Supporting Information

Cell-Selective Cytotoxicity of a Fluorescent Rhodium Metalloinsertor Conjugate Results from Irreversible DNA Damage at Base Pair Mismatches

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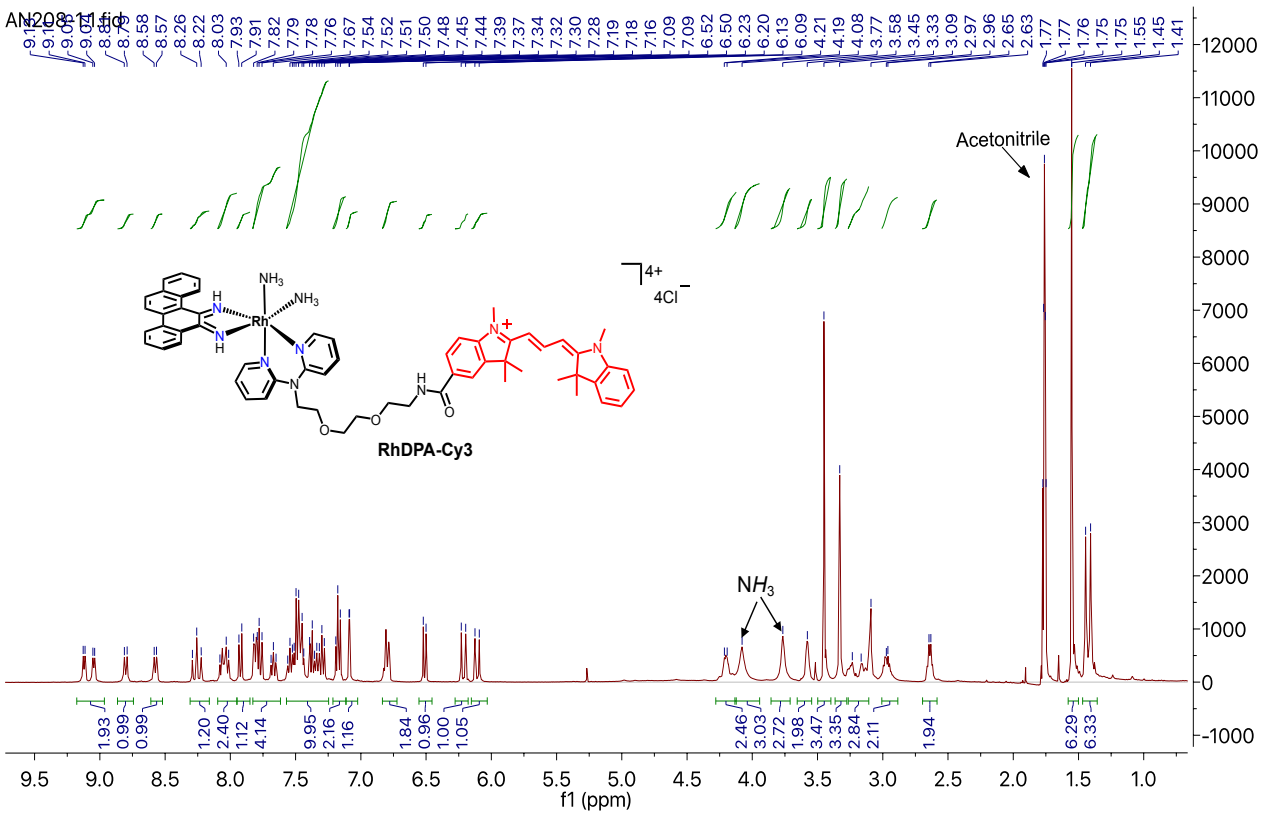

Figure S1. [1]H NMR spectrum of RhDPA-Cy3. The spectrum was collected in deuterated acetonitrile at room temperature.

