Mammalian Atg8 proteins and the autophagy factor IRGM control mTOR and TFEB at a regulatory node critical for responses to pathogens

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Autophagy is a homeostatic process with multiple functions in mammalian cells. Here, we show that mammalian Atg8 proteins (mAtg8s) and the autophagy regulator IRGM control TFEB, a transcriptional activator of the lysosomal system. IRGM directly interacted with TFEB and promoted the nuclear translocation of TFEB. An mAtg8 partner of IRGM, GABARAP, interacted with TFEB. Deletion of all mAtg8s or GABARAPs affected the global transcriptional response to starvation and downregulated subsets of TFEB targets. IRGM and GABARAPs countered the action of mTOR as a negative regulator of TFEB. This was suppressed by constitutively active RagB, an activator of mTOR. Infection of macrophages with the membrane-permeabilizing microbe Mycobacterium tuberculosis or infection of target cells by HIV elicited TFEB activation in an IRGM-dependent manner. Thus, IRGM and its interactors mAtg8s close a loop between the autophagosomal pathway and the control of lysosomal biogenesis by TFEB, thus ensuring coordinated activation of the two systems that eventually merge during autophagy.

TFEB is peripherally associated with lysosomes and is phosphorylated and regulated by mTOR25-28. It is kept in the cytoplasm but translocates to the nucleus and drives the expression of the lysosomal system, inflammation, and the control of autophagy as the lysosomal and autophagy pathways merge. IRGM and its murine orthologue Irgm1 bridge the immune system10 and the core autophagosomal and lysosomal and autophagy pathways merge19. TFEB is phosphorylated by kinases such as mTORC1, which prevents the translocation of TFEB to the nucleus; when phosphorylated, TFEB is bound to 14-3-3 proteins that retain it in the cytoplasm25. Dephosphorylation of TFEB by a calcineurin phosphatase, PPP3CB, is important for the release of TFEB from 14-3-3 (ref. 25) and its subsequent nuclear translocation27. The balance between phosphorylation and dephosphorylation of TFEB by mTORC1 and PPP3CB determines its cytoplasmic versus nuclear distribution.

We have previously shown that TFEB responds to endomembrane (for example, lysosomal) damage14 during infection with microbes such as Mycobacterium tuberculosis (Mtb)35, whereupon IRGM plays a protective role. Here, we show that IRGM, which bridges the immune system19 and the core autophagosomal and autolysosomal machinery21-24, interacts directly with TFEB and its phosphatase PPP3CB to control the activation of TFEB. We also show that Stx17 and mAtg8s influence TFEB nuclear translocation and that with IRGM, they affect mTOR, a kinase upstream of...
TFEB. We furthermore uncover that mAtg8s affect the expression of TFEB-controlled genes in a positive-feedback loop to regulate lysosomal gene expression.

Results

IRGM affects the nuclear translocation of TFEB. In the course of studying the role of IRGM in autophagy, we observed that it influenced the subcellular distribution of TFEB. Knockdown (KD) of IRGM reduced the nuclear translocation of TFEB under starvation conditions (Fig. 1a–c; Extended Data Fig. 1a–c) and in response to the pharmacological inhibition of mTOR by pp242 (Extended Data Fig. 1d,e). Following IRGM KD, subcellular fractionation showed reduced levels of TFEB in nuclear fractions (Fig. 1d,e). Primary bone-marrow-derived macrophages from Irgm knockout
Fig. 2 | IRGM and TFEB interact. A, Co-IP analysis of interactions between FLAG–TFEB and GFP–IRGM in 293T cells (n = 3 biologically independent experiments). B, Co-IP analysis of interactions between endogenous IRGM and TFEB in 293T cells (n = 3 biologically independent experiments). C, GST pull-down analysis of radiolabelled [35S]Myc–IRGM and [35S]Myc–Stx17 and [35S]Myc–LC3B with GST–TFEB (n = 3). CBB, Coomassie brilliant blue. D, Mapping of TFEB sites on IRGM. E, Co-IP image and analysis (f) of interactions between GFP–IRGM and different TFEB mutants. FL, full length. Data shown as the mean ± s.e.m. of intensities normalized to IP input (n = 3 biologically independent experiments), with paired t-test performed. Data shown as the mean ± s.e.m. of intensities normalized to IP input (n = 3 biologically independent experiments), with paired t-test performed. Uncropped blots for a, b, c, e, and g and numerical source data for f and h are provided as source data.

IRGM interacts directly with TFEB. IRGM tagged with green fluorescent protein (GFP–IRGM) co-immunoprecipitated (co-IPed) with FLAG–TFEB (Fig. 2a). GFP–IRGM and endogenous TFEB colocalized (Extended Data Fig. 2a), and endogenous IRGM and TFEB interacted (Fig. 2b). In glutathione S-transferase (GST) pull-down assays, [35S]Myc–IRGM directly interacted with GST–TFEB, whereas [35S]Myc–Stx17 and [35S]Myc–LC3B, used as a control, did not (Fig. 2c). The carboxy-terminal region of TFEB, which includes a hitherto functionally uncharacterized region termed DUF3371 (domain of unknown function 3371)37 (Extended Data Fig. 2b), was required for the binding of TFEB to IRGM (Fig. 2d–f). A GTPase mutant (S47N) of IRGM12 showed reduced TFEB binding (Fig. 2g,h). Wild-type (WT) IRGM rescued the effects of IRGM KD on TFEB nuclear translocation, whereas IRGM S47N did not (Extended Data Fig. 2c,d). IRGM interacted with other MiT/TEF members; for example, GFP–MiTF and GFP–TFE3 co-IPed with FLAG–IRGM (Extended Data Fig. 2e,f). GFP–IRGM S47N bound less efficiently than GFP–IRGM WT to MiTF (Extended Data Fig. 2g,h). Thus, IRGM interacts with MiTF/TFE members and directly binds TFEB.

IRGM affects TFEB phosphorylation status. Overexpression of GFP–IRGM caused an increased electrophoretic mobility of endogenous TFEB (Fig. 3a(i)), a result that is compatible with TFEB dephosphorylation36,37. Dephosphorylation of TFEB was detected (Fig. 3c,d) using a phospho-(Ser) 14-3-3 binding motif antibody25, which recognizes phospho-Ser-211 on TFEB, the site for 14-3-3 binding that keeps TFEB in the cytoplasm. Reduced TFEB phosphorylation caused by IRGM overexpression was confirmed using an anti-pS211–TFEB antibody (Fig. 3a(ii),b). Thus, IRGM promotes the dephosphorylation of TFEB.

IRGM affects mTOR activity. TFEB is phosphorylated by mTOR, which blocks the nuclear translocation of TFEB32,33. The activity of mTOR is inhibited during starvation, which is an effect that was diminished by IRGM KD as measured by p70S6K, pS6K1 and pS211 TFEB levels (Fig. 3e–h). IRGM KD also, albeit only partially, prevented a decrease in pS211 TFEB levels in cells treated with pp242, which is the catalytic inhibitor of mTOR (Extended Data Fig. 3a,b). Conversely, overexpression of IRGM reduced mTOR

(IRGm1KO) transgenic mice34 displayed reduced nuclear translocation of TFEB in response to starvation (Fig. 1f,g). Thus, IRGM is required for efficient TFEB nuclear translocation.
activity (Extended Data Fig. 3c–e). IRGM KD countered the desorption of mTOR from lysosomes during starvation44 (Fig. 3j,k; Extended Data Fig. 3f), whereas the number of LAMP2 puncta remained unaffected (Extended Data Fig. 3g,h). Paradoxically, the association of mTOR with lysosomes increased following IRGM KD under basal conditions (Fig. 3k). IRGM stabilizes and activates AMPK45, whereas AMPK inhibits mTOR activity36. IRGM KD reduced AMPK activity, as assessed using pS317–ULK1, in response to starvation (Fig. 3e,f). Thus, the activation of AMPK by IRGM45 may affect the basal state of mTOR. We next tested whether the known cysteine controlling mTOR activity, which includes Rag GTPases46, transduces IRGM effects to TFEB. Constitutively active RagB (RagB(D596)Ser) maintains mTOR in an active state even under starvation conditions46. The expression of GFP–IRGM increased TFEB nuclear translocation, and this effect was abrogated in cells stably expressing RagB(D596)Ser (Fig. 3f; Extended Data Fig. 3i). Thus, IRGM affects TFEB activation at least partially via mTOR.

