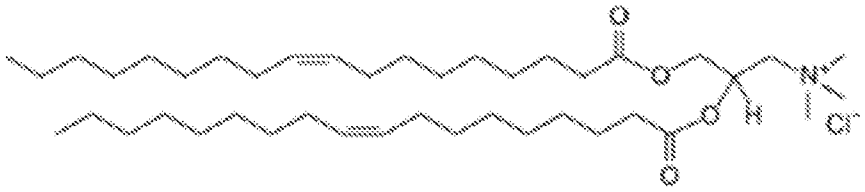
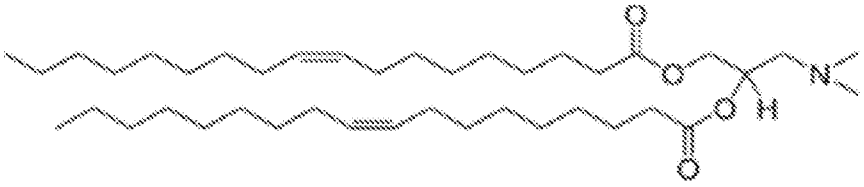
Fig. 1

DOTAP



DODAP



EPC

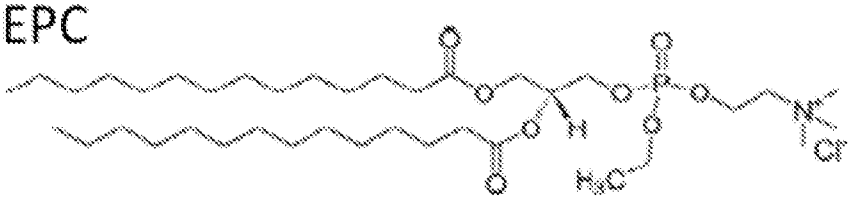


Fig. 2

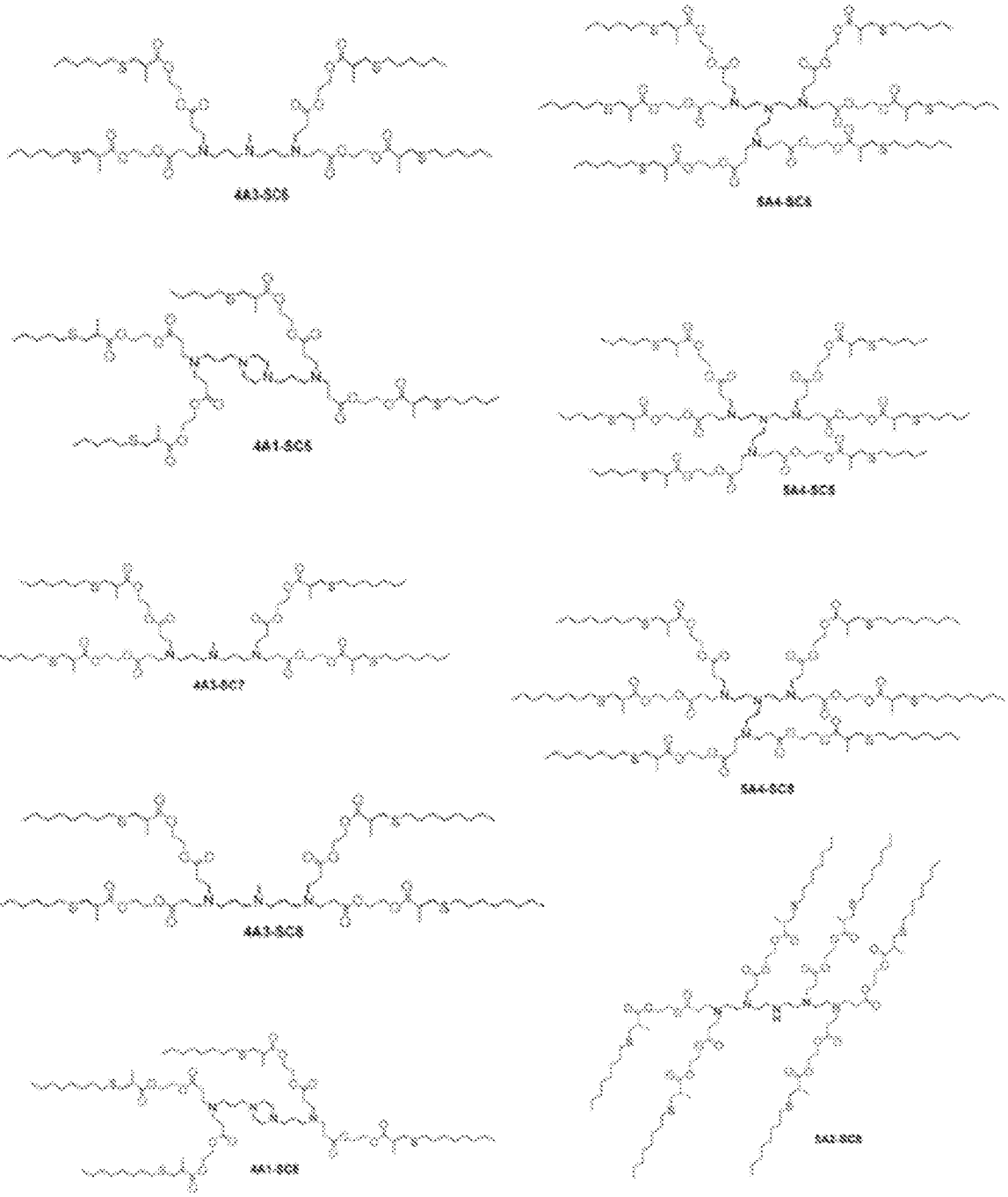
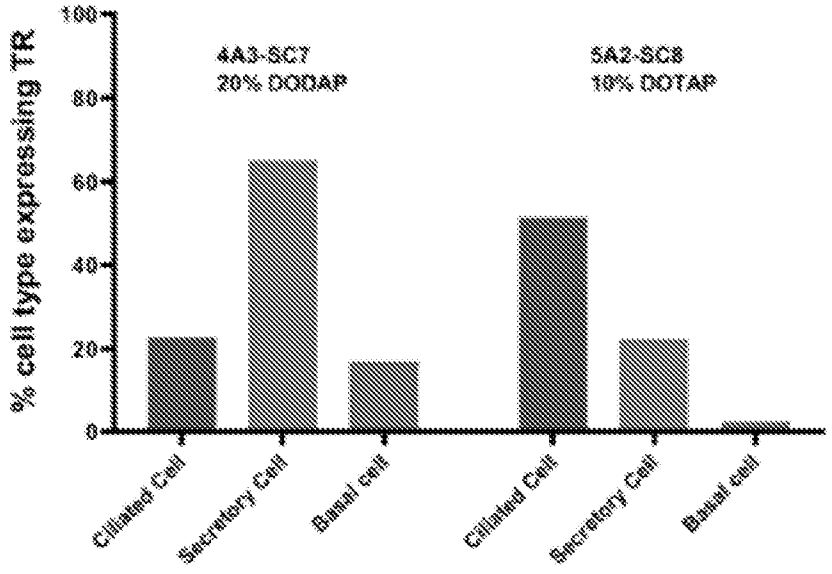


Fig. 3

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)

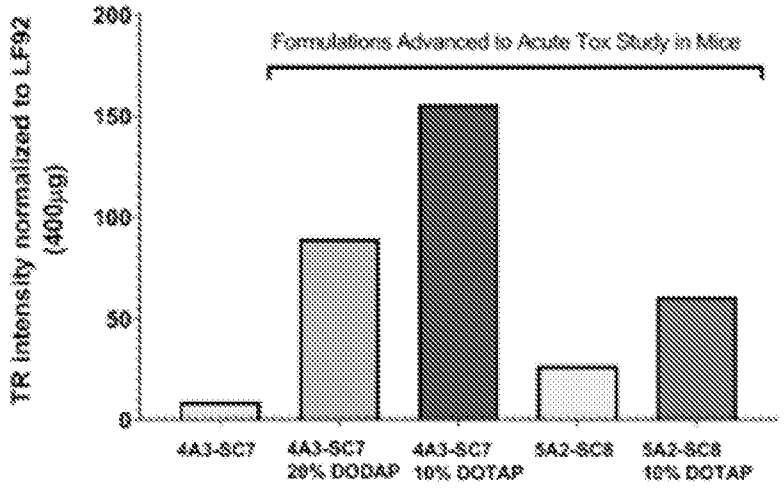


Fig. 4

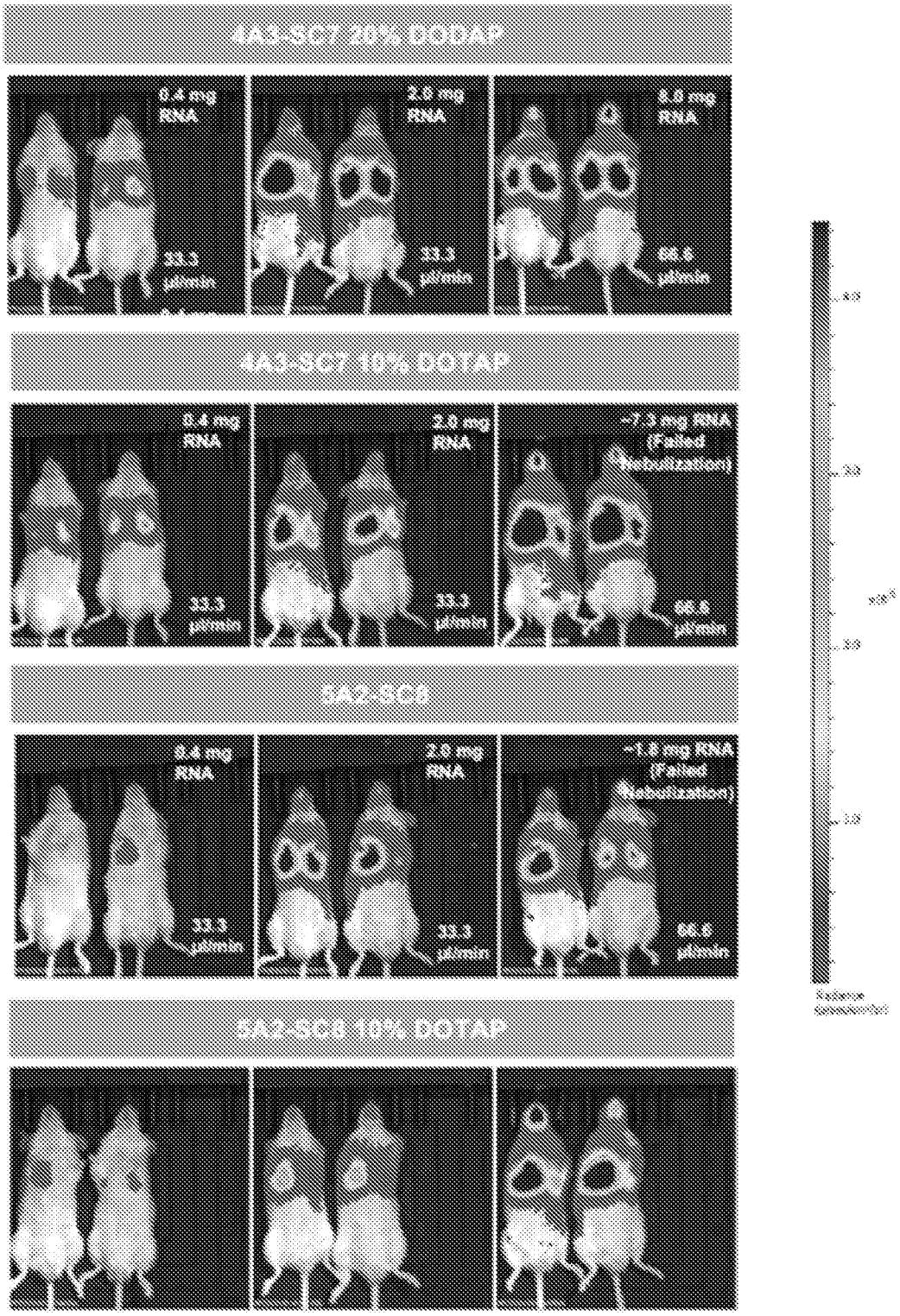

4A3-SC7 20% DODAP
0.4 mg RNA
2.0 mg RNA
~7.3 mg RNA (Failed Nebulization)
33.3 µl/min
66.6 µl/min
4A3-SC7 10% DOTAP
5A2-SC8
5A2-SC8 10% DOTAP

Fig. 5

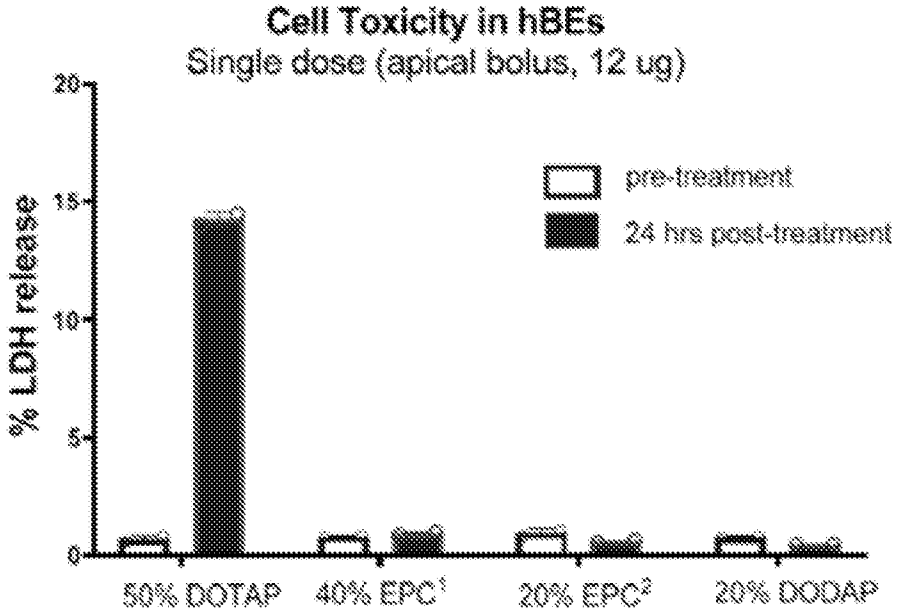

Cell Toxicity in hBEs
Single dose (apical bolus, 12 ug)
% LDH release
0
5
10
15
20
pre-treatment
24 hrs post-treatment
50% DOTAP
40% EPC1
20% EPC2
20% DODAP

Fig. 6

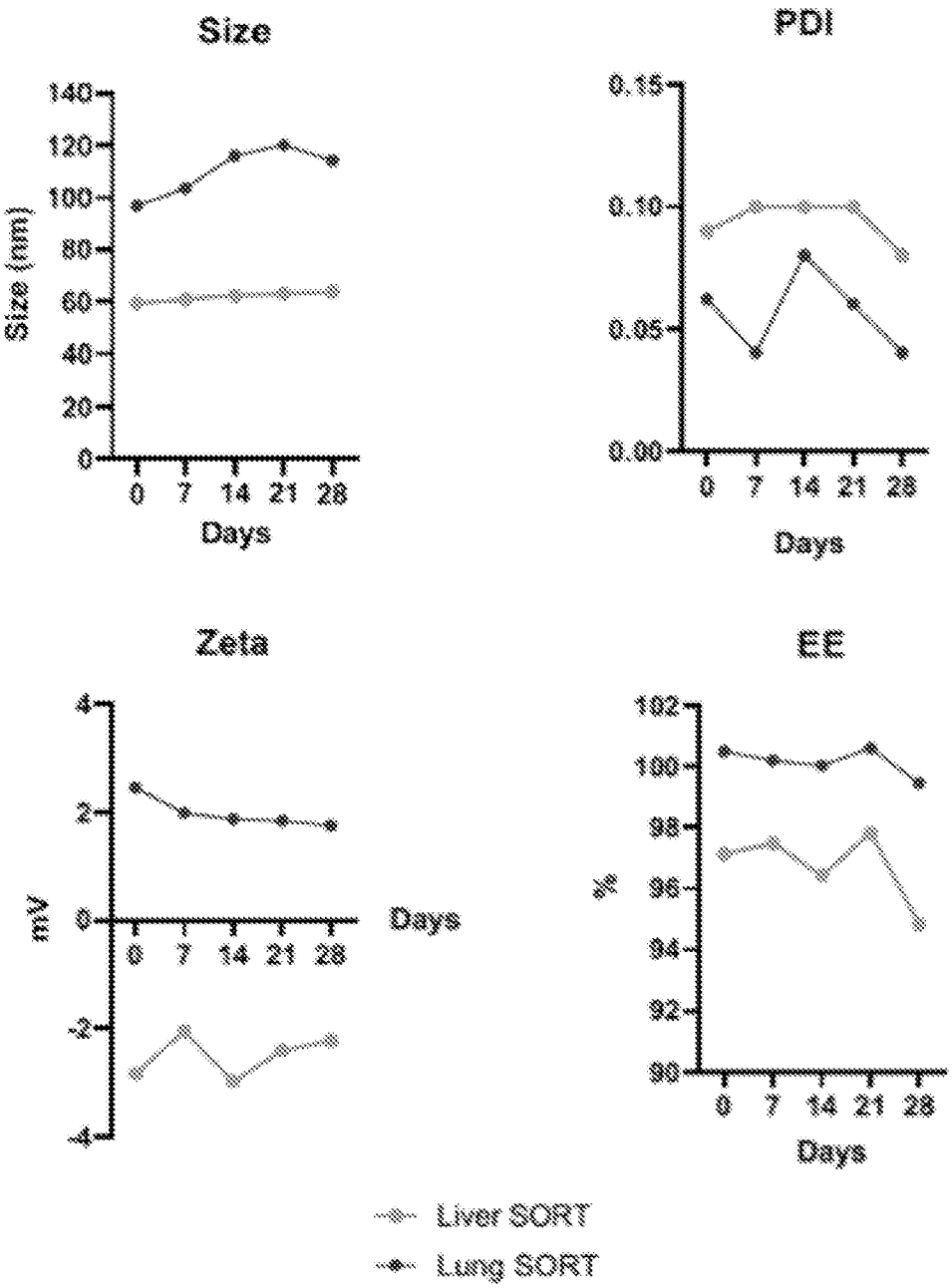

Size
Size (nm)
140
120
100
80
60
40
20
0
0 7 14 21 28
Days
PDI
0.15
0.10
0.05
0.00
0 7 14 21 28
Days
Zeta
mV
4
2
0
-2
-4
0 7 14 21 28
Days
EE
%
102
100
98
96
94
92
90
0 7 14 21 28
Days
Liver SORT
Lung SORT

Fig. 7

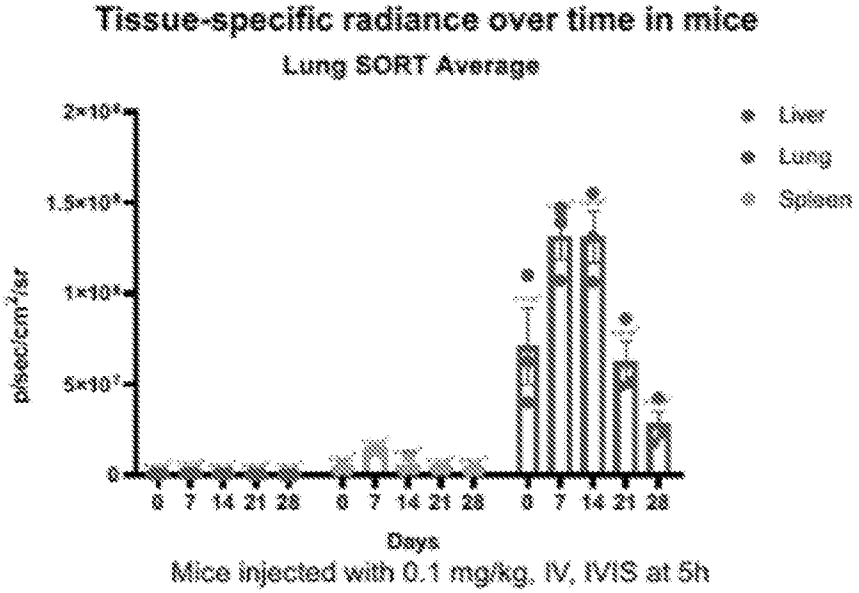

Tissue-specific radiance over time in mice
Lung SORT Average
Liver
Lung
Spleen
p/sec/cm²/sr
2×10⁴
1.5×10⁴
1×10⁴
5×10³
0
0 7 14 21 28 0 7 14 21 28 0 7 14 21 28
Days
Mice injected with 0.1 mg/kg, IV, IVIS at 5h

Fig. 8

Luminescence
1.4
1.2
1.0
0.8
0.6
0.4
0.2
x10^8
Day 0
Day 7
Day 14
Day 21
Day 28
SORT000016
(Liver SORT)
SORT000008
(Lung SORT)

