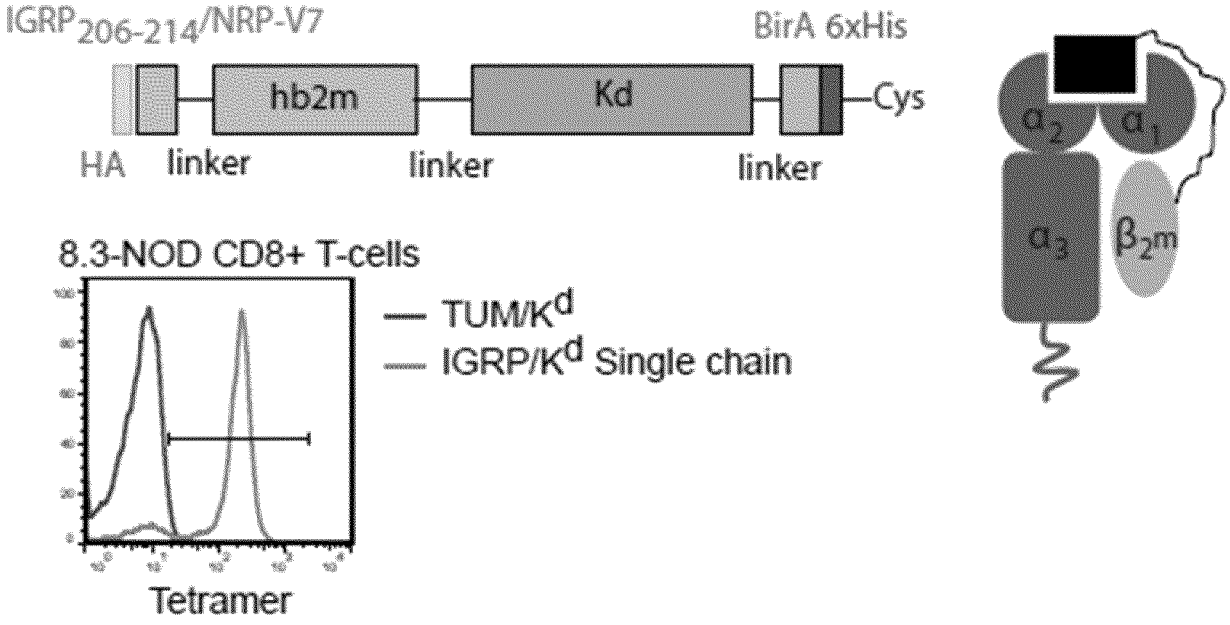

IGRP206-214/NRP-V7
BirA 6xHis
hb2m
Kd
Cys
HA
linker
linker
linker
α2
α1
α3
β2m
8.3-NOD CD8+ T-cells
TUM/Kd
IGRP/Kd Single chain
Tetramer

FIG. 1A

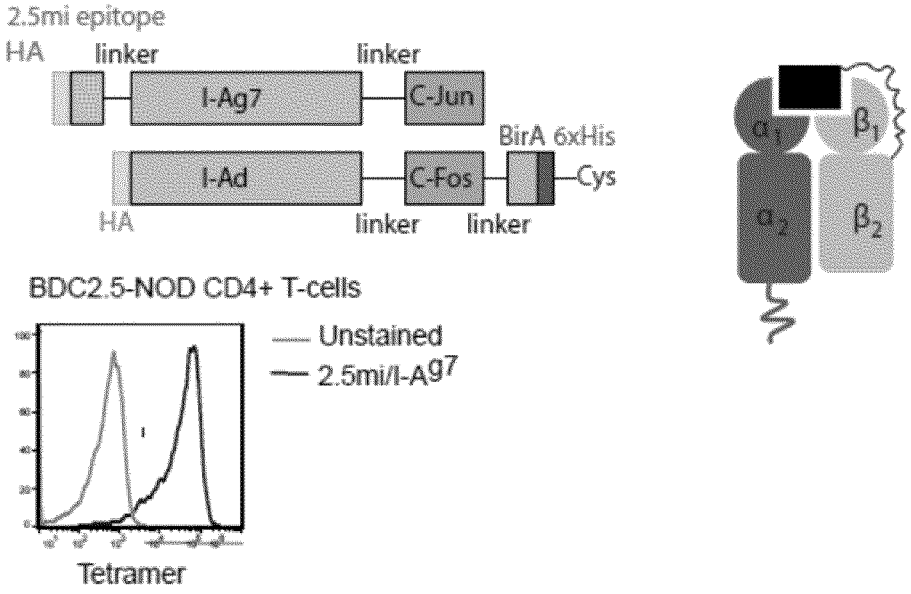

2.5mi epitope
HA
linker
linker
I-Ag7
C-Jun
BirA 6xHis
I-Ad
C-Fos
Cys
HA
linker
linker
α1
β1
α2
β2
BDC2.5-NOD CD4+ T-cells
Unstained
2.5mi/I-Ag7
Tetramer

FIG. 1B

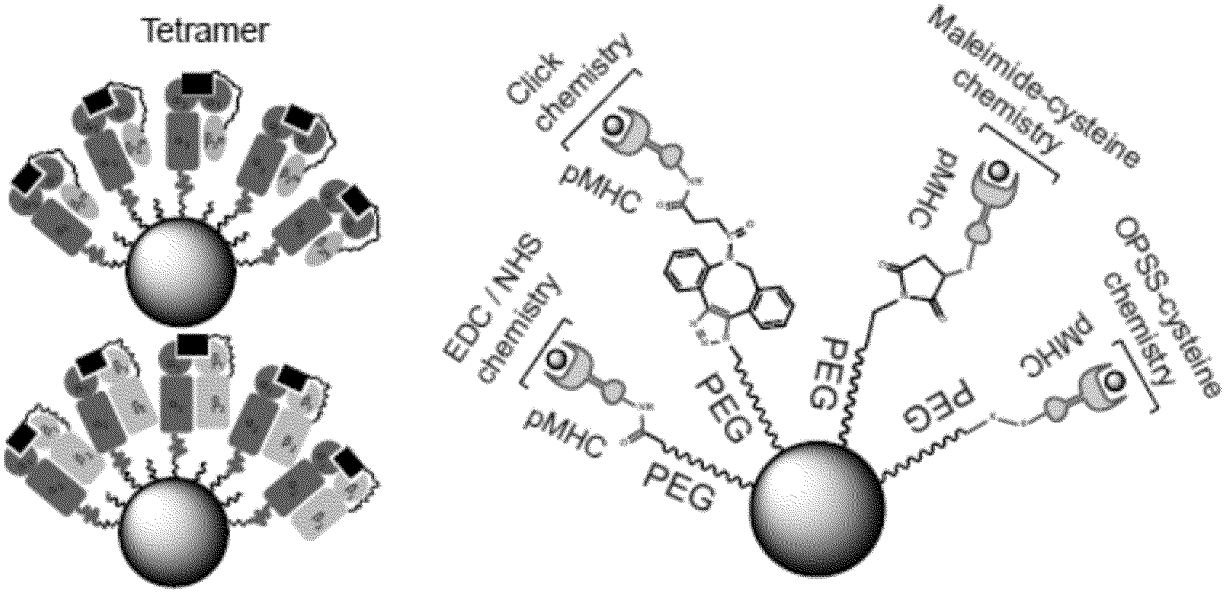

Tetramer
Click chemistry
pMHC
Maleimide-cysteine chemistry
pMHC
EDC / NHS chemistry
pMHC
PEG
PEG
PEG
PEG
pMHC
OPSS-cysteine chemistry

