innovations). (**D**) Membranous organelles from untreated (top)

or rapamycin-treated (bottom) HeLa cells expressing HA-TRIM5α were separated by isopycnic centrifugation in sucrose gradients. Arrow, shift upon rapamycin treatment. (**E**) Assessment of interaction between GFP-LC3B and HA-tagged TRIM5α in control cells or cells subjected to p62 knockdown by co-immunoprecipitation. Data, means ± SE; n ≥ 3 experiments *; $P < 0.05$ (t test).



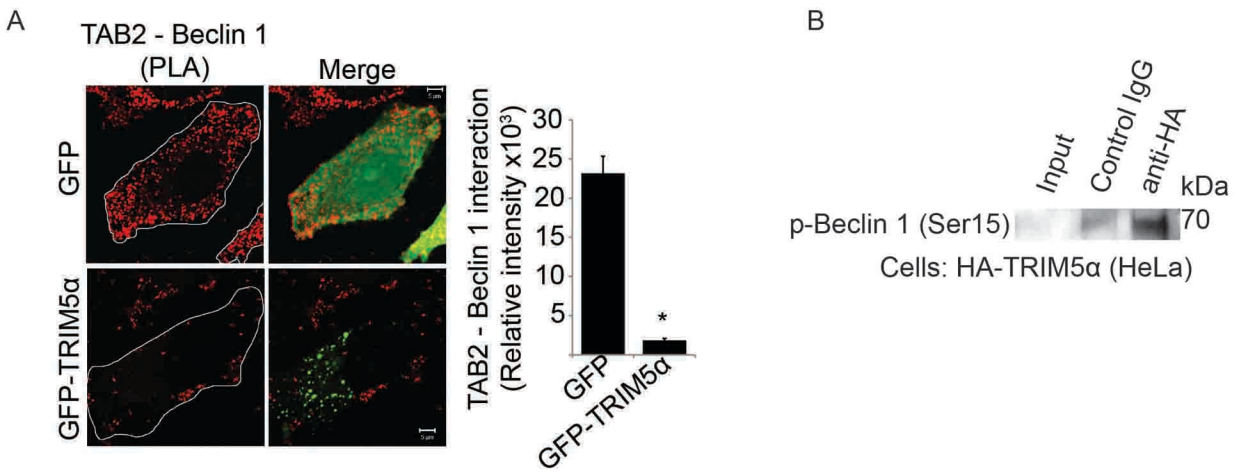
Supplementary Figure S3 related to Figure 3. TRIM5α promotes autophagosome formation and co-localizes and interacts with ULK1.

(**A**) Example of confocal microscopy images of GFP-TRIM5α-positive and -negative cells immunostained for endogenous LC3B, from samples used in quantitative high content analysis and quantification in Figure 3. (**B**) Fold induction of LC3 puncta area (relative to cells expressing GFP alone) in HeLa cells expressing GFP-TRIM5α and transfected with control or ATG7 siRNA. (**C**) Single color and merged images corresponding to the image shown in Figure 3C. (**D**) Single color and merged images corresponding to the image shown in Figure 3D. (**E**) Lysates from HA-TRIM5α-expressing cells were subjected to immunoprecipitation with either anti-HA or an isotype control and immunoblots probed with antibodies recognizing ULK1. (**F**) Immunoprecipitation analysis was performed as described for Figure 3G. See schematic of TRIM5α deletion constructs in Figure 5A.



