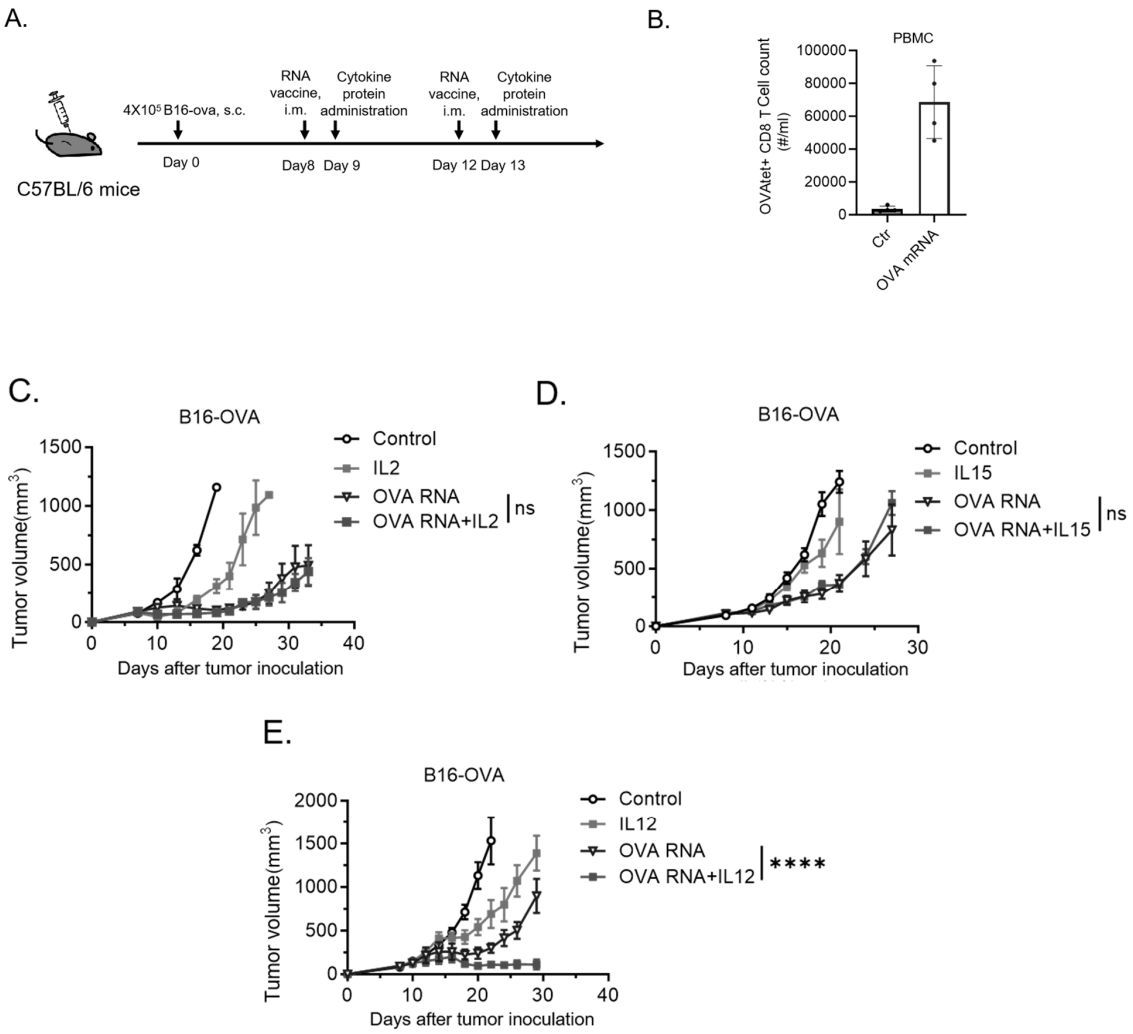
A.
4X10⁵ B16-ova, s.c.
RNA vaccine, i.m.
Cytokine protein administration
RNA vaccine, i.m.
Cytokine protein administration
Day 0
Day8
Day 9
Day 12
Day 13
C57BL/6 mice
B.
PBMC
OVAtet+ CD8 T Cell count (#/ml)
100000
80000
60000
40000
20000
0
Ctr
OVA mRNA
C.
B16-OVA
Tumor volume(mm³)
Days after tumor inoculation
Control
IL2
OVA RNA
OVA RNA+IL2
ns
D.
B16-OVA
Tumor volume(mm³)
Days after tumor inoculation
Control
IL15
OVA RNA
OVA RNA+IL15
ns
E.
B16-OVA
Tumor volume(mm³)
Days after tumor inoculation
Control
IL12
OVA RNA
OVA RNA+IL12
****

