Mitochondrial Transplantation is Feasible in an In Vitro Neuronal Cell Model and Ameliorates Ischemia-Reperfusion Injury

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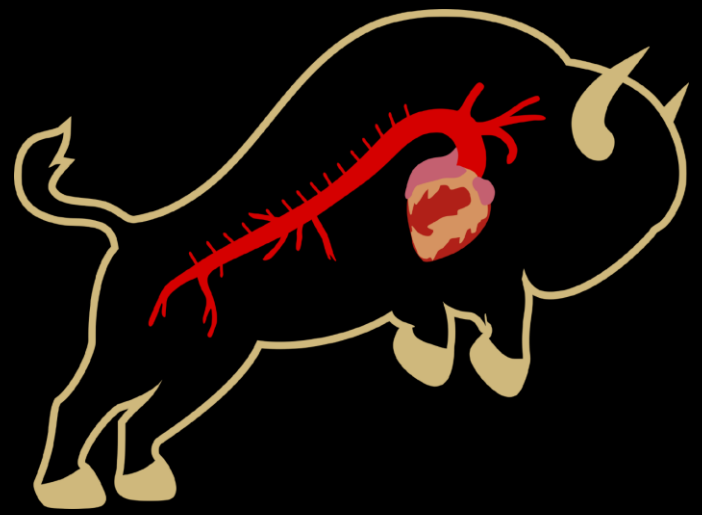
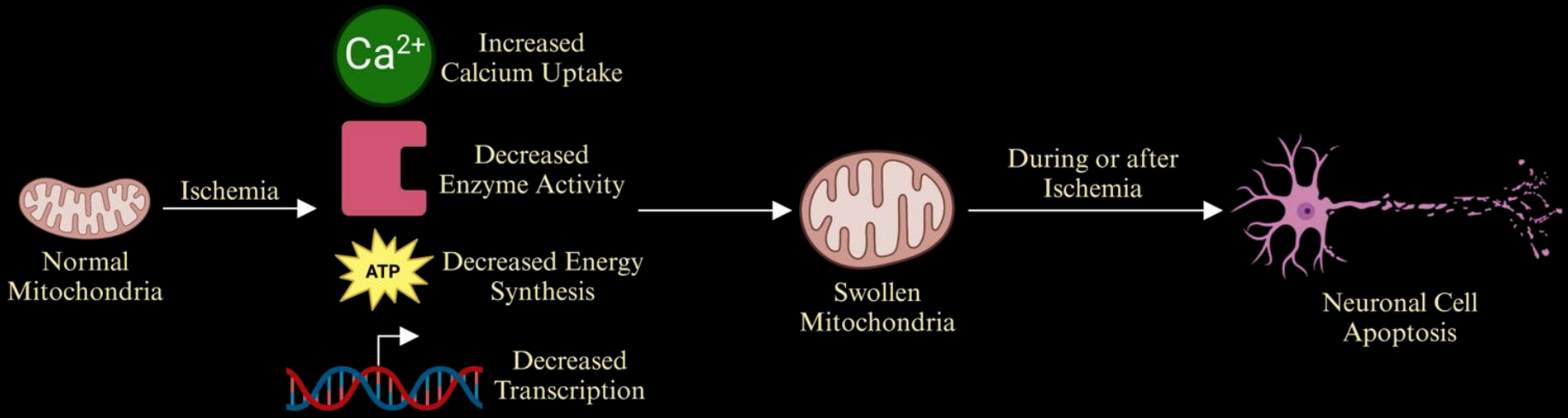
No disclosures

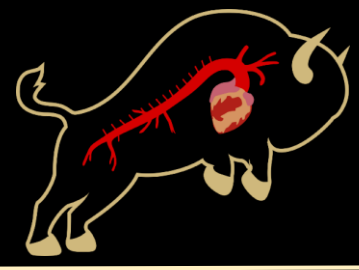


Introduction

- Ischemia-Reperfusion Injury (IRI) cause significant harm in aortic arch surgery, with potential to cause devastating neurologic outcomes
- Mitochondria (MT) play a key role in IRI, with initial oxygen-glucose deprivation depleting ATP, with subsequent production of reactive oxygen and nitrogen species leading to cell death
- Mitochondrial transplantation (MTR) has shown promise in other tissue models in attenuating ischemia-reperfusion injury



