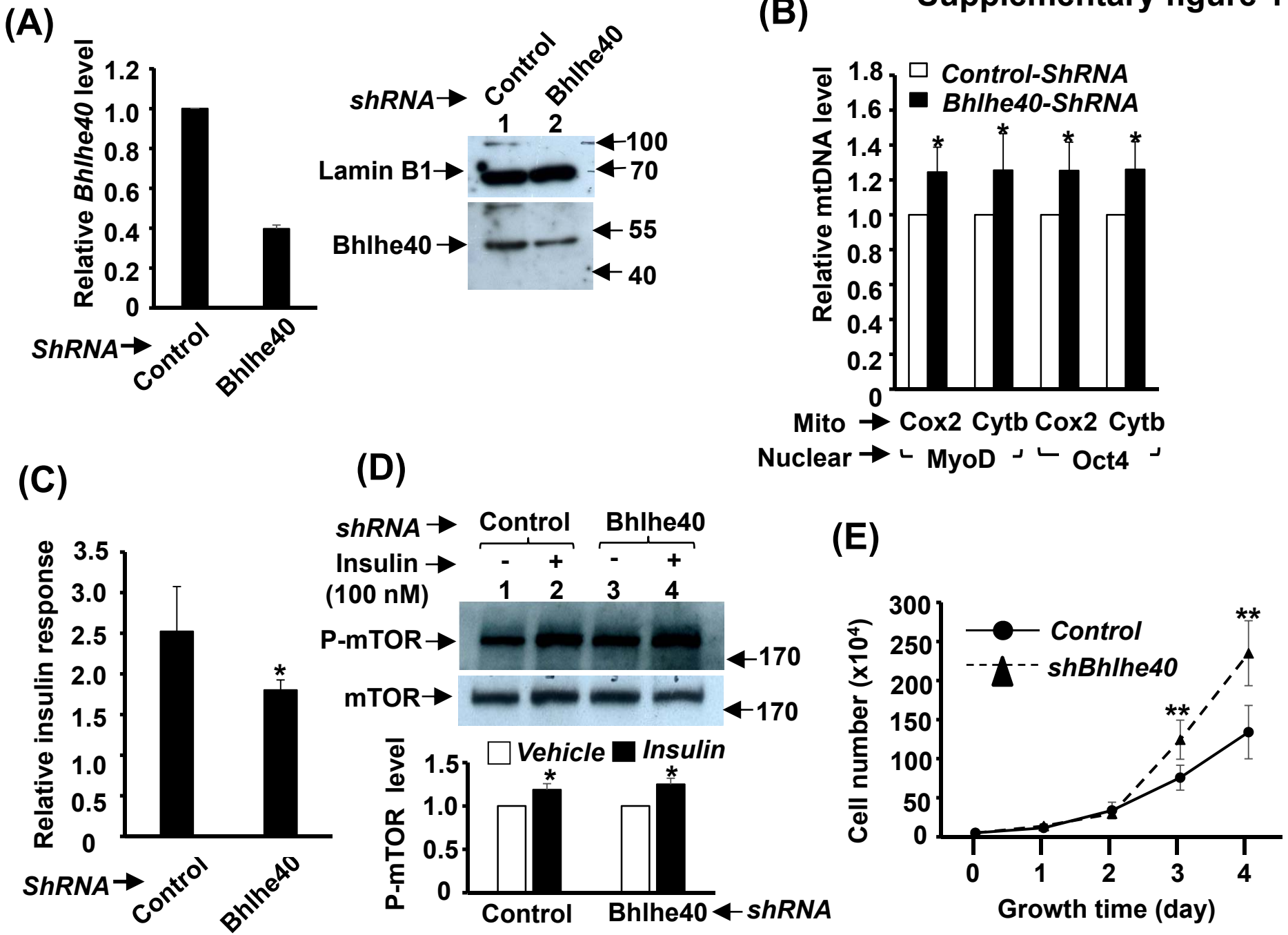