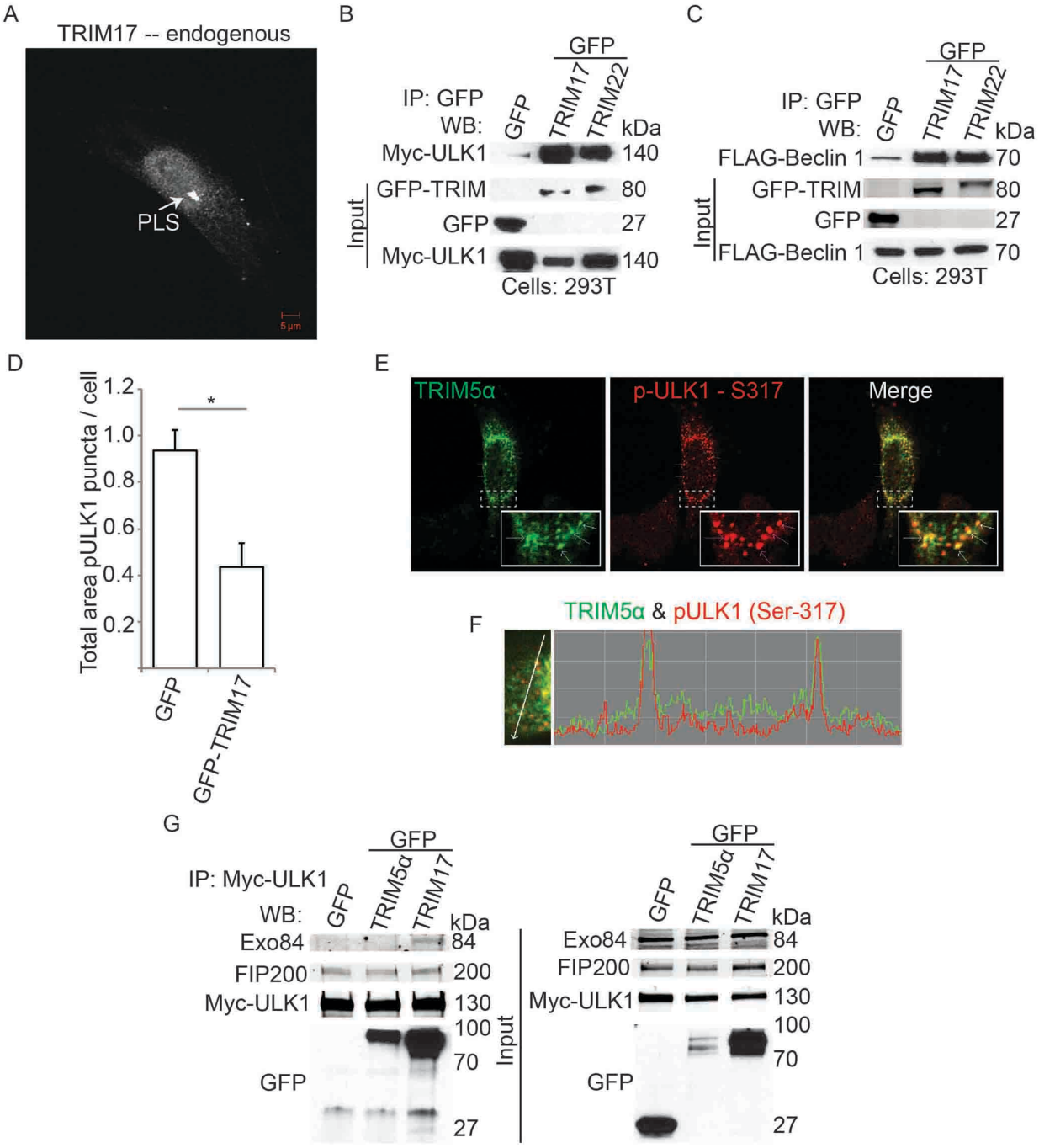
Supplementary Figure S4 related to Figure 4. TRIM5α is in complexes with Beclin 1.

(A) Lysates from HeLa cells stably expressing HA-TRIM5α were subjected to immunoprecipitation with anti-HA or isotype control and blots probed with anti-HA. Corresponds to blots shown in Figure 4E. (**B**) Proximity ligation assay (PLA) for direct in situ protein-protein interactions between HA-RhTRIM5α (antibody #1/Ab#1 to HA tag) and the indicated proteins (antibody #2/Ab#2 to endogenous Beclin 1, p62, TAB2 or TAK1). (**C**) Schematic of PLA assay: for directly interacting proteins (approximating FRET distances) the distance between Ab#1 and Ab#2 allows a PCR reaction to generate red fluorescent puncta (positive signal). Graph, quantitation of the average number of PLA puncta per cell (N = 200 cells). (**D**) Confocal immunofluorescence microscopy using the antibody pairs and cells as in (A) employed as a control showing that HA-tagged TRIM5α and TAK1 are recognized by Ab#1 (HA) and Ab#2 (TAK1) in HeLa cells stably expressing HA-TRIM5α. (**E**) Mapping of Beclin 1 regions interacting with HA-TRIM5α (see schematic in Figure 5A). 293T cells were transfected with the corresponding constructs (HA-TRIM5α; Beclin 1 domains tagged with FLAG epitope; 1-450, full size Beclin 1). Lysates were immunoprecipitated with anti-FLAG and immunoblots probed as indicated.



