

# QR-010, an RNA Therapy, Restores CFTR Function in $\Delta F508$ -CFTR Mice

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## Introduction

- Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR).
- The most common gene mutation is  $\Delta F508$ , which results in deletion of three nucleotides and results in a non-functional CFTR protein.  $\Delta F508$ -CFTR mice have mRNA homologous with human mRNA at the  $\Delta F508$  locus.
- Nasal potential difference (NPD) is an accepted tool for diagnosis of CF in humans as the basal potential difference and response to stimuli are specific to the CFTR dysfunction.  $\Delta F508$ -CFTR mice have NPD patterns similar to humans with CF<sup>1</sup>.
- The saliva secretion assay (SSA) is an investigational tool described in  $\Delta F508$ -CFTR mice, which is a surrogate of the sweat chloride test used in humans<sup>2</sup>.
- QR-010 is an investigational single-stranded, chemically modified RNA oligonucleotide designed to repair mRNA in CF patients with the  $\Delta F508$  mutation and result in translation of wild-type CFTR.
- To assess if QR-010 restored normal CFTR function in  $\Delta F508$ -CFTR mice, we assessed NPD before and after intranasal administration of QR-010 as well as SSA before and after oro-tracheal administration of QR-010.

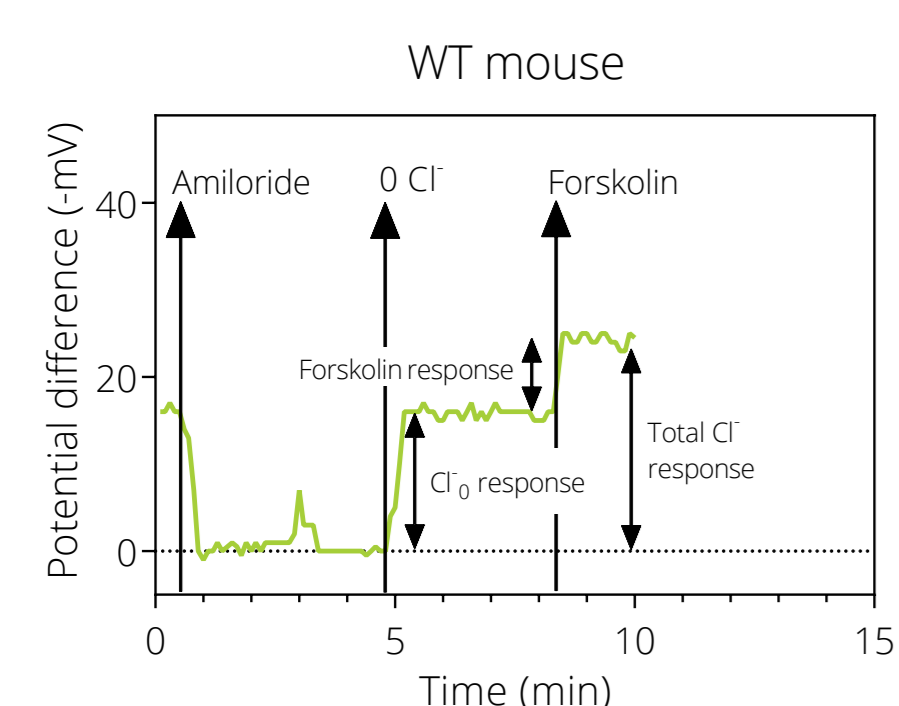
## Objectives

- Assess if topical administration of QR-010 could restore the CFTR-mediated chloride transport in  $\Delta F508$ -CFTR mice by measuring the nasal epithelial potential difference (NPD).
- Assess if oro-tracheal administration of QR-010, as a mimic of inhaled delivery, could restore the CFTR-mediated saliva secretion in  $\Delta F508$ -CFTR mice (SSA).

## Materials & Methods

### NPD:

- Mice:  $\Delta F508$ -CFTR mice (FVB-  $Cftr^{\Delta F508}$ ) male/female, 12w and older.
- Treatment: 6 intranasal (i.n.) QR-010 (2mg/kg) every other day.
- Readout: NPD before treatment and 48hrs after the last (6<sup>th</sup>) dose.
- Protocol: Leal T *et al.*<sup>4</sup>

