Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Material

Silica-coated $LiYF_4:Yb^{3+},Tm^{3+}$ upconverting nanoparticles are non-toxic and activate minor stress responses in mammalian cells

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Figure S1. Physicochemical properties of UCNPs used in this study.

Figure S2. Cell viability after incubation with UCNPs for 3 and 5 days.

Figure S3. Loss of PARP1 following endoplasmic reticulum stress.

Figure S4. Imaging of control samples incubated without UCNPs.

Figure S5. Impact of UCNPS on cell area, nuclear area, and the ratio nuclear area/cytoplasmic area.

Figure S6. Derailment of cellular homeostasis by established stressors.

Figure S7. NFκB accumulation in the nuclei of DTT-treated LLC-PK1 cells.

Raw data files for Western blots.

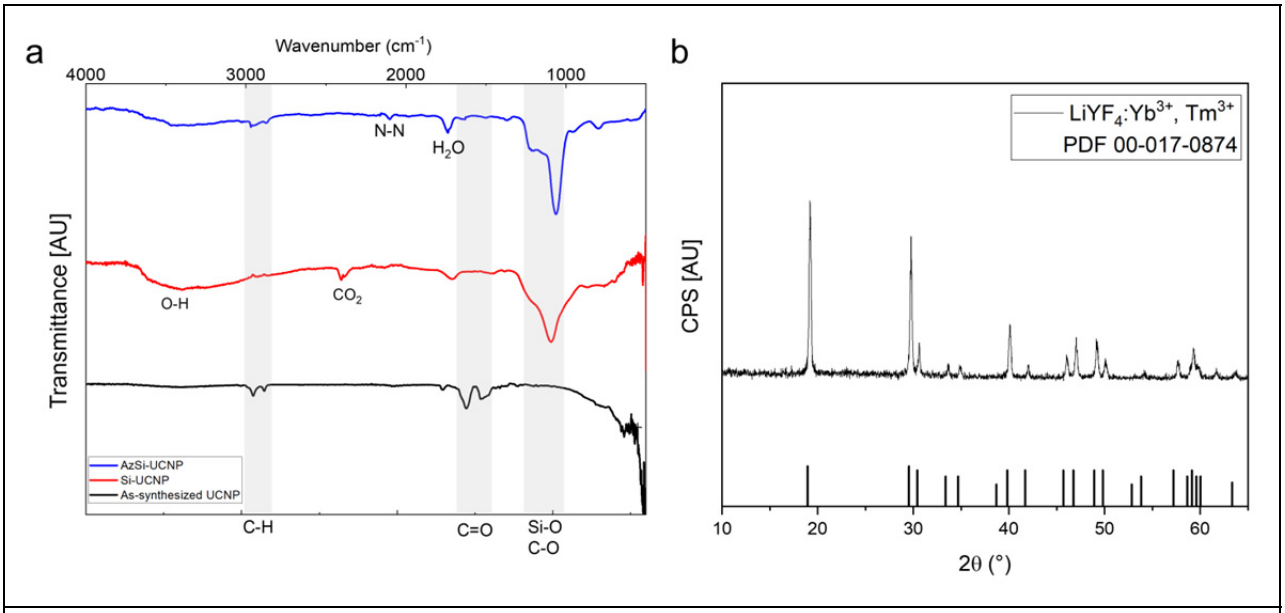


Figure S1. Physicochemical properties of UCNPs used in this study. (a) Infrared spectra of AzSi-UCNPs (blue), Si-UCNPs (red) and as-synthesized oleate-capped UCNPs (black) with the corresponding functional groups labelled on each band. (b) Powder X-ray diffractogram of as-synthesized oleate-capped $LiYF_4:Yb^{3+},Tm^{3+}$ UCNPs and the corresponding reference pattern (PDF 00-017-0874).