FIG. 1C

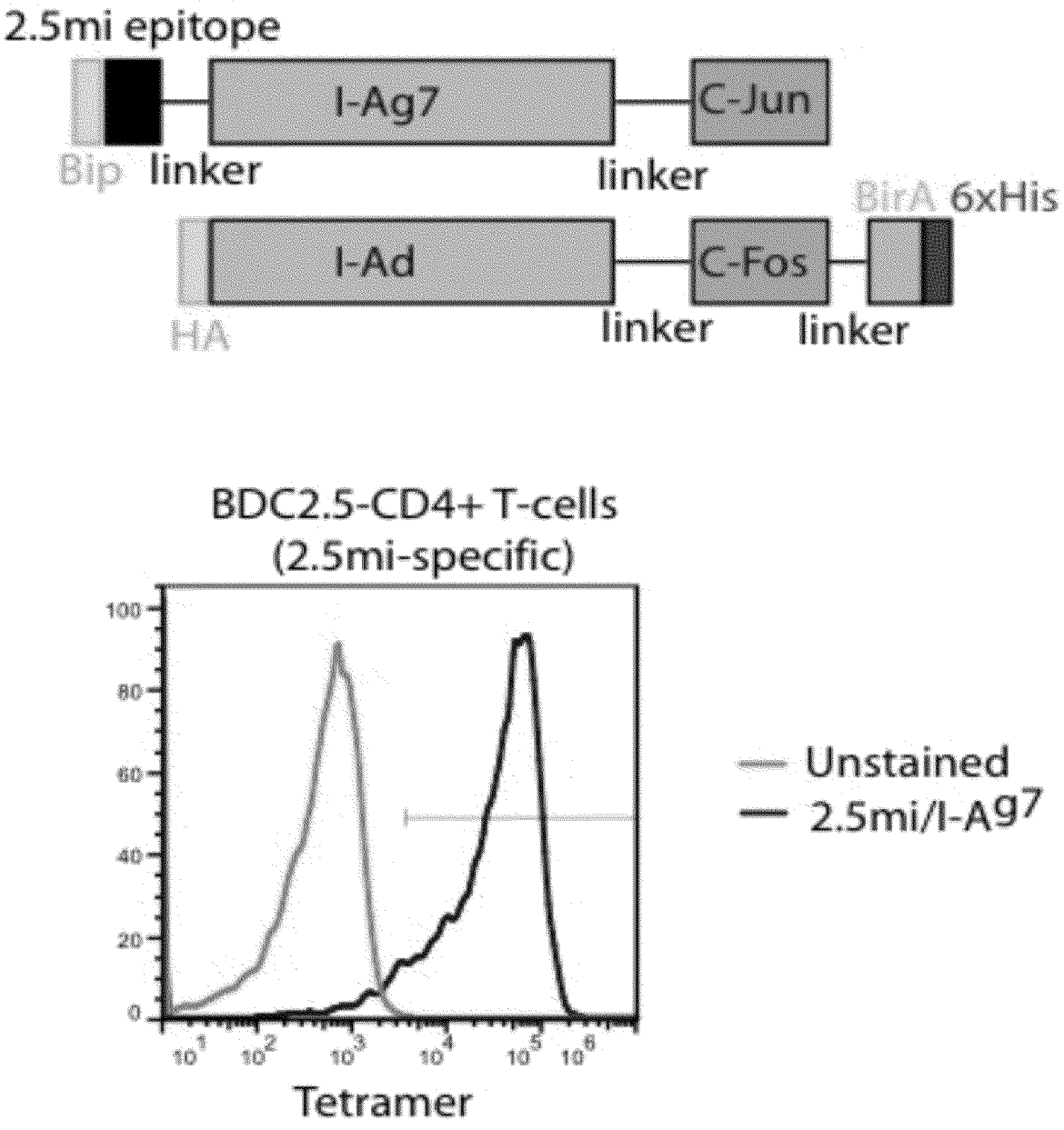

2.5mi epitope
I-Ag7
C-Jun
Bip
linker
linker
BirA
6xHis
I-Ad
C-Fos
HA
linker
linker
BDC2.5-CD4+ T-cells
(2.5mi-specific)
100
80
60
40
20
0
10^1
10^2
10^3
10^4
10^5
10^6
Tetramer
Unstained
2.5mi/I-A^{g7}

FIG. 2

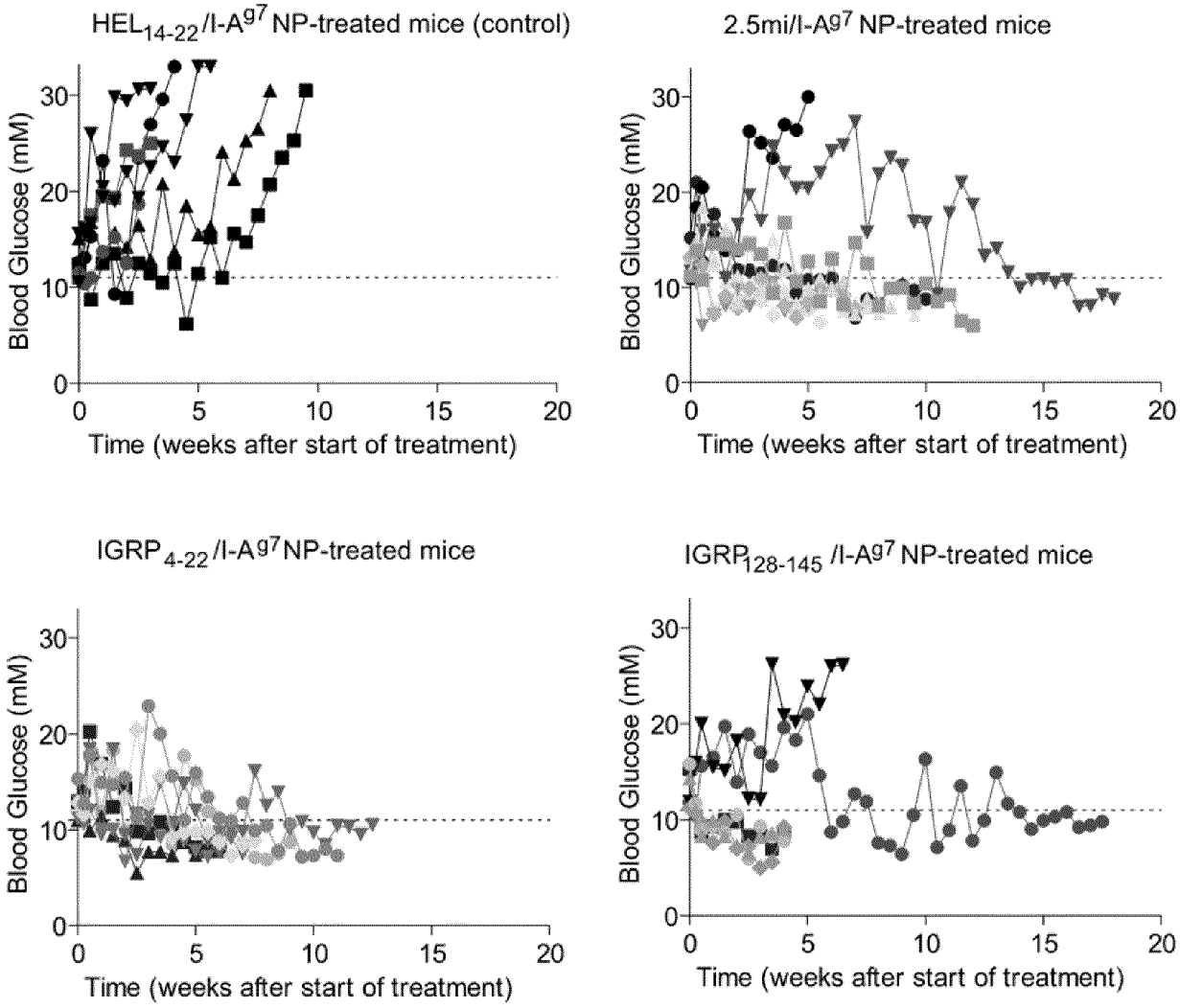

HEL14-22/I-Ag7 NP-treated mice (control)
2.5mi/I-Ag7 NP-treated mice
IGRP4-22/I-Ag7NP-treated mice
IGRP128-145 /I-Ag7 NP-treated mice
Blood Glucose (mM)
Time (weeks after start of treatment)
0
5
10
15
20
30

FIG. 3A

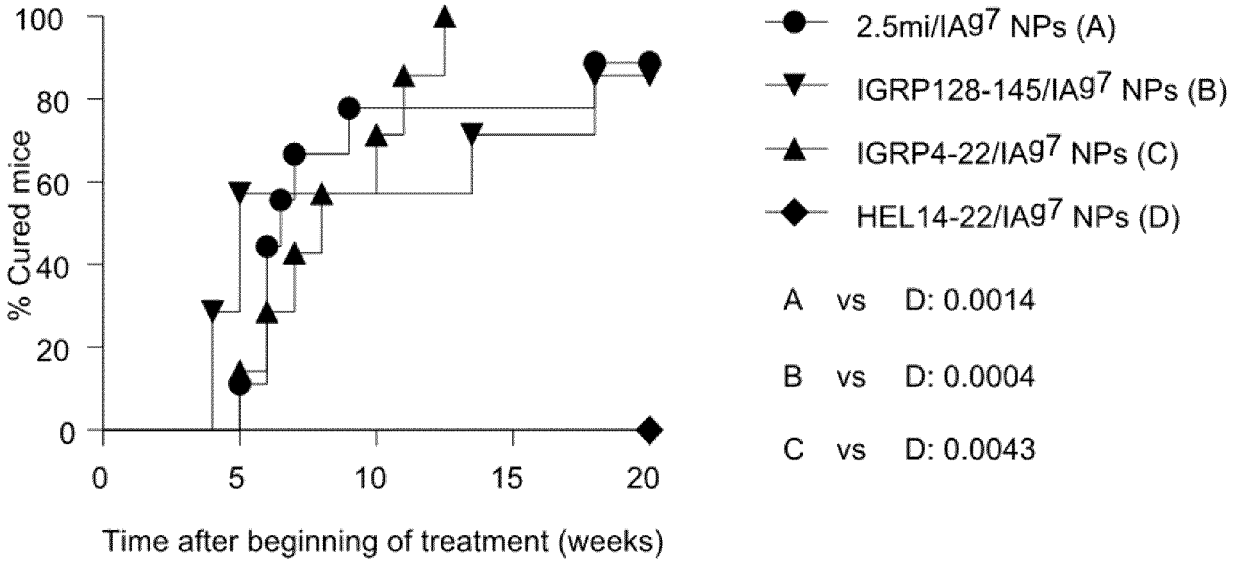

100
80
60
40
20
0
% Cured mice
0
5
10
15
20
Time after beginning of treatment (weeks)
2.5mi/IAg7 NPs (A)
IGRP128-145/IAg7 NPs (B)
IGRP4-22/IAg7 NPs (C)
HEL14-22/IAg7 NPs (D)
A vs D: 0.0014
B vs D: 0.0004
C vs D: 0.0043

