SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. PARP-1-mediated neuronal death and NAD^+ depletion.

(A) Neurons from wild-type or PARP-$1^{-/-}$ mice were exposed to 75 μM MNNG for 30 minutes or 2.5 mM SIN1 for 60 minutes, and cell death was assayed at 24 hours. The PARP inhibitors PJ34 (200 nM) or DPQ (25 μM) were present throughout the entire experiment until cell death determinations. n = 4; * $p< 0.01$, ** $p< 0.001$ vs MNNG or SIN1 alone.

(B) NAD^+ was measured in neurons from wt (filled bars) and PARP-$1^{-/-}$ mice (empty bars) 30 min after exposure to MNNG or SIN1 as in (A). n = 4; * $p< 0.01$, ** $p< 0.001$ vs treated (MNNG or SIN1).

(C,D) Total and mitochondrial NAD^+ were assessed 30 min after MNNG washout. n = 3; * $p< 0.01$, ** $p< 0.001$ vs MNNG alone. Total and mitochondrial NAD^+ were both near zero at the 3 h time point (not shown).

Supplemental Figure 2. MNNG-induced neuronal death is not blocked at intracellular nicotinamide concentrations below 4 nmol / mg protein.

(A) Effect of medium nicotinamide concentration on MNNG-induced neuronal death. Viability was assessed at 24 hours after exposure to MNNG, 75 μM for 30 min.

(B) Effect of medium nicotinamide concentration on neuronal intracellular nicotinamide. Measurements were performed after 30 min incubation. The intracellular nicotinamide concentration under the condition in which MNNG-induced cell death is significantly reduced in (A) is 4.8 ± 0.7 nmol/mg protein.

(C) Intracellular nicotinamide content measured in neurons 30 min after MNNG washout. The intracellular nicotinamide concentrations are in all conditions lower than required to prevent PARP-1-mediated cell death. PJ34 (200 nM) ,was co-incubated with MNNG, and NAD^+ 5 mM was added after MNNG washout. n = 3; * < 0.01, ** < 0.001.

Supplemental Figure 3. Experimental medium affects outcome of PARP-1-mediated cell death.

(A,B) Neurons were exposed to acetoacetate or hydroxybutyrate (filled) after washout of MNNG (75 μM, 30 min). The empty data point indicates MNNG alone. The dashed line indicates basal neuronal death with media exchange only. n =3, *p < 0.01, **p < 0.001 vs. MNNG alone.

(C-E) Experiments were carried out in either artificial CSF (aCSF) or in standard culture medium (C.M.) containing Neurobasal Medium with B27 Supplement and 0.25 mM Glutamax for neurons, or MEM with 10% FBS and 2 mM glutamine for astrocytes and mouse embryonic fibroblasts (MEF). Cell viability was assayed 24h after MNNG exposure (75 μM). Cell death was significantly reduced in neurons (C), astrocytes (D), and MEFs (E) by the PARP-1 inhibitor PJ34 (200 nM) in aCSF, and comparably reduced in C.M. without PJ34 Data are means ± SEM; n = 3 – 4; ** p<0.001.

