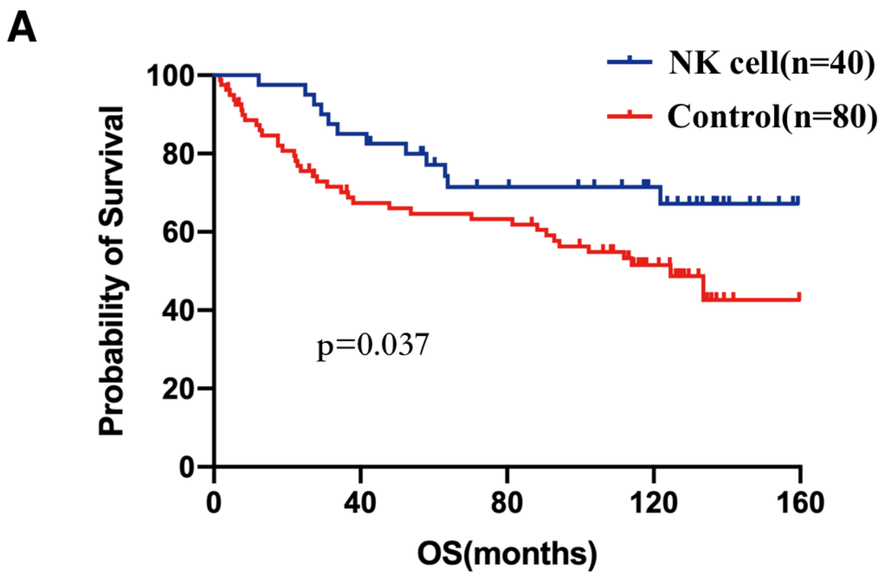
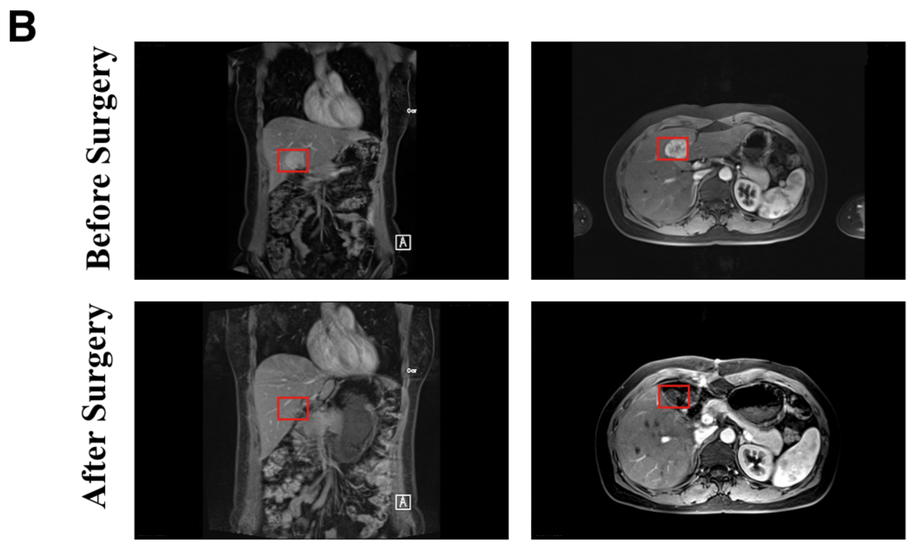
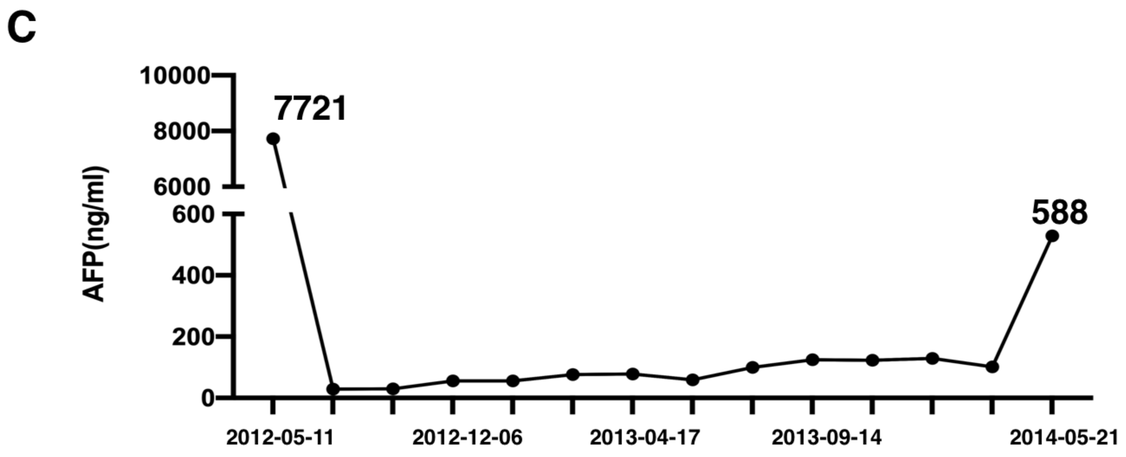
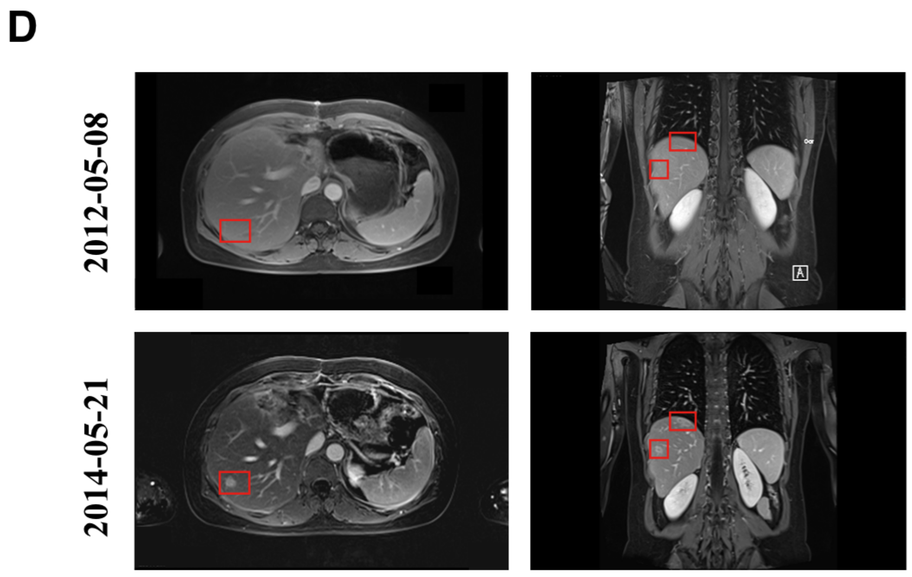
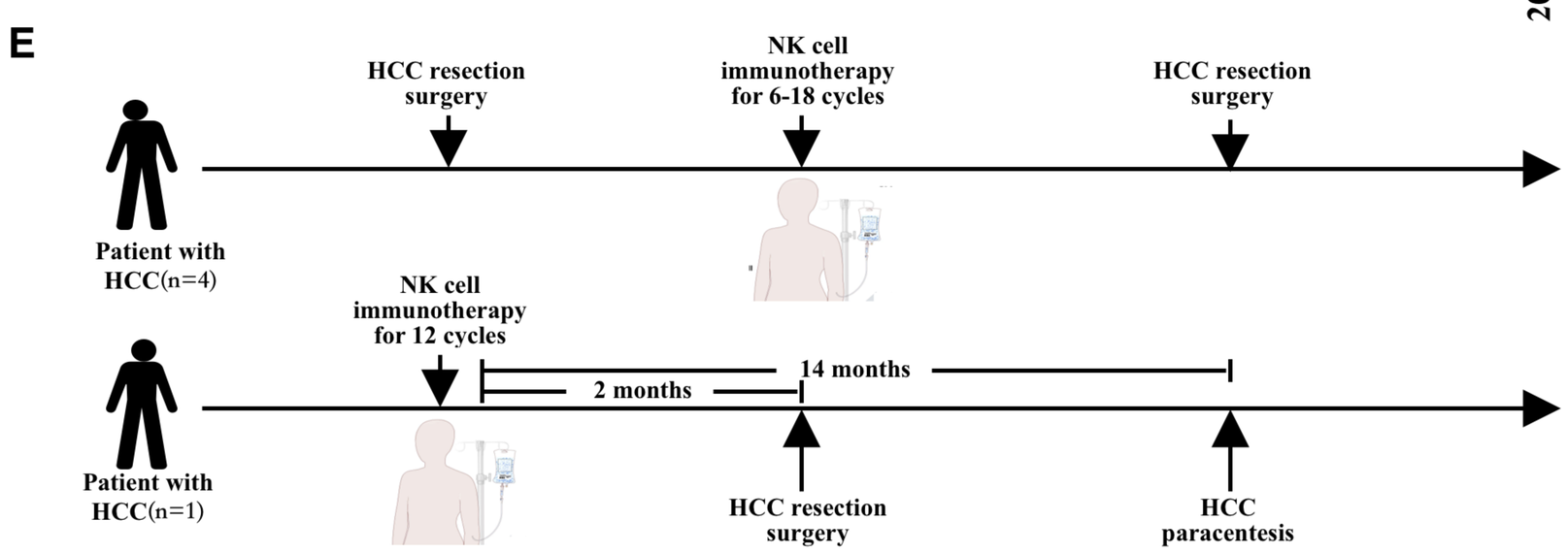
Fig.1

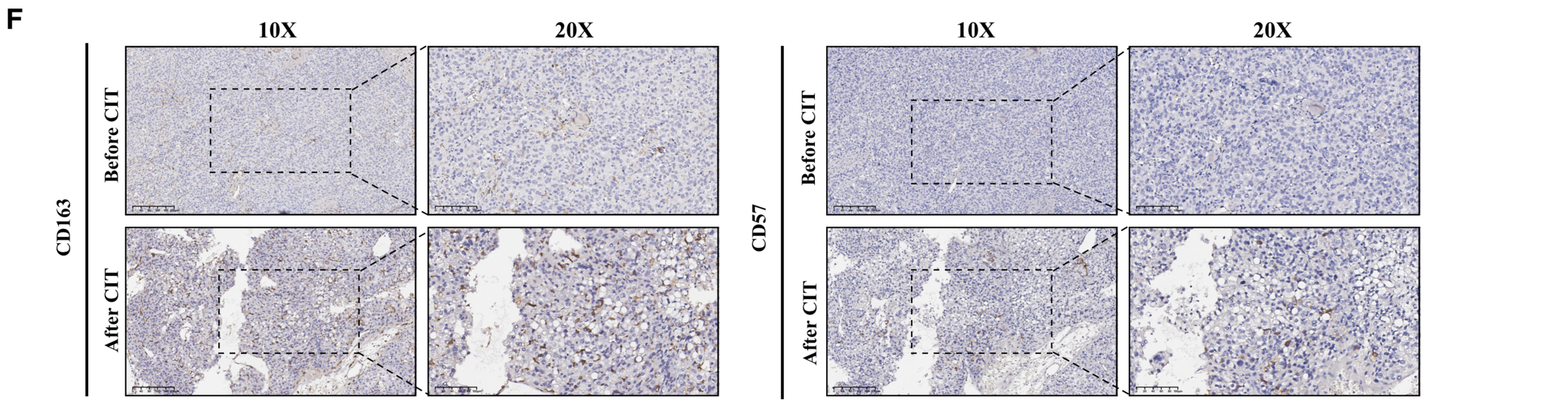

A
NK cell(n=40)
Control(n=80)
Probability of Survival
100
80
60
40
20
0
p=0.037
0
40
80
120
160
OS(months)

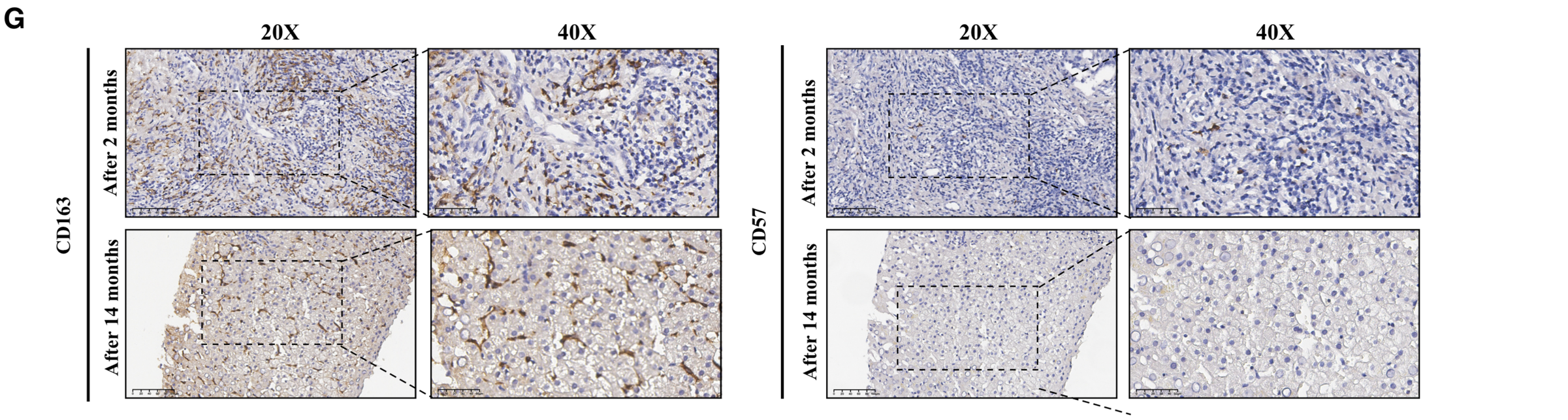

B
Before Surgery
After Surgery


C
AFP(ng/ml)
10000
8000
6000
600
400
200
0
7721
588
2012-05-11
2012-12-06
2013-04-17
2013-09-14
2014-05-21


D
2012-05-08
2014-05-21


E
HCC resection surgery
NK cell immunotherapy for 6-18 cycles
HCC resection surgery
Patient with HCC(n=4)
NK cell immunotherapy for 12 cycles
14 months
2 months
Patient with HCC(n=1)
HCC resection surgery
HCC paracentesis


F
10X
20X
10X
20X
CD163
Before CIT
After CIT
CD57
Before CIT
After CIT


G
20X
40X
20X
40X
CD163
After 2 months
After 14 months
CD57
After 2 months
After 14 months

