# Antisense oligonucleotide mediated increase in OPA1 improves mitochondrial function in fibroblasts derived from patients with autosomal dominant optic atrophy (ADOA)

Association for Research in Vision & Ophthalmology Meeting 2021

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# Authors and disclosures

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**Disclosures:**
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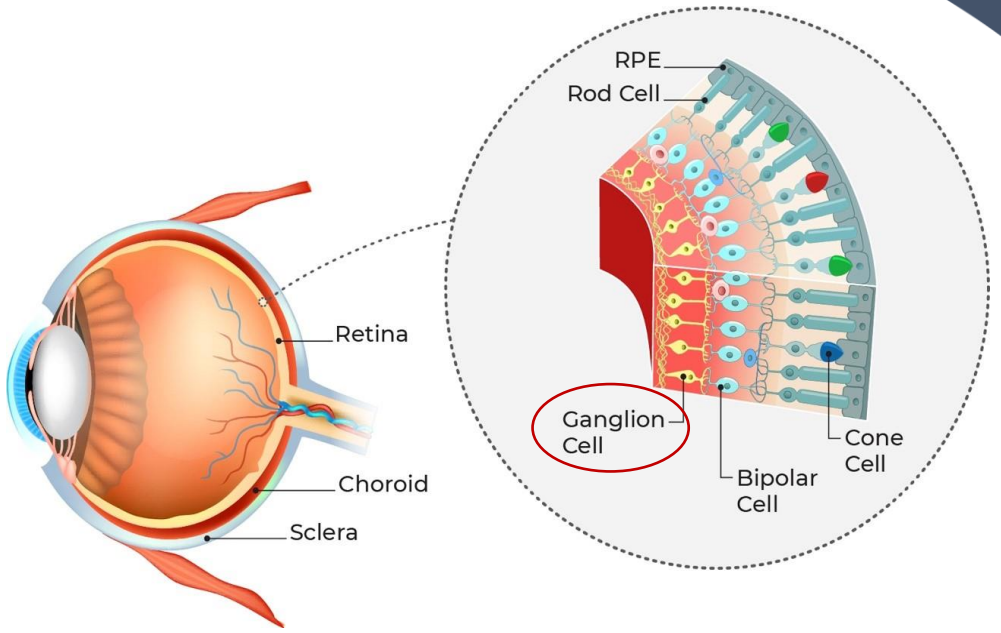
# OPA1 haploinsufficiency is the primary cause of autosomal dominant optic atrophy (ADOA)

- ADOA is the most common inherited optic nerve disorder and is characterized by retinal ganglion cell 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% decrease of normal OPA1 protein levels




- 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

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# Targeted Augmentation of Nuclear Gene Output

**Our compounds aim to restore protein levels by increasing protein production from the functional copy of a gene and:**

- Selectively boost expression only in tissues where the protein is normally expressed
- Offer one drug for diseases caused by many different mutations
- Apply to genes of diverse size: can be used to address small or large gene targets

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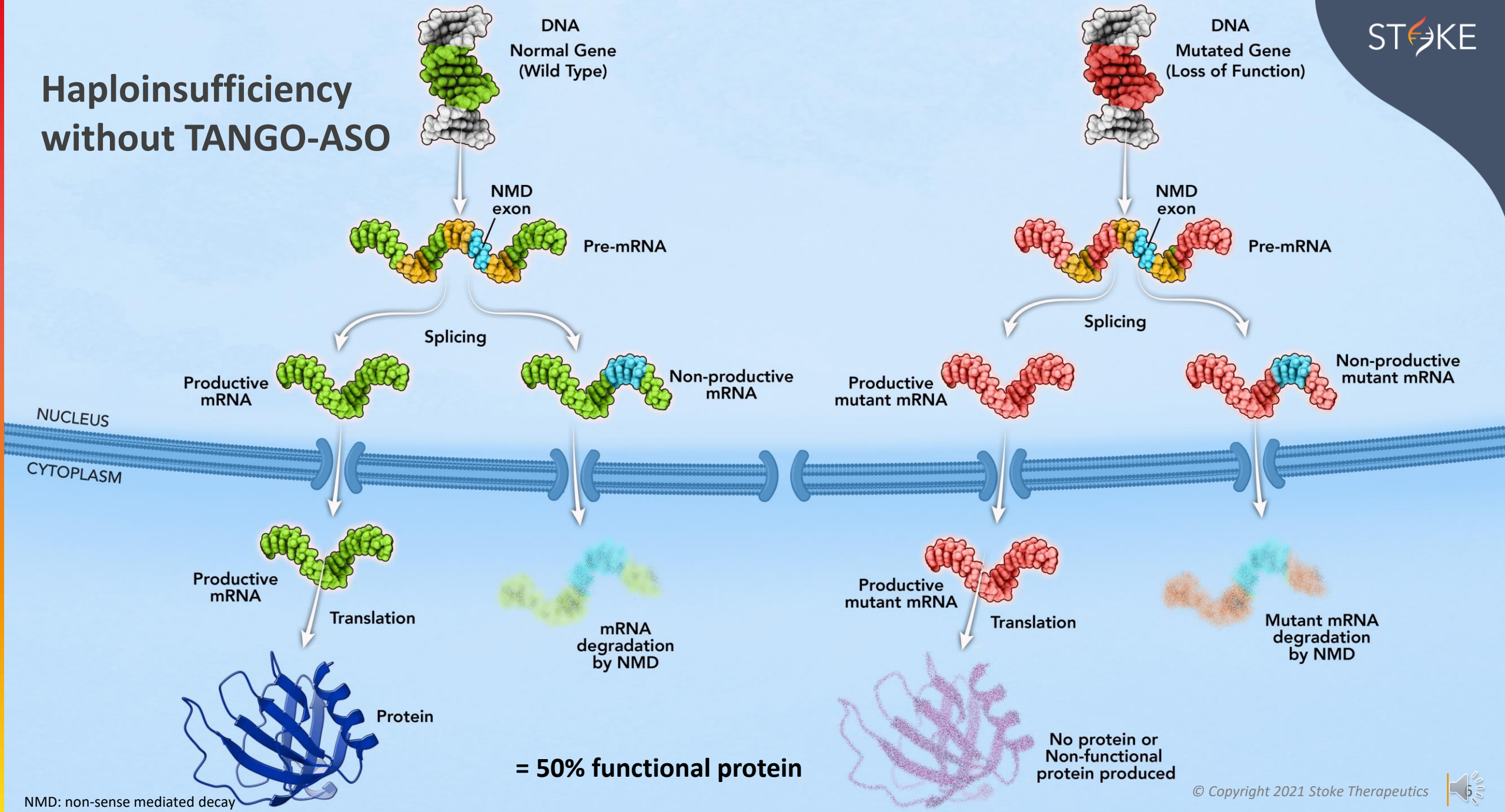
# Haploinsufficiency without TANGO-ASO