**IRGM interacts with the calcineurin PPP3CB.** TFEB is dephosphorylated by the calcineurin phosphatase PPP3CB22. GFP–IRGM and PPP3CB colocalized (Extended Data Fig. 3j–l), and the number of double-positive profiles increased during starvation (Extended Data Fig. 3k–l). Flag–IRGM co-IPed with GFP–PPP3CB (Fig. 3m). PPP3CB was found in protein complexes with endogenous IRGM (Fig. 3n). A direct interaction between IRGM and PPP3CB was established in GST pull-down assays (Fig. 3o). GFP–IRGM expression augmented the association of TFEB with PPP3CB, whereas IRGM KD reduced this association (Fig. 3p–s). IRGM was colocalized with TFEB and PPP3CB on lysosomes that were purified using the LysoIP technique46,47 (Extended Data Fig. 3n,o). IRGM overexpression promoted the dephosphorylation of another PPP3CB target, NFAT247 (Extended Data Fig. 3p). Thus, not only does IRGM control TFEB via mTOR but it also acts through calcineurin.

**Mammalian Atg8s control the nuclear translocation of TFEB.** IRGM and mAtg8s form a complex24. We wondered whether mAtg8s may contribute to the control of TFEB translocation. GFP–GABARAP co-IPed efficiently with Flag–TFEB (Extended Data Fig. 3q). TFEB directly bound GABARAP (Fig. 4a). The LIR docking site (LDS)48 was not required for binding of TFEB to GABARAP (Extended Data Fig. 3r), thus ruling out canonical LDS–LIR interactions.

We next used the previously characterized CRISPR knockouts in HeLa cells with inactivated mAtg8s as triple LC3TKO (LC3A,B,C KO), triple GABATKO (GABARAP, GAPARAPL1, GABARAPL2 KO) and total mAtg8 HexaKO (pan-mAtg8s KO)14. HexaKO displayed inefficient nuclear translocation of TFEB relative to the parental HeLa cells in response to starvation (Fig. 4b; Extended Data Fig. 3s). The nuclear translocation of GFP–MifT in response to starvation was also reduced in HexaKO cells (Extended Data Fig. 4a,b). The LC3TKO did not affect TFEB translocation (Fig. 4c–e; Extended Data Fig. 3s); however, KO of all GABARAPs (GABA7KO) reduced the nuclear translocation of TFEB (Fig. 4d–e; Extended Data Fig. 3s). Transfection of HexaKO cells with GFP–GABARAP or GFP–GABARAPL1, but not with GFP–GABARAPL2, rescued the effects on nuclear translocation of TFEB in response to starvation (Fig. 4f–g). Thus, GABARAP or GABARAPL1 are mAtg8s that promote TFEB translocation.

Previous studies have indicated that certain ATG proteins may affect TFEB translocation whereas others do not42. We detected only a partial reduction in the efficacy of TFEB nuclear translocation in HeLa ATG3KO cells relative to their parental HeLa WT cells (Fig. 4g,h). We next addressed the possibility that similar to the GABARAPs partner IRGM14, mTOR may be involved. GABARAP was found together with mTOR in lysosomal preparations (Extended Data Fig. 3n,o). KO of all three GABARAPs (GABA7KO) counteracted the starvation-induced inhibition of mTOR activity (Fig. 4i–k). Overexpression of GFP–GABARAP increased TFEB nuclear translocation in HEK293T (293T) cells but not in cells stably expressing RagB(D596)Ser (Fig. 4l). Thus, similar circuitries converging on mTOR are involved in the effects of IRGM and GABARAPs on TFEB.

**Mammalian Atg8s affect the expression of TFEB target genes.** TFEB functions as a transcriptional regulator42. We performed RNA sequencing (RNA-seq) analyses in pan-mAtg8 KO HeLa cells (HexaKO) and their parental HeLa cells induced for autophagy by starvation. This global analysis detected 451 downregulated genes in HexaKO cells compared with parental HeLa cells (Fig. 5a,b; Extended Data Fig. 5a,b), including 46 previously identified TFEB targets49 (Fig. 5c; Supplementary Table 1, tab 1). This group, marked on the volcano plot in Fig. 5c, consists of lysosomal hydrolases and other identified TFEB target genes such as CTSD, CSTF, CTSS, GUSB, HXEA and TPP1, as well as MMP12, FOLR1, AHNAK2, HLA-B, HOBX9, HKDC1, LPAR5 and SCPEP1. GABA7KO cells showed a partial overlap (50%; 29 out of 46 known TFEB-controlled genes20) relative to HexaKO cells (Fig. 5d). Additional TFEB targets, such as DEXI, VPS18, SYNJ2, SFN3, APBB3, HSPB8 and the key autophagy regulator ULK1, were reduced in GABA7KO cells (Supplementary Table 2, tab 1). A question arose of whether these changes were due to disabled autophagy or to other effector functions of mAtg8s.
To address this, we used ATG3KO HeLa cells, which cannot conjugate mAtg8s to lipids, which is a key step in autophagy. When we compared ATG3KO and HexaKO cells, the overlap with the previously identified TFEB targets in HexaKO cells was limited to 11 genes (Extended Data Fig. 5c; Supplementary Table 3). Thus, a substantial portion of the gene expression effects seen in HexaKO and GABAβKO cells exceed what could be ascribed to autophagy as a process.

HexaKO cells showed an altered expression of several genes associated with autophagy (Extended Data Fig. 5d; Supplementary Table 1, tab 1). PCR with reverse transcription (RT–PCR) showed reduced relative expression levels of SQSTM1 (also known as p62), ATG9B, and ULK1 in HexaKO cells (Extended Data Fig. 5e). The RNA-seq data, albeit not showing a cumulative decrease for SQSTM1, indicated downregulation of individual SQSTM1-specific transcripts in HexaKO cells (Supplementary Table 1, tab 2; transcript ID: ENST00000510187). Expression of TFEB in HexaKO cells did not change, which indicates that the effects of mAtg8s on TFEB are primarily at the protein level. In conclusion, mAtg8s affect global transcriptional activity, including the lysosomal system. This involves a partial overlap with the influence of the TFEB domain and additional systems that remain to be fully explored.