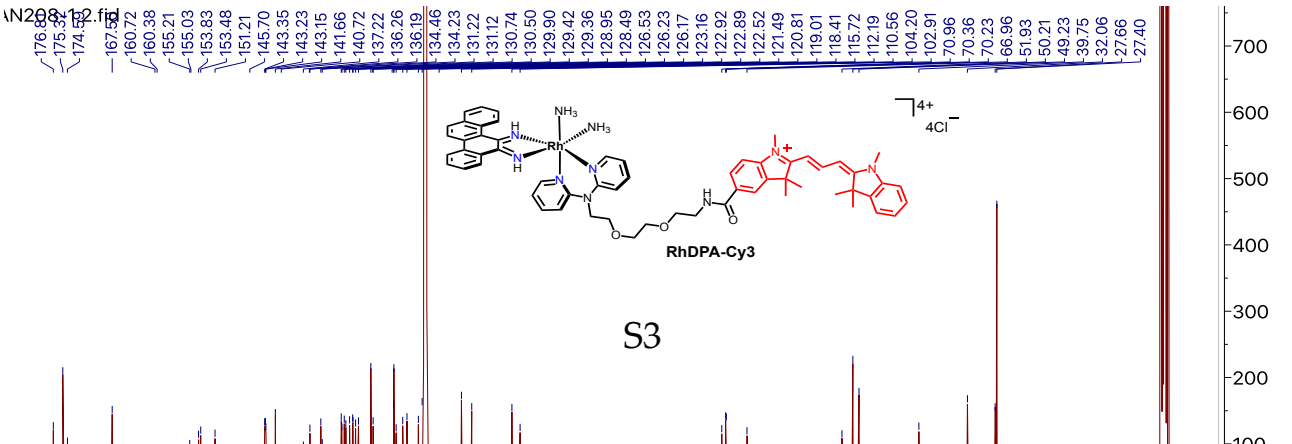

Figure S2. ^{13}C NMR spectrum of RhDPA-Cy3. The spectrum was collected in deuterated acetonitrile at room temperature

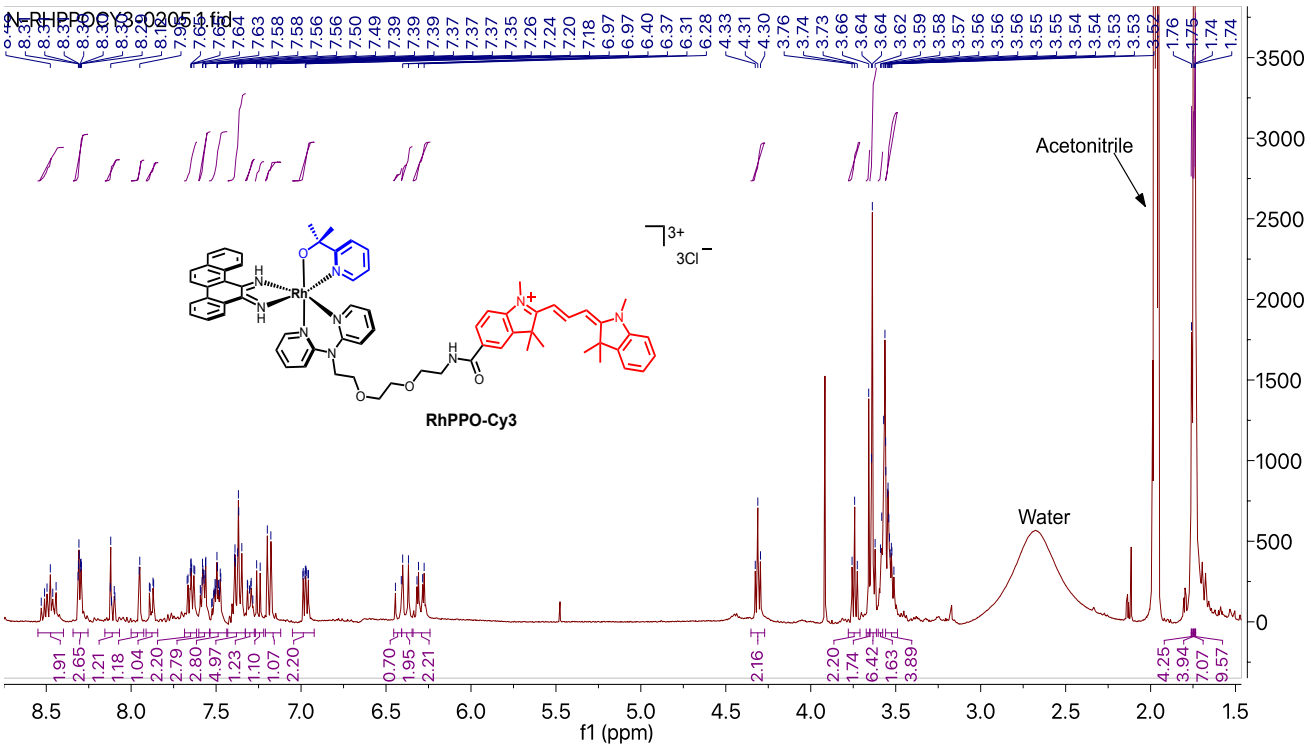

Figure S3. ^{13}H NMR spectrum of RhPPO-Cy3. The spectrum was collected in deuterated acetonitrile at room temperature. The compound was obtained as a diastereomeric mixture and characterized as such.

