Supporting Online Material

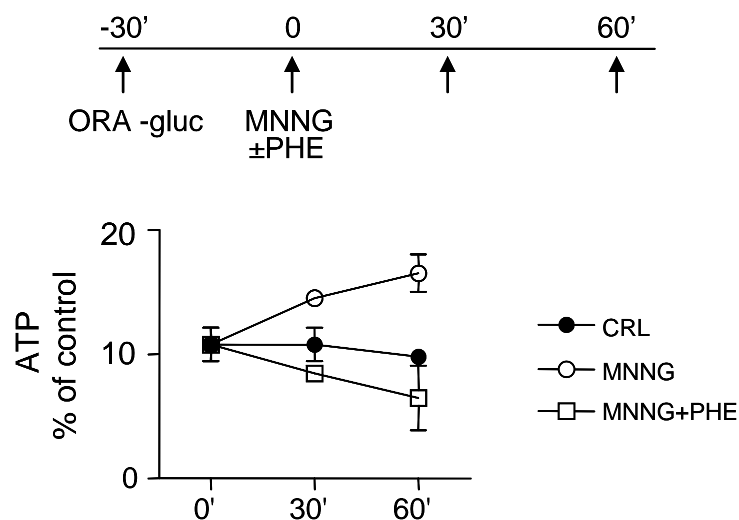

Fig. S1. Severe energy loss unmasks PARP-1 activation-dependent ATP production. HeLa cells exposed for 30 min to oligomycin, rotenone and antimycin (ORA, all compounds at 10 μM) in the absence of glucose (-gluc) undergo 90% reduction of the ATP content which remain stable for the following 60 min. Exposure of ATP-depleted cells to the PARP-1 activator MNNG (100 μM) leads to a significant increase of the ATP contents. This increase is prevented by the PARP-1 inhibitor phenanthridinone (PHE, 30 μM). The time frame of the experiment is shown. Each point represents the mean ± SEM of three experiments conducted in duplicate.

