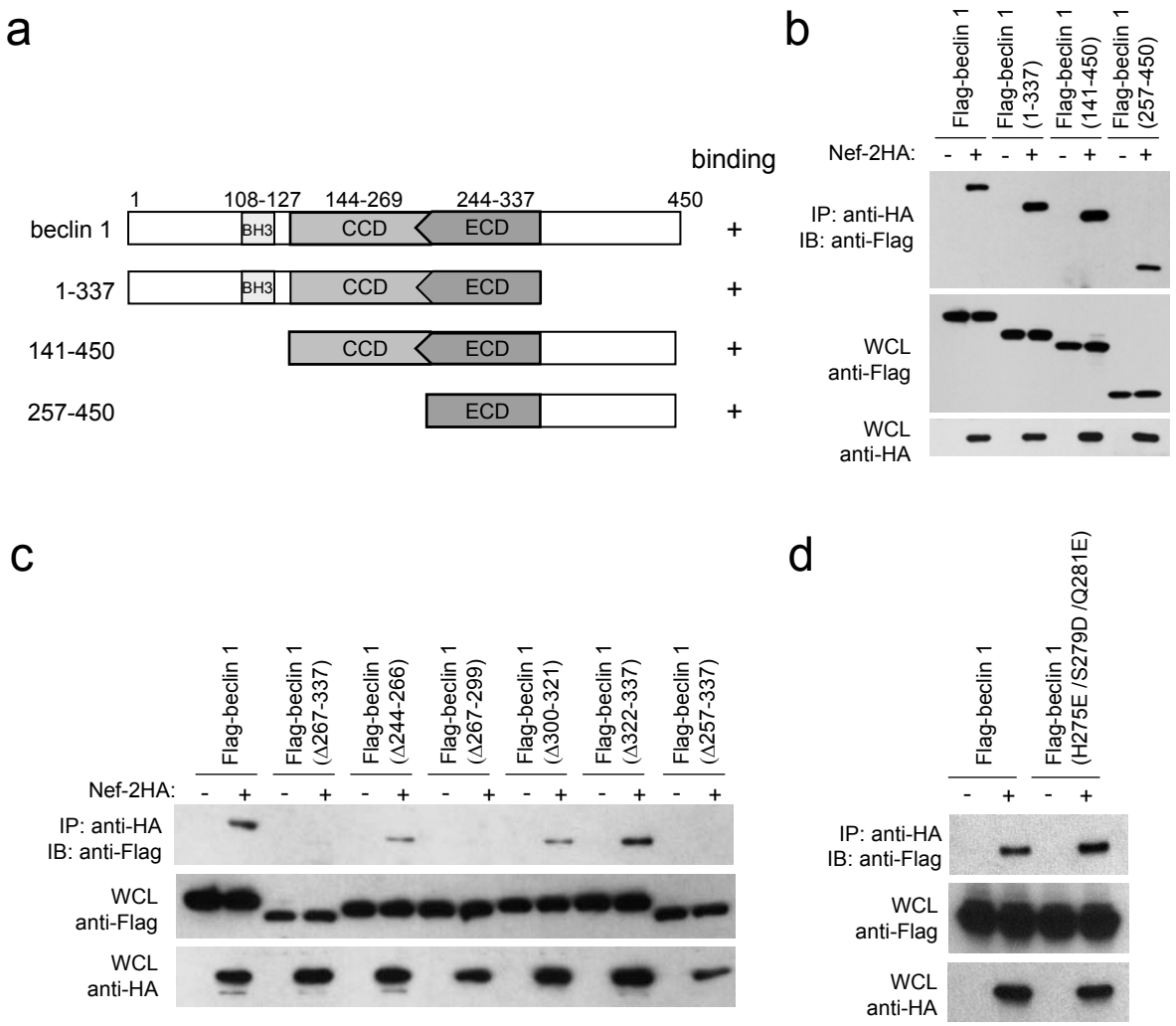
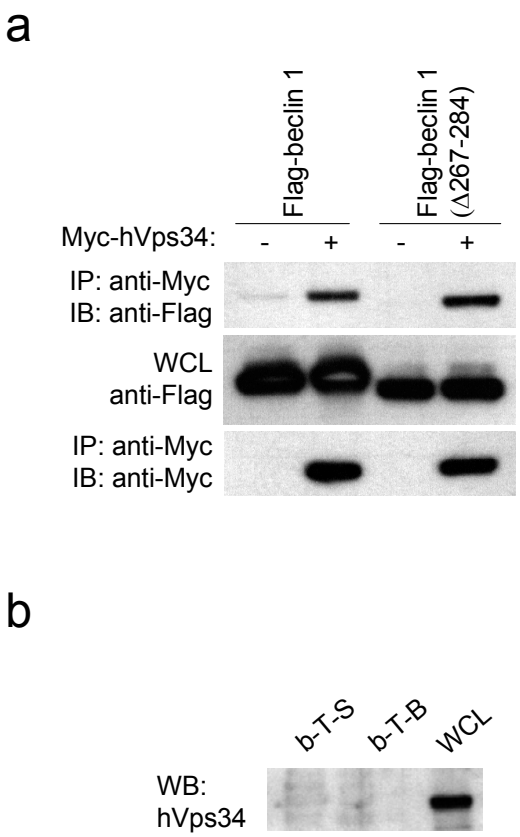
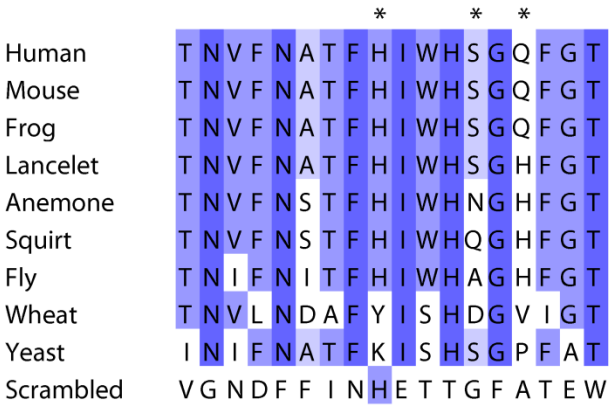
doi:10.1038/nature11866



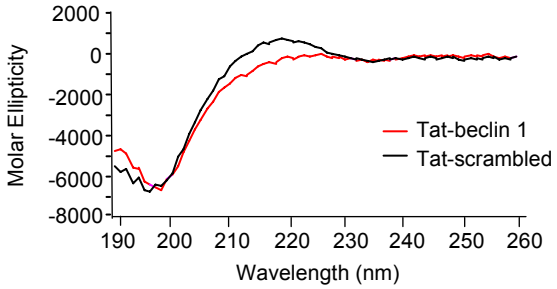
Supplementary Figure 1. Mapping of the Nef binding domain of beclin 1. a, Schematic map of Nef/beclin 1 co-immunoprecipitation results shown in (**b**). **b,** Immunoprecipitation of indicated Flag-beclin 1 constructs with the HIV-1 viral protein Nef-HA in COS-7 cells at 24 h post-transfection. **c,** Immunoprecipitation of beclin 1 mutants containing deletions within the ECD with Nef-HA in COS-7 cells at 24 h post-transfection. **d**, Immunoprecipitation of a Flag-beclin 1 mutant containing 3 amino acid substitutions (H275E/S279D/Q281E) with Nef-HA in COS-7 cells at 24 h post-transfection. BH3, Bcl-2-like homology domain 3; CCD, coiled coil domain; ECD, evolutionarily conserved domain.



