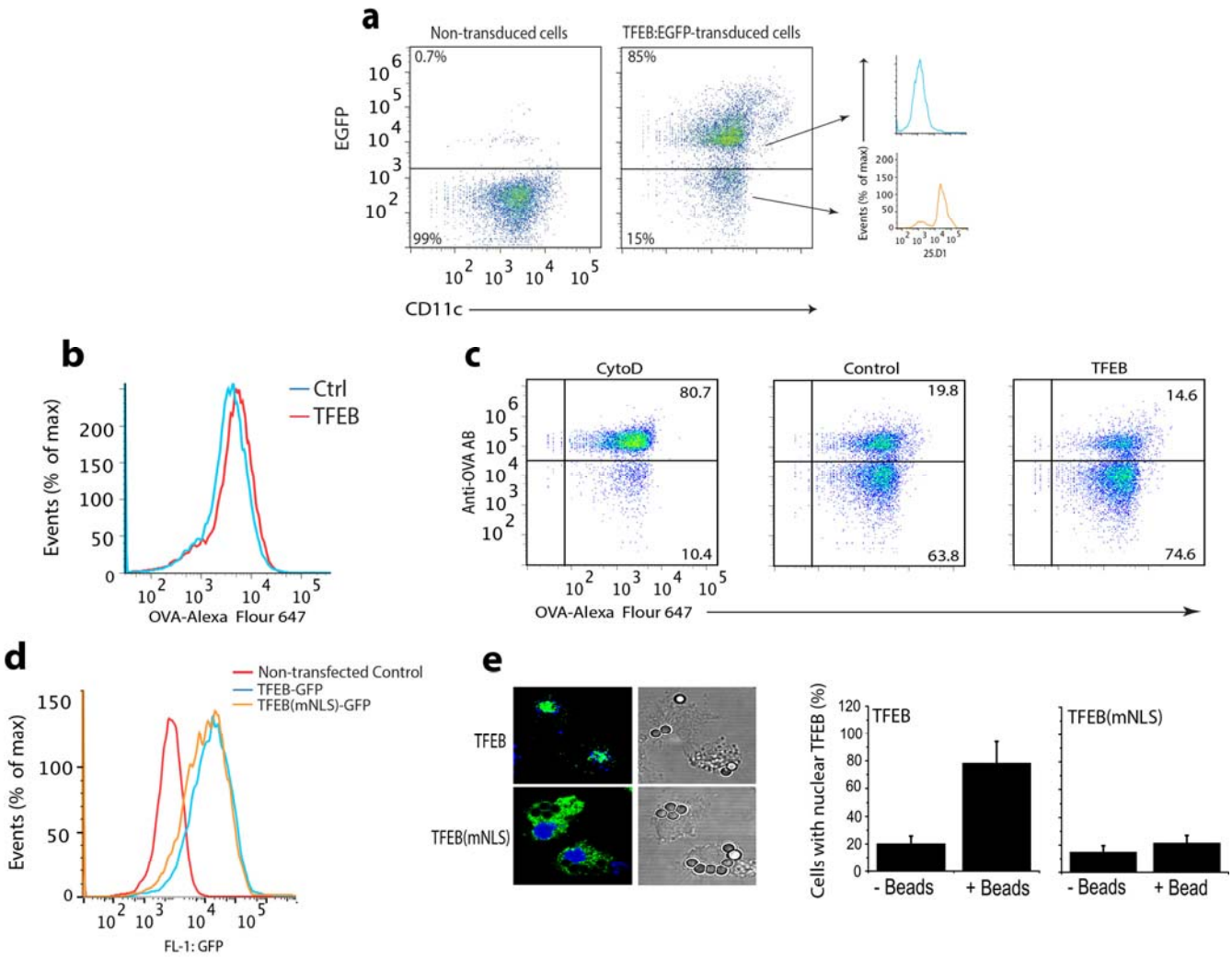