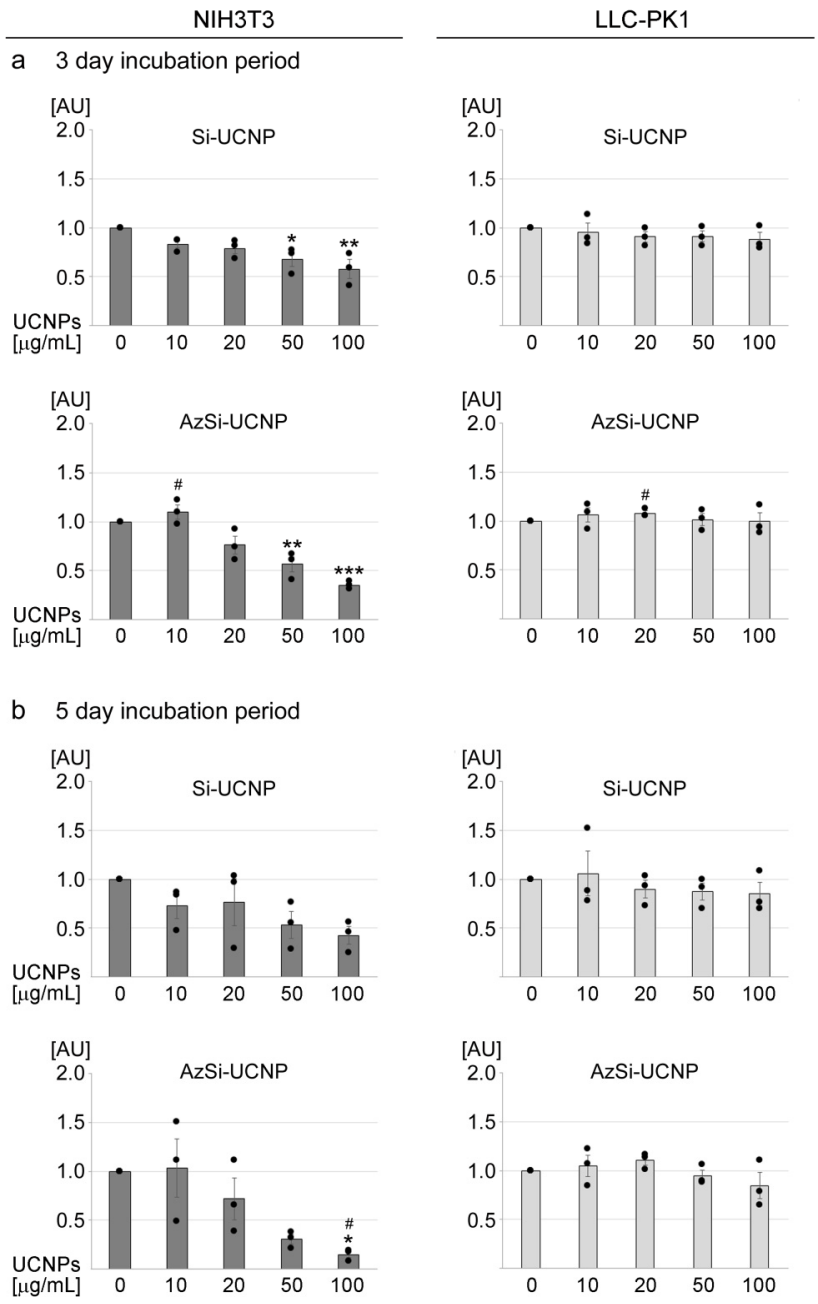


Figure S2. Cell viability after incubation with UCNPs for 3 and 5 days. Prolonged exposure to UCNPs reduces the viability of NIH3T3 fibroblasts, but not of LLC-PK1 proximal tubule cells. Cells were incubated with Si-UCNPs or AzSi-UCNPs for (a) 3 days or (b) 5 days. Cell viability was determined as described for Fig. 3. One-way ANOVA combined with Bonferroni correction identified significant differences between the control [0 µg/ml] and UCNP-treated samples for each experimental group; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Student's t-test was applied for pairwise comparisons between samples incubated with Si-UCNPs and AzSi-UCNPs; #, $p < 0.05$. AU, arbitrary units.

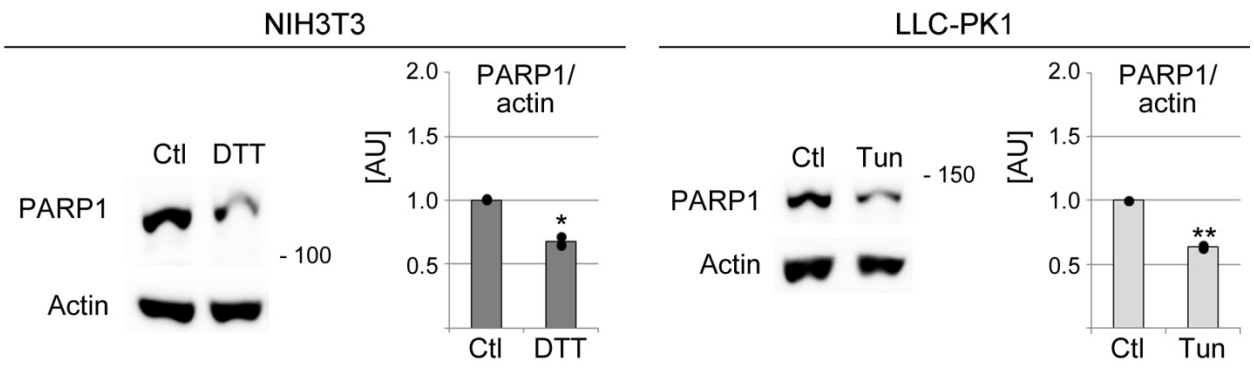


Figure S3. Loss of PARP1 following endoplasmic reticulum stress. NIH3T3 fibroblasts and LLC-PK1 cells were incubated with DTT or tunicamycin (Tun) to trigger an ER stress response. Changes in PARP1 abundance were determined by Western blotting. Molecular masses of marker proteins in kD are depicted at the right margins of the blots. Graphs show results for two independent experiments. Student's t-test was used for pairwise comparisons between vehicle controls (Ctl) and treated samples; *, $p < 0.05$; **, $p < 0.01$. AU, arbitrary units.

