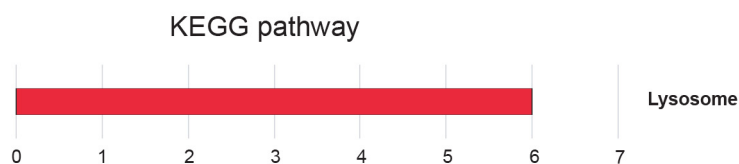


Expanded View Figures

A



B

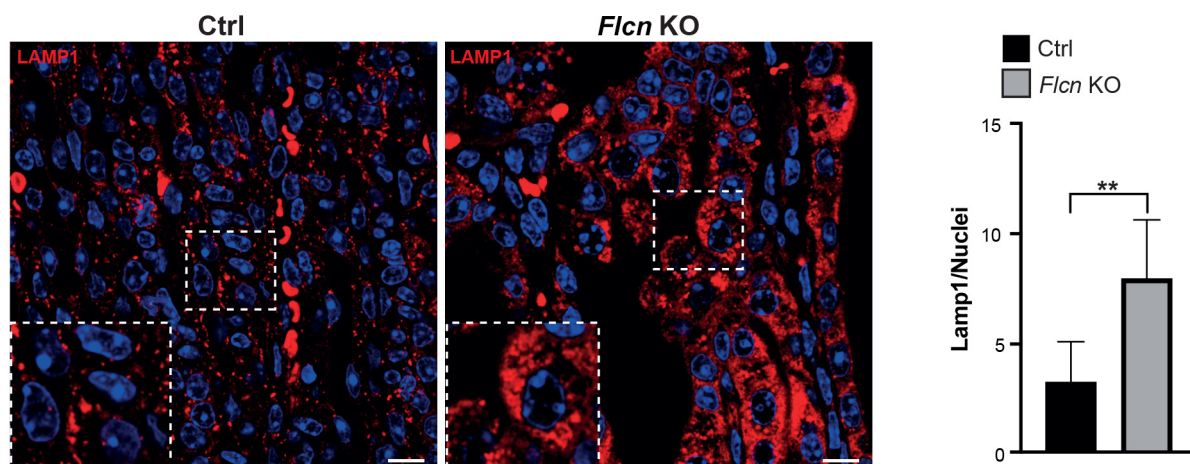


Figure EV1. Kidney tissues from *Flcn*-KO mice show upregulation of the lysosomal pathway.

A Kegg pathway associated with genes significantly upregulated in kidney tissues from *Flcn* KO mice relative to control mice at the precystic stage p2 (Dataset EV1).
 B Lamp1 immunostaining (in red) of renal tissues from control (Ctrl) and kidney-specific *Flcn* KO (*Flcn* KO) mice. Insets show magnification of the boxed area. Nuclei were stained with DAPI (blue). Scale bar 10 μ m. Bar graph shows quantification of Lamp1-positive vesicles/nuclei. Mean $\pm$ 95% of confidence interval ($n = 3$ biological replicates). Unpaired t -test $**P < 0.01$.

Source data are available online for this figure.

