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Supplemental Information

Lentivector Iterations and Pre-Clinical Scale-Up/Toxicity Testing: Targeting Mobilized $CD34^+$ Cells for Correction of Fabry Disease

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SUPPLEMENTARY MATERIAL

Table S1. Sequencing results of LV/FAB integration sites in IVIM study.

Sequence #	Clone #	Vector	c.MOI	Gene Symbol	EntrezGene	Chromosome	Distance to gene	TSS distance
3	3	FAB	**60**	Pde7b	29863	10	intronic	13070
14	5	FAB	**60**	Hey1	15213	3	-67574	-67716
17	5	FAB	**60**	Hey1	15213	3	-67552	-67583
18	4	FAB	**60**	Ahi1	52906	10	intronic	16678

Table S2. Biochemical parameters of NSF mice transplanted with mock or LV/AGA-transduced Fabry patient CD34$^+$ cells.

Mice group	**Mock group**		**LV/AGA group**	
Time after transplant	**Day 7**	**Day 28**	**Day 7**	**Day 28**
ALT (IU/L)	75.4 ± 29.66	91.6 ± 54.68	71.1 ± 62.83	59.4 ± 23.06
AST (IU/L)	270.0 ± 166.63	193.8 ± 106.91	170.5 ± 68.41	184.6 ± 78.00
ALP (IU/L)	46.7 ± 6.72	62.8 ± 12.90	47.2 ± 8.28	59.2 ± 9.69
TBIL (mg/dL)	0.20 ± 0.01	0.16± 0.05	0.16 ± 0.04	0.19 ± 0.05
TP (g/L)	45.8 ± 0.98	49.2 ± 0.94	48.4 ± 1.67	49.5 ± 1.67
ALB (g/L)	25.4 ± 1.53	27.1 ± 1.39	27.3 ± 1.34	27.4 ± 0.46
CHOL (mg/dL)	74.4 ± 8.29	101.2 ± 15.83	84.0 ± 13.51	98.9 ± 19.17
HDL (mg/dL)	35.7 ± 5.24	50.6 ± 11.34	41.2 ± 7.10	50.1 ± 10.35
TRIG (mg/dL)	93.7± 21.34	121.0 ± 78.78	164.7 ± 65.16	135.7 ± 49.84
PHOS (mg/dL)	8.2 ± 0.76	7.6 ± 0.77	7.7 ± 0.55	7.3 ± 0.53
CAL (mg/dL)	9.8 ± 0.27	10.1 ± 0.22	9.9 ± 0.15	10.0 ± 0.27
GLU (mg/dL)	178.2 ± 19.75	188.8 ± 18.38	193.8 ± 21.65	207.4 ± 30.73
CRE (mg/dL)	0.09 ± 0.05	0.09 ± 0.01	0.10 ± 0.02	0.10 ± 0.02
BUN (mg/dL)	35.8 ± 7.20	28.5 ± 4.23	27.1 ± 6.20	29.6 ± 6.00
Na (mmol/L)	150.6 ± 5.65	151.1 ± 3.29	151.0 ± 3.06	150.2 ± 2.36
K (mmol/L)	5.8 ± 0.28	6.3 ± 0.54	5.8 ± 0.47	6.2 ± 0.89
Cl (mmol/L)	114.6 ± 4.02	112.73 ± 2.24	112.6 ± 2.69	113.3 ± 1.52

ALT, Alanine aminotransferase; AST, Asparate aminotransferase; ALP, Alkaline phosphatase; TBIL, Total bilirubin; TP, Total protein; ALB, Albumin; CHOL, Total cholesterol; HDL, HDL Cholesterol; TRIG, Triglycerides; PHOS, Phosphorus; CAL, Calcium; GLU, Glucose; CRE, Creatinine; BUN, Blood urea nitrogen; Na, Sodium; K, Potassium; Cl, Chloride

Table S3. Hematological parameters of NSF mice transplanted with mock or LV/AGA-transduced Fabry patient CD34$^+$ hematopoietic cells at day 7 post-transplant.