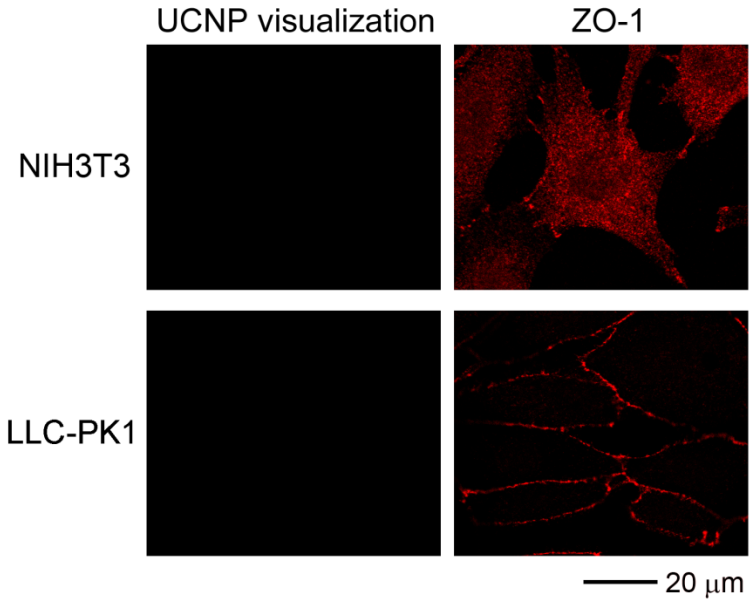


Figure S4. Imaging of control samples incubated without UCNPs. NIH3T3 and LLC-PK1 cells were cultured in the absence of UCNPs. Cell culture and imaging conditions were identical as described for Fig. 4. No signals were observed with the settings used to image UCNPs (UCNP visualization). Scale bar is 20 μm.

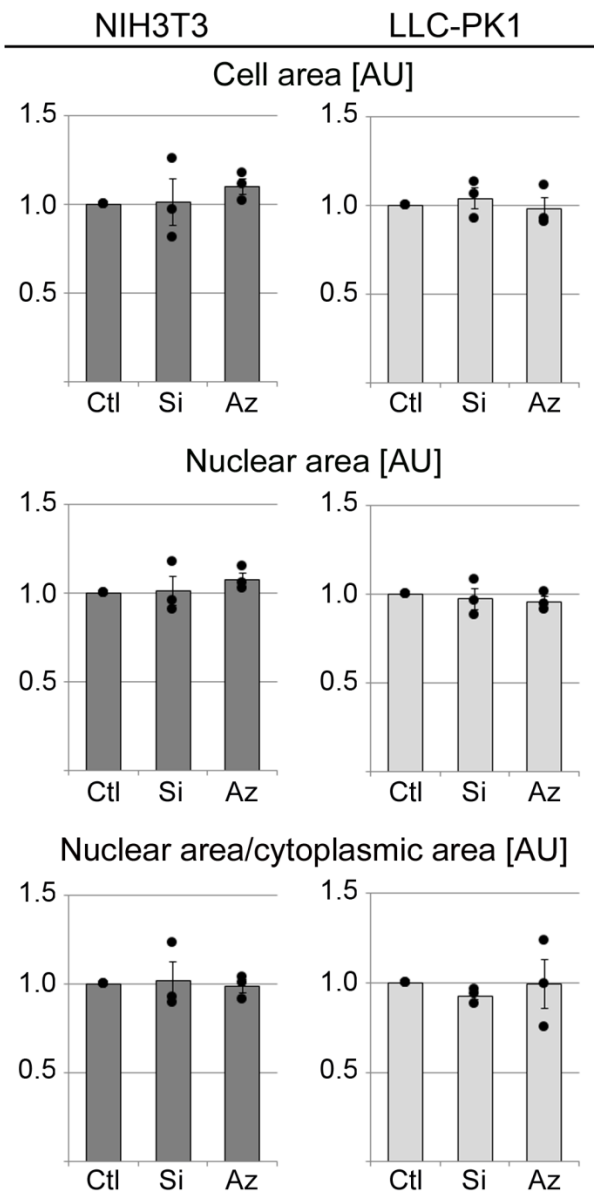


Figure S5. Impact of UCNPS on cell area, nuclear area, and the ratio nuclear area/cytoplasmic area. NIH3T3 or LLC-PK1 cells were treated for 24 hours with vehicle, 100 µg/mL Si-UCNPs or AzSi-UCNPs. Samples were fixed and processed for immunostaining. The size of cells and nuclei was quantified for three independent experiments. For each experiment, at least 30 cells were evaluated per condition. Results were normalized to vehicle controls. The graphs show averages ± SEM for the cell size, nuclear size, or the ratio of nuclear/cytoplasmic areas. UCNPs did not cause significant changes for any of these parameters. AU, arbitrary units.

