Martina and Puertollano, http://www.jcb.org/cgi/content/full/jcb.201209135/DC1

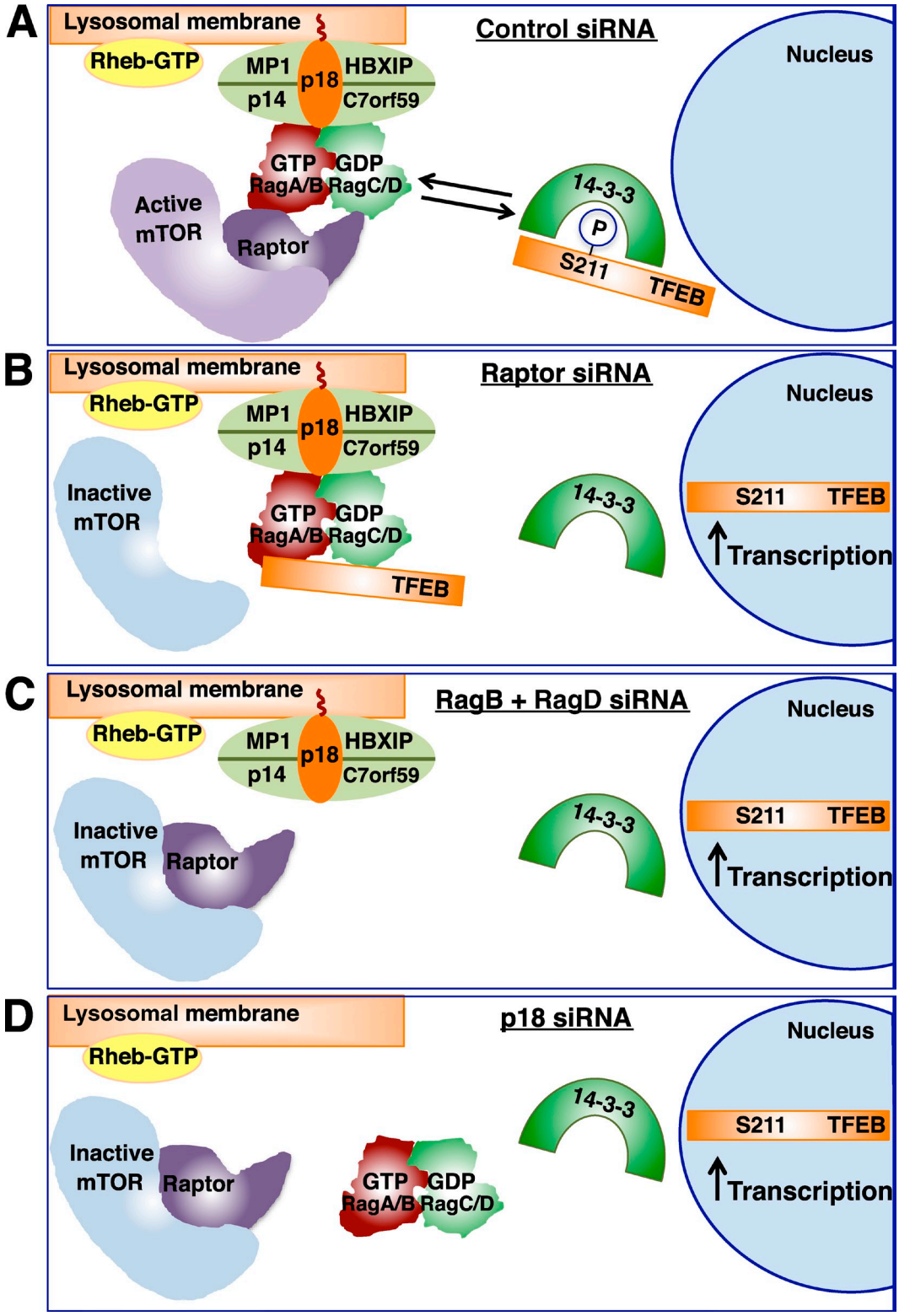

Figure S1. **Rag GTPases are required for association of TFEB with lysosomal membranes.** Schematic representation of changes in the distribution of TFEB, as well as alterations on the Ragulator–Rags–mTORC1 complex, induced by treatment with the indicated siRNAs. P, phosphorylation.

