MTMR4, a phosphoinositide-specific 3'-phosphatase, regulates TFEB activity and the endocytic and autophagic pathways.

List of Supplementary Data for

MTMR4, a phosphoinositide–specific 3'-phosphatase, regulates endocytic and autophagic pathways through mechanisms involving TFEB regulation

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Figure S5. MTMR4 knockdown causes accumulation of TFEB in PI(3)P-enriched vesicles.

Movie S1. Live-cell time-lapse imaging of mRFP-2xFYVE–expressing A549 cells. A549 cells co-transfected with the mRFP-2xFYVE expression vector and control siRNA (left) or MTMR4–specific siRNA (right) were monitored with confocal microscopy for 5 min (10-sec intervals) in normal growth medium. Note that the motility, fusion and fission of PI(3)P-enriched vesicles in MTMR4-depleted cells were impaired compared with those of control cells.

Movie S2. Live-cell time lapse imaging of mCherry-2xML1N ($PI(3,5)P_2$–expressing A549 cells. A549 cells co-transfected with the mCherry-2xML1N expression vector and control siRNA (left) or MTMR4–specific siRNA (right) were monitored with confocal microscopy for 5 min (10-sec intervals) in normal growth medium.

Movie S3. Live-cell time lapse imaging of GFP-TFEB–expressing control and MTMR4–depleted A549 cells. ***Upper)*** A549 cells were co-transfected with the GFP-TFEB expression vector and either control siRNA (left-upper cell) or MTMR4-siRNA (right-upper cell) and monitored with a confocal microscope for 5 min (5-sec intervals) in normal growth medium. Note that GFP-TFEB in the control cell was distributed largely in the cytoplasm in a diffuse homogenous pattern whereas GFP-TFEB in the MTMR4–depleted cell was distributed in the cytoplasm in both diffuse homogenous and coarse punctate patterns. ***Lower)*** A549 cells were co-transfected with the GFP-TFEB expression vector and either control siRNA (left-lower cell) or MTMR4-siRNA (right-lower cell) and monitored with a confocal microscope for 60 min (10-sec intervals) in normal growth medium. The mTOR inhibitor Torin 1 (1 μM) was added at 1 min. Note that Torin1 addition resulted in rapid nuclear translocation of GFP-TFEB in control cells whereas Torin1–induced nuclear TFEB translocation was marginal and delayed in MTMR4–depleted cells.

Table S1 qPCR primers used for determinations of human mRNA expression.

Gene	Forward (5' > 3')	Reverse (5' > 3')	Protein
MTMR4	GTGAATTCTGGTTGGCCAAACGAAG	CCTGATCAGCAACTCTATGACCCAG	Myotubularin-related protein 4 (MTMR4)
ATP6V0E1	CATTGTGATGAGCGTGTTCTGG	AACTCCCCGGTTAGGACCCTTA	ATPase, H+ transporting, V0 subunit E1
ATP6V1H	GGAAGTGTCAGATGATCCCCA	CCGTTTGCCTCGTGGATAAT	ATPase, H+ transporting, V1 Subunit H
MCOLN1	GAGTGGGTGCGACAAGTTTC	TGTTCTCTTCCCGGAATGTC	Mucolipin-1 (TRPML1)
CTSB	AGTGGAGAATGGCACACCCTA	AAGAGCCATTGTCACCCCA	Cathepsin B
CTSD	CTTCGACAACCTGATGCAGC	TACTTGGAGTCTGTGCCACC	Cathepsin D
TPP1	GATCCCAGCTCTCCTCAATAC	GCCATTTTTGCACCGTGTG	Tripeptidyl Peptidase 1
DPP7	GATTCGGAGGAACCTGAGTG	CGGAAGCAGGATCTTCTGG	Dipeptidyl Peptidase 7
LAMP1	ACGTTACAGCGTCCAGCTCAT	TCTTTGGAGCTCGCATTGG	Lysosomal-associated membrane protein 1
PPARGC1A	CATGCAAATCACAATCACAGG	TTGTGGCTTTTGCTGTTGAC	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)
ACTAB	TCTACAATGAGCTGCGTGTG	ATGGCTGGGGTGTTGAAG	beta-actin