**= 50% functional protein**

NMD: non-sense mediated decay

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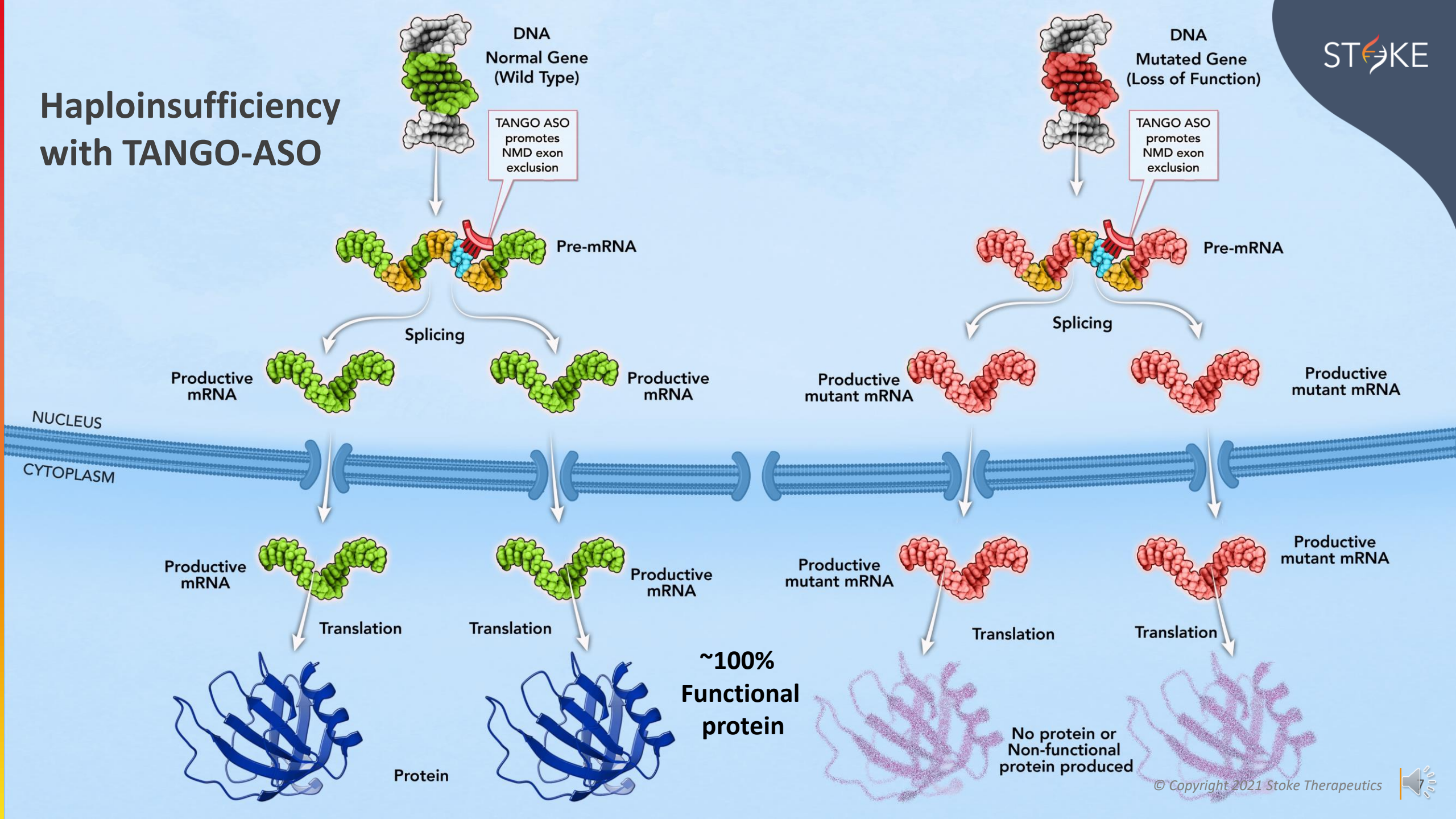
# Haploinsufficiency with TANGO-ASO

