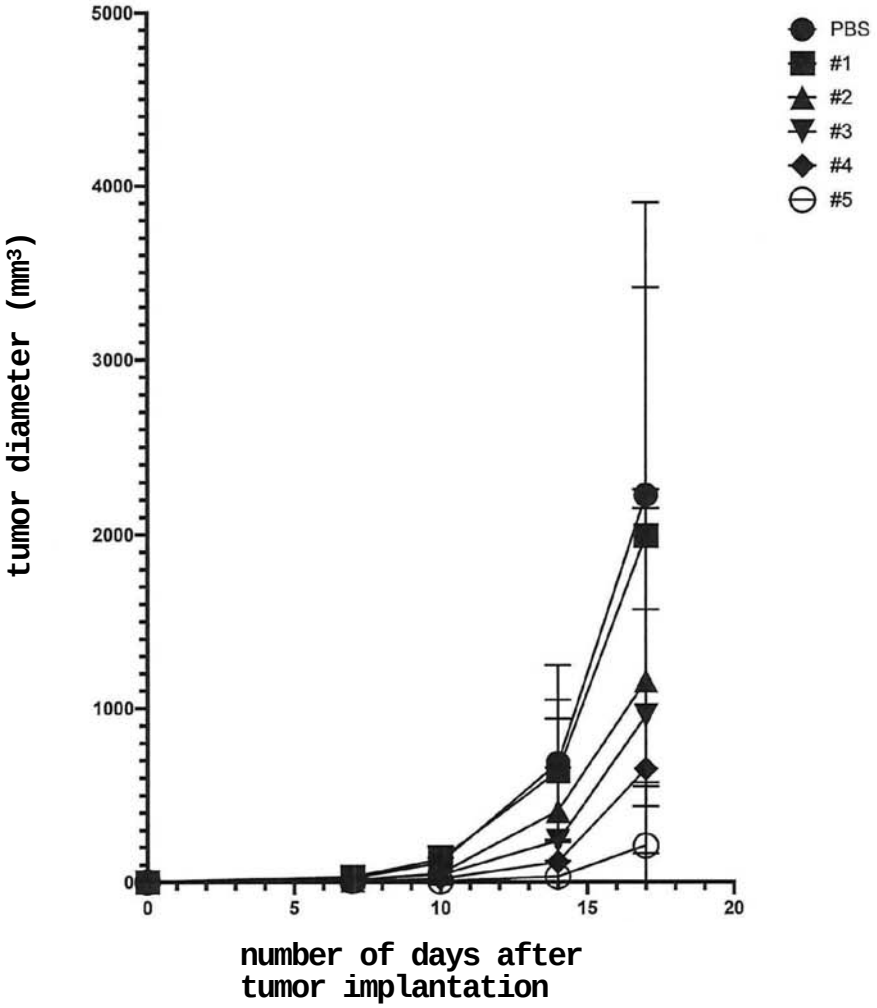
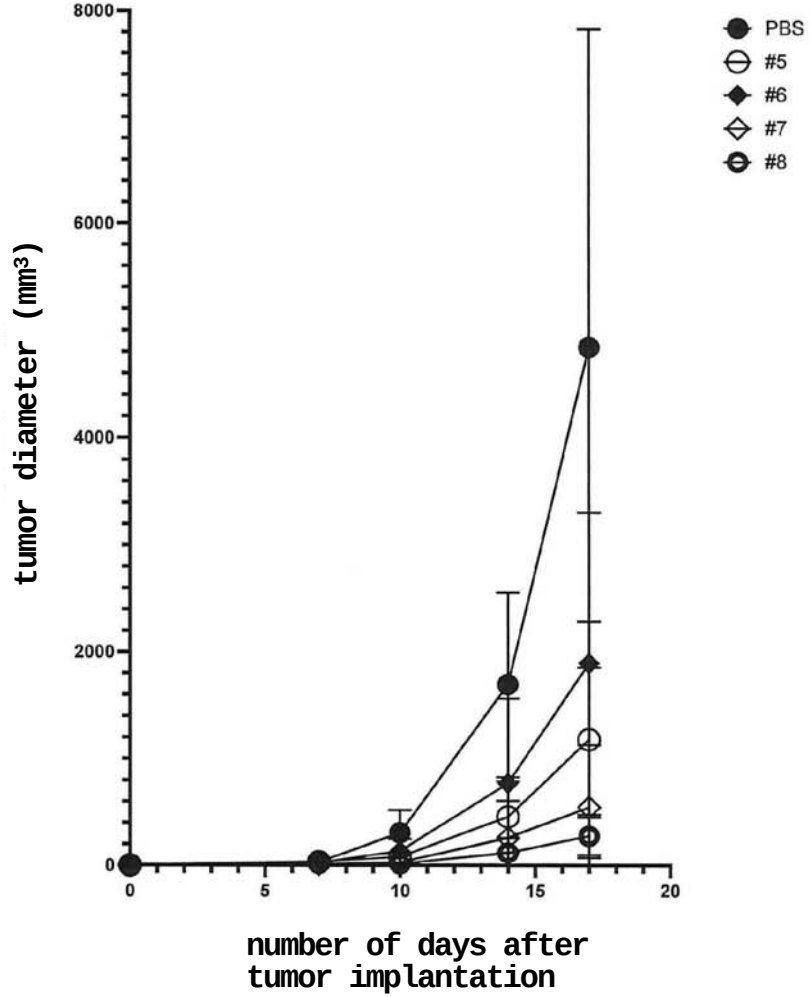
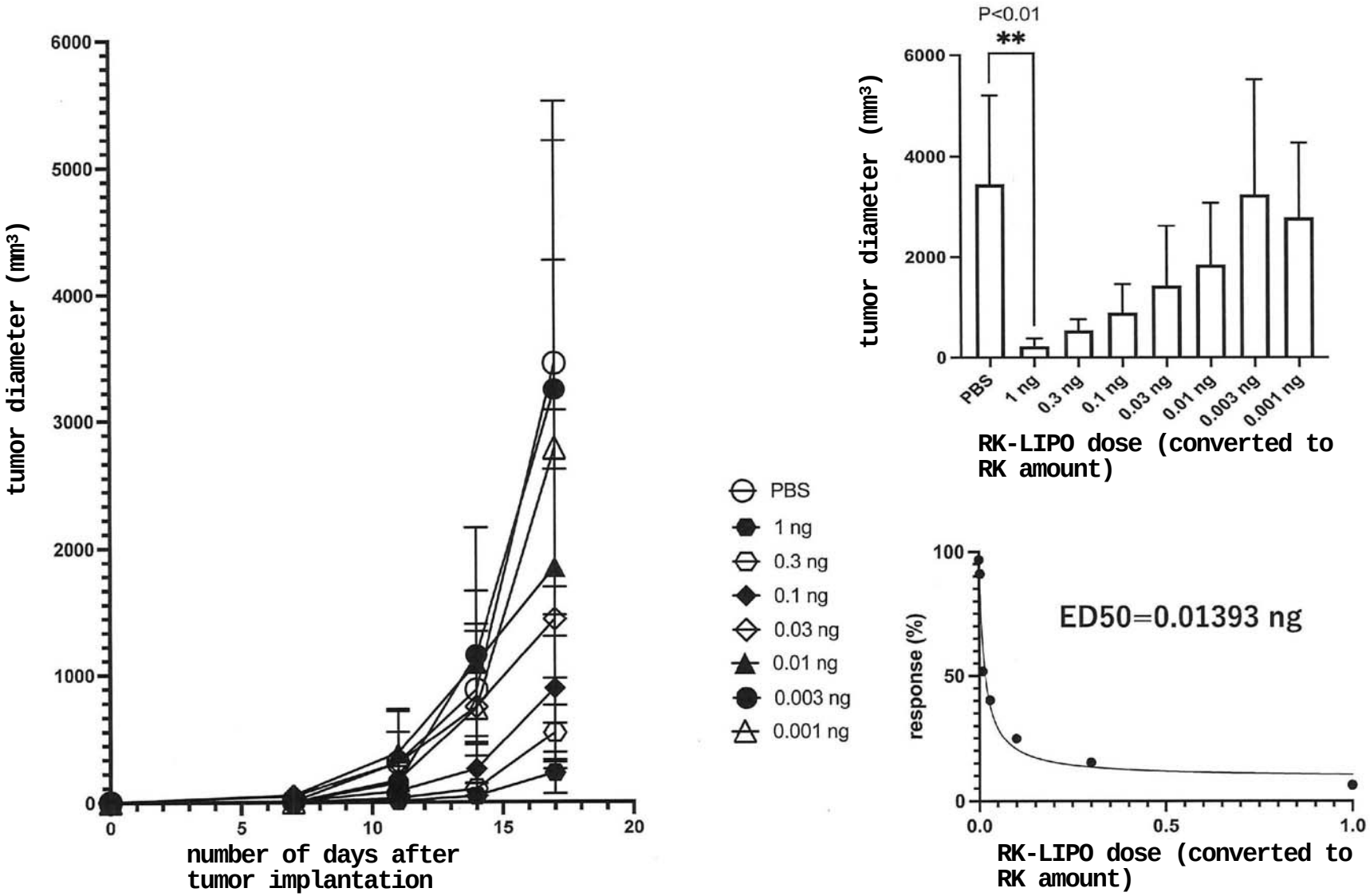
[DRAWINGS]

