Antisense oligonucleotide mediated increase of OPA1 expression using TANGO technology for the treatment of autosomal dominant optic atrophy

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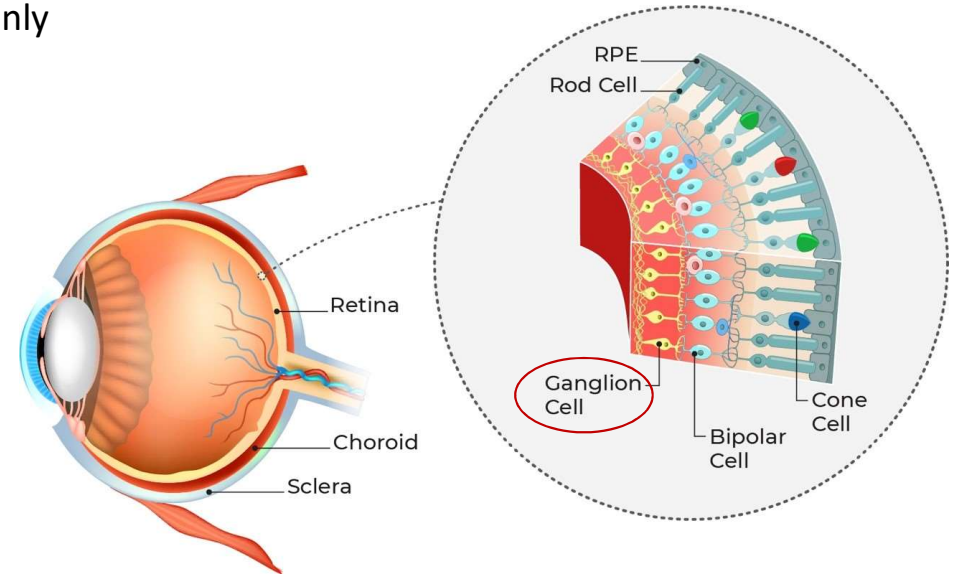
Autosomal dominant optic atrophy and *OPA1*

- Autosomal dominant optic atrophy (ADOA) is the most commonly diagnosed optic nerve disorder and is characterized by retinal ganglion cell loss
- Disease affects approximately 1 in 30,000 people and causes progressive and irreversible vision loss
- ADOA typically presents within the first decade of life
  - 80% of patients are symptomatic before 10 years of age
  - Mean age of onset is 7 years
- No therapeutic options available to ADOA patients



- 65-90% of cases are caused by mutations in the *OPA1* gene, which is a mitochondrial GTPase with a critical role in the maintenance of mitochondria structure and function
- Most *OPA1* mutations lead to haploinsufficiency resulting in a decrease to about 50% of the normal amount of OPA1 protein

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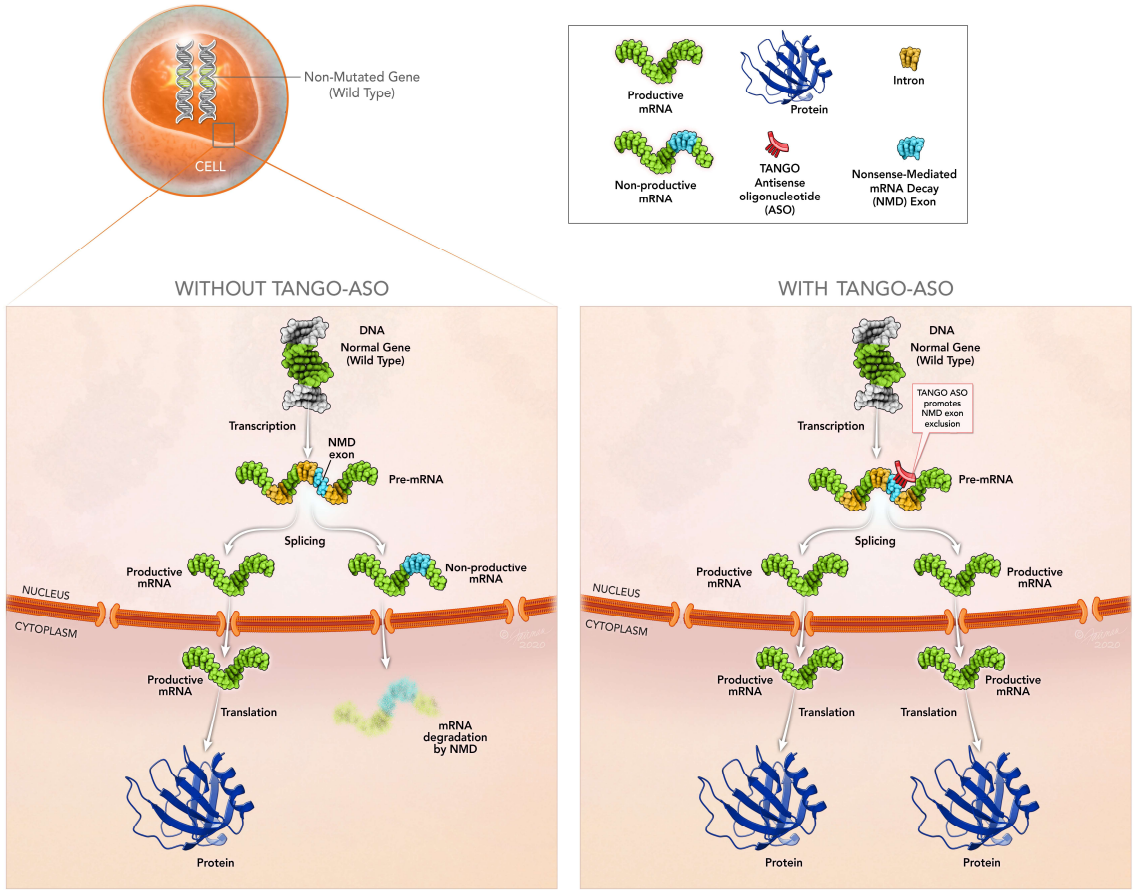
Applying TANGO for the treatment of autosomal dominant haploinsufficiency diseases

