Nezich et al., http://www.jcb.org/cgi/content/full/jcb.201501002/DC1

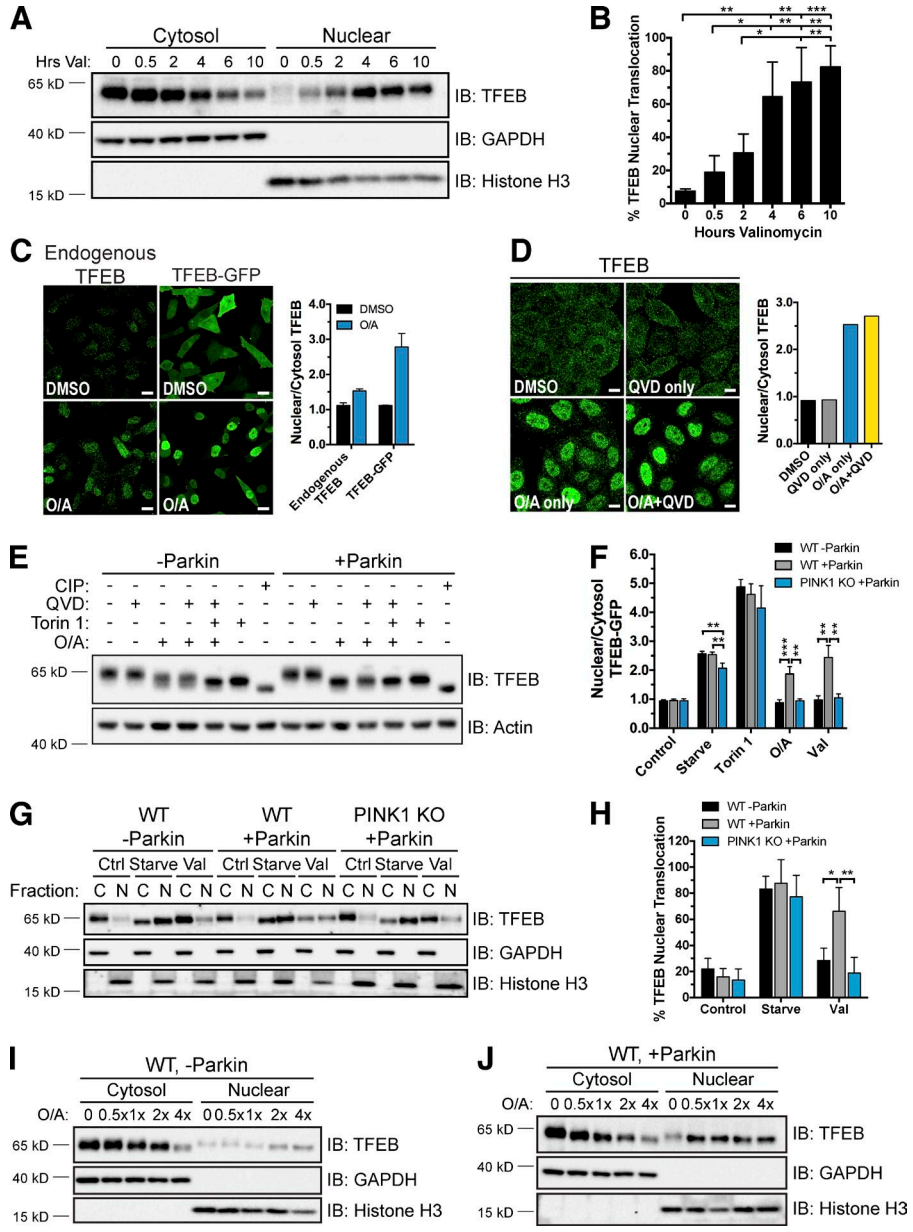


Figure S1. **Regulation of endogenous TFEB localization is Parkin dependent.** (A) YFP-Parkin HeLa cells were treated with valinomycin (Val) for up to 10 h, fractionated, and immunoblotted. (B) Quantification of data in A. Endogenous TFEB expression was normalized to GAPDH (cytosol) or histone H3 (nuclear) and nuclear TFEB was expressed as a percentage of total TFEB. Data are means ± SD (n = 3). (C) Subcellular localization of endogenous and ectopic TFEB was analyzed by immunofluorescence in fixed BE(2)-M17 cells treated with DMSO or O/A for 6 h. Bars, 20 µm. The nuclear/cytosol ratio for each condition was calculated from mean fluorescence intensity/volume measurements made for each compartment across a field (five to seven) of cells (~20 cells/field). Quantified data are means ± range (n = 2 experiments). (D) mCherry-Parkin HeLa cells were treated for 6 h as indicated, fixed, and immunostained for TFEB. Bars, 10 µm. Quantified data were generated as in C (four fields/condition, >40 cells/field, and n = 1 experiment). (E) WT and mCherry-Parkin HeLa cells were treated with torin 1 (2 h), CIP (1 h), O/A (6 h), and QVD (6 h) as indicated, lysed, and immunoblotted. (F) Quantification of TFEB-GFP nuclear localization in Fig. 1 F. Analysis was performed as in C (four to seven fields, 40–50 cells/field, and n = 3 