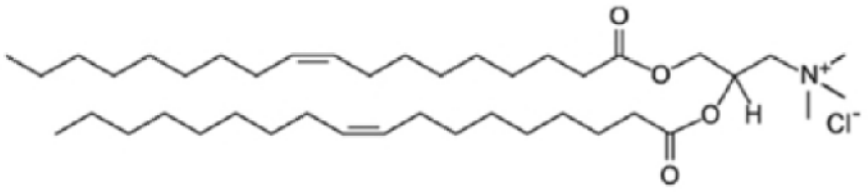
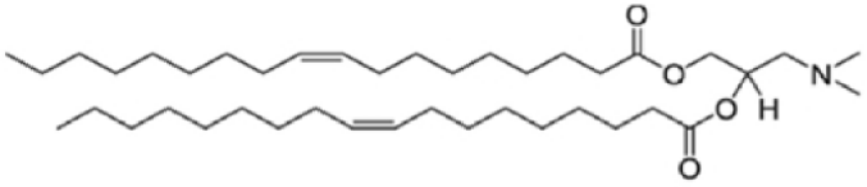
**Fig. 1**

DOTAP




DODAP




EPC

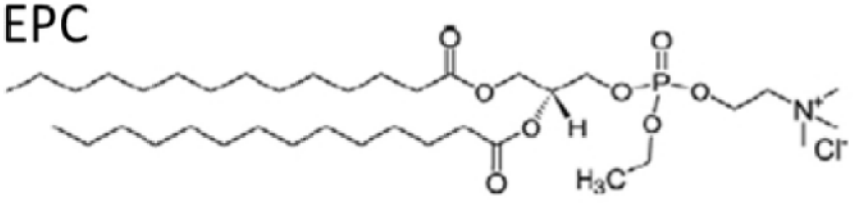


**Fig. 2**

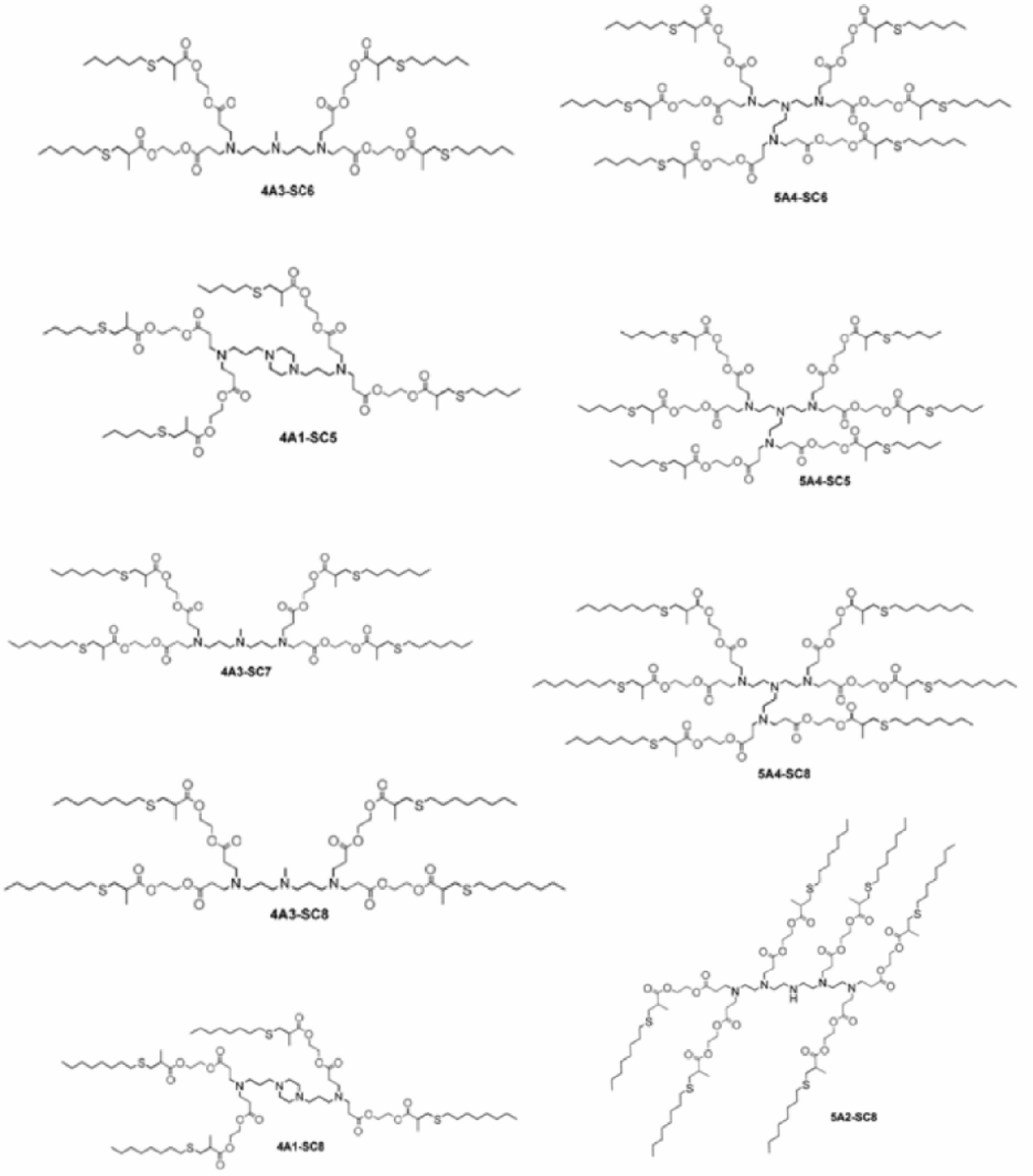
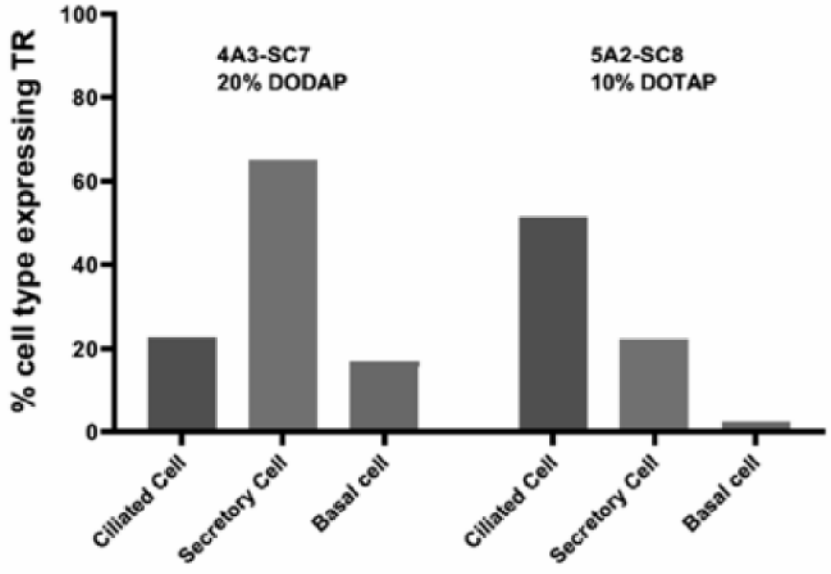

4A3-SC6
5A4-SC6
4A1-SC5
5A4-SC5
4A3-SC7
5A4-SC8
4A3-SC8
4A1-SC8
5A2-SC8

**Fig. 3**

Apical bolus dosing of well-differentiated human bronchial epithelial cultures with TR mRNA (12 ug)




Aerosol delivery to well-differentiated human bronchial epithelial cultures with TR mRNA (4 ug)

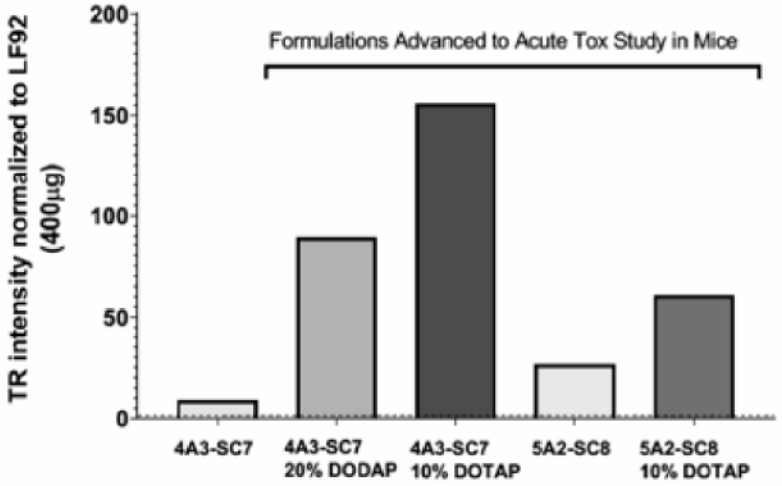


**Fig. 4**

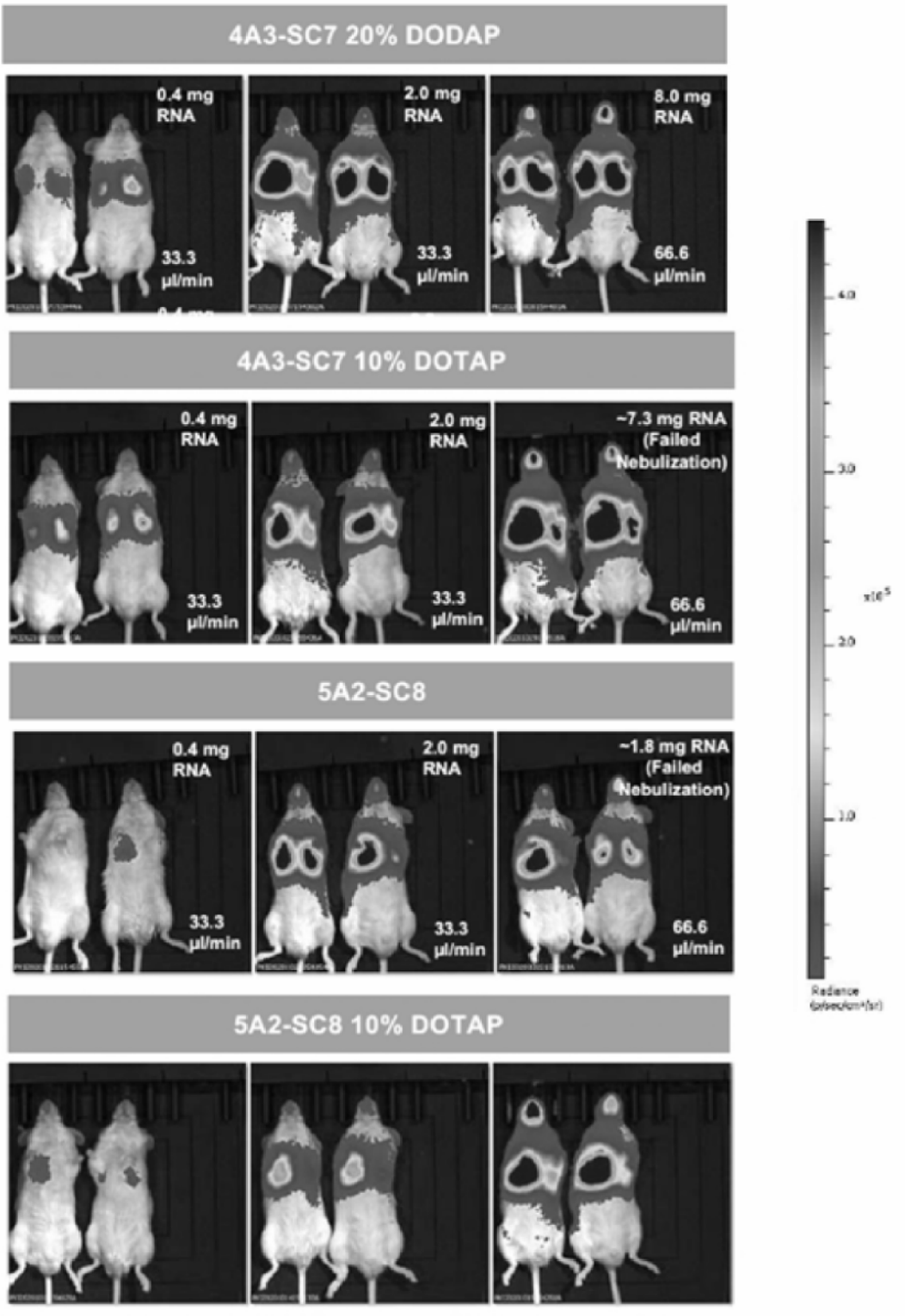

4A3-SC7 20% DODAP
0.4 mg RNA
33.3 µl/min
2.0 mg RNA
33.3 µl/min
8.0 mg RNA
66.6 µl/min
4A3-SC7 10% DOTAP
0.4 mg RNA
33.3 µl/min
2.0 mg RNA
33.3 µl/min
~7.3 mg RNA (Failed Nebulization)
66.6 µl/min
5A2-SC8
0.4 mg RNA
33.3 µl/min
2.0 mg RNA
33.3 µl/min
~1.8 mg RNA (Failed Nebulization)
66.6 µl/min
5A2-SC8 10% DOTAP
4.0
3.0
x10^5
2.0
1.0
Radiance (p/sec/cm²/sr)

**Fig. 5**

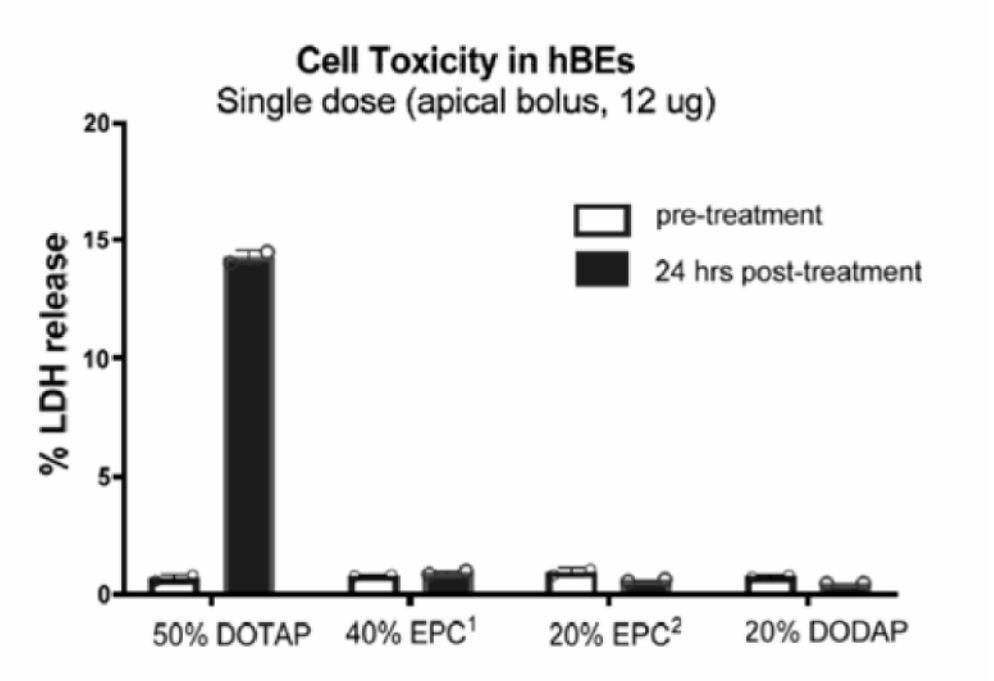

Cell Toxicity in hBEs
Single dose (apical bolus, 12 ug)
% LDH release
20
15
10
5
0
pre-treatment
24 hrs post-treatment
50% DOTAP
40% EPC1
20% EPC2
20% DODAP

**Fig. 6**

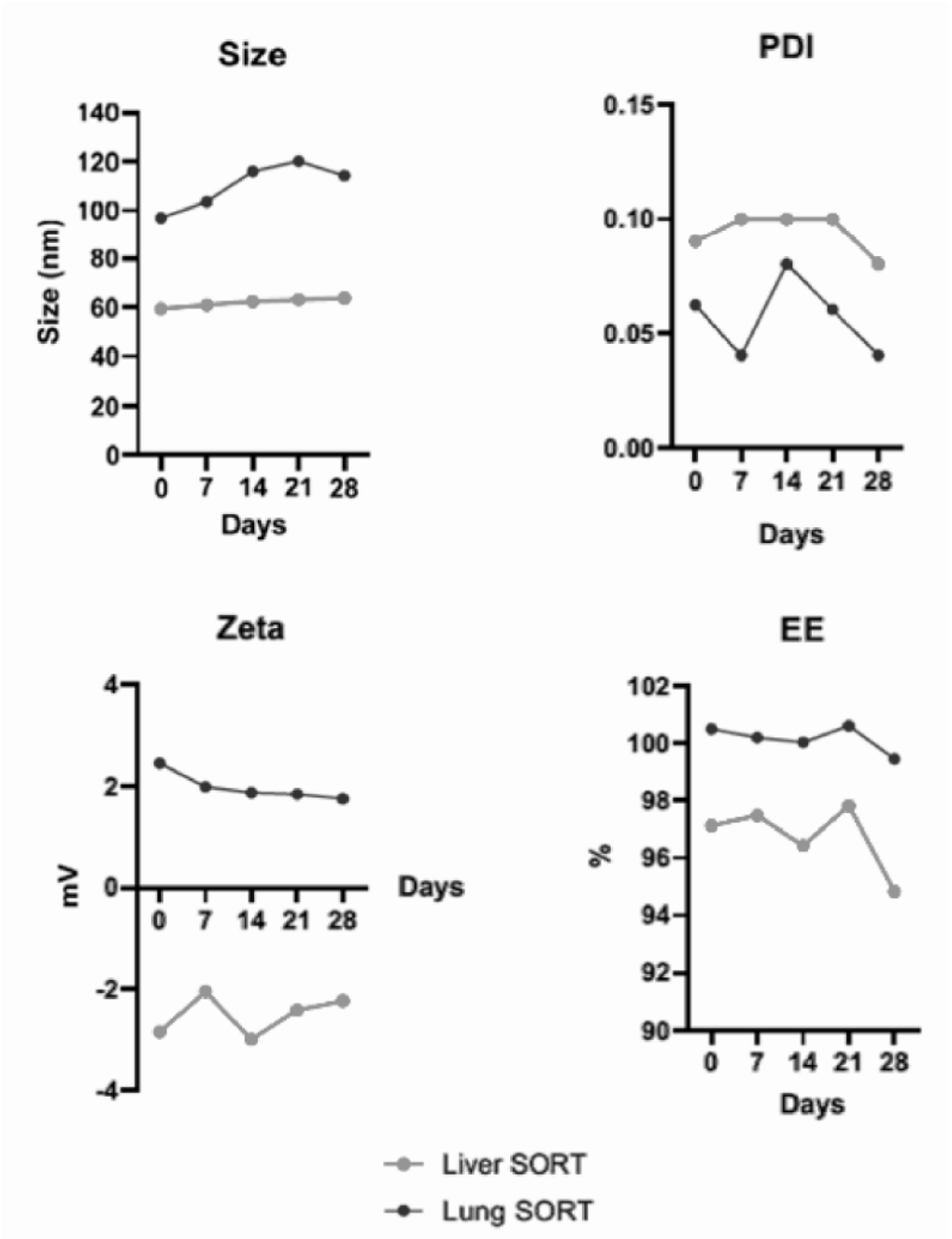

Size
Size (nm)
140
120
100
80
60
40
20
0
0 7 14 21 28
Days
PDI
0.15
0.10
0.05
0.00
0 7 14 21 28
Days
Zeta
mV
4
2
0
-2
-4
0 7 14 21 28
Days
EE
%
102
100
98
96
94
92
90
0 7 14 21 28
Days
Liver SORT
Lung SORT

**Fig. 7**

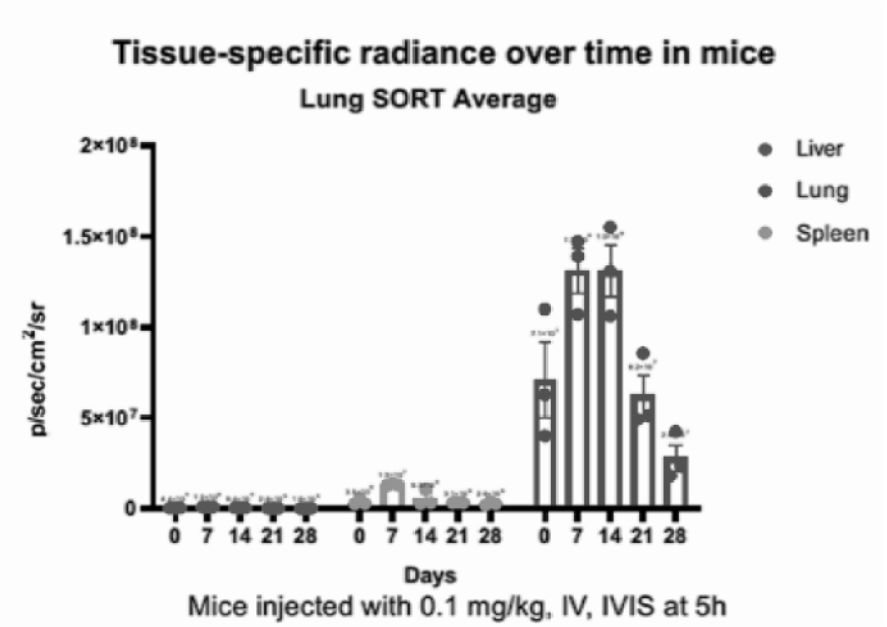
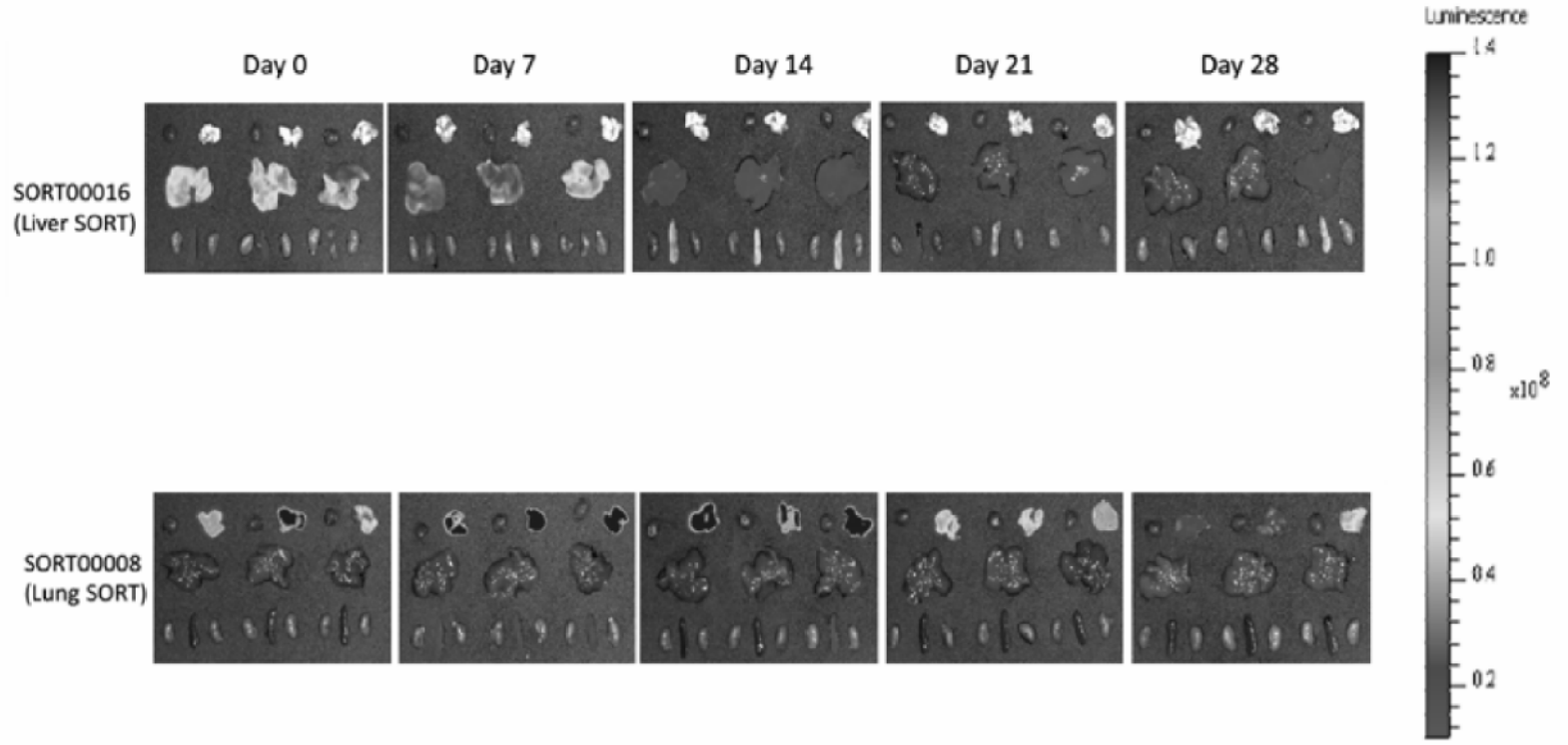

Tissue-specific radiance over time in mice
Lung SORT Average
Liver
Lung
Spleen
p/sec/cm²/sr
2×10⁸
1.5×10⁸
1×10⁸
5×10⁷
0
0 7 14 21 28 0 7 14 21 28 0 7 14 21 28
Days
Mice injected with 0.1 mg/kg, IV, IVIS at 5h

**Fig. 8**

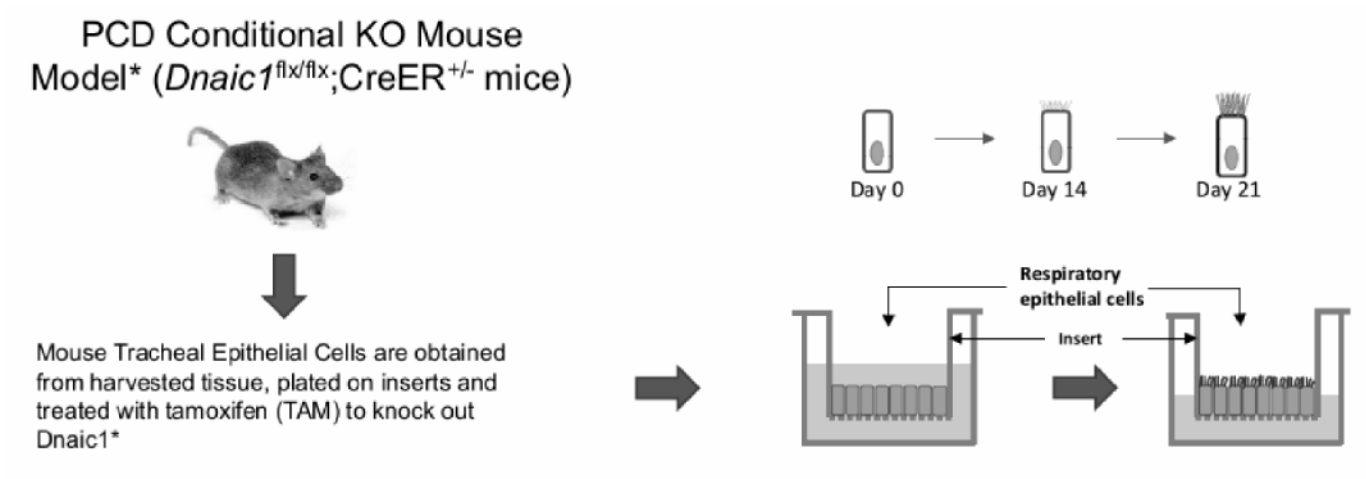

Day 0
Day 7
Day 14
Day 21
Day 28
SORT00016
(Liver SORT)
SORT00008
(Lung SORT)
Luminescence
1.4
1.2
1.0
0.8
0.6
0.4
0.2
x10^8

## Fig. 9A

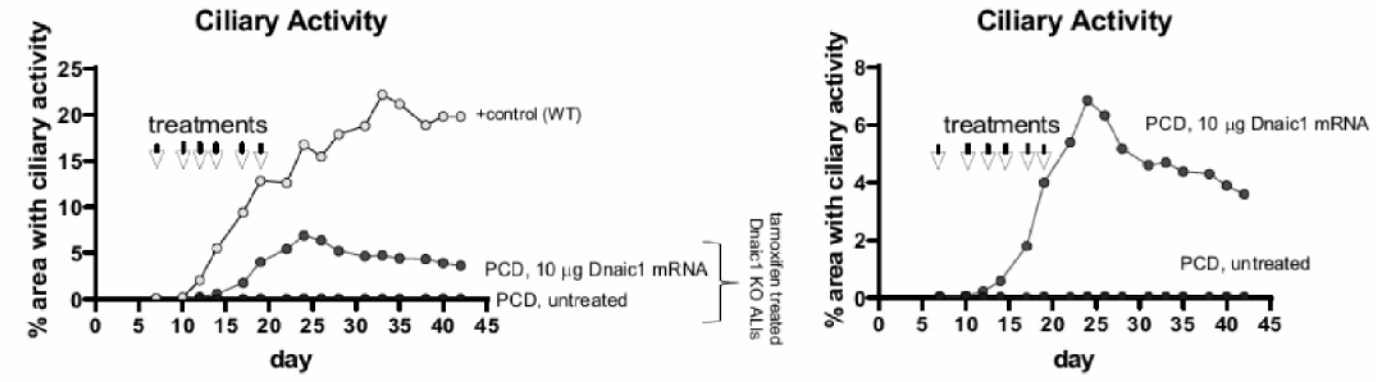
Examples

| Indication / Inclusion criteria | Phase 1/2 Study* | Primary Outcome Measures |
|---|---|---|
| **PCD Lead mRNA Program**<br>– Adults<br>– Pathogenic mutation in DNAI1<br>– FEV1 between 40% and 90% | SAD • MAD (6 mo.) • OLE (6 mo.)<br>Placebo<br>Low Dose<br>High Dose | – Safety and tolerability<br>– Absolute change in percent predicted FEV1 |