Supplementary Figure S5 related to Figure 5. TRIM5α promotes Beclin 1 activation.

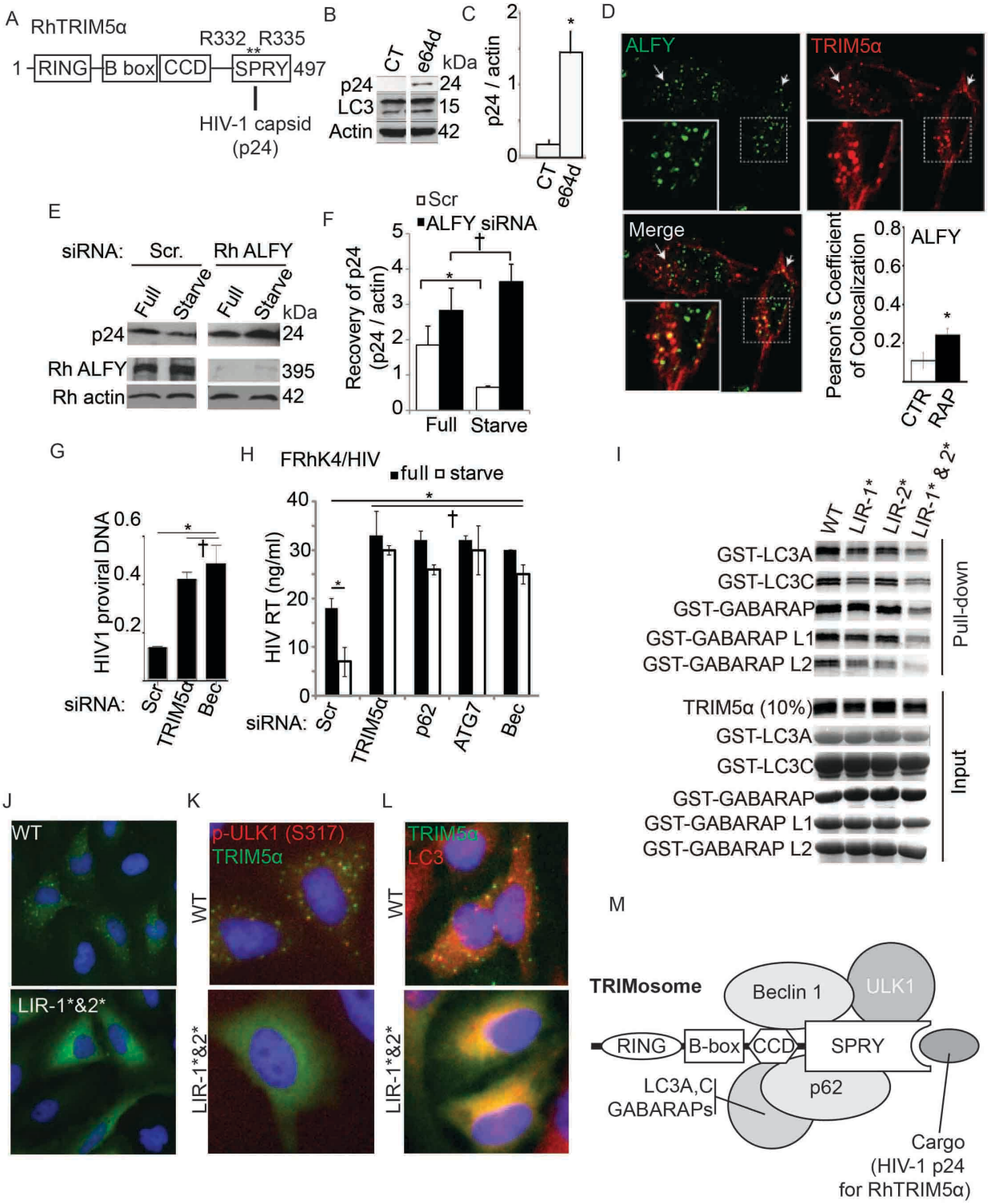
(**A**) PLA analysis of TAB2 - Beclin 1 interactions in HeLa cells expressing GFP-TRIM5α or GFP alone (white mask). PLA, red dots; diffuse (GFP) or punctate (GFP-TRIM5α), green fluorescence. PLA results reported is the total intensity of the PLA signal (sum of all puncta intensity) within green-fluorescent (transfected) cells using ImageJ software. (**B**) Lysates from HeLa cells stably expressing HA-TRIM5α and transiently over-expressing FLAG-Beclin 1 were immunoprecipitated with anti-HA antisera and immunoblots probed with antisera recognizing phospho-Beclin 1 (Ser-15). Data, means ± SE, n = 3 experiments, *, $P < 0.05$; (t test).