Mammalian Atg8s affect the differential expression of diverse genes. Additional global transcriptional changes in HexaKO cells, including the upregulation of 294 genes, were observed by RNA-seq (Fig. 5b; Supplementary Table 1, tab 1) that could not be fully explained by TFEB alone. RNA-seq showed changes in expression of NFATC2 and other calcium effectors such as CAMK2N1, CANA2D3, ORAI3 and PLCG2, which suggests a Ca2+-related theme (Extended Data Fig. 6a; Supplementary Table 1, tab 1). We examined whether the Ca2+ response is intact or affected in HexaKO cells, and found a diminished rise in cytosolic Ca2+ in HexaKO cells subjected to starvation in Hank’s balanced salt solution (HBSS) (Extended Data Fig. 6b,c). Thus, in addition to the very specific interactions with 14-3-3 proteins, mAtg8s affect the differential expression of diverse genes.

Stx17 affects the nuclear translocation of TFEB. Another member of the complex between IRGM and mAtg8s is Stx17 (ref. 14). Stx17 CRISPR-based KO (Stx17KO) cells displayed reduced TFEB translocation to the nucleus in response to starvation (Fig. 6a,b; Extended Data Fig. 6d). Mirroring this, a small fraction of residual TFEB, which remains localized to lysosomes even under starvation in WT cells, further increased in Stx17KO cells (Extended Data Fig. 6e–g). Likewise, the total number of TFEB puncta in the cytoplasm, which went down in WT cells subjected to starvation, increased in Stx17KO cells (Extended Data Fig. 6h). Complementation of Stx17KO HeLa cells with GFP–Stx17 rescued the nuclear translocation of TFEB (Fig. 6c,d). A LIR mutant of Stx17 (Stx17LIR−), which does not bind mAtg8s, did not efficiently rescue the nuclear translocation of TFEB (Fig. 6e,d).

How might Stx17 affect TFEB? Although there was no appreciable direct interaction between Stx17 and TFEB (Fig. 2c), using LysOpurified lysosome samples, we detected Stx17 on lysosomes together with other factors studied, including mTOR and TFEB (Extended Data Fig. 3n,o). We therefore tested whether TFEB and Stx17 coexisted in protein complexes even without direct interactions. Co-IP analyses showed that Stx17 and TFEB were in common protein complexes (Extended Data Fig. 6f,h). The three components, IRGM, Stx17 and TFEB, displayed interdependence. When GFP–IRGM was overexpressed, this increased Stx17 levels in FLAG–TFEB immunoprecipitates (Fig. 6f,e). IRGM KD reduced levels of Stx17 in FLAG–TFEB immunoprecipitates (Fig. 6g,h). Finally, overexpression of GFP–Stx17 increased levels of IRGM in FLAG–TFEB immunoprecipitates (Extended Data Fig. 6j,k). In proteomics studies, GFP–Stx17 was also found in protein complexes with a panel of 14–3–3 proteins (Extended Data Fig. 6l). Incidentally, mass spectrometry analyses also indicated interactions of 14–3–3 proteins with GFP–IRGM (Extended Data Fig. 6m; Supplementary Table 4). 14–3–3 proteins interact with TFEB and hold the phosphorylated TFEB in the cytoplasm, which may contribute to the effects of Stx17 on TFEB. Thus, Stx17 interacts with proteins that control TFEB localization and is required for the efficient nuclear translocation of TFEB.

Stx17 affects mTOR inhibition during starvation. As both IRGM and mAtg8s are required for efficient inhibition of mTOR in response to starvation, we also tested the role of Stx17. Inactivation of mTOR in response to starvation was reduced in Stx17KO HeLa cells, as evidenced by the persistent phosphorylation of S6K and ULK1 (Fig. 6i–k) and the presence of mTOR on lysosomes (Fig. 6l–n). GFP–Stx17 complemented these effects in Stx17KO cells (Extended Data Fig. 6p–o). Overexpression of GFP–Stx17 in 293T cells partially inhibited mTOR activity in full medium (Extended Data Fig. 6q–s), an effect that was suppressed by constitutively active Rag8KO (Extended Data Fig. 6q–s). Similar to IRGM and mAtg8s, Stx17 exerts effects on mTOR.

RagA and RagB are physiologically activated and loaded with GTP through the action of the cognate nucleotide exchange factor (GEF), which is a pantameric complex termed Ragulator and consists of LAMTOR1–5 (for example, LAMTOR1 (also known as p18), LAMTOR2 (also known as p14), and so on). The Ragulator–Rag interaction increases during amino acid starvation, an effect...
that is believed to reflect an increased affinity of GEFs (in this case, Ragulator) for inactive (GDP-bound) cognate GTPases\textsuperscript{46}, such as Rags\textsuperscript{38,45}. We used the Ragulator–Rag interaction as a readout\textsuperscript{45,47} of the activation state of RagA in cells lacking Stx17. Stx17\textsuperscript{KO} HeLa cells displayed lower RagA–p18 complexes than Stx17 WT HeLa cells (Extended Data Fig. 6t,u), which is consistent with an increased RagA activation state\textsuperscript{45,48}. Increased interactions of RagA with its effector Raptor were observed in Stx17\textsuperscript{KO} compared to WT HeLa cells (Extended Data Fig. 6v,w). Thus, Stx17 influences the state of the key Rag GTPase that activates mTOR.
Fig. 5 | mAtg8s control the transcriptional activity of TFEB. a, Principal component (PC) analysis from RNA-seq comparisons between parental HeLa WT and pan-mAtg8 mutant HeLa cells (Hexa⁰⁰; CRISPR-mediated pan-mAtg8 KO of LC3A, LC3B, LC3C, GABARAP, GABARAPL1 and GABARAPL2). RNA-seq was performed in triplicate. Cells were induced for autophagy in EBSS for 2 h. b, Number of upregulated (Up) and downregulated (Down) genes in HexaKO cells relative to HeLa WT cells. c, Volcano plot showing the effect of pan-mAtg8 KO on differential gene expression (log₂(fold change); ratio HexaKO/HeLa WT). Named genes are the previously identified TFEB target genes. Red circles indicate TFEB targets downregulated in HexaKO cells; green circles are TFEB targets upregulated in HexaKO cells. The dotted orange line indicates the significance cut-off ($P < 0.05$). d, Volcano plot showing the effect of GABA⁰⁰ on differential gene expression (log₂(fold change); ratio GABA⁰⁰/HeLa WT). Named genes are the previously identified TFEB target genes. Red circles indicate TFEB targets upregulated in GABA⁰⁰ cells; green circles are TFEB targets upregulated in GABA⁰⁰ cells. The dotted orange line indicate the significance cut-off ($P < 0.05$). For c and d, $P$ values were calculated using Fisher’s exact test adapted for overdispersed data; edgeR models read counts with NB distribution (see Methods for more). n = 3 biologically independent experiments.
IRGM affects TFEB nuclear translocation in pathological conditions. We tested the effects of IRGM on TFEB in a physiological setting of Mtb macrophage infection, which causes endomembrane damage that in turn affects TFEB nuclear translocation. TFEB was localized to the nucleus in infected macrophage-like THP-1 cells, and this was dependent on the ability of Mtb WT Erdman to disrupt the integrity of phagosomal membranes, since an ESX-1 mutant of Mtb Erdman, which is disabled for membrane permeabilization, had only 10% of cells with nuclear TFEB (Fig. 7a,b). The translocation of TFEB in response to Mtb Erdman was reduced following IRGM KO on mtOR activity (measured by phosphorylation of S6K (j) and ULK1 (l)) in response to starvation (EBSS, 2h). Data shown as the mean ± s.e.m. of normalized intensities (n = 3 biologically independent experiments), and ANOVA with tukey’s post-hoc test was performed. Confocal microscopy images (i) and HCM quantifications (m) of the effect of Stx17 on the colocalization between mtOR and LAMP2. Data shown as the mean ± s.e.m. (n = 3 biologically independent experiments), and ANOVA with Tukey’s post-hoc test was performed. Masks: white, algorithm-defined cell boundaries; yellow outline, computer-identified colocalization between mtOR and LAMP2. Scale bar, 10 μm. Uncropped blots for e, f and i and numerical source data for b, c, e, h, j, k and m are provided as source data.

