The antioxidant compound tert-butylhydroquinone activates Akt in myocardium, suppresses apoptosis and ameliorates pressure overload-induced cardiac dysfunction

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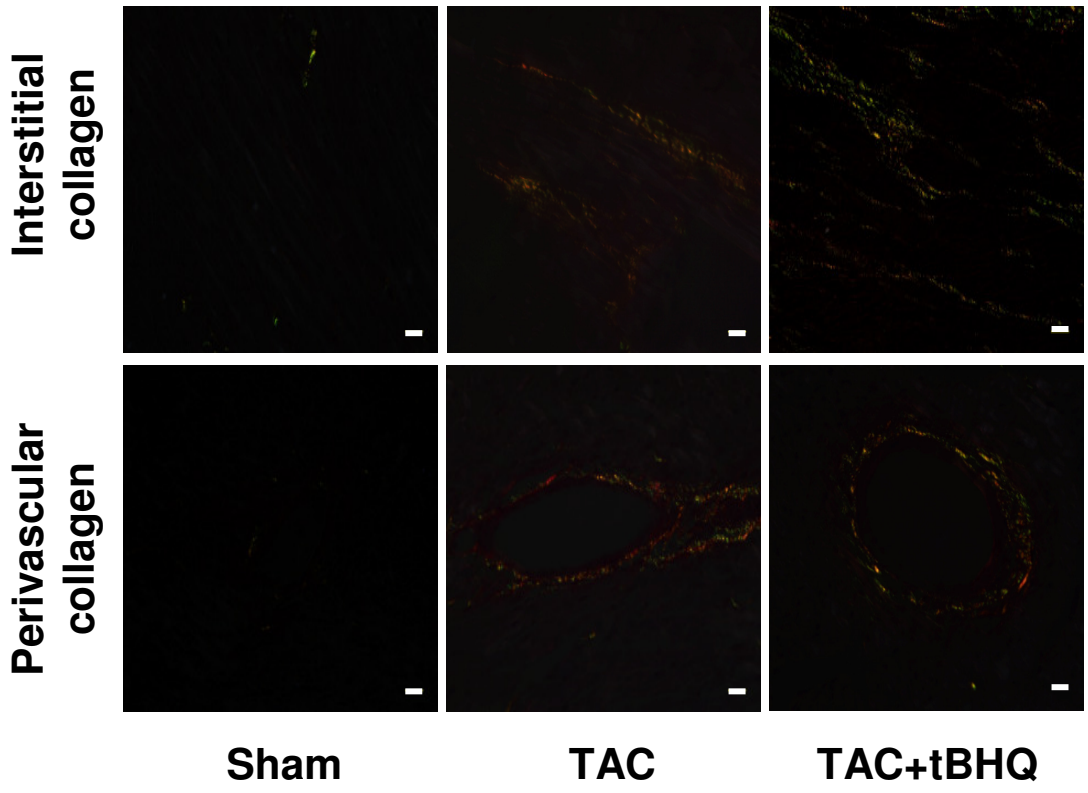
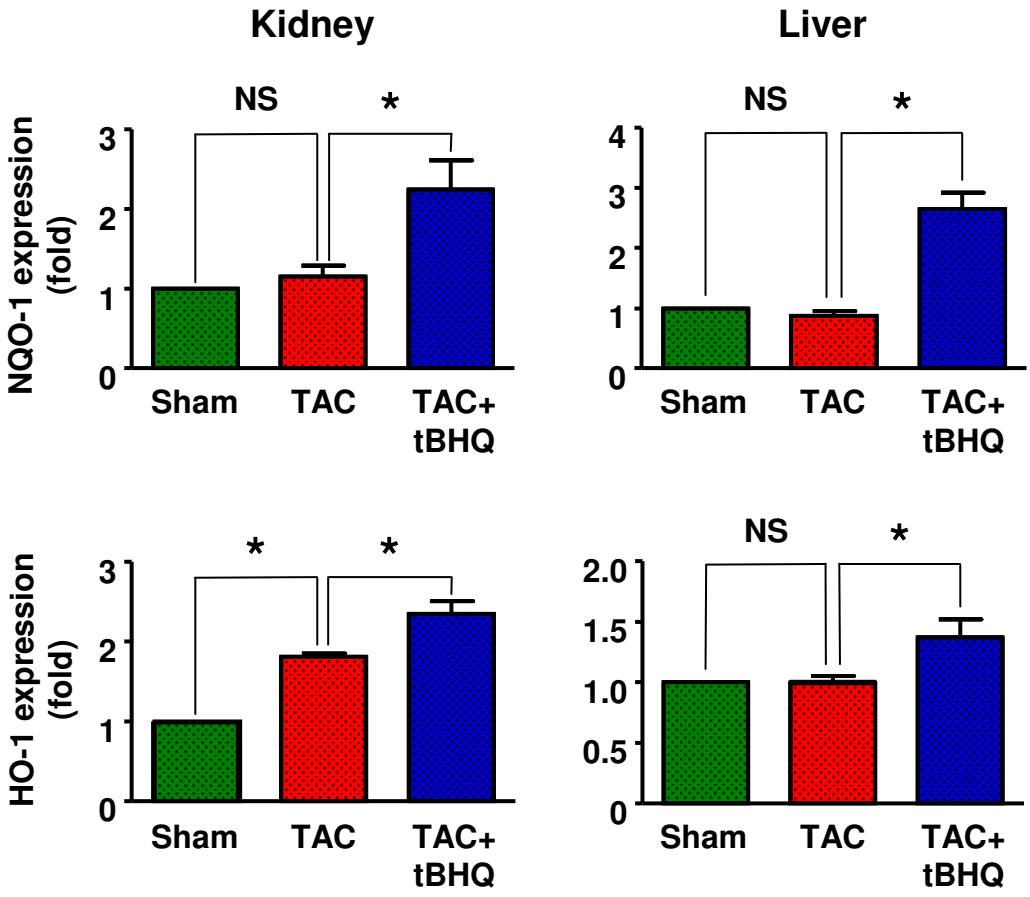
Supplemental Figure S1



