

Supplementary

Anticancer Effect of Pt^{II}PHENSS, Pt^{II}5MESS, Pt^{II}56MESS and Their Platinum(IV)-Dihydroxy Derivatives against Triple-Negative Breast Cancer and Cisplatin-Resistant Colorectal Cancer

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Method S1. Nano Proteomics Method

The digested protein samples were analyzed by UPLC-MS using a Waters nanoAcquity UPLC sample manager fitted with a binary solvent manager. Mass spectrometric detection was conducted using a Waters Synapt G2-Si. Separation consisted of two mobile phases. Mobile Phase A (0.1% formic acid in Milli-Q water) and Mobile Phase B (0.1% formic acid in ACN). The trapping column was a Waters nanoEase M/Z Symmetry C18 trap column (180 μ m x 20 mm) and the analytical column was a Waters nanoAcquity UPLC 1.7 μ m BEH130 C18 column (75 μ m x 100 mm) thermostatted to 40 °C. Elution was achieved at a flow rate of 0.3 μ L/min with each sample run for 55 minutes. The gradient was 0 min 1% B; 2 min 10% B; 40 min 40% B; 42 min 85% B and 50 min 85% B. System specific cleaning and equilibration protocols were run before each sample.

Mass spectrometry was conducted in positive ion mode with a capillary voltage of 3 kV and a sampling cone voltage of 30 V as well as a source offset of 30 V for electrospray ionizations. The source temperature was set at 80 °C. A desolvation source of nitrogen gas at 20 L/h and a desolvation temperature of 350 °C was used. Lock spray ion acquisition was conducted every 300 seconds with [Glu1]-fibrinopeptide B as the reference compound. Data acquisition was conducted over the mass to charge range of 50–2000. The data independent acquisition used an HDMSe experiment employing both low and high energy collision-induced dissociation of parent ions. Low energy collision was done at 6 V in the trap collision cell and at 4 V in the transfer collision cell. High energy collision used a collision energy ramp from 17 to 60 V in the transfer collision cell. Scan time was 0.5 seconds and after each scan the system would switch from high to low energy collision.

Protein identification was carried out using Progenesis QI for Proteomics and the UniProt Homo Sapiens reference database with the following conditions. The parent and fragment ion tolerances were set to automatic, the allowed maximum missed cleavages was set to 1, the allowed false discovery rate was set to 4% and the maximum protein size was set to 250 kDa. The peptide modifications were carbamidomethyl C (fixed) and oxidation M (variable). The ion matching requirements were fragments/peptide of 1 or more, fragments/protein of 3 or more and peptides/protein of 1 or more. Relative protein quantitation was performed with the Hi-N method using the top 3 peptides. Progenesis QI automatically selects unique reporter peptides to quantify a protein and measures their UPLC peak areas in the total ion chromatogram.

Method S2. Syntheses of Platinum(II) Complexes and Platinum(IV) Dihydroxy Complexes

The synthesis of [Pt(1*S*,2*S*-diaminocyclohexane)]Cl₂

Potassium tetrachloroplatinate (K₂PtCl₄; 500.8 mg, 1.21 mmol) and 1*S*,2*S*-diaminocyclohexane (1*S*,2*S*-dach, 1 mol eq.) were dissolved in deionised water (d.i.H₂O). The mixture was stored at 4 °C for 48 h. The solution turns opaque as the yellow precipitate forms. The solution was filtered and the product, [Pt(1*S*,2*S*-dach)Cl₂], was washed sequentially with d.i.H₂O, ethanol, ether, and then air dried. The product, [Pt(1*S*,2*S*-dach)Cl₂], was stored in a desiccator [1-5].

The general synthesis of [Pt(P_L)(1*S*,2*S*-diaminocyclohexane)](NO₃)₂, [Pt(1*S*,2*S*-dach)Cl₂] (490.5 mg, 1.42 mmol) and a polyaromatic ligand P_L (Phen, 5Mephen or 56Me₂phen) (P_L; 1.1 mol eq.) in d.i.H₂O (100 mL) were refluxed for 24 h at 100 °C. The refluxed solution was an opaque yellow mixture and became a clear yellow solution when the

reaction was completed. The reaction solution volume was reduced to roughly 20 mL *via* rotary evaporation for purification *via* a Vac 20cc (5 g) C₁₈ Sep-Pak[®] column connected to a Bio-Rad pump with UV detector (Bio-Rad, EM-1 Econo[™] UV Monitor). The C₁₈ Sep-Pak[®] column was activated by eluting initially with methanol (20 mL) and subsequently flushing with d.i.H₂O (20 mL) until the UV absorbance had equilibrated. The concentrated reaction product was loaded onto the column and eluted with H₂O. Fractions of the eluting yellow band were collected. The fractions, were reduced under vacuum using a rotary evaporator and then lyophilised, producing pale yellow solids.

To convert each platinum(II) complex from chloride to the nitrate salt, each solid was dissolved in a minimal volume of d.i.H₂O and silver nitrate (AgNO₃; 2 mol eq.) was then added. The solution was stirred in the dark overnight, and the resulting silver chloride (AgCl) precipitate was syringe filtered to isolate the solution containing the platinum(II) complex nitrate salt. The nitrate solutions of **Pt^{II}PHENSS**, **Pt^{II}5MESS** or **Pt^{II}56MESS** (Scheme 1) were then reduced under vacuum and lyophilised to produce pale yellow solid.

The successful synthesis and purity of the platinum(II) complexes was confirmed by high performance liquid chromatography (HPLC), ¹H nuclear magnetic resonance (NMR), ¹H-¹⁹⁵Pt heteronuclear multiple quantum coherence (HMQC) NMR and circular dichroism (CD) spectroscopy [1-5]. HPLC chromatograms were determined by eluting the platinum complexes through an Agilent Technologies Infinity HPLC machine, equipped with a Phenomenx Onyx[™] Monolithic C₁₈-reverse phase column (100 × 4.6 mm, 5 μm pore size). The mobile phase consisted of solvents, A (0.06% trifluoroacetic acid (TFA) in d.i.H₂O) and B (0.06% TFA in ACN/d.i.H₂O (90:10)). An injection volume of 5 μL was utilised and eluted with a 0 – 100% linear gradient over 15 min with a flow rate of 1 mL/min, within the set wavelengths of 214 and 254 nm. ¹H-MR was determined for each complex by dissolving the complex (5 mg) in deuterium oxide (D₂O) (500 μL). ¹H NMR was carried out on a 400 MHz Bruker Avance Spectrometer at 298 K. All samples were prepared to a concentration of 10 – 20 mM in 450 – 600 μL using D₂O. ¹H NMR was set to 10 ppm and 16 scans with a spectral width of 8250 Hz and 65536 data points. ¹H-¹⁹⁵Pt HMQC was carried out using a spectral width of 214436 Hz and 256 data points for ¹⁹⁵Pt nucleus, F1 dimension, also a spectral width of 4808 Hz with 2048 data points for ¹H nucleus, F2 dimension. All resonance recorded were presented as chemical shifts in parts per million (δ ppm). A Jasco J-810 CD spectrophotometer was used to measure the CD spectra of the purified platinum(II) and platinum(IV) complexes. The samples were prepared in d.i.H₂O, with a concentration of 0.05 mM and a 1 mm optical glass cuvette was used. CD experiments were performed in the range of 350–200 nm (20 accumulations) with a bandwidth of 1 nm, data pitch of 0.5 nm, a response time of 1 sec, and a 100 nm/min scan speed. The flowrate of nitrogen gas was 8 L/min. Excel) was used to process the spectral data.

The general synthesis of [Pt(P_L)(1*S*,2*S*-diaminocyclohexane)(OH)₂](NO₃)₂

Platinum(IV) complexes of the type [Pt^{IV}(P_L)(1*S*,2*S*-dach)(OH)₂]²⁺ were synthesised using platinum(II) products (**Pt^{II}PHENSS**, **Pt^{II}5MESS** or **Pt^{II}56MESS**) obtained previously after the nitrate conversion step. Oxidation was undertaken by adding 8 mL of 30% hydrogen peroxide to the solution, which was then heated at 70 °C for 3 h in the dark to obtain, **Pt^{IV}PHENSS (OH)₂**, **Pt^{IV}5MESS (OH)₂** or **Pt^{IV}56MESS(OH)₂** derivatives (Scheme 1). The resulting reaction solution was lyophilised overnight to obtain a yellow to brown precipitate. The crude lyophilised platinum(IV) product was dissolved in minimal amount of d.i.H₂O (3–5 mL) and excess ACN added to precipitate out the crude product which appears as a fine almost white powder when dried. The successful synthesis and purity of the platinum(IV) dihydroxy

complexes was confirmed with previously reported literature using HPLC and NMR [1-5]. Yields are reported in Table H.S1.

Result S2. Characterisation and Yields of Platinum(II) Complexes and Platinum(IV) Dihydroxy Complexes.

HPLC, NMR and CD were used to assess the structure and purity of the complexes for biological testing, passing for greater than 95% purity. The obtained yield and purity of the platinum (II) and their platinum(IV) dihydroxy derivatives were reported in Table H.S1 and chromatograms, which include the retention time (t_R) are reported in Figures HS1-HS6. The obtained ^1H and ^1H - ^{195}Pt -HMQC NMR spectra are tabulated in Table N.S1 and represented in Figures N.S7–S18. The obtained HPLC chromatograms (Figure H.S-S6) and NMR spectra (Figure N.S7–S18) matched those of previously published data, which confirmed the successful synthesis and isolation of our product of interest [1-3,5]. The CD experiments confirm the retention of chirality of the 1*S*,2*S*-dach. The obtained results for the platinum(II) and platinum(IV) complexes were comparable to what is reported in the literature [3]. All the characteristic peaks from CD spectra of the purified complexes are summarised in Table H.S1.

Table H.S1. Summary of the characterisation data of the platinum(II) and (IV) complexes.

| Complexes | Yields [mg] | Yields [%] | t _R [min] | Purity [%] | CD λ _{max} [nm] (mdeg.mol / L × 10 ¹) |
|--|-------------|------------|----------------------|------------|---|
| Pt^{II}PHENSS | 550 | 89 | 5.152 | 99.59 | 207 (−210.46), 228 (+168.45), 247 (−52.97), 257 (+15.56), 309 (−33.44), 334 (+127.32) |
| Pt^{II}5MESS | 620 | 84 | 5.599 | 99.03 | 208 (+361.54), 226 (+416.85), 247 (+296.63), 261 (+300.04), 290 (−92.00), 335 (+144.51) |
| Pt^{II}56MESS | 490 | 75 | 5.990 | 99.93 | 209 (−137.92), 228 (+150.21), 241 (−49.52), 268 (+71.07), 291 (−46.77), 310 (+42.38), 338 (+126.86) |
| Pt^{IV}PHENSS(OH)₂ | 307 | 90 | 4.092 | 99.99 | 202 (−596.81), 269 (−44.98), 286 (+45.70) |
| Pt^{IV}5MESS(OH)₂ | 380 | 88 | 4.132 | 99.43 | 205 (−463.98), 208 (−503.54), 253 (+9.05), 269 (−41.70), 298 (+57.97) |
| Pt^{IV}56MESS(OH)₂ | 257 | 92 | 4.441 | 99.55 | 202 (−517.15), 212 (−425.03), 255 (+28.35), 284 (−27.59) |

Table N.S1. Summary of NMR spectroscopy data of platinum(II) and (IV) complexes, showing the chemical shifts (ppm), integration, multiplicity and coupling constants. ^[a]

| Proton Labels | Complexes | | | | | |
|--------------------------------------|--|---|--|--|---|---|
| | Pt^{II}PHENSS | Pt^{II}5MESS | Pt^{II}56MESS | Pt^{IV}PHENSS(OH)₂ | Pt^{IV}5MESS(OH)₂ | Pt^{IV}56MESS(OH)₂ |
| H2/H9 | 8.8 (d, 2H, <i>J</i> = 5.4 Hz) | 8.80 (m, 3H) | 8.8 (d, 2H, <i>J</i> = 8.5 Hz) | 9.2 (d, 2H, <i>J</i> = 6.2 Hz) | 9.1 (d, 2H, <i>J</i> = 8.3 Hz) | 9.2 (d, 2H, <i>J</i> = 8.6 Hz) |
| H4/H7 | 8.8 (d, 2H, <i>J</i> = 8.3 Hz) | 9.1 (d, 2H, <i>J</i> = 8.3Hz), | 9.1 (d, 2H, <i>J</i> = 5.3 Hz) | 9.1 (d, 2H, <i>J</i> = 8.9 Hz) | 9.1 (d, 2H <i>J</i> = 8.5 Hz) | 9.1 (d, 2H, <i>J</i> = 5.5 Hz) |
| H5/H6 | 8.0 (s, 2H) | 7.59 (s, H6, 1H) | – | 8.3 (s, 2H) | 8.1 (s, H6, 1H), | – |
| H3/H8 | 7.9 (dd, 2H, <i>J</i> ₁ = 5.4 Hz, <i>J</i> ₂ = 8.3 Hz) | 7.9 (m, 2H, <i>J</i> ₁ = 5.4 Hz, <i>J</i> ₂ = 8.3 Hz) | 7.9 (dd, 2H, <i>J</i> ₁ = 5.4 Hz, <i>J</i> ₂ = 8.6 Hz) | 8.2 (dd, 2H, <i>J</i> ₁ = 5.5 Hz, <i>J</i> ₂ = 8.3 Hz) | 8.2 (dd, 2H, <i>J</i> ₁ = 5.5 Hz, <i>J</i> ₂ = 8.1Hz) | 8.2 (dd, 2H, <i>J</i> ₁ = 8.6 Hz <i>J</i> ₂ = 5.5 Hz) |
| CH ₃ | – | 2.65 (s, CH ₃ , 3H) | 2.59 (s, 6H) | – | 2.9 (s, CH ₃ , 3H) | 2.8 (s, 2 × CH ₃ , 6H), |
| H1'/H2' | 2.7 (m, 2H) | 2.7 (m, 2H) | 2.6 (m, 2H) | 3.2 (m, 2H) | 3.1 (m, 2H) | 3.1 (m, 2H) |
| H3'/H6' equatorial | 2.2 (d, 2H, <i>J</i> = 13.3 Hz) | 2.2 (d, 2H), | 2.22 (d, 2H, <i>J</i> = 13.3 Hz) | 2.39 (d, 2H, <i>J</i> = 10.8 Hz) | 2.38 (m, 2H; CH ₂) | 2.38 (d, 2H, <i>J</i> = 11 Hz) |
| H4'/H5' equatorial and H3'/H6' axial | 1.64 (d, 2H, <i>J</i> = 8.6 Hz) | 1.65 (d, 2H), 1.49 (d, 2H) | 1.66 (d, 2H, <i>J</i> = 8.6 Hz) | 1.69 (m, 4H) | 1.69 (m, 4H) | 1.68 (m, 4H) |
| H4'/H5' axial | 1.23 (m, 2H) | 1.24 (m, 2H). | 1.03 (m, 2H) | 1.30 (m, 2H) | 1.31 (m, 2H) | 1.30 (m, 2H) |
| ¹ H/ ¹⁹⁵ Pt | 8.88/−2821, 8.09/−2821 | 8.80/−2810, 7.95/−2810 | 8.87/−2822, 8.87/−2822 | 9.32/440; 8.27/440 | 9.10/422, 8.25/422 | 9.20/410, 8.23/410 |

[a] Experiments were performed in D₂O. Accordingly, ammine or hydroxido resonances are not observed due to proton exchange. [b] Symmetrical complexes, thus H2/9, H3/8, H4/7 and H5/6 are equivalent protons.

HPLC Chromatograms

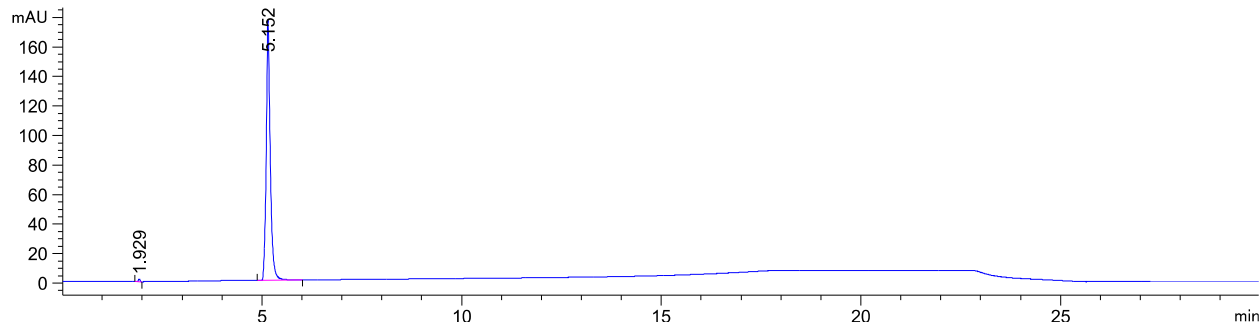


Figure H.S1. The HPLC chromatogram of [Pt^{II}PHENSS](NO₃)₂, at 254 nm and 298K was acquired by a Phenomenex OnyxTM Monolithic C18-reverse phase column (100 × 4.6 mm, 5 μm pore size). *t_R* at 5.152

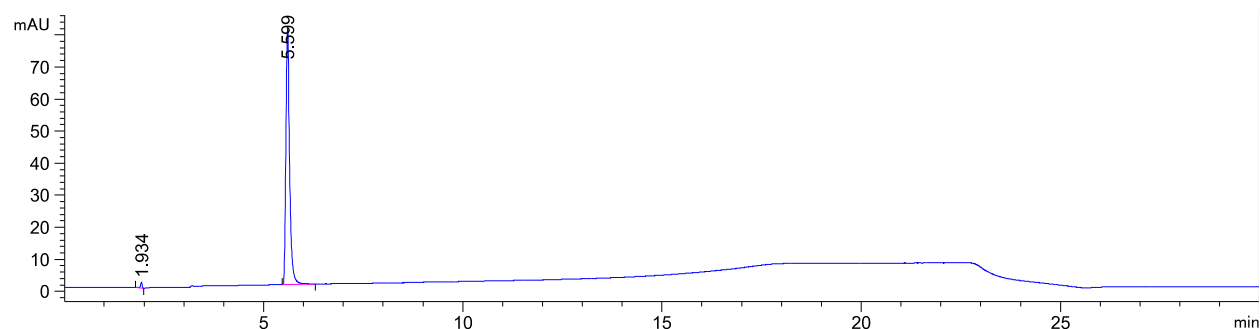


Figure H.S2. The HPLC chromatogram of [Pt^{II}5MESS](NO₃)₂, at 254 nm and 298K was acquired by a Phenomenex OnyxTM Monolithic C18-reverse phase column (100 × 4.6 mm, 5 μm pore size). *t_R* at 5.599 obtained.

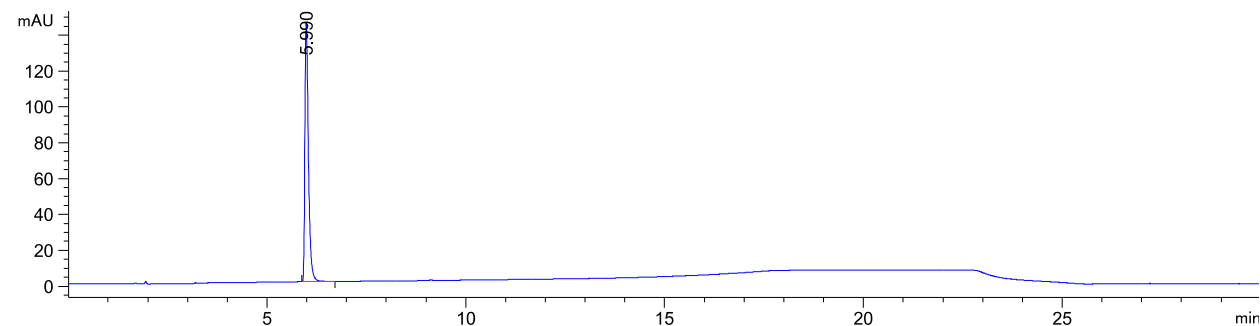


Figure H.S3. The HPLC chromatogram of [Pt^{II}56MESS](NO₃)₂, at 254 nm and 298K was acquired by a Phenomenex OnyxTM Monolithic C18-reverse phase column (100 × 4.6 mm, 5 μm pore size). *t_R* at 5.990.

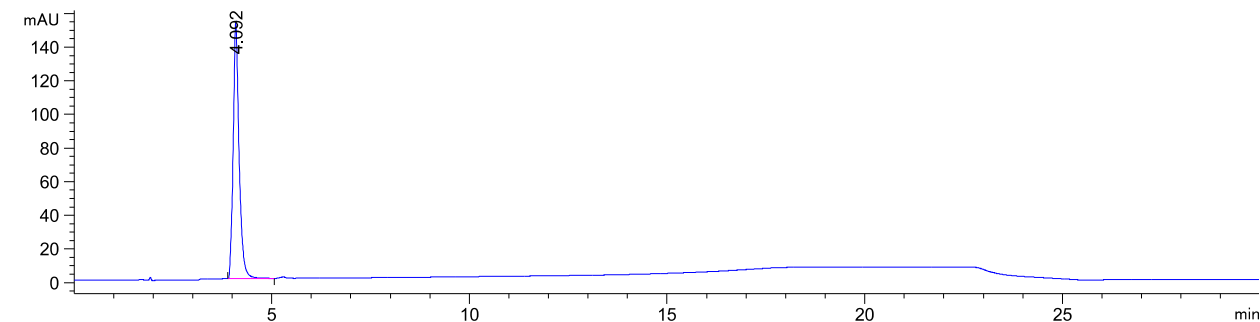


Figure H.S4. The HPLC chromatogram of [Pt^{IV}PHENSS(OH)₂](NO₃)₂, at 254 nm and 298K was acquired by a Phenomenex OnyxTM Monolithic C18-reverse phase column (100 × 4.6 mm, 5 μm pore size). *t_R* at 4.092.

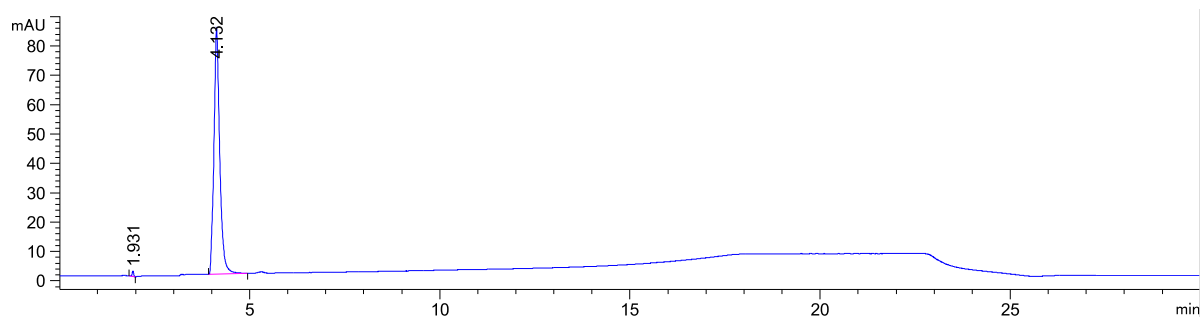


Figure H.S5. The HPLC chromatogram of $[\text{Pt}^{\text{IV}}5\text{MESS}(\text{OH})_2](\text{NO}_3)_2$, at 254 nm and 298K was acquired by a Phenomenex OnyxTM Monolithic C18-reverse phase column (100×4.6 mm, 5 μm pore size). t_{R} at 4.132.

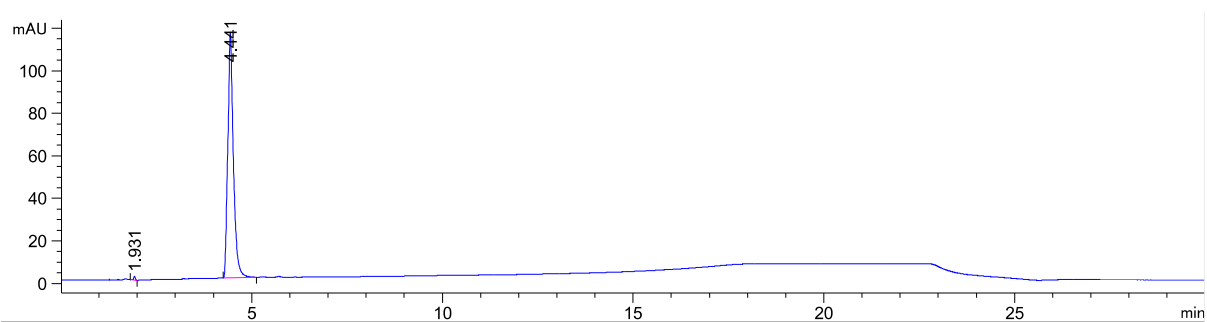


Figure H.S6. The HPLC chromatogram of $[\text{Pt}^{\text{IV}}56\text{MESS}(\text{OH})_2](\text{NO}_3)_2$, at 254 nm and 298K was acquired by a Phenomenex OnyxTM Monolithic C18-reverse phase column (100×4.6 mm, 5 μm pore size). t_{R} at 4.441.

^1H NMR and ^1H - ^{195}Pt HMQC Spectra

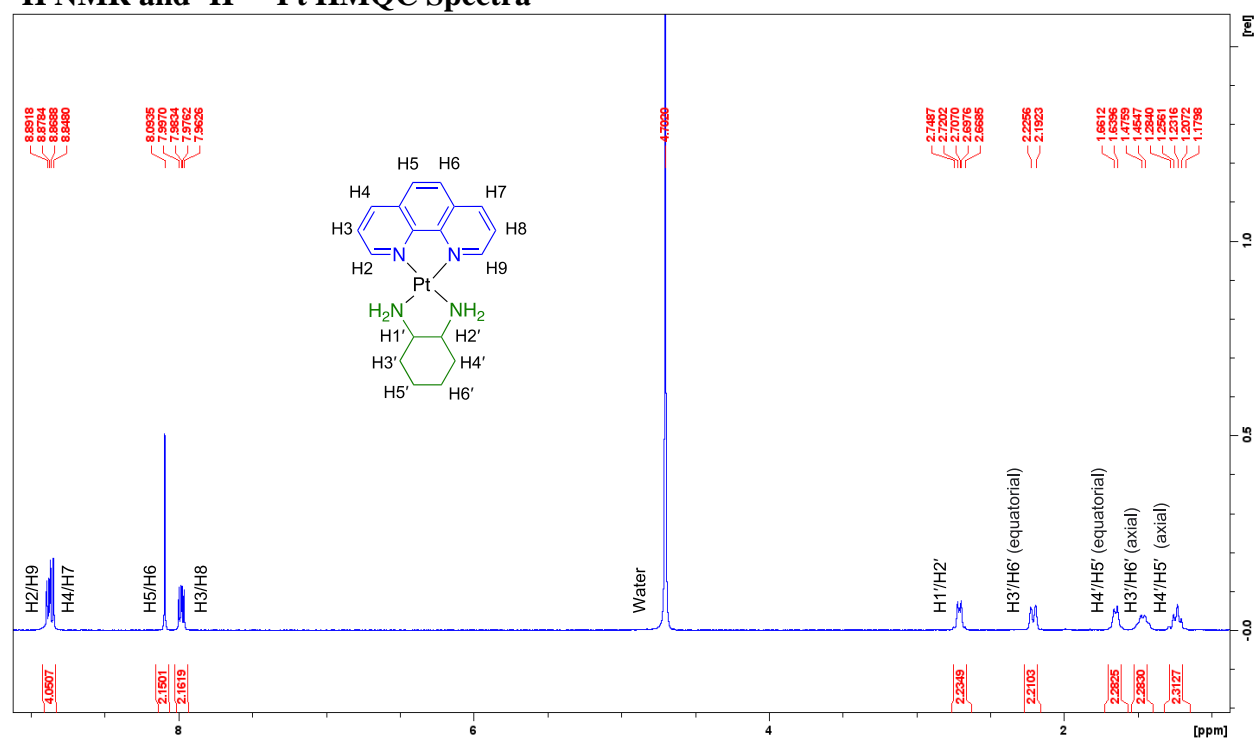


Figure N.S7. ^1H NMR spectra of Pt^{II} PHENSS in D_2O obtained at 298 K. Inset: structure of Pt^{II} PHENSS, with proton labelling system.

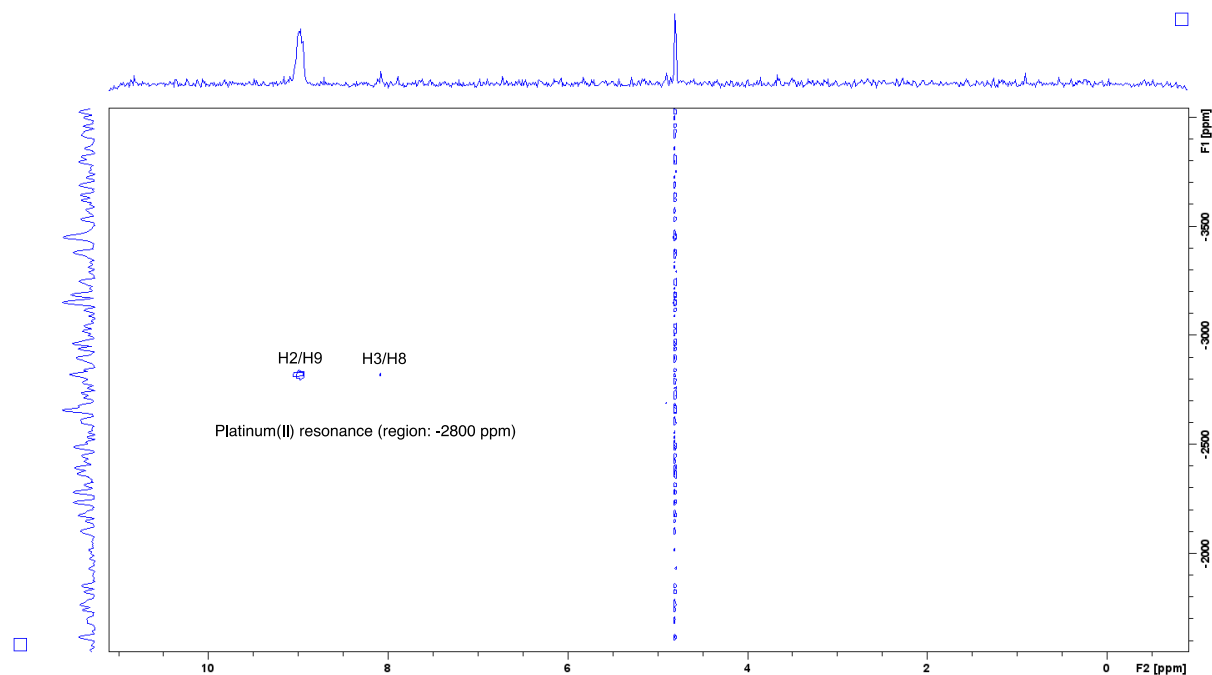


Figure N.S8. ^1H - ^{195}Pt HMQC spectra of Pt^{II} PHENSS, in D_2O obtained at 298 K.

