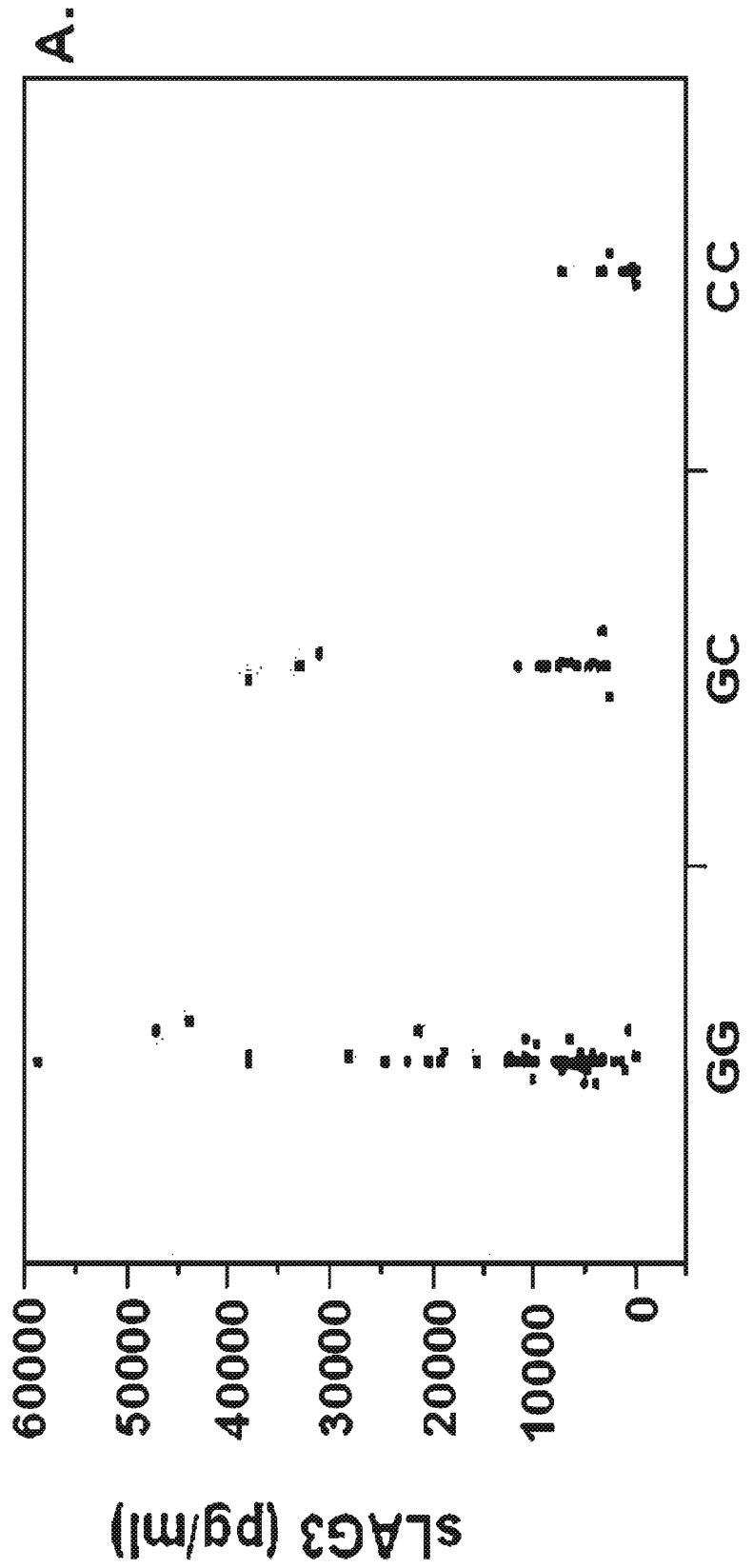

A.
sLAG3 (pg/ml)
60000
50000
40000
30000
20000
10000
0
GG
GC
CC
rs10846744

FIG. 1

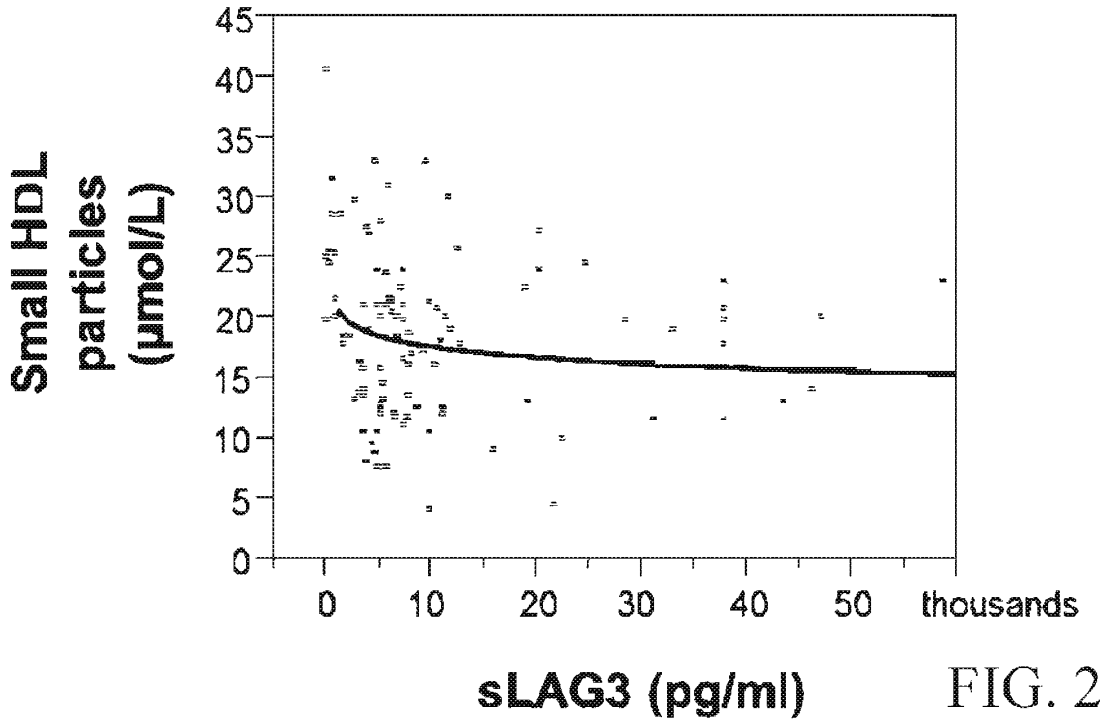


FIG. 2

Murine soluble Lag-3, extracellular, without signal peptide. Accession Number:

UniProtKB-Q61790

SGPGKELPVVWAQEGAPVHLPCSLKSPNLDPNFLRRGGVIWQHQPDSGQPTPIPALDLHQ
GMPSPRQPAPGRYTVLSVAPGGLRSGRQPLHPHVQLEERGLQRGDFSLWLRPALRTDAGE
YHATVRLPNRALSCSLRLRVGQASMIASPSGVLKLSDWVLLNCSFSRPDRPVSVHWFQGQ
NRVPVYNSPRHFLAETFLLLPQVSPLDSGTWGCVLTYRDGFNVSITYNLKVLGLEPVAPL
TVYAAEGSRVELPCHLPPGVGTPSLLIAKWTPPGGGPELPVAGKSGNFTLHLEAVGLAQA
GTYTCSIHLQGQQLNATVTLAVITVTPKSFGLPGSRGKLLCEVTPASGKERFVWRPLNNL
SRSCPGPVLEIQEARLLAERWQCQLYEGQRLLGATVYAAESSSGAHSARRISGDLKGGHL

FIG. 4

Human LAG-3 proteins. Accession number: NP_002277, 525 amino acids full length

mweaqflgll flqplwvapv kplqpgaevp vvwaqegapa qlpcsptipl qdlsllrrag
vtwqhqpdsg ppaaapghpl apgphpaaps swgprprryt vlsvgpgglr sgrlplqprv
qldergrgrg dfslwlrpar radageyraa vhlrdralsc rlrlrlgqas mtasppgslr
asdwvilncs fsrpdrpasv hwfrnrgqgr vpvresphhh laesflflpq vspmdsgpwg
ciltyrdgfn vsimynltvl glepptpltv yagagsrvgl pcrlpagvgt rsfltakwtp
pgggpdllvt gdngdftlrl edvsqaqagt ytchihlqeq qlnatvtlai itvtpksfgs
pgslgkllce vtpvsgqerf vwsaldtpsq rsfsgpwlea qeaqllsqpw qcqlyqgerl
lgaavyftel sspgaqrsgr apgalpaghl llfllilgvls llllvtgafg fhlwrrqwrp
rrfsaleqgi hppqaqskie eleqepepep epepepepep epeql