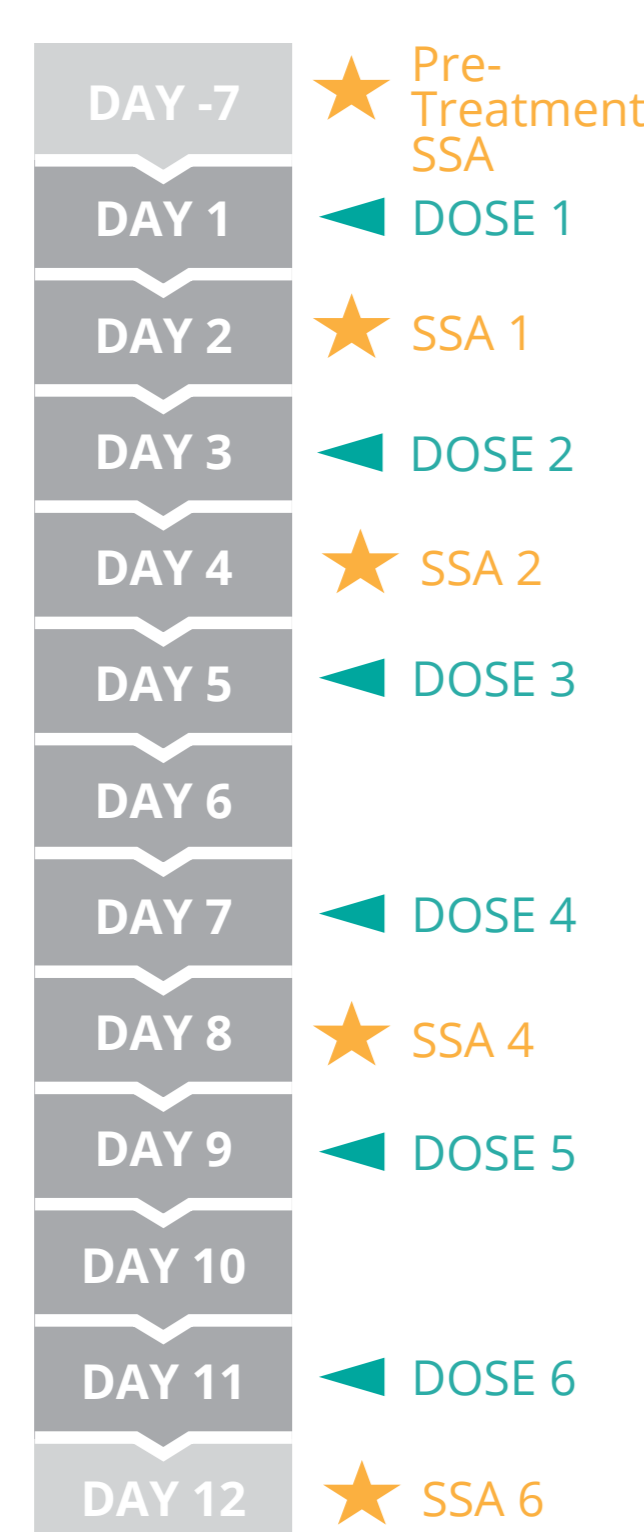


### SSA procedure:

- Modified from Best *et al.*<sup>2</sup>
- Use 2.5%/2.0% Isoflurane/air mixture for anesthesia.
- Subcutaneous (s.c.) injection (50 $\mu$ l, 1mM) atropine to block the cholinergic and adrenergic system.
- A cotton bud was used to absorb any remaining saliva.
- S.c. injection (50 $\mu$ l, 1mM) atropine and (100 $\mu$ M) isoprelanine to induce CFTR-mediated saliva production.
- Saliva was absorbed in pre-weighted pieces of filter paper and replaced every 3min for 30min.
- Total saliva production was calculated by weighing all filter papers, subtracting pre-weight and corrected for bodyweight.