FIG. 3B

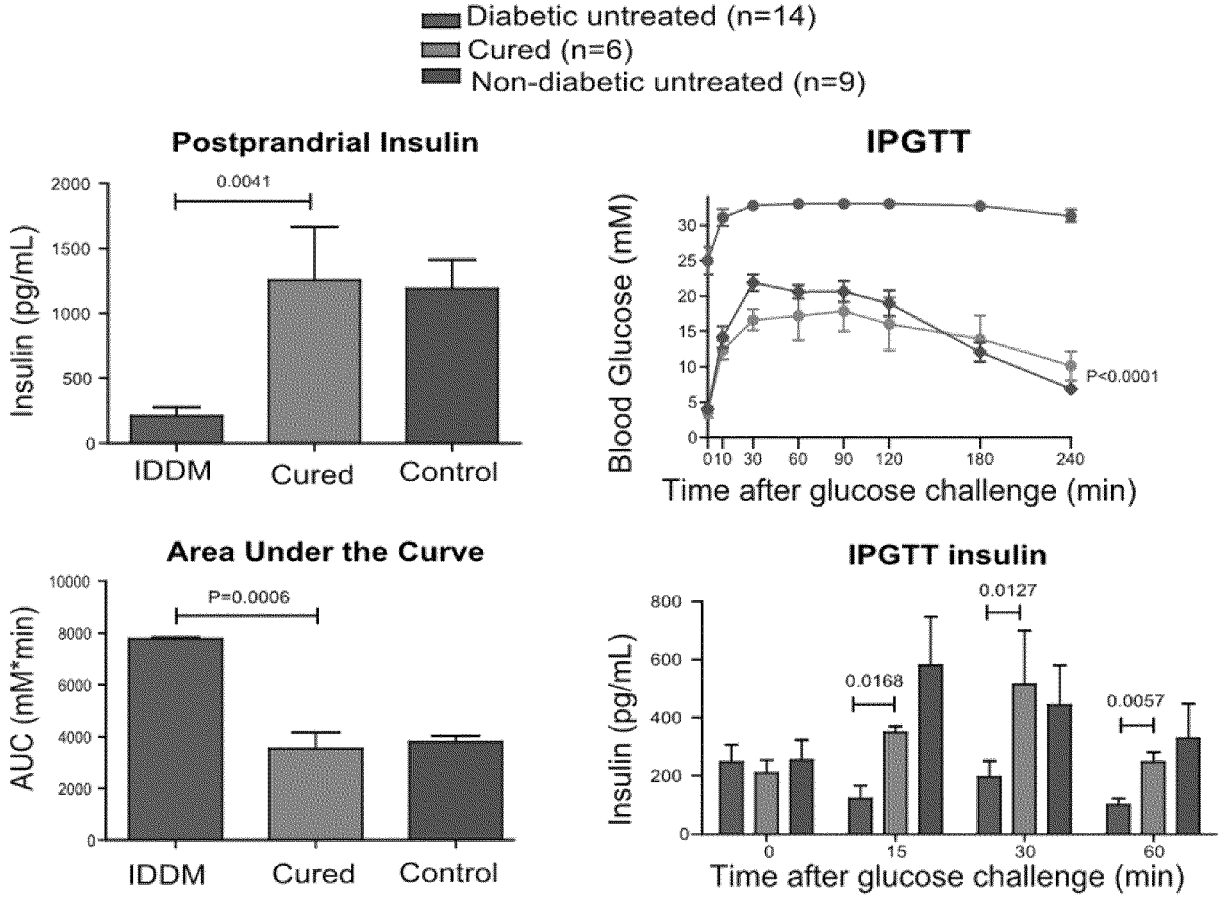

Diabetic untreated (n=14)
Cured (n=6)
Non-diabetic untreated (n=9)
Postprandrial Insulin
Insulin (pg/mL)
0.0041
IDDM
Cured
Control
IPGTT
Blood Glucose (mM)
P<0.0001
Time after glucose challenge (min)
Area Under the Curve
AUC (mM*min)
P=0.0006
IDDM
Cured
Control
IPGTT insulin
Insulin (pg/mL)
0.0168
0.0127
0.0057
Time after glucose challenge (min)

FIG. 4

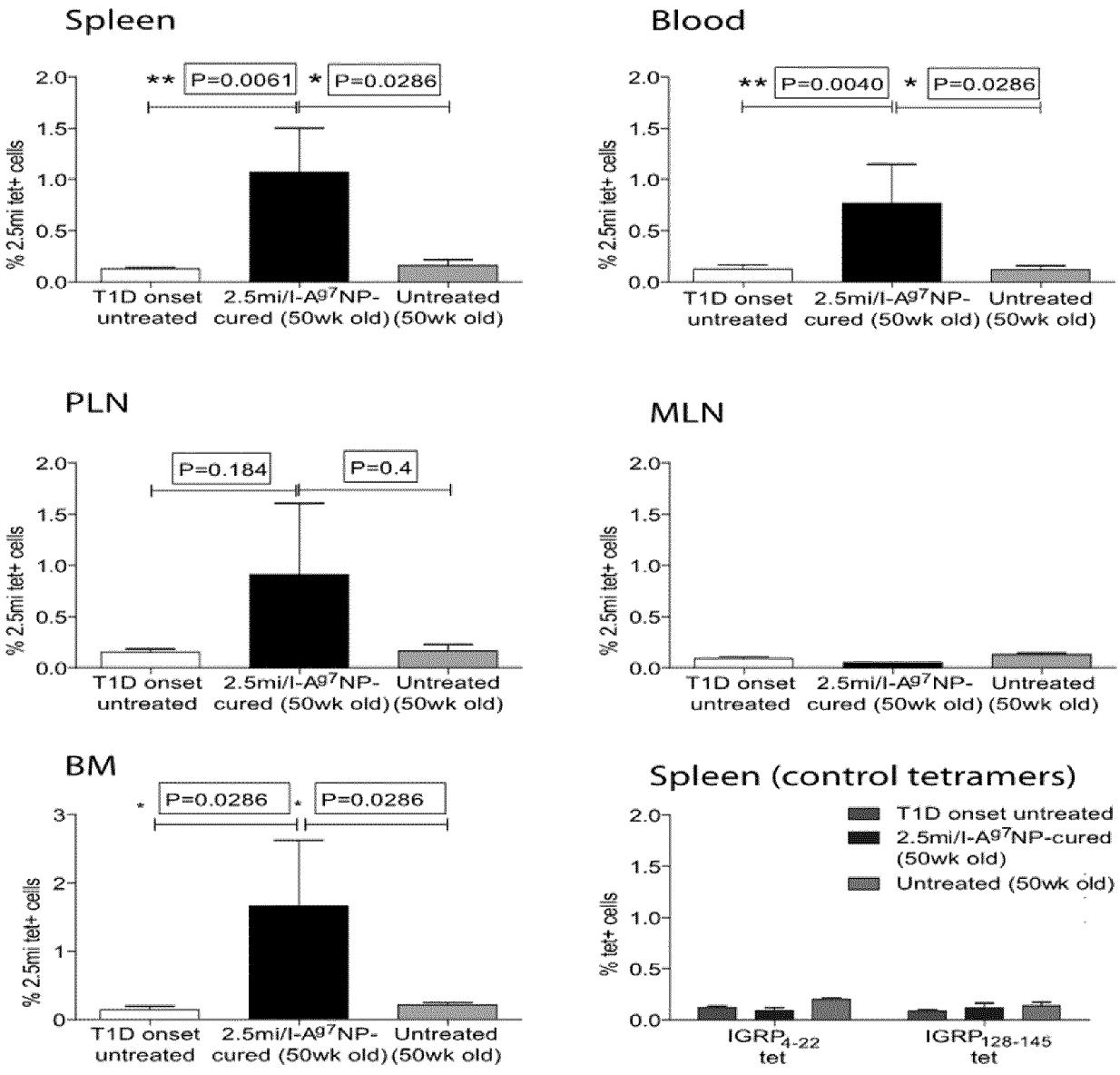

Spleen
** P=0.0061
* P=0.0286
% 2.5mi tet+ cells
2.0
1.5
1.0
0.5
0.0
T1D onset untreated
2.5mi/I-A^{g7}NP-cured (50wk old)
Untreated (50wk old)
Blood
** P=0.0040
* P=0.0286
PLN
P=0.184
P=0.4
MLN
BM
* P=0.0286
* P=0.0286
3
2
1
0
Spleen (control tetramers)
T1D onset untreated
2.5mi/I-A^{g7}NP-cured (50wk old)
Untreated (50wk old)
% tet+ cells
IGRP$_{4-22}$ tet
IGRP$_{128-145}$ tet

FIG. 5

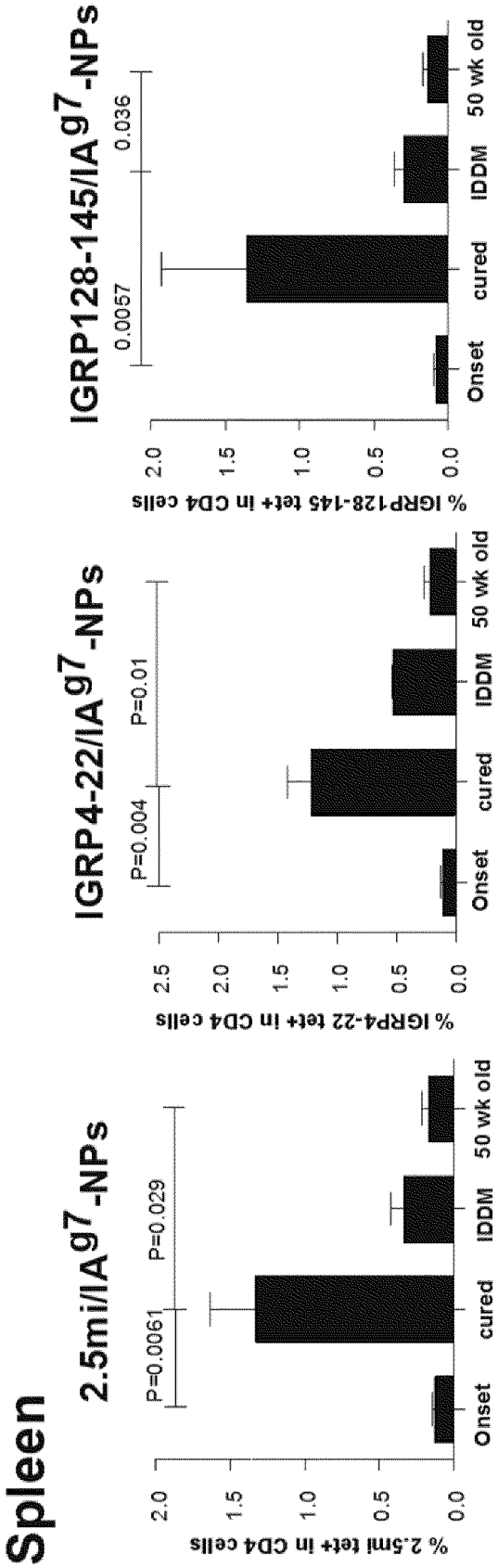

Spleen
2.5mi/IA^{g7}-NPs
% 2.5mi tet+ in CD4 cells
P=0.0061
P=0.029
Onset
cured
IDDM
50 wk old
IGRP4-22/IA^{g7}-NPs
% IGRP4-22 tet+ in CD4 cells
P=0.004
P=0.01
IGRP128-145/IA^{g7}-NPs
% IGRP128-145 tet+ in CD4 cells
0.0057
0.036