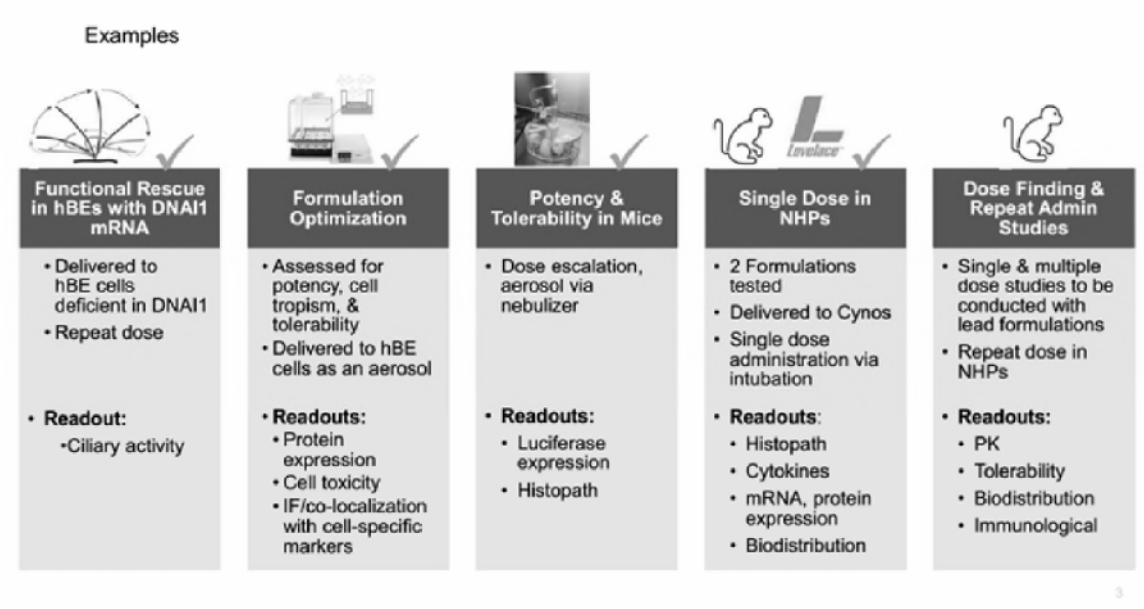
## Fig. 9B




## Fig. 9C



# Fig. 10A




# Fig. 10B

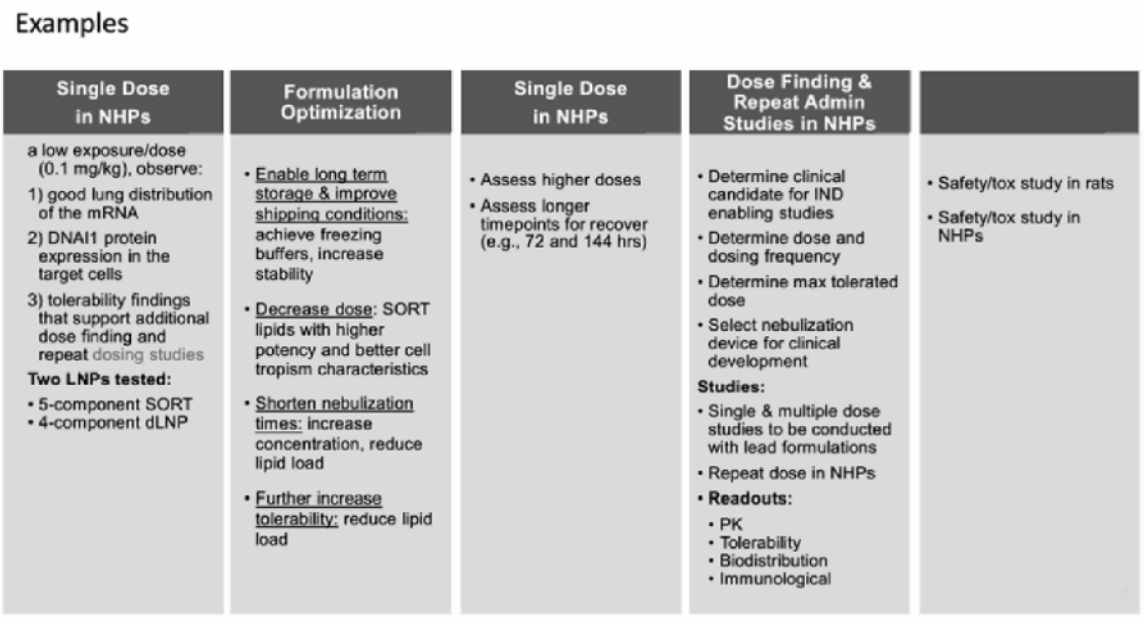


**Fig. 10C**

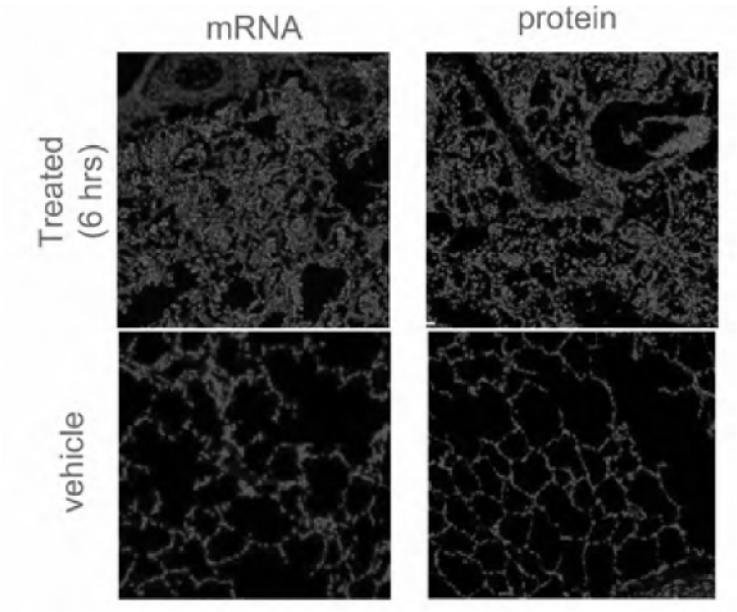



**Fig. 10D**

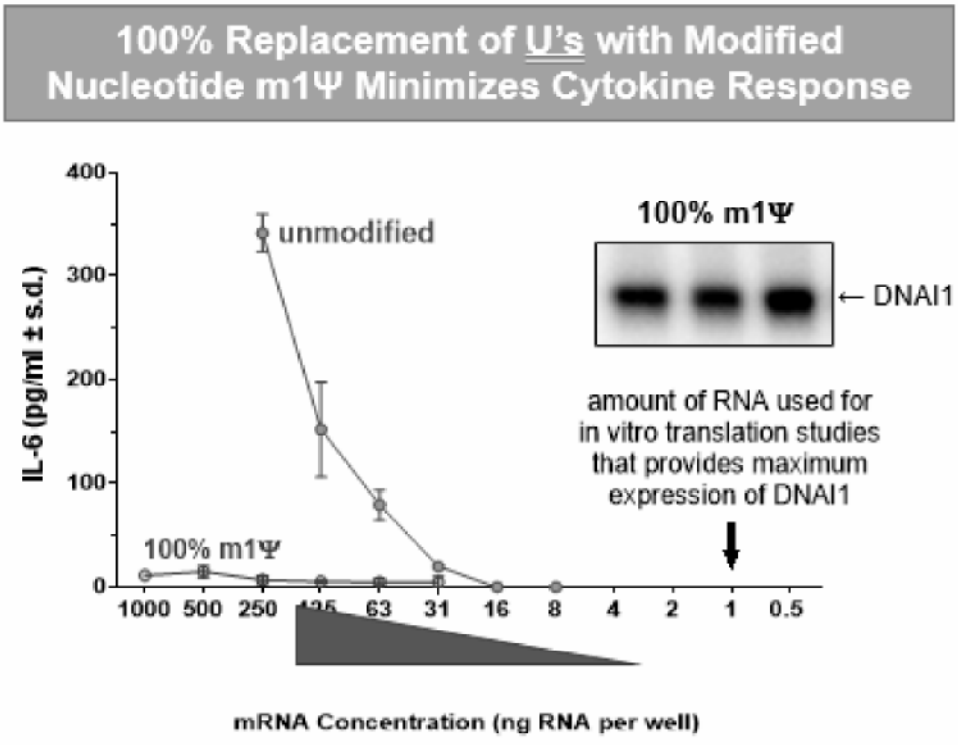


**Fig. 10E**

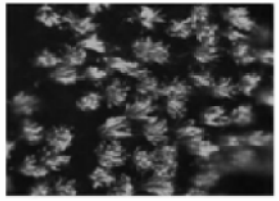
# Functional Restoration and Delivery to NHPs (non-human primate) Achieved, e.g.,

## FUNCTIONAL RESTORATION

- ✓Repeat administration well tolerated
- ✓Cellular uptake in the presence of mucus
- ✓Normal beat frequency recovered
- ✓Synchronized wave-like motion restored
- ✓Activity comparable to normal controls

Green: Acetylated alpha-tubulin (microtubules)

Red: HA tagged DNAI1

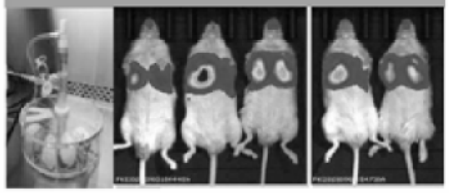


Human DNAI1 knock-out cells treated with a single dose of formulated DNAI1-HA and immuno-stained with anti-acetylated tubulin and anti-HA 72 hrs after dosing. [1]

## WELL-TOLERATED IN MICE

- ✓ Single administration, escalating dose well tolerated
- ✓ Good distribution and high levels of protein expression
- ✓ Histopathology results similar to vehicle

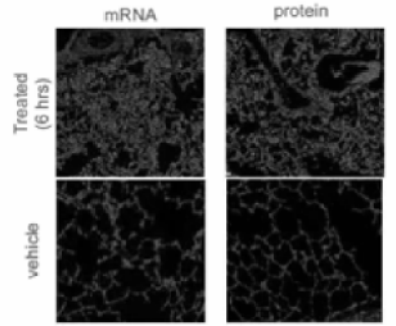
Whole-body images of mice treated with luciferase mRNA formulated in a ReCode LNP and nebulized using an Aerogen mesh nebulizer



## SUCCESSFUL DELIVERY IN NHPs

- ✓ Single dose, nebulization
- ✓ Target delivered dose achieved
- ✓ Well tolerated in all animals tested[1]
- ✓ Good distribution of mRNA
- ✓ DNAI1 protein detected

Positive lung labeling (red) for DNAI1 mRNA (left) and DNAI1 protein (right) in NHPs using a 5-component SORT LNP




[1] *Based on lack of acute response and initial blood, BAL fluid, cytokine and histopathology data.*

Fig. 11A

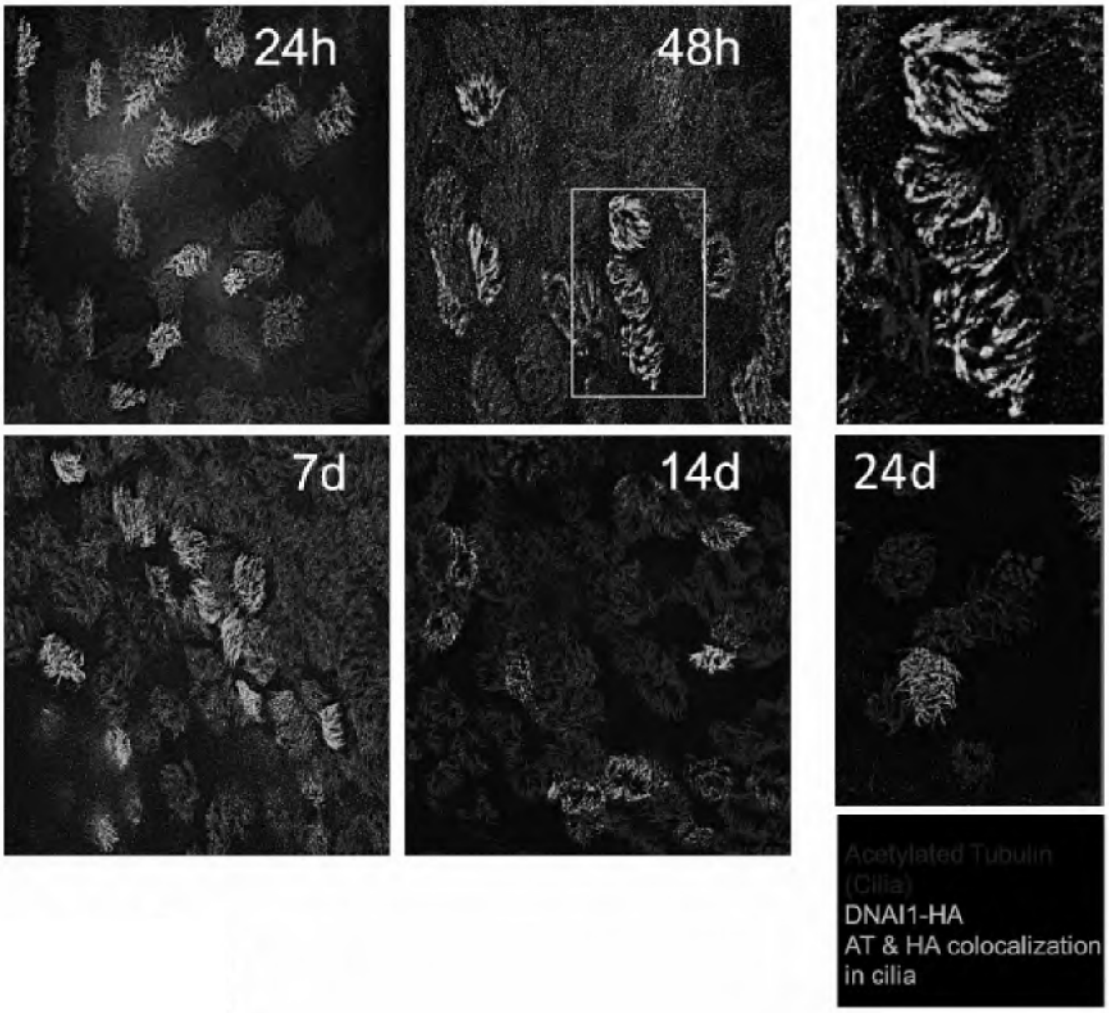



Fig. 11B

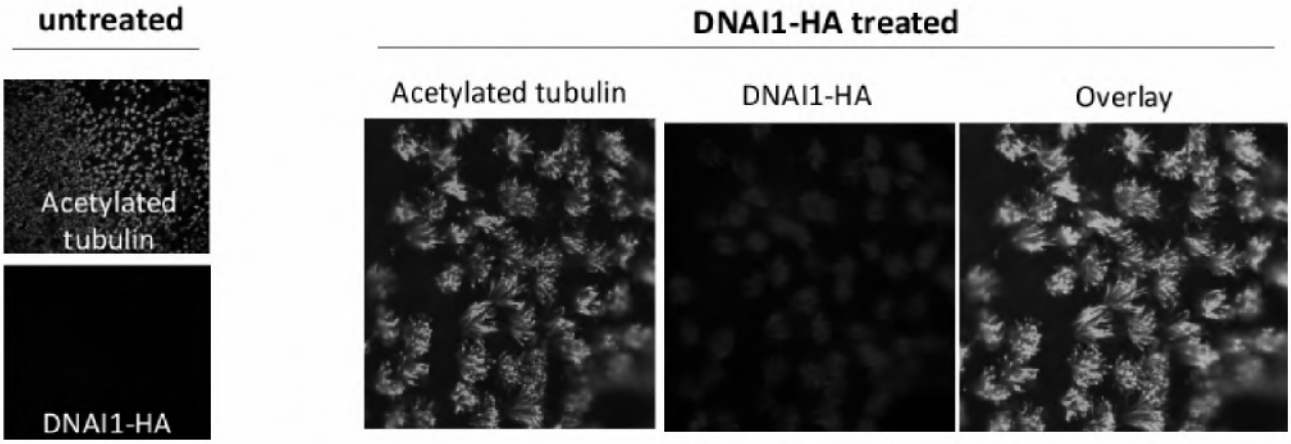


Fig. 12

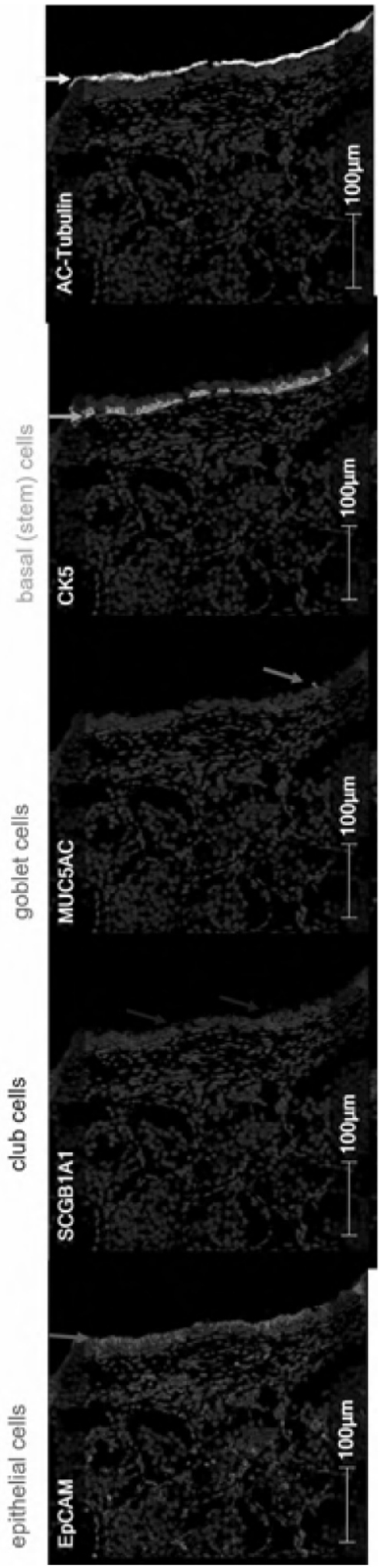

epithelial cells
club cells
goblet cells
basal (stem) cells
EpCAM
SCGB1A1
MUC5AC
CK5
AC-Tubulin
100µm
100µm
100µm
100µm
100µm

**Fig. 13A**

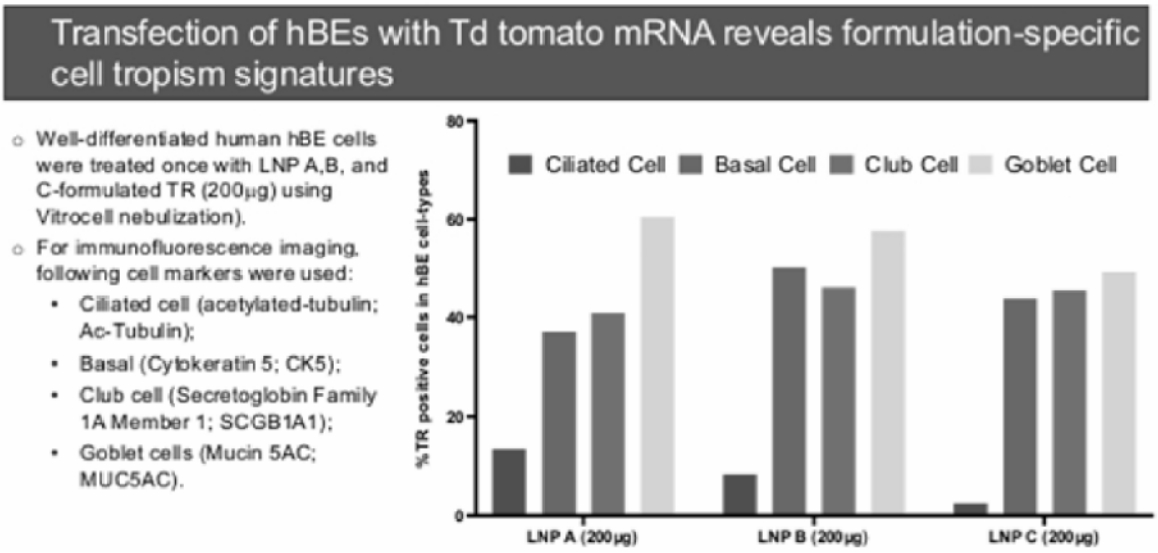



**Fig. 13B**

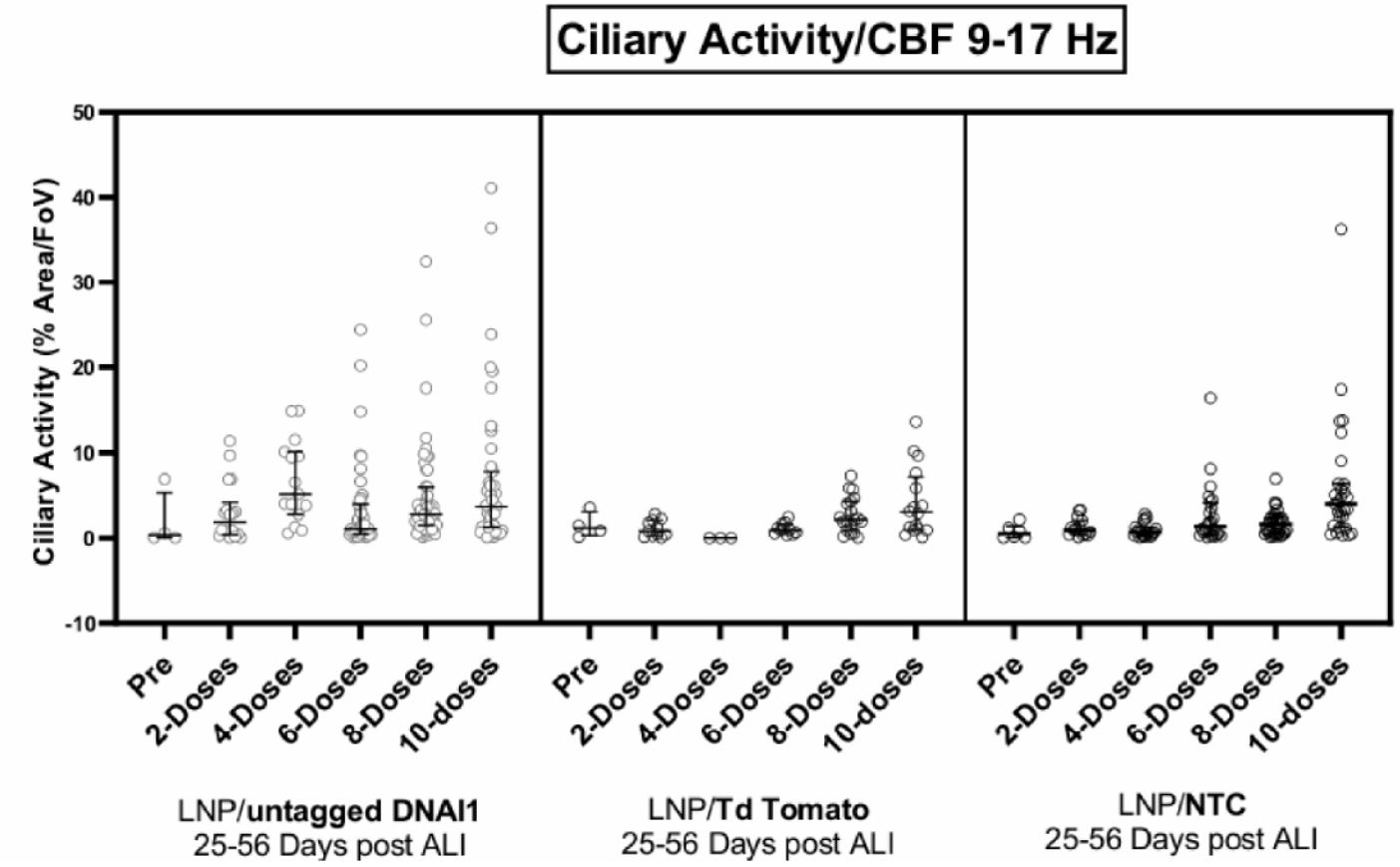


Fig. 14A

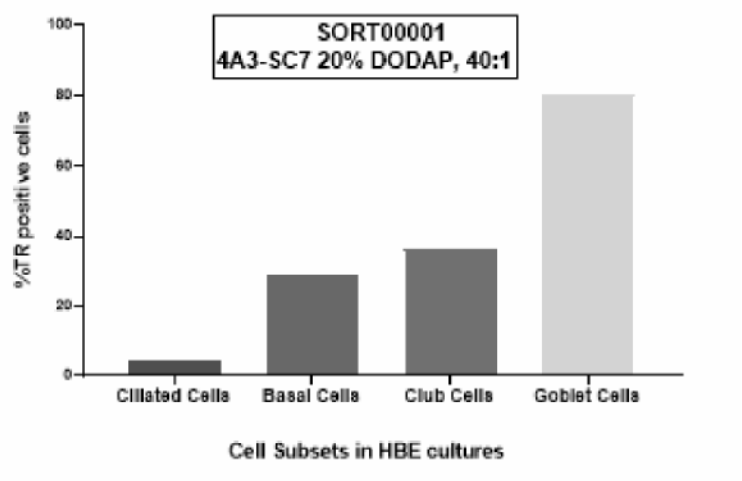



Fig. 14B

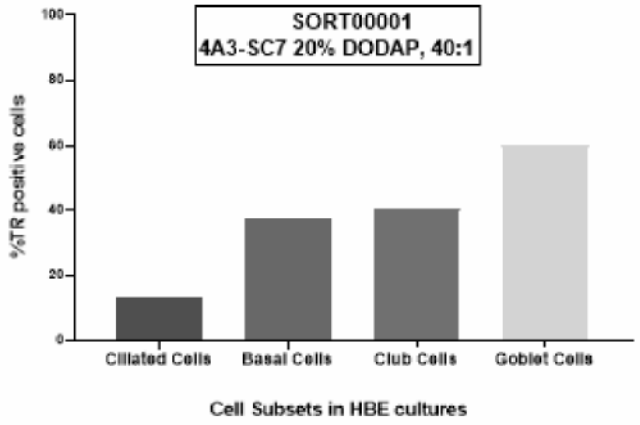



Fig. 14C

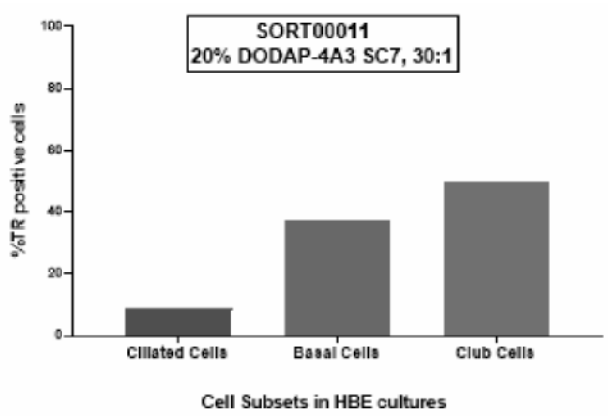



Fig. 14D

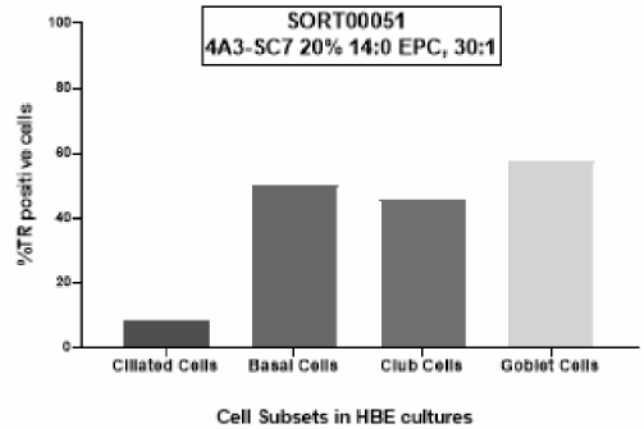



Fig. 14E

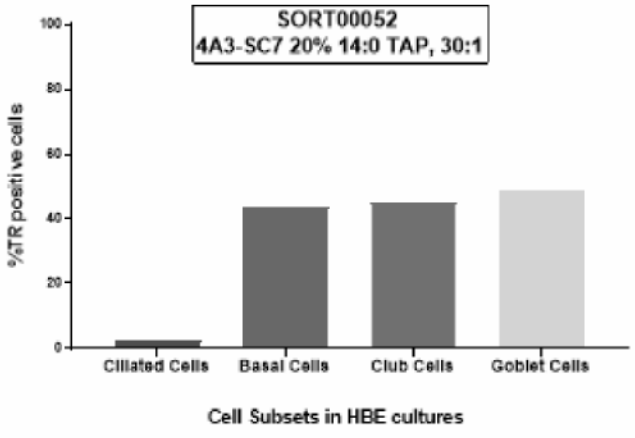
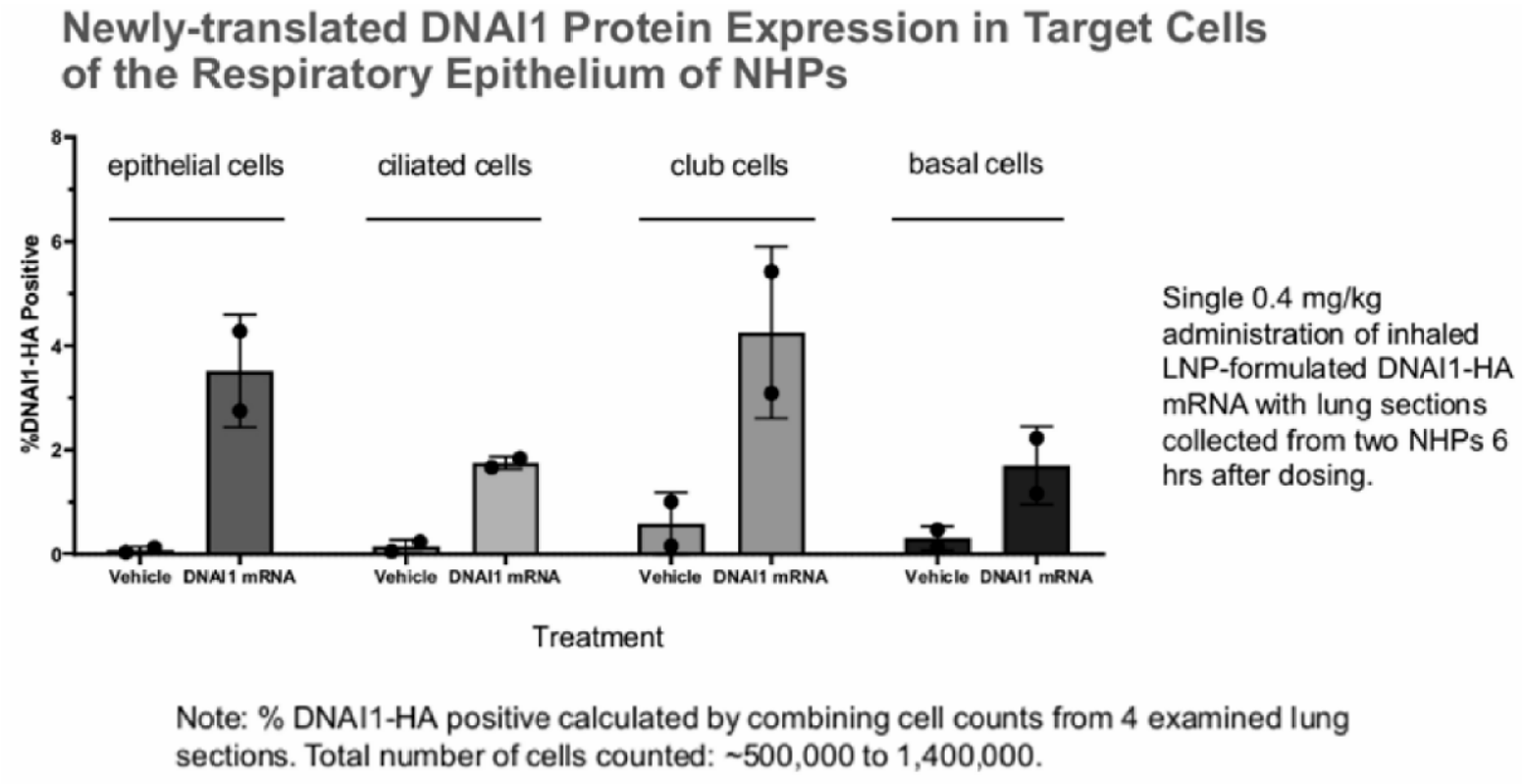
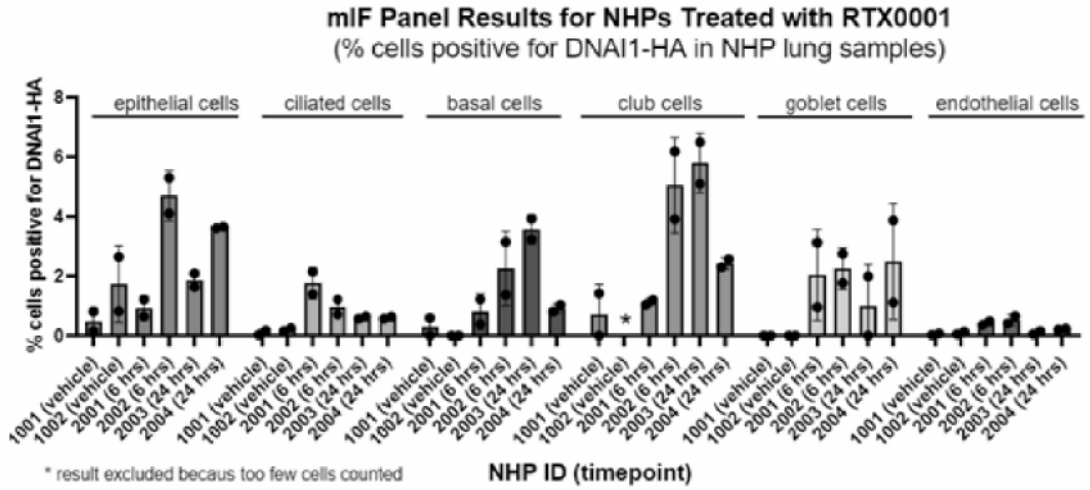


**Fig. 15**


Newly-translated DNAI1 Protein Expression in Target Cells of the Respiratory Epithelium of NHPs
epithelial cells
ciliated cells
club cells
basal cells
%DNAI1-HA Positive
0
2
4
6
8
Vehicle
DNAI1 mRNA
Treatment
Single 0.4 mg/kg administration of inhaled LNP-formulated DNAI1-HA mRNA with lung sections collected from two NHPs 6 hrs after dosing.
Note: % DNAI1-HA positive calculated by combining cell counts from 4 examined lung sections. Total number of cells counted: ~500,000 to 1,400,000.

