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The effect of Cisplatin on Human Diploid Fibroblast Cells expressing SV40 T-Antigen

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Can we predict Cisplatin resistance/sensitivity based on BIN1 protein isoform present?

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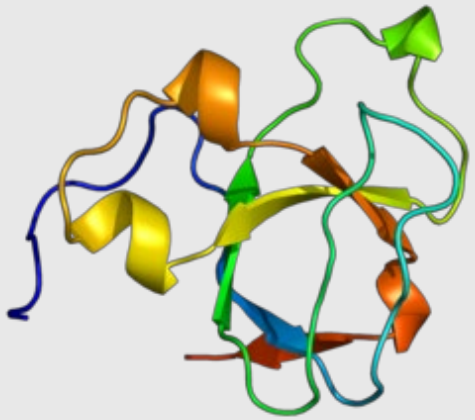
BIN1

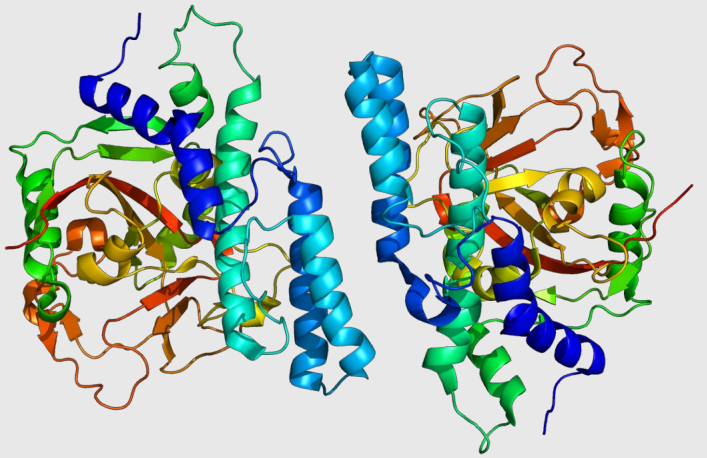
- Bridging integrator 1
- Nucleocytoplasmic tumor suppressor protein
  - Halt the cell cycle
- Ubiquitous expression in all cell types
- Variant isoforms across cell types
- Myc binding domain
  - Important for BIN1 mediated apoptosis
- Complete and partial losses of BIN1 documented in 60% breast cancer cases



c-Myc and PARP1

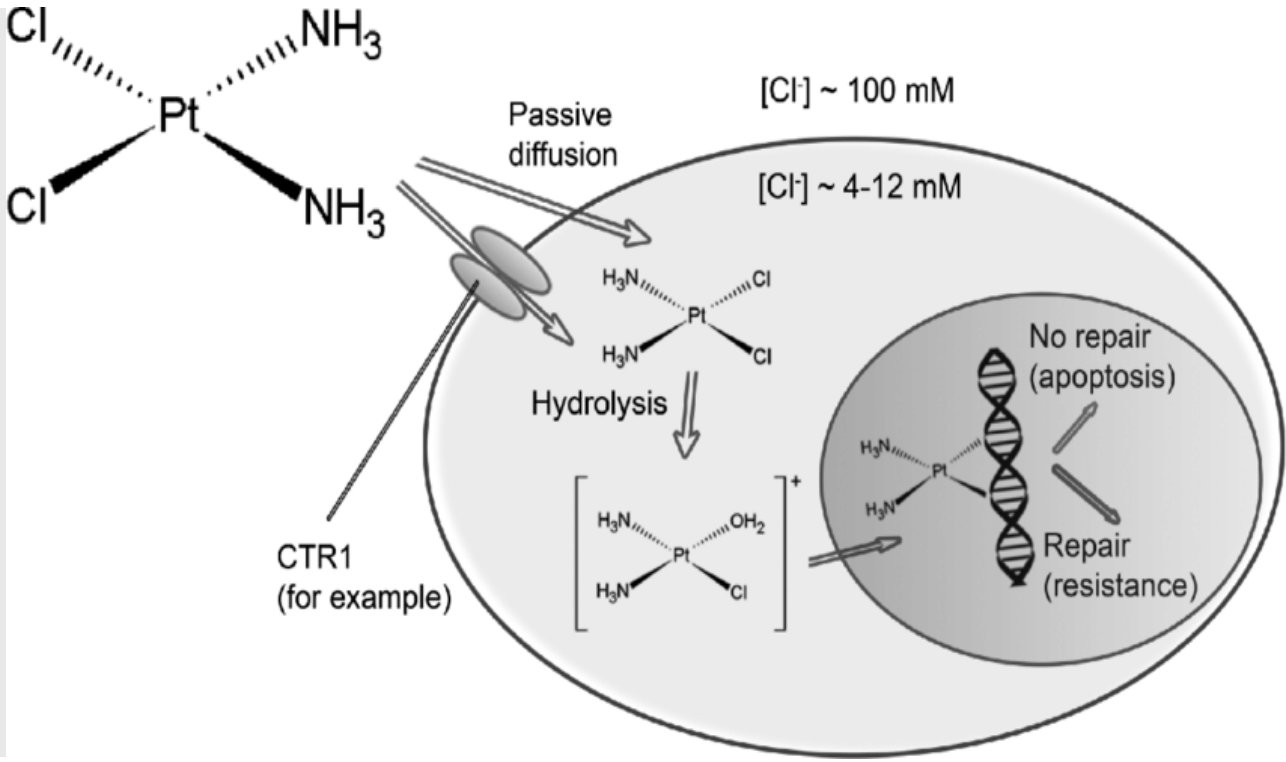
- cellular-Myc:
- Proto-oncogene
- Overexpression leads to increased cell proliferation

- Poly(ADP-ribose)polymerase-1
- Enzyme responsible for fixing double and single stranded DNA breaks
- Overexpressed in wide range of cancers