Discussion
In this study, we uncovered the role of mAtg8s as regulators of the lysosomal system acting upstream of TFEB14,15. The underlying regulatory circuitry is based on mAtg8 interactors (IRGM, Stx17 and TFEB), whereby mAtg8s act as a unifying platform. IRGM, Stx17 and mAtg8s affect mTOR, a kinase phosphorylating TFEB15-20. The action of IRGM extends to its partner calcineurin PPP3CB, a phosphatase that promotes TFEB translocation to the nucleus7, where TFEB initiates the lysosomal transcriptional programme. This study also unveiled a hitherto unappreciated inhibitory effect of IRGM and its interactors14 on mTOR activity. The expression of a constitutively active RagB can override the effects of mAtg8s and their interactors on mTOR. This indicates that their effects channel through the Rag-based control of mTOR, thus expanding the circuitry associated with mTOR regulation beyond the canonical nutrition-based control. A physical link between mAtg8s and TFEB is amplified by three factors: IRGM, which interacts with mAtg8s14; Stx17, which is known to bind members of the mAtg8 family11 and calcineurin, which binds IRGM as shown here. IRGM activates calcineurin, as evidenced by the dephosphorylation of non-TFEB substrates (NFAT). These protein interactions underlie the mechanism (Extended Data Fig. 7i) of how mAtg8s, IRGM and Stx17 control TFEB in addition to the upstream effects on mTOR.

The global gene expression changes during starvation in cells lacking all mAtg8s include a number of mTOR-dependent genes10. This relationship fits the general biological principle of feedback control, whereby mAtg8s feed-forward to stimulate lysosomal expression, which is reminiscent of the positive feedback between MCOLN1 and TFEB42. Using RNA-seq, we found fewer autophagy targets than previously described for TFEB42; however, a quantitative RT–PCR study showed that mAtg8s affected the expression of SQSTM1, ULK1 and ATG9B6. Mammalian Atg8s affect transcription more broadly, beyond the known TFEB targets15, including the lysosome-associated genes ARSD, DOC2A, SOD1, DENND3, TSPAN1 and ATP1A3, and others not related to lysosomes, including TGBI, KNYU, ZNF595, HPSE2, CADM1, IGBP6, SAGE1, MUC16 and NLRP1. One of the mAtg8s has been described as a nuclear protein that shuttles between the nucleus and cytosol53; thus, mAtg8s in the nucleus may have active roles in gene expression.

Autophagy immune functions include the direct elimination of intracellular microbes and control of inflammation. IRGM is necessary for a full response of TFEB to Mtb infection in macrophages,

IRGM affects TFEB nuclear translocation in pathological conditions. We tested the effects of IRGM on TFEB in a physiological setting of Mtb macrophage infection, which causes endomembrane damage that in turn affects TFEB nuclear translocation. TFEB was localized to the nucleus in infected macrophage-like THP-1 cells, and this was dependent on the ability of Mtb WT Erdman to disrupt the integrity of phagosomal membranes, since an ESX-1 mutant of Mtb Erdman, which is disabled for membrane permeabilization, had only 10% of cells with nuclear TFEB (Fig. 7a,b). The translocation of TFEB in response to Mtb Erdman was reduced following IRGM KO on mtOR activity (measured by phosphorylation of S6K (j) and ULK1 (l)) in response to starvation (EBSS, 2h). Data shown as the mean ± s.e.m. of normalized intensities (n = 3 biologically independent experiments), and ANOVA with tukey’s post-hoc test was performed. Confocal microscopy images (i) and HCM quantifications (m) of the effect of Stx17 on the colocalization between mtOR and LAMP2. Data shown as the mean ± s.e.m. (n = 3 biologically independent experiments), and ANOVA with Tukey’s post-hoc test was performed. Masks: white, algorithm-defined cell boundaries; yellow outline, computer-identified colocalization between mtOR and LAMP2. Scale bar, 10 μm. Uncropped blots for e, f and i and numerical source data for b, c, e, h, j, k and m are provided as source data.

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Autophagy immune functions include the direct elimination of intracellular microbes and control of inflammation. IRGM is necessary for a full response of TFEB to Mtb infection in macrophages,
whereas the HIV protein Nef affects TFEB in an IRGM-dependent fashion. The effects of IRGM on mTOR and AMPK may extend to immunometabolism and associated innate and T cell responses.

In summary, mAtg8s control the key regulator of lysosomal biogenesis TFEB, whereas IRGM together with Stx17 and mAtg8s governs nearly all stages of the autolysosomal pathway. Hence, a subset of mAtg8s act indirectly to complete the autophagy pathway to exert their function on autophagosomal maturation by regulating TFEB. The molecular complexes formed by IRGM participate in cellular responses to infectious and physiological processes such as starvation. With many functions converging on IRGM, as shown here and elsewhere, it is not surprising that IRGM has emerged as a medically important locus. Thus, IRGM and its complexes, as well as the functions of mAtg8s uncovered here, should be considered as potential drug targets.

Online content
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Methods

Antibodies and reagents. The following antibodies and dilutions were used for western blot (WB), immunofluorescence (IF) and IP assays: rabbit anti-Stx17 polyclonal antibody (Sigma, HPA001204, lot number (no.) F131989; 1:1,000 for WB; mouse anti-FLAG monoclonal (Sigma, F1804, lot no. SLB W512; 1:2000 dilution at 0.5 μg ml⁻¹ and 1:1,000 for WB); rabbit anti-IRGM monoclonal antibody (Abcam, catalogue (cat.) no. ab69494, lot no. GR33164D-1; 1:500 for WB); rabbit anti-anti-GFP polyclonal antibody (Abcam, cat. no. ab299; lot no. GR32226D-4; 1:5 μg ml⁻¹ for IP and 1:4,000 for WB); mouse anti-actin monoclonal antibody (Abgent, AM18298, lot no. AM18298-RAD; 1:1,000); mouse anti-PP35CB monoclonal antibody (Abcam, cat. no. ab58161; lot no. GR196202-3; 1:500 for WB); mouse anti-pan-14-3-3 monoclonal antibody (Santa Cruz Biotechnology, sc-13322 (B11), lot no. K0812; 1:500 for WB); rabbit anti-TFE3 monoclonal antibody (Cell Signaling Technology (CST), 4240, lot 2; 1:200 for IF and 1:1,000 for WB); rabbit anti-phospho (Ser) 14-3-3 binding motif polyclonal antibody (CST, 9017; 1:1,000 for WB); goat anti-TFE3 polyclonal antibody (Thermo Fisher, cat. no. PA1-31552, lot no. RD191941; 1:200 for IF); rabbit anti-phospho TFE3 (Ser211) (E98SN) monoclonal antibody (CST, 37681, lot no. 1; 1:1,000 for WB; rabbit anti-phospho p70S6K (T389) (108D2) monoclonal antibody (CST, 9112, lot no. 2; 1:5000 for WB); rabbit anti-p70S6K (9G7D) monoclonal antibody (CST, 2708, lot no. 20; 1:10,000 for WB); rabbit anti-phospho ULK1 (Ser757) (D700U) monoclonal antibody (CST, 14202, lot 2; 1:1,000 for WB; phospho-ULK1 (Ser317) (D2686) monoclonal antibody (CST, 12753, lot no. 1; 1:1,000 WB); rabbit anti-ULK1 (D97D) monoclonal antibody (CST, 6439, lot no. 1; 1:1,000 for WB); rabbit anti-mTOR (7C10) monoclonal antibody (CST, 2858, lot no. 16; 1:200 for IF; rabbit anti-p85α (24C12) monoclonal antibody (CST, 2230; 1:750 for WB); rabbit anti-RagA (DRB5) monoclonal antibody (CST, 4357, lot no. 2; 1:750 for WB; rabbit anti-NFAT monoclonal antibody (human; DSHB of University of Iowa), H4B4; 1:250 for IF); IRGM short interfering RNA (siRNA) (Dharmacon, 34561); Dynabeads Protein G (Thermo Fisher Scientific, 888161003D; 50 μl for IP); anti-HA magnetic beads (Thermo Fisher Scientific, 888361003D; 150 μl for IP).