Fig. 1

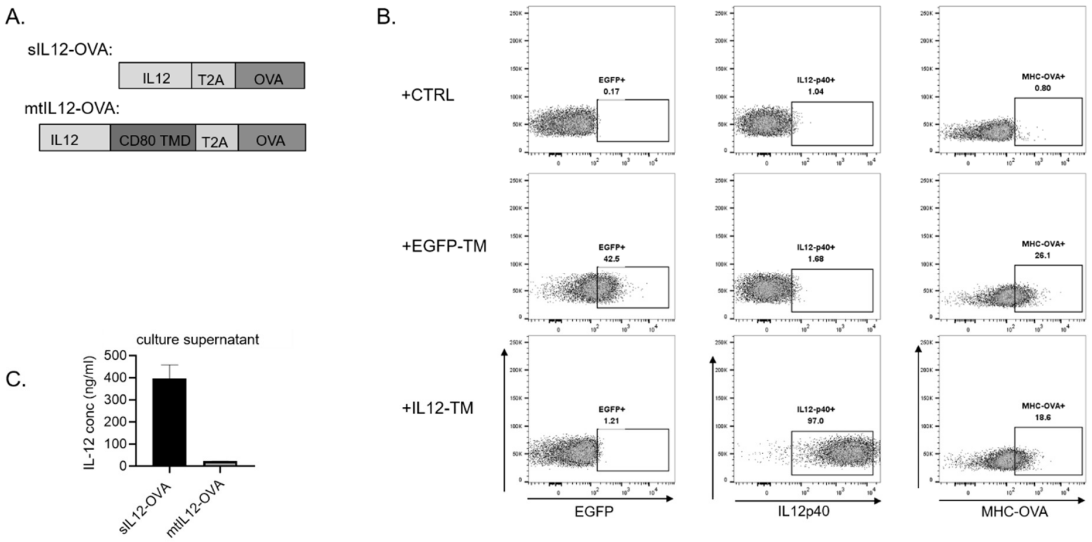
A.
sIL12-OVA:
IL12
T2A
OVA
mtIL12-OVA:
IL12
CD80 TMD
T2A
OVA
B.
+CTRL
+EGFP-TM
+IL12-TM
EGFP+
0.17
IL12-p40+
1.04
MHC-OVA+
0.80
EGFP+
42.5
IL12-p40+
1.68
MHC-OVA+
26.1
EGFP+
1.21
IL12-p40+
97.0
MHC-OVA+
19.6
EGFP
IL12p40
MHC-OVA
C.
culture supernatant
IL-12 conc (ng/ml)
500
400
300
200
100
0
sIL12-OVA
mtIL12-OVA