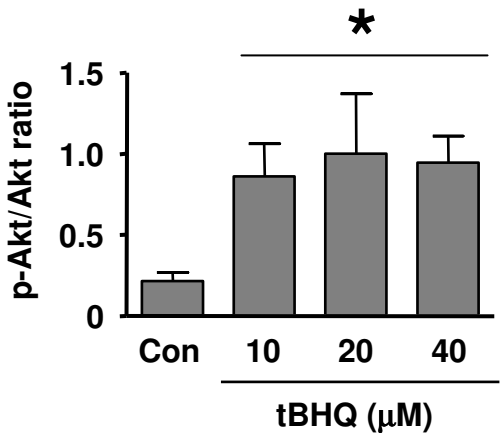
Polarization microscopy images of picrosirius red staining of LV tissues showing the effects of TAC and TAC+tBHQ on the degree of interstitial and perivascular fibrosis. Images were taken with a light microscope (Olympus BX-51). Bar = 20 μm.

Supplemental Figure S2



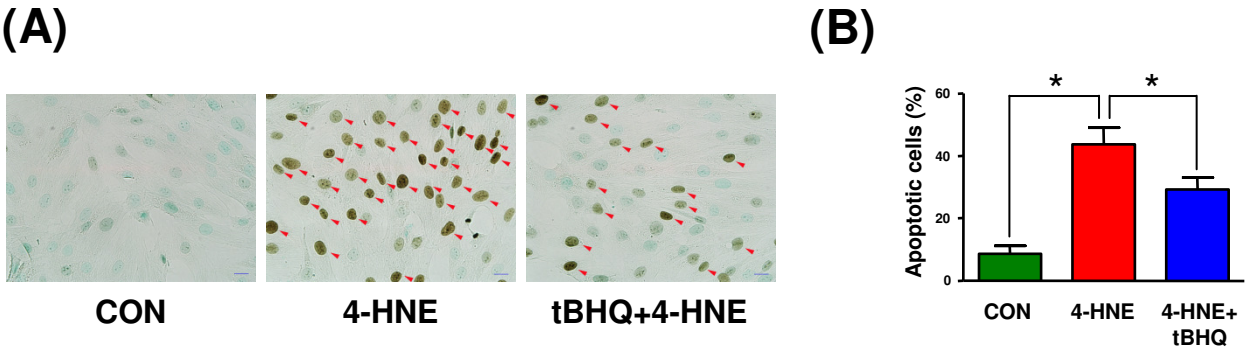
PCR results showing the expression levels of the Nrf2 target genes NQO1 and HO-1 in the kidney and liver in sham, TAC and TAC+TBHQ treated animals. * *P* < 0.05, one-way ANOVA (n = 6). NS, no significance.