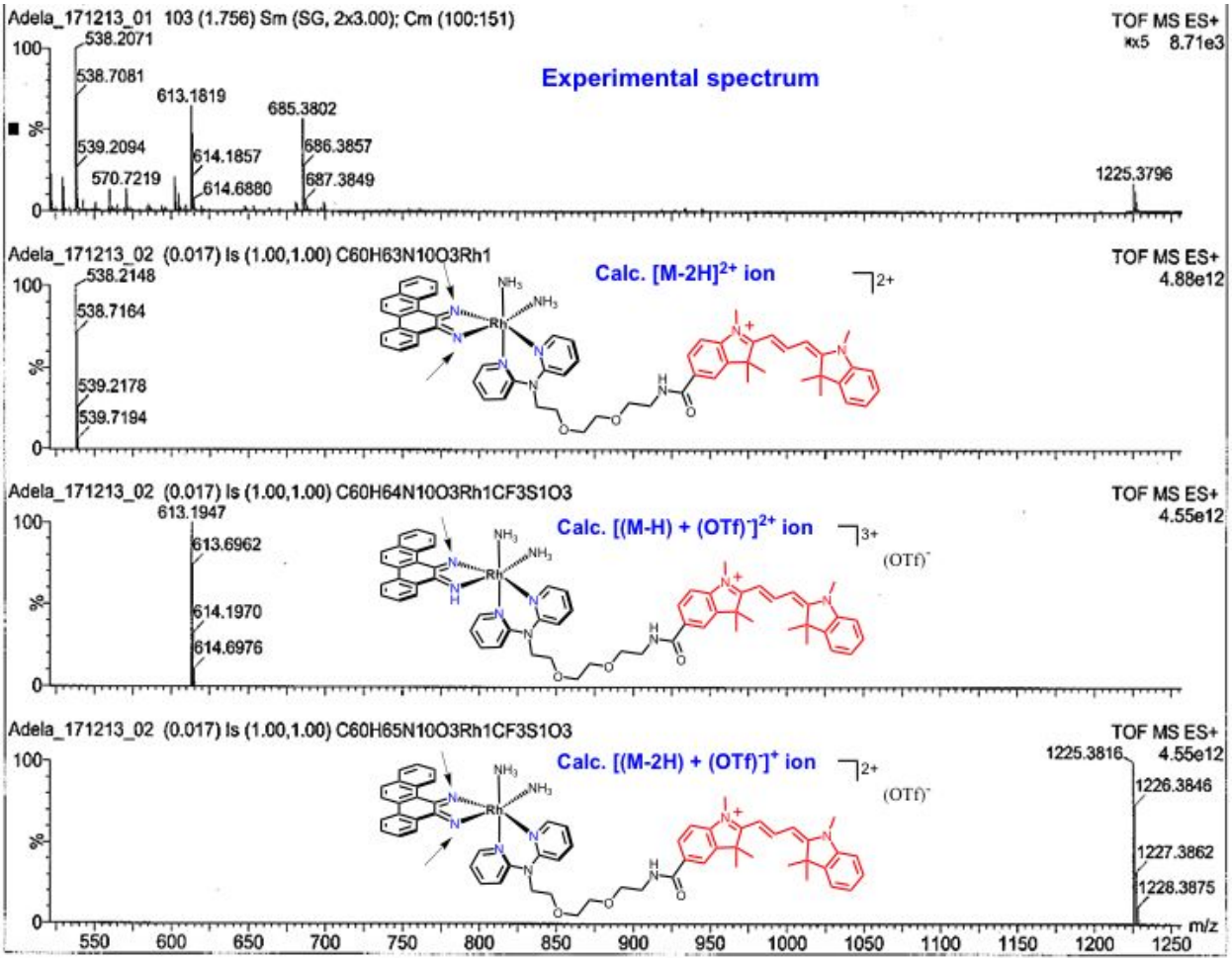

Figure S4. TOF-MS ES+ characterization of RhDPA-Cy3. (Upper) Experimental spectrum of RhDPA-Cy3; (Lower) Calculated spectra for *m*/*z* 538.2; 613.1; 1225.3. When the spectrum was recorded, the compound was not anion exchanged to its chloride salt. Therefore, we observe the triflate salt in the experimental spectrum.