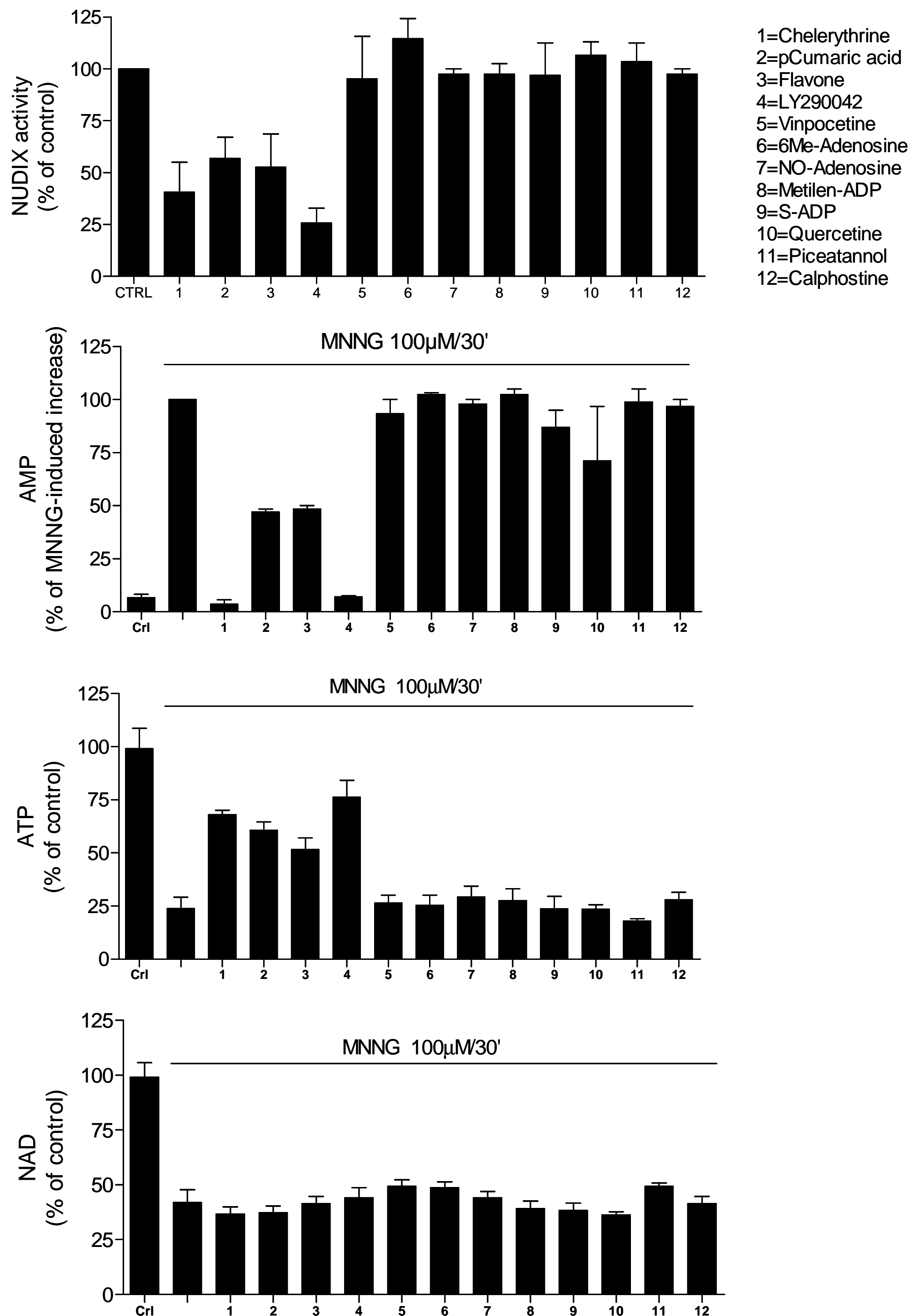

Fig. S2. NUDIX inhibition correlates with reduced AMP production and ATP loss in HeLa cells exposed to MNNG. A library of compounds were tested on NUDIX activity in HeLa cell extracts. Only those endowed with inhibitory potency reduce AMP increase and ATP loss prompted by a 30’ challenge with MNNG 100 µM. None of the tested compounds affects MNNG-dependent NAD depletion. Each bar is the mean of at least three experiments conducted in duplicate.