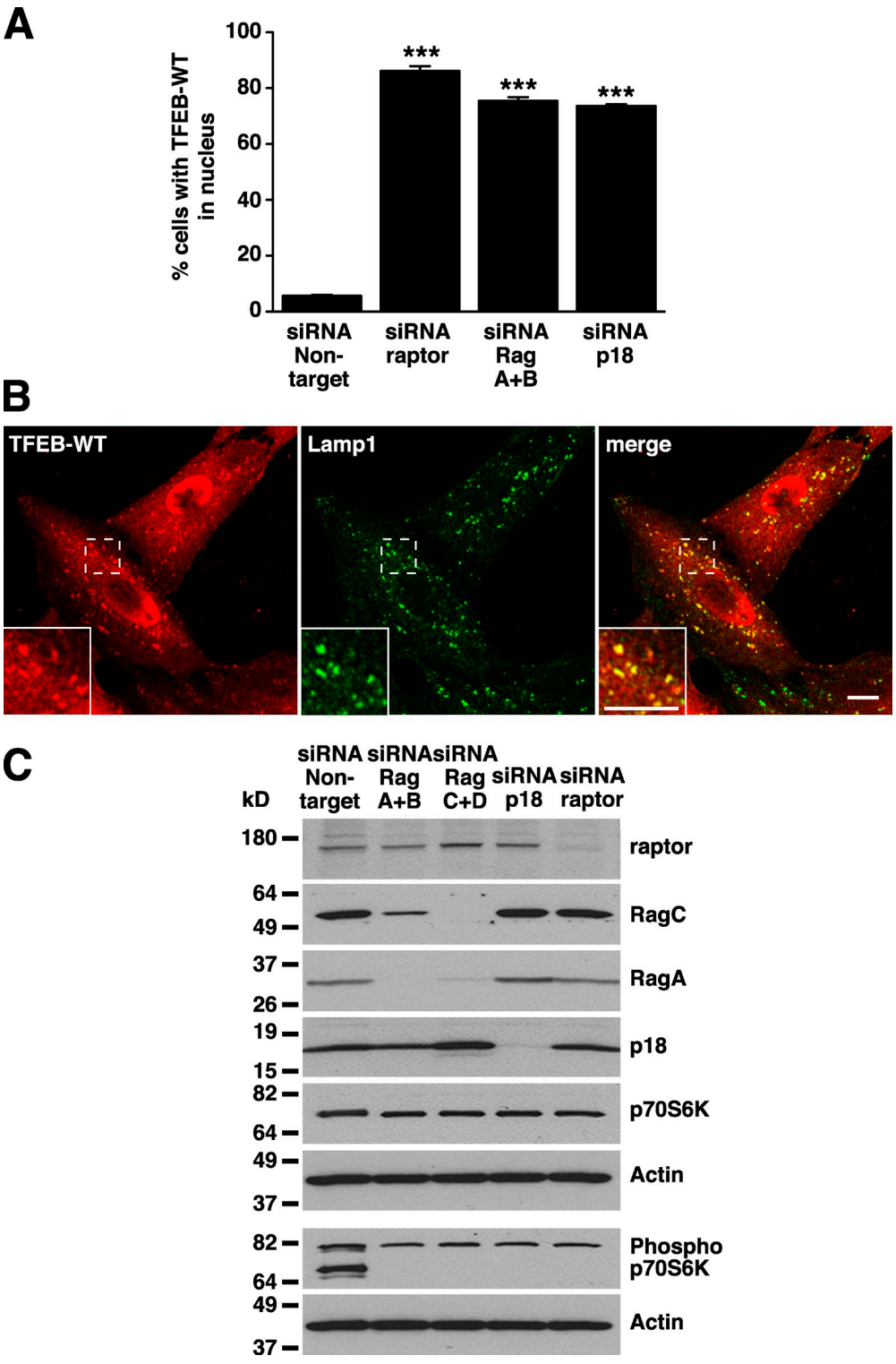

Figure S2. **Inactivation of mTORC1 by depletion of Rags, p18, or raptor causes accumulation of TFEB in the nucleus.** (A) ARPE-19 cells were transfected with siRNA duplexes to raptor, RagA+B, p18, or nontarget. 60 h after transfection, cells were infected with adenovirus expressing TFEB-WT. 12 h later, the cells were washed, fixed, permeabilized with 0.2% Triton X-100, and stained with antibodies against FLAG to assess the percentage of cells with nuclear localization of TFEB. Values are means ± SD of three independent experiments. ***, $P < 0.001$ to cells transfected with siRNA duplexes to nontarget. (B) Immunofluorescence confocal microscopy showing the subcellular distribution of TFEB-FLAG-WT in ARPE-19 cells depleted of raptor. ARPE-19 cells were transfected with siRNA duplexes to raptor. 60 h after transfection, cells were infected with adenovirus expressing TFEB-WT. 12 h later, cells were washed, fixed, permeabilized with 0.2% Triton X-100, and double stained with antibodies against FLAG (used to detect TFEB-WT) and Lamp1. Regions within the dotted boxes are magnified in the insets. Bars, 10 µm. (C) Immunoblotting