Mice group	**Mock group**		**Fabry group**	
Time after transplant	**Day 0**	**Day 7**	**Day 0**	**Day 7**
RBC (10^12/L)	8.9 ± 0.26	6.7 ± 0.21	9.0 ± 0.14	6.9 ± 0.42
Hgb (g/L)	147 ± 6.78	103.8 ± 2.95	149.3 ± 6.19	107.7 ± 6.88
HCT (L/L)	0.54 ± 0.01	0.39 ± 0.01	0.54 ± 0.01	0.40 ± 0.03
MCV (fL)	60.8 ± 0.69	57.5 ± 0.99	60.4 ± 0.68	58.7 ± 0.94
MCH (pg/cell)	16.4 ± 0.46	15.5 ± 0.29	16.6 ± 0.68	15.7 ± 0.50
MCHC (g/L)	270.2 ± 9.86	268.4 ± 1.82	274.6 ± 12.77	267.3 ± 5.97
PLT (10^9/L)	1050 ± 55.5[a]	**130.4 ± 27.2**[b]	963 ± 192.2[c]	**206 ± 36.8**[b]
WBC (10^9/L)	2.3 ± 1.03	0.4 ± 0.11	2.2 ± 0.77	0.5 ± 0.22
MPV (fL)	4.4 ± 0.14	5.9 ± 0.55	4.4 ± 0.15	5.9 ± 0.40
RDW (%)	17.6 ± 0.58	16.4 ± 0.57	17.6 ± 0.60	17.2 ± 0.59
NE (10^9/L)	1.37 ± 0.53	0.14 ± 0.08	1.47 ± 0.59	0.12 ± 0.08
LY (10^9/L)	0.59 ± 0.26	0.22 ± 0.05	0.48 ± 0.09	0.26 ± 0.10
MO (10^9/L)	0.28 ± 0.25	0.08 ± 0.02	0.13 ± 0.04	0.08 ± 0.04
BA (10^9/L)	0.02 ± 0.02	0.00	0.04 ± 0.06	0.00
EO (10^9/L)	0.07 ± 0.07	0.00	0.10 ± 0.09	0.00

RBC, red blood cells; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscle volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelets; WBC, white blood cells; MPV, mean platelet volume; NE, neutrophils; LY, lymphocytes; MO, monocytes; BA, basophils; EO, eosinophils

[a], one sample excluded due to hemolysis; [b], significant different between Mock and LV/AGA group; [c], one sample excluded due to hemolysis

Table S4. Hematological parameters of NSF mice transplanted with mock or LV/AGA-transduced Fabry patient CD34$^+$ hematopoietic cells at day 28 post-transplant.

Mice group	**Mock group**		**Fabry group**	
Time after transplant	**Day 0**	**Day 28**	**Day 0**	**Day 28**
RBC (10^12/L)	8.8 ± 0.27[a]	9.1 ± 0.30	9.1 ± 0.30	7.9 ± 0.24
Hgb (g/L)	141.8 ± 5.04	133.8 ± 8.57	147.3 ± 7.98	130.6 ± 5.93
HCT (L/L)	0.54 ± 0.02	0.54 ± 0.04	0.56 ± 0.02	0.51 ± 0.02
MCV (fL)	60.7 ± 0.74	65.4 ± 1.54	61.2 ± 0.78	64.4 ± 1.18
MCH (pg/cell)	16.0 ± 0.19	16.3 ± 0.75	16.2 ± 0.54	16.5 ± 0.45
MCHC (g/L)	263.3 ± 5.05	249.8 ± 8.84	264.4 ± 9.35	255.6 ± 5.90
RDW (%)	17.7 ± 0.18	21.2 ± 1.77	17.9 ± 0.35	20.9 ± 1.09
PLT (10^9/L)	976.5 ± 60.15[b]	622.5 ± 120.45	915.0 ± 188.64[c]	651.0 ± 141.97
WBC (10^9/L)	2.0 ± 0.52	0.8 ± 0.21	2.2 ± 0.75	1.4 ± 0.63
MPV (fL)	4.5 ± 0.23	4.9 ± 0.26	4.5 ± 0.27	4.9 ± 0.26
NE (10^9/L)	1.26 ± 0.48	**0.50 ± 0.18**[d]	1.31 ± 0.46	**0.84 ± 0.35**[d]
LY (10^9/L)	0.50 ± 0.07	0.25 ± 0.03	0.56 ± 0.17	0.34 ± 0.16
MO (10^9/L)	0.16 ± 0.06	0.05 ± 0.03	0.19 ± 0.10	0.08 ± 0.04
BA (10^9/L)	0.01 ± 0.02	0.00	0.05 ± 0.04	0.03 ± 0.04
EO (10^9/L)	0.04 ± 0.03	0.02 ± 0.01	0.08 ± 0.06	0.08 ± 0.08