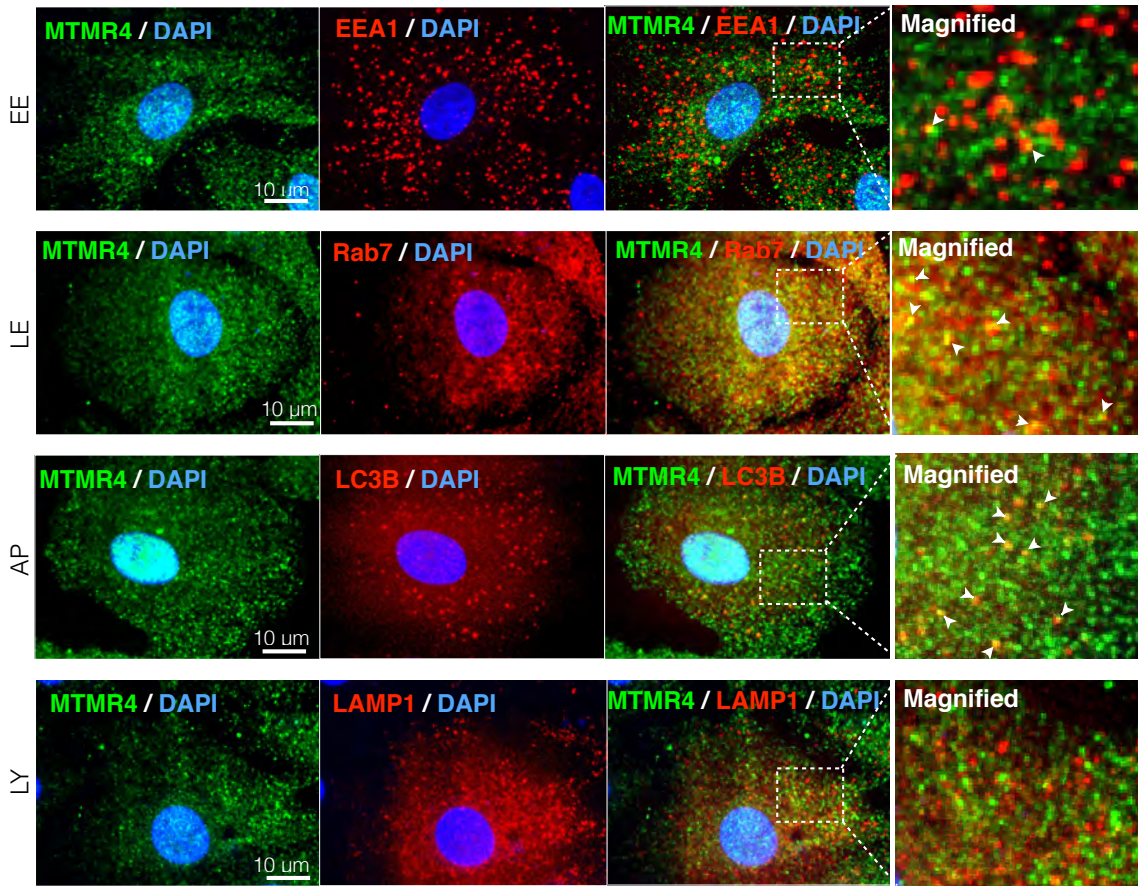

Figure S1. Localization of endogenous MTMR4 in late endosomes and autophagosomes. Double immunofluorescence staining using of endogenous MTMR4 and the organelle markers, EEA1 (early endosome, EE), Rab7 (late endosome, LE), LC3B (autophagosome, AP) and LAMP1 (lysosome, LY) in A549 cells. The white arrowheads in high-magnification views in insets indicate colocalization of MTMR4 and organelle markers. Nuclei are stained with DAPI (blue) staining. Scale bar, 10 μm.