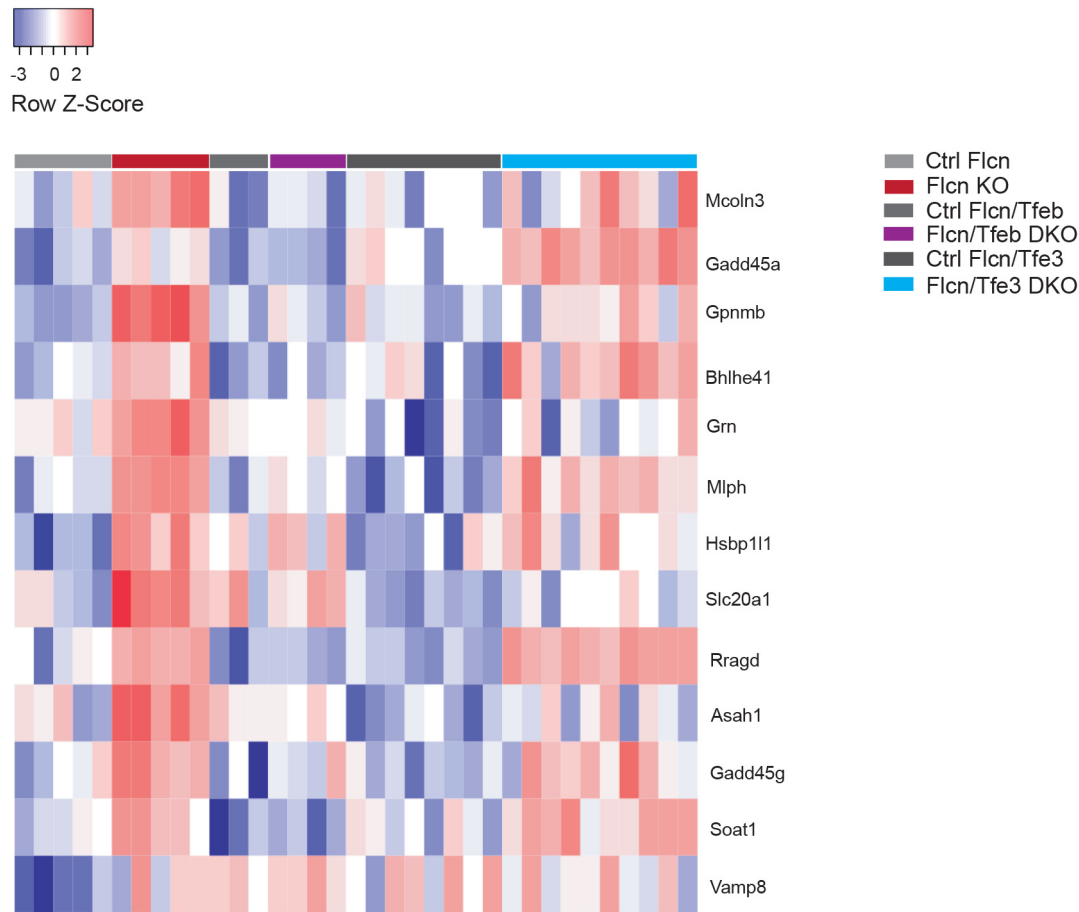


Figure EV2. Depletion of *Tfeb* but not *Tfe3* corrects the aberrant upregulation of validated *Tfeb*/*Tfe3* target genes in *Flcn*-KO mice. Heatmap showing validated TFEB/TFE3 target genes differentially expressed from kidney samples of the indicated murine groups (relative to Dataset EV1). Genes are ranked from the most significantly upregulated to the less significantly upregulated in the *Flcn* KO group. Each row shows the relative expression level of a single gene. Each column shows the expression level of a single sample. Upregulated transcripts are shown in red and downregulated transcripts are shown in blue.