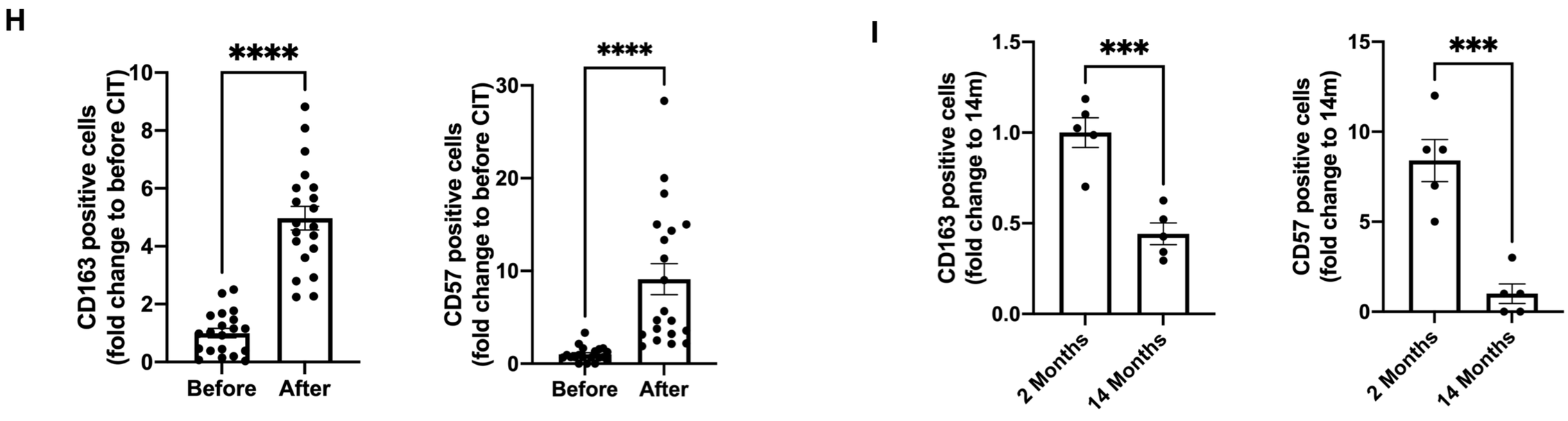
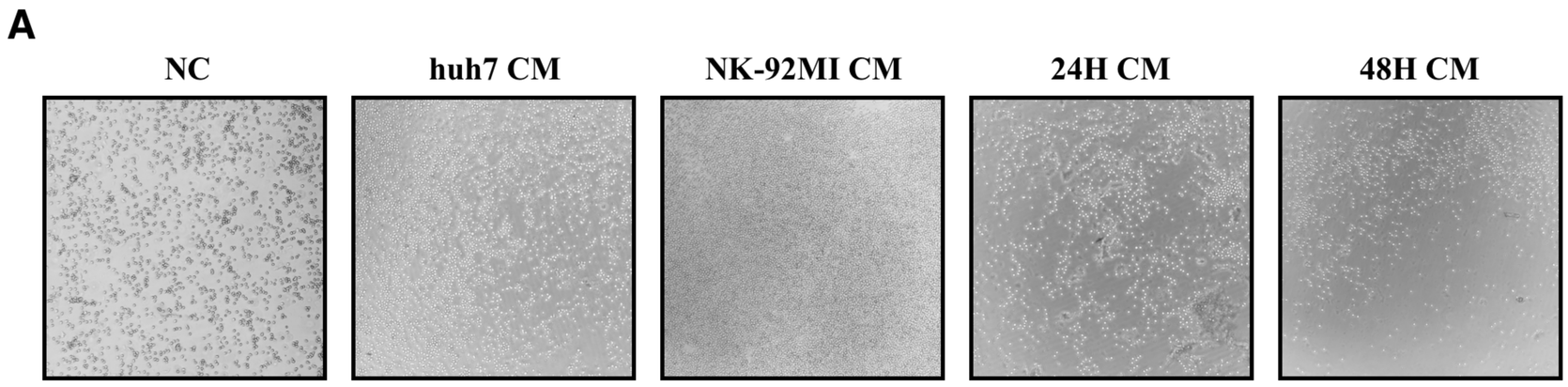
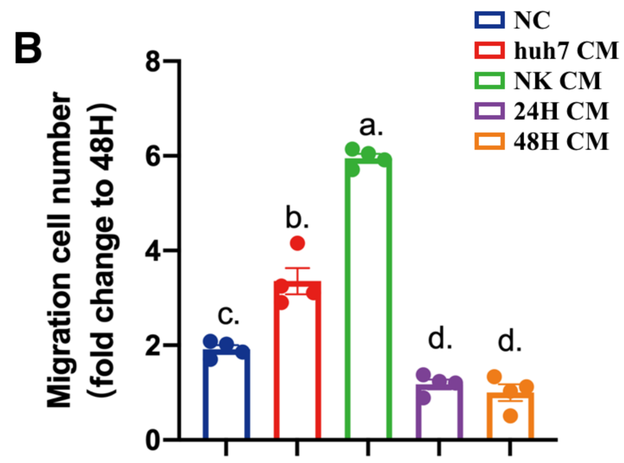
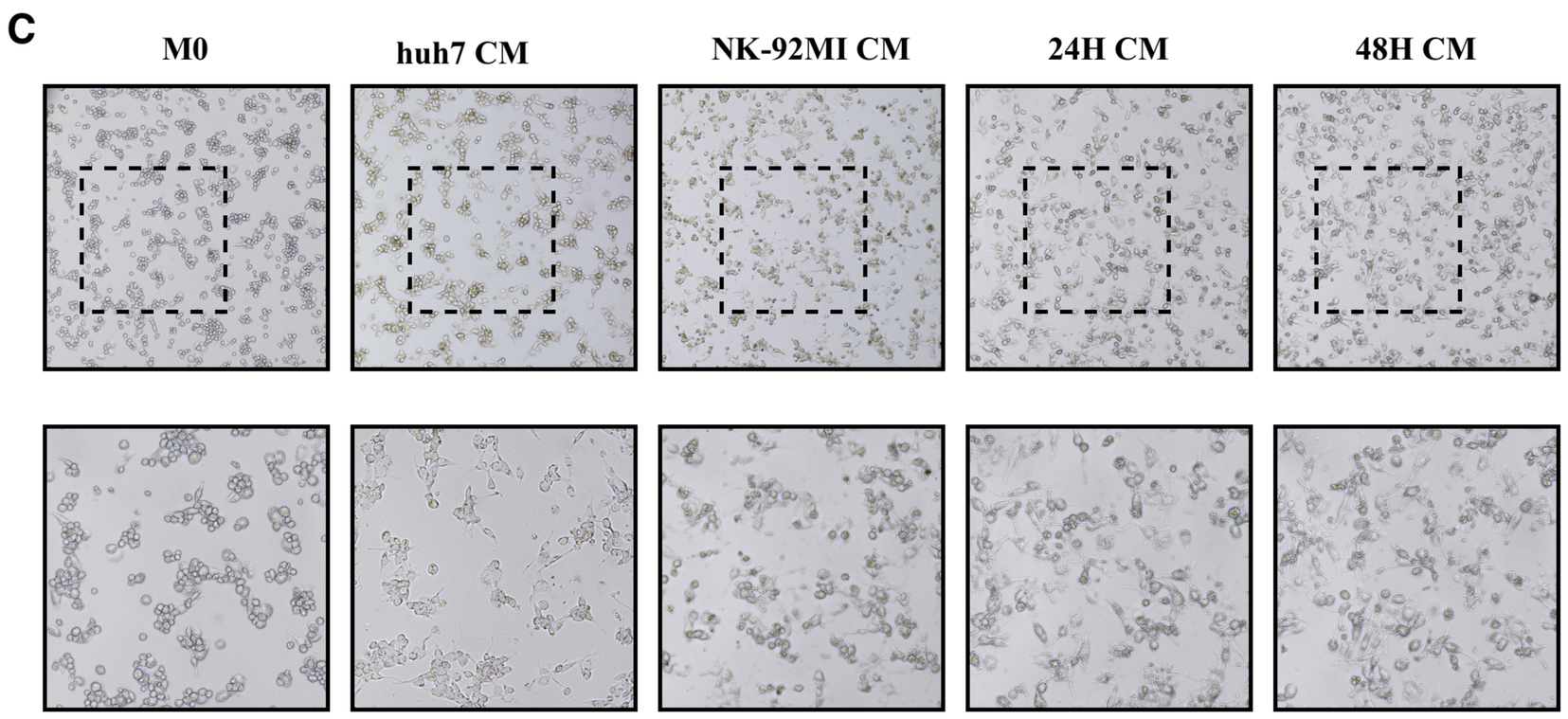
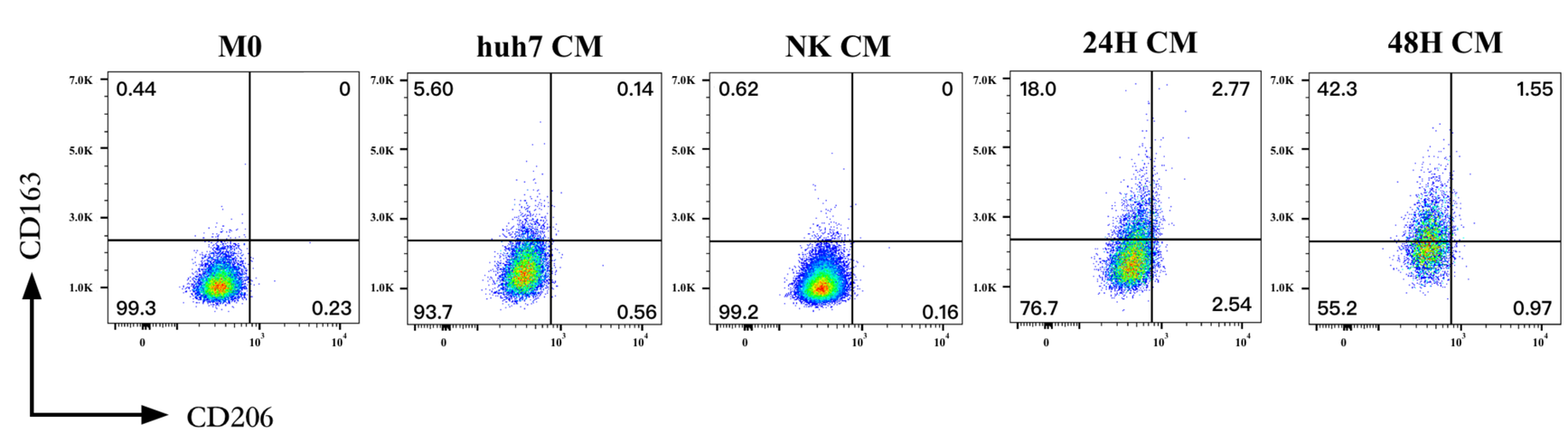

H
****
CD163 positive cells (fold change to before CIT)
Before
After
****
CD57 positive cells (fold change to before CIT)
Before
After
I
***
CD163 positive cells (fold change to 14m)
2 Months
14 Months
***
CD57 positive cells (fold change to 14m)
2 Months
14 Months

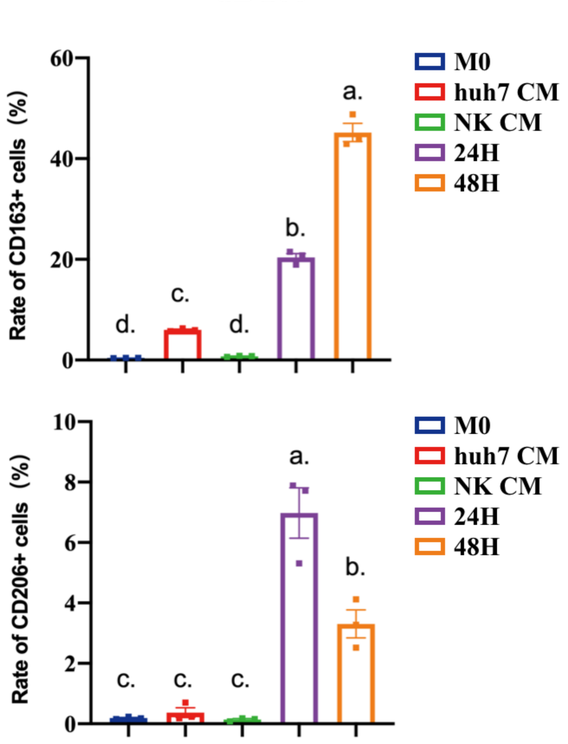
Fig.2

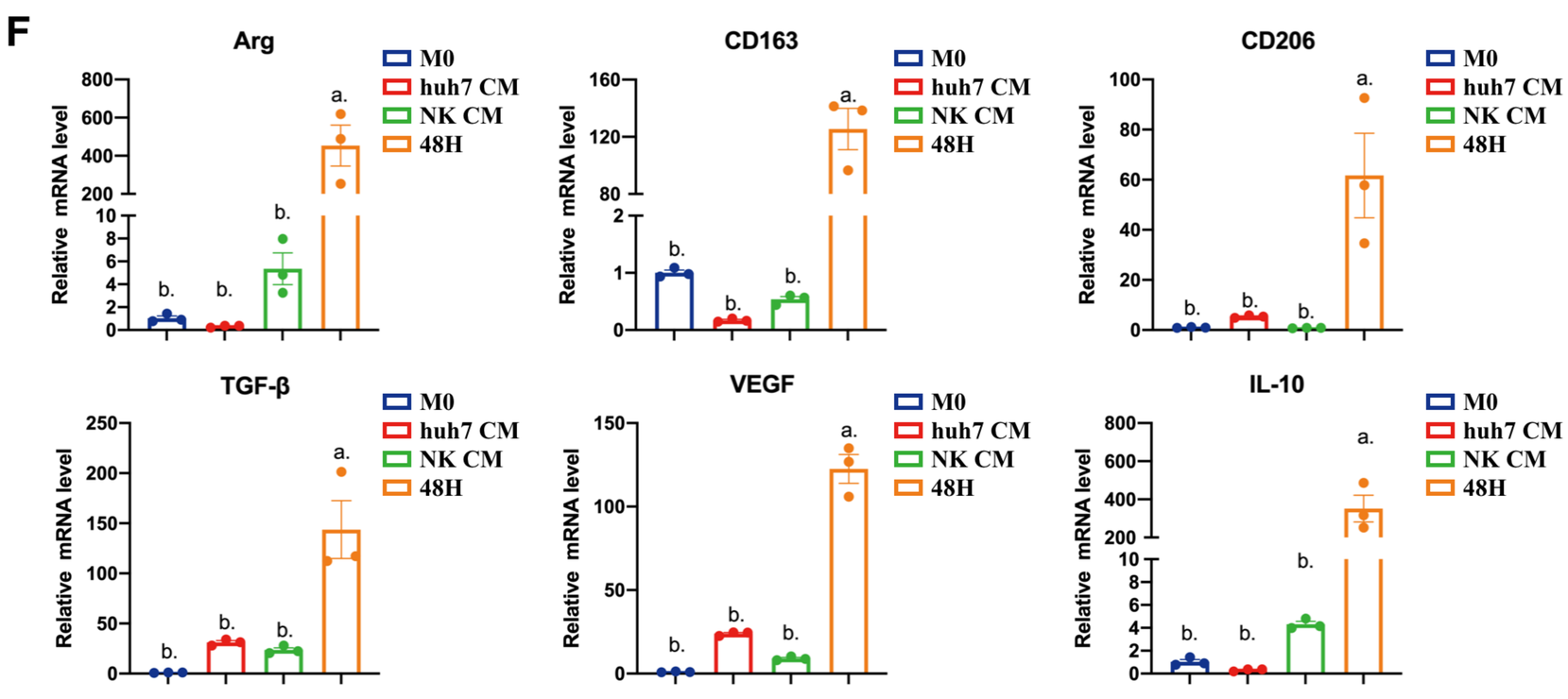

A
NC
huh7 CM
NK-92MI CM
24H CM
48H CM

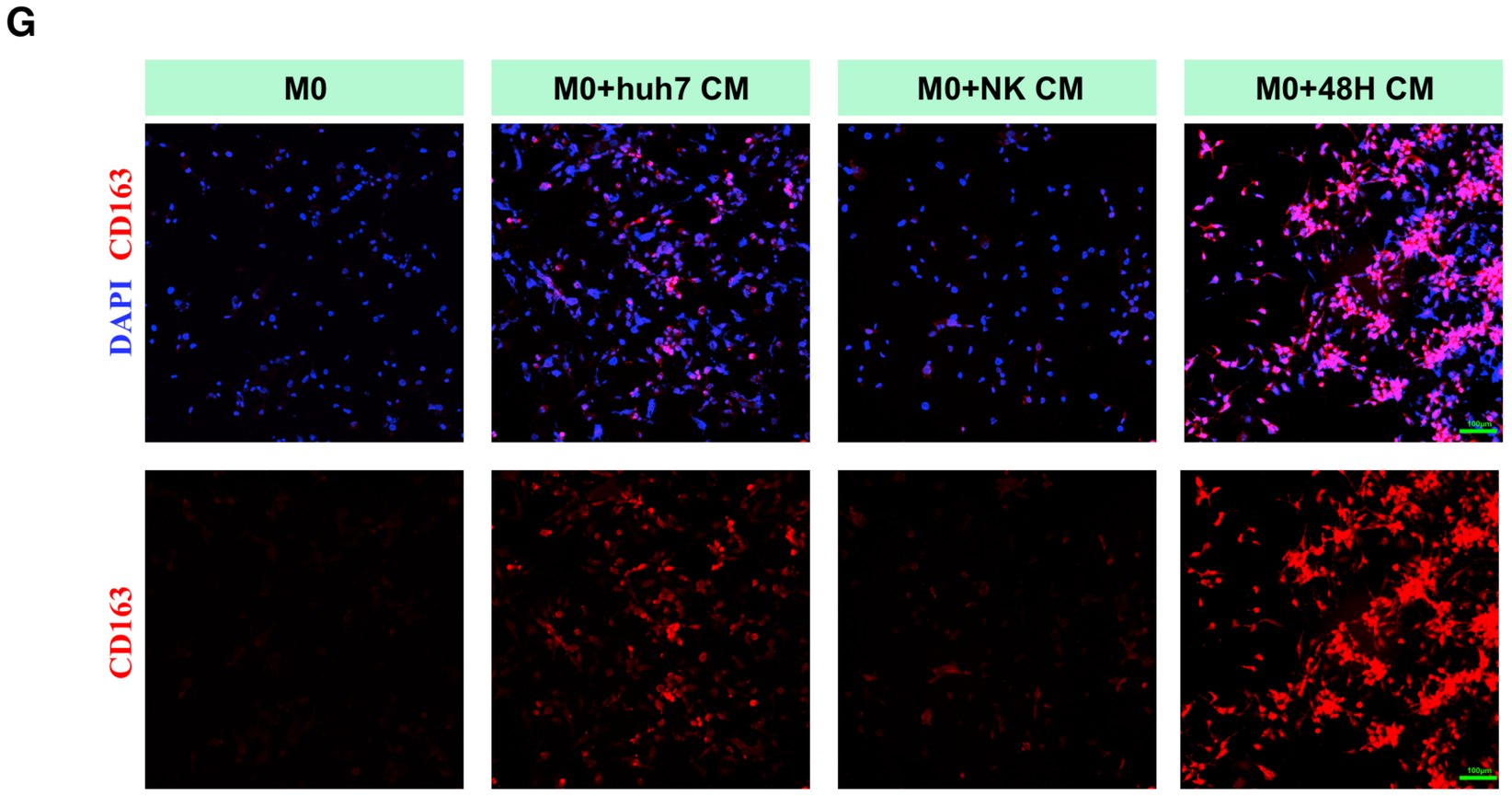

B
NC
huh7 CM
NK CM
24H CM
48H CM
Migration cell number (fold change to 48H)
0
2
4
6
8
a.
b.
c.
d.
d.