analysis of the indicated proteins in lysates of ARPE-19 cells depleted of raptor, RagA+B, RagC+D, or p18. Please note the images showing raptor, RagC, RagA, p18, p70S6K, and actin derive from the same gel/membrane. Phospho-p70S6K and its corresponding loading control (actin) derive from a different gel that was run in parallel.

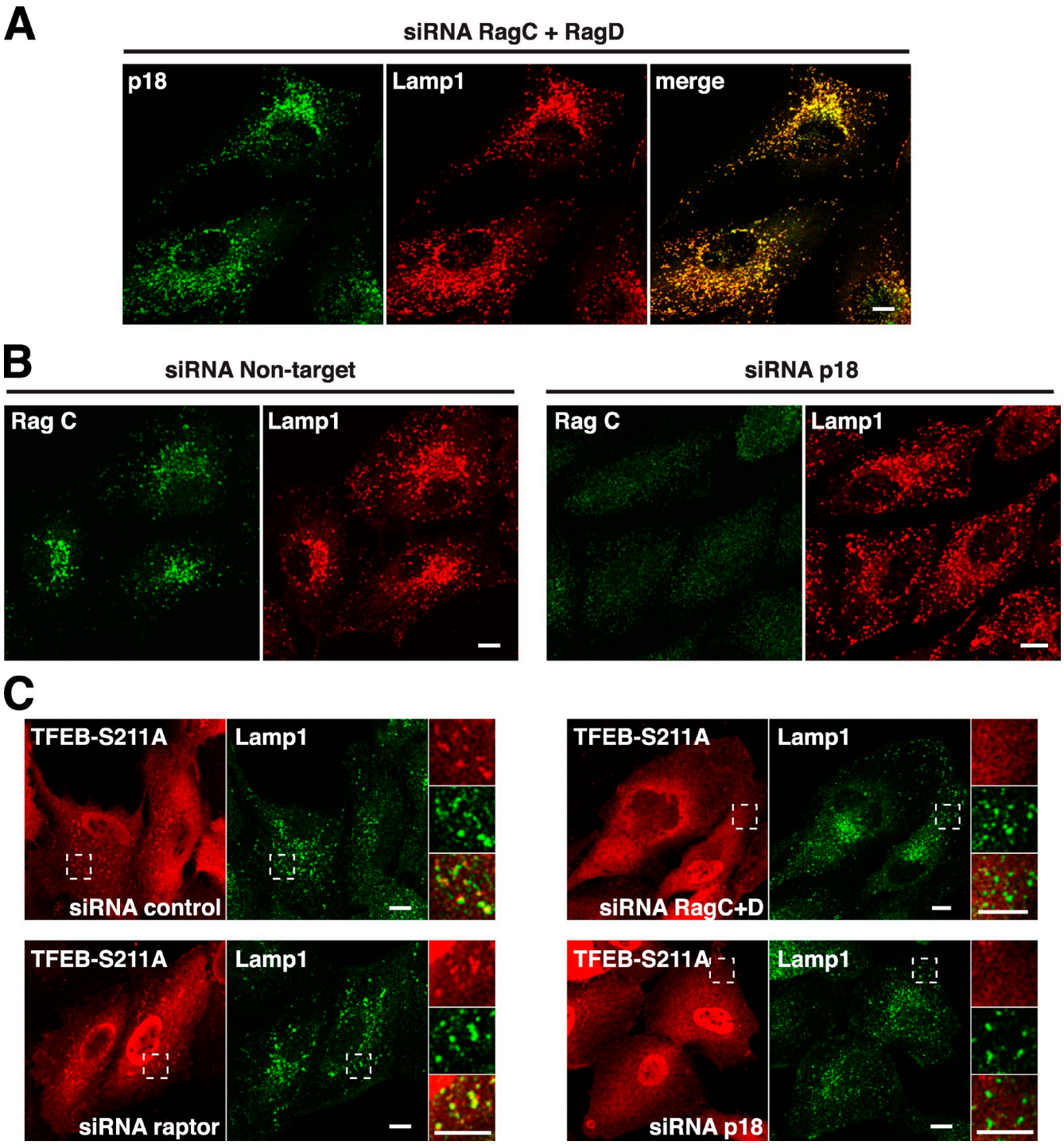

Figure S3. **Depletion of Rag GTPases and Ragulator complex protein p18.** (A) ARPE-19 cells were transfected with siRNA duplexes to RagC and RagD. 72 h after transfection, cells were washed, fixed, permeabilized, and double stained with antibodies against p18 and Lamp1. (B) ARPE-19 cells were transfected with either control (nontarget) siRNA duplexes or siRNA against p18. 72 h after transfection, cells were washed, fixed, permeabilized, and double stained with antibodies against RagC and Lamp1. (C) ARPE-19 cells were transfected with siRNA duplexes to raptor, RagC+D, p18, or nontarget. 60 h after transfection, cells were infected with adenovirus expressing TFEB-S211A. 12 h later, cells were washed, fixed, permeabilized with 0.2% Triton X-100, and double stained with antibodies against FLAG (used to detect TFEB-S211A) and Lamp1. Regions within the dotted boxes are magnified in the insets. Bars, 10 µm.