Fig. 2

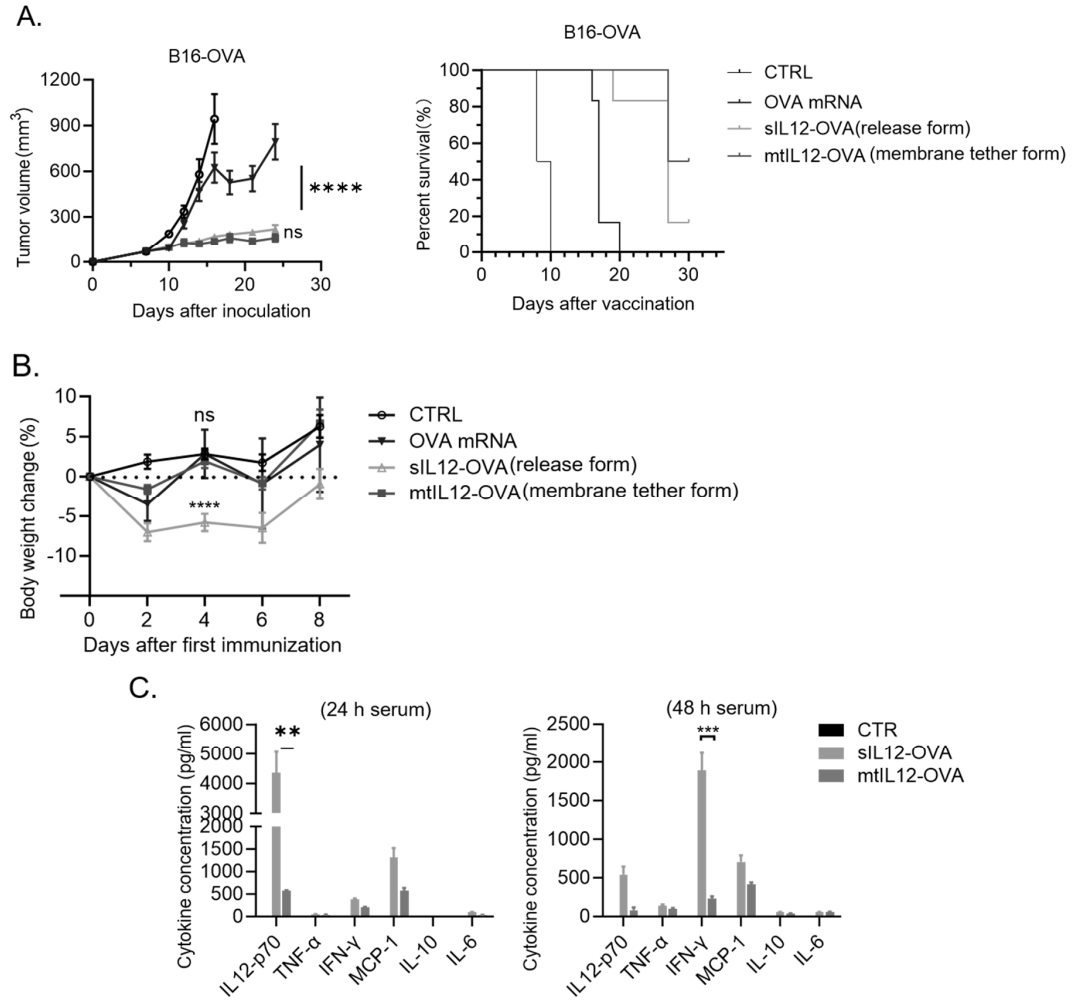
A.
B16-OVA
Tumor volume(mm3)
1200
900
600
300
0
****
ns
0
10
20
30
Days after inoculation
B16-OVA
Percent survival(%)
100
80
60
40
20
0
Days after vaccination
CTRL
OVA mRNA
sIL12-OVA(release form)
mtIL12-OVA (membrane tether form)
B.
Body weight change (%)
10
5
0
-5
-10
ns
****
0
2
4
6
8
Days after first immunization
CTRL
OVA mRNA
sIL12-OVA(release form)
mtIL12-OVA(membrane tether form)
C.
(24 h serum)
(48 h serum)
Cytokine concentration (pg/ml)
**
***
IL12-p70
TNF-α
IFN-γ
MCP-1
IL-10
IL-6
CTR
sIL12-OVA
mtIL12-OVA