experiments). Data are means ± SD. (G) WT and PINK1 KO HeLa cells expressing mCherry-Parkin as indicated were starved (2 h) or treated with DMSO (Ctrl) or valinomycin (Val) for 6 h. Cells lysates were fractionated and immunoblotted. C, cytosol; N, nuclear. (H) Quantification of endogenous TFEB nuclear localization in F, performed as in B. Data are means ± SD (n = 4–7). (I and J) HeLa cells lacking endogenous Parkin (I) or stably expressing mCherry-Parkin (J) were treated for 6 h with DMSO (0) or O/A doses ranging from 0.5 to 4 times the standard concentration, lysed, fractionated, and immunoblotted. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

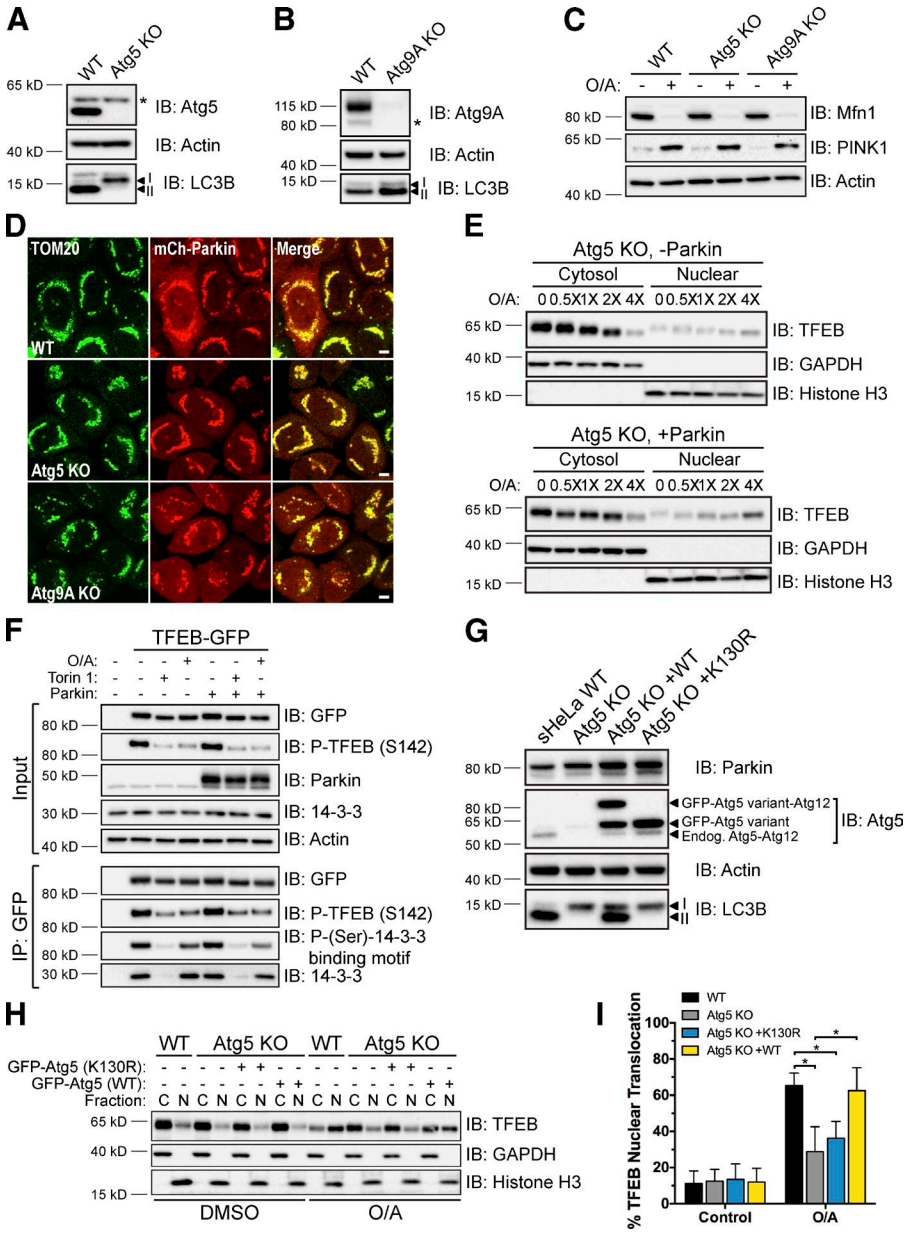


Figure S2. **Validation and supporting analysis of Atg5 and Atg9A KO cell lines.** (A and B) Cell lysates from untreated WT and Atg5 (A) or Atg9A (B) KO cells were subjected to immunoblotting. Asterisk denotes nonspecific band. (C) Cell lysates of WT, Atg5 KO, and Atg9A KO cells stably expressing mCherry-Parkin and treated for 3 h with DMSO or O/A were subjected to immunoblotting. (D) Cells from C were treated for 3 h with O/A, fixed, immunostained for TOM20, and analyzed by immunofluorescence. Bars, 5 µm. Images are representative of >95% of cells (>100 cells observed per cell line; *n* = 1 experiment). (E) Atg5 KO cells lacking endogenous Parkin (top) or stably expressing mCherry-Parkin (bottom) were treated for 6 h with DMSO (0) or O/A doses ranging from 0.5 to 4 times the standard concentration, lysed, fractionated, and immunoblotted. (F) Atg5 KO cells stably expressing TFEB-GFP were transfected with control or untagged Parkin plasmid DNA and treated the next day with DMSO (6 h), torin 1 (2 h), or O/A (6 h). Cells were lysed and TFEB-GFP was immunoprecipitated with anti-GFP beads. Cell lysates (Input) and immunoprecipitated proteins were analyzed by immunoblotting. Images are representative of *n* = 2 experiments. (G) Untreated WT and Atg5 KO cells stably expressing mCherry-Parkin and the indicated GFP-Atg5 variants were lysed and immunoblotted. (H) Cells from G were treated with DMSO or O/A (6 h), lysed, fractionated, and immunoblotted. C, cytosol; N, nuclear. (I) Quantification of data in H. Endogenous TFEB expression was normalized to GAPDH (cytosol) or histone H3 (nuclear) and nuclear TFEB was expressed as a percentage of total TFEB. Data are means ± SD (*n* = 3). *, P < 0.05.

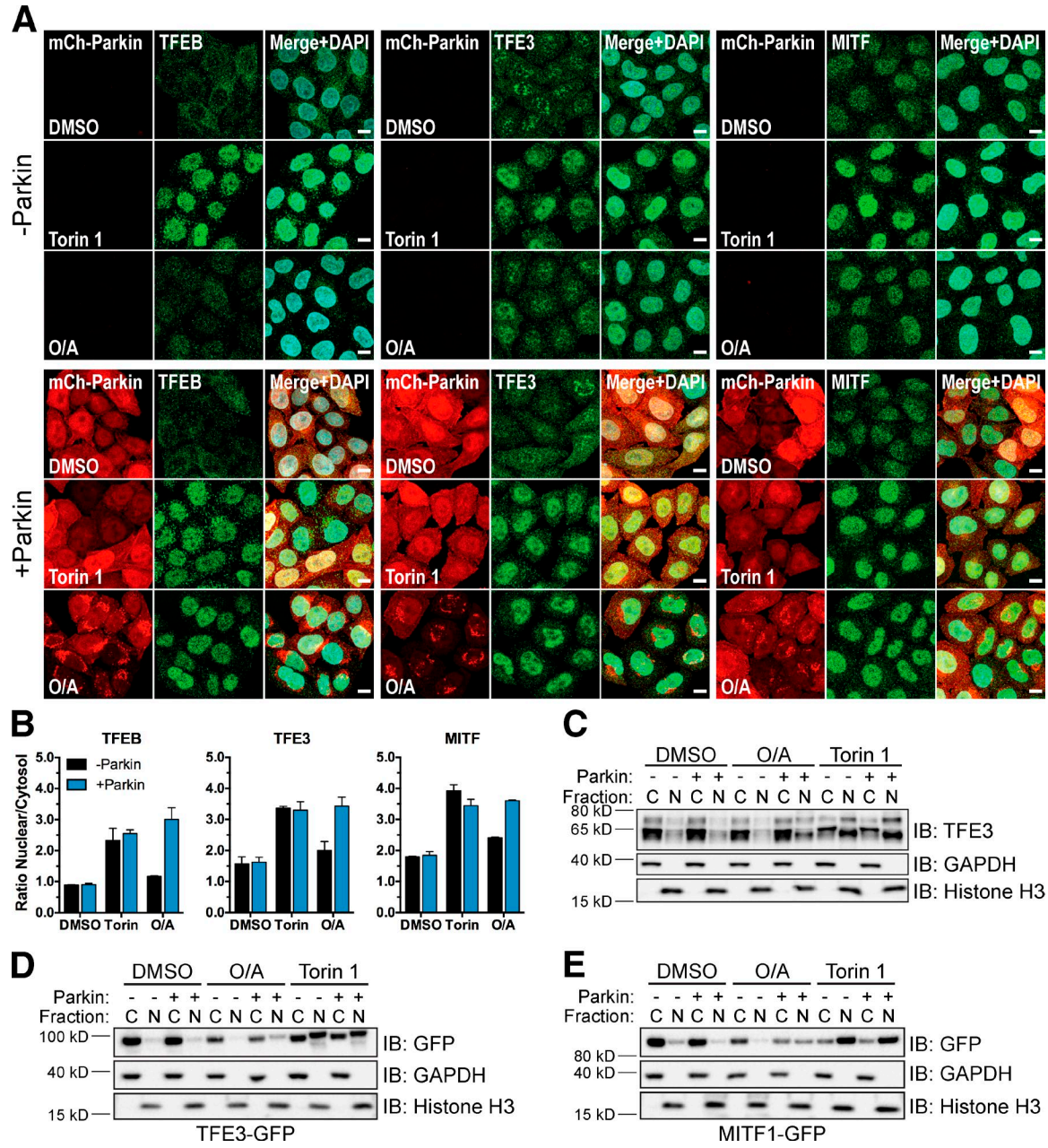
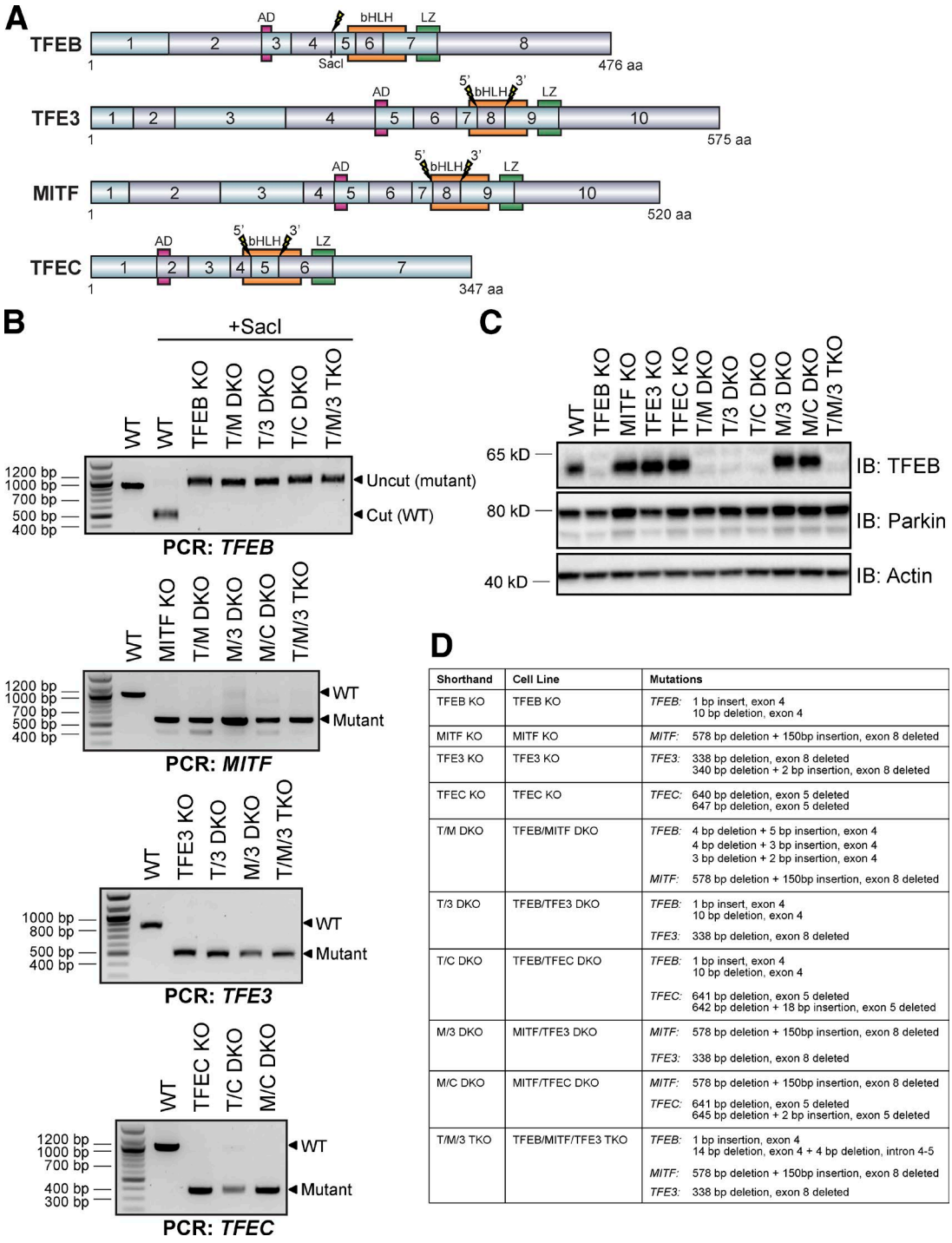


Figure S3. **Parkin is required for nuclear accumulation of endogenous MiT/TFE proteins during mitophagy.** (A) WT and stable mCherry-Parkin HeLa cells treated with DMSO (6 h), torin 1 (2 h), or O/A (6 h) were fixed; immunostained for TFEB (left), TFE3 (middle), or MITF (right); and analyzed by immunofluorescence. Bars, 10 µm. (B) Quantification of endogenous TFEB, TFE3, and MITF