Supplementary data

PARP-1 silencing in H9c2 cells

H9c2 cells were transfected with PARP-1 Silencer Select siRNA using Lipofectamine 2000 transfection reagent and PARP-1 expression was measured at the RNA (**Fig S1A**) and protein levels 24 (**Fig S1B**) and 48 hours (**Fig S1C**) following the transfection. PARP-1 mRNA level decreased by ~70% at 24 hours and no further reduction was measurable at 48 hours (**Fig. S1A**). At the protein level, 40% reduction was measured at 24 hours, further reduction was detected at 48 hours, reaching ~70% reduction (**Fig. S1D**)

NMNATs play minor roles in the recovery following oxidant-induced injury

Nicotinamide mononucleotide adenylyl transferases (NMNATs) catalyze the final step of NAD^+ biosynthesis. There are 3 NMNAT isoforms in mammals: the (predominantly) nuclear NMNAT1 and the mitochondrial NMNAT3 are ubiquitous, while NMNAT2 is localized to the Golgi and the cytoplasm but its expression is restricted to the brain. Thus we tested the role of NMNAT1 and NMNAT3 in oxidant induced injury model in cardiomyocytes.

We used siRNA mediated gene silencing to test whether NMNAT1 or NMNAT3 is essential for the recovery from oxidant induced injury. *NMNAT1* silencing decreased the expression of *NMNAT1*, but did not affect the expression of *NMNAT3* and *NamPRT* **(Fig. S2A)**. Cardiomyocytes exposed to hydrogen peroxide showed increased immediate sensitivity to oxidants **(Fig. S2B-D)**, but no enhancement of cellular injury was detected after 24 hours that could be associated with impairment of the recovery processes **(Fig. S2E-G)**.

Next we tested the role of NMNAT3 in a similar fashion. *NMNAT3* silencing selectively reduced the expression of *NMNAT3*, while no change was detectable in the expression of *NMNAT1* and *NamPRT* **(Fig. S3A)**. Decreased level of *NMNAT3* did not affect the cellular injury following oxidant exposure **(Fig. S3B-G)**, but reduced the basal ATP level of the cells **(Fig. S3E)**.

On the whole, these data suggest that NMNAT1 and NMNAT3 do not play essential roles in the bioenergetic recovery following oxidative stress, or alternatively, they may compensate for the loss of each other in this process.

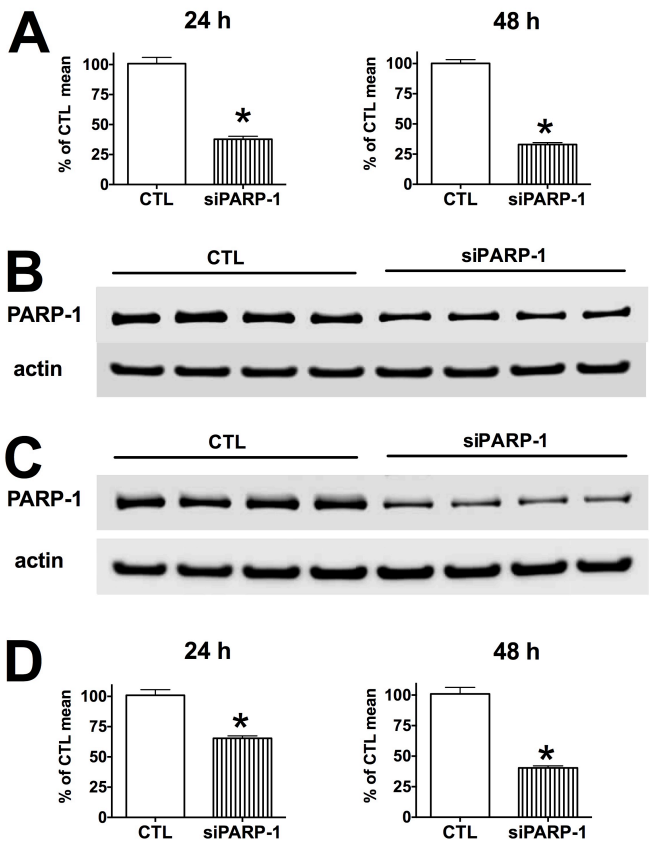

Fig. S1. PARP-1 silencing in H9c2 cells. PARP-1 expression was silenced by PARP-1 siRNA (siPARP-1) transfection in H9c2 cells. Control cells (CTL) were transfected with negative control siRNA. **A:** *PARP-1* mRNA expression was measured by Taqman assay 24 and 48 hours post-transfection and the relative expression was normalized to GAPDH expression. **B-D**: PARP-1 protein expression was measured by Western blotting 24 (**B**) and 48 (**C**) hours post-transfection and normalized to actin expression. Representative blots (**B, C**) and densitometric analysis results (**D**) are shown. At the mRNA level, PARP-1 silencing reaches its peak efficiency at 24 hours, but highest silencing efficiency requires 48 hours at the protein level. (n=3, *$p<0.05$ compared to CTL)