- 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



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Advantages of TANGO for the treatment of ocular diseases

- Intravitreal injection of ASOs permits diffusion throughout the eye and the ability to transduce retinal neurons
- Potential for long-term efficacy (up to 1 year in mouse retina) after a single intravitreal injection (Kach et al, ARVO Poster Presentation May 2019)
- No specialized formulation or encapsulation required for ASO therapy in the eye
- Potential to target genes with large coding domains that are not amenable to AAV-based gene therapy

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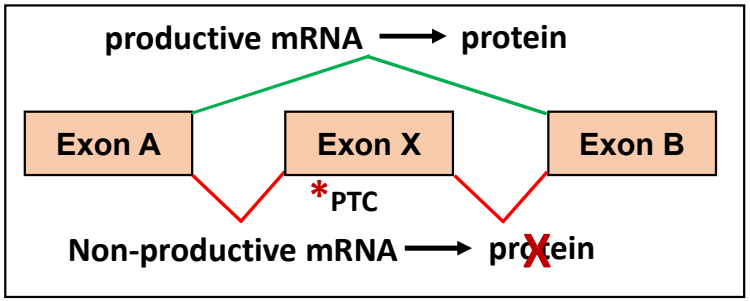
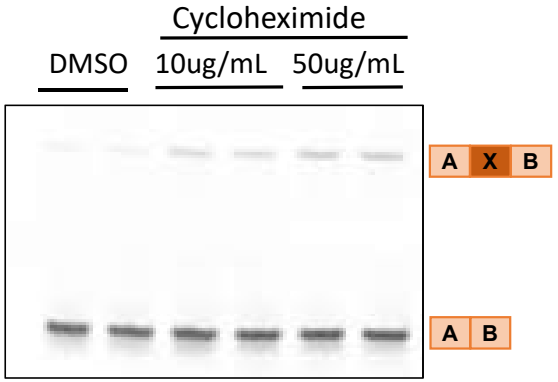
OPA1 non-productive splicing event identification and validation

- Novel exon inclusion event (Exon X) identified in the *OPA1* gene which leads to introduction of a premature termination codon (PTC)
- This produces a non-productive mRNA transcript that is degraded by non-sense mediated decay (NMD), producing no protein
- Inhibition of NMD with cycloheximide allows for evaluation of the true abundance of this event *in vitro*



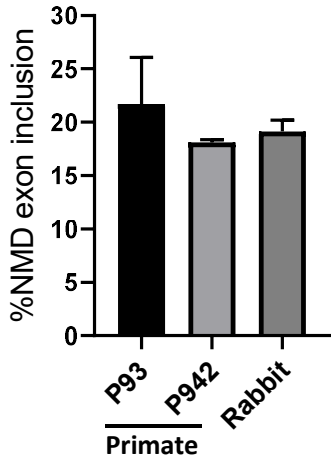


Human embryonic kidney 293 (HEK293) cells

***OPA1* non-productive splicing event was detected *in vitro* in various cell lines**

OPA1 non-productive splicing event conservation

- *OPA1* non-productive splicing event is conserved in non-human primates and rabbits
- Abundance of event was detected to be approximately 20% *in vivo* in primate and rabbit ocular tissue