FIG. 6

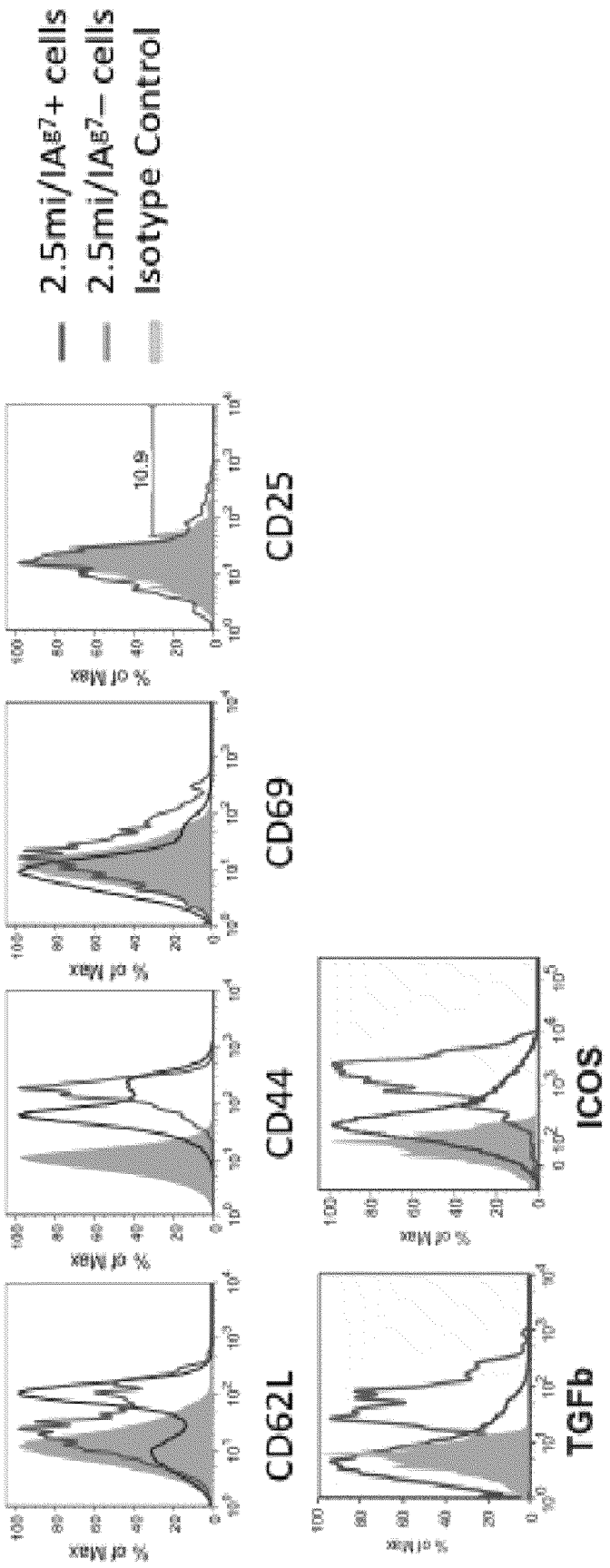

2.5mi/IA^{g7}+ cells
2.5mi/IA^{g7}− cells
Isotype Control
CD62L
CD44
CD69
CD25
TGFb
ICOS
% of Max
10.9

FIG. 7

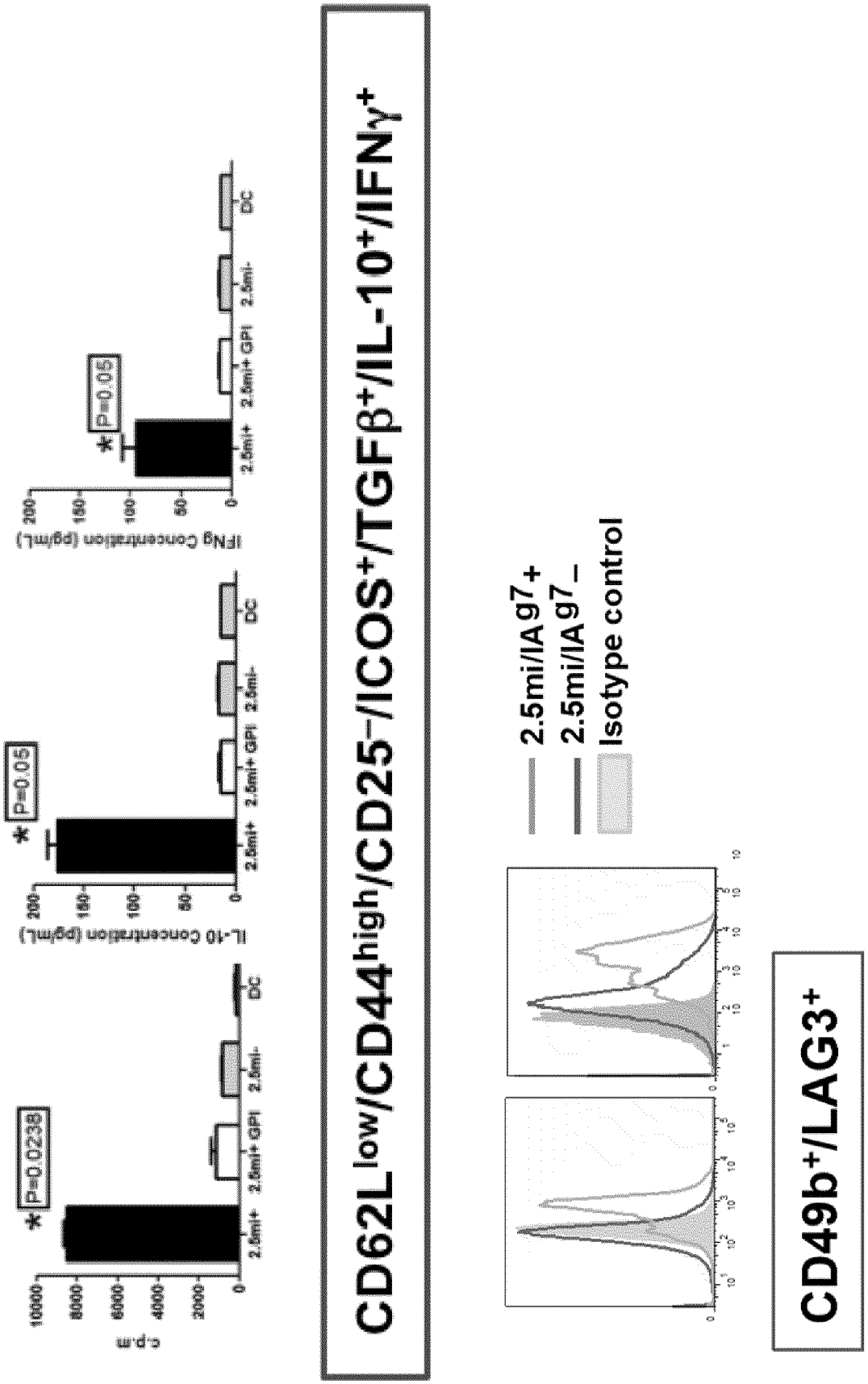

CD62L low/CD44high/CD25−/ICOS+/TGFβ+/IL-10+/IFNγ+
c.p.m
P=0.0238
2.5mi+
2.5mi+ GPI
2.5mi-
DC
IL-10 Concentration (pg/mL)
P=0.05
IFNg Concentration (pg/mL)
P=0.05
2.5mi/IA g7+
2.5mi/IA g7−
Isotype control
CD49b+/LAG3+

FIG. 7 (CONT'D)