[Fig. 1]

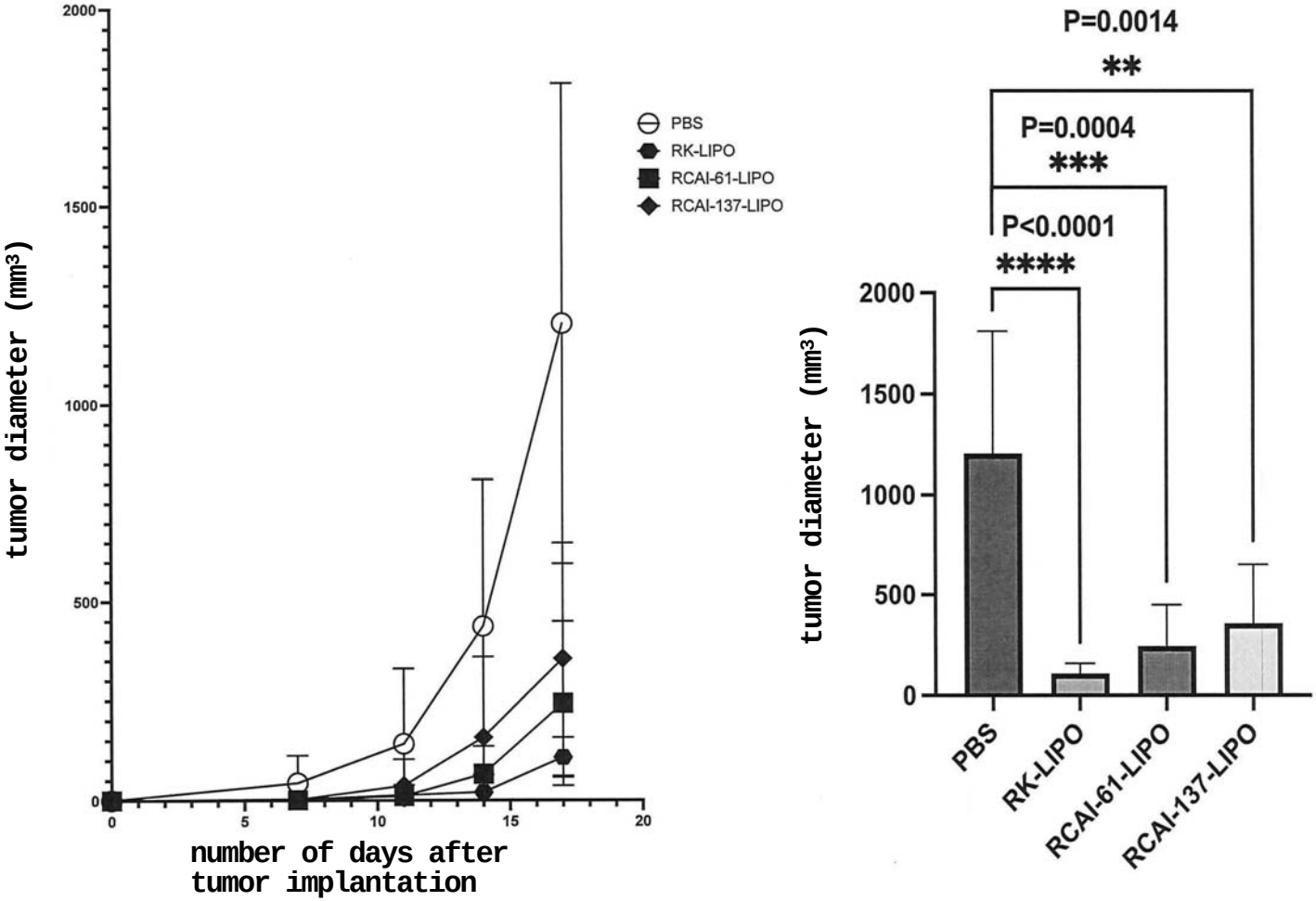




[Fig. 2]

