

Figure S2

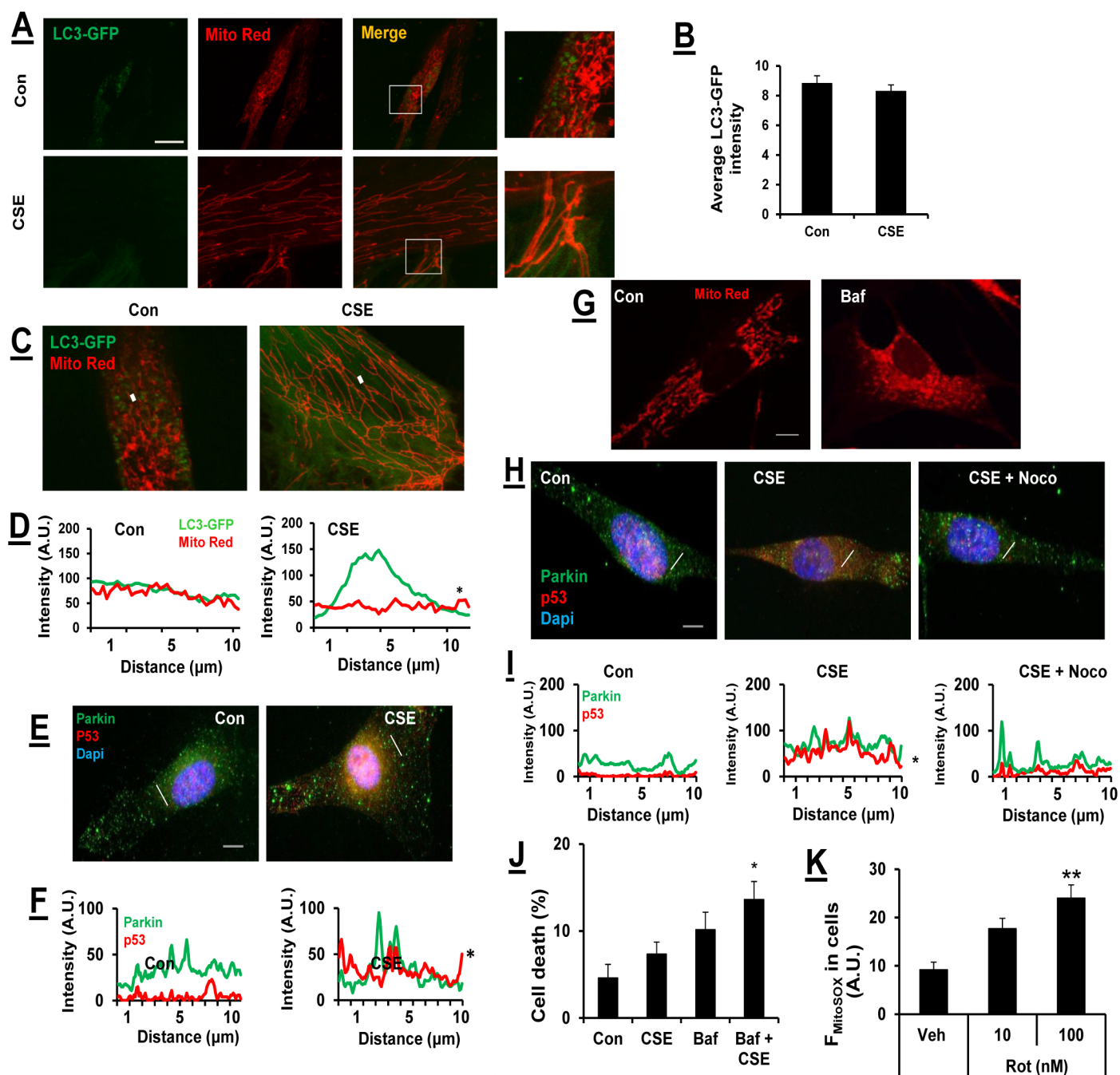


Figure S2. CSE treatment impairs mitophagy in fibroblasts

(A) Images showing LC3-GFP expressing cells stained with mitotracker red. Cells were treated with CSE (0.5%) for 15 days. Scale bars: 20 μ m. Areas in squares are zoomed as shown on right panel. (B) Average fluorescent intensity of LC3-GFP was measured by Metamorph. (C) Higher magnification of cells showing colocalization of LC3-GFP with mitochondria (green punctate seen in control slide). (D) Corresponding line scan showing the co-localization of LC3-GFP with mitochondria (red), $*P < 0.05$ vs control. (E) Representative images of Parkin (green) and p53 (red) in HFL1 cells treated with or without CSE (0.5%) for 15 days. Scale bars: 20 μ m. (F) Corresponding lines showed co-localization of Parkin (green) with p53 (red), $*P < 0.05$ vs control. (G) Representative images of mouse lung fibroblasts treated with bafilomycin (Baf, 10 nM for 10 days, alternatively). Cells were stained with mitotracker red (red), scale bars: 10 μ m. (H) Representative images of Parkin (green) and p53 (red) in HFL1 cells treated with nocadazole in the presence or absence of CSE (0.75%) for 24 h. Scale bars: 20 μ m. (I) Corresponding line scan showing co-localization of Parkin (green) with p53 (red), $*P < 0.05$ vs control. (J) Measurement of cell death using acridine orange and propidium iodide in cells treated with or without baf (10 nM for 10 days) and CSE (0.25% for 10 days, alternatively). $*P < 0.05$ vs control (Con). (K) Average fluorescent intensity (F_{MitoSOX}) of mitochondrial ROS (mtROS) in mouse lung fibroblasts treated with different concentrations of rotenone (Rot, 15 days alternate treatment). $**P < 0.01$ vs vehicle (Veh). Data are represented as mean \pm SEM, $n = 3$. Slanting lines on images (C, E and H) indicate the areas assessed for fluorescence intensity.