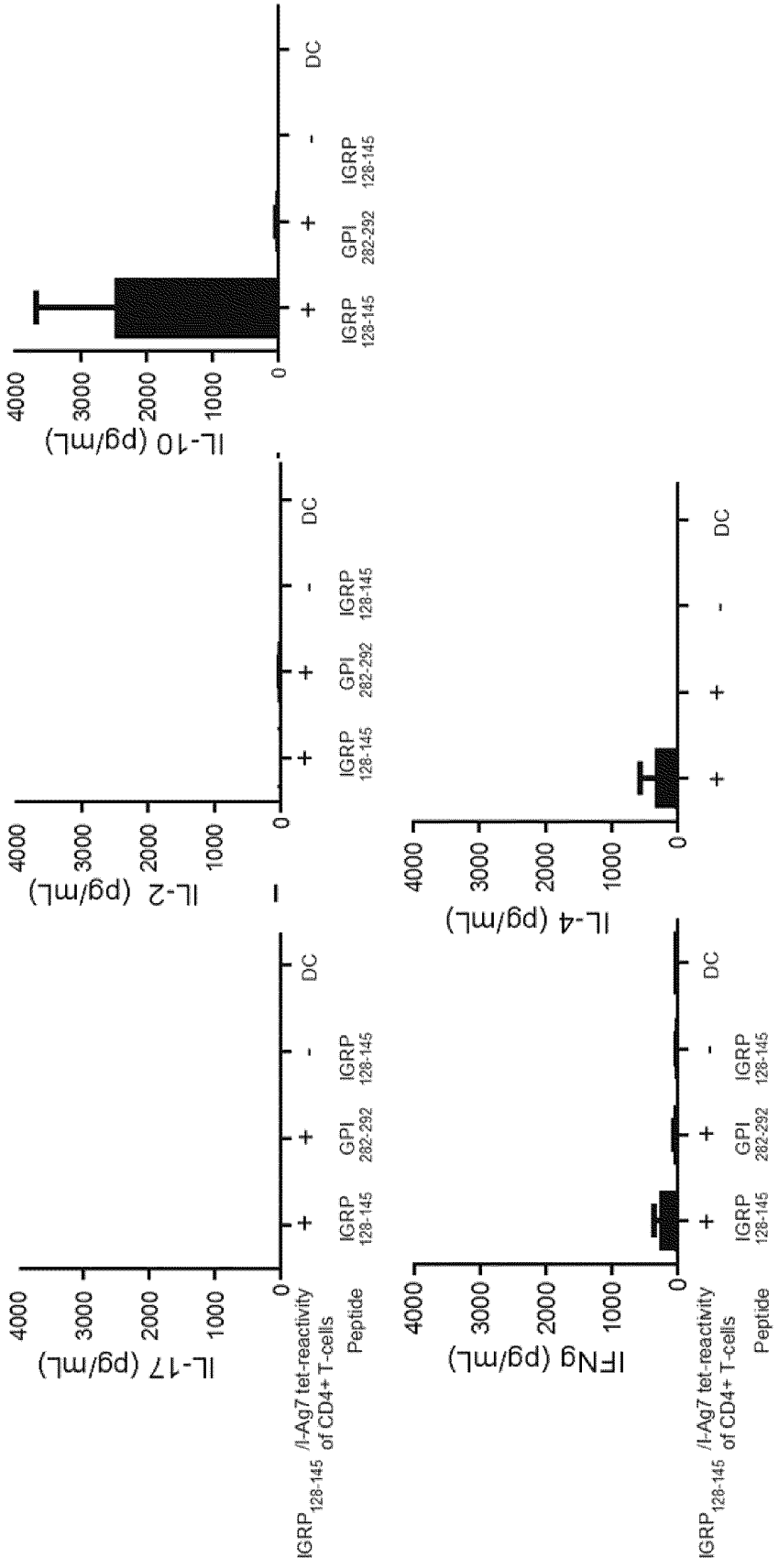

IL-17 (pg/mL)
IL-2 (pg/mL)
IL-10 (pg/mL)
IFNg (pg/mL)
IL-4 (pg/mL)
4000
3000
2000
1000
0
IGRP128-145 /I-Ag7 tet-reactivity of CD4+ T-cells
Peptide
IGRP 128-145
GPI 282-292
IGRP 128-145
DC
+
-

FIG. 8

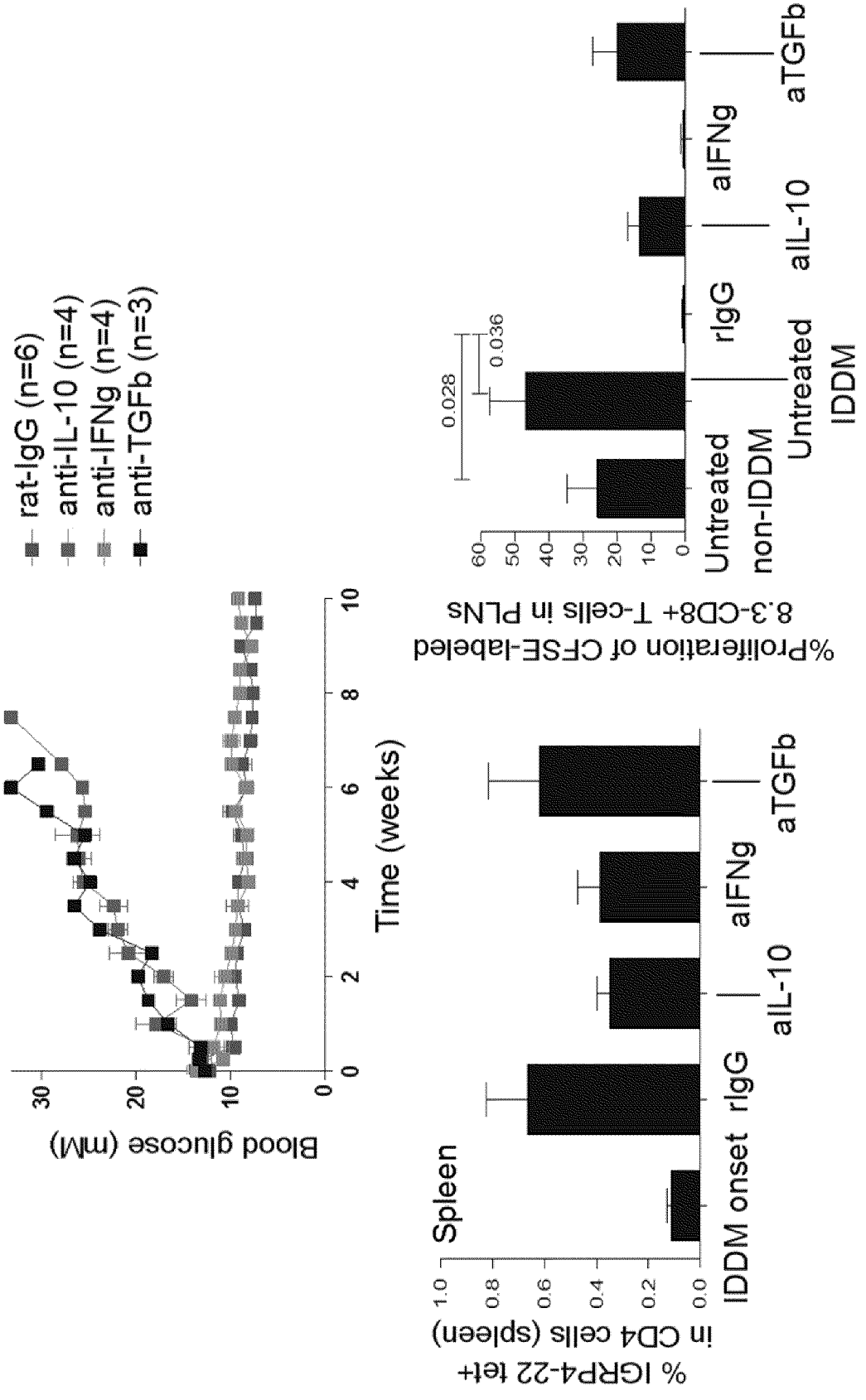

rat-IgG (n=6)
anti-IL-10 (n=4)
anti-IFNg (n=4)
anti-TGFb (n=3)
Blood glucose (mM)
Time (weeks)
%Proliferation of CFSE-labeled
8.3-CD8+ T-cells in PLNs
0.028
0.036
Untreated
rIgG
aIL-10
aIFNg
aTGFb
non-IDDM
IDDM
% IGRP4-22 tet+
in CD4 cells (spleen)
Spleen
IDDM onset