Fig. 9A

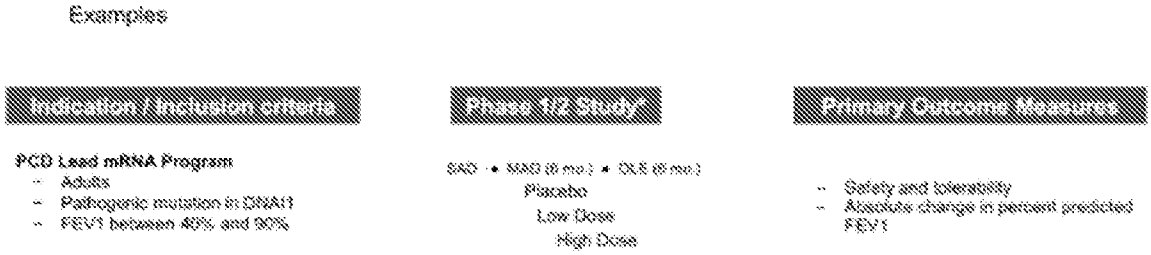


Fig. 9B

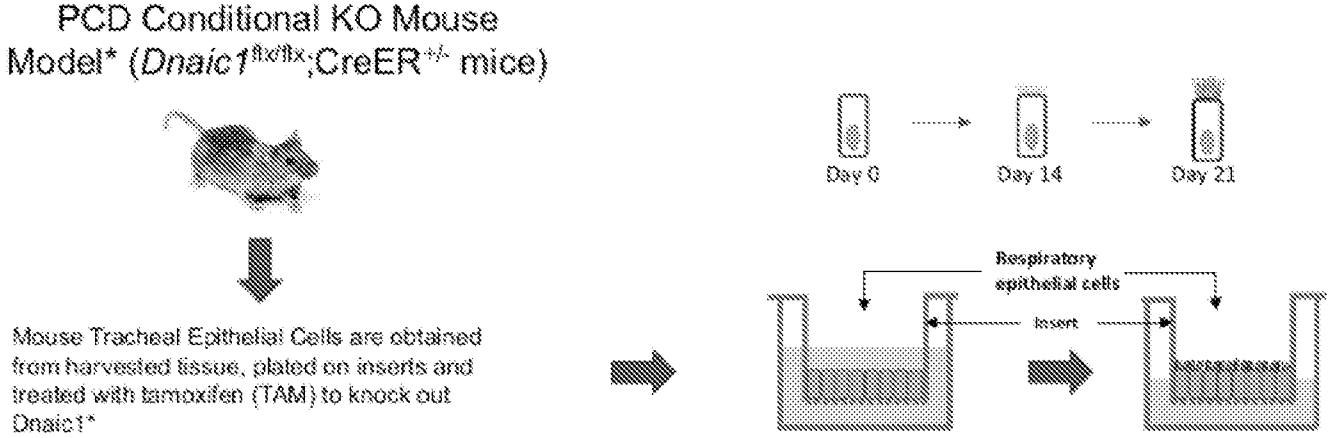


Fig. 9C

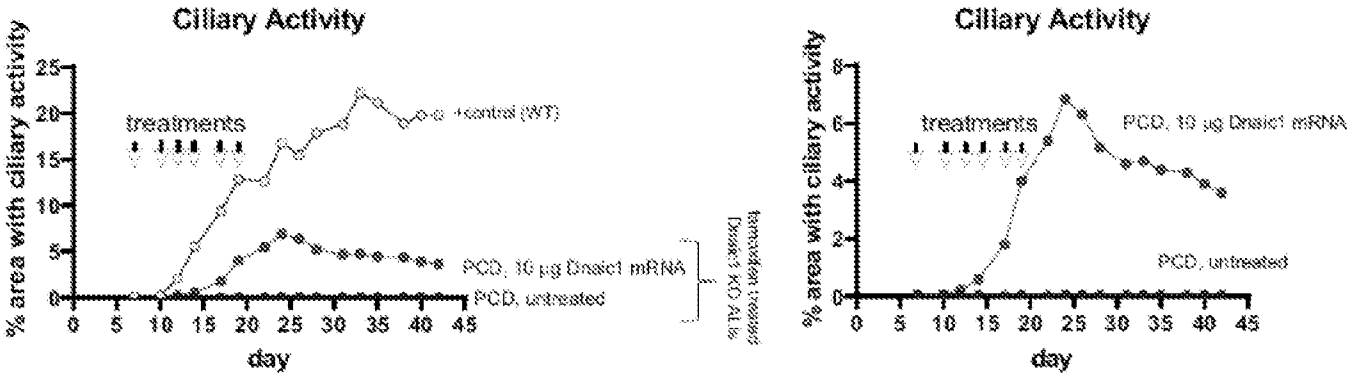


Fig. 10A

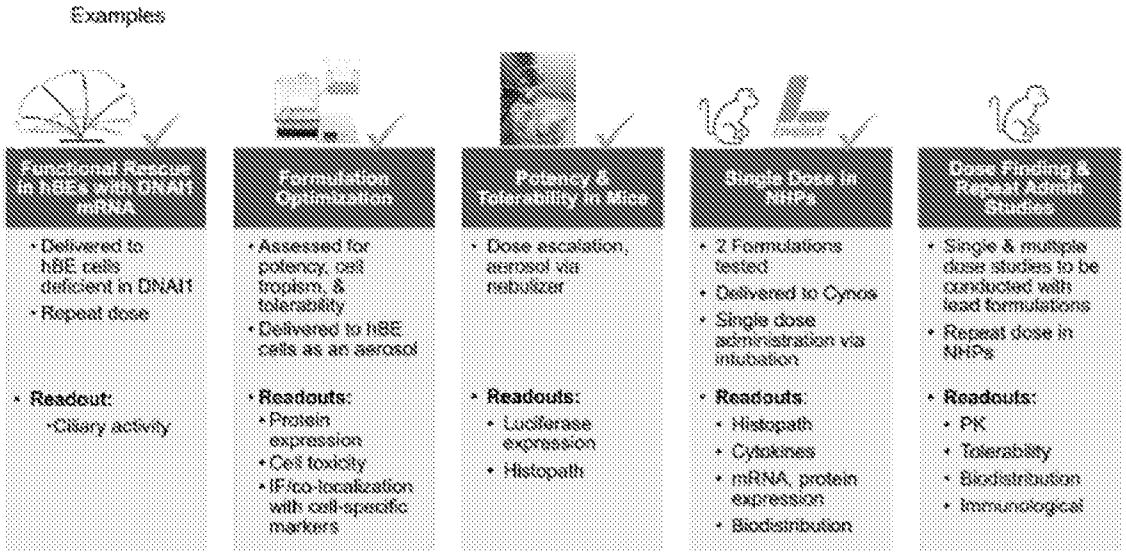


Fig. 10B

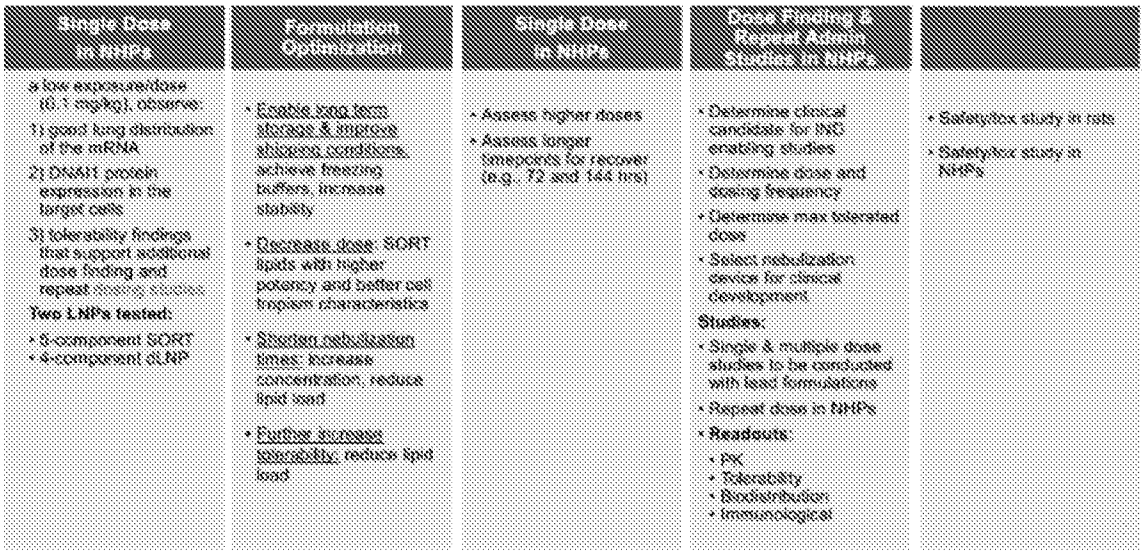


Fig. 10C

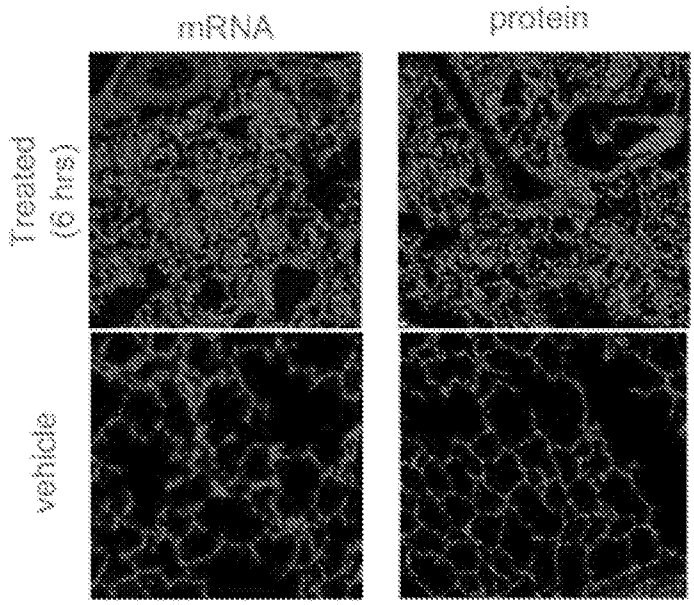


Fig. 10D

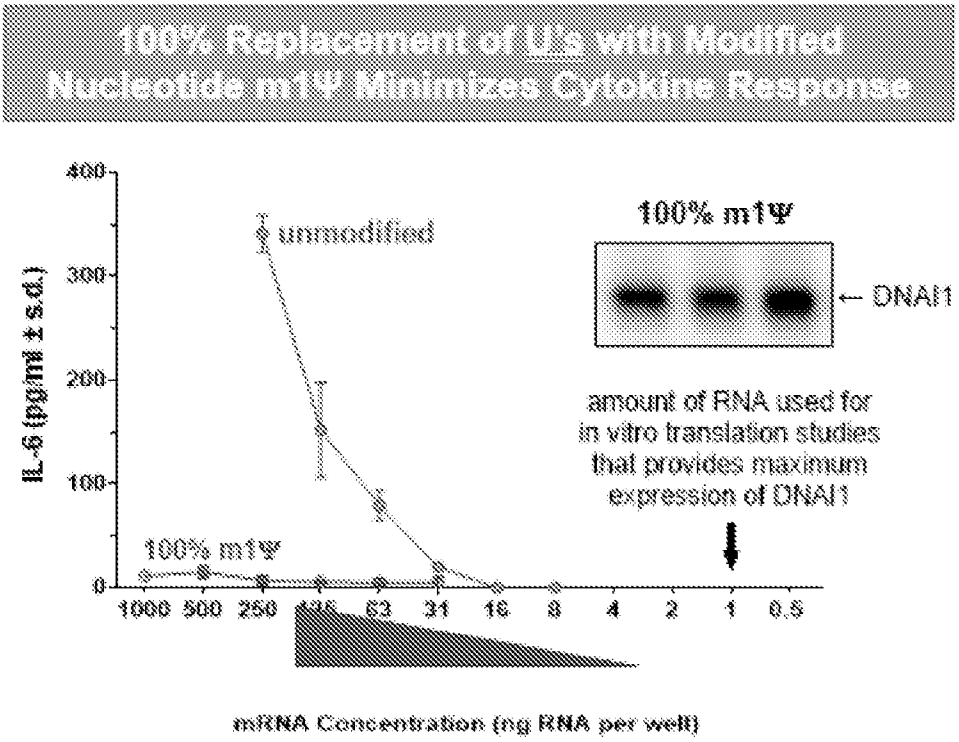


Fig. 10E

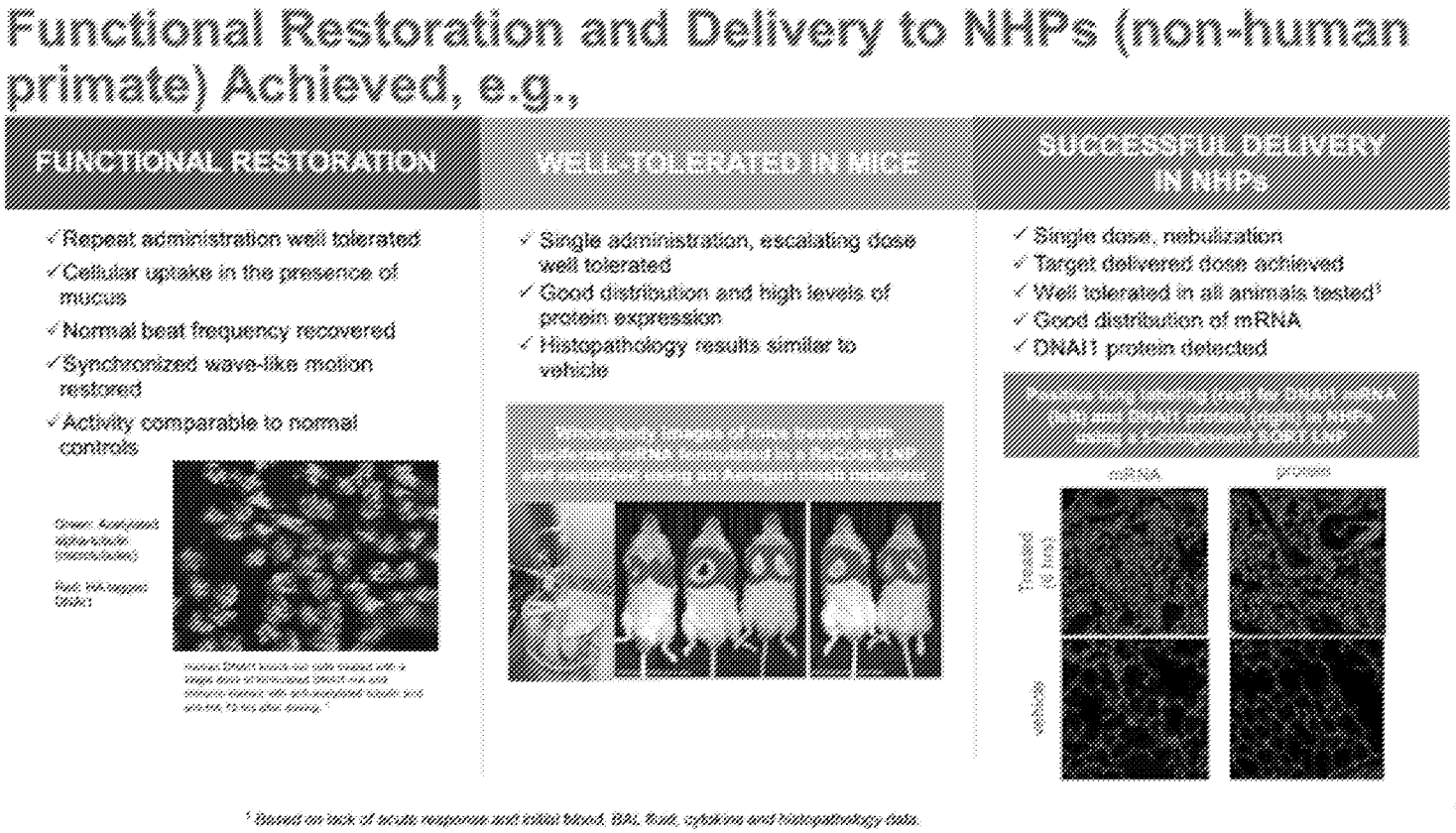

Functional Restoration and Delivery to NHPs (non-human primate) Achieved, e.g.,
FUNCTIONAL RESTORATION
✓Repeat administration well tolerated
✓Cellular uptake in the presence of mucus
✓Normal beat frequency recovered
✓Synchronized wave-like motion restored
✓Activity comparable to normal controls
WELL TOLERATED IN MICE
✓ Single administration, escalating dose well tolerated
✓ Good distribution and high levels of protein expression
✓ Histopathology results similar to vehicle
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
mRNA
protein
Treated
Vehicle
[1] Based on lack of acute response and initial blood, BAL fluid, cytokine and histopathology data.

Fig. 11A

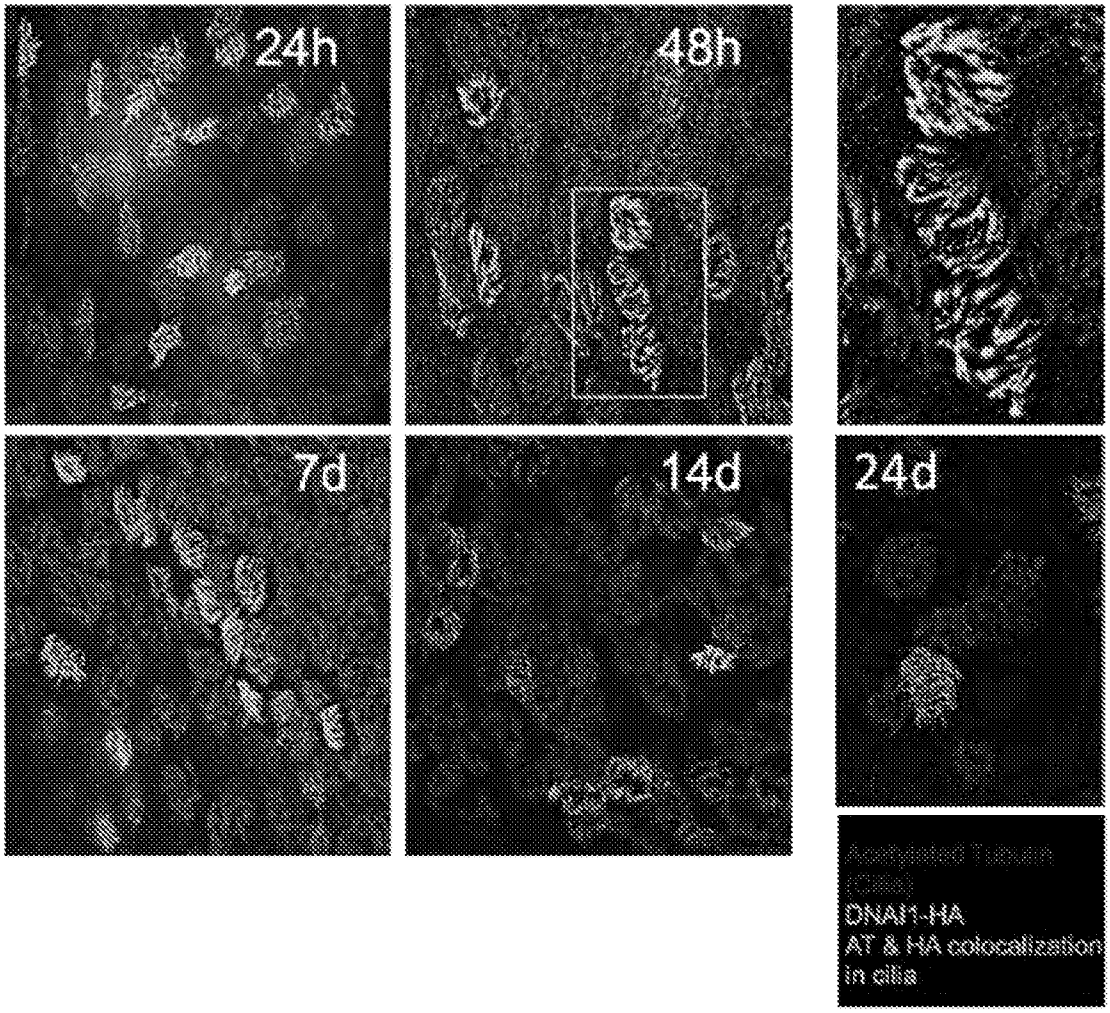


Fig. 11B

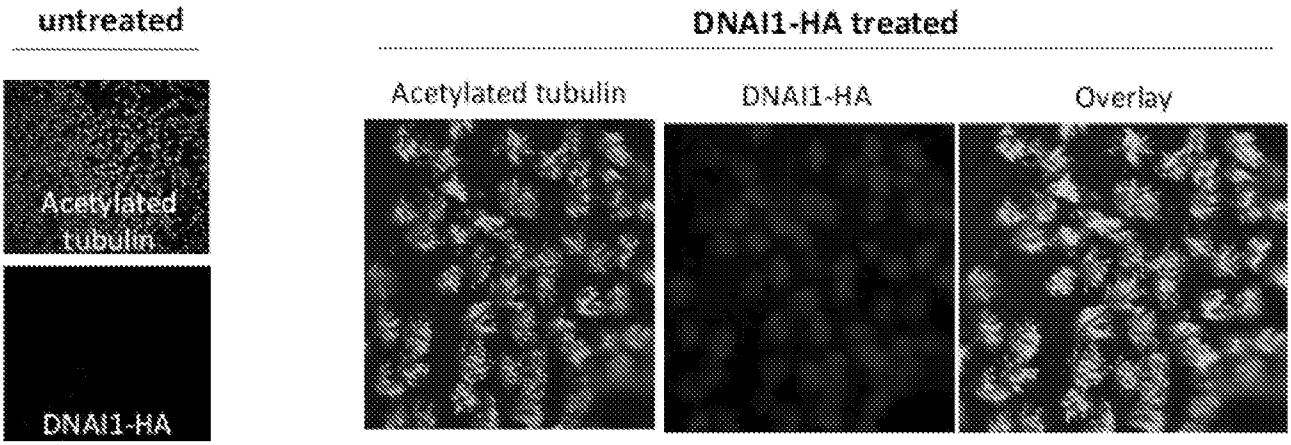


Fig. 12

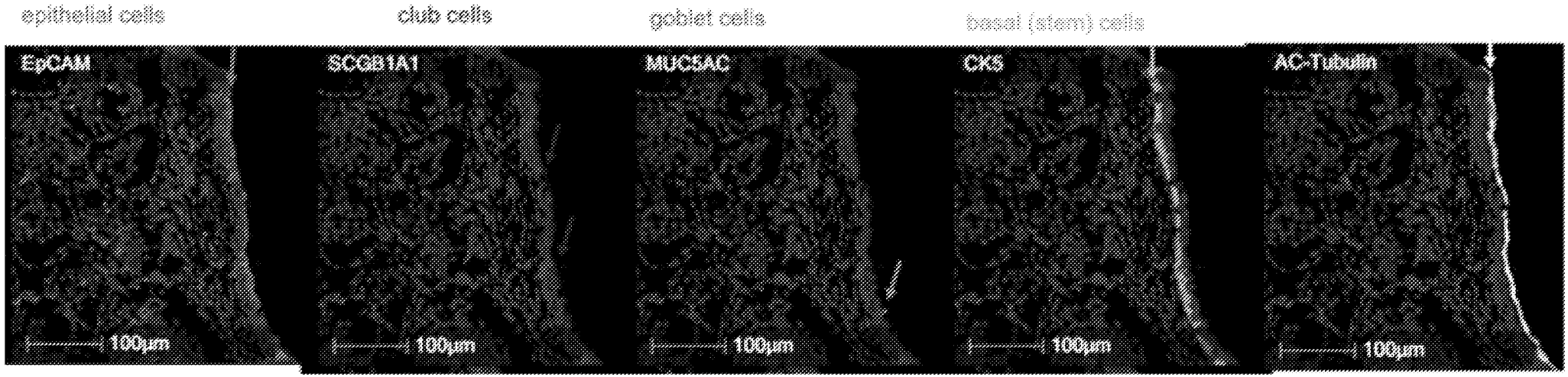

epithelial cells
club cells
goblet cells
basal (stem) cells
EpCAM
SCGB1A1
MUC5AC
CK5
AC-Tubulin
100µm
100µm
100µm
100µm
100µm