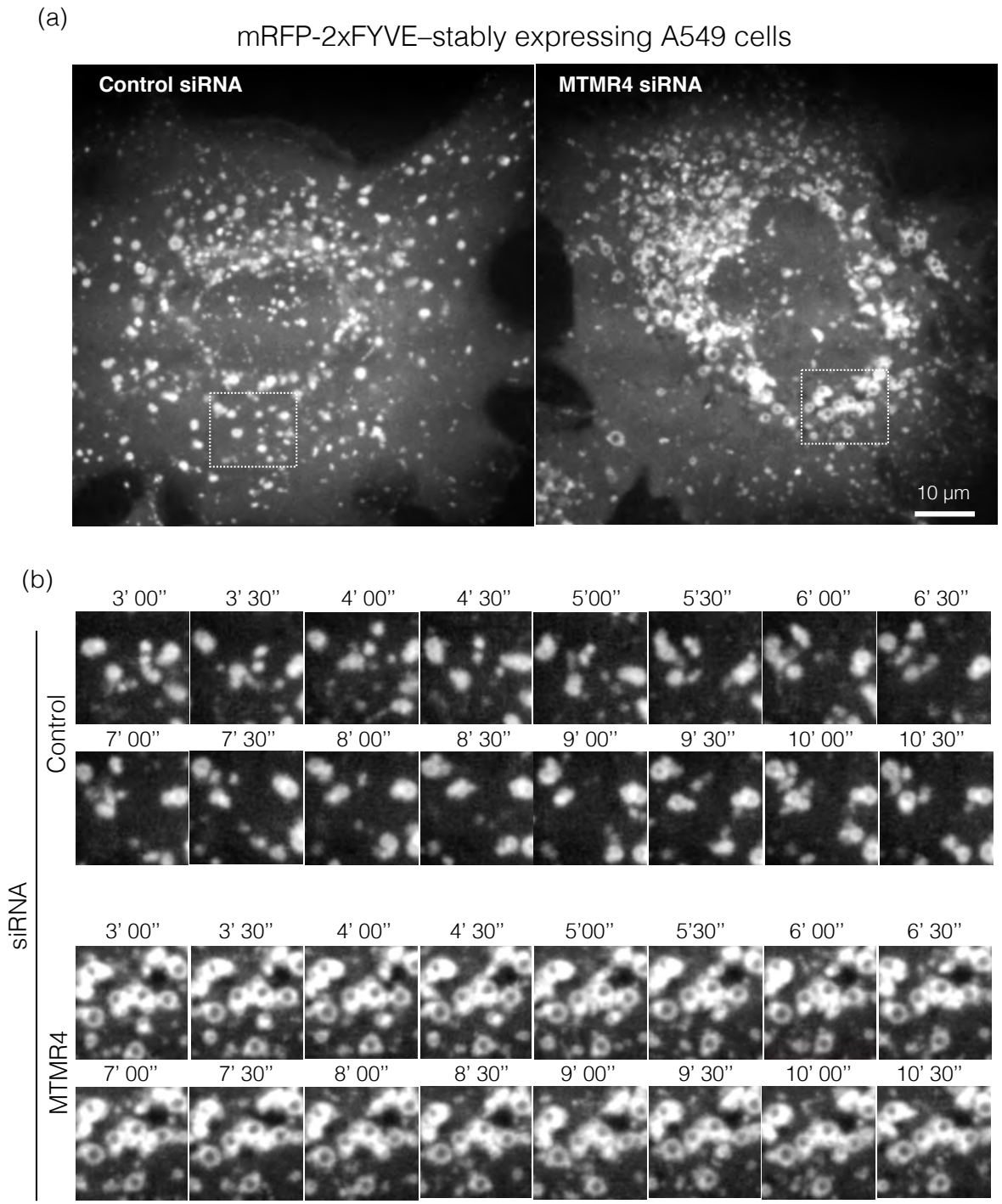

Figure S2. Live-cell time lapse imaging of A549 cells expressing mRFP-2xFYVE.
(a) Representative images of control (left panel) and MTMR4–depleted (right panel) A549 cells stably expressing mRFP-2xFYVE. (b) Insets of (a) shown as a montage depicting a time series of motility, fusion and fission of PI(3)P–enriched vesicles in MTMR4-depleted cells (lower panels) and control cells (upper panels).