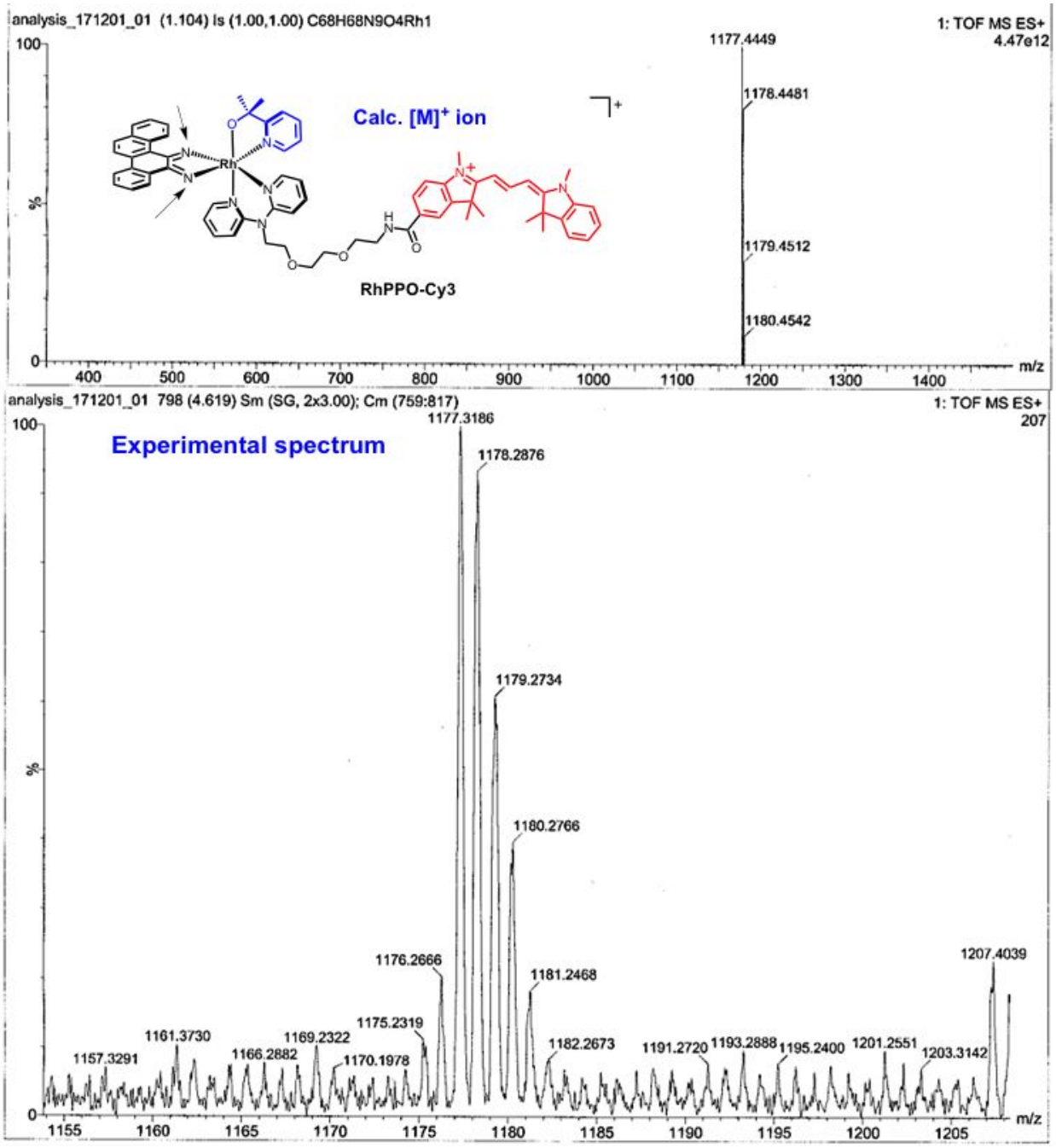

Figure S5. TOF-MS ES+ characterization of RhPPO-Cy3. (Upper) Calculated *m*/*z* = 1177.4449 for [M – 2H]+; (Lower) Experimental spectrum with *m*/*z* = 1177.3186. [M – 2H]$^{+}$ is assigned to the complex with both imines deprotonated, indicated with an arrow in the chemical structure.