Human LAG-3 proteins. Accession number: XXX, truncated soluble LAG-3

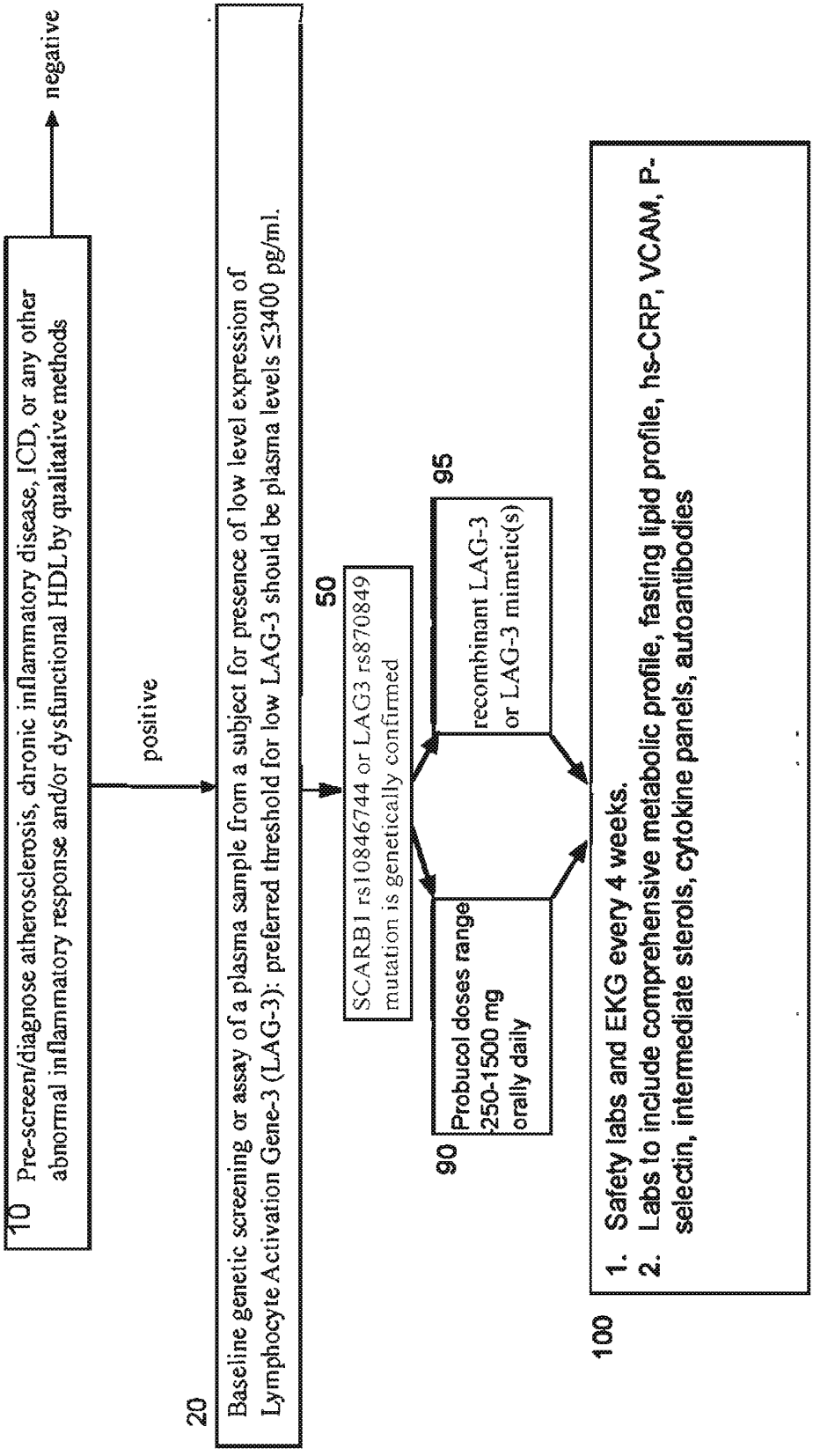

10
Pre-screen/diagnose atherosclerosis, chronic inflammatory disease, ICD, or any other abnormal inflammatory response and/or dysfunctional HDL by qualitative methods
negative
positive
20
Baseline genetic screening or assay of a plasma sample from a subject for presence of low level expression of Lymphocyte Activation Gene-3 (LAG-3): preferred threshold for low LAG-3 should be plasma levels ≤3400 pg/ml.
50
SCARB1 rs10846744 or LAG3 rs870849 mutation is genetically confirmed
90
Probucol doses range 250-1500 mg orally daily
95
recombinant LAG-3 or LAG-3 mimetic(s)
100
1. Safety labs and EKG every 4 weeks.
2. Labs to include comprehensive metabolic profile, fasting lipid profile, hs-CRP, VCAM, P-selectin, intermediate sterols, cytokine panels, autoantibodies

FIG. 3

Table 2. Multivariable regression analysis of independent predictors for plasma LAG-3 (A) and HDL-C (B): MESA.

A. Multivariable regression analysis of independent predictors for plasma LAG-3: MESA.

Covariates	N	Beta	SE	P value
	5137			
Rs10846744:				
GG:		183.98	91.1	0.04
GC:		-261.8	80.1	0.001
Race:				0.0005
White		-641.37	408.5	
Chinese		491.2	1061.6	
African American		862.7	450.2	
Age		-19.81	6.7	0.003
Lipid meds		153.22	70.5	0.03
Smoking		-10.95	2.72	<0.0001

Covariates included race, PCs of ancestry, age, study sites, sex, Hgb_{A1c}, BMI, lipid medications, lipids (TC, LDL-C, HDL-C), smoking pack years, systolic BP, and diastolic BP. LAG3 was log-transformed.

B. Multivariable regression analysis of independent predictors for HDL-C: MESA.

Covariates	N	Beta	SE	P value
	4953			
LAG3		-0.770	0.28	0.007
Age		0.056	0.02	0.006
Triglycerides		-0.062	0.002	<0.0001
Sex: Men		5.67	0.19	<0.0001
BMI		-0.623	0.035	<0.0001
Hgb_{A1c}		-0.450	0.181	0.01
Alcohol		3.28	0.370	<0.0001
Systolic BP		0.027	0.01	0.03

Covariates included race, PCs of ancestry, age, study sites, sex, Hgb_{A1c}, BMI, lipid medications, lipids (triglycerides), smoking pack years, current alcohol use, systolic BP, and diastolic BP. LAG3 was log-transformed.

FIG. 5

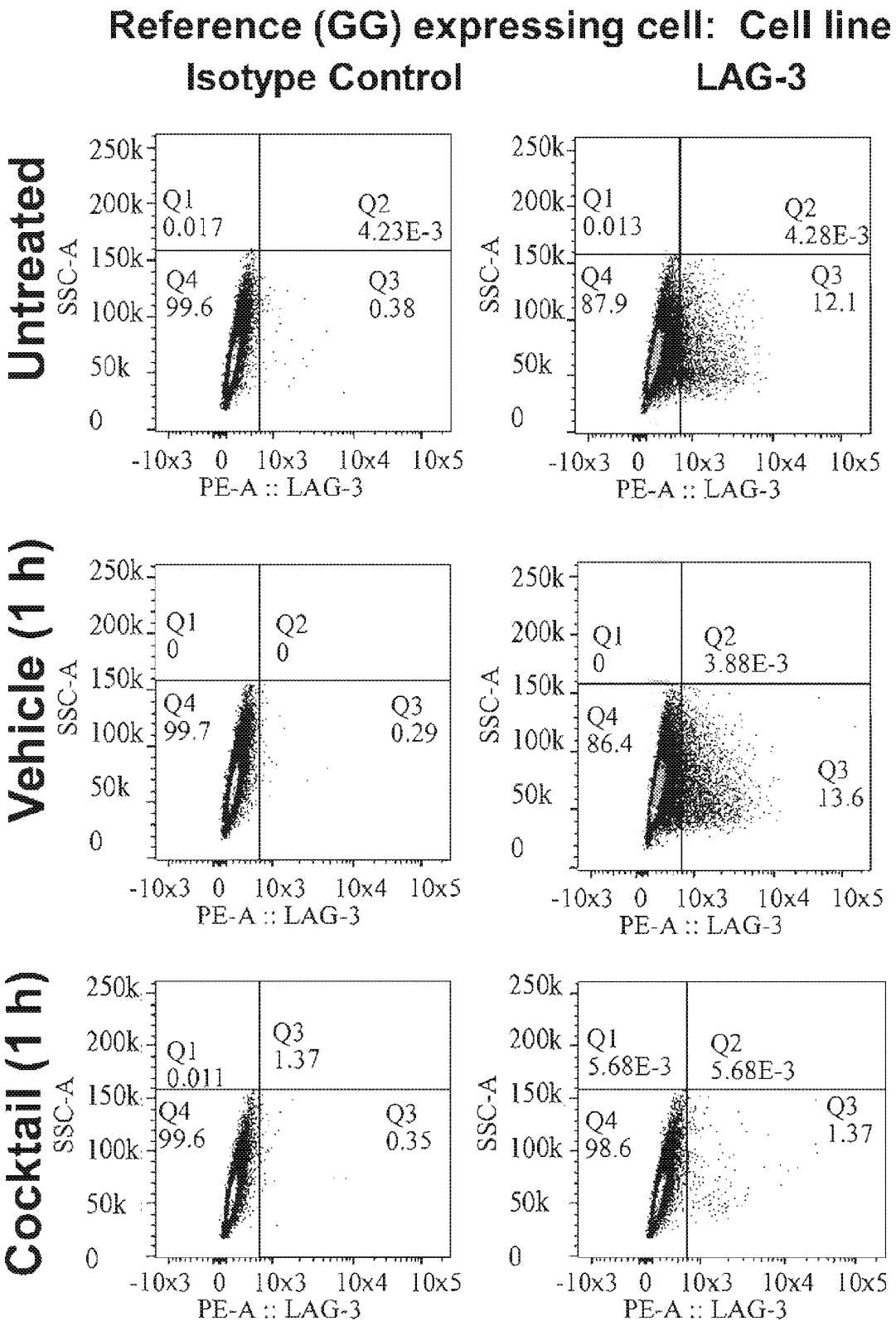

Reference (GG) expressing cell: Cell line
Isotype Control
LAG-3
Untreated
Vehicle (1 h)
Cocktail (1 h)
SSC-A
PE-A :: LAG-3
250k
200k
150k
100k
50k
0
-10x3 0 10x3 10x4 10x5
Q1 0.017
Q2 4.23E-3
Q4 99.6
Q3 0.38
Q1 0.013
Q2 4.28E-3
Q4 87.9
Q3 12.1
Q1 0
Q2 0
Q4 99.7
Q3 0.29
Q1 0
Q2 3.88E-3
Q4 86.4
Q3 13.6
Q1 0.011
Q3 1.37
Q4 99.6
Q3 0.35
Q1 5.68E-3
Q2 5.68E-3
Q4 98.6
Q3 1.37