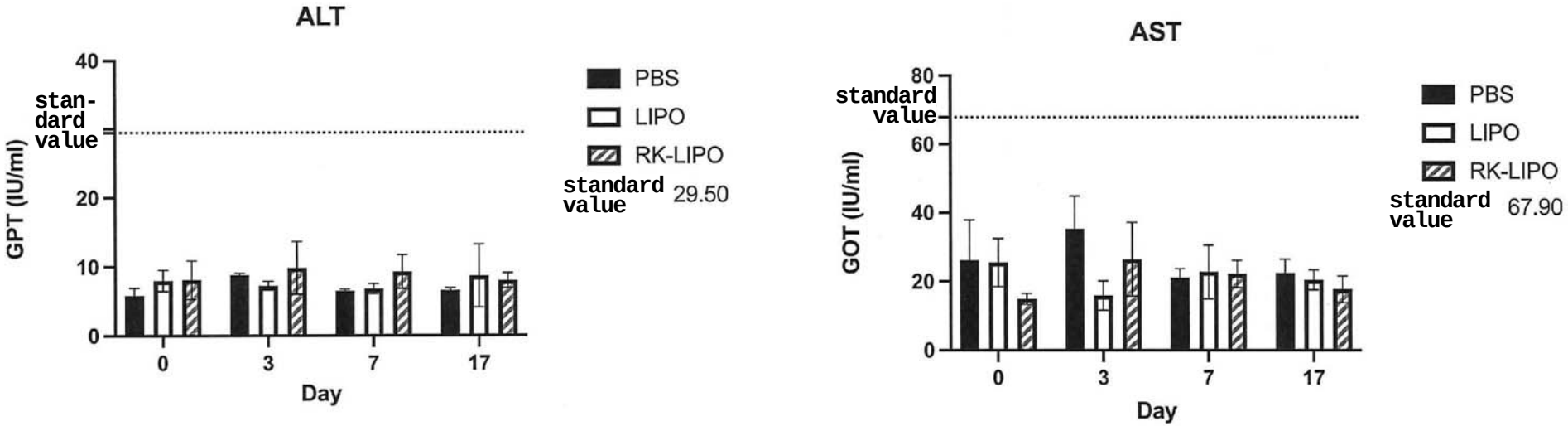

tumor diameter (mm³)
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
number of days after tumor implantation
PBS
1 ng
0.3 ng
0.1 ng
0.03 ng
0.01 ng
0.003 ng
0.001 ng
P<0.01
**
tumor diameter (mm³)
6000
4000
2000
0
PBS
1 ng
0.3 ng
0.1 ng
0.03 ng
0.01 ng
0.003 ng
0.001 ng
RK-LIPO dose (converted to RK amount)
response (%)
100
50
0
ED50=0.01393 ng
0.0
0.5
1.0
RK-LIPO dose (converted to RK amount)

[Fig. 3]

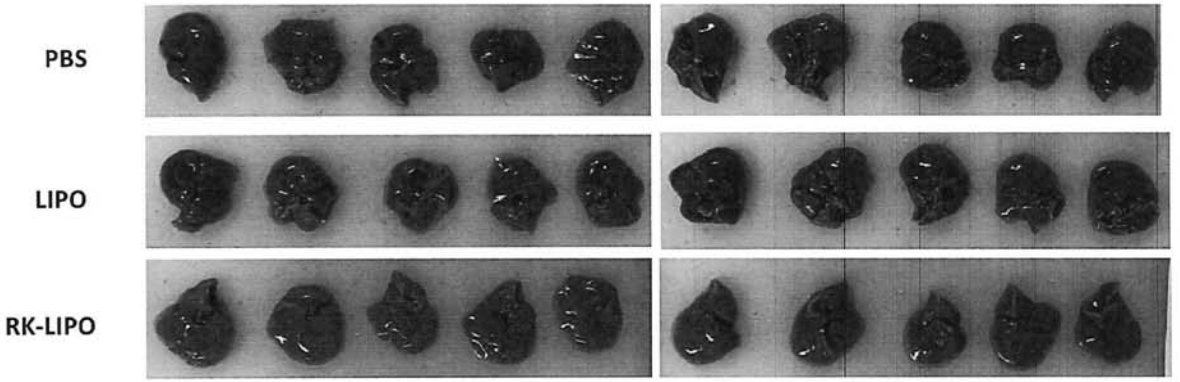

tumor diameter (mm³)
2000
1500
1000
500
0
0
5
10
15
20
number of days after tumor implantation
PBS
RK-LIPO
RCAI-61-LIPO
RCAI-137-LIPO
P=0.0014
**
P=0.0004
***
P<0.0001
****
tumor diameter (mm³)
2000
1500
1000
500
0
PBS
RK-LIPO
RCAI-61-LIPO
RCAI-137-LIPO

[Fig. 4]