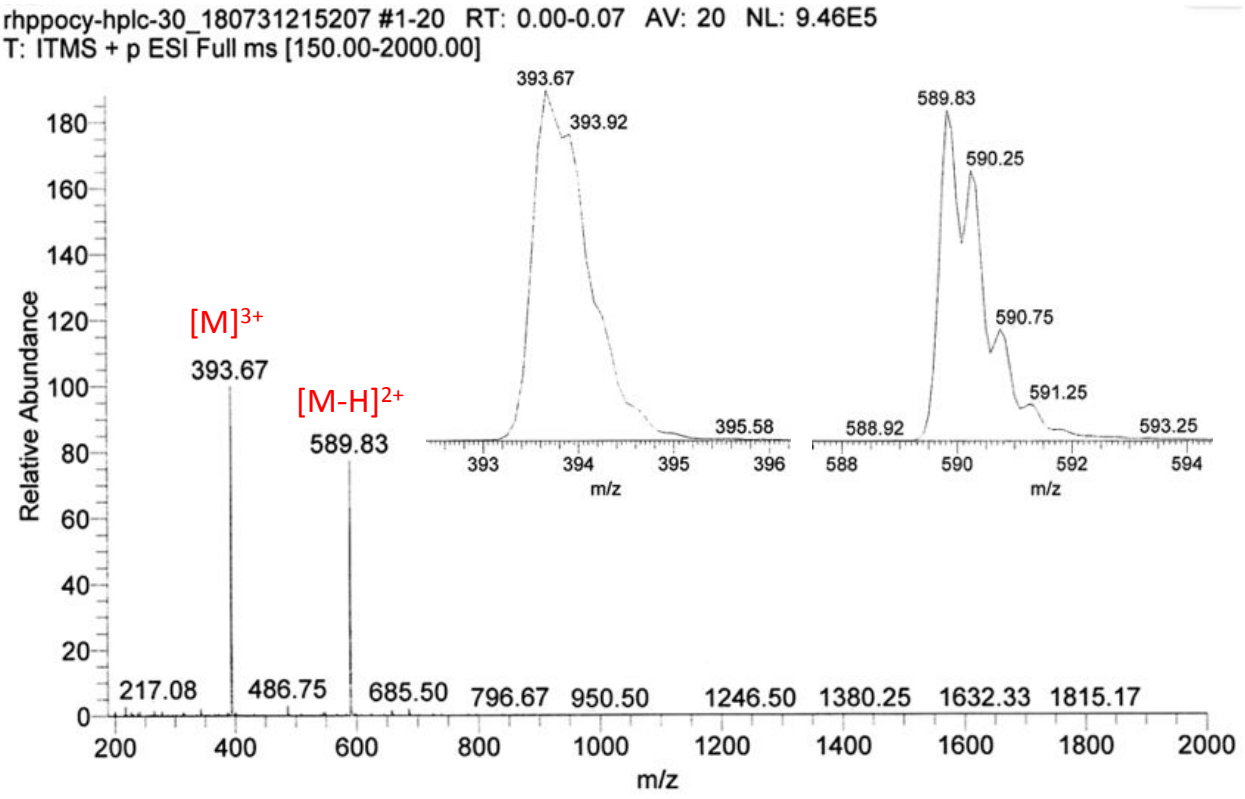

Figure S6. MS-ESI+ characterization of RhPPO-Cy3 using an LTQ spectrometer. The spectrum was recorded after HPLC purification of RhPPO-Cy3. Insets are zooms of *m*/*z* 393.67 and *m*/*z* 589.83 peak; *m*/*z* 393.67 (100%) corresponds to $[M]^{3+}$ (both imines on chrysi ligand are protonated); *m*/*z* 589.83 (77%) corresponds to $[M - H]^{2+}$ (only one imine is protonated).