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# TANGO is ideally suited to the treatment of ocular diseases

- Intravitreal injection permits diffusion throughout eye to deliver ASOs to retinal cells
- Potential for long-term efficacy (up to 1 year in mouse retina) after a single intravitreal injection[1]
- No specialized formulation or encapsulation required for ASO therapy
- Potential to target genes with large coding domains and/or multiple protein isoforms that are not amenable to AAV-based gene therapy

[1]Kach et al, ARVO Poster Presentation, May 2019.

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# Previous preclinical data[1] support the potential use of TANGO in ADOA

- ASO-mediated specific reduction in NMD exon inclusion splicing in *OPA1* pre-RNA
- Dose-dependent increase in total *OPA1* mRNA and protein levels *in vitro*
- Reduction in non-productive splicing and increase in OPA1 protein levels *in vivo* in wild-type rabbit retinae
- Well-tolerated in wild-type rabbit for up to 28 days after intravitreal injection (unpublished data)

**Is TANGO a disease modifying approach for ADOA?**

[1] Venkatesh et al, ARVO and ASGCT 2020 meeting presentations; NMD: non-sense mediated decay

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# Primary fibroblast cells were obtained from skin biopsies of three ADOA patients

*OPA1* mutations in diagnosed ADOA patients:

- F34: c.1608+1delGTGAGG (canonical splice mutation)
- F35: c.2873_2876del (frameshift mutation)
- F36: c.635_636delAA (frameshift mutation)

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# Questions addressed in the presentation

- Do the ADOA patient fibroblast lines show reduced OPA1 expression consistent with haploinsufficiency?
  - Can TANGO ASO modify OPA1 expression in ADOA patient cells?
- Is reduced OPA1 expression associated with a decrease in mitochondrial function?
  - Can TANGO ASO alter mitochondrial function in ADOA patient cells?

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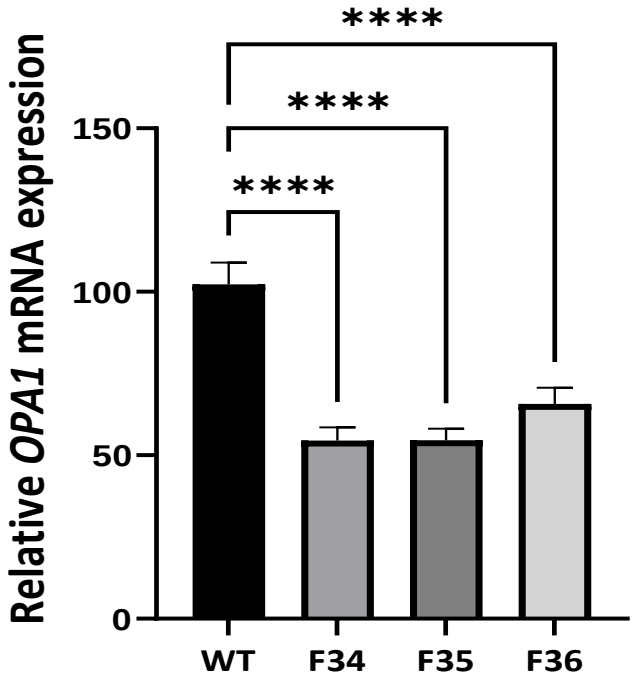
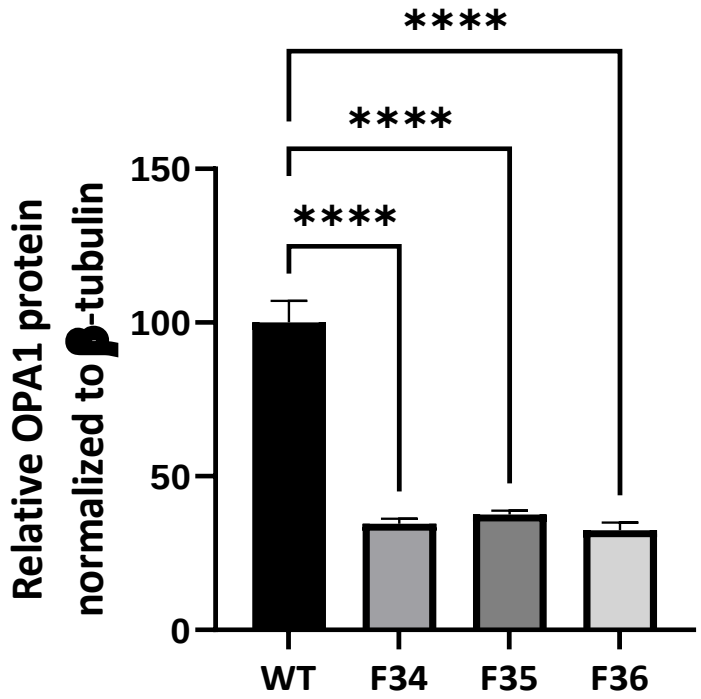
# All ADOA patient fibroblast lines show reduced *OPA1* mRNA & protein levels