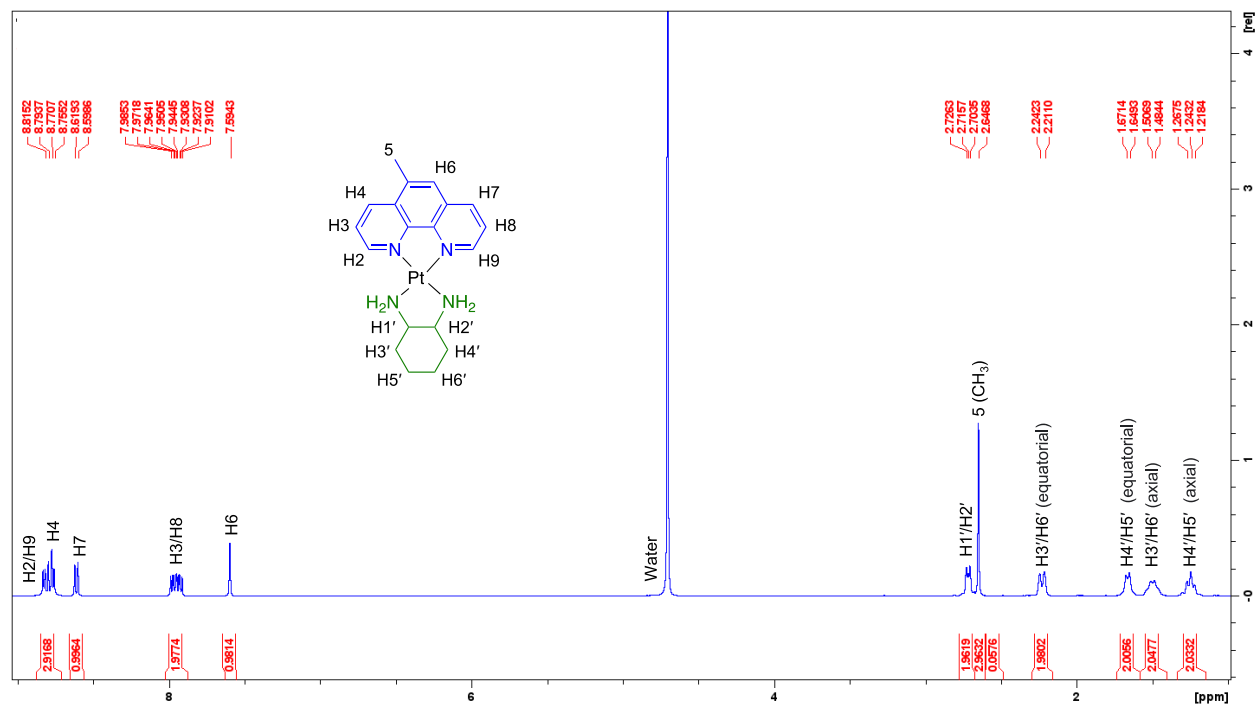


Figure N.S9. ^1H NMR spectra of $\text{Pt}^{\text{II}}5\text{MESS}$ in D_2O obtained at 298 K. Inset: structure of $\text{Pt}^{\text{II}}5\text{MESS}$, with proton labelling system.

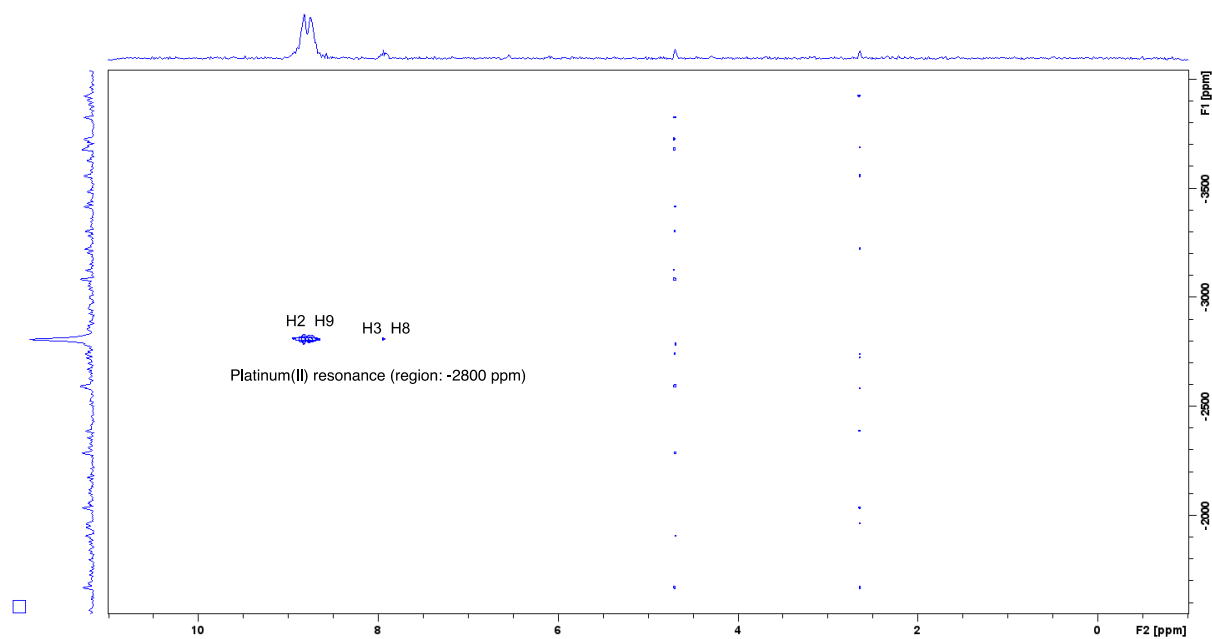


Figure N.S10. ^1H - ^{195}Pt HMQC spectra of $\text{Pt}^{\text{II}}5\text{MESS}$ in D_2O obtained at 298 K.

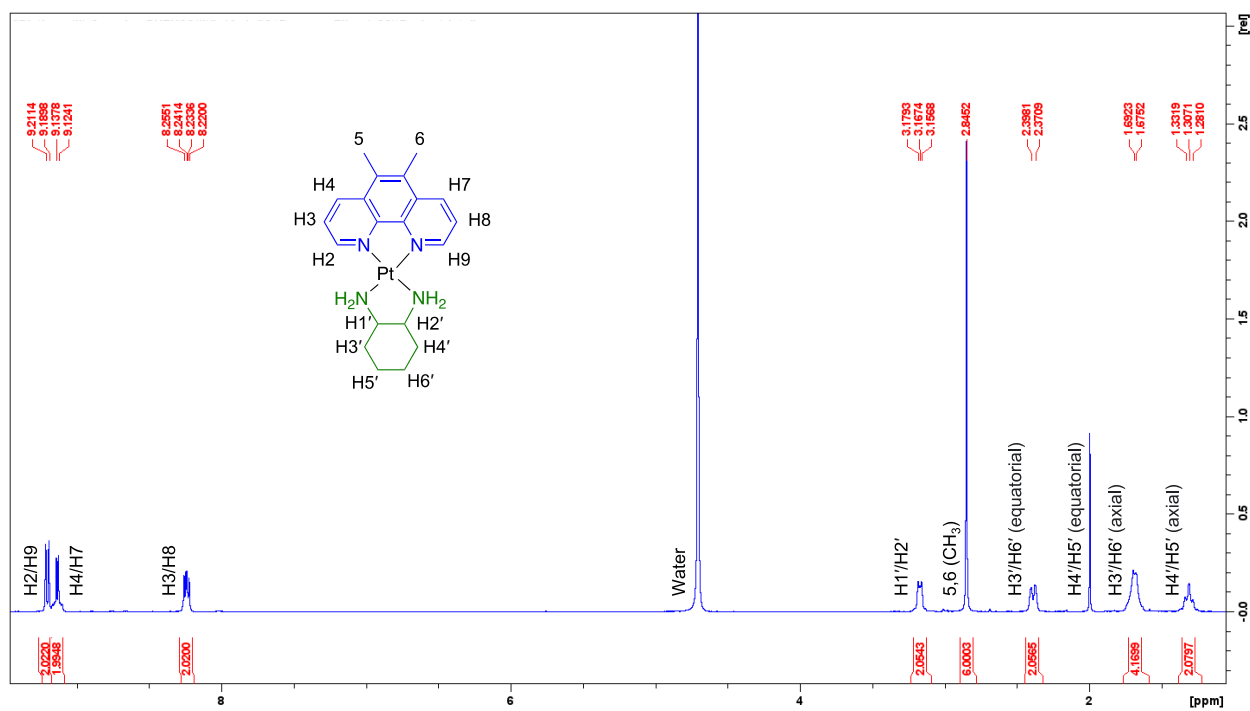


Figure N.S11. ^1H NMR spectra of **Pt^{II}56MESS** in D_2O obtained at 298 K. Inset: structure of **Pt^{II}56MESS**, with proton labelling system.

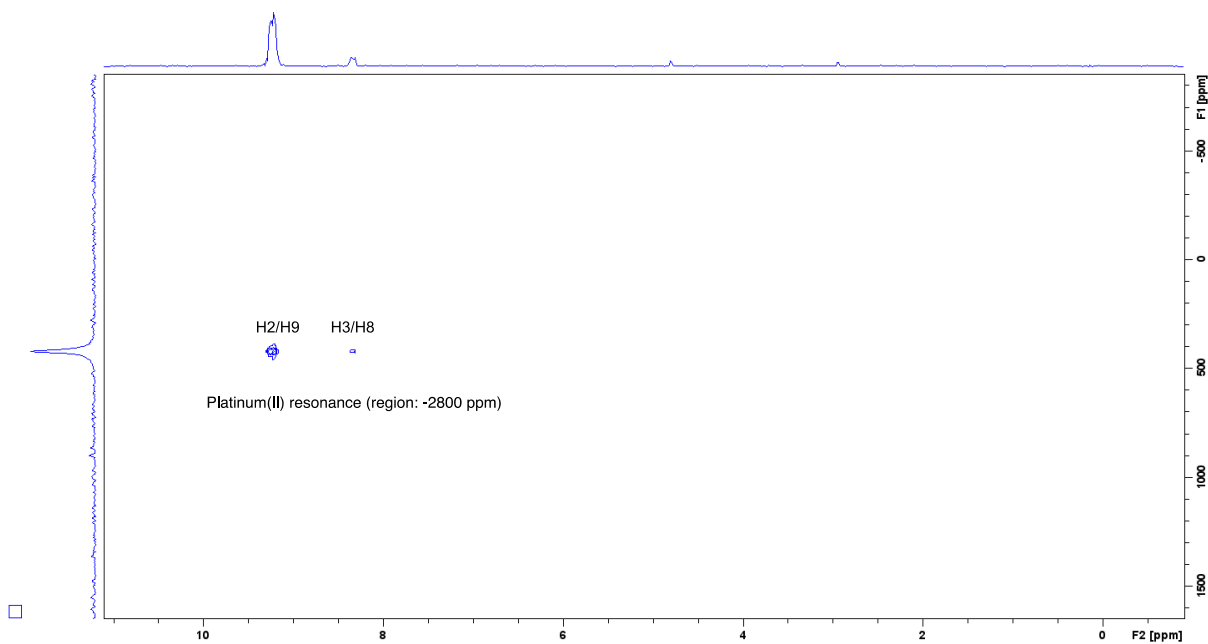


Figure N.S12. ^1H - ^{195}Pt HMQC spectra of **Pt^{II}56MESS** in D_2O obtained at 298 K.

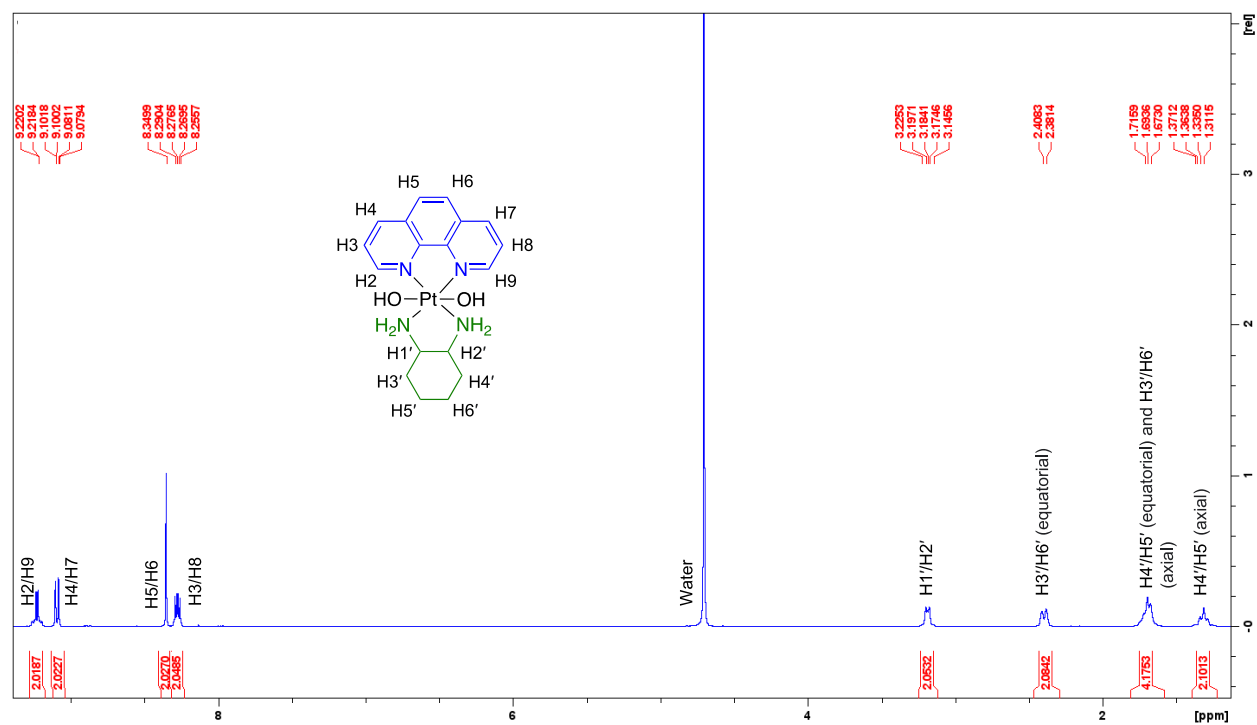


Figure N.S13. ^1H NMR spectra of $\text{Pt}^{\text{IV}}\text{PHENSS}(\text{OH})_2$ in D_2O obtained at 298 K. Inset: structure of $\text{Pt}^{\text{IV}}\text{PHENSS}(\text{OH})_2$, with proton labelling system.

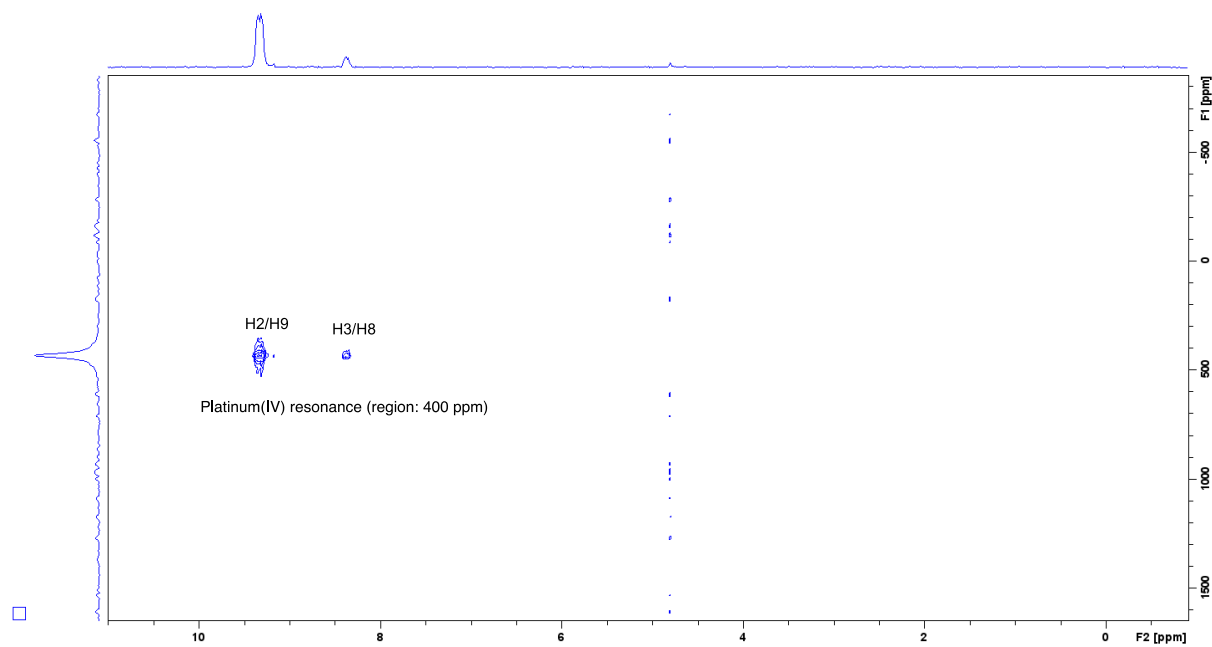


Figure N.S14. ^1H - ^{195}Pt HMQC spectra of $\text{Pt}^{\text{IV}}\text{PHENSS}(\text{OH})_2$ in D_2O obtained at 298 K.

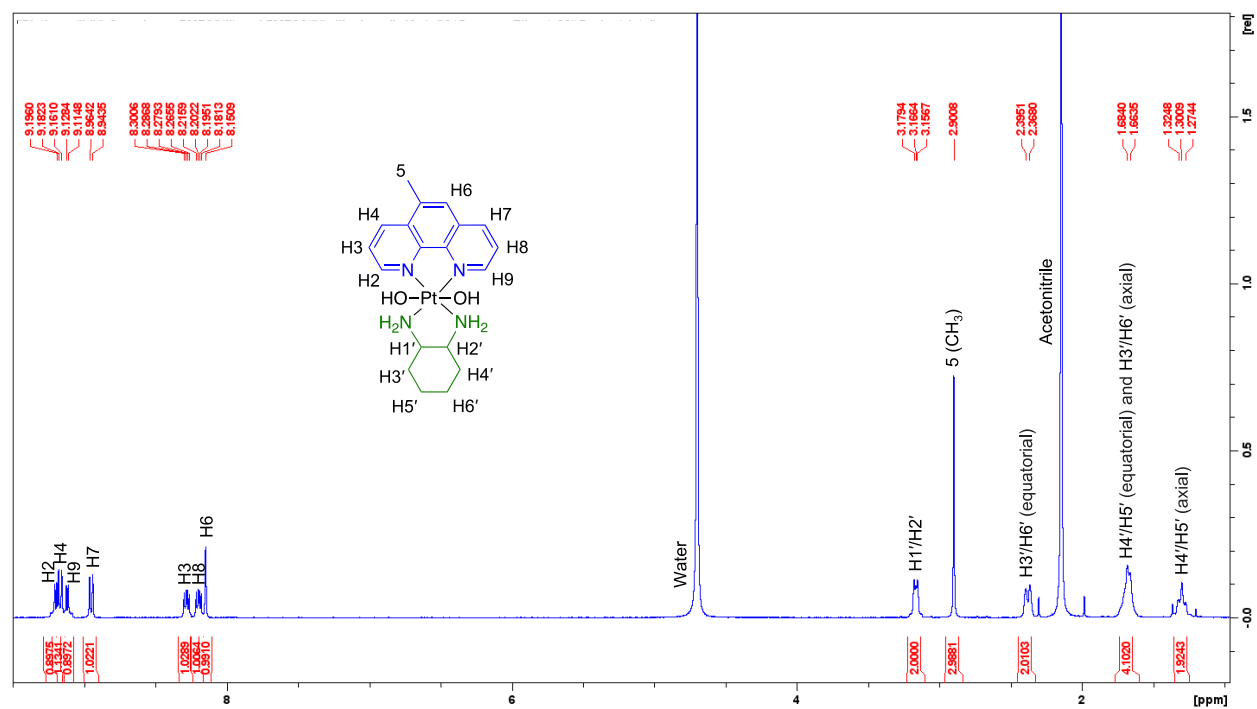


Figure N.S15. ^1H NMR spectra of $\text{Pt}^{\text{IV}}5\text{MESS}(\text{OH})_2$ in D_2O obtained at 298 K. Inset: structure of $\text{Pt}^{\text{IV}}5\text{MESS}(\text{OH})_2$, with proton labelling system.

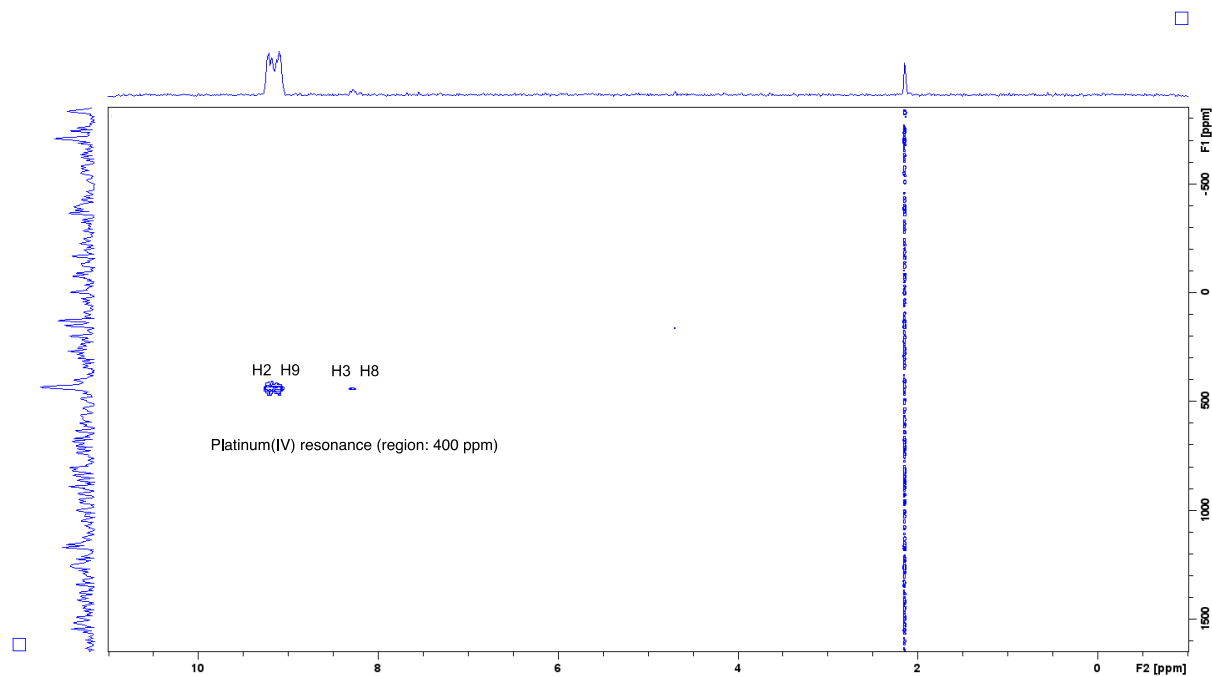
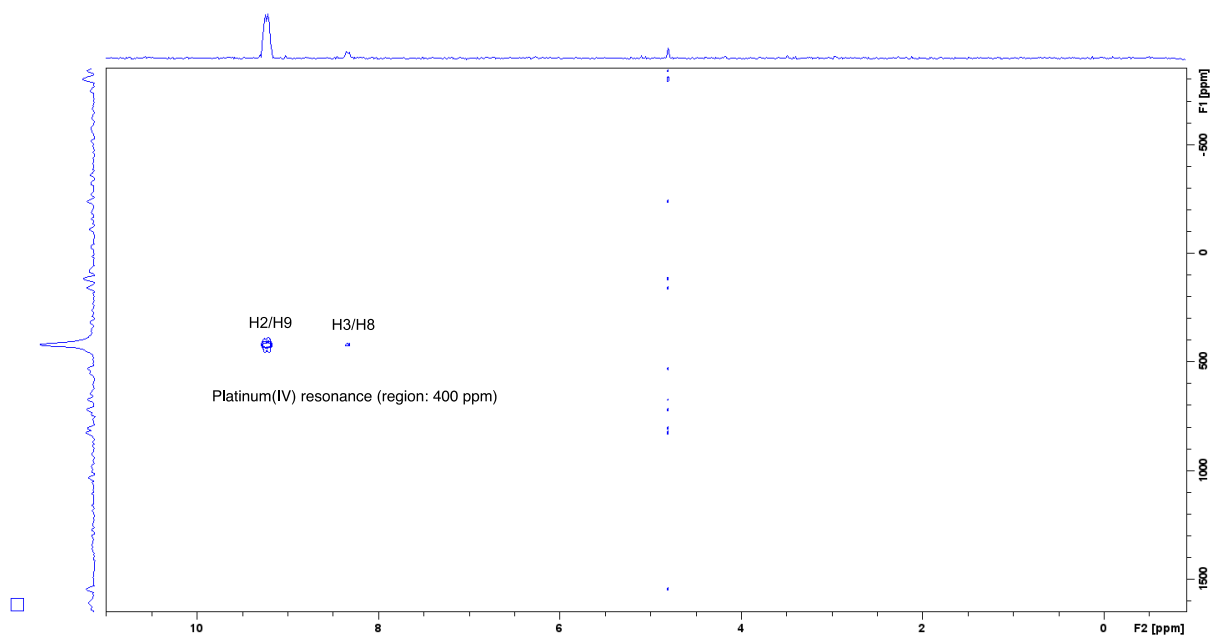
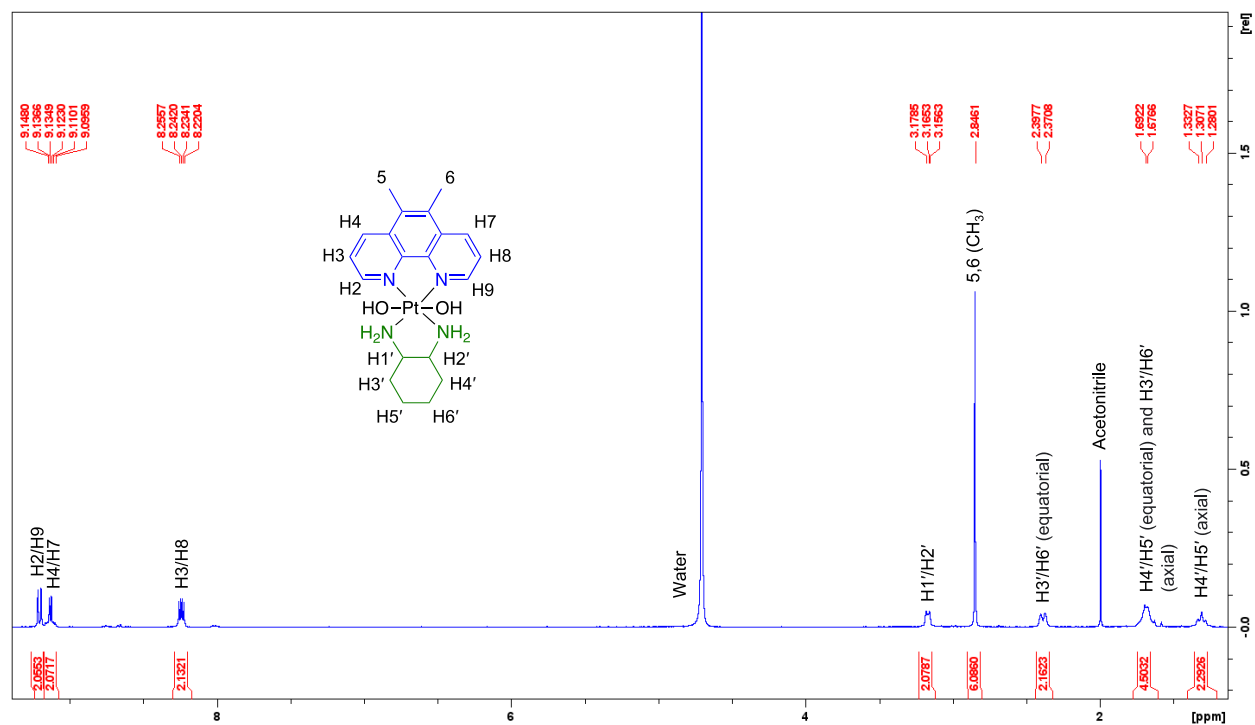


Figure N.S16. ^1H - ^{195}Pt HMQC spectra of $\text{Pt}^{\text{IV}}5\text{MESS}(\text{OH})_2$ in D_2O obtained at 298 K.



Method S3. Cellular Uptake of Platinum(II) and (IV) complexes

A final concentration of 10^6 cells/well of MDA-MB-231 and HT29 cells were seeded in 6-well plates and incubated overnight to adhere. The cells were then treated with a final concentration of $3\text{ }\mu\text{M}$ of either Pt^{II} or Pt^{IV} complexes. After 0, 0.5, 1, 3, 6, 12, 24 or 30 h, the medium was removed, and the cells were washed three times with cold PBS and allowed to dry. Then, $400\text{ }\mu\text{L}$ of 69% HNO_3 (Baseline grade nitric acid with Instrument Quality Grade Nitric acid with ppb level reported impurities, Seastar Chemicals, Sidney, Canada) was added to each well for 90 mins to ensure complete digestion. The digests were then moved to trace-metal free 15 mL centrifuge tubes (Labcon CA, USA), to which 7 mL milliQ water was then added which resulted in a final acid concentration of 3.5-4%. Iridium 193 (^{193}Ir), selected as internal standard, has similar first ionisation potential and mass to Platinum 195 (^{195}Pt). A concentration of 50 ppb ^{193}Ir in 2% HNO_3 solution was chosen for the Perkin Elmer NexION® Inductively Coupled Plasma Mass Spectrometer (ICP-MS) as it produced a steady state signal intensity of 600-800K counts per second. Mixing and injection of sample and internal standard solution was carried out using an ESI 7 port Valve system (Elemental Scientific, USA). A high-quality Solvent Flex 2 stop pump with 0.38 mm ID tubing was used in conjunction with the ICP-MS's Peristaltic pump set at 20 RPM (anti-clockwise direction) which resulted in the total Flow to Nebuliser (sample + internal standard both, at 0.25 mL/min) being 0.5 mL/min . The ICP-MS was tuned to high sensitivity settings to facilitate detection of low Pt concentrations in samples. This was done by adjusting the nebuliser gas flow between $0.92\text{--}0.94\text{ mL/min}$ to achieve maximum Indium 115 intensity whilst maintaining an acceptable average oxide and doubly charged ratio of 1.64-2.24% and 1.17-1.69% respectively. The analysis method was set to peak hopping mode with a dwell time of 50 milliseconds and 45 sweeps through the mass spectrum per sample read. This setting resulted in a peak integration time of 4500 milliseconds and enabled the collection of numerous data points for peak integration thereby increasing accuracy. The ICP-MS was tuned daily, and its performance optimised to a set of suppliers recommended settings. Analysis was only carried out when optimal oxide and doubly charged ratios with high ionic sensitivity was achieved. All ICP-MS parameters are described in Table S2. The results represent an average of three different experiments run in triplicates ($\pm\text{ SEM}$) and expressed as $\text{nmol}/10^6\text{ cells}$ or $\mu\text{M}/\text{cell}$. Quantification of the cellular uptake of Pt was based on external standards (Certified Standard from Sigma-Aldrich NSW, Australia) containing Internal standard Ir. The calibration curve is shown in Figure S1.