nuclear localization in A. The nuclear/cytosol ratio for each condition was calculated from mean fluorescence intensity/volume measurements made for each compartment across a field (n = 4) of cells (40–60 cells/field). Data are means ± range (n = 2 experiments). (C) WT and stable mCherry-Parkin HeLa cells were treated as in A, lysed, fractionated, and immunoblotted. (D and E) HeLa cells stably expressing TFE3-GFP (D) or MITF1-GFP (E) with mCherry-Parkin as indicated were treated as in A, lysed, fractionated, and immunoblotted. Images in C–E are representative of n = 2 experiments. C, cytosol; N, nuclear.



Shorthand	Cell Line	Mutations
TFEB KO	TFEB KO	*TFEB:* 1 bp insert, exon 4 10 bp deletion, exon 4
MITF KO	MITF KO	*MITF:* 578 bp deletion + 150bp insertion, exon 8 deleted
TFE3 KO	TFE3 KO	*TFE3:* 338 bp deletion, exon 8 deleted 340 bp deletion + 2 bp insertion, exon 8 deleted
TFEC KO	TFEC KO	*TFEC:* 640 bp deletion, exon 5 deleted 647 bp deletion, exon 5 deleted
T/M DKO	TFEB/MITF DKO	*TFEB:* 4 bp deletion + 5 bp insertion, exon 4 4 bp deletion + 3 bp insertion, exon 4 3 bp deletion + 2 bp insertion, exon 4 *MITF:* 578 bp deletion + 150bp insertion, exon 8 deleted
T/3 DKO	TFEB/TFE3 DKO	*TFEB:* 1 bp insert, exon 4 10 bp deletion, exon 4 *TFE3:* 338 bp deletion, exon 8 deleted
T/C DKO	TFEB/TFEC DKO	*TFEB:* 1 bp insert, exon 4 10 bp deletion, exon 4 *TFEC:* 641 bp deletion, exon 5 deleted 642 bp deletion + 18 bp insertion, exon 5 deleted