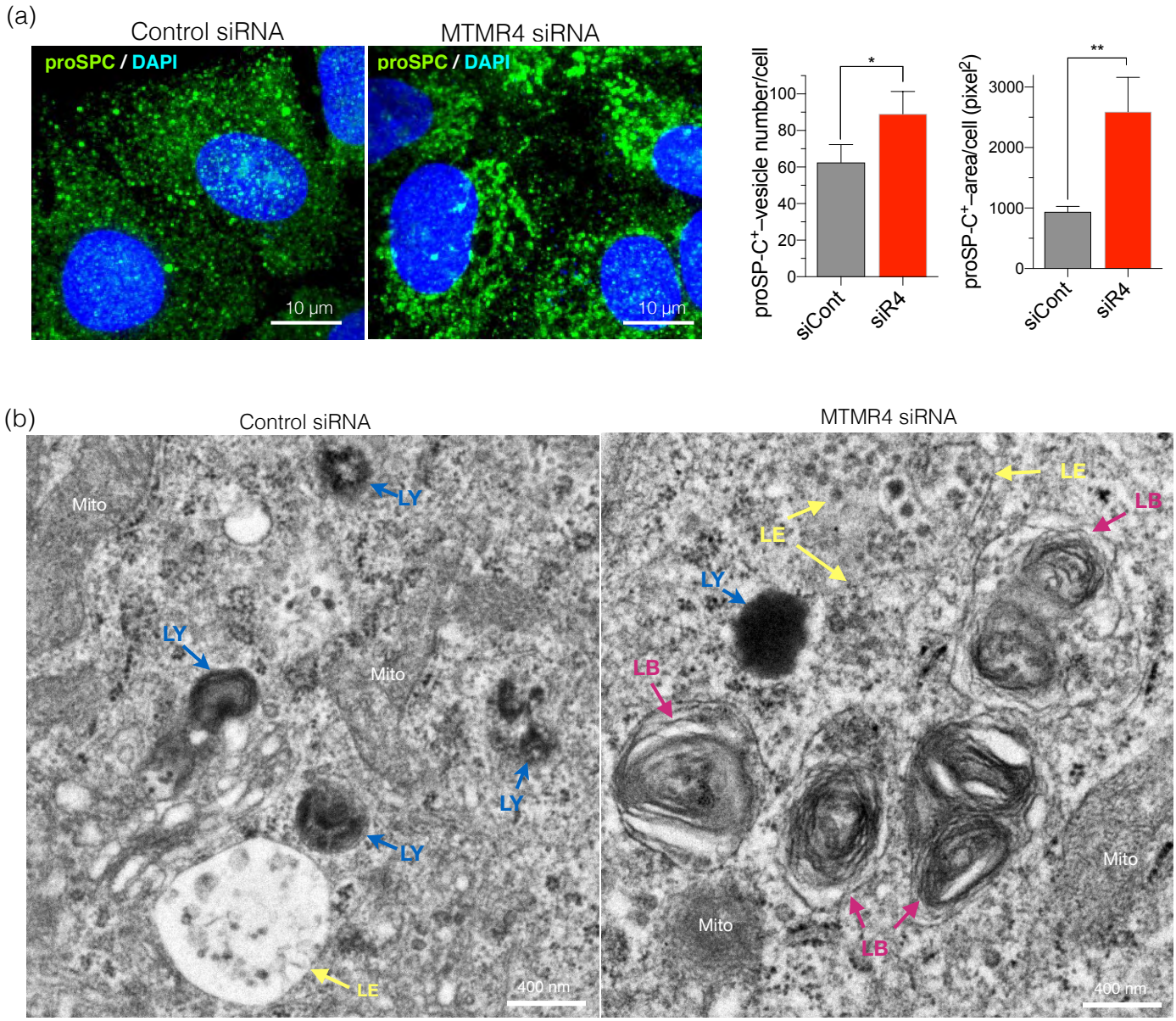

Figure S3. Lamellar bodies in control and MTMR4–depleted A549 cells.
(a) Immunofluorescence staining of the lamellar body (LB) marker proSPC in control (left panel) and MTMR4–depleted (right panel) A549 cells. Quantified data of proSPC–positive vesicle number and proSPC–positive area are shown in the right panels. Scale bar, 10 μm. (b) Transmission electron microscopic images of control (left panel) and MTMR4–depleted (right panel) A549 cells placed in normal growth medium. Note that MTMR4 depletion increased LBs.