Table S1. Matrix Conditions selected for RASTRUM bioprinting.

| Matrix Code | Formulation | Stiffness (kPa, storage modulus) | F-code (Bioink) | F-code (Activator) |
|-------------|-------------|----------------------------------|-----------------|--------------------|
| Px02.09 | GFOGER, RGD | 1.1 | F236 | F177 |
| Px02.31 | RGD | 1.1 | F242 | F177 |
| Px02.00 | N/A | 1.1 | F119 | F177 |

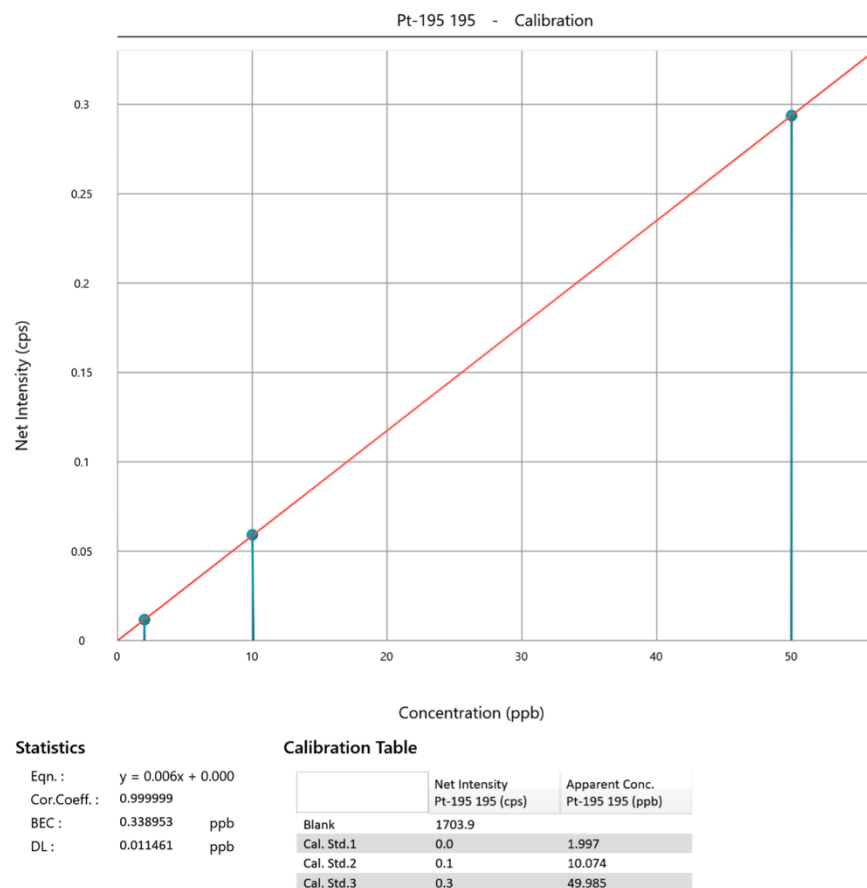


Figure S1. Calibration curve generated by plotting the peak areas measured by the ICP-MS against known concentrations ($^{195}\text{Pt}(\text{STD})$). This curve was used to quantify the cellular uptake of the Pt complexes; $y=0.006x + 0.000$ and $R^2= 0.999999$

Table S2. Conditions and parameters selected on the ICP-MS machine.

| Parameter | Value |
|--------------------------------|-------------------------------|
| Plasma RF power | 1500 W |
| Nebulizer gas flow rate | 0.92-0.94 L.min ⁻¹ |
| Auxiliary gas flow rate | 1.2 L.min ⁻¹ |
| Collision gas flow rate (He) | 4.5 L.min ⁻¹ |
| KED Cell Entrance/Exit voltage | -8/-25V |
| KED CRO/QRO Voltage | -15/-12V |
| Deflector Voltage | -9V |
| Isotope monitored | ^{195}Pt |
| Dwell times | 50ms |
| Integration Time | 4500ms |

RF, radio frequency; He, helium; KED, kinetic energy discrimination

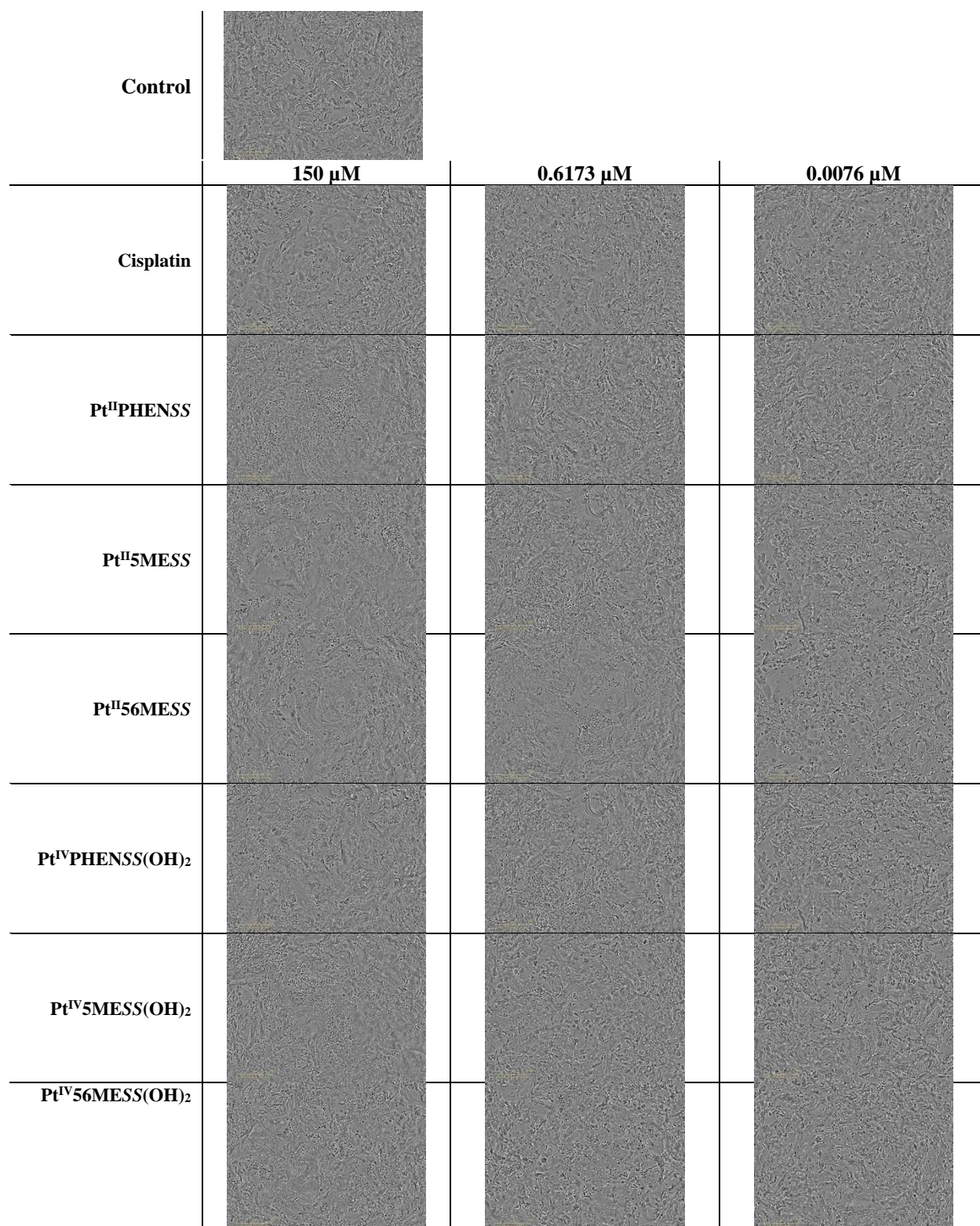


Figure S2. MDA–MB–231 networks. Cell viability upon treatment with platinum(II) (Pt^{II}PHENSS, Pt^{II}5MESS and Pt^{II}56MESS) and platinum(IV) (Pt^{IV}PHENSS(OH)₂, Pt^{IV}5MESS(OH)₂ and Pt^{IV}56MESS(OH)₂) complexes, as well as cisplatin in MDA–MB–231 networks at 72 h. Incucyte[®] phase contrast microscope used to collect bright-field live images using 10 \times objective.

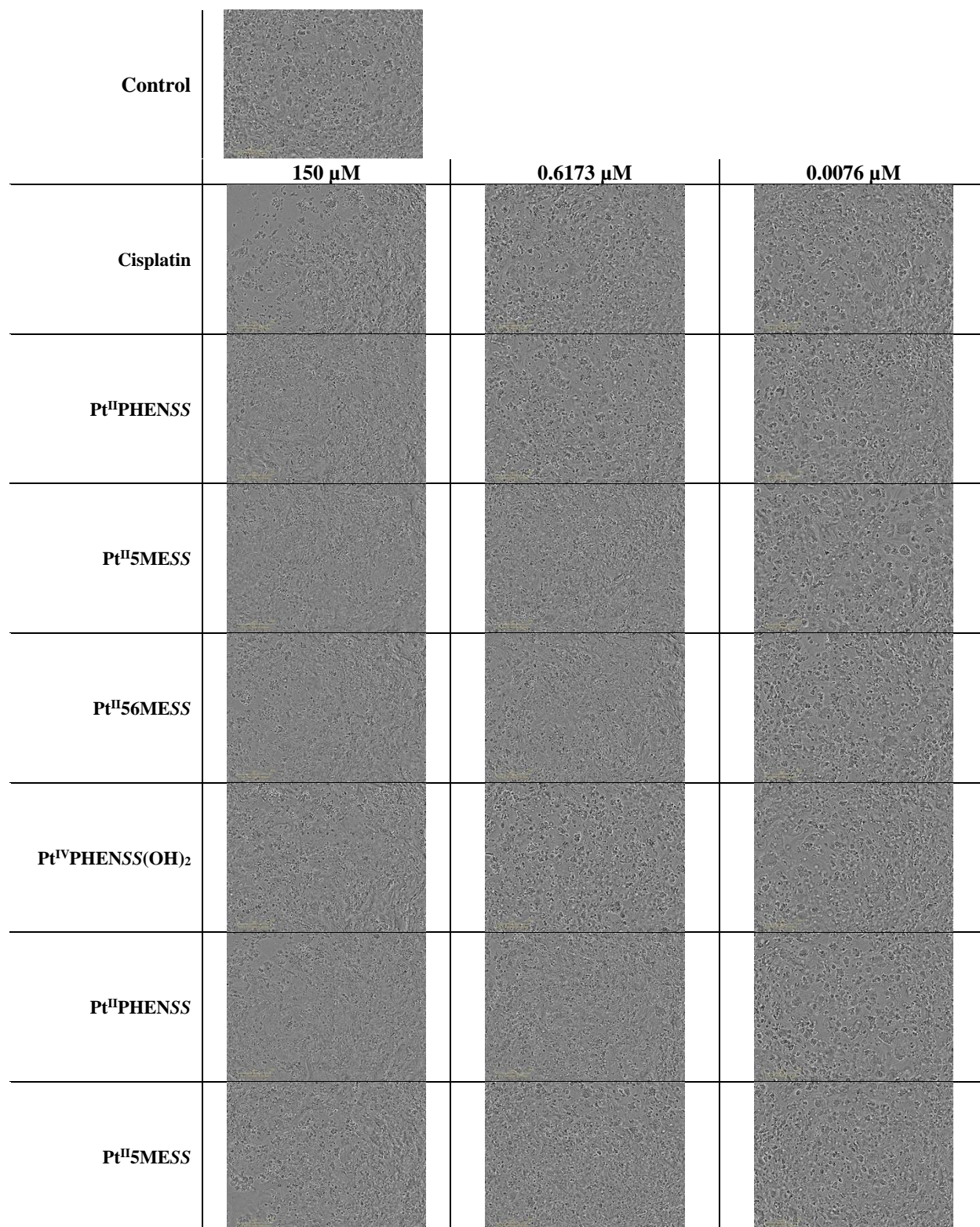


Figure S3. MDA-MB-231 spheroids. Cell viability upon treatment with platinum(II) (Pt^{II}PHENSS, Pt^{II}5MESS and Pt^{II}56MESS) and platinum(IV) (Pt^{IV}PHENSS(OH)₂, Pt^{IV}5MESS(OH)₂ and Pt^{IV}56MESS(OH)₂) complexes, as well as cisplatin in MDA-MB-231 spheroids at 72 h. Incucyte[®] phase contrast microscope used to collect bright-field live images using 10 \times objective.

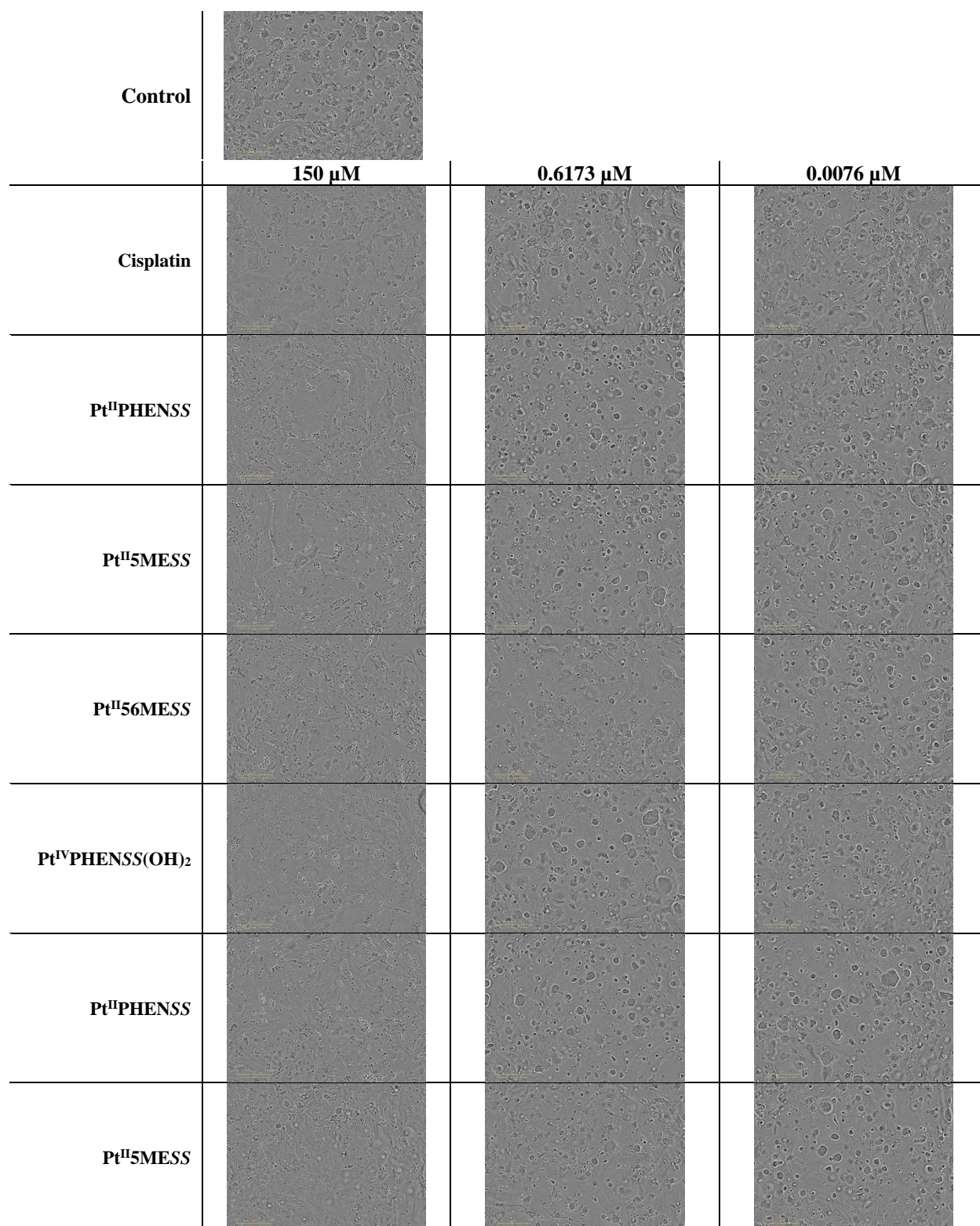
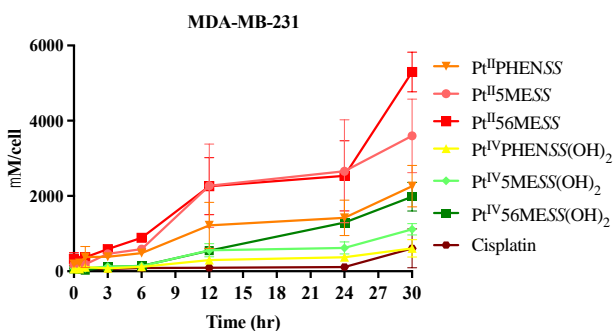


Figure S4. HT29 spheroids. Cell viability upon treatment with platinum(II) (Pt^{II}PHENSS, Pt^{II}5MESS and Pt^{II}56MESS) and platinum(IV) (Pt^{IV}PHENSS(OH)₂, Pt^{IV}5MESS(OH)₂ and Pt^{IV}56MESS(OH)₂) complexes, as well as cisplatin in HT29 spheroids at 72 h. Incucyte[®] phase contrast microscope used to collect bright-field live images using 10 \times objective.

A



B

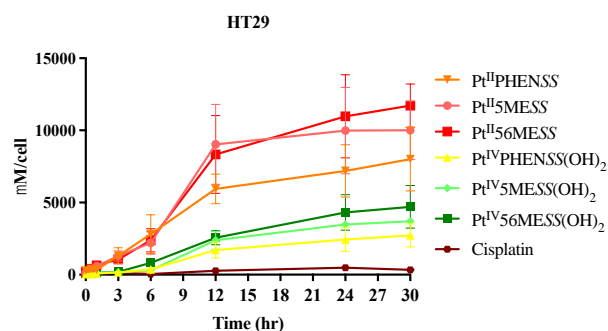


Figure S5. ICP-MS analysis for the uptake of Pt in MDA-MB-231 and HT29 at 0, 0.5, 1, 3, 6, 12, 24 and 30 h. The $\text{Pt}^{\text{II}}56\text{MESS}$ precursor has the greatest cellular uptake in both A. MDA-MB-231 and B. HT29. $n = 3$ from three independent experiments where samples were run in triplicates. Data points denote mean \pm SEM and expressed in $\mu\text{M}/\text{cell}$.

Table S3. Kinetic Cellular uptake of Pt complexes by MDA-MB-231. Table shows the average value of cellular concentration in $\text{nmol}/10^6$ cells (top) and the ratio of intracellular concentration to extracellular concentrations (bottom) for each complex. Data points denote mean \pm SEM. $n = 3$ from three independent experiments where samples were run in triplicates.

| | | Time (h) | | | | | | | | |
|---|---|------------------------|-----------------------|------------------------|-----------------------|-----------------------|-------------------------|-------------------------|-------------------------|--|
| | | 0 | 0.5 | 1 | 3 | 6 | 12 | 24 | 30 | |
| Intracellular Concentration ($\text{nmol}/10^6$ cells) | $\text{Pt}^{\text{II}}\text{PHENSS}$ | 0.40 ± 0.22 | 0.46 ± 0.23 | 0.80 ± 0.59 | 0.81 ± 0.14 | 1.03 ± 0.14 | 2.59 ± 1.29 | 3.01 ± 0.99 | 4.79 ± 1.18 | |
| | $\text{Pt}^{\text{II}}5\text{MESS}$ | 0.37 ± 0.17 | 0.44 ± 0.18 | 0.39 ± 0.05 | 0.98 ± 0.19 | 1.25 ± 0.20 | 4.82 ± 2.35 | 5.64 ± 2.90 | 7.63 ± 2.07 | |
| | $\text{Pt}^{\text{II}}56\text{MESS}$ | 0.68 ± 0.36 | 0.58 ± 0.18 | 0.78 ± 0.04 | 1.24 ± 0.13 | 1.88 ± 0.10 | 4.80 ± 1.61 | 5.38 ± 1.97 | 11.24 ± 1.12 | |
| | $\text{Pt}^{\text{IV}}\text{PHENSS}(\text{OH})_2$ | 0.13 ± 0.01 | 0.13 ± 0.03 | 0.223 ± 0.04 | 0.16 ± 0.01 | 0.25 ± 0.06 | 0.63 ± 0.25 | 0.78 ± 0.16 | 1.29 ± 0.49 | |
| | $\text{Pt}^{\text{IV}}5\text{MESS}(\text{OH})_2$ | 0.16 ± 0.03 | 0.22 ± 0.04 | 0.17 ± 0.07 | 0.25 ± 0.03 | 0.29 ± 0.09 | 1.19 ± 0.37 | 1.31 ± 0.34 | 2.37 ± 0.32 | |
| | $\text{Pt}^{\text{IV}}56\text{MESS}(\text{OH})_2$ | 0.14 ± 0.04 | 0.11 ± 0.02 | 0.05 ± 0.02 | 0.25 ± 0.04 | 0.30 ± 0.01 | 1.16 ± 0.11 | 2.74 ± 0.07 | 4.19 ± 0.81 | |
| | Cisplatin | 0.56 ± 0.40 | 0.15 ± 0.06 | 0.14 ± 0.05 | 0.12 ± 0.02 | 0.18 ± 0.07 | 0.19 ± 0.01 | 0.24 ± 0.04 | 1.29 ± 1.09 | |
| Intracellular/extracellular ratio | $\text{Pt}^{\text{II}}\text{PHENSS}$ | 63.45 ± 101.96 | 72.01 ± 107.27 | 126.21 ± 279.24 | 127.55 ± 65.28 | 161.09 ± 64.41 | 407.32 ± 612.67 | 473.33 ± 468.94 | 752.93 ± 554.54 | |
| | $\text{Pt}^{\text{II}}5\text{MESS}$ | 57.65 ± 80.43 | 69.63 ± 88.74 | 61.30 ± 24.80 | 154.59 ± 91.37 | 196.09 ± 95.27 | 757.65 ± 1106.77 | 885.63 ± 1367.55 | 1199.77 ± 977.40 | |
| | $\text{Pt}^{\text{II}}56\text{MESS}$ | 107.16 ± 170.69 | 92.36 ± 84.69 | 122.42 ± 23.52 | 195.59 ± 63.55 | 296.93 ± 48.82 | 754.52 ± 758.66 | 845.27 ± 931.54 | 1765.95 ± 526.75 | |
| | $\text{Pt}^{\text{IV}}\text{PHENSS}(\text{OH})_2$ | 21.21 ± 4.95 | 20.07 ± 12.01 | 35.47 ± 19.97 | 26.14 ± 2.33 | 38.82 ± 29.09 | 98.56 ± 116.92 | 123.87 ± 76.45 | 203.38 ± 233.85 | |
| | $\text{Pt}^{\text{IV}}5\text{MESS}(\text{OH})_2$ | 26.14 ± 14.63 | 35.23 ± 18.90 | 27.69 ± 34.32 | 40.45 ± 15.98 | 46.47 ± 42.96 | 186.97 ± 177.03 | 205.97 ± 161.43 | 371.97 ± 153.15 | |
| | $\text{Pt}^{\text{IV}}56\text{MESS}(\text{OH})_2$ | 64.92 ± 18.45 | 50.71 ± 7.93 | 26.14 ± 8.97 | 117.57 ± 16.99 | 142.93 ± 3.92 | 548.82 ± 52.42 | 1293.81 ± 33.95 | 1978.51 ± 381.47 | |
| | Cisplatin | 88.32 ± 189.97 | 23.57 ± 29.64 | 22.36 ± 25.77 | 19.47 ± 11.33 | 28.65 ± 30.59 | 30.78 ± 7.28 | 38.28 ± 22.01 | 202.79 ± 518.14 | |

Table S4. Kinetic Cellular uptake of Pt complexes by HT29. Table shows the average value of cellular concentration in nmol/10⁶ cells (top) and the ratio of intracellular concentration to extracellular concentrations (bottom) for each complex. Data points denote mean \pm SEM. *n* = 3 from three independent experiments where samples were run in triplicates.

| | | Time (h) | | | | | | | |
|--|--|------------------|-------------------|--------------------|--------------------|--------------------|---------------------|---------------------|----------------------|
| | Complex | 0 | 0.5 | 1 | 3 | 6 | 12 | 24 | 30 |
| Intracellular Concentration (nmol/10 ⁶ cells) | Pt ^{II} PHENSS | 0.61 ± 0.33 | 0.85 ± 0.25 | 0.72 ± 0.23 | 2.77 ± 1.16 | 5.95 ± 2.84 | 12.59 ± 2.18 | 15.26 ± 3.85 | 16.99 ± 4.69 |
| | Pt ^{II} 5MESS | 0.28 ± 0.06 | 0.79 ± 0.50 | 1.26 ± 0.22 | 2.75 ± 0.74 | 4.65 ± 1.64 | 19.18 ± 5.88 | 21.18 ± 6.35 | 21.24 ± 4.31 |
| | Pt ^{II} 56MESS | 0.45 ± 0.02 | 0.87 ± 0.27 | 1.38 ± 0.19 | 2.23 ± 0.45 | 5.05 ± 1.70 | 17.67 ± 5.72 | 23.28 ± 6.12 | 24.85 ± 3.20 |
| | Pt ^{IV} PHENSS(OH) ₂ | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.18 ± 0.02 | 0.33 ± 0.14 | 0.77 ± 0.53 | 3.64 ± 1.18 | 5.18 ± 1.73 | 5.79 ± 1.72 |
| | Pt ^{IV} 5MESS(OH) ₂ | 0.15 ± 0.02 | 0.17 ± 0.02 | 0.28 ± 0.05 | 0.33 ± 0.12 | 0.66 ± 0.32 | 5.05 ± 1.32 | 7.37 ± 0.10 | 7.85 ± 0.55 |
| | Pt ^{IV} 56MESS(OH) ₂ | 0.14 ± 0.01 | 0.18 ± 0.01 | 0.28 ± 0.01 | 0.42 ± 0.03 | 1.74 ± 0.17 | 5.43 ± 1.04 | 9.14 ± 2.61 | 9.98 ± 3.14 |
| | Cisplatin | 0.36 ± 0.04 | 0.94 ± 0.55 | 0.14 ± 0.02 | 0.28 ± 0.09 | 0.10 ± 0.01 | 0.57 ± 0.23 | 1.02 ± 0.57 | 0.71 ± 0.18 |
| Intracellular/ extracellular ratio | Pt ^{II} PHENSS | 95.39 ±157.34 | 133.55 ±119.40 | 112.44 ± 110.62 | 435.59 ± 544.47 | 935.29 ±1340.57 | 1979.17 ±1025.61 | 2397.59 ± 1814.7 | 26698.41 ±2208.68 |
| | Pt ^{II} 5MESS | 44.37 ± 26.94 | 125.00 ±236.39 | 197.63± 102.35 | 431.44 ± 348.12 | 730.89 ± 774.95 | 3013.21 ±2773.67 | 3327.94 ±2994.12 | 3337.20 ±2030.29 |
| | Pt ^{II} 56MESS | 70.93 ± 9.69 | 136.74 ±131.83 | 216.82 ± 89.98 | 350.73 ± 213.21 | 793.80 ± 803.03 | 2776.28 ±2694.76 | 3657.59 ±2886.20 | 3903.51 ±1509.01 |
| | Pt ^{IV} PHENSS(OH) ₂ | 16.45 ± 8.04 | 15.93 ± 3.81 | 28.08 ± 8.54 | 51.34 ± 67.56 | 121.19 ± 249.85 | 571.26 ± 554.55 | 813.09 ± 817.45 | 909.81 ± 808.35 |
| | Pt ^{IV} 5MESS(OH) ₂ | 25.03 ± 9.01 | 26.29 ± 8.01 | 44.59 ± 25.24 | 52.25 ± 56.49 | 104.08 ± 151.75 | 793.61 ± 622.21 | 1158.37 ± 47.58 | 1234.08 ± 258.11 |
| | Pt ^{IV} 56MESS(OH) ₂ | 21.81 ± 4.03 | 28.52 ± 6.13 | 43.51 ± 3.21 | 66.77 ± 12.84 | 273.54 ± 78.68 | 854.01 ± 488.39 | 1435.74 ±1229.28 | 1568.28 ±1478.83 |
| | Cisplatin | 57.31 ± 20.91 | 147.74 ±258.72 | 22.65 ± 9.82 | 43.31 ± 46.90 | 16.07 ± 6.78 | 89.42 ± 106.10 | 160.54 ± 271.72 | 112.14 ± 83.99 |

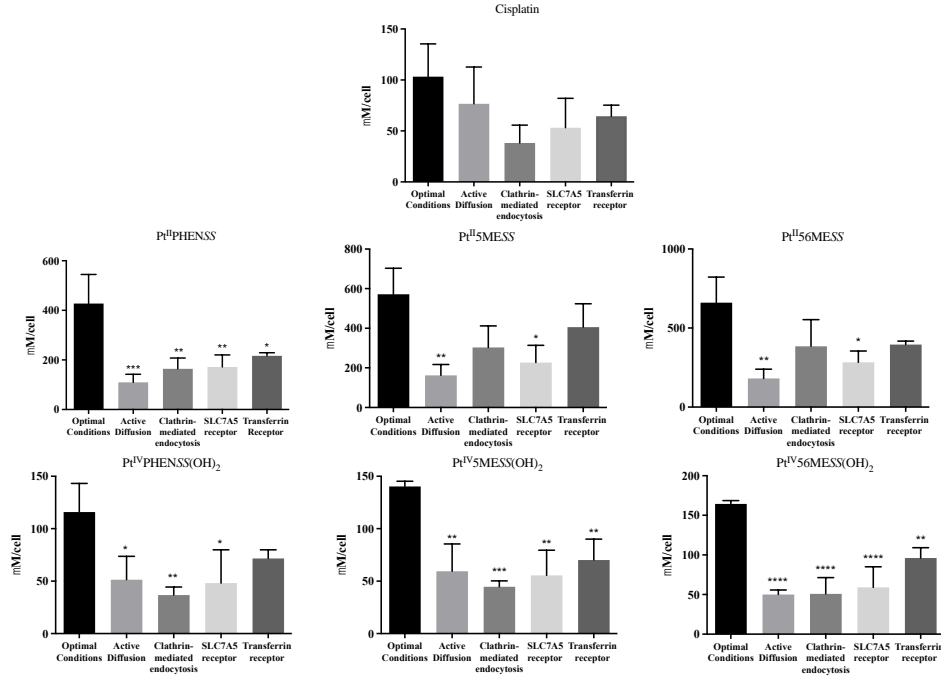


Figure S6. Mode of uptake of Platinum in MDA-MB-231. The intracellular amount of Pt was measured by ICP-MS after incubation at 37 °C or 4 °C, as well as following inhibition of the SLC7A5, transferrin receptor or clathrin-mediated endocytosis. Data denote mean \pm SEM of three independent experiments where samples were run in triplicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ in comparison to the optimal conditions.

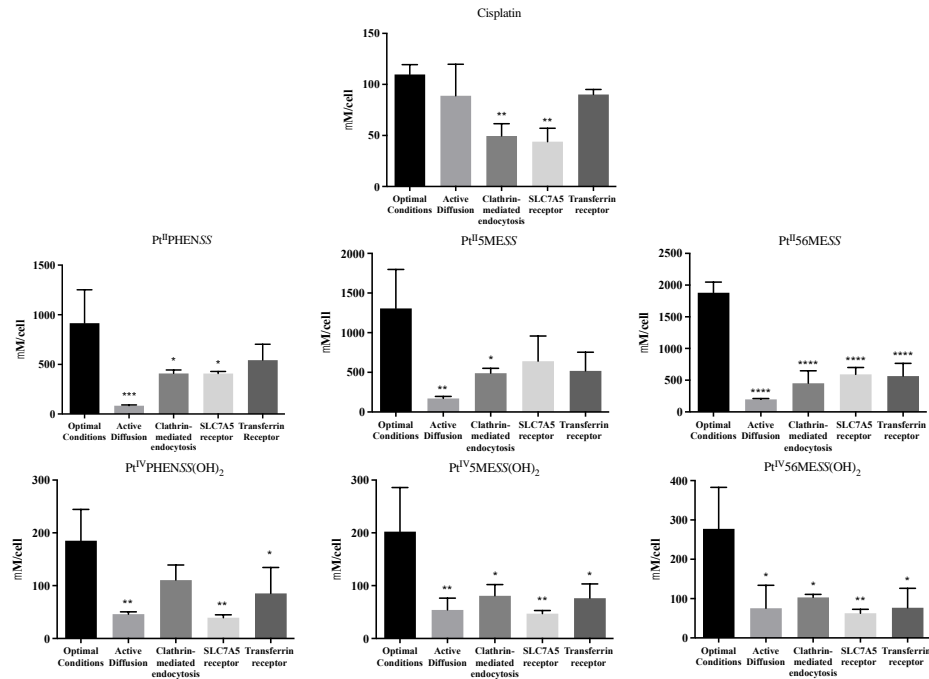


Figure S7. Mode of uptake of Platinum in HT29. The intracellular amount of Pt was measured by ICP-MS after incubation at 37 °C or 4 °C, as well as following inhibition of the SLC7A5, transferrin receptor or clathrin-mediated endocytosis. Data denote mean \pm SEM of three independent experiments where samples were run in triplicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ in comparison to the optimal conditions.

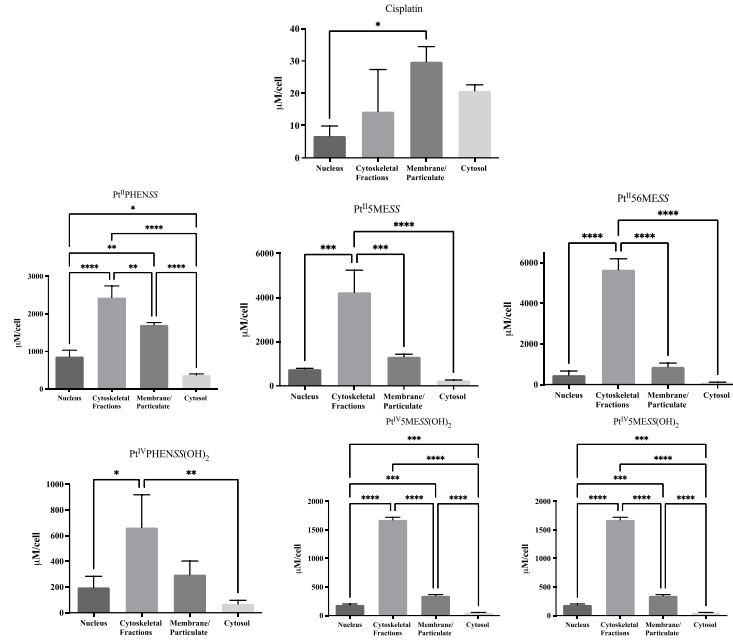


Figure S8. Cellular Localisation of Platinum in MDA-MB-231. The intracellular amount of Pt (µM/cell) was measured by ICP-MS after cellular fractionation. Data denote mean ± SEM of three independent experiments where samples were run in triplicates. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 and *****p* < 0.0001 in comparison to the fractions.