Fig. 13A

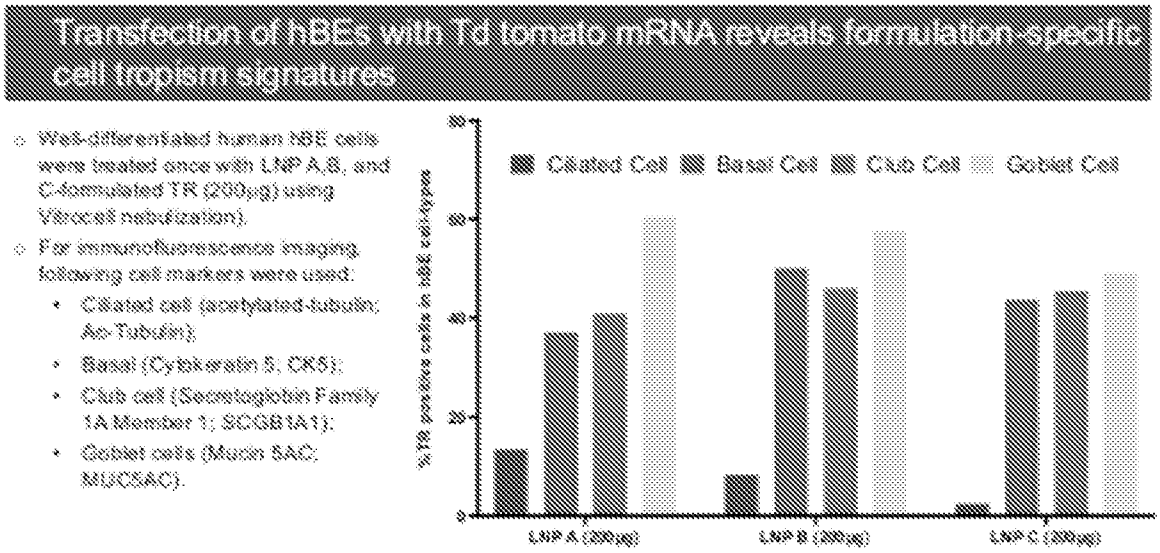


Fig. 13B

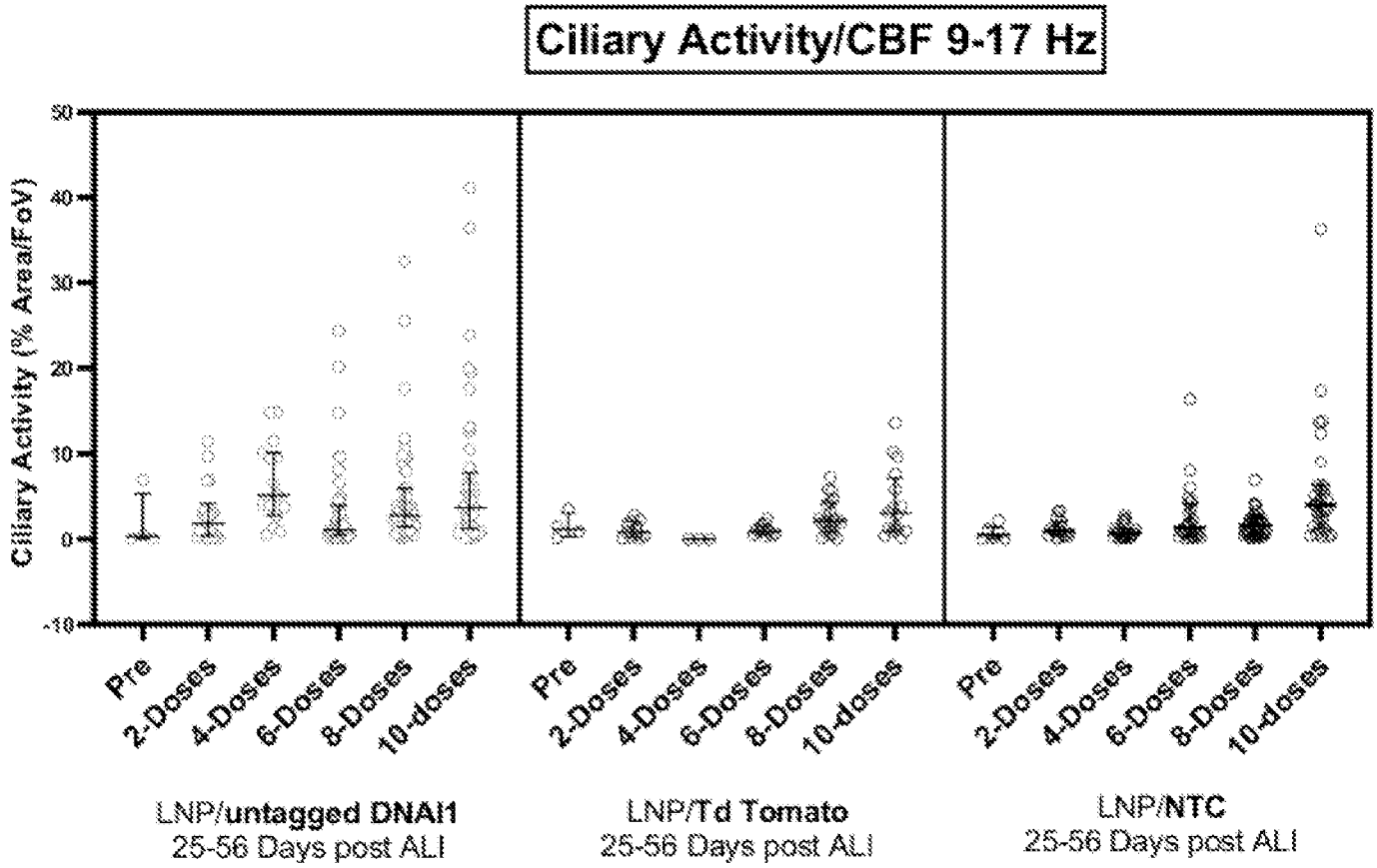


Fig. 14A

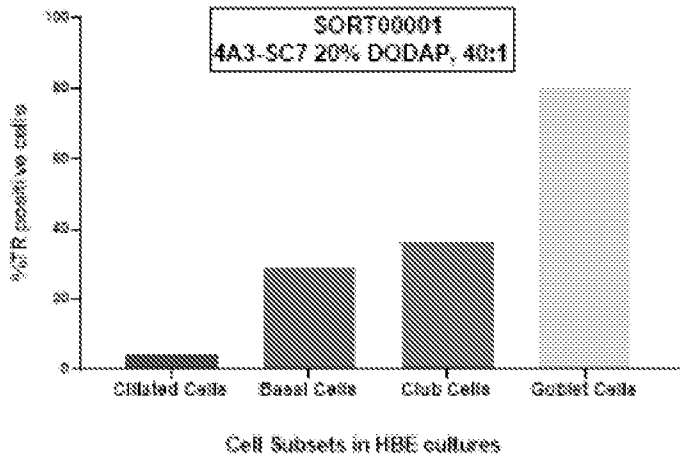


Fig. 14B

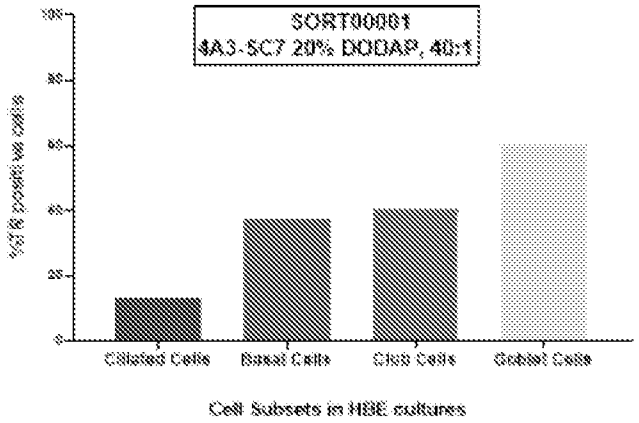


Fig. 14C

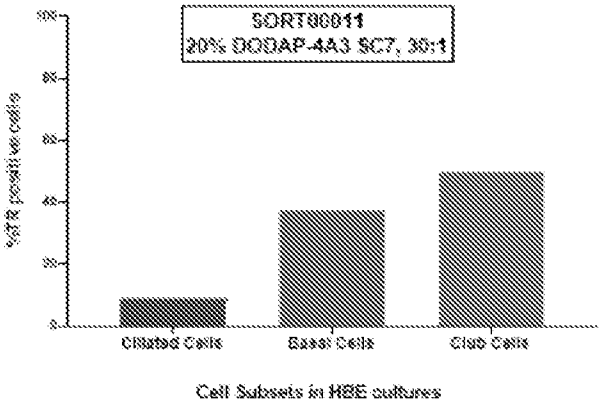


Fig. 14D

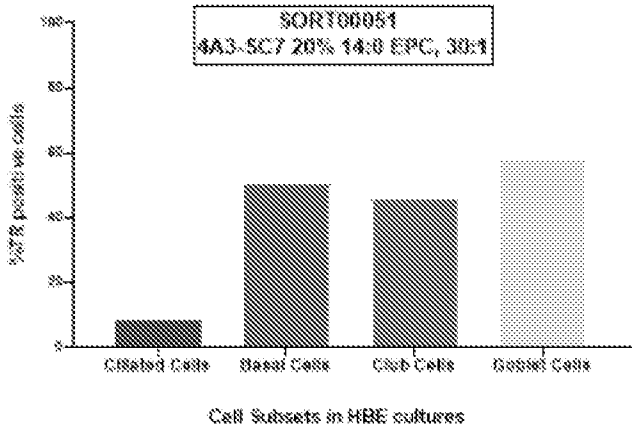


Fig. 14E

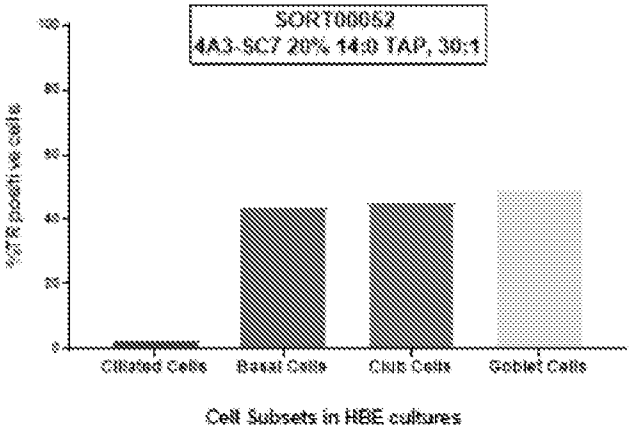
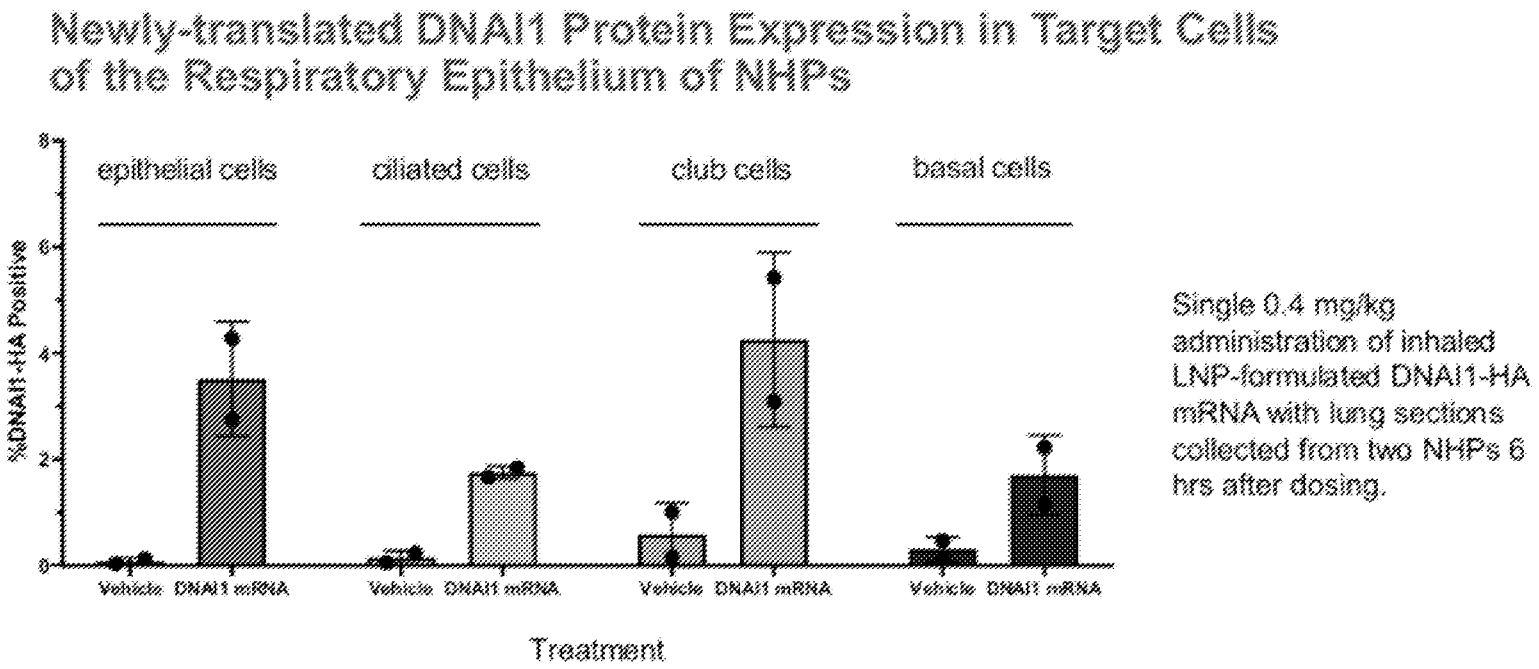


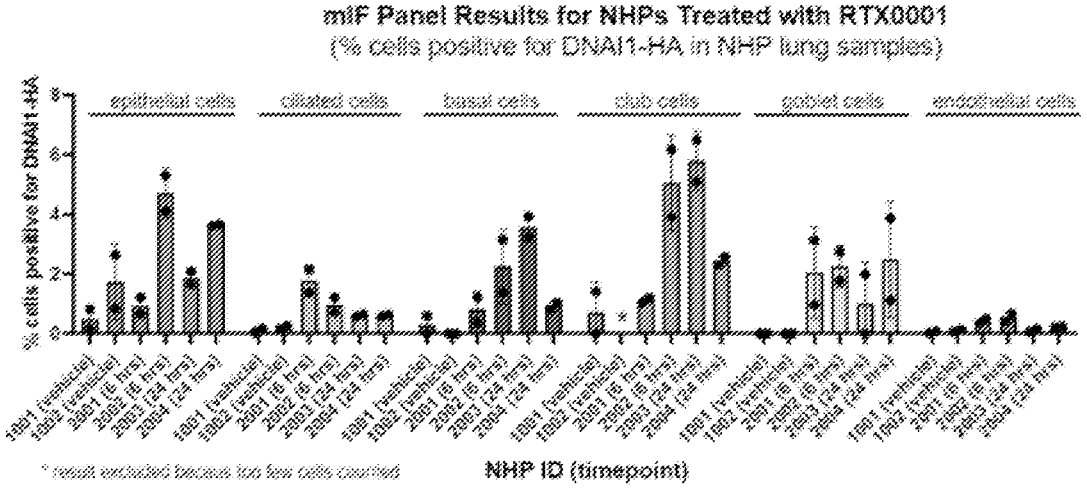
Fig. 15



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

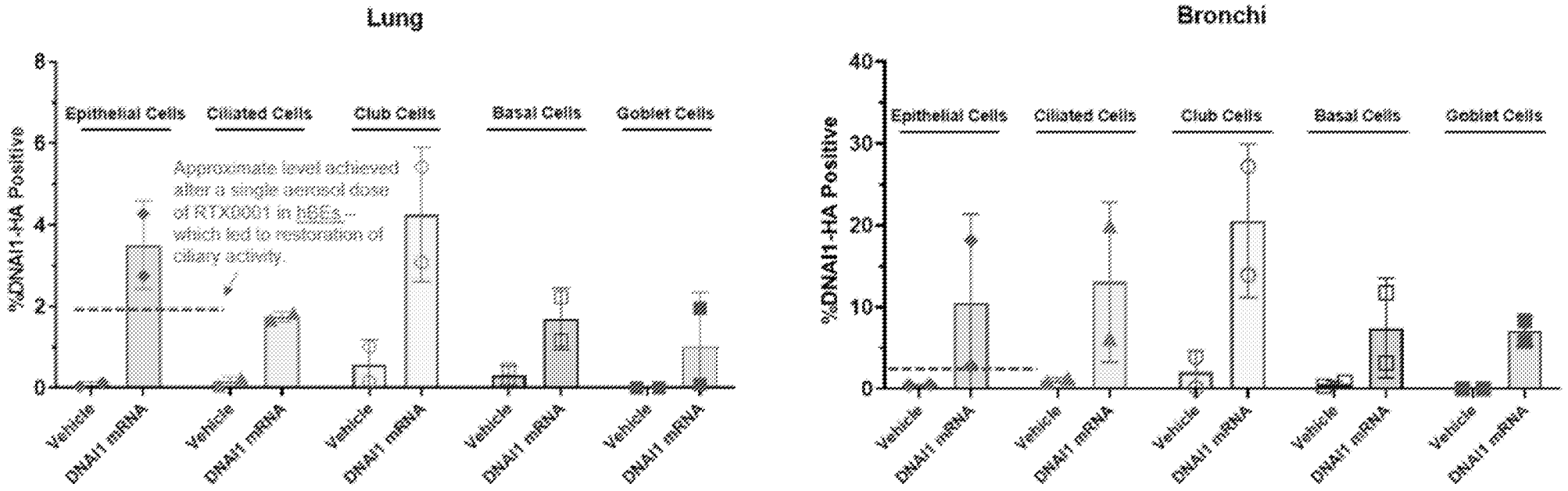

Lung
%DNAI1-HA Positive
Epithelial Cells
Ciliated Cells
Club Cells
Basal Cells
Goblet Cells
Approximate level achieved after a single aerosol dose of RTX0001 in hBEs – which led to restoration of ciliary activity.
Vehicle
DNAI1 mRNA
Bronchi
%DNAI1-HA Positive
Epithelial Cells
Ciliated Cells
Club Cells
Basal Cells
Goblet Cells
Vehicle
DNAI1 mRNA

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 DNAH1-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

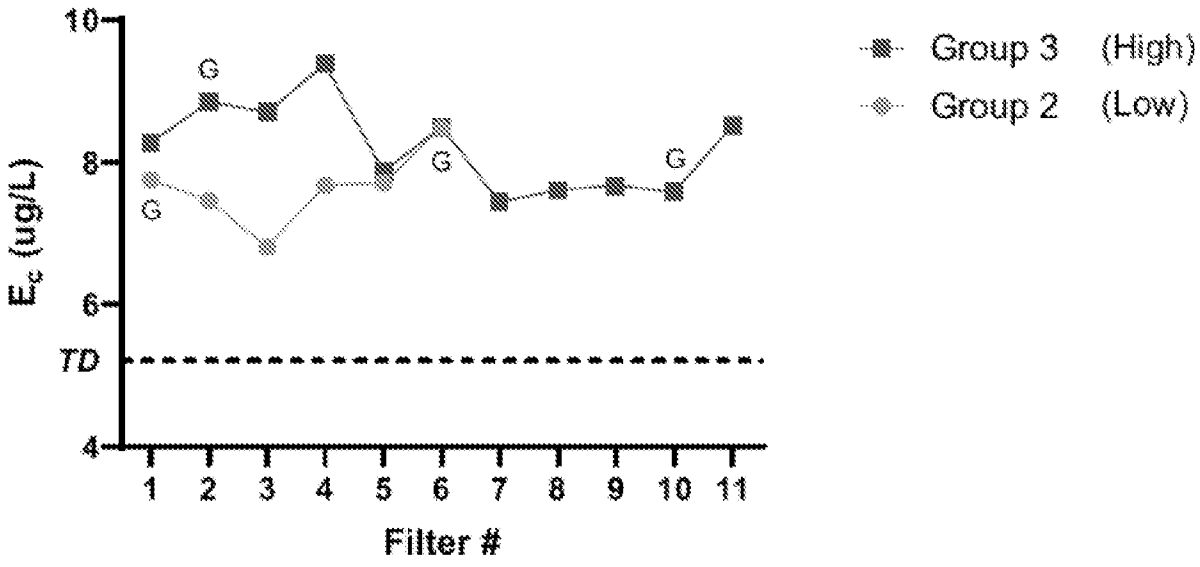


Fig. 18B

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

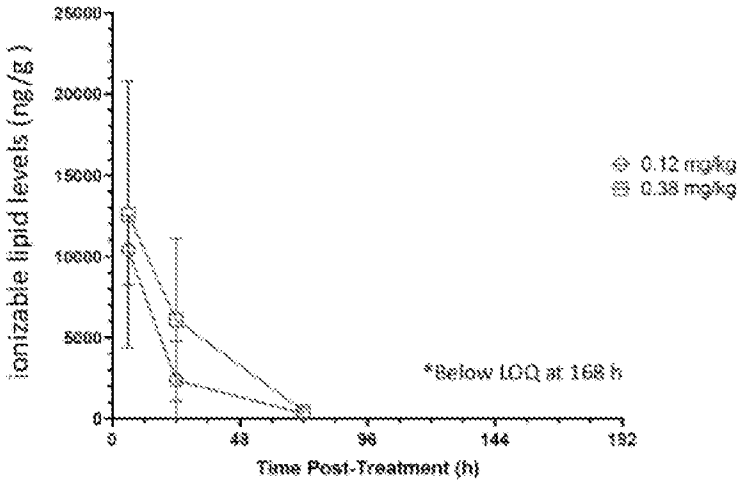


Fig. 19B

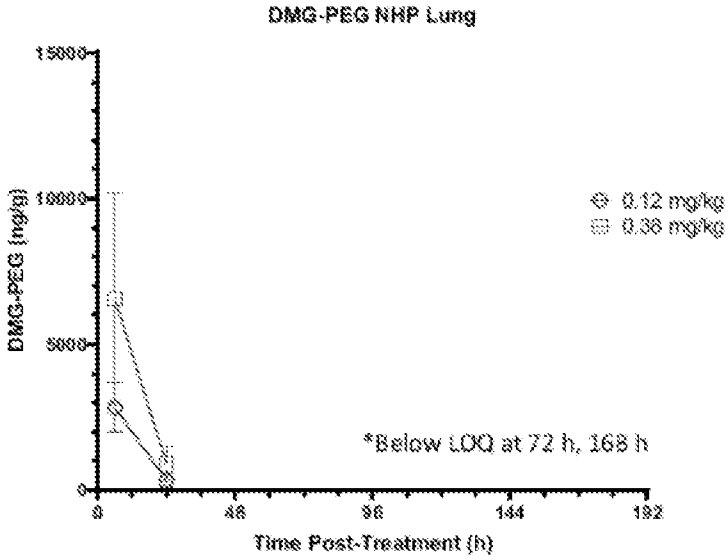


Fig. 19C

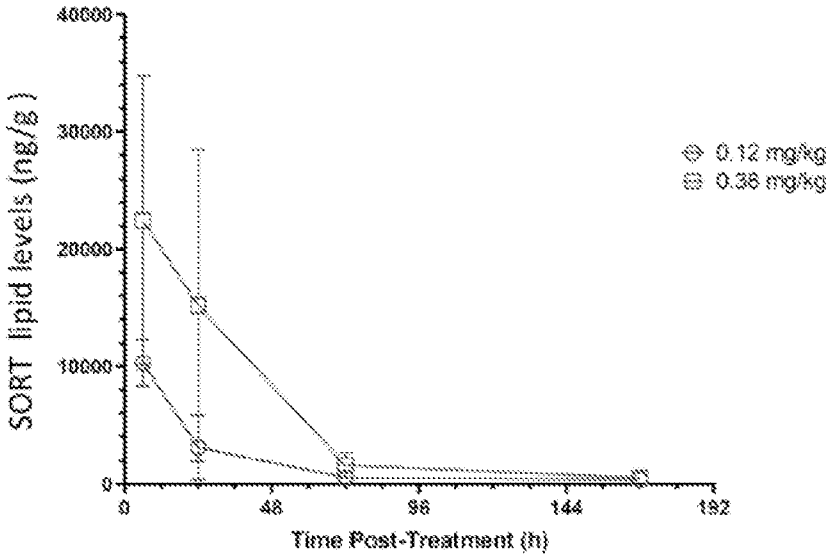


Fig. 20A

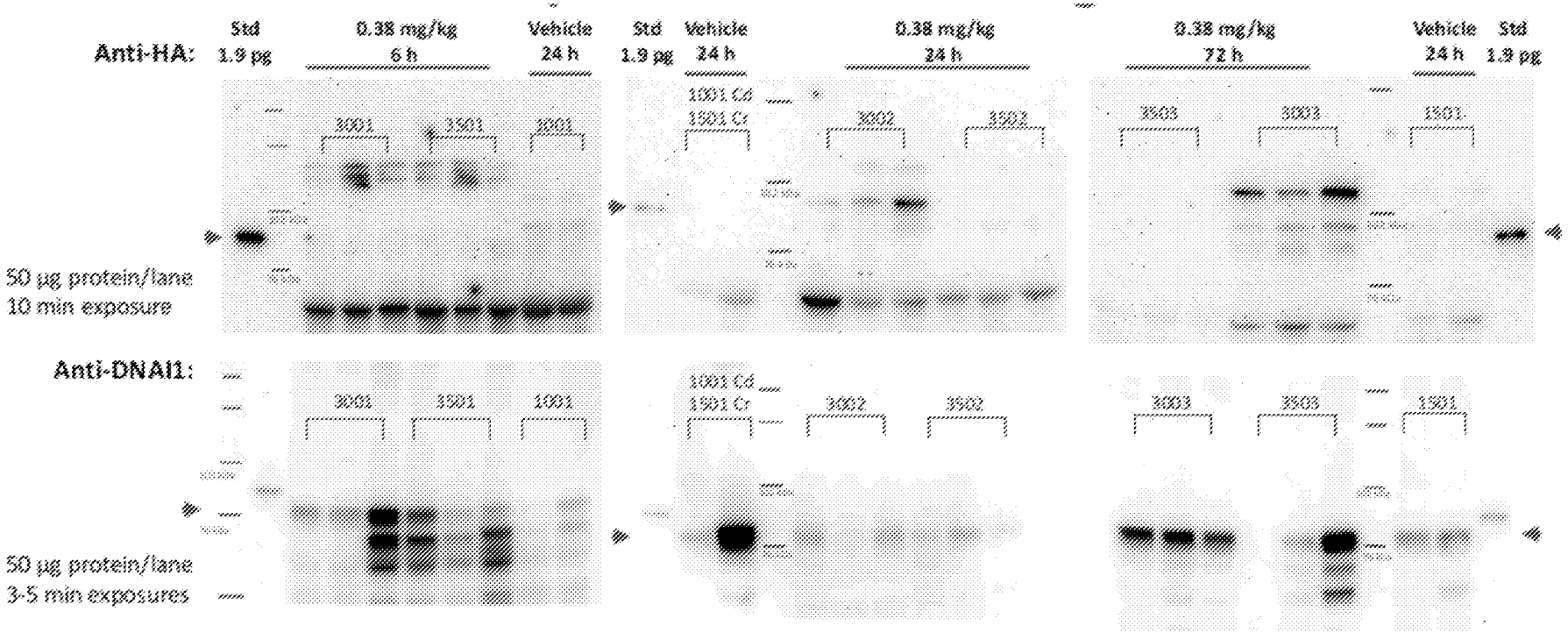
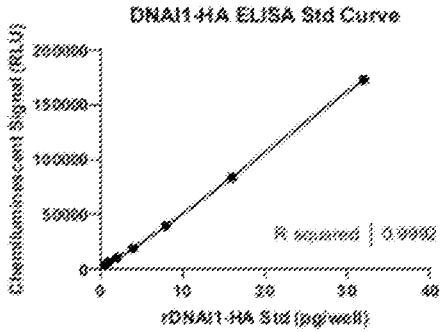

Anti-HA:
Std
1.9 pg
0.38 mg/kg
6 h
Vehicle
24 h
Std
1.9 pg
Vehicle
24 h
0.38 mg/kg
24 h
0.38 mg/kg
72 h
Vehicle
24 h
Std
1.9 pg
3001
3501
1001
1001 Cd
1501 Cr
3002
3502
3503
3003
1501
50 µg protein/lane
10 min exposure
Anti-DNAI1:
50 µg protein/lane
3-5 min exposures

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

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

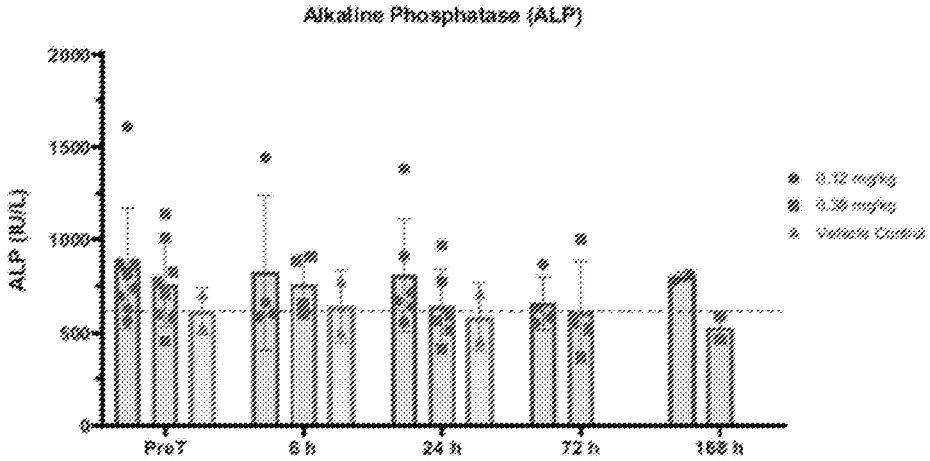
High Dose: 0.38 mg/kg 72 h

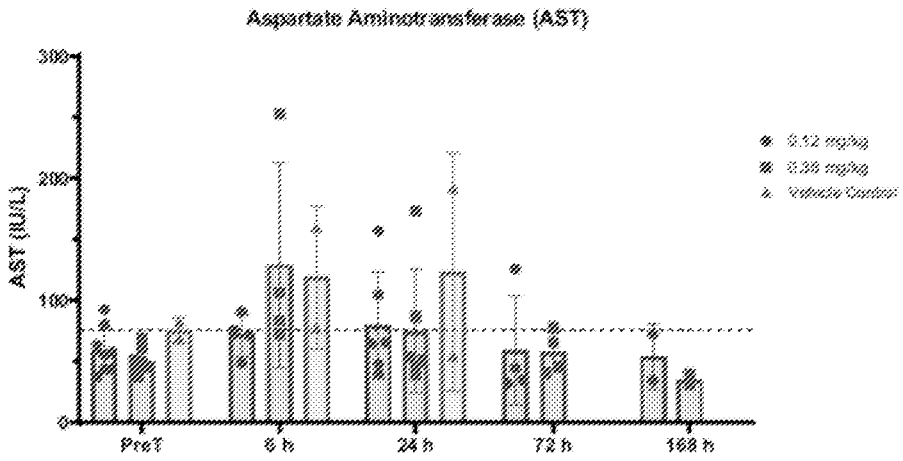
Animal Number	ELISA Result (pg/100 µg total protein)	Western Blot Signal (Y/N)
3003 Cr	n.d.	N
3003 Mi	n.d.	N
3003 Cd	n.d.	N
3503 Cr	n.d.	N
3503 Mi	n.d.	N
3503 Cd	n.d.	N