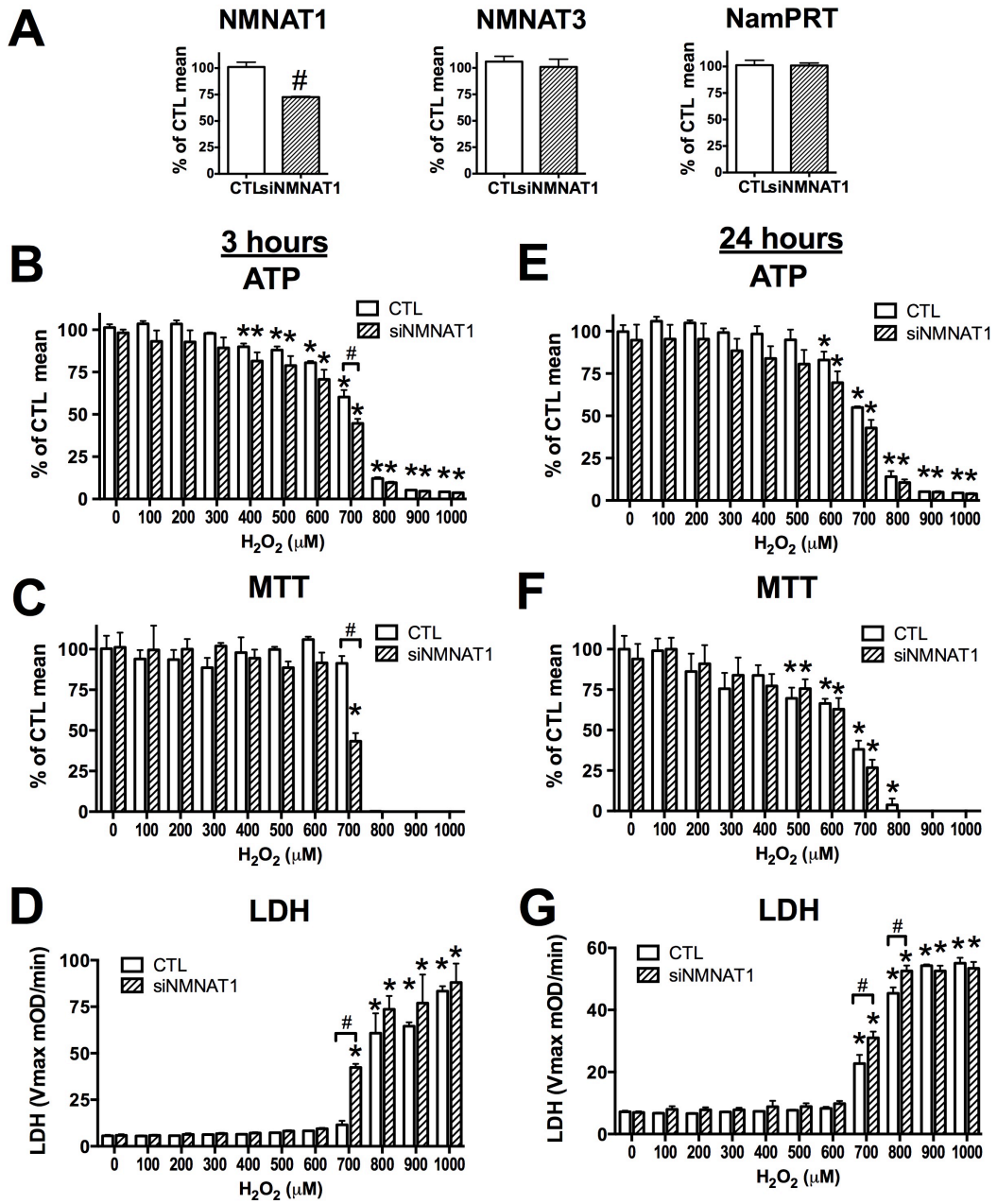

Fig. S2. NMNAT1 silencing cause enhanced sensitivity to oxidant-induced injury. **A-G**: H9c2 cells were transfected with NMNAT1 siRNA (siNMNAT1) or CTL siRNA (CTL). **A**: *NMNAT1*, *NMNAT3* and *NamPRT* expression was measured by realtime PCR 48 hours post-transfection. Relative expression values were normalized to *GAPDH* expression and are shown as % of CTL values. **B-G**: 48 hours following the siRNA transfection the cells were exposed to H_2O_2 (0-1000 μM) and the cellular ATP concentration (**B, E**), viability (**C, F**) and LDH release (**D, G**) were measured 3 hours (**B-D**) or 24 hours (**E-F**) after the addition of H_2O_2. NMNAT1 silencing results in increased cell death following 3 hours, but no difference following 24 hours. (n=3, *$p<0.05$ compared to cells not exposed to H_2O_2, #$p<0.05$ NMNAT1 silenced cells compared to respective CTL siRNA treated cells)