Cell culture. HEK293T, THP-1 and HeLa cells were obtained from the American Type Culture Collection (ATCC) and maintained in ATCC-recommended media. THP-1 cells were differentiated with 50 nM phorbol myristate acetate overnight for Cell culture. anti-HA magnetic beads (Thermo Fisher Scientific, 888361003D; 150 μl for IP); short interfering RNA (siRNA) (Dharmacon, 34561); western blotting for TFEB and other proteins (indicated in the figures, lysed in NP-40 buffer containing protease inhibitor cocktail and 1.5× SDS sample buffer). The radiolabeled proteins were detected using a PhosphorImager and a Fuji STORM 820 PhosphorImager (Bio-Rad).

IF confocal microscopy. For IF confocal microscopy, cells were plated onto coverslips in 12-well or 24-well plates. Cells were transfected with plasmids as indicated in the figures. The cells were incubated in full media or EBSS for 2 h and fixed in 4% PFA for 10 min followed by permeabilization with 0.1% saponin in 3% BSA. Cells were then blocked in 3% BSA and stained with primary antibodies followed by washings with PBS and then incubation with appropriate secondary antibodies for 1 h at room temperature. Coverslips were mounted using ProLong Gold Antifade mounting (Invitrogen) and analysed by confocal microscopy using a Zeiss LSM510 laser scanning microscope.

Plasmid, siRNA and miRNA transfections. IRGM constructs have been previously described44. The Stx17 construct was a gift from N. Mizushima. The MIIF and TFE3 constructs were from R. Poretz. The TFE3 constructs were provided by R. Puertollano. Plasmid constructs were verified by DNA sequencing. Plasmids were transfected using a Profection Mammalian Transfection system from Promega or Lipofectamine 2000 reagent from Thermo Fisher. All siRNAs were from Dharmacon. Cells were transfected with 10 pmol of siRNAs. For siRNA transfections, 10 pmol of siRNA was transfected in 100 μl of Nucleofector solution kit V (Amaxa), siRNAs were then added to the cell suspension and cells were nucleopored using an Amaxa Nucleofector apparatus with the program D-032. Cells were re-transfected with a second dose of siRNAs 24 h after the first transfection and assayed after 48 h. miR-196 (sequence: UAGUGUAGUUUCUGGUGU and mir-20 (sequence: UUAGUUUCUGUUCUGCUAG) were transfected using Lipotfectamine 2000 reagent. Cells were assayed 48 h after transfection.

Bacterial strains and procedures. Mtbc WT Erdman and its ESX-1 mutant were cultured as previously described14,49. For the TFE3 translocation experiments, differentiated THP-1 cells were transfected with miR-125 and its control WT cells were from R. Zoncu (UC Berkeley). HeLa cells stably expressing TMEM192–3KO and IrgmKO have been deposited in the MassIVE repository (https://massive.ucsd.edu). For co-IP, cells were transfected with plasmids as indicated in the figures, lysed in NP-40 buffer containing protease inhibitor cocktail and 1.5× SDS sample buffer. The radiolabeled proteins were detected using a PhosphorImager and a Fuji STORM 820 PhosphorImager (Bio-Rad).

HIV clones, viral production and cellular infection. The HIV molecular clones pNL4-3ΔEAv or control lentiviral vector were transfected in 293T cells together with vesicular stomatitis virus-G envelope61. After 24 h of transfection, supernatant was collected, filtered and normalized for viral budding by ELISA (ZeoProTix). Virus was titrated using TZM-bl cells and the X-Gal staining method62. HeLa cells were infected with a virus titre at a multiplicity of infection of 1 (ref. 11).
homogenized with 20 strokes of a homogenizer. The homogenate was then centrifuged at 1,000 g for 2 min at 4 °C. A total of 50 μl of samples was saved as input. The rest of the supernatant was incubated with 150 μl of anti-HA magnetic beads on a gentle rotator shaker for 10 min. Immunoprecipitates were then washed three times and eluted in SDS loading buffer. Western blotting for proteins indicated in the figures was done as described above.

**Quantitative RT–PCR.** HeLa WT or HexaA2, Stx17KO or the cells transfected with scrambled or IRGM siRNA were incubated in EBSS for 2 h. Cells were collected, and RNA was isolated using TRIzol reagent. Complementary DNA was generated using a high-capacity cDNA reverse transcriptase kit with RNase inhibitor and random hexamer primers (Applied Biosystems) on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). Quantitative real-time PCR was performed using a StepOne Plus instrument (Applied Biosystems) relative to the housekeeping gene 18S. For ULK1 (cat. no. Hs00177504_m1; 4331182; Thermo Fisher), ATG9B (cat. no. Hs01123449_g1; 4331182; Thermo Fisher) and p62 (cat. no. Hs02621445_s1; 4331182; Thermo Fisher) were used. Gene expression was quantified using QuantStudio Software (Applied Biosystems) relative to the housekeeping gene 18S.

**RNA-seq.** GABA1KO, HexaA2 and ATG3KO along with their parental WT HeLa cells were incubated with EBSS for 2 h. Total RNA was extracted using TRIzol reagent (Invitrogen) following the manufacturer’s protocol. Total RNA quantity and purity were assessed using a Bioanalyzer 2100 and a RNA 6000 Nano LabChip reagent (Agilent), with RNA integrity values of >2.0. Poly(A) RNA was purified from total RNA (5μg) using poly-T oligo-attached magnetic beads using two rounds of purification. Following purification, the mRNA was fragmented into small pieces using divalent cations at elevated temperature. Then the cleaved RNA fragments were reverse-transcribed to create the final cDNA library in accordance with the protocol for the TruSeq RNA Sample Preparation v2 (cat. no. RS-122-2001, RS-122-2002) (Illumina); the average insert size for the paired-end libraries was 300bp (±50bp). The paired-end sequencing was carried out on an Illumina NovaSeqTM 6000 at LC Sciences following the manufacturer’s recommended protocol. Using the Illumina paired-end RNA-seq approach, the transcriptome was sequenced, generating a total of 2×150 million bp paired-end reads. This yielded gigabases (Gb) of sequence. Before assembly, the low-quality reads (defined as (1) reads containing sequencing adaptors, (2) reads containing sequencing primers; and (3) nucleotide with ≤q quality score lower than 20) were removed. Sequencing reads were aligned to the reference genome using HISAT2 package. HISAT allows multiple alignments per read (up to 20 by default) and a maximum of two mismatches when mapping the reads to the reference. HISAT builds a database of potential splice junctions and confirms these by comparing the previously unmapped reads against the database of putative junctions. The mapped reads of each sample were assembled using StringTie. All transcripts from samples were merged to reconstruct a comprehensive transcriptome using perl scripts (LC Sciences). After the final transcriptome was generated, StringTie and edgeR were used to estimate the expression levels of all transcripts. StringTie was used to calculate the expression level for mRNAs via the FPKM (fragments per kilobase million) values. Differential gene expression was analysed using the R package edgeR, which takes into account dispersions (that is, variations) between biological replicates. P values were calculated using Fisher’s exact test adapted for overdispersed data; edgeR models read counts with negative binomial (NB) distribution. The differentially expressed mRNAs and genes were selected by R package with log2(fold change) values of ≥1 or log(fold change) values of ≤−1 and with statistical significance of P<0.05.