C
M0
huh7 CM
NK-92MI CM
24H CM
48H CM


D
M0
huh7 CM
NK CM
24H CM
48H CM
CD163
CD206
0.44
0
99.3
0.23
5.60
0.14
93.7
0.56
0.62
0
99.2
0.16
18.0
2.77
76.7
2.54
42.3
1.55
55.2
0.97


E
Rate of CD163+ cells (%)
Rate of CD206+ cells (%)
M0
huh7 CM
NK CM
24H
48H


F
Arg
CD163
CD206
TGF-β
VEGF
IL-10
Relative mRNA level
M0
huh7 CM
NK CM
48H


G
M0
M0+huh7 CM
M0+NK CM
M0+48H CM
DAPI CD163
CD163

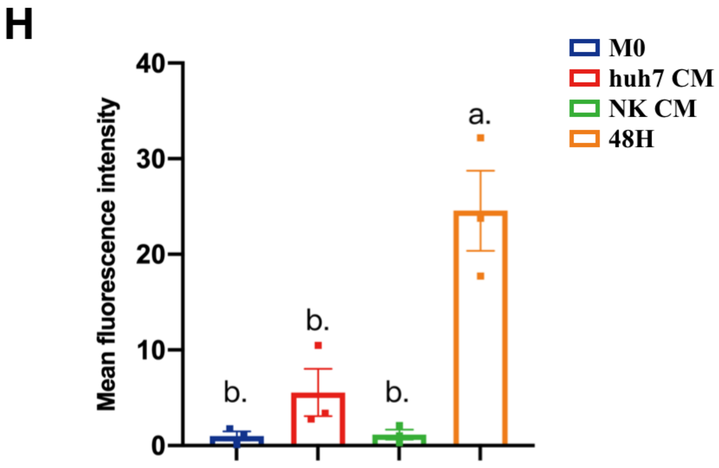
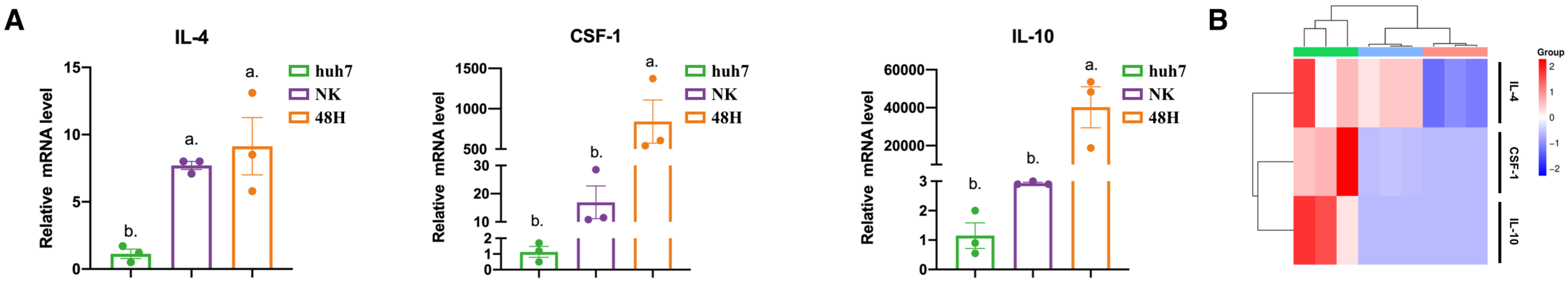
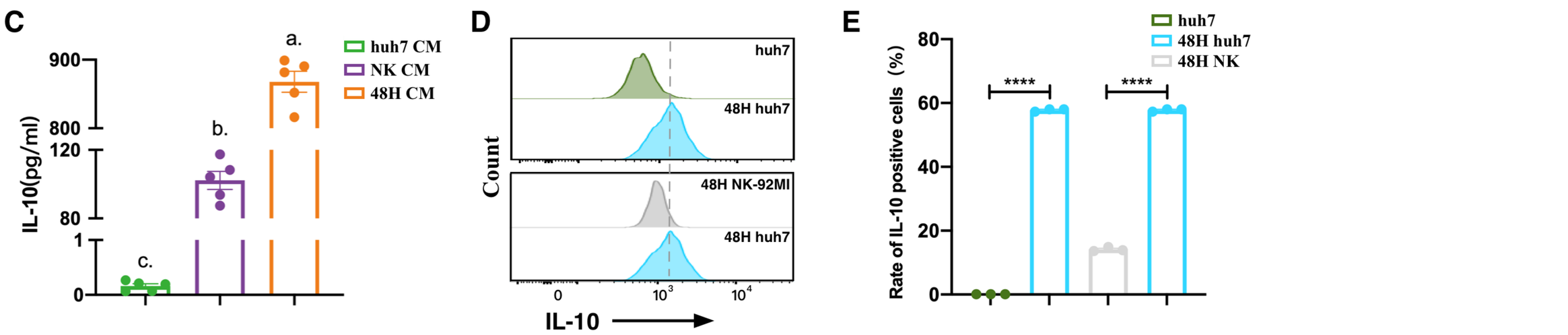
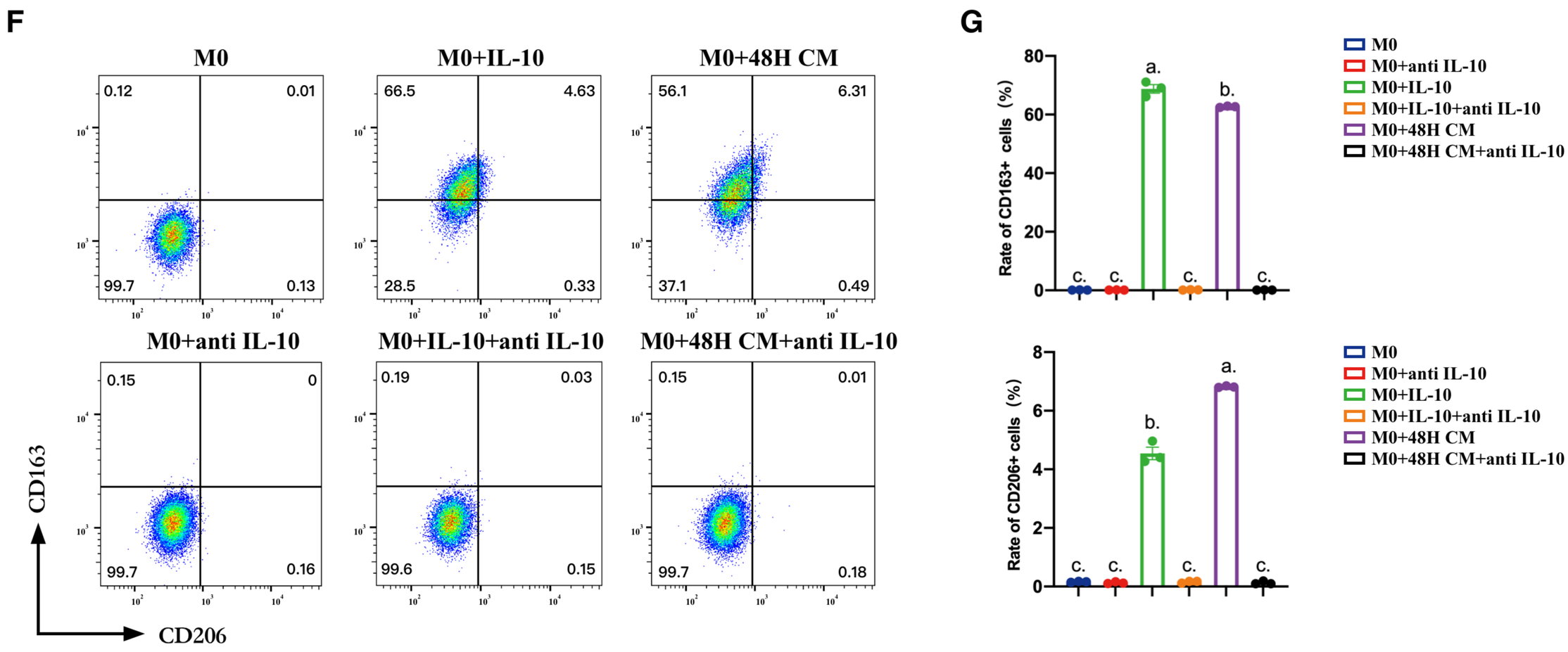
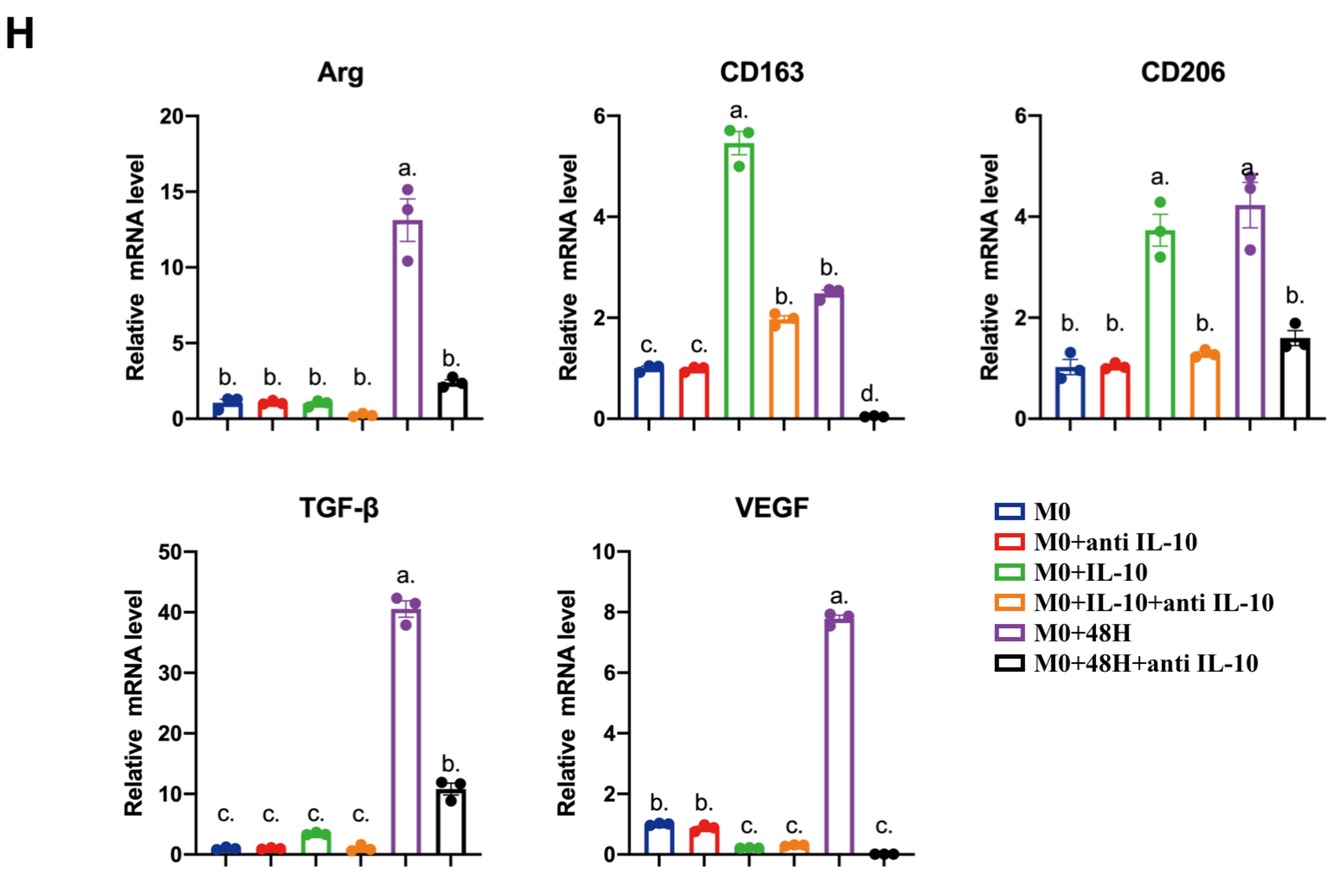

H
Mean fluorescence intensity
M0
huh7 CM
NK CM
48H

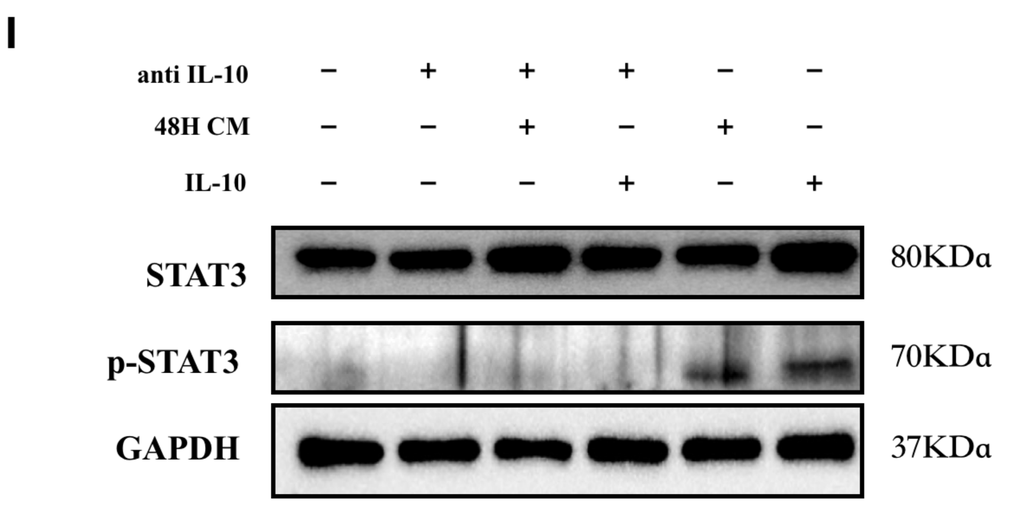
Fig.3


A
IL-4
CSF-1
IL-10
Relative mRNA level
huh7
NK
48H
B
Group
IL-4
CSF-1
IL-10


C
IL-10(pg/ml)
huh7 CM
NK CM
48H CM
D
Count
huh7
48H huh7
48H NK-92MI
IL-10
E
Rate of IL-10 positive cells (%)
huh7
48H huh7
48H NK
****


F
M0
M0+IL-10
M0+48H CM
M0+anti IL-10
M0+IL-10+anti IL-10
M0+48H CM+anti IL-10
CD163
CD206
G
Rate of CD163+ cells (%)
Rate of CD206+ cells (%)
M0
M0+anti IL-10
M0+IL-10
M0+IL-10+anti IL-10
M0+48H CM
M0+48H CM+anti IL-10


H
Arg
CD163
CD206
TGF-β
VEGF
Relative mRNA level
M0
M0+anti IL-10
M0+IL-10
M0+IL-10+anti IL-10
M0+48H
M0+48H+anti IL-10