FIG. 6

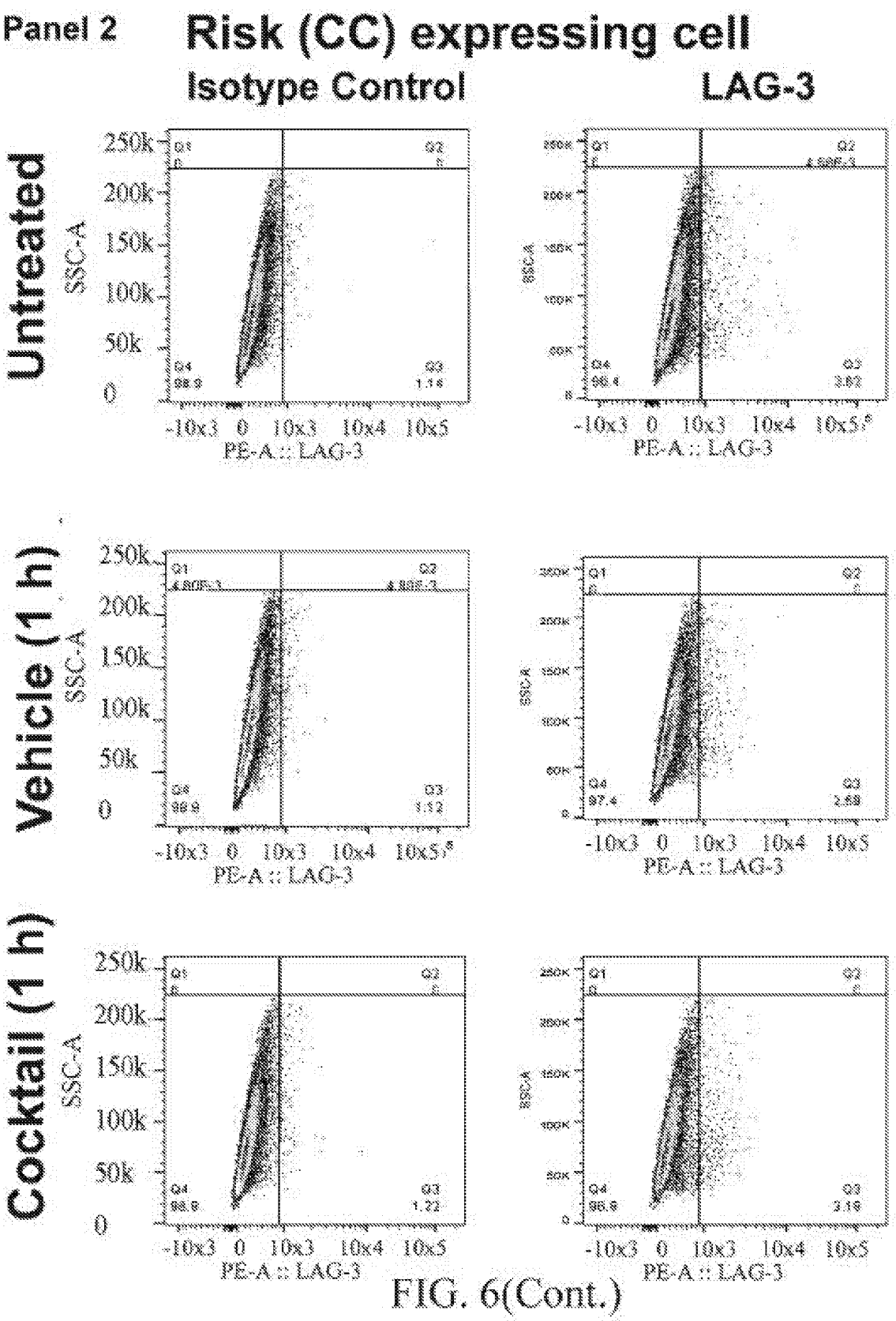

Panel 2
Risk (CC) expressing cell
Isotype Control
LAG-3
Untreated
Vehicle (1 h)
Cocktail (1 h)
SSC-A
PE-A :: LAG-3
250k
200k
150k
100k
50k
0
-10x3 0 10x3 10x4 10x5
Q1
Q2
Q3
Q4

FIG. 6(Cont.)

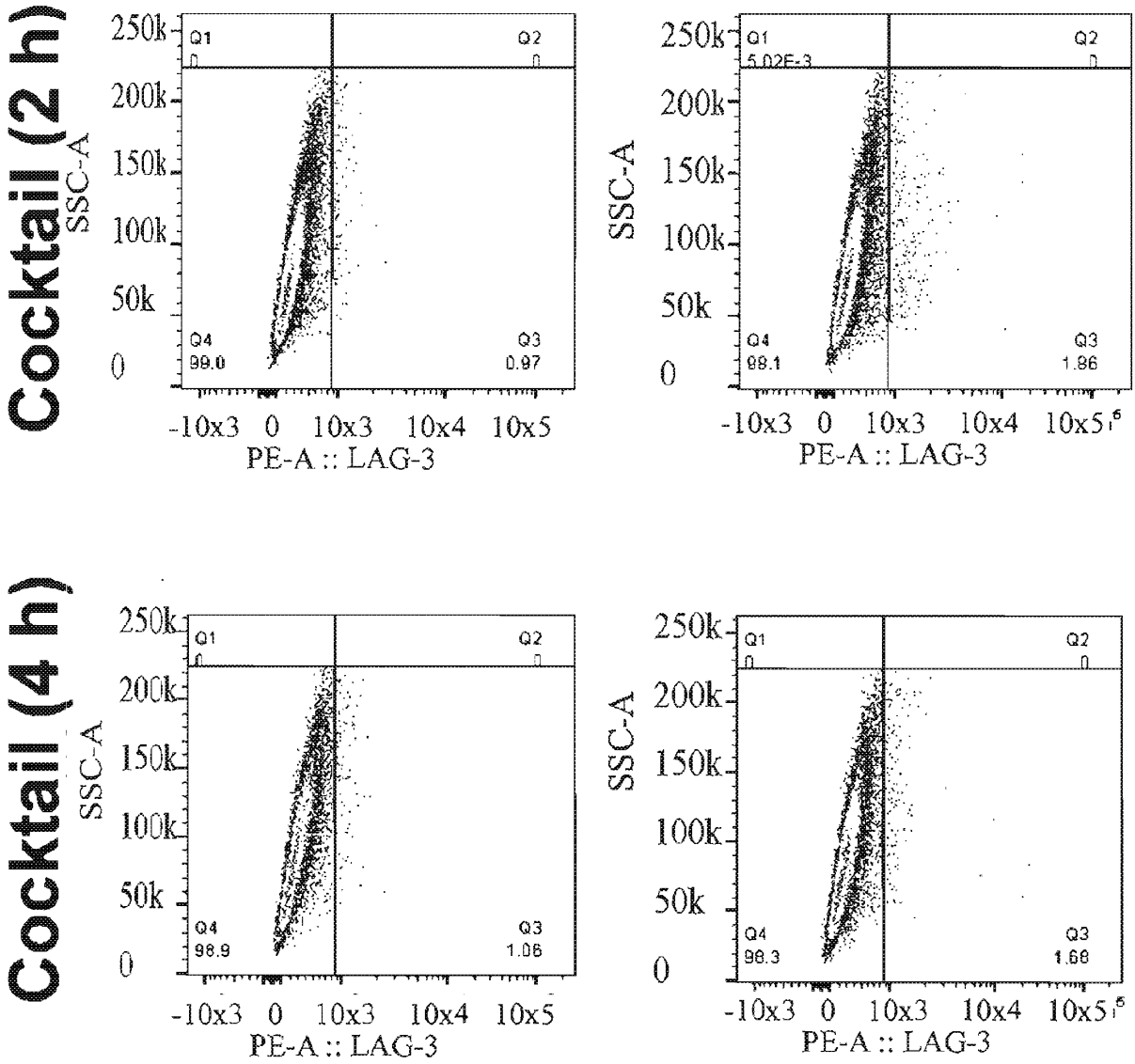

Panel 2 Risk (CC) expressing cell
Isotype Control
LAG-3
Cocktail (2 h)
Cocktail (4 h)
SSC-A
PE-A :: LAG-3
Q1
Q2
Q3
Q4
99.0
0.97
98.1
1.86
98.9
1.06
98.3
1.68
250k
200k
150k
100k
50k
0
-10x3
10x3
10x4
10x5

FIG. 6(Cont.)