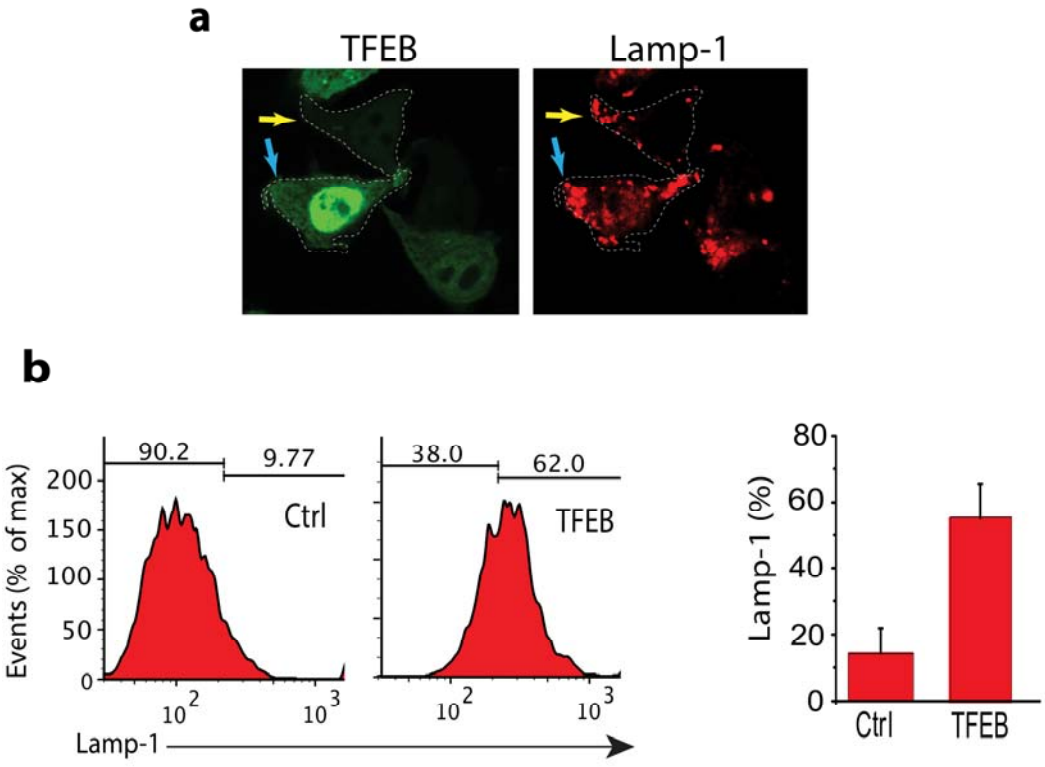