Aim



- Simulate in-vitro conditions mimicking ischemia-reperfusion injury
- Harvest viable mitochondria from animal specimens
  - Determine optimal tissue for harvesting
- Demonstrate successful in-vitro uptake of mitochondria in non-ischemic & ischemic conditions
- Apply mitochondrial transplantation to neuronal cells in an ischemia-reperfusion model to improve cell viability

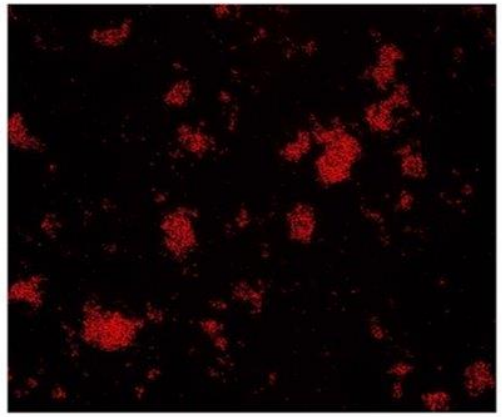
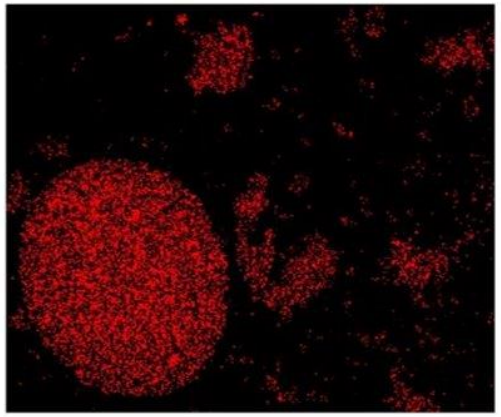
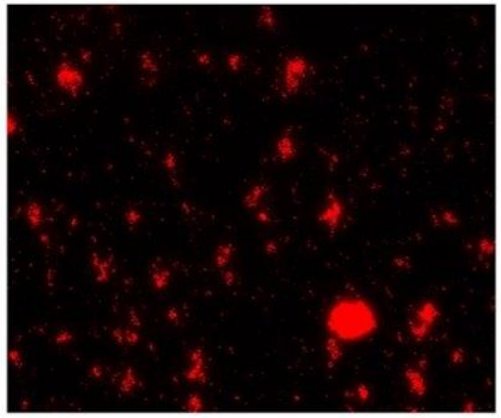
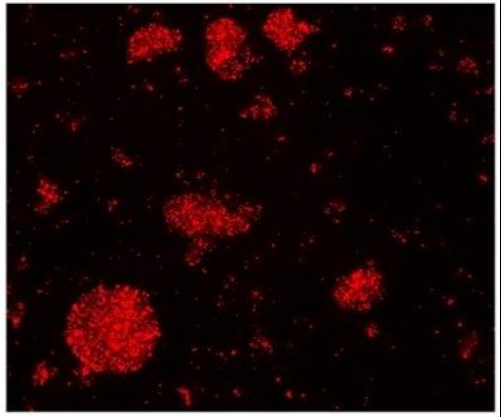
Methods & Results: IRI Condition Simulation

- HT-22, an immortalized mouse hippocampal cell line, cultured in 96 well plates
- Cells pre-cultured for 24 hours prior to ischemic conditions
- Ischemic conditions:
  - Oxygen-glucose deprived cell medium
  - Placement into hypoxia chamber at 0.1% O2
  - Control cells placed in new culture medium, returned to incubator
- Following oxygen-glucose deprivation (OGD) exposure, both groups of cells placed in new culture medium, returned to incubator for 24 hours
- Goal cell viability: 30-50% remaining cell viability as detected by MTS Assay
  - Tested at time points ranging from 8-48 hours
- 18 hours of OGD exposure was the optimal time point across multiple trials