M/3 DKO	MITF/TFE3 DKO	*MITF:* 578 bp deletion + 150bp insertion, exon 8 deleted *TFE3:* 338 bp deletion, exon 8 deleted
M/C DKO	MITF/TFEC DKO	*MITF:* 578 bp deletion + 150bp insertion, exon 8 deleted *TFEC:* 641 bp deletion, exon 5 deleted 645 bp deletion + 2 bp insertion, exon 5 deleted
T/M/3 TKO	TFEB/MITF/TFE3 TKO	*TFEB:* 1 bp insertion, exon 4 14 bp deletion, exon 4 + 4 bp deletion, intron 4-5 *MITF:* 578 bp deletion + 150bp insertion, exon 8 deleted *TFE3:* 338 bp deletion, exon 8 deleted

Figure S4. **Generation of MiT/TFE transcription factor gene KO cell lines by CRISPR/Cas9 gene editing.** (A) Schematic representation of TFEB, MITF, TFE3, and TFEC transcription factor proteins. Coding exons are numbered and functional domains are colored. CRISPR target sites are indicated by yellow bolts and a SacI digestion site is shown for TFEB. AD, activation domain; bHLH, basic helix-loop-helix domain; LZ, leucine zipper domain. (B) A portion of the specified gene was amplified by PCR for WT cells and the indicated CRISPR clones from D and analyzed by electrophoresis. TFEB PCR products were further digested with the SacI enzyme as indicated before analysis. (C) Cell lysates from untreated WT cells and the indicated CRISPR clones from D stably expressing mCherry-Parkin were subjected to immunoblotting. (D) Table of the CRISPR-generated MiT/TFE KO cells used in this study. Allelic mutations for each gene were determined by DNA sequencing.