Supplementary Figure 1

Comparable uptake of soluble OVA and of OVA-coated beads in TFEB-transduced or non-transduced DCs.

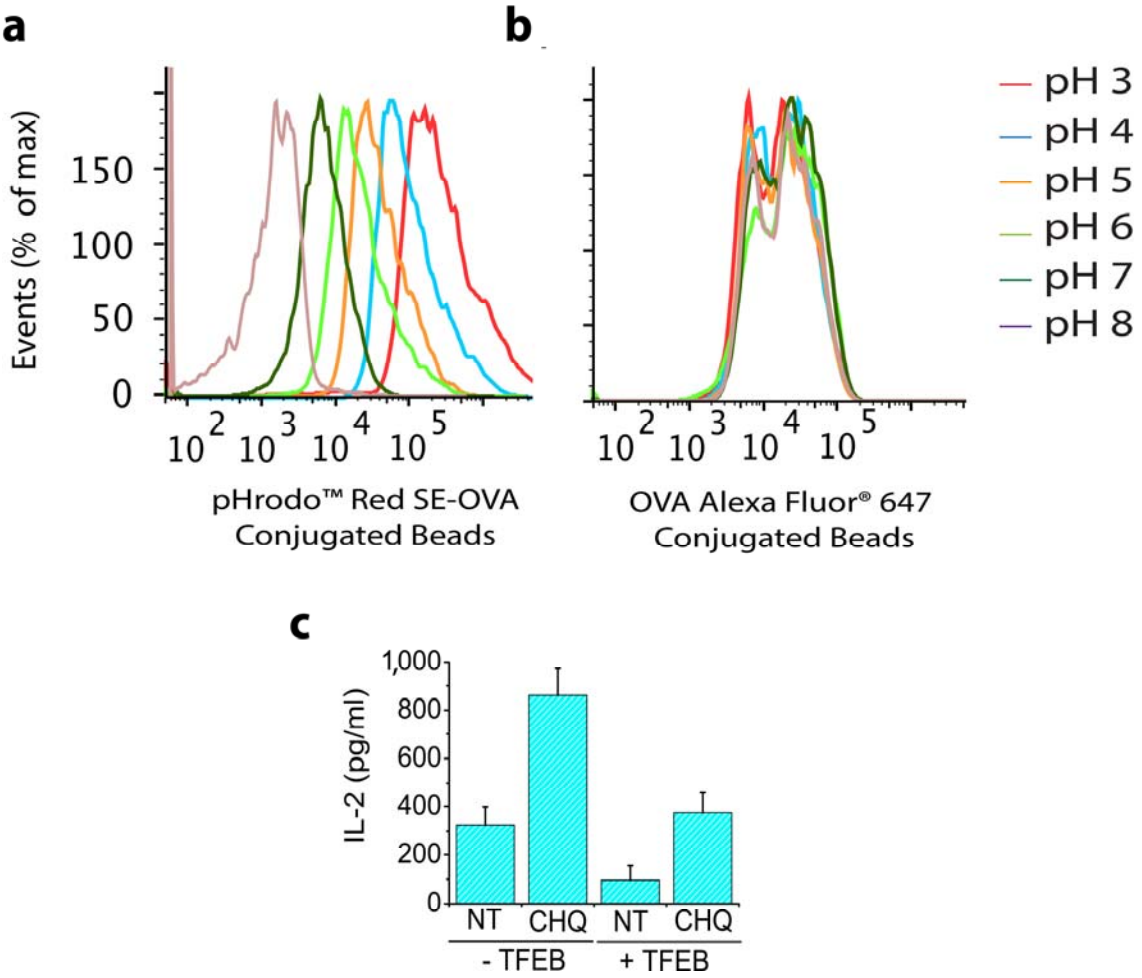
(a) The efficiency of TFEB viral transduction is displayed. The surface expression of K^b- SIINFEKL in TFEB-transduced and non-transduced population is evaluated by 25.D1 antibody. (b) TFEB transduced and non-transduced DCs were fed with 2 mg/ml soluble OVA conjugated with alexa fluor 647 for 1 hour. Cells were then collected and the MFI of ingested OVA was evaluated by flow cytometry (c) Uptake of OVA coated beads in TFEB-EGFP-transduced and control-EGFP-transduced BMDCs were investigated using flow cytometry. (d) TFEB-EGFP and TFEB(mNLS)-EGFP constructs are comparably expressed in transduced DCs. (e) DCs were transduced with TFEB-EGFP or TFEB(mNLS)-EGFP and then exposed to OVA coated beads for 30 minutes. The un-ingested beads were washed away and cells were processed for confocal microscopy to investigate the nuclear translocation of TFEB.



Supplementary Figure 2

TFBE induces lysosomal biogenesis in DCs.