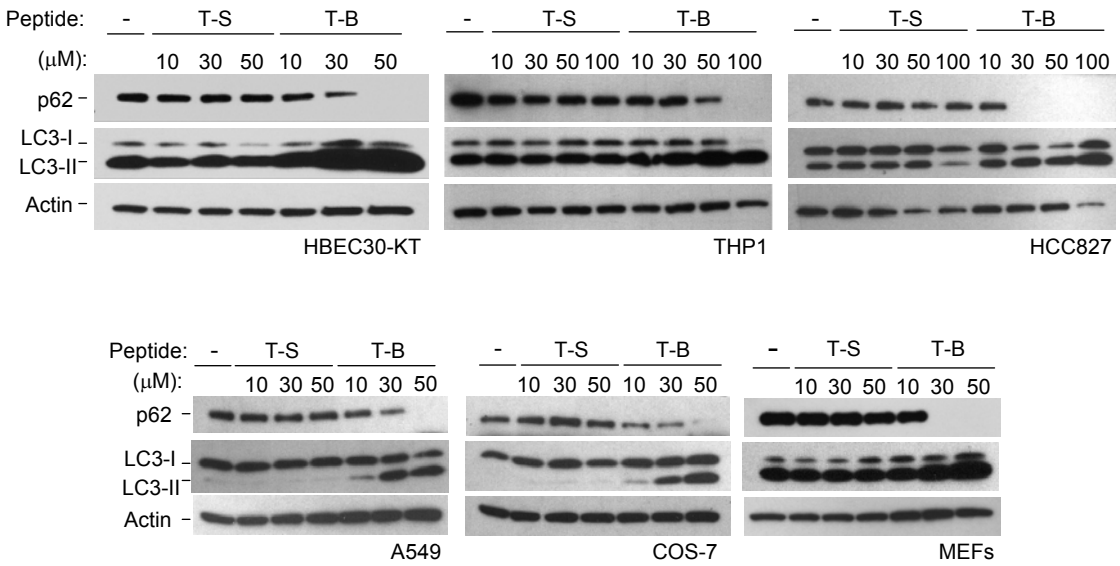
Supplementary Figure 2. Amino acids 267-284 of beclin 1 are not required for binding to hVps34. a, Immunoprecipitation of indicated Flag-beclin 1 constructs with Myc-hVps34 in HeLa cells 24 h after co-transfection of plasmids expressing indicated Flag-beclin 1 constructs and Myc-hVps34. **b,** HeLa cells were treated with biotin-conjugated peptides (30 μM, 3 h); cell lysates were incubated with streptavidin beads; and proteins bound to the biotin-conjugated peptides were subjected to immunoblot analysis with an anti-hVps34 antibody. b-T-S, biotin-conjugated Tat-scrambled peptide; b-T-B, biotin-conjugated Tat-beclin 1 peptide; WCL, whole cell lysate.



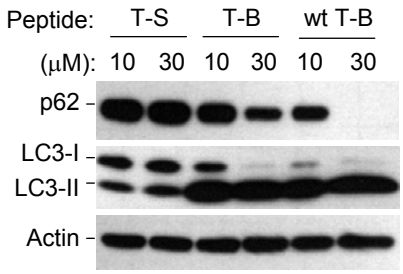
Supplementary Figure 3. Alignment of the amino acid sequence of human beclin 1 amino acids 267 to 284 with orthologs in other species. Residue positions are highlighted according to identity. Asterisks above the alignment indicate amino acids positions in which mutations were made in the Tat-beclin 1 peptide to enhance hydrophilicity.



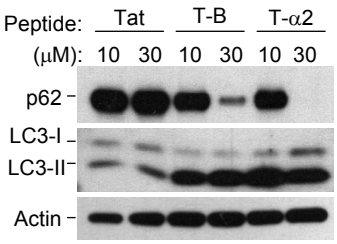
Supplementary Figure 4. Circular dichroism spectroscopic analysis of Tat-scrambled and Tat-beclin 1 peptides.



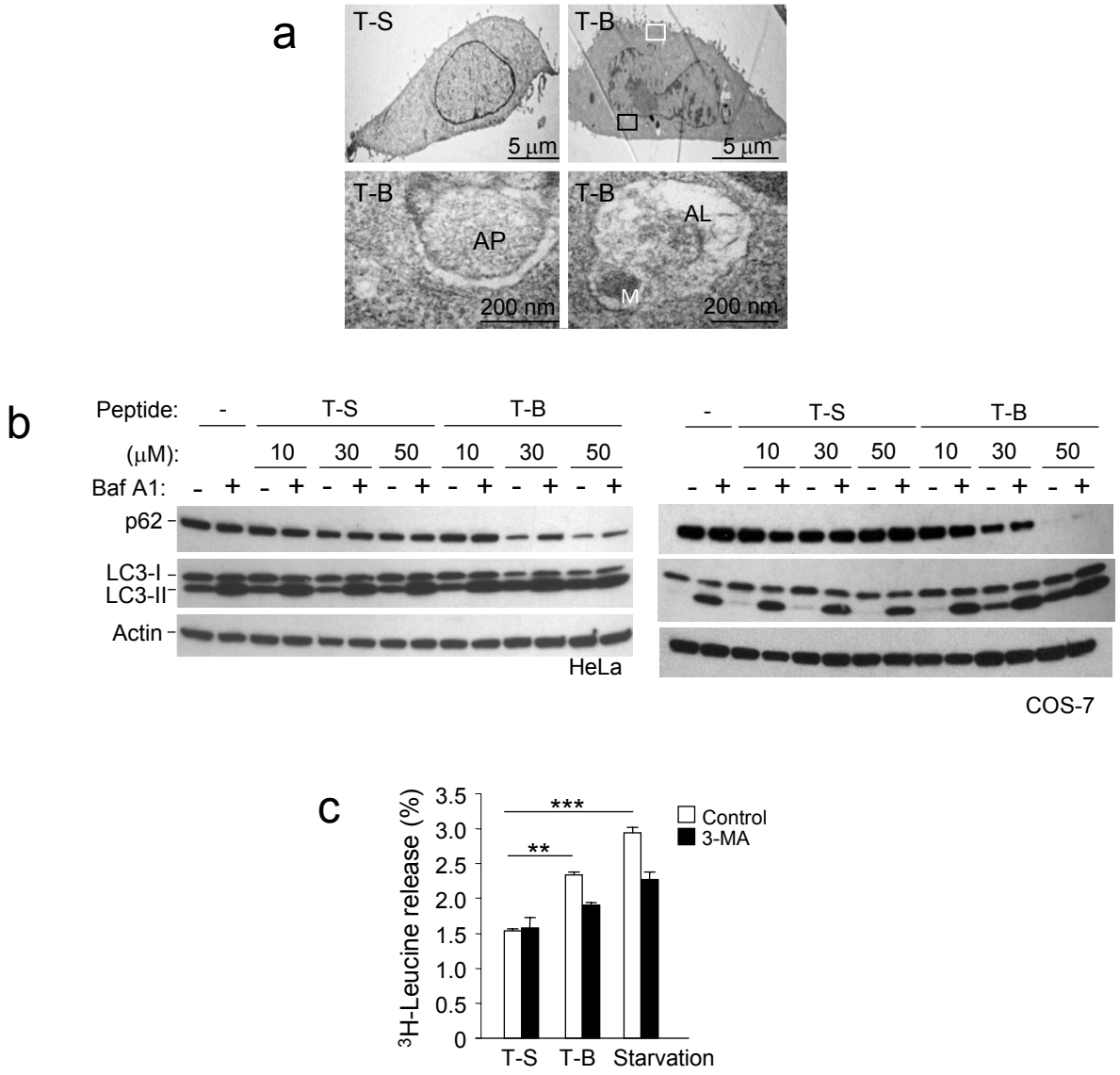
Supplementary Figure 5. Tat-beclin 1 peptide induces p62 degradation and conversion of LC3-I to LC3-II in multiple different cell lines. Immunoblot analysis of p62 and LC3 protein levels in HBEC30-KT, THP1, HCC827, A549, and COS-7 cells, and primary murine embryonic fibroblasts (MEFs) treated with indicated peptide at indicated dose for 3 h. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



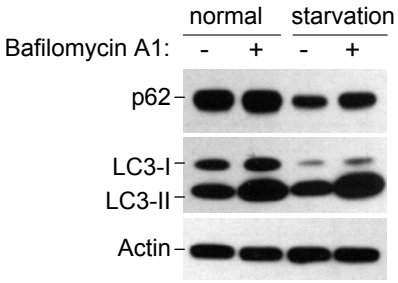
Supplementary Figure 6. Comparison of autophagy induction by Tat-beclin 1 and wild-type Tat-beclin 1 peptides. Immunoblot analysis of p62 and LC3 protein levels in HeLa cells treated with indicated peptide at indicated dose for 3 h. T-S, Tat-scrambled; T-B, Tat-beclin 1; wt T-B, a peptide consisting of Tat fused to the wild-type beclin aa sequence 267-284.