**Flow cytometry to analyse intracellular calcium.** Intracellular calcium was analysed using FLUO-3 AM fluorescence on the FL-1 channel of a flow cytometer (BD FACScan). WT HeLa or HexaA2 cells were left unstimulated and incubated in HBSS for 2 h. Cells were incubated with 5μM of FLUO-3 AM for 30 min, followed by analysis on the flow cytometer. The authors declare no competing interests.

**Competing interests**
Extended Data Fig. 1 | IRGM affects nuclear translocation of TFEB. 
a, confocal microscopy analysis of effects of IRGM KD on TFEB nuclear translocation in response to 2 h starvation. Scale bar 5 μm, (n = 3 biologically independent experiments).

b,c, HCM images and quantification to test the effect of IRGM KD on nuclear translocation of TFEB. Cells were permeabilized with Triton. Data, means ± SEM (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test; high content microscopy, >500 cells counted per well; minimum number of valid wells 9. Masks; white: algorithm-defined cell boundaries; yellow outline: computer-identified colocalization between TFEB and Hoechst-33342 nuclear stain). Scale bar 10 μm.

d,e, HCM images and quantifications to test the effect of IRGM KD on nuclear translocation of TFEB in cells treated with DMSO or pp242. Data, means ± SEM (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test; high content microscopy, >500 cells counted per well; minimum number of valid wells. Masks; white: algorithm-defined cell boundaries; yellow outline: computer-identified colocalization between TFEB and Hoechst-33342 nuclear stain). Scale bar 10 μm. Numerical source data for panels b and d are provided in Source Data Extended Data Fig. 1.
Extended Data Fig. 2 | Interactions and localization analyses of IRGM with MiT/TFE family of transcriptional regulators. **a.** Confocal microscopy analysis of co-localization of GFP-IRGM and endogenous TFEB. Scale bar 5 μm, (n = 3 biologically independent experiments). **b.** A screenshot from NCBI showing domain of unknown function (DUF3371) in TFEB. c,d. HCM images and quantifications to analyze the effect of complementation of IRGM KD with GFP-IRGM Wt or GFP-IRGM S47N on nuclear translocation of TFEB. Data, means ± SEM (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test; HCM, >500 cells counted per well; minimum number of valid wells 9, 3 independent experiments. Masks; white: algorithm-defined cell boundaries and computer-identified GFP positive cells; blue outline: computer-identified nuclear stain; yellow outline: computer-identified colocalization between TFEB and Hoechst-33342 nuclear stain). Scale bar 10 μm. The masks in gray scale panels are cloned from the merged images. Inset: western blot showing GFP-IRGM expression IRGM KD cells. e,f, Co-IP analysis of interactions between GFP-MiT (H isoform) and FLAG-IRGM in 293t cells, (n = 3 biologically independent experiments). f, Co-IP analysis of interactions between GFP-TFE3 and FLAG-IRGM in 293t cells. Data, means ± SEM of normalized intensities (n = 3 biologically independent experiments) paired t-test. Uncropped blots for panels e, f and g and numerical source data for panels c and h are provided in Source Data Extended Data Fig. 2.
Extended Data Fig. 3 | IRGM effects on mTOR and calcineurin and mAtg8s interactions with and effects on TFEB. a, b, Western blot analysis and quantifications of the effects of IRGM KD on pTFEB (S211) levels in cells treated with pp242. Data, means ± SEM of normalized intensities (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. c–e, western blots analysis of the effects of IRGM on mTOR substrates pS6K and pULK1. Data, means ± SEM of normalized intensities (n = 3 biologically independent experiments) paired t-test. f, HCM image analysis of co-localization between mTOR and LAMP2. Scale bar 10 µm. g, HCM analysis of the effects of IRGM KD on LAMP2 puncta. Data, means ± SEM; (n = 3 biologically independent experiments) paired t-test. Scale bar 10 µm. h, HCM analysis of the effect of IRGM expression on nuclei translocation of TFEB, (n = 3 biologically independent experiments). Scale bar 10 µm. i, j, confocal microscopy analysis of co-localization between GFP-IRGM and endogenous PPP3CB in HeLa cells (n = 3 biologically independent experiments). Scale bar 5 µm. k, l, HCM analysis of the effect of starvation on colocalization between GFP-IRGM and PPP3CB. Data, means ± SEM (n = 3 biologically independent experiments) paired t-test. Scale bar 10 µm. m, western blot showing PPP3CB KD in HeLa cells (n = 3 biologically independent experiments). n, schematics of LysoIP technique. o, LysoIP to detect indicated proteins on lysosomes (n = 3 biologically independent experiments). p, western blot analysis of the effects of IRGM expression on NFAT mobility shift (n = 3 biologically independent experiments). q, Co-IP analysis of GFP-LC3B and GFP-GABARAP with FLAG-TFEB in 293 T cells. r, GST pull-down analysis of TFEB with WT or LDS mutant of GABARAP. s, HCM images in WT or indicated KO cells in full medium (n = 3 biologically independent experiments). Scale bar 10 µm. Uncropped blots for panels a, c, o, p, q and r and numerical source data for panels b, d, e, g and l are provided in Source Data Extended Data Fig. 3.
Extended Data Fig. 4 | GABARAP and GABARAPL1 but not GABARAPL2 control nuclear translocation of TFEB. a,b, HCM images and quantifications to test the role of mAtg8s on nuclear translocation of GFP-MiTF in response to autophagy induction (EBSS 2 h). Data, means ±SEM (n = 3 biologically independent experiments) paired t-test. Masks; white: algorithm-defined cell boundaries; blue outline: computer-identified nuclear stain; yellow outline: computer-identified colocalization between TFEB and Hoechst-33342 nuclear stain). Scale bar 10 µm. The masks in gray scale panels are cloned from the merged images, (n = 3 biologically independent experiments). c, HCM image analysis of effects of complementation of HexaKO with GFP-GABARAP on nuclear translocation of TFEB. Scale bar 10 µm. d-g, HCM analysis of the effect of complementation of HexaKO cells with GABARAPL1 or GABARAPL2 on nuclear translocation of TFEB. Data, means ±SEM, ANOVA, Tukey’s post hoc test; HCM, >500 cells counted per well; minimum number of valid wells 9, (n = 3 biologically independent experiments). Scale bar 10 µm. h, HCM analysis of effect of expression of GABARAP in 293T cells expressing RagBQ99L or parental 293T cells on nuclear translocation of TFEB. Masks in c, e, g, h; white: algorithm-defined cell boundaries in GFP positive cells; blue outline: computer-identified nuclear stain; yellow outline: computer-identified co-localization between TFEB and Hoechst-33342 nuclear stain), (n = 3 biologically independent experiments). Scale bar 10 µm. Numerical source data for panels a, d, and f are provided in Source Data Extended Data Fig. 4.
Extended Data Fig. 5 | mAtg8s affect global gene expression. a, Volcano plot (RNAseq) showing the effect of pan-mAtg8 knockout on differential gene expression (log2 fold change; ratio HeLa HeLaKO/HeLaWt). Red points: down-regulated genes in HexaKO cells. Green points: up-regulated in HexaKO cells. A subset of genes not identified as TFEB targets are named. Dotted orange line, significance cuttof (p value < 0.05). P values were calculated using Fisher’s exact test adapted for over-dispersed data; edgeR models read counts with negative binomial (NB) distribution (see Methods). (n = 3 biologically independent experiments). b, Heat map representation of genes upregulated or downregulated in HeLaKO vs. HeLaWt cells. c, A volcano plot showing RNAseq analysis of HeLaKO vs. ATG3KO cells. P values were calculated using Fisher’s exact test using R package. Named genes are previously identified TFEB targets those were also down-regulated in HexaKO shown in Fig. 5c. (n = 3 biologically independent experiments). d, A volcano plot (RNAseq) listing upregulated and downregulated autophagy-related genes in HeLaKO vs. HeLaWt cells. P values were calculated using Fisher’s exact test adapted for over-dispersed data; edgeR models read counts with negative binomial (NB) distribution (see Methods). (n = 3 biologically independent experiments). e, qRT-PCR analysis of p62, ATG9B and ULK1 in HeLaKO vs. HexaKO cells induced for autophagy in EBSS for 2h; 18 S was used as an internal control, Data, means ± SEM (n = 3 biologically independent experiments). Numerical source data for panel e are provided in Source Data Extended Data Fig. 5.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | mAtg8s affect calcium fluxes and Stx17 affects mTOR and TFEB. 

