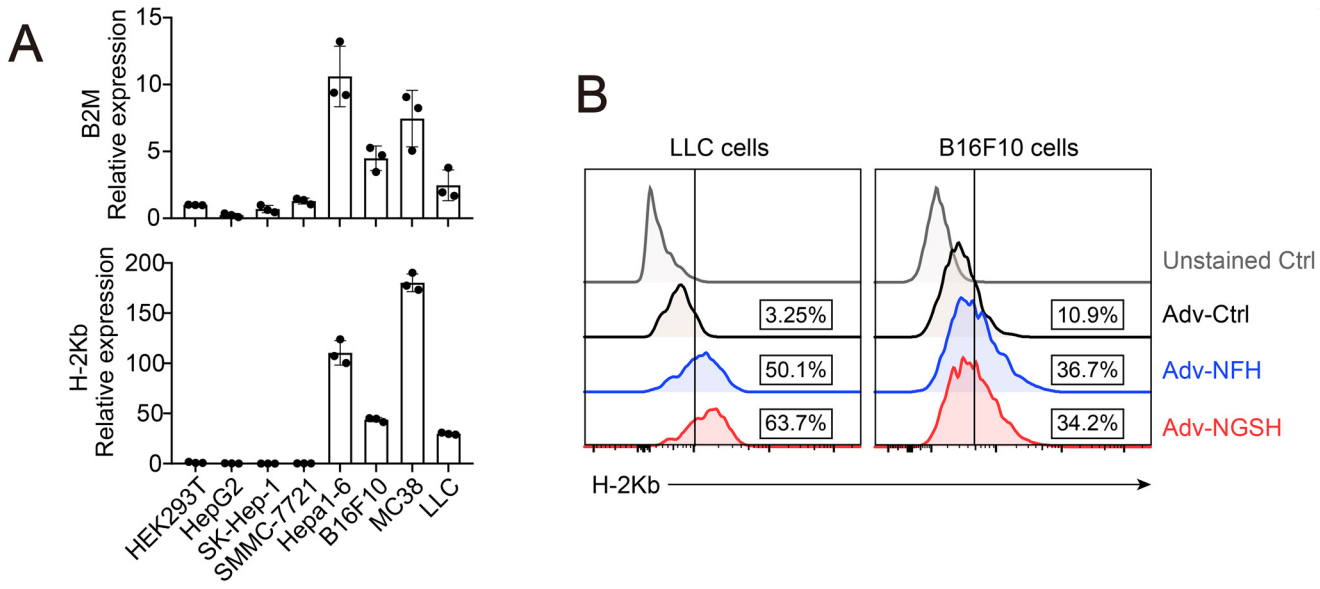
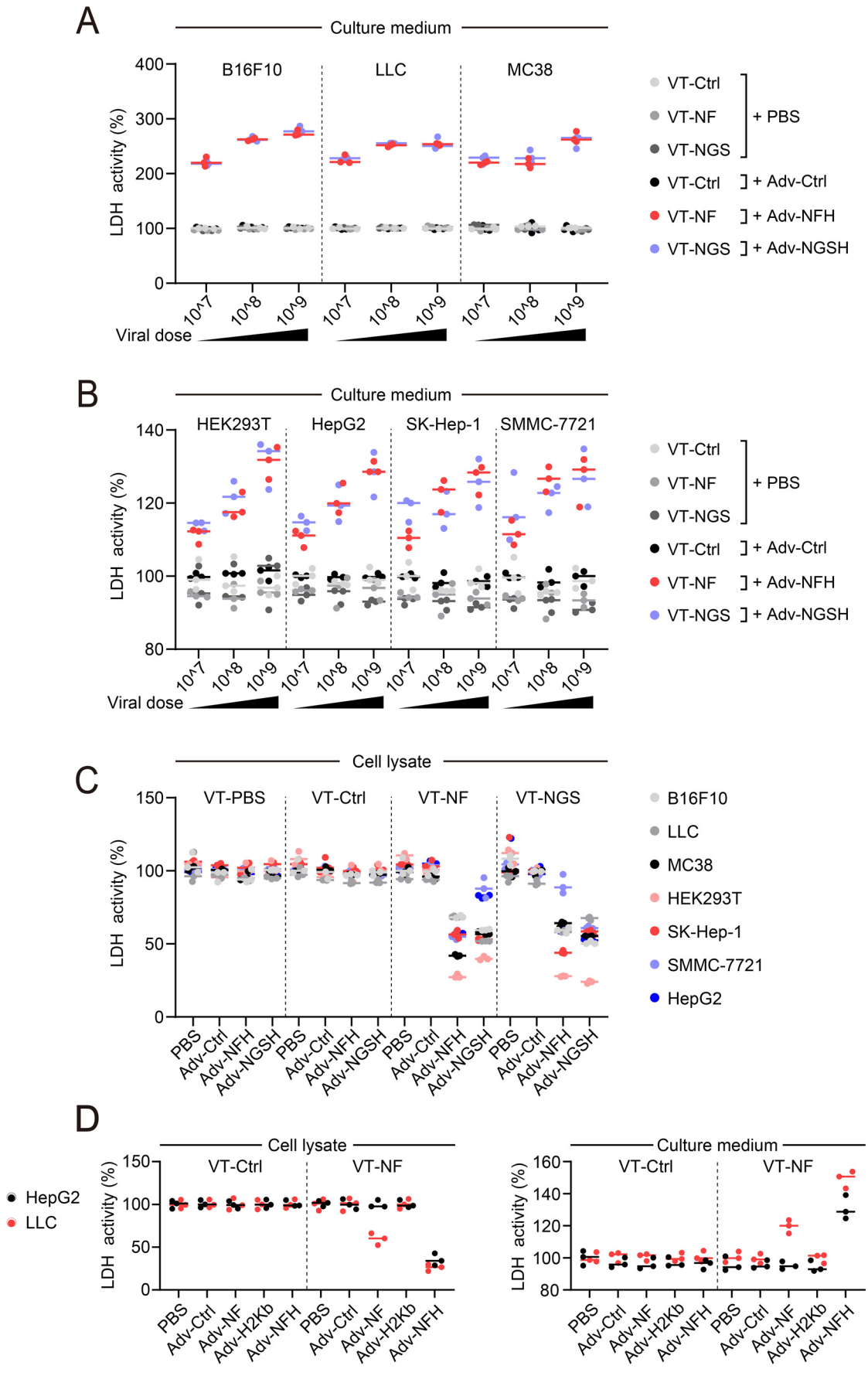
# Expanded View Figures