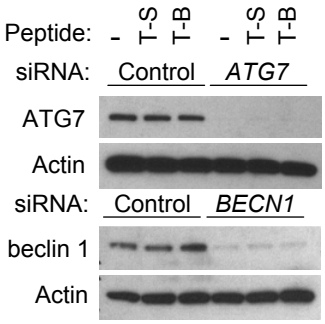
Supplementary Figure 7. Tat-beclin 1 and Tat-vFLIP α2 peptide induce p62 degradation and conversion of LC3-I to LC3-II. Immunoblot analysis of p62 and LC3 protein levels in HeLa cells after treatment with indicated peptide at indicated dose for 3 h. T-S, Tat-scrambled; T-B, Tat-beclin 1, T-α2; Tat-vFLIP α2 peptide.



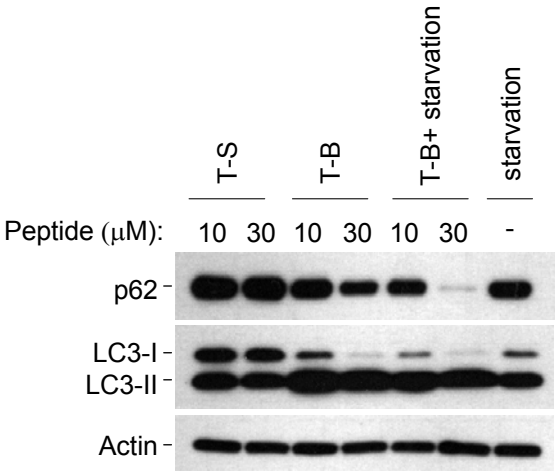
Supplementary Figure 8. Tat-beclin 1 peptide induces autophagic flux and autophgic degradation of long-lived proteins. a, Representative electron micrographs of HeLa cells treated with 20 μM of indicated peptide for 3 h. Lower panels, high magnification images of insets in upper right panel; left panel corresponds to white inset and right panel corresponds to black inset. AP, autophagosome; AL, autolysosome; M, mitochondrion. **b**, Immunoblot analysis of p62 and LC3 levels in HeLa and COS-7 cells treated with indicated peptide at the indicated dose for 3 h ± 100 nM bafilomycin A1. **c,** Degradation of long-lived proteins in COS-7 cells treated with 30 μM of indicated peptide or cultured in EBSS for 2 h ± 3-methyladenine (3-MA). Values represent mean ± s.e.m. for triplicate wells per condition. Similar results were observed in 3 independent experiments. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



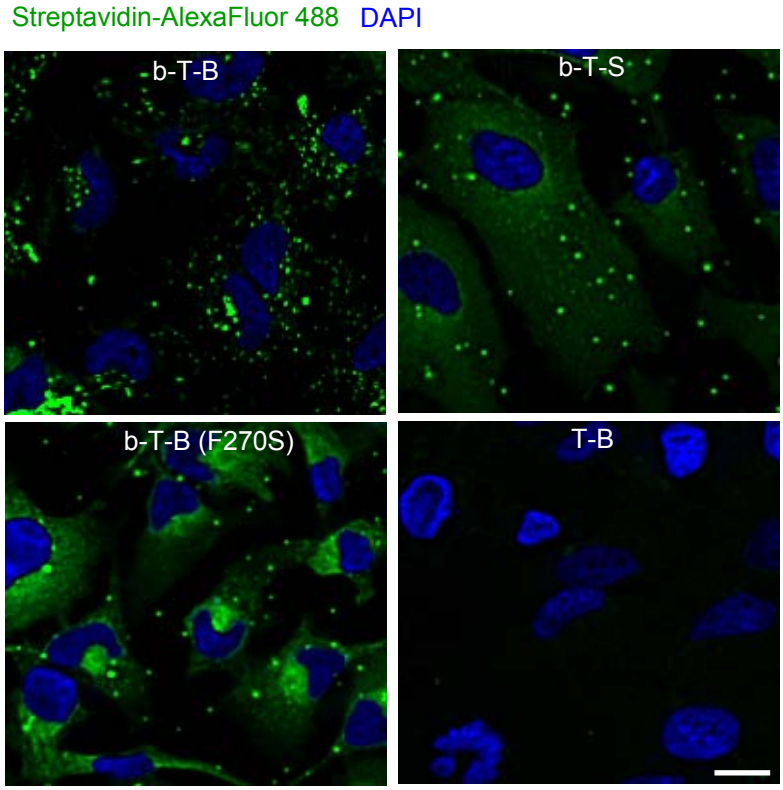
Supplementary Figure 9. Effect of bafilomcyin A1 on p62 and LC3 protein levels in HeLa cells incubated in normal or starvation medium. HeLa cells were grown in normal medium or starved (in EBSS) for 4 h ± 100 nM bafilomycin A1, and then subjected to immunoblot analyses with the indicated antibodies.



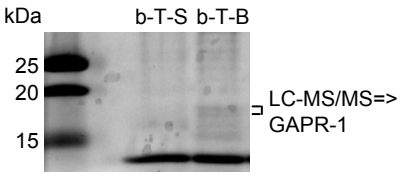
Supplementary Figure 10. Effects of *ATG7* and *BECN1* siRNA on ATG7 and beclin 1 protein expression. Immunoblot analysis of ATG7 and beclin 1 protein levels in HeLa cells transfected with indicated siRNA for 72 h and treated with 30 μM indicated peptide for 3 h. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



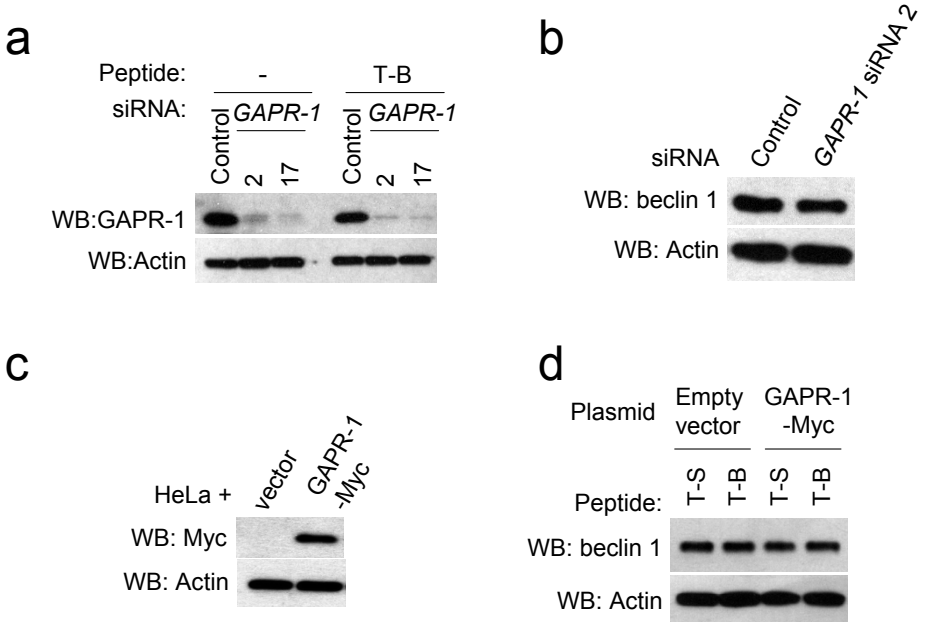
Supplementary Figure 11. Effect of dual Tat-beclin 1 peptide treatment and starvation on autophagy. Immunoblot analysis of p62 and LC3 protein levels in HeLa cells treated with indicated peptide at indicated dose for 3 h in normal medium or starvation medium (EBSS). T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



