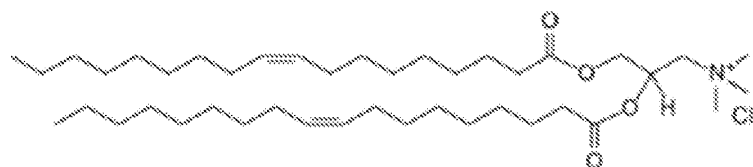
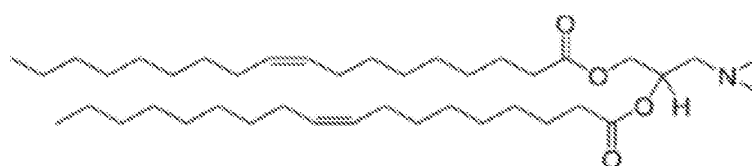


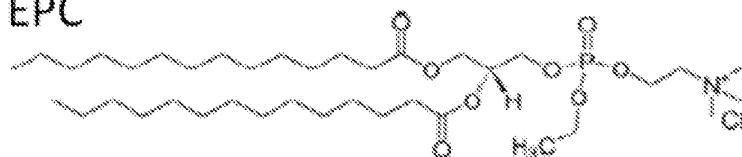
DOTAP



DODAP



EPC



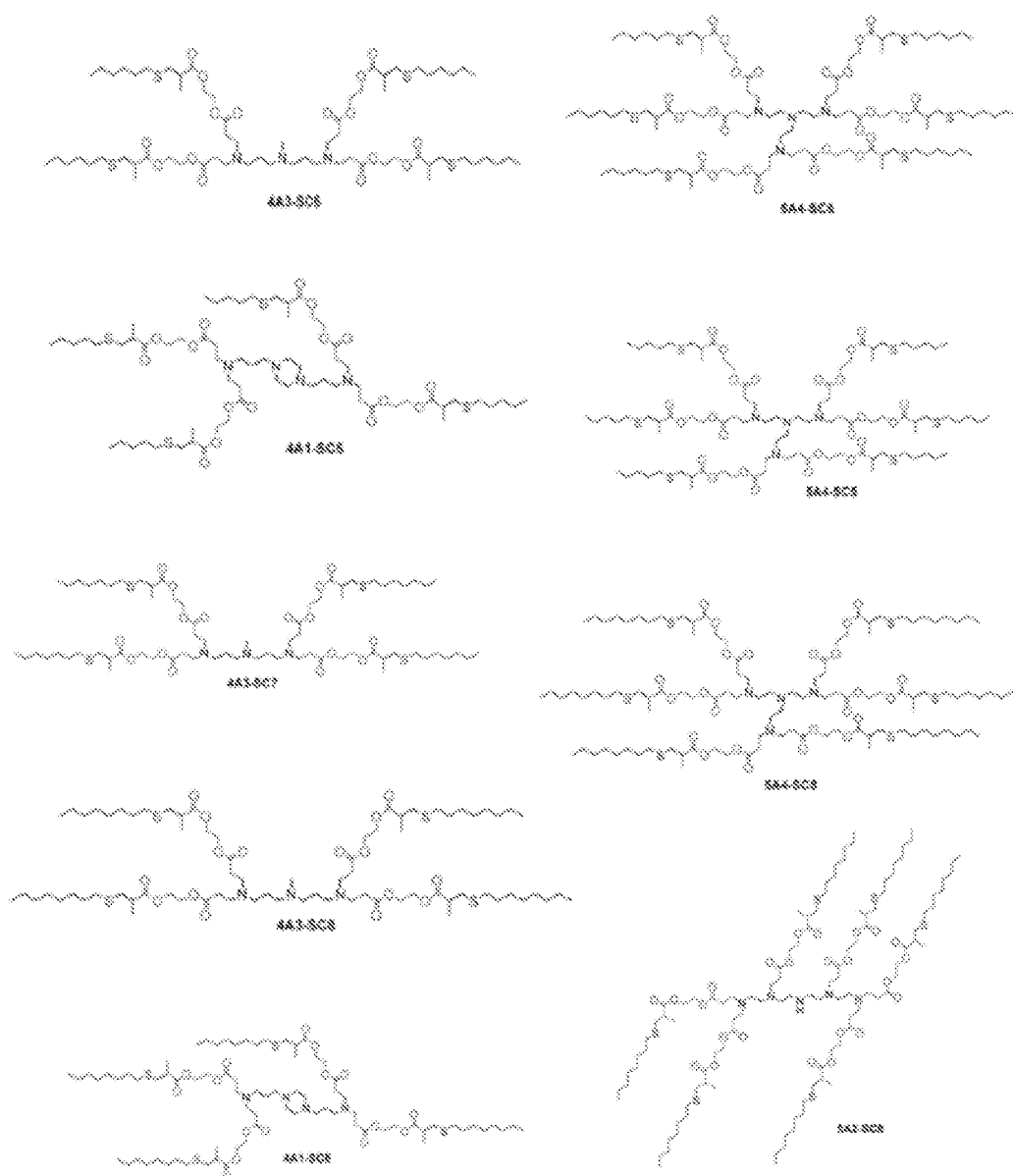
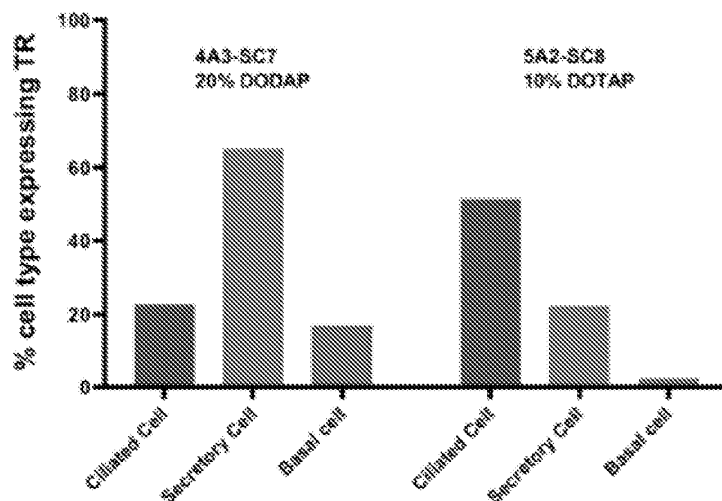


Fig. 3

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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)

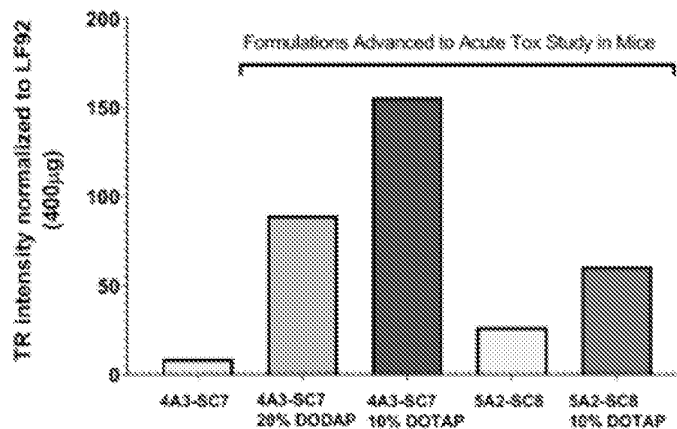
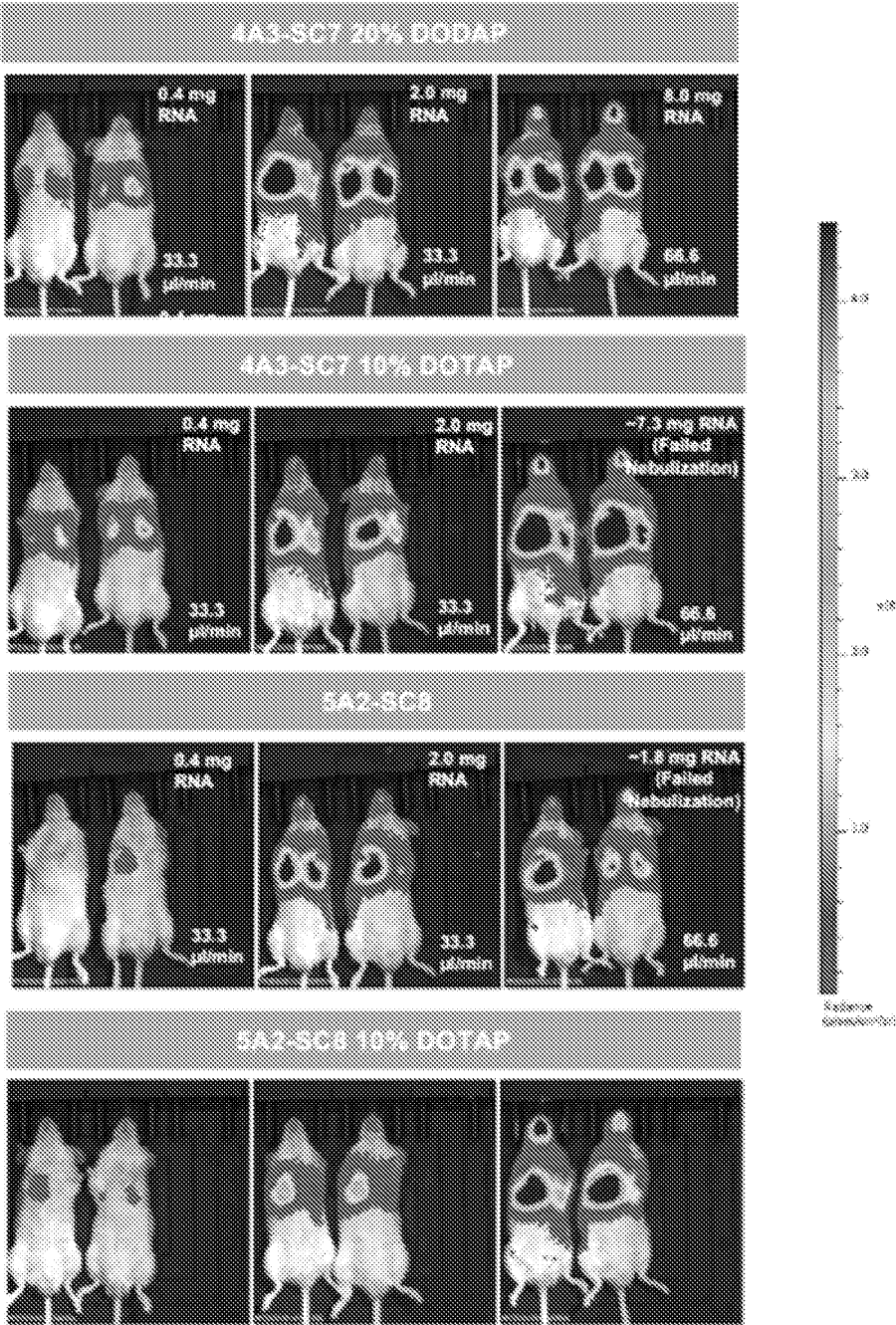


Fig. 4



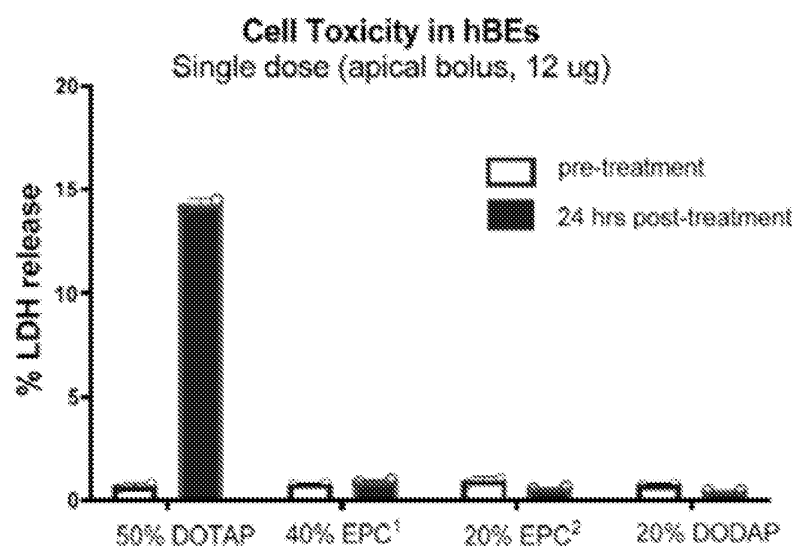


Fig. 6

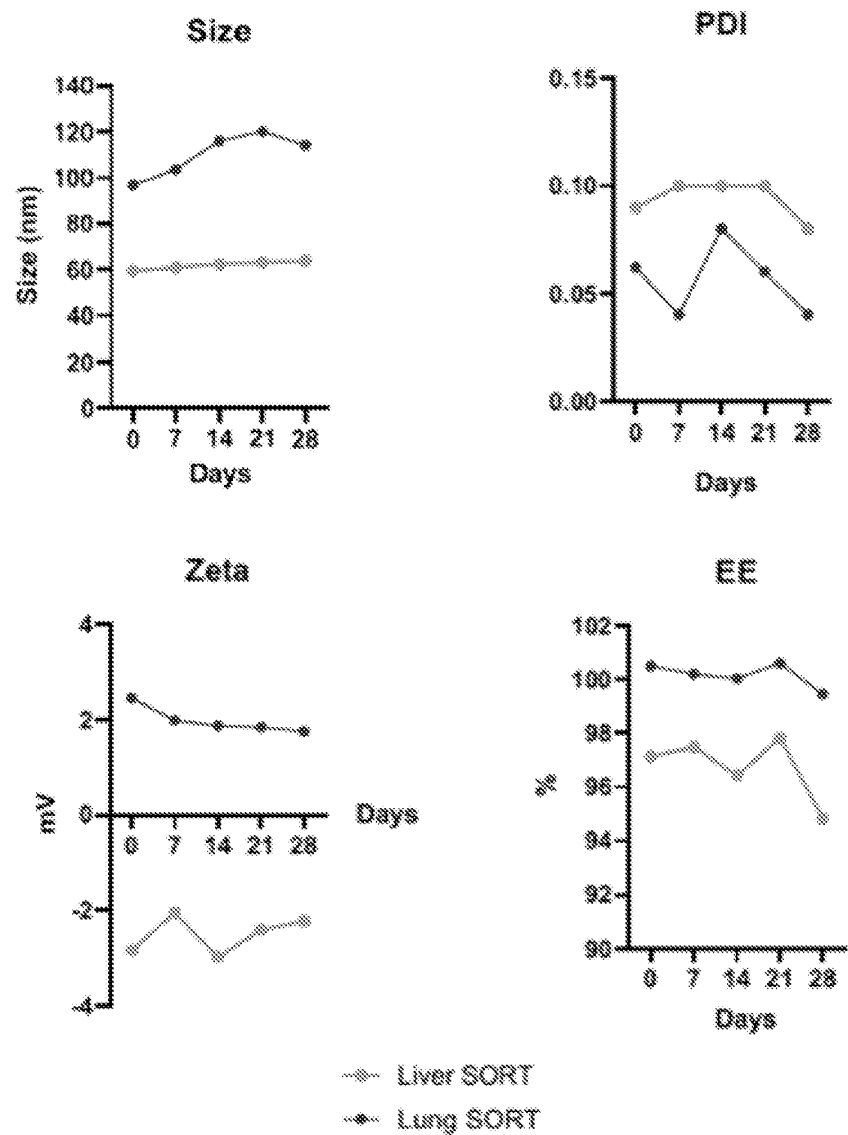


Fig. 7

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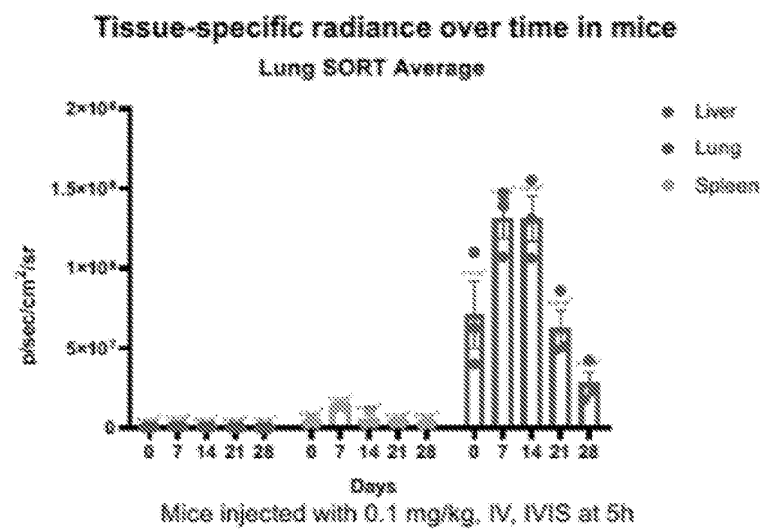


Fig. 8

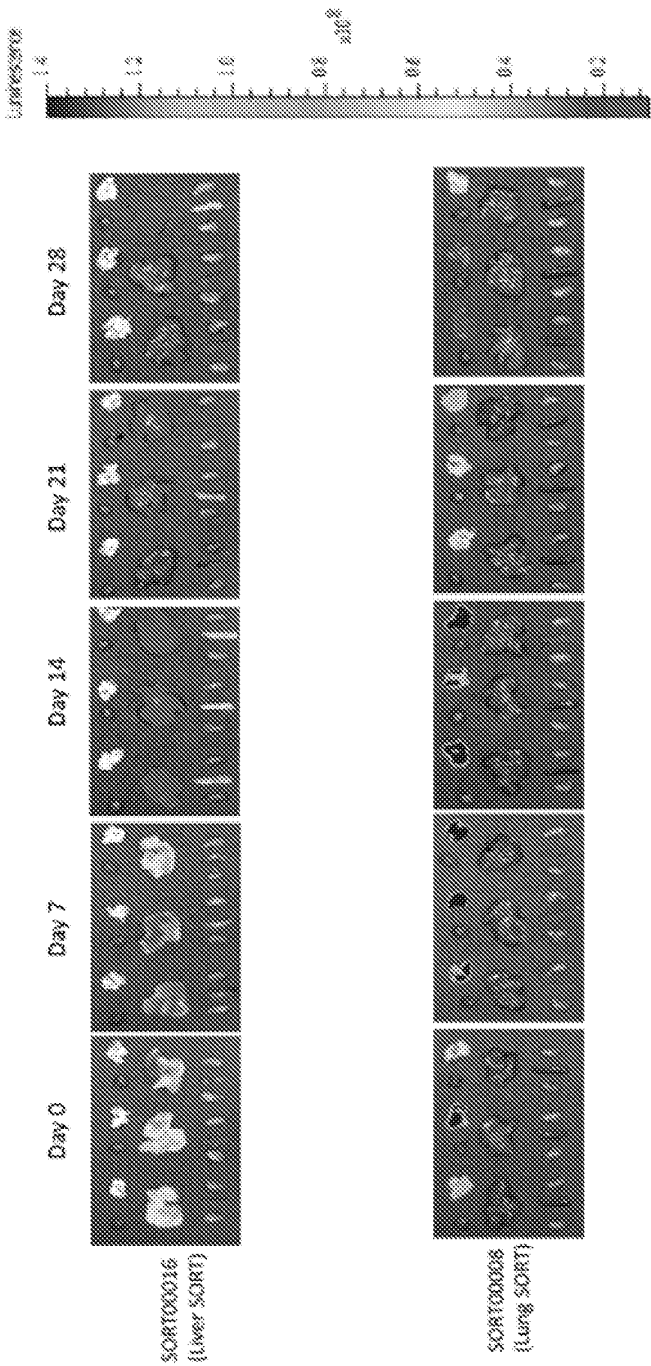


Fig. 9A

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PCT/US2022/021437

Examples

Indication / Inclusion criteria	Phase 1/2 Study	Primary Outcome Measures
PCD Lead mRNA Program - Adults - Pathogenic mutation in DNAAF1 - FEV1 between 40% and 90%	SAD → MAD (6 mo) → DLE (6 mo) Placebo Low Dose High Dose	- Safety and tolerability - Absolute change in percent predicted FEV1

Fig. 9B

PCD Conditional KO Mouse Model* (*Dnaic1^{flx/flx}; CreER^{+/-}* mice)



Mouse Tracheal Epithelial Cells are obtained from harvested tissue, plated on inserts and treated with tamoxifen (TAM) to knock out *Dnaic1**

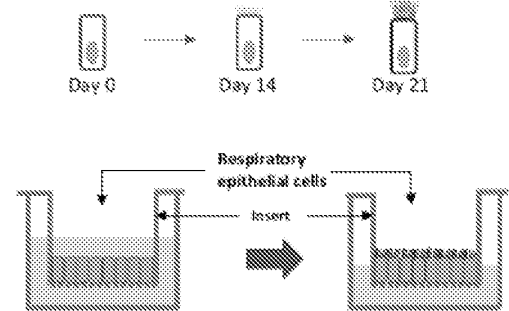
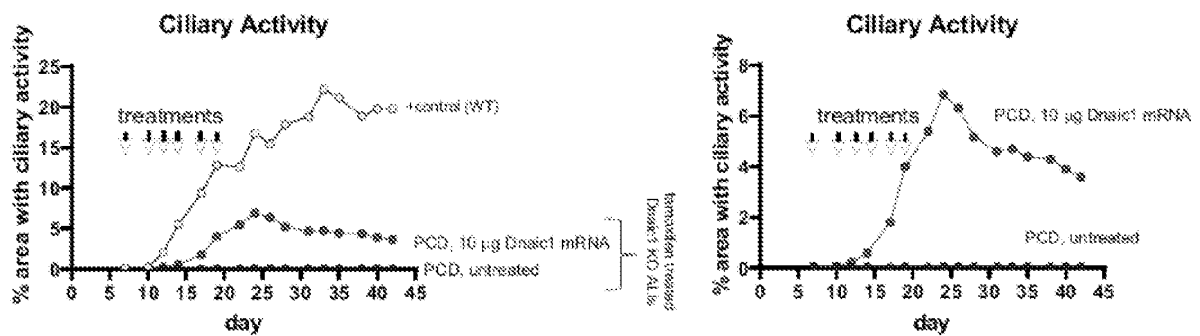