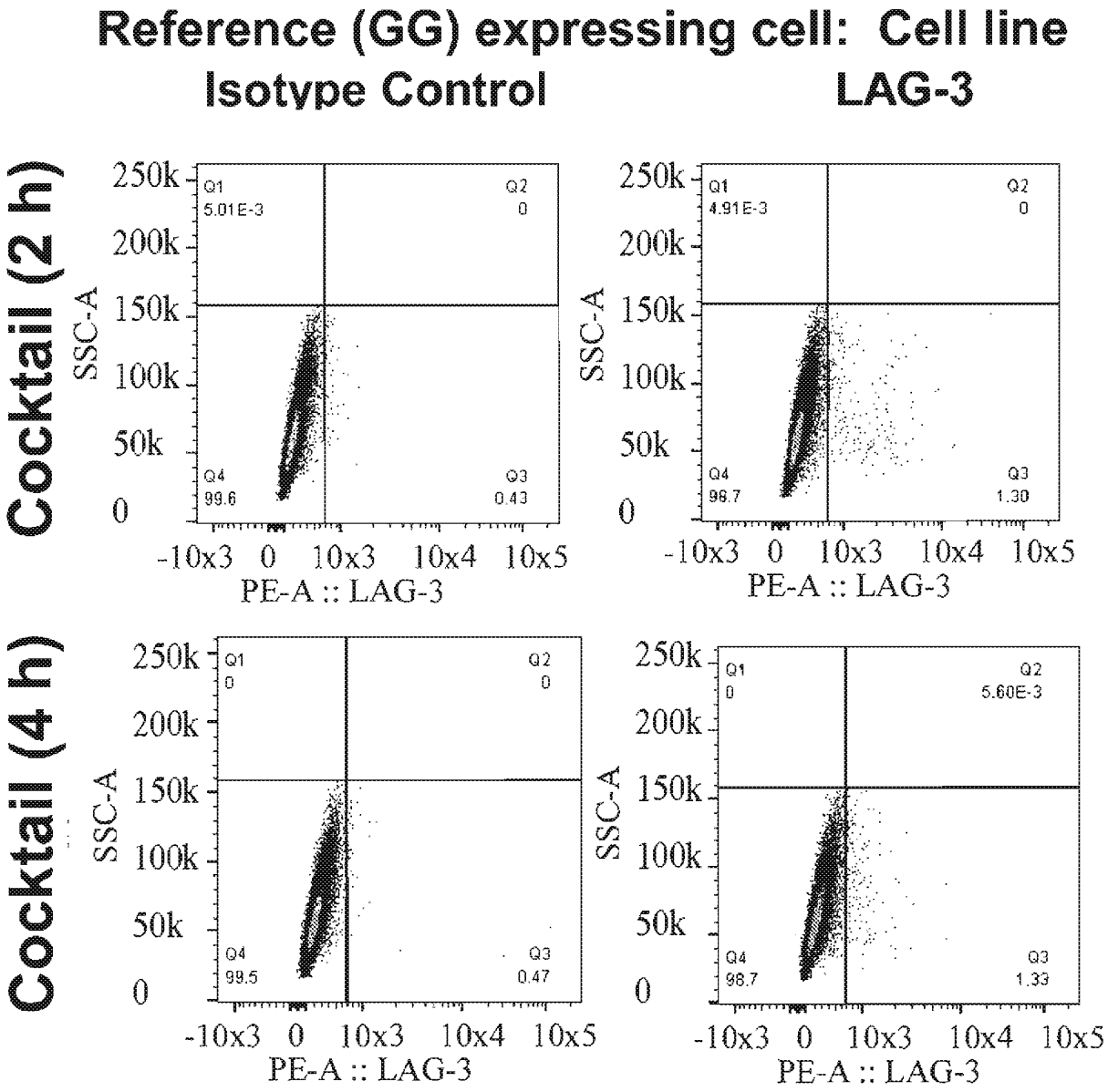

Reference (GG) expressing cell: Cell line
Isotype Control
LAG-3
Cocktail (2 h)
Cocktail (4 h)
SSC-A
PE-A :: LAG-3
Q1
5.01E-3
Q2
0
Q4
99.6
Q3
0.43
Q1
4.91E-3
Q2
0
Q4
98.7
Q3
1.30
Q1
0
Q2
0
Q4
99.5
Q3
0.47
Q1
0
Q2
5.60E-3
Q4
98.7
Q3
1.33
250k
200k
150k
100k
50k
0
-10x3
0
10x3
10x4
10x5

FIG. 6(Cont.)

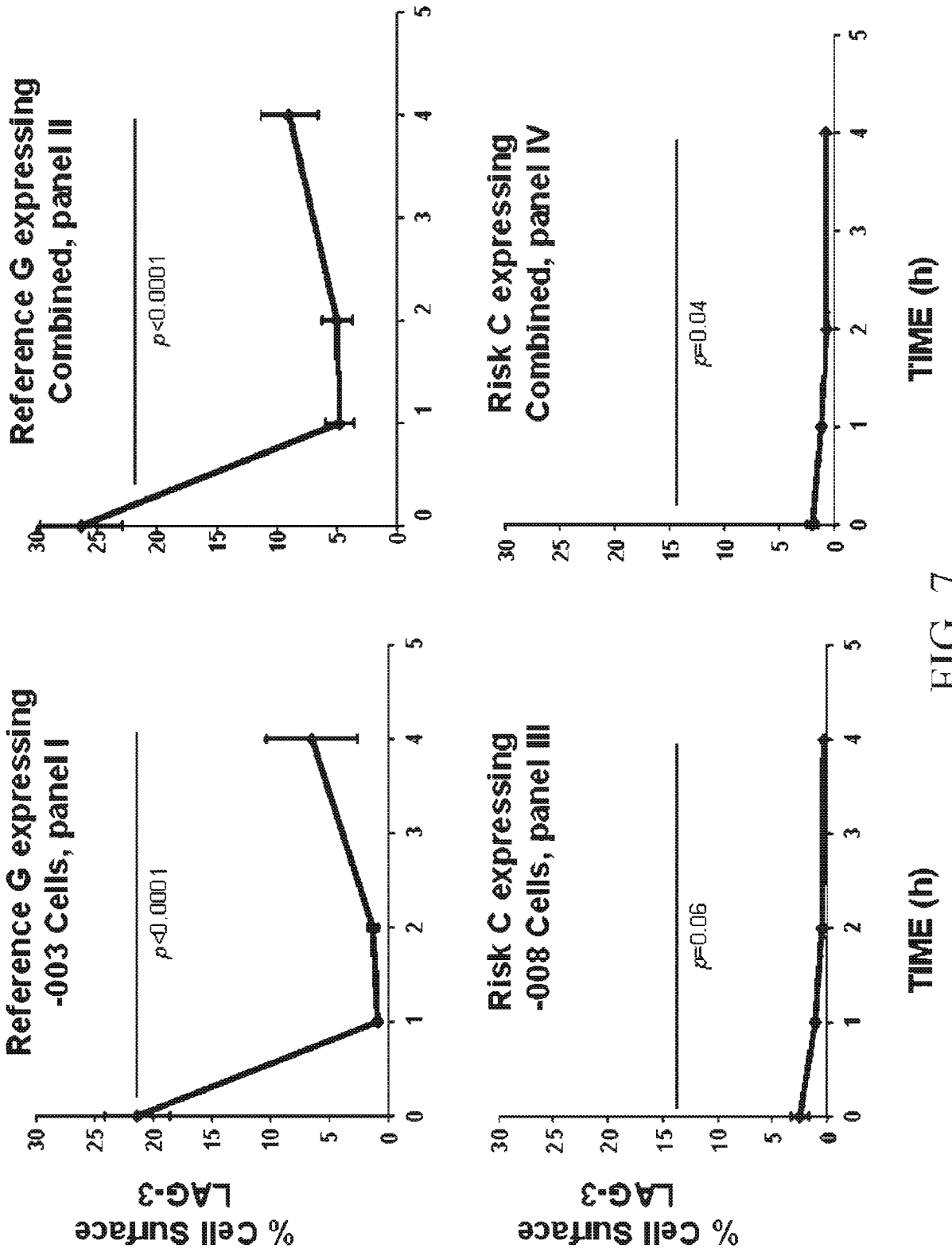

Reference G expressing
-003 Cells, panel I
p<0.0001
% Cell Surface LAG-3
TIME (h)
Reference G expressing
Combined, panel II
p<0.0001
Risk C expressing
-008 Cells, panel III
p=0.06
% Cell Surface LAG-3
TIME (h)
Risk C expressing
Combined, panel IV
p=0.04
TIME (h)

FIG. 7

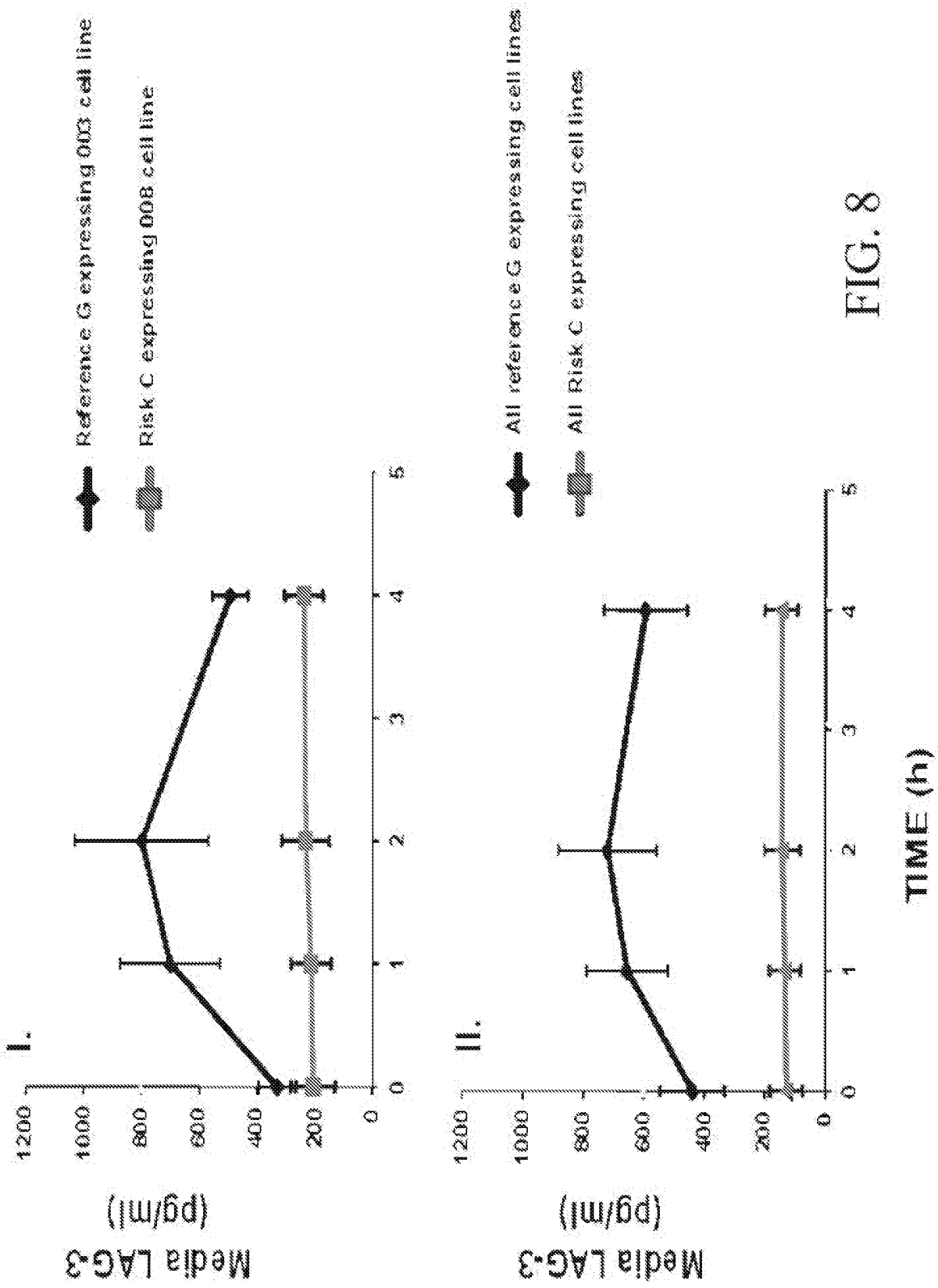

I.
Reference G expressing 003 cell line
Risk C expressing 008 cell line
Media LAG-3 (pg/ml)
1200
1000
800
600
400
200
0
0
1
2
3
4
5
II.
All reference G expressing cell lines
All Risk C expressing cell lines
Media LAG-3 (pg/ml)
TIME (h)