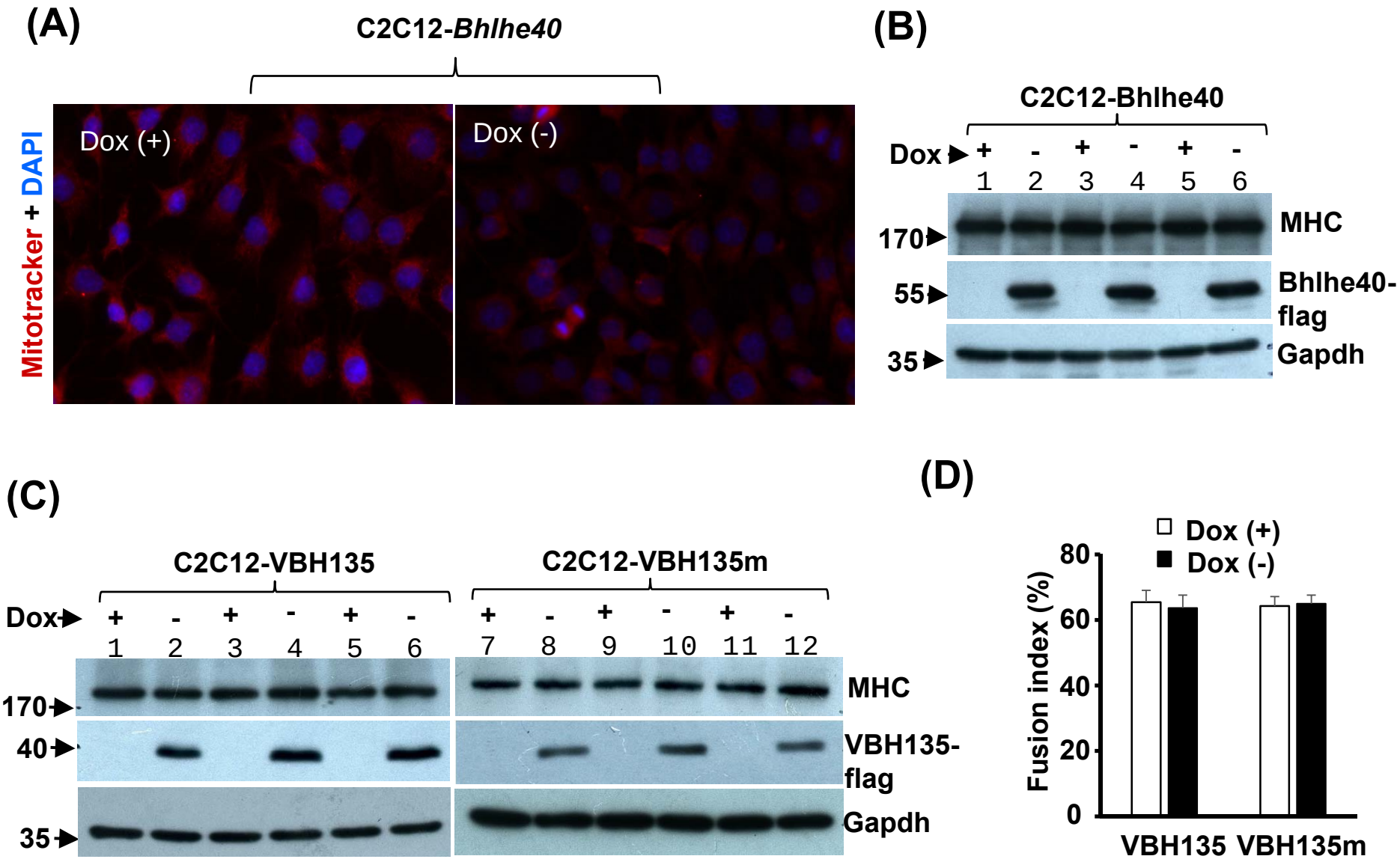