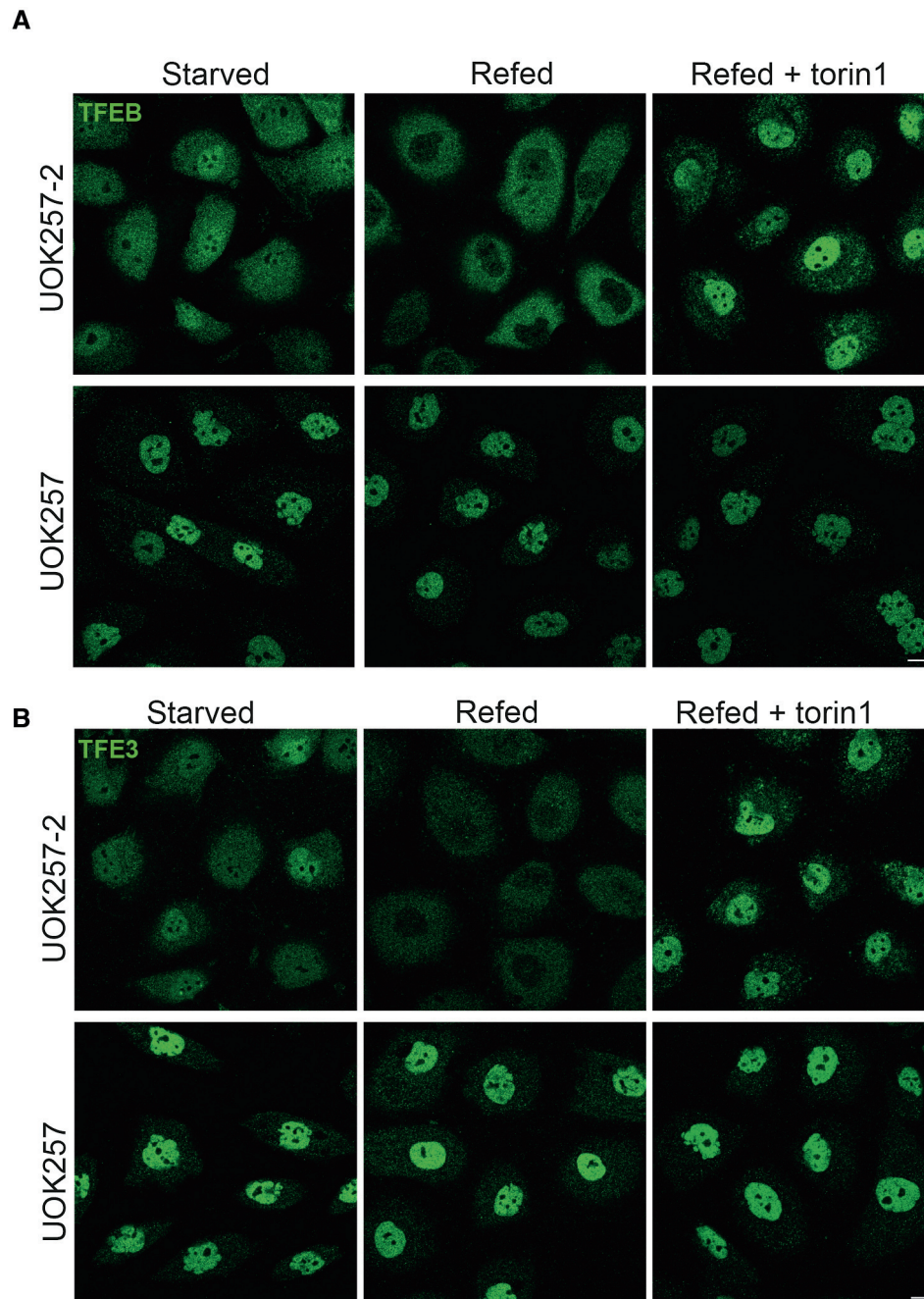


Figure EV3. Both TFEB and TFE3 are constitutively nuclear in UOK257 cells.

A, B Representative immunofluorescence analysis of TFEB (A) or TFE3 (B) localization in BHD patient-derived UOK257 cells and control UOK257-2 cells, obtained by stable transfection of UOK257 cells with exogenous FLCN. Cells were deprived of amino acids for 2 h (starved) and then restimulated with amino acids (refed) for 1 h in the presence or absence of 300 nM torin1. Scale bar, 10 μ m.

Source data are available online for this figure.

Figure EV4. Silencing of TFEB or TFE3 abrogates the activation of target genes in UOK257 cells.

- A Immunoblot analysis of the indicated proteins in UOK257 cells silenced for Luciferase (shLUC) or TFEB (shTFEB) or TFE3 (shTFE3).
- B Heatmap showing validated TFEB target genes differentially expressed from UOK257 cells infected with the indicated shRNA (relative to Dataset EV3). Genes are ranked from the most significantly upregulated to the less significantly upregulated in the cells infected with control shRNA (shLUC). Each row shows the relative expression level of a single gene. Each column shows the expression level of a single sample. Upregulated transcripts are shown in red and downregulated transcripts are shown in blue.
- C Kegg pathways associated with TFEB target genes significantly downregulated in (B).