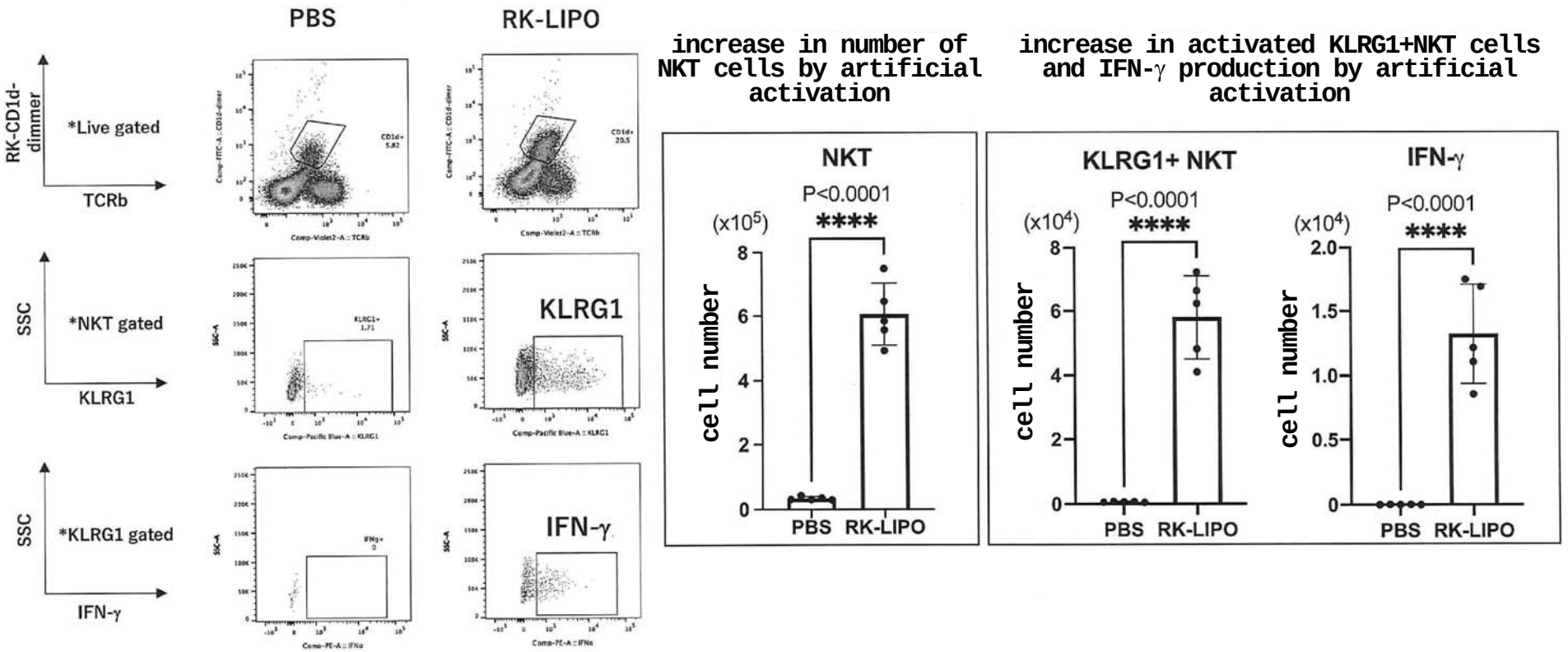
no acute liver toxicity



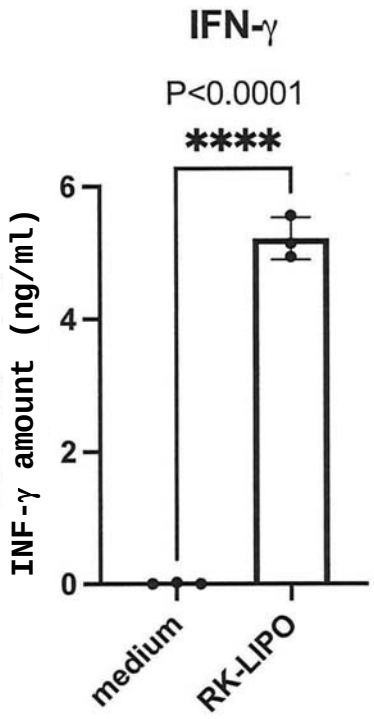
no white spots on the liver



[Fig. 5-1]


PBS
RK-LIPO
RK-CD1d-dimmer
*Live gated
TCRb
SSC
*NKT gated
KLRG1
SSC
*KLRG1 gated
IFN-γ
KLRG1
IFN-γ
increase in number of NKT cells by artificial activation
NKT
P<0.0001
****
(x10^5)
cell number
PBS
RK-LIPO
increase in activated KLRG1+NKT cells and IFN-γ production by artificial activation
KLRG1+ NKT
IFN-γ
P<0.0001
****
(x10^4)
cell number
PBS
RK-LIPO

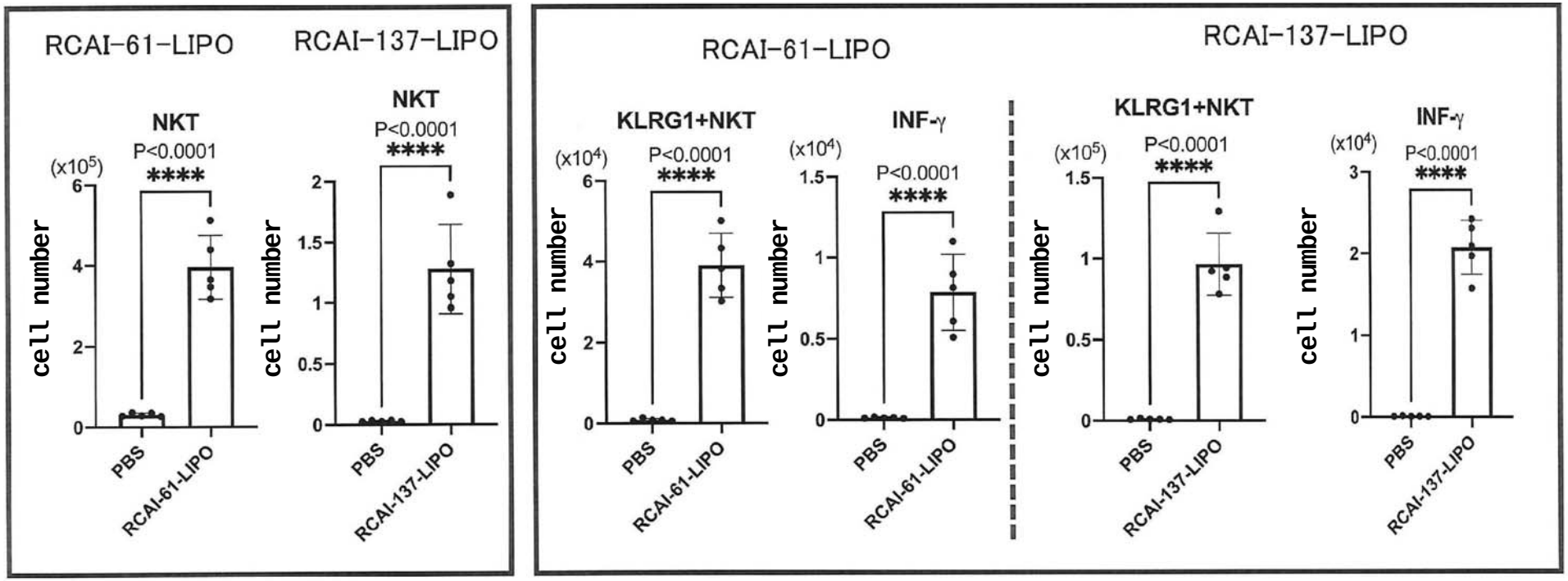
[Fig. 5-2]