I
anti IL-10
48H CM
IL-10
STAT3
p-STAT3
GAPDH
80KDa
70KDa
37KDa

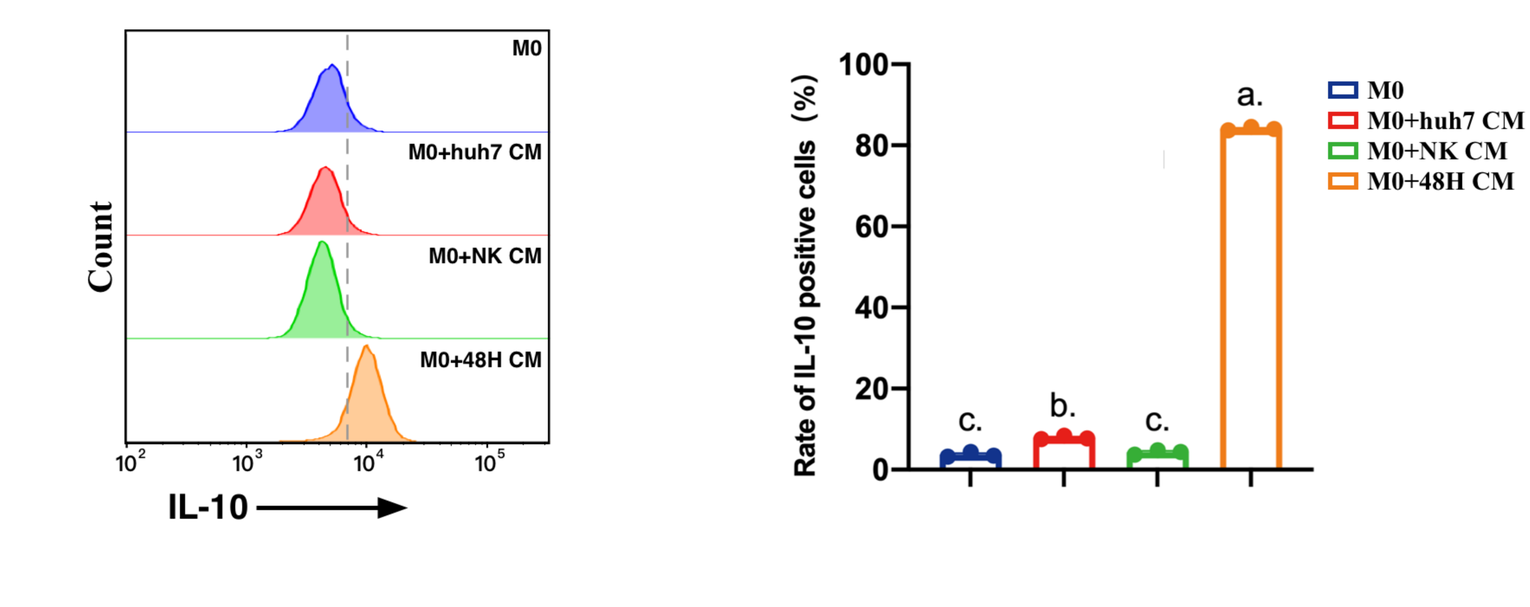
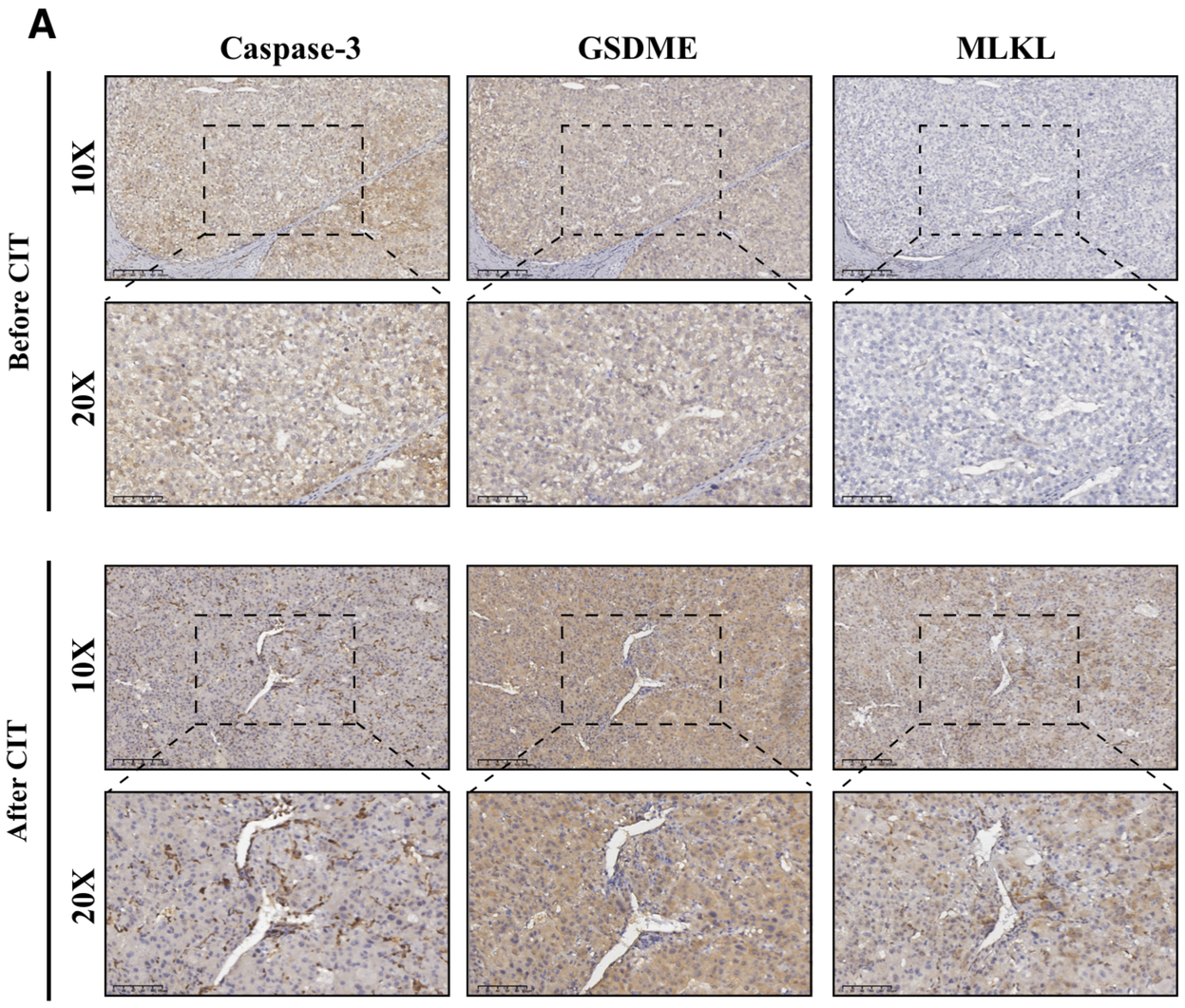
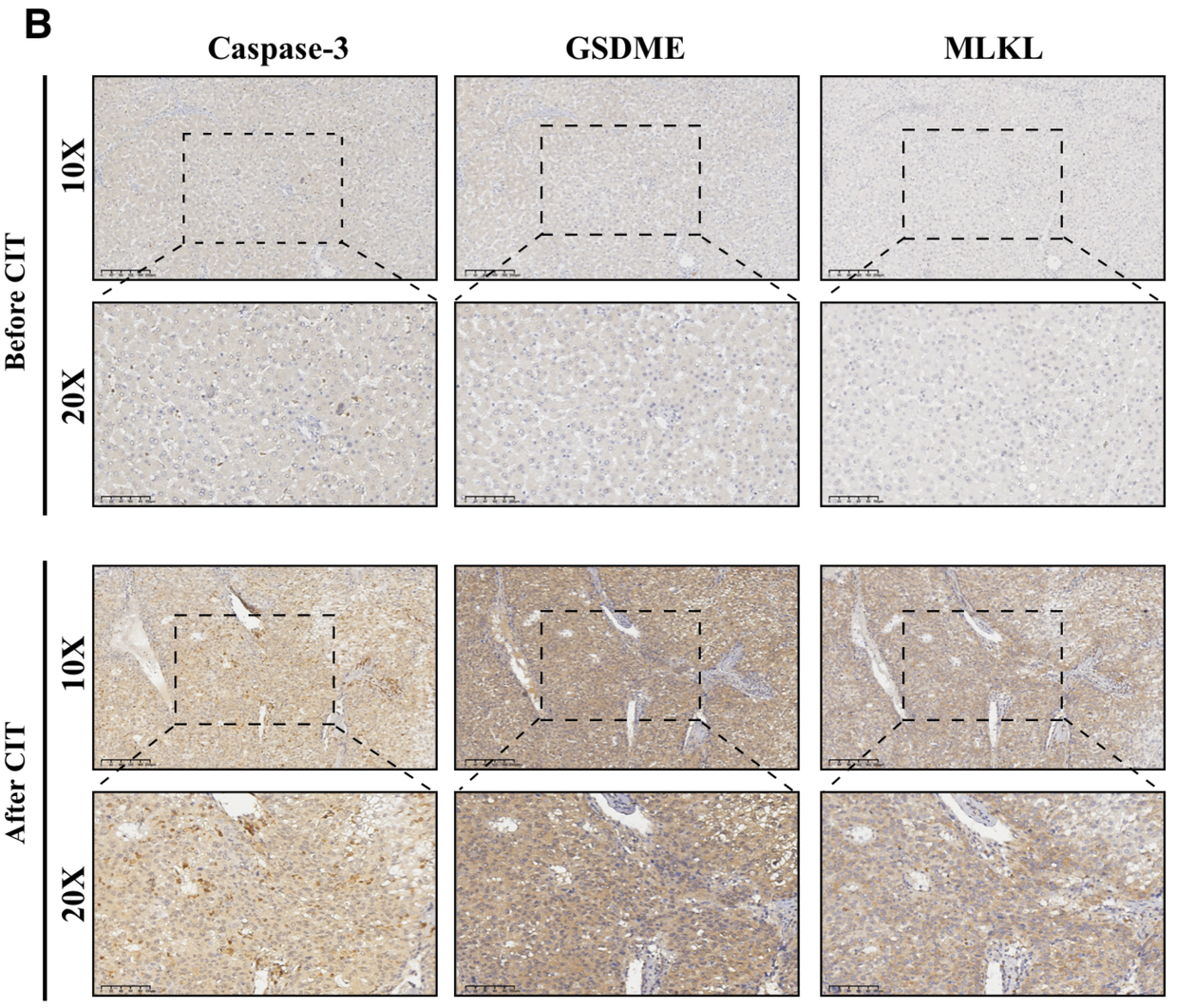
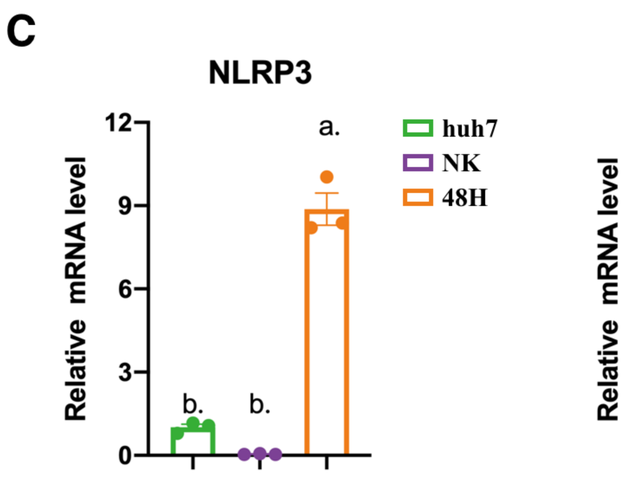
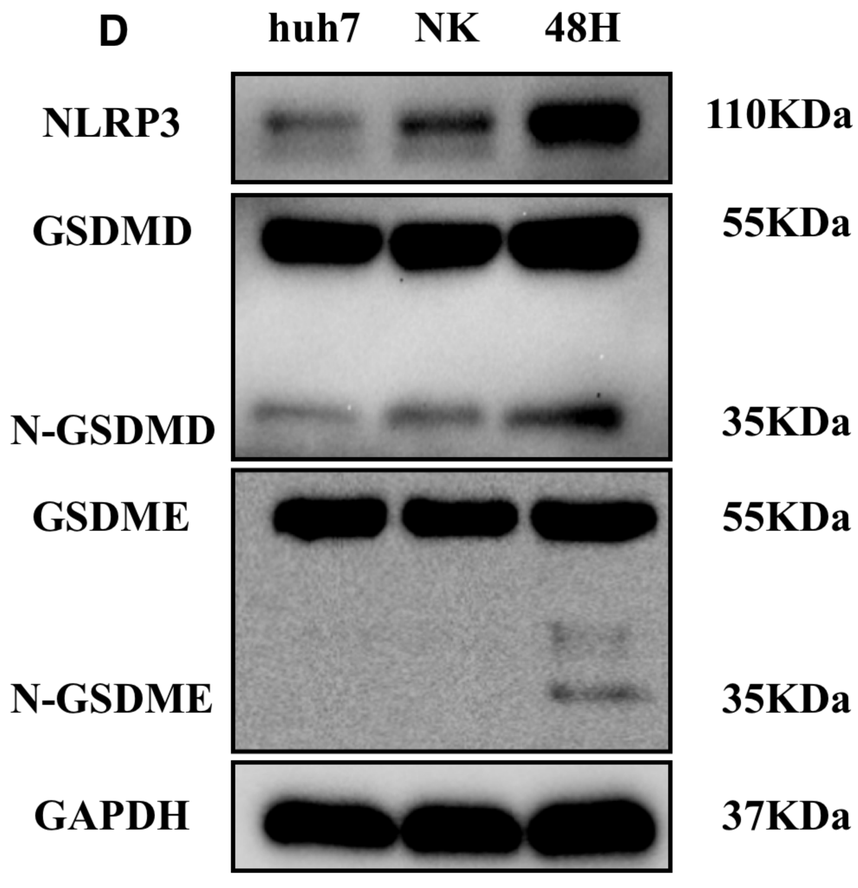

J
K
L
Count
IL-10
M0
M0+huh7 CM
M0+NK CM
M0+48H CM
Rate of IL-10 positive cells (%)
IL-10 (pg/ml)