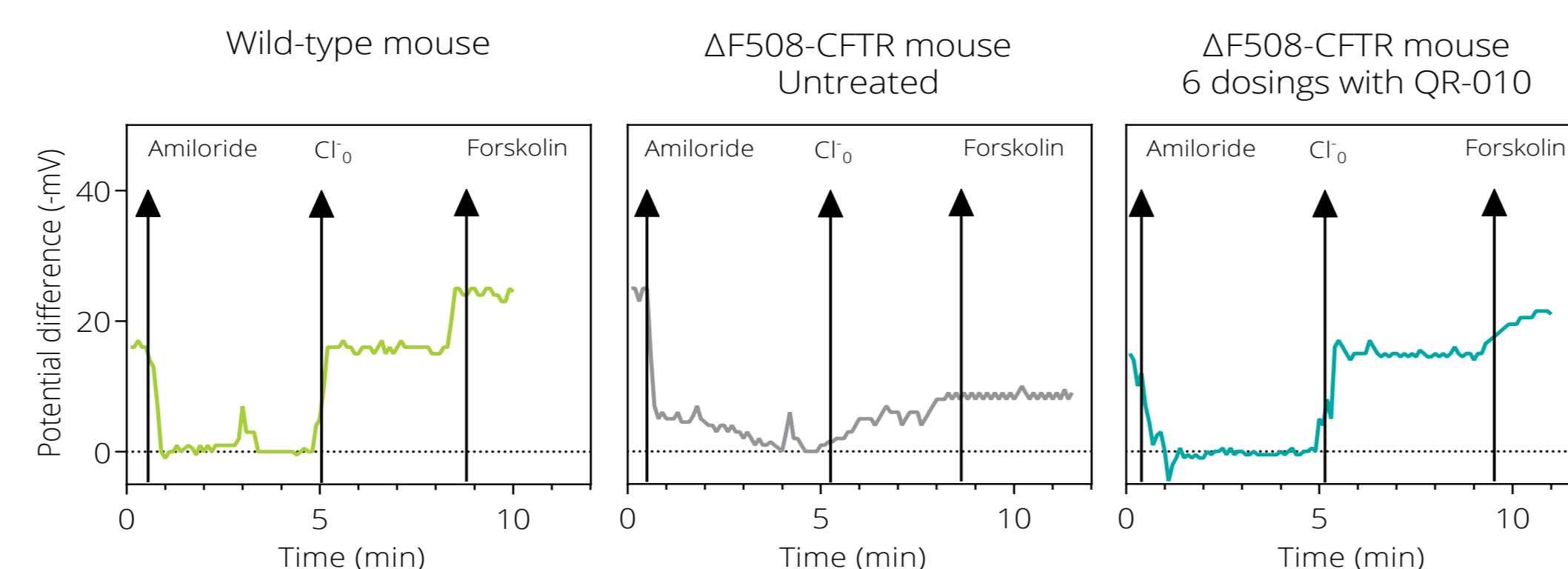
### SSA:

- Mice:  $\Delta F508$ -CFTR mice (FVB-  $Cftr^{\Delta F508}$ ) male/female, 12w and older.
- Treatment: 6x oro-tracheal (OT) QR-010 (10mg/kg) every other day.
- Readout: SSA before treatment and 24hrs after 1, 2, 4 and 6 doses.



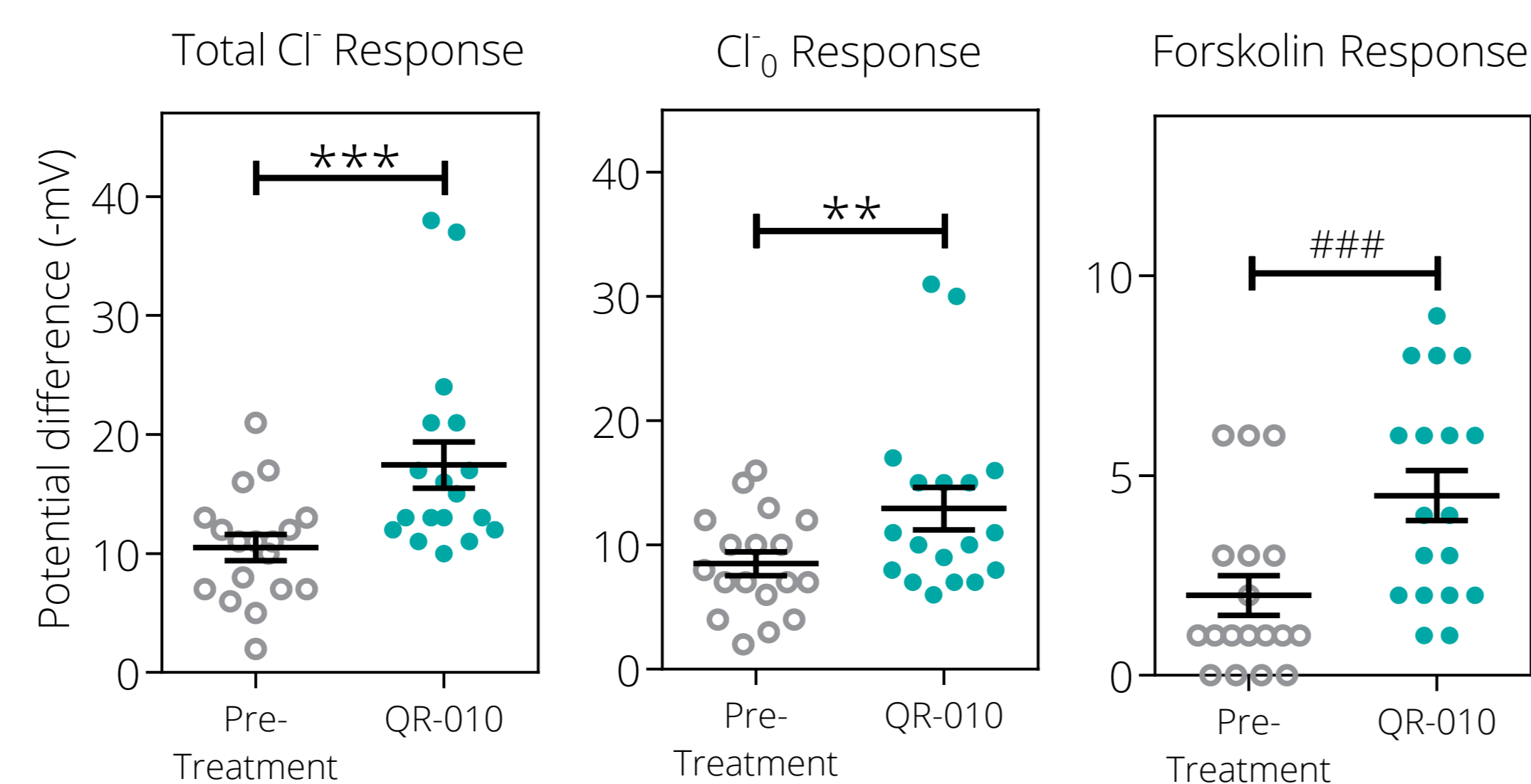
## QR-010 Restores CFTR Function as Measured by NPD