# Fig. 16A

## mIF Panel Results for NHP Lung Samples: DNAI1-HA Is Expressed in Cells of the Respiratory Epithelium




- DNAI1-HA expression detected in lung samples from NHPs treated with RTX0001.
- DNAI1-HA expression co-localized with markers for epithelial cells, including the club, basal and ciliated cells (club and basal cells are precursors for ciliated cells).
- No staining detected in lung samples from NHPs treated with RTX0004.

Fig. 16B

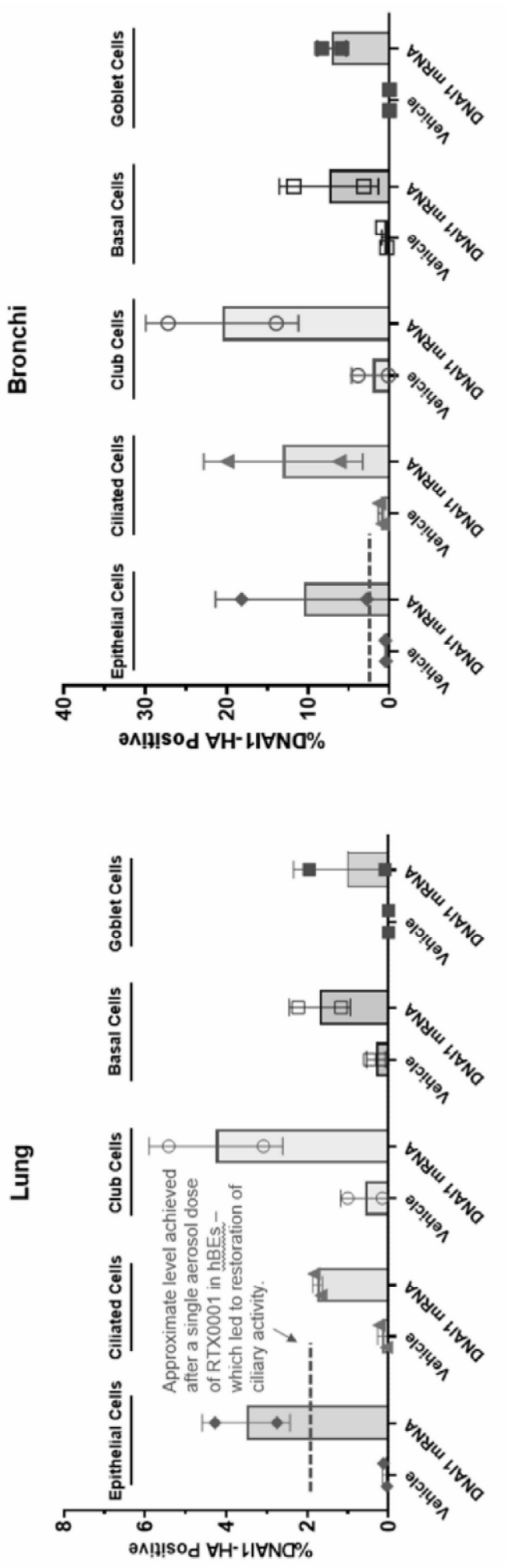

Bronchi
%DNAI1-HA Positive
0
10
20
30
40
Epithelial Cells
Ciliated Cells
Club Cells
Basal Cells
Goblet Cells
Vehicle
DNAI1 mRNA
Lung
%DNAI1-HA Positive
0
2
4
6
8
Approximate level achieved after a single aerosol dose of RTX0001 in hBEs – which led to restoration of ciliary activity.

**Fig. 17**

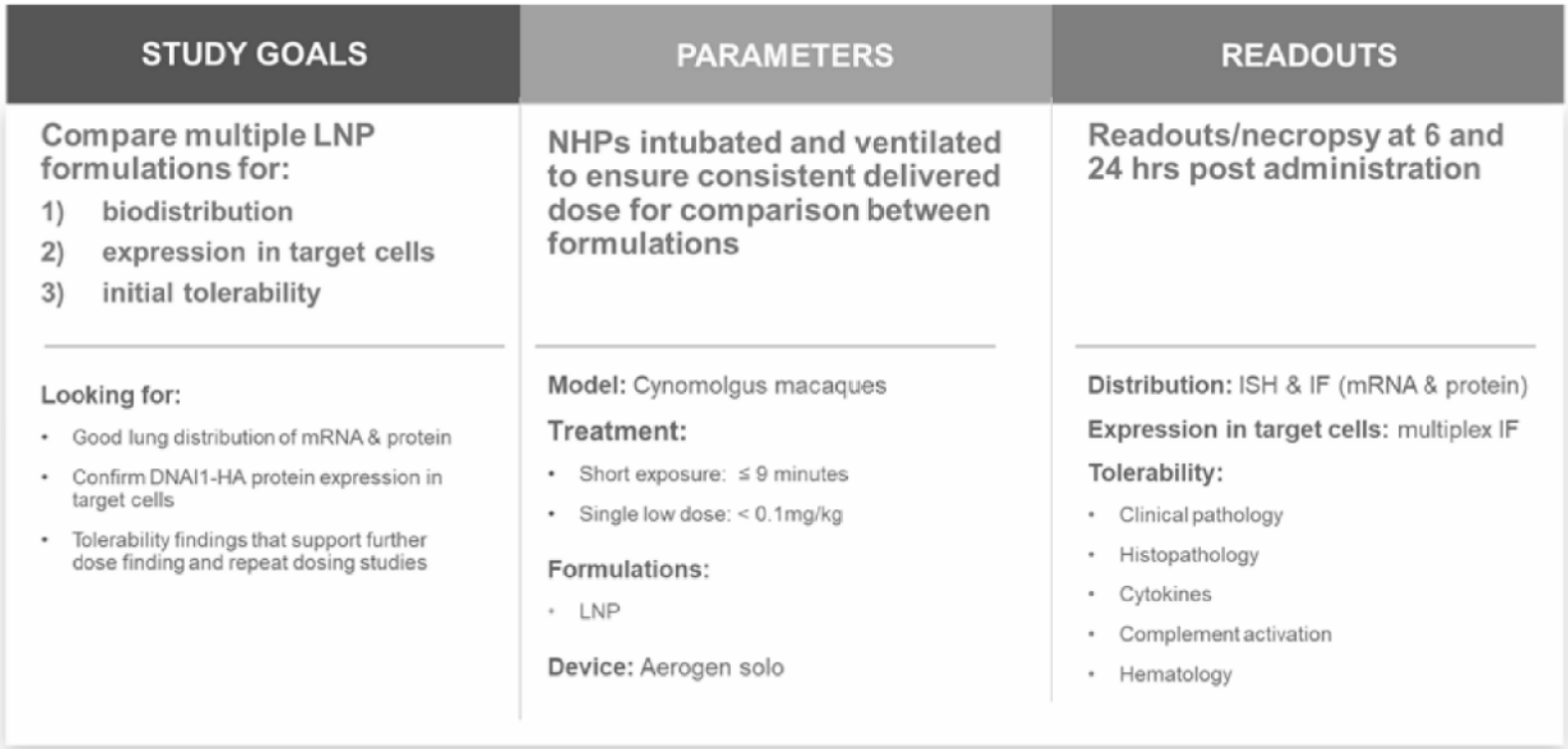

STUDY GOALS
PARAMETERS
READOUTS
Compare multiple LNP formulations for:
1) biodistribution
2) expression in target cells
3) initial tolerability
NHPs intubated and ventilated to ensure consistent delivered dose for comparison between formulations
Readouts/necropsy at 6 and 24 hrs post administration
Looking for:
• Good lung distribution of mRNA & protein
• Confirm DNAI1-HA protein expression in target cells
• Tolerability findings that support further dose finding and repeat dosing studies
Model: Cynomolgus macaques
Treatment:
• Short exposure: ≤ 9 minutes
• Single low dose: < 0.1mg/kg
Formulations:
• LNP
Device: Aerogen solo
Distribution: ISH & IF (mRNA & protein)
Expression in target cells: multiplex IF
Tolerability:
• Clinical pathology
• Histopathology
• Cytokines
• Complement activation
• Hematology

**Fig. 18A**

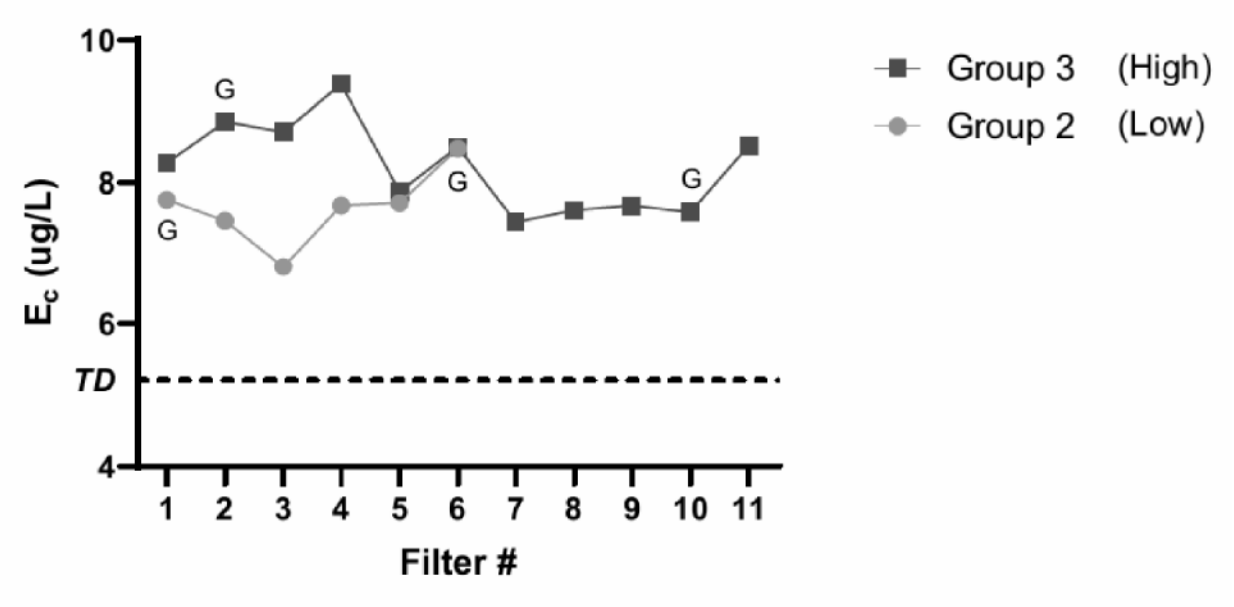



**Fig. 18B**

| No# | Designation (min) | Target dose *TD* (mg/kg) | Aerosol concentration $E_c$ (ug/L) | *RMV* (L) | *BW* (kg) | Delivered dose (mg/kg) | Animals |
|---|---|---|---|---|---|---|---|
| 1 | Vehicle 90 | 0 | 0 | 1.638 | 3.2 | 0 | Male |
| | | | | 1.417 | 2.7 | 0 | Female |
| 2 | Low 30 | 0.08 | 7.62 +/- 0.369 (5.2) | 1.550 | 3.0 | 0.12 | Male |
| | | | | 1.462 | 2.8 | 0.12 | Female |
| 4 | High 90 | 0.24 | 8.22 +/- 0.741 (5.2) | 1.594 | 3.1 | 0.38 | Male |
| | | | | 1.506 | 2.9 | 0.38 | Female |

0.38 mg/kg → 1.14 mg -25%-> 285 µg (RNA) → 8.55 mg total lipids

**Fig. 18C**

| | Impactor (Mercer) | |
|---|---|---|
| RTX-00052/DNAI1 | MMAD (µm) | GSD |
| 2 | 1.7 | 1.88 |
| 3 | 2.3 | 1.92 |

**Fig. 19A**

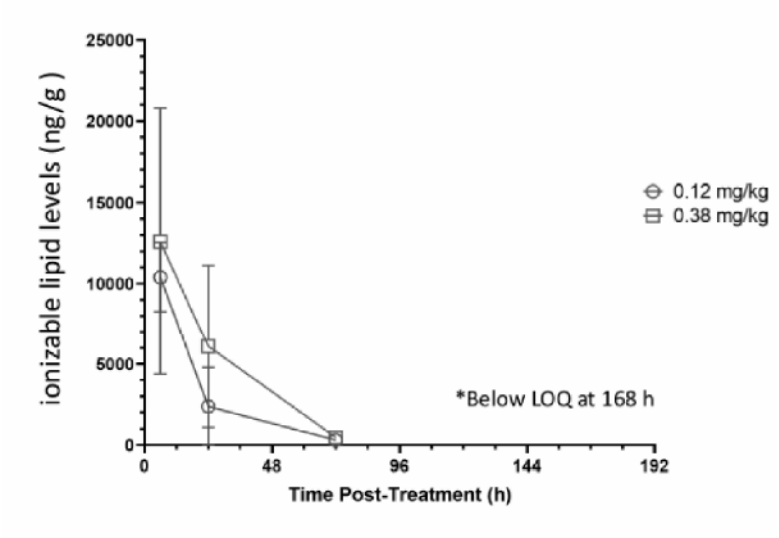



**Fig. 19B**

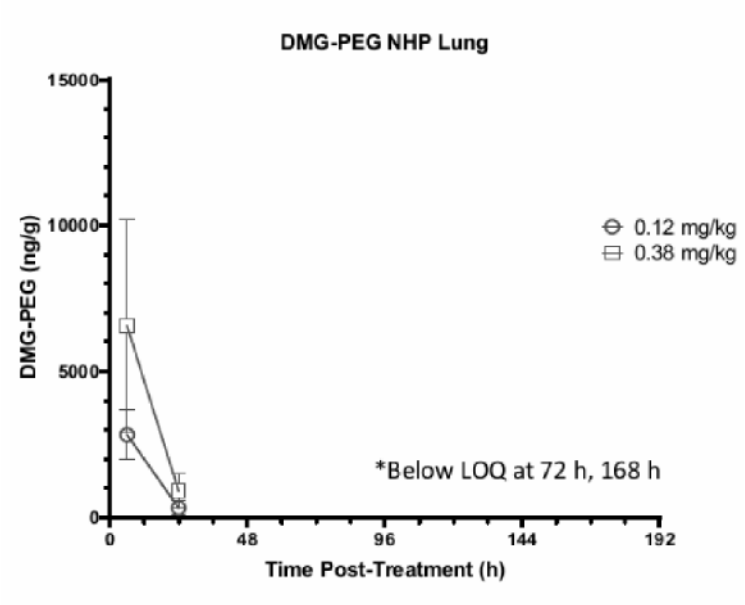



**Fig. 19C**

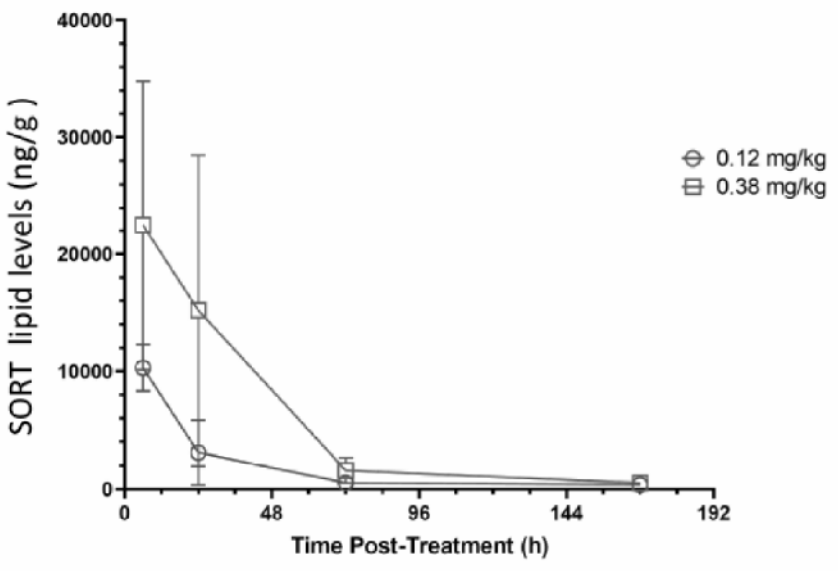
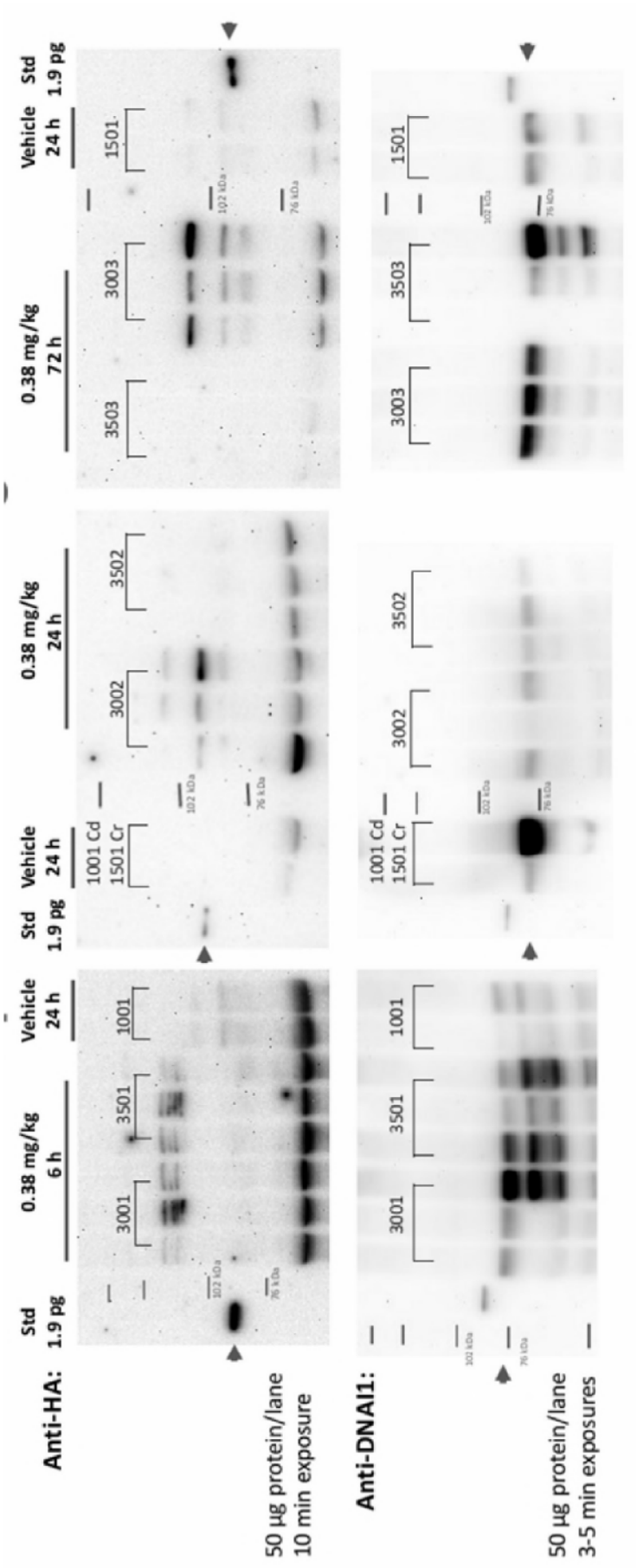


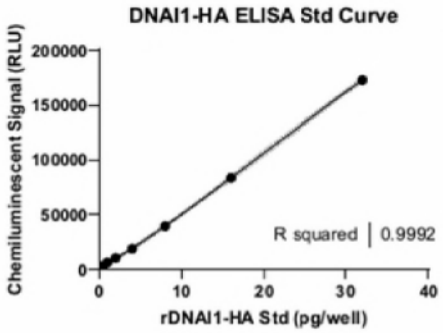
Fig. 20A


Anti-HA:
Std 1.9 pg
0.38 mg/kg 6 h
Vehicle 24 h
3001
3501
1001
50 µg protein/lane
10 min exposure
Std 1.9 pg
Vehicle 24 h
1001 Cd
1501 Cr
0.38 mg/kg 24 h
3002
3502
Vehicle 24 h
Std 1.9 pg
0.38 mg/kg 72 h
3503
3003
1501
Anti-DNAI1:
3001
3501
1001
50 µg protein/lane
3-5 min exposures
1001 Cd
1501 Cr
3002
3502
3003
3503
1501

# Fig. 20B

**Vehicle 24 h**

| Animal Number | ELISA Result (pg/100 µg total protein) | Western Blot Signal (Y/N) |
|---|---|---|
| 1001 Cr | n.d. | N |
| 1001 Mi | n.d. | N |
| 1501 Cr | n.d. | N |




Est. LOQ: 5 pg/ml, 0.5 pg/well

**High Dose: 0.38 mg/kg 6 h**

| Animal Number | ELISA Result (pg/100 µg total protein) | Western Blot Signal (Y/N) |
|---|---|---|
| 3001 Cr | n.d. | N |
| 3001 Mi | n.d. | N |
| 3001 Cd | n.d. | N |
| 3501 Cr | n.d. | N |
| 3501 Mi | n.d. | N |
| 3501 Cd | 0.43* | N |

*Below LOQ

**High Dose: 0.38 mg/kg 24 h**

| Animal Number | ELISA Result (pg/100 µg total protein) | Western Blot Signal (Y/N) |
|---|---|---|
| 3002 Cr | n.d. | N |
| 3002 Mi | n.d. | N |
| 3002 Cd | n.d. | N |
| 3502 Cr | n.d. | N |
| 3502 Mi | n.d. | N |
| 3502 Cd | n.d. | N |

**High Dose: 0.38 mg/kg 72 h**

| Animal Number | ELISA Result (pg/100 µg total protein) | Western Blot Signal (Y/N) |
|---|---|---|
| 3003 Cr | n.d. | N |
| 3003 Mi | n.d. | N |
| 3003 Cd | n.d. | N |
| 3503 Cr | n.d. | N |
| 3503 Mi | n.d. | N |
| 3503 Cd | n.d. | N |

- Only detected signal in one 6 h sample (Below LOQ of assay)
- Did not correlate with western blot results

Fig. 21A

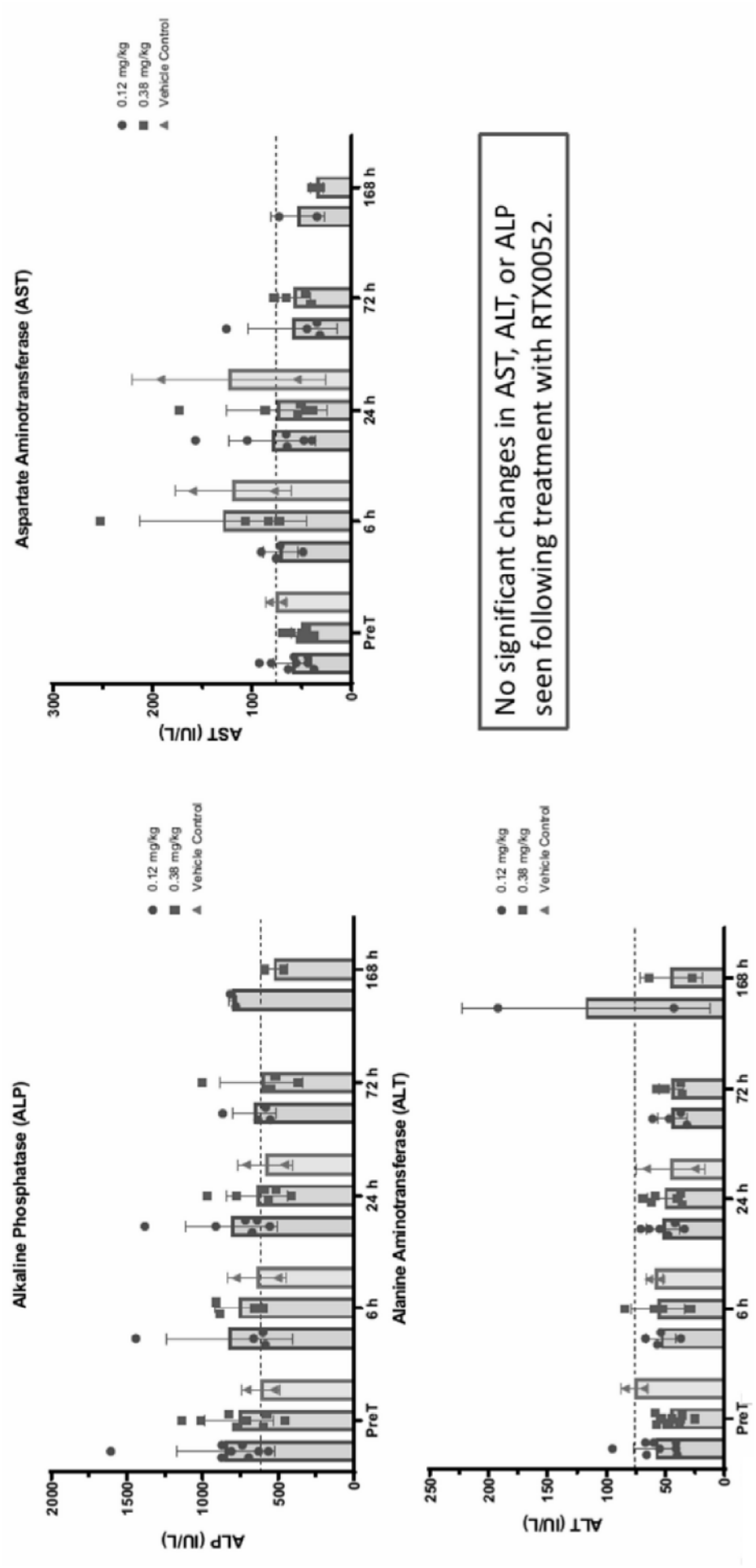

Aspartate Aminotransferase (AST)
AST (IU/L)
0.12 mg/kg
0.38 mg/kg
Vehicle Control
PreT
6 h
24 h
72 h
168 h
No significant changes in AST, ALT, or ALP seen following treatment with RTX0052.
Alkaline Phosphatase (ALP)
ALP (IU/L)
Alanine Aminotransferase (ALT)
ALT (IU/L)

