*Supplementary Materials*

# Autophagy alteration in ApoA-I related systemic amyloidosis

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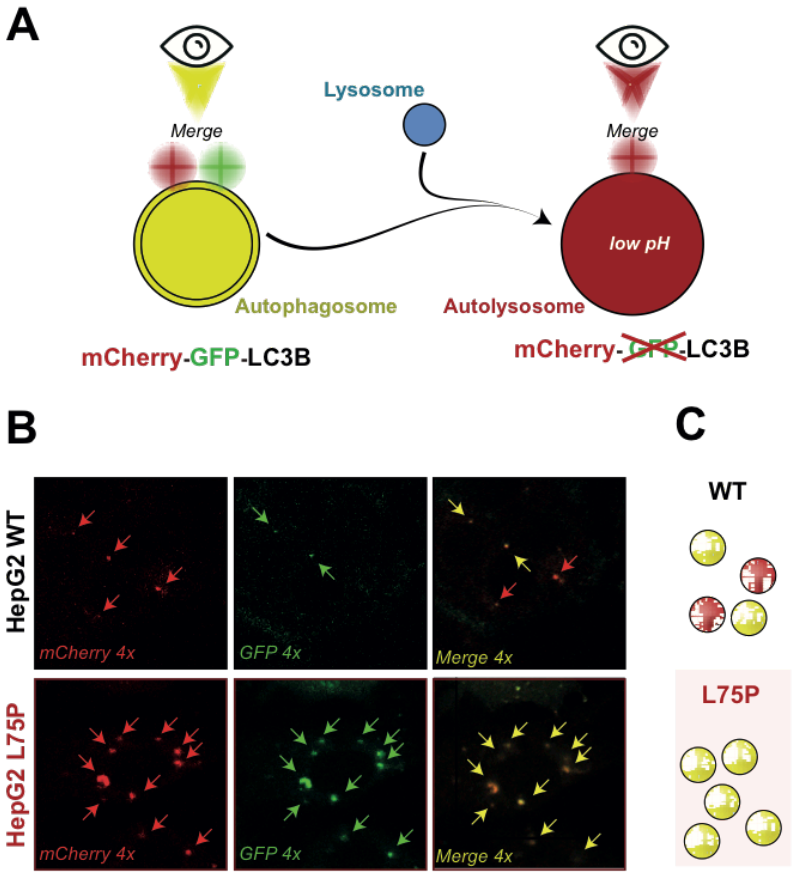


**Figure S1. The mCherry-GFP-LC3 tandem fluorescent probe** (**A**). Schematic representation of mCherry-GFP-LC3 tandem fluorescent probe emit yellow fluorescence (green-red merged) in non-acidic structures (phagophores or autophagosomes) and emit in red in the autolysosomes due to quenching of GFP in low pH conditions. (**B, C**). 4x magnification of the pictures shown in the Figure 2K (**B**) and summary (**C**) of the results showed in **Figure 2L**. The red arrows show the fluorescent signal of mCherry, the green arrows show the fluorescent signal of GFP and the yellow arrows show both signals merged. In merged image, the red arrows indicate autolysosomes (green signal has been lost in contact with acidic pH) and the yellow arrows indicate the presence of autophagosomes (green signal is intact).