Abundance of the event is likely to be higher *in vivo*, given that NMD is presumed active in the tissue

- Primate tissue: Posterior segment of eye from two stages of *Chlorocebus sabaeus* (green monkey) at 3 months and 2.6 years of age; N=1 animal/age. Graphed data represents average of OD and OS values for each animal
- Rabbit tissue: Retinae from adult female New Zealand white rabbits; N=3 animals. Graphed data represents average of OD and OS values for the three animals
- OD: oculus dextrus (right eye); OS: oculus sinister (left eye); P: post-natal day

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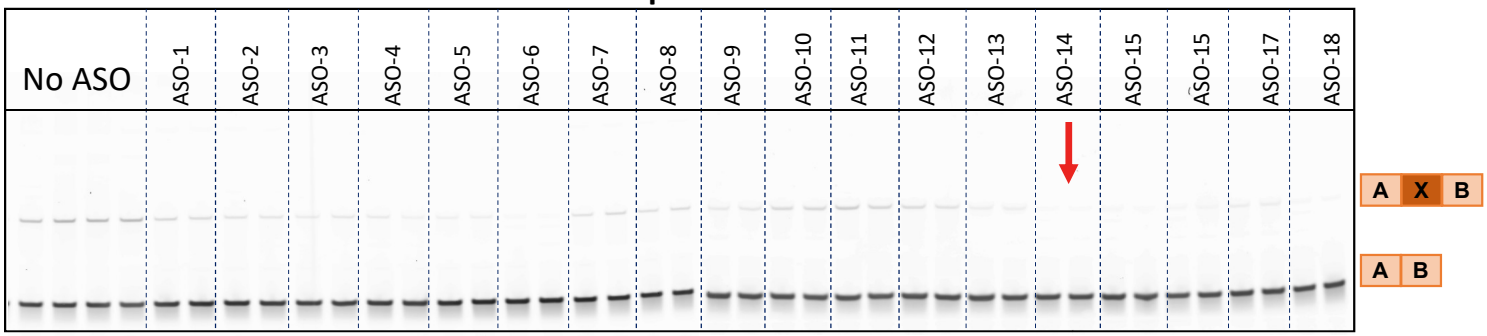
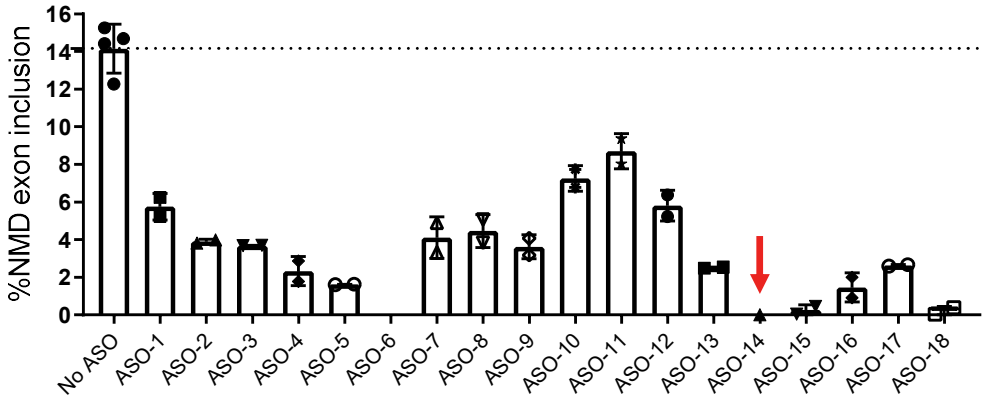
Selected *OPA1*-targeting ASOs reduce non-productive mRNA and increase productive mRNA *in vitro*

- Cells: HEK293 cells
- ASO conc.: 80nM
- Delivery method: Transfection (Lipofectamine RNAiMax)
- Time course: 24 hours
- Cells treated with cycloheximide (50ug/mL, 3hrs) for non-productive mRNA evaluation

