Supplementary Data: Jianhui Zhu, Aaron M Gusdon, Huseyin Cimen, Bennett Van Houten, Emine Koc & Charleen T. Chu**.** *Impaired mitochondrial biogenesis contributes to depletion of functional mitochondria in chronic MPP+ toxicity: dual roles for ERK1/2*
Cell Death and Disease (2012) 3, e□; doi:10.1038/cddis.2012.46

Fig S1

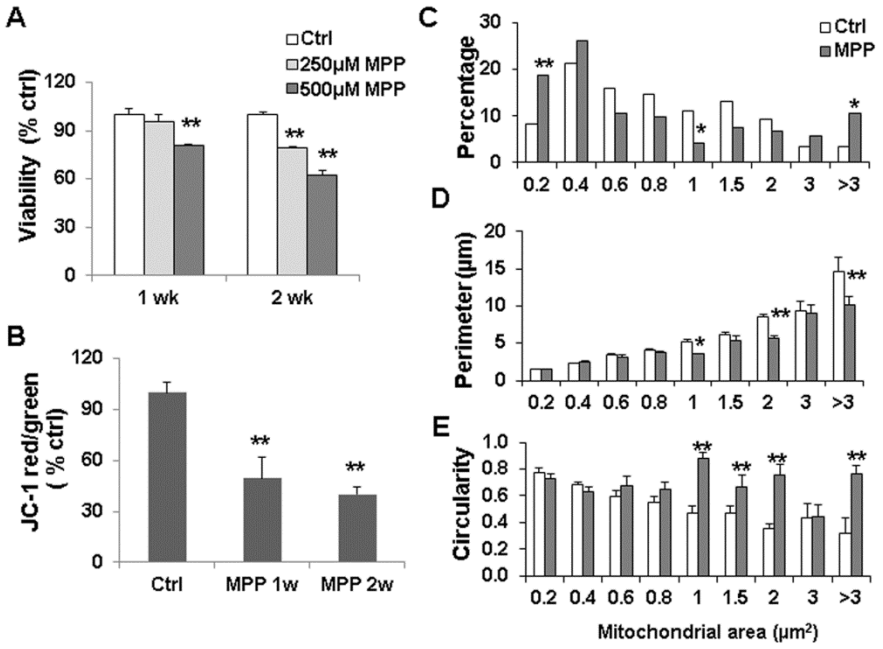

Fig S1. Progressive, dose-dependent cell death in a 2 wk model of MPP+ toxicity. Differentiated SH-SY5Y cells were treated three times a week with the indicated doses of MPP+ (A). Mitochondrial membrane potential was analyzed by JC-1 staining (B). Quantitative analysis of mitochondrial contours showed chronic MPP treatment significantly increased the percentage of both shortened/fragmented and giant mitochondria (C). These mitochondria generally showed significant decreases in perimeter (D) with increased circularity (E). ** $P < 0.01$, *$P < 0.05$, MPP^+ vs Ctrl.

Fig S2

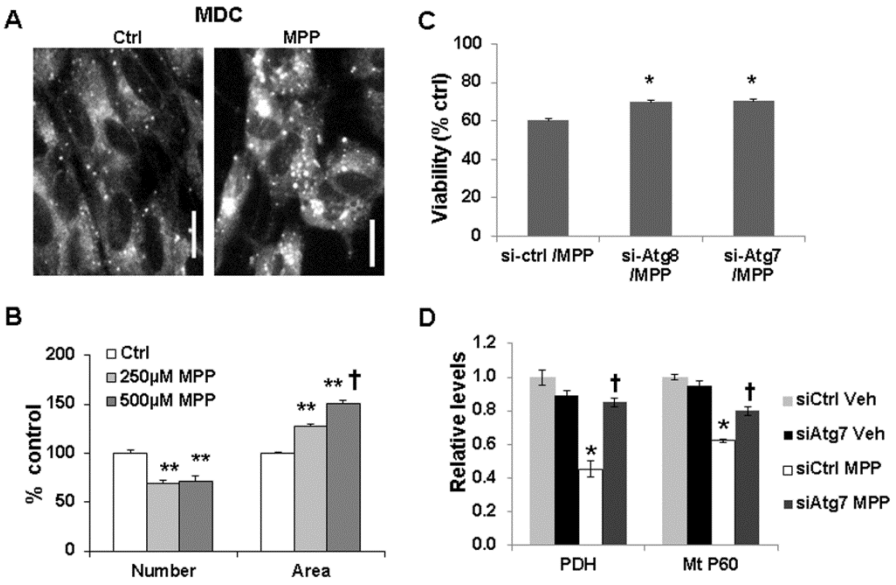

Fig S2. Representative images showed that 250μM MPP^+ x 2 weeks increased the size of MDC granules compared with vehicle-treated control (A) (scale bar: 20μm). Quantitative analysis reveal a decrease in numbers of granules per cell after treatment, but with an increase in size (B, ** $P < 0.01$ MPP^+ vs Ctrl. † $P < 0.01$ 500uM MPP^+ vs 250uM MPP^+) consistent with lysosomal activation. RNAi knockdown of either Atg8 (LC3B) or Atg7 conferred partial protection from MPP^+ toxicity (C, * $P < 0.05$ versus siCtrl). Atg7 siRNA attenuates the MPP^+ induced loss of the mitochondrial proteins PDH and mitochondrial p60 (D, *$p<0.05$ vs siCtrl-Veh, † $p<0.05$ vs siCtrl-MPP. n=4).