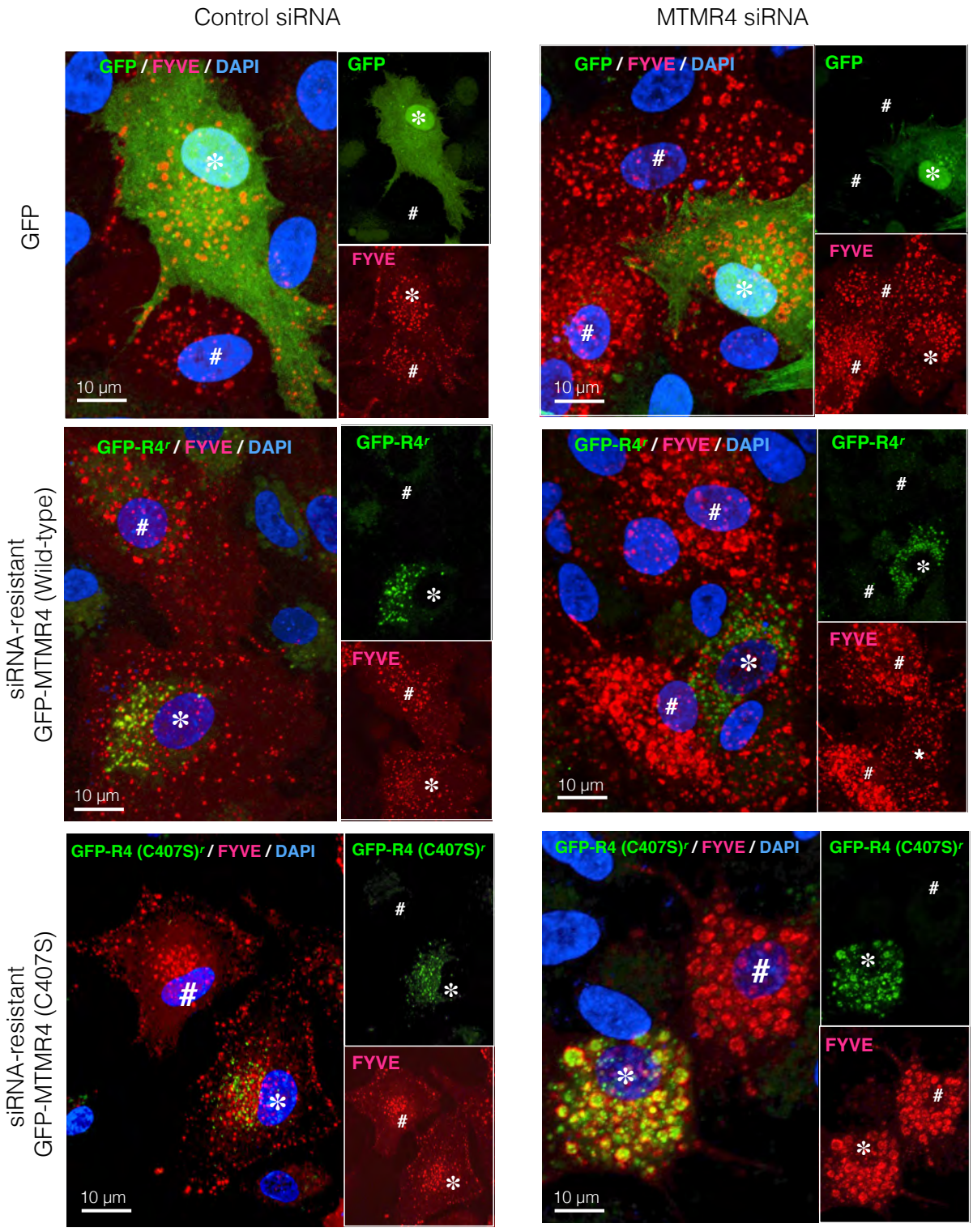

Figure S4. The effects of the expression of siRNA-resistant wild-type MTMR4 and catalytically inactive mutant on the MTMR4 knockdown phenotype.

mRFP-2xFYVE–expressing A549 cells were transfected with the expression vectors for GFP, the siRNA–resistant GFP-MTMR4 (GFP-R4^{r}) or the siRNA–resistant, catalytically inactive MTMR4 mutant GFP-MTMR4^{C407S} (GFP-R4(C407S)^{r}), and either control– or MTMR4–siRNA. The cells were then processed for confocal microscopy. Nuclei are stained with DAPI (blue) staining. *, nuclei of GFP–, GFP-R4^{r}–, and GFP-R4(C407S)^{r}–transfected cells. $^{\#}$, DAPI–stained nuclei. Scale bar, 10 μm.

