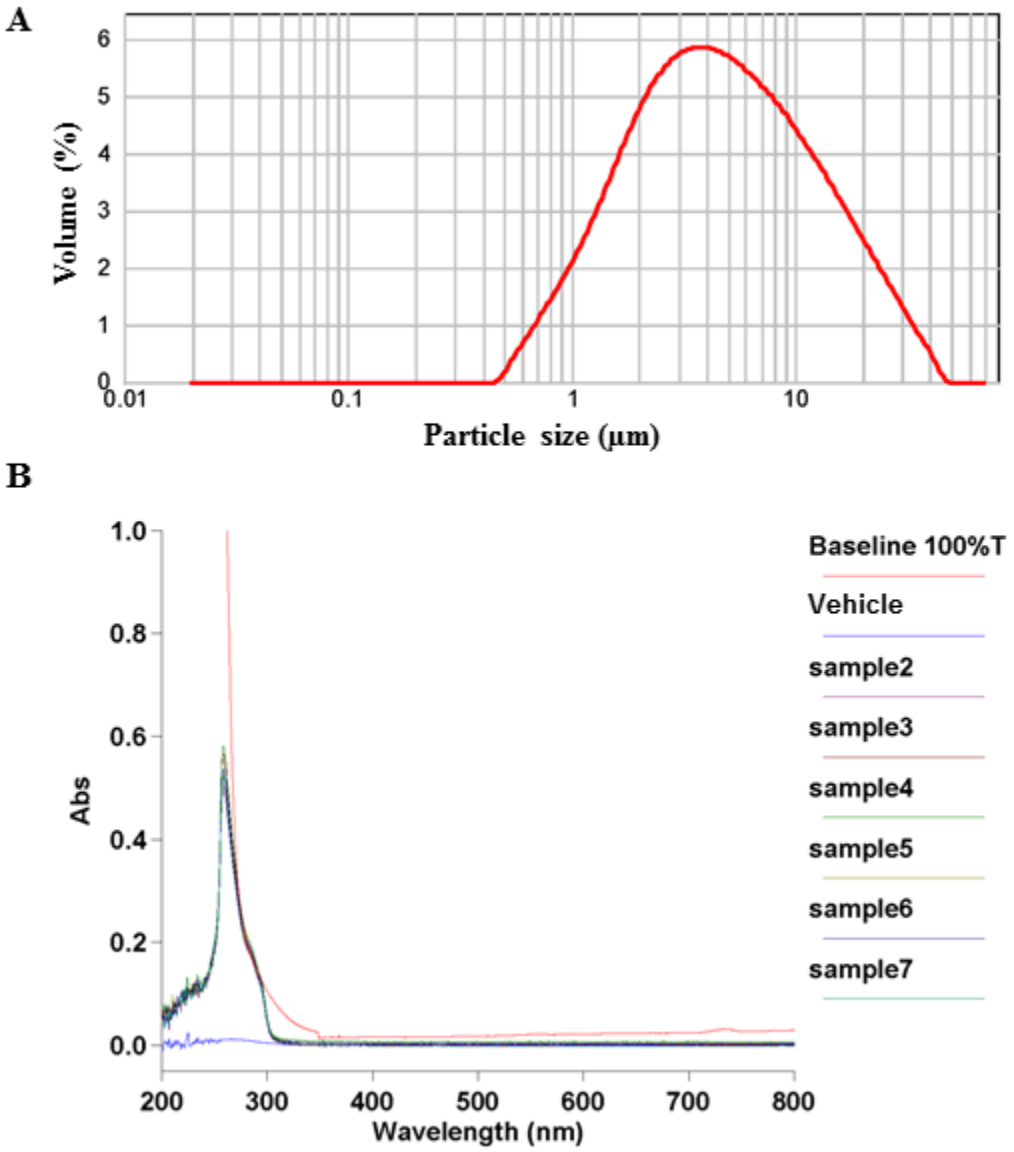
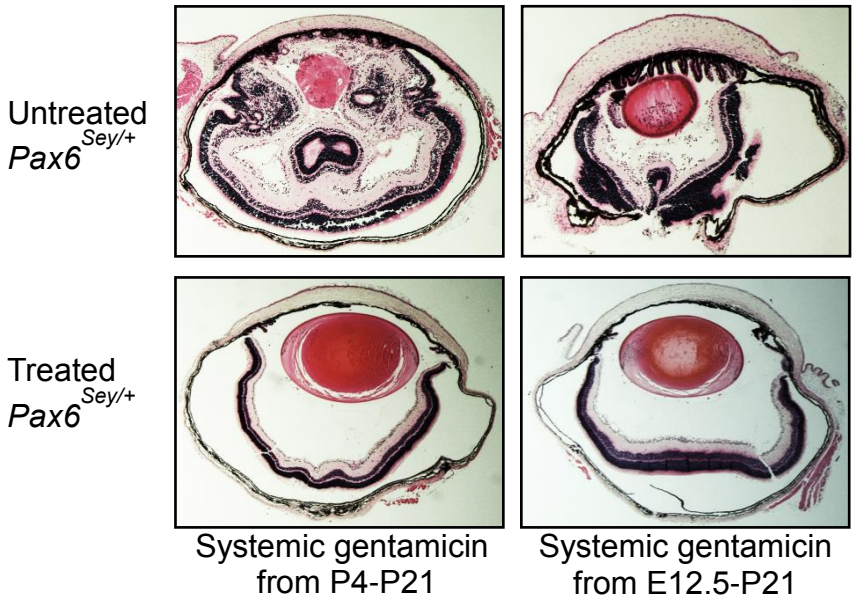
Supplemental Figures



Supplemental Figure 1. Physical-chemical characterization of the novel ophthalmic START suspension formulation. (**A**) representative laser diffraction particle size read-out of the START formulation with mean peak particle size of 4.49±0.03 µm (n=3). (**B**) correlation of UV absorbance of START formulation for 6 independent samples after re-suspension, demonstrating the actual concentration of the drug within the formulation and the absence of any drug metabolism or chemical breakdown.