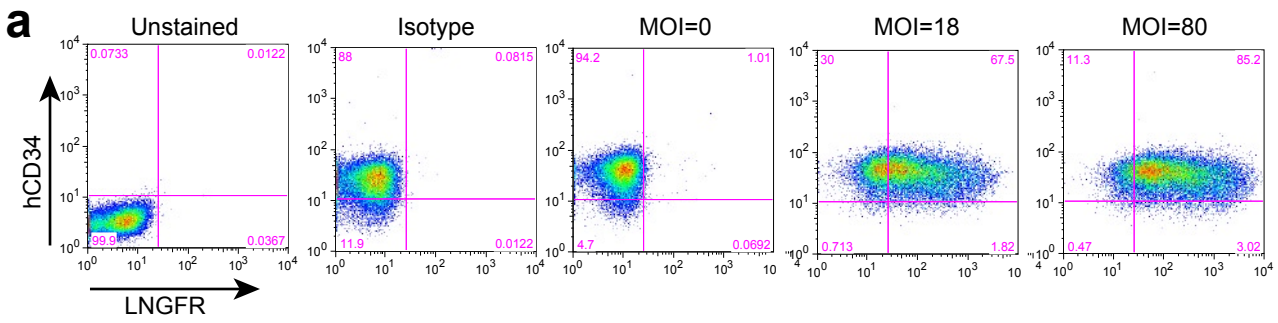
[a], one sample excluded due to hemolysis; [b], one sample excluded due to hemolysis; [c], one sample excluded due to hemolysis; [d], significant different between Mock and LV/AGA group

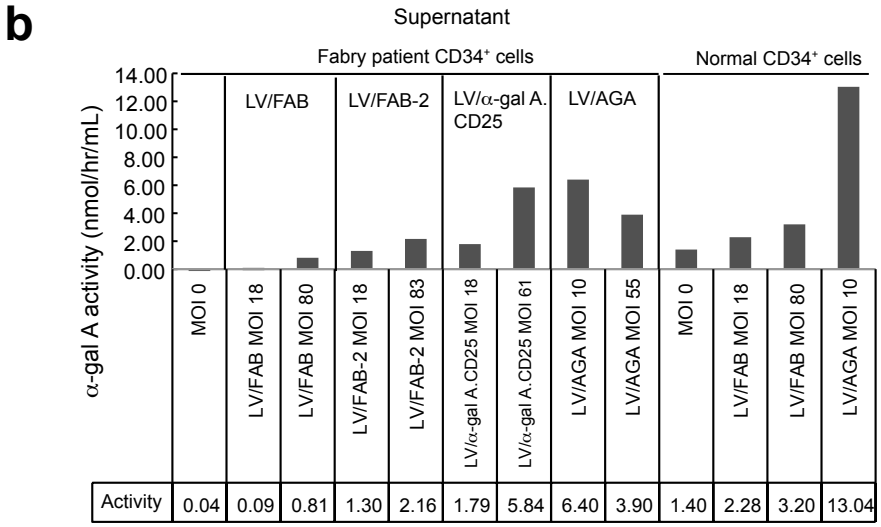
Supplementary Figures Legend

Figure S1. α-Gal A activity in supernatants and cell lysates of $CD34^+$ hematopoietic cells transduced with multiple LVs. $CD34^+$ hematopoietic cells isolated from healthy donor (normal) or Fabry patient #14-159 were transduced independently with each LV preparation. After overnight transduction, cells were washed and cultured for 2 additional days to minimize pseudo-transduction effects. **(a)** Transduction efficiency in LV/FAB-transduced Fabry patient $CD34^+$ cells were determined by staining cells with a monoclonal antibody against human LNGFR (i.e. CD271) and flow cytometry analyses. Cells were also stained with an antibody against human CD34 to confirm phenotype. Cell culture was harvested, and secreted (supernatant) **(b)** and intracellular (cell lysate) **(c)** α-gal A activity was measured as described in the Materials and Methods section.