Fig. 3

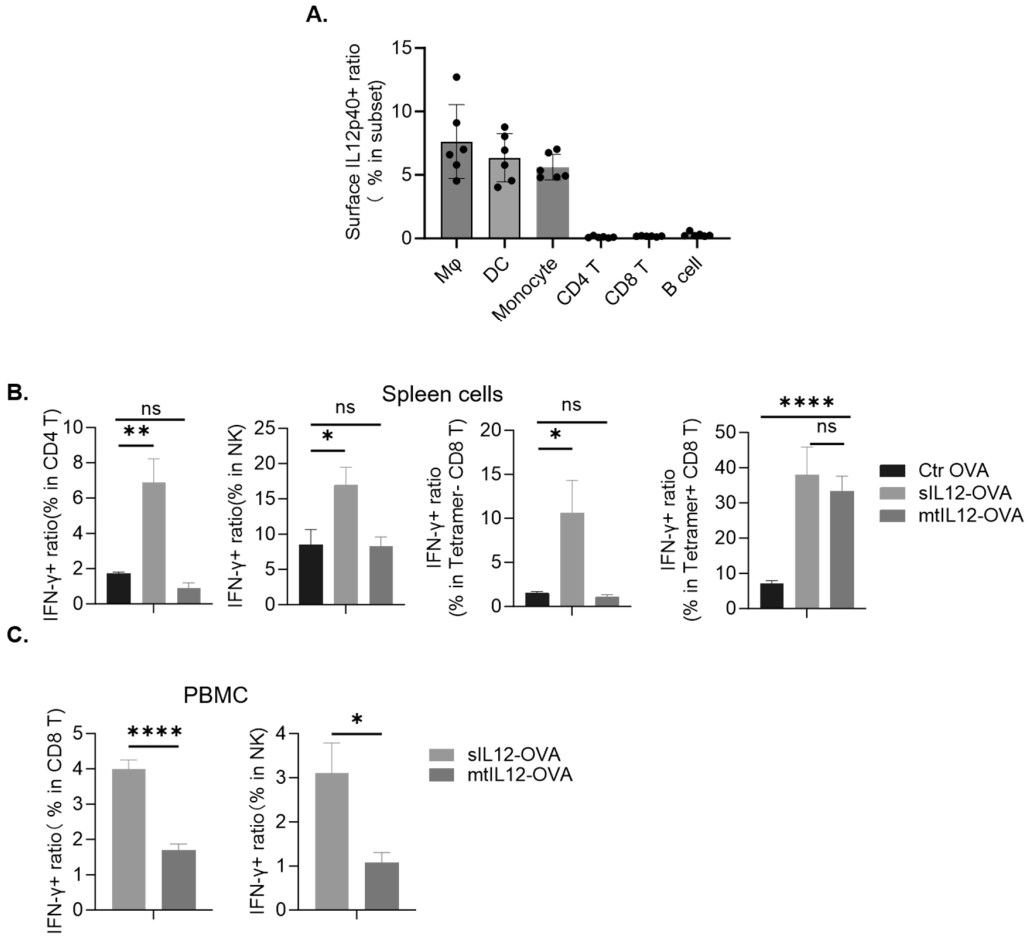
A.
Surface IL12p40+ ratio (% in subset)
Mφ
DC
Monocyte
CD4 T
CD8 T
B cell
B.
Spleen cells
IFN-γ+ ratio(% in CD4 T)
IFN-γ+ ratio(% in NK)
IFN-γ+ ratio (% in Tetramer- CD8 T)
IFN-γ+ ratio (% in Tetramer+ CD8 T)
Ctr OVA
sIL12-OVA
mtIL12-OVA
C.
PBMC
IFN-γ+ ratio (% in CD8 T)
IFN-γ+ ratio (% in NK)
sIL12-OVA
mtIL12-OVA

Fig. 4

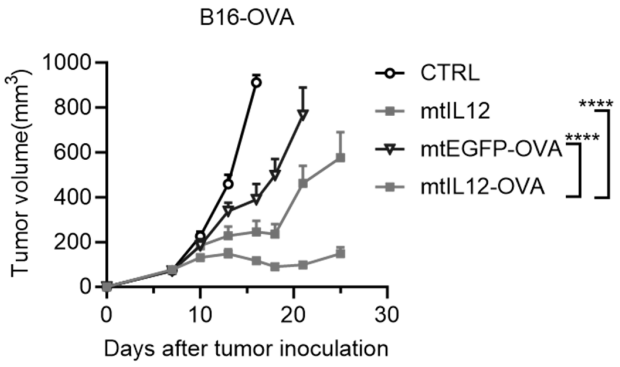
B16-OVA
Tumor volume(mm3)
Days after tumor inoculation
CTRL
mtIL12
mtEGFP-OVA
mtIL12-OVA

Fig. 5

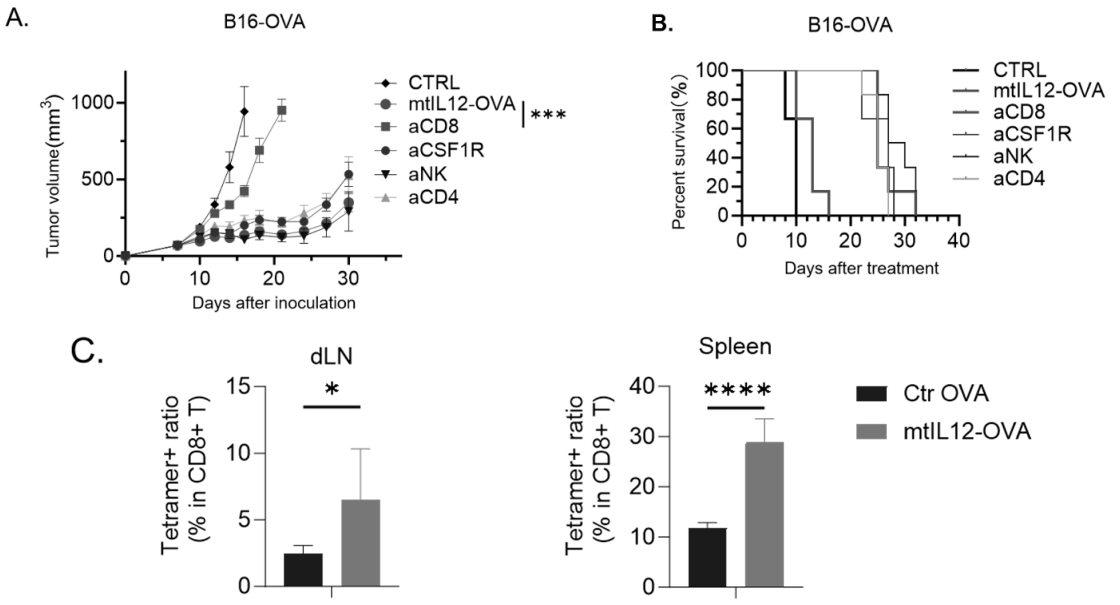
A.
B16-OVA
Tumor volume(mm3)
Days after inoculation
CTRL
mtIL12-OVA
aCD8
aCSF1R
aNK
aCD4
***
B.
B16-OVA
Percent survival(%)
Days after treatment
C.
dLN
Tetramer+ ratio (% in CD8+ T)
*
Spleen
****
Ctr OVA
mtIL12-OVA