Methods & Results: Mitochondrial Harvesting

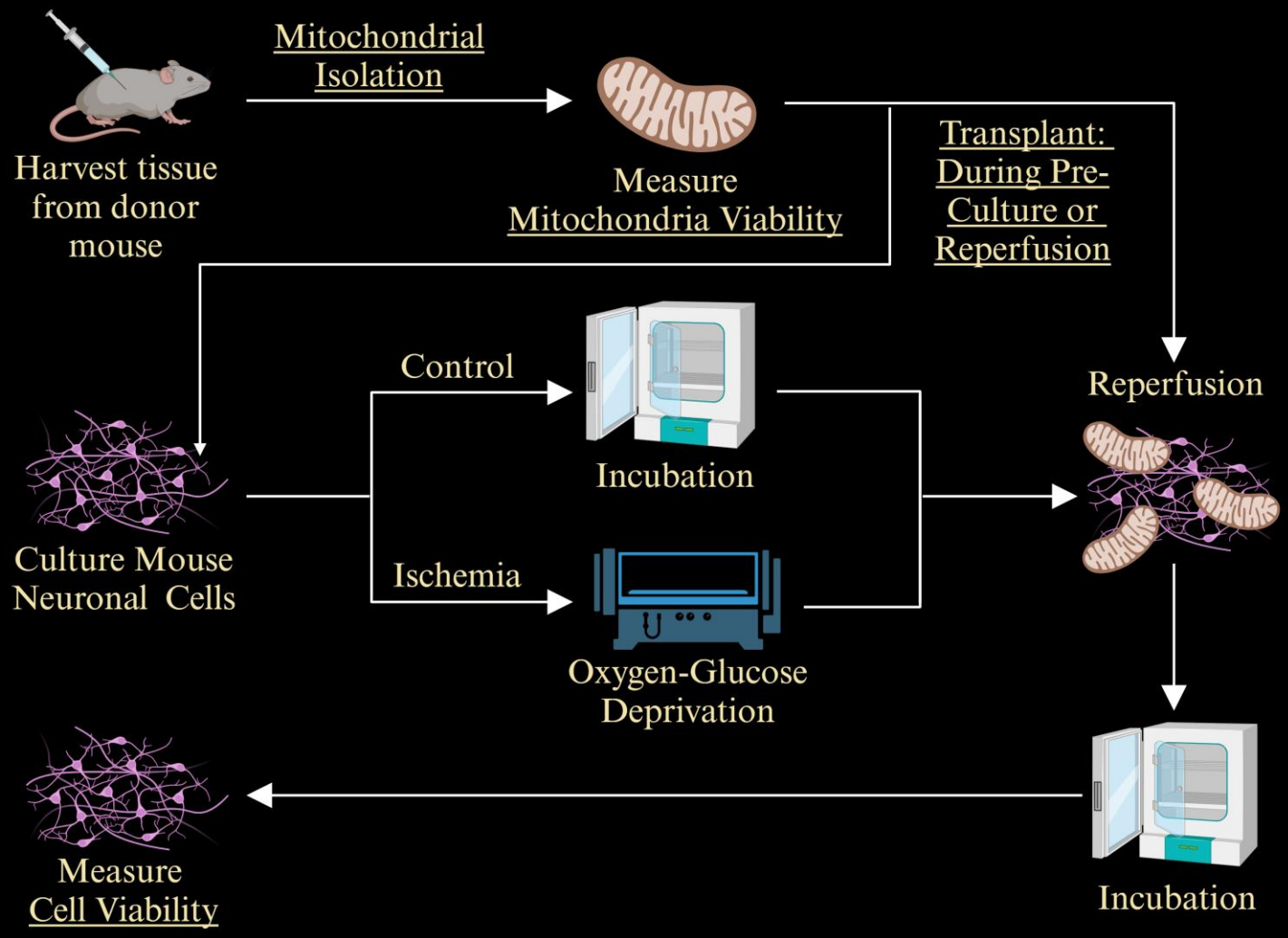
- Tissue harvested from male mice at four different tissue sites: brain, heart, liver, skeletal muscle
- Tissue samples homogenized at varying time points, isolated with mitochondrial buffer
  - Optimal homogenization time point 30-40 seconds
- Excellent yield demonstrated from all tissue types
- MT stained with MitoTracker, successful uptake demonstrated viable mitochondria