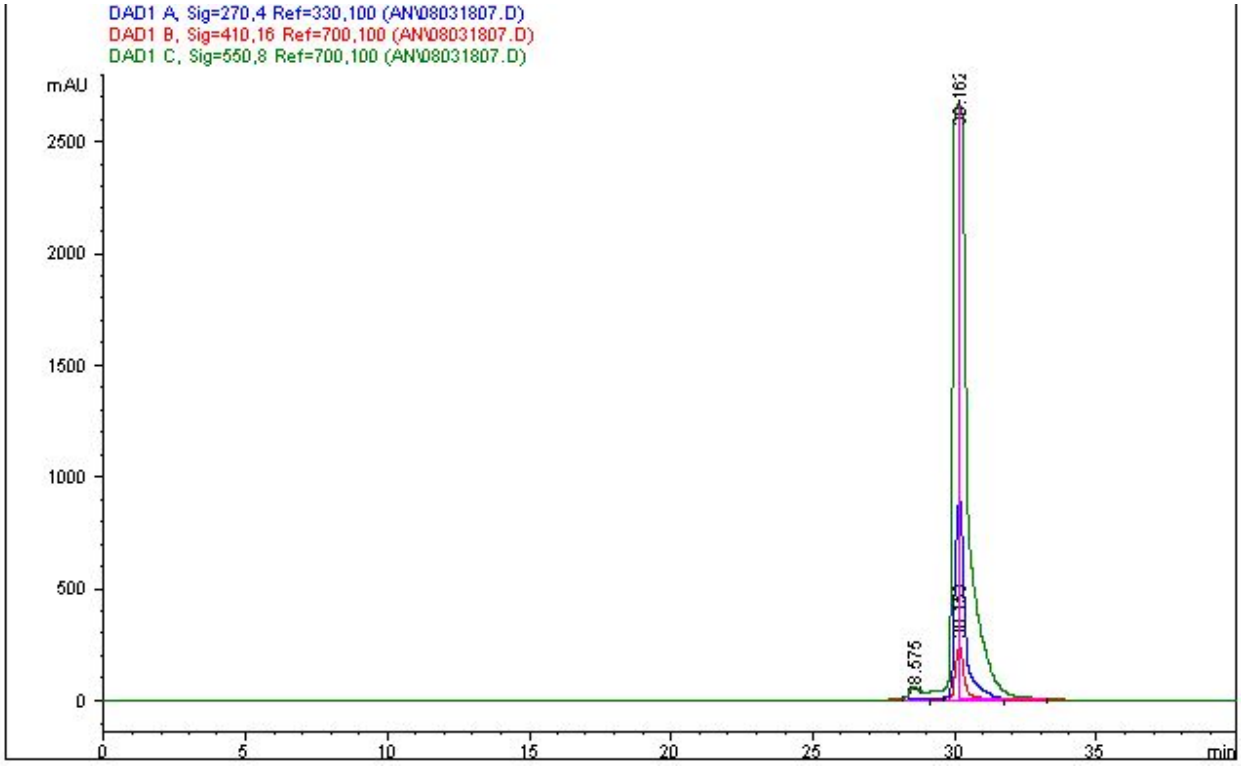

Figure S7. HPLC trace of RhPPO-Cy3 after purification. The HPLC purification was followed using three different wavelengths: at 270 nm, 410 nm (marking the absorption of rhodium complex center), and 550 nm (marking the absorption of Cy3 dye). Mobile phase: 85/15 to 50/50 to 85/50 (0.1% trifluoroacetic acid in water)/acetonitrile eluting over 40 minutes.