Fig. 6

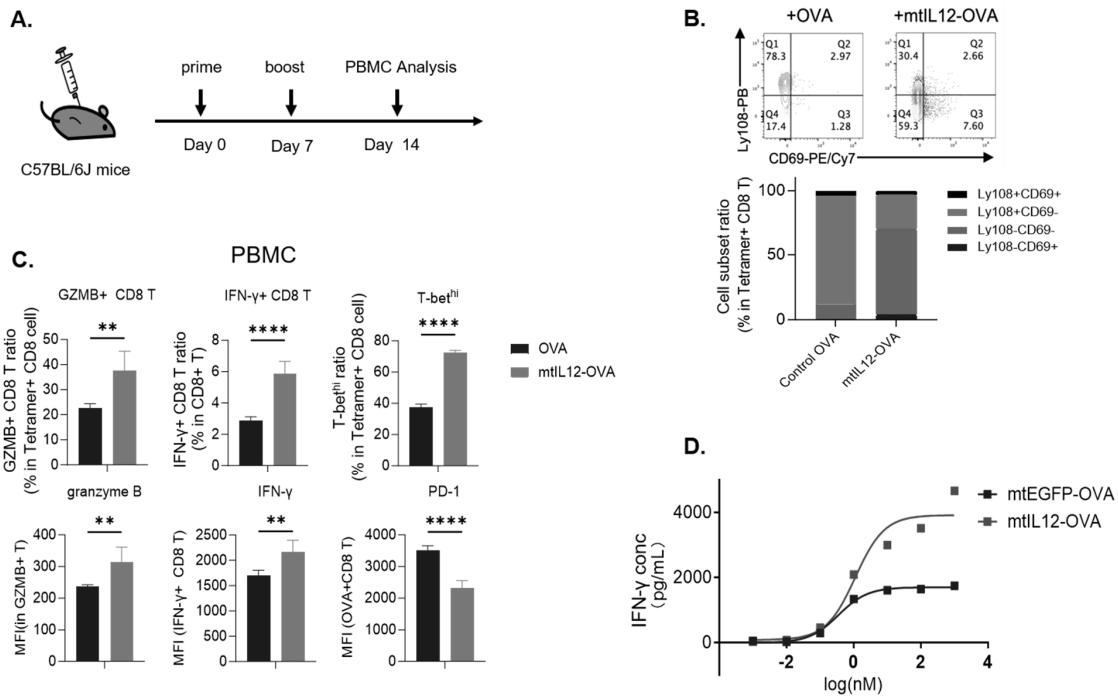
A.
prime
boost
PBMC Analysis
Day 0
Day 7
Day 14
C57BL/6J mice
B.
+OVA
+mtIL12-OVA
Ly108-PB
CD69-PE/Cy7
Cell subset ratio (% in Tetramer+ CD8 T)
Ly108+CD69+
Ly108+CD69-
Ly108-CD69-
Ly108-CD69+
Control OVA
mtIL12-OVA
C.
PBMC
GZMB+ CD8 T
IFN-γ+ CD8 T
T-bethi
OVA
mtIL12-OVA
granzyme B
IFN-γ
PD-1
D.
IFN-γ conc (pg/mL)
mtEGFP-OVA
mtIL12-OVA
log(nM)