Supplemental Figure S3



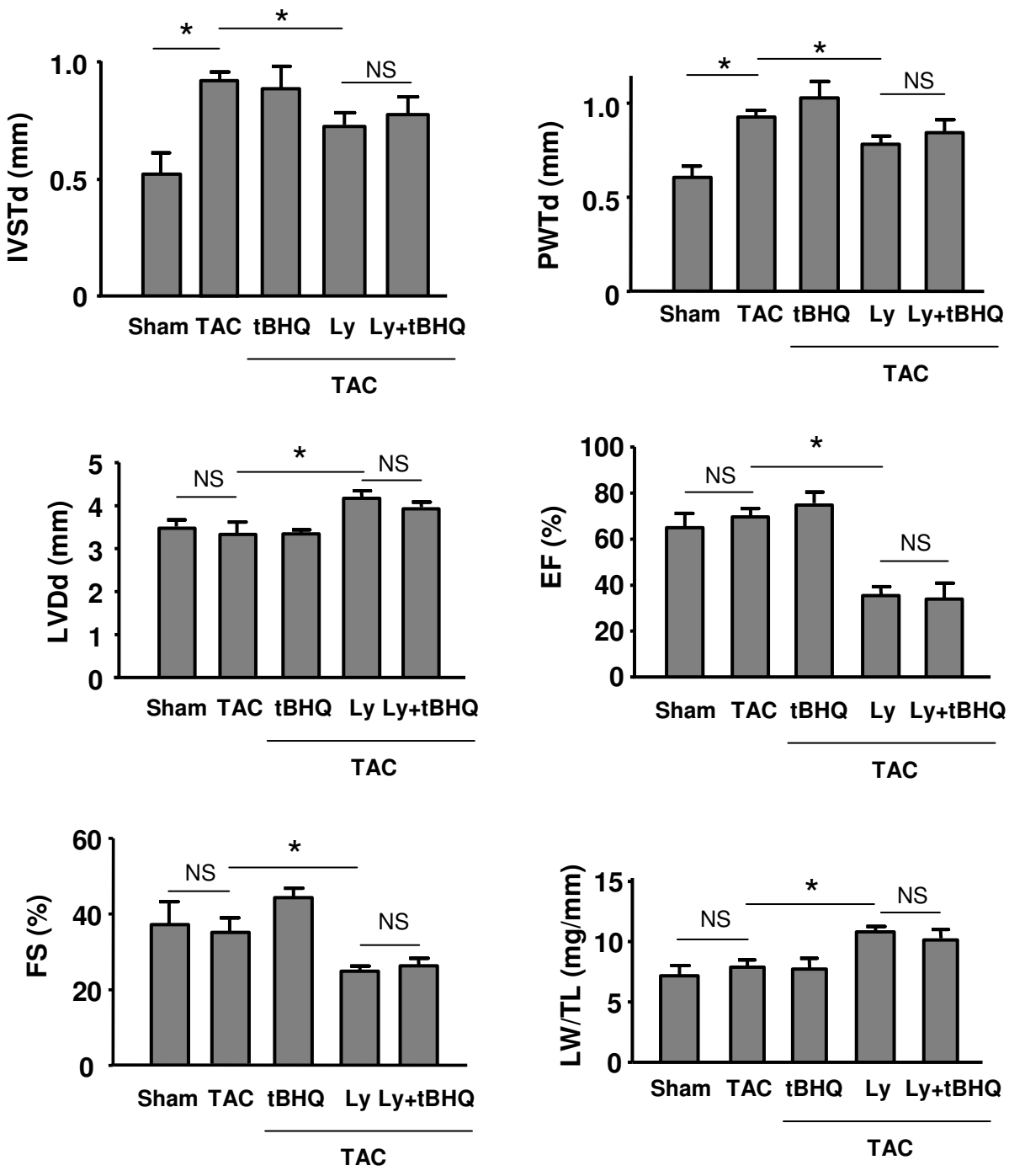
Quantitative densitometry data of the western blot in Figure 8A showing the effects of TBHQ treatment at different concentrations on Akt phosphorylation in cultured H9c2 cells. * *P* < 0.05, one-way ANOVA versus control (*n* = 4).