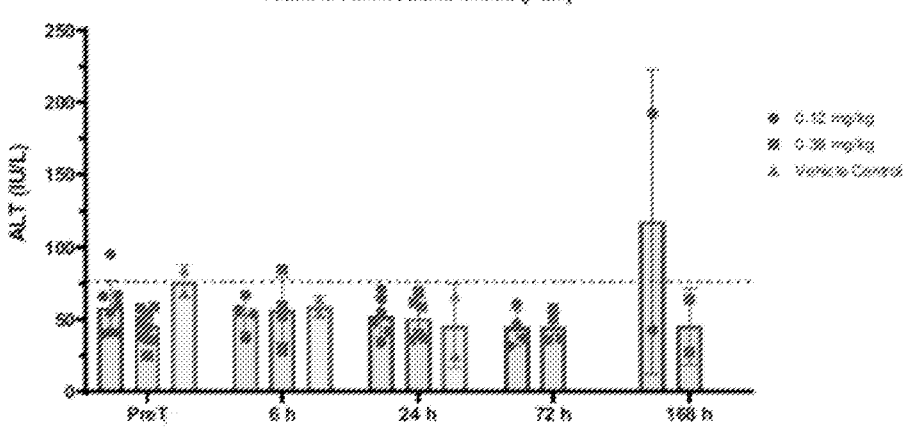
*Below LOQ

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

Fig. 21A







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

Fig. 21B

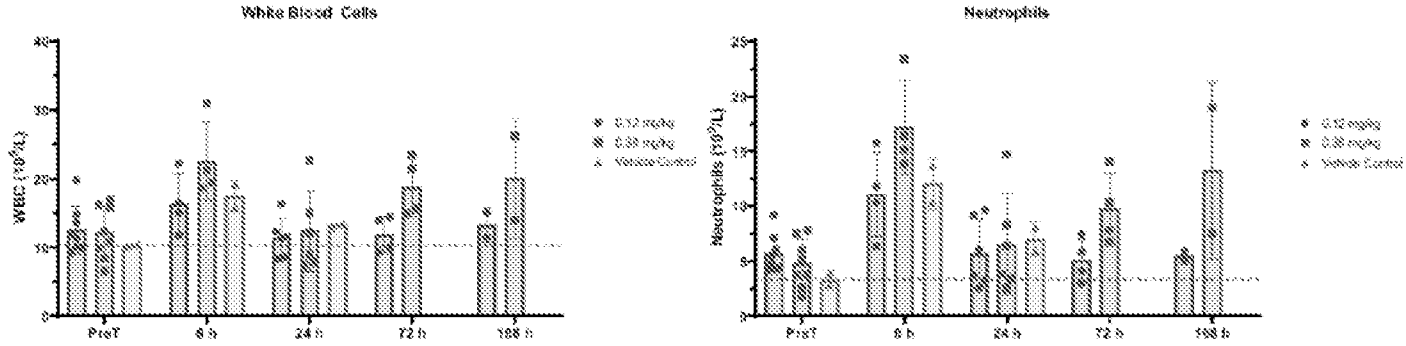


Fig. 21C

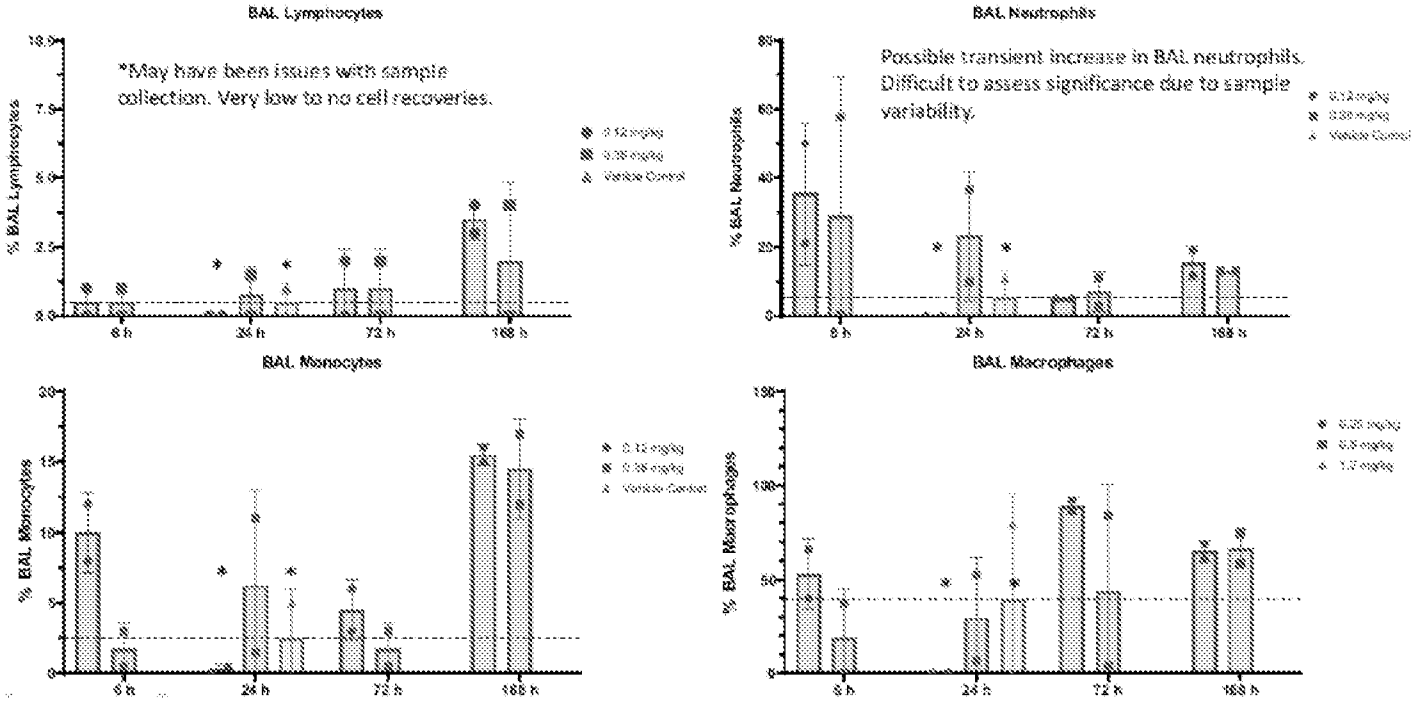
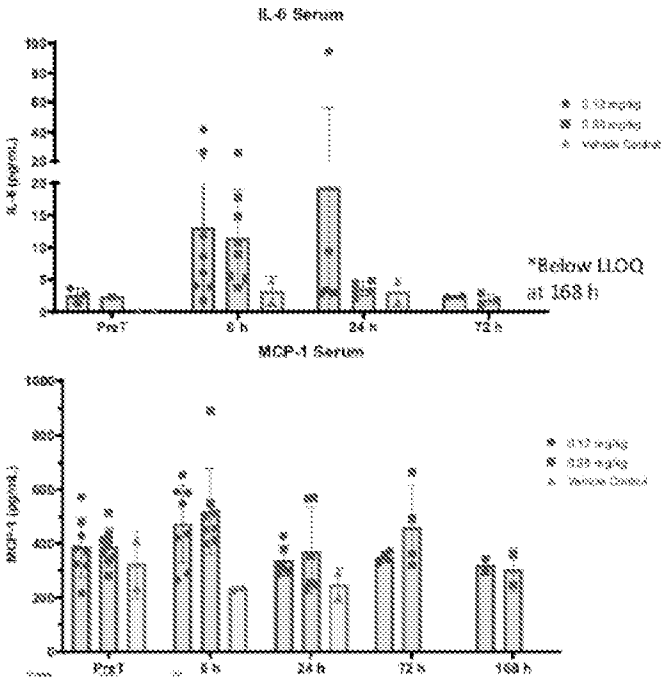
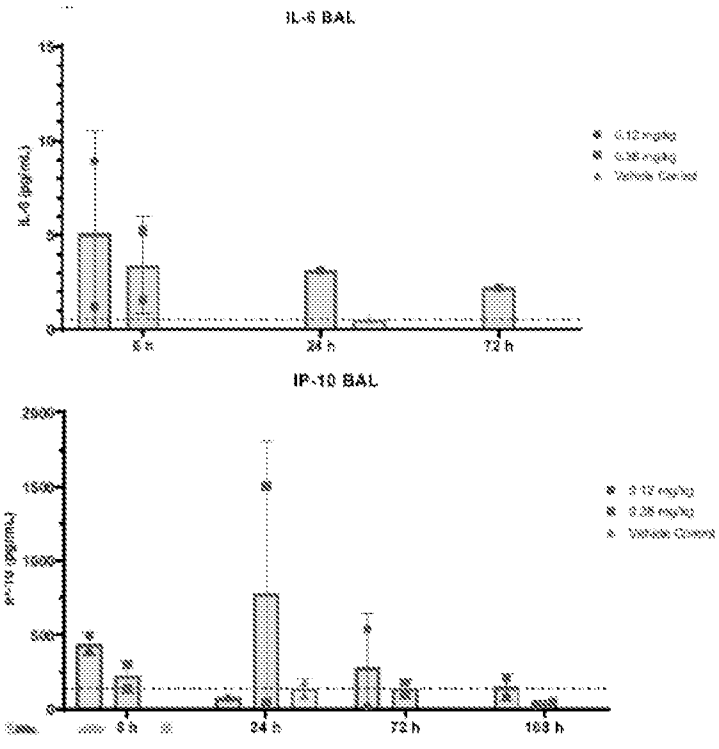


Fig. 21D



- 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

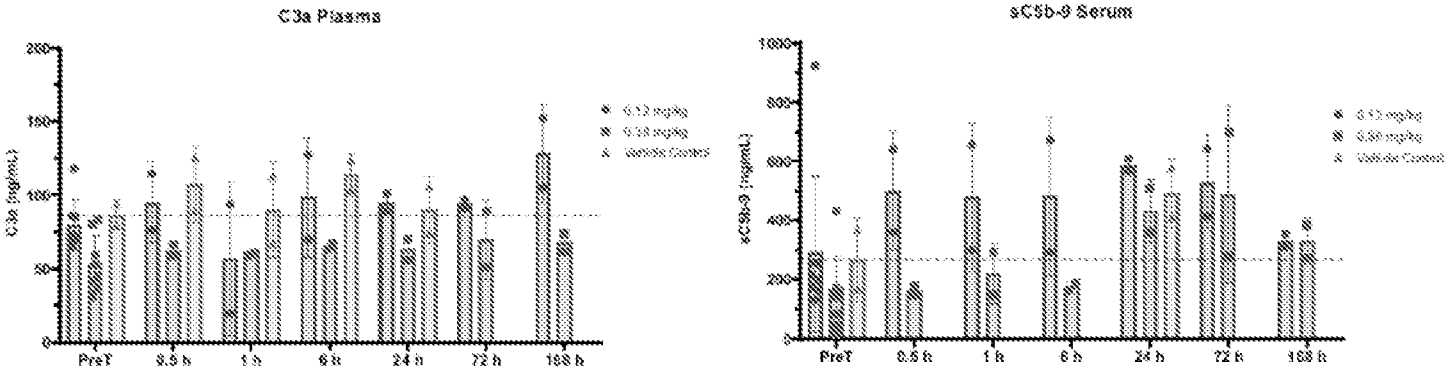


Fig. 21G

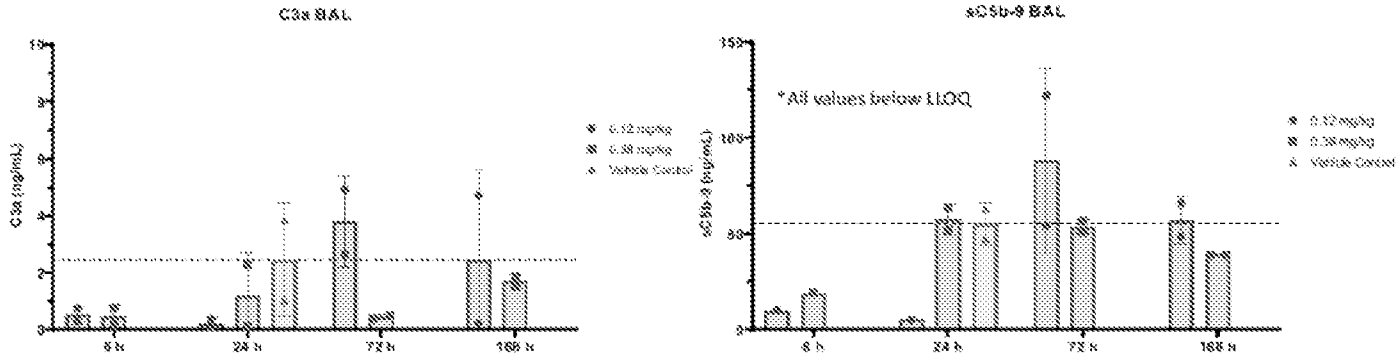
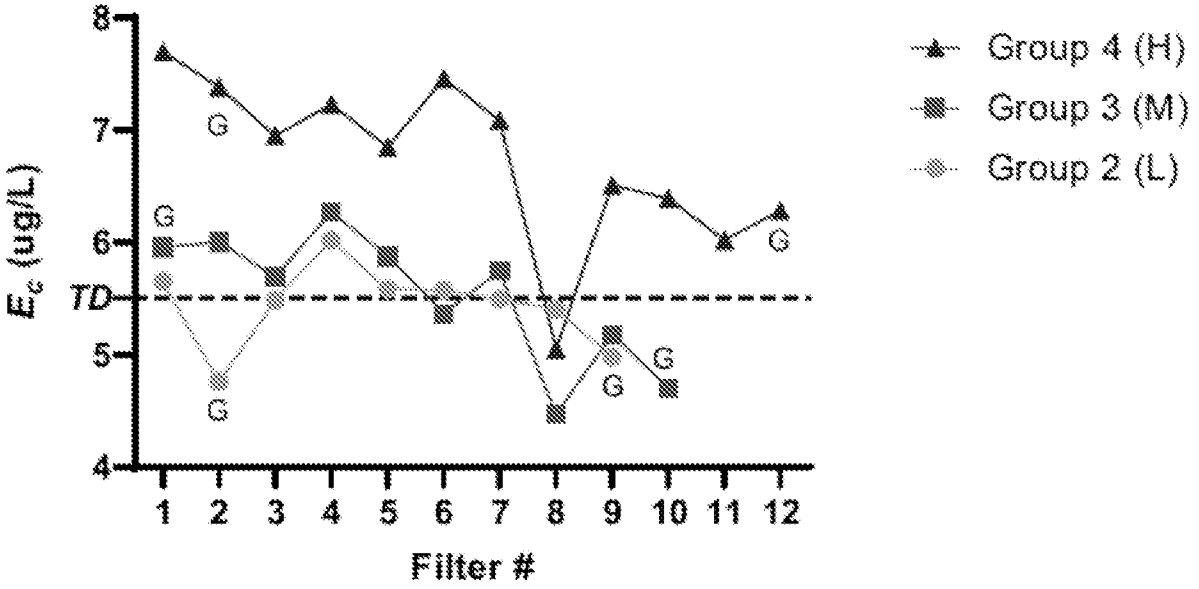


Fig. 22A



G = gravimetric analysis

Fig. 22B

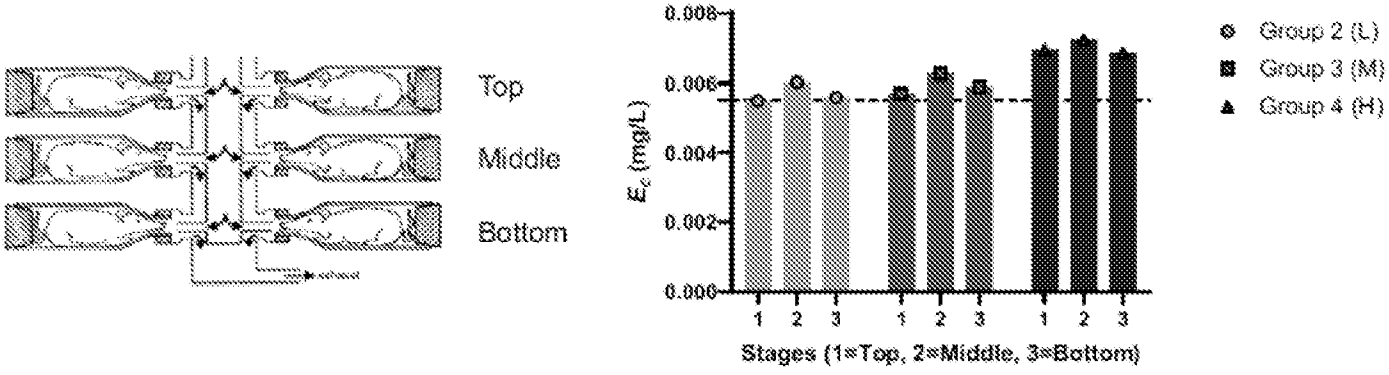


Fig. 22C

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

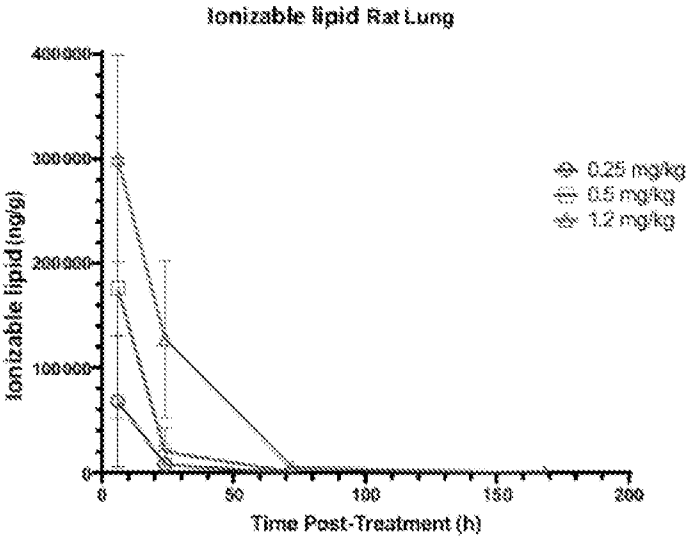


Fig. 23B

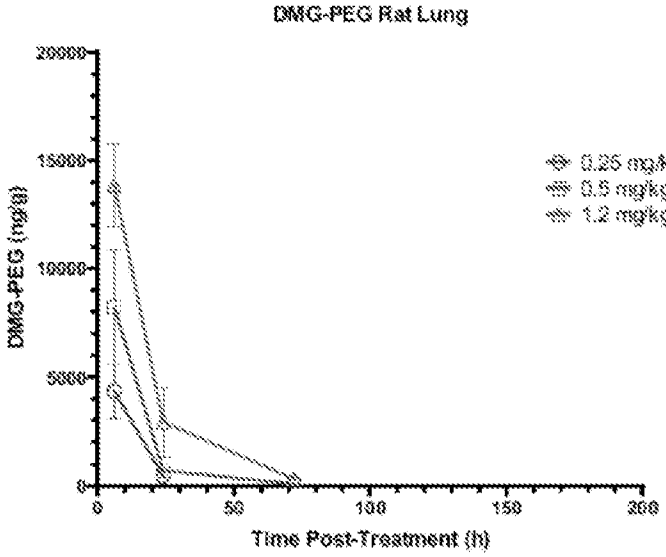


Fig. 23C

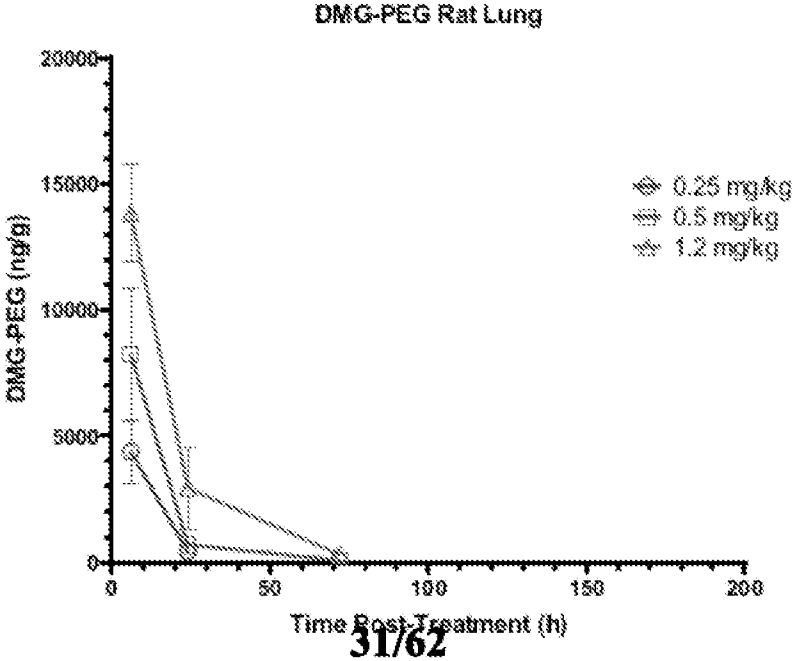


Fig. 24A

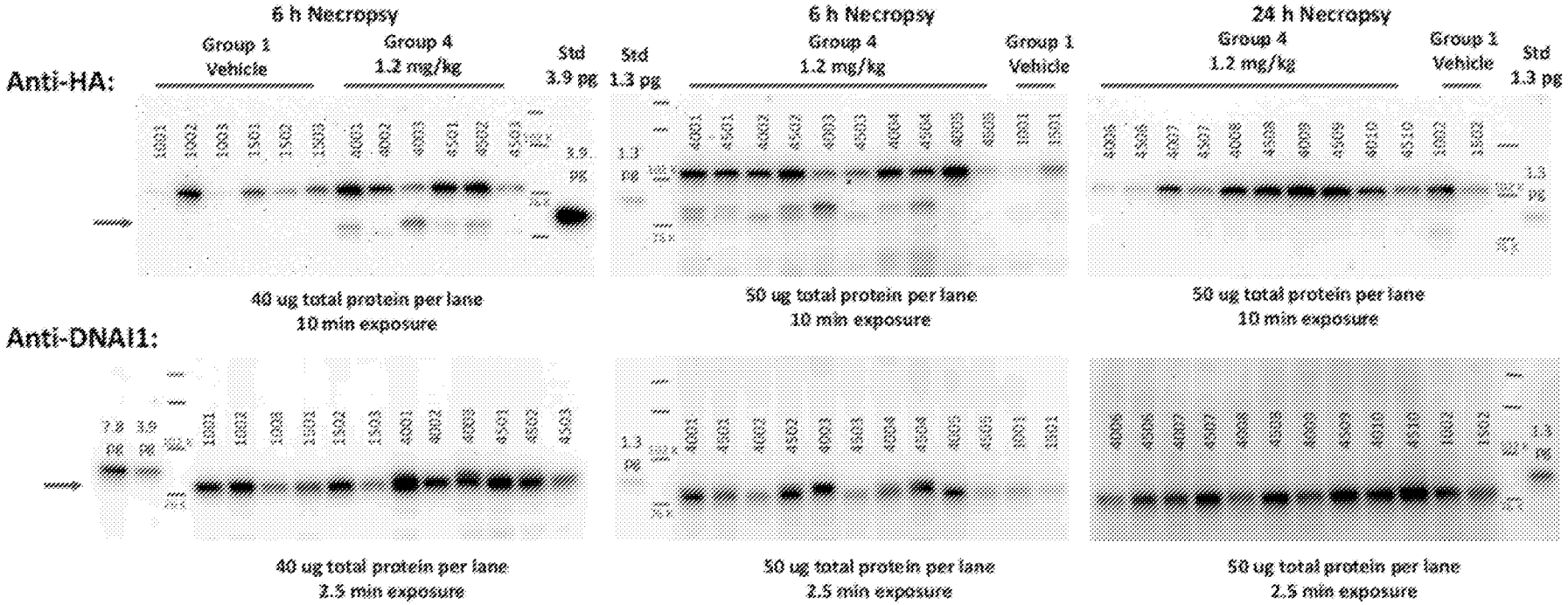
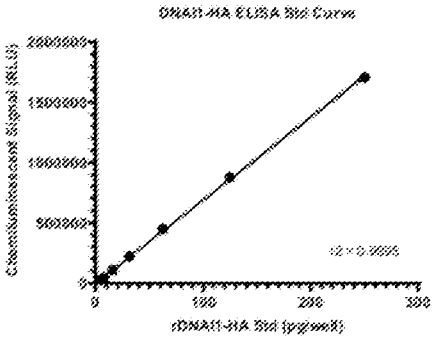

6 h Necropsy
Group 1 Vehicle
Group 4 1.2 mg/kg
Std 3.9 pg
Std 1.3 pg
6 h Necropsy
Group 4 1.2 mg/kg
Group 1 Vehicle
24 h Necropsy
Group 4 1.2 mg/kg
Group 1 Vehicle
Std 1.3 pg
Anti-HA:
40 ug total protein per lane
10 min exposure
50 ug total protein per lane
10 min exposure
50 ug total protein per lane
10 min exposure
Anti-DNAI1:
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

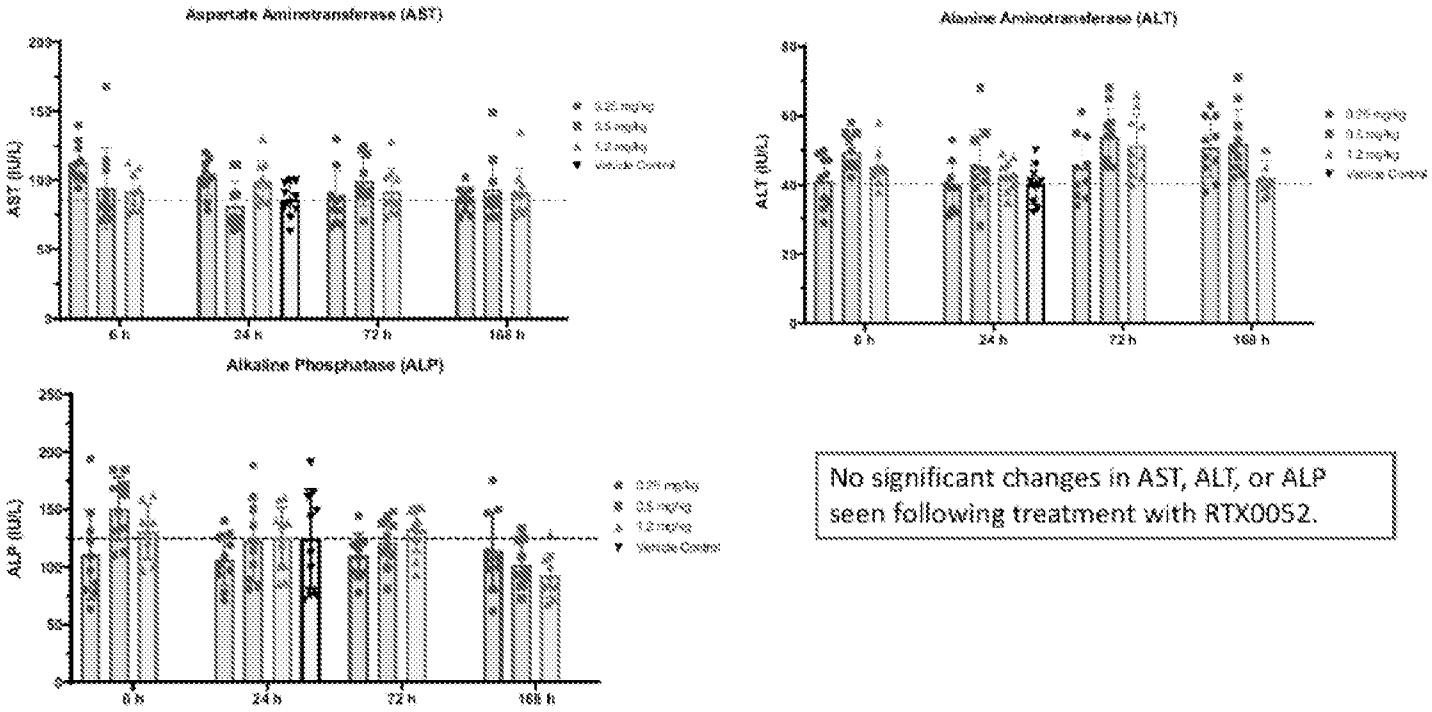
*Below LOQ



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

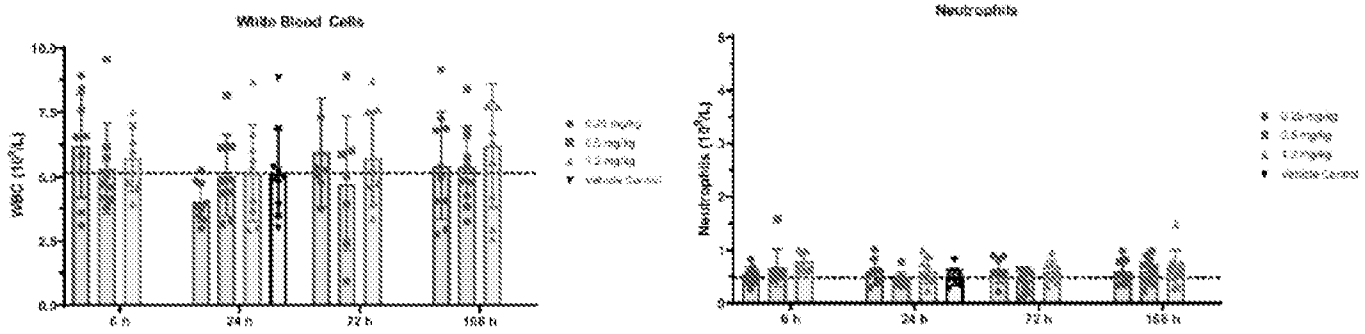
Good correlation with WB results: 5/6 WB+ samples are also ELISA+