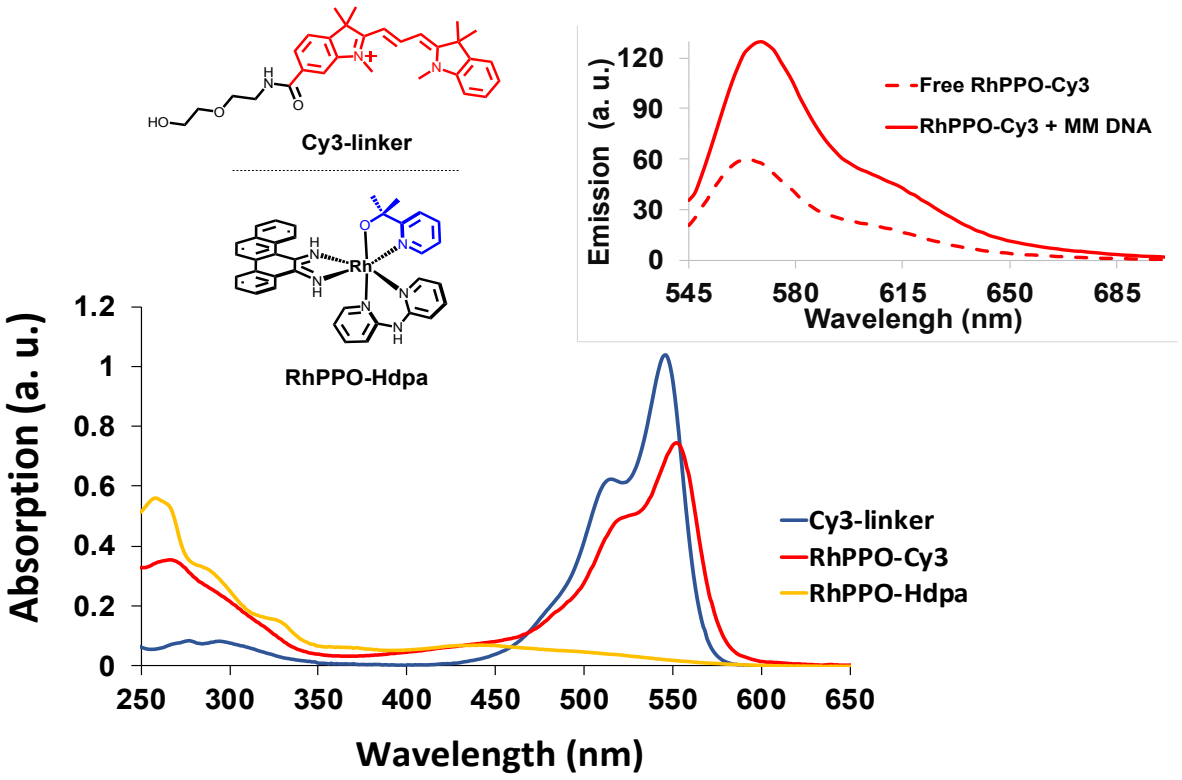

Figure S8. Absorption profile of RhPPO-Cy3, and its models Cy3-linker and RhPPO-Hdpa. Spectra collected in Tris buffer (5mM Tris, 200 mM NaCl, pH 7.4) at room temperature. [RhPPO-Cy3]; [Cy3-linker]; [RhPPO-Hdpa] = 10 μM. Absorption maxima for RhPPO-Cy3 (in Tris 5 mM, 200 mM NaCl, pH 7.4): λ(552nm) ε = 61,000 M^{-1} cm^{-1}; λ(267nm) ε = 28,000 M^{-1} cm^{-1}. Inset: Emission of RhPPO-Cy3 at 1μM free in Tris buffer solution (5mM Tris, 200 mM NaCl, pH 7.4) with λmax = 565 nm, or in the presence of 1

eq. MM dsDNA with λmax = 570 nm. The synthesis and characterization of RhPPO-Hdpa and Cy3-linker have been previously reported.[1,2]

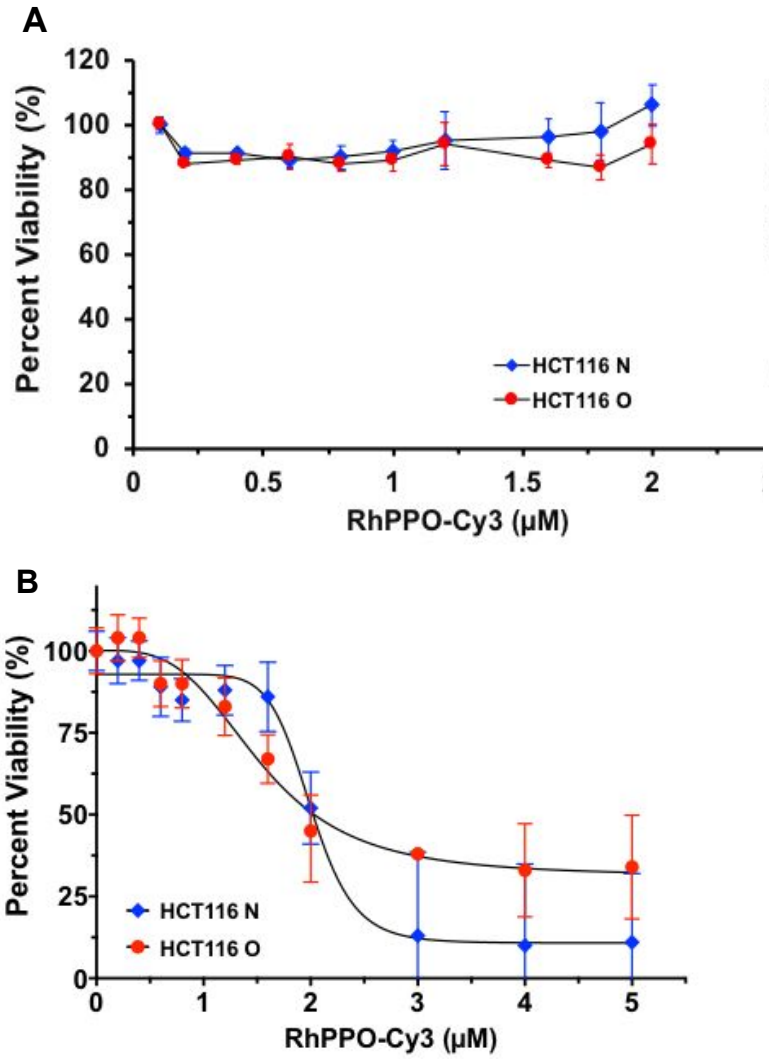

Figure S9. Evaluation of cytotoxicity of RhPPO-Cy3 at different time points. The cells were treated with RhPPO-Cy3 at the concentrations indicated, and then cell viability was assessed with a Cell Titer-glo assay. (A) Dose response curve of cell viability assessed after 24 hours incubation with RhPPO-Cy3; (B) dose response curve assessed after 48 hours incubation with RhPPO-Cy3. Error bars are representative of three replicates.