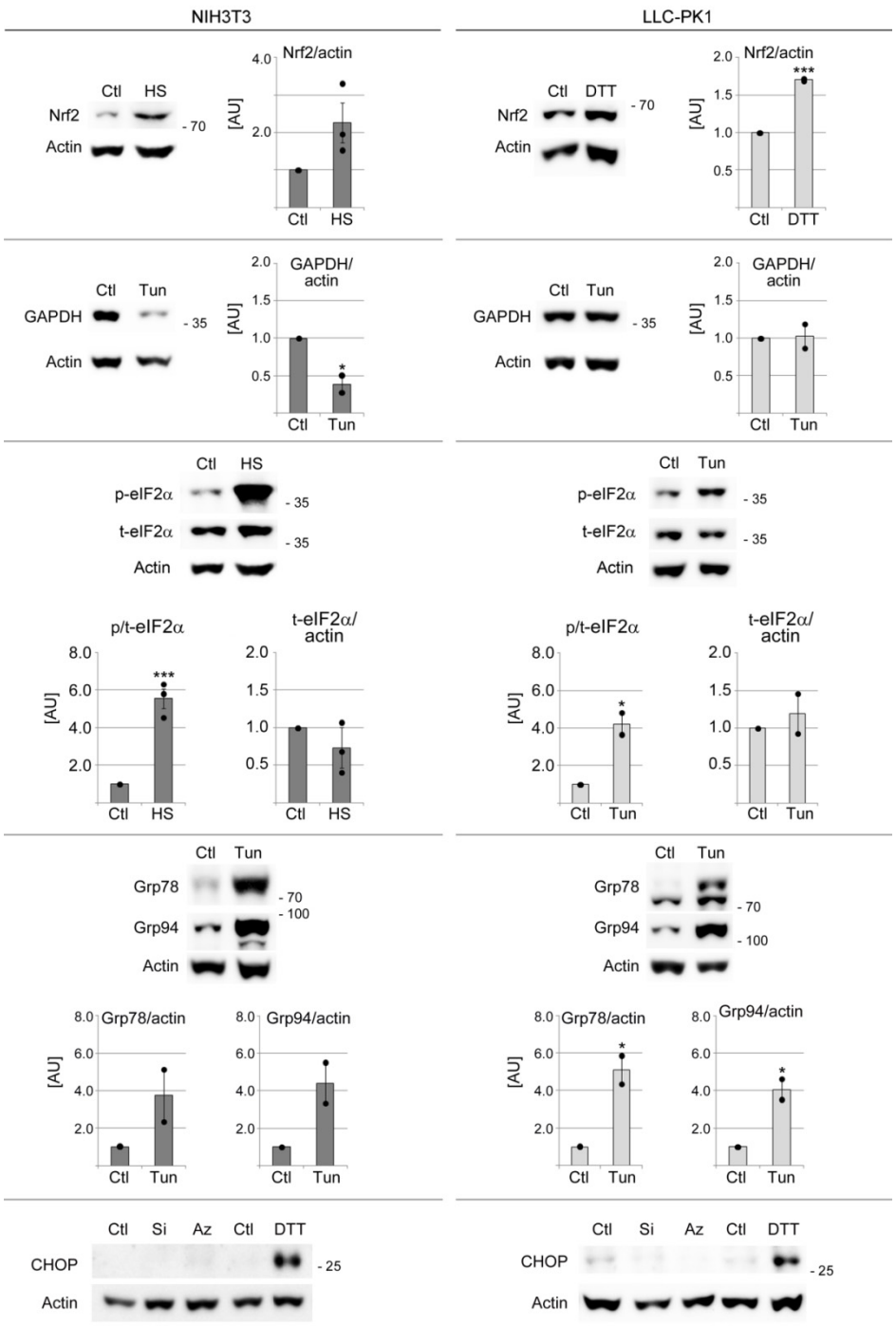


Figure S6. Derailment of cellular homeostasis by established stressors. NIH3T3 and LLC-PK1 cells were incubated with vehicle (Ctl), DTT, tunicamycin (Tun) or heat stress (HS) to impair normal cell physiology. Western blotting assessed Nrf2 and GAPDH. Components of the integrated stress response, p/t-eIF2α, Grp78, Grp94, and CHOP, were also evaluated. Graphs represent Western blotting results for two independent experiments. Student's t-test identified significant differences; *, $p < 0.05$; ***, $p < 0.001$. Molecular masses of marker proteins in kD are depicted at the right margins of the blots. AU, arbitrary units. The same blot was used to assess Nrf2 and eIF2α in NIH3T3 cells. The panels for actin are therefore identical.