Fig. 9C



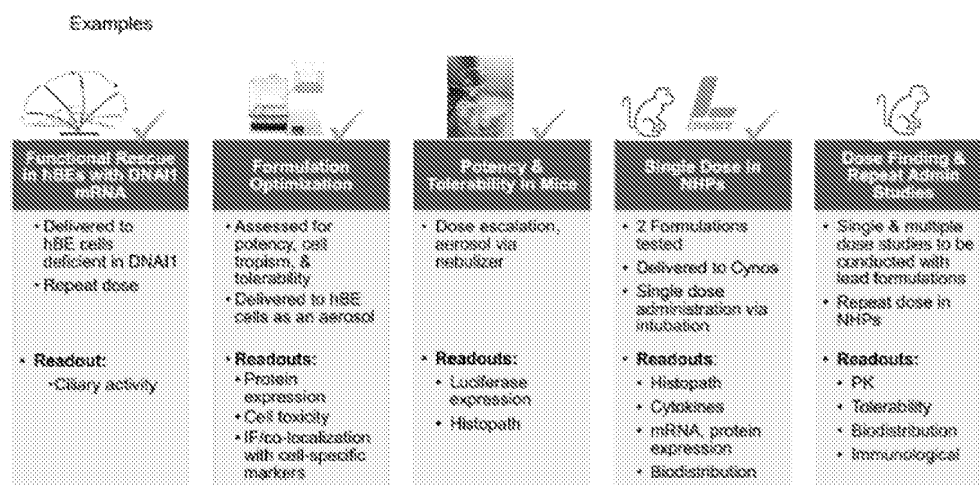


Fig. 10B

Examples

Single Dose in NHPs	Formulation Optimization	Single Dose in NHPs	Dose Finding & Repeat Admin Studies in NHPs	
a low exposure/dose (0.1 mg/kg), observe: - good lung distribution of the mRNA - DNAM1 protein expression in the target cells - tolerability findings that support additional dose finding and repeat dosing studies Two LNPs tested: 5-component SORT 4-component cLNPs	<u>Create long term storage & improve shipping conditions</u>, achieve freezing buffers, increase stability <u>Decrease dose</u>: SORT lipids with higher potency and better cell tropism characteristics <u>Shorten nebulization times</u>: increase concentration, reduce lipid load <u>Further increase stability</u>: reduce lipid load	- Assess higher doses - Assess longer timepoints for recovery (e.g., 72 and 144 hrs)	- Determine clinical candidate for IND enabling studies - Determine dose and dosing frequency - Determine max tolerated dose - Select nebulization device for clinical development Studies: - Single & multiple dose studies to be conducted with lead formulations - Repeat dose in NHPs Readouts: - PK - Tolerability - Biodistribution - Immunological	- Safety/tox study in rats - Safety/tox study in NHPs

Fig. 10C

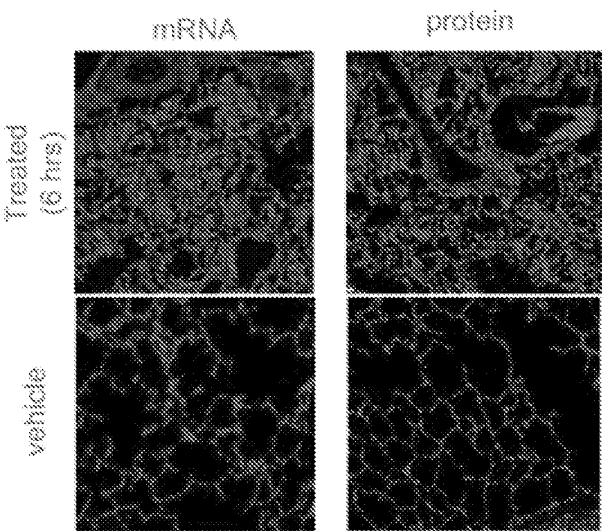


Fig. 10D

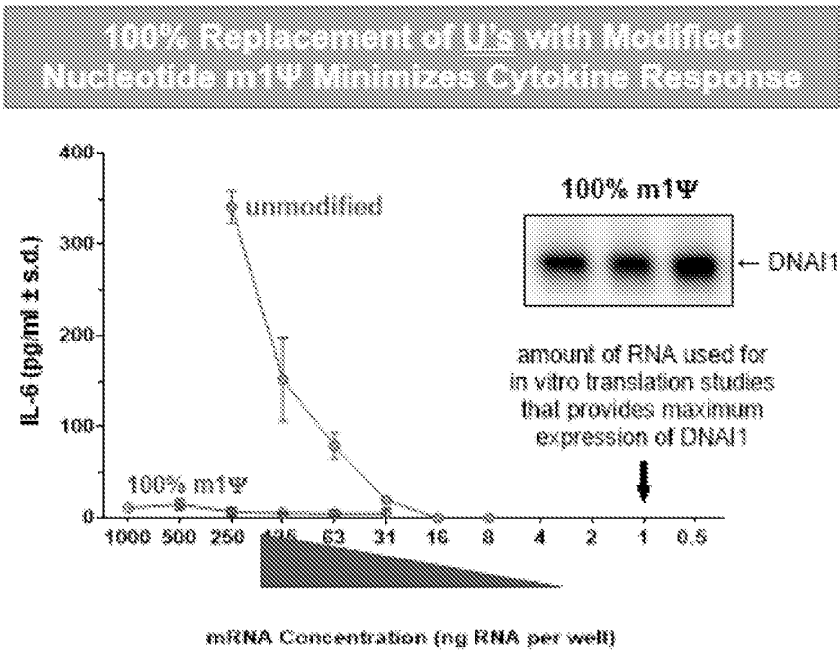
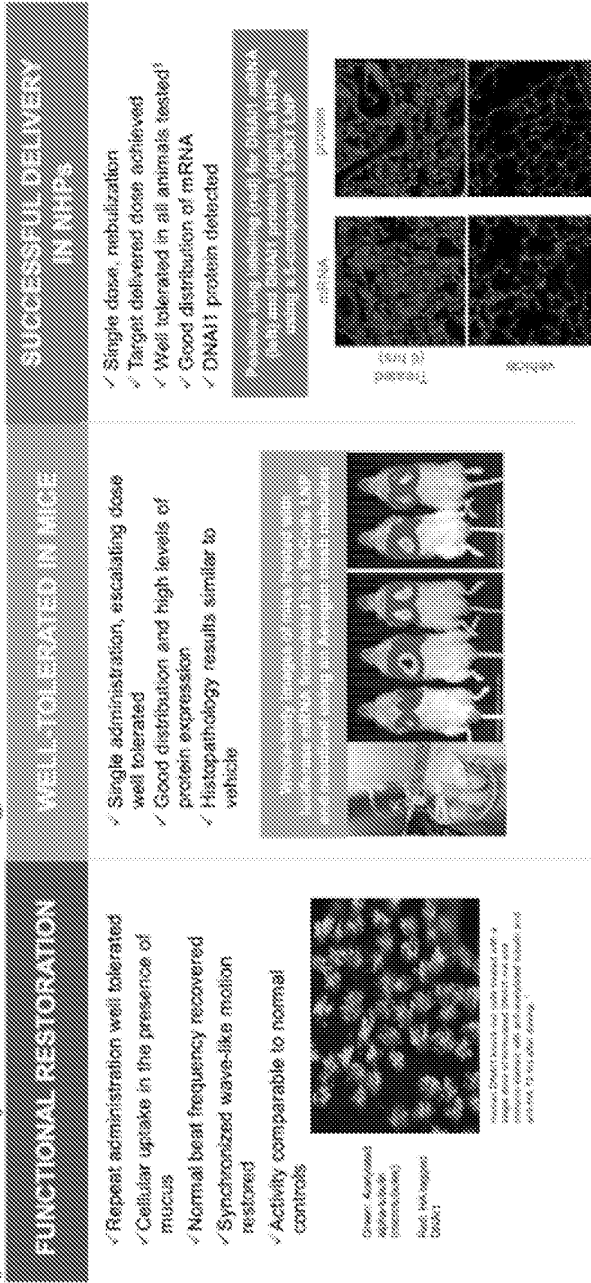


Fig. 10E

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



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

Fig. 11A

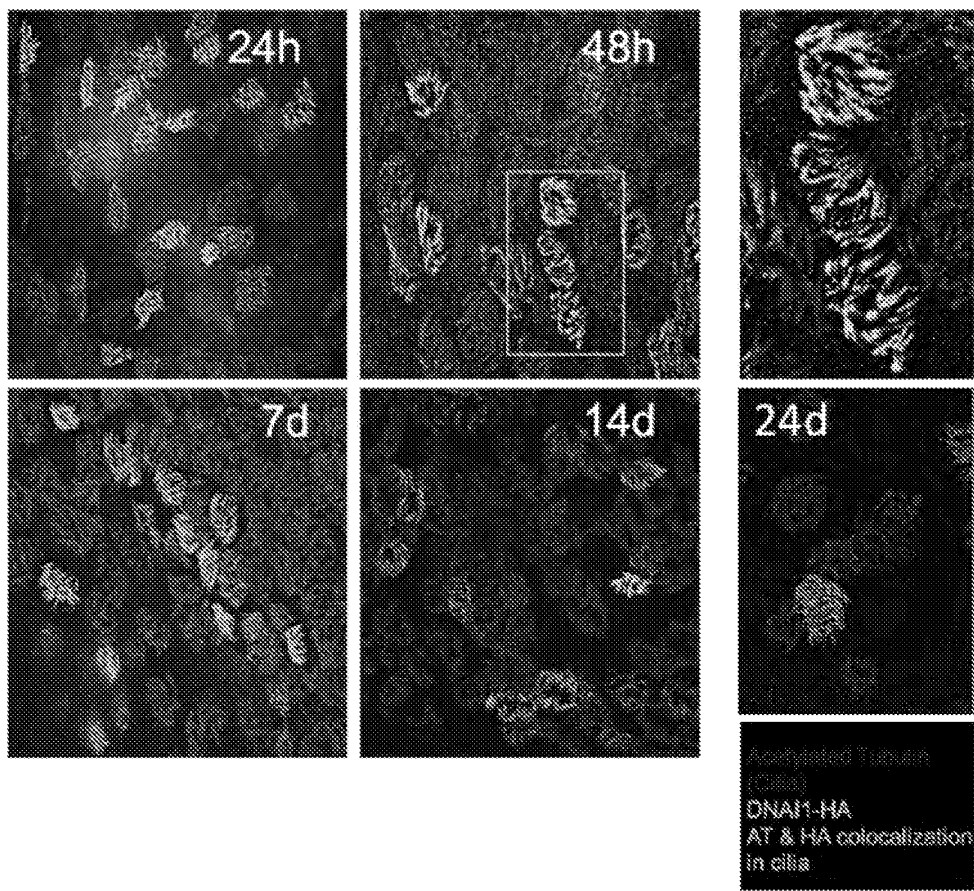


Fig. 11B

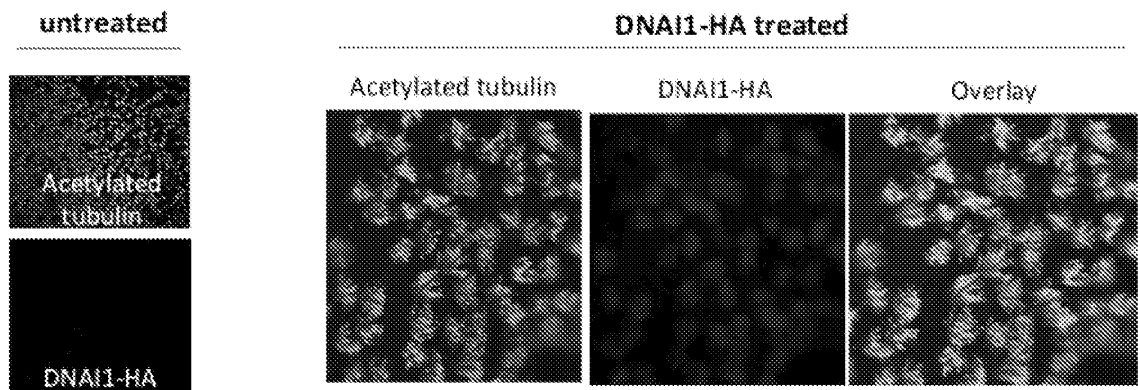
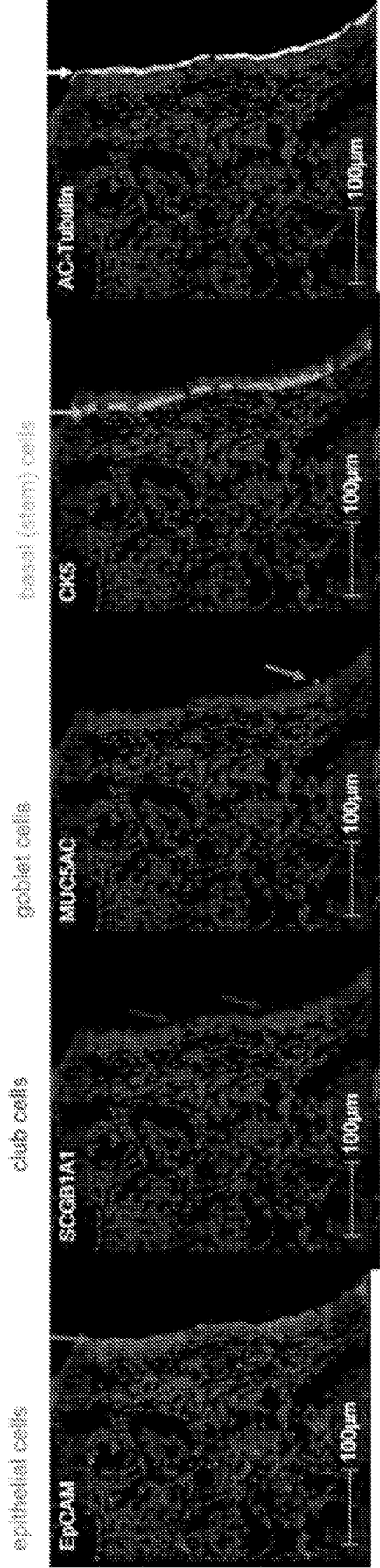


Fig. 12



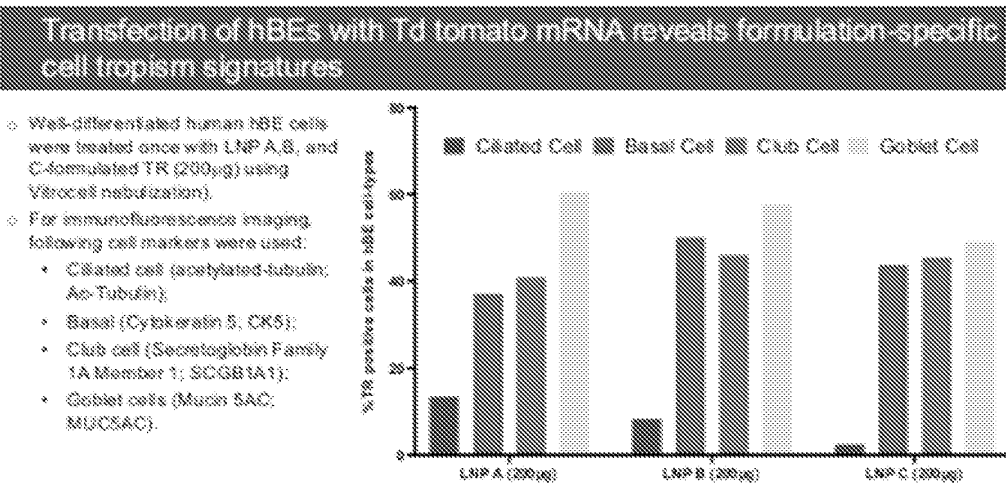


Fig. 13B

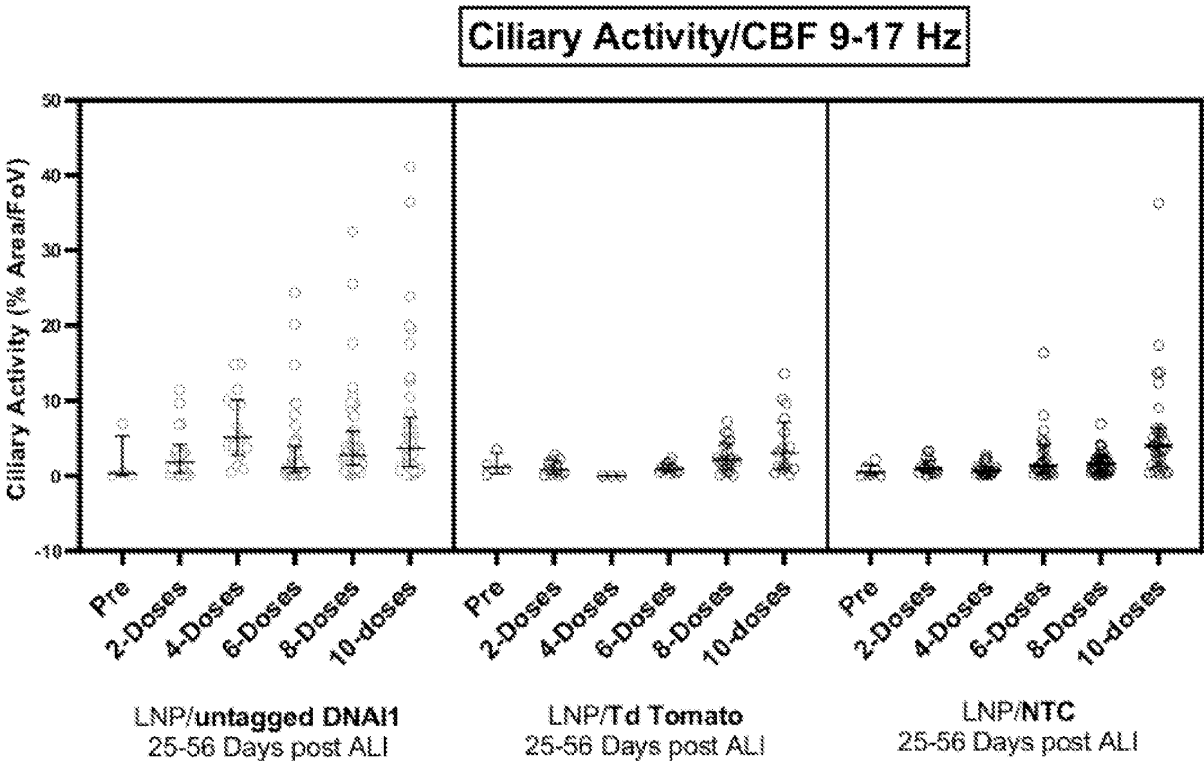


Fig. 14A

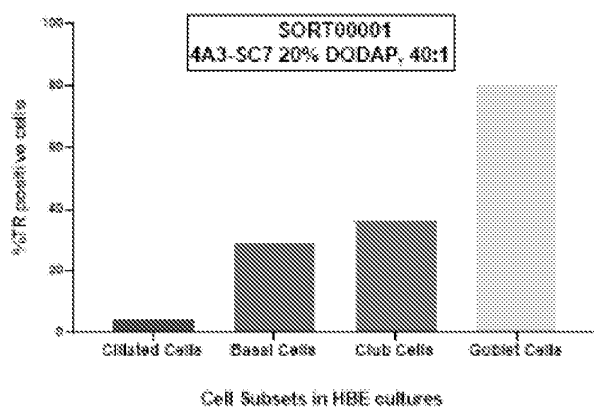


Fig. 14B

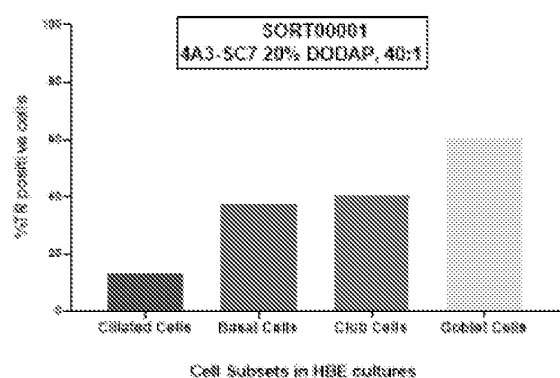


Fig. 14C

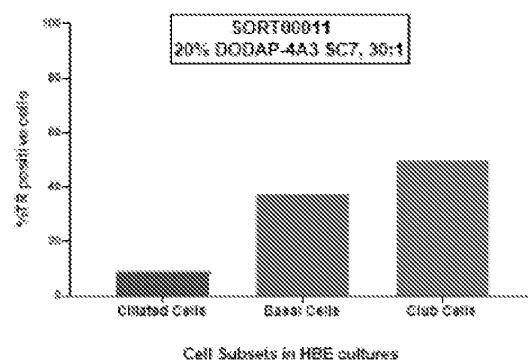


Fig. 14D

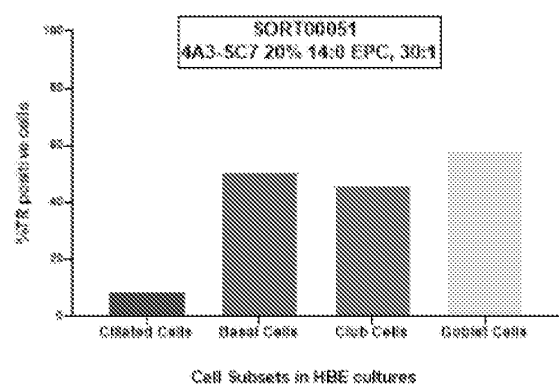


Fig. 14E

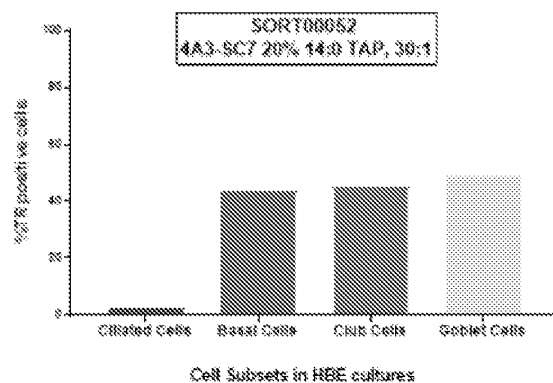
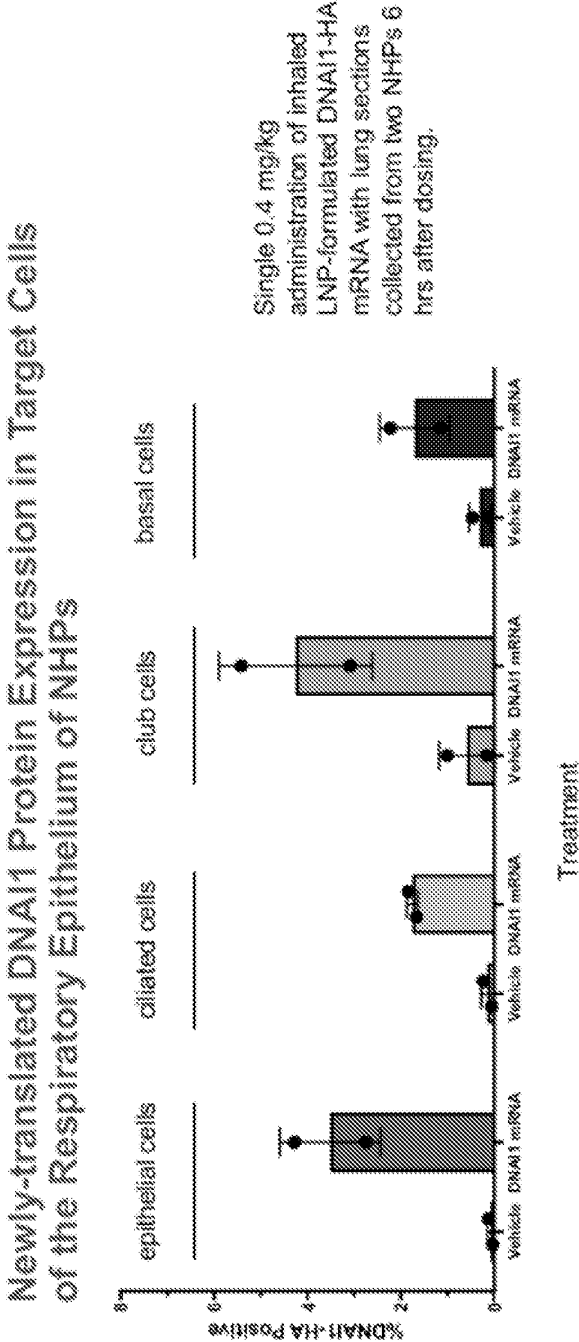