Figure S2. **Sample ion chromatograms of 22 Gb_3 isoforms analyzed by UPLC-MS/MS from mouse spleen tissue samples. (a)** High abundance Gb_3 isoforms; **(b)** Low abundance Gb_3 isoforms. Cps = counts per second

Figure S3. Platelet and neutrophil levels in NSF mice following the pre-clinical Toxicology study. (a) Mice from the LV/AGA group showed significantly higher levels of platelets (PLT) than mice from the Mock group at Day 7 after transplant (Day+7). **(b)** Mice from the LV/AGA group showed significantly higher levels of neutrophils (NE) than mice from the Mock group at Day 28 after transplant (Day+28).


a
Unstained
Isotype
MOI=0
MOI=18
MOI=80
hCD34
LNGFR
0.0733
0.0122
99.9
0.0367
88
0.0815
11.9
0.0122
94.2
1.01
4.7
0.0692
30
67.5
0.713
1.82
11.3
85.2
0.47
3.02


b
Supernatant
Fabry patient CD34⁺ cells
Normal CD34⁺ cells
α-gal A activity (nmol/hr/mL)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
LV/FAB
LV/FAB-2
LV/α-gal A. CD25
LV/AGA
MOI 0
LV/FAB MOI 18
LV/FAB MOI 80
LV/FAB-2 MOI 18
LV/FAB-2 MOI 83
LV/α-gal A.CD25 MOI 18
LV/α-gal A.CD25 MOI 61
LV/AGA MOI 10
LV/AGA MOI 55
MOI 0
LV/FAB MOI 18
LV/FAB MOI 80
LV/AGA MOI 10
Activity
0.04
0.09
0.81
1.30
2.16
1.79
5.84
6.40
3.90
1.40
2.28
3.20
13.04

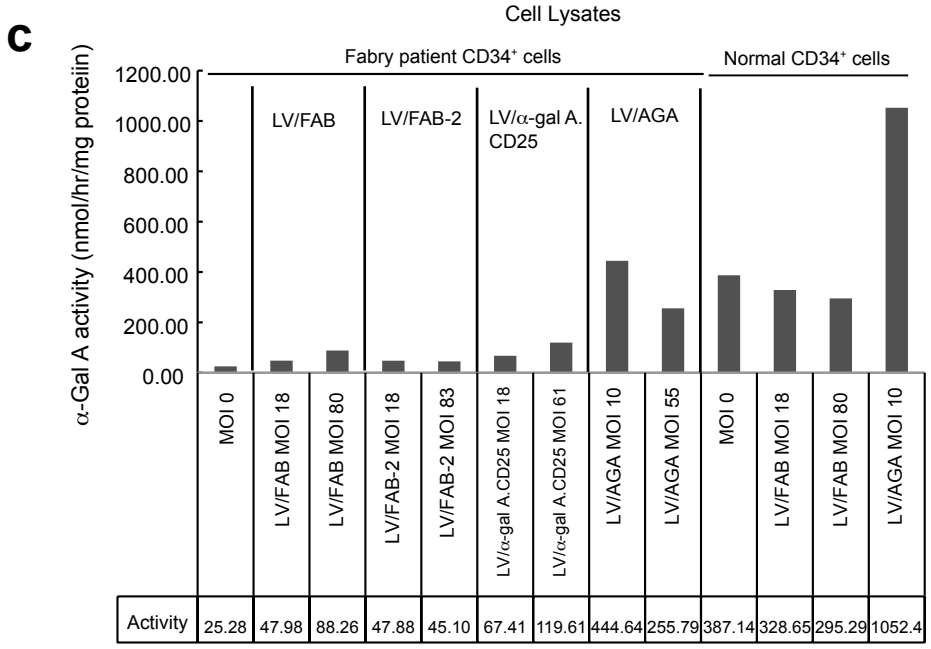

c
Cell Lysates
Fabry patient CD34⁺ cells
Normal CD34⁺ cells
α-Gal A activity (nmol/hr/mg protein)
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
LV/FAB
LV/FAB-2
LV/α-gal A. CD25
LV/AGA
MOI 0
LV/FAB MOI 18
LV/FAB MOI 80
LV/FAB-2 MOI 18
LV/FAB-2 MOI 83
LV/α-gal A.CD25 MOI 18
LV/α-gal A.CD25 MOI 61
LV/AGA MOI 10
LV/AGA MOI 55
MOI 0
LV/FAB MOI 18
LV/FAB MOI 80
LV/AGA MOI 10
Activity
25.28
47.98
88.26
47.88
45.10
67.41
119.61
444.64
255.79
387.14
328.65
295.29
1052.4

