STK-002, an Antisense Oligonucleotide (ASO) for the Treatment of Autosomal Dominant Optic Atrophy (ADOA), is Taken Up by Retinal Ganglion Cells (RGCs) and Upregulates OPA-1 Protein Expression After Intravitreal Administration to Non-human Primates (NHPs)

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Stoke Therapeutics

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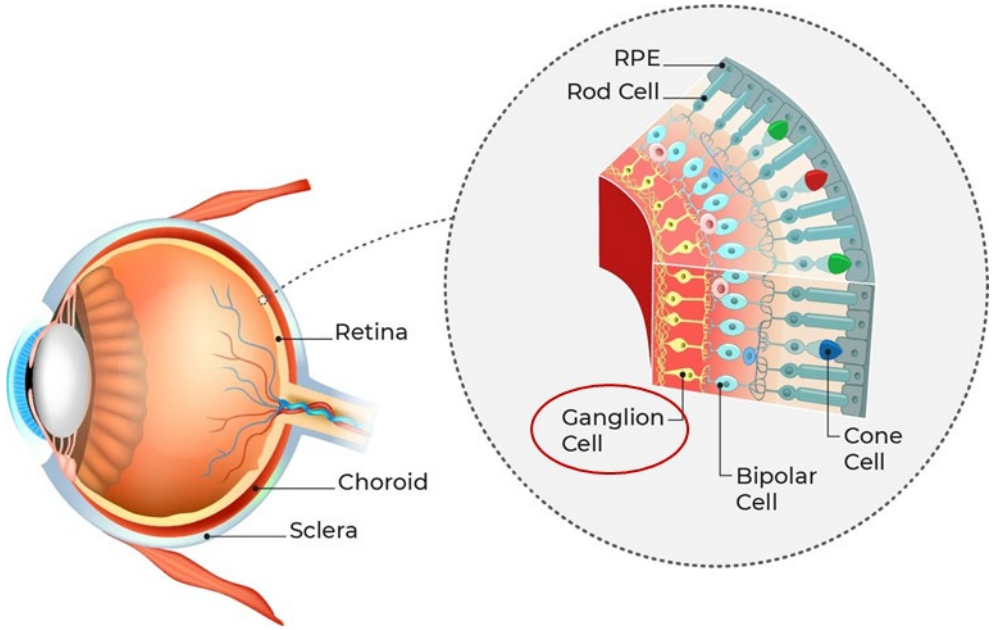
Autosomal Dominant Optic Atrophy (ADOA) and *OPA1*