**Figure EV1. Expression of B2M, H-2Kb and the neoantigens in murine and human cell lines.**

(**A**) With indicated primers, the relative expression of B2M and H-2Kb was quantitatively analyzed in HEK293T, HepG2, SK-Hep-1, SMMC-7721, Hepa1-6, B16F10, MC38, and LLC cells ($n = 3$). (**B**) LLC and B16F10 cells were treated with PBS, Adv-Ctrl (1 MOI), Adv-NFH (1 MOI), or Adv-NGSH (1 MOI) for 24 h. The cells were collected and stained by a H-2Kb antibody (Biolegend, 116505, clone AF6-88.5, 5 μg/ml) for 30 min before subjecting to flow cytometry analysis. (**C**) Sanger sequencing results demonstrated the existence of the neoantigens in indicated cell lines. Data are shown as mean ± SD for 3 biological replicates.

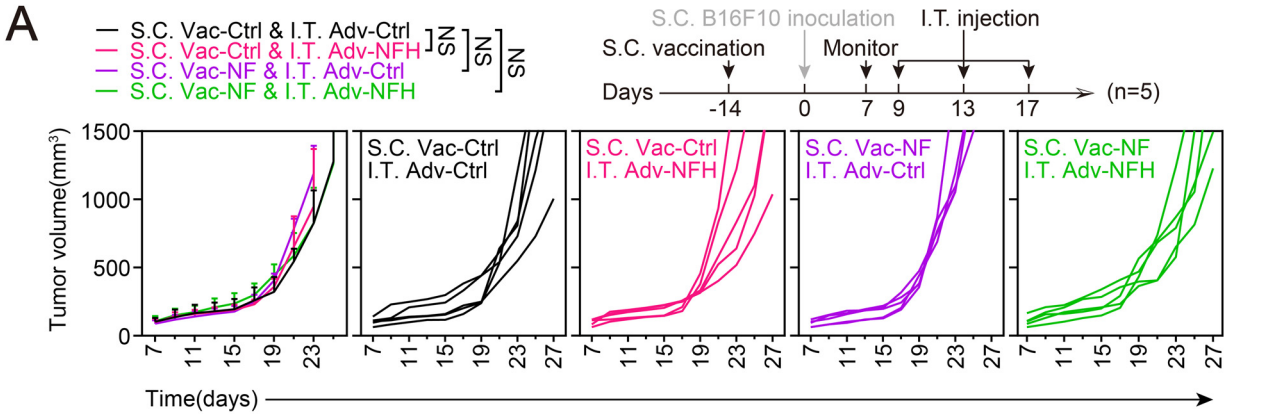

A
B2M
Relative expression
15
10
5
0
H-2Kb
Relative expression
200
150
100
50
0
HEK293T
HepG2
SK-Hep-1
SMMC-7721
Hepa1-6
B16F10
MC38
LLC
B
LLC cells
B16F10 cells
Unstained Ctrl
Adv-Ctrl
Adv-NFH
Adv-NGSH
3.25%
50.1%
63.7%
10.9%
36.7%
34.2%
H-2Kb