a, A volcano plot showing expression of calcium effectors in HexaKO cells. P values were calculated using Fisher’s exact test adapted for over-dispersed data (see Methods) (n = 3 biologically independent experiments). 

b,c Flow cytometry using FLUO-3AM to detect intracellular calcium in HeLaWT or HexaKO: Data, means ± SEM; (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. 

d, Confocal microscopy analysis of the effects of Stx17KO on TFEB localization, (n = 3 biologically independent experiments). Scale bar 5 µm. 

e-g, confocal microscopy (e) and HCM (f,g) analyses of the effects of Stx17KO on colocalization between TFEB and LAMP2. Scale bar 5 µm (e). Scale bar 10 µm (f). Data, means ± SEM; (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. 

h, HCM analysis of the effects of Stx17KO on TFEB puncta. Data, means ± SEM; (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. 

i, Co-IP analysis of interactions between GFP-Stx17 and FLAG-TFEB in 293 T cells (n = 3 biologically independent experiments). 

j,k, Co-IP analysis of effects of GFP-Stx17 on FLAG-TFEB and IRGM complexes. Data, means ± SEM (n = 3 biologically independent experiments) paired t-test. 

l,m, MS analysis showing 14-3-3 peptides those interacted with GFP or GFP-Stx17 and GFP-IRGM (n = 3 biologically independent experiments). 

n-p, Western blot analysis and quantification of the effect of GFP-Stx17 in HeLaWT (full media) or in Stx17KO cells (EBSS 2 h) on mTOR activity. Data, means ± SEM; (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. 

q-s, Western blot analysis and quantification of pULK1 and pS6K to test the effects of GFP-Stx17 expression in WT 293 T cells and cells expressing RagBQ99G. Data, means ± SEM; (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. 

t-w, Co-IP analysis of interactions between RagA and FLAG-p18 (t,u) and Raptor and FLAG-RagA (v-w) in Stx17KO or parental HeLa cells. Data, means ± SEM of normalized intensities (n = 3 biologically independent experiments) paired t-test. Uncropped blots for panels b, f, h, k, p, o, r, s, u and w are provided in Source Data Extended Data Fig. 6.


Extended Data Fig. 7 | mIR196B affects protective CD variant of IRGM in its role in nuclear translocation of TFEB. 

a,b, HCM analysis of the effects of mIR196B (shown to downregulate CD protective IRGM variant) and mIR20 (control) transfection on TFEB nuclear localization in 293T cells (c.313 C). HCM (n = 3 biologically independent experiments); >500 primary objects examined per well; minimum number of wells, 9). Masks; white: algorithm-defined cell boundaries; blue: computer-identified nucleus; yellow outline: computer-identified colocalization between TFEB and Hoechst-33342 nuclear stain). Images, a detail from a large database of machine-collected and computer-processed images. Data, means ± SEM; (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. Scale bar 10 µm. 

c, HCM image analysis of the effects of IRGM KD on AIEC LF82 influenced nuclear translocation of TFEB. K12 was used as control, (n = 3 biologically independent experiments). Scale bar 10 µm. 

d,e, HC microscopy and quantifications to analyze the effect of HIV infection on TFEB localization in HeLa cells transfected with scramble siRNA or IRGM siRNA. HC microscopy (n = 3 biologically independent experiments; >500 primary objects examined per well; minimum number of wells, 12). Masks; white: algorithm-defined cell boundaries; blue: computer-identified nucleus; yellow outline: computer-identified colocalization between TFEB and Hoechst-33342 nuclear stain). Images, a detail from a large database of machine-collected and computer-processed images. Data, means ± SEM; (n = 3 biologically independent experiments) ANOVA, Tukey’s post hoc test. Scale bar 10 µm. 

f, the model summarizes the effects of IRGM, Stx17 and mAtg8s/GABARAPs on mTOR inhibition and calcineurin (CN) activation promoting nuclear translocation of TFEB. L, lysosome. Numerical source data for panels a and d are provided in Source Data Extended Data Fig. 7.
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  Give P values as exact values whenever possible.
- □ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- □ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- □ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Images were collected by LSM5 (Zeiss), Cellomics iDEV (Thermo Fisher), QuantStudio Software (Applied Biosystems), Calibur (BD BioSciences), Q Exactive (Thermo Fisher).

Data analysis

- Fj (Image J 2.0) was used for quantifying western blot bands
- Prism 5 (GraphPad) was used for statistical analysis
- AIM software (LSM 5 Carl Zeiss Version 4.2) was used for confocal microscopy.
- IDEV version 6.6, Thermo Fisher software was used for high content microscopy analysis.
- Image Studio lite version 5.2 Software (LI-COR) was used for analyzing Western blots.
- Adobe Illustrator CC 2015 was used for images composing and exhibition.
- Adobe Photoshop CC 2015 was used for preliminary figure preparation.
- Scaffold software (Proteome Software Inc, version 2.1.1) was used to analyze proteomics data
- RNA-seq informatics was performed by the company (LC Sciences, Houston) carrying out RNA-seq analysis on our samples using industry standards

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Policy information about availability of data. All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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- A list of figures that have associated raw data
- A description of any restrictions on data availability

We have submitted RNA-seq data to GEO with accession number: GSE149533; Mass spectrometry proteomics data for IRGM interactors has been deposited to MassIVE repository (https://massive.ucsd.edu) with the accession number MSV000085401; Mass spectrometry proteomics data for Stx1/7 interactors has been published Kumor et al., Dev Cell. 2019 Apr 8;49(1):130-144.e6 and deposited to MassIVE repository (https://massive.ucsd.edu) with the accession number MSV000083251.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size For High Content Microscopy, a professional statistician (letter can be provided) carried out power analysis based on published data and determined based on medium effect size the number of repeats. No statistical method was used to determine the correct sample size for Western blot quantifications. For all the experiments, we followed the routine practice in the similar studying fields.

