Supplementary information

Polyethylenimine, an Autophagy-Inducing Platinum-Carbene-Based Drug Carrier with Potent Toxicity towards Glioblastoma Cancer Stem Cells

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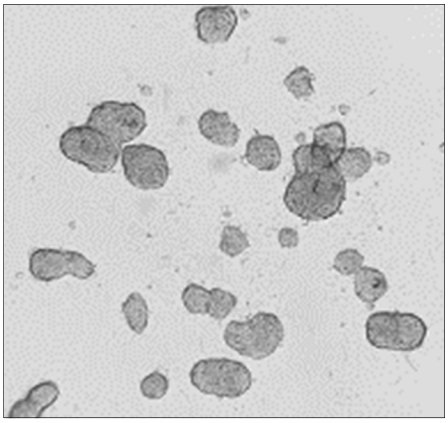

Figure S1. **DPSC spheroid cell culture.** Inverted light microscope image of DPSC spheroids grown in U-bottomed suspension 96 well culture dishes for 24 h before imaging at 4x*10x = 400x magnification using an Axio Vert A1 inverted light microscope (Zeiss) coupled to a ProgRes C5 cool (Jenoptik) camera.

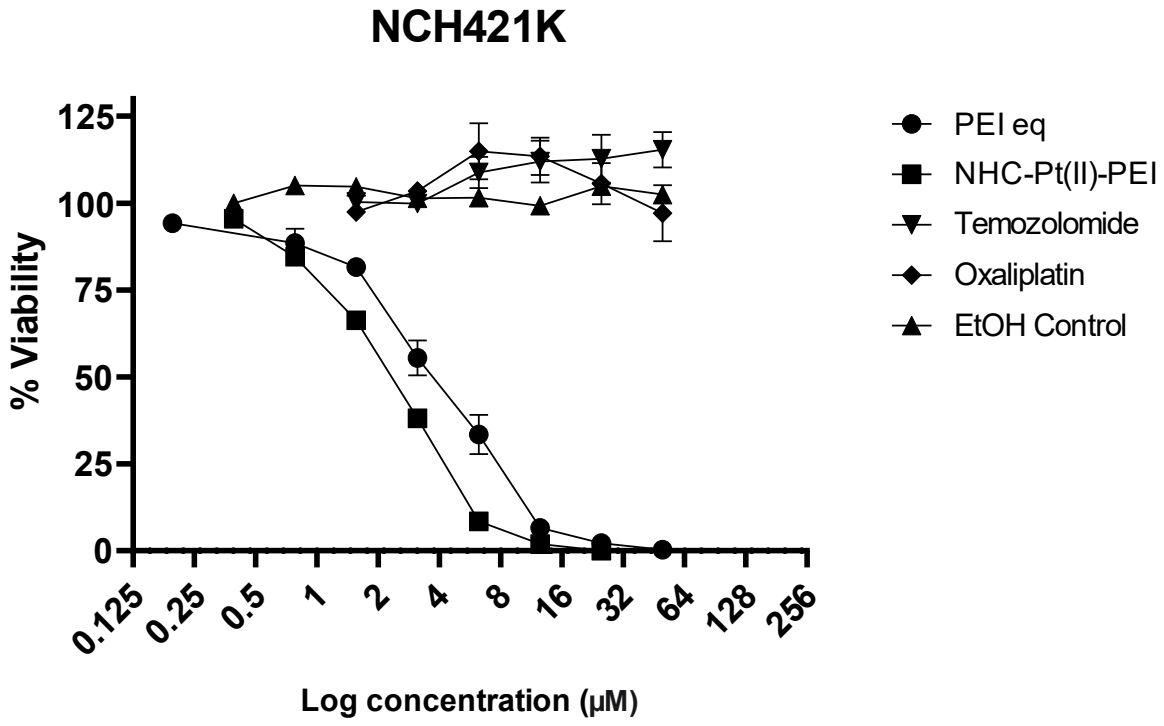

Figure S2. **IC_{50} for the GSC cell line NCH421K.** CelltiterGlo 3D viability dose-response of 24 h treated NCH421K cells. Values represent the mean of at least n=3 independent replicates. Error bars represent +/- one SEM.