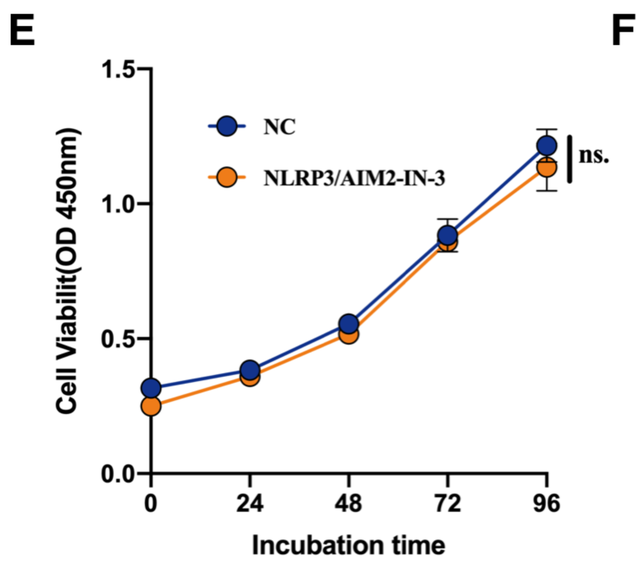
Fig.4

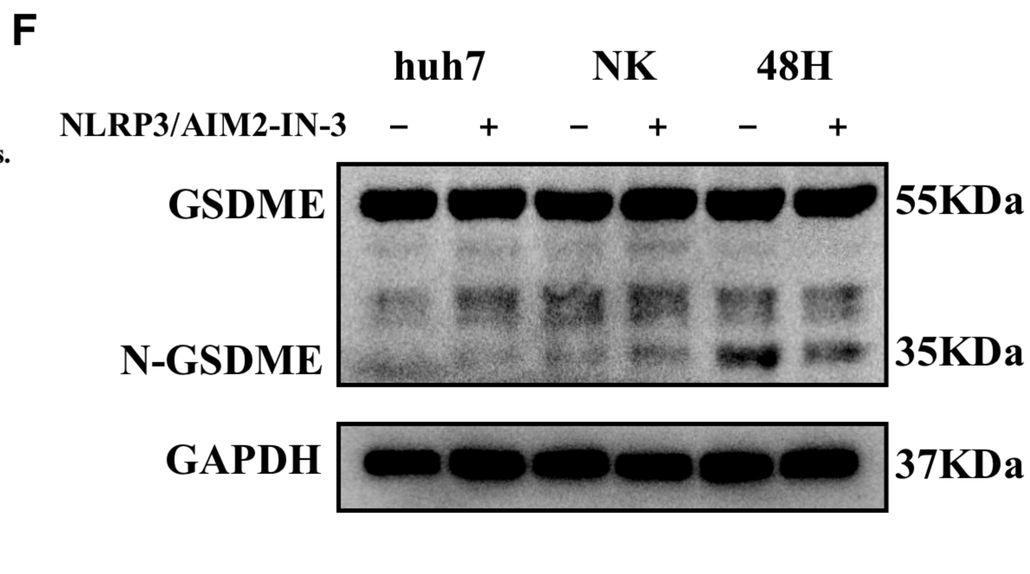

A
Caspase-3
GSDME
MLKL
Before CIT
After CIT
10X
20X

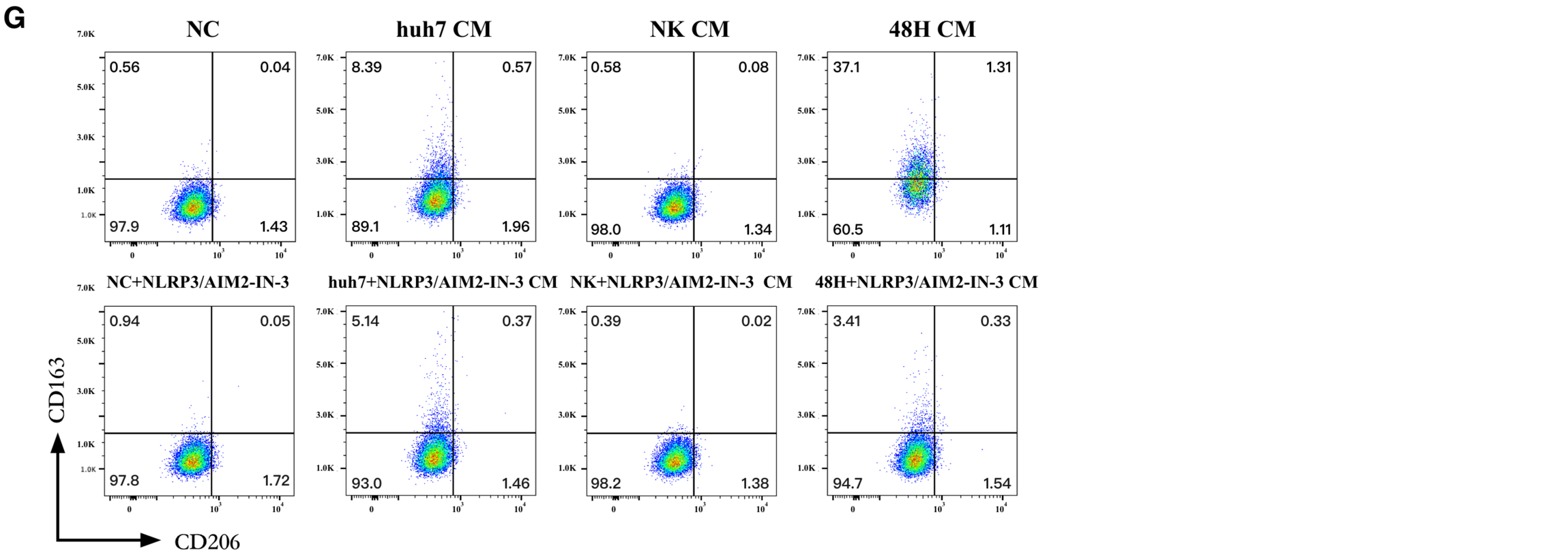

B
Caspase-3
GSDME
MLKL
Before CIT
After CIT
10X
20X


C
NLRP3
GSDMD
GSDME
Relative mRNA level
huh7
NK
48H
a.
b.
c.


D
huh7
NK
48H
NLRP3
110KDa
GSDMD
55KDa
N-GSDMD
35KDa
GSDME
55KDa
N-GSDME
35KDa
GAPDH
37KDa


E
Cell Viabilit(OD 450nm)
NC
NLRP3/AIM2-IN-3
ns.
Incubation time


F
huh7
NK
48H
NLRP3/AIM2-IN-3
GSDME
55KDa
N-GSDME
35KDa
GAPDH
37KDa


G
NC
huh7 CM
NK CM
48H CM
NC+NLRP3/AIM2-IN-3
huh7+NLRP3/AIM2-IN-3 CM
NK+NLRP3/AIM2-IN-3 CM
48H+NLRP3/AIM2-IN-3 CM
CD163
CD206

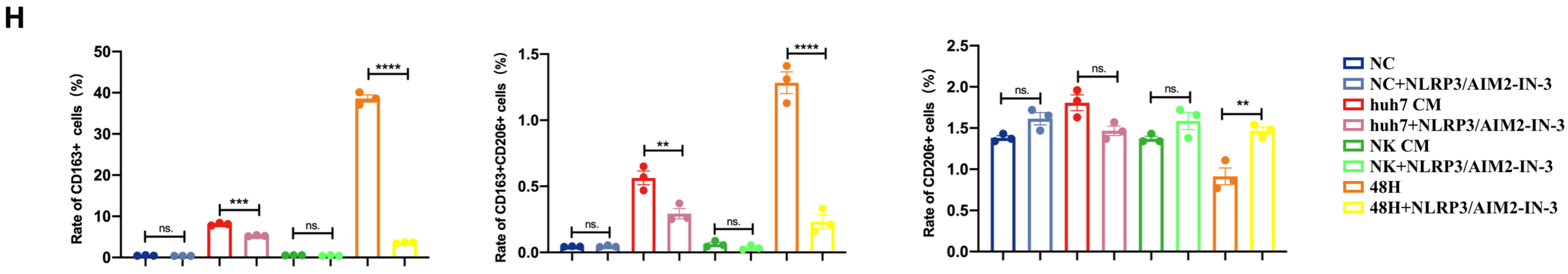
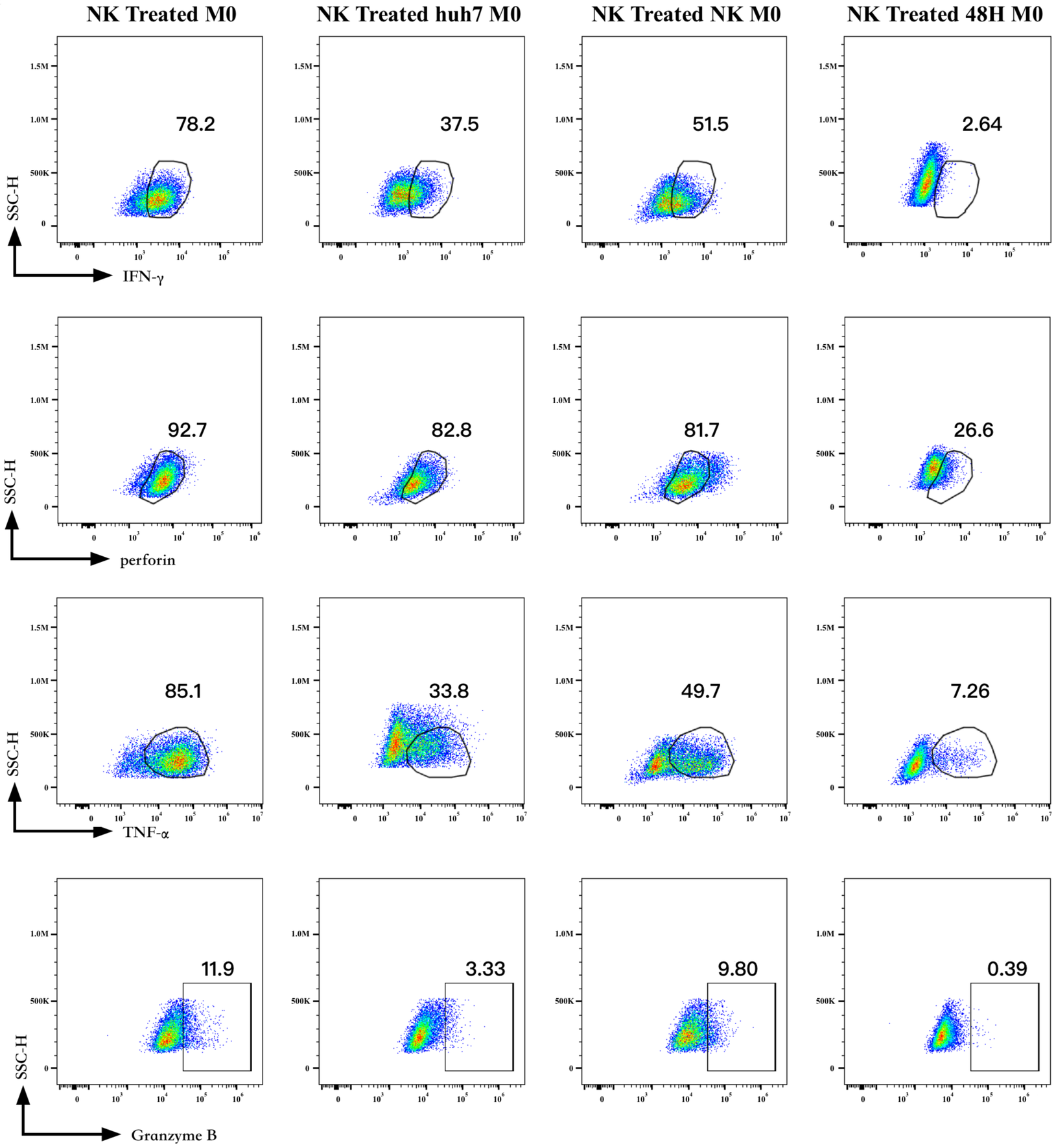
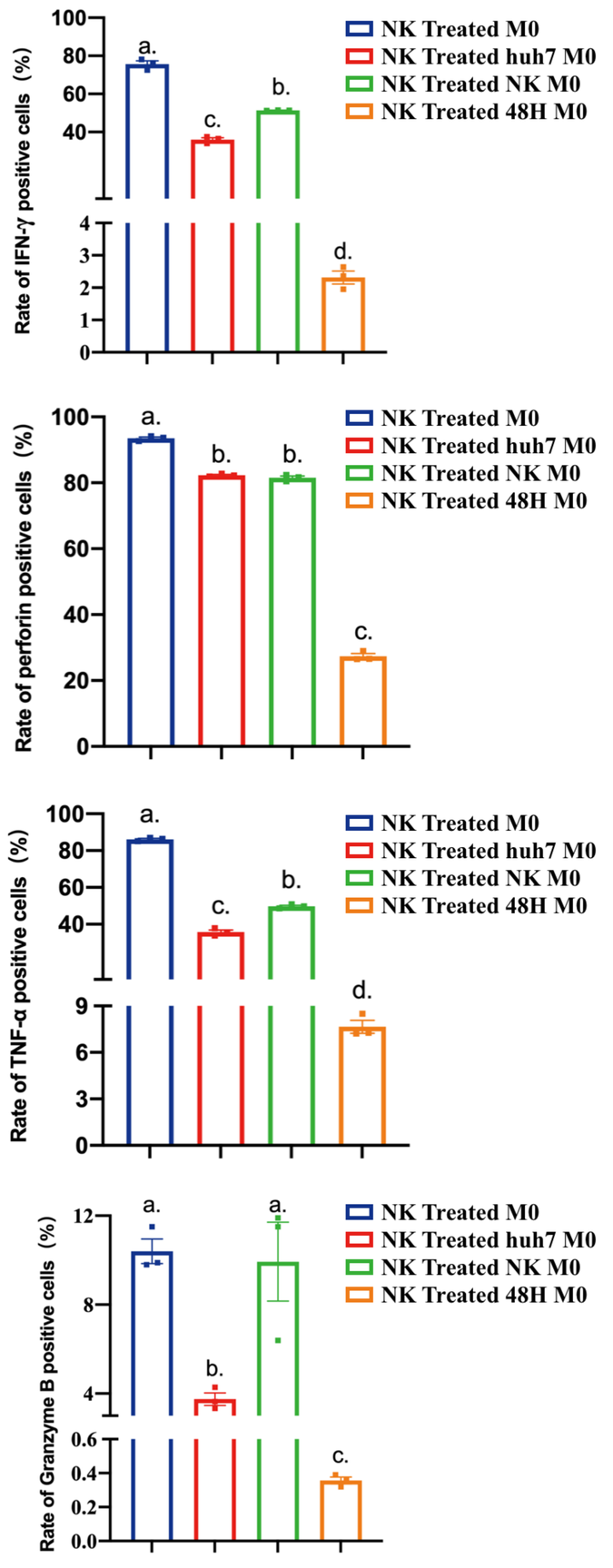

H
Rate of CD163+ cells (%)
Rate of CD163+CD206+ cells (%)
Rate of CD206+ cells (%)
ns.
NC
NC+NLRP3/AIM2-IN-3
huh7 CM
huh7+NLRP3/AIM2-IN-3
NK CM
NK+NLRP3/AIM2-IN-3
48H
48H+NLRP3/AIM2-IN-3