Figure S1

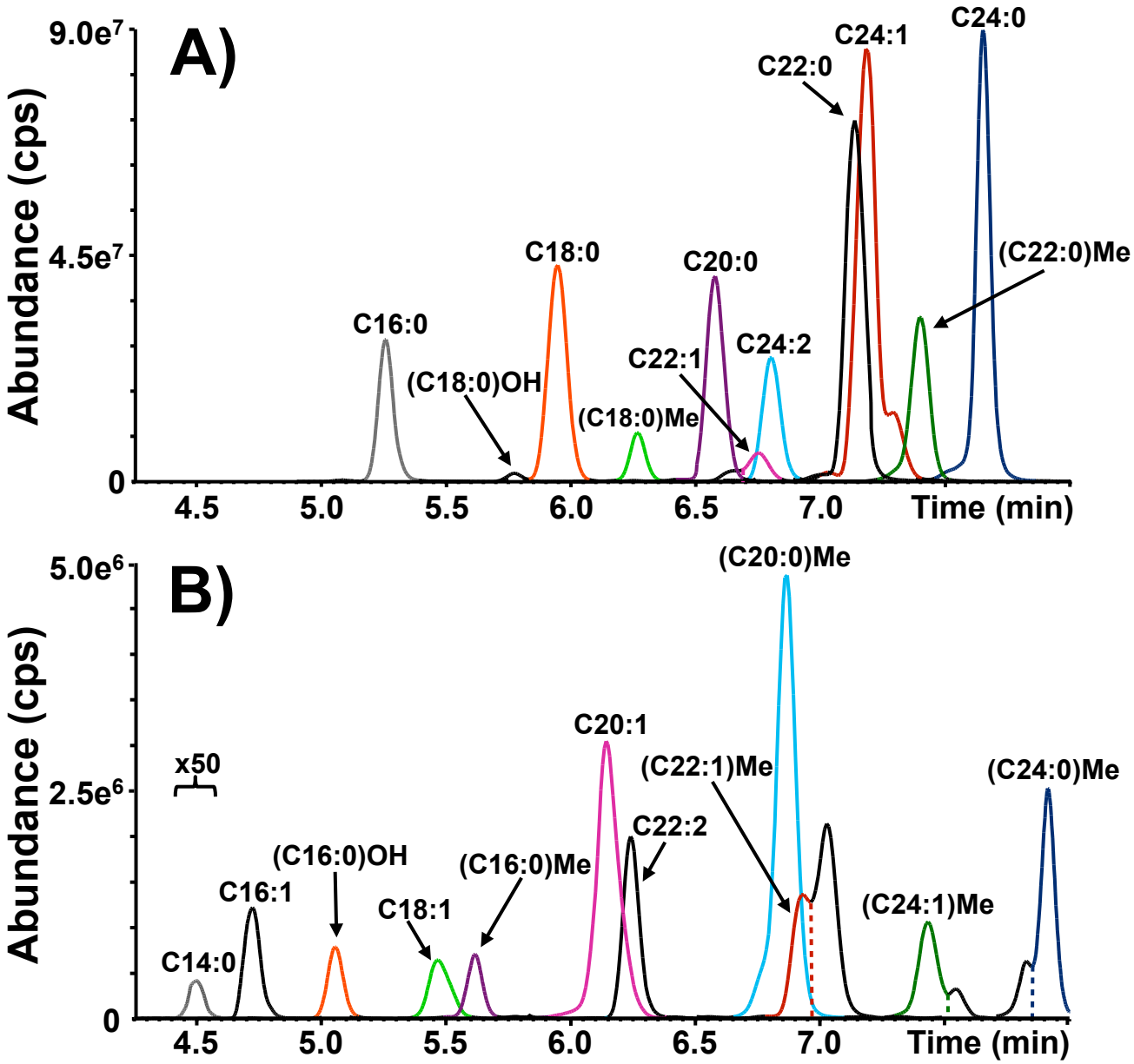

A)
9.0e7
4.5e7
0
Abundance (cps)
C16:0
(C18:0)OH
C18:0
(C18:0)Me
C20:0
C22:1
C24:2
C22:0
C24:1
C24:0
(C22:0)Me
4.5
5.0
5.5
6.0
6.5
7.0
Time (min)
B)
5.0e6
2.5e6
0
Abundance (cps)
x50
C14:0
C16:1
(C16:0)OH
C18:1
(C16:0)Me
C20:1
C22:2
(C20:0)Me
(C22:1)Me
(C24:1)Me
(C24:0)Me
4.5
5.0
5.5
6.0
6.5
7.0
Time (min)