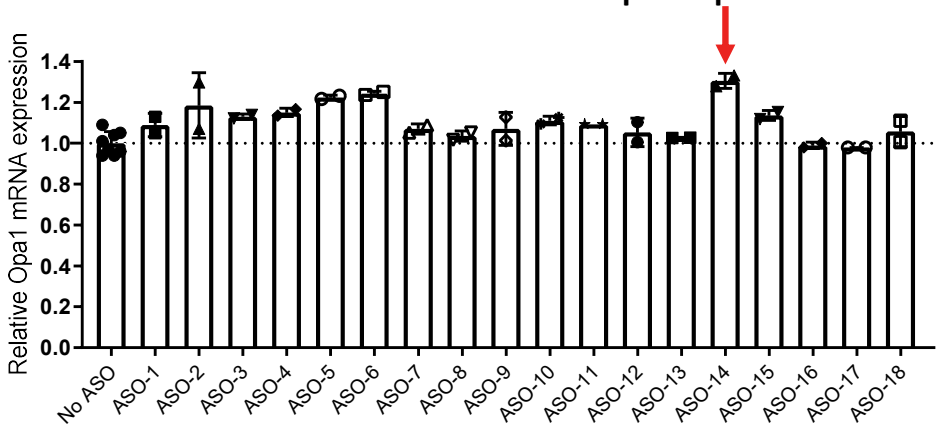
RT-PCR for non-productive *OPA1* mRNA



Non-productive *OPA1* mRNA Quantification



Productive *OPA1* mRNA – Taqman qPCR



ASO-14 (red arrow) reduces non-productive splicing and produces the most increase in *OPA1* mRNA levels (30%)

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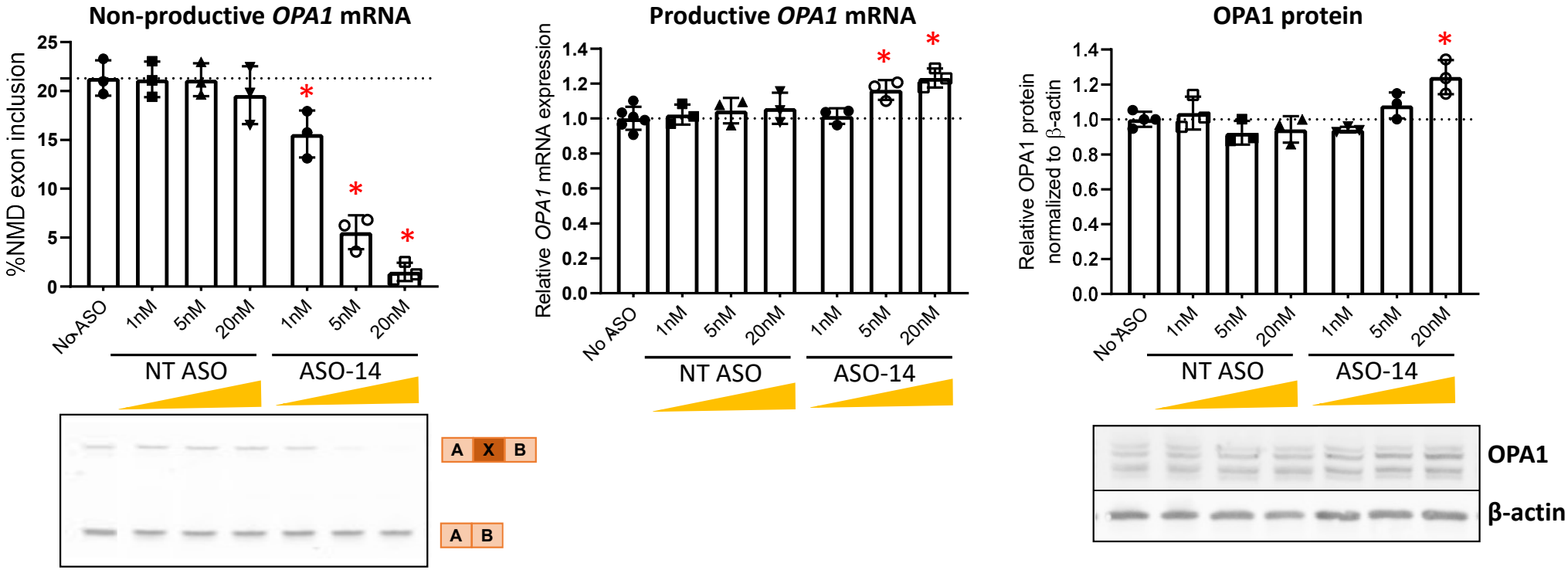
ASO-14 decreases non-productive *OPA1* mRNA and increases OPA1 expression in a dose-dependent manner *in vitro*