FIG. 9

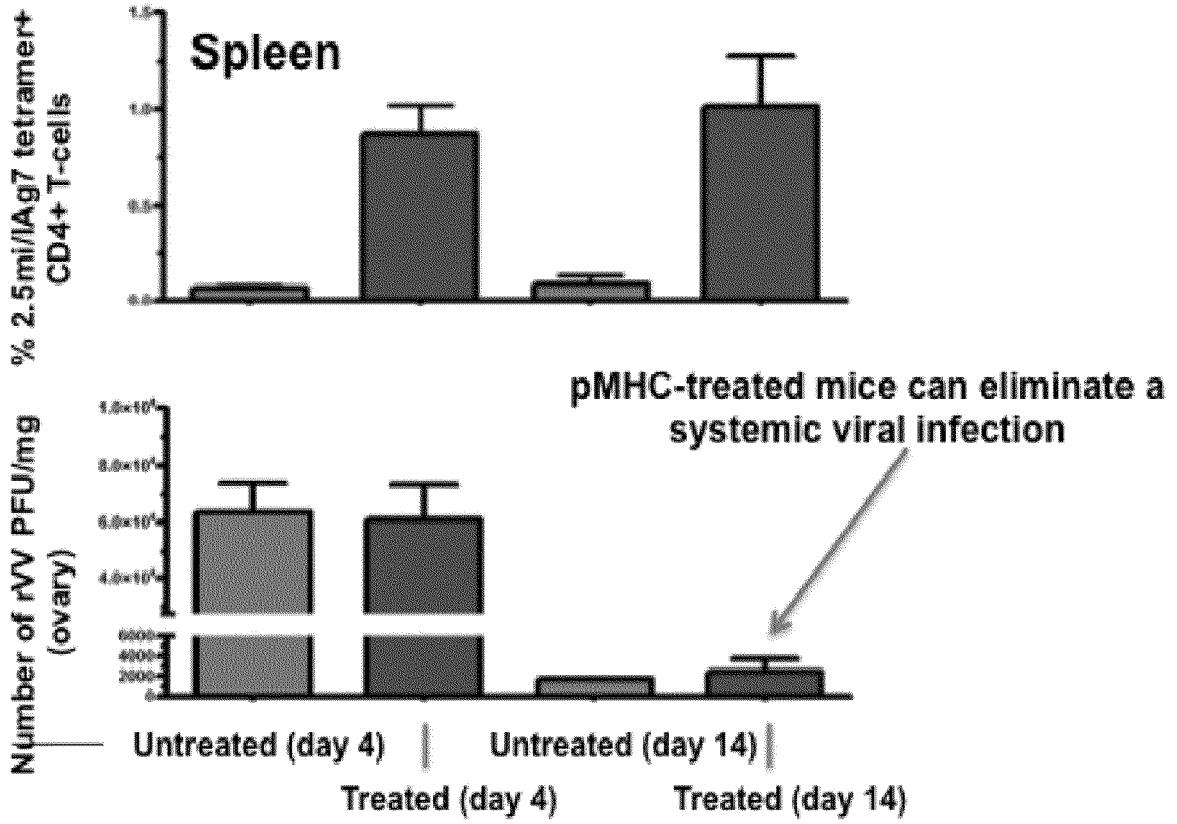


FIG. 10A

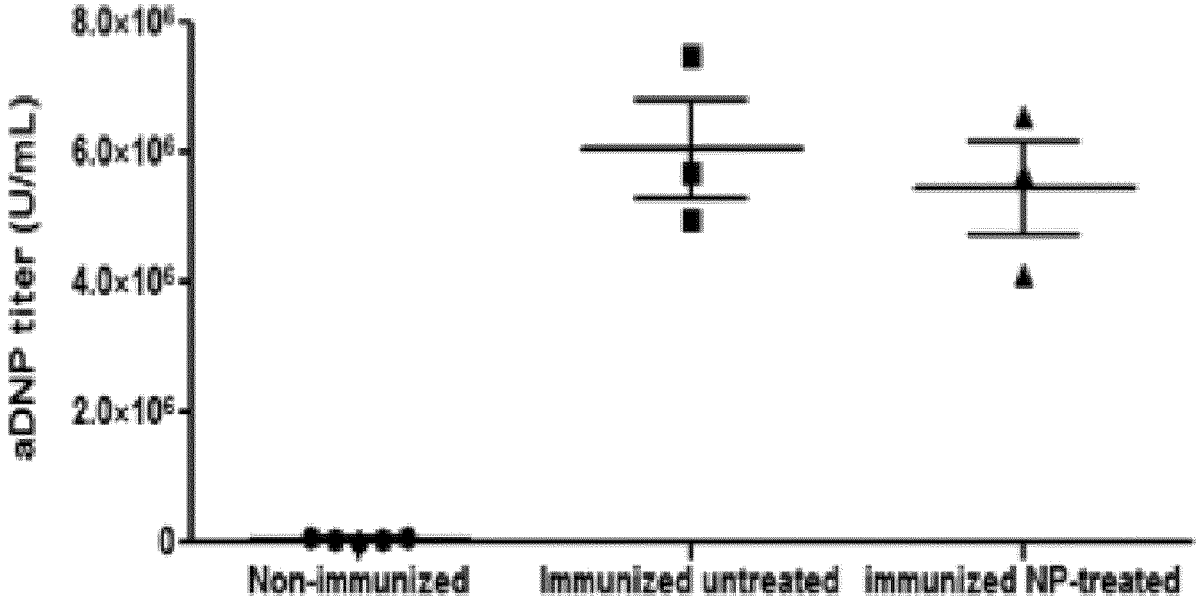


FIG. 10B

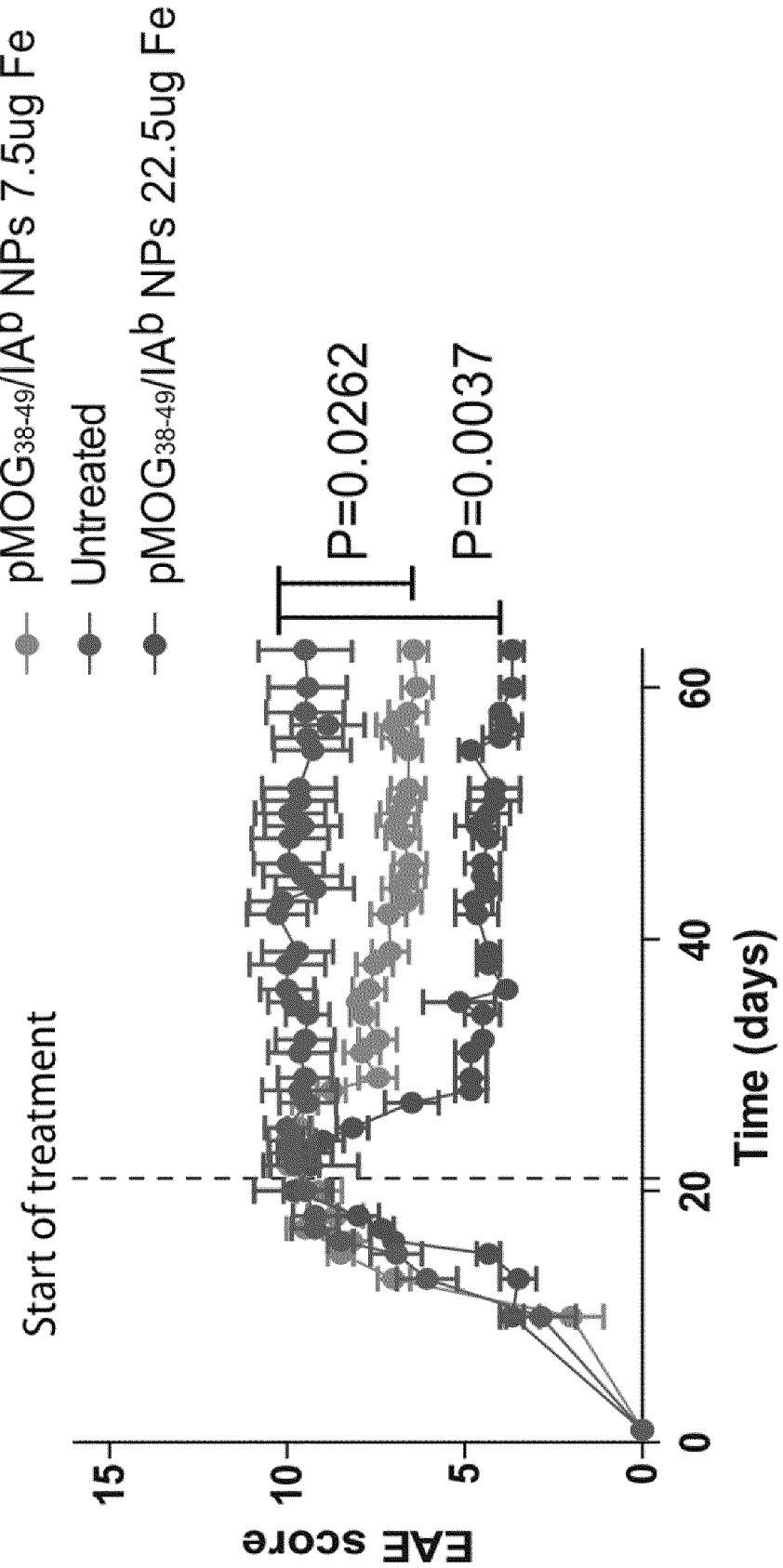

pMOG38-49/IAb NPs 7.5ug Fe
Untreated
pMOG38-49/IAb NPs 22.5ug Fe
P=0.0262
P=0.0037
Start of treatment
EAE score
Time (days)
0
5
10
15
0
20
40
60