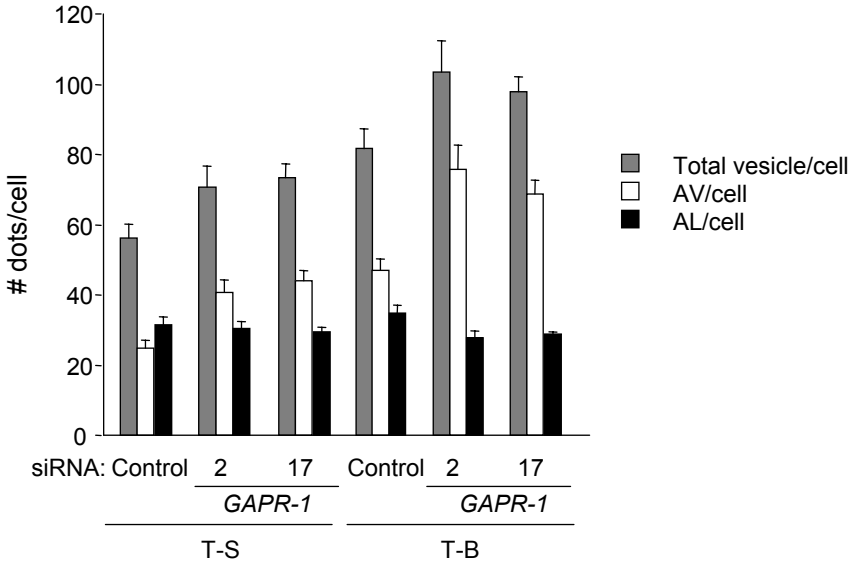
Supplementary Figure 12. Intracellular staining pattern of biotin-conjugated peptides. HeLa cells were treated for 1 h with indicated peptide, fixed, and biotin-conjugated peptides were detected by staining with Streptavidin-AlexaFluor 488. Scale bar, 20 μm. b-T-B; biotin-conjugated Tat-beclin 1 peptide; b-T-S, biotin-conjugated Tat-scrambled peptide; b-T-B (F270S), biotin-conjugated Tat-beclin 1(F270S) peptide.



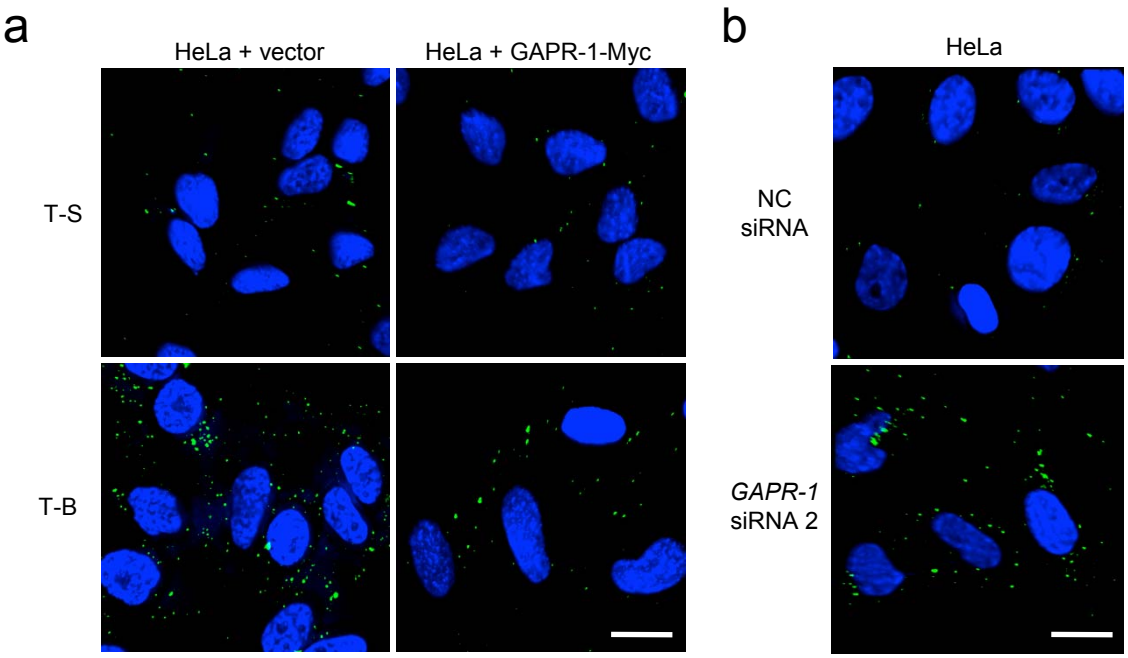
Supplementary Figure 13. Identification of GAPR-1 as a Tat-Beclin1 binding protein by LC-MS/MS. HeLa cells were treated with biotin-conjugated peptides (30 μM, 3 h) and proteins bound to the peptides were isolated and detected by SDS-PAGE and GelCode Blue stain reagent staining. b-T-S, biotin-conjugated Tat-scrambled peptide; b-T-B; biotin-conjugated Tat-beclin 1 peptide.



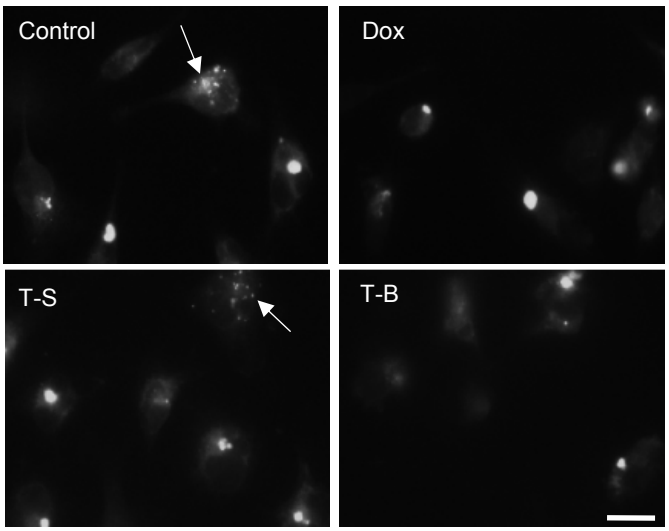
Supplementary Figure 14. Expression of GAPR-1 and beclin 1 in HeLa cells with *GAPR-1* siRNA knockdown or GAPR-1 overexpression. **a-b,** HeLa cells were transfected with non-silencing control or indicated *GAPR-1* siRNA for 72 h, and cell lysates were immunoblotted to detect GAPR-1 (**a**) or beclin 1 (**b**). **c-d**, HeLa cells were stably transduced with indicated expression vector and cell lysates were immunoblotted to detect GAPR-1 (**c**) or beclin 1 (**d**). In a and d, cells were treated with 20 μM indicated peptide for 1 h prior to lysis. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



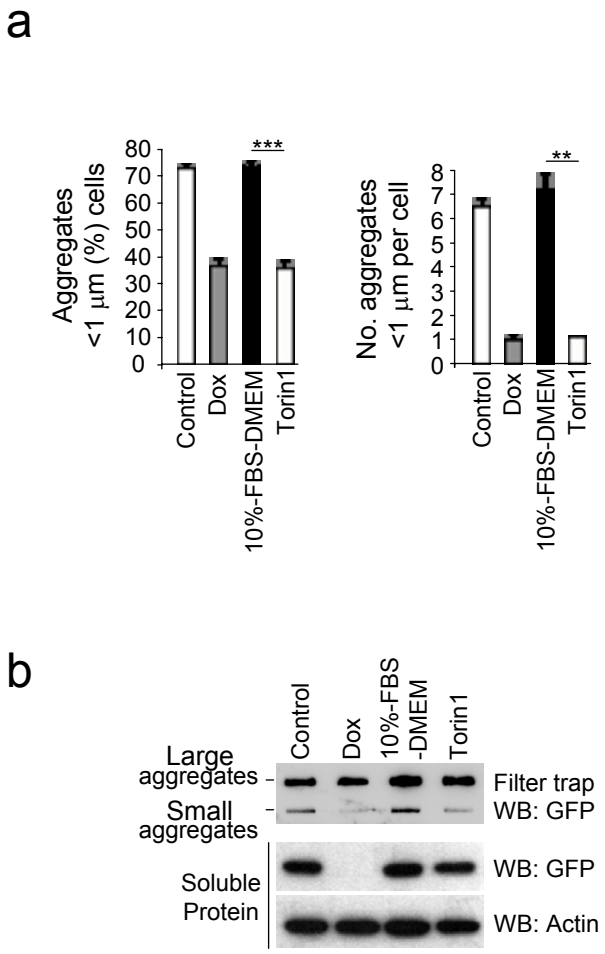
Supplementary Figure 15. The effect of GAPR-1 knockdown on autophagosome and autolysosome formation. Quantification of total number of vesicles/cell, autophagosomes/cell (AV/cell; LC3-GFP$^+$/mRFP$^+$), and autolysosomes/cell (AL/cell; LC3-GFP$^-$/mRFP$^+$). Values represent mean $\pm$ s.e.m. for triplicate samples (with at least 40 cells analyzed per sample). Similar results observed in 3 independent experiments.



