

Abstract

Autophagy is an evolutionarily conserved cell renewal process that depends on phosphatidylinositol 3-phosphate (PtdIns(3)P). In metazoans, autophagy is inhibited by PtdIns(3,4,5)P₃, the product of Class IA PI3Ks, which mediates the activation of the Akt/TOR kinase cascade. However, a direct role of Class IA PI3Ks in autophagy remains undetermined. Class IA PI3Ks are heterodimeric proteins consisting of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. Here we show that the Class IA p110β catalytic subunit is a positive regulator of autophagy. Gene deletion of p110β results in impaired autophagy in murine embryonic fibroblasts, liver, and heart. p110β does not promote autophagy by affecting the Akt/TOR pathway. Rather, it associates with the autophagy-promoting Vps34-Vps15-Beclin 1-Atg14L complex and facilitates the generation of cellular PtdIns(3)P. Our results unveil a previously unknown function of p110β as a positive regulator of autophagy in multicellular organisms.

p110α and p110β differentially regulate autophagy

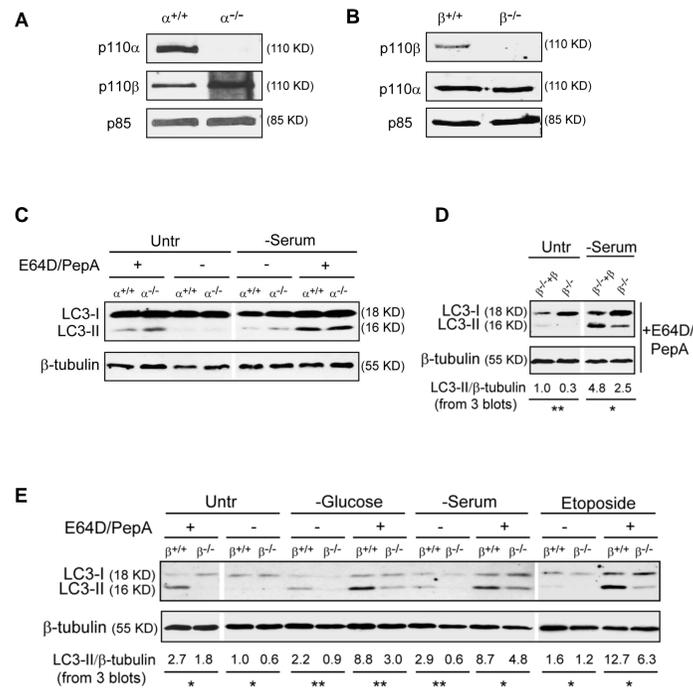


Figure 1. (A and B) MEFs with indicated genotypes were subjected to immunoprecipitation using phosphotyrosine peptide-conjugated agarose. The precipitates were analyzed with p110α, p110β, and p85 antibodies. (C-E) MEFs were subjected to indicated treatments, in the presence or absence of lysosomal inhibitors E64D and PepA. Cell lysates were probed for LC3 and β-tubulin. Quantification of LC3-II/β-tubulin from three independent immunoblots is shown in (D) and (E). Data presented are the mean values normalized to β^{+/+} untreated condition. (* p<0.05, ** p<0.005)

p110β knockout MEFs are deficient in autophagy

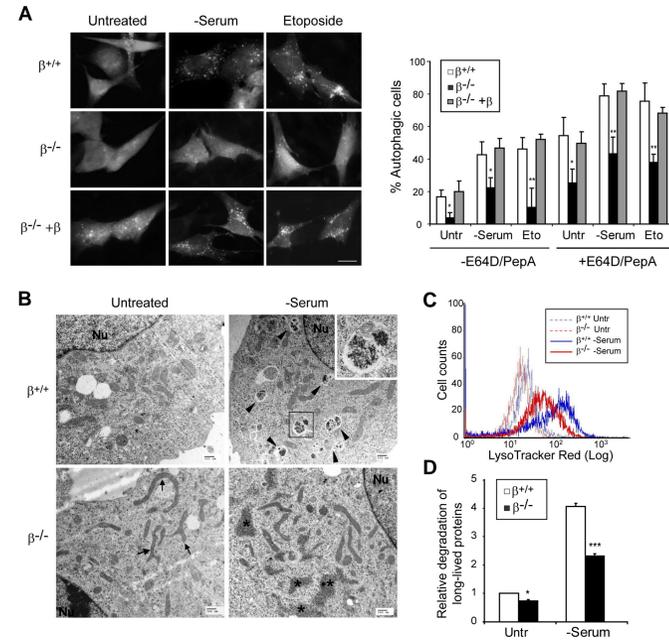


Figure 2. (A) MEFs expressing GFP-LC3 were subjected to indicated treatments. Representative images were taken, and the quantification is shown. Scale bar: 20 μm. (B) Electron microscope images of MEFs. Note the appearance of autophagosomes in serum-deprived β^{+/+} MEFs, indicated by arrowheads. In β^{-/-} MEFs, deformed mitochondria are indicated by arrows, and aggregated ribosomes are indicated by asterisks. (C) Cells were stained with LysoTracker Red, and subjected to flow cytometry analysis. (D) Degradation of long-lived proteins, measured by ¹⁴C-Valine labeled medium. (* p<0.05, ** p<0.005, *** p<0.0001)

