Expanded View Figures

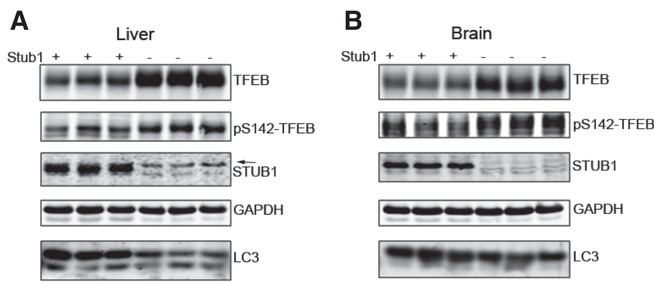

Figure EV1. Accumulation of TFEB in STUB1 knockout mice.

A, B Liver (A) and brain (B) tissues from wild-type (+/+) or STUB1$^{-/-}$ mice were analyzed by Western blot analysis using indicated antibodies. Arrow denotes a previously described non-specific band (Sha *et al*, 2009).

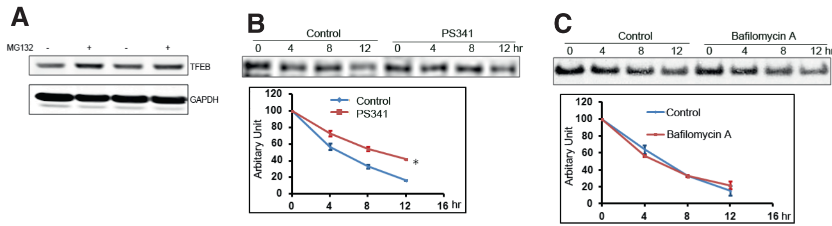

Figure EV2. TFEB is predominantly degraded by proteasome.

A HeLa cells were treated for 2 h with proteasome inhibitor MG132 (50 μM), and cell lysates were analyzed by Western blot.

B, C HeLa cells were mock-treated or treated with proteasome inhibitor PS341 (10 μM; B), or the lysosome inhibitor bafilomycin (10 μM; C), and TFEB half-life was measured using pulse-chase analysis. Data are mean ± SD, $n = 3$. * denotes $P < 0.05$ using two-way ANOVA.

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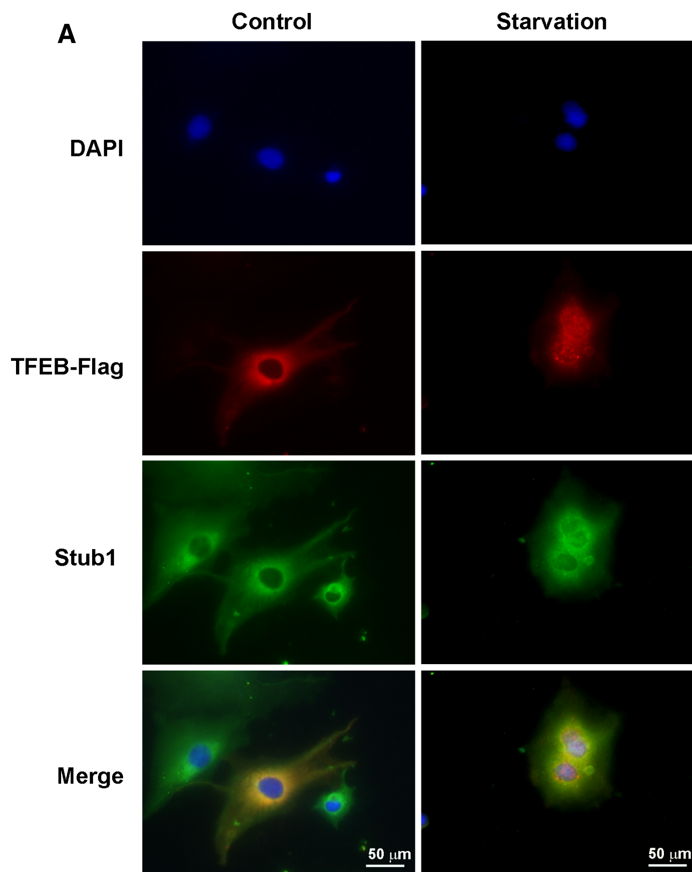