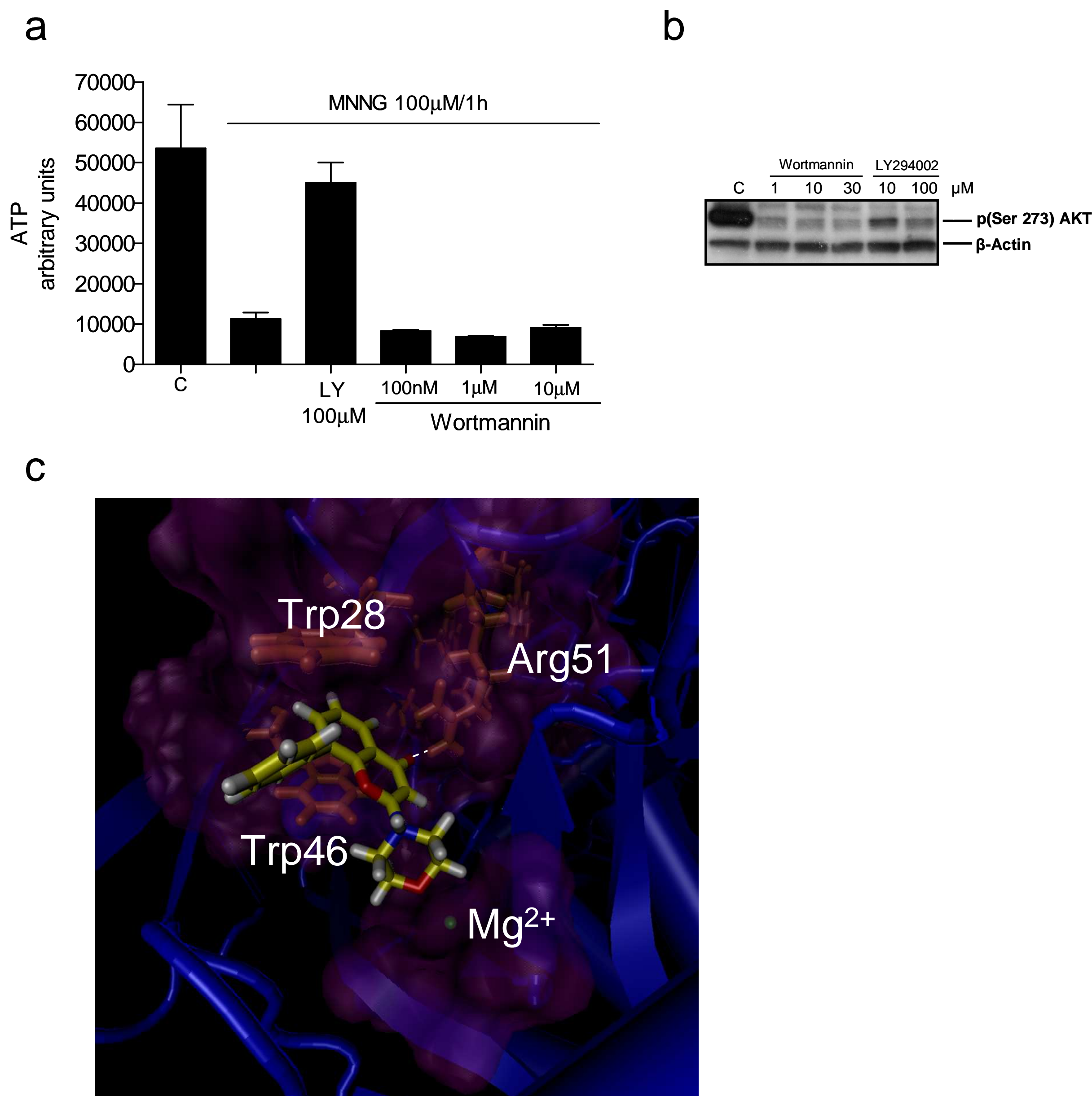

Fig. S3. Inhibition of phosphoinositide-3 kinase (PI3K) does not underlie the ATP-rescuing effects of LY290042. (**a**), LY290042 (LY) but not the other potent PI3K inhibitor worthmannin prevents ATP depletion in HeLa cells exposed to MNNG. (**b**) Both drugs reduce constitutive phosphorylation of the PI3K substrate AKT. (**c**) Docking pose of LY290042 into the catalytic site of NUDT5. The compound occupies the adenine binding site of ADPR with the chromane nucleus being in π-π stacking with the side chains of Trp28 and Trp46, the carbonyl group interacting with Arg51 through hydrogen bonding, and the oxygen atom of the morpholine group contacting the magnesium ion of the catalytic site. In (**a**) bars represent the mean ± SEM of three experiments conducted in duplicate. In (**b**) one blot representative of two is shown.