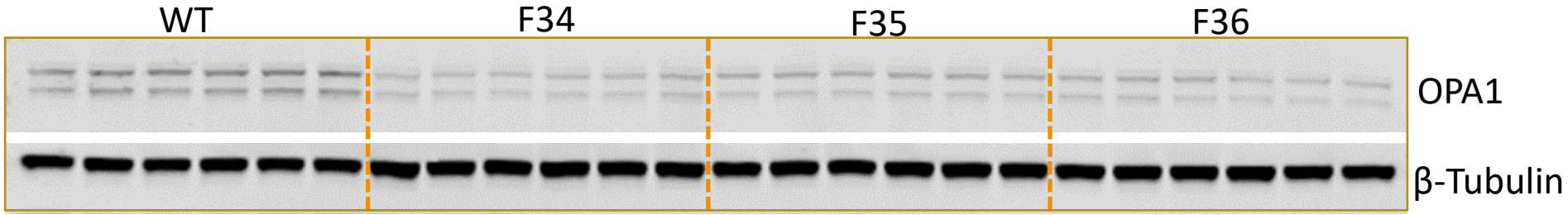
**A**




**B**




**C** Representative immunoblot of OPA1 protein expression level in *OPA1* +/- fibroblast cells




WT: Wild-type; histograms show mean ± SEM of 3 independent experiments; one-way ANOVA compared to WT group (****$P$<.0001).

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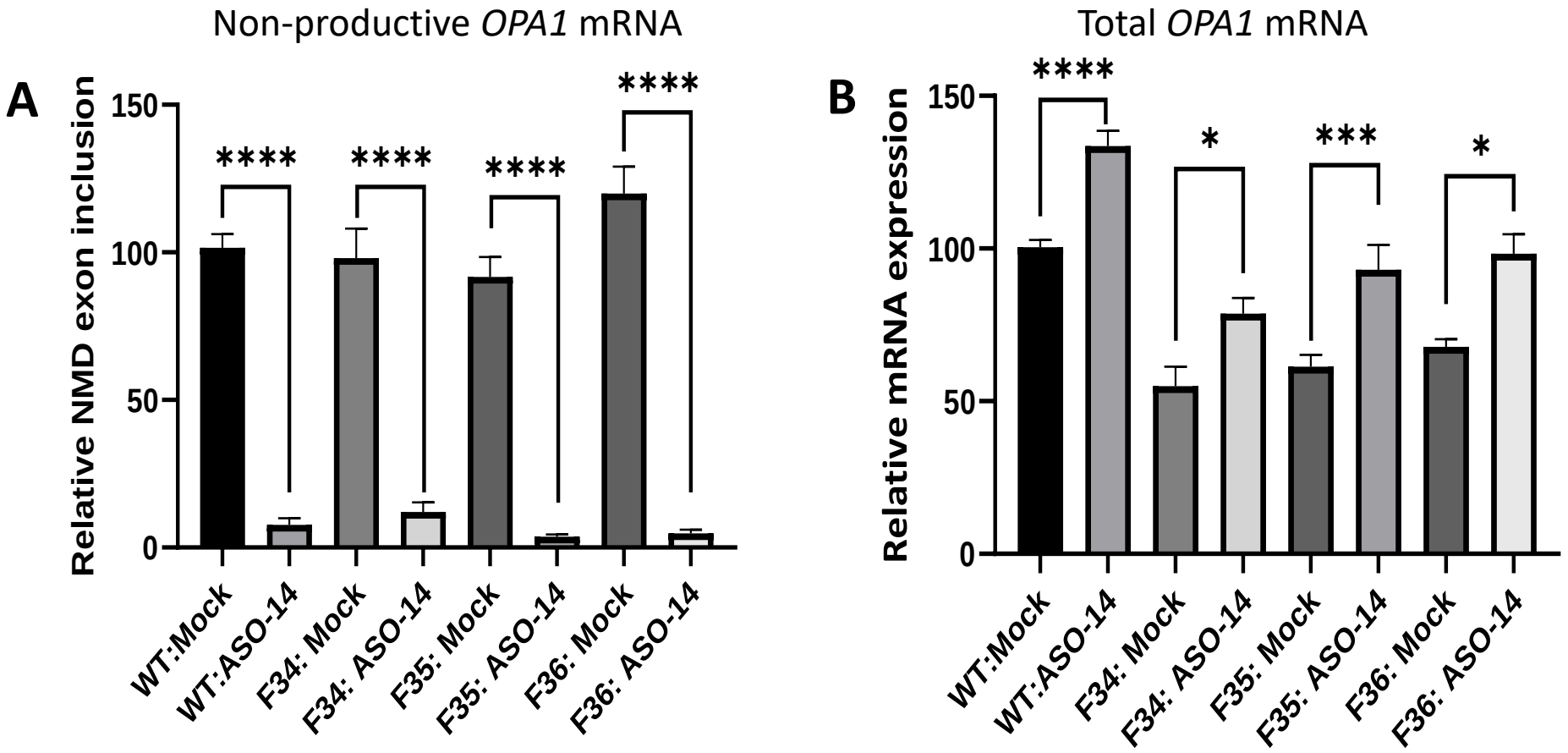
# TANGO ASO reduces non-productive exon inclusion and increases *OPA1* mRNA expression in all cell lines