Fig. 15



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: DNA11-HA Is Expressed in Cells of the Respiratory Epithelium

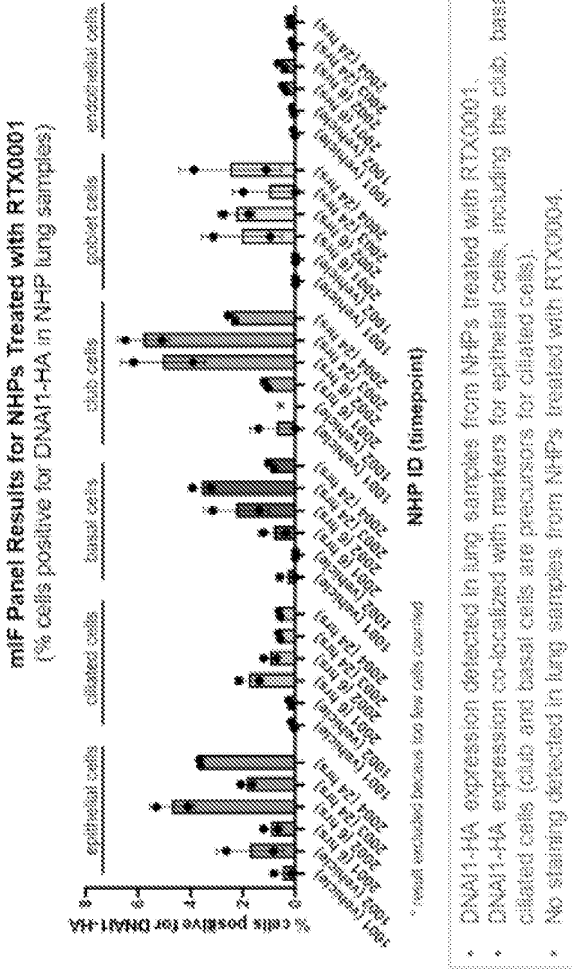


Fig. 16B

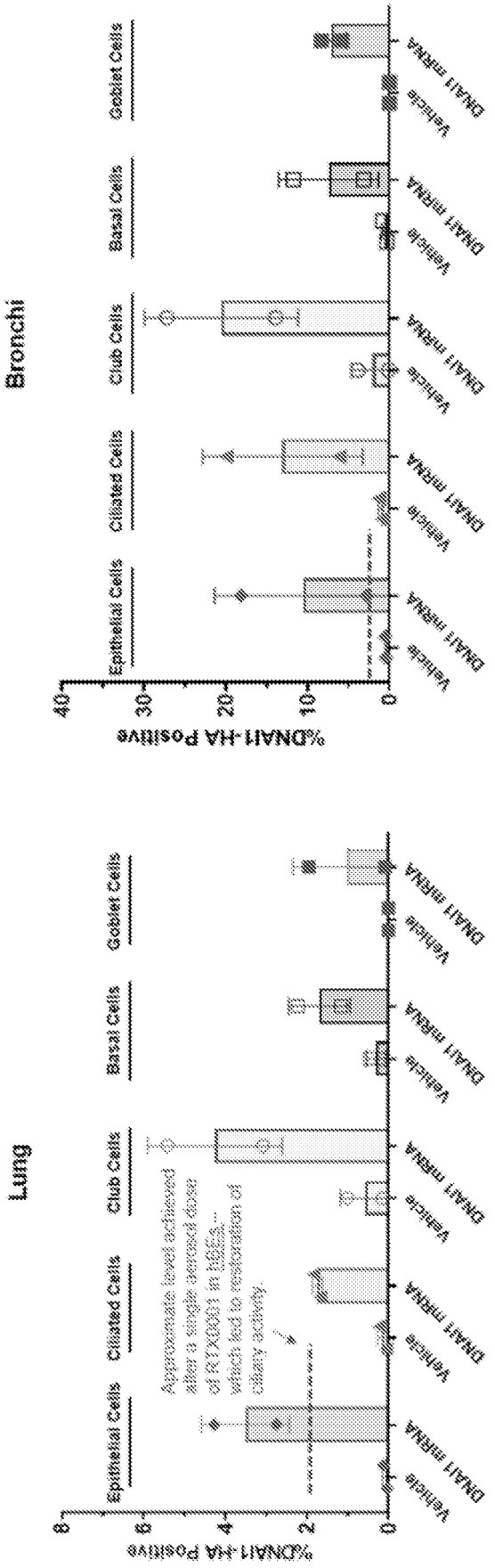


Fig. 17

STUDY GOALS	PARAMETERS	READOUTS
Compare multiple LNP formulations for: 1) biodistribution 2) expression in target cells 3) initial tolerability	NLPs 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 DAA11-4A protein expression in target cells • Toxicity findings that support further dose finding and repeat dosing studies	Model: Cynomolgus macaques Treatment: • Short exposure: 30 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

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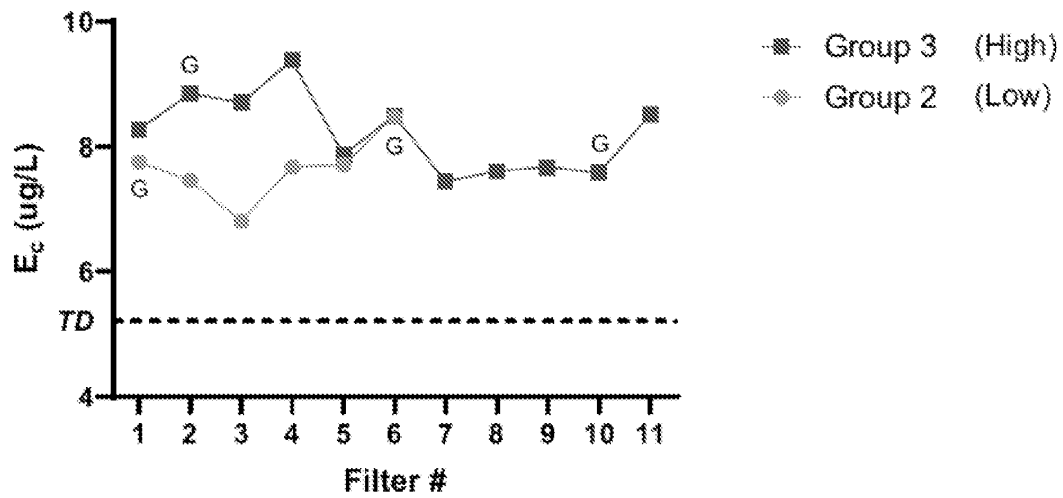


Fig. 18B

No#	Designation (min)	Target dose TD (mg/kg)	Aerosol concentration E_c ($\mu\text{g/L}$)	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 $\pm$ 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 $\pm$ 0.741 (5.2)	1.594	3.1	0.38	Male
				1.506	2.9	0.38	Female

0.38 mg/kg $\rightarrow$ 1.14 mg -25% $\rightarrow$ 285 μg (RNA) $\rightarrow$ 8.55 mg total lipids

Fig. 18C

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

Fig. 19A

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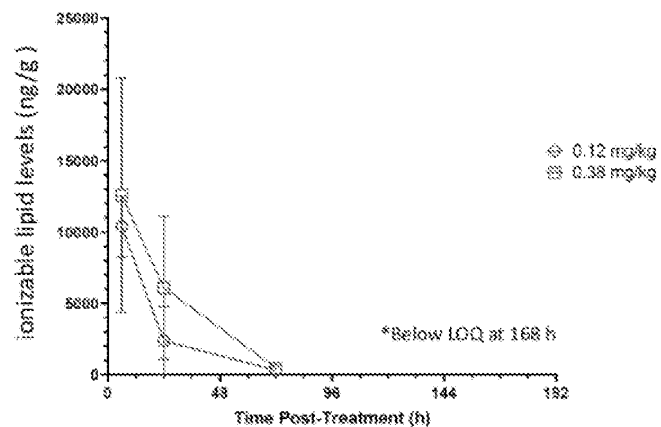


Fig. 19B

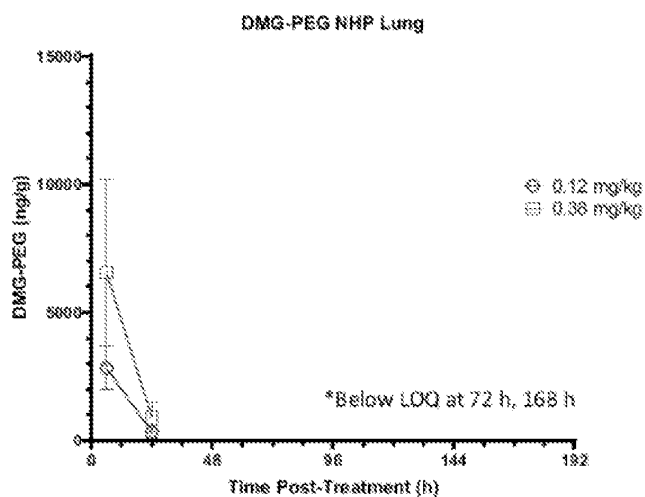


Fig. 19C

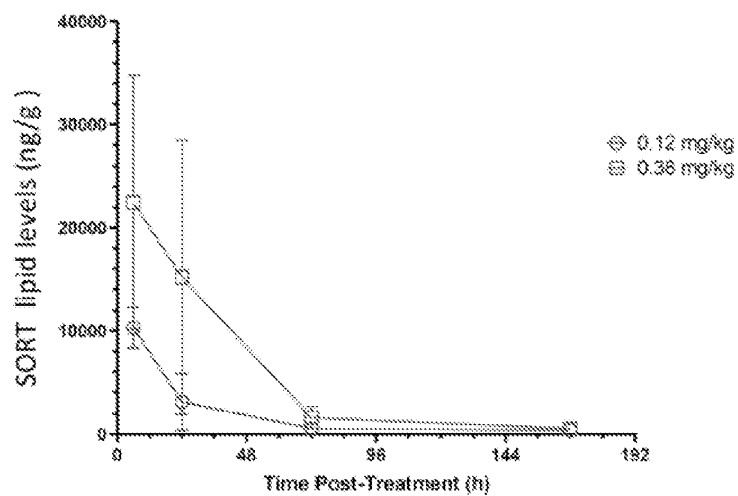


Fig. 20A

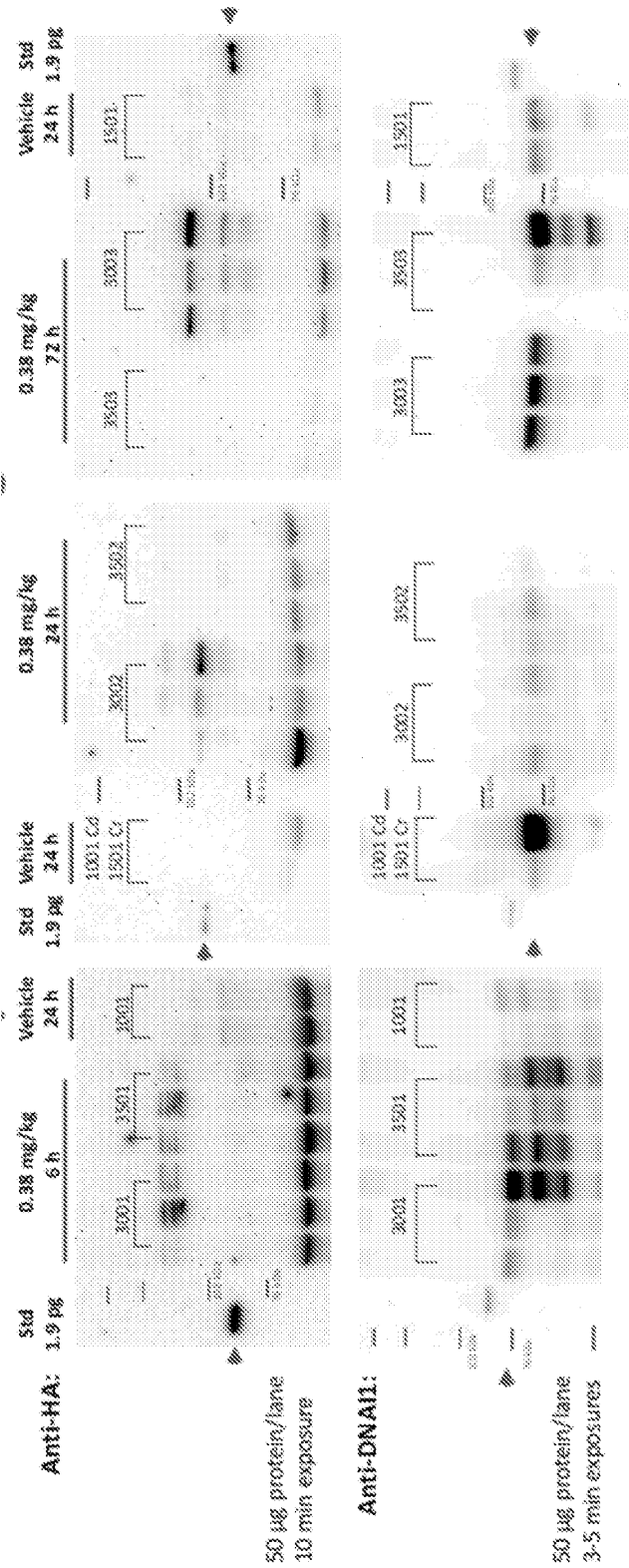
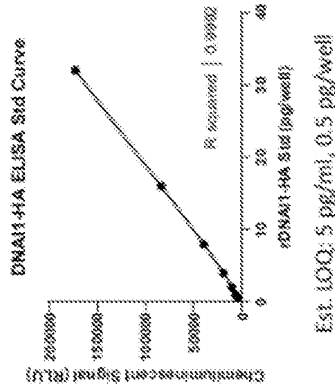


Fig. 20B

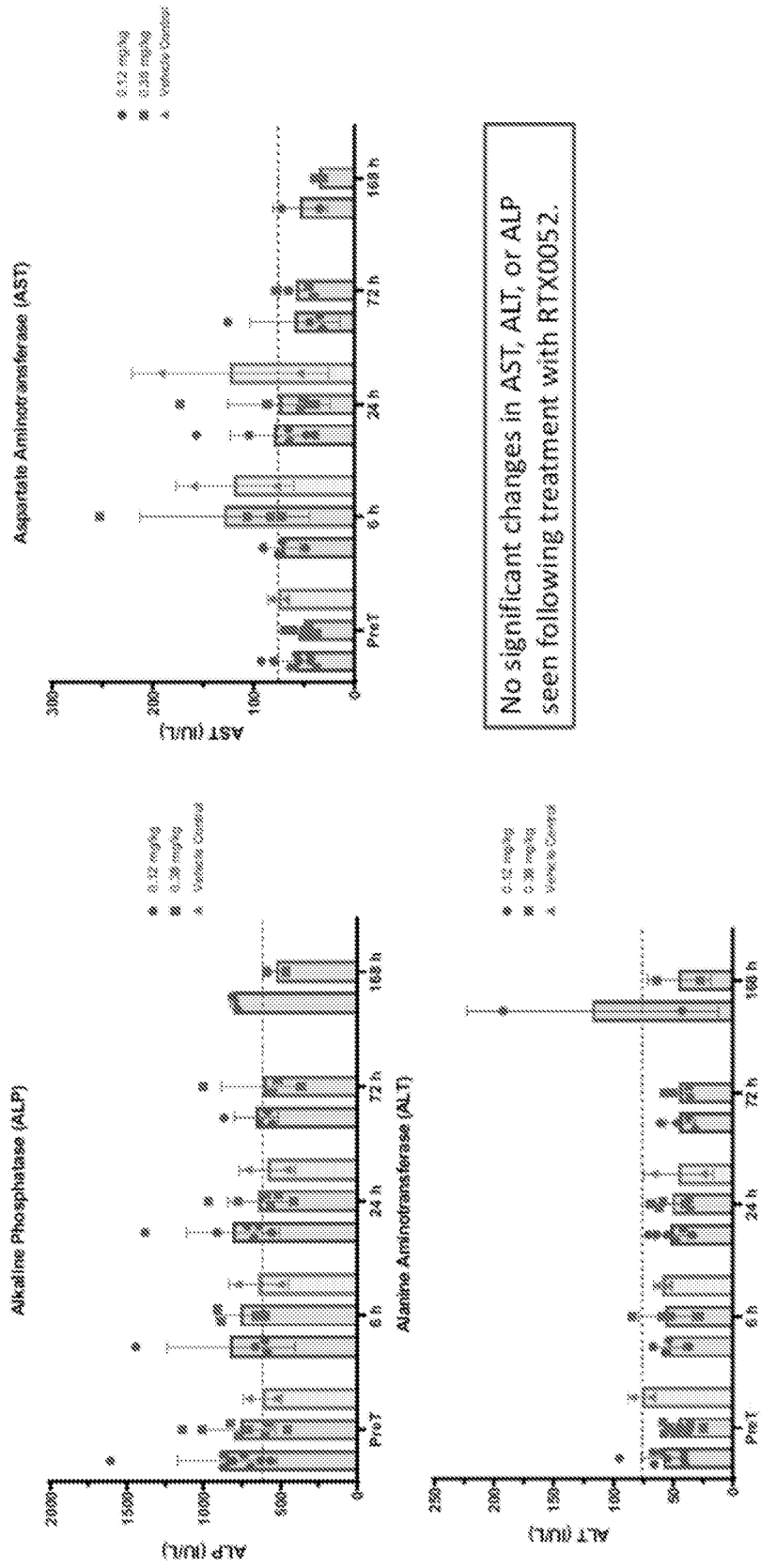
Vehicle 24 h				High Dose: 0.38 mg/kg 6 h				High Dose: 0.38 mg/kg 24 h				High Dose: 0.38 mg/kg 72 h			
Animal Number	ELISA Result (pg/100 µg total protein)	Western Blot Signal (V/N)		Animal Number	ELISA Result (pg/100 µg total protein)	Western Blot Signal (V/N)		Animal Number	ELISA Result (pg/100 µg total protein)	Western Blot Signal (V/N)		Animal Number	ELISA Result (pg/100 µg total protein)	Western Blot Signal (V/N)	
1001 Cr	n.d.	N		3001 Cr	n.d.	N		3002 Cr	n.d.	N		3003 Cr	n.d.	N	
1001 Mi	n.d.	N		3001 Mi	n.d.	N		3002 Mi	n.d.	N		3003 Mi	n.d.	N	
1501 Cr	n.d.	N		3001 Cd	n.d.	N		3002 Cd	n.d.	N		3003 Cd	n.d.	N	
				3501 Cr	n.d.	N		3502 Cr	n.d.	N		3503 Cr	n.d.	N	
				3501 Mi	n.d.	N		3502 Mi	n.d.	N		3503 Mi	n.d.	N	
				3501 Cd	0.43*	N		3502 Cd	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



