SUPPLEMENTAL INFORMATION

Erastin-like Anti-Warburg Agents Prevent Mitochondrial Depolarization Induced by Free Tubulin and Decrease Lactate Formation in Cancer Cells

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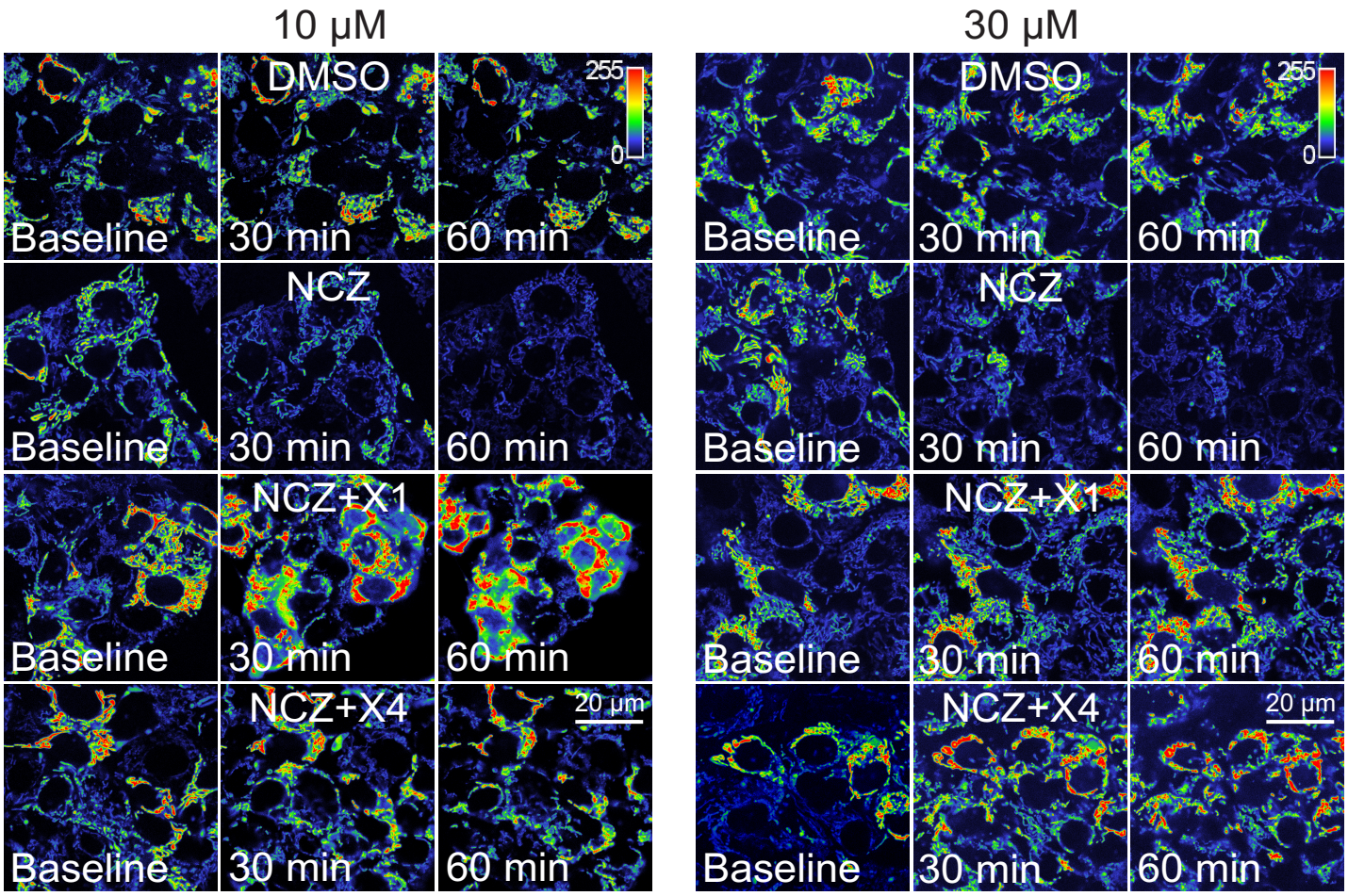
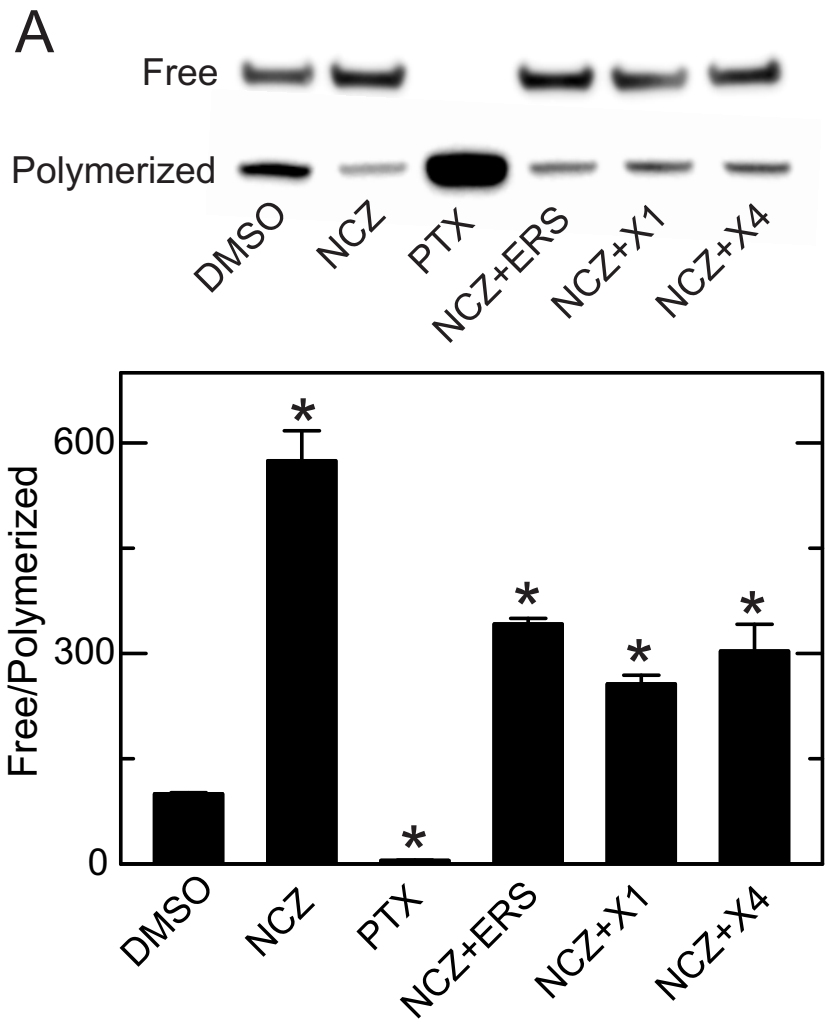


Suppl. Fig. 1. Lead validation by confocal microscopy in HeG2 hepatoma cells. HepG2 cells were untreated (DMSO) or treated with NCZ (10 µM) or NCZ (10 µM) plus X1 or X4 (10-30 µM), and TMRM fluorescence was imaged at 0, 30 and 60 min. This figure extends Fig. 5 by showing baseline and intermediate imaging time points for each treatment. Note that TMRM fluorescence was stable in untreated cells and that NCZ decreased TMRM intensity relative to baseline with the greatest decrease after 60 min. At 10 and 30 µM, X1 and X4 both blocked NCZ induced mitochondrial depolarization and actually increased TMRM intensity relative to baseline.



Suppl. Fig. 2 Free to polymerized tubulin ratios after treatment with erastin and lead compounds in the presence of NCZ. In **A**, Western blotting of tubulin was performed after separating free and polymerized tubulin, as described in **Materials and Methods**. HepG2 cells were treated with vehicle (DMSO), NCZ or 10 μM X1 or X4 in the presence of NCZ. Representative blots are shown from 3 independent experiments. In **B**, free/polymerized tubulin ratios were calculated using relative band densities. *$p < 0.05$ compare to DMSO (n=3).