Supplemental Figure S4



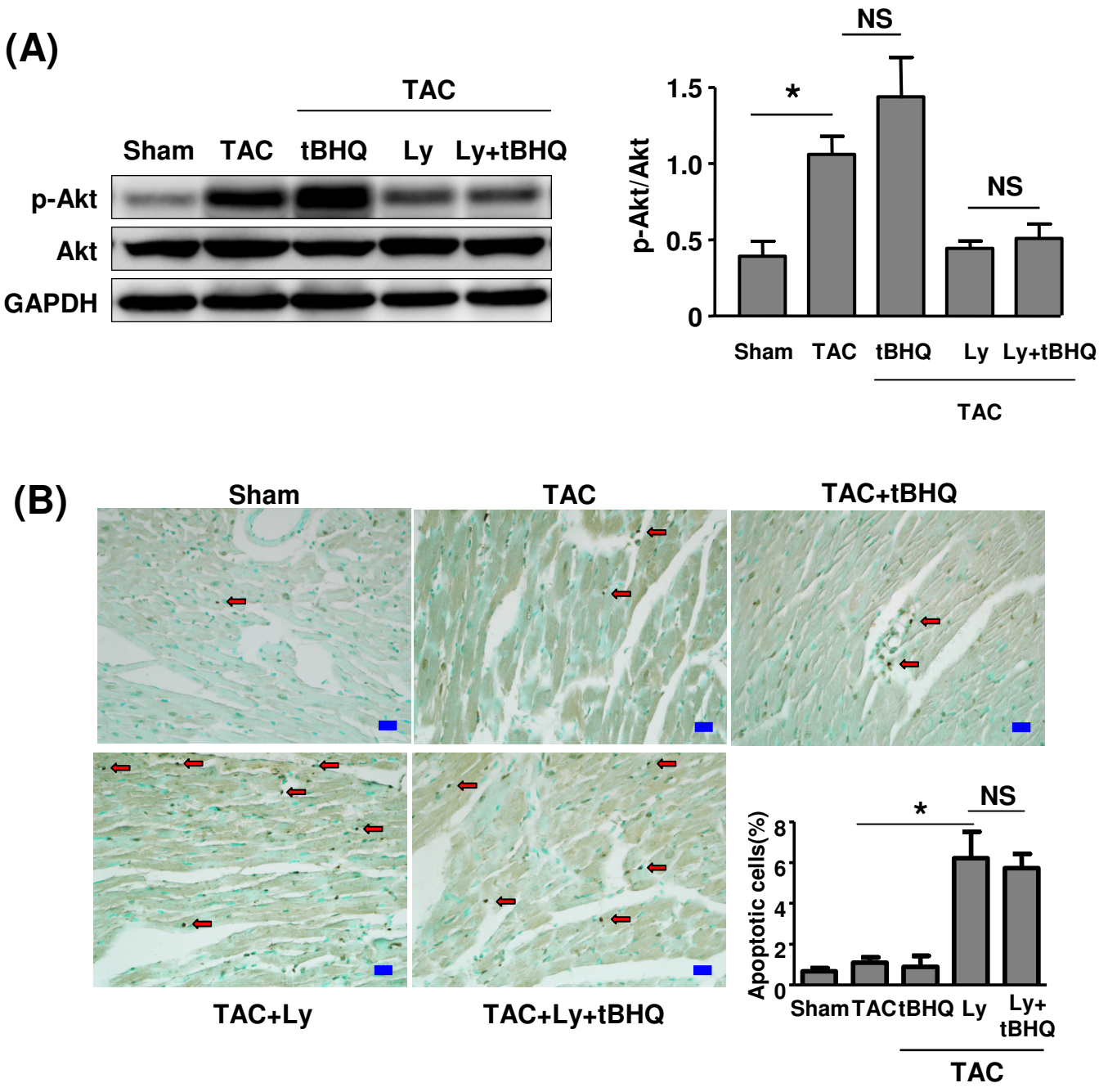
TUNEL staining in cultured H9c2 cells showing the effect of tBHQ (20 μM) pretreatment on 4-HNE (20 μM for 24 hr)-induced apoptosis. (A) Arrow heads indicate the nuclei of apoptotic cells. (B) Cell counting data for (A). * *P* < 0.05, one-way ANOVA, *n* = 3 independent experiments.