**Reduction in non-productive splicing increases total *OPA1* mRNA levels in all patient cell lines**

Fibroblast cells were transfected with ASO-14 (40nM). RNA was isolated 24 hrs. after transfection and analyzed. For non-productive OPA1 mRNA measurement, cells were treated with cycloheximide (50µg/mL) for 3 hrs. prior to RNA isolation.

NMD: non-sense mediated decay, WT: Wild-type; histograms show mean ± SEM of 2-3 independent experiments; one-way ANOVA vs. Mock for respective cell line (*$P$<.05; ***$P$<.001; ****$P$<.0001).

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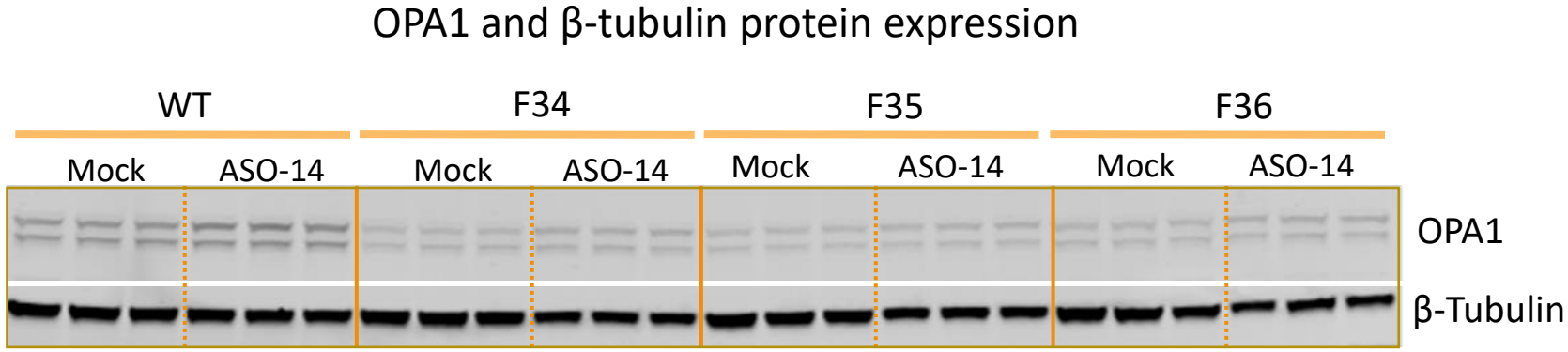
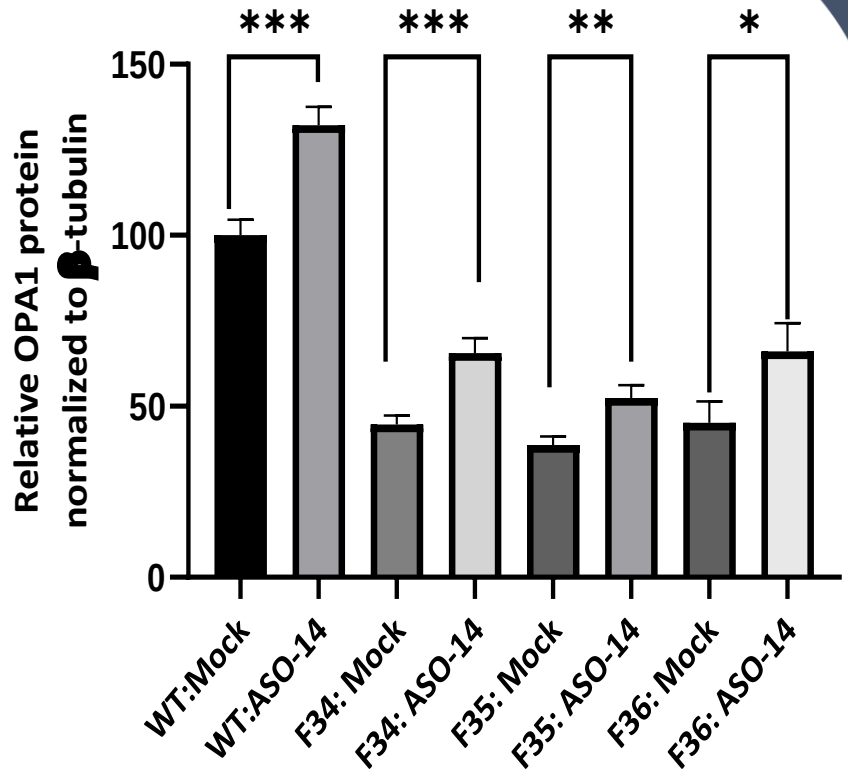
# TANGO ASO increases OPA1 protein expression for all three mutations