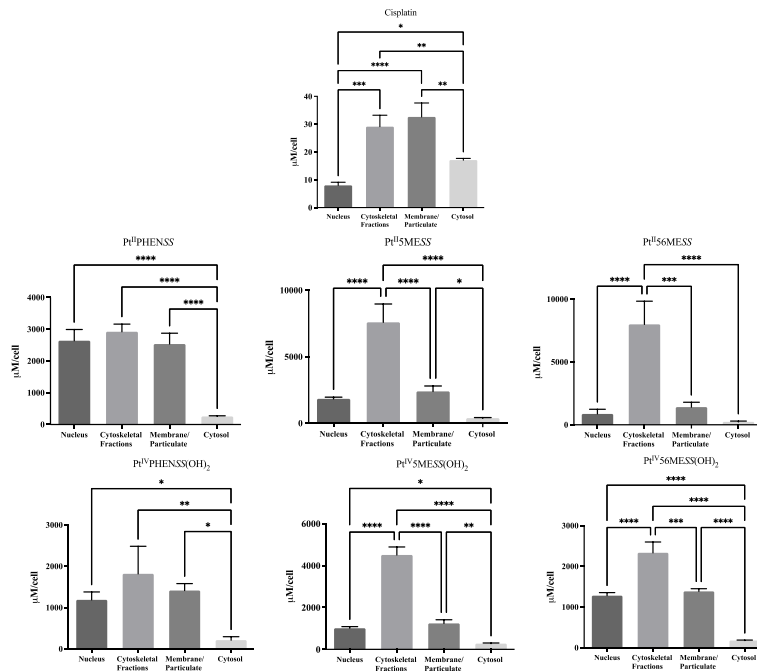


Figure S9. Cellular Localisation of Platinum in HT29. The intracellular amount of Pt (µM/cell) was measured by ICP-MS after cellular fractionation. Data denote mean ± SEM of three independent experiments where samples were run in triplicates. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 and *****p* < 0.0001 in comparison to the fractions.

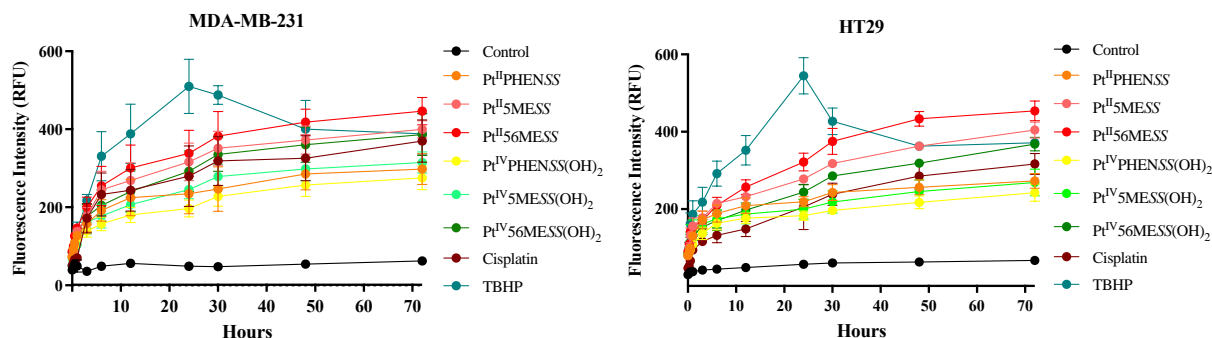


Figure. S10. ROS production upon treatment with platinum(II) and (IV) complexes in MDA–MB–231 and HT29 at 0, 0.25, 0.5, 1, 3, 6, 12, 24, 48 and 72 h. Pt^{II}PHENSS, Pt^{II}5MESS, Pt^{II}56MESS, Pt^{IV}PHENSS(OH)₂, Pt^{IV}5MESS(OH)₂, Pt^{IV}56MESS(OH)₂, cisplatin and TBHP: t-butyl hydroperoxide. Data points denote mean ± SEM. *n* = 3 from three independent experiments where samples were run in triplicate.

Table S5. ROS production upon treatment with complexes Pt^{II}PHENSS, Pt^{II}5MESS, Pt^{II}56MESS, Pt^{IV}PHENSS(OH)₂, Pt^{IV}5MESS(OH)₂, Pt^{IV}56MESS(OH)₂, Cisplatin and TBHP: t-butyl hydroperoxide, in MDA–MB–231 and HT29 cells at 24, 48 and 72 h.

| Complex | ROS production in different time intervals (RFU) | | | | | |
|--|--|----------------|----------------|----------------|---------------|---------------|
| | MDA–MB–231 | | | HT29 | | |
| | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| Control | 48.77 ± 1.16 | 54.55 ± 1.14 | 62.33 ± 0.69 | 57.22 ± 0.34 | 63.22 ± 0.30 | 67.33 ± 0.21 |
| Cisplatin | 279.82 ± 14.88 | 325.96 ± 11.09 | 369.97 ± 10.43 | 205.39 ± 11.26 | 285.11 ± 6.49 | 316.55 ± 5.21 |
| TBHP | 509.88 ± 13.38 | 400.11 ± 14.21 | 388.41 ± 10.04 | 544.67 ± 9.04 | 362.55 ± 1.84 | 371.44 ± 1.74 |
| Pt ^{II} PHENSS | 234.82 ± 9.88 | 285.31 ± 7.84 | 297.81 ± 7.62 | 219.00 ± 2.52 | 256.22 ± 3.67 | 273.00 ± 3.71 |
| Pt ^{II} 5MESS | 316.23 ± 9.22 | 372.49 ± 6.94 | 399.97 ± 4.28 | 278.00 ± 2.18 | 362.89 ± 0.88 | 404.44 ± 3.93 |
| Pt ^{II} 56MESS | 338.49 ± 11.45 | 418.08 ± 6.35 | 446.09 ± 6.81 | 321.56 ± 4.44 | 433.67 ± 3.65 | 453.89 ± 4.91 |
| Pt ^{IV} PHENSS(OH) ₂ | 196.62 ± 4.17 | 256.83 ± 5.57 | 275.09 ± 5.81 | 182.77 ± 2.29 | 217.33 ± 3.18 | 242.44 ± 4.28 |
| Pt ^{IV} 5MESS(OH) ₂ | 245.26 ± 4.55 | 264.00 ± 5.91 | 314.78 ± 5.21 | 234.55 ± 3.09 | 278.33 ± 4.39 | 296.67 ± 4.85 |
| Pt ^{IV} 56MESS(OH) ₂ | 292.21 ± 10.00 | 360.12 ± 8.69 | 386.28 ± 10.27 | 244 ± 3.67 | 318.56 ± 1.15 | 368.11 ± 3.38 |

Table S6. Mitochondrial membrane potential upon treatment with platinum(II) (Pt^{II}PHENSS, Pt^{II}5MESS, Pt^{II}56MESS) and platinum (IV) (Pt^{IV}PHENSS(OH)₂, Pt^{IV}5MESS(OH)₂, Pt^{IV}56MESS(OH)₂,) complexes, and cisplatin in MDA–MB–231 and HT29 cells at 24, 48 and 72 h.

| Complex | MtMp in different time intervals (RFU) | | | | | |
|--|--|----------------|----------------|----------------|----------------|----------------|
| | MDA–MB–231 | | | HT29 | | |
| | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| Control | 411.10 ± 13.62 | 368.86 ± 19.21 | 367.15 ± 12.30 | 471.52 ± 19.62 | 398.14 ± 15.77 | 409.06 ± 4.52 |
| Cisplatin | 208.24 ± 11.41 | 177.91 ± 11.37 | 154.83 ± 15.32 | 270.02 ± 10.56 | 237.68 ± 4.61 | 188.59 ± 10.43 |
| FCCP | 215.89 ± 6.12 | 123.38 ± 13.15 | 99.10 ± 8.58 | 184.32 ± 9.51 | 143.31 ± 4.61 | 83.58 ± 3.54 |
| Pt^{II}PHENSS | 258.17 ± 8.25 | 218.19 ± 16.07 | 169.95 ± 14.30 | 321.85 ± 21.92 | 256.63 ± 8.58 | 236.73 ± 10.29 |
| Pt^{II}5MESS | 230.62 ± 12.03 | 189.85 ± 7.61 | 150.71 ± 14.76 | 253.81 ± 6.85 | 211.88 ± 6.22 | 143.93 ± 5.49 |
| Pt^{II}56MESS | 155.45 ± 21.62 | 131.53 ± 18.06 | 86.51 ± 2.83 | 159.93 ± 9.67 | 151.44 ± 13.23 | 102.76 ± 7.13 |
| Pt^{IV}PHENSS(OH)₂ | 297.56 ± 7.26 | 257.95 ± 17.97 | 179.38 ± 16.49 | 312.19 ± 14.04 | 261.95 ± 4.88 | 215.58 ± 7.74 |
| Pt^{IV}5MESS(OH)₂ | 250.80 ± 11.01 | 201.07 ± 11.03 | 155.85 ± 10.56 | 304.45 ± 1.60 | 224.23 ± 8.71 | 198.24 ± 3.77 |
| Pt^{IV}56MESS(OH)₂ | 190.39 ± 20.15 | 156.37 ± 16.19 | 92.94 ± 5.17 | 258.69 ± 15.37 | 172.39 ± 5.78 | 124.84 ± 12.34 |

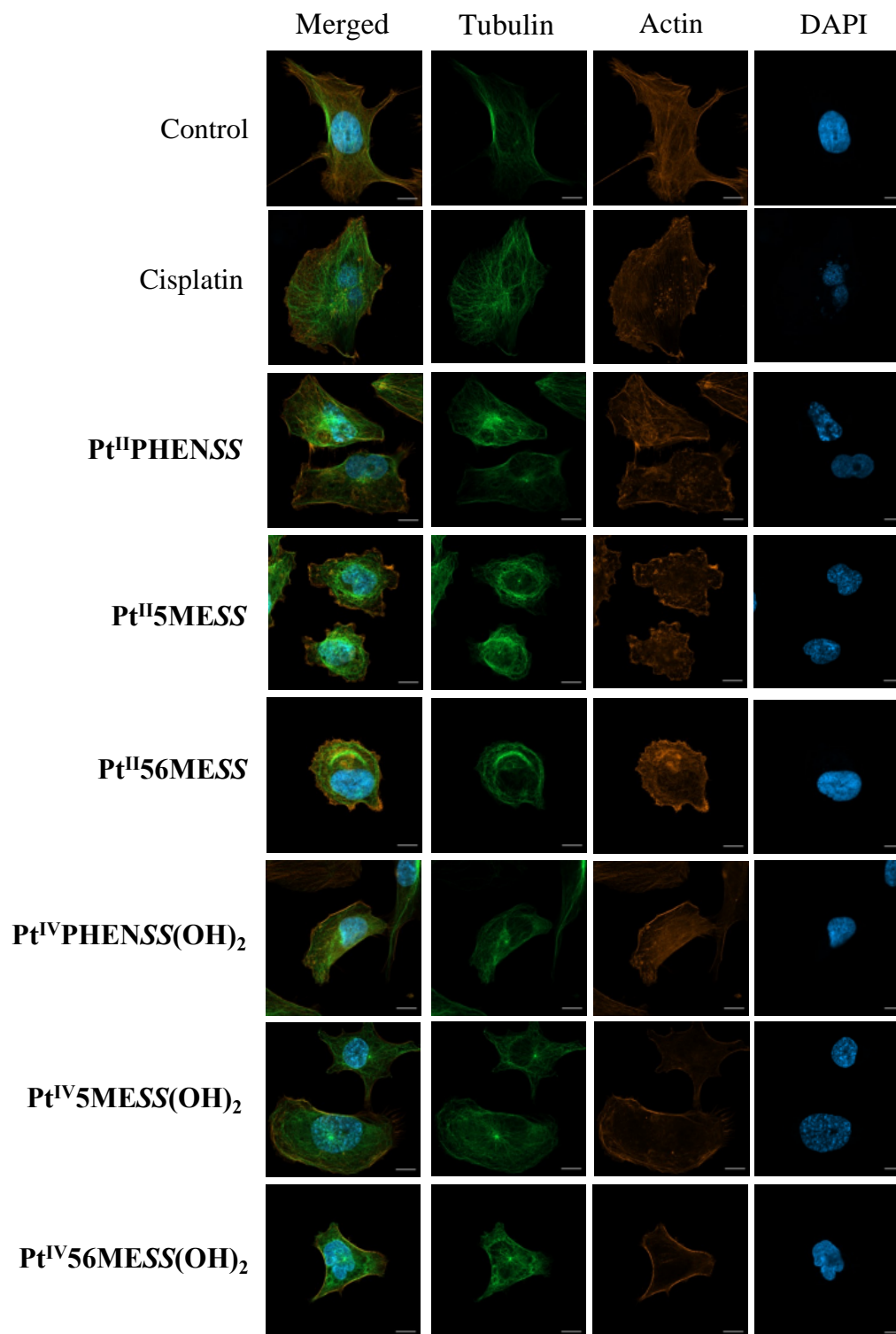


Figure S11. Effect of platinum complexes and cisplatin on β -tubulin and F-actin. Immunofluorescence upon treatment with platinum(II) and platinum(IV) complexes, as well as cisplatin in MDA-MB-231. Airyscan images were collected at 63 \times . Confocal microscope parameters were constant across all treatments for comparison in expression. $n = 50$ cells.

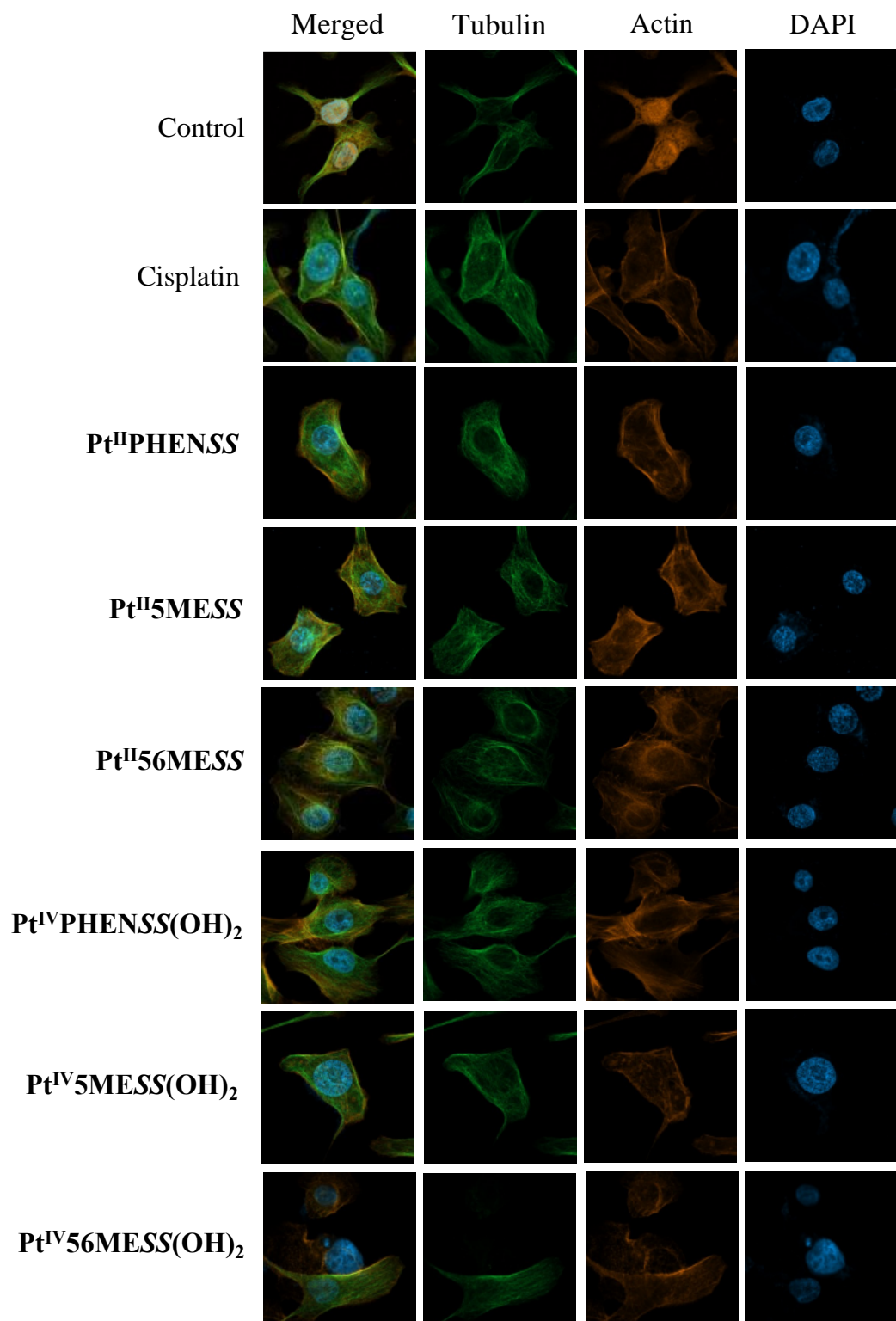


Figure S12. Effect of platinum complexes and cisplatin on β -tubulin and F-actin. Immunofluorescence upon treatment with platinum(II) and platinum(IV) complexes, as well as cisplatin in MCF10A. Airyscan images were collected at 63 \times . Confocal microscope parameters were constant across all treatments for comparison in expression. $n = 50$ cells.

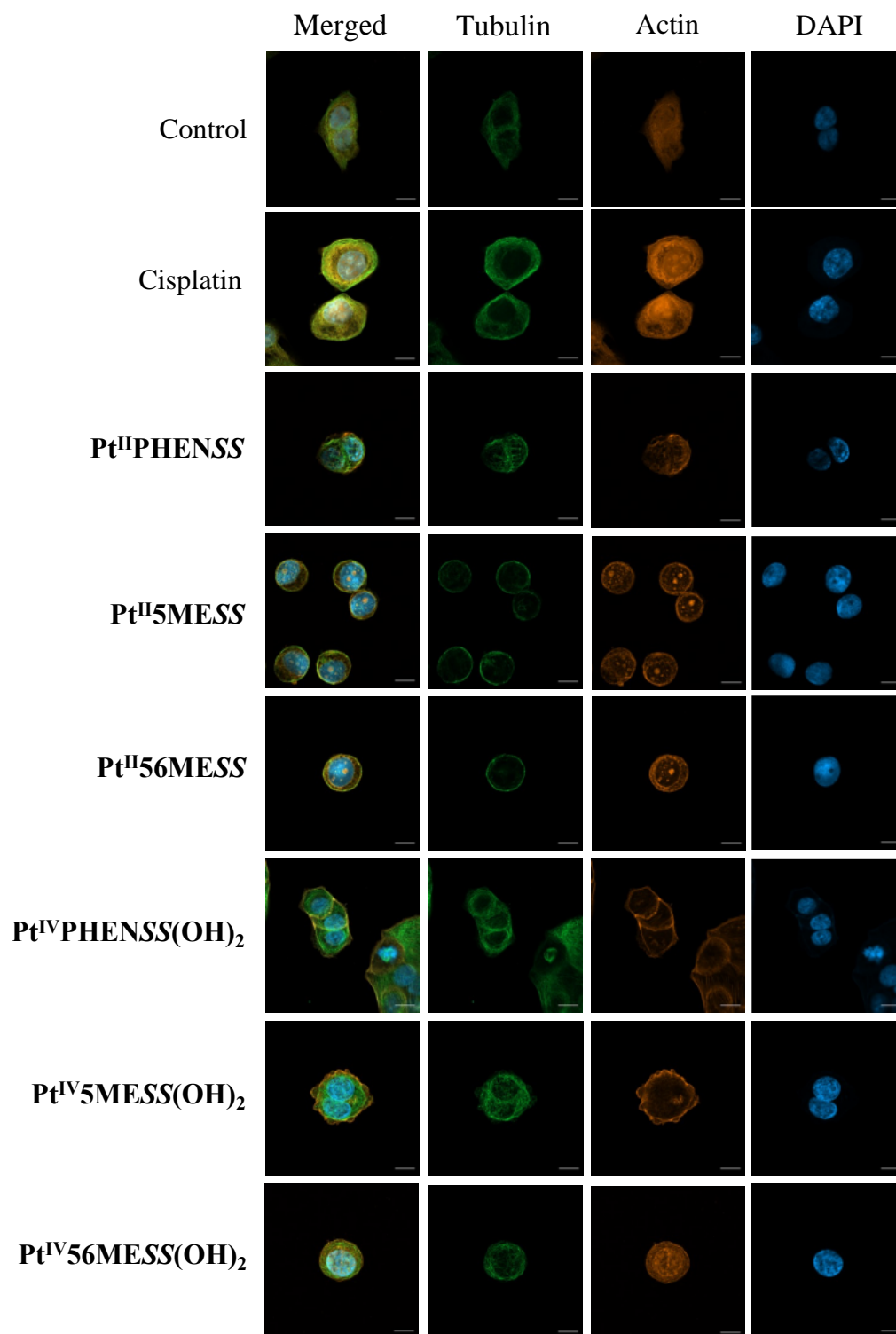


Figure S13. Effect of platinum complexes and cisplatin on β -tubulin and F-actin. Immunofluorescence upon treatment with platinum(II) and platinum(IV) complexes, as well as cisplatin in HT29. Airyscan images were collected at 63 \times . Confocal microscope parameters were constant across all treatments for comparison in expression. $n = 50$ cells.

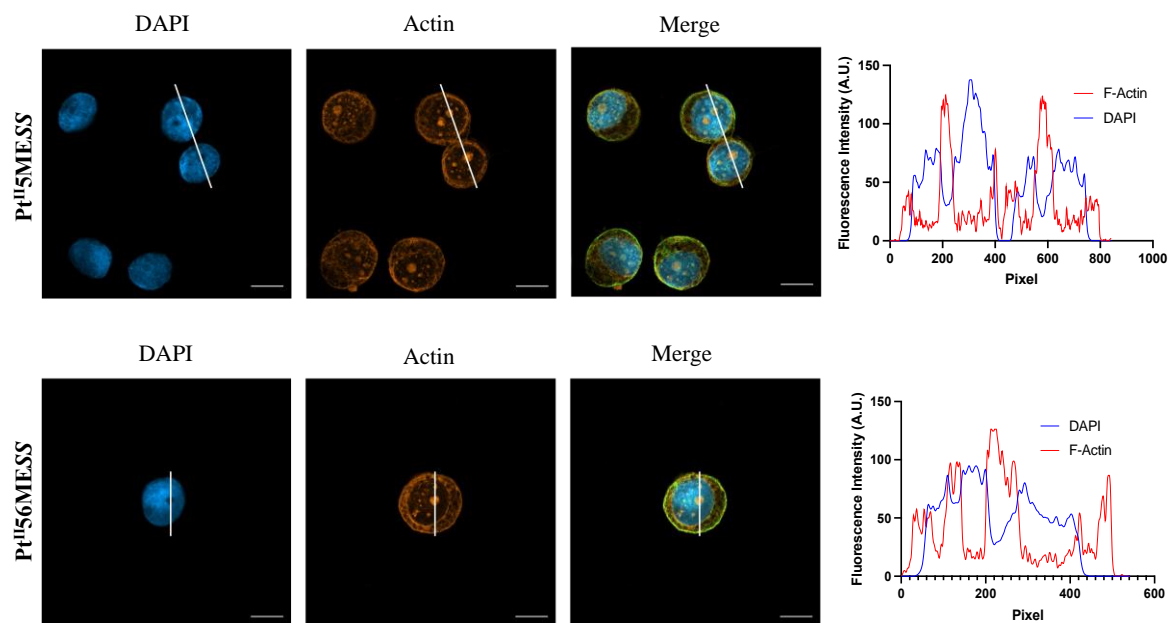


Figure S14. Relation between phalloidin and DAPI after $\text{Pt}^{\text{II}}5\text{MESS}$ and $\text{Pt}^{\text{II}}56\text{MESS}$ treatment. Immunofluorescence upon treatment with platinum(II) in HT29. Airyscan images were collected at 63 \times . Fluorescent Intensity profile of Actin (red) and DAPI (blue) over line selection (white).

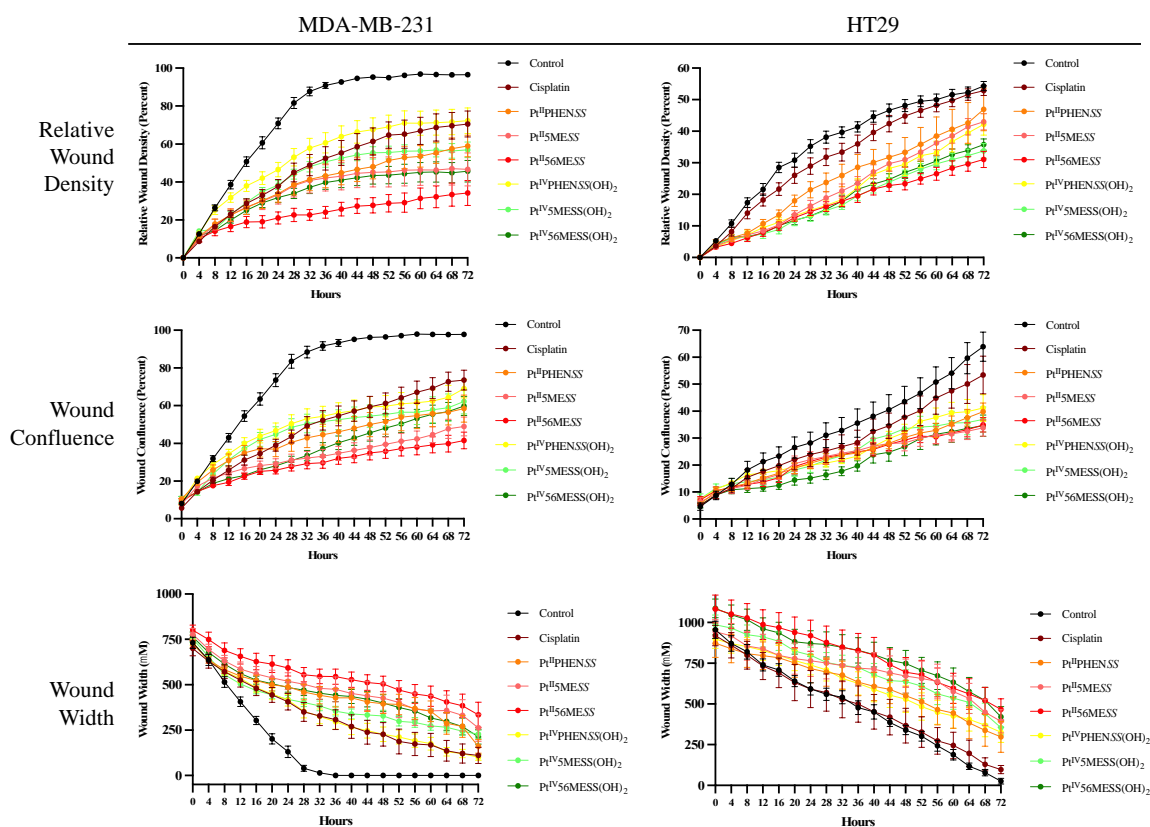


Figure S15. Cell migration (scratch wound healing assay). Cell Migration upon treatment with platinum(II) ($\text{Pt}^{\text{II}}\text{PHENSS}$, $\text{Pt}^{\text{II}}5\text{MESS}$ and $\text{Pt}^{\text{II}}56\text{MESS}$) and platinum(IV) ($\text{Pt}^{\text{IV}}\text{PHENSS}(\text{OH})_2$, $\text{Pt}^{\text{IV}}5\text{MESS}(\text{OH})_2$ and $\text{Pt}^{\text{IV}}56\text{MESS}(\text{OH})_2$) complexes, as well as cisplatin in MDA-MB-231 and HT29 cells quantified every 4 h for up to 72 h.

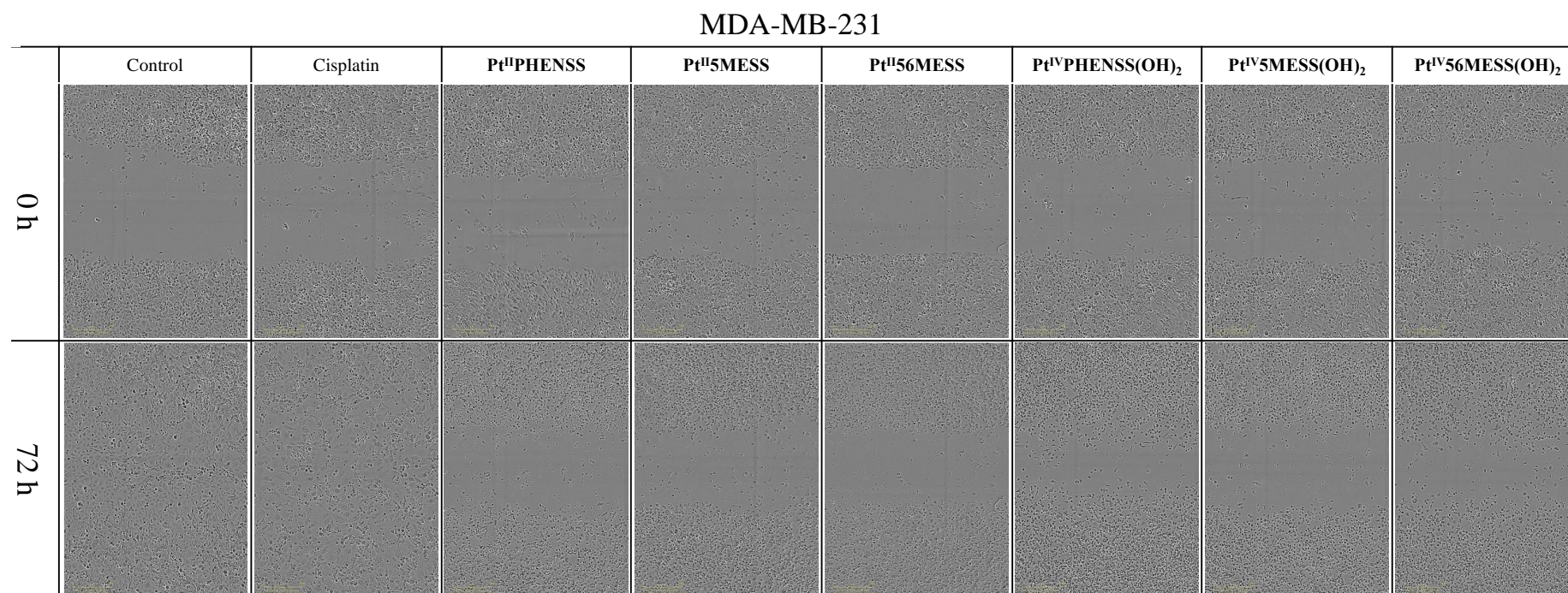


Figure S16. Cell migration. Cell Migration upon treatment with platinum(II) (**Pt^{II}PHENSS**, **Pt^{II}5MESS** and **Pt^{II}56MESS**) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂**, **Pt^{IV}5MESS(OH)₂** and **Pt^{IV}56MESS(OH)₂**) complexes, as well as cisplatin in MDA-MB-231 cells at 0 and 72 h. Incucyte[®] phase contrast microscope used to collect bright-field live images using 10× objective.