Supplemental Figure S5



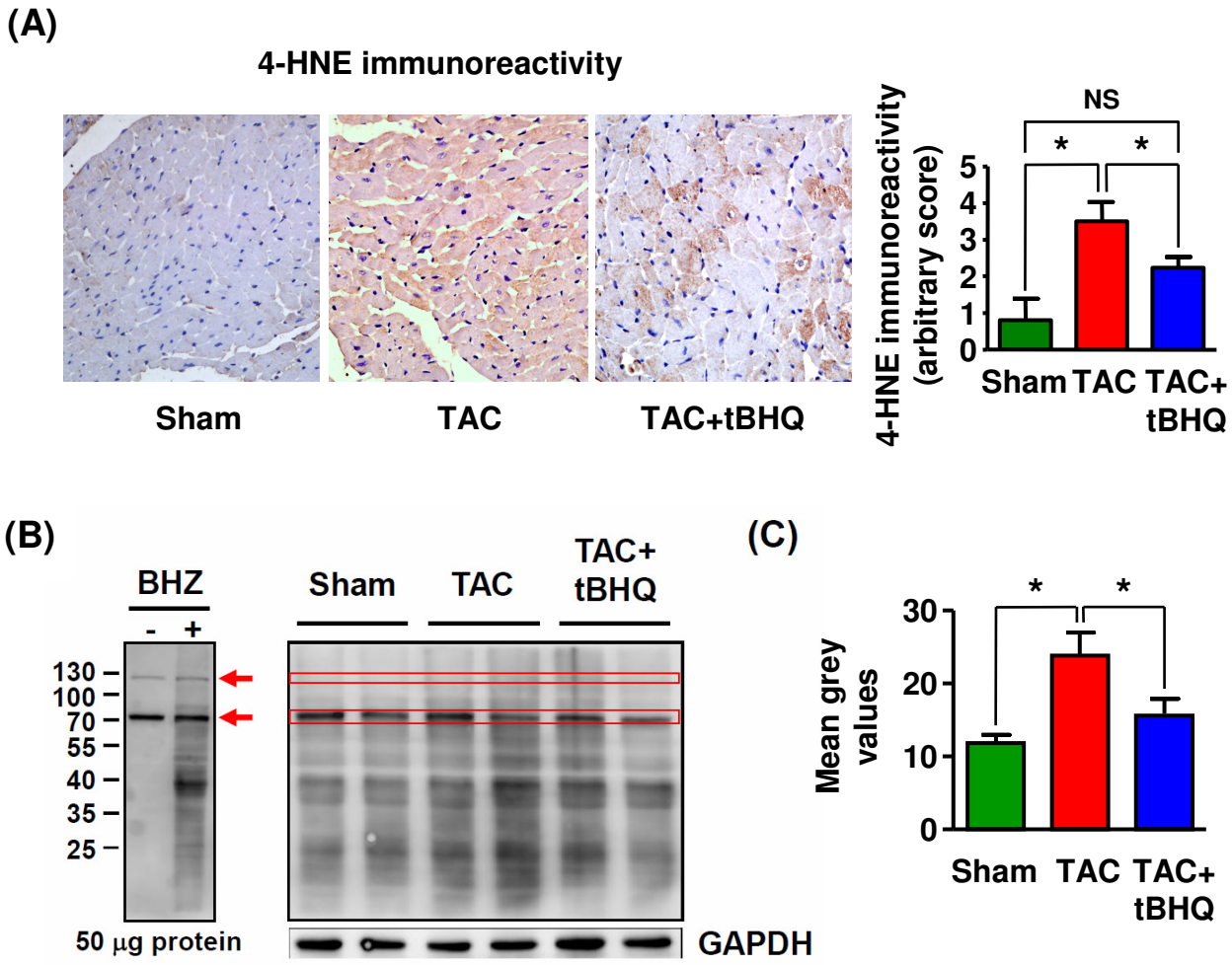
Effects of LY294002 co-treatment in vivo on the actions of TBHQ on echocardiography parameters of cardiac structure and function, and the relative lung weight at 2 weeks after TAC. * $P < 0.05$, one-way ANOVA ($n = 6$). NS, no significance. LW/TL, lung weight to tibia length ratio.

Supplemental Figure S6



Effects of LY294002 co-treatment in vivo on the actions of TBHQ on (A) Akt phosphorylation level and (B) cell apoptosis at 2 weeks after TAC. * $P < 0.05$, one-way ANOVA ($n = 6$). NS, no significance.

Supplemental Figure S7



Effects of TBHQ on reactive aldehyde production and protein carbonylation in LV tissues. (A) Immunohistochemistry staining for 4-HNE (brown color) and the corresponding semi-quantitative data. Sections were counter stained with hematoxylin. (B) Carbonylated proteins in the myocardium detected by biotin-hydrazide (BHZ) labeling followed by western blotting. Left panel shows protein samples without and with BHZ labeling. Arrows and boxes indicated the non-specific endogenous biotin-containing proteins. (C) Quantitative analysis of the blots. The mean grey density values for each lane were obtained by selecting the entire area excluding the non-specific signals enclosed by the red boxes, followed by densitometry measurements using Image J software. * $P < 0.05$, one-way ANOVA ($n = 6$).