Figure S2

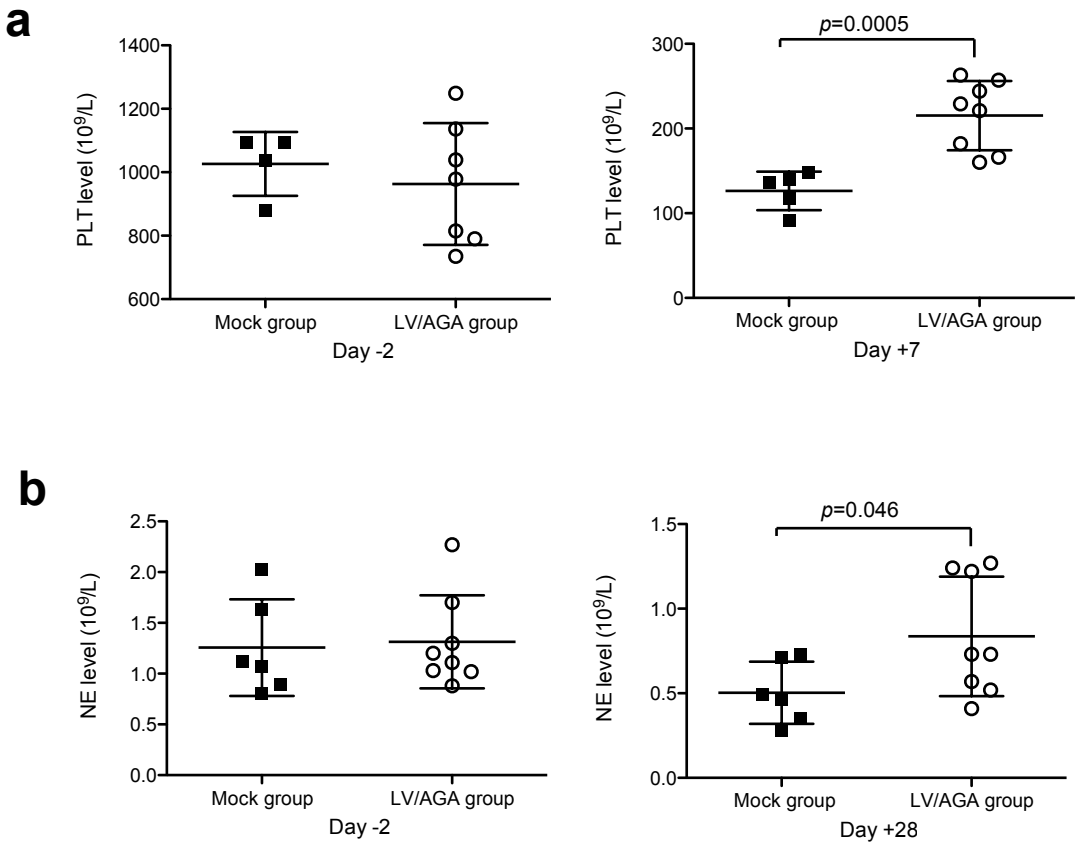

a
1400
1200
1000
800
600
PLT level (10^9/L)
Mock group
LV/AGA group
Day -2
p=0.0005
300
200
100
0
PLT level (10^9/L)
Mock group
LV/AGA group
Day +7
b
2.5
2.0
1.5
1.0
0.5
0.0
NE level (10^9/L)
Mock group
LV/AGA group
Day -2
p=0.046
1.5
1.0
0.5
0.0
NE level (10^9/L)
Mock group
LV/AGA group
Day +28

Figure S3