HT29

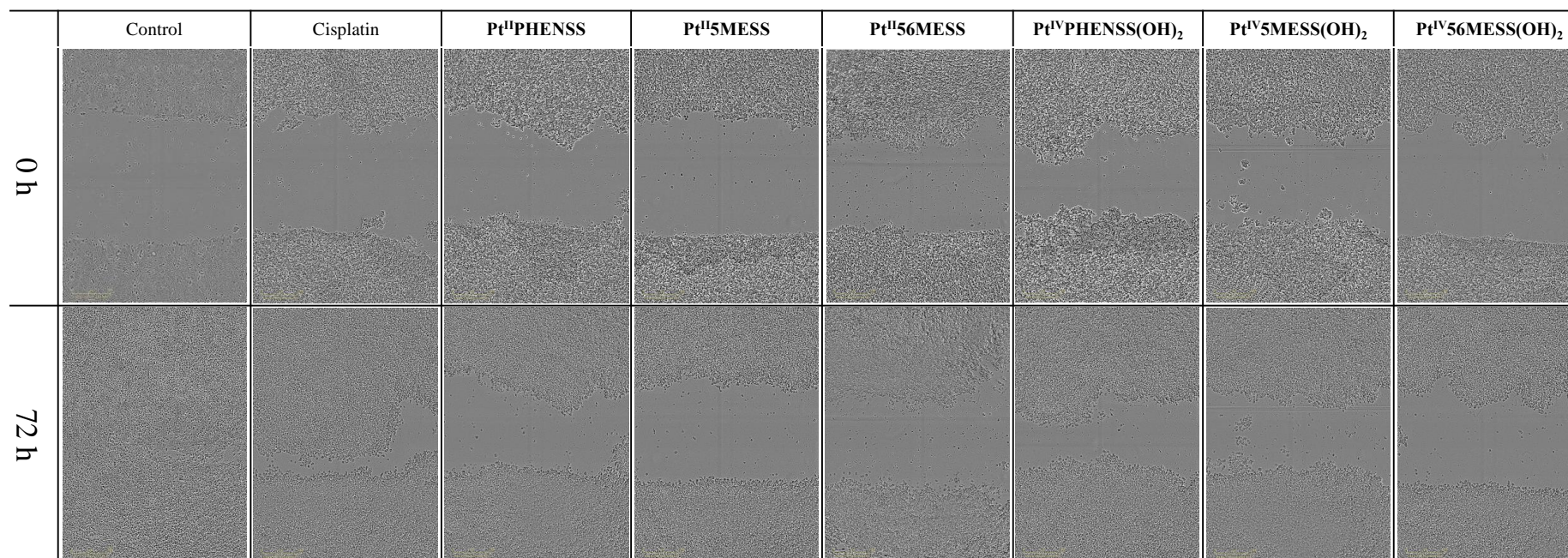


Figure S17. Cell migration. Cell Migration upon treatment with platinum(II) (Pt^{II}PHENSS, Pt^{II}5MESS and Pt^{II}56MESS) and platinum(IV) (Pt^{IV}PHENSS(OH)₂, Pt^{IV}5MESS(OH)₂ and Pt^{IV}56MESS(OH)₂) complexes, as well as cisplatin in HT29 cells at 0 and 72 h. Incucyte[®] phase contrast microscope used to collect bright-field live images using 10× objective.

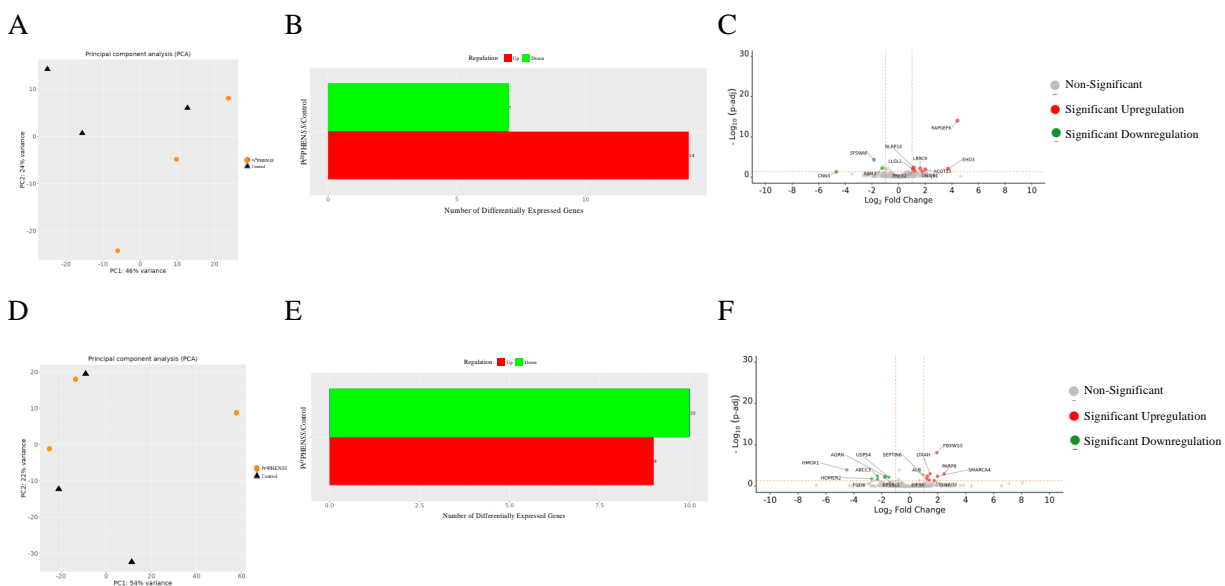


Figure S18. Proteomic analysis of MDA-MB-231 and HT29 upon treatment with $\text{Pt}^{\text{IV}}\text{PHENSS}$. A. MDA-MB-231 principal component analysis. B. Number of differentially expressed proteins (DEPs) in MDA-MB-231. C. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in MDA-MB-231. D. HT29 principal component analysis. E. Number of differentially expressed proteins (DEPs) in HT29. F. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in HT29.

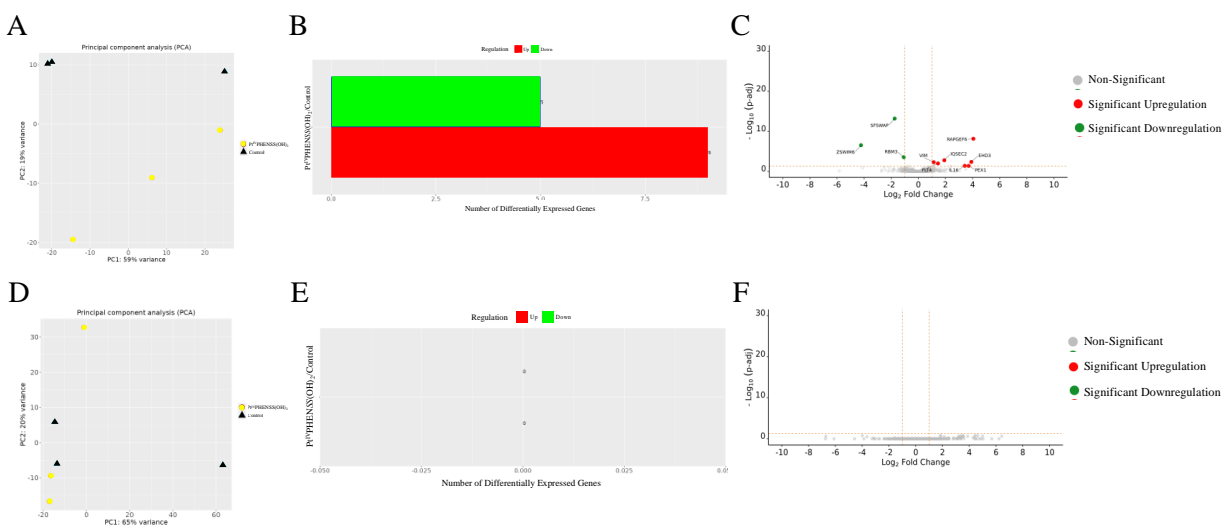


Figure S19. Proteomic analysis of MDA-MB-231 and HT29 upon treatment with $\text{Pt}^{\text{IV}}\text{PHENSS}(\text{OH})_2$. A. MDA-MB-231 principal component analysis. B. Number of differentially expressed proteins (DEPs) in MDA-MB-231. C. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in MDA-MB-231. D. HT29 principal component analysis. E. Number of differentially expressed proteins (DEPs) in HT29. F. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in HT29.

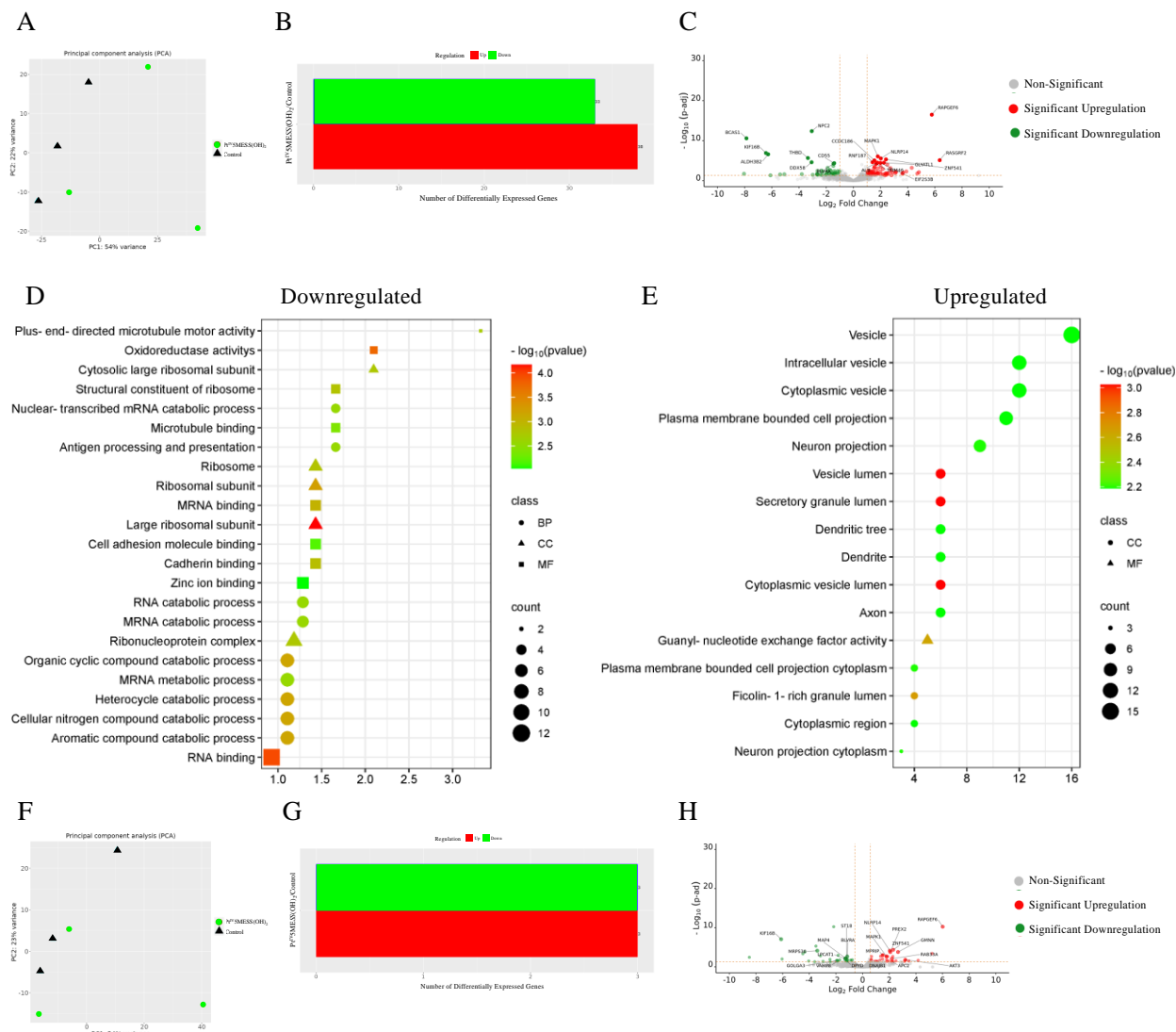


Figure S20. Proteomic analysis of MDA-MB-231 and HT29 upon treatment with Pt^{IV}5MESS(OH)₂. A. MDA-MB-231 principal component analysis. B. Number of differentially expressed proteins (DEPs) in MDA-MB-231. C. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in MDA-MB-231. D. MDA-MB-231 GO enriched biological processes, cellular components, and molecular function in MDA-MB-231. E. downregulated and F. upregulated proteins. G. HT29 principal component analysis. H. Number of differentially expressed proteins (DEPs) in HT29. I. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in HT29.

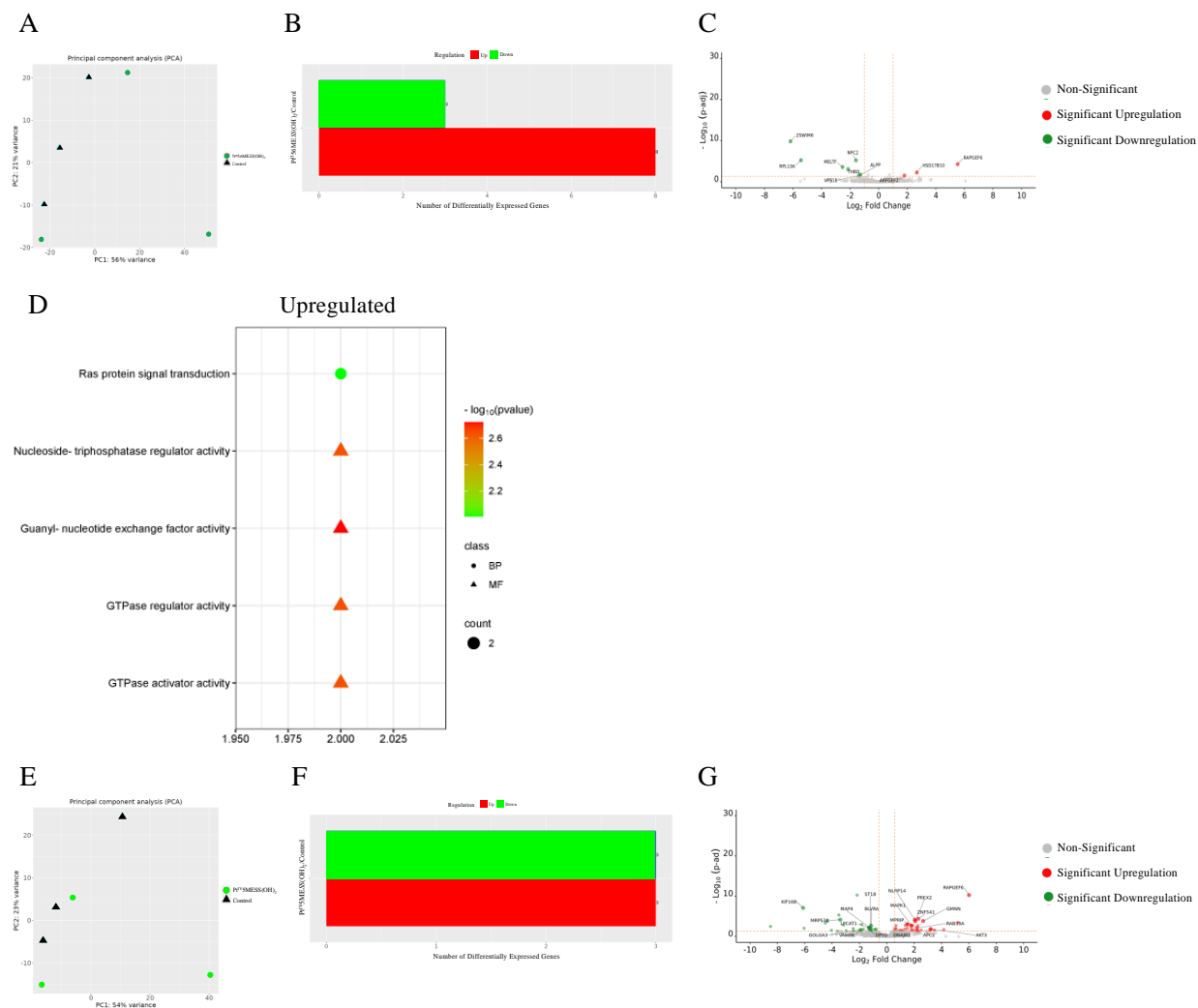


Figure S21. Proteomic analysis of MDA-MB-231 and HT29 upon treatment with Pt^{IV}56MESS(OH)₂. A. MDA-MB-231 principal component analysis. B. Number of differentially expressed proteins (DEPs) in MDA-MB-231. C. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in MDA-MB-231. D. MDA-MB-231 GO enriched biological processes, cellular components, and molecular function in upregulated proteins. E. HT29 principal component analysis. F. Number of differentially expressed proteins (DEPs) in HT29. G. Volcano plot of DEPs upregulated (red) and down regulated (green) proteins in HT29.

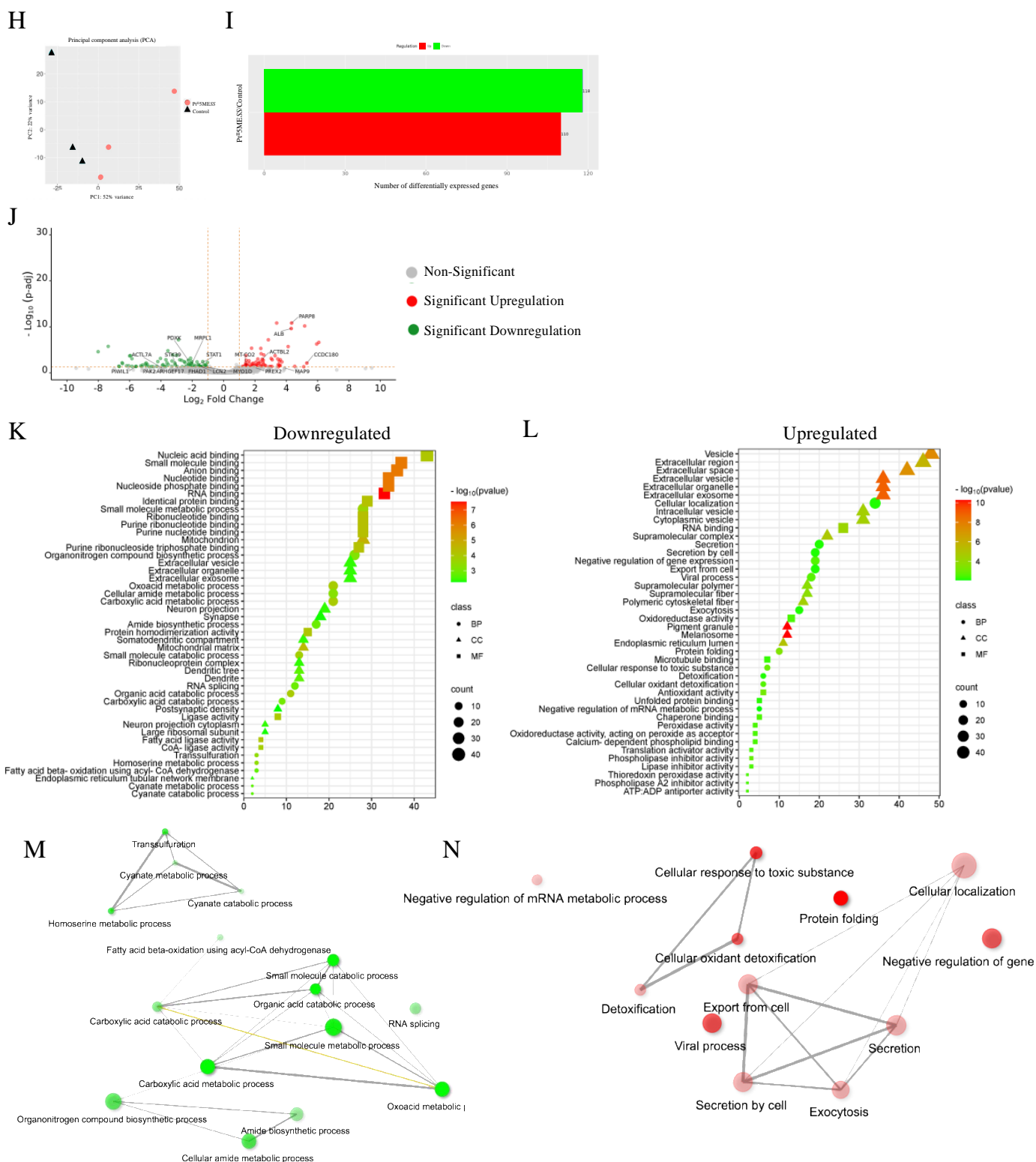
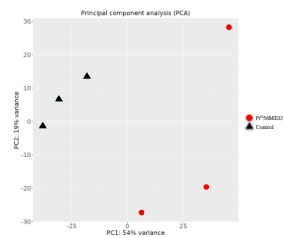


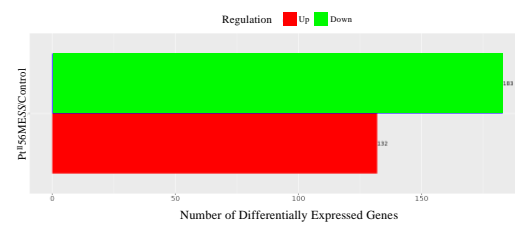
Figure S22. Proteomic analysis of MDA-MB-231 and HT29 upon treatment with **Pt^{II}5MESS** as described in Section 2.15. A. MDA-MB-231 principal component analysis. B. MDA-MB-231 number of differentially expressed proteins (DEPs). C. MDA-MB-231 volcano plot of DEPs upregulated (red) and down regulated (green). GO enriched biological processes, cellular components, and molecular function in MDA-MB-231, D. Downregulated and E. Upregulated proteins. MDA-MB-231 pathway enrichment and gene act network analysis in most significance of F.

Downregulated pathways; mRNA metabolic process, GTP binding and nucleoside activity and G. Upregulated pathways; nucleotide binding and pyrophosphatase activity. H. HT29 principal component analysis. I. HT29 number of differentially expressed proteins (DEPs). J. HT29 volcano plot of DEPs upregulated (red) and down regulated (green). GO enriched biological processes, cellular components, and molecular function in HT29, K. Downregulated and L. Upregulated proteins. HT29 pathway enrichment and gene act network analysis in most significance of M. Downregulated pathways; molecular metabolic and catabolic processes and RNA splicing and N. Upregulated pathways; protein folding, response to toxic substance, detoxification, secretion, and exocytosis activity. Data points denote mean \pm SEM. $n = 3$ from three independent experiments.

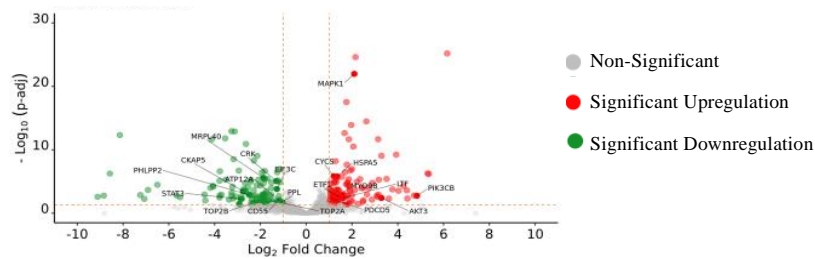
A



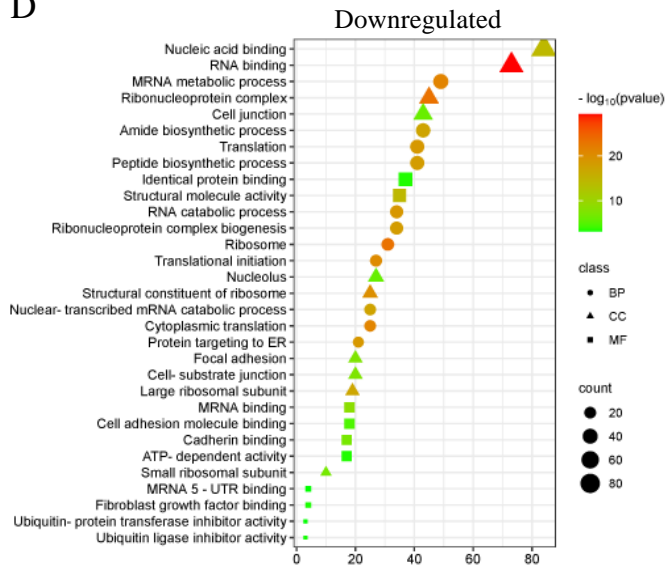
B



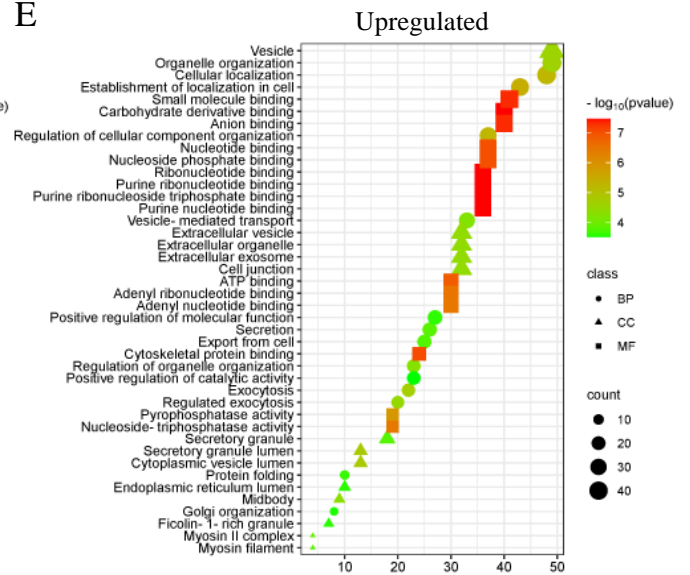
C



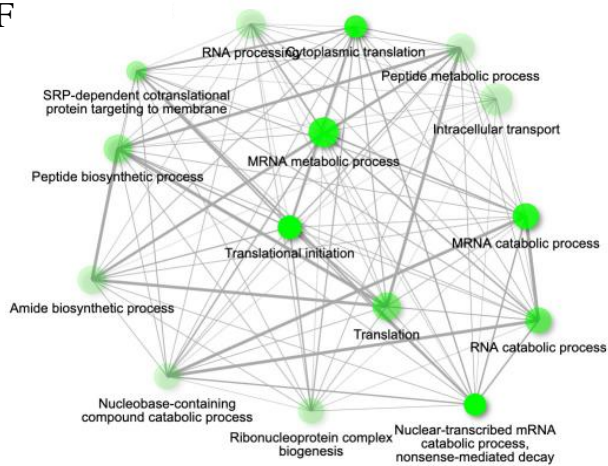
D



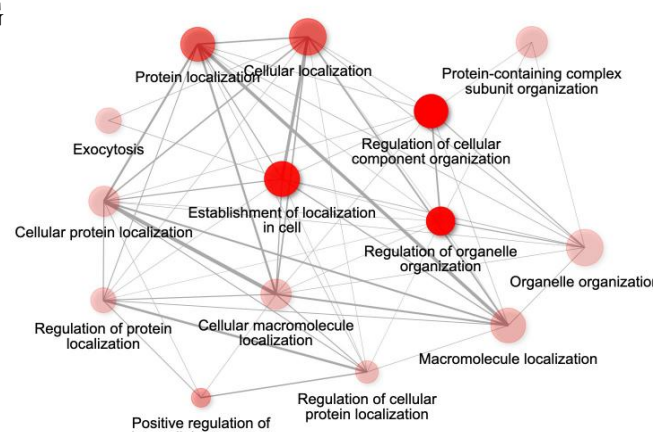
E



F



G



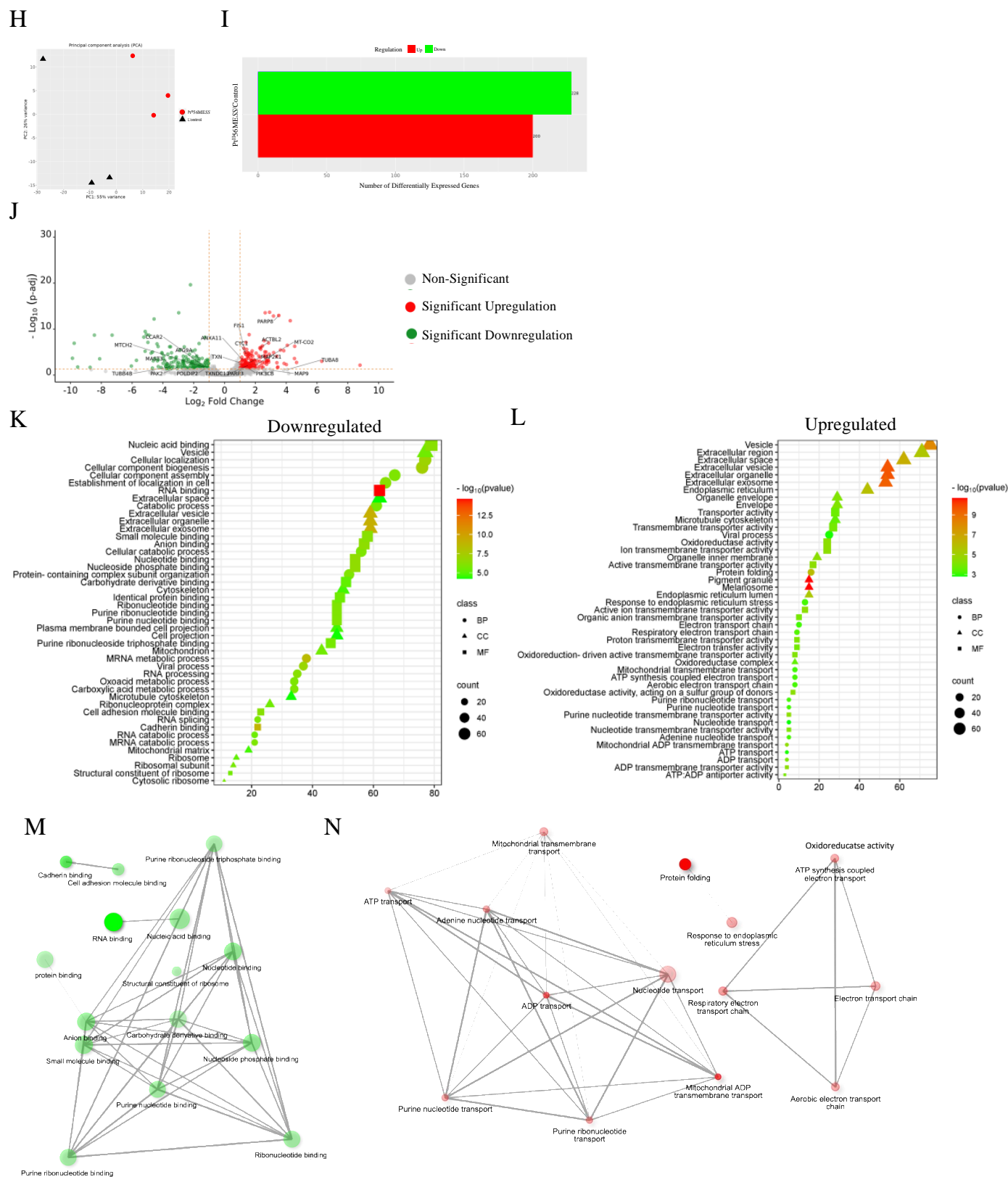


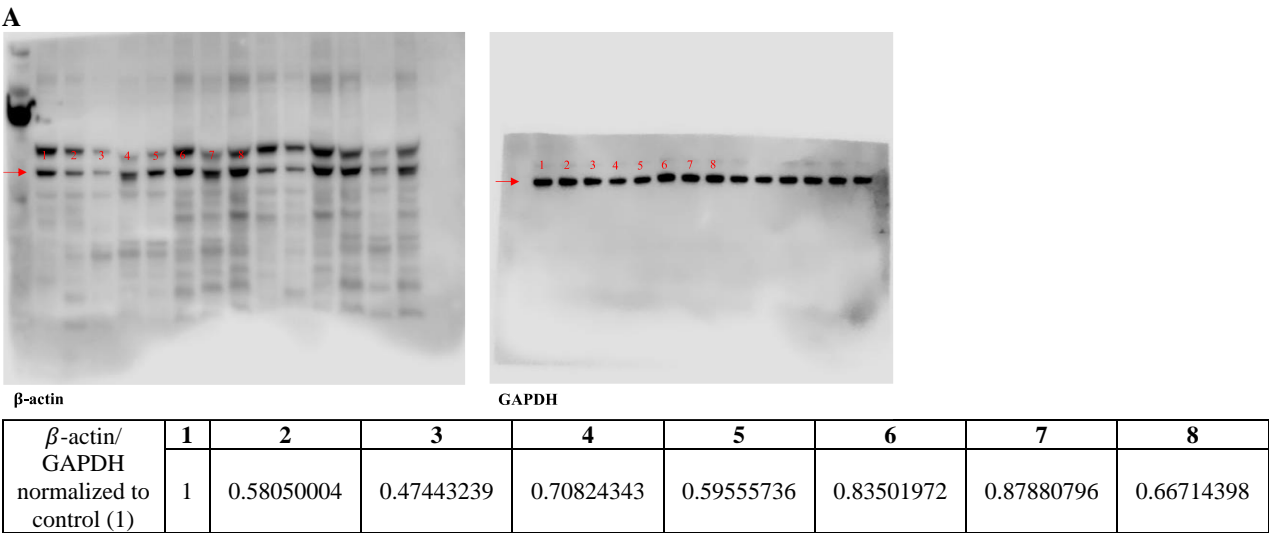
Figure S23. Proteomic analysis of MDA-MB-231 and HT29 upon treatment with **Pt^{II}56MESS** as described in Section 2.15. A. MDA-MB-231 principal component analysis. B. MDA-MB-231 number of differentially expressed proteins (DEPs). C. MDA-MB-231 volcano plot of DEPs upregulated (red) and down regulated (green). GO enriched

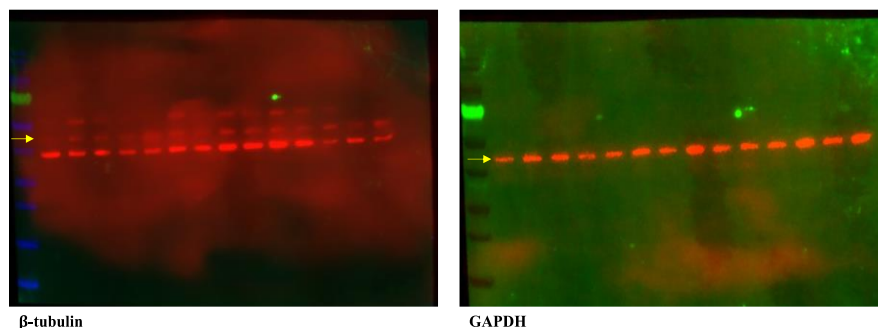
biological processes, cellular components, and molecular function in MDA-MB-231, D. Downregulated and E. Upregulated proteins. MDA-MB-231 pathway enrichment and gene act network analysis in most significance of F. Downregulated pathways; translational initiation and mRNA metabolic process and G. Upregulated pathways; localisation in cell, cellular organisation, protein localisation and exocytosis. H. HT29 principal component analysis. I. HT29 number of differentially expressed proteins (DEPs). J. HT29 volcano plot of DEPs upregulated (red) and down regulated (green). GO enriched biological processes, cellular components, and molecular function in HT29, K. Downregulated and L. Upregulated proteins. HT29 pathway enrichment and gene act network analysis in most significance of M. Downregulated pathways; RNA binding and nucleotide binding and N. Upregulated pathways; ion transmembrane transport, active transport, and oxidoreductase activity. Data points denote mean \pm SEM. $n = 3$ from three independent experiments.