FIG. 8

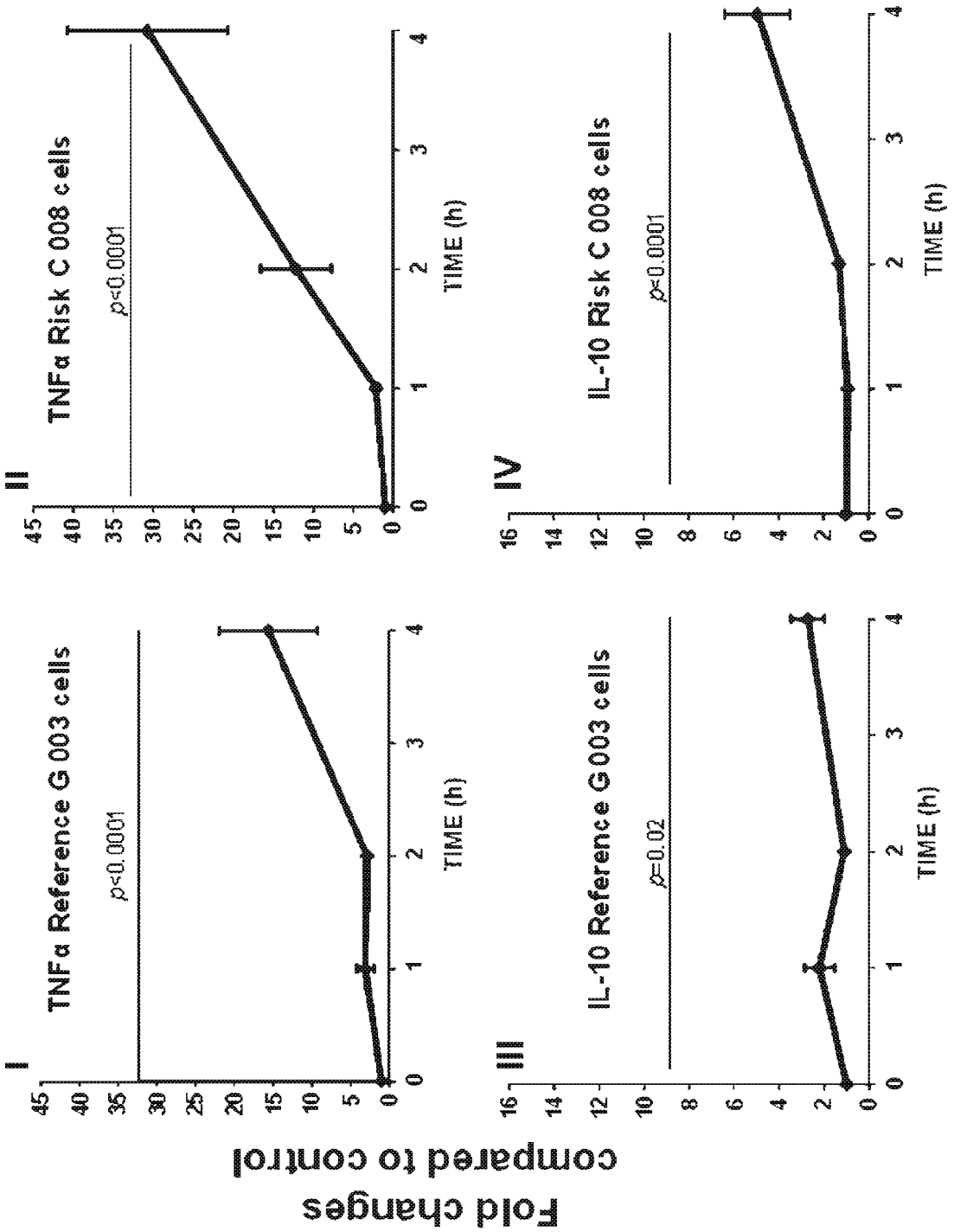

I
TNFα Reference G 003 cells
p<0.0001
II
TNFα Risk C 008 cells
p<0.0001
III
IL-10 Reference G 003 cells
p=0.02
IV
IL-10 Risk C 008 cells
p<0.0001
TIME (h)
Fold changes compared to control

FIG. 9

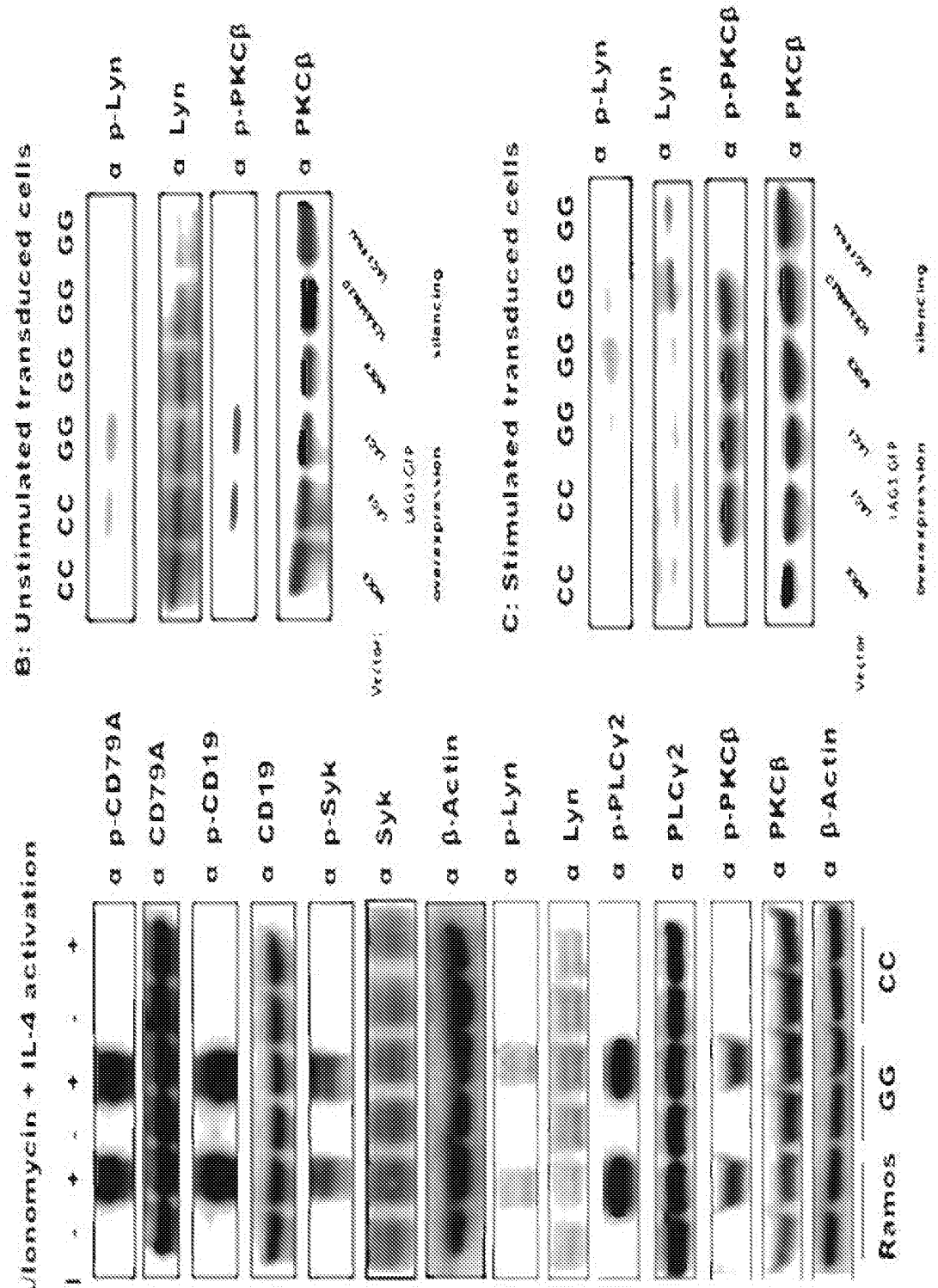

A: PMA/Ionomycin + IL-4 activation
Cocktail - + - + - +
α p-CD79A
α CD79A
α p-CD19
α CD19
α p-Syk
α Syk
α β-Actin
α p-Lyn
α Lyn
α p-PLCγ2
α PLCγ2
α p-PKCβ
α PKCβ
α β-Actin
Ramos GG CC
B: Unstimulated transduced cells
CC CC GG GG GG GG
α p-Lyn
α Lyn
α p-PKCβ
α PKCβ
Vector
LAG3 GP
overexpression
silencing
C: Stimulated transduced cells
CC CC GG GG GG GG
α p-Lyn
α Lyn
α p-PKCβ
α PKCβ
Vector
LAG3 GP
overexpression
silencing