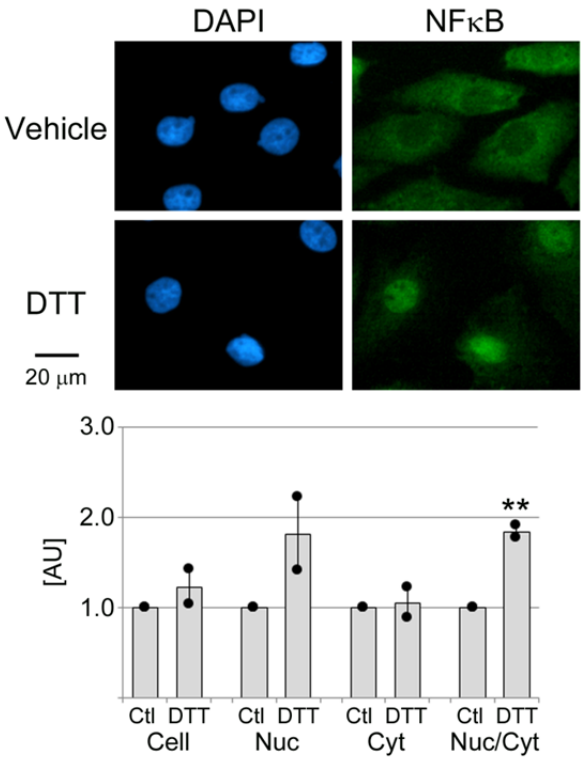
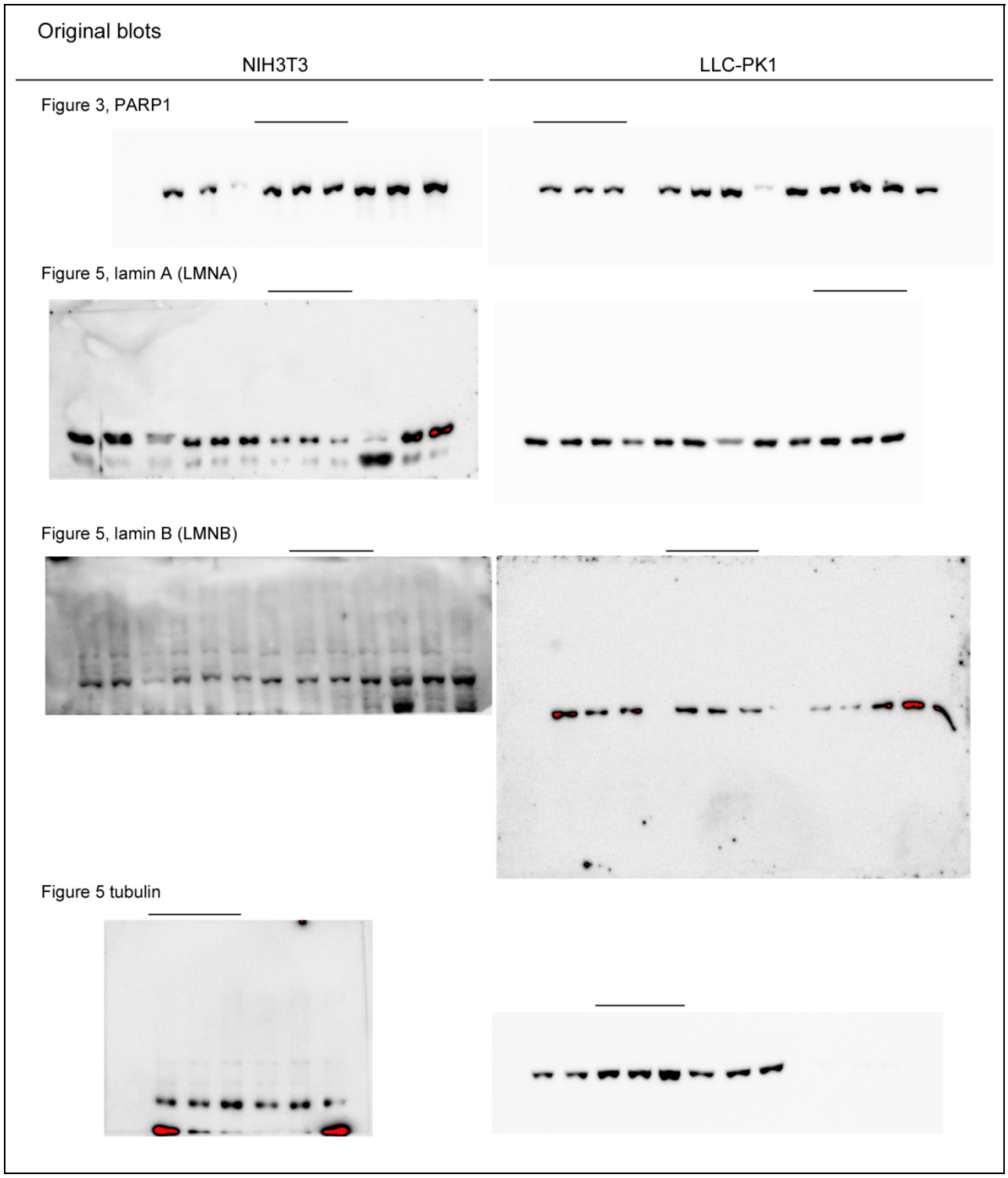


Figure S7. NFκB accumulation in the nuclei of DTT-treated LLC-PK1 cells. LLC-PK1 cells were incubated with vehicle or DTT as described in Materials and Methods. Following immunostaining, the subcellular localization of NFκB was determined for at least 29 cells per condition for each experiment. Results for two independent experiments are shown as average ± SEM. Student's t-test uncovered a significant increase of the nuc/cyt ratio for DTT-treated cells; **, $p < 0.01$. Scale bar is 20 μm. AU, arbitrary units.