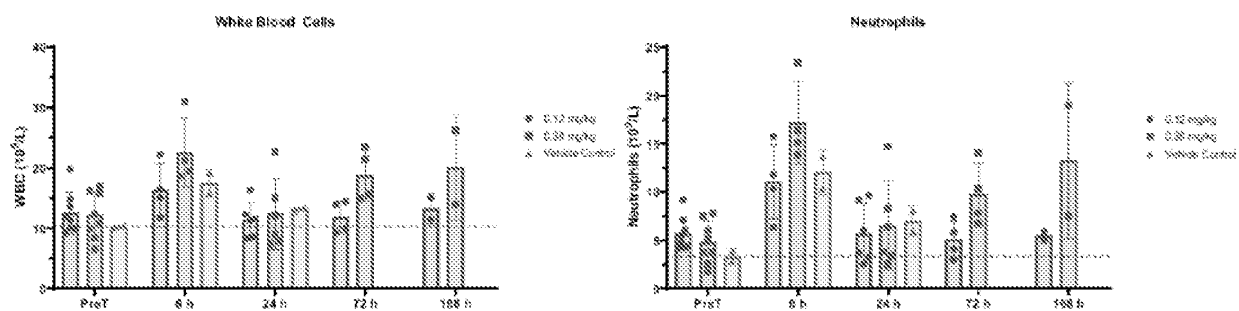
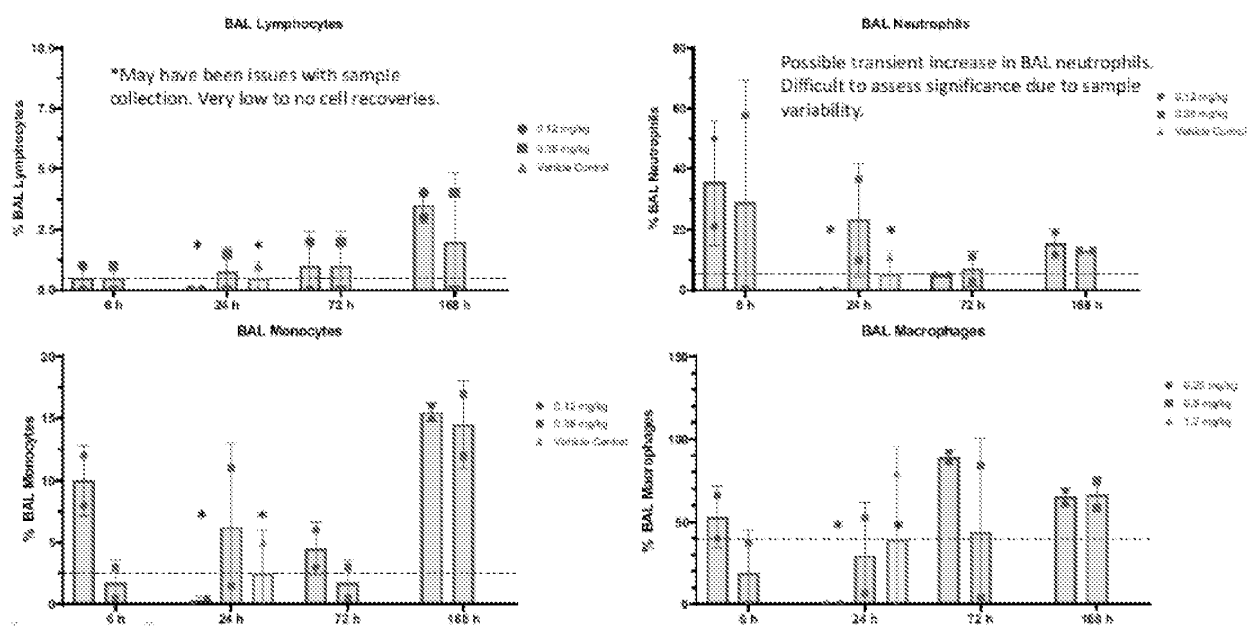


Fig. 21C



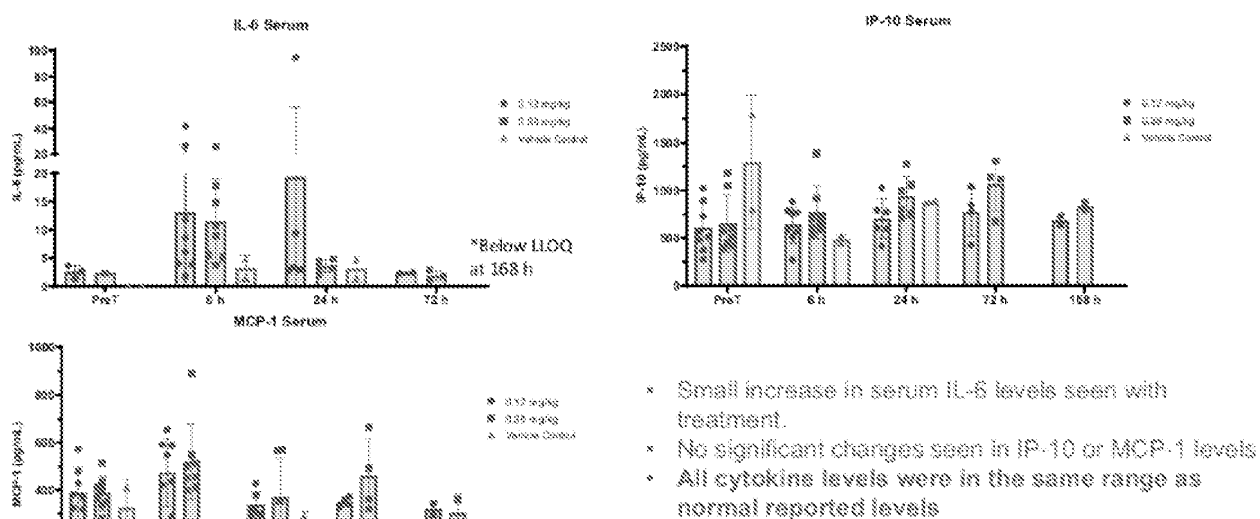
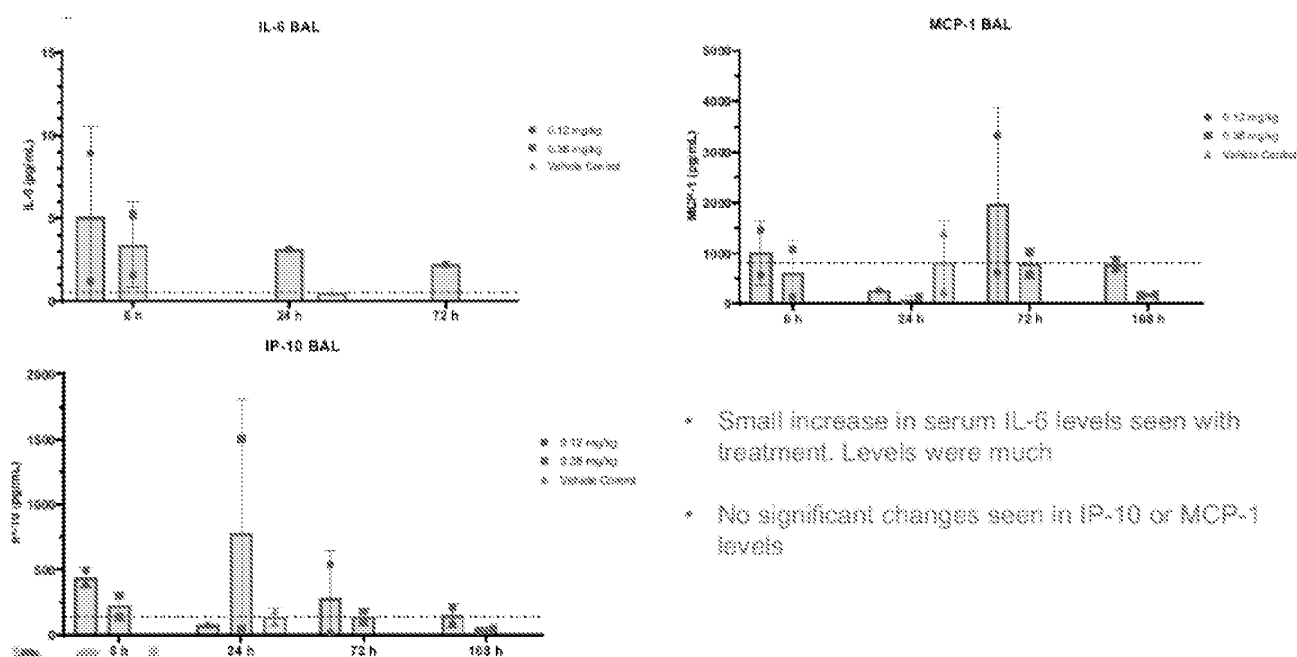


Fig. 21E



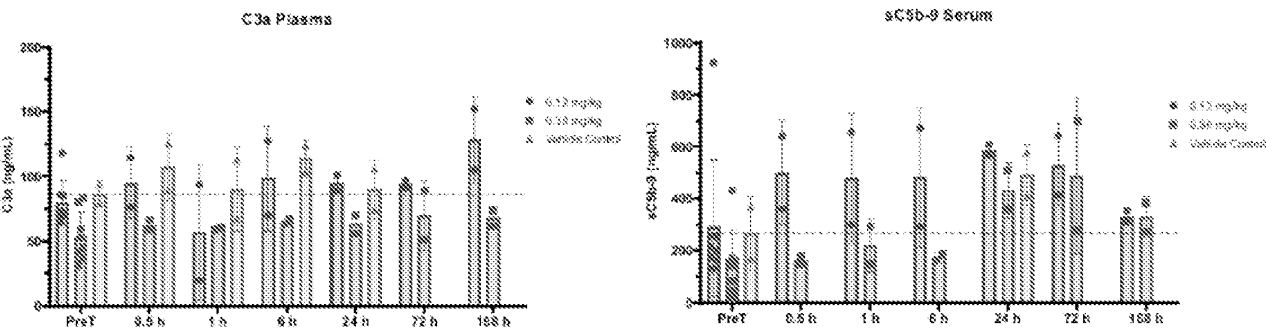


Fig. 21G

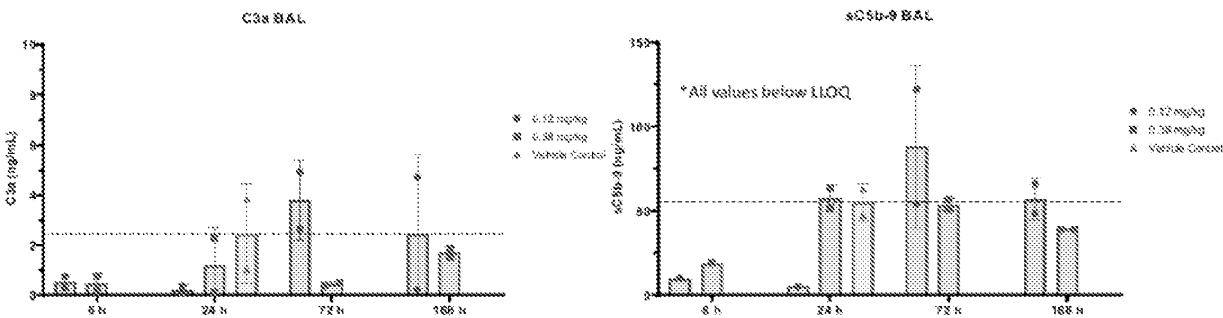
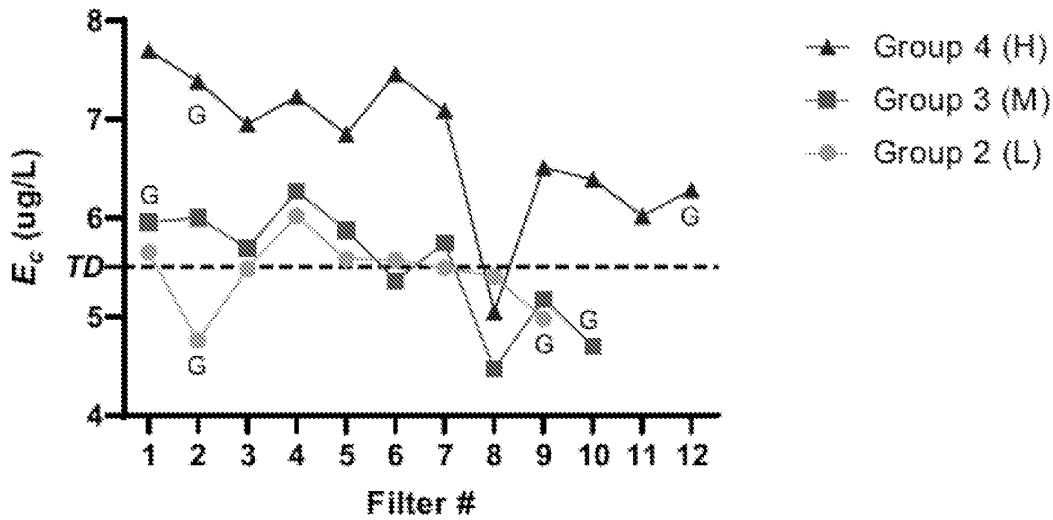
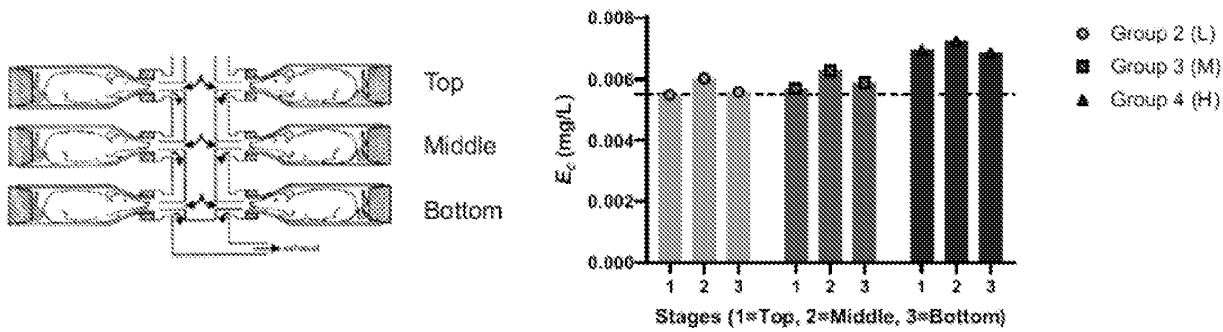


Fig. 22A



G = gravimetric analysis

Fig. 22B



No#	Designation (min)	Target dose TD (mg/kg)	Aerosol concentration E _a (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

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

Fig. 23A

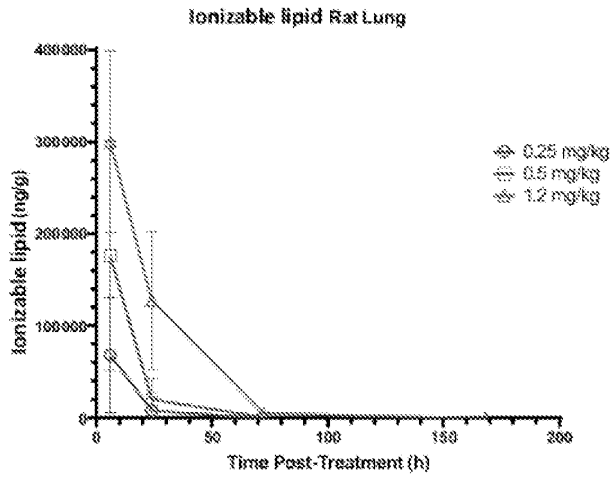


Fig. 23B

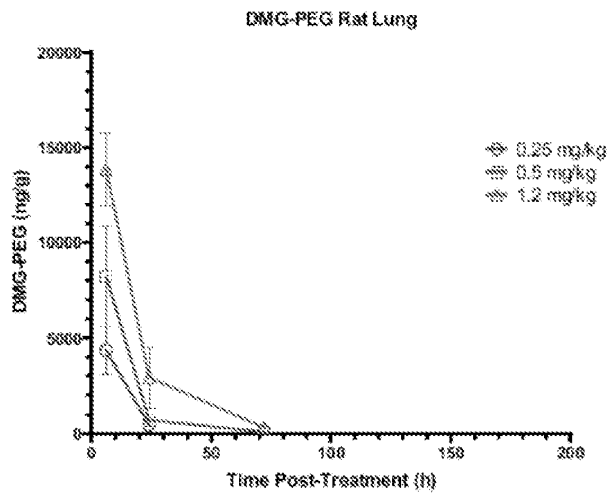


Fig. 23C

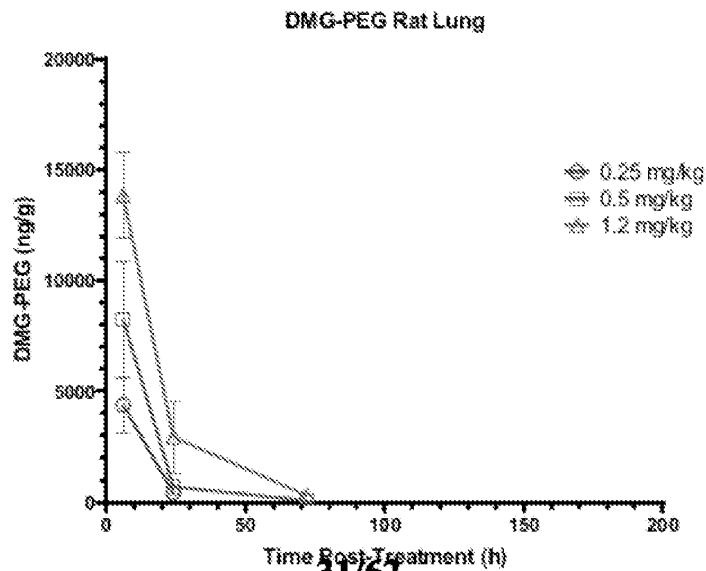
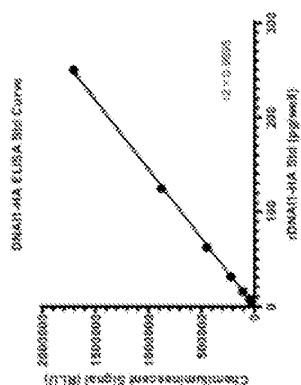