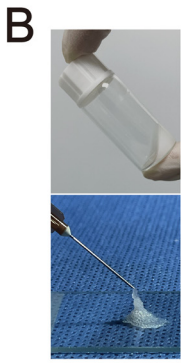

C
Mapk3
Lmf1
Samd91
Traf7
Dtnb
Lbr
Ptpn2
Ref.
CAGTCTCTG
285
Gln Ser Leu
AAGTTCAGC
Lys Phe Ser
CAGAAGTTT
Gln Lys Phe
AAGTGCCAA
Lys Cys Gln
TGCAAGTTA
40
Cys Lys Leu
TTGGCACTG
340
Leu Ala Leu
CCTATTCTC
Pro Ile Leu
Hepa1-6
CAGTCTCTG
AAGGTCAGC
CAGAAGTTT
AAGTGCCAA
TGCACGTTA
TTGCCACTG
CCTACTCTC
B16F10
CAGTCTCTG
AAGTTCAGC
CAGAAGTTT
AAGTGCCAA
TGCAAGTTA
TTGGCACTG
CCTATTCTC
LLC
CAGTCTCTG
AAGTTCAGC
CAGAAGTTT
AAGTGCCAA
TGCAAGTTA
TTGGCACTG
CCTATTCTC
MC38
CAGTCTCTG
AAGTTCAGC
CAGAAGTTT
AAGTGCCAA
TGCAAGTTA
TTGGCACTG
CCTATTCTC
HEK293T
CAGTCTCTG
AAGTTCAGC
AAAAAACTTC
AAGTGTCAG
TGCAAATTA
TTTGCACTT
CCTATTCTC
HepG2
CAGTCTCTG
AAGTTCAGC
AAAAAACTTC
AAGTGTCAG
TGCAAATTA
TTTGCACTT
CCTATTCTC
SK-Hep-1
CAGTCTCTG
AAGTTCAGC
AAAAAACTTC
AAGTGTCAG
TGCAAATTA
TTTGCACTT
CCTATTCTC
SMMC-7721
CAGTCTCTG
AAGTTCAGC
AAAAAACTTC
AAGTGTCAG
TGCAAATTA
TTTGCACTT
CCTATTCTC

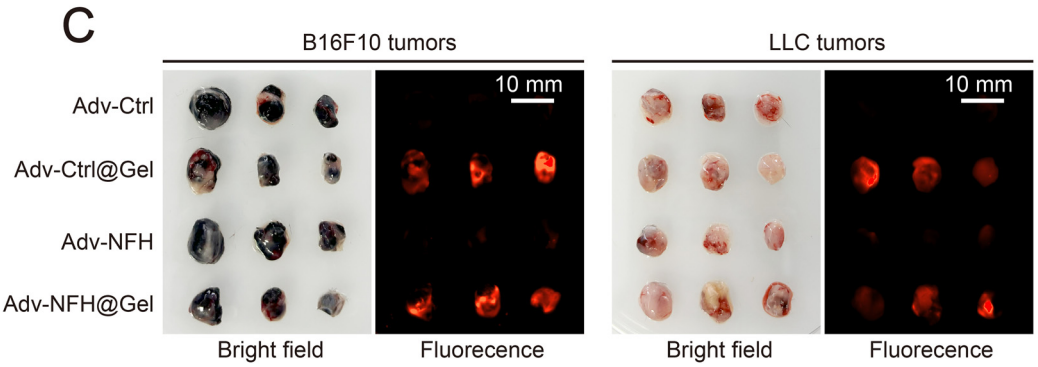

A
Culture medium
B16F10
LLC
MC38
LDH activity (%)
400
300
200
100
0
10^7
10^8
10^9
Viral dose
VT-Ctrl
VT-NF
VT-NGS
+ PBS
VT-Ctrl
VT-NF
VT-NGS
+ Adv-Ctrl
+ Adv-NFH
+ Adv-NGSH
B
Culture medium
HEK293T
HepG2
SK-Hep-1
SMMC-7721
LDH activity (%)
140
120
100
80
Viral dose
C
Cell lysate
VT-PBS
VT-Ctrl
VT-NF
VT-NGS
LDH activity (%)
150
100
50
0
PBS
Adv-Ctrl
Adv-NFH
Adv-NGSH
B16F10
LLC
MC38
HEK293T
SK-Hep-1
SMMC-7721
HepG2
D
HepG2
LLC
Cell lysate
VT-Ctrl
VT-NF
LDH activity (%)
150
100
50
0
PBS
Adv-Ctrl
Adv-NF
Adv-H2Kb
Adv-NFH
Culture medium
VT-Ctrl
VT-NF
LDH activity (%)
160
140
120
100
80
PBS
Adv-Ctrl
Adv-NF
Adv-H2Kb
Adv-NFH