FIG. 10

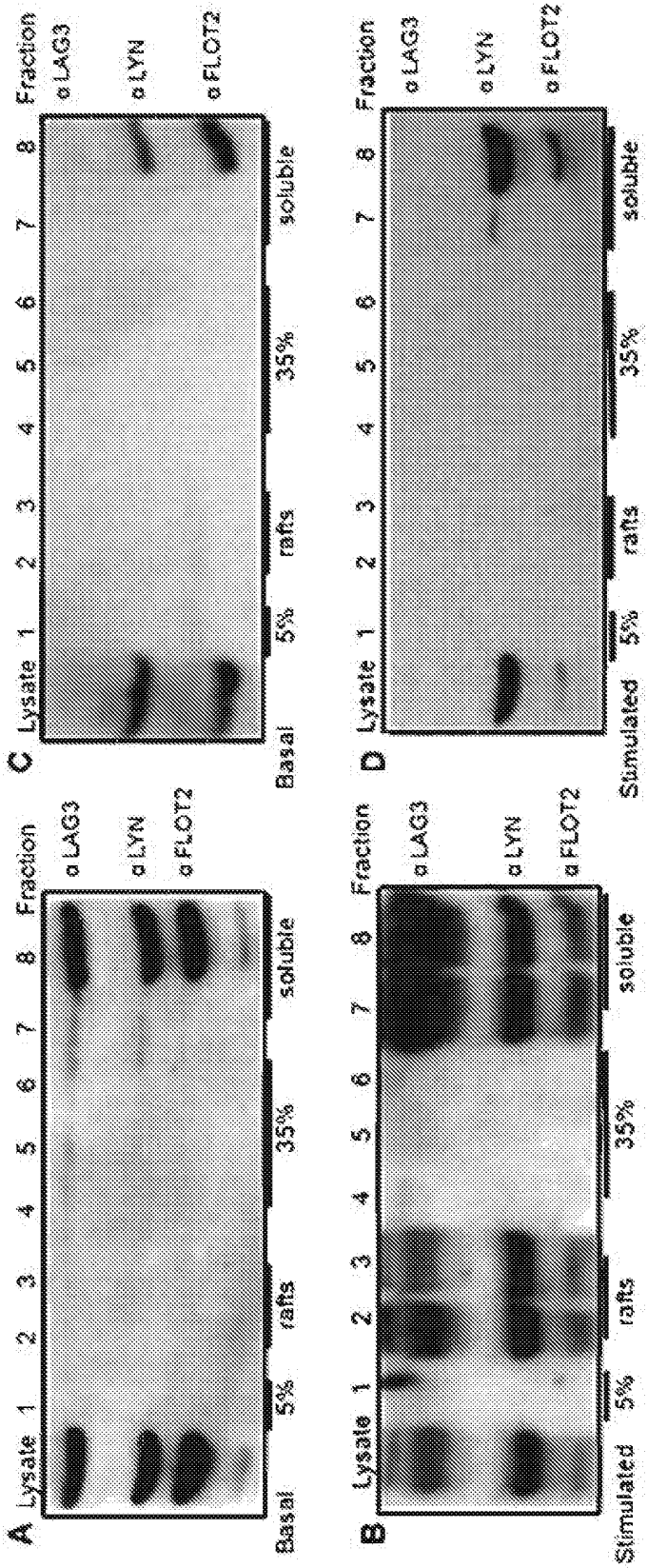

A
Lysate 1 2 3 4 5 6 7 8 Fraction
αLAG3
αLYN
αFLOT2
Basal 5% rafts 35% soluble
B
Lysate 1 2 3 4 5 6 7 8 Fraction
αLAG3
αLYN
αFLOT2
Stimulated 5% rafts 35% soluble
C
Lysate 1 2 3 4 5 6 7 8 Fraction
αLAG3
αLYN
αFLOT2
Basal 5% rafts 35% soluble
D
Lysate 1 2 3 4 5 6 7 8 Fraction
αLAG3
αLYN
αFLOT2
Stimulated 5% rafts 35% soluble

FIG. 11

Table 3. Association of plasma LAG3 with CHD in MESA participants.

Variable	Odds Ratio	95% CI
**LAG3 (log scale)	1.15	1.02-1.28
*Age	2.41	1.81-3.21
[+]Sex	2.10	1.50-2.94
[+]Systolic blood pressure	1.60	1.25-2.06
**LDL cholesterol	1.79	1.11-2.87
[++]Total cholesterol	0.60	0.37-0.97
[&]Lipid medication	1.60	1.18-2.17
[&&]Hgb_{A1c}	1.09	1.01-1.14
[#]Smoking pack-years	1.07	1.00-1.14

Table 4. Association of plasma LAG3 with CHD in MESA participants with HDL-C ≥ 60 mg/dl.

Variable	Odds Ratio	95% CI
*LAG3 (log scale)	1.45	1.12-1.85
**Age	2.79	1.35-5.78
[+]Sex	3.80	1.68-8.56
[++]Diastolic blood pressure	0.49	0.26-0.92

Table 5. Plasma LAG3 as a CHD risk predictor compared with Framingham risk score.

	Beta	SE	P-value
Model 1: (N=5468)			
Framingham risk score (log scale)	0.872	0.093	< 0.0001
Model 2: (N=5468)			
Framingham risk score (log scale)	0.875	0.094	< 0.0001
LAG3 (log scale)	-0.065	0.031	0.039
Model 3: (N=5468)			
Framingham risk score (log scale)	0.891	0.095	< 0.0001
Model 4: (N=5468)			
Framingham risk score (log scale)	0.893	0.095	< 0.0001
LAG3 (log scale)	-0.064	0.031	0.044

Table 6. Association of plasma LAG3 with inflammatory markers in MESA participants.

Outcomes	N	Beta	SE	P-value
IL-2	2386	-0.002	0.004	0.624
IL-6	5414	-0.006	0.044	0.203
IL-10	2350	0.035	0.007	< 0.0001
CD40 ligand	851	-0.0004	0.010	0.966
sTNFαR	2396	-0.001	0.003	0.824
hs-CRP	5533	-0.013	0.007	0.087

FIG. 12

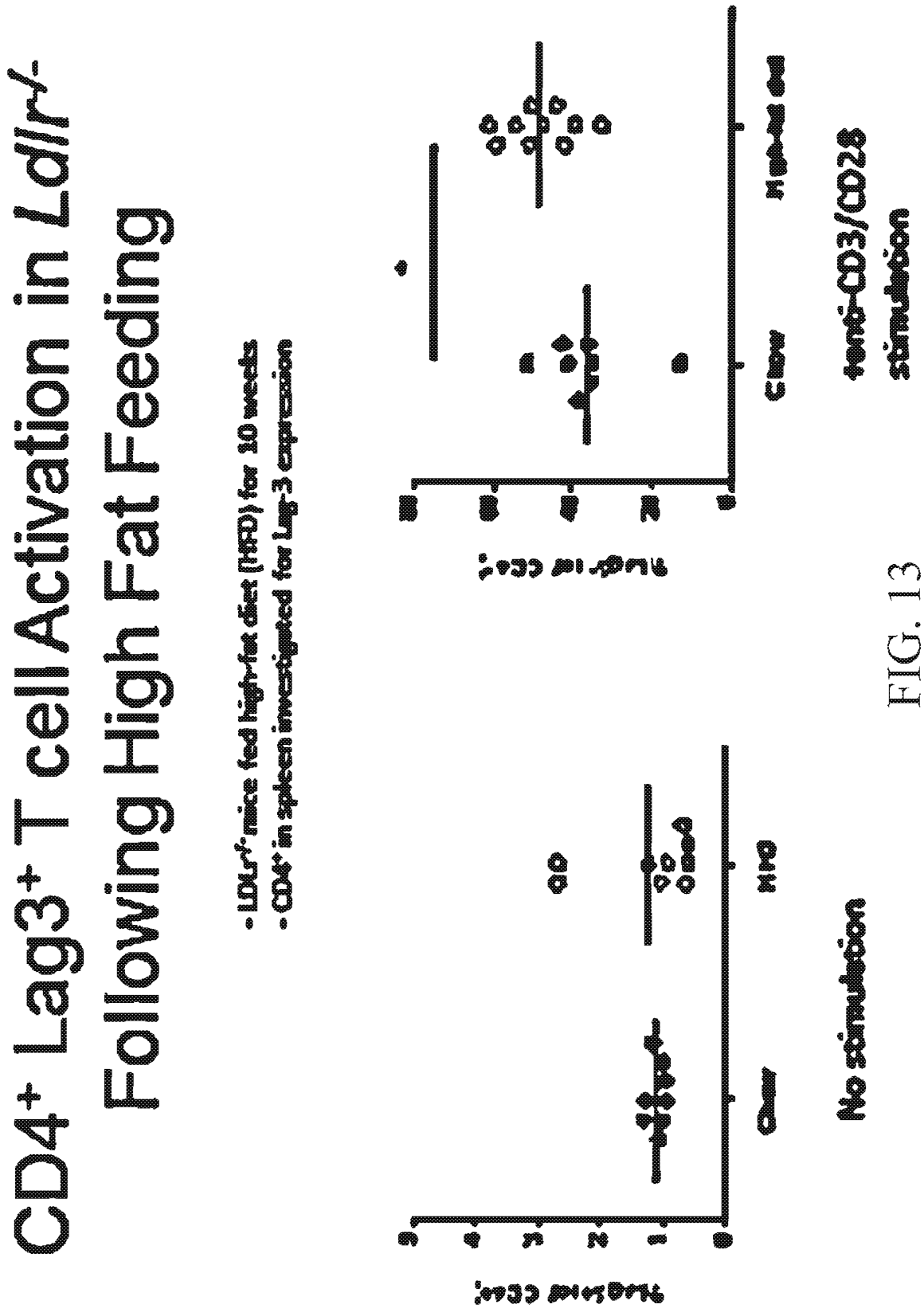

CD4+ Lag3+ T cell Activation in Ldlr-/- Following High Fat Feeding
• Ldlr-/- mice fed high-fat diet (HFD) for 10 weeks
• CD4+ in spleen investigated for Lag-3 expression
No stimulation
anti-CD3/CD28 stimulation
Chow
HFD

FIG. 13

Bone Marrow Transfer of *Lag-3*$^{-/-}$ Cells Significantly Associated with Aortic Root Activated CD4+ T Cells