Fig. 24B

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

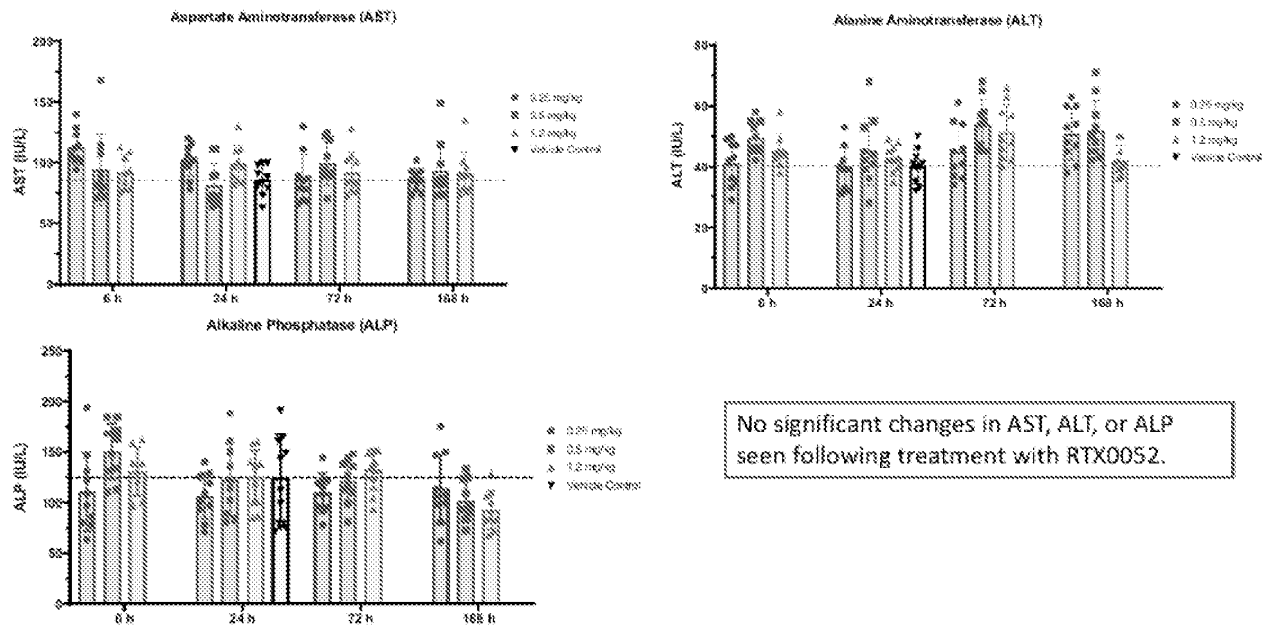


Fig. 25B

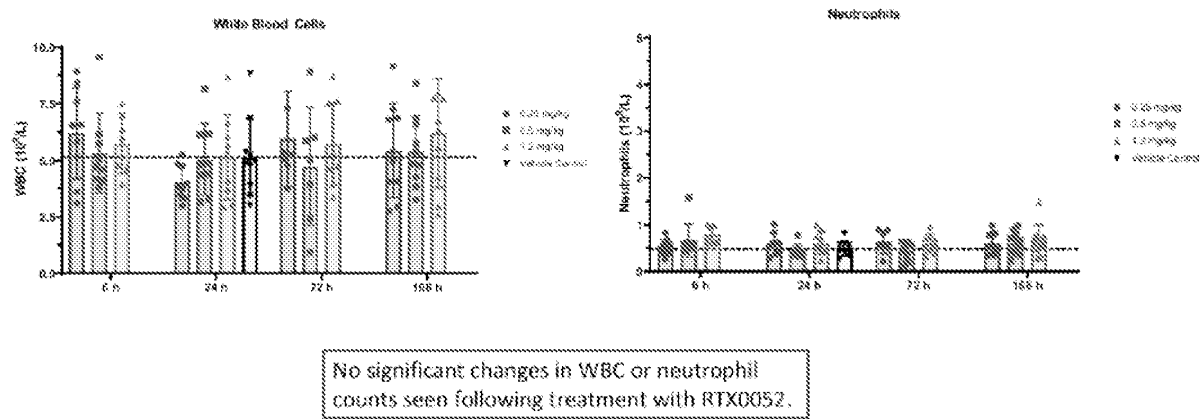


Fig. 25C

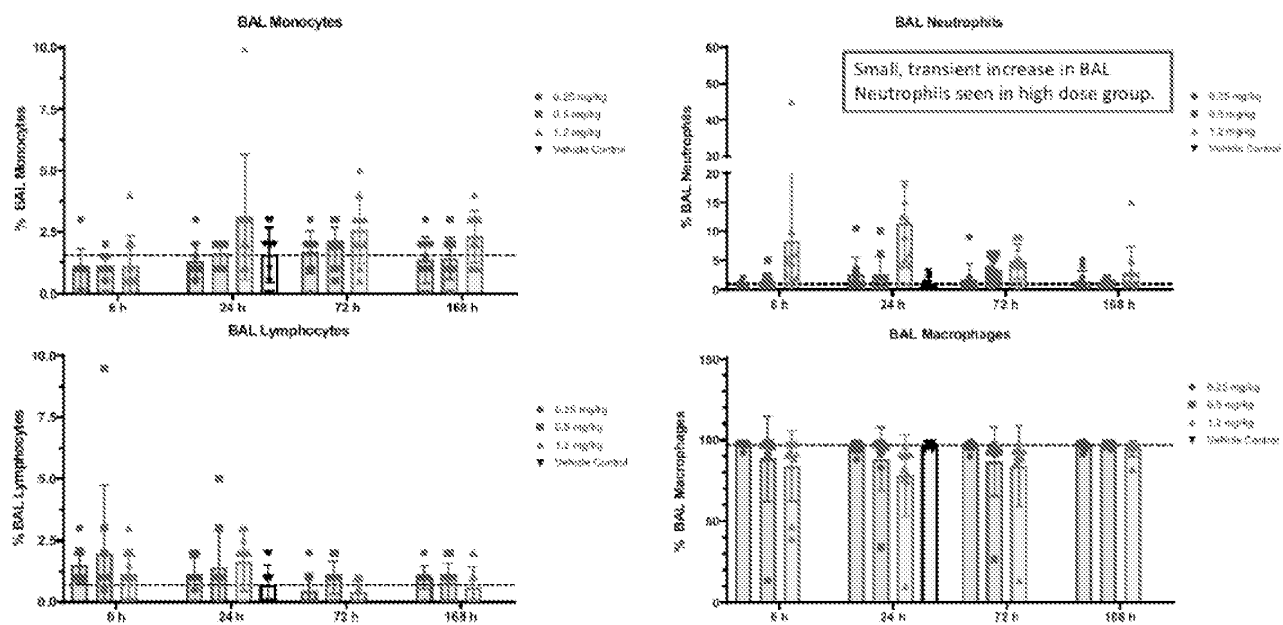


Fig. 25D

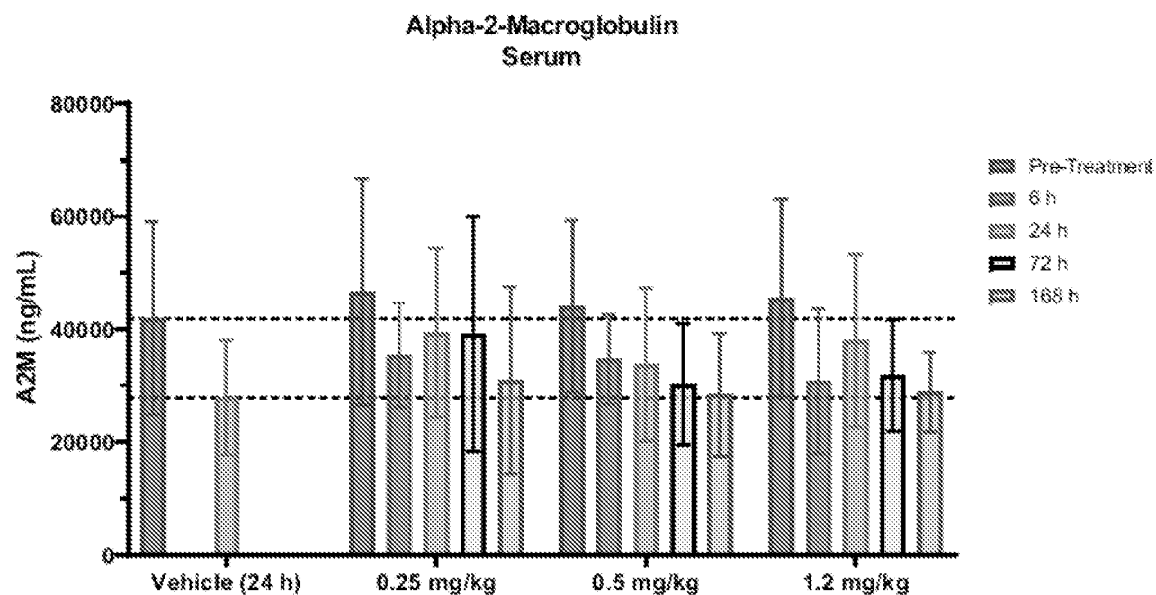


Fig. 26A

Group	Formulation	Animals	Delivery	[RNA] (mg/mL)	Total Dose	aerosol delivery of substrate	# animals	Data Collection
1	Control vehicle	Albino B6J, naive	Nebulization 2 h 66.6 μ L/min	none	4 mL buffer 27	-	n = 12	For groups 2 to 7 – once a week (Mondays at 11am) and after last dose. a) 2 mice @ 4h post-dosing = 1/15
2	RTX00001 (95% DMAT-HA + 5% luciferase mRNA)	Albino B6J, naive	Nebulization 2 h 66.6 μ L/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	For all groups – after last dose a) 2 mice @ 4h post-last dose and post-RTS = perfuse and excise liver, spleen and lungs. Hold for RNAlater: half for protein assay
3	RTX00001 (95% DMAT-HA + 5% luciferase mRNA)	Albino B6J, naive	Nebulization 2 h 66.6 μ L/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	b) Histopath at 72h post-last dosing (5 mice) and 7 days post-last dosing (5 mice)
4	RTX00002 (95% DMAT-HA + 5% luciferase mRNA)	Albino B6J, naive	Nebulization 2 h 66.6 μ L/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

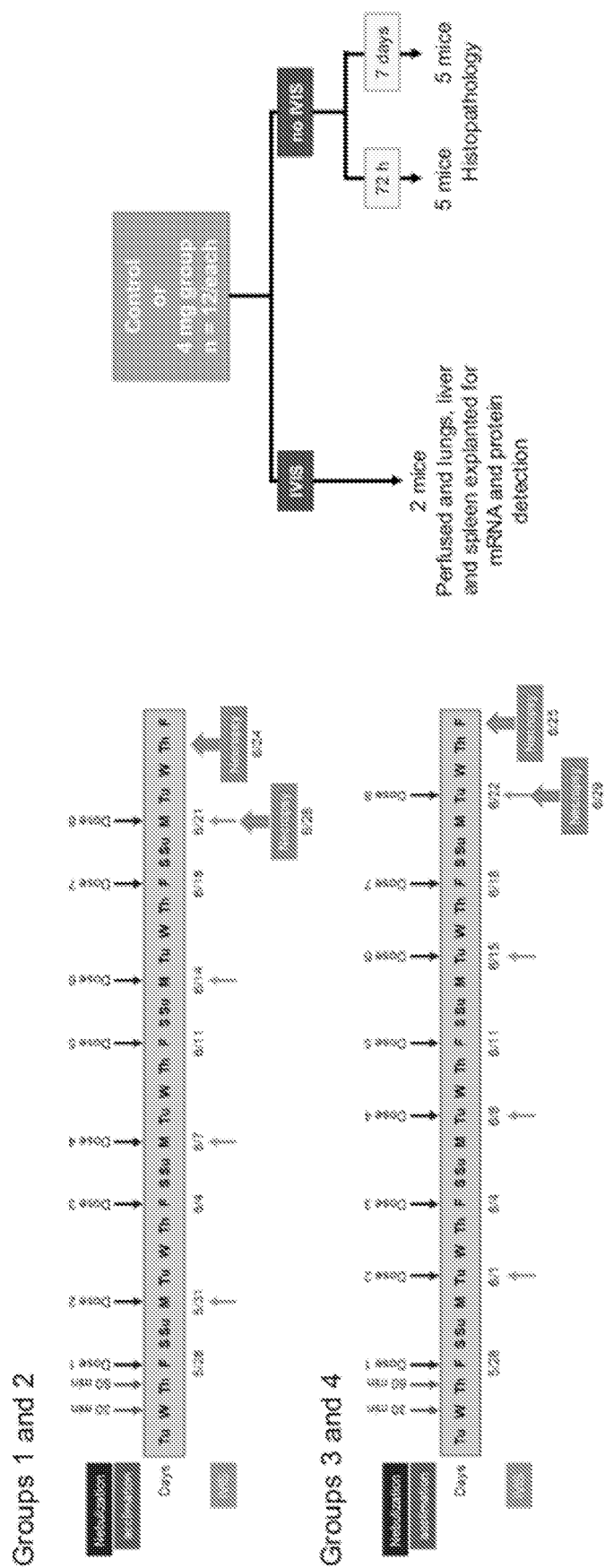


Fig. 27A

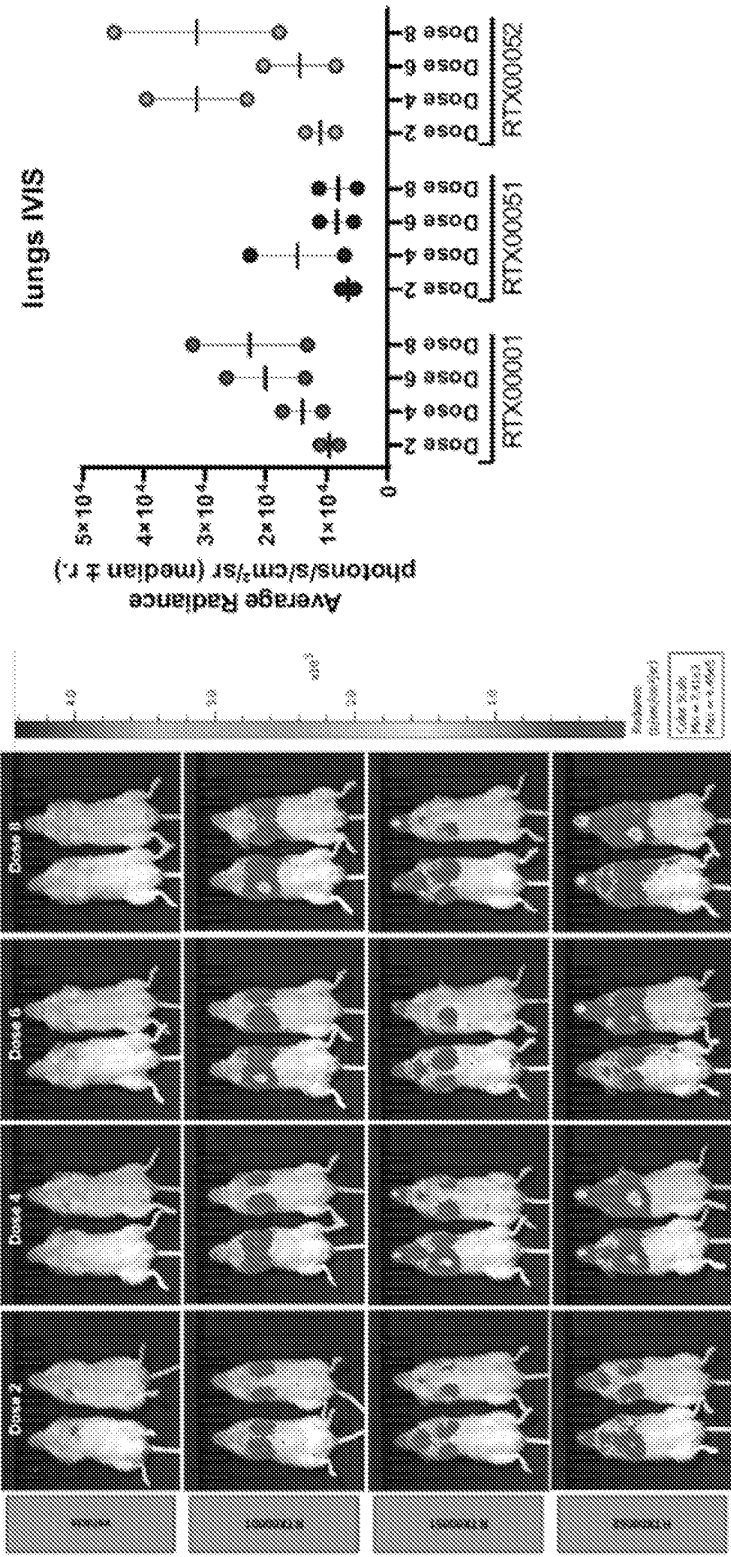


Fig. 27B

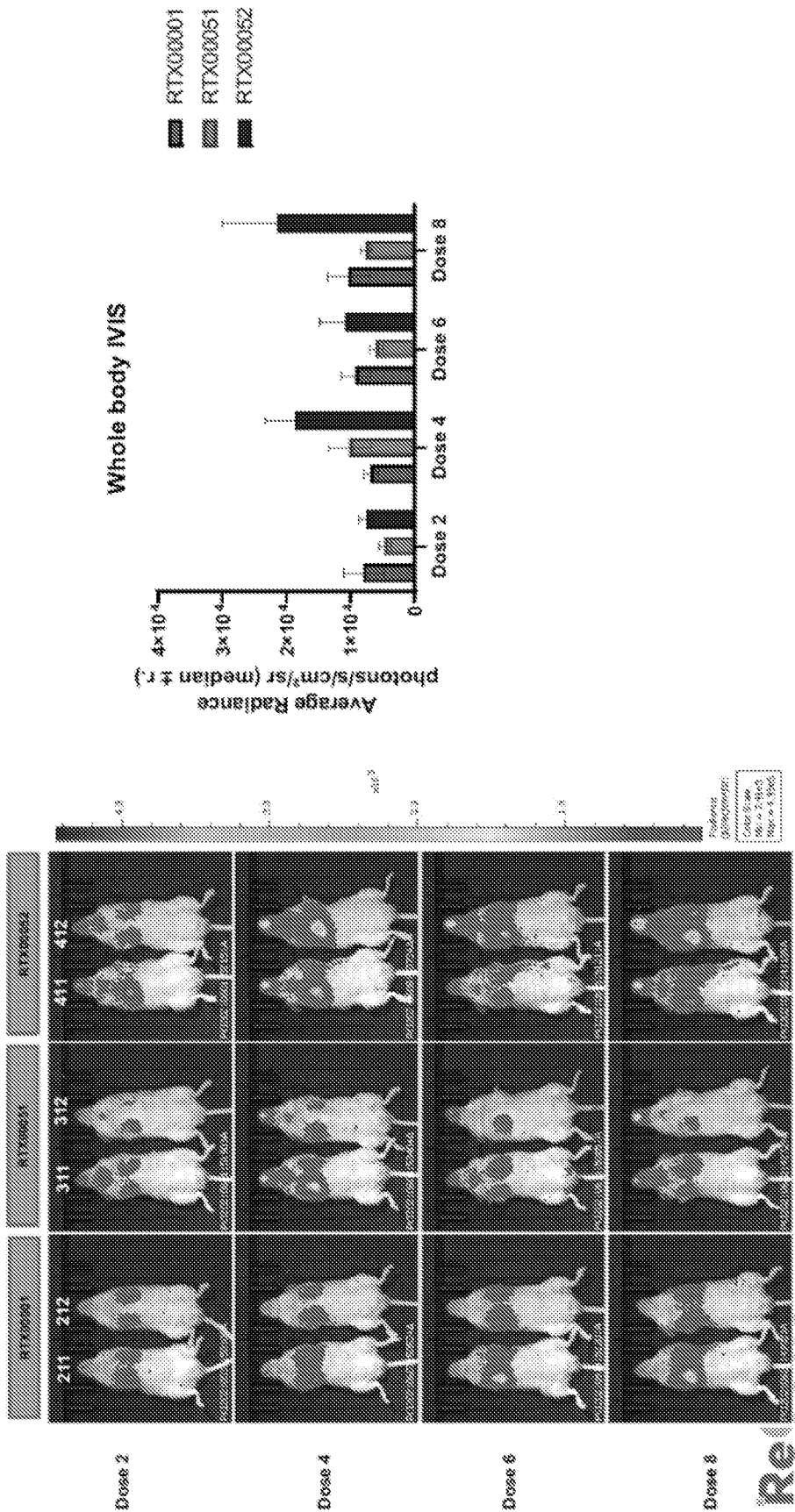


Fig. 27C

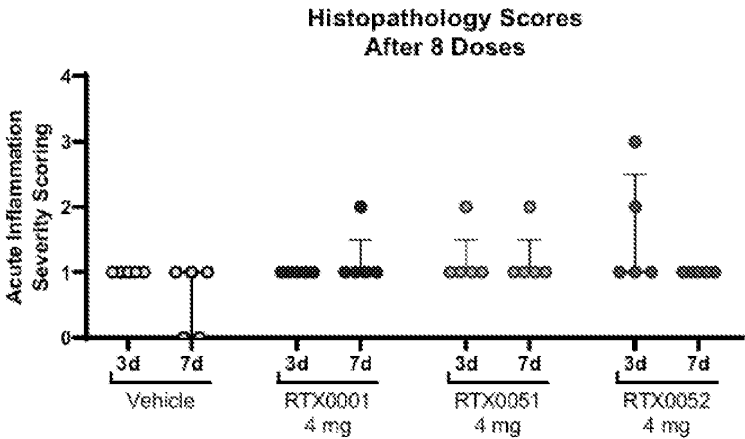


Fig. 27D

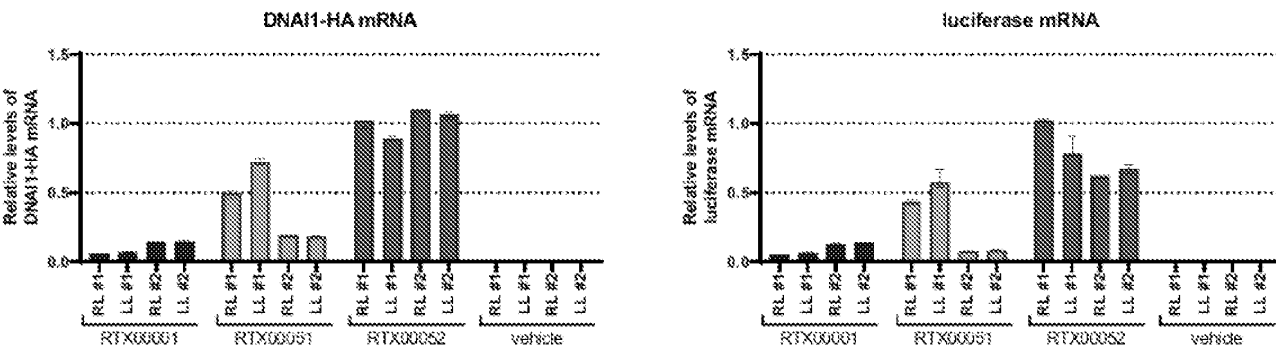
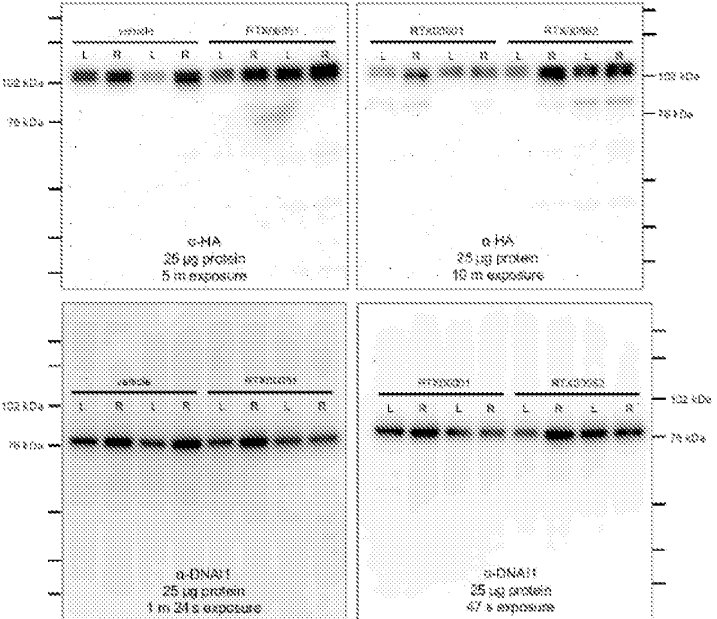