**Fig. 21B**

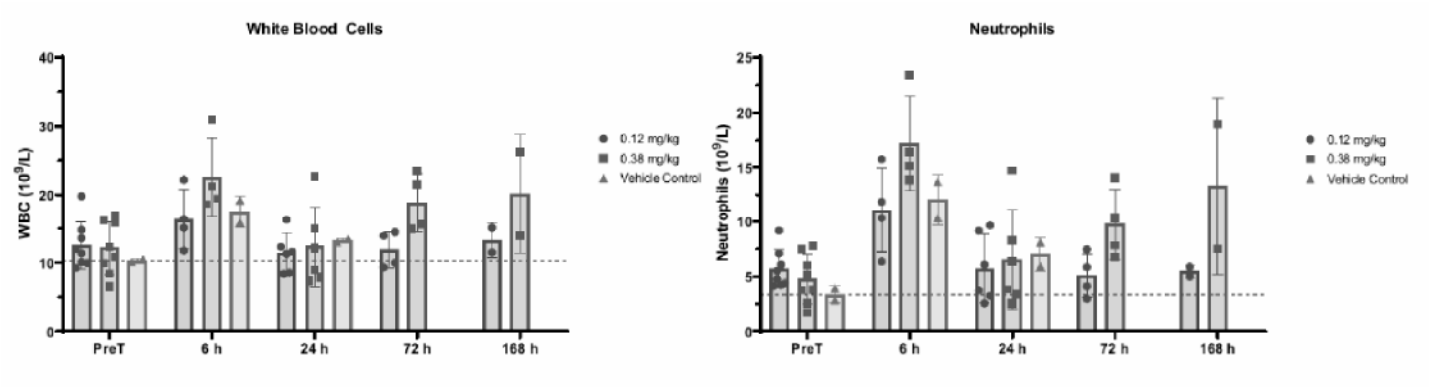
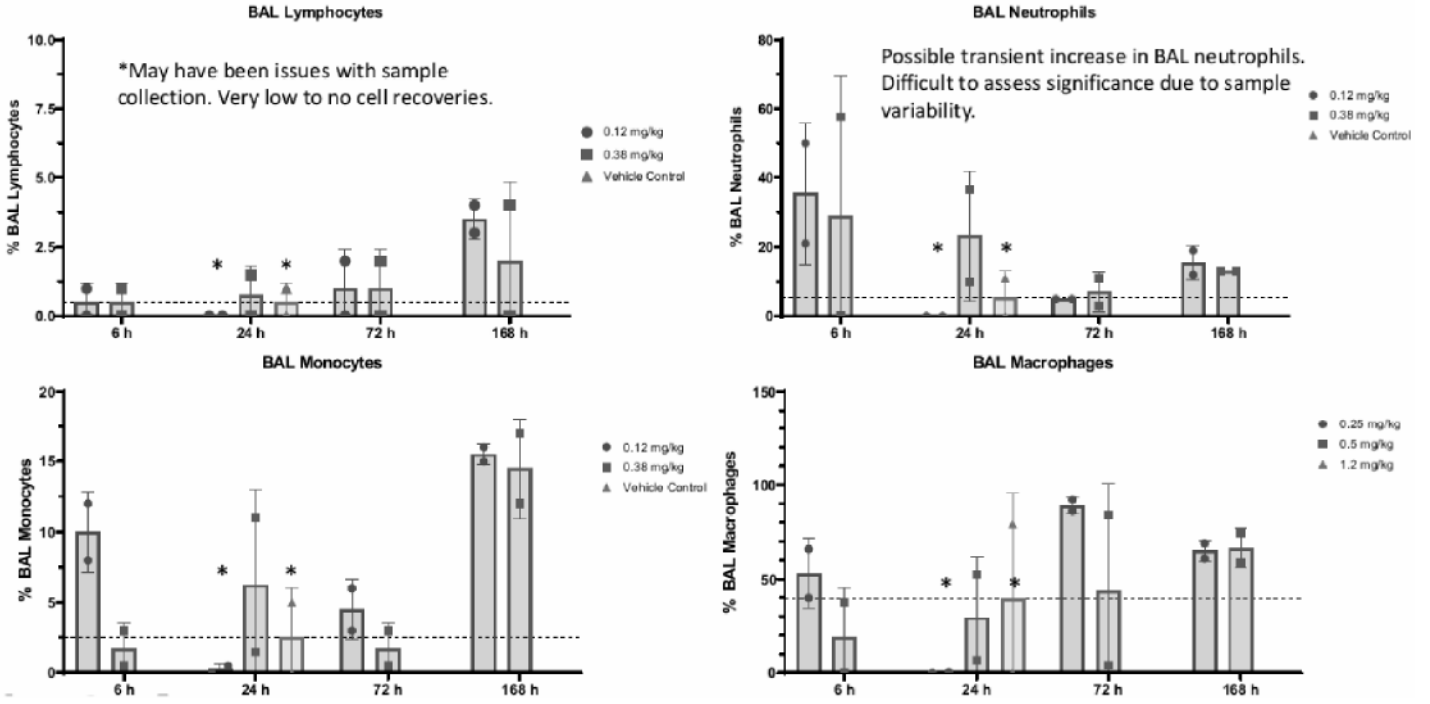
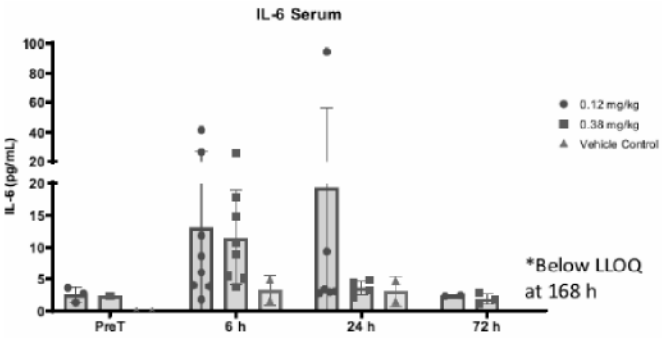
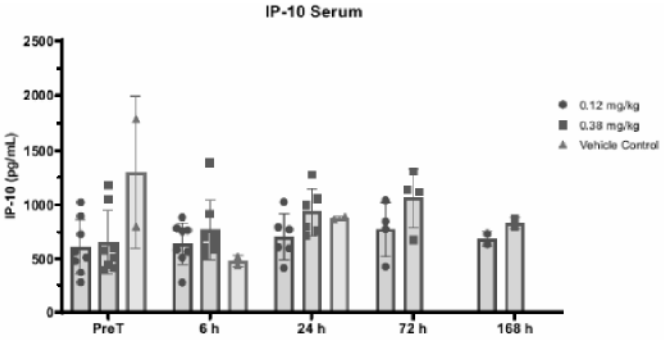


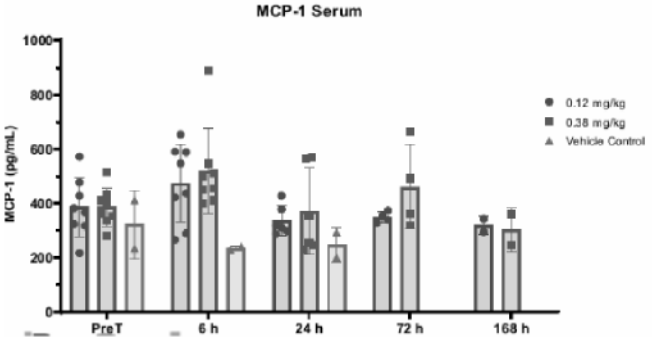

**Fig. 21C**



# Fig. 21D

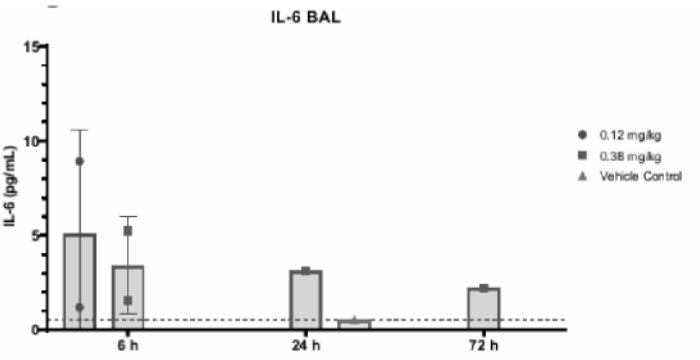


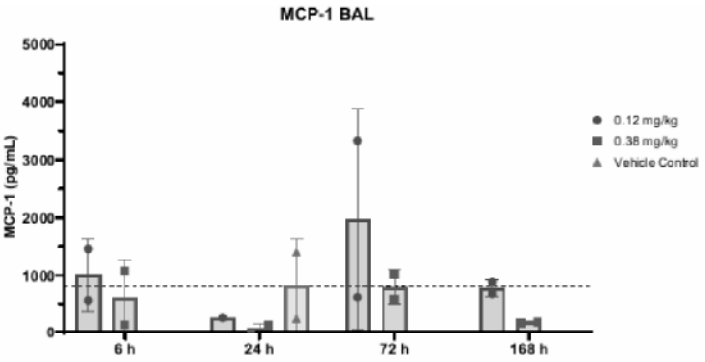



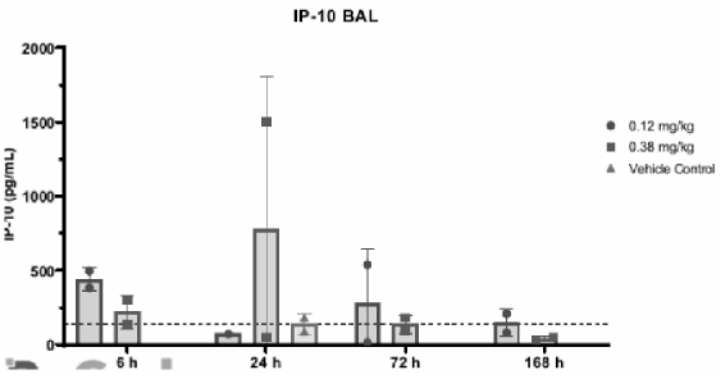




- Small increase in serum IL-6 levels seen with treatment.
- No significant changes seen in IP-10 or MCP-1 levels
- **All cytokine levels were in the same range as normal reported levels**

# Fig. 21E

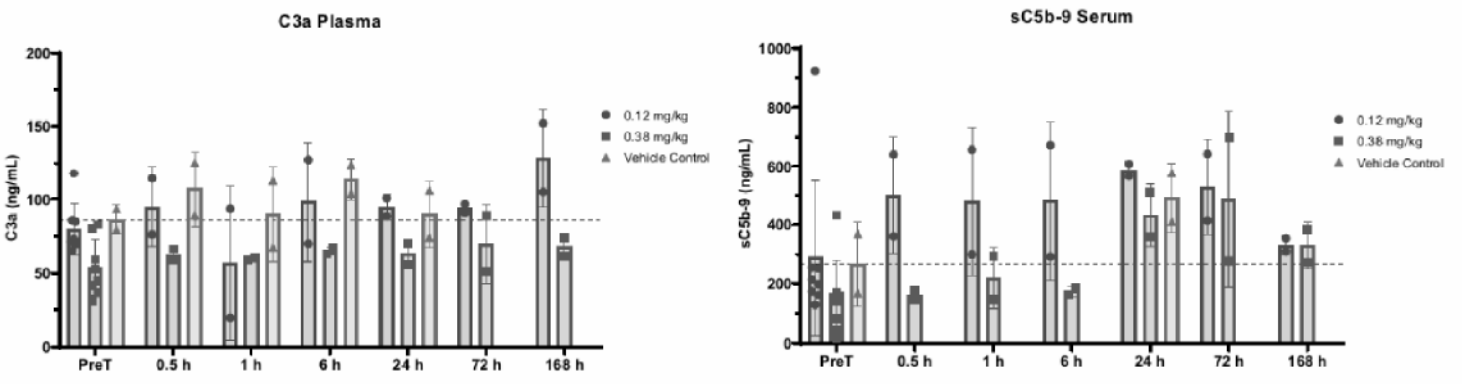



- Small increase in serum IL-6 levels seen with treatment. Levels were much
- No significant changes seen in IP-10 or MCP-1 levels

# Fog. 21F




# Fig. 21G

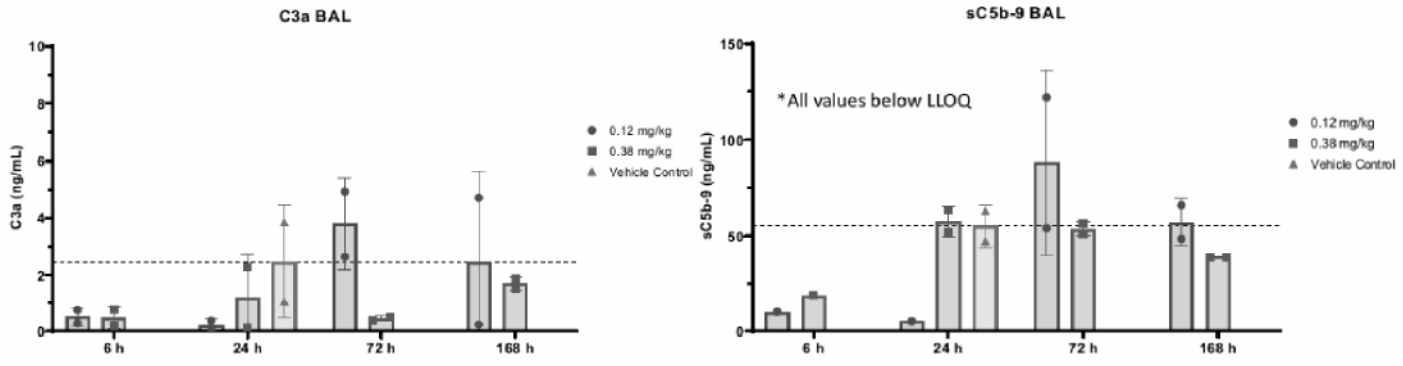


**Fig. 22A**

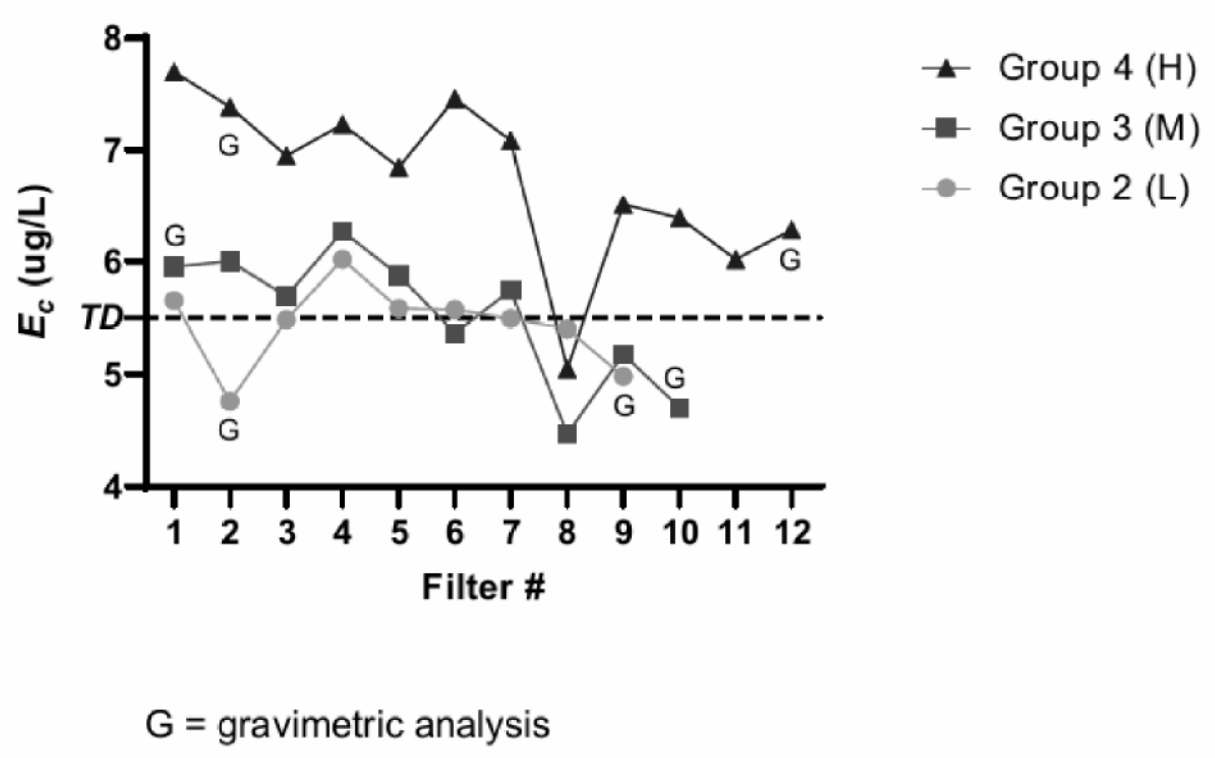



**Fig. 22B**

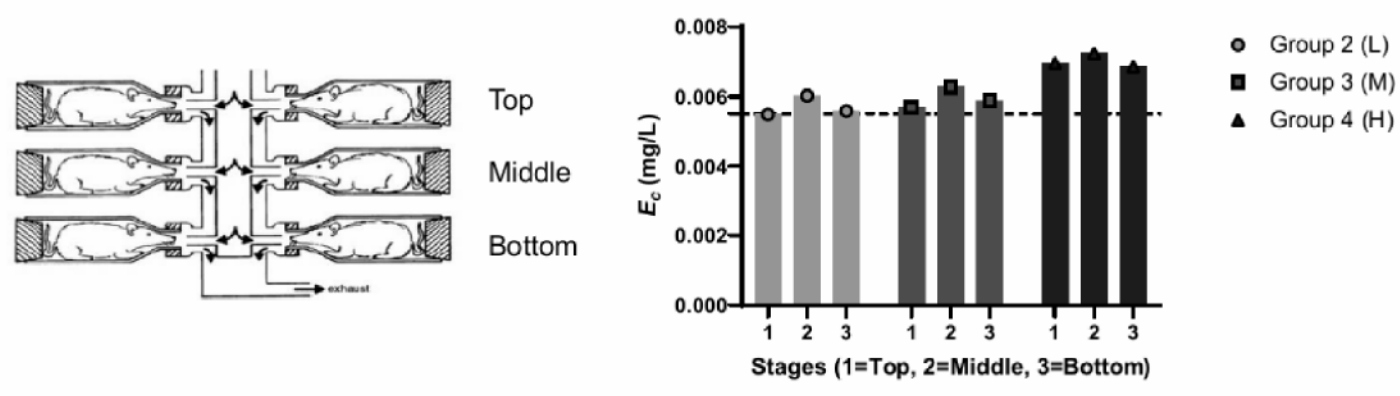


**Fig. 22C**

| No# | Designation (min) | Target dose *TD* (mg/kg) | Aerosol concentration $E_c$ (ug/L) | *RMV* (L) | *BW* (kg) | Delivered dose (mg/kg) | Animals |
|---|---|---|---|---|---|---|---|
| 1 | Vehicle 240 | 0 | 0 | 0.224 | 0.310 | 0 | Male |
| | | | | 0.158 | 0.205 | 0 | Female |
| 2 | Low 60 | 0.25 | 5.44 +/- 0.369 (5.5) | 0.223 | 0.308 | 0.24 | Male |
| | | | | 0.153 | 0.198 | 0.25 | Female |
| 3 | Mid 120 | 0.49 | 5.50 +/- 0.585 (5.5) | 0.255 | 0.311 | 0.48 | Male |
| | | | | 0.156 | 0.203 | 0.51 | Female |
| 4 | High 240 | 0.99 | 6.70 +/- 0.741 (5.5) | 0.227 | 0.315 | 1.16 | Male |
| | | | | 0.161 | 0.210 | 1.23 | Female |

**Fig. 22D**

| | Impactor (Mercer) | |
|---|---|---|
| RTX00052/DNAI1-HA | MMAD (μm) | GSD |
| 2 | 1.3 | 1.97 |
| 3 | 1.1 | 2.00 |
| 4 | 1.4 | 1.89 |

Fig. 23A

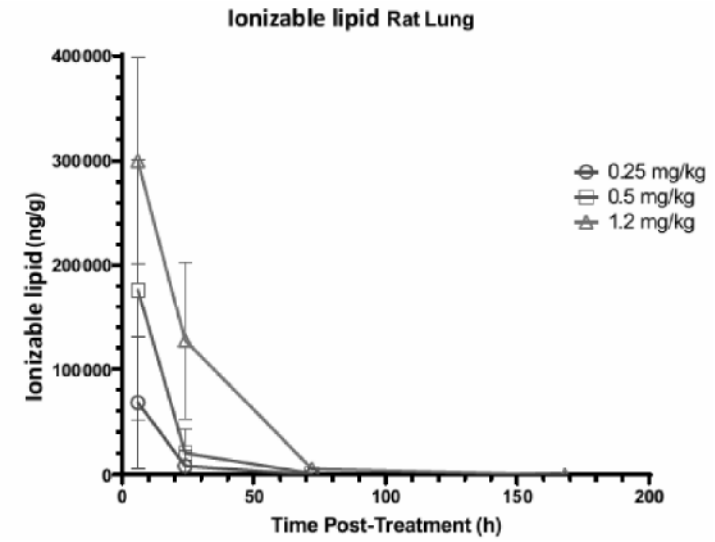



Fig. 23B

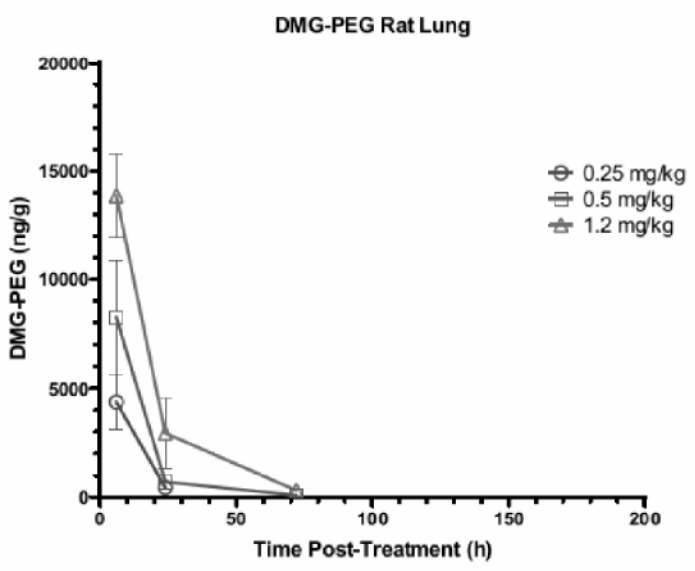
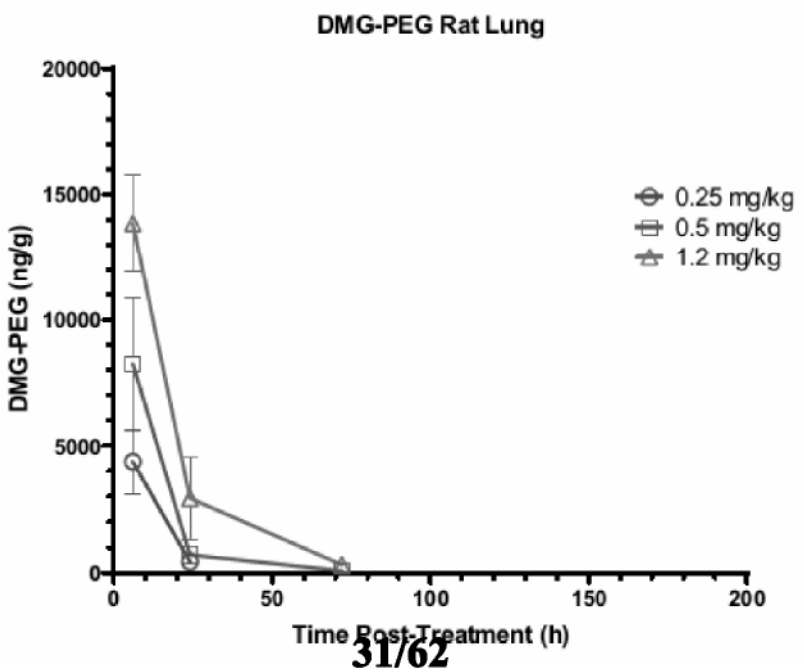
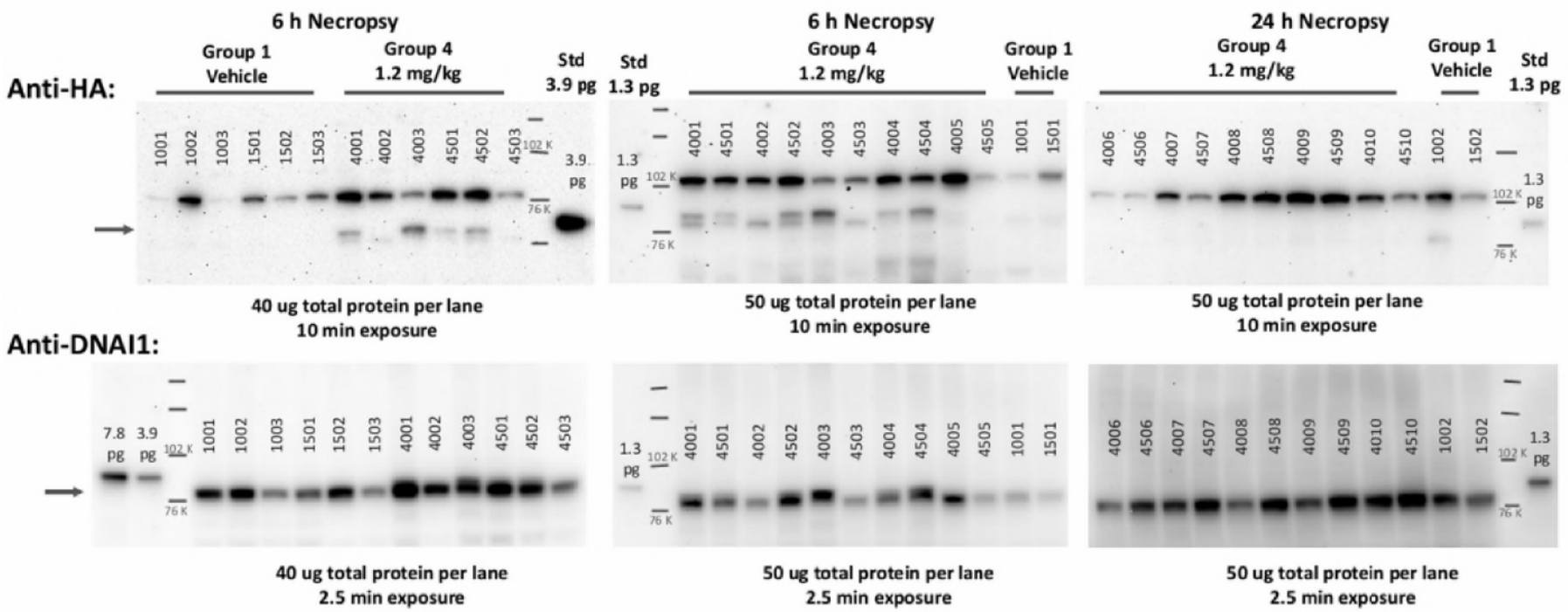



Fig. 23C



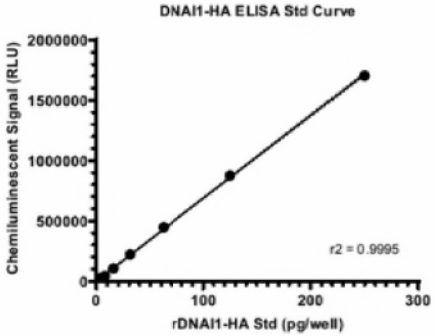
# Fig. 24A


6 h Necropsy
Group 1 Vehicle
Group 4 1.2 mg/kg
Std 3.9 pg
Std 1.3 pg
6 h Necropsy
Group 4 1.2 mg/kg
Group 1 Vehicle
24 h Necropsy
Group 4 1.2 mg/kg
Group 1 Vehicle
Std 1.3 pg
Anti-HA:
1001 1002 1003 1501 1502 1503 4001 4002 4003 4501 4502 4503
3.9 pg
102 K
76 K
1.3 pg
4001 4501 4002 4502 4003 4503 4004 4504 4005 4505 1001 1501
4006 4506 4007 4507 4008 4508 4009 4509 4010 4510 1002 1502
40 ug total protein per lane
10 min exposure
50 ug total protein per lane
10 min exposure
50 ug total protein per lane
10 min exposure
Anti-DNAI1:
7.8 pg
3.9 pg
40 ug total protein per lane
2.5 min exposure
50 ug total protein per lane
2.5 min exposure
50 ug total protein per lane
2.5 min exposure

# Fig. 24B

| Vehicle 24 h | | | High Dose: 1.2 mg/kg 6 h | | | High Dose: 1.2 mg/kg 24 h | | |
|---|---|---|---|---|---|---|---|---|
| Animal Number | ELISA Result (pg/100 µg total protein) | Western Blot Signal (Y/N) | Animal Number | ELISA Result (pg/100 µg total protein) | Western Blot Signal (Y/N) | Animal Number | ELISA Result (pg/100 µg total protein) | Western Blot Signal (Y/N) |
| 1001 | n.d. | N | 4001 | 0.47 | Y | 4006 | n.d. | N |
| 1002 | n.d. | N | 4501 | n.d. | Y | 4506 | n.d. | N |
| 1003 | n.d. | N | 4002 | n.d. | N | 4007 | n.d. | N |
| 1501 | n.d. | N | 4502 | 1.10 | Y | 4507 | n.d. | N |
| 1502 | n.d. | N | 4003 | 3.23 | Y | 4008 | n.d. | N |
| 1503 | n.d. | N | 4503 | n.d. | N | 4508 | n.d. | N |
| | | | 4004 | 0.34* | Y | 4009 | n.d. | N |
| | | | 4504 | 1.01 | Y | 4509 | n.d. | N |
| | | | 4005 | 0.09* | N | 4010 | n.d. | N |
| | | | 4505 | n.d. | N | 4510 | n.d. | N |

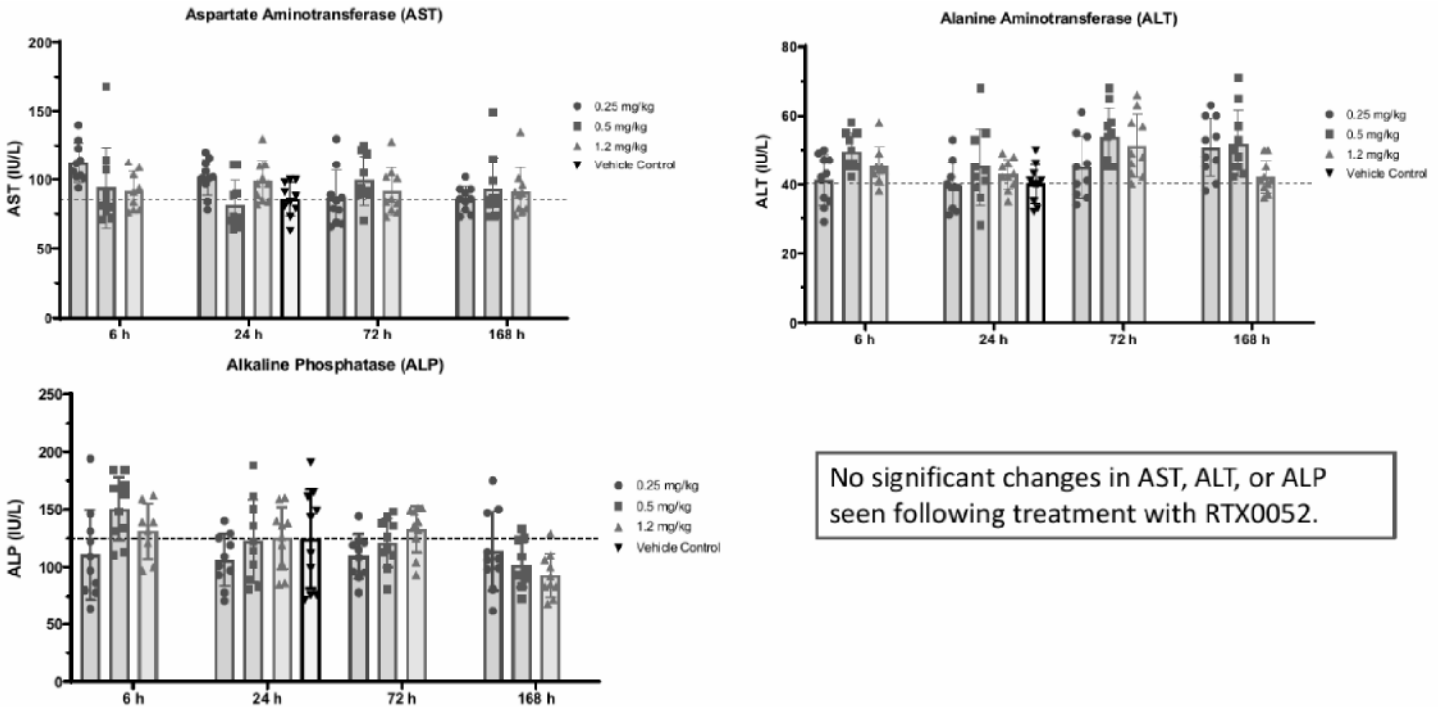
*Below LOQ




Est. LOQ: 5 pg/ml, 0.5 pg/well

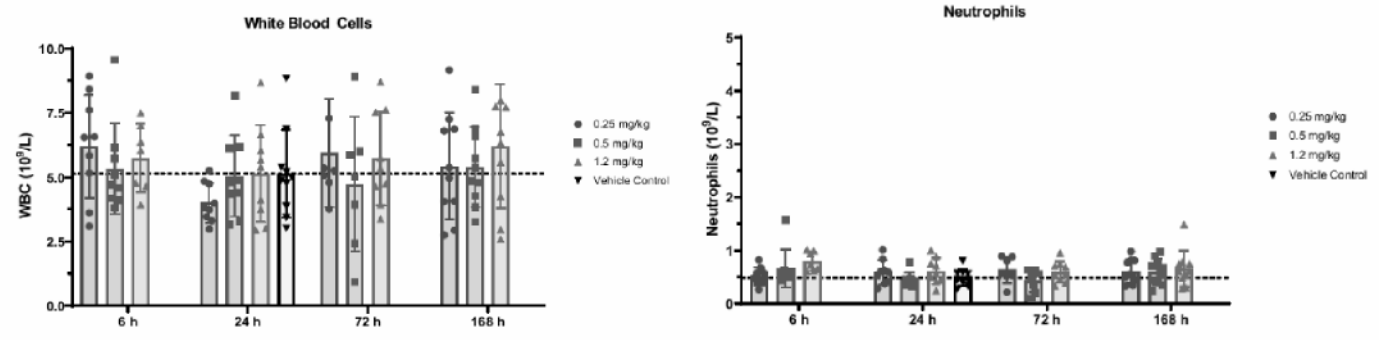
**Good correlation with WB results: 5/6 WB+ samples are also ELISA+**

# Fig. 25A




No significant changes in AST, ALT, or ALP seen following treatment with RTX0052.