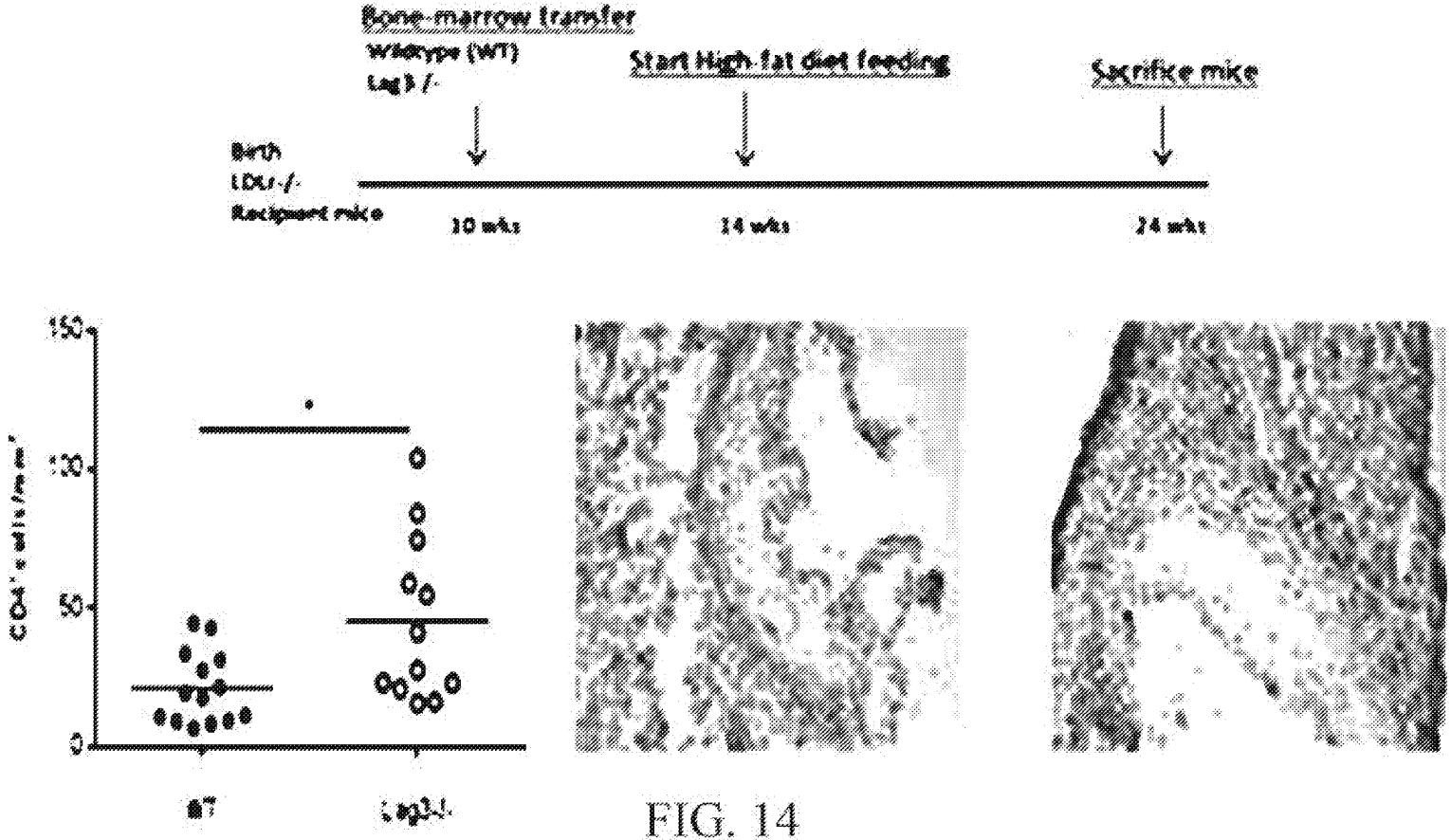


FIG. 14

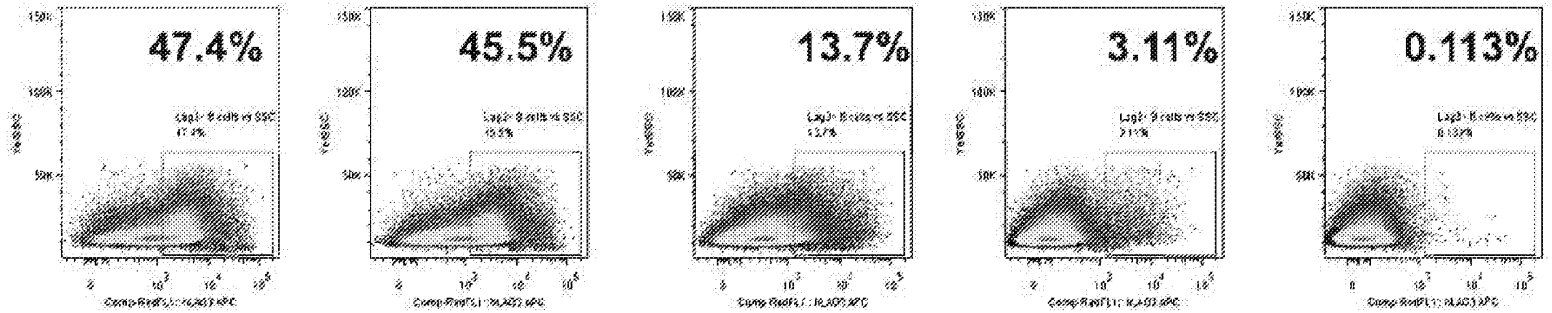
Lag-3-/- Deficiency Significantly Associated with Impaired Tregs (exTregs)
Significant Association of Para-Aortic Tregs and Lesion Size
WT
Lag-3-/-
Lesion Size (um2)
% Tregs (Foxp3+CD25+ of CD4+)

FIG. 15

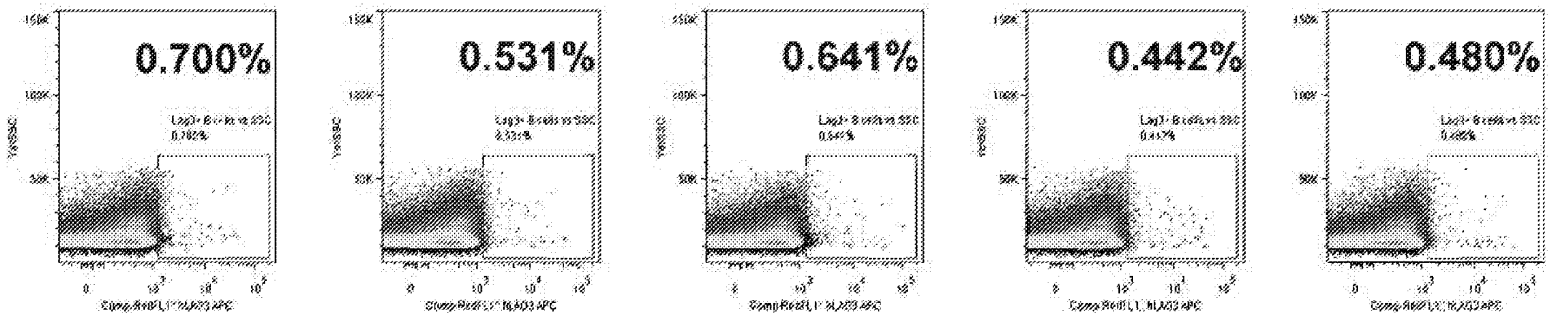
Recombinant human monomeric LAG3

162 µg protein/ml | 32.4 µg/ml | 3.24 µg/ml | 0.32 µg/ml | 0 µg/ml

Anti-human LAG3 APC



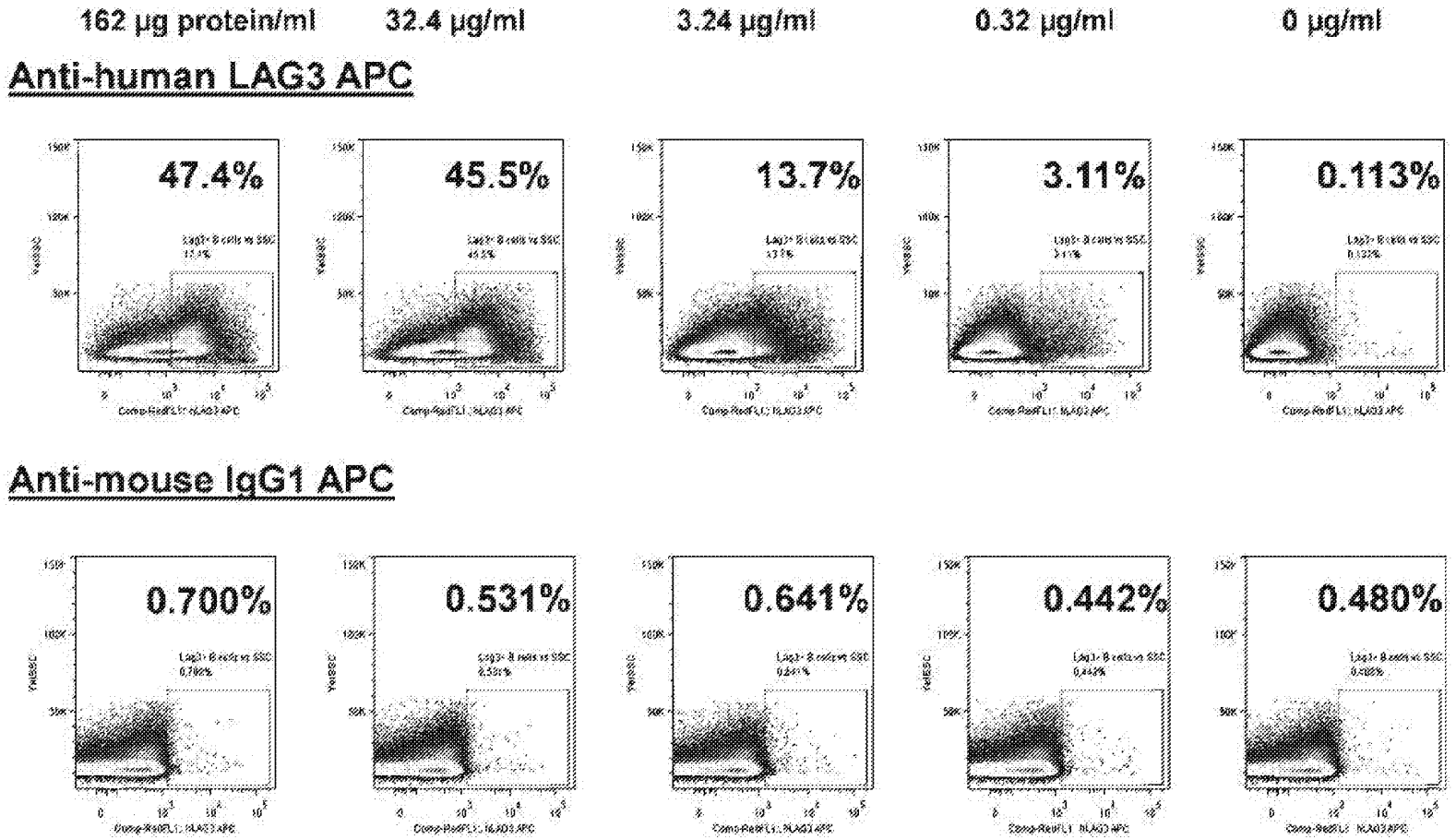
Anti-mouse IgG1 APC



Wild-type mouse B splenocytes (approximately 1 million) were incubated with varying concentrations of recombinant human LAG3 (µg protein/ml) and then stained with anti-human LAG3 (upper panels, 5 µl per condition) or control mouse IgG1 antibodies and then analyzed by flow cytometry.