Fig. S3

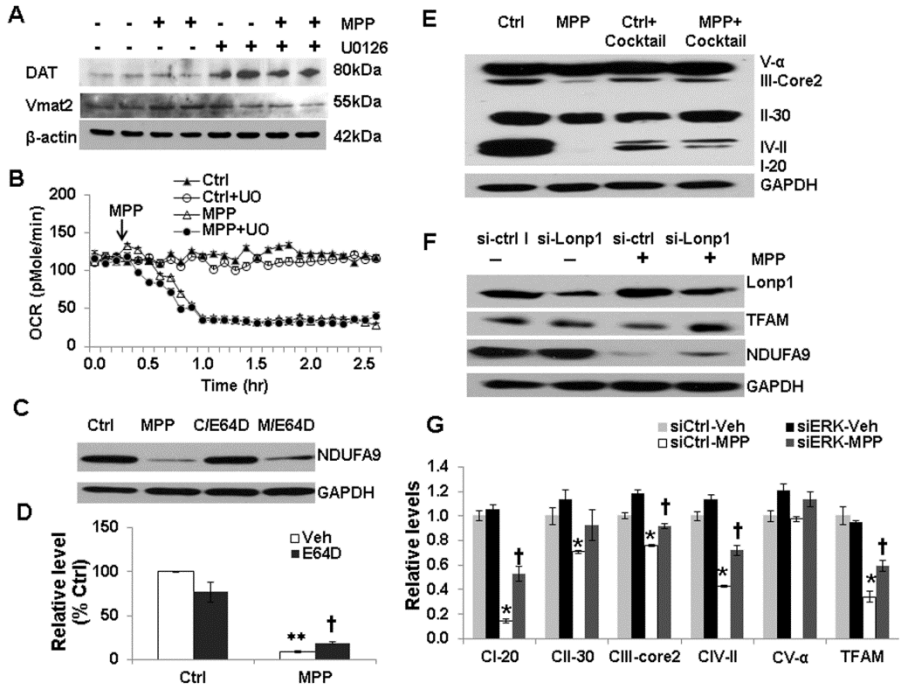

Fig. S3. Western blot data showed that administration of U0126 on a chronic basis mimicking rescue conditions caused an increase in expression of the dopamine transporter (DAT) and decreased expression of vesicular monoamine transporter 2 (Vmat 2) protein levels (A). Mitochondrial respiration assay showed that U0126 had no effect on the acute MPP+-induced inhibition of oxygen consumption (B). Inhibiting lysosomal activity by E64D partially, but significantly, reversed the NDUFA9 reduction in MPP^+ treated cells (250 µM x 2wks) (C,D). ** $p < 0.01$, MPP vs ctrl. † $p<0.01$ MPP^++E64D vs MPP^++Veh. A cocktail containing proteasomal (2.5µM lactacystin) and lysosomal [20µM Pepstatin A(Cat D); 5 µM CA074ME (Cat B) and 10 µM E64D-Cat B,L] inhibitors moderately reduced the decrease in mitochondrial respiratory complex I, III and IV subunits elicited by MPP^+ treatment (E). The decrease in TFAM and NDUFA9 levels are also reduced by siRNA knockdown of the mitochondrial LON peptidase 1 (F). Densitometric analysis of western blots show that siRNA to ERK1/2 attenuates the loss in TFAM and the indicated subunits of the mitochondrial respiratory complexes (G, *$p<0.05$ vs siCtrl-Veh, † $p<0.05$ vs siCtrl-MPP, n=4)

