Figure S1

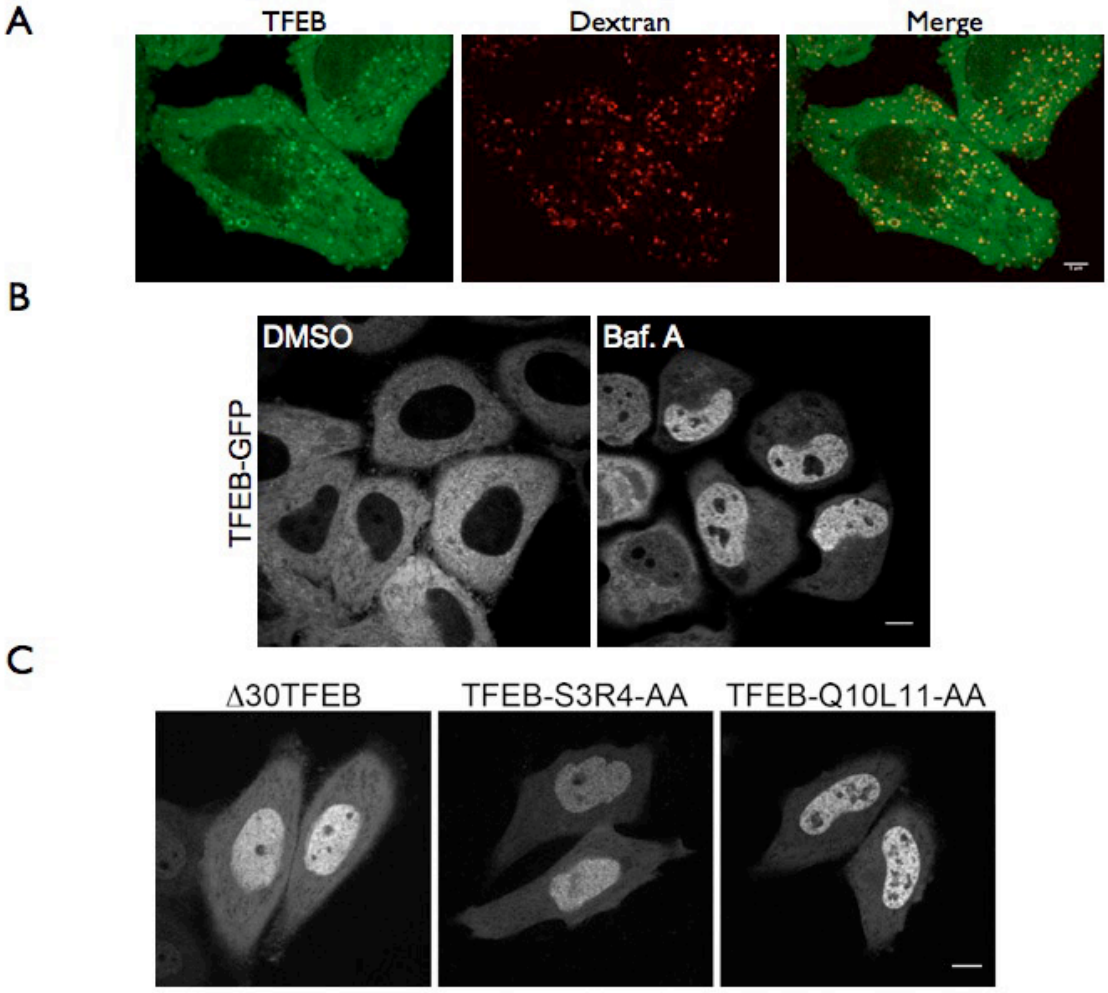

Fig. S1. Regulation of TFEB localization by lysosomal status. **(A)** Spinning disk confocal images showing the co-localization between TFEB-GFP and lysosomes labeled by Alexa 594-dextran. N=2 experiments, images are representative of the majority of cells observed. Scale bar = 5 μm. **(B)** Spinning disk confocal images of HeLa M cells stably expressing TFEB-GFP following incubation (15 hours) with vehicle control (0.1% DMSO, left) or bafilomycin A (100 nM, right). N=3, images are representative of the majority of cells observed. **(C)** Spinning disk confocal imaging of HeLa M cells

transiently transfected with the indicated TFEB-GFP mutants. N =3 experiments, images are representative of the majority of cells observed. Scale bars= 10 μm for B and C.