**ASO-14 produces ~35-47% increase in multiple OPA1 protein isoforms**

Fibroblast cells were transfected with ASO-14 (40 nM). Immunoblot was performed 72 hrs. post transfection with antibodies targeting OPA1 and β-tubulin
WT: Wild-type; histograms show mean ± SEM of 3 independent experiments; unpaired t-test vs. Mock for respective cell line (*$P$<0.05, ** $P$<0.01, ***< 0.001)

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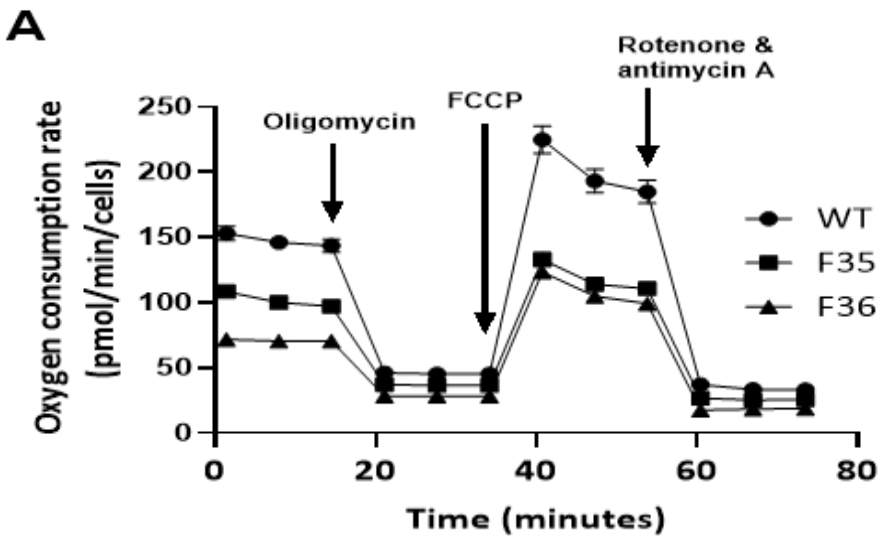
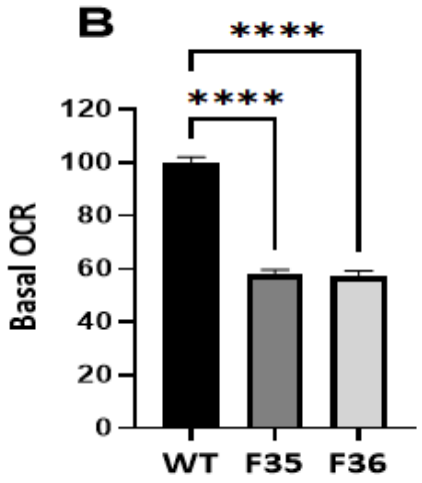
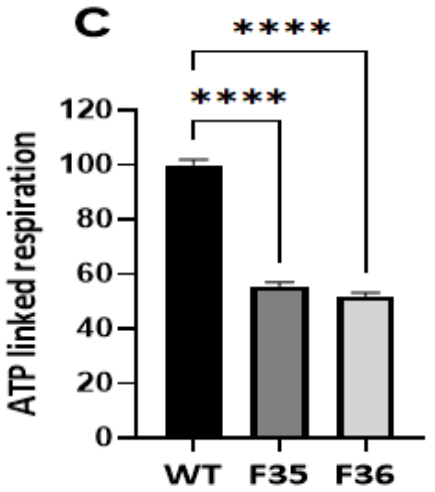
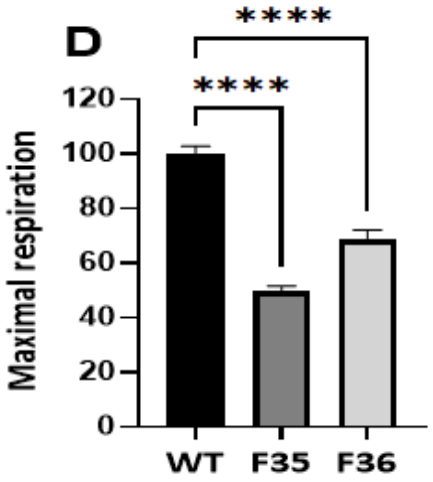
# Patient fibroblast cell lines show deficiencies in mitochondrial bioenergetics

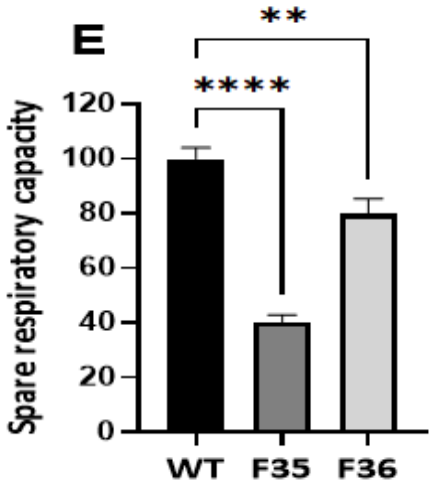
## ADOA patient cell lines show impaired mitochondrial bioenergetics




Units are pmol/min/cells. Oxygen Consumption Rates (OCR) normalized to total cell count and plotted relative to wild-type (WT);
Histograms show mean ± SEM of >18 individual measurements from 2 independent experiments; one-way ANOVA vs. WT (** *P*<.01; **** *P*<.0001).