ASO-14 produces a dose-dependent increase in *OPA1* mRNA and protein levels *in vitro*

- Cells: HEK293 cells
- Delivery method: Transfection (Lipofectamine RNAiMax)
- Time course: 24 hours for RNA, 48 hours for protein
- Cells treated with cycloheximide (50ug/mL, 3hrs) for non-productive mRNA evaluation
- NT: non-targeting ASO; *P<0.05 by one-way ANOVA compared to "No ASO" group

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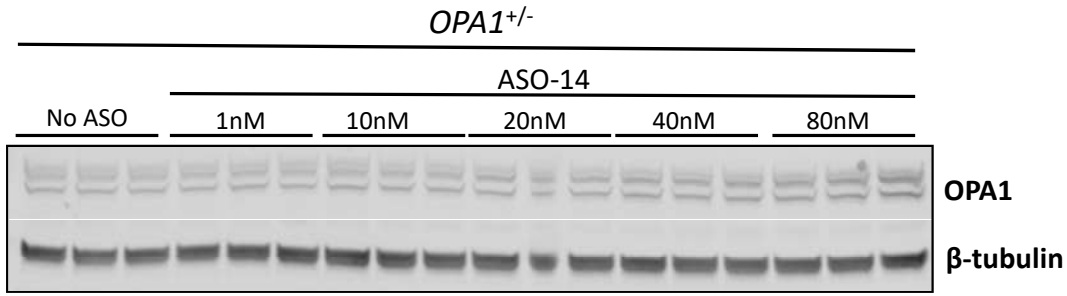
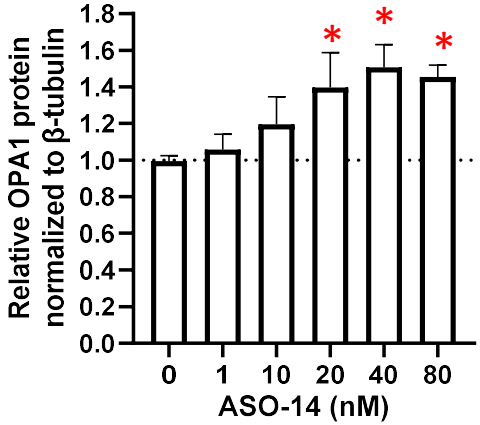
ASO-14 increases OPA1 protein expression in an *OPA1* haploinsufficient (*OPA1*$^{+/-}$) cell line

ASO-14 increases OPA1 protein levels in *OPA1*$^{+/-}$ HEK293 cells by ~50%, which translates to 75% of wild-type levels





- Cells: *OPA1* $^{+/-}$ HEK293 cells
- ASO conc.: 1, 10, 20, 40 and 80nM
- Delivery method: Transfection (Lipofectamine RNAiMax)
- Time course: 72 hours
- **P*<0.05 by one-way ANOVA compared to "No ASO" group

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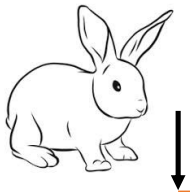
In vivo proof of concept study in wild-type rabbits

Study Design



Female NZW rabbits

Day 1
Single intravitreal (IVT) injection of vehicle/ASO (n=3/group)

Day 16
Euthanize animals and collect retinal tissue

Group	N	Treatment	Dose (ug/eye)	Dose volume (uL/eye)	Final conc. expected in vitreous* (uM)	Matrices collected (OU)
1	3	Vehicle (PBS)	N/A	30	N/A	Retinae split along the nasal-temporal axes, temporal half used for RNA, nasal for protein
2	3	NT ASO	110		12	
3	3	Test ASO – Low Dose	39		4	
4	3	Test ASO – High Dose	116		12	

*Final ASO conc. assumes vitreal volume of 1.5mL in rabbits
NT: non-targeting, OU: oculus utereque (both eyes)