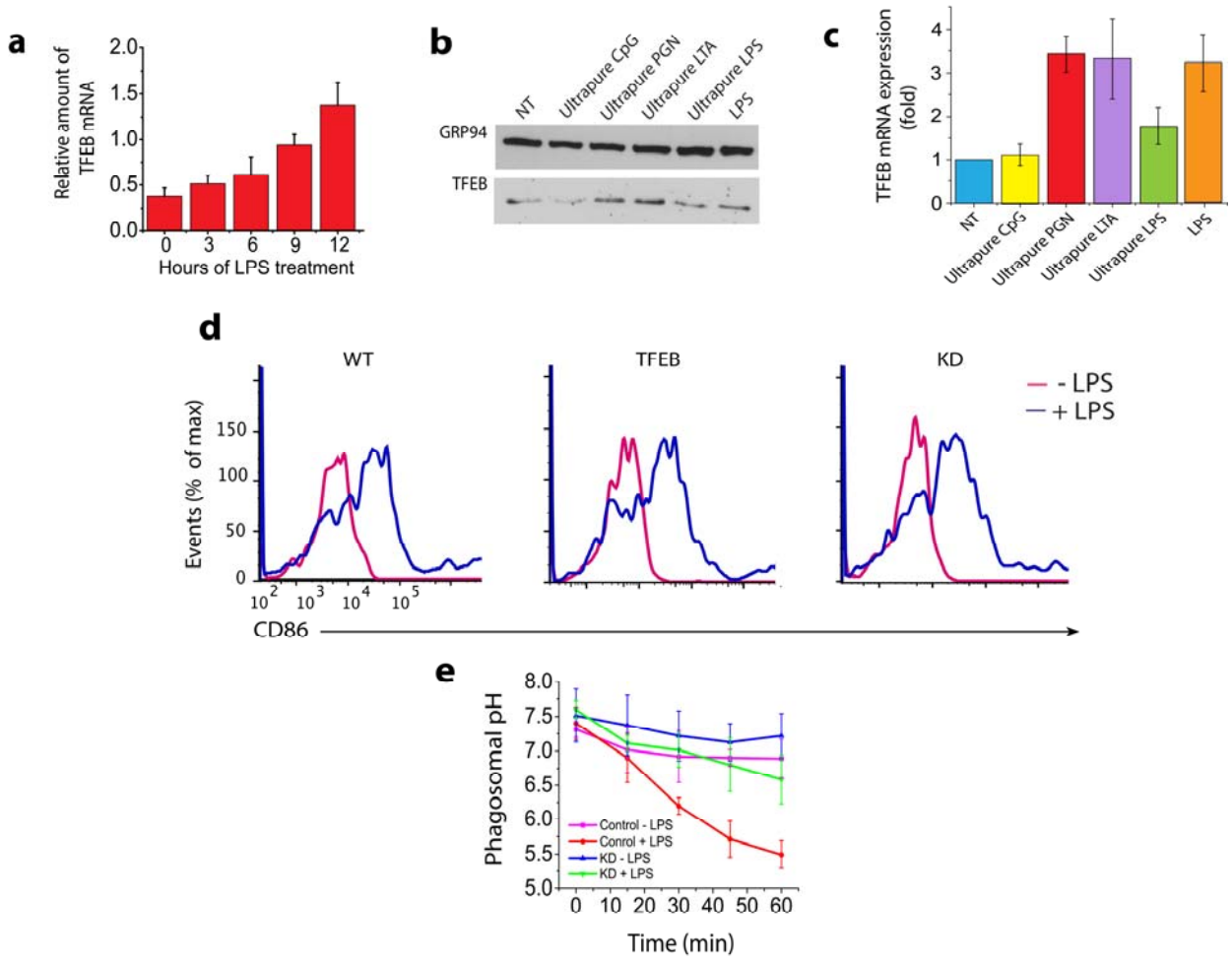
(a) Immunofluorescence confocal microcopy images illustrating Lamp-1 positive compartments in TFEB-EGFP transduced and non-transduced BMDCs. (b-c) Histograms showing flow cytometry analysis of Lamp1 positive compartments in BMDCs transduced with TFEB.



Supplementary Figure 3

Analysis of phagosomal pH.

