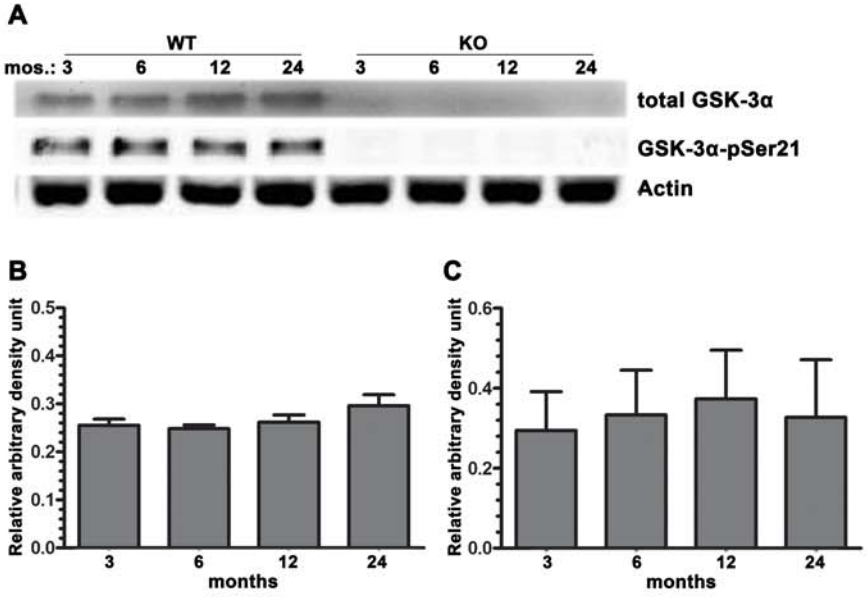
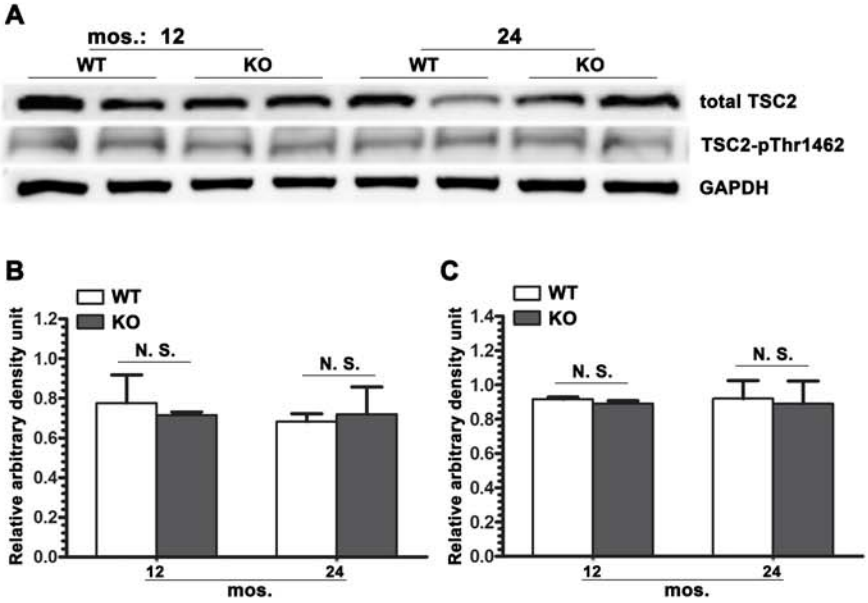
Supplemental Figure 1



Supplemental Figure 1. GSK-3α expression and activity in WT mice with advancing age

1a) Representative immunoblot images for total and phospho-Ser21 GSK-3α of WT or GSK-3α KO mice at 3, 6, 12 and 24 mos. of age. 1b, c) Quantitative analysis of total (1b) or phospho-Ser21 (1c) GSK-3α in WT mice at 3, 6, 12 and 24 mos. of age. There was no significant difference between any two time points for both total and phospho-GSK-3α.