Raw data files for Western blots.

Raw data files for enhanced chemiluminescence are depicted below. Black bars on top of the blots identify the lanes that are shown in the figures. For some of the raw data files, the position of the protein of interest is indicated with an arrowhead. Alternatively, the name of the protein is depicted at the margin of the blot. Original blots are shown side-by-side for NIH3T3 and LLC-PK1 cells.

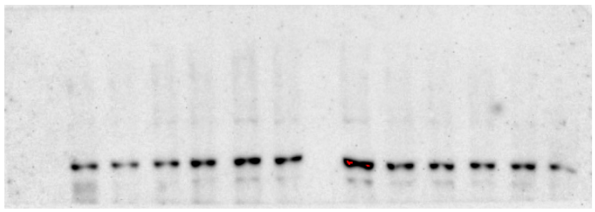


Original blots

NIH3T3

LLC-PK1

Figure 6, Nrf2



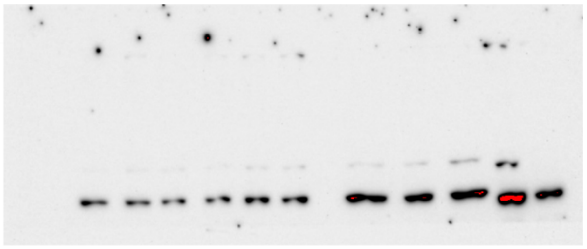


Figure 6, NFκB

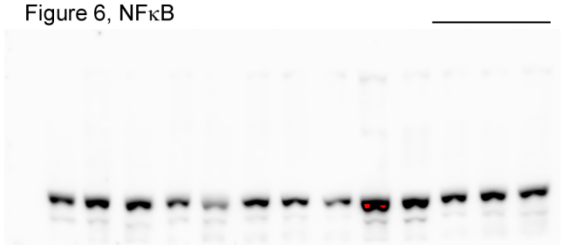
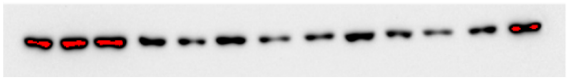
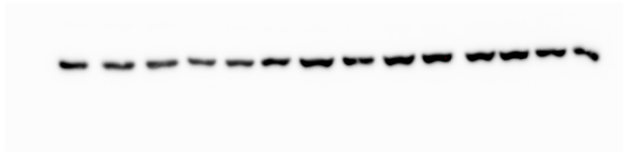