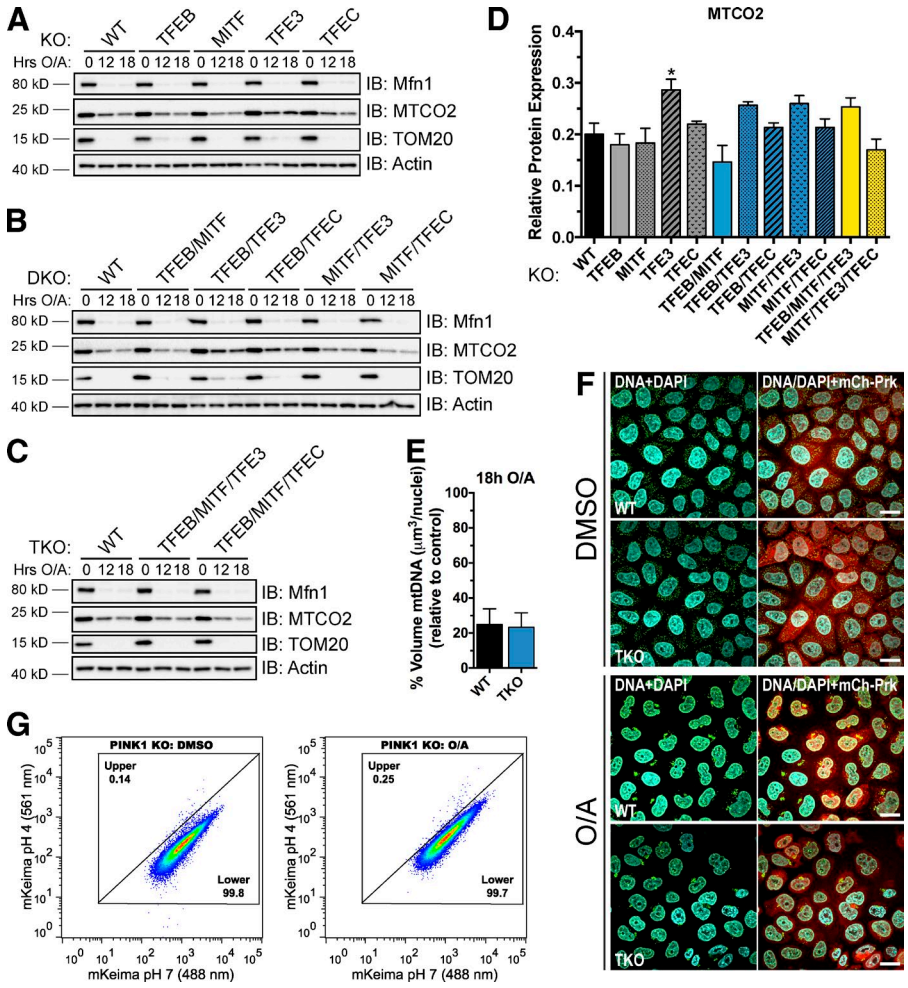


Figure S5. **Single, double, and triple MiT/TFE family member KO cell lines exhibit a partial deficit in Parkin-mediated mitophagy.** (A–C) WT and MiT/TFE single KO (A), DKO (B), and TKO (C) HeLa cell lines stably expressing mCherry-Parkin were treated with DMSO or O/A as indicated, lysed, and immunoblotted. (D) Quantification of MTCO2 levels in A–C after 18-h O/A treatment. MTCO2 expression was normalized to actin and expressed relative to 0-h treatment. Data are means ± SD (n = 3). Statistical comparisons are in reference to WT. *, $P < 0.05$. (E) Quantification of anti-DNA stain in F after 18-h O/A treatment. Z stacks of five to seven fields per condition (20–30 cells/field) were analyzed per experiment (n = 3). Data are means ± SD (F) WT and TFEB/MITF/TFE3 TKO (TKO) cells stably expressing mCherry-Parkin treated for 18 h with DMSO or O/A were fixed, immunostained for DNA, and analyzed by immunofluorescence. Bars, 20 µm. (G) PINK1 KO cells stably expressing YFP-Parkin and mt-mKeima were treated with DMSO or O/A for 18 h and subjected to FACS analysis. Plots are representative of n = 3 experiments.

Table S1. **CRISPR and primer sequences used for generation and screening of KO cell lines**

Gene	Primer	Sequence	PCR size	Screening method
			bp	
PINK1	Exon 1–7 screening forward	5′-GCCCCAAGTTTGTTGTGAC-3′	16,155	Truncated PCR product: ~791 bp