### QR-010 restores NPD to normal in $\Delta F508$ -CFTR mice



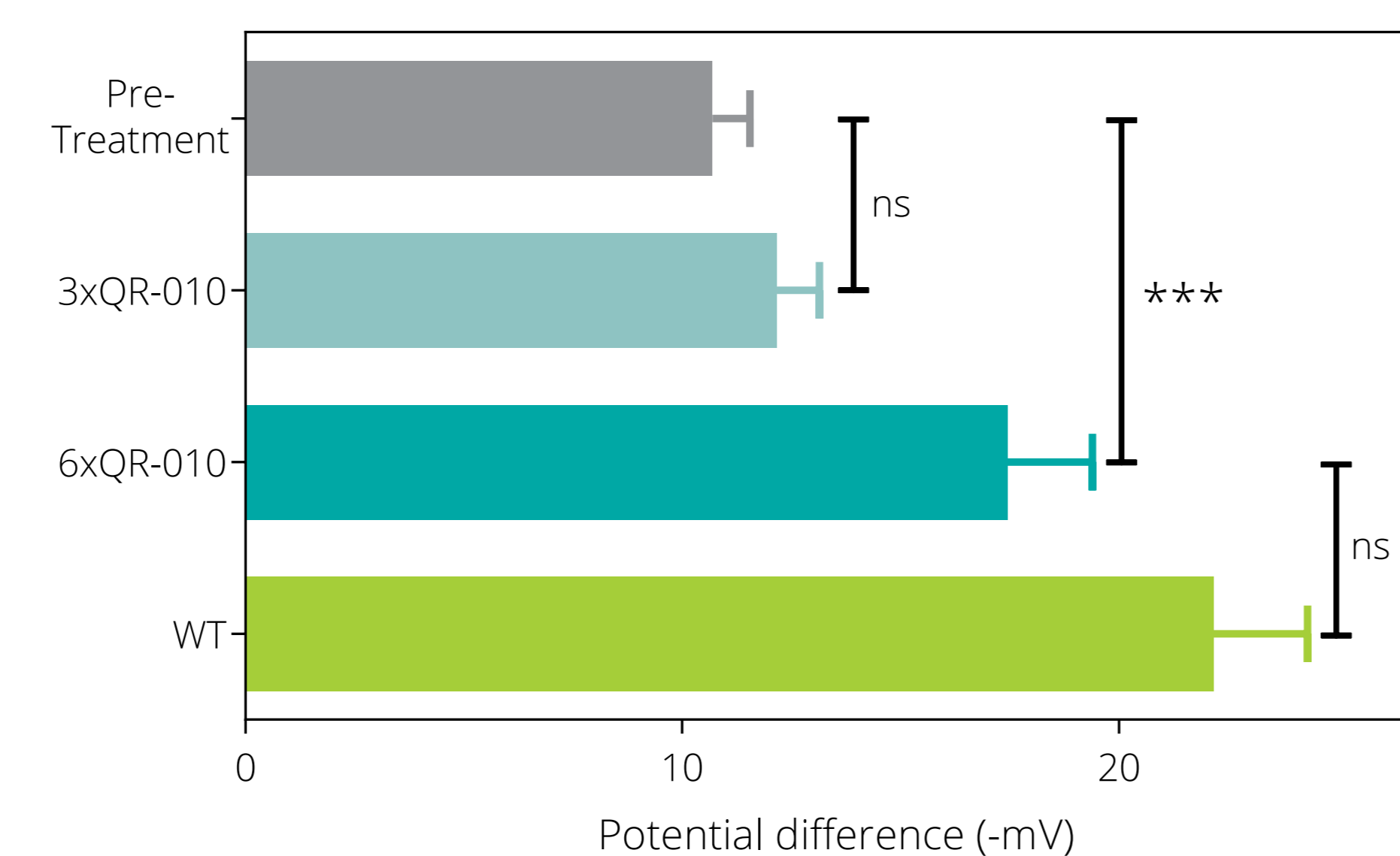
Representative NPD traces of a wild-type mouse and a  $\Delta F508$ -CFTR mouse before treatment and after 6 doses of QR-010 are shown. Both amplitude of the response and response-time to stimuli revert toward wild-type after QR-010 treatment.