# Fig. 25B




No significant changes in WBC or neutrophil counts seen following treatment with RTX0052.

Fig. 25C

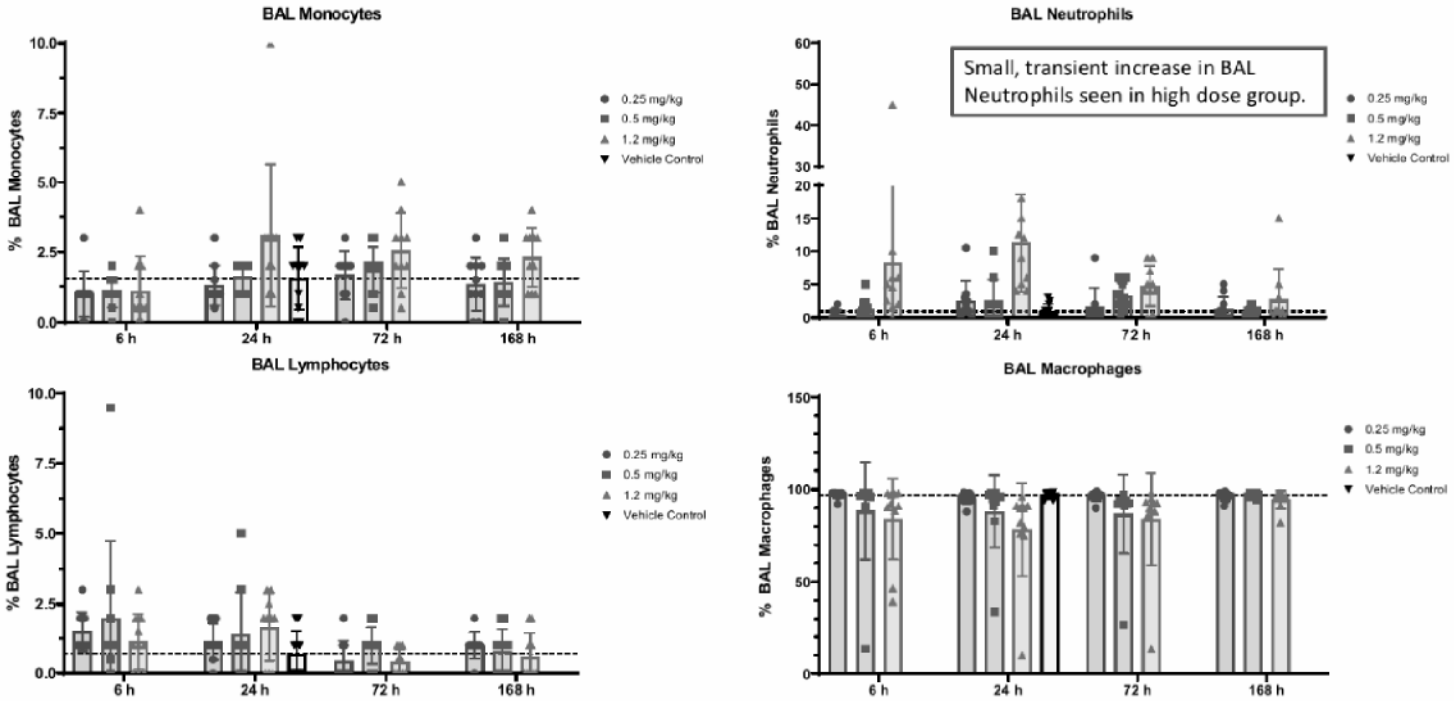
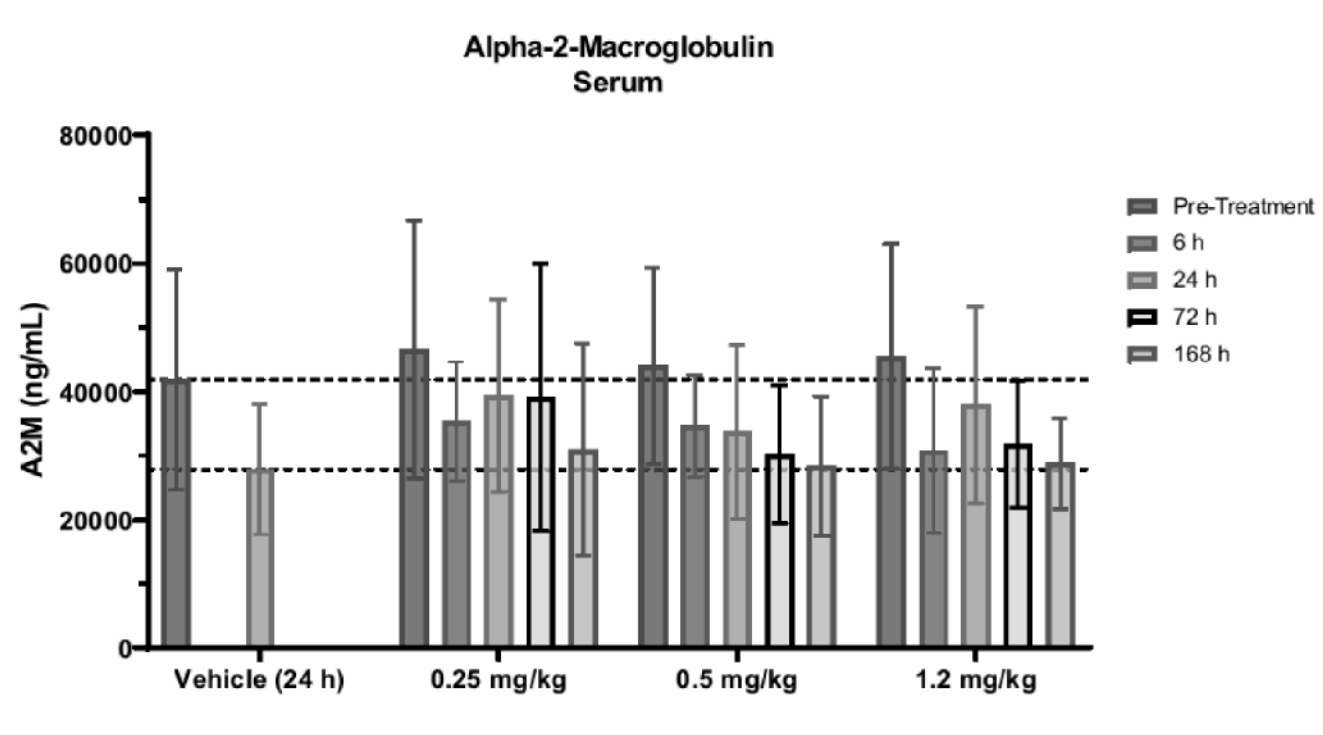



Fig. 25D



# Fig. 26A

| Group | Formulation | Animals | Delivery | [RNA] (mg/mL) | Total Dose | aerosol delivery of Substrate | # animals | Data Collection |
|---|---|---|---|---|---|---|---|---|
| 1 | Control vehicle | Albino B6[1], naive | Nebulization 2 h 66.6 µL/min | **none** | **4 mL buffer 27** | - | **n = 12** | **<u>For groups 2 to 7 – once a week (Mondays or Tuesdays) and after last dose</u>**:<br>a) 2 mice @ 4h post-dosing = IVIS<br><br>**<u>For all groups – after last dose</u>**:<br>a) 2 mice @ 4h post-last dose and post-IVIS = perfuse and explant liver, spleen and lungs. Half for RNAlater; half for protein assay<br><br>b) Histopath at<br>72h post-last dosing<br>(5 mice) and<br>7 days post-last dosing<br>(5 mice) |
| 2 | RTX00001 (95% DNAI1-HA + 5% luciferase mRNA) | Albino B6[1], naive | Nebulization 2 h 66.6 µL/min | **0.5 mg/mL neb: 0.5 mg/mL** | **4.0 mg per chamber** | **For 2 mice: 2 mL Luciferin @ 95 mM (30 mg/mL)** | **n = 12** | |
| 3 | RTX00051 (95% DNAI1-HA + 5% luciferase mRNA | Albino B6[1], naive | Nebulization 2 h 66.6 µL/min | **0.5 mg/mL neb: 0.5 mg/mL** | **4.0 mg per chamber** | **For 2 mice: 2 mL Luciferin @ 95 mM (30 mg/mL)** | **n = 12** | |
| 4 | RTX00052 (95% DNAI1-HA + 5% luciferase mRNA | Albino B6[1], naive | Nebulization 2 h 66.6 µL/min | **0.5 mg/mL neb: 0.5 mg/mL** | **4.0 mg per chamber** | **For 2 mice: 2 mL Luciferin @ 95 mM (30 mg/mL)** | **n = 12** | |

**Fig. 26B**

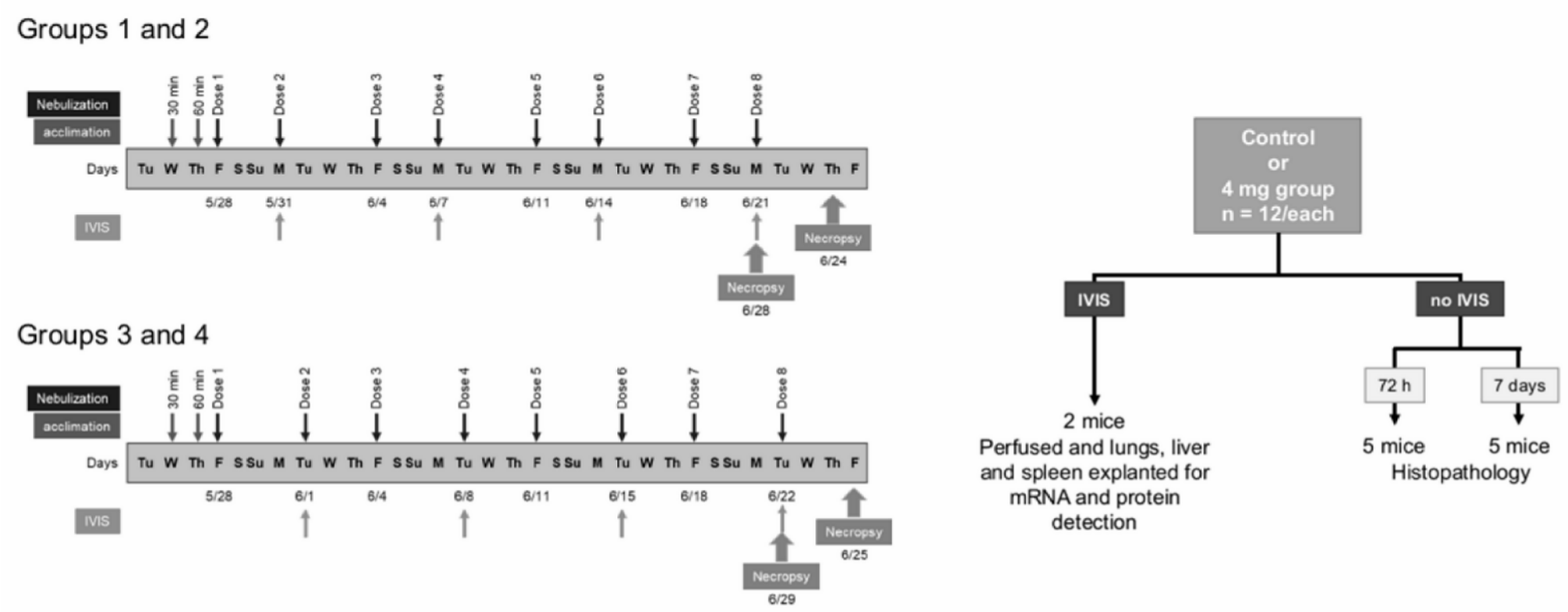
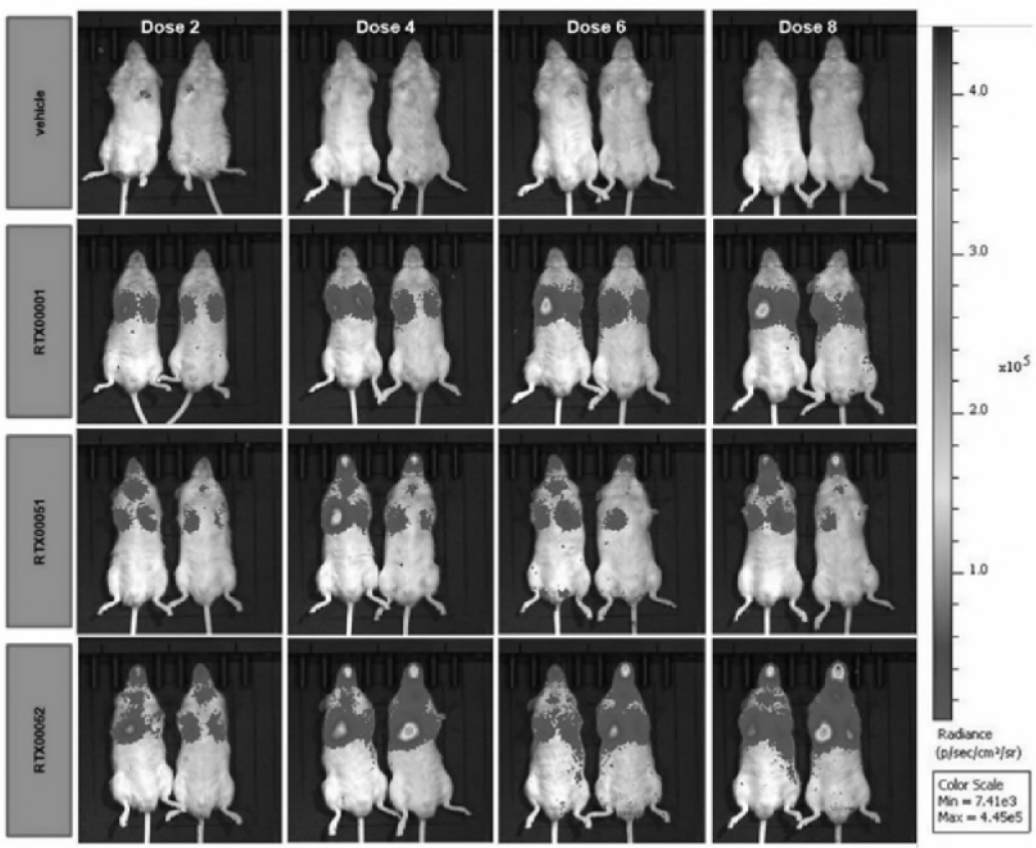
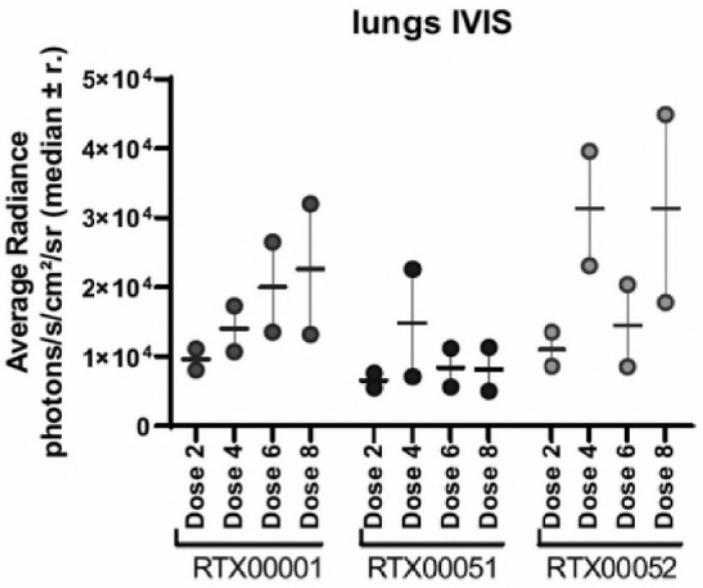
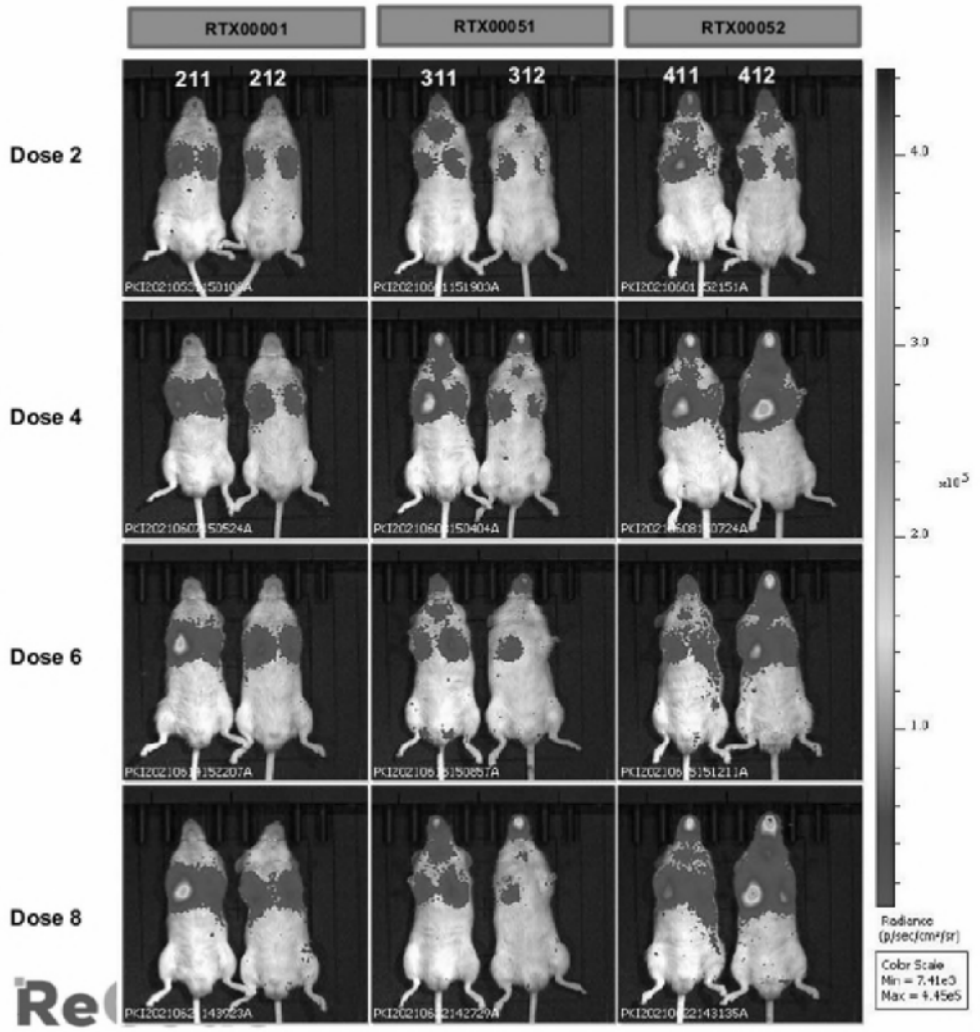

Groups 1 and 2
Nebulization
acclimation
30 min
60 min
Dose 1
Dose 2
Dose 3
Dose 4
Dose 5
Dose 6
Dose 7
Dose 8
Days
Tu W Th F S Su M Tu W Th F S Su M Tu W Th F S Su M Tu W Th F S Su M Tu W Th F
5/28
5/31
6/4
6/7
6/11
6/14
6/18
6/21
IVIS
Necropsy
6/24
Necropsy
6/28
Groups 3 and 4
Nebulization
acclimation
30 min
60 min
Dose 1
Dose 2
Dose 3
Dose 4
Dose 5
Dose 6
Dose 7
Dose 8
Days
Tu W Th F S Su M Tu W Th F S Su M Tu W Th F S Su M Tu W Th F S Su M Tu W Th F
5/28
6/1
6/4
6/8
6/11
6/15
6/18
6/22
IVIS
Necropsy
6/25
Necropsy
6/29
Control
or
4 mg group
n = 12/each
IVIS
no IVIS
2 mice
Perfused and lungs, liver and spleen explanted for mRNA and protein detection
72 h
7 days
5 mice
5 mice
Histopathology

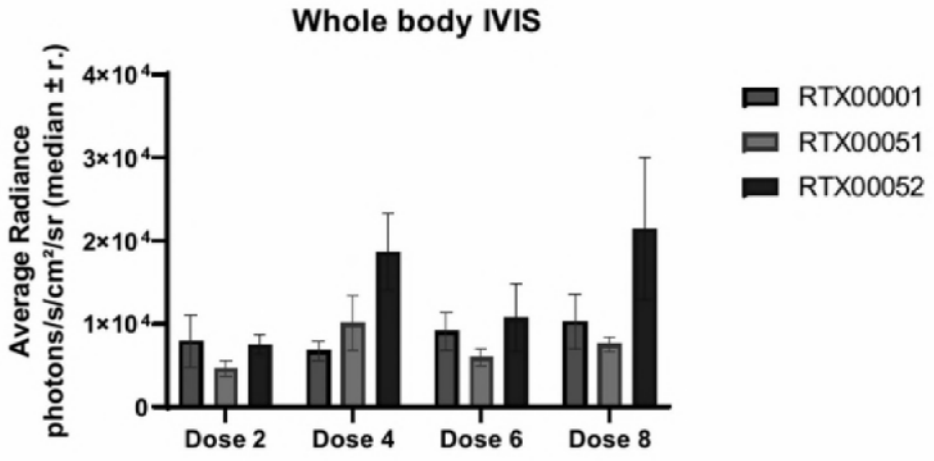
Fig. 27A


Dose 2
Dose 4
Dose 6
Dose 8
vehicle
RTX00001
RTX00051
RTX00052
4.0
3.0
x10^5
2.0
1.0
Radiance (p/sec/cm²/sr)
Color Scale
Min = 7.41e3
Max = 4.45e5

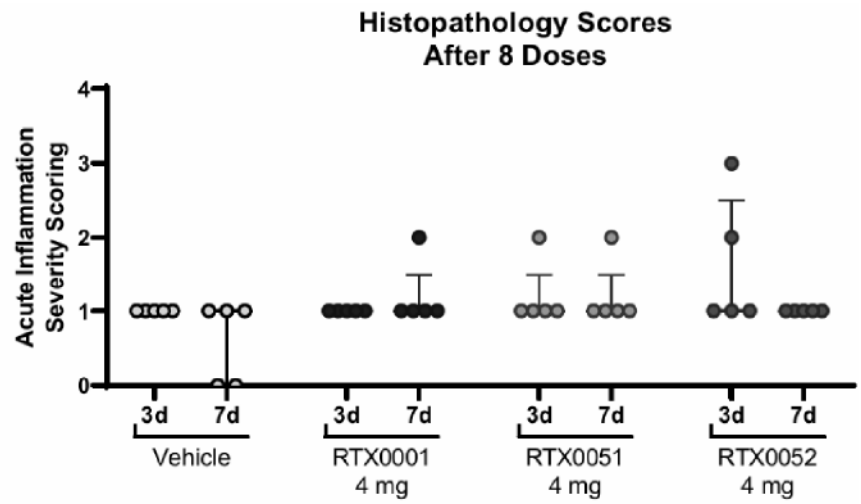

lungs IVIS
Average Radiance photons/s/cm²/sr (median ± r.)
5×10⁴
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
Dose 2
Dose 4
Dose 6
Dose 8
RTX00001
RTX00051
RTX00052

# Fig. 27B

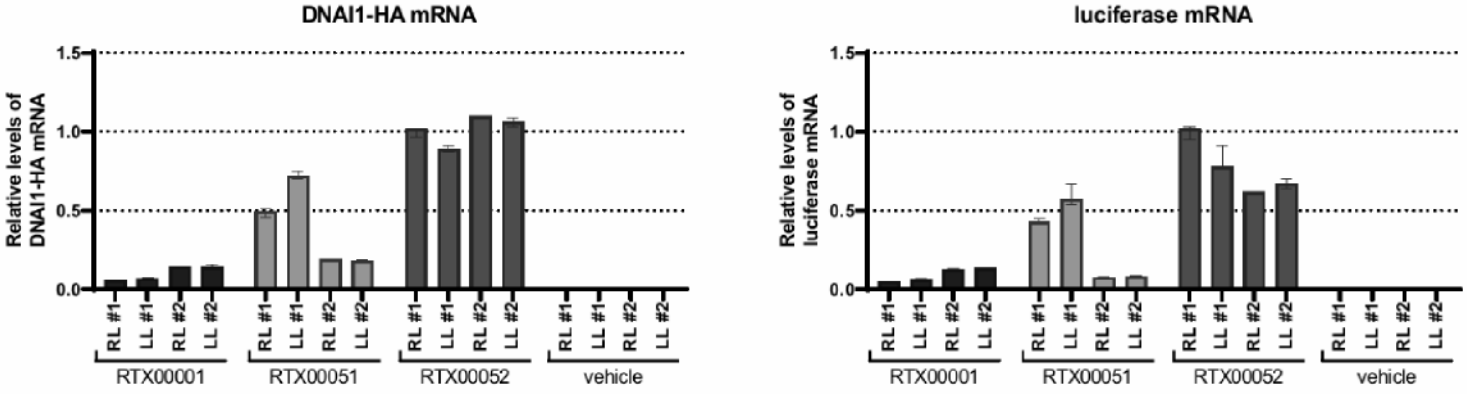

RTX00001
RTX00051
RTX00052
211
212
311
312
411
412
Dose 2
Dose 4
Dose 6
Dose 8
4.0
3.0
2.0
1.0
Radiance (p/sec/cm²/sr)
Color Scale
Min = 7.41e3
Max = 4.45e5


Whole body IVIS
Average Radiance photons/s/cm²/sr (median ± r.)
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
Dose 2
Dose 4
Dose 6
Dose 8
RTX00001
RTX00051
RTX00052