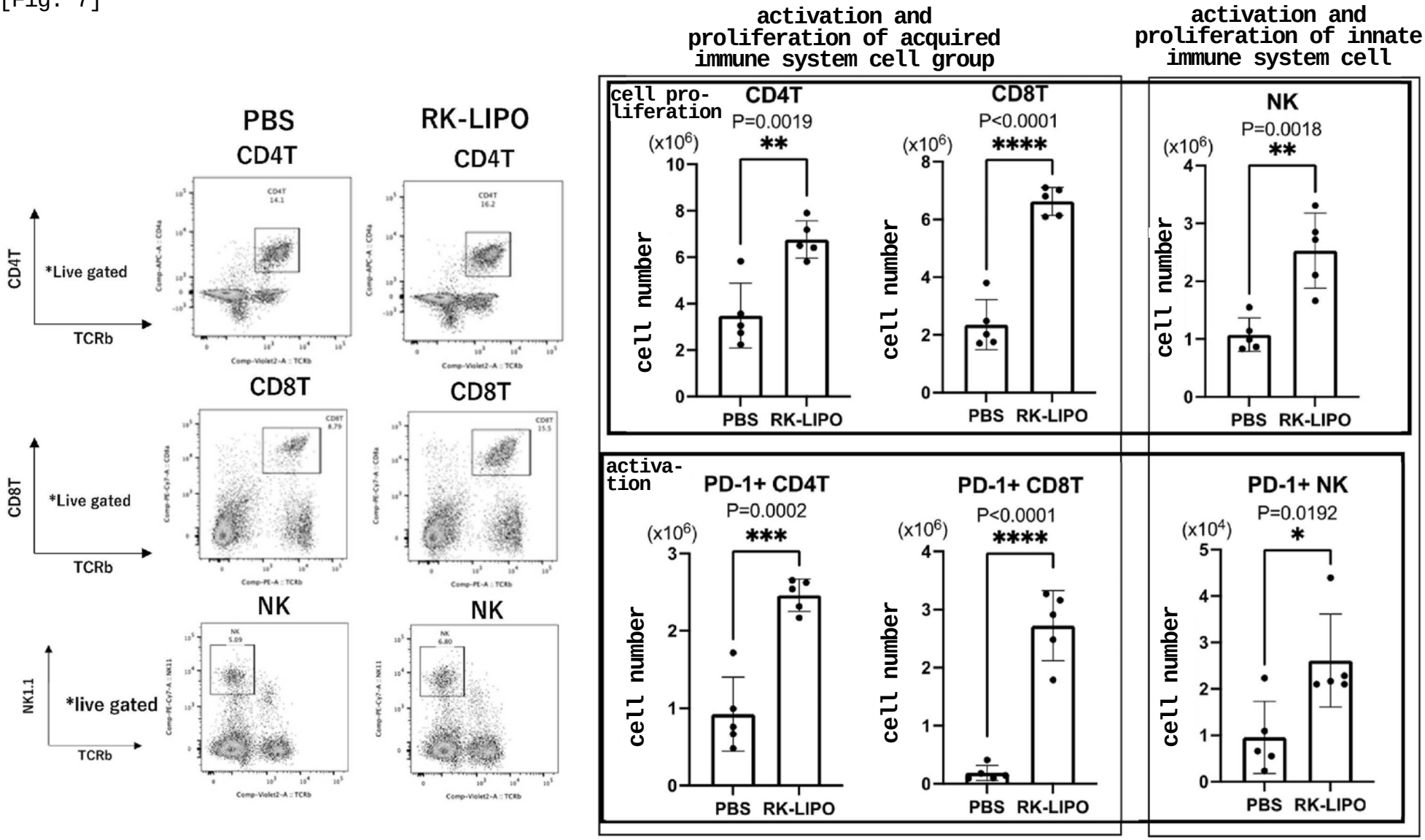
[Fig. 6]

increase in number of NKT cells by artificial activation

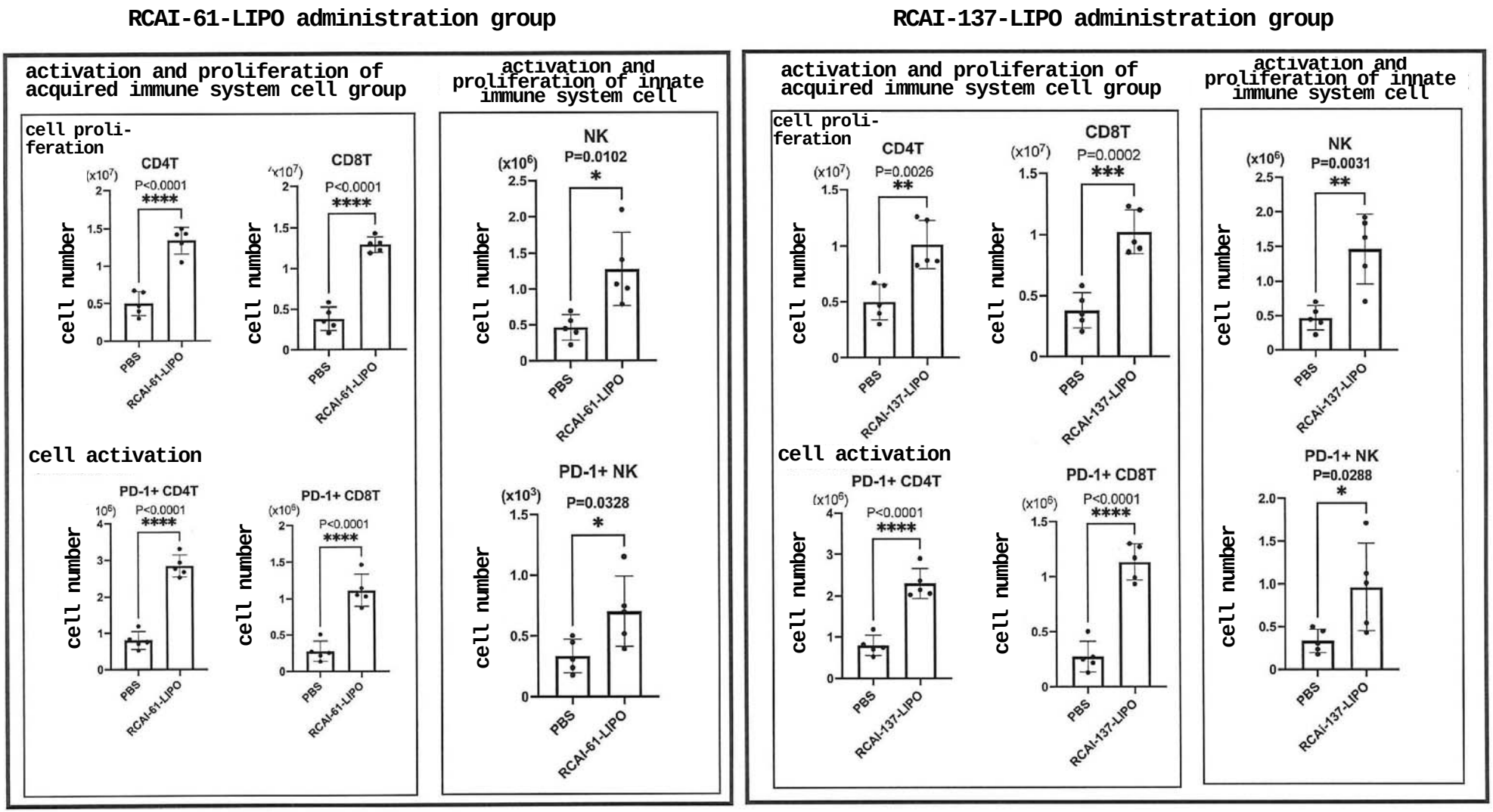
increase in number of activated KLRG1+NKT cells and IFN-γ production by artificial activation