### QR-010 improves CFTR-specific NPD parameters



Dots depict individual mice pre- and post-treatment with 6 i.n. doses of QR-010 (2mg/kg). Both the zero-chloride as well as the forskolin response were improved after QR-010 treatment. Line with error bars show mean $\pm$ SEM, paired T-test, n=18. \*\*\*p=0.0005, \*\*p=0.0030, ###p=0.0001.

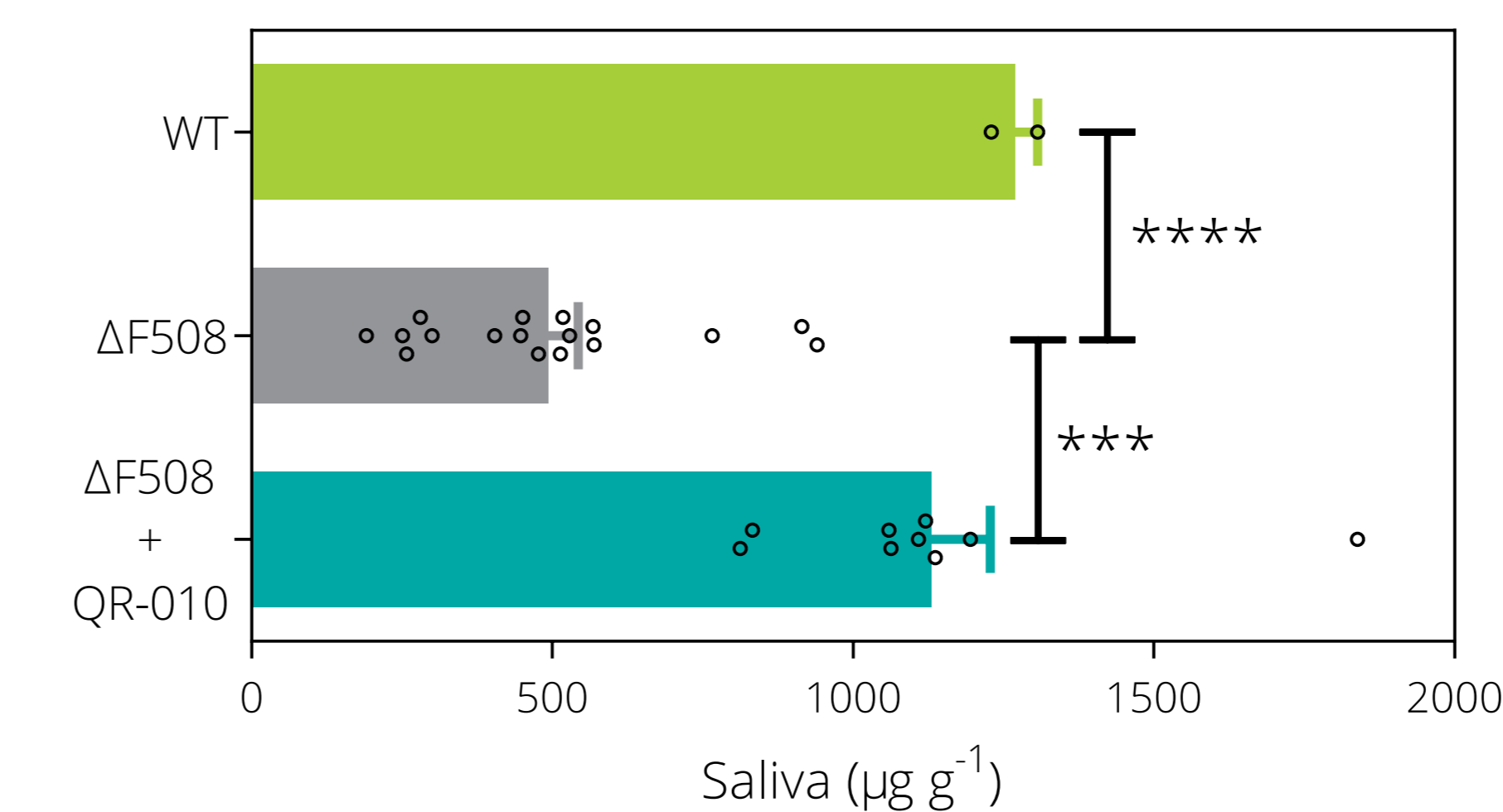
### Total Chloride response is dose dependent