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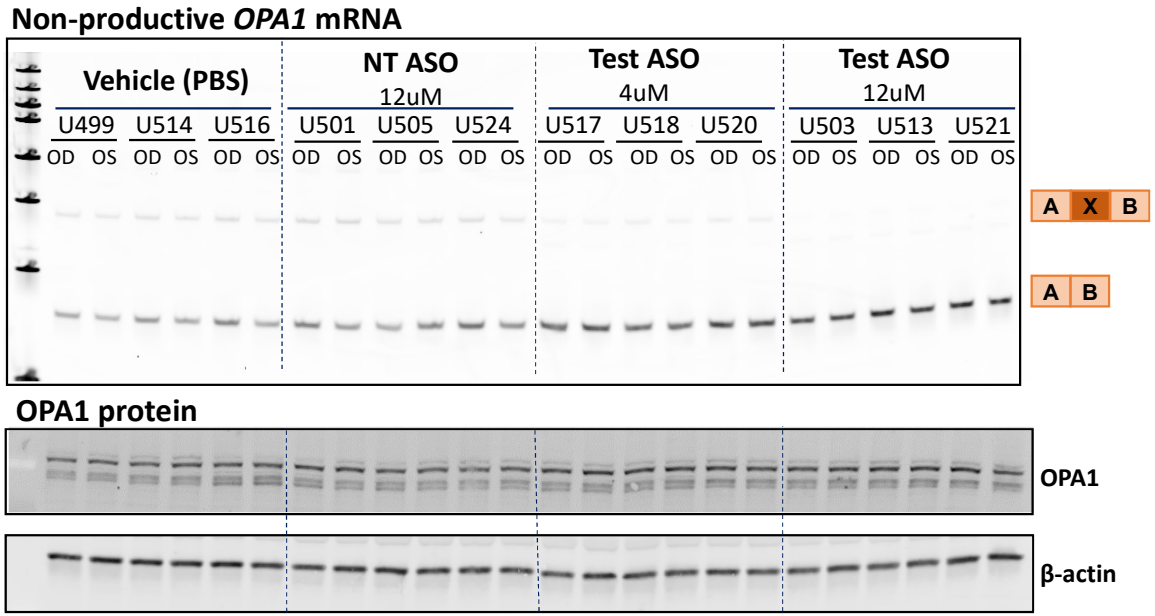
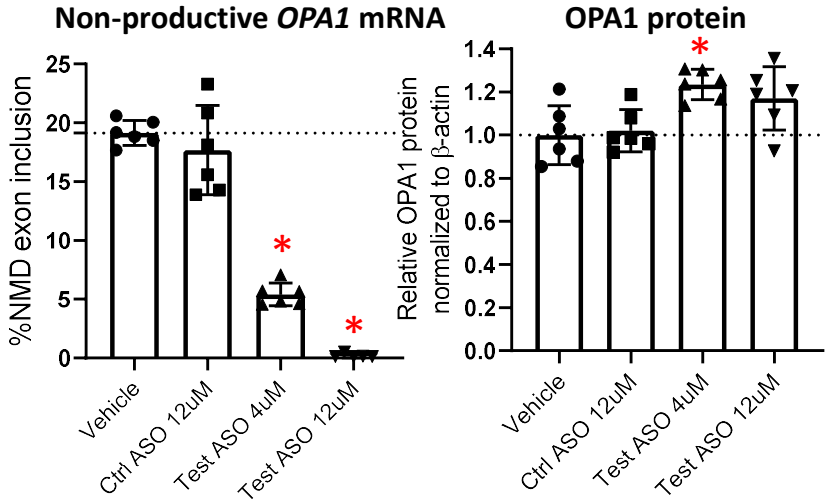
ASO increases OPA1 protein expression *in vivo* in wild-type rabbit retinae

Rabbit surrogate ASO decreases non-productive splicing and increases OPA1 protein expression in wild-type rabbit retinae following intravitreal injection. The test ASO was well tolerated for up to 15 days after injection





NT: non-targeting; OD: oculus dextrus (right eye), OS: oculus sinister (left eye)
*$P<0.05$ by one-way ANOVA compared to "No ASO" group

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Conclusions

Preclinical data support the potential use of Stoke's TANGO technology in ADOA

- ASO-mediated specific reduction in non-productive *OPA1* mRNA, increase in productive OPA1 mRNA and increase in OPA1 protein in a dose-dependent manner *in vitro*
- 50% increase in OPA1 protein levels in *OPA1*$^{+/-}$ cells, which translates to 75% of wild-type levels
- Reduction in non-productive mRNA and increase in protein *in vivo* in rabbit retinae
- Well-tolerated in wild-type rabbit for up to 15 days after intravitreal injection
- Approach allows leverage of the wild-type allele and can be used to potentially treat ADOA in a mutation-independent manner

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Questions?

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