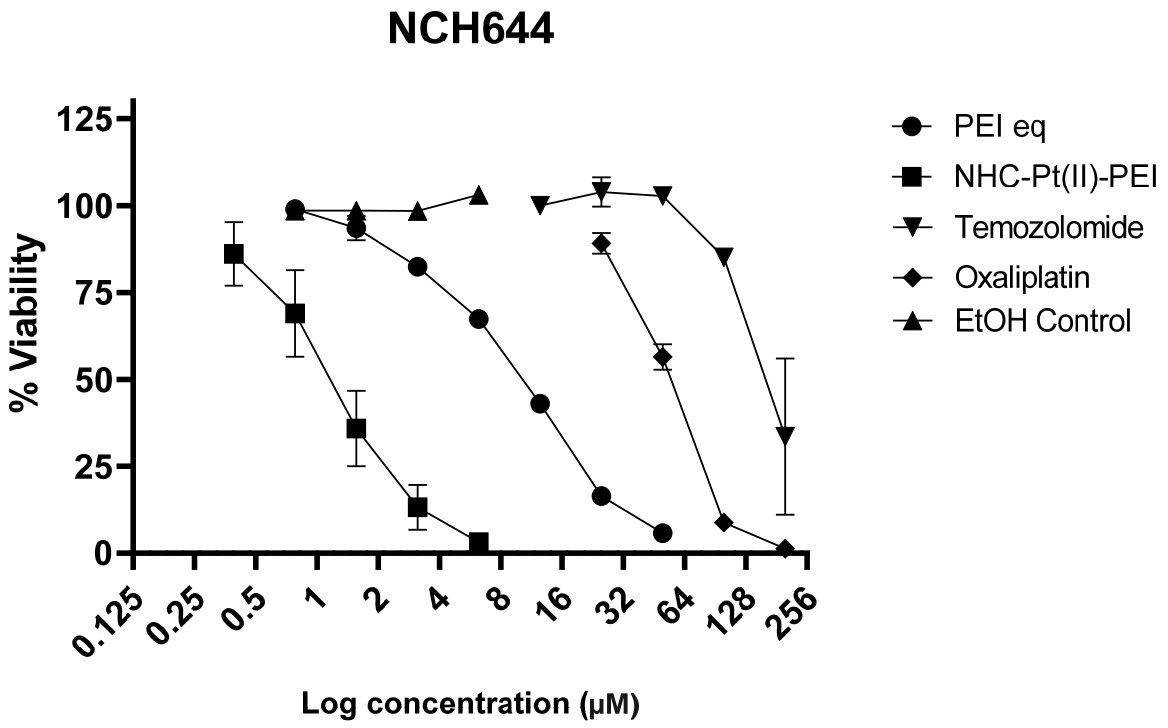

Figure S3. **IC_{50} for the GSC cell line NCH644.** CelltiterGlo 3D viability dose-response of 24 h treated NCH644 cells. Values represent the mean of at least n=3 independent replicates. Error bars represent +/- one SEM.