Supplemental Figure 2. Effect of systemic aminoglycoside treatment on *Pax6*$^{Sey/+}$ eyes. Representative images of eyes prepared for histology at P21. Time-mated pregnant females (E12.5-P21) or postnatal pups (from P4-P21) were treated by daily subcutaneous injections of an aqueous solution of gentamicin (6.25 μg/g). Malformation defects affecting the retina, lens and cornea are normalized compared to untreated *Pax6*$^{Sey/+}$ controls.

Supplemental Tables

Supplemental Table 1. Physical-chemical characterizations of the ophthalmic START suspension formulation

Parameter	**Mean value ± SD**
pH	5.58 ± 0.04 (n=3)
Viscosity (cP)	25.8 ± 0.3 (n=3)
Mean particle size (µm)	4.49 ± 0.03 (n=3)
Uniformity	1.23 ± 0.25 (n=3)
Sedimentation volume	0.1
Ease of re-dispersion (sec)	8.3 ± 0.6 (n=3)
Correlation of stability of START suspension (%)	107 ± 6 (n=6)

Supplemental Methods

Genotyping. The *Pax6*$^{Sey\text{-}1Neu}$ allele is a splice site mutation leading to a truncated protein product, that has a novel 22 amino acid peptide replacing exons 11, 12 and 13. The *Pax6*$^{Sey+/-}$ mouse mutant is a semi-dominant allele caused by a G:T transversion which replaces the glycine 194 codon (GGA) with a TGA stop codon. *Pax6*^{Sey} offspring were identified by genotyping of genomic tail DNA prepared using a REDExtract-N-Amp Tissue PCR kit (Sigma). Each 25 µl PCR reaction contained 1 X PCR master mix (Promega), 250 nM of each primer and 4 µl extracted DNA tissue mix. PCR was amplified: first cycle: 95°C, 3 min; 55°C, 1 min; 72°C, 1 min, and then for 35 cycles of 1 min at 95°C, 40 seconds at 55°C, and 40 seconds at 72°C. Primers and restriction digest for *Pax6*$^{Sey\text{-}1Neu}$ allele were are previously described (1). Primers

for *Pax6*$^{Sey/+}$ were as follows: forward SP1 (annealing to *Sey* allele): 5'-GAGAACACCAACTCCATCAGTTCTAAGT-3'; forward SP2 (annealing to wildtype allele): 5'-AGCAACAGGAAGGAGGGGGAACGAACACCAACTCCATCAGTTCTTACG-3'; reverse MC130 for both PCR reactions: 5'-CTTTCTCCAGAGCCTCAATCTG-3'. PCR amplified DNA was analyzed on a 3% TBE agarose gel. The wild-type allele produces a 148 bp SP2/MC130 band and the *Pax6*^{sey} allele generates a 129 bp SP1/MC130 band (2).

Drug formulation. Since Ataluren is not soluble in H_20 a 1% aqueous suspension was prepared. After topical administration we noted a strong irritation response in the mice (eye rubbing immediately upon drug administration), we therefore reformulated Ataluren using a variety of FDA approved excipients acceptable for ophthalmic delivery. Each formulation was assessed for homogenous particle dispersion and a lack of aggregation upon re-dispersion. We finally selected the 'START' formulation (0.9% **S**odium chloride, 1% **T**ween 80, 1% powdered **A**taluren, 1% ca**R**boxy me**T**hylcellulose) without preservatives. To improve particle dispersion Ataluren was ground into a very fine powder resulting in a mean particle size of 4.49 ±0.03 μm (Supplemental Figure 1A) measured using a laser diffraction particle sizer (Mastersizer, Malvern, UK). The ground powder was added to the Tween 80 and NaCl solution and sonicated for 300 sec. Residual particulate material was removed on passing the suspension through a #325 mesh screen, prior to adding carboxy methylcellulose which increased the viscosity of the formulation. The time for re-dispersion was determined by the time it took for the sediment to be fully shaken up manually after standing the container in an upright position for 5 days at 25°C. Viscosity measurements (Brookfield Synchro-lectric Dial reading viscometer) and particle size uniformity (Mastersizer, Malvern, UK) were determined using standard protocols. The presence

of Ataluren in the formulation was assayed by correlation of UV absorbance between samples after re-suspension (Supplemental Figure 1B). The physical-chemical properties of this ophthalmic suspension formulation were found to meet current pharmacopoeia standards (Supplemental Table 1**)**.

Supplemental References

1. Quinn JC, West JD, Hill RE. Multiple functions for Pax6 in mouse eye and nasal development. *Genes Dev.* 1996;10(1):435-446.
2. Collinson JM, Hill RE, West JD. Different roles for Pax6 in the optic vesicle and facial epithelium mediate early morphogenesis of the murine eye. *Development.* 2000;127(1):945-956.