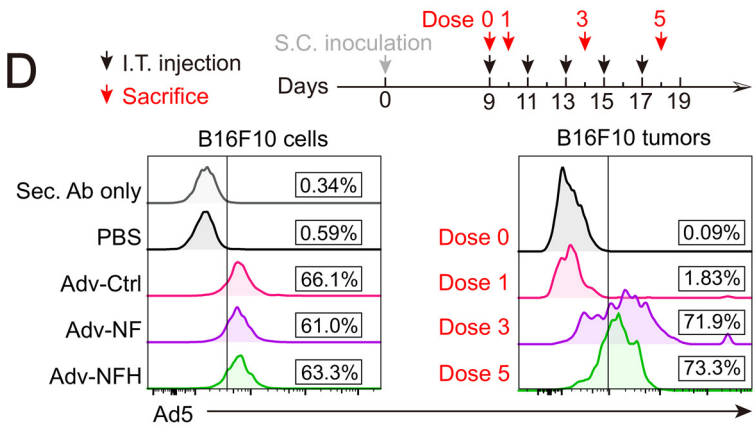
◀ **Figure EV2. Delivery of neoantigen peptide-MHC complexes redirected the cytotoxicity of neoantigen vaccine-primed T cells.**

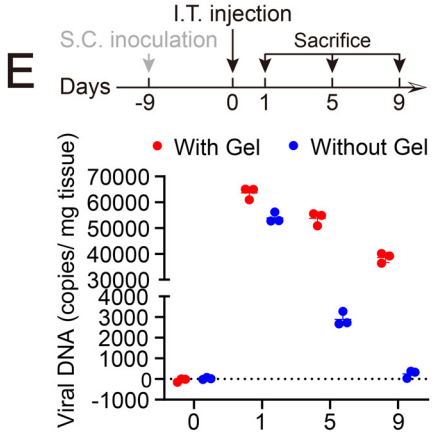
(**A**) LDH assay demonstrated the dose-dependent cytotoxicity of adenovirus-primed T cells in murine tumor cells that infected with Adv-NFH or Adv-NGSH. Samples were the culture medium of the murine cells assessed in Fig. 2E ($n = 3$). (**B**) Dose-dependent cytotoxicity of adenovirus-primed T cells in human tumor cells infected by Adv-NFH or Adv-NGSH. Samples were the culture medium of the human cells used in Fig. 2E ($n = 3$). (**C**) LDH assay in remnant tumor cells showed the specificity of the redirected cytotoxicity ($n = 3$). The assay was performed by following the experimental protocol in Fig. 2E. Mice were vaccinated with $2 \times 10^9$ VP of adenoviruses for 14 d before harvesting the SMNCs to prepare related T cells (VT-PBS, VT-Ctrl, VT-NF, and VT-NGS). Tumor cells, including B16F10, LLC, MC38, HEK293T, SK-Hep-1, SMMC-7721, and HepG2, were pre-treated with 1 MOI of indicated viruses and co-cultured with the prepared T cells at an E:T ratio of 1:3 for 48 h. (**D**) LDH assay demonstrated the functionality of MHC-I overexpression. After 48 h infection with 1 MOI of indicated viruses, HepG2 and LLC cells were co-cultured with VT-Ctrl or VT-NF at a ratio of 1:3 for 48 h before assessing the cell viability ($n = 3$). Data are shown as mean ± SD for 3 biological replicates.

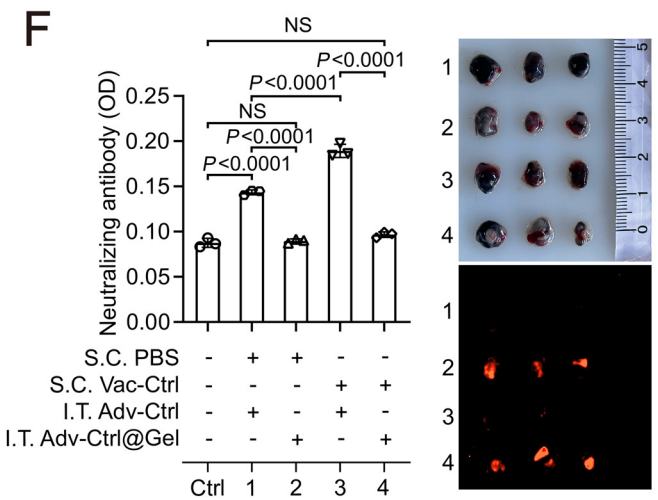

A
S.C. Vac-Ctrl & I.T. Adv-Ctrl
S.C. Vac-Ctrl & I.T. Adv-NFH
S.C. Vac-NF & I.T. Adv-Ctrl
S.C. Vac-NF & I.T. Adv-NFH
NS
NS
NS
S.C. B16F10 inoculation
I.T. injection
S.C. vaccination
Monitor
Days
-14 0 7 9 13 17
(n=5)
Tumor volume(mm3)
1500
1000
500
0
S.C. Vac-Ctrl
I.T. Adv-Ctrl
S.C. Vac-Ctrl
I.T. Adv-NFH
S.C. Vac-NF
I.T. Adv-Ctrl
S.C. Vac-NF
I.T. Adv-NFH
7 11 15 19 23 27
Time(days)