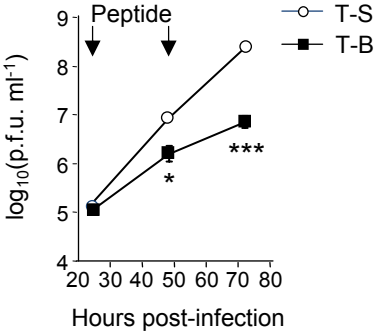
Supplementary Figure 16. Representative images of WIPI2 staining in HeLa cells stably transduced with indicated expression construct and treated with indicated peptide (a) or in HeLa cells treated with indicated siRNA (b). Similar images were used for quantitation in Fig. 2g of main text (background staining used to define cell boundaries not shown here). Scale bars, 20 μm. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



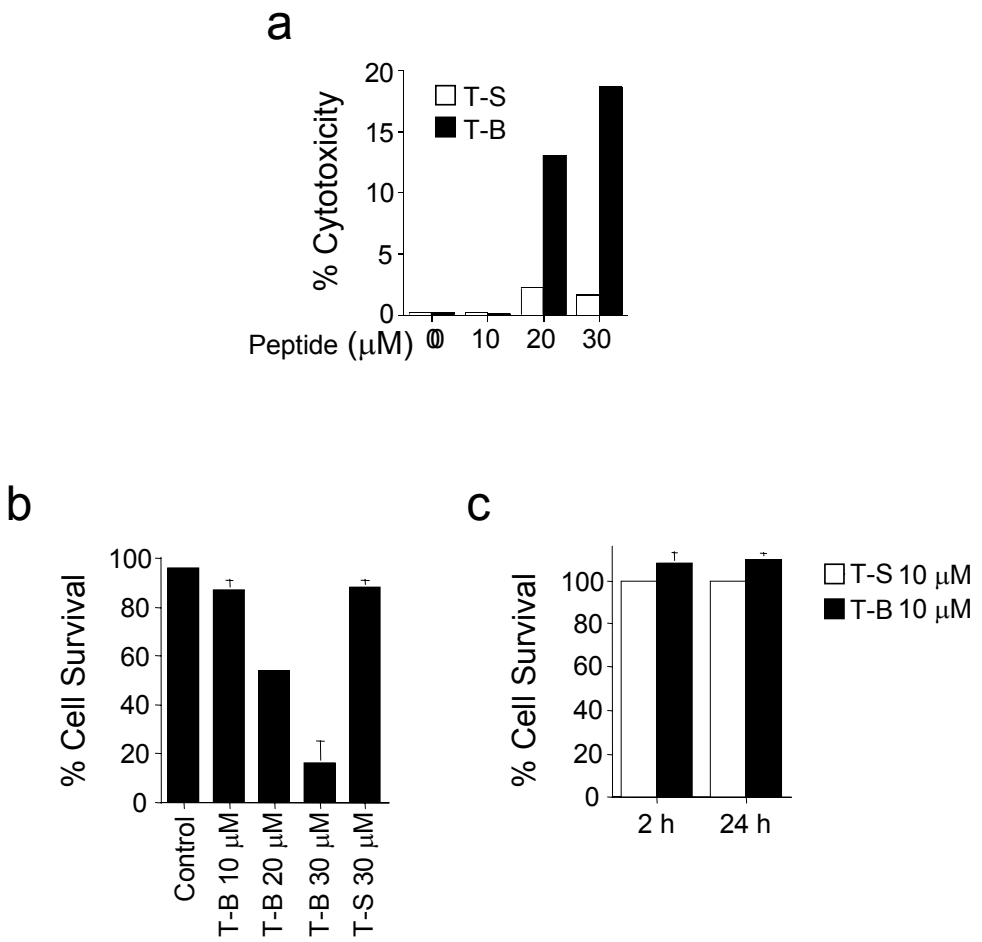
Supplementary Figure 17. Representative micrographs of HeLa/htt103Q cells expressing doxycycline-repressible CFP-htt103Q. Cells were treated daily with doxycycline (Dox) or with 10 μM of indicated peptide for 4 h per day for 2 days. Arrows denote representative small aggregates (<1 μm). T-S, Tat-scrambled peptide, T-B, Tat-beclin 1 peptide.



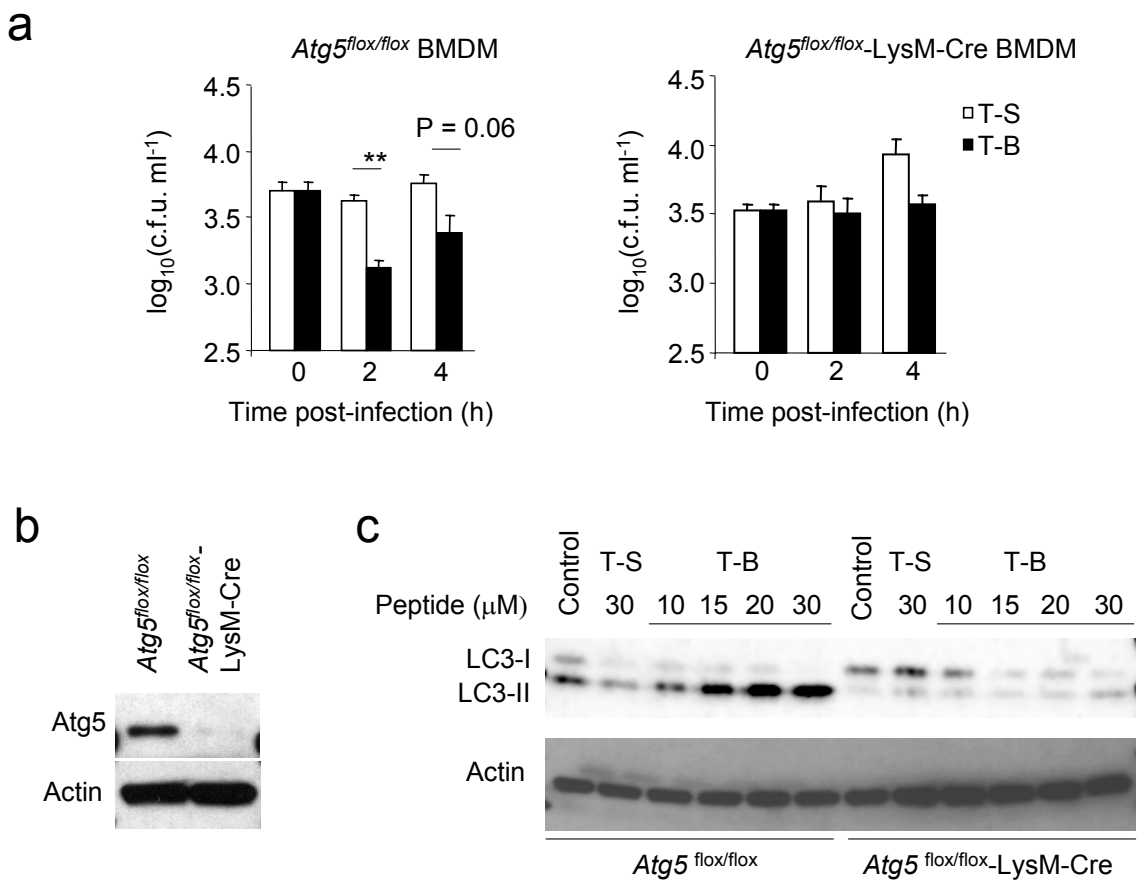
Supplementary Figure 18. Torin1 decreases levels of a mutant huntintin protein fragment. a, Quantitation of cells with small htt103Q aggregates (left graph) and number of htt aggregates per cell (right graph) in HeLa cells expressing doxycycline(Dox)-repressible CFP-htt103Q treated with Dox or with 250 nM of Torin1 for 3 h per day for 2 days. Bars represent mean $\pm$ s.e.m. of triplicate samples (60-120 cells analyzed per sample). Similar results were observed in 3 independent experiments. **b,** Filter trap assays to detect large and small htt103Q aggregates in HeLa/htt103Q cells subjected to indicated treatment.