Table S7. Antibody Concentrations used in Western Blot

| Supplier | Clone no. | Clone | Primary Antibody Target | Molecular Weight |
|----------|-----------|-------------------|-------------------------|---|
| Abcam | ab32503 | Rabbit monoclonal | BAX | 21 KDa |
| Abcam | ab196495 | Rabbit polyclonal | Bcl2 | 26 KDa |
| Abcam | ab32138 | Rabbit monoclonal | PARP-1 | pro-form 113 Kda and 25 Kda (N-terminal catalytic domain) cleaved form of PARP1 |
| Abcam | ab133504 | Rabbit monoclonal | Cytochrome C | 11 or 14 KDa |
| Abcam | ab214430 | Rabbit monoclonal | Caspase 3 | 32 Kda and cleaved fragments at 17, 19 or 24 Kda |
| Abcam | ab108333 | Rabbit monoclonal | Caspase 8 | 55 KDa |
| Abcam | ab202068 | Rabbit monoclonal | Caspase 9 | 46, KDa |
| Abcam | ab8227 | Rabbit polyclonal | Beta Actin | 42 KDa |
| Abcam | ab7291 | Mouse monoclonal | Alpha Tubulin | 50 KDa |
| Abcam | ab6046 | Rabbit Polyclonal | Beta Tubulin | 50 KDa |
| Abcam | ab32389 | Rabbit monoclonal | p53 | 44 KDa |
| Abcam | ab109520 | Rabbit monoclonal | p21 | 21 KDa |
| Abcam | ab192591 | Rabbit polyclonal | p-ERK | 41-44 KDa |
| Abcam | ab184699 | Rabbit monoclonal | ERK | 42 KDa |
| Abcam | ab38449 | Rabbit polyclonal | p-AKT | 56 KDa |
| Abcam | ab8805 | Rabbit polyclonal | AKT | 56 KDa |
| Abcam | ab8245 | Mouse monoclonal | GAPDH | 36 or 40 KDa |
| Abcam | ab228668 | Rabbit polyclonal | APG5L/ATG5 | predicted at 32 KDA but seen at 55 Kda |
| Abcam | ab228525 | Rabbit polyclonal | ATG16L1 - N-terminal | 68-70 Kda |
| | | | ATG4B | 44 Kda |
| | | | ATG9A | 94-100 Kda |
| | | | Beclin-1 | 52 Kda |
| | | | LC3B | 14-16 Kda |
| Abcam | ab10640 | Rabbit polyclonal | Bid Cleavage Site | 15 KDa |

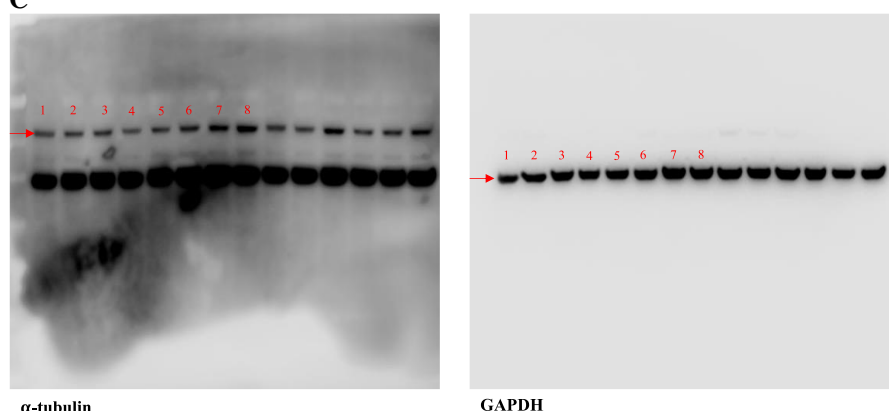
The representative full blots below include proteins from several projects run in parallel. Proteins related to this study are labelled lane 1-8 for the relevant treatment.



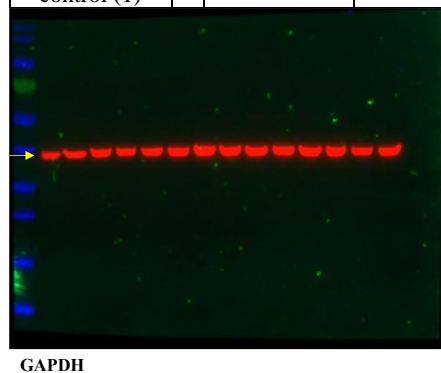


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

C



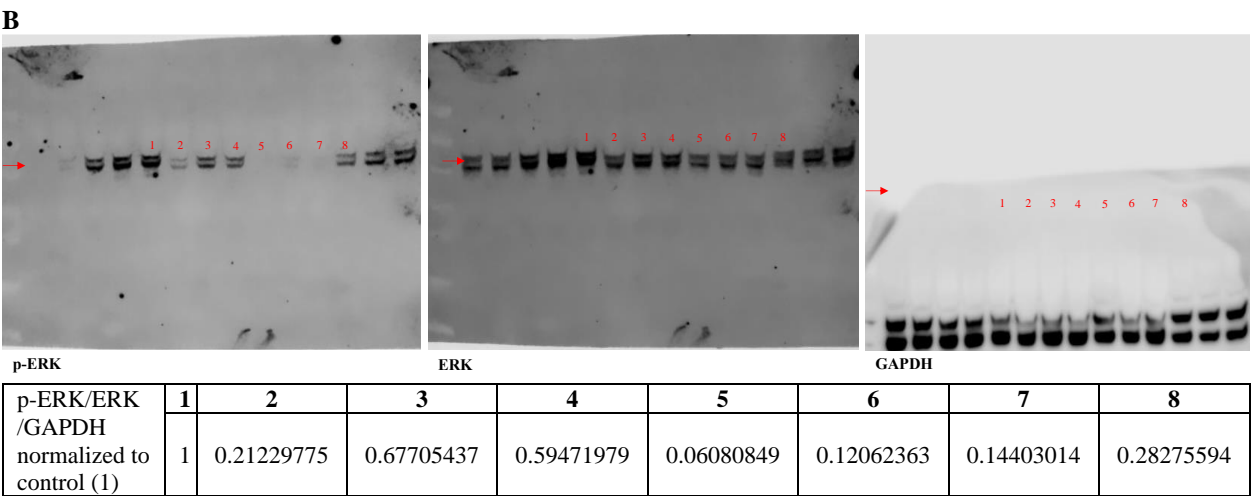
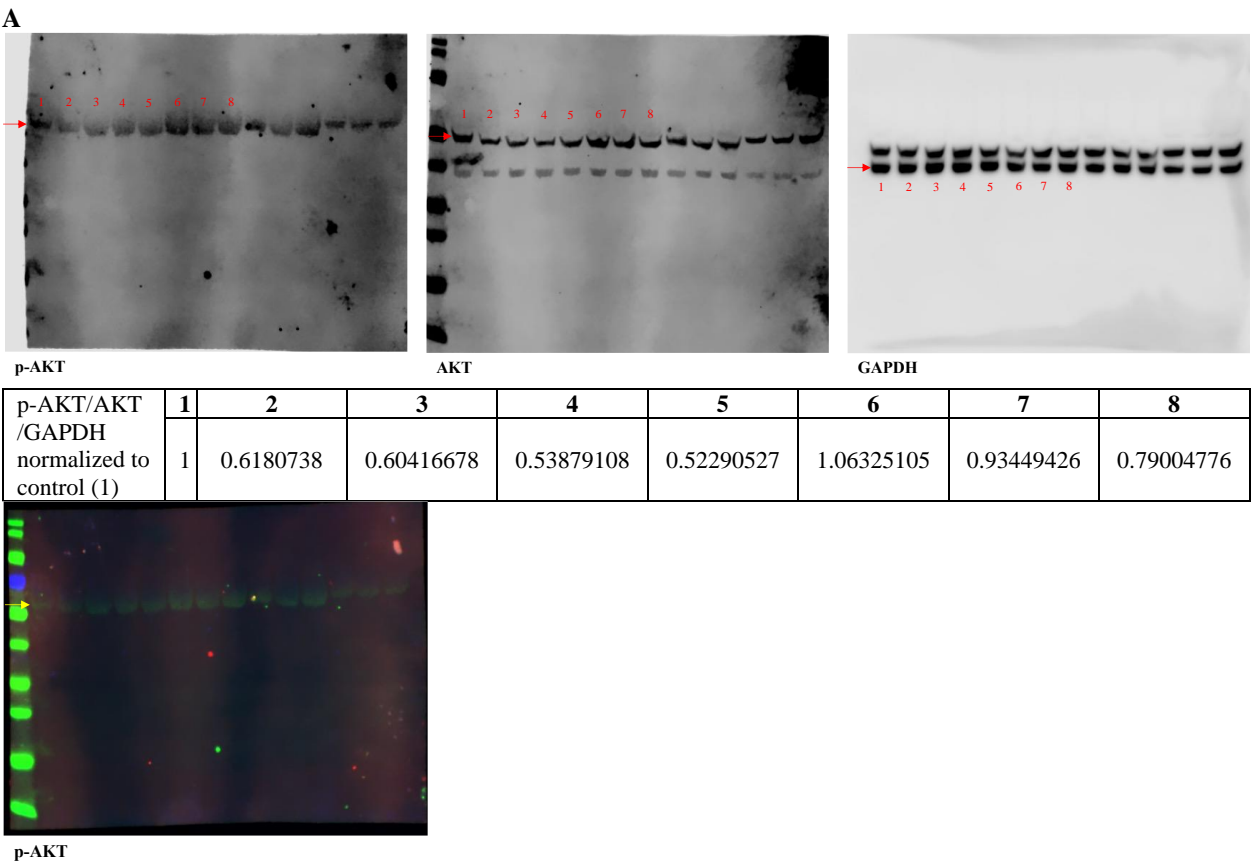
| α -tubulin/ GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|------------|------------|------------|-----------|------------|------------|------------|
| | 1 | 0.94863864 | 0.93546771 | 0.63992484 | 0.7886653 | 0.92790063 | 1.17680214 | 1.35918472 |

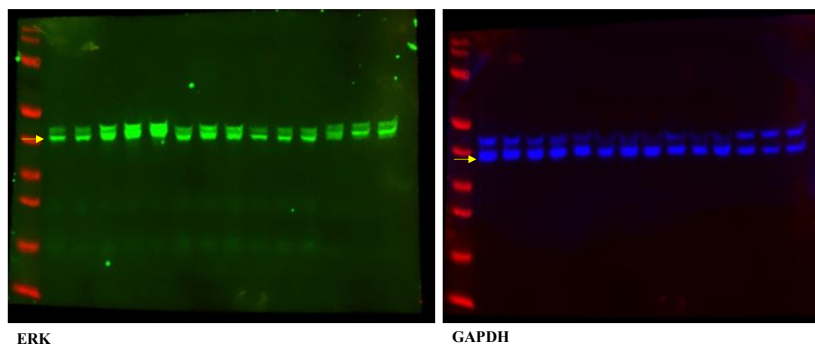


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of GAPDH with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. α -tubulin molecular marker can be seen on the chemiluminescence blot and tracked using the GAPDH fluorescent blot, given α -tubulin falls above GAPDH.

Figure S24. Full representative western blot of microtubule cytoskeleton protein markers in MDA-MB-231. Represented data normalized to GAPDH relative to control (lane 1). A. β -actin, B. β -tubulin and C. α -tubulin. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5))

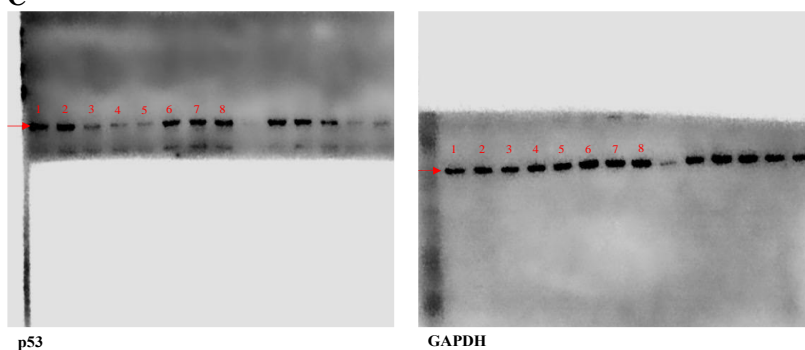
and platinum(IV) (**Pt^{II}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (lane 8)) complexes, as well as cisplatin (lane 2) in MDA–MB–231 cells at 72 h compared with control (lane 1).



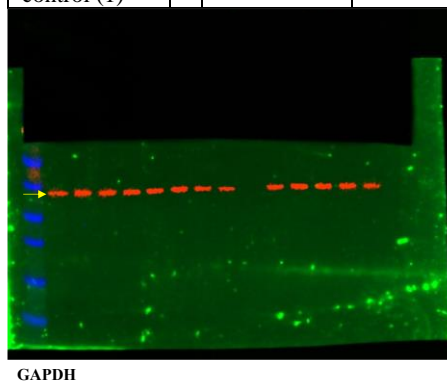


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of ERK and GAPDH with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. p-ERK molecular marker can be seen on the chemiluminescence blot and tracked using the ERK fluorescent blot, given p-ERK will fall at the same molecular weight.

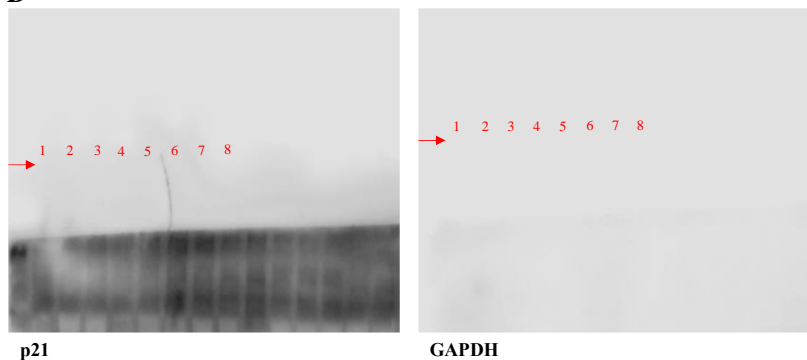
C



| p53/GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|------------|------------|------------|---|
| 1 | 1.58464538 | 1.10500528 | 0.78093946 | 0.61872139 | 1.17637615 | 1.18271952 | 1.18294515 | |



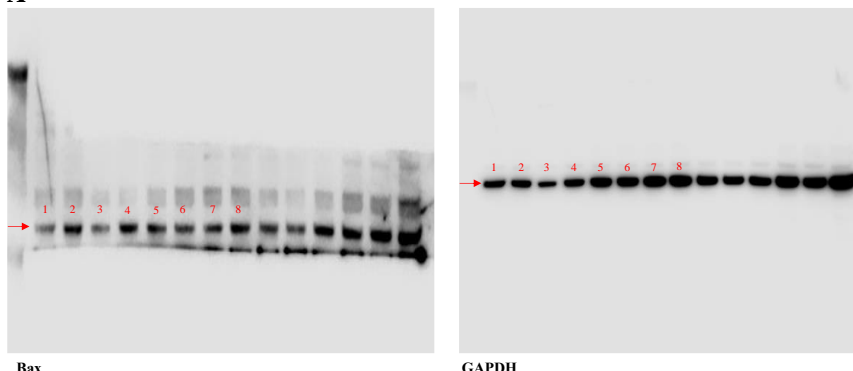
Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of GAPDH with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. p53 molecular marker can be tracked on the chemiluminescence blot using the GAPDH fluorescent blot, given p53 will fall above GAPDH.

D

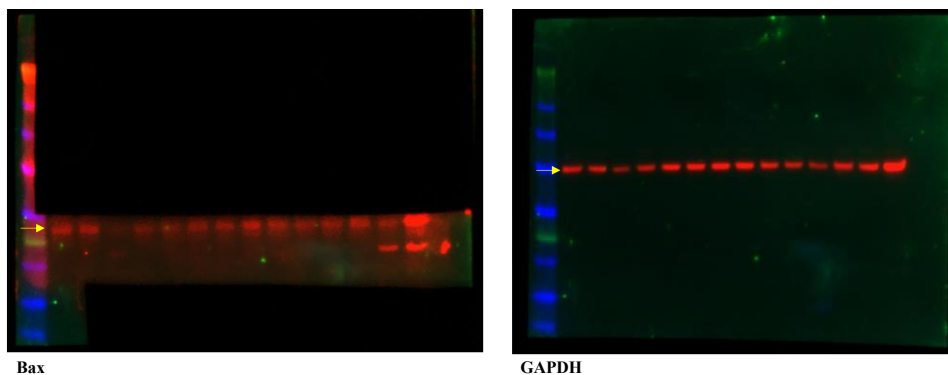
| p21/GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|------------|-----------|-----------|---|
| 1 | 1.35060326 | 1.81212007 | 2.47783829 | 2.59969343 | 1.34603696 | 1.0418355 | 1.0525943 | |

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. Molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was still loaded to the left of the gel and can still be observed in p21 chemiluminescent blot. Protein markers p21 and GAPDH were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

Figure S25. Full representative western blot of cell proliferation protein markers in MDA-MB-231. Represented data normalized to GAPDH relative to control (lane 1). A. p-AKT/AKT, B. p-ERK/ERK, C. p53 and D. p21. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5)) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (8)) complexes, as well as cisplatin (lane 2) in MDA-MB-231 cells at 72 h compared with control (lane 1).

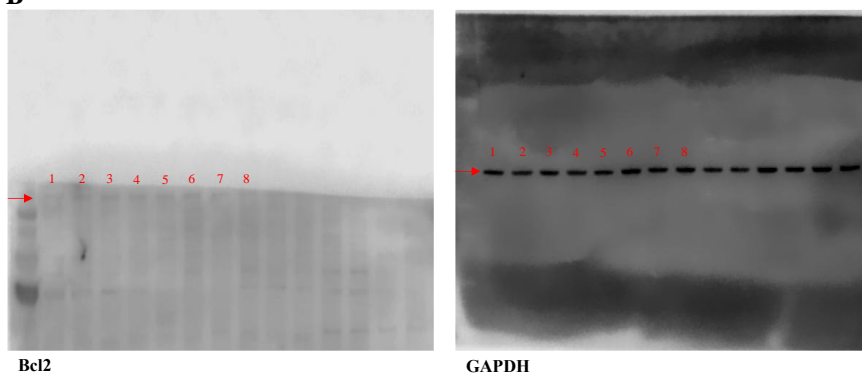
A

| Bax/GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|------------|------------|------------|---|
| 1 | 1.83635249 | 1.16069247 | 1.88631075 | 1.27646318 | 1.39794735 | 1.84635944 | 2.60047266 | |

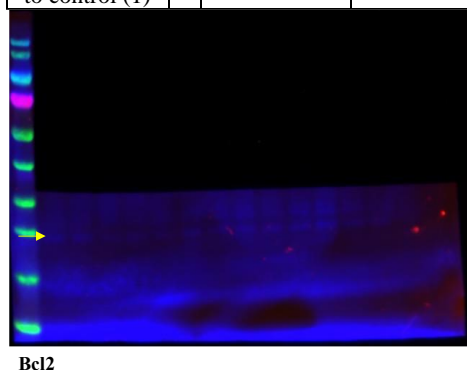


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of Bax and GAPDH with molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

B

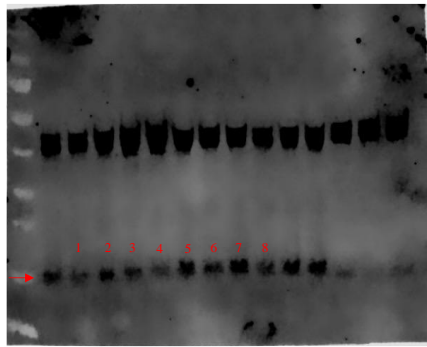


| Bcl2/GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--------------------------------------|------------|-----------|------------|------------|------------|------------|-----------|---|
| 1 | 0.75285082 | 0.8361108 | 0.50739129 | 0.68460454 | 0.78430242 | 0.79136982 | 0.8651495 | |



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of Bcl2 with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. GAPDH molecular marker fluorescent blot was not obtained but can be seen on the chemiluminescence blot and tracked using the Bcl2 fluorescent blot, given GAPDH will fall above.

C

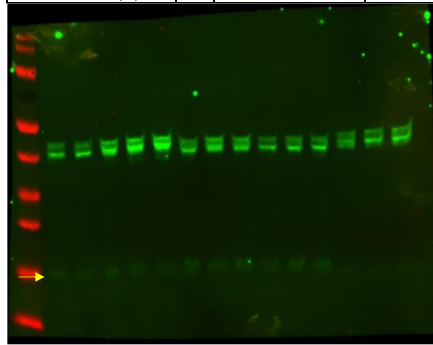


Cytochrome C

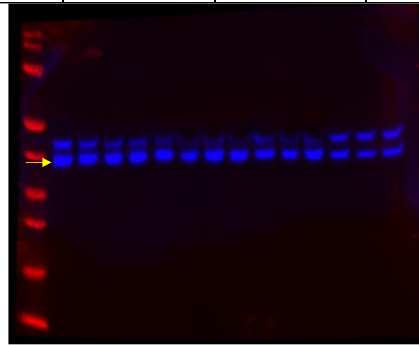


GAPDH

| Cytochrome C /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|------------|------------|------------|------------|------------|------------|------------|---|
| 1 | 2.37118767 | 1.82721713 | 1.51269724 | 3.10869364 | 2.37833562 | 2.79548123 | 2.18464815 | |



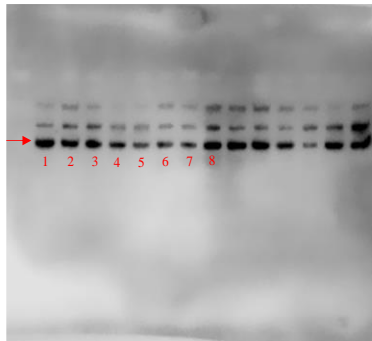
Cytochrome C



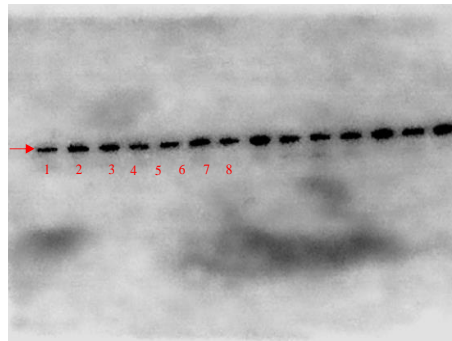
GAPDH

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 on the Odyssey® FC imaging system and protein of interest directed with a yellow arrow.

D

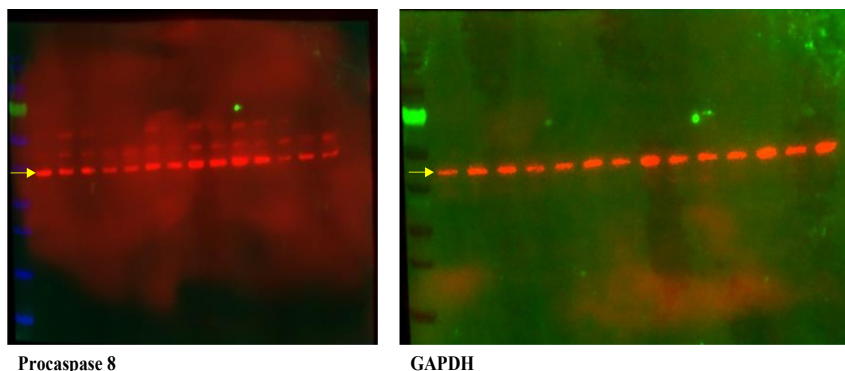


Procaspase 8



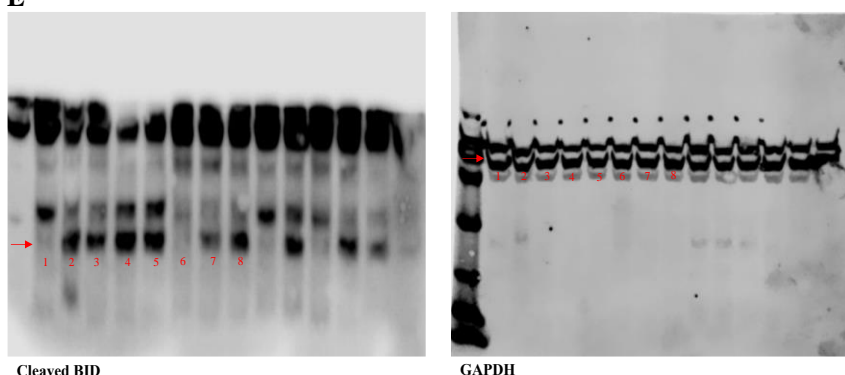
GAPDH

| Procaspase 8 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|------------|------------|------------|------------|------------|------------|------------|---|
| 1 | 0.56815789 | 0.51949587 | 0.50994632 | 0.35839375 | 0.28884874 | 0.35460896 | 0.56804293 | |



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

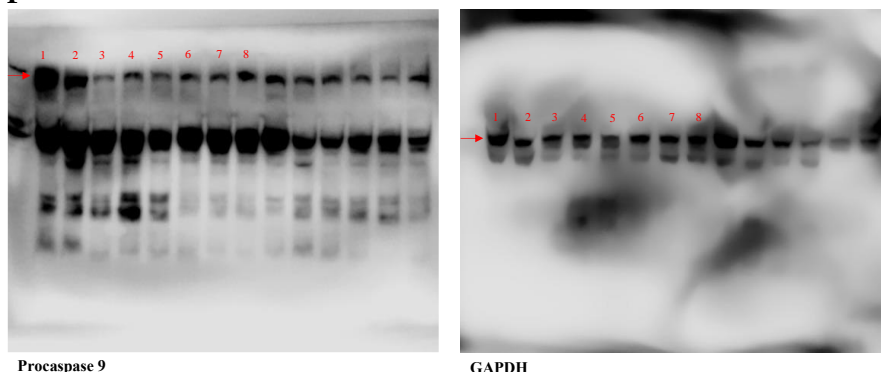
E



| Cleaved BID /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|-----------|-----------|-----------|---|
| 1 | 11.0692064 | 13.3905609 | 14.2890745 | 14.7401877 | 1.3298651 | 5.9481588 | 8.5326365 | |

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. However, the appearance of the molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) on the chemiluminescence blot in cleaved BID and GAPDH blots confirm the detected band position. Protein markers were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

F



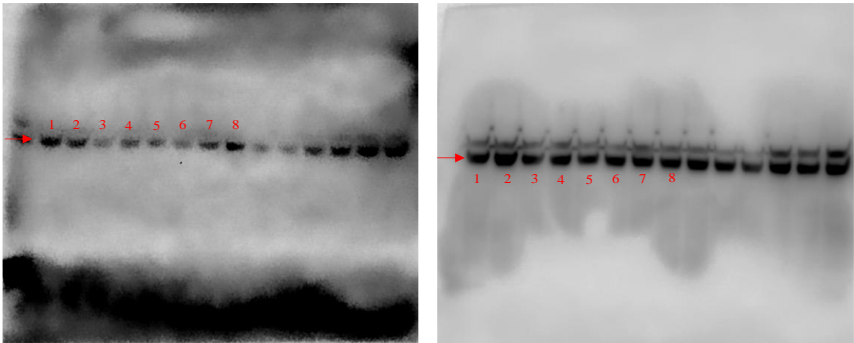
Procaspase 9

GAPDH

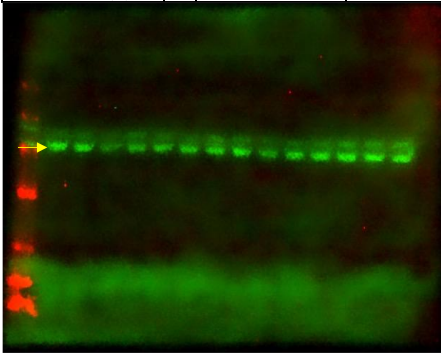
| | | | | | | | | |
|--|------------|------------|------------|------------|------------|------------|------------|----------|
| Procaspase 9 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 1.12199504 | 0.20416262 | 0.31011077 | 0.47547078 | 0.26748363 | 0.30218544 | 0.45565747 | |

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. Molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was still loaded to the left of the gel and can still be somewhat observed in procaspase 9 chemiluminescent blot. Protein markers procaspase 9 and GAPDH were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

G

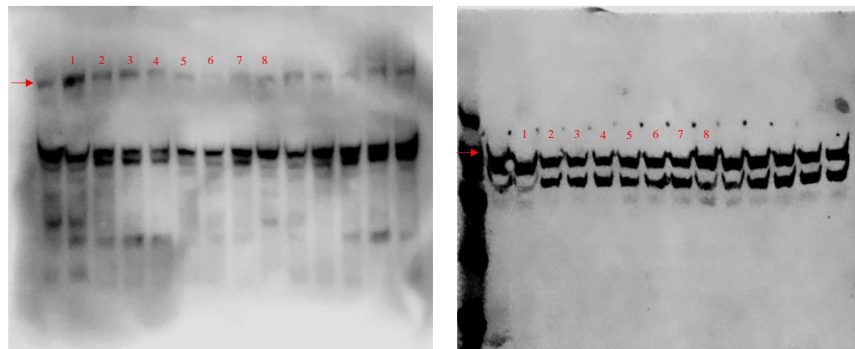


| | | | | | | | | |
|--|-----------|------------|------------|-----------|------------|------------|------------|----------|
| Procaspase 3 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 0.8252645 | 0.32070402 | 0.54050899 | 0.4243036 | 0.40928799 | 0.71667593 | 0.94708058 | |

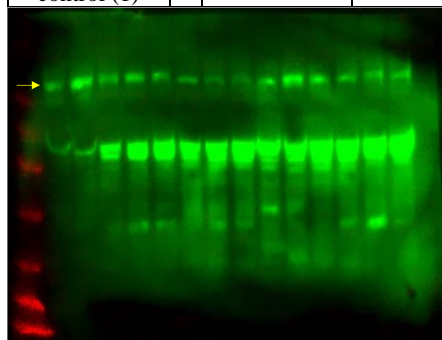


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of Procaspase 3 with molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) was detected using laser 600 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. GAPDH molecular marker fluorescent blot was not obtained but can be seen on the chemiluminescence blot and tracked using the Procaspase 3 fluorescent blot, given GAPDH will in the same molecular range.