FIG. 11

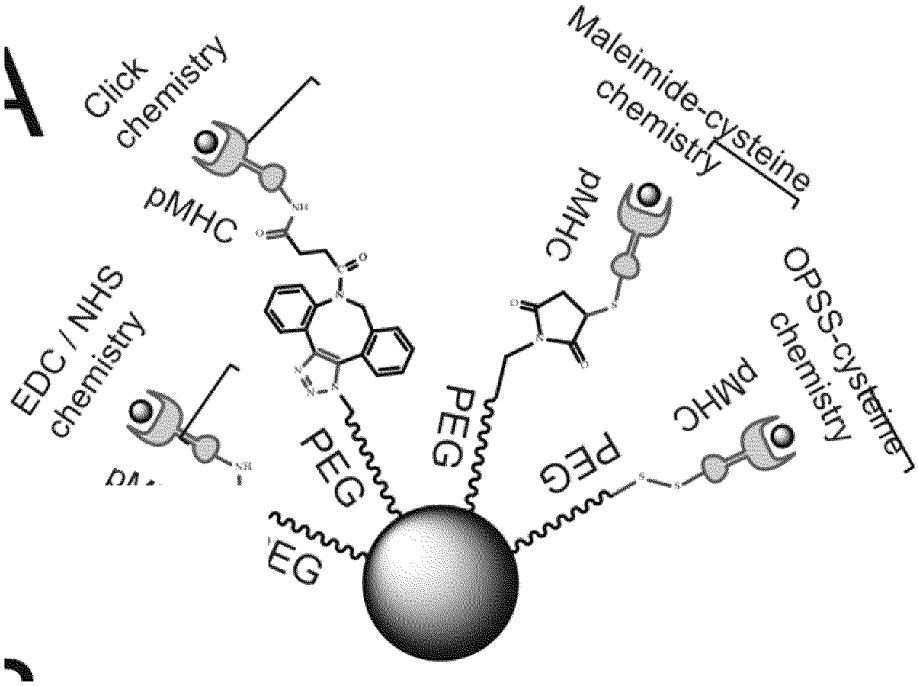


FIG. 12A

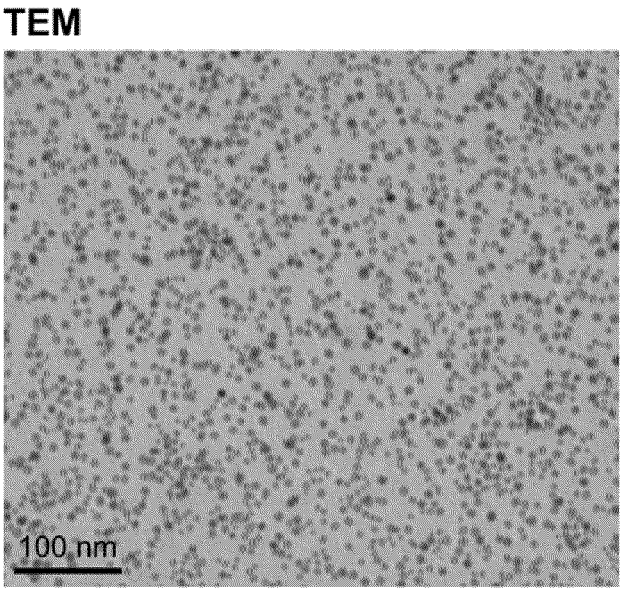


FIG. 12B

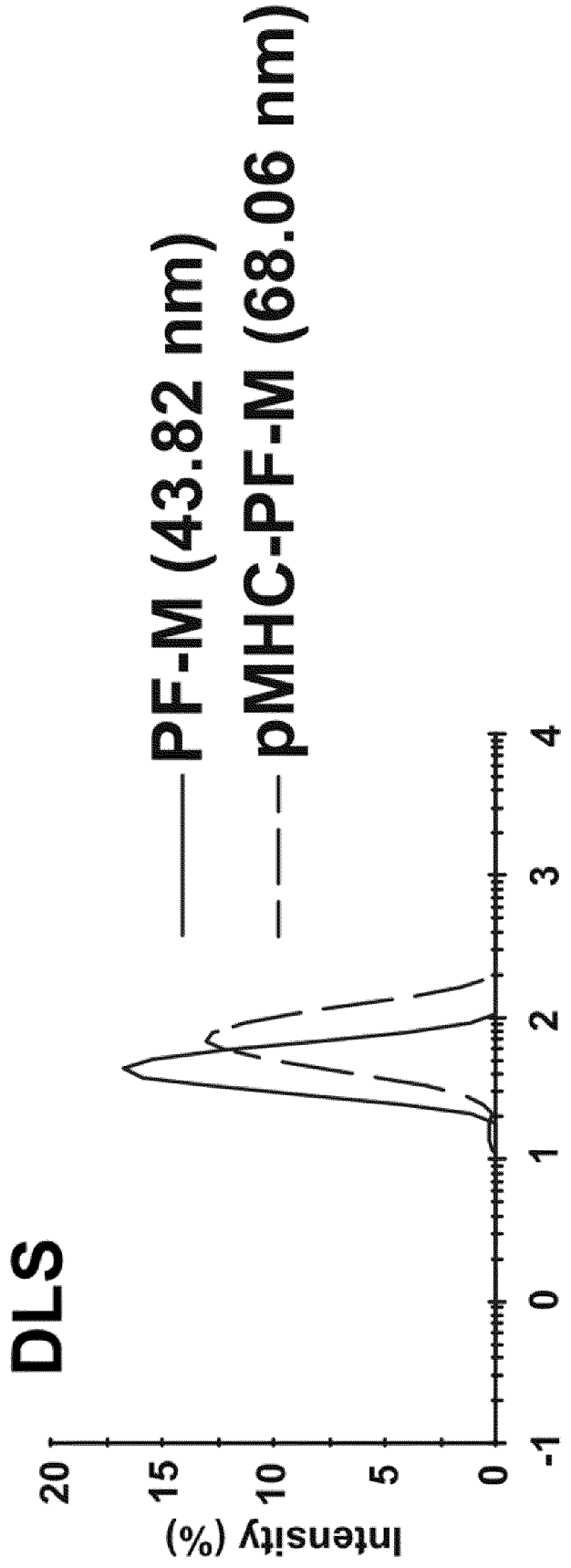

DLS
PF-M (43.82 nm)
pMHC-PF-M (68.06 nm)
Intensity (%)
20
15
10
5
0
-1
0
1
2
3
4
Size (10^n nm)

FIG. 12C

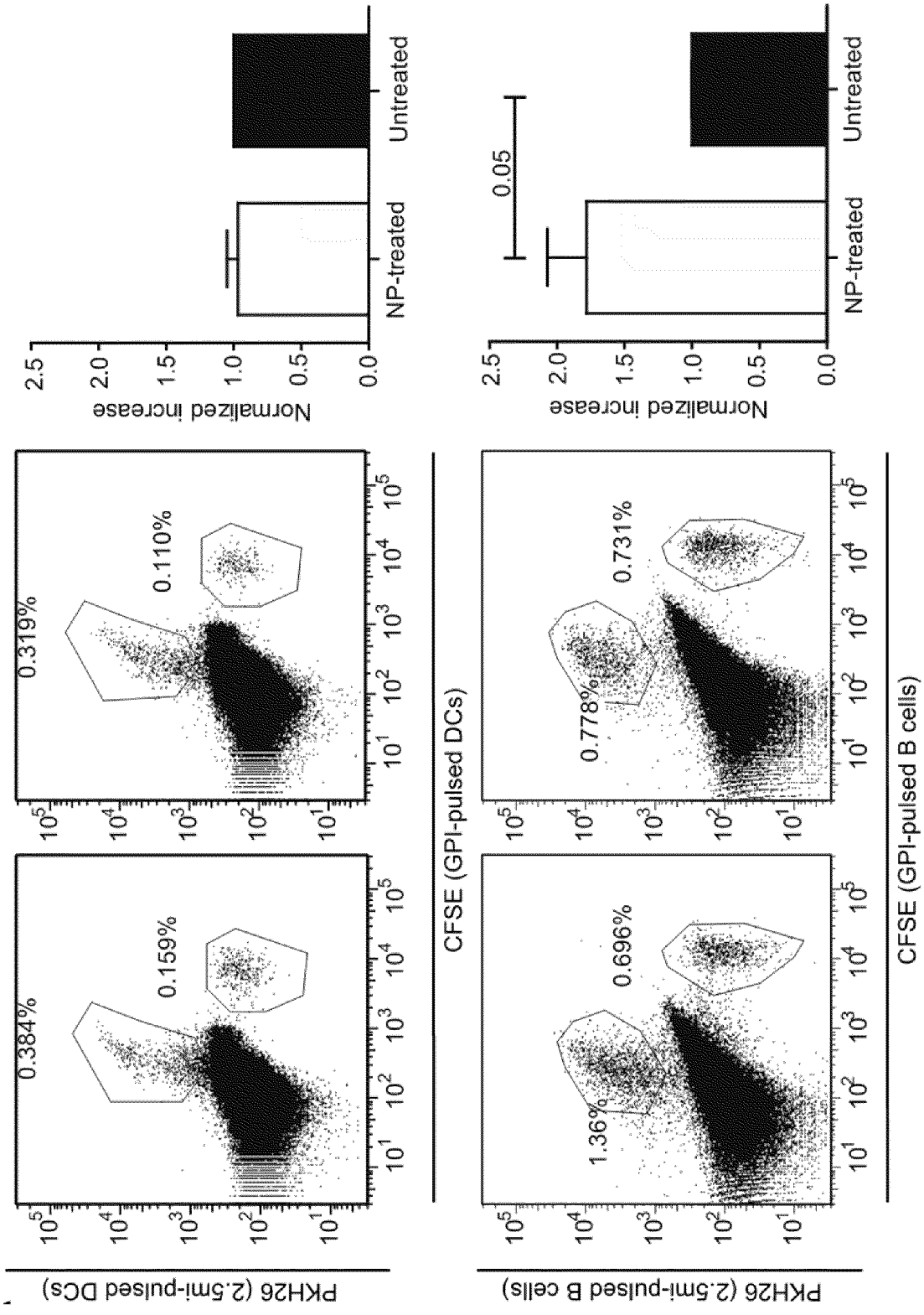

PKH26 (2.5mi-pulsed DCs)
CFSE (GPI-pulsed DCs)
0.384%
0.159%
0.319%
0.110%
PKH26 (2.5mi-pulsed B cells)
CFSE (GPI-pulsed B cells)
1.36%
0.696%
0.778%
0.731%
Normalized increase
NP-treated
Untreated
0.05

FIG. 13A

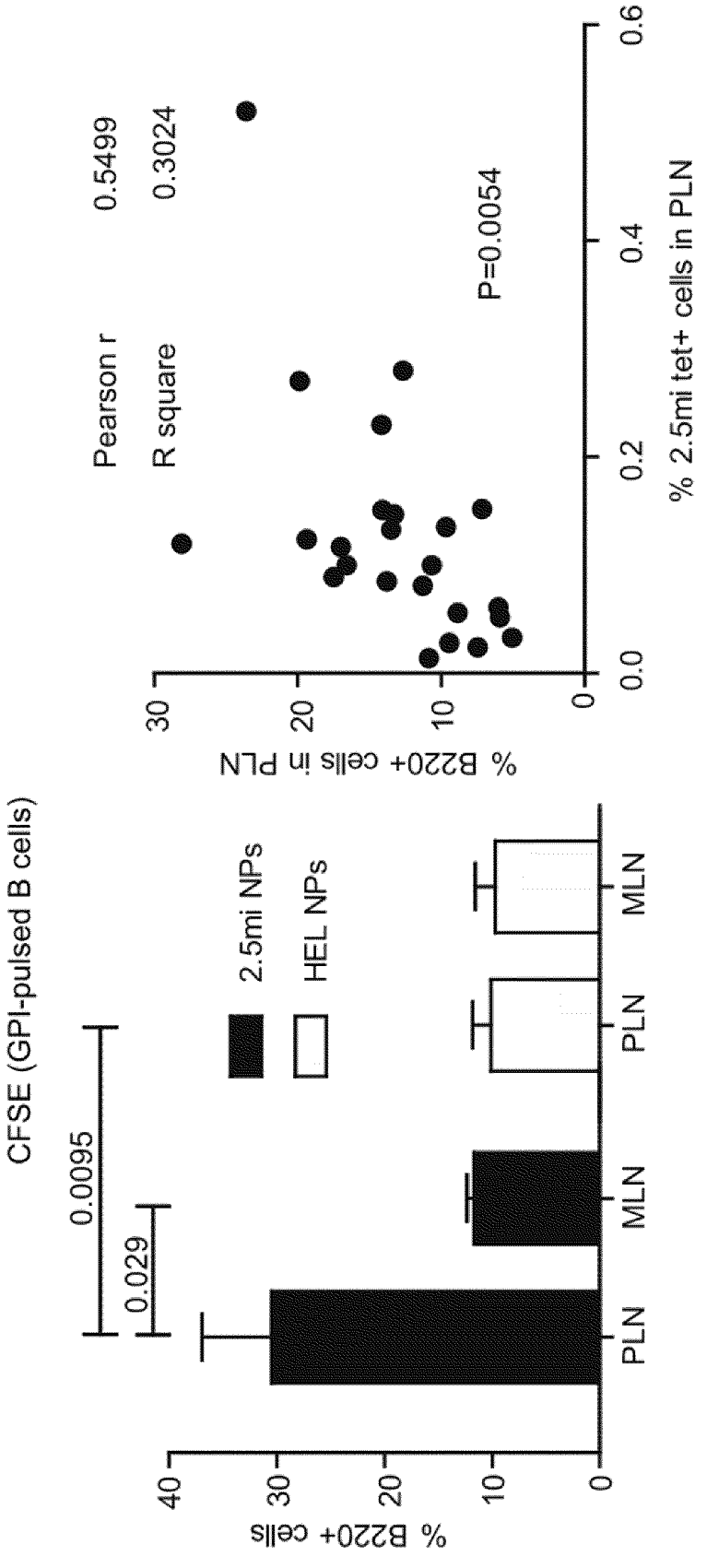

CFSE (GPI-pulsed B cells)
0.0095
0.029
2.5mi NPs
HEL NPs
% B220+ cells
PLN
MLN
PLN
MLN
% B220+ cells in PLN
% 2.5mi tet+ cells in PLN
Pearson r 0.5499
R square 0.3024
P=0.0054