Fig. S4

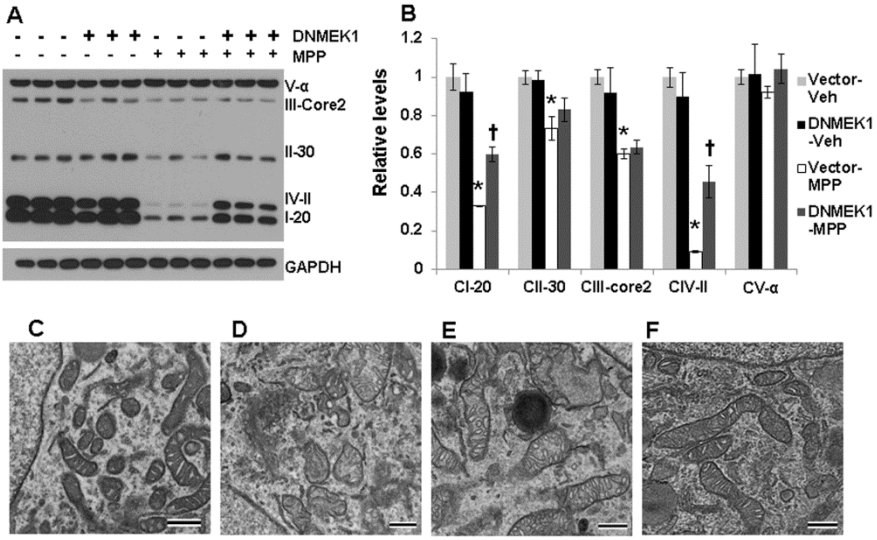

Fig. S4. Preventing ERK1/2 activation through molecular or pharmacologic inhibition of MEK reverses MPP+ induced mitochondrial injury. Overexpressing DN-MEK1 prevented the decrease in mitochondrial complex I and IV subunits in MPP^+ treated cells (A, B. *$p<0.05$ vs Vector-Veh, † $p<0.05$ vs Vector-MPP, n=5 for B). Representative electron micrographs of control cells (C) compared to cells treated with 250μM MPP^+ for 1 week (D), for 2 weeks (E) or for 2 weeks with MPP+ with addition of 5μM U0126 during the second week only (F). (scale bar: 500nm)

Fig S5

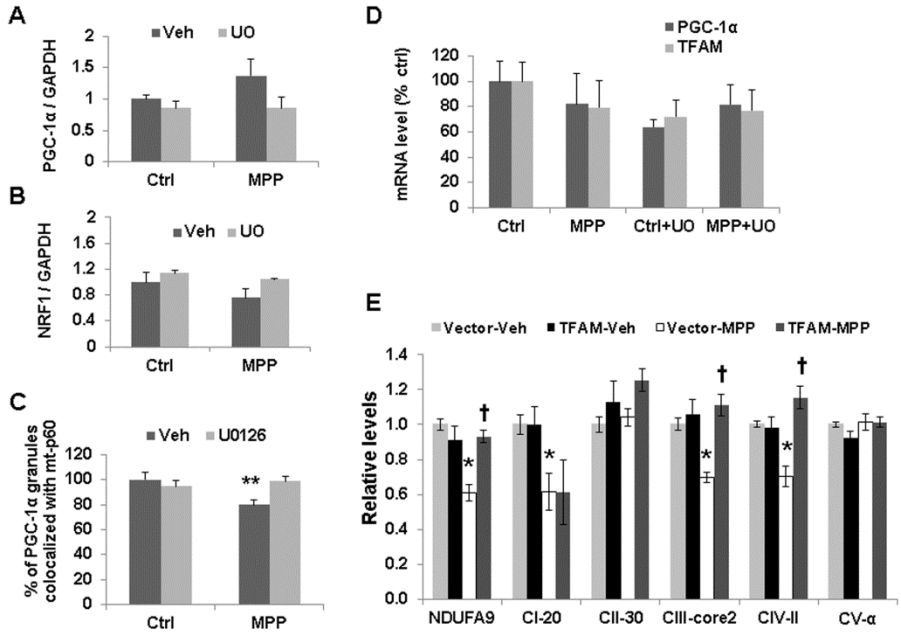

Fig. S5. Quantitative analysis of Western blots showed that MPP^+ treatment (250μM, 2wks) did not significantly change the total PGC-1α and NRF1 protein levels (A,B). The degree of PGC-1α colocalization with immunostained mitochondria was analyzed using NIH Image J (C). ** p< 0.01 compared with other groups. The mRNA levels of TFAM and PGC-1α, as analyzed by qRT-PCR (normalized to GAPDH), showed no significant changes in MPP^+ (250μM x 1wk)-treated cells +/- U0126 (D). TFAM over-expression protected against MPP+ induced alterations in mitochondrial complexes (E, *p<0.05 vs Vector-Veh, † p<0.05 vs Vector-MPP, n=4)

Fig S6

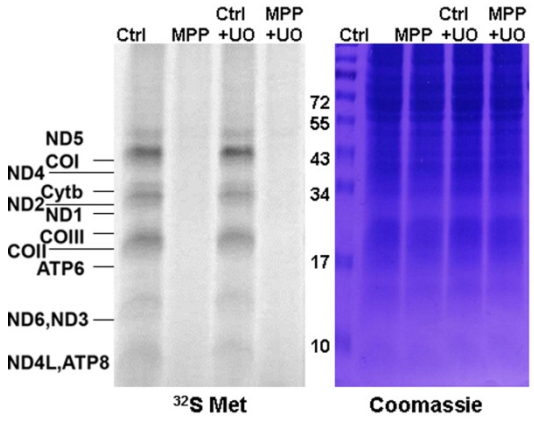

Fig S6. Acute 2.5mM MPP+ treatment for 24hr inhibits synthesis of mtDNA coded respiratory subunits; however, inhibiting MEK-ERK1/2 signaling had no significant effect on mitochondrial translation in this high-dose acute model. Coomassie Brilliant Blue stain was used as a loading control.