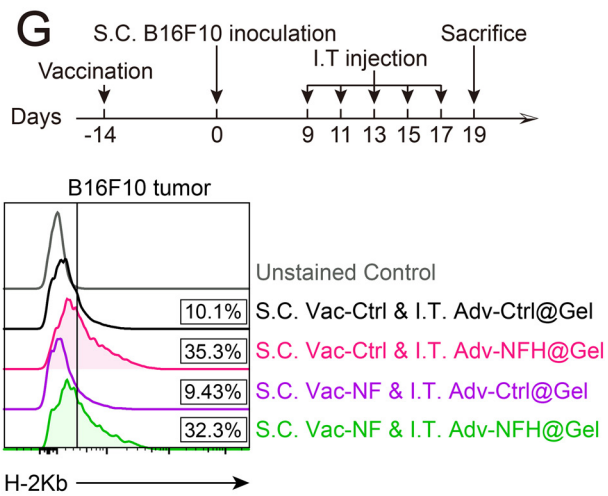

B


C
B16F10 tumors
LLC tumors
Adv-Ctrl
Adv-Ctrl@Gel
Adv-NFH
Adv-NFH@Gel
10 mm
10 mm
Bright field
Fluorecence
Bright field
Fluorecence


D
I.T. injection
Sacrifice
S.C. inoculation
Dose 0 1 3 5
Days
0 9 11 13 15 17 19
B16F10 cells
B16F10 tumors
Sec. Ab only
PBS
Adv-Ctrl
Adv-NF
Adv-NFH
0.34%
0.59%
66.1%
61.0%
63.3%
Dose 0
Dose 1
Dose 3
Dose 5
0.09%
1.83%
71.9%
73.3%
Ad5