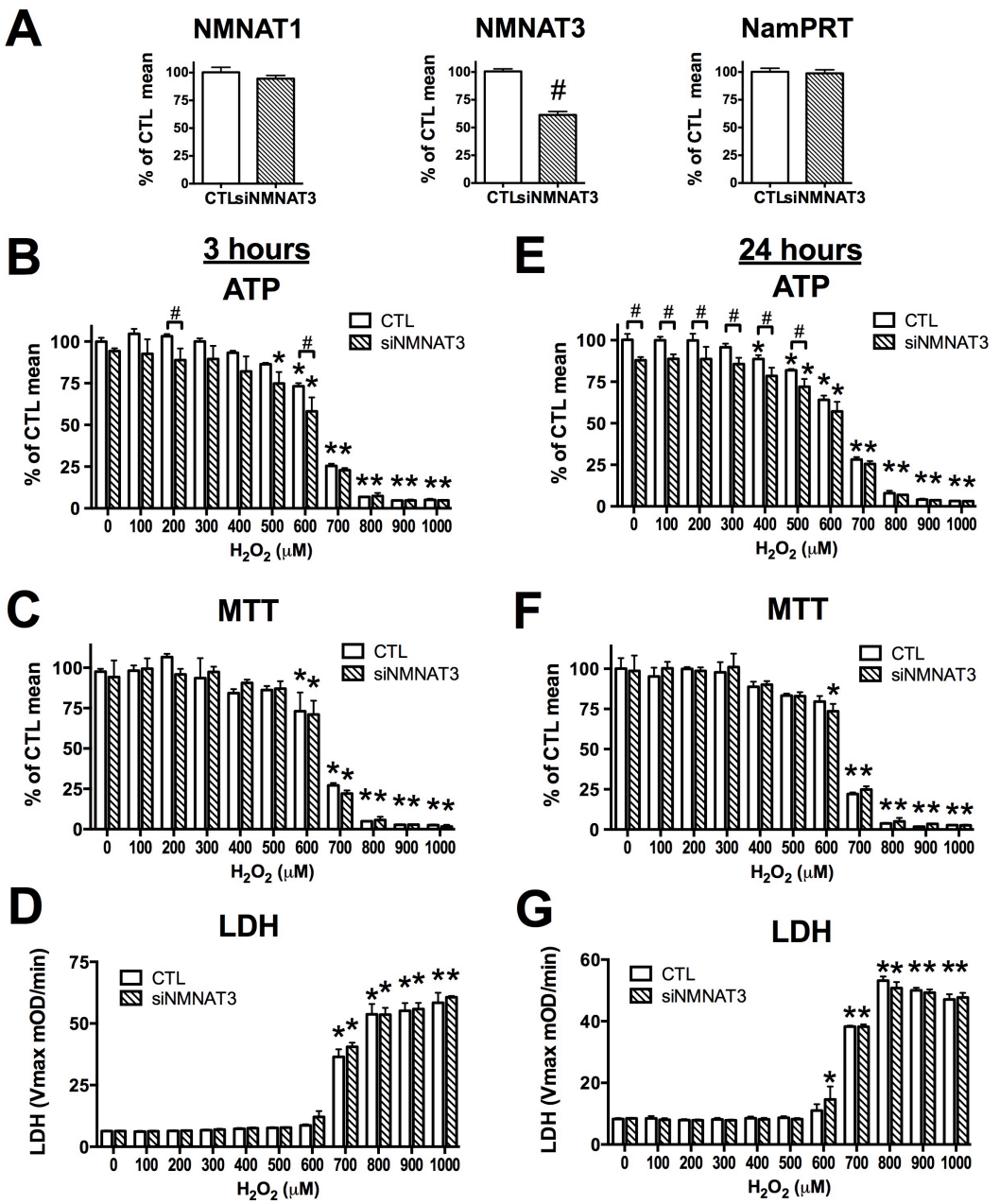

Fig. S3. **NMNAT3 silencing does not affect the oxidant-induced injury**. **A-G**: H9c2 cells were transfected with NMNAT3 siRNA (siNMNAT3) or CTL siRNA (CTL). **A**: *NMNAT1*, *NMNAT3* and *NamPRT* expression was measured by realtime PCR 48 hours post-transfection. Relative expression values were normalized to *GAPDH* expression and are shown as % of CTL values. **B-G**: 48 hours following the siRNA transfection the cells were exposed to H_2O_2 (0-1000 μM) and the cellular ATP concentration (**B, E**), viability (**C, F**) and LDH release (**D, G**) were measured 3 hours (**B-D**) or 24 hours (**E-F**) after the addition of H_2O_2. NMNAT3 silencing does not inhibit the recovery following oxidant exposure. (n=3, *$p<0.05$ compared to cells not exposed to H_2O_2, #$p<0.05$ NMNAT3 silenced cells compared to respective CTL siRNA treated cells)