## Fig. 27C




## Fig. 27D




## Fig. 27E

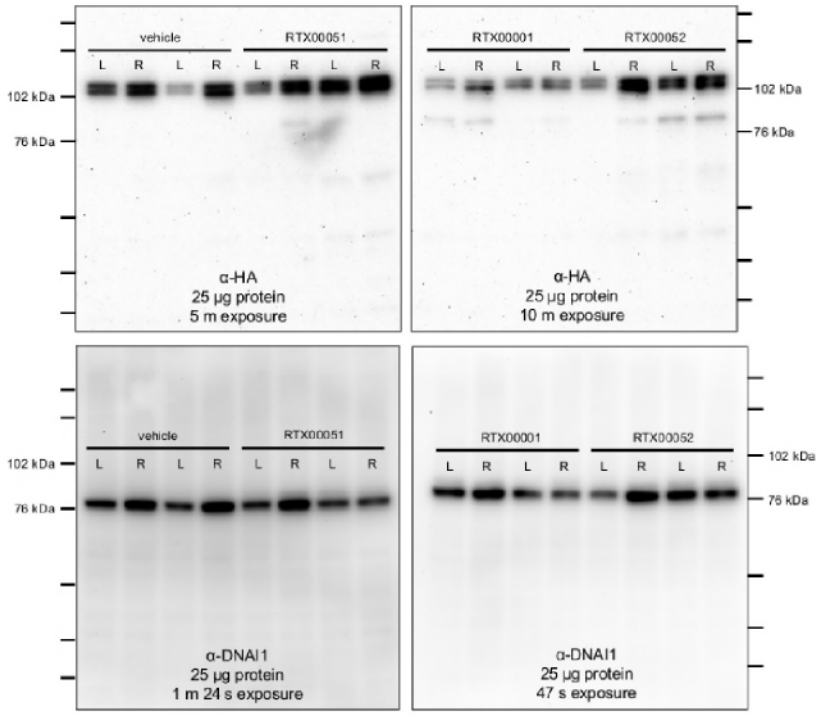


**Fig. 28A**

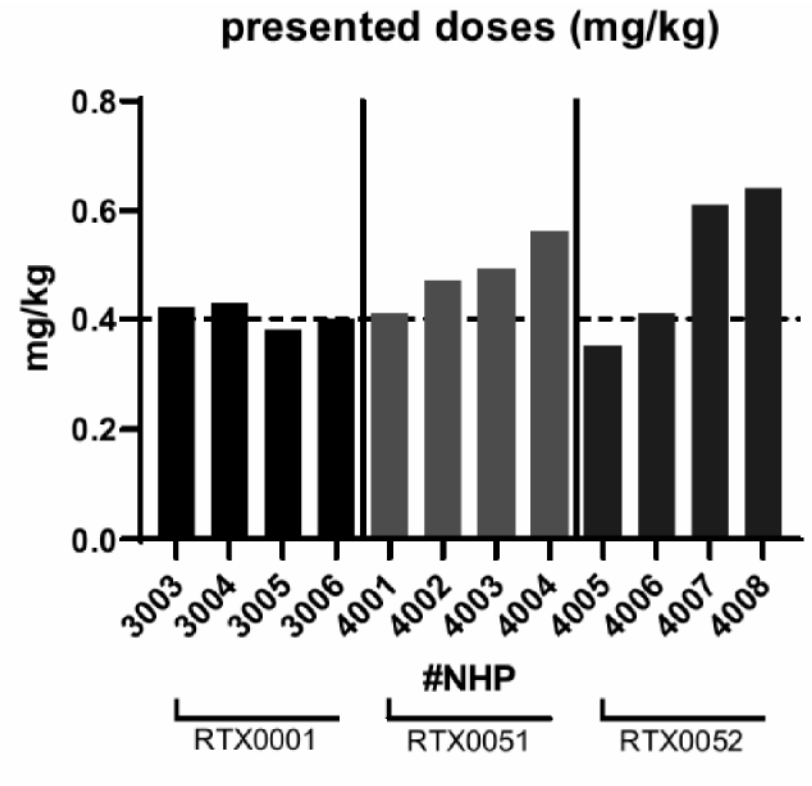
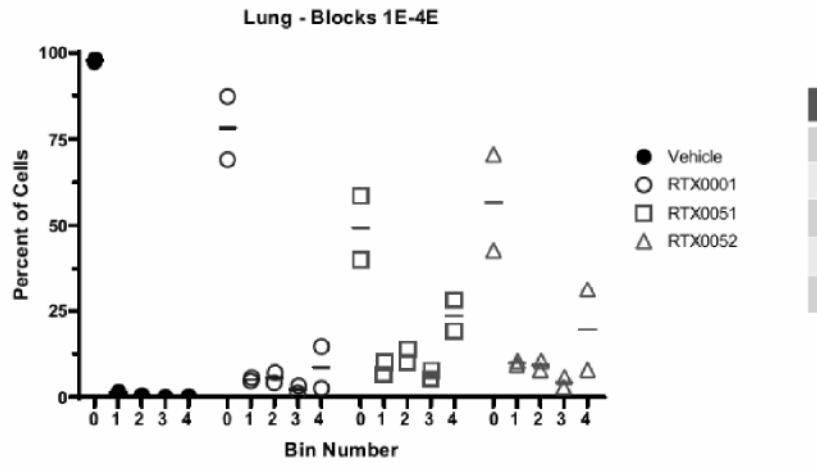



**Fig. 28B**

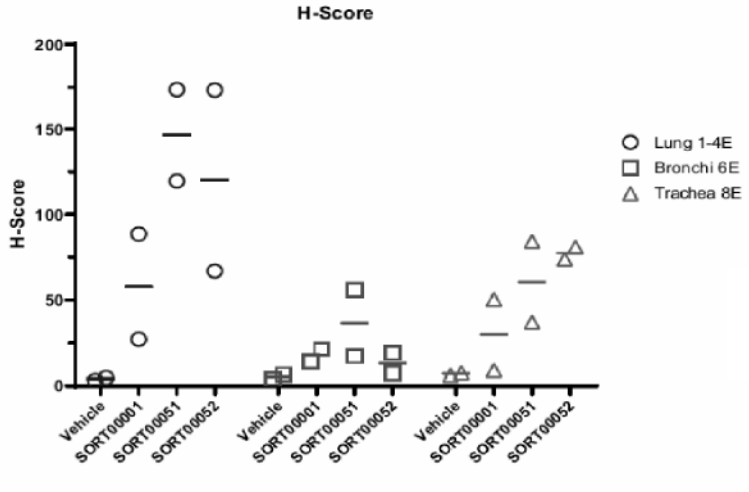
| | APS (Aerodynamic particle sizer) | | | | Impactor (Mercer) | |
|---|---|---|---|---|---|---|
| | MMAD (μm) | | GSD | | MMAD (μm) | GSD |
| | Pre | Post | Pre | Post | | |
| RTX0001 | 2.59 | 2.21 | 1.69 | 2.23 | 2.55 | 1.86 |
| RTX0051 | 3.33 | 1.95/2.16 | 1.93 | 1.77/2.22 | n.d. | n.d. |
| RTX0052 | 2.96 | 2.99 | 1.83 | 1.86 | 2.57 | 1.80 |

## Fig. 28C




| Bin # | Scoring |
|---|---|
| Bin 0 | 0 copies/cell or no staining |
| Bin 1 | 1-3 copies/cell |
| Bin 2 | 4-9 copies/cell and none/few clusters |
| Bin 3 | 10-15 copies/cell and/or <10% dots in clusters |
| Bin 4 | >15 copies/cell and/or >10% dots in clusters |

## Fig. 28D




**H-Score Definition:**
H-score = Σ[Bin 0-4] (bin number x percentage of cells per bin)

Range: 0 to 400

Fig. 29A

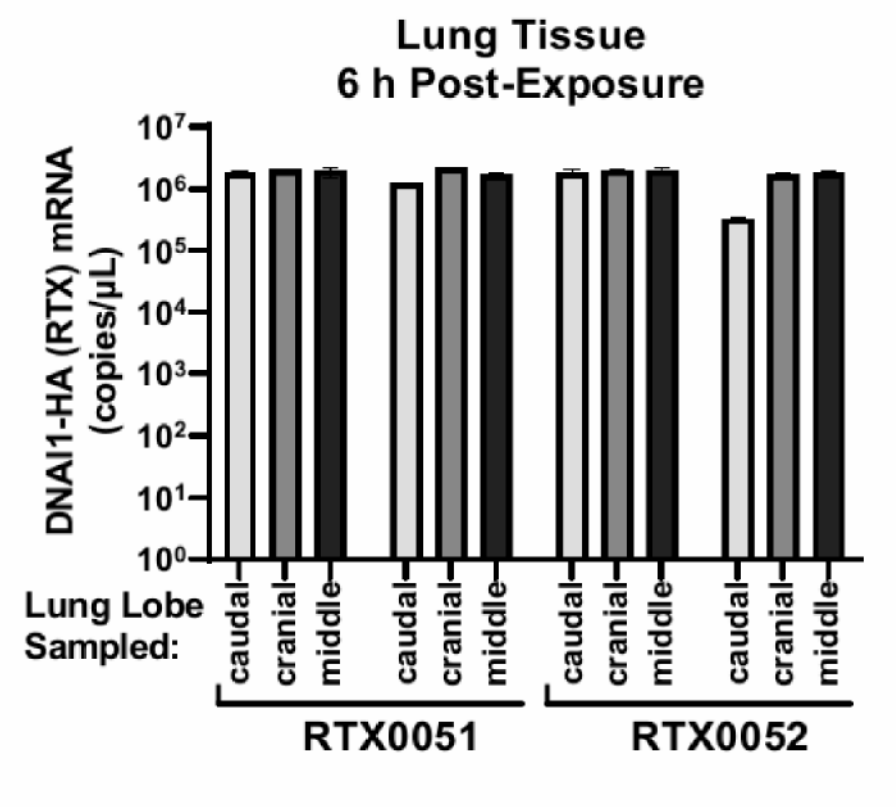



Fig. 29B

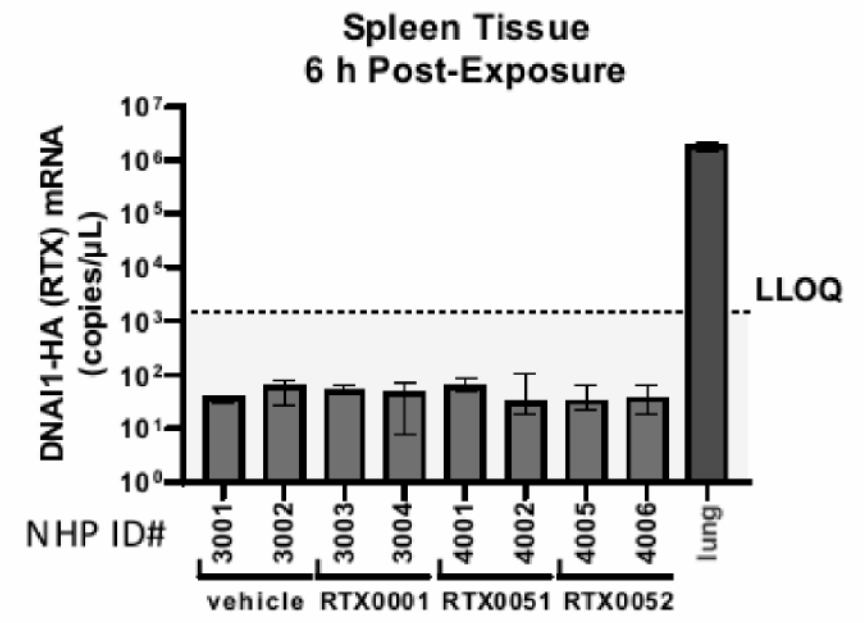


Fig. 29C

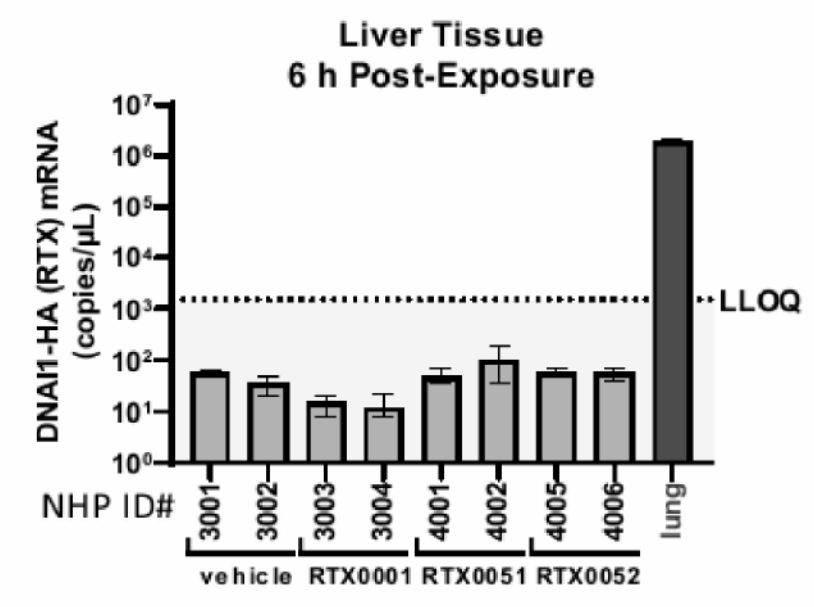



Fig. 29D

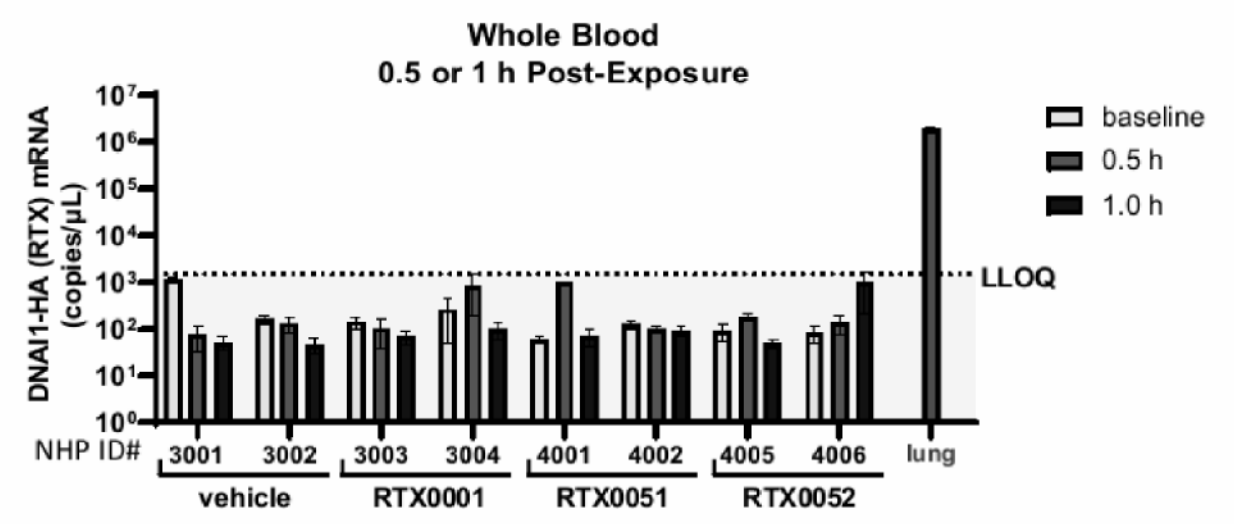


Fig. 30A

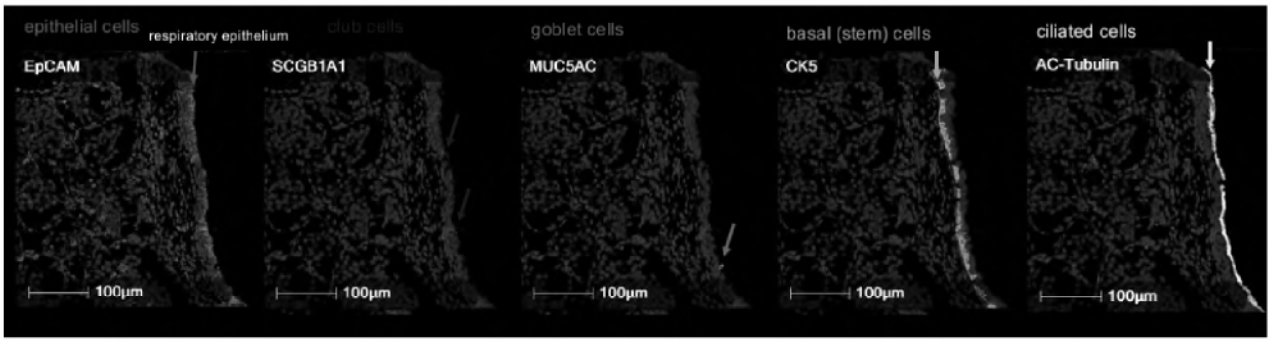



Fig. 30B

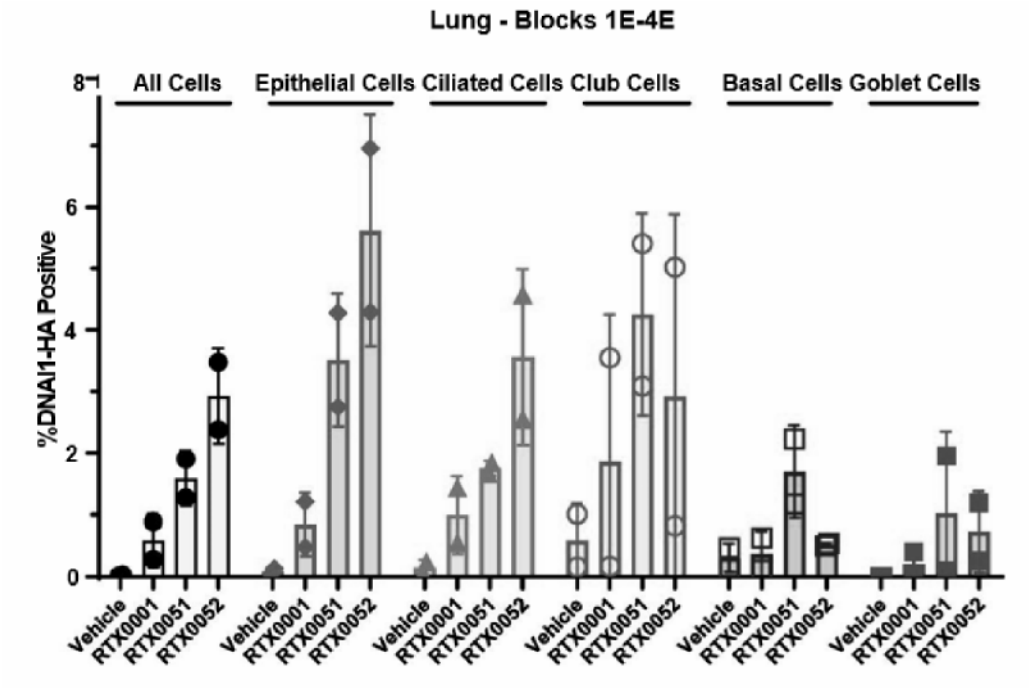



Fig. 30C

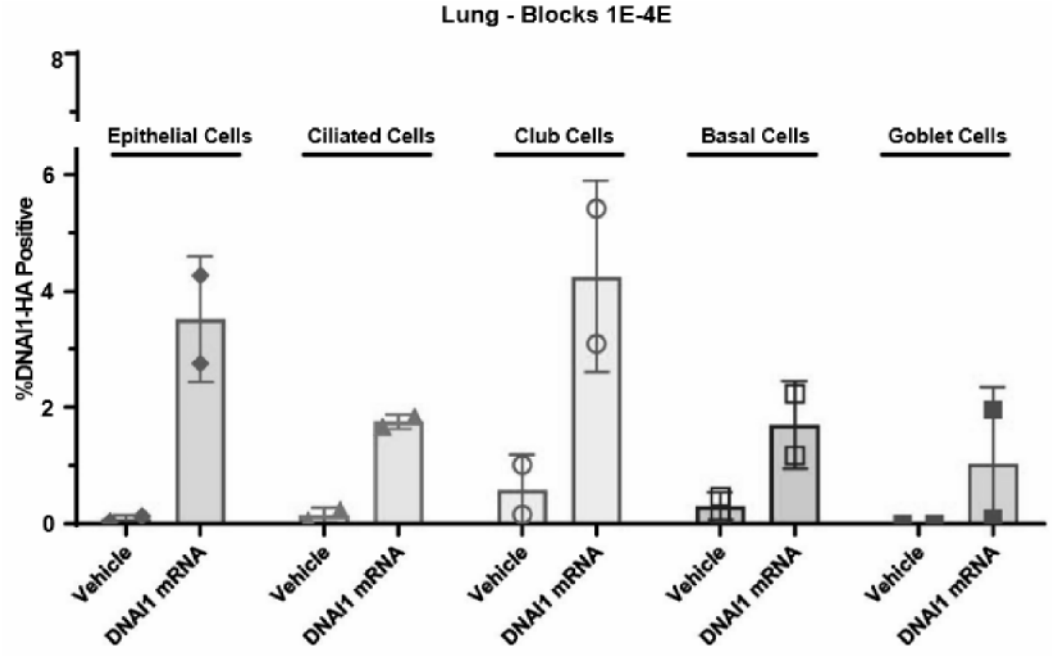


**Fig. 30D**

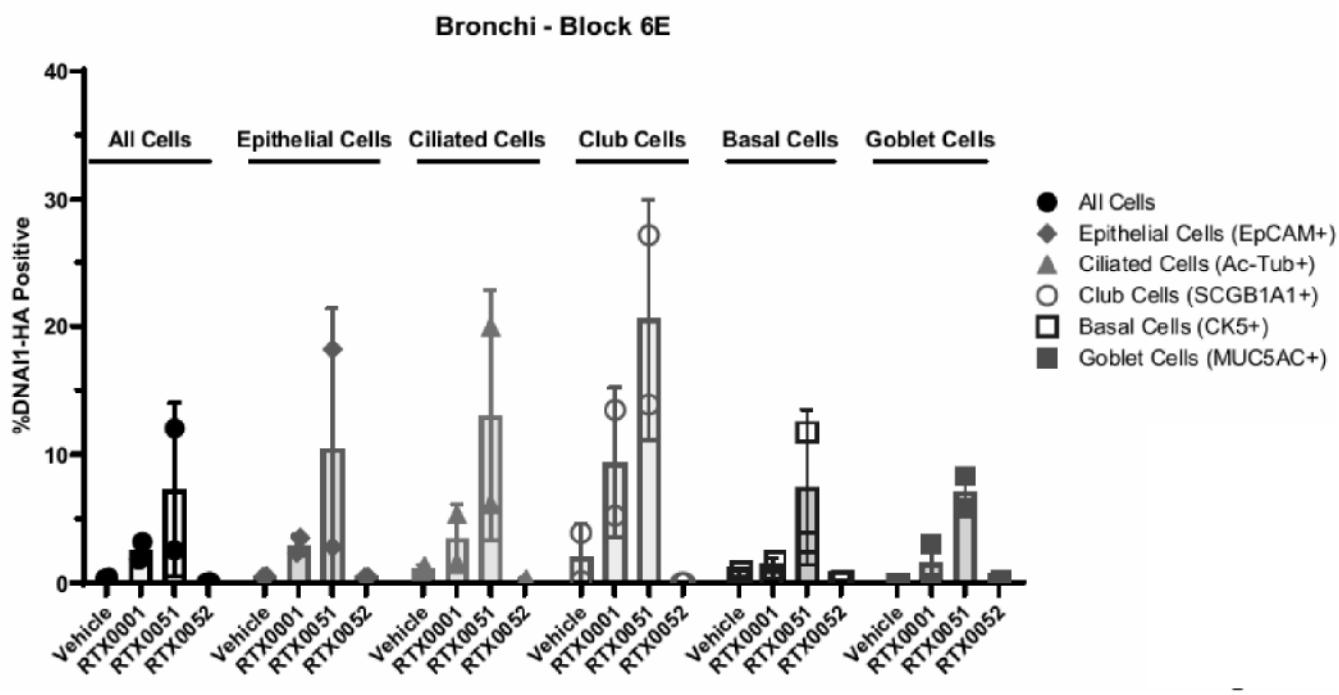



**Fig. 30E**

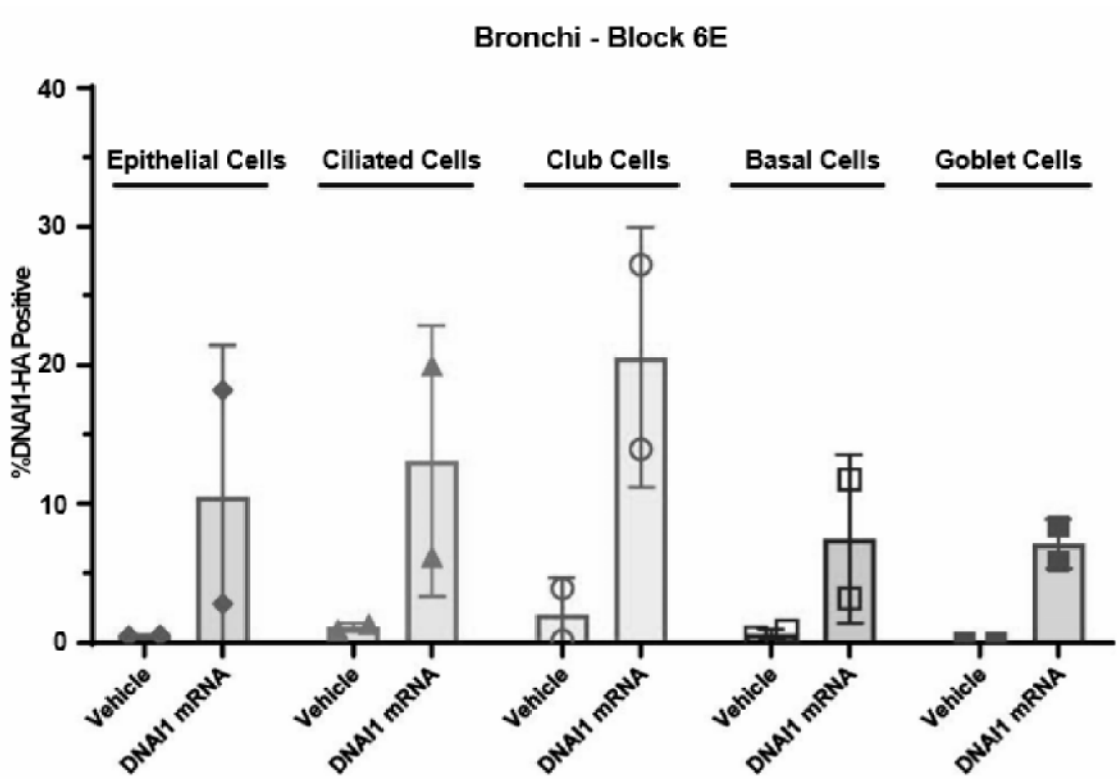
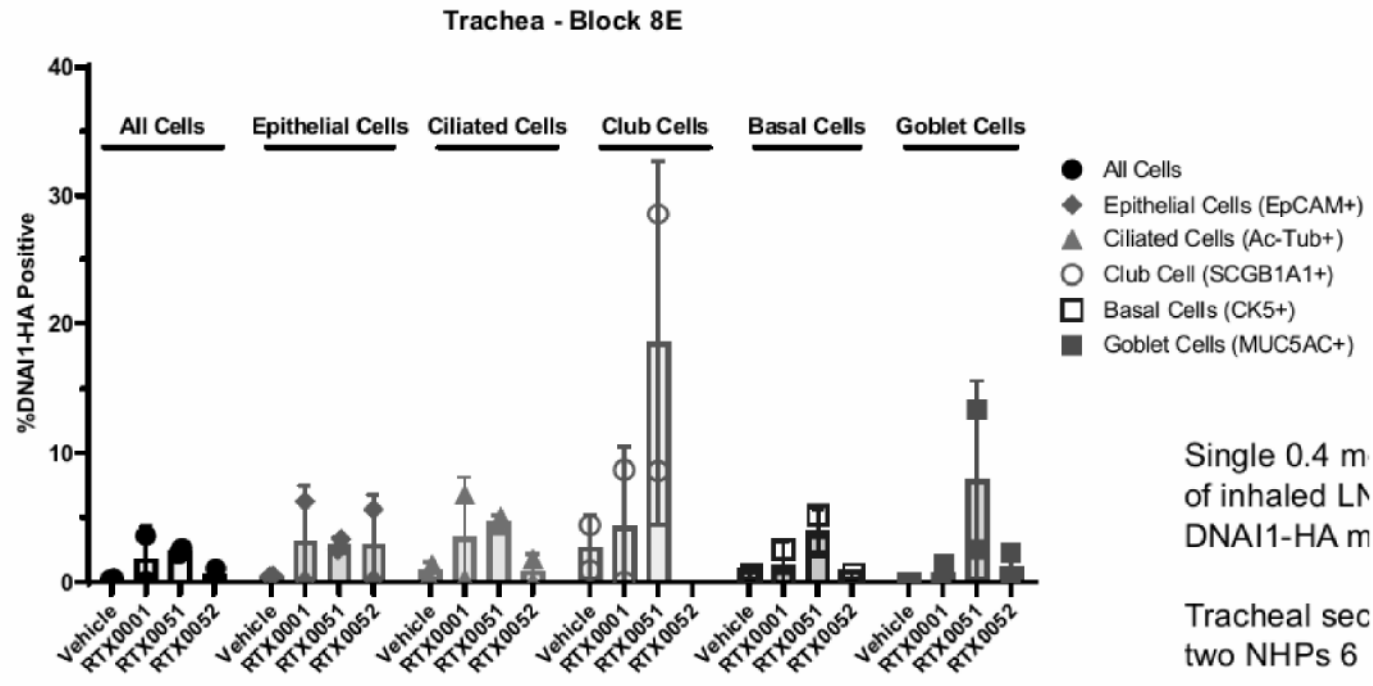
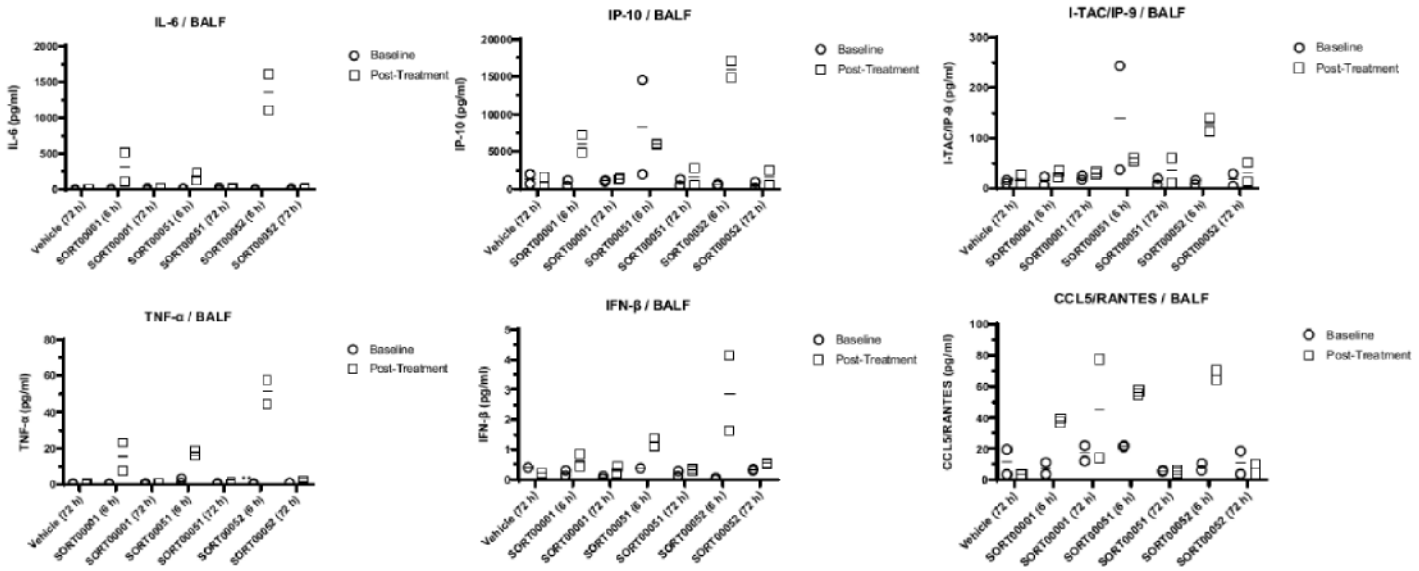