Supplementary Figure S6 related to Figure 6. TRIM17 interacts with autophagy regulators.

(**A**) HeLa cells stained with anti-TRIM17 show prominent large structure profiles (PLS). (**B,C**) Lysates from 293T cells expressing Myc-ULK1 (B) or FLAG-Beclin 1 (C) and GFP or GFP-TRIM fusions were subjected to immunoprecipitation with anti-GFP and blots were probed with the indicated antibodies. (**D**) High content imaging analysis of

the abundance of p-ULK1 (Ser-317) puncta (PULKA assay) in HeLa cells expressing GFP or GFP-TRIM17. (**E**) Intracellular distribution of endogenous p-ULK1 (p-Ser 317) in HeLa cells expressing GFP-TRIM5α. (**F**) Fluorescence intensity line tracings showing phospho-ULK1 localization relative to GFP-TRIM5α. (**G**) Co-immunoprecipitation analysis of the interaction between Myc-ULK1 and endogenous Exo84 or endogenous FIP200 in lysates from 293T cells expressing GFP or GFP-TRIMs.



Supplementary Figure S7 related to Figure 7. TRIM5α cooperates with ALFY and mAtg8s in targeting its cognate retroviral core for autophagic destruction.

(**A**) Schematic of rhesus TRIM5α (RhTRIM5α), emphasizing HIV-1 capsid protein (p24) binding domain and key residues (asterisks). (**B,C**) Levels of intracellular p24 were determined by immunoblotting from rhesus cells (FRhK4) that had been exposed to pseudotyped virus including HIV-1 p24 for 4 h in the presence or absence of lysosomal protease inhibitors e64d and pepstatin A (e64d). Bands shown are non-adjacent lanes from the same immunoblot (developed simultaneously). (**D**) Co-localization analysis (graph, Pearson's colocalization coefficient) for ALFY and HA-RhTRIM5α in HeLa cells. RAP, autophagy induced with rapamycin. CTRL, control (vehicle). Arrows, examples of colocalization between ALFY and HA-RhTRIM5α. See legend Suppl. Figure S2C. (**E,F**) Effects of rhesus ALFY knockdown on p24 levels in FRhK4 cells following exposure to pseudotyped HIV-1. Cells were incubated in full or starvation media following infection. (**G**) HIV-1 proviral DNA in FRhK4 cells subjected to TRIM5α or Beclin 1 (Bec) knockdowns and infected with VSVG-pseudotyped HIV-1 for 4 h. (**H**) HIV-1 reverse transcriptase (RT) activity in fed or starved rhesus cells (FRhK4) knocked down for indicated factors and infected for 4 h with VSVG-pseudotyped HIV-1. (**I**) GST pull-down analysis of the role of LIR-1 and LIR-2 in TRIM5α binding to the indicated mAtg8s. (**J**) Representative epifluorescent image of the localization of WT or LIR-1*&2* RhGFP-TRIM5α in HeLa cells. (**K**) Representative epifluorescent image of the effect of WT or LIR-1*&2* GFP-RhTRIM5α expression on the intracellular distribution of p-ULK1 (Ser 317). (**L**) Representative epifluorescent image of the effect of WT or LIR-1*&2* GFP-RhTRIM5α expression on the abundance of autophagosomes detected as punctate LC3B. (**M**) Model of TRIM-based assembly (TRIMosome) of autophagy regulators and effectors directing selective autophagy. Depiction based on SPRY-containing TRIMs and TRIM5α-based recognition of cargo. Data, means ± SE; *, $P < 0.05$; †, $P \geq 0.05$ (Student's t test; n≥3).