Fig. 27E



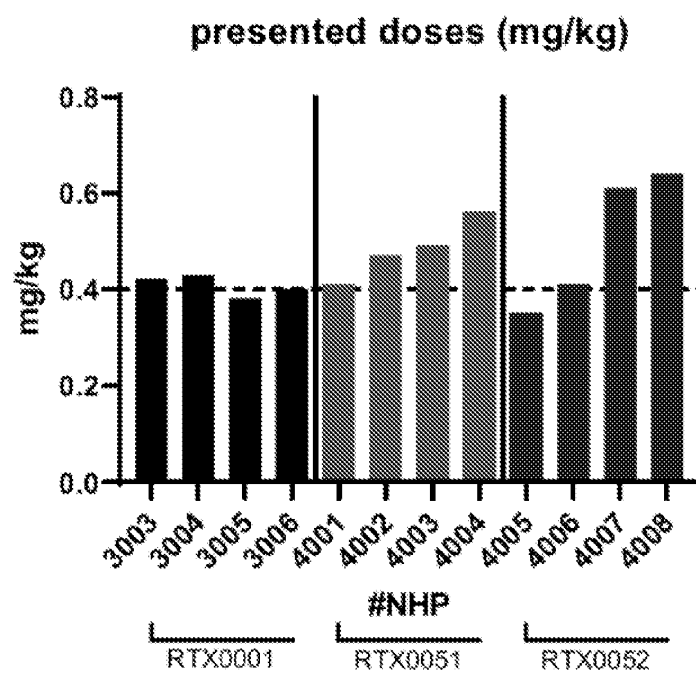


Fig. 28B

	APS (Aerodynamic particle sizer)				Impactor (Merson)	
	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

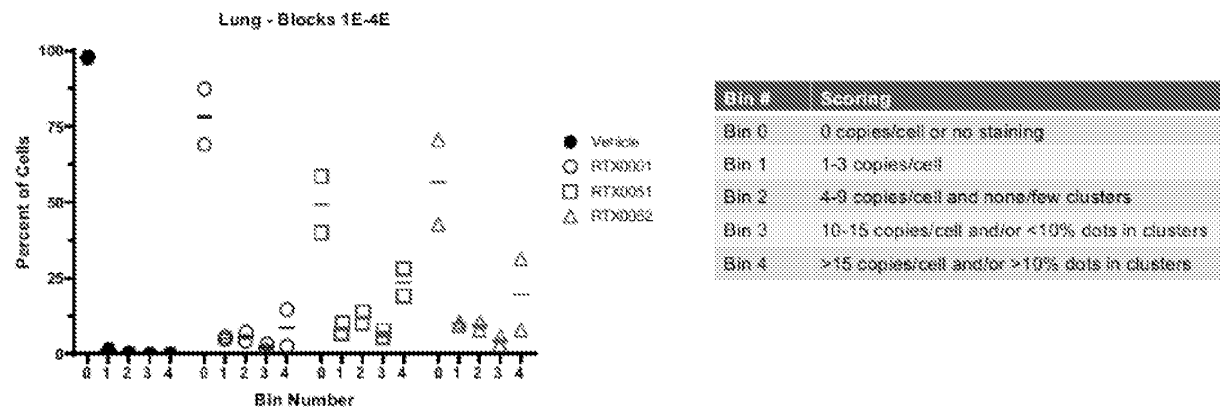


Fig. 28D

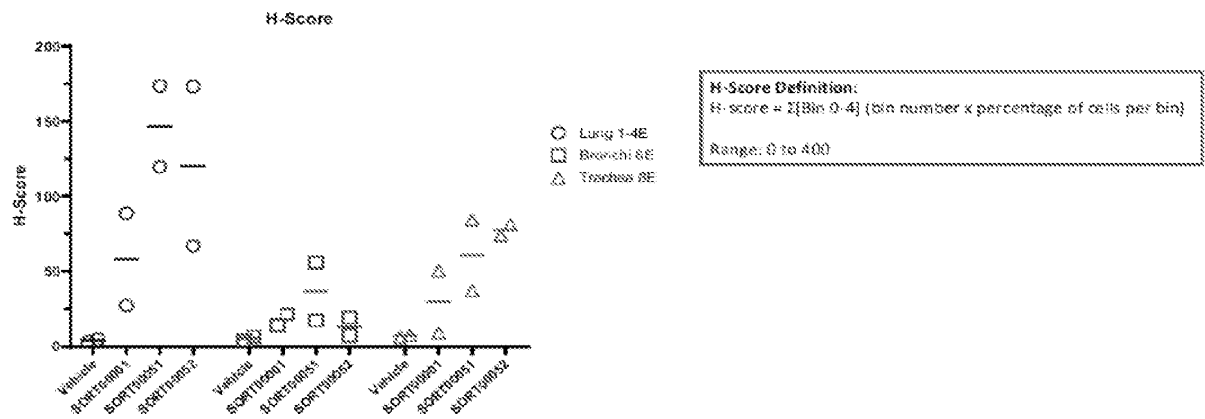


Fig. 29A

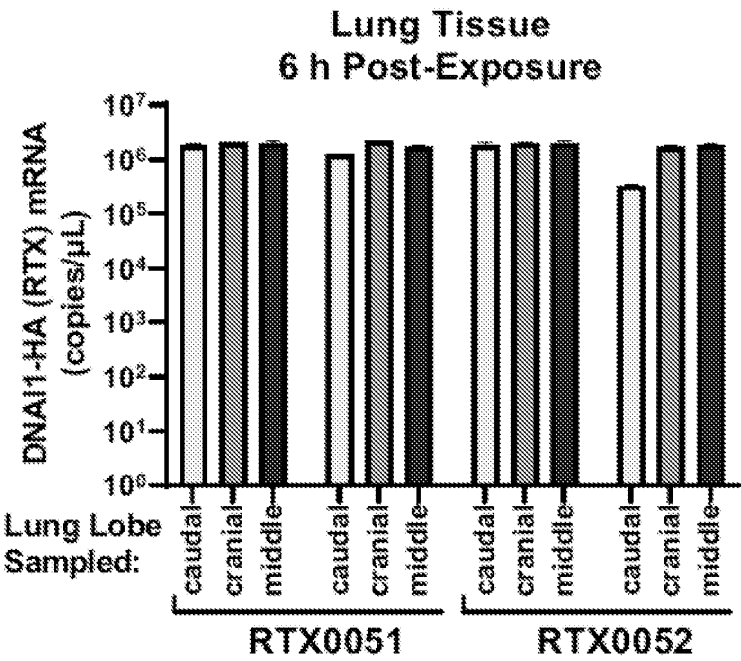
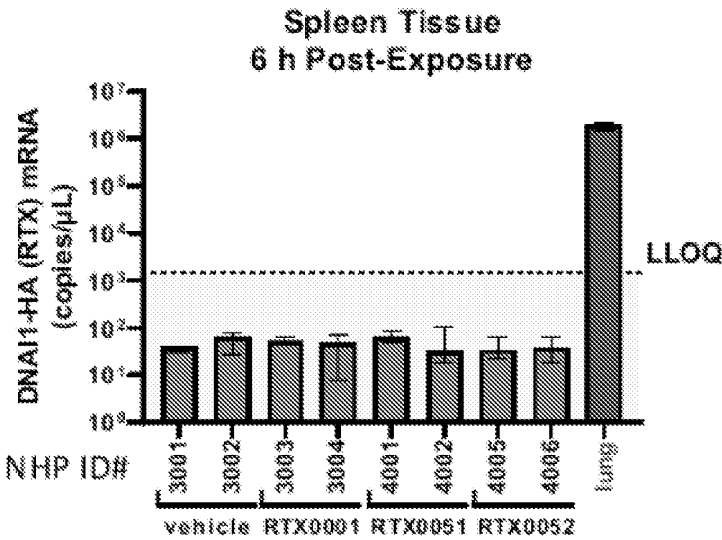


Fig. 29B



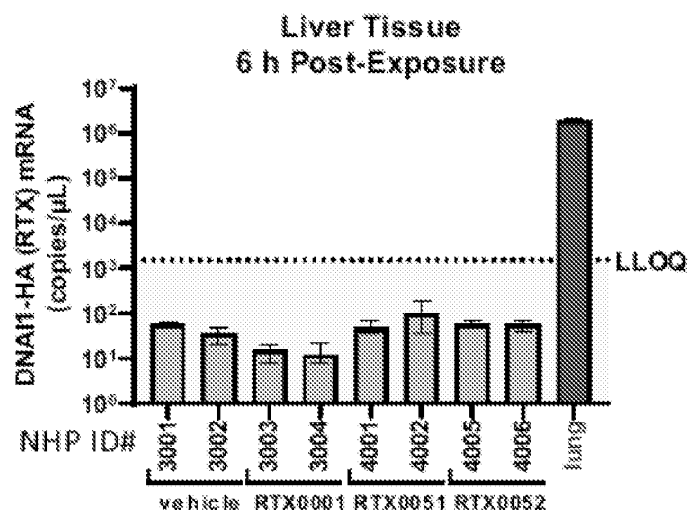
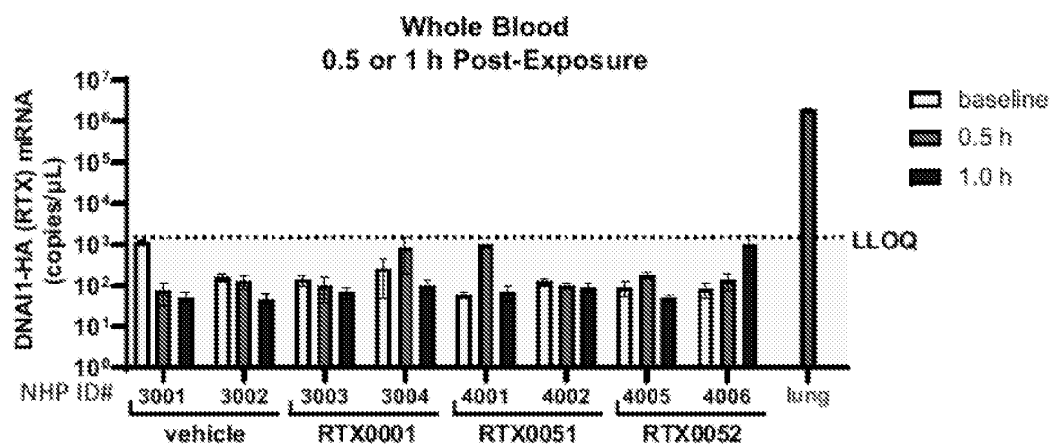


Fig. 29D



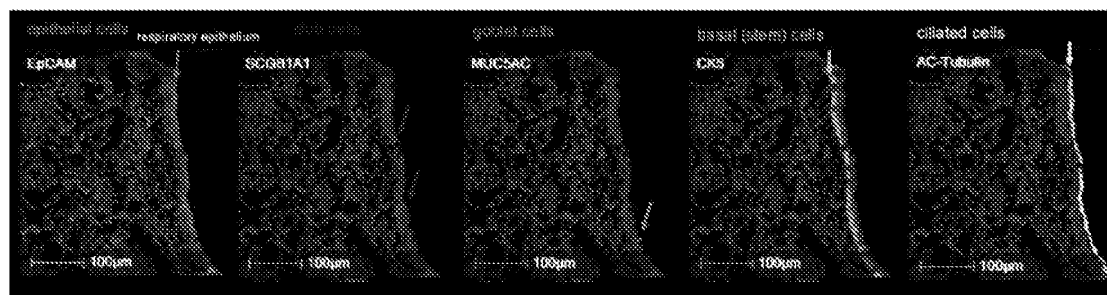


Fig. 30B

Lung - Blocks 1E-4E

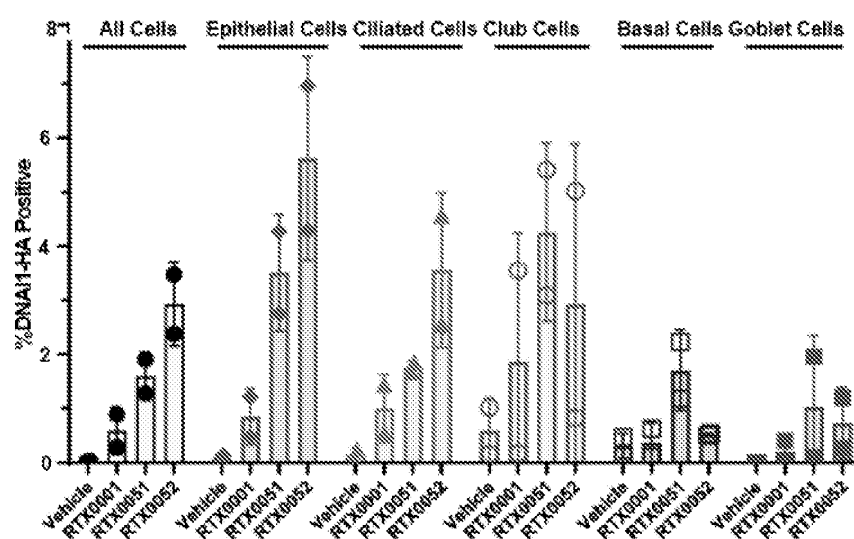
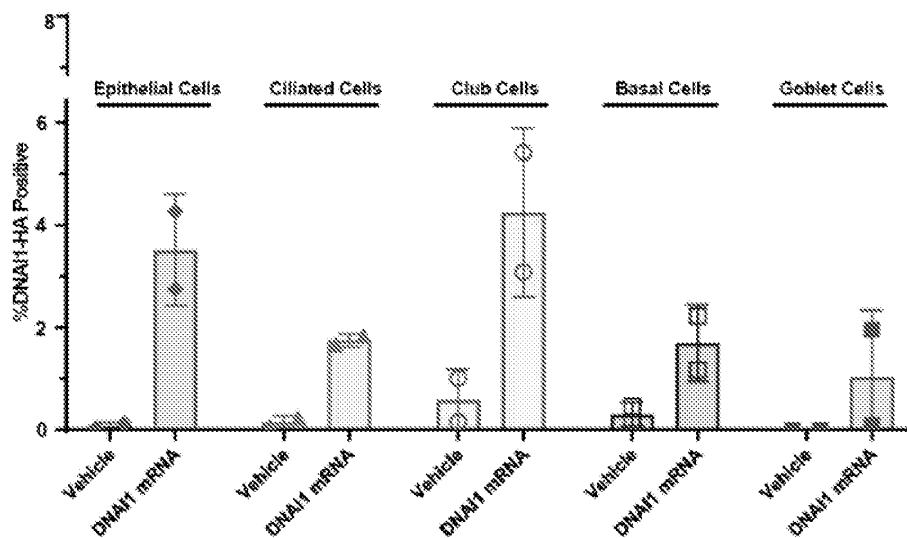


Fig. 30C

Lung - Blocks 1E-4E



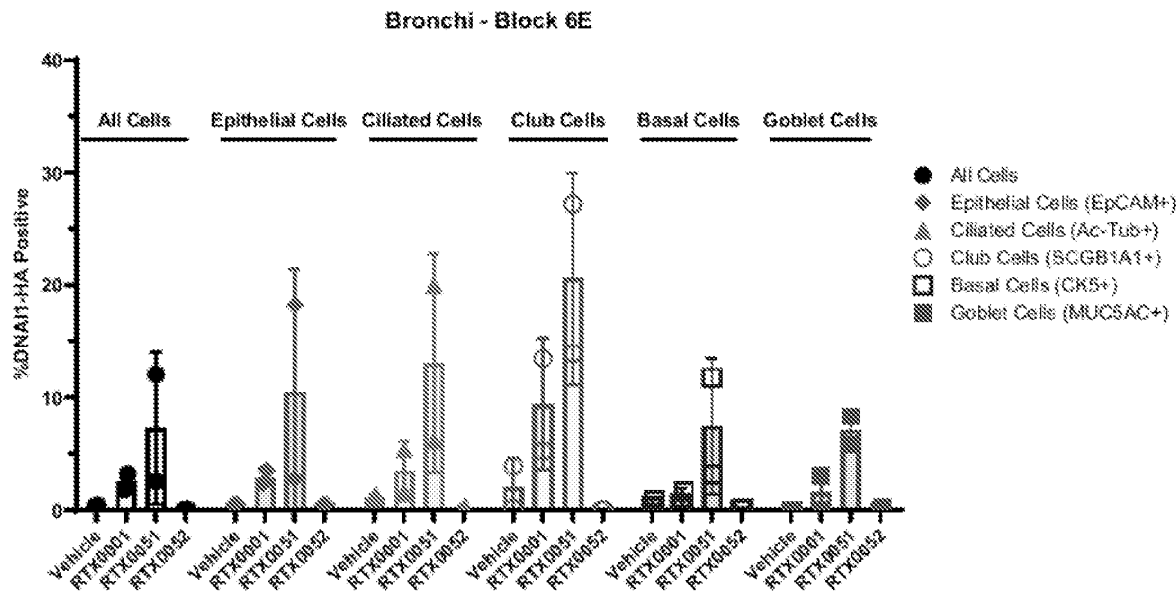
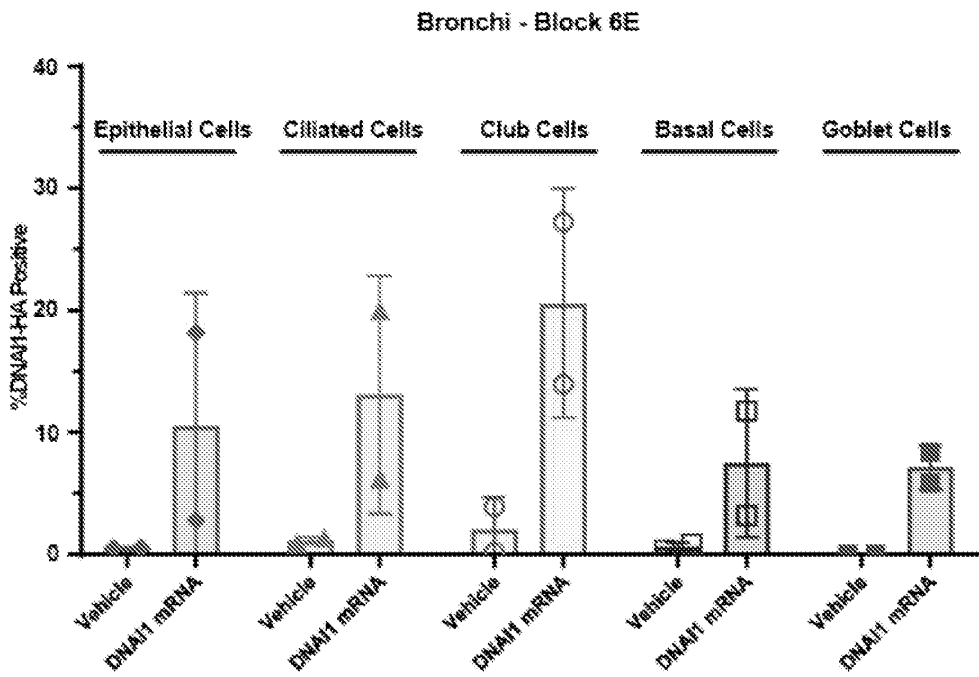
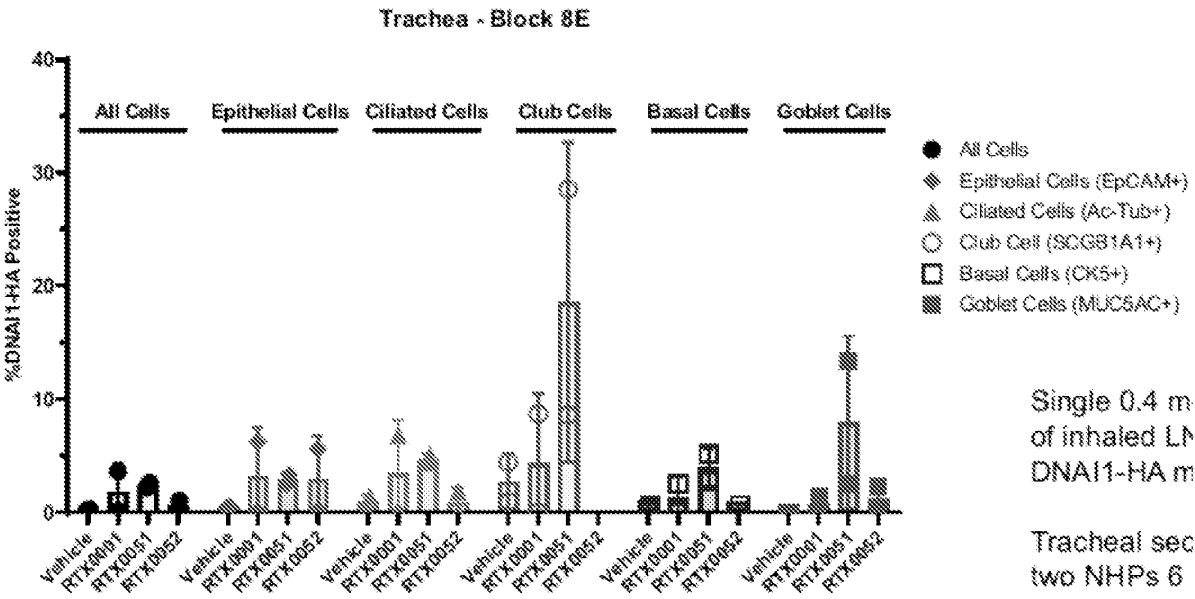