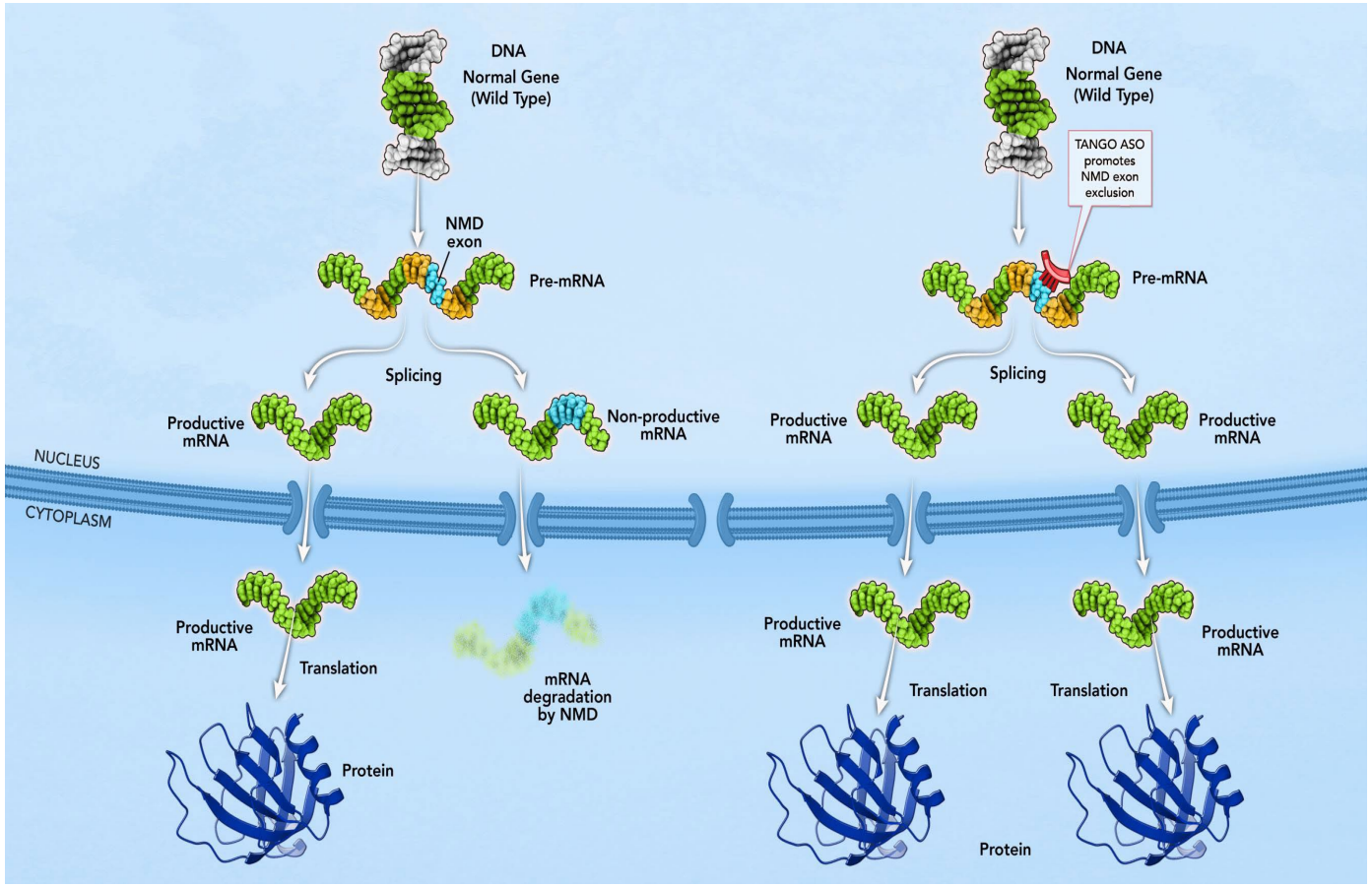
- ADOA is the most common inherited optic nerve disorder and is characterized by retinal ganglion cell (RGC) loss
- 65-90% of cases are caused by mutations in one allele of the *OPA1* gene, a mitochondrial GTPase with a critical maintenance role in mitochondria structure and function
- Most *OPA1* mutations lead to a haploinsufficiency, resulting in about a 50% of normal OPA1 protein levels in patient cells



- Approximately 1 out of 30,000 people are affected globally with a higher incidence of ~1 out of 10,000 in Denmark due to a founder effect
- ADOA typically presents within the first decade of life. 80% of patients are symptomatic before 10 years of age
- The disease causes progressive and irreversible vision loss and up to 46% of patients are registered as legally blind. No therapeutic options are available to patients with ADOA

Applying TANGO for the Treatment of Autosomal Dominant Haploinsufficiency Diseases

- Targeted Augmentation of Nuclear Gene Output (TANGO) uses antisense oligonucleotides (ASOs) to modulate splicing to precisely upregulate protein expression
- TANGO ASOs reduce or prevent the generation of naturally occurring non-productive mRNA and increase productive mRNA, resulting in increased production of functional protein
- Leverages the wild type allele to increase protein expression
- Provides a mutation-independent approach to treat autosomal dominant haploinsufficiency diseases



Previous Preclinical Data Support the Potential Use of TANGO in ADOA[1,2]

- STK-002 mediated dose-dependent reduction in Nonsense Mediated Decay (NMD) exon inclusion, increase in total *OPA1* mRNA and OPA1 protein levels in human cells *in vitro*[1]
- Reduction in non-productive splicing and increase in OPA1 protein levels *in vivo* in wild type rabbit retina[1]
- Well-tolerated in the rabbit for up to 4 weeks after intravitreal injection (unpublished data)
- STK-002 reduced NMD exon inclusion, increased OPA1 protein and increased mitochondrial respiratory function in fibroblasts derived from patients who each had different ADOA-associated *OPA1* mutations[2]