H

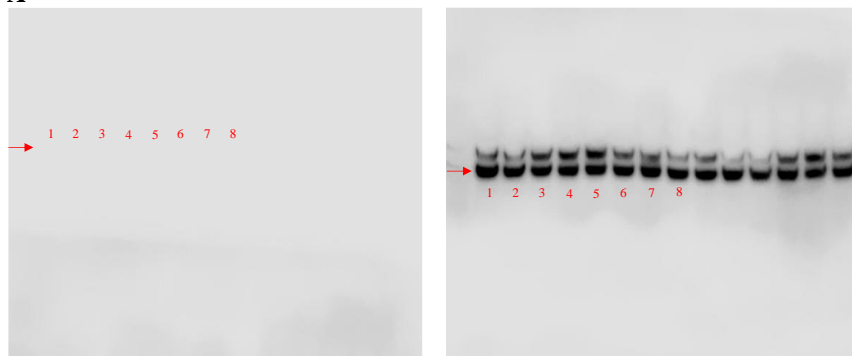


| PARP-1 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|---|------------|------------|------------|------------|------------|------------|------------|
| | 1 | 0.78666175 | 0.77749498 | 0.76929938 | 0.63398891 | 0.57165935 | 0.51867466 | 0.61815111 |

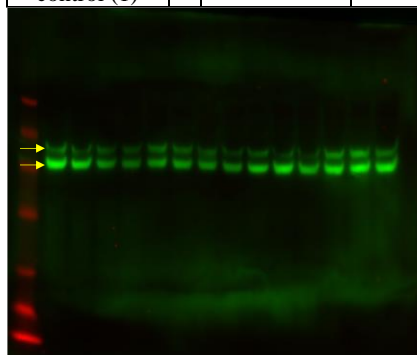


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of PARP-1 with molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) was detected using laser 600 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. GAPDH molecular marker fluorescent blot was not obtained but can be seen on the chemiluminescence blot and tracked using the PARP-1 fluorescent blot for marker reference, given previous observational knowledge of GAPDH.

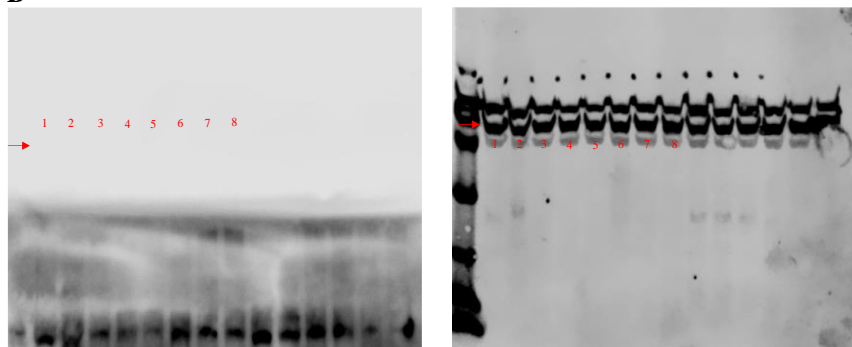
Figure S26. Full representative western blot of intrinsic and extrinsic apoptotic cell death markers in MDA–MB–231. Represented data normalized to GAPDH relative to control (lane 1). A. Bax, B. Bcl2, C. Cytochrome C, D. Procaspase 8, E. Cleaved BID, F. Procaspase 9, G. Procaspase 3 and H. PARP-1. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5)) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (lane 8)) complexes, as well as cisplatin (lane 2) in MDA–MB–231 cells at 72 h compared with control (lane 1).

A**Beclin1****GAPDH**

| Beclin1 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|-----------|------------|------------|------------|---|
| 1 | 0.92112095 | 1.91053049 | 2.58803175 | 3.1497487 | 1.40825406 | 0.93739607 | 0.95917922 | |

**Beclin1 -top
GAPDH -bottom**

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

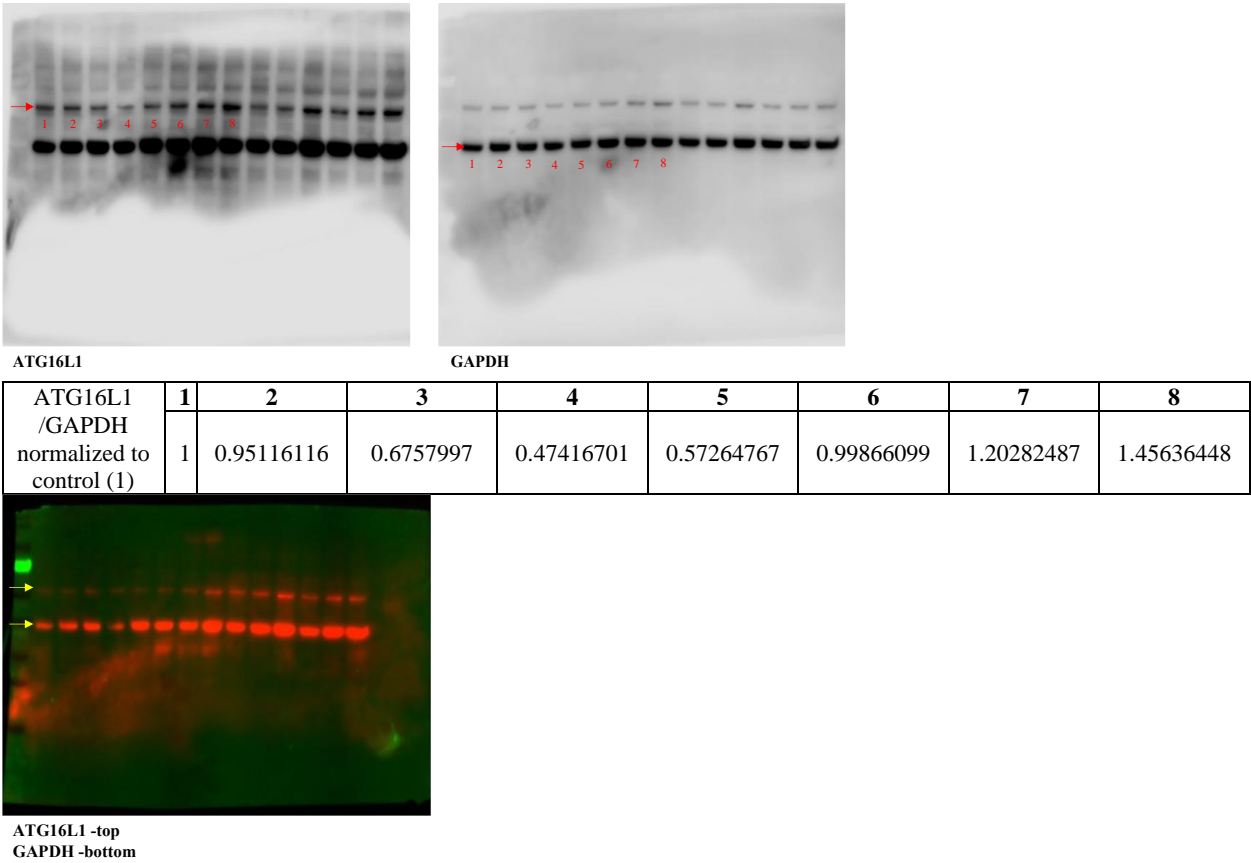
B**APGSL/ATG5****GAPDH**

| ATG5 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|------------|-----------|-----------|------------|------------|------------|------------|---|
| 1 | 0.82158927 | 0.7761884 | 0.5948127 | 0.40117138 | 1.12853361 | 1.06548285 | 0.89236817 | |

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. However, the appearance of the molecular

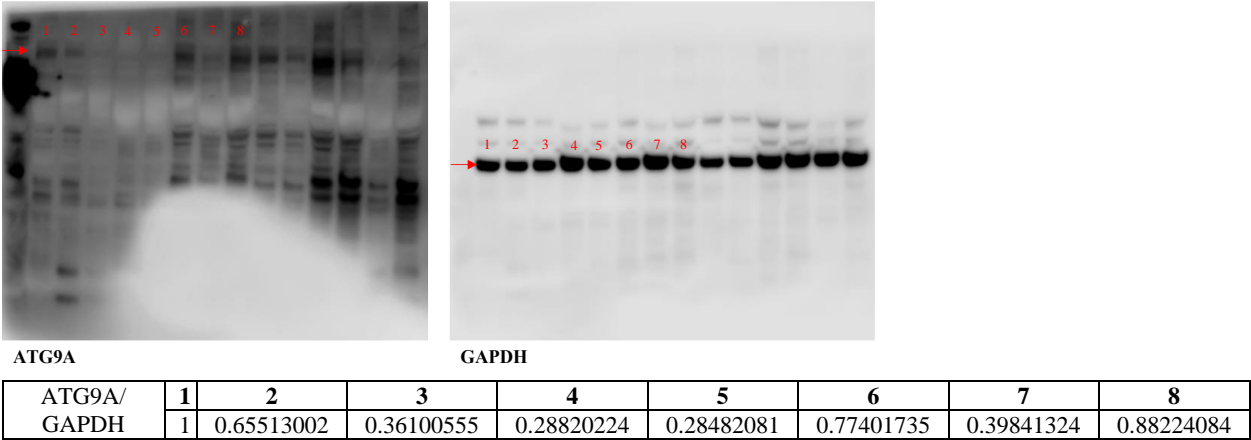
marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) on the chemiluminescence blot in APG5L/ATG5 and GAPDH blots confirm the detected band position. Protein markers were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

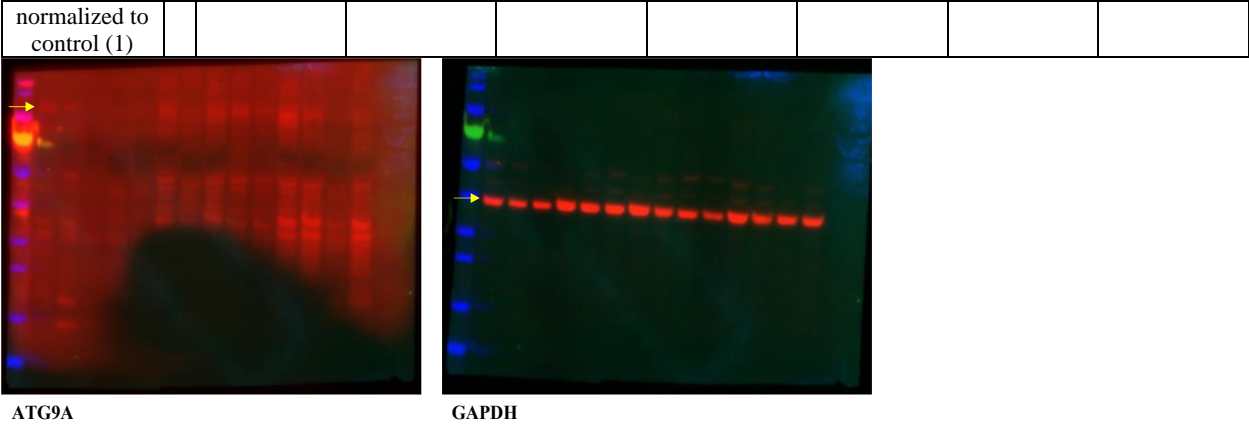
C



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

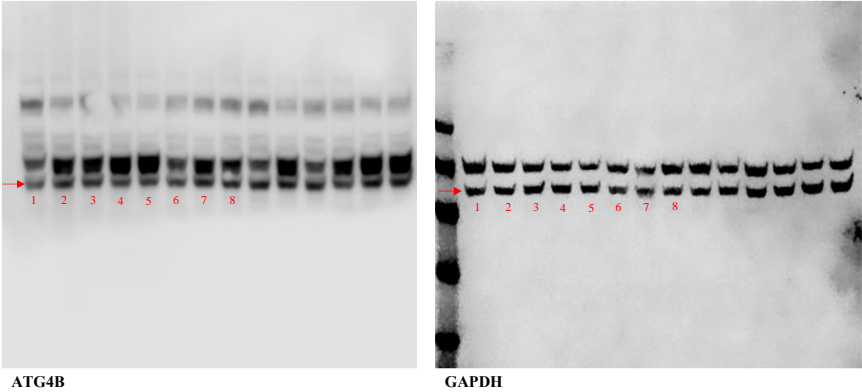
D



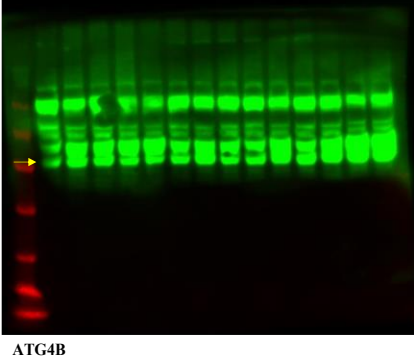


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

E

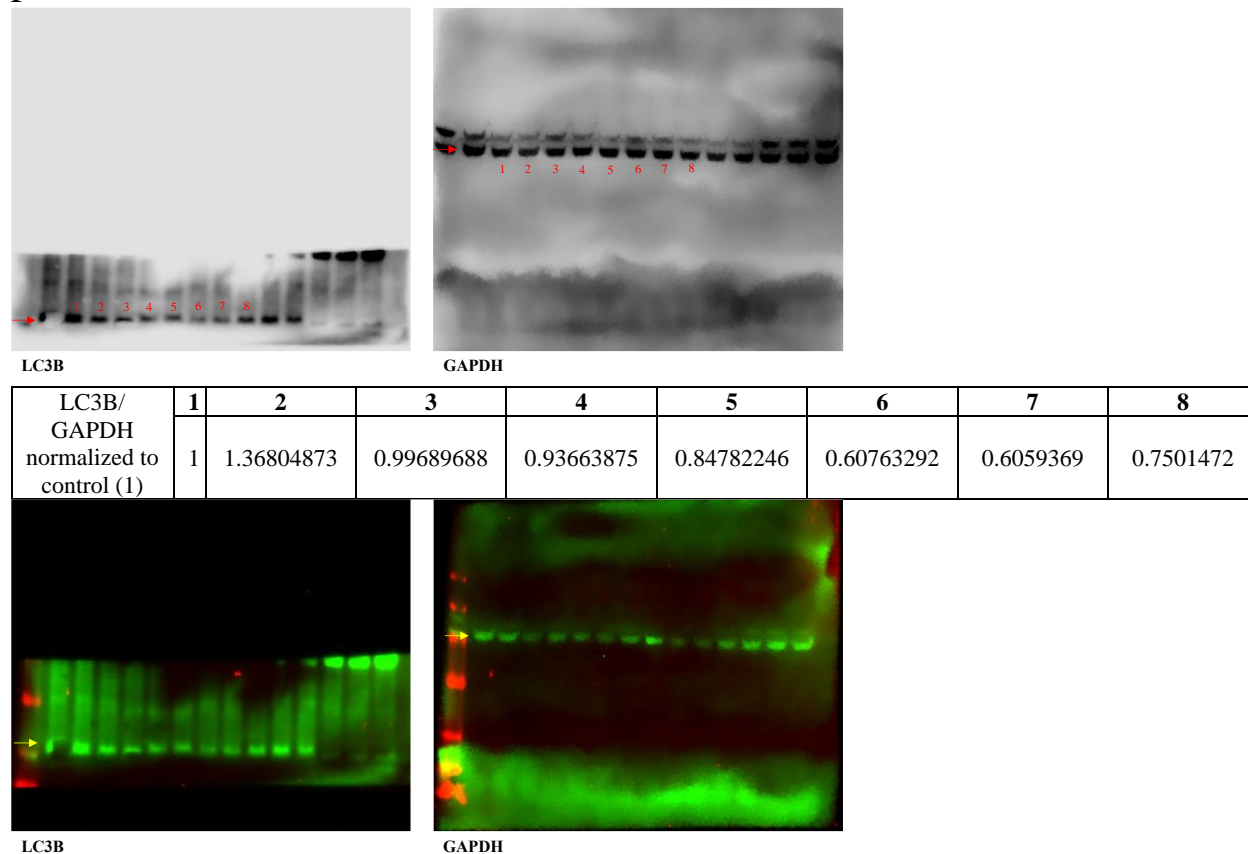


| ATG4B/ GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|------------|------------|------------|------------|------------|-----------|------------|
| | 1 | 1.55113158 | 1.54556964 | 1.83877154 | 2.10690916 | 1.58129772 | 2.7974247 | 2.17850114 |



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. Fluorescent western blot has not been obtained for GAPDH, however, the appearance of the molecular marker in GAPDH chemiluminescence blot is observed.

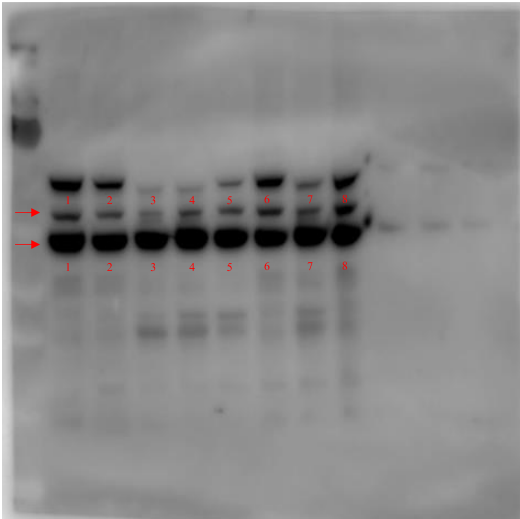
F



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

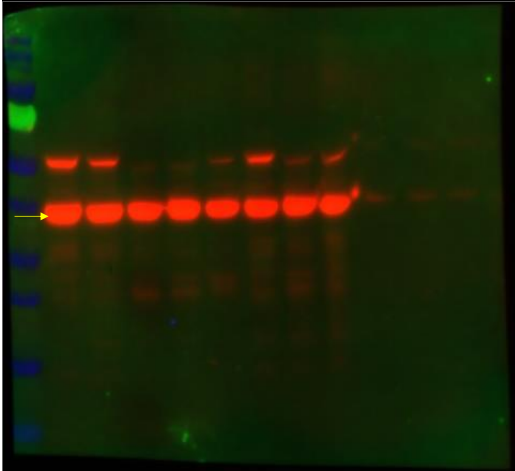
Figure S27. Full representative western blot of autophagy markers in MDA-MB-231. Represented data normalized to GAPDH relative to control (lane 1). A. Beclin1, B. APG5L/ATG5, C. ATG16L1, D. ATG9A, E. ATG4B and F. LC3B. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5)) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (lane 8)) complexes, as well as cisplatin (lane 2) in MDA-MB-231 cells at 72 h compared with control (lane 1).

A



β -actin -top
GAPDH -bottom

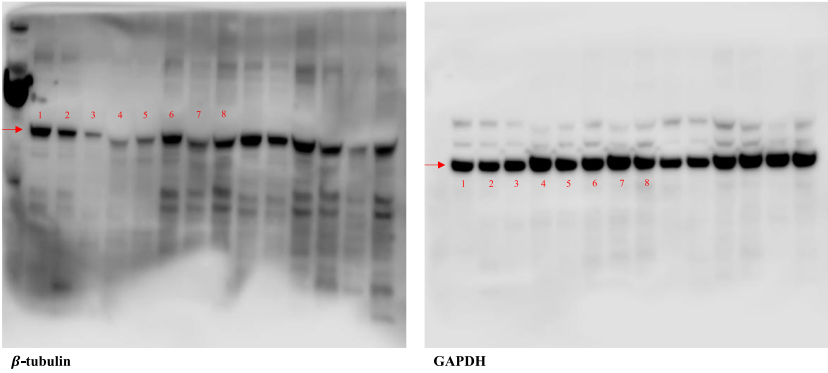
| β -actin/ GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|------------|------------|-----------|------------|------------|------------|------------|---|
| 1 | 0.63447655 | 0.12673082 | 0.2979228 | 0.49425744 | 0.89432236 | 0.29479307 | 1.20892896 | |



GAPDH

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. Fluorescent western blot has not been obtained for β -actin, however, the appearance of the molecular marker in GAPDH chemiluminescence blot is observed. While the top observed protein marker (above GAPDH) is procaspase 9, β -actin can be seen to fall between procaspase 9 and GAPDH on the chemiluminescence blot and accordingly can be estimated and confirmed on the fluorescence blot.

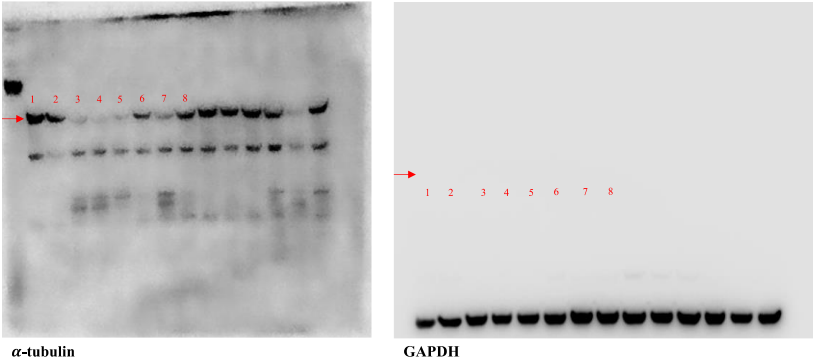
B



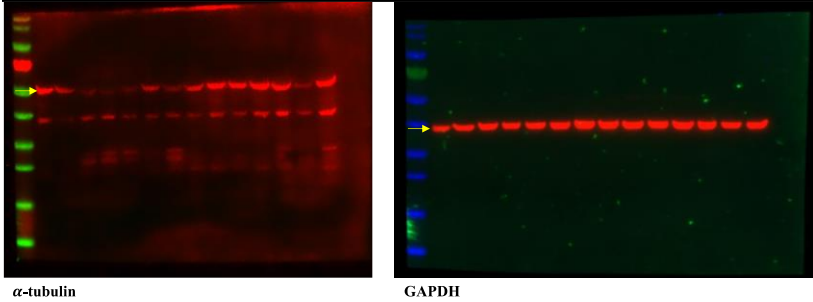
| β -tubulin/ GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|------------|------------|-------------|------------|------------|------------|------------|---|
| 1 | 0.80027005 | 0.23201888 | 0.123819852 | 0.21816259 | 0.81366556 | 0.16113688 | 0.70747627 | |

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. Molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was still loaded to the left of the gel and can be observed in β -tubulin chemiluminescence blot. Protein markers β -tubulin and GAPDH were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

C

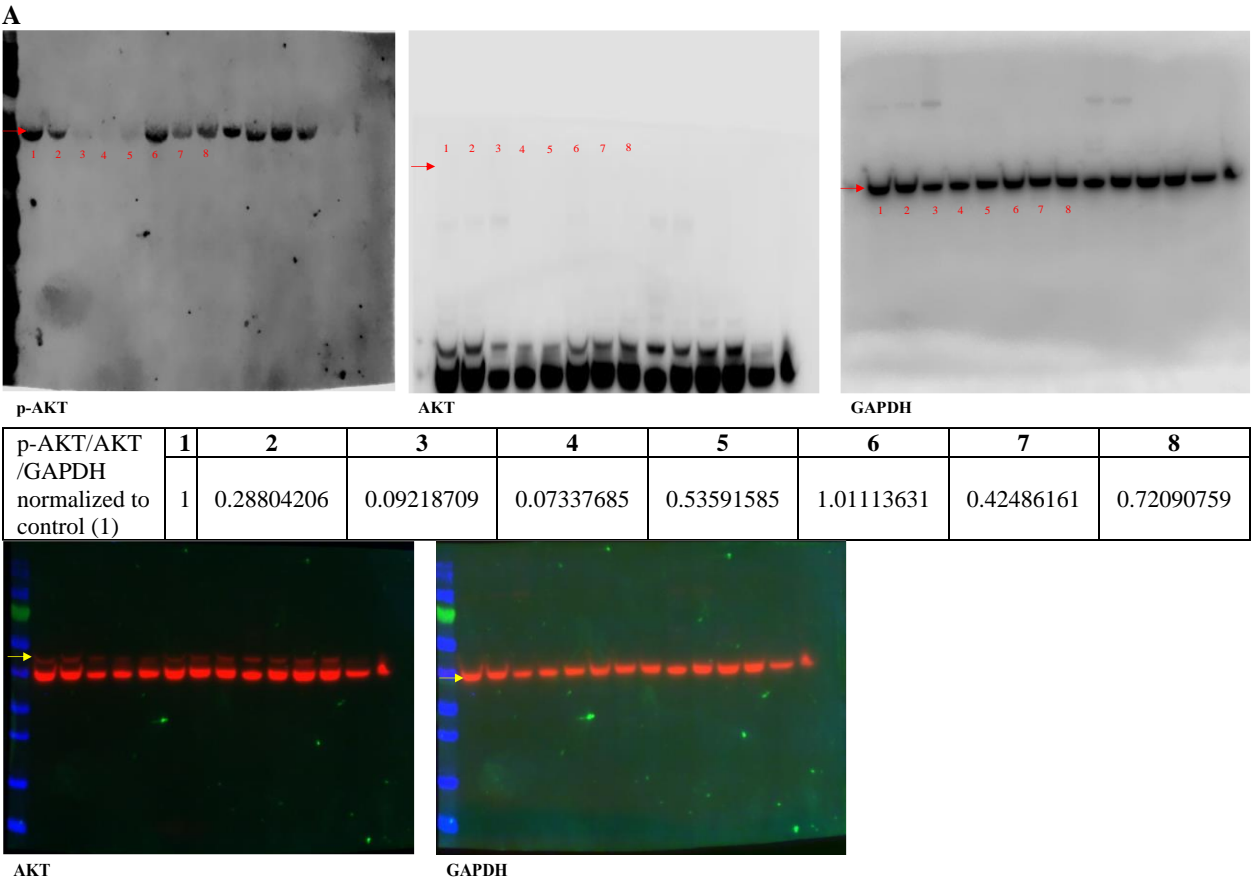


| α -tubulin/ GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|-------------|-------------|------------|------------|------------|---|
| 1 | 0.82006592 | 0.09086078 | 0.067885741 | 0.049057164 | 0.40539325 | 0.15181101 | 0.57191859 | |

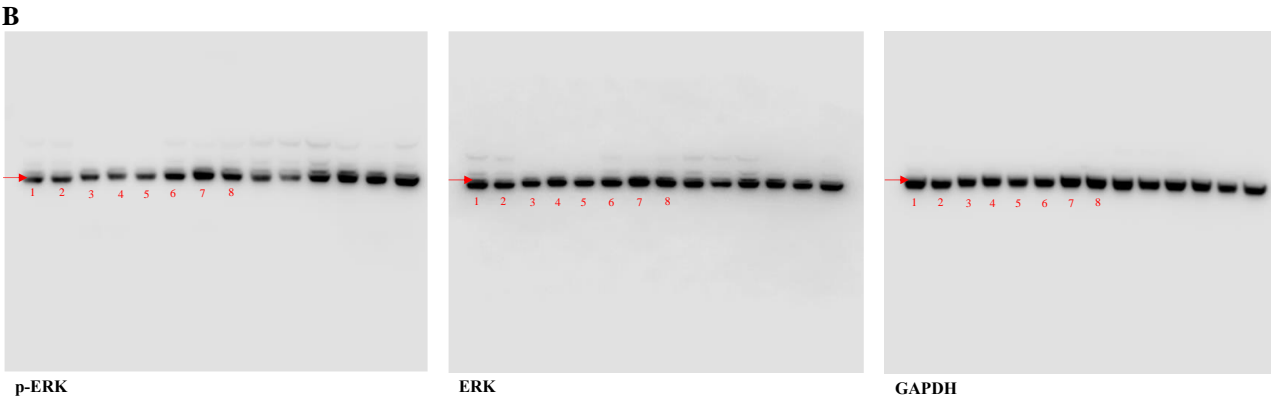


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

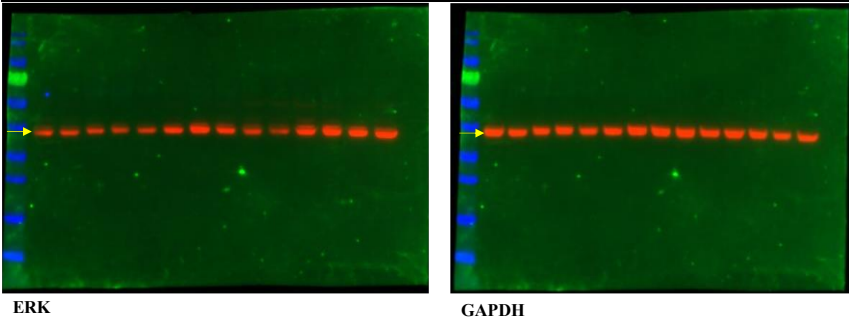
Figure S28. Full representative western blot of microtubule cytoskeleton protein markers in HT29. Represented data normalized to GAPDH relative to control (lane 1). A. β -actin. B. β -tubulin C. α -tubulin. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5)) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (lane 8)) complexes, as well as cisplatin (lane 2) in HT29 cells at 72 h compared with control (lane 1).



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of AKT and GAPDH with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. p-AKT molecular marker can be seen on the chemiluminescence blot and tracked using the AKT fluorescent blot, given p-AKT will fall at the same molecular weight.

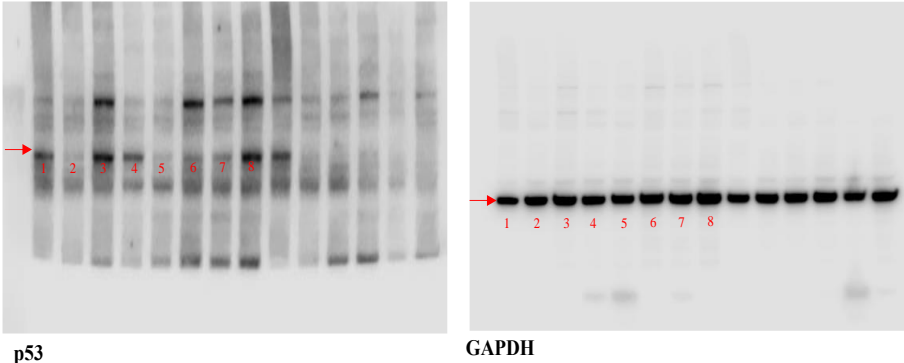


| p-ERK/ERK /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|-----------|------------|------------|------------|------------|-----------|------------|---|
| 1 | 1.1248135 | 0.95088206 | 0.70761589 | 0.83252105 | 1.22923095 | 1.2973034 | 1.28018524 | |

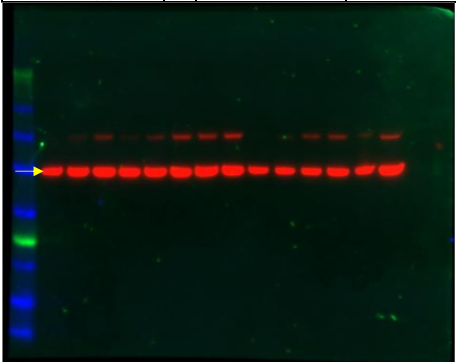


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of ERK and GAPDH with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. p-ERK molecular marker can be seen on the chemiluminescence blot and tracked using the ERK fluorescent blot, given p-ERK will fall at the same molecular weight.

C



| p53/GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|------------|------------|-----------|---|
| 1 | 0.95073342 | 4.79055651 | 1.27530306 | 1.01424683 | 4.54269273 | 3.34882757 | 5.0467805 | |



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot of GAPDH with molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. p53 molecular marker can be tracked on the

chemiluminescence blot using the GAPDH fluorescent blot, given p53 will fall above GAPDH as slightly detected on the fluorescent blot.