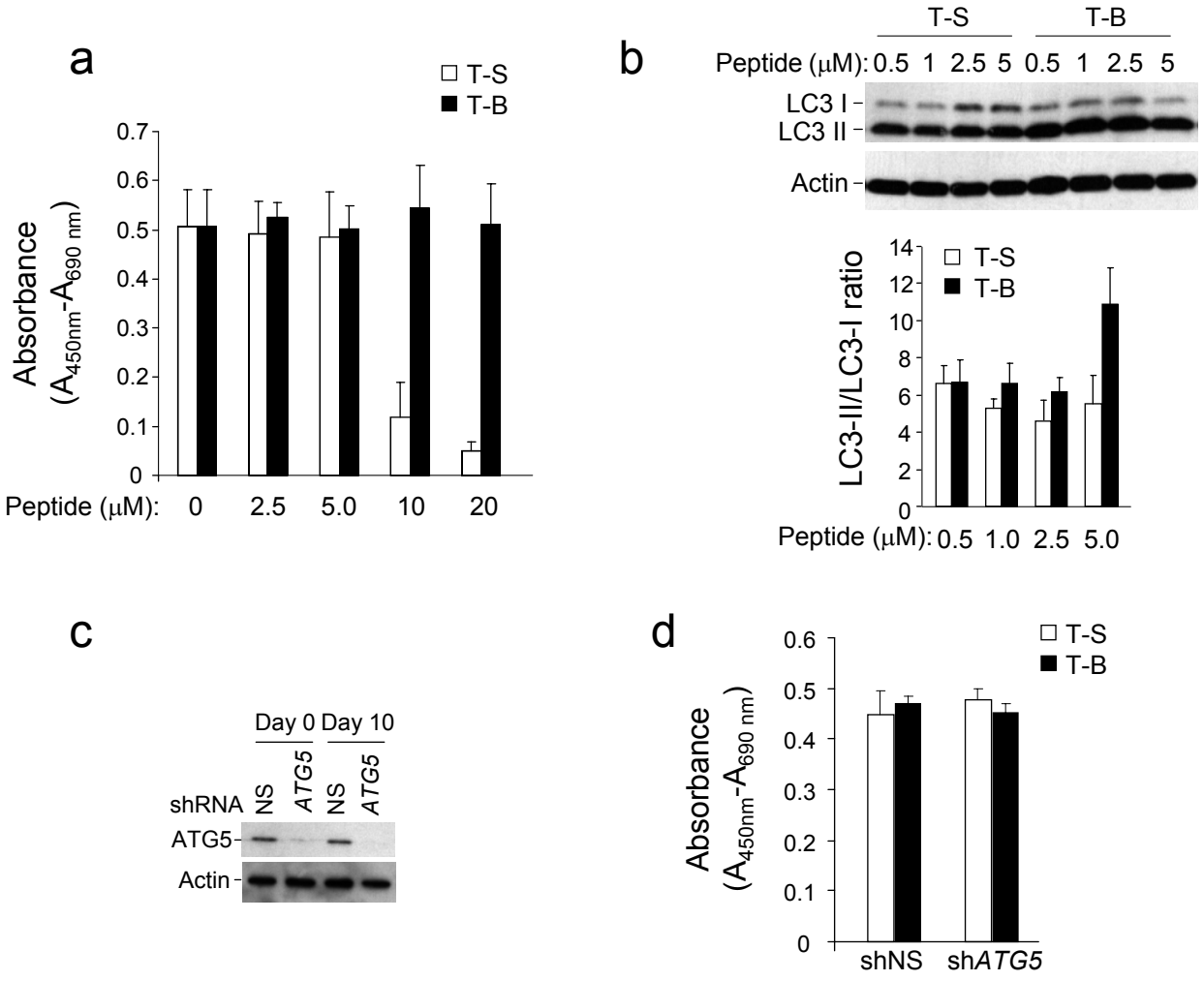
Supplementary Figure 19. Tat-beclin 1 peptide decreases CHIKV replication at serial time points after infection in HeLa cells. Viral titers in supernatants of HeLa cells infected with CHIKV at an MOI of 0.1, and treated with 10 μM of indicated peptide for 3 h at 24 and 48 h post-infection. Values represent geometric mean ± s.e.m. of triplicate samples. Similar results were observed in 3 independent experiments.



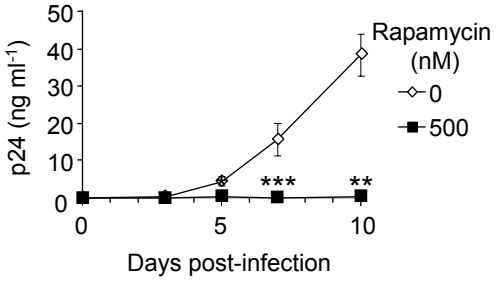
Supplementary Figure 20. Effects of Tat-beclin 1 peptide on cell survival. a, Percentage of cytotoxicity measured using LDH cytotoxicity assay in HeLa cells 24 h after treatment with indicated dose of indicated peptide for 4 h. No toxicity of 10 μM T-B was observed in other cell death assays including trypan blue staining and Cell Titer Glo (data not shown). **b**, Percentage of cell survival as assessed by trypan blue exclusion in primary murine BMDMs after 2 h treatment with indicated dose of indicated peptide. **c**, Percentage of BMDM cell survival as measured by Cell Titer Glo assay either immediately after or 24 after 2 h treatment with 10 μM of indicated peptide. For **a-c**, bars represent mean ± s.em. of triplicate wells. Similar results were observed in 3 independent experiments. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



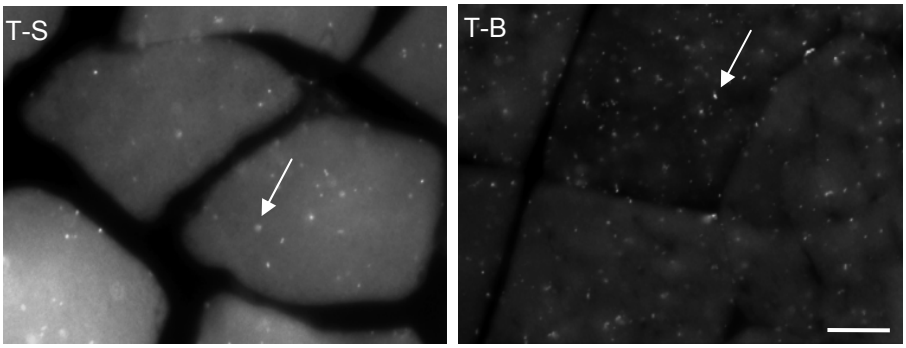
Supplementary Figure 21. Effects of macrophage *Atg5* deletion on Tat-beclin 1 peptide-induced antibacterial effects. a, Bacteria CFUs in control (*Atg5*$^{flox/flox}$) and *Atg5*$^{flox/flox}$-LysM-Cre primary murine bone-marrow derived macrophages (BMDMs) at serial times after infection with *Listeria monocytogenes* Δ*actA* mutant strain DPL-4029. Cells were infected for 30 min prior to time point labeled "0" and then treated with 15 μM peptide from 0-2 h post-infection. Bars represent mean ± s.e.m. of triplicate samples. Similar results were observed in 3 independent experiments. In BMDMs prepared from (*Atg5*$^{flox/flox}$) and *Atg5*$^{flox/flox}$-LysM-Cre mice, no toxicity was observed in uninfected cells treated with 15 μM peptide (data not shown). **b,** Immunoblot analysis of Atg5 protein levels in control (*Atg5*$^{flox/flox}$) and *Atg5*-deficient (*Atg5*$^{flox/flox}$-LysM-Cre) primary BMDMs after 8 days in culture. **c**, Analysis of LC3-II conversion in control (*Atg5*$^{flox/flox}$) and *Atg5*-deficient (*Atg5*$^{flox/flox}$-LyzM-Cre) BMDMs 2 h after treatment with indicated peptide at indicated dose. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