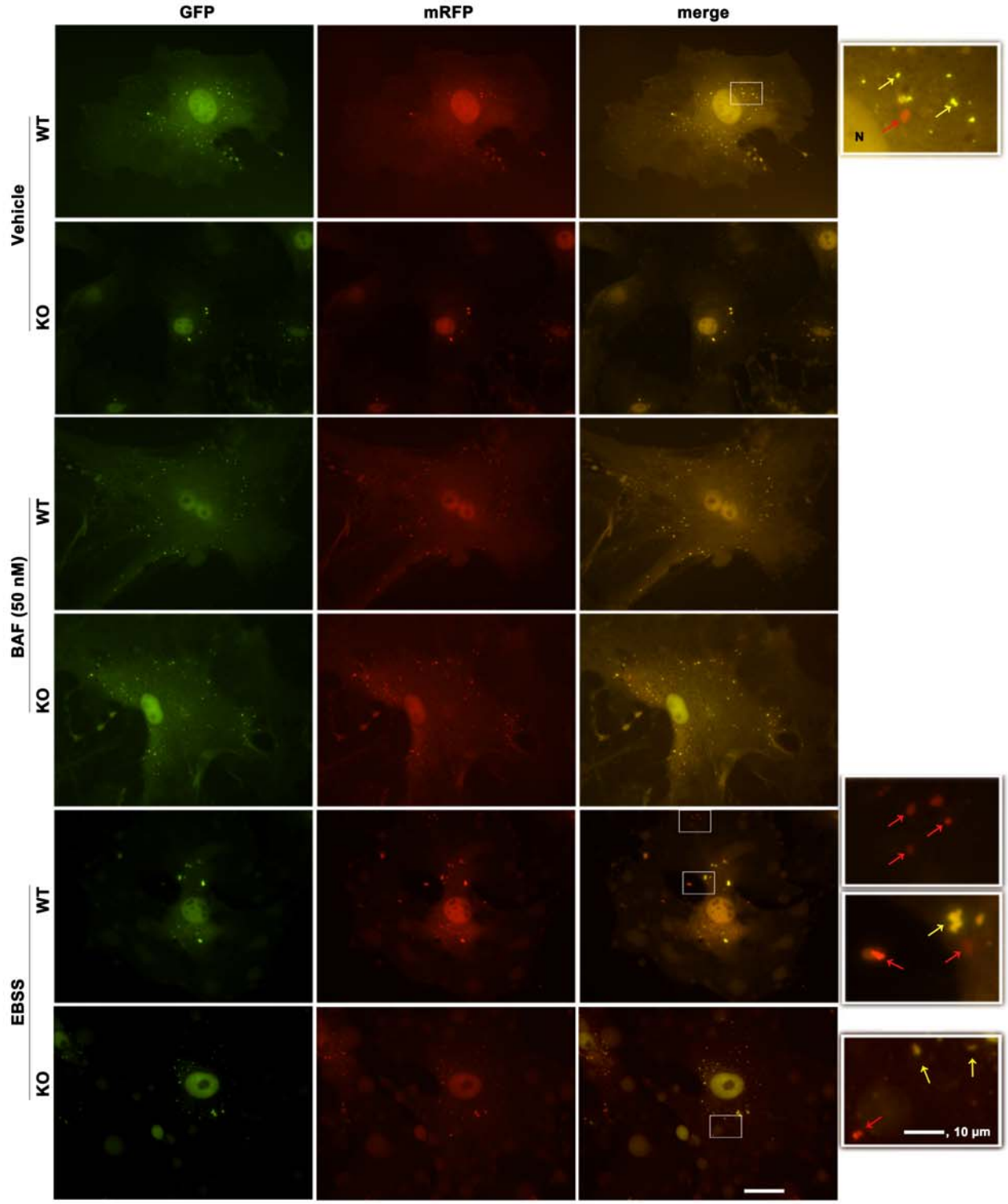
Supplemental Figure 2



Supplemental Figure 2. TSC2 level in GSK-3α KO mice vs. WT at 12 and 24 mos. of age.

2a) Representative immunoblot images for total and phospho-Thr1462 TSC2 of GSK-3α KO and WT mice at 12 and 24 mos. of age. 2b,c) Quantitative analysis of total (2b) or phospho-Thr1462 (2c) of TSC2 of GSK-3α KO vs. WT mice at 12 and 24 mos. of age. There was no significant differences between GSK-3α KO and WT mice for both total and phospho-TSC2.

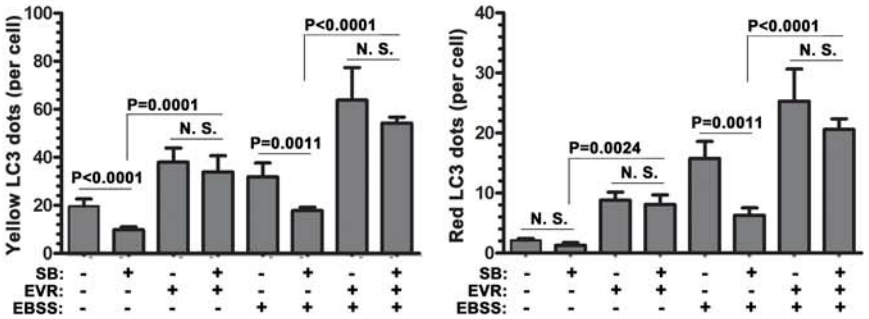
Supplemental Figure 3



Supplemental Figure 3. Starvation-induced autophagic flux is impaired in adult cardiac fibroblasts from GSK-3α KO mice.

Representative fluorescence images of WT or GSK-3α KO adult cardiac fibroblasts transfected with the tandem mRFP-GFP-LC3 adenovirus then starved in EBSS buffer or treated with bafilomycin-A1. The greater magnified images on the far right show arrows indicating yellow and red LC3 dots. Original magnification, ×400; bar: 50 μm.

Supplemental Figure 4



Supplemental Figure 4. mTOR inhibitor rescues impaired autophagy due to inhibition of GSK-3

MEFs were transfected with the tandem mRFP-GFP-LC3 adenovirus. Cells were starved in EBSS buffer in the presence or absence of GSK-3 inhibitor SB216763 (10 μM) and/or mTORC1 inhibitor everolimus (EVR; 100 nM). After four hours, the number of yellow and red LC3 dots per cell was quantified by fluorescence microscopy.