Figure S2

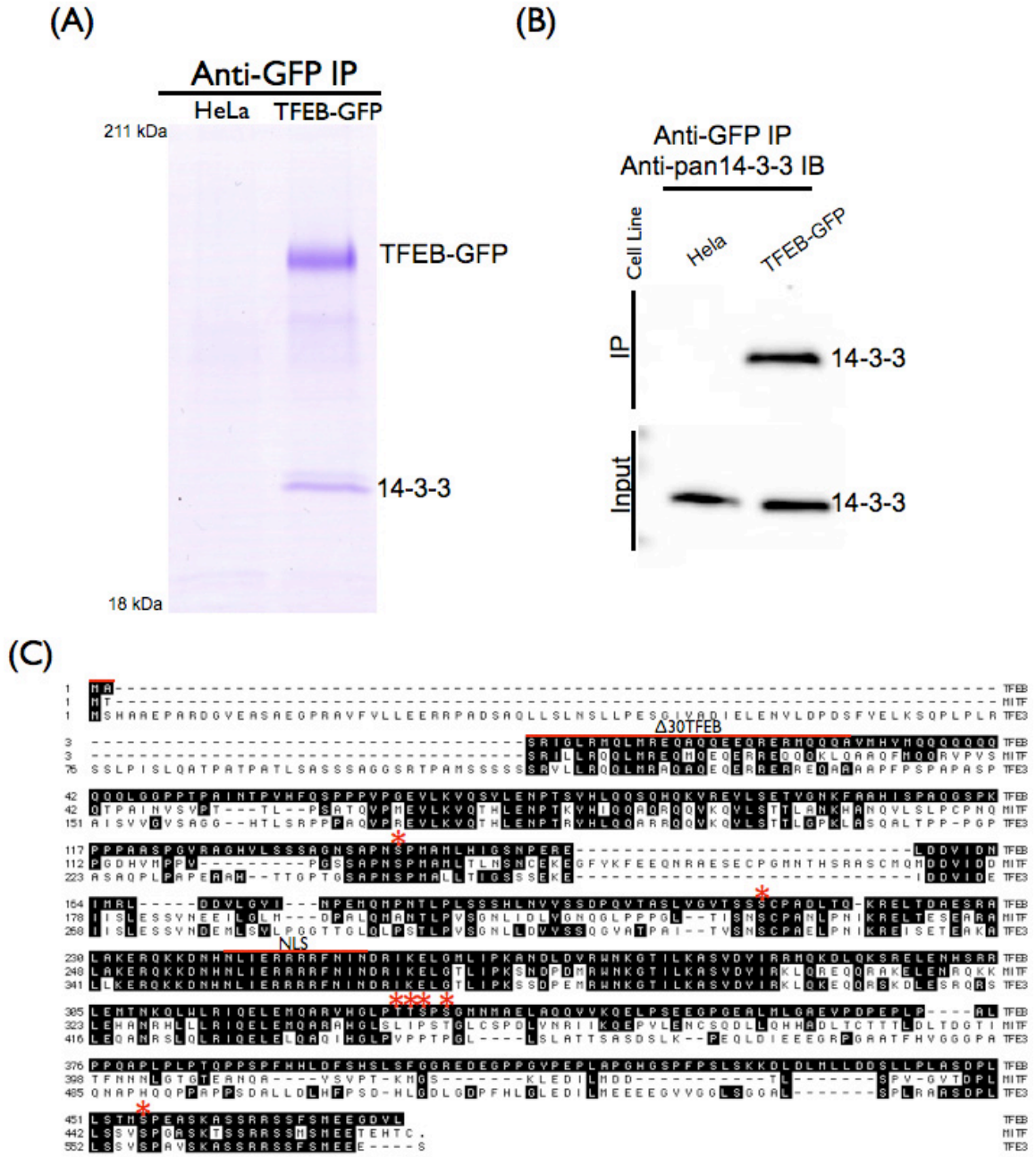

Fig. S2. 14-3-3 proteins are major binding partners of TFEB. **(A)** Coomassie stained SDS-PAGE gel showing the results of anti-GFP immunoprecipitations from control HeLa M cells or a TFEB-GFP expressing stable HeLa M cell line (n=3). **(B)** Immunoblot of anti-GFP immunoprecipitates using a pan-14-3-3 antibody shows the selective

enrichment of 14-3-3 isoforms in the material isolated from the TFEB-GFP stable cell line. The total fraction lanes were loaded with the equivalent of 1% of the material that was used for the corresponding immunoprecipitations (n=3). **(C)** Alignment of human TFEB, MITF (isoform D) and TFE3 sequences. Shading indicates sequences matching TFEB. The regions comprising the Δ30 mutant of TFEB and the NLS are highlighted by red lines and the locations of Ser^{142}, Ser^{211}, Thr^{330}, Thr^{331}, Ser^{332}, Ser^{334} and Ser^{455} in TFEB are highlighted by red asterisks.