Data exclusions No data were excluded from the analysis.

Replication All experimental replicates were performed independent of each other and all replication attempts were successful.

Randomization Microscopic images were acquired randomly and for high content microscopy were collected and analyzed in operator-independent machine/computer-driven acquisition and data analysis mode.

Blinding Because all the data were conducted based on random sampling and the high content microscopy data were acquired and analyzed in an operator-independent mode no blinding was needed.

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Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies           |
| ☑   | Eukaryotic cell lines|
| ☑   | Paleontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data        |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging|

Antibodies

The following antibodies and dilutions were used: Rabbit anti Stx1/7 polyclonal antibody (Sigma; #HPA001204; lot # F11598; 1:1000 [WB]); mouse anti FLAG monoclonal (Sigma; #F1804, lot #S1B W5142; used at 0.5 μg/ml and 1:1000 for [WB]); Rabbit anti IRGM polyclonal antibody (Abcam; cat. # ab66949; lot no. GR3316406-1; 1:500 for western blots [WB]); rabbit anti GFP polyclonal antibody (Abcam; cat. no. ab2390; lot no. GR3222604-1; 0.5 μg/ml IP and 1:4,000 [WB]), mouse anti-actin monoclonal antibody (Abgent; #AM1829B, lot #SG100806A; used at 1:4,000); mouse anti PPP3CB polyclonal antibody (Abcam, cat. #ab58161; lot # GR19620-3; 1:500 [WB]); mouse anti pan 14-3-3 monoclonal antibody (Santa Cruz Biotechnology, Inc. #sc-133232 [B11], lot #K0812 1:500 [WB]); rabbit anti TFEB polyclonal antibody (Cell Signaling CST #4240, lot # 2; 1:200 (IF); 1:1000 [WB]); rabbit anti phospho (Ser) 14-3-3 binding motif polyclonal antibody (Cell Signaling CST #9601; 1:1000 [WB]); goat
Validation

Antibodies were validated by western blot.
Sxo1/7 antibody was validated by CRISPR Cas9 knock out in this study and in https://www.sigmaaldrich.com/catalog/product/sigma/ha001204?lang=en&region=US
Flag was validated by western blot of IF after transfection with Flag tagged plasmids and https://www.sigmaaldrich.com/catalog/product/sigma/18047?lang=en&region=US
IRGM was validated using siRNA in this study and https://www.abcam.com/irgm-antibody-ab669494.html
GFP was validated using western blot and and immunofluorescence after transfection GFP-tagged plasmids in this study and https://www.abcam.com/gfp-antibody-chip-grade-ab250.html
Actin was validated by manufacturer: https://www.abcam.com/beta-actin-antibody-mabs-cam-8226-loading-control-ab8226.html
PPP93CR1 was validated by manufacturer: file://C:/users/Suresh/downloads/datasheet_36573.pdf
pan 14-3-3 was validated by manufacturer: https://www.scbt.com/scbt/product/pan-14-3-3-antibody-b-11
TFE3, validated by western blot and IF; changed cellular distribution (translocation from cytosol to nucleus) after induction of autophagy by starvation and https://www.cellsignal.com/products/primary-antibodies/tfeb-antibody/4240
TFEB pS211, validated by western blots, the expression is decreased in starved media and https://www.cellsignal.com/products/primary-antibodies/phospho-tfeb-ser211-e5s68-rabbit-mab/37681
TFEB for BMM staining was validated by IF, cellular localization changed from cytosolic to nuclear in starved cells and https://www.thermoscientific.com/antibody/product/TFEB-Antibody-Polyclonal/PA01-31552
phospho (Ser) 14-3-3 was validated by manufacturer: https://www.cellsignal.com/products/primary-antibodies/phospho-ser14-3-3-binding-motif-antibody/36017?site-search-type=Products
p70S6K (T389), validated by western blots; Starvation decreased the levels and https://www.cellsignal.com/products/primary-antibodies/phospho-p70-s6-kinase-thr389-108d2-rabbit-mab/9234
p70S6K, validated by manufacturer: https://www.cellsignal.com/products/primary-antibodies/p70-s6-kinase-49d7-rabbit-mab/2708
pULK1 [5317], validated by western blot and https://www.cellsignal.com/product/productDetail.jsp?productid=12753
pULK1 [5757], validated by western blots, incubation in EBSS for 2h decreased the levels and https://www.cellsignal.com/products/primary-antibodies/phospho-ULK1-1757-d764-rabbit-mab/14202
ULK1, validated by manufacturer: https://www.cellsignal.com/products/primary-antibodies/ULK1-d867-rabbit-mab/6439
mTOR, validated by IF, lysosomal localization decreased in 2h starvation and https://www.cellsignal.com/products/primary-antibodies/mtor-7c10-rabbit-mab/29837?site-search-type=Products
RagA, validated by manufacturer: https://www.cellsignal.com/products/primary-antibodies/RagA-d85-rabbit-mab/4357
Raptor, validated by manufacturer: https://www.cellsignal.com/products/primary-antibodies/raptor-24c12-rabbit-mab/2280?site-search-type=Products
LAMP2, validated by manufacturer: https://dshb.biology.uiowa.edu/core/media/media.nl?id=15239553&c=57157&h=2004571d1beced804488_xt=.pdf
NFA1, validated by manufacturer: https://www.cellsignal.com/products/primary-antibodies/nfat1-antibody/4389

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
- HeLa (ATCC) CCL-2
- HEK-293T (ATCC) CR-CL-11268
- THP-1 (ATCC) TIB-202
HeLa cells knocked out for LC3s (LC3A, LC3B and LC3C), GABARAPs (GABARAP, GABARAPL1 and GABARPL2), six mAtg8s (LC3A, LC3B, LC3C, GABARAP, GABARAPL1 and GABARPL2) and parental HeLa cells have been previously described (Nguyen et al.; J. Cell. Biol. 215, 857–874 (2016))
HeLa cells knocked out for Sxo17 (parental cells; HeLa (ATCC) CCL-2)
- HeLa cells knocked out for ATG5 (parental cells; HeLa (ATCC) CCL-2)
- 293T stably expressing RagBQ291L and control wild type cells were from Roberto Zoncu (UC Berkeley).
- HeLa cells stably expressing TMEM192-2xFLAG or TMEM192-3xHA are described previously (Jia et al., Dev Cell. 2020 Jan 6,52(1):69-87.e8)
- T2Z-bi Cells (NIH AIDS Reagent Program) 8129

Authentication
Cell lines were not authenticated but parental lines were from ATCC.

Mycoplasma contamination
The cell lines were not tested for mycoplasma. However, our facility has no mycoplasma contamination which is periodically monitored.
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals | Mus musculus: C57BL/6 Mouse; irgm1KO (25 week older female) and wild type litter mate (22 week old male)
Wild animals | Study did not involve any wild animal
Field-collected samples | Study did not collect samples from field
Ethics oversight | Protocols approved by Institutional Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation | Wild type HeLa or HeLa-KO cells were left unstimulated or incubated in HBSS for 2h. Cells were incubated with 5μM of FLUO-3AM for 30 minutes, followed by analysis on flow cytometer.
Instrument | BD FACSscan
Software | FACSscan
Cell population abundance | 2 million cells were used for each sample
Gating strategy | No gating was done as a single channel was used to analyze FLUO 3AM florescence.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.