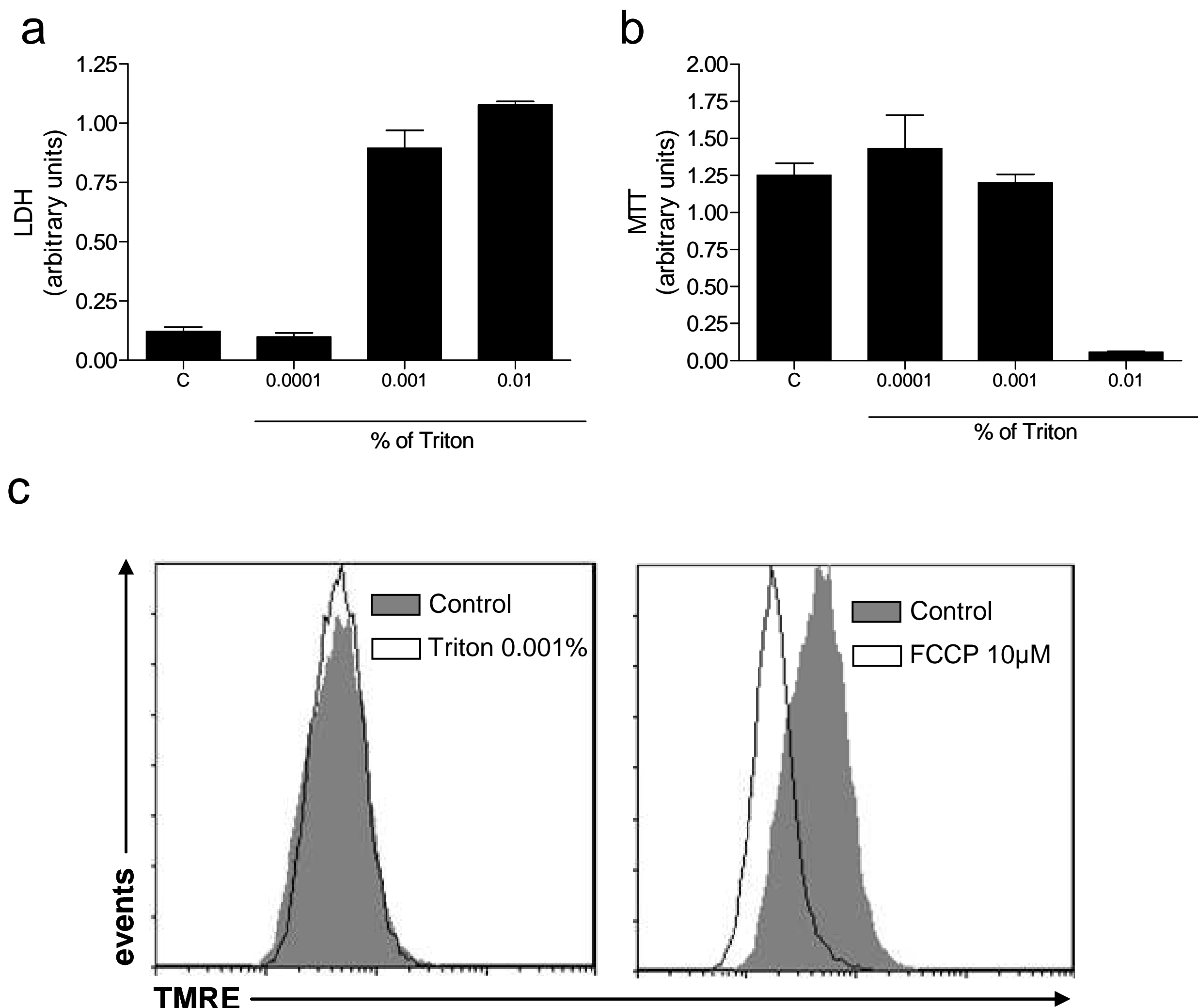

Fig. S4. Effect of different concentrations of Triton X100 on membrane permeability, mitochondrial respiration and membrane potential. A 5 min exposure to 0.001% Triton X100 prompts leakage of cytoplasmic lactic dehydrogenase (LDH) into the culture medium (**a**), indicating membrane permeabilization. Under these conditions, (**b**) MTT reduction (an index of cell viability and mitochondrial respiration) as well as (**c**) tetramethylrhodamine ethyl ester (TMRE, 2,5 nM) fluorescence (an index of mitochondrial membrane potential) are not affected. The protonophore FCCP is used as a positive control for mitochondrial depolarization. In (a) and (b) bars represent the mean ± SEM of at least 3 experiments. In (c) representative plots of flow cytometric analysis are shown.