



SUPPLEMENTARY FIG. S6. Effect of overexpression of BOLA1 on intracellular levels of glutathione and NAD(P)H and the effect of overexpression of BOLA3 on the BSO-induced mitochondrial morphology change. (A) Fibroblasts transfected to overexpress BOLA1-GFP were treated with the indicated concentrations of BSO, loaded with MCB, a fluorescent indicator of intracellular GSH, and subjected to digital-imaging microscopy for quantification of intracellular GSH. The value obtained with nontransfected, vehicle-treated cells was set at 100%. The results depicted show that BOLA1 overexpression does not interfere with BSO-induced GSH depletion. Notice that BOLA1-GFP negative and positive cells were present on the same coverslip. Numerals indicate the number of cells analyzed. (B) Fibroblasts transfected to overexpress BOLA3-GFP were treated with 12.5 μ M BSO for 72 h, loaded with TMRM and subjected to digital-imaging microscopy of GFP and TMRM fluorescence. Quantitative image analysis revealed that the BSO-induced decrease in mitochondrial length and degree of branching (form factor, *F*) was the same for BOLA3-GFP negative and positive cells. Numerals indicate the number of cells analyzed. The value obtained with vehicle-treated cells was set at 100%. a, significantly different from vehicle-treated ($p < 0.05$). (C) Fibroblasts transfected to overexpress either COX8-RFP or BOLA1-RFP, were treated with 12.5 μ M BSO for 72 h and subjected to digital-imaging microscopy of NAD(P)H autofluorescence. This type of measurement does not allow one to distinguish between NADH and NADPH. BSO increased the NAD(P)H signal to the same extent in nontransfected control cells, in COX8-RFP positive control cells and in BOLA1-RFP positive cells, indicating that BOLA1 overexpression does not counter the BSO-induced increase in NAD(P)H. The autofluorescence signal of vehicle-treated cells was the same for all three conditions, demonstrating that BOLA1 overexpression has no effect on basal NAD(P)H levels. Numerals indicate the number of cells analyzed. The value obtained with nontransfected, vehicle-treated cells was set at 100%. a, b and c, significantly different from corresponding vehicle-treated control ($p < 0.05$). MCB, monochlorobimane.