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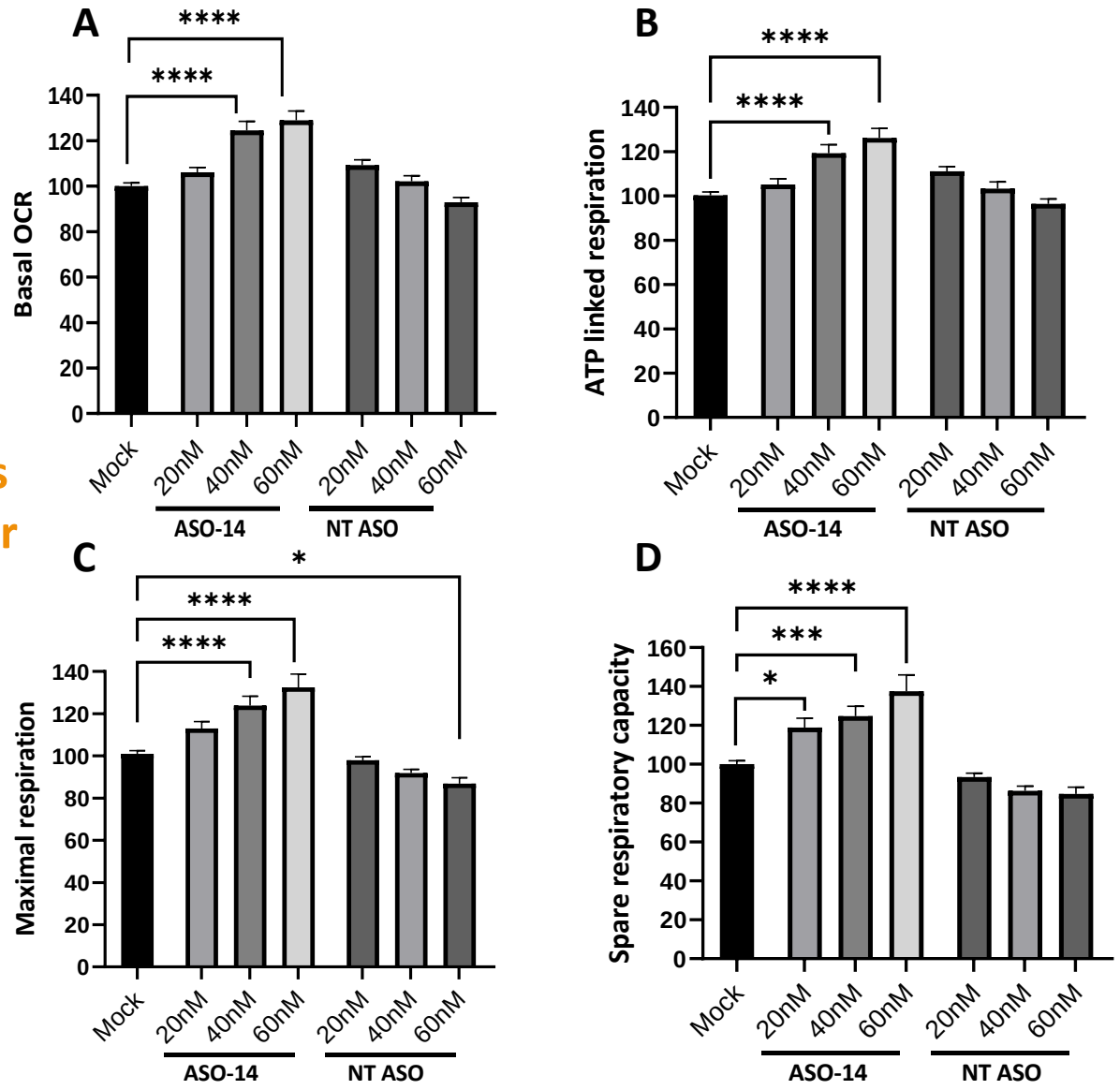
# TANGO ASO increases mitochondrial bioenergetics in F35 patient cell line

**ASO-14 increases mitochondrial bioenergetics in a dose-dependent manner**




Units are pmol/min/cell; Oxygen Consumption Rates (OCR) normalized to total cell count and plotted relative to Mock (No ASO); NT: Non-targeting.
Histograms show mean ± SEM of >20 individual measurements from at least 3 independent experiments; one-way ANOVA vs. Mock (*$P$<.05; ***$P$<.001; ****$P$<.0001); NT ASO targets an unrelated gene.