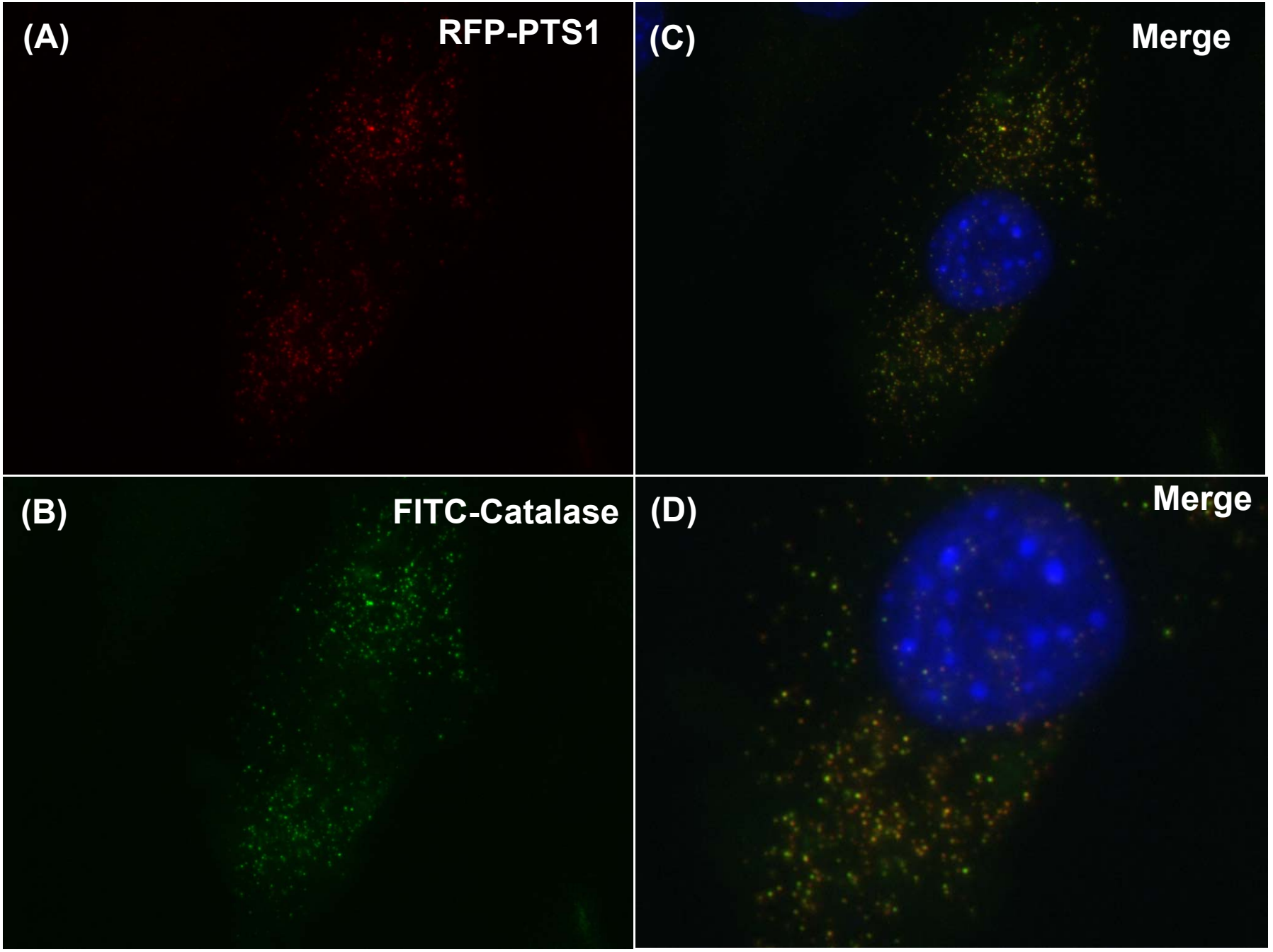
Supplementary figure 1