Fluorescent Microscope Imaging

	20x Zoom	40x Zoom
1:10 dilution in PBS		
1:5 dilution in PBS		

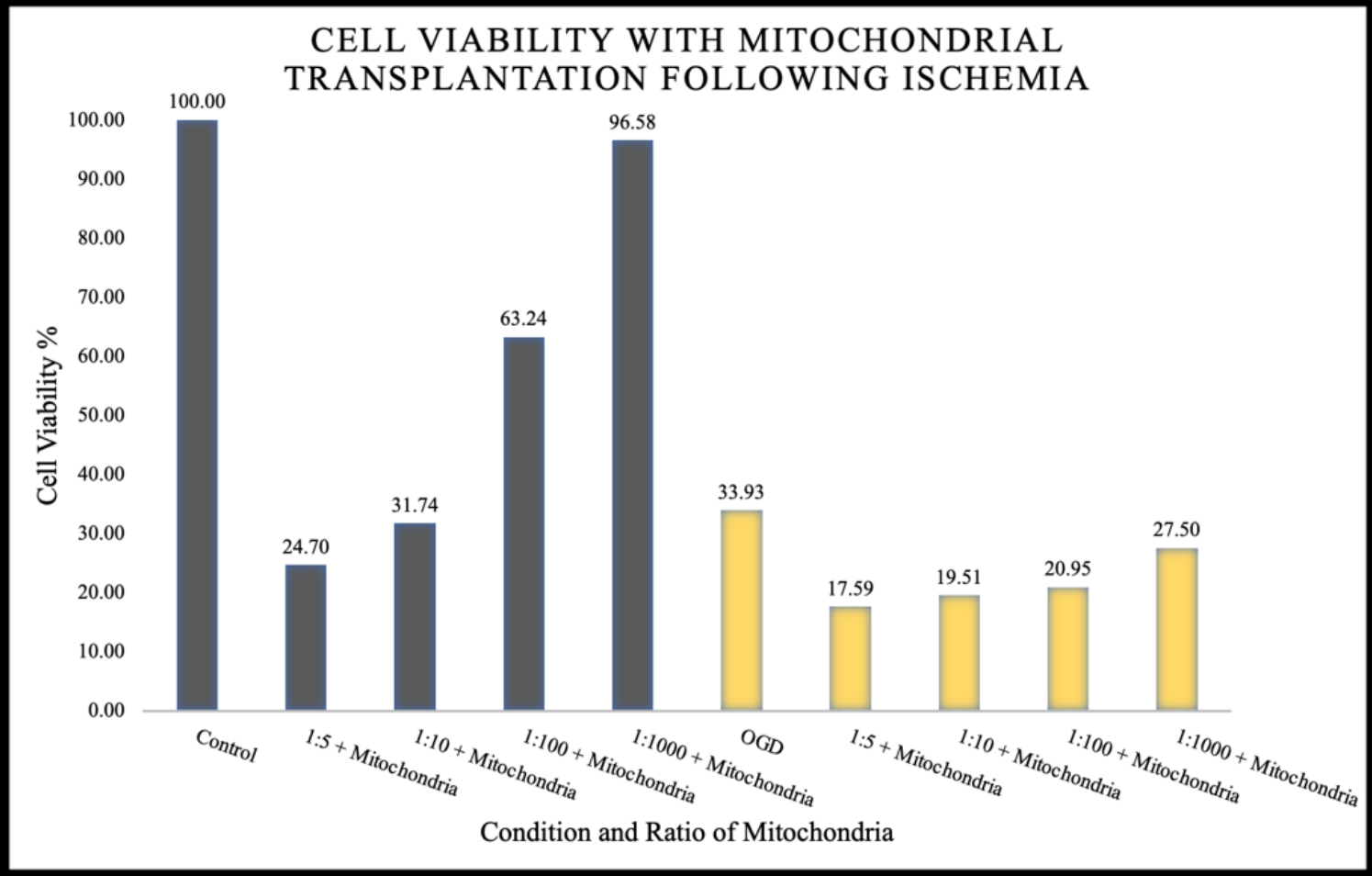
Methods & Results: Mitochondrial Transplantation

- Tested mitochondrial transplantation of different tissue sources at two time points:
  - Pre-culture phase
  - Reperfusion phase