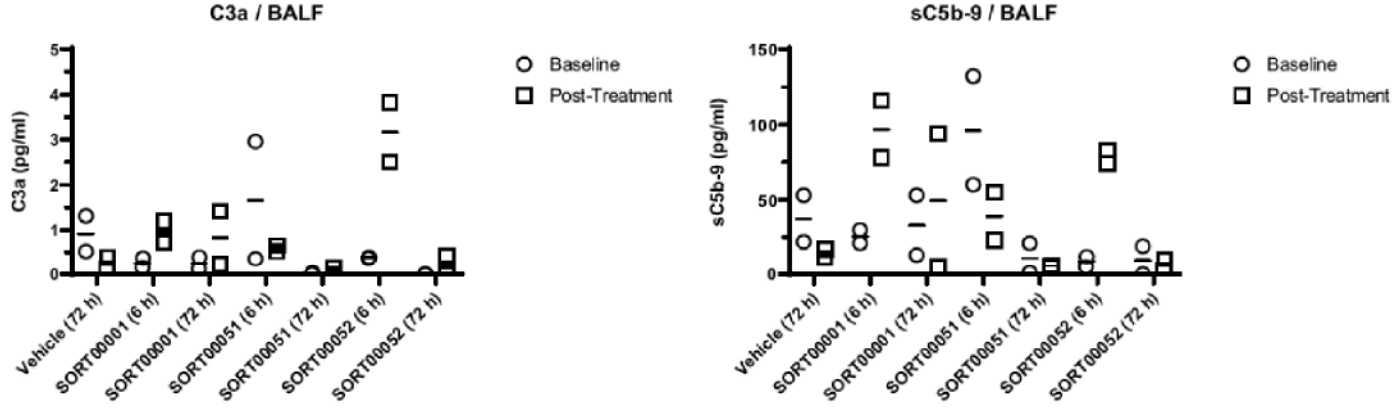
**Fig. 30F**


Trachea - Block 8E
%DNAI1-HA Positive
40
30
20
10
0
All Cells
Epithelial Cells
Ciliated Cells
Club Cells
Basal Cells
Goblet Cells
Vehicle
RTX0001
RTX0051
RTX0052
All Cells
Epithelial Cells (EpCAM+)
Ciliated Cells (Ac-Tub+)
Club Cell (SCGB1A1+)
Basal Cells (CK5+)
Goblet Cells (MUC5AC+)
Single 0.4 m
of inhaled LN
DNAI1-HA m
Tracheal sec
two NHPs 6

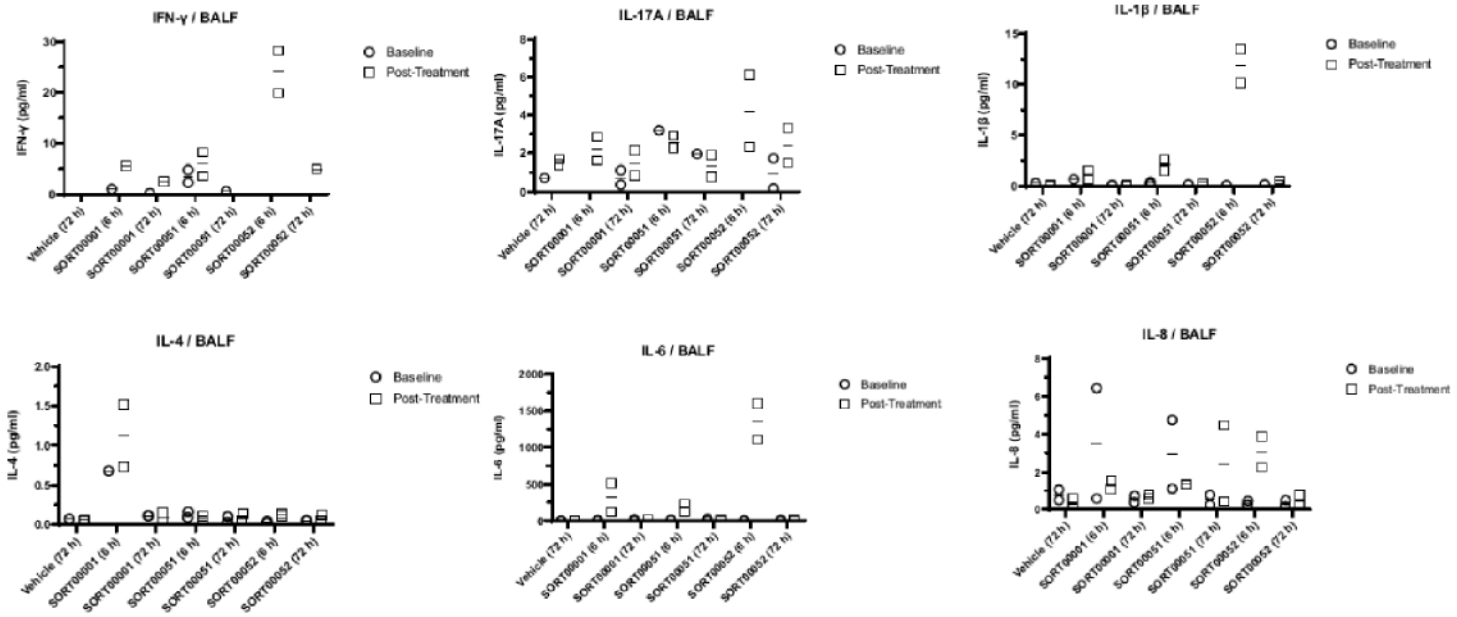
# Fig. 31A



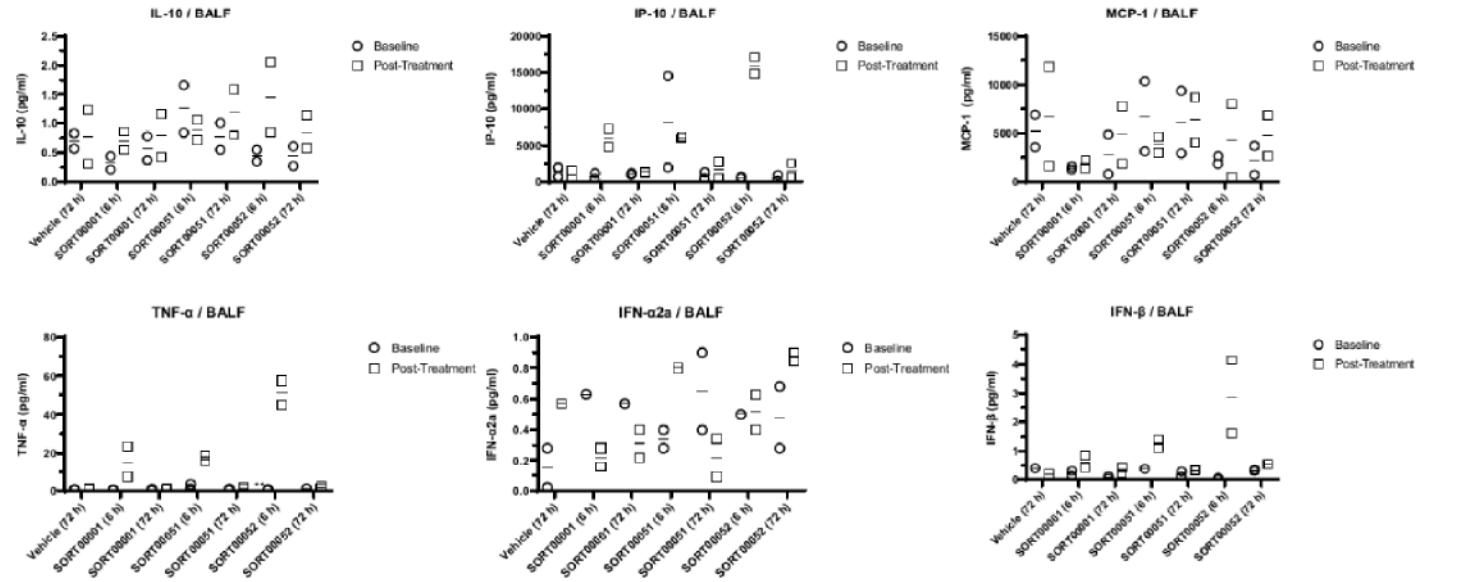

# Fig. 31B



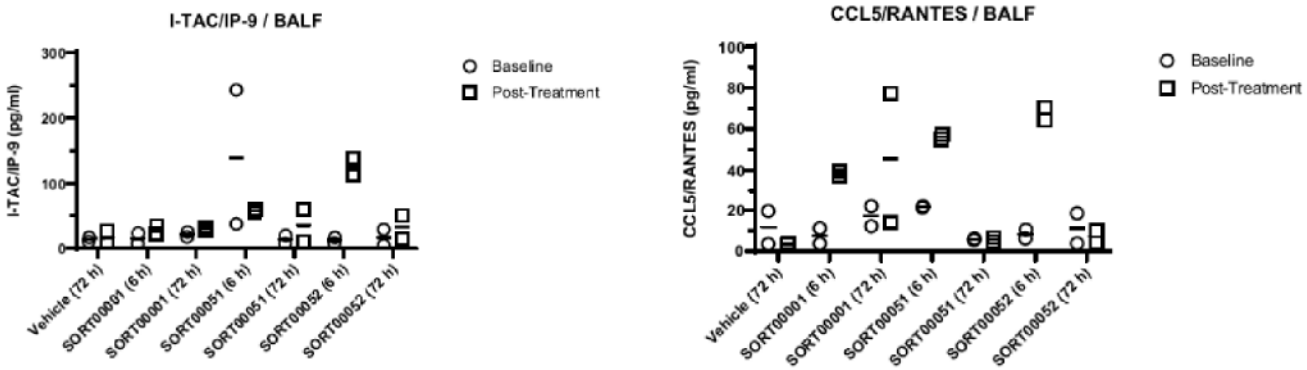
# Fig. 31C



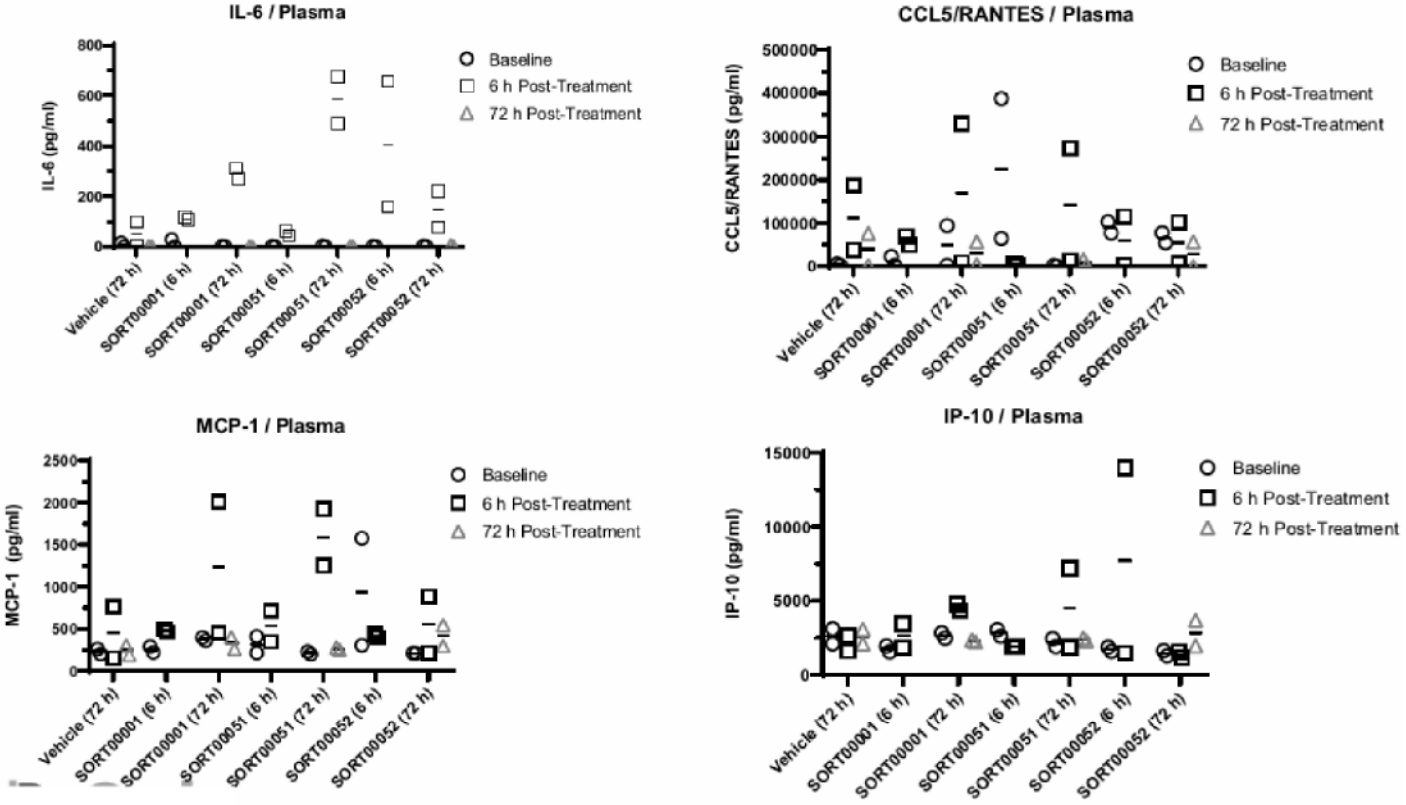

# Fig. 31D




# Fig. 31E



# Fig. 32A




# Fig. 32B

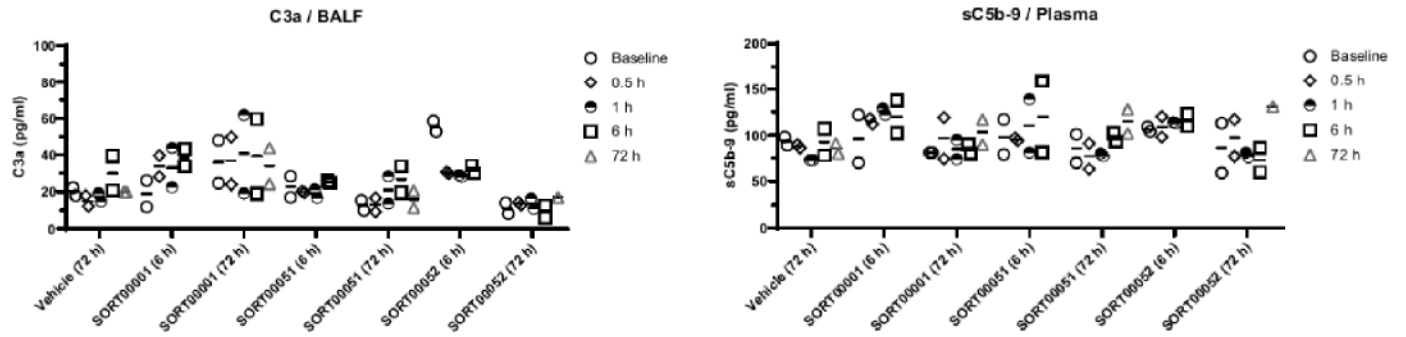


Fig. 32C

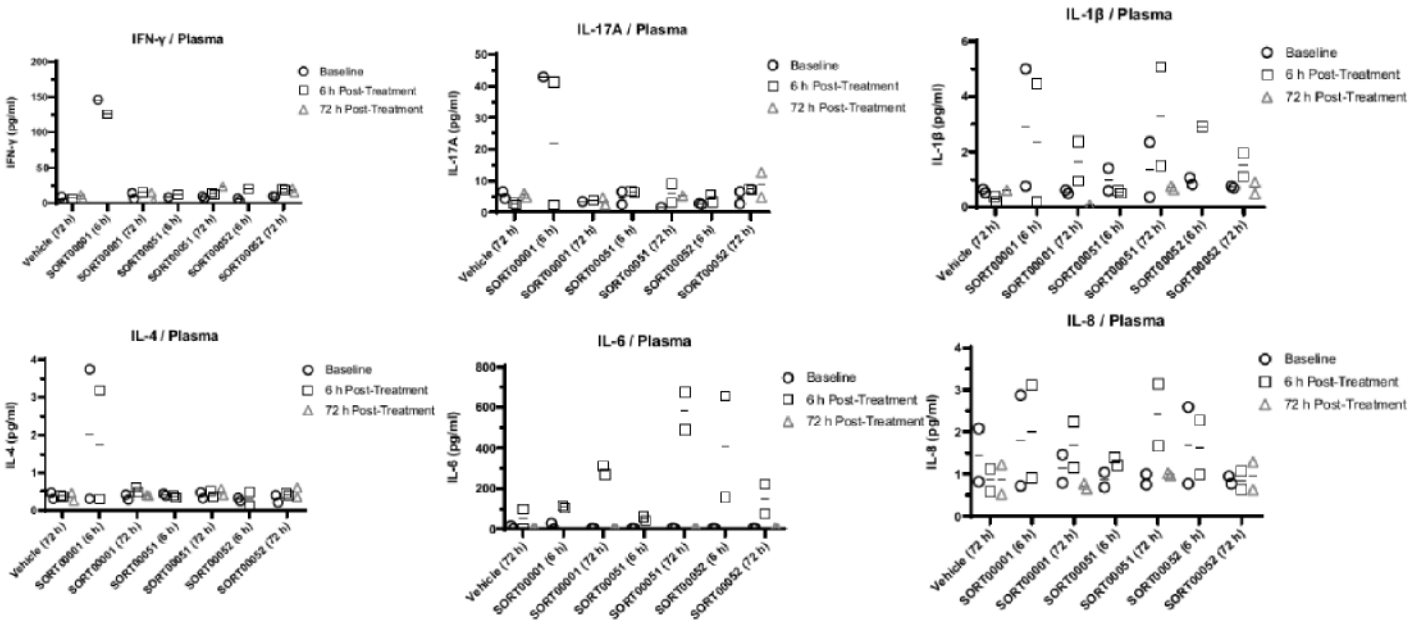



Fig. 32D

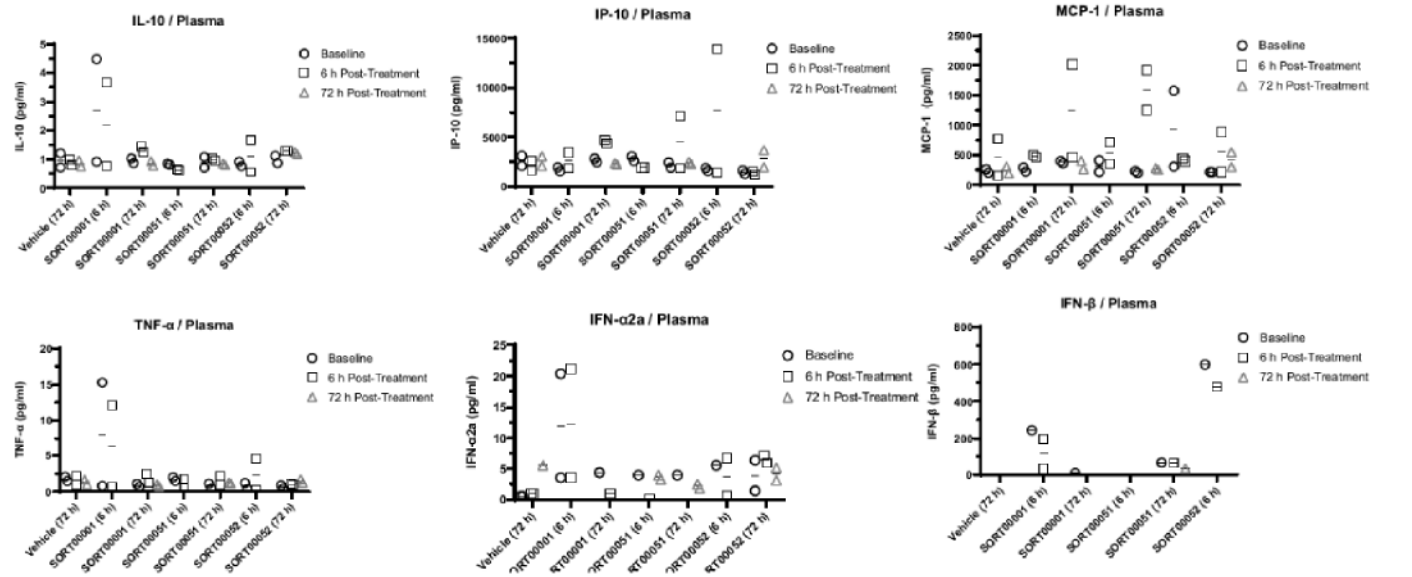
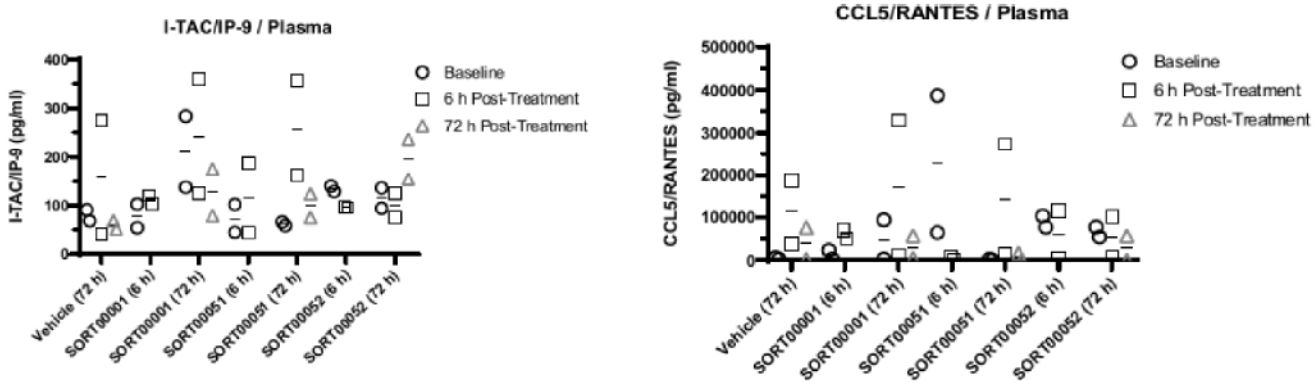
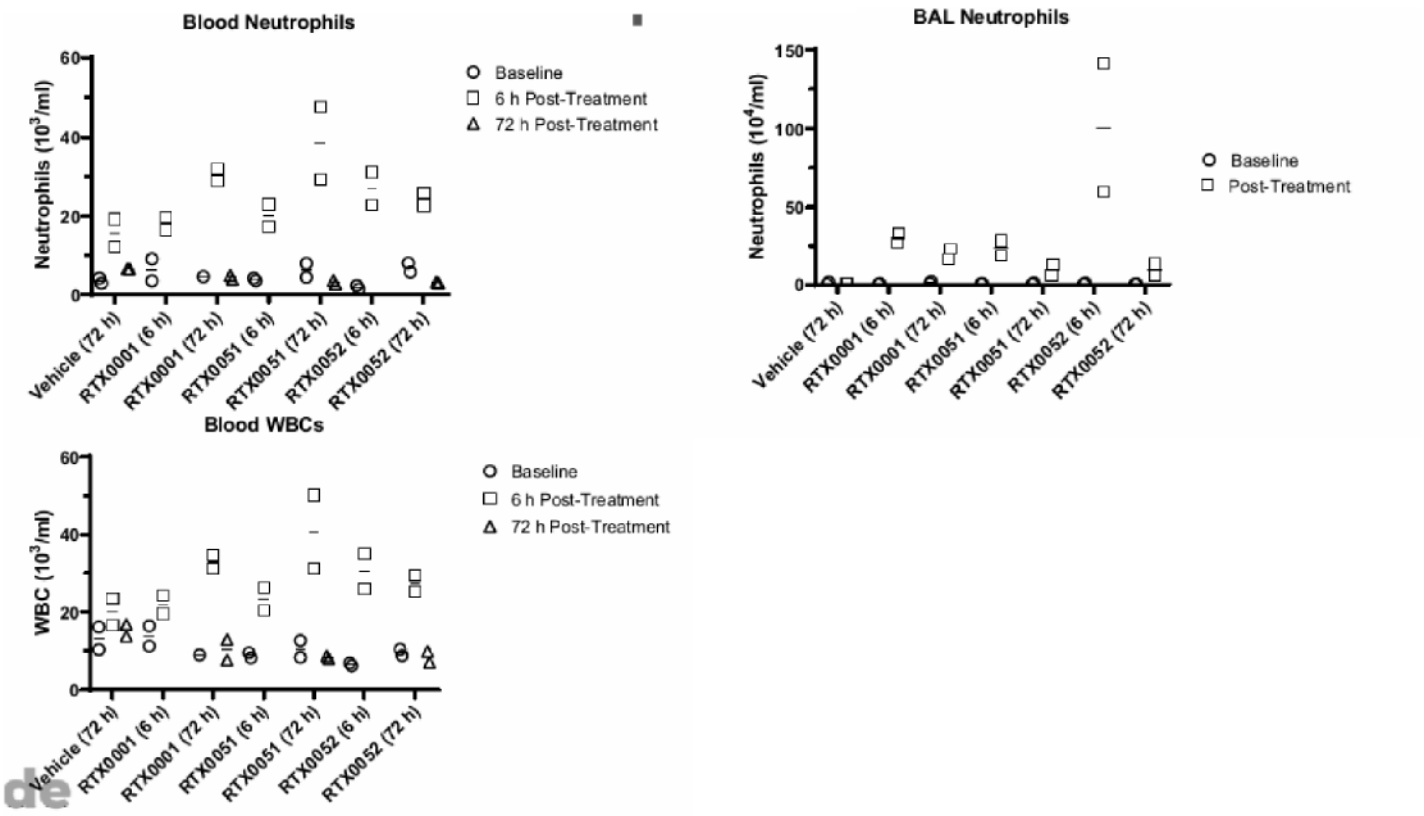


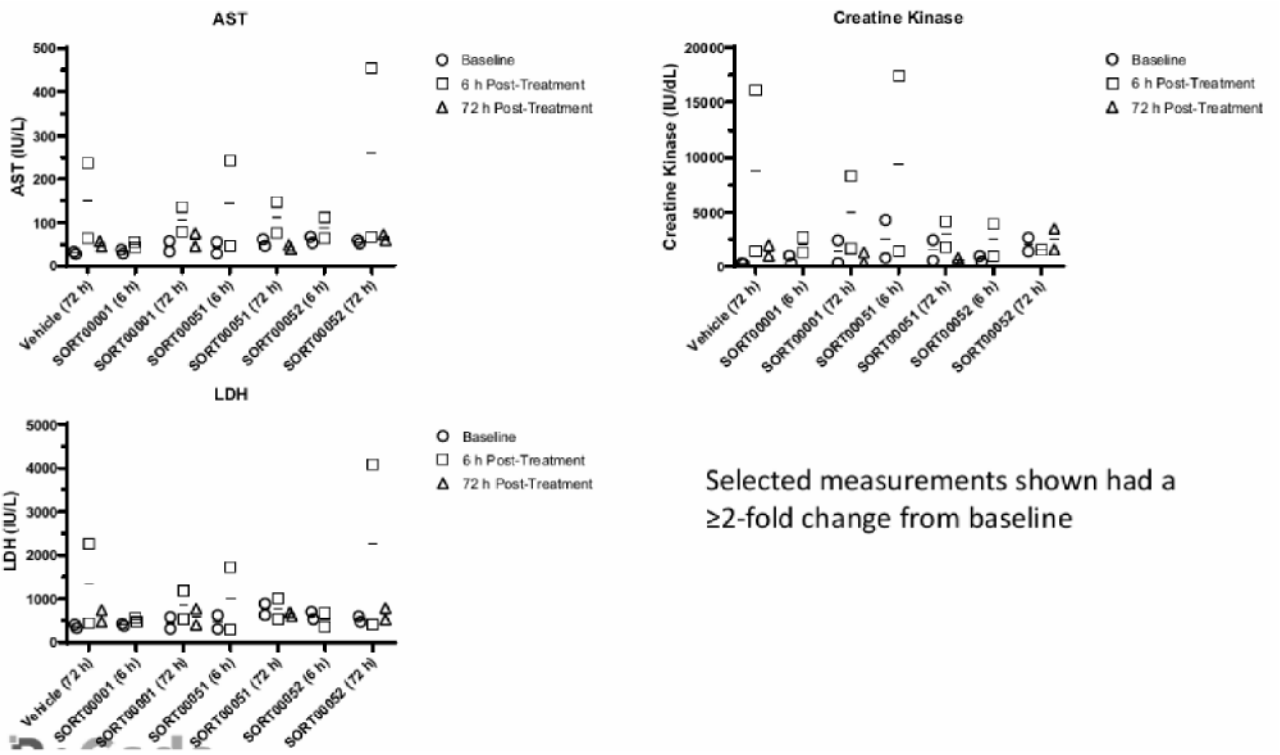

Fig. 32E



# Fig. 33


Blood Neutrophils
Neutrophils ($10^3$/ml)
60
40
20
0
Vehicle (72 h)
RTX0001 (6 h)
RTX0001 (72 h)
RTX0051 (6 h)
RTX0051 (72 h)
RTX0052 (6 h)
RTX0052 (72 h)
Baseline
6 h Post-Treatment
72 h Post-Treatment
BAL Neutrophils
Neutrophils ($10^4$/ml)
150
100
50
0
Baseline
Post-Treatment
Blood WBCs
WBC ($10^3$/ml)
60
40
20
0
Baseline
6 h Post-Treatment
72 h Post-Treatment