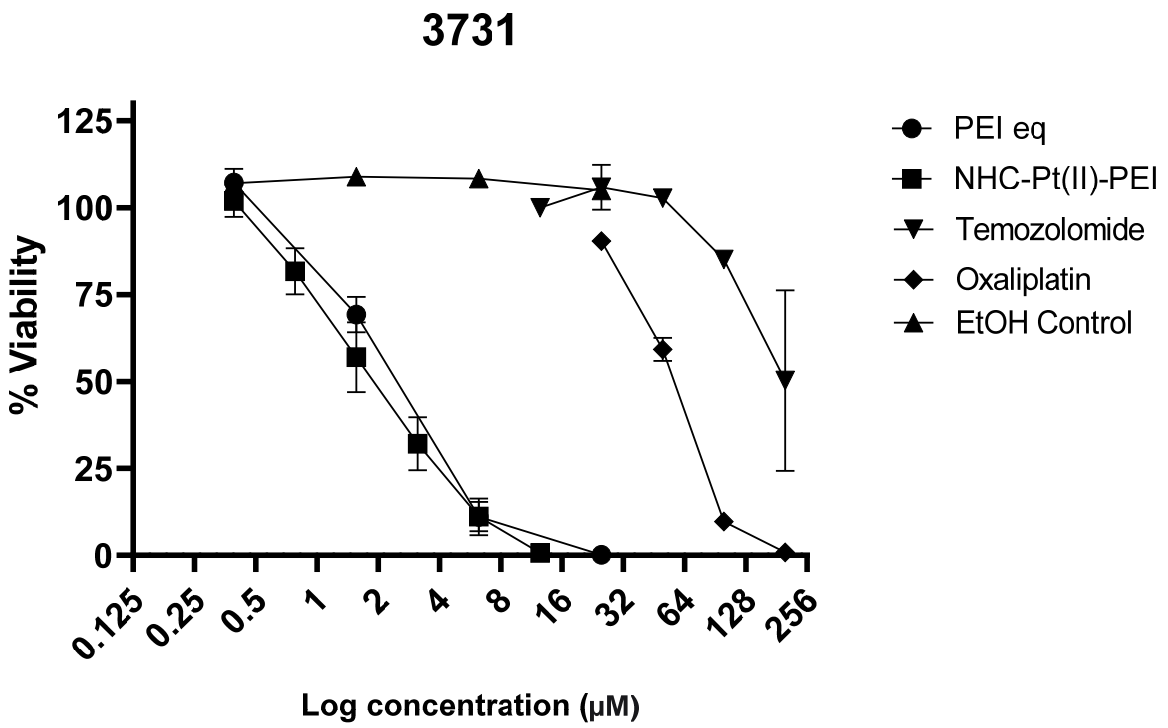

Figure S4. **IC_{50} for the GSC cell line 3731.** CelltiterGlo 3D viability dose-response of 24 h treated 3731 cells. Values represent the mean of at least n=3 independent replicates. Error bars represent +/- one SEM

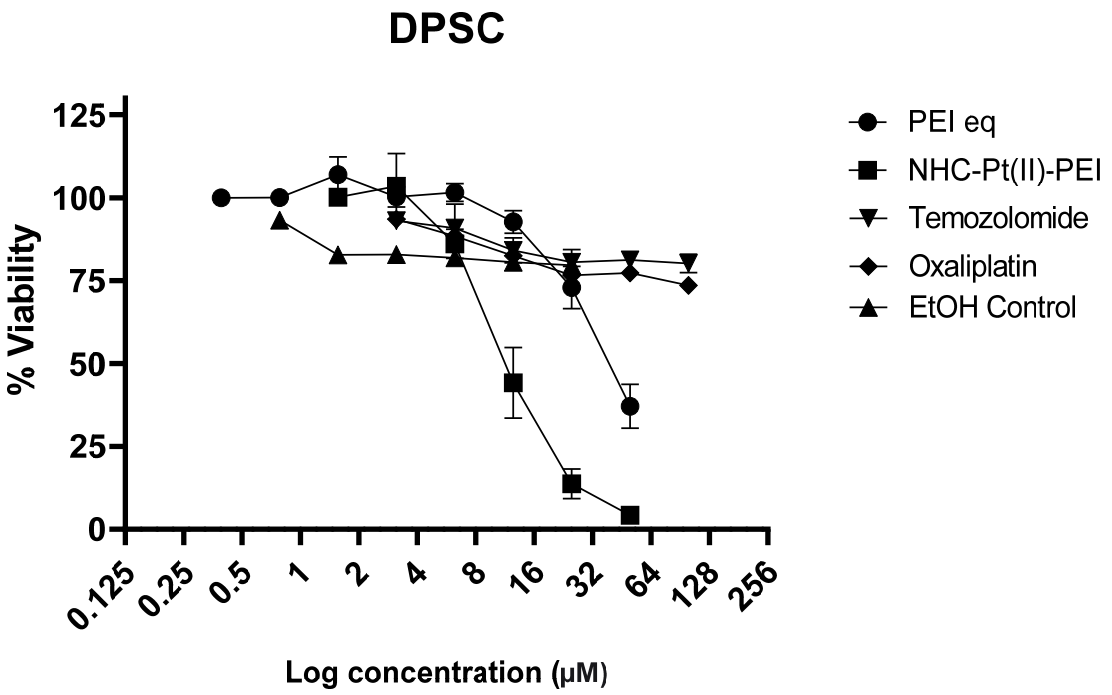

Figure S5. **IC_{50} for the non-cancerous stem cells, DPSCs.** CelltiterGlo 3D viability dose-response of 24 h treated DPSC cells. Values represent the mean of at least n=3 independent replicates. Error bars represent +/- one SEM.