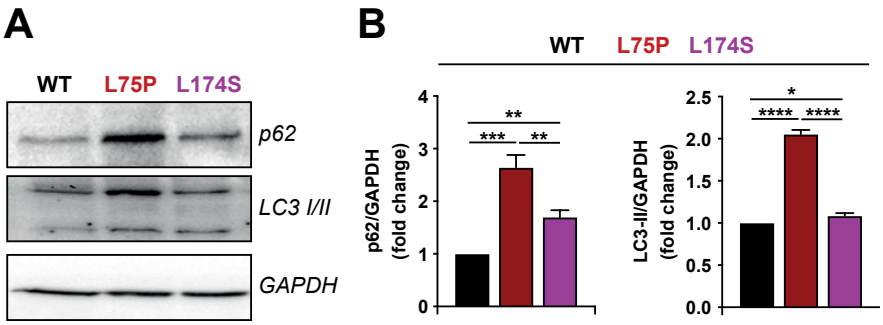


**Figure S2. Measurement of autophagy in L174S-ApoA-I expressing cells.** Wild type (WT), L75P-ApoA-I (L75P) and L174S-ApoaA-I (L174S) HepG2 cells were maintained in control conditions. Thereafter, the LC3 lipidation and p62 expression levels were evaluated by immunoblot (**A**). GAPDH levels were monitored to ensure equal loading of lanes, and densitometry was employed to quantify the abundance of lipidated LC3 (LC3-II) and p62 (both normalized to GAPDH levels) (**B**). One representative experiment out of three performed is showed. Data are means ± SD of at least three independent experiments; *p< 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001.

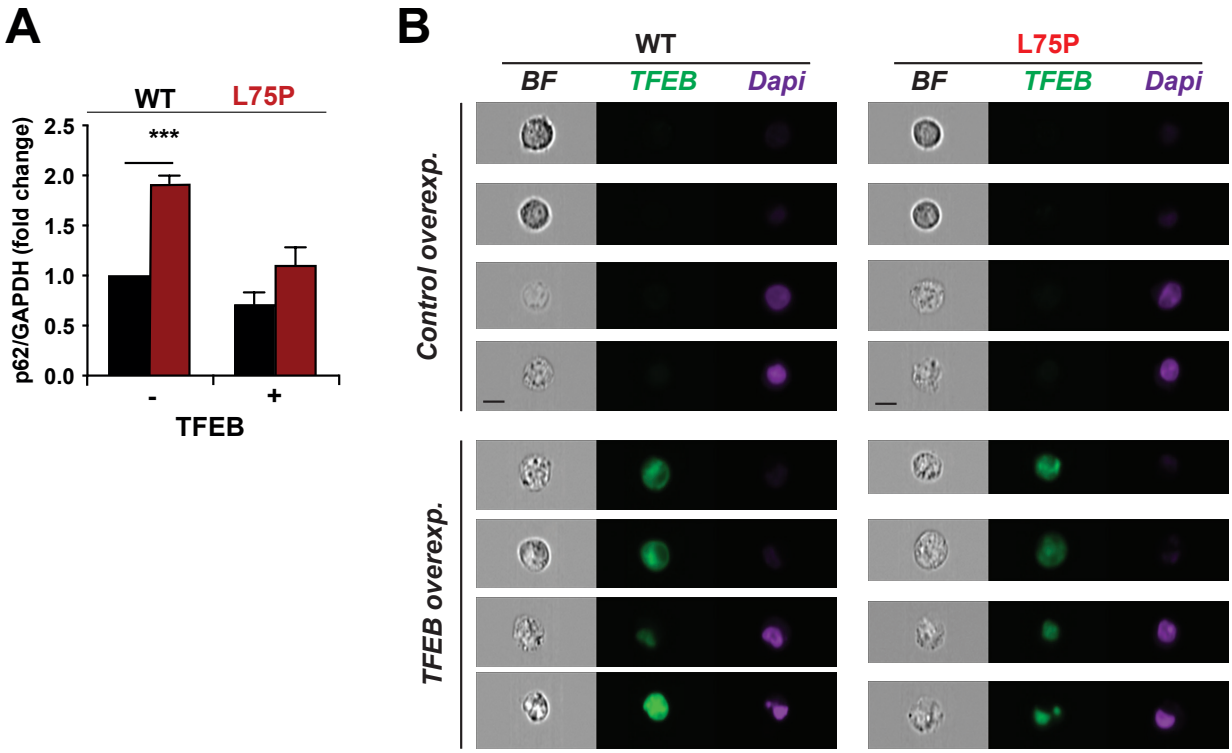


**Figure S3. GFP-TFEB plasmid transfection.** (**A**) Densitometry of immunoblot showed in **Figure 3F**, employed to quantify the abundance of p62 (normalized to GAPDH levels). Data are means ± SEM of at least three independent experiments; ***p < 0.001. (**B**) Representative images showing Wild type (WT) and L75P-ApoA-I (L75P) HepG2 cells transfected with the empty vector or GFP-TFEB plasmid.