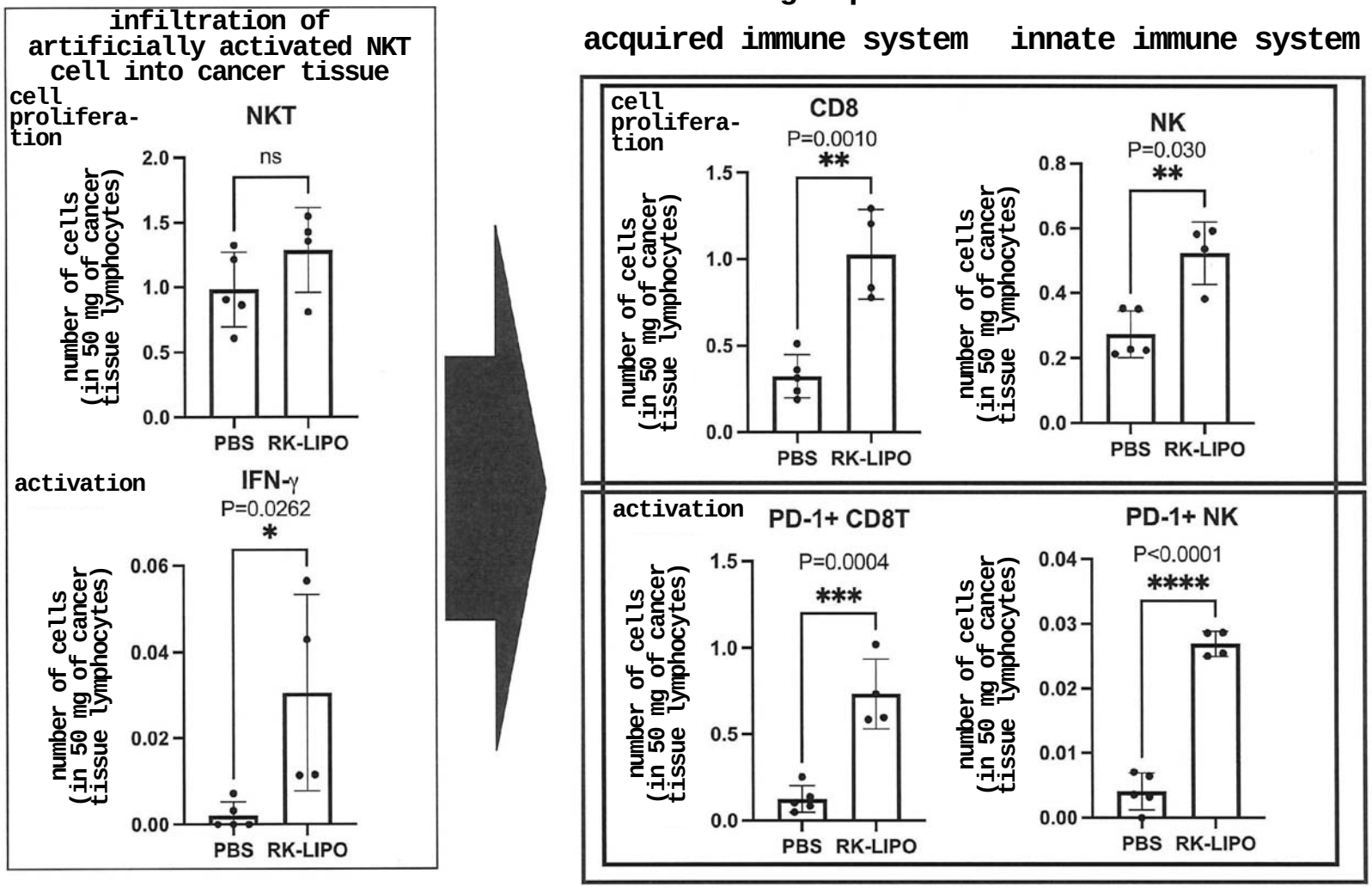
[Fig. 7]


PBS
RK-LIPO
CD4T
CD4T
CD4T
*Live gated
TCRb
CD8T
CD8T
CD8T
*Live gated
TCRb
NK
NK
NK1.1
*live gated
TCRb
activation and proliferation of acquired immune system cell group
activation and proliferation of innate immune system cell
cell pro-liferation
CD4T
P=0.0019
**
CD8T
P<0.0001
****
NK
P=0.0018
**
(x10^6)
cell number
PBS RK-LIPO
activa-tion
PD-1+ CD4T
P=0.0002
***
PD-1+ CD8T
P<0.0001
****
PD-1+ NK
P=0.0192
*
(x10^4)

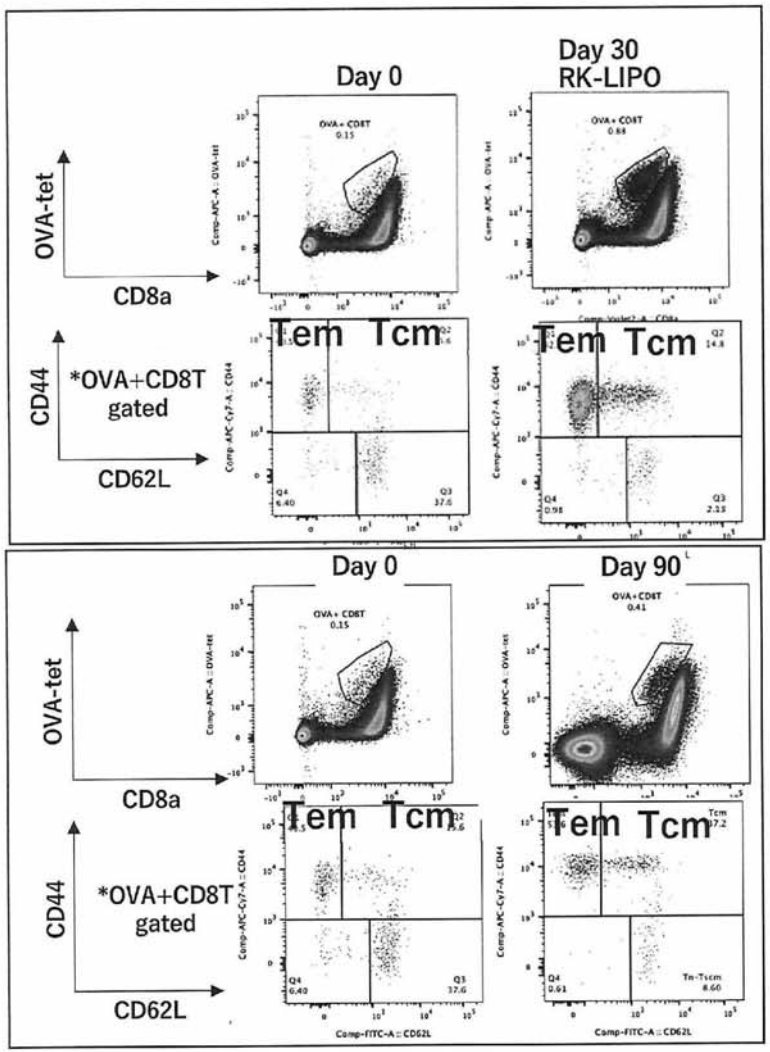
[Fig. 8]