Figure S3

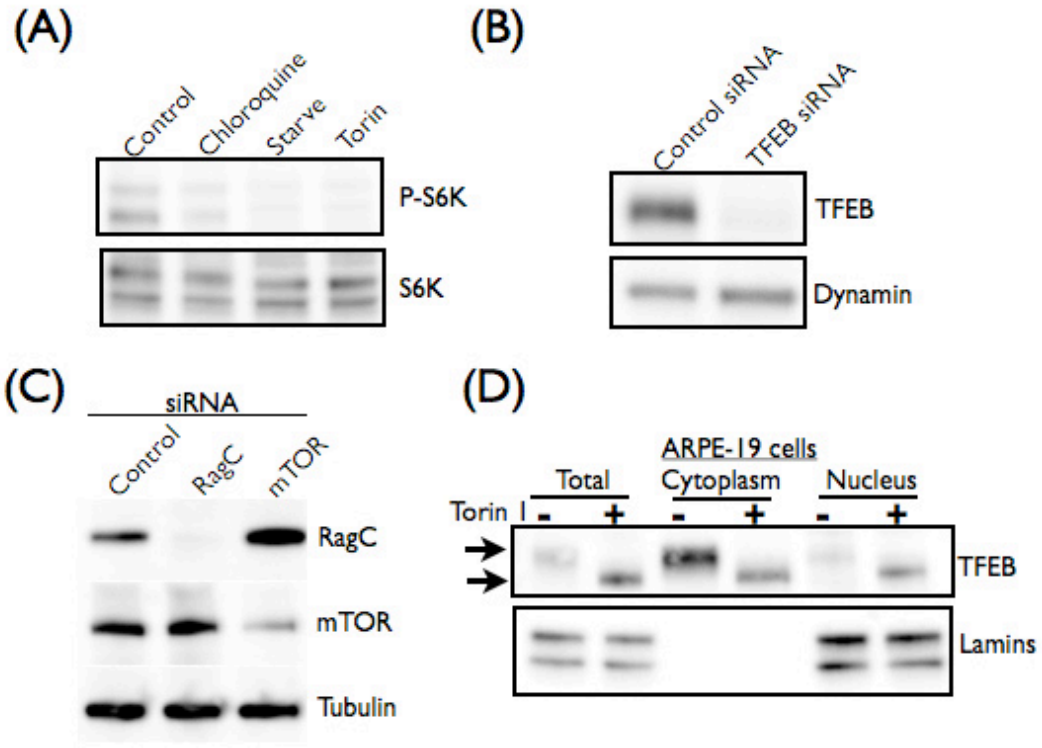

Fig. S3. Relationship between TFEB, mTOR and lysosomes. **(A)** Phospho-S6 kinase (P-S6K, Thr^{389}) and total S6K immunoblots on lysates from cells receiving the indicated treatments (as described for Fig. 3D, n=2). **(B)** Immunoblot from HeLa cells transfected with control or TFEB siRNA demonstrates the specificity of the TFEB antibody purchased from Bethyl Laboratories (n=3). The blot was re-probed for dynamin to demonstrate equal sample loading. **(C)** Immunoblots showing efficiency of RagC and mTOR knockdowns (n=3). **(D)** Immunoblotting of subcellular fractions of ARPE-19 cells treated ± torin 1 (2 μM, 2 hours). Arrows highlight the migration difference that arises due to torin 1 treatment (n=3).