# Fig. 34


AST
AST (IU/L)
500
400
300
200
100
0
Vehicle (72 h)
SORT00001 (6 h)
SORT00001 (72 h)
SORT00051 (6 h)
SORT00051 (72 h)
SORT00052 (6 h)
SORT00052 (72 h)
Baseline
6 h Post-Treatment
72 h Post-Treatment
Creatine Kinase
Creatine Kinase (IU/dL)
20000
15000
10000
5000
0
Vehicle (72 h)
SORT00001 (6 h)
SORT00001 (72 h)
SORT00051 (6 h)
SORT00051 (72 h)
SORT00052 (6 h)
SORT00052 (72 h)
Baseline
6 h Post-Treatment
72 h Post-Treatment
LDH
LDH (IU/L)
5000
4000
3000
2000
1000
0
Vehicle (72 h)
SORT00001 (6 h)
SORT00001 (72 h)
SORT00051 (6 h)
SORT00051 (72 h)
SORT00052 (6 h)
SORT00052 (72 h)
Baseline
6 h Post-Treatment
72 h Post-Treatment
Selected measurements shown had a
≥2-fold change from baseline

# Fig. 35

## 6 hr Necropsy Scores

| 6 HRS | 01 | | 51 | | 52 | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| Alveolar macrophages, increased | 0 | 0 | 0 | 0 | 0 | 1 |
| Infiltrate, mixed, interstitial | 1 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| Inflammation, neutrophilic, alveolar, multifocal | 3 | 2 | 1 | 2 | 3 | 3 |
| Inflammation, neutrophilic, terminal bronchiole | 1 | 1 | 1 | 0 | 2 | 2 |
| Ulceration, bronchiole | 0 | 0 | 0 | 0 | 1 | 0 |

## 72 hr Necropsy Scores

| 72 HRS | vehicle | | 01 | | 51 | | 52 | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Alveolar macrophages, increased | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, mixed, interstitial | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 0 |
| Infiltrate, mixed, terminal bronchiole | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Inflammation, mixed, alveolar, multifocal | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 2 |
| Inflammation, mixed, interstitial, multifocal | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 2 |
| Inflammation, mixed, terminal bronchiole | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Inflammation, neutrophilic, alveolar, multifocal | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 0 |
| Inflammation, neutrophilic, terminal bronchiole | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| | | | | | | | | |

**Fig. 36A**

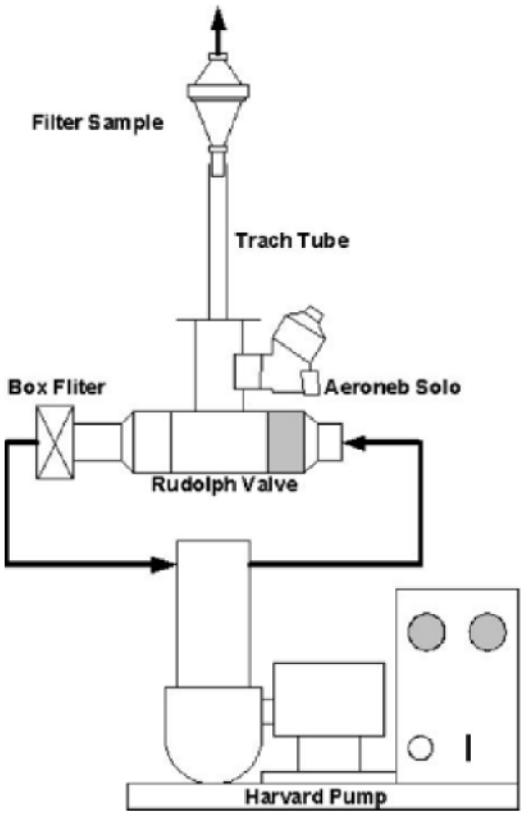
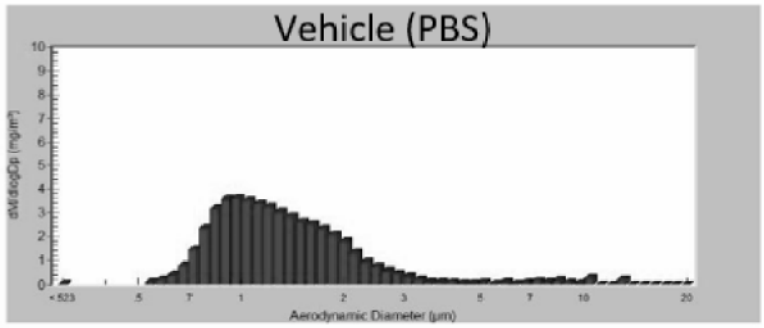
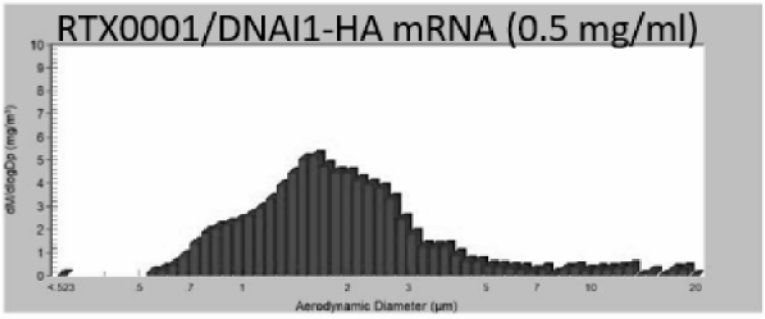


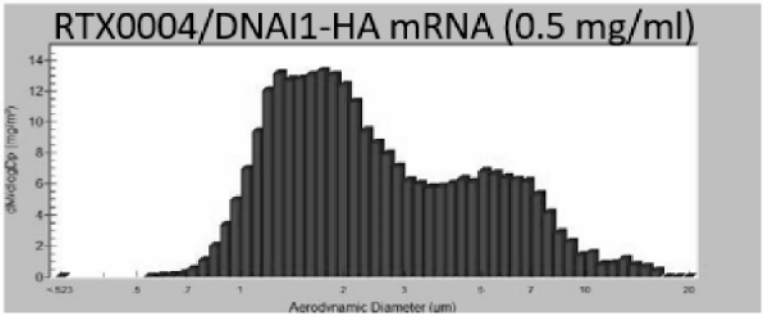

**Fig. 36B**




| APS | | |
|---|---|---|
| **Sample** | **MMAD (µm)** | **GSD** |
| 1 | 1.22 | 1.64 |
| 2 | 1.21 | 1.64 |
| 3 | 1.2 | 1.68 |
| **Average** | **1.21** | **1.65** |
| **Std Dev** | **0.01** | **0.02** |
| **%RSD** | **1%** | **1%** |

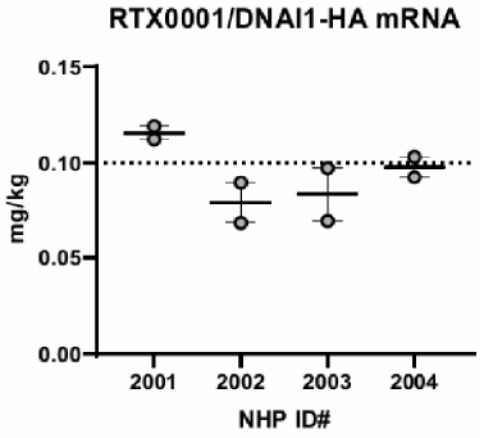


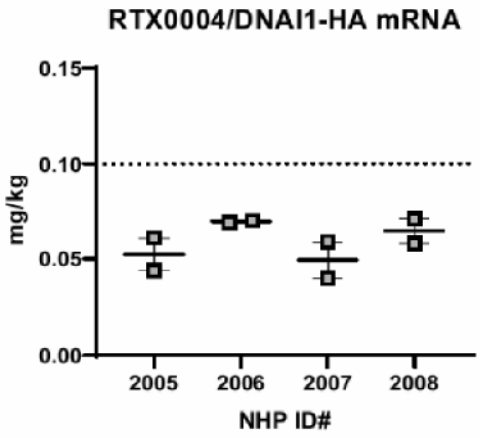

| APS | | |
|---|---|---|
| **Sample** | **MMAD (µm)** | **GSD** |
| 1 | 1.72 | 1.82 |
| 2 | 1.72 | 1.76 |
| 3 | 1.78 | 1.83 |
| **Average** | **1.74** | **1.80** |
| **Std Dev** | **0.03** | **0.04** |
| **%RSD** | **2%** | **2%** |



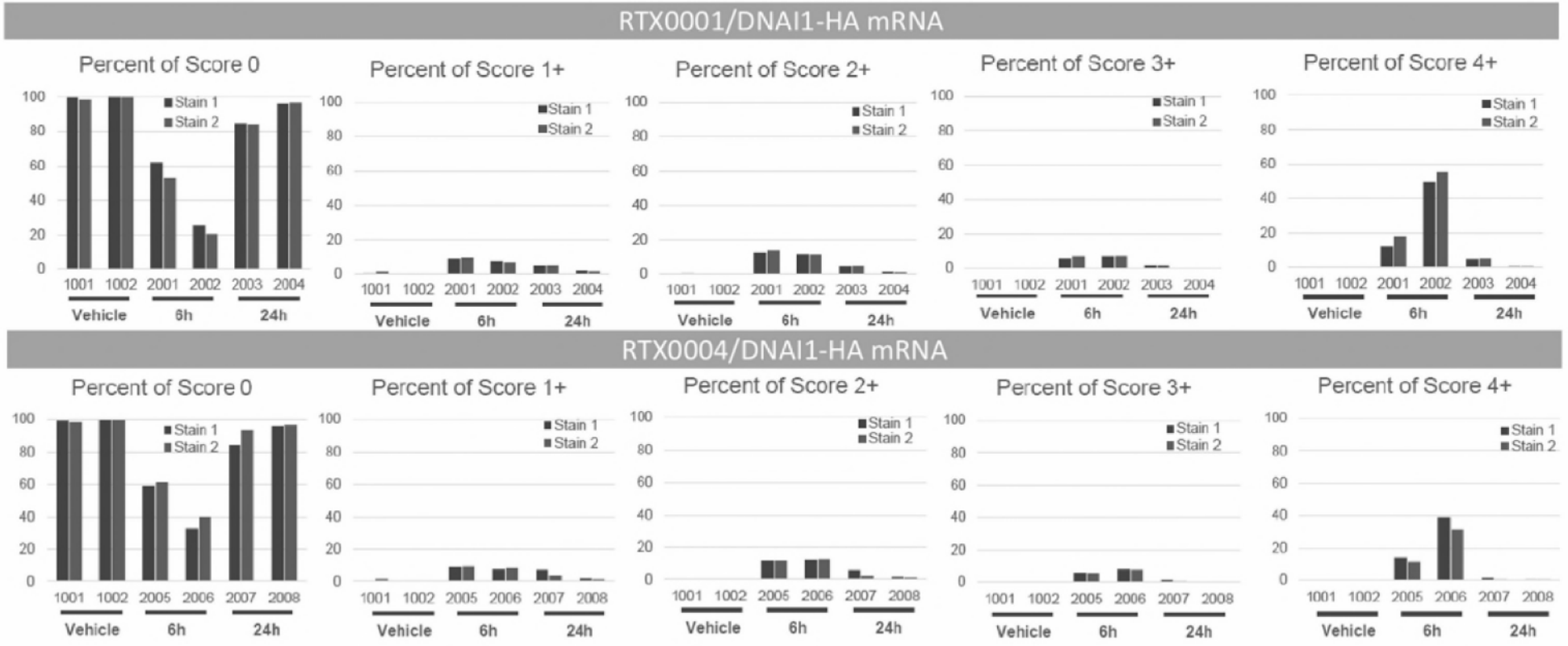

| APS | | |
|---|---|---|
| **Sample** | **MMAD (µm)** | **GSD** |
| 1 | 2.34 | 2.13 |
| 2 | 2.23 | 2.03 |
| 3 | 2.12 | 1.95 |
| **Average** | **2.23** | **2.04** |
| **Std Dev** | **0.11** | **0.09** |
| **%RSD** | **5%** | **4%** |

**Fig. 37**

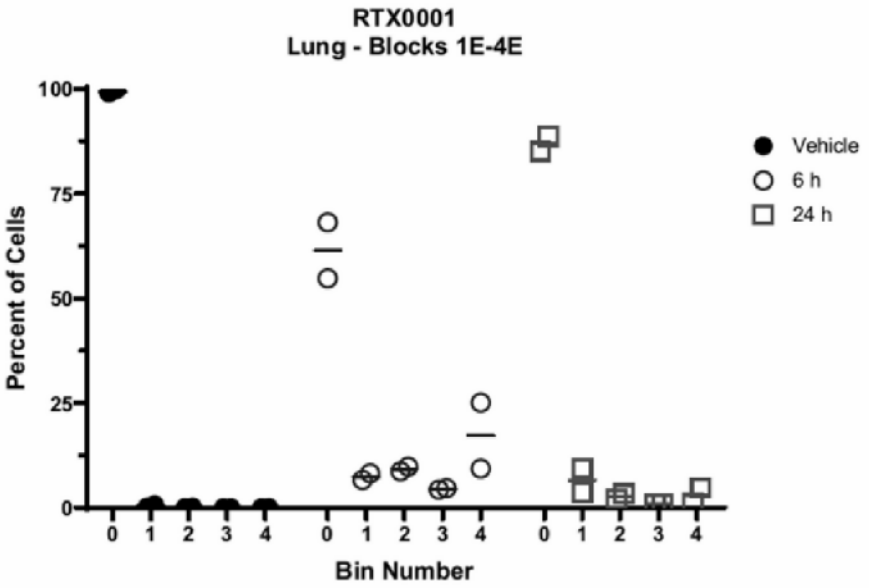

RTX0001/DNAI1-HA mRNA
0.15
0.10
0.05
0.00
mg/kg
2001
2002
2003
2004
NHP ID#


RTX0004/DNAI1-HA mRNA
0.15
0.10
0.05
0.00
mg/kg
2005
2006
2007
2008
NHP ID#

# Fig. 38A


RTX0001/DNAI1-HA mRNA
Percent of Score 0
Percent of Score 1+
Percent of Score 2+
Percent of Score 3+
Percent of Score 4+
Stain 1
Stain 2
100
80
60
40
20
0
1001 1002 2001 2002 2003 2004
Vehicle
6h
24h
RTX0004/DNAI1-HA mRNA
Percent of Score 0
Percent of Score 1+
Percent of Score 2+
Percent of Score 3+
Percent of Score 4+
Stain 1
Stain 2
100
80
60
40
20
0
1001 1002 2005 2006 2007 2008
Vehicle
6h
24h

# Fig. 38B




UPDATED – FINAL ISH RESULTS FOR RTX0001

| Bin # | Scoring |
|---|---|
| Bin 0 | 0 copies/cell or no staining |
| Bin 1 | 1-3 copies/cell |
| Bin 2 | 4-9 copies/cell and none/few clusters |
| Bin 3 | 10-15 copies/cell and/or <10% dots in clusters |
| Bin 4 | >15 copies/cell and/or >10% dots in clusters |

**Fig. 38C**

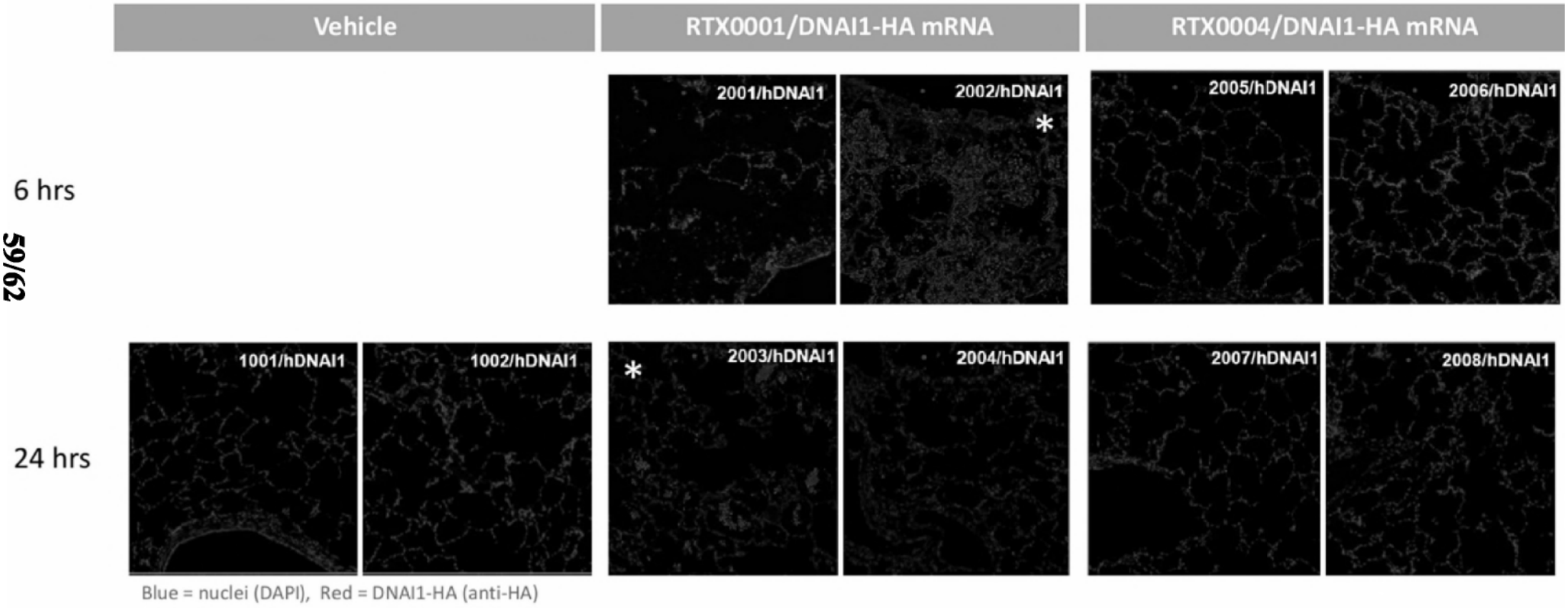

Vehicle
RTX0001/DNAI1-HA mRNA
RTX0004/DNAI1-HA mRNA
6 hrs
24 hrs
1001/hDNAI1
1002/hDNAI1
2001/hDNAI1
2002/hDNAI1
2003/hDNAI1
2004/hDNAI1
2005/hDNAI1
2006/hDNAI1
2007/hDNAI1
2008/hDNAI1
*
*
Blue = nuclei (DAPI), Red = DNAI1-HA (anti-HA)

Fig. 39A

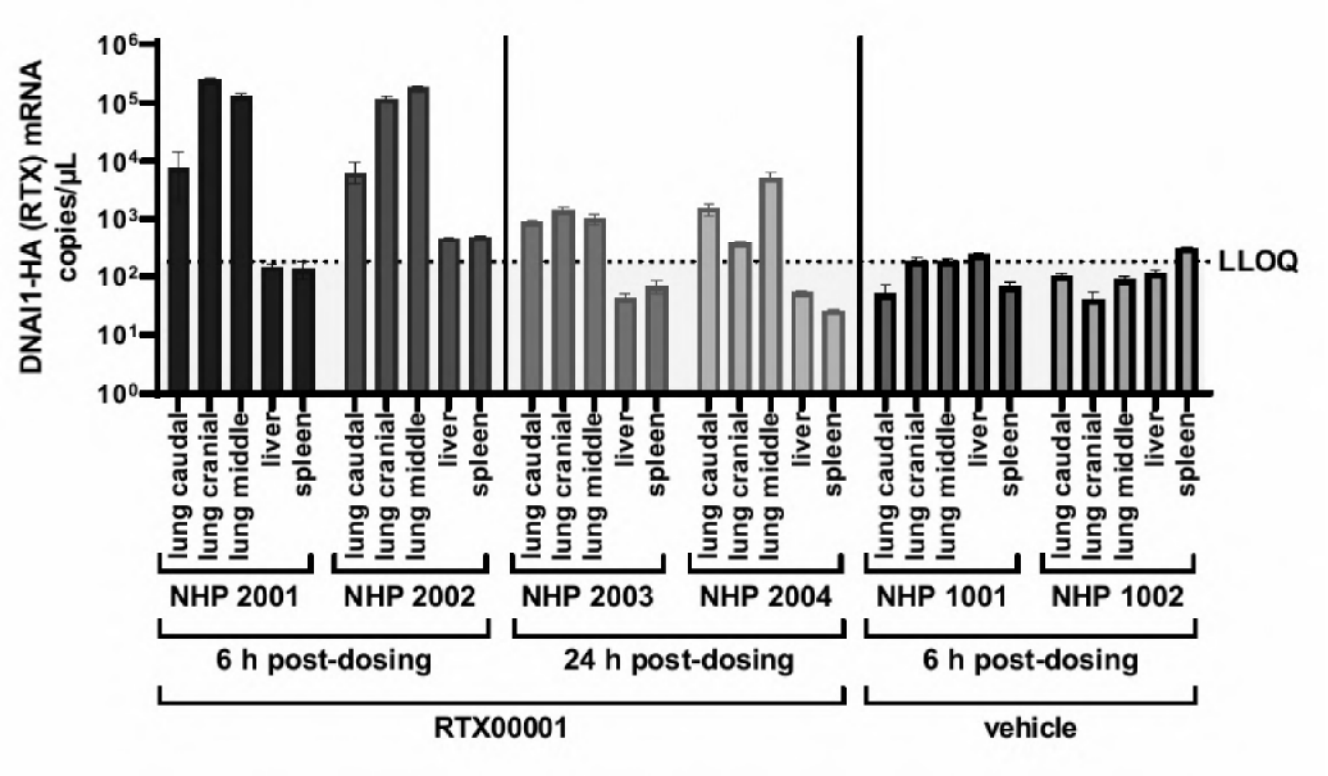



Fig. 39B

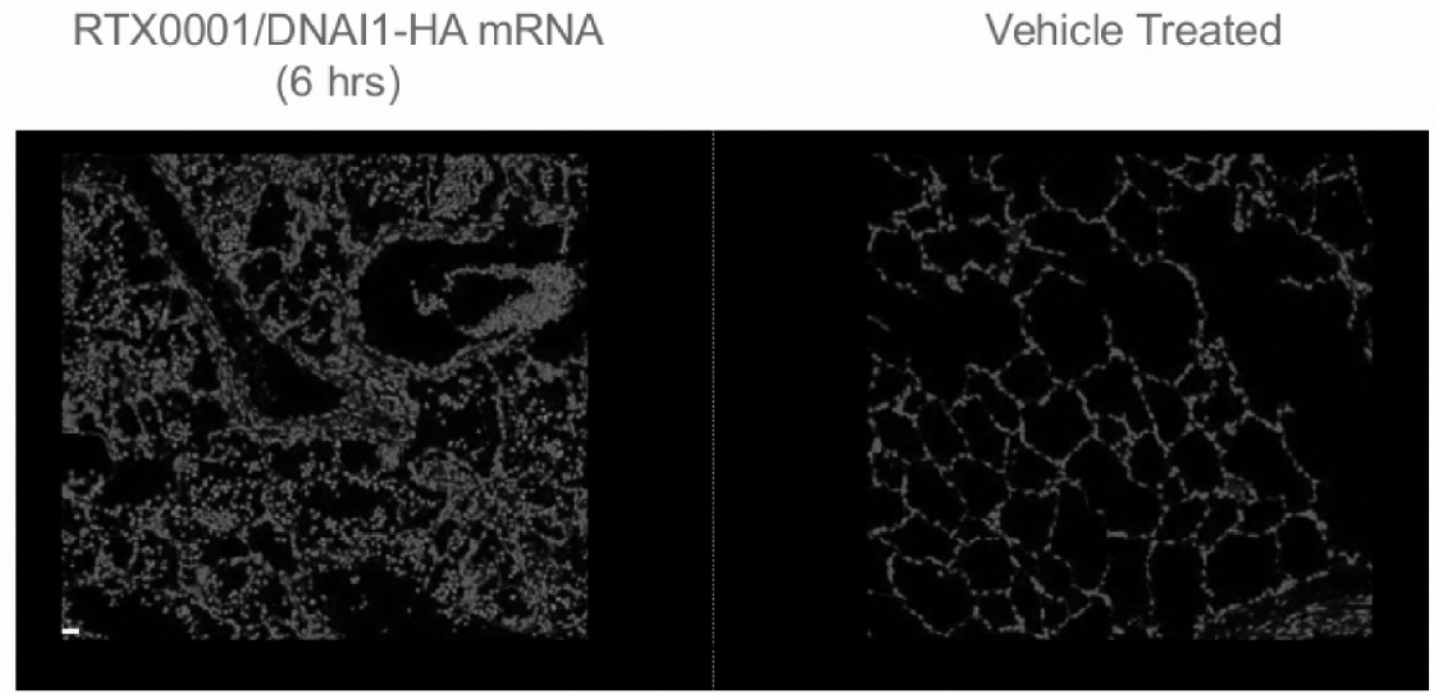


**Fig. 39C**

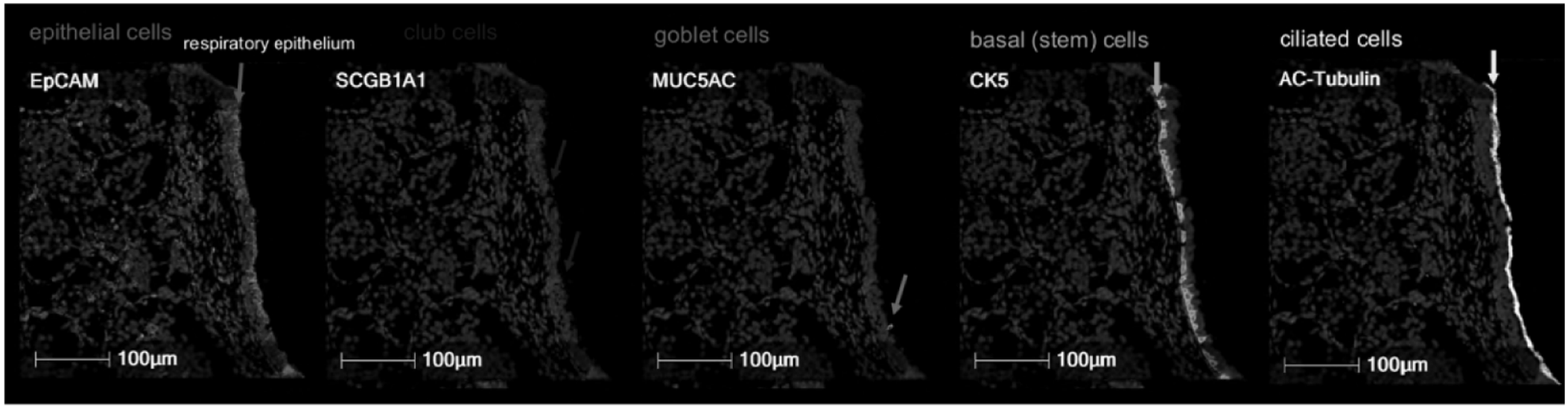

epithelial cells
respiratory epithelium
club cells
goblet cells
basal (stem) cells
ciliated cells
EpCAM
SCGB1A1
MUC5AC
CK5
AC-Tubulin
100µm
100µm
100µm
100µm
100µm

Fig. 40A

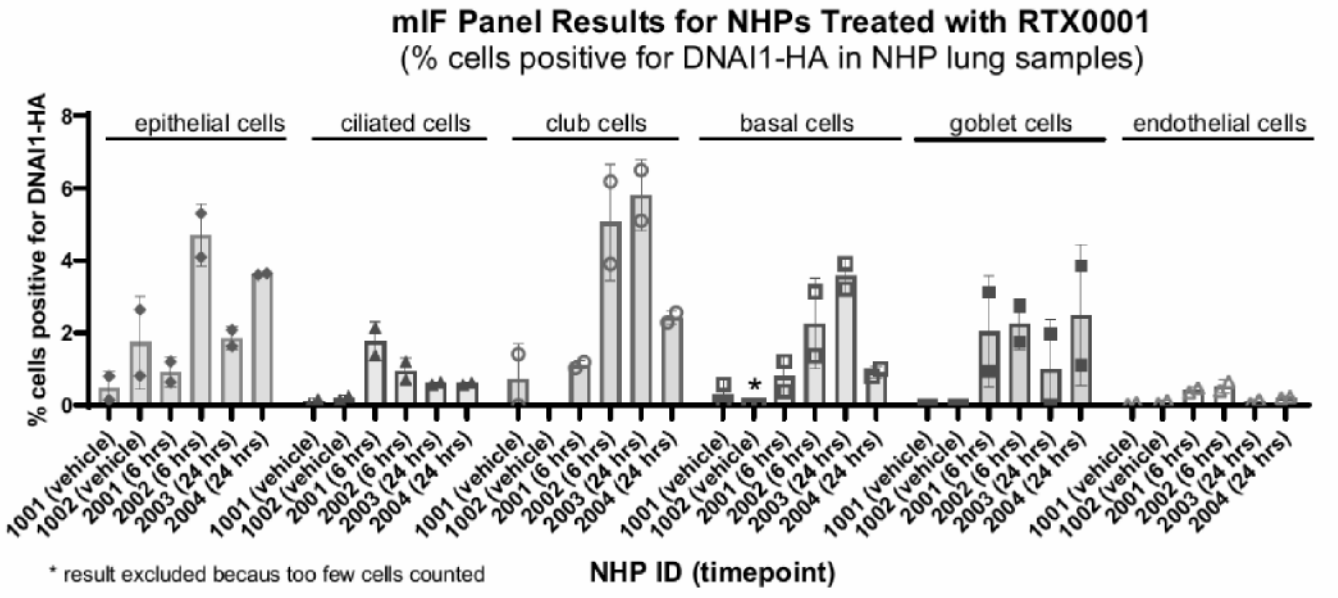



Fig. 40B