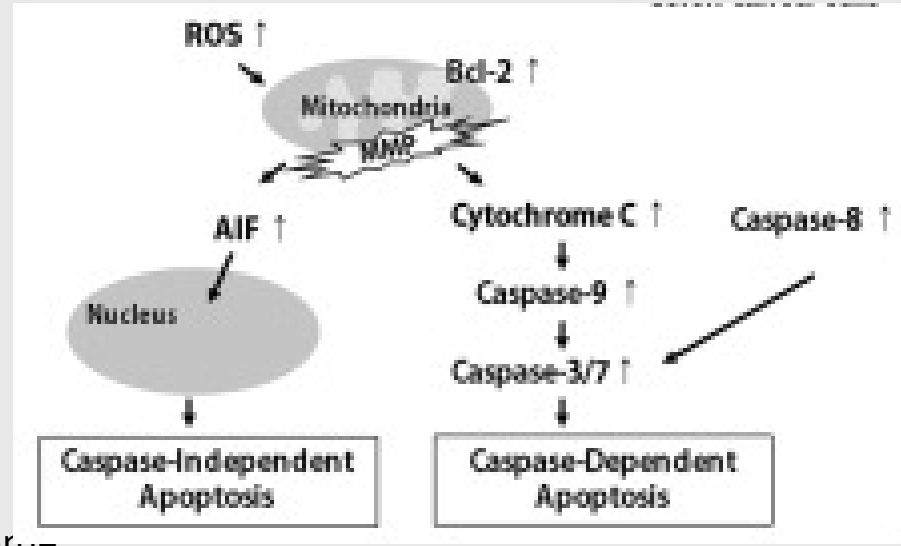
Cisplatin

- Chemotherapeutic drug
- Activated once taken up by cell
  - Water ions in the cytoplasm displace Cl^-
  - Binds to N7 reactive centers on purine to cause DNA damage
- Very effective against a wide range of cancers
- Drug resistance and side effects
  - Nephro- and hepatotoxicity

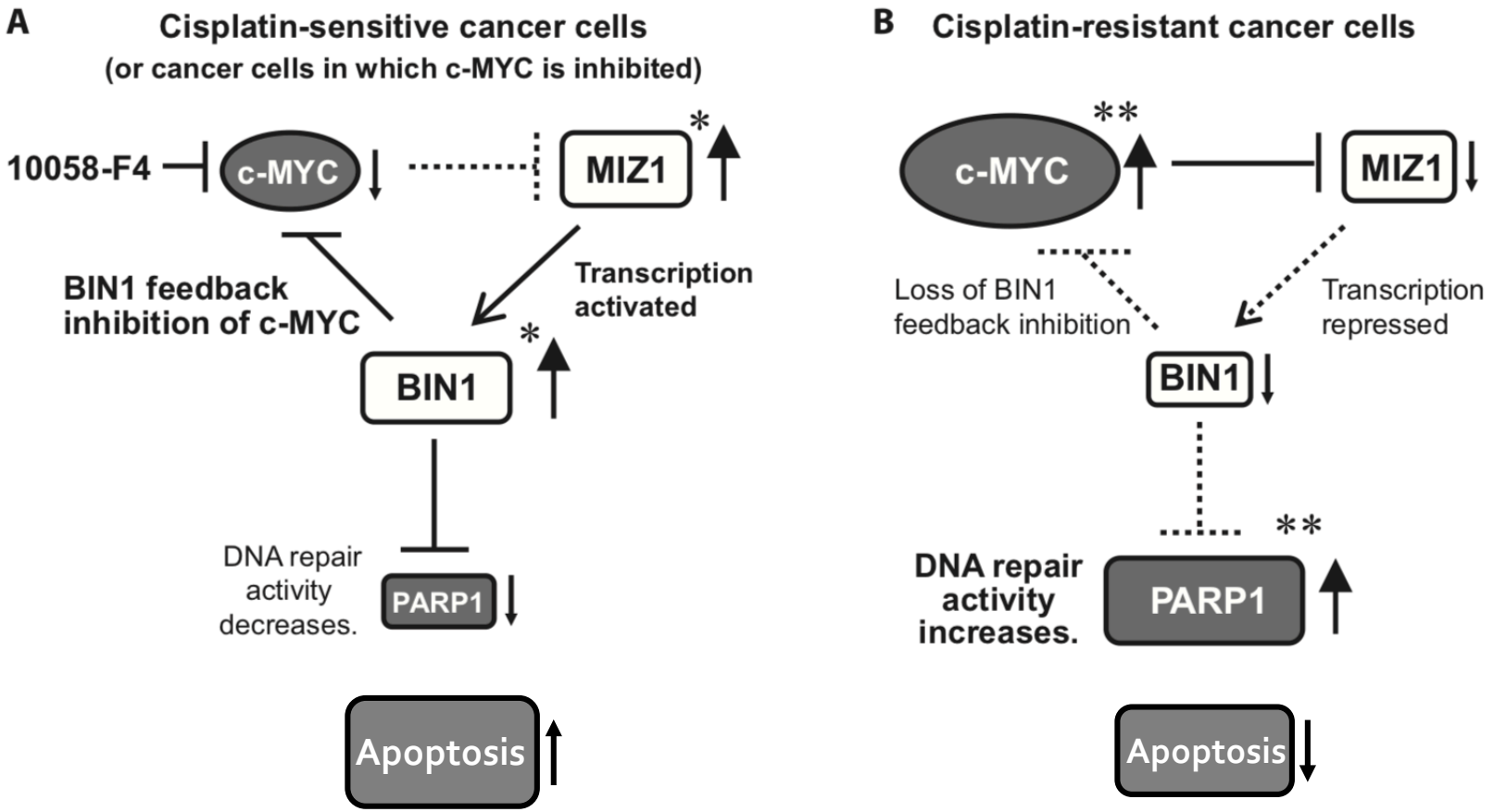


Apoptosis

- Programmed cell death
- Caspase independent (protein damage)
  - Inner mitochondrial membrane dissipates
  - Release apoptosis inducing factor (AIF)
- Caspase-dependent (DNA damage)
  - Extrinsic
    - Received signal, activate Caspase 8
    - Effector caspases cause nuclear damage
  - Intrinsic (p-53 independent)
    - Reactive Oxygen species activate Bcl-2 family releasing cytochrome c from the mitochondria