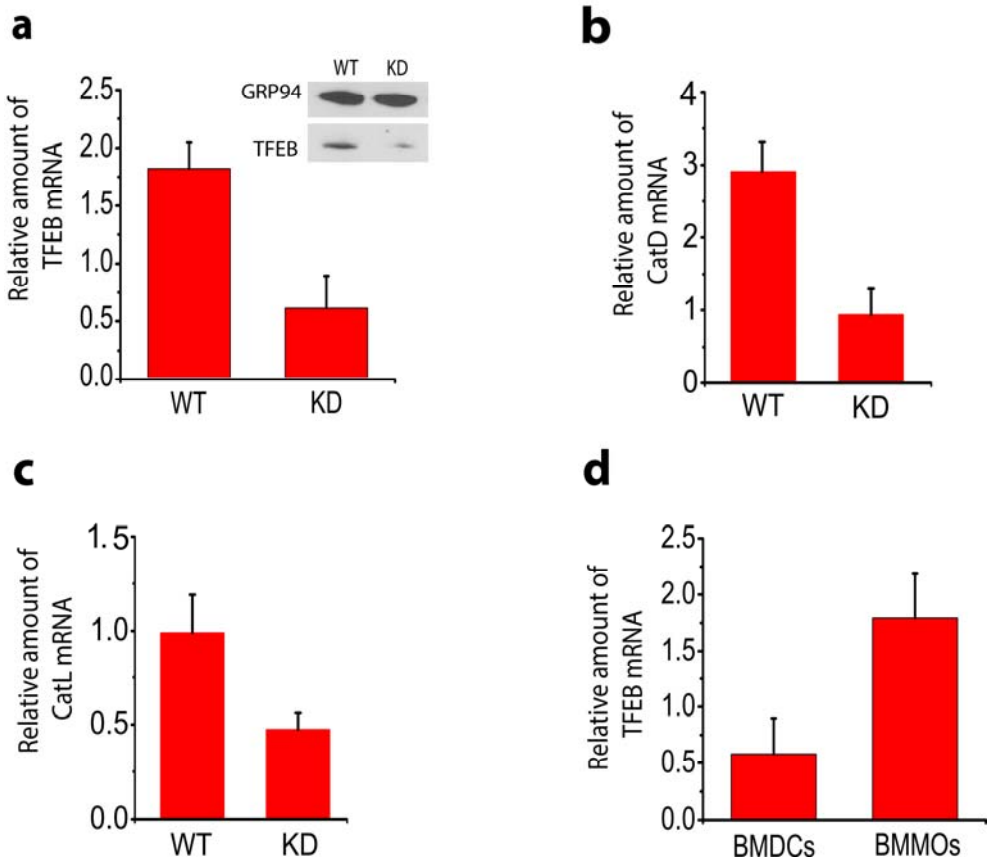
(a-b) DCs were allowed to uptake polystyrene beads coated with OVA-pHrodo (pH sensitive) and OVA-647 (pH insensitive) fluorescent probes for 30 min. Un-ingested beads were washed away and cells were incubated for an additional 60 minutes. Cells were then resuspended in media resembling intra-lysosomal ionic composition with fixed pH, ranging from 3 to 8, containing 0.1% Triton X100. Graphs represent flow cytometry analysis for phagosomal pH calibration, showing MFI of the OVA-SE dye (a) and OVA-647 (b) at different pH. (c) The TFEB-mediated decrease in cross-presentation depends on reduced lysosomal acidification. Cross-presentation was partially rescued in TFEB-transduced BMDCs after CHQ treatment.



Supplementary Figure 4

TLR ligands induce TFEB expression, while TFEB has no effect on TLR-induced maturation of BMDCs.