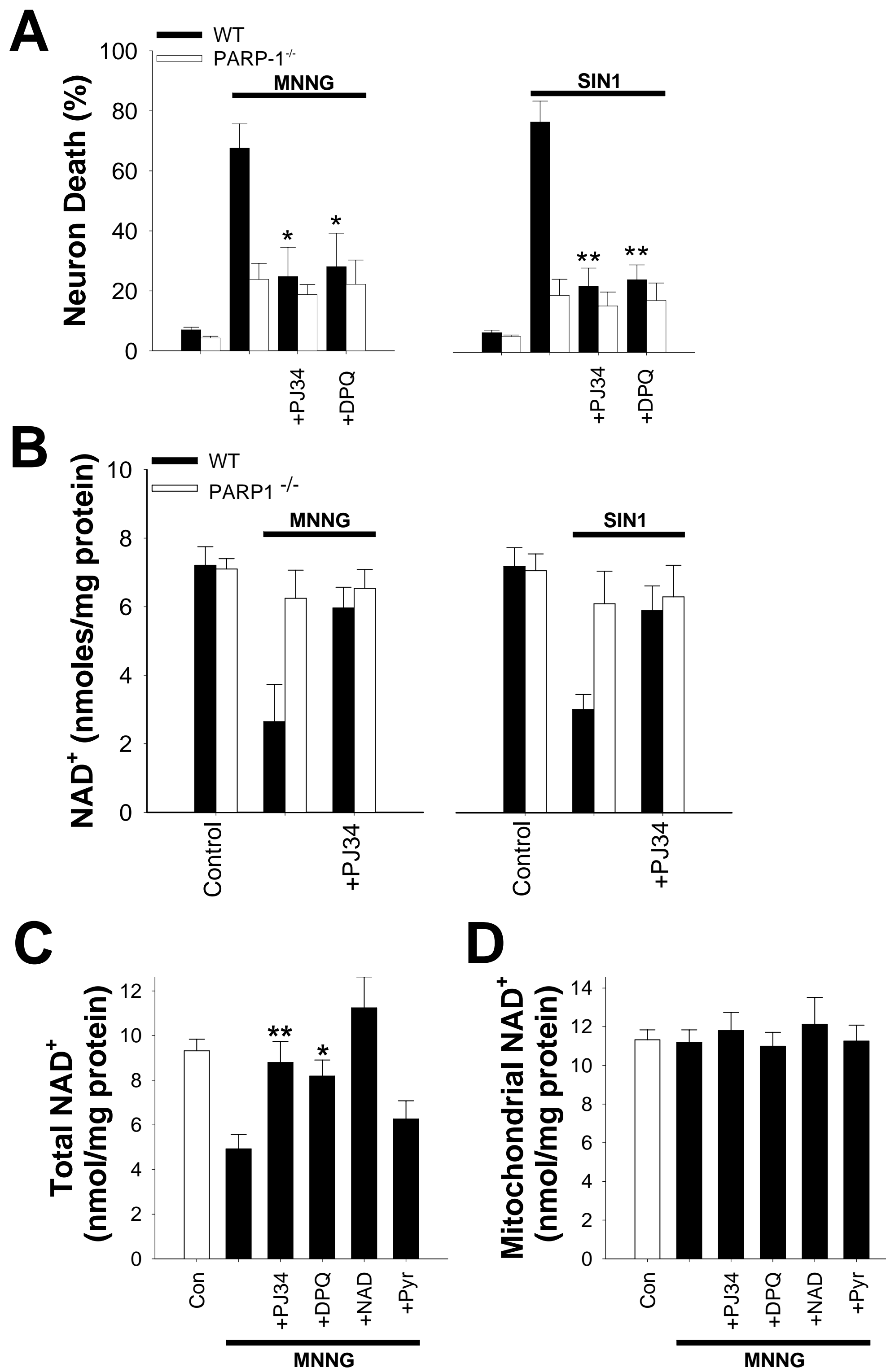
Supplemental Figure 4. NADase causes a block in neuronal glycolysis

(A) NADase transfection blocked glycolysis in wt and PARP-$1^{-/-}$ neurons, measured 3 hours after Bioporter transfection. The block in glycolysis was prevented by nicotinamide (Nam; 200 μM), but not by pyruvate (2.5 mM).

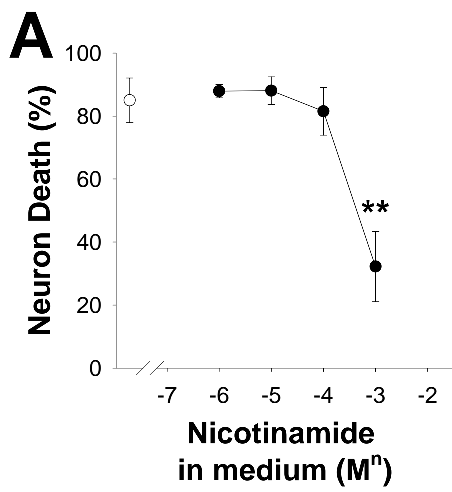
(B) Neuronal pyruvate content was restored by the addition of pyruvate (2.5 mM) or Nam (200 μM).

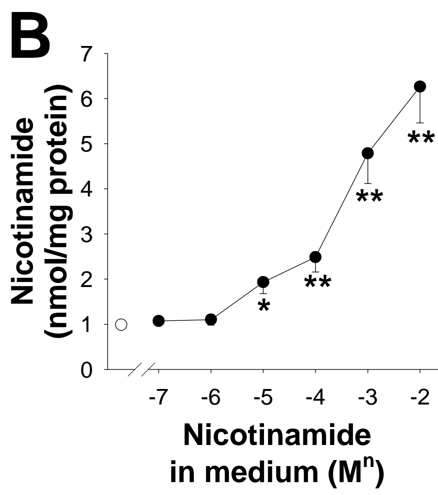
(C) Neuronal death after NADase treatment was prevented by either pyruvate (2.5 mM) or NAM (200 μM). n =3, **p < 0.001 vs. NADase alone (black bar)