Cisplatin-sensitive vs Cisplatin-resistant

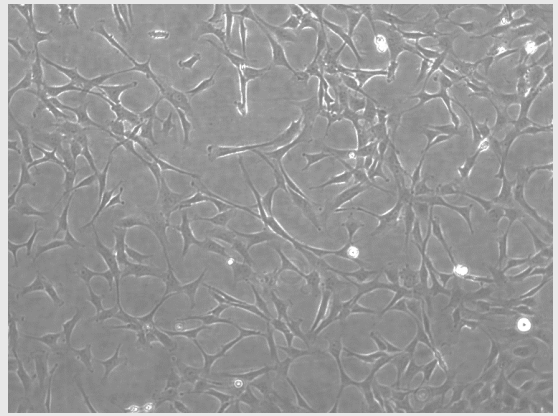


Hypothesis

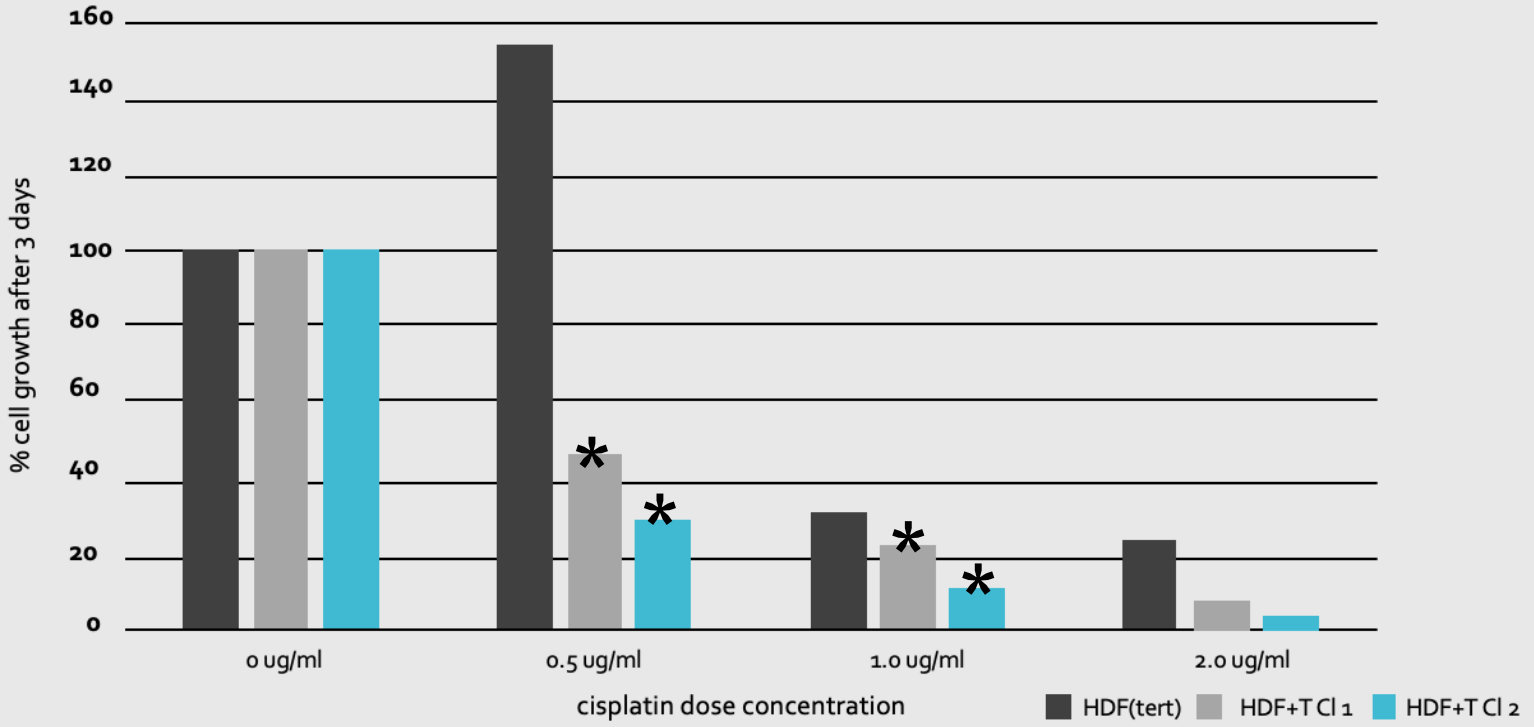
- Unsure of BIN1 isoform present
- Determine functionally if there was a difference in cisplatin sensitivity across variant isoforms

Methods

- Human diploid fibroblast (HDF) cells
  - Immortalized with telomerase
  - Stably transfected with SV40 T-antigen (HDF+T)
    - 2 Clones
- Seeded cells of each 3 lines in triplicate and conducted a dose curve
  - 0.0, 0.5, 1.0, 2.0 ug/ml
- Counted cells 3 days after dosage to determine percentage of cell death
- Immunofluorescence and Western blot analysis to determine presence of BIN1