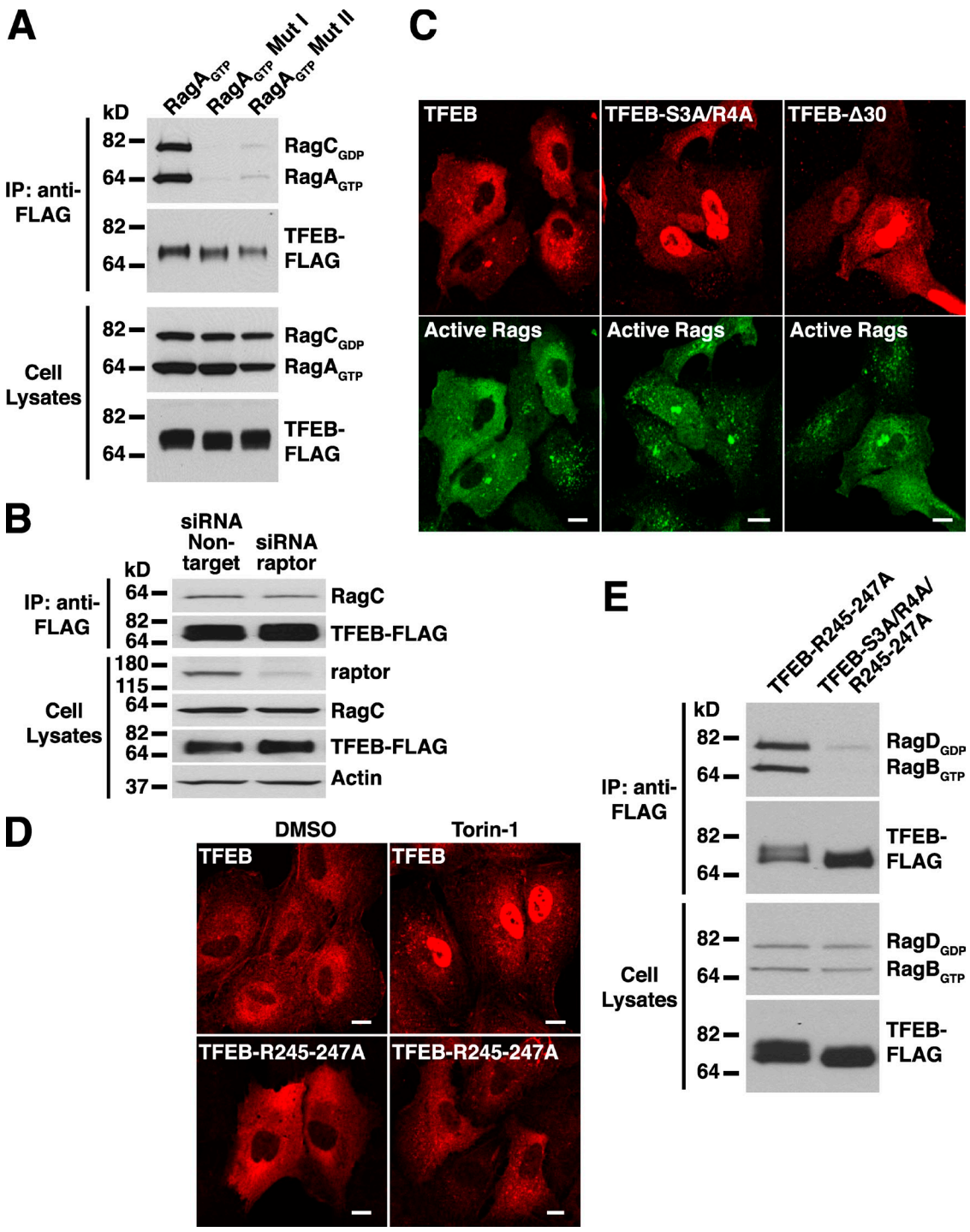

Figure S4. **Mapping of the interaction between TFEB and Rags.** (A) Immunoblotting analysis showing impaired interaction of TFEB with Rag heterodimers carrying mutations in the switch I (R34A/D35A/R37A/R38A) or switch II (N55A/V57A/N59A/W61A) regions of RagA. ARPE-19 cells were nucleofected with the indicated Rag-expressing plasmids. 6 h after nucleofection, cells were infected with adenovirus expressing TFEB-FLAG-WT. 12 h later, cells were immunoprecipitated with the anti-FLAG antibody and immunoblotted with antibodies against GST and FLAG (used to detect Rag proteins and TFEB-WT, respectively). (B) ARPE-19 cells were transfected with siRNA duplexes to raptor or nontarget. 60 h after transfection, cells were infected with adenovirus expressing TFEB-FLAG-WT. 12 h later, cells were lysed and subjected to immunoprecipitation with the anti-FLAG antibody. The immunoprecipitates were analyzed by immunoblotting with antibodies against FLAG (used to detect TFEB-WT) and RagC. (C) Immunofluorescence confocal microscopy showing the subcellular distribution of TFEB amino acid or deletion mutants in ARPE-19 cells overexpressing active RagB/D heterodimers. ARPE-19 cells were nucleofected with the indicated Rag- and TFEB-expressing plasmids. 12 h later, cells were washed, fixed, permeabilized with 0.2% Triton X-100, and double stained with antibodies against FLAG and GST (used to detect TFEB and Rag proteins, respectively). (D) Identification of a novel TFEB nuclear localization signal. ARPE-19 cells were nucleofected with either TFEB- or TFEB-R245A/R246A/R247A–expressing plasmids. 12 h later, cells were treated with Torin-1 for 1 h, washed, fixed, permeabilized with 0.2% Triton X-100, and stained with antibodies against FLAG (used to detect TFEB). (E) Immunoblotting analysis of coimmunoprecipitated active RagB/D heterodimer with TFEB amino acid mutants. ARPE-19 cells were nucleofected with the indicated Rag- and TFEB-expressing plasmids. 18 h later, cells were lysed and subjected to immunoprecipitation with the anti-FLAG antibody. Immunoprecipitates were analyzed by immunoblotting with antibodies against FLAG and GST (used to detect TFEB and Rag proteins, respectively). IP, immunoprecipitation. Bars, 10 µm.