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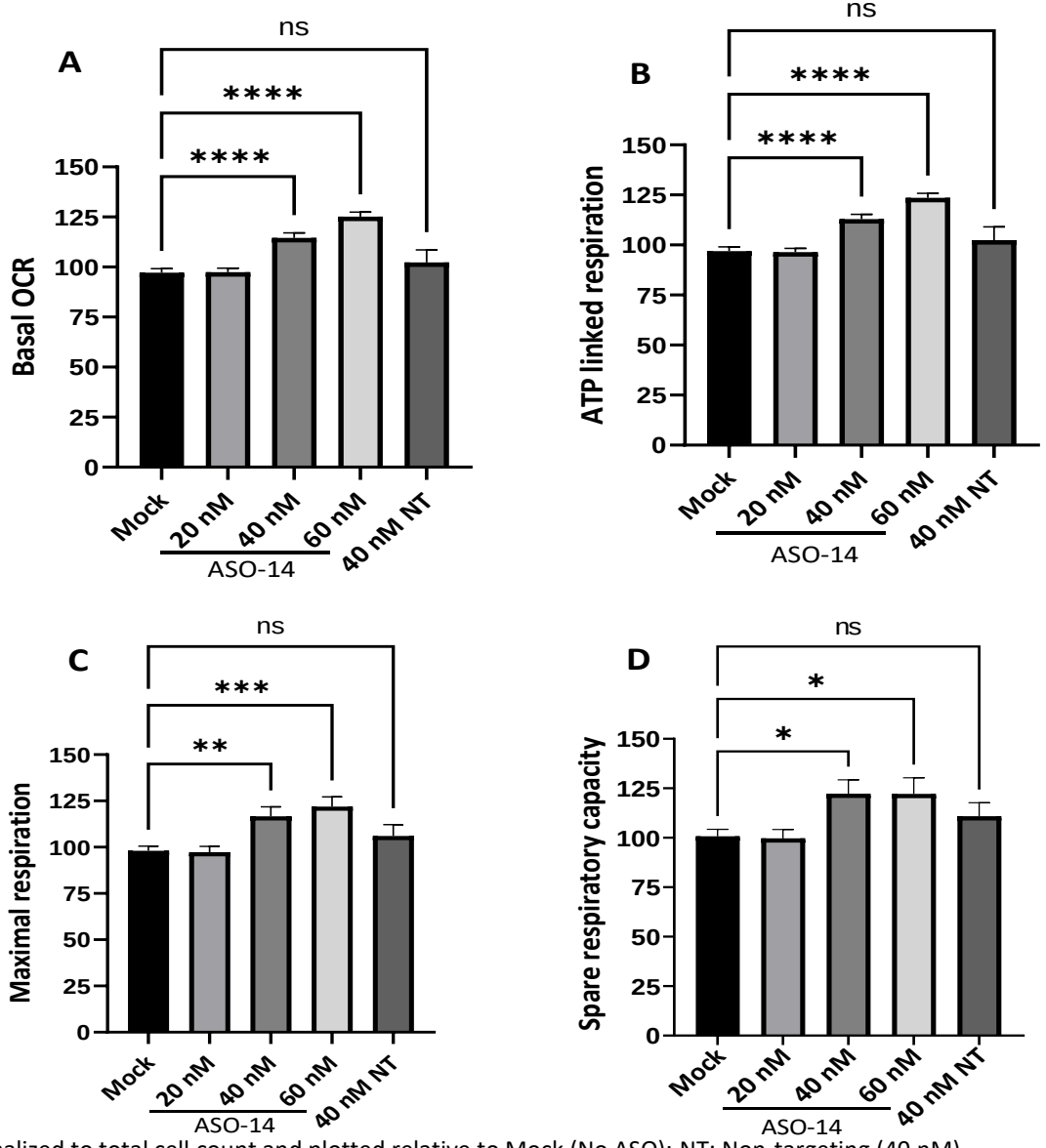
# TANGO ASO increases mitochondrial bioenergetics in F36 patient cell line

**Dose-dependent increase in mitochondrial bioenergetics is mutation-independent**




Units are pmol/min/cells; Oxygen Consumption Rates (OCR) normalized to total cell count and plotted relative to Mock (No ASO); NT: Non-targeting (40 nM).
Histograms show mean ± SEM of >20 individual measurements from 2-5 independent experiments; one-way ANOVA vs. Mock (*$P$<.05; ** $P$<.01; *** $P$<.001 **** $P$<.0001).

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# Preclinical data support TANGO as a potential disease modifying approach to treat ADOA

- TANGO ASO reduced non-productive exon inclusion and increased total *OPA1* mRNA expression in three patient fibroblast cell lines with different mutations.
- TANGO ASO increased expression of multiple OPA1 protein isoforms in patient fibroblast cell lines.
- TANGO ASO supported a dose-dependent increase in mitochondrial respiration in patient fibroblast cell lines.

**TANGO upregulation of OPA1 potentially provides a disease modifying approach to treat ADOA in a mutation-independent manner.**

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# Questions?

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