MKK3 deletion improves mitochondrial quality

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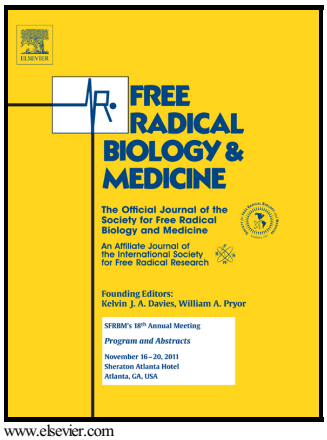

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Running Title: MKK3 regulates mitochondrial quality

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ABSTRACT:

Sepsis, a severe response to infection, leads to excessive inflammation and is the major cause of mortality in intensive care units. Mitochondria have been shown to influence the outcome of septic injury. We have previously showed that MKK3$^{-/-}$ mice are resistant to septic injury and MKK3$^{-/-}$ macrophages have improved mitochondrial function. In this study we examined processes that lead to improved mitochondrial quality in MKK3$^{-/-}$ mouse embryonic fibroblasts (MEFs) and specifically the role of mitophagy in mitochondrial health. MKK3$^{-/-}$ MEFs had lower inflammatory cytokine release and oxidant production after LPS stimulation, confirming our earlier observations. MKK3$^{-/-}$ MEFs had better mitochondrial function as measured by mitochondrial membrane potential (MMP) and ATP, even after LPS treatment. We observed higher mitophagy in MKK3$^{-/-}$ MEFs compared to WT. Transmission electron microscopy studies showed longer and larger mitochondria in MKK3$^{-/-}$ MEFs indicative of healthier mitochondria. We performed a SILAC (stable isotope labeling by/with amino acids in cell culture) study to assess differences in mitochondrial proteome between WT and MKK3$^{-/-}$ MEFs and observed increased expression of tricarboxylic acid cycle (TCA) enzymes and respiratory complex subunits. Further, inhibition of mitophagy by Mdivi1 lead to loss in MMP and increased cytokine secretion after LPS treatment in MKK3$^{-/-}$ MEFs. In conclusion, this study demonstrates that MKK3 influences mitochondrial quality by affecting the