Source data are available online for this figure.

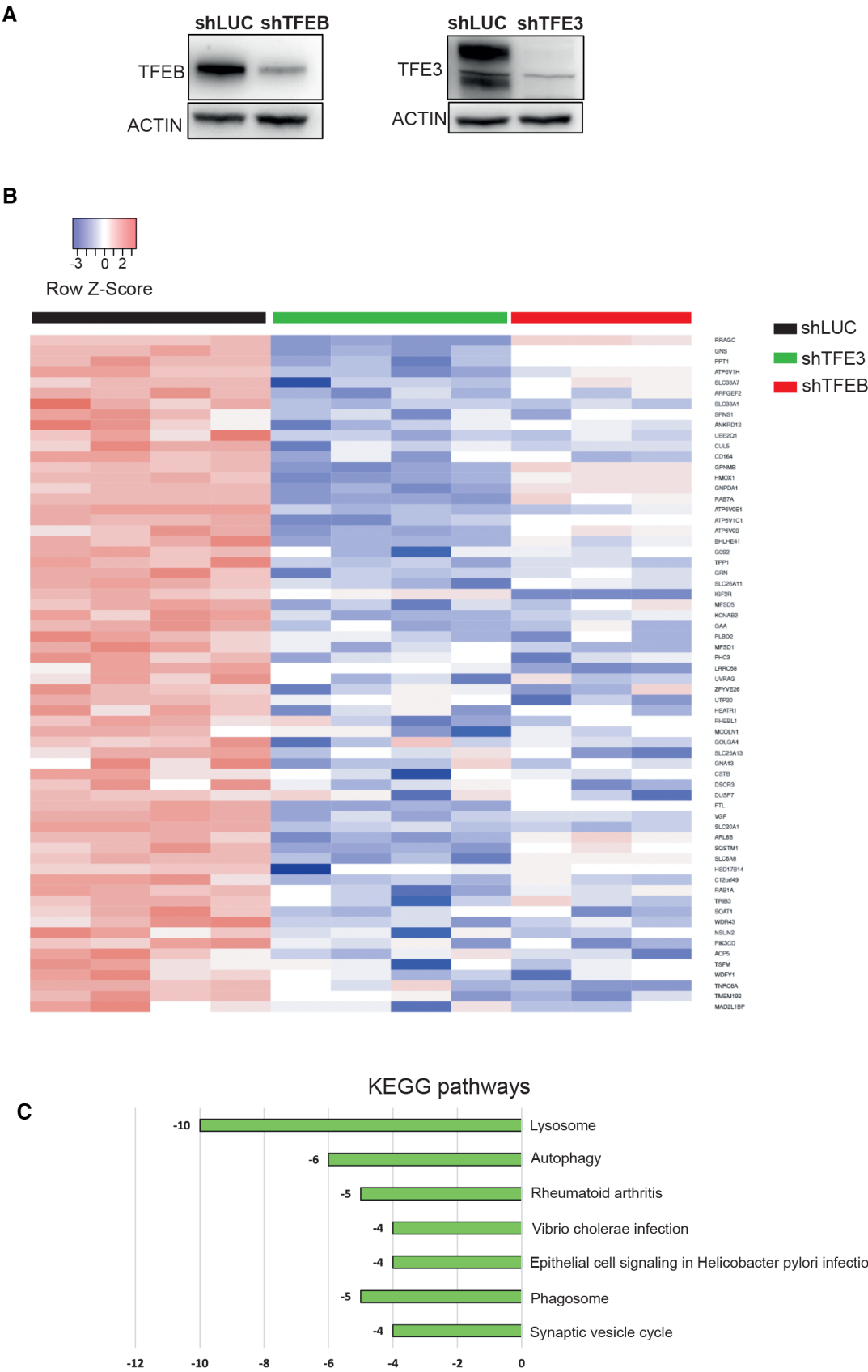


Figure EV4.

