SUPPLEMENTARY METHODS

Oligonucleotides

Stereopure oligonucleotides were synthesized as described previously[1-3]. Stereorandom oligonucleotides (MALAT1-181) were synthesized using standard procedures. The non-targeting control (NTC) used in this study was a stereorandom oligonucleotide with 2′-O-methyl-modified ribose in the terminal 5 nucleotides and 2'-deoxyribose in the central 10 nucleotides with the sequence 5′- CCUUCCCTGAAGGTTCCUCC -3′. Endotoxin levels for all oligonucleotides used in in vivo studies are in Table S1.

Measurement of Initial Velocity

RNase H1 activity was assessed with the substrate: enzyme ratio of 250:1 as described previously.[1] The sequence for the *Malat1* RNA surrogate used in these experiments was 5′- CUGAGUCAUAACCAGCCUGGCA -3′.

Cell Lines

iCell neurons (Cellular Dynamics, Madison, WI) were cultured at 400,000 cells/well. After 48 h, fresh medium was added containing 10, 30, 100, 300, 1000, or 3000 nM oligonucleotides, either stereopure or stereorandom, in duplicate. Four days after treatment, cells were lysed, RNA was extracted, and *MALAT1* expression was determined by quantitative polymerase chain reaction (qPCR). RNA isolation, cDNA synthesis, quantification, and normalization of RNA were performed according to routine practices.

Human Donor Eyes

Human eyes were procured from Lions Gift of Sight (Saint Paul, MN) with the written consent of the donor or the donor's family for use in medical research in accordance with the Declaration of Helsinki. Lions Gift of Sight is licensed by the Eye Bank Association of America (accreditation #0015204) and accredited by the FDA (FDA Established Identifier 3000718528). Donor tissue is considered a pathological specimen and is, therefore, exempt from the process of Institutional Review Board approval.

Ex Vivo Human Retina Culture

Whole human eyes were procured (Lions Gift of Sight, Saint Paul, MN) and immediately placed in Dulbecco’s modified eagle medium (DMEM) with 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin. Approximately 15-24 hours post-enucleation, retinas were dissected into pieces of approximately equal size, added to a 96-well dish containing media described above, and treated with PBS or antisense oligonucleotide under free uptake (gymnotic) conditions for 48 hours. Specifically, retinal dissection was performed by removal of the cornea, iris and lens to expose the eye cup with surgical scissors. The eye cup was inverted over the lid of a 96-well dish, and using sterile forceps, the vitreous and retina were teased away from the choroid and placed onto the lid of the dish. A sterile scalpel was used to slice through the vitreous (avoiding the retina) to dissociate the vitreous gel matrix. A surgical forcep and scissors were then used to pick up and cut, respectively, tear-drop sized portions of vitreous containing retina and added to each treatment well of the 96-well dish. Retinal pieces were cut at random across the retinal quadrants since *MALAT1* is expressed ubiquitously. Dose levels were chosen based on in vitro activity experiments. RNA was extracted and RNA levels were evaluated as described below.

Quantification of RNA

qPCR was performed in a reaction mixture including diluted sample (4 µL from a mix of 20 µL of cDNA and 30 µL of water) using either mouse *Malat1* primers (Thermo Fisher Mm01227912_s1) for rodent samples or human *MALAT1* primers (Thermo Fisher Hs00273907-s1) for NHP and human samples in addition to the normalizer primers for mouse *Hprt* (IDT, forward primer CAA ACT TTG CTT TCC CTG GTT, probe sequence ACC AGC AAG CTT GCA ACC TTA ACC, reverse primer TGG CCT GTA TCC AAC ACT TC) or human *HPRT* (Thermo Fisher Hs02800695_m1). The thermocycling conditions for PCR include 40 cycles of 95°C for 3 minutes; 95°C for 10 seconds; and 60°C for 30 seconds.

Statistical Analysis

All statistical analyses were conducted using R version 3.6.3[4]. Linear models were built using the *stats* package. Sigmoidal dose-response models were built using the *drc* package. Analysis of variance was performed using the *aov* package; for analysis of covariance a same-slope model was employed. Summary statistics for data or model parameters were given as the mean or median with 95% confidence intervals. We report *P* values for all null-hypothesis significance tests in the text. Graphs were generated using the package *ggplot2*, and model fits were accompanied by their 95% confidence intervals whenever possible.