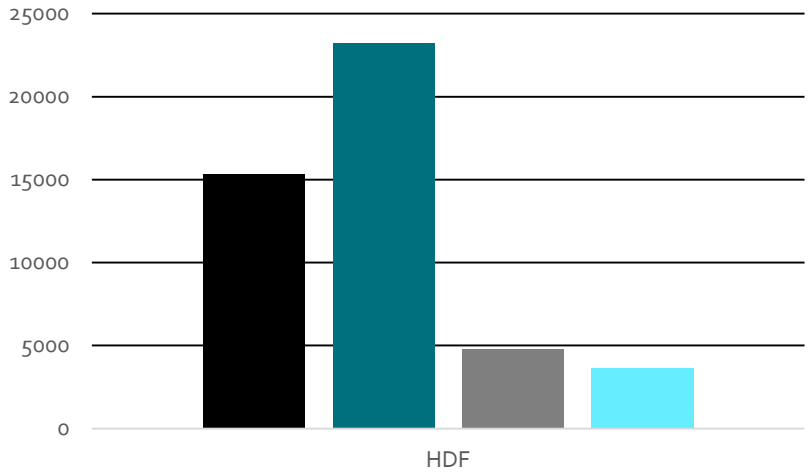


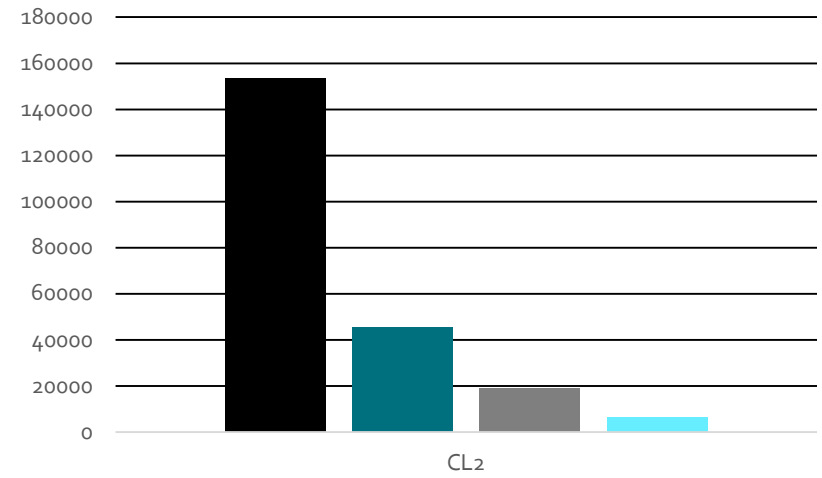
Percent change over 3 days

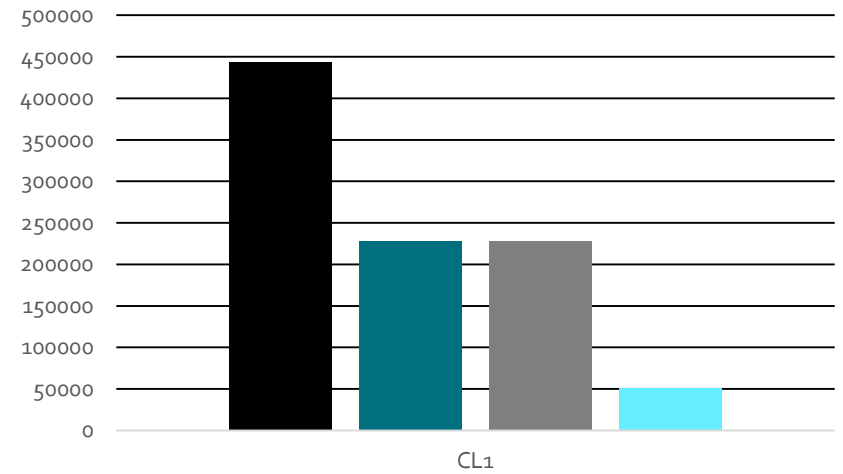


* Significant p-value

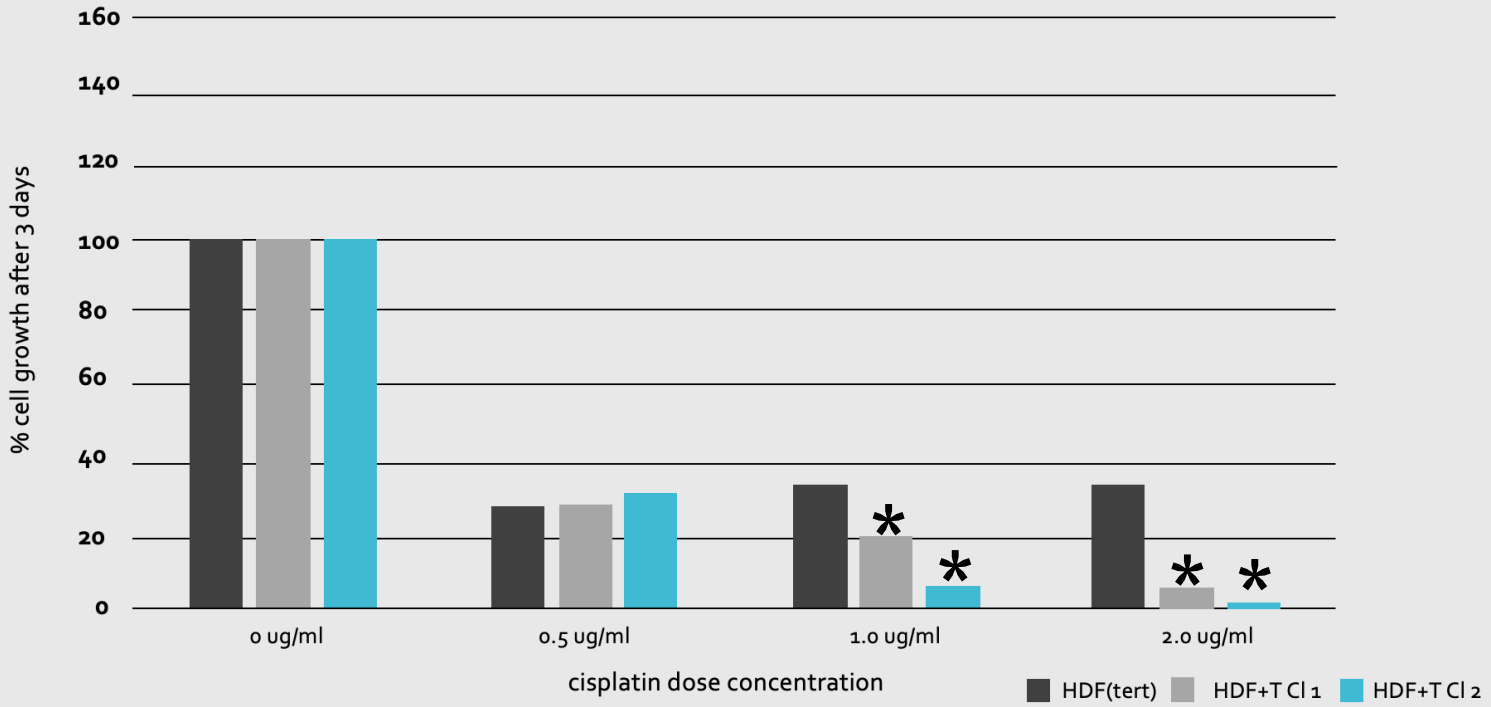
Cell Counts after 3 days