RCAI-61-LIPO administration group
RCAI-137-LIPO administration group
activation and proliferation of acquired immune system cell group
activation and proliferation of innate immune system cell
cell proli-feration
CD4T
CD8T
NK
P<0.0001
****
P=0.0102
*
P=0.0026
**
P=0.0002
***
P=0.0031
**
cell number
PBS
RCAI-61-LIPO
RCAI-137-LIPO
cell activation
PD-1+ CD4T
PD-1+ CD8T
PD-1+ NK
P<0.0001
****
P=0.0328
*
P=0.0288
*

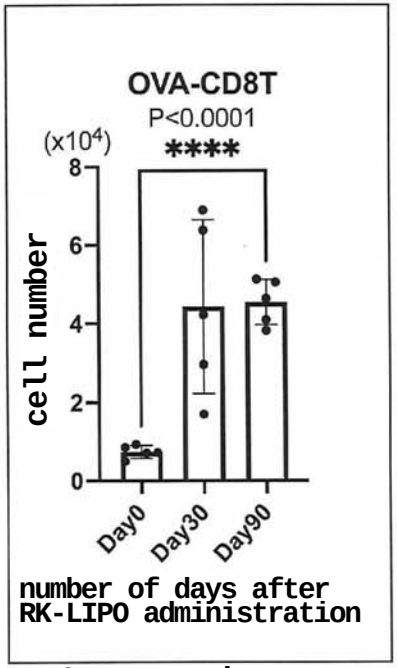
[Fig. 9]


infiltration of artificially activated NKT cell into cancer tissue
cell proliferation
NKT
ns
number of cells (in 50 mg of cancer tissue lymphocytes)
2.0
1.5
1.0
0.5
0.0
PBS
RK-LIPO
activation
IFN-γ
P=0.0262
*
0.06
0.04
0.02
0.00
activation and proliferation of immune cell group in cancer tissue
acquired immune system
innate immune system
CD8
P=0.0010
**
NK
P=0.030
**
0.8
0.6
0.4
0.2
PD-1+ CD8T
P=0.0004
***
PD-1+ NK
P<0.0001
****
0.04
0.03
0.02
0.01

[Fig. 10]