Fig. 7

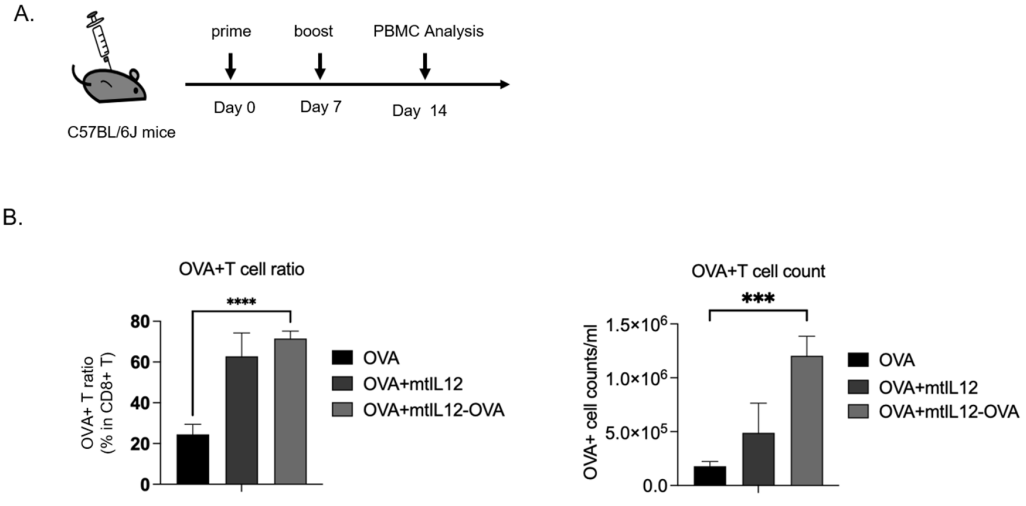

Fig. 8

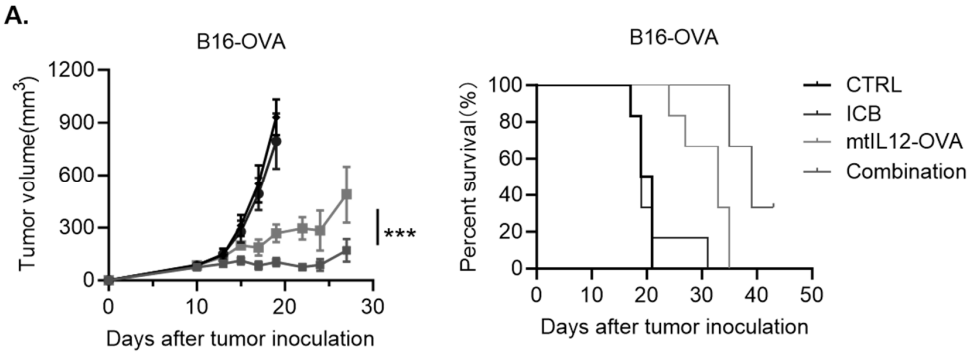

Fig. 9

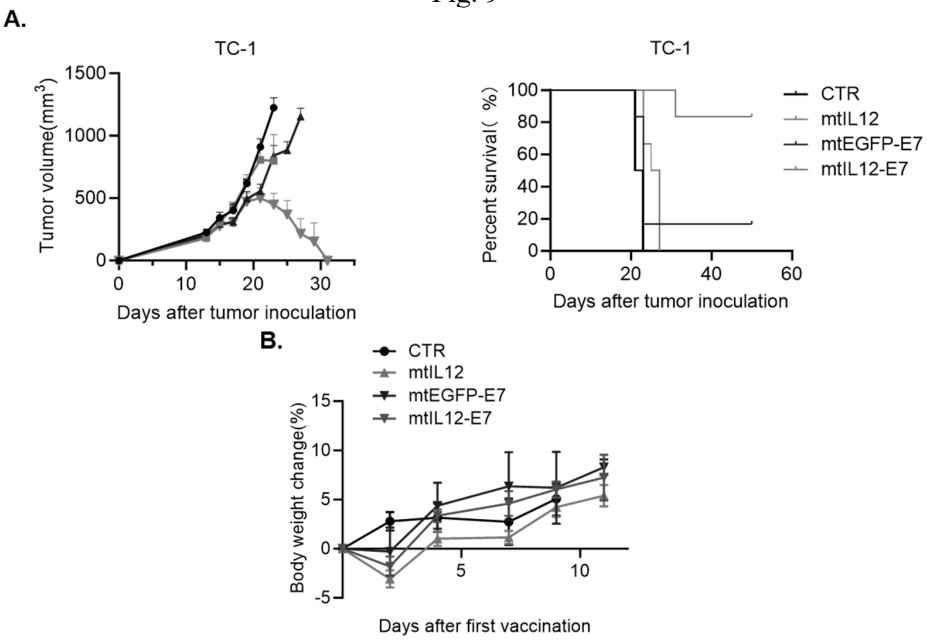

Fig. 10

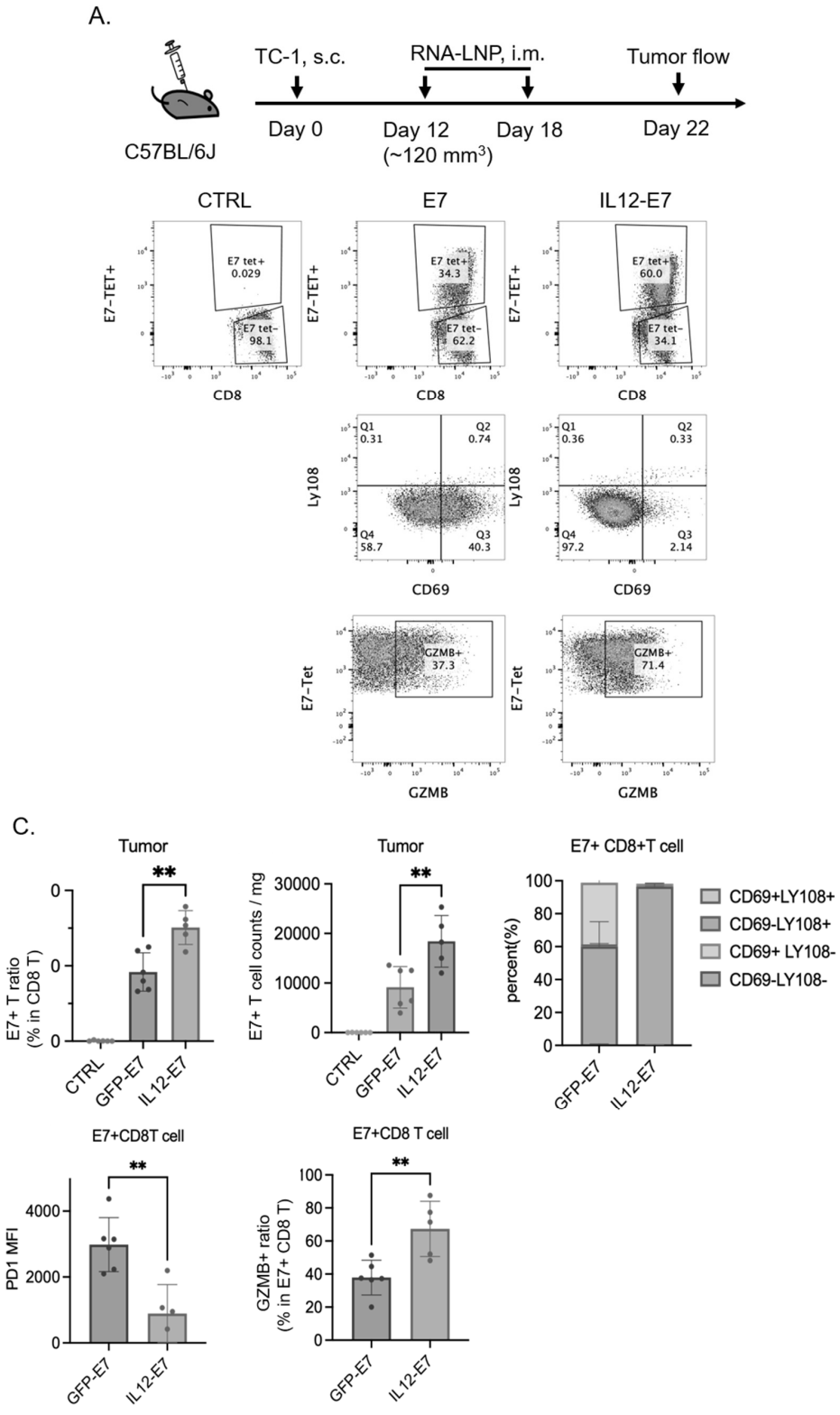
A.
TC-1, s.c.
RNA-LNP, i.m.
Tumor flow
C57BL/6J
Day 0
Day 12
(~120 mm3)
Day 18
Day 22
CTRL
E7
IL12-E7
E7-TET+
E7 tet+
0.029
E7 tet-
98.1
E7 tet+
34.3
E7 tet-
62.2
E7 tet+
60.0
E7 tet-
34.1
CD8
Ly108
CD69
Q1
0.31
Q2
0.74
Q4
58.7
Q3
40.3
Q1
0.36
Q2
0.33
Q4
97.2
Q3
2.14
E7-Tet
GZMB+
37.3
GZMB+
71.4
GZMB
C.
Tumor
E7+ T ratio
(% in CD8 T)
E7+ T cell counts / mg
E7+ CD8+T cell
percent(%)
CD69+LY108+
CD69-LY108+
CD69+ LY108-
CD69-LY108-
CTRL
GFP-E7
IL12-E7
E7+CD8T cell
PD1 MFI
E7+CD8 T cell
GZMB+ ratio
(% in E7+ CD8 T)