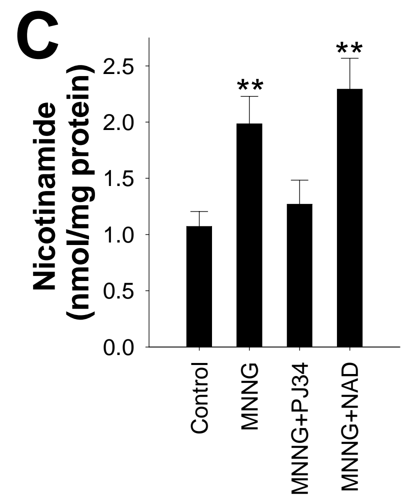
Supplemental Figure 1



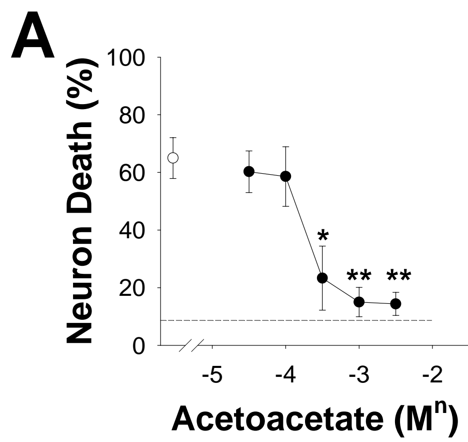
Supplemental Figure 2

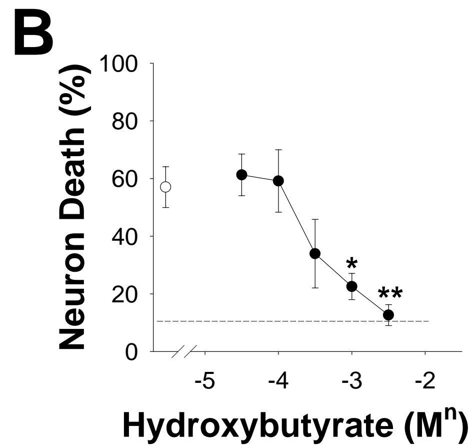


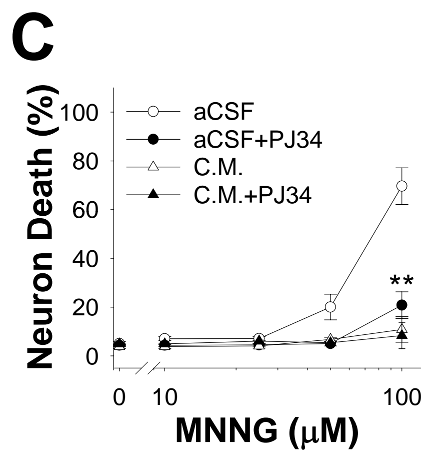


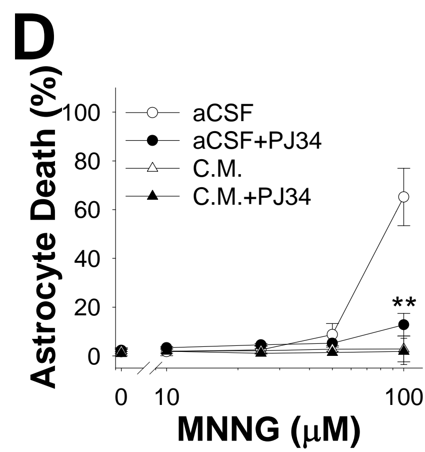


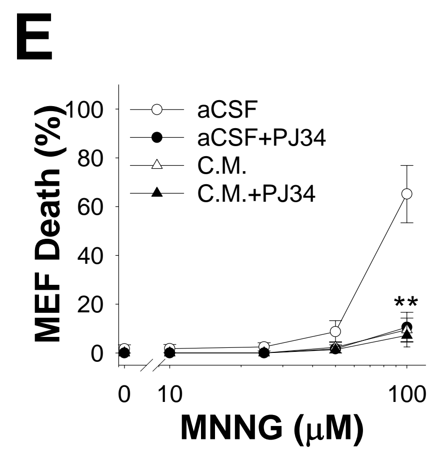
Supplemental Figure 3











Supplemental Figure 4