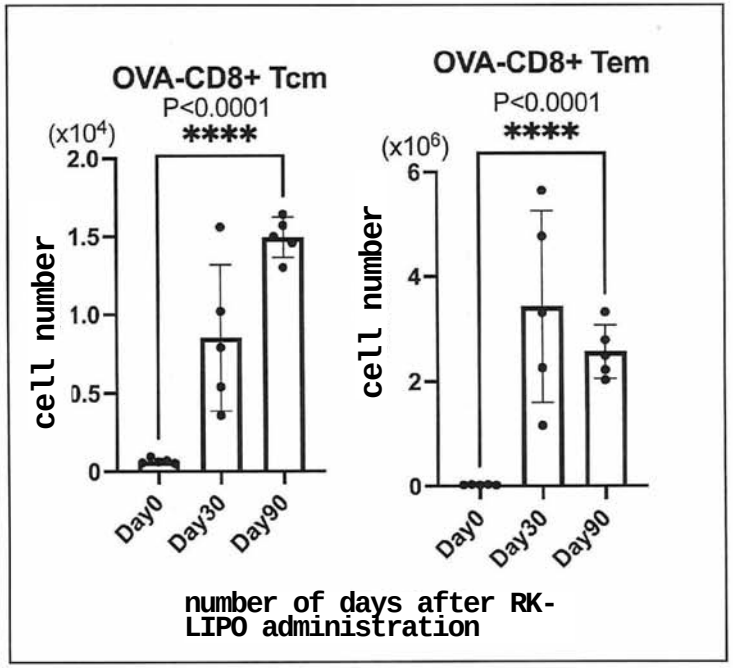


long-term proliferation maintenance of antigen-specific CD8T cell population

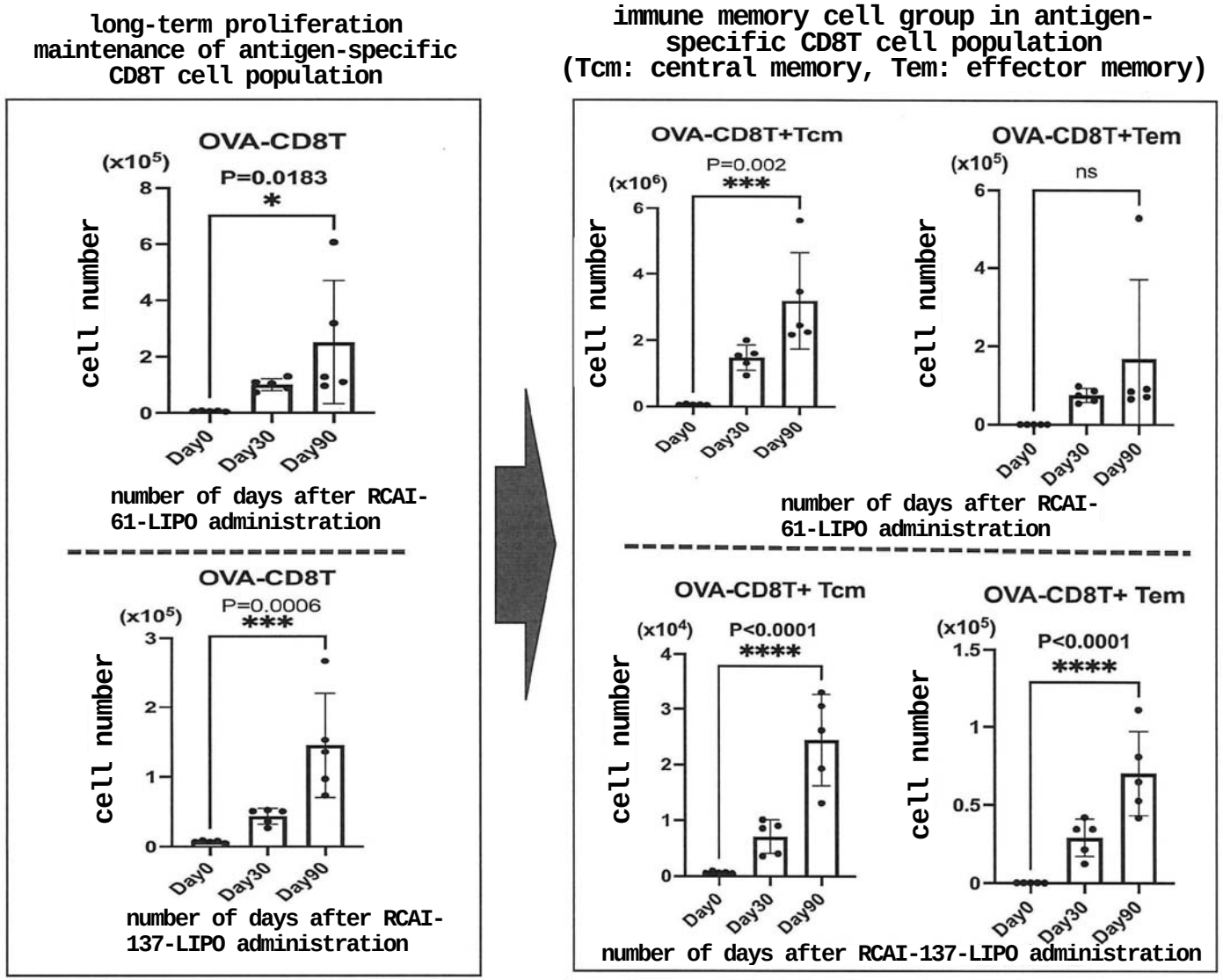


OVA: pseudo-cancer antigen

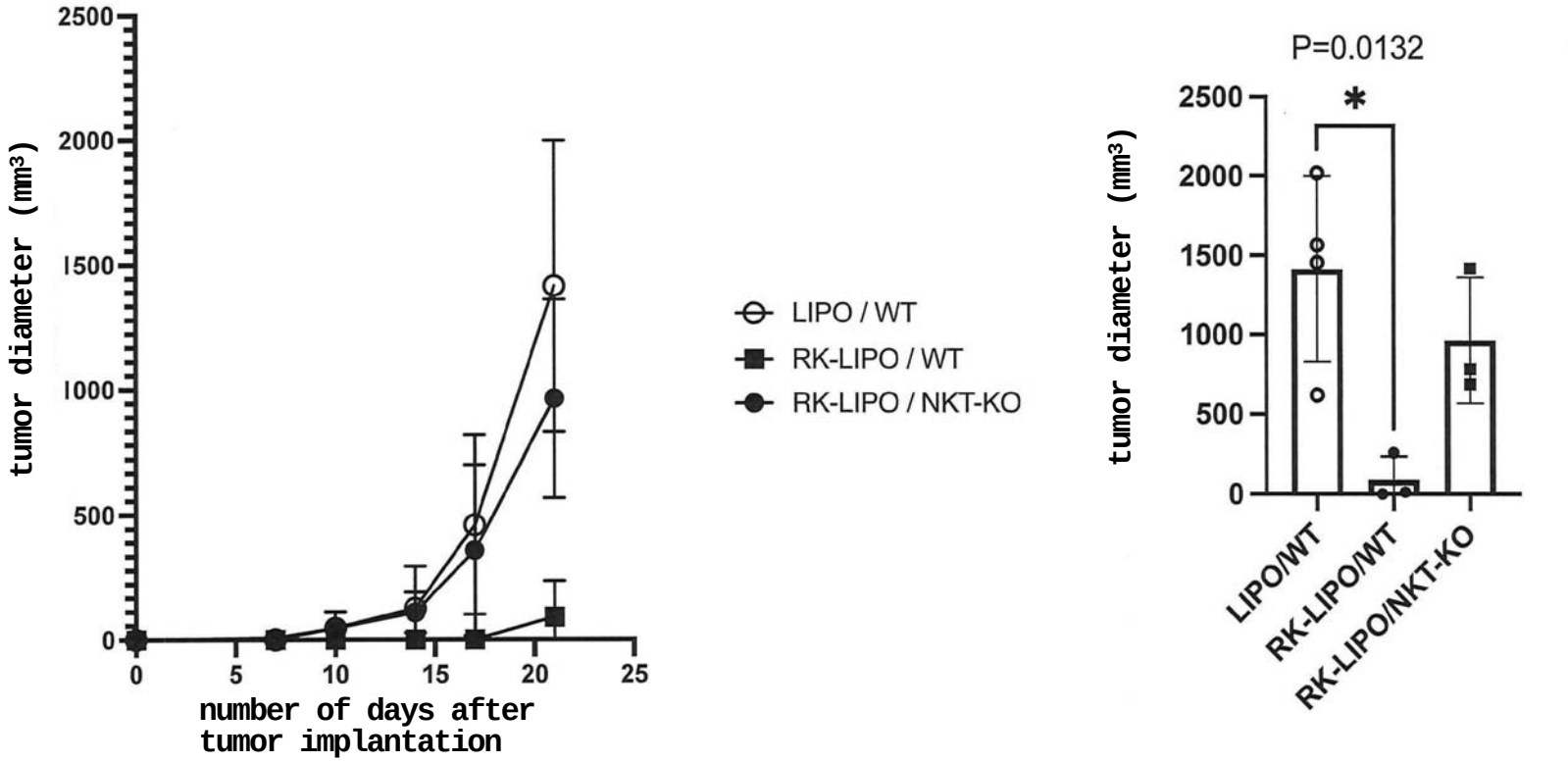
immune memory cell group in antigen-specific CD8T cell population (Tcm: central memory, Tem: effector memory)



[Fig. 11]


long-term proliferation maintenance of antigen-specific CD8T cell population
immune memory cell group in antigen-specific CD8T cell population (Tcm: central memory, Tem: effector memory)
OVA-CD8T
(x10^5)
P=0.0183
*
cell number
Day0
Day30
Day90
number of days after RCAI-61-LIPO administration
OVA-CD8T
(x10^5)
P=0.0006
***
cell number
number of days after RCAI-137-LIPO administration
OVA-CD8T+Tcm
(x10^6)
P=0.002
***
OVA-CD8T+Tem
(x10^5)
ns
number of days after RCAI-61-LIPO administration
OVA-CD8T+ Tcm
(x10^4)
P<0.0001
****
OVA-CD8T+ Tem
(x10^5)
P<0.0001
****
number of days after RCAI-137-LIPO administration

[Fig. 12]


tumor diameter (mm³)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
number of days after tumor implantation
LIPO / WT
RK-LIPO / WT
RK-LIPO / NKT-KO
P=0.0132
*
tumor diameter (mm³)
2500
2000
1500
1000
500
0
LIPO/WT
RK-LIPO/WT
RK-LIPO/NKT-KO