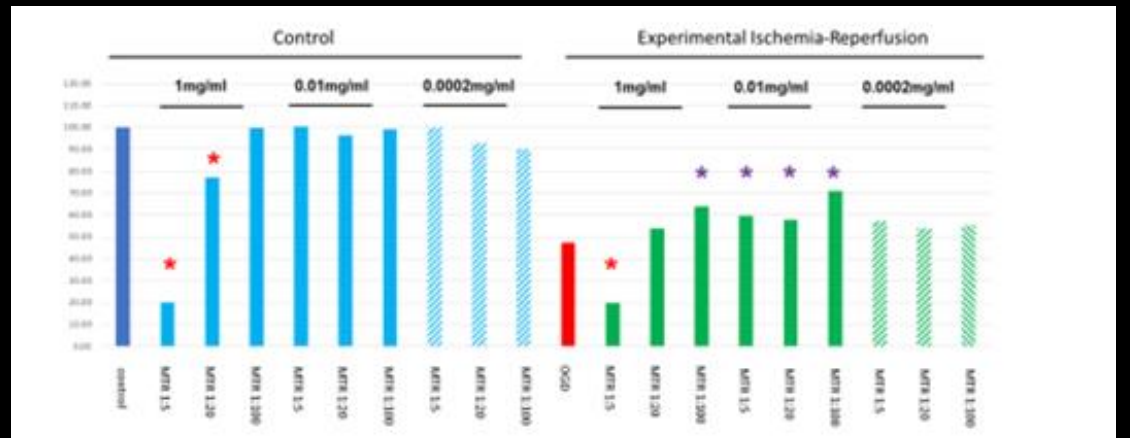
Methods & Results: Reperfusion MTR

Regardless of tissue type, MTR during reperfusion resulted in dose-dependent loss of viable cells for both control and OGD cells

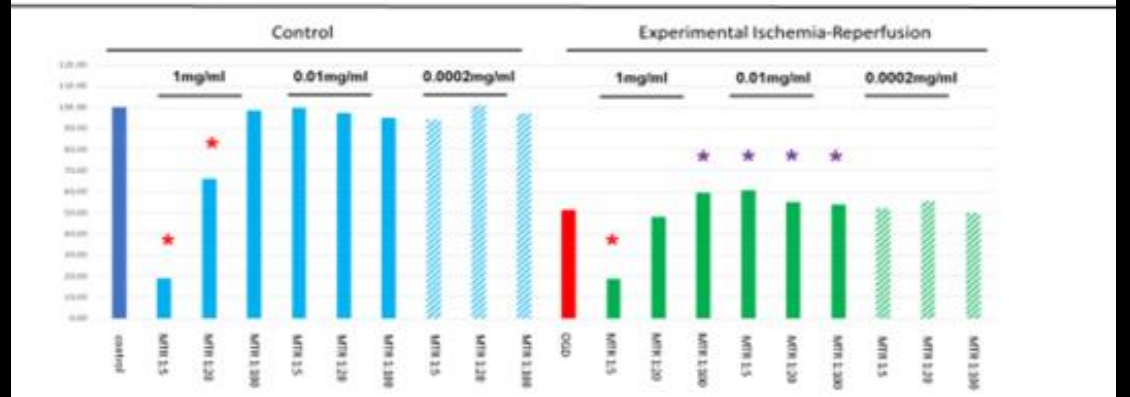


Methods & Results: Pre-culture MTR

- At high doses, MTR killed both control and OGD cells
- However, at optimal concentrations MTR increased post-OGD cell viability
  - Repeat experiments performed with heart and muscle tissue (selected due to clinical potential) reinforced results