Second Cisplatin Dose Curve (% decrease)



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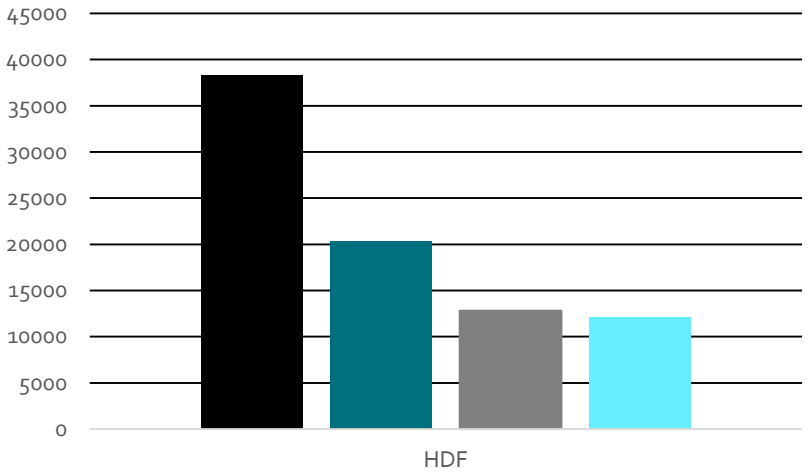
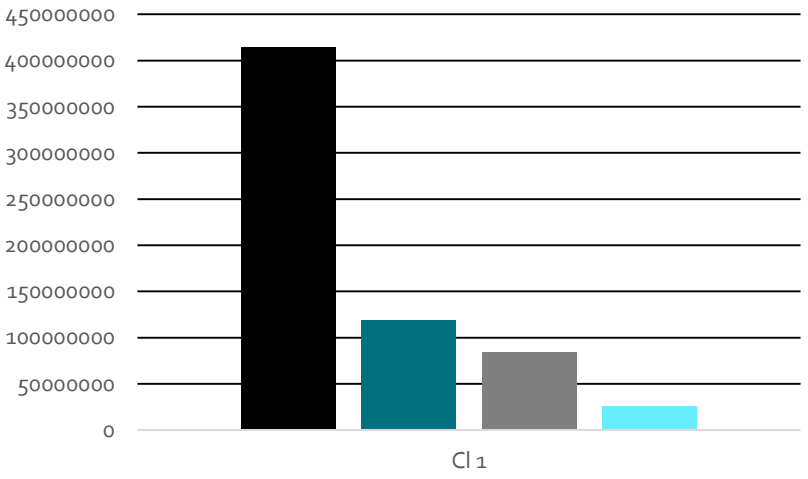
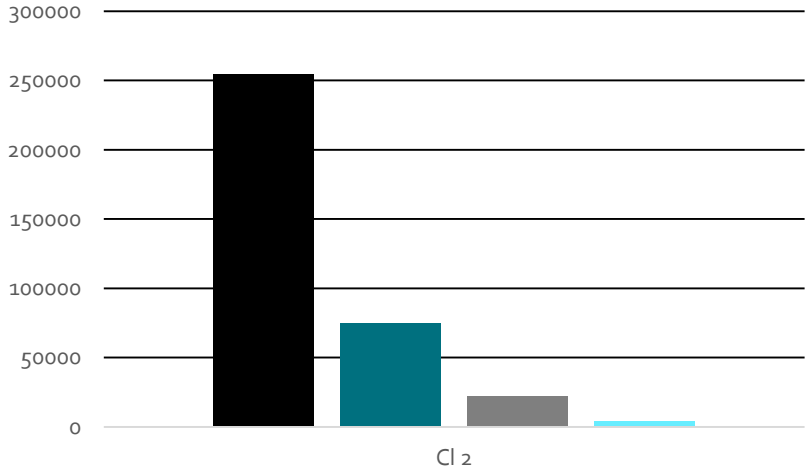
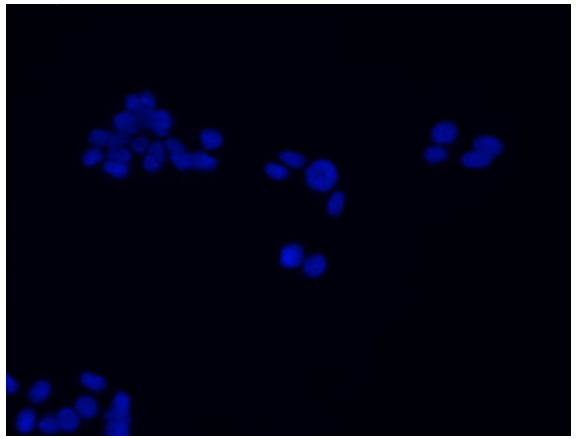