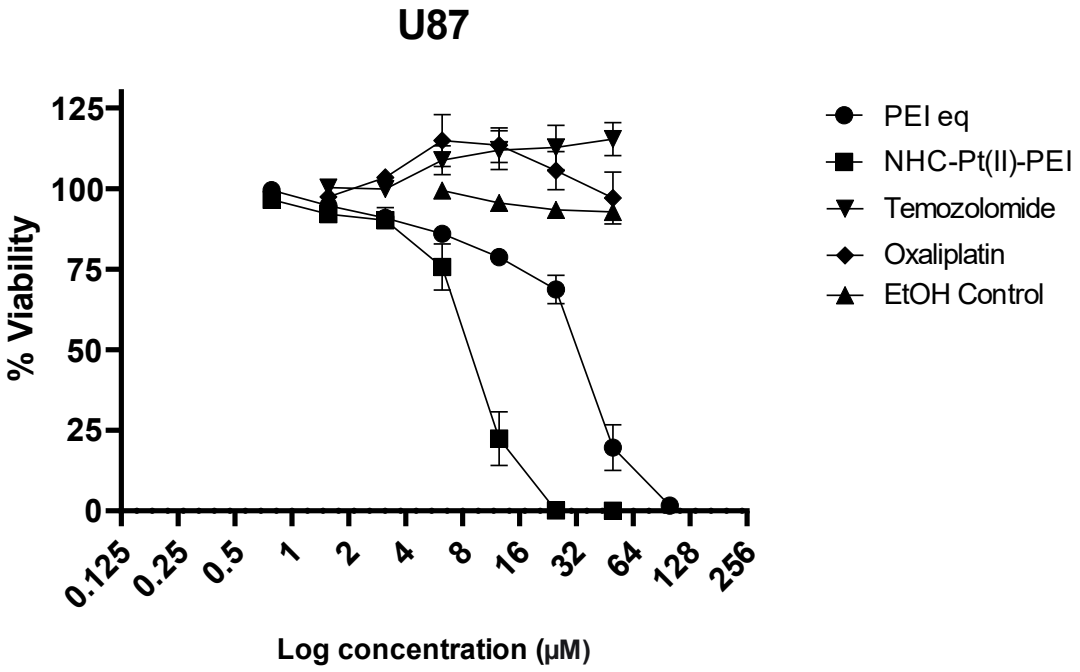

Figure S6. IC_{50} for the non-stem glioma cell line U87-MG. CelltiterGlo 3D viability dose-response of 24 h treated U87 cells. Values represent the mean of at least n=3 independent replicates. Error bars represent +/- one SEM.