A. Venkatesh et al., 2020[1] and 2021[2], ARVO and ASGCT presentations

Intravitreal (IVT) Administration of STK-002 to Cynomolgus Monkeys

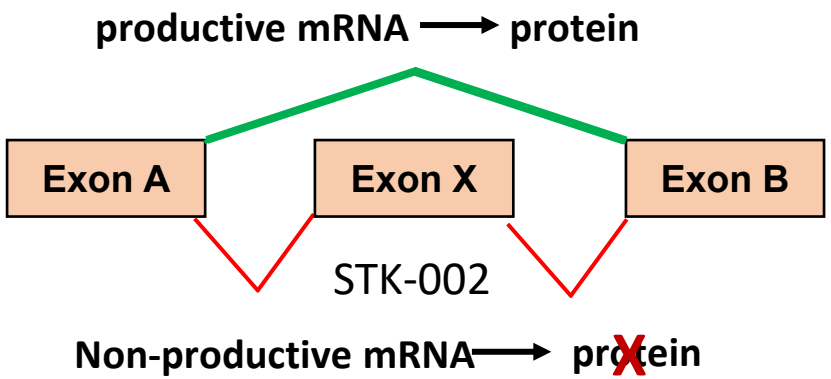
TANGO leverages wild type allele so wild type monkeys were used to show *in vivo* proof of mechanism

Group	Test Material	Dose Level	Necropsy	
			4 Weeks	8 Weeks
			Male/Female	Male/Female
1	PBS	N/A	2/2	-
2	STK-002	Low dose/eye	2/2	-
3	STK-002	Mid dose/eye	2/2	1/1
4	STK-002	High dose/eye	2/2	1/1
Drug in 50 µl volume				

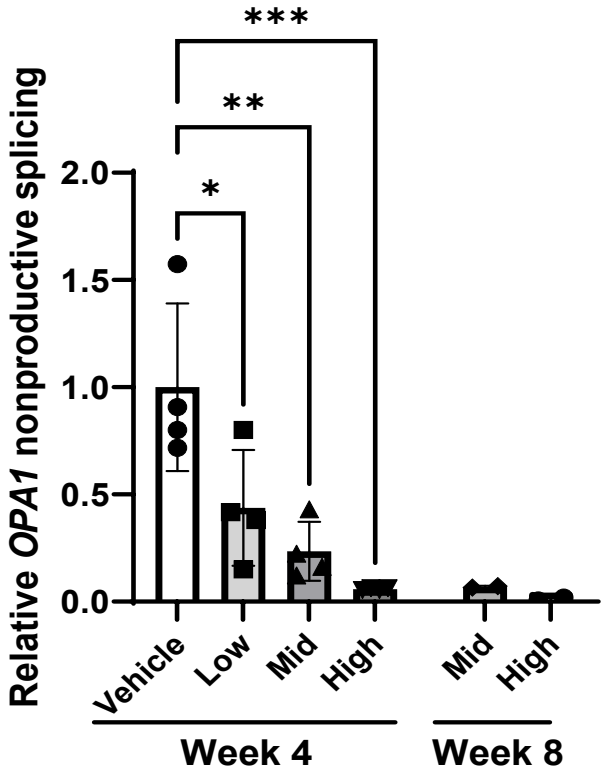
- Equal numbers of 18-24 month old male and female monkeys
- Bilateral IVT injections of PBS or STK-002 (low, mid, or high dose)
- Timepoints at 4 weeks (all treatments) and 8 weeks (mid and high doses)
- Retina from left eye was harvested and dissected into three parts for mRNA, protein and ASO quantitation
- Right eye was fixed and processed for histology

Robust Dose-Related Target Engagement and OPA1 Protein Upregulation in the Cynomolgus Retina Following IVT STK-002 injection