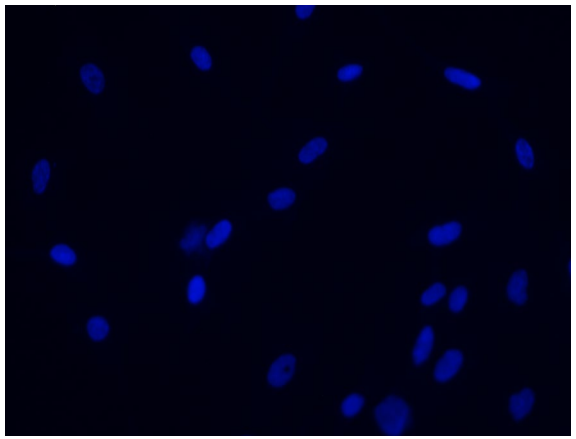


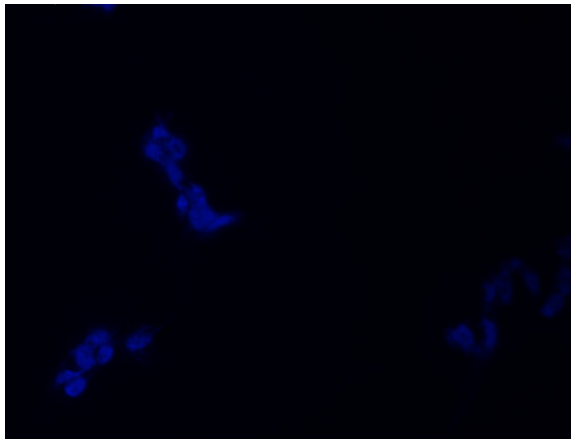


Immunofluorescence

Immuno-fluorescence





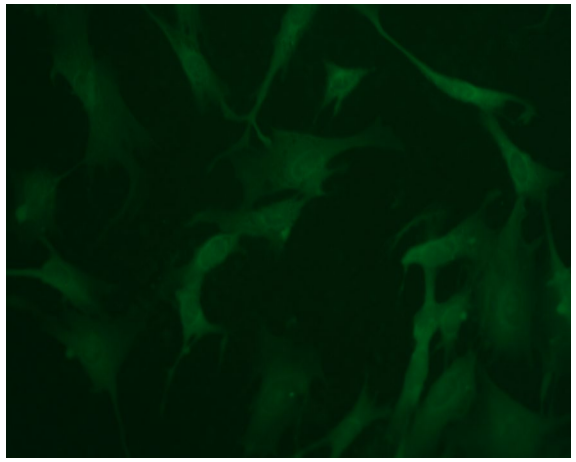


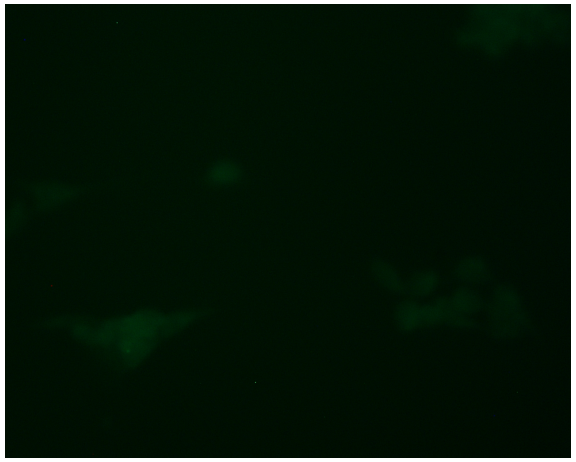