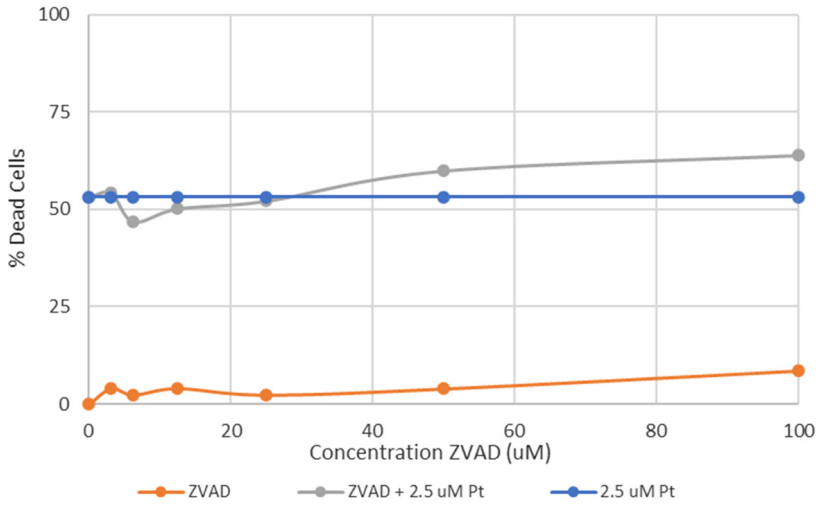

Figure S7. Cell death proportion of 24 h NHC-Pt(II)-PEI and zVAD-fmk co-treated NCH421K cells. The proportion of propidium iodide positive (dead) cells measured by flow cytometry following 24 h treatment with 2.5 µM NHC-Pt(II)-PEI (noted Pt), a cascade of concentrations of zVAD-fmk (noted ZCAD) or 2.5 µM NHC-Pt(II)-PEI + ZVAD.

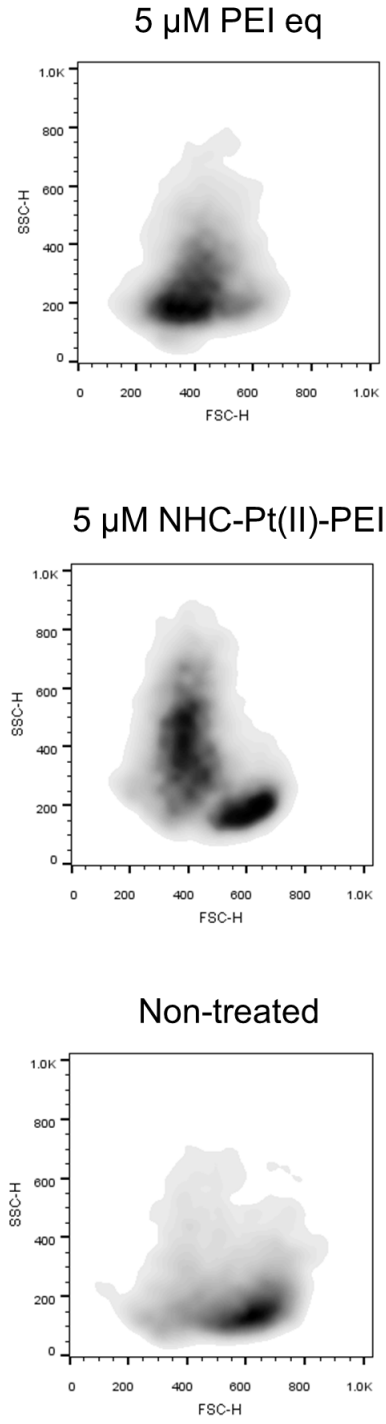

Figure S8. **The granularity of 24 h 5 µM PEI eq and NHC-Pt(II)-PEI treated NCH421K spheroids.** Flow cytometry FSC and SSC dot plots (FlowJo) of treated cells.

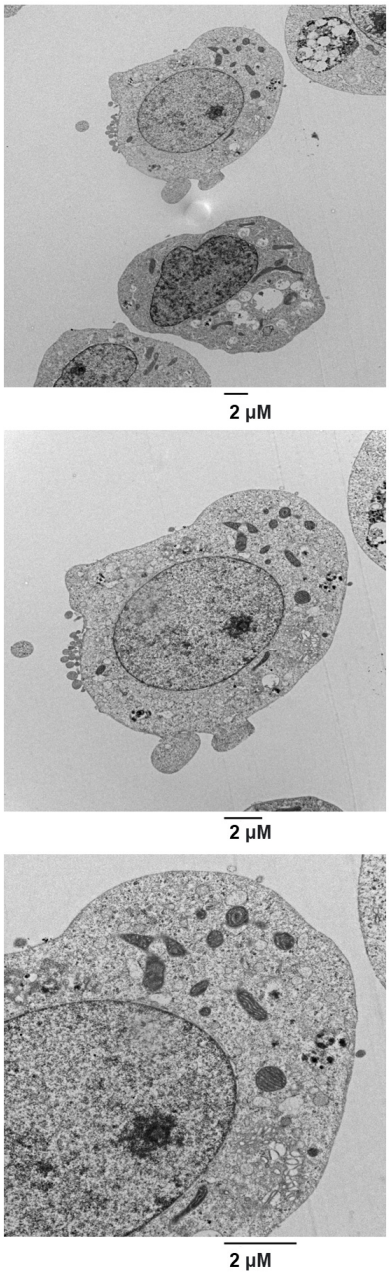

Figure S9. **Transmission electron microscopy of 6 h 60 µM chloroquine treated NCH421K cells.** Top = 5000x magnification. Middle = 8000x magnification. Bottom = 15,000x magnification.