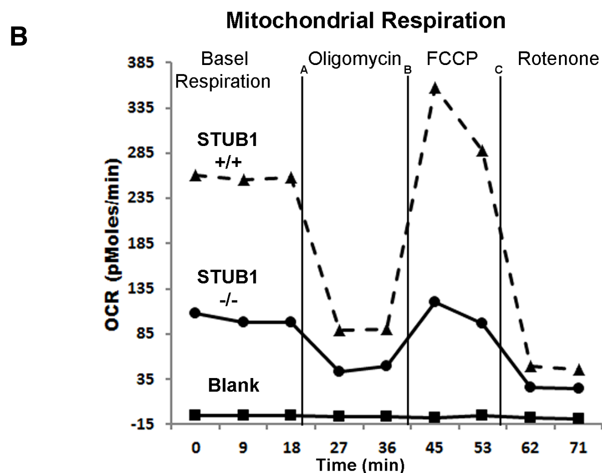

Figure EV3. STUB1 regulates TFEB and mitochonrial respiration.

A TFEB colocalizes with STUB1 in cytosol. HeLa cells stably expressing Flag-TFEB were mock-treated or starved for 2 h and analyzed by immunofluorescence using Flag or Stub1 antibodies. Scale bar, 20 µm.

B STUB1 deficiency reduced mitochondrial biogenesis. Wild-type (+/+) or STUB1$^{-/-}$ MEFs were subjected to mitochondrial respiration analysis.

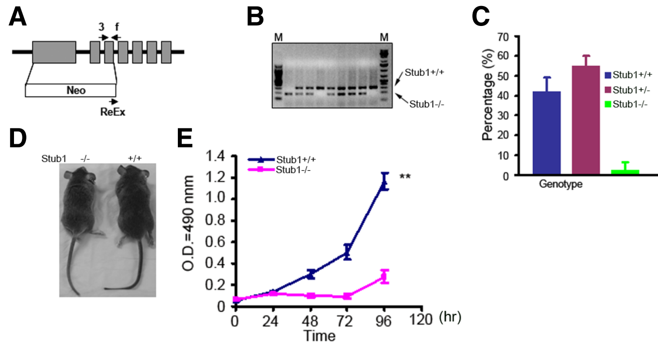

Figure EV4. STUB1 knockout mice exhibit partial neonatal lethality.

A Schematic diagram of generating STUB1 knockout (−/−) mice. Arrows denotes the primers used for genotyping the transgenic mice.

B Genotyping of STUB1$^{-/-}$ mice using PCR.

C Homozygous STUB1 knockout mice are significantly lower than the expected Mendelian inheritance.

D STUB1$^{-/-}$ mice are smaller than wild-type (+/+) mice.

E Proliferation assay of MEFs from STUB1$^{-/-}$ and +/+ mice.

Data information: Data are mean ± SD, $n = 3$ (C) or 6 (E). ** denotes $P < 0.01$, using Student's t-test analysis.

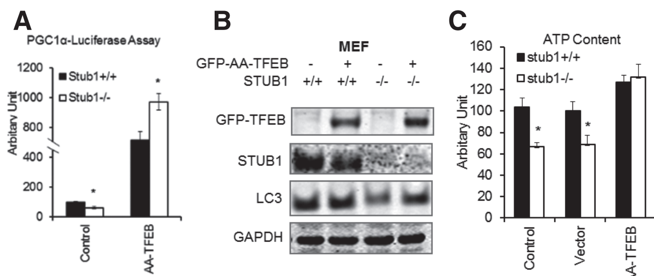

Figure EV5. Overexpression of the non-phosphorylatable TFEB mutant rescues autophagy and mitochondrial biogenesis in STUB1 knockout MEFs.

MEF cells from wild-type (+/+) or STUB1$^{-/-}$ mice were used.

A MEFs were transfected for 24 h with vector only (control) or TFEB-S142A/S211A mutant (GFP-AA-TFEB) and then transfected for another 24 h with PGC1α promoter–luciferase before luciferase activity was analyzed.

B MEFs were transfected for 48 h with vector only or GFP-AA-TFEB, and cell lysates were analyzed by Western blot.

C MEFs were treated with transfection reagent only (control) or transfected with vector only or GFP-AA-TFEB. ATP content in cell lysates was analyzed 48 h post-transfection.

Data information: Data are mean ± SD, $n = 3$. * denotes $P < 0.05$ using Student's t-test analysis.

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