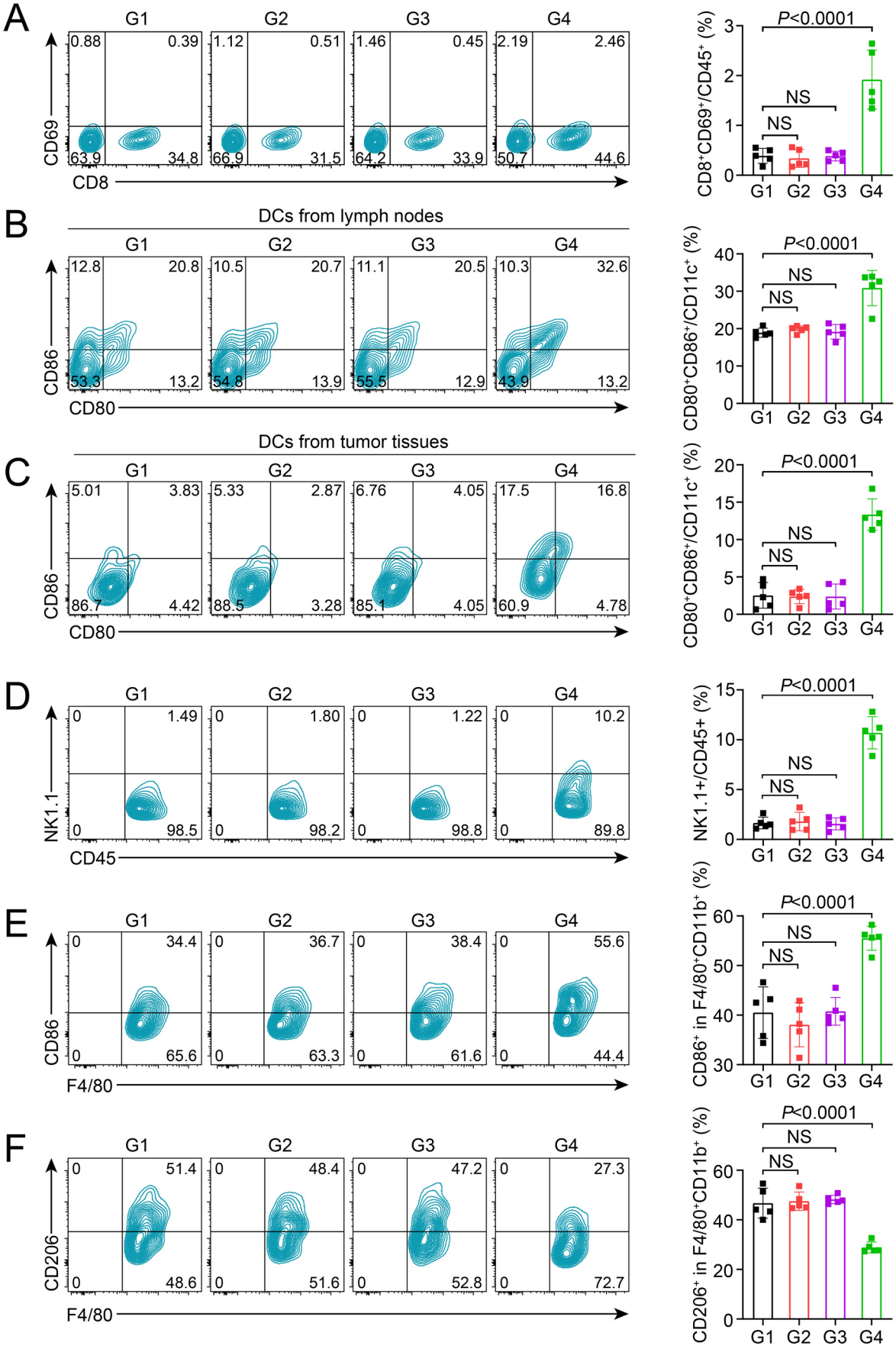

E
I.T. injection
S.C. inoculation
Sacrifice
Days
-9 0 1 5 9
With Gel
Without Gel
Viral DNA (copies/ mg tissue)
70000
60000
50000
40000
30000
4000
3000
2000
1000
0
-1000
0 1 5 9


F
NS
P<0.0001
P<0.0001
NS
P<0.0001
P<0.0001
Neutralizing antibody (OD)
0.25
0.20
0.15
0.10
0.05
0.00
S.C. PBS - + + - -
S.C. Vac-Ctrl - - - + +
I.T. Adv-Ctrl - + - + -
I.T. Adv-Ctrl@Gel - - + - +
Ctrl 1 2 3 4
1 2 3 4
1 2 3 4


G
S.C. B16F10 inoculation
Sacrifice
Vaccination
I.T injection
Days
-14 0 9 11 13 15 17 19
B16F10 tumor
Unstained Control
10.1%
S.C. Vac-Ctrl & I.T. Adv-Ctrl@Gel
35.3%
S.C. Vac-Ctrl & I.T. Adv-NFH@Gel
9.43%
S.C. Vac-NF & I.T. Adv-Ctrl@Gel
32.3%
S.C. Vac-NF & I.T. Adv-NFH@Gel
H-2Kb

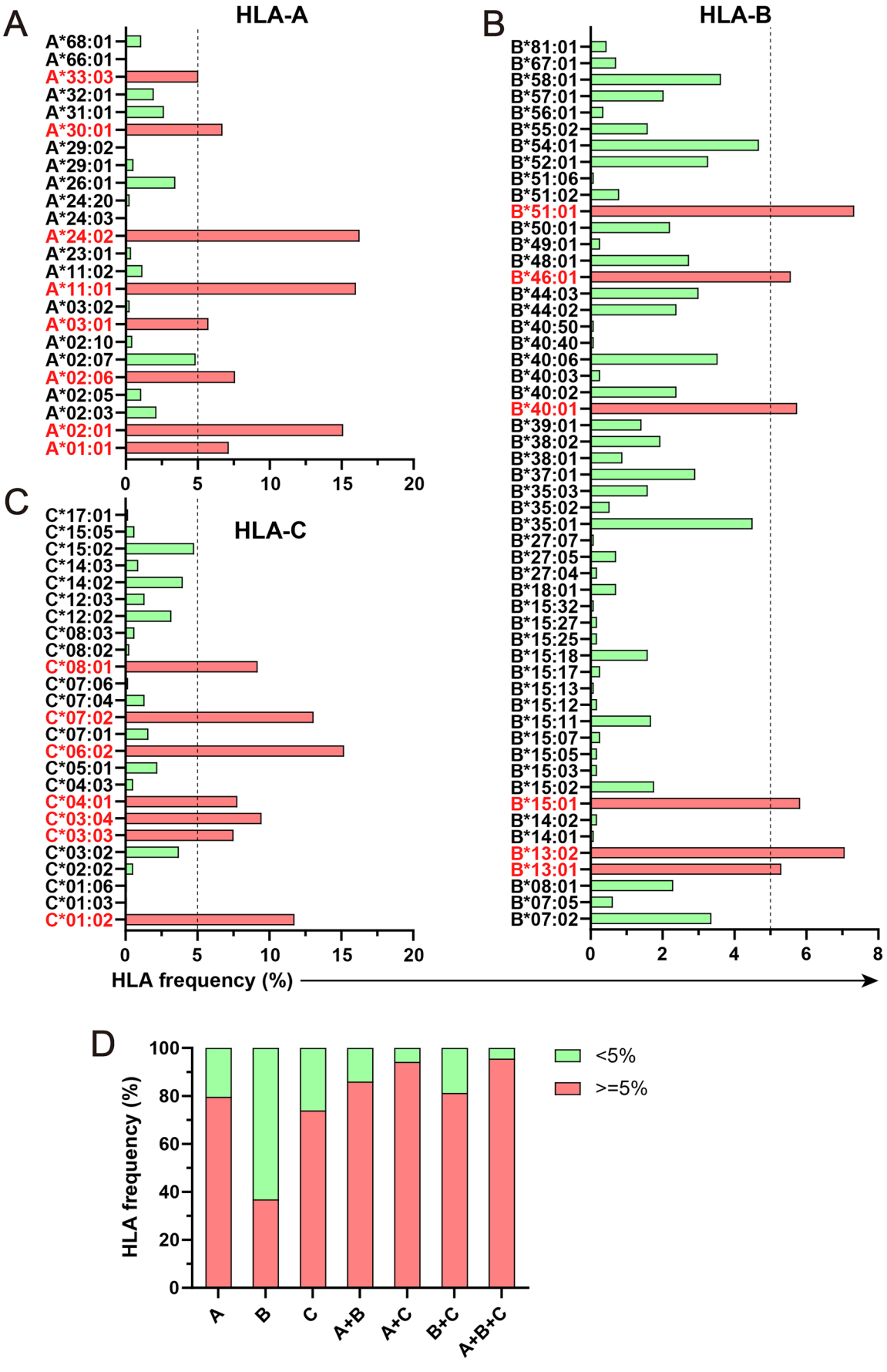
◀ **Figure EV3. Improving the efficacy of virotherapy with hydrogel encapsulation.**