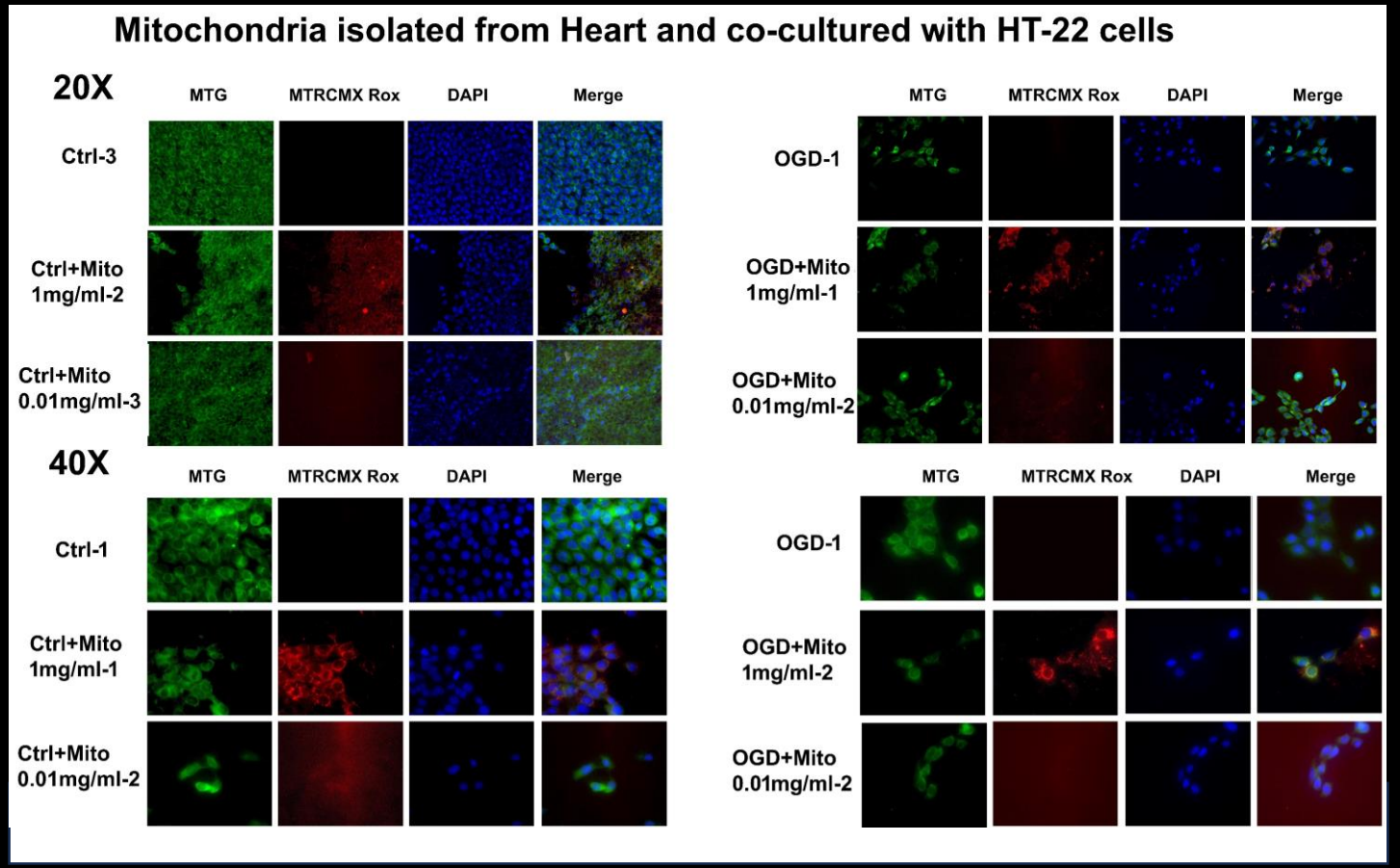
Neuronal cell viability post ischemia-reperfusion as detected from BCA assay. Mitochondrial transplantation (MTR) performed during pre-culture with mitochondria extracted from **Heart** at specified concentrations and ratios above.



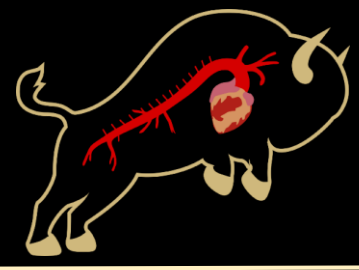
Neuronal cell viability post ischemia-reperfusion as detected from BCA assay. Mitochondrial transplantation (MTR) performed during pre-culture with mitochondria extracted from **Muscle** at specified concentrations and ratios above.

Methods & Results: Mitochondrial Incorporation

- To ensure mitochondria were successfully incorporated, performed immunofluorescence
- Red-stained mitochondria transplanted during pre-culture phase at different concentrations
- Following co-culture wells stained with DAPI to dye cell nucleus blue, and with MitoTracker Green to dye endogenous mitochondria green
- Demonstrated successful incorporation of exogenous mitochondria in both OGD and control conditions



Conclusions



- Mitochondria transplantation is feasible in an in-vitro neuronal cell model
- MTR performed during reperfusion results in dose-dependent loss in cell viability
- MTR during pre-culture increases cell viability after exposure to ischemic conditions
- Future Directions:
  - Attenuating response with introduction of pharmacologic agents targeting signaling mechanisms

Questions???