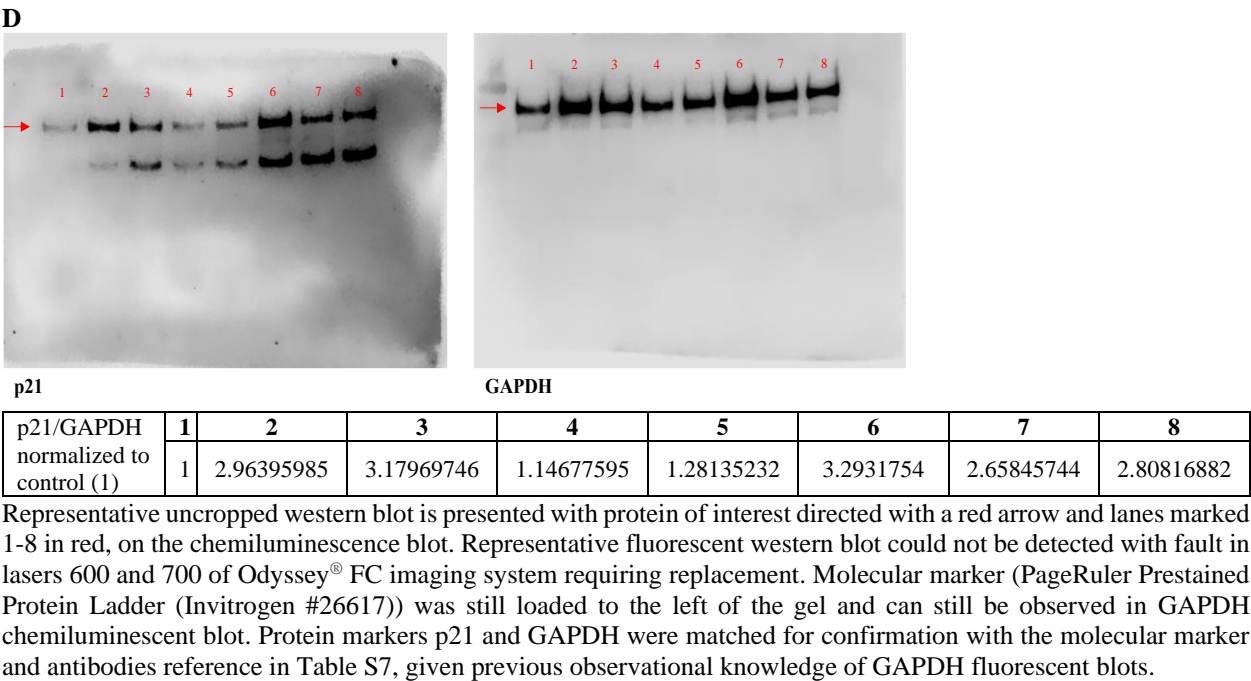
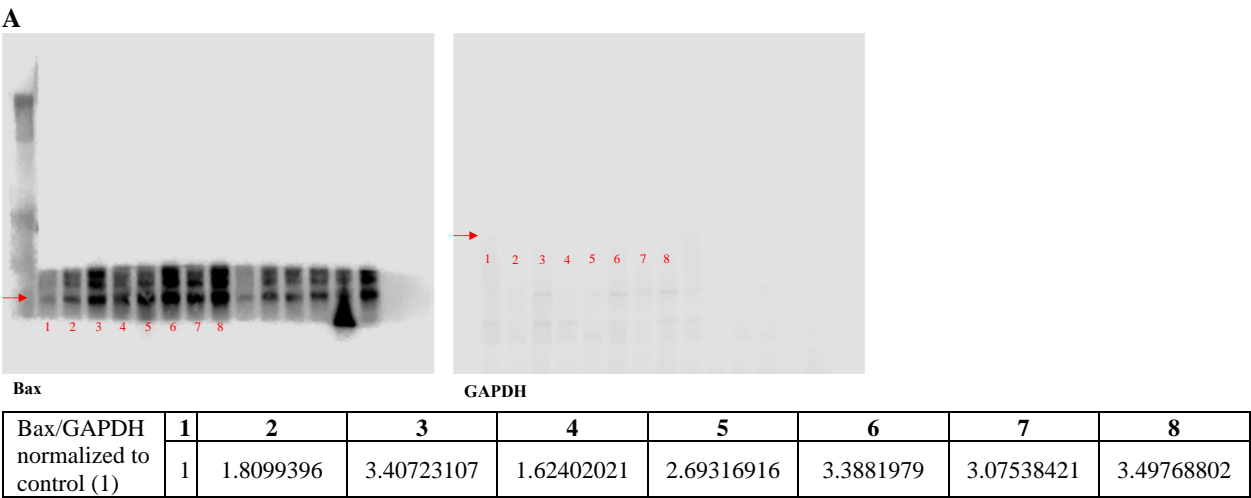
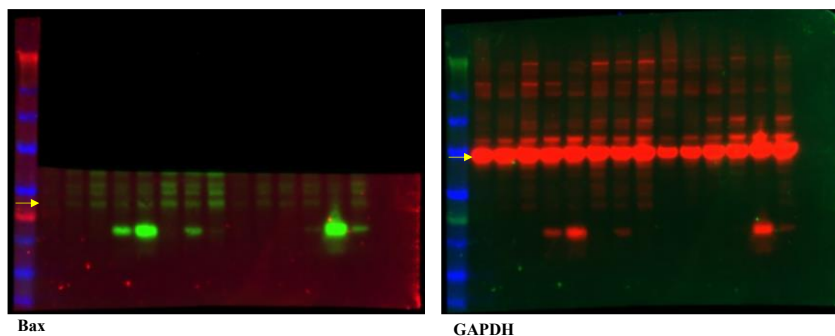


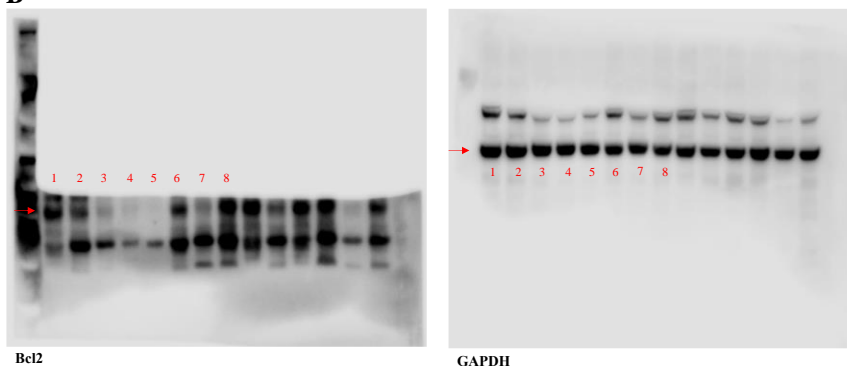
Figure S29. Full representative western blot of cell proliferation protein markers in HT29. Represented data normalized to GAPDH relative to control (lane 1). A.p-AKT/AKT, B. p-ERK/ERK, C. p53 and D. p21. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5)) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (lane 8)) complexes, as well as cisplatin (lane 2) in HT29 cells at 72 h compared with control (lane 1).



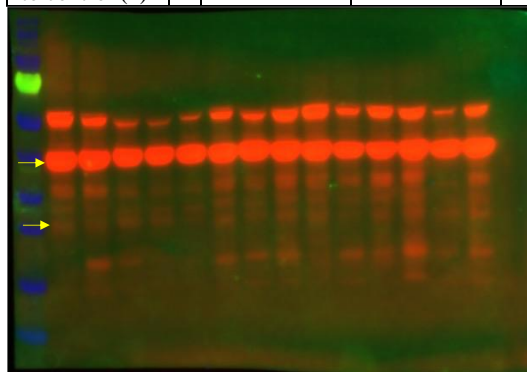


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

B

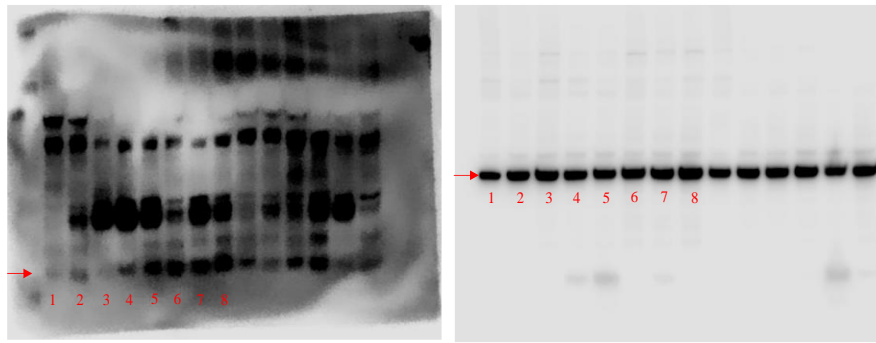


| Bcl2/GAPD H normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|------------|------------|------------|------------|------------|------------|------------|
| | 1 | 0.56594514 | 0.28723981 | 0.09591015 | 0.04575617 | 1.19447984 | 0.43808048 | 1.03376818 |



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

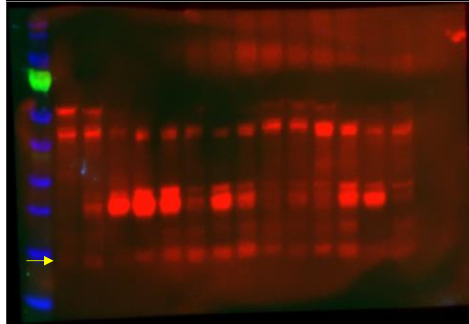
C



Cyt C

GAPDH

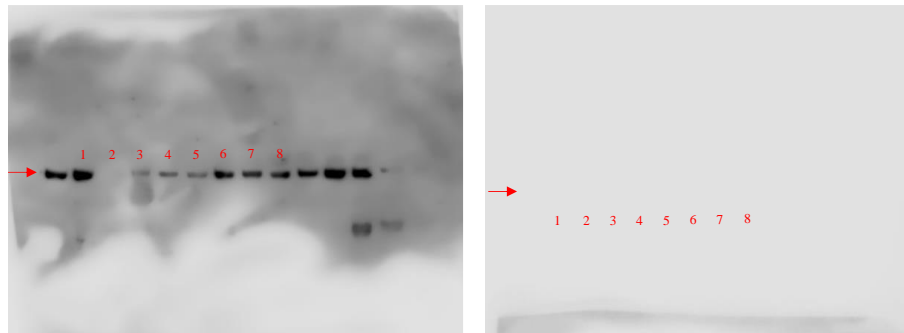
| Cyt C /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|-----------|-----------|------------|---|
| 1 | 2.41871468 | 1.69154667 | 3.74101488 | 6.70558815 | 7.0905811 | 6.1838904 | 7.66289382 | |



Cyt C

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

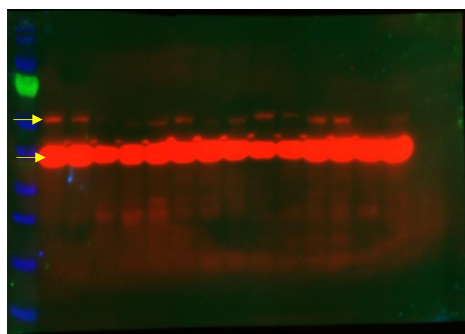
D



Procaspase 8

GAPDH

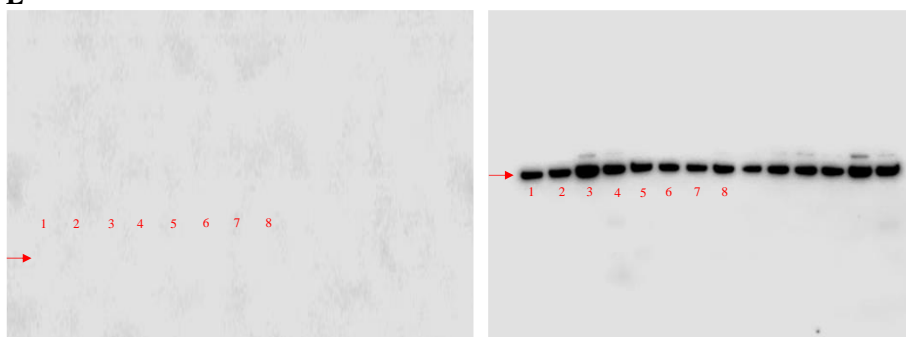
| Procaspase 8 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|------------|------------|------------|------------|------------|------------|------------|---|
| 1 | 0.00402925 | 0.16334698 | 0.29343619 | 0.25047865 | 0.94737332 | 0.43718733 | 0.40350856 | |



Procaspase 8 -top
GAPDH -bottom

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

E



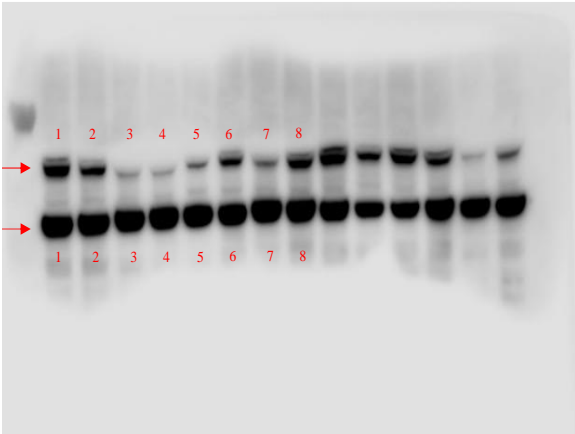
Cleaved BID

GAPDH

| Cleaved BID /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|------------|------------|------------|------------|-----------|------------|------------|
| | 1 | 2.52691567 | 1.61137382 | 1.68964872 | 2.82106515 | 2.7111347 | 2.71169915 | 2.65145596 |

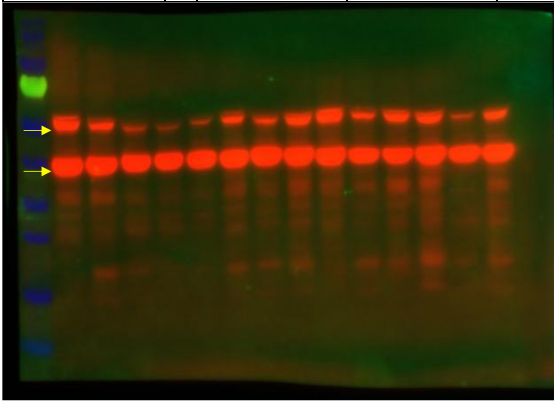
Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. Molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was still loaded to the left of the gel. Protein markers cleaved BID and GAPDH were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

F



Procaspase 9 -top
GAPDH -bottom

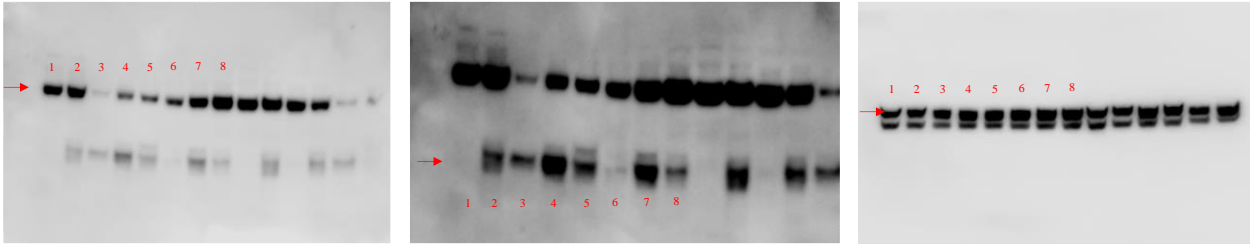
| Procaspase 9 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|------------|------------|------------|---|
| 1 | 0.63876707 | 0.19809129 | 0.13933932 | 0.27172406 | 0.59273615 | 0.20260478 | 0.49638347 | |



Procaspase 9 -top
GAPDH -bottom

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

G



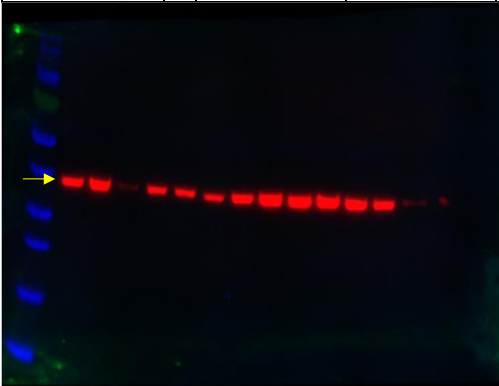
Procaspase 3

Cleaved Caspase 3

GAPDH

| Procaspase 3 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------|------------|------------|------------|------------|------------|------------|---|
| 1 | 1.20012485 | 0.48743248 | 0.76777954 | 0.89362428 | 1.14123935 | 1.23755226 | 1.29742903 | |

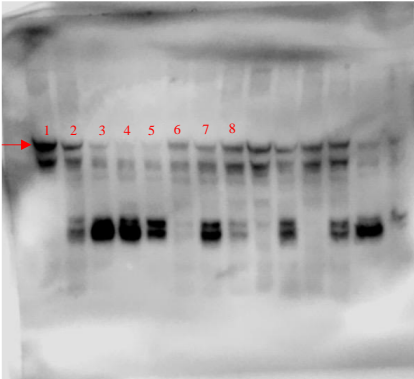
| | | | | | | | | |
|--|---|------------|------------|------------|------------|------------|-----------|------------|
| Cleaved caspase 3 /GAPDH normalized to control (1) | 1 | 11.8149621 | 17.4647582 | 10.8813144 | 5.09893053 | 1.01684366 | 7.8873937 | 1.74578195 |
|--|---|------------|------------|------------|------------|------------|-----------|------------|



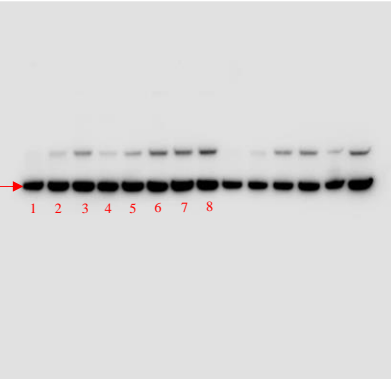
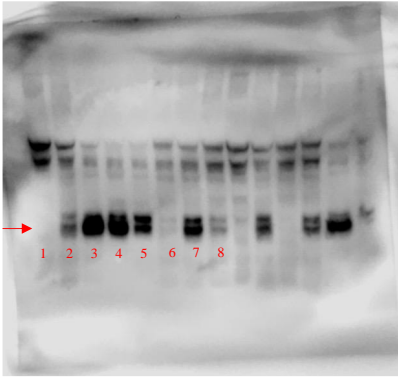
Procaspase 3

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. While cleaved caspase 3 and GAPDH were not detected in the fluorescent blot, they were both manually confirmed and estimated with reference to the procaspase 3 and known antibodies reference in Table S7.

H



PARP-1

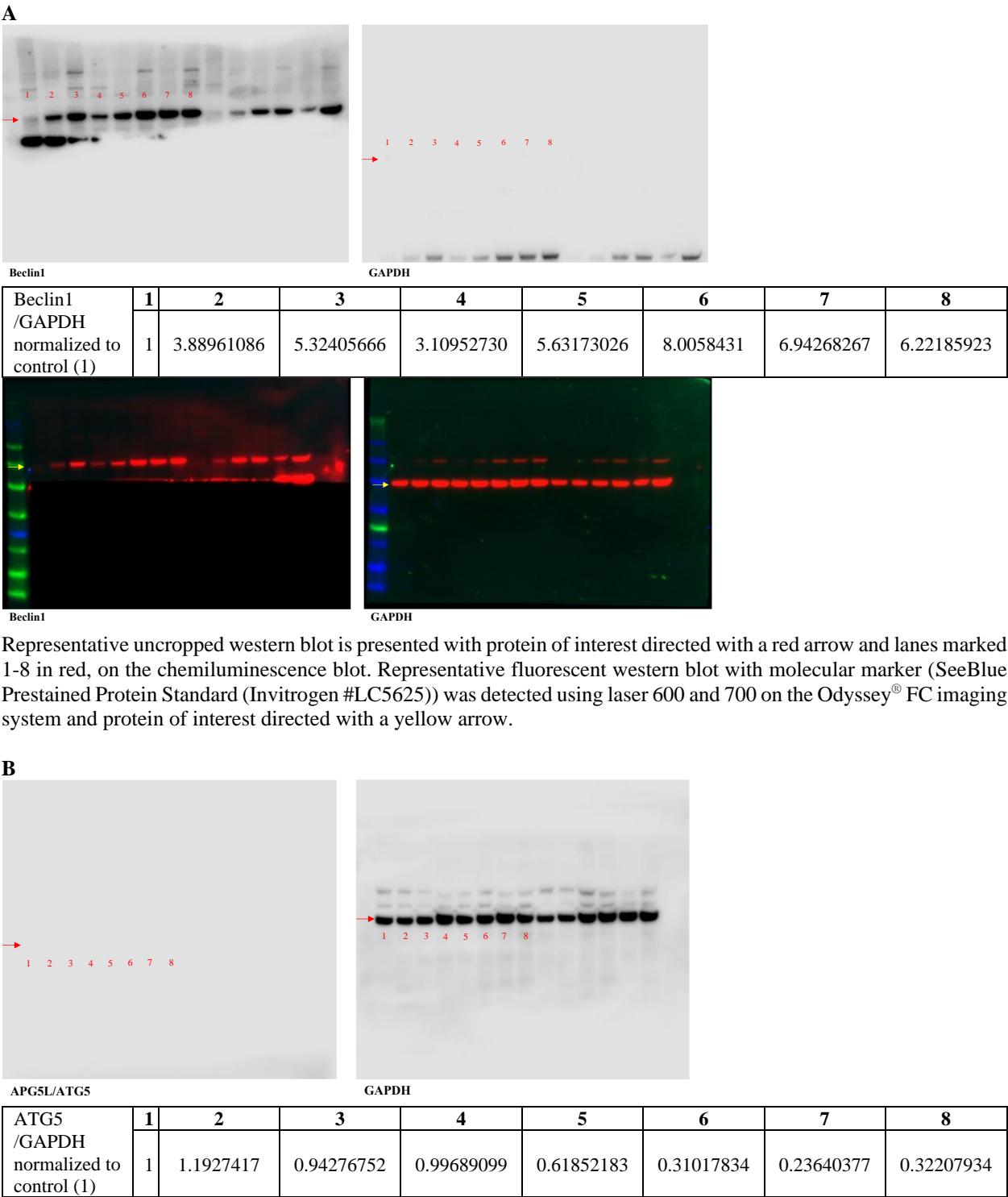


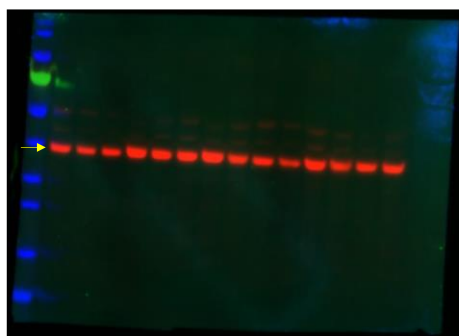
GAPDH

| | | | | | | | | |
|---|---|------------|------------|------------|------------|------------|------------|------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| PARP-1 /GAPDH normalized to control (1) | 1 | 0.82364919 | 0.26115596 | 0.25541081 | 0.31700455 | 0.64751417 | 0.62066593 | 0.71718936 |
| Cleaved PARP-1 /GAPDH normalized to control (1) | 1 | 3.4836068 | 8.49620931 | 11.420335 | 7.0644866 | 2.23930102 | 5.7612047 | 2.7242088 |

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. Molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was still loaded to the left of the gel. Protein markers PARP-1 and GAPDH were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

Figure S30. Full representative western blot of intrinsic and extrinsic apoptotic cell death markers in HT29. Represented data normalized to GAPDH relative to control (lane 1). A. Bax, B. Bcl2, C. Cyt C, D.Procaspase 8, E. Cleaved BID, F. Procaspase 9, G. Procaspase 3 and H. PARP-1. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5)) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (lane 8)) complexes, as well as cisplatin (lane 2) in HT29 cells at 72 h compared with control (lane 1).

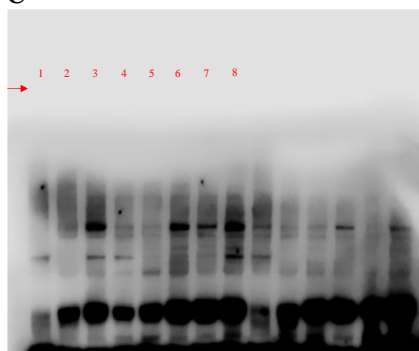




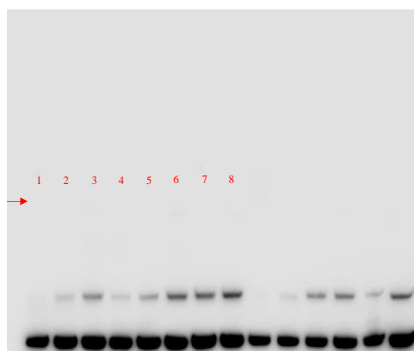
GAPDH

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow. While APG5/ATG5 fluorescent blot was not detected, its fluorescent bands fall at the same level as GAPDH. We observed two band rows above the APG5/ATG5 in the chemiluminescent blot and the same bands can be seen above GAPDH in the fluorescence blot. This confirms the correct observation of APG5/ATG5 at its correct molecular marker.

C



ATG16L1

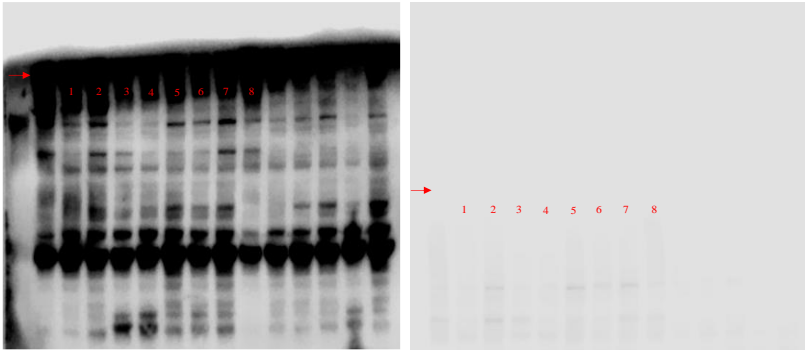


GAPDH

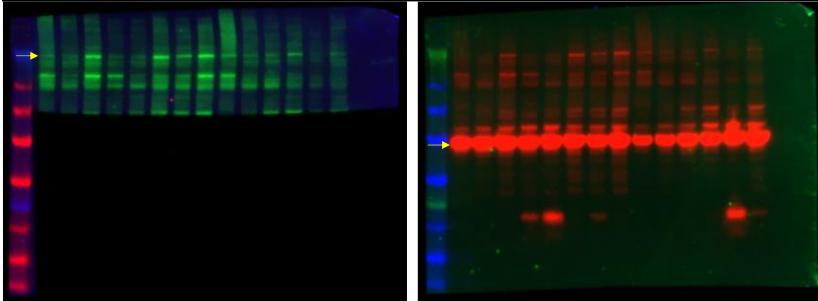
| | | | | | | | | |
|---|---|----------|---------|-----------|----------|----------|----------|----------|
| ATG16L1 /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | 1 | 1.173333 | 3.84385 | 1.2845093 | 1.309065 | 4.092945 | 2.006203 | 4.523285 |

Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot could not be detected with fault in lasers 600 and 700 of Odyssey[®] FC imaging system requiring replacement. Molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was still loaded to the left of the gel. Protein markers ATG16L1 and GAPDH were matched for confirmation with the molecular marker and antibodies reference in Table S7, given previous observational knowledge of GAPDH fluorescent blots.

D

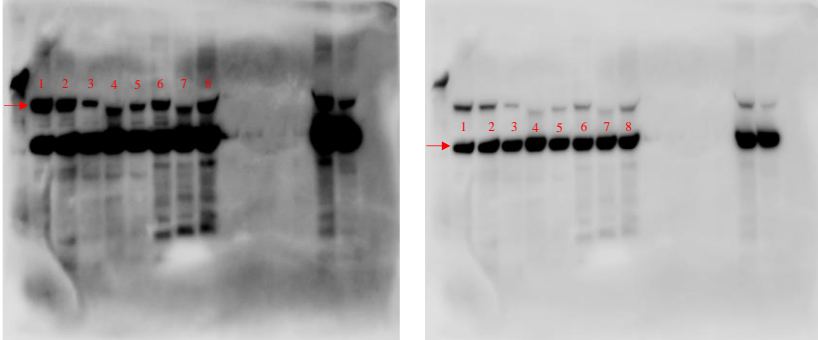


| ATG9A | | GAPDH | | | | | | |
|--|------------|------------|------------|------------|------------|------------|------------|---|
| ATG9A /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 2.21216263 | 0.46496635 | 0.44859831 | 1.51595621 | 1.20296996 | 2.33441205 | 0.90437915 | |

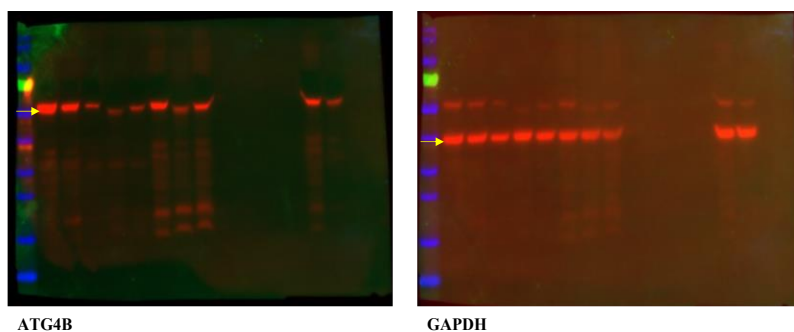


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (SeeBlue Prestained Protein Standard (Invitrogen #LC5625)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

E

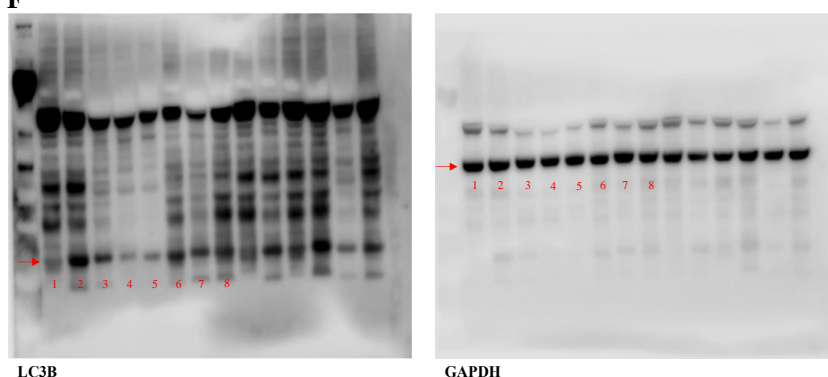


| ATG4B | | GAPDH | | | | | | |
|--|------------|------------|------------|------------|------------|-----------|------------|---|
| ATG4B /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 1.10064684 | 0.68440434 | 0.73063446 | 0.71064903 | 0.90489986 | 0.5921638 | 0.92231009 | |

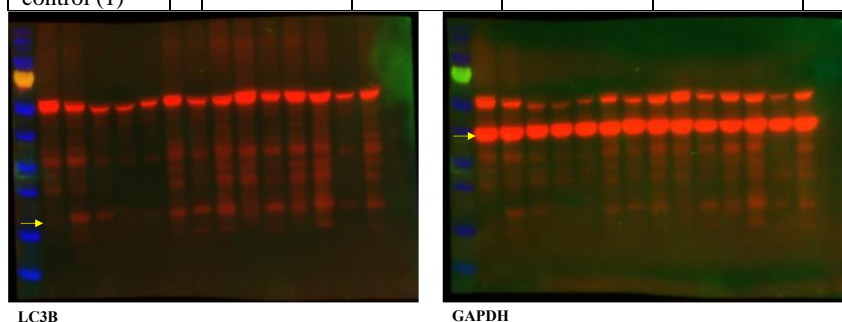


Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

F



| LC3B /GAPDH normalized to control (1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--|---|------------|------------|------------|------------|------------|------------|------------|
| | 1 | 3.23330908 | 2.12095134 | 1.20741902 | 1.04367288 | 2.23203726 | 1.71266931 | 1.97638983 |



Representative uncropped western blot is presented with protein of interest directed with a red arrow and lanes marked 1-8 in red, on the chemiluminescence blot. Representative fluorescent western blot with molecular marker (PageRuler Prestained Protein Ladder (Invitrogen #26617)) was detected using laser 600 and 700 on the Odyssey[®] FC imaging system and protein of interest directed with a yellow arrow.

Figure S31. Full representative western blot of autophagy markers in HT29. Represented data normalized to GAPDH relative to control (lane 1). A. Beclin1, B. APG5L/ATG5, C. ATG16L1, D. ATG9A, E. ATG4B and F. LC3B. Protein expression upon treatment with platinum(II) (**Pt^{II}PHENSS** (lane 3), **Pt^{II}5MESS** (lane 4) and **Pt^{II}56MESS** (lane 5)) and platinum(IV) (**Pt^{IV}PHENSS(OH)₂** (lane 6), **Pt^{IV}5MESS(OH)₂** (lane 7) and **Pt^{IV}56MESS(OH)₂** (lane 8)) complexes, as well as cisplatin (lane 2) in HT29 cells at 72 h compared with control (lane 1).

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