

Fig. S1, Feng et al.

Figure S1. RIP3 overexpression in HK-2 cells increases cell death and reduces cell viability. (A) Image of HK-2 cells transfected with an RIP3-overexpressed plasmid and control vector (pCMV6), a' and b' are the regions magnified in a, and b, respectively. (B) Bar graph showing reductions in cell viability in RIP3-overexpressed, Mitofilin-overexpressed, and RIP3-overexpressed plus Mitofilin-overexpressed plasmids compared to the control (Ctrl) vector. The graph shows a significant reduction in cell viability in RIP3-overexpressed cells compared to the control plasmid. However, in cells co-transfected with both RIP3-overexpressed and Mitofilin-overexpressed plasmids, the level of cell viability was reduced when compared to cells transfected with RIP3-overexpressed vector alone. Note that there was no difference in the levels of cell viability in Mitofilin-overexpressed plasmid versus control plasmid. Values are expressed as means \pm SEM; * $P < 0.05$ versus control (Ctrl) vector group ($n = 5$ /group).

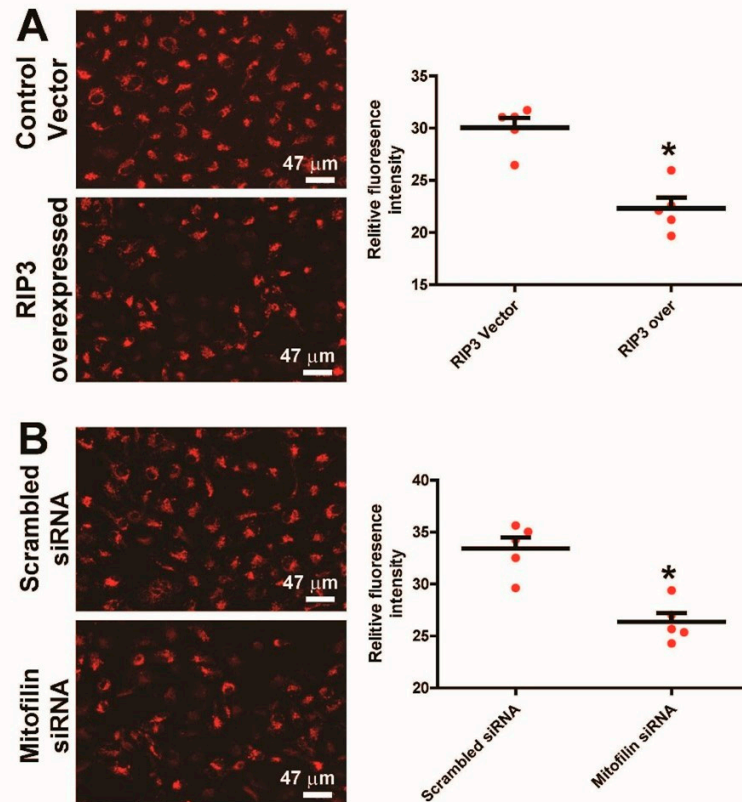


Figure S2. RIP3 overexpression or Mitofilin knockdown in HK-2 cells decreases mitochondrial membrane potential. **(A)** Left: Image of live HK-2 cells transfected with a control vector (pCMV6) or RIP3-overexpressed plasmid labeled with MitoTracker Red dye, respectively. Right: Bar graphs showing a reduction in mitochondrial membrane potential in cells transfected with RIP3-overexpressed plasmid compared to those transfected with control vector. Values are expressed as means \pm SEM; * $P < 0.05$ versus control vector group ($n = 5/\text{group}$). **(B)** Left: Image of live HK-2 cells transfected with scrambled siRNA or with Mitofilin siRNA labeled with MitoTracker Red dye, respectively. Right: Bar graphs showing a reduction in mitochondrial membrane potential in cells transfected with Mitofilin siRNA compared to those transfected with scrambled siRNA. Values are expressed as means \pm SEM; * $P < 0.05$ versus scrambled siRNA group ($n = 5/\text{group}$). Note that MitoTracker Red is a red fluorescent dye that stains mitochondria in live cells, and its accumulation is dependent upon membrane potential.