Summary of Total Chloride Response. 3 doses of QR-010 did not significantly improve chloride response. In contrast, 6 doses did improve chloride response to levels not significantly different from wild-type (WT). Mean $\pm$ SEM is shown for  $\Delta F508$ -CFTR mice pre-treatment, after 3 (n=6) and 6 (n=18) i.n. doses of QR-010 (2mg/kg) and WT mice (n=6). Results were compared by unpaired T-test (vs. WT) and paired T-test (vs. pre-treatment), n=18. \*\*\*p=0.0005, ns=not significant.

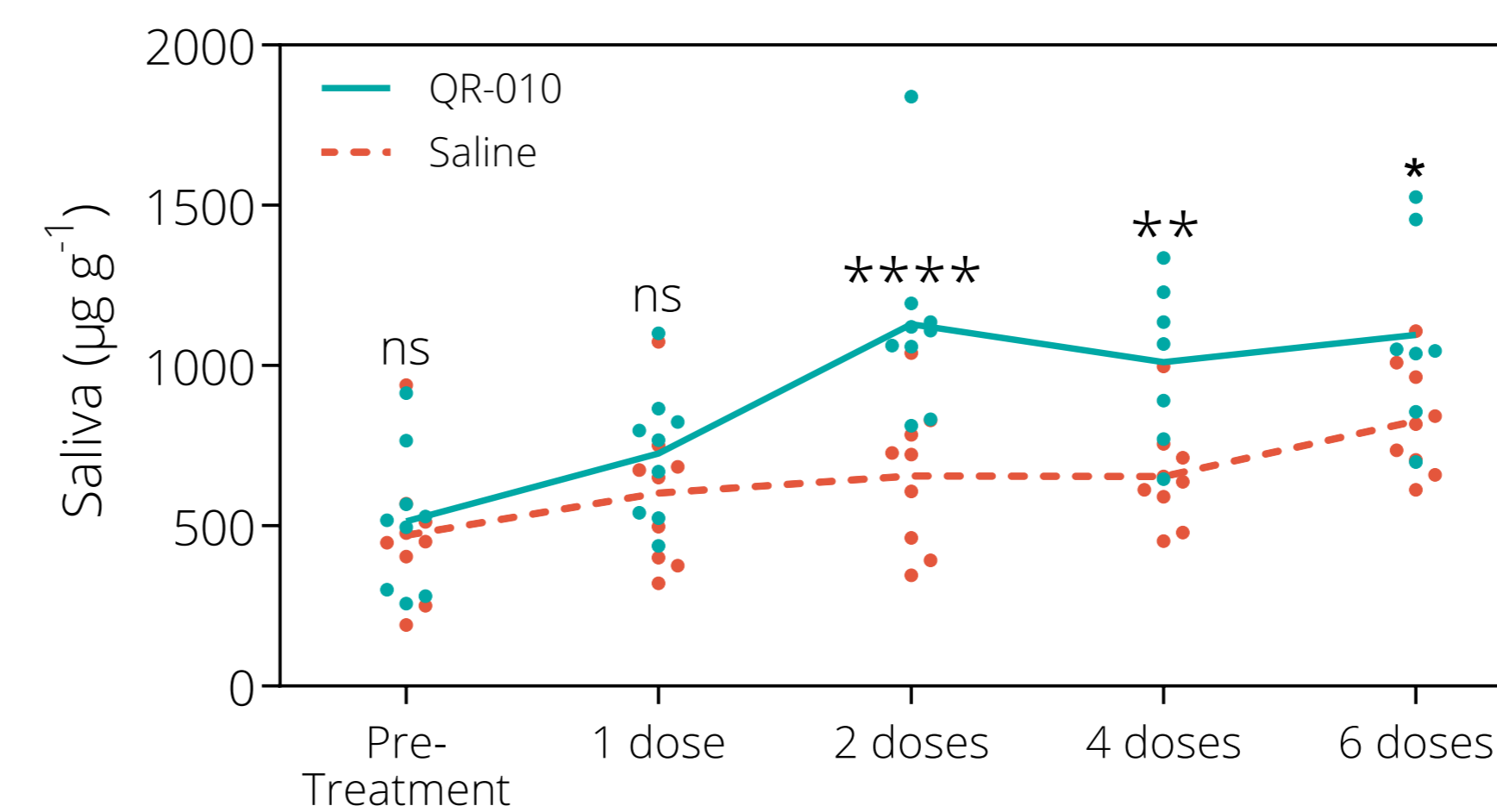
## QR-010 Restores CFTR Function via Systemic Exposure as Measured in SSA