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

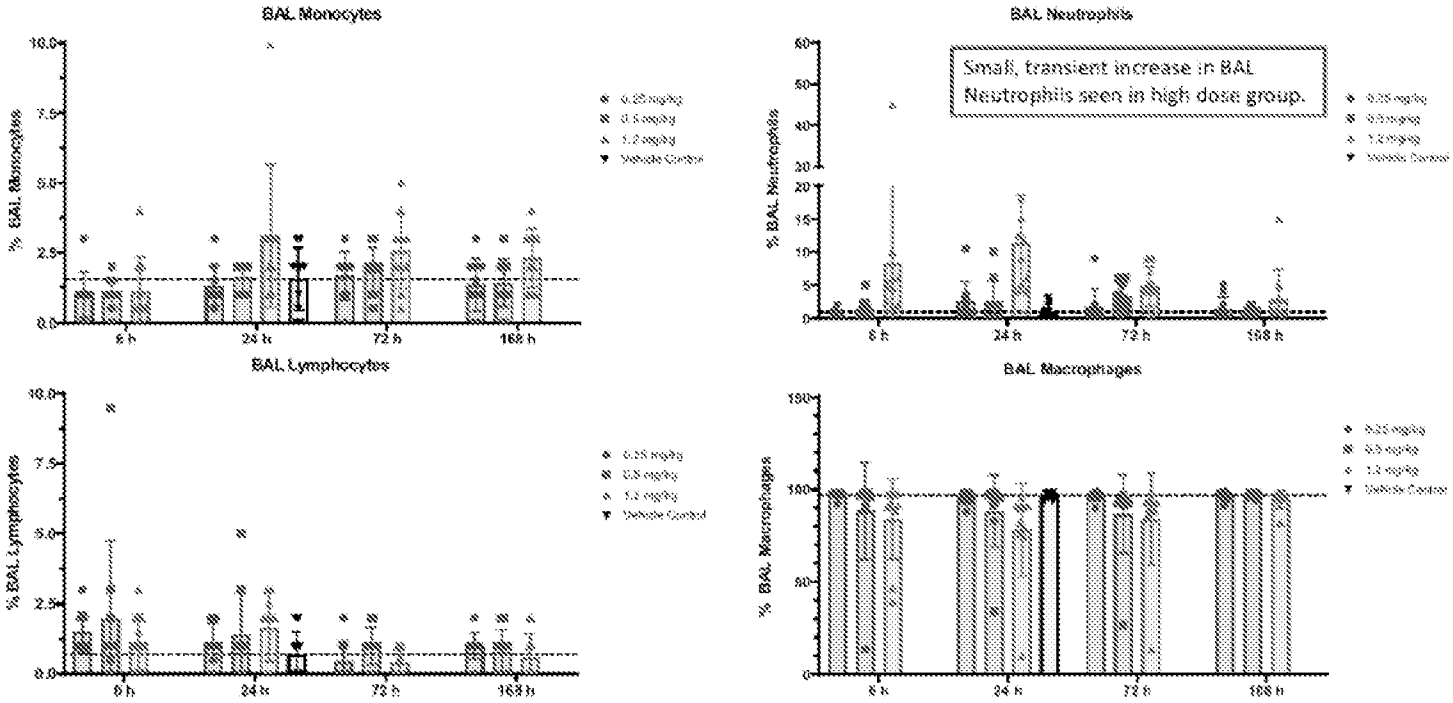


Fig. 25D

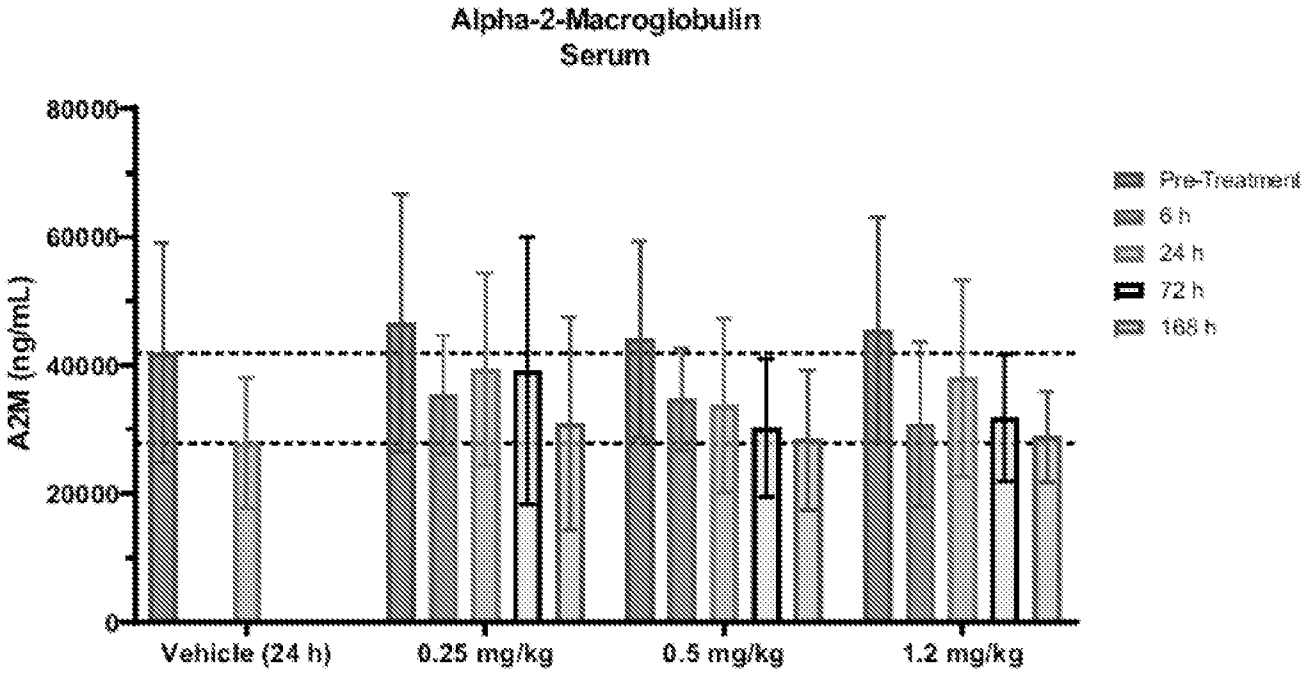
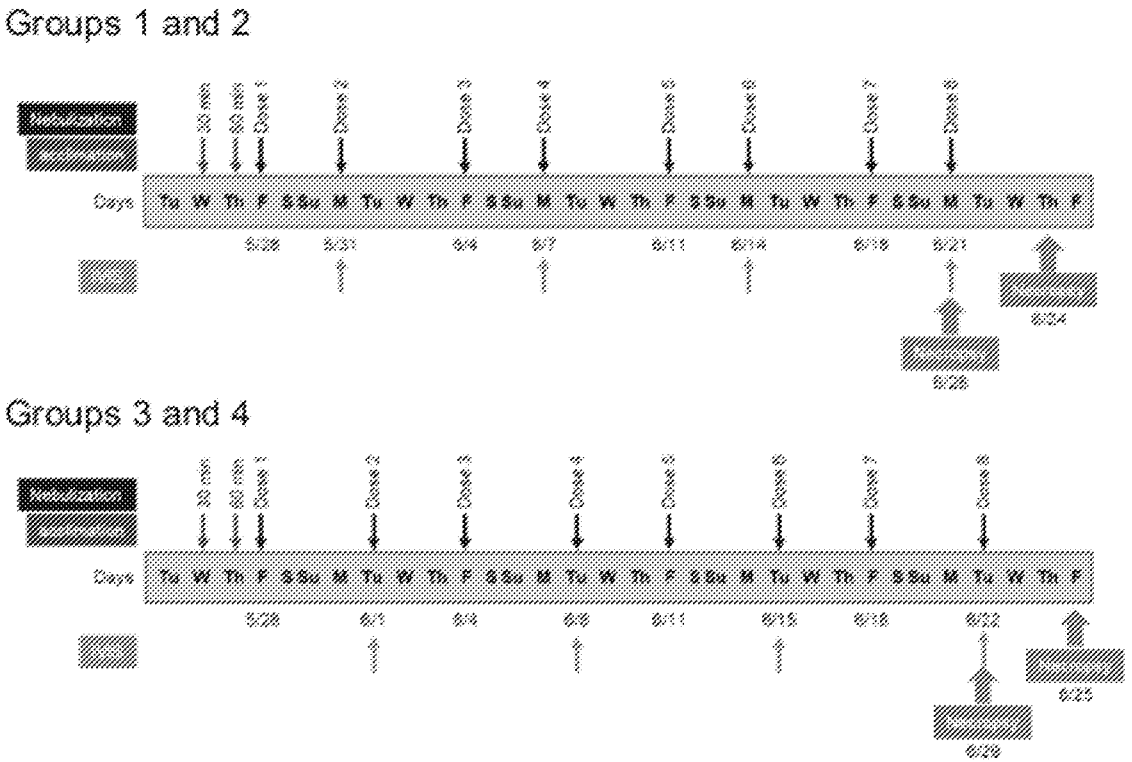


Fig. 26A

Group	Formulation	Animals	Delivery	[RNA] (mg/mL)	Total Dose	aerosol delivery of Substrate	# animals	Data Collection
1	Control vehicle	Albino B6', 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>: a) 2 mice @ 4h post-dosing = IVIS <u>For all groups – after last dose</u>: a) 2 mice @ 4h post-last dose and post-IVIS = perfuse and explant liver, spleen and lungs. Half for RNAlater; half for protein assay b) Histopath at 72h post-last dosing (5 mice) and 7 days post-last dosing (5 mice)
2	RTX00001 (95% ONAT-HA + 5% luciferase mRNA)	Albino B6', 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% ONAT-HA + 5% luciferase mRNA	Albino B6', 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% ONAT-HA + 5% luciferase mRNA	Albino B6', 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
Groups 3 and 4

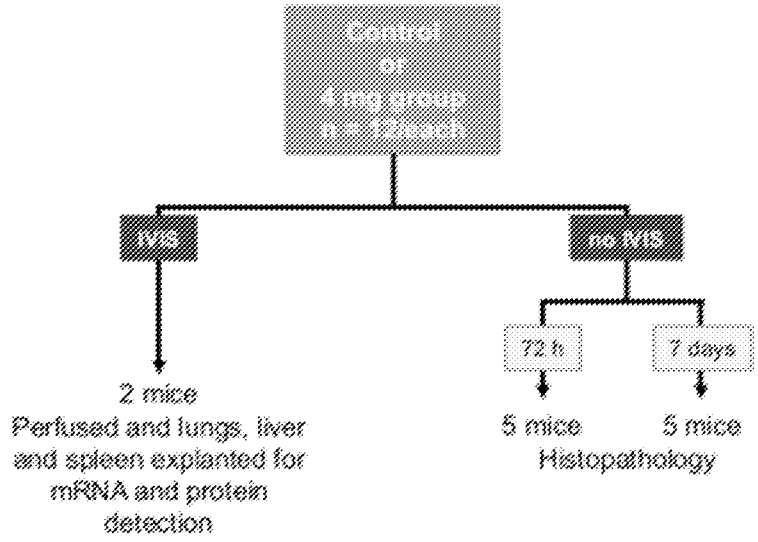
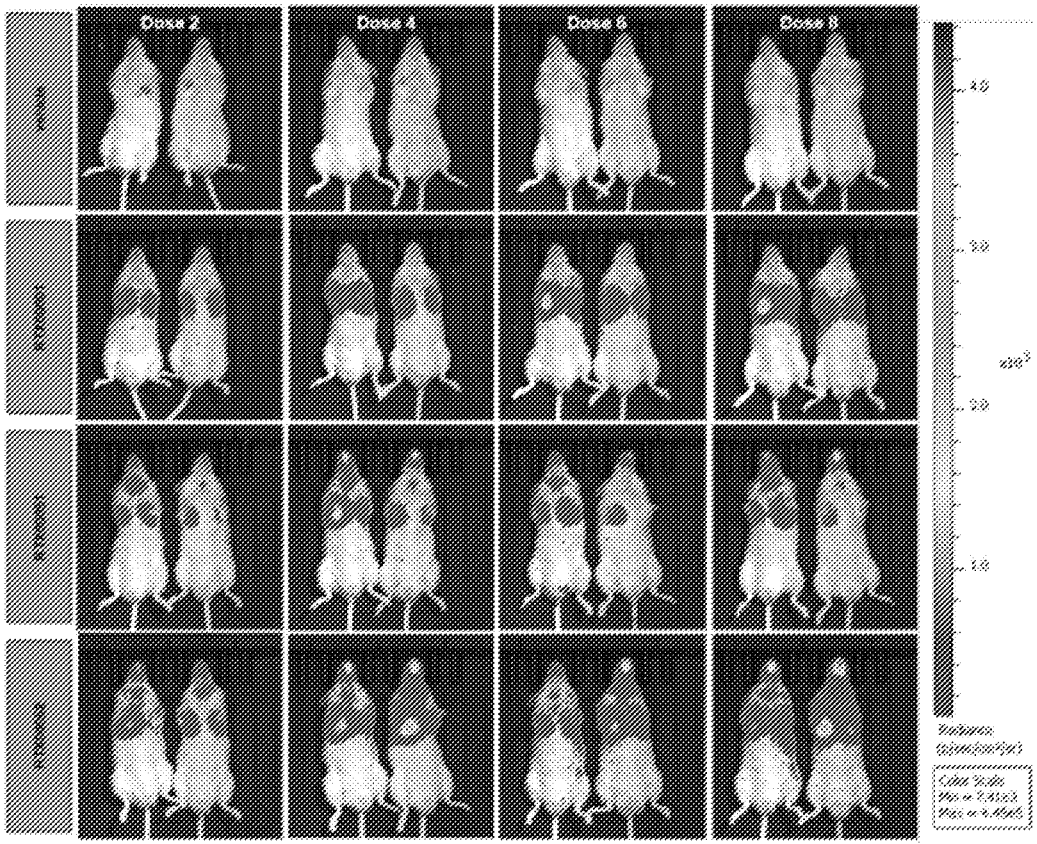

Control
or
4 mg group
n = 12/each
IVIS
no IVIS
72 h
7 days
2 mice
Perfused and lungs, liver and spleen explanted for mRNA and protein detection
5 mice
5 mice
Histopathology

Fig. 27A


Dose 2
Dose 4
Dose 6
Dose 8

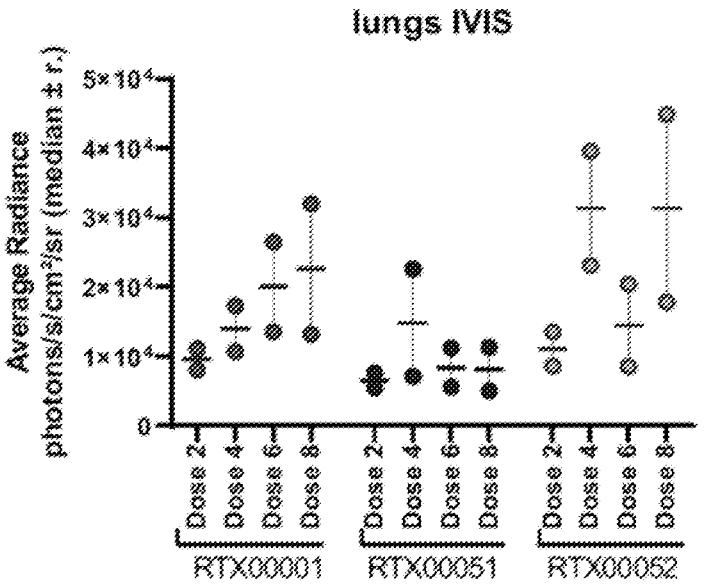
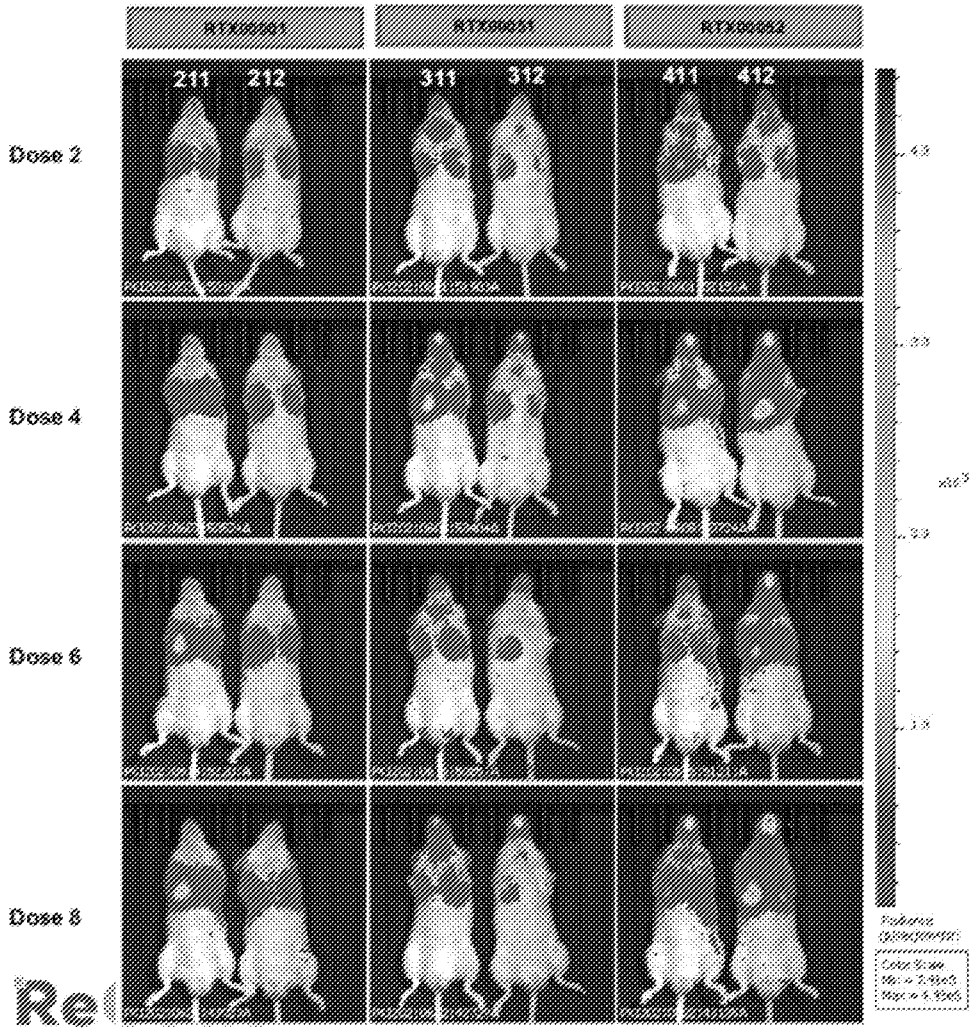

lungs IVIS
Average Radiance
photons/s/cm²/sr (median ± r.)
5×10⁴
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
Dose 2
Dose 4
Dose 6
Dose 8
RTX00001
RTX00051
RTX00052

Fig. 27B


211
212
311
312
411
412
Dose 2
Dose 4
Dose 6
Dose 8

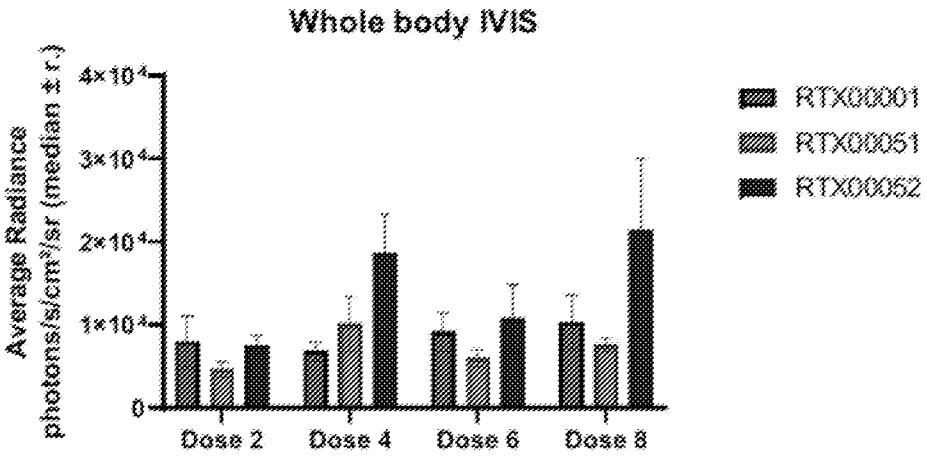

Whole body IVIS
Average Radiance
photons/s/cm²/sr (median ± r.)
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
Dose 2
Dose 4
Dose 6
Dose 8
RTX00001
RTX00051
RTX00052

Fig. 27C

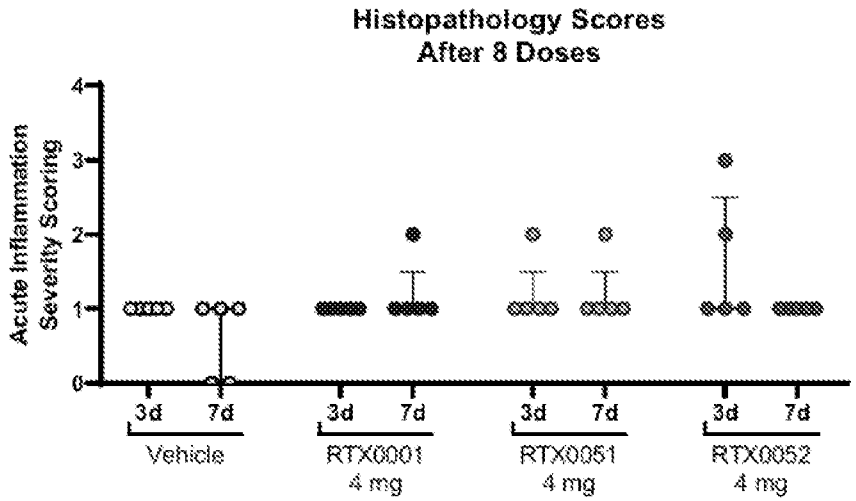


Fig. 27D

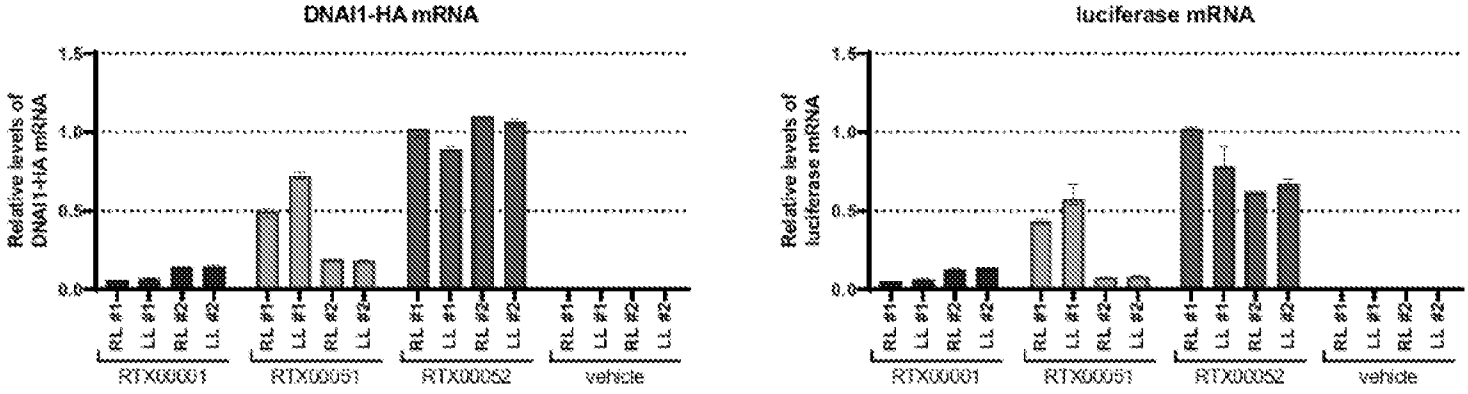


Fig. 27E

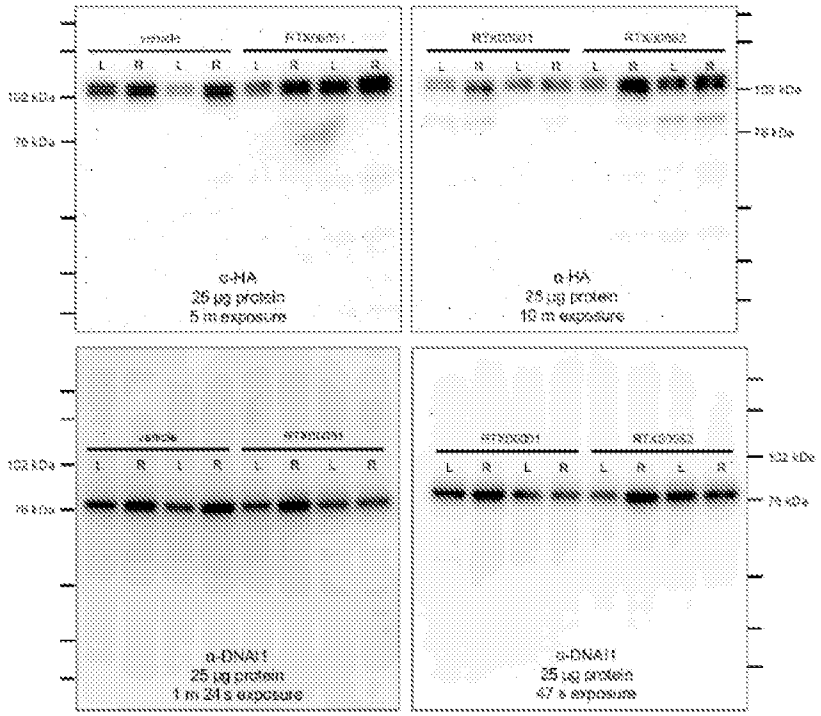


Fig. 28A

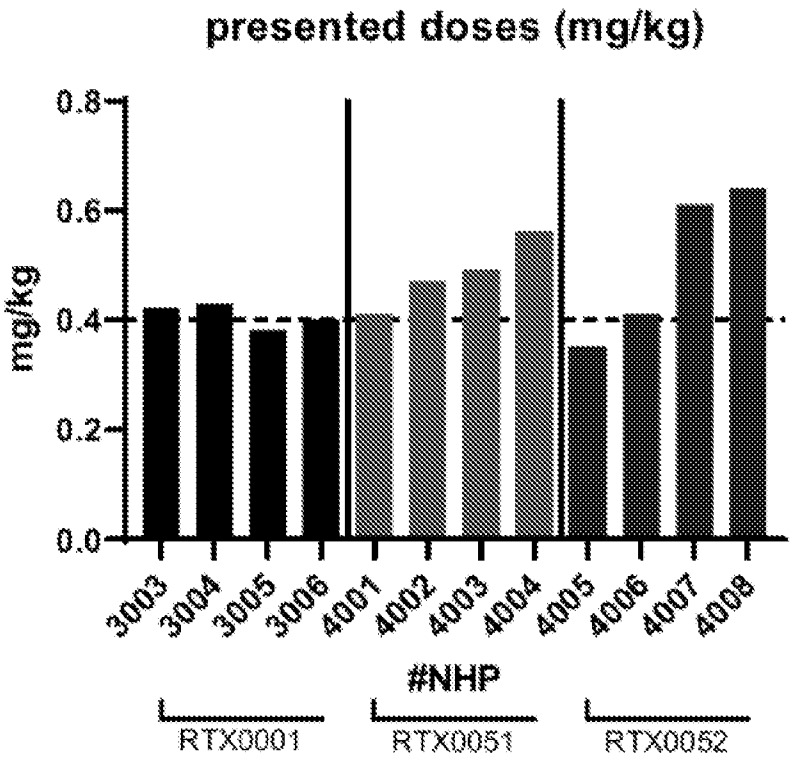
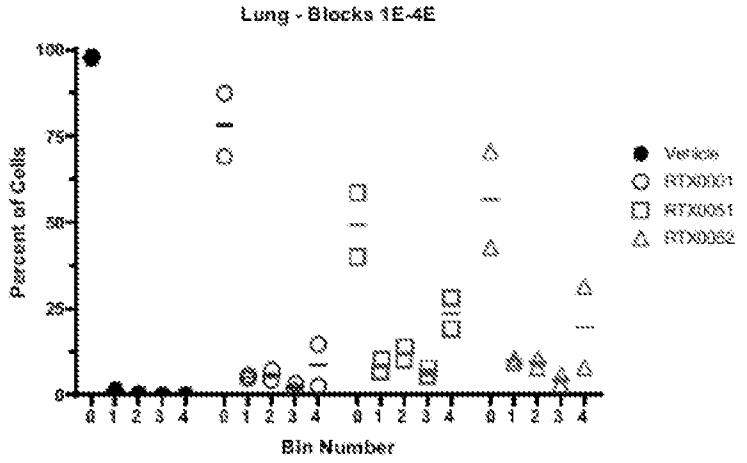