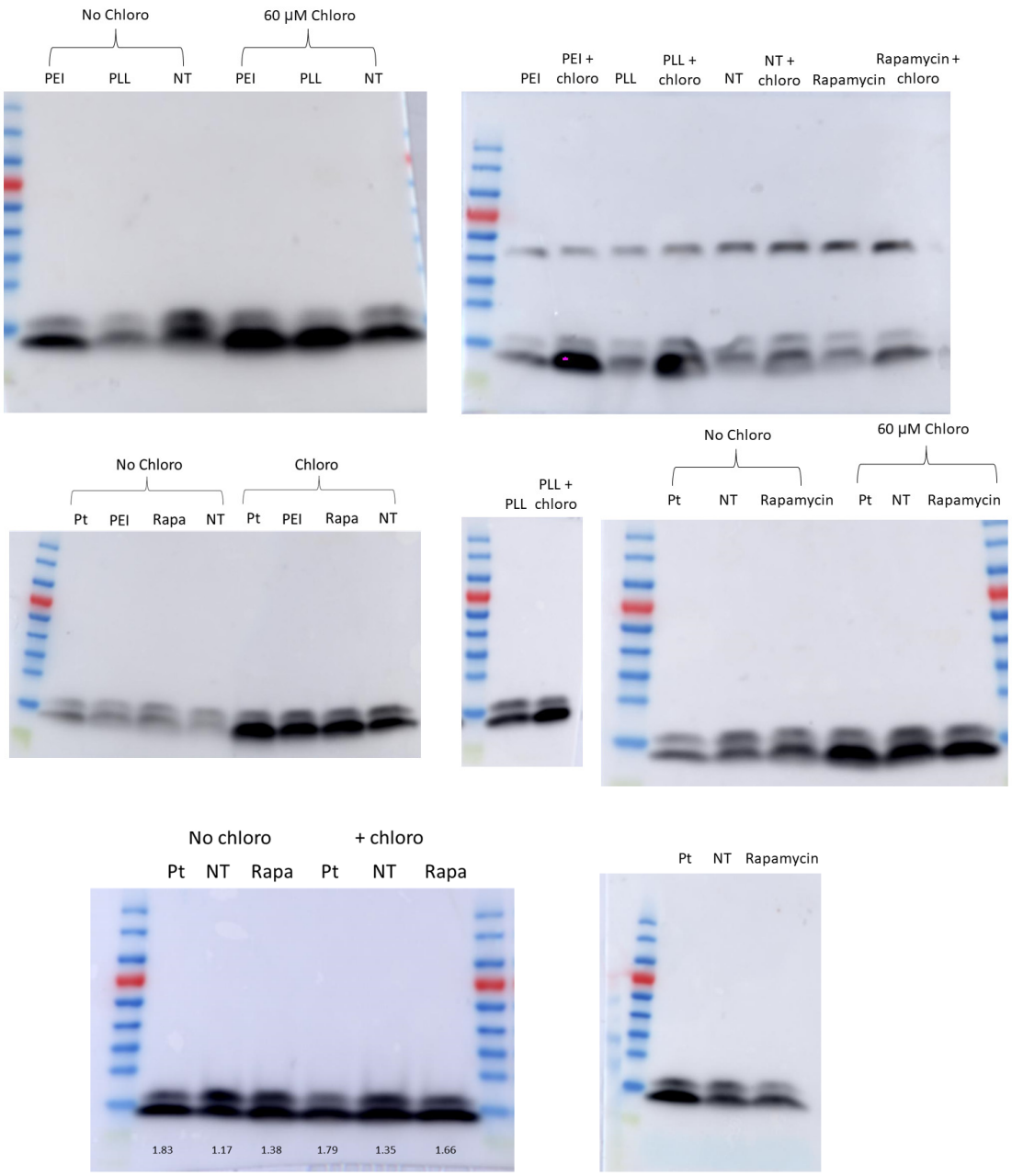

Figure S10. **Unedited LC3 western blots.** All treatments 6 h except chloroquine (3 h). Pt = NHC-Pt(II)-PEI. Rapamycin (Rapa) = 10 µM. Poly-L-Lysine (PLL) = 5 µM Pt eq (294 nM polymer). Polyethylenimine (PEI) = 5 µM Pt eq (294 nM polymer). Non-treated = NT.