p110β positively regulates PI(3)P production and Vps34 kinase activity

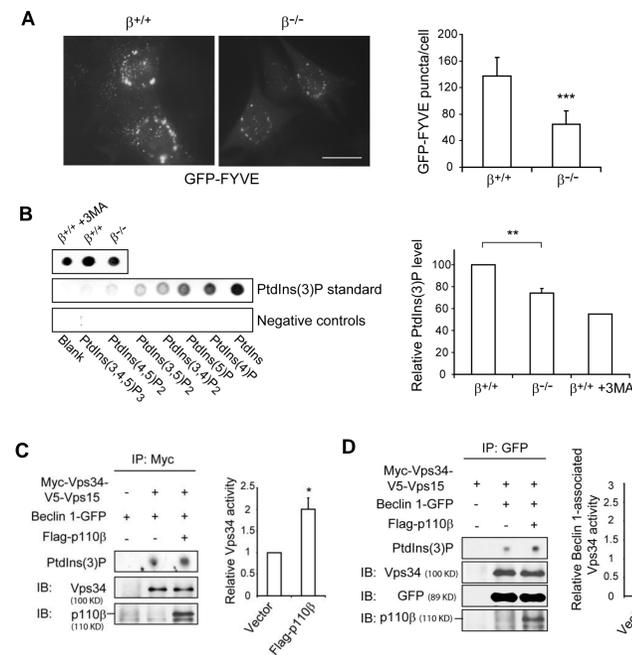


Figure 3. (A) Representative images of MEFs stably expressing GFP-FYVE (left) and quantification of GFP-FYVE puncta per cell (right) are shown. Scale bar: 20 μm. (B) Total lipids were extracted and subjected to protein-lipid overlay analysis for PtdIns(3)P content. PtdIns(3)P standard and other PtdIns species were used as controls. Quantification of total cellular PtdIns(3)P normalized against total protein is shown on the right. (C and D) HEK293T cells were transfected with indicated plasmids. 48 h post transfection, cell lysates were subjected to immunoprecipitation using Myc (C) or GFP antibody (D). Vps34 activity is calculated as the amount of PtdIns(3)P generated normalized against the amount of Vps34 present in the precipitates. (* p<0.05, ** p<0.05, *** p<0.001; error bars: S.E.M.)

p110β associates with the autophagy-essential Vps34-Vps15-Beclin 1-Atg14L complex

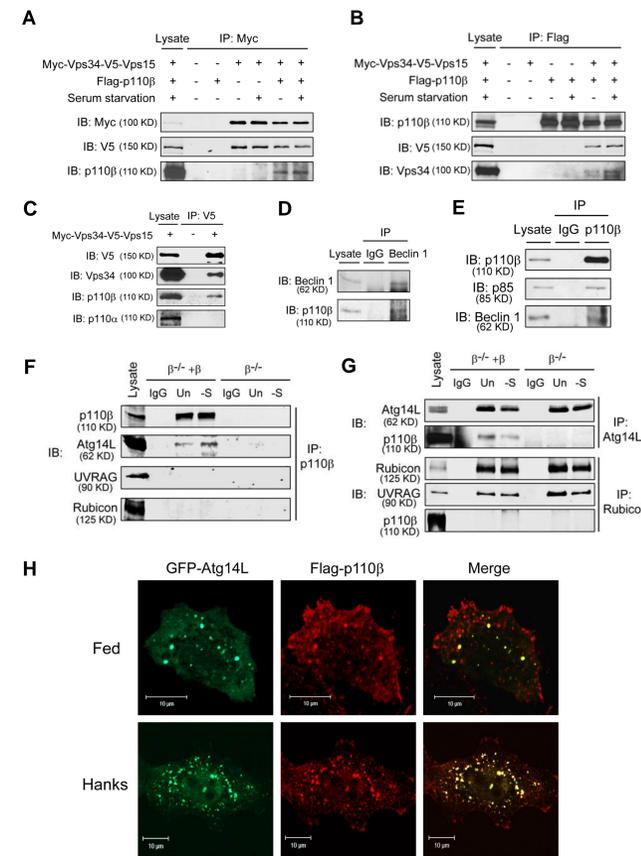


Figure 4. (A-C) HEK293T cells were transfected with indicated constructs. The lysates were subjected to immunoprecipitation and immunoblotting using indicated antibodies. (D and E) Beclin 1 and p110β association at endogenous levels in HEK293T (D) or MCF10A (E). (F and G) MEFs with indicated genotypes were subjected to immunoprecipitation and immunoblotting using antibodies as indicated. (H) HeLa cells transfected with Flag-p110β together with GFP-Atg14L constructs were left untreated or starved in Hanks buffer. The cells were fixed and stained with Flag and Alexa594-conjugated antibodies, and observed under a confocal fluorescence microscope.