Fig.5

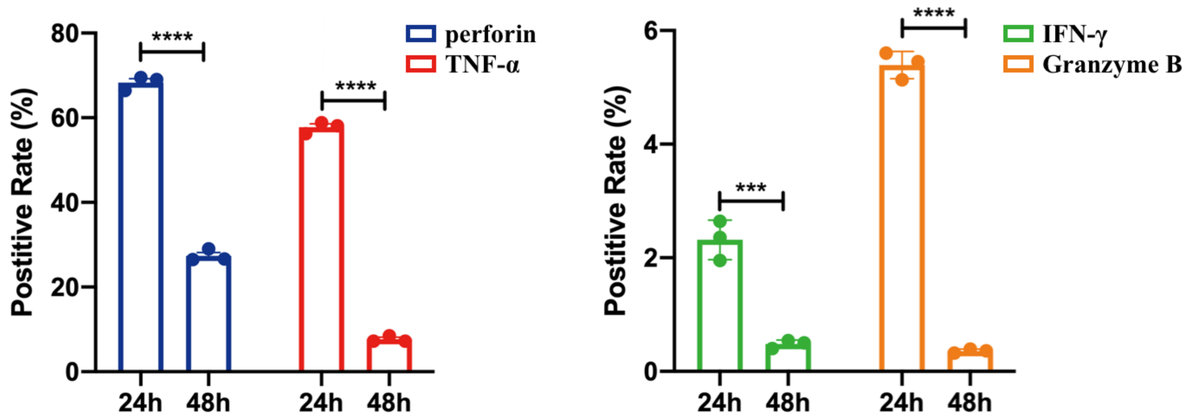
A



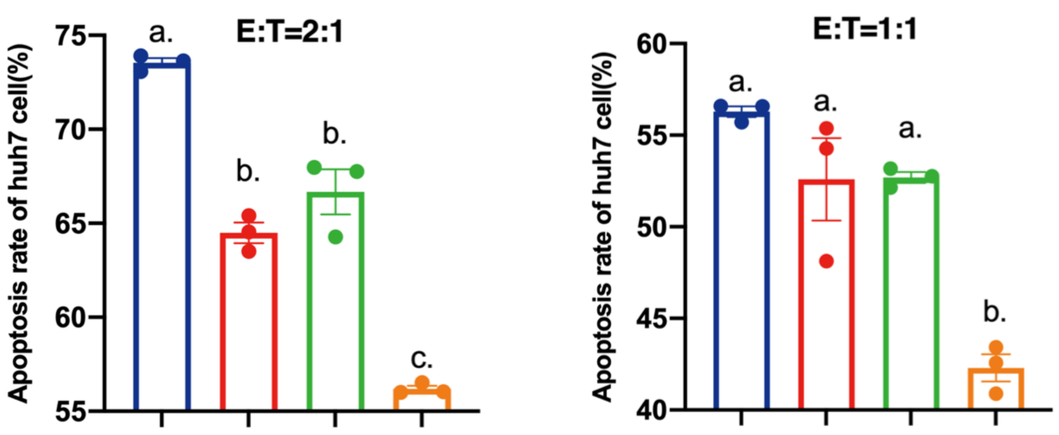
B



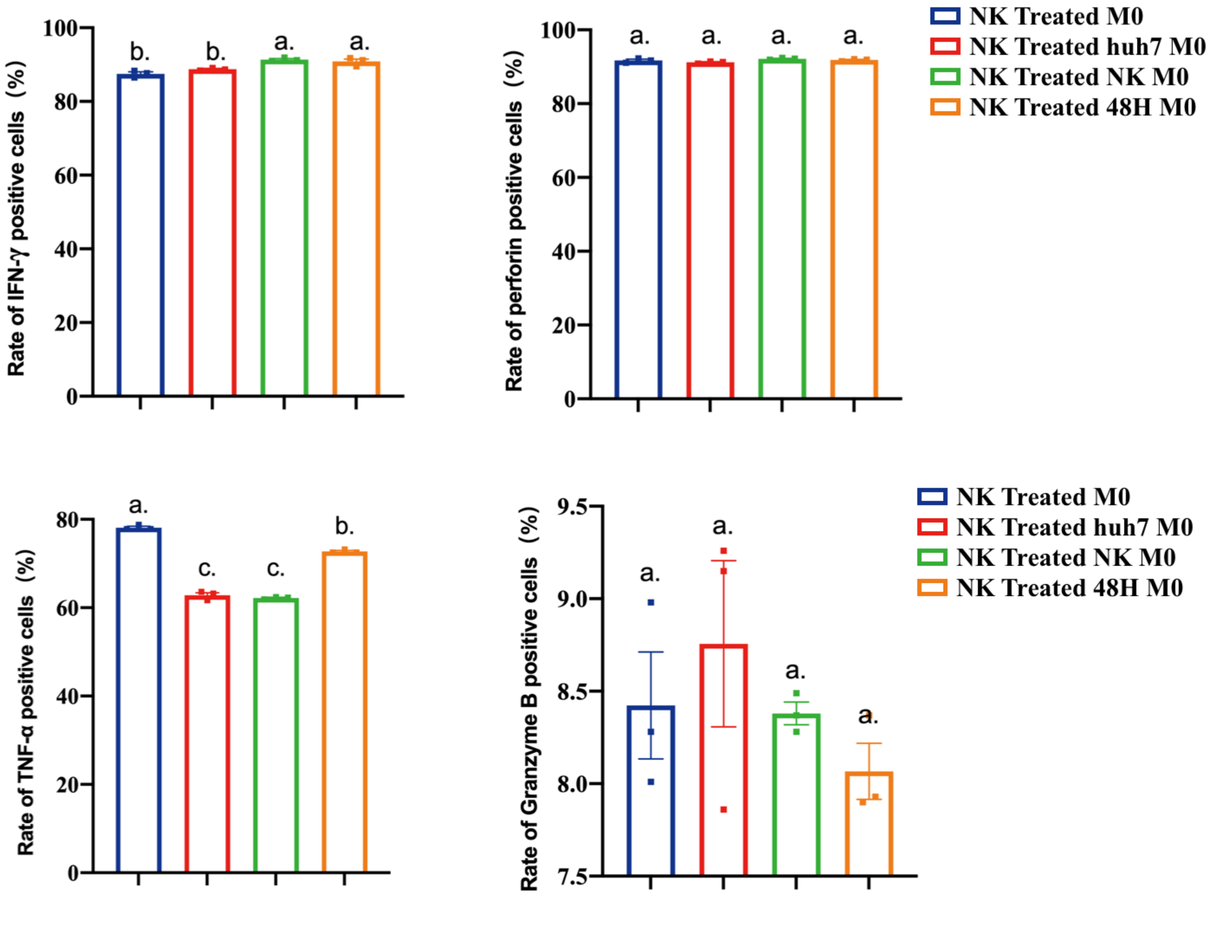
C



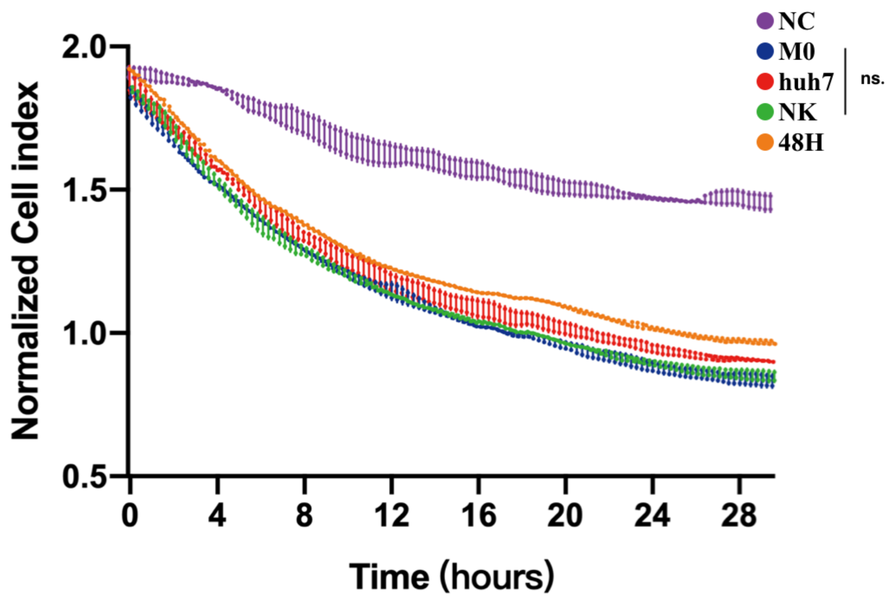
D



E



F



G

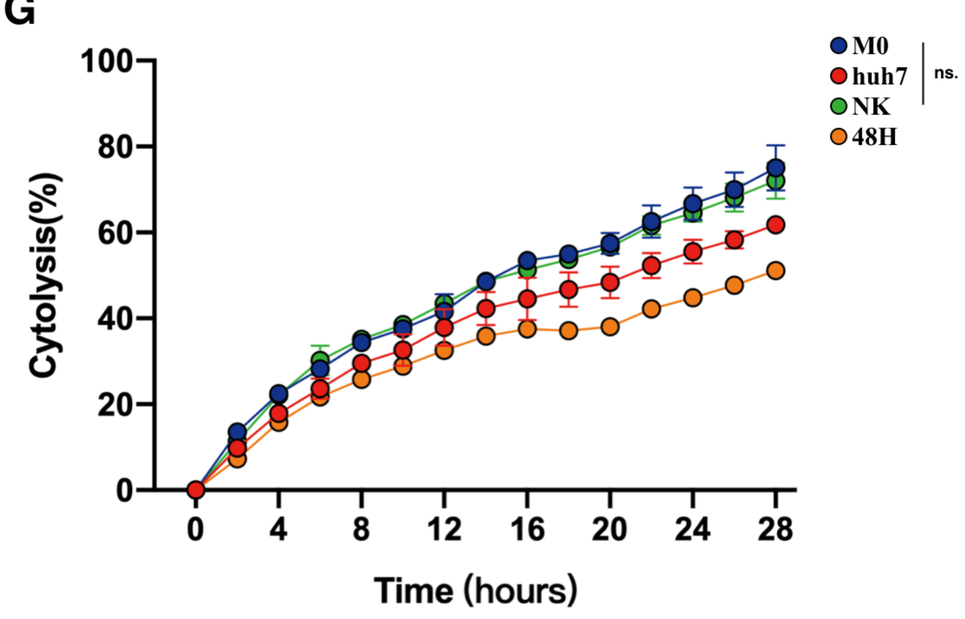
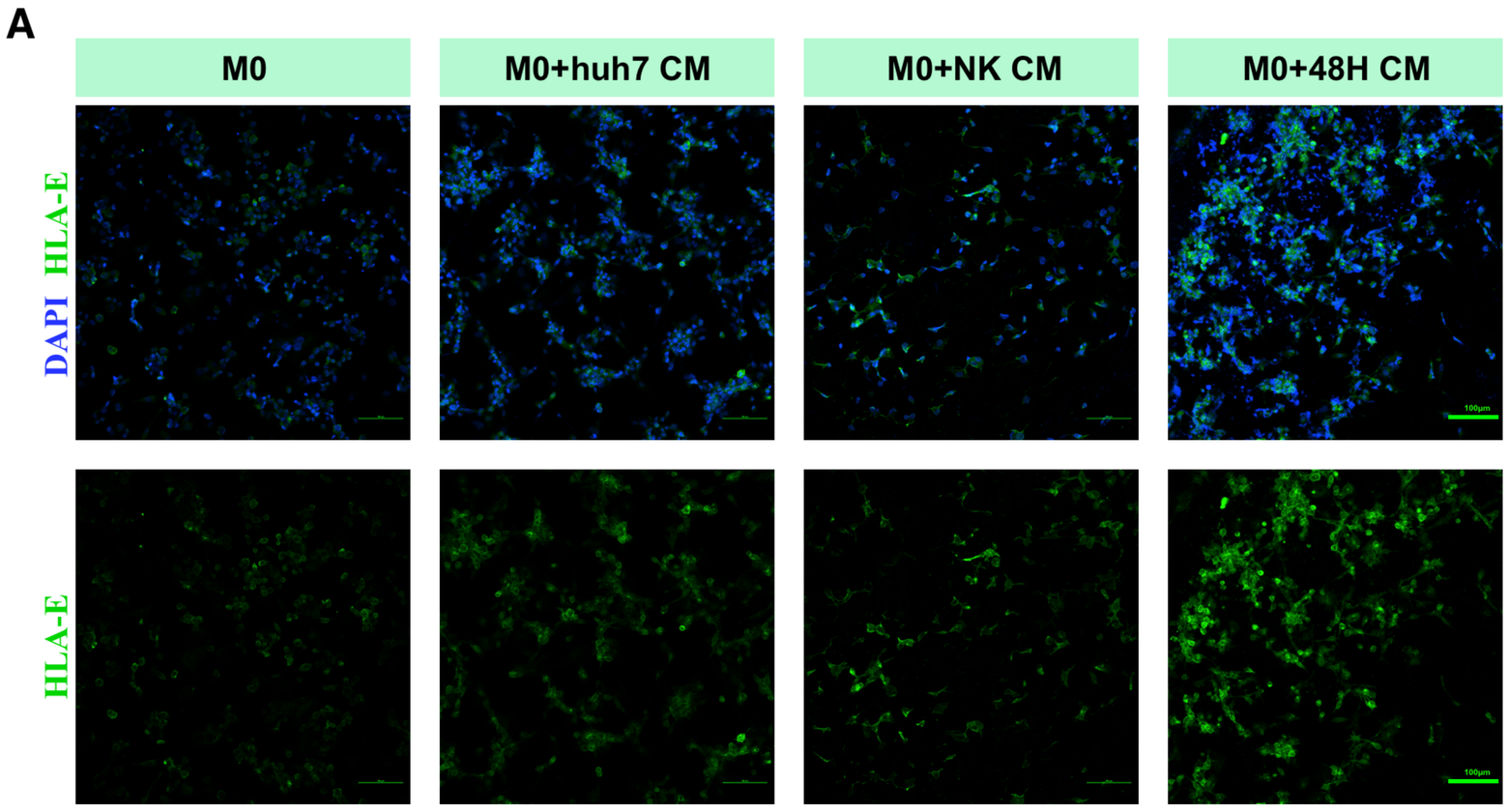
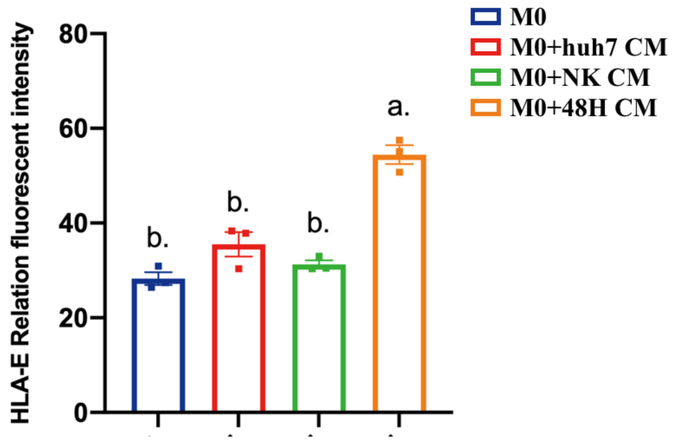
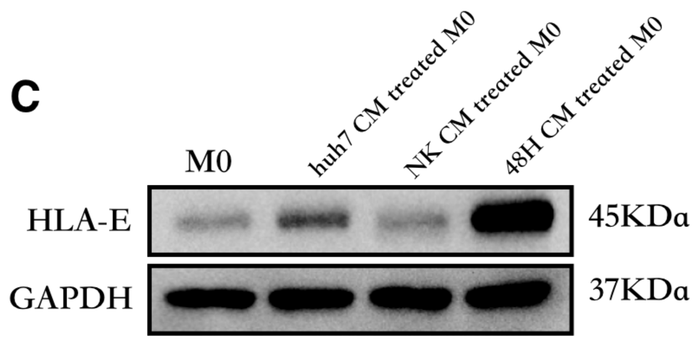
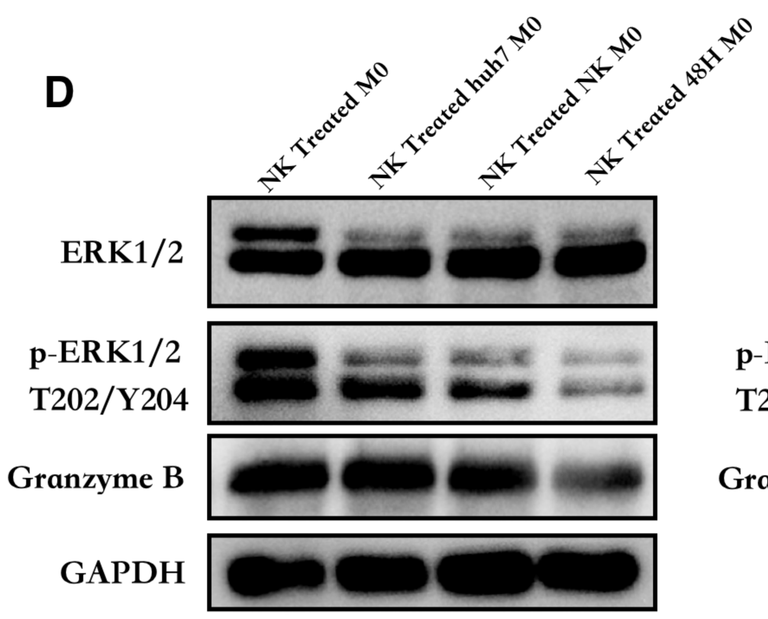


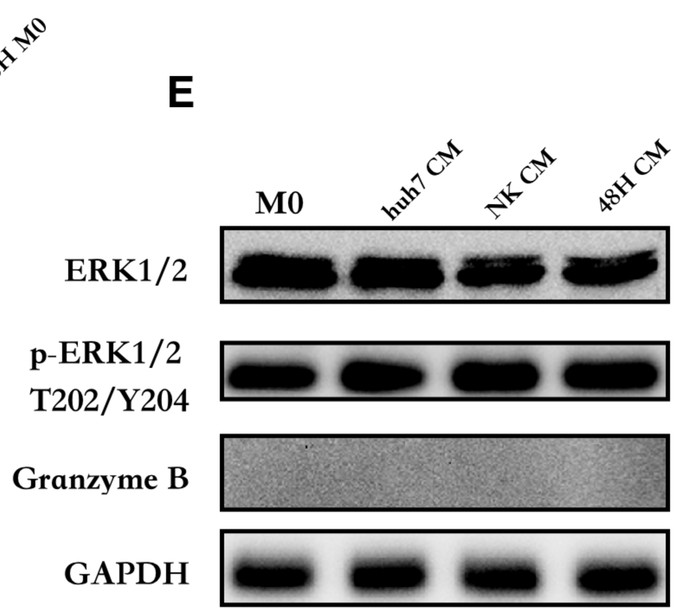
Fig.6

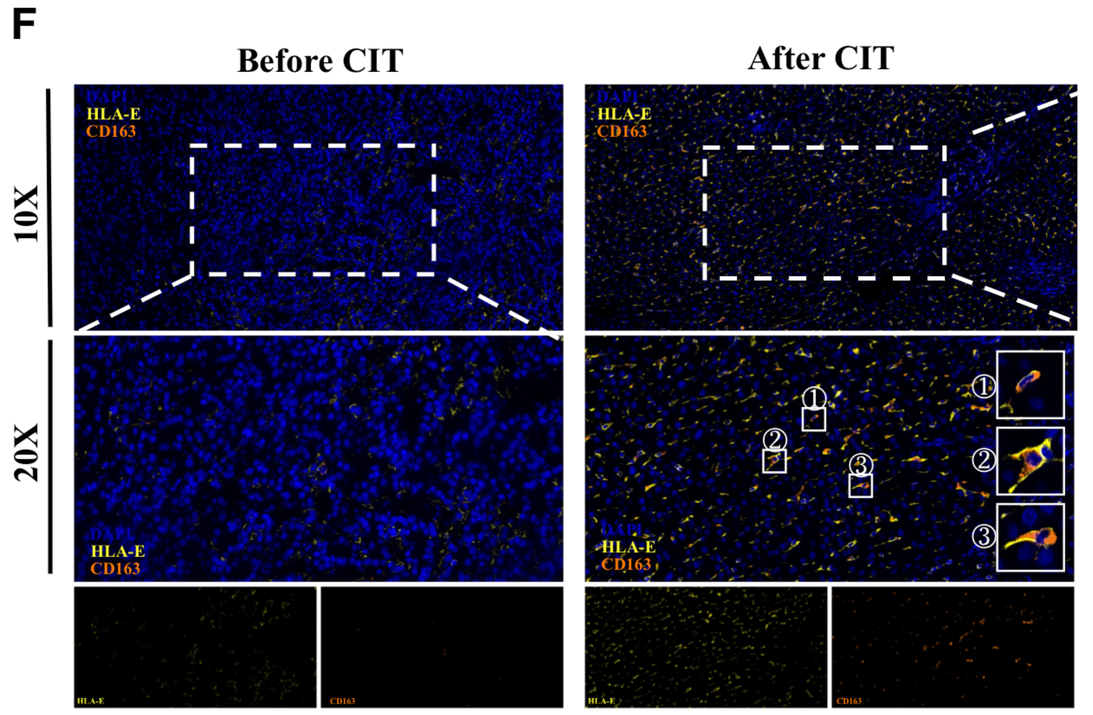

A
M0
M0+huh7 CM
M0+NK CM
M0+48H CM
DAPI HLA-E
HLA-E

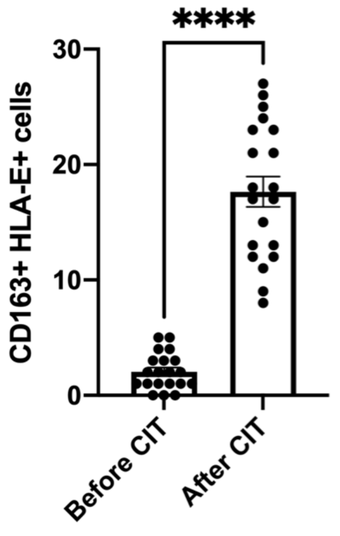

B
HLA-E Relation fluorescent intensity
M0
M0+huh7 CM
M0+NK CM
M0+48H CM
a.
b.