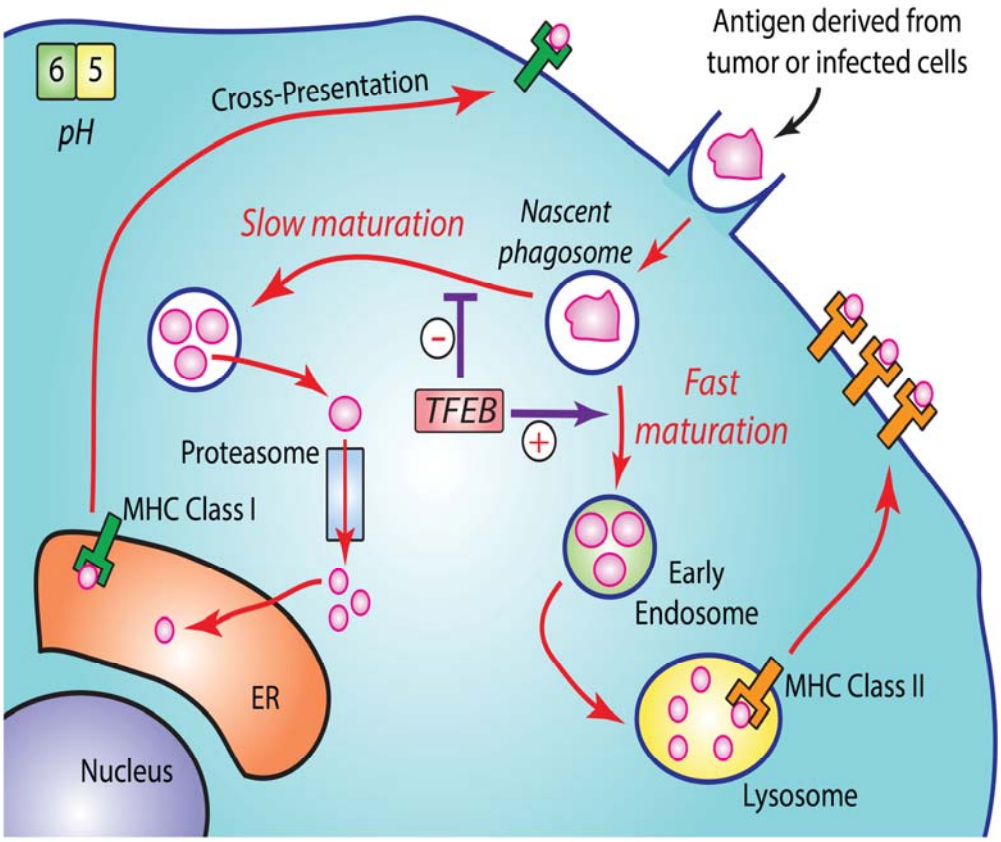
(**a**) BMDCs were treated with LPS for different time periods. After each time point cells were collected and their mRNA was extracted. The amount of cDNA synthesized form mRNA was quantified by using qRTPCR using primers specific for TFEB. **(b-c**) TFEB expression was analyzed by western blot and qRTPCR after cells were exposed to crude LPS extracted from E.coli, ultrapure LPS, ultrapure PGN, ultrapure LTA, and ultrapure CpG for 24 hours. (**d**) DCs were cultured overnight with LPS and analyzed by flow cytometry to evaluate maturation. CD86 expression. (**e**) Kinetics of phagosomal acidification in TFEB-KD or control BMDCs with or without LPS treatment.



Supplementary Figure 5

The expression of cathepsins D and L is downregulated in BMMs in which TFEB is knocked down.

Cells were collected and their mRNA was extracted from silenced TFEB BMMs. The amount of cDNA synthesized form mRNA was quantified by using QPCR using primers specific for (**a**) TFEB (**b**) Cat D (**c**) Cat L (**d**) The mRNA levels of TFEBin BMMs and BMDCs.



Supplementary Figure 6

TFEB regulates exogenous antigen presentation by inducing phagosomal maturation.

Upon phagosome formation in DCs, the newly formed phagosomes can potentially go through two different maturation routes, slow or fast maturation pathways. Slow maturation leads to antigen escape from the endosomes and MHC class I antigen cross-presentation. In contrast, fast maturation pathway leads to lysosomal degradation and MHC class II antigen presentation. TFEB promotes fast maturation pathway by inducing lysosomal overall function and trafficking. This leads to the inhibition of cross-presentation pathway and the induction of antigen presentation through MCH class II.

Supplemental Table 1.

List of primers used for qRTPCR for indicated genes.

Name of the gene	Forward primer sequence	Reverse primer sequence
TFEB	CAAGGAGCGGCAGAAGAAAG	GCTGCTTGTTGTCATCTCC
Cathepsin D	AAGGTATCGCAGGGTGGAAA	CTGACAGTGGAGAAGGAGCA
Cathepsin L	AGACCGGCAAACTGATCTCA	ATCCACGAACCCTGTGTCAT
Cathepsin S	CATTGCCTGACACTGTGGAC	CGCCTCCACAGCCTTTATTC
LMP2	CCAACTCAGAAATCCGCCTG	CGGGTGGTAAGGAAGACAGT
TAP2	GGCTCCTTCCTCTTCACCAT	AAGAAGTAGAGCCCCACCAC
L32	TGGTGAAGCCCAAGATCGTC	CTTCTCCGCACCCTGTTGTC