Fig. 11

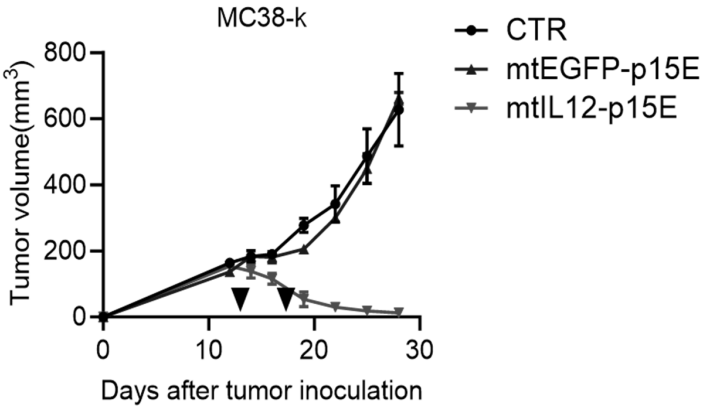
MC38-k
CTR
mtEGFP-p15E
mtIL12-p15E
Tumor volume(mm3)
800
600
400
200
0
0
10
20
30
Days after tumor inoculation

Fig. 12

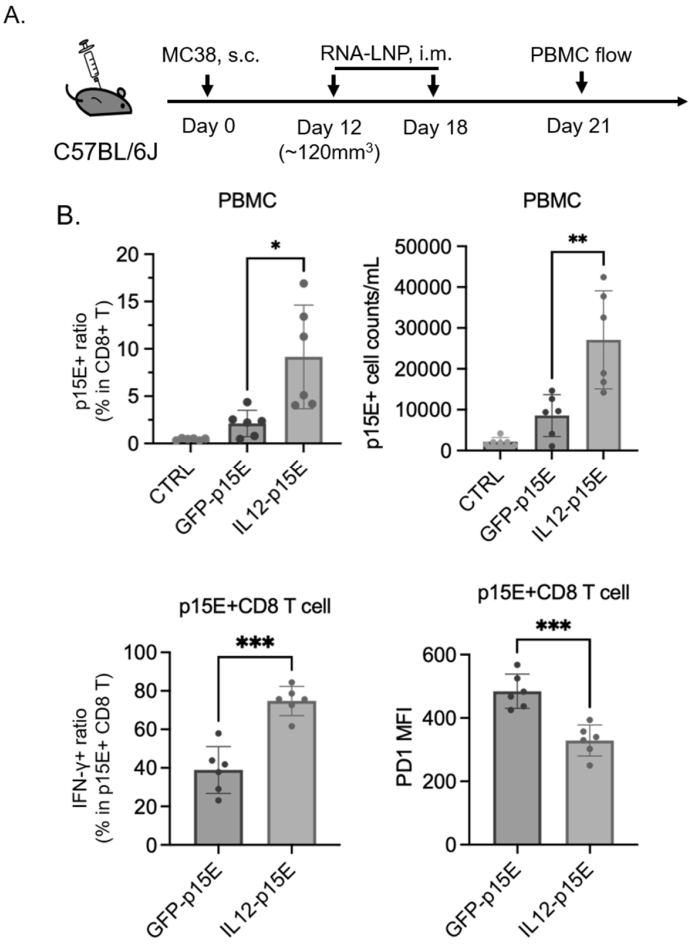
A.
MC38, s.c.
RNA-LNP, i.m.
PBMC flow
Day 0
Day 12
(~120mm3)
Day 18
Day 21
C57BL/6J
B.
PBMC
PBMC
p15E+ ratio
(% in CD8+ T)
p15E+ cell counts/mL
CTRL
GFP-p15E
IL12-p15E
p15E+CD8 T cell
p15E+CD8 T cell
IFN-γ+ ratio
(% in p15E+ CD8 T)
PD1 MFI

Fig. 13