FIG. 13B

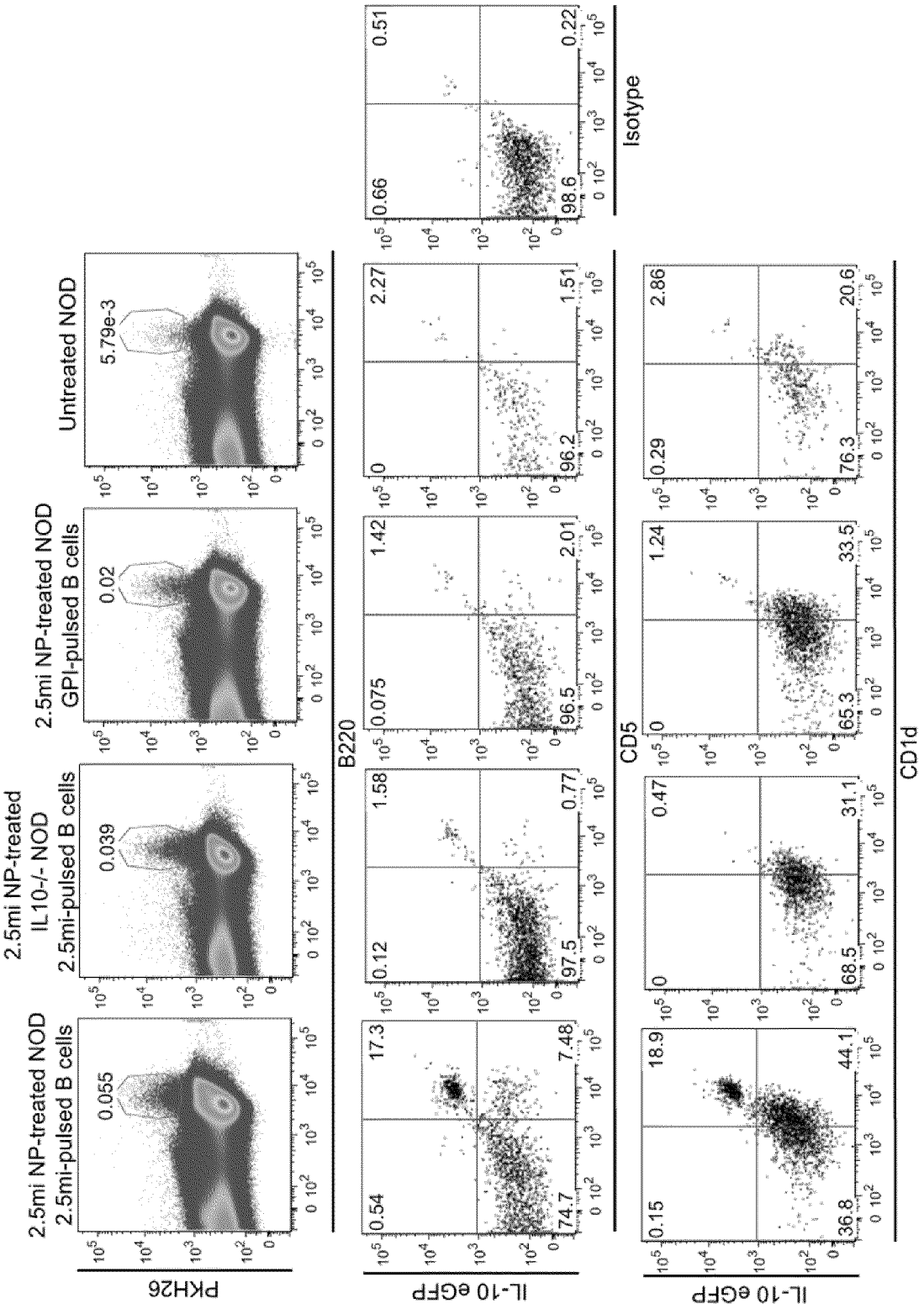

2.5mi NP-treated NOD
2.5mi-pulsed B cells
2.5mi NP-treated IL10-/- NOD
2.5mi-pulsed B cells
2.5mi NP-treated NOD
GPI-pulsed B cells
Untreated NOD
PKH26
0.055
0.039
0.02
5.79e-3
B220
IL-10 eGFP
0.54
17.3
74.7
7.48
0.12
1.58
97.5
0.77
0.075
1.42
96.5
2.01
0
2.27
96.2
1.51
0.66
0.51
98.6
0.22
CD5
CD1d
0.15
18.9
36.8
44.1
0
0.47
68.5
31.1
0
1.24
65.3
33.5
0.29
2.86
76.3
20.6
Isotype

FIG. 13C

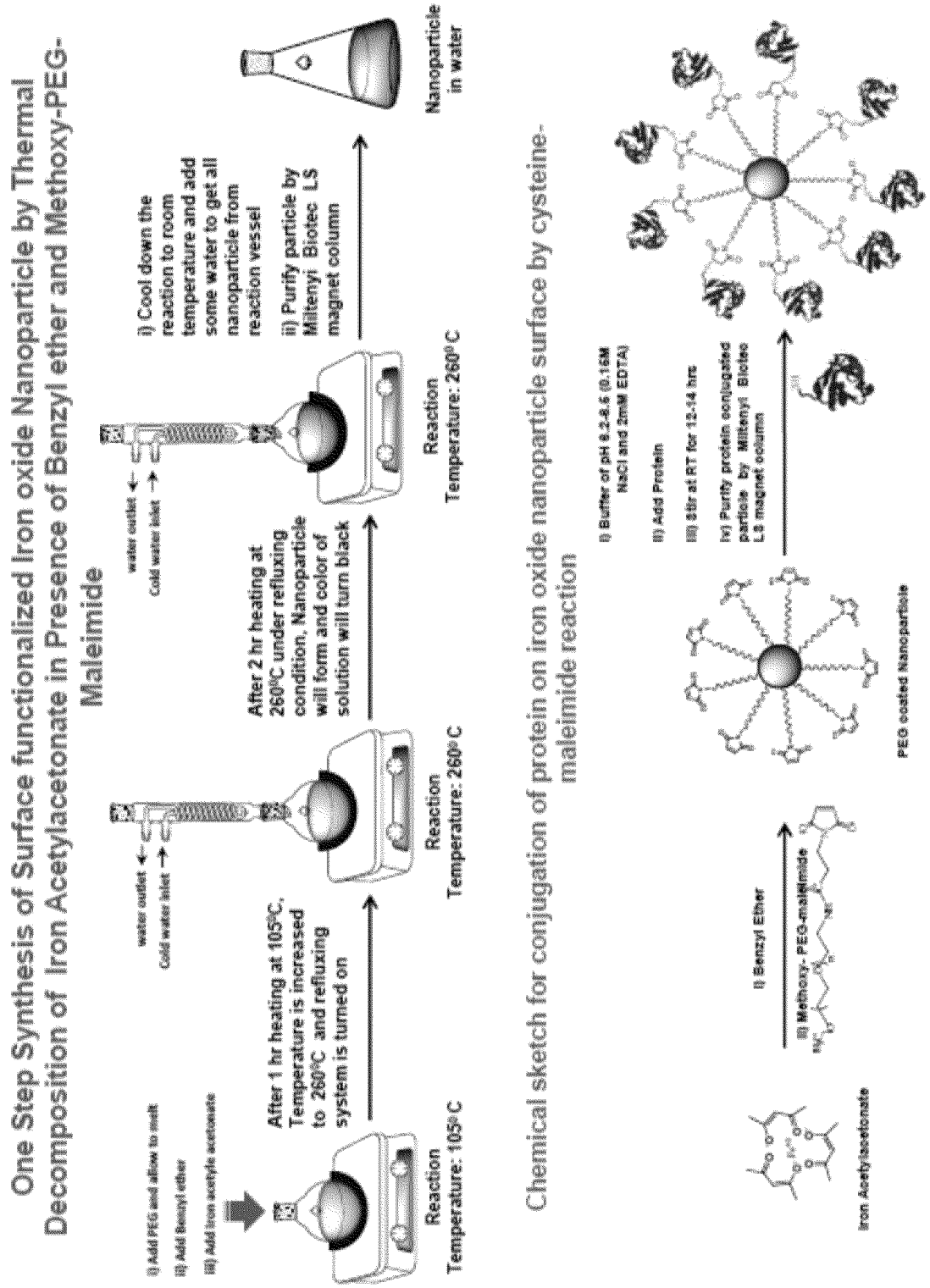

One Step Synthesis of Surface functionalized Iron oxide Nanoparticle by Thermal Decomposition of Iron Acetylacetonate in Presence of Benzyl ether and Methoxy-PEG-Maleimide
i) Add PEG and allow to melt
ii) Add Benzyl ether
iii) Add Iron acetyle acetonate
Reaction Temperature: 105°C
After 1 hr heating at 105°C, Temperature is increased to 260°C and refluxing system is turned on
water outlet
Cold water inlet
Reaction Temperature: 260°C
After 2 hr heating at 260°C under refluxing condition, Nanoparticle will form and color of solution will turn black
water outlet
Cold water inlet
Reaction Temperature: 260°C
i) Cool down the reaction to room temperature and add some water to get all nanoparticle from reaction vessel
ii) Purify particle by Miltenyi Biotec LS magnet column
Nanoparticle in water
Chemical sketch for conjugation of protein on iron oxide nanoparticle surface by cysteine-maleimide reaction
i) Benzyl Ether
iii) Methoxy- PEG-maleimide
Iron Acetylacetonate
PEG coated Nanoparticle
i) Buffer of pH 6.2-6.5 (0.1M NaCl and 2mM EDTA)
ii) Add Protein
iii) Stir at RT for 12-14 hrs
iv) Purify protein conjugated particle by Miltenyi Biotec LS magnet column

FIG. 14