### QR-010 increases total saliva secretion volume in female $\Delta F508$ -CFTR mice



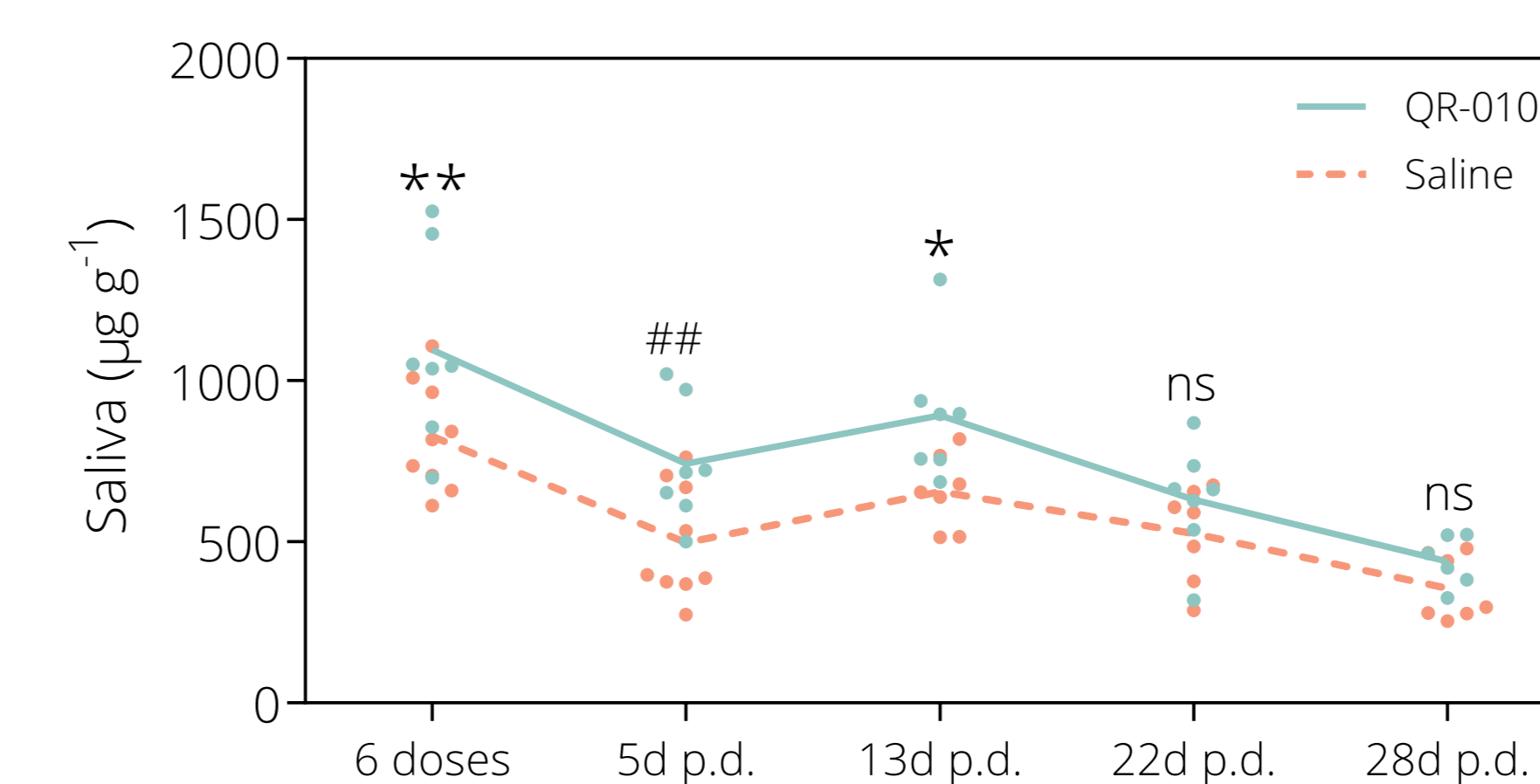
Total CFTR-mediated saliva production (corrected for bodyweight) in female and male WT and  $\Delta F508$ -CFTR mice (pre-treatment and after treatment with 2 doses of QR-010). Bars with error bars show mean with SEM. Both male and female  $\Delta F508$ -CFTR mice have significantly lower CFTR-mediated saliva production compared to WT (\*\*\*\*p<0.0001, Unpaired T-test). 2 doses of QR-010 significantly improved CFTR-mediated saliva production in female  $\Delta F508$ -CFTR mice to WT levels (\*\*\*p=0.0001, Paired T-test). QR-010 had no effect in males (ns: non-significant).