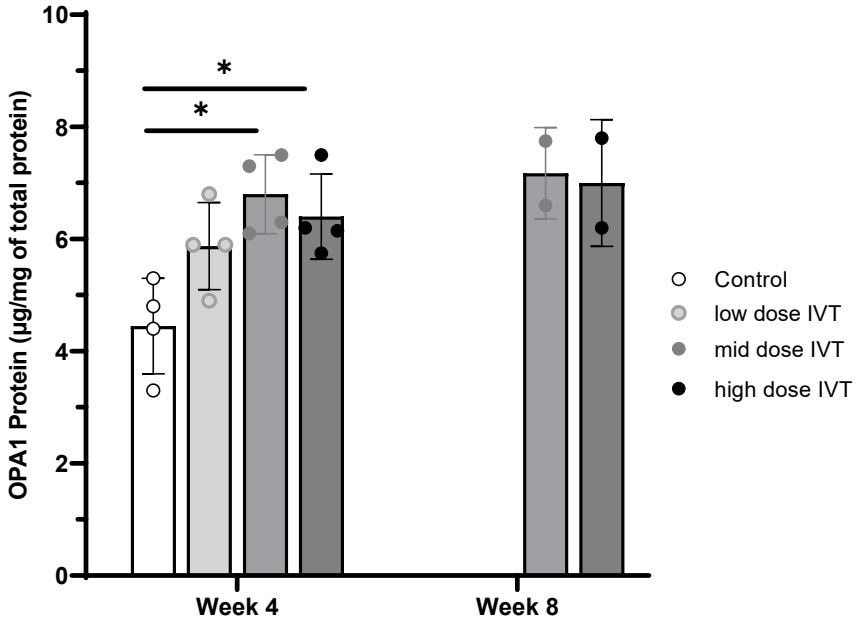
Normal *OPA1* pre-mRNA



Target Engagement

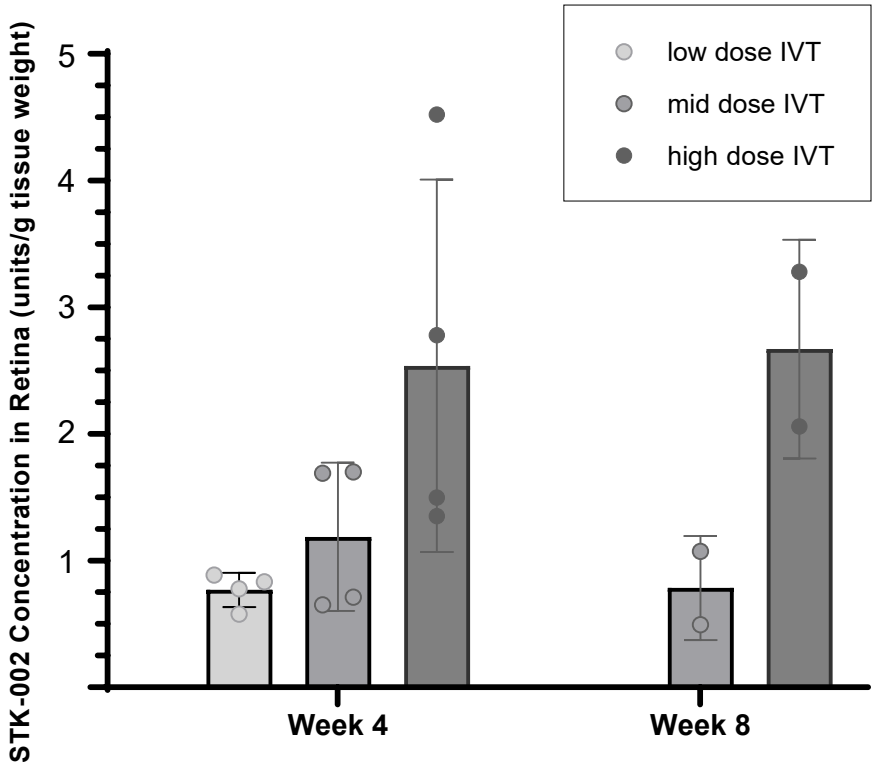


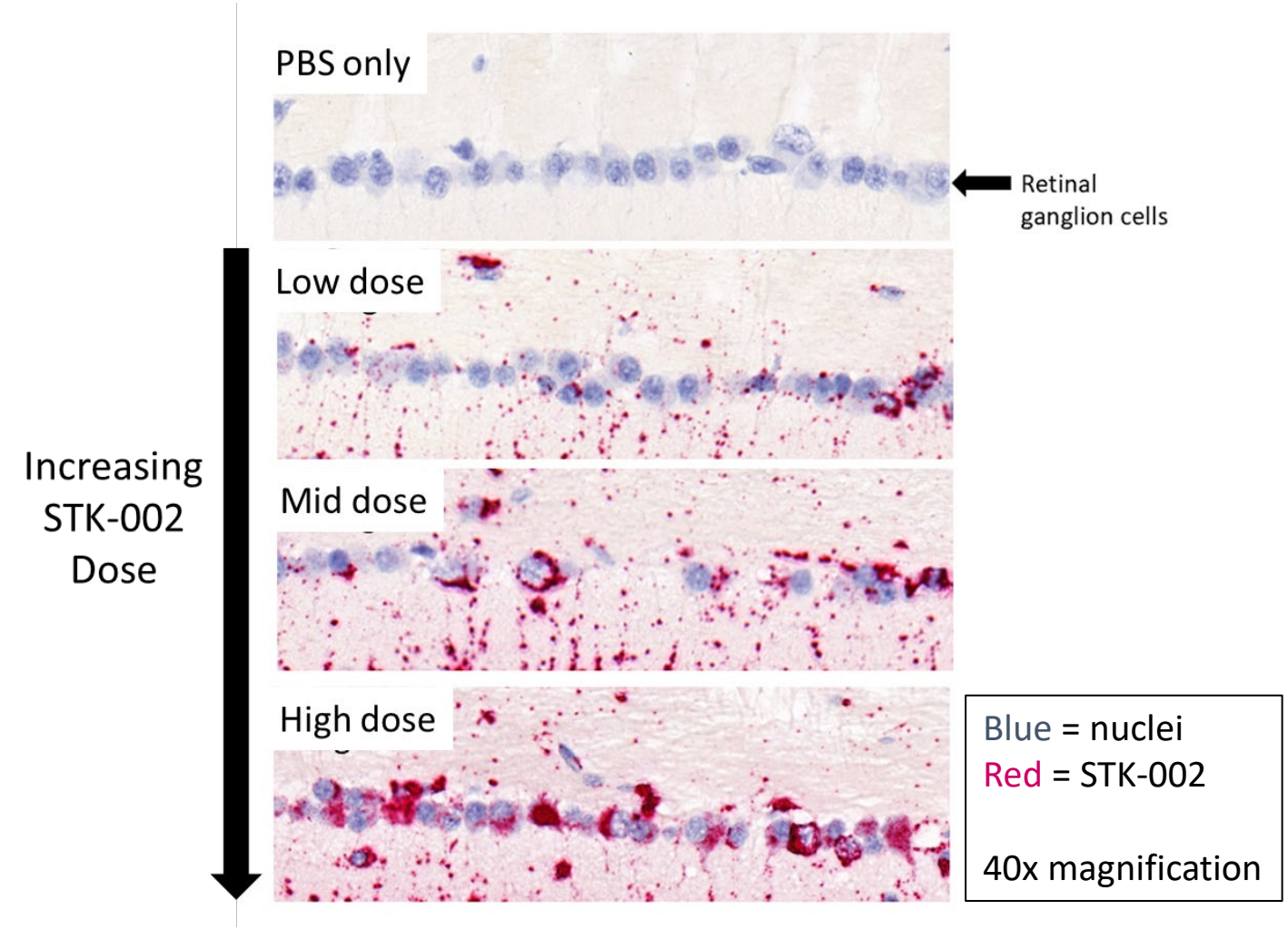
OPA1 Protein



- Significant reduction in retinal NMD transcripts (target engagement) by quantitative PCR seen at 4 and 8 weeks
- Significant increase in retinal OPA1 protein quantitated by ELISA at mid (47.1%) and high dose (43.8%) at 4 weeks that persisted at 8 weeks

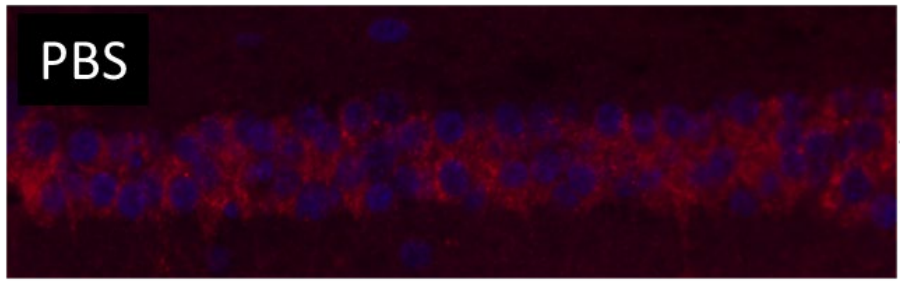
STK-002 Signal Increases in Cynomolgus RGCs with Increasing IVT Doses