p110β positively regulates autophagy in vivo

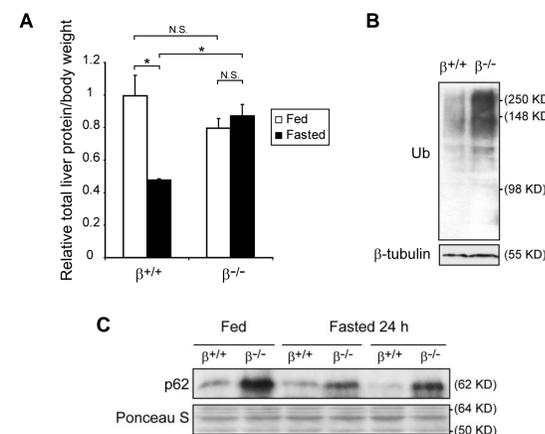


Figure 5. (A) Gender and age-paired 8 to 10 week-old mice with indicated genotypes were fed or fasted for 24 h. Liver weight, body weight, and liver protein concentrations were measured. Data presented are the normalized average values ± S.E.M.; n=5 within each group; * p<0.05; N.S., non-significant. (B) Total lysates were made from β^{+/+} and β^{-/-} livers, and probed for ubiquitin and β-tubulin. (C) Total liver lysates from fed or 24 h-fasted mice with indicated genotypes were generated, and immunoblotted for p62. Ponceau S staining is shown for equal loading.

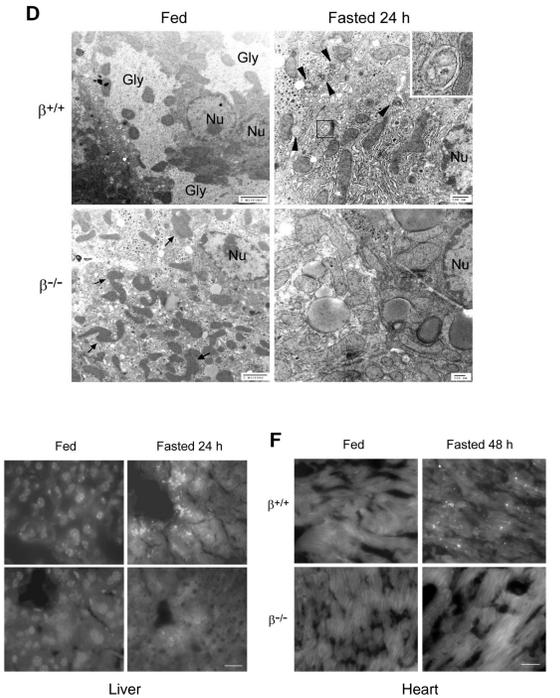


Figure 5-continued. (D) Electron micrographic images of livers from 8-week old fed or 24 h-fasted mice. Deformed mitochondria are indicated by arrow in the β^{-/-} liver. In fasted livers, autophagosomes are labeled with arrowheads. Gly: glycogen area, Nu: nucleus. (E) 8-week-old GFP-LC3 transgenic mice with indicated liver genotypes were fed or fasted for 24 h. Cryosections of the livers were observed under a deconvolution fluorescence microscope. (F) GFP-LC3 transgenic mice with indicated heart genotypes were fed or fasted for 48 h. Heart cryosections were observed and representative images are shown. Scale bar: 20 μm.

Schematic model of p110β in the regulation of autophagy

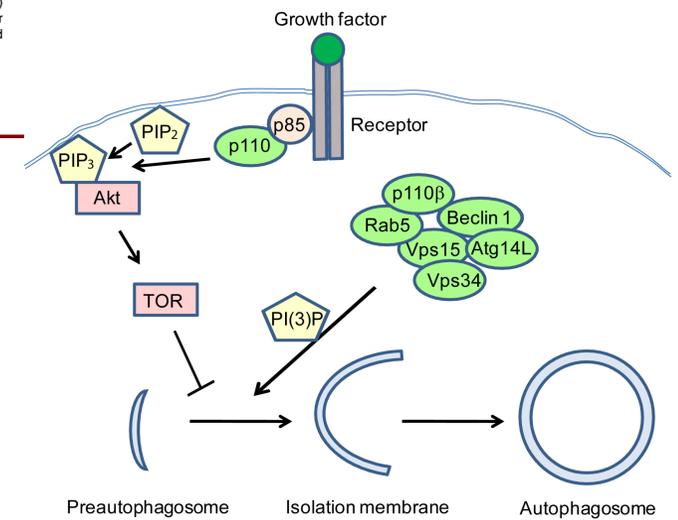


Figure 6. Growth factors activate membrane receptors (such as RTK and GPCR), which recruit p85-p110 and stimulates the production of PtdIns(3,4,5)P₃, which in turn activates the Akt/TOR pathway and inhibits autophagy. On the other hand, p110β (but not p110α) localizes in the autophagy-promoting complex that contains Rab5, Vps34, Vps15, Beclin 1, and Atg14L. The complex produces PtdIns(3)P and facilitates the formation of autophagosomes.