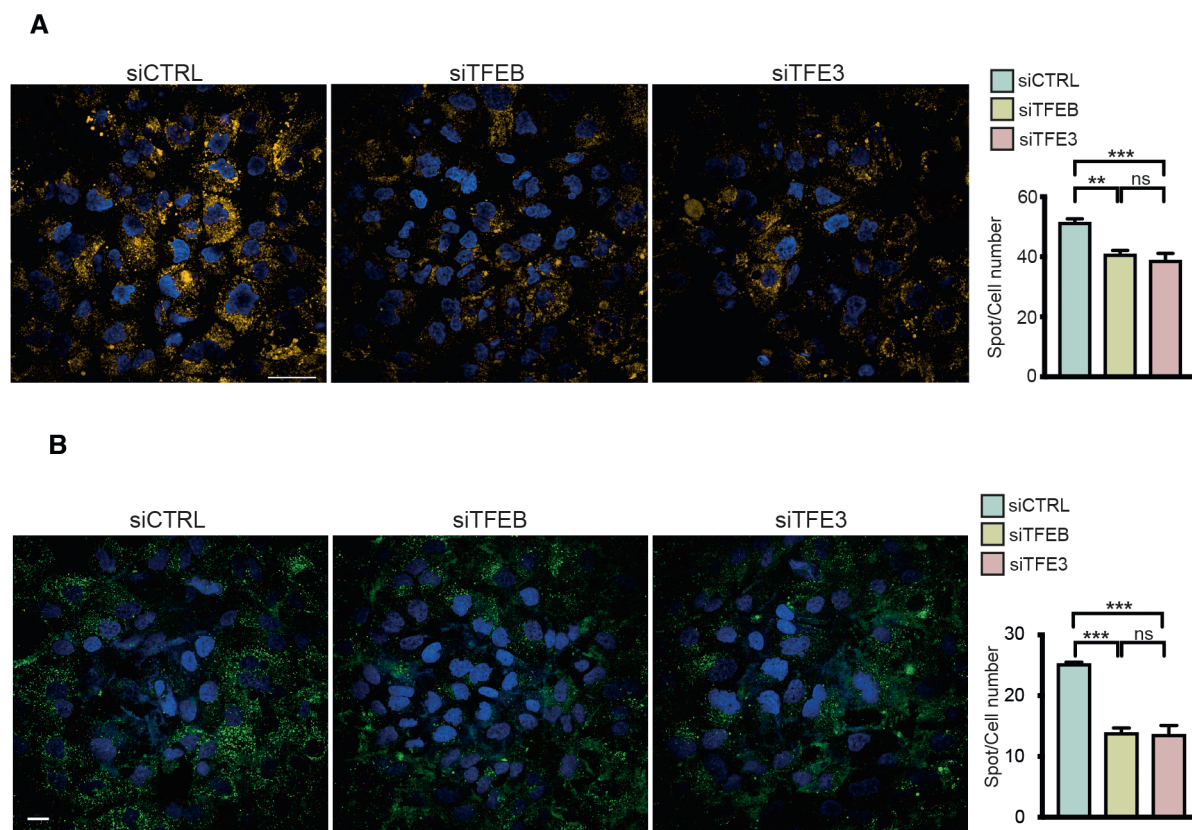


Figure EV5. Silencing of TFEB or TFE3 significantly reduces lysosomal number and degradative ability.

A, B LysoTracker (A) and DQ-BSA (B) analysis of UOK257 cells silenced for TFEB or TFE3 or scramble siRNA (CTRL). Scale bars: 50 μ m in (A) and 20 μ m in (B). Plots represent number of spots/cell and are expressed as mean $\pm$ SE ($n = 3$ biological replicates), ordinary one-way ANOVA, Tukey's multiple comparisons test.

Data information: ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Source data are available online for this figure.