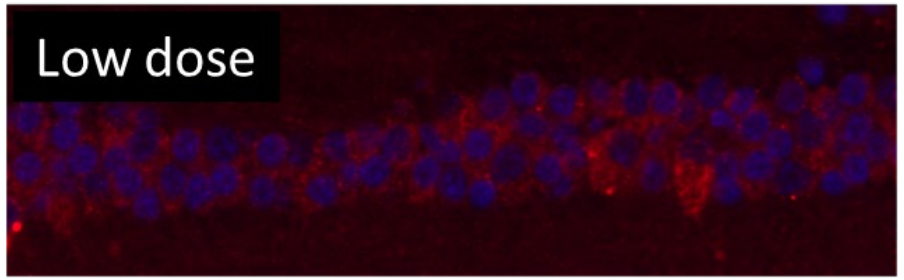


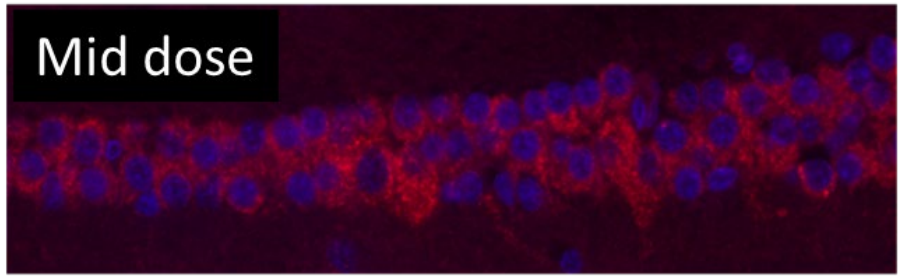
- STK-002 retinal tissue concentration quantitated by hybridization ELISA persisted at substantial levels at 4 and 8 weeks after mid and high dose/eye
- Dose-related increase in STK-002 signal observed by *in situ* hybridization with specific probe

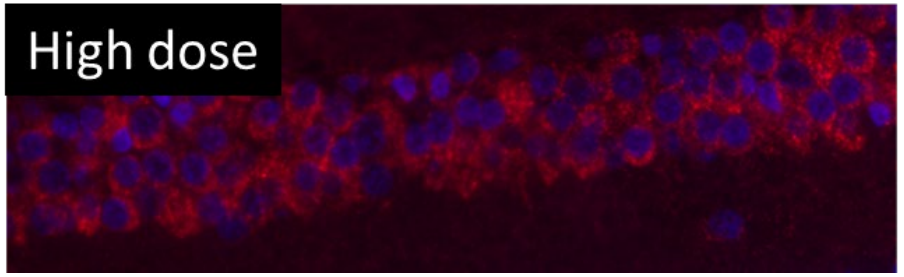
OPA1 Protein Qualitatively Increased in Cynomolgus RGCs



RGCs







Increasing STK-002 Dose

- Presented here are representative images taken in/near fovea
- Dose-related increase in OPA1 protein in RGCs was detected using immuno-fluorescence

Blue = nuclei
Red = OPA1 protein

40x magnification

Conclusions

- As previously shown, STK-002 induced reduction in inclusion of the NMD exon, increased productive *OPA1* mRNA and OPA1 protein in engineered human haploinsufficient cells and ADOA patient-derived fibroblasts, and increased mitochondrial respiration in the latter[1,2]

- IVT administration of STK-002 was well-tolerated in wild type cynomolgus monkeys and resulted in:
  - Dose-related increase in STK-002 in RGCs (the cells that are affected in ADOA)
  - Sustained ASO level in retinal tissue (at least 8 weeks after injection)
  - Dose-related reduction in NMD exon inclusion (evidence of target engagement) in retinal tissue
  - Dose-related increase in OPA1 protein in retinal tissue
  - OPA1 protein increase visualized in RGCs

- The current preclinical pharmacology studies support continued development of STK-002 for treatment of patients with ADOA

A. Venkatesh et al., 2020[1] and 2021[2], ARVO and ASGCT presentations