	Exon 1–7 screening reverse	5′-TGTGGTGGCTAGTGCTCCTA-3′		
	Exon 1 CRISPR	5′-GGAAGAAGCGGAGACGGTT-3′		
	Exon 7 CRISPR	5′-TCAATCCCTTCTACGGCCA-3′		
Atg5	Exon 3 screening forward	5′-TGCCTTTGCCCACAATCCTAT-3′	513	SapI cuts into 212 and 301 bp
	Exon 3 screening reverse	5′-AGTGTTTCTGCCAAGAGTTAGG-3′		
	Exon 3 CRISPR	5′-ATCAAGTTCAGCTCTTCCT-3′		
Atg9A	Exon 8 screening forward	5′-CCTCTTTTGACACTGCTGGC-3′	252	PshAI cuts into 117 and 135 bp
	Exon 8 screening reverse	5′-CCGGGATAAAGGACCTAGTGG-3′		
	Exon 8 CRISPR	5′-GACCCCCAGGAGTGTGACGG-3′		
TFEB	Exon 4 screening forward	5′-TTGCCTCACATCTCTGCTCA-3′	933	SacI cuts into 446 and 487 bp
	Exon 4 screening reverse	5′-AGGTTCCATGCCTTAACCCAG-3′		
	Exon 4 CRISPR	5′-CCCAGAAGCGAGAGCTCAC-3′		
MITF	Exon 8 screening forward	5′-TTGTTGGATGTCCTCGCCAA-3′	998	Truncated PCR product: ~435 bp
	Exon 8 screening reverse	5′-AAGGGGACAGGGTGGGTAAA-3′		
	5′ exon 8 CRISPR	5′-GGTTTCCGTTGTCATGACC-3′		
	3′ exon 8 CRISPR	5′-TTGTACAGTACCTCTAAAT-3′		
TFE3	Exon 8 screening forward	5′-AGGTCTAGAGATGGGCCTGA-3′	832	Truncated PCR product: ~494 bp
	Exon 8 screening reverse	5′-TATCCTACGGGGACCAGATG-3′		
	5′ exon 8 CRISPR	5′-GGGCAATGCACACGCTCTC-3′		
	3′ exon 8 CRISPR	5′-TGCCTGGGCCGAGACTGCC-3′		
TFEC	Exon 5 screening forward	5′-ATTGCGTTAGGCTTTGAGGA-3′	1,009	Truncated PCR product: ~368bp
	Exon 5 screening Rreverse	5′-AGTAATTTGTTTGATGGACATTTTG-3′		
	5′ exon 5 CRISPR	5′-TCAGGATTGTAATCATTCG-3′		
	3′ exon 5 CRISPR	5′-TTACTTCTTATAGCTCAAG-3′		