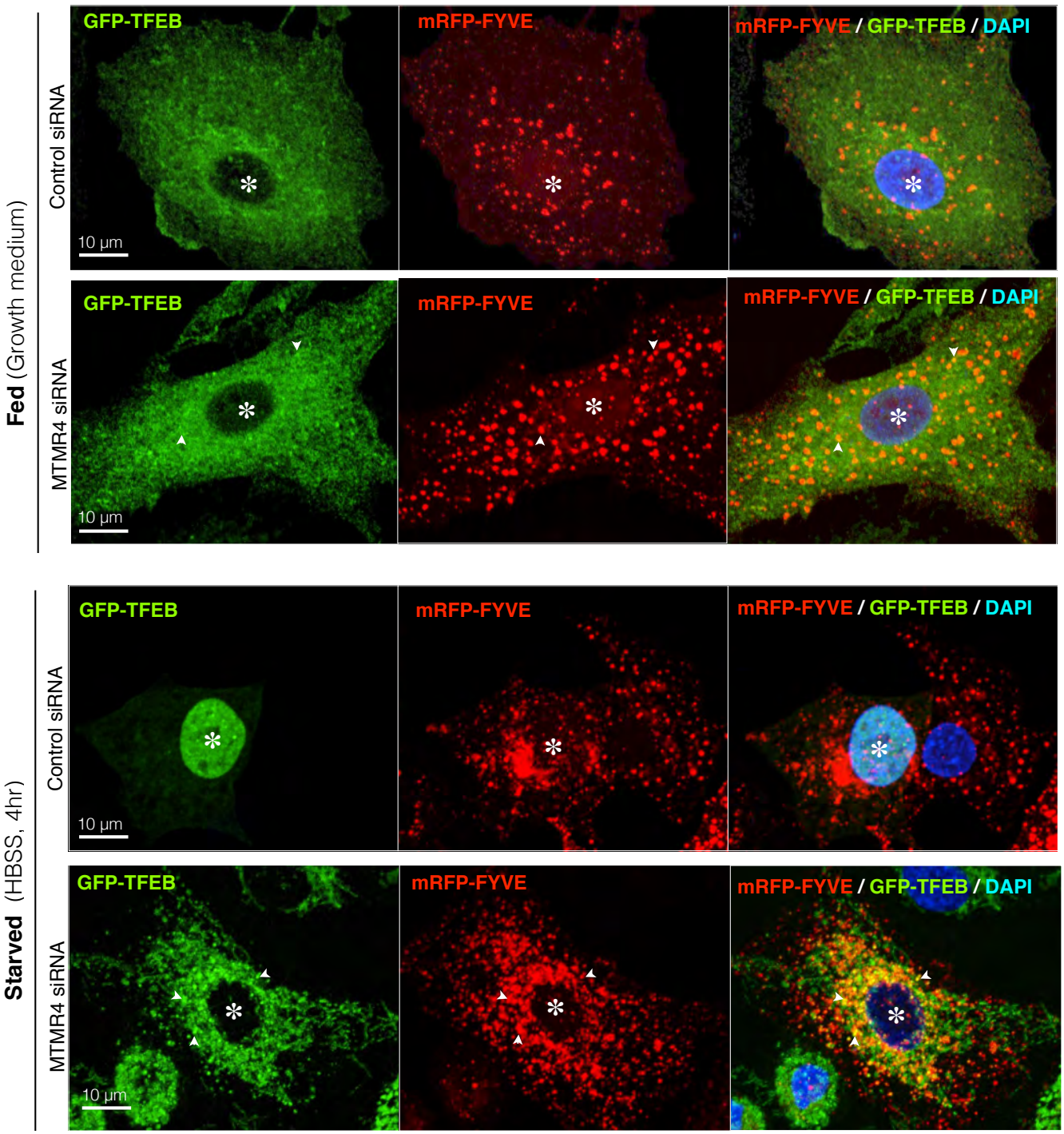

Figure S5. MTMR4 knockdown causes accumulation of TFEB in PI(3)P-enriched vesicles. mRFP-2xFYVE domain-expressing A549 cells were transfected with the GFP-TFEB expression vector and either control– or MTMR4–siRNA, and were placed in growth medium (fed) or HBSS (starved) for 4h. The cells were then processed for confocal microscopy. Nuclei are stained with DAPI (blue) staining. *, nuclei of GFP-TFEB–transfected cells. Scale bar, 10 μm.