Fig. 28B

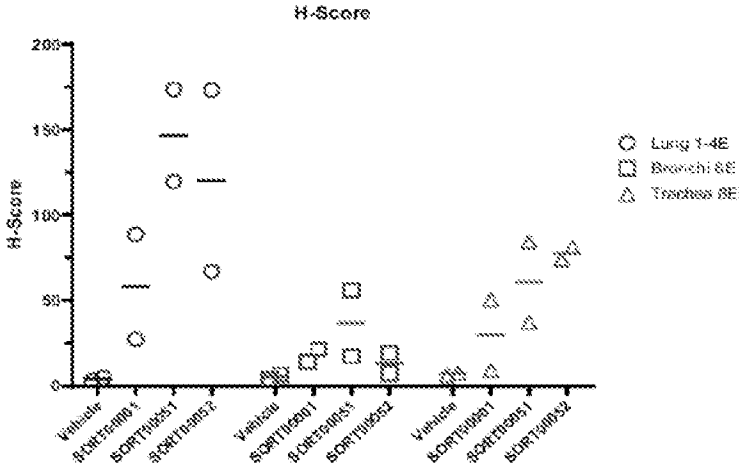
	APS (Aerodynamic particle sizer)				Impactor (Mercer)	
	MMAD (μm)		GSD		MMAD (μm)	GSD
	Pre	Post	Pre	Post		
RTX0001	2.59	2.21	1.69	2.23	2.55	1.86
RTX0051	3.33	1.95/2.16	1.93	1.77/2.22	n.d.	n.d.
RTX0052	2.96	2.99	1.83	1.86	2.57	1.80

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

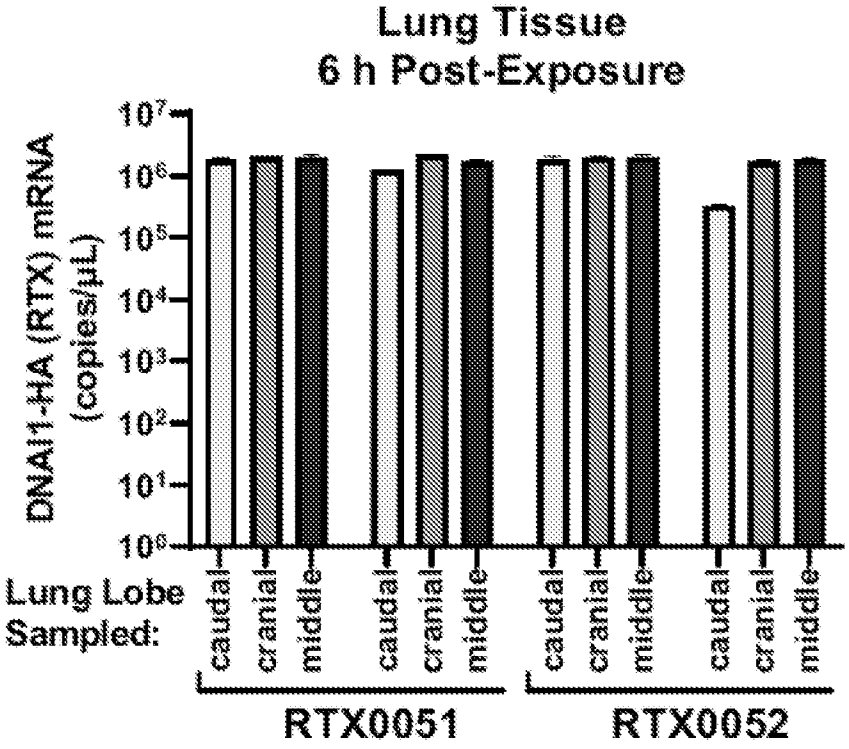


Fig. 29B

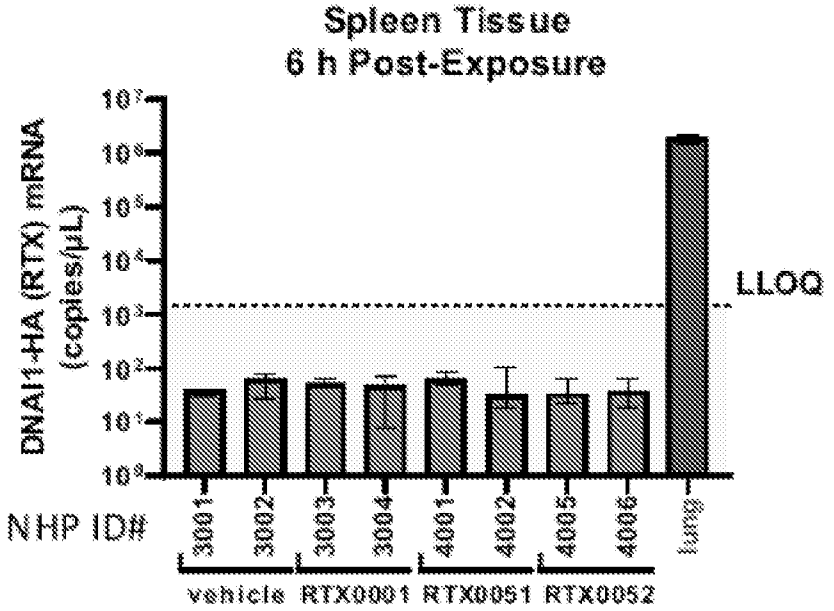


Fig. 29C

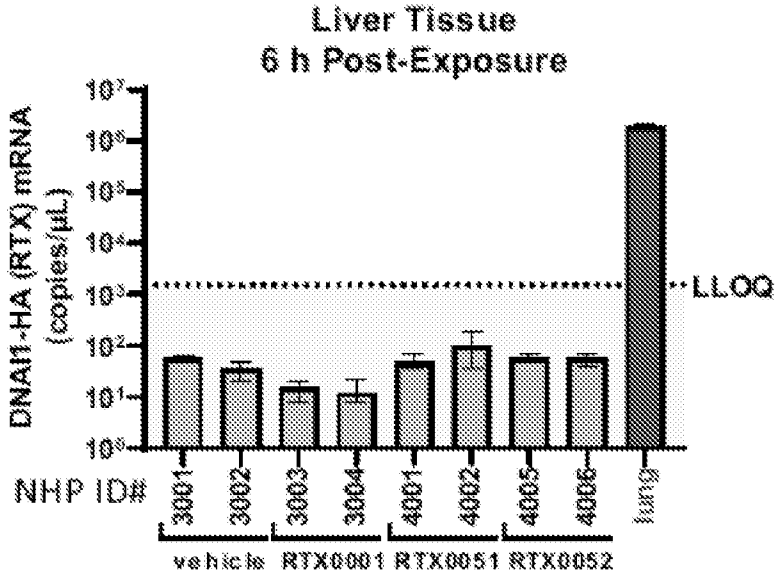


Fig. 29D

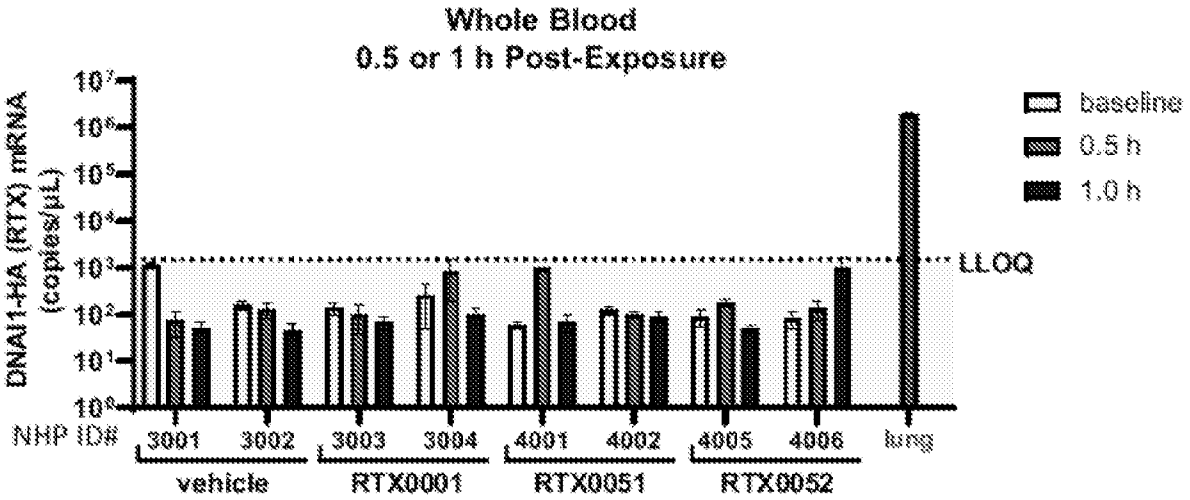


Fig. 30A

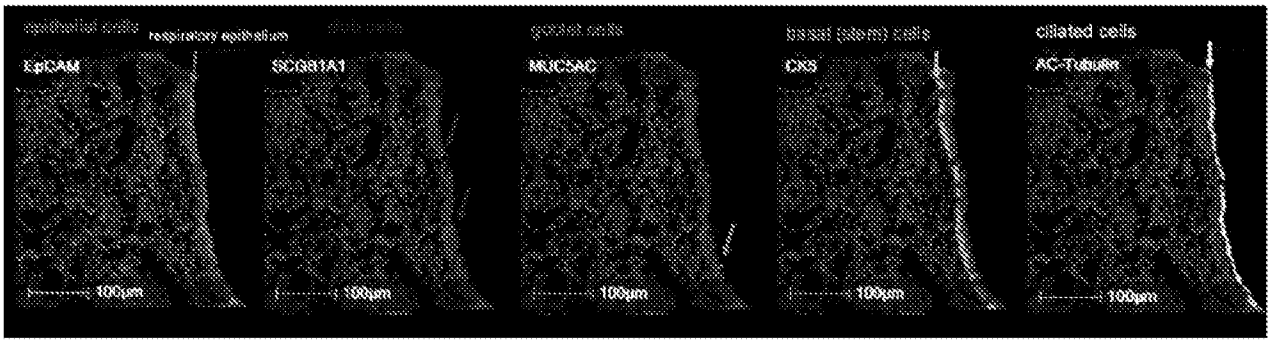


Fig. 30B

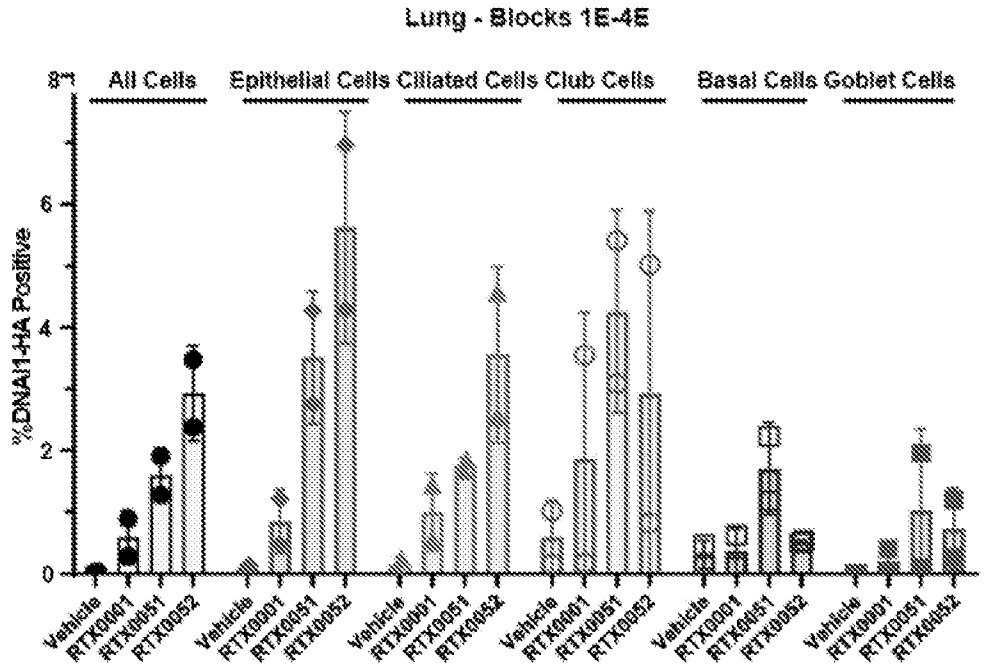


Fig. 30C

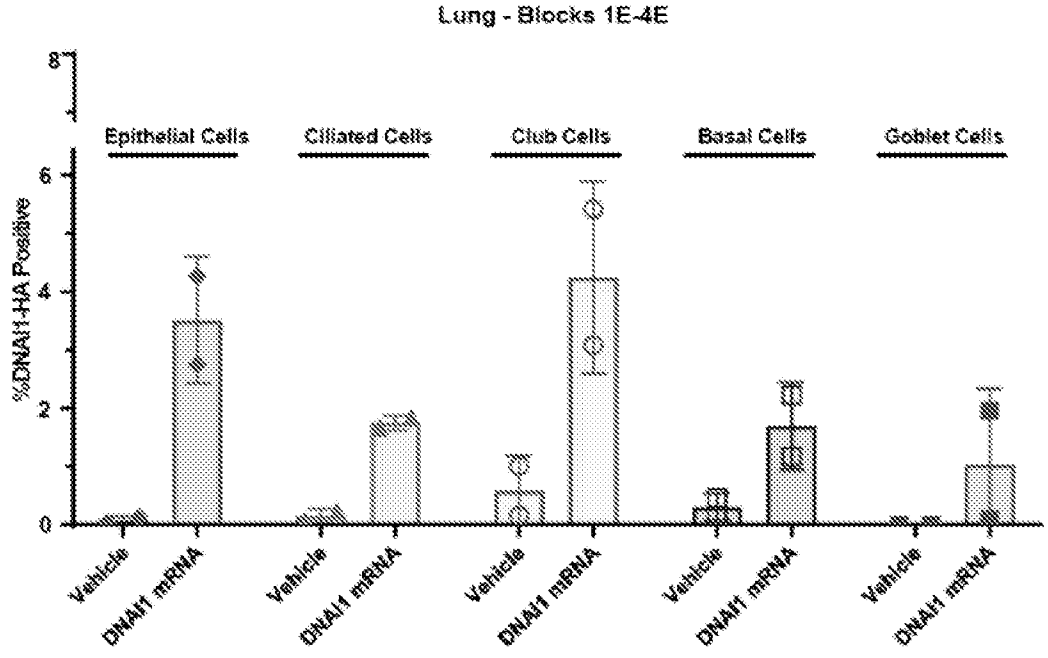


Fig. 30D

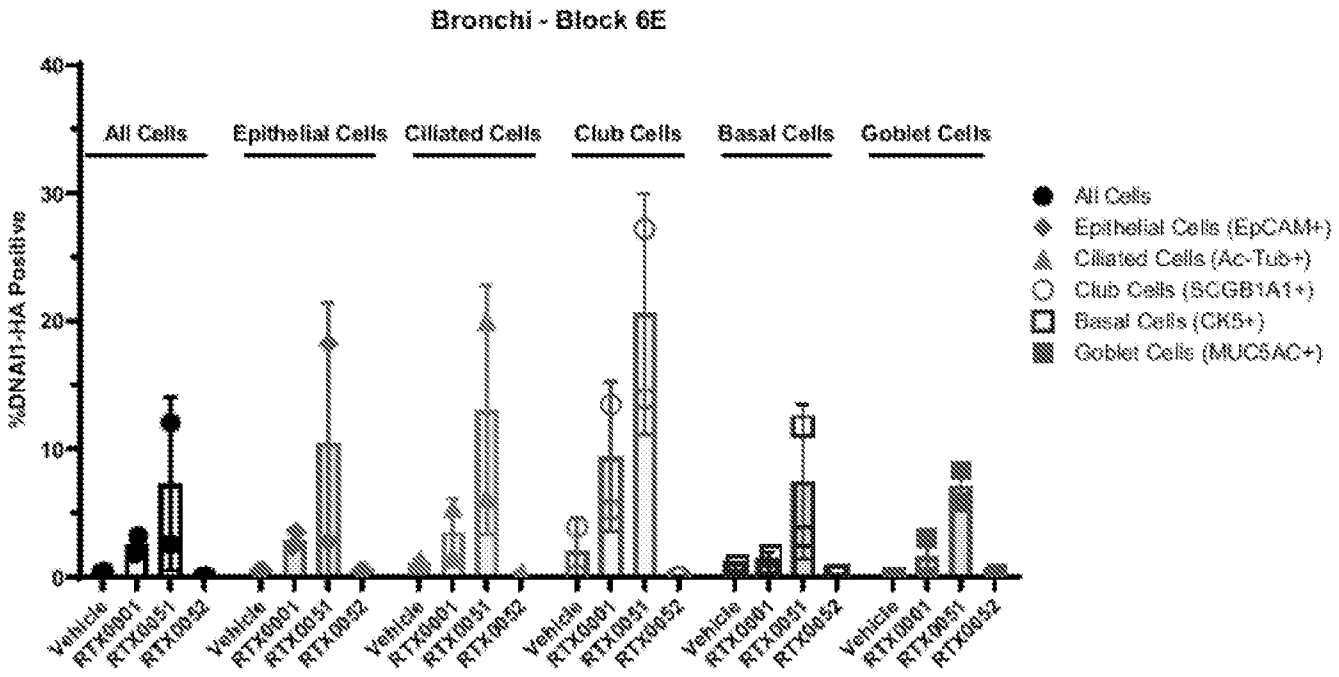


Fig. 30E

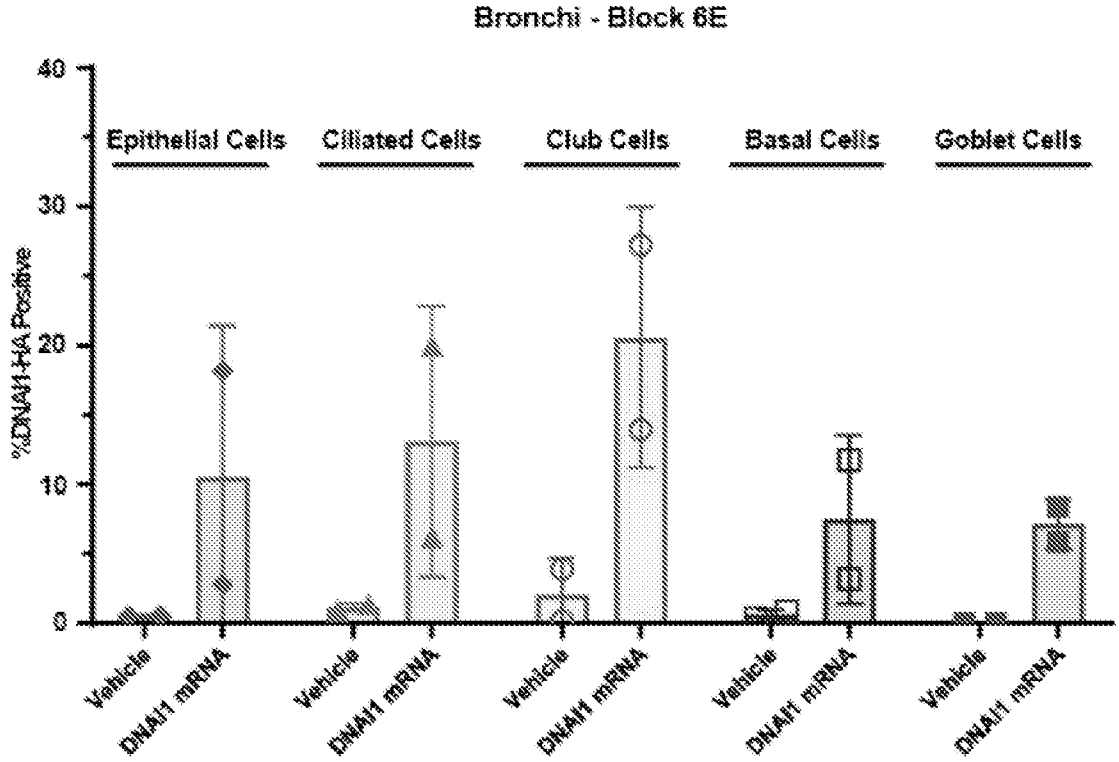
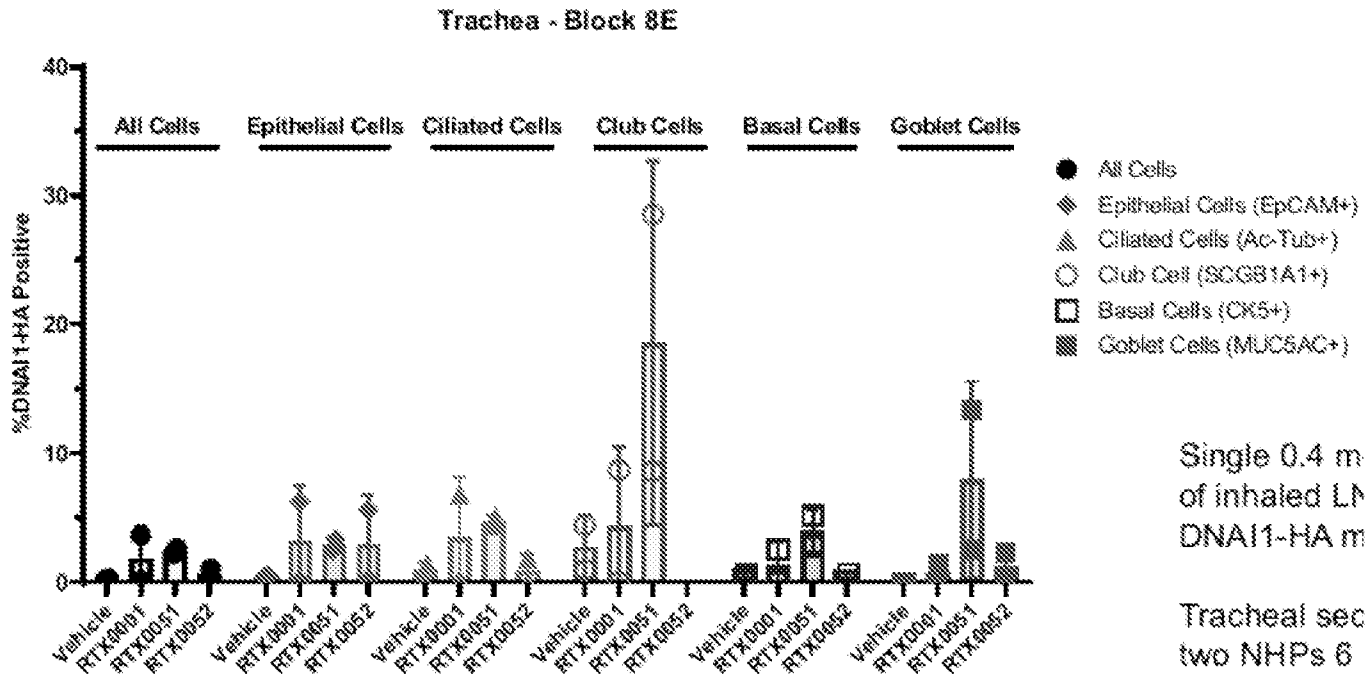