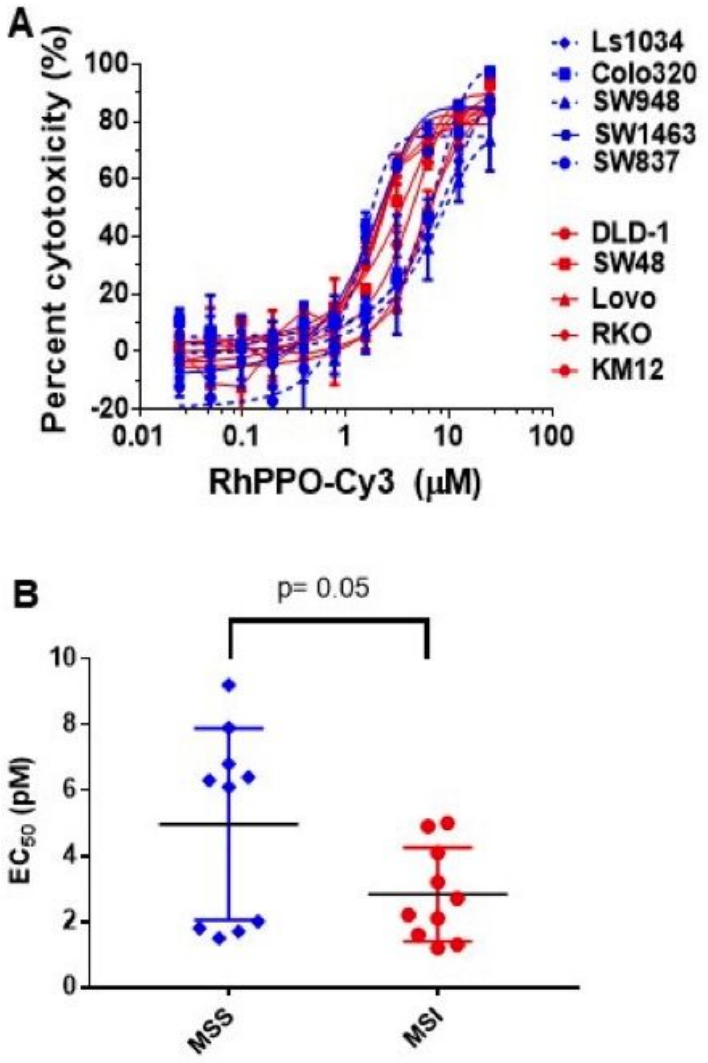

Figure S10. RhPPO-Cy3 shows preferential cytotoxicity for MMR-deficient CRC cell lines. A panel of MMR-deficient and MMR-proficient CRC cell lines was treated with RhPPO-Cy3 in a dose response. After 72 hours incubation, cell viability was assessed with a Cell Titer-glo assay. Duplicate samples were analyzed in each experiment. (A) Dose response curves from a representative experiment are shown. In blue are MSS cell lines; in red are MSI cell lines. (B) Comparison of EC_{50} values for the MSS and MSI cell lines.

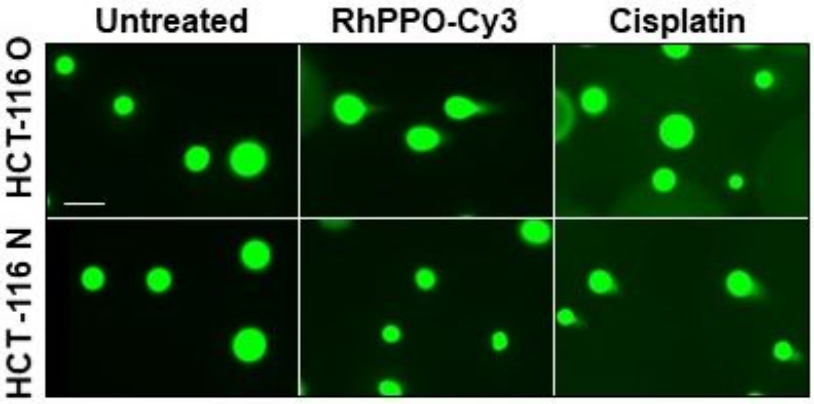

Figure S11. RhPPO-Cy3 causes DNA damage. HCT-116 O and HCT-116 N cells were treated with 5 μM RhPPO-Cy3 or cisplatin for 24 hours, and then analyzed for DNA damage using a neutral Comet assay. Cells were embedded in agarose, denatured and analyzed by TBE gel electrophoresis. Cell nuclei and comet tails were visualized with a Vista Green fluorescent dye. Representative images are shown. Scale bar, 20 μM.

REFERENCES

1. Boyle, K. M.; Barton, J. K. (**2018**) A Family of Rhodium Complexes with Selective Toxicity toward Mismatch Repair-Deficient Cancers. *J. Am. Chem. Soc. 140*, 5612-5624.
2. Nano, A.; Boynton, A.; Barton, J.K. (**2017**) A Rhodium-Cyanine Fluorescent Probe: Detection and Signaling of Mismatches in DNA. *J. Am. Chem. Soc. 139*, 17301-17304.