### Saliva secretion improves after 2 doses of QR-010



Lines show the mean CFTR-mediated saliva secretion corrected for bodyweight ( $\mu$ g saliva/g bodyweight) and circles show individual measurements for saline (dotted purple) and QR-010 (in teal) treated females in  $\Delta F508$ -CFTR mice. Treatment groups were compared by two-way ANOVA with Fisher's LSD test. QR-010 improved CFTR-mediated saliva production significantly compared to saline 2, 4 and 6 doses in female  $\Delta F508$ -CFTR mice. There was no effect of QR-010 observed in male  $\Delta F508$ -CFTR mice. ns=non-significant, \*\*\*\*p<0.00001, \*\*p=0.0029, \*p=0.0233.

### Effect of QR-010 on CFTR mediated saliva secretion remains up to 13 days post-dosing



Lines show the mean CFTR-mediated saliva secretion corrected for bodyweight ( $\mu$ g saliva/g bodyweight) and circles show individual measurements post-dose (p.d.) for saline (light purple dotted) and QR-010 (light teal) treated female  $\Delta F508$ -CFTR mice. Treatment groups were compared by two-way ANOVA with Fisher's LSD test: ns=non-significant, \*\*p=0.0038, ##p=0.0076, \*p=0.0149.

## Discussion

### Topical (intranasal) administration QR-010 restores CFTR function in $\Delta F508$ -CFTR mice as assessed by nasal potential difference measurements

- Topical application of QR-010 (2mg/kg, intranasal) restores nasal potential difference to 80% of wild-type levels to  $\Delta F508$ -CFTR mice.
- Inter-animal variation is observed which may reflect variable uptake of dose, route of administration, or factors not yet characterized.
- A significant improvement of total chloride transport was seen after 6 but not 3 doses suggesting a threshold response to therapy.

### Oro-tracheal administration of QR-010 restores CFTR function in female $\Delta F508$ -CFTR mice as assessed by the saliva secretion assay

- Systemic absorption of QR-010 significantly improves CFTR-mediated saliva secretion volume in female  $\Delta F508$ -CFTR mice compared to placebo (saline).
- Two OT doses of QR-010 (10mg/kg) restored CFTR-induced saliva secretion to 80% of WT level in female  $\Delta F508$ -CFTR mice which was maintained but did not increase after 4 or 6 doses.
- The effect of QR-010 remained until 13 days after dosing completed.
- There was no effect of QR-010 observed in male  $\Delta F508$ -CFTR mice. Different responses between male and females in the SSA is consistent with previous publications<sup>2</sup>.
- The impact of repeated measurements of SSA is not as well characterized, but may explain differences in magnitude of response over time.

## Conclusion

QR-010 restores CFTR function in  $\Delta F508$ -CFTR mice as measured in two independent assays.

## References

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## Thank you

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QR-010, an RNA Therapy, is Taken up by Airway Epithelial Cells Showing Systemic Exposure After Oro-Tracheal Dosing

QR-010, an RNA Therapy, Restores CFTR Function in  $\Delta F508$  Cell Cultures