Fig. 30F



Single 0.4 m
of inhaled LN
DNAI1-HA m

Tracheal sec
two NHPs 6

Fig. 31A

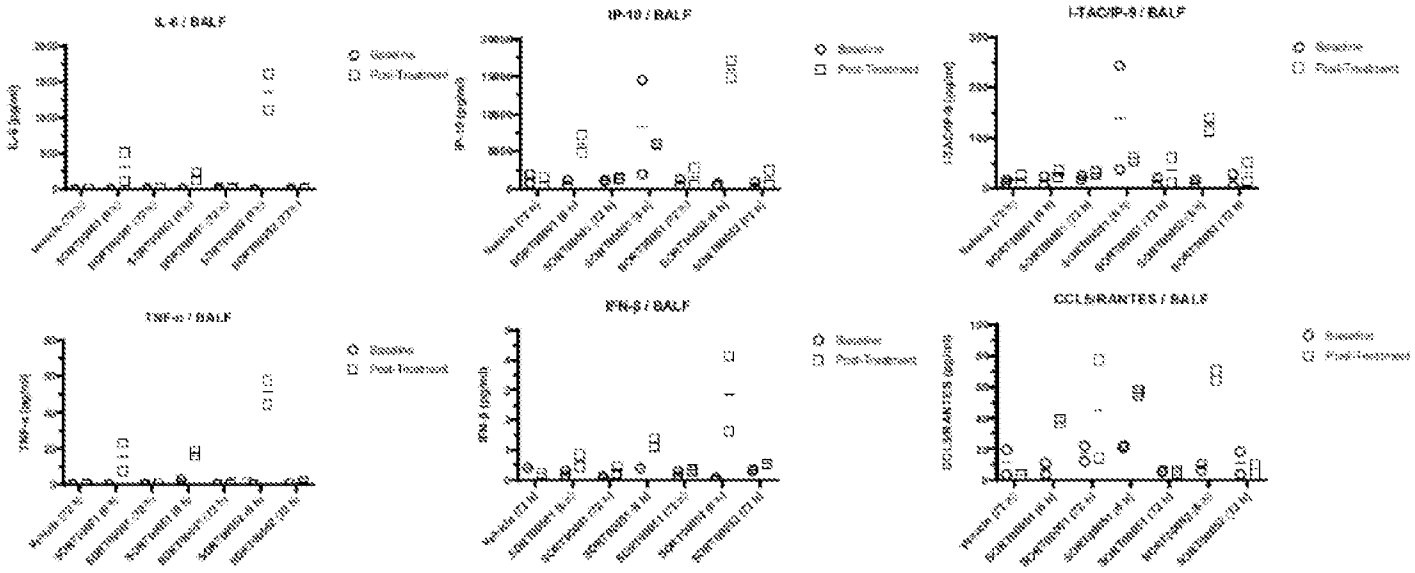


Fig. 31B

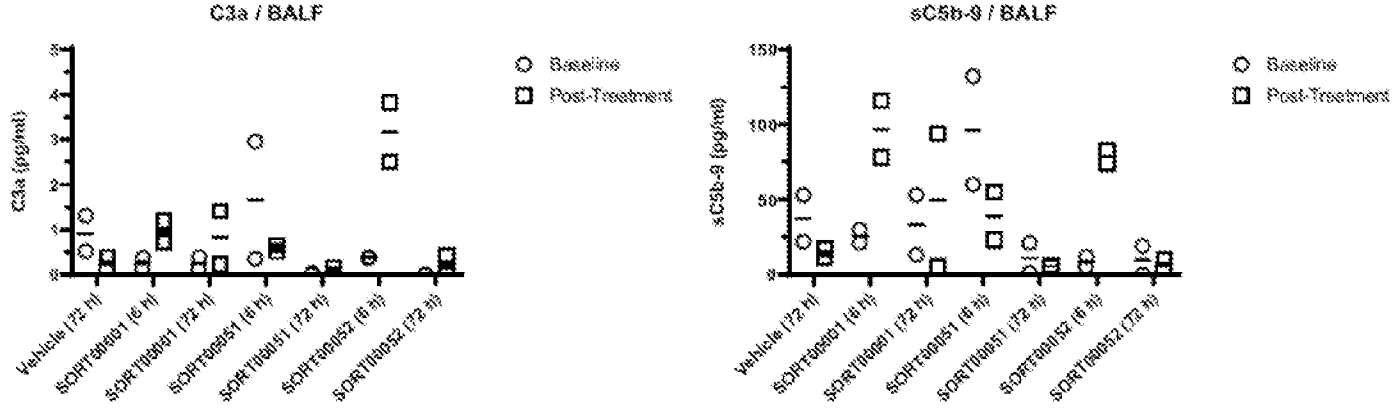


Fig. 31C

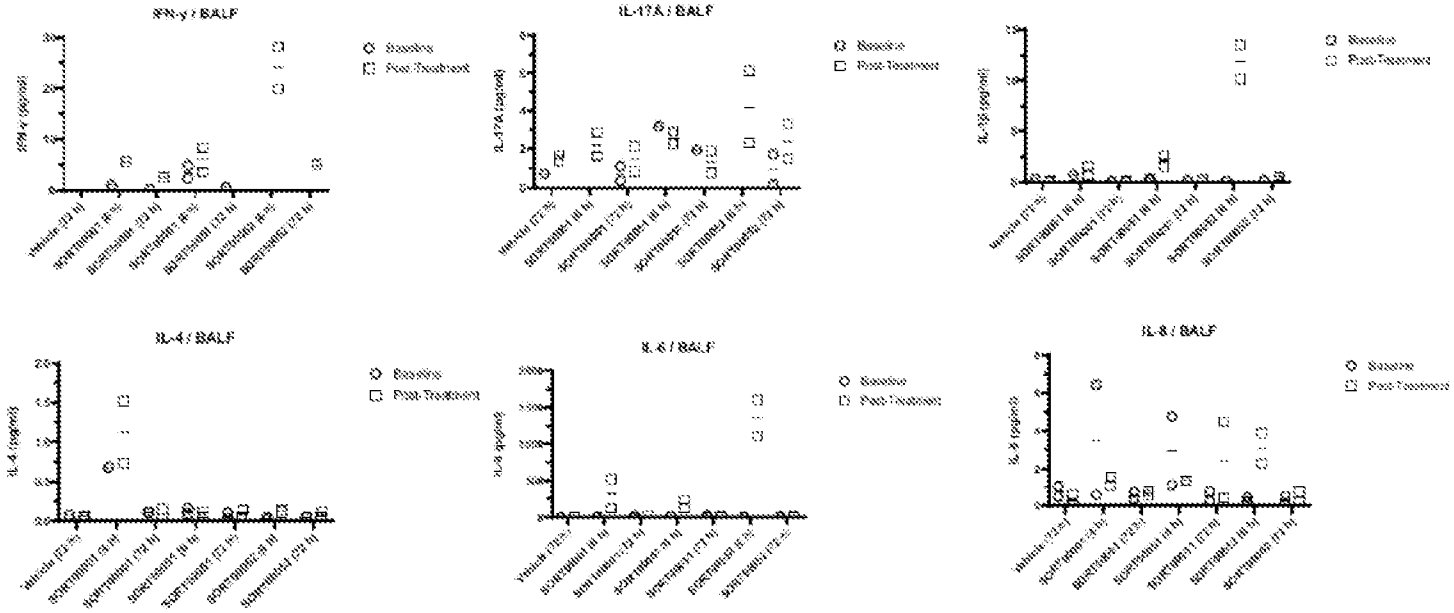


Fig. 31D

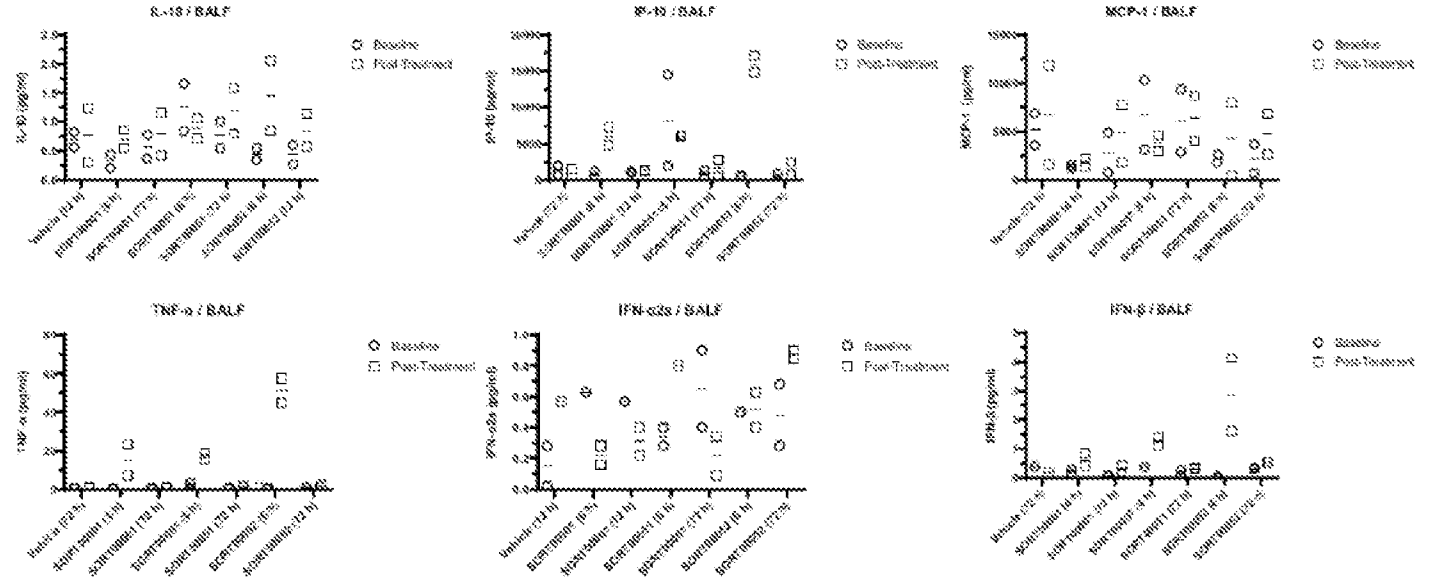


Fig. 31E

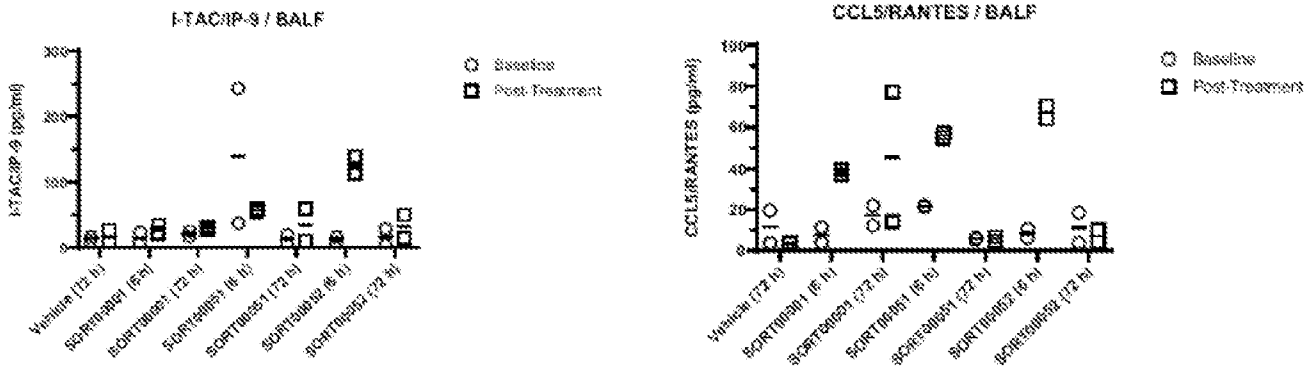


Fig. 32A

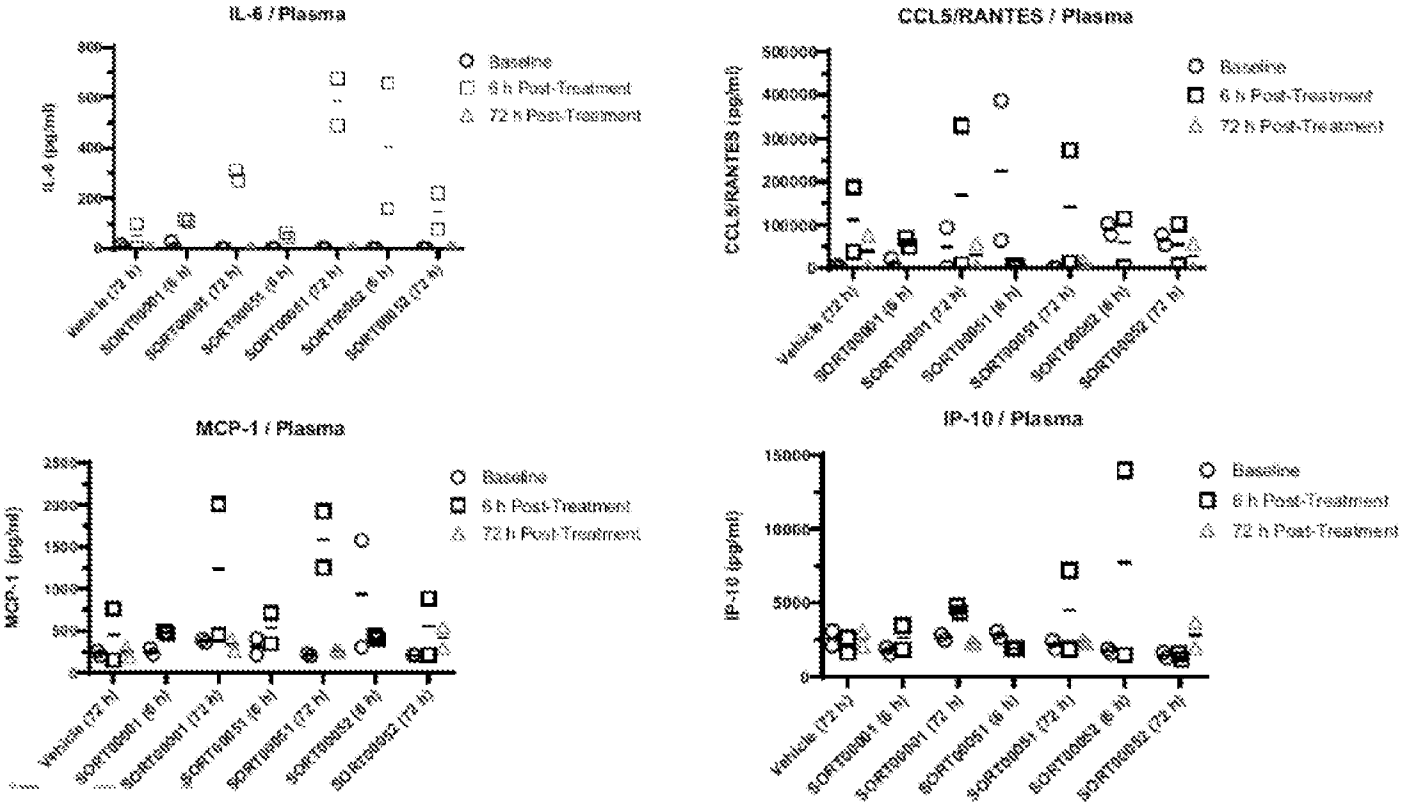


Fig. 32B

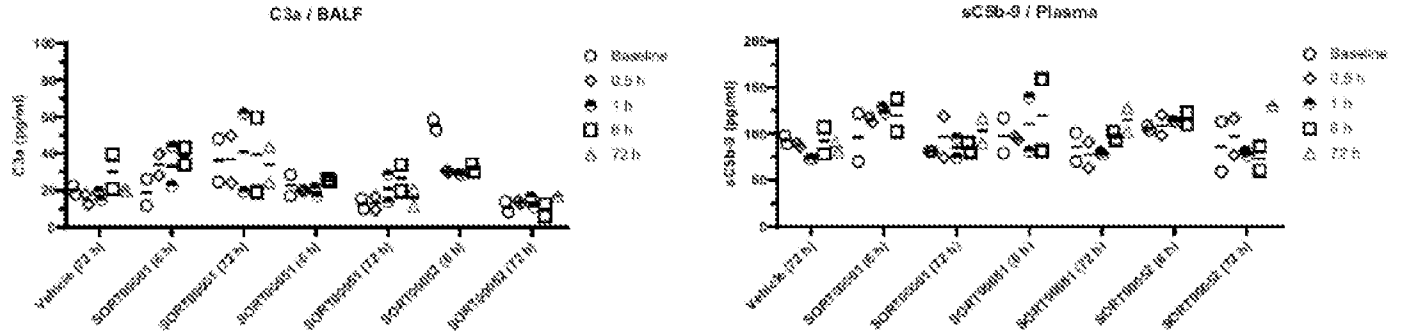


Fig. 32C

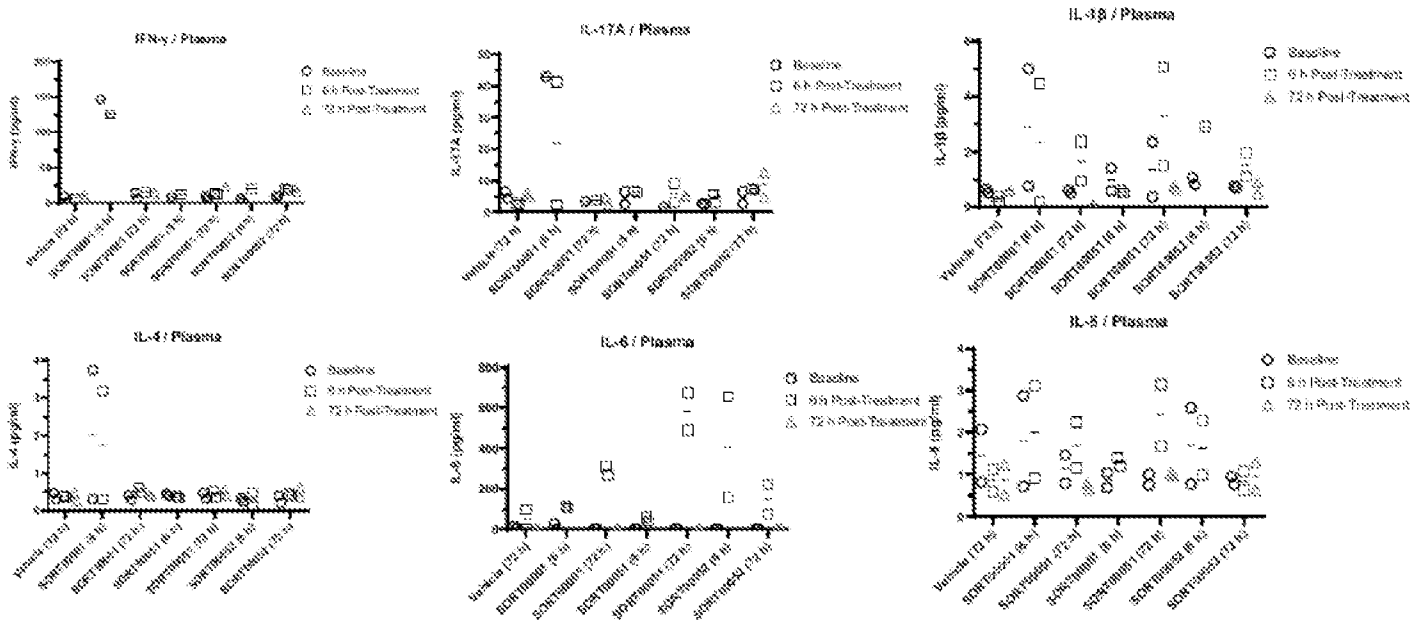


Fig. 32D

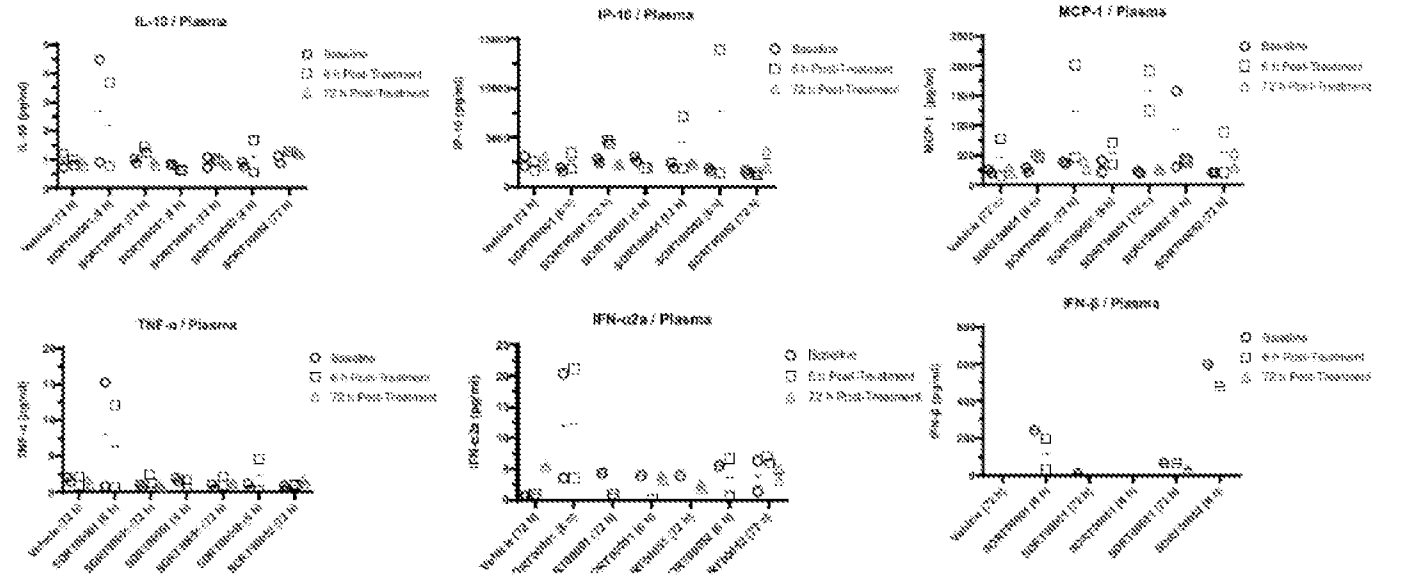


Fig. 32E

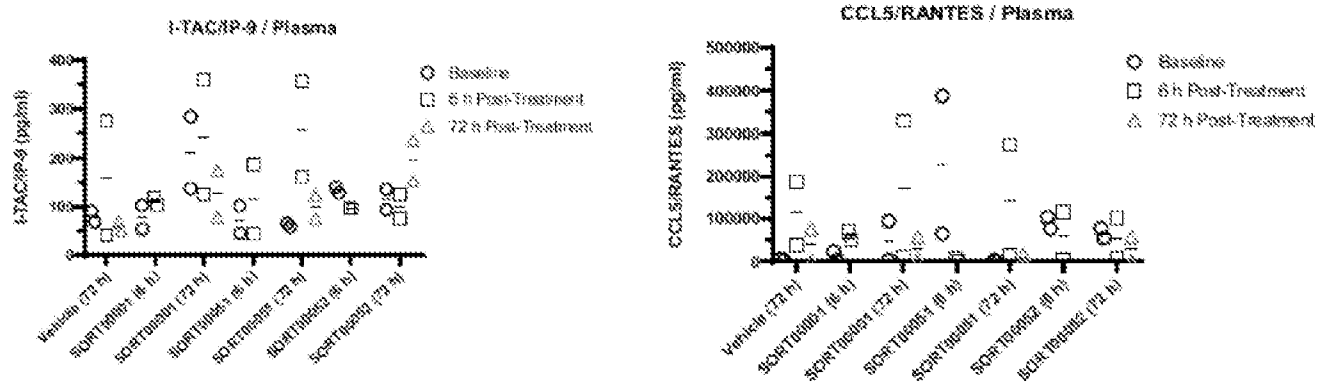


Fig. 33

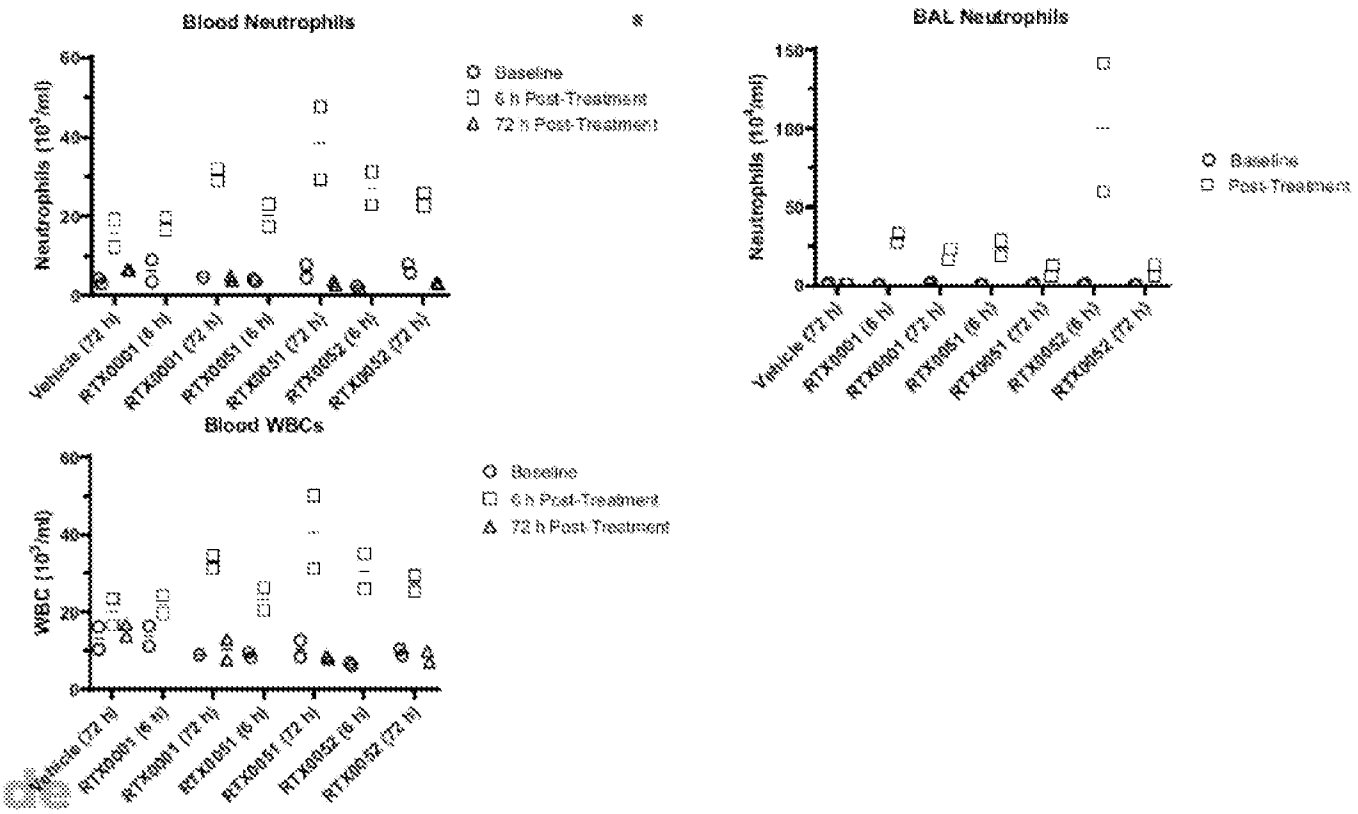
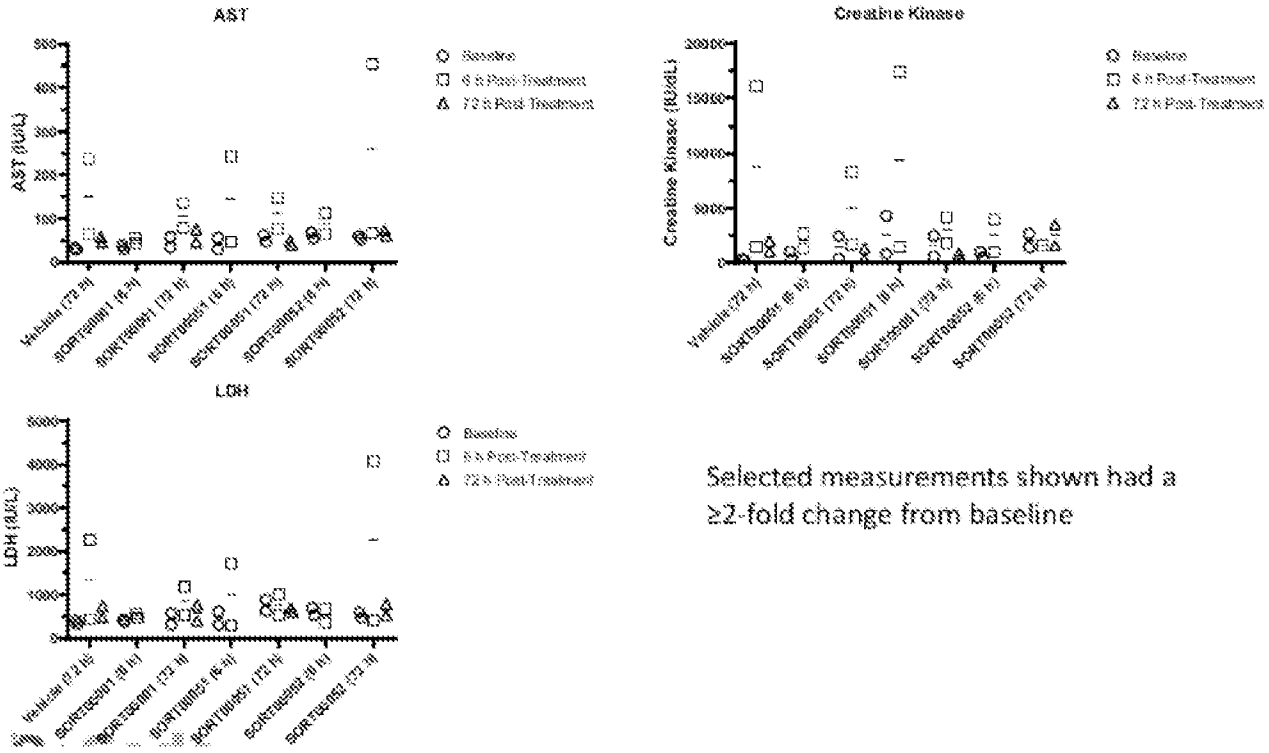