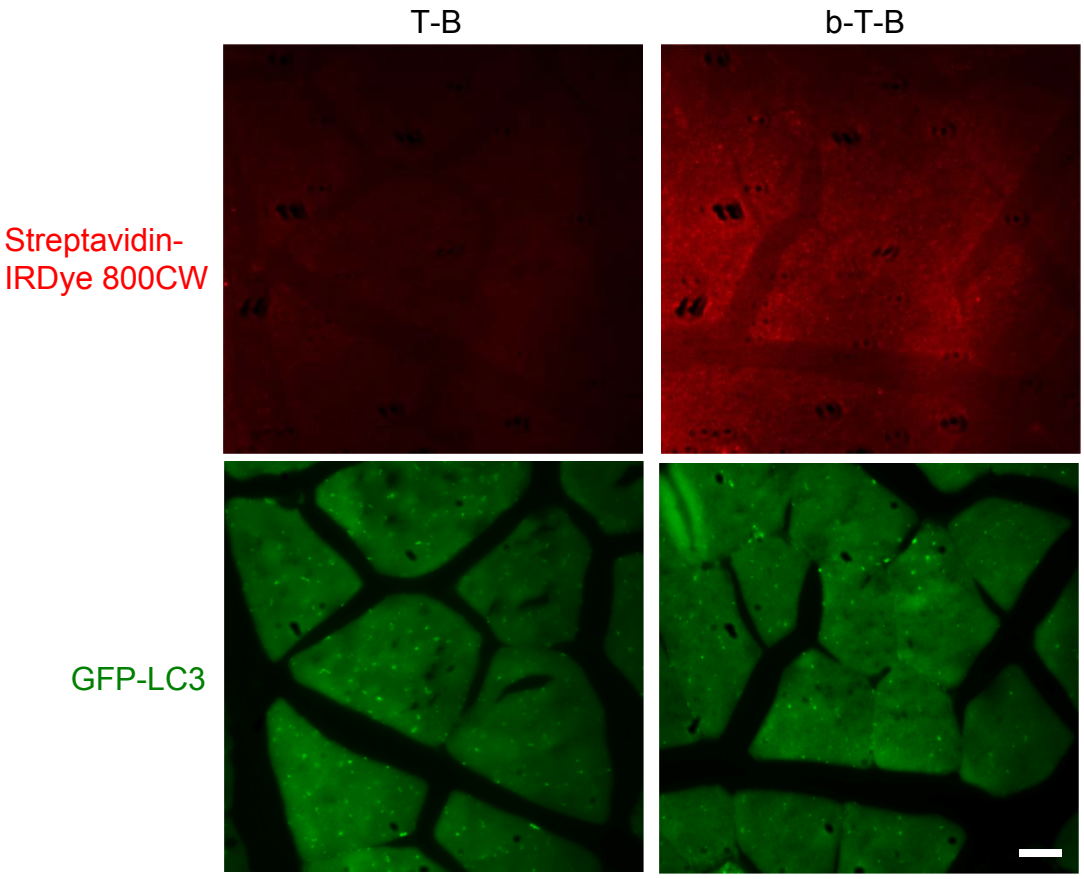
Supplementary Figure 22. Effects of Tat-beclin 1 peptide treatment on survival of primary human monocyte-derived macrophages (MDMs) and on LC3-II conversion. a, Analysis of cell survival of MDMs treated with indicated peptide at indicated dose for 24 h as measured by a WST-1 assay. Values represent mean ± s.e.m. of triplicate wells of MDMs from one donor. Similar results were observed in 3 independent experiments. **b**, Upper panel, representative immunoblot detection of LC3 protein levels in MDMs at day 1 after treatment with indicated dose of indicated peptide from one donor. Lower panel, graph showing densitometric quantification of LC3-II/LC3-I ratios for immunoblots performed on samples from 3 donors. Bars represent mean ± s.e.m. for three donors. **c,** Immunoblot detection of ATG5 in MDMs transduced with non-specific scrambled shRNA (shNS) or *ATG5* shRNA (sh*ATG5*) at day 0 and day 10 after HIV-1 infection. **d**, MDMs transduced with shNS or *shATG5* were treated with indicated peptide at 5 μM for 24 h prior to HIV infection and for 10 days after HIV infection and a WST-1 assay was performed. Bars represent mean ± s.e.m. of triplicate wells of macrophages from one donor. Similar results were observed in 2 independent experiments from independent donors. Note the absence of differences in cell viability at day 10 in any of the conditions; the lower levels of actin in T-B-treated samples in Fig. 3g of main text is due to autophagy-induced protein degradation and technical difficulties loading equal protein concentrations in each lane (due to limited total protein concentrations in primary MDM samples) rather than differences in surviving cell numbers.



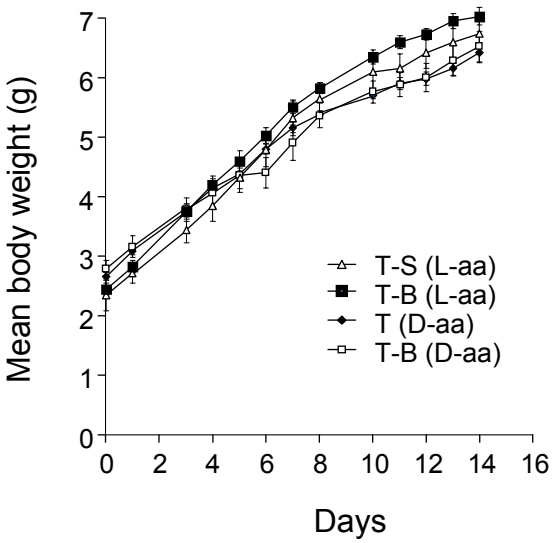
Supplementary Figure 23. Effect of rapamycin on HIV p24 antigen release in primary human monocyte-derived macrophages (MDMs) at serial time points after HIV infection. Values represent mean ± s.e.m. of triplicate wells. Similar results were observed in 3 independent experiments from 3 independent donors. ***P*<0.01, ****P*<0.001; t-test.