RNA Isolation and cDNA Synthesis

Frozen tissue was added to 700 µL of TRIzol and homogenized for 3 minutes. Bromochloropropane was added to each sample, which was shaken vigorously and

centrifuged at 4000g for 5 minutes. Supernatant (250 µL) was transferred to the binding plate from SV96 total RNA extraction kit (Promega) and RNA was extracted per protocol. cDNA was synthesized by adding 3 µL of total RNA to a 20 µL reverse-transcription reaction using the High-Capacity cDNA Reverse-Transcription Kit (Thermo Fisher #4368814).

Quantification/Normalization of RNA

qPCR was performed in reaction mixture including diluted sample (4 µL from a mix of 20 µL of cDNA and 30 µL of water), *Malat1* primers (Thermo Fisher Mm01227912_s1), and the normalizer primers for *HPRT* (IDT, forward primer CAA ACT TTG CTT TCC CTG GTT, reverse primer TGG CCT GTA TCC AAC ACT TC). The thermocycling conditions for PCR include 40 cycles of 95°C for 3 minutes, 95°C for 10 seconds, and 60°C for 30 seconds.

Animals and IVT Injections

All animal work was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the institutional animal care and use committee of Biomere - Biomedical Research Models, Inc. (Worcester, MA). Both C57BL/6 mice and non-human primates (NHPs) were given oligonucleotide via IVT injection. Mice and NHPs were on a 12-hour light-dark cycle. Food (monkey diet 5038 or lab diet 5001) and water were available ad libitum. All injections were performed by Ora, Inc. or Biomere biomedical by veterinary staff (mice) or board-certified veterinary ophthalmologist (NHP).

C57BL/6 female mice (The Jackson Laboratory, Bar Harbor, ME) were ~8 weeks of age at the time of dosing. Mice were anesthetized with 30-40 mg/kg ketamine and 0.5-10 mg/kg xylazine. While mice were anesthetized, a 3 µL drop of 0.5% proparacaine hydrochloride was applied to both eyes. A 100 µL NanoFil syringe with a 33-gauge needle was inserted into the vitreous, 3 mm posterior to the limbus taking care to avoid the iris or lens. The test article (1 µL) was injected into the vitreous of the eye by using a micromanipulator and microinjection pump. Following injection, the needle was left in place for approximately 30 seconds. Test article was injected into each eye of all mice. Antibiotic ointment was applied to the eyes after injection. After the procedure, mice were monitored until recovered. Mice received either 0.5, 5, 15, or 50 µg per injection depending on the experiment.

Male NHPs used in this study were non-naïve cynomolgus macaques weighing ~2.5-5.0 kg at the time of dosing. All animals had at least a 30-day washout period prior to dosing. NHPs were anesthetized with ketamine (5-15 mg/kg) via intramuscular injection. While the animals were anesthetized, 2-5 drops of 0.5% proparacaine was applied to both eyes for anesthesia. After ~2 minutes, 1-2 drops of Betadine (5%) were added to each eye and left for ~5 minutes. Excess was wicked away with an ocular absorbent spear and rinsed with saline. The eyes were held open with a speculum and positioned into place with a cotton-tipped applicator. A 28-30-guage insulin syringe (U-100) was used to inject 50 µL into the eye at a 45-degree angle pointed toward the optic nerve (caution was taken to avoid touching the lens). Following the injection, the needle was slowly removed, and the eye was monitored for efflux. Antibiotic ointment was applied immediately after injection, and animals were monitored until recovery. NHPs received

45, 150 or 450 μg per injection. The lowest active dose in our mouse studies was 0.5 μg. The equivalent NHP dose (assuming a NHP vitreous volume of 3 mL, 600-times greater volume than mouse) is 300 μg. We chose two doses below and one dose above the lowest mouse-active dose.