(**A**) Overall and individual tumor growth curves demonstrated an integrative therapy of subcutaneous B16F10 tumor model with S.C. vaccination (Vac-Ctrl or Vac-NF, $2 \times 10^9$ VP) and I.T. virotherapy (Adv-Ctrl or Adv-NFH, $10^9$ VP) ($n = 5$). (**B**) Representative images demonstrated the successful preparation of injectable silk hydrogel. (**C**) Hydrogel encapsulation potentiated the intratumoral infection of adenoviruses ($n = 3$). (**D**) Quantification of intratumoral infection rate of hydrogel encapsulated Adv-Ctrl at early and late time point with an anti-adenovirus antibody (Merck, AB1056, 1:500) and Alexa FluorTM 488 secondary antibody (Thermo Fisher, A-11055, 2 μg/ml). Before the analysis, the functionality of the primary antibody was validated by staining B16F10 cells that have been infected with adenoviruses (Adv-Ctrl, Adv-NF, or Adv-NFH) in vitro at a MOI of 1 for 24 h (left panel). (**E**) qPCR assay quantified the viral DNA levels in the tumor tissues. Adv-Ctrl was prepared either in hydrogel (With Gel) or PBS (Without Gel) and injected into the tumors. Tumor tissues were subsequently collected at various time points for analysis. (**F**) Adenovirus neutralizing antibody assay and fluorescent imaging showed the protection of adenoviral vectors from host immunity by hydrogel encapsulation ($n = 3$). *P* value: Ctrl vs. 1, $P = 3.380 \times 10^{-7}$; Ctrl vs. 2, $P = 0.982$; Ctrl vs. 4, $P = 0.199$; 1 vs. 2, $P = 4.752 \times 10^{-7}$; 1 vs. 3, $P = 2.034 \times 10^{-6}$; 3 vs. 4, $P = 1.457 \times 10^{-9}$. (**G**) MHC status of B16F10-established tumors following the intratumoral injection of hydrogel encapsulated adenoviruses. Statistical analysis was performed using one-way ANOVA (**F**) and two-way ANOVA (**A**). Data are shown as mean ± SD for 3 biological replicates.


A
G1
G2
G3
G4
0.88
0.39
63.9
34.8
1.12
0.51
66.9
31.5
1.46
0.45
64.2
33.9
2.19
2.46
50.7
44.6
CD69
CD8
CD8+CD69+/CD45+ (%)
P<0.0001
NS
NS
B
DCs from lymph nodes
12.8
20.8
53.3
13.2
10.5
20.7
54.8
13.9
11.1
20.5
55.5
12.9
10.3
32.6
43.9
13.2
CD86
CD80
CD80+CD86+/CD11c+ (%)
C
DCs from tumor tissues
5.01
3.83
86.7
4.42
5.33
2.87
88.5
3.28
6.76
4.05
85.1
4.05
17.5
16.8
60.9
4.78
D
1.49
98.5
1.80
98.2
1.22
98.8
10.2
89.8
NK1.1
CD45
NK1.1+/CD45+ (%)
E
34.4
65.6
36.7
63.3
38.4
61.6
55.6
44.4
F4/80
CD86+ in F4/80+CD11b+ (%)
F
51.4
48.6
48.4
51.6
47.2
52.8
27.3
72.7
CD206
CD206+ in F4/80+CD11b+ (%)