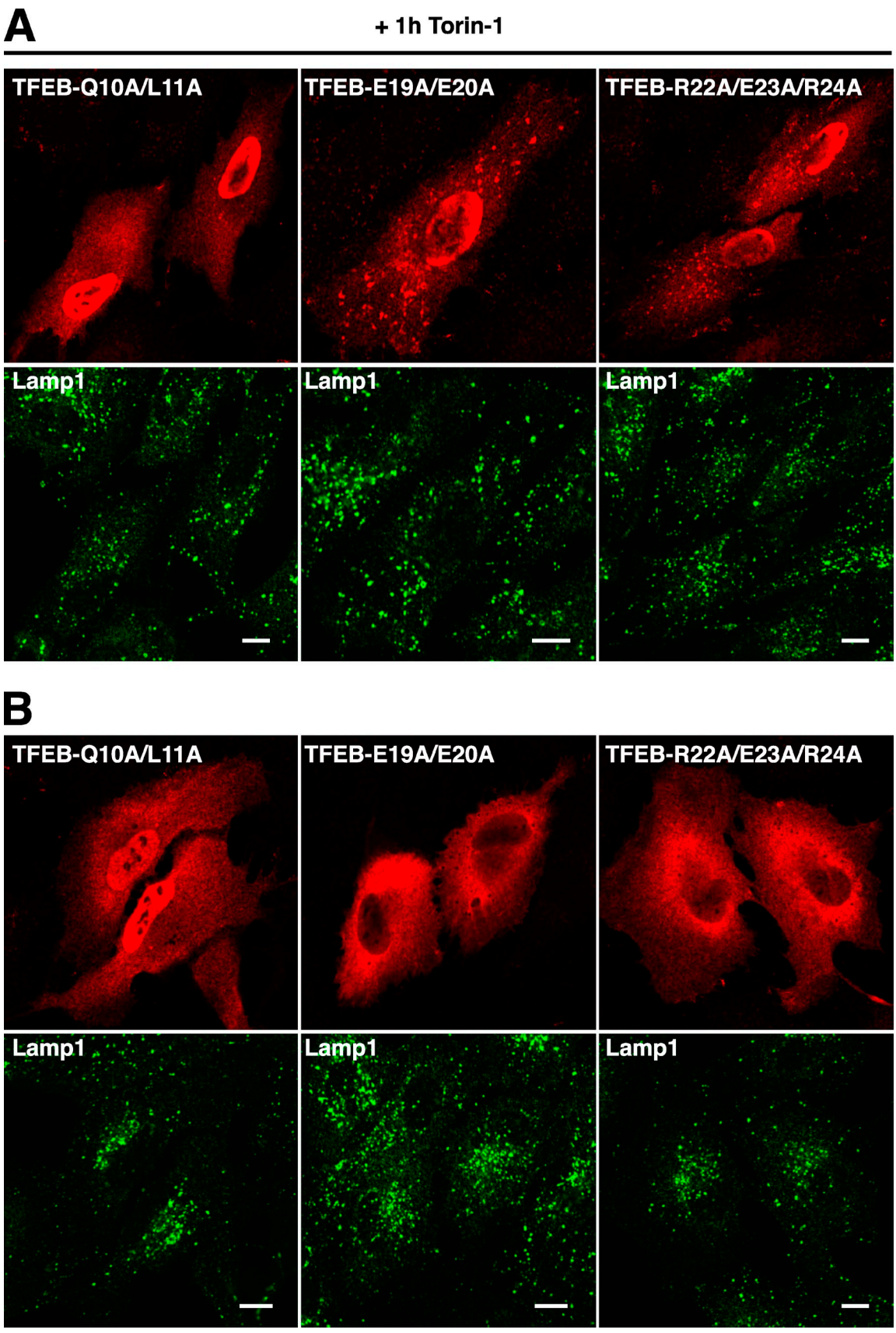

Figure S5. **Gln10 and Leu11 are required for proper regulation and localization of TFEB.** (A and B) ARPE-19 cells were nucleofected with the indicated TFEB amino acid mutants. 12 h after nucleofection, cells were incubated with 250 nM Torin-1 for 1 h (A) or DMSO (B). Cells were then washed, fixed, permeabilized with 0.2% Triton X-100, and double stained with antibodies against FLAG (used to detect TFEB-FLAG) and Lamp1. Bars, 10 µm.