In vivo endpoints

In vivo experiments were evaluated for potency, efficacy and durability. Potency is defined as to the relative decrease of *Malat1* RNA following treatment with one oligonucleotide compared to another. For example, if 5 μg of oligo1 leads to a greater depletion of *Malat1* RNA than 50 μg of oligo2, then oligo1 is more potent than oligo2. Efficacy is defined as the relative amount of *Malat1* RNA that is depleted at a given concentration of oligonucleotide. For example, if 5 μg oligo1 leads to a greater depletion of Malat1 RNA than 5 μg of oligo2, then oligo1 is more efficacious than oligo2. Durability is defined as the length of time *Malat1* RNA remains at or below 50% of its normal level of expression.

Tissue Collection

Animal eyes were enucleated and immediately frozen on dry ice. For mouse studies, each frozen globe was bisected along the coronal plane to separate what we are calling the “anterior portion” (cornea, iris, lens, partial sclera) and the “posterior portion” (retina, choroid, sclera) of each eye. For NHPs, the enucleated eyes were dissected either as anterior and posterior portions (as described above) or were dissected to isolate retina, choroid/sclera, cornea, iris, and vitreous tissues. Each tissue was placed in a pre-labeled, pre-weighed 1.5 mL Eppendorf tube and stored at –80°C until processed.

Histology

Whole animal eyes were enucleated, placed in Davidson's fixative for 24 hours, transferred to 70% ethanol, and then processed and embedded in paraffin. The tissue was cut into 5 µm sections and evaluated by using ViewRNA Tissue Core Kit 96 assay (Thermo Fisher, cat#:19942) for *MALAT1* RNA (Thermo Fisher, cat#:VB6-17323) or oligonucleotide sequence–specific ViewRNA (Thermo Fisher, custom design) to detect oligonucleotides.

Quantification of *MALAT1* oligonucleotides by hybridization ELISA

We utilized the following probes to selectively quantify the oligonucleotides by hybridization ELISA: Malat1-Univ-Capture probe /5AmMC12/G+A+G+T+C+A+T+AA; Malat1-Univ-Detection probe CC+AGC+CT+GG/3BioTEG/ (Integrated DNA Technologies, Coralville, IA). We coated maleic anhydride-activated 96-well plates (Pierce 15110) with 50 µL of capture probe at 500 nM in 2.5% $NaHCO_3$ (Gibco, 25080-094) for 2 h at 37°C. The plate was then washed 3 times with PBST (PBS + 0.1% Tween-20), blocked with 5% fat free milk-PBST at 37°C for 1 h. Payload oligonucleotide was serially diluted into matrix. This standard together with original samples were diluted with lysis buffer so that the oligonucleotide amount in all samples was less than 50 ng/mL. 20 µL of diluted samples were mixed with 180 µL of 333 nM detection probe diluted in PBST, then denatured (65°C, 10 minutes, 95°C, 15 minutes, 4°C, ∞). 50 µL of the denatured samples were distributed in blocked ELISA plates in duplicates, and incubated overnight at 4°C. After 3 washes with PBST, 50 µL of 1:2000 streptavidin-AP (Southern Biotech, 7100-04) in PBST was added, 50 µL per well and incubated at room temperature for 1 h. After extensive washes with PBST, 100 µL of AttoPhos (Promega

S1000) was added, incubated at room temperature in the dark for 10 minutes and read on the plate reader (Molecular Device, M5) fluorescence channel: Excitation: 435 nm, Emission: 555 nm. The oligonucleotide in samples were calculated according to standard curve by 4-parameter regression. The lower limit of detection was 1.25 µg oligonucleotide/g of tissue.

Pharmacokinetic (PK)-pharmacodynamic (PD) analysis

For PK-PD analyses, the concentration of oligonucleotide detected in a specific tissue or portion (eg, posterior portion of mouse eye) as measured by hybridization ELISA (see above) were plotted with respect to quantified and normalized expression of *Malat1* RNA (see above) in the same tissue at the same time.

SUPPLEMENTARY REFERENCES

1. Iwamoto N, Butler DCD, Svrzikapa N, et al. Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. *Nature biotechnology.* 2017;35(9):845-851.
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