Figure 6, GAPDH





Original blots

NIH3T3

LLC-PK1

Figure 7, p53

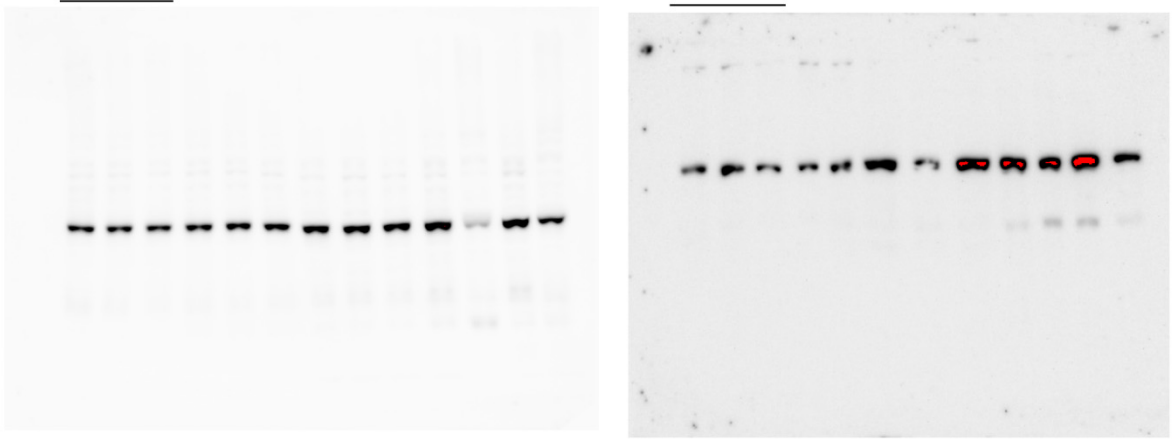


Figure 7, RPA194

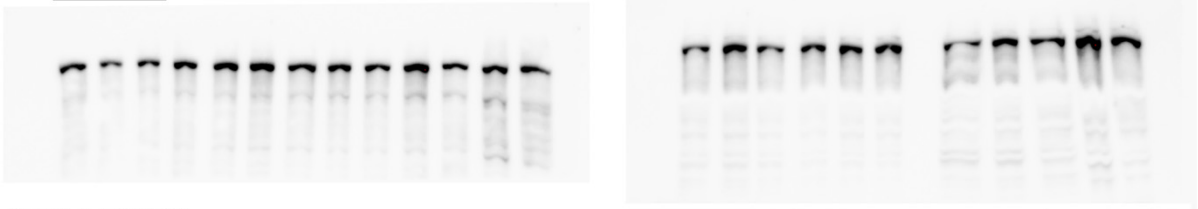


Figure 7, Fibrillarin

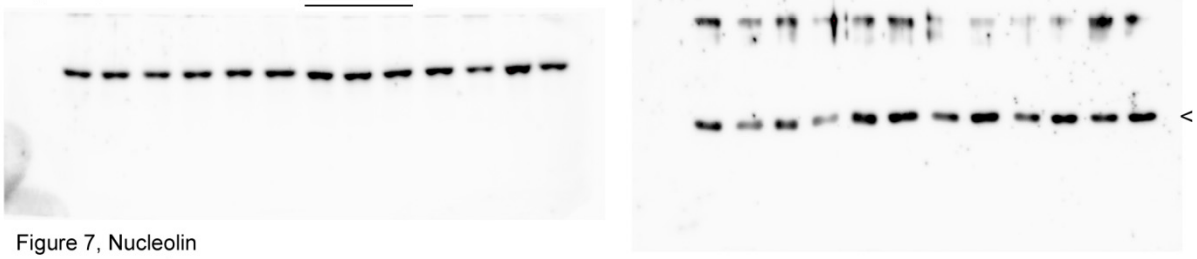
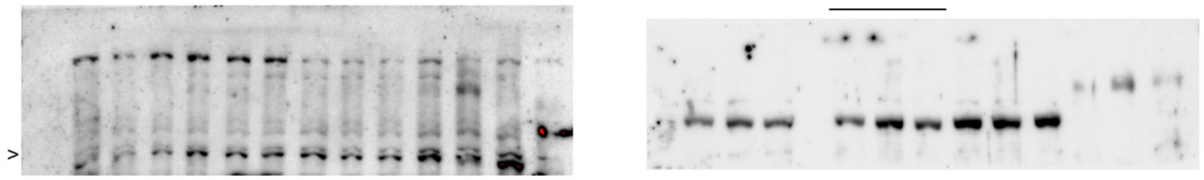


Figure 7, Nucleolin



Original blots

NIH3T3 LLC-PK1

Figure 8, p-eIF2α

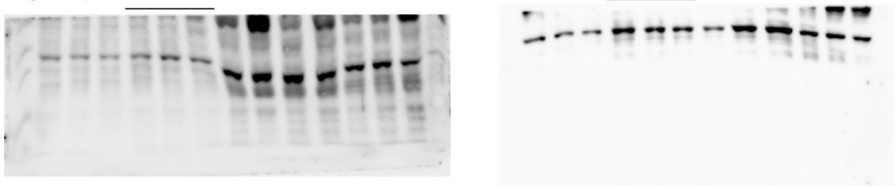


Figure 8, t-eIF2α

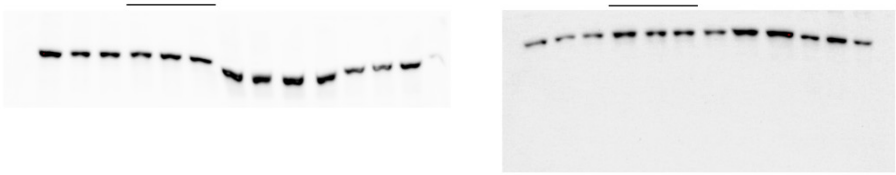
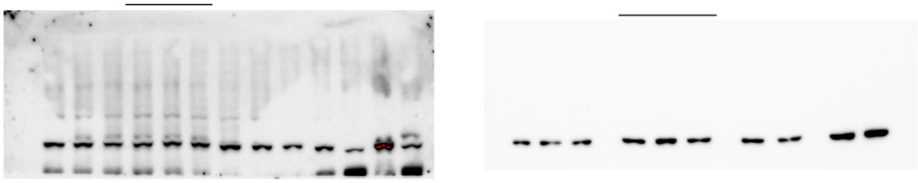


Figure 8, Grp78



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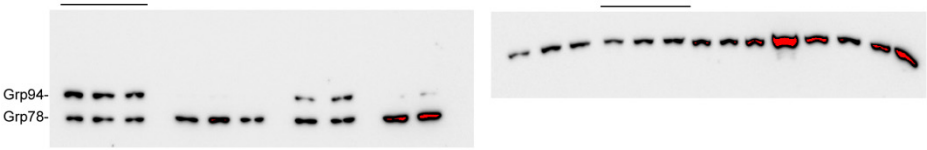