DAPI

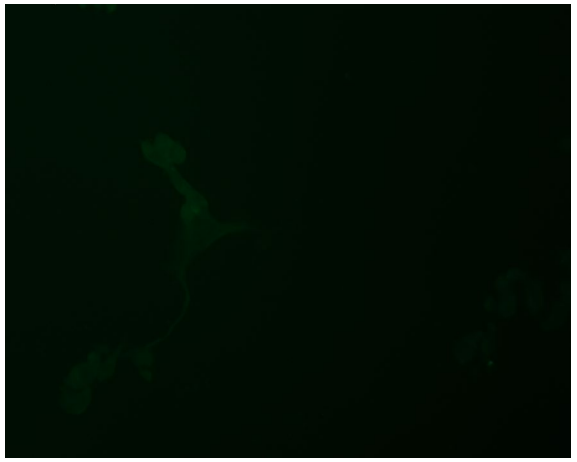
HDF

Cl1

Cl2

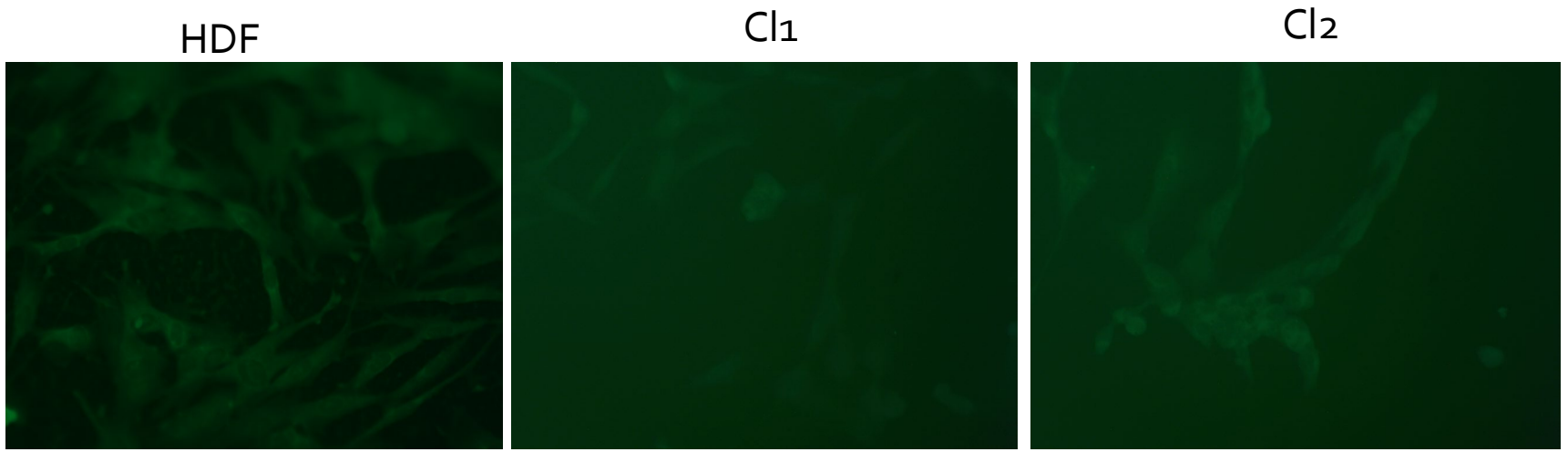






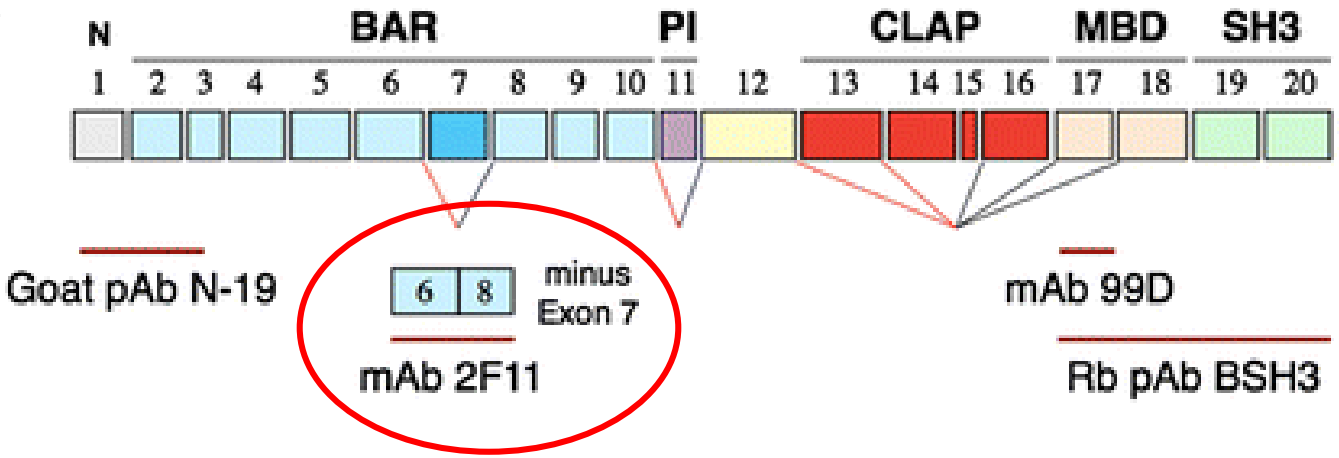
BIN1

IF Negative Control (PBS)