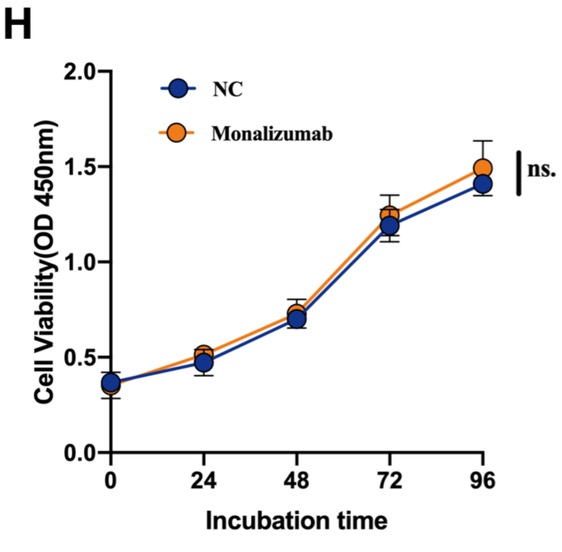

C
M0
huh7 CM treated M0
NK CM treated M0
48H CM treated M0
HLA-E
GAPDH
45KDa
37KDa

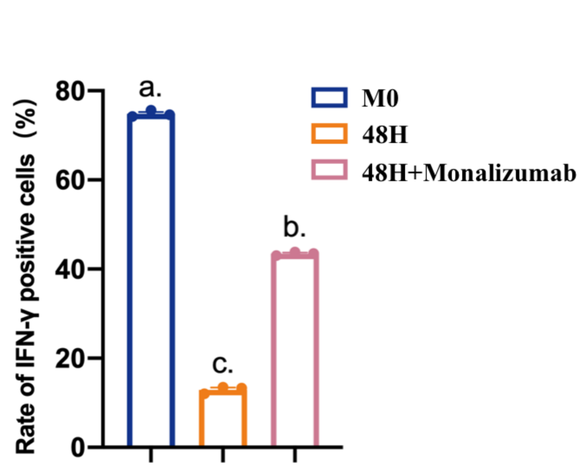

D
NK Treated M0
NK Treated huh7 M0
NK Treated NK M0
NK Treated 48H M0
ERK1/2
p-ERK1/2 T202/Y204
Granzyme B
GAPDH

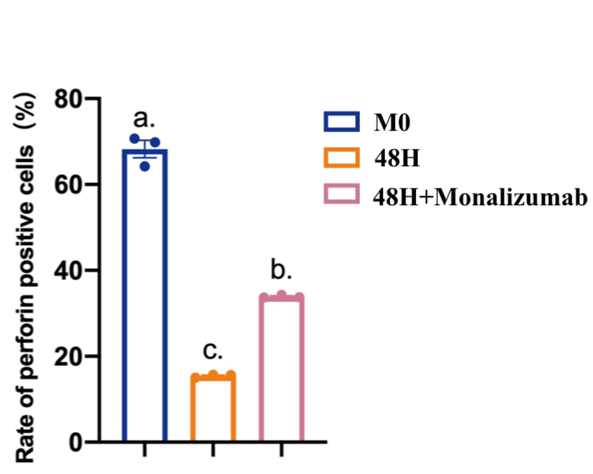

E
M0
huh7 CM
NK CM
48H CM
ERK1/2
p-ERK1/2 T202/Y204
Granzyme B
GAPDH

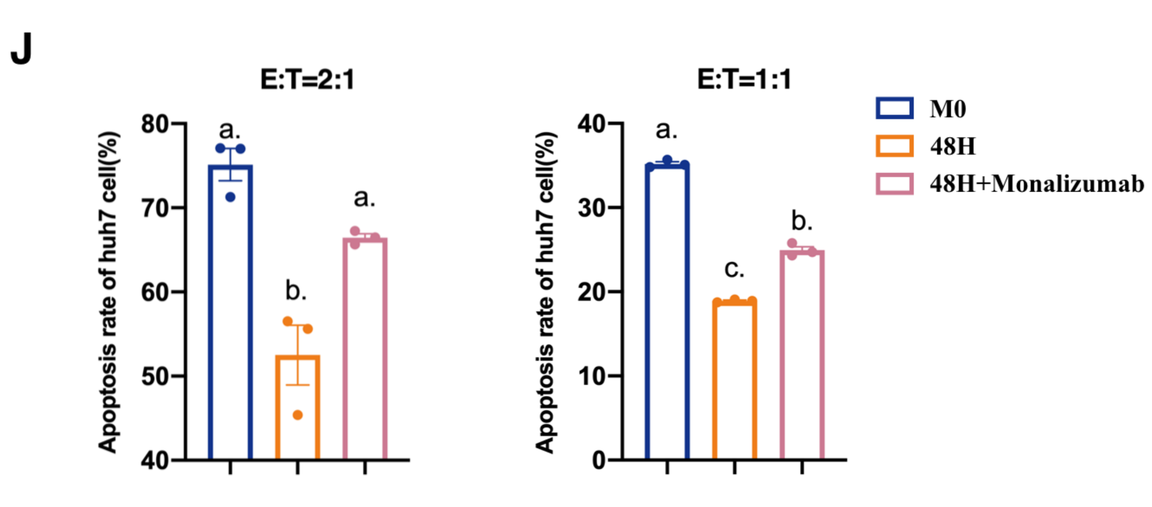

F
Before CIT
After CIT
10X
20X
DAPI
HLA-E
CD163

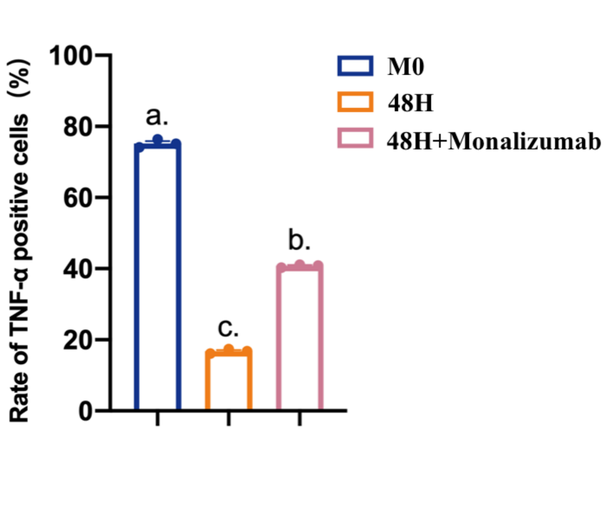

G
****
CD163+ HLA-E+ cells
Before CIT
After CIT

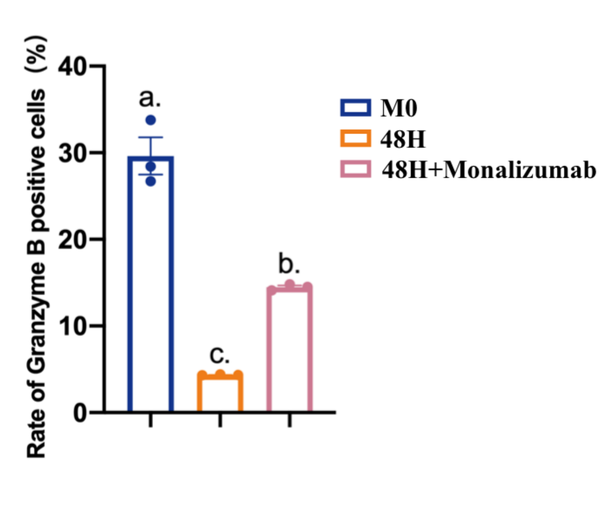

H
NC
Monalizumab
ns.
Cell Viability(OD 450nm)
Incubation time

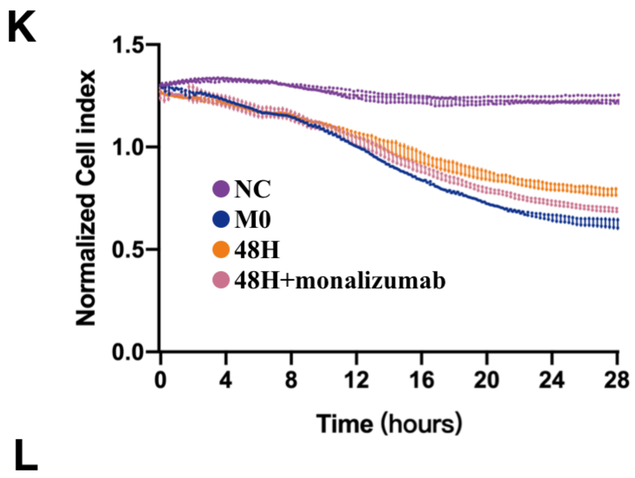

I
M0
48H
48H+Monalizumab
Rate of IFN-γ positive cells (%)

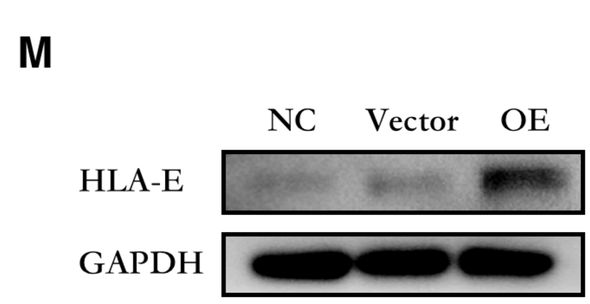

M0
48H
48H+Monalizumab
Rate of perforin positive cells (%)

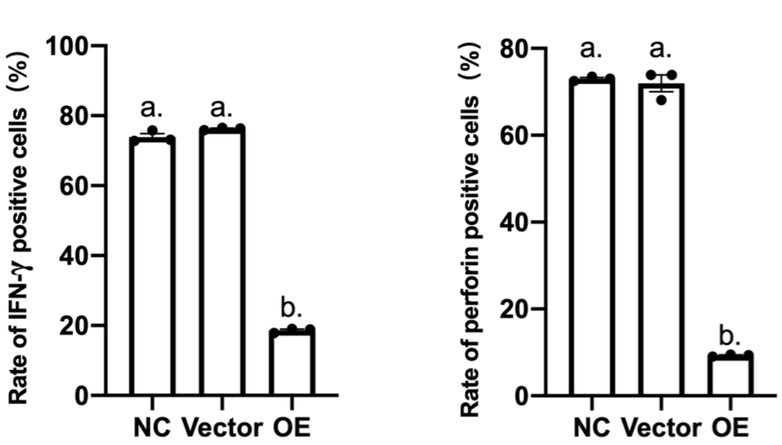

J
E:T=2:1
E:T=1:1
M0
48H
48H+Monalizumab
Apoptosis rate of huh7 cell(%)

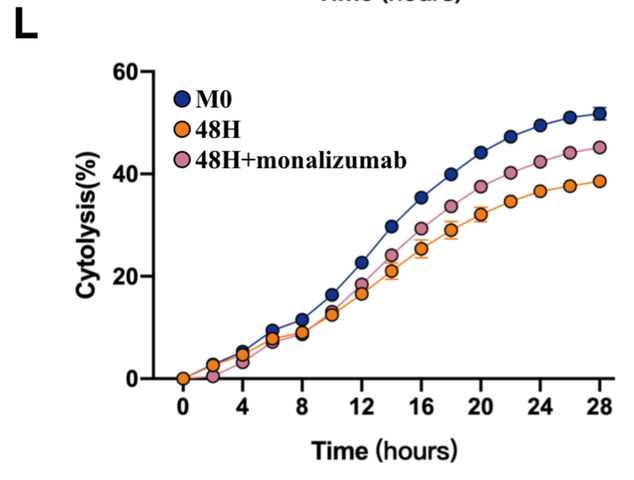

M0
48H
48H+Monalizumab
Rate of TNF-α positive cells (%)


M0
48H
48H+Monalizumab
Rate of Granzyme B positive cells (%)


K
NC
M0
48H
48H+monalizumab
Normalized Cell index
Time (hours)


M
NC
Vector
OE
HLA-E
GAPDH


N
Rate of IFN-γ positive cells (%)
Rate of perforin positive cells (%)
NC Vector OE


L
M0
48H
48H+monalizumab
Cytolysis(%)
Time (hours)