Figure 8, Hsp70

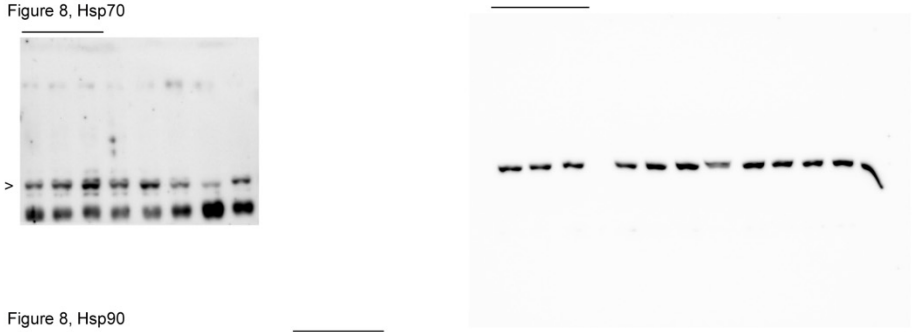


Figure 8, Hsp90

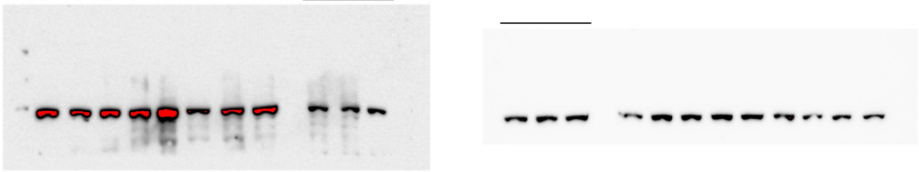


Figure 8, VCP

Original blots

NIH3T3 LLC-PK1

Figure S3, PARP1

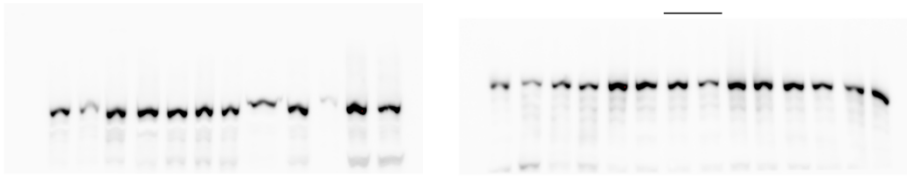


Figure S6, Nrf2

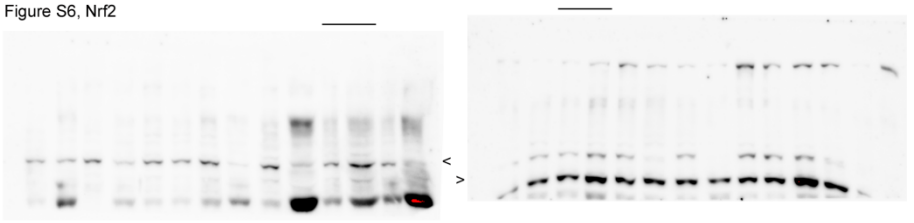


Figure S6, GAPDH

Figure S6, p-eIF2α

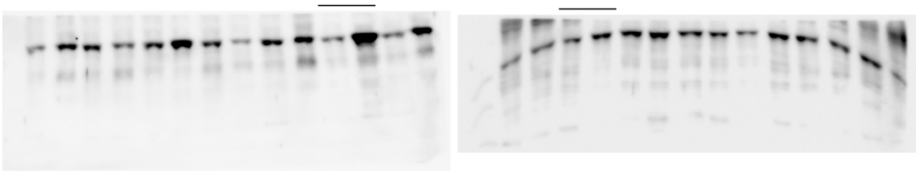
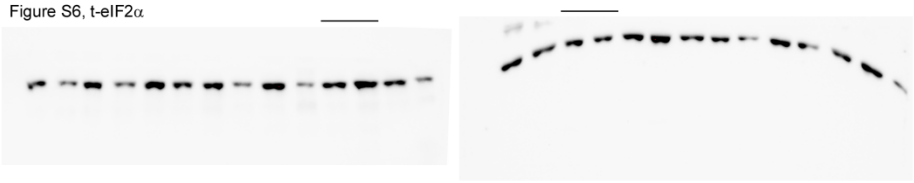


Figure S6, t-eIF2α



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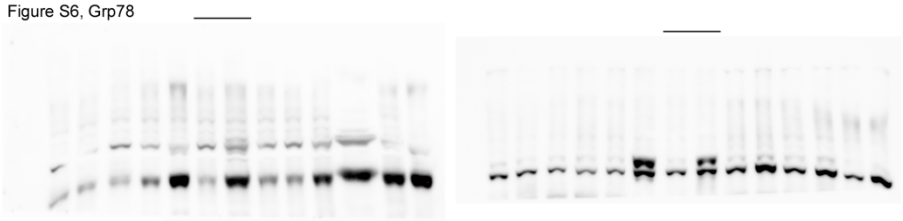

Figure S6, Grp94

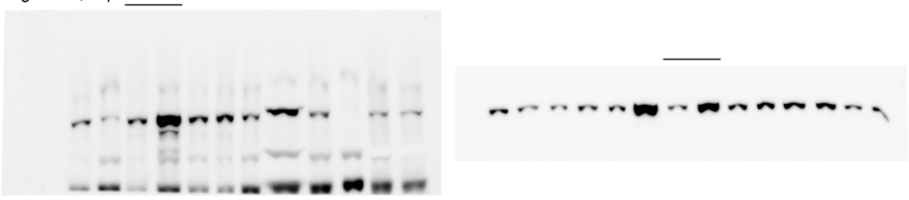

Figure S6, CHOP