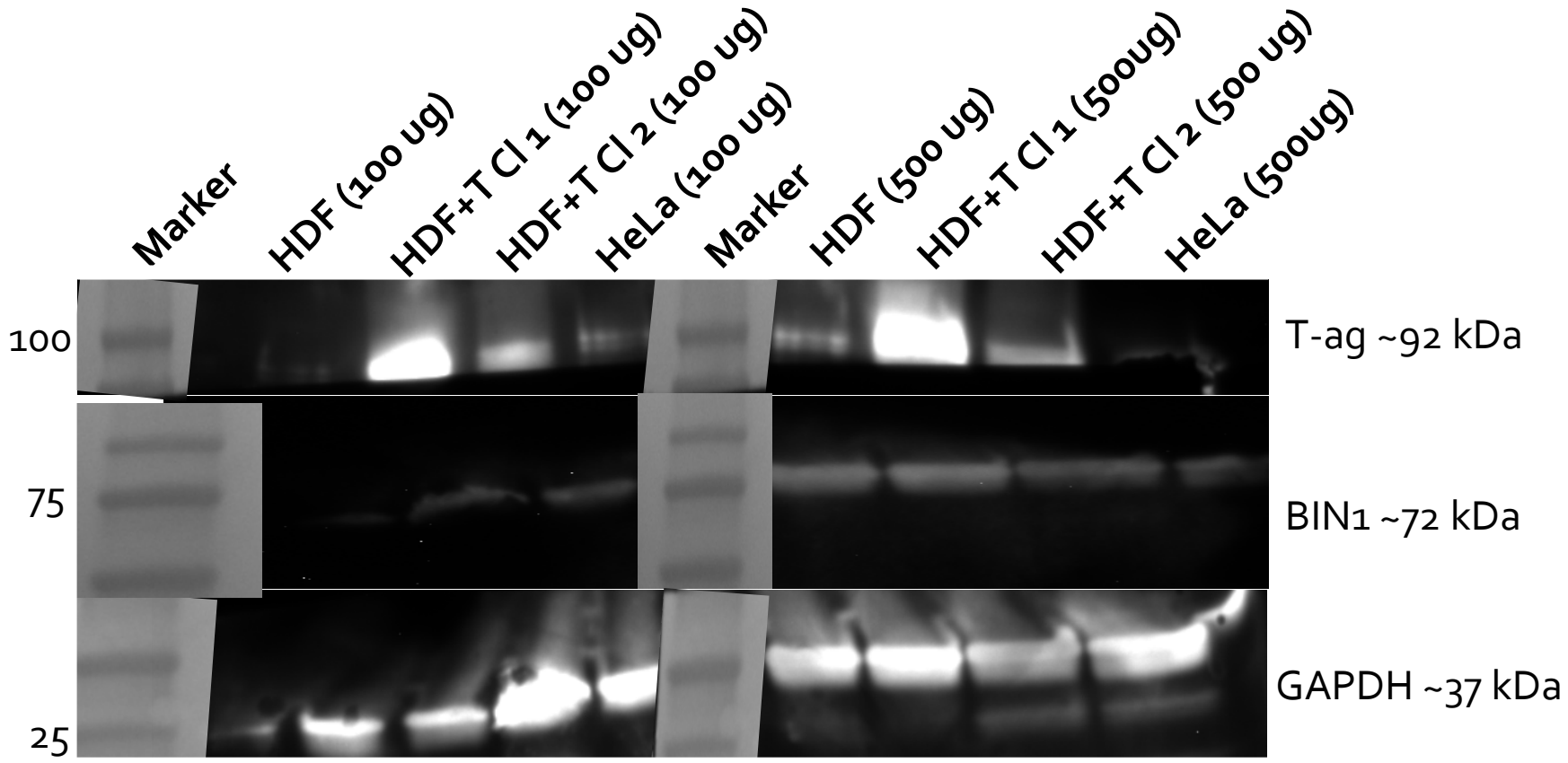


Western Blot Analysis

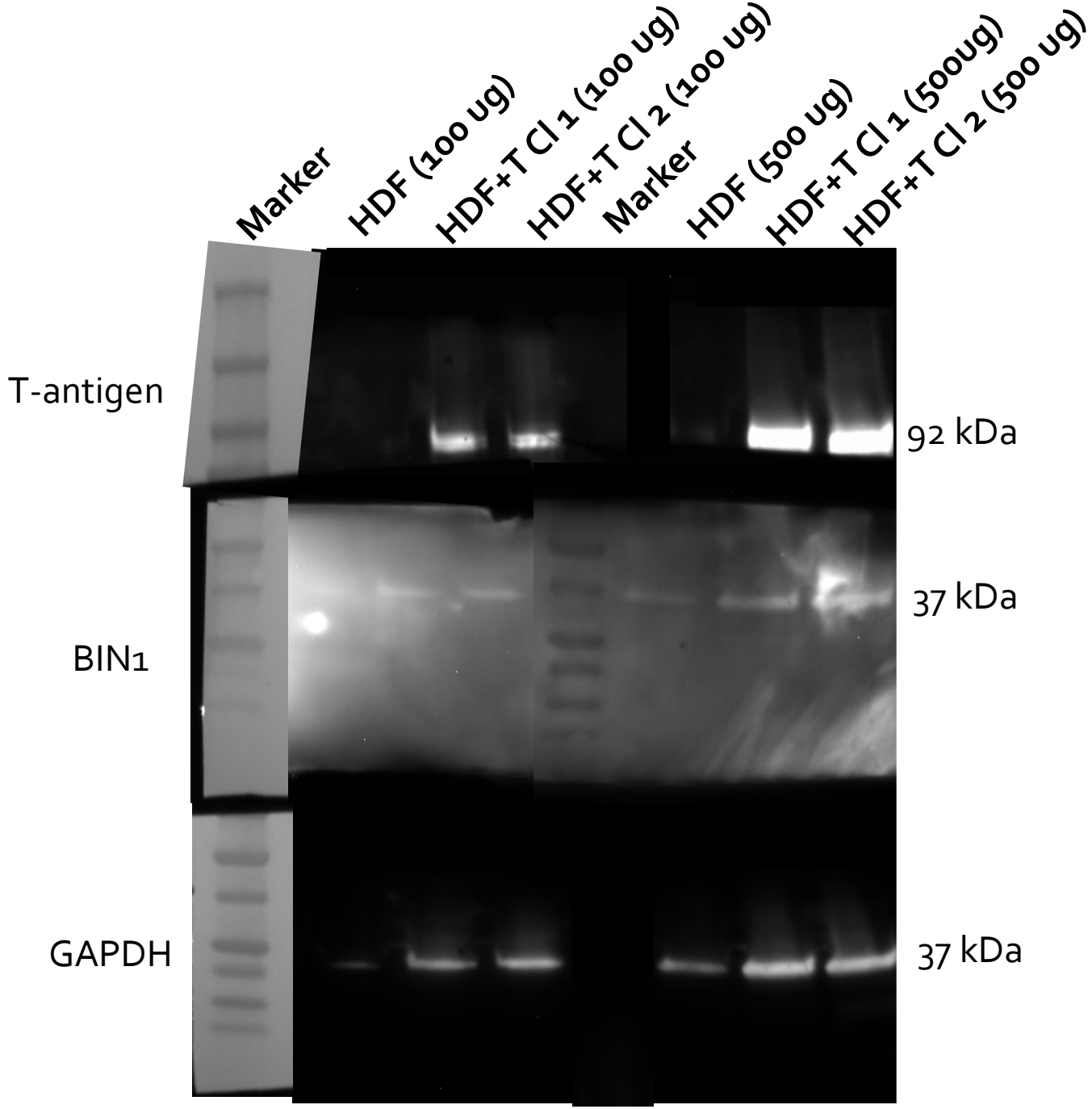
BIN1 Antibody Binding



2F11 BIN1 Antibody using



Western Blot



Future Experiments

- Determine apoptosis vs necrosis
- Test known Cisplatin resistant cells and determine their isoform
- Probe Western blot for PARP1
- Treat Cisplatin resistant cells with PARP1-inhibitors

Acknowledgements

- Dr. Jane Cavender and the Elizabethtown College Biology Department
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