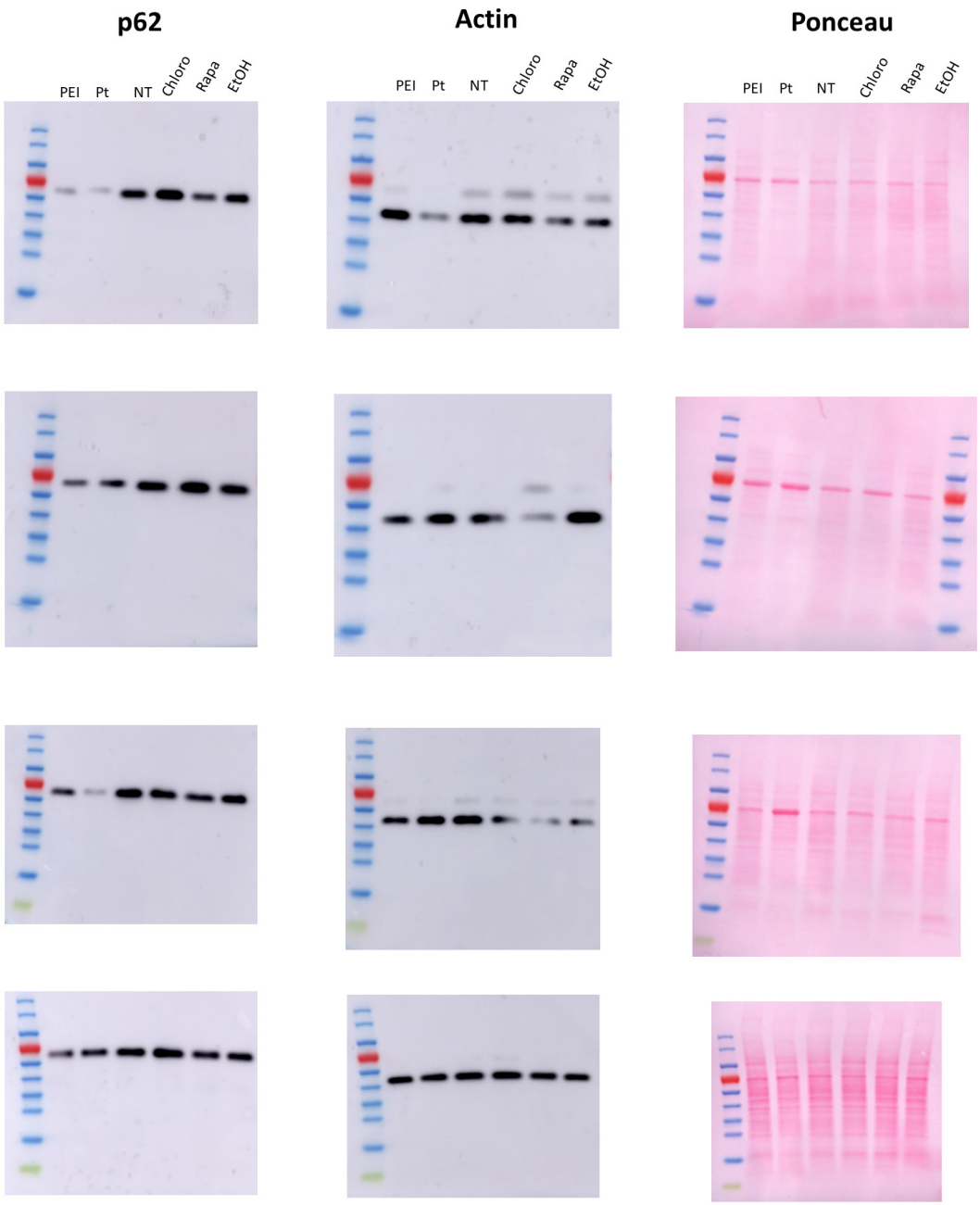

Figure S11. Unedited p62 western blots. Pt (NHC-Pt(II)-PEI) = 5 μM. Polyethylenimine (PEI) = 5 μM Pt eq (294 nM polymer). Non-treated = NT. Chloro (chloroquine) = 60 μM. Rapamycin (Rapa) = 10 μM. Ethanol control (EtOH) = Equivalent EtOH quantity corresponding to NHC-Pt(II)-PEI condition. All treatments 6 h.

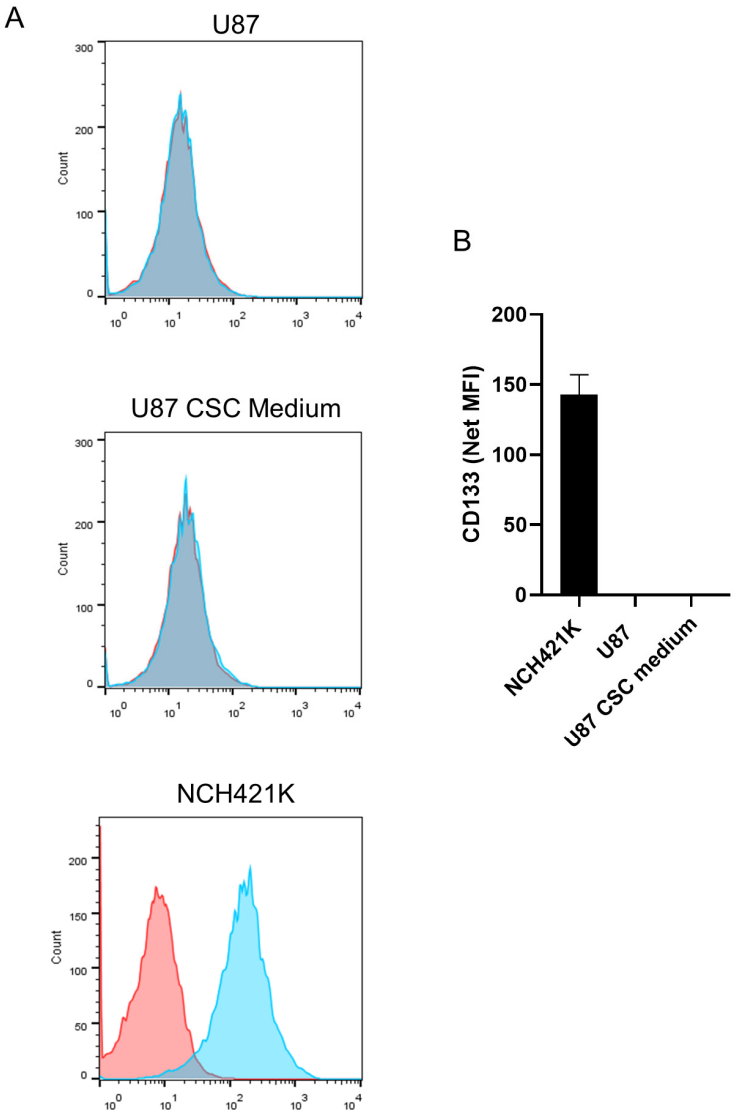

Figure S12. **U87 CD133 expression.** A) Representative flow cytometry histograms (FlowJo) of CD133 expression on U87 cells, U87 cells cultured for 24 h in CSC medium, and NCH421K cell. Red = isotype. Blue = CD133. B) Histograms of net MFI (geometric mean fluorescence intensity) (MFI CD133 – MFI isotype) CD133 expression. Values represent the mean of n=3 independent experiments ± one SEM.