Fig. 30E





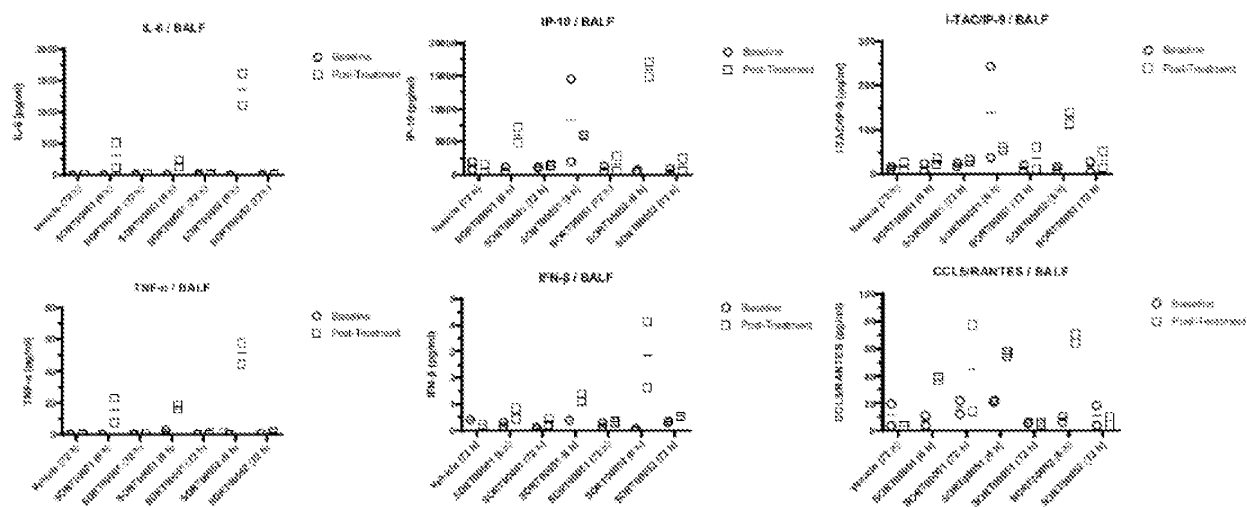
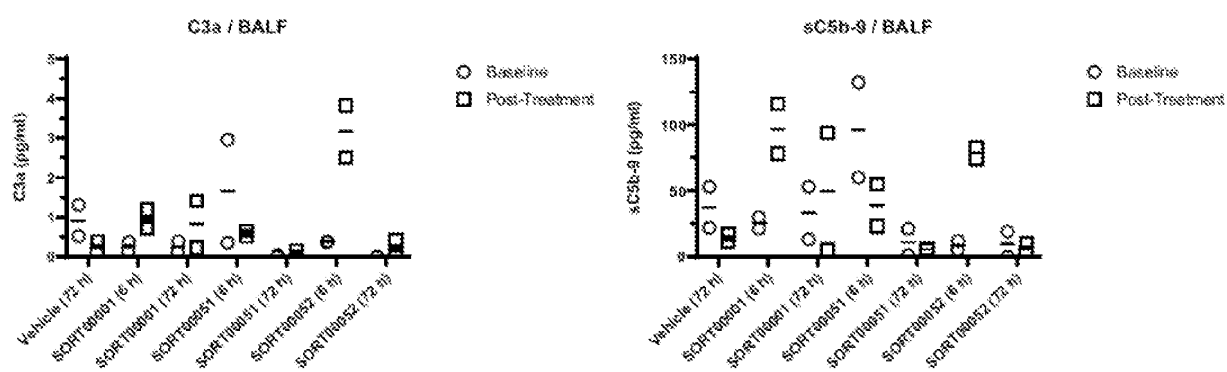
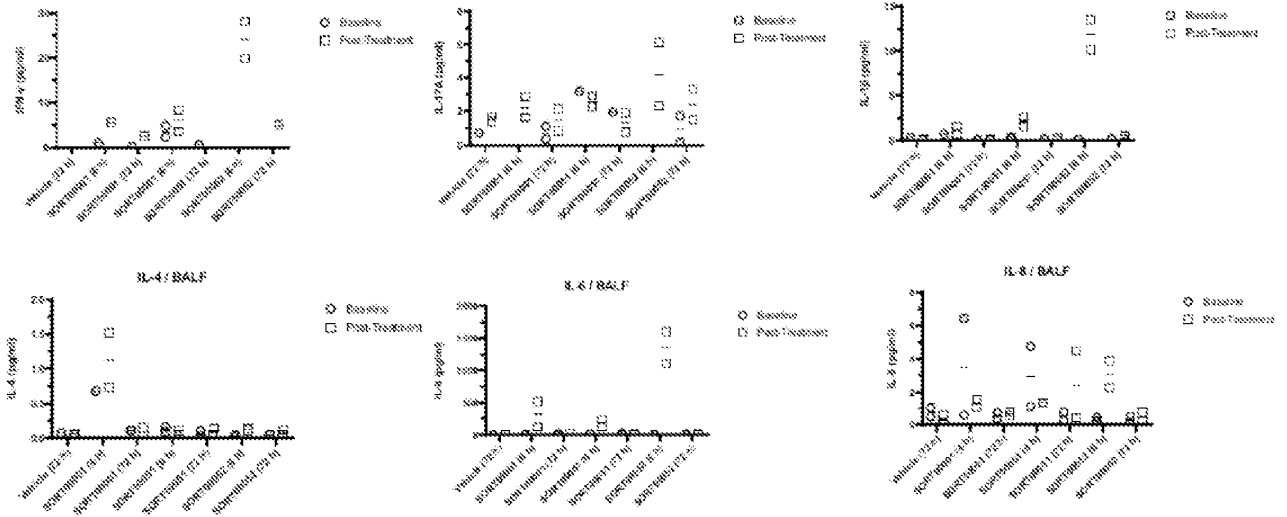


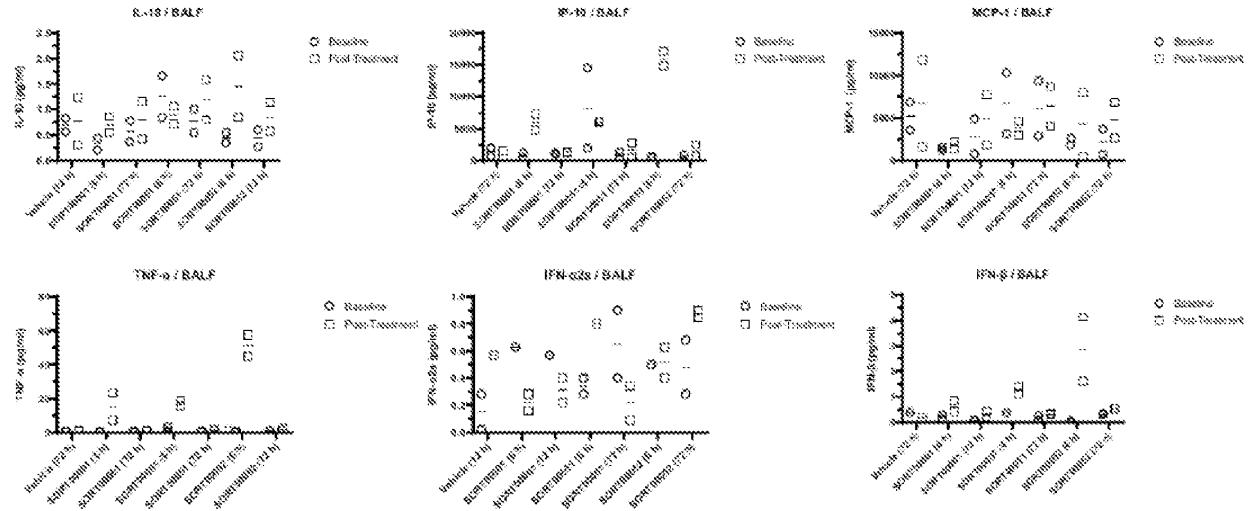
Fig. 31B



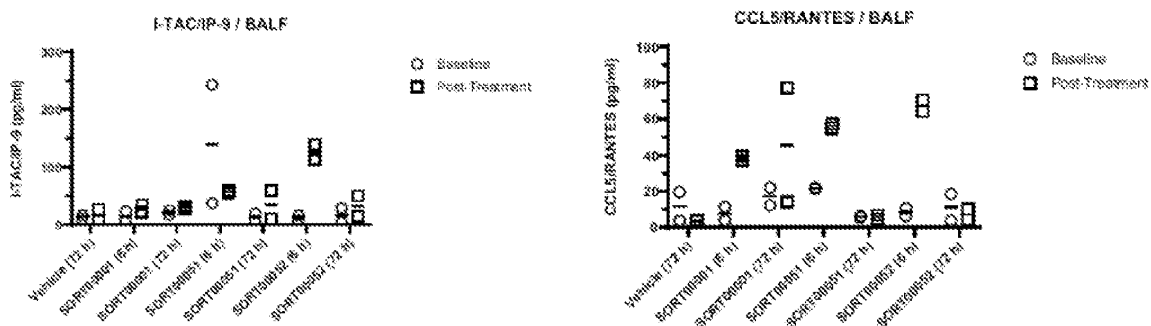
PCT/US2022/021437



8.18.3 BALF



LTACBP-9 / BALF



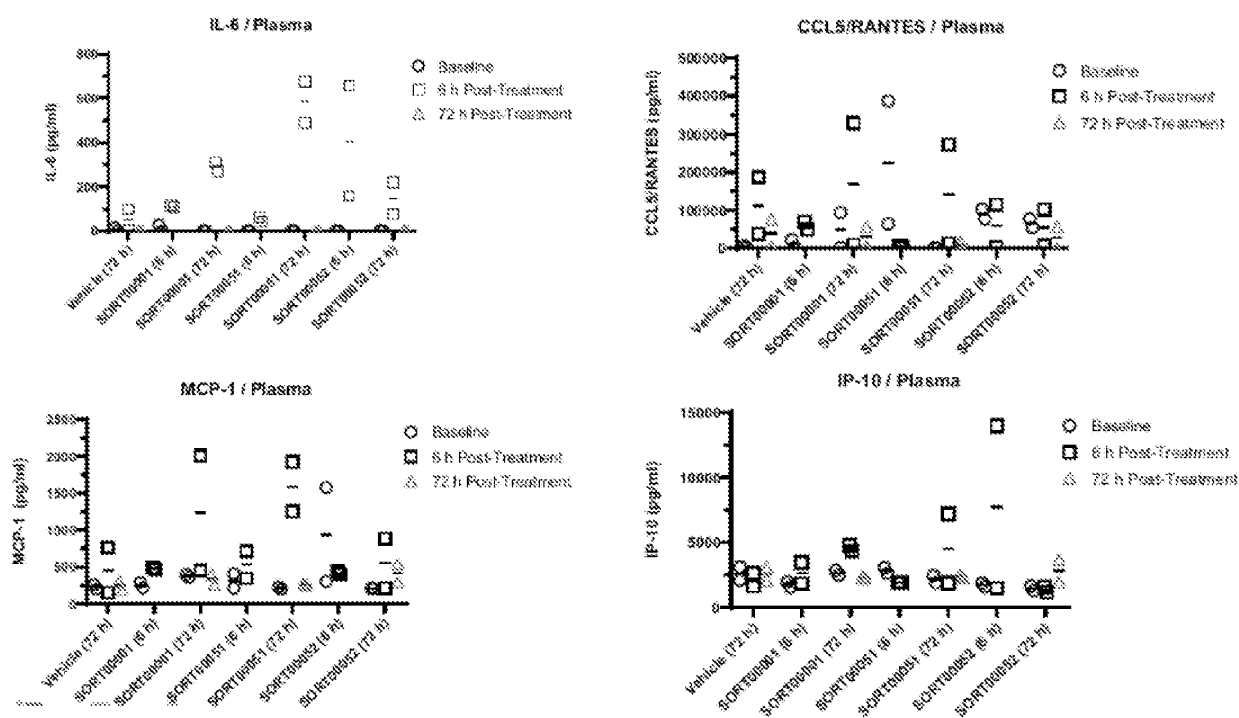
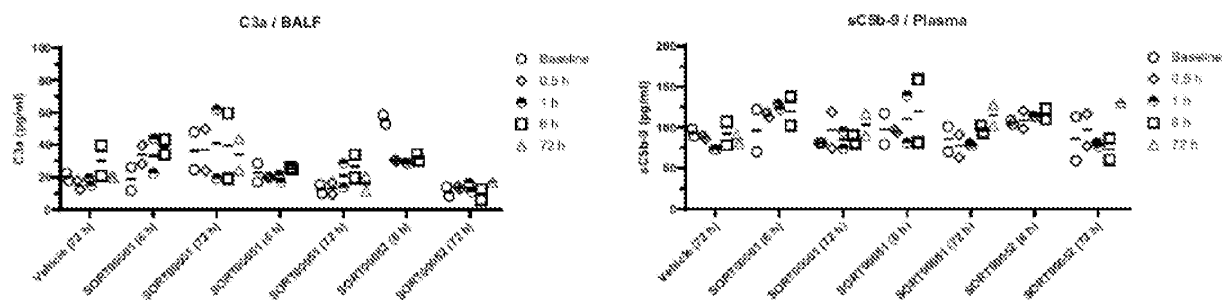


Fig. 32B



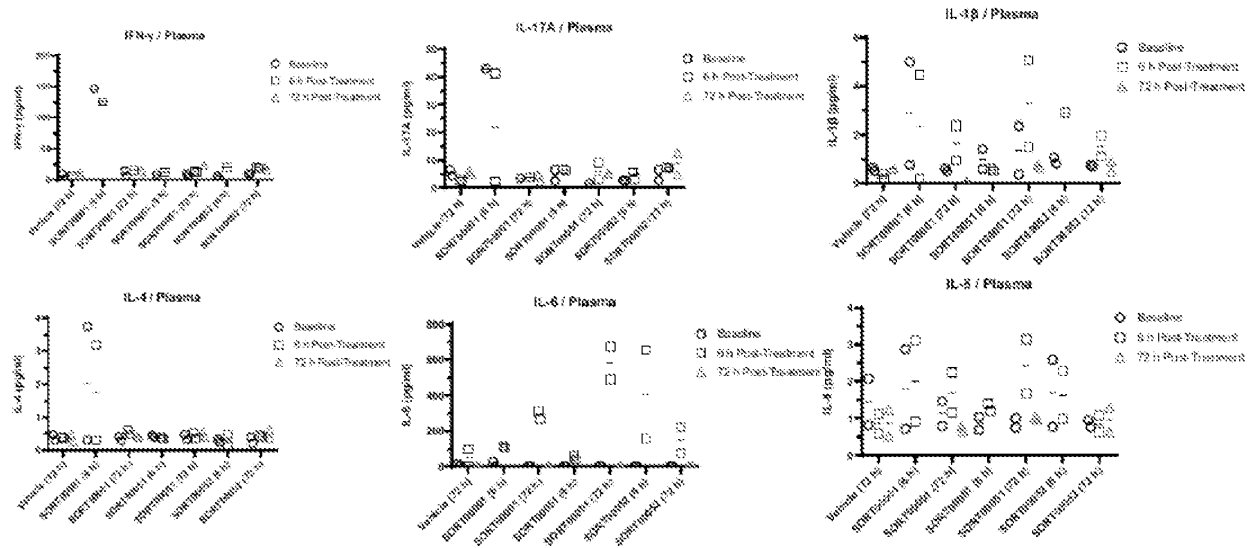


Fig. 32D

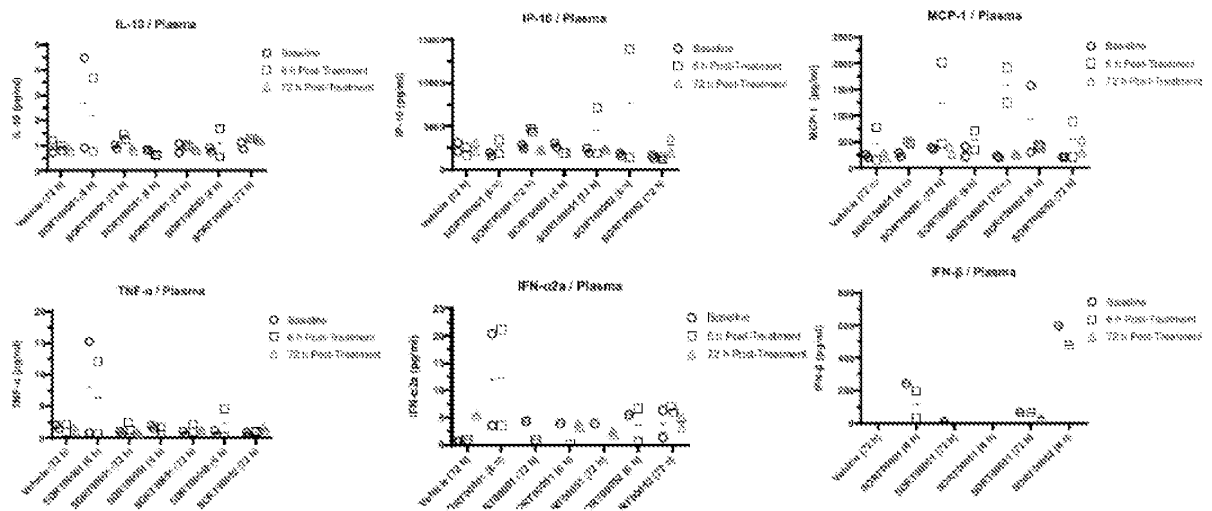
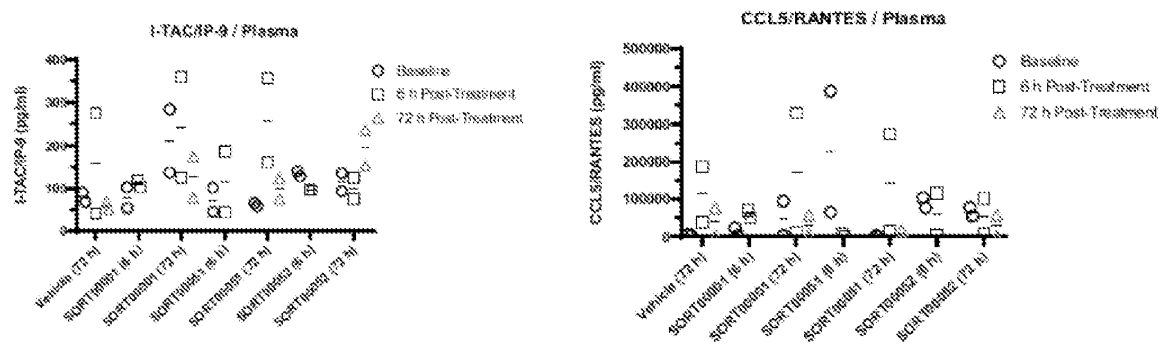
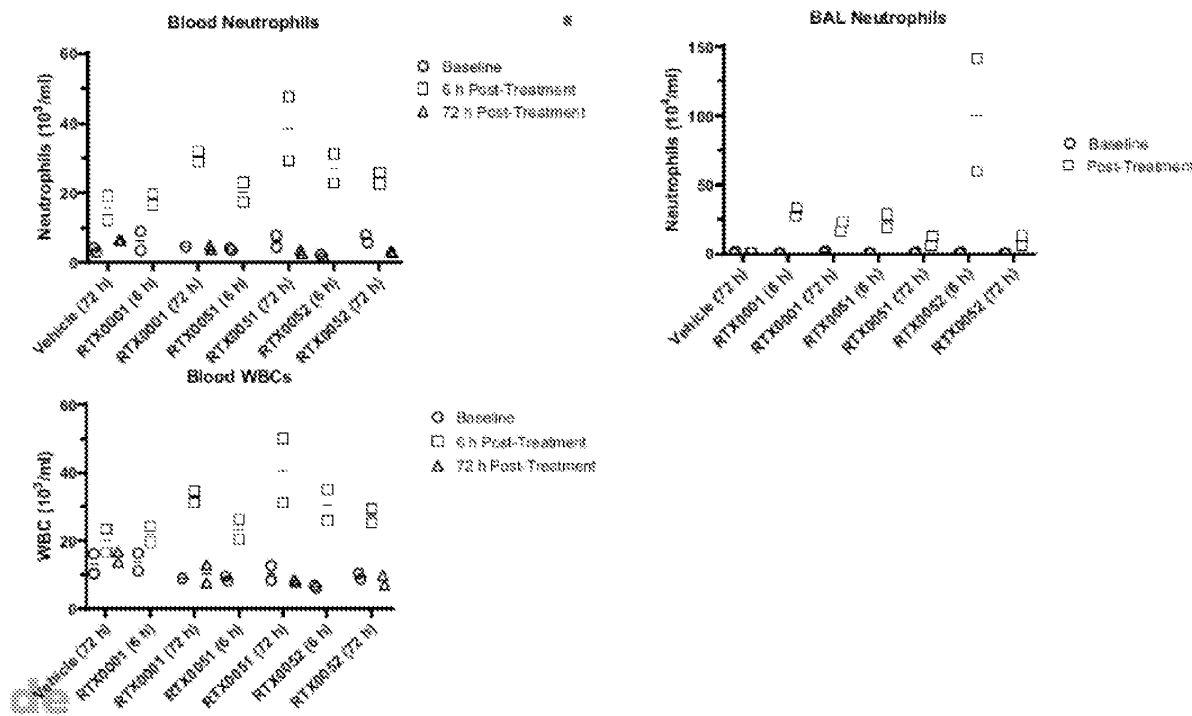