Figure S4

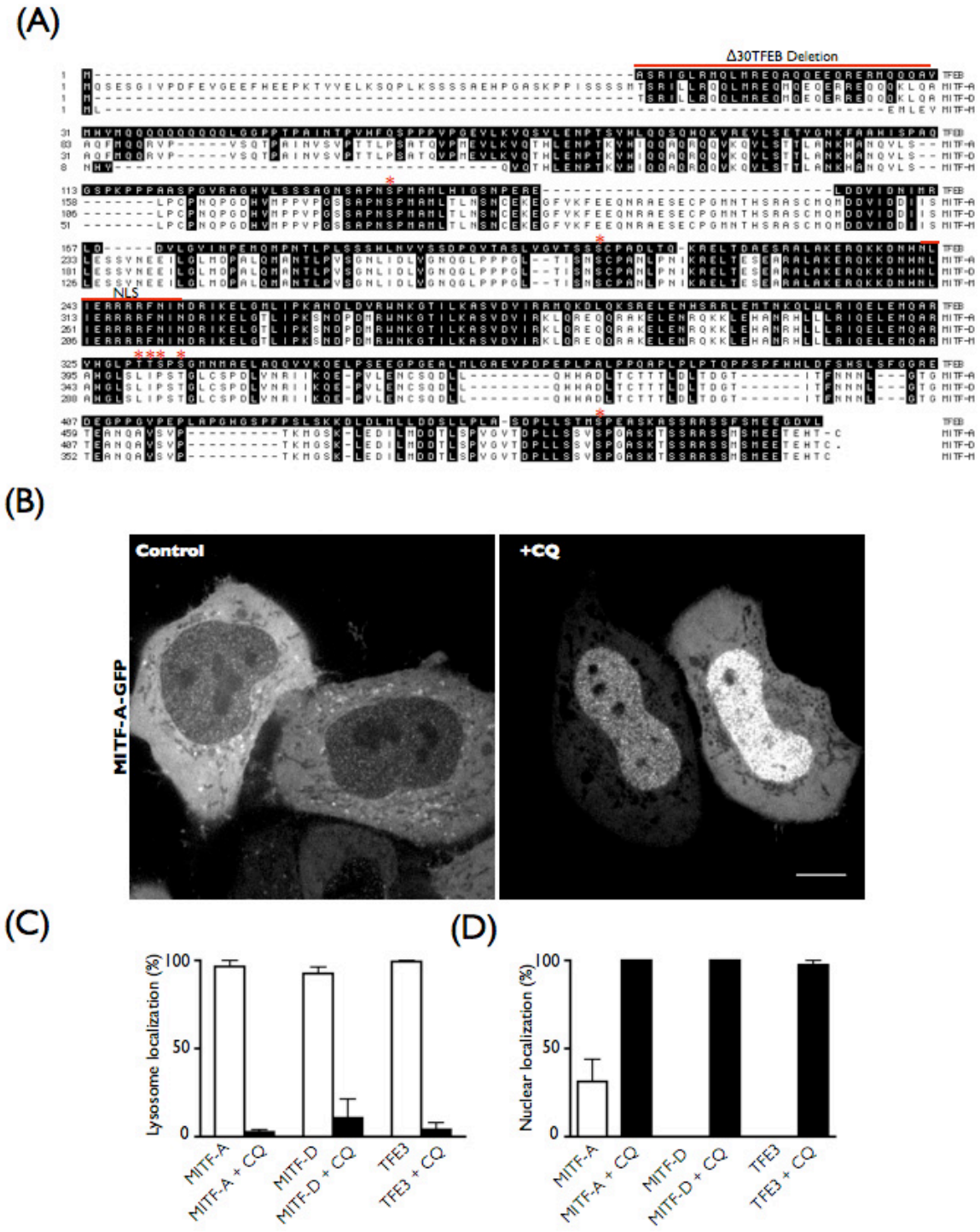

Fig. S4. MITF and TFE3 localization is sensitive to lysosome status. **(A)** Alignment of TFEB with MITF-A, D and M isoforms. Shading indicates sequences matching TFEB.

The regions comprising the Δ30 mutant of TFEB and the NLS are highlighted by red lines and the locations of Ser^{142}, Ser^{211}, Thr^{330}, Thr^{331}, Ser^{332}, Ser^{334} and Ser^{455} in TFEB are highlighted by red asterisks. **(B)** Spinning disk confocal imaging of HeLa M cells expressing MITF-A-GFP ± CQ treatment (50 μM, 15 hours). Scale bar = 10 μm. **(C)** Percentage of cells exhibiting lysosomal localization. n=3 experiments, 30-40 cells/condition/experiment. **(D)** Percentage of cells showing nuclear enrichment. n=3 experiments, 30-40 cells/condition/experiment.

Movie S1. Imaging of TFEB-GFP under basal conditions. Images from transfected HeLa M cells were acquired by spinning disk confocal imaging at 0.25 Hz and played back at 15 frames/second. Scale bar = 10 μm.