Nickel enhances zinc-induced neuronal cell death by priming the endoplasmic reticulum stress response

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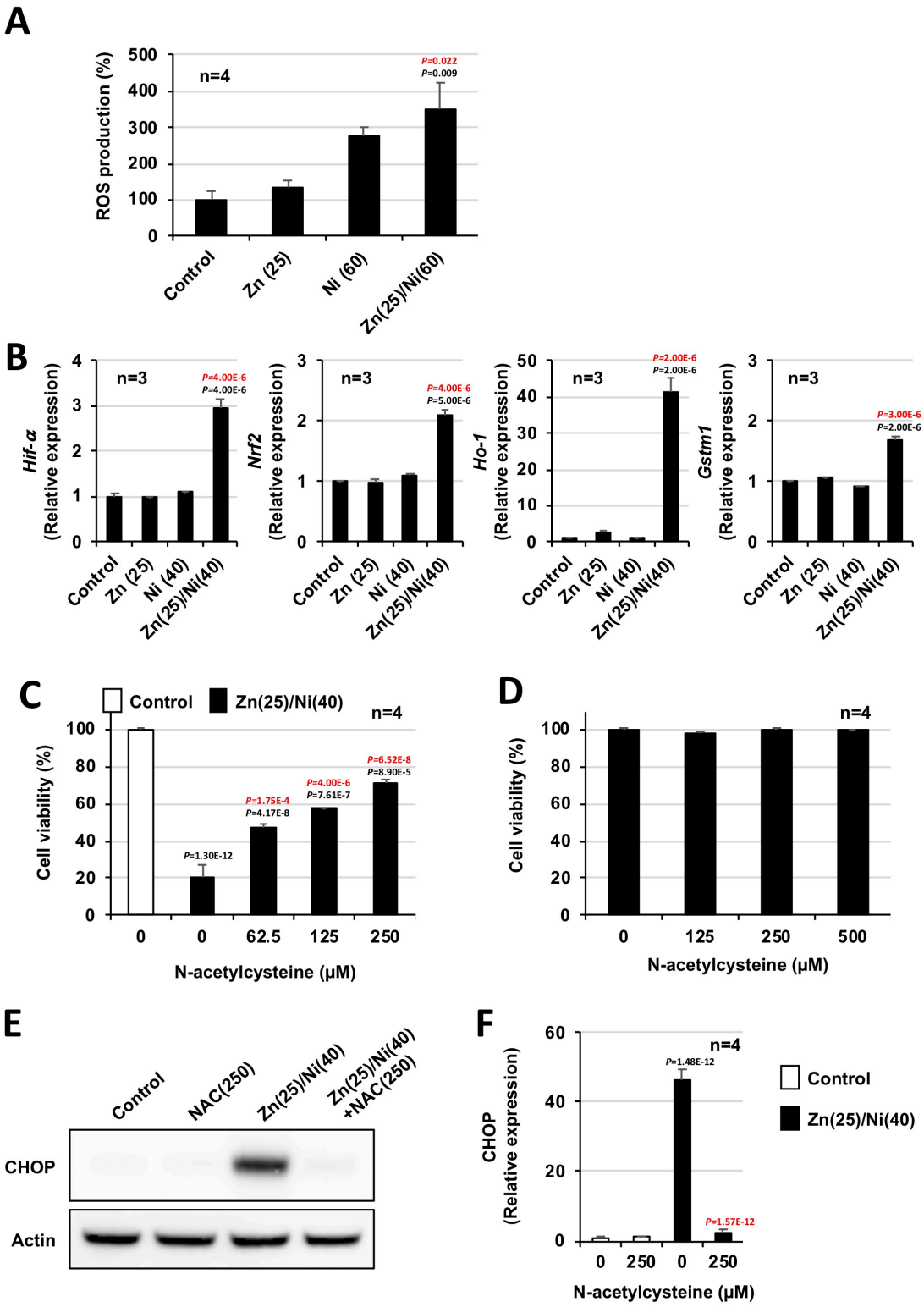
A
ROS production (%)
n=4
P=0.022
P=0.009
Control
Zn (25)
Ni (60)
Zn(25)/Ni(60)
B
Hif-α
Nrf2
Ho-1
Gstm1
(Relative expression)
n=3
P=4.00E-6
P=4.00E-6
P=4.00E-6
P=5.00E-6
P=2.00E-6
P=2.00E-6
P=3.00E-6
P=2.00E-6
Control
Zn (25)
Ni (40)
Zn(25)/Ni(40)
C
Control
Zn(25)/Ni(40)
n=4
Cell viability (%)
P=1.30E-12
P=1.75E-4
P=4.17E-8
P=4.00E-6
P=7.61E-7
P=6.52E-8
P=8.90E-5
N-acetylcysteine (μM)
D
n=4
Cell viability (%)
N-acetylcysteine (μM)
E
Control
NAC(250)
Zn(25)/Ni(40)
Zn(25)/Ni(40)
+NAC(250)
CHOP
Actin
F
n=4
CHOP
(Relative expression)
P=1.48E-12
P=1.57E-12
Control
Zn(25)/Ni(40)
N-acetylcysteine (μM)

Supplementary Figure S1

GT1-7 cells (6-well culture plates at a density of 7.5×10^5 cells per well) were incubated with DCFHDA (100 μM), an indicator of ROS, in the absence (Control) or presence of $NiCl_2$ (Ni, 60 μM) and/or $ZnCl_2$ (Zn, 25 μM) for 2 h. DCFHDA fluorescence (ex 485 nm, em 530 nm) was measured using a fluorescence microplate reader (A). GT1-7 cells (6-well culture plates at a density of 7.5×10^5 cells per well) were incubated in the absence (Control) or presence of $NiCl_2$ (Ni, 40 μM) and $ZnCl_2$ (Zn, 25 μM) for 4 h. Total RNA was extracted and subjected to real-time RT-PCR using primer sets specific for each gene. Values were normalized to *Gapdh* and expressed relative to the control (B). GT1-7 cells (96-well culture plates at a density of 3×10^4 cells per well (C, D), 6-well culture plates at a density of 7.5×10^5 cells per well (E, F)) were pre-treated with the indicated concentrations (μM) of N-acetylcysteine just before Ni^{2+}/Zn^{2+} treatment. Next, GT1-7 cells were incubated in the absence (Control) or presence of $NiCl_2$ (40 μM) and $ZnCl_2$ (25 μM) for 24 h (C) or 7 h (E, F). GT1-7 cells were treated with the indicated concentrations of N-acetylcysteine for 24 h (D). Cell viability was determined using CellTiter-Glo® 2.0 (C, D). Whole-cell extracts were analyzed by immunoblotting with an antibody against CHOP or actin (E). The band intensity of CHOP was determined using ImageJ software (F). Values represent mean ± S.E.M. *P* values are described in the figure when $P < 0.05$. [Black: vs. Control, Red: vs. $ZnCl_2$ alone] (A, B) or [Black: vs. Control, Red: vs. Zn(25)/Ni(40)] (C, F).