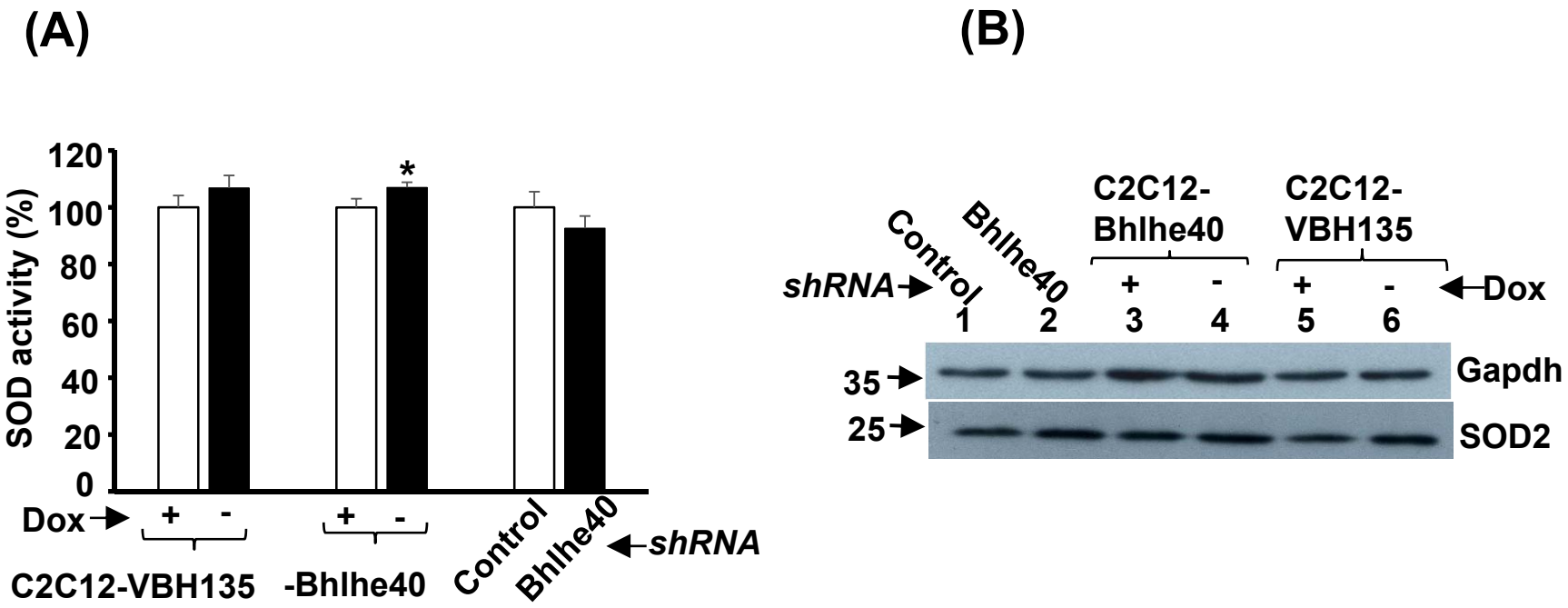
Supplementary figure 2



Supplementary figure 3



Supplementary figure 4



Supplementary figure 1: The differences between *Control* and *Bhlhe40* knockdown cells

(A) The expression levels of *Bhlhe40* were determined by qRT-PCR (left panel) and Western blot (right panel). *Gapdh* and Lamin B1 signals are used as input controls for mRNA and protein, respectively. **(B)** The levels of mitochondrial DNA were examined with quantitative PCR, in which nuclear genes *MyoD* and *Oct4* were used as input control for determining the levels of mitochondrial genes *cytochrome b* and *Cox2*. **(C)** The relative insulin responses of control and *Bhlhe40* knockdown cells. **(D)** Detection of Phospho- and total mTOR in vehicle and insulin treated myoblasts by Western blot. Relative levels of phospho-mTOR are shown at the bottom panel. **(E)** The growth curve of control and Bhlhe40 knockdown cells established during a 4 day culture on 6-well dishes. * and **: $p<0.05$ and $p<0.01$, respectively, vs. control cells.

Supplementary figure 2: The influences of Bhlhe40 or VBH135 on cells

(A) Images of Mitotracker and DAPI stained C2C12-*Bhlhe40* myoblasts. **(B, C)** The protein levels of myosin heavy chain (MHC), Bhlhe40-flag, and VBH135-flag in myotubes (triplicates) of *Tet-off* regulated stable clones were determined by Western blot. **(D)** The fusion indexes of C2C12-VBH135 and -VBH135m after in DM for 4 days.

Supplementary figure 3: The RFP-PTS1 specially marks peroxisomes

Immunofluorescent detection of Catalase was performed on C2C12 cells stably expressing RFP-PTS1 (C2C12-RFP-PTS1). The RFP **(A)** and FITC-labeled Catalase **(B)** images were merged in **(C)** to demonstrate co-localization of both signals. A higher magnification image is shown in **(D)**. All images were taken at 400X magnification.

Supplementary figure 4: SOD activity and *SOD2* expression

Total SOD activity in C2C12-*VBH135* and –*Bhlhe40* myotubes and in C2C12-*Control/-shBhlhe40* myoblasts was determined **(A)**, The SOD2 protein levels under the same condition were determined by Western blot and is shown in **(B)**.