◀ **Figure EV4. Integrative therapy remodeled the tumor microenvironment.**

Results of flow cytometry analysis showed the composition of $CD69^+CD8^+$ T cells in tumors (**A**), $CD80^+CD86^+$ DCs in lymph nodes (**B**), $CD80^+CD86^+$ DCs (**C**), $NK1.1^+$ NK cells (**D**), $CD86^+$ macrophages (**E**) and $CD206^+$ macrophages (**F**) in tumors ($n = 5$). PE-Cyanine7-conjugated anti-NK1.1 (eBioscience, 25-5941-82, 2.5 μg/ml), FITC-conjugated anti-F4/80 (BioLegend, 123107, 2.5 μg/ml), APC-conjugated anti-CD11b (eBioscience, 17-0112-82, 1.25 μg/ml), and PE-conjugated anti-CD206 (eBioscience, 12-2061-82, 1.25 μg/ml) antibodies were used for analysis. *P* value (**A**): G2, $P = 0.995$; G3, $P = 0.999997$; G4, $P = 6.875 \times 10^{-6}$. *P* value (**B**): G2, $P = 0.946$; G3, $P = 0.999$; G4, $P = 1.359 \times 10^{-5}$. *P* value (**C**): G2, $P = 0.999$; G3, $P = 0.999$; G4, $P = 1.063 \times 10^{-7}$. *P* value (**D**): G2, $P = 0.996$; G3, $P = 0.999$; G4, $P = 1.202 \times 10^{-9}$. *P* value (**E**): G2, $P = 0.755$; G3, $P = 0.9996$; G4, $P = 8.186 \times 10^{-5}$. *P* value (**F**): G2, $P = 0.987$; G3, $P = 0.924$; G4, $P = 8.309 \times 10^{-6}$. Statistical analysis was performed using one-way ANOVA (**A–F**). Data are shown as mean ± SD for 3 biological replicates.


A
HLA-A
A*68:01
A*66:01
A*33:03
A*32:01
A*31:01
A*30:01
A*29:02
A*29:01
A*26:01
A*24:20
A*24:03
A*24:02
A*23:01
A*11:02
A*11:01
A*03:02
A*03:01
A*02:10
A*02:07
A*02:06
A*02:05
A*02:03
A*02:01
A*01:01
0 5 10 15 20
B
HLA-B
B*81:01
B*67:01
B*58:01
B*57:01
B*56:01
B*55:02
B*54:01
B*52:01
B*51:06
B*51:02
B*51:01
B*50:01
B*49:01
B*48:01
B*46:01
B*44:03
B*44:02
B*40:50
B*40:40
B*40:06
B*40:03
B*40:02
B*40:01
B*39:01
B*38:02
B*38:01
B*37:01
B*35:03
B*35:02
B*35:01
B*27:07
B*27:05
B*27:04
B*18:01
B*15:32
B*15:27
B*15:25
B*15:18
B*15:17
B*15:13
B*15:12
B*15:11
B*15:07
B*15:05
B*15:03
B*15:02
B*15:01
B*14:02
B*14:01
B*13:02
B*13:01
B*08:01
B*07:05
B*07:02
0 2 4 6 8
C
HLA-C
C*17:01
C*15:05
C*15:02
C*14:03
C*14:02
C*12:03
C*12:02
C*08:03
C*08:02
C*08:01
C*07:06
C*07:04
C*07:02
C*07:01
C*06:02
C*05:01
C*04:03
C*04:01
C*03:04
C*03:03
C*03:02
C*02:02
C*01:06
C*01:03
C*01:02
0 5 10 15 20
HLA frequency (%)
D
HLA frequency (%)
0 20 40 60 80 100
A B C A+B A+C B+C A+B+C
<5%
>=5%

◀ **Figure EV5. Coverage of HLA molecules indicates the applicability of the integrative therapy in human.**

Allele frequencies of HLA-A (**A**), HLA-B (**B**), and HLA-C (**C**) in the 741 individuals from Han population residing in Shanxi Province of China. The data was downloaded from PGG.MHC website: https://pog.fudan.edu.cn/pggmhc/#/population, and all the alleles were plotted. The 5% cut-off value was indicated by the dotted line. Alleles with frequencies <5% and >=5% were, respectively, indicated by green and red columns. The coverage of alleles with frequencies >=5% was calculated and demonstrated as cumulative histogram (**D**), in which HLA-A, HLA-B, and HLA-C were, respectively, abbreviated as A, B, and C. The cumulative results (>=5%) were as follows: A (79.59%), B (36.84%), C (73.94%), A + B (85.94%), A + C (94.17%), B + C (81.27%), A + B + C (95.58%).