FIG. 16

Recombinant human monomeric LAG3



Wild-type mouse B splenocytes (approximately 1 million) were incubated with varying concentrations of recombinant human LAG3 (µg protein/ml) and then stained with anti-human LAG3 (upper panels, 5 µl per condition) or control mouse IgG1 antibodies and then analyzed by flow cytometry.

FIG. 16(cont.)

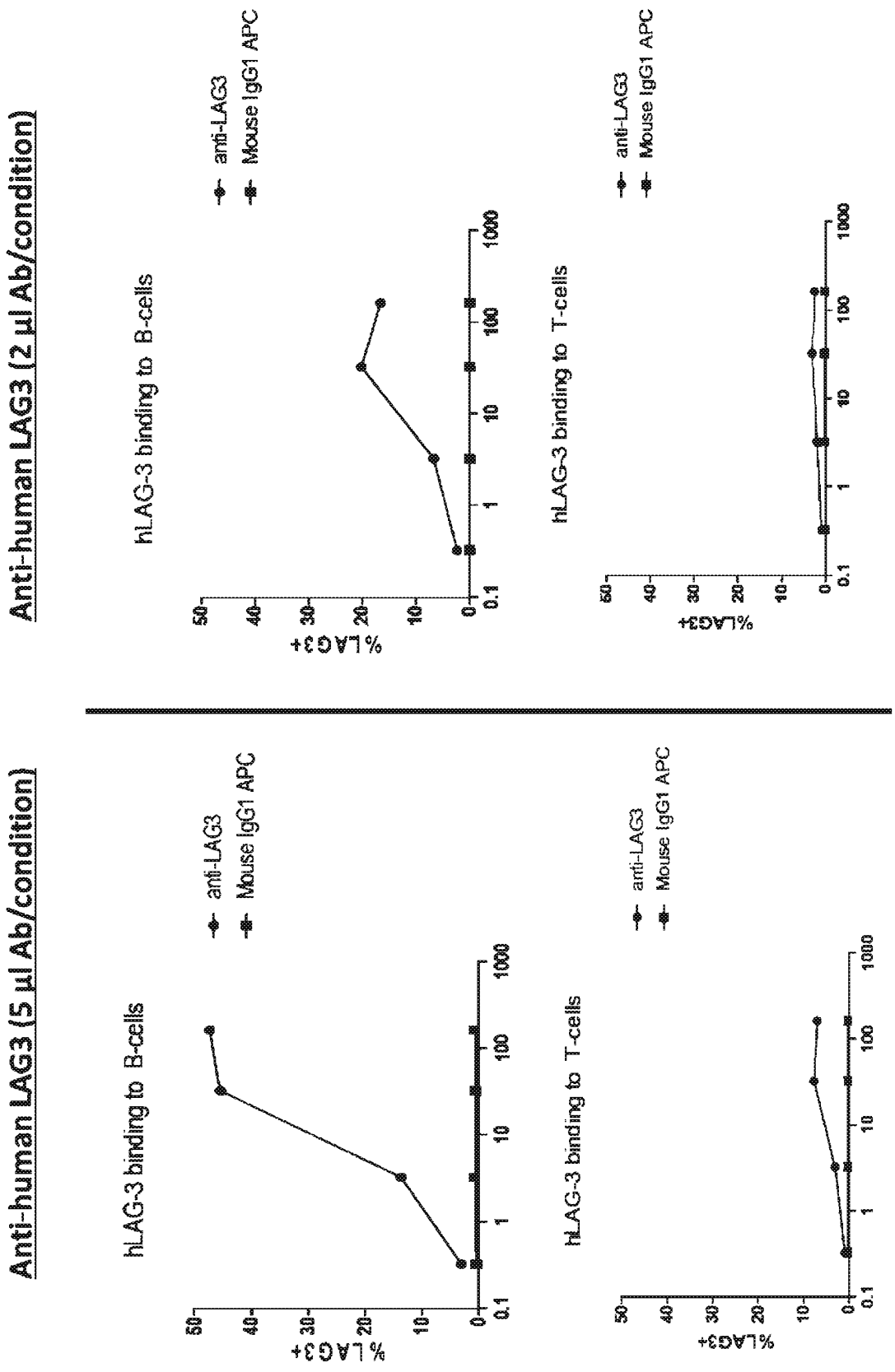

Anti-human LAG3 (2 µl Ab/condition)
hLAG-3 binding to B-cells
anti-LAG3
Mouse IgG1 APC
%LAG3+
hLAG-3 binding to T-cells
Anti-human LAG3 (5 µl Ab/condition)

FIG. 17

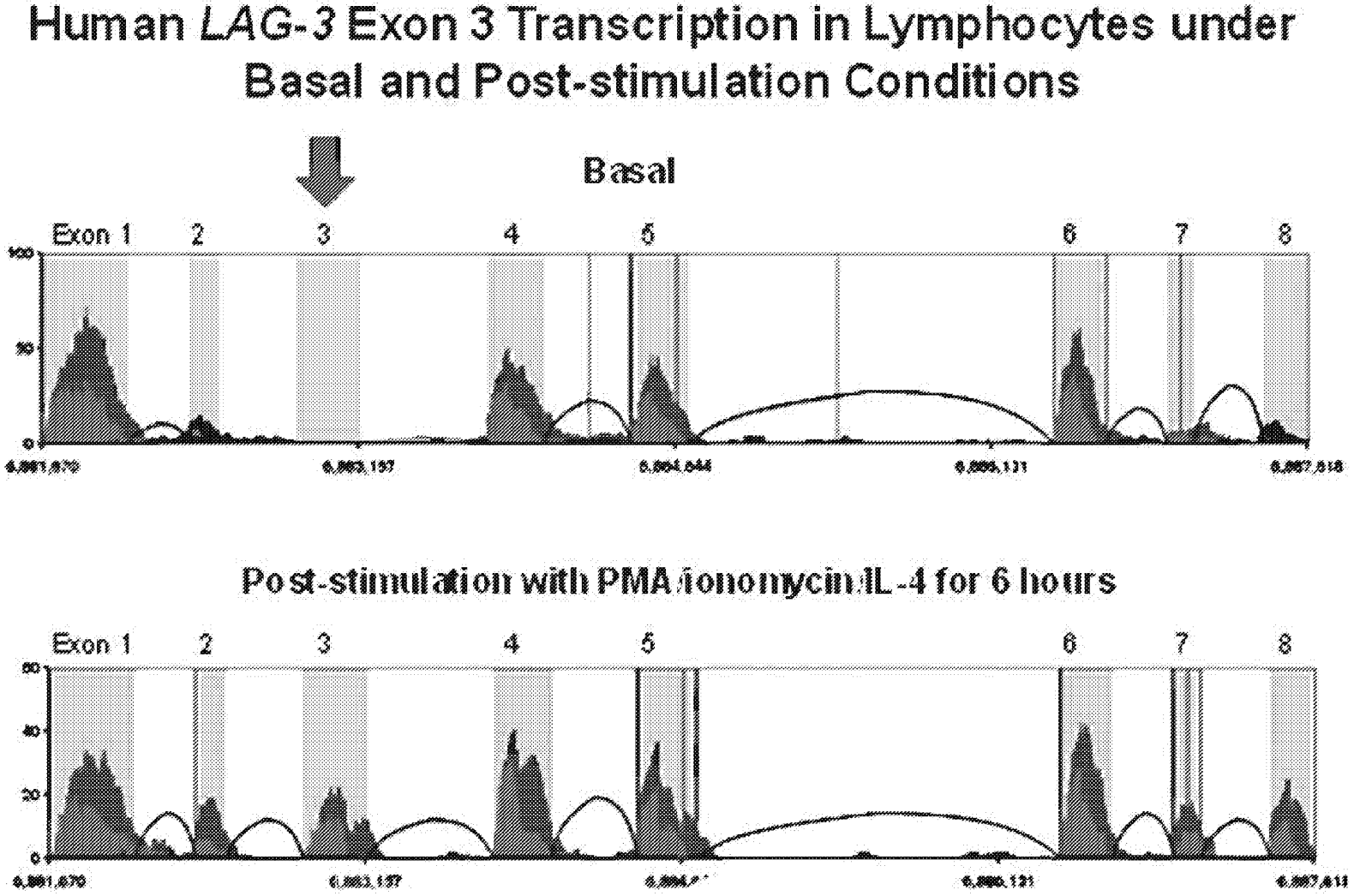

Human LAG-3 Exon 3 Transcription in Lymphocytes under Basal and Post-stimulation Conditions
Basal
Exon 1
2
3
4
5
6
7
8
Post-stimulation with PMA/ionomycin/IL-4 for 6 hours
Exon 1
2
3
4
5
6
7
8

FIG. 18