Fig. 32E





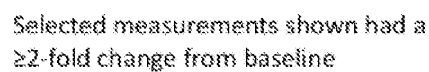


Fig. 35

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

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

Fig. 36A

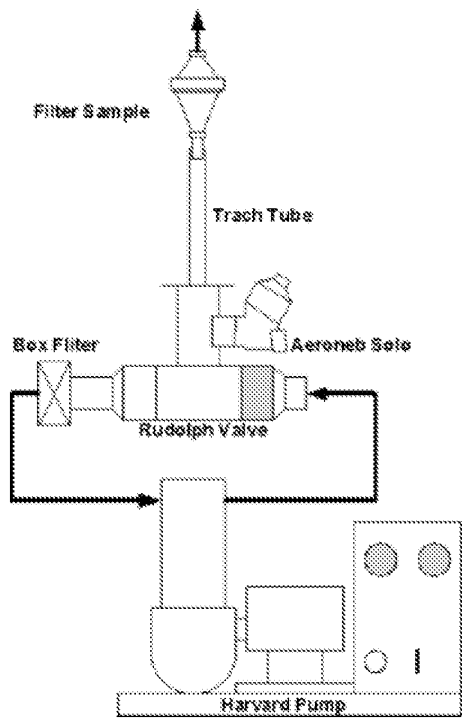


Fig. 36B

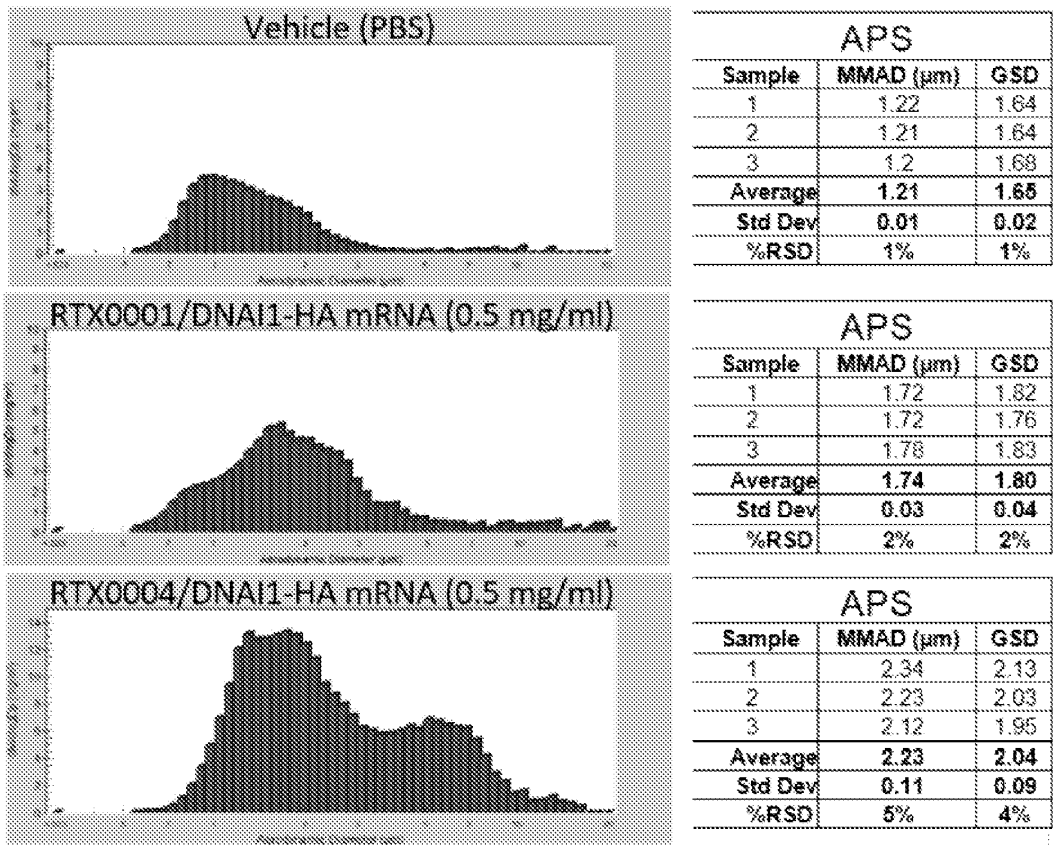


Fig. 37

WO 2022/204215

PCT/US2022/021437

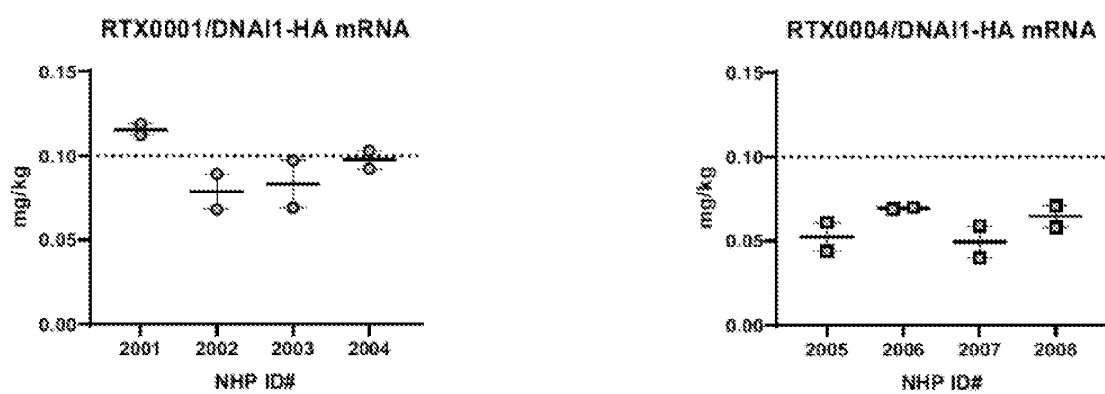


Fig. 38A

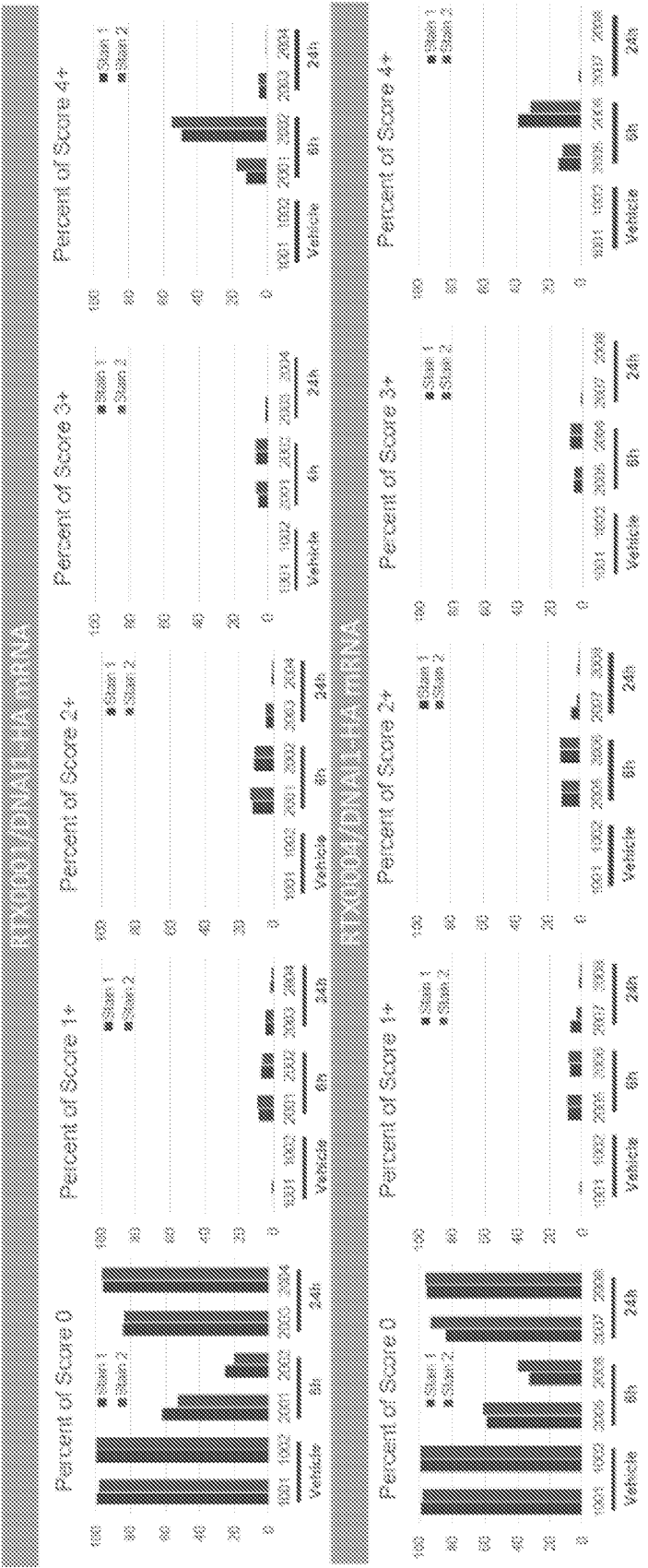


Fig. 38B

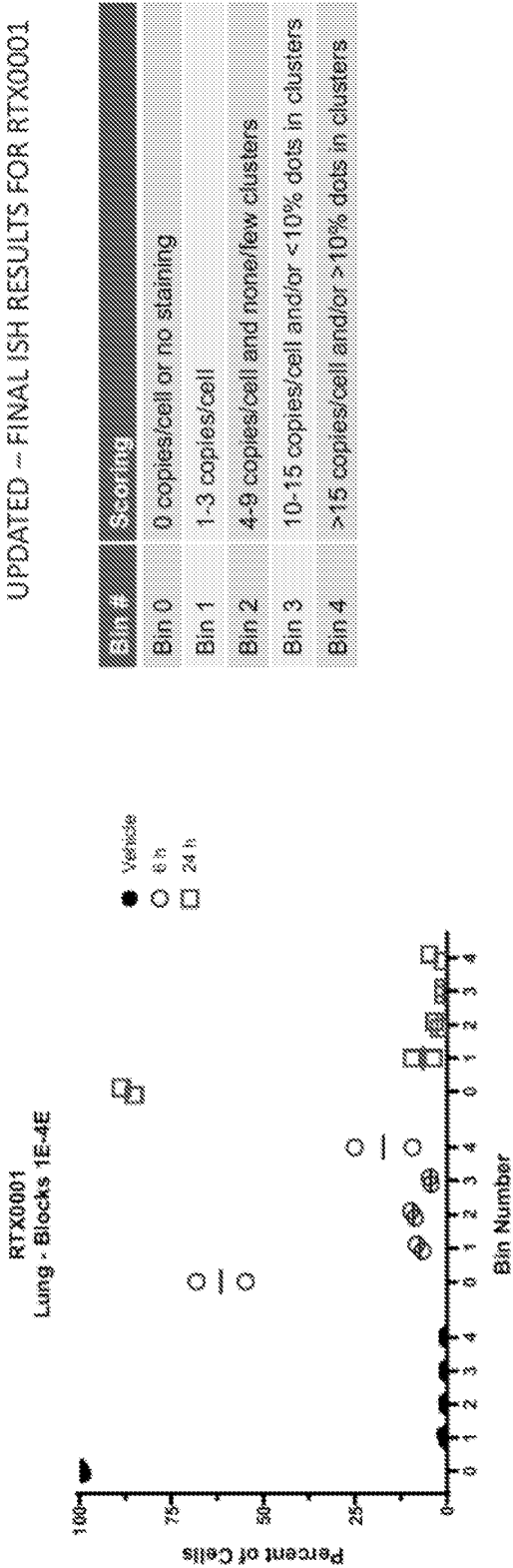
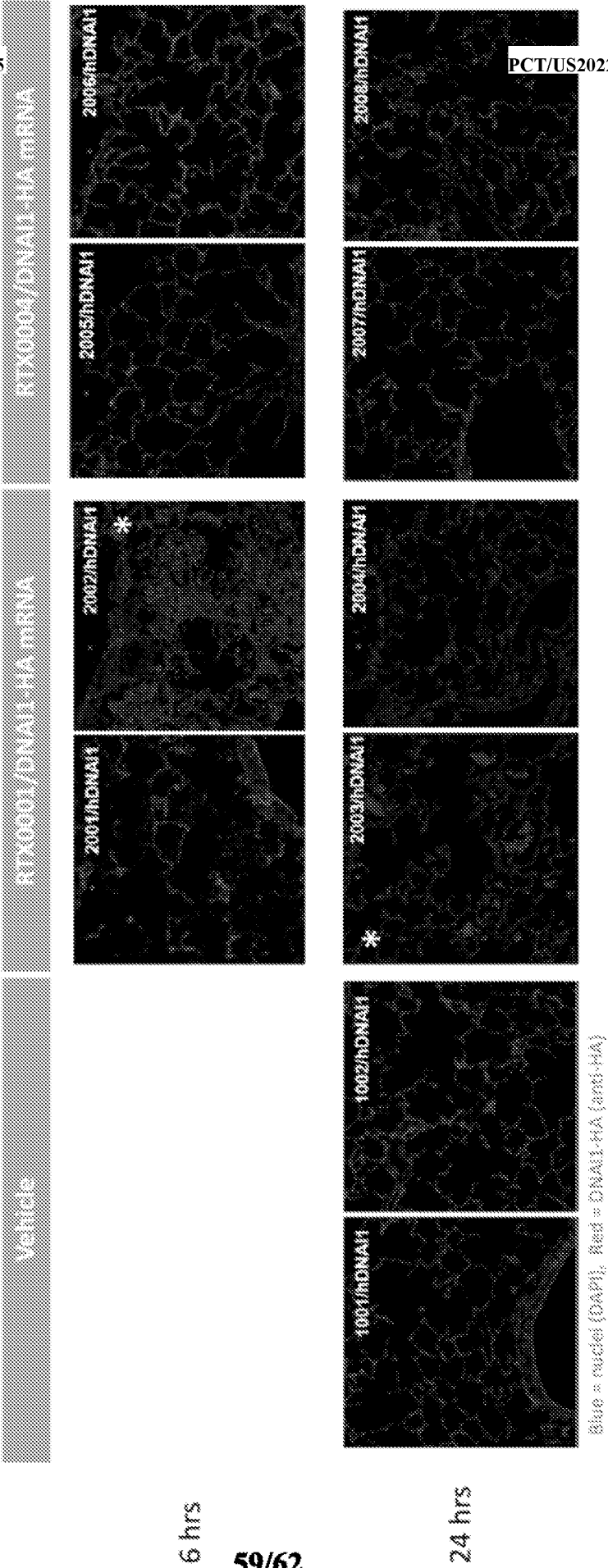


Fig. 38C



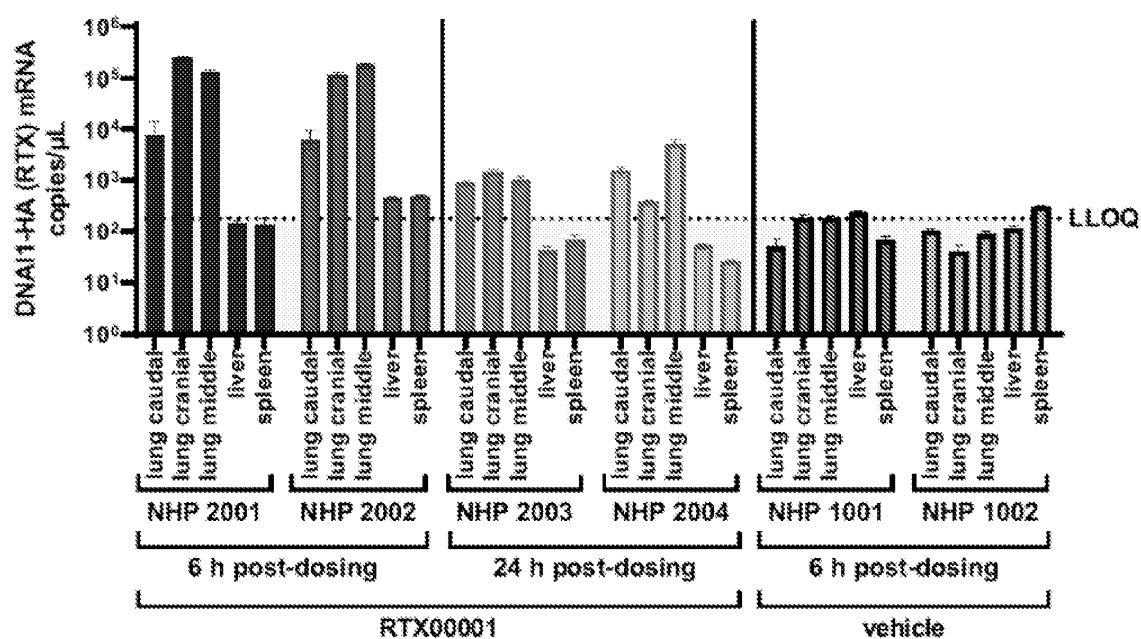
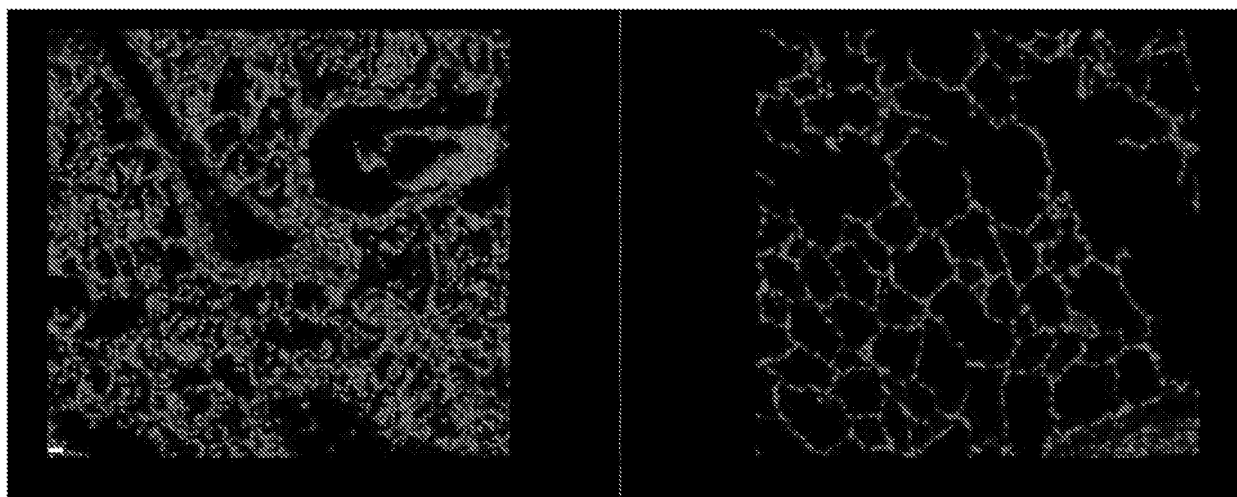


Fig. 39B

RTX0001/DNAI1-HA mRNA
(6 hrs)

Vehicle Treated



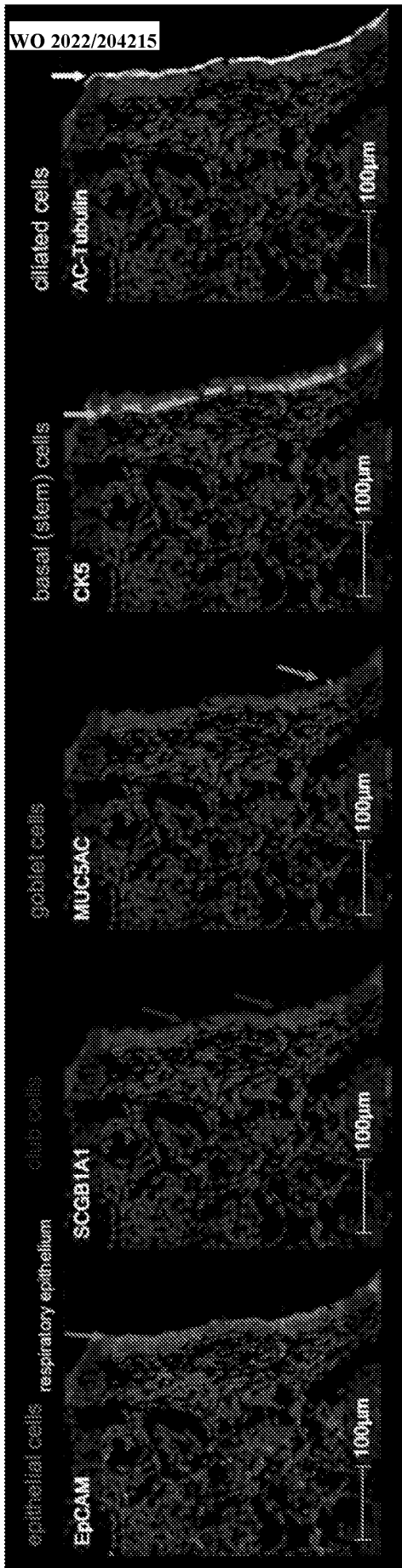


Fig. 39C

PCT/US2022/021437

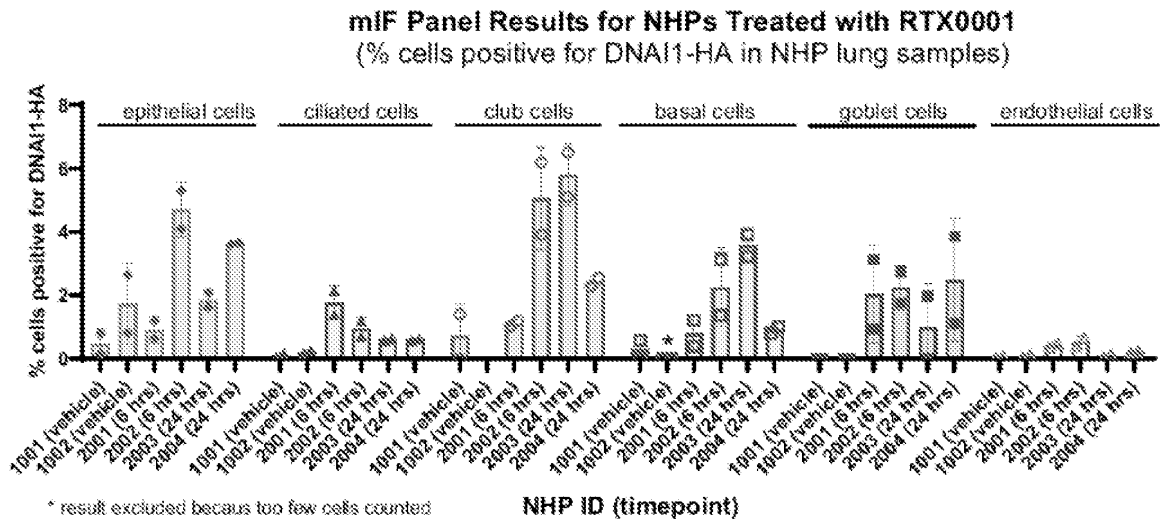


Fig. 40B