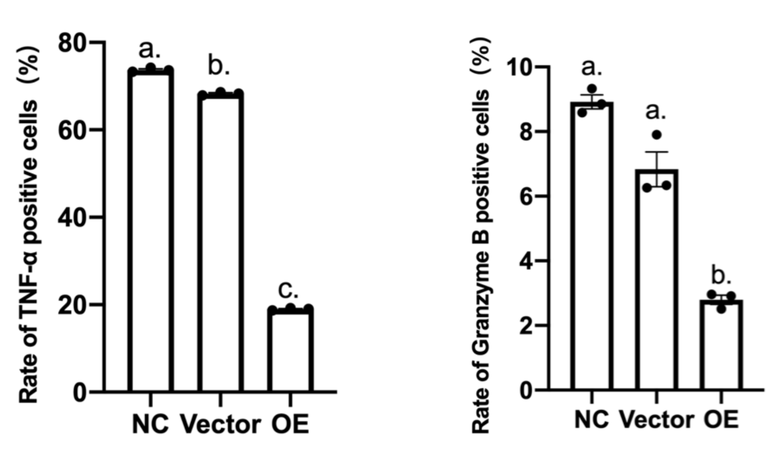
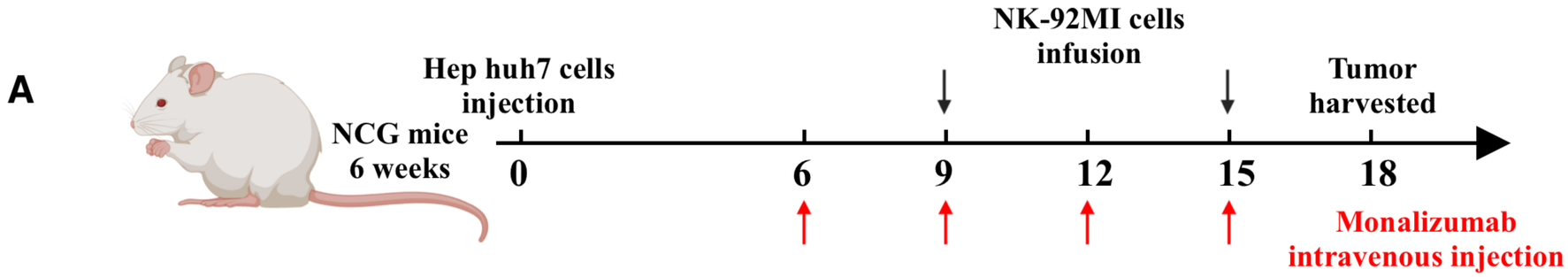
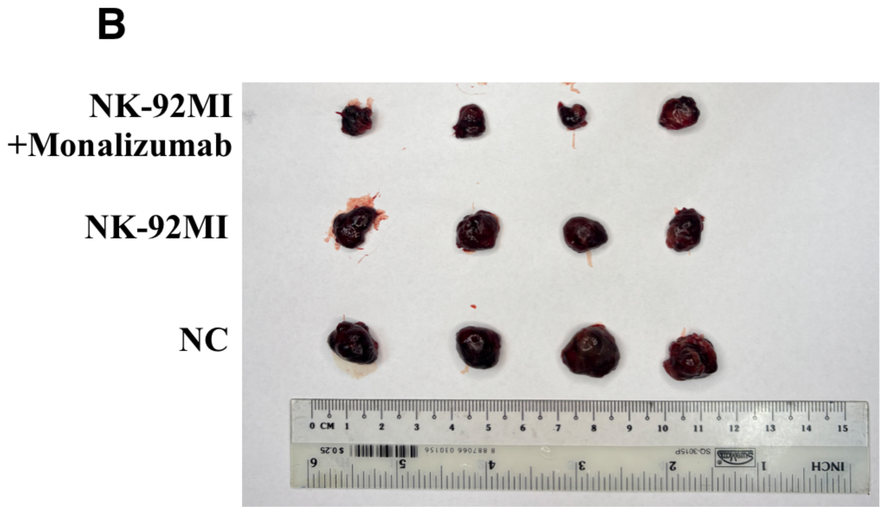
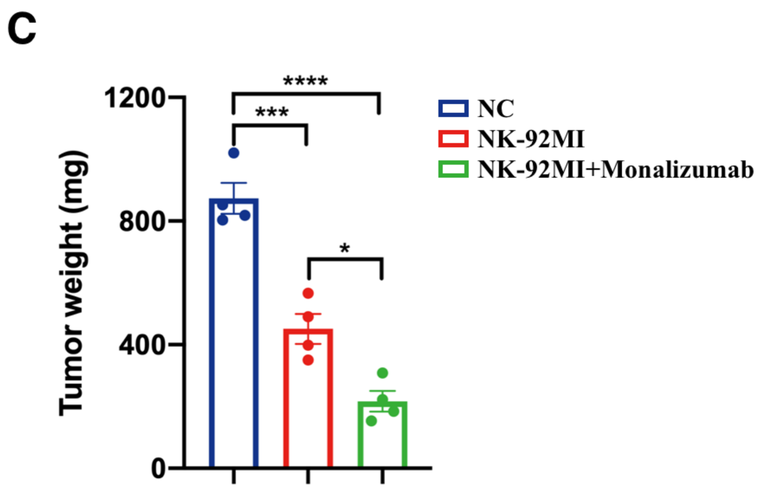
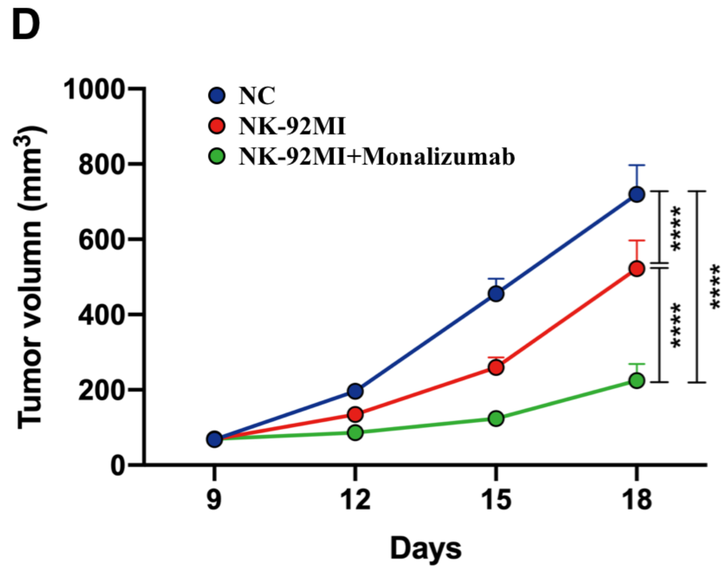

Rate of TNF-α positive cells (%)
Rate of Granzyme B positive cells (%)
NC Vector OE

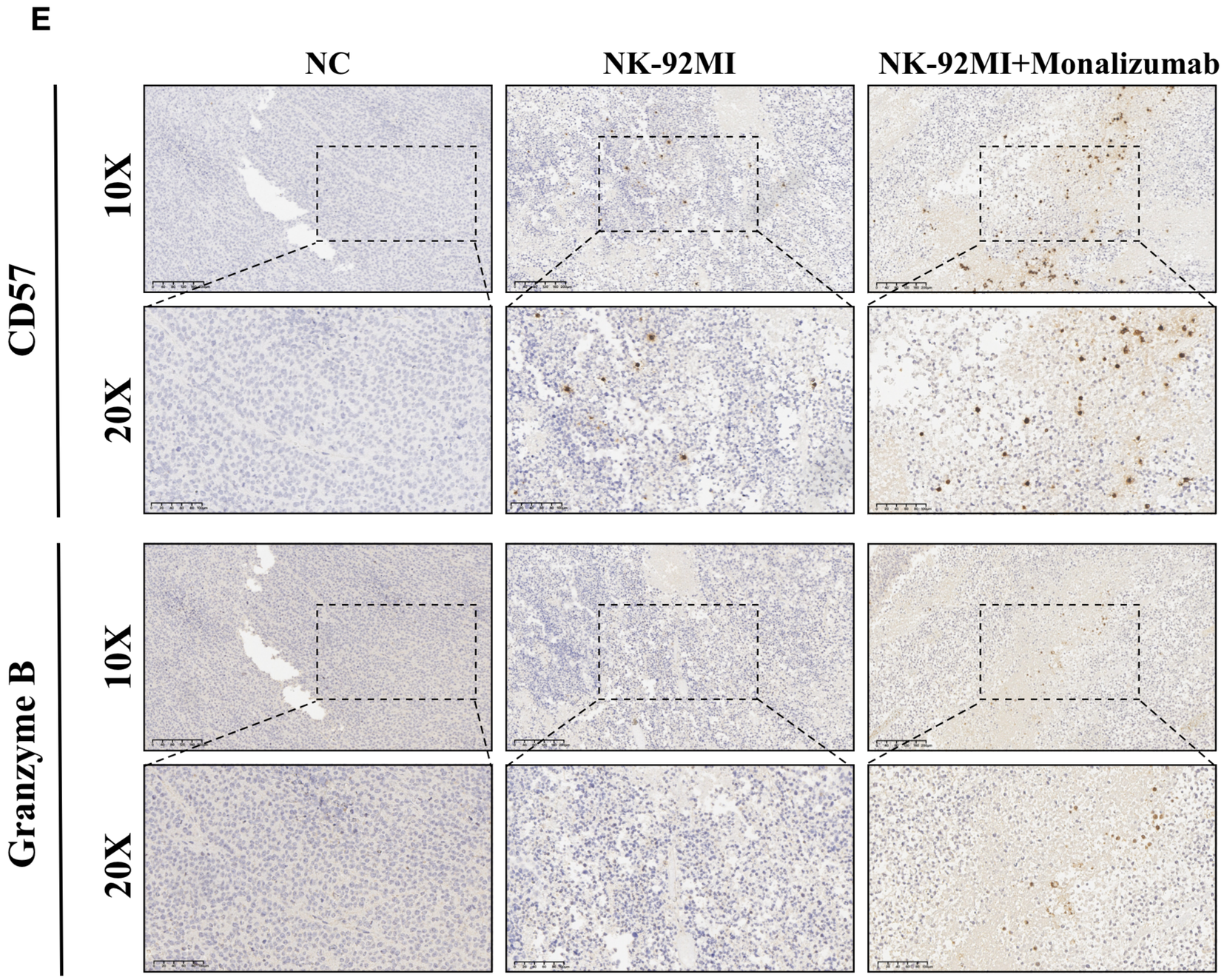
Fig.7

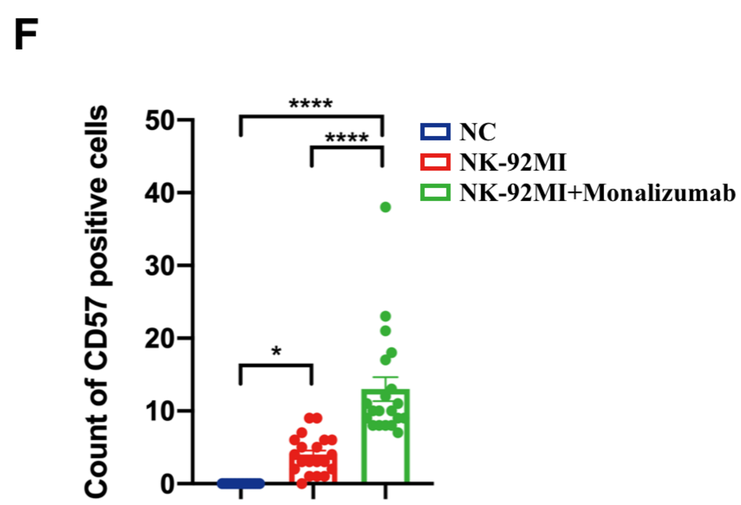

A
NCG mice
6 weeks
Hep huh7 cells
injection
NK-92MI cells
infusion
Tumor
harvested
0
6
9
12
15
18
Monalizumab
intravenous injection

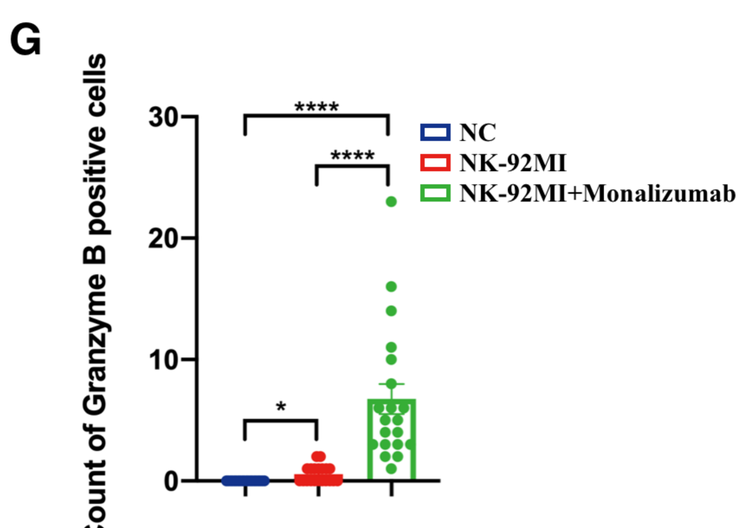

B
NK-92MI
+Monalizumab
NK-92MI
NC

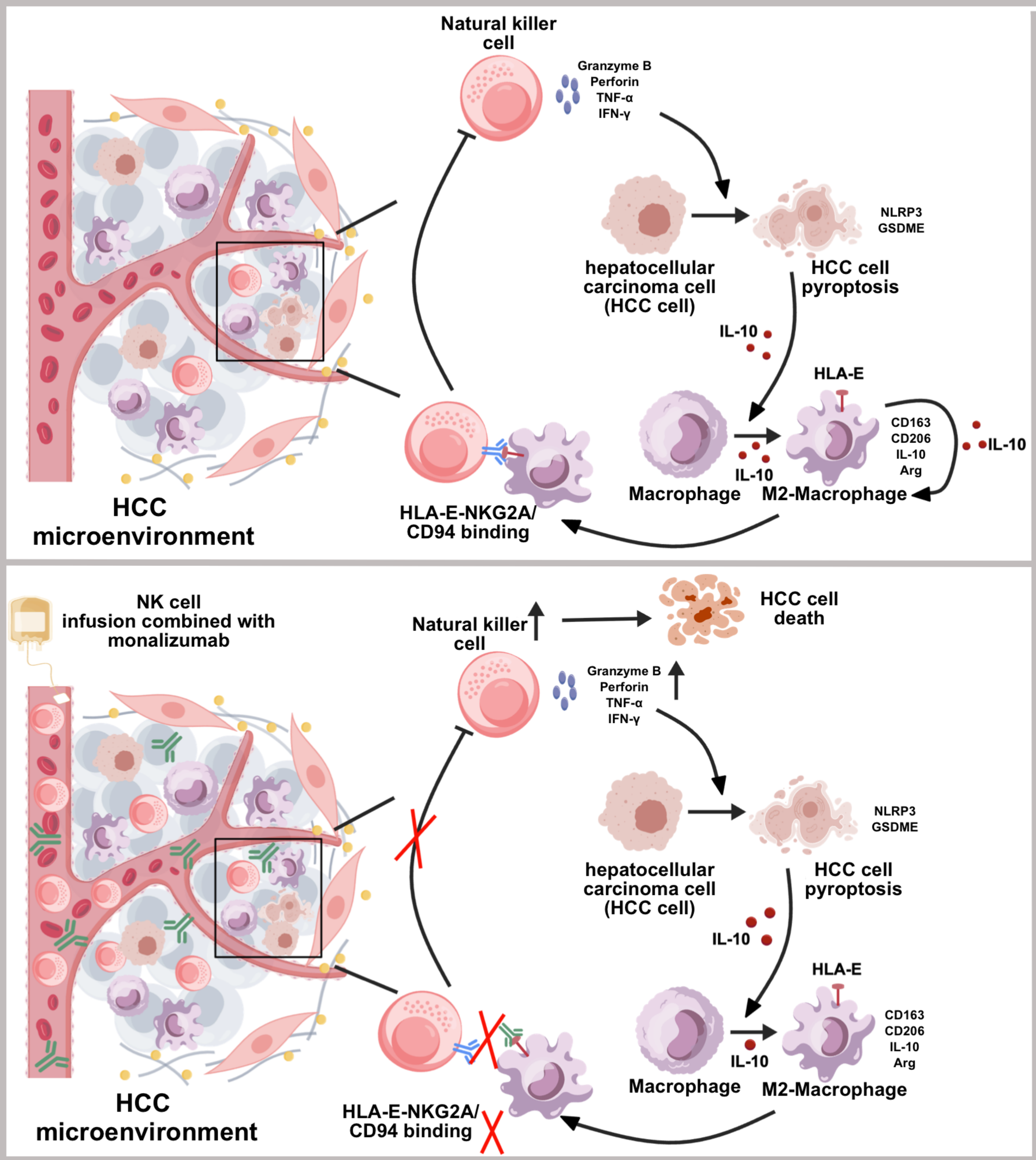

C
Tumor weight (mg)
1200
800
400
0
****
***
*
NC
NK-92MI
NK-92MI+Monalizumab


D
Tumor volumn (mm3)
1000
800
600
400
200
0
9
12
15
18
Days
NC
NK-92MI
NK-92MI+Monalizumab
****
****
****


E
NC
NK-92MI
NK-92MI+Monalizumab
CD57
10X
20X
Granzyme B
10X
20X


F
Count of CD57 positive cells
50
40
30
20
10
0
****
****
*
NC
NK-92MI
NK-92MI+Monalizumab


G
Count of Granzyme B positive cells
30
20
10
0
****
****
*
NC
NK-92MI
NK-92MI+Monalizumab


Natural killer cell
Granzyme B
Perforin
TNF-α
IFN-γ
hepatocellular carcinoma cell (HCC cell)
NLRP3
GSDME
HCC cell pyroptosis
IL-10
HLA-E
CD163
CD206
IL-10
Arg
IL-10
IL-10
Macrophage
M2-Macrophage
HLA-E-NKG2A/ CD94 binding
HCC microenvironment
NK cell infusion combined with monalizumab
Natural killer cell
HCC cell death
Granzyme B
Perforin
TNF-α
IFN-γ
hepatocellular carcinoma cell (HCC cell)
NLRP3
GSDME
HCC cell pyroptosis
IL-10
HLA-E
CD163
CD206
IL-10
Arg
IL-10
Macrophage
M2-Macrophage
HLA-E-NKG2A/ CD94 binding
HCC microenvironment
NK cell
HCC cell
Macrophage
M2-Macrophage
NKG2A/ CD94
HLA-E
Monalizumab
NK cell effector molecules
HCC cell pyroptosis
HCC cell death
IL-10
block