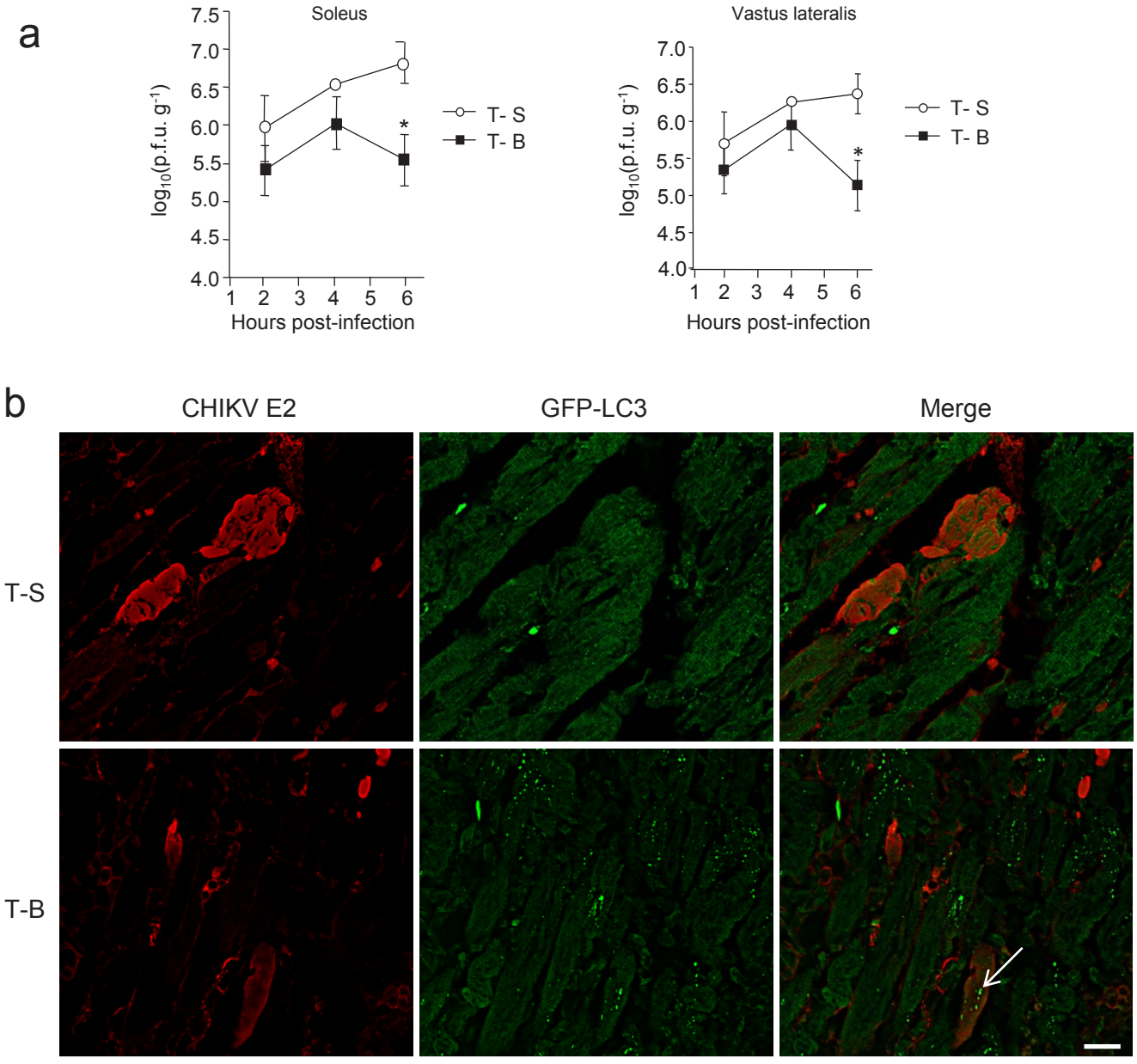
Supplementary Figure 24. Tat-beclin 1 induces GFP-LC3-positive puncta in muscle. Representative photomicrographs of vastus lateralis muscle sections from 6 week-old GFP-LC3 mice treated with 20 mg kg^{-1} of indicated peptide used for quantitative analysis in Fig. 4a of main text. Arrows denote representative autophagosomes. Scale bar, 20 μm.



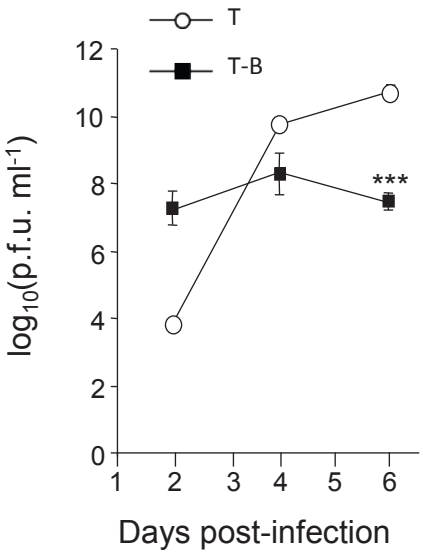
Supplementary Figure 25. Intracellular staining of biotin-Tat-beclin 1 peptide in the vastus lateralis muscle of a GFP-LC3 transgenic mouse 2 h after 20 mg kg^{-1} i.p. of peptide treatment. Biotin-Tat-beclin 1 (b-T-B) was detected by streptavidin-conjugated IRDye 800 CW. Tat-beclin 1 without biotin (T-B) was used as a control for endogenous biotin staining. Similar results were observed in muscles from 3 mice per group. Scale bar, 20 μm.



Supplementary Figure 26. Effect of daily peptide treatment on body weight of neonatal mice. Five day-old C57/BL6 mice were treated daily with 15 mg kg^{-1} i.p. of indicated L-amino acid peptide or 20 mg kg^{-1} i.p. of indicated D-amino acid peptide (n = 5 per group). T-S, Tat-scrambled peptide; T, Tat peptide; T-B, Tat-beclin 1 peptide. No significant differences were observed among the four treatment groups.



Supplementary Figure 27. CHIKV titers and GFP-LC3-positive puncta in CHIKV-infected muscle. a, Viral titers in indicated muscle groups in 5 day-old C57BL/6J mice infected with CHIKV (10^6 p.f.u. s.c.) and treated daily with 15 mg kg^{-1} i.p. of indicated peptide beginning day one post-infection. Values represent geometric mean ± s.e.m. for 3 mice per group. **b,** GFP-LC3 mice were infected with CHIKV (10^6 p.f.u. s.c.) and treated with 20 mg kg^{-1} of indicated peptide i.p. at 4 days post-infection, and vastus lateralis muscle was harvested 6 h later. Representative micrographs of muscles sections stained with antibody against CHIKV envelope E2 protein. White arrow, representative autophagosome in CHIKV-infected muscle cell. T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide.



Supplementary Figure 28. Effect of Tat-beclin 1 peptide treatment on WNV brain titers. Five day-old C57BL/6J mice were infected with 1 p.f.u. i.c. of Egypt strain 101, and treated daily with 20 mg kg^{-1} i.p. of indicated peptide beginning day one post-infection. Values represent geometric mean $\pm$ s.e.m. for 3-5 mouse brains per treatment group per time point. T, Tat peptide (D-amino acid); T-B, Tat-beclin 1 peptide (D-amino acid).

Supplementary Table 1. Serum chemistries and hematological data from C57BL/6 mice treated daily with 20 mg kg^{-1} i.p. of indicated peptide for two weeks.

	Suckling mice (5 days-old)				Adult mice (3 month-old)	
Group	T-S (L-aa)	T-B (L-aa)	T-S (D-aa)	T-B (D-aa)	T-S (L-aa)	T-B (L-aa)
Number of mice	5	4	4	5	3	3*
Renal function						
Creatinine (μmol/l)	0.64 ± 0.08	0.54 ± 0.04	0.60 ± 0.06	0.64 ± 0.08	QNS	0.24
Liver function						
AST (IU/l)	QNS	QNS	QNS	QNS	122 ± 31.5	67.0 ± 11.1
ALT (IU/l)	"	"	"	"	40.3 ± 5.0	35.3 ± 1.7
Alk Phos (IU/l)	"	"	"	"	118 ± 14.8	111.3 ± 13.7
Hematological						
Hb (g/dl)	"	"	"	"	13.3 ± 0.5	14.3 ± 0.3
HCT (%)	"	"	"	"	40.1 ± 1.2	43.8 ± 0.4

T-S, Tat-scrambled peptide; T-B, Tat-beclin 1 peptide; AST, aspartate amino transferase; ALT, alanine amino transferase; Alk Phos, alkaline phosphatase; Hb, hemoglobin; HCT, hematocrit; QNS, quantity not sufficient. Data represent mean values ± S.E. M.

*Sera was only available for two mice for creatinine measurement.