

## Appendix

### **STUB1 Regulates TFEB-Induced Autophagy-Lysosome Pathway**

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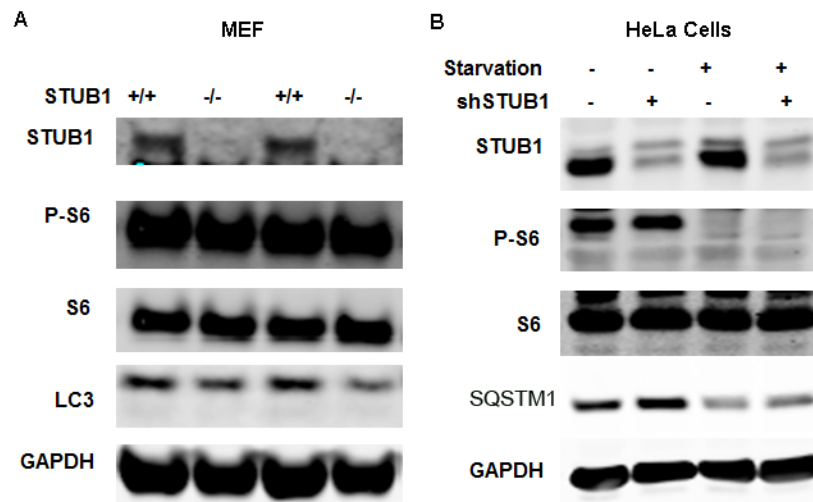
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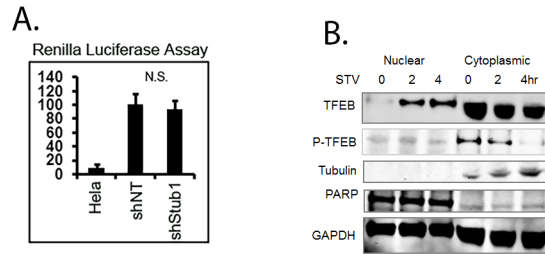
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## Appendix Supplementary Figures

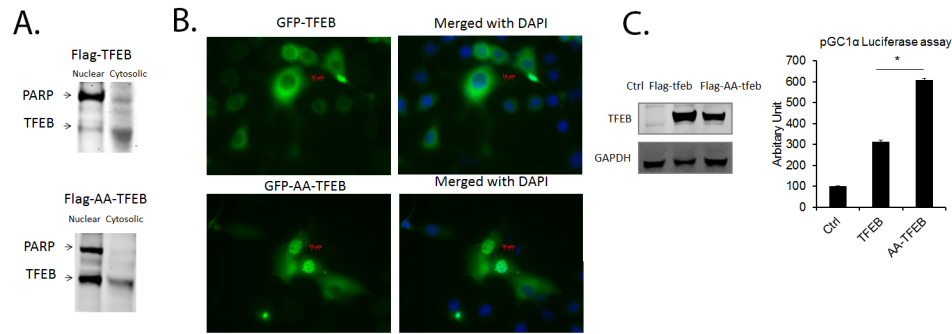


### Appendix Figure S1. mTOR signal pathway is not affected by STUB1 deficiency.

(A) MEF cells from wild type (+/+) or STUB1<sup>-/-</sup> mice were analyzed by Western blot analysis using indicated antibodies. (B) HeLa cells stably expressing control shRNA or STUB1 specific shRNA were mock-treated or treated by starvation. Cell lysates were then analyzed by Western blot using indicated antibodies.



**Appendix Figure S2. A. STUB1 deficiency reduced TFEB activity.** HeLa cells, stably expressing shNT or shSTUB1, were transfected for 24 hr with a Renilla luciferase vector and luciferase activity was analyzed. N.S. denotes non significant. **B. Starvation increases nuclear translocation of non-phosphorylated TFEB.** HeLa cells were mock-treated or starved for 2 or 4 hr. Cell lysates, were then fractionated into nuclear and cytoplasmic fractions and were analyzed by Western blot. Data are representative of three independent experiments



**Appendix Figure S3. Non-phosphorylatable TFEB (Ala-TFEB) have increased translocation to the nucleus and activity towards PGC1 $\alpha$  promoter.** HeLa cells were transfected, for 48 hr, with Flag-TFEB or TFEB-S142A/S211A nonphosphorylatable mutant (Flag-AA-TFEB). Cell lysates, were then fractionated into nuclear and cytoplasmic fractions and were analyzed by Western blot (**A**) or by fluorescence microscopy (**B**). HeLa cells stably expressing Flag-TFEB or Flag-AA-TFEB were co-transfected for 48 hr with PGC1 $\alpha$ -Luciferase and Luciferase activity assay was performed (**C**). \*Denotes  $p < 0.05$  using student t-test analysis. Data are representative of three independent experiments