Blood Neutrophils
Neutrophils (10³/ml)
Baseline
6 h Post-Treatment
72 h Post-Treatment
Vehicle (72 h)
RTX00051 (6 h)
RTX00051 (72 h)
RTX00051 (6 h)
RTX00051 (72 h)
RTX00052 (6 h)
RTX00052 (72 h)
BAL Neutrophils
Neutrophils (10³/ml)
Baseline
Post-Treatment
Blood WBCs
WBC (10³/ml)
Baseline
6 h Post-Treatment
72 h Post-Treatment

Fig. 34



Selected measurements shown had a ≥2-fold change from baseline

Fig. 35

6 hr Necropsy Scores

6 HRS	01		S1		S2	
	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		S1		S2	
	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

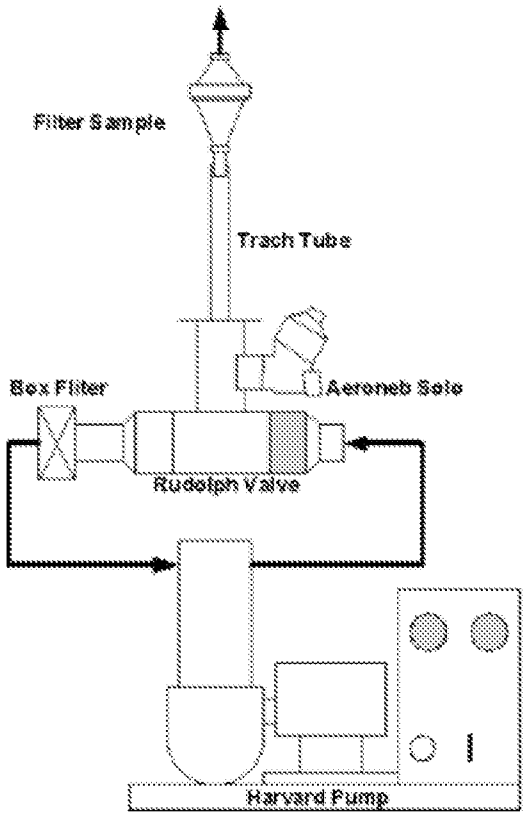
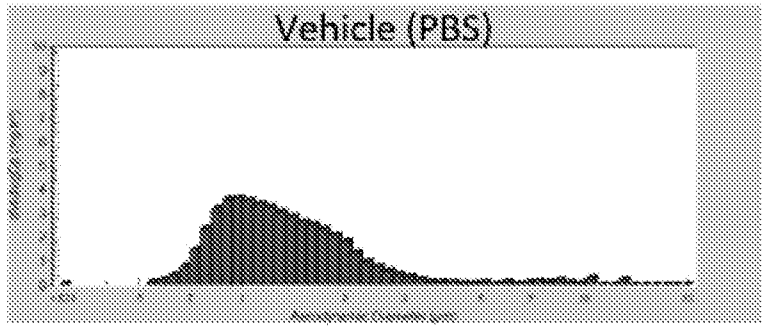
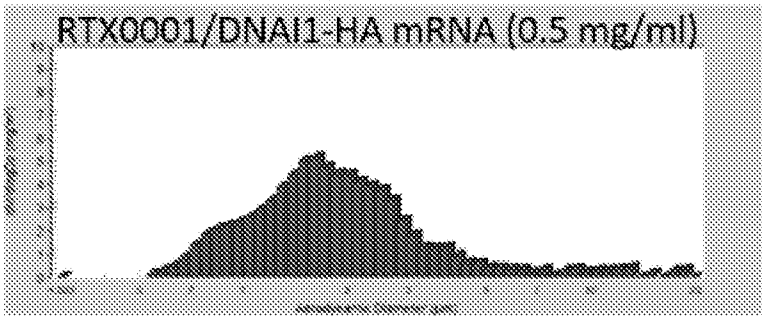


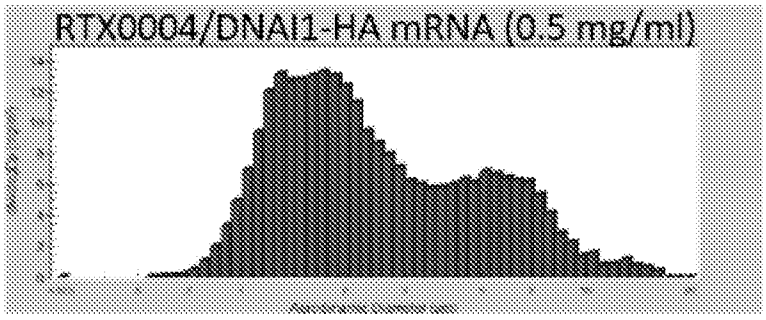
Fig. 36B



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%

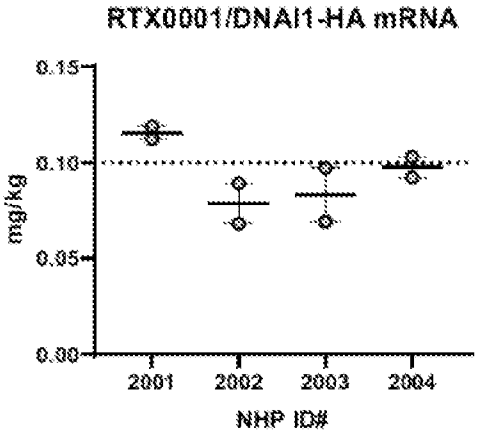


APS		
Sample	MMAD (µm)	GSD
1	1.72	1.82
2	1.72	1.76
3	1.78	1.83
Average	1.74	1.80
Std Dev	0.03	0.04
%RSD	2%	2%



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#

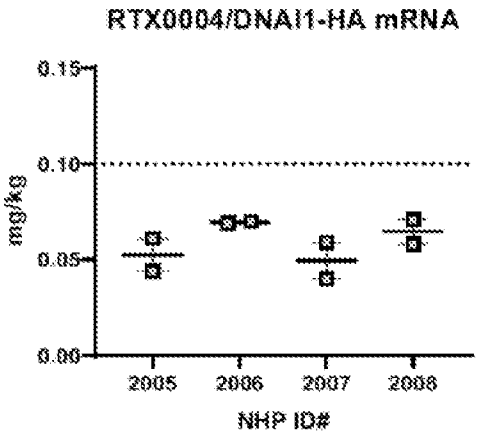
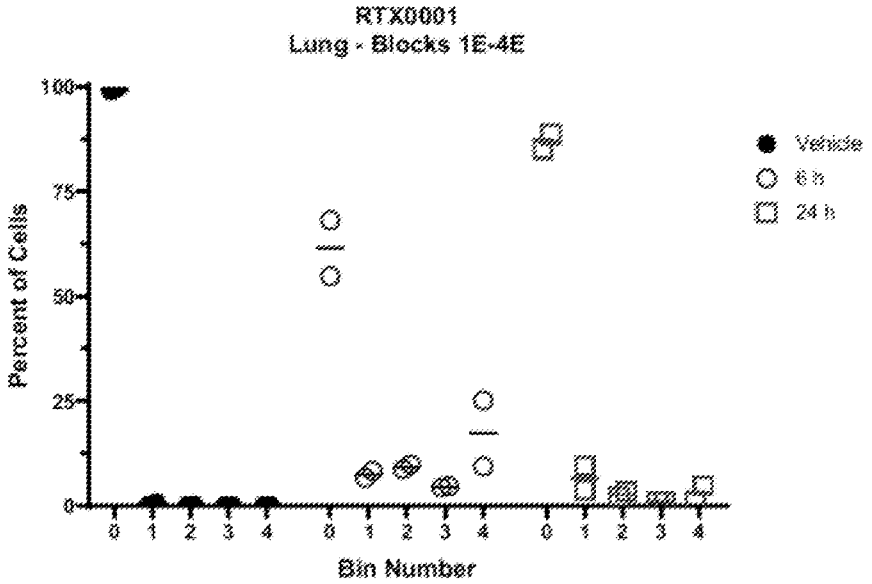

RTX0004/DNAI1-HA mRNA
0.15
0.10
0.05
0.00
mg/kg
2005
2006
2007
2008
NHP ID#

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
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
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

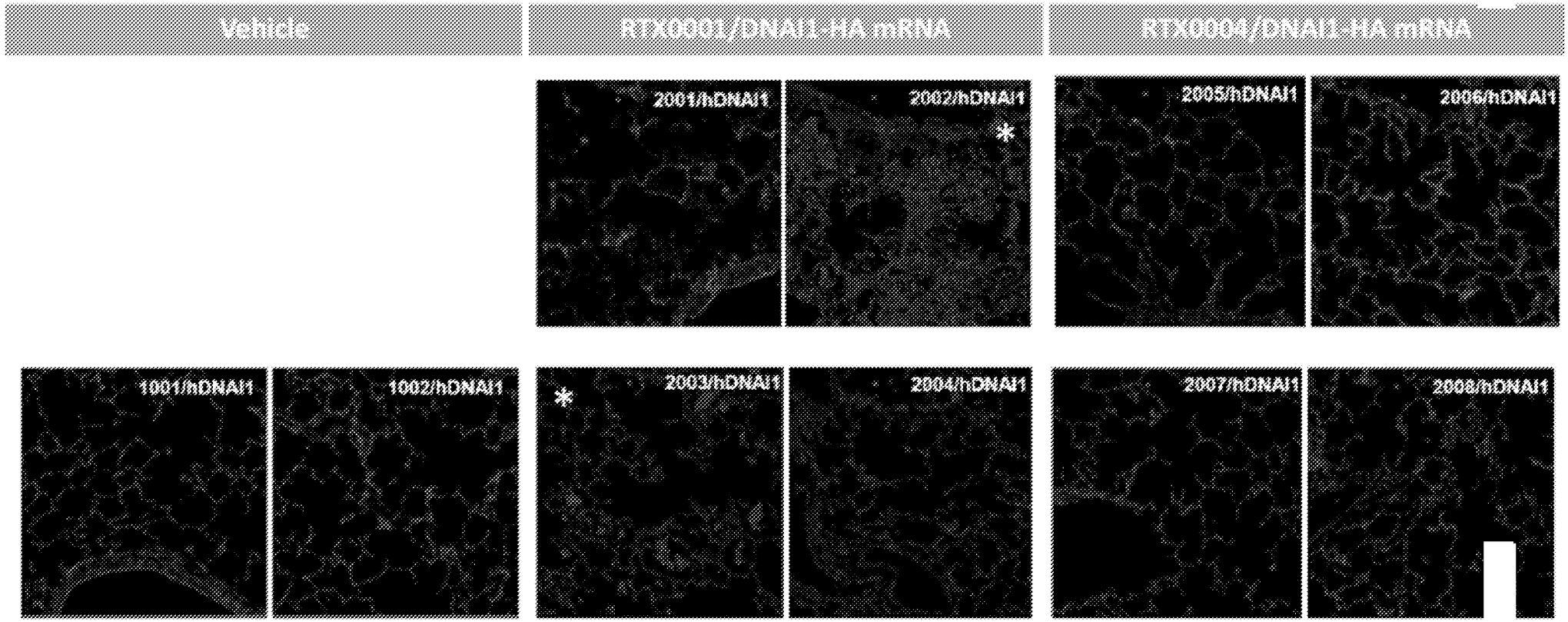

Vehicle
RTX0001/DNAI1-HA mRNA
RTX0004/DNAI1-HA mRNA
6 hrs
24 hrs
2001/hDNAI1
2002/hDNAI1
2005/hDNAI1
2006/hDNAI1
1001/hDNAI1
1002/hDNAI1
2003/hDNAI1
2004/hDNAI1
2007/hDNAI1
2008/hDNAI1
Blue = nuclei (DAPI), Red = DNAI1-HA (anti-HA)

Fig. 39A

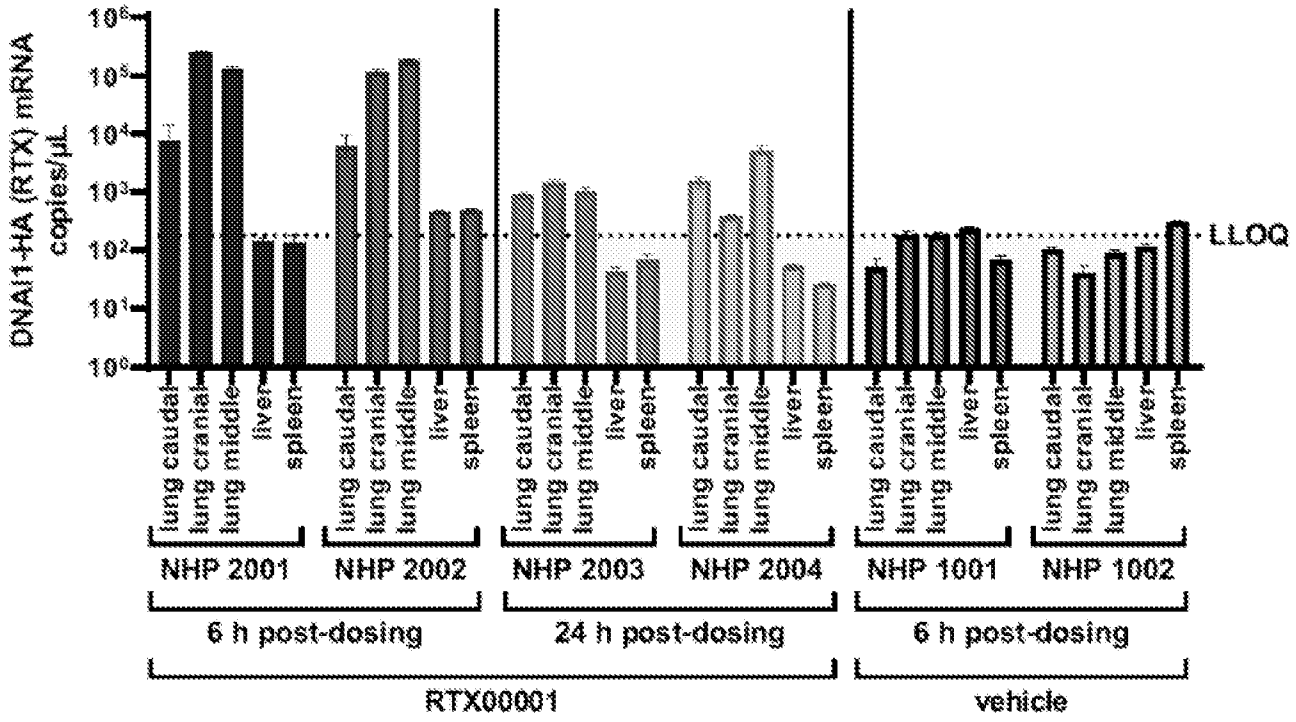


Fig. 39B

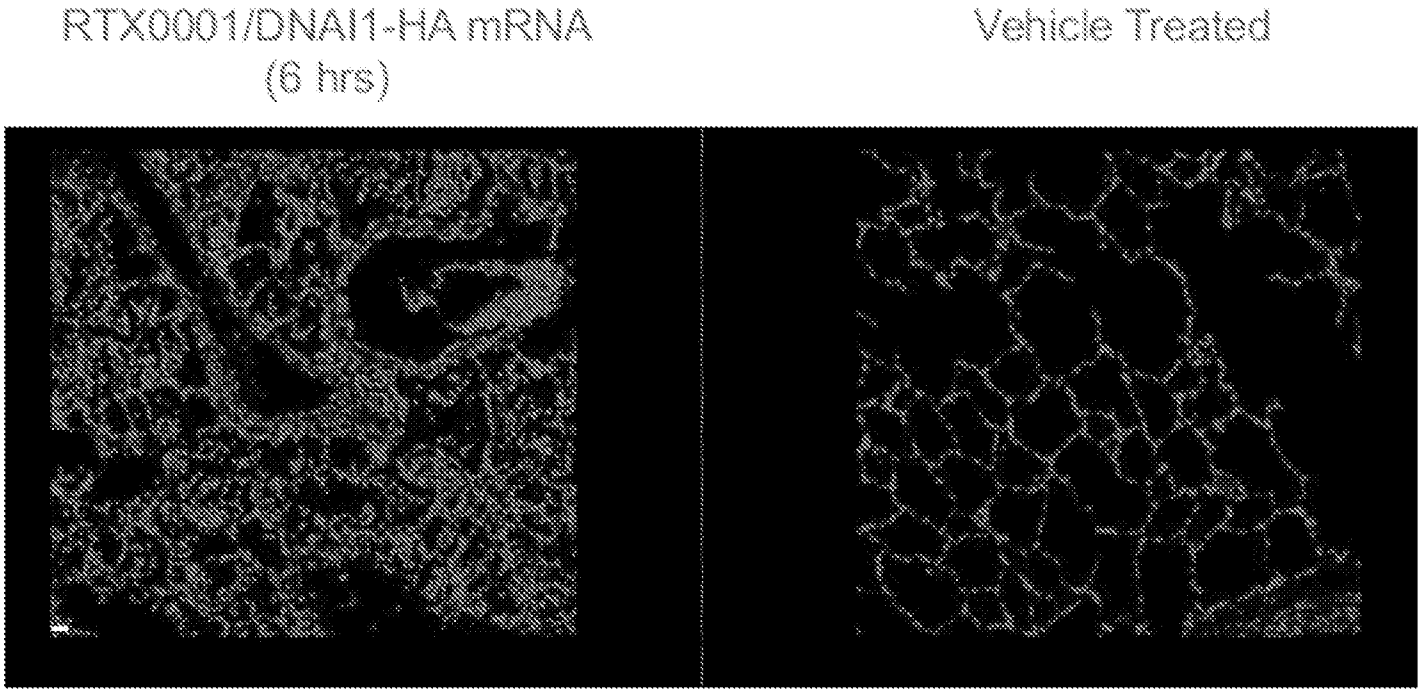


Fig. 39C

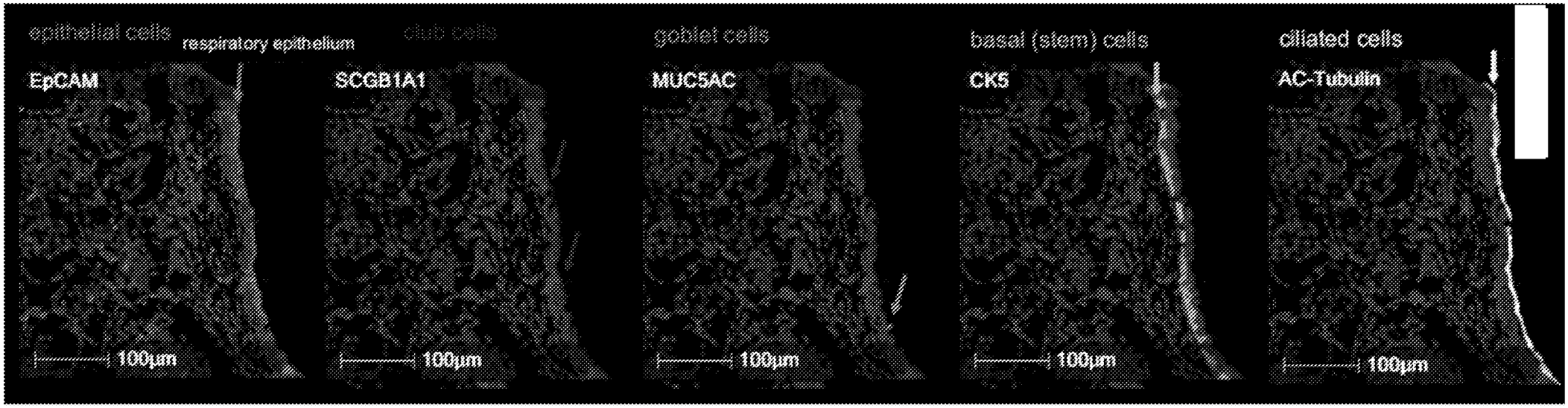

epithelial cells
respiratory epithelium
club cells
goblet cells
basal (stem) cells
ciliated cells
EpCAM
SCGB1A1
MUC5AC
CK5
AC-Tubulin
100µm
100µm
100µm
100µm
100µm

Fig. 40A

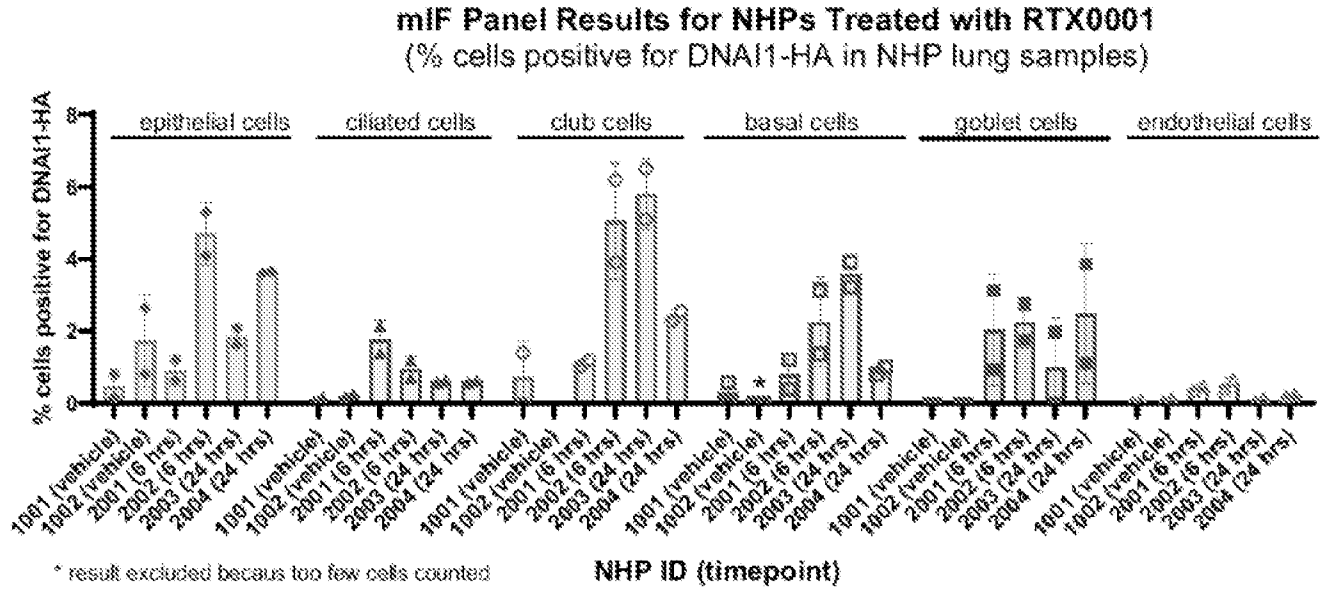


Fig. 40B

